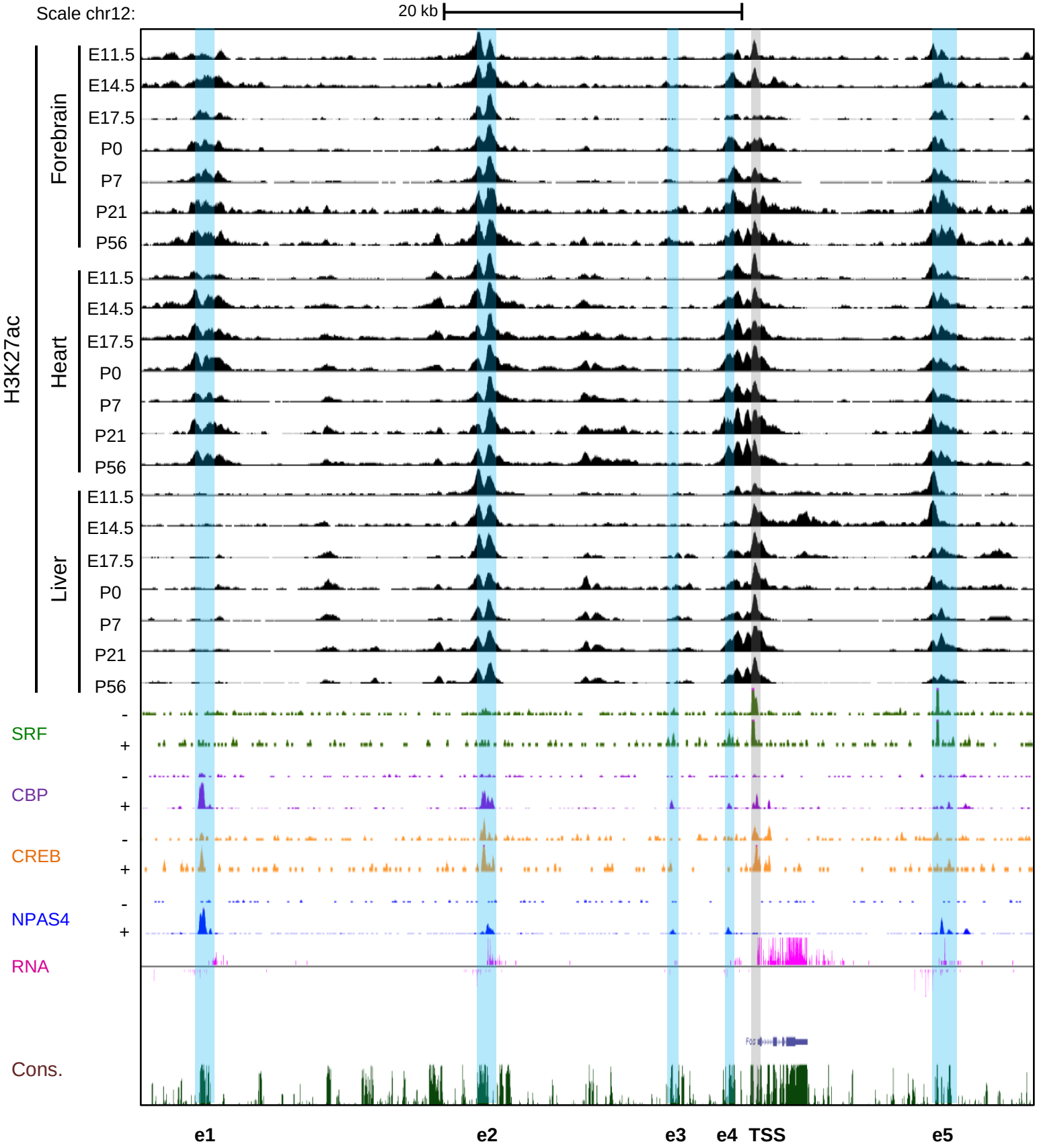
Supplementary Figure 1



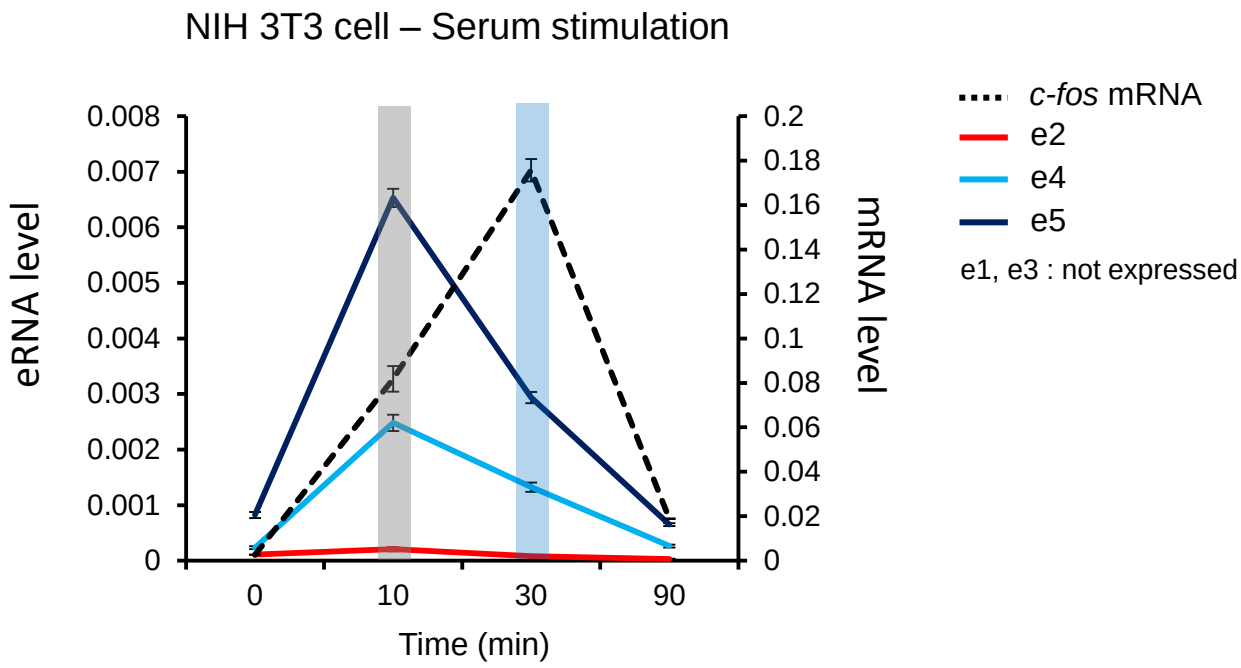
Supplementary Figure 1

Binding profiles of the H3K27ac mark at the *c-fos* enhancers and the promoter in various tissues throughout development

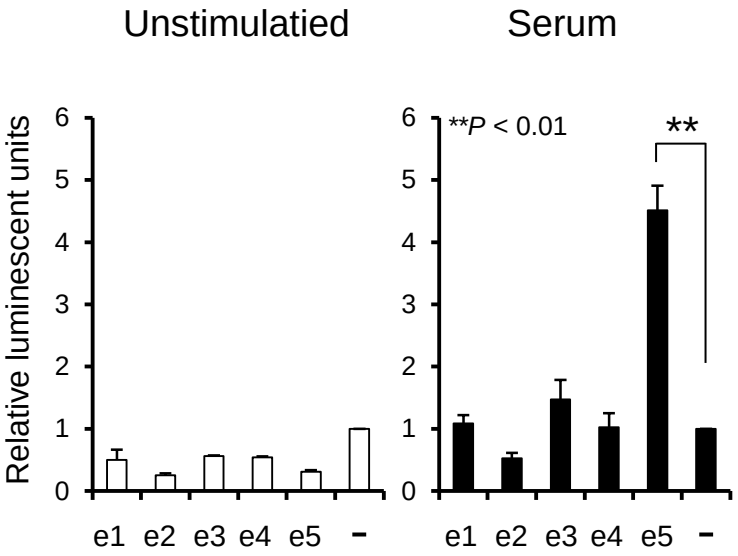
H3K27ac binding profiles were adapted from the data generated by Nord *et al*. 2013. Blue and gray vertical bars indicate the locations of the *c-fos* enhancers and the promoter, respectively.

