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Supplemental information

Prestin amplifies cardiac motor functions

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Supplementary Information

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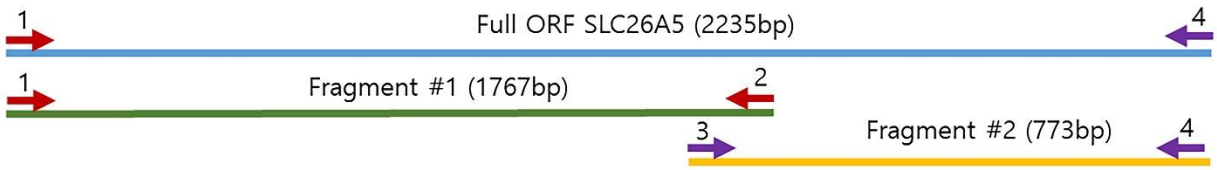
Figures S1-S5

Figure S1



5full hA5 (F)	ATGGATCATGCTGAAGAAAATG	1
3hA5P (R)	GTTGGCCATATTTGCATTTC	2
hA5P (F)	CTGCTGTGATCATTGCTCTG	3
hPrestin (R)	CATCTATGCCTCAGGAGTGGC	4

Fig. S1. The strategy and cloning of human cardiac prestin (~2.2 kb). Related to STAR Methods and METHOD DETAILS. For amplifying the full-length coding sequence of human cardiac *SLC26A5*, fragments 1 (1767 bp) and 2 (773 bp) were first amplified using two separate sets of primers.

Figure S2

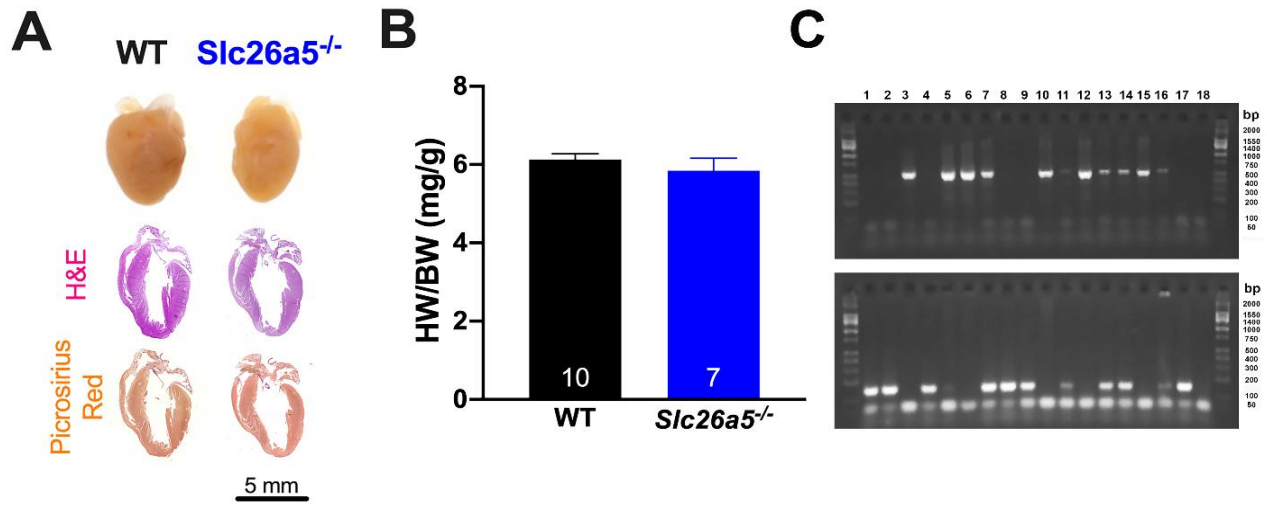


Fig. S2. Characterization and genotype analysies of *Slc26a5*$^{-/-}$ mice. Related to Figure 1, STAR Methods, and METHOD DETAILS. (**A**) Photomicrographs of the whole heart of WT and *Slc26a5*$^{-/-}$ mice together with histological staining using H&E and Picrosirius Red, demonstrating no evidence of fibrosis in the knockout mice (scale bar, 5 mm). (**B**) Summary data for heart/body weight ratio in mg/g. (**C**) Photomicrographs of agarose gel from genotype analyses using primers to amplify the mutant and WT bands shown in the upper and lower panels, respectively. The leftmost and rightmost lanes are All-Purpose LO^{TM} DNA Marker 50-2000 bp (Bionexus). Lanes 1-14 is PCR products amplified from genomic DNA samples from 2 different

