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Supplemental Information

SorCS2 Controls Functional Expression of Amino Acid Transporter EAAT3 and Protects Neurons from Oxidative Stress and Epilepsy-Induced Pathology

Anna R. Malik, Kinga Szydlowska, Karolina Nizinska, Antonino Asaro, Erwin A. van Vliet, Oliver Popp, Gunnar Dittmar, Raphaela Fritsche-Guenther, Jennifer A. Kirwan, Anders Nykjaer, Katarzyna Lukasiuk, Eleonora Aronica, and Thomas E. Willnow

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SUPPLEMENTAL INFORMATION

SUPPLEMENTAL TABLES

Table S1. Related to Figure 1. Clinical features of patients involved in the study.

Pathology	*n*	Gender m/f	Age	Duration of epilepsy (years)	Age at onset	Number of seizures (per month)
Control	6	3/3	43.8 (31-60)	-	-	-
TLE – HS	6	3/3	38.8 (25-62)	22.8 (15-61)	9.0 (1-27)	12.8 (2-25)

TLE, temporal lobe epilepsy; HS, hippocampal sclerosis. Values are given as mean (range).

Table S2. Related to Figure 4. List of selected proteins with altered cell surface exposure in SorCS2-deficient neurons.

GENE	PROTEIN	Ratio (S2KO/WT)	*p*-value	BH-adjusted *p*-value (FDR 0.15)
Sorting receptors, cell surface receptors				
Sorcs2	SorCS2	0	0.001	0.044
Gpr98	G-protein coupled receptor 98	>10	0.001	0.048
Sort1	Sortilin	2.97	0.013	0.123
Grm3	Metabotropic glutamate receptor 3	2.35	<0.001	0.004
Egfr	Epidermal growth factor receptor	2.35	0.014	0.124
Endolysosomal system, intracellular trafficking				
Lamp1	Lysosome-associated membrane glycoprotein 1	0.11	0.001	0.044
Igf2r	Cation-independent mannose-6-phosphate receptor	>10	<0.001	<0.001
Aak1	AP2-associated protein kinase 1	>10	<0.001	<0.001
Tom1l2	TOM1-like protein 2	>10	<0.001	<0.001
Stx12	Syntaxin-12	>10	0.002	0.051
Tfg	TFG	>10	0.012	0.121
Rph3a	Rabphilin-3A	>10	0.013	0.123
Sh3gl1	Endophilin-A2	>10	0.016	0.130
Cyth2	Cytohesin-2	3.46	0.007	0.108

Ap3d1	AP-3 complex subunit delta-1	3.14	0.004	0.081
Snx16	Sorting nexin-16	2.87	0.015	0.124
Flot1	Flotillin-1	2.29	0.021	0.144
Adhesion				
Cd99l2	CD99 antigen-like protein 2	>10	<0.001	<0.001
Efnb1	Ephrin-B1	>10	0.013	0.123
Adam22	Disintegrin and metalloproteinase domain-containing protein 22	2.28	<0.001	<0.001
Signal transduction				
Src	Neuronal proto-oncogene tyrosine-protein kinase Src	0.14	0.004	0.079
Mras	Ras-related protein M-Ras	>10	0.010	0.120
Dab2ip	Disabled homolog 2-interacting protein	>10	0.012	0.122
Transporters, channels				
Slc17a7	Vesicular glutamate transporter 1	0.45	0.011	0.121
Cacng8	Voltage-dependent calcium channel gamma-8 subunit	3.14	0.011	0.121
Scn3b	Sodium channel subunit beta-3	2.57	0.011	0.121
Other				
Tmem65	Transmembrane protein 65	0	0.012	0.122
Serpine2	Glia-derived nexin	0	0.013	0.123
Arl6ip5	PRA1 family protein 3, JWA	>10	<0.001	0.014
Csmd1	CUB and sushi domain-containing protein 1	>10	<0.001	0.043
Tapt1	Transmembrane anterior posterior transformation protein 1	>10	0.002	0.051
Palm	Paralemmin-1	2.29	0.003	0.074

