Mechanosensing defects and YAP-signaling in LMNA-related congenital muscular dystrophy

Dissertation

in fulfillment of the
requirements for the
Joint Degree (Cotutelle)

“Doctor rerum naturalium (Dr. rer. nat.)”

integrated in the International Graduate School for Myology “MyoGrad”
in the Department of Biology, Chemistry, Pharmacy at Freie Universität Berlin and in
Cotutelle Agreement with the
Ecole Doctorale 515 “Complexité du Vivant” at the
Université Pierre et Marie Curie Paris 6

Submitted by

Martina Fischer

Supervised by
Catherine Coirault and Prof. Dr. Petra Knaus

Thesis defense: June 28th, 2017

Thesis jury:
Thesis supervisor, UPMC: Catherine Coirault, PhD
Thesis supervisor, first reviewer of Freie Universität Berlin: Prof. Dr. Petra Knaus
Reviewer UPMC: Jean-Thomas Vilquin, PhD
Second reviewer, Freie Universität Berlin: Prof. Dr. Sigmar Stricker
External Expert: Prof. Dr. Henning Wackerhage
External Expert: Prof. Dr. Michael Gotthardt
External Expert: Prof. Dr. Markus Schülke-Gerstenfeld
Postdoctoral Fellow, Freie Universität Berlin: Dr. Maria Reichenbach

ACKNOWLEDGEMENTS

Firstly, I would like to express my sincere gratitude to my advisors Catherine Coirault and Prof. Dr. Petra Knaus for the continuous support of my PhD study, for their example, effort and knowledge. Their guidance was essential in all the time of my research and writing.

Besides my advisors, I would like to thank the members of my former thesis committee and todays defense committee: Prof. Dr. Sigmar Stricker, Prof. Dr. Michael Gotthardt, Prof. Dr. Henning Wackerhage, Jean-Thomas Vilquin, Dr. Maria Reichenbach, Danièle Lacasa, Athanassia Sotiropoulos, Sigolène Meilhac and Dr. Christian Hiepen for their insightful comments and support.

I also own thanks to the founders and coordinators of MyoGrad, especially Gisèle Bonne and Simone Spuler for creating this fruitful environment.

My sincere thanks also go to my collaboration partners and colleagues. Marc Osterland, guided by Dr. Steffen Prohaska and Prof. Dr. Christof Schütte, from the Zuse Institute Berlin, for a very inspiring and productive but just as cheerful collaboration.

I would also very much like to thank my fellow labmates in Berlin and Paris, especially Dr. Christian Hiepen, Dr. Gina Dörpholz und Dr. Maria Reichenbach for their skilled advice. Tsolere Arakelian, Paul Rikeit, Daniel Owens and Nada Essawy for their contribution and hands on support. And of course, I also thank the other lab members for stimulating discussions, motivation, friendship and all the fun I had with you in the last years.

Besonders möchte ich auch meiner Familie danken: Meiner Mutter Conny und meiner Schwester Andrea, die sich immer wieder selbst zurückgenommen haben um mir den nötigen Raum für diese Arbeit zu geben. Dabei danke ich besonders meiner Mutter, für ihren unerschütterlichen Glauben in mich und meiner Schwester, für ihr Verständnis, ihre Begleitung und dass es zwischen Paris und Berlin immer einen Platz für mich gab.

Last but not least, I would like to thank my friends for their encouragement throughout all my study and dissertation time. Jorien, Wanda, Ines, Ronja, Rui, Conny and Tim for distraction, confidence, loyalty, criticism and loads of fun.

This work would not have been possible without all your precious support!

TABLE OF CONTENTS

TABLE OF FIGURES

1. INTRODUCTION

1.1. LAMINOPATHIES

Mutations in the LMNA gene cause more than ten different disorders, commonly named laminopathies. Laminopathies are characterized by a great clinical as well as genetical variability. The first *LMNA* mutation associated with a genetic disorder was identified in autosomal dominant Emery-Dreifuss Muscular Dystrophy (EDMD) (Bonne et al. 1999). Since then, it has been shown that laminopathies can affect different tissues including adipose tissue, nervous system and skeletal and/or cardiac muscles but, also cause systemic disease, the premature aging syndromes. However, the most frequent diseases associated with LMNA mutations are characterized by skeletal and cardiac muscle involvement. Different phenotypes have been reported with skeletal muscle involvement: limb-girdle muscular dystrophy type 1B (LGMD1B), autosomal dominant Emery-Dreifuss muscular dystrophy (EDMD2) and a form of congenital muscular dystrophy (L-CMD) (Maggi, Carboni, and Bernasconi 2016). These disorders differ in term of age at onset, first symptoms and pattern of affected muscles. More than 200 mutations were identified to cause these disorders, which are spread throughout the whole *LMNA* gene. However, relationships between genotypes and phenotypes remain poorly understood (Bertrand et al, 2011). Mutations in lamin-binding proteins, including emerin and nesprins can also caused striated muscular dystrophies. These diseases are often refered as nuclear envelopathies.

LMNA-RELATED CONGENITAL MUSCULAR DYSTROPHY

LMNA-related congenital muscular dystrophy, or L-CMD, is a rare but especially severe early-onset form of laminopathies. Children, suffering from L-CMD, show a severe weakness and wasting of skeletal muscles with a characteristic development of a “dropped head” syndrome phenotype. This clinical phenotype is further described with selective axial weakness, wasting of the cervicoaxial muscles, proximal involvement of the upper limbs, distal involvement in lower limbs, an early development of talipes feet and a rigid spine with thoracic lordosis. Later, patients develop proximal contractures, sparing elbows and most children require ventilatory support. L-CMD patients frequently develop a cardiac dysfunction and ventricular arrhythmias in their second decade (Quijano-Roy et al. 2008). The onset of this disease is in the first years of life and rare cases

have been reported where children evolved no motor-development and already showed fetal immobility. Histopathological studies on patient biopsies revealed dystrophic changes and an abnormal variability of fiber size (Fig.1).

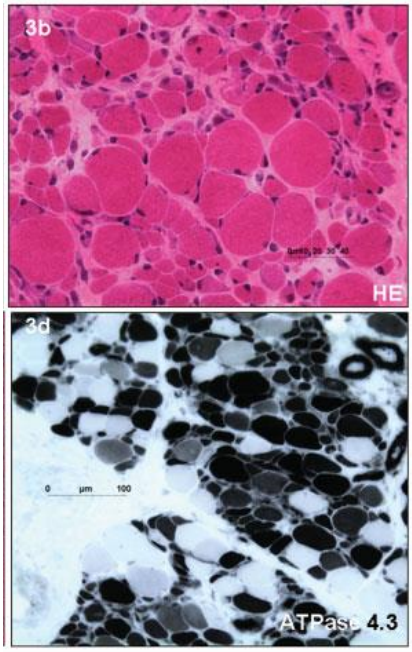

FIGURE 1-1: H/E and ATPase stained muscle sections from L-CMD patients. Modified from Quijano-Roy et al, 2008

L-CMD is caused by autosomal dominant *de novo* heterozygous mutations of the *LMNA* gene. The disease has been first described in 2008, by Quijano-Roy et al. In the 15 reported cases, more than 10 different *LMNA* mutations were identified to cause L-CMD (Quijano-Roy et al. 2008). Two of those patients have been identified to carry the p.delK32 mutation (ΔK32) (Fig. 2). A knock-in mouse model has been generated for this mutation as a model for L-CMD (Azibani et al. 2014). Heterozygous ΔK32 mice die between the ages of 10 and 20 months from dilated cardiomyopathy (CMD), a common late symptom of laminopathies. However, surprisingly, these mice do not show any spontaneous skeletal muscle defects (Cattin et al. 2013). In contrast, homozygous $LMNA^{\Delta K32/\Delta K32}$ mice exhibit a severe delay in striated muscle maturation and growth retardation. These mice die in their second week of life, most likely from metabolic defects (Bertrand et al 2012). The expression of lamin A/C protein is severely reduced in homozygous $LMNA^{\Delta K32/\Delta K32}$ mice (only 20% of Lamin A/C levels compared to WT). Moreover, mutant A-type lamins are mainly localized in the nucleoplasm rather than the nuclear envelope in $LMNA^{\Delta K32/\Delta K32}$ mice (Bertrand et al. 2012).

However, unlike the ΔK32 mouse model, which requires homozygous expression of the mutant lamin A/C (Bertrand et al., 2011), human cells from L-CMD patients carry only one mutated LMNA allele. Recent data show that L-CMD causing LMNA mutations impair the ability of muscle cell precursors to sense tissue stiffness and to respond to mechanical challenges (Bertrand et al. 2014). Moreover, LMNA-mutated myoblasts show enhanced activity of Yes-Associated protein (YAP)-dependent signaling which is paradoxically reduced after cyclic stretch (Bertrand et al. 2014). Furthermore, L-CMD mutant myoblasts failed to align in 3D soft matrix (Bertrand et al. 2014).

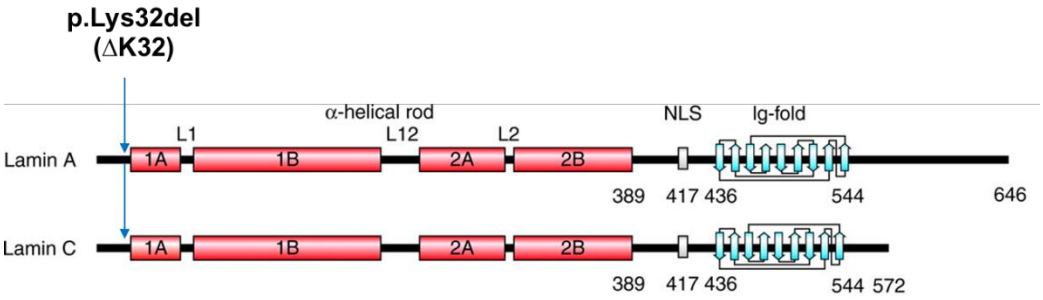

FIGURE 1-2: LaminA/C protein structure. The blue array indicated the side of the (ΔK32) mutation site. Modified from (Dechat et al. 2010)

A-TYPE LAMINS

L-CMD causing mutations are found all along the LMNA gene which codes for A-type lamins. A-type lamins contain at least three isoforms; lamin A, lamin C, and lamin AΔ10, which result from alternative splicing. A fourth isoform, lamin C2, has been found in rodents (Link et al. 2013). The two main isoforms are lamin A and lamin C (Fig. 2). The N-terminal domain of lamin A and C are identical for the first 566 amino acids whereas they differ at the C-terminal end. Lamin A derives out of the full-length transcript containing all 12 exons, whereas the lamin C transcript contains only the first 10 exons. The mature lamin A protein is synthesized through a precursor, prelaminA, which is farnesylated and corboxymethylated at the cystein residue at the C-terminal CAAX box. The C-terminal residues, including the farnesylated and carboxymethylated cysteine, are then cleaved off by Zmpste24/FACE1 to generate the mature lamin A. Mature lamin A and lamin C proteins share a N-terminal head domain, a central α-helical rod domain, a nuclear localization signal sequence (NLS) and an Ig-fold. Lamin A and Lamin C differ at their C-terminal end, with an 80 amino acids long unique region for lamin A. Lamin C has a unique six amino acids long region in the C terminal domain. (Dechat et al, 2008).

A-type lamins are type V intermediate filaments nuclear envelope. Together with B-type lamins they form the nuclear lamina, which is adjacent to the inner nuclear membrane. B-type lamins are farnesylated and form independent but interconnected networks with A-type lamins (Schermelleh et al. 2008; Goldberg et al. 2008; Shimi et al. 2008; Kolb et al. 2011). B-type lamins form a thin meshwork, which is associated with the inner nuclear membrane (INM) and the nuclear

pore complexes (NPCs), whereas A-type lamins form a thicker network (Davidson and Lammerding 2014)

A and B-type lamins differ in their solubility during cell mitosis as well as in their expression patterns. B-type lamins are expressed throughout development and differentiation, beginning in the unfertilized egg. In contrast, A-type lamins are only expressed at very low levels in early development and their expression increases with cell differentiation (Burke and Stewart 2002; Eckersley-Maslin et al. 2013). LaminA is expressed ubiquitously in vertebrate cells, however, expression levels vary largely between different tissues. LaminA expreesion levels are very low in the central nervous system whereas they are particularly high in muscle and other tissues derived from mesenchymal cells (Davidson and Lammerding 2014; Hanif et al. 2009; Yang et al. 2011)

To form the two-dimensional supramolecular structure of the nuclear lamina, mature lamin protein first assemble into dimers. These dimers polymerize into a polar head-to-tail polymer. A non-polar protofilaments is formed through lateral assembly of the dimers. This non polar protofilaments in turn assembles in an antiparallel order into intermediate filaments (Dechat et al. 2008). The laminA network is reversibly disassembled during mitosis in early G1 phase (Adam and Goldman 2012). The disassembly of the A-type lamina is regulated by phosphorylation of laminA (Dechat et al. 2000; Moir et al. 2000; Naetar et al. 2008). Two phospho-acceptor sites flank the central rod domain of A-type lamins at serine 22 and serin 392. Their phosporylation via the mitosis specific kinase Cdk1 drives the disassembly during nuclear envelope breakdown in the early stages of mitosis (Dessev, Iovcheva-Dessev, and Goldman 1990; Kochin et al. 2014).

The LMNA ΔK32 mutation corresponds to a deletion of a lysine on position 32, which is located in the highly-conserved coil 1A region at the N terminus of the first lamin rod domain. The ΔK32 muation in human corresponds to the ΔK46 in *c.elegans*. The ΔK46 mutation alters the lateral assembly of head to tail assembly polymers, which results in increased mobility and solubility of mutant lamins as well as in the formation of A-type lamins aggregates in the nuclear interior (Bank et al. 2012; Zwerger et al. 2013).

Several functions of A-type lamins have already been proposed. A-type lamins are crucial for the structural stability of the nucleus, the maintenance of nuclear architecture and nuclear

positioning. A-type lamins have further been implicated in the regulation of chromatin organization, epigenetics, gene transcription, cell cycle regulation, cell apoptosis and differentiation (Broers et al. 2006). In particular, A-type lamins have multiple functions through associations with chromatin, nuclear histones and various transcription factors to maintain chromatin structure and regulate gene expression (Meier et al. 1991; Verstraeten et al. 2007; Dechat et al. 2008). A-type lamins also directly bind to promoters both at the nuclear periphery and the nuclear interior (Lund and Collas 2013). According to the so called "gene regulation hypothesis", A-type mutations alter the regulation of different genes. This in turn may contribute to the clinical variability of laminopathies. The differentially expression of transcription factors in distinct tissues can thereby determine the affected tissue (Azibani et al., 2014)

Another hypothesis explaining LMNA related muscular dystrophies is the "structural hypothesis". The major functions attributed to nuclear lamins include the regulation of the nuclear size and shape by maintaining the structural integrity of the nuclear scaffold and by defining the mechanical properties of the nucleus (Dechat et al. 2008). Particularly, A-type lamins define the viscosity and stiffness of a nucleus (Davidson et al. 2014; Jevtić and Levy 2014). Nuclear deformability is sensitive to A-type lamin expression and laminopathies are associated with altered nuclear shape (Swift et al. 2013; Lammerding et al. 2006; Sullivan et al. 1999). An increased nuclear fragility in response to physical stress has been demonstrated in LMNA deficient cells (Lammerding et al. 2004; Broers et al. 2005; Nikolova et al. 2004; De Vos et al. 2011).

In contracting tissues, such as skeletal or cardiac muscles, disrupted nuclei may promote tissue damage and apoptosis. EDMD and DCM disease causing mutations led to decreased nuclear stiffness and increased nuclear deformation and ruptures *in vitro* and *in vivo* (Zwerger et al. 2013; De Vos et al. 2011; Gupta et al. 2010).

NUCLEO-CYTOSKELETAL COUPLING AND NUCLEAR MECHANOTRANSDUCTION

A third pathophysiological model has been proposed after the discovery of the nucleo-cytoskeletal coupling by the LINC complex (Linker of Nucleoskeleton to Cytoskeleton Complex). This multiprotein complex is composed of Sun and Nuclear Envelope Spectrin Repeat (NESPRIN) proteins, which span the nuclear envelope. At the inner surface of the inner nuclear membrane, Sun proteins bind to A-type lamin and emerin. In the luminal space, Sun proteins interact with Nesprins (Salpingidou et al. 2007; Östlund et al. 2009; Liang et al. 2011). At the cytoplasmic side, nesprinsconnect with actin-, microtubule- and intermediate filament networks of the cytoskeleton. Thereby, the LINC complex builds a physical connection between the nucleo- and cytoskeleton (Fig. 3). Although the molecular dynamics of the LINC complex remain largely unknown, critical functions of the LINC complex have been proposed. The positioning of the nucleus insight the cell during cell migration and the localization of synaptic nuclei in muscle fibers dependent on the LINC complex (Lombardi et al. 2011; Roux et al. 2009; Luxton et al. 2010; Lei et al. 2009; Yu et al. 2011).

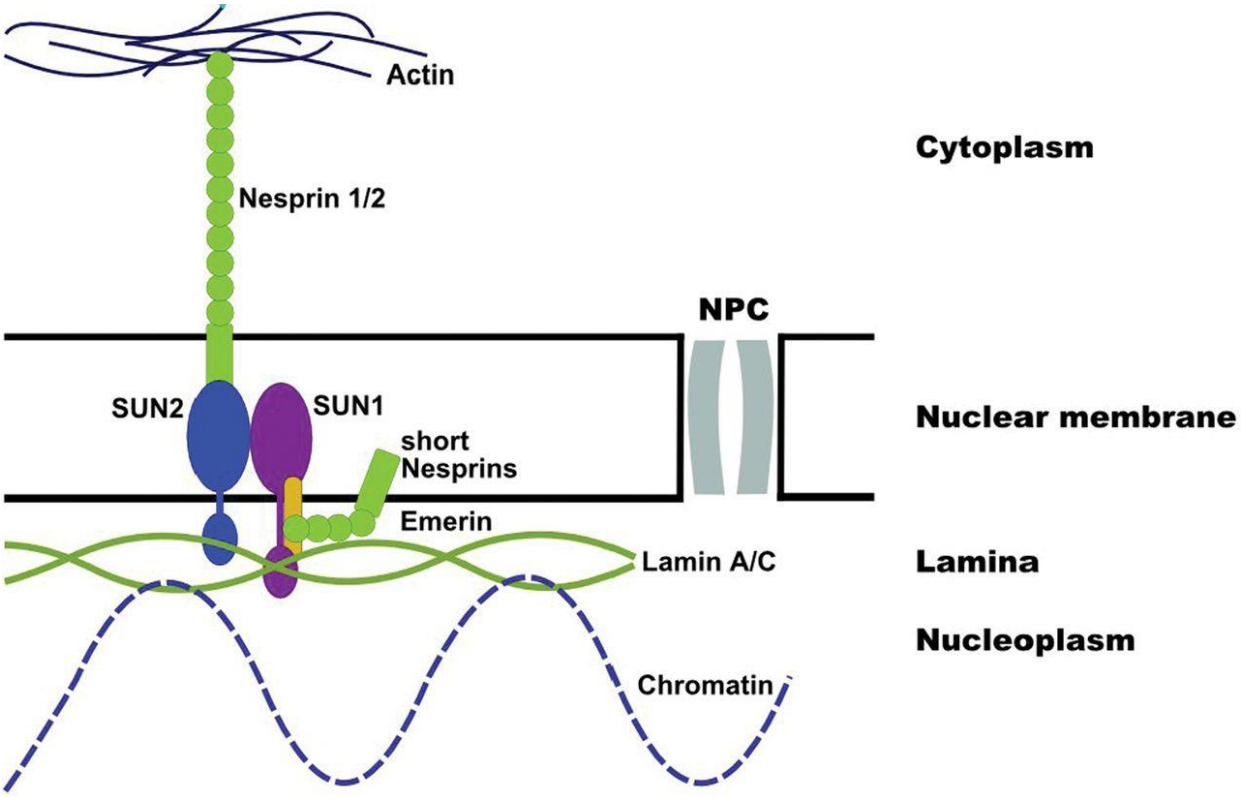

FIGURE 1-3: The LINC complex. Modified from (Meinke, Nguyen, and Wehnert 2011)

Furthermore, the LINC complex transmits mechanical forces between the cytoskeleton and the interior of the nucleus (Lombardi and Lammerding 2011; Zwerger et al. 2013). In a subset of muscular dystrophy causing LMNA mutations, the interaction between A-type lamin and Sun is disrupted (Haque et al. 2010). The force transmission between the nucleus and cytoskeleton is impaired in several myopathic lamin mutations (Zwerger et al. 2013). Furthermore, mutations in nesprin proteins cause muscular dystrophy. Taken as a whole, these studies strongly support the hypothesis that an impaired nucleo-cytoskeletal coupling contributes to the pathophysiology of LMNA related muscular dystrophies (Zhang et al. 2007). A possible mechanism regulating the nuclear resistance against mechanical rupture has recently been proposed. It has been shown that A-type lamin protein levels depend on the stiffness of the ECM and accumulate at the nuclear envelope after strain (Swift et al., 2013)

Lamin muations also affect the physical properties of the cytoskeleton, most likely due to defects in the nucleo-skeletal coupling. Lamin A/C-deficient cells have a reduced cytoskeletal stiffness and an aberrant organization of cytoskeleton networks. The actin cytoskeleton of LMNA deficient cells shows a severe disorganization in the perinuclear region, as found in cells with disrupted LINC complex (Broers et al. 2005; Lee et al. 2007; Khatau et al. 2009; Lammerding et al. 2004). A subset of actin fibers in the perinuclear region of adherent cells is called the “actin cap”. The actin cap consists of thick, actomyosin filament bundles which cover the top of the nucleus and are mostly aligned with the overall cell orientation (Khatau et al. 2009). These actin fibers are structurally and functionally distinct from dorsal and basal actin fibers, which do not directly connect to the nucleus (Hotulainen and Lappalainen 2006).

Altered actin dynamics have been reported in LMNA deficient and LMNA mutant cells (Chambliss et al. 2013; Ho et al. 2013). Moreover, the microtubule-, vimentin- and desmin network are also disorganized in LMNA deficient cells (Nikolova et al. 2004; Hale et al. 2008). This suggests that lamins do not only define the architecture of the nucleus, but also impact on the architecture of the whole cell through LINC complex mediated coupling between the nucleus and the cytoskeleton.

In addition, mechanical forces imposed on the nucleus modify the stiffness of the nuclear envelope (Guilluy et al., 2014) and modify the organisation of the nuclear lamina (Swift et al., 2013). These mechanical inputs can be the ECM stiffness or an applied strain generated by the cell environment (Guilak 1995; Maniotis, Chen, and Ingber 1997; Lombardi et al. 2011; Anno,

Sakamoto, and Sato 2012). Furthermore, altered signaling of mechanosensitive pathways have been reported in lamin A/C and emerin mutated cells. Expression of an emerin mutant alters serum response factor (SRF) dependent transcription (Guilluy et al. 2014). Consistently, lamin A-C and emerin regulate the nuclear localization of megakaryoblastic leukemia 1 (MKL1) and SRF-dependent transcription (Ho et al. 2013). Moreover, myoblasts carrying L-CMD causing mutations show enhanced YAP signalling activity (Bertrand et al. 2014). These results indicate that the nuclear mechanosensing may impact on regulating mechanosensitive pathways (Guilluy and Burridge 2015).

1.2. SKELETAL MUSCLE

Skeletal muscle is the most abundant tissue in our body and represents 40%-50% of the human body mass. It is a dynamic tissue which is able to adapt to exercise training or disuse. Furthermore, it has one of the best-studied stem cell-dependent regenerative process. After injury, a complex orchestrated regenerative response restores the cytoarchitecture (Cossu and Biressi 2005; Hawke and Garry 2001; Dhawan and Rando 2005). In contrast, skeletal muscle is subject to many degenerative disorders with only a few therapeutic options.

MYOGENESIS

In mice, embryonic myoblasts fuse and form the primary myofibers during primary myogenesis. Later, fetal myoblasts, which develop from distinct but related progenitors (Hutcheson et al. 2009), fuse with each other and form the secondary myofibers (Duxson, Usson, and Harris 1989). During secondary myogenesis, myofibers express different myosin heavy chain isoforms. This differential expression leads to future fast- and slow- muscles of the adult, which ultimately, defines the type, form and location of muscle fibers (Lyons et al. 1990). A third type of muscle precursor cells are the adult satellite cells which function as the precursors during muscle growth and regeneration (Lepper and Fan 2010; Murphy and Courtneidge 2011; Sambasivan et al. 2011). In postnatal muscle growth, activated satellite cells fuse with the ends of existing myofibers and thereby increase the size of the existing fibers (Kitiyakara and Angevine 1963; Williams and Goldspink 1971; Snow 1977; Cusella-De Angelis et al. 1994; Edom-Vovard et al. 1999; Tseng and Levin 2008).

SKELETAL MUSCLE REGENERATION

Regeneration in the skeletal muscle recapitulates some of the processes which occur during embryonic muscle development. In response to muscle injury, satellite cells start to proliferate, migrate, differentiate and fuse (Fig. 4) to regenerate the parent myofiber (Robertson et al. 1990; Schmalbruch 1976; Snow 1977; Papadimitriou et al. 1990; Robertson, Papadimitriou, and Grounds 1993). Newly regenerated myofibers are easily identified by the presence of centrally located nuclei. Part of satellite cells return to their normal position underneath the basal lamina of muscle fibres.

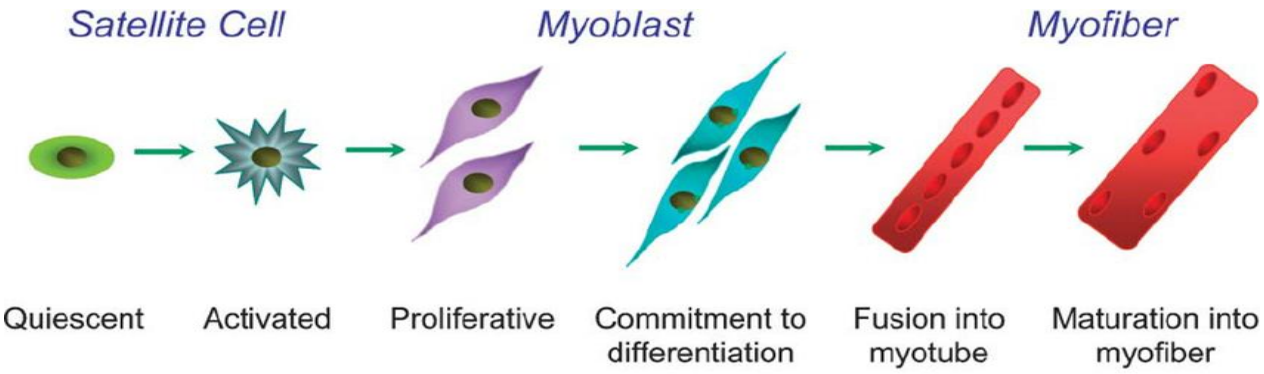

FIGURE 1-4: Satellite cell myogenesis (Zammit, Partridge, and Yablonka-Reuveni 2006).

SATELLITE CELLS

The mononucleated satellite cells gained their name based on their unique satellite position around the polynucleated myofiber (Mauro 1961). Satellite cells have a specialized niche in the adult skeletal muscle, localized between the basal lamina and the sarcolemma. The number of satellite cells is dependent on species, age, and muscle fiber type. Satellite cells are characterized by their small size, high nuclear to cytoplasm ratio, relative absence of cytoplasmic organelles, and increased nuclear heterochromatin. Moreover, these characteristics represent a quiescent state of the cell. Satellite cells are the adult stem cells of skeletal muscle tissue as they are able to rebuild their pool of quiescent satellite cells by asymmetric cell divisions (Moss and Leblond 1971; Schultz and Jaryszak 1985; Bischoff 1994; Kuang et al. 2006). When satelite cells are activated, for example in response to injury, they leave quiescence and proliferate as skeletal myoblasts

(symmetric divisions). Satellite cells are tightly regulated by transcription factors including Pax7, the most frequently used marker of satellite cells (Seale et al. 2000; Zammit et al. 2006). Beyond others, Pax7 controls the activation of satellite cells through induction of specific Myogenic Regulatory Factors (MRFs) (Sassoon 1993; Cornelison and Wold 1997a; Nicolas, Gallien, and Chanoine 1998; McKinnell et al. 2008). MyoD is one of the first transcription factors expressed in myogenic progenitors whereas myogenin expression is induced later (Davis, Weintraub, and Lassar 1987; Hopwood, Pluck, and Gurdon 1989; Kuang et al. 2007; Rhodes and Konieczny 1989; Wright, Sassoon, and Lin 1989; Jennings 1992).

MYOBLAST CELL-CELL-CONTACT SIDES

Cell-contact sites between myoblasts are formed by a macromolecular complex, which includes cadherins and catenins. Cadherins are membrane proteins, which contain two or more extracellular cadherin domains. The extracellular domains form a homotypical Ca2+-dependent binding with cadherins of neighboring cells, forming cell–cell adhesion (Leckband and Prakasam 2006; Pokutta and Weis 2007). Cell-cell junctions of myoblasts are distinct from the general adherence junction present in other cells, because myoblasts can fuse with each other. Two distinct stages are defined in cell-cell contact formation between myoblasts: Adherens Junction1 (AJ1) is a cell-cell contact, which is still in formation, whereas AJ2 corresponds to an already established cell-cell contact (Causeret et al. 2005). AJ2 is further characterised by the presence of actin stress fibers between adjacent cells and the presence of cadherin/catenin complexes which acuumulate at the intercellular junction (Fig 5).

The formation of cadherin clusters at intercellular junctions requires a myosin II dependent coupling of actin to cadherins (Shewan et al. 2005). The association between cadherin and actin stabilizes the cadherin clusters, thus preventing rupture. Cadherin cluster formation is a dynamic process, regulated by a dynamic competition between cluster formation, breakup, and endocytosis
.

CADHERINS

Cadherins provide the mechanical stability of cell-cell contacts. Cadherin regulation in skeletal muscle has mainly been studied during myoblast fusion. M-cadherin is expressed in a subpopulation of quiescent satellite cells. It is strongly up-regulated in activated satellite cells suggesting a potential function in skeletal muscle regeneration (Donalies et al. 1991; Irintchev et al. 1994; Cornelison and Wold 1997b). In myofibers *in vivo* and after completion of myotube formation in vitro, M-cadherin expression is downregulated (Beauchamp et al. 2000; Kuch et al. 1997; Pouliot, Gravel, and Holland 1994). Overexpression of M-cadherin in cadherin-deficient mouse fibroblasts resulted in enhanced calcium-dependent cell adhesion (Kostetskii et al. 2001). The M-cadherin mediated cell-cell interaction is necessary for the fusion of embryonic myoblasts and for the fusion of myoblasts with myofibers (Zeschnigk et al. 1995; Kaufmann et al. 1999; Wernig et al. 2004; Charrasse et al. 2006). Additionally, inhibition of M-cadherin homophilic cell-cell interactions inhibits myoblast fusion in a dose-dependent manner, without affecting the expression of differentiation markers (Zeschnigk et al. 1995).

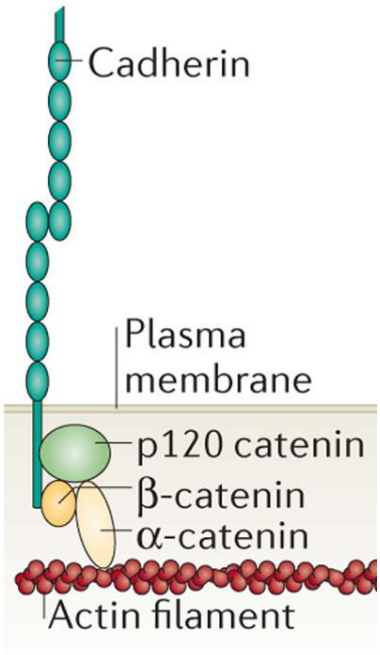

FIGURE 1-5: Cadherin/catenin complex. Modified from (Ratheesh and Yap 2012)

However, M-cadherin knockout mice showed a normal muscle development and regeneration. In this animal model, N-cadherin protein levels are increased which suggests that N-cadherin can compensate for the absence of M-cadherin (Hollnagel et al. 2002). In contrast, N-cadherin knockout mice die before E10, with disorganized somites. Therefore, skeletal muscle development cannot be investigated in N-cadherin knockout mice (Radice et al. 1997).

Primary N-cadherin null myoblasts differentiate and fuse normally. This is most likely due to the increased expresssion of other cadherins including M-cadherin (Charlton et al. 1997). N-cadherin is found at the cell-cell contacts in AJ1 where it accumulates during AJ2. Furthermore, N-cadherin mediated-adhesion has a major role in the induction of skeletal muscle differentiation

(Knudsen, Myers, and McElwee 1990; Mege et al. 1992; George-Weinstein et al. 1997; Redfield, Nieman, and Knudsen 1997; Goichberg and Geiger 1998).

Cadherins are also adhesion-activated signaling receptors. Rho-family GTPases are regulated by cadherins (Wheelock and Johnson 2003). In turn, RhoA activity regulates M-cadherin stability (Charrasse et al. 2006). Rho GTPases are potent regulators of actin dynamics and are involved in the regulation of myoblast fusion and the induction of myogenesis (Luo et al. 1994; Hakeda-Suzuki et al. 2002; Charrasse et al. 2003; Fernandes et al. 2005). The coordinated regulation of RhoA and Rac1 during myogenesis induction has been shown to be orchestrated by N-cadherin (Charrasse et al. 2002). Furthermore, M-cadherin also regulate Rac1 activity during myoblast fusion (Charrasse et al. 2007).

CATENINS

The cytoplasmic domains of cadherins interact with β-catenin, which is a central structural adaptor protein that links cadherins to the actin cytoskeleton (Fig 5). Furthermore, β-catenin is a key transcription co-factor in the Wnt signaling pathway (Gottardi and Gumbiner 2001). β-catenin localizes to myoblast cell-cell contact sites. In muscle cells, the M-cadherin/ β-catenin complex also interacts with microtubules (Vasyutina et al. 2009).

