

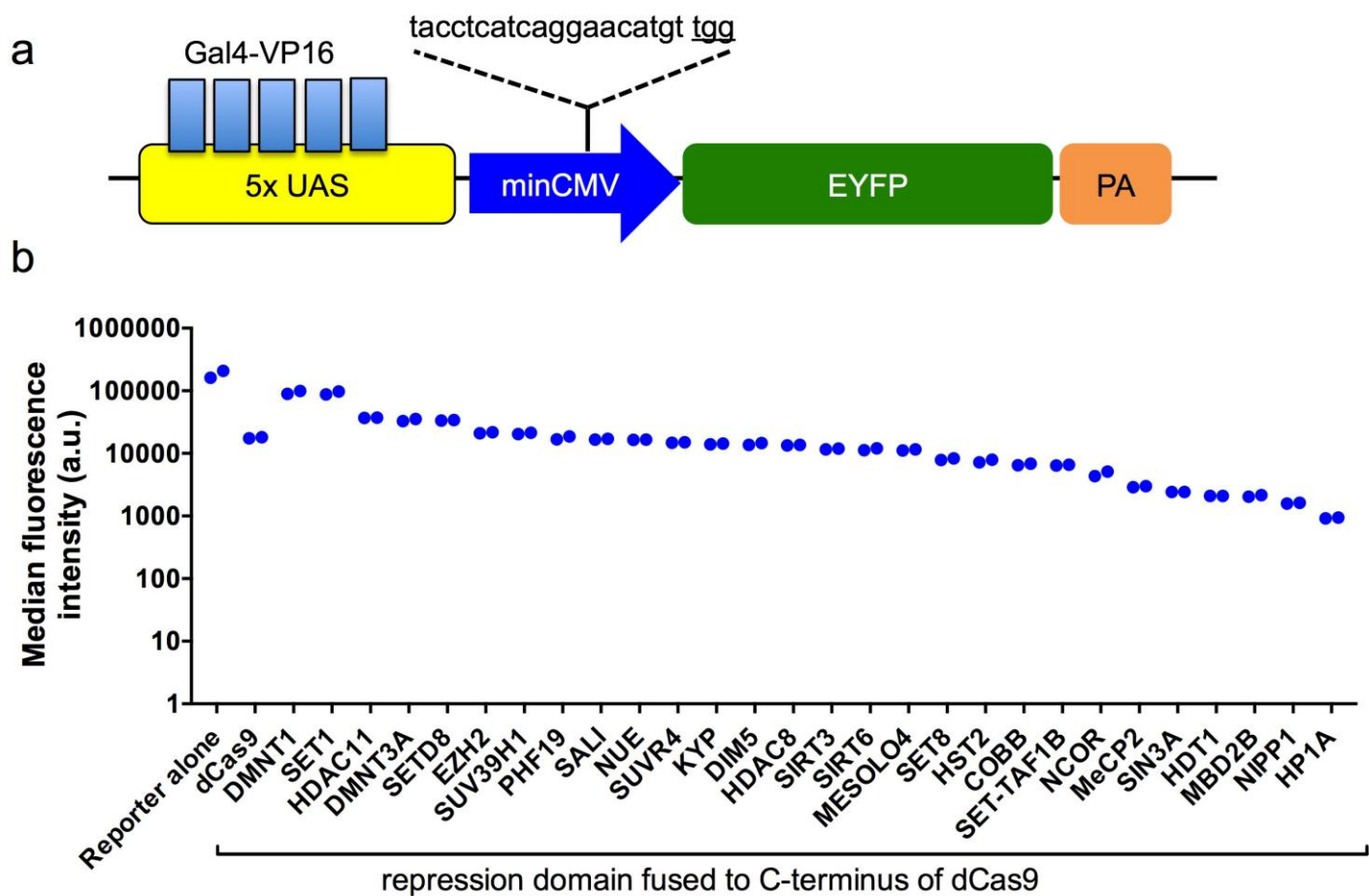
In the format provided by the authors and unedited.

An enhanced CRISPR repressor for targeted mammalian gene regulation

Nan Cher Yeo ^{1,2,14}, Alejandro Chavez ^{3,14*}, Alissa Lance-Byrne¹, Yingleong Chan^{1,2}, David Menn⁴, Denitsa Milanova^{1,2}, Chih-Chung Kuo^{5,6}, Xiaoge Guo^{1,2}, Sumana Sharma⁷, Angela Tung¹, Ryan J. Cecchi¹, Marcelle Tuttle¹, Swechchha Pradhan⁴, Elaine T. Lim^{1,2}, Noah Davidsohn^{1,2}, Mo R. Ebrahimkhani^{4,8}, James J. Collins^{1,9,10,11,12}, Nathan E. Lewis ^{5,6,13}, Samira Kiani ^{4*} and George M. Church ^{1,2*}

¹Wyss Institute for Biologically Inspired Engineering, Harvard University, Cambridge, MA, USA. ²Department of Genetics, Harvard Medical School, Boston, MA, USA. ³Department of Pathology and Cell Biology, Columbia University College of Physicians and Surgeons, New York, NY, USA. ⁴School of Biological and Health Systems Engineering, Ira A. Fulton Schools of Engineering, Arizona State University, Tempe, AZ, USA. ⁵Department of Bioengineering, University of California, San Diego, San Diego, CA, USA. ⁶Novo Nordisk Foundation Center for Biosustainability, University of California, San Diego, San Diego, CA, USA. ⁷Cell Surface Signalling Laboratory, Wellcome Trust Sanger Institute, Cambridge, UK. ⁸Division of Gastroenterology and Hematology, Mayo Clinic College of Medicine and Science, Phoenix, AZ, USA. ⁹Institute for Medical Engineering & Science, Massachusetts Institute of Technology, Cambridge, MA, USA. ¹⁰Synthetic Biology Center, Massachusetts Institute of Technology, Cambridge, MA, USA. ¹¹Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA. ¹²Broad Institute of MIT and Harvard, Cambridge, MA, USA. ¹³Department of Pediatrics, University of California, San Diego, San Diego, CA, USA. ¹⁴These authors contributed equally: Nan Cher Yeo, Alejandro Chavez.

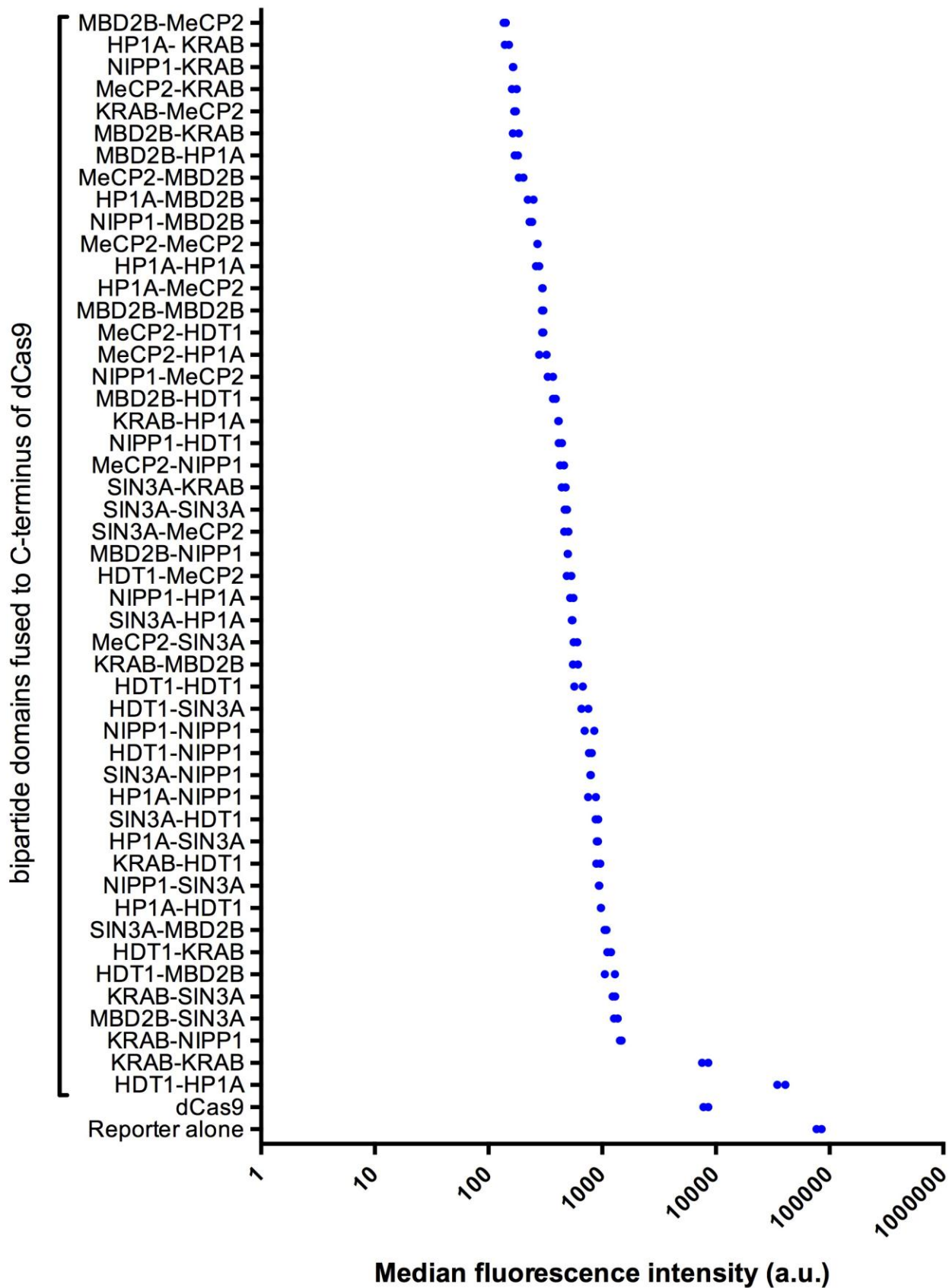
*e-mail: ac4304@cumc.columbia.edu; samira.Kiani@asu.edu; gchurch@genetics.med.harvard.edu



Supplementary Figure 1

Targeted screen to identify repression domains that function with dCas9.

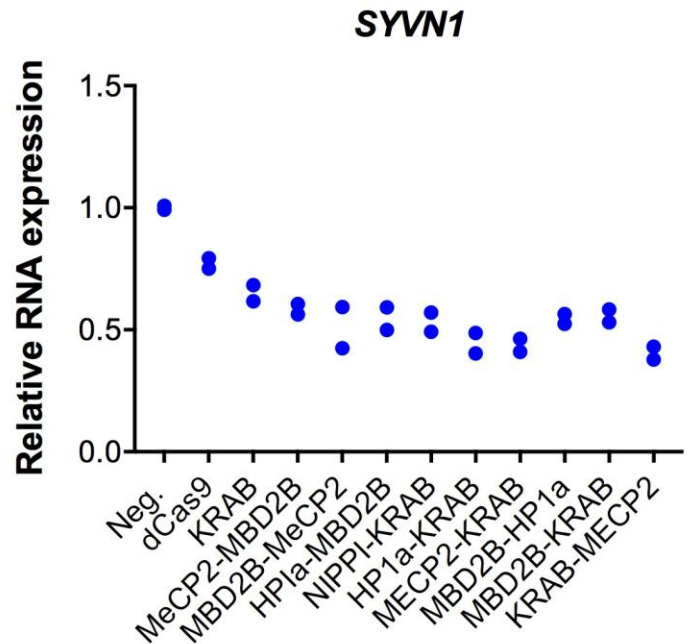
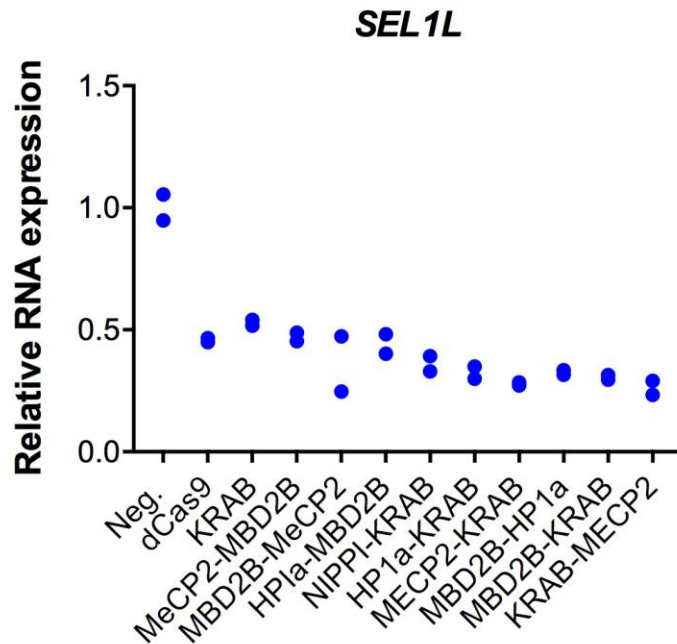
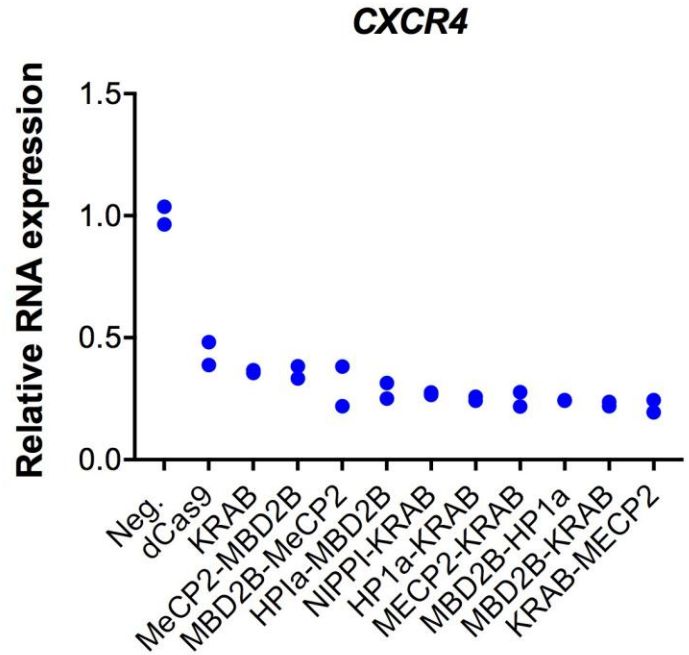
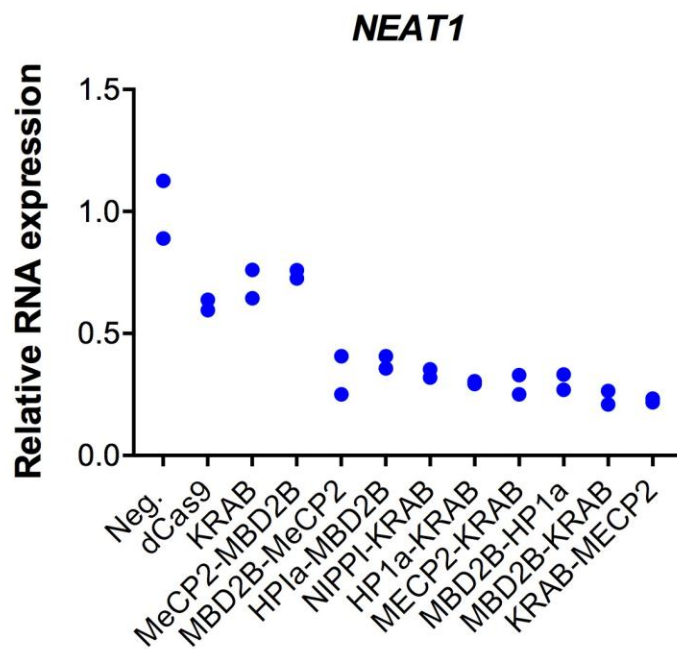
(a) Schematic of the EYFP fluorescent reporter construct used in the targeted screen. A protospacer sequence followed by TGG PAM is placed within the minimal CMV (minCMV) promoter upstream of the EYFP reporter gene. Transcription of the EYFP reporter is driven upon binding of a GAL4-VP16 protein to the UAS sequences present upstream of the minCMV promoter. (b) The fluorescent reporter and sgRNA were co-transfected with the indicated repressors into HEK293T cells. At 2 days post-transfection, EYFP fluorescence levels were measured to quantify the amount of repression from the various dCas9 fusion proteins. The name of the protein from which the transcriptional regulatory domain was isolated is listed on the x-axis. All domains were fused to the C-terminus of the dCas9 protein. KRAB and the six top-performing domains were used for subsequent engineering. $n=2$ biologically independent samples (cell cultures).



Supplementary Figure 2

Reporter screen of 49 dCas9 bipartite repressors.

