**Membralin deficiency dysregulates astrocytic glutamate homeostasis, leading to ALS-like impairment**

**Supplemental Materials**
(Figure S1 – S6, Tables S1-S2, Video S1)

**Other supplemental items:**
Table S1. Genes with altered expression in Astro-mem KO (GC) mouse motor cortex
Table S2. Human ALS patient information

Video S1, related to Figure 1. Righting reflex impairment in mem KO animals

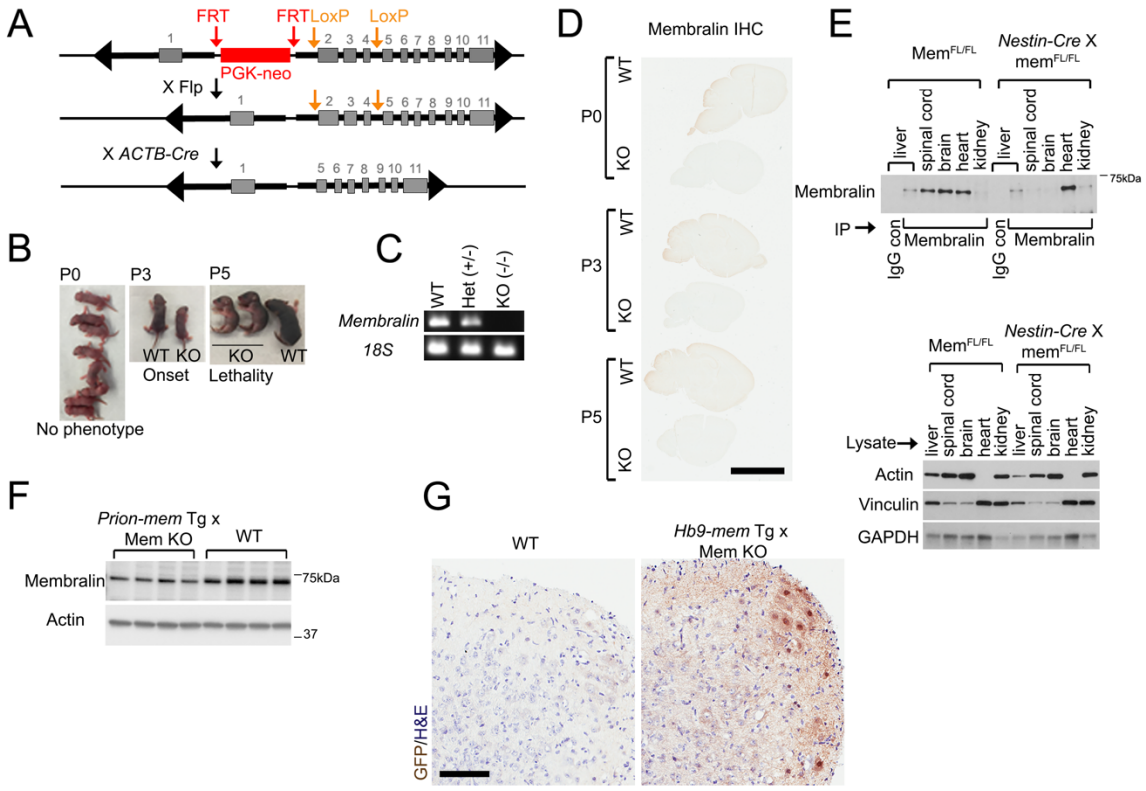


**Figure S1. Characterizing membralin deletion in the CNS.** (A) Schematic depicting deletion constructs used to achieve membralin whole body deletion by membralin exon 2 through exon 4 excision. (B) Membralin whole body deletion at P0 does not manifest in any noticeable phenotypes compared to littermates. At postnatal 3 (P3) day, membralin KO animals appear to be smaller compared to WT littermates (shown in Figure 1A), and mem KO animals die by ~P5 day. (C) qRT-PCR analysis demonstrates that *membralin* mRNA is undetectable in membralin KO samples. (D) Membralin IHC staining in WT and mem KO brain collected at P0, P3 and P5; immunopositive signals indicate no membralin staining in mem KO brain (bar = 3 μm). (E) Upper blots: membralin was immunoprecipitated using membralin antibodies (or IgG control) from mem $^{FL/FL}$ and *Nestin-Cre* x mem $^{FL/FL}$ brain, spinal cord, heart, kidney, and liver tissue lysates. Membralin is undetectable in the spinal cord and brain samples from *Nestin-Cre* x mem $^{FL/FL}$ animals. Lower blots: Lysates from organs indicated were immunoblotted for β-actin, vinculin, and GAPDH as loading controls. (F) Western blots from *Prion-mem* Tg x mem KO and WT astrocyte lysates (n=4 biological repeats) were probed to detect membralin expression. (G) Spinal cord from 3 day old WT and *Hb9-mem* Tg x mem KO animals, stained for GFP as an indicator of Tg (*Hb9-Membralin-IRES-GFP*) expression (brown) (bar = 100 μm).

