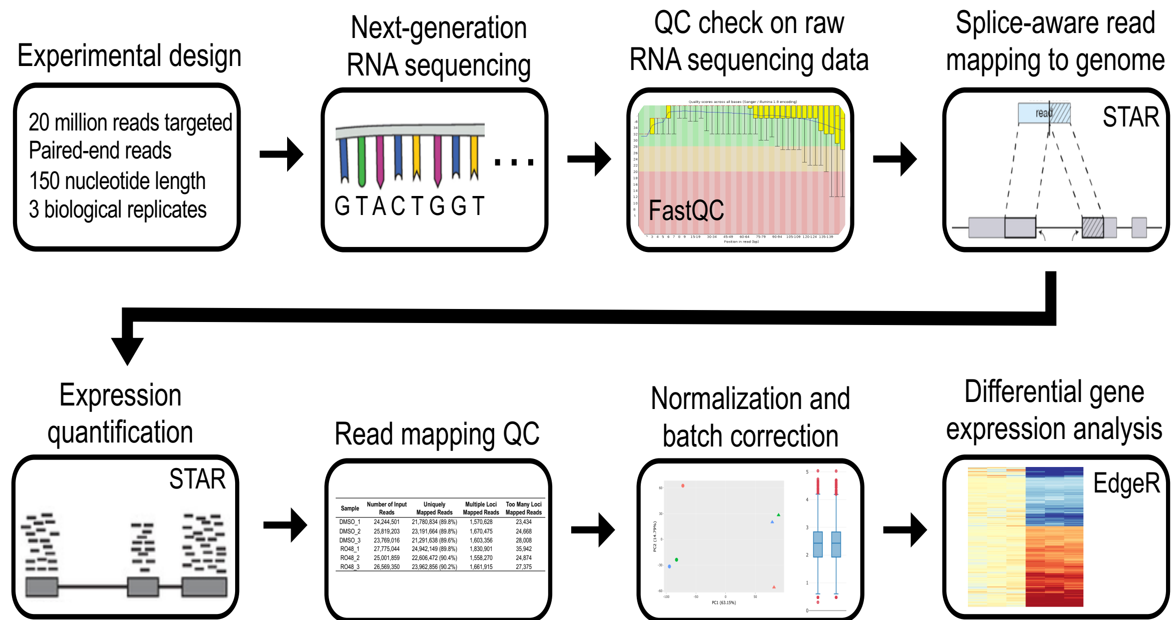
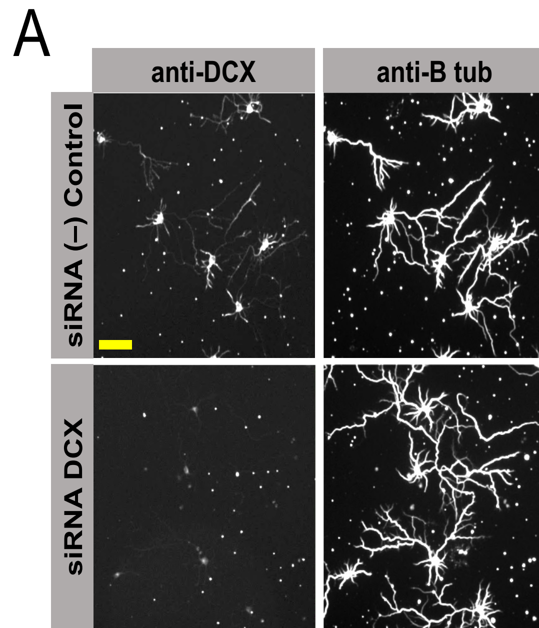
Supplemental Figure 1

