Supplementary Information

A HaloTag-TEV genetic cassette for mechanical phenotyping of native proteins

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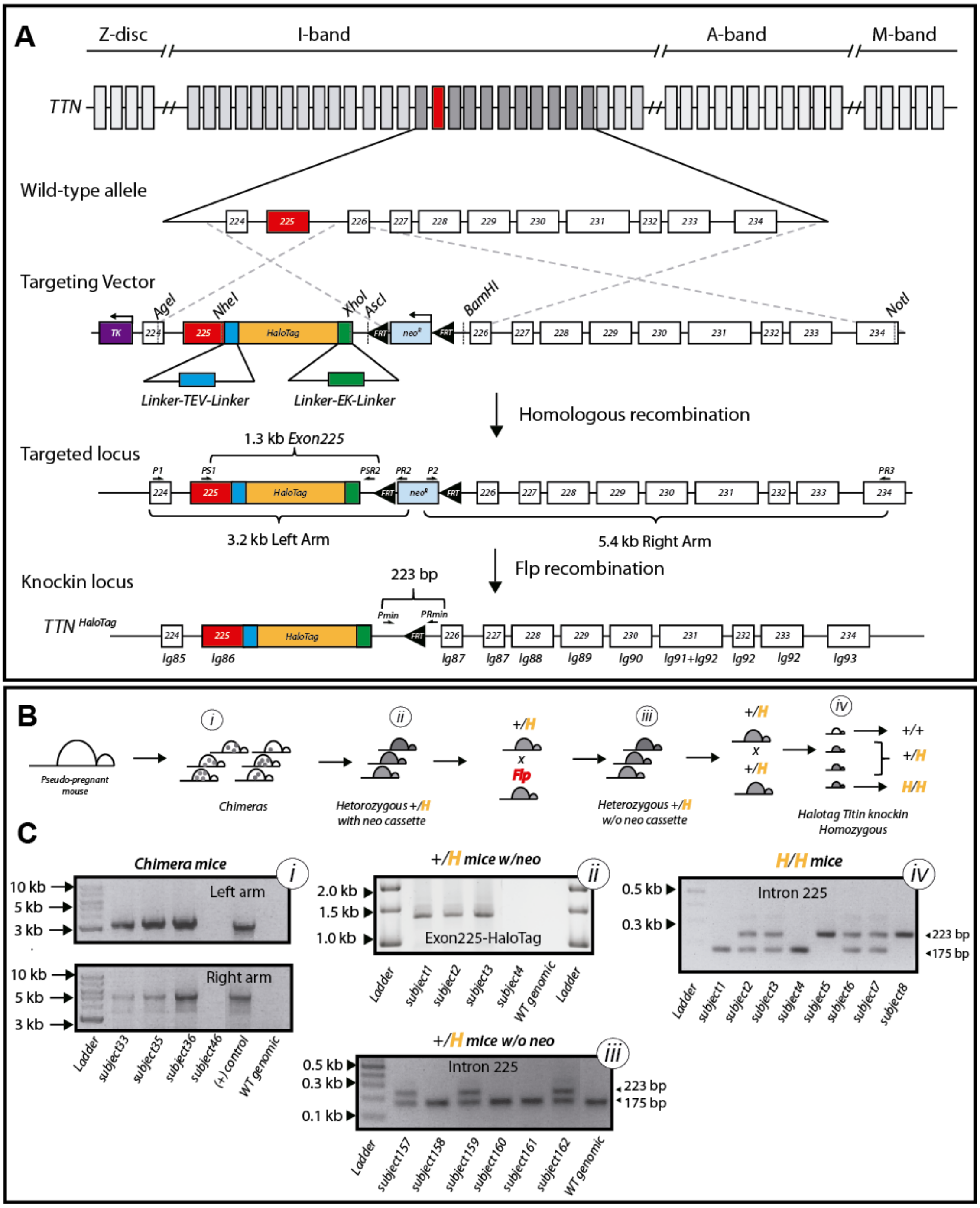
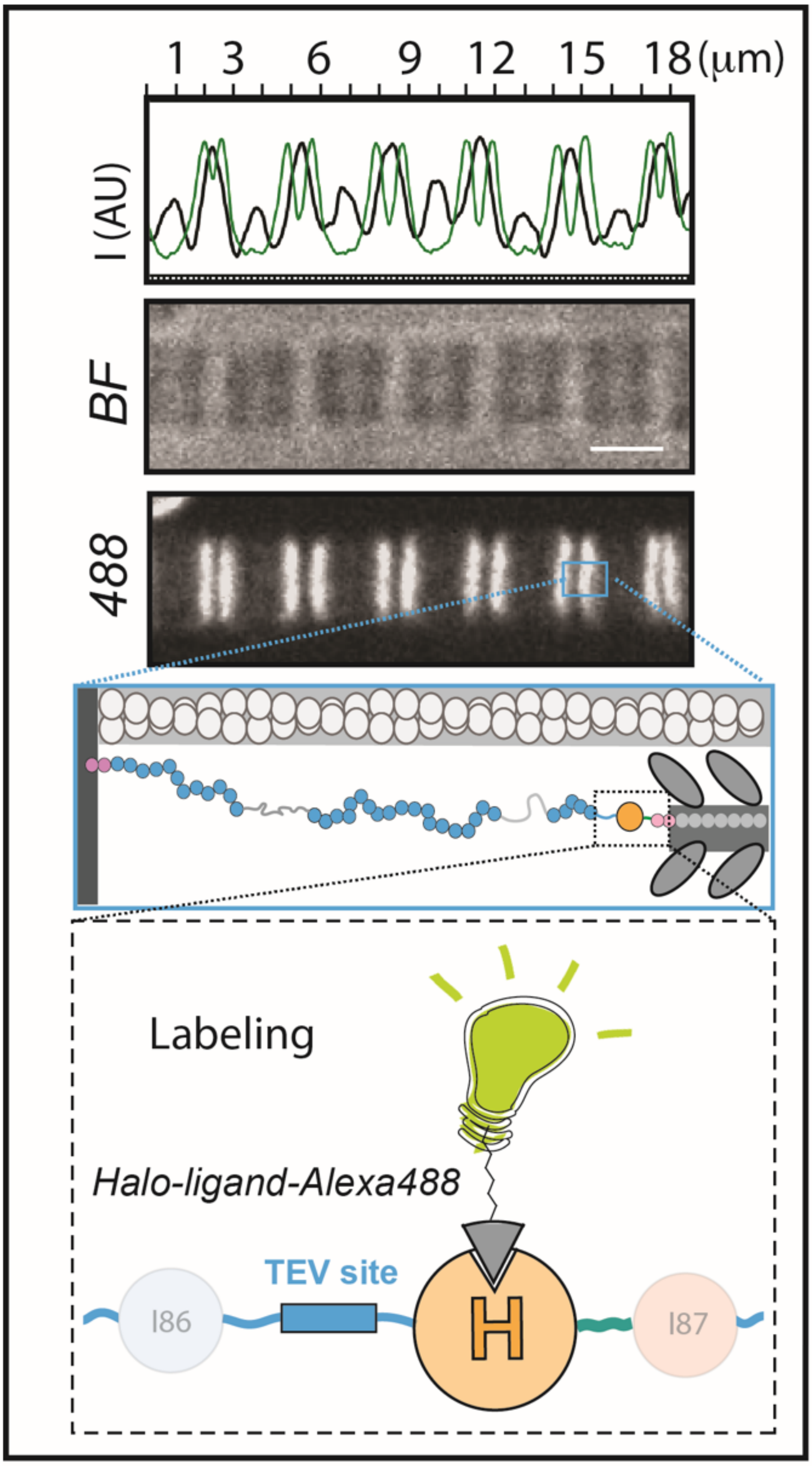
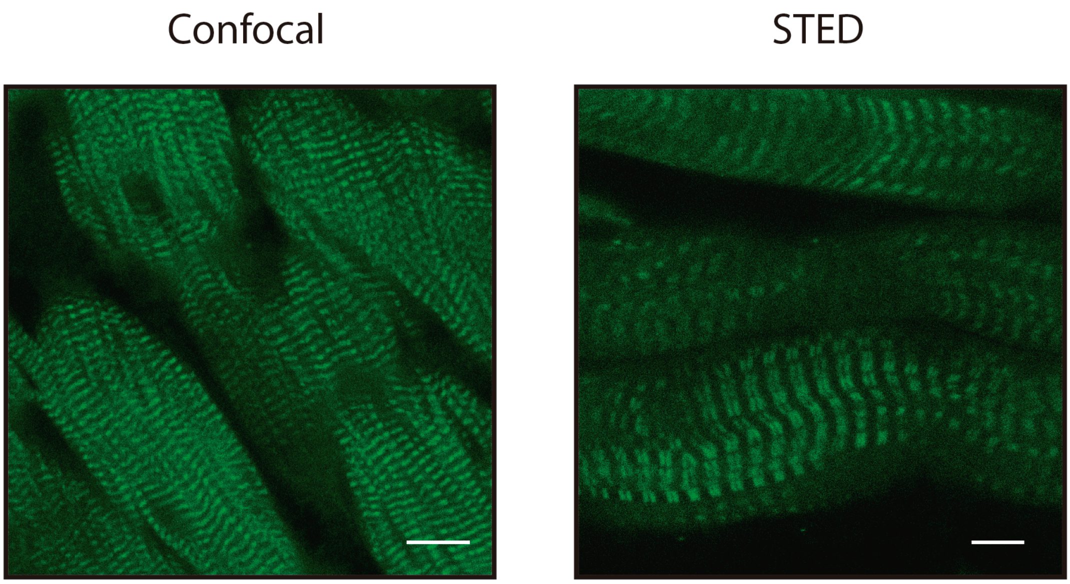


