

Supplementary Material for

Disruption of the sodium-dependent citrate transporter SLC13A5 in mice causes alterations in brain citrate levels, neuronal network excitability and increased seizure propensity

Christine Henke, Kathrin Töllner, R. Maarten van Dijk, Nina Miljanovic, Thekla Cordes, Friederike Twele, Sonja Bröer, Vanessa Ziesak, Marco Rohde, Stefanie M. Hauck, Charlotte Vogel, Lisa Welzel, Tina Schumann, Diana M. Willmes, Anica Kurzbach, Nermeen N. El-Agroud, Stefan R. Bornstein, Susanne A. Schneider, Jens Jordan, Heidrun Potschka, Christian M. Metallo, Rüdiger Köhling, Andreas L. Birkenfeld, Wolfgang Löscher

Corresponding author name: **Wolfgang Löscher** (wolfgang.loescher@tiho-hannover.de)

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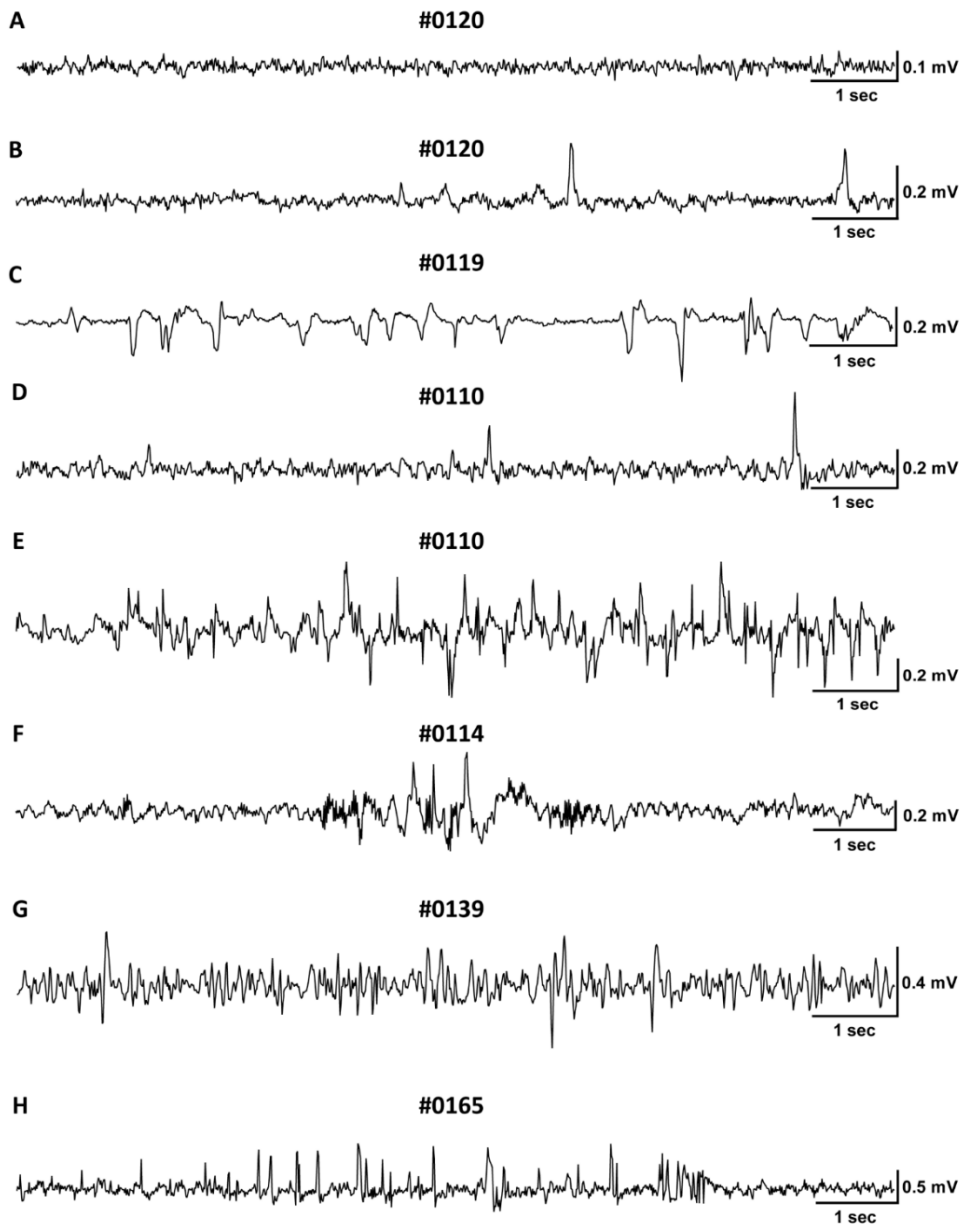


Fig. S1

Representative intrahippocampal EEG recordings in *Slc13a5*^{+/+} and *Slc13a5*^{-/-} mice.

The recordings are 10 sec cutouts of the 30 sec EEG recordings shown in Fig. 1, but are illustrated here with higher time resolution. Note the different amplitudes (in mV) of the recordings. See Fig. 1 legend for more details. Representative examples of the electroclinical seizures recorded in *Slc13a5*^{+/+} and *Slc13a5*^{-/-} mice are shown in movies S1-S4.

