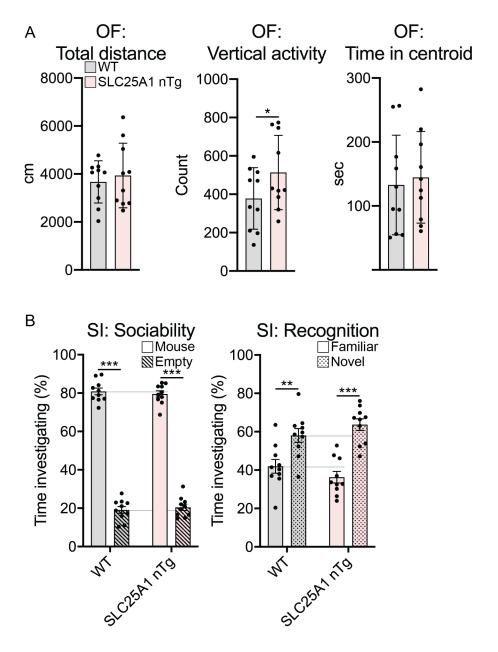
## SUPPLEMENTARY MATERIAL

Increased expression of SLC25A1/CIC causes an autistic-like phenotype with altered neuron morphology

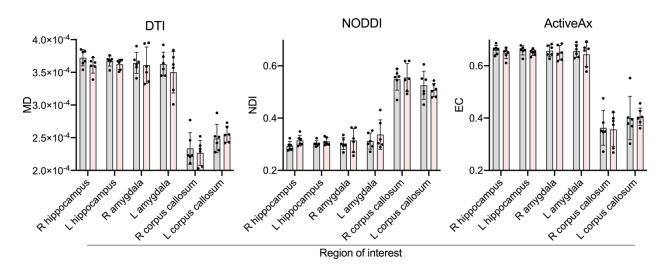
Michael J. Rigby, Nicola Salvatore Orefice, Alexis J. Lawton, Min Ma, Samantha L. Shapiro, Sue Y. Yi, Inca A. Dieterich, Alyssa Frelka, Hannah N. Miles, Robert A. Pearce, John Paul J. Yu, Lingjun Li, John M. Denu, Luigi Puglielli

## **CONTENTS:**

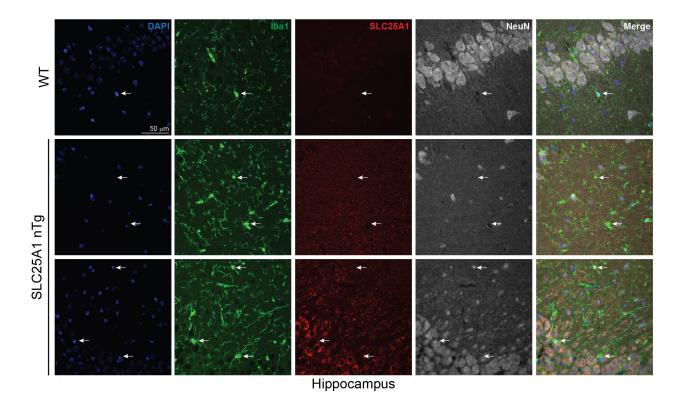
- Supplementary Figures
- Supplementary Figure Legends
- Supplementary Movie Legend



Supplementary Figure 1. SLC25A1 nTg mice exhibit normal open field activity and social interaction. (A) Open field (OF) assay. WT, n = 10; Tg, n = 10; equal males/females. \*p < 0.05 via 2-way ANOVA (genotype x sex). (B) Social interaction (SI) assay. WT, n = 10; Tg, n = 10; equal males/females. \*\*p < 0.005, \*\*\*p < 0.0005 via 2-way ANOVA (condition x sex). All data are displayed as mean  $\pm$  SD.

