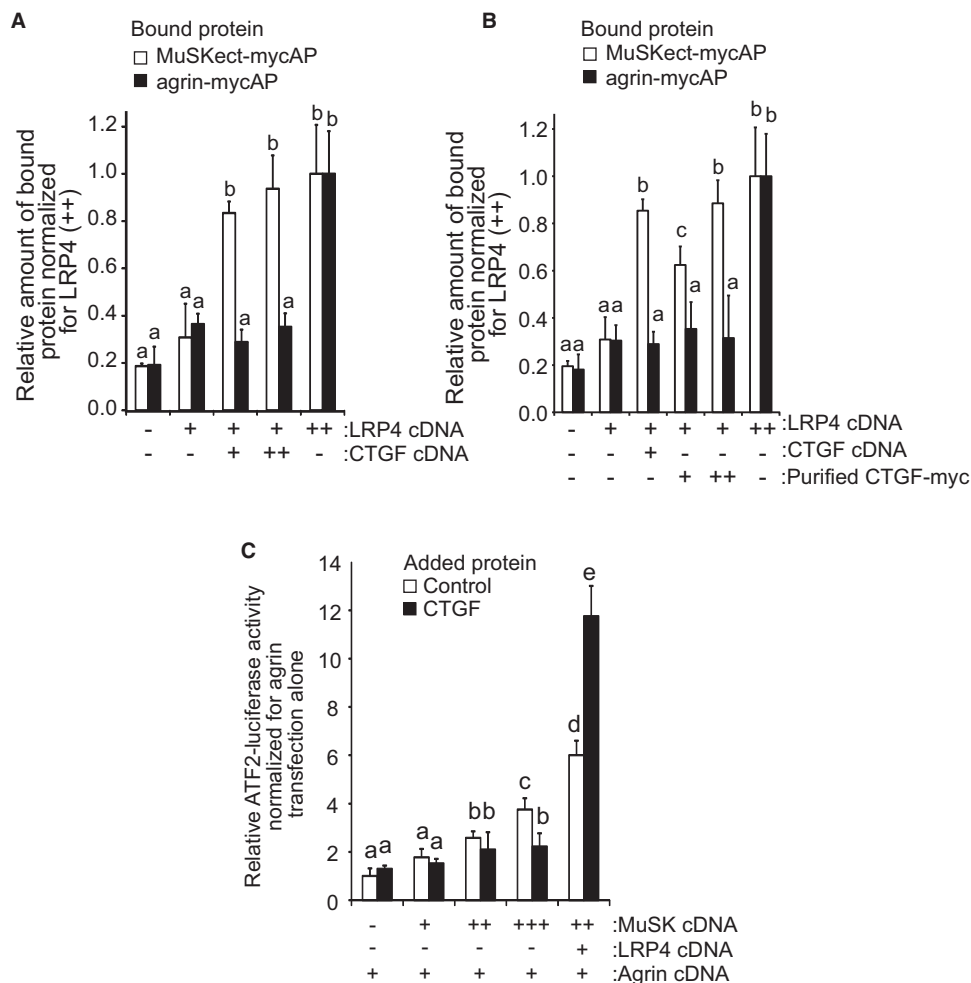


Expanded View Figures

**Figure EV1. CTGF functions with LRP4.**

- A, B Cell surface-binding assays. Variable concentrations of LRP4 cDNA and CTGF cDNA were introduced to HEK293 cells. Purified MuSKect-mycAP (30 nM) or purified agrin-mycAP (30 nM), both of which carried alkaline phosphatase in fusion, was added to the cultured medium. In (B), 30 (+) or 60 (++) nM CTGF was also added as indicated. Bound protein was quantified by measuring alkaline phosphatase activity. CTGF enhanced binding of LRP4 to MuSKect-mycAP but not to Agrin-mycAP. Mean and SD ($n = 9$ wells; 3 wells in 3 independent experiments) are indicated. P -value < 0.05 by two-way repeated measures ANOVA for both (A and B). $P < 0.05$ by *post hoc* Tukey test is indicated by a single letter representing each group.
- C ATF-2 Luciferase assay. HEK293 cells were transfected with 1 (+), 2 (++) or 5 (+++) ng pExpress-1-Musk encoding full-length MuSK, 5 ng pHLRP4 encoding full-length LRP4, 5 ng Agrin/pAptag-5 encoding partial agrin retaining AChR clustering activity and 5 ng ATF-2 Luciferase reporter to monitor MuSK phosphorylation. The cells were then treated with 250 pM BSA (Control) or CTGF for 24 h. Purified recombinant CTGF enhanced MuSK activation in the presence of LRP4. Mean and SD ($n = 9$ wells; 3 wells in 3 independent experiments) are indicated. P -value < 0.05 by two-way repeated measures ANOVA. $P < 0.05$ by *post hoc* Tukey test is indicated by a single letter representing each group.

