Supplementary Materials for

Genetic reduction of eEF2 kinase alleviates pathophysiology in Alzheimer's disease model mice

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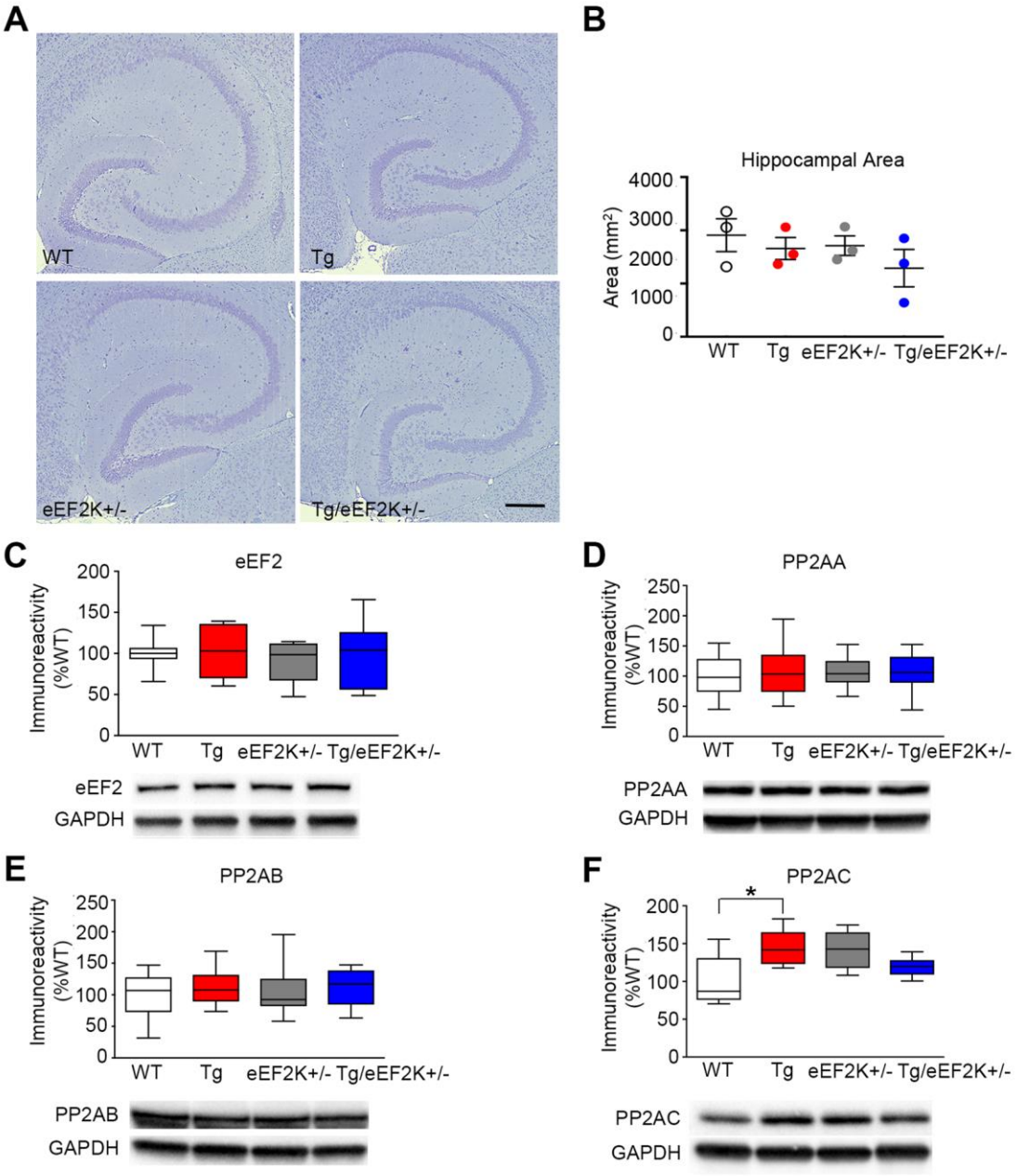
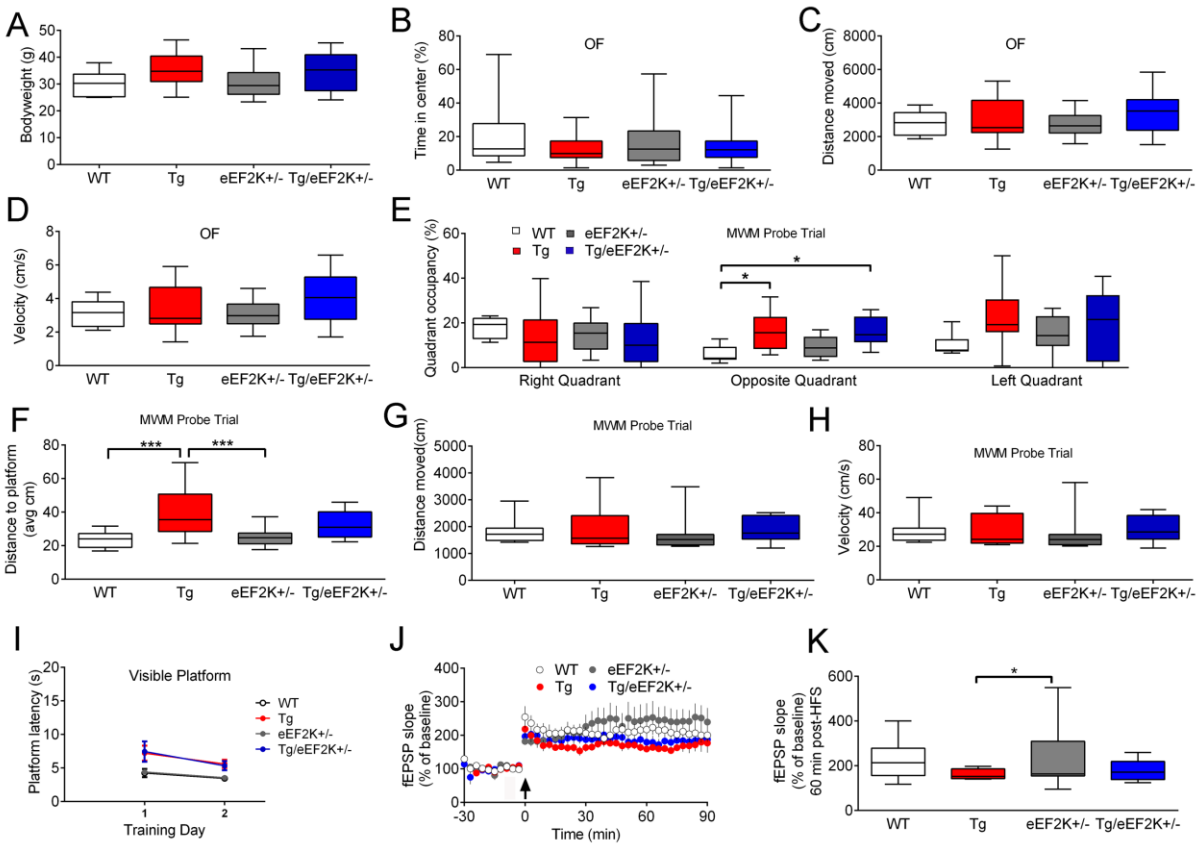
Supplementary Figures Legends

