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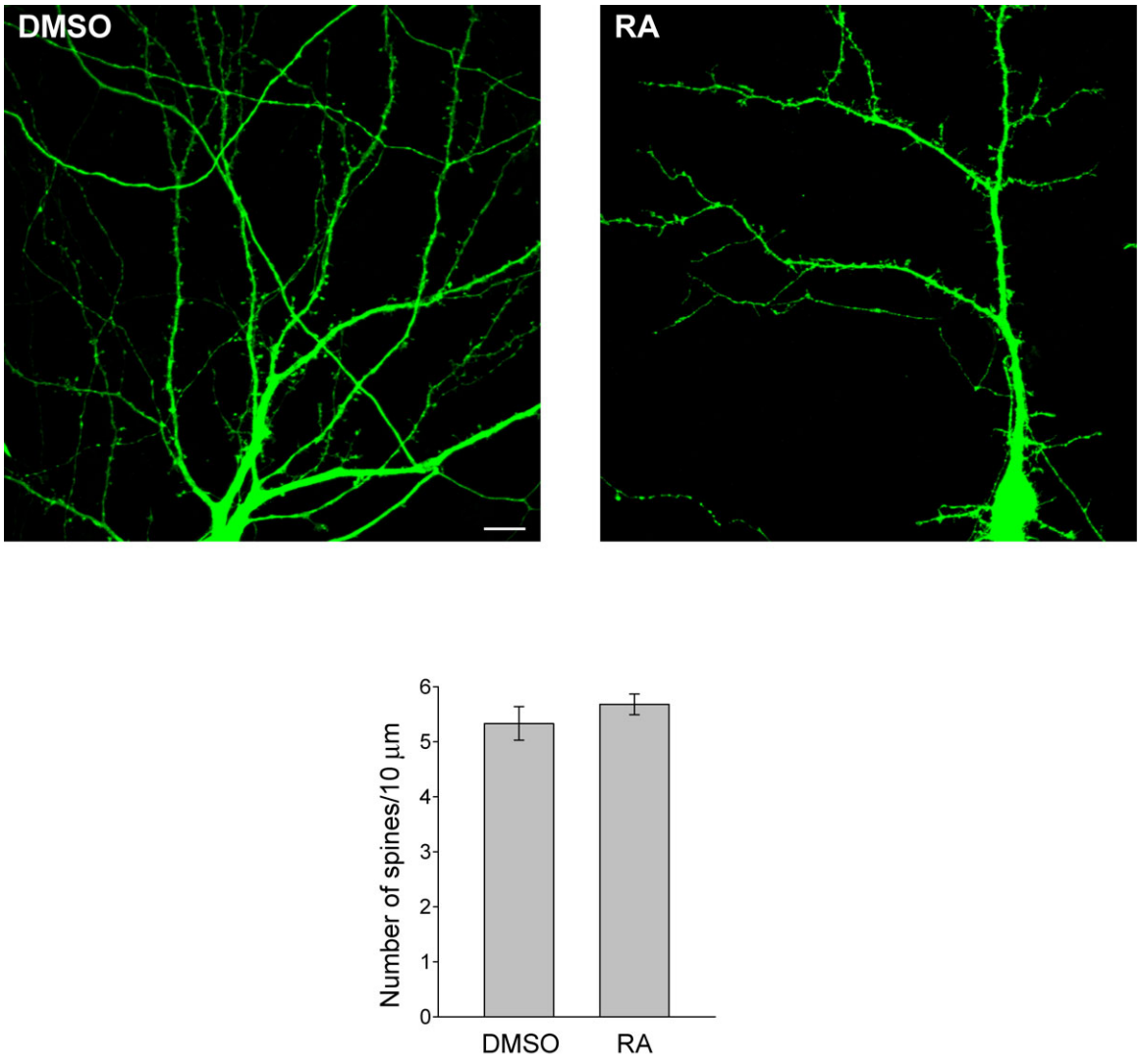
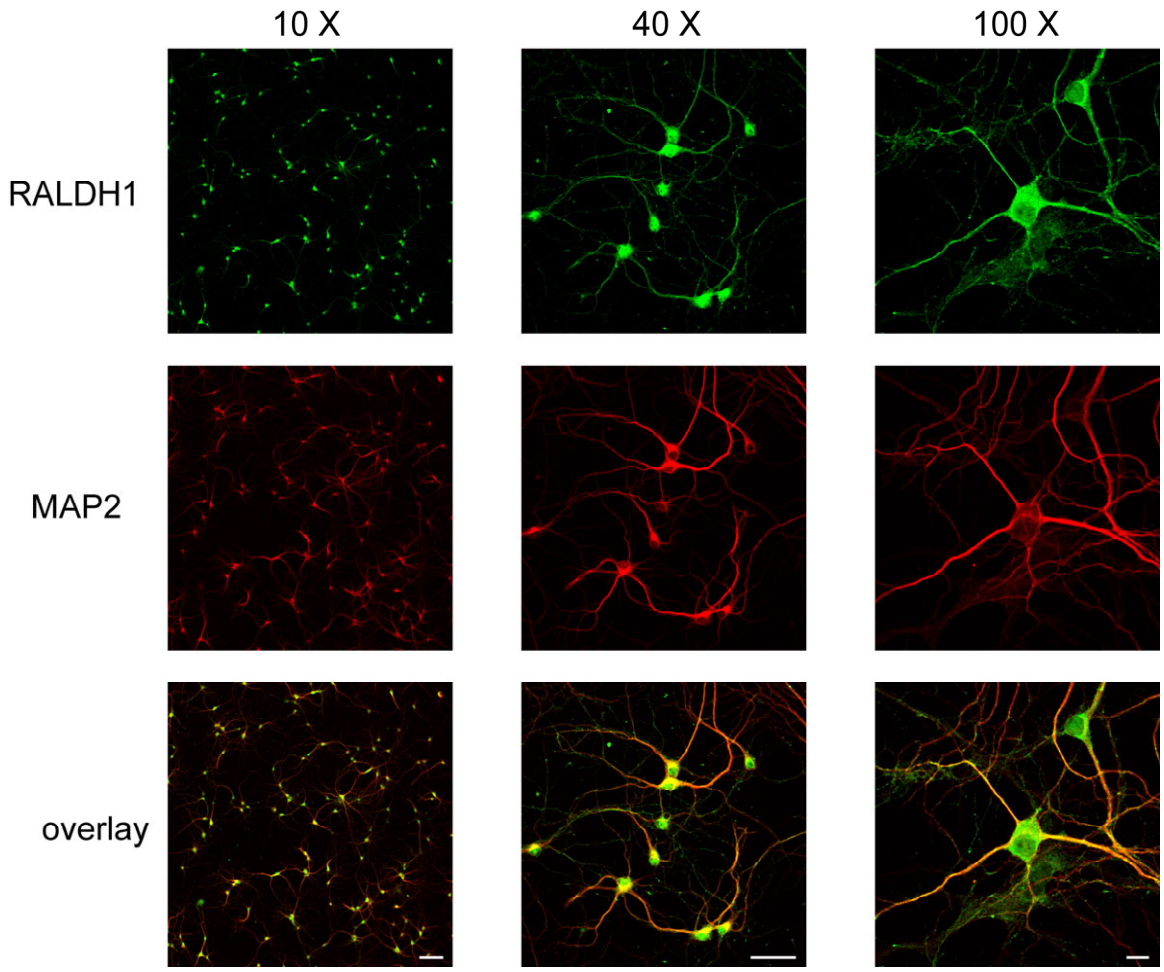
Supplemental Data

Synaptic Signaling by All-*Trans* Retinoic Acid in Homeostatic Synaptic Plasticity

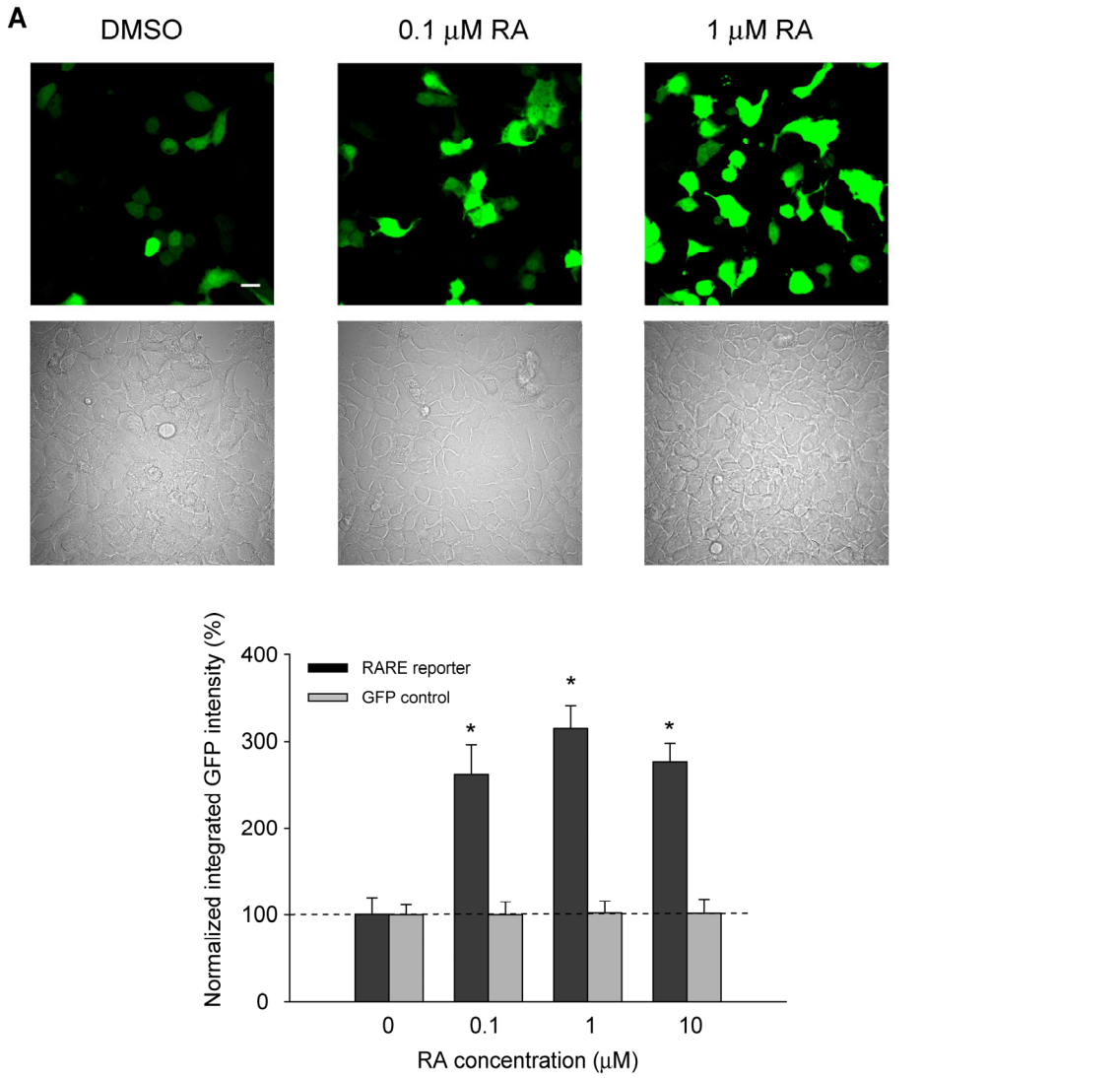
Jason Aoto, Christine I. Nam, Michael M. Poon, Pamela Ting, and Lu Chen



