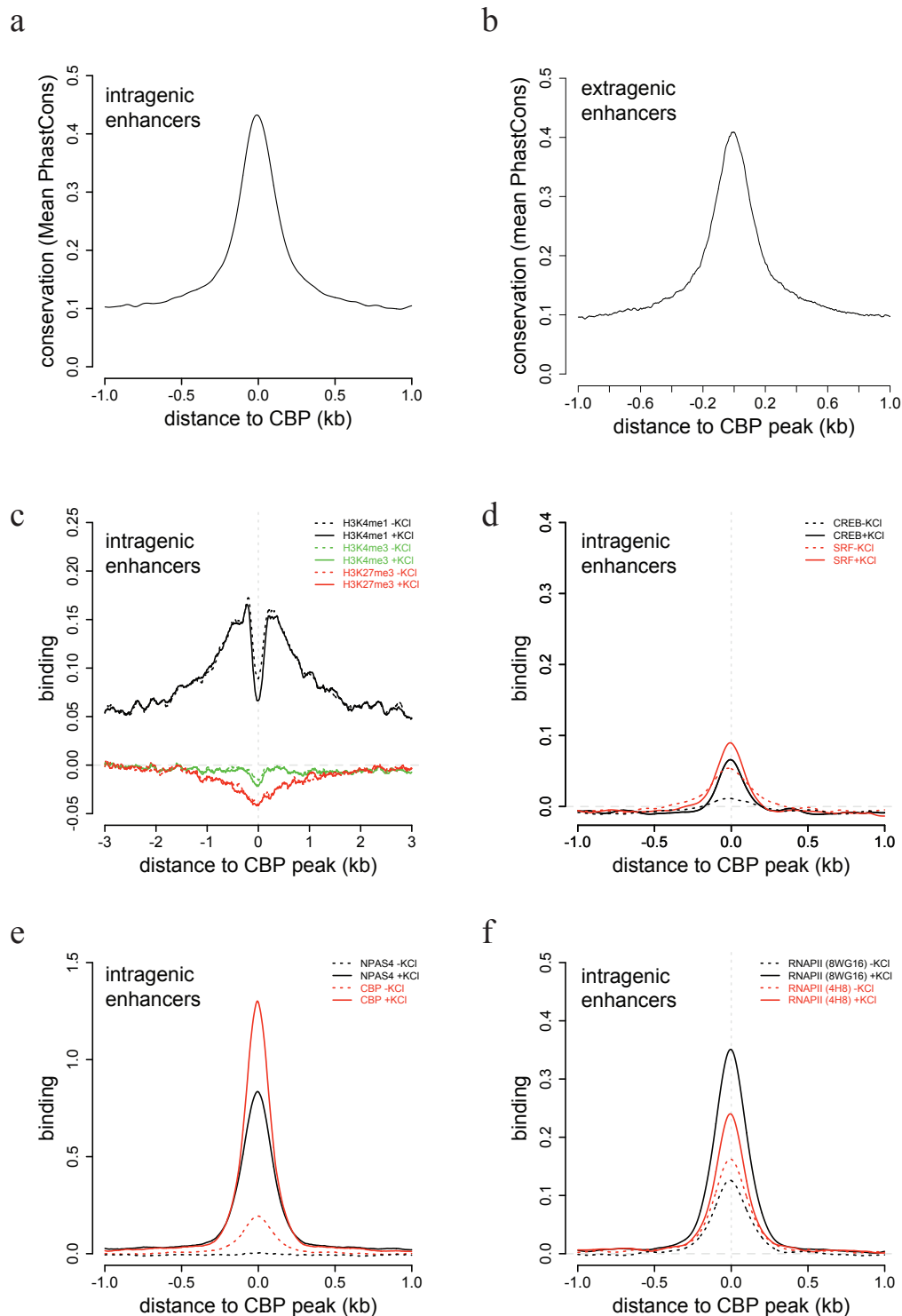
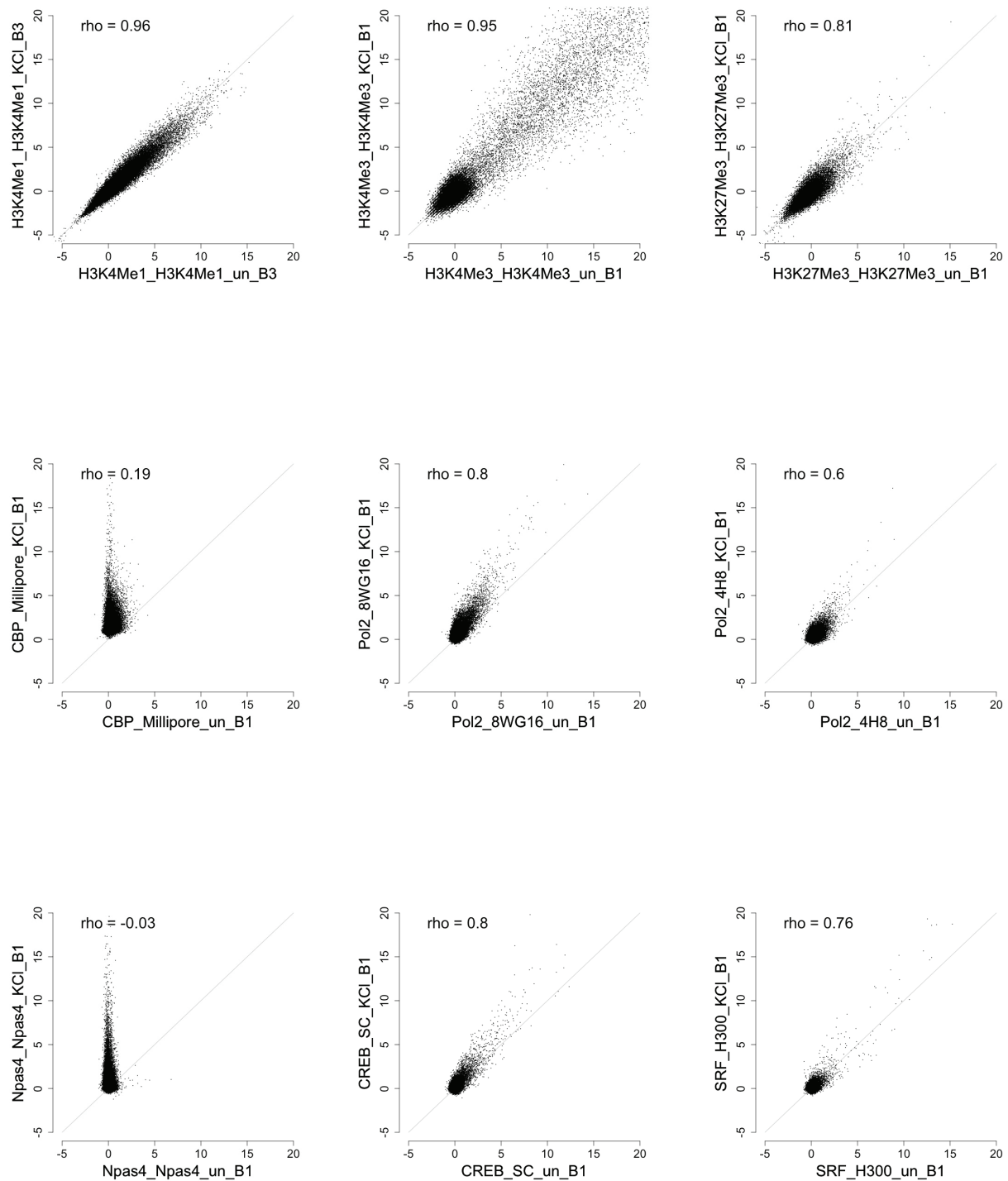


