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Supplemental Information

Enhancer Histone Acetylation Modulates Transcriptional Bursting Dynamics of Neuronal Activity-Inducible Genes

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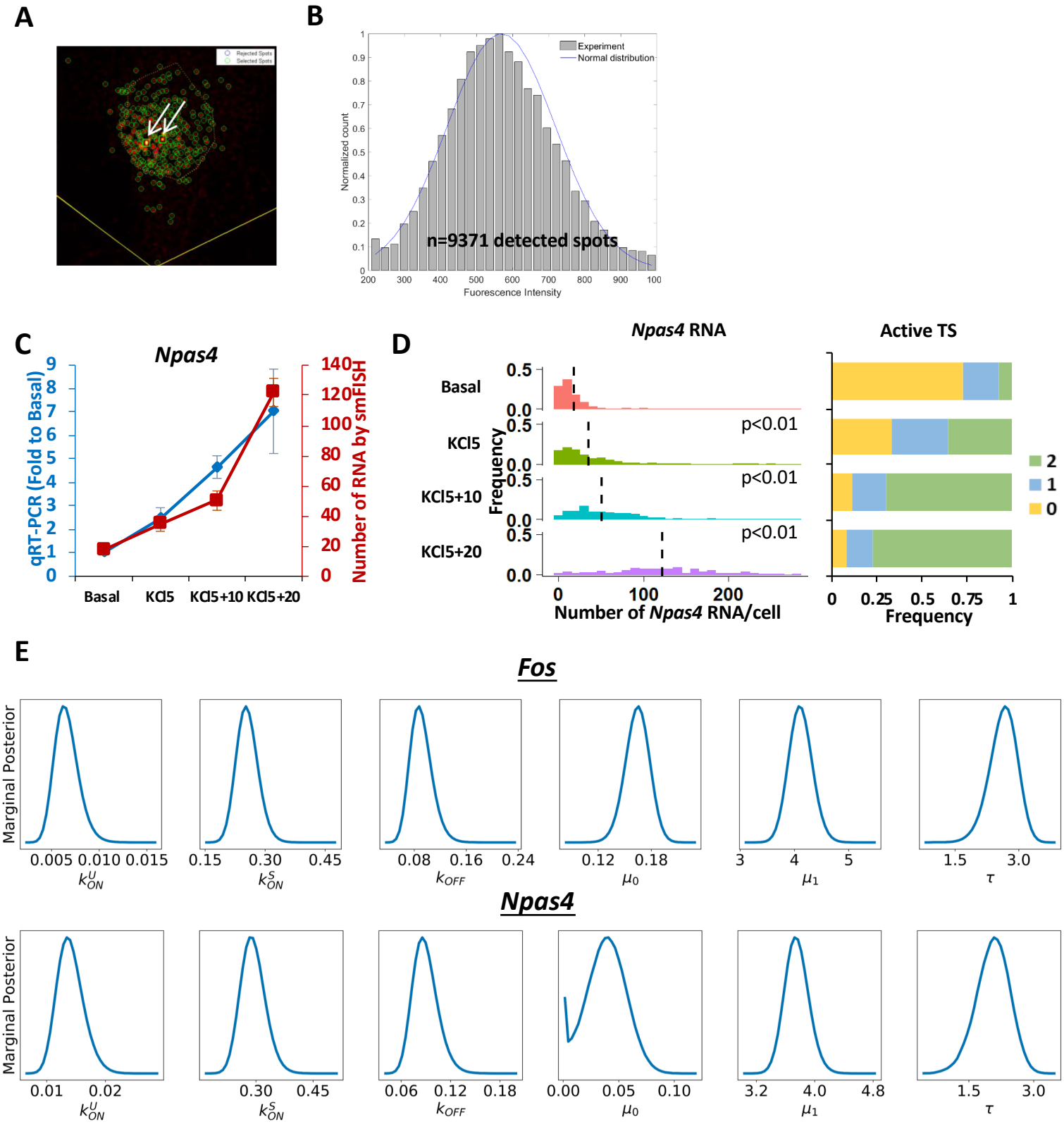

Figure S1: Validation and modeling of smFISH data from neurons, related to Figure 2. **(A)** Representative image of smFISH quantification by FISH-quant. **(B)** Representative distribution of fluorescence intensity of the detected spots by FISH-quant. **(C)** Average *Npas4* RNA levels at different time points before (Basal) and after a 5min pulse of KCl by smFISH (red line) and qRT-PCR (blue line). Error bars are 95% CI for smFISH and SEM for qRT-PCR. n=3 biological replicates/condition. **(D)** Observed distributions of *Npas4* RNA and active transcription sites (TSs) per single cell by smFISH. Dashed lines show average RNA number for each group. Data were analyzed by Kolmogorov–Smirnov test. $p<0.01$ Basal vs. KCl5, $p<0.01$ Basal vs. KCl5+10, $p<0.01$ Basal vs. KCl5+20. Basal n=209, KCl5 n=232, KCl5+10 n=107, KCl5+20 n=151 neurons from 3 biological replicates. *Npas4* data were originally reported in (Gómez-Schiavon et al., 2017) and are differently graphed and analyzed here. Marginal posterior distributions of kinetic parameters inferred by BayFish from **(E)** *Fos* and **(F)** *Npas4* smFISH data, respectively. The posterior distributions quantify the uncertainty in the kinetic parameters in the mathematical model. We also list the best-fit (i.e. most likely) parameters listed in Table S1.

