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Supplemental Information

Local miRNA-Dependent Translational Control of $GABA_AR$ Synthesis during Inhibitory Long-Term Potentiation

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Figure S1

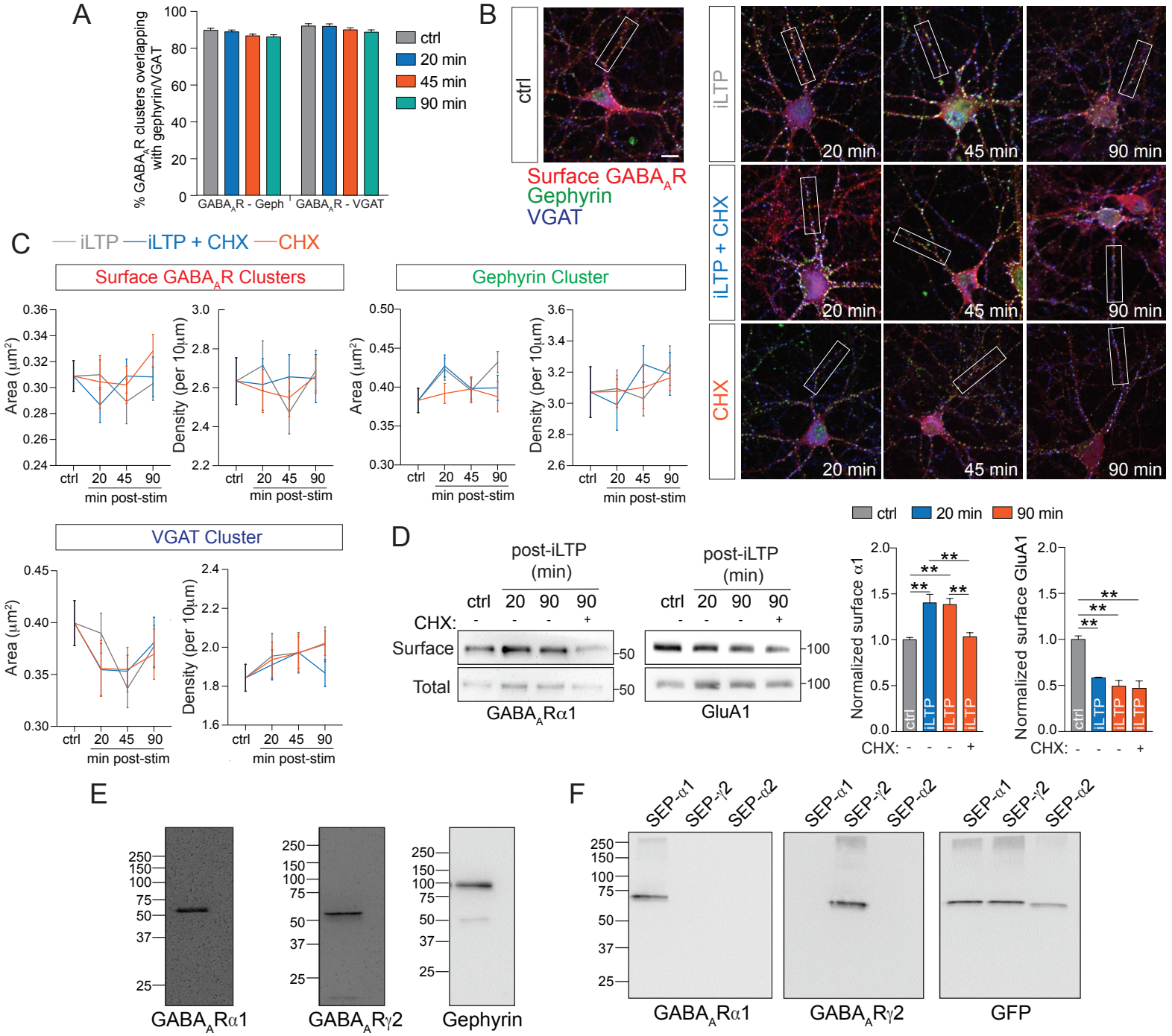

Figure S1, related to Figure 1. Protein synthesis is required to maintain dendritic surface GABA$_A$Rs following iLTP. A) Percentage dendritic surface GABA$_A$Rγ2 overlapping with gephyrin or VGAT in resting neurons and post-iLTP induction. Quantification based on images from Figure 1A, n=16 cells per condition from 3 independent experiments. **B**) Representative soma images labeled with surface GABA$_A$Rγ2 and intracellular gephyrin and VGAT. Neurons were fixed at 20, 45 and 90 min post-treatment: iLTP induction, iLTP induction in the presence of cycloheximide (CHX), or CHX alone. Dendritic segments shown in Figure 1A are from the boxed white areas shown. Scale bar = 10μm. **C**) Quantification of somatic surface GABA$_A$Rγ2, gephyrin and VGAT cluster area and density from B), n=15-16 cells per condition from 3 independent experiments.**D**) WBs and quantification of GABA$_A$Rα1 and GluA1 from surface biotinylation assays following iLTP in the presence or absence of CHX. Surface levels were normalized to total levels, n=5. **E**) Full-length WBs showing anti-GABA$_A$Rα1 and anti-GABA$_A$Rγ2 recognize a single band at ~50kDa from hippocampal neuronal lysates and anti-gephyrin recognizes a major band at ~100 kDa. Predicted molecular weights for α1, γ2 and gephyrin are 52 kDa, 54 kDa and 84.4 kDa respectively. Gephyrin runs higher due to post-translational modifications. **F**) Lysates from HEK293T cells expressing SEP-tagged GABA$_A$Rα1, GABA$_A$Rγ2 or GABA$_A$Rα2 were probed with anti-GABA$_A$Rα1, anti-GABA$_A$Rγ2 and anti-GFP antibodies. Anti-GABA$_A$Rα1 only detects GABA$_A$Rα1. Anti-GABA$_A$Rγ2 only detects GABA$_A$Rγ2. Anti-GFP detects all three subunits.

All values represent mean ± SEM. **p < 0.01, by one-way ANOVA, Bonferroni post hoc test (A,D) or two-way ANOVA, Bonferoni post hoc test (C).