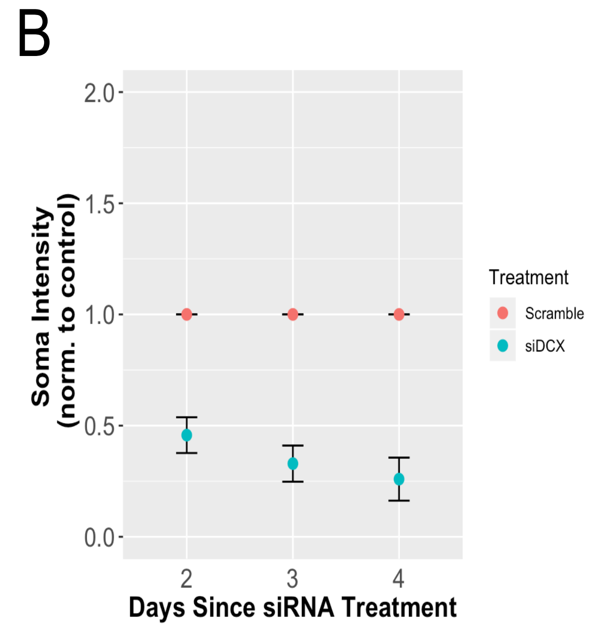


Sample	Number of Input Reads	Uniquely Mapped Reads	Multiple Loci Mapped Reads	Too Many Loci Mapped Reads
DMSO_1	24,244,501	21,780,834 (89.8%)	1,570,628	23,434
DMSO_2	25,819,203	23,191,664 (89.8%)	1,670,475	24,668
DMSO_3	23,769,016	21,291,638 (89.6%)	1,603,356	28,008
RO48_1	27,775,044	24,942,149 (89.8%)	1,830,901	35,942
RO48_2	25,001,859	22,606,472 (90.4%)	1,558,270	24,874
RO48_3	26,569,350	23,962,856 (90.2%)	1,661,915	27,375

- **Supplemental Figure 1**. RNA sequencing analysis pipeline. Experimental design incorporates selecting the number of reads, read length, single- or paired-end reads, and number of biological replicates necessary for reliably detecting differences in gene expression. Sequencing of isolated RNA is performed using a high-throughput next-generation platform, such as the NovaSeq 6000 system from Illumina. Raw sequencing output undergoes quality control (QC) checks using the FastQC java tool to evaluate for errors during sequencing. Raw sequencing data are then mapped to the appropriate species genome using a splice-aware mapping tool, such as STAR (Spliced Transcripts Alignment to a Reference). Subsequently, mapped reads are quantified as raw counts of expression of a given gene. Mapped gene counts undergo further quality control analysis based on mapping statistics including percent of unmapped reads and the nature of mapped reads (singly mapping or multiple mappings per read). Raw gene counts are then normalized and batch corrected to produce gene counts that can be compared for differential expression using a differential expression analysis tool, such as EdgeR. Differentially expressed genes can then be used for functional analysis or downstream bioinformatics analysis.

