

Supplementary Figure S1

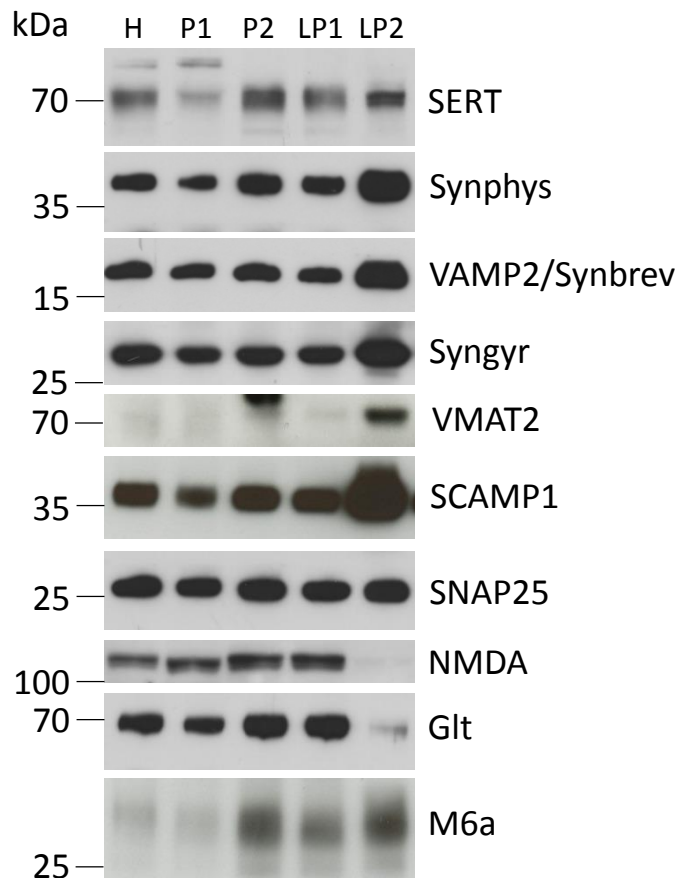
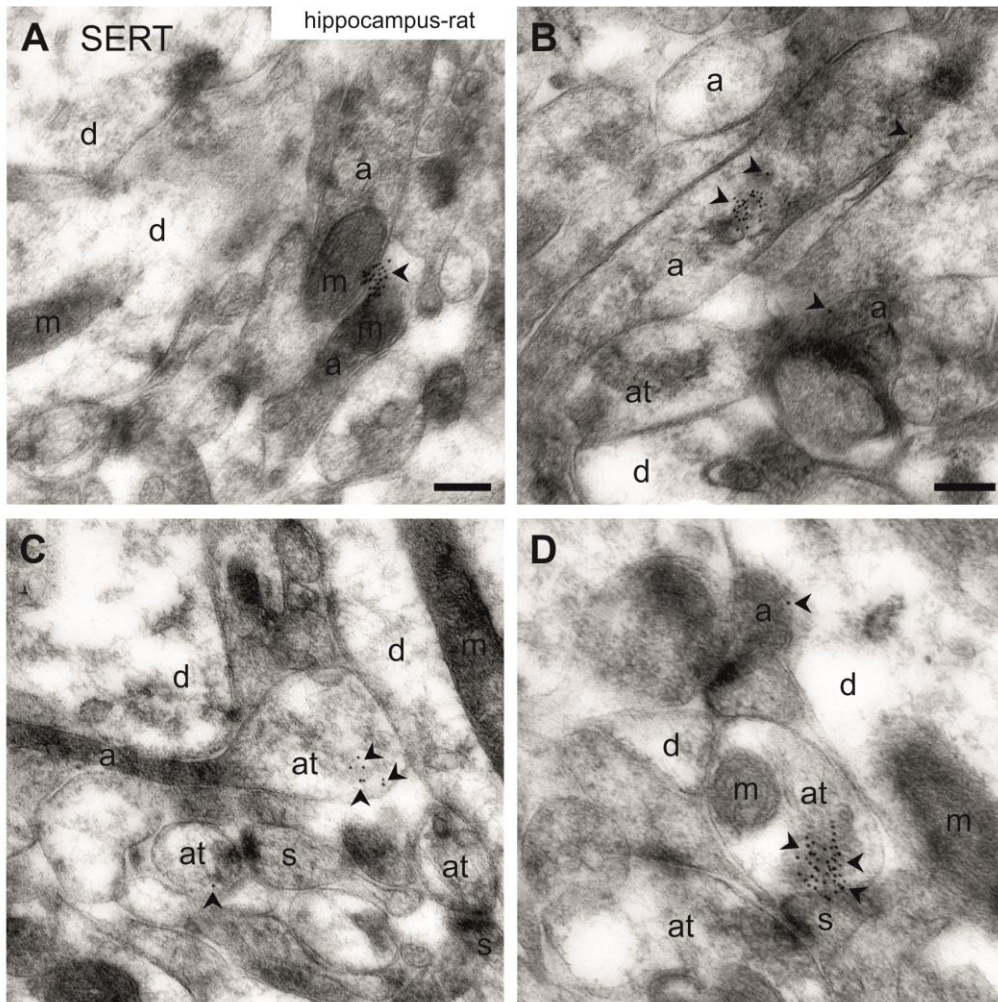