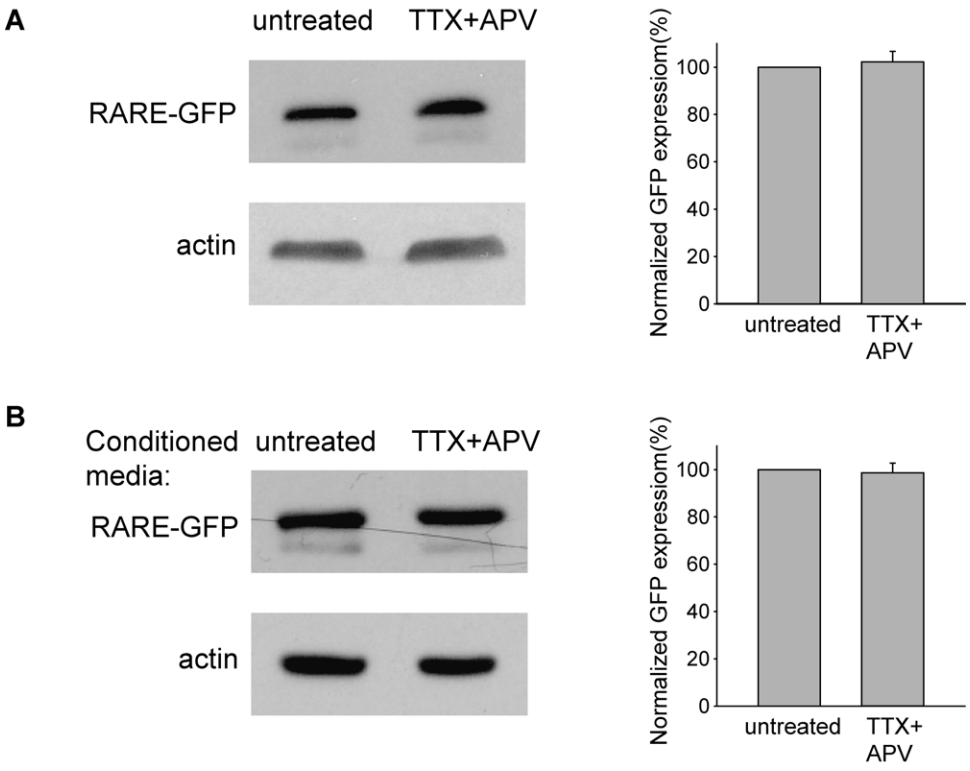
Supplementary Figure 1. Acute RA treatment does not induce spine formation. 13-14 DIV hippocampal neurons were transfected with EGFP, treated with DMSO or 1 µM RA for an hour and fixed. Spine density was not changed by RA treatment (n = 17/group, $p > 0.25$). Scale bar: 10 µm.