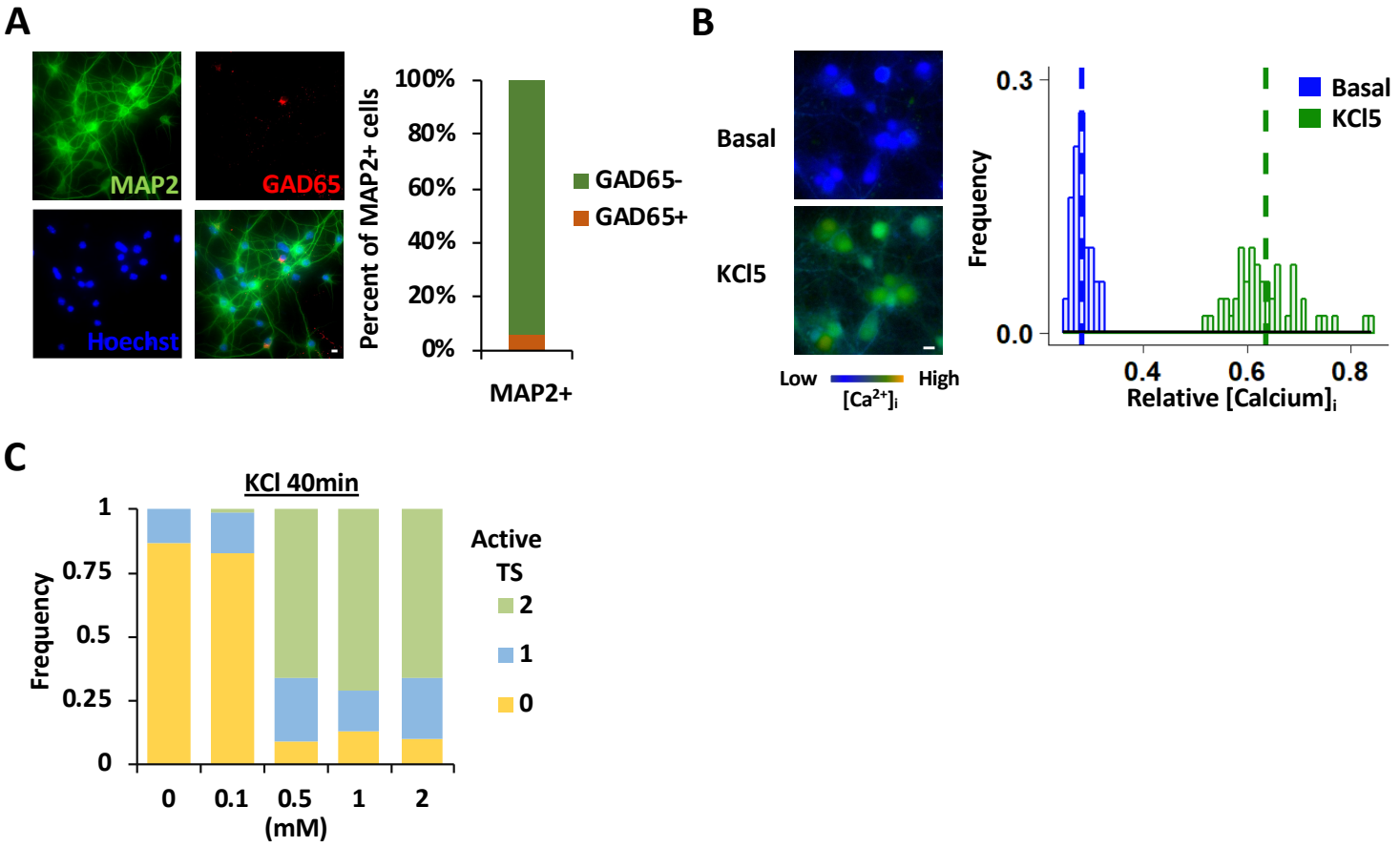

Figure S2: Assessment of extrinsic factors contributing to transcriptional variation between neurons, related to Figure 3. **(A)** Fraction of presumed excitatory (GAD65-) and inhibitory (GAD65+) neurons (MAP2+) in primary cultured hippocampal neurons. n=1531 neurons from 2 biological replicates. Scale bar= 10µm. **(B)** Intracellular calcium concentration in single primary mouse hippocampal neurons measured by Fura-2, 1 min before (Basal, blue) and 1 min after (KCl5, green) 5 min exposure to 55 mM KCl. Dashed lines show average for each group. n=50 neurons per time point from 2 biological replicates. Data were analyzed by Kolmogorov–Smirnov test. Basal vs. KCl5 $p<0.01$ n=50 neurons/condition. scale bar= 5µm. **(C)** Distribution of active TSs measured by smFISH in single neurons at 40 min exposure to 55mM KCl with the indicated extracellular calcium concentration. 0mM n=62, 0.1mM n=68, 0.5mM n=70, 1mM n=76, 2mM n=70 neurons from 2 biological replicates.