Supplementary Figure S1. Generation of knock-in mice with the HaloTag-TEV cassette inserted into the titin gene. (**A**) The targeting vector contains the *TTN* sequence between exons 224 and 234. The HaloTag gene is inserted downstream of exon 225, flanked by linkers including TEV and EK sites. A Neo resistance gene flanked by FRT elements was inserted in intron 225. Restriction sites and primers used during the different genetic engineering steps are shown, together with the size in base pairs (bp) of relevant fragments. (**B**) Strategy followed to get homozygous knock-in mice (H/H) from recombinant ES cells. The different experimental stages are labeled i-iv, see below. (**C**) We used PCR amplification of genomic DNA to confirm (i) the presence of the HaloTag in the TTN gene in chimera mice (primers *P1* and *PR2, left arm;* primers *P2* and *PR3*, *right arm*), (ii and iii) the presence and subsequent removal of the neo resistance by crossing with Flp mice (primers *PS1* and *PSR2,* Exon225-HaloTag*; Pmin* and *PRmin,* Intron225), and (iv) the generation of the homozygous H/H titin mouse (*Pmin* and *PRmin*). We used the vector construct and wild-type genomic DNA as controls.



Supplementary Figure S2. Spinning disk microscopy. Under bright field illumination, the A- and I-band sections are easily distinguished along the myofibril as dark and clear regions, respectively (middle panel, BF). The fluorescent signal coming from Alexa488-labeled HaloTag-TEV titin appears as doublets at the I/A band interface (bottom panel, 488). The top panel shows intensity profiles (Alexa488 fluorescence in green and brightfield intensity in black). *Insets:* Cartoons showing the location of the HaloTag-labeled titin. Scale bar, 2.5 μm.



Supplementary Figure S3. The HaloTag-TEV cassette is correctly inserted in cardiac titin. The heart of a homozygous HaloTag-TEV titin mouse was incubated with HaloTag Oregon Green ligand, fixed and clarified. Although these samples show higher autofluorescence than skeletal preparations (**Figure 1B**), there is strong labeling in bands as expected from the location of the HaloTag insertion in titin (**Figure 1A**). Staining in doublets can also be observed, although to a lesser extent than in skeletal muscle, probably reflecting shorter I-bands in cardiac sarcomeres. Scale bars: 10 µm (Confocal) and 5 µm (STED).

