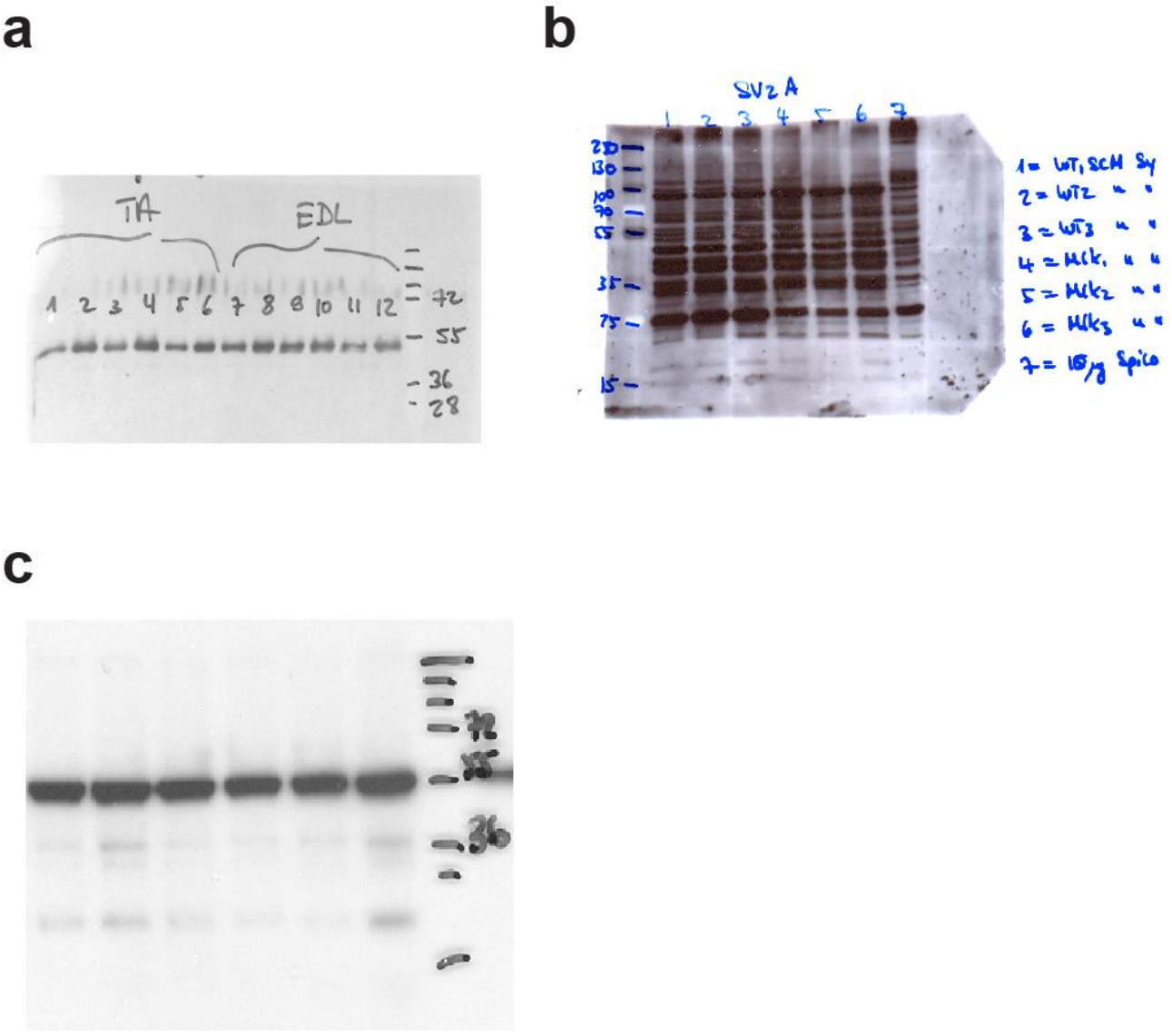