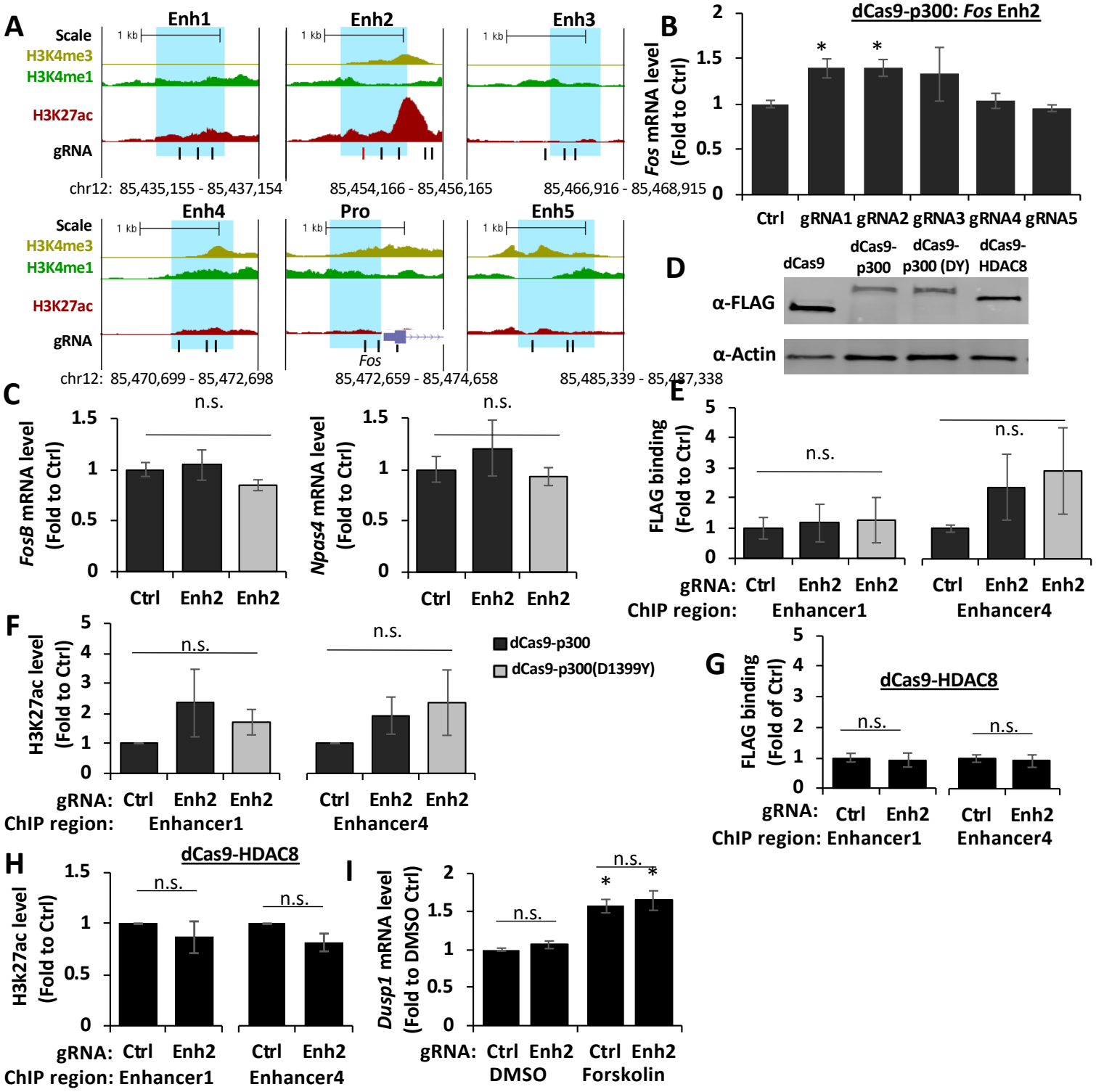

Figure S3: Validation of gRNAs for dCas9-dependent Fos regulation, related to Figure 4. **(A)** gRNAs used in this study. Blue bars show *Fos* enhancers and promoter. **(B)** *Fos* mRNA in N2A cells co-transfected with dCas9-p300 and single gRNAs targeting to *Fos* Enh2, n=4/condition. **(C)** *Fosb* and *Npas4* mRNA in N2A cells co-transfected with either dCas-p300 or an acetyltransferase dead (D1933Y) version of dCas-p300 with either a control gRNA plasmid or a pool of gRNAs targeted to *Fos* Enh2. n=4/condition. **(D)** Western blot of FLAG in N2A cells transfected with the indicated fusion dCas9 variants. Actin is shown as a loading control. **(E)** FLAG binding at *Fos* enhancer 1 and enhancer 4 in N2A cells co-transfected with either a control gRNA plasmid or a pool of gRNAs targeted to *Fos* Enh2 and the indicated dCas-p300 proteins. n=3/condition. **(F)** H3K27ac ChIP-qPCR enrichment at *Fos* enhancer1 and enhancer4 in N2A cells co-transfected with either a control gRNA plasmid or a pool of gRNAs targeted to *Fos* Enh2 and the indicated dCas-p300 proteins. n=5/condition. **(G)** FLAG binding at *Fos* promoter and Enh2 in N2A cells co-transfected with either a control gRNA plasmid or a pool of gRNAs targeted to *Fos* Enh2 and the indicated FLAG fusion dCas9-HDAC8. n=6/condition. **(H)** H3K27ac ChIP-qPCR enrichment at *Fos* promoter and enhancer2 in N2A cells co-transfected with either a control gRNA plasmid or a pool of gRNAs targeted to *Fos* Enh2 and the indicated dCas9-HDAC8 proteins, n=5/condition. **(I)** *Dusp1* mRNA levels in N2A cells co-transfected with dCas-HDAC8 along with either a control gRNA plasmid or a pool of gRNAs targeted to *Fos* Enh2 and treated with the indicated drugs. n=8/condition. Data are represented as mean ± SEM. Two-tailed Student's t test, n.s., not significant. *p<0.05.

