SI Supplementary information for

Impaired tonic inhibition in *SLC1A3*-associated episodic ataxia

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SI Material and Methods

Animal provenance. *Slc1a3*$^{P290R/+}$ mice were initially generated within C57BL6/N background by Polygene (Switzerland) from genetic modification of ES-cells. Due to their extremely mortal and strong phenotype, we backcrossed the mutation into 129S6/SvEvTac background to reduce mortality[1]. The strain was cryopreserved from 2020 onwards and rederivated (Taconic Biosciences, Denmark) for this study. All bred animals were genotyped from tail samples or ear mark pieces.

Sodium imaging of RGLs. Intracellular $[Na^+]_{int}$ were determined by ratiometric wide-field imaging at an epifluorescence microscope[2,3] (Nikon Eclipse FN 1, Nikon Europe, the Netherlands), equipped with a Fluor 60×/1.0 NA water immersion objective (Nikon), a Poly-V monochromator (Till Photonic) and an ORCA FLASH 4.0LT camera (Hamamatsu Photonics). Imaging was performed on wt and mutant animals (*N* = 5/4) from both sexes. Cells were bolus-loaded with the chemical indicator sodium-binding benzofuran isophthalate-AM (SBFI-AM, 116.7 µM, ION Biosciences), as described before[1]. SBFI was excited at 340 nm (Na^+-insensitive wavelength) and 380 nm (Na^+-sensitive), and its emission was collected using a 409 nm dichroic mirror and a 510/84 nm band-pass emission filter. The fluorescence ratio (F340 / F380 nm) of SR101-positive radial glia-like cells was calculated from regions of interest (ROIs) manually drawn around cell bodies using NIS Elements 5.21 (Nikon) and OriginPro 2021 (OriginLab). For overview images, z-stacks were generated from SBFI- and SR101-stained acute slices by multi-photon microscopy at a Nikon A1-R system (Nikon Europe), equipped with a water-immersion objective (NIR Apo 60×/NA 1.0, Nikon), and a mode-locked Titan Sapphire laser (Mai Tai DeepSee, Newport, Spectra Physics) operated at 790 nm and 80 MHz. Fluorescence emission was collected using non-descanned detection at 525/50 nm (SBFI, 560 nm dichroic mirror) and at 575/25 nm (SR101, 593 nm dichroic mirror).

Fluorescence lifetime imaging of $[Cl^-]_{int}$. $[Cl^-]_{int}$ measurements were performed with two different imaging systems: an upright fluorescence microscope (A1 MP, Nikon) equipped with a 25× water immersion objective and a mode-locked Titan-Sapphire laser (Mai Tai DeepSee, Newport Spectra Physics; output power 2.3 W at 750 nm), and a Zeiss laser scanning microscope 880 equipped with a 20× water immersion objective (NA 1.0, WD 2.1 mm, Zeiss), coupled to a tunable laser (InSight X3, Newport Spectra Physics, output power 1.9 W at 750 nm). Fluorophores were excited using short pulses of photons (100 fsec or 120 fsec light pulses of 750 nm, two-photon excitation), with identical results for both methods. Fluorescence was detected with a GaAsP hybrid photodetector (HPM-100−40; Becker and Hickl) after passing through a bandpass filter at 445 ± 45 nm

(445BP90, Omega Optical).

