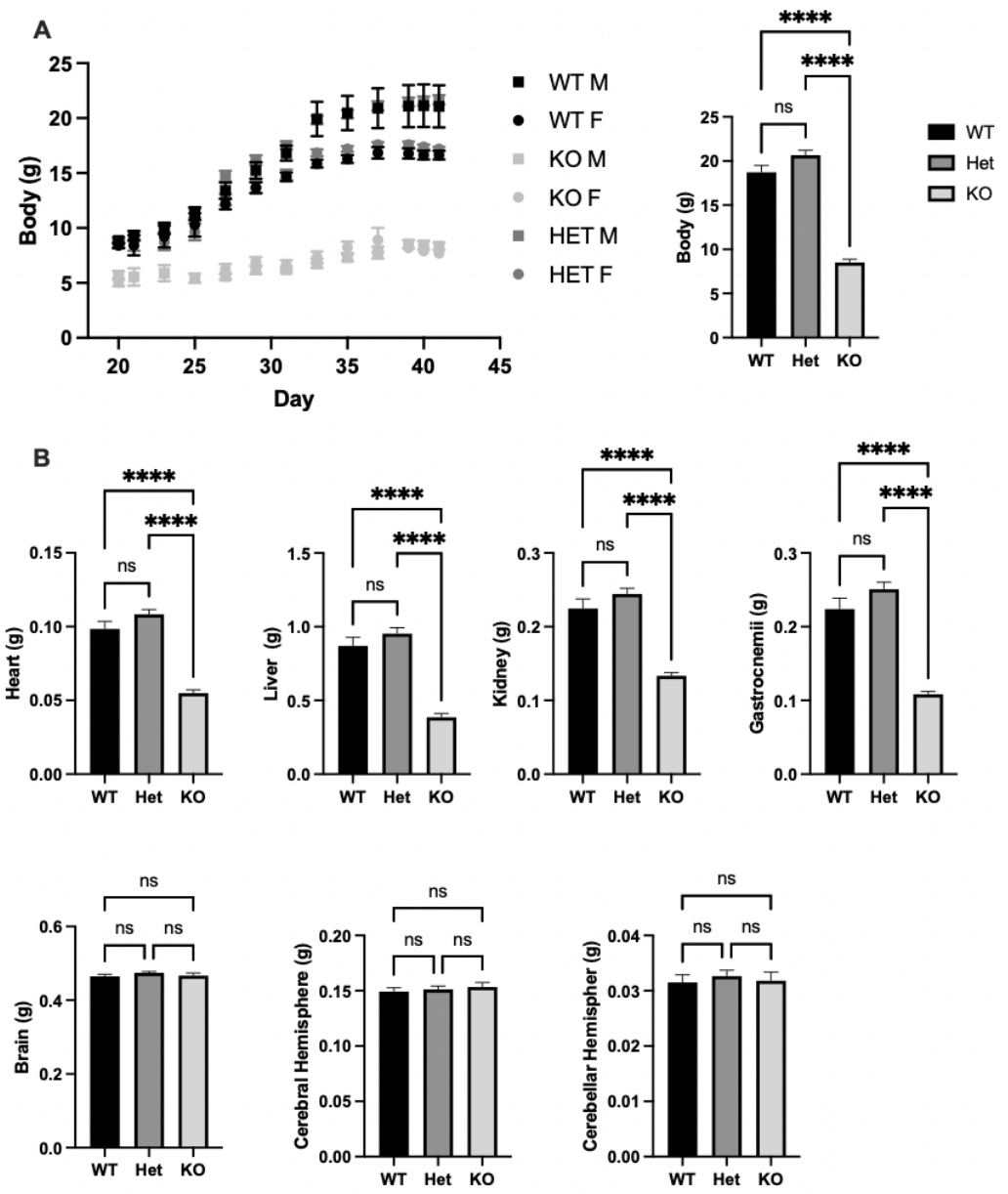
SUPPLEMENTARY FIGURES

Potential glutamatergic and GABAergic false neurotransmitters in models of excitation-inhibition imbalance

