

7 Anhang

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7.3 Verzeichnis der Abkürzungen

BAC	Bacterial artificial chromosome
BDM	2,4-Butanedione monoxime
bp	Basenpaare
BSA	Bovine Serum Albumin
cDNA	Komplementäre DNA, aus RNA durch reverse Transkription gewonnen
dH ₂ O	Destilliertes Wasser
ES-Zellen	Embryonale Stammzellen
FKS	Fetales Kälber Serum
HBSS	Henks ausgeglichene Salzlösung

HCl	Salz der Chlorwassersäure
IF	Immunfluoreszenz
IH	Immunhistochemie
ISO	Isoproterenol hydrochlorid
IVC	Individually Ventilated Cages / Einzeln belüftete Käfighaltung
kb	Kilobasen
KO	Knockout
LiCl	Lithiumchlorid
MCS	Multiple cloning site
min	Minute
ms	Millisekunde
NaCl	Natriumchlorid
ORF	Offener Leseramen (Open reading frame)
PBS	Phosphat gepufferte Salzlösung
Pen/strep	Penicillin/ Steptavidin
PFA	Paraformaldahyd
RT	Raumtemperatur
SDS	Natrium Dodecyl Sulfat
sec	Sekunde
TBS	Tris gepufferte Salzlösung
TE	Tris-EDTA
U	Einheit (Unit)
upm	Umdrehungen pro Minute
Vol	Volumen
WB	Westernblot
WT	Wildtyp
X-Gal	5-Brom-4-Chlor-3-Indolyl-b-D-Galaktosid

8 Verzeichnis der erfolgten Publikationen

Radke M., Peng J., Wu Y., McNabb M., Nelson L., Granzier H., Gotthardt M. Targeted deletion of Titin's N2B-Region leads to diastolic dysfunction and cardiac atrophy. PNAS submitted 28.09.2006

9 Literaturverzeichnis:

Aihara,Y., Kurabayashi,M., Saito,Y., Ohyama,Y., Tanaka,T., Takeda,S., Tomaru,K., Sekiguchi,K., Arai,M., Nakamura,T., and Nagai,R. (2000). Cardiac ankyrin repeat protein is a novel marker of cardiac hypertrophy: role of M-CAT element within the promoter. Hypertension 36, 48-53.

Arber,S., Hunter,J.J., Ross,J.J., Hongo,M., Sansig,G., Borg,J., Perriard,J.C., Chien,K.R., and Caroni,P. (1997). MLP-deficient mice exhibit a disruption of cardiac cytoarchitectural organization, dilated cardiomyopathy, and heart failure. Cell 88, 393-403.

Austin,C.P., Battey,J.F., Bradley,A., Bucan,M., Capecchi,M., Collins,F.S., Dove,W.F., Duyk,G., Dymecki,S., Eppig,J.T., Grieder,F.B., Heintz,N., Hicks,G., Insel,T.R., Joyner,A., Koller,B.H., Lloyd,K.C., Magnuson,T., Moore,M.W., Nagy,A., Pollock,J.D., Roses,A.D., Sands,A.T., Seed,B., Skarnes,W.C., Snoddy,J., Soriano,P., Stewart,D.J., Stewart,F., Stillman,B., Varmus,H., Varticovski,L., Verma,I.M., Vogt,T.F., von,M.H., Witkowski,J., Woychik,R.P., Wurst,W., Yancopoulos,G.D., Young,S.G., and Zambrowicz,B. (2004). The knockout mouse project. Nat. Genet. 36, 921-924.

Ayoob,J.C., Turnacioglu,K.K., Mittal,B., Sanger,J.M., and Sanger,J.W. (2000). Targeting of cardiac muscle titin fragments to the Z-bands and dense bodies of living muscle and non-muscle cells. Cell Motil Cytoskeleton 45, 67-82.

Bang,M.L., Centner,T., Fornoff,F., Geach,A.J., Gotthardt,M., McNabb,M., Witt,C.C., Labeit,D., Gregorio,C.C., Granzier,H., and Labeit,S. (2001a). The complete gene sequence of titin, expression of an unusual approximately 700-kDa titin isoform, and its interaction with obscurin identify a novel Z-line to I-band linking system. Circ Res 89, 1065-1072.

Bang,M.L., Mudry,R.E., McElhinny,A.S., Trombitas,K., Geach,A.J., Yamasaki,R., Sorimachi,H., Granzier,H.L., Gregorio,C.C., and Labeit,S. (2001b). Myopalladin, a novel 145-kilodalton sarcomeric protein with multiple roles in z-disc and i-band protein assemblies. J Cell Biol 153, 413-428.