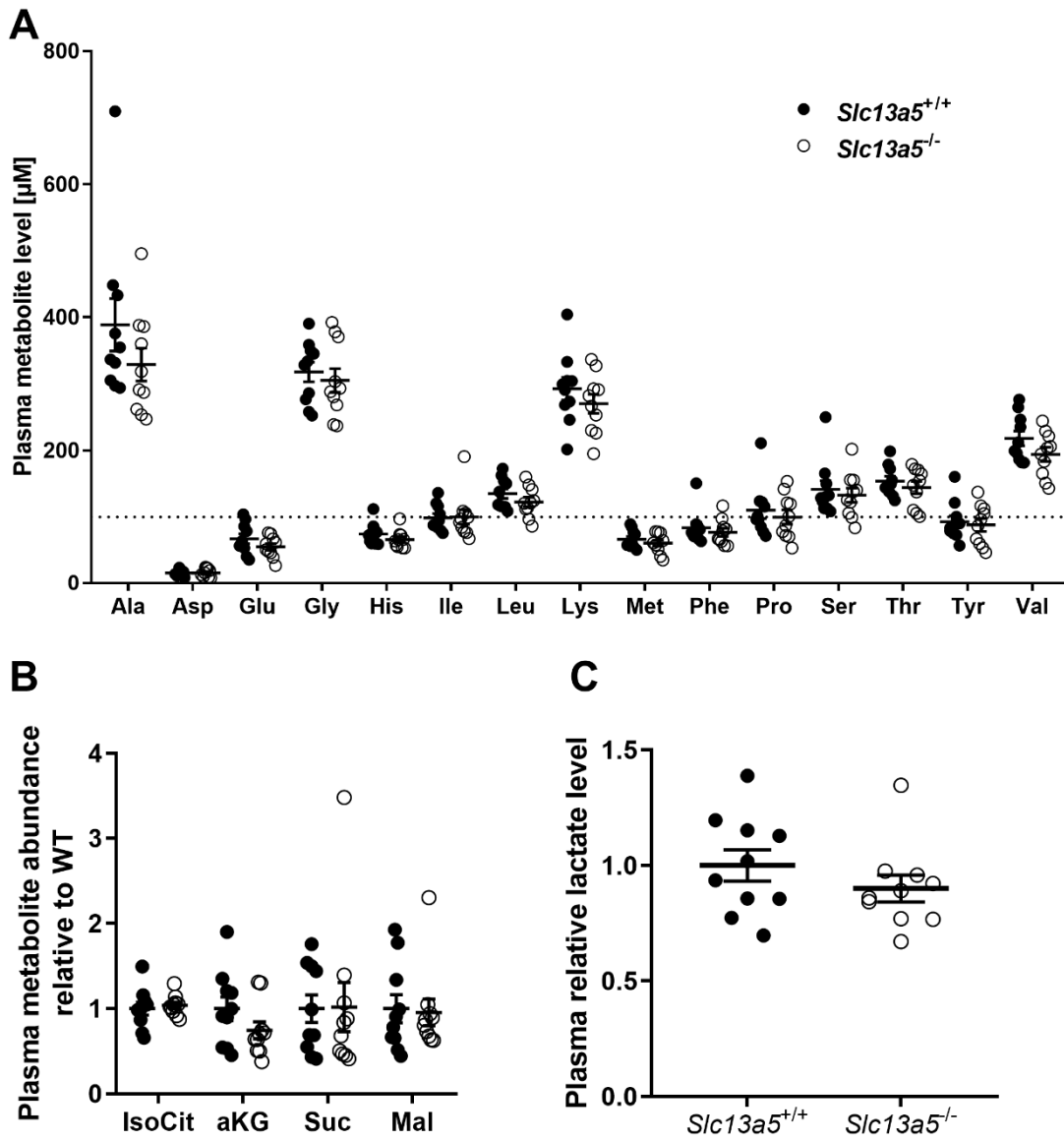


Fig. S2

Metabolomics in plasma. Amino acids (A), TCA-cycle intermediates (B) and lactate (C) in plasma from *Slc13a5*^{+/+} (closed circles) and *Slc13a5*^{-/-} (open circles) were analyzed by GC-MS. No significant differences were observed.

