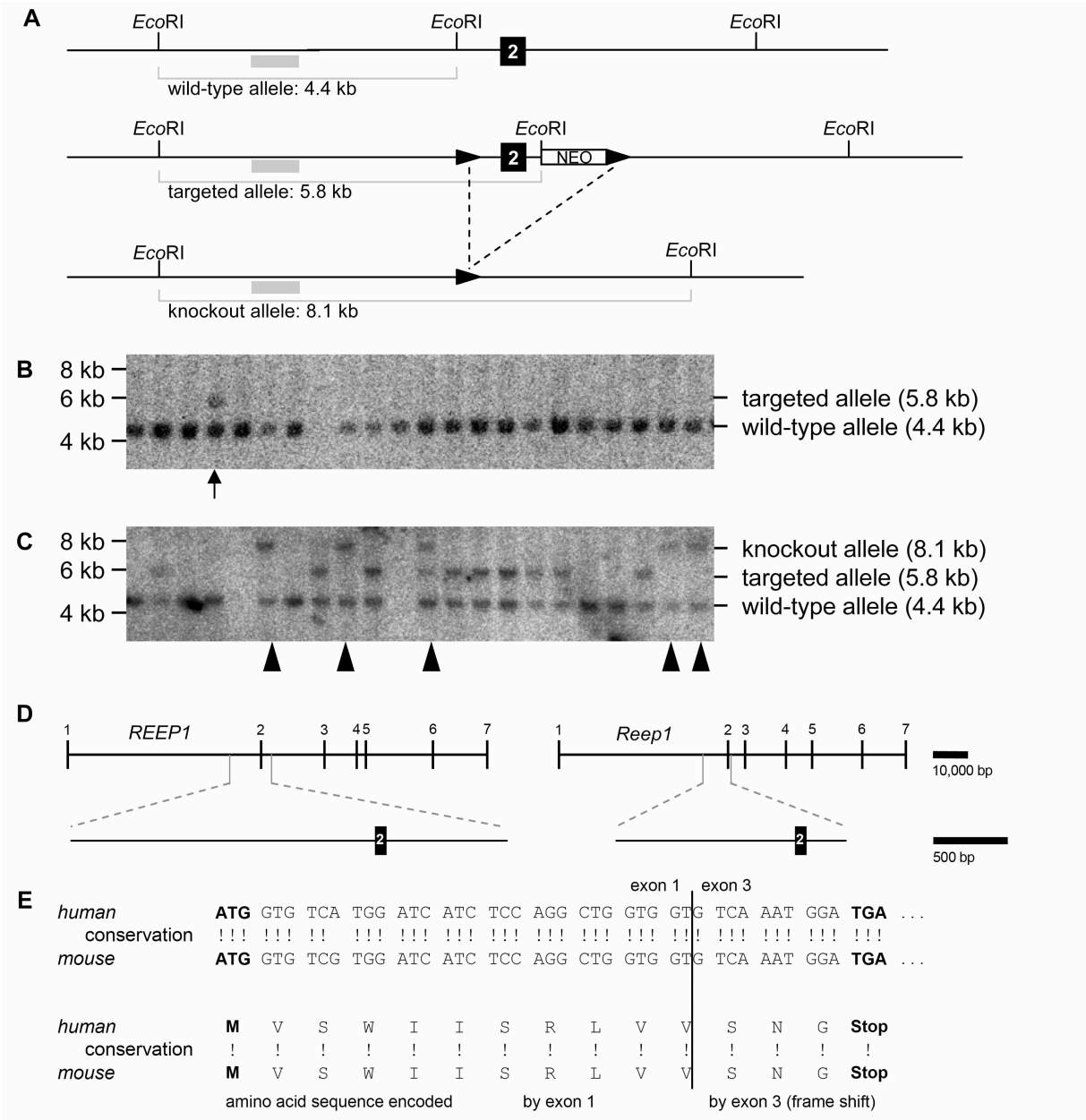
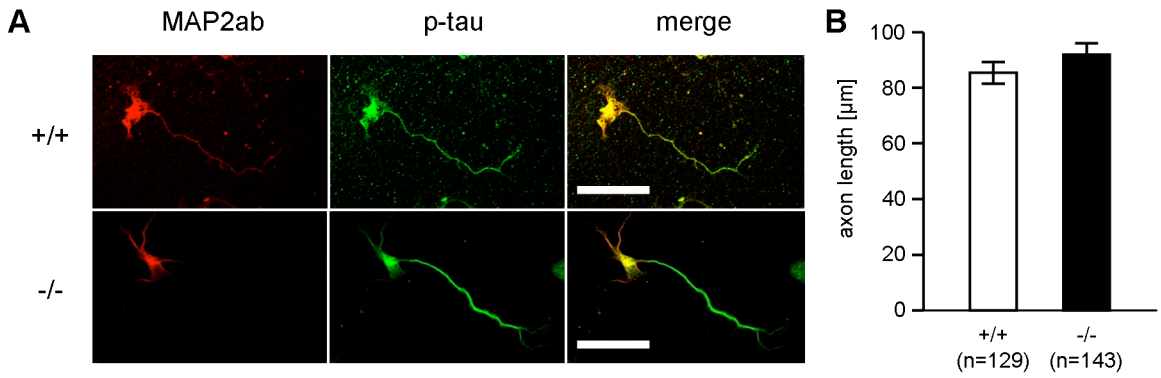


