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Supplemental information

Intracellular TMEM16A is necessary for myogenesis of skeletal muscle

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Supplementary Figures and Tables

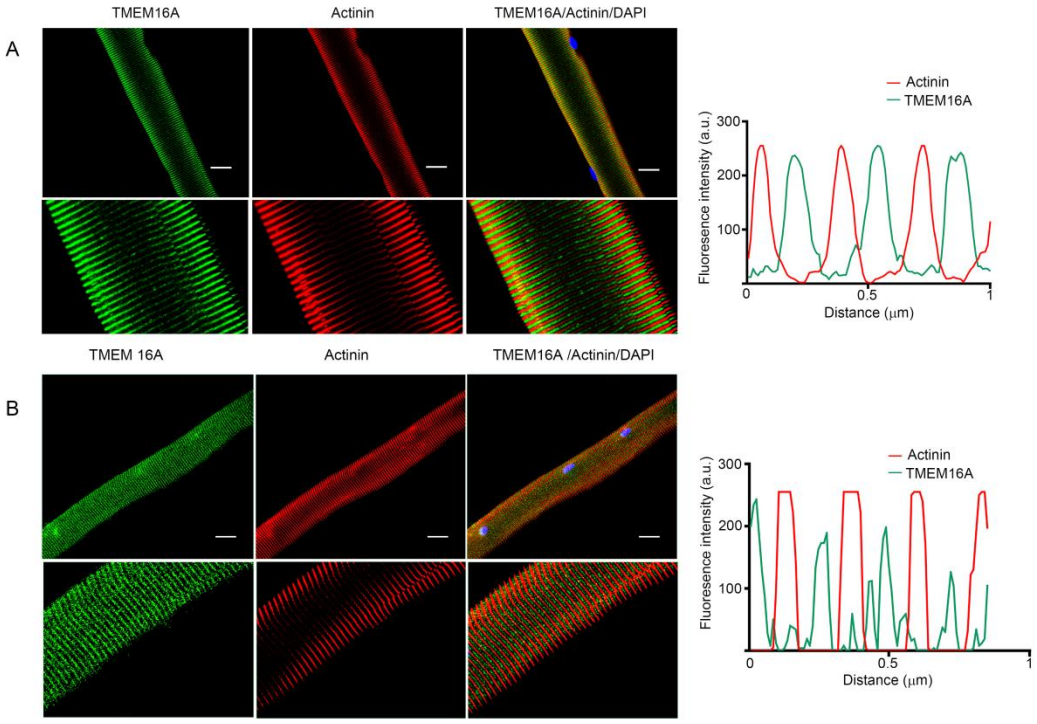

Figure S1 TMEM16A and Actinin staining in mature (A) and immature myofibers (B), related to Figure 1. Images were obtained using a confocal fluorescence microscope and processed using Photoshop software. Scale bars, 10 μm.

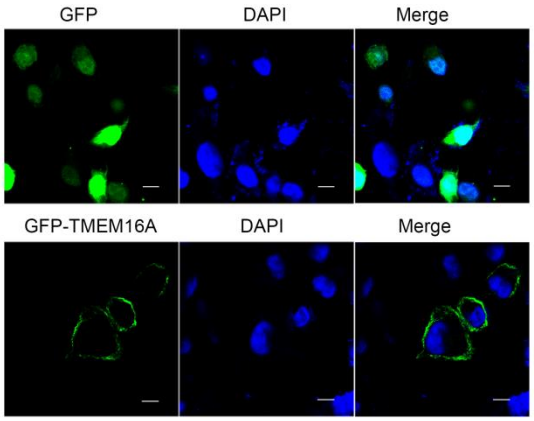

Figure S2 GFP-TMEM16A is localized in the plasma membrane of Chinese hamster ovary cells (CHO), related to Figure 2. CHO cells were transfected with *Gfp-Tmem16a* recombinant plasmid; the green fluorescence signals of GFP-TMEM16A fusion proteins were examined under a confocal fluorescence microscope (Nikon A1). The *Gfp* plasmid transfected CHO cells were used as a control. Scale bars, 10 μm.

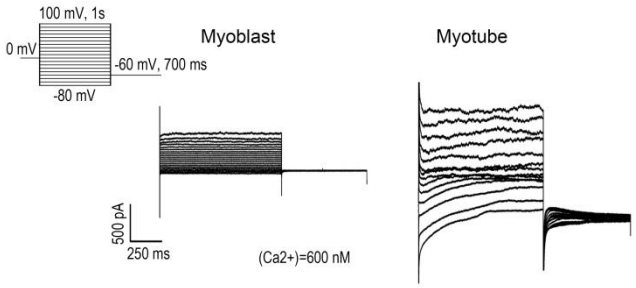

Figure S3 Ca^{2+}-activated Cl^- currents in myoblasts and myotubes, related to Figure 2. The Ca2+-activated Cl- currents were recorded in the whole-cell voltage clamp configuration.

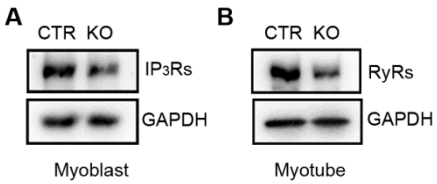

Figure S4 Expression of IP_3Rs and RyRs in myoblasts and myotubes. A, related to Figure 3. Western blotting analysis of the expression of IP3Rs in myoblasts, GAPDH was used as loading control. B. Western blotting analysis of the expression of RyRs in myotubes, GAPDH was used as loading control.