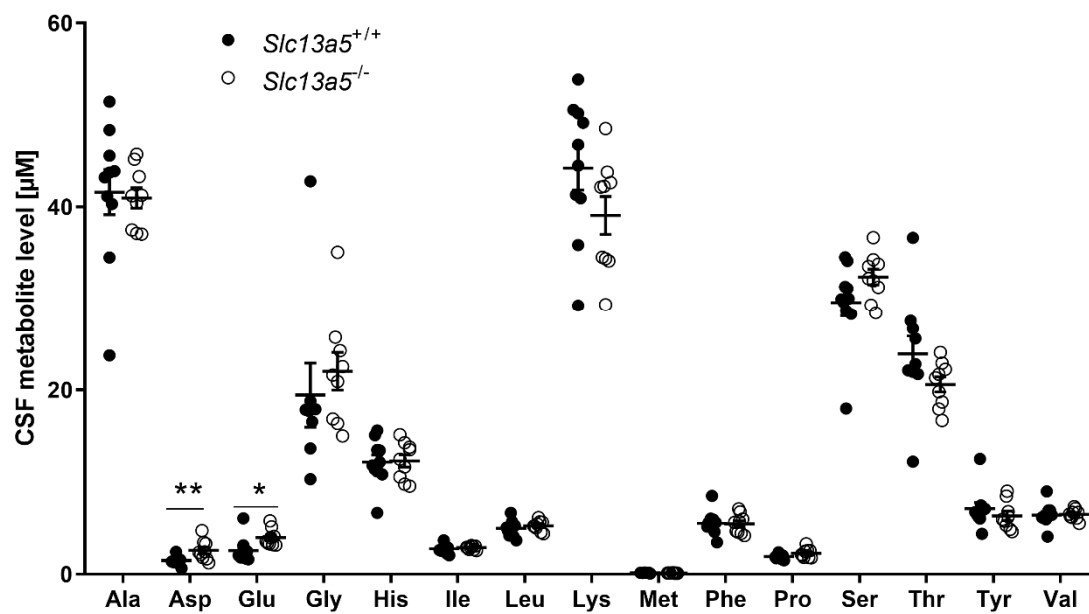


Fig. S3

Metabolomics in CSF. Amino acid analysis in CSF from *Slc13a5*^{+/+} (closed circles) and *Slc13a5*^{-/-} (open circles) were analyzed by GC-MS. No significant differences were observed.

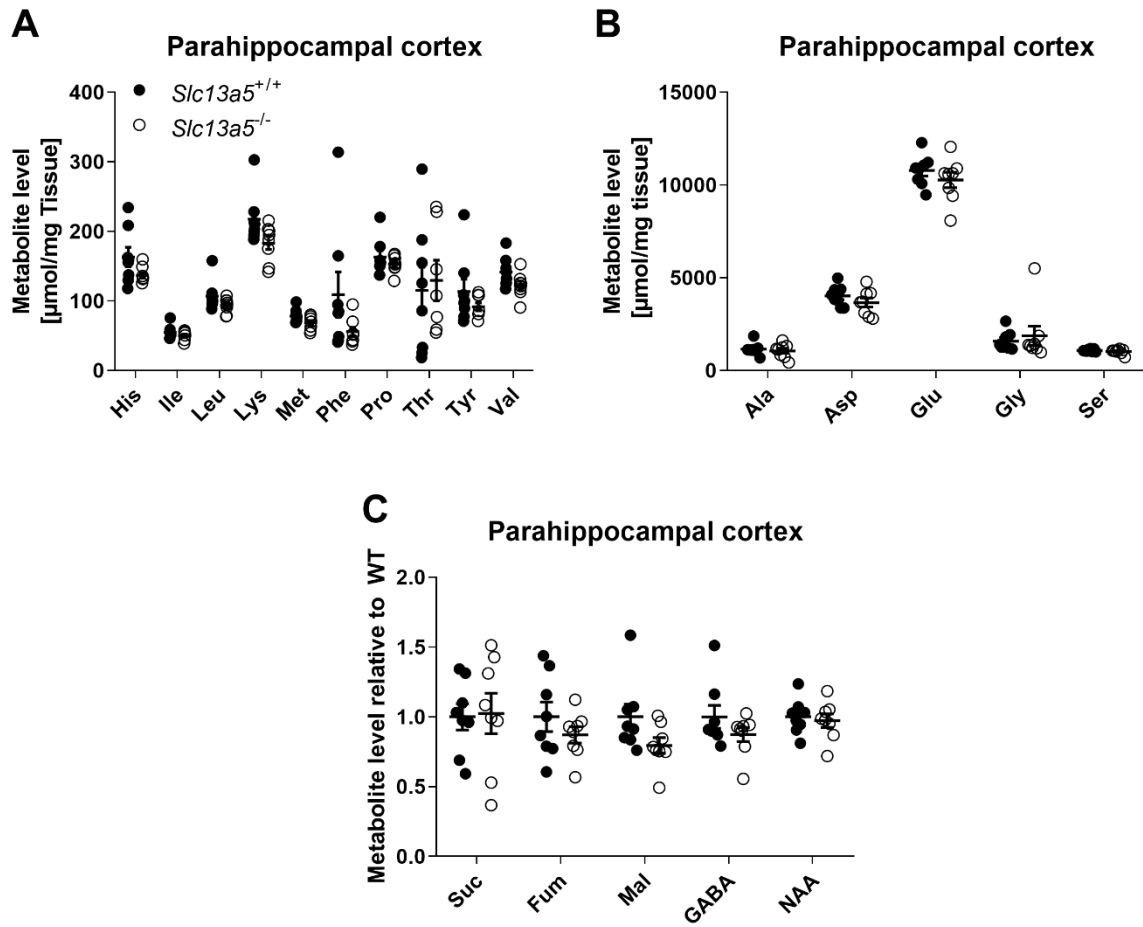


Fig. S4

Metabolomics in parahippocampal cortex. (A, B) Amino acids and (C) TCA Cycle intermediates, GABA and NAA in parahippocampal cortex (PHC) from *Slc13a5*^{+/+} (closed circles) and *Slc13a5*^{-/-} (open circles) were analyzed by GC-MS.