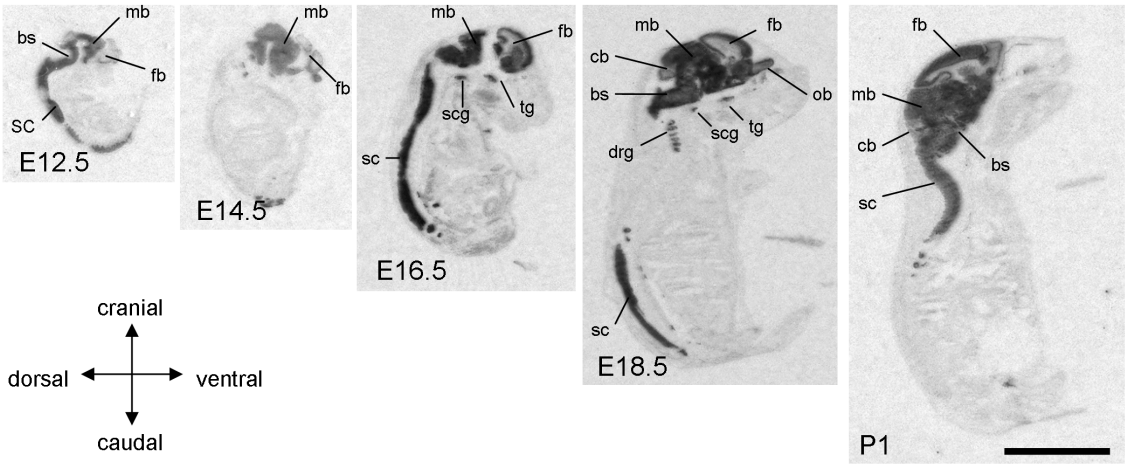
Supplemental Figure S1: Identification of the targeted and the *Reep1* knockout allele, and analogy of the exon 2 deletion detected in humans and the exon 2 deletion created in mice.

(A) Strategy to identify the different *Reep1* alleles by Southern blotting of *Eco*RI-digested genomic DNA (see also Figure 1C). The grey box represents the target sequence of the probe. (B) Southern blot analysis of individual neomycin-resistant embryonic stem (ES) cell clones after electroporation with the targeting construct. One clone harbors the targeted allele in addition to the wild-type allele (arrow). (C) Southern blot analysis of ES cell clones derived from the positive clone shown in (B) after transfection with Cre-recombinase. In some clones recombination between the loxP sites resulted in the desired knockout allele (arrowheads). (D) To-scale schemes of the human and the murine *REEP1*/*Reep1* genes (upper illustrations) and visualization of the extent of the 2,890 bp deletion in the SPG31 patient and the 1,518 bp *Reep1* deletion in mouse. Vertical lines represent exons. Scale bar: 10,000 bp (for whole gene schemes), 500 bp (for magnifications). (E) Comparison of the predicted effects at the cDNA and protein level. The murine fusion cDNA sequence was verified by sequencing. Exclamation marks indicate nucleotide/amino acid identity.