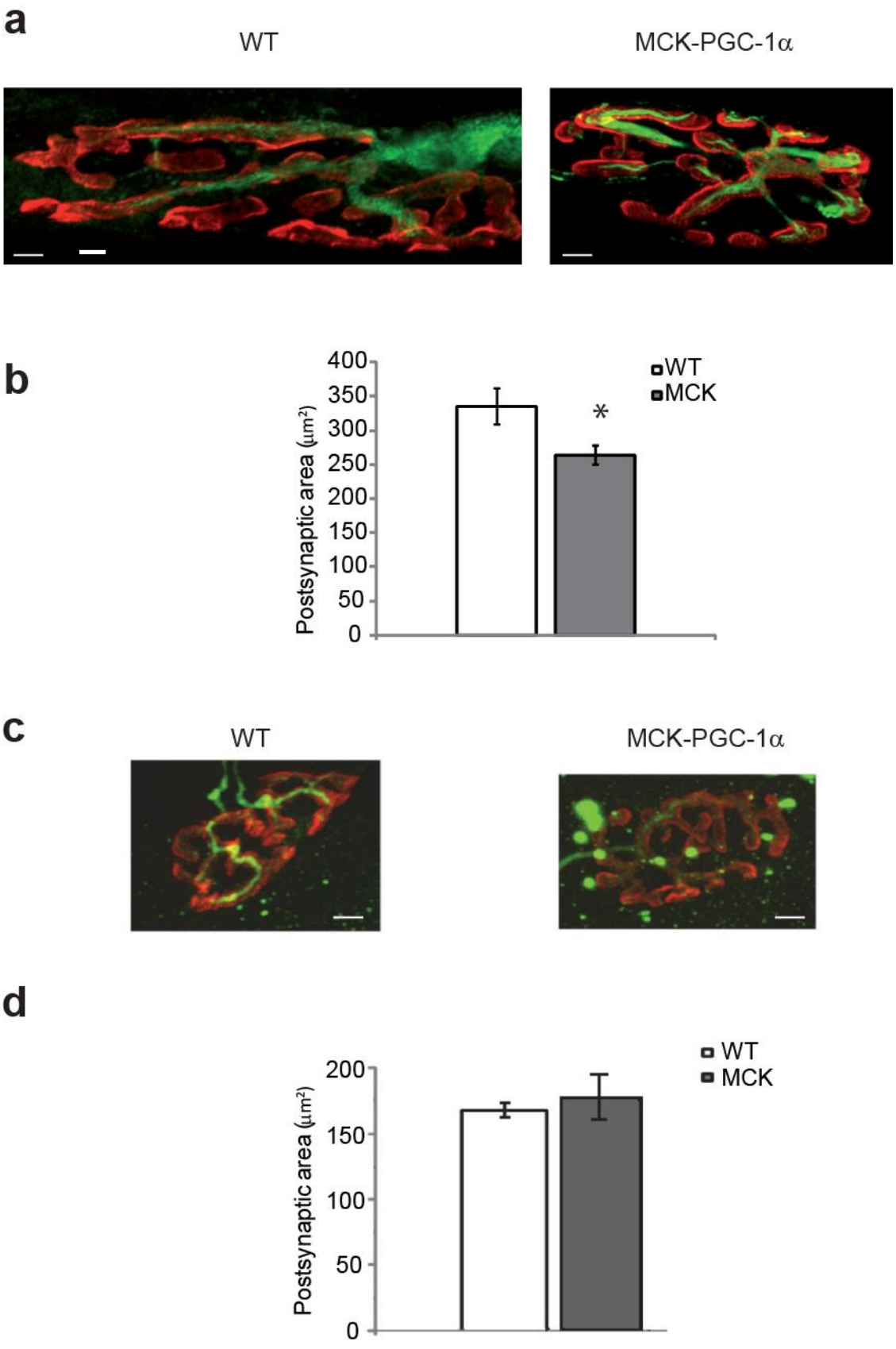
Supplementary Figures

