

Figure S1. Supplement to Figure 1

(A) Western blot (WB) of AGO2 immunoprecipitated from neurons following control treatment (Ctrl) or 90 min post-iLTP stimulation (iLTP).

(B) qRT-PCR of *Gphn* mRNA bound to AGO2 in neurons from (A). AGO2-bound *Gphn* was normalized to total *Gphn* mRNA expression, and fold change from Ctrl was quantified for each condition. $n = 4$.

(C) qRT-PCR of total *Gphn* mRNA levels in Ctrl and iLTP-90 neurons. *Gphn* mRNA levels were normalized to U6 expression, and fold change from Ctrl was quantified for each condition. $n = 3$.

(D) Western blots of GABA_AR subunits β3 (synaptic) and α5 (extrasynaptic), AMPAR subunit GluA1, GAPDH, and GFP protein levels in neuron overexpressing miRCon or miR153 (left), and miRCon inhibitor or miR153 inhibitor (right). miRNA overexpression (OE) constructs contain a GFP reporter.

(E) Quantification of β3, α5, and GluA1 in miRCon or miR153 OE neurons. Protein levels were normalized to GAPDH, and the data quantified as relative change in normalized protein expression. $n = 5$.

(F) Quantification of β3, α5, and GluA1 in neurons expressing miRCon or miR153 inhibitors. $n = 4$.

All values represent mean ± SEM. * $p < 0.05$ and ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.0001$; one-sample t-test (B), Wilcoxon signed rank test (C), and Mann-Whitney test (E,F).

