Supplementary Information for

Divergent roles of astrocytic versus neuronal EAAT2 deficiency on cognition and overlap with aging and Alzheimer's molecular signatures

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This PDF file includes:

Figs. S1 to S4

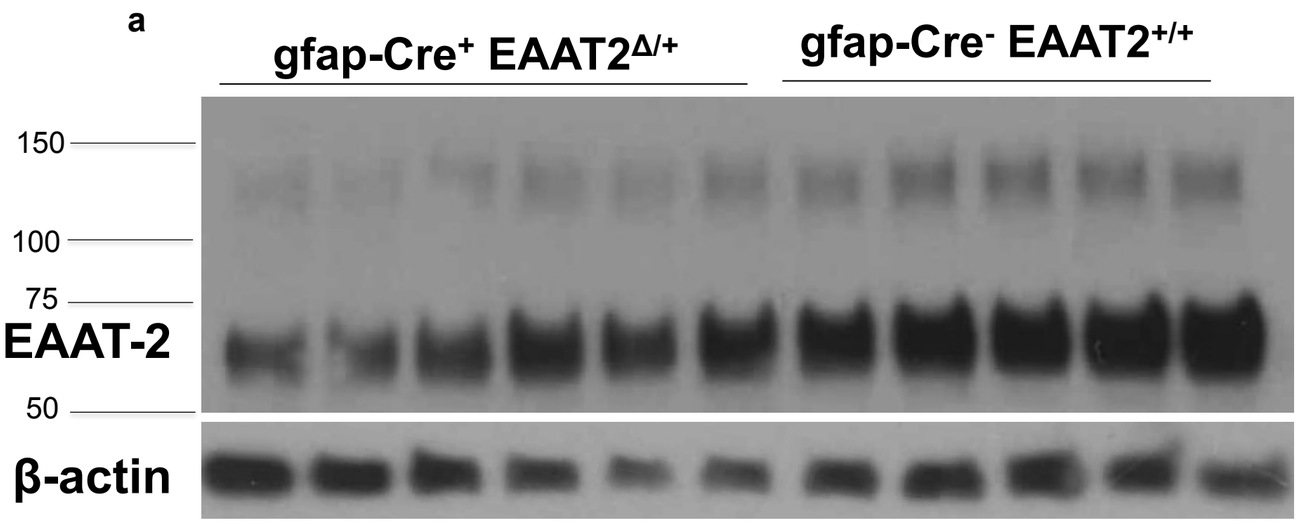
Fig. S1. Conditional heterozygous astrocytic EAAT2 knockout (gfap-Cre^{+} EAAT2$^{\Delta/+}$) mice exhibited significant reduction (22%) in EAAT2 expression while homozygous neuronal EAAT2 knock out (syn-Cre^{+} EAAT2$^{-/-}$) mice displayed a non-significant reduction (14%) in EAAT2 expression.

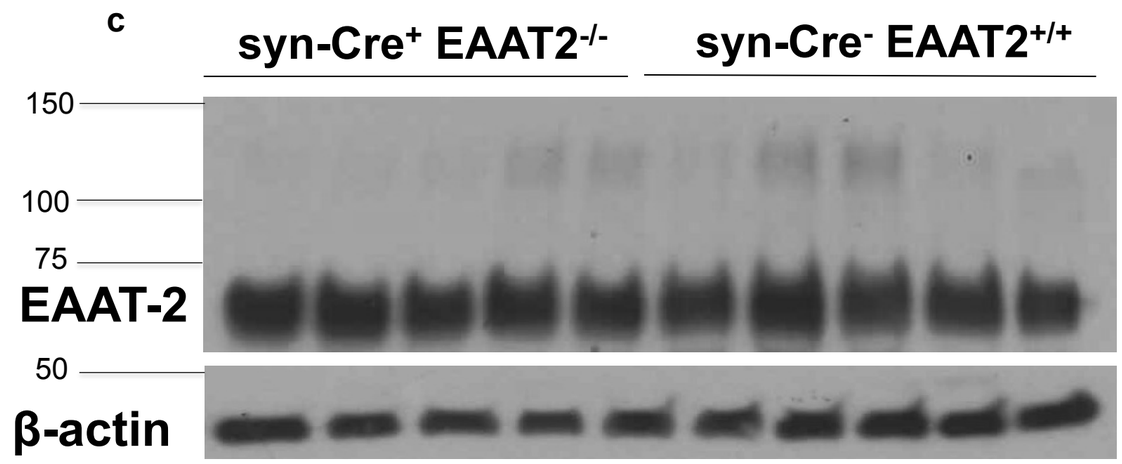
a, Representative protein immunoblots from cortical plasma membrane vesicle fractions of gfap-Cre^{+} EAAT2$^{\Delta/+}$ and gfap-Cre^{-} EAAT2$^{-/-}$ mice probed with antibodies against EAAT2 and βactin.