Source data are available online for this figure.

Figure EV2. CTGF enhances agrin- and LRP4-mediated AChR clustering in C2C12 myotubes.

- A, B C2C12 myotubes were infected with lentivirus expressing shControl, shCtgf-1, or shCtgf-2. Doxycycline was added for 2 days to induce shRNA expression. (A) Quantitative RT-PCR. (B) Immunoblotting. Mean and SD ($n = 3$ independent experiments) are indicated. P -value < 0.05 by two-way repeated measures ANOVA for both (A and B). $P < 0.05$ by *post hoc* Tukey test is indicated by a single letter representing each group.
- C, D C2C12 myotubes were infected with GFP-expressing lentivirus carrying shControl or shCtgf-1. Doxycycline was added for 2 days to induce shRNA expression. C2C12 myotubes were treated with indicated concentrations of purified agrin-mycAP and/or purified LRP4ect-Flag for 3 h. (C) Total MuSK was immunoprecipitated (IP) with anti-MuSK antibody, and phosphorylated MuSK was immunoblotted with anti-phosphotyrosine (p-Tyr) antibody. Quantification of immunoblots is shown in the right panel. *Ctgf* knockdown decreased MuSK phosphorylation, which was rescued by adding LRP4ect-Flag. (D) AChRs were visualized with Alexa 594-conjugated α -bungarotoxin (red signals) in the infected myotube (green GFP signals). AChR cluster with an axis length of 4 μm or more (arrowheads) was recognized and measured by the MetaMorph software. Analysis was performed in a blinded manner. *Ctgf* knockdown decreased the number and the length of AChR clusters, which was partially rescued by adding LRP4ect-Flag. Scale bar = 20 μm . Mean and SD are indicated in the right panels ($n = 60$ –75 myotubes in 3 independent experiments). P -value < 0.05 by one-way ANOVA for the three panels in (C and D). $P < 0.05$ by *post hoc* Tukey test is indicated by a single letter representing each group.

Source data are available online for this figure.