Supplementary Figure 1



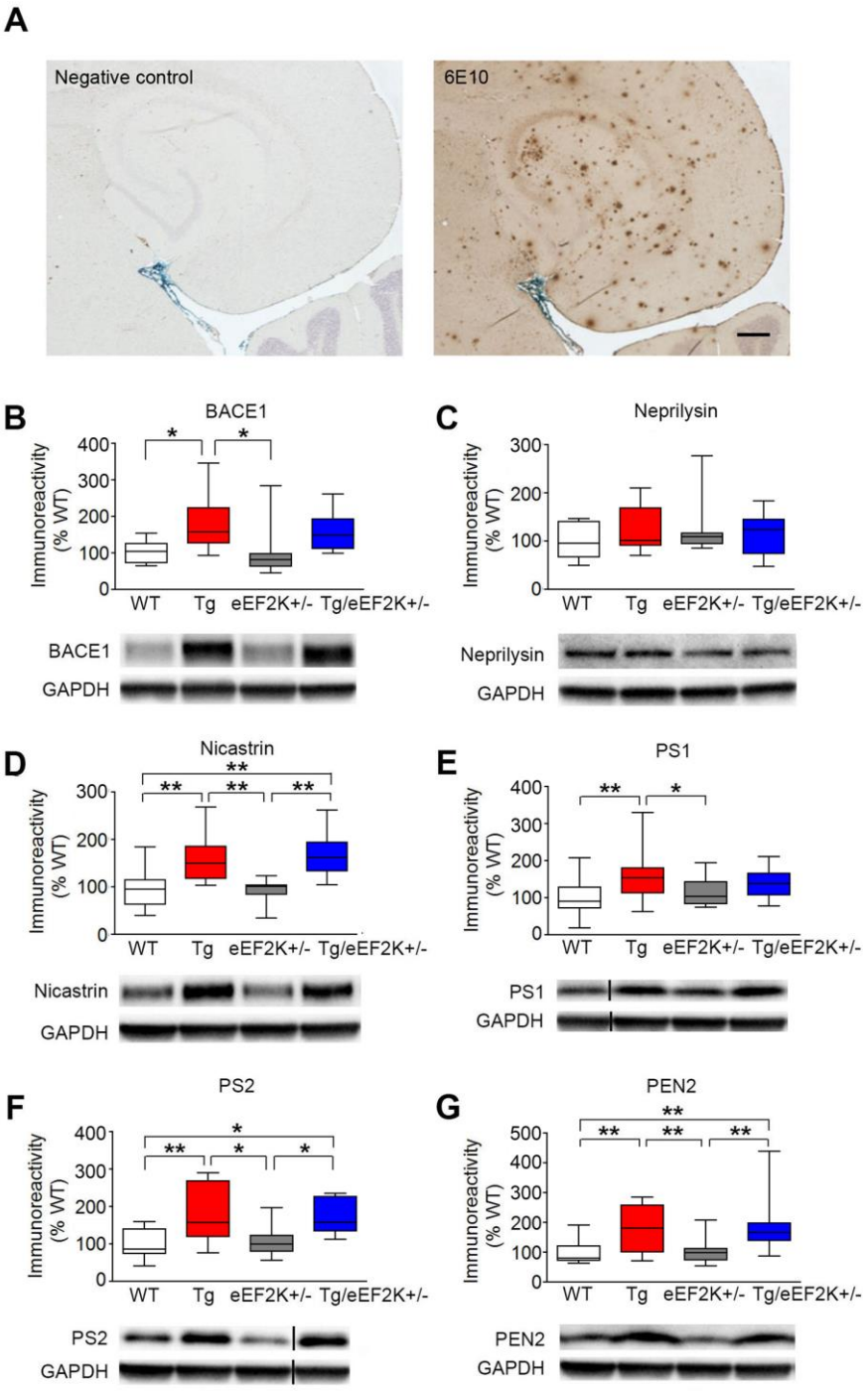
Supplementary Figure 1. Gross hippocampal morphology, total eEF2 levels, and eEF2 phosphatase expression are unchanged with genetic eEF2K reduction. (**A**) Representative images showing Nissl stain in hippocampal sections (20X, scale bar 300 µm). (**B**) Quantification of hippocampal area from Nissl stained sections (WT *n*=7 sections, Tg *n*=8, eEF2K+/- *n*=3, Tg/eEF2K+/- *n*=7). Error bars represent ± SEM. (**C**) No differences in total eEF2 levels were detected in hippocampal lysates of WT (*n*=7), Tg19959 (*n*=10), eEF2K+/- (*n*=8), and Tg19959/eEF2K+/- (*n*=11) mice. (**D**) Expression of the A subunit of protein phosphatase 2A (PP2A) did not change among the four genotypes. (**E**) No differences were detected in expression of the B subunit of PP2A. (**F**) Expression of the C subunit of PP2A was significantly increased in Tg19959 hippocampal lysates compared to WT lysates. **p*<0.05, one-way ANOVA with Tukey's *post hoc* tests. Box and whisker plots represent the interquartile range with the line across the box indicating the median. Whiskers show the highest and lowest values detected.