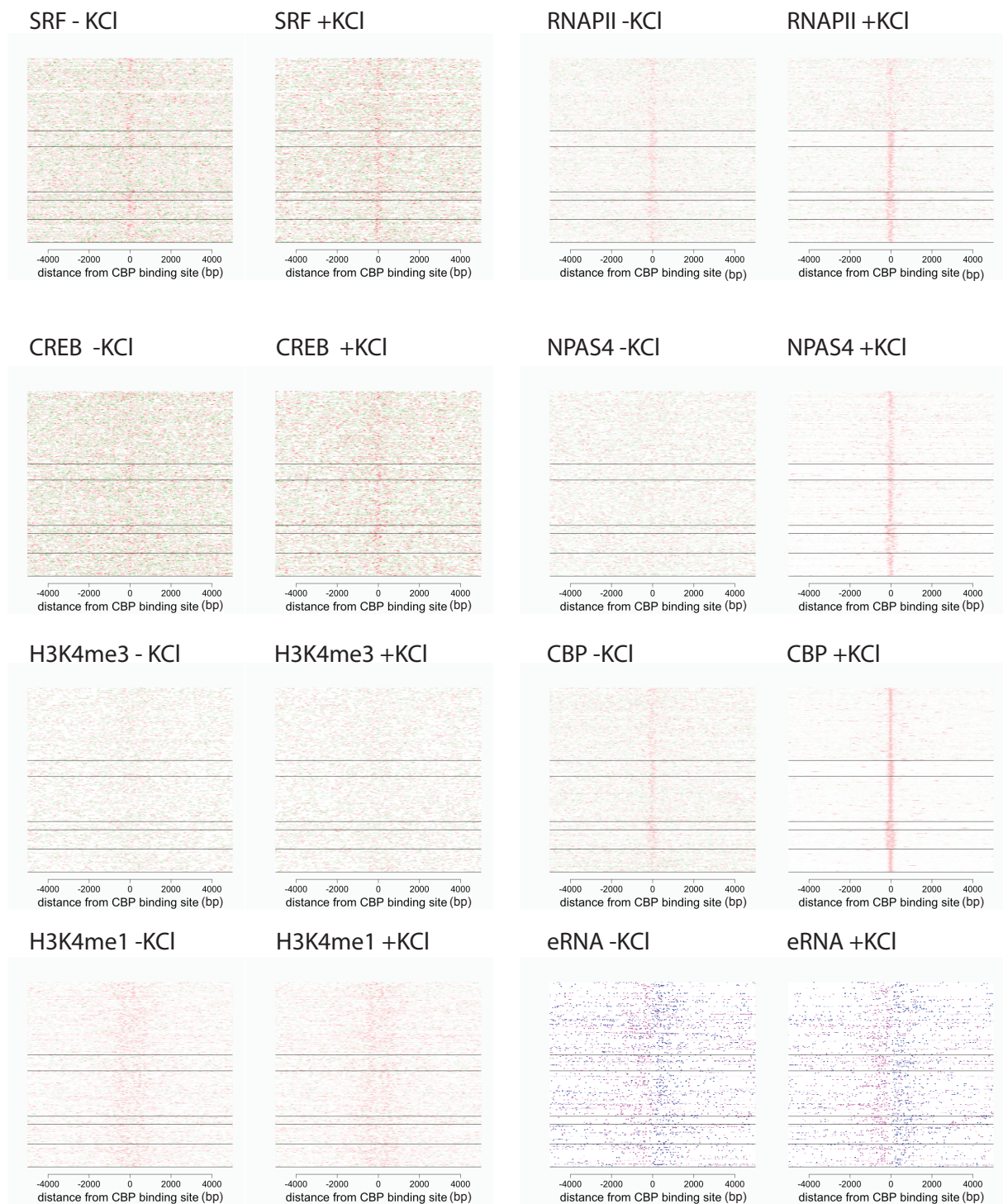
SUPPLEMENTARY INFORMATION



Supplementary Figure 1. ChIP-Seq profiles for histone modifications, TFs, and RNAPII at intragenic enhancers. **a-b**, Conservation profiles at 6,718 intragenic (**a**) and 5,117 extragenic (**b**) enhancers. **c-f**, Profiles of histone modifications and TFs at enhancers located within intragenic regions are displayed as in Figs. 2b and 4b.