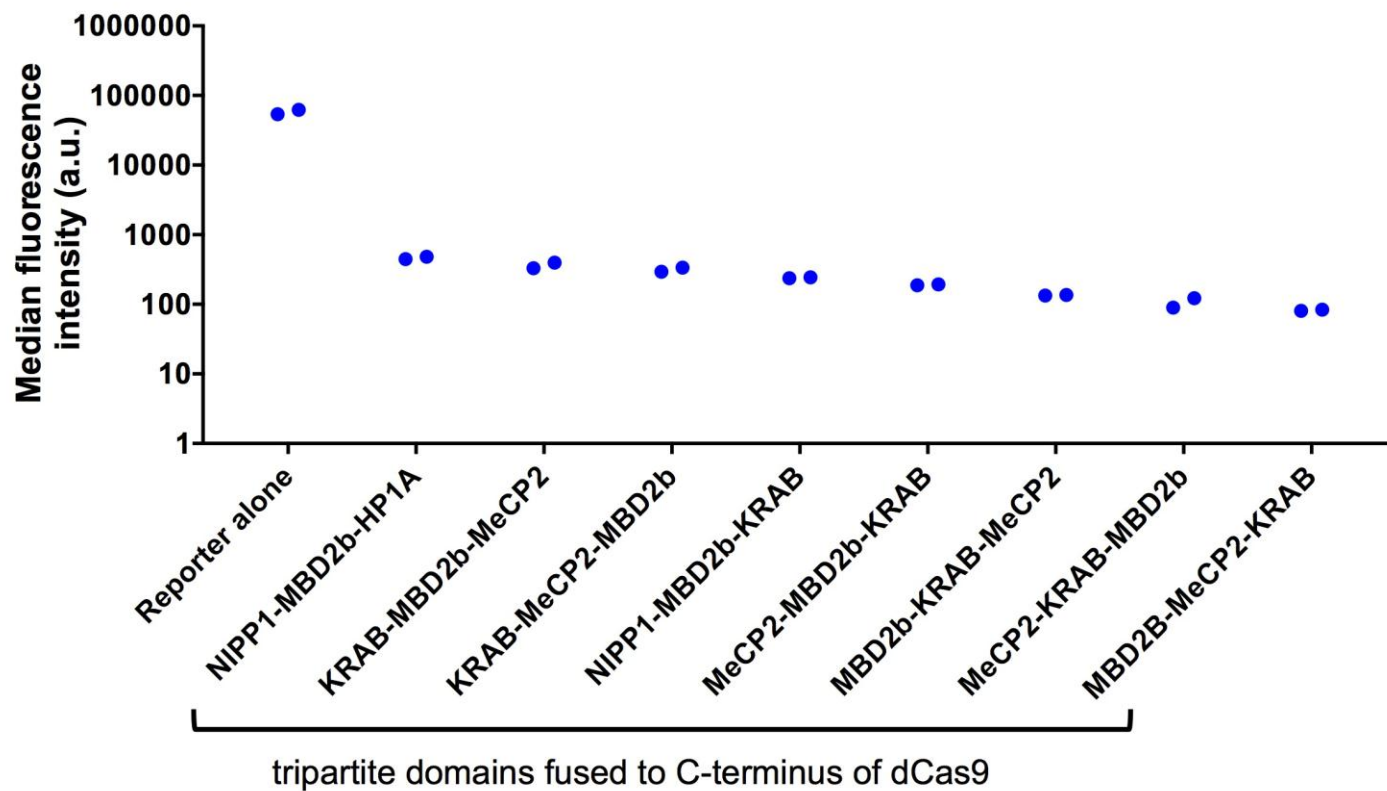
A series of all pairwise repeating and non-repeating bipartite repressors were generated using KRAB and the six top-performing domains identified from the initial screen (MeCP2, SIN3A, HDT1, MBD2B, NIPP1, and HP1a). Each repressor was tested using the same fluorescent reporter assay as in Supplementary Figure 1. n=2 biologically independent samples (cell cultures).



Supplementary Figure 3

Repression of endogenous genes using top bipartite fusions.

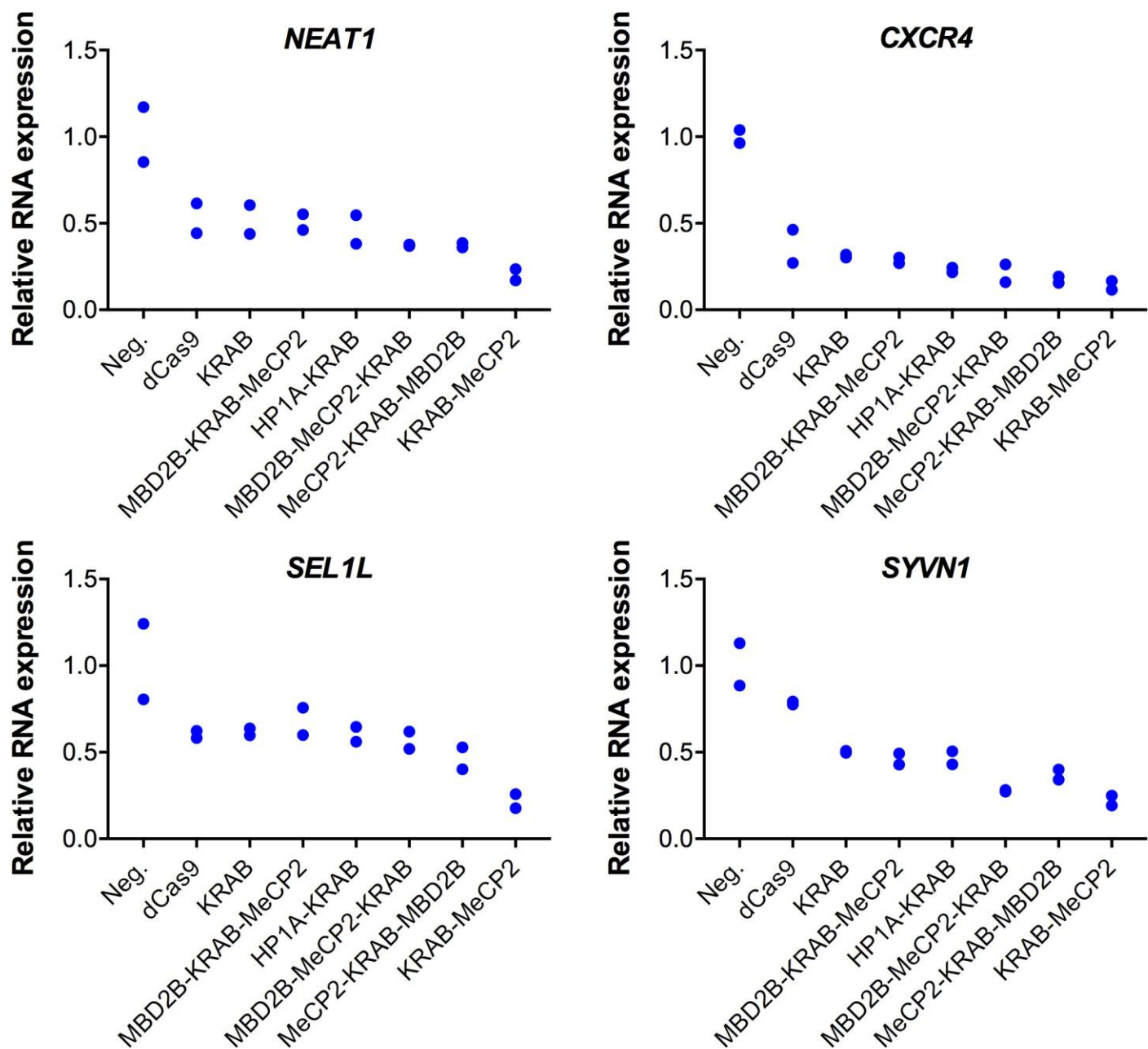
HEK293T cells were co-transfected with gRNAs against the indicated four genes simultaneously with the labeled repressors. Samples were collected for RNA extraction at 4 days post-transfection. The expression levels of targeted genes were measured by RT-qPCR. n=2 biologically independent samples (cell cultures).



Supplementary Figure 4

Reporter screen of eight dCas9 tripartite repressors.

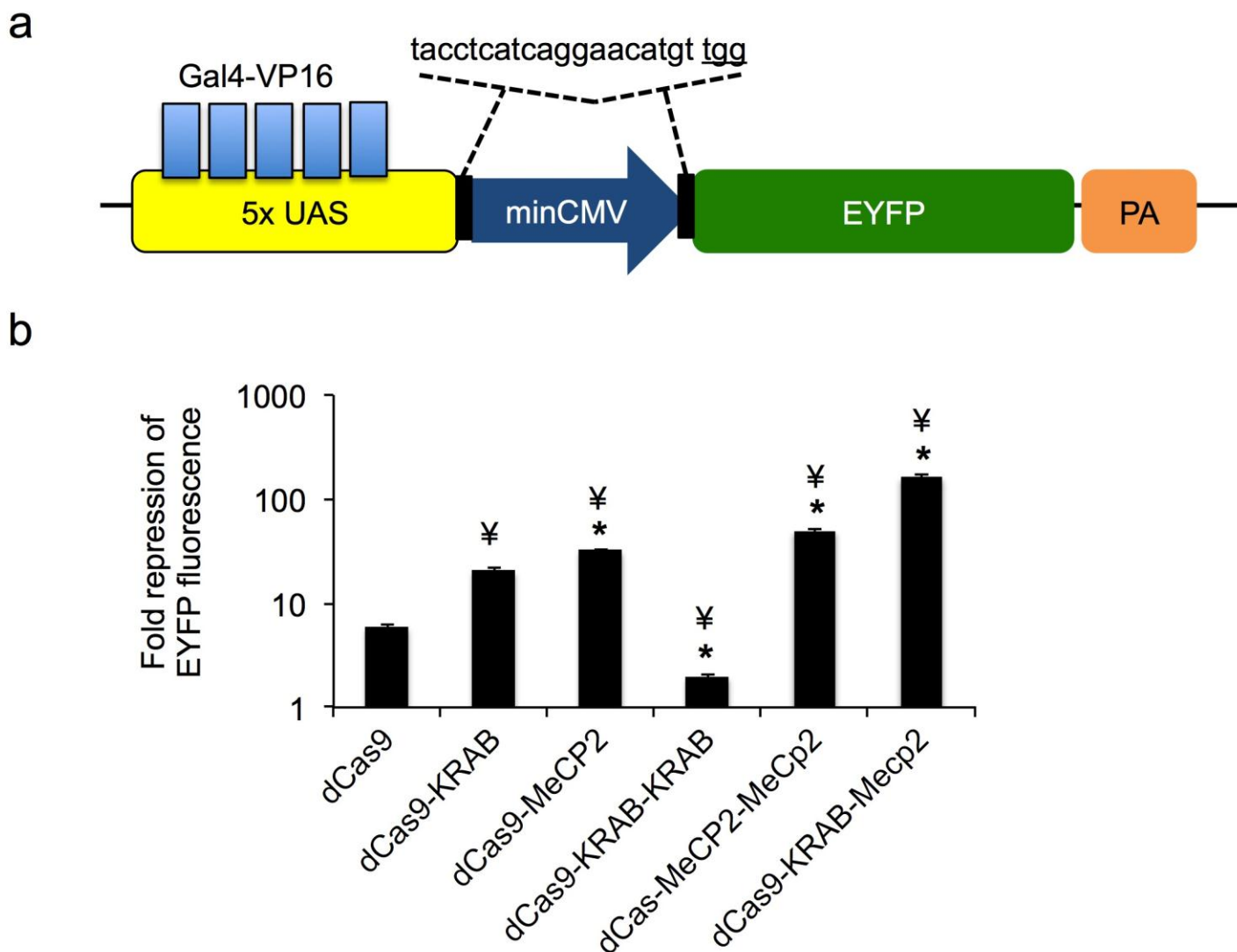
A series of rationally designed tripartite repressors were generated based on the top-performing bipartite repression domains. Each repressor was tested using the same fluorescent reporter assay as in Supplementary Figure 1. n=2 biologically independent samples (cell cultures).



Supplementary Figure 5

Repression of endogenous genes with the top tripartite fusions compared with that observed with the bipartite KRAB-MeCP2 repressor.

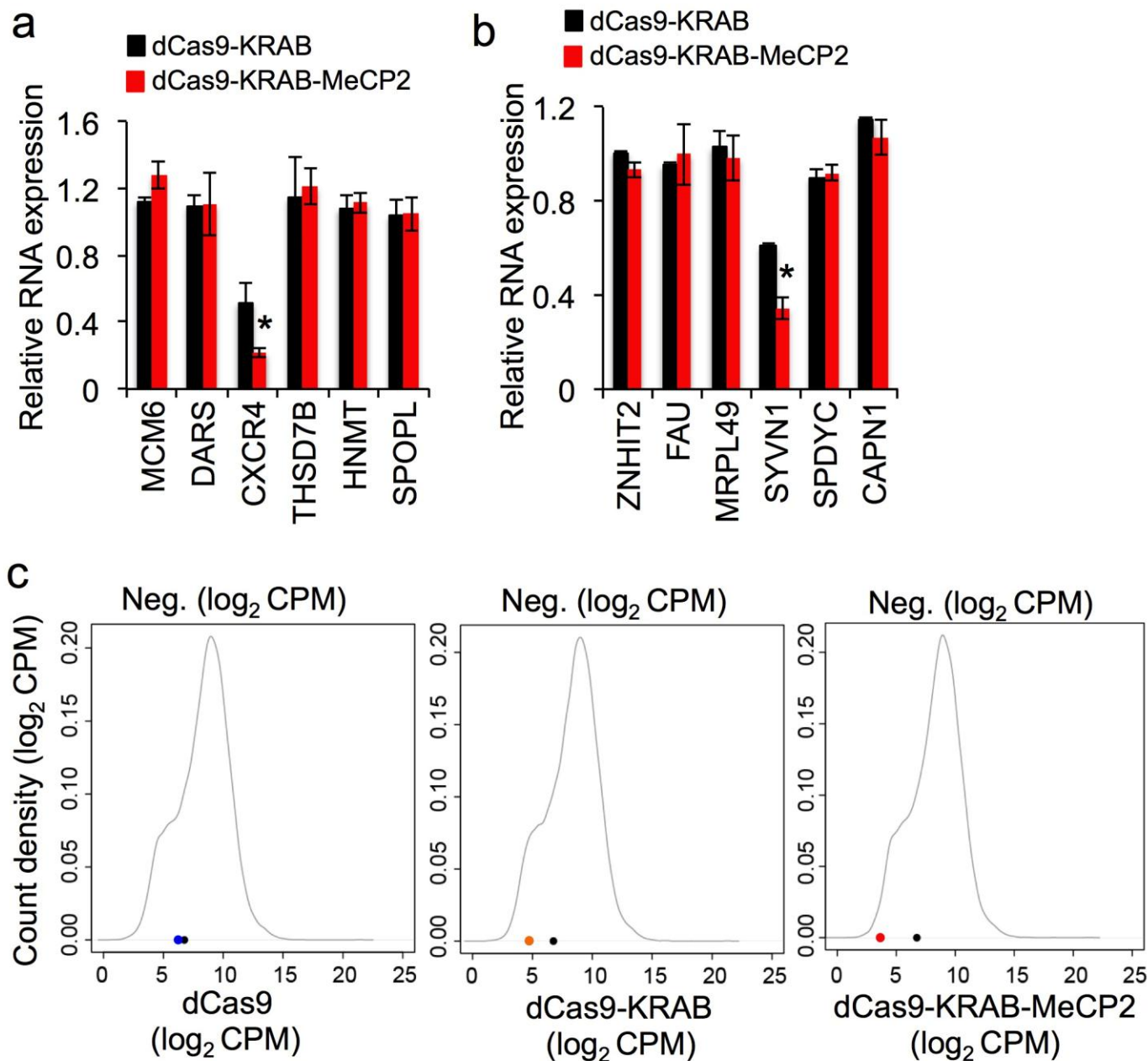
The three best performing tripartite repressors based on the reporter screen were selected and tested against a series of endogenous loci, along with HP1A-KRAB and KRAB-MeCP2. RNA levels of the four indicated genes from a multiplexed repression experiment were measured using RT-qPCR. n=2 biologically independent samples (cell cultures).



Supplementary Figure 6

Reporter screen of different dCas9 fusion proteins containing KRAB or MeCP2.

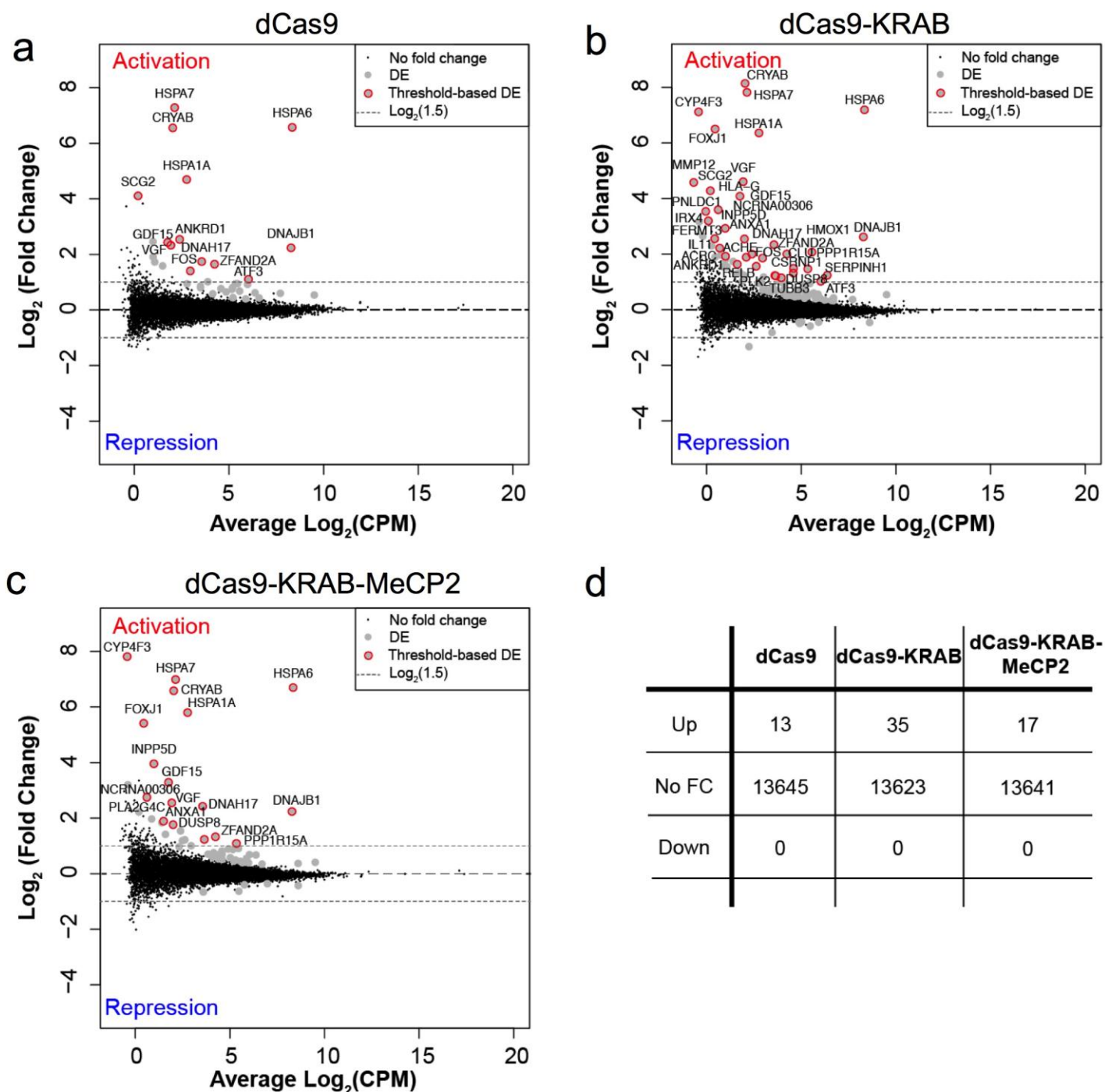
(a) Schematic of CRP repression device. dCas9 repressors coupled with a U6-driven gRNA regulate EYFP output through binding two targets sites within the CRP promoter. (b) dCas9-KRAB-MeCP2 outperformed either KRAB or MeCP2 either as single or double fusions to dCas9 when paired with sgRNA in repressing EYFP output. Data are presented as mean $\pm$ s.e.m. $n=3$ biologically independent samples (cell cultures). One-sided Student's T-test was used for statistical comparison. ¥ indicates $p < 0.05$ vs. dCas9, * indicates $p < 0.05$ vs. dCas9-KRAB.



