Supporting Information Figure Legends

Supporting Information Fig. 1. Malin KO bodies are MGS- and PAS-positive (Skeletal Muscle and Heart). Presence of MGS- and PAS-positive inclusions in skeletal muscle and heart of 11-month-old WT mice, and of 4- and 11-month-old malin KO mice. Scale bar 100μm. 4x = 4-fold magnification.

Supporting Information Figure 2. Generation and validation of the malin KO mouse. (**A**) Schematic representation of the targeted disruption. Arrows indicate the position of the primers used for genotyping. (**B**) 5'-end and 3'-end genotyping. Bands corresponding to the WT and the KO allele are indicated. (**C**) RT-PCR confirming the absence of malin mRNA in the KO.

Supporting Information Figure 3. Analysis of GP and glycogenin in the insoluble fraction. Brain extracts from 11-month-old wild-type (WT) and malin knock-out (KO) mice were analyzed. Soluble and insoluble fractions resulting from low speed centrifugation were used for the biochemical analysis. (**A**) Western blotting for muscle glycogen phosphorylase (MGP) and brain glycogen phosphorylase (BGP). (**B**) Western blotting for glycogenin in amylase-treated insoluble fraction. Actin was used as loading control.

Supporting Information Figure 4. Gliosis in malin KO hippocampus. Representative images of 11-months WT and KO hippocampus are shown. Antibodies were used against GFAP (green) and polyglucosan (red). Hoechst (blue) was used for nuclear staining. Scale bar 100μm.

Supporting Information Figure 5. Open field test. WT and KO mice were tested for open field activity for 30 min. Percentage of time spent in the center of the arena, distance run and number of rearings were scored. Data are expressed as mean ± SEM. *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$. WT (n=9), KO (n=9).

Supporting Information Methods

Open Field Test. A square open field arena with an area of 40cm×40 cm and walls 30 cm high was used. A mouse was placed in the center and allowed to move freely for 30 min while being recorded by a video camera mounted above the open field. The recordings were scored later by a motion-recognition software that detects and analyzes mouse movements (Smart Junior, Panlab). At the end of each trial the surface of the arena was cleaned with 90% ethanol. A square central area accounting for 16% of the total area was defined as 'center'.

Operant conditioning procedures. Following early descriptions from some of us (Gottlieb et al., 2006; Madronal et al., 2010)**,** training took place in Skinner box modules measuring 12.5×13.5×18.5 cm (MED Associates, St. Albans, VT, USA). Each Skinner box was housed within a sound-attenuating chamber, which was constantly illuminated (19 W lamp) and exposed to a 45 dB white noise (Cibertec, S.A., Madrid, Spain). Each Skinner box was equipped with a food dispenser from which pellets (Noyes formula P; 45 mg; Sandown Scientific, Hampton, UK) could be delivered by pressing a lever. Before training, mice were handled daily for 7 days and food-deprived to 80% of their free-feeding weight. For operant conditioning, animals were trained to press the lever to receive pellets from the feeder using a fixed-ratio (1:1) schedule. Sessions lasted for 20 min. Animals were maintained on this 1:1 schedule until they reached the selected criterion, namely when they obtained ≥ 20 pellets/session for two successive sessions. Conditioning programs, lever presses, and delivered reinforcements were monitored and recorded by a computer, using a MED-PC program (MED Associates, St. Albans, VT, USA).