Supplemental Figure 2

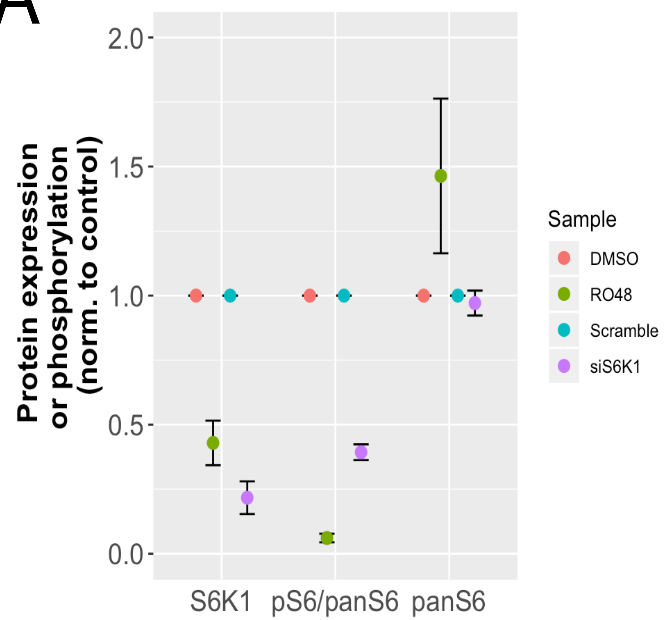




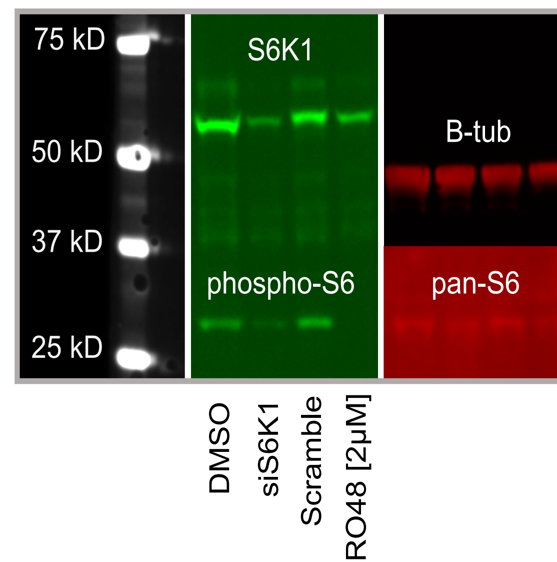
- **Supplemental Figure 2**. Doublecortin (DCX)-targeted siRNA effectively reduces DCX expression by two days after siRNA treatment and maintains reduced DCX expression for at least four days after siRNA treatment. E18 hippocampal neurons were treated with Doublecortin (DCX)-targeted siRNA or non-targeting (negative control) siRNA ("Scramble") the same day or 24 hours after being plated. Neurons were fixed two, three, and four days after respective siRNA treatment. **(A)** Neurons were stained for DCX, BIII-tubulin, and with Hoechst and then imaged. Images depict neurons four days after siRNA treatment, at the period of maximum knockdown for the length of time tested. Scale bar indicates 100 µm. **(B)** Quantification of neuronal soma fluorescence intensity representing DCX expression staining at two, three, and four days after siRNA treatment. The soma intensity of neurons treated with DCX-targeted siRNA (siDCX) is normalized to the soma intensity of the non-targeting siRNA for the respective timepoint. N=2, with at least 379 neurons counted per replicate. Error bars indicate the range.

