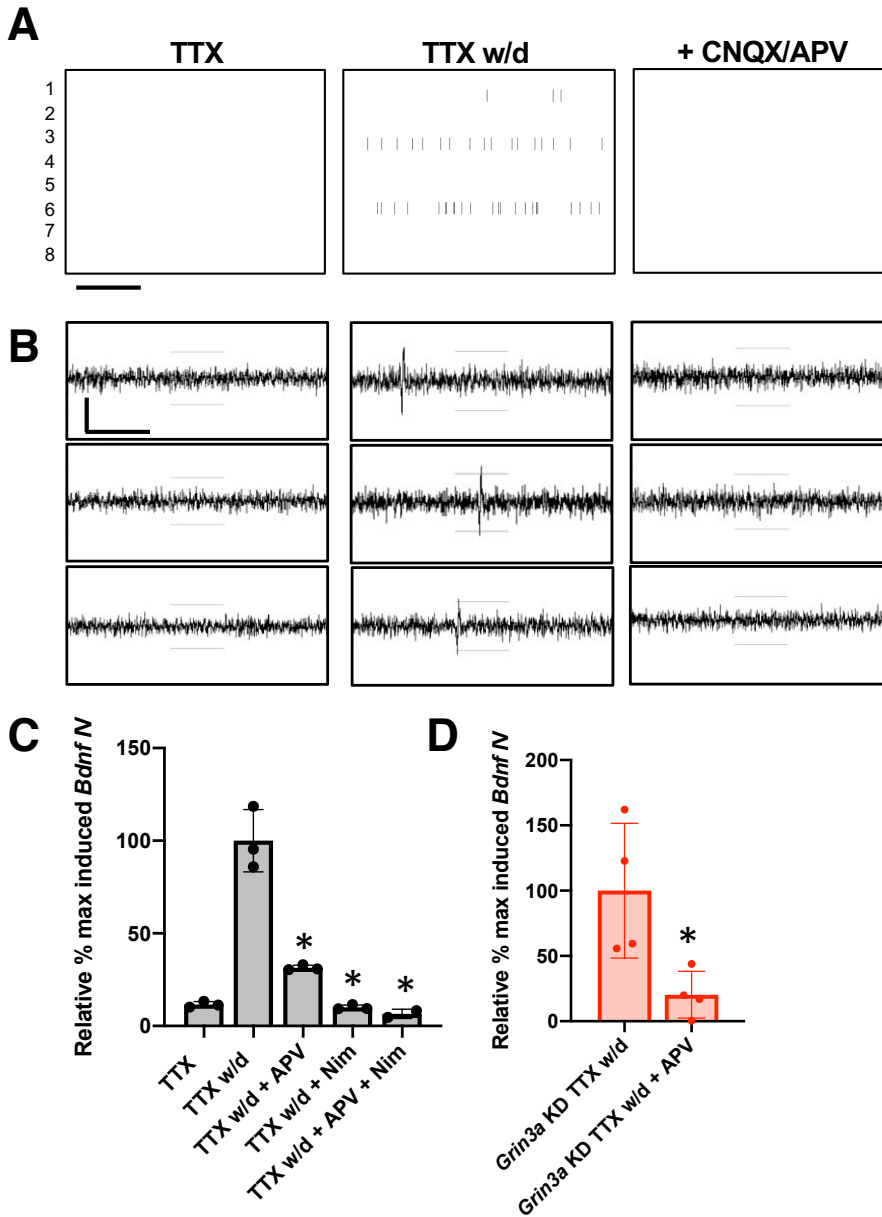
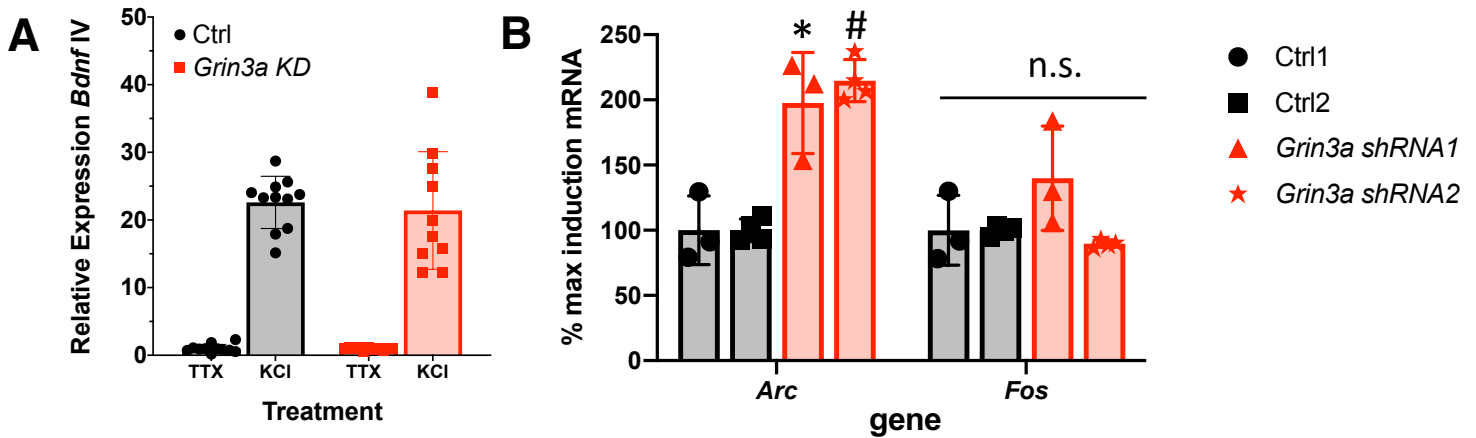