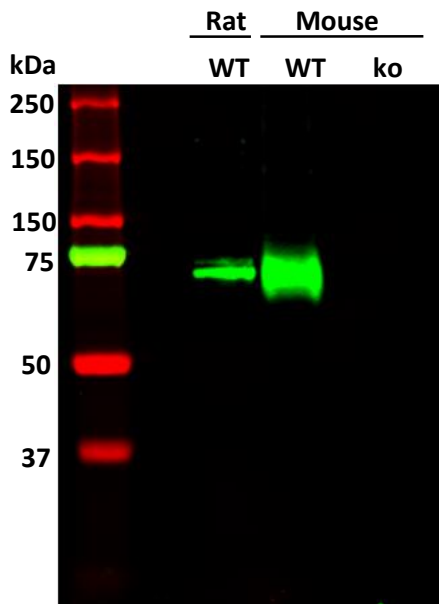
Figure S1: Subcellular fractionation of mouse brain tissue was carried out similar to rat brain tissue as described in the Method section. Resident synaptic vesicle proteins, such as synaptophysin, synaptobrevin (VAMP2) or the vesicular monoamine transporter VMAT2, show enrichment in the LP2 fraction relative to P2 and are less abundant in LP1 (compare to Fig. 4A). Typical plasma membrane proteins, such as the NMDA receptor and Glt, are de-enriched in LP2 relative to P2, and more abundant in LP1. SERT and the SERT interacting protein GPM6a are both detected at similar level in P2 and LP2.

Supplementary Figure S2



Supplementary Figure 2. Ultrastructural localization of SERT in rat hippocampus. A: Electron micrograph illustrates axonal (a, longitudinal section) localization of SERT (10 nm immunogold particles, forked arrowhead) in the CA1 area of the rat hippocampus. Note that the gold labeling is in close apposition to the mitochondrial membrane (m). Scale bar, A: 500 nm. B: Serotonergic axon (a) showing immunogold labeling (10 nm gold; forked arrowheads) for SERT found in cytoplasmic structures and occasionally on axonal membrane in CA1 area of rat hippocampus. Axon terminal (at) making symmetric contact to the dendritic shaft (d) was not labelled. C: A serotonergic terminal (at) forming symmetric contact onto the dendritic shaft (d) in the CA1 area of rat hippocampus. The postembedding immunogold labeling for SERT (10 nm gold; forked arrowheads) was found at lower level presynaptically on synaptic vesicle membrane and in the plasma membrane of the axon terminal. Forked arrowhead shows also gold particle on presynaptic membrane in axon terminal (at) making asymmetric contact to the spine (s). D: Serotonergic axon terminal (at) making symmetric contact with the dendritic shaft (d) in CA1 area of the rat hippocampus. Forked arrowheads indicate immunogold labeling for SERT (10 nm gold) found on the membrane of synaptic vesicles. Glutamatergic axon terminal (at) making asymmetric contact to the spine (s) was not labeled. Scale bar, B-D: 200 nm. Abbreviations: a, axon; at, axon terminal; d, dendritic shaft; m, mitochondrion; s, spine.

Supplementary Figure S3



Supplementary Figure S3: Western blot analysis verification of SERT antibody specificity.

P2 membrane fractions were prepared from frozen wildtype and SERT knockout mouse brain, as well as wildtype rat brain, and loaded onto a 10% SDS polyacrylamide gel as indicated. Western blot analysis was carried out using rabbit anti-SERT antibody (Synaptic Systems) and fluorescence labelled secondary IgG antibody DyLight 800 Goat anti-Rabbit (gree, Thermo Scientific). The membrane was scanned using the LICOR Odyssey system (LICOR Biosciences). [This figure was generated by Eric Brown, University College Dublin; SERT knockout brain tissue was a generous gift of Harald Sitte, University of Vienna]