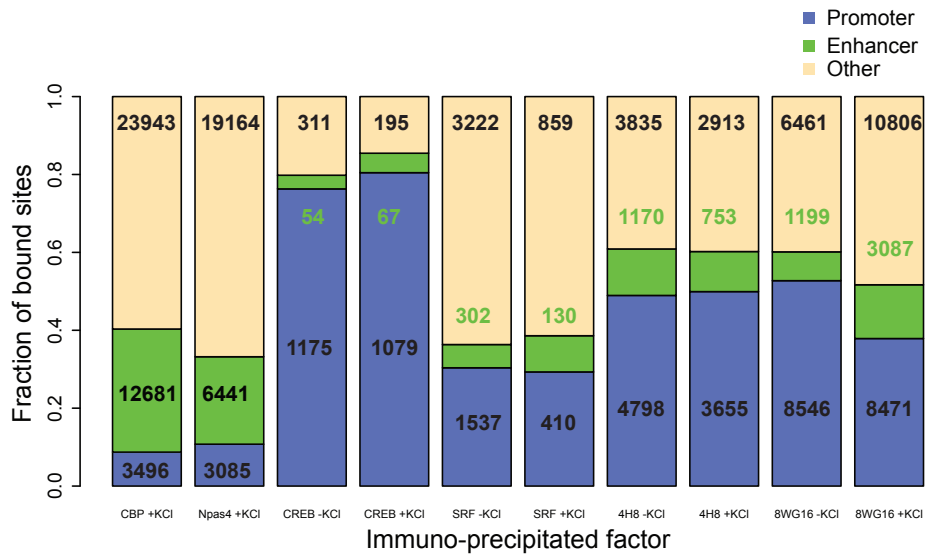


Supplementary Figure 2. Changes in TF binding at individual CBP peaks upon membrane depolarization. In each plot, each dot represents one or more of 41,148 CBP peaks called using the Millipore CBP antibody after 2 hours KCl treatment. The position of each dot on the x-axis denotes the number of ChIP-Seq reads in the unstimulated condition, while the position of each dot on the y-axis denotes the number of ChIP-Seq reads in the stimulated condition. For histone methylation marks, reads within 1 kb of the CBP-bound enhancer center are included. For the TFs, reads within 100 bp of the enhancer center are included. Read numbers are normalized and input-subtracted as described in Methods. Rho is the Spearman correlation coefficient. *BN* refers to biological replicate *N*.

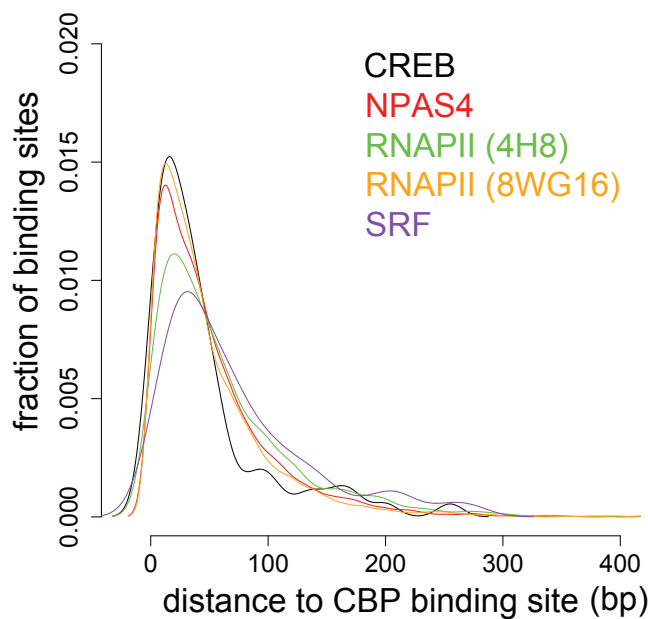


Supplementary Figure 3. Heatmap showing the binding profiles of methylated histones and transcription factors at 315 selected extragenic enhancers. The same 315 enhancers shown in fig. 5a are repeated in the same order for each factor. The color represents the strength of binding, from dark green (strongly de-enriched compared to input) to dark red (strongly enriched compared to input). A similar plot for eRNAs displays forward (blue) and reverse (magenta) reads from the total RNA fraction across the same set of 315 enhancers, as also shown in fig. 5a. Note that in the RNAPII experiments, 8WG16 antibody was used.