Ratio(S2KO/WT): 0, protein detected only in WT; >10, protein detected only in S2KO

SUPPLEMENTAL FIGURES

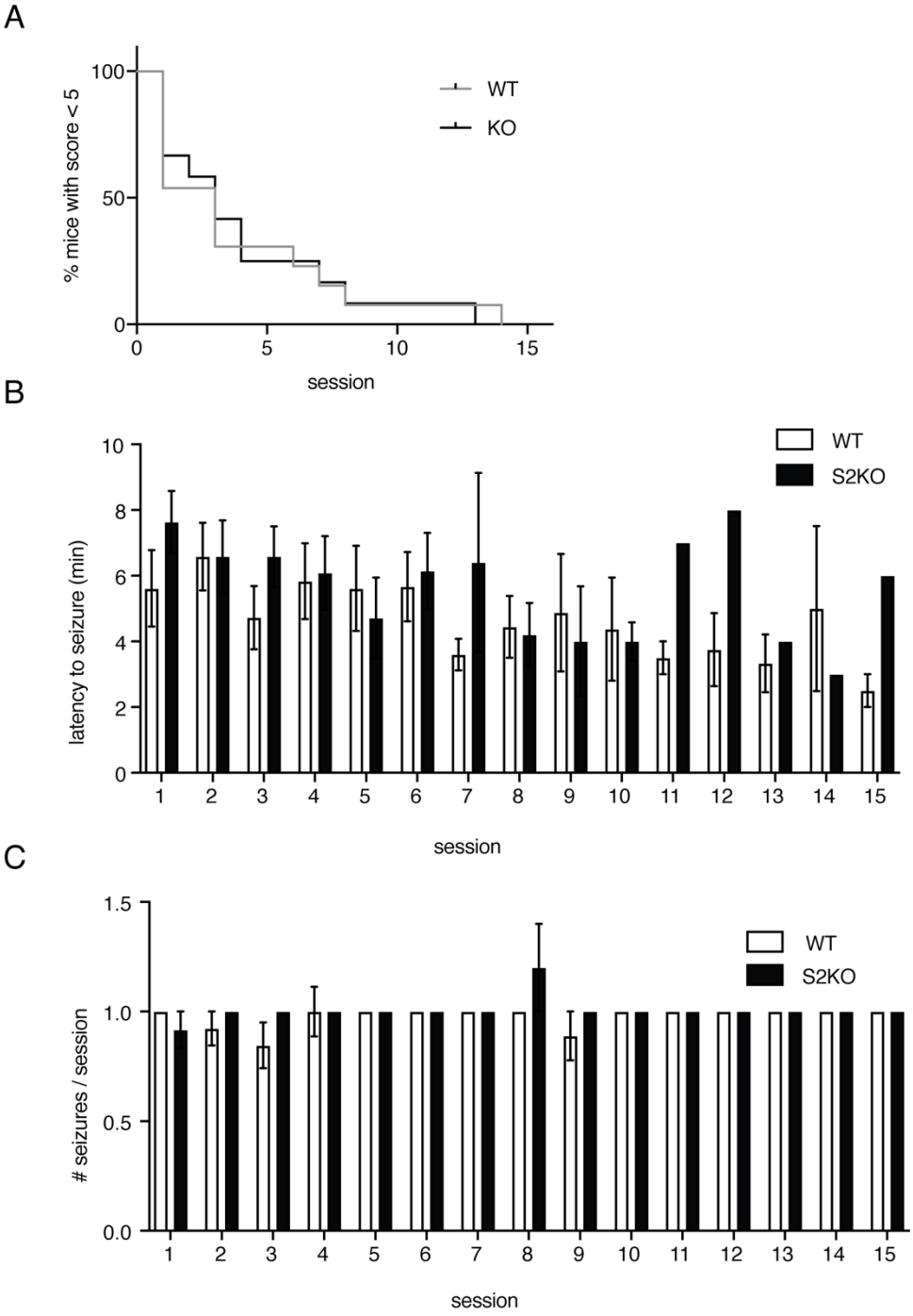

Figure S1. Related to Figure 1. WT and S2KO mice show similar epileptic phenotypes upon PTZ kindling.