The cadherin/ β-catenin complex is involved in the alignment of myoblasts during fusion (Kaufmann et al. 1999). β-catenin-mediated Wnt signaling is involved in muscle development (Cossu and Borello 1999; Zhang et al. 2012). Moreover, β-catenin is essential for myoblast differentiation (Wróbel, Brzóska, and Moraczewski 2007). In non-proliferating satellite cells, β-catenin is located at the cell membrane. In proliferating cells β--catenin translocates to the nucleus where it acts as transcriptional coactivator in the canonical Wnt signaling pathway (Otto et al. 2008; Zammit 2008). β-catenin/Wnt signaling regulates the expression of myogenic regulatory factors (MRFs) such as MyoD and Inhibitor of Differentiation 3 (ID3) (Zhang et al. 2012). Blocking Wnt/β-catenin signaling in proliferating cells decreases proliferation and inhibits myoblast fusion (Suzuki, Pelikan, and Iwata 2015). Cadherins can inhibit the activity of β-catenin/canonical Wnt signaling (Simcha et al. 2001; Stockinger et al. 2001; Kuphal and Behrens 2006; Sadot et al. 1998). Specifically, knockdown of M-cadherin in C2C12 myoblasts increases the phosphorylation of β-

catenin at Ser33/37/Thr41 by GSK-3β. The increased phosphorylation enhances myogenic differentiation induced by canonical Wnt signaling (Wang, Mohamed, and Alway 2013).

1.1. MIGRATION

MYOBLAST MIGRATION

Myoblast migration is crucial for the achievement of cell–cell adhesion and for the formation and growth of myotubes *in vitro* (Kang et al. 2004; Bae et al. 2008; Jansen and Pavlath 2006; Mylona et al. 2006; O'Connor et al. 2007; Griffin et al. 2010b). Myoblast fusion generally occurs between muscle cells that are initially positioned at a distance from each other. *In vivo*, during development in mice, myoblasts often migrate long distances to form muscles in the limbs (Dietrich 1999; Christ and Brand-Saberi 2002; Birchmeier and Brohmann 2000). During muscle regeneration, myoblasts migrate towards a myotube target (Fig.6). In *vivo*, live-cell imaging of satellite cells provides direct evidence for myoblast migration in adult muscles (Ishido and Kasuga 2011). Myoblast migration is guided by attractants secreted by the damage myofiber (Bondesen et al. 2007; Griffin et al. 2010b). Proper cell fusion requires precise regulation and localization of myogenic progenitor cells (Krauss et al. 2005).

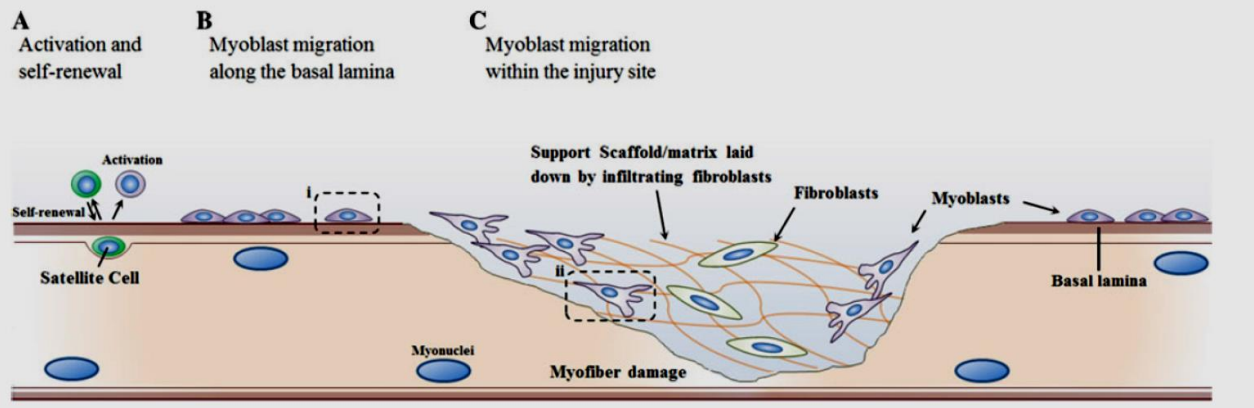

FIGURE1-6: Myoblast migration during muscle repair (Goetsch, Myburgh, and Niesler 2013).

In vitro, satellite cells migrate extensively on their associated myofibers and concurrently express receptors for chemorattractants (Siegel et al., 2009). Moreover, myoblasts cultured on artificial substrates are motile and migrate in response to different chemokines and growth factors (Corti et al. 2001; Ödemis et al. 2007; Griffin et al. 2010b; Robertson, Papadimitriou, and Grounds

1993; Bischoff 1997; Lee et al. 1999; Villena and Brandan 2004; Nedachi et al. 2009; Tokura et al. 2011). Thereby, specific chemoattractants have been identified which modulate the velocity or direction of migrating cells (Horsley et al. 2003; Jansen and Pavlath 2006; Lafreniere et al. 2006; Griffin, Kafadar, and Pavlath 2009). Moreover, cell differentiation modulates cell motility. Myocytes are less motile and less senitive to many inducers of migration than myoblasts (Powell 1973; Nowak et al. 2009; Griffin et al. 2010a).

Collective cell migration is a type of migration in which a group of cells move together without completely losing their cell-cell contacts. To move in a collective manner, individual cells need to align and synchronize their movements relative to that of their neighbouring cells. To date, the role of cell-cell interactions during myoblast migration and the collective character of myoblast migration has rarely been studied. C2C12 cells do not endogenously exhibit a collective cell migration as epithel or endothel cells do (Plutoni et al. 2016). However, live-cell imaging of C2C12 cells revealed that fibronectin promotes a directional collective migratory behavior (Vaz et al. 2012). In addition, transplanted myoblasts *in vivo* migrate as groups of cells (El Fahime et al. 2000).

ROLE OF CADHERINS IN COLLECTIVE MIGRATION

Classic cadherins play a central role in cell–cell contact and adherence junction formation. Therefore they are important factors of collective cell migration (Halbleib and Nelson 2006). It has been shown that collective cell migration depends on both growth factor-mediated regulation and mechanically-stimulated regulation (Theveneau and Mayor 2013; Wang et al. 2010; Reffay et al. 2014; Hidalgo-Carcedo et al. 2011; Das et al. 2015; Bianco et al. 2007). Self-generated chemokine gradients appear to be essential to guide cells through this process (Dona et al. 2013).

Mechanical stress is produced at cell-cell junctions during collective cell migration (Tambe et al., 2011). Moreover, it has been shown that neighbouring cells join forces together to migrate along a minimum of intercellular shear stress. Thus, collective cell migration is guided by intercellular mechanical stress. This intercellular stress has been shown to be cadherin dependent (Tambe et al. 2011). Moreover, it has been proposed that mechanical coupling between migratory cells induce force-dependent signals, which may in turn contribute to the orchestration of collective cell movement (Hirashima et al. 2013; Mertz et al. 2012; Tambe et al. 2011).

However, precise mechanosensitive pathway, which translates cadherin mediated cell–cell adhesion into cell motion, remains to be elucidated. Recent data indicate that cadherin rich pertrusions (cadherin fingers) guide cells during collective cell migration from (Hayer et al., 2016). Local actin polymerization and membrane tension regulate the formation of cadherin fingers. Furthermore, P-Cadherin, another cadherin isoform, has been shown to promote collective cell migration (Plutoni et al. 2016). P-Cadherin is not expressed in C2C12 cells or satellite cell-derived myoblasts. However, P-cadherin is expressed in muscle progenitor of mice embryos during early myogenesis which suggests a collective migration of muscle precursor cells during early myogenesis.

As stated before, cadherins are largely regulated by Rho family GTPases. Rho GTPases play a key role in the coordination of collective migration (Weber, Bjerke, and DeSimone 2012; Das et al. 2015). RhoA and RhoE activity seem to be involved in decreasing the contractility at cell–cell contact sites between migrating cells, which guides collective cell migration (Hidalgo-Carcedo et al. 2011; Omelchenko and Hall 2012; Tanbe et al., 2011). Moreover, a correlation between E-cadherins and Rac1 activity has been shown in border cell migration, which hints to a Rac1 dependent formation of cell-cell conatcts during migration (Cai et al. 2014). The development of a front-to-rear polarity in the cytoskeletal architecture is crucial for both single and collective cell migration. Polarity is regulated by zone-specific activitivation of small Rho GTPases (Das and Spatz 2016). Furthermore, expression of P-cadherin in C2C12 cells increases the collective character of migrating C2C12 cells through activation of another Rho GTPase, Cdc42 (Plutoni et al., 2016).

MECHANOBIOLOGY

Mechanobiology is an emerging scientific field which describes how cell and tissues sense and respond to physical forces. Mechanobiology comprises the processes of mechanosensing and mechanotransduction. “Mechanosensing” refers to the sensing of mechanical properties of the environment by the cell, while “mechanotransduction” refers to the conversion of mechanical signals into biochemical signals. Mechanical stimuli obviously regulate skeletal muscle tissue as muscle grows in response to exercise and degrades if not used. Therefore, the importance of “mechanotransduction” in skeletal muscle homeostasis was first described more than 30 years ago

(Goldberg 1968; Vandenburgh and Kaufman 1979). More recent studies reveal that aside from biochemical inputs, all tissues and cells are regulated by the physical properties of their environment. In recent years, mechanotransduction has emerged as a major field in biomedical studies.

Several models have been developed to describe mechanisms involved in mechanotransduction. In one of them, the cell itself is considered as a compartmentalized mechanical body with given physical properties such as its viscosity, elasticity or stiffness. These cellular mechanical properties are primarily defined through the organization and dynamics of the cyto- and nucleoskeleton. The cytoskeleton, in turn, is connected to the extracellular matrix (ECM) and to neighboring cells by cell surface multiprotein complexes such as focal adhesions or cell-cell junctions. Through these connections, cellular mechanics are in a permanent coordination with extracellular constraints. Actin fibers, microtubules and intermediate filaments are components of the cytoskeleton. Thereof, actin dynamics evolved to be the most important modulators of mechanotransduction (Ramaekers and Bosman 2004). Changes in extracellular mechanics are simultaneously translated into various cell processes which are regulated by cytoskeletal dynamics including cell morphology or the activation of signaling pathways (Fischer et al. 2016).

1.2.YAP

The transcriptional co-activator YAP (Yes-Associated Protein) is one of the most prominent signaling proteins involved in mechanobiology. In its unphosphorylated active confirmation, YAP, together with its paralog TAZ (Transcriptional co-activator with PDZ-binding motif), localizes to the nucleus and regulates the activity of several transcription factors, the most important being TEAD family transcription factors (Zhao et al. 2008) (Fig.7). Prominent target genes of YAP include CTGF, Cyclin D1, myogenic transcription factor Myf5 and also the expression of contractile proteins like β-myosin heavy chain or skeletal α-actin (Dong et al. 2007; Watt et al. 2010).

YAP controls a wide range of cellular functions. During embryogenesis in mice, YAP is expressed at all stages. Homozygous disruption of the YAP allele in mice results in embryonic lethality at E8.5 (Morin-Kensicki et al. 2006). Nuclear YAP activity typically drives proliferation and survival and inhibits apoptosis (Dong et al. 2007). YAP mediates cell contact inhibition. Therefore, YAP activity regulates organ size *in vivo* (Zhao et al. 2007; Camargo et al. 2007; Dong

et al. 2007). As a regulator of the cell-cycle, aberrant regulation of YAP can lead to tumorigenesis including skeletal muscle cancer (Dong et al. 2007; Tremblay et al. 2014) Furthermore, YAP is involved in cell fate decisions in different progenitor cell pools, including satellite cells of skeletal muscle, in which YAP activity promotes proliferation and blocks differentiation (Camargo et al. 2007; Cao, Pfaff, and Gage 2008; Watt et al. 2010; Schlegelmilch et al. 2011).

YAP also influences cell migration. It was shown that YAP overexpression in MCF10A or HEK293 cells increases migration. Moreover, YAP knockdown abolishes migration in T47D cells and renal carcinoma cell lines (Haskins, Nguyen, and Stern 2014; Schutte et al. 2014; Sorrentino et al. 2014; Moroishi et al. 2015). As regulator of proliferation, apoptosis and migration, YAP is an important player in regenerative processes in different tissues including heart muscle tissue, where YAP knockdown severely impairs the regenerative capacity (Xin et al. 2013; Mateus et al. 2015). In brief, YAP is a regulator of the cell cycle and cell fate decisions and consequently, a regulator of development, organ size and tumorigenesis.

YAP is overexpressed in many human cancers. Different YAP overexpression systems are used to analyze YAP function or regulation. However, the regulation of YAP gene expression itself is nearly completely unknown. YAP is ubiquitously expressed in a wide range of tissues, except in peripheral blood leukocytes (Komuro et al. 2003). Regarding YAP protein level regulation, a few transcription factors or binding sites have been identified in the YAP promotor region (Wu et al. 2013; Danovi et al. 2008). This includes the β-catenin/TCF4 complex which can control YAP gene expression by binding of this complex to a DNA enhancer element within the first intron of the YAP gene (Konsavage et al. 2012). Above that, microRNAs target YAP mRNA and have been shown to suppress YAP mRNA and protein levels (Liu, Poon, and Luk 2010).

UPSTREAM REGULATION

Canonical regulation of YAP activity depends on the Hippo pathway activity. At the core of the mammalian Hippo pathway is a kinase cassette containing Mammalian Ste20-like 1/2 kinase (MST1/2) and large tumor suppressor 1/2 kinase (LATS1/2) (Fig. 7). YAP activity is regulated by phosphorylation at five different phosphorylation sites which are located in HXRXXS consensus motifs for LATS1/2 kinases. The widely studied LATS mediated phosphorylation is at Serine 127. This phosphorylation leads to binding of YAP to the 14-3-3 proteins, and consequently sequestration of YAP in the cytoplasma (Zhao et al. 2007). In addition, phosphorylation at Serine

381 by LATS1/2, primes YAP for further phosphorylation by casein kinases CK1δ or CK1ε and subsequent ubiquitination via SCFβTRCP E3 ubiquitin ligase and proteasomal degradation (Zhao et al. 2010). LATS1/2 kinases are canonically activated by phosphorylation of activated MST1/2 kinases (Visser and Yang 2010). YAP activity is balanced through a negative feedback loop. YAP dependent TEAD activity induces LATS2 kinase expression and activation which, in turn, leads to phosphorylation and inactivation of YAP (Moroishi et al. 2015).

A specific Hippo receptor, as the primary trigger of the Hippo signaling cascade, has not been identified. Moreover, the dependence of YAP regulation on Hippo signaling in several contexts including mechanotransduction has been questioned (Aragona et al. 2013). However, a great number of other YAP activators and inhibitors are known, although their interplays are not completely uncovered today (Fig. 7).

GPCR RECEPTORS

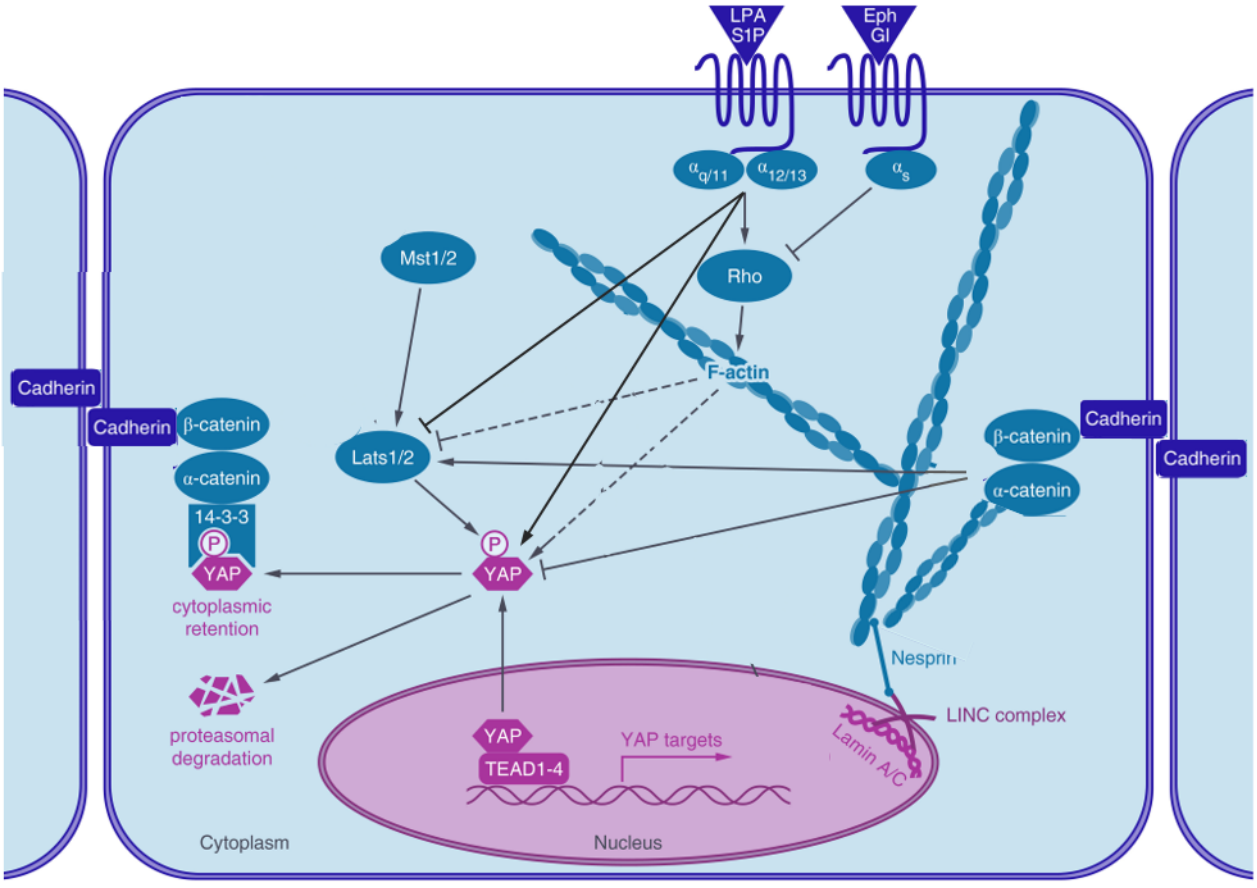

FIGURE 1-7: Regulation of YAP activity. Modified from (Fischer et al. 2016)

YAP can be regulated through G-protein coupled receptors (GPCRs). Lysophosphatidic acid (LPA) or sphingosine-1-phosphate (S1P) are components of fetal calf serum which is a supplement of most culture media. LPA and S1P can activate G12/13-, Gq/11-, and Gi/o-coupled receptors. Hence, fetal calf serum can activate YAP/TAZ so that serum starvation inhibits YAP activity via reduced GPCR signaling (Yu et al. 2012) (Fig. 7).

The exact pathway by which GPCRs act on YAP is still under review. Regulation of YAP by G-proteins has been shown to be either mediated by the Rho family of GTPases, actin dynamics and LATS (Yu et al. 2012) or by the PI3-kinase (PI3K) and phosphoinositide-dependent kinase (PDK1) (Gumbiner and Kim 2014).

CONTACT INHIBITION OF PROLIFERATION (CIP)

Cells grown at low density show a nuclear localization of YAP, whereas, in high cell density, YAP is sequestered in the cytoplasm (Zhao et al. 2011). This has been consistently shown in several cell types including, epithelial and mesenchymal cells (Zhao et al. 2007; Schlegelmilch et al. 2011; Kim et al. 2011). In *drosophila,* overexpression of YAP can overcome contact inhibition of proliferation (CIP), leading confluent cells to over-proliferate. Thereby, overexpression of the wildtype YAP drosophila analogue Yki led to an in transcriptional activity but only a slight increase in organ size. In contrast, the overexpression of the S127A phosphorylation-deficient mutant of Yki lead to significant increase in organ size (Zhao et al. 2007).

The canonical Hippo signaling in mammals has been first described in the context of CIP. It has been shown that YAP inactivation and cytoplasmic retention is mediated by activation of the Hippo pathway. More precisely, LATS1/2 mediated phosphorylation of YAP at Serine 127 induced ist inactivation (Zhao et al. 2007). Consistently, overexpression of LATS1 induces the cytoplasmic translocation of YAP even in sparse cells. Moreover, LATS knockdown induces a nuclear localization of YAP even in dense culture (Kim et al. 2011). However, the involvement of LATS in CIP has been more recently questioned (Silvis et al., 2011; Schlegelmilch et al.,2011, see below).

CADHERIN-CATENIN MEDIATED YAP INACTIVATION

The cadherin-catenin protein complex at cell-cell contact sites has been identified as the key transmitter of cell-cell contact into YAP inactivation during CIP. The cytoplasmic tail of cadherins interacts with catenins, β-catenin, p120-catenin, γ-catenin and α-catenin (Fig 5). Catenins therefore connect cadherins to the actin cytoskeleton (Perez-Moreno and Fuchs 2006; Pokutta and Weis 2007; Nishimura and Takeichi 2009).

Catenins also serve as signaling molecules (Perez-Moreno and Fuchs 2006). β-catenin has a dual role in cell adhesion and transcriptional coactivator of the WNT-signaling pathway (Nelson and Nusse 2004; Bienz 2005). The p120-catenin, β-catenin and γ-catenin are armadillo family proteins, whereas α-catenin differs notably in both sequence and structural organization. The armadillo family catenins bind directly to the cytoplasmic tail of cadherins, whereas, α-catenin connects to the complex via binding to β-catenin or γ-catenin. Only α-catenin binds to actin, thus, providing the link to the actin cytoskeleton (Fig. 5).

Most studies have investigated the effects of E-cadherin, the prototypical epithelial cadherin on YAP (McClatchey and Yap, 2012). However, a similar inhibition of proliferation has been reported for VE-cadherin, suggesting that other members of the cadherin family share the role of E-cadherin in CIP (Caveda et al. 1996; Lampugnani et al. 2006).

Expression of E-cadherins and their association with α- and β-catenin are required for density dependent nuclear exclusion of YAP (St Croix et al. 1998). The absence of E-cadherin in cancer cell lines correlated with decreased inhibition of proliferation. Vice versa, expression of E-cadherin in cadherin-deficient cell lines inhibited cell proliferation (St Croix et al. 1998). *In vivo*, immunostaining of sections of human keratoacanthoma tumors for YAP revealed a significant correlation between low E-catenin abundance and nuclear Yap1 localization (Silvis et al. 2011).

Furthermore, YAP relocates to the nucleus in confluent monolayers *in vitro* when cadherin dependent cell–cell connections are disrupted by removing extracellular calcium (Schlegelmilch et al. 2011). It has been demonstrated that protein levels of E-cadherin tune the efficacy of CIP (Kim et al. 2009). Moreover, expression of a mutant E-cadherin lacking the β-catenin binding domain, is not sufficient to inactivate YAP and a knockdown of β-catenin prevents YAP nuclear exclusion. In addition, ß-catenin knockdown decreases YAP phosphorylation at Serine 127 and its nuclear accumulation. Finally, it has been shown YAP inactivation by homophilic ligation of E-Cadherins was shown to further require α-catenin and LATS, but not MST (Kim et al. 2011) (Fig. 7).

Several studies indicate that cell-cell contact-induced inactivation of YAP occurs downstream of LATS, independently of the Hippo pathway. An indirect interaction between α-catenin and YAP has been shown which involves the binding of YAP to the 14-3-3 protein and subsequently, the cytoplasmic retention of YAP (Silvis et al., 2011; Schlegelmilch et al.,2011). Accordingly, α-catenin has been shown to be crucial for nuclear exclusion of YAP in high contact conditions. Knockout of α-catenin increases nuclear localization of YAP and cell proliferation, which can only be abolished by knockdown of YAP but not LATS or MST (Silvis et al., 2011) Moreover, Schlegelmilch et. al. do not find a reactivation of YAP after depletion of MST or LATS in keratinocytes (2011). In both studies, increased LATS activity does not correlate with YAP inhibition. These findings lead to the proposal that α-catenin controls YAP activity by modulating the interaction between YAP and the 14-3-3 protein. Thus, cytoplasmic retention of YAP in high contact conditions occurs independent of LATS activity (Schlegelmilch et al., 2011) (Fig. 7). Conflicting data also exist regarding the phosphorylation of YAP at serine 127 during CIP.

After knockdown of α-catenin, decreased levels of phosphorylated YAP are found alongside stable total YAP levels by Schlegelmilch and colleagues. Further investigations revealed a dephosphorylation of YAP after α-catenin knockdown by the protein phosphatase PP2A (Schlegelmilch et al., 2011). In contrast, Silvis et. al. find that α-catenin knockout cells have reduced levels of phosphorylated but also total YAP protein. They conclude that the phosphorylation of YAP at serine 127 is not involved in CIP (Silvis et al.,2011). Not only do cell-cell contacts regulate YAP activity but, interestingly, adherence junction formation has been recently shown to be regulated by YAP activity. In detail, YAP overexpression alters the assembly of adherence junction *in vivo*. Moreover, in primary hepatocytes *in vitro*, YAP overexpression was shown to antagonized E-cadherin junction assembly by regulating actin cytoskeleton architecture through myosin light chain expression (Bai et al. 2016).

YAP REGULATION IN MECHANOTRANSDUCTION

Increasing evidence points to YAP as the key regulator of mechanotransduction. Piccolo and collegues have shown that mechanical forces can serve as inputs for the regulation of YAP. YAP localization and transcriptional response is regulated by ECM stiffness or cell-spreading (Dupont et al. 2011) (Fig.8). It has been proposed that actin dynamics play a critical role of in the regulation

of YAP by mechanical cues. In particular, YAP activity has been correlated with the presence of actin stress fiber. YAP is inactivated after F-actin depolymerization or Rho inhibition but not after disruption of the microtubules network (Dupont et al., 2011; (Halder, Dupont, and Piccolo 2012; Zhao et al. 2012). Similarly, *in vivo*, in Drosophila, increased actin stress fiber assembly correlates with YAP nuclear localization and overgrowth of the wing disc (Sansores-Garcia et al. 2011; Fernandez et al. 2011). However, the specificity of this effect and the mechanism linking stress fiber formation to YAP activity is controversial and the focus of ongoing research.

YAP regulation by cell morphology and cell-contact inhibition has been further investigated in epithelial cells. It has been shown that mechanical forces overarch the regulation of YAP by CIP. YAP can be inactivated by a reduced cell spreading area, independent of cell-cell contacts. Using microdomain culture system, in which the cell area is defined whereas the formation of cell-cell contacts are prevented, the group of Sasaki has proposed a model where cell morphology alone modulates YAP activity (Wada et al. 2011). Moreover, the requirement of focal adhesion sites for the regulation of YAP by cell morphology was excluded by seeding epithelial cells on poly-lysine (Zhao et al. 2012).

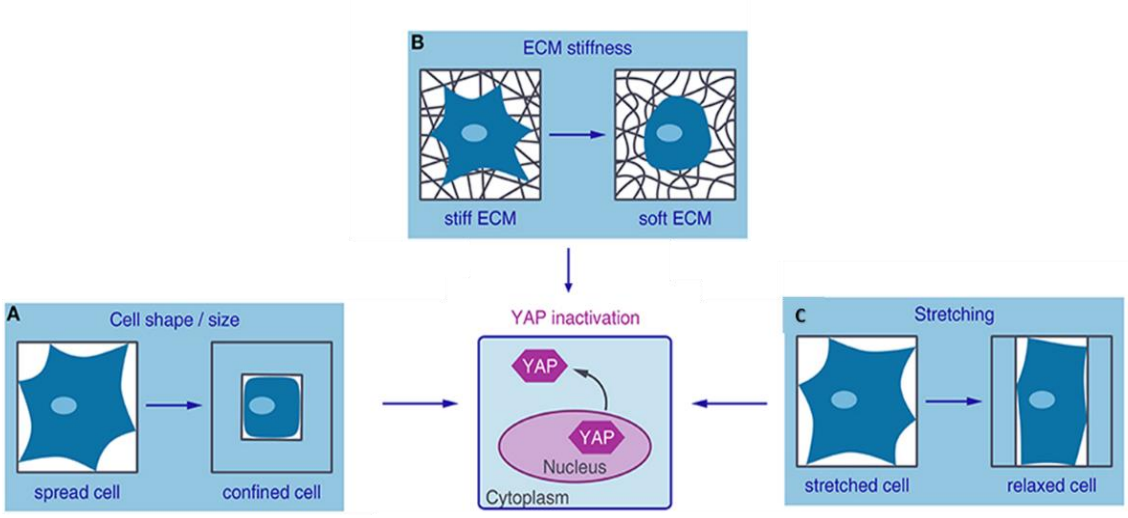

FIGURE 1-8: YAP regulation in mechanotransduction. Modified from (Fischer et al. 2016)

The actin-capping and -severing proteins; Cofilin, GapZ, and Gelsolin have been identified as gatekeepers by limiting YAP activity in cells which experience low mechanical stress. By depleting actin-capping/severing proteins, actin stress fiber formation increases and YAP activity in dense monolayers is restored (Aragona et al. 2013). In addition, YAP activity can be reactivated in postconfluent culture conditions by stretching the cells while preventing cell-cell contact loose

(Aragona et al., 2013). Cyclic stretching can reactivate YAP on soft surfaces and is associated with an increase in cell spreading, stress fiber formation and proliferation (Cui et al. 2015) (fig. 8).

YAP nuclear translocation is further dependent on nuclear mechanotransduction through the LINC-complex. Transfer of the strain to the nucleus is essential for YAP localization and activity. YAP nuclear re-localization after strain can be prevented by knocking down Nesprin, a protein of the LINC-complex (Driscoll et al. 2015). Consistently, satellite cell-derived myoblasts carrying a mutation in A-type lamins were unable to activate YAP after cyclic stretch (Bertrand et al. 2014).

YAP SIGNALING IN SKELETAL MUSCLE MOGENESIS AND HOMEOSTASIS

YAP transcriptional activity is important in myogenesis, muscle homeostasis and muscle disorders. YAP activity in muscle tissue activates the muscle promoter elements MCAT. MCATs are regulated by TEAD family transcription factors and are found in promotors of genes coding for contractile proteins suchas β-myosin heavy chain or skeletal α-actin and regulators of myogenic differentiation (Myf5, Mrf4, myogenin) (Mar and Ordahl 1988; Yoshida 2008; Ribas et al. 2011; Benhaddou et al. 2012).

In muscle development, regeneration and homeostasis activated satellite cells expand, migrate, differentiate and fuse with existing myofibers (Zhang and McLennan 1994). A differential regulation of YAP activity during satellite cell maturation has been shown, in vitro and ex vivo, on murine myoblasts. High YAP activity promotes proliferation of activated muscle progenitor cells whereas, YAP inactivation is needed for myogenic differentiation. YAP localization was primarily nuclear in proliferative culture. After myogenic differentiation YAP localization was cytoplasmic, and differentiated muscle fibers showed decreased Yap mRNA, YAP protein levels and increased YAP phosphorylation (Watt et al. 2010; Judson et al. 2012).

Moreover, YAP knockdown reduced proliferation of satellite cell-derived myoblasts. However, it had no impact on the progression of myoblast differentiation (Nagata et al. 2006). Furthermore, YAP overexpression led to inhibition of MyoD expression (Gee et al. 2011). In skeletal myofibers, YAP levels were reduced during postnatal maturation (Watt et al. 2015). Mechanistically, overexpression of constitutively active YAP *in vitro,* in myoblast precursors, resulted in increased Cyclin D1 and Myf5 expression as well as in decreased expression of

myogenin, which inhibits terminal myogenic differentiation (Ishibashi et al. 2005; De Falco and De Luca 2006; Watt et al. 2010).

Interestingly, standard culture conditions for myoblast differentiation show striking similarities with those for YAP inactivation. Indeed, myoblast differentiation preferentially occurs at high cell density, reduced serum concentration and substrates softer than standard cell culture plastic (Yaffe and Saxel 1977; Kaushik and Engler 2014).

In sections of healthy muscle tissue, YAP staining is weak and predominantly cytoplasmic (Crose et al. 2014). This suggests that YAP activity does not play a transcriptional role in the function of adult muscle. However, conflicting data on the role of YAP in adult muscle homeostasis and muscle mass regulation have been reported (Judson et al. 2013) (Watt et al. 2015) (Goodman et al. 2015). High levels of a constitutively active YAP led to degeneration, atrophy and necrosis of skeletal muscle fibers after use of a skeletal muscle fiber but not satellite cell specific knock-in mouse model. Interestingly, this muscle wasting phenotype is largely reversible. Gene expression profiling of these mice show similarities to muscles from mdx mice, a model for Duchenne muscular dystrophy (Hoffman, Brown, and Kunkel 1987). In contrast, it was found that YAP is a positive regulator of skeletal muscle size through a TEAD-dependent but mTOR-independent regulation of protein synthesis.

In a chronic mechanical overload model in mice, YAP expression and phosphorylation is increased, supporting the hypertrophic role of YAP in muscle. Vice versa, overexpression of YAP in the mouse tibialis anterior lead to hypertrophy. In addition, increasing muscle mass, through the blocking of myostatin and activin signaling in mice *in vivo*, increases total YAP and YAP phosphorylation. Finally, also physical exercises increase YAP phosphorylation in mouse limb muscles (Hulmi et al. 2013). Together, these results suggest that YAP is inactivated in healthy adult skeletal muscle tissue and that YAP is reactivated, most probably in satellite cells, for muscle growth and regeneration.

YAP signaling defects have been implicated in other skeletal muscle diseases. Rhabdomyosarcomas are cancers of skeletal muscle tissue that are divided into different subtypes. These include embryonal rhabdomyosarcoma (eRMS) and alveolar rhabdomyosarcoma (aRMS). Levels of YAP phosphorylation show high variability between different RMS cell lines. However, total YAP protein levels were elevated in RMS cells and histological RMS tumor sections show

increased nuclear YAP stainings (Crose et al. 2014). Overexpression of constitutively active YAP in activated, but not quiescent satellite cells, lead to muscle tumors similar to those found in eRMS. *In vitro* and *in vivo*, YAP knockdown experiments reveal that lowering YAP expression in human eRMS can rescue tumorigenicity (Tremblay et al. 2014). Interestingly, overexpression of consititive active YAP in skeletal muscle fibers in vivo induced muscle atrophy (Judson et al., 2013). Finally, in myoblasts from L-CMD patients, YAP-dependent signaling is increased in soft environment and paradoxically reduced after cyclic stretch (Bertrand et al. 2014).