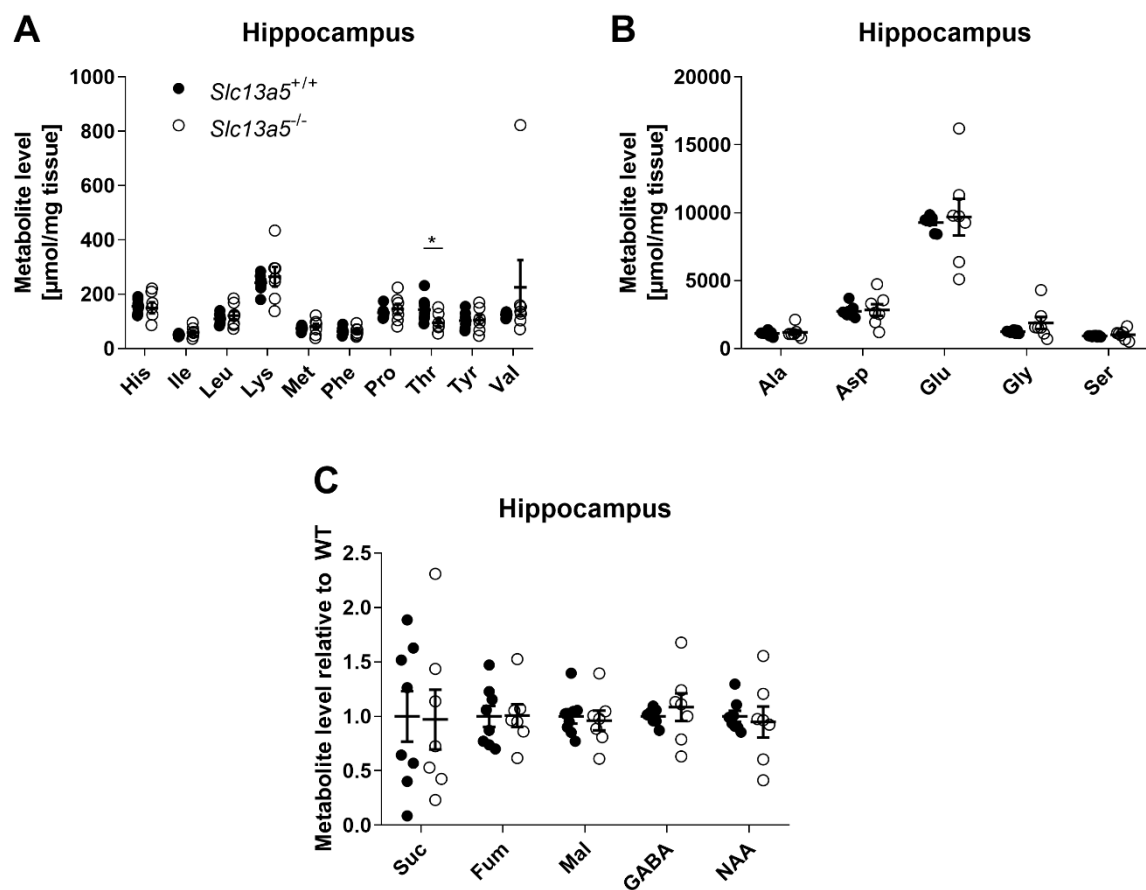


Fig. S5

Metabolomics in hippocampus. (A, B) Amino acids and (C) TCA Cycle intermediates, GABA and NAA in hippocampus (HC) from *Slc13a5*^{+/+} (closed circles) and *Slc13a5*^{-/-} (open circles) were analyzed by GC-MS. Threonin levels were significantly reduced in *Slc13a5*^{-/-}. * $p < 0.05$.

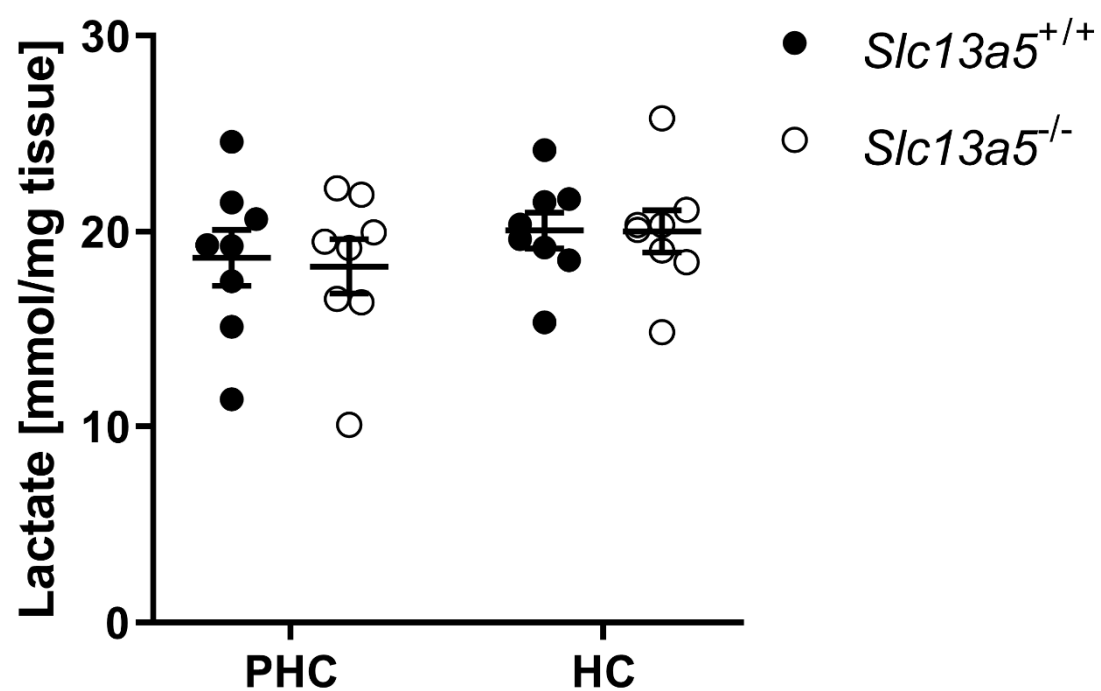


Fig. S6

Lactate in brain tissue. Lactate in parahippocampal cortex (PHC) and hippocampus (HC) from *Slc13a5*^{+/+} (closed circles) and *Slc13a5*^{-/-} (open circles) was analyzed by GC-MS. No significant differences.