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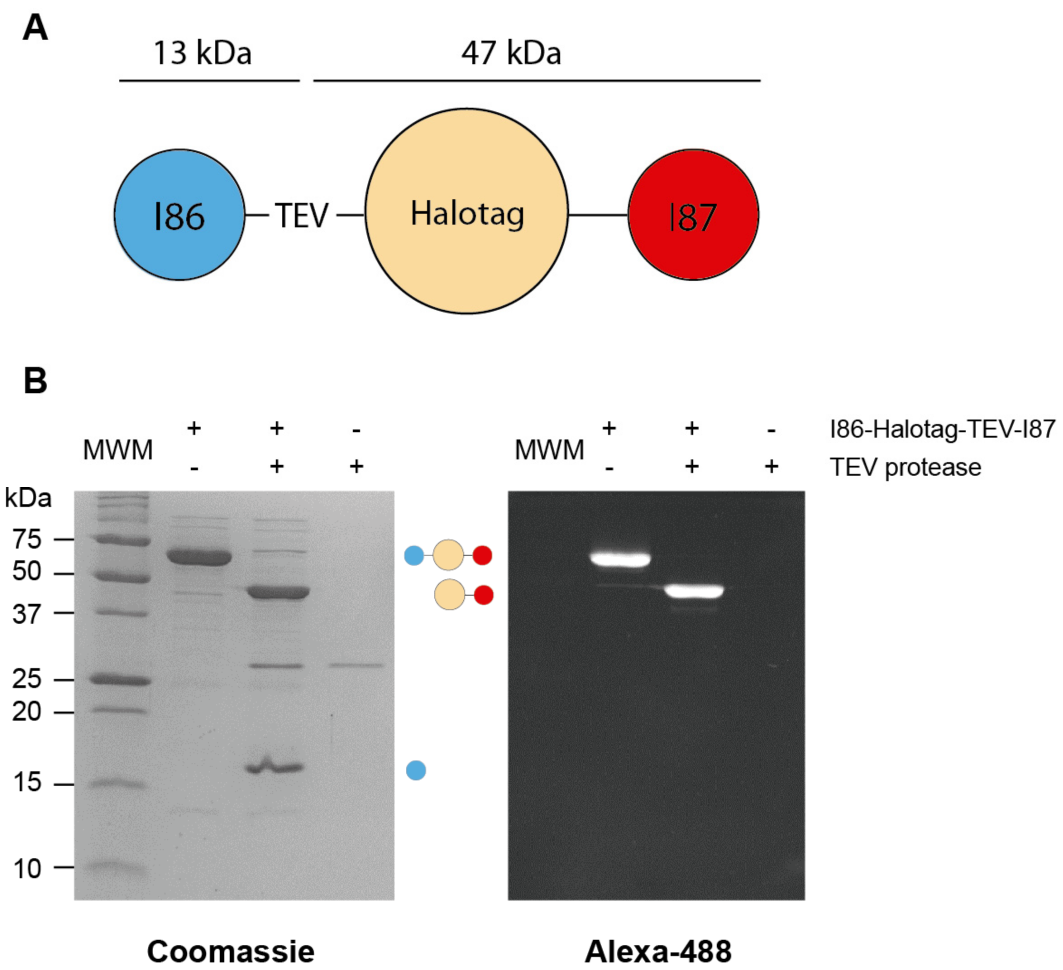
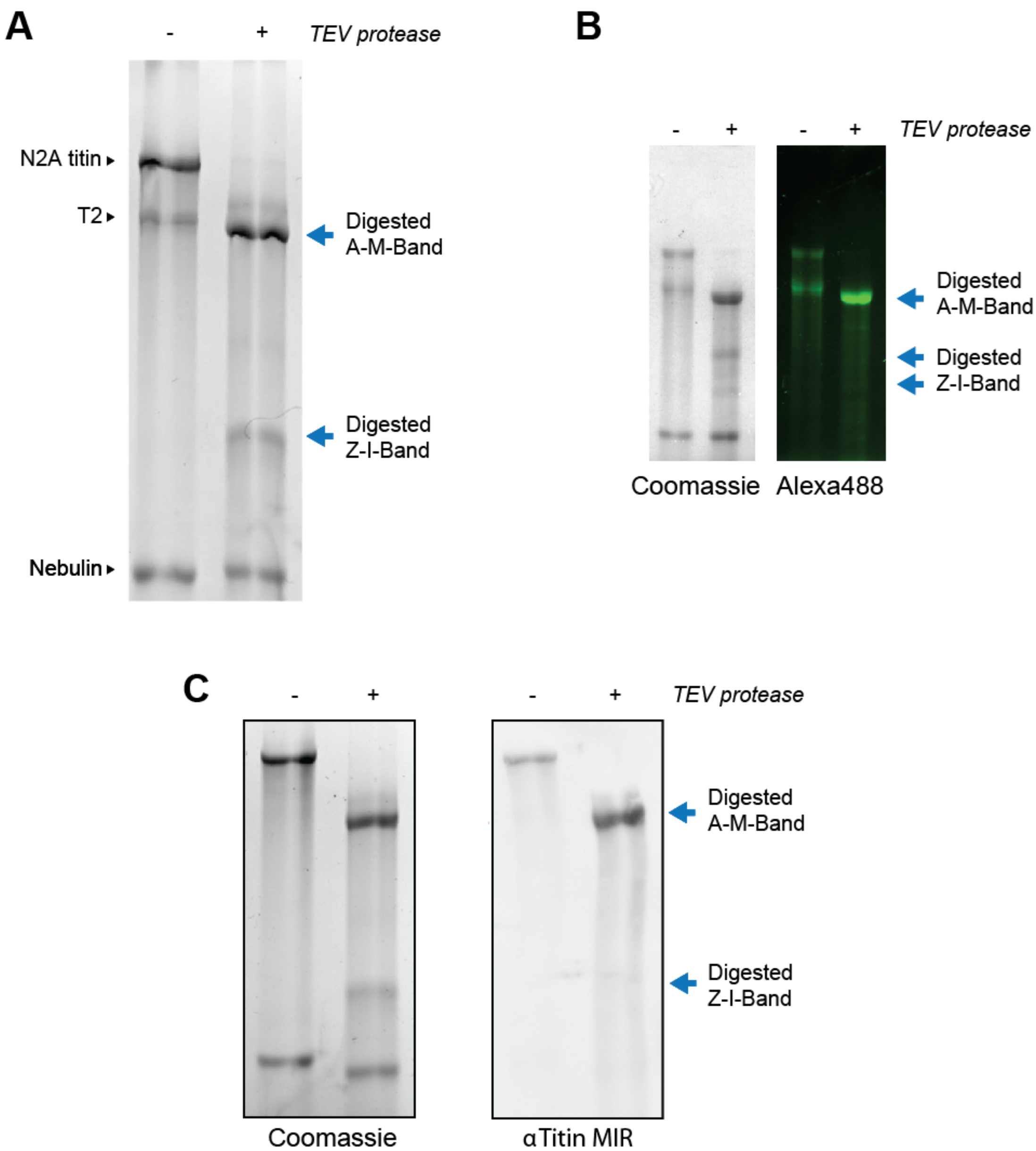