The regulation of YAP in skeletal muscle remains incompletely characterized. In adult skeletal muscle, major Hippo pathway components including YAP are expressed in fast and slow muscles (Watt et al., 2010). In a neurogenic atrophy model, MST1 expression was found to be upregulated in fast- but not in slow-dominant muscle. Furthermore, knockout of MST1 attenuated fast-dominant skeletal muscle wasting (Wei et al. 2013). In addition, MST1 was found to be activated during myoblast differentiation by caspase3 and active MST1 is needed for proper myoblast differentiation (Fernando et al. 2002). Understanding YAP regulation in skeletal muscle may be a key to understand and possibly treat skeletal muscle disease.

1.3. BMP SIGNALING

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor beta (TGFβ) superfamily of cytokines. BMPs compose a group of secreted growth factors which regulate many cellular functions including cell differentiation, proliferation, survival/apoptosis and cell migration (Sieber et al. 2009). Bone morphogenetic protein-2 (BMP-2) stimulates osteoblast differentiation but inhibits myogenic differentiation in C2C12 myoblasts (Katagiri et al., 1994). BMP-2 activates intracellular signaling by binding to a type I and type II receptor complex. Upon ligand binding, the constitutive active type II receptor activates the type I receptor by transphosphorylation. The activated type I receptor then in turn phosphorylates receptor-bound R-Smads (Smad1/5/8) which, leads to a release of activated Smads into the cytoplasm. These activated Smads form a complex with the co-Smad, smad 4. Together, this complex is translocated into the nucleus where it induces transcription via binding to BMP responsive elements or transcriptional coactivators (Chen and Massague 1999; Massague, Seoane, and Wotton 2005; Sieber et al. 2009). BMP-2 induces the transcription of Inhibitor of Differentiation (IDs) proteins. ID proteins are antagonists of the basic

helix–loop–helix family of transcription factors which, positively regulate differentiation of different tissuse, including skeletal muscle tissue (Ruzinova and Benezra 2003).

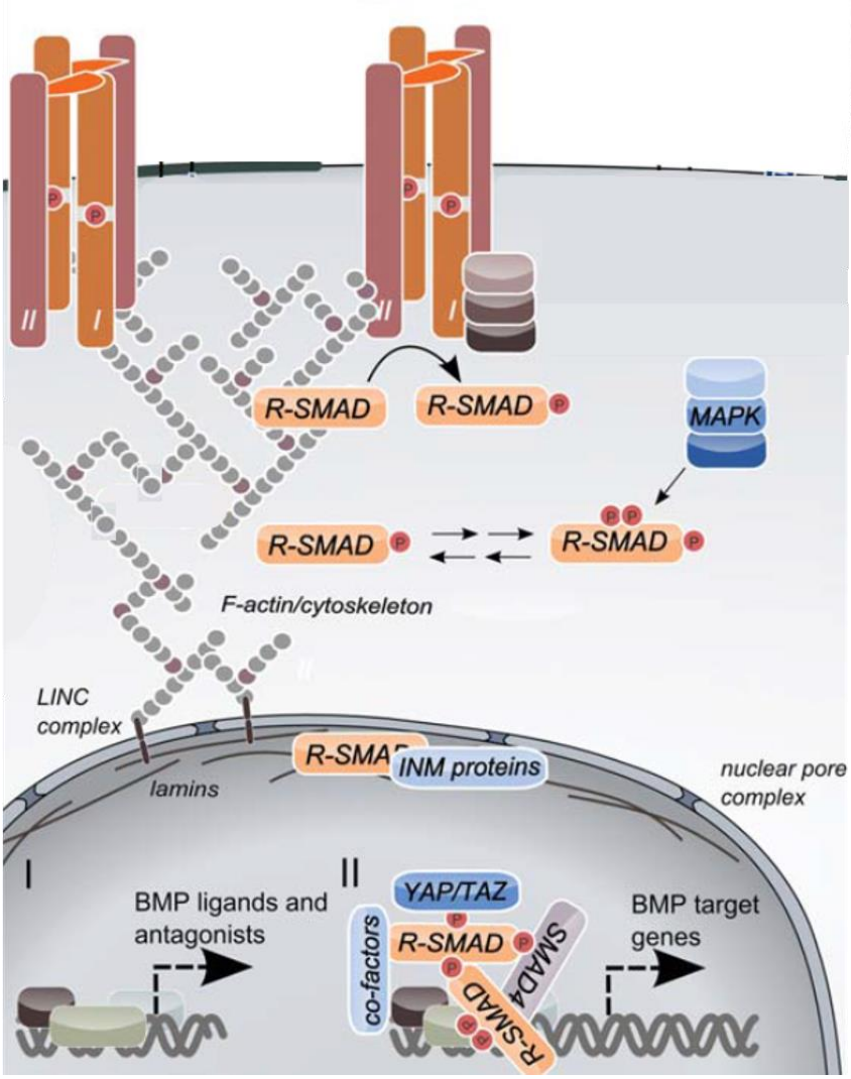

FIGURE 1-9: BMP/smad signalling cascades. Modified from (Kopf et al. 2012).

BMP signaling has been implicated in mechanobiology (Maeda et al. 2011; Kopf et al. 2014). BMP-2 and mechanical force induce bone fomation. Experiments which combine BMP-2 and mechanical stimulation of osteoblasts showed that those two factors act synergistically on Smad 1/5/8 activation. The phosphorylation of R-Smads is increased in intensity and duration after BMP2 stimulation and simultaneous mechanical loading (Kopf et al. 2012). The mechanism, which incorporates mechanical signals into Smad signaling, remains to be elucidated. However, different hypotheses are currently tested which include the regulation of BMP receptor presentation at the plasma membrane or integration of mechanical signals into BMP signaling through crosstalks with other mechanosensitive pathways like Hippo/YAP Signaling (Kopf et al., 2014)

Several crosstalks between YAP and Smad signaling have been identified, including interactions with Smad2/3, Smad7 and Smad 1 (Grannas et al. 2015; Varelas et al. 2010; Narimatsu et al. 2015; Ferrigno et al. 2002). Generally, the stability of R-Smads is regulated by phosphorylation in their linker-region by MAPKs and GSK3-β (fig. 1.9). This phosporylation

pattern can either prime Smad for degradation or stabilize Smads through additional binding of transcriptional coactivators (Aragon et al. 2011). In particular, YAP interacts with Smad1, as YAP can bind to the linker region of Smad1 and thereby stabilizes the transcriptionl active smad complex (Alarcon et al. 2009). Interestingly, also crosstalks between lamins and BMP signaling have been identified. MAN1, a protein of the nuclear envelope which binds to lamins can bind R-Smads and thereby affect BMP signaling activity (Pan et al. 2005). Furthermore, Smad4 binds to Otefin, another protein of the inner-nuclear membrane, and therfore regulates Smad4 transcriptional activity (Jiang et al. 2009).

2. AIMS

L-CMD is characterized by excessive muscle weakness and loss of skeletal muscle mass (Quijano-Roy et al. 2008). L-CMD is a severe and early onset muscular dystrophy caused by mutations in the LMNA gene. The LMNA gene codes for A-type lamins, intermediate filaments of the nucleoskeleton, which contribute to the nuclear structure and stability. As stated previously in this manuscript, mechanosensing defects have already been reported in L-CMD patient cells on soft surfaces (Betrandt et al., 2014). YAP is a key regulator in mechanotransduction (Dupont et al., 2011). Canonically, YAP is regulated by the HIPPO pathway with its core kinase Lats. Active Lats inactivates YAP through phosphorylation at Serine 127. Lats, in turn, is activated by phosphorylation at Threonine 1079 by Mst. Phosphorylated YAP is retained in the cytoplasm through interactions with the 14-3-3 protein (Zaoh et al., 2007).

As transcriptional co-activator non-phophorylated, active YAP localizes to the nucleus, where it induces transcription, mainly through TEAD family transcription factors (Zhao et al., 2008). In ΔK32 mutant myoblasts, increased YAP activity has been reported on soft surfaces (Betrandt et al., 2014). However, YAP activity in standard culture conditions as well as mechanisms leading to aberrant YAP activity in ΔK32 mutant myoblasts remain to be described. Therefore, I first aimed to analyze the activity of the mechanosensitive signaling pathways Hippo/YAP in ΔK32 mutant myoblasts in standard culture conditions (hard surface, sparsely seeded).

The BMP pathway is another signaling pathway implicated in mechanotransduction (Maeda et al., 2011; Kopf et al., 2014). Mechanical cues have been reported to act synergistically with BMP receptor-mediated signaling on Smad transcriptional activity (Kopf et al., 2012). Canonical BMP signaling acts through a receptor mediated phosphorylation of R-smads. Phosphorylated R-smads associate into a transcriptional active smad complex, enter the nucleus and activate transcription by binding to the BMP responsive element (BRE) in promotor regions. Moreover, YAP can bind Smad1, thereby, stabilizing the transcriptional active smad complex (Alarcón et al., 2009). Therefore, I aimed to analyze if the activity of the mechanosensitive BMP signaling pathways in also impaired in ΔK32 mutant myoblasts.

Although YAP has been widely shown to be involved in mechanotransduction, the mechanism by which YAP senses mechanical cues only begins to be elucidated. Therefore, here I aimed to dissect and describe the regulation of YAP in ΔK32 mutant myoblasts compared to WT myoblasts. Beside canonical Hippo signaling, different routes of YAP regulation have been discovered, involving or not the Hippo core kinase Lats (fig. 7). For example, YAP activity is regulated by growth factor induced GPCR signaling and by cell contact inhibition (Yu et al., 2012; Zhao et al., 2007). Therefore, I aimed to dissect aberrant regulation of YAP in ΔK32 mutant myoblasts by investigating total YAP levels, cellular YAP localization, YAP phosphorylation and the transcriptional activity of YAP in different conditions including serum-starved condition as well as in dense culture conditions.

In dense culture conditions cell-cell contacts are formed, when membrane bound-cadherins of adjacent myoblasts bind to each other. As myoblasts can undergo fusion, cell-cell contact sides in myoblasts differ from those in other cells. Two stages, AJ1 and AJ2, have been identified during the formation of cell-cell contacts (Causeret et al., 2005). In the latter stage, AJ2, the cadherin/catenin protein complexes are located along the tips of actin stress fibers, creating the characteristic, zipper-like staining pattern at cell-cell junctions. Cadherin/catenin complexes at cell-cell junctions are stabilized through α-catenin mediated binding to the actin cytoskeleton. YAP is inactivated in dense culture conditions by 14-3-3 protein mediated sequestration of YAP into the cytoplasma. The inactivation of YAP in dense conditions can be mediated by 1) the formation of cadherin/catenin complexes at cell-cell contact sides or 2) independently of cell-cell contacts, through reduced spreading area and F-actin depolymerisation (Zaoh et al., 2007; Schlegelmilchetal., 2011; Silvisetal., 2011; Dupont et al, 2011; Halder et al., 2012; Zhao et al., 2012; Fernández et al., 2011; Sansores- Garcia et al., 2011).

According to the 1st hypothesis, α-catenin mediates YAP cytoplasmic retention and binding of YAP to the 14-3-3 protein (Schlegelmilch, 2011). β-catenin was also crucial for YAP inactivation by cell-cell contact as knockdown of β-catenin prevented YAP nuclear exclusion (Kim et al., 2011). According to to the 2nd hypothesis, a reduced spreading area inactivates YAP, independently of the Hippo core kinases and independently of cell-cell contact formation (Dupont et al., 2011). Therefore, I next aimed to investigate whether defective regulation of YAP in K32

mutant myoblasts was related to a defective organization of the actin cytoskeleton and/or imapired formation of cell-cell contact sites.

Cadherin-dependent mechanotransduction is crucial for cell-migration (Hirashima et al. 2013; Mertz et al. 2012; Tambe et al. 2011). Moreover, YAP and BMP signaling were shown to influence cell migration (Haskins et al., 2014; Schütte et al., 2014; Sorrentino et al., 2014; Moroishi et al., 2015). In addition, myoblast migration is a critical step in skeletal muscle myogenesis and regeneration (Goetsch, Myburgh, and Niesler 2013). Myogenic progenitor cells must be precisely regulated and positioned for proper cell fusion. Thus, migration is crucial to achieve cell–cell adhesion (Kang et al., 2004; Bae et al., 2008; Jansen and Pavlath, 2006; Mylona et al., 2006; O'Connor et al., 2007). Moreover, striated muscle disease causing LMNA mutations have been implicated in migration defects (Folker et al. 2010). Therefore I finally aimed to investigate if the mechanotransduction defects in ΔK32 mutant myoblasts affect the migration pattern of ΔK32 mutant myoblast.

SUMMARY

i. First, I aimed to analyze the activity of the mechanosensitive signaling pathways Hippo/YAP and BMP/smads in ΔK32 mutant myoblasts in standard culture conditions (hard surface, sparsely seeded).
ii. Then, I aimed to dissect and briefly describe the aberrations in the regulation of YAP. Therefore, I aimed to investigate total YAP levels, cellular YAP localization, YAP phosphorylation and the transcriptional activity of YAP in standard culture conditions, in serum-starved conditions and dense culture conditions.
iii. Thereafter, I aimed to investigate the mechanism which lead to a defective regulation of YAP in K32 mutant myoblasts. Hence, I focused on the organization of the actin cytoskeleton and the formation of cell-cell contact sites in ΔK32 mutant myoblasts.
iv. Finally, I aimed to investigate if the mechanotransduction defects in ΔK32 mutant myoblast affect the migration pattern of ΔK32 mutant myoblast.

3. MATERIALS AND METHODS

HUMAN MYOBLASTS CULTURE

Experiments were performed on immortalized human myoblasts. Human myoblasts were derived from patients carrying a heterozygous p.Lys32del LMNA (ΔK32) mutation (Quijano-Roy et al. 2008). Myoblasts from a male control subject without muscular disorders were used as control (WT). Following muscular biopsy, cells were first expanded and enriched in myoblasts by magnetic cell sorting with the use of anti-CD56/NCAM to reach a purity of at least 95%. Immortalized lines from WT and ΔK32 myoblasts were obtained by transducing cells with pBABE retroviral vectors carrying Cdk4 and hTERT (Mamchaoui et al. 2011). Puromycin and neomycin were used as selection markers.

CELL CULTURE

Immortalized myoblasts were cultured in growth medium consisting of 1 vol 199 Medium to 4 vol DMEM (Life technologies, Carlsbad, CA, USA) supplemented with 20% fetal calf serum (Life technologies, Carlsbad, CA, USA), 5 ng/ml hEGF (Life technologies, Carlsbad, CA, USA), 0.5 ng/ml bFGF, 0,1mg/ml Dexamethasone (Sigma-Aldrich, St. Louis, Missouri, USA), 50 µg/ml fetuin (Life technologies, Carlsbad, CA, USA), 5 µg/ml insulin (Life technologies, Carlsbad, CA, USA) and 50mg/ml Gentamycin (GibcoTM, Life technologies, Carlsbad, CA, USA) or DMEM. Cell cultures were performed on 2D conventional rigid substrates or on soft hydrogels (12 kPa, Matrigen, Brea, California, USA). All experiments on soft hydrogels and its controls were performed with fibronectin coated surfaces at a concentration of 10 µg/ml (Sigma-Aldrich, St. Louis, Missouri, USA). On rigid substrates cells were grown on uncoated cell culture plastic unless stated otherwise. Dense or sparse culture conditions were achieved by seeding 1x10^5 or 1*10^4 cells per cm^2 growth area.

COATINGS

Fibronectin coatings were performed by diluting Fibronectin (Sigma Aldrich, St. Louis, Missouri, USA) in PBS to a final concentration of 1-10 µg/ml. Cell culture plastic was covered with the fibronectin solution and incubated for 1h at 37°C. Then, the solution was removed and the cell culture plastic was dried for several hours in the sterile bank.

CHEMICALS, RECOMBINANT GROWTH FACTORS AND INHIBITORS

Recombinant human BMP2 was kindly provided by Walter Sebald (University of Würzburg, Würzburg, Germany). All chemicals were purchased from Sigma Aldrich unless stated otherwise. Myosin II inhibitor blebbistatin, ROCK inhibitor Y27632, the microtubule assembly inhibitor nocodazole (Sigma-Aldrich, St. Louis, Missouri, USA), LatrunculinA (Milipor, Billerica, MA, USA) were diluted to final concentrations of 25 µM; 10 µM; 1 µg/ml and 5µM in the culture medium. Cells were incubated with each drug for the indicated times. The actin stabilizing drug Jasplakinolide (Santa Cruz, Dallas, Texas, USA) was added at a concentration of 500nM. Controls were performed with the according concentrations of DMSO. Eukaryotic translation inhibitor Cycloheximid (CHX) and proteasome inhibitor MG-132 (both Sigmar Aldrich, St. Louis, Missouri, USA) were diluted in growth Medium to a final concentration of 30 µg/ml and 25µM and added to the adherent myoblasts for 2, 4, 6, and 8h. Proliferation inhibitor Mitomycin C (Sigma-Aldrich, St. Louis, Missouri, USA) was diluted in growth medium to a final concentration of 30µg/ml and added to the adherent cells for 1h before the migration experiments.

LUCIFERASE REPORTER ASSAYS

Myoblasts were transfected with Lipofectamin® 2000 or Lipofectamin® 3000 (both Invitrogen, Carlsbad, CA, USA) reagents in growth media without antibiotics in solution at RT for 6h, otherwise according to manufacturer's instructions. TBS (Tead Binding Sequence: 14 times GGAATG)- and BRE (BMP Response Element)- Firefly Luciferase reporter constructs were used at a 1:5 ration to the co-reporter vector for the weak constitutive expression of wild-type Renilla luciferase (pRL-TK, Promega GmbH, Mannhein, Germany). Transfected cells were seeded into 24-,48- or 96-well plates and recovered overnight in growth medium. For the BRE-assay, cells were starved in growth Medium or DMEM with 0,5% FCS for 3h and stimulated with the indicated concentrations of BMP2 for 24h, or as indicated. For the TBE, assay cells were cultivated for 24h after transfection under the stated conditions. The cells were lysed with passive lysis buffer (PJK GmbH, Kleinblittersdorf, Germany). Luciferase activity was measured with a Mithras LB940 (Wildbad, Germany) by addition of firefly Luciferase substrate Beetle Juice (PJK GmbH, Kleinblittersdorf, Germany) and Renilla Juice (PJK GmbH, Kleinblittersdorf, Germany).

IMMUNOCYTOCHEMISTRY

Experiments have been performed on fibronectin coated cell culture plastics (1µg/ml fibronectin in PBS, 1h at 37°C). Myoblasts were fixed for 10 min with 4% formaldehyde, permeabilized for 15 min with 0.5% Triton X100 and blocked with 5% BSA or normal goat serum (St. Louis, Missouri, USA) diluted in PBS. Actin was stained with fluorescent labelled phalloidin (Interchim, Mannheim, Germany or SantaCruz, Dallas, Texas, USA). Primary antibodies were diluted in PBS with 1% BSA and incubated overnight at 4°C and secondary antibodies were incubated for 1h at room temperature. The preparations were mounted with fluorescent mounting medium containing DAPI (Vectashield, Vector Labs, Burlingame, CA, USA) or incubated with 300nM DAPI (Sigma Aldrich, St. Louis, Missouri, USA) solution for five minutes and mounted in Fluorount-G (Southern Biotech, Birmingham, AL, USA). Primary antibodies used are listed in table (Table 1). As secondary antibodies, the Alexa-conjugated secondary antibody system (Invitrogen, Carlsbad, CA, USA) was utilized.

CELL PROLIFERATION ASSAY

Cells were grown on glass coverslips in growth medium at different densities for 24h. Proliferation was measured with the help of the Click-iT® EdU Alexa Fluor® 488 kit (Life Technologies, St. Louis, Missouri, USA). EdU was added to the culture media in concentrations of 1µM for durations of 16h. Cells were permeabilized and fixed with 4% formaldehyde. The fixed cells were stored at 4°C and stained the next day according to manufactors instruction. The staining mix was prepared fresh each time. After staining, the cells on coverslips were counterstained and mounted with fluorescent mounting medium containing DAPI (Vectashield, Vector Labs, Burlingame, CA, USA) and imaged by fluorescence microscopy.

TABLE 3-1: ANTIBODIES

Target protein	Company	Dilution
GAPDH (cs-2118)	Cell Signaling Technology	WB 1:1000
Lats-1 (cs-3477)	Cell Signaling Technology	WB 1:1000
M-cadherin (ab65157)	Abcam	WB 1:1000
M-cadherin (bd-11100)	BD	WB 1:1000
N-cadherin (ab19348)	Abcam	WB 1:1000
pSmad1(Ser463/465)/pSmad5(Ser463/465)/ pSmad9(Ser465/467) (D5B10)	Cell Signaling Technology	WB 1:1000
pLats 1(Thr1079) (CS-8654)	Cell Signaling Technology	WB 1:1000
pSmad 1/5 (Ser463/465) (cs-9516)	Cell Signaling Technology	WB 1:1000 IF 1:250
pYAP(Ser127) (cs-4911)	Cell Signaling Technology	WB 1:1000
Smad1 XP (cs-D5907)	Cell Signaling Technology	WB 1:1000
YAP/TAZ (sc-10119s)	Santa Cruz Biotechnology	WB 1:1000 IF 1:200
α-Tubulin	Home made (AG Knaus)	WB 1:1000
β -Catenin (cs-9581)	Cell Signaling Technology	WB 1:1000 IF 1:200
Syntaxin-6 (C34B2)	Cell Signaling Technology	IF 1:50
Pan-cadherin (ab6529)	Abcam	IF 1:100

WOUND HEALING ASSAY

The scratch wound healing assay was performed using cell culture inserts (ibidi GmbH, Martinsried, Germany) according to the manufacturer's instructions on fibronectin coated (5µg/ml, 1h at 37°C) tissue culture plastic, if not indicated differently. $25x10^5$ Myoblasts were seeded into every insert 24h before insert removal. 1 hour prior to insert removal, cells were incubated with 30 µg/ml mitomycin C to block cell proliferation. After insert removal, cells were washed with PBS and further cultivated in growth media. For life cell imaging, the wound closure was permitted to proceed in the live cell imaging, heating and CO2 chamber (ibidi GmbH, Martinsried, Germany). Cells were imaged every 5 minutes for the indicated periods by fluorescence and/or phase-contrast

microscopy using the AxioVision 4.9 software. Cell movements and contacts were tracked by analyzing time-lapse images. For Immunocytochemistry, the wound healing assay, as described above, was performed in Chamber slides (Nunc™ Lab-Tek™, Thermo Fischer, Waltham, MA, USA). Closure was permitted to proceed under standard incubation conditions for the indicated periods. Cells were then fixed and immuno-stained as described above.

CELL TRACKING

Cell tracking was performend by Marc Osterland at the Zuse Institute Berlin. The image analyses were performed using Python 2.7.12, OpenCV 3.0, and scikit-image 0.12.3 In the interest of a clear differentiation between background and foreground, the optical flow was computed. The foreground is defined as the area with non-zero optical flow. The relatively darker cell bodies, which are surrounded by a bright halo in phase contrast microscopy, were segmented by an inverse adaptive threshold. Based on this cell body mask a distance map was computed. On these distance maps Watershed segmentation was applied to approximate the whole cell area. Therefore, the connected components from the cell body mask were used as seeds and the foreground mask as a restrictive mask. The neighborhood was then determined by counting adjacent cell areas. To calculate the cell speed, an overlap heuristic was applied on the cell areas. For each cell in a time step the cell of the preceding time step with the largest overlapping area was determined and added to a trajectory. In a post-processing step, tracking gaps due to segmentation failures in single time steps were closed using extrapolation of that track. The resulting tracks then formed the basis for the velocity analysis.

CELL ORIENTATION ANALYSIS

Cell orientation analysis were performend by Marc Osterland at the Zuse Institute Berlin. The images were taken in a 9 by 8 grid with approx. 10% overlap. Using the ImageJ plugin by Preibisch et al (2009), these images were stitched to a single, large image. The nuclei were segmented using adaptive thresholding on the DAPI channel. Clustered nuclei were separated using Watershed segmentation on the inner distance map of the nuclei with distance peaks as seeds. The Syntaxin 6 channel was smoothened using a Gaussian kernel after high intensity outliers were filtered. Then, centers of the nuclei were connected to an orientation vector with the closest intensity peak on the Syntaxin 6 channel. All connections were manually checked and corrected, where necessary. Based

on the orientation vectors, the orientation angle for each cell was calculated. The Local Orientation Variance (LOV) was computed with a sliding circular window on the image area, computing the variance with Von Mises distance of the angles of all cells within this window, resulting in the LOV map. The LOV was omitted if less than three cells were in the window. Given almost equal image dimensions for each experiment, the LOV map were scaled to the mean image size. The average LOV map for each condition was determined by computing the mean over all experiments for each position.

MICROSCOPY

Fluorescent signals were visualized using a Leica SP2 (Leica Microsystems, Wetzlar, Germany) microscope and an inverted fluorescence Axiovert 200 microscope (Carl Zeiss, Oberkochen, Germany). Life-cell imaging was performed with the Axiovert 200 equipped with a live cell imaging heating and CO2 chamber mounted to a CoolSnapHQ CCD camera (Roper Scientific, Trenton, NJ, USA). Mosaic pictures have been generated with the help of the AxioVision 4 Module Mark & Find software. Confocal images were taken with an Olympus FV 1000 (Olympus, Hamilton, Bermuda).

IMAGE ANALYSIS

Standard image analyses and quantitative immunofluorescent analysis were performed using ImageJ software. Cell spreading area was measured by quantifying the area of Pholliodin -positive areas from > 50 cells per cell line.

ACTIN FIBER ORIENTATION ANALYSIS

Actin fiber orientation analysis was performed on z-stacks of confocal images. Therefore, a maximum projection of the z-layers of each image was generated with imageJ. Direction analysis with the ImageJ plugin OrienationJ were then applied every projected image.

TABLE 3-2: SEQUENCES OF PRIMERS USED IN RT-QPCR

Name	Abbreviation	Fw/rev	Sequence
human Inhibitor of Differentiation1	hID-1	fw	GCTGCTCTACGACATGAACG
		rev	CCAACTGAAGGTCCCTGATG
human Inhibitor of Differentiation2	hID-2	fw	GTGGCTGAATAAGCGGTGTT
		rev	TGTCCTCCTTGTGAAATGGTT
human Inhibitor of Differentiation3	hID3	fw	CTTCCGGCAGGAGAGGTT
		rev	AAAGGAGCTTTTGCCACTGA
human Myogenin	hMyoG	fw	CAGGGGTGCCCAGCGAATGC
		rev	ATCTGTAGGGTCAGCCGTGA
human Myogenic differentiation	hMyoD	fw	CCGACGGCATGATGGACTAC
		rev	GCGACTCAGAAGGCACGTC
human Myosin heavy chain IIa	hMHC_IIa	fw	GCAACTCACTTACCAAACTG
		rev	GACCTGGGACTCAGCAATGT
human Myosin heavy chain IIb	hMHC_IIb	fw	GCTGAAGAGGCTGAGGAACA
		rev	TTTGTGTGAACCTCCCGACT
human Glycerinaldehyd-3-phosphat-Dehydrogenase	hGAPDH	fw	GAAGGTGAAGGTCGGAGTC
		rev	GAAGATGGTGATGGGATTTC
human Yess-associated protein 1	hYAP1	fw	GCTACAGTGTCCCTCGAACC
		rew	CCGGTGCATGTGTCTCCTTA
human connective tissue growth factor	h-CTGF	fw	ACCGACTGGAAGACACGTTTG
		rew	CCAGGTCAGCTTCGCAAGG
human connective tissue growth factor	h-RPLPO	fw	CTCCAAGCAGATGCAGCAGA
		rew	ATAGCCTTGCGCATCATGGT
human Glycerinaldehyd-3-phosphat-Dehydrogenase	h-GAPDH	fw	TGC-CAT-GTA-GAC-CCC-TTG-AA
		rew	TGG-TTG-AGC-ACA-GGG-TAG-TT
human myosin light chain 9	h-Myl9	fw	CGA-ATA-CCT-GGA-GGG-CAT-GAT
		rew	AAA-CCT-GAG-GCT-TCC-TCG-TC

SDS-PAGE AND PROTEIN ANALYSIS

Cells were lyzed in total protein extraction buffer (50 mM Tris-HCl, pH 7.5, 2% SDS, 250 mM sucrose, 75 mM urea, 1 mM DTT) with protease inhibitor (25 µg/ml Aprotinin, 10 µg/ml Leupeptin, 1 mM 4-[2-aminoethyl]-benzene sulfonylfluoride hydrochloride and 2mM Na3VO4) or directly in 2x Lämmli buffer. Nuclear-/cytosolic fractioning was performed with the help of NE-PER™ Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher, Waltham, MA, USA) and according to manufactors instructions. Protein lysates were subjected to SDS-PAGE and transferred to PVDF or nitrocellulose membranes by Western blotting. Membranes were blocked for 1 h in 0.1% TBS-T containing 5% BSA, washed three times in 0.1% TBS-T and incubated with indicated primary antibodies overnight at 4°C (see table 1). For HRP-based detection, goat anti-mouse, goat anti-rat or donkey anti-goat HRP conjugates were used. Detection of adsorbed HRP-coupled secondary antibodies was performed by ECL reaction with Immobilon Western Chemiluminescent HRP Substrate (Millipore, Billerica, Massachusetts, USA). HRP signals were detected using a CCD-based detection system (Vilber Lourmat). Membranes subjected to a second round of immunoblotting were stripped with stripping buffer (62.5mM Tris-HCL pH 6.8, 2%SDS, 100mM β-mercaptoethanol) and incubated at 55°C for 30 minutes with mild shaking before excessive washing with tap water and re-blocking. Quantification was performed using ImageJ.

STATISTICAL ANALYSIS

SPSS (IBM Corporation, Armonk, New York, USA) was used to calculate means and standard error of the mean (SEM) of measured quantities. Statistical significances were assessed by two-tailed unpaired t-tests when comparing means of two groups. Differences between conditions were considered significant at $p < 0.05$.

QUANTIFICATION OF GENE EXPRESSION

The mRNA was isolated from cell lysates using the RNeasy mini kit (Qiagen, Hilden, Germany) with the Proteinase K step, according to the manufacturer instruction for YAP response genes, or using the NucleoSpin RNA II Kit (Machery Nagel, Düren, Germany) for BMP receptor and IDs expression analysis. The complementary DNA (cDNA) was transcribed by SuperscriptIII (Life Technologies, Carlsbad, CA, USAEuro) or qScriptTMcDNA SuperMix (Quanta Biosciences, Gaithersburg, MD, USA).

Gene expression was quantified by using PerfeCTa®SYBR®Green SuperMix (Quanta, Biosciences, Gaithersburg, USA) with the help of LightCycler 480 II (Roche Diagnostics GmbH, Mannheim, Germany). The primers were designed by Primer-BLAST (NCBI) and synthesized by Eurogentec (Liège, Belgium) or TIB Molbiol (Berlin, Germany). Expression of all target genes was normalized to the expression of house-keeping genes hypoxanthine-guanine phosphoribosyltransferase (HPRT), GAPDH or RPLP0 as indicated which accounts for potential changes in cell numbers. Primer sequences are listed in Table 2.

4. RESULTS

INCREASED YAP ACTIVITY IN ΔK32 MUTANT MYOBLASTS

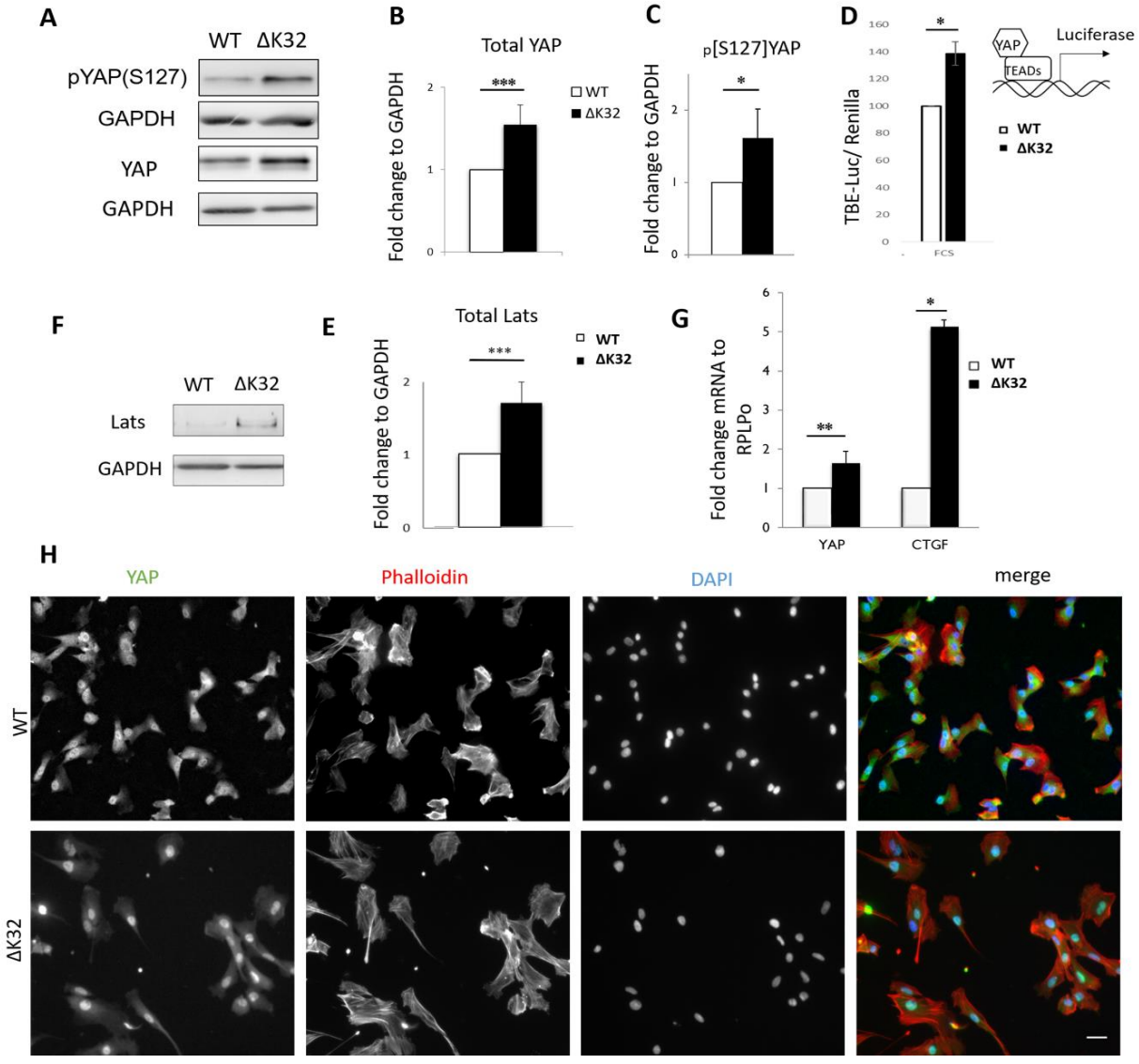

Figure 4-1: Increased YAP signaling in ΔK32 mutant myoblast. *Significantly increased protein levels of YAP, p-YAP(S127) and Lats in ΔK32 mutant myoblast compared to WT myoblasts (A, B, C, F, E). Tead-dependent dual luciferase reporter assay shows a significantly increased transcriptional activity of YAP in ΔK32 mutant myoblast compared to WT (D). Significantly increased mRNA levels of YAP and the YAP response gene ctgf (G). YAP (green) cellular localization is nuclear in WT and ΔK32 mutant myoblasts. Cells were counterstained with dapi (blue) and phalloidin (red) (H). Representative western blots are shown. Quantifications represent means +/- SEM of ≥ 3 independent experiments. Values are foldchanges, normalized to WT. (*) p-value ≤0.05; (**) p-value ≤0.005, (***) p-value ≤0.0005. scale bar= 30µm.*

To analyze the activity of mechanosensitive YAP signaling in LMNA mutant myoblasts, YAP activity was first analyzed in WT and ΔK32 mutant myoblasts cultured on conventional hard

surface and plated in sparse conditions. YAP protein and mRNA levels were significantly higher in ΔK32 compared to WT myoblasts (fig. 4-1 A, B, G). Also, protein levels of the hippo core kinase Lats were significantly increased in ΔK32 mutant myoblast compared to WT (fig. 4-1F, E). In addition, the phosphorylation of YAP at Serine 127 was significantly increased in ΔK32 compared with WT myoblasts (fig. 4-1 A, C). YAP cellular localization was mainly nuclear in both WT and ΔK32 mutant myoblasts (fig. 4-1 E). However, the transcriptional activity of YAP was increased in ΔK32 myoblasts compared to WT myoblasts, as attested by the TEAD Luciferase reporter gene assay and the increased expression of the YAP response gene *ctgf* (fig. 4-1 D, G).