Supplementary tables

Table S1: Stereotaxic coordinates for EEG electrode implantation into the right CA1 of the dorsal hippocampus of mice.

Genotype	Sex	Stereotaxic coordinates (in mm from bregma)		
		AP	L	DV
Wildtype (<i>Slc13a5</i> ^{+/+})	Male	-2.00	-1.50	-1.70
Wildtype (<i>Slc13a5</i> ^{+/+})	Female	-1.90	-1.50	-1.70
Knock out (<i>Slc13a5</i> ^{-/-})	Male	-1.80	-1.50	-1.70
Knock out (<i>Slc13a5</i> ^{-/-})	Female	-1.80	-1.50	-1.60

Table S2: Body weight

Genotype	Sex	n	Mean weight (range) [g]
Wildtype (<i>Slc13a5</i> ^{+/+})	Male	10	28.59 (26.5 - 30.0)***
Wildtype (<i>Slc13a5</i> ^{+/+})	Female	6	22.48 (20.3 - 2.8)***
Heterozygote (<i>Slc13a5</i> ^{+/-})	Male	8	29.80 (22.8 - 29.8)**
Heterozygote (<i>Slc13a5</i> ^{+/-})	Female	9	21.41 (19.7 - 23.2)**
Knock out (<i>Slc13a5</i> ^{-/-})	Male	8	24.26 (22.7 - 26.0)
Knock out <i>Slc13a5</i> ^{-/-})	Female	10	18.68 (15.7 - 20.5)

***p<0.001 vs. *Slc13a5*^{-/-}; ** p<0.01vs. *Slc13a5*^{-/-}

Table S3: PTZ seizure threshold determination

Parameter (mean \pm SD)	<i>Slc13a5</i> ^{+/+}			<i>Slc13a5</i> ^{+/-}			<i>Slc13a5</i> ^{-/-}		
	Male and female	Male	Female	Male and female	Male	Female	Male and female	Male	Female
First myoclonic twitch (mg/kg PTZ)	49.28 \pm 13.25	51.29 \pm 16.7	46.08 \pm 4.23	52.04 \pm 11.5	53.62 \pm 14.53	51.16 \pm 10.35	54.95 \pm 13.67	52.07 \pm 10.78	56.03 \pm 15.13
Clonus without loss of righting reflex (mg/kg PTZ)	58.44 \pm 12.67	60.34 \pm 16.47	55.78 \pm 4.38	58.46 \pm 12.65	61.12 \pm 15.35	56.8 \pm 11.47	67.95 \pm 16.46	59.23 \pm 8.04	72.94 \pm 18.43
Highest seizure severity (score)	4.2 \pm 1.4	4.6 \pm 1.6	3.4 \pm 0.5	3.9 \pm 1.2	3.6 \pm 0.5	4 \pm 1.5	5.1 \pm 1.5	4 \pm 0	5.5 \pm 1.6*
Mortality	2/13	2/8	0/5	1/14	0/5	1/9	4/11	0/3	4/8

*Significantly different from female *Slc13a5*^{+/+} mice (p=0.0163).

Table S4: Novelty-induced hypophagia test results

Parameter (mean \pm SD)	<i>Slc13a5</i> ^{+/+}	<i>Slc13a5</i> ^{+/-}	<i>Slc13a5</i> ^{-/-}
Distance moved (cm)	2755 \pm 656.3	3299 \pm 699.4*	2385 \pm 493.1
Velocity (cm/s)	9.2 \pm 2.2	11.0 \pm 2.3*	8.0 \pm 1.6
Latency to center (s)	87.3 \pm 65.5	83.3 \pm 70.6	75.3 \pm 57.5
Duration (s) in			
- center	4.0 \pm 4.2	2.7 \pm 2.1	3.6 \pm 2.8
- inner zone	43.1 \pm 15.0	42.7 \pm 25.9	48.0 \pm 25.4
- outer zone	252.9 \pm 18.4	254.7 \pm 27.0	248.4 \pm 26.4
Rearing	18 \pm 7.1	26 \pm 9.7*	14 \pm 7.9
Grooming	2 \pm 0.9	1.5 \pm 0.5	2 \pm 0.8
Food intake	0/16	0/16	1/14