Supplemental Figure 3

A

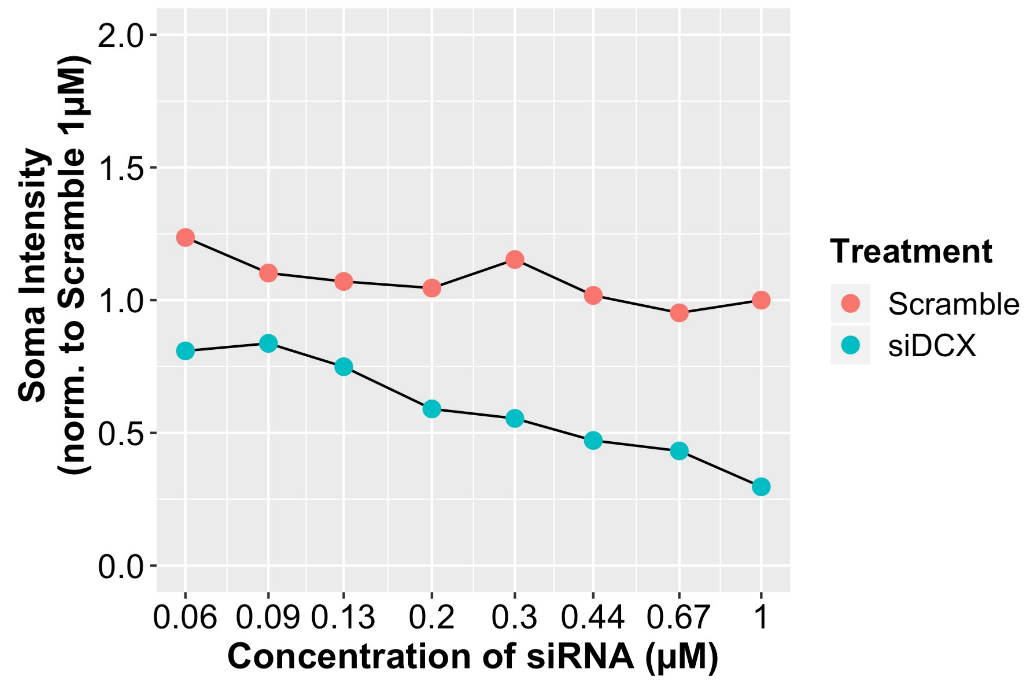


B



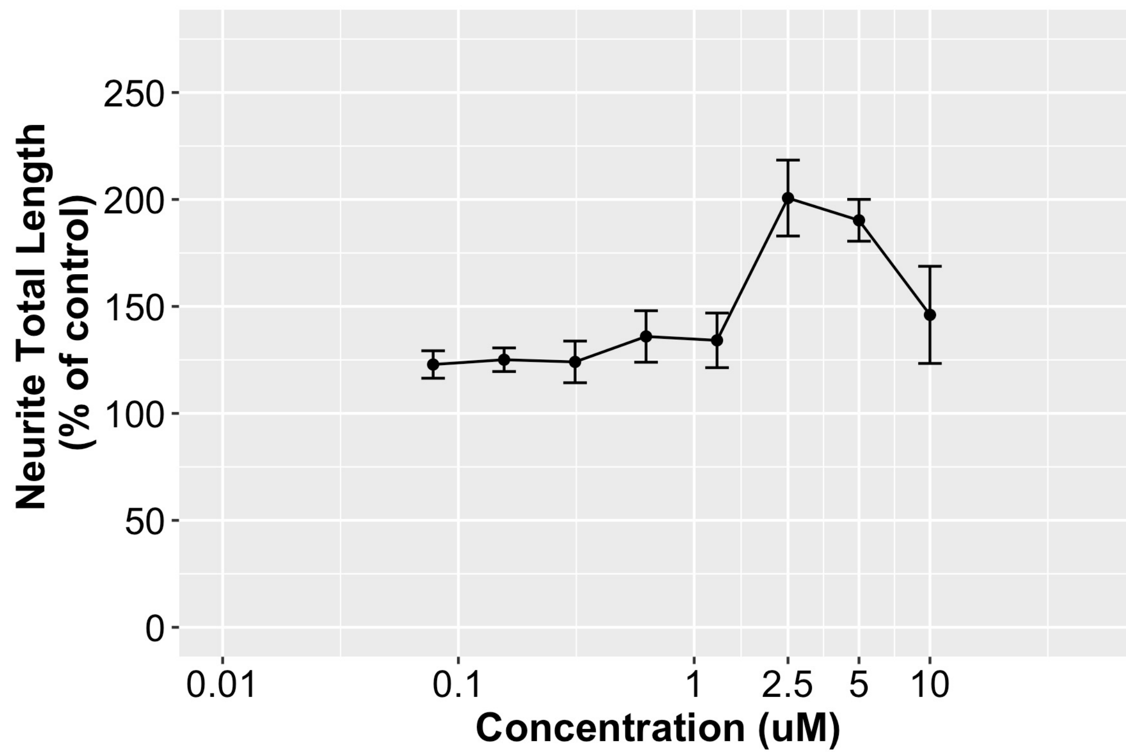
- **Supplemental Figure 3**. Ribosomal protein S6 kinase 1 (S6K1)-targeted siRNA and kinase inhibitor RO48 each reduces S6K1 protein expression and levels of phosphorylated ribosomal S6 protein. Hippocampal neurons were treated with either DMSO or RO48 [2 µM] the day they were plated, or with S6K1-targeted siRNA (siS6K1) or non-targeting (negative control) siRNA ("Scramble") one day after the neurons were plated. Neurons were grown for a total of five days before cellular lysates were collected for western blot quantification of protein expression. **(A)** Protein expression is calculated relative to total BIII-tubulin expression, and phosphorylated-ribosomal S6 levels are calculated relative to the total ribosomal S6 expression (pS6/panS6). The final expression or phosphorylation values for RO48 and siS6K1 are normalized to either DMSO or non-targeting siRNA values, respectively. N=3, each experiment performed in triplicate. Error bars indicate SEM. **(B)** Representative western blot image containing each of the treatment conditions to show the relevant band intensities and protein sizes.