Figure S2

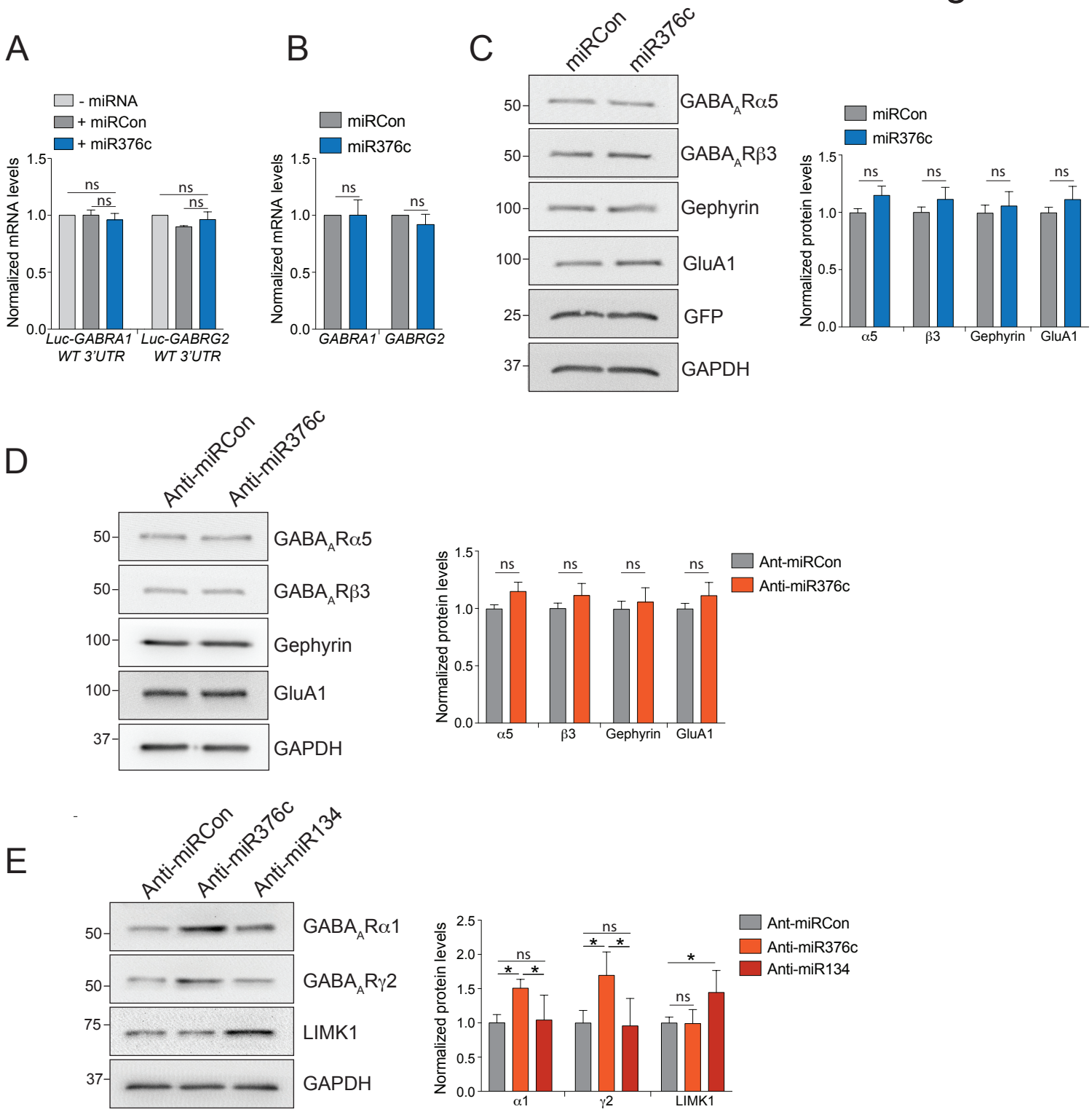

Figure S2, related to Figure 2. miR376c specifically regulates translational repression of *GABRA1* and *GABRG2*. **A**) qRT-PCR measuring mRNA levels of Luc-*GABRA1*WT and Luc-*GABRG2*WT from HEK-293T cells. All readings were normalized to Renilla levels, n=3. **B**) miR376c overexpression does not change *GABRA1* and *GABRG2* levels in hippocampal neurons. mRNA levels were normalized to snRNA U6, n=3. **C**) WBs showing miR376c overexpression does not change protein levels of GABA$_A$Rα5, GABA$_A$Rβ3, Gephyrin or GluA1 in hippocampal neurons. miRNA overexpression constructs contain a GFP reporter, which shows equal expression of miRCon and miR376c. GFP and GAPDH blots are the same blots as those used in Figure 2E. Protein levels were normalized to GAPDH, n=6. **D**) WBs showing that anti-miR376c does not change protein levels of GABA$_A$Rα5, GABA$_A$Rβ3, Gephyrin or GluA1 in hippocampal neurons. GAPDH blot is the same as used in Figure 2F. Protein levels were normalized to GAPDH. N=5. **E**) WBs showing that anti-miR134 does not change protein levels of GABA$_A$Rα1 and GABA$_A$Rγ2, and anti-miR376c does not change protein levels of LIMK1, n=4.

*p=<0.05, by one-way ANOVA, Bonferroni post hoc test. All values represent mean ± SEM. *p < 0.05 by T-test (B,C,D) or one-way ANOVA, Bonferroni post hoc test (A,E).

