Supplementary Information

Figure S1 Methods:

The Dual Bait Hybrid Hunter (Invitrogen, Catalog No. K5200-01) system was used to screen for protein-protein interactions. The mutant G85R ($SOD1^{G85R}$) was constructed in the plasmid pHybLex/Zeo and wild-type SOD1 ($SOD1^{WT}$) in the pHybcI/HK as the baits. The bait plasmids were introduced to the yeast strain with the confirmation that there was no self-activation with the bait construct. Yeast SKY48/pLacGUS (genotype, MATα *ura3 trp1 his3* 6*lex*Aop-*LEU2* 3*cI*op-*LYS2 pLacGUS* (*URA3*); Phenotype, $Ura^{+}Trp^{-}His^{-}Leu^{-}Lys^{-}$) was used as the host strain for the screening of the adult human brain cDNA library (Invitrogen, Catalog No. A204-01). Transformants were plated in YC-UHWK Z200 Gal/Raf or YC-UHWL Z200 Gal/Raf medium to assay for leucine or lysine prototrophy. Cells that contain an interactor with the LexA fusion construct (LexA-G85R) will grow in the absence of leucine (L) and exhibit β-galactosidase activity, while cells that contain an interactor with the cI fusion construct (cI-WT) will grow in the absence of lysine (K) and exhibit β-glucuronidase activity. The interaction was further confirmed by co-transformation of the purified prey and bait to the yeast SKY48/pLacGUS.

Figure S1. Confirmation of the specific interaction between adaptor-associated kinase 1 (AAK1) and G85R in yeast. Yeast co-transformed with AAK1 together with wild type (WT) and mutant SOD1 (G85R) grew selectively on the YC-UHWL200 Raf/Gal but not YC-UHWK200 Raf/Gal medium and exhibited β-galactosidase activity indicating that mutant SOD1 (G85R) but not the wild type interacted with AAK1. Krev/Fos2 instead of G85R/WT used as baits and other controls showed no growth further confirming the specificity of the interaction between G85R and AAK1.

Medium	YC-UHWLZ 200 Raf/Gal	YC-UHWKZ 200 Raf/Gal	YC-UWL Raf/Gal	YC-UHLZ 200 Raf/Gal	YC-UHWLZ 200 Raf/Gal	YC-UWL Raf/Gal	YC-UHWLZ 200 Raf/Gal
AAK1	+	+	+	-	+	-	+
G85R/WT	+/+	+/+	-/+	+/+	Krev/Fos2	-/+	+/+
L	-	+	-	-	-	-	-
K	+	-	+	+	+	+	+
X-gal	-	-	-	-	-	-	+

Figure S2 Methods:

Clathrin-coated vesicle (CCV) preparation was performed. Briefly, mouse brain tissues were rinsed and homogenized in the buffer of 100 mM MES (2-(*N*-morpholino)ethanesulfonic acid) pH 6.5, 0.5 mM $MgCl_2$, 1 mM EGTA (Ethylene glycol tetraacetic acid), 1 mM PMSF (phenylmethylsulfonyl fluoride), 0.8 mM DTT (Dithiothreitol), and 0.02% NaN_3 (HB). The homogenate was centrifuged at 15,000× *g* for 30 min at 4 °C to result in P1 fraction. The supernatant was further centrifuged at 100,000× *g* for 1 h at 4 °C to result in P2, the crude microcomal faction containing CCVs. The supernatant was collected as S100 fraction. The immunoprecipitation (IP) methodology was used to assess whether there is a stable interaction with AAK1 and mutant SOD1 with all three fractions: P1, P2 and S100. Multiple antibodies that interact with human $SOD1^{G85R}$ ($hSOD1^{G85R}$) but not the mouse $SOD1^{WT}$ ($mSOD1^{WT}$) were used to pull down SOD1 in the buffer of 50 mM Tris pH 7.5/150 mM NaCl/0.2% Triton. Protein G beads were used to isolate out the antibody-precipitated proteins which were further subjected to immunoblot analysis probed with the AAK1 antibody.