Barbee,R.W., Perry,B.D., Re,R.N., Murgo,J.P., and Field,L.J. (1994). Hemodynamics in transgenic mice with overexpression of atrial natriuretic factor. Circ. Res 74, 747-751.

Bradley,A., Evans,M., Kaufman,M.H., and Robertson,E. (1984). Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines. Nature 309, 255-256.

- Braz,J.C., Bueno,O.F., De Windt,L.J., and Molkentin,J.D. (2002). PKC alpha regulates the hypertrophic growth of cardiomyocytes through extracellular signal-regulated kinase1/2 (ERK1/2). *J Cell Biol.* 156, 905-919.
- Bullard,B., Ferguson,C., Minajeva,A., Leake,M.C., Gautel,M., Labeit,D., Ding,L., Labeit,S., Horwitz,J., Leonard,K.R., and Linke,W.A. (2004). Association of the chaperone alphaB-crystallin with titin in heart muscle. *J Biol. Chem.* 279, 7917-7924.
- Cazorla,O., Freiburg,A., Helmes,M., Centner,T., McNabb,M., Wu,Y., Trombitas,K., Labeit,S., and Granzier,H.L. (2000). Differential expression of cardiac titin isoforms and modulation of cellular stiffness. *Circ Res* 86, 59-67.
- Clark,K.A., McElhinny,A.S., Beckerle,M.C., and Gregorio,C.C. (2002). Striated Muscle Cytoarchitecture: An Intricate Web of Form and Function. *Annu Rev Cell Dev Biol.*
- Deng,C. and Capecchi,M.R. (1992). Reexamination of gene targeting frequency as a function of the extent of homology between the targeting vector and the target locus. *Mol. Cell Biol.* 12, 3365-3371.
- Doetschman,T., Gregg,R.G., Maeda,N., Hooper,M.L., Melton,D.W., Thompson,S., and Smithies,O. (1987). Targetted correction of a mutant HPRT gene in mouse embryonic stem cells. *Nature* 330, 576-578.
- Du,X., Hublitz,P., Gunther,T., Wilhelm,D., Englert,C., and Schule,R. (2002a). The LIM-only coactivator FHL2 modulates WT1 transcriptional activity during gonadal differentiation. *Biochim. Biophys. Acta* 1577, 93-101.
- Du,X.J., Cole,T.J., Tenis,N., Gao,X.M., Kontgen,F., Kemp,B.E., and Heierhorst,J. (2002b). Impaired cardiac contractility response to hemodynamic stress in S100A1-deficient mice. *Mol Cell Biol* 22, 2821-2829.
- Evans,M.J. and Kaufman,M.H. (1981). Establishment in culture of pluripotential cells from mouse embryos. *Nature* 292, 154-156.
- Freiburg,A., Trombitas,K., Hell,W., Cazorla,O., Fougerousse,F., Centner,T., Kolmerer,B., Witt,C., Beckmann,J.S., Gregorio,C.C., Granzier,H., and Labeit,S. (2000). Series of exon-skipping events in the elastic spring region of titin as the structural basis for myofibrillar elastic diversity. *Circ Res* 86, 1114-1121.
- Fujita,H., Labeit,D., Gerull,B., Labeit,S., and Granzier,H.L. (2004). Titin isoform-dependent effect of calcium on passive myocardial tension. *Am. J Physiol Heart Circ Physiol* 287, H2528-H2534.
- Fukuda,N., Sasaki,D., Ishiwata,S., and Kurihara,S. (2001). Length dependence of tension generation in rat skinned cardiac muscle: role of titin in the Frank-Starling mechanism of the heart. *Circulation* 104, 1639-1645.
- Furst,D.O., Osborn,M., Nave,R., and Weber,K. (1988). The organization of titin filaments in the half-sarcomere revealed by monoclonal antibodies in immunoelectron microscopy: a map of ten nonrepetitive epitopes starting at the Z line extends close to the M line. *J Cell Biol* 106, 1563-1572.