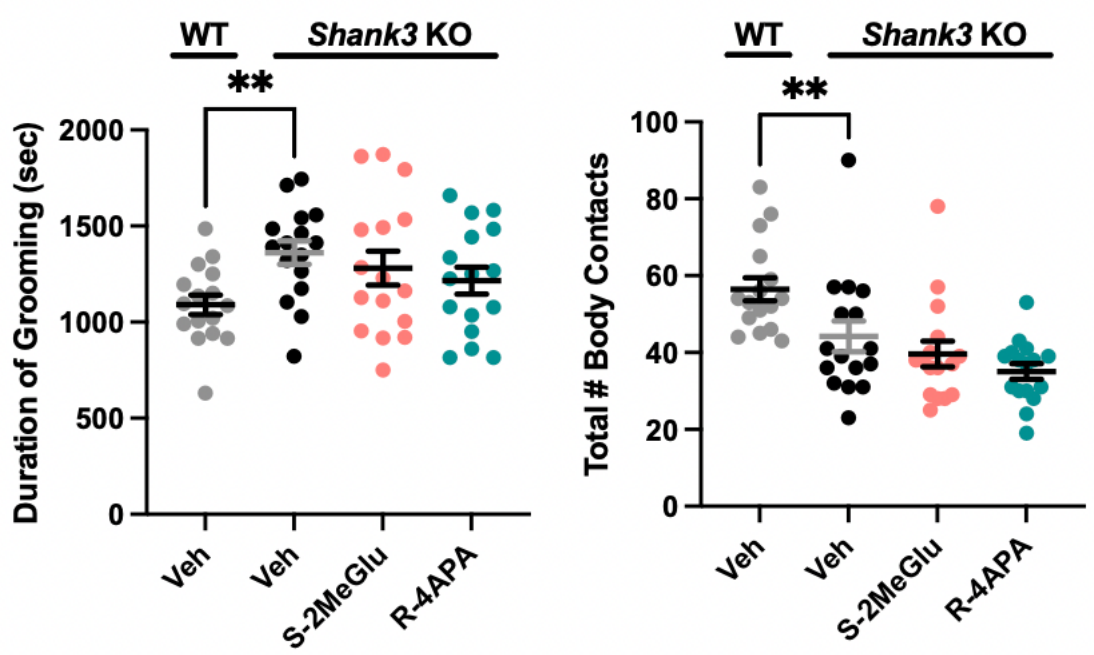
Amalia Perna[1*], Jing Zhao[1], Chandresh R. Gajera[1], Marcus Schonemann[1], Nay L. Saw[2], Mehrdad Shamloo[2,3], Kathleen S. Montine[1], Albert Garofalo[1], and Thomas J. Montine[1]

Affiliations: [1]Department of Pathology, Stanford University School of Medicine; Stanford, California, 94305, USA. [2]Behavioral and Functional Neuroscience Laboratory, Stanford University School of Medicine, Stanford, California, 94305, USA. [3]Department of Neurosurgery, Stanford University School of Medicine, Stanford, California, 94305, USA