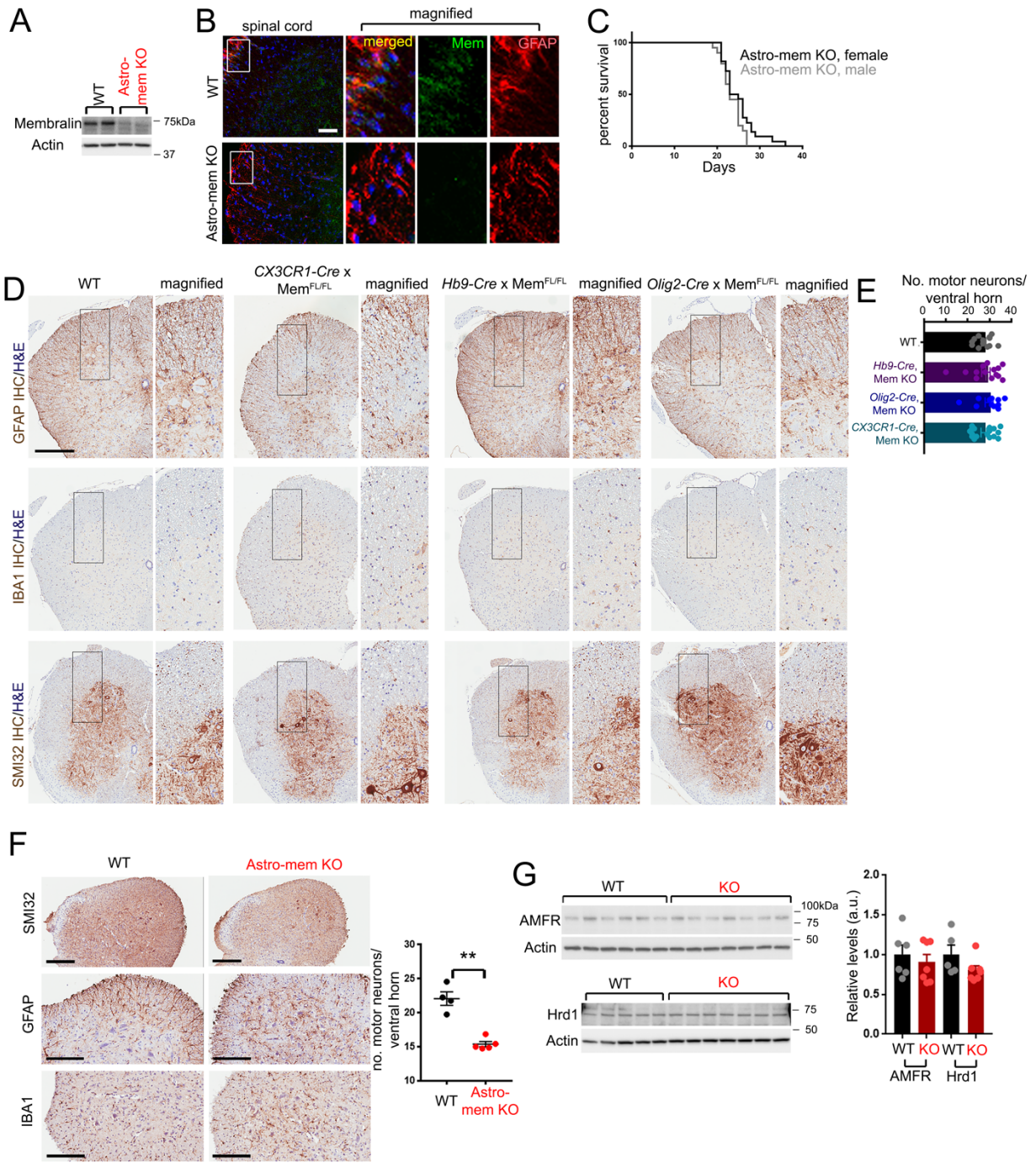


**Figure S2.** (A) Immunoblot to detect membralin levels in spinal cord from WT and Astro-mem KO mice. (B) Histological examination of membralin levels in spinal cord from WT and litter-matched Astro-mem KO mouse lines; tissues were stained for membralin (green), GFAP (red) and DAPI (blue) (bar = 50 µm). (C) Kaplan-Meier survival curves for Astro-mem KO female mice (black) and male mice (grey) show no gender-dependent differences in Astro-mem KO mice survival. (D, E) Histological analysis of spinal cord from a Cre-membralin deletion mouse panel. WT spinal cord (left panels) and spinal cord tissue from mouse lines with membralin deletion in

microglia (*CX3CR1-Cre*, middle left panels), motor neurons (*Hb9-Cre*, middle right panels), and oligodendrocytes (*Olig2-Cre*, right panels) were stained for GFAP (top panels), IBA1 (middle panels), and SMI32 (bottom panels) as indicated (bar = 300 μm). (E) Number of motor neurons within the ventral horn of the lumbar sections were counted based on SMI32 staining as shown in (D). (F) Histological analysis of spinal cord tissue collected from WT and Astro-mem KO mice: SMI32 (motor neurons), GFAP (astrogliosis), and IBA1 (microgliosis) staining is depicted. Motor neuron number in the lumbar sections (adjacent graph) was significantly reduced in Astro-mem KO (n=4) compared to WT (n=3), **p< 0.01, Student's t test (bar = 300 μm). (G) AMFR and Hrd1 levels from mouse spinal cord were detected by immunoblot. Normalized AMFR or Hrd1 levels (to actin) in WT (black bars) and mem KO (red bars) samples (WT sample mean value set to 1.0) were calculated and depicted in the adjacent graph (mean±SE).