Western blotting. Western blotting was performed using hippocampal or cortical lysates from at least three animals per genotype and age group. Dissected hippocampi and samples from the somatosensory and motor cortex were snap-frozen in liquid nitrogen and homogenised at 4°C. Hippocampal homogenates were centrifuged at 40,000 *g* for 45 min at 4°C. The resulting pellets were resuspended in 10 mM sodium phosphate buffer (pH 8.0) containing 1% (w/v) sodium dodecyl sulfate (SDS), 40 µl/ml sodium deoxycholate, and protease inhibitors. After incubation for 5 min at RT, samples were centrifuged again at 13,000 *g* for 10 min at 15°C. Protein samples were separated on 10%–12% SDS-polyacrylamide gels and transferred to polyvinylidene fluoride (PVDF) membranes. Membranes were blocked for 60 min at RT in PBS containing 0.1% Tween-20 and 3% bovine serum albumine (BSA, PBS-T), and then incubated with primary antibodies against doublecortin X (DCX), $GABA_AR$ δ subunit, GAT-3, or GLAST (RRIDs: AB_1586992, AB_2107256, AB_304437, AB_10829302) for 1 hour at RT. Frozen samples of the cortex were homogenised in ice-cold lysis buffer (20 mM Tris, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 2.5 mM Na-pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 10 mM DTT, 1% Triton X-100) supplemented with EDTA-free protease inhibitors. Lysates were incubated on ice for 10 min, subjected to six freeze–thaw cycles, and centrifuged at 14,000 rpm for 20 min at 4 °C. Protein concentration was determined by BCA assay (Bio-Rad Laboratories, Hercules, USA). Equal amounts of protein (15 µg) were resolved by SDS-PAGE (8%) and transferred to nitrocellulose membranes. Membranes were blocked with 5% milk in PBS-T and incubated overnight at 4 °C with antibodies against $GABA_AR$ δ subunit (1:5000) and mouse anti-vinculin (1:5000). After washing with PBS, 0.1% Tween-20, membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (RRIDs: AB_10981814, AB_228415, AB_228333) for 45 min at RT. Following additional washes, bands were visualised and quantified. Signal intensities were normalised to background-corrected grayscale values of α-actin or Na^+/K^+-ATPase. Original western blots for figures 3D, 5G, and 6G are provided below, and are also reposited at https://github.com/peterkovermann/episodic_ataxia_6_II/.

Quantitative receptor autoradiography. The mean densities of glutamatergic and GABAergic receptors were quantified in different brain regions on brains from P42–P47 mice (*N* = 10/10, WT/*Slc1a3*$^{P290R/+)}$ and visualised via color-coding[4]. Receptor densities (in pmol/mg protein) were measured using the original, non-contrast-enhanced datasets in seven regions: the caudate putamen, the motor and somatosensory (S) cortices, the hippocampal cornu ammonis 1 (CA1), the dentate gyrus (DG) and the cerebellum (Cb). For

each animal, brain area, and receptor type, three regions of interest (ROIs) were measured and their average was used to represent the mean binding site density. Fig. 8 in the manuscript shows only AMPAR data. The data from all tested receptor types are available at https://github.com/peterkovermann/episodic_ataxia_6_II/.:

Dendritic spine quantification. Golgi-Cox staining was performed as previously describes[3]. For quantitative analysis, three to 11 individual dendritic segments (10 µm in length) were evaluated per hippocampus (N = 3/3, WT/*Slc1a3*$^{P290R/+}$). Only spines with clearly distinguishable heads and necks, located on secondary and tertiary dendrites of DG granule cells or CA1 pyramidal neurons, were included. Linear spine density was calculated as the number of spines divided by the length of the corresponding dendritic segment.