(A) Fraction of WT and S2KO mice that did not respond with severe seizure (score 5) to PTZ injection in course of the PTZ kindling experiment. n=12-13 mice per group.

(B) Latency from PTZ injection to seizure in all sessions of PTZ kindling experiments. Mean ± SEM; n=12-13 mice per group.

(C) Number of seizures in each session of the PTZ kindling experiments. Mean ± SEM; n=12-13 mice per group.

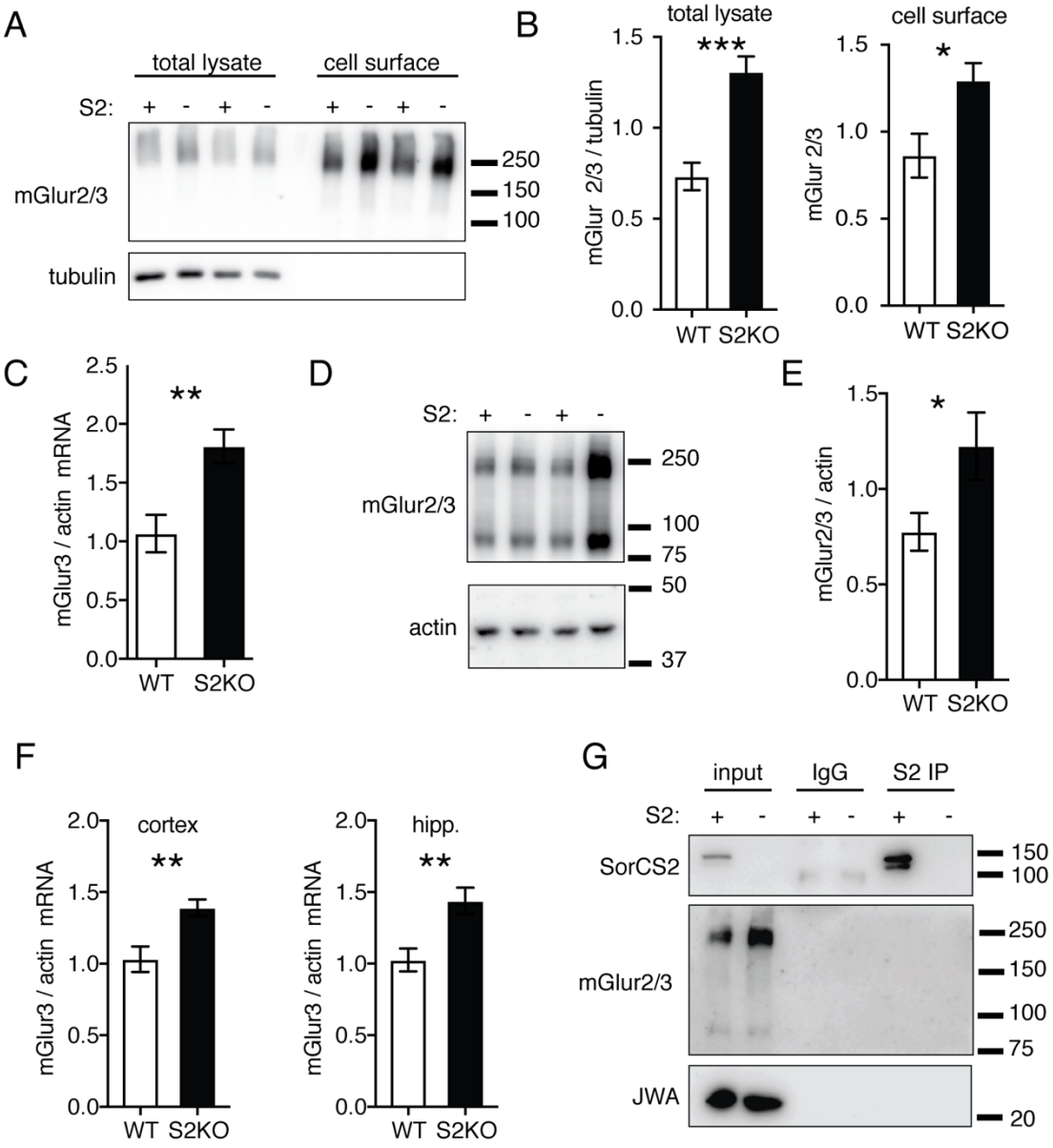

Figure S2. Related to Figure 4. mGlur3 and JWA are indirect targets of SorCS2.

(A) Western blot analysis of total and cell surface levels of mGlur2/3 in WT (S2+) and S2KO (S2-) primary neurons (mixed cortical/hippocampal cultures). The primary antibody detects both mGlur2 and mGlur3 (multimers, >200kDa, predominant form in cultured neurons). Tubulin is shown as a loading control for total cell lysates.

(B) Quantification of mGlur2/3 levels in neuronal lysates and cell surface fractions of WT and S2KO primary neurons as exemplified in (A). Mean ± SEM; n=5-6 independent neuronal preparations per genotype; *, $p<0.05$; **, $p<0.01$ (unpaired t-test).