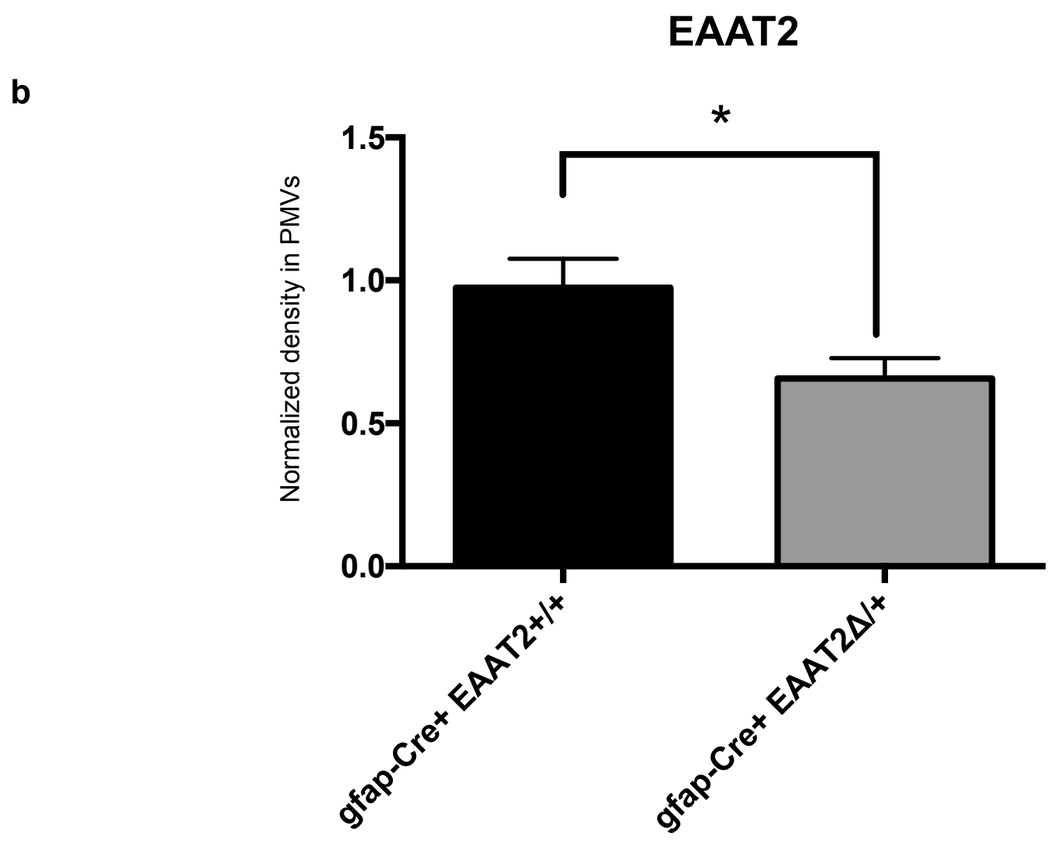
b, Quantification of Western blot data are shown as mean ± S.E.M. and are based on gfap-Cre^{+} EAAT2$^{\Delta/+}$ (n = 6) and gfap-Cre^{-} EAAT2$^{+/+}$ (n = 5).

c, Representative protein immunoblots from cortical plasma membrane vesicle fractions of syn-Cre^{+} EAAT2$^{-/-}$ and syn-Cre^{-} EAAT2$^{+/+}$ mice probed with antibodies against EAAT2 and βactin.

d, Quantification of Western blot data are shown as mean ± S.E.M. and are based on syn-Cre^{+} EAAT2$^{-/-}$ (n = 5) and syn-Cre^{-} EAAT2$^{+/+}$ (n = 5).