- Furst,D.O., Vinkemeier,U., and Weber,K. (1992). Mammalian skeletal muscle C-protein: purification from bovine muscle, binding to titin and the characterization of a full-length human cDNA. *J Cell Sci 102 (Pt 4)*, 769-778.
- Gautel,M., Leonard,K., and Labeit,S. (1993). Phosphorylation of KSP motifs in the C-terminal region of titin in differentiating myoblasts. *EMBO J 12*, 3827-3834.
- Gerull,B., Gramlich,M., Atherton,J., McNabb,M., Trombitas,K., Sasse-Klaassen,S., Seidman,J.G., Seidman,C., Granzier,H., Labeit,S., Frenneaux,M., and Thierfelder,L. (2002). Mutations of TTN, encoding the giant muscle filament titin, cause familial dilated cardiomyopathy. *Nat Genet 30*, 201-204.
- Golenhofen,N., Arbeiter,A., Koob,R., and Drenckhahn,D. (2002). Ischemia-induced association of the stress protein alpha B-crystallin with I-band portion of cardiac titin. *J Mol Cell Cardiol. 34*, 309-319.
- Golenhofen,N., Ness,W., Koob,R., Htun,P., Schaper,W., and Drenckhahn,D. (1998). Ischemia-induced phosphorylation and translocation of stress protein alpha B-crystallin to Z lines of myocardium. *Am. J Physiol 274*, H1457-H1464.
- Granzier,H.L., Helmes,M., and Trombitas,K. (1996). Nonuniform elasticity of titin in cardiac myocytes: a study using immunoelectron microscopy and cellular mechanics. *Biophys J 70*, 430-442.
- Granzier,H.L. and Irving,T.C. (1995). Passive tension in cardiac muscle: contribution of collagen, titin, microtubules, and intermediate filaments. *Biophys J 68*, 1027-1044.
- Granzier,H.L., Kellermayer,M., Helmes,M., and Trombitas,K. (1997). Titin elasticity and mechanism of passive force development in rat cardiac myocytes probed by thin-filament extraction. *Biophys J 73*, 2043-2053.
- Granzier,H.L. and Labeit,S. (2004). The giant protein titin: a major player in myocardial mechanics, signaling, and disease. *Circ Res 94*, 284-295.
- Grater,F., Shen,J., Jiang,H., Gautel,M., and Grubmuller,H. (2005). Mechanically induced titin kinase activation studied by force-probe molecular dynamics simulations. *Biophys. J 88*, 790-804.
- Gregorio,C.C., Granzier,H., Sorimachi,H., and Labeit,S. (1999). Muscle assembly: a titanic achievement? *Curr Opin Cell Biol 11*, 18-25.
- Gregorio,C.C., Trombitas,K., Centner,T., Kolmerer,B., Stier,G., Kunke,K., Suzuki,K., Obermayr,F., Herrmann,B., Granzier,H., Sorimachi,H., and Labeit,S. (1998). The NH₂ terminus of titin spans the Z-disc: its interaction with a novel 19-kD ligand (T-cap) is required for sarcomeric integrity. *J Cell Biol 143*, 1013-1027.
- Gutierrez-Cruz,G., Van Heerden,A.H., and Wang,K. (2001). Modular motif, structural folds and affinity profiles of the PEVK segment of human fetal skeletal muscle titin. *J Biol Chem 276*, 7442-7449.
- Hayashi,D., Kudoh,S., Shiojima,I., Zou,Y., Harada,K., Shimoyama,M., Imai,Y., Monzen,K., Yamazaki,T., Yazaki,Y., Nagai,R., and Komuro,I. (2004). Atrial natriuretic

peptide inhibits cardiomyocyte hypertrophy through mitogen-activated protein kinase phosphatase-1. *Biochem. Biophys. Res Commun.* 322, 310-319.

Hein,S. and Schaper,J. (2002). Weakness of a giant: mutations of the sarcomeric protein titin. *Trends Mol Med.* 8, 311-313.

Helmes,M., Trombitas,K., Centner,T., Kellermayer,M., Labeit,S., Linke,W.A., and Granzier,H. (1999). Mechanically driven contour-length adjustment in rat cardiac titin's unique N2B sequence: titin is an adjustable spring. *Circ Res* 84, 1339-1352.

Helmes,M., Trombitas,K., and Granzier,H. (1996). Titin develops restoring force in rat cardiac myocytes. *Circ Res* 79, 619-626.

Houmeida,A., Holt,J., Tskhovrebova,L., and Trinick,J. (1995). Studies of the interaction between titin and myosin. *J Cell Biol* 131, 1471-1481.

Isaacs,W.B., Kim,I.S., Struve,A., and Fulton,A.B. (1989). Biosynthesis of titin in cultured skeletal muscle cells. *J Cell Biol* 109, 2189-2195.

Itoh-Satoh,M., Hayashi,T., Nishi,H., Koga,Y., Arimura,T., Koyanagi,T., Takahashi,M., Hohda,S., Ueda,K., Nouchi,T., Hiroe,M., Marumo,F., Imaizumi,T., Yasunami,M., and Kimura,A. (2002). Titin Mutations as the Molecular Basis for Dilated Cardiomyopathy. *Biochem Biophys Res Commun* 291, 385-393.