Input/output curves, paired pulse facilitation and long-term potentiation (LTP) in behaving mice. Animals were prepared following procedures described elsewhere (Gruart et al., 2006). Under deep anesthesia (ketamine, 35 mg/kg and xylazine, 2 mg/kg, i.p.) mice were implanted with bipolar electrodes aimed at the right Schaffer collateral-commissural pathway of the dorsal hippocampus (2 mm lateral and 1.5 mm posterior to Bregma; depth from brain surface, 1.0-1.5 mm; Paxinos and Franklin, 2001) and with two recording electrodes aimed at the ipsilateral CA1 area (1.2 mm lateral and 2.2 mm posterior to Bregma; depth from brain surface, 1.0-1.5 mm). Electrodes were made from 50 μm, Teflon-coated, tungsten wire (Advent Research, Eynsham, UK). The final location of the recording electrode in the CA1 area was determined according to the field potential depth profile evoked by single pulses presented to the Schaffer collateral pathway (Gruart et al., 2006). A bare silver wire was affixed to the bone as ground. Implanted wires were soldered to a six-pin socket (RS Amidata, Madrid, Spain), which was fixed to the skull with dental cement (see (Gruart et al., 2006) for details).

For input/output curves, mice were stimulated at the Schaffer collaterals with paired pulses (40 ms of inter-stimulus interval) at increasing intensities (0.02-0.4 mA). We also checked the effects of paired pulses at a range of (10, 20, 40, 100, 200, and 500 ms) inter-stimulus intervals when using intensities corresponding to 40% and 60% of the amount required to evoke a saturating response. In all the cases, the pair of pulses of a given intensity was repeated ≥ 5 times with time intervals ≥ 30 s, to avoid as much as possible interferences with slower short-term potentiation (augmentation) or depression processes (Zucker & Regehr, 2002). Moreover, to avoid any cumulative effect, intensities and intervals were presented at random.

To evoke LTP in behaving mice, we followed procedures described previously (Gruart et al., 2006). Field EPSP baseline values were collected 15 min prior to LTP induction using single 100 μs, square, biphasic pulses. Pulse intensity was set at 30–40% of the amount required to evoke a maximum fEPSP response (0.15–0.25 mA) ─ i.e., well below the threshold for evoking a

population spike. For LTP induction, animals were presented with a high-frequency stimulation (HFS) session consisting of five 200 Hz, 100 ms trains of pulses at a rate of 1/s repeated six times, at intervals of 1 min. Thus, a total of 600 pulses were presented during the HFS session. In order to avoid evoking large population spikes and/or the appearance of EEG seizures, the stimulus intensity during HFS was set at the same as that used for generating baseline recordings. After each HFS session, the same single stimuli were presented every 20 s for 60 additional min and for 30 min the followings five days.

Electroencephalographic (EEG) recordings. EEG recordings were carried out with the awake animal placed in a small (5 × 5 × 5 cm) box, to avoid over walking movements. Recordings were carried out for 5 min. The power spectrum of the hippocampal EEG activity was computed with Mat Lab 7.4.0 software (MathWorks, Natick, MA, USA), using the fast Fourier transform with a Hanning window, expressed as relative power and averaged across each recording session (Munera et al., 2000).

Kainate injection and recording of seizure activities. To study the propensity of WT and KO mice to generate convulsive seizures, animals were injected (i.p.) with the AMPA/kainate receptor agonist kainic acid (8 mg/kg; Sigma, Saint Louis, Missouri, USA) dissolved in 0,1 M phosphate buffered saline (PBS) pH = 7.4. The electrocorticographic activity of the hippocampal pyramidal CA1 area was recorded for 2 h after the injection. Injected animals were presented with a stimulus session (five 200 Hz, 100 ms trains of pulses at a rate of 1/s) 1 h after the injection (see Rangel et al., 2009 for details).