a

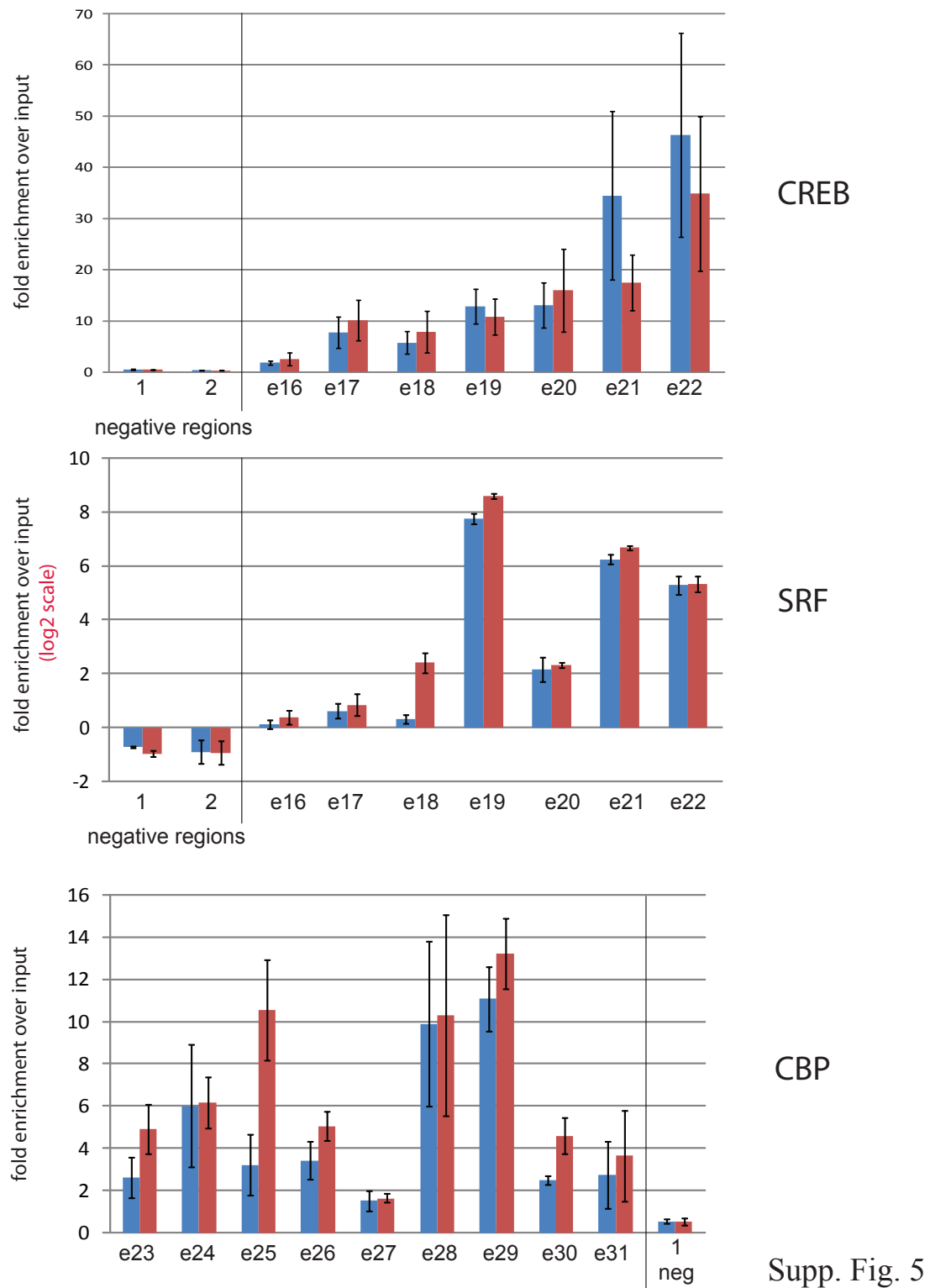


b

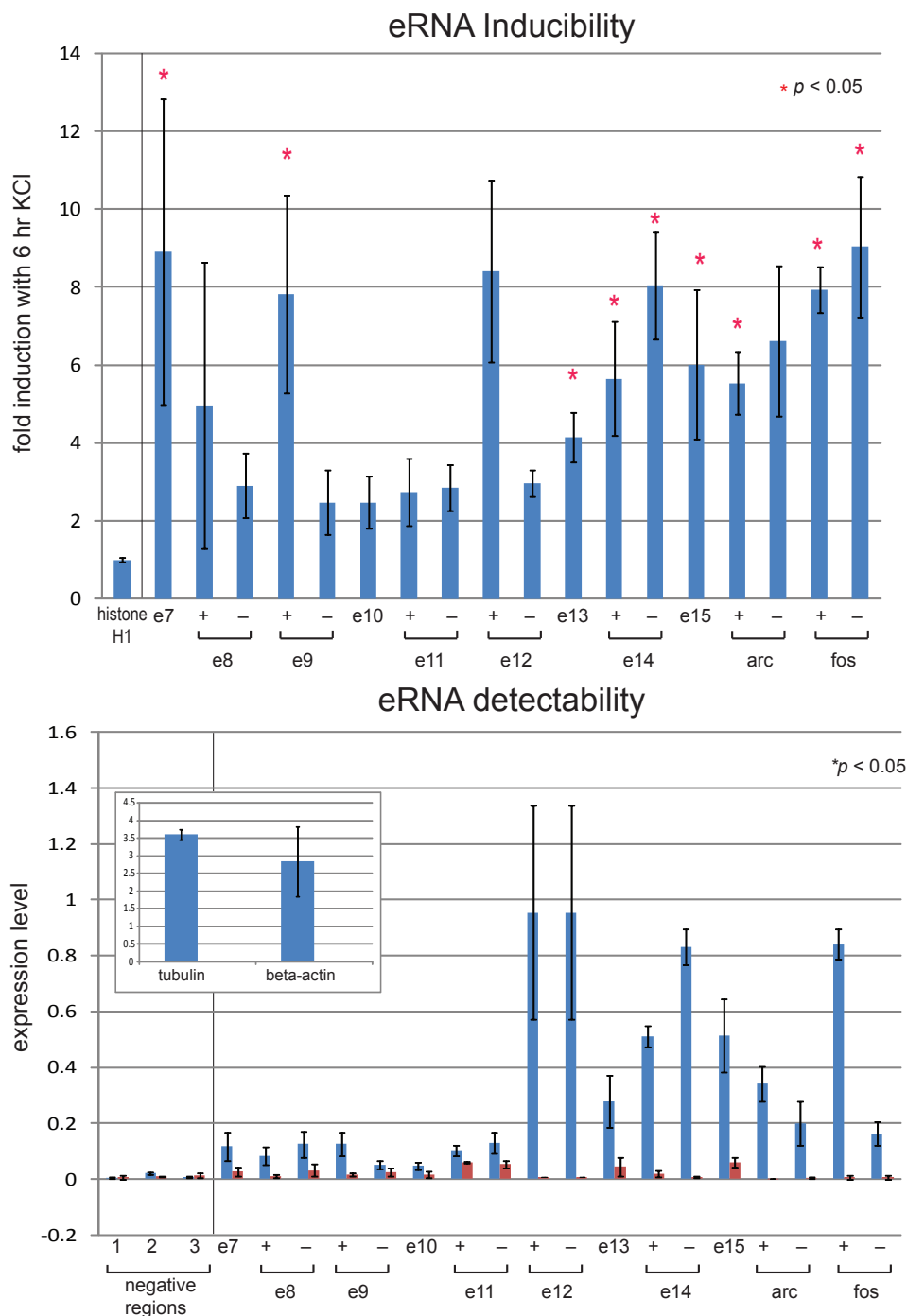


Supp. Fig. 4

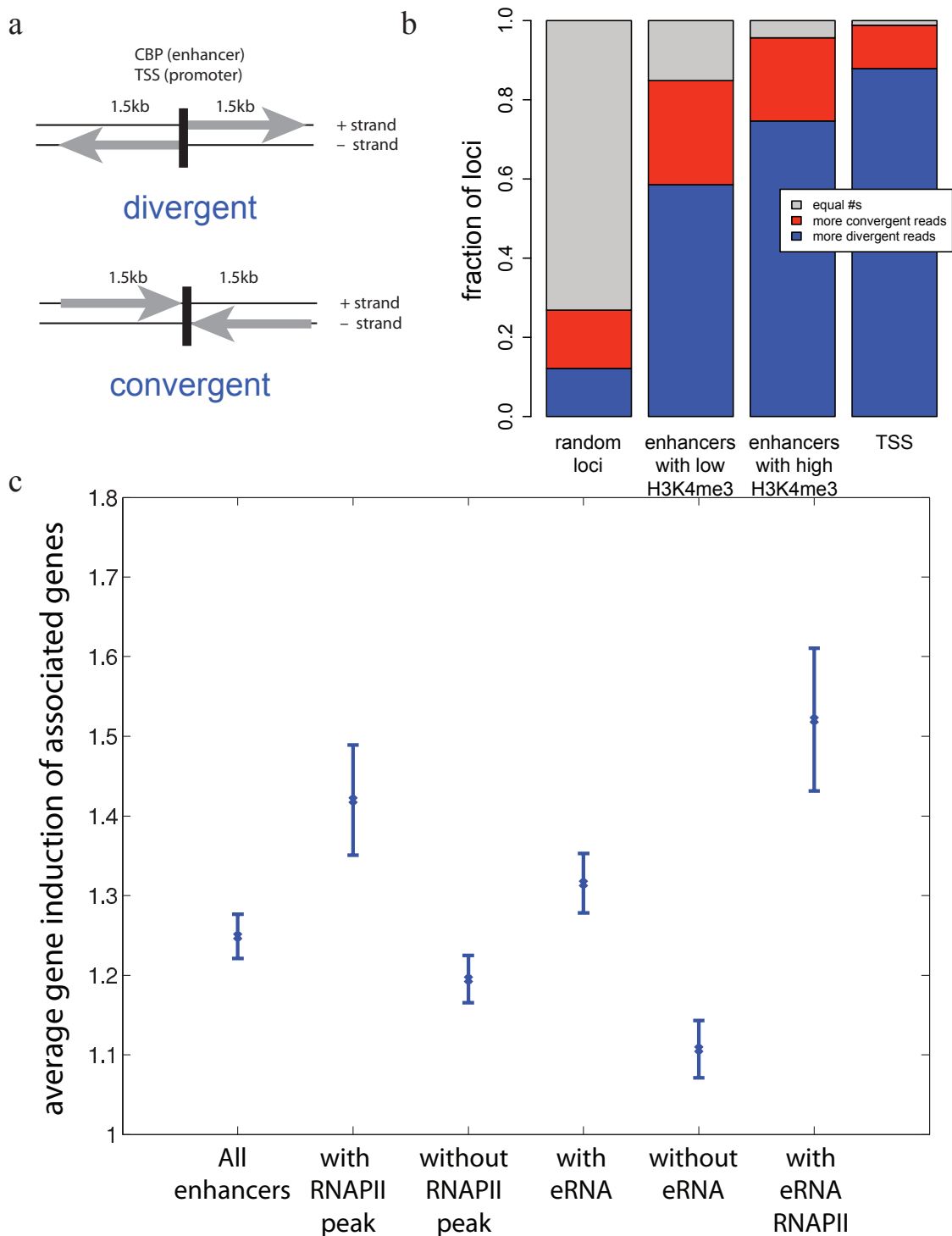
Supplementary Figure 4. Locations of TF and RNAPII binding sites. **a**, Binding sites for each TF (or RNAPII) are grouped into three categories: promoter-proximal (within 1 kb of 25,562 annotated RefSeq TSSs), enhancer-proximal (within 1kb of 11,835 CBP-bound enhancer centers), or other (*i.e.*, not in the previous two categories). The actual number of peaks in each category is also shown. 4H8 and 8WG16 are anti-RNAPII antibodies. **b**, Distribution of the distances from each TF or RNAPII binding site to the CBP peak center. The x-axis denotes the distance (bp) between the factor binding site and the CBP binding site. The y-axis denotes the fraction of binding sites of a given TF at any given distance to CBP.