Figure S3

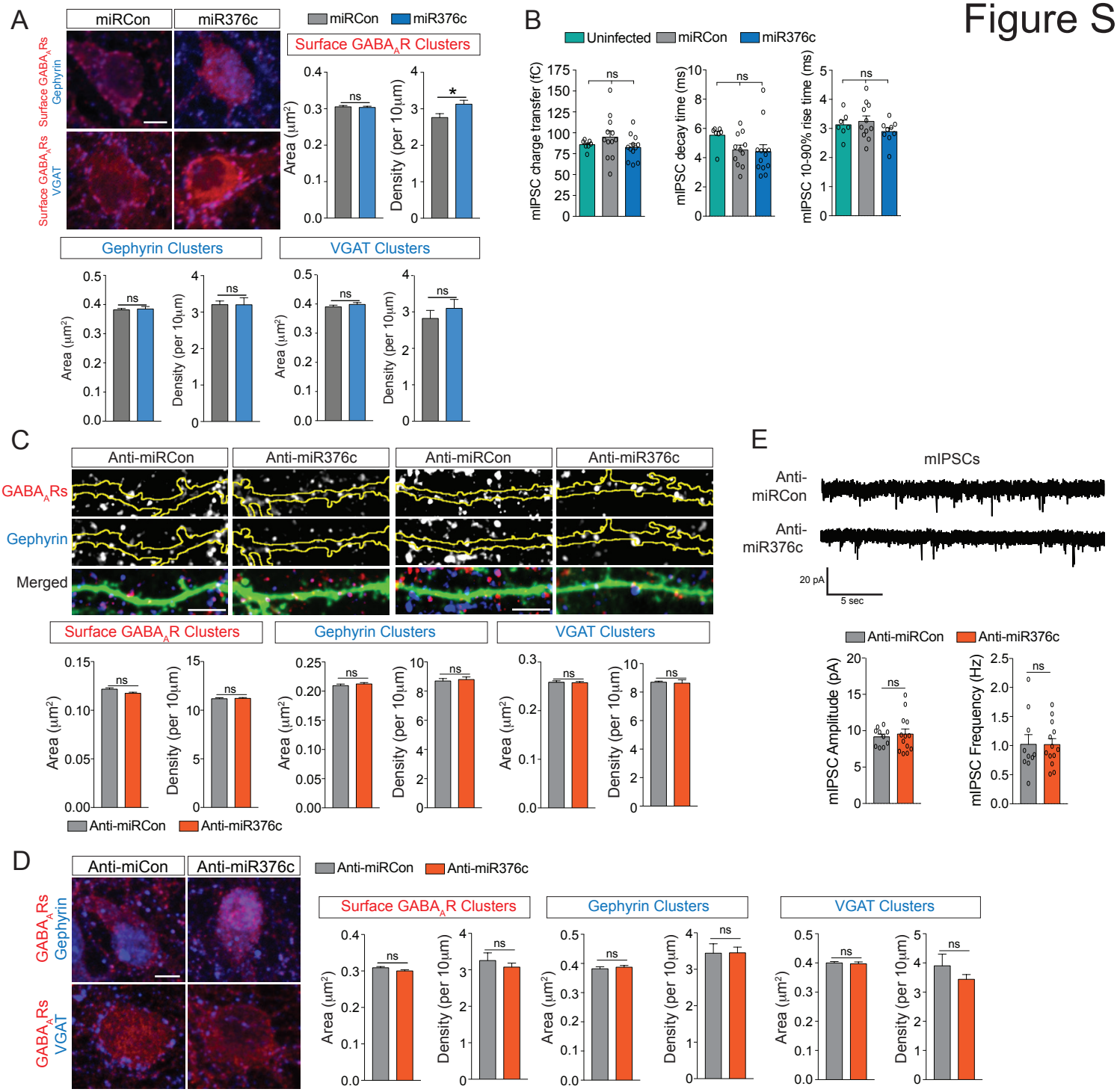

Figure S3, related to Figure 3. miR376c does not influence clustering of somatic GABA$_A$Rs. A) Representative soma images from neurons expressing miRCon or miR376c labeled with surface GABA$_A$Rγ2 and gephyrin or GABA$_A$Rγ2 and VGAT. GFP is not shown to aid visualization of somatic clusters. Graphs show quantification of GABA$_A$Rγ2, gephyrin and VGAT cluster area and density, n=7-18 neurons per condition from 3 independent experiments. Scale bar=5μm. **B**) Quantification of mIPSC charge transfer, decay time and 10-90% rise time from uninfected neurons or neurons overexpressing miRCon or miR376c, n=7-12 cells from 3 independent experiments. **C**) Representative dendritic images from neurons transfected with anti-miRCon or anti-miR376c labeled with surface GABA$_A$Rγ2 and intracellular gephyrin or GABA$_A$Rγ2 and VGAT. Yellow outlines are constructed from GFP fill. Graphs show quantification of GABA$_A$Rγ2, gephyrin and VGAT cluster area and density, n=9-18 neurons per condition from 3 independent experiments. Scale bar = 5μm. **D**) Representative soma images from neurons expressing anti-miRCon or anti-miR376c labeled with surface GABA$_A$Rγ2 and intracellular gephyrin or GABA$_A$Rγ2 and VGAT. GFP is not shown to aid visualization of somatic clusters. Graphs show quantification of GABA$_A$Rγ2, gephyrin and VGAT cluster area and density, n=9-18 neurons per condition from 3 independent experiments. Scale bar=5μm. **E**) Representative mIPSC traces from neurons expressing anti-miRCon or anti-miR376c. Graphs show quantified mIPSC amplitude and frequency, n=10-13 cells from 3 independent experiments. All values represent mean ± SEM. Statistical significance determined by t-test (A,C,D,E) or one-way ANOVA, Bonferoni *post hoc* test (B).