Supplemental Figure 4



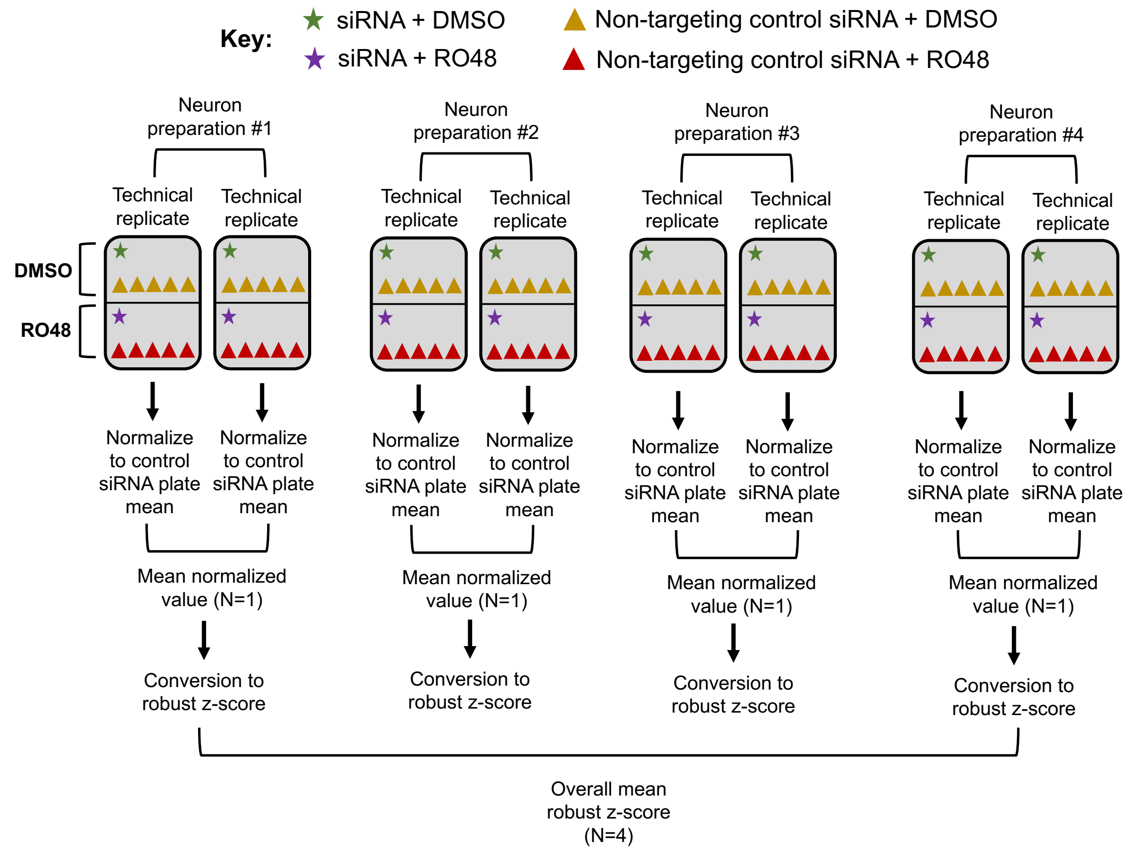
- **Supplemental Figure 4.** Doublecortin (DCX)-targeted siRNA produces optimum DCX knockdown at 1 µM and this concentration is within the linear range of protein knockdown. Hippocampal neurons were plated at 2000 cells per well in a poly-D-lysine-coated 96-well plate. Neurons were treated with serially diluted Doublecortin (DCX)-targeting siRNA (siDCX) or non-targeting (negative control) siRNA ("Scramble") one day after being plated, and were fixed four days after siRNA addition. Neurons were stained for DCX, BIII-tubulin, and nuclei (Hoechst) and then imaged. Soma intensity was measured by quantification of fluorescence derived from secondary antibody binding to anti-DCX at the neuron cell body, and intensity values were normalized to the intensity in non-targeting siRNA wells at the 1 µM concentration. N=1, at least 315 neurons counted per treatment.

Supplemental Figure 5



- **Supplemental Figure 5.** Dose-response with delayed RO48 treatment relative to day of plating. Rat embryonic hippocampal neurons were plated at 2000 cells per well in a poly(D-lysine)-coated 96-well plate. To mimic screening conditions, neurons were treated with either DMSO or serially diluted RO48 after three days in culture and were fixed two days later for a total of five days in culture. Neurons were stained with anti-BIII-tubulin to visualize neurites, and neurite outgrowth was quantified with automated neurite tracing. Neurite total length values were normalized to the value of control DMSO-treated neurons. N=4, performed in triplicate with at least 40 neurons counted per replicate. Error bars indicate SEM.