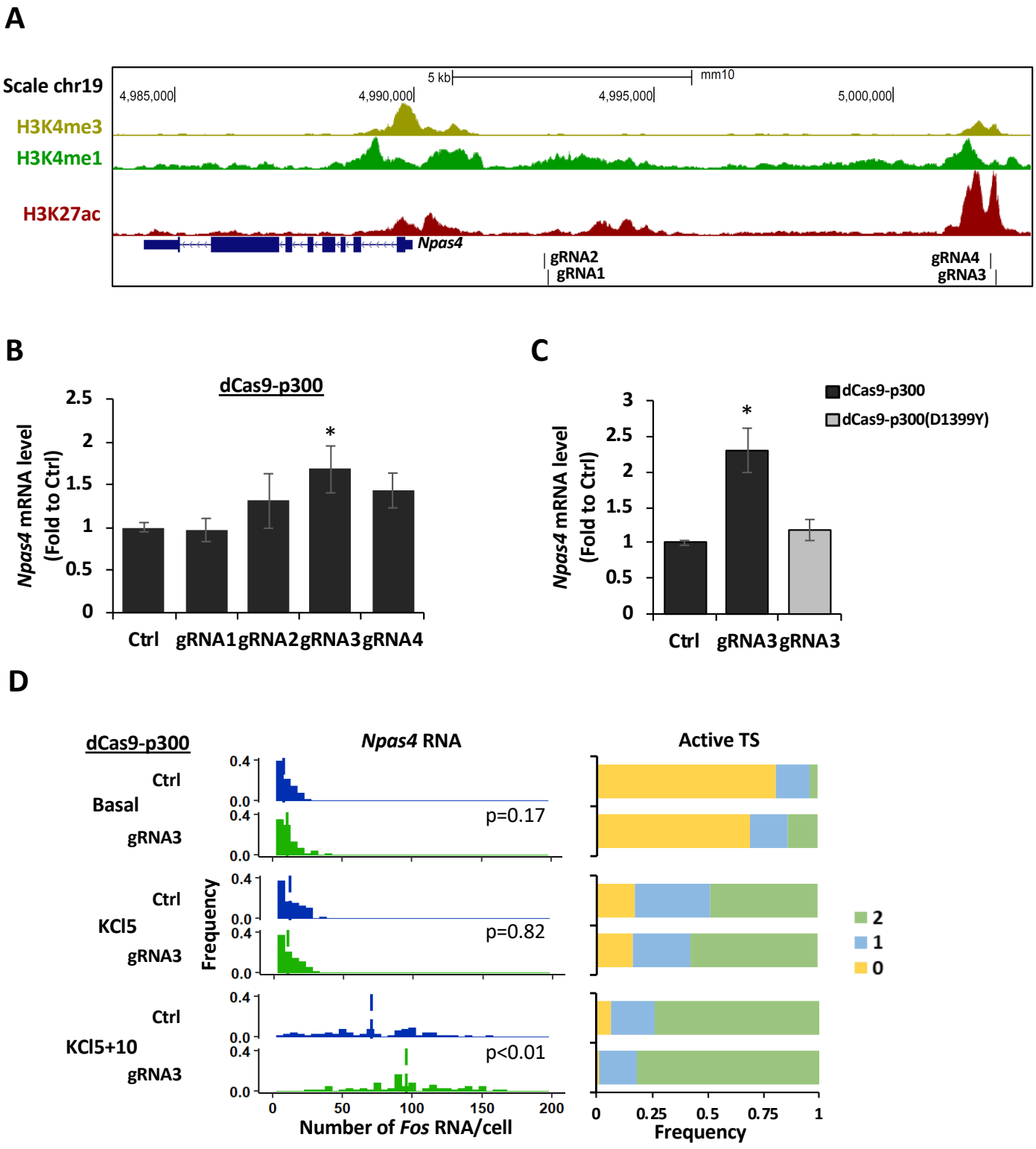

Figure S4: Identification of gRNAs for enhancer-recruited dCas9-p300 dependent regulation of *Npas4*, related to Figure 4. **(A)** The chromatin landscape of the *Npas4* genomic locus in embryonic 16.5 days mouse forebrain. The ENCODE data GSE82453, GSE82464, and GSE82690 and locations of gRNAs are shown. **(B)** Level of *Npas4* mRNA in N2A cells co-transfected with dCas-p300 and the indicated single gRNAs. gRNA1: p=0.83, gRNA2: p=0.37, gRNA3: p=0.04, gRNA4: p=0.08, compared with Ctrl. n=5/condition. **(C)** Level of *Npas4* mRNA in N2A cells co-transfected with either the dCas-p300 or an acetyltransferase dead (D1933Y) version of dCas-p300 along with either a control gRNA plasmid or *Npas4* gRNA3. p<0.01 control vs dCas9-p300 with gRNA3, p=0.26 control vs. dCas9-p300DY with gRNA3, Ctrl n=6, dCas9-p300 with gRNA3 n=5, dCas9-p300DY with gRNA3 n=6. *p<0.05 compared with Ctrl. **(D)** Observed distributions of *Npas4* RNA and active TSs measured by smFISH in single neurons at different time points before (Basal), after a 5 min pulse of KCl (KCl5), and 10 minutes after the pulse (KCl5+10). Cultured mouse hippocampal neurons were co-transfected with dCas-p300 and gRNA3. Dashed lines show average RNA number for each group. Data were analyzed by Kolmogorov–Smirnov test. Basal: p=0.17 Ctrl n=74, Enh2 n=68, KCl5: p=0.82 Ctrl n=65, Enh2 n=54, KCl5+10: p<0.01 Ctrl n=64, Enh2 n=61 neurons per group from 3 biological replicates.

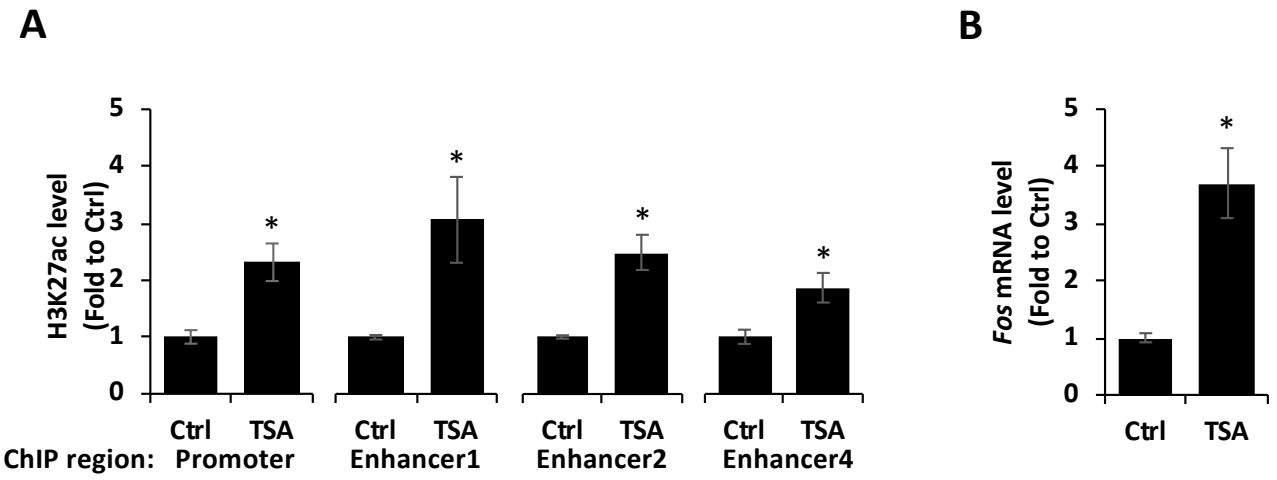

Figure S5: HDAC inhibition promotes *Fos* enhancer H3K27ac and *Fos* expression in cultured mouse hippocampal neurons, related to Figure 5. **(A)** H3K27ac ChIP-qPCR enrichment at *Fos* promoter and enhancers in cultured mouse primary cortical neurons with (TSA) or without (Ctrl) 30 nM TSA treatment for 20 hour before harvesting. Pro p=0.01, Enh1 p=0.03, Enh2 p<0.01, Enh4 p=0.02, n=4/condition. Data are represented as mean ± SEM. Two-tailed Student's t test, *p<0.05 compared with Ctrl. **(B)** Level of *Fos* mRNA in cultured mouse primary cortical neurons with (TSA) or without (Ctrl) 30 nM TSA treatment for 20 hour before harvesting. p<0.01, n=5/condition.