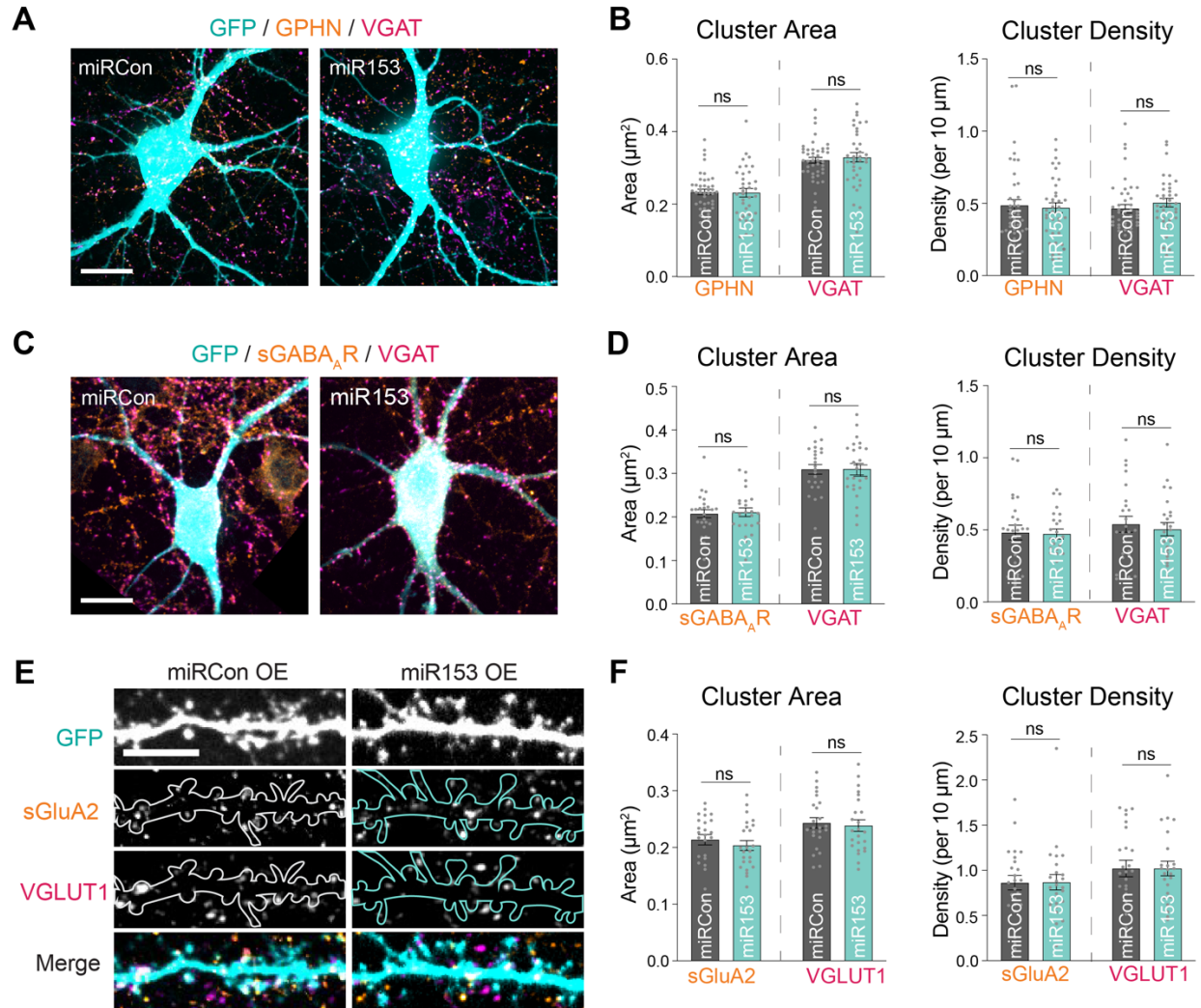


Figure S2. Supplement to Figure 2

(A) Representative somata of miRCon or miR153 OE-expressing neurons labeled with antibodies to GPHN and VGAT. Scale bar, 80 μm .

(B) Quantification of GPHN and VGAT cluster area (left) and cluster density (right) in neurons from (A). $n = 37$ - 43 neurons per condition.

(C) Representative somata of miRCon or miR153 OE-expressing neurons labeled with antibodies to surface GABA_AR $\gamma 2$ subunit (sGABA_AR) and VGAT. Scale bar, 80 μm .

(D) Quantification of sGABA_AR and VGAT cluster area (left) and cluster density (right) in neurons as shown in (C). $n = 24$ neurons in each condition.

(E) Representative dendritic segments of miRCon or miR153 OE neurons labeled with antibodies to surface AMPAR subunit GluA2 (sGluA2) and VGLUT1.

Scale bar, 80 μm .

(F) Quantification of surface GluA2 and VGLUT1 cluster area (left) and cluster density (right) in neurons from (C). $n = 24$ neurons per condition.

All values represent mean \pm SEM. * $p < 0.05$ and ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.0001$; Mann-Whitney test.

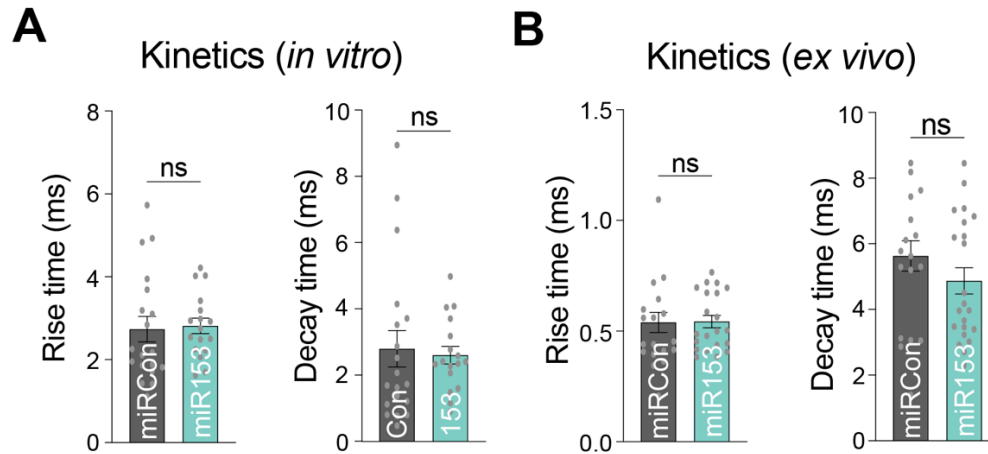


Figure S3. Supplement to Figure 3

(A) Quantification of mIPSC rise time (left) and decay time (right) from miRCon and miR153 OE-expressing neurons in culture. $n = 17-19$ neurons per condition.

(B) Quantification of mIPSC rise time (left) and decay time (right) from miRCon and miR153 OE-expressing neurons in slice. $n = 17-21$ neurons per condition.

All values represent mean \pm SEM. * $p < 0.05$ and ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.0001$; Mann-Whitney test.