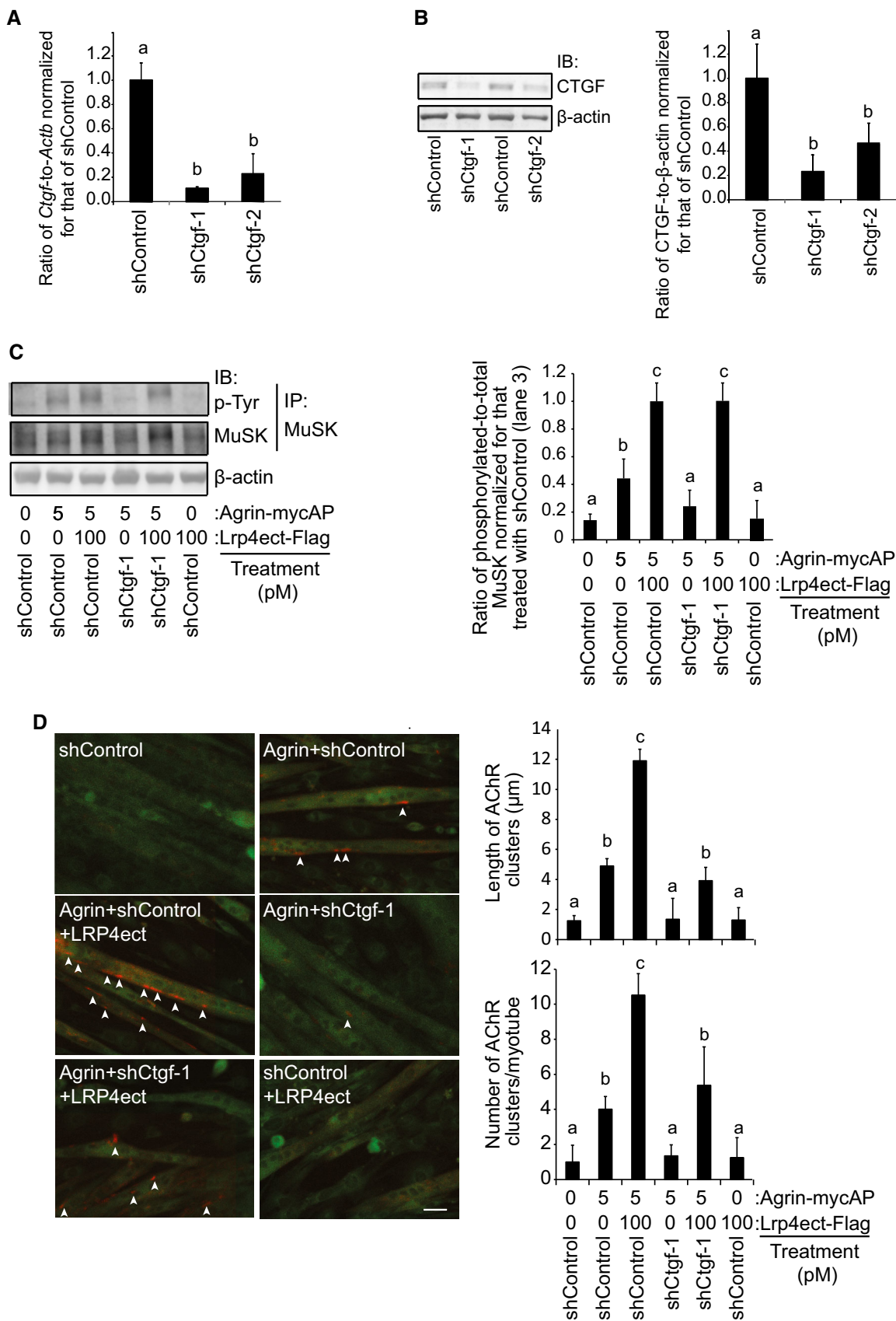


Figure EV2.

Figure EV3. *Ctgf*^{-/-} diaphragm shows no significant differences in myogenic gene expressions, myofibril ultrastructure, width of the AChR band, or axonal branches of the phrenic nerves.

- A Quantitative RT-PCR of myogenic genes (*Myod* and *Myh1*) and genes expressed at the NMJs (*MuSK* and *Lrp4*) in the mouse embryonic diaphragm. Mean and SD ($n = 6$ mice) are indicated. P -value > 0.05 by two-way repeated measures ANOVA. Lack of statistical difference is indicated by a single letter "a."
- B Representative electron micrographs of muscle fibers in the diaphragm of wild-type and *Ctgf*^{-/-} mice at embryonic day (E) 18.5. Scale bar = 1 μ m.
- C-F Representative low (C) and high (D) magnification images of the whole-mount left diaphragms at E18.5 stained for peripherin (anti-peripherin antibody, upper panels) and AChR (α -bungarotoxin, lower panels). Scale bar = 1 mm. (E) Blinded morphometry of the endplate bandwidth of AChR clusters in (C). (F) Blinded morphometry of the 2nd and 3rd branches in (C). Mean and SD ($n = 6$ left diaphragms) are indicated. P -value > 0.05 by one-way ANOVA for the three panels in (E and F). Lack of statistical difference is indicated by a single letter "a."