litters. Lanes 15, 16, and 17 are control samples from mutant, heterozygous, and WT mice, respectively. Lane 18 is a no template control. The PCR products' expected sizes are 500 and 175 bp for mutant and WT bands, respectively.

Figure S3

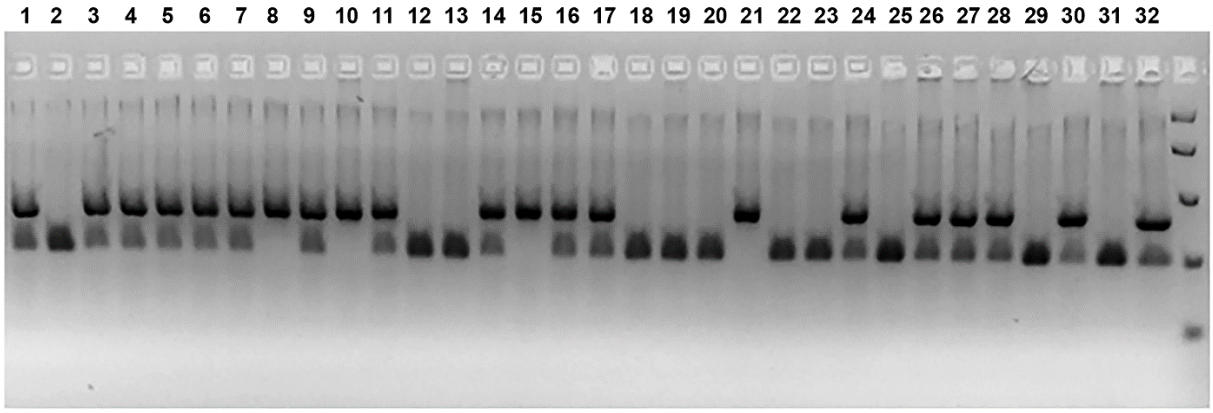


Fig. S3. Photomicrographs of agarose gel from genotype analyses using primers to amplify the mutant and WT bands for *Prestin-YFP* knockin mice. Related to Figure 2, STAR Methods, and METHOD DETAILS. The rightmost lane is LMW DNA Ladder. Lanes 2, 12, 13, 18, 19, 20, 22, 23, 25, 29, and 31 are PCR products amplified from genomic DNA samples from WT mice. Lanes 8, 10, 15, and 21 are PCR products amplified from genomic DNA samples from *Prestin-YFP* knockin mice. The rest of the lanes are from heterozygous knockin mice. The PCR products' expected sizes are 387 and 247 bp for mutant and WT bands, respectively.