Supplementary Figure 2

a



b



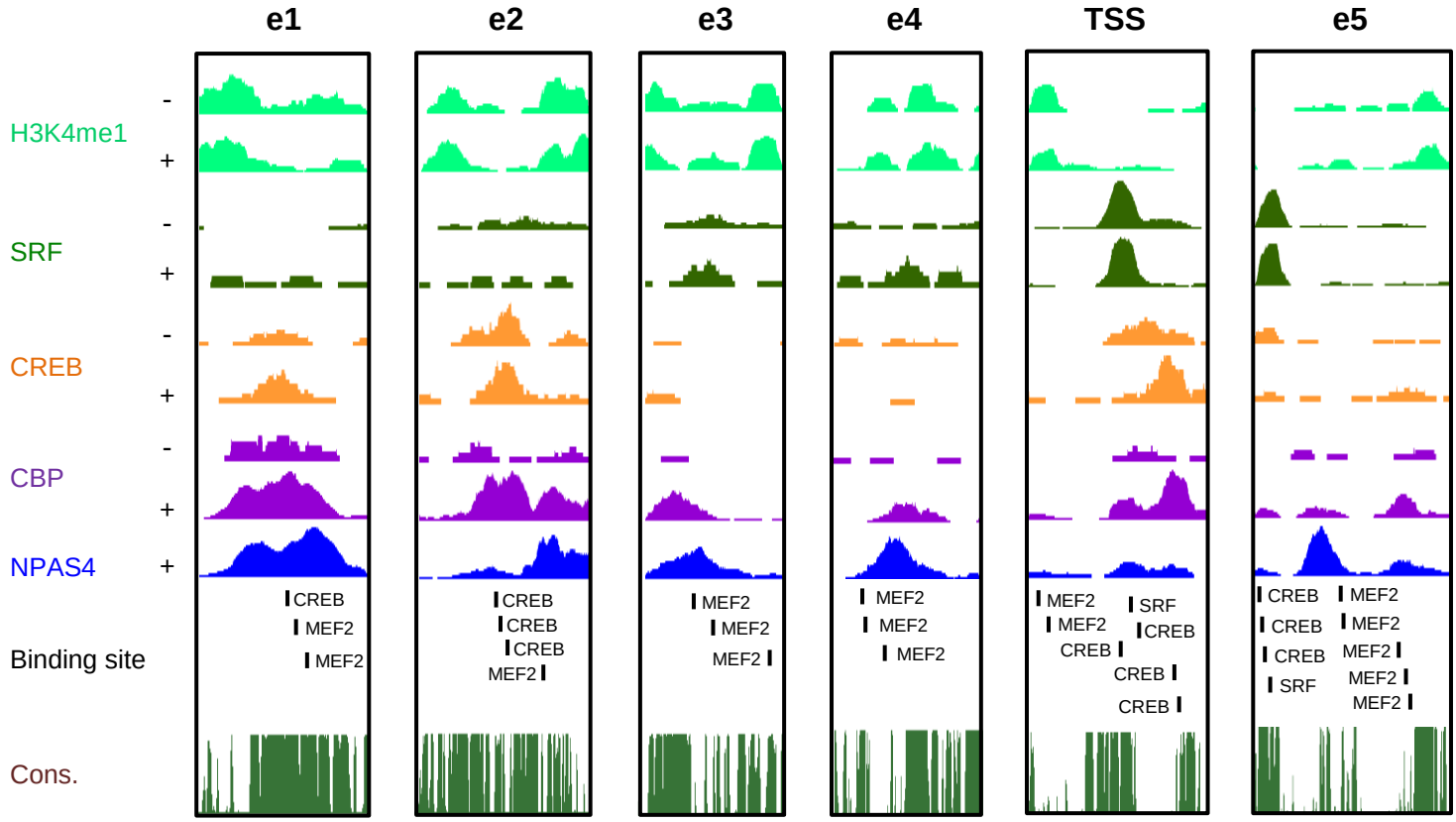
Supplementary Figure 2

The *c-fos* enhancer activity in NIH 3T3 cells during serum stimulation.

(**a**) Time course analysis of *c-fos* mRNA and eRNA in NIH 3T3 cells following serum stimulation. Expression levels of *c-fos* mRNA and five eRNAs were measured at indicated time points after serum stimulation and normalized to *Gapdh* mRNA (n = 3 biological replicates). (**b**) Individual enhancer activities measured by luciferase reporter assay. The luciferase activity in NIH 3T3 cells transfected with each enhancer reporter construct was measured after 6 h serum stimulation (P = 0.0075, $t(2)$ = 11.513, n = 3 biological replicates, unpaired t-test with Welch correction). Error bars indicate SEM; p values are from a two-tailed t test.

Supplementary Figure 3

a



b

Blue : CREB **Red** : MEF2 **Orange** : SRF

e1

CAAAACACAGTCAGGCTTATCTGTTTGCAGAAAGAGGTTTCAAAGGGGCCAGCCTTCCCACTCCTTGGGTCAGAGAGAAGT
GTGTGGGTCCGAGAAAAGAAACTGCAAGGTAAACTTGAGACCGGTACGGGAACCAACTTCCTTCCAAATAGCCTGAAACAT
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GTCTGAAAAATCTGGCAGCCTTCTCAGCTGATGGCAGAGTGTTGAGTCTGTTCTGTCTCCCTGGAGTCCCTCTGCACACGC
AGGTGCACGTCAGCGCCTGCTCTAATTTTATCCCTGGCTGATGGAGGAGACTAACTGAGCAGCAGGGAAGATGCTATTTT
GGGTCTCTCTGCAGCTCTGGGTTATATAAAGCCTATTGCCGTGACCTGCATTGCTTTGAGGCTGAGTCTCACTCTTGAGCA
TGCTGTCATAATATGTCATCAGCTCAGACACACACACACACACACACAGAGCTCTTGGGGAATGAGGAGAGGTGTGTGCT
GAGGAGGAGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTTCCTGCCAGCTTGACTATACTATCCGGTAAG
GGCATTGTAAGGGAAAGAGGCTTGTTCTGGAAGGAAATAGAAAGCTCTTTCTGATGGAGAAACATGAGGGTCTGGCTTTGA
GCTTTAGGACAGAATGTTTTGTGAGCTGATGTTGTAGGTCTGCTGTTGGCATGGTGATAACAA

e2

TCCAACAGTGGGAGTTCTCTAAGCTGGGTTTGATTTTAGCCTCCTGGGCAAGGTAGTTTGGTTAGTGGCTGTTCTGTGGTC
TACTGTCTGAGGAGAAGTGGTTAGGGAGGTCACCCAGACAGTTATGGTCCACTGAGGGTGGAGGGAGTGGTCTAGGTTTG
GCCTCAGTCTGGAGTCCCAGGCTCACAGACAGATCTTGGCTCAGGACATGAGGCTGAGGCGTGCTGGGACAGCCTAGGG
GGTGTCCTGGATTTCCCAGGGCTGGGGAACCCTCGCTGGCCCGGGGGCGGTAGACACTGTTCCAGAATCTGTTCTGGCT
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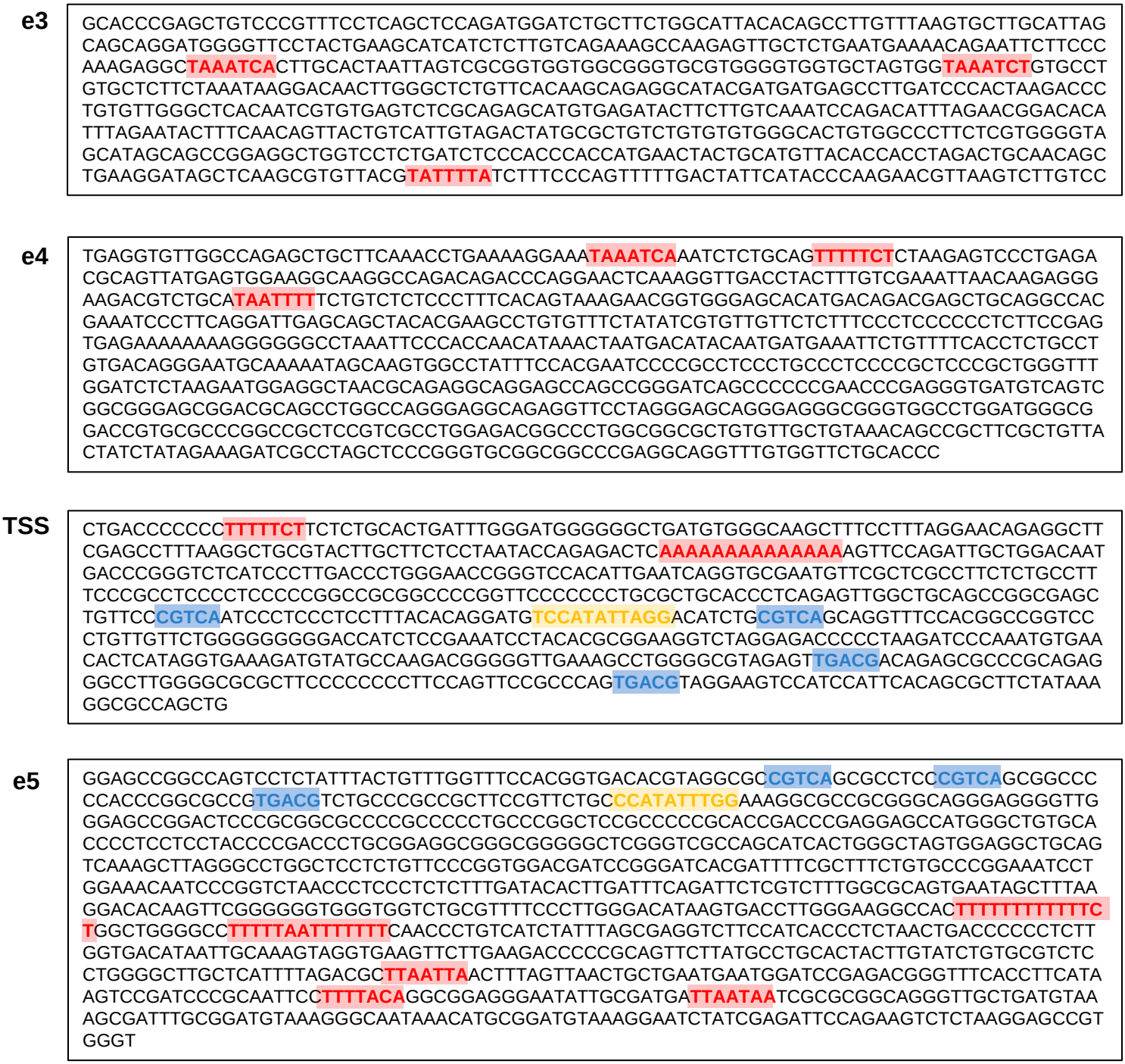
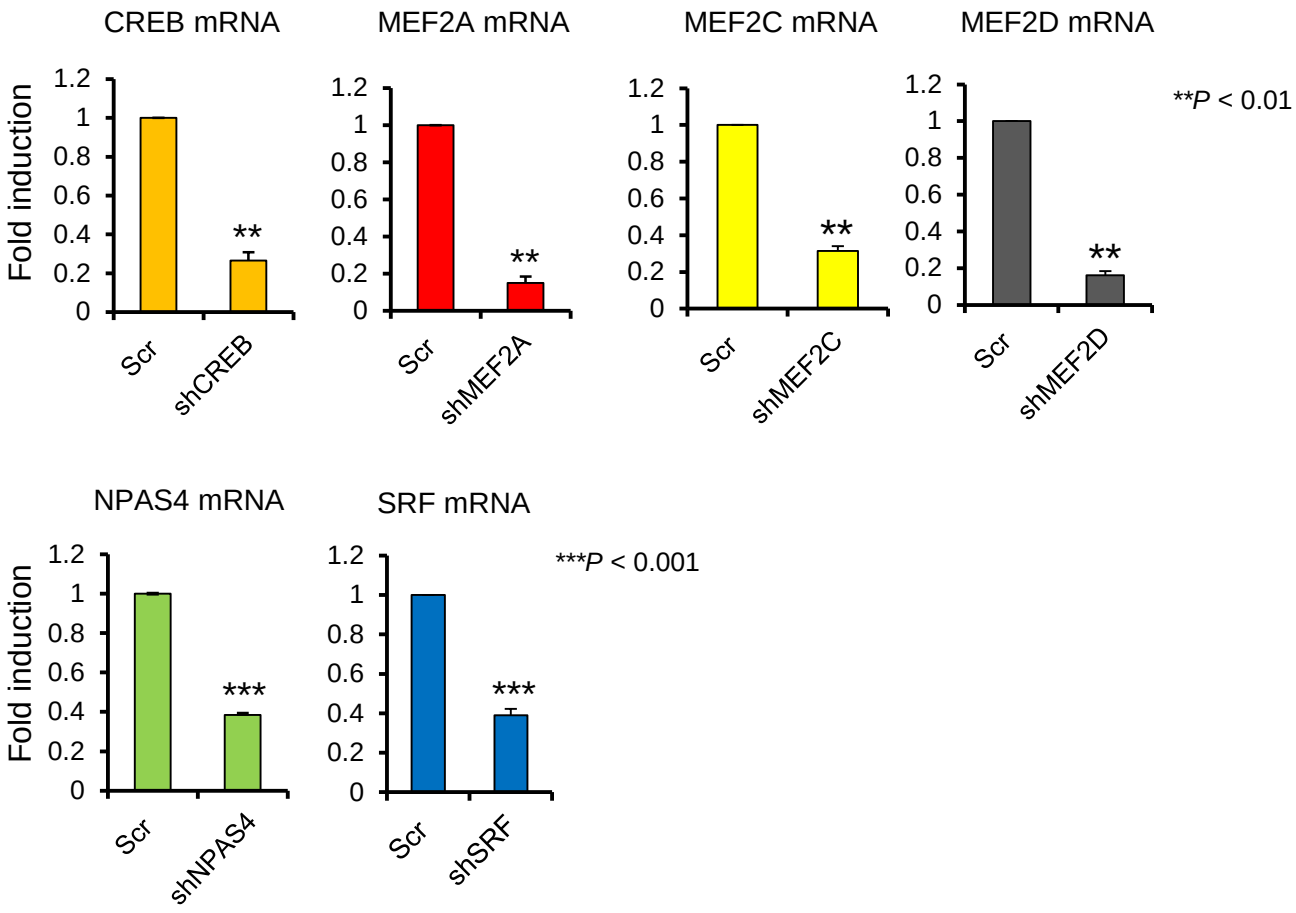