Supplementary Figure 2



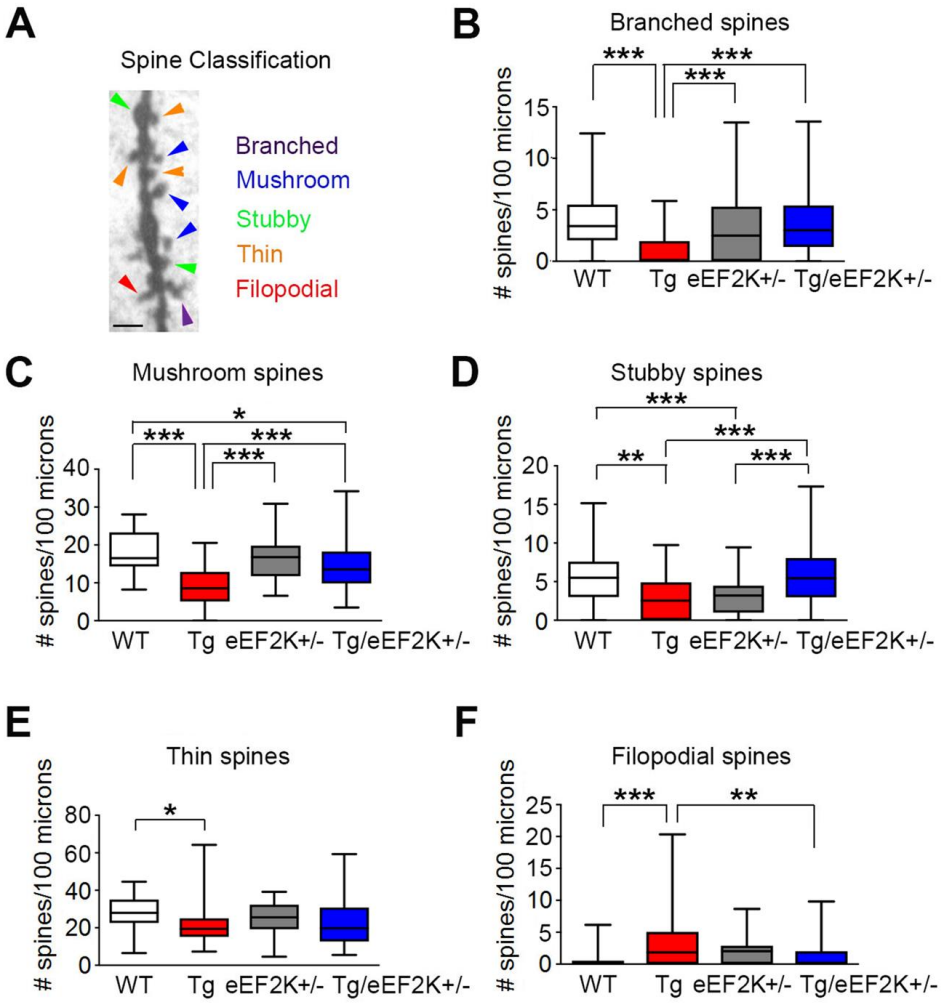
Supplementary Figure 2. Control behavioral data for Tg19959/eEF2K+/- cohorts. (**A**) Body weights at the beginning of behavioral testing for WT (*n*=11), Tg19959 (*n*=12), eEF2K+/- (*n*=16), and Tg19959/eEF2K+/- (*n*=11) experimental mice. (**B**) Percent of time spent in the center arena in the open field assay (OF) (WT *n*=14, Tg19959 *n*=14, eEF2K+/- *n*=20, Tg19959/eEF2K+/- *n*=12). (**C**) Distance moved in cm in the open field assay. (**D**) Velocity of movement in cm/s for open field assay. (**E**) Percent quadrant occupancy for non-target quadrants in Morris Water Maze (MWM) probe trial. Labels are based on position relevant to target quadrant (**p*<0.05, one-way ANOVA with Tukey's *post hoc* tests). (**F**) Average distance to platform in cm during MWM probe trial (****p*<0.01, one-way ANOVA with Tukey's *post hoc* tests). (**G**) Distance moved in cm for MWM probe trial. (**H**) Velocity moved in cm/s for probe trial. (**I**) Visible platform version of MWM. Tg19959 and Tg19959/eEF2K+/- performed significantly slower on both days of training than WT or eEF2K+/- mice (*p*<0.05, one-way repeated measures ANOVA with Tukey's *post hoc* tests). (**J**) Acute hippocampal slices from 4-5-month old WT (*n*=14), Tg19959 (*n*=5), eEF2K+/- (*n*=11), and Tg19959/eEF2K+/- (*n*=7) mice were stimulated with high frequency stimulation (HFS) to induce LTP at the CA3-CA1 synapse. (**K**) Field excitatory postsynaptic potential (fEPSP) slope 60 minutes after HFS (**p*<0.05, one-way ANOVA with Tukey's *post hoc* tests). Box and whisker plots represent the interquartile range with the line across the box indicating the median. Whiskers show the highest and lowest values detected.