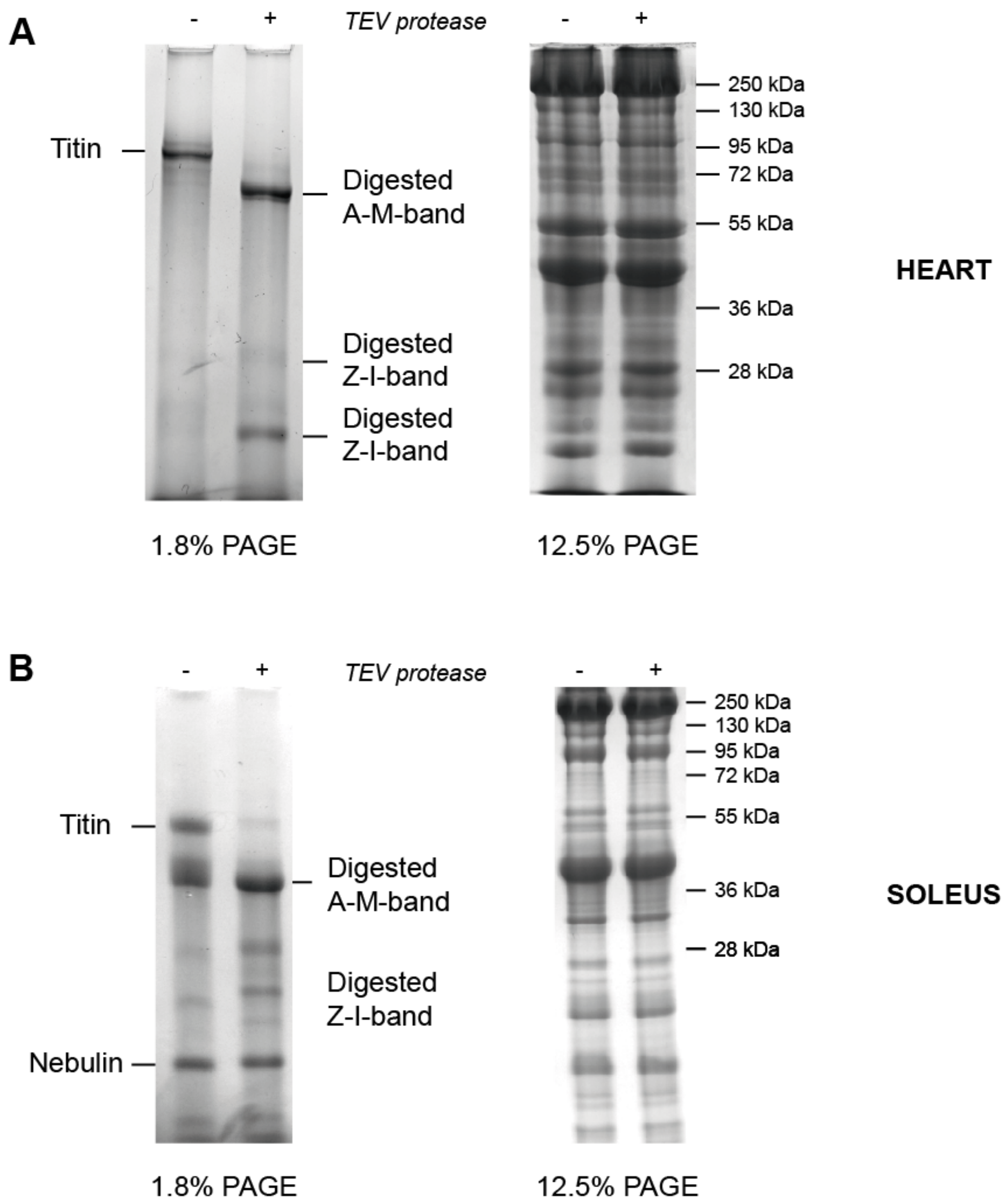