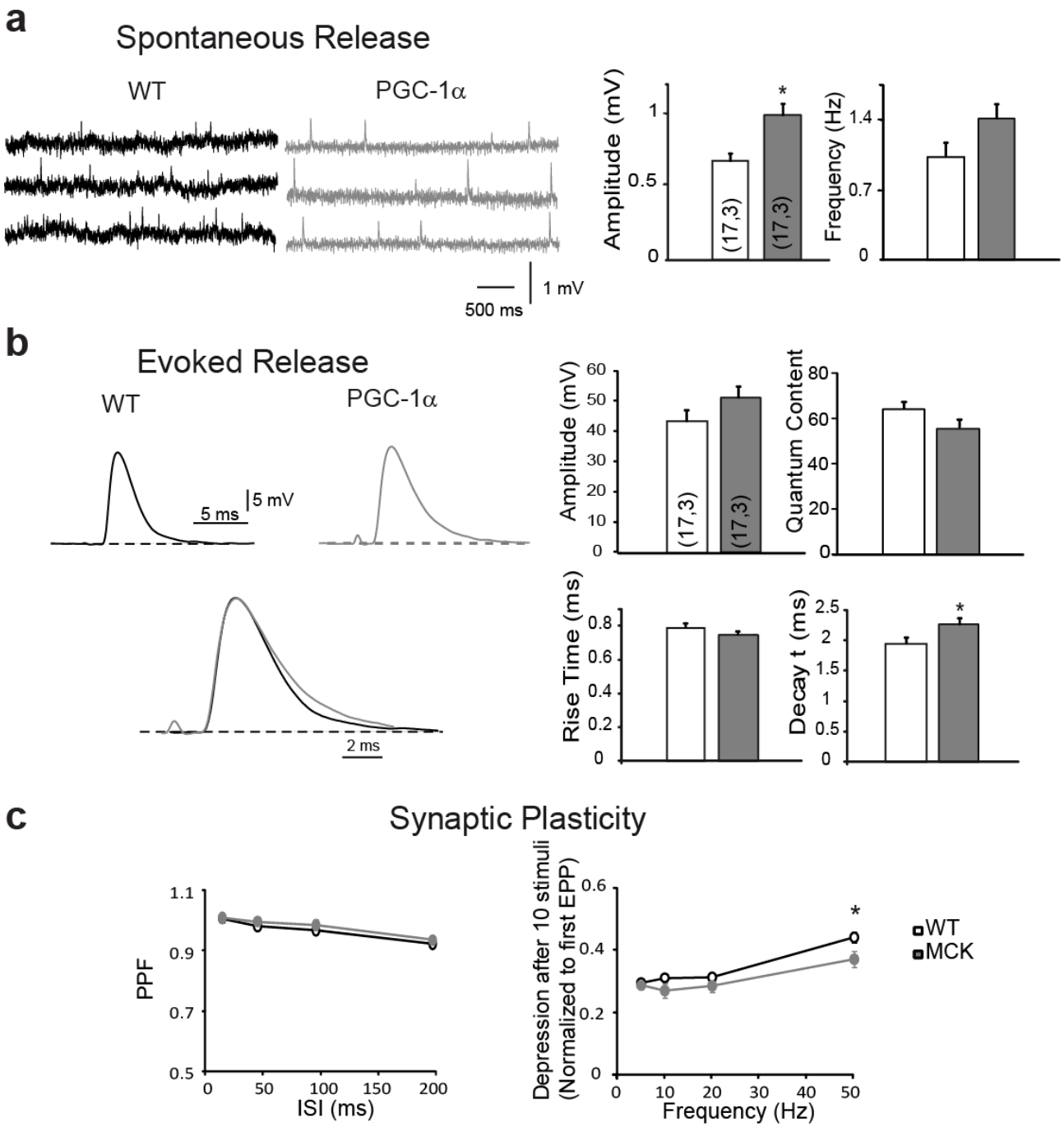


Supplementary Figure 1. Representativ full-sized Western blots. (a) Western blot for synaptophysin in the TA and EDL muscles of WT (lanes 2, 4, 6, 8, 10 and 12) and MCK mice (lanes 1, 3, 5, 7, 9, 11).(b) Western blot for SV2A. (c) Western blot for tubulin.