Supplementary Figure 3

Transcription factor binding motifs present at the *c-fos* enhancers and the promoter.

(**a**) Transcription factor binding motifs present at each *c-fos* enhancer. **(b)** Transcription factor binding motifs present in each of the *c-fos* enhancers and the promoter. Binding motifs for CREB, MEF2, and SRF are indicated by blue, red, and orange letters, respectively.

Supplementary Figure 4

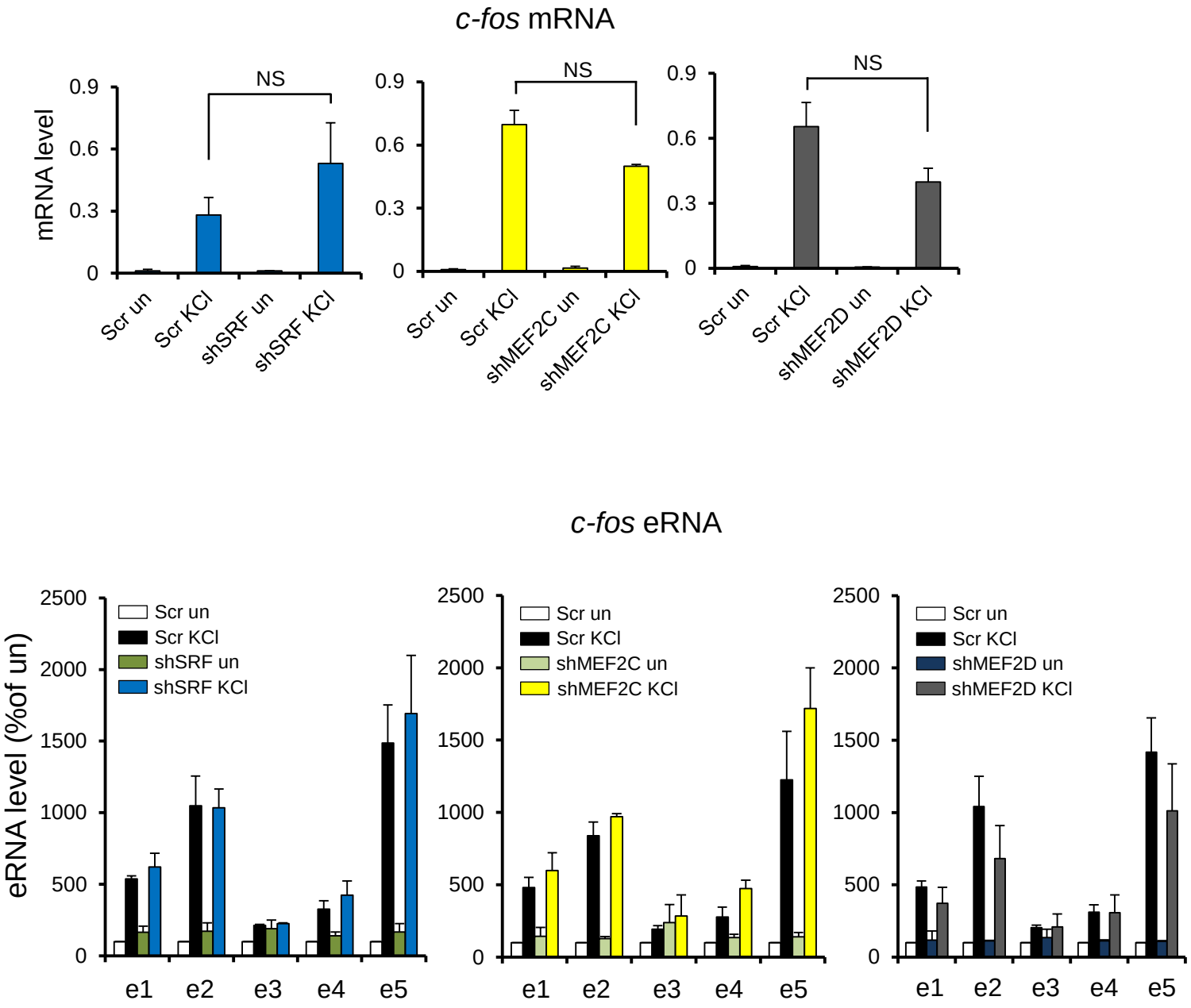


Supplementary Figure 4

Demonstration of the efficiency of knockdown.

RT-qPCR analysis of *Creb*, *Mef2a*, *Mef2c*, *Mef2d*, *Npas4, and Srf* mRNA expression after knockdown in cortical neurons, related to **Fig. 4** and **Supplementary Fig. 5** (CREB mRNA: $P = 0.0031$, $t(2) = 18.040$, n = 3; MEF2A mRNA: $P = 0.0029$, $t(2) = 18.616$, n = 3; MEF2C mRNA: $P = 0.0019$, $t(2) = 22.975$, n = 3; MEF2D mRNA: $P = 0.0012$, $t(2) = 28.633$, n = 3; NPAS4 mRNA: $P = 0.0019$, $t(2) = 22.709$, n = 3; SRF mRNA: $P = 0.0014$, $t(2) = 26.942$, n = 3 biological replicates). All unpaired *t*-test with Welch correction. Error bars indicate SEM; p values are from a two-tailed *t* test.

Supplementary Figure 5

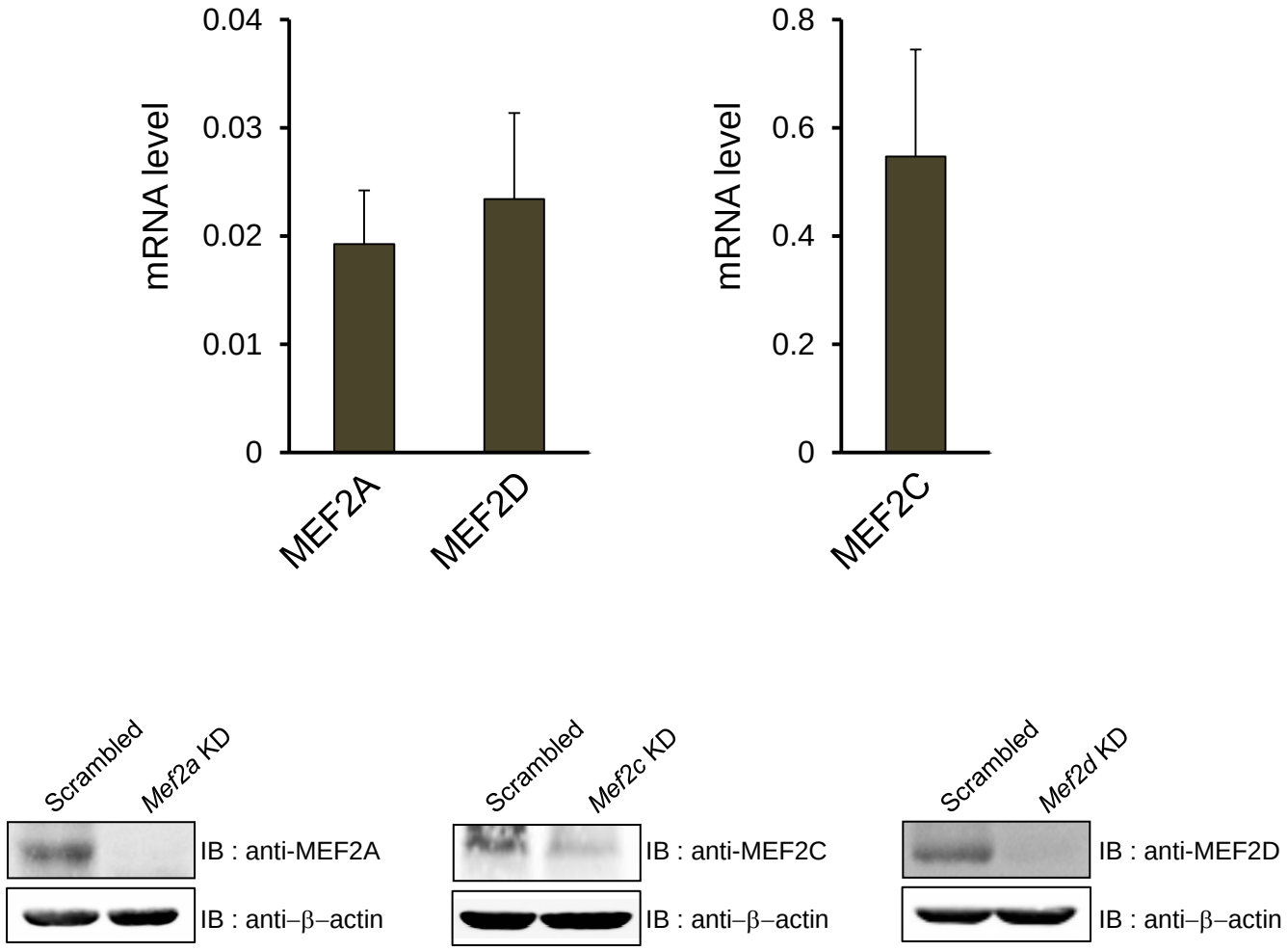


Supplementary Figure 5

Effect of SRF, MEF2C, and MEF2D knockdown on KCl-mediated induction of *c-fos* mRNA and eRNAs.

