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A

|                | anti-lba1 | anti-GFAP | Merge |
|----------------|-----------|-----------|-------|
| CreER;Glut1<sup>+</sup> | ![Image](image1) | ![Image](image2) | ![Image](image3) |
| CreER;Glut1<sup>FL/+</sup> | ![Image](image4) | ![Image](image5) | ![Image](image6) |
| Tie2-Cre;Glut1<sup>FL/+</sup> | ![Image](image7) | ![Image](image8) | ![Image](image9) |

70μm

B

![Graph](image10)

C

|                | anti-lba1 | anti-GFAP | Merge |
|----------------|-----------|-----------|-------|
| Glut1<sup>+</sup> | ![Image](image11) | ![Image](image12) | ![Image](image13) |
| Glut1<sup>-/-</sup> | ![Image](image14) | ![Image](image15) | ![Image](image16) |

70μm

Legend:
- Glut1<sup>+</sup> (1 wk)
- Glut1<sup>-/-</sup> (1 wk)
- CreER;Glut1<sup>+/+</sup> (5 mo)
- CreER;Glut1<sup>FL/+</sup> (5 mo)
- Tie2;Glut1<sup>FL/+</sup> (5 mo)
Supplementary Figure 6 – Glut1 haploinsufficiency triggers early-onset brain neuro-inflammation in model mice. (A) Severe gliosis featuring activated microglia and reactive astrocytes in thalamic brain tissue (ventral posteromedial nucleus – VPM) of 5-month old mutants ubiquitously depleted of Glut1 at PND2 or selectively depleted of the protein in ECs during embryonic development. (B) Enumeration of reactive astrocytes and activated microglia; ***, $P < 0.001$, t test or one-way ANOVA, $n \geq 9$ thalamic regions from each of $N=3$ mice of each genotype assessed. (C) Neuro-inflammation was detected as early as 1 week of age in Glut1$^{+/+}$ mutants.
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Supplementary Figure 9 – Depleting Glut1 in adult mice below heterozygous levels triggers disease. (A) Marked reduction in Glut1 transcripts in brain tissue of 5-month old CreER;Glut1^{FL/FL} mutants administered tamoxifen at 8 weeks of age to inactivate the floxed alleles; *** , P < 0.001, t test, n=3 controls and n=7 mutant mice. (B) Western blot of Glut1 protein in brain tissue of CreER;Glut1^{FL/FL} mutants treated with tamoxifen at 8 weeks of age. (C) Quantified result of Glut1 protein in brain tissue of tamoxifen-treated CreER;Glut1^{FL/FL} mutants; *** , P < 0.001, t test, n=3 mice of each cohort. (D) Depleting Glut1 below 50% in adult mice severely impairs motor performance on the rotarod. Note precipitous decline in performance 4 weeks after tamoxifen administration; *, ***, P < 0.05, P < 0.001 respectively, t test, n≥6 mice of each cohort analyzed. (E) Kaplan-Meier survival curves depict a modest reduction in lifespan of CreER;Glut1^{FL/FL} mutants depleted of Glut1 below heterozygous levels during adult life; n=10 mice of each cohort.
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**Supplementary Information**

**Rotarod test.** To administer the rotarod test, mice were subjected to a training period of 5 minutes on an accelerating rotarod (Ugo Basile Inc., Italy) three times a day for four consecutive days. Measurements were recorded on the fifth day at a setting of 25rpm. Duration of time on the rotating rod was recorded and the experiment terminated if a mouse surpassed 1000s.