Figure S2. Stable interaction between human $SOD1^{G85R}$ and AAK1 is not detected. The CCV preparation (P2) as well as the S100 fraction from G85R mouse brain was analyzed. The crude P2 fraction was washed with the buffer and centrifuged at 100,000× *g* for 1 h. Both the supernatant (P2, peripheral) and the pellet (P2, washed) were analyzed. IP was performed using two different monoclonal antibodies (Ab1 and Ab2) against human SOD1 or a control monoclonal antibody which does not recognize SOD1 (cont.). Immunoprecipitated proteins were analyzed via immunoblot using both SOD1 and AAK1 antibodies. T.I.: Total input; Ab1: Monoclonal antibody NCL from Novocastra Laboratories (UK); Ab2: Monoclonal antibody from Biodesign International (Saco, ME, USA).

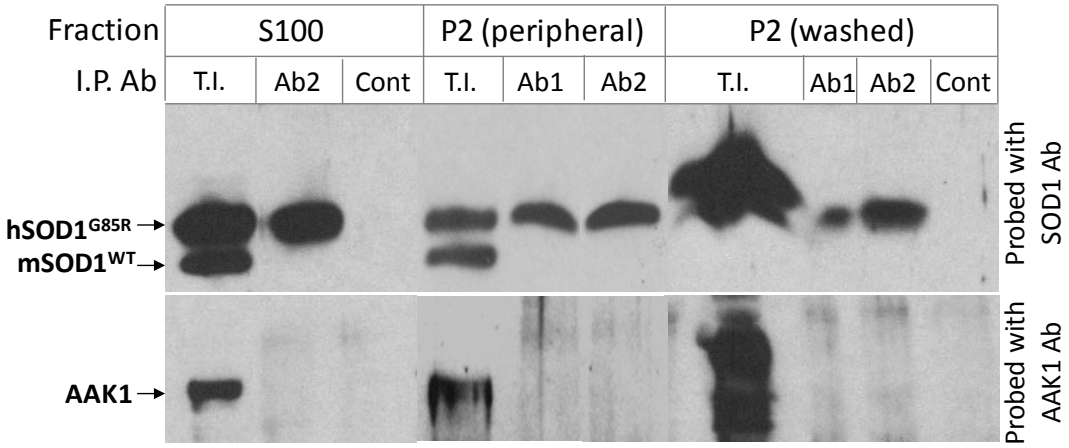

Figure S3 Methods:

Paraffin sections of less than 10 μm thickness from mouse and rat spinal cords were used for double immunofluorescent staining for AAK1 with another molecular marker synaptophysin (Syn) or phosphorylated neurofilament protein (SMI31). All sections were deparaffinized, heat-boiled by microwaving to expose the epitopes, and blocked in 5% normal serum in PBS/0.01% Triton for 1.0 h at room temperature. Subsequently, sections were incubated with primary antibodies in PBS/1% normal serum overnight at 4 °C. The sections were continued for incubation with Cy3 or Cy5-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA). All images were captured on the upright Nikon microscope equipped with a CCD (charge coupled device) camera.

Figure S3. AAK1 is partially colocalized with the presynaptic protein marker synaptophysin, but not with the phosphorylated neurofilament protein. Double immunofluorescent staining was performed using spinal cord sections for both AAK1 (red, **A**,**D**,**G**,**J**) and another protein marker (green): Synaptophysin (Syn, **B**,**E**); Phosphorylated neurofilament protein (SMI31, **H**,**K**); The superimposed images of the two protein signals were shown in the most right panel for each section with DAPI-staining was shown in blue (**C**,**F**,**I**,**L**); Some superimposed AAK1 and synaptophysin signals on the cell surfaces were indicated by the arrows (**C**,**I**); Spinal cord tissues were from three month old non-transgenic mouse (mNtg, **A**–**F**) and one month old non-transgenic rat (rNtg, **G**–**L**). Scale bars = 40 μm apply to all images.