Effect of transcription factor knockdown in KCl-mediated *c-fos* mRNA and eRNA induction (n = 3 biological replicates). Error bars indicate SEM; p values are from a two-tailed *t* test. NS, not significant.

Supplementary Figure 6

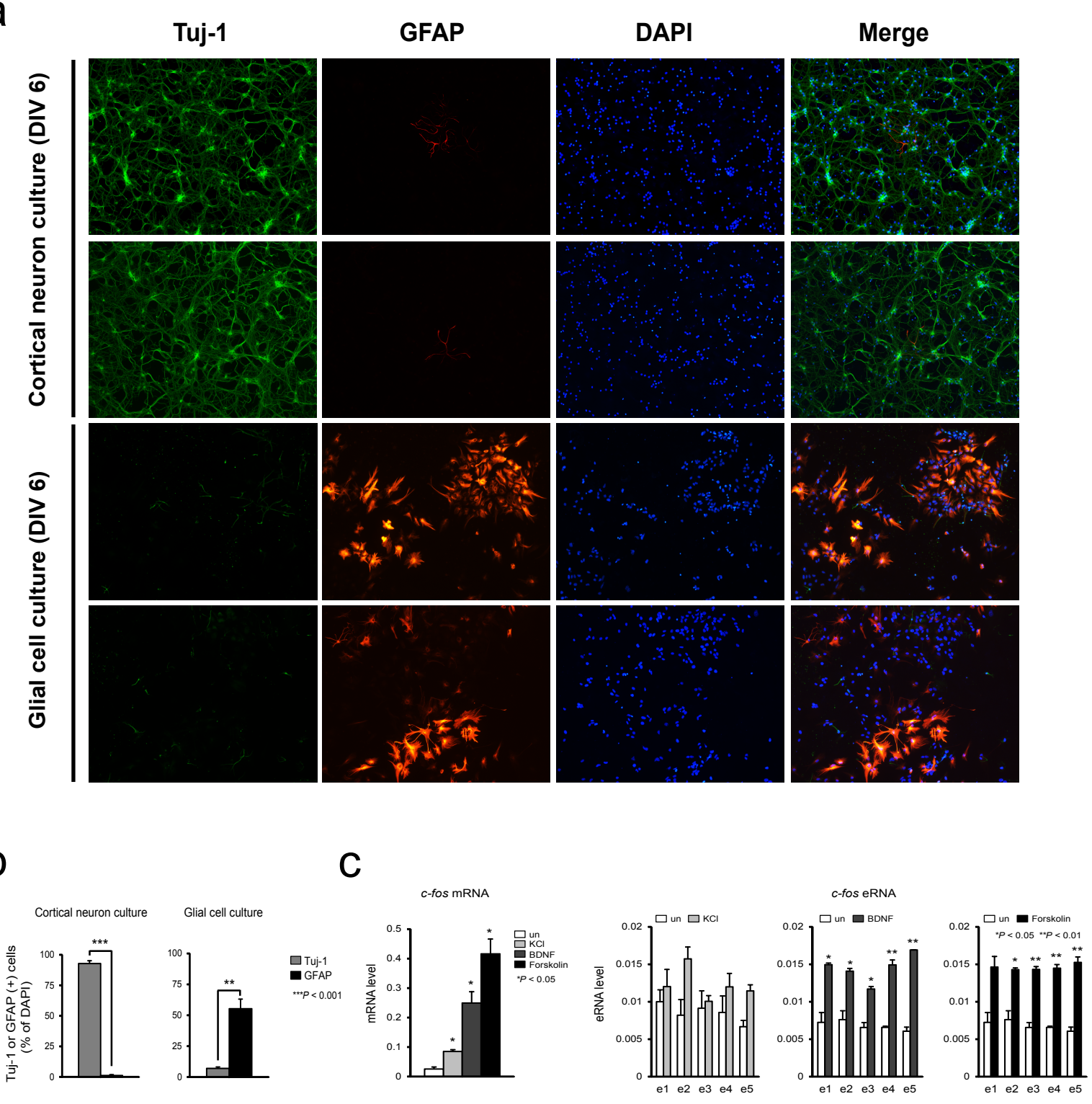


Supplementary Figure 6

Expression levels of MEF2 family member mRNAs and proteins in cortical neurons.

Mef2a, *Mef2c*, and *Mef2d* mRNAs were measured at DIV 6, and normalized to the level of *Gapdh* mRNA (top, n = 3 biological replicates). Cortical neurons infected with lentivirus encoding either a scrambled shRNA or shRNA against *Mef2a*, *Mef2c*, and *Mef2d*. Beta (β)-actin protein was blotted as a loading control (bottom). Western blot experiment was only run once. The original western blot images are presented in Supplementary Figure 16. Error bars indicate SEM. (Note that the y axes are scaled differently).

Supplementary Figure 7

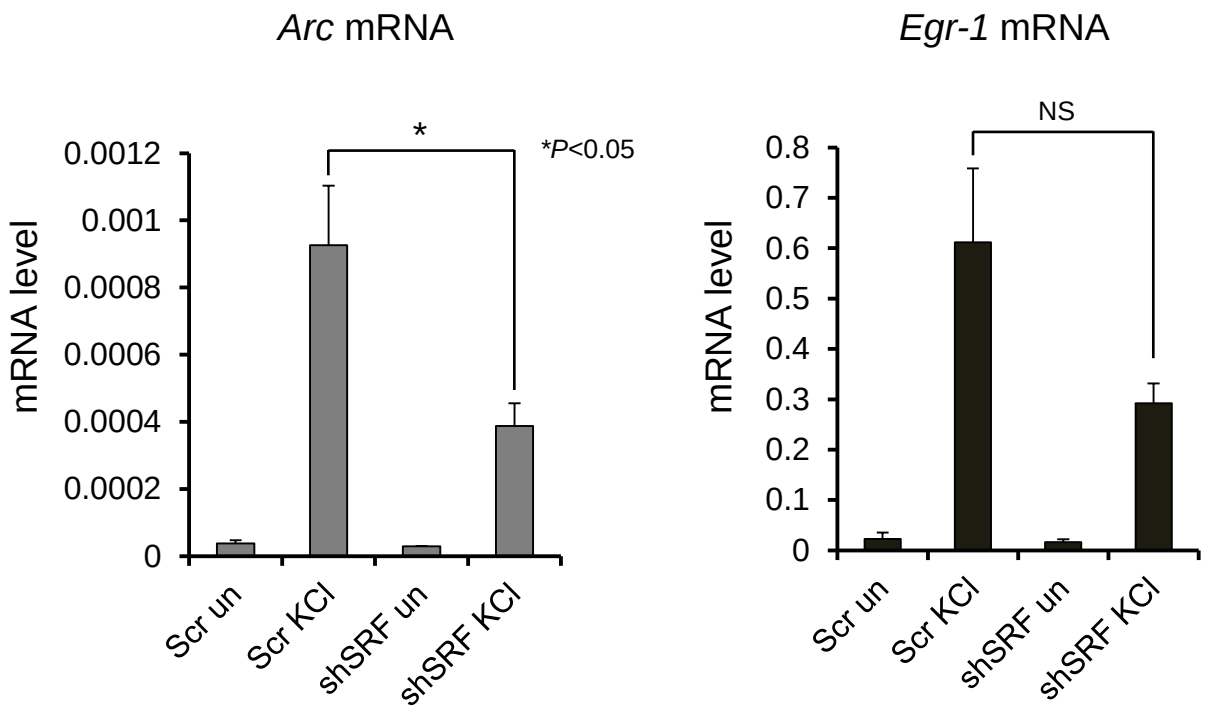


Supplementary Figure 7

The *c-fos* enhancer activities measured by eRNA analysis in glial cells.

(**a,b**) Cultured DIV 6 cortical neurons and glial cells were double immunostained by Tuj-1 and GFAP. Tuj-1 (+) or GFAP (+) cells were counted, and numbers are expressed as the percentage of DAPI (+) cells (Cortical neuron culture: $P = 0.0001$, $t(3) = 37.044$, n = 4; Glial cell culture: $P = 0.0089$, $t(3) = 6.084$, n = 4, unpaired *t*-test with Welch correction unpaired *t*-test with Welch correction). (**c**) Expression of *c-fos* mRNA and eRNA in glial cells induced by KCl, BDNF, and Forskolin. The induction levels of the five *c-fos* eRNAs and mRNA were measured using RT-qPCR and normalized to *Gapdh* mRNA (KCl stimulation: $P = 0.0239$, $t(2) = 6.349$, $F = 1.356$ [$_{SD}P = 0.4517$], n = 2; BDNF stimulation: $P = 0.0286$, $t(2) = 5.787$, $F = 28.667$ [$_{SD}P = 0.1175$], n = 2; Forskolin stimulation: $P = 0.0165$, $t(2) = 7.690$, $F = 50.055$ [$_{SD}P = 0.0894$], n = 2; BDNF stimulation e1: $P = 0.0283$, $t(2) = 5.817$, $F = 36.979$ [$_{SD}P = 0.1038$], n = 2, e2: $P = 0.0345$, $t(2) = 5.240$, $F = 11.137$ [$_{SD}P = 0.1853$], n = 2, e3: $P = 0.0195$, $t(2) = 7.063$, $F = 3.634$ [$_{SD}P = 0.3076$], n = 2, e4: $P = 0.0064$, $t(2) = 12.464$, $F = 17.529$ [$_{SD}P = 0.1493$], n = 2, e5: $P = 0.0025$, $t(2) = 19.845$, $F = 961.36$ [$_{SD}P = 0.0571$], n = 2; Forskolin stimulation: e2: $P = 0.0312$, $t(2) = 5.527$, $F = 26.532$ [$_{SD}P = 0.1221$], n = 2, e3: $P = 0.0092$, $t(2) = 10.368$, $F = 2.896$ [$_{SD}P = 0.3395$], n = 2, e4: $P = 0.0042$, $t(2) = 15.298$, $F = 10.024$ [$_{SD}P = 0.1948$], n = 2, e5: $P = 0.0096$, $t(2) = 10.109$, $F = 1.775$ [$_{SD}P = 0.4099$], n = 2 biological replicates, unpaired *t*-test). Error bars indicate SEM; p values are from a two-tailed *t* test. $_{SD}P$ is the P value from comparing the standard deviations for both groups. $_{SD}P > 0.05$ means the two SDs are not significantly different and can be used for an unpaired *t*-test.