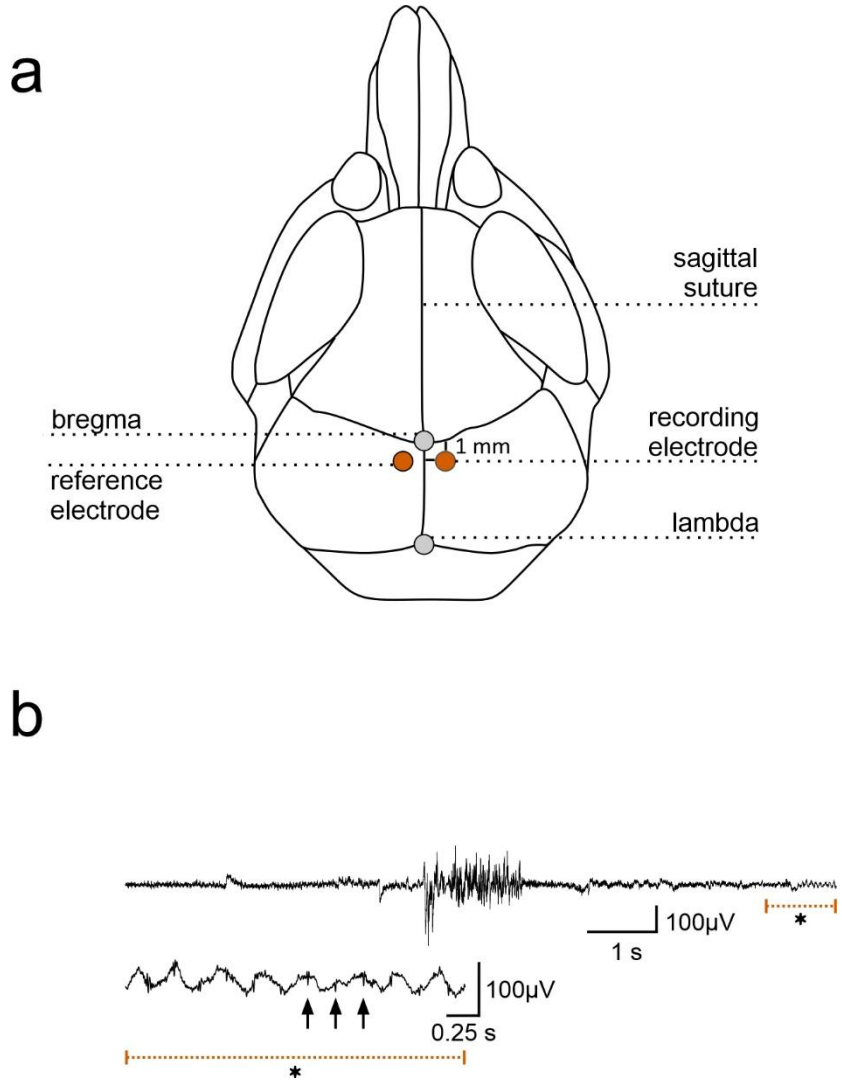
References for SI Materials and Methods

[1]Kovermann P, Untiet V, Kolobkova Y, Engels M, Baader S, Schilling K, Fahlke C. Increased glutamate transporter-associated anion currents cause glial apoptosis in episodic ataxia 6. *Brain Comms.* 2020; 2(1):fcaa022.

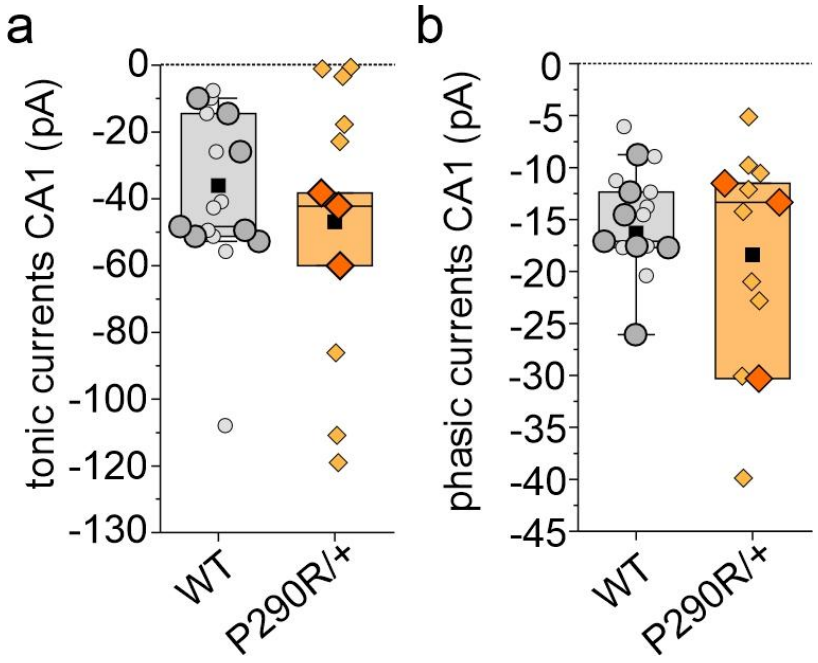
[2]Langer J, Rose CR. Synaptically induced sodium signals in hippocampal astrocytes in situ. *J Physiol*. 2009;587(Pt 24):5859-77.