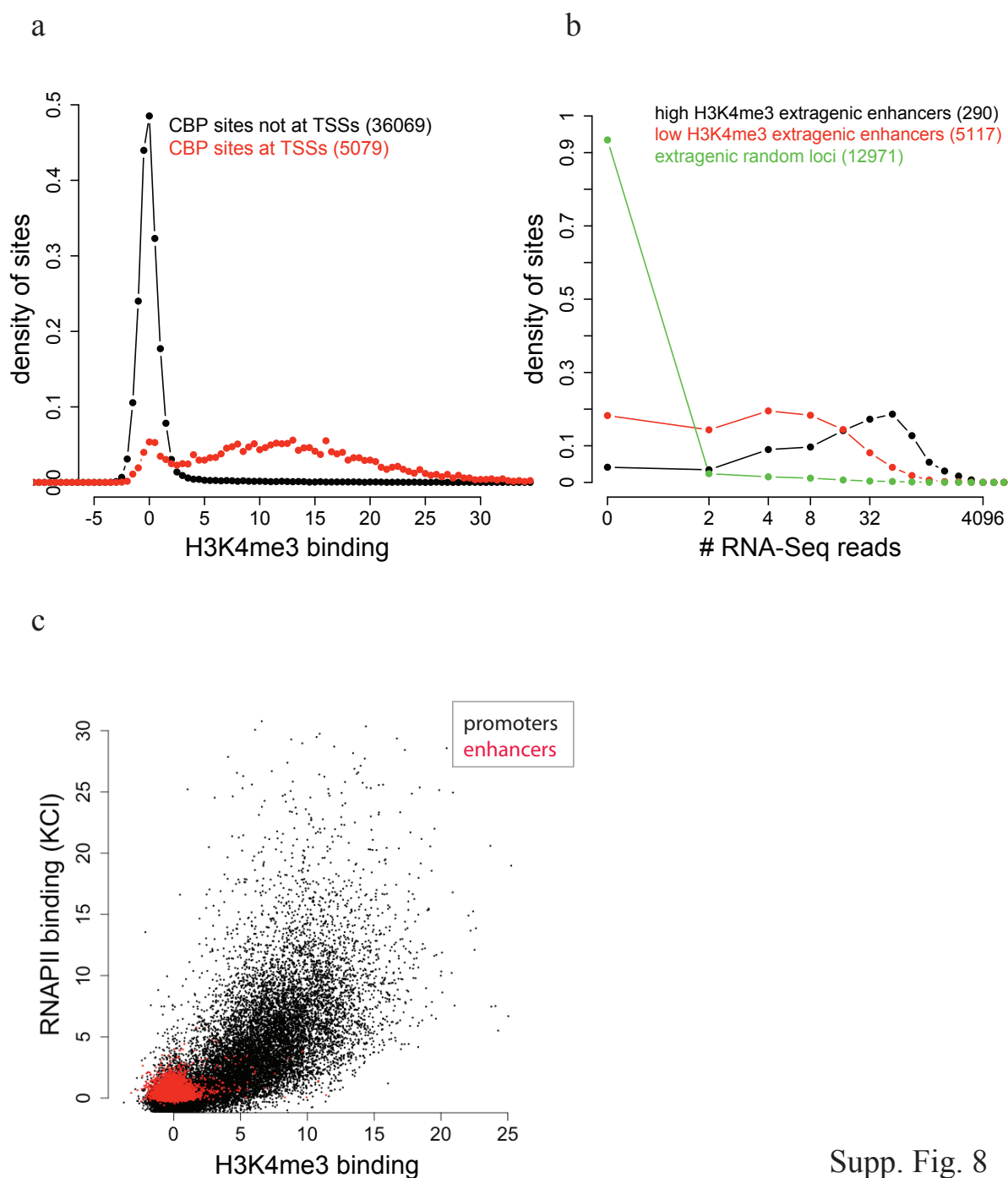
Supplementary Figure 5. ChIP-qPCR validation of randomly chosen CREB, SRF, and CBP binding sites. Blue and red bars represent qPCR signal from immunoprecipitated DNA over input DNA from 0 and 2 hour KCl-treated neurons, respectively. Labels e16-e31 designate the binding sites identified in our ChIP-Seq experiments (Methods, Supplementary Table 10). Two negative regions were chosen for an absence of binding in ChIP-Seq experiments. Error bars, s.e.m. (n=4 biological replicates).



Supplementary Figure 6. eRNA Validation after DNase I treatment. eRNA inducibility and detectability as measured by RT-qPCR using random hexamer-primed reverse transcription. Candidate eRNA transcripts were selected randomly with the exceptions of the *c-fos* and *arc* enhancers. In the inducibility plot, histone mRNA transcript whose expression is not induced by membrane-depolarization was included as a negative control. + and – refer to regions upstream (–) or downstream (+) of the CBP-bound enhancer center, where upstream is defined as 3' on the – genomic strand and downstream is defined as 3' on the + genomic strand. For the enhancers e7, e10, e13 and e15, only the downstream regions (+) were tested for the presence of eRNA. In the detectability plot, blue bars indicate qPCR signal of each eRNA transcript relative to a genomic DNA standard used across all samples. Thus, the values in y-axis represent a relative measure of gene expression. The red bars indicate RT- controls in which the RT reaction lacked the RT enzyme. All tested eRNAs were detectable significantly above the mean background level observed in the negative regions (1-3) based on a *t*-test. Error bars, s.e.m. (n=3 biological replicates)