Supporting Information References

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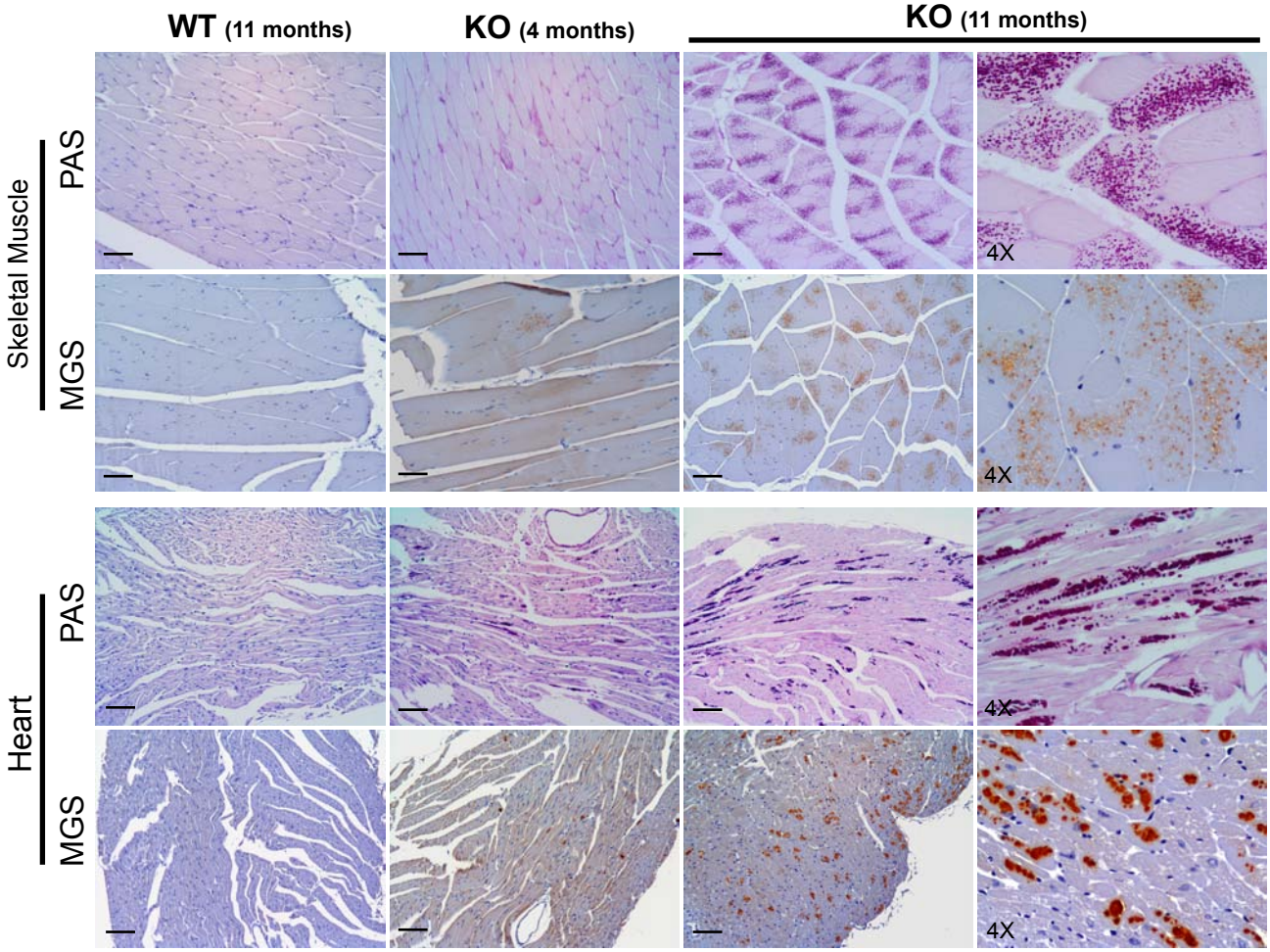
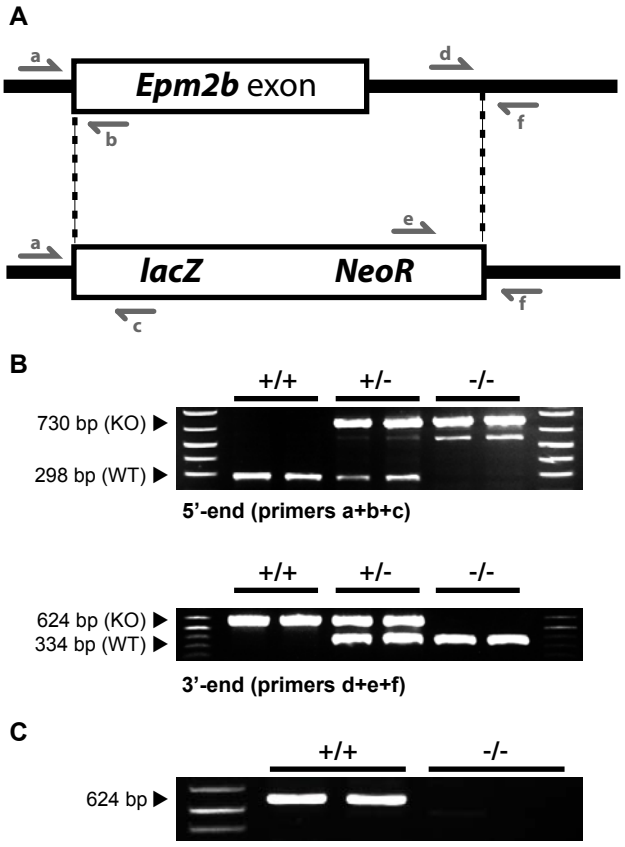
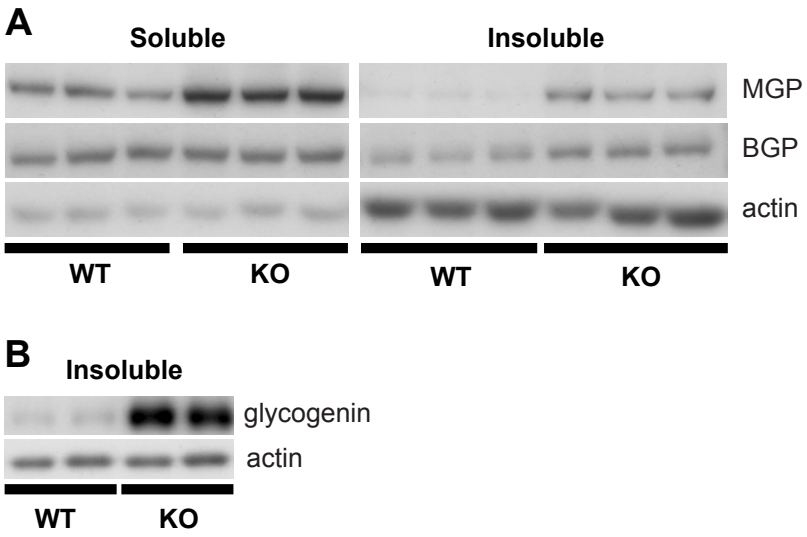
Supporting Information Figure 1