Johannessen,M., Moller,S., Hansen,T., Moens,U., and Ghelue,M.V. (2006). The multifunctional roles of the four-and-a-half-LIM only protein FHL2. *Cell Mol. Life Sci.* 63, 268-284.

Johannessen,M., Olsen,P.A., Johansen,B., Seternes,O.M., and Moens,U. (2003). Activation of the coactivator four-and-a-half-LIM-only protein FHL2 and the c-fos promoter through inhibition of protein phosphatase 2A. *Biochem. Pharmacol.* 65, 1317-1328.

Kaul,A., Koster,M., Neuhaus,H., and Braun,T. (2000). Myf-5 revisited: loss of early myotome formation does not lead to a rib phenotype in homozygous Myf-5 mutant mice. *Cell* 102, 17-19.

Knoll,R., Hoshijima,M., Hoffman,H.M., Person,V., Lorenzen-Schmidt,I., Bang,M.L., Hayashi,T., Shiga,N., Yasukawa,H., Schaper,W., McKenna,W., Yokoyama,M., Schork,N.J., Omens,J.H., McCulloch,A.D., Kimura,A., Gregorio,C.C., Poller,W., Schaper,J., Schultheiss,H.P., and Chien,K.R. (2002). The Cardiac Mechanical Stretch Sensor Machinery Involves a Z Disc Complex that Is Defective in a Subset of Human Dilated Cardiomyopathy. *Cell* 111, 943-955.

Kong,Y., Shelton,J.M., Rothermel,B., Li,X., Richardson,J.A., Bassel-Duby,R., and Williams,R.S. (2001). Cardiac-specific LIM protein FHL2 modifies the hypertrophic response to beta-adrenergic stimulation. *Circulation* 103, 2731-2738.

Koretz,J.F., Irving,T.C., and Wang,K. (1993). Filamentous aggregates of native titin and binding of C-protein and AMP-deaminase. *Arch Biochem Biophys* 304, 305-309.

Kramerova,I., Kudryashova,E., Tidball,J.G., and Spencer,M.J. (2004). Null mutation of calpain 3 (p94) in mice causes abnormal sarcomere formation in vivo and in vitro. *Hum. Mol Genet* 13, 1373-1388.

Kulke,M., Fujita-Becker,S., Rostkova,E., Neagoe,C., Labeit,D., Manstein,D.J., Gautel,M., and Linke,W.A. (2001). Interaction between PEVK-titin and actin filaments: origin of a viscous force component in cardiac myofibrils. *Circ. Res.* 89, 874-881.

Kurzban,G.P. and Wang,K. (1988). Giant polypeptides of skeletal muscle titin: sedimentation equilibrium in guanidine hydrochloride. *Biochem. Biophys. Res Commun.* 150, 1155-1161.

Labeit,S., Barlow,D.P., Gautel,M., Gibson,T., Holt,J., Hsieh,C.L., Francke,U., Leonard,K., Wardale,J., Whiting,A., and et,a. (1990). A regular pattern of two types of 100-residue motif in the sequence of titin. *Nature* 345, 273-276.

Labeit,S. and Kolmerer,B. (1995). Titins: giant proteins in charge of muscle ultrastructure and elasticity. *Science* 270, 293-296.

Laemmli,U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680-685.

Lange,S., Auerbach,D., McLoughlin,P., Perriard,E., Schafer,B.W., Perriard,J.C., and Ehler,E. (2002). Subcellular targeting of metabolic enzymes to titin in heart muscle may be mediated by DRAL/FHL-2. *J. Cell Sci.* 115, 4925-4936.

Lange,S., Xiang,F., Yakovenko,A., Vihola,A., Hackman,P., Rostkova,E., Kristensen,J., Brandmeier,B., Franzen,G., Hedberg,B., Gunnarsson,L.G., Hughes,S.M., Marchand,S., Sejersen,T., Richard,I., Edstrom,L., Ehler,E., Udd,B., and Gautel,M. (2005). The Kinase Domain of Titin Controls Muscle Gene Expression and Protein Turnover. *Science* 308, 1599-1603.

Leake,M.C., Grutzner,A., Kruger,M., and Linke,W.A. (2006). Mechanical properties of cardiac titin's N2B-region by single-molecule atomic force spectroscopy. *J Struct. Biol.*

Linke,W.A. and Granzier,H. (1998). A spring tale: new facts on titin elasticity. *Biophys J* 75, 2613-2614.

Linke,W.A., Ivemeyer,M., Mundel,P., Stockmeier,M.R., and Kolmerer,B. (1998a). Nature of PEVK-titin elasticity in skeletal muscle. *Proc Natl Acad Sci U S A* 95, 8052-8057.