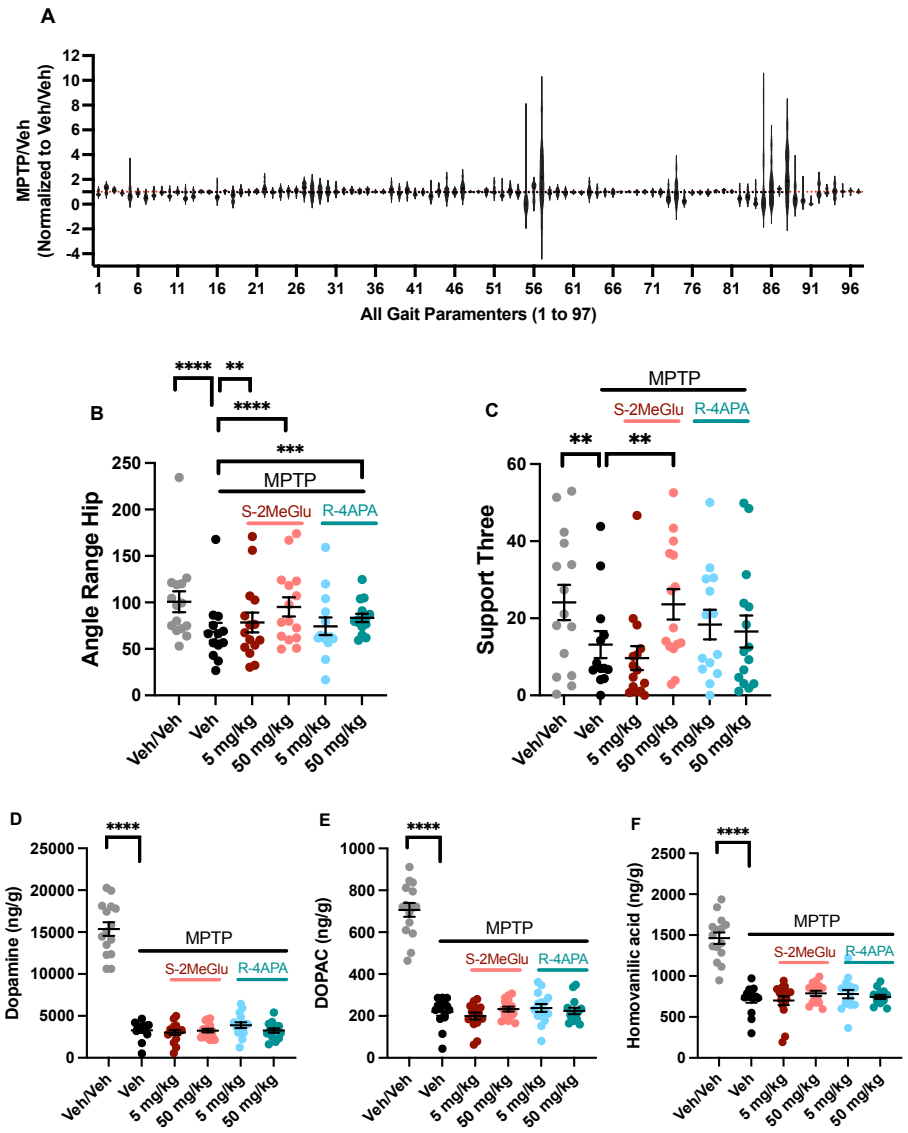
*Corresponding author: aperna@stanford.edu



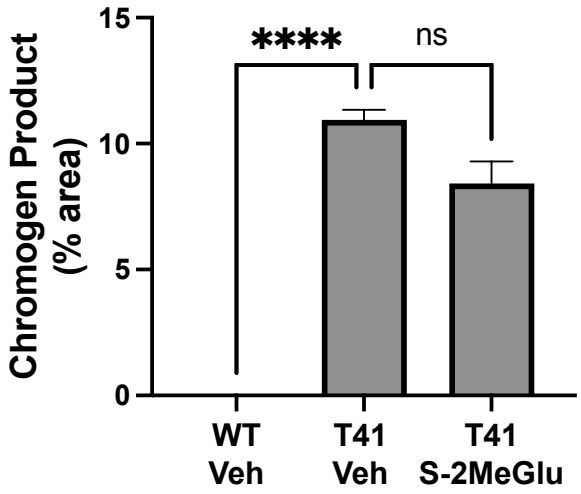
Supplementary Fig. 1. *Aldh5a1* deficient mouse model of severe epilepsy. Body and organ weights were collected from the subset of wild type (WT, *Aldh5a1*$^{+/+}$; n=13), heterozygous (Het, *Aldh5a1*$^{+/-}$; n=23), and knockout (KO, *Aldh5a1*$^{-/-}$; n=6) mice of both sexes (male = 21 and female = 21) mice aged 44 ± 3 (range 31 to 46) days. Approximately one-third of each genotype was treated with vehicle or 50 mg/kg/ QOD rac-2MeGlu or rac-4APA. At the time of sacrifice, Racine seizure score was 5 or 6 of 6 for each KO mouse and 0 of 6 for each WT and Het mouse regardless of treatment group. (**A**) Change in body weight over the course of the experiment was significantly different by sex (P<0.001) but not by treatment group. When stratified by genotype at the end of the experiment, WT and Het mice were significantly heavier than KO mice (****P<0.0001) but not different from each other. (**B**) There was no significant difference in organ weight by sex or treatment group. Weights of heart, liver, kidneys, and gastrocnemii in KO mice were about one-half of WT or Het (****P<0.0001 for each); however, weights of whole brain, one cerebral hemisphere, and one cerebellar hemisphere were not significantly different among the three genotypes. Data are presented as mean ± SEM.