INCREASED SMAD TRANSCRIPTIONAL ACTIVITY IN ΔK32 MYOBLASTS

To determine whether the activity of another mechanosensitive pathway, the BMP pathway, was also impaired in ΔK32 myoblasts, Smad 1/5/8 transcriptional activity in ΔK32 myoblasts was analyzed using a dual luciferase reporter assay on the BMP-responsive-element. In sparse conditions, there was a strong and significant increase of Smad transcriptional activity in ΔK32 compared to WT myoblasts in starved condition (fig. 4-2A). After stimulation with BMP2, Smad transcriptional activity was enhanced in both cell types, while there was still a significant higher transcriptional activity of Smads in ΔK32 mutant myoblasts compared to WT (fig. 4-2A). Moreover, mRNA levels of Smad response genes *id2* and *id3* were significantly increased in ΔK32 compared to WT myoblasts in starved condition and after stimulation with BMP2 [3nM] for 6h (fig. 4-2C). Total Smad 1 and pSmad 1/5 protein levels were analyzed by western blot in starved condition and after stimulation with BMP2 for 15 min (fig. 4-22D). The phosphorylation of Smad 1/5 was slightly but significantly increased in ΔK32 compared to WT myoblasts in starved condition but, not after stimulation with BMP2 (fig. 4-2E). There was no significant difference in total Smad1 protein levels between WT and ΔK32 myoblasts, in starved condition or after stimulation (fig. 4-2F).

The cellular localization of pSmad1/5 was analyzed by immunofluorescence in starved condition and after stimulation with BMP2 for 15 min. The cellular localization of pSmad1/5 was equally distributed in the cytoplasm and the nucleus in starved conditions in WT and ΔK32 myoblasts. The localization of pSmad1/5 was mainly nuclear after stimulation with BMP2 (fig. 4-2B). Taken together, these results showed an increased transcriptional activity of the BMP pathway

in ΔK32 compared to WT myoblasts. If this increased activity is caused by mechanosensing defects of ΔK32 mutant myoblasts, remains to be investigated.

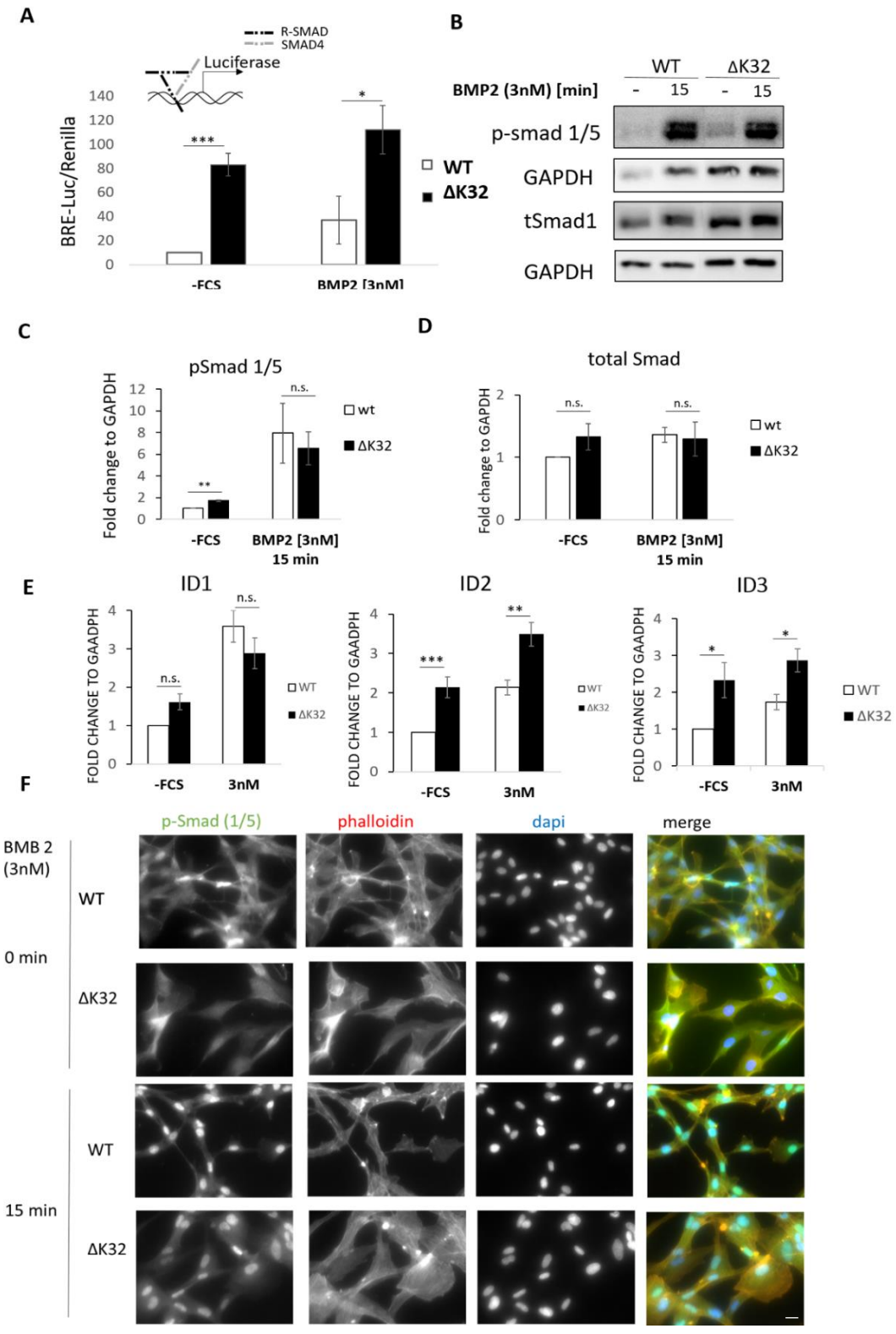

Figure 4-2: Increased smad transcriptional activity. *ΔK32 mutant myoblasts showed an increased Smad transcriptional activity in starved condition and after stimulation with BMP2 [3nM] (A). ΔK32 mutant myoblasts also showed significantly increased mRNA levels of Smad response genes ID2 and ID3 in starved condition and after stimulation with 3nM BMP2 for 6h compared to WT myoblasts. ID1 mRNA expression was not significantly increased (E). pSmad 1/5 protein levels were significantly increased in ΔK32 mutant compared to WT myoblasts in starved but not stimulated condition, while total Smad1 levels remained stable (B, C, D). A representative western blot is shown. Myoblasts were stained for pSmad (green) and counterstained with phalloidin (red) and DAPI (blue) to visualize cellular localization. In ΔK32 and WT myoblasts, pSmad 1/5 localization was cytoplasmic and nuclear in starved condition and became mainly nuclear after stimulation with 3nM BMP2 (F). Quantifications represent mean values +/- SEM from ≥ 3 independent experiments. Values are fold changes, normalized to WT-FCS. (*) p-value ≤0.05; (**) p-value ≤0.005, (***) p-value ≤0.0005; (n.s) not significant. Scale bar= 10µm*

YAP OVER-ACTIVITY IS INHIBITED BY GROWTH FACTOR STARVATION

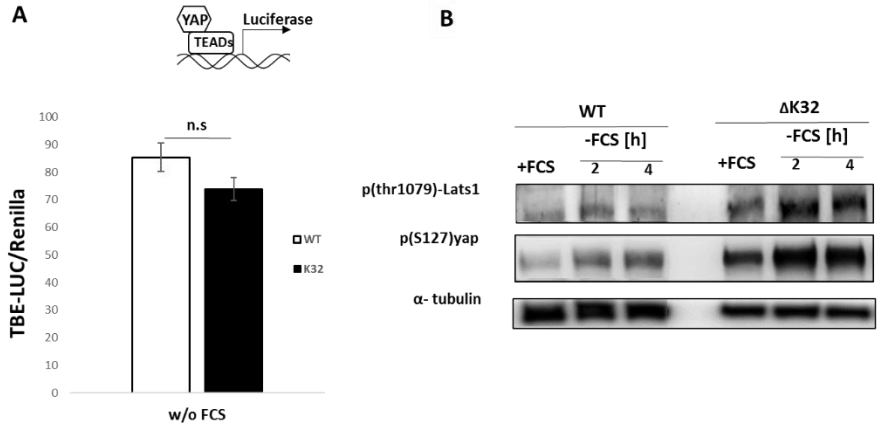

Figure 4-3: Regulation of YAP in ΔK32 mutant myoblast*. No significant difference in the transcriptional activity between WT and ΔK32 mutant myoblasts, after starvation with 0% FCS (A). After starvation with 0% FCS for the indicated time, p(S127) YAP and p(Thr1079) levels increased in WT and ΔK32 mutant myoblasts compared to unstarved condition, while total YAP levels remained stable (B). A representative western blot is shown (B). Tead- dependent luciferase assay was quantified as mean +/- SEM ≥ 3 independent experiments. Values are fold changes to renilla lucifersase activity. (n.s) not significant.*

To dissect the regulation of YAP signaling in ΔK32 mutant myoblasts, YAP transcriptional activity and phosphorylation were analyzed after starvation of cells with 0 % FCS. Interestingly, there was no significant difference in the tead-dependent transcriptional activity of YAP in ΔK32 and WT myoblasts in starved conditions (fig. 4-3A). Starvation induced an increase in the phosphorylation of Lats (thr1079) and YAP (S127) in both WT and ΔK32 mutant myoblasts compared to full-medium condition (fig. 4-3B). However, protein levels of pYAP remained significantly increased in ΔK32 compared to WT myoblasts after starvation. These results showed that the increased transcriptional activity of YAP, but not the increased protein levels, can be abolished by growth factor mediated phosphorylation of YAP.

INCREASED NUCLEAR LOCALIZATION OF YAP IN ΔK32 MUTANT MYOBLASTS IN DENSE CONDITION

YAP is normally retained in the cytoplasm in dense culture conditions (Zhao et al.,2007). To further dissect the regulation of YAP in ΔK32 myoblasts, immunofluorescence studies of YAP cellular localization in dense culture conditions were performed. In dense condition, YAP was excluded from the nucleus in WT myoblasts (90 %) whereas, YAP remained predominantly nuclear in ΔK32 mutant myoblasts (fig. 4-4A, B). Increased nuclear localization of YAP in mutant cells correlated with a slight but significant increase of YAP transcriptional activity, as indicated by increased

TEAD-dependent Luciferase reporter assay activity and increased expression of the YAP response genes ctgf and myl9 (fig. 4-4C, D).

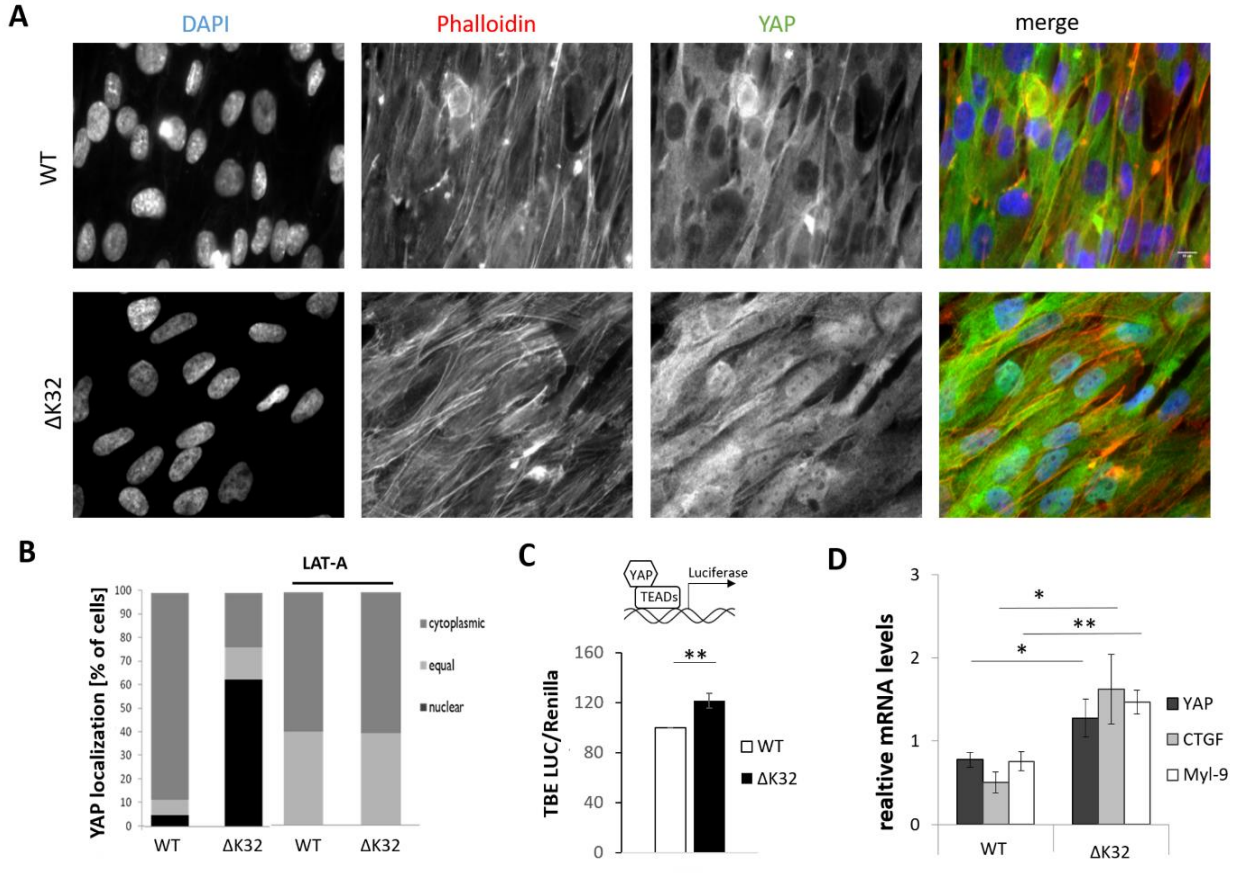

Figure 4-4: Increased nuclear localization of YAP in dense condition. *YAP (green) cellular localization was analyzed in dense culture conditions 24h after plating and counterstained with phalloidin (red) and DAPI (blue) (A). In WT myoblasts, YAP is mainly cytoplasmic, while in ΔK32 mutant myoblasts, YAP remains predominantly nuclear. Quantifications show the number of cells (%) with a nuclear (black), equally distributed (light-grey) or only cytoplasmic (dark grey) YAP staining (B). A dual reporter assay on TEAD-dependent Luciferase expression shows an increased YAP transcriptional activity for ΔK32 mutant myoblasts (D). mRNA levels of YAP response genes Myl-9 and ctgf, as well as YAP were increased (E). Quantifications represent means +/- SEM of ≥ 3 independent experiments. Values are fold changes. C is normalized to WT. (*) p-value ≤0.05; (**) p-value ≤0.005; (***) p-value ≤0.0005; (n.s) not significant. Scale bar= 10µm.*

These results show a defect in the inactivation of YAP in ΔK32 mutant myoblasts in dense conditions. However, cell starvation abolished the increased transcriptional activity of YAP in

ΔK32 mutant myoblasts plated in dense conditions. There was no significant difference in YAP activity between WT and ΔK32 mutant after removal of FCS (fig. 4-5).

REGULATION OF YAP AT INCREASING CELL DENSITY

YAP cytoplasmic retention in dense conditions was shown to be either regulated through contact inhibition of proliferation (CIP) or through a reduced cell size and decreased F-actin formation, independent of cell-cell contacts (Zaoh et al., 2007; Schlegelmilchetal., 2011; Silvisetal., 2011; Dupont et al, 2011). To investigate if YAP nuclear localization in ΔK32 mutant myoblasts remains dependent on F-actin polymerization in dense culture conditions, WT and ΔK32 mutant myoblasts were treated with the F-actin polymerization inhibitor Latrunculin A. Inhibition of actin polymerization in dense condition caused an exclusion of YAP from the nucleus in ΔK32 mutant myoblasts (fig. 4-4B). In WT myoblasts, treatment with Latrunculin A led to a further increase of YAP nuclear exclusion. This demonstrates that ΔK32 mutant myoblasts preserved the general ability to exclude YAP from the nucleus.

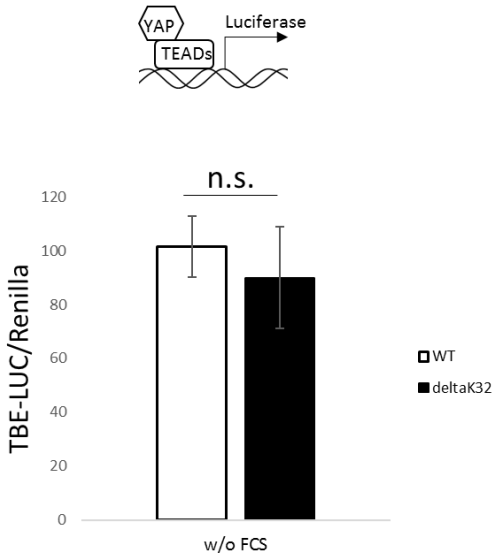

Figure 4-5 No significant difference of YAP activity between ΔK32 and WT cells after starvation. *Tead-dependent luciferase assay in dense condition after starvation with 0% FCS. Quantifications are means +/- SEM of ≥ 3 independent experiments. Values are foldchanges to renilla lucifersase activity. (n.s) not significant.*

During cell contact inhibition, YAP cytoplasmic retention was reported to be regulated by increased phosphorylation of YAP at Serine 127 (Zaoh et al., 2007). To analyze the effects of cell-cell contact on YAP phosphorylation, the p(S127) phosphorylation of YAP was determined with growing cell density (fig. 4-6A, B). p(S127) YAP protein levels decreased with increased density in both WT and ΔK32 mutant myoblasts (fig. 4-6 B). In addition, total YAP levels decreased from sparse to dense culture conditions in both WT and ΔK32 mutant myoblasts (fig. 4-6B). Interestingly, in dense culture conditions, total YAP and p(S127) YAP protein levels did not differ in ΔK32 mutant compared to WT myoblasts (fig. 4-6C,D). YAP transcriptional inactivation through cell-contact is associated with a decrease in cell proliferation (Zaoh et al, 2007). To determine if the increased YAP activity

in ΔK32 mutant myoblast led to an increased cell proliferation, WT and ΔK32 mutant myoblasts proliferation.

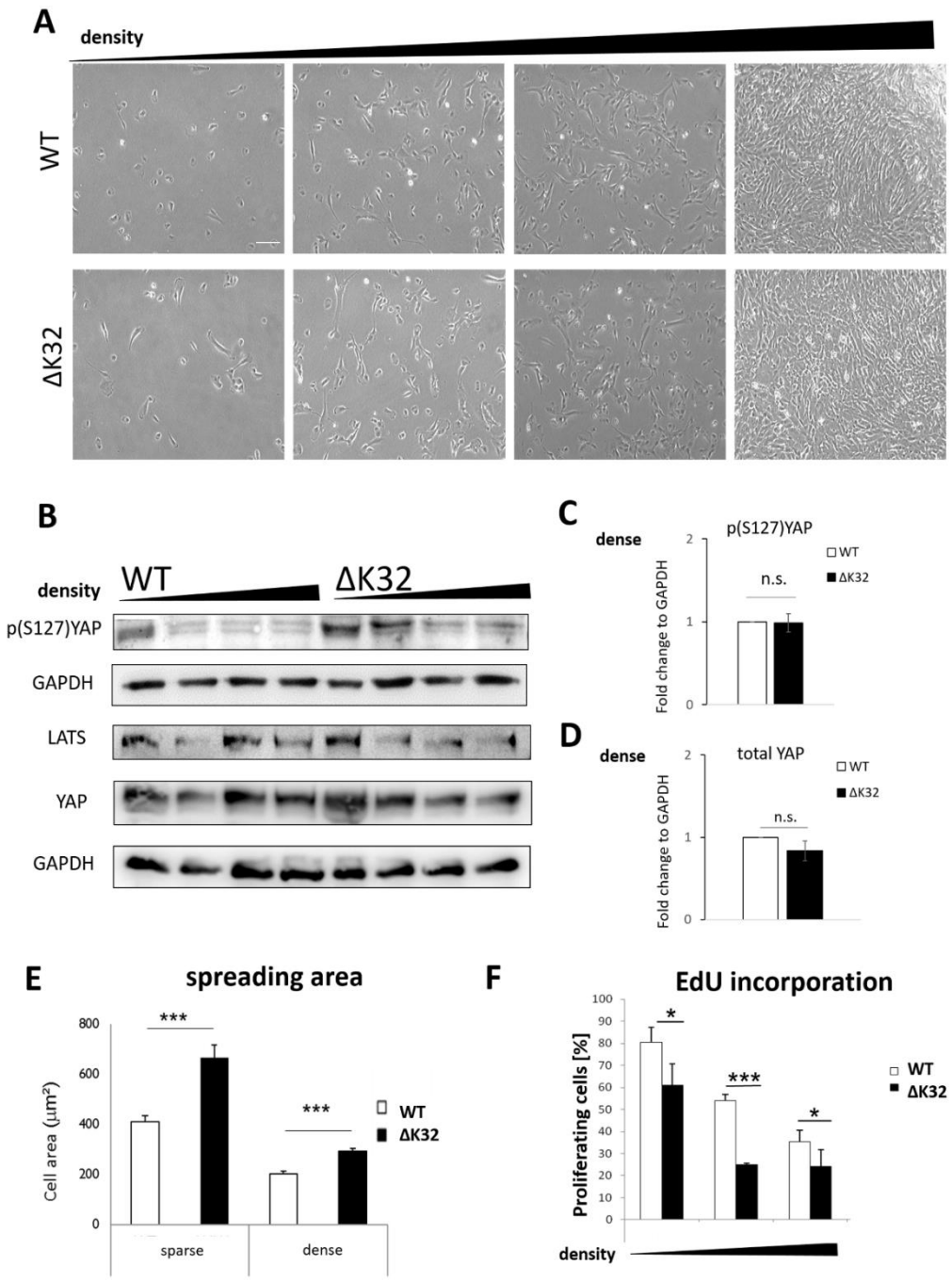

Figure 4-6: Regulation of YAP with increasing density. *To analyze YAP phosphor-ylation kinetics, ΔK32 and WT myoblasts were seeded with increasing density (A). Representative western blot and quantification of total YAP and p(S127) YAP with increasing density (B). Total YAP and p(S127) YAP levels decreased with increasing density. The increased YAP and P(S127) YAP levels of ΔK32 mutant myoblasts were abolished in dense condition compared to WT myoblasts (C, D). ΔK32 mutant myoblasts have an increased spreading area in sparse and dense conditions compared to WT myoblasts (H)). ΔK32 mutant myoblasts had a reduced proliferation in all densities compared to WT cells (I).. (D, E, F) Quantifications represent means +/- SEM of ≥ 3 independent experiments. D and E are shown as foldchanges, normalized to WT. (*) p-value ≤0.05; (**) p-value ≤0.005, (***)p-value ≤0.0005; (n.s)not significant. Scale bar= 100µm*

Altogether, these results indicated that YAP transcriptional activity was increased in ΔK32 mutant myoblasts in both, sparse and dense condition, but increased total YAP and p (S127) YAP protein levels were only observed in ΔK32 myoblasts plated in sparse conditions. Increased YAP activity was not associated with an increased proliferation of ΔK32 compared to WT myoblasts. Finally, YAP over-activity in ΔK32 mutant could be abolished by growth factor starvation.

To determine if the increased YAP activity of ΔK32 mutant myoblasts in sparse and dense conditions could be related to an increased cell size, the spreading area of ΔK32 mutant myoblasts was measured. Compared to WT myoblasts, the spreading area of ΔK32 mutant was significantly higher in both sparse and dense culture conditions (fig. 4-6E).

DISORGANIZED ACTIN FIBER NETWORK IN ΔK32 MYOBLASTS

Actin organization plays a crucial role in YAP regulation (Dupont et al., 2011; Halder et al., 2012; Zhao et al., 2012). Moreover, disorganized perinuclear actin stress fibers (actin cap) have been reported in A-type lamin deficient (Lee at al., 2007; Chambliss et al.,2013; Khatau et al 2009) and A-type lamin mutant cells (Schwartz et al., submitted). We investigated the organization of actin fibers in ΔK32 mutant myoblasts in sparse and dense culture conditions (fig. 4-7 and 4-8).

Supranuclear actin of WT myoblasts was composed of parallel aligned actin fibers, which cover the nucleus (top) in sparse and dense conditions. The number of actin bundles on the top of the nucleus was lower in ΔK32 mutant myoblasts in sparse and dense conditions (fig. 4-7A left). In sparse conditions, the basal actin cytoskeleton (bottom) of WT myoblasts was characterized by the presence of actin bundles as well as several dots, suggestive of actin depolymerization. The presence of these dots apparently increased in dense condition in WT myoblasts. In contrast, ΔK32 mutant myoblasts did not show this dotted phalloidin staining pattern in sparse or dense conditions. Instead, ΔK32 mutant myoblasts had polymerized actin fibers beneath the nucleus in sparse and dense conditions (fig. 4-7A right). These results strongly suggest a depolymerization of actin fibers from sparse to dense conditions beneath the nucleus in WT, but not in ΔK32 mutant myoblasts.

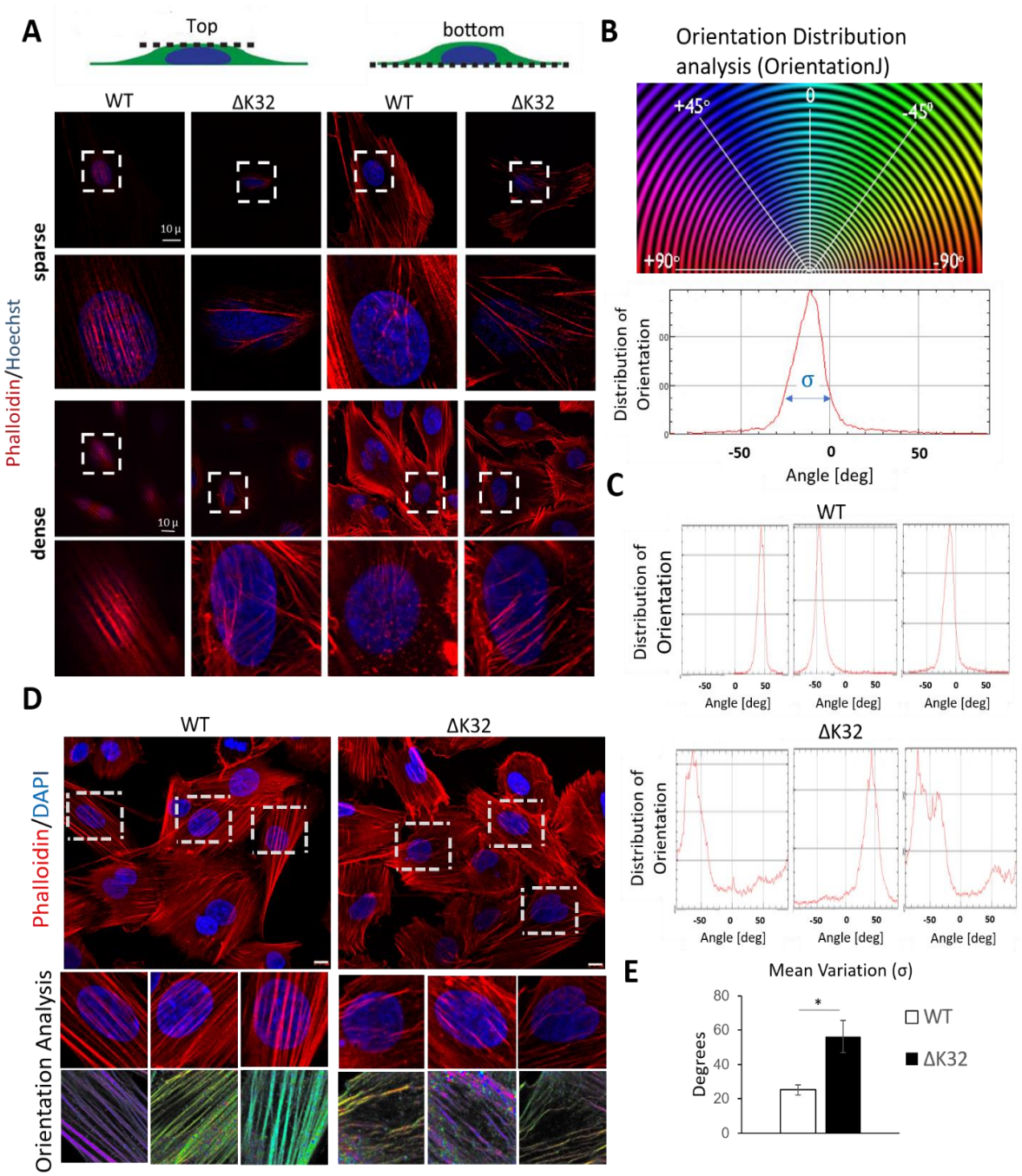

FIGURE 4-7: Defective organization of perinuclear actin in ΔK32 mutant myoblasts. *WT and ΔK32 mutant myoblasts were stained with phalloidin (red) and Hoechst or dapi (blue). Confocal images of the top and the bottom of perinuclear actin show an abnormal organization of the perinuclear actin in ΔK32 mutant myoblasts(A). Perinuclear F-actin was analyzed for Orientation Distrubution with OrientationJ (ImageJ)(B-E). Actin fibers were colored dependent on their orientation angle (D) and histograms represent the number of pixels for each orientation angle (C). The mean variation of the distribution of the orientation of perinuclear f-actin fibers is significantly increased in ΔK32 mutant myoblasts (E). Quantifications represent means of ≥ 3 independent analysis, +/- SEM(*) p-value ≤0.05; (**)p-value ≤0.005, (***)p-value ≤0.0005; (n.s)not significant. Scale bar = 10µm*

To further characterize the organization of actin fibers, we analyzed their orientation using maximum projections of z-stacks. The orientation angle of each actin fiber was determined and each fiber was colored, so that similar angles will have similar colors. In addition, the distribution of the orientation angles was quantified and depicted as histogram. The mean variation of the distribution of the orientation is defined as sigma (σ) (fig. 4-7B).

WT myoblast had a parallel organization of actin stress fibers in the perinuclear region, which is reflected in a monotonous coloration of actin fibers. In contrast, ΔK32 myoblasts showed multicolored actin fibers in the perinuclear region (fig. 4-7D). Histograms of WT actin fibers showed narrow shaped peaks whereas distribution peaks of ΔK32 myoblasts were more wide (fig. 4-7C). The mean variation (Ω) of the distribution was significantly increased in ΔK32 mutant myoblasts (fig. 4-7E). Taken together, these results suggested quantitative and qualitative defects of perinuclear actin fibers in ΔK32 mutant myoblasts.

Actin fibers reorganized in confluent WT myoblasts, forming parallel actin fibers, which span the whole cell body and connect to cell-cell junctions. In contrast, the actin cytoskeleton of ΔK32 mutant myoblasts was less frequently organized in parallel bundles and mostly did not span the whole cell body (fig. 4-8A, B). Orientation analysis of actin fibers revealed that the orientation of actin fibers was lower in ΔK32 compared to WT myoblasts. The orientation of actin fibers in WT myoblasts was parallel, as reflected in mainly monotonous colored fibers of one cell. In contrast, orientation analysis of ΔK32 myoblasts actin revealed multicolored fibers in each cell (fig. 4-8B), attested by a wider shaped distribution peak of the actin cytoskeleton in ΔK32 than in WT myoblasts (fig. 4-8D). Together these results indicate a defective orientation of perinuclear and whole cell actin fibers in ΔK32 myoblasts compared to WT myoblasts.