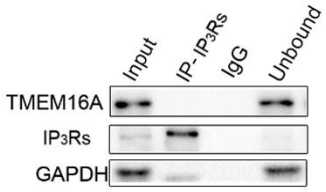

Figure S5 TMEM16A does not interact with IP_3Rs, related to Figure 3. Western Blot analysis showed that TMEM16A was not co-immunoprecipitated with IP_3Rs. Input: the total cell lysate control; IgG: non-specific IgG, used as a negative control; Unbound: proteins were not pulled down.

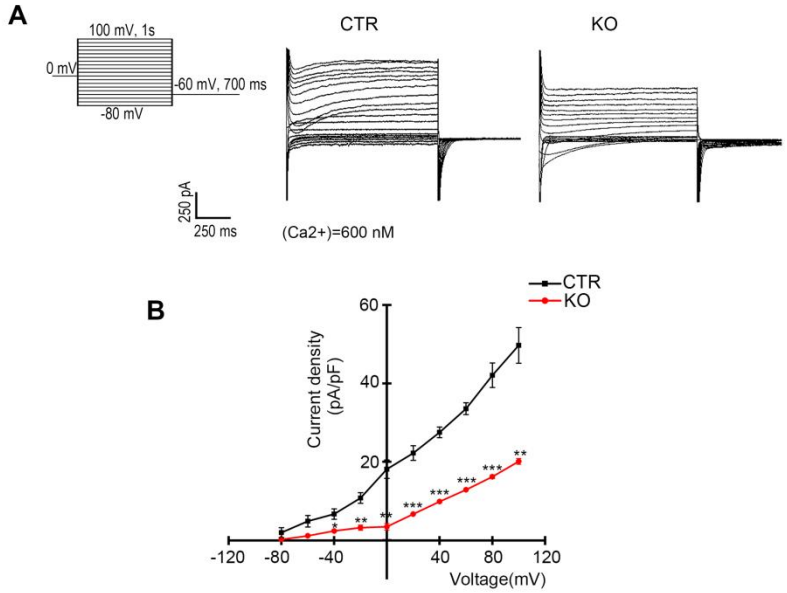

Figure S6 The CaCC currents is declined in TMEM16A KO myotubes (DM4), related to Figure 4. (A) The primary myoblasts were induced to differentiate for 4 days, the CaCC was recorded using clamp patch. (B) I-V curves of $I_{Cl.Ca}$ from the experiments shown in A. The currents were significantly declined in TMEM16A KO myotubes compared with CTR. n=6. Data are mean ±SD. **P*<0.05, ***P*<0.01, ****P*<0.001.

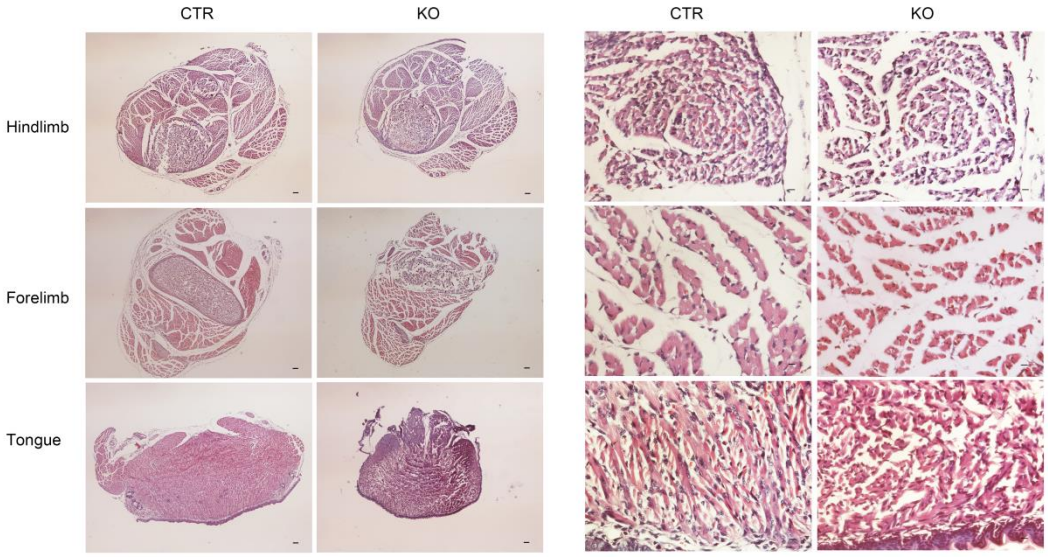

Figure S7 Muscles of P1 mice, related to Figure 4. A: H/E staining of the cross sections of hind limb, forelimb, and tongue muscles. Scale bars, 400 μm. B: Zoom in of part regions in A panel. Scale Bars, 50 μm.

Gene	Primer Sequences	
	Forward	Reverse
Tmem16a	5'-CTGGCCCCTCTCCTCATTTTCCACAGACAT-3'	5'-AGAATTACCCCCTTGGTCTACTATGGCTT-3'
Myogenin	5'-CATCCAGTACATTGAGCGCCTA-3'	5'-GAGCAAATGATCTCCTGGGTTG-3'
GAPDH	5'-AGGTCGGTGTGAACGGATTTG-3'	5'-TGTAGACCATGTAGTTGAGGTCA-3'

Table S1: The primer sequences used this study, related to STAR Methods.