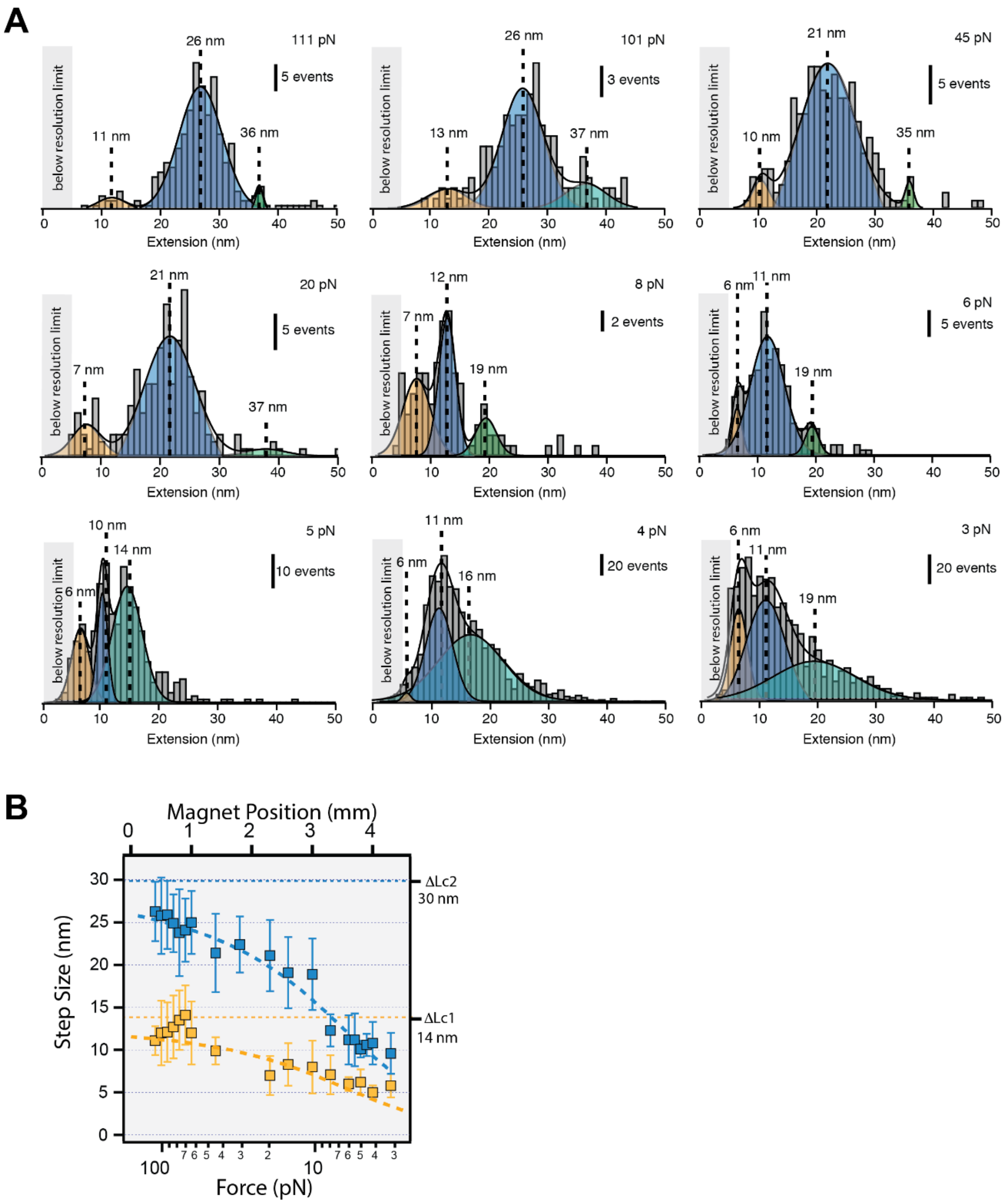
Supplementary Figure S4. Digestion of recombinant HaloTag-TEV titin fragment. (A) Scheme of I86-HaloTag-TEV-I87 (60 kDa) showing the size of the fragments that result from TEV digestion. **(B)** I86-HaloTag-TEV-I87 was treated or not with TEV protease (28 kDa) at 34ºC for 1 hour, and results were analyzed by 17% SDS-PAGE. Digestion resulted in the appearance of two new bands at the expected mobility (*left*, Coomassie staining). Specificity of TEV-cleavage is demonstrated by labeling with HaloTag Alexa488 ligand, which only reacts with HaloTag-containing bands (*right*, Alexa488 fluorescence).