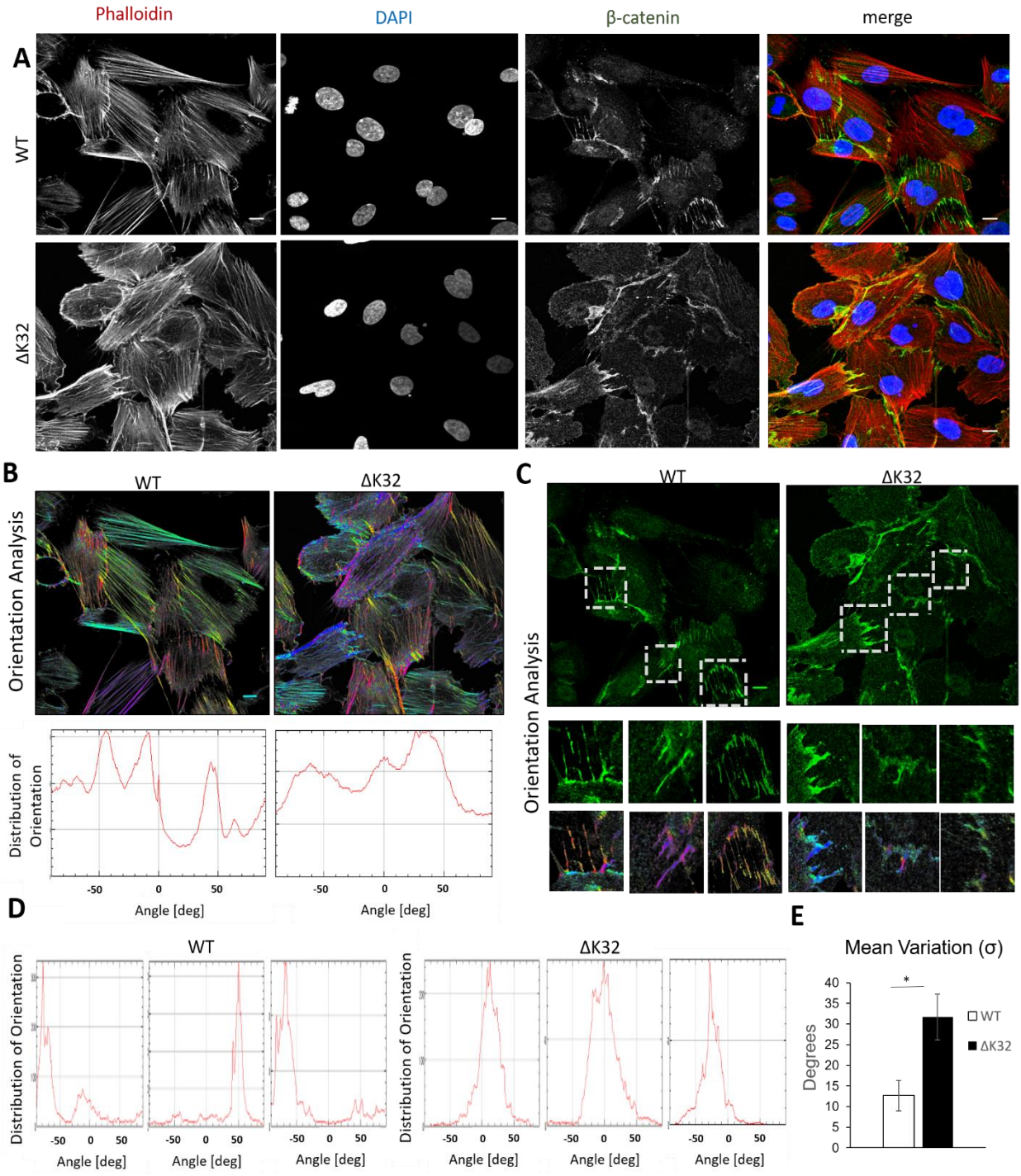

Figure 4-8*: *Disorganization of actin stress fibers in ΔK32 mutant myoblasts affects the organization of adherence junctions. *WT and ΔK32 mutant myoblasts were stained for F-actin (Phalloidin, red), ß-catenin (green) and dapi (blue) (A,C). Whole cell F-actin (B) and ß-catenin stainings (C) were analyzed for Orientation Distrubution with OrientationJ (ImageJ). Actin fibers were colored dependent on their orientation angle (B,C) and histograms represent the number of pixels for each orientation angle (B,D). The mean variation of the distribution of the orientation of the zipper-like ß-catenin staining is significantly increased in ΔK32 mutant myoblasts (E). Scale bar = 10µm. Quantifications represent means of ≥ 3 independent image analysis, +/- SEM. (*) p-value ≤0.05; (**)p-value ≤0.005, (***)p-value ≤0.0005; (n.s)not significant. Scale bar = 10µm*

DISORGANIZED CELL-CELL CONTACT SIDES IN ΔK32 MYOBLASTS

As the organization of actin fibers in ΔK32 myoblasts may affect the organization of cell- cell contact sites, cell-cell contact formation was analyzed (fig. 4-8A,C). In stage AJ2, cell-cell junctions between myoblasts show a zipper-like structure at the tip of actin fibers. In WT myoblasts, the ß-catenin staining pattern was zipper-like and parallel organized. In contrast, the ß-catenin staining pattern in ΔK32 myoblasts was more diffuse, indicating a reduced formation of AJ2 stage cell-cell junctions in ΔK32 myoblasts. Orientation analysis of ß-catenin stainings are depicted in figure (fig. 4-8C,D). The mean variation (σ) of ß-catenin stained cell-cell contacts was significantly increased in ΔK32 myoblasts compared to WT myoblasts (fig. 4-8E). These results supported our hypothesis that disorganization of actin fibers in ΔK32 myoblasts contribute to the disorganization of cell-cell contact sites.

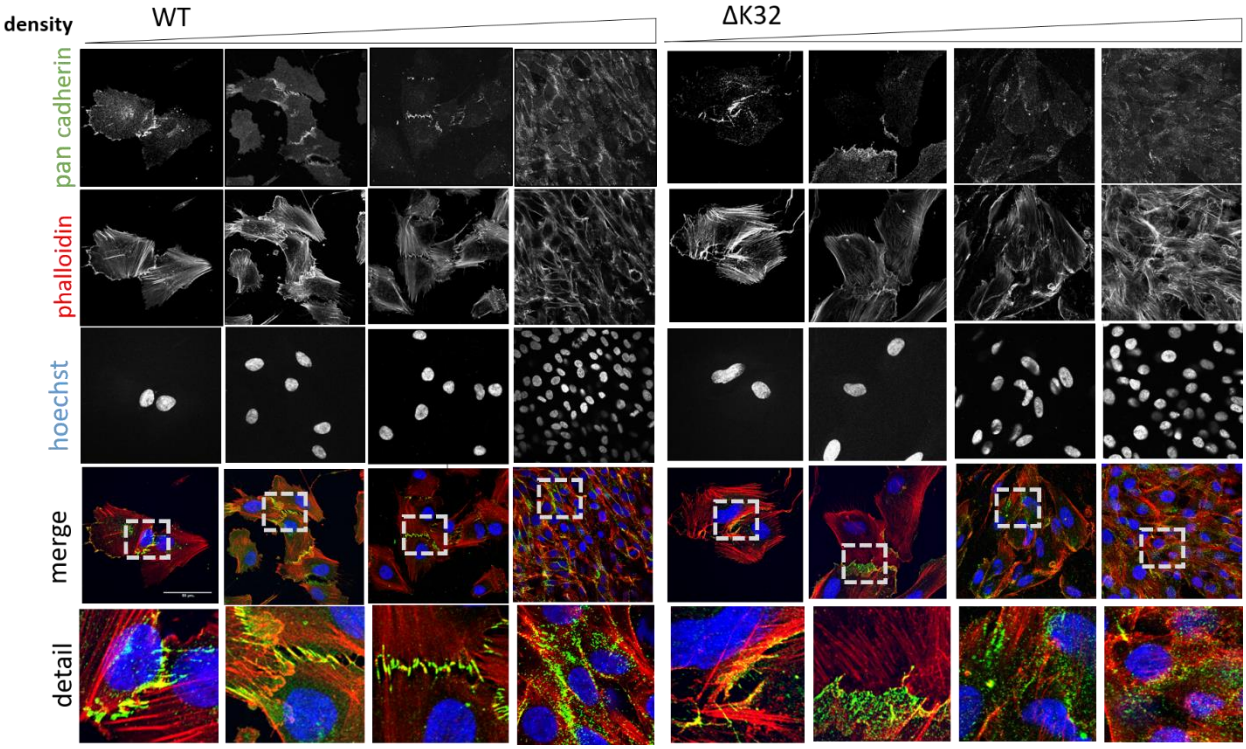

Figure 4-9: Defecive recruitment of cadherins to cell-cell junctions in ΔK32 myoblasts. *WT and ΔK32 mutant myoblasts were stained with an anti-pan-cadherin antibody (green) at different densities and counterstained with Phalloidin (red) and DAPI (blue)(A). WT myoblasts showed the typical zipper-like cadherin staining pattern, while ΔK32 mutant myoblasts showed no or a diffuse cadherin staining pattern at cell-cell junctions. Scale bar = 50µm*

DEFECTIVE FORMATION OF CELL-CELL CONTACT SITES

We hypothetized that the increased YAP activity affects the expression and localization of M- and N-cadherin, which are the predominantly expressed cadherins in myoblasts. In human myoblasts, we were unable to obtain a specific immunostaining of M-cadherin. Immunofluorescence labelling was obtained using a pan-cadherin antibody. Whereas WT myoblasts showed the characteristic zipper-like cadherin pattern at cell-cell junctions, ΔK32 mutant myoblasts showed no or only a weak and diffuse staining (fig. 4-9).

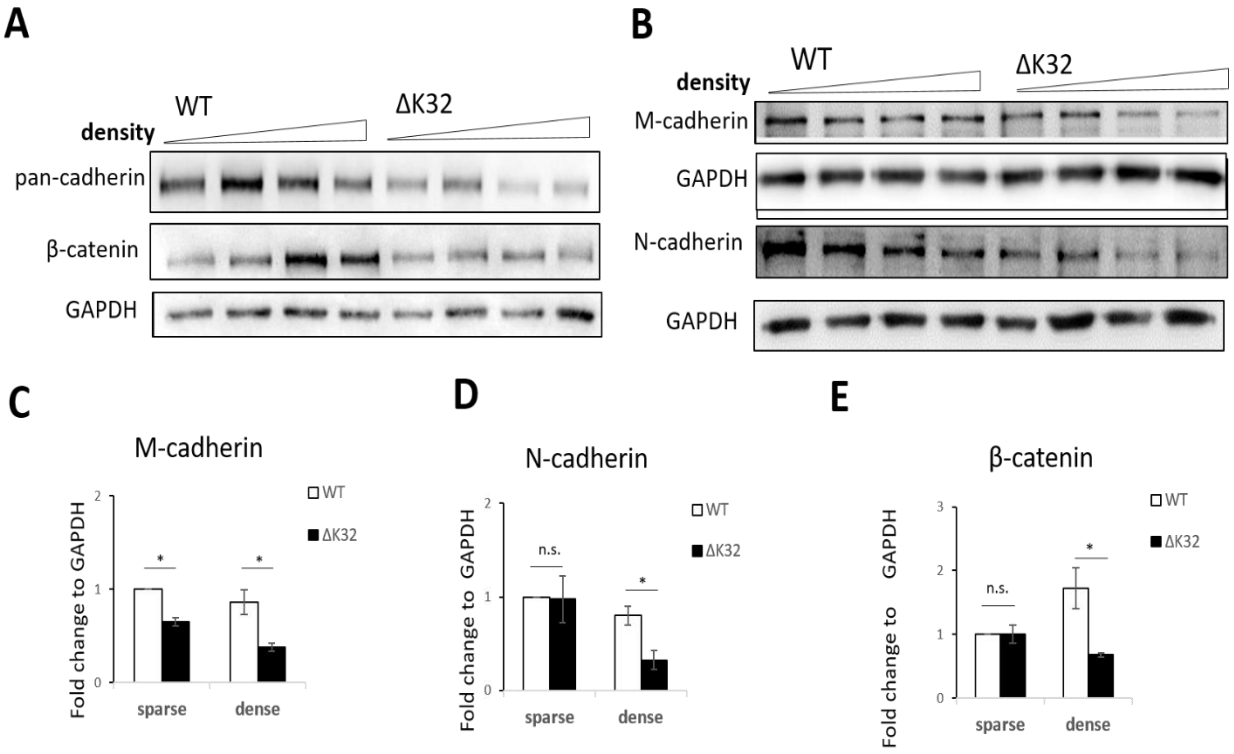

Figure 4-10: Decreased cadherin protein levels in ΔK32 myoblasts. Protein levels of different members of the cell junction were analyzed at different *cell densities (A,B). In sparse condition M-cadherin but, not N-cadherin protein levels were significantly lower in ΔK32 mutant compared to WT myoblasts(C,D). ΔK32 mutant myoblasts had significantly reduced M- and N-cadherin levels in dense condition (C,D). While ß-catenin protein level increased in WT myoblasts with growing density, it decreased in ΔK32 mutant myoblasts (E). Quantifications represent means of ≥ 3 independent experiments, +/- SEM, shown as fold changes, normalized to WT sparse. (*) p-value ≤0.05; (**) p-value ≤0.005, (***), p-value ≤0.0005; (n.s) not significant.*

We then analyzed cadherin protein levels at various cell densities (fig. 4-10A,B). ΔK32 myoblasts had significantly lower M- cadherin protein levels in sparse and dense conditions compared to WT myoblasts (fig. 4-10C). In addition, N-cadherin protein levels were significantly lower in ΔK32 mutant compared to WT myoblasts in dense condition (fig. 4-10D).

The α- and ß-catenin protein complexes regulate the connection between actin fibers and cadherins at cell-cell contact sides. In sparse condition, ß-catenin protein levels did not differ in WT and ΔK32 myoblasts. With increasing cell density, β-catenin protein levels increased in WT myoblasts but did not change in ΔK32 myoblasts (fig. 4-10A). As a result, the protein level of β-catenin was significantly higher in WT compared to ΔK32 myoblasts in dense condition (fig. 4-10D). These results point to a reduced expression of cadherins in leading to a reduced recruitment of cadherins to form cell-cell contacts. Additonal experiments are on-going to determine the role of YAP in cell-cell contact defects of ΔK32 myoblasts.

LOSS OF CELL-CELL CONTACT IN ΔK32 MUTANT MYOBLASTS MIGRATION

We next sought to determine whether the defective cell-cell contact formation of ΔK32 mutant myoblasts affected the migration pattern in ΔK32 mutant myoblasts. Migrating myoblasts were recorded by life-cell imaging in a wound healing assay and analyzed in regard to cell-cell interactions (fig. 4-11A). The histogram in figure 4-11B depicts the percentage of migrating cells without interaction partner as a function of time. Compared to WT, the number of ΔK32 mutant myoblasts which lost cell-cell contact and migrated without interaction partner was significantly increased (fig. 4-11C). After 12h, ΔK32 mutant myoblasts were spread throughout the whole migration area, whereas WT myoblasts remained in groups (fig. 4-11D). These results suggest that defects in cell-cell contact formation in ΔK32 mutant myoblasts modified the migration pattern from a sheet like to a single cell migration pattern.

Conflicting results regarding the migration speed of LMNA-deficient cells have been reported (Lee et al., 2007; Davidson et al., 2014). Here, migration speed was measured as the migrated distance per time for each tracked cell. Additionally, the number of neighbor cells was determined for each migrating cell at every timepoint. We found that the mean migration speed of WT and ΔK32 mutant myoblasts depended on the number of neighbors migrating together. In both, WT and ΔK32 mutant myoblasts, migration speed increased with an increasing number of neighbors. However, with the same number of neighbors, preliminary data suggest, that the mean migration speed of ΔK32 mutant myoblasts was reduced compared to WT myoblasts (fig. 4-11E).

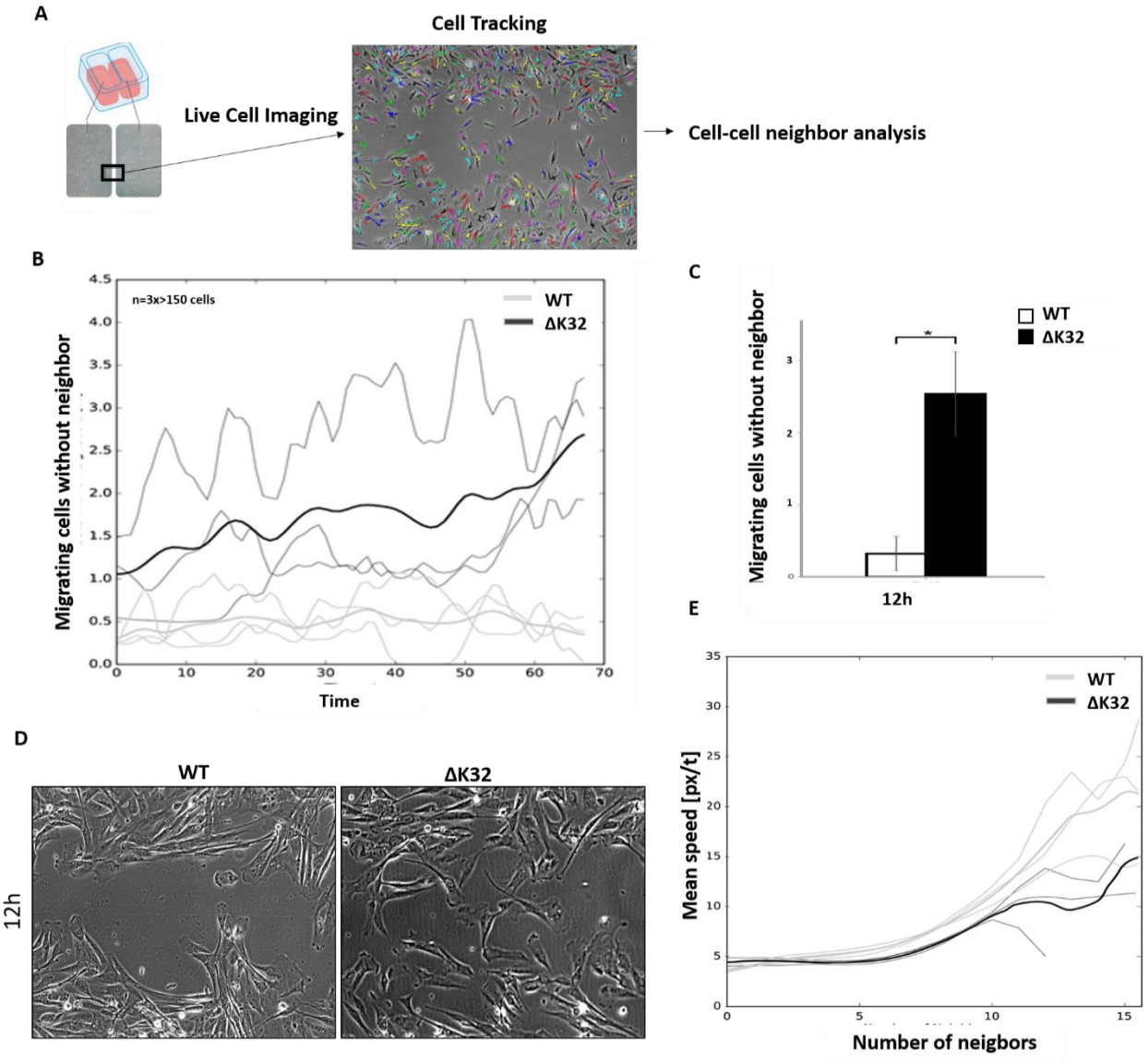

Figure 4-11: Loss of cell-cell contact during migration in ΔK32 mutant myoblasts. *Time-lapse videos of WT and ΔK32 mutant myoblasts migrating in a wound healing assay were recorded for 12h. Videos were analyzed with a software written by Marc Osterland (A). Cell-cell interaction analysis revealed that a significant higher percentage of ΔK32 mutant myoblasts lose cell-cell contact during migration and migrate as a single cell. In contrast, WT myoblasts migrated in groups of cells (B-D). (B) shows the percentage of each cell type, which migrate without interaction partner for every timepoint. After 12h the number of ΔK32 mutant myoblasts migrating without interaction partner is significantly increased(C). In the endpoint image, after 12h migration, ΔK32 mutant myoblasts are more spread in the original gab space than WT myoblasts (D). Migration speed was quantified as migrated distance per time for each tracked cell. The histogram shows the migration speed depending on the number of neighbors. WIth similar quantities of neighbors, WT myoblasts migrate faster than ΔK32 mutant myoblasts. Quantifications represent means of ≥ 3 independent experiments, +/- SEM, shown as fold changes, normalized to WT sparse. (*) p-value ≤0.05; (**) p-value ≤0.005, (***), p-value ≤0.0005; (n.s) not significant.*

ΔK32 MUTANT MYOBLASTS FAILED TO SYNCHRONIZE THEIR ORIENTATION TO EACH OTHER

Previous studies report that the ability of lamin deficient cells to polarize at the wound edge is defective (Lee et al, 2007; Houben et al., 2009). I analyzed nuclear repositioning in migrating myoblasts but did not found significant defects in migrating ΔK32 myoblasts. To extend this analysis, the relative orientation of ΔK32 mutant myoblasts to each other during migration was determined. ΔK32 and WT myoblasts were stained with a trans-golgi marker (Syntaxin-6) following 12h migration in the wound healing assay. Cell-orientation vectors were generated starting at the nuclear center and ending at the trans-golgi (fig. 4-12A).

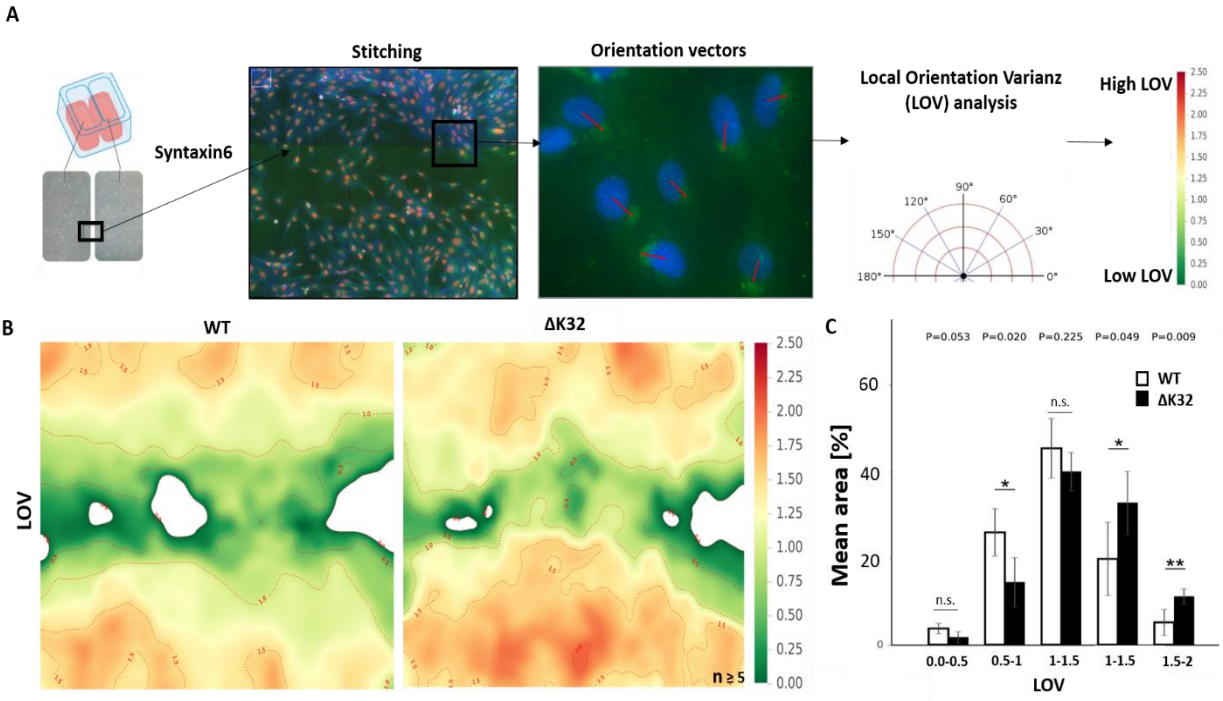

Figure 4-12: Defective synchronization of cell-orientation in ΔK32 mutant myoblasts*. WT and ΔK32 mutant myoblasts were stained with syntaxin6 after migrating in a wound healing assay for 12h (E). Orientation vectors were generated and used to calculate the local orientation variance (LOV). F shows mean LOVs quantified from ≥ 5 independent experiments and color-coded as indicated. ΔK32 mutant myoblasts show another distribution of the LOV than WT myoblasts (F). LOVs were grouped in 0.,5 steps and the covered area for each LOV group was quantified for each cell type (G). In wound healing assays of ΔK32 mutant myoblasts larger areas have high LOVs (1 - 2,5). Quantification represent means of ≥ 5 independent experiments. (*) p-value ≤0.05; (**)p-value ≤0.005, (***)p-value ≤0.0005; (n.s) not significant.*

The Local Orientation Variance (LOV) was then calculated, based on the orientation vectors of neighboring cells. The LOV index quantifies if cells in proximity to each other are orientated in a similar direction. Figure 10F depicts the mean LOVs (≥ 5 experiments) in WT and

ΔK32 mutant myoblasts. The LOV is coded as color gradient from green to red for low to high LOVs.

WT myoblasts showed a large area with a very low LOV (green) around the original scratch area. Further away from the original scratch area, the LOV increases into a light green and orange area, which code for intermediate LOVs. Only a small area, far from the original scratch area, showed a high LOV (red). As a consequence, WT myoblasts only had a high LOV in the middle of the cell-layer, where cells did not migrate yet. In areas where WT cells migrated, neighboring WT cells had a similar orientation. In contrast, ΔK32 mutant myoblasts showed an area of high LOV (red) close to the original scratch area. This showed, that neighboring, migrating ΔK32 mutant myoblasts did not have a similar orientation (fig. 4-12B).

Finally, the covered area for each LOV was quantified for WT and ΔK32 mutant myoblasts (fig. 4.12C). The mean area covered with low LOVs (LOV= 0 - 1,5) was increased in WT myoblasts, while mean areas of high LOVs (LOV = 1,5 - 2,5) were significantly increased in ΔK32 mutant myoblasts (fig. 4.12C). Altogether, these results suggest that neighboring ΔK32 mutant myoblasts fail to synchronize their orientation to each other during migration, which leads to a more single than sheet-like migration pattern.

5. DISCUSSION

This PhD project aims to analyze the role of YAP deregulation and mechanosensing defects in the pathophysiology of LMNA-related congenital muscular dystrophy. Using immortalized human myoblasts carrying the LMNA p.Lys32del (ΔK32) mutation, we showed, that defective inactivation of YAP in confluent K32 mutant myoblasts was associated with a defect in the maturation of cell-cell contacts and a defect in collective migration. Increased YAP activity in ΔK32 mutant myoblasts was not associated with impaired inactivation of YAP by canonical Hippo pathway signaling. Overall, the data strongly suggested that YAP deregulation in ΔK32 mutant myoblasts impairs mechanosensing at cell-cell junctions through a Hippo independent mechanisms and contributes to a defective migration pattern.

Mutations of the LMNA gene, which codes for A-type lamins, cause laminopathies, a highly heterogeneous group of disorders, including muscular dystrophies and cardiomyopathies (Worman and Bonne 2007; Bertrand et al. 2011). While the disease mechanisms underlying LMNA-related muscular dystrophy remains somewhat elusive, recent discoveries point to key interactions between A-type lamins and stress response pathways. There is growing evidence that the integrity of the nucleus and mechanotransduction signaling may be impaired in diseases caused by mutations in A-type lamins and lamin-associated proteins (Lammerding et al. 2004; Hale et al. 2008; Lammerding et al., 2005; Emerson et al. 2009). The mechanosensibility of myoblasts from L-CMD patients has recently been determined in 3D soft microenvironment as well as on 2D substrates of various stiffness (Bertrand et al, 2014; Schwartz et al., in revision). Severe defects in the capacity of lamin A/C mutated cells to sense their environment stiffness and to respond to mechanical stress have been reported and related to a deregulation of YAP (Bertrand et al 2014). However, the molecular mechanisms responsible for the increased YAP activity remains unknown.

INCREASED YAP ACTIVITY IN ΔK32 MUTANT MYOBLASTS: ROLE OF THE HIPPO PATHWAY

We found that the transcriptional activity of YAP was significantly increased in ΔK32 mutant compared to WT myoblasts plated on conventional hard surface. Therefore, YAP overactivity in lamin A/C mutated cells was not restricted to cultivation in a soft environment as previously

reported (Bertrand et al, 2014), but rather appeared as a constitutive abnormality of myoblasts with a L-CMD causing mutation.

Because YAP is canonically regulated by the Hippo pathway with its core kinase LATS, my 1st aim was to determine whether impaired Hippo pathway activity caused the increased YAP activity. With an active Hippo pathway, activated MST activates LATS, which in turn phosphorylates YAP. Phosphorylated YAP is sequestered in the cytoplasm and degraded (Zhao et al., 2007; Zhao et al., 2010). Therefore, activation of Hippo signaling prevents YAP transcriptional activity.

In standart human myoblast medium, which contains 20% of fetal calf serum, total and active LATS (phospho(thr1079)LATS) as well as phosphorylated YAP(S127) protein levels were significantly increased in ΔK32 compared to WT myoblasts. This indicates that the Hippo pathway is active in ΔK32 mutant myoblasts. We concluded that the overactivation of YAP in ΔK32 mutant myoblasts was not due to a decreased Hippo pathway activity.

A recent report shows that YAP transcriptional activity induces the expression of LATS which, in turn, phosphorylates YAP (Moroishi et al., 2015). This intrinsic negative feedback mechanism may contribute to explain the increased levels of phosphorylated YAP in ΔK32 myoblasts.

YAP can be inactivated by growth factor starvation, through a LATS-mediated phosphorylation of YAP at serine 127 (Yu et al., 2012). Our results showed that growth factor starvation increased the activity of LATS, the phosphorylation of YAP (S127) and consequently decreased the transcriptional activity of YAP in both ΔK32 mutant and WT myoblasts. We concluded, that the inactivation of YAP through growth factor mediated HIPPO activation was not impaired in ΔK32 mutant myoblasts.

Taken together, we concluded that the overactivation of YAP in ΔK32 mutant myoblasts was not due to impaired Hippo signaling pathway but, that the increased activity of YAP in ΔK32 mutant myoblasts occured through Hippo-independent regulation of YAP. Consistently, the regulation of YAP by the Hippo pathway in the context of mechanotransduction has been questioned (Aragona et al., 2013). Analyzing YAP phosphorylation at Serine127 is widely used as an indicator for Hippo pathway activity. However, it has been shown that LATS can be activated

through non canonical Hippo pathway signaling (fig. 1.7). Therefore, it is possible that YAP phosphorylation at Ser127 occurs through non canonical Hippo pathway signaling.

INCREASED YAP ACTIVITY IN ΔK32 MUTANT MYOBLASTS: ROLE OF CELL SPREADING AREA

The cell spreading area and cell shape regulate YAP activity (Wada et al., 2011). ΔK32 mutant myoblasts have increased total YAP protein levels as well as an increased spreading area compared to WT myoblasts. Total YAP levels decreased together with the spreading area from sparse to dense conditions in both cell lines. Interestingly, protein levels of phosphorylated YAP increased by growth factor mediated YAP inactivation, whereas they decreased through density mediated inactivation of YAP. However, in dense culture conditions, the spreading area and YAP transcriptional activity remained higher in ΔK32 mutant compared to WT myoblasts. These results suggest that total YAP levels may depend on the cell spreading area and therefore, may tune the sensitivity to YAP activation. To validate this hypothesis, we propose to analyze YAP protein levels in cells plated in restricted areas, using micropatterning techniques.

REGULATION OF YAP BY CELL CONTACT INHIBITION IS DEFECTIVE IN ΔK32

In dense culture conditions, YAP was excluded from the nucleus in WT but not ΔK32 mutant myoblasts. This in turn may contribute to the increased transcriptional activity of YAP in ΔK32 myoblasts at high density. In non-cancer cells, cell-cell contact and high cell density activate the Hippo pathway to inhibit YAP activity (Zaoh et al., 2007). Further studies revealed that the formation of cadherin/catenin complexes at cell-cell junctions modulate YAP inhibition in high cell density (Zaoh et al., 2007; Kim et al., 2011; Schlegelmilch et al., 2011, Zaoh et al., 2012).

Expression of E-cadherins, as well as their association with α- and β-catenin, are required for density dependent nuclear exclusion of YAP (Kim et al., 2009). Furthermore, YAP relocates to the nucleus in confluent monolayers when cadherin dependent cell–cell connections are disrupted (Schlegelmilch et al., 2011). It has been demonstrated that protein levels of E-cadherin can even tune the efficacy of contact mediated inhibition of proliferation (Kim, Kushiro et al. 2009). Furthermore, knockdown of β-catenin prevents YAP nuclear exclusion (Kim, Koh et al. 2011).

ΔK32 mutant myoblasts showed a defective formation of cell-cell contacts in dense conditions. M-, N-cadherin and ß-catenin protein levels were significantly reduced in confluent ΔK32 mutant compared to WT myoblasts. Also, the characteristic zipper-like staining pattern of cell-cell junctions was only found in WT myoblasts. Taken as a whole, our data strongly suggested a link between reduced M-cadherin, N-cadherin and ß-catenin protein levels and the increased YAP nuclear localization and YAP activity in ΔK32 mutant myoblasts at high cell density. To unveil the link between YAP over activity and the reduced cadherin/catenin protein levels in ΔK32 mutant myoblasts, cadherin/catenin levels after YAP overexpression and siRNA mediated YAP silencing should be investigated. Additional experiments are on-going to dissect potential mechanisms.

