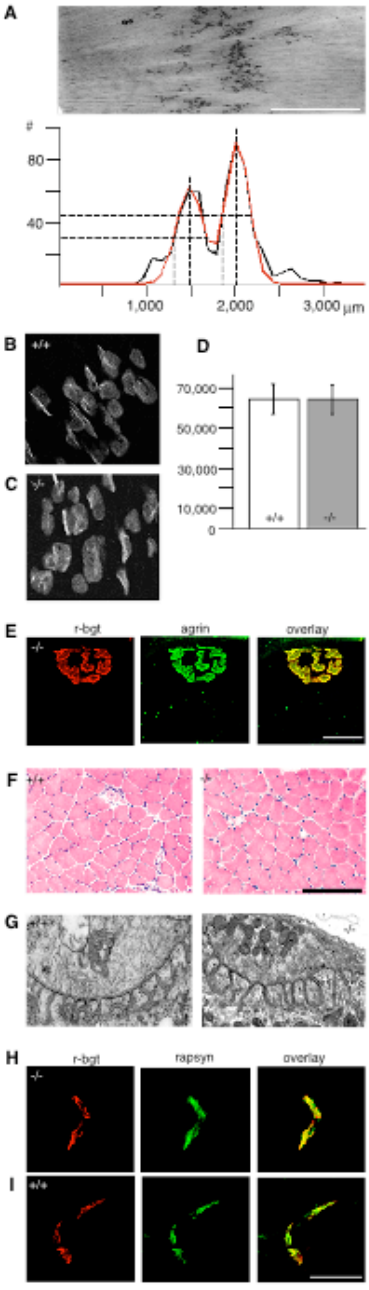
Supplementary information



(A) In segment 4 of one mutant diaphragm muscle at P6, endplate distribution appeared in shape of a double peak fitted by the sum of two Gaussians (red) their means were separated by 519 µm (Values±SD; peak A_1=62.0±4.33 µm, A_2=91.87±6.42 µm; X_{01}=1504±12.1, X_{02}=2023.2±7.66; width at half-maximal level =203.81±18.3, and =176.44±11.4). The appearance of multiple peaks might reflect increased branching of the phrenic nerve. (# = number of synapses/100 µm). Calibration bar 1000 µm. The location of endplates in the diaphragm was measured in AChE stained muscle as the distance from the tendinous muscle insertion using IP lab (Scanalytics). Data were copied to Excel and the distribution was calculated using Igor (WaveMetrics). (B,C) Quantitative fluorescence measurements of subsynaptic AChR densities at 6 days of age (P6) in wildtype (B, +/+) and mutant (C, -/-) diaphragm muscle stained with Rhodamine-labelled α-Bungarotoxin. (D) 228 control (+/+) and 226 mutant (-/-) postsynapses where measured using Image J. Mean gray value per pixel of wildtype (white column; +/+): 64.543±15.343 (Values ±SD) and mutant (grey column; -/-): 64.631±14.869. (& = mean gray value per pixel). (E) Comparison of mutant (-/-) and wildtype (+/+) endplates revealed no differences in secondary postsynaptic folds. (F, G) Rapsyn (green), shown to be critical for AChR clustering is unaffected and protein levels appeared comparable in cross sections through soleus muscle of $AChR\gamma^{(\varepsilon/\varepsilon)}$ (-/-) (F) and control (+/+) animals (G). Rhodamine-labelled α-Bungarotoxin (r-bgt, red). Calibration bar 20 µm.

(H) Double staining with r-bgt (red) and antibodies directed against agrin (green) revealed no altered appearance of agrin in $AChR\gamma^{(\varepsilon/\varepsilon)}$ mutants. Calibration bar 20 µm.

(I) Muscle morphology of mutant mice, analysed by cross sections of $AChR\gamma^{(\varepsilon/\varepsilon)}$ (-/-) and control (+/+) soleus muscle stained with Hematoxylin/Eosin at P70, displayed no differences in size, density and number of fibres. Calibration bar 500 µm.