* *Slc13a5*^{+/+} vs. *Slc13a5*^{+/-}, p<0.05

Table S5. Brain structural findings in Slc13a5^{-/-} and Slc13a5^{+/-} mice

Mouse #	Sex	Genotype	Morphological findings	Affected cerebral ventricles	Seizures in video/EEG (see Table 1)
0120	f	+/+	Moderate to profound enlargement of ventricles; no obvious displacement of hippocampus by enlarged ventricles	Lateral and third ventricles and aqueduct	No
0105	f	+/+	Moderate enlargement of ventricles; deformation/displacement of hippocampus by enlarged ventricles	Lateral and third ventricles and aqueduct	No
0119	f	+/+	Moderate enlargement of ventricles; moderate displacement of right hippocampus by enlarged ventricles	Lateral and third ventricles and aqueduct	Yes
0099	m	+/+	Profound enlargement of ventricles; deformation/displacement of hippocampus by enlarged ventricles	Lateral and third ventricles and aqueduct	No
0123	m	+/+	Profound enlargement of ventricles; partial deformation/displacement of hippocampus by enlarged ventricles	Lateral and third ventricles and aqueduct	No
0142	m	+/+	Moderate enlargement of ventricles; moderate/partial deformation/displacement of hippocampus by enlarged ventricles	Lateral and third ventricles and aqueduct	No
0163	m	+/+	Moderate to profound enlargement of ventricles; moderate displacement of hippocampus by enlarged ventricles	Lateral and third ventricles and aqueduct	No
0167	m	+/+	Moderate enlargement of ventricles; partial deformation/displacement of hippocampus by enlarged ventricles	Lateral and third ventricles and aqueduct	No
0107	f	-/-	Moderate to profound enlargement of ventricles; moderate/partial deformation/displacement of hippocampus by enlarged ventricles	Lateral and third ventricles and aqueduct	No
0114	f	-/-	Moderate to profound enlargement of ventricles; moderate/partial deformation/displacement of hippocampus by enlarged ventricles	Lateral and third ventricles and aqueduct	Yes
0129	f	-/-	Moderate to profound enlargement of ventricles; mild to moderate displacement of hippocampus by enlarged ventricles	Lateral and third ventricles and aqueduct	No
0139	m	-/-	Moderate to profound enlargement of ventricles; no obvious displacement of hippocampus by enlarged ventricles	Lateral and third ventricles and aqueduct	Yes
0154	m	-/-	Moderate to profound enlargement of ventricles; moderate/partial deformation/displacement of hippocampus by enlarged ventricles	Lateral and third ventricles and aqueduct	No
0165	m	-/-	Moderate to profound enlargement of ventricles; moderate/partial deformation and profound displacement of hippocampus by enlarged ventricles	Lateral and third ventricles and aqueduct	Yes

Table S6. Intrinsic properties of CA1 rat neurons with and without SLC13A blocker PF-06761281

	Control (n=18; mean \pm SEM)	PF-06761281 (2 μ M) (n=20; mean \pm SEM)
Resistance R (M Ω)	44.45 \pm 3.50	42.31 \pm 4.30
Time constant τ (ms)	13.23 \pm 1.14	16.80 \pm 0.84
AHP at +1nA (mV)	-7.17 \pm 0.87	-8.33 \pm 0.75
Voltage sag at -1nA (mV)	4.38 \pm 0.62	3.75 \pm 0.54

Table S7A: Overrepresented pathways, hippocampus – ConsensusPathDB

P-value	Pathway	Source	Overlap members
0.002	<i>Tryptophan metabolism</i>	EHMN	MAOA; ADHFE1; ALDH2; GSTK1
0.003	<i>GABA shunt</i>	HumanCyc	ALDH5A1; ABAT; GAD2
0.003	<i>4-aminobutyrate degradation</i>	HumanCyc	ALDH5A1; ABAT
0.008	<i>serotonin degradation</i>	HumanCyc	MAOA; ALDH2
0.000	<i>beta-Alanine metabolism - Homo sapiens (human)</i>	KEGG	MLYCD; ALDH2; ACADM; ABAT; HIBCH; GAD2
0.003	<i>Prion diseases - Homo sapiens (human)</i>	KEGG	C1QC; MAP2K1; FYN; PRKACA; PRNP
0.009	<i>N-Glycan biosynthesis - Homo sapiens (human)</i>	KEGG	DOLK; ALG2; ALG10B; DPM3
0.003	<i>Metformin Pathway, Pharmacodynamic</i>	PharmGKB	MLYCD; NDUFS1; NDUFS2; GPAM; NDUFV1
0.008	<i>Nicotine Pathway (Dopaminergic Neuron), Pharmacodynamics</i>	PharmGKB	STX1A; STX8; PRKACA; VAMP4; STX6
0.006	<i>Arf6 signaling events</i>	PID	IPCEF1; ARRB1; ACAP2; GNA11
0.010	<i>SHP2 signaling</i>	PID	MAP2K1; NTRK3; PRKACA; SOS1; IL6ST
0.000	<i>Complex I biogenesis</i>	Reactome	NDUFA6; NDUFV1; NDUFAF4; NDUFB6; NDUFB5; NDUFAF7; NDUFS1; NDUFS2; NDUFAF2; NDUFS8
0.002	<i>GABA synthesis, release, reuptake and degradation</i>	Reactome	STX1A; DNAJC5; GAD2; ABAT; ALDH5A1
0.003	<i>Metabolism of serotonin</i>	Reactome	MAOA; ALDH2
0.003	<i>Serotonin clearance from the synaptic cleft</i>	Reactome	MAOA; ALDH2
0.003	<i>Degradation of GABA</i>	Reactome	ALDH5A1; ABAT
0.003	<i>Respiratory electron transport</i>	Reactome	NDUFA6; NDUFV1; NDUFAF4; NDUFB6; NDUFB5; NDUFA4; NDUFS2; NDUFS1; NDUFAF7; NDUFAF2; NDUFS8
0.005	<i>Cell surface interactions at the vascular wall</i>	Reactome	SIRPA; SOS1; FYN; SLC7A5; ATP1B2; GRB7; L1CAM; PTPN6; F11R
0.007	<i>Nucleotide-binding domain, leucine rich repeat containing</i>	Reactome	TXNIP; IKBKB; SUGT1; CASP9