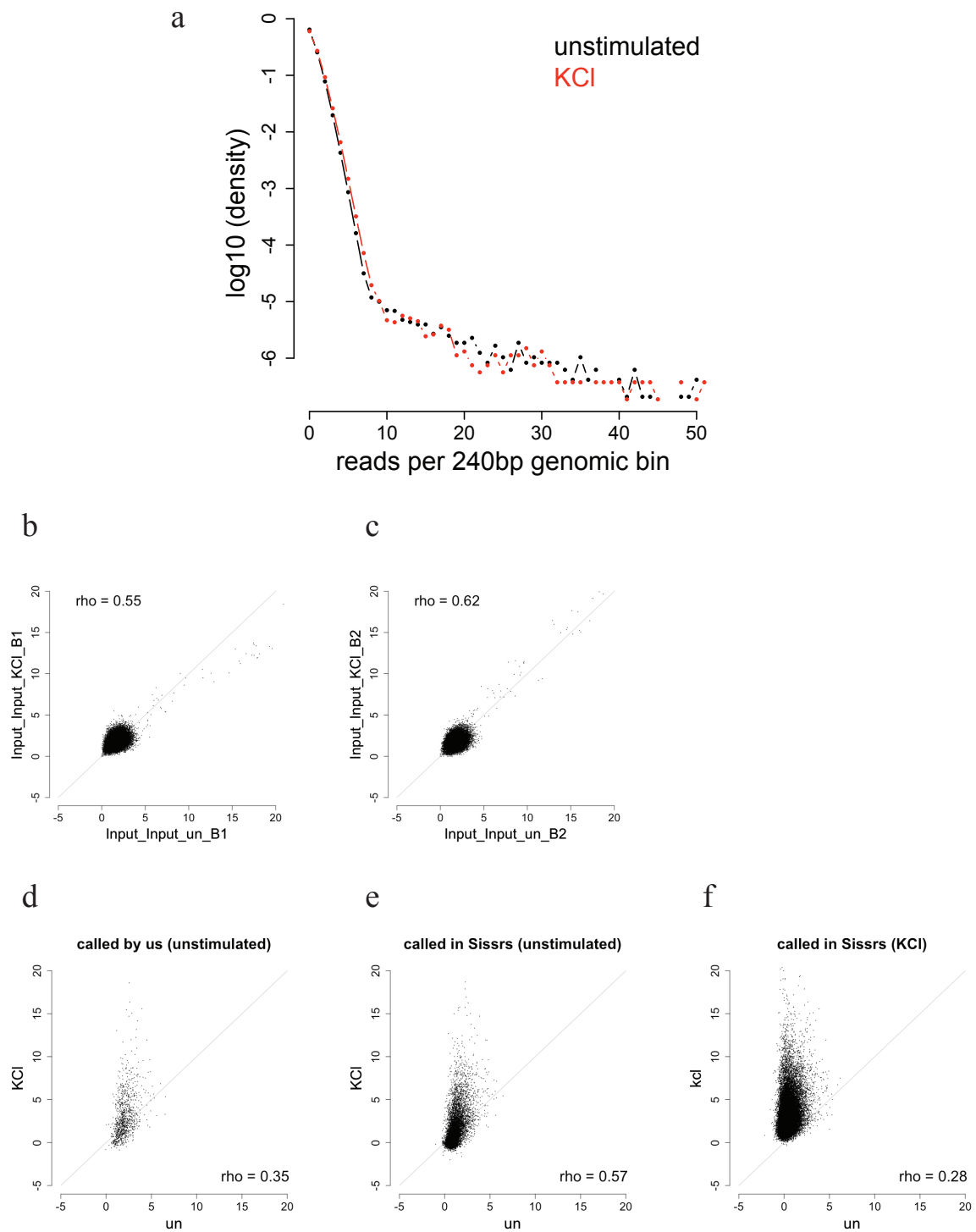


Supplementary Figure 7. Transcription at enhancers appears to initiate from the CBP-bound enhancer center. **a**, Schematic illustration of convergent and divergent reads with respect to a given locus. “Transcriptional noise” resulting from transcription initiated at other loci is predicted to result in equal numbers of divergent and convergent reads with respect to enhancer centers or TSSs. Transcription initiated from a CBP-bound enhancer center or TSS will be mostly divergent. **b**, Enhancers have more divergent than convergent reads, which is consistent with the notion that they initiate transcription. Only extragenic enhancers and random regions were included in this analysis. **c**, The average induction level for genes associated with various sets of enhancers.



Supp. Fig. 8

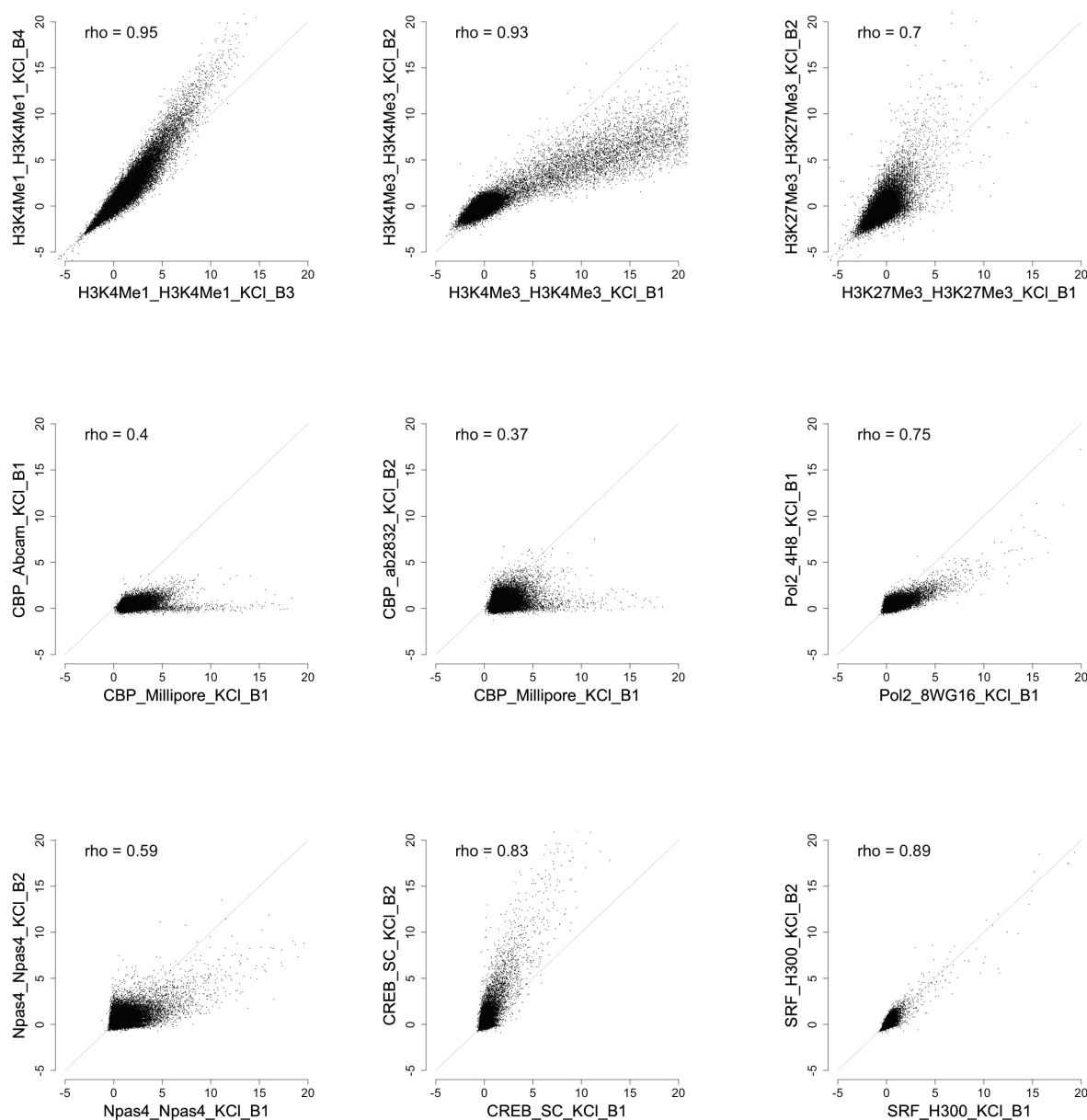
Supplementary Figure 8. Characterization of H3K4me3 detected at enhancers. **a**, Binding profiles of H3K4me3 near CBP (Millipore) peaks (Supp. Tbl 5). The CBP peaks found near RefSeq, Ensembl, or UCSC-annotated TSSs (red) exhibit a bimodal distribution of H3k4me3 levels, whereas most of the other CBP sites exhibit low levels of H3K4me3 binding. The cut-off for separating high and low H3Kme3 peaks at enhancers is defined as the local minimum between the two modes of the red curve found at $x = 2$. **b**, The distribution of the numbers of RNA-Seq reads found within 1.5 kb of random loci (green) or extragenic enhancers with low (red) or high (black) levels of H3K4me3, as in Fig. 5c. **c**, Levels of H3K4me3 and RNAPII binding at promoters. Each black dot represents a TSS, and each red dot represents one of 5,117 extragenic enhancers with low H3K4me3 or one of 290 extragenic enhancers with high H3K4me3. The anti-RNAPII antibody used was 8WG16.



