Supplemental Methods

Plasmids:

N-terminal myc-tagged human SorCS2 was generated by subcloning human SorCS2 into pcDNA3.1(+) vector as previously described (Anastasia et al., 2014) and served as the source plasmid for generating all SorCS2 expression constructs by PCR based cloning. All constructs were verified by sequencing. SorCS2 deletion constructs contain the following sequences: ΔECD = minus 51-1078 amino acids, ΔICD = minus 1100 – 1159 amino acids. To make the N-terminal deletion construct (ΔECD), the SorCS2 signal peptide sequence was fused to the transmembrane domain by two polymerase chain reaction amplification steps. In the first step, two partially overlapping FS fragments were generated using the fused sequence oligonucleotide (5'-CGTCTGCTGGGCGCCTGCGGGGCGGCGGGGGGCTACTGGGCGGTAGTGGTGCTGTTTGTC-3') as a forward primer and a reverse primer with a C-terminal HA tag (5'-TGCTTATCTAGATCAAGCGTAATCTGGAACATCGTATGGGTAGCTCACCAGGTAGCTGTGC-3') in one reaction, and a signal peptide forward primer (5'-TAAGCAAAGCTTGCCACCATGGCGCACCGGGGGCCCTCGCGCGCCTCGAA-3') with the complementary oligonucleotide of the fused sequence as a reverse primer in a separate concurrent reaction. The two overlapping products were fused and amplified using the signal peptide forward primer and the reverse primer with HA-sequence in the second polymerase chain reaction step. Following restriction digestion, the final mutated polymerase chain reaction product was purified and cloned back into pcDNA3.1(+). For full-length (FL) and ΔICD SorCS2 constructs, a HA tag was added to the C-terminal of the protein sequence by PCR-based cloning.

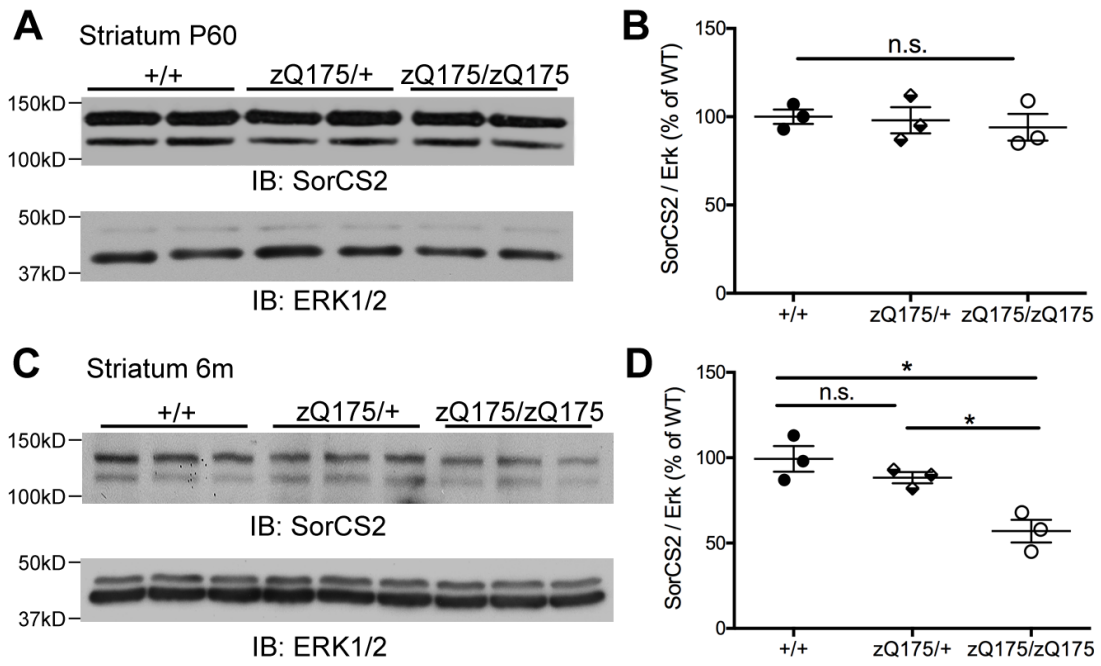
HEK293T pull-down assay:

For pull-down assays on HEK293T cells, cells were grown in 10 cm dishes in DMEM (Invitrogen) supplemented with 10% FBS (Invitrogen) and penicillin/streptomycin, and transfected with either pcDNA3.1(+) empty vector or SorCS2 expression constructs using Lipofectamine-2000 (Invitrogen). 72 hours after transfection, cells were lysed in lysis buffer (1% NP-40, 1% Triton X-100, 1mM PMSF, 10% glycerol and protease inhibitor cocktail (Sigma) in Tris-buffered saline) for 1 hr at 4°C. Lysates were spun at 15,000 rpm for 5 min at 4°C. The supernatant (600µg total protein) was then incubated with either mouse anti-myc antibody (Santa Cruz, sc-40) or mouse anti-HA.11 antibody (BioLegend, Clone 16B12, #901501), together with Protein-G Sepharose beads (Invitrogen, #101241) for 18 hr at 4°C. Beads were then washed three times in lysis buffer, and incubated with 600ug of cortical tissue lysate from SorCS2$^{-/-}$ adult mice for 18 hr at 4°C. Beads were again washed three times in lysis buffer, and immune-complexes were resolved by SDS-PAGE and then probed for NR2A using Western blot analysis.

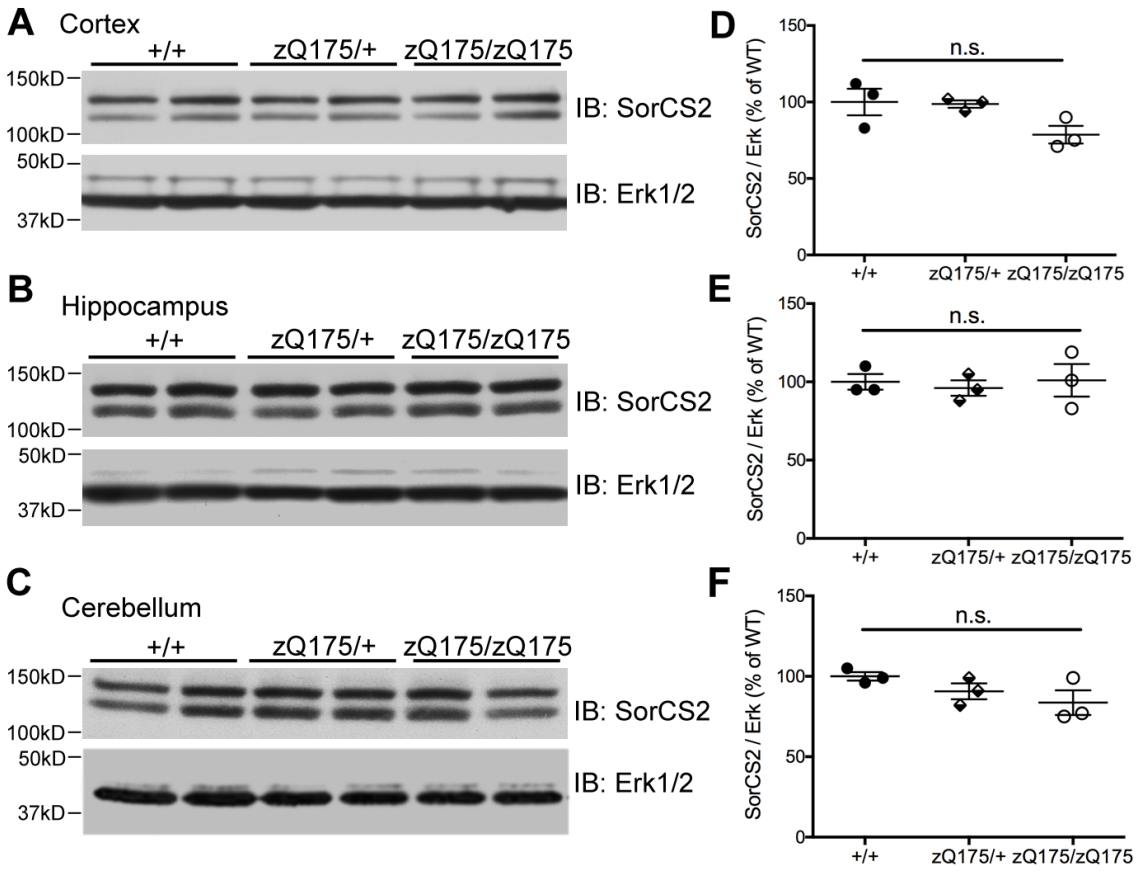
RNA isolation, reverse transcription, and real-time quantitative PCR analysis:

Total RNA from brain tissues was isolated utilizing TRIZOL reagent (Life Technologies, 15596108). RNA was treated with DNase I (Life Technologies, 18068015) to remove DNA contamination. RNA was then reverse transcribed into single-stranded cDNA using SuperScript III First-Strand Synthesis System (Life Technologies, 18080051) as described by the manufacturer.

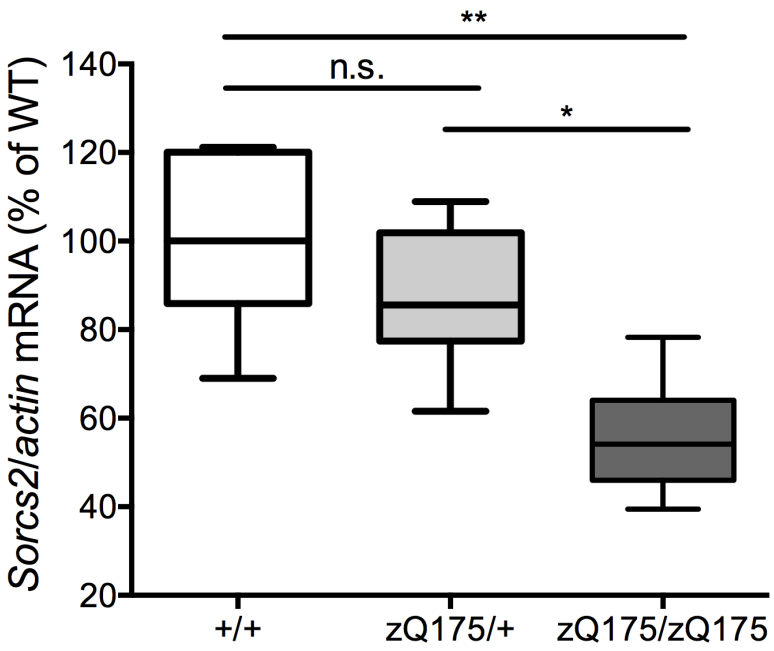
For real-time PCR analyses, Mastercycler Realplex^2 real-time PCR machine (Eppendorf) was used, and all reactions were performed in a total volume of 25 µl containing 50 ng of cDNA, QuantiTect SYBR Green PCR kit (Qiagen, #204143) and 0.4 µM of forward and reverse primers. Amplification cycles were carried out as follows: an initial denaturing cycle at 95 °C for 15min, followed by 40 cycles of 95 °C for 30s, 60 °C for 30s and 72 °C for 30s, for all of the genes analyzed. C_t was quantified during the 60 °C annealing step. Amounts of *Sorcs2* mRNA were normalized to a reference gene (β-actin). Each sample was repeated in triplicate for statistical analyses. Primer sequences used are as follows: *Sorcs2* exon 2 forward, 5'-TGCAGACATGGGCAAGGTTCT-3'; *Sorcs2* exon 3 reverse, 5'-GTTGTCAATGACAGTGGTGACA-3'; β -actin forward, 5'-AGTGTGACGTTGACATCCGTA-3'; β-actin reverse, 5'-GCCAGAGCAGTAATCTCCTTCT-3'. N=3 animals of each genotype were analyzed.