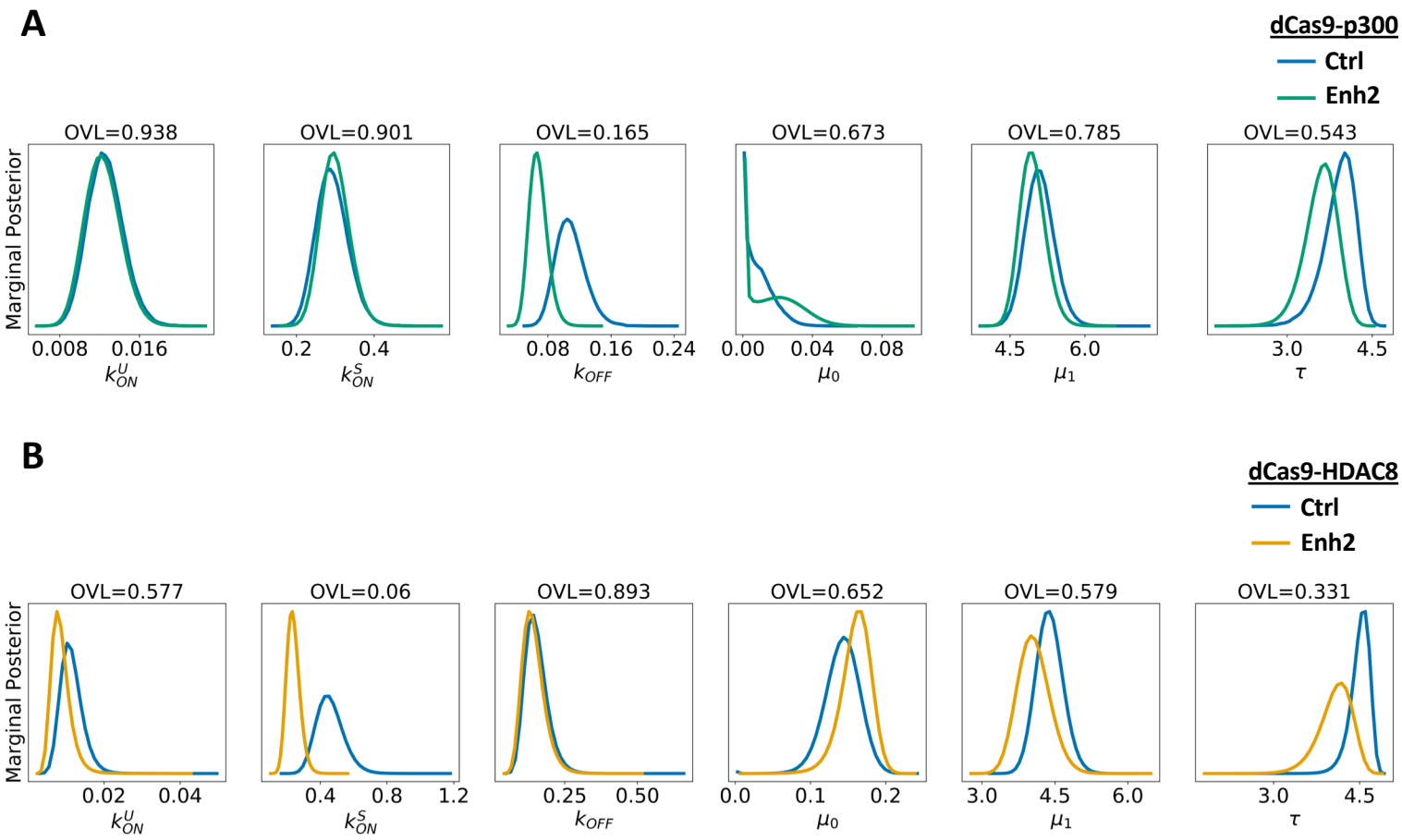

Figure S6: Regulation of *Fos* enhancer 2 H3K27ac tunes transcriptional bursting kinetics, related to Figure 5. **(A)** Marginal posterior distributions of kinetic parameters inferred by BayFish for dCas9-p300 Enh2 and control data set. **(B)** Marginal posterior distributions of kinetic parameters inferred by BayFish for dCas9-HDAC8 Enh2 and control data set. Posterior distributions quantify the uncertainty in the kinetic parameters in the mathematical model. We also list the best-fit (i.e. most likely) parameters listed in Table S2. The overlap coefficient (OVL) quantifies the degree to which the distribution of inferred parameters are similar between (de)acetylated Enh2 and corresponding controls; see Methods. Kinetic parameters (k_{OFF} or stimulated k^{S}_{ON}) with the lowest overlap between control and Enh2 were most likely to have been changed by histone modifications (dCas9-p300 or dCas9-HDAC8, respectively).

