# 2 Abstract

Cardiac diseases such as hypertrophic or dilated cardiomyopathy and coronary artery disease are the major cause of death in developed nations. The underlying causes are complex and not understood in detail, but eventually lead to altered contractile or elastic properties of the heart.

The heart consists of cardiomyocytes, which contract as a result of the energy dependent sliding of the actin and myosin filaments in the sarcomere, the smalles functional unit of muscle. The elastic properties of the sarcomere are based on titin, a giant protein with over 3 MDa that spans the half-sarcomere overlapping at the Z-disk and M-band. Thus, titin forms a continuous filament system along the myofibril. Due to its unique domain structure, titin provides support and elasticity. Based on its integration into the sarcomere and the presence of elastic and signalling domains, titin has been proposed to play a role in the length dependent contaction of the heart (Frank-Starling-Mechanism) and the conversion of mechanical stimuli into biochemical signals.

To study the influence of titin on the mechanical properties of the sarcomere and cardiac function *in vivo*, I have generated two titin knockout models removing its elastic spring elements, the heart specific N2B domain and the PEVK-region. In addition to their elastic properties, the N2B domain is involved in hypertrophic signal transduction via FHL2, while the PEVK-Region acts as a molecular brake through interaction with actin.

In both knockouts embryonic development proceeds normally, which suggests that the elastic domains do not interfere with sarcomerogenesis. Anatomically, the knockout hearts are normal, except for the reduced size of the N2B knockout heart, which is associated with altered hypertrophy signalling (reduced levels of FHL2 and increased ANP expression). Histology and ultrastructure of the N2B knockout heart are unchanged compared to littermate controls, except for the reduced slack length of the sarcomere. Electron microscopy revealed that in response to the loss of titin's N2B-Region extension of the PEVK-Region and the immunoglobulin domains is increased. This leads to an elevated stiffness of isolated muscle fibers from N2B deficient animals. Thus, titin's N2B-Region determines the slack length and the mechanical properties of the sarcomere.

The increased stiffness of cardiomyocytes is partly compensated through the increased expression of the more compliant N2BA isoform. Nevertheless, the N2B deletion results in diastolic dysfunction, as documented by echocardiography. The smaller and stiffer ventricle produces a cardiac output comparable to wildtype controls by increased fractional shortening. The systolic function of the N2B deficient ventricle is not affected as show in the isolated heart setup. Thus, the deletion of the N2B-Region does not influence the length-dependent contractile force of cardiac muscle (Frank-Starling-Law). Echocardiography of the PEVK knockout reveals a trend to diastolic dysfunction compensated by an increased heart rate.

Cardiomyocytes derived from N2B or PEVK knockouts display altered contracting properties. While the fractional shortening in the PEVK knockout cells increases, the contractility in the N2B knockout cardiomyocytes is reduced, possibly via FHL2 and its influence on the sarcomere metabolism.

Deletion of the N2B or PEVK-Region did not change the localisation of the respective binding proteins. However, FHL2 protein levels are altered in both knockouts. FHL2 expression is decreased in the N2B knockout, but increased after deletion of the PEVK-Region. Thus, the regulation of FHL2, which is altered in heart disease, is dependent on titin's I-band.

The animal models presented here demonstrate a role of titin in both hypertrophy signalling and the elastic function of the heart. They provide the first genetic model to study diastolic dysfunction and could be a valuable tool to develop novel therapeutic strategies for diastolic heart failure.