	<i>receptor (NLR) signaling pathways</i>		
0.008	<i>Dectin-2 family</i>	Reactome	FYN; MUC4
0.010	<i>EGF-Ncore</i>	Signalink	FYN; SPAG9; PRKACA; SIRPA; ARRB1
0.004	<i>Malonyl-coa decarboxylase deficiency</i>	SMPDB	MLYCD; HIBCH; ABAT; ACADM
0.004	<i>Malonic Aciduria</i>	SMPDB	MLYCD; HIBCH; ABAT; ACADM
0.004	<i>Propanoate Metabolism</i>	SMPDB	MLYCD; HIBCH; ABAT; ACADM
0.004	<i>Methylmalonic Aciduria Due to Cobalamin-Related Disorders</i>	SMPDB	MLYCD; HIBCH; ABAT; ACADM
0.008	<i>Folate malabsorption, hereditary</i>	SMPDB	ALDH1L1; MTHFD1L
0.008	<i>Methylenetetrahydrofolate Reductase Deficiency (MTHFRD)</i>	SMPDB	ALDH1L1; MTHFD1L
0.008	<i>Methotrexate Action Pathway</i>	SMPDB	ALDH1L1; MTHFD1L
0.008	<i>Folate Metabolism</i>	SMPDB	ALDH1L1; MTHFD1L
0.002	<i>Oxidative phosphorylation</i>	Wikipathways	NDUFA6; NDUFV1; NDUFB6; NDUFB5; NDUFA4; NDUFS1; NDUFS2; NDUFS8
0.008	<i>Interleukin-11 Signaling Pathway</i>	Wikipathways	IKBKB; MAP2K1; FYN; ITGA2; IL6ST

Table S7B: Overrepresented pathways, hippocampus – Genomatix

p-value	Pathways	Source	Overlap members
0.003	<i>Gamma-aminobutyric acid synthesis</i>	Genomatix	ABAT; ALDH5A1; GAD2
0.004	<i>Arf6 signaling events</i>	Genomatix	GNA11; ACAP2; IPCEF1; ARRB1
0.009	<i>il 3 signaling pathway</i>	Genomatix	PTPN6; SOS1
0.009	<i>Response to metal ions</i>	Genomatix	MT3; CSRP1

Table S8A: Overrepresented pathways, parahippocampal cortex – ConsensusPathDB

p-value	Pathway	Source	Overlap members
0.002	<i>extrinsic prothrombin activation pathway</i>	BioCarta	FGG; FGB
0.002	<i>fibrinolysis pathway</i>	BioCarta	FGG; FGB
0.003	<i>intrinsic prothrombin activation pathway</i>	BioCarta	FGG; FGB
0.002	<i>Statin Pathway, Pharmacodynamics</i>	PharmGKB	LDLR; APOA1
0.003	<i>Platelet Aggregation Inhibitor Pathway, Pharmacodynamics</i>	PharmGKB	ITGA2; FGG; FGB
0.001	<i>Beta1 integrin cell surface interactions</i>	PID	LAMB3; FGG; ITGA2; FGB
0.000	<i>Metabolism of fat-soluble vitamins</i>	Reactome	LDLR; GC; APOA1; VKORC1L1
0.002	<i>Scavenging of heme from plasma</i>	Reactome	ALB; APOA1
0.002	<i>O-linked glycosylation of mucins</i>	Reactome	WBSCR17; MUC4
0.002	<i>Extracellular matrix organization</i>	Reactome	ITGA2; NCSTN; CD151; LAMB3; FGG; FGB
0.002	<i>Platelet degranulation</i>	Reactome	TF; FGG; ALB; APOA1; FGB
0.002	<i>Chylomicron-mediated lipid transport</i>	Reactome	LDLR; APOA1
0.002	<i>Regulation of TLR by endogenous ligand</i>	Reactome	FGG; FGB
0.002	<i>Steroid hormones</i>	Reactome	FDXR; GC
0.003	<i>Response to elevated platelet cytosolic Ca²⁺</i>	Reactome	TF; FGG; ALB; APOA1; FGB
0.003	<i>Type I hemidesmosome assembly</i>	Reactome	CD151; LAMB3
0.003	<i>Prolactin receptor signaling</i>	Reactome	STAT5B; SH2B1
0.003	<i>Lipoprotein metabolism</i>	Reactome	LDLR; ALB; APOA1
0.004	<i>HDL-mediated lipid transport</i>	Reactome	ALB; APOA1
0.006	<i>Common Pathway of Fibrin Clot Formation</i>	Reactome	FGG; FGB
0.007	<i>Assembly of collagen fibrils and other multimeric structures</i>	Reactome	CD151; LAMB3
0.008	<i>Lipid digestion, mobilization, and transport</i>	Reactome	LDLR; ALB; APOA1