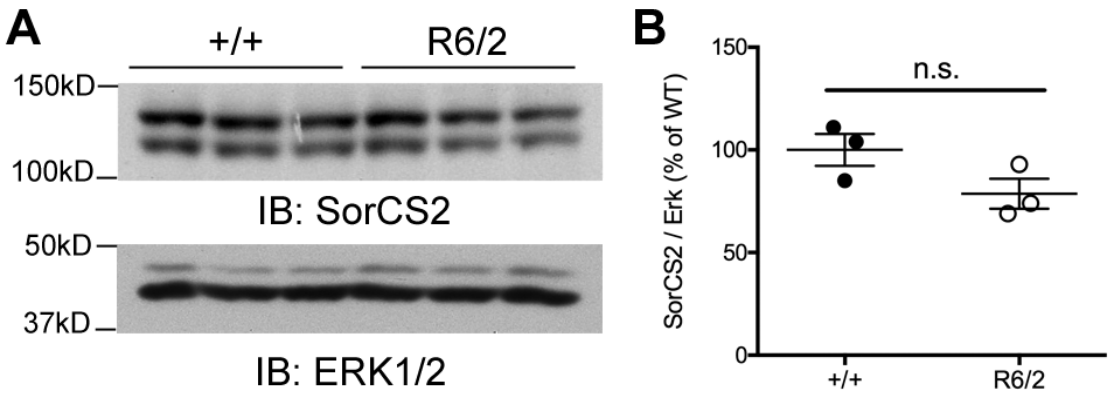
Supplemental Figure 1. SorCS2 levels in the striatum of zQ175 mice at postnatal day 60 (P60) and 6 months (6m). (**A, B**) Western blot and analysis showing that total SorCS2 protein level in the striatal lysate of wild type, zQ175 heterozygous and homozygous mice are comparable at P60. N=3 animals per genotype. Data represents mean ± S.E.M. n.s., non-significant (p>0.05; One-way ANOVA). (**C, D**) Western blot and analysis showing that total SorCS2 protein level is significantly decreased in the striatal lysate of zQ175 homozygous mice at the age of 6 months compared to wild type and zQ175 heterozygous mice. N=3 animals per genotype. Data represents mean ± S.E.M. *, p<0.05; n.s., non-significant (p>0.05; One-way ANOVA).



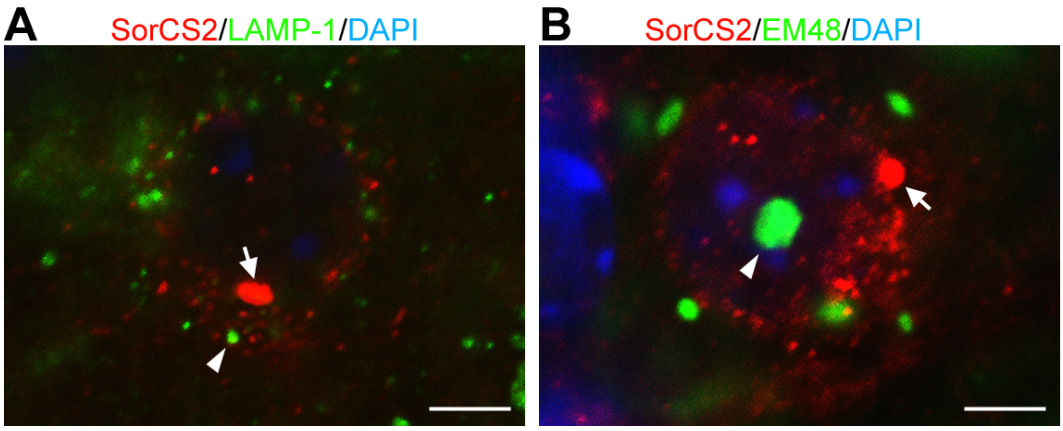
Supplemental Figure 2. SorCS2 levels are not altered in the cortex, the hippocampus and the cerebellum. Western blot and analysis showing total SorCS2 protein level in the cortical (**A, D**), hippocampal (**B, E**), and cerebellar (**C, F)** lysate of wild type, zQ175 heterozygous and homozygous mice at the age of 12 months. Statistical analysis revealed no significant differences of total SorCS2 levels among genotypes at this time point. N=3 animals per genotype. Data represents mean ± S.E.M. n.s., non-significant (p>0.05; One-way ANOVA).