Supplementary Figure 8

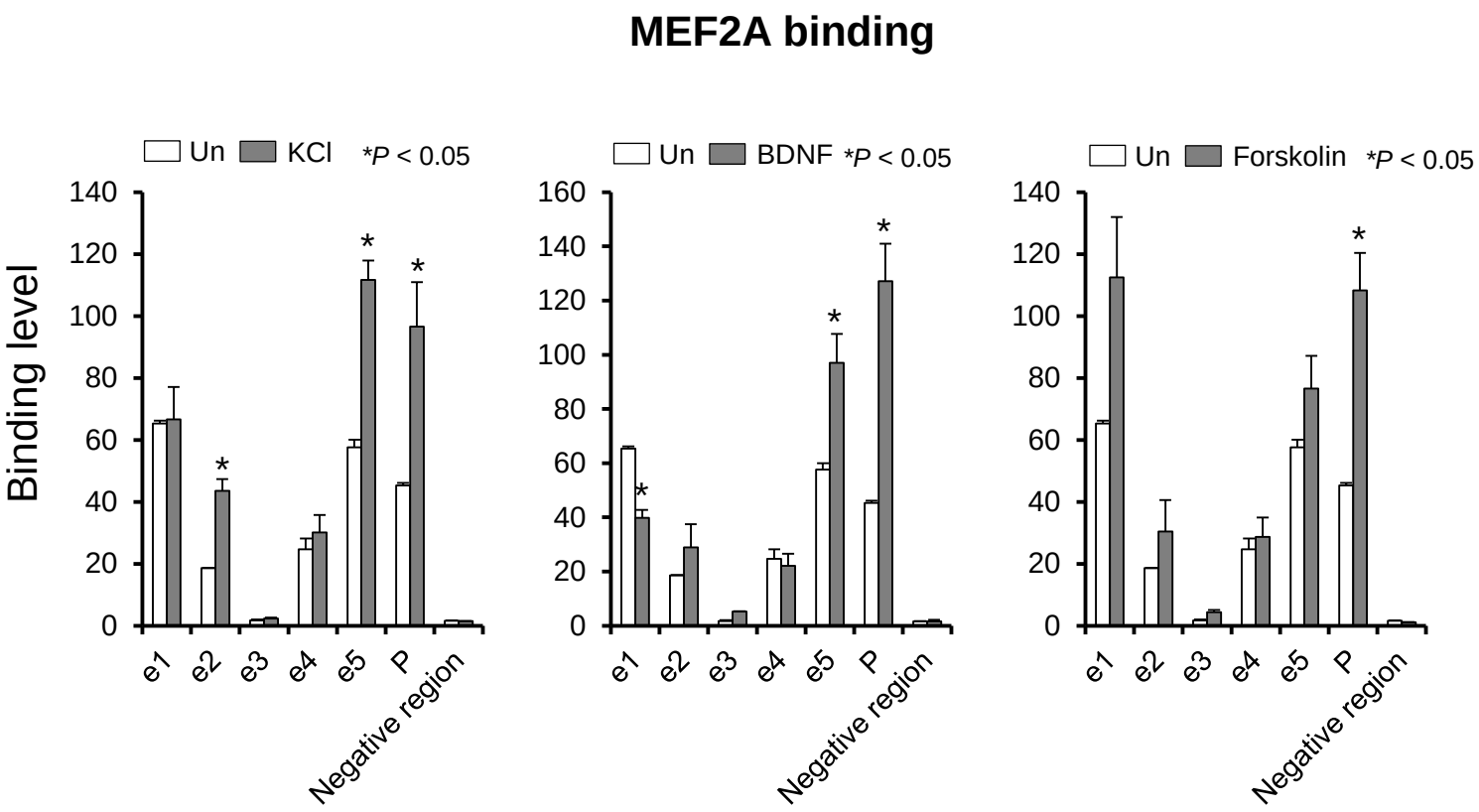


Supplementary Figure 8

SRF regulates KCl-mediated induction of *Arc* and *Egr-1* mRNAs.

SRF was knocked down by shRNAs in cortical neurons, and then the induction levels of *Arc* and *Egr-1* mRNA were measured by RT-qPCR (*Arc* mRNA: $P = 0.0481$, $t(4) = 2.814$, $F = 6.896$ [$_{SD}P = 0.1267$], n = 3 biological replicates, unpaired *t*-test). Error bars indicate SEM; p values are from a two-tailed *t* test. NS, not significant. $_{SD}P$ is the P value from comparing the standard deviations for both groups. $_{SD}P > 0.05$ means the two SDs are not significantly different and can be used for an unpaired *t*-test.

Supplementary Figure 9

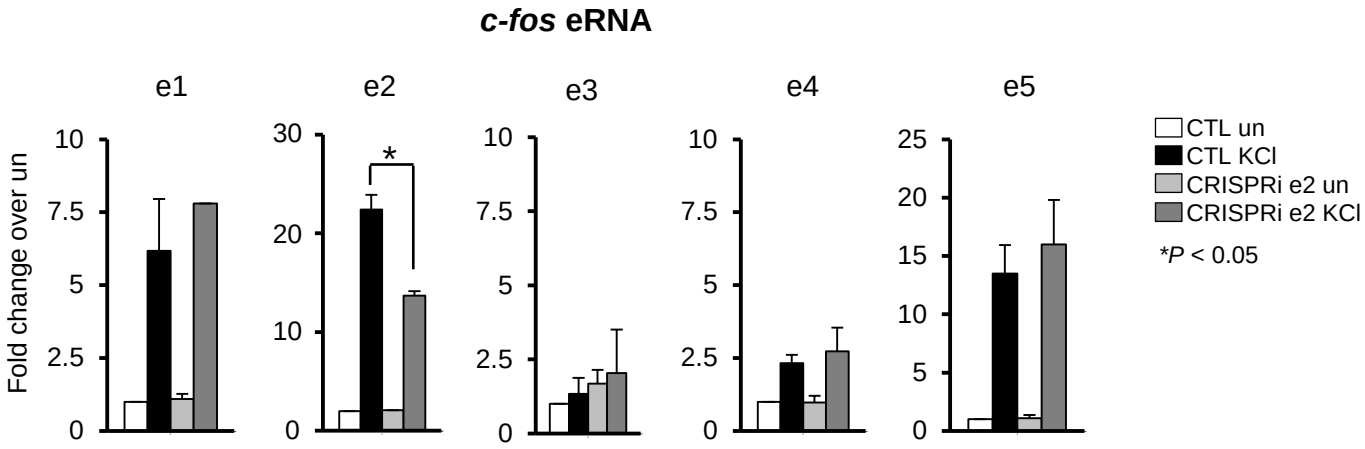


Supplementary Figure 9

MEF2A binding profile at the *c-fos* promoter and five enhancers by KCl, BDNF, and Forskolin stimulation.

Binding was determined at the following locations along the *c-fos* gene: *c-fos* enhancer 1 (e1), enhancer 2 (e2), enhancer 3 (e3), enhancer 4 (e4), enhancer 5 (e5), or promoter (P) (KCl stimulation: e2, $P = 0.0466$, $t(1) = 13.632$, n = 2, unpaired *t*-test with Welch correction, e5, $P = 0.0180$, $t(2) = 7.355$, $F = 6.548$ [$_{SD}P = 0.2372$], n = 2, P, $P = 0.0228$, $t(2) = 6.514$, $F = 150.94$ [$_{SD}P = 0.0517$], n = 2, unpaired *t*-test; BDNF stimulation: e1, $P = 0.0135$, $t(2) = 8.507$, $F = 10.418$ [$_{SD}P = 0.1913$], n = 2, e5, $P = 0.0419$, $t(2) = 4.733$, $F = 19.313$ [$_{SD}P = 0.1432$], n = 2, P, $P = 0.0121$, $t(2) = 8.997$, $F = 136.89$ [$_{SD}P = 0.0543$], n = 2, unpaired *t*-test; Forskolin stimulation: P, $P = 0.0182$, $t(2) = 7.313$, $F = 90.055$ [$_{SD}P = 0.0668$], n = 2 biological replicates, unpaired *t*-test). Error bars indicate SEM; p values are from a two-tailed *t* test. $_{SD}P$ is the P value from comparing the standard deviations for both groups. $_{SD}P > 0.05$ means the two SDs are not significantly different and can be used for an unpaired *t*-test.

Supplementary Figure 10

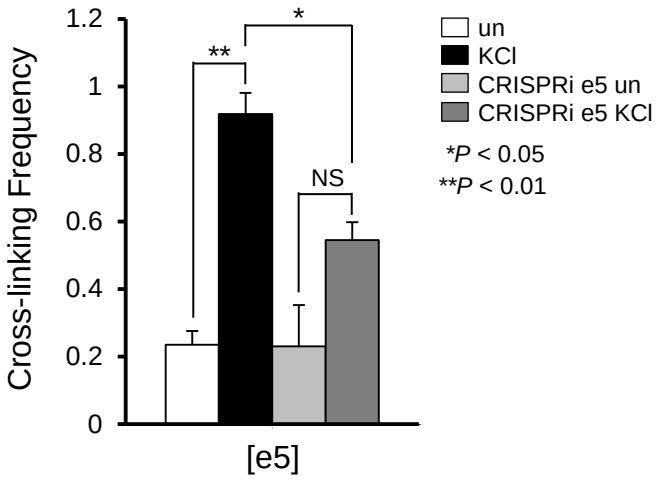


Supplementary Figure 10

Specific e2 enhancer-targeted CRISPRi does not affect other *c-fos* eRNA.