Supplementary Figure 7

dCas9-KRAB-MeCP2-mediated repression is highly specific in human cells.

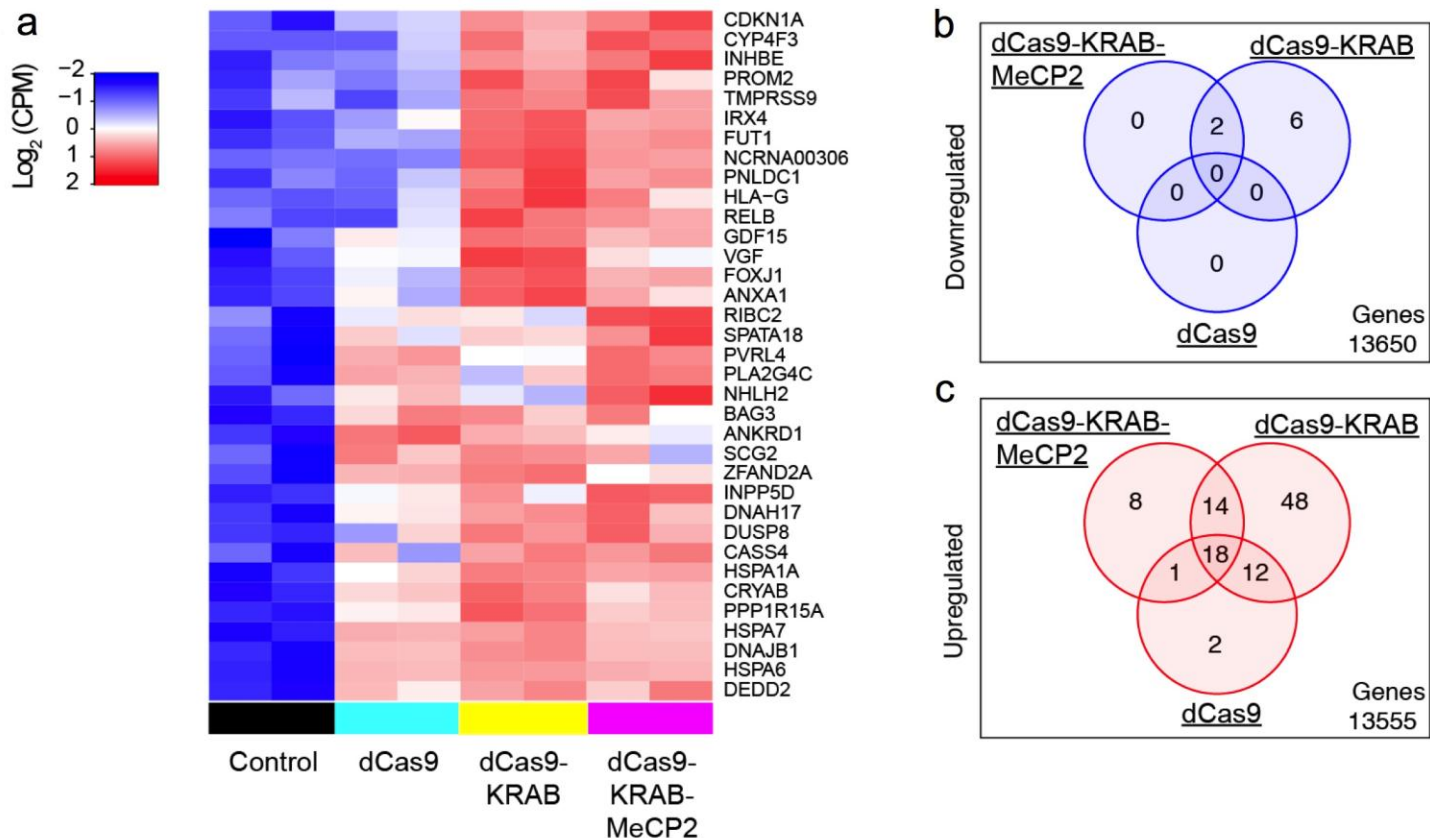
(a) HEK293T cells were transfected with a gRNA targeting the *CXCR4* gene along with the indicated dCas9 repressor. Expression of the target gene *CXCR4* and several nearest neighboring genes, including *MCM6*, *DARS*, *THSD7B*, *HNMT*, and *SPOPL*, were examined. These genes are located at approximately -274, -208, 651, 1849, and 2387 kb, respectively, away from *CXCR4*. (b) Expression of the target gene *SYVN1* and several nearest neighboring genes, including *ZNHIT2*, *FAU*, *MRPL49*, *SPDYC*, and *CAPN1*, were examined. These genes are located at approximately -10, -6, -5, 42, and 53 kb, respectively, away from *SYVN1*. For a-b, data are shown as mean $\pm$ s.e.m. n=4 biologically independent samples (cell cultures). One-sided Student T-test was used to perform statistical comparison. * $p < 0.05$ v.s. dCas9-KRAB. (c) RNA-seq analysis of HEK293T cells transfected with gRNA targeting *CXCR4* along with dCas9, dCas9-KRAB or dCas9-KRAB-MeCP2. Shown are density plots indicating the fold changes in gene expression from different groups. *CXCR4* expression is indicated with blue, orange, or red dot and control is indicated with a black dot. n=2 biologically independent samples (cell cultures).



Supplementary Figure 8

Differentially expressed (DE) genes in an RNA-seq experiment.

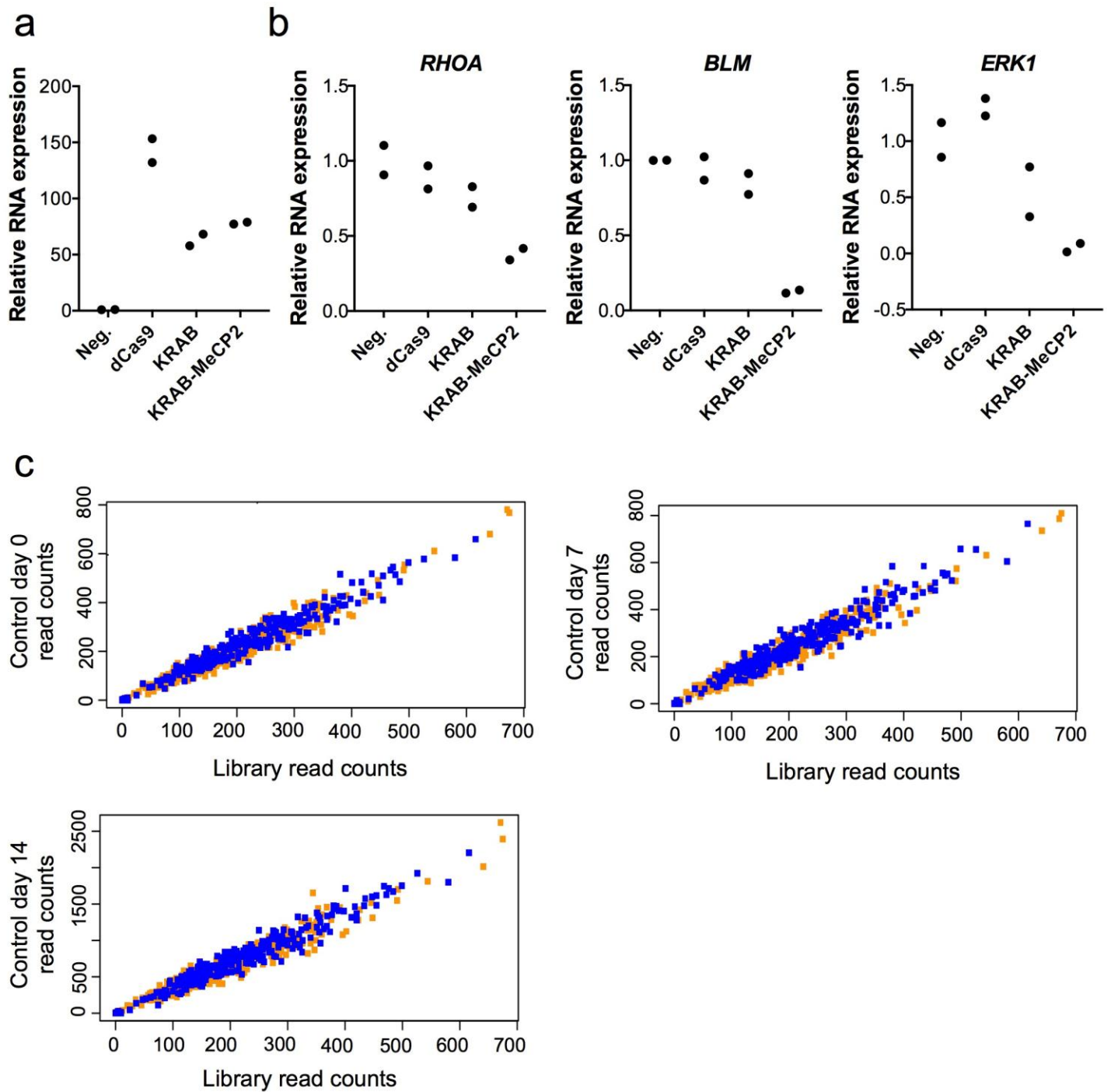
(a-c) Shown are log₂ fold change (FC) versus average log₂ count per millions (CPM) for dCas9, dCas9-KRAB and dCas9-KRAB-MeCP2 relative to the negative control. Genes with no fold change are marked in black, DE genes are marked in grey, and DE genes above log₂ FC of 1.5 threshold are marked in red. Positive log₂ FC represents transcriptional activation while negative values indicate repression. (d) Shown is a summary of the number of DE genes showing up- or down-regulation with log₂ FC > 1.5 in different groups. (Methods associated with this figure are described in Supplementary Note 2)



Supplementary Figure 9

Cluster analysis of the top 35 DE genes in negative control, dCas9, dCas9–KRAB, and dCas9–KRAB–MeCP2 groups.

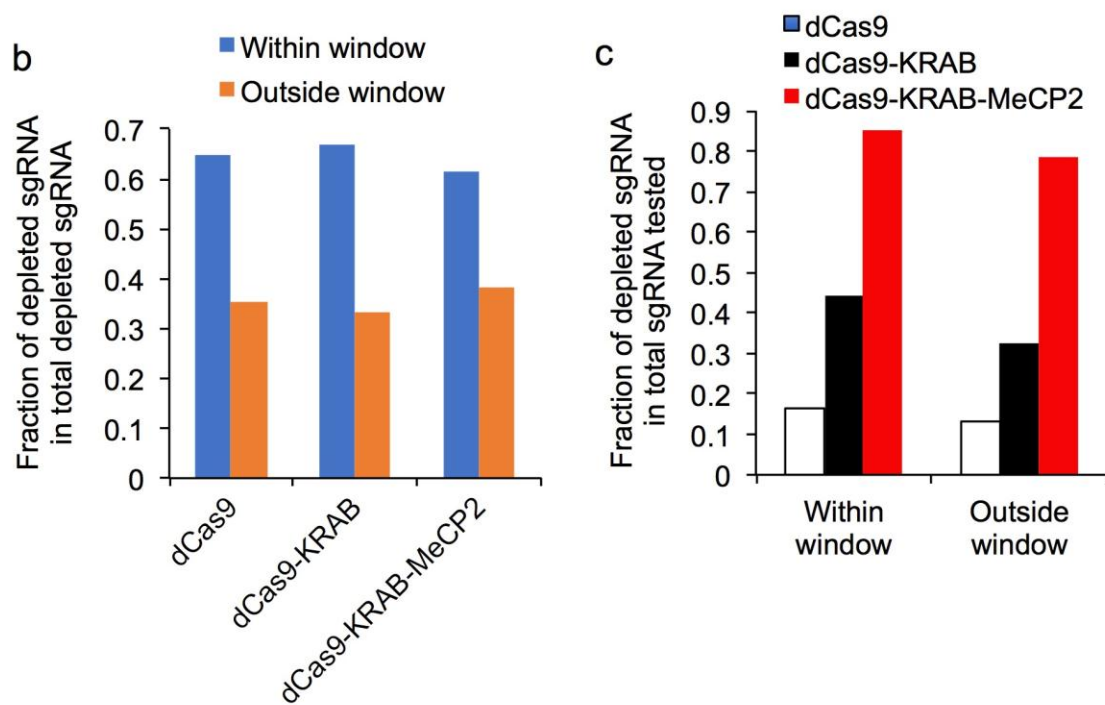
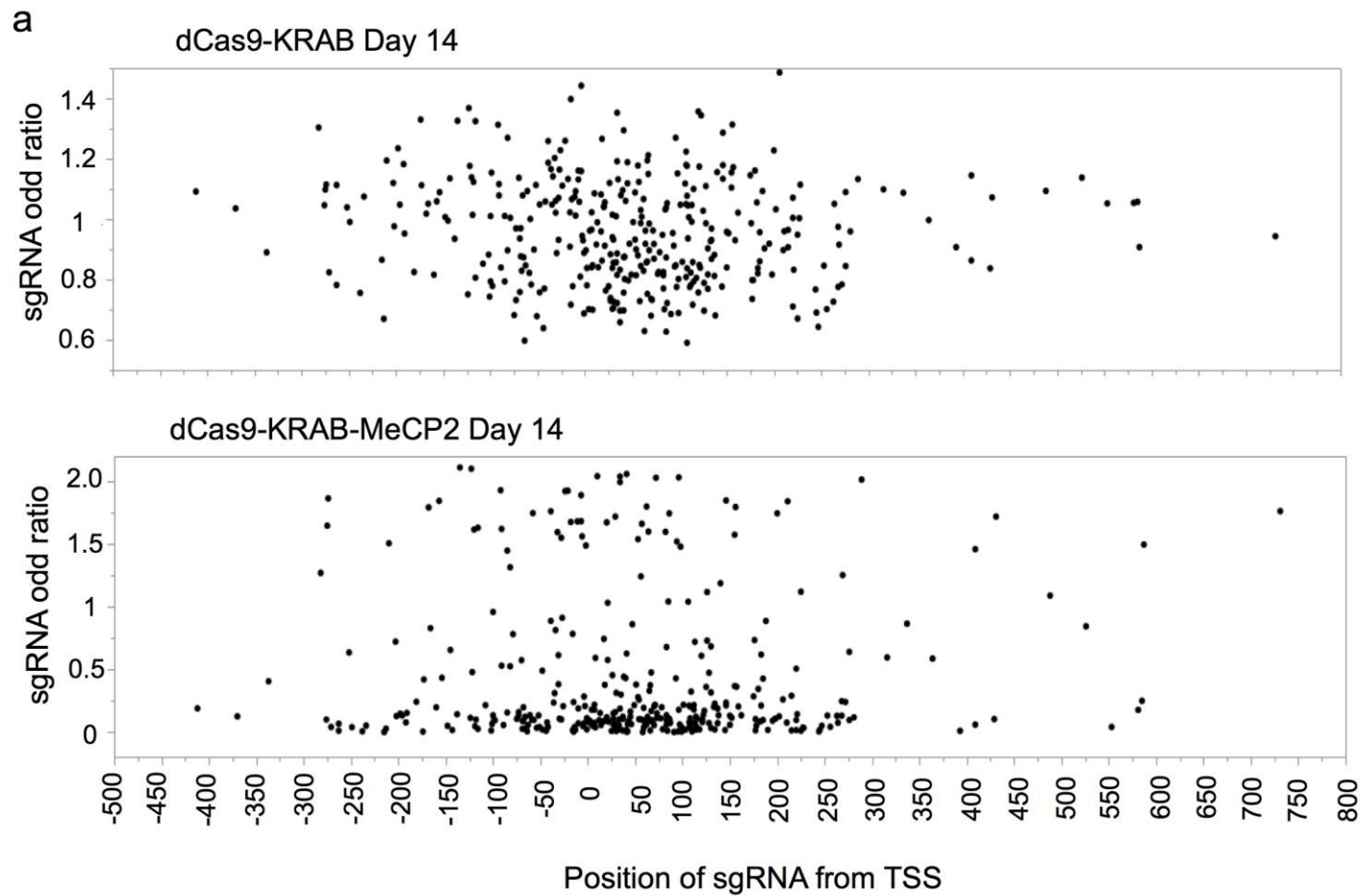
a) Clustering of genes with correlated expression provides insights into the biological effects of repressor's activity. Heatmap representing distances between each gene pair is calculated based on Euclidean distance, $(1-R)/2$ where R represents the Pearson's correlation of two genes. A scale key bar of normalized log₂ CPM shows large negative (colored in blue) and positive (colored in red) correlations. Genes with large positive correlations correspond to small Euclidean distances and cluster together. (b-c) Shown are Venn diagrams comparing transcriptome-wide downregulated or upregulated genes among the different repressors. (Methods associated with this figure are described in Supplementary Note 2)



Supplementary Figure 10

HAP1 cells stably expressing dCas9 repressors.