Supplementary Figure S5. TEV digestion of skeletal muscles from homozygous HaloTag-TEV titin mice, as analyzed using 1.8% acrylamide SDS-PAGE gels. (A) Results of TEV-digestion of isolated soleus myofibrils (Coomassie staining). **(B)** Analysis of the digestion of psoas myofibrils with TEV. Both Coomassie staining and HaloTag Alexa488 ligand are used to visualize proteins. The HaloTag-specific Alexa488 ligand only labels the digested A-M-band fragment **(C)** Equivalent results are obtained in TEV digestions of soleus myofibrils, as analyzed by western blot using the MIR antibody, which recognizes the A-band segment of titin.



Supplementary Figure S6. Effect of TEV treatment on the protein composition of striated muscle from homozygous HaloTag-TEV titin mice. (A) *Left*: 1.8% SDS-PAGE shows TEV-induced specific digestion of cardiac titin. *Right*: the pattern of protein bands in 12.5% SDS-PAGE gels remains unaffected by TEV treatment. **(B)** Equivalent results are obtained with soleus samples. In this particular experiment, specific assignment of Z-I-band fragments is hindered by some non-specific degradation of titin already present in the –TEV sample.



Supplementary Figure S7. (Un)folding step sizes. (A) Single titin molecules extracted from gastrocnemius muscles were pulled at different forces between 3 and 111 pN, and the size of the unfolding and refolding events was measured. We found three main populations of step sizes (solid lines are Gaussian fits to the data). **(B)** Force-dependency of the step sizes for the two populations corresponding to single-domain unfolding events, and fits to the worm like chain model of polymer elasticity (dashed lines). We obtain $\Delta L_C = 30 \pm 1$ nm (blue) and $\Delta L_C = 14 \pm 1$ nm (yellow).

Supplementary Text S1. Sequence of the targeting vector to introduce Halotag-TEV in titin. Exons and Introns are shown in grey and white background, respectively.

Exon224—Exon225-**TEV**-HaloTag-**EK**—NeoFRT—Exon226—Exon227—Exon228—
Exon229—Exon230—Exon231—Exon232—Exon233—Exon234

Exon224

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Exon225

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HaloTag

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Exon232

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Exon233

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Exon234

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Supplementary Text S2. Sequence of the I86-HaloTag-TEV-I87 recombinant construct. The QS peptide bond cleaved by TEV is indicated. Please note that DNA sequence do not match the murine DNA because it was codon-optimized for optimal protein expression in *E.coli*.

I86-TEV site-Halotag-EK site-I87

cDNA:

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AGGTGCAGTGGTTTAAAGATGGTGCCGAAATCAAAAAAGGCAAAAAATACGACATCATCTCCAA
AGGTGCCGTTCGTATTCTGGTTATTAACAAATGTCTGCTGAACGATGAAGCCGAATATAGCTGT
GAAGTTCGTACCGCACGTACCAGCGGTATGCTGACCAGATCTTAA

Protein:

MRGSHHHHHHGSPPVEFTKPLEDQTVEEEATAVLECEVSRENAKVKWFKNGTEILKSKKYEIVA
DGRVRKLIIHGCTPEDIKTYTCDAKDFKTSCNLNVVLASDNTTPEEDLYFQ’SDNTTPEAEIGT
GFPFDPHYVEVLGERMHYVDVGPRDGTPVLFLHGNPTSSYVWRNIIPHVAPTHRCIAPDLIGMG
KSDKPDLGYFFDDHVRFMDAFIEALGLEEVVLVIHDWGSALGFHWAKRNPERVKGIAFMEFIRP
IPTWDEWPEFARETFQAFRTTDVGRKLIIDQNVFIEGTLPMGVVRPLTEVEMDHYREPFLNPVD
REPLWRFPNELPIAGEPANIVALVEEYMDWLHQSPVPKLLFWGTPGVLIPPAEAARLAKSLPNC
KAVDIGPGLNLLQEDNPDLIGSEIARWLSTLEISGDNTTPEDDDDKDNTTPETRAPHVEFLRPL
TDLQVKEKETARFECEISKENEKVQWFKDGAEIKKGKKYDIISKGAVRILVINKCLLNDEAEYS
CEVRTARTSGMLTRS

Supplementary Video S1. 3D reconstruction of HaloTag-TEV clarified muscle fibers. Following specific labeling of gastrocnemius muscle with Oregon Green Halo ligand, fixation and clarification, a 250-µm-deep Z-stack was obtained using multiphoton microscopy (127 images). Individual images were intensity corrected and the software Imaris was used to produce the 3D animation.