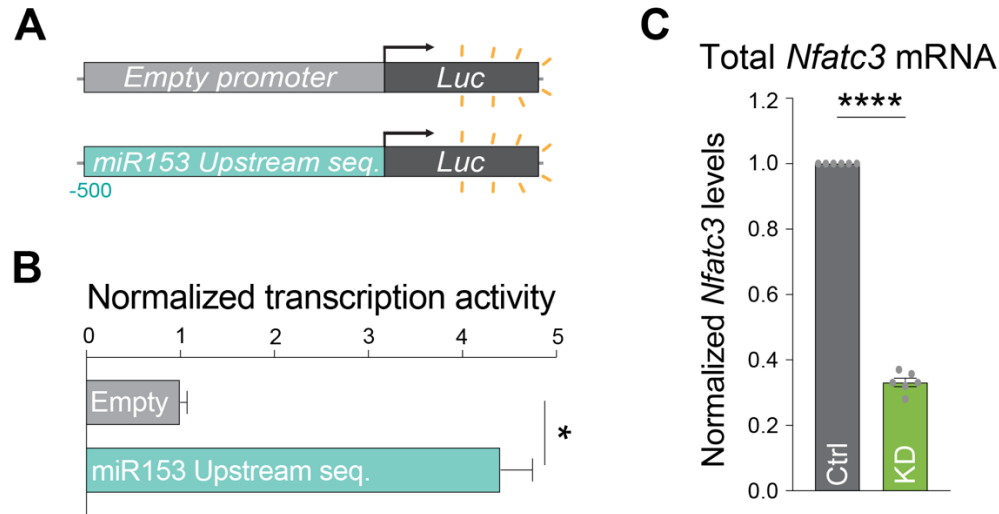


Figure S4. Supplement to Figure 4

- (A) Schematic of the Empty-Luc (no promoter) and miR153⁻⁵⁰⁰-Luc luciferase reporters, designed to test transcriptional activity of the sequence 500 bp upstream of miR153.
- (B) Quantification of Luc activities in neurons expressing reporters containing no promoter (Empty) or the sequence upstream of pri-miR153 coding region (miR153 Upstream seq.). Firefly was normalized to Renilla, and the data quantified as relative change in normalized Luc activity with error-corrected control values. n = 4.
- (C) qRT-PCR of total *Nfatc3* mRNA levels in Ctrl and NFATc3 knockdown (NFAT KD) neurons. *Nfatc3* mRNA levels normalized to *Gapdh* mRNA expression, and fold change from Ctrl was quantified for each condition. n = 6.
- All values represent mean ± SEM. *p<0.05 and **p<0.01, ***p<0.005, ****p<0.0001; Mann-Whitney test (B) and one-sample t-test (C).