Figure S4

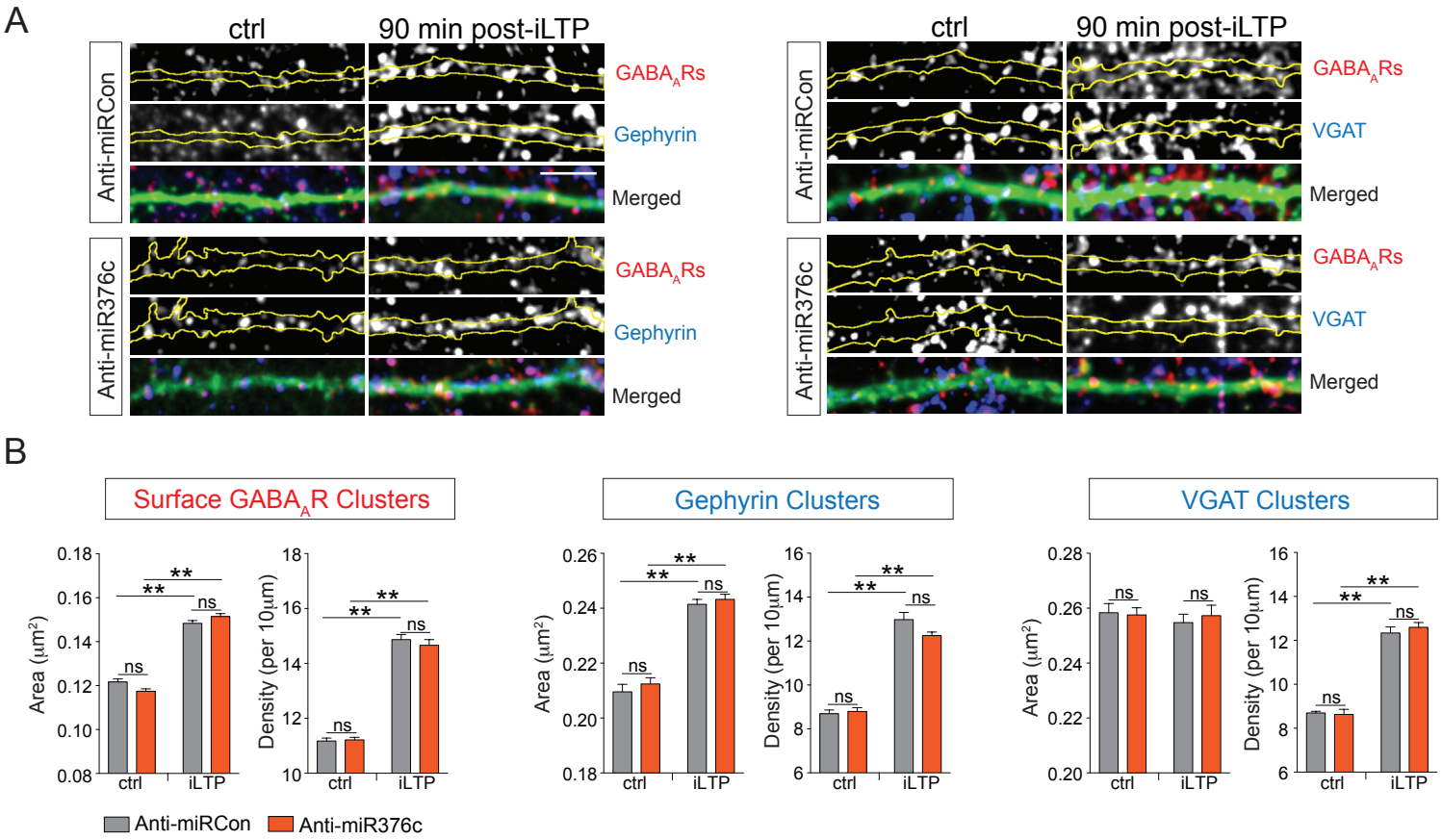

Figure S4, related to Figure 4. miR376c inhibition does not occlude the upregulation of synaptic GABA$_A$Rs following iLTP stimulation.

A) Representative dendritic images from resting and iLTP induced (90 min) neurons expressing anti-miRCon or anti-miR376c, labeled with surface GABA$_A$Rγ2 and intracellular gephyrin or GABA$_A$Rγ2 and VGAT. Yellow outlines are constructed from GFP fill. Scale bar = 5μm.

B) Graphs show quantification of GABA$_A$Rγ2, gephyrin and VGAT cluster area and density.n=9-18 neurons per condition from 3 independent experiments. All values represent mean ± SEM. **p < 0.01, by two-way ANOVA, Bonferroni *post hoc* test.