Expression levels of e1, e2, e3, e4, and e5 eRNA were measured using RT-qPCR (*c-fos* e2 eRNA, , $P = 0.0393$, $t(4) = 3.015$, $F = 3.461$ [$_{SD}P = 0.2242$], n = 3 biological replicates). Error bars indicate SEM; p values are from a two-tailed *t* test. $_{SD}P$ is the P value from comparing the standard deviations for both groups. $_{SD}P > 0.05$ means the two SDs are not significantly different and can be used for an unpaired *t*-test.

Supplementary Figure 11

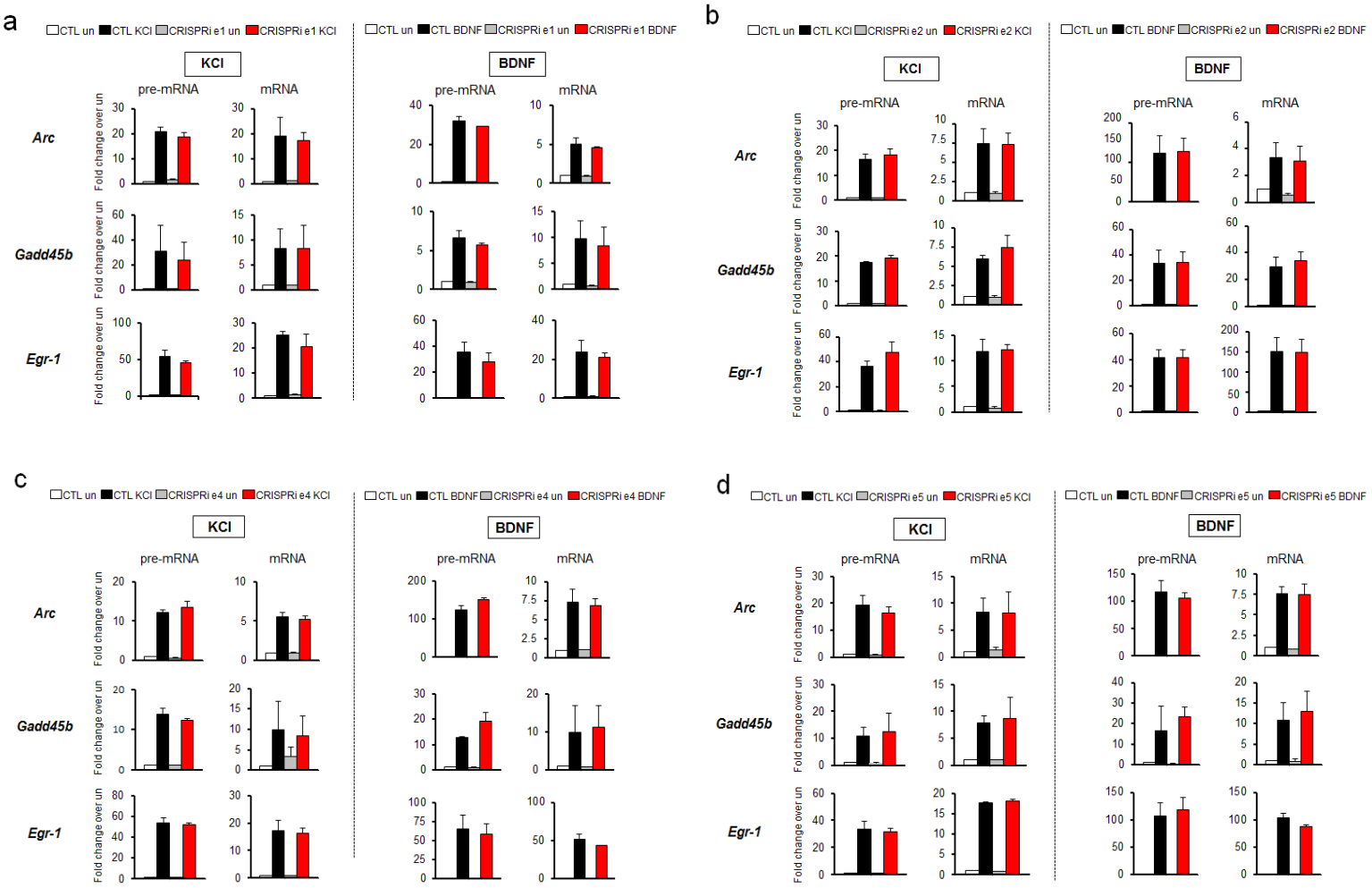


Supplementary Figure 11

The *c-fos* enhancer 5 and promoter interaction was decreased by suppression of the e5 enhancer

Cortical neurons were infected with an sgRNA targeting e5 and stimulated with KCl. (KCl e5: $P = 0.0117$, $t(2) = 9.144$, $F = 2.420$ [$_{SD}P = 0.3637$], n = 2; CRISPRi e5 KCl: $P = 0.0453$, $t(2) = 4.539$, $F = 1.398$ [$_{SD}P = 0.4470$], n = 2 biological replicates). All unpaired *t*-test. Error bars indicate SEM; p values are from a two-tailed *t* test. $_{SD}P$ is the P value from comparing the standard deviations for both groups. $_{SD}P > 0.05$ means the two SDs are not significantly different and can be used for an unpaired *t*-test.

Supplementary Figure 12



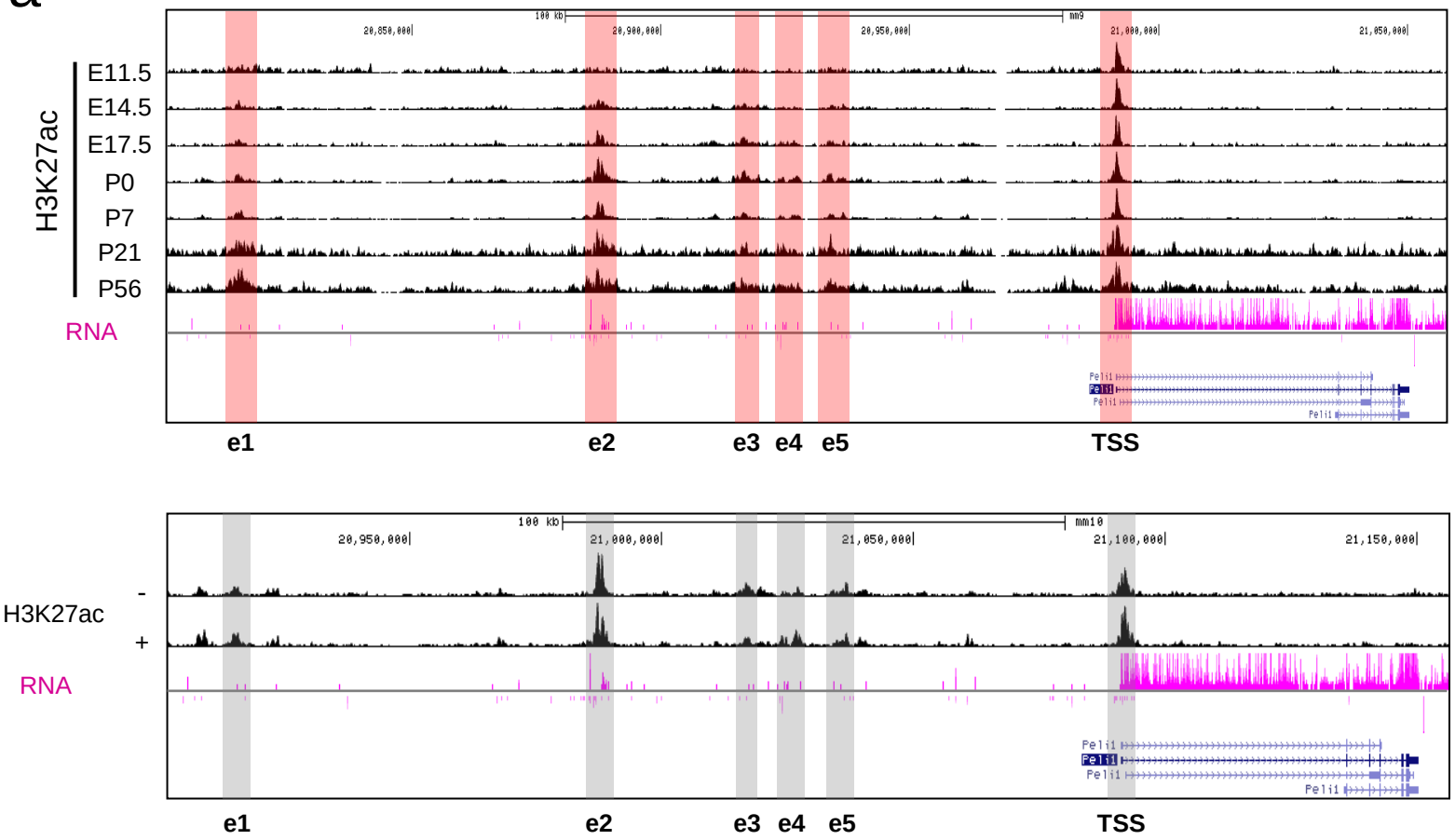
Supplementary Figure 12

Effect of e1, e2, e4, and e5 enhancer-targeted CRISPRi on various IEGs.

(**a-d**) Following the suppression of e1, e2, e4, and e5 enhancer by CRISPRi, cortical neurons were stimulated by KCl or BDNF, and expression levels of *Arc*, *Gadd45b*, and *Egr-1* pre-mRNA and mRNA were measured using RT-qPCR (KCl stimulation: n = 3 biological replicates, BDNF stimulation: n = 2 biological replicates). Error bars indicate SEM; p values are from a two-tailed *t* test.