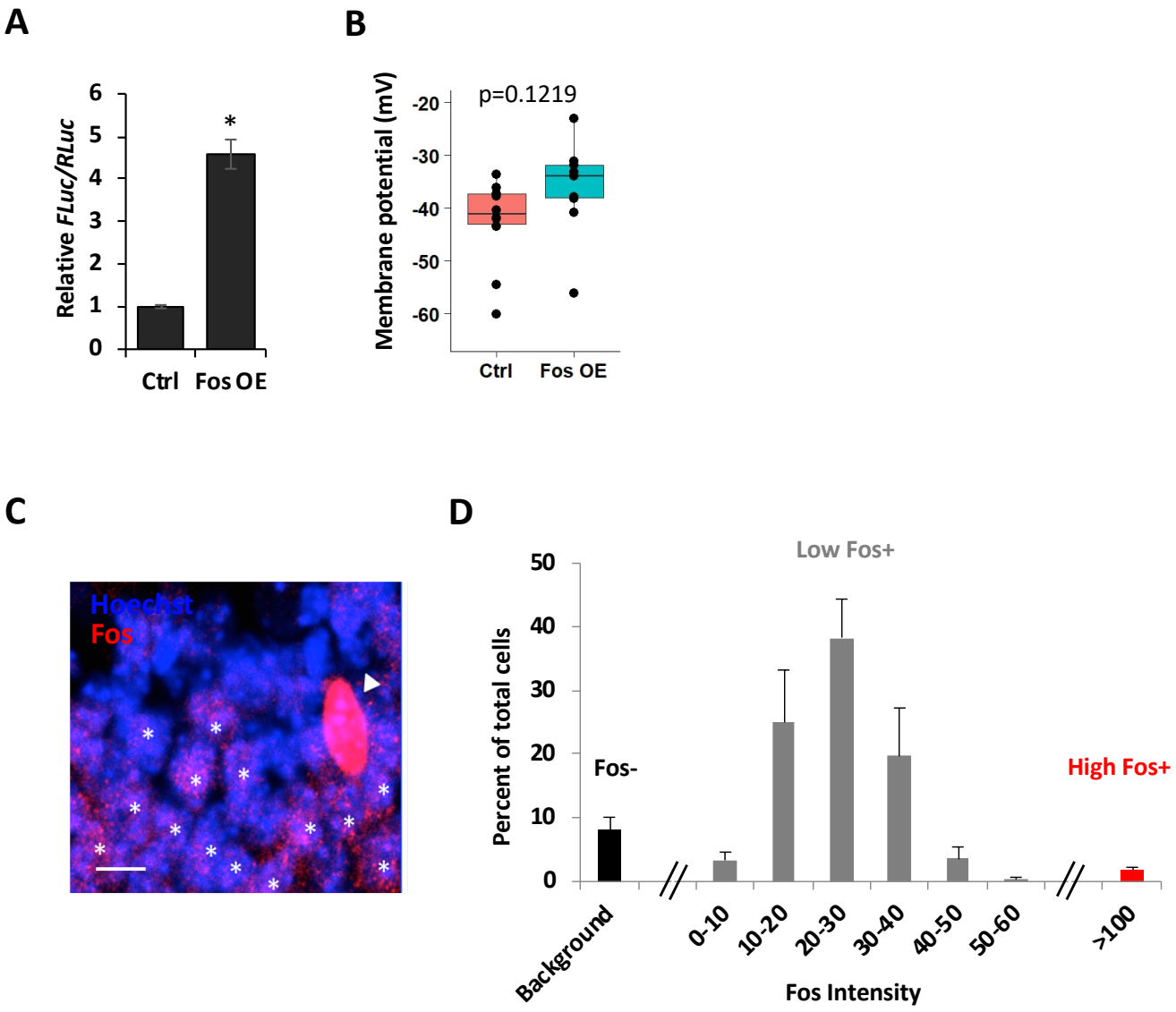

Figure S7: Validation of Fos protein expression and function, related to Figure 7. **(A)** FLuc mRNA to RLuc mRNA levels in N2A cells co-transfected with 3xAP1-FLuc, pTK-renilla luciferase and control (Ctrl) or Fos overexpression plasmid (Fos OE). The FLuc mRNA levels were normalized for each well to co-transfected RLuc mRNA levels. $p<0.01$, n=6/condition. *$p<0.05$ compared with Ctrl. **(B)** Box and whiskers plot of resting membrane potential (mV) in mouse hippocampal neurons that were transfected with either control (Ctrl) or Fos overexpression plasmid (Fos OE). Ctrl n=10, Fos OE n=9 neurons from 2 biological replicates. The lower and upper hinges correspond to the first and third quartiles. The whiskers extend from the hinges to the values no further than 1.5 times the inter-quartile range(IQR) from the hinges. Dots are data points of individual neurons. Two-tailed Student's t test. **(C)** Immunofluorescence for Fos on coronal sections through the dentate gyrus of mice 2 hr after exposure to a novel environment. Many cells (nuclei labeled blue with Hoechst dye) are immunoreactive for Fos (red). Only a small number of cells show high Fos levels (white arrowhead) whereas a larger number show lower Fos immunoreactivity (white asterisks). Scale bar = 10µm. **(D)** Quantification of Fos immunoreactivity as shown in A. n= 100 cells/section, 1 section per hemisphere from each of three mice (600 cells total). If Fos intensity over the nucleus was indistinguishable from background, these cells were called Fos- (black bar). Fos intensities above background occurred in a bimodal pattern that we classified as Low Fos+ (gray bars) and High Fos+ (red bar).