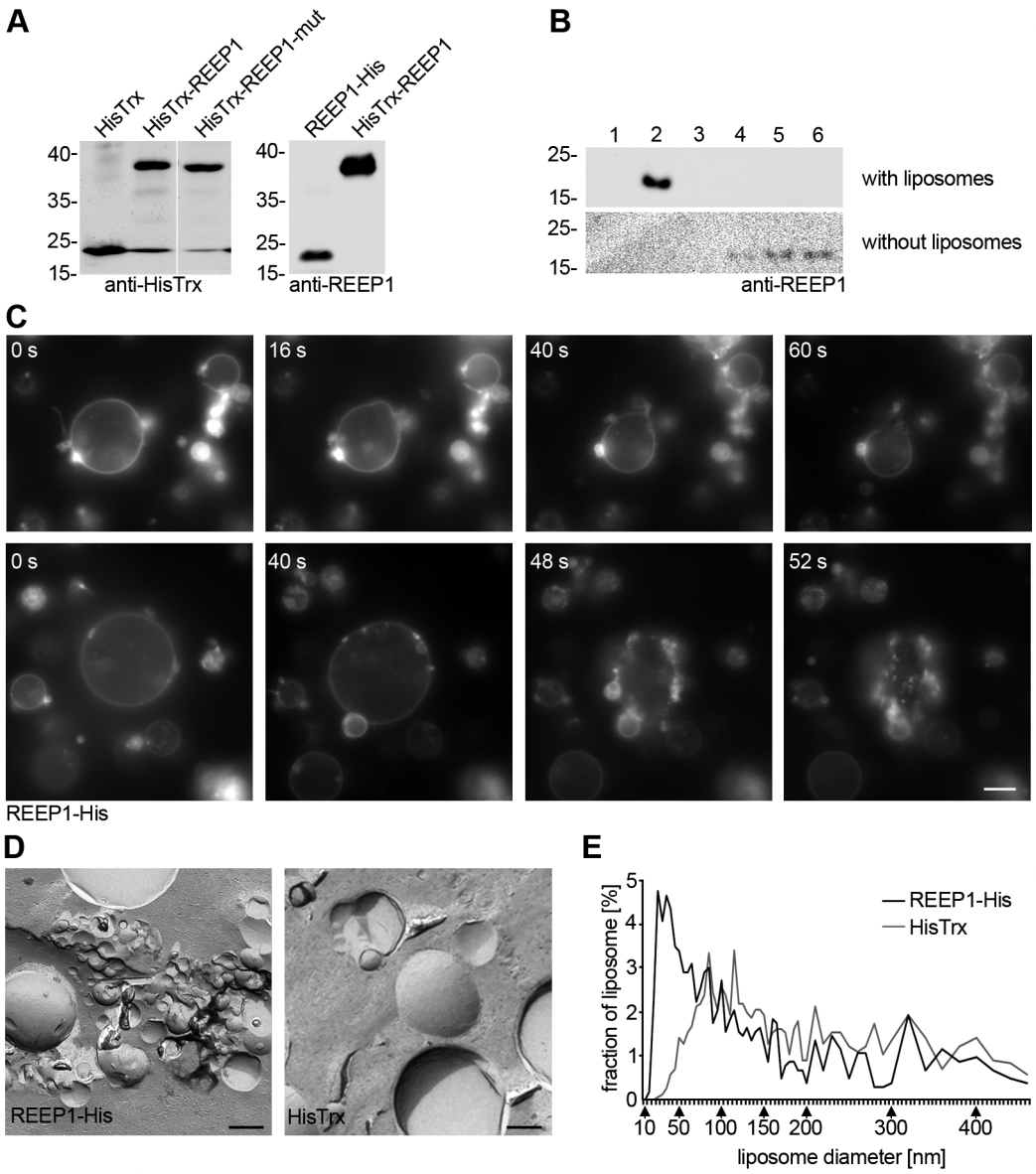
Supplemental Figure S2. Generation and characterization of a REEP1-specific antibody. (A) Alignment of the murine REEP family of proteins. Asterisks mark residues identical for all sequences and colons residues identical in more than half of the sequences. The residues of the peptide used for immunization of rabbits are highlighted in bold red. (B) Alignment of mouse REEP1 (REEP1_MM) and human REEP1 (REEP1_HS). Asterisks mark residues identical for both sequences. Residues of the peptide used for immunization of rabbits are highlighted in bold red. (C) Characterization of anti-REEP1 antibodies by Western blotting. A band of the expected size (~20 kDa) is detected in murine brain lysates and in lysates of HeLa cells overexpressing untagged REEP1. The immunosignal is abolished upon preincubation of the antiserum with the peptide used for immunization. B-actin immunoblotting served as loading control.



Supplemental Figure S3: Knockout of REEP1 does not affect outgrowth of lower motor neuron axons in vitro.
(A) Lower motor neurons were obtained from E12.5 mouse embryos and cultured for 5 days. Dendrites and axons were identified by staining against microtubule-associated protein 2ab (MAP2ab) and against phospho-tau (p-tau), respectively. Bar: 50 µm. (B) Quantification of axon length, as measured based on p-tau staining. Error bars represent SEM.