Figure S5

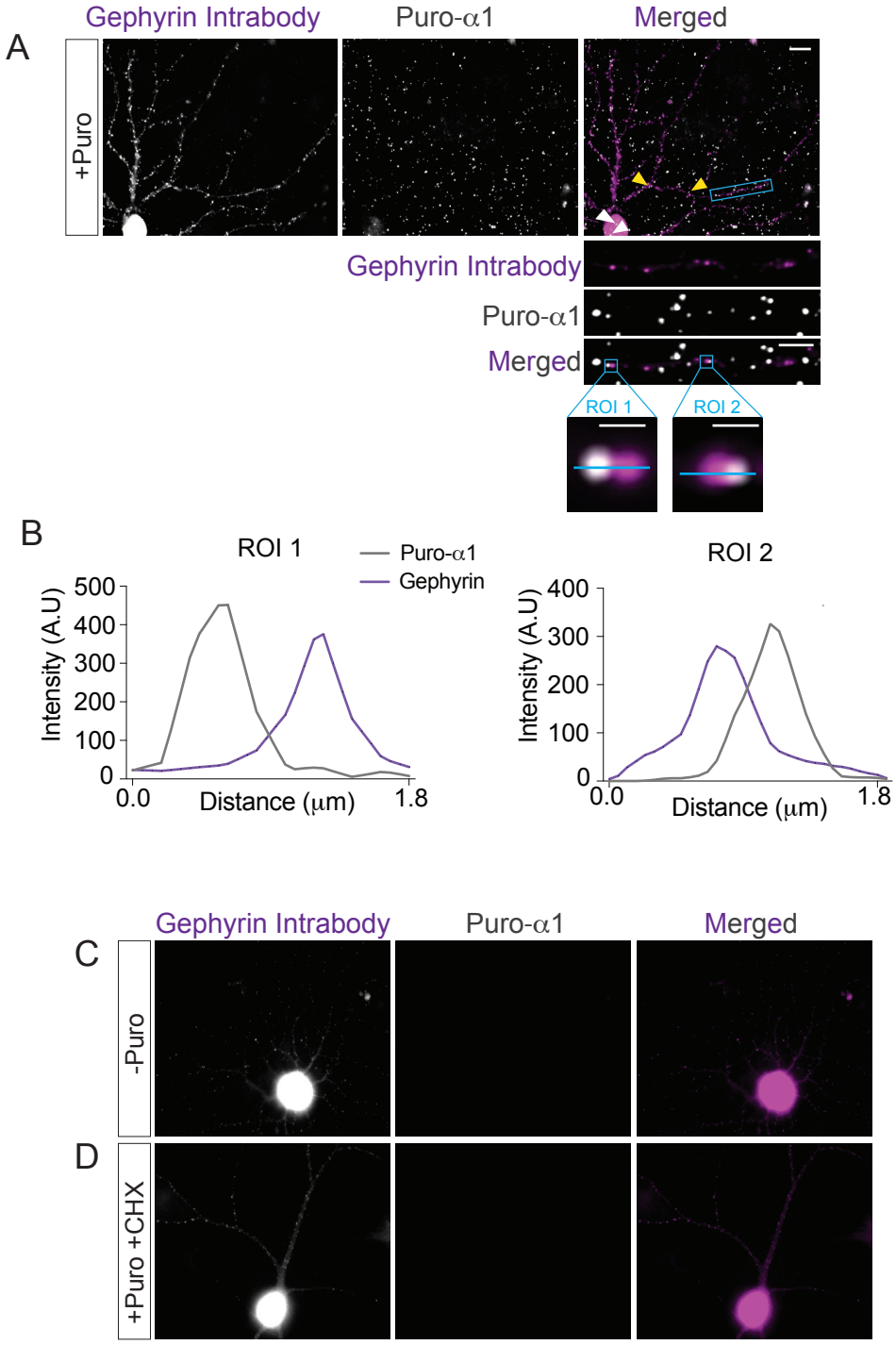

Figure S5, related to Figure 5. *De novo* synthesis of $GABA_AR\alpha1$ occurs at sites close to inhibitory synapses

A) Puromycin-proximity ligation assay (Puro-PLA) for $GABA_AR\alpha1$ performed in the presence of puromycin. Nascent $GABA_AR\alpha1$ (Puro-α1) in the soma (white arrow heads) and dendrites (yellow arrowheads) of hippocampal neurons. Puro-α1 puncta not within the cell-fill is labeling from neurons not transfected with the gephyrin intrabody. Neurons were fed with 1μM of puromycin for 10 minutes prior to fixation. Dendritic enlargement of boxed area in the merged image is shown. Scale bar = 10μm for the whole cell image, 5μm for dendritic enlargement, 1μm for ROIs.

B) Line-scan analysis from 2 regions of interest (ROIs) marked show puro-α1 adjacent to gephyrin labeled inhibitory synapses.

C) Puro-PLA for $GABA_AR\alpha1$ performed in the absence of puromycin.

D) Puro-PLA for $GABA_AR\alpha1$ performed in the presence of puromycin and cycloheximide (CHX).