(a) Shown is the expression level of dCas9 repressors in the different stable cell lines. n=2 biologically independent samples (cell cultures). (b) Stable cells were transduced with lentiviruses containing a gRNA targeting the indicated genes. dCas9-KRAB-MeCP2-containing cell line induced stronger suppression of most target genes. For a-b, n=2 biologically independent samples (cell cultures). (c) Shown are distributions of sgRNA constructs transduced into HAP1 wild-type cells over time. The sgRNA counts remained similar to that of the initial DNA library used to generate lentivirus. Blue squares represent non-essential gene-targeting constructs and orange squares represent essential gene-targeting constructs.



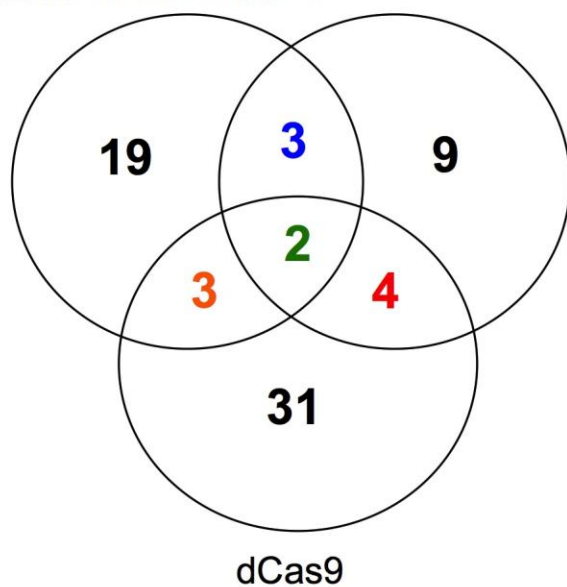
Supplementary Figure 11

dCas9–KRAB–MeCP2 outperformed dCas9–KRAB regardless of targeting position.

(a) Using the data from our pooled library screens, we plot sgRNA odd ratio (<1 indicates depletion) as a function of position from the transcription start site (TSS) for 370 essential gene-targeting sgRNAs. Shown are results from HAP1 cells using dCas9-KRAB or dCas9-KRAB-MeCP2 in the screen at day 14. (b) Of the total sgRNAs depleted by each repressor, we quantified the fraction of significantly depleted sgRNAs within or outside of the optimal window. sgRNAs located within the optimal targeting window showed a higher likelihood of being depleted as compared to sgRNAs positioned outside of the window across all repressors. (c) Of the total sgRNAs tested within or outside of the previously defined targeting window (-50bp to +200 bp from TSS), we quantified the fraction of significantly depleted sgRNAs when combined with the different repressors. dCas9-KRAB-MeCP2 showed the highest performance regardless of sgRNA targeting window. Similar results were observed in SH-SY5Y and HEK293T screens (figures not shown). See Supplementary Table 7 for a summary of the data in all three cell lines.

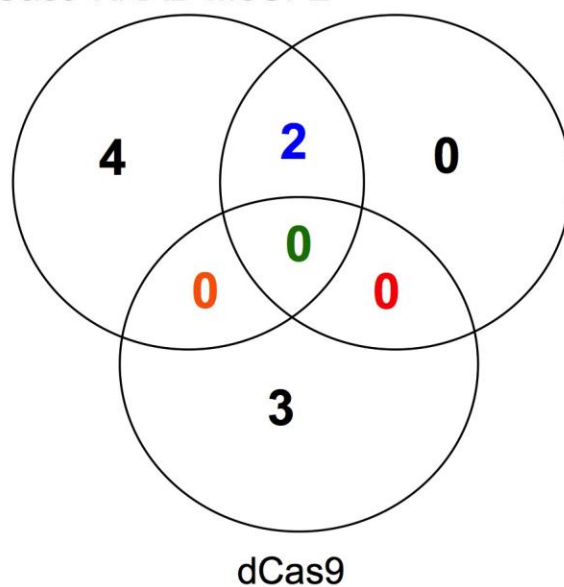
a HAP1 screen

dCas9-KRAB-MeCP2 dCas9-KRAB



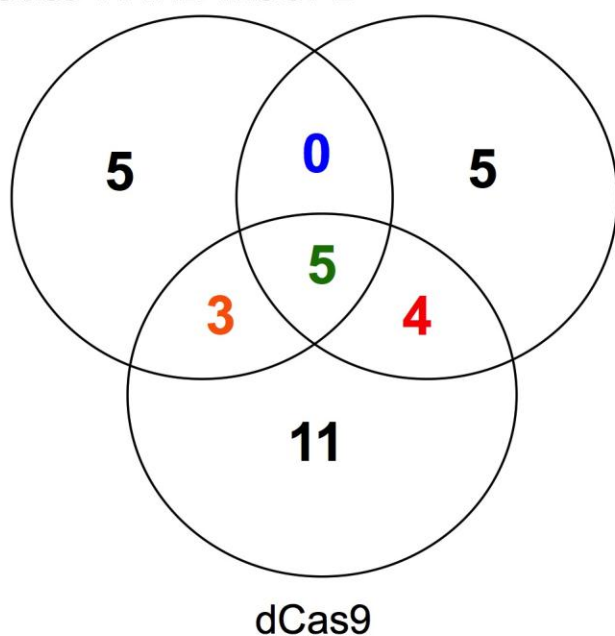
b SH-SY5Y screen

dCas9-KRAB-MeCP2 dCas9-KRAB



c HEK293T screen

dCas9-KRAB-MeCP2 dCas9-KRAB

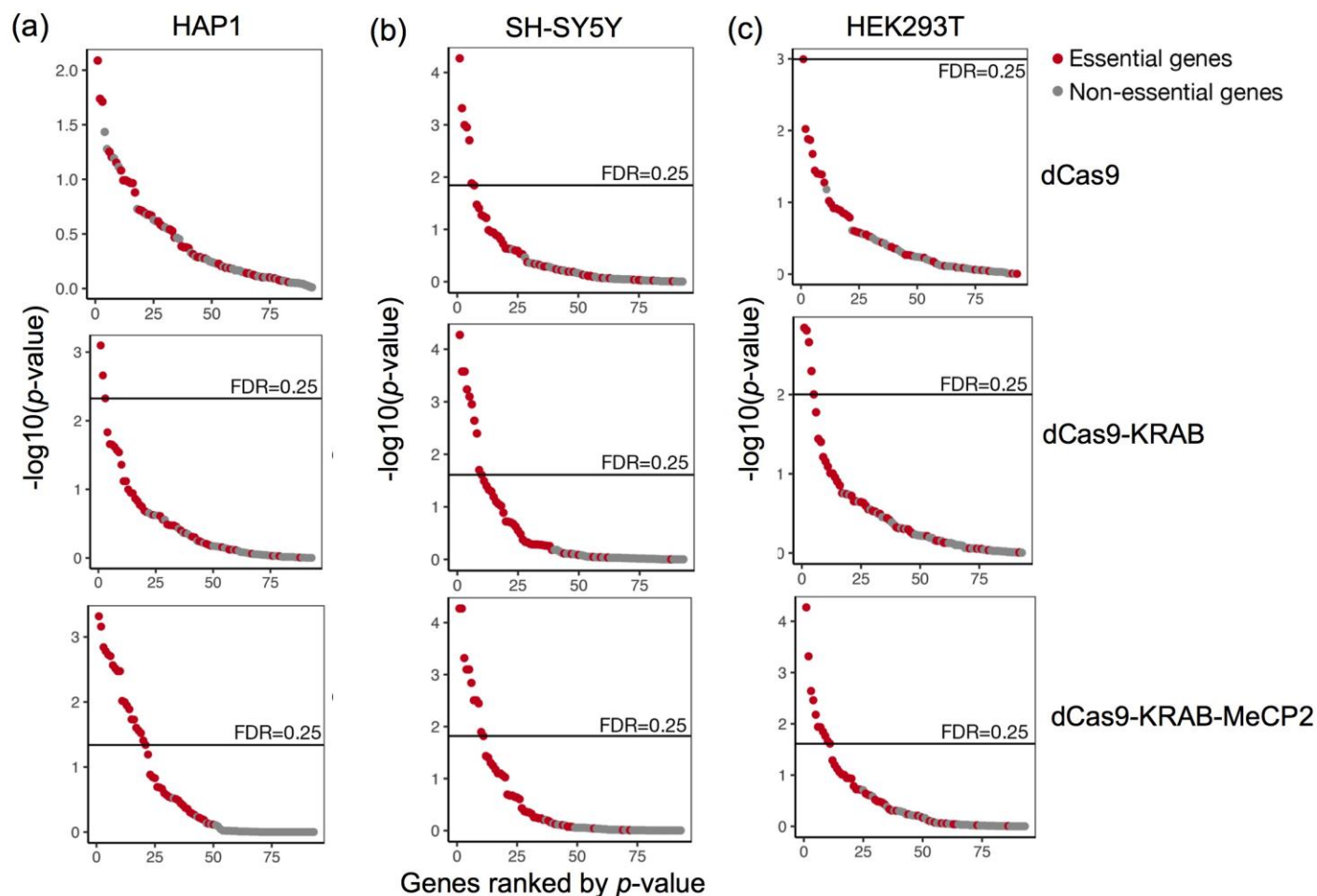


Supplementary Figure 12

Overlap of non-essential (NE) gene-targeting sgRNAs showing depletion in HAP1, SH-SY5Y, and HEK293T cells.

Venn diagrams show the numbers of NE-targeting sgRNAs exhibiting depletion that are unique to a repressor or common among repressors. (a) In HAP1 cells, PLA2G2E-19, sgRNA with the strongest depletion score, was depleted by both dCas9-KRAB-MeCP2 and dCas9-KRAB. Other top depleted sgRNAs seen in KRAB-MeCP2 screen, including HTR3D-6, LHX5-8, MRGPRD-2, and POU4F2-15,

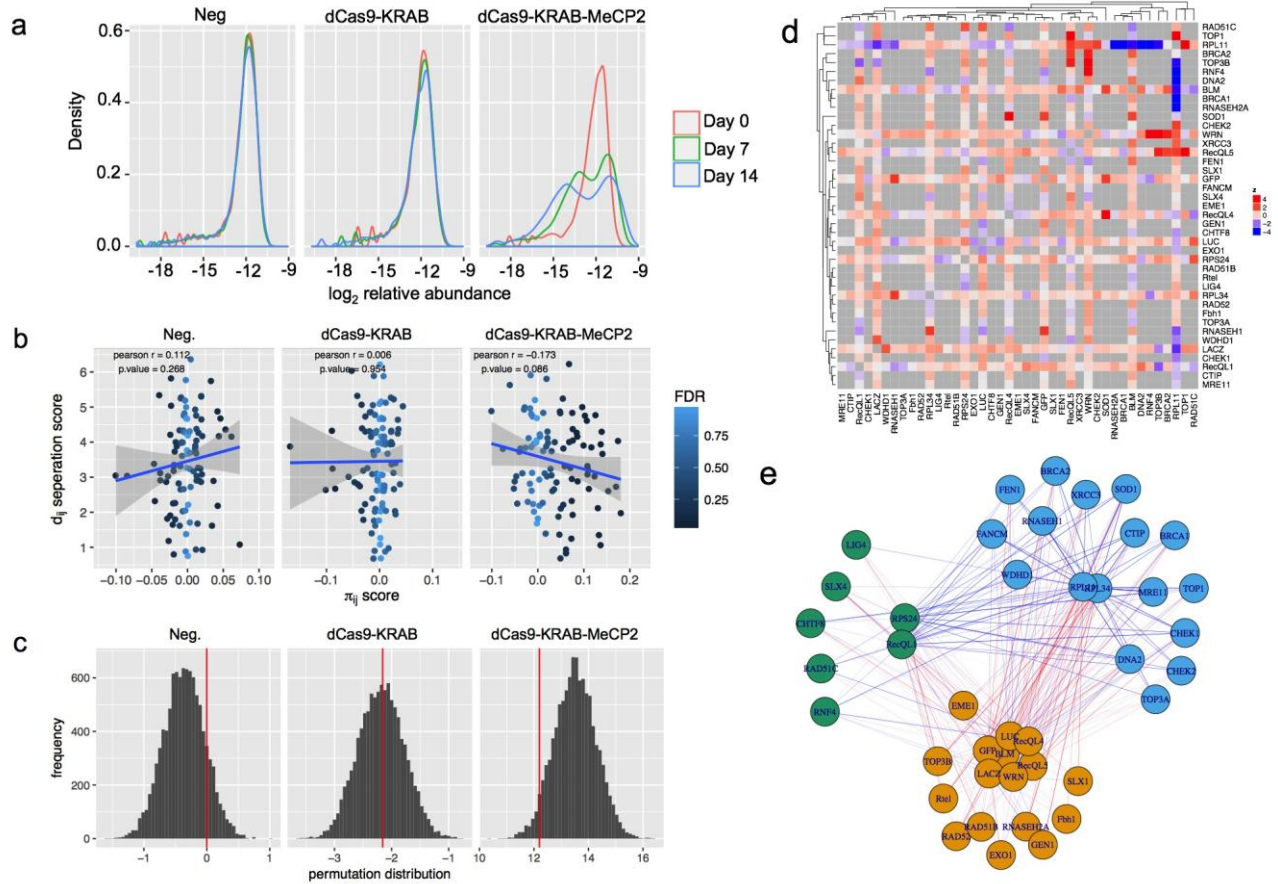
are also common to dCas9-KRAB or dCas9. (b) In SH-SY5Y screen, the top two NE-targeting sgRNAs, NPHS2-7 and PLA2G2E-19, seen with dCas9-KRAB-MeCP2 were also depleted with dCas9-KRAB. (c) Overlap of NE-targeting sgRNAs observed in HEK293T was shown. See **Supplementary Data 5** for a full list of NE guides exhibiting depletion when combined with different repressors.



Supplementary Figure 13

Performance of different dCas9 repressors in pooled essentiality screens.

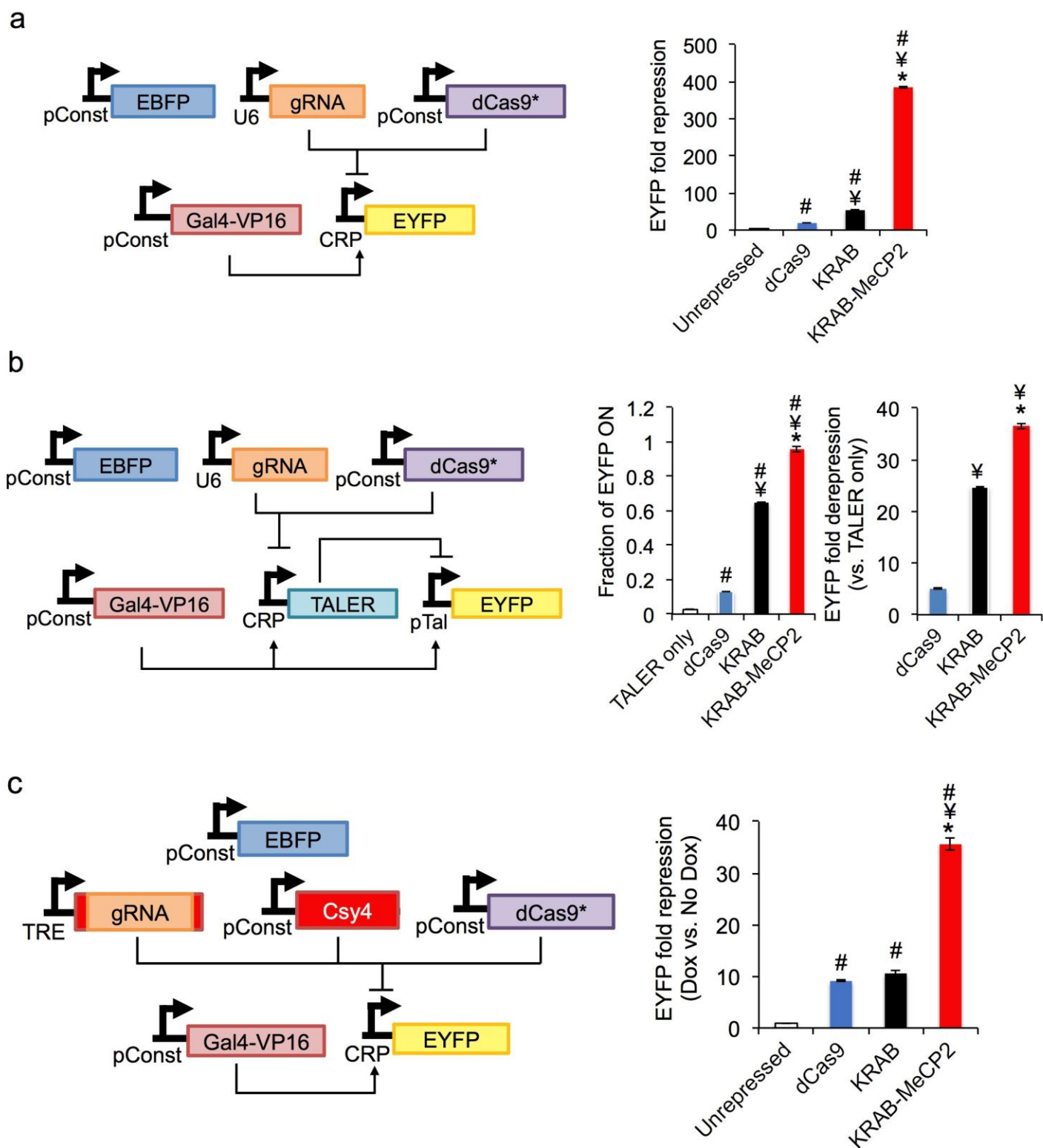
Shown are rank-ordered genes identified from sgRNA enrichment analysis performed using MAGeCK analysis pipeline in (a) HAP1 (day 14), (b) SH-SY5Y (day 14), and (c) HEK293T (day 14) with the indicated repressors. Genes were plotted according to the p-values obtained from MAGeCK and marked the FDR threshold at 0.25 (25%). The genes are colored by essentiality (red indicates essential genes and gray indicates non-essential genes). The horizontal lines indicate the FDR threshold and where there is no line indicates no genes were identified at the given threshold.