IMPAIRED INTEGRITY OF THE ACTIN CYTOSKELETON IN ΔK32 MUTANT MYOBLASTS

The actin cytoskeleton is coupled to A-type lamins of the nucleoskeleton via the LINC-complex (Salpingidou. et al., 2007). To date, all LMNA mutations that cause striated muscle disease compromise the Nesprin/Sun/lamin interactions, resulting in dysfunctional nucleo-cytoskeletal linkages (Haque et al. 2010; Folker et al., 2010, Chang et al., 2013, Chang et al., 2015). Accordingly, the ΔK32 lamin mutation most likely impairs the integrity of the nucleo-cytoskeletal linkages. A Co-Immunoprecipitation with the Sun proteins of the LINC-complex and the ΔK32 mutant A-type Lamins remain to be performed in order to support this hypothesis.

Although the detailed mechanism remains to be determined, there is growing evidence that a dysfunctional LINC complex may impair the dynamics and organization of the actin cytoskeleton (Lammerding et al., 2004; Hale et al., 2008; Khatau et al. 2009; Luxton et al., 2010). Functional loss of A-type lamins alters actin structures around the nucleus in cells cultured on rigid substrates (Khatau et al, 2009; Chambliss et al 2013; Kim et al 2012), In addition, cells from $LMNA^{-/-}$ mice exhibit abnormal actin fiber localization and polymerization (Broers at al., 2005; Lee at al., 2007; Khatau et al.,2009; Lammerding et al., 2004). The cytoplasm of LMNA deficient MEFs is softer, less elastic, and more compliant than the cytoplasm of WT cells (Lee et al., 2006).

Consistently, we reported alteration in the orientation of perinuclear actin fibers in ΔK32 mutant myoblasts. Furthermore, only WT myoblasts reorganized their actin fibers into parallel aligned fibers in dense culture conditions, while the orientation of actin fibers of ΔK32 mutant myoblasts was less parallel. ΔK32 mutant myoblasts have already been reported to fail to adapt

their cytoskeletal organization to a mechanical challenge (Betrandt et al., 2014). Here, we can extend this observation and report that ΔK32 mutant myoblasts fail to adapt their actin cytoskeleton to high cell-cell contact condition.

Mechanotransduction depends on actin dynamics (Dupont et al., 2011). We found an increased variation of the orientation of actin fibers in the perinuclear region and in basal actin fibers of ΔK32 mutant myoblasts. In addition, the expression of a myosin light chain protein (myl9), which regulates the formation of actin stress fibers, was increased in ΔK32 mutant myoblasts. Furthermore, force transmission between the nucleus and the cytoskeleton is impaired in several myopathic lamin mutations (Zwerger et al., 2013; Bertrand et al.; 2014). Therefore, the defective orientation of actin fibers in ΔK32 mutant myoblasts may impair the transmission of forces from the cell periphery. This, in turn, may lead to the aberrant mechanosensitive signaling activity. Additionnal studies are required to precise the molecular mechanisms which regulates the interplay between YAP activity and actin-mediated intercellular stress in ΔK32 myoblasts given that a reduced actin contractility was expected to reduce YAP activity. Furthermore, fluorescence microscopy can not provide information about the mechanical forces which are generated by actin fibers. To study mechanical forces generated by ΔK32 mutant myoblasts another method like traction force microscopy need to be performend.

DEFECTIVE FORMATION OF CELL-CELL CONTACTS

YAP overexpression was recently found to antagonize E-cadherin junction assembly by regulating actin cytoskeleton architecture through increased expression of myosin light chain 9 (myl) (Bai et al., 2016). Consistently, ΔK32 mutant myoblasts had an increased expression of YAP and myl9 in dense condition, which may cause the defective formation of cell-cell junctions.

Alternatively, actin disorganization may directly affect the formation of cell-cell contacts in ΔK32 mutant myoblasts. The characteristic zipper-like staining pattern of cell-cell junctions was only found in WT myoblasts. Mature cell-cell junctions are formed by cadherin/catenin complexes at the tips of actin fibers, to which they connect via α-catenin mediated binding (Causeret et al. 2005). Cadherin clusters have been shown to be stabilized at cell-cell junctions by binding to actin fibers (Truong et al., 2013). Therefore, the defective formation of cell-cell junctions in ΔK32 mutant myoblasts may be caused by the impaired orientation of actin fibers in ΔK32 mutant myoblasts.

To further investigate if the decreased formation of cell-cell contacts in ΔK32 mutant myoblasts is caused by the impaired actin cytoskeleton or by the increased YAP activity, cell-cell contact formation in ΔK32 mutant myoblasts after knockdown and overexpression of YAP needs to be analyzed. Alternatively, the formation of cell-cell contacts after actin destabilization in WT myoblasts can be analyzed.

ABERRANT MIGRATION BEHAVIOUR OF ΔK32 MUTANT MYOBLASTS

Our data showed an increased loss of cell-cell contacts during migration of ΔK32 mutant myoblasts compared to WT myoblasts. As a result, an increased number of ΔK32 mutant myoblasts migrated alone whereas a sheet like migration was observed in WT myoblasts. Further we reported an increased variance in the polarization between neighboring ΔK32 mutant myoblasts compared to WT myoblasts.

Cell–cell contacts are critical for the coordination of cell movements during collective migration (Arboleda-Estudillo et al., 2010; Murrell et al., 2011; Tambe et al., 2011). Cadherin-dependent adhesion contributes to the regulation of cell polarization and directionality during collective cell migration (Cai et al., 2014). Moreover, cadherin-dependent propagation of mechanical stresses at cell-cell junctions orchestrates collective cell migration. Thereby, neighbouring cells join their forces together to then migrate along a minimum of intercellular shear stress. Hence, collective migration is guided by mechanical stress (Tambe et al. 2011; Trepat et al., 2011).

Knockdown of P-cadherin or overexpression of dominant negative E-cadherin reduces the coordination of cells orientation (Ng et al., 2012). Recent data indicate that formation of cadherin rich pertrusions (cadherin fingers) guides cells during collective cell migration (Hayer et al., 2016). Therefore, the reduced collective migration pattern of ΔK32 mutant myoblasts is most likely caused by a defective cadherin-dependent transmission of forces between cells. Therefore, we provided, herein, the first evidence that a LMNA mutation causes a defective mechanotransduction at cell-cell contact sides, which, causes a defect in the coordination of collective cell migration.

Further data are needed to confirm this hypothesis. For instance, expression of M-cadherin with a tension sensor module could be used to determine intercellular force transmission, as already developed for VE-cadherin (Conway et al., 2013). Alternatively, intercellular stress maps of

migrating myoblasts could be used to assess a disturbed transmission of forces between migrating ΔK32 mutant myoblasts (Tambe et al., 2011).

Notably, defects in the polarization of LMNA deficient cells have already been reported at early time points after initiation of migration; i.e., 30min, 1h and 3h after the onset of migration. (Lee et al., 2007; Houben et al., 2009; Chang et al., 2015; Folker et al., 2010). Polarization defects have been attributed to a defect of nuclear repositioning in LMNA deficient cells. Nuclear repositioning is achieved by retrograde flow of 'transmembrane actin associated nuclear (TAN) lines, which are defective in lamin A/C deficient cells (Folker et al., 2010).

Interestingly, at later time points, the orientation of LMNA mutant cells towards the wound edge is nearly similar to WT cells, (Houben et al., 2009). We analyzed nuclear repositioning in ΔK32 mutant myoblasts during random cell migration. No significant difference in nuclear repositioning was observed between ΔK32 and WT myoblats. However, to finally exclude a defect in nuclear repositioning in ΔK32 mutant myoblasts, a computer based time-lapse video analysis needs to be performed.

In L-CMD patients, a strong variation of muscle fiber size has been reported (Quijano-Roy et al., 2008). Myoblast migration is a critical step in myogenesis and skeletal muscle regeneration. Sub-optimal migration can reduce the number of myoblasts which enter fusion or reduce myoblast alignment and consequently impair adaptation or repair of skeletal muscle tissue. Thus, the reported collective cell polarization and migration defects may contribute to the pathophysiology of L-CMD. Further experiments need to be performed to understand the consequences of migration defects in the pathophysiology of L-CMD. Although the collective character of migrating myoblasts *in vivo* has not been shown so far, cell density is known to be a critical factor of myoblast fusion *in vitro*. In the classical fusion assay myoblasts need to be cultivated in dense condition. The presented results provide some evidence, that ΔK32 mutant myoblasts may fail to achieve high cell confluence *in vivo*, as they increasingly loose cell-cell contact during migration. To test if the migration defect of ΔK32 mutant myoblasts affects myoblast fusion, an alternative fusion assay, which includes myoblast migration, would need to be developed.

ΔK32 MUTANT MYOBLASTS PROLIFERATION

YAP is a positive regulator of the cell cycle (Dong et al. 2007; Zhao et al., 2007). Surprisingly, ΔK32 mutant myoblasts did not have an increased proliferation capacity, although, YAP transcriptional activity was increased in sparse and dense conditions. On the contrary, ΔK32 mutant myoblasts proliferation was slightly but significantly reduced compared to WT, at all tested densities. This apparent contradiction may be due to the fact that A-type lamins play important role in cell proliferation. Therefore, the ΔK32 LMNA mutation may cause a disturbance during mitosis which overrules the YAP-mediated cell-cycle regulation.

INCREASED TRANSCRIPTIONAL SMAD ACTIVITY IN ΔK32 MUTANT MYOBLASTS

Lastly, our results pointed to potential defects in BMP signaling. Here, we reported a strong increase in the transcriptional activity of Smads in ΔK32 mutant compared to WT myoblasts in starved condition as well as after stimulation with BMP2. Consitently, BMP stimulation has been shown to act synergistically with mechanical signals on Smad activity (Kopf et al., 2012). However, we only found a slight increase in the phosphorylation of Smad1/5 in ΔK32 mutant compared to WT myoblasts in starved condition. Moreover, after stimulation with BMP2 there was no difference in the level of phosphorylation of Smads between ΔK32 mutant and WT myoblasts. If the. These results point to an enhanced transcriptional activity of Smads which, may be independent of Smad phosphorylation.

Nuclear YAP can bind and stabilize the Smad complex, leading to an increased transcriptional activity (Alarcón et al.,2009). Therefore, the increased transcriptional activity of Smads, in ΔK32 mutant myoblasts, may be caused by the increased pool of nuclear YAP. Hence, YAP has been proposed as a mechanically activated transcriptional co-regulation which, may integrate mechanical stimulations into BMP signaling (Kopf et al., 2014). Further investigations are needed to determine whether the increased Smad transcriptional activity of Smads is caused by mechanosensing defects of ΔK32 mutant myoblasts and if these mechanosensing defects are integrated in the BMP pathway by YAP. For example, an increased activation of the BMP pathway by increased expression of BMPs by ΔK32 mutant myoblasts needs to be excluded. Furthermore, Smad transcriptional activity after knockdown of YAP in ΔK32 mutant myoblasts should be investigated. More direct evidence could be achieved through a quantitative co-

immunoprecipitation of YAP and Smad or a FRET based visualization of the YAP/Smad interaction.

In addition, further studies are needed to determine the role of YAP and BMP signaling defects in lamin mutant myoblasts. BMP signaling has been shown to affect planar cell polarity and cortical actin organization during the migration of mesenchymal progenitor cells (Hiepen et al., 2014). Furthermore, BMP signaling was recently shown to regulate the presentation of cadherins on the cell surface (Benn et al., 2016). Aditionally, also YAP has been implicated in the regulation of cell migration (Haskins et al., 2014; Schutte et al. 2014; Sorrentino et al. 2014; Moroishi et al. 2015). Moreover, Merlin, an upstream regulator of the Hippo pathway, is critical for cortical actin dynamics, which have been shown to contribute to the orchestration of collective migration (Zhou and Hanemann 2012; Cooper and Giancotti 2014; Das et al., 2015). To further investigate the role of YAP and Smad over-activity in the defective coordination of migration in ΔK32 mutant myoblasts, cell-cell interaction and cell orientation analysis during migration of ΔK32 mutant myoblasts should be analyzed after inhibition of YAP and Smad activity via siRNA or small molecule inhibotors.

CONCLUSION

In conclusion, this PhD thesis shows that YAP deregulation impairs mechanosensing at cell-cell junctions and contributes to a defective migration pattern in ΔK32 mutant myoblasts through a Hippo independent mechanisms. Although additional experiments are needed, we propose the following mecahnisms to explain the pathogenic feature in ΔK32 mutant myoblasts.

LMNA mutation causes a defective anchorage of actin fibers to the nucleus which in turn impairs the integrity of the actin cytoskeleton and alters the force transmission of mechanical cues from the cell periphery, including cell-cell contacts.

Mechanosensing defects of ΔK32 mutant myoblasts increased the activity of mechanosensitive YAP and Smad signaling. In dense culture conditions, ΔK32 mutant myoblasts failed to inactivate YAP. Interestingly, the growth factor mediated inactivation of YAP through phosphorylation by LATS was not impaired in ΔK32 mutant myoblasts. Thus, the translation of cell-cell contacts into YAP regulation but, not the translation of growth factor stimulation into YAP regulation is severely impaired in ΔK32 mutant myoblasts. Furthermore, we provided some

evidence that the regulation of YAP by mechanical cues includes a regulation of total YAP protein levels, which, may tune the sensitivity to YAP activation.

Impaired force transmission by the actin cytoskeleton and decreased formation of stable, actin-associated cell-cell contacts impacted on migration, from a sheet like to a single cell like migration pattern. ΔK32 mutant myoblasts lost cell-cell contact during migration and showed a reduced synchronization of their polarization. Because myoblast migration is a critical step in myogenesis and skeletal muscle regeneration, these defects may contribute to the pathophysiology of L-CMD.

6. ABSTRACTS

ENGLISH

Mechanotransduction is critical for tissue development, homeostasis and diseases. YAP (Yes-Associated Protein) signaling has emerged as a particularly important regulator of the mechano-response. A defective mechanosensing response, including aberrant YAP signaling, has been recently reported in human myoblasts from patients suffering from LMNA related congenital dystrophy (L-CMD) (Bertrand et al., 2014). L-CMD is a severe early-onset form of muscular dystrophies caused by mutations in A-type lamins. My PhD project aims to further dissect mechanosensing defects of immortalized muscle precursor cells which carry the L-CMD causing ΔK32 mutation.

My results showed that ΔK32 mutant myoblasts had a defective translation of mechanical forces at cell-cell contact sides. ΔK32 mutant myoblasts failed to inactivate YAP in high cell-cell contact conditions, as attested by an increased transcriptional activity of YAP and a persistent nuclear localization. YAP overactivity in ΔK32 mutant myoblasts was not related to an impaired activation of the Hippo signaling pathway. Defective YAP signaling was associated with a disorganization of different subsets of the actin cytoskeleton, including the supranuclear actin, the basal actin and the actin fibers at cell-cell junction. The formation of mature cell-cell contacts in ΔK32 myoblats was defective, and the protein expressions of both M- and N-cadherins were significantly reduced in high cell-cell contact conditions. Moreover, ΔK32 mutant myoblasts showed an increased loss of cell-cell contact during migration, which caused a shift from a sheet-like to a single cell migration pattern. Finally, we reported an increased transcriptional activity of mechanosensitive Smad 1/5/8 signaling in ΔK32 mutant myoblasts. Taken together, these results suggest that mechanosensing defects in ΔK32 mutant myoblasts affect the ability of myoblast to form cell-cell contacts and to migrate collectively. These mechanosensing defects may contribute to the pathophysiology of L-CMD.

DEUTSCH

Mechanotransduktion ist kritisch für die Entwicklung von Geweben, Homöostase und in der Entstehung von Krankheiten. YAP (Yes-Associated Protein) hat sich als besonders wichtiger Regulator der Mechanotransduktion herausgestellt. Eine fehlerhafte Mechanotransduktion, einschließlich abweichender YAP-Signaltransduktion, wurde kürzlich in menschlichen Myoblasten von Patienten mit Kongenitaler Muskeldystrophie mit LMNA-Mutation (L-CMD) (Bertrand et al., 2014) beschrieben. L-CMD ist eine schwere, früh einsetzende Form der Kongenitalen Muskeldystrophien, die durch Mutationen in A-Typ-Laminen verursacht wird. Mein PhD-Projekt zielt darauf ab, Mechanotranduktionsdefekte in immortalisierten Muskelvorläuferzellen, die die L-CMD verursachende ΔK32 Mutation tragen, weiter zu analysieren.

Meine Ergebnisse zeigten, dass ΔK32 mutierte Myoblasten eine defekte Übertragung von mechanischen Kräften an Zell-Zell-Kontakten aufwiesen. In ΔK32 mutierten Myoblasten konnte YAP unter hohen Zell-Zell-Kontaktbedingungen nicht inaktiviert werden. Dies wurde durch eine nukleare Lokalisation und eine erhöhte transkriptionelle Aktivität von YAP gezeigt. Die erhöhte Aktivität von YAP in ΔK32 mutierten Myoblasten war nicht mit einer beeinträchtigten Aktivierung des Hippo-Signalwegs verbunden. Die aberrante YAP-Signaltransduktion war hingegen mit einer veränderten Organisation von verschiedenen Teilen des Aktin-Zytoskeletts, einschließlich des supranuklearen Aktins, des basalen Aktins und der Aktinfasern am Zell-Zell-Übergang, verbunden. Die Ausbildung von reifen Zell-Zell-Kontakten in ΔK32-Myoblasten war gestört und die Proteinlevel von M- und N-Cadherin waren signifikant reduziert in hoher Zell-Zell-Kontaktbedingung. Darüber hinaus zeigten ΔK32 mutierte Myoblasten einen verstärkten Verlust von Zell-Zell-Kontakten während der Migration, was eine Verschiebung von einem „sheet-like“ zu einem „single-like“ Zellmigrationsmuster verursachte. Abschließend zeigten wir eine erhöhte transkriptionelle Aktivität der mechano-sensitiven Smad1/5/8 in ΔK32 mutierten Myoblasten. Zusammengefasst deuten diese Ergebnisse darauf hin, dass Defekte in der Mechanotransduktion in ΔK32 mutierten Myoblasten die Fähigkeit dieser Zellen beeiträchtigt Zell-Zell-Kontakte auszubilden und kollektiv zu migrieren. Diese Defekte in der Mechanotransduktion können zur Pathophysiologie von L-CMD beitragen.

FRANÇAIS

La mécanotransduction est une propriété essentielle au développement des tissus, leur homéostasie et leur physiopathologie. La voie de signalisation YAP (Yes-Associated Protein) est apparue comme un régulateur particulièrement important de la mécano-réponse. Un défaut de mécanosensibilité défectueuse, associant une signalisation aberrante de la voie YAP, a récemment été rapportée dans des myoblastes humains de patients souffrant de dystrophie musculaire congénitale liée à des mutations du gène de la lamine (L-CMD) (Bertrand et al., 2014). La L-CMD est une forme grave de dystrophie musculaire à début précoce. Mon projet de doctorat visait à disséquer les défauts de la mécanosensibilité de cellules précurseurs du muscle présentant la mutation ΔK32.

Mes résultats ont montré que les myoblastes mutants ΔK32 présentaient des un défaut de contact cellule-cellul, attestant d'anomalies de transmission des forces mécaniques entre cellules. Contrairement à ce que l'on observe dans les cellules contrôles à confluence, la voie YAP reste activée dans les myoblastes mutants ΔK32. Cela s'est traduit par une activité transcriptionnelle accrue de YAP et une localisation nucléaire persistante de YAP dans les myoblastes ΔK32. La suractivité de YAP dans les myoblastes mutants ΔK32 n'était pas liée à une altération de la voie Hippo, voie de signalisation canonique qui régule YAP. La signalisation YAP défectueuse a été associée à une désorganisation de différents sous-ensembles du cytosquelette d'actine, incluant l'actine supranucléaire, l'actine basale et les fibres d'actine de la jonction cellule-cellule. La formation et la maturation de jonctions cellule-cellule était défectueuse dans les myoblates ΔK32, et les expressions protéiques des deux principales cadhérines, M et N-cadhérins, étaient significativement réduites à confluence. De plus, les myoblastes mutants ΔK32 présentaient une perte accrue de contact cellule-cellule pendant la migration, responsable d'une perte de la migration collective dans les cellules mutantes. Enfin, nous avons rapporté une augmentation de l'activité transcriptionnelle de la signalisation Smad 1/5/8 dans les myoblastes mutants ΔK32. En conclusion, ces résultats de thèse suggèrent que les défauts de mécanosensibilité dans les myoblastes mutants ΔK32 affectent la capacité des myoblastes à former des contacts cellule-cellule et à migrer collectivement. Ces défauts de mécanosensibilité peuvent contribuer à la physiopathologie de la L-CMD.

7. REFERENCES

Adam, Stephen A., and Robert D. Goldman. 2012. 'Insights into the differences between the A- and B-type nuclear lamins', *Advances in Biological Regulation*, 52: 108-13.

Alarcon, C., A. I. Zaromytidou, Q. Xi, S. Gao, J. Yu, S. Fujisawa, A. Barlas, A. N. Miller, K. Manova-Todorova, M. J. Macias, G. Sapkota, D. Pan, and J. Massague. 2009. 'Nuclear CDKs drive Smad transcriptional activation and turnover in BMP and TGF-beta pathways', *Cell*, 139: 757-69.

Anno, Toshiro, Naoya Sakamoto, and Masaaki Sato. 2012. 'Role of nesprin-1 in nuclear deformation in endothelial cells under static and uniaxial stretching conditions', *Biochemical and Biophysical Research Communications*, 424: 94-99.

Aragon, E., N. Goerner, A. I. Zaromytidou, Q. Xi, A. Escobedo, J. Massague, and M. J. Macias. 2011. 'A Smad action turnover switch operated by WW domain readers of a phosphoserine code', *Genes Dev*, 25: 1275-88.

Aragona, M., T. Panciera, A. Manfrin, S. Giulitti, F. Michielin, N. Elvassore, S. Dupont, and S. Piccolo. 2013. 'A mechanical checkpoint controls multicellular growth through YAP/TAZ regulation by actin-processing factors', *Cell*, 154: 1047-59.

Arboleda-Estudillo Y, Krieg M, Stühmer J, Licata NA, Muller DJ, Heisenberg CP.2010. 'Movement directionality in collective migration of germ layer progenitors', Curr Biol, 20(2):161-9.

Azibani, Feriel, Antoine Muchir, Nicolas Vignier, Gisèle Bonne, and Anne T. Bertrand. 2014. 'Striated muscle laminopathies', *Seminars in Cell & Developmental Biology*, 29: 107-15.

Azzolin, L., T. Panciera, S. Soligo, E. Enzo, S. Bicciato, S. Dupont, S. Bresolin, C. Frasson, G. Basso, V. Guzzardo, A. Fassina, M. Cordenonsi, and S. Piccolo. 2014. 'YAP/TAZ incorporation in the beta-catenin destruction complex orchestrates the Wnt response', *Cell*, 158: 157-70.

Bae, Gyu-Un, Ursula Gaio, Youn-Joo Yang, Hye-Jin Lee, Jong-Sun Kang, and Robert S. Krauss. 2008. 'Regulation of Myoblast Motility and Fusion by the CXCR4-associated Sialomucin, CD164', *Journal of Biological Chemistry*, 283: 8301-09.

Bai, H., Q. Zhu, A. Surcel, T. Luo, Y. Ren, B. Guan, Y. Liu, N. Wu, N. E. Joseph, T. L. Wang, N. Zhang, D. Pan, G. Alpini, D. N. Robinson, and R. A. Anders. 2016. 'Yes-associated protein impacts adherens junction assembly through regulating actin cytoskeleton organization', *Am J Physiol Gastrointest Liver Physiol*, 311: G396-411.

Bank, Erin M., Kfir Ben-Harush, Naomi Feinstein, Ohad Medalia, and Yosef Gruenbaum. 2012. 'Structural and physiological phenotypes of disease-linked lamin mutations in C. elegans', *Journal of Structural Biology*, 177: 106-12.

Beauchamp, J. R., L. Heslop, D. S. Yu, S. Tajbakhsh, R. G. Kelly, A. Wernig, M. E. Buckingham, T. A. Partridge, and P. S. Zammit. 2000. 'Expression of CD34 and Myf5 defines the majority of quiescent adult skeletal muscle satellite cells', *J Cell Biol*, 151: 1221-34.

Benhaddou, A., C. Keime, T. Ye, A. Morlon, I. Michel, B. Jost, G. Mengus, and I. Davidson. 2012. 'Transcription factor TEAD4 regulates expression of myogenin and the unfolded protein response genes during C2C12 cell differentiation', *Cell Death Differ*, 19: 220-31.

Benn, Andreas, Clara Bredow, Isabel Casanova, Slobodan Vukičević, and Petra Knaus. 2016. 'VE-cadherin facilitates BMP-induced endothelial cell permeability and signaling', *Journal of Cell Science*, 129: 206.

Bertrand AT, Chikhaoui K, Yaou RB, Bonne G. 2011. 'Clinical and genetic heterogeneity in laminopathies'. Biochem Soc Trans. 39(6):1687-92.

Bertrand, A. T., L. Renou, A. Papadopoulos, M. Beuvin, E. Lacene, C. Massart, C. Ottolenghi, V. Decostre, S. Maron, S. Schlossarek, M. E. Cattin, L. Carrier, M. Malissen, T. Arimura, and G. Bonne. 2012. 'DelK32-lamin A/C has abnormal location and induces incomplete tissue maturation and severe metabolic defects leading to premature death', *Hum Mol Genet*, 21: 1037-48.

Bertrand, A. T., S. Ziaei, C. Ehret, H. Duchemin, K. Mamchaoui, A. Bigot, M. Mayer, S. Quijano-Roy, I. Desguerre, J. Laine, R. Ben Yaou, G. Bonne, and C. Coirault. 2014. 'Cellular microenvironments reveal defective mechanosensing responses and elevated YAP signaling in LMNA-mutated muscle precursors', *J Cell Sci*, 127: 2873-84.

Bianco, A., M. Poukkula, A. Cliffe, J. Mathieu, C. M. Luque, T. A. Fulga, and P. Rorth. 2007. 'Two distinct modes of guidance signalling during collective migration of border cells', *Nature*, 448: 362-5.

Bienz, M. 2005. 'beta-Catenin: a pivot between cell adhesion and Wnt signalling', *Curr Biol*, 15: R64-7.

Birchmeier, Carmen, and Henning Brohmann. 2000. 'Genes that control the development of migrating muscle precursor cells', *Current Opinion in Cell Biology*, 12: 725-30.

Bischoff, Richard. 1994. 'The satellite cell and muscle regeneration', *Myology*, 1: 97-118.

———. 1997. 'Chemotaxis of skeletal muscle satellite cells', *Developmental Dynamics*, 208: 505-15.

Bondesen, Brenda A., Kristen A. Jones, Wayne C. Glasgow, and Grace K. Pavlath. 2007. 'Inhibition of myoblast migration by prostacyclin is associated with enhanced cell fusion', *The FASEB Journal*, 21: 3338-45.

Bonne, Gisele, Marina Raffaele Di Barletta, Shaida Varnous, Henri-Marc Becane, El-Hadi Hammouda, Luciano Merlini, Francesco Muntoni, Cheryl R. Greenberg, Francoise Gary, Jon-Andoni Urtizberea, Denis Duboc, Michel Fardeau, Daniela Toniolo, and Ketty Schwartz. 1999. 'Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy', *Nat Genet*, 21: 285-88.

Broers, J. L. V., F. C. S. Ramaekers, G. Bonne, R. Ben Yaou, and C. J. Hutchison. 2006. 'Nuclear Lamins: Laminopathies and Their Role in Premature Ageing', *Physiological Reviews*, 86: 967-1008.

Broers, Jos L. V., H. J. H. Kuijpers, C. Östlund, H. J. Worman, J. Endert, and F. C. S. Ramaekers. 2005. 'Both lamin A and lamin C mutations cause lamina instability as well as loss of internal nuclear lamin organization', *Experimental Cell Research*, 304: 582-92.

Burke, Brian, and Colin L. Stewart. 2002. 'Life at the edge: the nuclear envelope and human disease', *Nat Rev Mol Cell Biol*, 3: 575-85.

Cai, Danfeng, Shann-Ching Chen, Mohit Prasad, Li He, Xiaobo Wang, Valerie Choesmel-Cadamuro, Jessica K Sawyer, Gaudenz Danuser, and Denise J Montell. 2014. 'Mechanical Feedback through E-Cadherin Promotes Direction Sensing during Collective Cell Migration', *Cell*, 157: 1146-59.

Camargo, F. D., S. Gokhale, J. B. Johnnidis, D. Fu, G. W. Bell, R. Jaenisch, and T. R. Brummelkamp. 2007. 'YAP1 increases organ size and expands undifferentiated progenitor cells', *Curr Biol*, 17: 2054-60.

Cao, X., S. L. Pfaff, and F. H. Gage. 2008. 'YAP regulates neural progenitor cell number via the TEA domain transcription factor', *Genes Dev*, 22: 3320-34.

Cattin, Marie-Elodie, Anne T. Bertrand, Saskia Schlossarek, Marie-Catherine Le Bihan, Søren Skov Jensen, Christiane Neuber, Claudia Crocini, Sophia Maron, Jeanne Lainé, Nathalie Mougenot, Shaïda Varnous, Yves Fromes, Arne Hansen, Thomas Eschenhagen, Valérie Decostre, Lucie Carrier, and Gisèle Bonne. 2013. 'Heterozygous LmnadelK32 mice develop dilated cardiomyopathy through a combined pathomechanism of haploinsufficiency and peptide toxicity', *Human Molecular Genetics*, 22: 3152-64.

Causeret, M., N. Taulet, F. Comunale, C. Favard, and C. Gauthier-Rouviere. 2005. 'N-Cadherin Association with Lipid Rafts Regulates its Dynamic Assembly at Cell-Cell Junctions in C2C12 Myoblasts', *Mol Biol Cell*.

Caveda, L., I. Martin-Padura, P. Navarro, F. Breviario, M. Corada, D. Gulino, M. G. Lampugnani, and E. Dejana. 1996. 'Inhibition of cultured cell growth by vascular endothelial cadherin (cadherin-5/VE-cadherin)', *J Clin Invest*, 98: 886-93.

Chambliss, A. B., S. B. Khatau, N. Erdenberger, D. K. Robinson, D. Hodzic, G. D. Longmore, and D. Wirtz. 2013. 'The LINC-anchored actin cap connects the extracellular milieu to the nucleus for ultrafast mechanotransduction', *Sci Rep*, 3: 1087.

Chang, W., S. Antoku, C. Ostlund, H. J. Worman, and G. G. Gundersen. 2015. 'Linker of nucleoskeleton and cytoskeleton (LINC) complex-mediated actin-dependent nuclear positioning orients centrosomes in migrating myoblasts', *Nucleus*, 6: 77-88.

Chang, Wakam, Eric S. Folker, Howard J. Worman, and Gregg G. Gundersen. 2013. 'Emerin organizes actin flow for nuclear movement and centrosome orientation in migrating fibroblasts', *Molecular Biology of the Cell*, 24: 3869-80.

Charlton, Carol A., William A. Mohler, Glenn L. Radice, Richard O. Hynes, and Helen M. Blau. 1997. 'Fusion Competence of Myoblasts Rendered Genetically Null for N-Cadherin in Culture', *The Journal of Cell Biology*, 138: 331-36.

Charrasse, S., M. Causeret, F. Comunale, A. Bonet-Kerrache, and C. Gauthier-Rouviere. 2003. 'Rho GTPases and cadherin-based cell adhesion in skeletal muscle development', *J Muscle Res Cell Motil*, 24: 309-13.

Charrasse, S., F. Comunale, M. Fortier, E. Portales-Casamar, A. Debant, and C. Gauthier-Rouviere. 2007. 'M-cadherin activates Rac1 GTPase through the Rho-GEF trio during myoblast fusion', *Mol Biol Cell*, 18: 1734-43.

Charrasse, S., F. Comunale, Y. Grumbach, F. Poulat, A. Blangy, and C. Gauthier-Rouviere. 2006. 'RhoA GTPase regulates M-cadherin activity and myoblast fusion', *Mol Biol Cell*, 17: 749-59.

Charrasse, S., M. Meriane, F. Comunale, A. Blangy, and C. Gauthier-Rouviere. 2002. 'N-cadherin-dependent cell-cell contact regulates Rho GTPases and beta-catenin localization in mouse C2C12 myoblasts', *J Cell Biol*, 158: 953-65.

Chen, Y. G., and J. Massague. 1999. 'Smad1 recognition and activation by the ALK1 group of transforming growth factor-beta family receptors', *J Biol Chem*, 274: 3672-7.

Christ, B., and B. Brand-Saberi. 2002. 'Limb muscle development', *Int J Dev Biol*, 46: 905-14.

Cooper, J., and F. G. Giancotti. 2014. 'Molecular insights into NF2/Merlin tumor suppressor function', *FEBS Lett*, 588: 2743-52.

Conway, Daniel E. , Mark T. Breckenridge, Elizabeth Hinde, Enrico Gratton, Christopher S. Chen, Martin A. Schwartz. 2013. 'Fluid Shear Stress on Endothelial Cells Modulates Mechanical Tension across VE-Cadherin and PECAM-1', Current Biology, 11:1024-1030.

Cornelison, D. D. W., and Barbara J. Wold. 1997a. 'Single-Cell Analysis of Regulatory Gene Expression in Quiescent and Activated Mouse Skeletal Muscle Satellite Cells', *Developmental Biology*, 191: 270-83.

Cornelison, D. D., and B. J. Wold. 1997b. 'Single-cell analysis of regulatory gene expression in quiescent and activated mouse skeletal muscle satellite cells', *Dev Biol*, 191: 270-83.

Corti, S., S. Salani, R. Del Bo, M. Sironi, S. Strazzer, M. G. D'Angelo, G. P. Comi, N. Bresolin, and G. Scarlato. 2001. 'Chemotactic Factors Enhance Myogenic Cell Migration across an Endothelial Monolayer', *Experimental Cell Research*, 268: 36-44.

Cossu, G., and U. Borello. 1999. 'Wnt signaling and the activation of myogenesis in mammals', *EMBO J*, 18: 6867-72.

Cossu, Giulio, and Stefano Biressi. 2005. 'Satellite cells, myoblasts and other occasional myogenic progenitors: Possible origin, phenotypic features and role in muscle regeneration', *Seminars in Cell & Developmental Biology*, 16: 623-31.