Figure S6

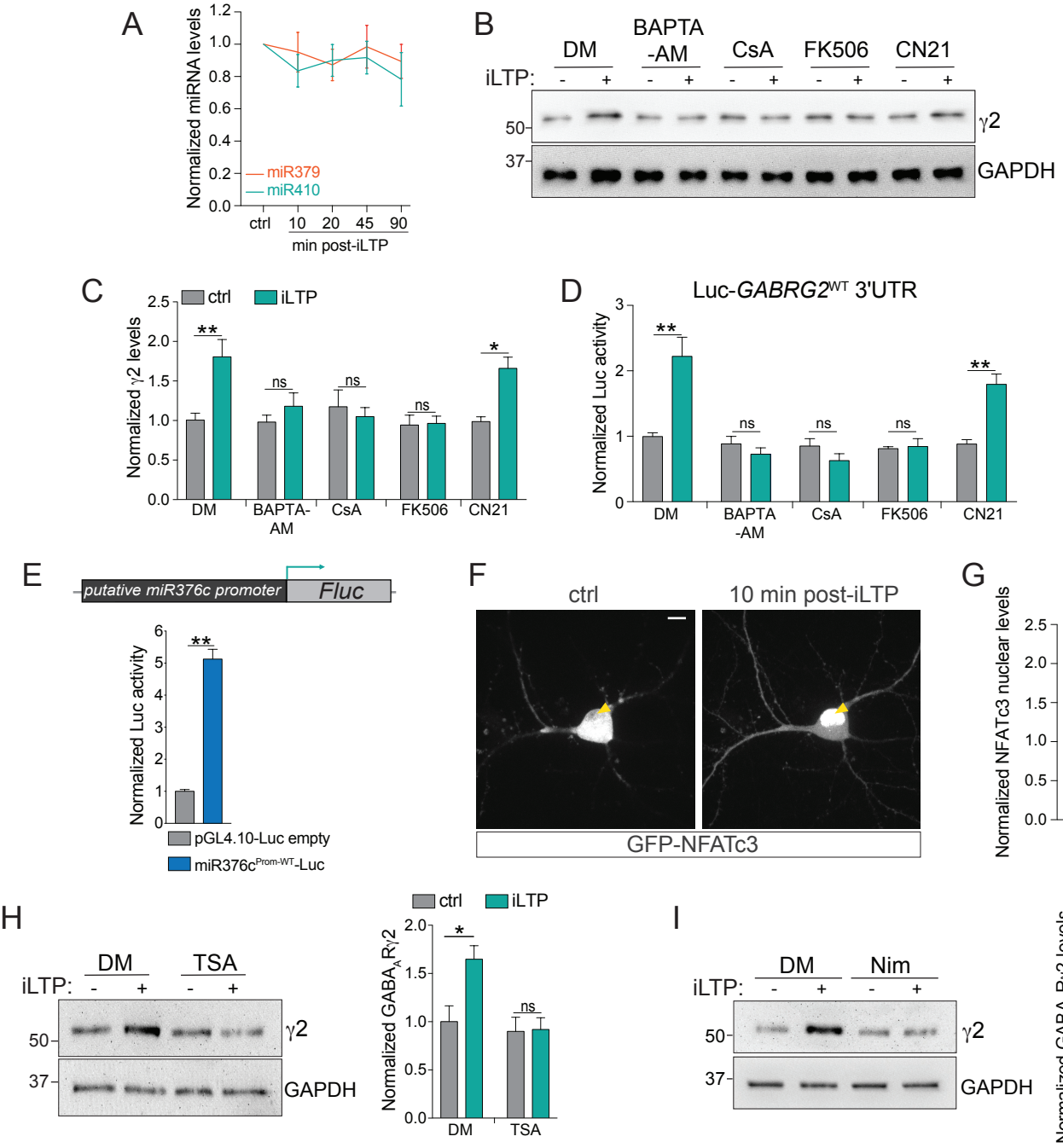

Figure S6, related to Figure 7. L-type Ca^{2+} channels, calcineurin and NFATc3 signaling is involved in promoting *de novo* synthesis of $GABA_AR$ subunits α1 and γ2 following iLTP. A) qRT-PCR showing expression levels of mature miR379 and miR410 do not change following iLTP. miRNA levels were normalized to U6 snRNA, n=5. **B**) WBs showing levels of $GABA_AR\gamma2$ from resting neurons (ctrl) and from neurons 90 min post-iLTP-induction in the presence of DMSO (DM), BAPTA-AM, CsA, FK506 or TAT-CN21 (CN21).GAPDH blot is the same as Fig. 7C. **C**) Quantification of γ2 protein levels from B), n=6. **D**) Luciferase reporter readings of Luc-*GABRG2* WT in resting neurons or in neurons 90 min post-iLTP-induction in the presence of DMSO (DM), BAPTA-AM, CsA, FK506 or TAT-CN21 (CN21), n = 5. **E**) Luciferase assay showing the putative miR376c promoter (miR376cProm-WT-Luc) has significantly greater luciferase activity compared to a promoter-less empty luciferase reporter construct (pGL4.10-Luc empty). The putative miR376c promoter is the first 500bp of genomic sequence upstream of the pre-miR376c coding sequence. **F**) Live imaging showing GFP-NFATc3 translocates to the nucleus (yellow arrow heads) within 10 min of iLTP induction in hippocampal neurons. Scale bar = 10μm. **G**) Quantification of nuclear GFP-NFATc3 levels. Nuclear expression was normalized to somatic expression. Scale bar = 10μm, n=40 neurons from 5 independent experiments. **H**) WBs and quantification