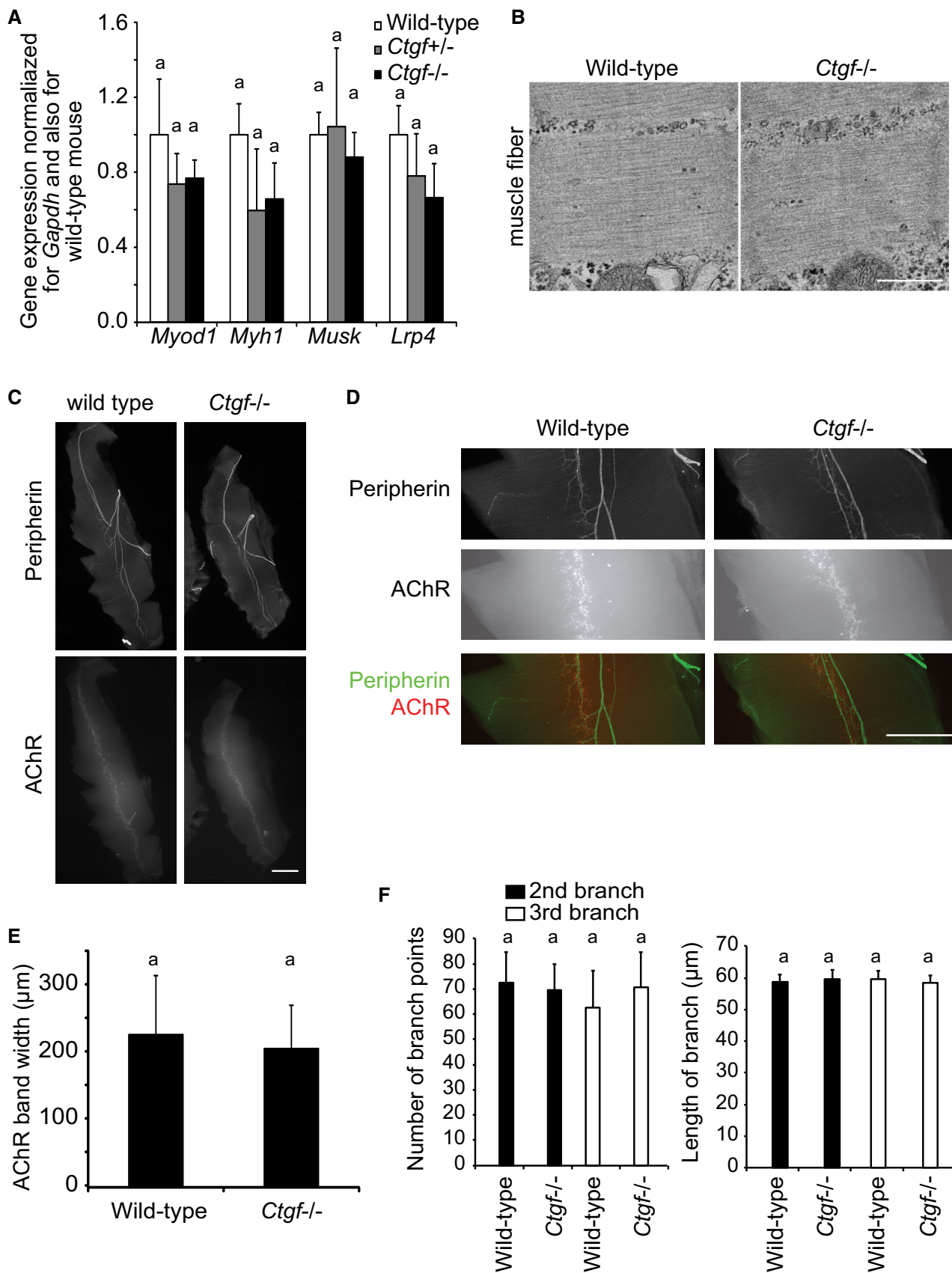


Figure EV3.

Figure EV4. *Ctgf*^{-/-} diaphragm shows no significant differences in number of Schwann cells at the NMJ.

- A–C Representative low (A) and high (B) magnification images of the whole-mount left diaphragms at E18.5 stained for S100 β (green) and AChR (α -bungarotoxin, red). (C) Blinded morphometry to examine the ratio of AChR signals adjacent to S100 β signals. Mean and SD ($n = 30$ NMJs in 5 left diaphragms) are indicated. Scale bar = 20 μ m (A) and 2 μ m (B). P -value > 0.05 by Student's t -test. Lack of statistical difference is indicated by a single letter "a."
- D Representative electron micrographs of the NMJs in the diaphragm of wild-type and *Ctgf*^{-/-} mice at embryonic day (E) 18.5. Nerve terminal (red) is juxtaposed with Schwann cells (yellow). Scale bar = 1 μ m.