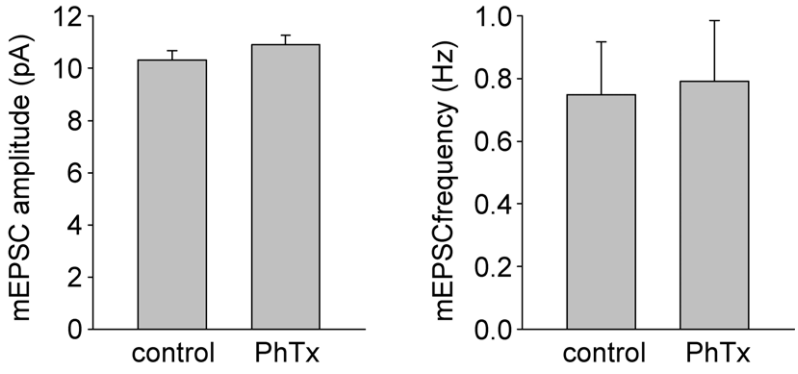
Supplementary Figure 2. RALDH1 expression in cultured hippocampal neurons. RALDH1 immunoreactivity (green) was examined in 13 DIV hippocampal neurons labeled with MAP2 (red). Images were taken at three magnifications. Almost all hippocampal neurons (MAP2-positice) express RALDH1 (see images at 10x and 40x). Within each neuron, RALDH1 is present in both dendrites and axon (see images at 100x). In addition, glia cells exhibited faint stainings, reflecting either background staining or low expression level. Scale bars: 10x, 100 μm; 40x, 50 μm; 100x, 10 μm.