(C) mGlur3 mRNA levels in primary WT and S2KO neurons (mixed cortical/hippocampal cultures) as assessed by quantitative (q) RT-PCR (relative to actin mRNA). Mean ± SEM; n=5-6 independent neuronal preparations per genotype; **, $p<0.01$ (unpaired t-test).

(D) Western blot analysis of mGlur2/3 levels in P2 brain fractions (crude membranes including plasma membrane) from WT (S2+) and S2KO (S2-) mice. The antibody detects both mGlur2 and mGlur3 (monomers, 90 kDa; multimers, >200 kDa). Actin is shown as loading control.

(E) Quantification of mGlur2/3 levels in P2 brain fractions (normalized to actin levels) as exemplified in (D). Mean ± SEM; n=7 mice per genotype; *, $p<0.05$ (unpaired t-test).

(F) mGlur3 mRNA levels in brain cortex and hippocampus (hipp.) of WT and S2KO mice as assessed by qRT-PCR (relative to actin). Mean ± SEM; n=9 mice per group; **, $p<0.01$ (unpaired t-test).

(G) Western blot analysis of co-immunoprecipitation experiments. SorCS2 was immunoprecipitated from mouse hippocampal lysates of wild-type (S2+) but not S2KO (S2-) mice (S2 IP). No immunoprecipitation was seen in wild-type brain tissue using non-immune IgG (IgG). Although detected in the input samples using specific antisera, mGlur2/3 and JWA were not co-immunoprecipitated with anti-SorCS2 antibodies (S2 IP).

