Legends to supplementary figures and video recording

Figure S1. Immunoblots showing endogenous myotubularin in the nervous system and skeletal muscle. **a)** Myotubularin in soleus muscle, sciatic nerve and brain of 6 week-old mice. Additional bands of about 70-75 kDa (arrow) are not myotubularin isoforms, as shown in tissues from *Mtm1* knockout (KO) mice, but cross-reacting bands (75 µg of protein extract was loaded in each lane). **b)** Myotubularin expression during skeletal muscle development both *in vitro* (myoblasts and myotubes) and *in vivo* (muscles from the hindlimb of embryonic day (E) 14.5 and 17.5 embryos and quadriceps from mice at postnatal (P) day 0, 7, 21, 28 and at 7 weeks of age) (50 µg of protein extract per lane). **c)** Expression of myotubularin in striated muscles from adult mouse by western blotting (75 µg of proteins per lane). The entire membrane was incubated with p1947 to show the specificity of the antibody.

Figure S2. Immunostaining of wild-type muscle cross sections with antibodies against MTM1, laminin alpha 2 (Lama2, 4H8-2, Alexis Biochemicals), dystrophin (Dys, kindly provided by Prof. Koenig, IGBMC), caveolin-3 (CAV3, sc-7665, Santa Cruz Biotechnologies) and dihydropyridine receptor alpha 1-subunit (DHPR-α, sc-8160, Santa Cruz Biotechnologies). Vacuoles and membrane aggregates are negative for laminin but positive for the other markers. The bars represent 2 µm.

Figure S3. Staining of *Mtm1* deficient (mKO, top panels) muscle cross sections with antibodies directed against caveolin 3 (left) and DHPR-α□(right). Dystrophin, but not laminin alpha 2, was also present at the membrane of the vacuoles (not shown). The distribution of caveolin 3 and DHPR-α is normal in mKO-AAV muscle sections (bottom panels). Bars represent 15 µm.

Figure S4. Immunolabelling of an AAV-transduced wild-type semithin section with antibodies against the MTM1 protein. Note that myotubularin accumulates in subsarcolemmal regions (arrows).

Video recording. Images show that an *Mtm1* mutant mouse uses the AAV-treated left hindlimb to climb on racks and fingers of the treated but not the untreated leg are able to grasp on a pipette (video).

Figure S1

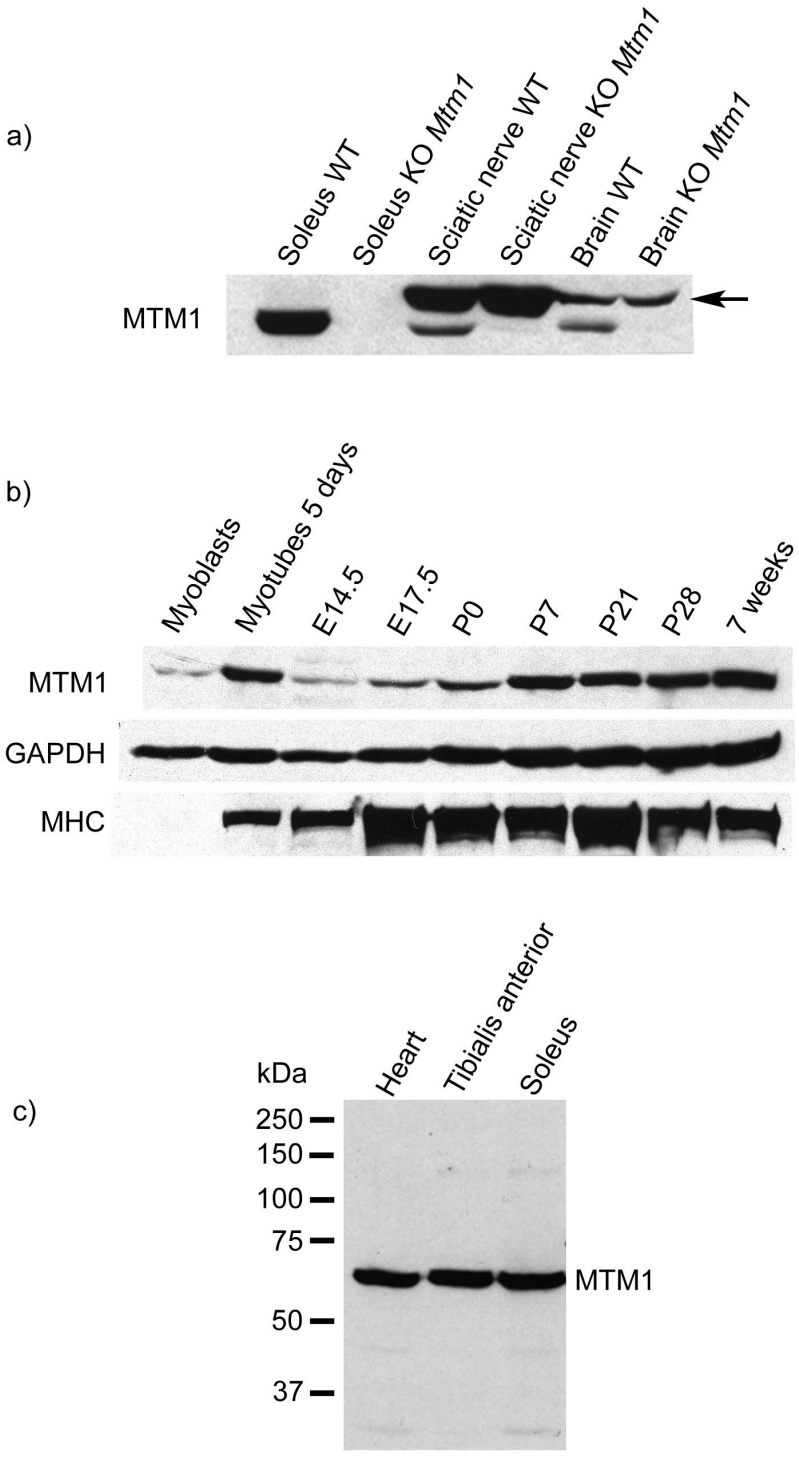
a)
Soleus WT
Soleus KO Mtm1
Sciatic nerve WT
Sciatic nerve KO Mtm1
Brain WT
Brain KO Mtm1
MTM1
b)
Myoblasts
Myotubes 5 days
E14.5
E17.5
P0
P7
P21
P28
7 weeks
MTM1
GAPDH
MHC
c)
kDa
Heart
Tibialis anterior
Soleus
250
150
100
75
50
37
MTM1