Supplementary Figure 13

a



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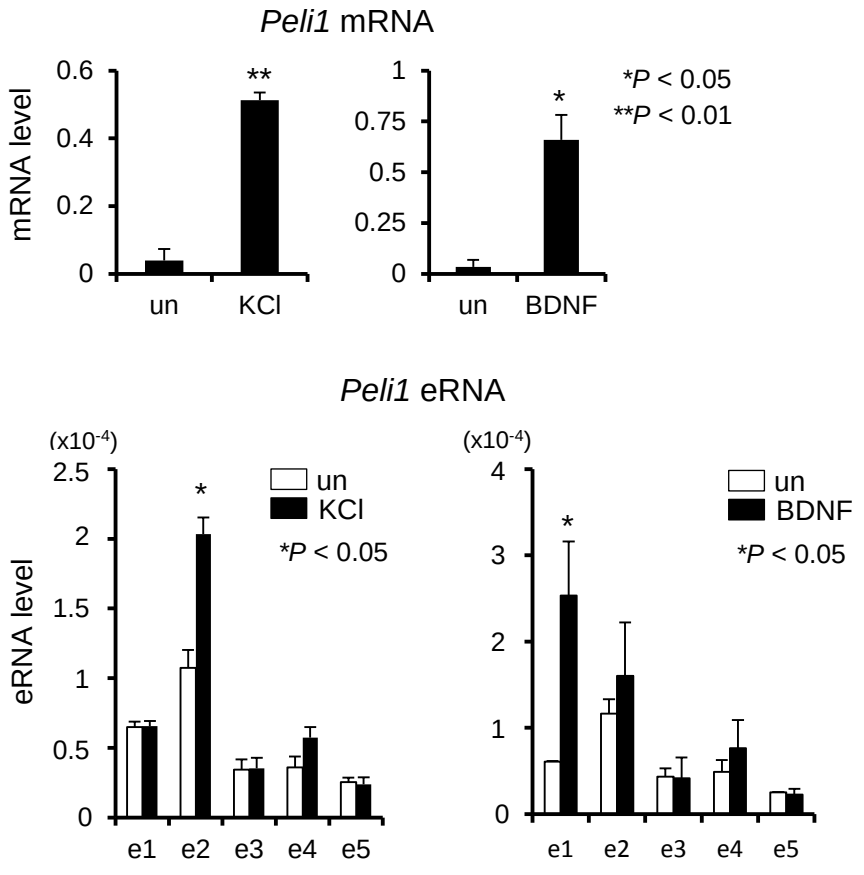


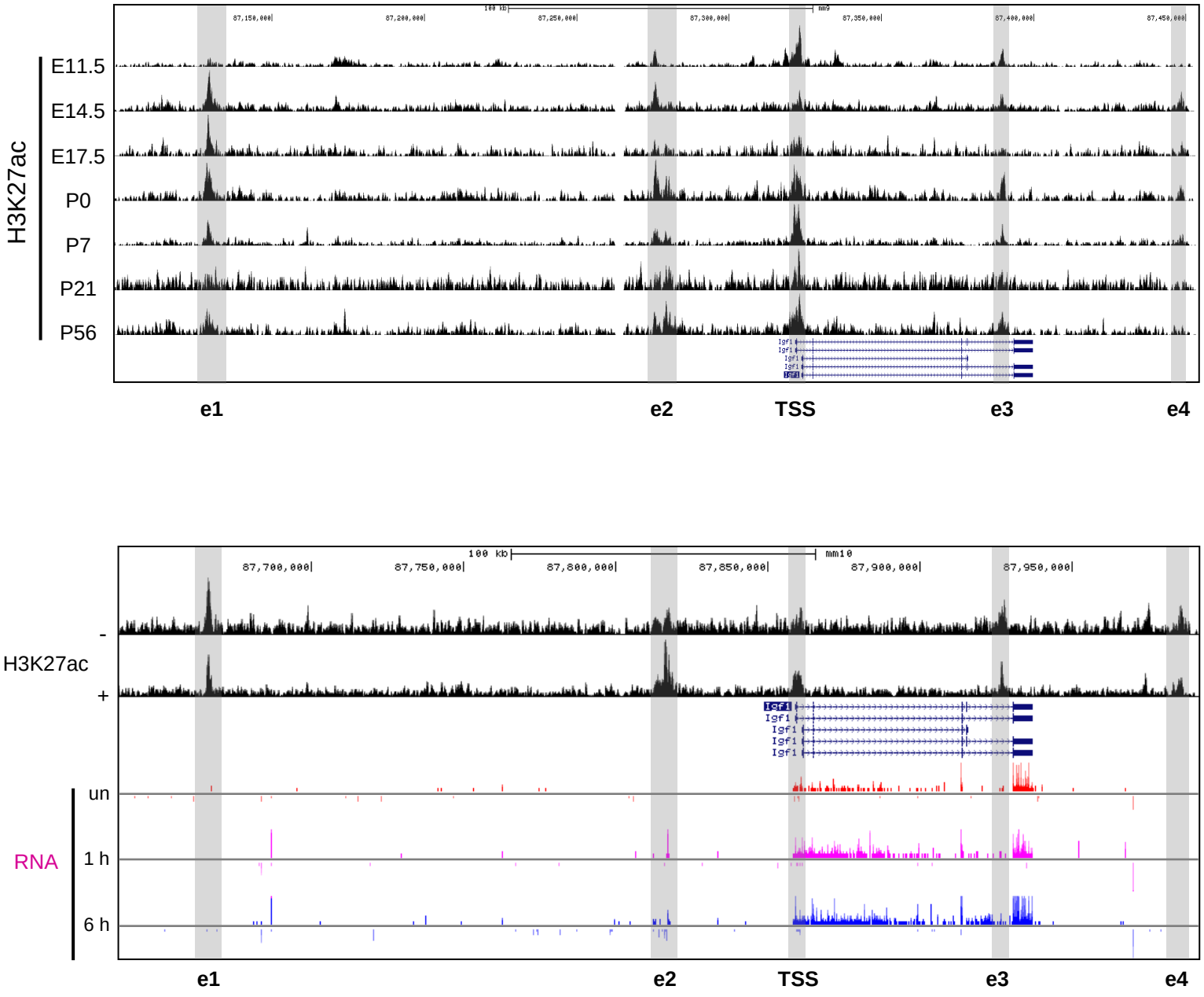
Supplementary Figure 13

Binding profiles of the H3K27ac mark at the *Peli1* enhancer and promoter and enhancer activities measured by eRNA analysis.

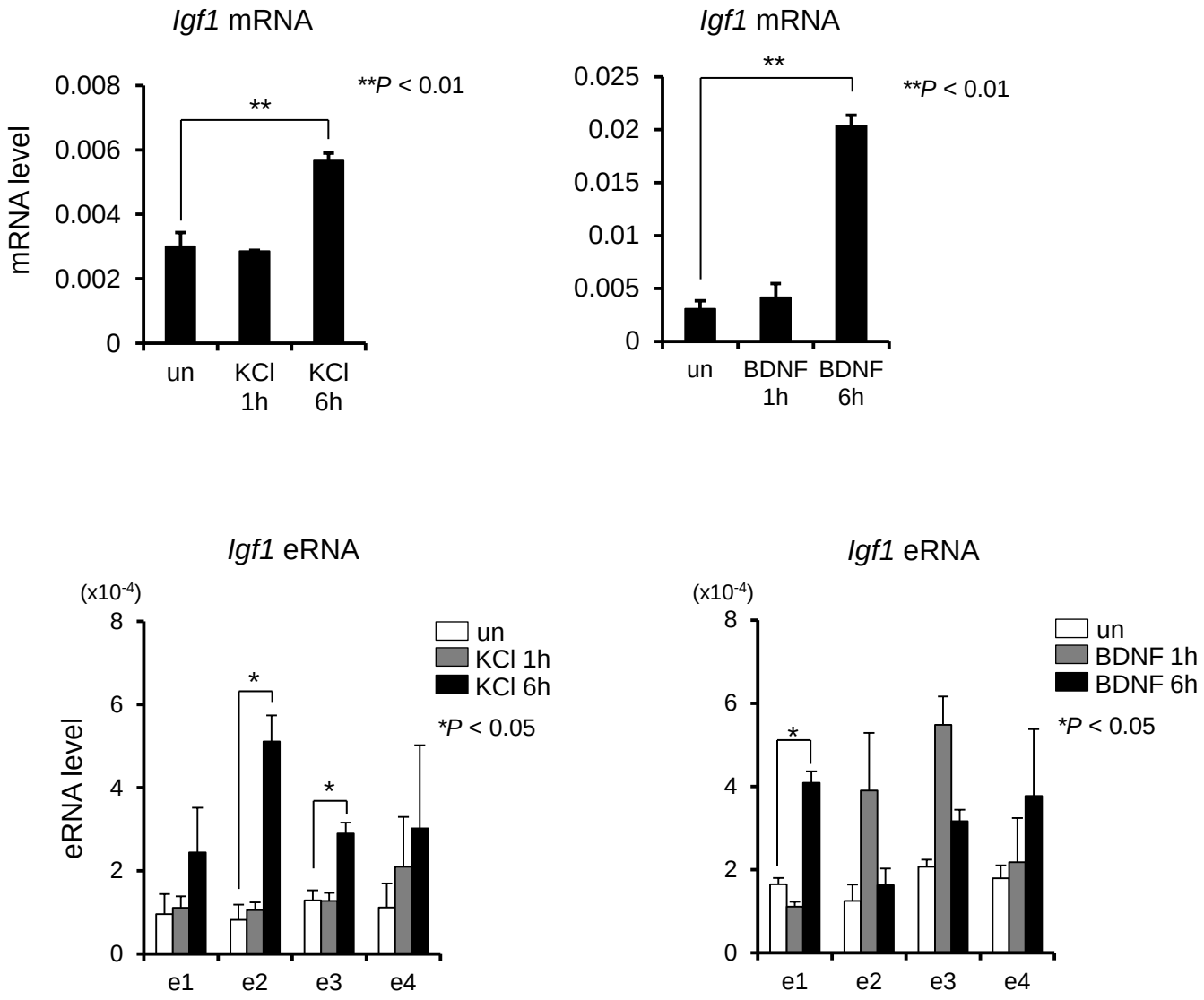
(**a**) H3K27ac binding profiles throughout the mouse forebrain development and KCl stimulation were adapted from the data generated by Nord *et al*. 2013 and Malik *et al*. 2014. Total RNA-seq data (1 h) was adapted from previous study by Kim et al 2010. Gray vertical bars indicate the locations of the *Peli1* enhancers and the promoter, respectively. + and – indicate the presence or absence of KCl. (**b**) Expression of *Peli1* mRNA and eRNA in cortical neurons induced by KCl and BDNF. The induction levels of the five *Peli1* eRNAs and mRNA were measured using RT-qPCR and normalized to *Gapdh* mRNA (KCl stimulation: *Peli1* mRNA, $P = 0.0076$, $t(2) = 11.430$, $F = 2.346$ [$_{SD}P = 0.3682$], n = 2, *Peli1* e2, $P = 0.0318$, $t(2) = 5.477$, $F = 1.154$ [$_{SD}P = 0.4773$], n = 2; BDNF stimulation: *Peli1* mRNA, $P = 0.0388$, $t(4) = 4.930$, $F = 13.471$ [$_{SD}P = 0.1693$], n = 2, *Peli1* e1, $P = 0.0392$, $t(2) = 4.900$, $F = 63.601$ [$_{SD}P = 0.080$], n = 2 biological replicates). All unpaired *t*-test. Error bars indicate SEM; p values are from a two-tailed *t* test. $_{SD}P$ is the P value from comparing the standard deviations for both groups. $_{SD}P > 0.05$ means the two SDs are not significantly different and can be used for an unpaired *t*-test.