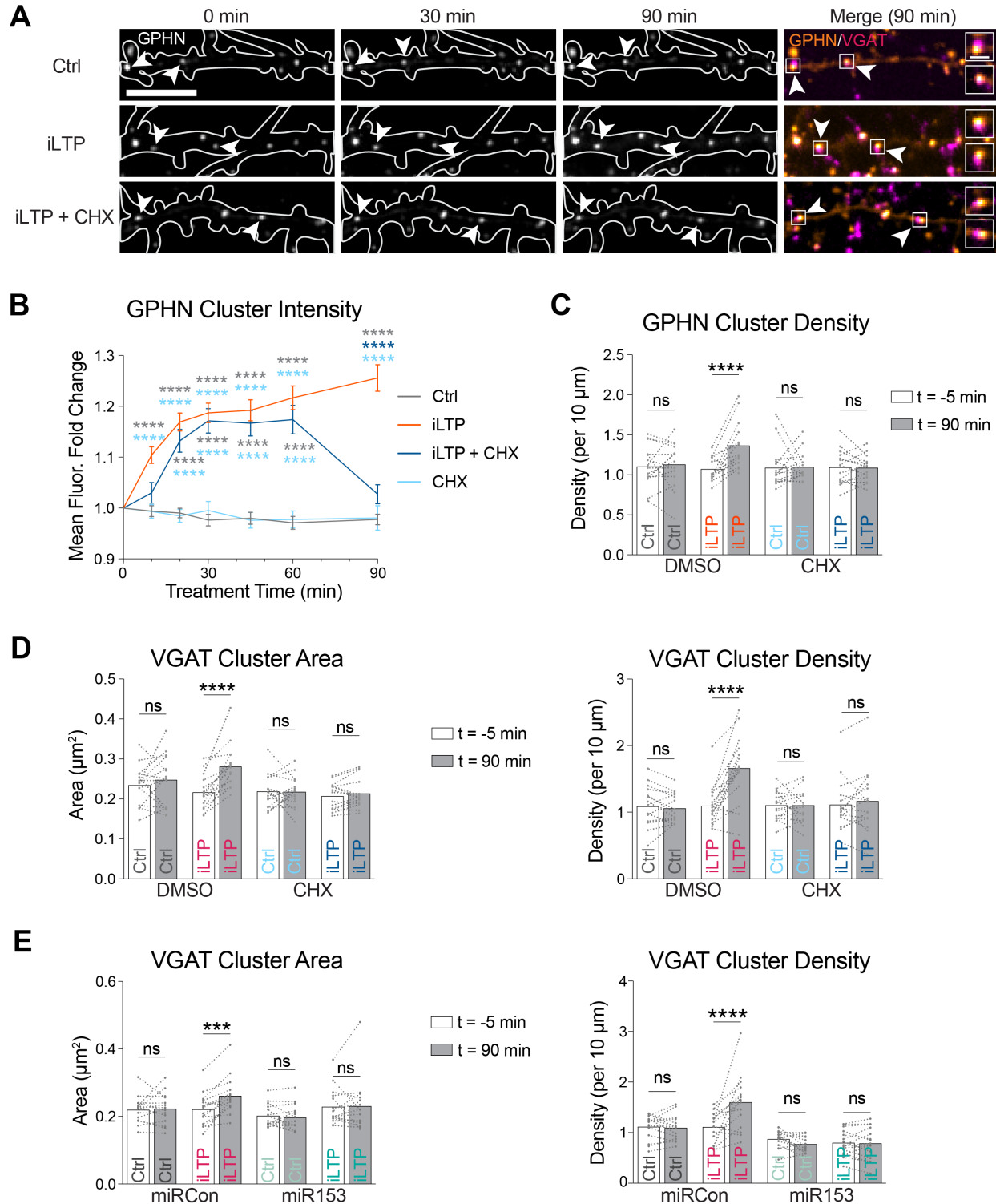


Figure S5. Supplement to Figure 5

(A) Representative dendritic segments of neurons expressing GPHN IB and labeled with an antibody to VGAT, imaged over time in control and iLTP conditions in the presence or absence of translational inhibitor cycloheximide (CHX). Puncta are labeled with filled arrowheads when the fluorescence is unchanged and open arrowheads when

fluorescence increases over time. Boxes indicate the fluorescent puncta enlarged in the merged images (dendrite scale bar, 10 μm ; synapse scale bar, 2 μm).

- (B) Quantification of fold change in GPHN puncta fluorescence intensity over time following treatment in neurons from A). n = 15 neurons per condition, 10 puncta per neuron.
- (C) Paired measurements of GPHN cluster density in dendrites prior to (-5 min) and 90 min following treatment. n = 15 neurons per condition.
- (D) Paired measurements of VGAT cluster area (left) and density (right) in dendrites prior to (-5 min) and 90 min following treatment. n = 15 neurons per condition.
- (E) Paired measurements of VGAT cluster area (left) and density (right) in miRCon or miR153 OE neurons (as seen in Figure 5A) prior to (-5 min) and 90 min following treatment. n = 15 neurons per condition.

All values represent mean \pm SEM. * $p < 0.05$ and ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.0001$; RM two-way ANOVA with Geisser-Greenhouse correction (B) and Šidák's multiple comparisons post-hoc test.

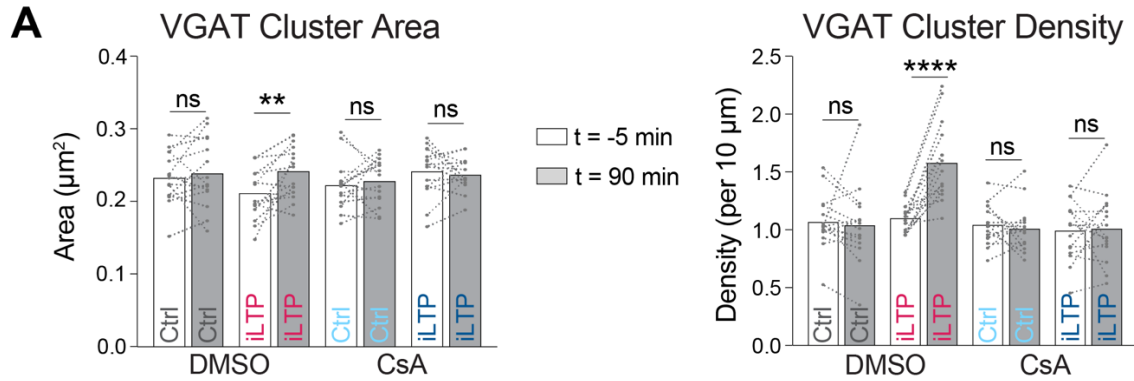


Figure S6. Supplement to Figure 6

(A) Paired measurements of VGAT cluster area (left) and density (right) in treated neurons (as seen in Figure 6D) prior to (-5 min) and 90 min following treatment. $n = 15$ neurons per condition.

All values represent mean \pm SEM. * $p < 0.05$ and ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.0001$; RM two-way ANOVA with Šidák's multiple comparisons post-hoc test.