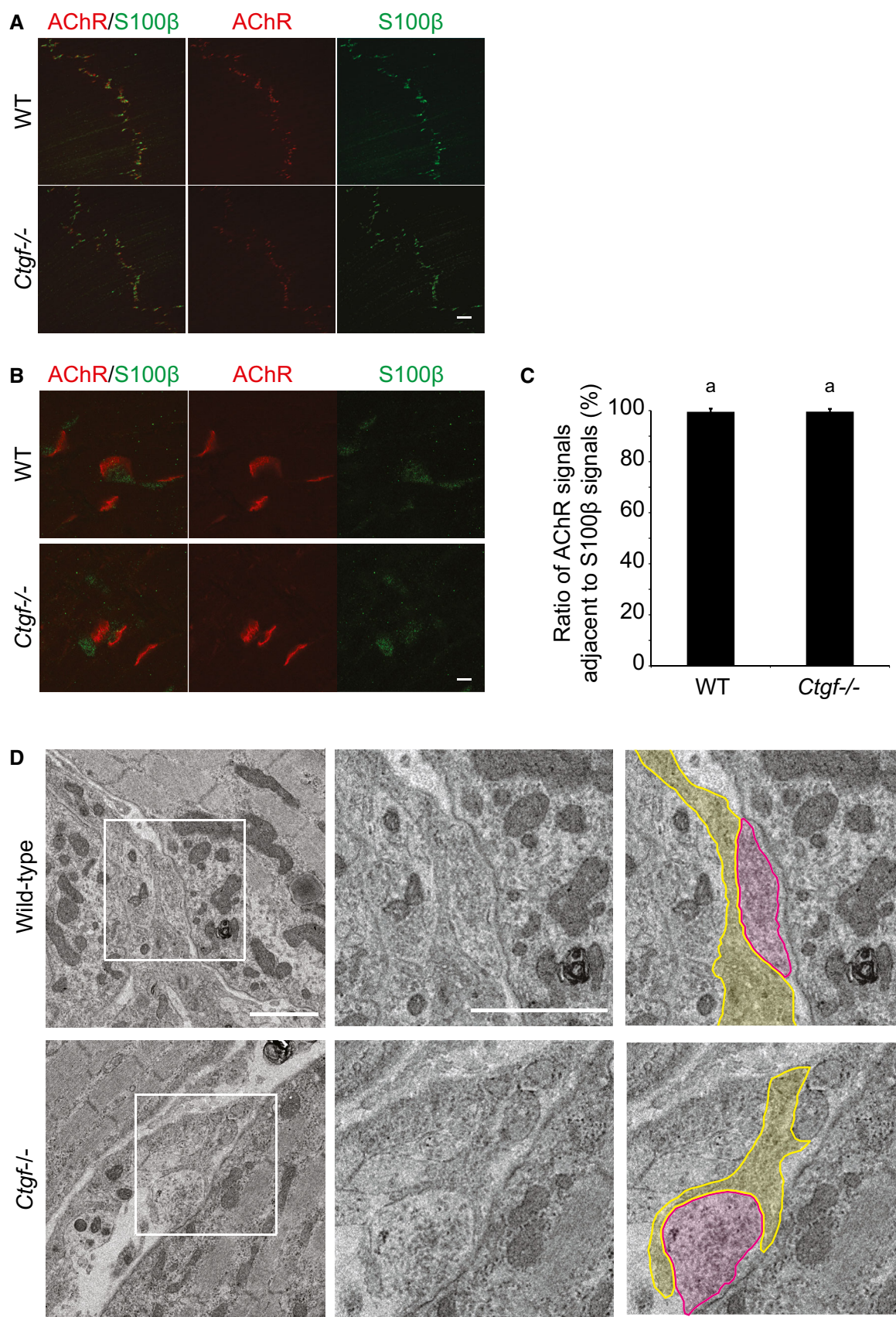


Figure EV4.

Figure EV5. *Ctgf*^{-/-} diaphragm shows mild to moderate electrophysiological abnormalities and different expression patterns for CCN family genes.

- A Representative low magnification images of the whole-mount left diaphragms at E18.5 stained for synaptophysin (green) and AChR (α -bungarotoxin, red). Confocal images of the NMJ are indicated in Fig 5B. Scale bar = 20 μ m.
- B Representative raw MEPP traces for 7 s of wild-type and *Ctgf*^{-/-} mice at E18.5.
- C Representative averaged MEPP recordings of wild-type and *Ctgf*^{-/-} mice at E18.5.
- D Representative CMAP traces of wild-type and *Ctgf*^{-/-} mice at E18.5 in response to repetitive nerve stimulation at 2 Hz. Quantifications of MEPP and CMAP recordings are indicated in Table 2.
- E, F Quantitative RT-PCR of the CCN family genes, *Ccn1-6*, in the mouse diaphragm at E13.5 and E18.5. (F) Expressions of *Ccn1-3* are indicated by additional normalization for the mean value of wild-type mice. Note that *Ccn3* is highly expressed in E13.5 *Ctgf*^{-/-} embryos. Mean and SD are indicated ($n = 9$ including 3 independent experiments for 3 diaphragm). P -value < 0.05 by two-way repeated measures ANOVA for both (E and F). $P < 0.05$ by *post hoc* Tukey test is indicated by a single letter representing each group.