Linke,W.A., Ivemeyer,M., Olivieri,N., Kolmerer,B., Ruegg,J.C., and Labeit,S. (1996). Towards a molecular understanding of the elasticity of titin. *J Mol Biol* 261, 62-71.

Linke,W.A., Rudy,D.E., Centner,T., Gautel,M., Witt,C., Labeit,S., and Gregorio,C.C. (1999). I-band titin in cardiac muscle is a three-element molecular spring and is critical for maintaining thin filament structure. *J Cell Biol* 146, 631-644.

- Linke,W.A., Stockmeier,M.R., Ivemeyer,M., Hosser,H., and Mundel,P. (1998b). Characterizing titin's I-band Ig domain region as an entropic spring. *J Cell Sci* 111 (Pt 11), 1567-1574.
- Liu,J.P., Schlosser,R., Ma,W.Y., Dong,Z., Feng,H., Liu,L., Huang,X.Q., Liu,Y., and Li,D.W. (2004). Human alphaA- and alphaB-crystallins prevent UVA-induced apoptosis through regulation of PKCalpha, RAF/MEK/ERK and AKT signaling pathways. *Exp. Eye Res* 79, 393-403.
- Ma,K., Kan,L.L., and Wang,K. (2001). Polyproline II Helix Is a Key Structural Motif of the Elastic PEVK Segment of Titin. *Biochemistry* 40, 3427-3438.
- Makarenko,I., Opitz,C.A., Leake,M.C., Neagoe,C., Kulke,M., Gwathmey,J.K., del,M.F., Hajjar,R.J., and Linke,W.A. (2004). Passive stiffness changes caused by upregulation of compliant titin isoforms in human dilated cardiomyopathy hearts. *Circ. Res* 95, 708-716.
- Maruyama,K., Kimura,S., Yoshidomi,H., Sawada,H., and Kikuchi,M. (1984). Molecular size and shape of beta-connectin, an elastic protein of striated muscle. *J Biochem. (Tokyo)* 95, 1423-1433.
- Maruyama,K., Murakami,F., and Ohashi,K. (1977). Connectin, an elastic protein of muscle. *Comparative Biochemistry. J Biochem. (Tokyo)* 82, 339-345.
- Matsumoto,Y., Hayashi,T., Inagaki,N., Takahashi,M., Hiroi,S., Nakamura,T., Arimura,T., Nakamura,K., Ashizawa,N., Yasunami,M., Ohe,T., Yano,K., and Kimura,A. (2006). Functional analysis of titin/connectin N2-B mutations found in cardiomyopathy. *J Muscle Res Cell Motil.* 1-8.
- Mayans,O., van der Ven,P.F., Wilm,M., Mues,A., Young,P., Fnrst,D.O., Wilmanns,M., and Gautel,M. (1998). Structural basis for activation of the titin kinase domain during myofibrillogenesis. *Nature* 395, 863-869.
- Miller,M.K., Bang,M.L., Witt,C.C., Labeit,D., Trombitas,C., Watanabe,K., Granzier,H., McElhinny,A.S., Gregorio,C.C., and Labeit,S. (2003). The Muscle Ankyrin Repeat Proteins: CARP, ankrd2/Arpp and DARP as a Family of Titin Filament-based Stress Response Molecules. *J Mol Biol* 333, 951-964.
- Morlon,A. and Sassone-Corsi,P. (2003). The LIM-only protein FHL2 is a serum-inducible transcriptional coactivator of AP-1. *Proc. Natl. Acad. Sci U. S. A* 100, 3977-3982.
- Morrison,L.E., Hoover,H.E., Thuerauf,D.J., and Glembotski,C.C. (2003). Mimicking phosphorylation of alphaB-crystallin on serine-59 is necessary and sufficient to provide maximal protection of cardiac myocytes from apoptosis. *Circ Res* 92, 203-211.
- Morrison,L.E., Whittaker,R.J., Klepper,R.E., Wawrousek,E.F., and Glembotski,C.C. (2004). Roles for alphaB-crystallin and HSPB2 in protecting the myocardium from ischemia-reperfusion-induced damage in a KO mouse model. *Am. J Physiol Heart Circ. Physiol* 286, H847-H855.