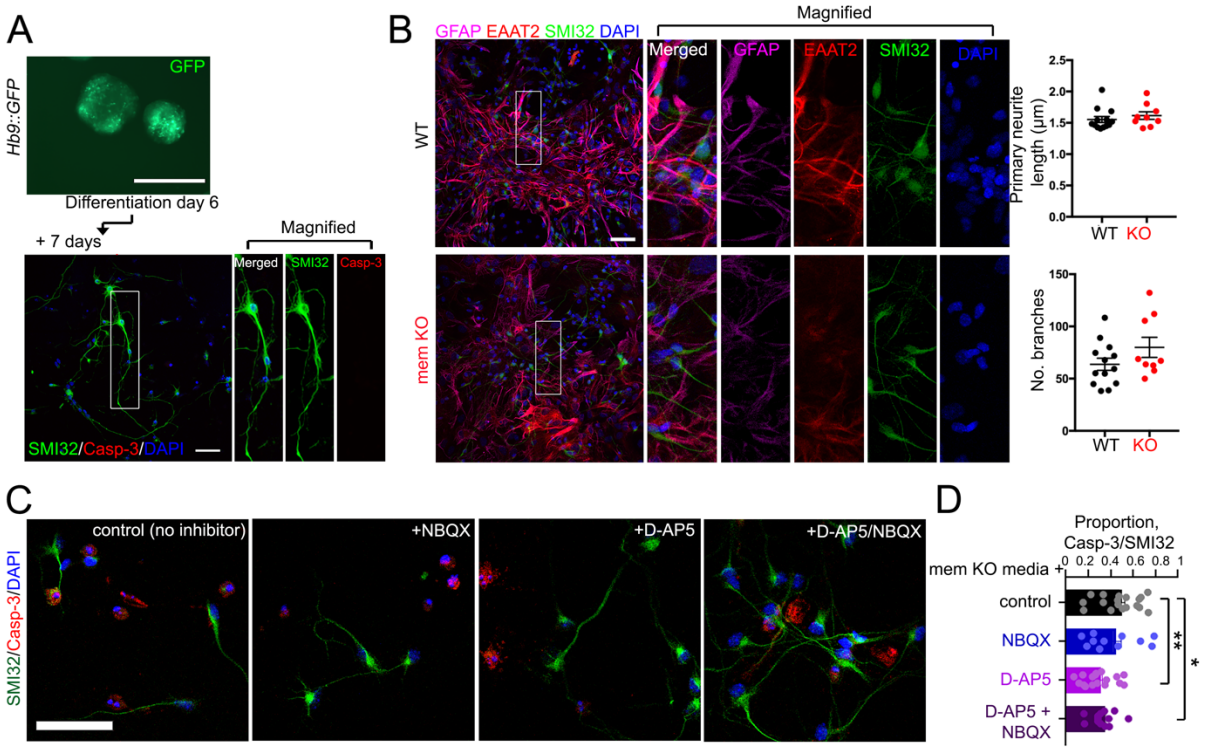


**Figure S3.** (A) Embryonic bodies derived from *Hb9:: GFP* mouse embryonic stem (ES) cells at differentiation day 6 prior to dissociation into single cells (bar = 400 µm). Lower pannel: Motor neurons cultured for 7 days in non-conditioned medium (motor neuron media) for 24 hrs, stained with SMI32 (green), active casp-3 (red) and DAPI (blue). Little caspase 3 activation was observed. (B) WT or mem KO astrocytes were co-cultured with WT motor neurons derived from mouse ES cells (*Hb9::GFP*) for 24 hrs. Neurites were visualized by SMI32 (motor neurofilament, green) staining. Astrocytes (GFAP, pink; EAAT2, red) were visualized in co-cultures, DAPI is indicated in blue (bar = 50 µm). SMI32-stained neurite tracings were subject to analysis for primary filament length (upper graph) and branch no. (lower graph) using Imaris software; no significant differences were observed in motor neurons co-cultured with WT or mem KO astrocytes after 24 hrs in co-culture. (C) ES-derived motor neurons were incubated with conditioned media from mem KO astrocytes in the absence of inhibitor, or D-AP5 (100 µM), NBQX (100 µM) or D-AP5/NBQX in combination for 24 hrs as indicated. Motor neurons were fixed and stained for SMI32 (green), active Caspase-3 (red) or DAPI (blue) and visualized by confocal microscopy (bar = 50 µm). (D) The ratio of cells stained for active Caspase-3 SMI32 positive motor neurons were calculated as a ratio over DAPI (mean±SE), significance was determined by One-way ANOVA with Tukey's multiple comparison tests, *$p<0.05$, **$p<0.01$.