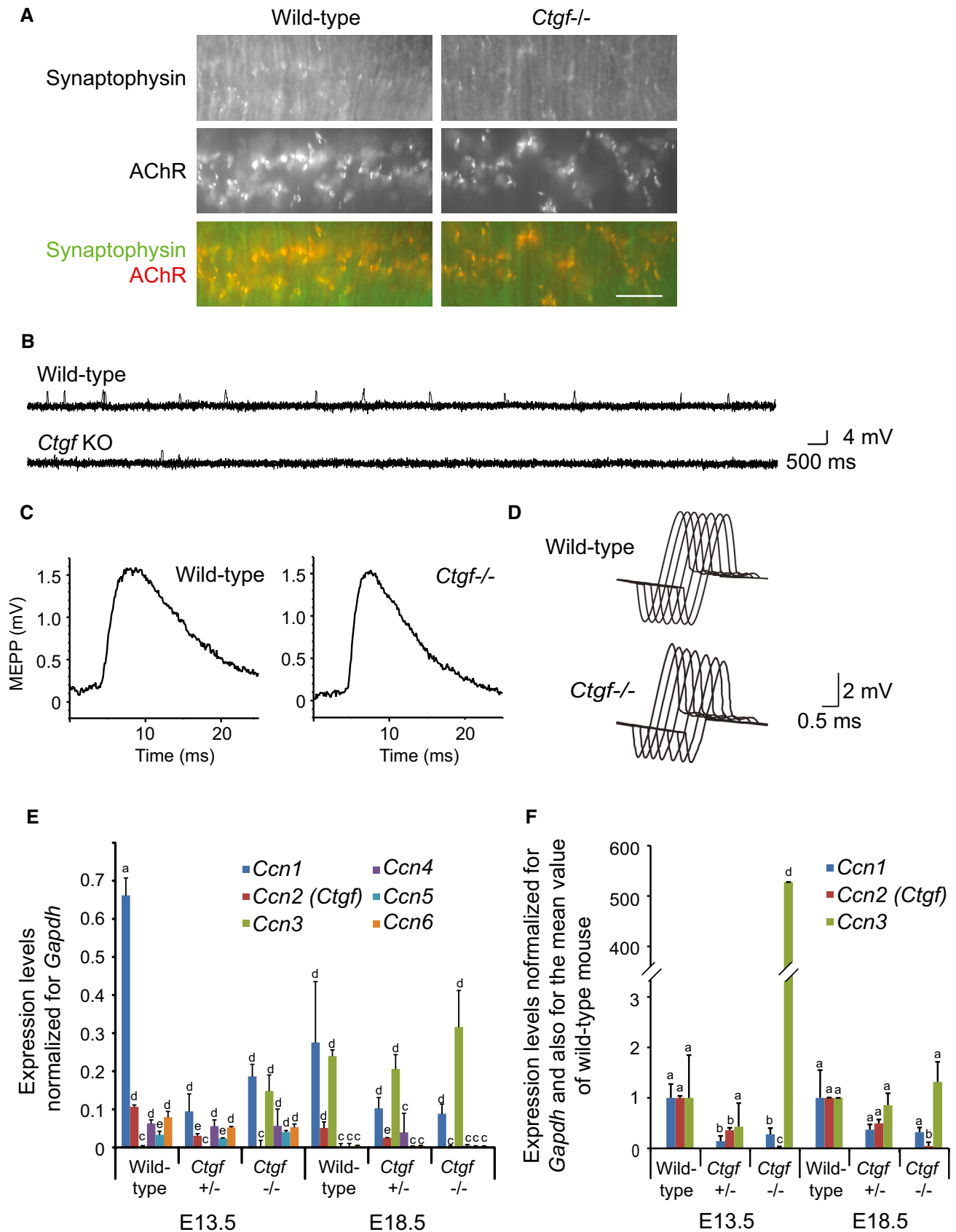


Figure EV5.