**Brain parenchymal and vessel fractions.** Mice were perfused with 1X PBS, whole brains extracted and the tissue gently homogenized by means of Dounce-type glass homogenizer in 1ml PBS. The extract was centrifuged at 1000g for 5min, the resulting pellet re-suspended once again in 1ml PBS and the centrifugation step repeated. The supernatant was then removed and the pellet re-suspended in 1ml of an 18% dextran solution in PBS. This suspension was centrifuged at 10,000g for 1min, the pellet saved and the supernatant containing the parenchymal fraction transferred to new tube. The pellet was once again re-suspended in 1ml of the 18% dextran and the centrifugation repeated. This process was repeated a third time and the pellets containing the vessel fractions were pooled and stored at -80°C until use. The supernatant fractions – containing the neuropil – were similarly combined and stored until use.

**Quantification of neurons and activated glia in thalamic brain.** Thalamic brain in the region of the ventral posteromedial (VPM) nucleus was imaged at a magnification of 10X and the external medullary lamina used as an anatomical marker to demarcate identical regions of the nucleus in the different mice. Neurons within the demarcated (also see fig. S7) dorsal two-thirds of the nucleus (dorsal VPM) were enumerated. Counts were conducted by the ImageJ software suite (NIH, Bethesda, MD). Activated glial cells were enumerated manually in images of the dorsal VPM acquired at a magnification of 63X.

**Live-imaging of brain microvasculature.** Five month old mice were anesthetized (1500 mg/kg urethane and 500 mg/kg glycopyrrolate, administered I.P.). Next, a craniotomy on the right hemisphere between bregma and lamda was performed and then fluorescein-conjugated dextran (2000 kDa, 0.1 ml from 25 mg/ml) injected into the tail vein to enable visualization of the capillaries. Images were acquired using a home-built two-photon laser scanning microscope (41, 42) equipped with a 20X, 0.95 NA objective lens (XLUMPLanFl,
Olympus). Stacks of angiograms (~ 510 x 510µm) were constructed beginning at the cortical surface down to a depth of ~ 500µm. Images were acquired every 2µm in the z-axis. Microvascular length was quantified by modifying an image processing pipeline previously described (42). Image analysis was performed using ImageJ and MATLAB. Briefly, three sub-regions (510 x 510 x 20µm) at depths of 200, 300 and 400µm were selected from the stack. The mean image of the sub-region was first pre-processed with a tubeness filter to enhance the features of the vessels. Then, an automatic intensity thresholding was applied to segment the vessels. Capillary diameter was further determined by skeletonization and Euclidean distance map. Only blood vessel segments with diameters of < 6µm were included in the final result.