showing levels of $GABA_AR\gamma2$ from resting neurons (ctrl) or from neurons 90 min post-iLTP-induction in the presence of DMSO (DM) or Trichostatin A (TSA), n=6. GAPDH blot is the same as Fig. 7O. **I**) WBs and quantification showing L-type Ca^{2+} channel blockade with Nimodipine (Nim;10μm) inhibits the iLTP-induced upregulation of $GABA_AR\gamma2$. $GABA_AR\gamma2$ protein levels were normalized to GAPDH levels, n=3.GAPDH blot is the same as Fig. 7Q.

All values represent mean ± SEM. *p < 0.05 and **p < 0.01, by T-test (E,G), one-way ANOVA, Bonferroni *post hoc* test (A) or two-way ANOVA, Bonferoni *post hoc* test (C,D,H,I).

Figure S7

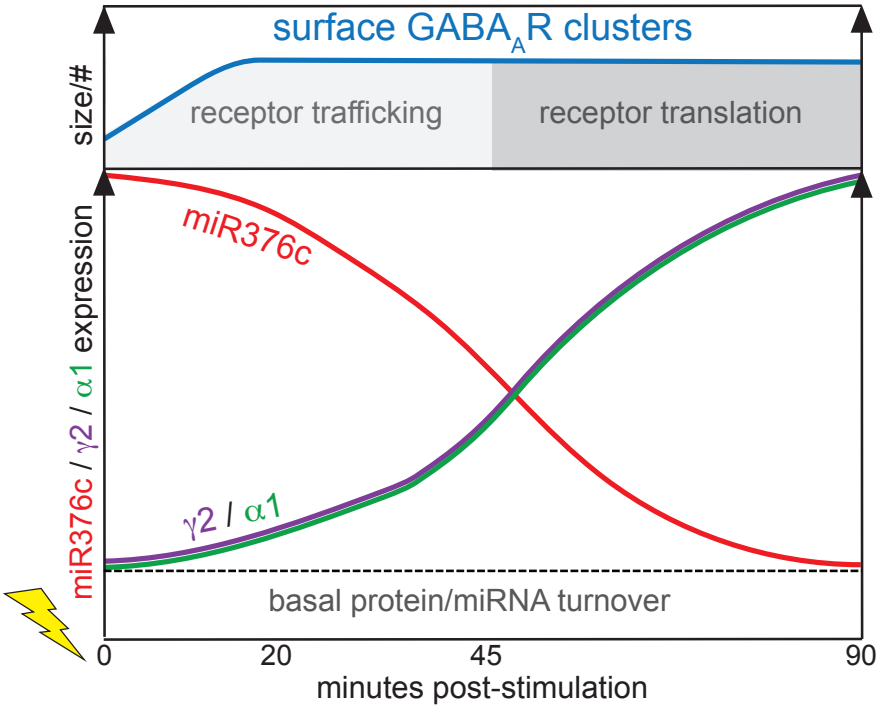

Figure S7, related to Figures 1 and 4. miR376c reduction parallels increased *de novo* synthesis of synaptic GABA$_A$R subunits following NMDAR activation.
Following NMDAR-mediated iLTP stimulation, pre-existing assembled GABA$_A$Rs are forward trafficked to the synapse to increase inhibitory synaptic strength (Marsden *et al*., 2007, Petrini *et al*., 2014). Maximal steady-state surface expression of GABA$_A$Rs is achieved within the first 20 min of stimulation. During this time-frame, transcriptional-repression of the miR376c gene leads to a reduction in mature functional miR376c over time. This leads to an increase in *de novo* protein synthesis of α1 and γ2 GABA$_A$R subunits in dendrites, which assemble into functional synaptic GABA$_A$Rs that can be incorporated into synapses, thereby maintaining potentiated GABAergic synapses.