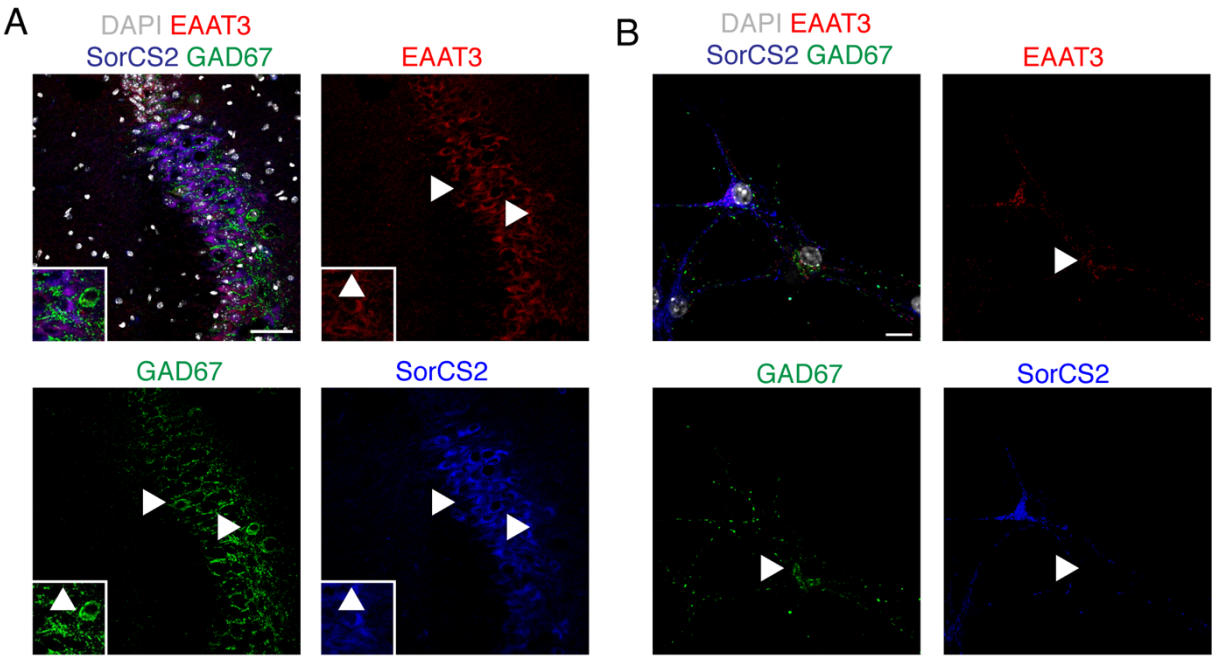

Figure S3. Related to Figure 4. SorCS2 is not expressed in GAD67-positive inhibitory neurons.

(A) Representative confocal images showing immunodetection of SorCS2 (blue), EAAT3 (red), and the GABA-ergic neurons marker GAD67 (green) in the CA2 region of the mouse hippocampus. Sections were counterstained with DAPI (white). Both individual channels and merged immunostainings are shown. SorCS2 is not expressed in GAD67-positive neurons (indicated by arrowheads). Scale bar: 50 μm.

(B) Representative images showing immunodetection of SorCS2 (blue), EAAT3 (red), and GABA-ergic neuron marker GAD67 (green) in cultured hippocampal neurons. Cells were counterstained with DAPI (white). EAAT3 is detected in GAD67-positive neurons (indicated by arrowheads), but SorCS2 is only expressed in GAD67-negative neurons. Scale bar: 10 μm.