- Moss,R.L. and Fitzsimons,D.P. (2002). Frank-Starling relationship: long on importance, short on mechanism. *Circ. Res.* 90, 11-13.
- Most,P., Pleger,S.T., Volkers,M., Heidt,B., Boerries,M., Weichenhan,D., Loffler,E., Janssen,P.M., Eckhart,A.D., Martini,J., Williams,M.L., Katus,H.A., Remppis,A., and Koch,W.J. (2004). Cardiac adenoviral S100A1 gene delivery rescues failing myocardium. *J Clin. Invest* 114, 1550-1563.
- Muller,J.M., Isele,U., Metzger,E., Rempel,A., Moser,M., Pscherer,A., Breyer,T., Holubarsch,C., Buettner,R., and Schule,R. (2000). FHL2, a novel tissue-specific coactivator of the androgen receptor. *EMBO J* 19, 359-369.
- Muller-Seitz,M., Kaupmann,K., Labeit,S., and Jockusch,H. (1993). Chromosomal localization of the mouse titin gene and its relation to "muscular dystrophy with myositis" and nebulin genes on chromosome 2. *Genomics* 18, 559-561.
- Nagueh,S.F., Shah,G., Wu,Y., Torre-Amione,G., King,N.M., Lahmers,S., Witt,C.C., Becker,K., Labeit,S., and Granzier,H.L. (2004). Altered Titin Expression, Myocardial Stiffness, and Left Ventricular Function in Patients With Dilated Cardiomyopathy. *Circulation*.
- Neagoe,C., Kulke,M., del Monte,F., Gwathmey,J.K., de Tombe,P.P., Hajjar,R.J., and Linke,W.A. (2002). Titin isoform switch in ischemic human heart disease. *Circulation* 106, 1333-1341.
- Neagoe,C., Opitz,C.A., Makarenko,I., and Linke,W.A. (2003). Gigantic variety: expression patterns of titin isoforms in striated muscles and consequences for myofibrillar passive stiffness. *J Muscle Res Cell Motil.* 24, 175-189.
- Obermann,W.M., Gautel,M., Steiner,F., van der Ven,P.F., Weber,K., and Furst,D.O. (1996). The structure of the sarcomeric M band: localization of defined domains of myomesin, M-protein, and the 250-kD carboxy-terminal region of titin by immunoelectron microscopy. *J Cell Biol* 134, 1441-1453.
- Ohtsuka,H., Yajima,H., Maruyama,K., and Kimura,S. (1997a). Binding of the N-terminal 63 kDa portion of connectin/titin to alpha-actinin as revealed by the yeast two-hybrid system. *FEBS Lett* 401, 65-67.
- Ohtsuka,H., Yajima,H., Maruyama,K., and Kimura,S. (1997b). The N-terminal Z repeat 5 of connectin/titin binds to the C-terminal region of alpha-actinin. *Biochem Biophys Res Commun* 235, 1-3.
- Opitz,C.A., Leake,M.C., Makarenko,I., Benes,V., and Linke,W.A. (2004). Developmentally regulated switching of titin size alters myofibrillar stiffness in the perinatal heart. *Circ Res* 94, 967-975.
- Pan,J., Singh,U.S., Takahashi,T., Oka,Y., Palm-Leis,A., Herbelin,B.S., and Baker,K.M. (2005). PKC mediates cyclic stretch-induced cardiac hypertrophy through Rho family GTPases and mitogen-activated protein kinases in cardiomyocytes. *J Cell Physiol* 202, 536-553.