Supplementary Table S1: Extended mass spectrometry data

Protein_ID	Affinity purification run 1				Affinity purification run 2				GST-CSERT				GST-N100-SERT				GST-N115-SERT			
	Score	#Pep	#Uniq	%Cov	Score	#Pep	#Uniq	%Cov	Score	#Pep	#Uniq	%Cov	Score	#Pep	#Uniq	%Cov	Score	#Pep	#Uniq	%Cov
AT1B1					109.84	4	4	20.72					68.32	1	1	4.61	46.03	1	1	3.59
1433T					101.02	4	1	16.33									106.02	5	2	20.82
PPIA	81.54	3	2	21.95													157.55	6	6	34.15
GPM6B	132.5	7	7	18.9	141.1	5	4	17.99												
CNTN2	110.98	7	1	8.17																
AT1B2	237.43	18	0	18.83	77	2	2	4.83												
FYN	72.66	2	2	4.84																
CNRP1	66.2	1	1	9.76																
PGM1	25.84	1	1	2.14																
STXB1	272.48	30	30	50.17	210.48	23	22	39.56	104.11	7	7	13.97								
TBB2A					259.3	22	4	66.52					221.69	17	3	34.83				
ACTB					247.02	20	1	66.4					126.55	4	2	14.13	219.09	21	6	42.00
EAA2	245.64	18	18	23.04	209.65	11	11	25.31	31.96	3	3	5.24								
GBB1					242.3	13	1	57.35	49.14	1	1	2.94	104.67	2	1	6.18	124.74	5	2	15.00
TBB3					237.8	17	3	42.89					217.74	12	2	32				
TBA4A					223.12	15	3	44.2					214.7	13	1	32.88	155.97	9	2	24.00
GRP75	223.01	16	1	27.84					217.31	35	2	45.21								
VPP1	220.09	18	1	24.46	148.04	11	11	14.08	33.45	3	3	4.65								
SYT1	188.38	11	9	28.5	219.38	14	10	42.28	150.37	9	8	24.47								
DPYL2	212.69	18	16	41.96					210.43	27	20	56.82								
TBB6					209.25	13	1	32.66					189.75	6	1	17.9	159.48	8	1	21.00
AT2B4	203.92	9	3	10.22	123.79	3	0	3.57	145.16	8	1	6.65								
GBB2					202.62	10	4	43.82									112.01	5	2	15.00
AT2B3	197.53	10	3	10.41	117.89	4	1	3.97									36.8	1	1	1.00
DDAH2	189.03	8	8	35.79					32.64	2	2	7.72								
VAMP2	188.89	6	3	54.31													108.98	2	2	20.69
1433Z					184.03	9	7	46.53									115.25	8	5	29.00
G3P	106.24	3	1	18.02	170.35	5	0	26.43					155.38	5	2	20.12				
S6A11	170.08	10	2	16.59	43.63	1	1	2.55	48.66	7	4	5.58					145.85	3	3	4.00
STX1B	169.07	9	8	33.33	146.93	7	6	28.47									28.47	1	1	3.47
GNAI2	128.39	6	0	18.31	167.32	8	3	29.01	98.92	6	2	18.31								
SNP25	161.02	9	9	42.72	108.85	4	4	24.27									77.36	2	2	12.14
NIPS1					159.45	10	10	44.37									125.96	6	5	27.00
GPM6A	94.55	3	3	11.51	155.63	9	8	26.26									60.67	1	1	4.00
SEPT7	154.96	8	7	18.35					43.88	2	1	4.36								
SHPS1	150.4	10	10	19.65													53.73	1	1	2.00
ODPA	147.35	5	5	12.31									68.51	1	1	2.31				
ADT1	146.73	9	3	29.19					154.09	17	12	46.98								
NIPS2					145.91	5	5	23.84									92.24	3	2	12.10
SV2A	142.69	5	4	8.49	88.61	3	3	4.72	46.81	3	3	5.26					80.26	2	2	3.00
CMC1	142.59	6	3	10.64													45.37	1	1	2.00
ATPO	136.85	6	2	37.56									173.94	5	2	33.33	136.39	6	3	29.11
GNAQ	135.11	7	5	19.22	119.51	7	4	24.51	55.51	2	2	8.36								
STX1A	134.72	7	6	27.78	94.25	3	2	12.5									46.08	1	1	4.86