# Figure S1



**Figure S1: TTX withdrawal drives synaptic activity and NMDAR-dependent induction of *Bdnf IV* expression.** (A) Representative recording from hippocampal neurons plated on multielectrode arrays and treated with the drugs indicated. Raster plots from 8 individual electrodes are shown. Scale bar 30sec. (B) Zoomed view of electrodes 1, 3, and 6 from (A). Scale bar, 10 $\mu$ V vertical, 10msec horizontal. (C) Expression of *Bdnf IV* in hippocampal neurons treated with the drugs indicated. Expression is scaled to 100% for the TTX w/d values to show % inhibition by pharmacological blockade. ANOVA for treatment  $F(4, 9) = 69.08$ ,  $p=0<0.0001$ .  $n=3$ /condition except APV + Nim = 2. All conditions vs TTX w/d  $p<0.0001$ . (D) Expression of *Bdnf IV* in hippocampal neurons infected *Grin3a* shRNA1 and treated with TTX w/d +/- APV.  $n=4$ /condition. TTX w/d vs TTX w/d + APV  $p= 0.027$ . \* $p<0.05$  compared with TTX w/d.

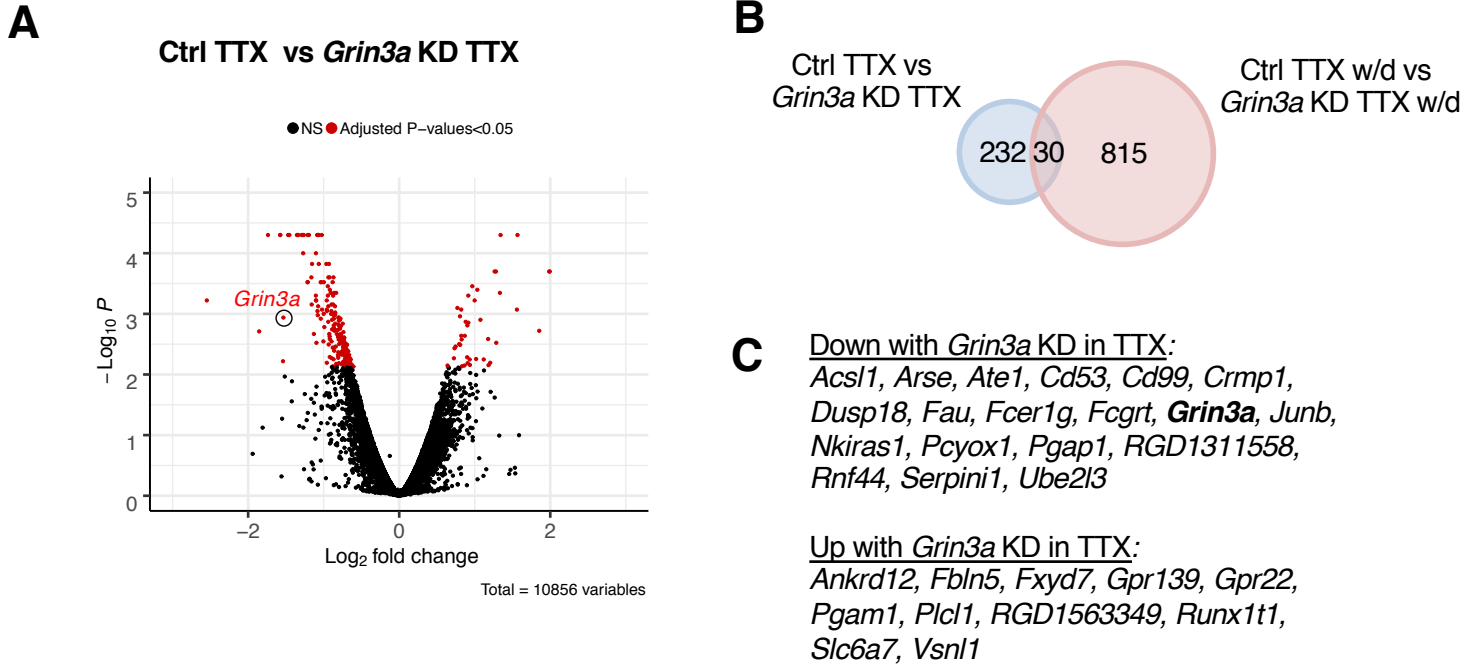