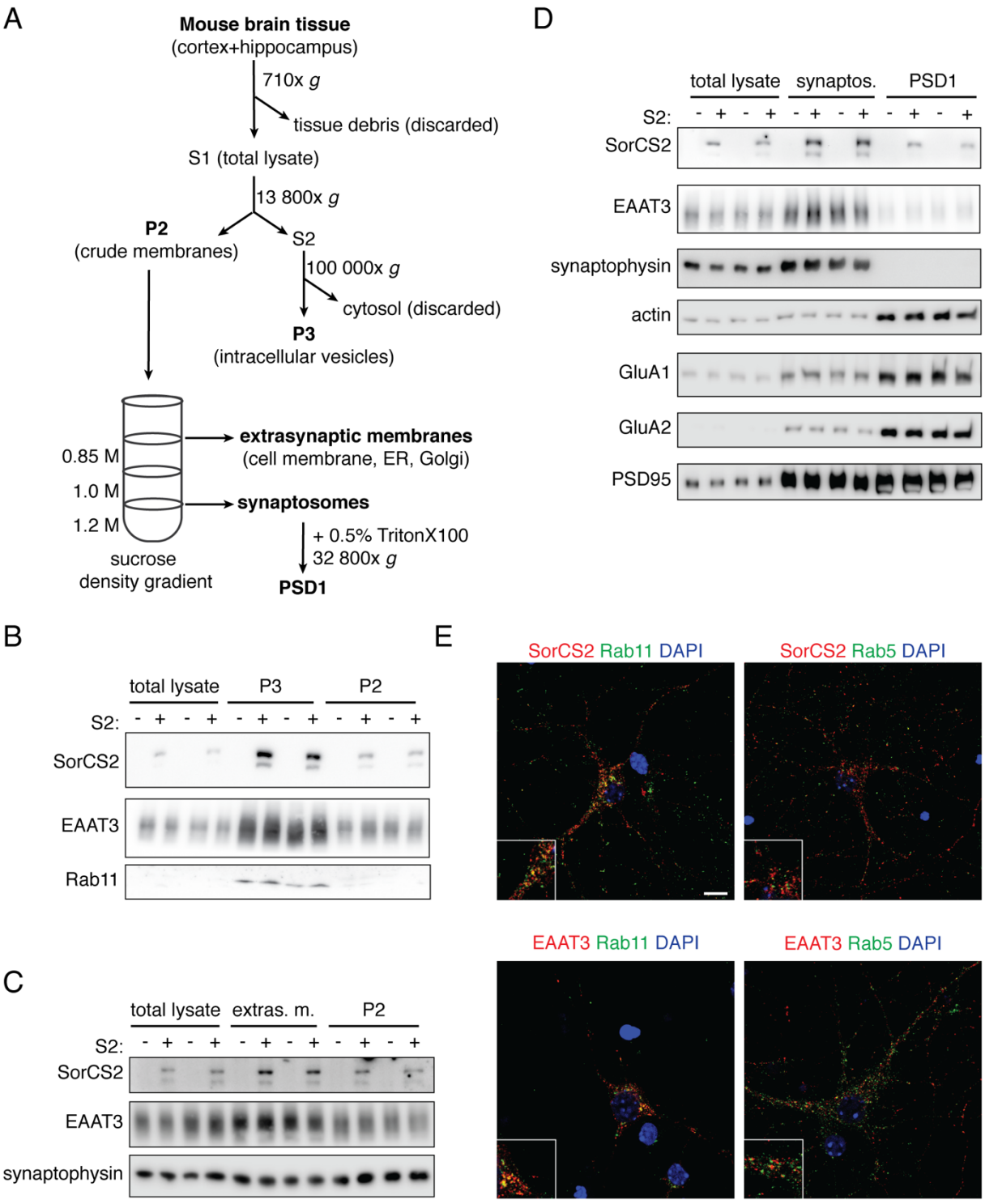

Figure S4. Related to Figure 5. SorCS2 and EAAT3 are enriched in the same subcellular compartments in the mouse brain.

(A) Schematic depiction of subcellular fractionation procedure. Mouse brain tissue (cortex and hippocampus) was subjected to serial centrifugations to obtain P2 (crude membranes), intracellular vesicles (P3), extrasynaptic membranes (including plasma membrane), synaptosomes, as well as post-synaptic density (PSD1).

(B-D) Western blot analyses of distribution of SorCS2 and EAAT3 in subcellular brain fractionations from wild-type (S2+) and S2KO mice (S2-). In tissue lysates and fractions, only monomeric EAAT3 is present. SorCS2 and EAAT3 are enriched in intracellular vesicle fraction P3 (B), extrasynaptic membranes (extras. m.; C), as well as synaptosomes (synaptos; D). The efficiency of fractionation is documented by enrichment of marker proteins Rab11 (P3), synaptophysin (synaptosomes), and GluA1, GluA2, and PSD95 (PSD1). Actin served as loading control.

(E) Immunodetection of SorCS2 (red, upper panels) and EAAT3 (red, lower panels) together with markers of recycling endosome (Rab11, green) and early endosome (Rab5, green) in primary WT hippocampal neurons. Cell were counterstained with DAPI (blue). Higher magnification insets are given. Scale bar: 10 μm.