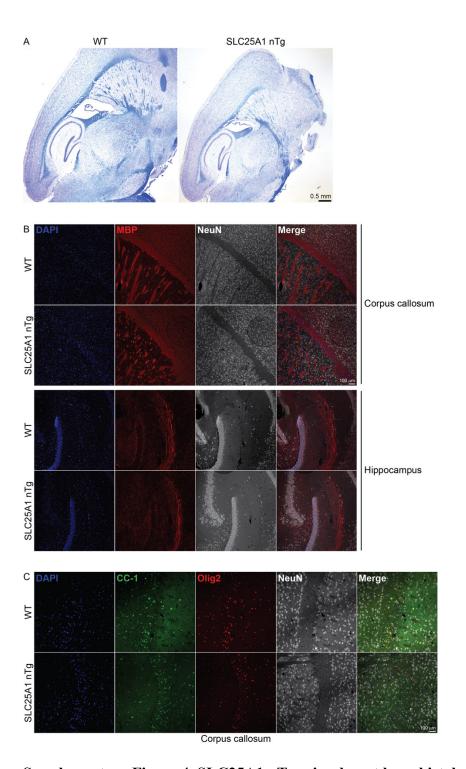


Supplementary Figure 2. Diffusion weighted imaging on SLC25A1 nTg mice reveal no changes in DTI MD, NODDI NDI, and ActiveAx EC parameters in a ROI analysis.  $Ex\ vivo$  diffusion weighted imaging of male WT and SLC25A1 nTg mice at 4 months old, n=6 mice per genotype. Data are mean  $\pm$  SD.

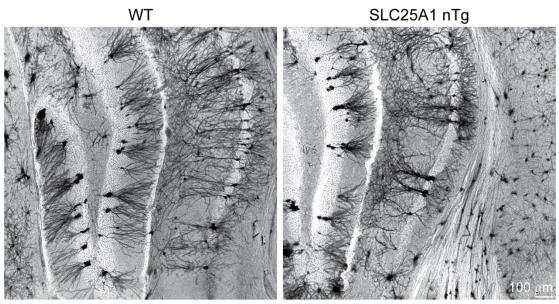


Supplementary Figure 3. Microglia do not overexpress SLC25A1 in SLC25A1 nTg mice.

Paraffin-embedded brain immunostaining. Arrows point to microglial cell bodies. Mice are male at 10-12 months of age.

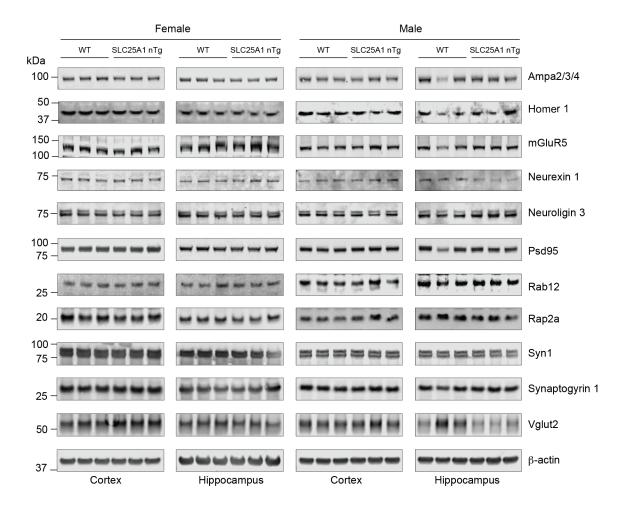


Supplementary Figure 4. SLC25A1 nTg mice do not have histologic evidence of altered white matter integrity or oligodendrocyte abundance. (A) Klüver-Barerra staining of paraffin-embedded brain. (B-C) Paraffin-embedded brain immunostaining. All mice are 3-month-old males.