## Figure S2



### Figure S2: Stimulus- and target-specificity of transcriptional regulation by *Grin3a* knockdown.

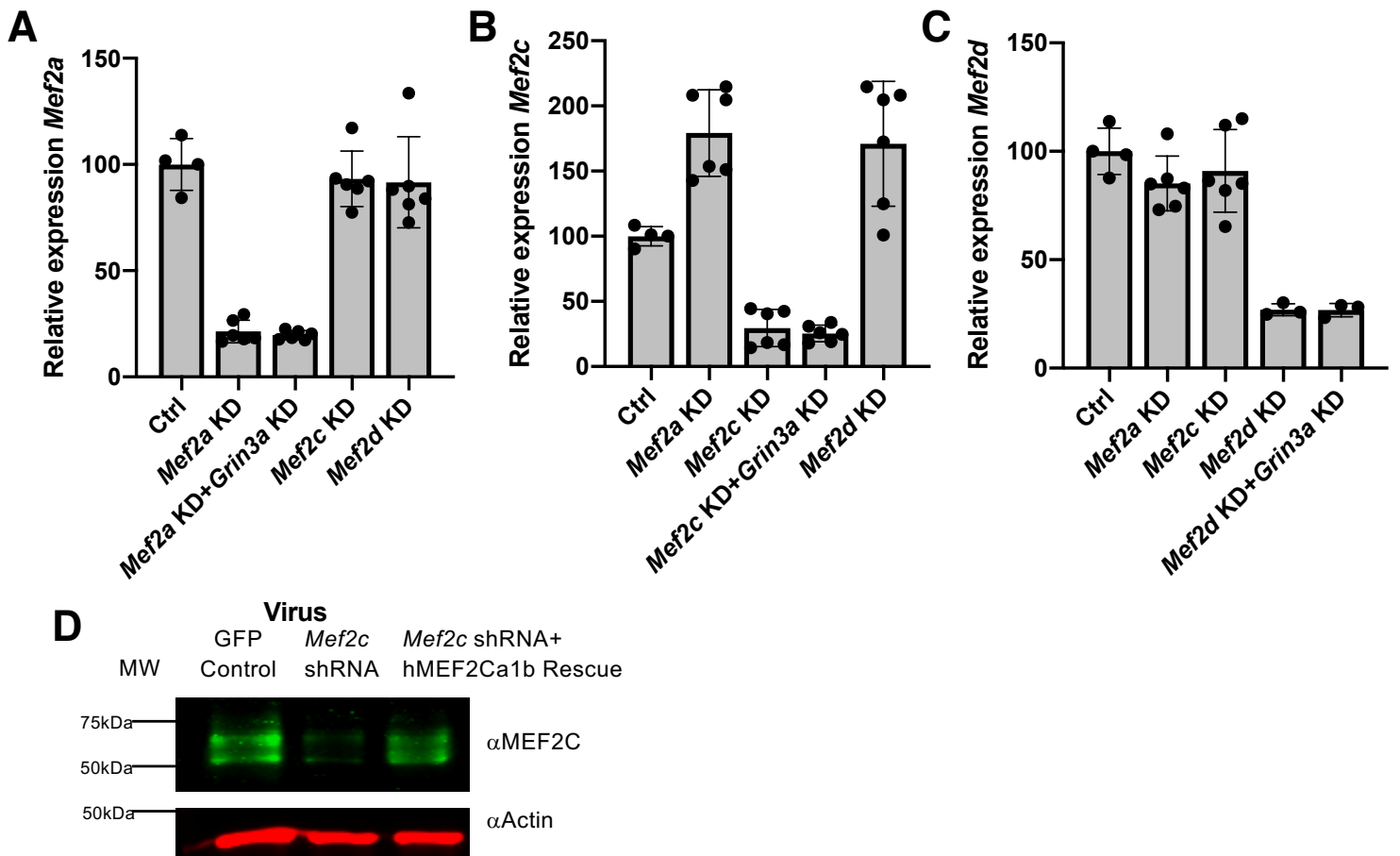
**(A)** Levels of *Bdnf* IV mRNA in hippocampal neurons infected with the indicated lentiviruses in the presence of TTX or 6hrs following KCl stimulation. Two-way ANOVA for treatment  $F(1,39)=221.8$   $p<0.0001$ , for virus  $F(1,39)=0.19$   $p=0.67$ , and treatment x virus interaction  $F(1,39)=0.17$   $p=0.68$ . **(B)** Levels of *Arc*, and *Fos* mRNA in hippocampal neurons infected with the indicated lentiviruses then stimulated with 30min TTX w/d. Induced mRNA levels for neurons infected with each shRNA targeting *Grin3a* (shRNA1, shRNA2) are reported as percentages of induction relative to their respective control vectors (Ctrl1 or Ctrl2). Two-way ANOVA for virus  $F(3,20)=16.92$ ,  $p<0.0001$ , for gene  $F(1,20)=28.41$ ,  $p<0.0001$ , and virus x gene interaction  $F(3,20)=13.28$ ,  $p<0.0001$ . \* $p=0.0007$  for shRNA1 compared with Ctrl1 for *Arc*; # $p<0.0001$  for shRNA2 compared with Ctrl2 for *Arc*. n.s., not significant.