Supplementary Figure 14

Genetic interactions captured through the use of dCas9-KRAB-MeCP2.

(a) A density plot showing negative and positive selection pressure against gRNA pairs over time. (b) Proteins that are in the same complex are expected to have positive genetic interactions, while pairs of proteins in parallel pathways are expected to show a negative genetic interaction and be more distant from each other in a protein complex network. The negative control (Neg.) and dCas9-KRAB samples failed to capture this expected behavior, and their π -scores (quantifying genetic interactions) were smaller in general. However, the dCas9-KRAB-MeCP2 samples demonstrated a wider range of π -scores, and these were moderately consistent with the expected behavior. Specifically, gene pairs that were more closely connected in the protein complex network tended to show more positive genetic interactions. (c) A permutation test was used to assess the significance of this correlation. Specifically, the genetic interaction scores and protein complex network distances were multiplied for each gene pair, and then all such scores were summed across all genetic interactions. This was repeated 10000 times after randomizing the gene identifiers. Only the dCas9-KRAB-MeCP2 showed a significant trend. (permutation p-value: 0.8654, 0.53, and 0.041 for Neg., dCas9-KRAB, and dCas9-KRAB-MeCP2, respectively) (d) The hierarchical clustered heatmap of genetic interactions for negative control (wild-type HAP1 without repressor). (e) Resulting network diagram derived from dCas9-KRAB-MeCP2 screening data.



Supplementary Figure 15

Superiority of dCas9–Krab–MeCP2 in regulating synthetic circuits.

(a) When paired with dCas9-KRAB-MeCP2, gRNA expressed from a U6 promoter shows improved repression of EYFP over those paired with dCas9 or dCas9-KRAB. (b) In a two-tier repressor cascade comprised of a gRNA repressing a TALER which in turn inhibits EYFP expression, dCas9-KRAB-MeCP2 improves the transfer of information. While dCas9 and dCas9-KRAB repressors propagate some of the signal through the circuit, dCas9-KRAB-MeCP2 increases signal fidelity, making the full circuit indistinguishable from an unrepressed EYFP. (c) When expressed from a doxycycline inducible Pol II promoter and edited by Csy4, gRNA showed improved repression of EYFP when co-expressed with dCas9-KRAB-MeCP2, relative to dCas9 or dCas9-KRAB. For a-c, n=4 biologically independent samples (cell cultures). Data are presented as mean $\pm$ s.e.m. One-sided Student T-test was performed for all statistical comparison. # $p < 0.05$ vs. unrepressed or TALER only control, ¥ $p < 0.05$ v.s. dCas9, and * $p < 0.05$ v.s. dCas9-KRAB.

An enhanced CRISPR repressor for targeted mammalian gene regulation

Nan Cher Yeo^{1,2, †}, Alejandro Chavez^{3,†,*}, Alissa Lance-Byrne¹, Yingleong Chan^{1,2,Φ}, David Menn^{4,Φ}, Denitsa Milanova^{1,2,Φ}, Chih-Chung Kuo^{5,6,Φ}, Xiaoge Guo^{1,2}, Sumana Sharma⁷, Angela Tung¹, Ryan J. Cecchi¹, Marcelle Tuttle¹, Swechchha Pradhan⁴, Elaine T Lim^{1,2}, Noah Davidsohn^{1,2}, Mo R. Ebrahimkhani^{4,8}, James J. Collins^{1,9,10,11,11}, Nathan E. Lewis^{5,6,13}, Samira Kiani^{4,*}, and George M. Church^{1,2,*}

¹Wyss Institute for Biologically Inspired Engineering, Harvard University, Cambridge, Massachusetts, USA.

²Department of Genetics, Harvard Medical School, Boston, Massachusetts, USA.

³Department of Pathology and Cell Biology, Columbia University College of Physicians and Surgeons, New York, New York, USA

⁴School of Biological and Health Systems Engineering, Ira. A Fulton Schools of Engineering, Arizona State University, Tempe, Arizona, USA.

⁵Department of Bioengineering, University of California, San Diego, USA.

⁶Novo Nordisk Foundation Center for Biosustainability at the University of California, San Diego, USA.

⁷Cell Surface Signalling Laboratory, Wellcome Trust Sanger Institute, Cambridge CB101SA, United Kingdom.

⁸Division of Gastroenterology and Hematology, Mayo Clinic College of Medicine and Science, Phoenix, Arizona, USA.

⁹Institute for Medical Engineering & Science, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA.

¹⁰Synthetic Biology Center, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA.

¹¹Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA.

¹²Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA.

¹³Department of Pediatrics, University of California, San Diego, USA

[†]Co-first authors

^ΦThese authors contributed equally to the manuscript

*Correspondence can be addressed to:

Alejandro Chavez

E-mail: ac4304@cumc.columbia.edu

Samira Kiani

E-mail: samira.Kiani@asu.edu

George M. Church

E-mail: gchurch@genetics.med.harvard.edu

Supplementary Table 1 Sequences of dCas9-Krab and dCas9-KRAB-MeCP2

>dCas9-KRAB

Labels: **dCas9**; **SV40 nucleus localization signal (NLS)**; **KRAB**; glycine serine-rich linker (**bold**); stop codon (*italic*)

```

ATGGACAAGAAGTACTCCATTGGGCTCGCTATCGGCACAAACAGCGTCGGCTGGGCCGTCA
TTACGGACGAGTACAAGGTGCCGAGCAAAAAATTCAAAGTTCTGGGCAATACCGATCGCCA
CAGCATAAAGAAGAACCCTCATTGGCGCCCTCCTGTTTCTGACTCCGGGGAGACGGCCGAAGC
CACGCGGCTCAAAAGAACAGCACGGCGCAGATATACCCGCAGAAAGAATCGGATCTGCTAC
CTGCAGGAGATCTTTAGTAATGAGATGGCTAAGGTGGATGACTCTTTCTTCCATAGGCTGGA
GGAGTCCTTTTTGGTGGAGGAGGATAAAAAGCACGAGCGCCACCCAATCTTTGGCAATATC
GTGGACGAGGTGGCGTACCATGAAAAGTACCCAACCATATATCATCTGAGGAAGAAGCTTG
TAGACAGTACTGATAAGGCTGACTTGCGGTTGATCTATCTCGCGCTGGCGCATATGATCAAA
TTTCGGGGACACTTCCTCATCGAGGGGGACCTGAACCCAGACAACAGCGATGTGCAAAAACCT
CTTTATCCAAGTGGTTTCAAGTCTTACAATCAGCTTTTTCGAAGAGAACCCGATCAACGCATCCG
GAGTTGACGCCAAAGCAATCCTGAGCGCTAGGCTGTCCAATCCCGGCGGCTCGAAAACCT
CATCGCAGCAGCTCCCTGGGGAGAAGAAGACGGCCTGTTTGTAATCTTATCGCCCTGTCA
CTCGGGCTGACCCCAACTTTAAATCTAACTTCGACCTGGCCGAAGATGCCAAGCTTCAACT
GACCAAAGACACCTACGATGATGATCTCGACAATCTGCTGGCCAGATCGGCGCAGTAGTAC
GCAGACCTTTTTTTGGCGGCAAAGAACCTGTGACGACGCCATTCTGCTGAGTGATATTCTGCG
AGTGAACACGGAGATCACCAAAGCTCCGCTGAGCGCTAGTATGATCAAGCGCTATGATGAG
CACCACCAAGACTTGACTTTGCTGAAGGCCCTTGTGACAGCAACTGCCTGAGAAGTACAA
GGAAATTTTCTTCGATCAGTCTAAAAATGGCTACGCCGGATACATTGACGGCGGAGCAAGCC
AGGAGGAATTTTACAAATTTATTAAGCCCATCTTGAAAAAATGGACGGCACCGAGGAGCTG
CTGGTAAAGCTTAACAGAGAAGATCTGTTGCGCAAACAGCGCACTTTGACAATGGAAGCAT
CCCCCACCAGATTCACCTGGGCGAACTGCACGCTATCCTCAGGCGGCAAGAGGATTTCTAC
CCCTTTTTGAAAGATAACAGGGGAAAAGATTGAGAAAATCCTCACATTTTCGGATACCCTACTAT
GTAGGCCCCCTCGCCCGGGGAAAATTCCAGATTGCGCTGGATGACTCGCAAATCAGAAGAGA
CCATCACTCCCTGGAACCTTCGAGGAAGTCGTGGATAAGGGGGCCTCTGCCAGTCCTTCAT
CGAAAGGATGACTAACTTTGATAAAAAATCTGCCTAACGAAAAGGTGCTTCCTAAACACTCTCT
GCTGTACGAGTACTTCACAGTTTATAACGAGCTCACCAAGGTCAAATACGTCACAGAAGGGA
TGAGAAAGCCAGCATTCTGTCTGGAGAGCAGAAGAAAGCTATCGTGGACCTCCTCTTCAA
GACGAACCGGAAAAGTTACCGTGAAACAGCTCAAAGAAGACTATTTCAAAAAGATTGAATGTT
TCGACTCTGTTGAAATCAGCGGAGTGGAGGATCGCTTCAACGCATCCCTGGGAACGTATCA
CGATCTCCTGAAAATCATTAAAGACAAGGACTTCCTGGACAATGAGGAGAACGAGGACATTC
TTGAGGACATTGTCCTCACCTTACGTTGTTTGAAGATAGGGAGATGATTGAAGAACGCTTG
AAAACCTACGCTCATCTCTTCGACGACAAAGTCATGAAACAGCTCAAGAGGCGCCGATATAC
AGGATGGGGGCGGCTGTCAAGAAAAGTATCAATGGGATCCGAGACAAGCAGAGTGGAAG
GACAATCCTGGATTTTCTTAAGTCCGATGGATTTGCCAACCAGGAACTTCATGCAGTTGATCC
ATGATGACTCTCTCACCTTTAAGGAGGACATCCAGAAAGCACAAGTTTCTGGCCAGGGGGA
CAGTCTTCACGAGCACATCGCTAATCTTGCAAGGTAGCCAGCTATCAAAAAGGGAATACTGC
AGACCGTTAAGGTCGTGGATGAATCTGTCAGGTAATGGGAAGGCATAAGCCCGAGAATAT
CGTTATCGAGATGGCCCGAGAGAACCAAACCTACCCAGAAGGGACAGAAGAACAGTAGGGAA
AGGATGAAGAGGATTGAAGAGGGGTATAAAAGAACTGGGGTCCCAAATCCTTAAGGAACACC
CAGTTGAAAACACCCAGCTTCAGAATGAGAAGCTCTACCTGTACTACCTGCAGAACGGCAG
GGACATGTACGTGGATCAGGAACTGGACATCAATCGGCTCTCCGACTACGACGTGGCTGCT
ATCGTGCCCCAGTCTTTTCTCAAAGATGATTCTATTGATAATAAAGTGTTGACAAGATCCGAT
AAAGcTAGAGGGAAGAGTGATAACGTCCCCTCAGAAGAAGTTGTCAAGAAAATGAAAAATTAT
TGCGGCGCAGCTGCTGAACGCCAACTGATCACACAACGGAAGTTGATAATCTGACTAAGG
CTGAACGAGGTGGCCTGTCTGAGTTGGATAAAGCCGGCTTCATCAAAGGCAGCTTGTTGA
GACACGCCAGATCACCAAGCACGTGGCCCAAATTCTCGATTACGCATGAACACCAAGTAC
GATGAAAATGACAACTGATTTCGAGAGGTGAAAGTTATTACTCTGAAGTCTAAGCTGGTCTC
AGATTTTCAGAAAGGACTTTTCAAGTTTATAAGGTGAGAGAGATCAACAATTACCACCATGCGC
ATGATGCCTACCTGAATGCAGTGGTAGGCACTGCACCTTATCAAAAATATCCCAAGCTTGAA

```

TCTGAATTTGTTTACGGAGACTATAAAGTGTACGATGTTAGGAAAATGATCGCAAAGTCTGAG
 CAGGAAATAGGCAAGGCCACCGCTAAGTACTTCTTTTACAGCAATATTATGAATTTTTTCAAG
 ACCGAGATTACACTGGCCAATGGAGAGATTCGGAAGCGACCACTTATCGAAACAAACGGAG
 AAACAGGAGAAAATCGTGTGGGACAAGGGTAGGGATTTTCGCGACAGTCCGGAAGGTCTGTCT
 CATGCCGCAGGTGAACATCGTTAAAAAGACCGAAGTACAGACCGGAGGCTTCTCCAAGGAA
 AGTATCCTCCCAGAAAAGGAACAGCGACAAGCTGATCGCACGCAAAAAAGATTGGGACCCCA
 AGAAATACGGCGGATTCTGATTCTCTACAGTCGCTTACAGTGTACTGGTTGTGGCCAAAGTG
 GAGAAAGGGAAGTCTAAAAAACTCAAAAGCGTCAAGGAAGTCTGGGCATCACAATCATGG
 AGCGATCAAGCTTCGAAAAAAACCCCATCGACTTTCTCGAGGCGAAAGGATATAAAGAGGTCT
 AAAAAAGACCTCATCATTAAGCTTCCCAAGTACTCTCTCTTTGAGCTTGAAAACGGCCGGAA
 ACGAATGCTCGCTAGTGCGGGCGAGCTGCAGAAAGGTAACGAGCTGGCACTGCCCTCTAAA
 TACGTTAATTTCTTGTATCTGGCCAGCCACTATGAAAAGCTCAAAGGGTCTCCCGAAGATAAT
 GAGCAGAAGCAGCTGTTCTGGAACAACACAAACACTACCTTGATGAGATCATCGAGCAAAT
 AAGCGAATTCTCCAAAAGAGTGATCCTCGCCGACGCTAACCTCGATAAGGTGCTTTCTGCTT
 ACAATAAGCACAGGGATAAGCCCATCAGGGAGCAGGCAGAAAACATTATCCACTTGTCTTACT
 CTGACCAACTTGGGCGCGCTGCAGCCTTCAAGTACTTCGACACCACCATAGACAGAAAGC
 GTTACACCTCTACAAAGGAGGTCTTGACGCCACACTGATTCATCAGTCAATTACGGGGCT
 CTATGAAACAAGAATCGACCTCTCTCAGCTCGGTGGAGACAGCAGGGCTGAC**CCCAAGAAG**
AAGAGGAAGGTG**AGTGGTGGAGGAAGTGGCGGGTCAGGGTCG**ATGGACGCGAAATCACTT
 ACGGCATGGTCGAGAACAACCTGTTACGTTCAAGGACGTGTTTGTGGACTTTACACGTGAGG
 AGTGGAAATTGCTGGATACTGCGCAACAAATTGTGTATCGAAATGTCATGCTTGAGAATTAC
 AAGAACCTCGTCAGTCTCGGATACCAGTTGACGAAACCGGATGTGATCCTTAGGCTCGAAAA
 GGGGGAAGAACCTTGGCTGGTA*TAG*

>dCas9-KRAB-MeCP2

Labels: dCas9; SV40 nucleus localization signal (NLS); KRAB; TRD domain of MeCP2; glycine serine-rich linker (bold); stop codon (italic)

ATGGACAAGAAGTACTCCATTGGGCTCGCTATCGGCACAAACAGCGTCGGCTGGGCCGTCA
 TTACGGACGAGTACAAGGTGCCGAGCAAAAAATTCAAAGTTCTGGGCAATACCGATCGCCA
 CAGCATAAAGAAGAACCTCATTGGCGCCCTCTGTTGACTCCGGGGAGACGGCCGAAGC
 CACGCGGCTCAAAAGAACAGCACGGCGCAGATATACCCGCAGAAAGAATCGGATCTGCTAC
 CTGCAGGAGATCTTTAGTAATGAGATGGCTAAGGTGGATGACTCTTTCTTCATAGGCTGGA
 GGAGTCCTTTTTGGTGGAGGAGGATAAAAAGCACGAGCGCCACCCAATCTTTGGCAATATC
 GTGGACGAGGTGGCGTACCATGAAAAGTACCCAACCATATATCATCTGAGGAAGAAGCTTG
 TAGACAGTACTGATAAGGCTGACTTGCGGTTGATCTATCTCGCGCTGGCGCATATGATCAAA
 TTTTCGGGGACACTTCCTCATCGAGGGGGACCTGAACCCAGACAACAGCGATGTGCAAAACT
 CTTTATCCAACCTGGTTTCACTTACAATCAGCTTTTTCGAAGAGAACCCGATCAACGCATCCG
 GAGTTGACGCCAAAGCAATCCTGAGCGCTAGGCTGTCCAAATCCCGGGCGGCTCGAAAACCT
 CATCGCACAGCTCCCTGGGGAGAAGAAGAACGGCCTGTTTGGTAATCTTATCGCCCTGTCA
 CTCGGGCTGACCCCAACTTTAAATCTAACTTCGACCTGGCCGAAGATGCCAAGCTTCAACT
 GAGCAAAGACACCTACGATGATGATCTCGACAATCTGCTGGCCAGATCGGCGACCAAGTAC
 GCAGACCTTTTTTGGCGGCAAAAGAACCTGTCAGACGCCATTCTGCTGAGTGATATTCTGCG
 AGTGAACACGGAGATCACCAAAGCTCCGCTGAGCGCTAGTATGATCAAGCGCTATGATGAG
 CACCACCAAGACTTGACTTTGCTGAAGGCCCTTGTGACAGCAACTGCCTGAGAAGTACAA
 GGAAATTTCTTCGATCAGTCTAAAAATGGCTACGCCGGATACATTGACGGCGGAGCAAGCC
 AGGAGGAATTTTACAAATTTTAAAGCCATCTGTTGCGCAACAGCGCACTTTTCGACAATGGAAGCAT
 CTGGTAAAGCTTACAGAGAAGATCTGTTGCTGCAACAGCGCACTTTTCGACAATGGAAGCAT
 CCCCCACCAGATTACCTGGGCGAACTGCACGCTATCCTCAGGCGGCAAGAGGATTCTAC
 CCTTTTTTGAAGATAACAGGGAAAAAGATTGAGAAAATCCTCACATTTTCGGATACCCTACTAT
 GTAGGCCCCCTCGCCCGGGGAAATTCCAGATTTCGCGTGGATGACTCGCAAATCAGAAGAGA