**Supplemental Information**

**PCR primers.**

| Primer                                      | SOURCE | SEQUENCE                                      |
|---------------------------------------------|--------|-----------------------------------------------|
| **Primers for PCR**                         |        |                                               |
| Cre-ER<sub>T2</sub> Mutant Rvs             | IDT    | 5’-CGGTTATTCAACTTGCACCA-3’                    |
| Cre-ER<sub>T2</sub> Common Fwd             | IDT    | 5’-AAGGGAGCTGCAGTGGAGTA-3’                    |
| Cre-ER<sub>T2</sub> Wild type Rvs          | IDT    | 5’-CCGAAAATCTGTGGAAGTC-3’                     |
| LoxP Fwd                                    | IDT    | 5’-CTGTGAGTTCCGAGACCCCTG-3’                   |
| LoxP Rvs                                    | IDT    | 5’-CCCAGGCAAGGAAGTGGTTCC-3’                   |
| Glut-1<sup>Δ/Δ</sup> Fwd                   | IDT    | 5’-CTGTGAGTTCCGAGACCCCTG-3’                   |
| Glut-1<sup>Δ/Δ</sup> Rvs                   | IDT    | 5’-GAAGGCACATATGAAACAAATG-3’                  |
| Zp3 Cre transgene Fwd                      | IDT    | 5’-CGAGATTGAGGGAAGGAGG-3’                     |
| Zp3 Cre transgene Rvs                      | IDT    | 5’-CAGGTTCCTTGCAACCTCAT-3’                    |
| Zp3 Cre Internal Positive Control Fwd       | IDT    | 5’-AGTGGGCTCTTCAGAAGAG-3’                     |
| Zp3 Cre Internal Positive Control Rvs       | IDT    | 5’-TGCGACTGTAGTCTGATTTC-3’                    |
| Tie2-Cre Fwd                                | IDT    | 5’-GGCAATTTTGGTACGGTC-3’                      |
| Tie2-Cre Rvs                                | IDT    | 5’-CCTGTGACTACAGAAATG-3’                      |
| **Primers for qRT-PCR**                     |        |                                               |
| Glut-1 Fwd                                  | IDT    | 5’-CTTGAGTCTACAGAAGATC-3’                     |
| Glut-1 Rvs                                  | IDT    | 5’-CAGTGATCCGAGCAGCTGC-3’                     |
| BDNF Fwd                                    | IDT    | 5’-TGGCCCTTGAGGCTAAGT-3’                      |
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies for Western blotting** | | |
| Glut1 (1:5000) | Millipore | Cat#07-1401 |
| Vinculin (1:2000) | Abcam | Cat#ab129002 |
| β-actin (1:5000) | Sigma | Cat#A5441 |
| Donkey anti-rabbit IgG (1:10,000) | Santa Cruz | Cat#sc-2313 |
| Goat anti-mouse IgG (1:10,000) | Jackson Immunoreas. | Cat#115-035-003 |
| phospho-AMPKα (Thr172) (1:1000) | Cell Signaling | Cat#2531 |
| GAPDH | Santa Cruz Biotech. | Sc-32233 |
| **Antibodies for Immunostaining** | | |
| Lectin (1:1000) | Vector Laboratories | Cat#FL-1171 |
| Glut1 (1:500) | Abcam | Cat#ab40084 |
| GFAP (1:500) | Abcam | Cat#ab134436 |
| Antibody                              | Manufacturer      | Catalog Number |
|---------------------------------------|-------------------|----------------|
| GFAP (1:500)                          | Sigma             | Cat#G3893      |
| Iba-1 (1:500)                         | Wako              | Cat#019-19741  |
| CD11b (1:500)                         | BD Biosciences    | Cat#553308     |
| NeuN (1:500)                          | Synaptic Systems  | Cat#266006     |
| BDNF (1:500)                          | ABclonal          | Cat# A1307     |
| Alexa Fluor 488 goat α-rabbit (1:1000) | Abcam              | Cat#ab150085   |
| Alexa Fluor 488 goat α-mouse (1:1000) | Invitrogen        | Cat#A11029     |
| Alexa Fluor 568 goat α-rat (1:1000)   | Invitrogen        | Cat#A-11077    |
| Alexa Fluor 568 donkey α-mouse (1:1000) | Invitrogen        | Cat#A10037     |
| Alexa Fluor 647 goat α-chicken (1:1000) | Abcam              | Cat#ab150171   |

**Experimental Models: Organisms/Strains**

| Organism                              | Source            | Stock Number |
|---------------------------------------|-------------------|--------------|
| Glut-1 FL/FL mouse                    | Young et al., 2011 | N/A          |
| Zp3-Cre mouse                         | Jackson Laboratory| Stock #003651|
| R26-CreERT2 mouse                     | Jackson Laboratory| Stock #008463|
| Tie2-Cre mouse                        | Jackson Laboratory| Stock #008863|

**Software and Algorithms**

| Software                              | Manufacturer      | Website                     |
|---------------------------------------|-------------------|-----------------------------|
| GraphPad Prism                        | Graph Pad Software| https://www.graphpad.com/scientificsoftware/prism/|
| ImageJ                                | NIH               | https://imagej.nih.gov/ij/  |
| MATLAB                                | MathWorks         | https://www.mathworks.com/products/matlab.html |
| ImageQuantTL                          | GE Healthcare     | https://www.gelifesciences.com/ |
| Leica LAS X                           | Leica             | https://www.leica-microsystems.com/ |
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