Supplementary Figure 3



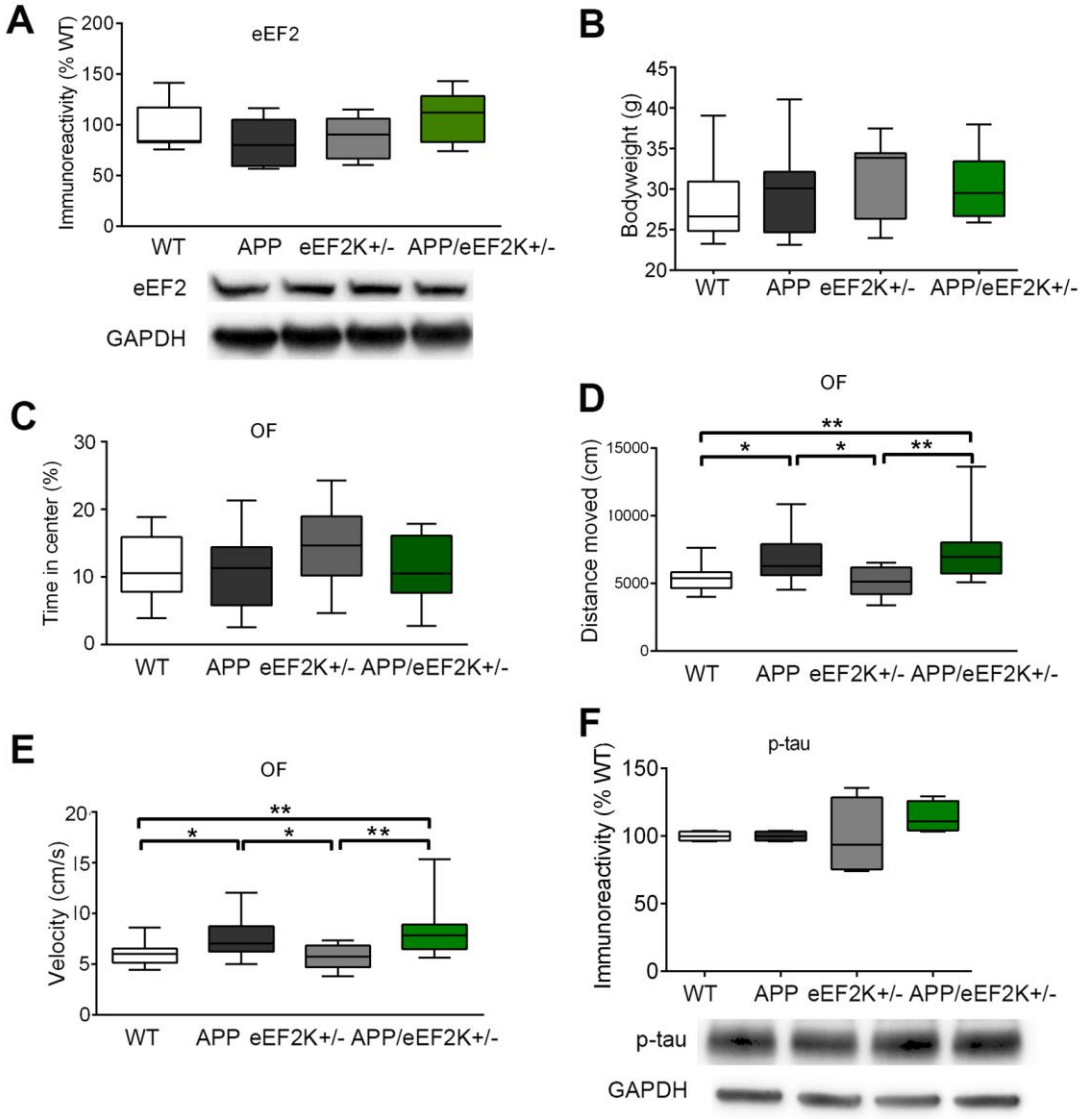
Supplementary Figure 3. Amyloid beta (Aβ) processing is not affected by eEF2K reduction in Tg19959 mice. (**A**) Representative images of negative control staining for 6E10 Aβ antibody in Tg19959 sections (2X, scale bar 300 μm). (**B**) Expression of the β-secretase BACE1 was increased in Tg19959 hippocampal lysates compared to WT and eEF2K+/- lysates. (**C**) Neprilysin expression was unchanged across the four genotypes. (**D**) Tg19959 and Tg19959/eEF2K+/- mice had significantly greater expression of the γ-secretase subunits nicastrin, (**E**) presenilin-1 (PS1), (**F**) presenilin-2 (PS2), and (**G**) presenilin enhancer 2 (PEN2) than WT or eEF2K+/- mice. Black lines in representative images indicate that lanes were run on the same gel but were noncontiguous. n=10, *$p<0.05$; **$p<0.01$, one-way ANOVA with Tukey's *post hoc* tests. Box and whisker plots represent the interquartile range with the line across the box indicating the median. Whiskers show the highest and lowest values detected.