- Piedrahita,J.A., Zhang,S.H., Hagaman,J.R., Oliver,P.M., and Maeda,N. (1992). Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. Proc. Natl. Acad. Sci. U. S. A 89, 4471-4475.
- Purcell,N.H., Darwis,D., Bueno,O.F., Muller,J.M., Schule,R., and Molkentin,J.D. (2004). Extracellular signal-regulated kinase 2 interacts with and is negatively regulated by the LIM-only protein FHL2 in cardiomyocytes. Mol Cell Biol 24, 1081-1095.
- Rodriguez,C.I., Buchholz,F., Galloway,J., Sequerra,R., Kasper,J., Ayala,R., Stewart,A.F., and Dymecki,S.M. (2000). High-efficiency deleter mice show that FLPe is an alternative to Cre-loxP. Nat Genet 25, 139-140.
- Rossi,E., Faiella,A., Zeviani,M., Labeit,S., Floridia,G., Brunelli,S., Cammarata,M., Boncinelli,E., and Zuffardi,O. (1994). Order of six loci at 2q24-q31 and orientation of the HOXD locus. Genomics 24, 34-40.
- Sambrook, J. and Russell, D. W. Molecular Cloning: A Laboratory Manual. Third Edition. 2001. Cold Spring Harbor Laboratory Press.
Ref Type: Serial (Book,Monograph)
- Satoh,M., Takahashi,M., Sakamoto,T., Hiroe,M., Marumo,F., and Kimura,A. (1999). Structural analysis of the titin gene in hypertrophic cardiomyopathy: identification of a novel disease gene. Biochem Biophys Res Commun 262, 411-417.
- Schaffner,W. and Weissmann,C. (1973). A rapid, sensitive, and specific method for the determination of protein in dilute solution. Anal. Biochem. 56, 502-514.
- Sebestyen,M.G., Wolff,J.A., and Greaser,M.L. (1995). Characterization of a 5.4 kb cDNA fragment from the Z-line region of rabbit cardiac titin reveals phosphorylation sites for proline-directed kinases. J Cell Sci 108 (Pt 9), 3029-3037.
- Smith,A.G., Heath,J.K., Donaldson,D.D., Wong,G.G., Moreau,J., Stahl,M., and Rogers,D. (1988). Inhibition of pluripotential embryonic stem cell differentiation by purified polypeptides. Nature 336, 688-690.
- Song,Q., Schmidt,A.G., Hahn,H.S., Carr,A.N., Frank,B., Pater,L., Gerst,M., Young,K., Hoit,B.D., McConnell,B.K., Haghghi,K., Seidman,C.E., Seidman,J.G., Dorn,G.W., and Kranias,E.G. (2003). Rescue of cardiomyocyte dysfunction by phospholamban ablation does not prevent ventricular failure in genetic hypertrophy. J Clin. Invest 111, 859-867.
- Sorimachi,H., Freiburg,A., Kolmerer,B., Ishiura,S., Stier,G., Gregorio,C.C., Labeit,D., Linke,W.A., Suzuki,K., and Labeit,S. (1997). Tissue-specific expression and alpha-actinin binding properties of the Z-disc titin: implications for the nature of vertebrate Z- discs. J Mol Biol 270, 688-695.
- Sorimachi,H., Kinbara,K., Kimura,S., Takahashi,M., Ishiura,S., Sasagawa,N., Sorimachi,N., Shimada,H., Tagawa,K., and Maruyama,K. (1995). Muscle-specific calpain, p94, responsible for limb girdle muscular dystrophy type 2A, associates with connectin through IS2, a p94-specific sequence. J Biol Chem 270, 31158-31162.

Starling,E.H. and Visscher,M.B. (1927). The regulation of the energy output of the heart. *J Physiol (Camb)* 62, 243-261.

Thomas,K.R. and Capecchi,M.R. (1987). Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. *Cell* 51, 503-512.

Towbin,H., Staehelin,T., and Gordon,J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. U. S. A* 76, 4350-4354.

Trombitas,K., Freiburg,A., Centner,T., Labeit,S., and Granzier,H. (1999). Molecular dissection of N2B cardiac titin's extensibility. *Biophys J* 77, 3189-3196.

Trombitas,K., Freiburg,A., Greaser,M., Labeit,S., and Granzier,H. (2000a). From connecting filaments to co-expression of titin isoforms. *Adv Exp Med Biol* 481, 405-418.

Trombitas,K., Greaser,M., French,G., and Granzier,H. (1998a). PEVK extension of human soleus muscle titin revealed by immunolabeling with the anti-titin antibody 9D10. *J Struct Biol* 122, 188-196.

Trombitas,K., Greaser,M., Labeit,S., Jin,J.P., Kellermayer,M., Helmes,M., and Granzier,H. (1998b). Titin extensibility in situ: entropic elasticity of permanently folded and permanently unfolded molecular segments. *J Cell Biol* 140, 853-859.

Trombitas,K., Redkar,A., Centner,T., Wu,Y., Labeit,S., and Granzier,H. (2000b). Extensibility of isoforms of cardiac titin: variation in contour length of molecular subsegments provides a basis for cellular passive stiffness diversity [In Process Citation]. *Biophys J* 79, 3226-3234.

Turnacioglu,K.K., Mittal,B., Dabiri,G.A., Sanger,J.M., and Sanger,J.W. (1997). An N-terminal fragment of titin coupled to green fluorescent protein localizes to the Z-bands in living muscle cells: overexpression leads to myofibril disassembly. *Mol Biol Cell* 8, 705-717.

Udy,G.B., Parkes,B.D., and Wells,D.N. (1997). ES cell cycle rates affect gene targeting frequencies. *Exp. Cell Res* 231, 296-301.

Vitarelli,A. and Gheorghiade,M. (1998). Diastolic heart failure: standard Doppler approach and beyond. *Am. J Cardiol.* 81, 115G-121G.

Wan,S., Yim,A.P., Wong,C.K., Arifi,A.A., Yip,J.H., Ng,C.S., Waye,M.M., and Lam,C.W. (2002). Expression of FHL2 and cytokine messenger RNAs in human myocardium after cardiopulmonary bypass. *Int. J Cardiol.* 86, 265-272.

