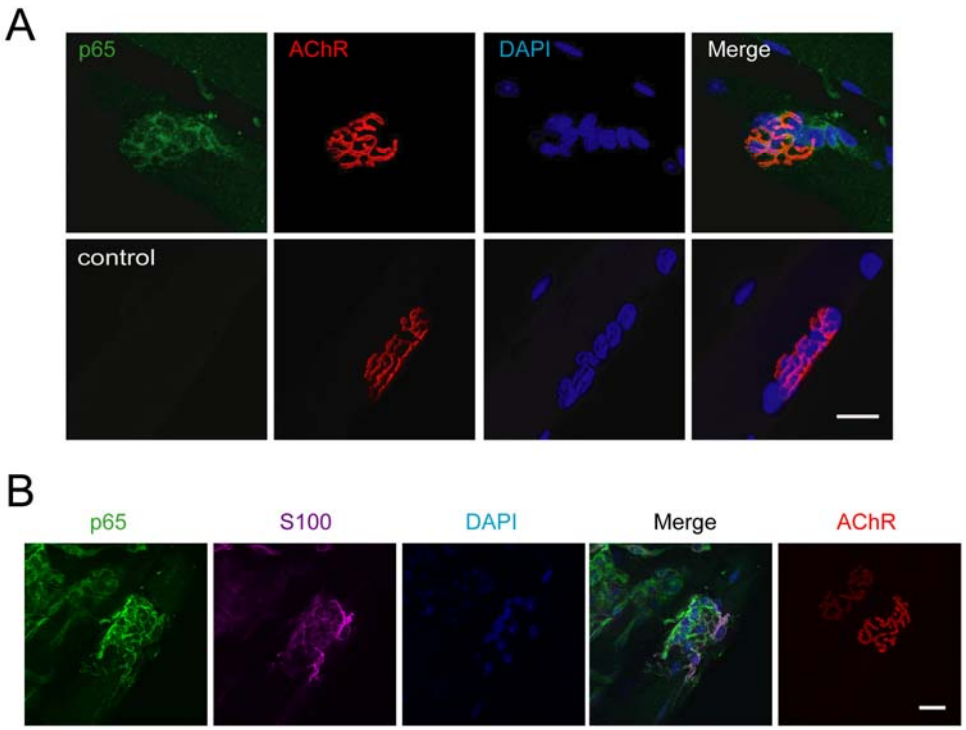


Fig. S1

Supplemental Figure S1. Localization of RelA/p65 at the NMJ. ***A,*** Teased soleus muscles from adult mice were stained with R-BTX and anti-RelA/p65 antibody. DAPI was used to mark nuclear. Rabbit IgG was used as a negative control. Scale bar, 20 μm. ***B,*** Soleus muscles from adult mice were co-stained with RelA/p65 (rabbit antibody), R-BTX, and Schwann cell marker S100 (mouse antibody). Alexa Fluor® 488 goat anti-Rabbit IgG and Alexa Fluor® 647 goat anti-mouse IgG were used as second antibodies. Scale bar, 20 μm.

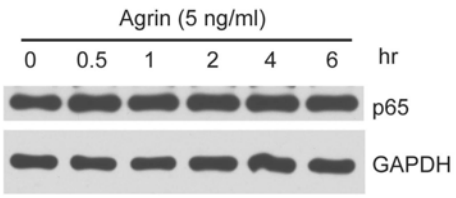
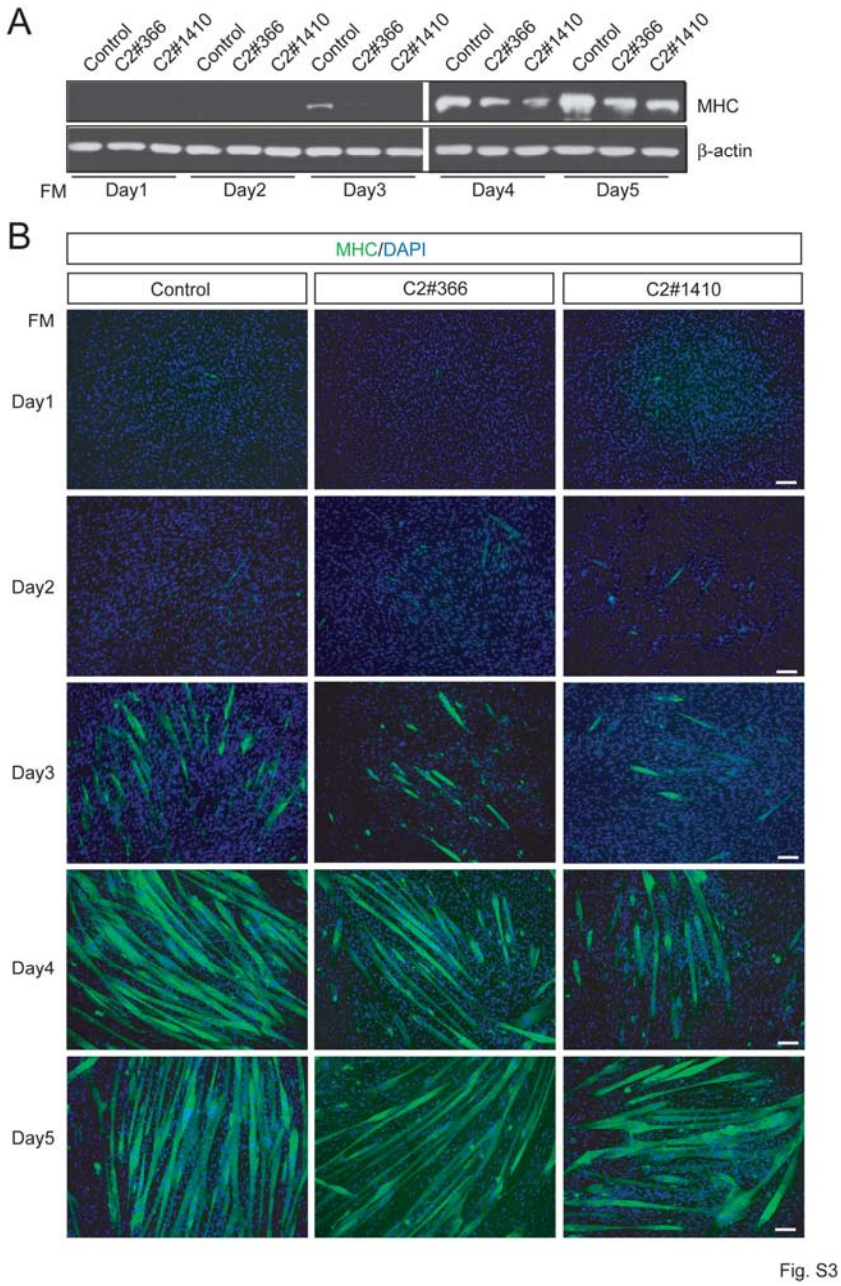