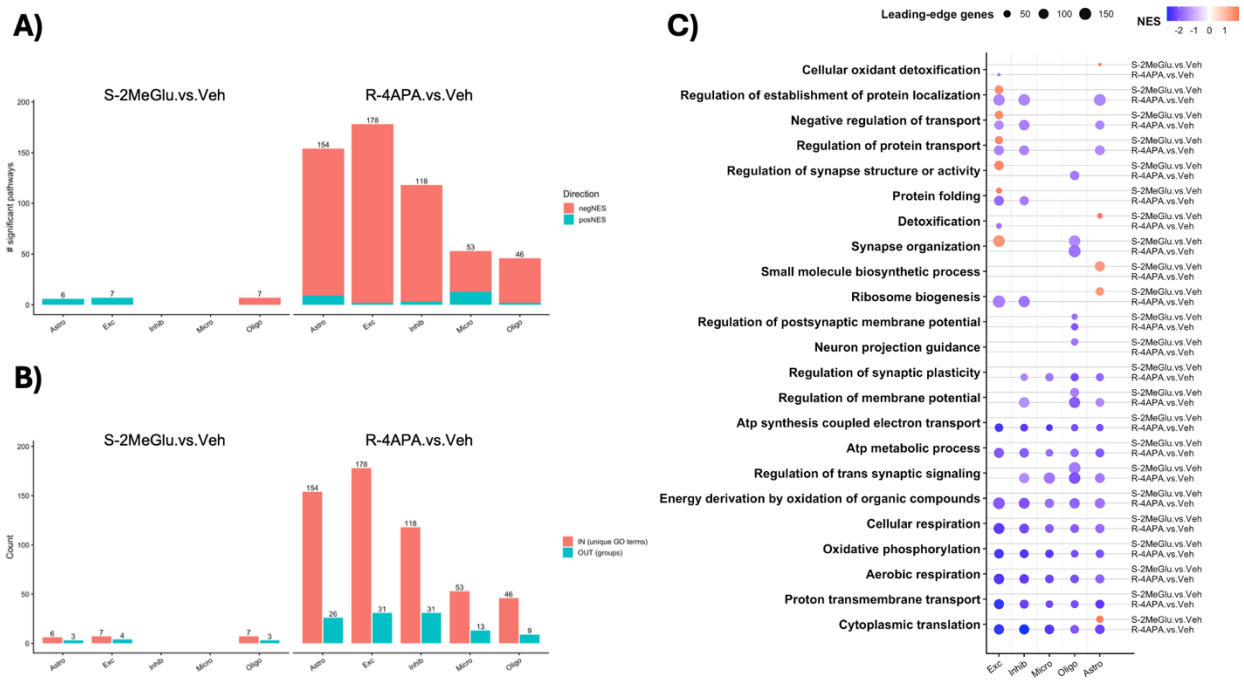
Supplementary Fig. 2. *Shank 3*$^{-/-}$ model of autism. One group of male wild type (WT) and three groups of male *Shank3*$^{-/-}$ (KO) mice (n=16 per group) were injected IP once per week with vehicle (Veh) or 50 mg/kg S-2MeGlu or R-4APA on the day of behavioral testing starting at 10 weeks of age. Two behavioral tests showed a significant change in *Shank3* KO/Veh compared to WT/Veh mice: increased duration of grooming (P=0.0074) and decreased number of total body contacts (P=0.0083); however, neither was significantly changed by treatment with S-2MeGlu or R-4APA. Data presented are mean ± SEM.