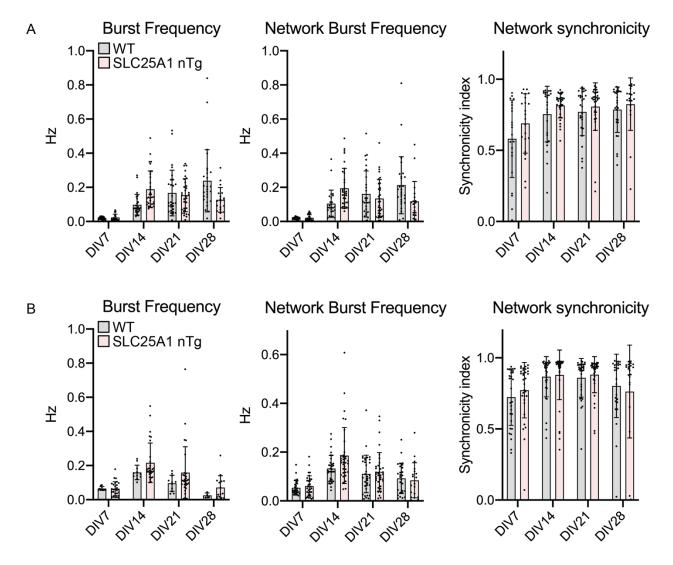
Hippocampus

Supplementary Figure 5. SLC25A1 nTg mice do not have histologic evidence of altered dendritic branching *in vivo*. Mice are male at 3 months of age.

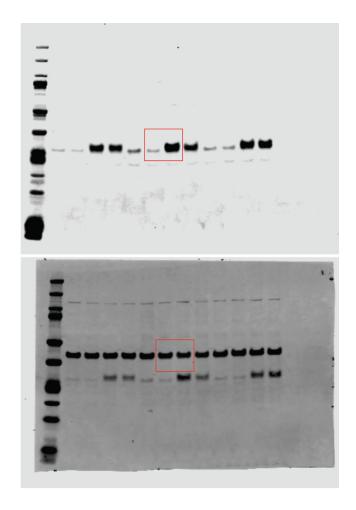


Supplementary Figure 6. SLC25A1 nTg crude synaptosomes do not show widespread changes in expression of proteins involved in neuronal outgrowth and synaptic plasticity.

Mice are 9-10 months of age.

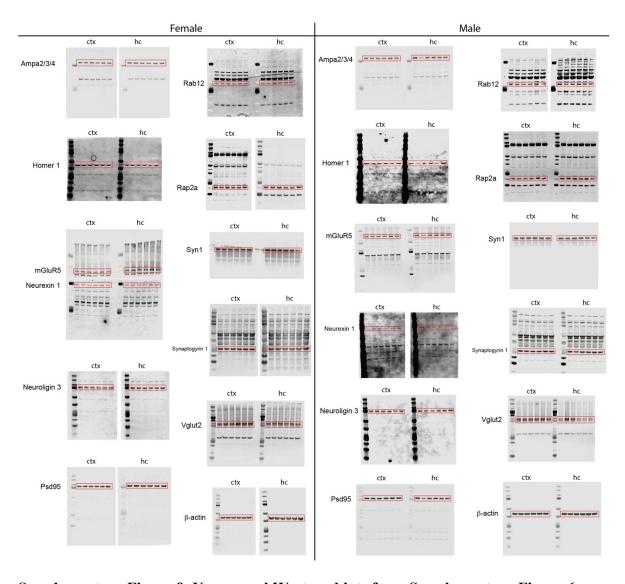


**Supplementary Figure 7. SLC25A1 nTg primary neurons do not exhibit changes in network synchrony.** Multi-electrode array spontaneous activity. Each data point is an independent network of cultured neurons with at least 8 of 16 active electrodes. Data are from 6 embryos per genotype. (A) Cortical primary neurons. (B) Hippocampal primary neurons.



**Supplementary Figure 8. Uncropped Western blots from Figure 1A.** Top: anti-Slc25a1.

Bottom: anti- $\beta$ -actin.



Supplementary Figure 9. Uncropped Western blots from Supplementary Figure 6.

**Supplementary Movie 1. SLC25A1 nTg exhibit repetitive jumping.** The movie shows the aberrant repetitive jumping behavior of one SLS25A1 nTg mouse. WT littermates display no such behavior. The video is available from figshare: doi.org/10.6084/m9.figshare.15130062