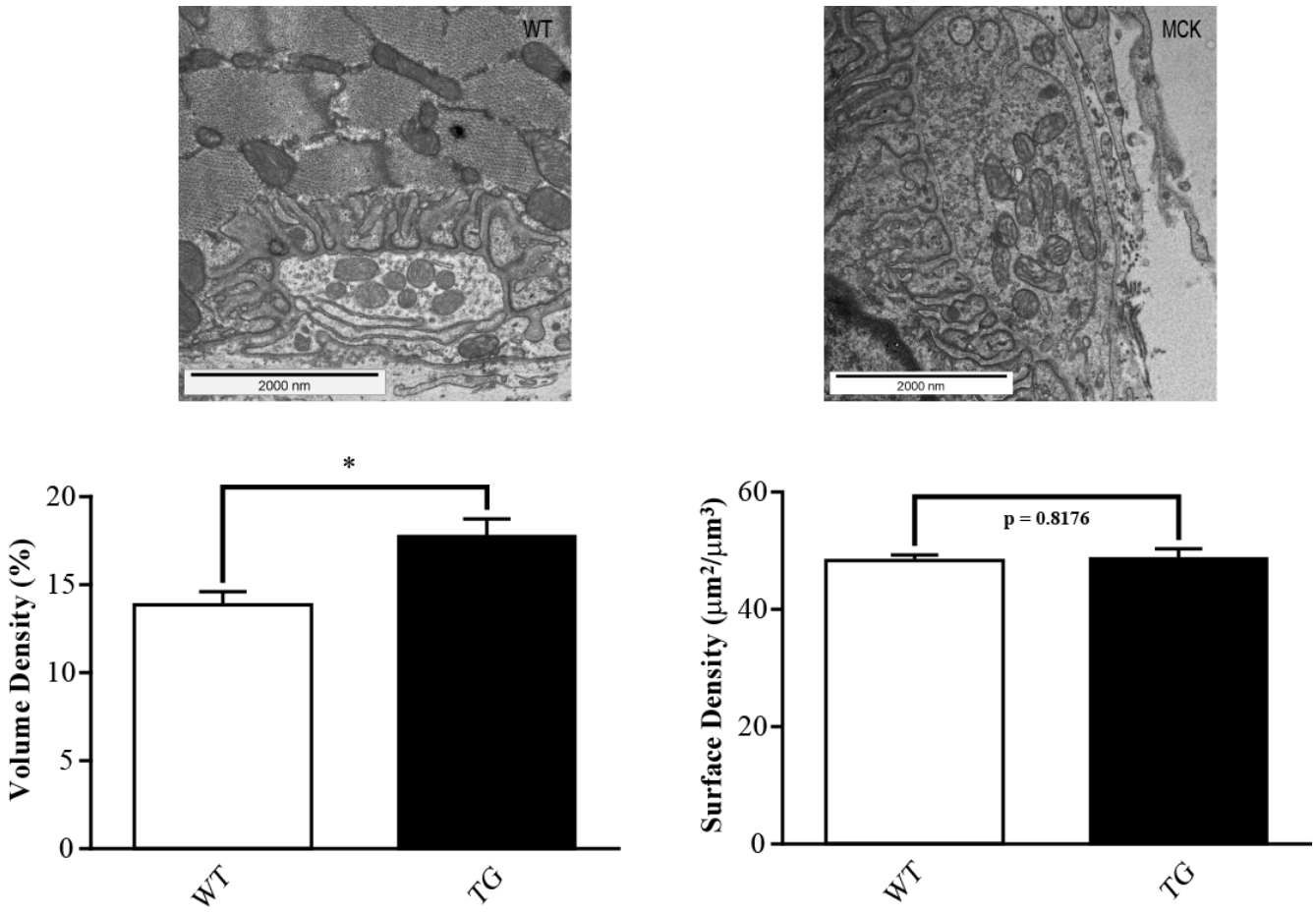
Supplementary Figure 2. NMJ morphology of the LAL and TVA muscles. (**a**) Representative confocal stack image of fluorescently labeled NMJ in the LAL muscle. Muscles were stained with rhodamine-coupled α-bungarotoxin to visualize AChRs (red) and anti-neurofilament antibodies to

stain the nerve part (green). Calibration bar: 5 µm (**b**) Mean size of post-synaptic terminal areas in WT (White bars, n=32 terminals, 3 mice) and MCK (Grey bar, n=35 terminals, 3 mice). Each point represents mean ± SEM. (**c**) Representative confocal stack image of fluorescently labeled NMJ in the TVA muscle. Calibration bar: 3 µm (**d**) Mean size of pretzel areas in WT (White bars, n=106 terminals, 3 mice) and MCK (Grey bar, n=113 terminals, 3 mice). Each point represents mean ± SEM. *$p<0.05$, t test two-tails.