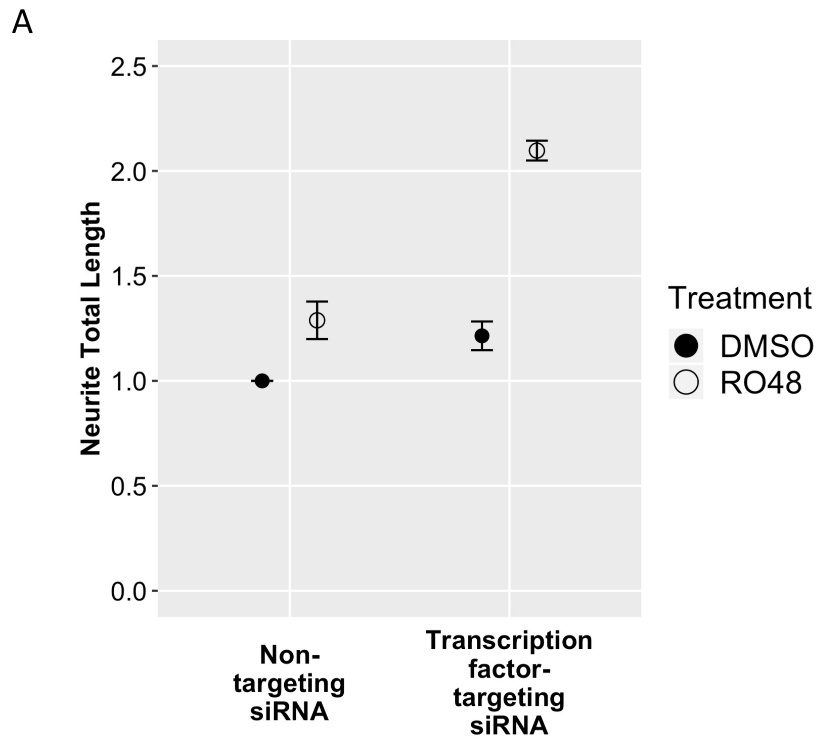
Supplemental Figure 6



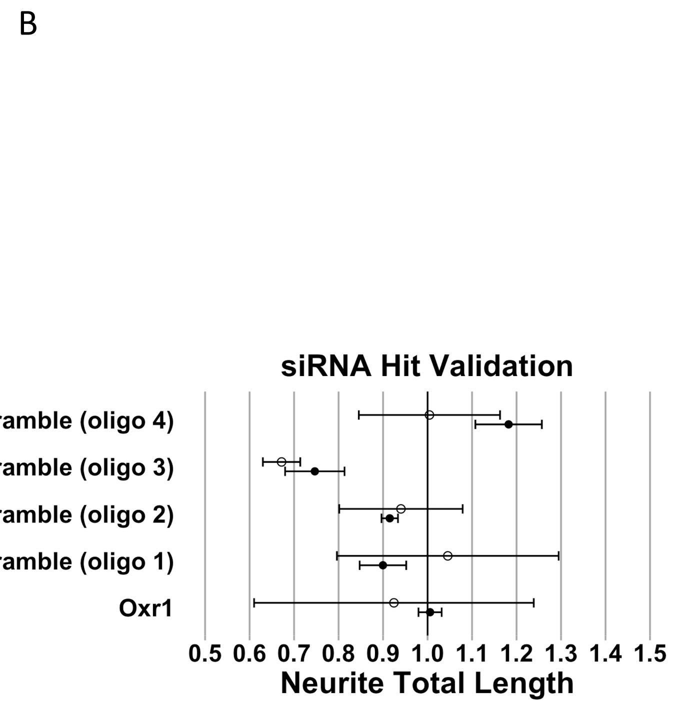
- **Supplemental Figure 6. siRNA screen experimental setup and analysis method.** Rat embryonic hippocampal neurons were plated in 96-well plates and treated with either siRNA targeting a transcription factor or a non-targeting control siRNA one day after being plated. All siRNA treatments are duplicated on a given plate to give two identical halves (shown oblong boxes containing triangles on the plate drawings). Two days after siRNA treatment, each half of a plate was treated with either DMSO or RO48 [2 μM]. For a given neuronal preparation of hippocampi from one litter of embryos, six different sets of two identical treatment plates are created such that each siRNA + DMSO (green star) or siRNA + RO48 (purple star) treatment has two technical replicates. The two technical replicates are spread across two plates such that each plate has only one of the two replicates. Each siRNA + DMSO or siRNA + RO48 plate treatment is normalized to the five non-targeting control siRNA + DMSO (yellow triangle) or non-targeting control siRNA + RO48 (red triangle) replicates on the same plate. The two technical replicates for a given preparation, after normalization to the appropriate control siRNA group, are averaged to produce a single value for that hippocampal neuron preparation. The normalized assay value is converted to a robust Z-score using the values of all the other respective siRNA + DMSO or siRNA + RO48 treatments for that hippocampal neuron preparation. Finally, the four robust Z-scores acquired from the four different hippocampal neuron preparations are averaged to attain the mean robust Z-score for an siRNA + DMSO or siRNA + RO48 treatment.