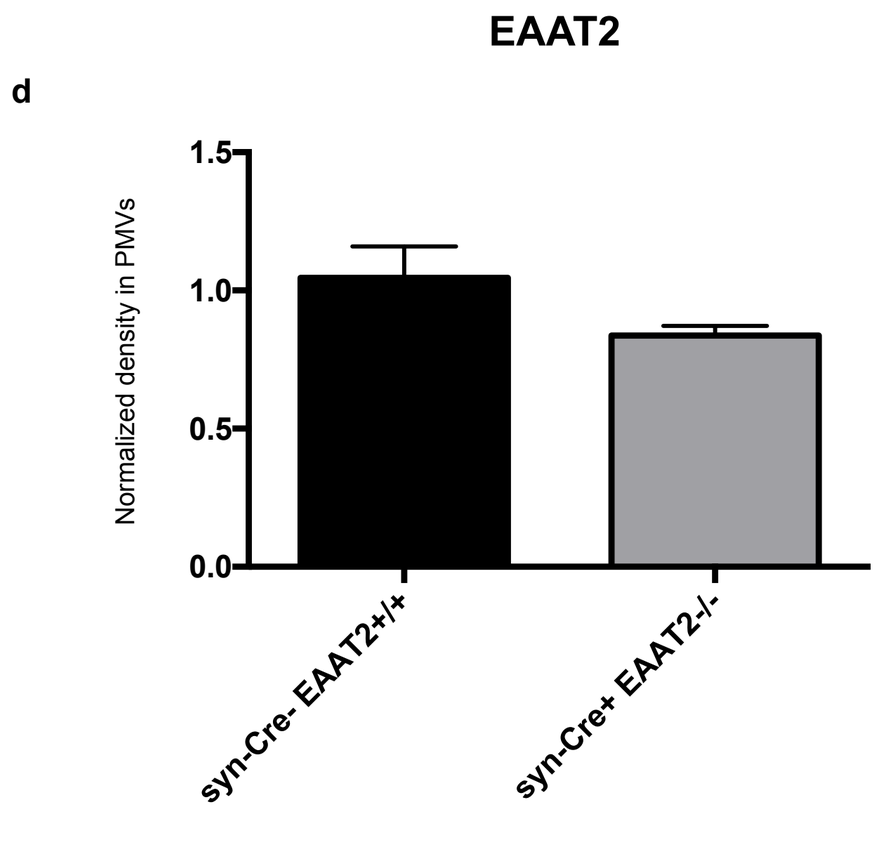


Fig. S2. Conditional heterozygous astrocytic EAAT2 knockout (gfap-Cre^{+} EAAT2$^{\Delta/+}$) and homozygous neuronal EAAT2 knock out (syn-Cre^{+} EAAT2$^{-/-}$) mice do not display changes in expression of synaptic markers.

a, Representative protein immunoblots from plasma membrane vesicle fractions of gfap-Cre^{+} EAAT2$^{\Delta/+}$ and gfap-Cre^{-} EAAT2$^{-/-}$ mice probed with antibodies against synaptophysin, PSD95 and βactin.

b,c Quantification of Western blot data are shown as mean ± S.E.M. and are based on gfap-Cre^{+} EAAT2$^{\Delta/+}$ (n = 6) and gfap-Cre^{-} EAAT2$^{+/+}$ (n = 5).

d, Representative protein immunoblots from plasma membrane vesicle fractions of syn-Cre^{+} EAAT2$^{-/-}$ and syn-Cre^{-} EAAT2$^{+/+}$ mice probed with antibodies against synaptophysin, PSD95 and βactin.

e,f Quantification of Western blot data are shown as mean ± S.E.M. and are based on syn-Cre^{+} EAAT2$^{-/-}$ (n = 5) and syn-Cre^{-} EAAT2$^{+/+}$ (n = 5).