Figure S2

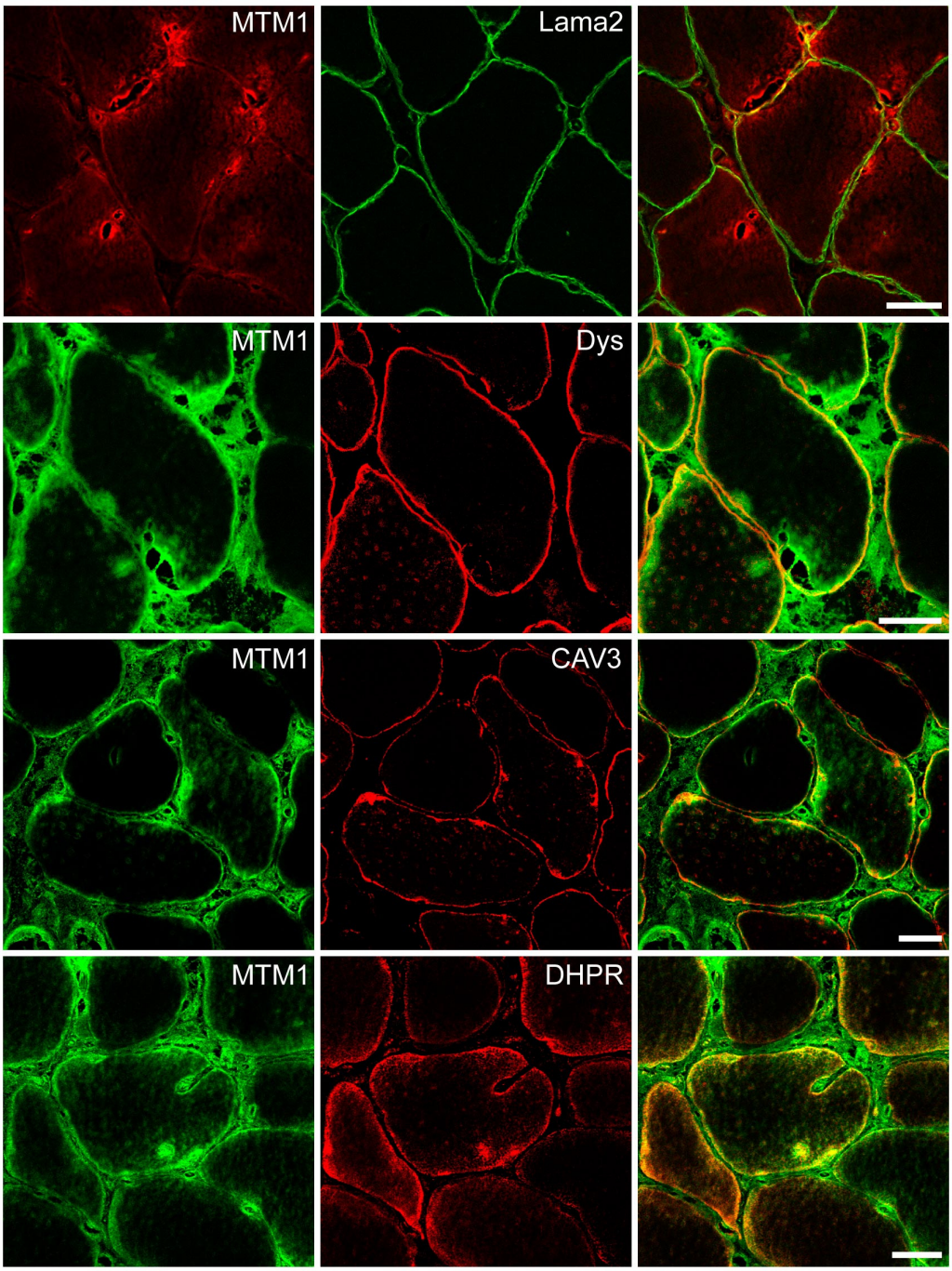