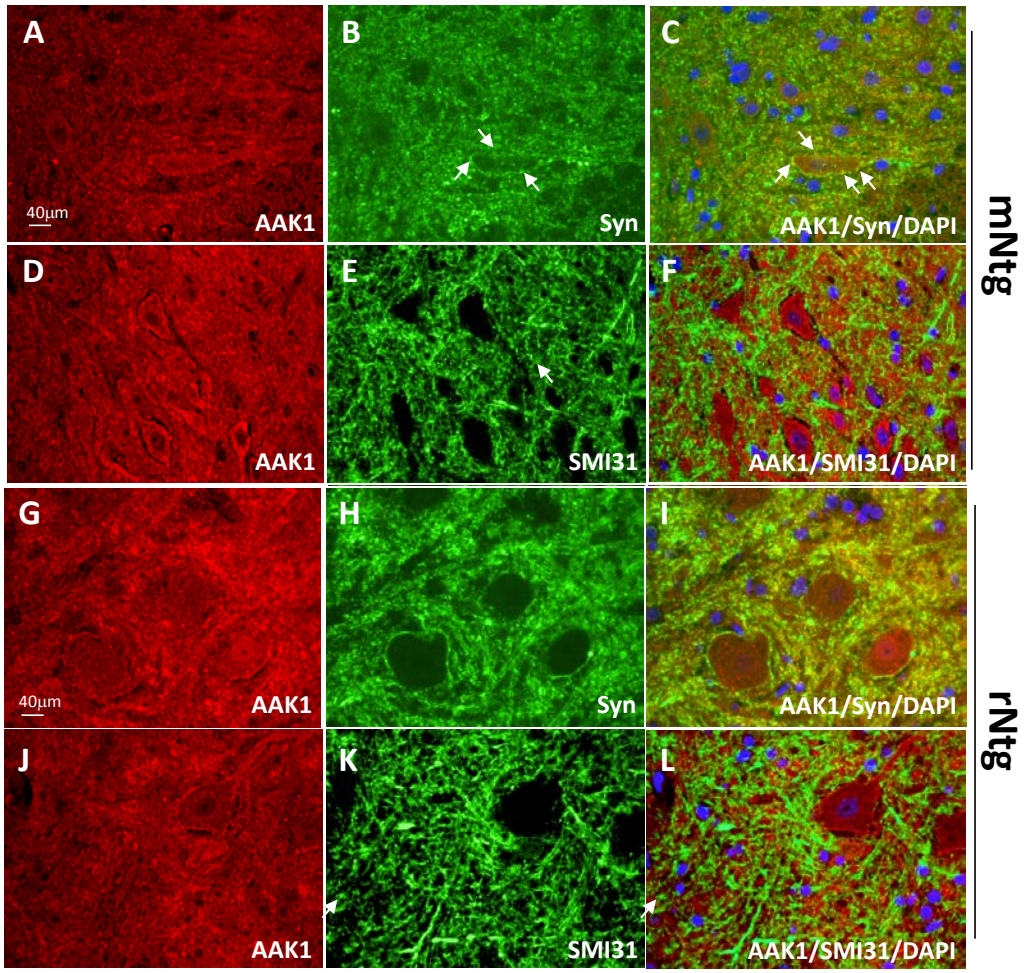

Figure 4S Methods:

Figure 4S. AAK1 is enriched in the microsomal fraction from mouse spinal cord. Spinal cord tissues from normal, non-transgenic and SOD1^{G93A} transgenic mice were obtained. Tissue fractionation was performed as described (Liu *et al*. [37]). Protein concentrations were measured for the following fractions: total extract (T), nuclear (N), mitochondrial (mito), microsomal (mv) and cytosolic (cyto). An equal amount (50 μg) of proteins from each fraction was loaded on the gel for Western analysis. The antibody against AAK1 was used and image was obtained using the ECL (Enhanced chemiluminescence) method.