A
DMSO
0.1 μM RA
1 μM RA
Normalized integrated GFP intensity (%)
400
300
200
100
0
RARE reporter
GFP control
*
*
*
0
0.1
1
10
RA concentration (μM)
B
RARE-GFP reporter
RA (μM)
0
0.1
1
10
GFP
actin
control GFP
RA (μM)
0
0.1
1
10
GFP
actin
Normalized GFP expression (%)
700
600
500
400
300
200
100
0
RARE reporter
GFP control
*
*
*
0
0.1
1
10
RA concentration (μM)

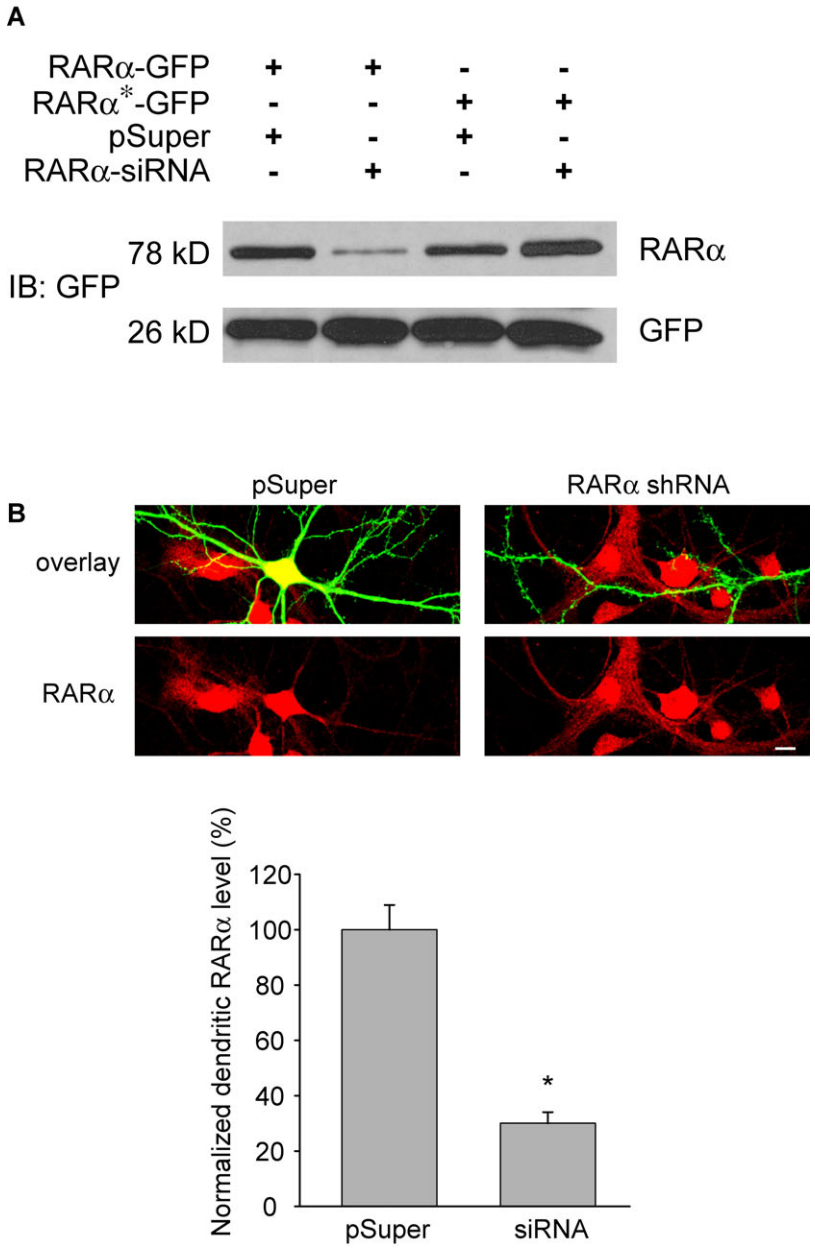
Supplementary Figure 3. *In vitro* RA detection assay. **A,** The 3xDR5-RARE-GFP reporter in HEK293 cells is responsive to direct RA treatment. Upper panels are fluorescent and DIC images of RARE-GFP-transfected HEK cells. The expression of a control GFP construct lacking the RARE sequence was not affected by RA (images not shown). Lower graph show that RA significantly enhanced the expression of RARE-GFP but not control GFP (n = 10 fields/group; *, $p < 0.0005$).Scale bar: 10 μm. **B,** Western blot of lysates from HEK293 cells transfected with either 3xDR5-RARE-GFP reporter construct or a control GFP construct. RA treatment induced increase in 3xDR5-RARE-GFP (n = 3; *, $p < 0.001$), but not control GFP expression (n = 3; $p > 0.05$).