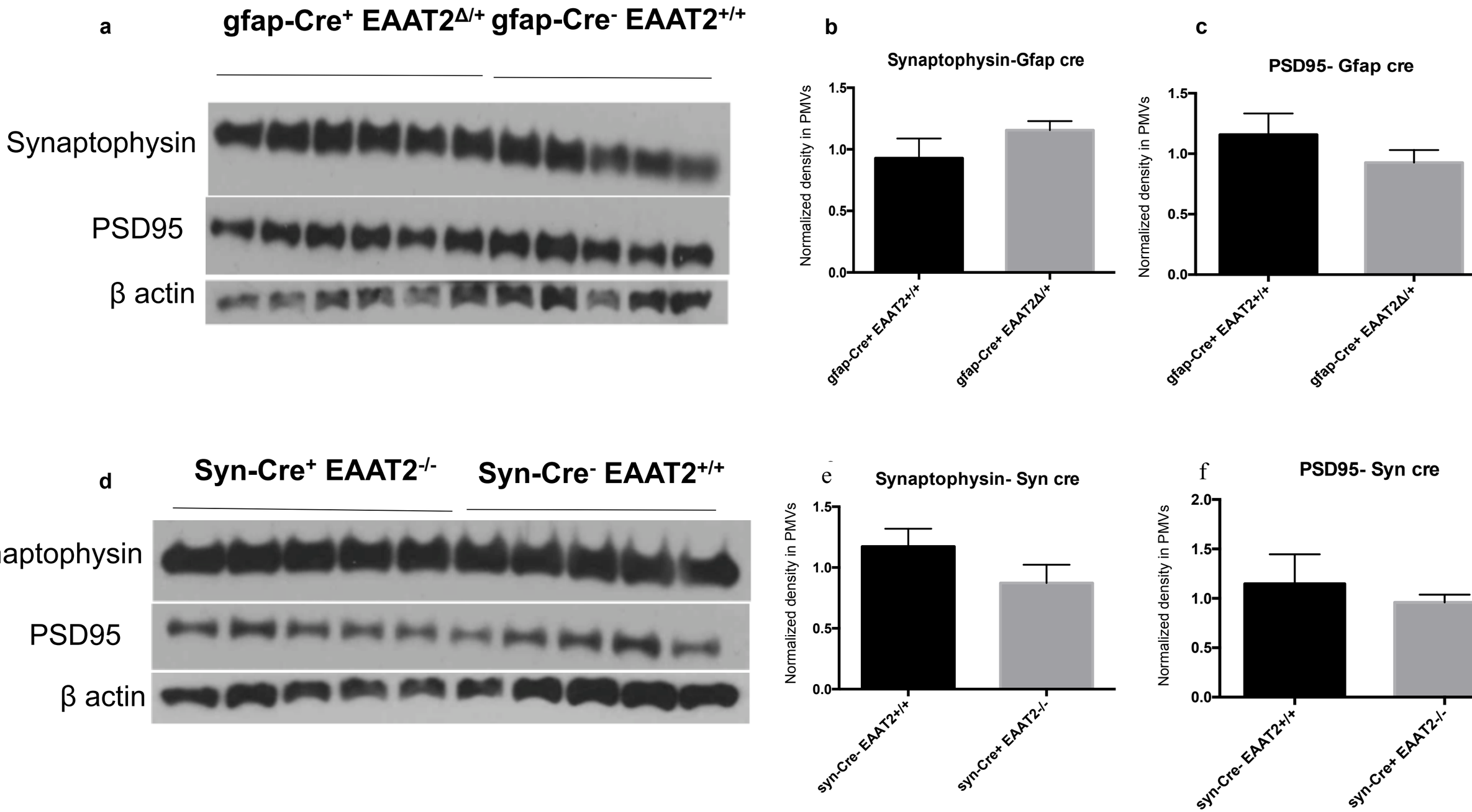


Fig. S3. Age-matched gfap-Cre+ control (without EAAT2 deletion) mice did not exhibit deficit in spatial reference memory in Y-maze and MWM at 13 and 17 months of age respectively.