Figure S3

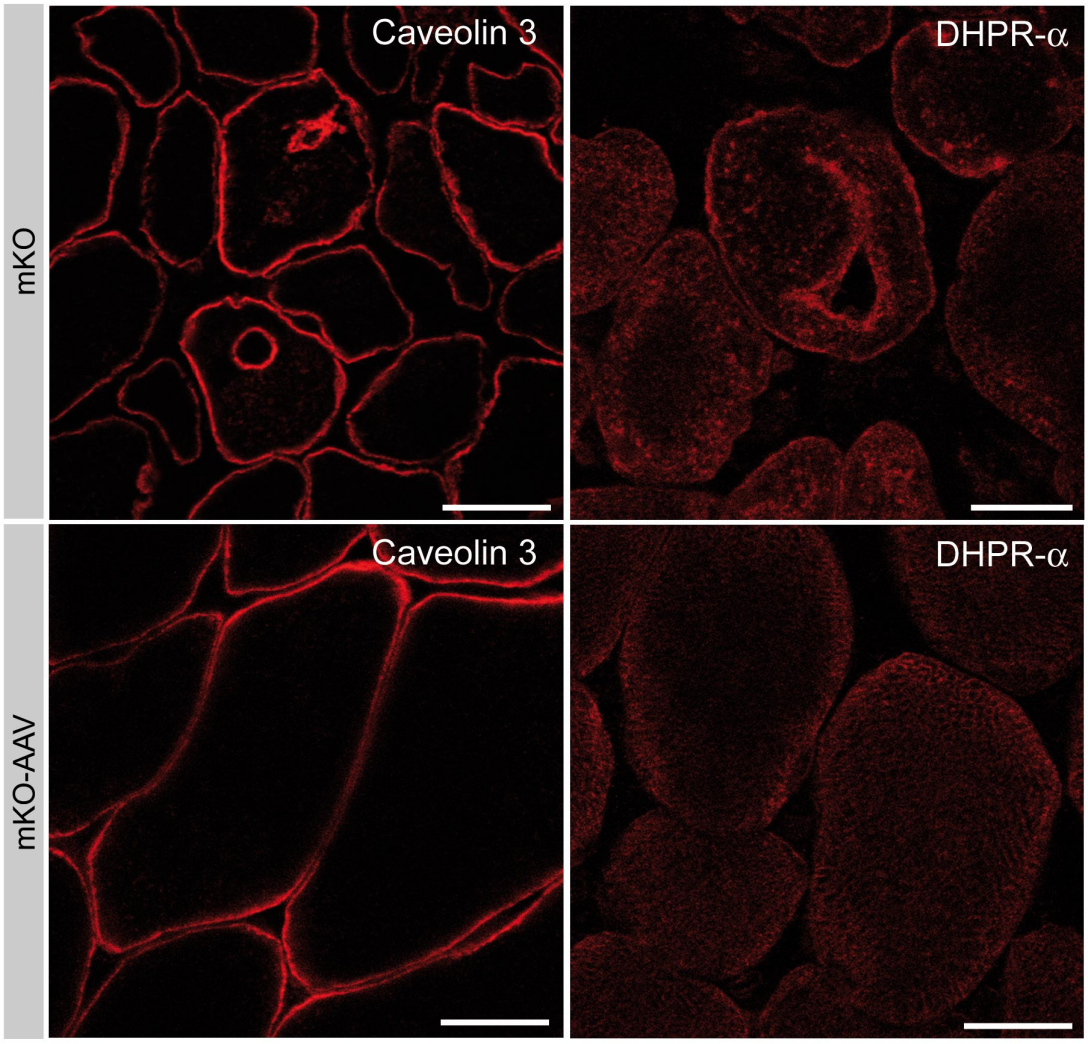

Figure S4