Fos	6.58E-03	2.53E-01	8.85E-02	1.63E-01	4.08E+00	2.63E+00
Npas4	1.19E-02	2.90E-01	8.58E-02	4.64E-02	3.80E+00	2.31E+00

Table S1. Best-fit kinetic parameters for *Fos* and *Npas4* induction by KCl-mediated membrane depolarization, related to Figure 2. BayFish software determined that these best-fit kinetic parameters had the highest-likelihood of generating each set of observed data.

dCas9-p300:Ctrl	1.18E-02	3.08E-01	1.15E-01	1.07E-02	5.32E+00	4.04E+00
dCas9-p300:Enh2	1.22E-02	3.10E-01	6.51E-02	2.72E-02	4.86E+00	3.71E+00
dCas9-HDAC8:Ctrl	1.22E-02	4.80E-01	1.58E-01	1.41E-01	4.44E+00	4.56E+00
dCas9-HDAC8:Enh2	8.13E-03	2.43E-01	1.44E-01	1.67E-01	4.15E+00	4.14E+00

Table S2: Best-fit kinetic parameters for *Fos* induction upon increased or decreased acetylation at Enh2, related to Figure 5. BayFish software determined that these best-fit kinetic parameters had the highest-likelihood of generating each set of observed data.

Gene	Element	Guide	Sequence
Fos	enh1	gRNA1	CATACACACACGGCTCCGTC
Fos	enh1	gRNA2	GCAATGCAGGTCACGGCAAT
Fos	enh1	gRNA3	CTTGACTATACTATCCGGTA
Fos	enh2	gRNA1	GGAACAGTGTCTACCGCCCC
Fos	enh2	gRNA2	ACGTCTATGCGTTTTAGCCA
Fos	enh2	gRNA3	AGATCTTGGAGGCTGCGGTC
Fos	enh2	gRNA4	GTGCTACCCCCTGCAGGATC
Fos	enh2	gRNA5	TACGCCGGCTAGAAGAAATC
Fos	enh3	gRNA1	CGGGCGTGGGATTCTGCCGC
Fos	enh3	gRNA2	CTTGCACTAATTAGTCGCGG
Fos	enh3	gRNA3	GCCCAACACAGGGTCTTAGT
Fos	enh4	gRNA1	TCCACTCATAACTGCGTCTC
Fos	enh4	gRNA2	AGGCGGGGATTCGTGGAAAT
Fos	enh4	gRNA3	CGAGGGTGATGTCAGTCGGC
Fos	enh5	gRNA1	CTGTTCCCGGTGGACGATCC
Fos	enh5	gRNA2	TGATTAATAATCGCGCGGCA
Fos	enh5	gRNA3	TTGCGGGATCGGACTTATGA
Fos	promoter	gRNA1	TCCGAAATCCTACACGCGGA
Fos	promoter	gRNA2	GGATGGACTTCCTACGTCAC
Fos	promoter	gRNA3	GGGTTTCAACGCCGACTACG
Npas4	enh	gRNA1	GGACTAGGGATAAGGAACGT
Npas4	enh	gRNA2	TGTCAAGACTCCTCGGTTCT
Npas4	enh	gRNA3	GTCTAGGCCCAATAGCCCCC
Npas4	enh	gRNA4	GTCTTGTTATTTGACCGCAG.

Table S3: Guide RNA sequences for CRISPR/dCas9, related to STAR methods.

Gene	**Primer Sequence**
Gapdh F	5’-CATGGCCTTCCGTGTTCCT-3’
Gapdh R	5’-TGATGTCATCATACTTGGCAGGTT-3’
Fos F	5’-TTTATCCCCACGGTGACAGC-3’
Fos R	5’-CTGCTCTACTTTGCCCCTTCT-3’
Fosb F	5’-GTGAGAGATTTGCCAGGGTC-3’
Fosb R	R 5’-AGAGAGAAGCCGTCAGGTTG-3’
Npas4 F	5’-GCTATACTCAGAAGGTCCAGAAGGC-3’
Npas4 R	R 5’-TCAGAGAATGAGGGTAGCACAGC-3’
Dusp1 F	5’-GGCCAGCTGCTGCAGTTTGAG-3’
Dusp1 R	R 5’-AGGTGCCCCGGTCAAGGACA-3’
firefly luciferase F	5’- GAGGTGGACATCACTTACGCT -3’
firefly luciferase R	5’-AAGAGAGTTTTCACTGCATACGACG-3’
renilla luciferase F	5'-GCTTATCTACGTGCAAGTGATGATTT-3’
renilla luciferase R	R 5’-GAAACTTCTTGGCACCTTCAACA-3’

Table S4: Primers for qPCR, related to STAR methods.

Fos Promoter F	5’-CAAGACGGGGGTTGAAAGCC-3’
Fos Promoter R	5’-TCACTGCTCGTTCGCGGAAC-3’
Fos 3’UTR F	5’-TGACACCTGAGAGCTGGTAGTTAG-3’
Fos 3’UTR R	5’-ATCAGCTGCACTAGATACAATCCA-3'
Fos Enh1 F	5’-TAAAGCCTATTGCCGTGACCTG-3’
Fos Enh1 R	R 5’-TCTTTCCCTTACAATGCCCTTACC-3’
Fos Enh2 F	5’-GTCTACTGTCTGAGGAGAAGTGGTTAG-3’
Fos Enh2 R	5’-AGAACAGATTCTGGAACAGTGTCTAC-3’
Fos Enh4 F	5’-GGCCTAAATTCCCACCAACATAAA-3’
Fos Enh4 R	5’-GAGGGCAGGGAGGCGGGGATTC-3’

Table S5: Primers for ChIP-qPCR, related to STAR methods.