## Figure S3



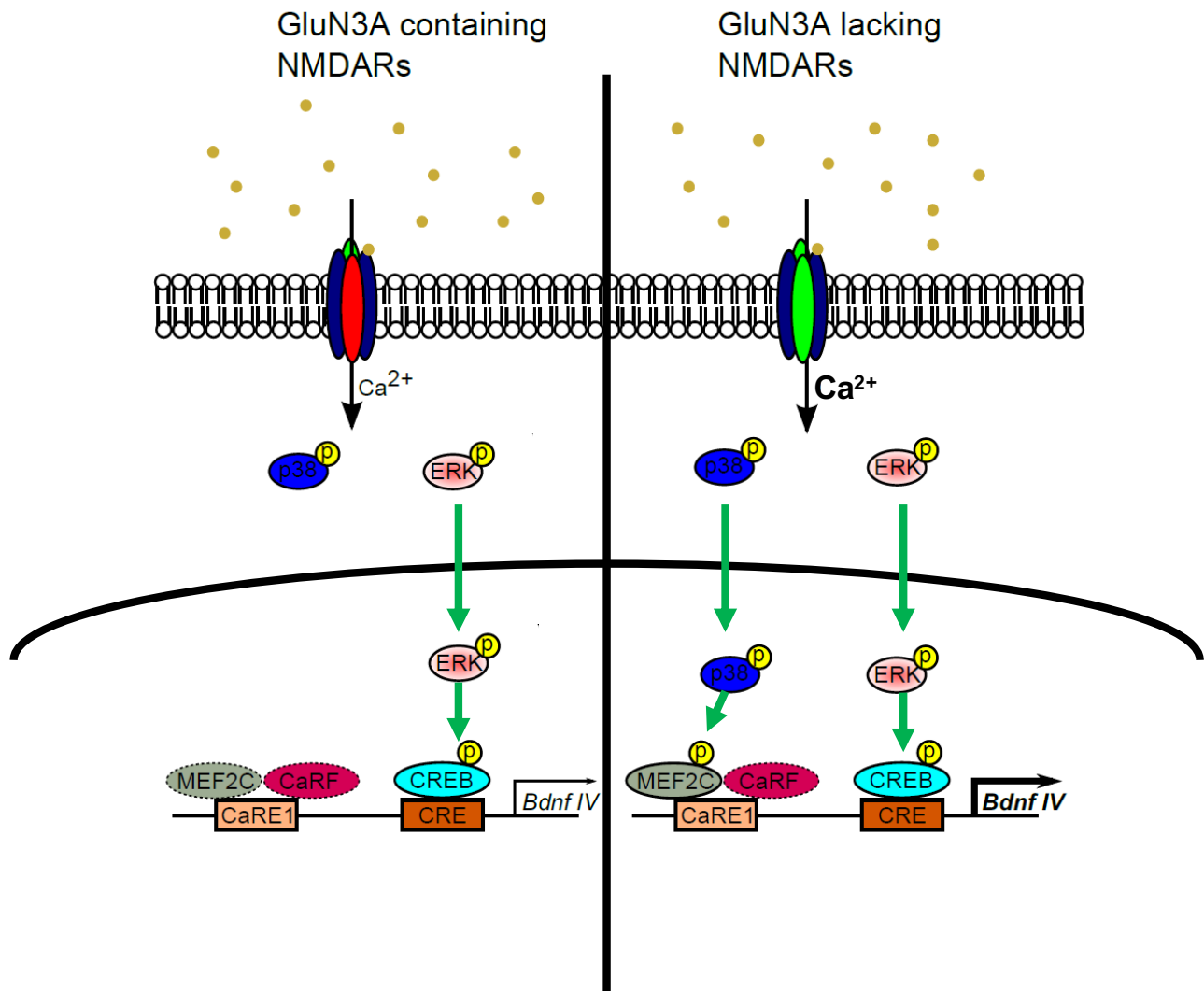
**Figure S3: Limited effects of GluN3A knockdown on gene expression in TTX-silenced neurons.** (A) Volcano plot showing genes significantly different at FDR-adjusted  $p < 0.05$  (red dots) comparing Ctrl TTX with GluN3A KD TTX.  $n=3$  biological replicates per sample. *Grin3a* is shown for reference. (B) Venn diagram showing genes significantly different in Ctrl vs GluN3A KD neurons in the presence of TTX (blue) compared with genes significantly different in Ctrl vs GluN3A KD neurons after TTX w/d (red). (C) Identity of the 30 genes affected by knockdown of GluN3A in both TTX and TTX w/d comparisons divided by their direction of regulation with *Grin3a* KD alone.

## Figure S4



**Figure S4: Selective viral manipulation of *Mef2a*, *Mef2c*, and *Mef2d* in cultured rat hippocampal neurons.** Levels of (A) *Mef2a* (B) *Mef2c* and (C) *Mef2d* mRNA in hippocampal neurons infected with the indicated shRNA lentiviruses. mRNA levels are shown normalized to levels in cells infected with the control pLKO virus. (D) Nuclear fractions from cultured neurons infected with the indicated lentiviruses were analyzed by Western blot using an antibody that detects the MEF2C protein (MW~51kDa). Actin is shown as a loading control.

Figure S5



**Figure S5: Model for the GluN3A-dependent regulation of NMDAR-induced and MEF2C dependent transcription.** Activation of both GluN3A-containing (left) and GluN3A-lacking (right) NMDARs leads to the Erk MAPK-dependent phosphorylation (P) of the transcription factor CREB and the activation of CREB-dependent genes including *Bdnf*. Both receptors also promote the phosphorylation and activation of p38 MAP kinase in the cytoplasm, but only GluN3A-lacking NMDARs can drive p38 activation in the nucleus of the neurons. In the nucleus, p38 MAP kinase phosphorylates and activates the transcription factor MEF2C, which collaborates with CREB to potentiate the transcription of *Bdnf*. Transcription of *Bdnf IV* is indicated by the black arrow at the transcription start site and the potentiated transcription is indicated by the increased thickness of the arrow. CREB is shown bound to its regulatory element (CRE) and MEF2C as well as the transcription factor CaRF bind Calcium-Response Element 1 (CaRE1) in *Bdnf* promoter IV.