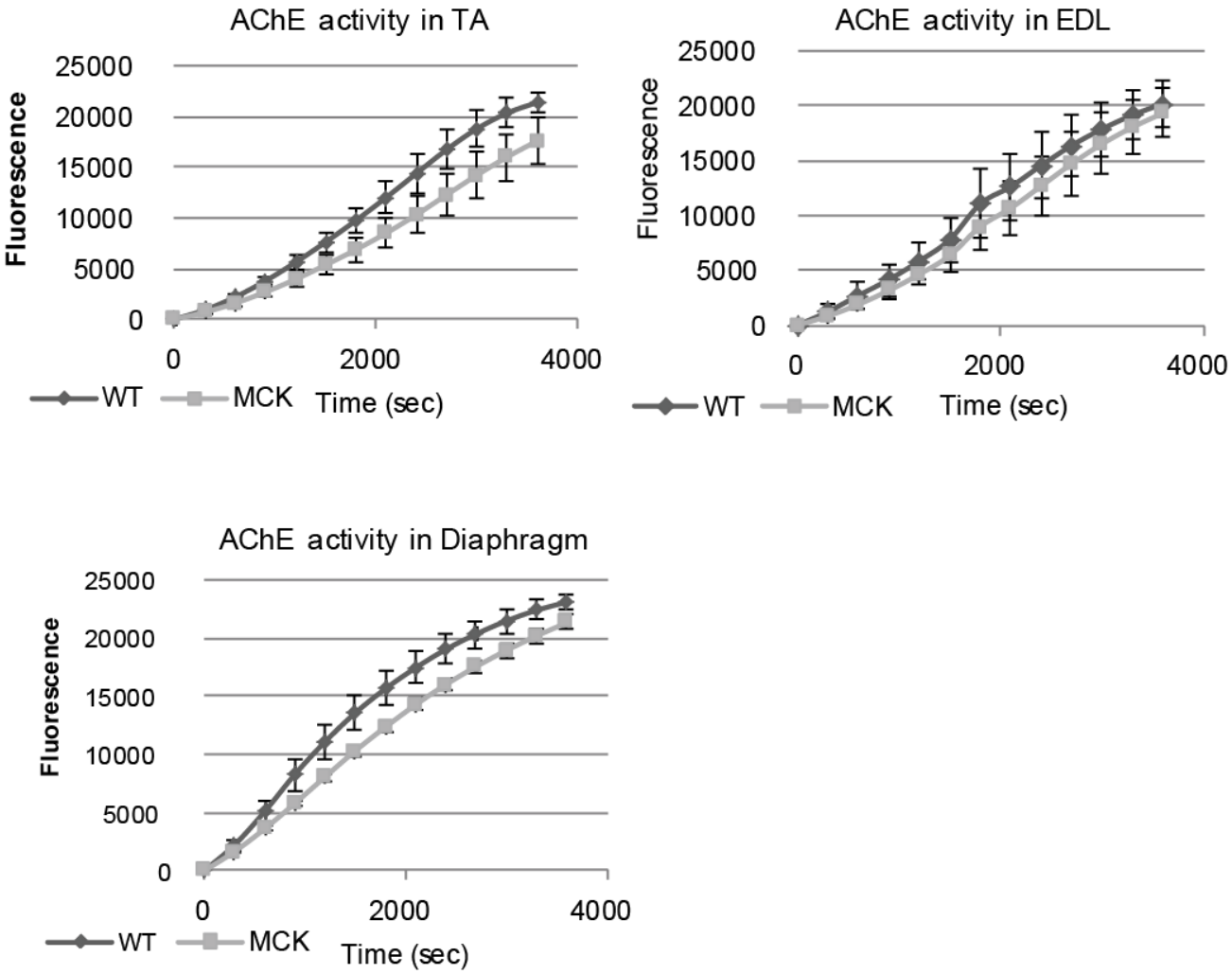


Supplementary Figure 3. Electrophysiological properties of TVA muscles from WT or transgenic mice. (**a**) The spontaneous neurotransmitter release of LAL muscle resembled the representative traces of mEPP in WT (black) and MCK-PGC-1α (gray) NMJs. Amplitude, frequency of mEPP and their rise and decay times were determined. (**b**) Evoked neurotransmitter release resembled the representative traces of EPP in WT (black) and MCK-PGC-1α (gray) NMJs. Amplitude, quantum content of EPP and their rise and decay times were determined. (**c**) The synaptic plasticity shows a representative trace of steady-state depression in WT (black) and MCK-PGC-1α (gray) mice. The pair-pulse-facilitation (PPF) at inter-stimulus-intervals(ISI) ranging from