[3]Risher WC, Ustunkaya T, Singh Alvarado J, Eroglu C. Rapid Golgi analysis method for efficient and unbiased classification of dendritic spines. *PloS One*. 2014;9:e107591.

[4]Zilles K, Palomero-Gallagher N, Grefkes C, Scheperjans F, Boy C, Amunts K, Schleicher A. Architectonics of the human cerebral cortex and transmitter receptor fingerprints: reconciling functional neuroanatomy and neurochemistry. *Eur Neuropsychopharmacol*. 2002;12:587-99.



Supplementary Fig. S1 Scheme showing the sites for electrode implantation (*a*) and a typical interictal activity (*b*). Subfigure 9a illustrates, how recording and reference electrodes were implanted through holes ~ 1mm posterior to the bregma. In 9b a typical interictal EEG activitv is shown, which indicates occurrence of status epilepticus in young *Slc1a3*$^{P290R/+}$ mice



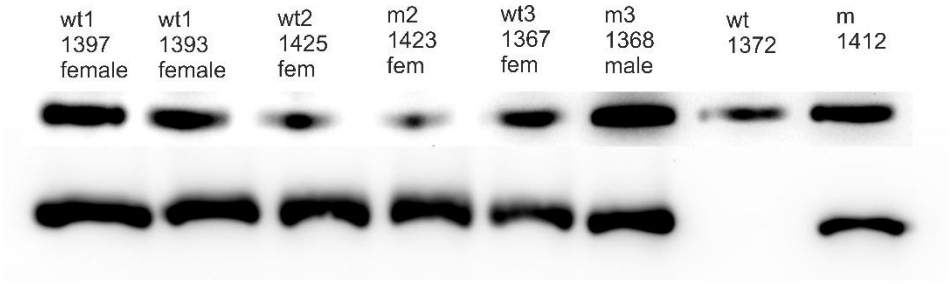
Supplementary Fig. S2 Tonic and phasic currents do not differ between genotypes in pyramidal neurons from CA1 region at P20.

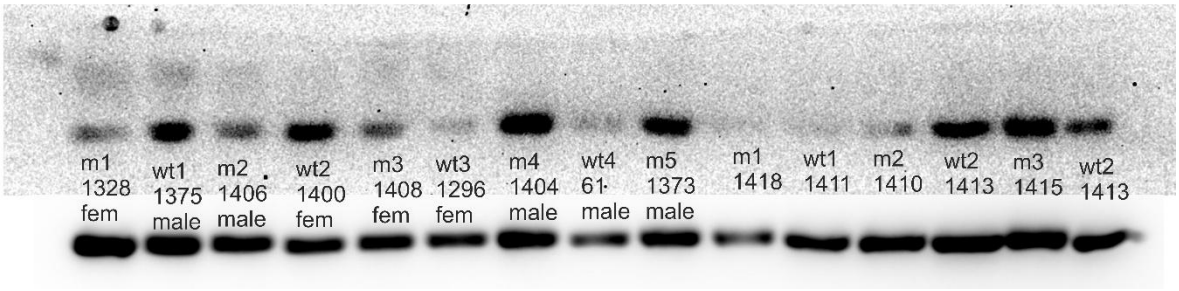
a Current amplitudes from tonic current amplitudes recorded from pyramidal neurons in the CA1 region (p = 0.67, N = 7/3). ***b*** Current amplitudes from mIPSCs recorded from pyramidal neurons in CA1 (p = 0.39, N = 7/3, WT/*Slc1a3*$^{P290R/+}$, *two-tailed t*-tests)