Crose, L. E., K. A. Galindo, J. G. Kephart, C. Chen, J. Fitamant, N. Bardeesy, R. C. Bentley, R. L. Galindo, J. T. Chi, and C. M. Linardic. 2014. 'Alveolar rhabdomyosarcoma-associated PAX3-FOXO1 promotes tumorigenesis via Hippo pathway suppression', *J Clin Invest*, 124: 285-96.

Cui, Y., F. M. Hameed, B. Yang, K. Lee, C. Q. Pan, S. Park, and M. Sheetz. 2015. 'Cyclic stretching of soft substrates induces spreading and growth', *Nat Commun*, 6: 6333.

Cusella-De Angelis, M.G., S. Molinari, A. Le Donne, M. Coletta, E. Vivarelli, M. Bouche, M. Molinaro, S. Ferrari, and G. Cossu. 1994. 'Differential response of embryonic and fetal myoblasts to TGF beta: a possible regulatory mechanism of skeletal muscle histogenesis', *Development*, 120: 925-33.

D'Amico, A., G. Haliloglu, P. Richard, B. Talim, S. Maugenre, A. Ferreiro, P. Guicheney, I. Menditto, S. Benedetti, E. Bertini, G. Bonne, and H. Topaloglu. 2005. 'Two patients with ‘Dropped head syndrome’ due to mutations in LMNA or SEPN1 genes', *Neuromuscular Disorders*, 15: 521-24.

Danovi, S. A., M. Rossi, K. Gudmundsdottir, M. Yuan, G. Melino, and S. Basu. 2008. 'Yes-associated protein (YAP) is a critical mediator of c-Jun-dependent apoptosis', *Cell Death Differ*, 15: 217-9.

Das, T., K. Safferling, S. Rausch, N. Grabe, H. Boehm, and J. P. Spatz. 2015. 'A molecular mechanotransduction pathway regulates collective migration of epithelial cells', *Nat Cell Biol*, 17: 276-87.

Das, T., and J. P. Spatz. 2016. 'Getting a grip on collective cell migration', *Nat Cell Biol*, 18: 1265-67.

Davidson, P. M., C. Denais, M. C. Bakshi, and J. Lammerding. 2014. 'Nuclear deformability constitutes a rate-limiting step during cell migration in 3-D environments', *Cell Mol Bioeng*, 7: 293-306.

Davidson, P. M., and J. Lammerding. 2014. 'Broken nuclei--lamins, nuclear mechanics, and disease', *Trends Cell Biol*, 24: 247-56.

Davis, Robert L., Harold Weintraub, and Andrew B. Lassar. 1987. 'Expression of a single transfected cDNA converts fibroblasts to myoblasts', *Cell*, 51: 987-1000.

De Falco, M., and A. De Luca. 2006. 'Involvement of cdks and cyclins in muscle differentiation', *Eur J Histochem*, 50: 19-23.

De Vos, Winnok H., Frederik Houben, Miriam Kamps, Ashraf Malhas, Fons Verheyen, Juliën Cox, Erik M.M. Manders, Valerie L.R.M. Verstraeten, Maurice A.M. van Steensel, Carlo L.M. Marcelis, Arthur van den Wijngaard, David J. Vaux, Frans C.S. Ramaekers, and Jos L.V. Broers. 2011. 'Repetitive disruptions of the nuclear envelope invoke temporary loss of cellular compartmentalization in laminopathies', *Human Molecular Genetics*, 20: 4175-86.

Dechat, T., B. Korbei, O.A. Vaughan, S. Vlcek, C.J. Hutchison, and R. Foisner. 2000. 'Lamina-associated polypeptide 2alpha binds intranuclear A-type lamins', *Journal of Cell Science*, 113: 3473-84.

Dechat, Thomas, Stephen A. Adam, Pekka Taimen, Takeshi Shimi, and Robert D. Goldman. 2010. 'Nuclear Lamins', *Cold Spring Harbor Perspectives in Biology*, 2.

Dechat, Thomas, Katrin Pfleghaar, Kaushik Sengupta, Takeshi Shimi, Dale K. Shumaker, Liliana Solimando, and Robert D. Goldman. 2008. 'Nuclear lamins: major factors in the structural organization and function of the nucleus and chromatin', *Genes & Development*, 22: 832-53.

Dessev, G N, C Iovcheva-Dessev, and R D Goldman. 1990. 'Lamin dimers. Presence in the nuclear lamina of surf clam oocytes and release during nuclear envelope breakdown', *Journal of Biological Chemistry*, 265: 12636-41.

Dhawan, Jyotsna, and Thomas A. Rando. 2005. 'Stem cells in postnatal myogenesis: molecular mechanisms of satellite cell quiescence, activation and replenishment', *Trends in Cell Biology*, 15: 666-73.

Dietrich, Susanne. 1999. 'Regulation of hypaxial muscle development', *Cell and Tissue Research*, 296: 175-82.

Dona, E., J. D. Barry, G. Valentin, C. Quirin, A. Khmelinskii, A. Kunze, S. Durdu, L. R. Newton, A. Fernandez-Minan, W. Huber, M. Knop, and D. Gilmour. 2013. 'Directional tissue migration through a self-generated chemokine gradient', *Nature*, 503: 285-9.

Donalies, M., M. Cramer, M. Ringwald, and A. Starzinski-Powitz. 1991. 'Expression of M-cadherin, a member of the cadherin multigene family, correlates with differentiation of skeletal muscle cells', *Proc Natl Acad Sci U S A*, 88: 8024-8.

Dong, J., G. Feldmann, J. Huang, S. Wu, N. Zhang, S. A. Comerford, M. F. Gayyed, R. A. Anders, A. Maitra, and D. Pan. 2007. 'Elucidation of a universal size-control mechanism in Drosophila and mammals', *Cell*, 130: 1120-33.

Driscoll, T. P., B. D. Cosgrove, S. J. Heo, Z. E. Shurden, and R. L. Mauck. 2015. 'Cytoskeletal to Nuclear Strain Transfer Regulates YAP Signaling in Mesenchymal Stem Cells', *Biophys J*, 108: 2783-93.

Dupont, S., L. Morsut, M. Aragona, E. Enzo, S. Giulitti, M. Cordenonsi, F. Zanconato, J. Le Digabel, M. Forcato, S. Bicciato, N. Elvassore, and S. Piccolo. 2011. 'Role of YAP/TAZ in mechanotransduction', *Nature*, 474: 179-83.

Duxson, M.J., Y. Usson, and A.J. Harris. 1989. 'The origin of secondary myotubes in mammalian skeletal muscles: ultrastructural studies', *Development*, 107: 743-50.

Eckersley-Maslin, Melanie A., Jan H. Bergmann, Zsolt Lazar, and David L. Spector. 2013. 'Lamin A/C is Expressed in Pluripotent Mouse Embryonic Stem Cells', *Nucleus*, 4: 53-60.

Edom-Vovard, F., V. Mouly, J.P. Barbet, and G.S. Butler-Browne. 1999. 'The four populations of myoblasts involved in human limb muscle formation are present from the onset of primary myotube formation', *Journal of Cell Science*, 112: 191-99.

El Fahime, E., Y. Torrente, N. J. Caron, M. D. Bresolin, and J. P. Tremblay. 2000. 'In vivo migration of transplanted myoblasts requires matrix metalloproteinase activity', *Exp Cell Res*, 258: 279-87.

Emerson LJ, Holt MR, Wheeler MA, Wehnert M, Parsons M, Ellis JA. 2009. 'Defects in cell spreading and ERK1/2 activation in fibroblasts with lamin A/C mutations', Biochim Biophys Acta, 1792(8):810-21.

Fernandes, J. J., K. B. Atreya, K. M. Desai, R. E. Hall, M. D. Patel, A. A. Desai, A. E. Benham, J. L. Mable, and J. L. Straessle. 2005. 'A dominant negative form of Rac1 affects myogenesis of adult thoracic muscles in Drosophila', Dev Biol, 285: 11-27.

Fernandez, B. G., P. Gaspar, C. Bras-Pereira, B. Jezowska, S. R. Rebelo, and F. Janody. 2011. 'Actin-Capping Protein and the Hippo pathway regulate F-actin and tissue growth in Drosophila', Development, 138: 2337-46.

Fernando, P., J. F. Kelly, K. Balazsi, R. S. Slack, and L. A. Megeney. 2002. 'Caspase 3 activity is required for skeletal muscle differentiation', Proc Natl Acad Sci U S A, 99: 11025-30.

Ferrigno, O., F. Lallemand, F. Verrecchia, S. L'Hoste, J. Camonis, A. Atfi, and A. Mauviel. 2002. 'Yes-associated protein (YAP65) interacts with Smad7 and potentiates its inhibitory activity against TGF-beta/Smad signaling', *Oncogene*, 21: 4879-84.

Fischer, M., P. Rikeit, P. Knaus, and C. Coirault. 2016. 'YAP-Mediated Mechanotransduction in Skeletal Muscle', *Front Physiol*, 7: 41.

Folker, E. S., C. Ostlund, G. W. G. Luxton, H. J. Worman, and G. G. Gundersen. 2010. 'Lamin A variants that cause striated muscle disease are defective in anchoring transmembrane actin-associated nuclear lines for nuclear movement', *Proceedings of the National Academy of Sciences*, 108: 131-36.

Gee, S. T., S. L. Milgram, K. L. Kramer, F. L. Conlon, and S. A. Moody. 2011. 'Yes-associated protein 65 (YAP) expands neural progenitors and regulates Pax3 expression in the neural plate border zone', *PLoS One*, 6: e20309.

George-Weinstein, M., J. Gerhart, J. Blitz, E. Simak, and K. A. Knudsen. 1997. 'N-cadherin promotes the commitment and differentiation of skeletal muscle precursor cells', *Dev Biol*, 185: 14-24.

Goetsch, K. P., K. H. Myburgh, and C. U. Niesler. 2013. 'In vitro myoblast motility models: investigating migration dynamics for the study of skeletal muscle repair', *J Muscle Res Cell Motil*, 34: 333-47.

Goichberg, P., and B. Geiger. 1998. 'Direct involvement of N-cadherin-mediated signaling in muscle differentiation', *Mol Biol Cell*, 9: 3119-31.

Goldberg, A. L. 1968. 'Protein synthesis during work-induced growth of skeletal muscle', *J Cell Biol*, 36: 653-8.

Goldberg, Martin W., Irm Huttenlauch, Christopher J. Hutchison, and Reimer Stick. 2008. 'Filaments made from A- and B-type lamins differ in structure and organization', *Journal of Cell Science*, 121: 215-25.

Goodman, C. A., J. M. Dietz, B. L. Jacobs, R. M. McNally, J. S. You, and T. A. Hornberger. 2015. 'Yes-Associated Protein is up-regulated by mechanical overload and is sufficient to induce skeletal muscle hypertrophy', *FEBS Lett*, 589: 1491-7.

Gottardi, C. J., and B. M. Gumbiner. 2001. 'Adhesion signaling: how beta-catenin interacts with its partners', *Curr Biol*, 11: R792-4.

Grannas, K., L. Arngarden, P. Lonn, M. Mazurkiewicz, A. Blokzijl, A. Zieba, and O. Soderberg. 2015. 'Crosstalk between Hippo and TGFbeta: Subcellular Localization of YAP/TAZ/Smad Complexes', *J Mol Biol*, 427: 3407-15.

Griffin, C. A., L. H. Apponi, K. K. Long, and G. K. Pavlath. 2010a. 'Chemokine expression and control of muscle cell migration during myogenesis', *J Cell Sci*, 123: 3052-60.

Griffin, Christine A., Luciano H. Apponi, Kimberly K. Long, and Grace K. Pavlath. 2010b. 'Chemokine expression and control of muscle cell migration during myogenesis', *Journal of Cell Science*, 123: 3052-60.

Griffin, Christine A., Kimberly A. Kafadar, and Grace K. Pavlath. 2009. 'MOR23 Promotes Muscle Regeneration and Regulates Cell Adhesion and Migration', *Developmental Cell*, 17: 649-61.

Guilak, Farshid. 1995. 'Compression-induced changes in the shape and volume of the chondrocyte nucleus', *Journal of Biomechanics*, 28: 1529-41.

Guilluy, C., L. D. Osborne, L. Van Landeghem, L. Sharek, R. Superfine, R. Garcia-Mata, and K. Burridge. 2014. 'Isolated nuclei adapt to force and reveal a mechanotransduction pathway in the nucleus', *Nat Cell Biol*, 16: 376-81.

Guilluy, Christophe, and Keith Burridge. 2015. 'Nuclear mechanotransduction: Forcing the nucleus to respond', *Nucleus*, 6: 19-22.

Gumbiner, B. M., and N. G. Kim. 2014. 'The Hippo-YAP signaling pathway and contact inhibition of growth', *J Cell Sci*, 127: 709-17.

Gupta, Pallavi, Zofia T. Bilinska, Nicolas Sylvius, Emilie Boudreau, John P. Veinot, Sarah Labib, Pierrette M. Bolongo, Akil Hamza, Tracy Jackson, Rafal Ploski, Michal Walski, Jacek Grzybowski, Ewa Walczak, Grzegorz Religa, Anna Fidzianska, and Frédérique Tesson. 2010. 'Genetic and ultrastructural studies in dilated cardiomyopathy patients: a large deletion in the lamin A/C gene is associated with cardiomyocyte nuclear envelope disruption', *Basic Research in Cardiology*, 105: 365-77.

Hakeda-Suzuki, Satoko, Julian Ng, Julia Tzu, Georg Dietzl, Yan Sun, Matthew Harms, Tim Nardine, Liqun Luo, and Barry J. Dickson. 2002. 'Rac function and regulation during Drosophila development', *Nature*, 416: 438-42.

Halbleib, J. M., and W. J. Nelson. 2006. 'Cadherins in development: cell adhesion, sorting, and tissue morphogenesis', *Genes Dev*, 20: 3199-214.

Halder, G., S. Dupont, and S. Piccolo. 2012. 'Transduction of mechanical and cytoskeletal cues by YAP and TAZ', *Nat Rev Mol Cell Biol*, 13: 591-600.

Hale, C. M., A. L. Shrestha, S. B. Khatau, P. J. Stewart-Hutchinson, L. Hernandez, C. L. Stewart, D. Hodzic, and D. Wirtz. 2008. 'Dysfunctional connections between the nucleus and the actin and microtubule networks in laminopathic models', *Biophys J*, 95: 5462-75.

Hanif, Mubashir, Ylva Rosengardten, Hanna Sagelius, Björn Rozell, and Maria Eriksson. 2009. 'Differential Expression of A-Type and B-Type Lamins during Hair Cycling', *PLoS One*, 4: e4114.

Haque, Farhana, Daniela Mazzeo, Jennifer T. Patel, Dawn T. Smallwood, Juliet A. Ellis, Catherine M. Shanahan, and Sue Shackleton. 2010. 'Mammalian SUN Protein Interaction Networks at the Inner Nuclear Membrane and Their Role in Laminopathy Disease Processes', *Journal of Biological Chemistry*, 285: 3487-98.

Haskins, Jonathan W., Don X. Nguyen, and David F. Stern. 2014. 'Neuregulin 1-activated ERBB4 interacts with YAP to induce Hippo pathway targetgenes and promote cell migration', *Science signaling*, 7: ra116-ra16.

Hawke, Thomas J., and Daniel J. Garry. 2001. 'Myogenic satellite cells: physiology to molecular biology', *Journal of Applied Physiology*, 91: 534-51.

Hidalgo-Carcedo, C., S. Hooper, S. I. Chaudhry, P. Williamson, K. Harrington, B. Leitinger, and E. Sahai. 2011. 'Collective cell migration requires suppression of actomyosin at cell-cell contacts mediated by DDR1 and the cell polarity regulators Par3 and Par6', *Nat Cell Biol*, 13: 49-58.

Hiepen, Christian, Andreas Benn, Agnieszka Denkis, Ilya Lukonin, Christoph Weise, Jan H. Boergermann, and Petra Knaus. 2014. 'BMP2-induced chemotaxis requires PI3K p55γ/p110α-dependent phosphatidylinositol (3,4,5)-triphosphate production and LL5β recruitment at the cytocortex', *BMC Biology*, 12: 43.

Hirashima, T., Y. Hosokawa, T. Iino, and M. Nagayama. 2013. 'On fundamental cellular processes for emergence of collective epithelial movement', *Biol Open*, 2: 660-6.

Ho, C. Y., D. E. Jaalouk, M. K. Vartiainen, and J. Lammerding. 2013. 'Lamin A/C and emerin regulate MKL1-SRF activity by modulating actin dynamics', *Nature*, 497: 507-11.

Hoffman, Eric P., Robert H. Brown, and Louis M. Kunkel. 1987. 'Dystrophin: The protein product of the duchenne muscular dystrophy locus', *Cell*, 51: 919-28.

Hollnagel, A., C. Grund, W. W. Franke, and H. H. Arnold. 2002. 'The Cell Adhesion Molecule M-Cadherin Is Not Essential for Muscle Development and Regeneration', *Molecular and Cellular Biology*, 22: 4760-70.

Hopwood, N. D., A. Pluck, and J. B. Gurdon. 1989. 'MyoD expression in the forming somites is an early response to mesoderm induction in Xenopus embryos', *EMBO J*, 8: 3409-17.

Horsley, Valerie, Katie M. Jansen, Stephen T. Mills, and Grace K. Pavlath. 2003. 'IL-4 Acts as a Myoblast Recruitment Factor during Mammalian Muscle Growth', *Cell*, 113: 483-94.

Hotulainen, Pirta, and Pekka Lappalainen. 2006. 'Stress fibers are generated by two distinct actin assembly mechanisms in motile cells', *The Journal of Cell Biology*, 173: 383-94.

Hulmi, J. J., B. M. Oliveira, M. Silvennoinen, W. M. Hoogaars, H. Ma, P. Pierre, A. Pasternack, H. Kainulainen, and O. Ritvos. 2013. 'Muscle protein synthesis, mTORC1/MAPK/Hippo signaling, and capillary density are altered by blocking of myostatin and activins', *Am J Physiol Endocrinol Metab*, 304: E41-50.

Hutcheson, David A., Jia Zhao, Allyson Merrell, Malay Haldar, and Gabrielle Kardon. 2009. 'Embryonic and fetal limb myogenic cells are derived from developmentally distinct progenitors and have different requirements for β-catenin', *Genes & Development*, 23: 997-1013.

Irintchev, A., M. Zeschnigk, A. Starzinski-Powitz, and A. Wernig. 1994. 'Expression pattern of M-cadherin in normal, denervated, and regenerating mouse muscles', *Dev Dyn*, 199: 326-37.

Ishibashi, J., R. L. Perry, A. Asakura, and M. A. Rudnicki. 2005. 'MyoD induces myogenic differentiation through cooperation of its NH2- and COOH-terminal regions', *J Cell Biol*, 171: 471-82.

Ishido, Minenori, and Norikatsu Kasuga. 2011. 'In situ real-time imaging of the satellite cells in rat intact and injured soleus muscles using quantum dots', *Histochemistry and Cell Biology*, 135: 21-26.

Jansen, Katie M., and Grace K. Pavlath. 2006. 'Mannose receptor regulates myoblast motility and muscle growth', *The Journal of Cell Biology*, 174: 403-13.

Jennings, Charles G. B. 1992. 'Expression of the myogenic gene MRF4 during Xenopus development', *Developmental Biology*, 150: 121-32.

Jevtić, Predrag, and Daniel L Levy. 2014. 'Nuclear Size Scaling during Xenopus Early Development Contributes to Midblastula Transition Timing', *Current Biology*, 25: 45-52.

Jiang, Q., D. Liu, Y. Gong, Y. Wang, S. Sun, Y. Gui, and H. Song. 2009. 'yap is required for the development of brain, eyes, and neural crest in zebrafish', *Biochem Biophys Res Commun*, 384: 114-9.

Judson, R. N., S. R. Gray, C. Walker, A. M. Carroll, C. Itzstein, A. Lionikas, P. S. Zammit, C. De Bari, and H. Wackerhage. 2013. 'Constitutive expression of Yes-associated protein (Yap) in adult skeletal muscle fibres induces muscle atrophy and myopathy', *PLoS One*, 8: e59622.

Judson, R. N., A. M. Tremblay, P. Knopp, R. B. White, R. Urcia, C. De Bari, P. S. Zammit, F. D. Camargo, and H. Wackerhage. 2012. 'The Hippo pathway member Yap plays a key role in influencing fate decisions in muscle satellite cells', *J Cell Sci*, 125: 6009-19.

Kang, Jong-Sun, Min-Jeong Yi, Wei Zhang, Jessica L. Feinleib, Francesca Cole, and Robert S. Krauss. 2004. 'Netrins and neogenin promote myotube formation', *The Journal of Cell Biology*, 167: 493-504.

Katagiri T, Yamaguchi A, Komaki M, Abe E, Takahashi N, Ikeda T, Rosen V, Wozney JM, Fujisawa-Sehara A, Suda T. 1994. 'Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage'. J CellBiol. 127(6 Pt 1):1755-66

Kaufmann, U., J. Kirsch, A. Irintchev, A. Wernig, and A. Starzinski-Powitz. 1999. 'The M-cadherin catenin complex interacts with microtubules in skeletal muscle cells: implications for the fusion of myoblasts', Journal of Cell Science, 112: 55-68.

Kaushik, G., and A. J. Engler. 2014. 'From stem cells to cardiomyocytes: the role of forces in cardiac maturation, aging, and disease', Prog Mol Biol Transl Sci, 126: 219-42.

Khatau, Shyam B., Christopher M. Hale, P. J. Stewart-Hutchinson, Meet S. Patel, Colin L. Stewart, Peter C. Searson, Didier Hodzic, and Denis Wirtz. 2009. 'A perinuclear actin cap regulates nuclear shape', *Proceedings of the National Academy of Sciences*, 106: 19017-22.

Kim, Jin-Hong, Keiichiro Kushiro, Nicholas A. Graham, and Anand R. Asthagiri. 2009. 'Tunable interplay between epidermal growth factor and cell–cell contact governs the spatial dynamics of epithelial growth', *Proceedings of the National Academy of Sciences*, 106: 11149-53.

Kim, N. G., E. Koh, X. Chen, and B. M. Gumbiner. 2011. 'E-cadherin mediates contact inhibition of proliferation through Hippo signaling-pathway components', *Proc Natl Acad Sci U S A*, 108: 11930-5.

Kitiyakara, Amara, and D. Murray Angevine. 1963. 'A study of the pattern of postembryonic growth of M. gracilis in mice', *Developmental Biology*, 8: 322-40.

Knudsen, Karen A., Linda Myers, and Sheila A. McElwee. 1990. 'A role for the Ca2+-dependent adhesion molecule, N-cadherin, in myoblast interaction during myogenesis', *Experimental Cell Research*, 188: 175-84.

Kochin, Vitaly, Takeshi Shimi, Elin Torvaldson, Stephen A. Adam, Anne Goldman, Chan-Gi Pack, Johanna Melo-Cardenas, Susumu Y. Imanishi, Robert D. Goldman, and John E. Eriksson. 2014. 'Interphase phosphorylation of lamin A', *Journal of Cell Science*, 127: 2683-96.

Kolb, Thorsten, Kendra Maaß, Michaela Hergt, Ueli Aebi, and Harald Herrmann. 2011. 'Lamin A and lamin C form homodimers and coexist in higher complex forms both in the nucleoplasmic fraction and in the lamina of cultured human cells', *Nucleus*, 2: 425-33.

Komuro, A., M. Nagai, N. E. Navin, and M. Sudol. 2003. 'WW domain-containing protein YAP associates with ErbB-4 and acts as a co-transcriptional activator for the carboxyl-terminal fragment of ErbB-4 that translocates to the nucleus', *J Biol Chem*, 278: 33334-41.

Konsavage, W. M., Jr., S. L. Kyler, S. A. Rennoll, G. Jin, and G. S. Yochum. 2012. 'Wnt/beta-catenin signaling regulates Yes-associated protein (YAP) gene expression in colorectal carcinoma cells', *J Biol Chem*, 287: 11730-9.

Kopf, J., P. Paarmann, C. Hiepen, D. Horbelt, and P. Knaus. 2014. 'BMP growth factor signaling in a biomechanical context', *Biofactors*, 40: 171-87.

Kopf, J., A. Petersen, G. N. Duda, and P. Knaus. 2012. 'BMP2 and mechanical loading cooperatively regulate immediate early signalling events in the BMP pathway', *BMC Biol*, 10: 37.

Kostetskii, I., R. Moore, R. Kemler, and G. L. Radice. 2001. 'Differential adhesion leads to segregation and exclusion of N-cadherin-deficient cells in chimeric embryos', *Dev Biol*, 234: 72-9.

Krauss, Robert S., Francesca Cole, Ursula Gaio, Giichi Takaesu, Wei Zhang, and Jong-Sun Kang. 2005. 'Close encounters: regulation of vertebrate skeletal myogenesis by cell-cell contact', *Journal of Cell Science*, 118: 2355-62.

Kuang, Shihuan, Sophie B. Chargé, Patrick Seale, Michael Huh, and Michael A. Rudnicki. 2006. 'Distinct roles for Pax7 and Pax3 in adult regenerative myogenesis', *The Journal of Cell Biology*, 172: 103-13.

Kuang, Shihuan, Kazuki Kuroda, Fabien Le Grand, and Michael A. Rudnicki. 2007. 'Asymmetric Self-Renewal and Commitment of Satellite Stem Cells in Muscle', *Cell*, 129: 999-1010.

Kuch, C., D. Winnekendonk, S. Butz, U. Unvericht, R. Kemler, and A. Starzinski-Powitz. 1997. 'M-cadherin-mediated cell adhesion and complex formation with the catenins in myogenic mouse cells', *Exp Cell Res*, 232: 331-8.

Kumar, A., N. Khandelwal, R. Malya, M. B. Reid, and A. M. Boriek. 2004. 'Loss of dystrophin causes aberrant mechanotransduction in skeletal muscle fibers', *FASEB J*, 18: 102-13.

Kuphal, F., and J. Behrens. 2006. 'E-cadherin modulates Wnt-dependent transcription in colorectal cancer cells but does not alter Wnt-independent gene expression in fibroblasts', *Exp Cell Res*, 312: 457-67.

Lafreniere, J. F., P. Mills, M. Bouchentouf, and J. P. Tremblay. 2006. 'Interleukin-4 improves the migration of human myogenic precursor cells in vitro and in vivo', *Experimental Cell Research*, 312: 1127-41.

Lammerding, Jan, Loren G. Fong, Julie Y. Ji, Karen Reue, Colin L. Stewart, Stephen G. Young, and Richard T. Lee. 2006. 'Lamins A and C but Not Lamin B1 Regulate Nuclear Mechanics', *Journal of Biological Chemistry*, 281: 25768-80.

Lammerding, Jan, P. Christian Schulze, Tomosaburo Takahashi, Serguei Kozlov, Teresa Sullivan, Roger D. Kamm, Colin L. Stewart, and Richard T. Lee. 2004. 'Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction', *The Journal of Clinical Investigation*, 113: 370-78.

Lammerding J, Hsiao J, Schulze PC, Kozlov S, Stewart CL, Lee RT. 2005. 'Abnormal nuclear shape and impaired mechanotransduction in emerin-deficient cells', J Cell Biol, 170(5):781-91.

Lampugnani, M. G., F. Orsenigo, M. C. Gagliani, C. Tacchetti, and E. Dejana. 2006. 'Vascular endothelial cadherin controls VEGFR-2 internalization and signaling from intracellular compartments', *J Cell Biol*, 174: 593-604.

Leckband, D., and A. Prakasam. 2006. 'Mechanism and dynamics of cadherin adhesion', *Annu Rev Biomed Eng*, 8: 259-87.

Lee, J. S., C. M. Hale, P. Panorchan, S. B. Khatau, J. P. George, Y. Tseng, C. L. Stewart, D. Hodzic, and D. Wirtz. 2007. 'Nuclear lamin A/C deficiency induces defects in cell mechanics, polarization, and migration', *Biophys J*, 93: 2542-52.

Lee, K. K. H., C. C. Wong, S. E. Webb, M. K. Tang, A. K. C. Leung, P. F. Kwok, D. Q. Cai, and K. M. Chan. 1999. 'Hepatocyte growth factor stimulates chemotactic response in mouse embryonic limb myogenic cells in vitro', *Journal of Experimental Zoology*, 283: 170-80.

Lei, Kai, Xiaochang Zhang, Xu Ding, Xue Guo, Muyun Chen, Binggen Zhu, Tian Xu, Yuan Zhuang, Rener Xu, and Min Han. 2009. 'SUN1 and SUN2 play critical but partially redundant roles in anchoring nuclei in skeletal muscle cells in mice', *Proceedings of the National Academy of Sciences*, 106: 10207-12.

Lepper, Christoph, and Chen-Ming Fan. 2010. 'Inducible lineage tracing of Pax7-descendant cells reveals embryonic origin of adult satellite cells', *genesis*, 48: 424-36.

Liang, Ying, Peng Hang Chiu, Kit Yan Yip, and Siu Yuen Chan. 2011. 'Subcellular Localization of SUN2 Is Regulated by Lamin A and Rab5', *PLoS One*, 6: e20507.

Link, Jana, Daniel Jahn, Johannes Schmitt, Eva Göb, Johannes Baar, Sagrario Ortega, Ricardo Benavente, and Manfred Alsheimer. 2013. 'The Meiotic Nuclear Lamina Regulates Chromosome Dynamics and Promotes Efficient Homologous Recombination in the Mouse', *PLOS Genetics*, 9: e1003261.

Liu, A. M., R. T. Poon, and J. M. Luk. 2010. 'MicroRNA-375 targets Hippo-signaling effector YAP in liver cancer and inhibits tumor properties', *Biochem Biophys Res Commun*, 394: 623-7.

Lombardi, M. L., D. E. Jaalouk, C. M. Shanahan, B. Burke, K. J. Roux, and J. Lammerding. 2011. 'The interaction between nesprins and sun proteins at the nuclear envelope is critical for force transmission between the nucleus and cytoskeleton', *J Biol Chem*, 286: 26743-53.

Lombardi, M. L., and J. Lammerding. 2011. 'Keeping the LINC: the importance of nucleocytoskeletal coupling in intracellular force transmission and cellular function', *Biochem Soc Trans*, 39: 1729-34.

Low, B. C., C. Q. Pan, G. V. Shivashankar, A. Bershadsky, M. Sudol, and M. Sheetz. 2014. 'YAP/TAZ as mechanosensors and mechanotransducers in regulating organ size and tumor growth', *FEBS Lett*, 588: 2663-70.

Lund, Eivind, and Philippe Collas. 2013. 'Nuclear lamins', *Nucleus*, 4: 424-30.

Luo, L, Y J Liao, L Y Jan, and Y N Jan. 1994. 'Distinct morphogenetic functions of similar small GTPases: Drosophila Drac1 is involved in axonal outgrowth and myoblast fusion', *Genes & Development*, 8: 1787-802.

Luxton, G. W. Gant, Edgar R. Gomes, Eric S. Folker, Erin Vintinner, and Gregg G. Gundersen. 2010. 'Linear Arrays of Nuclear Envelope Proteins Harness Retrograde Actin Flow for Nuclear Movement', *Science*, 329: 956-59.

Maeda, T., T. Sakabe, A. Sunaga, K. Sakai, A. L. Rivera, D. R. Keene, T. Sasaki, E. Stavnezer, J. Iannotti, R. Schweitzer, D. Ilic, H. Baskaran, and T. Sakai. 2011. 'Conversion of mechanical force into TGF-beta-mediated biochemical signals', *Curr Biol*, 21: 933-41.

Maggi, Lorenzo, Nicola Carboni, and Pia Bernasconi. 2016. 'Skeletal Muscle Laminopathies: A Review of Clinical and Molecular Features', *Cells*, 5.

Mamchaoui, K., C. Trollet, A. Bigot, E. Negroni, S. Chaouch, A. Wolff, P. K. Kandalla, S. Marie, J. Di Santo, J. L. St Guily, F. Muntoni, J. Kim, S. Philippi, S. Spuler, N. Levy, S. C. Blumen, T. Voit, W. E. Wright, A. Aamiri, G. Butler-Browne, and V. Mouly. 2011. 'Immortalized pathological human myoblasts: towards a universal tool for the study of neuromuscular disorders', *Skelet Muscle*, 1: 34.

Maniotis, Andrew J., Christopher S. Chen, and Donald E. Ingber. 1997. 'Demonstration of mechanical connections between integrins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure', *Proceedings of the National Academy of Sciences*, 94: 849-54.

Mar, J. H., and C. P. Ordahl. 1988. 'A conserved CATTCCT motif is required for skeletal muscle-specific activity of the cardiac troponin T gene promoter', *Proc Natl Acad Sci U S A*, 85: 6404-8.

Marjoram, R. J., E. C. Lessey, and K. Burridge. 2014. 'Regulation of RhoA activity by adhesion molecules and mechanotransduction', *Curr Mol Med*, 14: 199-208.

Massague, J., J. Seoane, and D. Wotton. 2005. 'Smad transcription factors', *Genes Dev*, 19: 2783-810.

Mateus, R., R. Lourenco, Y. Fang, G. Brito, A. Farinho, F. Valerio, and A. Jacinto. 2015. 'Control of tissue growth by Yap relies on cell density and F-actin in zebrafish fin regeneration', *Development*, 142: 2752-63.

Mauro, A. 1961. 'Satellite cell of skeletal muscle fibers', *J Biophys Biochem Cytol*, 9: 493-5.

McKinnell, Iain W., Jeff Ishibashi, Fabien Le Grand, Vincent G. J. Punch, Gregory C. Addicks, Jack F. Greenblatt, F. Jeffrey Dilworth, and Michael A. Rudnicki. 2008. 'Pax7 activates myogenic genes by recruitment of a histone methyltransferase complex', *Nat Cell Biol*, 10: 77-84.

Mege, R.M., D. Goudou, C. Diaz, M. Nicolet, L. Garcia, G. Geraud, and F. Rieger. 1992. 'N-cadherin and N-CAM in myoblast fusion: compared localisation and effect of blockade by peptides and antibodies', *Journal of Cell Science*, 103: 897-906.