Figure S4

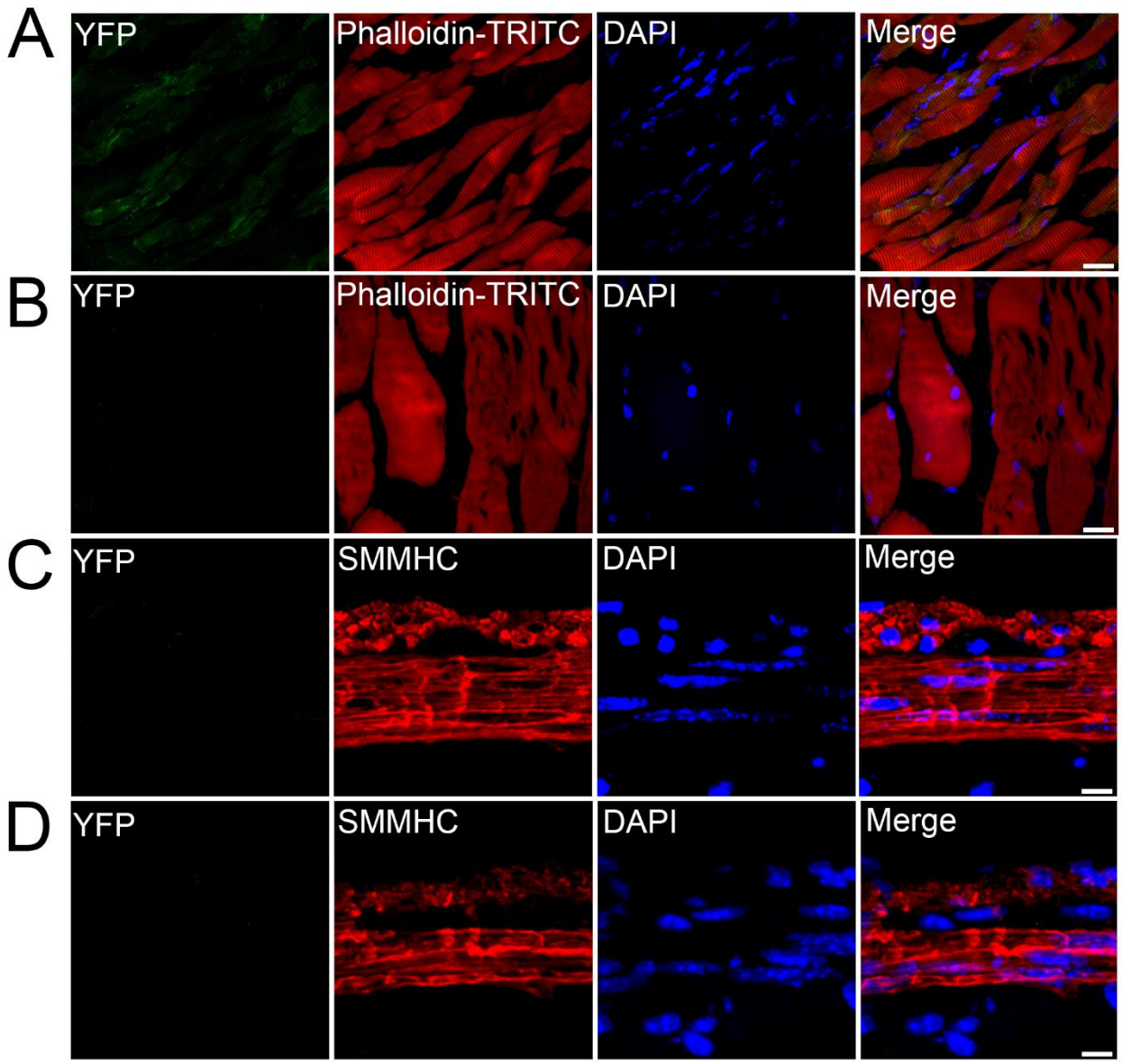


Fig. S4. Expression of prestin in skeletal muscle tissues from Prestin-YFP KI mice. Related to Figure 2. (**A**) Expression of prestin-YFP in skeletal muscles. Phalloidin-TRITC stained skeletal muscles. Scale bar: 30 μm. (**B**) YFP signals were not detected in **c**ontrol skeletal muscles from WT mice. Scale bar: 30 μm. (**C**) The prestin-YFP signals in gut smooth muscles are not detected. The gut smooth muscles were labeled by the anti-smooth muscle myosin heavy chain 11(SMMHC). Scale bar: 7 μm. (**D**) YFP signals were not detected in control gut smooth muscles from WT mice. Scale bar: 7 μm.