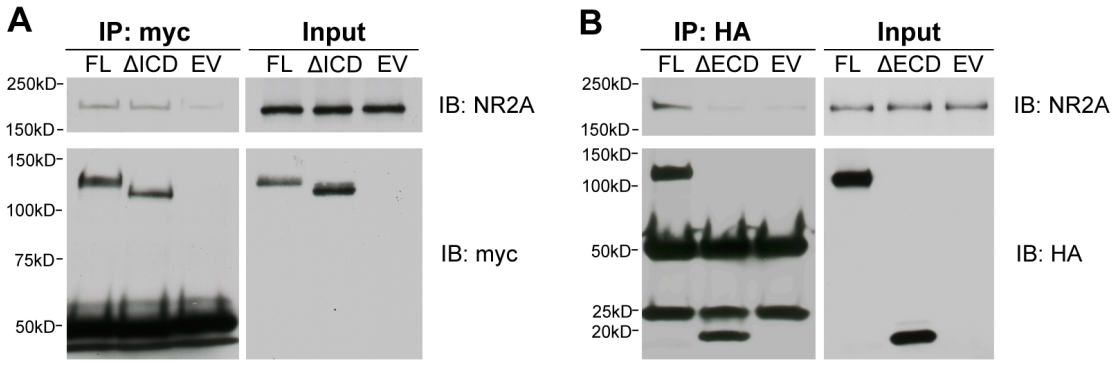
Supplemental Figure 3. Reduced *Sorcs2* gene transcription in the striatum of 12-month-old zQ175 homozygous mice by quantitative PCR analysis. Total *Sorcs2* mRNA from the striatum of zQ175 homozygous mice is significantly decreased compared to wild type and zQ175 heterozygous mice at the age of 12 months, as measured by real-time quantitative RT-PCR. All samples were normalized to *β-actin* mRNA level, and then the ratios were calculated as percentage of the ratio of wild type (N=3 of each genotype. *, $p<0.05$; **, $p<0.01$; n.s., non-significant, one-way ANOVA). In this box and whisker plot, the boxes extend from the 25th to 75th percentile. The line in the middle of the box is plotted at the median. The whiskers go down to the minimum value and up to the maximum.



Supplemental Figure 4. SorCS2 levels in the cortex of R6/2 mice at the age of 13 weeks. Western blot (**A**) and analysis (**B**) showing that total SorCS2 protein level in the cortical lysate of R6/2 mice and their wild type littermates are not statistically different at the age of 13 weeks. N=3 animals per genotype. Data represents mean ± S.E.M. n.s., non-significant (p>0.05; unpaired t-test with Welch correction).



Supplemental Figure 5. SorCS2 is not mislocalized to lysosomes or mutant huntingtin aggregates in the striatum of zQ175 homozygous animals at the age of 12 months. Double-labeling immunofluorescence microscopy shows that SorCS2 (red, arrow) does not colocalize with LAMP-1 (green, arrowhead) - a lysosomal marker (**A**) or EM48 (green, arrowhead) - the marker for mutant huntingtin aggregates (**B**) in the striatum of zQ175 homozygous mice at the age of 12 months. Scale bar, 5 μ m. Three independent experiments were conducted.



Supplemental Figure 6. Mapping the interaction of NR2A with SorCS2 mutants. HEK293T cells were transfected with cDNAs encoding full-length SorCS2 (FL), or a mutant truncated SorCS2 either lacking the ectodomain (ΔECD) or the intracellular domain (ΔICD). The ΔICD construct has a N-terminal myc tag, and the ΔECD construct has a C-terminal HA tag, while the FL construct carries both epitope tags. Full-length or mutant SorCS2 were pulled down from HEK293T cell lysate first using protein-G beads and antibodies against myc tag (**A**) or HA tag (**B**). These beads were then incubated with SorCS2$^{-/-}$ brain tissue lysate, allowing protein complexes to form between SorCS2 and endogenously expressed NR2A. Following co-precipitation, protein complexes were immunoblotted with NR2A antibody using Western blot analysis, which shows that the ectodomain of SorCS2 is required (**B**), where as the intracellular domain is dispensable (**A**) for its interaction with NR2A.