0.009	<i>Integrin cell surface interactions</i>	Reactome	ITGA2; FGG; FGB
0.009	<i>Metabolism of vitamins and cofactors</i>	Reactome	LDLR; GC; APOA1; VKORC1L1
0.010	<i>p130Cas linkage to MAPK signaling for integrins</i>	Reactome	FGG; FGB
0.010	<i>GRB2:SOS provides linkage to MAPK signaling for Integrins</i>	Reactome	FGG; FGB
0.010	<i>MET activates PTK2 signaling</i>	Reactome	LAMB3; ITGA2
0.010	<i>O-linked glycosylation</i>	Reactome	WBSCR17; MUC4
0.010	<i>Visual phototransduction</i>	Reactome	LDLR; APOA1; RDH12
0.007	<i>Coagulation</i>	SMPDB	FGG; FGB
0.007	<i>Bivalirudin Action Pathway</i>	SMPDB	FGG; FGB
0.007	<i>Argatroban Action Pathway</i>	SMPDB	FGG; FGB
0.007	<i>Ximelagatran Action Pathway</i>	SMPDB	FGG; FGB
0.007	<i>Lepirudin Action Pathway</i>	SMPDB	FGG; FGB
0.007	<i>Aminocaproic Acid Action Pathway</i>	SMPDB	FGG; FGB
0.007	<i>Tranexamic Acid Action Pathway</i>	SMPDB	FGG; FGB
0.007	<i>Urokinase Action Pathway</i>	SMPDB	FGG; FGB
0.007	<i>Retepase Action Pathway</i>	SMPDB	FGG; FGB
0.007	<i>Streptokinase Action Pathway</i>	SMPDB	FGG; FGB
0.007	<i>Tenecteplase Action Pathway</i>	SMPDB	FGG; FGB
0.007	<i>Alteplase Action Pathway</i>	SMPDB	FGG; FGB
0.007	<i>Anistreplase Action Pathway</i>	SMPDB	FGG; FGB
0.007	<i>Aprotinin Action Pathway</i>	SMPDB	FGG; FGB
0.007	<i>Phenindione Action Pathway</i>	SMPDB	FGG; FGB
0.007	<i>Dicoumarol Action Pathway</i>	SMPDB	FGG; FGB
0.007	<i>Warfarin Action Pathway</i>	SMPDB	FGG; FGB
0.007	<i>Acenocoumarol Action Pathway</i>	SMPDB	FGG; FGB
0.007	<i>Phenprocoumon Action Pathway</i>	SMPDB	FGG; FGB
0.007	<i>Dicumarol Action Pathway</i>	SMPDB	FGG; FGB
0.008	<i>Ardeparin Action Pathway</i>	SMPDB	FGG; FGB
0.008	<i>Heparin Action Pathway</i>	SMPDB	FGG; FGB
0.008	<i>Fondaparinux Action Pathway</i>	SMPDB	FGG; FGB
0.008	<i>Enoxaparin Action Pathway</i>	SMPDB	FGG; FGB

0.000	<i>Human Complement System</i>	Wikipathways	ITGA2; FGG; ALB; APOA1; FGB
0.003	<i>Vitamin B12 Metabolism</i>	Wikipathways	LDLR; ALB; APOA1
0.003	<i>Statin Pathway</i>	Wikipathways	LDLR; APOA1
0.004	<i>Blood Clotting Cascade</i>	Wikipathways	FGG; FGB
0.004	<i>Dengue-2 Interactions with Blood Clotting Cascade</i>	Wikipathways	FGG; FGB
0.005	<i>Folate Metabolism</i>	Wikipathways	LDLR; ALB; APOA1
0.009	<i>Selenium Micronutrient Network</i>	Wikipathways	LDLR; ALB; APOA1

Table S8B: Overrepresented pathways, parahippocampal cortex – Genomatix

p-value	Pathways	Source	Overlap members
0.001	<i>Beta1 integrin cell surface interactions</i>	Genomatix	FGG; LAMB3; ITGA2; FGB
0.001	<i>Plasminogen activating cascade</i>	Genomatix	FGG; FGB
0.002	<i>extrinsic prothrombin activation pathway</i>	Genomatix	FGG; FGB
0.003	<i>intrinsic prothrombin activation pathway</i>	Genomatix	FGG; FGB
0.004	<i>fibrinolysis pathway</i>	Genomatix	FGG; FGB
0.005	<i>Visual phototransduction</i>	Genomatix	LDLR; RDH12; APOA1
0.005	<i>Extracellular matrix organization</i>	Genomatix	FGG; LAMB3; ITGA2; FGB; NCSTN
0.006	<i>Blood coagulation</i>	Genomatix	FGG; FGB
0.007	<i>Beta2 integrin cell surface interactions</i>	Genomatix	FGG; FGB
0.008	<i>Signaling Pathways</i>	Genomatix	LDLR; FGG; LAMB3; PSMB8; PREX1; ARHGAP21; PTPRJ; ITGA2; SH2B1; RRAGA; RRAGB; FGB; RDH12; APOA1; NCSTN

Legends for supplementary movies

Movie S1

Electroclinical seizure in mouse #0119. The movie shows videos of four mice. Mouse #0119 is located in the right bottom cage. The hippocampal EEG of this mouse is directly linked to simultaneous digital video recording. Note the onset of myoclonic twitches and jumping during abnormal EEG activity, characterized by an irregular, high-amplitude spike cluster.

Movie S2

Electroclinical seizure in mouse #0110. The movie shows videos of four mice. Mouse #0110 is located in the right top cage. The hippocampal EEG of this mouse is directly linked to simultaneous digital video recording. Note the behavioral arrest and myoclonic twitches during abnormal EEG activity, characterized by high-amplitude run of spikes.

Movie S3

Electroclinical seizure in mouse #0114. The movie shows videos of four mice. Mouse #0110 is located in the left bottom cage. The hippocampal EEG of this mouse is directly linked to simultaneous digital video recording. Note the polymorphic high-amplitude spikes and sharp waves, during which the mouse starts to twitch, then stretches and flips over, resembling a generalized convulsive seizure. The rapid flipping-over can be better seen when slowing the movie.

Movie S4

Electroclinical seizure in mouse #0165. The movie shows videos of four mice. Mouse #0110 is located in the right top cage. Note the myoclonic twitches during abnormal EEG activity, characterized by an irregular, low- and high-amplitude run of spikes.