Supplemental Fig. 3: 1-Methyl-4-phenyltetrahydropyridine (MPTP) model of dopaminergic toxicity. Six groups of male wild type (WT) C57Bl/6 mice (n=13 to 15 per group, 9 weeks-old) were injected IP with Veh or MPTP (40 mg/kg on days 6 and 7) during a 19 day period when mice received daily IP injection with Veh or with 5 mg/kg/d or 50 mg/kg/d S-2MeGlu or R-4APA and then underwent automated assessment of 97 gait and balance parameters. (**A**) Violin plots show each gait and balance parameter in MPTP/Veh mice normalized to Veh/Veh mice. Secondary analysis by two-way ANOVA of all individual gait parameters (gait parameters P<0.0001, treatment group P=0.0412, interaction P<0.0001) highlighted (**B**) Angle Range Hip with significant post hoc tests for MPTP/Veh vs. Veh/Veh (****P<0.0001), vs. 5 mg/kg S-2MeGlu (**P<0.0078), vs. 50 mg/kg S-2MeGlu (****P<0.0001), and vs. 50 mg/kg R-4APA (***P<0.001) as well as (**C**) Support Three with significant post hoc tests for MPTP/Veh vs. Veh/Veh (****P<0.0032) and vs. 50 mg/kg S-2MeGlu (****P<0.0047). Mice were sacrificed on day 19 and striatum assayed for (**D**) dopamine and its major metabolites, (**E**) 3,4-dihydroxyphenylacetate (DOPAC) and (**F**) homovanillic acid (HVA), using HPLC with electrochemical detection. Results were analyzed by one way ANOVA for dopamine and each metabolite with post hoc tests for each Veh/Veh group vs. MPTP/Veh group (****P<0.0001 for each); there was no significant difference among MPTP/Veh and any of the MPTP mice also treated with either dose on S-2MeGlu or R-4APA. Data presented are mean ± SEM.



Supplementary Fig. 4. **T41 transgenic mouse model of cerebral Aβ amyloidosis**. Immunohistochemistry with antibody to human Aβ was performed on male WT and T41 mouse brains, and hippocampal area occupied by chromogen product determined at 100X. Representative examples show IHC results in WT and T41 mice. Percent hippocampal area occupied by chromogen product was not different between T41/Veh and T41/S-2MeGlu groups (4–6 mice/group were randomly selected for histological analysis, P=0511). Data presented are mean ± SEM.



Supplementary Figure 5: GSEA enrichment and redundancy reduction across cell types. (**A**) Number of significantly enriched Gene Ontology Biological Process (GO:BP) terms identified by FGSEA (adjusted P < 0.05) for S-2MeGlu vs. vehicle and R-4APA vs. vehicle comparisons within each major cell class. Bars are stacked by enrichment direction (positive (pos)and negative (neg) normalized enrichment score (NES); numbers above bars indicate total significant terms per class. (**B**) Summary of term redundancy reduction based on leading-edge gene overlap (Jaccard similarity) followed by Louvain clustering, aggregated across enrichment direction. IN (unique terms) indicates the number of non-redundant representative GO:BP terms retained after reduction, whereas OUT (term modules) indicates the number of term modules identified by clustering. (**C**) Dot plot showing the top five terms per cell class per comparison (adjusted P < 0.05). Rows correspond to S-2MeGlu vs. vehicle and R-4APA vs. vehicle for each term. Dot color denotes NES, and dot size represents the number of leading-edge genes contributing to each term.