Supplementary Figure 14

a



b

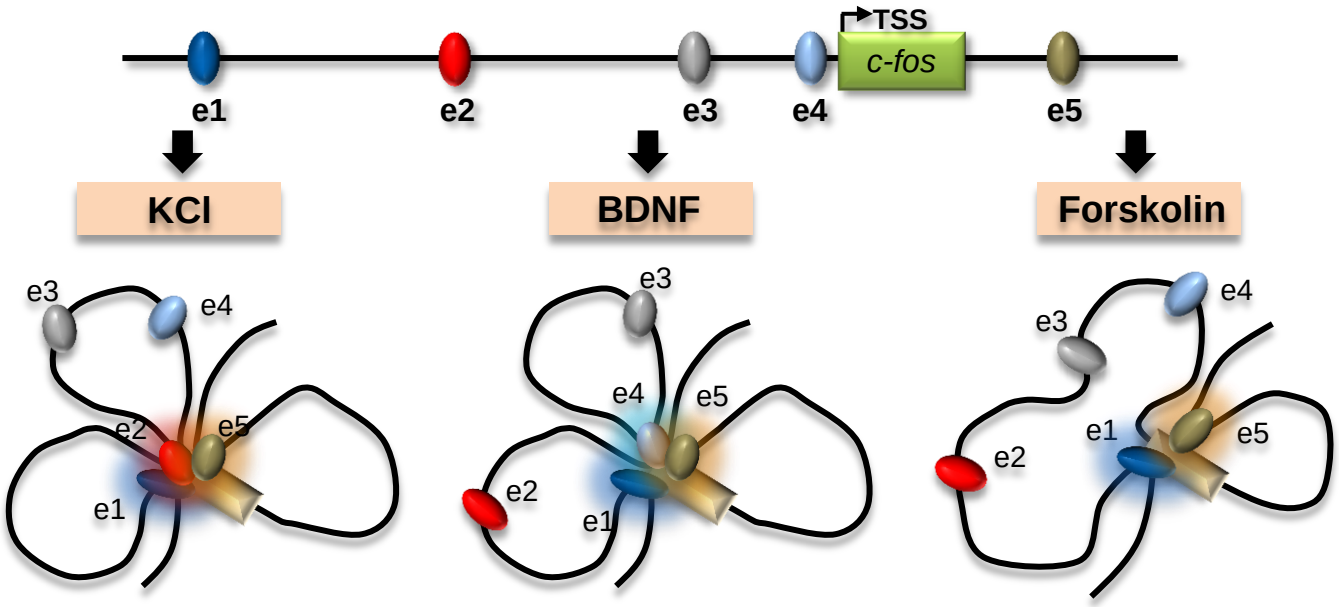


Supplementary Figure 14

Binding profiles of the H3K27ac mark at the *Igf-1* enhancer and promoter and enhancer activities measured by eRNA analysis.

(**a**) H3K27ac binding profiles throughout the mouse forebrain development and KCl stimulation were adapted from the data generated by Nord *et al*. 2013 and Malik *et al*. 2014. Total RNA-seq data (unstimulated, 1 h, 6 h) were adapted from previous study by Kim et al 2010. Gray vertical bars indicate the locations of the *Igf-1* enhancers and the promoter, respectively. + and – indicate the presence or absence of KCl. (**b**) Expression of *Igf-1* mRNA and eRNA in cortical neurons induced by KCl, BDNF. The induction levels of the five *Igf-1* eRNAs and mRNA were measured using RT-qPCR and normalized to *Gapdh* mRNA (KCl stimulation: *Igf1* mRNA, P = 0.0317, $t(2)$ = 5.486, F = 3.435 [$_{SD}P$ = 0.3150], n = 2, *Igf1* e2, P = 0.0280, $t(2)$ = 5.852, F = 2.903 [$_{SD}P$ = 0.3379], n = 2, *Igf1* e3, P = 0.0465, $t(2)$ = 4.520, F = 1.161 [$_{SD}P$ = 0.4762], n = 2, BDNF stimulation: *Igf1* mRNA, P = 0.0050, $t(2)$ = 14.092, F = 1.686 [$_{SD}P$ = 0.4178], n = 2, *Igf1* e1, P = 0.0224, $t(2)$ = 6.570, F = 3.354 [$_{SD}P$ = 0.3182], n = 2 biological replicates). All unpaired *t*-test. Error bars indicate SEM; p values are from a two-tailed *t* test. $_{SD}P$ is the P value from comparing the standard deviations for both groups. $_{SD}P > 0.05$ means the two SDs are not significantly different and can be used for an unpaired *t*-test.

Supplementary Figure 15

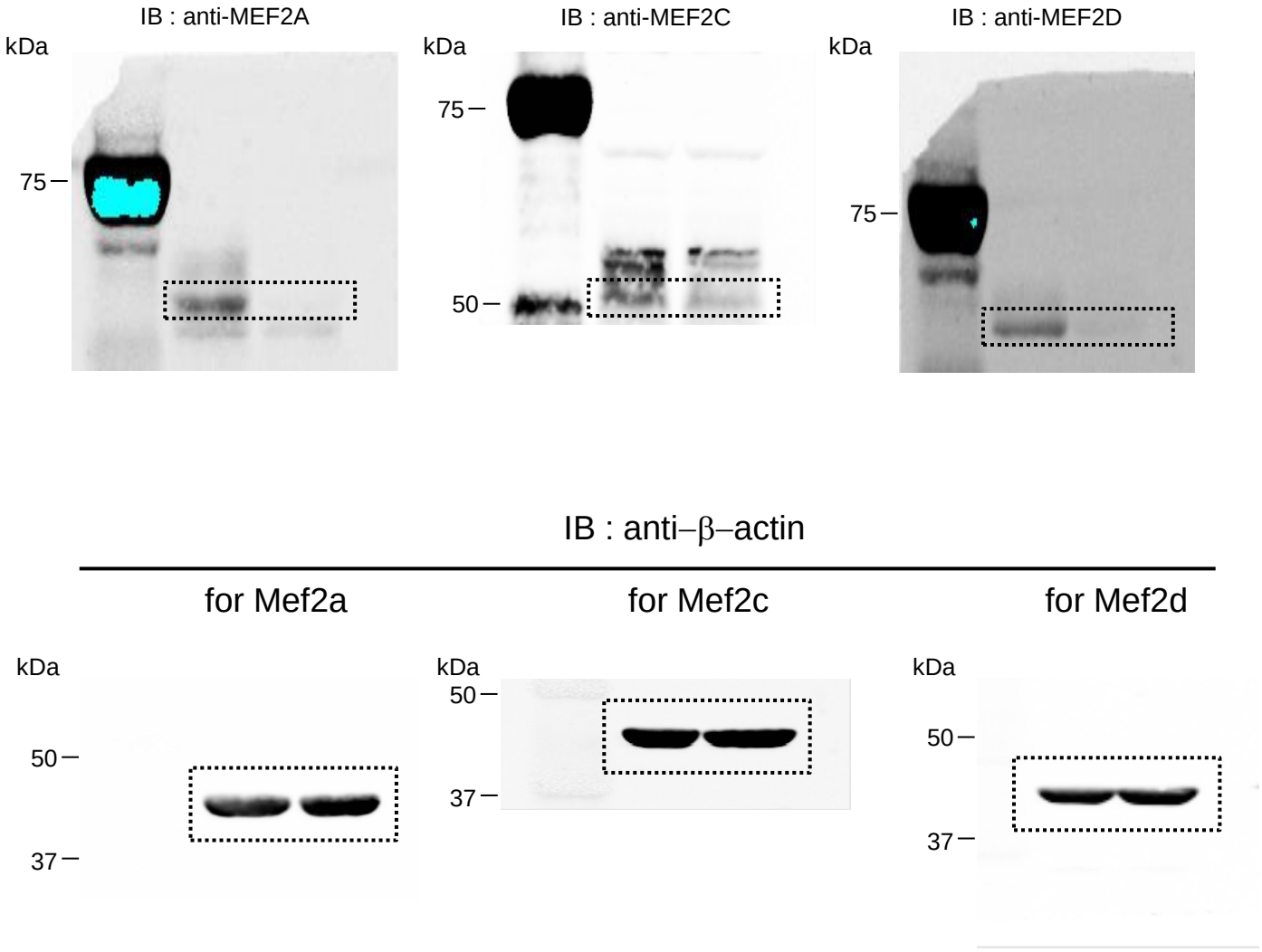


Supplementary Figure 15

Stimulus-specific combinatorial activation of multiple *c-fos* enhancers.

In response to different stimuli, distinct subsets of the surrounding enhancers are dynamically assembled with the promoter to mediate robust *c-fos* transcription.

Supplementary Figure 16



Supplementary Figure 16

Full blots of the western blots shown in the figure

This Figure contains the full blots of the western blots shown in the Supplementary Figure 6.