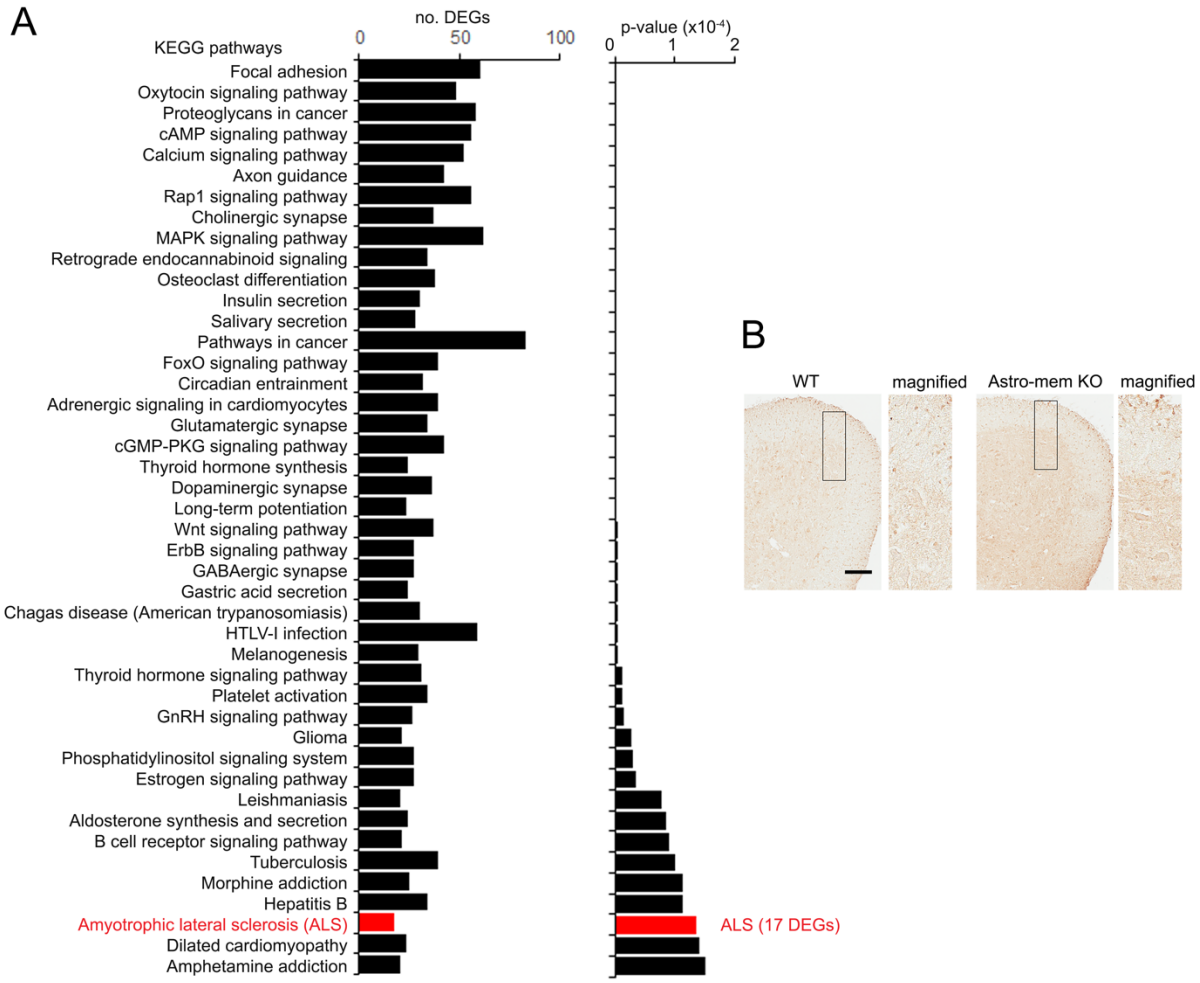


**Figure S4, ALS components are perturbed with Astrocytic membralin deletion in motor cortex.** (A) KEGG pathways and number of DEGs observed in each category in Astro-mem KO motor cortex identified by RNAseq compared to WT. The KEGG ALS pathway (mmu05014) is highlighted in red. (B) WT and Astro-mem KO spinal cord (22 day old) were stained for TNFR1 as indicated (bar = 150 μm).

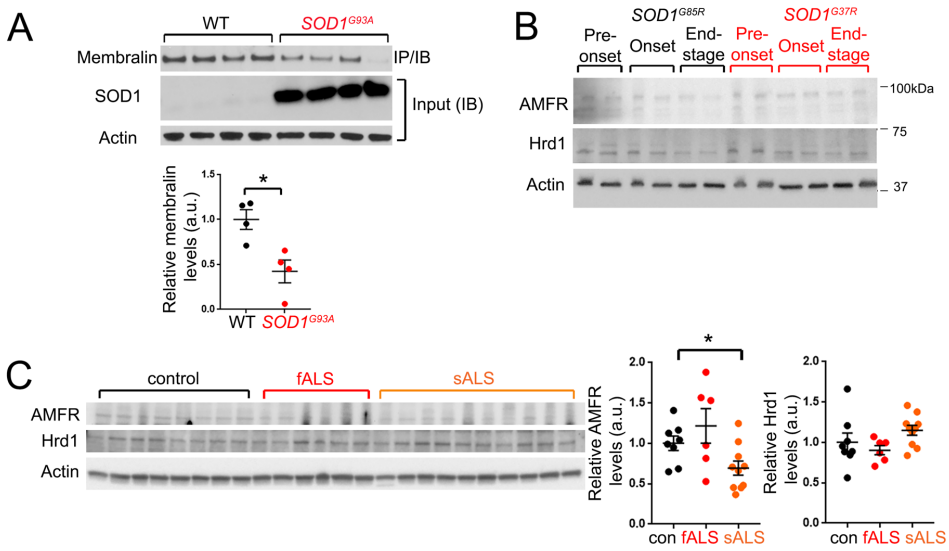


**Figure S5,** (A) Lysates from WT or *SOD1*$^{G93A}$ mouse spinal cord at 5 months (end stage) were immunoblotted for SOD1 or actin, or immunoprecipitated/immunoblotted for membralin as indicated. Relative membralin levels were determined (normalized to actin), where membralin levels were compared relative to WT animals (set to 1.0, lower graph). Statistical significance is determined by Student's t test. (B) AMFR and Hrd1 levels were characterized by western blot as indicated in spinal cord from *SOD1*$^{G85R}$ and *SOD1*$^{G37R}$ mice at pre-onset, onset and end-stage. (C) Characterizing ERAD levels in human ALS spinal cord. AMFR and Hrd1 levels were examined by immunoblot in control, familial ALS (fALS, red) and sporadic ALS (sALS, orange) spinal cord as indicated. Graphs on the right represent AMFR and Hrd1 levels normalized to actin, compared to control (set to 1.0). Values are mean±SE, statistical differences are determined by One-way ANOVA with Tukey's multiple comparison tests, *p<0.05.