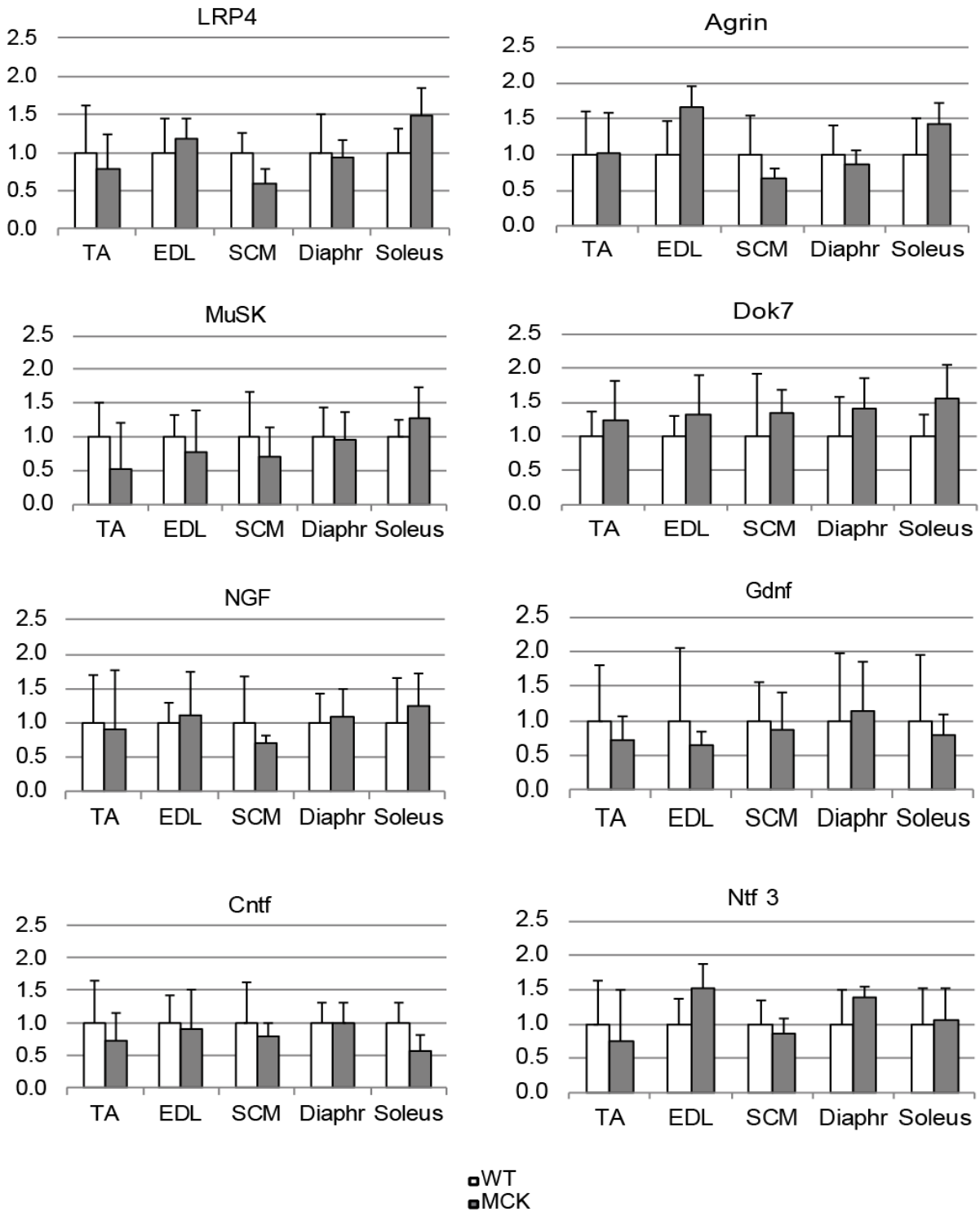
10 to 200 ms and the steady-state depression were measured at the end of the train and normalized to the amplitude of the first EPP. The normalized depression after 10 repetitive stimuli was measured between 0 and 100 Hz. The results are represented as mean ± SEM. The numbers in the bars are n,N: number of fibers, number of mice. T test (*$p<0.05$).