CCATCACTCCCTGGAACCTTCGAGGAAGTCGTGGATAAGGGGGCCTCTGCCCAGTCCTTCAT
 CGAAAGGATGACTAACTTTGATAAAAATCTGCCTAACGAAAAGGTGCTTCCTAAACACTCTCT
 GCTGTACGAGTACTTCACAGTTTATAACGAGCTCACCAAGGTCAAATACGTCACAGAAGGGA
 TGAGAAAGCCAGCATTCTGTCTGGAGAGCAGAAGAAAGCTATCGTGGACCTCCTCTTCAA
 GACGAACCGGAAAAGTTACCGTGAAACAGCTCAAAGAAGACTATTTCAAAAAGATTGAATGTT
 TCGACTCTGTTGAAATCAGCGGAGTGGAGGATCGCTTCAACGCATCCCTGGGAACGTATCA
 CGATCTCCTGAAAATCATTAAAGACAAGGACTTCCTGGACAATGAGGAGAACGAGGACATTG
 TTGAGGACATTGTCCTCACCTTACGTTGTTTGAAGATAGGGAGATGATTGAAGAACGCTTG
 AAACTTACGCTCATCTCTTCGACGACAAAGTCATGAAACAGCTCAAGAGGCGCCGATATAC
 AGGATGGGGGCGGCTGTCAAGAAAAGTATGATCAATGGGATCCGAGACAAGCAGAGTGGAAA
 GACAATCCTGGATTTTCTTAAGTCCGATGGATTTGCCAACCAGGAACTTCATGCAGTTGATCC
 ATGATGACTCTCTCACCTTTAAGGAGGACATCCAGAAAGCACAAGTTTCTGGCCAGGGGGA
 CAGTCTTCACGAGCACATCGCTAATCTTGCAAGTAGCCAGCTATCAAAAAGGGAATACTGC
 AGACCGTTAAGGTCGTGGATGAACTCGTCAAAGTAATGGGAAGGCATAAGCCCGAGAATAT
 CGTTATCGAGATGGCCCGAGAGAACCAGTAACTACCCAGAAAGGACAGAAAGACAGTAGGGAA
 AGGATGAAGAGGATTGAAGAGGGGTATAAAAGAACTGGGGTCCCAAATCCTTAAGGAACACC
 CAGTTGAAAACACCCAGCTTCAGAATGAGAAGCTCTACCTGTACTACCTGCAGAACGGCAG
 GGACATGTACGTGGATCAGGAAGTGGACATCAATCGGCTCTCCGACTACGACGTGGCTGCT
 ATCGTGCCCCAGTCTTTTCTCAAAGATGATTCTATTGATAATAAAGTGTTGACAAGATCCGAT
 AAAgcTAGAGGGAAGAGTGATAACGTCCCCTCAGAAGAAGTTGTCAAGAAAATGAAAAATTAT
 TGGCGGCAGCTGCTGAACGCCAACTGATCACACAACGGAAGTTCGATAATCTGACTAAGG
 CTGAACGAGGTGGCCTGTCTGAGTTGGATAAAGCCGGCTTCATCAAAGGCAGCTTGTTGA
 GACACGCCAGATCACCAAGCACGTGGCCCAAATTCTCGATTACGCATGAACACCAAGTAC
 GATGAAAATGACAACTGATTGAGAGGTGAAAGTTATTACTCTGAAGTCTAAGCTGGTCTC
 AGATTTGAGAAAGGACTTTTCTGTTTTATAAGGTGAGAGAGATCAACAATTACCACCATGCGC
 ATGATGCCTACCTGAATGCAGTGGTAGGCACTGCACCTTATCAAAAATATCCCAAGCTTGAA
 TCTGAATTTGTTTACGGAGACTATAAAGTGTACGATGTTAGGAAAATGATCGCAAAGTCTGAG
 CAGGAAATAGGCAAGGCCACCGCTAAGTACTTCTTTTACAGCAATATTATGAATTTTTTCAAG
 ACCGAGATTACACTGGCCAATGGAGAGATTGGAAGCGACCACTTATCGAAACAAACGGAG
 AAACAGGAGAAATCGTGTGGGACAAGGGTAGGGATTTGCGGACAGTCCGGAAGGTCTCTGTC
 CATGCCGCAGGTGAACATCGTTAAAAAGACCGAAGTACAGACCGGAGGCTTCTCCAAGGAA
 AGTATCCTCCCGAAAAGGAACAGCGACAAGCTGATCGCACGCAAAAAAGATTGGGACCCCA
 AGAAATACGGCGGATTCTGATTCTCTACAGTCGCTTACAGTGTACTGGTTGTGGCCAAAGTG
 GAGAAAGGGAAGTCTAAAAAACTCAAAGCGTCAAGGAAGTCTGGGCATCACATCATGG
 AGCGATCAAGCTTCGAAAAAAACCCCATCGACTTTCTCGAGGCGAAAGGATATAAAGAGGTG
 AAAAAAGACCTCATCATTAAGCTTCCCAAGTACTCTCTCTTTGAGCTTGAAAACGGCCGGAA
 ACGAATGCTCGCTAGTGCGGGCGAGCTGCAGAAAGGTAACGAGCTGGCACTGCCCTCTAAA
 TACGTTAATTTCTTGATCTGGCCAGCCACTATGAAAAGCTCAAAGGGTCTCCCGAAGATAAT
 GAGCAGAAGCAGCTGTTCTGTGGAACAACACAAACACTACCTTGATGAGATCATCGAGCAAAT
 AAGCGAATTCTCCAAAAGAGTGATCCTCGCCGACGCTAACCTCGATAAGGTGCTTTCTGCTT
 ACAATAAGCACAGGGATAAGCCCATCAGGGAGCAGGCAGAAAACATTATCCACTTGTTTACT
 CTGACCAACTTGGGCGCGCCTGCAGCCTTCAAGTACTTCGACACCACCATAGACAGAAAGC
 GGTACACCTCTACAAAGGAGGTCCTGGACGCCACACTGATTCATCAGTCAATTACGGGGCT
 CTATGAAACAAGAATCGACCTCTCTCAGCTCGGTGGAGACAGCAGGGCTGACCCCAAGAAG
 AAGAGGAAGGTGAGTGGTGGAGGAAGTGGCGGGTCAGGGTCGATGGACGCGAAATCACTT
 ACGGCATGGTCGAGAACACTGGTTACGTTCAAGGACGTGTTTGTGGACTTTACACGTGAGG
 AGTGGAATTTGCTGGATACTGCGCAACAAATTGTGTATCGAAATGTCATGCTTGAGAATTAC
 AAGAACCTCGTCAGTCTCGGATACCAGTTGACGAAACCGGATGTGATCCTTAGGCTCGAAAA
 GGGGGAAGAACCTTGGCTGGTATCGGGAGGTGGTTTCGGGTGGCTCTGGATCAAGCCAAA
 GAAGAAACGGAAGGTGGAAGCCTCAGTGCAGGTGAAAAGGGTGCTGGAAAAATCCCCGG
 CAACTCCTCGTGAAGATGCCCTTCCAGGCTTCCCCTGGCGGAAAAGGTGAAGGGGGTGG
 CGCAACCACATCTGCCAGGTCATGGTCATCAAGCGACCTGGAAGGAAAAGAAAGGCCGAG
 GCTGACCCTCAGGCCATTCCAAAGAAACGGGGACGCAAGCCAGGGTCCGTGGTTCGAGCT
 GCAGCAGCTGAGGCTAAGAAAAAGGCAGTGAAGGAAAGCTCCATCCGACGTGTGCAGGAG
 ACTGTCCTGCCCATCAAGAAGAGGAAGACTAGGGAGACCGTGTCCATCGAGGTCAAAGAAG
 TGGTCAAGCCCCTGCTCGTGTCCACCCTGGGCGAAAAATCTGGAAAGGGGCTCAAAACATG

CAAGTCACCTGGACGGAAAAGCAAGGAGTCTAGTCCAAAGGGGCGCTCAAGCTCCGCTTCT
AGTCCCCCTAAAAAGGAACACCATCACCATCACCATCACGCCGAGTCTCCTAAGGCTCCTAT
GCCACTGCTCCACACCTCCACACCTGAGCCACAGTCAAGCGAAGACCCCATCAGCCCA
CCCGAGCCTCAGGATCTGTCCTCTAGTATTTGCAAAGAGGAAAAGATGCCCAGAGCAGGCA
GCCTGGAGAGTGATGGCTGTCCAAAAGAACCCGCCAAGACCCAGCCTATGGTGGCAGCCG
CTGCAACTACCACCACAACCACAACCTACCACAGTGGCCGAAAAATACAAGCATCGCGGCGA
GGCGAACGAAAGGACATTGTGTCAAGCTCCATGCCCAGACCTAACCGGGAGGAACCAGT
CGATAGTAGGACACCCGTGACTGAGAGAGTCTCA TAG

Supplementary Table 2 A list of differentially expressed (DE) genes with log₂ FC above 1.5 threshold in RNA-seq experiment. (Note that all of these genes have positive log₂ FC, which corresponds to increased transcriptional activity).

dCas9				
	Log₂FC	Log₂CPM	P-Value	FDR
HSPA6¹	6.57	8.3400	2.00E-25	2.74E-21
DNAJB1¹	2.24	8.2800	6.09E-23	4.16E-19
HSPA7¹	7.28	2.1290	9.68E-16	4.41E-12
HSPA1A¹	4.69	2.7660	4.75E-13	1.62E-09
CRYAB¹	6.55	2.0320	4.09E-12	1.12E-08
ANKRD1²	2.54	2.3950	4.02E-11	9.16E-08
ZFAND2A¹	1.65	4.2370	3.74E-10	7.29E-07
ATF3²	1.11	6.0300	2.30E-07	3.93E-04
DNAH17¹	1.74	3.5590	2.92E-07	4.44E-04
SCG2	4.11	0.1960	1.35E-05	1.75E-02
VGF¹	2.33	1.9290	1.41E-05	1.75E-02
FOS²	1.41	2.9560	1.65E-05	1.88E-02
GDF15¹	2.43	1.7580	2.68E-05	2.82E-02
dCas9-KRAB				
	Log₂FC	Log₂CPM	P-Value	FDR
HSPA6¹	7.19	8.3398	2.26E-26	2.65E-22
DNAJB1¹	2.62	8.2801	3.88E-26	2.65E-22
HSPA1A¹	6.36	2.7657	8.82E-21	4.02E-17
HMOX1	2.07	5.5632	1.31E-20	4.48E-17
CRYAB¹	8.14	2.0315	2.86E-18	7.82E-15
HSPA7¹	7.82	2.1293	5.79E-18	1.32E-14
VGF¹	4.6	1.9288	4.29E-16	8.37E-13
ZFAND2A¹	2	4.2367	6.13E-14	1.05E-10
PPP1R15A³	1.47	5.3442	2.88E-12	4.36E-09
GDF15¹	4.08	1.7576	6.69E-12	9.13E-09
DNAH17¹	2.34	3.5595	2.04E-11	2.53E-08
CLU	1.49	4.588	6.38E-11	7.26E-08
SERPINH1	1.24	6.3749	1.08E-10	1.14E-07
FOXJ1³	6.5	0.4447	1.47E-10	1.43E-07
ANXA1	2.55	1.9991	1.02E-09	9.33E-07
FOS²	1.86	2.9564	3.62E-09	3.09E-06
NCRNA00306	3.6	0.6114	1.87E-08	1.50E-05
CSRNP1	1.32	4.5789	2.82E-08	2.14E-05
ANKRD1²	2	2.3946	1.28E-07	9.22E-05

ACHE	1.89	2.0935	2.28E-06	1.56E-03
SCG2	4.28	0.1963	4.42E-06	2.75E-03
ATF3²	1.02	6.0304	4.42E-06	2.75E-03
HLA-G	3.54	-0.0433	5.02E-06	2.98E-03
INPP5D³	2.93	0.983	5.69E-06	3.24E-03
RELB	1.56	2.6216	6.66E-06	3.64E-03
PNLDC1	3.2	0.0906	8.19E-06	4.15E-03
CYP4F3	7.12	-0.4274	8.21E-06	4.15E-03
MMP12	4.58	-0.6846	9.52E-06	4.64E-03
TUBB3	1.15	3.9455	1.39E-05	6.53E-03
PLK2	1.23	3.6057	1.52E-05	6.92E-03
IRX4	2.55	0.425	2.43E-05	1.07E-02
DUSP8	1.22	3.6476	3.69E-05	1.57E-02
FERMT3	2.22	0.6882	5.66E-05	2.34E-02
ACRC	1.63	1.6191	7.17E-05	2.88E-02
IL11	1.92	0.9971	9.89E-05	3.86E-02
dCas9-KRAB-MeCP2				
	Log₂FC	Log₂CPM	P-Value	FDR
HSPA6¹	6.7	8.34	1.27E-25	1.73E-21
DNAJB1¹	2.24	8.28	6.26E-23	4.28E-19
HSPA1A¹	5.8	2.766	4.26E-18	1.94E-14
HSPA7¹	6.99	2.129	2.26E-14	7.71E-11
CRYAB¹	6.59	2.032	4.73E-12	1.29E-08
DNAH17¹	2.43	3.559	8.96E-12	2.04E-08
INPP5D³	3.96	0.983	1.65E-09	3.23E-06
GDF15¹	3.29	1.758	2.00E-08	3.41E-05
CYP4F3	7.81	-0.427	3.40E-07	5.16E-04
FOXJ1³	5.41	0.445	4.53E-07	6.19E-04
ZFAND2A¹	1.33	4.237	1.24E-06	1.54E-03
PPP1R15A³	1.09	5.344	1.46E-06	1.66E-03
VGF¹	2.55	1.929	2.43E-06	2.56E-03
NCRNA00306	2.75	0.611	2.56E-05	2.50E-02
DUSP8³	1.23	3.648	3.32E-05	2.92E-02
PLA2G4C	1.89	1.495	3.55E-05	2.92E-02
ANXA1	1.76	1.999	3.64E-05	2.92E-02

Note: ¹ indicates genes common to all three repressor groups, ² indicates common genes between dCas9 and dCas9-KRAB, and ³ indicates common genes between dCas9-KRAB and dCas9-KRAB-MeCP2. FC = fold-change, CPM = counts per million, FDR = false discovery rate. n=2 biologically independent samples. For DE analyses, see **Supplementary Note 2**.

Supplementary Table 3. A list of down-regulated genes identified in RNA-seq experiment. Note that all of these genes have log₂ FC below 1.5 threshold.

dCas9-KRAB				
	Log₂FC	Log₂CPM	P-Value	FDR
EIF5B¹	-0.59	5.4743	6.88E-07	2.54E-04
TMEM44	-1.329	2.2374	5.03E-06	1.37E-03
HNRNPA2B1¹	-0.463	8.6101	1.72E-05	3.86E-03
HNRNPD	-0.451	5.9149	2.69E-05	5.74E-03
HIST1H2AE	-0.822	3.4547	4.20E-05	8.44E-03
TAF15	-0.497	4.9187	1.88E-04	3.02E-02
NFXL1	-0.444	5.0202	2.40E-04	3.62E-02
ODZ3	-0.452	4.8766	2.44E-04	3.62E-02
dCas9-KRAB-MeCP2				
	Log₂FC	Log₂CPM	P-Value	FDR
EIF5B¹	-0.63	5.474	2.87E-07	1.87E-04
HNRNPA2B1¹	-0.427	8.61	5.75E-05	1.96E-02
LRRFIP1	-0.398	5.767	1.54E-04	4.34E-02
PRPF19	-0.359	6.983	1.55E-04	4.34E-02
RNU6ATAC	-0.662	3.595	1.67E-04	4.36E-02

Note: ¹ indicates genes down-regulated in both dCas9-KRAB and dCas9-KRAB-MeCP2 groups. None of the down-regulated genes showed a near sequence match to the *CXCR4* targeting sgRNA. No downregulated genes were observed in the dCas9 group. FC = fold-change, CPM = counts per million, FDR = false discovery rate. n=2 biologically independent samples. For DE analyses, see **Supplementary Note 2**.

Supplementary Table 4 Mean fold changes of all essential gene-targeting sgRNAs and *p*-values for statistical comparison between essential and non-essential guides in HAP1 lethality screen.

Experiment	Mean essential guides log ₂ OR	P-value [#]
dCas9_day0	-0.008	0.1329
dCas9_day7	0.001	0.669
dCas9_day14	-0.001	0.6289
dCas9-KRAB_day0	-0.015	0.005708
dCas9-KRAB _day7	-0.056	1.83E-07
dCas9-KRAB _day14	-0.081	5.41E-19
dCas9-KRAB-MeCP2_day0	0.031	0.9996
dCas9-KRAB-MeCP2_day7*	-2.358	4.87E-78
dCas9-KRAB-MeCP2_day14**	-2.586	3.52E-80

[#] *p*-value is derived from one-tailed Welch T-test comparing the log₂ OR of the essential guides versus the log₂ OR of the non-essential guides. See **Supplementary Note 3** for detailed analysis and statistical test.

* Log₂ Odds-ratio (log₂ OR) that was -Infinity, i.e. complete depletion, were replaced by the least finite OR for day 7, i.e. -7.607176

** log₂ Odds-ratio (log₂ OR) that were -Infinity, i.e. complete depletion, were replaced by the least finite OR for day 14, i.e. -8.867043

Supplementary Table 5 Mean fold changes of all essential gene-targeting sgRNAs and *p*-values for statistical comparison between essential and non-essential guides in SH-SY5Y lethality screen.

Experiment	mean essential guides log ₂ OR	P-value [#]
dCas9_day0	-0.0517	0.0009321
dCas9_day7	-0.10179	9.92E-16
dCas9_day14	-0.12011	3.76E-20
dCas9_day22	-0.149066	1.31E-23
dCas9-KRAB_day0	-0.1341297	3.79E-22
dCas9-KRAB _day7	-0.3476919	4.72E-37
dCas9-KRAB _day14	-0.3733395	2.16E-44
dCas9-KRAB _day22	-0.386887	2.24E-52
dCas9-KRAB-MeCP2_day0	-0.1937839	5.27E-26
dCas9-KRAB-MeCP2_day7	-0.4740388	2.55E-55
dCas9-KRAB-MeCP2_day14	-0.3774654	1.19E-63
dCas9-KRAB-MeCP2_day22	-0.525382	3.08E-70

[#] *p*-value is derived from one-tailed Welch T-test comparing the log₂ OR of the essential guides versus the log₂ OR of the non-essential guides. See **Supplementary Note 3** for detailed analysis and statistical test.