Wang,K. (1984). Cytoskeletal matrix in striated muscle: the role of titin, nebulin and intermediate filaments. *Adv Exp Med Biol* 170, 285-305.

Wang,K., McClure,J., and Tu,A. (1979). Titin: major myofibrillar components of striated muscle. *Proc. Natl. Acad. Sci. U. S. A* 76, 3698-3702.

- Warren,C.M., Jordan,M.C., Roos,K.P., Krzesinski,P.R., and Greaser,M.L. (2003a). Titin isoform expression in normal and hypertensive myocardium. *Cardiovasc. Res* 59, 86-94.
- Warren,C.M., Krzesinski,P.R., Campbell,K.S., Moss,R.L., and Greaser,M.L. (2004). Titin isoform changes in rat myocardium during development. *Mech. Dev.* 121, 1301-1312.
- Warren,C.M., Krzesinski,P.R., and Greaser,M.L. (2003b). Vertical agarose gel electrophoresis and electroblotting of high-molecular-weight proteins. *Electrophoresis* 24, 1695-1702.
- Watanabe,K., Nair,P., Labeit,D., Kellermayer,M., Greaser,M., Labeit,S., and Granzier,H. (2002). Molecular mechanics of cardiac titin's PEVK and N2B spring elements. *J Biol Chem.*
- Weinert,S., Bergmann,N., Luo,X., Erdmann,B., and Gotthardt,M. (2006). M line-deficient titin causes cardiac lethality through impaired maturation of the sarcomere. *J. Cell Biol.* 173, 559-570.
- Willnow,T.E. and Herz,J. (1994). Homologous recombination for gene replacement in mouse cell lines. *Methods Cell Biol* 43 Pt A, 305-334.
- Wu,Y., Bell,S.P., Trombitas,K., Witt,C.C., Labeit,S., LeWinter,M.M., and Granzier,H. (2002). Changes in titin isoform expression in pacing-induced cardiac failure give rise to increased passive muscle stiffness. *Circulation* 106, 1384-1389.
- Wu,Y., Cazorla,O., Labeit,D., Labeit,S., and Granzier,H. (2000). Changes in Titin and Collagen Underlie Diastolic Stiffness Diversity of Cardiac Muscle. *J Mol Cell Cardiol* 32, 2151-2162.
- Xu,X., Meiler,S.E., Zhong,T.P., Mohideen,M., Crossley,D.A., Burggren,W.W., and Fishman,M.C. (2002). Cardiomyopathy in zebrafish due to mutation in an alternatively spliced exon of titin. *Nat Genet* 30, 205-209.
- Yamasaki,R., Berri,M., Wu,Y., Trombitas,K., McNabb,M., Kellermayer,M.S., Witt,C., Labeit,D., Labeit,S., Greaser,M., and Granzier,H. (2001). Titin-actin interaction in mouse myocardium: passive tension modulation and its regulation by calcium/S100A1. *Biophys. J.* 81, 2297-2313.
- Yamasaki,R., Wu,Y., McNabb,M., Greaser,M., Labeit,S., and Granzier,H. (2002). Protein kinase A phosphorylates titin's cardiac-specific N2B domain and reduces passive tension in rat cardiac myocytes. *Circ Res* 90, 1181-1188.
- Yan,J., Zhu,J., Zhong,H., Lu,Q., Huang,C., and Ye,Q. (2003). BRCA1 interacts with FHL2 and enhances FHL2 transactivation function. *FEBS Lett.* 553, 183-189.
- Yang,Y., Hou,H., Haller,E.M., Nicosia,S.V., and Bai,W. (2005). Suppression of FOXO1 activity by FHL2 through SIRT1-mediated deacetylation. *EMBO J.*
- Yates,L.D. and Greaser,M.L. (1983). Quantitative determination of myosin and actin in rabbit skeletal muscle. *J Mol. Biol.* 168, 123-141.

Young,P., Ferguson,C., Banuelos,S., and Gautel,M. (1998). Molecular structure of the sarcomeric Z-disk: two types of titin interactions lead to an asymmetrical sorting of alpha-actinin. *EMBO J* 17, 1614-1624.

Zou,P., Pinotsis,N., Lange,S., Song,Y.H., Popov,A., Mavridis,I., Mayans,O.M., Gautel,M., and Wilmanns,M. (2006). Palindromic assembly of the giant muscle protein titin in the sarcomeric Z-disk. *Nature* 439, 229-233.

Zou,Y., Evans,S., Chen,J., Kuo,H.C., Harvey,R.P., and Chien,K.R. (1997). CARP, a cardiac ankyrin repeat protein, is downstream in the Nkx2-5 homeobox gene pathway. *Development* 124, 793-804.