Supplementary Figure 4. Pre-synaptic mitochondrial morphology in soleus muscles. Representative TEM picture illustrating the synaptic vesicle number and the mitochondria structure in the synaptic region of the soleus muscle from WT and MCK mice. Volume density (left panel) and surface density (right panel) in WT (white bars) and MCK mice (black bars). Volume density of mitochondria within the NMJ was calculated according to Weibel et al. using a D64 grid (q^2=16, P_T=64, P'_T=1024) at 8500x magnification. The surface density of mitochondria (*right*) was calculated according to Weibel et al. using a customized D576 Grid (q^2=16, P_T=576, P'_T=9216).at 8500x magnification. Individual NMJ data (N=9-16 per mouse) were averaged per mouse and subsequently between mice at the same genotype (WT=4, MCK=3). Unpaired t test (*p<0.05).



Supplementary Figure 5. **Acetylcholine esterase activity in different muscles.** For each muscle, a kinetic of AChE activity was measured indirectly by detecting the fluorescence emitted at 590 nm for 3600 sec. Each point represents mean ± SEM. (N=3).



Supplementary Figure 6. Level of gene expression in different muscles from WT and MCK mice. Total RNA was reverse transcribed and the level of expression of the genes of interest was determined by real-time PCR, relative to the TATA-Binding Protein (TBP) expression level, analyzed according to the ΔΔCt method. The expression level in the WT samples was set to 1. Each bar represents mean ± SEM. (n=3, t test two tails).

Supplementary Tables

Supplementary Table 1: Muscle resting membrane potential in LAL and TVA muscles from WT and MCK mice.

Vm (mV)	**WT**	**MCK**	**p**
LAL	-66.2 ± 3.6 (18, 3)	-61 ± 1.4 (24, 4)	0.22
TVA	-66.6 ± 2.3 (18, 3)	-68.1 ± 1.4 (17, 3)	0.56

The results are represented as mean ± SEM. The numbers in the parenthesis are n,N: number of fibers, number of mice. T-test.

Supplementary Table 2: Cumulated released vesicles during 100 Hz, 1s train of stimuli in the LAL muscle from WT and MCK mice.

WT	MCK	p
3285 ± 272 vesicles (n, N=17, 3)	2840 ± 141 vesicles (n, N=24, 4)	0.159

The results are represented as mean ± SEM. The numbers in the parenthesis are n,N: number of fibers, number of mice. T-test.

Supplementary Table 3: Size of the Readily-Releasable Pool (RRP) of synaptic vesicles in LAL and TVA muscles from WT or MCK mice.

RRP	WT	MCK	p
LAL	1744 ± 158 (n, N=17, 3)	1750 ± 126 (n, N=24, 4)	0.98
TVA	1583 ± 98 (n, N=15, 3)	1565 ± 160 (n, N=15, 3)	0.93

The results are represented as mean ± SEM. The numbers in the parenthesis are n,N: number of fibers, number of mice. T test.

Supplementary Table 4: Semiquantitative real-time PCR primer list.

Gene Symbol	Reference	Forward primer	Reverse primer
mPpargc1a	NM_008904.2	GTGACATAGAGTGTGCTGCTC	ACTTCAATCCACCCAGAAAG
mTBP	NM_013684.3	ATATAATCCCAAGCGATTTGC	GTCCGTGGCTCTCTTATTCTC
mLrp4	NM_172668	GGCAAAAAGCAGGAACTTGT	TCTACCCAGTGGCCAGAACT
mAgrn	NM_021604.2	GGCCTGGATCCTCAACACTA	AACCTGGGATCCACTTCACA
mMuSK	NM_001037127.2	GAACATCTATTCCGCAGACTACTACAA	TCGGGCGGCATCCA
mDok7	NM_172708.3	CCAGCACCTCCCGCTATG	ACCCCGACGCGATGTG
mGdnf	NM_010275.2	TCTCGAGCAGGTTCGAATGG	AAGAACCGTCGCAAACTTTACC
mNGF	NM_013609.2	TCTGTGTACGGTTCTGCCTG	CAGCTTTCTATACTGGCCGC
mCntf	NM_170786.2	CCATCCACTGAGTCAAGGCT	CTGTAGCCGCTCTATCTGGC
mNtf3	NM_008742.3	GCCACGGAGATAAGCAAGAA	ACGGATGCCATGGTTACTTC