Materials and Methods

Rodent colonies and genotyping

*Reeler* mice (B6C3Fe-a/a-Rehrl+/+) were obtained from The Jackson Laboratory and genotyped as described (1) except that *Reln* primers were: forward, TAATCTGTTCCTCCTCTCGCC, reverse wild type, ACAGTTGACATACCTTAATC and reverse reeler, TGCATTAATGTGCAGTGTTG. Dab1 knock out mice (2) and double VLDLR/ApoER2 knock out mice (3) were genotyped as described. Sprague-Dawley rats were obtained from Harlan.

In situ hybridization

Mice were perfused with 4 % paraformaldehyde in 0.1 M PBS. Tissues were sectioned using a cryostat at 20 µm thickness. Digoxigenin-labeled riboprobes were synthesized using T7 or T3 RNA polymerase and a labeling kit (Roche Biochemicals). *reelin* riboprobes correspond to nucleotides 5269-5980 of the GenBank sequence U24703. For Dab1 riboprobes, sense and antisense templates were generated by RT-PCR using the following primers with or without a T7 promoter tag: GGATCCTAATACGACTCAGTTAGGAGACGAGTCTGGGAGGCC (sense) and GGATCCTAATACGACTCAGTTAGGAGACGAGTCTGGGAGGCC (antisense). The hybridization was carried out according to the manufacturer’s instructions.

Immunofluorescence

Immunofluorescence was performed using 20 µm spinal cord or muscle sections. Anti-Reln mouse monoclonal E4 (4) or CR50 (5) antibodies were purified from hybridoma cell lines and diluted in PBS containing 0.3 %Triton X-100 and 3 % horse serum (Vector Laboratories). Goat polyclonal antibodies directed against the C terminus of Dab1 were used as described (6). The following commercial antibodies were used: anti-choline acetyltransferase (Chemicon, 1:1,000), anti-β-synuclein (Chemicon; 1:1000), anti-neurofilament 200 (NF, Sigma, 1:150) or SM312 (Sternberger Monoclonals), anti S-100 (Sigma; 1:160). The sections were blocked in 10 % horse serum, 0.1 % Triton X-100 in PBS and then incubated overnight at 4 °C with the primary antibodies, followed by a 30-60 min incubation with Alexa 488- or Alexa 594-conjugated secondary antibodies (Molecular Probes). To label motor end plates, TRITC-α-bungarotoxin (Molecular Probes; 2 µg/ml) was used. Sections were mounted using Vectastain (Vector Laboratories). Brightfield and epifluorescence images were acquired using a Nikon E800 microscope using a CoolSNAPfx digital camera and MetaView Imaging software (Universal Imaging). Confocal images were acquired using a FluoView FV300 confocal laser-scanning microscope (Olympus). Images stacks of double-labeled sections were acquired in the z plane, overlaid and rotated to determine co-localization.

Electron Microscopy

Diaphragm muscles from postnatal day 30 mice were fixed in 4 % paraformaldehyde-PBS and incubated with TRITC-α-bungarotoxin to identify the end plates. The tissue was incubated with 0.2 % osmium tetroxide pH 7.2, dehydrated, treated propylene oxide and transferred in block mold in Spurr’s resin at 60 °C. 60 nm sections were cut and mounted on copper grids. To avoid repeated analysis of the same NMJ, ultra-thin sections cut every 20 nm were analyzed using a
JEOL 100C TEMSCAN electron microscope. Micrographs were developed using Kodak 4489 Electron Microscope film.

**Western blot analysis**

Muscle tissue was homogenized in 50 mM Tris pH 7.5, 150 mM NaCl and 1 % Triton X-100, and clarified by centrifugation at 10,000 g for 30 min at 4 °C. Samples were analyzed by SDS-PAGE using 4-12 % gels and transferred to nitrocellulose. Blots were blocked in TBS containing 0.1% Tween and 3 % dry milk, and incubated with antibodies against laminin (Sigma, 1:1000), synaptophysin (Sigma, 1:5000), α--dystroglycan (Upstate Biotechnologies, 1:500) and β-actin (Chemicon, 1:1000). Following incubation with HRP-conjugated secondary antibodies, blots were developed by chemiluminescence (ECL Plus, Amersham Pharmacia).

**Data collection and analysis**

The numbers of synapses and synaptic areas were determined on serial sections from hind limb or from whole-mount diaphragm, stained with TRITC-α-bungarotoxin, using fluorescence microscopy. The percentage of multiply innervated NMJs was determined by analysing similar sections by confocal microscopy Ultrastructural analysis was performed using the MetaVue software (Universal Imaging). Statistical analysis was performed using Student t test.

**Intracellular recordings of end-plate potentials**

Mice were anesthetized, and the extensor digitorum longus muscle transferred to a chamber perfused with oxygenated (95% O2/5% CO2) saline containing (in mmol/L): NaCl 145, KCl 2, MgSO4 5, CaCl2 0.5, NaHEPES 10, glucose 10 (pH 7.1, 19-21°C). The muscle nerve was stimulated using bipolar platinum electrodes connected to a stimulus isolation unit (WPI) triggered by pClamp software at low frequency (≤ 0.5/sec) to minimize stimulation-induced changes in release. Signals from muscle fibers impaled with KCl-filled micropipettes (15-40 MΩ) were amplified (Axoclamp-2A), and digitized at 30 µs for analysis. Fibers with resting membrane potential below -70 mV, stable EPP morphology with rise time < 1 ms, and low stimulation thresholds for evoking EPPs (typically ≤ 2 mA, allowing for supramaximal stimulation at 3-5X threshold) were selected for analysis.

**Methods References**

**Supplementary Figure 1**

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**Supplementary Fig. 1.** A and B, spinal cord (ventral horn) sections showing Reln expression in motor neurons. C and D, muscle sections of wild type (C) or reeler (D) mice labeled with nerve marker NF. E and F, muscle sections of wild type mice hybridized with reelin antisense (E) or sense (F) probes. G, non-permeabilized muscle tissue stained for extracellular Reln. H, NMJ labeled with nerve and Schwann cell markers. I-K, NMJ labeled with Reln and a Schwann cell marker. Arrow indicates lack of colocalization. Bars =50 µm (A and B), 100 µm (C-F) and 10 µm (G-K).
Supplementary Figure 2
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**Supplementary Fig.2.** In situ hybridization of a cerebellum (A) or a lumbar spinal cord section (B) with a Dab1 antisense probe. Arrow indicates expression in Purkinje cells. C, Overlay image of a NMJ from a Dab1 knock out mouse section demonstrating specificity of the Dab1 antibody. Bars =100 µm (A) and 10 µm (B-D).
Supplementary Figure 3
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Supplementary Fig. 3. A-C, brightfield images of muscle fibers. D-H, confocal images of NMJs in muscle sections of adult wild type (wt), reeler, or Dab1 knock out mice stained with TRITC-α-bungarotoxin. Bars= 100 μm (A-C) and 10 μm (D-H).
Supplementary Figure 4
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**Supplementary Fig 4.** Muscle sections from wild type (A), *reeler* (B), Dab1 knock out (C) or VLDLR/ApoER2 knock out (D) mice. Arrows show multiple axons innervating the same NMJ in *reeler*. Bars = 10 µm.