Supplemental Figure 7

A



B



- **Supplemental Figure 7.** Non-targeting control siRNA treatment adversely affects neurite outgrowth relative to other transcription factor-targeting siRNA treatments. **(A)** Neurons were treated with either siRNA targeting a transcription factor or a non-targeting control siRNA one day after being plated. Two days after siRNA treatment neurons were treated with either DMSO or RO48 [2 µM]. Neurons were fixed after a total of five days in culture. Biological replicates were considered the mean of all plates in a given neuron preparation such that each plate is a technical replicate (12 plates per neuron preparation) after grouping wells by DMSO or RO48 treatment (24-25 wells per treatment per plate). A technical replicate was the mean of the non-targeting control siRNA or targeting siRNA wells on a plate after normalizing all wells on a plate to the DMSO non-targeting control siRNA plate average. N=4, errors bars indicate SEM. **(B)** Hippocampal neurons were plated in quintuplicate in 96-well plates. After 1 day in culture neurons were treated with targeting or non-targeting (“Scramble”) siRNA, and two days later neurons were treated with either DMSO (0.02%; black) or RO48 [2 µM] (white). Neurons were fixed after five total days in culture. Neurite total length was normalized separately by DMSO or RO48 treatment to the mean neurite total length of wells that received Scramble oligos 1, 2, or 4. N=3 for Scramble oligos, N=2 for Oxr1 siRNA. Error bars indicate 95%CI.

Supplemental Table 1. Per sample genome mapping quality of RNA sequencing output.

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DMSO_1	24,244,501	21,780,834 (89.8%)	1,570,628	23,434
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Supplemental Table 2:
Transcription factor binding site enrichment scoring within the upregulated gene set or downregulated gene set, determined by differential expression in RO48-treated hippocampal neurons compared to DMSO-treated neurons.

Transcription factors with binding sites enriched within upregulated gene set

TF Name	TF ID	Z-score	Fisher score	Motif
CTCFL	MA1102.1	7.415	3.081	CNNCAGGGGGCNNN
DMRT3	MA0610.1	6.216	2.581	NNTGTANCNNN
DUX4	MA0468.1	6.527	2.452	TAANNNAATCA
EGR2	MA0472.2	5.89	2.34	NCGCCCACGCN
FIGLA	MA0820.1	5.486	2.256	NNCACCTGNN
FOS::JUND	MA1141.1	6.949	2.749	NNNTGAGTCANNN
FOXD1	MA0031.1	6.646	3.829	GTAAACAN
FOXI1	MA0042.2	7.509	5.478	GTAAACA
FOXK1	MA0852.2	6.627	2.477	NNNGTAAACANNNN
FOXK2	MA1103.1	9.331	5.166	NNNTAAACANN
FOXP1	MA0481.2	9.336	4.582	NNGTAAACANNN
HIC2	MA0738.1	9.891	3.324	NTGCCCNNN
HSF4	MA0771.1	5.229	2.148	TTCNNGAANNTTC
ID4	MA0824.1	10.06	4.983	NNCACCTGNN
INSM1	MA0155.1	5.715	2.301	TGNNNGGGGNNN
JUN	MA0488.1	17.013	7.493	NNNATGANGTNAT
JUND	MA0491.1	5.786	2.768	NNTGANTCATN
JUND(var.2)	MA0492.1	13.631	5.069	NNNNATGANGTCATN
Klf1	MA0493.1	9.82	4.943	NNCCACACCCN
KLF4	MA0039.3	13.435	5.584	NNACACCCNNN
Myod1	MA0499.1	12.854	4.373	NNCAGCTGNNNNN
Myog	MA0500.1	13.474	9.166	NNCAGCTGNNN
MZF1(var.2)	MA0057.1	6.064	2.341	NNANGGGNNN
NFIC	MA0161.2	6.484	2.163	NNCTTGGCANN
NFIX	MA0671.1	10.278	2.701	NNNGCCANN
NR3C1	MA0113.3	17.511	4.248	NNGNACANNNTGTNCNN
NR3C2	MA0727.1	21.826	5.445	NNGNACANNNTGTNCNN
POU1F1	MA0784.1	17.89	5.396	NNTATGCNAATNAN
POU2F1	MA0785.1	11.649	5.31	NNTATGNNAATN
POU2F2	MA0507.1	10.134	4.659	NNNATTTGCATNN
POU3F1	MA0786.1	13.273	6.041	NTATGNNAATNN
POU3F2	MA0787.1	12.717	5.48	NTATGNNAATNN
POU3F3	MA0788.1	10.402	3.912	NNTATGNNAATNN
POU3F4	MA0789.1	9.204	4.109	TATGCNAAT
POU4F1	MA0790.1	8.034	2.082	NTNNATNATTNATN
POU5F1	MA1115.1	12.355	7.105	NNATGCAAANN