Supplementary Table 6 Mean fold changes of all essential gene-targeting sgRNAs and *p*-values for statistical comparison between essential and non-essential guides in HEK293T lethality screen.

Experiment	mean essential guides log ₂ OR	P-value [#]
dCas9_day0	0.003201572	0.12
dCas9_day7	-0.03381157	1.18E-06
dCas9_day14	-0.05741197	2.43E-08
dCas9-KRAB_day0	-0.07741853	0.02
dCas9-KRAB _day7	-0.06196394	1.53E-14
dCas9-KRAB _day14	-0.007220968	4.15E-15
dCas9-KRAB-MeCP2_day0	-0.03050618	2.34E-06
dCas9-KRAB-MeCP2_day7	-0.120637	7.67E-38
dCas9-KRAB-MeCP2_day14	-0.1226212	4.63E-34

[#] *p*-value is derived from one-tailed Welch T-test comparing the log₂ OR of the essential guides versus the log₂ OR of the non-essential guides. See **Supplementary Note 3** for detailed analysis and statistical test.

Supplementary Table 7. Shown is the total number of sgRNAs showing depletion within or outside of the optimal targeting window previously defined for repression. Data was based on our pooled essentiality screens where 370 essential gene-targeting sgRNAs were tested.

a. Summary of depleted sgRNAs in HAP1 cells at day 14

Targeting window	Total number of essential sgRNAs	Number of significantly depleted sgRNAs with dCas9	Number of significantly depleted sgRNAs with dCas9-KRAB	Number of significantly depleted sgRNAs with dCas9-KRAB-MeCP2
Within -50 to +200bp from TSS	213	35	94	181
Outside -50 to +200bp from TSS	144	19	47	113

b. Summary of depleted sgRNAs in SH-SY5Y cells at day 22

Targeting window	Total number of essential sgRNAs	Number of significantly depleted sgRNAs with dCas9	Number of significantly depleted sgRNAs with dCas9-KRAB	Number of significantly depleted sgRNAs with dCas9-KRAB-MeCP2
Within -50 to +200bp from TSS	213	75	117	149
Outside -50 to +200bp from TSS	144	27	58	77

c. Summary of depleted sgRNAs in 293T cells at day 14

Targeting window	Total number of essential sgRNAs	Number of significantly depleted sgRNAs with dCas9	Number of significantly depleted sgRNAs with dCas9-KRAB	Number of significantly depleted sgRNAs with dCas9-KRAB-MeCP2
Within -50 to +200bp from TSS	213	50	64	84
Outside -50 to +200bp from TSS	144	26	29	49

Supplementary Table 8 Sequence of oligos used to construct dual guide RNA library

Target genes	Sequence of oligos used for cloning	Note
BLM	[TTTTCGTCTCTCACCG] AGGAAACGGAAGAACCCGAG [gttttagagctatgctgaaaagca]	first spacer BLM_1
WRN	[TTTTCGTCTCTCACCG] CCGGCTTGACTCGGCAGCG [gttttagagctatgctgaaaagca]	first spacer WRN_1
RECQL1	[TTTTCGTCTCTCACCG] GCTGAACGGACCGACCCGGA [gttttagagctatgctgaaaagca]	first spacer RecQL1_1
RECQL4	[TTTTCGTCTCTCACCG] TCGCTGGACGATCGCAAGCG [gttttagagctatgctgaaaagca]	first spacer RecQL4_1
RECQL5	[TTTTCGTCTCTCACCG] CGACGGATATAAGATTGCGT [gttttagagctatgctgaaaagca]	first spacer RecQL5_1
BLM	[TTTTCGTCTCTCACCG] CCTCGCACGCAGACTCCTAG [gttttagagctatgctgaaaagca]	first spacer BLM_2
WRN	[TTTTCGTCTCTCACCG] CTAGCACTATAGATACCCCG [gttttagagctatgctgaaaagca]	first spacer WRN_2
RECQL1	[TTTTCGTCTCTCACCG] GAGATCGGAGAGTCGGACAC [gttttagagctatgctgaaaagca]	first spacer RecQL1_2
RECQL4	[TTTTCGTCTCTCACCG] TGGAGCGGCTGCGGGACGTG [gttttagagctatgctgaaaagca]	first spacer RecQL4_2
RECQL5	[TTTTCGTCTCTCACCG] TGAGTTGGGTTGTGTATAG [gttttagagctatgctgaaaagca]	first spacer RecQL5_2
BLM	[TTTTCGTCTCTCACCG] CCGCTAGGAGTCTGCGTGCG [gttttagagctatgctgaaaagca]	first spacer BLM_3
WRN	[TTTTCGTCTCTCACCG] GATGTGTAAGTGTGCGCCG [gttttagagctatgctgaaaagca]	first spacer WRN_3
RECQL1	[TTTTCGTCTCTCACCG] AAGATTTTACTCCCGAGTAG [gttttagagctatgctgaaaagca]	first spacer RecQL1_3
RECQL4	[TTTTCGTCTCTCACCG] CTGGACGATCGCAAGCGCGG [gttttagagctatgctgaaaagca]	first spacer RecQL4_3
RECQL5	[TTTTCGTCTCTCACCG] TTAATTCTTGGGCGGACCA [gttttagagctatgctgaaaagca]	first spacer RecQL5_3
GFP	[TTTTCGTCTCTCACCG] CAAGTTCAGCGTGTCCGGCG [gttttagagctatgctgaaaagca]	first spacer GFP_1
LACZ	[TTTTCGTCTCTCACCG] AGGTAGCAGAGCGGGTAAAC	first spacer LACZ_1

	[gttttagagctatgctgaaaagca]	
LUC	[TTTTCGTCTCTCACCG] AACGCCTTGATTGACAAGGA [gttttagagctatgctgaaaagca]	first spacer LUC_1
RPL34 (ESSENTIAL GENE)	[TTTTCGTCTCTCACCG] TGGTGAGCTGTGGCTACTCA [gttttagagctatgctgaaaagca]	first spacer RPL34_1
RPL11 (ESSENTIAL GENE)	[TTTTCGTCTCTCACCG] TCTCTTCCTGCTCTCCATCA [gttttagagctatgctgaaaagca]	first spacer RPL11_1
RPS24 (ESSENTIAL GENE)	[TTTTCGTCTCTCACCG] CCATCATGGTGAGTCTCCCT [gttttagagctatgctgaaaagca]	first spacer RPS24_1
GFP	[TTTTCGTCTCTCACCG] CAGCTCGATGCGGTTACCA [gttttagagctatgctgaaaagca]	first spacer GFP_2
LACZ	[TTTTCGTCTCTCACCG] TTTGTGGACGAAGTACCGAA [gttttagagctatgctgaaaagca]	first spacer LACZ_2
LUC	[TTTTCGTCTCTCACCG] ACAACTTTACCGACCGCGCC [gttttagagctatgctgaaaagca]	first spacer LUC_2
RPL34 (ESSENTIAL GENE)	[TTTTCGTCTCTCACCG] CTCCTCGGATGGCAGCCGAT [gttttagagctatgctgaaaagca]	first spacer RPL34_2
RPL11 (ESSENTIAL GENE)	[TTTTCGTCTCTCACCG] CCAGCTACTACCGCCATGA [gttttagagctatgctgaaaagca]	first spacer RPL11_2
RPS24 (ESSENTIAL GENE)	[TTTTCGTCTCTCACCG] TCCGTGCGCGTTGATATGAT [gttttagagctatgctgaaaagca]	first spacer RPS24_2
GFP	[TTTTCGTCTCTCACCG] CATGCCGAGAGTGATCCCGG [gttttagagctatgctgaaaagca]	first spacer GFP_3
LACZ	[TTTTCGTCTCTCACCG] AGGGCGGCTTCGTCTGGGAC [gttttagagctatgctgaaaagca]	first spacer LACZ_3
LUC	[TTTTCGTCTCTCACCG] AGCTATTCTGATTACACCG [gttttagagctatgctgaaaagca]	first spacer LUC_3
RPL34 (ESSENTIAL GENE)	[TTTTCGTCTCTCACCG] GAATGCAGCAAAGTCCCGGG [gttttagagctatgctgaaaagca]	first spacer RPL34_3
RPL11 (ESSENTIAL GENE)	[TTTTCGTCTCTCACCG] CGGCCTGCCATGGATGGCGA [gttttagagctatgctgaaaagca]	first spacer RPL11_3
RPS24 (ESSENTIAL GENE)	[TTTTCGTCTCTCACCG] CCGCGTATCCGAGCCATCCG [gttttagagctatgctgaaaagca]	first spacer RPS24_3
BLM	[TTTTCGTCTCTAAAC] CTCGGGTTCTTCGTTTCCT[cggtgACCCAGGCGG CGCACAAG]	second spacer BLM_1
WRN	[TTTTCGTCTCTAAAC] CGCTGCCGAGTACAAGCCGG[cggtgACCCAGGCG GCGCACAAG]	second spacer WRN_1

RECQL1	[TTTTCGTCTCTAAAC] TCCGGGTCGGTCCGTTCAGC[cggtgACCCAGGCG GCGCACAAG]	second spacer RecQL1_1
RECQL4	[TTTTCGTCTCTAAAC] CGCTTGCGATCGTCCAGCGA[cggtgACCCAGGCG GCGCACAAG]	second spacer RecQL4_1
RECQL5	[TTTTCGTCTCTAAAC] ACGCAATCTTATATCCGTCTG[cggtgACCCAGGCGG GCGCACAAG]	second spacer RecQL5_1
CHEK1	[TTTTCGTCTCTAAAC] CCTGGTACCATTCTCCACC[cggtgACCCAGGCGG GCGCACAAG]	second spacer CHEK1_1
CHEK2	[TTTTCGTCTCTAAAC] CCTGGAGCCGCACACTCTCC[cggtgACCCAGGCG GCGCACAAG]	second spacer CHEK2_1
SLX4	[TTTTCGTCTCTAAAC] CCCGGGTGCCGACTCCCAGC[cggtgACCCAGGCG GCGCACAAG]	second spacer SLX4_1
DNA2	[TTTTCGTCTCTAAAC] CCGGTTCGCTGTCTTTTCT[cggtgACCCAGGCGG GCGCACAAG]	second spacer DNA2_1
EME1	[TTTTCGTCTCTAAAC] CTATCAGGAGATCTACTTCC[cggtgACCCAGGCGG GCGCACAAG]	second spacer EME1_1
GEN1	[TTTTCGTCTCTAAAC] CTCGGCTTTCCCTTGCCGGC[cggtgACCCAGGCG GCGCACAAG]	second spacer GEN1_1
RNF4	[TTTTCGTCTCTAAAC] ACTTCCGCTTCGGAGGCCTC[cggtgACCCAGGCG GCGCACAAG]	second spacer RNF4_1
SLX1	[TTTTCGTCTCTAAAC] GTCGGCGAGCGCGTACCATT[cggtgACCCAGGCG GCGCACAAG]	second spacer SLX1_1
TOP3A	[TTTTCGTCTCTAAAC] CCTCAGCACC GAATCCAGTA[cggtgACCCAGGCG GCGCACAAG]	Second spacer TOP3A_1
TOP3B	[TTTTCGTCTCTAAAC] CTATTTCCGGGTCCAGCCGC[cggtgACCCAGGCG GCGCACAAG]	second spacer TOP3B_1
WDHD1	[TTTTCGTCTCTAAAC] CAGTGGCGGAGGCTCGGTCA[cggtgACCCAGGCG GCGCACAAG]	second spacer WDHD1_1
CHTF8	[TTTTCGTCTCTAAAC] CGCGGCAACGGGCGACAAC[cggtgACCCAGGCG GCGCACAAG]	second spacer CHTF8_1
SOD1	[TTTTCGTCTCTAAAC] CGTCTCCGCGACTACTTTAT[cggtgACCCAGGCGG GCGCACAAG]	second spacer SOD1_1
GFP	[TTTTCGTCTCTAAAC] CGCCGGACACGCTGAACTTG[cggtgACCCAGGCG GCGCACAAG]	second spacer GFP_1
LACZ	[TTTTCGTCTCTAAAC] GTTTACCCGCTCTGCTACCT[cggtgACCCAGGCGG GCGCACAAG]	second spacer LACZ_1

LUC	[TTTTCGTCTCTAAAC] TCCTTGTCATCAAGGCGTT[cggtgACCCAGGCGG GCGACAAG]	second spacer LUC_1
RPL34 (ESSENTIAL GENE)	[TTTTCGTCTCTAAAC] TGAGTAGCCACAGCTCACCA[cggtgACCCAGGCG GCGACAAG]	second spacer RPL34_1
RPL11 (ESSENTIAL GENE)	[TTTTCGTCTCTAAAC] TGATGGAGAGCAGGAAGAGA[cggtgACCCAGGCG GCGACAAG]	second spacer RPL11_1
RPS24 (ESSENTIAL GENE)	[TTTTCGTCTCTAAAC] AGGGAGACTCACCATGATGG[cggtgACCCAGGCG GCGACAAG]	second spacer RPS24_1
BLM	[TTTTCGTCTCTAAAC] CTAGGAGTCTGCGTGCGAGG[cggtgACCCAGGCG GCGACAAG]	second spacer BLM_2
WRN	[TTTTCGTCTCTAAAC] CGGGGTATCTATAGTGCTAG[cggtgACCCAGGCG GCGACAAG]	second spacer WRN_2
RECQL1	[TTTTCGTCTCTAAAC] GTGTCCGACTCTCCGATCTC[cggtgACCCAGGCGG GCGACAAG]	second spacer RecQL1_2
RECQL4	[TTTTCGTCTCTAAAC] CACGTCCCGCAGCCGCTCCA[cggtgACCCAGGCG GCGACAAG]	second spacer RecQL4_2
RECQL5	[TTTTCGTCTCTAAAC] CTATACACAACCCCAACTCA[cggtgACCCAGGCGG GCGACAAG]	second spacer RecQL5_2
CHEK1	[TTTTCGTCTCTAAAC] TCCCTCACTAATCTAGACCC[cggtgACCCAGGCGG GCGACAAG]	second spacer CHEK1_2
CHEK2	[TTTTCGTCTCTAAAC] CTCTGCTGGCTGAGGCTGCG[cggtgACCCAGGCG GCGACAAG]	second spacer CHEK2_2
SLX4	[TTTTCGTCTCTAAAC] CGGAGCCAGCGAGGGAGACG[cggtgACCCAGGC GGCGACAAG]	second spacer SLX4_2
DNA2	[TTTTCGTCTCTAAAC] CTCCGCTCACAGCTCCGCCG[cggtgACCCAGGCG GCGACAAG]	second spacer DNA2_2
EME1	[TTTTCGTCTCTAAAC] CCTGAACACCGCTCTGCAGA[cggtgACCCAGGCG GCGACAAG]	second spacer EME1_2
GEN1	[TTTTCGTCTCTAAAC] CCCGTGCTACCAGCTTCCC[cggtgACCCAGGCG GCGACAAG]	second spacer GEN1_2
RNF4	[TTTTCGTCTCTAAAC] CGAGAAAGATGCCGCCGCCT[cggtgACCCAGGCG GCGACAAG]	second spacer RNF4_2
SLX1	[TTTTCGTCTCTAAAC] CGGTACCGGGGCCGCGTCTA[cggtgACCCAGGCG GCGACAAG]	second spacer SLX1_2
TOP3A	[TTTTCGTCTCTAAAC] CGCTTCGGTCACGTCCCCAC[cggtgACCCAGGCG GCGACAAG]	second spacer TOP3A_2

TOP3B	[TTTTCGTCTCTAAAC] GAGCTGGATCCGCGGTGCGG[cggtgACCCAGGCG GCGCACAAG]	second spacer TOP3B_2
WDHD1	[TTTTCGTCTCTAAAC] CTAGGGCCCGTTCTCCGCAG[cggtgACCCAGGCG GCGCACAAG]	second spacer WDHD1_2
CHTF8	[TTTTCGTCTCTAAAC] CTCGGCTCGCCATTCTTCTC[cggtgACCCAGGCGG CGCACAAG]	second spacer CHTF8_2
SOD1	[TTTTCGTCTCTAAAC] TCGTGCGCCATAACTCGCTAG[cggtgACCCAGGCGG CGCACAAG]	second spacer SOD1_2
GFP	[TTTTCGTCTCTAAAC] TGGTGAACCGCATCGAGCTG[cggtgACCCAGGCG GCGCACAAG]	second spacer GFP_2
LACZ	[TTTTCGTCTCTAAAC] TTCGGTACTTCGTCCACAAA[cggtgACCCAGGCGG CGCACAAG]	second spacer LACZ_2
LUC	[TTTTCGTCTCTAAAC] GGCGCGGTGCGTAAAGTTGT[cggtgACCCAGGCG GCGCACAAG]	second spacer LUC_2
RPL34 (ESSENTIAL GENE)	[TTTTCGTCTCTAAAC] ATCGGCTGCCATCCGAGGAG[cggtgACCCAGGCG GCGCACAAG]	second spacer RPL34_2
RPL11 (ESSENTIAL GENE)	[TTTTCGTCTCTAAAC] TCATGGCGGTGAGTAGCTGG[cggtgACCCAGGCG GCGCACAAG]	second spacer RPL11_2
RPS24 (ESSENTIAL GENE)	[TTTTCGTCTCTAAAC] ATCATATCAACGCGCACGGA[cggtgACCCAGGCG GCGCACAAG]	second spacer RPS24_2
BLM	[TTTTCGTCTCTAAAC] CGCACGAGACTCCTAGCGG[cggtgACCCAGGCG GCGCACAAG]	second spacer BLM_3
WRN	[TTTTCGTCTCTAAAC] CGGCGCACACAGTACACATC[cggtgACCCAGGCG GCGCACAAG]	second spacer WRN_3
RECQL1	[TTTTCGTCTCTAAAC] CTACTCGGGAGTAAAATCTT[cggtgACCCAGGCGG CGCACAAG]	second spacer RecQL1_3
RECQL4	[TTTTCGTCTCTAAAC] CCGCGCTTGCGATCGTCCAG[cggtgACCCAGGCG GCGCACAAG]	second spacer RecQL4_3
RECQL5	[TTTTCGTCTCTAAAC] TGGTCCGCCCAAGAATTAAA[cggtgACCCAGGCGG CGCACAAG]	second spacer RecQL5_3
CHEK1	[TTTTCGTCTCTAAAC] CTCTGAATGTCGGCGGCTCC[cggtgACCCAGGCG GCGCACAAG]	second spacer CHEK1_3
CHEK2	[TTTTCGTCTCTAAAC] CATATGACTCACCGCGTGAG[cggtgACCCAGGCG GCGCACAAG]	second spacer CHEK2_3
SLX4	[TTTTCGTCTCTAAAC] CCGCGGAGCATTGCCTGCGC[cggtgACCCAGGCG GCGCACAAG]	second spacer SLX4_3