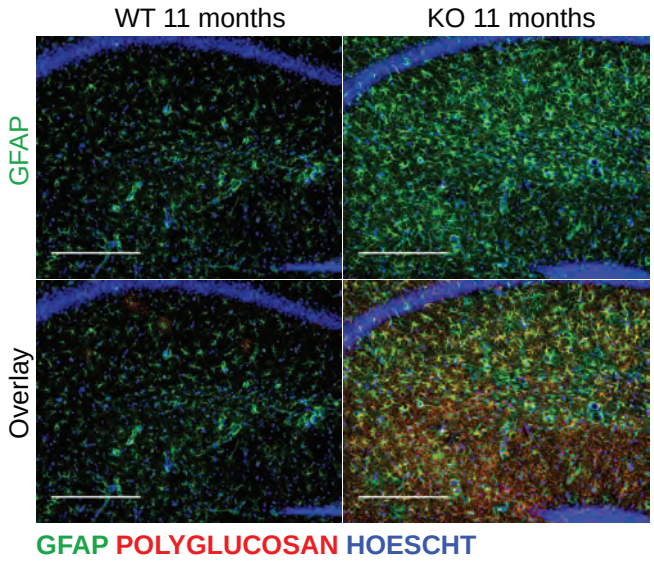
Supporting Information Figure 2



Supporting Information Figure 3



Supporting Information Figure 4



Supporting Information Figure 5