maximal force, but abolished the initial difference in $Ca^{2+}$-sensititivy between $MYBPC3_{mut}$ ($pCa_{50}$=5.46 ± 0.03) and donor ($pCa_{50}$=5.48 ± 0.02).
***Conclusions*** Truncating *MYBPC3* mutations cause haploinsufficiency, deranged phosphorylation of contractile proteins and reduced maximal force generating capacity of cardiomyocytes. The enhanced $Ca^{2+}$-sensititivy in $MYBPC3_{mut}$ is due to hypophosphorylation of troponin I secondary to mutation-induced dysfunction.

**1909-Plat**
**Truncation Of Titin'S Elastic PEVK Region Leads To Cardiomyopathy With Diastolic Dysfunction**
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The giant protein titin is important for proper myofilament assembly and structure, as well as the passive mechanical properties of the sarcomere. Within the sarcomeric I-band region of titin reside two elastic elements - the cardiac specific N2B element and the PEVK element. Unlike the N2B element that has been linked to metabolism and signal transduction, the PEVK region has so far solely been regarded as a mechanical spring. Here we have used a loss of function approach to delete exons 219-225 from the mouse titin gene, which encode all residues of the PEVK element present in N2B titin, the main titin isoform expressed in the heart. Homozygous PEVK knockout (KO) mice survived to adulthood and were fertile. Titin-based passive tension was highly increased, accompanied by diastolic dysfunction, as determined by echocardiography, isolated heart experiments, and muscle mechanics. Surprisingly, PEVK-KO mice had dilated and hypertrophied hearts and had increased FHL2 expression, contrasting the cardiac atrophy and decreased FHL2 levels that result from the deletion of the N2B element. This work indicates a concerted action of titin's elastic elements in providing proper diastolic function, but a distinct effect on the trophic properties of the heart.

**1910-Plat**
**Protein Kinase G Modulates Human Myocardial Passive Stiffness By Phosphorylation Of The Titin Springs**
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The sarcomeric titin springs influence myocardial distensibility and passive stiffness. Titin-isoform composition and protein kinase-A-dependent titin phosphorylation are variables contributing to diastolic heart function. However, diastolic tone is lowered, left ventricular extensibility is increased, and relaxation is accelerated also by activating protein kinase-G (PKG). Here we studied using back-phosphorylation assays whether PKG can phosphorylate titin and affect titin-based stiffness in skinned myofibers and isolated myofibrils. PKG in the presence of 8-pCPT-cGMP (cGMP) phosphorylated the two main cardiac titin isoforms, N2BA and N2B, in human donor and dog left ventricle. In human myofibers/myofibrils dephosphorylated prior to mechanical analysis, passive stiffness dropped 10-20% upon application of cGMP-PKG. Autoradiography and anti-phosphoserine blotting of recombinant human I-band-titin domains established that PKG phosphorylates titin's N2-B and N2-A domains. Using site-directed mutagenesis, a serine residue near the COOH-terminus of the cardiac N2-B-unique sequence (N2-Bus) was identified as a PKG-phosphorylation site. To address the mechanism of the PKG-effect on titin stiffness, single-molecule AFM force-extension experiments were performed on engineered N2-Bus-containing constructs. The presence of cGMP-PKG increased the bending rigidity of the N2-Bus to a degree that explained the overall PKG-mediated decrease in cardiomyofibrillar stiffness. Thus, the mechanically relevant site of PKG-induced titin phosphorylation is most likely in the N2-Bus, whereas phosphorylation of other titin sites could affect protein-protein interactions. Results suggest that reducing titin stiffness through PKG-dependent phosphorylation of the N2-Bus can benefit diastolic function. Since failing human hearts revealed lower PKG-mediated basal titin phosphorylation than donor hearts, titin-phosphorylation deficits may contribute to diastolic dysfunction in heart failure.

**1911-Plat**
**Proteasome Dysfunction in Troponin Related Cardiomyopathies**
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Hypertrophic cardiomyopathy (HCM) is a disease characterized by small to significant increases in calcium sensitivity of force development. Our results suggest that patients with mutations which are associated with large increases in calcium sensitivity of force development show poor prognosis. Investigation of two cardiac troponin T (cTnT) transgenic mice, I79N and F110I, which are associated with HCM using a functional proteomics approach showed that several key proteins which are known to be degraded by the proteasome are altered when compared to wild-type cTnT transgenic mice. Skinned fiber studies from I79N and F110I transgenic mice showed that these mutations both caused similar increases in calcium sensitivity of force development (change in pCa50 = approx. 0.22) when compared to wild-type transgenic mice. The 179N and F110I mice both showed significant decreases in 20S proteasome activities related to wild-type cTnT transgenic mice suggesting that proteasomal dysfunction may be a contributing factor to the pathogenesis of HCM. The levels of polyubiquitinated proteins present in the I79N and F110I were also increased relative to wild-type cTnT transgenic mice. Transgenic mice expressing cTnT I79N or cTnT F110I do not develop significant hypertrophy or ventricular fibrosis even after chronic exercise challenge. This suggests that increased heart size may not the critical factor for causing proteasome dysfunction in some cardiomyopathies. These results suggest that the UPS is an important system involved in the pathogenesis of troponin related HCM which is initially caused by significant increases in calcium sensitivity of force development.

**1912-Plat**
**Engineering Cardiac Contractility from the Sarcomere to Tissue-Scale**
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Engineering myocardium with specific contractile properties is a major goal of cardiac regenerative medicine, both for in vivo therapy and in vitro disease models. Yet maintaining the multiscale coupling and uniaxial orientation from nanometer-scale actin-myosin motors to centimeter-scale muscle tissue remains a major obstacle. Inspired by the collagen fibrillar network and capillary beds in myocardium, we hypothesized that these microscale heterogeneities act as boundary conditions that direct cardiomyocyte alignment and functional coupling. Specifically, our interest is the role these boundary conditions play in the sub-cellular alignment of the sarcomeres. To tackle this problem we developed a cardiac tissue engineering methodology that allows us to control and quantify sub-micron sarcomere orientation and measure macroscale contractile force and electrical conduction. We evaluate the deformation of 2-dimensional (2D) myocardial sheets that mimic the lamellar layers of the ventricular wall. Cardiomyocytes are grown onto a free-standing film of polydimethylsiloxane elastomer, referred to as a muscular thin film (MTF). Microscale heterogeneities are created using microcontact printing to direct 2D myogenesis, either (i) isotropic with no cell or sarcomere alignment, (ii) anisotropic with uniaxial cell and sarcomere alignment or (iii) an array of 20 micrometer wide myocardial strands with enhanced uniaxial cell and sarcomere alignment. For contractility experiments, MTFs are fashioned into cantilevers, mounted in an organ bath and electrically stimulated at 0.5 Hz. Results demonstrate that isotropic myocardium generates ~1 kPa peak systolic stress. In stark contrast the anisotropic myocardium generates an order-of-magnitude greater peak systolic stress of ~10 kPa. The 1D myocardial strands generate the greatest peak systolic stress of ~17 kPa, or ~35 kPa when normalized for muscle mass. This demonstrates that microscale heterogeneities can have a profound effect on sarcomere alignment and muscle contractility, defining new strategies for optimizing electro-mechanical function in engineered muscle.