Meier, J., K.H. Campbell, C.C. Ford, R. Stick, and C.J. Hutchison. 1991. 'The role of lamin LIII in nuclear assembly and DNA replication, in cell-free extracts of Xenopus eggs', *Journal of Cell Science*, 98: 271-79.

Meinke, Peter, Thuy Duong Nguyen, and Manfred S. Wehnert. 2011. 'The LINC complex and human disease', *Biochemical Society Transactions*, 39: 1693-97.

Mertz, A. F., S. Banerjee, Y. Che, G. K. German, Y. Xu, C. Hyland, M. C. Marchetti, V. Horsley, and E. R. Dufresne. 2012. 'Scaling of traction forces with the size of cohesive cell colonies', *Phys Rev Lett*, 108: 198101.

Moir, Robert D., Miri Yoon, Satya Khuon, and Robert D. Goldman. 2000. 'Nuclear Lamins a and B1', *Different Pathways of Assembly during Nuclear Envelope Formation in Living Cells*, 151: 1155-68.
Morin-Kensicki, E. M., B. N. Boone, M. Howell, J. R. Stonebraker, J. Teed, J. G. Alb, T. R. Magnuson, W. O'Neal, and S. L. Milgram. 2006. 'Defects in yolk sac vasculogenesis, chorioallantoic fusion, and embryonic axis elongation in mice with targeted disruption of Yap65', *Mol Cell Biol*, 26: 77-87.
Moroishi, Toshiro, Hyun Woo Park, Baodong Qin, Qian Chen, Zhipeng Meng, Steven W. Plouffe, Koji Taniguchi, Fa-Xing Yu, Michael Karin, Duojia Pan, and Kun-Liang Guan. 2015. 'A YAP/TAZ-induced feedback mechanism regulates Hippo pathway homeostasis', *Genes & Development*, 29: 1271-84.
Moss, F. P., and C. P. Leblond. 1971. 'Satellite cells as the source of nuclei in muscles of growing rats', *The Anatomical Record*, 170: 421-35.
Murphy, Danielle A., and Sara A. Courtneidge. 2011. 'The 'ins' and 'outs' of podosomes and invadopodia: characteristics, formation and function', *Nat Rev Mol Cell Biol*, 12: 413-26.
Murrell M, Kamm R, Matsudaira P. 2010. 'Tension, free space, and cell damage in a microfluidic wound healing assay', PLoS One, 6(9): e24283
Mylona, Eleni, Kristen A. Jones, Stephen T. Mills, and Grace K. Pavlath. 2006. 'CD44 regulates myoblast migration and differentiation', *Journal of Cellular Physiology*, 209: 314-21.
Naetar, Nana, and Roland Foisner. 2009. 'Lamin complexes in the nuclear interior control progenitor cell proliferation and tissue homeostasis', *Cell Cycle*, 8: 1488-93.
Naetar, Nana, Barbara Korbei, Serguei Kozlov, Marc A. Kerenyi, Daniela Dorner, Rosana Kral, Ivana Gotic, Peter Fuchs, Tatiana V. Cohen, Reginald Bittner, Colin L. Stewart, and Roland Foisner. 2008. 'Loss of nucleoplasmic LAP2[alpha]-lamin A complexes causes erythroid and epidermal progenitor hyperproliferation', *Nat Cell Biol*, 10: 1341-48.
Nagata, Y., T. A. Partridge, R. Matsuda, and P. S. Zammit. 2006. 'Entry of muscle satellite cells into the cell cycle requires sphingolipid signaling', *J Cell Biol*, 174: 245-53.
Narimatsu, M., P. Samavarchi-Tehrani, X. Varelas, and J. L. Wrana. 2015. 'Distinct polarity cues direct Taz/Yap and TGFbeta receptor localization to differentially control TGFbeta-induced Smad signaling', *Dev Cell*, 32: 652-6.
Nedachi, Taku, Hiroyasu Hatakeyama, Tatsuyoshi Kono, Masaaki Sato, and Makoto Kanzaki. 2009. 'Characterization of contraction-inducible CXC chemokines and their roles in C₂C₁₂ myocytes', *American Journal of Physiology - Endocrinology And Metabolism*, 297: E866-E78.
Nelson, W. J., and R. Nusse. 2004. 'Convergence of Wnt, beta-catenin, and cadherin pathways', *Science*, 303: 1483-7.
Ng, Mei Rosa, Achim Besser, Gaudenz Danuser, and Joan S. Brugge. 2012. 'Substrate stiffness regulates cadherin-dependent collective migration through myosin-II contractility', *The Journal of Cell Biology*, 199: 545.
Nicolas, Nathalie, Claude-Louis Gallien, and Christophe Chanoine. 1998. 'Expression of myogenic regulatory factors during muscle development of Xenopus: Myogenin mRNA accumulation is limited strictly to secondary myogenesis', *Developmental Dynamics*, 213: 309-21.
Nikolova, Vesna, Christiana Leimena, Aisling C. McMahon, Ju Chiat Tan, Suchitra Chandar, Dilesh Jogia, Scott H. Kesteven, Jan Michalicek, Robyn Otway, Fons Verheyen, Stephen Rainer, Colin L. Stewart, David Martin, Michael P. Feneley, and Diane Fatkin. 2004. 'Defects in nuclear structure and function promote dilated cardiomyopathy in lamin A/C–deficient mice', *The Journal of Clinical Investigation*, 113: 357-69.
Nishimura, T., and M. Takeichi. 2009. 'Remodeling of the adherens junctions during morphogenesis', *Curr Top Dev Biol*, 89: 33-54.

Nowak, Scott J., Patrick C. Nahirney, Anna-Katerina Hadjantonakis, and Mary K. Baylies. 2009. 'Nap1-mediated actin remodeling is essential for mammalian myoblast fusion', *Journal of Cell Science*, 122: 3282-93.

O'Connor, Roddy S., Stephen T. Mills, Kristen A. Jones, Steffan N. Ho, and Grace K. Pavlath. 2007. 'A combinatorial role for NFAT5 in both myoblast migration and differentiation during skeletal muscle myogenesis', *Journal of Cell Science*, 120: 149-59.

Ödemis, Veysel, Karina Boosmann, Maja Theresa Dieterlen, and Jürgen Engele. 2007. 'The chemokine SDF1 controls multiple steps of myogenesis through atypical PKCζ', *Journal of Cell Science*, 120: 4050-59.

Omelchenko, Tatiana, and Alan Hall. 2012. 'Myosin-IXA Regulates Collective Epithelial Cell Migration by Targeting RhoGAP Activity to Cell-Cell Junctions', *Current Biology*, 22: 278-88.

Onishi, M., Y. Fujita, H. Yoshikawa, and T. Yamashita. 2013. 'Inhibition of Rac1 promotes BMP-2-induced osteoblastic differentiation', *Cell Death Dis*, 4: e698.

Östlund, Cecilia, Eric S. Folker, Jason C. Choi, Edgar R. Gomes, Gregg G. Gundersen, and Howard J. Worman. 2009. 'Dynamics and molecular interactions of linker of nucleoskeleton and cytoskeleton (LINC) complex proteins', *Journal of Cell Science*, 122: 4099-108.

Otto, Anthony, Corina Schmidt, Graham Luke, Steve Allen, Petr Valasek, Francesco Muntoni, Diana Lawrence-Watt, and Ketan Patel. 2008. 'Canonical Wnt signalling induces satellite-cell proliferation during adult skeletal muscle regeneration', *Journal of Cell Science*, 121: 2939-50.

Pan, D., L. D. Estevez-Salmeron, S. L. Stroschein, X. Zhu, J. He, S. Zhou, and K. Luo. 2005. 'The integral inner nuclear membrane protein MAN1 physically interacts with the R-Smad proteins to repress signaling by the transforming growth factor-{beta} superfamily of cytokines', *J Biol Chem*, 280: 15992-6001.

Papadimitriou, J. M., T. A. Robertson, C. A. Mitchell, and M. D. Grounds. 1990. 'The process of new plasmalemma formation in focally injured skeletal muscle fibers', *J Struct Biol*, 103: 124-34.

Perez-Moreno, M., and E. Fuchs. 2006. 'Catenins: keeping cells from getting their signals crossed', *Dev Cell*, 11: 601-12.

Piccolo, S., S. Dupont, and M. Cordenonsi. 2014. 'The biology of YAP/TAZ: hippo signaling and beyond', *Physiol Rev*, 94: 1287-312.

Plutoni, C., E. Bazellieres, M. Le Borgne-Rochet, F. Comunale, A. Brugues, M. Seveno, D. Planchon, S. Thuault, N. Morin, S. Bodin, X. Trepat, and C. Gauthier-Rouviere. 2016. 'P-cadherin promotes collective cell migration via a Cdc42-mediated increase in mechanical forces', *J Cell Biol*, 212: 199-217.

Pokutta, S., and W. I. Weis. 2007. 'Structure and mechanism of cadherins and catenins in cell-cell contacts', *Annu Rev Cell Dev Biol*, 23: 237-61.

Pouliot, Y., M. Gravel, and P. C. Holland. 1994. 'Developmental regulation of M-cadherin in the terminal differentiation of skeletal myoblasts', *Dev Dyn*, 200: 305-12.

Powell, Jeanne A. 1973. 'Development of normal and genetically dystrophic mouse muscle in tissue culture', *Experimental Cell Research*, 80: 251-64.

Quijano-Roy, S., B. Mbieleu, C. G. Bonnemann, P. Y. Jeannet, J. Colomer, N. F. Clarke, J. M. Cuisset, H. Roper, L. De Meirleir, A. D'Amico, R. Ben Yaou, A. Nascimento, A. Barois, L. Demay, E. Bertini, A. Ferreiro, C. A. Sewry, N. B. Romero, M. Ryan, F. Muntoni, P. Guicheney, P. Richard, G. Bonne, and B. Estournet. 2008. 'De novo LMNA mutations cause a new form of congenital muscular dystrophy', *Ann Neurol*, 64: 177-86.

Radice, Glenn L., Helen Rayburn, Hiroaki Matsunami, Karen A. Knudsen, Masatoshi Takeichi, and Richard O. Hynes. 1997. 'Developmental Defects in Mouse Embryos Lacking N-Cadherin', *Developmental Biology*, 181: 64-78.

Ramaekers, F. C., and F. T. Bosman. 2004. 'The cytoskeleton and disease', *J Pathol*, 204: 351-4.

Ratheesh, Aparna, and Alpha S. Yap. 2012. 'A bigger picture: classical cadherins and the dynamic actin cytoskeleton', *Nat Rev Mol Cell Biol*, 13: 673-79.
Redfield, A., M. T. Nieman, and K. A. Knudsen. 1997. 'Cadherins promote skeletal muscle differentiation in three-dimensional cultures', *J Cell Biol*, 138: 1323-31.
Reffay, M., M. C. Parrini, O. Cochet-Escartin, B. Ladoux, A. Buguin, S. Coscoy, F. Amblard, J. Camonis, and P. Silberzan. 2014. 'Interplay of RhoA and mechanical forces in collective cell migration driven by leader cells', *Nat Cell Biol*, 16: 217-23.
Rhodes, S J, and S F Konieczny. 1989. 'Identification of MRF4: a new member of the muscle regulatory factor gene family', *Genes & Development*, 3: 2050-61.
Ribas, R., N. Moncaut, C. Siligan, K. Taylor, J. W. Cross, P. W. Rigby, and J. J. Carvajal. 2011. 'Members of the TEAD family of transcription factors regulate the expression of Myf5 in ventral somitic compartments', *Dev Biol*, 355: 372-80.
Robertson, T. A., M. D. Grounds, C. A. Mitchell, and J. M. Papadimitriou. 1990. 'Fusion between myogenic cells in vivo: an ultrastructural study in regenerating murine skeletal muscle', *J Struct Biol*, 105: 170-82.
Robertson, T. A., J. M. Papadimitriou, and M. D. Grounds. 1993. 'Fusion of myogenic cells to the newly sealed region of damaged myofibres in skeletal muscle regeneration', *Neuropathol Appl Neurobiol*, 19: 350-8.
Roux, Kyle J., Melissa L. Crisp, Qian Liu, Daein Kim, Serguei Kozlov, Colin L. Stewart, and Brian Burke. 2009. 'Nesprin 4 is an outer nuclear membrane protein that can induce kinesin-mediated cell polarization', *Proceedings of the National Academy of Sciences*, 106: 2194-99.
Ruzinova, M. B., and R. Benezra. 2003. 'Id proteins in development, cell cycle and cancer', *Trends Cell Biol*, 13: 410-8.
Sadot, E., I. Simcha, M. Shtutman, A. Ben-Ze'ev, and B. Geiger. 1998. 'Inhibition of beta-catenin-mediated transactivation by cadherin derivatives', *Proc Natl Acad Sci U S A*, 95: 15339-44.
Salpingidou, Georgia, Andrei Smertenko, Irena Hausmanowa-Petrucewicz, Patrick J. Hussey, and Chris J. Hutchison. 2007. 'A novel role for the nuclear membrane protein emerin in association of the centrosome to the outer nuclear membrane', *The Journal of Cell Biology*, 178: 897-904.
Sambasivan, Ramkumar, Roseline Yao, Adrien Kissenpfennig, Laetitia Van Wittenberghe, Andràs Paldi, Barbara Gayraud-Morel, Hind Guenou, Bernard Malissen, Shahragim Tajbakhsh, and Anne Galy. 2011. 'Pax7-expressing satellite cells are indispensable for adult skeletal muscle regeneration', *Development*, 138: 3647-56.
Sansores-Garcia, L., W. Bossuyt, K. Wada, S. Yonemura, C. Tao, H. Sasaki, and G. Halder. 2011. 'Modulating F-actin organization induces organ growth by affecting the Hippo pathway', *EMBO J*, 30: 2325-35.
Sassoon, David A. 1993. 'Myogenic Regulatory Factors: Dissecting Their Role and Regulation during Vertebrate Embryogenesis', *Developmental Biology*, 156: 11-23.
Schermelleh, Lothar, Peter M. Carlton, Sebastian Haase, Lin Shao, Lukman Winoto, Peter Kner, Brian Burke, M. Cristina Cardoso, David A. Agard, Mats G. L. Gustafsson, Heinrich Leonhardt, and John W. Sedat. 2008. 'Subdiffraction Multicolor Imaging of the Nuclear Periphery with 3D Structured Illumination Microscopy', *Science*, 320: 1332-36.
Schlegelmilch, K., M. Mohseni, O. Kirak, J. Pruszak, J. R. Rodriguez, D. Zhou, B. T. Kreger, V. Vasioukhin, J. Avruch, T. R. Brummelkamp, and F. D. Camargo. 2011. 'Yap1 acts downstream of alpha-catenin to control epidermal proliferation', *Cell*, 144: 782-95.
Schmalbruch, H. 1976. 'The morphology of regeneration of skeletal muscles in the rat', *Tissue and Cell*, 8: 673-92.
Schultz, Edward, and Debra L. Jaryszak. 1985. 'Effects of skeletal muscle regeneration on the proliferation potential of satellite cells', *Mechanisms of Ageing and Development*, 30: 63-72.

Schutte, U., S. Bisht, L. C. Heukamp, M. Kebschull, A. Florin, J. Haarmann, P. Hoffmann, G. Bendas, R. Buettner, P. Brossart, and G. Feldmann. 2014. 'Hippo signaling mediates proliferation, invasiveness, and metastatic potential of clear cell renal cell carcinoma', *Transl Oncol*, 7: 309-21.

Schwartz, C., M. Fischer, K. Mamchaoui, A. Bigot, T. Lok, C. Verdier, A. Duperray,.R. Michel, I. Holt, T. Voit, S. Quijano-Roy, G. Bonne, C. Coirault. 'Nuclear-cytoskeletal linkages mediate inside-out mechanical coupling in muscle', Sci Rep, in revison

Seale, Patrick, Luc A. Sabourin, Adele Girgis-Gabardo, Ahmed Mansouri, Peter Gruss, and Michael A. Rudnicki. 2000. 'Pax7 Is Required for the Specification of Myogenic Satellite Cells', Cell, 102: 777-86.

Shewan, A. M., M. Maddugoda, A. Kraemer, S. J. Stehbens, S. Verma, E. M. Kovacs, and A. S. Yap. 2005. 'Myosin 2 is a key Rho kinase target necessary for the local concentration of E-cadherin at cell-cell contacts', *Mol Biol Cell*, 16: 4531-42.

Shimi, Takeshi, Katrin Pfleghaar, Shin-ichiro Kojima, Chan-Gi Pack, Irina Solovei, Anne E. Goldman, Stephen A. Adam, Dale K. Shumaker, Masataka Kinjo, Thomas Cremer, and Robert D. Goldman. 2008. 'The A- and B-type nuclear lamin networks: microdomains involved in chromatin organization and transcription', *Genes & Development*, 22: 3409-21.

Sieber, C., J. Kopf, C. Hiepen, and P. Knaus. 2009. 'Recent advances in BMP receptor signaling', *Cytokine Growth Factor Rev*, 20: 343-55.

Silvis, M. R., B. T. Kreger, W. H. Lien, O. Klezovitch, G. M. Rudakova, F. D. Camargo, D. M. Lantz, J. T. Seykora, and V. Vasioukhin. 2011. 'alpha-catenin is a tumor suppressor that controls cell accumulation by regulating the localization and activity of the transcriptional coactivator Yap1', *Sci Signal*, 4: ra33.

Simcha, Inbal, Catherine Kirkpatrick, Einat Sadot, Michael Shtutman, Gordon Polevoy, Benjamin Geiger, Mark Peifer, and Avri Ben-Ze'ev. 2001. 'Cadherin Sequences That Inhibit β-Catenin Signaling: A Study in Yeast and Mammalian Cells', *Molecular Biology of the Cell*, 12: 1177-88.

Snow, Mikel H. 1977. 'Myogenic cell formation in regenerating rat skeletal muscle injured by mincing I. A fine structural study', *The Anatomical Record*, 188: 181-99.

Sorrentino, G., N. Ruggeri, V. Specchia, M. Cordenonsi, M. Mano, S. Dupont, A. Manfrin, E. Ingallina, R. Sommaggio, S. Piazza, A. Rosato, S. Piccolo, and G. Del Sal. 2014. 'Metabolic control of YAP and TAZ by the mevalonate pathway', *Nat Cell Biol*, 16: 357-66.

St Croix, B., C. Sheehan, J. W. Rak, V. A. Florenes, J. M. Slingerland, and R. S. Kerbel. 1998. 'E-Cadherin-dependent growth suppression is mediated by the cyclin-dependent kinase inhibitor p27(KIP1)', *J Cell Biol*, 142: 557-71.

Stockinger, Andreas, Andreas Eger, Julia Wolf, Hartmut Beug, and Roland Foisner. 2001. 'E-cadherin regulates cell growth by modulating proliferation-dependent β-catenin transcriptional activity', *The Journal of Cell Biology*, 154: 1185-96.

Sullivan, Teresa, Diana Escalante-Alcalde, Harshida Bhatt, Miriam Anver, Narayan Bhat, Kunio Nagashima, Colin L. Stewart, and Brian Burke. 1999. 'Loss of a-Type Lamin Expression Compromises Nuclear Envelope Integrity Leading to Muscular Dystrophy', *The Journal of Cell Biology*, 147: 913-20.

Suzuki, Akiko, Richard C. Pelikan, and Junichi Iwata. 2015. 'WNT/β-Catenin Signaling Regulates Multiple Steps of Myogenesis by Regulating Step-Specific Targets', *Molecular and Cellular Biology*, 35: 1763-76.

Swift, J., I. L. Ivanovska, A. Buxboim, T. Harada, P. C. Dingal, J. Pinter, J. D. Pajerowski, K. R. Spinler, J. W. Shin, M. Tewari, F. Rehfeldt, D. W. Speicher, and D. E. Discher. 2013. 'Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation', *Science*, 341: 1240104.

Tambe, D. T., C. C. Hardin, T. E. Angelini, K. Rajendran, C. Y. Park, X. Serra-Picamal, E. H. Zhou, M. H. Zaman, J. P. Butler, D. A. Weitz, J. J. Fredberg, and X. Trepat. 2011. 'Collective cell guidance by cooperative intercellular forces', *Nat Mater*, 10: 469-75.

Theveneau, E., and R. Mayor. 2013. 'Collective cell migration of epithelial and mesenchymal cells', *Cell Mol Life Sci*, 70: 3481-92.
Tokura, Yuka, Yuki Nakayama, So-ichiro Fukada, Noriko Nara, Hiroshi Yamamoto, Ryoichi Matsuda, and Takahiko Hara. 2011. 'Muscle injury-induced thymosin β4 acts as a chemoattractant for myoblasts', *Journal of Biochemistry*, 149: 43-48.
Tremblay, A. M., E. Missiaglia, G. G. Galli, S. Hettmer, R. Urcia, M. Carrara, R. N. Judson, K. Thway, G. Nadal, J. L. Selfe, G. Murray, R. A. Calogero, C. De Bari, P. S. Zammit, M. Delorenzi, A. J. Wagers, J. Shipley, H. Wackerhage, and F. D. Camargo. 2014. 'The Hippo transducer YAP1 transforms activated satellite cells and is a potent effector of embryonal rhabdomyosarcoma formation', *Cancer Cell*, 26: 273-87.
Trepat, Xavier, and Jeffrey J. Fredberg. 2011. 'Plithotaxis and emergent dynamics in collective cellular migration', *Trends in Cell Biology*, 21: 638-46.
Truong Quang, B. A., M. Mani, O. Markova, T. Lecuit, and P. F. Lenne. 2013. 'Principles of E-cadherin supramolecular organization in vivo', *Curr Biol*, 23: 2197-207.
Tseng, A.-S., and M. Levin. 2008. 'Tail Regeneration in Xenopus laevis as a Model for Understanding Tissue Repair', *Journal of Dental Research*, 87: 806-16.
Vandenburgh, H., and S. Kaufman. 1979. 'In vitro model for stretch-induced hypertrophy of skeletal muscle', *Science*, 203: 265-8.
Varelas, X., P. Samavarchi-Tehrani, M. Narimatsu, A. Weiss, K. Cockburn, B. G. Larsen, J. Rossant, and J. L. Wrana. 2010. 'The Crumbs complex couples cell density sensing to Hippo-dependent control of the TGF-beta-SMAD pathway', *Dev Cell*, 19: 831-44.
Vasyutina, Elena, Benedetta Martarelli, Cord Brakebusch, Hagen Wende, and Carmen Birchmeier. 2009. 'The small G-proteins Rac1 and Cdc42 are essential for myoblast fusion in the mouse', *Proceedings of the National Academy of Sciences*, 106: 8935-40.
Vaz, R., G. G. Martins, S. Thorsteinsdottir, and G. Rodrigues. 2012. 'Fibronectin promotes migration, alignment and fusion in an in vitro myoblast cell model', *Cell Tissue Res*, 348: 569-78.
Verstraeten, V. L. R. M., J. L. V. Broers, F. C. S. Ramaekers, and M. A. M. van Steensel. 2007. 'The Nuclear Envelope, a Key Structure in Cellular Integrity and Gene Expression', *Current Medicinal Chemistry*, 14: 1231-48.
Villena, Joan, and Enrique Brandan. 2004. 'Dermatan sulfate exerts an enhanced growth factor response on skeletal muscle satellite cell proliferation and migration', *Journal of Cellular Physiology*, 198: 169-78.
Visser, S., and X. Yang. 2010. 'LATS tumor suppressor: a new governor of cellular homeostasis', *Cell Cycle*, 9: 3892-903.
Vytopil, Michal, Enzo Ricci, Antonio Dello Russo, Frank Hanisch, Stephan Neudecker, Stephan Zierz, Roberta Ricotti, Laurence Demay, Pascale Richard, Manfred Wehnert, Gisèle Bonne, Luciano Merlini, and Daniela Toniolo. 2002. 'Frequent low penetrance mutations in the Lamin A/C gene, causing Emery Dreifuss muscular dystrophy', *Neuromuscular Disorders*, 12: 958-63.
Wada, K., K. Itoga, T. Okano, S. Yonemura, and H. Sasaki. 2011. 'Hippo pathway regulation by cell morphology and stress fibers', *Development*, 138: 3907-14.
Wang, X., L. He, Y. I. Wu, K. M. Hahn, and D. J. Montell. 2010. 'Light-mediated activation reveals a key role for Rac in collective guidance of cell movement in vivo', *Nat Cell Biol*, 12: 591-7.
Wang, Yan, Junaith S. Mohamed, and Stephen E. Alway. 2013. 'M-cadherin-inhibited phosphorylation of β-catenin augments differentiation of mouse myoblasts', *Cell and Tissue Research*, 351: 183-200.
Watt, K. I., R. Judson, P. Medlow, K. Reid, T. B. Kurth, J. G. Burniston, A. Ratkevicius, C. De Bari, and H. Wackerhage. 2010. 'Yap is a novel regulator of C2C12 myogenesis', *Biochem Biophys Res Commun*, 393: 619-24.

Watt, K. I., B. J. Turner, A. Hagg, X. Zhang, J. R. Davey, H. Qian, C. Beyer, C. E. Winbanks, K. F. Harvey, and P. Gregorevic. 2015. 'The Hippo pathway effector YAP is a critical regulator of skeletal muscle fibre size', *Nat Commun*, 6: 6048.

Weber, Gregory F, Maureen A Bjerke, and Douglas W DeSimone. 2012. 'A Mechanoresponsive Cadherin-Keratin Complex Directs Polarized Protrusive Behavior and Collective Cell Migration', *Developmental Cell*, 22: 104-15.

Wei, B., W. Dui, D. Liu, Y. Xing, Z. Yuan, and G. Ji. 2013. 'MST1, a key player, in enhancing fast skeletal muscle atrophy', *BMC Biol*, 11: 12.

Wernig, Anton, Michael Bone, Andrey Irintchev, R Schafer, and Michael J Cullen. 2004. 'M-cadherin is a reliable marker of quiescent satellite cells in mouse skeletal muscle', *Basic Appl Myol*, 14: 161-68.

Wheelock, M. J., and K. R. Johnson. 2003. 'Cadherin-mediated cellular signaling', *Curr Opin Cell Biol*, 15: 509-14.

Williams, Pamela E., and G. Goldspink. 1971. 'Longitudinal Growth of Striated Muscle Fibres', *Journal of Cell Science*, 9: 751-67.

Worman HJ, Bonne G. 2007. ' "Laminopathies": a wide spectrum of human diseases'. Exp Cell Res. 313(10):2121-33.

Wright, Woodring E., David A. Sassoon, and Victor K. Lin. 1989. 'Myogenin, a factor regulating myogenesis, has a domain homologous to MyoD', *Cell*, 56: 607-17.

Wróbel, Edyta, Edyta Brzóska, and Jerzy Moraczewski. 2007. 'M-cadherin and β-catenin participate in differentiation of rat satellite cells', *European Journal of Cell Biology*, 86: 99-109.

Wu, H., Y. Xiao, S. Zhang, S. Ji, L. Wei, F. Fan, J. Geng, J. Tian, X. Sun, F. Qin, C. Jin, J. Lin, Z. Y. Yin, T. Zhang, L. Luo, Y. Li, S. Song, S. C. Lin, X. Deng, F. Camargo, J. Avruch, L. Chen, and D. Zhou. 2013. 'The Ets transcription factor GABP is a component of the hippo pathway essential for growth and antioxidant defense', *Cell Rep*, 3: 1663-77.

Xin, M., Y. Kim, L. B. Sutherland, M. Murakami, X. Qi, J. McAnally, E. R. Porrello, A. I. Mahmoud, W. Tan, J. M. Shelton, J. A. Richardson, H. A. Sadek, R. Bassel-Duby, and E. N. Olson. 2013. 'Hippo pathway effector Yap promotes cardiac regeneration', *Proc Natl Acad Sci U S A*, 110: 13839-44.

Yaffe, D., and O. Saxel. 1977. 'Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle', *Nature*, 270: 725-7.

Yang, Shao H., Hea-Jin Jung, Catherine Coffinier, Loren G. Fong, and Stephen G. Young. 2011. 'Are B-type lamins essential in all mammalian cells?', *Nucleus*, 2: 562-69.

Yoshida, T. 2008. 'MCAT elements and the TEF-1 family of transcription factors in muscle development and disease', *Arterioscler Thromb Vasc Biol*, 28: 8-17.

Yu, F. X., B. Zhao, N. Panupinthu, J. L. Jewell, I. Lian, L. H. Wang, J. Zhao, H. Yuan, K. Tumaneng, H. Li, X. D. Fu, G. B. Mills, and K. L. Guan. 2012. 'Regulation of the Hippo-YAP pathway by G-protein-coupled receptor signaling', *Cell*, 150: 780-91.

Yu, Juehua, Kai Lei, Min Zhou, Cheryl M. Craft, Gezhi Xu, Tian Xu, Yuan Zhuang, Rener Xu, and Min Han. 2011. 'KASH protein Syne-2/Nesprin-2 and SUN proteins SUN1/2 mediate nuclear migration during mammalian retinal development', *Human Molecular Genetics*, 20: 1061-73.

Zammit, P. S., T. A. Partridge, and Z. Yablonka-Reuveni. 2006. 'The skeletal muscle satellite cell: the stem cell that came in from the cold', *J Histochem Cytochem*, 54: 1177-91.

Zammit, Peter S. 2008. 'All muscle satellite cells are equal, but are some more equal than others?', *Journal of Cell Science*, 121: 2975-82.

Zammit, Peter S., Frederic Relaix, Yosuke Nagata, Ana Pérez Ruiz, Charlotte A. Collins, Terence A. Partridge, and Jonathan R. Beauchamp. 2006. 'Pax7 and myogenic progression in skeletal muscle satellite cells', *Journal of Cell Science*, 119: 1824-32.

Zegers, M. M., and P. Friedl. 2014. 'Rho GTPases in collective cell migration', *Small GTPases*, 5: e28997.

Zeschnigk, M., D. Kozian, C. Kuch, M. Schmoll, and A. Starzinski-Powitz. 1995. 'Involvement of M-cadherin in terminal differentiation of skeletal muscle cells', *Journal of Cell Science*, 108: 2973-81.

Zhang, L. L., J. J. Liu, F. Liu, W. H. Liu, Y. S. Wang, B. Zhu, and B. Yu. 2012. 'MiR-499 induces cardiac differentiation of rat mesenchymal stem cells through wnt/beta-catenin signaling pathway', *Biochem Biophys Res Commun*, 420: 875-81.

Zhang, M., and I. S. McLennan. 1994. 'Use of antibodies to identify satellite cells with a light microscope', *Muscle Nerve*, 17: 987-94.

Zhang, Qiuping, Cornelia Bethmann, Nathalie F. Worth, John D. Davies, Christina Wasner, Anja Feuer, Cassandra D. Ragnauth, Qijian Yi, Jason A. Mellad, Derek T. Warren, Matthew A. Wheeler, Juliet A. Ellis, Jeremy N. Skepper, Matthias Vorgerd, Beate Schlotter-Weigel, Peter L. Weissberg, Roland G. Roberts, Manfred Wehnert, and Catherine M. Shanahan. 2007. 'Nesprin-1 and -2 are involved in the pathogenesis of Emery–Dreifuss muscular dystrophy and are critical for nuclear envelope integrity', *Human Molecular Genetics*, 16: 2816-33.

Zhao, B., L. Li, Q. Lu, L. H. Wang, C. Y. Liu, Q. Lei, and K. L. Guan. 2011. 'Angiomotin is a novel Hippo pathway component that inhibits YAP oncoprotein', *Genes Dev*, 25: 51-63.

Zhao, B., L. Li, K. Tumaneng, C. Y. Wang, and K. L. Guan. 2010. 'A coordinated phosphorylation by Lats and CK1 regulates YAP stability through SCF(beta-TRCP)', *Genes Dev*, 24: 72-85.

Zhao, B., L. Li, L. Wang, C. Y. Wang, J. Yu, and K. L. Guan. 2012. 'Cell detachment activates the Hippo pathway via cytoskeleton reorganization to induce anoikis', *Genes Dev*, 26: 54-68.

Zhao, B., X. Wei, W. Li, R. S. Udan, Q. Yang, J. Kim, J. Xie, T. Ikenoue, J. Yu, L. Li, P. Zheng, K. Ye, A. Chinnaiyan, G. Halder, Z. C. Lai, and K. L. Guan. 2007. 'Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control', *Genes Dev*, 21: 2747-61.

Zhao, B., X. Ye, J. Yu, L. Li, W. Li, S. Li, J. Yu, J. D. Lin, C. Y. Wang, A. M. Chinnaiyan, Z. C. Lai, and K. L. Guan. 2008. 'TEAD mediates YAP-dependent gene induction and growth control', *Genes Dev*, 22: 1962-71.

Zhou, L., and C. O. Hanemann. 2012. 'Merlin, a multi-suppressor from cell membrane to the nucleus', *FEBS Lett*, 586: 1403-8.

Zwerger, M., D. E. Jaalouk, M. L. Lombardi, P. Isermann, M. Mauermann, G. Dialynas, H. Herrmann, L. L. Wallrath, and J. Lammerding. 2013. 'Myopathic lamin mutations impair nuclear stability in cells and tissue and disrupt nucleo-cytoskeletal coupling', *Hum Mol Genet*, 22: 2335-49.

8. PUBLICATIONS

Technau C and Fischer M, Mamchaoui K, Bigot A, Lok T, Verdier C, Duperray A, Michel R, Holt I, Voit T, Quijano-Roy S, Bonne G, Coirault C (in press). Nuclear-cytoskeletal linkages mediate inside-out mechanical coupling in muscle cell precursors through FHOD1. Scientific Reports.

Contribution: I contributed to the acquisition and analysis of the data on the role of FHOD1. Furthermore and critically revised the manuscript.

Fischer M, Rikeit P, Knaus P, Coirault C. 2016. 'YAP-Mediated Mechanotransduction in Skeletal Muscle', Front Physiol, 7:41. Review

Contribution: I conceptualized the article, selected the literature and wrote manuscript.

Fischer M, Osterland M, Christian Hiepen, Daniel Owens, Arakelian T, Knaus P and Coirault C. Mechanosensing defects and YAP signaling in LMNA-related congenital muscular dystrophy. Manuscript in preparation.

Contribution: I designed and performed the great majority of this work, analyzed and interpreted the data and drafted the manuscript.