Protein_ID	Affinity purification run 1				Affinity purification run 2				GST-CSERT				GST-N100-SERT				GST-N115-SERT			
	Score	#Pep	#Uniq	%Cov	Score	#Pep	#Uniq	%Cov	Score	#Pep	#Uniq	%Cov	Score	#Pep	#Uniq	%Cov	Score	#Pep	#Uniq	%Cov
GNAI1					132.82	6	1	16.95									72.78	2	1	6.00
AT2B2	237.43	18	0	18.83	132.73	4	1	4.59	165.87	12	6	10.22								
COX41	129.42	7	5	36.69													135.22	3	3	20.71
HCD2	120.76	2	0	14.18	67.17	2	1	9.2									49.36	1	1	4.60
CH60	119.89	6	6	11.17					256.12	49	1	59.69					140.65	6	6	10.00
RTN1	118.98	4	1	8.1	88.25	1	1	1.67	35.43	2	2	2.96								
NOMO1	117.89	4	4	4.12					46.04	4	4	3.87								
COF1	116.87	3	3	25.9													99.44	3	3	13.86
ATP5H	116.83	5	2	44.72													92.87	2	2	18.63
TOM70	114.6	4	1	8.69													103.54	5	1	6.00
CAMKV	113.92	4	4	14.09					126.27	7	7	20.83								
SNAB	109.8	4	4	16.44													142.93	7	4	20.13
VISL1					107.38	4	4	24.61									36.23	1	1	5.76
S12A5	107.15	6	3	5.27													48.84	1	1	0.97
ODPB	103.69	3	3	12.26					208.91	19	1	44.29								
MPCP	102.06	5	0	16.01	44.75	1	1	3.37	95.78	6	1	20.22								
RAB2A	96.96	3	3	20.28					103.15	2	2	14.15					128.02	4	4	18.87
PYGM	93.33	4	1	5.58													48.44	1	1	1.00
BDH	89.4	1	1	5.83													164.28	8	3	25.66
RAB1B	84.85	5	3	27.36	46.04	1	0	5.47									101.46	3	1	14.93
RL12	83.88	4	4	28.48	61.76	2	2	15.15									28.07	1	1	5.45
RTN3	81.45	3	1	3.19	70.12	2	0	2.45									88.9	2	1	2.00
SEP11					81.31	3	1	6.03	67.96	4	3	12.3								
1433B	104.34	5	0	17.07	79.04	4	1	16.26									109.7	4	1	12.00
PHB	79.04	2	2	8.82													133.11	6	6	22.43
KPYM	76.68	2	2	4.9													53.78	2	1	3.00
SYNPO	76.33	2	2	2.91					101.77	5	5	6.98								
CAZA2	75.16	2	2	8.74					82.55	4	4	25.52					35.49	1	1	3.00
HPCA	74.47	2	1	10.88													28.33	1	1	5.18
PRDX1	72.09	2	1	10.55	35.28	1	1	4.02									66.16	2	1	10.55
VAPA	65.66	2	2	10.44													88.96	2	2	11.24
MPP2	63.21	2	2	3.99					52.93	3	3	5.8								
H2A2B	60.69	2	1	12.31													47.16	1	1	6.92
SNA A					56.81	1	1	3.73									131.54	5	2	20.00
SDHB	59.17	1	0	4.26	56.29	1	1	4.26									164.83	8	5	27.00
PP14C	54.12	3	3	12.2					53.54	8	8	23.78								
SYN2					52.63	2	1	6.31	154.88	10	8	30.38								
NDUB5	49.8	1	1	5.82													41.06	1	1	5.82
SNG1	49.67	1	1	5.13													77.04	2	2	8.00
ODO1	49.61	1	1	1.37					119.07	6	6	7.14								
RAP1B	47.9	1	1	6.52									86.09	1	1	5.98				
TPIS					45.19	1	1	5.02									222.31	17	6	76.71
SIR2					42.94	1	1	3.34	53.7	2	2	6.43								
EVX2					41.4	1	1	4	40.21	9	9	13.47								
SYNJ1	38.98	1	1	0.76					82.65	7	2	4.13								
RP3A	38.63	1	1	3.08					30.99	2	2	5.56								
HSP7C													209.62	7	3	10.99				

Protein_ID	Affinity purification run 1				Affinity purification run 2				GST-CSERT				GST-N100-SERT				GST-N115-SERT			
	Score	#Pep	#Uniq	%Cov	Score	#Pep	#Uniq	%Cov	Score	#Pep	#Uniq	%Cov	Score	#Pep	#Uniq	%Cov	Score	#Pep	#Uniq	%Cov
NDUA4													117.38	4	4	47.56				
VDAC2																	116.16	5	4	18.64
DYHC1									88.83	9	9	2.26								
MAP1A									66.71	8	1	3.6								
COX2																	65.29	2	2	7.49
MCCB									64.44	2	2	4.8								
PDIA3																	28.15	1	1	2.00
NSF					158.37	8	1	11.29												
MARCS	89.94	3	2	13.92																
SCAM5	64.77	2	2	8.51																
SCAM1					37.3	1	1	6.8												
SC24A	31.88	1	1	0.73																
NOS2					23.72	1	1	0.87												
PP2BA									106.94	7	4	16.89					68.4	1	1	2.00

Column definitions

Protein_ID Uniprot/Swissprot protein identifier
Score protein identification confidence score
#Pep number of total peptides contributing to protein identification
#Uniq number of unique peptides
%Cov percentage of the protein sequence covered by identified peptides