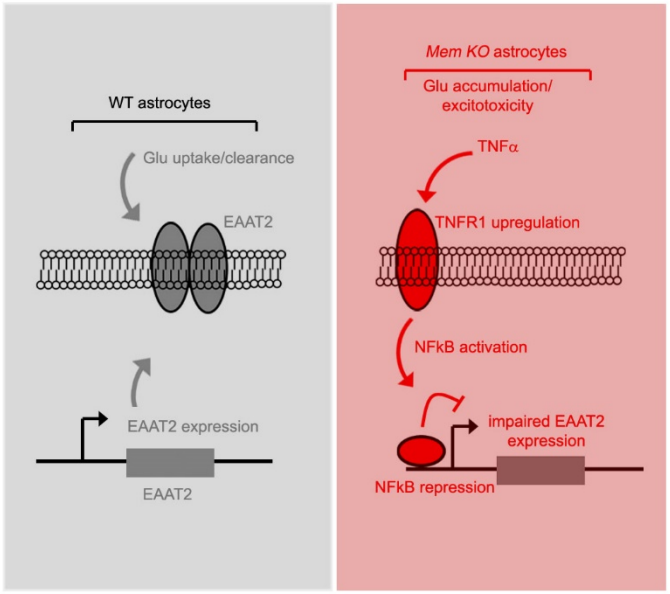


**Figure S6. Model, a role astrocytic role for membralin in glutamate homeostasis.** WT astrocytes normally express EAAT2 which facilitate glutamate uptake and clearance (left panel). Membralin deletion in astrocytes results in enhanced TNFR1 expression, resulting in increased NFκB-mediated repression of EAAT2 expression, consequent glutamate accumulation and excitotoxicity (right panel).

**Video S1, Righting reflex impairment in mem KO animals.** WT animals at P3 are able to right themselves when turned over (animal on the right), whereas age-matched membralin KO animals (left) are unable to roll into an upright position within 30 sec.

**Table S2. Human ALS patients used in this study**

| Patient | Primary diagnosis | Pathology | Date of sample received (Death Date) | Death Age | Gender |
|---|---|---|---|---|---|
| 19 | Control | Pakinson's Disease | 3/2/3005 | 80 | Female |
| 20 | Control | Basilar CVA | 3/4/2005 | 38 | Male |
| 65 | Control | | 7/20/2007 | 82 | Male |
| 67 | Control | | 10/9/2007 | 77 | Male |
| 70 | Control | Multiple medical | 2/14/2008 | 59 | Female |
| 78 | Control | Vasculitis, cerebral hem | 2/4/2010 | 58 | Female |
| 83 | Control | Sepsis, myeloma, ARF, | 9/9/2010 | 63 | Female |
| 88 | Control | CVA, multisystem failure | 3/9/2011 | 78 | Female |
| 13 | FALS | Non SOD | 8/29/2004 | 67 | Female |
| 15 | FALS | SOD1 (A4V) | 8/29/3004 | 65 | Female |
| 24 | FALS | SOD1 (A4V) | 7/1/2005 | 21 | Female |
| 101 | FALS | SOD1 (A4V) | 10/27/2014 | 68 | Male |
| 124 | FALS | SOD1(c.317C>t;p.Ser10) | 2/16/2018 | 45 | Female |
| 114 | FALS | SOD1 (A4V) | 12/3/2016 | 48 | Male |
| 16 | SALS | | 12/14/2004 | 61 | Male |
| 21 | SALS | | 3/17/2005 | 84 | Male |
| 27 | SALS | | 8/21/2005 | 74 | Male |
| 29 | SALS | | 10/4/2005 | 77 | Female |
| 33 | SALS | | 1/2/2006 | 54 | Male |
| 112 | SALS | | 6/13/2016 | 54 | Female |
| 113 | SALS | Ventilator (short or long term?) | 7/1/2016 | 66 | Male |
| 120 | SALS | | 11/28/2017 | 64 | Male |
| 127 | SALS | | 7/25/2018 | 67 | Male |
| 130 | SALS | | 8/27/2018 | 63 | Male |
| 128 | SALS | | 8/1/2018 | 69 | Female |