Figure S5

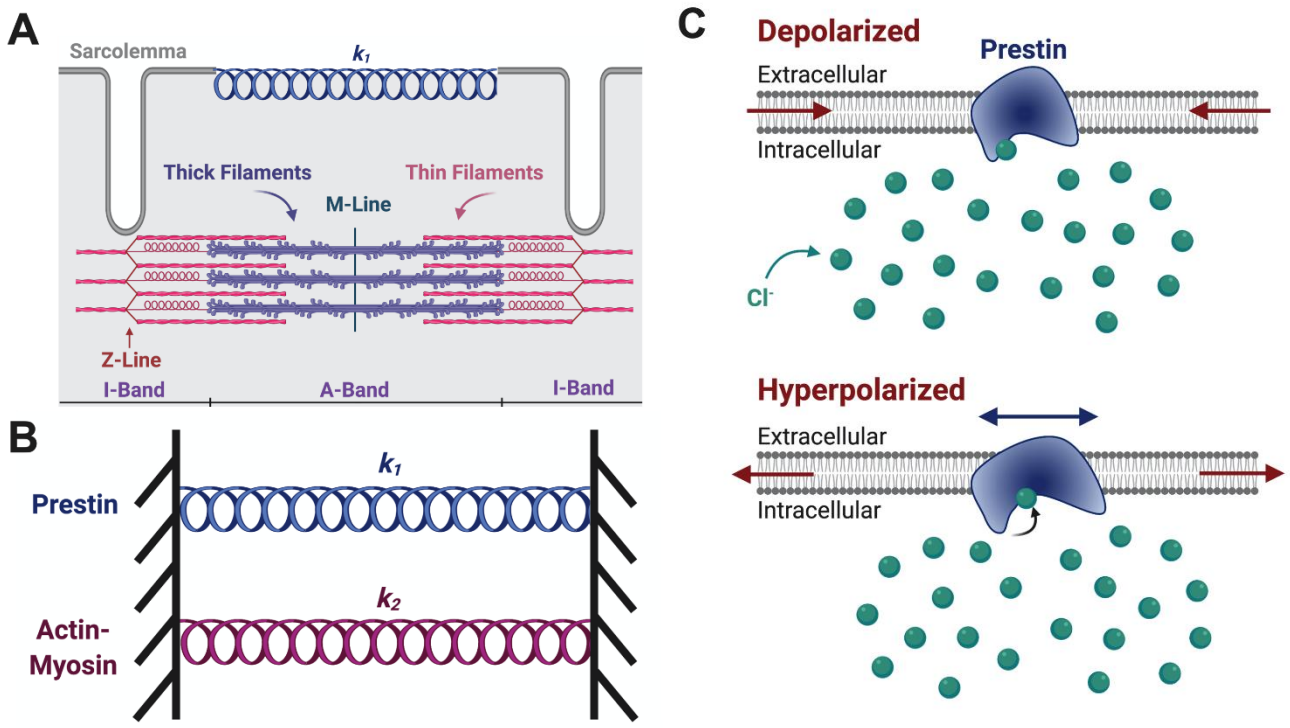


Fig. S5. A conceptual model of prestin function in cardiomyocytes. Related to Figure 2 and Figure 5. (**A**) Schematic diagram of a cardiac sarcomere with the added parallel spring. The schematic depicts the thick (myosin) and thin filaments (actin) with Z line, M line, A band, and I band indicated. (**B**) Prestin serves as a spring in parallel to the actin-myosin with a spring constant of the assembly of $k = k_1$ (prestin) + k_2 (actin-myosin). (**C**) A conceptual model of prestin in cardiomyocytes. During membrane depolarization, intracellular anion Cl^- increases and binds to the cytoplasmic face of the prestin molecule. Prestin is expected to assume a contracted conformation (short conformation). When the membrane hyperpolarizes, intracellular Cl^- is translocated across the protein towards the external side, and prestin is expected to assume an expanded conformation (long conformation). We speculate that prestin may co-localize with Cl^- channels or transporters, generating a local Cl^- microdomain necessary for providing the Cl^- to prestin function. Images were generated using BioRender.