Supplementary Figure 4



Supplementary Figure 4. Golgi-Cox analysis of dendritic spine type in area CA1. (**A**) Diagram depicting spine classification based on previous publication (28) (100X, scale bar 3 μm). (**B**) Tg19959 animals had significantly fewer branched spines than WT, eEF2K+/-, or Tg19959/eEF2K+/- mice. (**C**) Tg19959 animals had significantly fewer mushroom spines than WT, eEF2K+/-, or Tg19959/eEF2K+/- mice. (**D**) Tg19959 mice had significantly fewer stubby spines than WT, eEF2K+/-, or Tg19959/eEF2K+/- mice. (**E**) Tg19959 mice had significantly fewer thin spines than WT mice. (**F**) Tg19959 mice had significantly more filopodial spines than WT or Tg19959/eEF2K+/- mice. WT n=34 total dendrites, Tg19559 n=58, eEF2K+/- n=40, Tg19959/eEF2K+/- n=50; *p<0.05, **p<0.01, ***p<0.001, one-way ANOVA with Tukey's *post hoc* tests. Box and whisker plots represent the interquartile range with the line across the box indicating the median. Whiskers show the highest and lowest values detected.

Supplementary Figure 5



Supplementary Figure 5. Control data for APP/PS1/eEF2K+/- cohorts. (**A**) Total levels of eEF2 did not vary across WT (*n*=7), APP/PS1 (*n*=10), eEF2K+/- (*n*=8), or APP/PS1/eEF2K+/- (*n*=9) hippocampal lysates. (**B**) Body weight data across the four genotypes showed no significant differences between groups. (**C**) Percent of time spent in the center arena in open field assay (OF). WT *n*=18, APP/PS1 *n*=16, eEF2K+/- *n*=11, APP/PS1/eEF2K+/- *n*=15. (**D**) Distance moved in cm in open field assay. APP/PS1 and APP/PS1/eEF2K+/- animals moved significantly more than WT or eEF2K+/- mice. * $p<0.05$, ** $p<0.01$; one-way ANOVA with Tukey's *post hoc* tests. (**E**) Velocity of movement (cm/s) in open field assay. APP/PS1 and APP/PS1/eEF2K+/- mice moved significantly faster than WT or eEF2K+/- mice. WT *n*=18, APP/PS1 *n*=16, eEF2K+/- *n*=11, APP/PS1/eEF2K+/- *n*=15; * $p<0.05$, ** $p<0.01$; one-way ANOVA with Tukey's *post hoc* tests. (**F**) Levels of phosphorylated tau (Ser416) did not vary across WT, APP/PS1, eEF2K+/-, or APP/PS1/eEF2K+/- brain lysates (*n*=4; $p=0.31$, one-way ANOVA). Box and whisker plots represent the interquartile range with the line across the box indicating the median. Whiskers show the highest and lowest values detected.