Supplementary Figure 9. Characterization of the input control and CBP peak finding

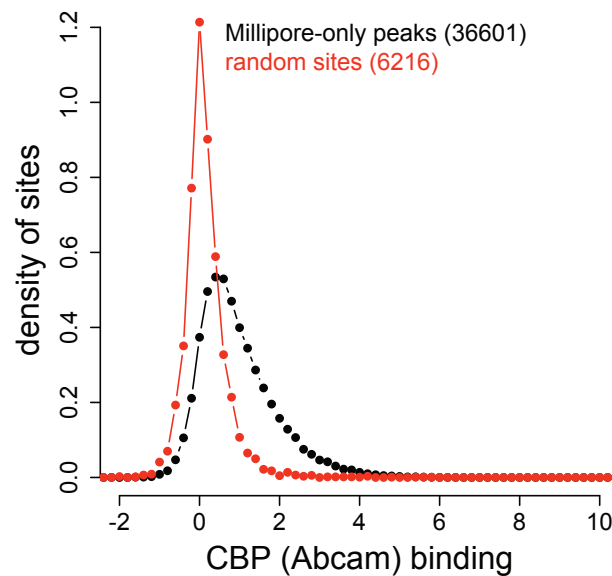
using Sissrs. **a**, The distributions of input ChIP-Seq read numbers from unstimulated and KCI-treated neurons are very close to a Poisson distribution with deviations appearing only in 240 bp bins containing more than 10 reads. **b-c**, The number of input reads found near the 41,148 CBP peaks before and after membrane depolarization for two biological replicates (B1, B2). **d**, Levels of CBP before and after membrane depolarization at the 1,100 CBP peaks detected in the unstimulated condition using our peak finding method on the Millipore CBP data (Methods, Supp. Tbl 5). (These peaks were not included on our enhancer list unless they were

independently detected in the membrane-depolarized condition). **e**, Levels of CBP before and after membrane depolarization at the 8,980 CBP peaks detected in the unstimulated condition (Millipore) using Sissrs. **f**, Levels of CBP before and after membrane depolarization at the 32,656 CBP peaks detected in the stimulated condition (Millipore) using Sissrs. In all three cases for **d-f**, it is clear that the peaks are strongly inducible (e.g., compare to CREB and SRF in Supplementary Figure 2).

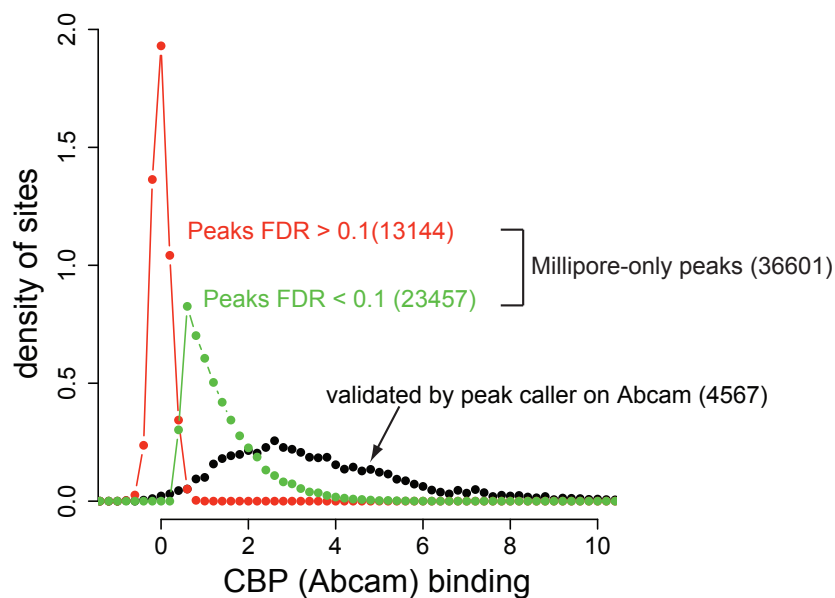


Supplementary Figure 10. Biological replicates of ChIP-Seq experiments. Each dot represents the normalized number of reads found near one of the 41,148 CBP peaks for each of the biological replicates. Rho is the Spearman correlation. The axes represent normalized, input-subtracted ChIP-Seq read counts. BN refers to biological replicate *N*.

a



b



Supplementary Figure 11. Reproducibility of CBP peaks. **a**, CBP binding levels determined by ChIP-Seq with the Abcam CBP antibody at 36,601 loci, which were initially defined as CBP binding sites by the Millipore CBP antibody but that were not replicated by peak-calling on the Abcam CBP antibody (black). Many of these loci are still significantly enriched compared to 6,216 random regions (red), suggesting that they are *bona fide* CBP binding sites but were not called by our peak calling method (from the Abcam data) due to insufficient sequencing depth (Methods). **b**, Using a 0.1 FDR for the distribution in (a), 23,457 out of 36,601 CBP Millipore-only peaks are statistically validated, and the figure shows the distribution of the level of Abcam binding for the different peak categories.