a, In the acquisition trial of Y-maze at 13 months of age, gfap-Cre+ control (without EAAT2 deletion) mice explored Start arm (SA) and Familiar arm (FA) equally. $p = 0.28$

b, In the retention trial, gfap-Cre+ control mice spent more time exploring the novel arm (NA) than FA, suggesting absence of any spatial reference memory deficit at 13 months of age. $p = 0.0134$

c, Spatial learning curve of gfap-Cre+ control mice in MWM. The escape latency during the training phase (Blocks 1-4, D1-8) of gfap-Cre+ control mice. The gfap-Cre+ control mice exhibited statistically significant spatial learning over the learning trials ($F(2.6) = 5.854$, $p = 0.0018$, repeated measures two-way ANOVA).

d, Spatial reference memory evaluation of gfap-Cre+ control mice in MWM. The amount of time spent in TQ by gfap-Cre+ control mice in the probe trial (D9, 24-hr retention trial) is shown.

The data are shown as mean ± S.E.M. and are based on gfap-Cre+ control mice (n = 9).

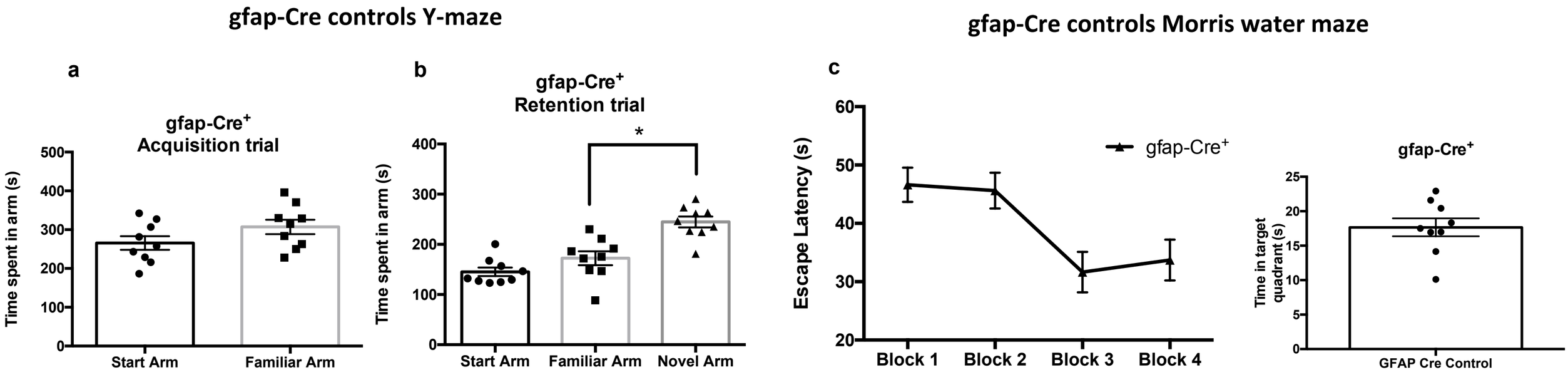


Fig. S4. Visible platform trial in MWM suggested lack of visual acuity deficit in gfap-Cre^{+} EAAT2$^{\Delta/+}$ and syn-Cre^{-} EAAT2$^{+/+}$ mice and their respective controls at 17 months of age.

Latency to find the visible platform in study mice assessed in a single trial conducted on D9 after probe trial is shown. The study groups did not differ significantly in the latency (s) to find the visible platform (p = 0.5268, Kruskal-Wallis test followed by Dunn's multiple comparison test).
The data are depicted as box and whisker plots (median with interquartile range, and upper and lower extremes), and based on gfap-Cre^{+} EAAT2$^{\Delta/+}$, n = 7; gfap-Cre^{-} EAAT2$^{+/+}$, n = 13; syn-Cre^{+} EAAT2$^{-/-}$, n = 11; syn-Cre^{-} EAAT2$^{+/+}$, n = 12.