Original western blots for Figs 3d, 5g, and 6g*

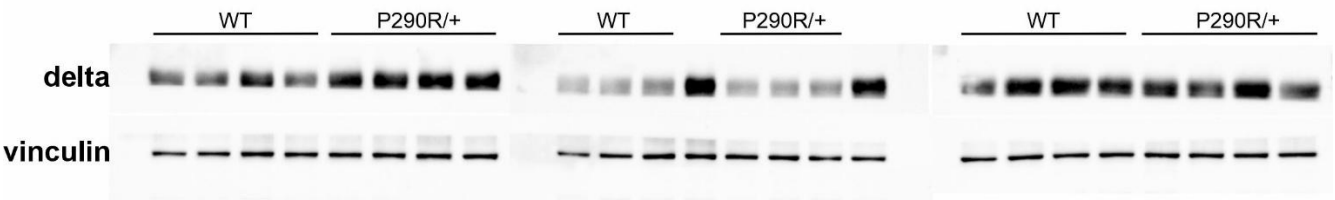
*larger sized images are reposited at: https://github.com/peterkovermann/episodic_ataxia_6_II/

$GABA_AR$ delta (Fig. 3D) - Hippocampus

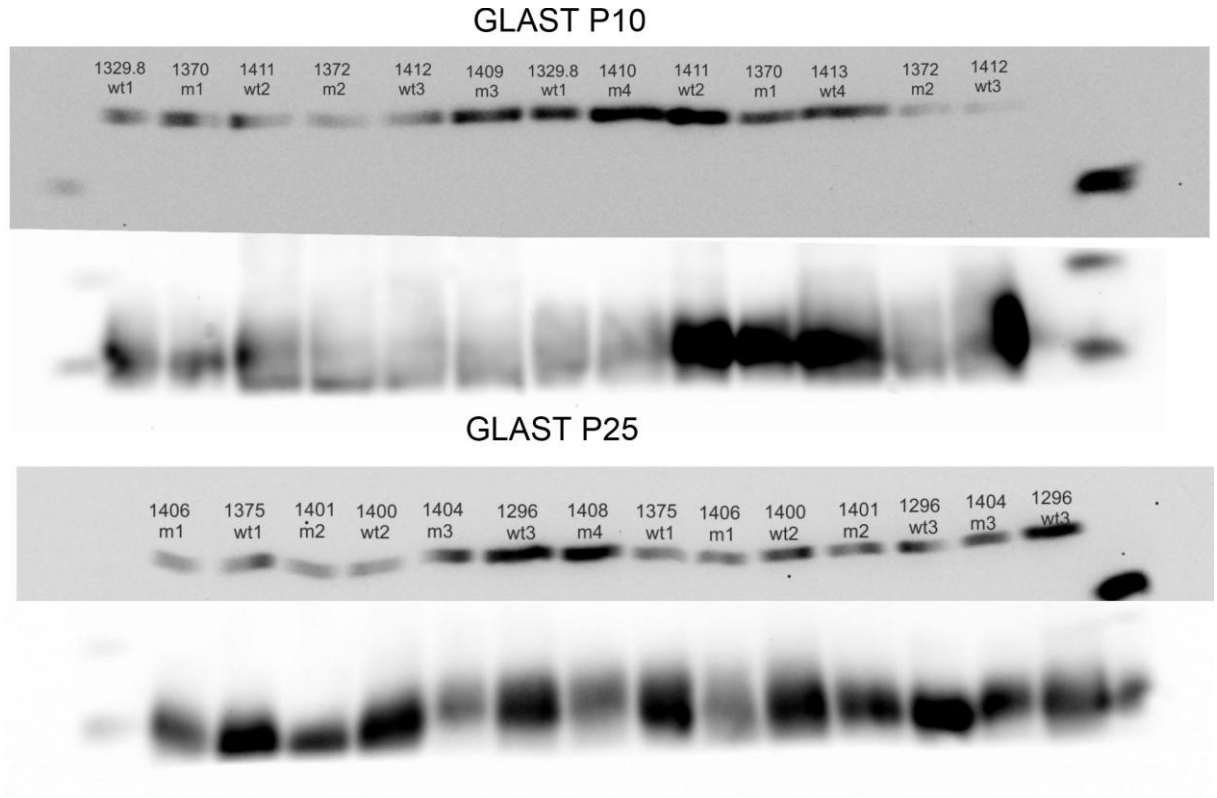




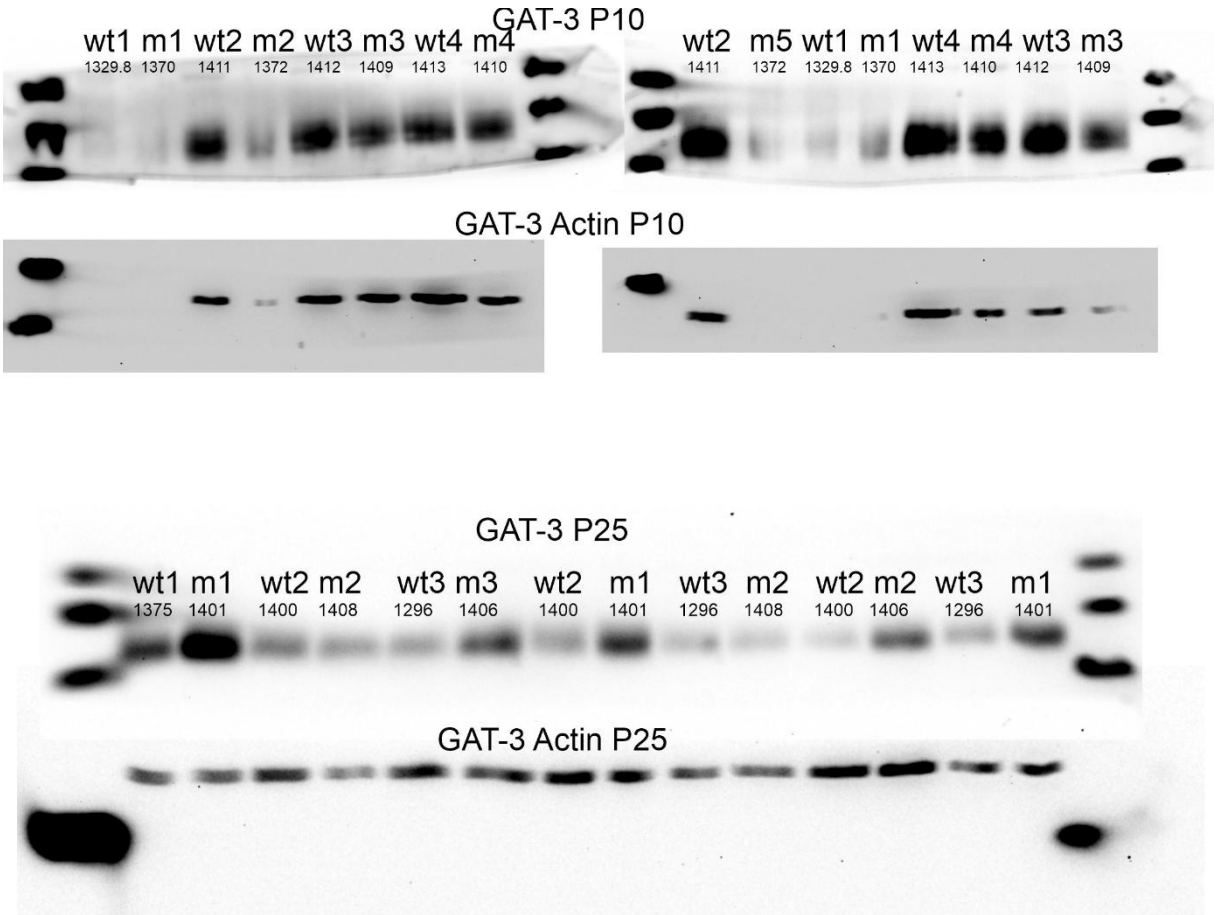
$GABA_AR$ delta (Fig.3D) - Cortex



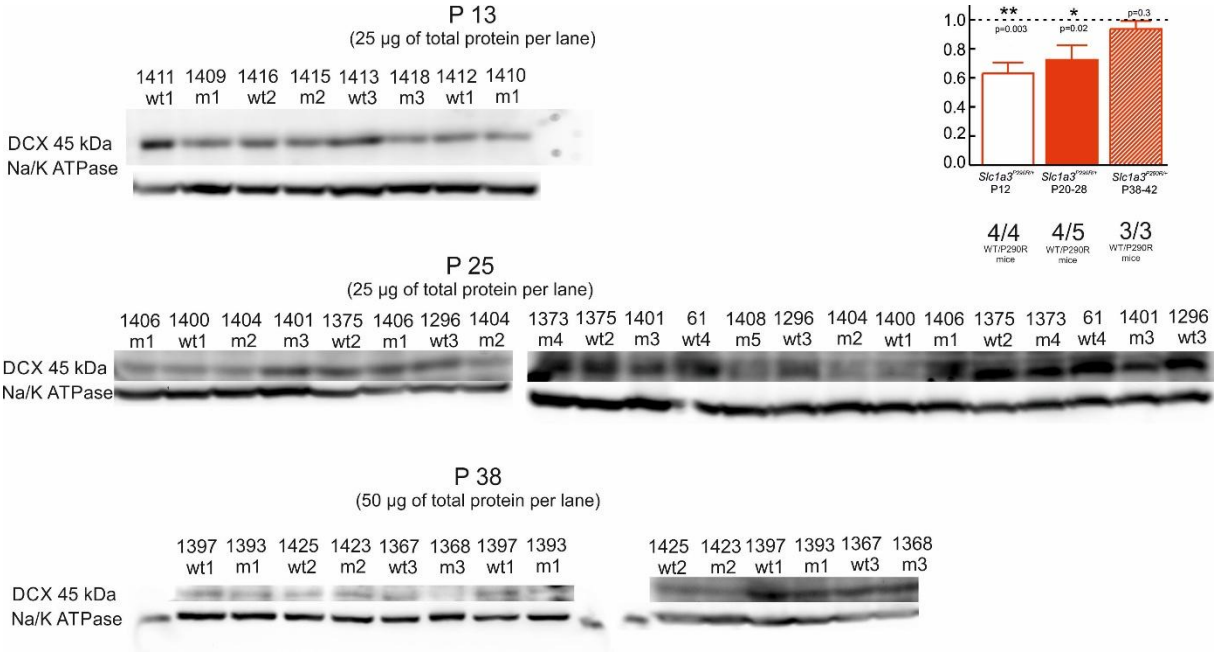
GLAST (Fig. 5G)



GAT-3 (Fig. 5G)



DCX (Fig. 6G)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(25 µg of total protein per lane)
1411 wt1
1409 m1
1416 wt2
1415 m2
1413 wt3
1418 m3
1412 wt1
1410 m1
DCX 45 kDa
Na/K ATPase
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p=0.003
*
p=0.02
p=0.3
1.0
0.8
0.6
0.4
0.2
0.0
P12
P20-28
P38-42
4/4
4/5
3/3
WT/P290R mice
P 25
(25 µg of total protein per lane)
1406 m1
1400 wt1
1404 m2
1401 m3
1375 wt2
1406 m1
1296 wt3
1404 m2
1373 m4
1375 wt2
1401 m3
61 wt4
1408 m5
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1404 m2
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1296 wt3
DCX 45 kDa
Na/K ATPase
P 38
(50 µg of total protein per lane)
1397 wt1
1393 m1
1425 wt2
1423 m2
1367 wt3
1368 m3
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1393 m1
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DCX 45 kDa
Na/K ATPase