Fig. S2

Supplemental Figure S2. Agrin has no effect on RelA/p65 expression. Fully differentiated C2C12 myotubes were treated with Agrin (5 ng/ml) for indicated time, and cell lysates (10 μg proteins) were subjected to IB with indicated antibodies.



Supplemental Figure S3. Myotube formation in Rel A/P65 knock-down cells. Stable cell lines (C2#366 and #1410) cultured in growth media were switched to fusion media at 50% confluence for different days (Day1 to 5). During this period, cells were lysed and subjected to western blot with anti-MHC antibody, using β-actin as loading control (***A***) or stained with MHC and DAPI to examine myotube formation (***B***). Note that at day 5, normal myotubes were formed in all three groups. Scale bar, 100 μm.

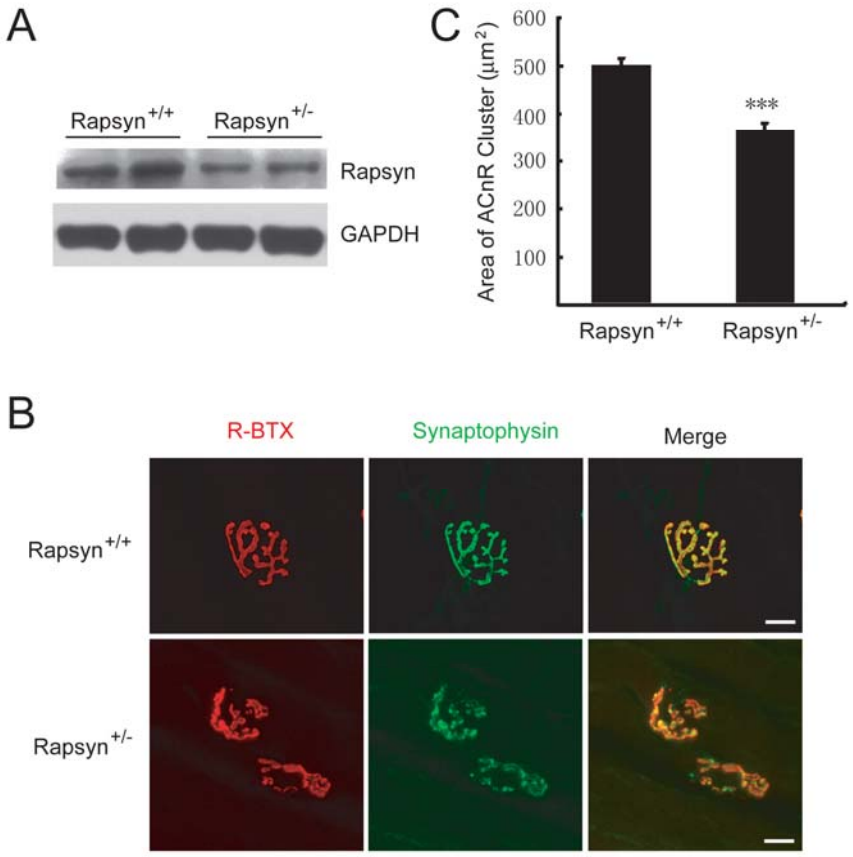


Fig. S4

Supplemental Figure S4. Aberrant AChR clustering in adult Rapsyn$^{+/-}$ mutant mice. ***A,*** Muscle homogenates (20 μg protein) of Rapsyn$^{+/-}$ or littermate control mice at P60 were subjected to IB with indicated antibodies. Note that Rapsyn was down-regulated in Rapsyn$^{+/-}$ mice. ***B,*** Sternomastoid muscles of Rapsyn$^{+/-}$ or littermate controls (Rapsyn$^{+/+}$) at P60 were stained with R-BTX and anti-Synaptophysin antibody. Shown are representative images. Scale bar, 20 μm. ***C***, Quantification for the average area of individual NMJs (n=35 for Rapsyn$^{+/+}$; n=25 for Rapsyn$^{+/-}$). ***p<0.001, Student's t test.