POU5F1B	MA0792.1	9.091	3.548	TATGNNAAT
RELB	MA1117.1	9.625	5.208	NNATTCCCCNN
RUNX1	MA0002.2	10.793	4.92	NNNTGTGGNNN
SNAI2	MA0745.1	10.181	4.159	NNCAGGTGN
SREBF1	MA0595.1	6.329	2.868	NTCACNCCAN
TAL1::TCF3	MA0091.1	6.926	2.833	NNANCATCTGNT
TBP	MA0108.2	7.313	2.371	NTATANANNNNNNNN
Tcf12	MA0521.1	9.45	4.629	NNCAGCTGNNN
TCF3	MA0522.2	14.544	4.961	NNCACCTGNN
TCF4	MA0830.1	12.382	2.734	NNCACCTGNN
TFAP2B	MA0811.1	6.502	2.708	NNCCNNNNGGCN
TFAP2B(var.3)	MA0813.1	6.501	2.333	NGCCCNNNGGGCN
TWIST1	MA1123.1	8.229	3.3	NNNCCAGATGTNN
ZBTB18	MA0698.1	6.915	3.376	NNNCCAGATGTNN
Znf423	MA0116.1	8.206	2.196	NNNNCCNANGGNNNN
ZNF740	MA0753.1	7.505	3.434	NCCCCCCCNN

Transcription factors with binding sites enriched within downregulated gene set

TF Name	TF ID	Z-score	Fisher score	Motif
ATF4	MA0833.1	16.931	6.134	NNNTGANGCAANN
Bcl6	MA0463.1	8.195	2.852	NTTCCTNGANNNNN
Dux	MA0611.1	4.291	2.048	NCAATCAN
E2F1	MA0024.3	13.244	4.416	NNNGGCGCCNNN
E2F4	MA0470.1	15.384	9.77	NNGCGGGANNN
E2F6	MA0471.1	11.607	3.815	NNGCGGGANNN
E2F8	MA0865.1	10.993	2.099	TTTCCCGCCAAA
EN2	MA0642.1	4.369	2.389	NNNAATTANN
Esrra	MA0592.2	6.467	2.999	NTCAAGGTCAT
FOSL1::JUNB	MA1137.1	9.252	2.798	NNNTGANTNANNN
Gfi1	MA0038.1	6.907	3.247	NNAATCNNNN
GSC	MA0648.1	4.959	2.588	NNTAATCCNN
GSC2	MA0891.1	7.464	2.367	NNTAATCNNN
HMBOX1	MA0895.1	6.694	3.939	NNTAGTTANN
Lhx8	MA0705.1	8.582	5.389	NTAATTAN
LIN54	MA0619.1	8.405	2.454	NTTTNAANN
MXI1	MA1108.1	7.044	2.603	NNNCCACGTGNNN
MYC	MA0147.3	7.178	2.418	NNCCACGTGNNN
MYCN	MA0104.4	5.478	2.623	NNCCACGTGGNN
MZF1(var.2)	MA0057.1	4.859	2.191	NNANGGGNNN
NFAT5	MA0606.1	7.33	2.556	NNTTTCCANN
NFATC2	MA0152.1	7.529	3.053	TTTTCCN

NFIX	MA0671.1	5.75	2.095	NNNGCCANN
NFKB2	MA0778.1	6.626	2.215	NGGGGANTCCCCN
NFYA	MA0060.3	20.884	12.26	NNCCAATCANN
NFYB	MA0502.1	21.552	12.392	NNNNNNNCCAATNAG
NKX2-3	MA0672.1	6.95	2.189	NNCACTTNNN
NKX2-8	MA0673.1	8.57	4.128	NCACTTNNN
NR4A1	MA1112.1	4.052	2.596	NNAAGGTCAN
NR4A2::RXRA	MA1147.1	8.993	2.095	NNGGNCNNTGACCNN
PITX3	MA0714.1	5.486	2.435	NNTAATCCN
POU6F2	MA0793.1	5.606	2.442	NNCTNATTAN
RHOXF1	MA0719.1	4.807	3.35	NTNANCCN
RORA	MA0071.1	5.028	2.832	NNNNAGGTCA
RORB	MA1150.1	6.009	2.906	ANNNNGGTCAN
SIX1	MA1118.1	5.269	2.324	GNAACCTGANN
SMAD3	MA0795.1	9.225	3.633	NGTCTAGACN
Sox2	MA0143.3	4.726	2.488	CCNTTGTN
STAT3	MA0144.2	5.269	2.014	NTTCNNGGAAN
TFDP1	MA1122.1	19.554	11.926	NNGCGGGAANN

Graphical abstract