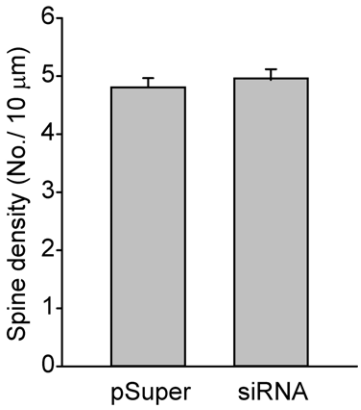
Supplementary Figure 4. 3xDR5-RARE-GFP reporter expression in HEK 293 cells. **A,** Direct treatment with TTX+APV (24 hrs) in HEK cells did not affect the expression of the GFP reporter ($n = 3$, $p > 0.5$). **B,** HEK cells that have been treated with conditioned media obtained from TTX+APV-treated or untreated neuronal cultures showed a similar level of GFP reporter expression ($n = 3$, $p > 0.5$).



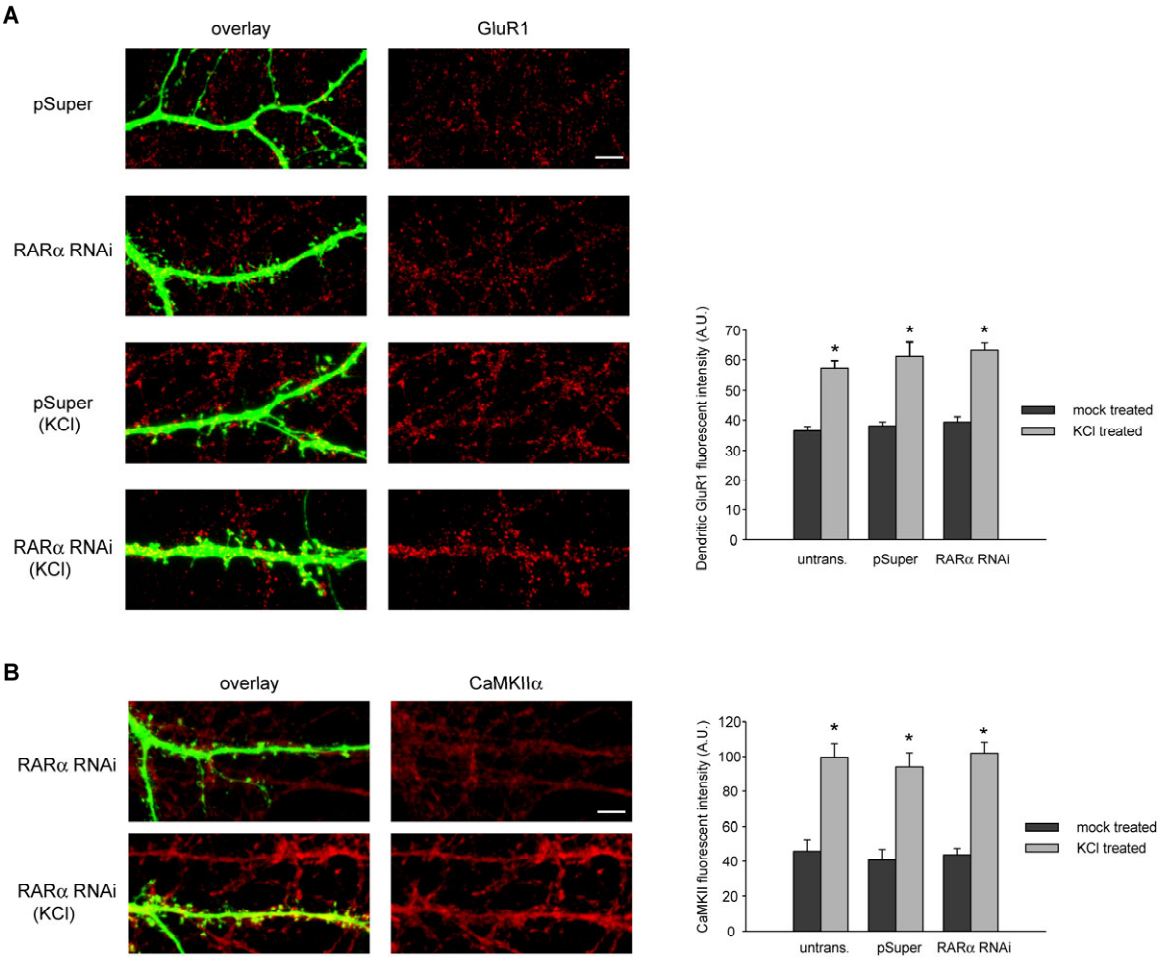
Supplementary Figure 5. Philanthotoxin does not affect basal synaptic transmission. PhTx or vehicle control was bath-applied to cultured hippocampal neurons and mEPSCs were recorded at least ten minutes after the application. Neither amplitude nor frequency of mEPSCs was changed by PhTx ($n = 10$/group, $p > 0.2$).



Supplementary Figure 6. Efficiency of RARα knockdown by RARα siRNA. **A,** RARα siRNA significantly reduced RARα protein expression in HEK293 cells. A rescue RARα construct bearing a silent point mutation (RARα*-GFP) is resistant to the siRNA knockdown. **B,** Expression of RARα siRNA significantly reduced the endogenous RARα expression in neurons. (n = 7/group; *, $p < 1 \times 10^{-4}$).



Supplementary Figure 7. Knocking down RARα expression with RARα siRNA did not alter spine density (n = 50 dendrites from 17 neurons/group, $p > 0.5$).



Supplementary Figure 8. Local translation of GluR1 and CaMKIIα in neuronal dendrites induced by a high K^+ protocol is not affected by RARα siRNA. **A,** Empty vector (pSuper) or RARα siRNA expression did not change basal GluR1 protein level in the dendrites. High KCl stimulation induced equally robust increase in local synthesis of GluR1 in dendrites of neurons from all groups (n = 28 dendrites from 10 neurons/group; *, $p < 1x10^{-5}$). Scale bar, 5 µm. **B,** The CaMKIIα protein level in dendrites was not affected by pSuper or RARα siRNA, and was increased to a similar level by high K^+ in all groups (n = 24 dendrites from 8 neurons/group; *, $p < 1x10^{-8}$). Scale bar, 5 µm.