Supplemental Figure S4: Expression of *Reep1* during embryonic mouse development. Autoradiography of sagittal sections from the indicated embryonic (E) stages and postnatal day (P) 1 subjected to in situ-hybridization with a *Reep1*-specific probe. At all developmental stages analyzed *Reep1*-expression was specific to the nervous system. The image for E18.5 is the same as shown in Fig. 3A. Bar: 5 mm. bs: brain stem; cb: cerebellum; drg: dorsal root ganglia; fb: forebrain; mb: midbrain; ob: olfactory bulb; sc: spinal cord; scg: superior cervical ganglion; tg: trigeminal ganglion.



Supplemental Figure S5. N-terminally untagged REEP1 binds to membranes and promotes membrane curvature similarly to HisTrx-REEP1.

(A) Anti-REEP1 and anti-HisTrx immunoblotting analyses show the correct size and the integrity of purified recombinant proteins used for membrane binding studies. (B) Liposome binding assay showing that N-terminally untagged REEP1 binds to liposomes and thus floats to fraction 2 in density gradient centrifugations of in vitro-reconstitutions of REEP1/liposome associations. Proteins were detected using anti-REEP1 antibodies in immunoblottings of sucrose gradient fractions. (C) TEM images of freeze-fractured incubations of liposomes with the indicated recombinant proteins. Scale bar: 500 nm. (D) Distribution of liposome diameters observed by TEM of freeze-fractured liposome incubations incubated with HisTrx control protein and N-terminally untagged REEP1-His, respectively. Note that incubation with REEP1 (black) leads to an increase in the frequency of 20-40 nm structures, which are largely absent in the control incubations (grey). (E) Sequential video frames (see also Supplemental Videos 5 and 6) of liposomes incubated with N-terminally untagged, recombinant REEP1. Similar to the consequences of HisTrx-REEP1 addition, larger liposomes are rapidly converted into smaller, more highly curved membrane structures upon addition of N-terminally untagged REEP1. Bar: 5 μm.

application	name	sequence (5' ➔ 3')	size of PCR product (bp)	comment
amplification and sequencing of the fusion sequence for a deletion of *REEP1* exon 2 (compare Fig. 1A,B)	REEP1_int1_f40	GCTTGAGCCTGCTTCCTTC	1,201	patient-specific
	REEP1_int2_r01	GGCAAAGGCATGGCTATTAC		
genotyping of *Reep1* mouse line	Reep1KO_wt_f	CTGCAGGCTTATATTTGGCACCCTTTATCCTGAATATTATTCATACAAGG	358	specific to wild-type *Reep1* allele
	Reep1KO_wt_r	CCCGGGGATATCGGCGCGCCTGAGGGAACTGGCCAGAGAG		
	Reep1KO_mut_f	TTAAAAATACCTATTAGGCTGTG	163	specific to mutant *Reep1* allele
	Reep1KO_mut_r	GGAAGAAGGTGGTCTGTG		

Supplemental Table 1. Primers used to analyze the *REEP1* mutant allele in a family suffering of SPG31 and primers used for genotyping of the corresponding mouse line.

Supplemental Video 1.
A representative sequence of a spontaneously walking 12-month-old *Reep1*$^{-/-}$ mouse. In contrast to the normal alternating step cycles in wild-type mice, the *Reep1*$^{-/-}$ mouse displayed frequent hind limb "hopping", simultaneous forward movements of both hind limbs, suggesting impaired inter-limb coordination and/or muscle weakness partially compensated by use of the trunk musculature.

Supplemental Video 2.
Skilled walking was tested by climbing of an inclined ladder. In 12-month-old *Reep1*$^{-/-}$ mice, spastic cloni were often initiated during swing phases leading to subsequent inability to place the paws onto the rungs.

Supplemental Video 3. Example from Figure 5F upper panel. Rhodamin-PE labelled liposomes incubated with control protein HisTrx did not change in size and curvature over time.

Supplemental Video 4.
Movie corresponding to Figure 5F, lower panel. Rhodamin-PE labelled liposomes incubated with N-terminally untagged recombinant REEP1. Addition of recombinant N-terminally HisTrx-tagged REEP1 leads to constriction and release of smaller vesicles.

Supplemental Video 5.
Movie corresponding to Supplemental Figure S5E upper panel. Rhodamin-PE labelled liposomes incubated with N-terminally untagged recombinant REEP1. Larger liposomes are rapidly converted into smaller, more highly curved membrane structures.

Supplemental Video 6.
Movie corresponding to Supplemental Figure S5E lower panel. Rhodamin-PE labelled liposomes incubated with N-terminally untagged recombinant REEP1. Larger liposomes are rapidly converted into smaller, more highly curved membrane structures.