DNA2	[TTTTCGTCTCTAAAC] CGCGTCCAGGATGGAGCAGC[cggtgACCCAGGCG GCGCACAAG]	second spacer DNA2_3
EME1	[TTTTCGTCTCTAAAC] CAGGCCTGCGACCGGGGACG[cggtgACCCAGGCG GCGCACAAG]	second spacer EME1_3
GEN1	[TTTTCGTCTCTAAAC] CCGAGTCCGGTCACTGCGGA[cggtgACCCAGGCG GCGCACAAG]	second spacer GEN1_3
RNF4	[TTTTCGTCTCTAAAC] CGCAGCGCGGCTCCCCAAG[cggtgACCCAGGCG GCGCACAAG]	second spacer RNF4_3
SLX1	[TTTTCGTCTCTAAAC] TACTAAGGCGTACGTCAACG[cggtgACCCAGGCG GCGCACAAG]	second spacer SLX1_3
TOP3A	[TTTTCGTCTCTAAAC] CACAGCGACCTGGA ACTACA[cggtgACCCAGGCG GCGCACAAG]	second spacer TOP3A_3
TOP3B	[TTTTCGTCTCTAAAC] CCCCGGGAACAAGGACCGGA[cggtgACCCAGGCG GCGCACAAG]	second spacer TOP3B_3
WDHD1	[TTTTCGTCTCTAAAC] GAGTGGGACTCACCCGGGT[cggtgACCCAGGCG GCGCACAAG]	second spacer WDHD1_3
CHTF8	[TTTTCGTCTCTAAAC] CCAATCCCGGCTCGGCCCTC[cggtgACCCAGGCG GCGCACAAG]	second spacer CHTF8_3
SOD1	[TTTTCGTCTCTAAAC] TTCAGCACGCACACGGCCTT[cggtgACCCAGGCG GCGCACAAG]	second spacer SOD1_3
GFP	[TTTTCGTCTCTAAAC] CCGGGATCACTCTCGGCATG[cggtgACCCAGGCG GCGCACAAG]	second spacer GFP_3
LACZ	[TTTTCGTCTCTAAAC] GTCCCAGACGAAGCCGCCCT[cggtgACCCAGGCG GCGCACAAG]	second spacer LACZ_3
LUC	[TTTTCGTCTCTAAAC] CGGGTGTAATCAGAATAGCT[cggtgACCCAGGCGG CGCACAAG]	second spacer LUC_3
RPL34 (ESSENTIAL GENE)	[TTTTCGTCTCTAAAC] CCCGGGACTTTGCTGCATTG[cggtgACCCAGGCG GCGCACAAG]	second spacer RPL34_3
RPL11 (ESSENTIAL GENE)	[TTTTCGTCTCTAAAC] TCGCCATCCATGGCAGGCCG[cggtgACCCAGGCG GCGCACAAG]	second spacer RPL11_3
RPS24 (ESSENTIAL GENE)	[TTTTCGTCTCTAAAC] CGGATGGCTCGGATACGCGG[cggtgACCCAGGCG GCGCACAAG]	second spacer RPS24_3
TOP1	[TTTTCGTCTCTAAAC] AAGTTCGCATTTGGGCTCAC[cggtgACCCAGGCGG CGCACAAG]	second spacer TOP1_1
FEN1	[TTTTCGTCTCTAAAC] CCGGGAGCGACGGGGTCCGC[cggtgACCCAGGC GGCGCACAAG]	second spacer FEN1_1

EXO1	[TTTTCGTCTCTAAAC] TTCGCGCGCTGTGTAGGCAA[cggtgACCCAGGCG GCGCACAAG]	second spacer EXO1_1
RNASEH1	[TTTTCGTCTCTAAAC] CGCCGGTGACGGAAGTGCGG[cggtgACCCAGGCG GCGCACAAG]	second spacer RNASEH1_1
LIG4	[TTTTCGTCTCTAAAC] CCGGTCTGTTGCCCCACAGA[cggtgACCCAGGCG GCGCACAAG]	second spacer LIG4_1
BRCA1	[TTTTCGTCTCTAAAC] TCTGTCAGCTTCGGAATCC[cggtgACCCAGGCGG CGCACAAG]	second spacer BRCA1_1
MRE11	[TTTTCGTCTCTAAAC] CGGGAGAGAACGGCGTCCGT[cggtgACCCAGGCG GCGCACAAG]	second spacer MRE11_1
CTIP	[TTTTCGTCTCTAAAC] CCGAGATTGCCTCGGGATTC[cggtgACCCAGGCG GCGCACAAG]	second spacer CTIP_1
RNASEH2A	[TTTTCGTCTCTAAAC] CATCGACGCCAGGACGCAA[cggtgACCCAGGCG GCGCACAAG]	second spacer RNASEH2A_1
RAD51B	[TTTTCGTCTCTAAAC] CCTTAAGACTCGGGATCGTC[cggtgACCCAGGCG GCGCACAAG]	second spacer RAD51B_1
XRCC3	[TTTTCGTCTCTAAAC] CCCGCGGGTTCCGCACTCCT[cggtgACCCAGGCG GCGCACAAG]	second spacer XRCC3_1
RAD51C	[TTTTCGTCTCTAAAC] CCGAGCTTAGCAAAGGTAAC[cggtgACCCAGGCG GCGCACAAG]	second spacer RAD51C_1
BRCA2	[TTTTCGTCTCTAAAC] CCTAGTTTCAGAAGCTCGCG[cggtgACCCAGGCG GCGCACAAG]	second spacer BRCA2_1
RAD52	[TTTTCGTCTCTAAAC] TCTTGTTACTCCCTAGCAGT[cggtgACCCAGGCGG CGCACAAG]	second spacer RAD52_1
RTEL	[TTTTCGTCTCTAAAC] CGGCGAACCTTCCAGAACCG[cggtgACCCAGGCG GCGCACAAG]	second spacer Rtel_1
FBH1	[TTTTCGTCTCTAAAC] CGTCTGCGGCTCACGCACT[cggtgACCCAGGCG GCGCACAAG]	second spacer Fbh1_1
FANCM	[TTTTCGTCTCTAAAC] CTACGGTTCCGATCCCATC[cggtgACCCAGGCGG CGCACAAG]	second spacer FANCM_1
TOP1	[TTTTCGTCTCTAAAC] CCGCTTACCTGCGCCTCCTC[cggtgACCCAGGCG GCGCACAAG]	second spacer TOP1_2
FEN1	[TTTTCGTCTCTAAAC] CCCGCCGCTAAGCTGAGAAG[cggtgACCCAGGCG GCGCACAAG]	second spacer FEN1_2
EXO1	[TTTTCGTCTCTAAAC] GTGTTCTGCGTTGCCGGCCG[cggtgACCCAGGCG GCGCACAAG]	second spacer EXO1_2

RNASEH1	[TTTTCGTCTCTAAAC] CCGGCGCTCAACACCGCACT[cggtgACCCAGGCG GCGCACAAG]	second spacer RNASEH1_2
LIG4	[TTTTCGTCTCTAAAC] GCGTGCTTGAGCCCGGTGAC[cggtgACCCAGGCG GCGCACAAG]	second spacer LIG4_2
BRCA1	[TTTTCGTCTCTAAAC] TCCAGGAAGTCTCAGCGAGC[cggtgACCCAGGCG GCGCACAAG]	second spacer BRCA1_2
MRE11	[TTTTCGTCTCTAAAC] TGGGTGCGGATTGTGGGGCT[cggtgACCCAGGCG GCGCACAAG]	second spacer MRE11_2
CTIP	[TTTTCGTCTCTAAAC] CCGAGTGTAGCCCGGGCCCG[cggtgACCCAGGCG GCGCACAAG]	second spacer CTIP_2
RNASEH2A	[TTTTCGTCTCTAAAC] CGGGCACAGGCGAACTCAGG[cggtgACCCAGGCG GCGCACAAG]	second spacer RNASEH2A_2
RAD51B	[TTTTCGTCTCTAAAC] CCAATATCGAAACCCACGAG[cggtgACCCAGGCG GCGCACAAG]	second spacer RAD51B_2
XRCC3	[TTTTCGTCTCTAAAC] CGGGTTCGCACTCCTCTTC[cggtgACCCAGGCG GCGCACAAG]	second spacer XRCC3_2
RAD51C	[TTTTCGTCTCTAAAC] CGCTGGGGCGTGCGGCGTGA[cggtgACCCAGGCG GCGCACAAG]	second spacer RAD51C_2
BRCA2	[TTTTCGTCTCTAAAC] CGGGTGTCTTTTGC GGCGGT[cggtgACCCAGGCG GCGCACAAG]	second spacer BRCA2_2
RAD52	[TTTTCGTCTCTAAAC] TTCATTTCTTGACATCCGG[cggtgACCCAGGCGG CGCACAAG]	second spacer RAD52_2
RTEL	[TTTTCGTCTCTAAAC] TTGCTTTGTGCTCCCGGCGG[cggtgACCCAGGCG GCGCACAAG]	second spacer Rtel_2
FBH1	[TTTTCGTCTCTAAAC] CCGTGTGGAAACTTAACCT[cggtgACCCAGGCGG CGCACAAG]	second spacer Fbh1_2
FANCM	[TTTTCGTCTCTAAAC] TCGGTGGTTGTGCGCCTAAT[cggtgACCCAGGCG GCGCACAAG]	second spacer FANCM_2
TOP1	[TTTTCGTCTCTAAAC] CACAGGCCGTTTCGCGTCT[cggtgACCCAGGCG GCGCACAAG]	second spacer TOP1_3
FEN1	[TTTTCGTCTCTAAAC] CGAACCAAGCTTTAGCCGCC[cggtgACCCAGGCG GCGCACAAG]	second spacer FEN1_3
EXO1	[TTTTCGTCTCTAAAC] CTGGGCGGGGCCGCAAGGAA[cggtgACCCAGGCG GCGCACAAG]	second spacer EXO1_3
RNASEH1	[TTTTCGTCTCTAAAC] ACAGAGTCGCCTTGGCCGCC[cggtgACCCAGGCG GCGCACAAG]	second spacer RNASEH1_3

LIG4	[TTTTCGTCTCTAAAC] CACAGACTTCTCGCCGCCTG[cggtgACCCAGGCG GCGCACAAG]	second spacer LIG4_3
BRCA1	[TTTTCGTCTCTAAAC] CCCGTCAAAGAATACCCATC[cggtgACCCAGGCGG CGCACAAG]	second spacer BRCA1_3
MRE11	[TTTTCGTCTCTAAAC] CGGAATTCAGGTTTACGGCC[cggtgACCCAGGCG GCGCACAAG]	second spacer MRE11_3
CTIP	[TTTTCGTCTCTAAAC] CGGTGGGAAAGCCGACCCCT[cggtgACCCAGGCG GCGCACAAG]	second spacer CTIP_3
RNASEH2A	[TTTTCGTCTCTAAAC] AGACCCGCTCCTGCAGTATT[cggtgACCCAGGCGG CGCACAAG]	second spacer RNASEH2A_3
RAD51B	[TTTTCGTCTCTAAAC] CACAACGGCACCCACATGA[cggtgACCCAGGCG GCGCACAAG]	second spacer RAD51B_3
XRCC3	[TTTTCGTCTCTAAAC] CGGGTCTCCATTGCCCGAGC[cggtgACCCAGGCG GCGCACAAG]	second spacer XRCC3_3
RAD51C	[TTTTCGTCTCTAAAC] CTGCATTTCAAAGCGGAACG[cggtgACCCAGGCG GCGCACAAG]	second spacer RAD51C_3
BRCA2	[TTTTCGTCTCTAAAC] CGCCGGTCACAAATCTGTCC[cggtgACCCAGGCG GCGCACAAG]	second spacer BRCA2_3
RAD52	[TTTTCGTCTCTAAAC] CCGGGGTGGTTCTAGCCGTG[cggtgACCCAGGCG GCGCACAAG]	second spacer RAD52_3
RTEL	[TTTTCGTCTCTAAAC] TCGACTGGAGTCGGTTGAGT[cggtgACCCAGGCG GCGCACAAG]	second spacer Rtel_3
FBH1	[TTTTCGTCTCTAAAC] CAGTGCGTGAGGCCGCAGAC[cggtgACCCAGGCG GCGCACAAG]	second spacer Fbh1_3
FANCM	[TTTTCGTCTCTAAAC] CACGTCTGAAAAAGCGTTCT[cggtgACCCAGGCGG CGCACAAG]	second spacer FANCM_3

Note: Sequences in bracket '[']' are homologous to guide expression vector. First spacer represents oligo sequence cloned into the first guide position, while second spacer represents oligo sequence cloned into the second guide position in the vector.

Supplementary Table 9 Sequence of sgRNAs used in the studies

Target gene	sgRNA sequence
EYFP reporter ¹	TACCTCATCAGGAACATGT
NEAT1 sgRNA1	GCGACAGGGAGGGATGCGCGCC
NEAT1 sgRNA2	GCGCGCCTGGGTGTAGTTGT
NEAT1 sgRNA3	GAAGTGGCTAGCTCAGGGCTTC
CXCR4	CAGGTAGCAAAGTGACGCCGA
SEL1L sgRNA1	GCAGGAAGAGCAGCGGCGAGG
SEL1L sgRNA2	GGGGGCGGATACTGACCCG
SEL1L sgRNA3 ²	GATACTGACCCGAGGACGCCG
ARPC2 sgRNA1 ²	TGTCGGTGAAGCGGCAGTGG
ARPC2 sgRNA2	CAGGCGGGTTCAGGCTTCGG
ERK1 sgRNA1 ²	GGGAGCCCCGTAGAACCGAG
ERK1 sgRNA2	CACCGCCCTCCTCCCCACGG
BRCA1 sgRNA1	GGATTTCCGAAGCTGACAGA
BRCA1 sgRNA2 ²	GCTCGCTGAGACTTCCTGGA
BLM sgRNA1	AGGAAACGGAAGAACCCGAG
BLM sgRNA2	CCTCGCACGCAGACTCCTAG
MET1 sgRNA1	TGAGCAGATGCGGAGCCGAG
MET1 sgRNA2	ACTGGTTCCTGGGCACCGAA
RHOA sgRNA1	AGTTCCCGTGATGCCCCACG
RHOA sgRNA2	GCGCGCCTCCGAGTGCCCAG
CHK2 sgRNA1	GGAGAGTGTGCGGCTCCAGG
CHK2 sgRNA2	CGCAGCCTCAGCCAGCAGAG
CHK1 sgRNA1	GGTGGAGGAATGGTACCAGG
CHK1 sgRNA2 ²	GGGTCTAGATTAGTGAGGGA
CANX ₋₇₀₀ *	TGAAGTGAGATTAGGTGTCA
CANX ₋₃₃₅ *	GTTGGGTTGGAACGCCCCGA
CANX ₋₅₀₅ *	GGTTCTGCTCACGCCCGTAG
CANX ₋₂₂ *	GCTCGCTCGCGCGGCAGCGG
CANX ₋₁ ² *	GGCCGAGGCCTCTTGTTCTG
CANX ₄₇ *	GCGCCGCAGTAAAGAGAGAGG
CANX ₁₅₅ *	TCGGGCCTGTGAGGACCTCG
CANX ₂₆₃ *	CGACGCGCCCGCCGTGAGCG
CANX ₄₇₂ *	GAGTAACTGGGTAAAAGTAT
CANX ₆₄₂ *	ACCAGAAGGAGAACACGCAG
SYVN1 ₋₁₀₃₂ ^{3,4} *	GGAAAACGCAAGGCACAAAG
SYVN1 ₋₇₃₄ ^{3,4} *	AACGTTCCCGGAGGCCAGCC
SYVN1 ₋₆₀₁ ^{3,4} *	ACCTTTGCTGGCCTATAGAA
SYVN1 ₋₅₅₅ ^{3,4,6} *	AACTTATCGCAACCAATCAG
SYVN1 ₋₃₃₉ ^{3,4,6} *	CAGGTGGTACAGCCCCGAAG

SYVN1_-194 ^{3,4*}	ATTACCTTCCGACCACCTCT
SYVN1_-116 ^{3,4,6*}	CCTACGTGGGCCCATAGCAA
SYVN1_-43 ^{3,4*}	ACACCTCACTTCCGGCGGCG
SYVN1_19 ^{3,5*}	CCGCTCAATCCGCGCGACTG
SYVN1_45 ^{3,5*}	GGCGCTGGGTTCCTGGTGAGT
SYVN1_183 ^{3,5*}	GCACCGGCGTCTGAGGTCTC
SYVN1_228 ^{2,3,5,6*}	GTTGCGGGCGTCGCAGGCA
SYVN1_292 ^{3,5*}	GAGAGCAGCAGCGGGACGGG
SYVN1_480 ^{3,5*}	TGAGAGCAGCCAAGGCACAG
SYVN1_702 ^{3,5*}	TAAGTGATCACACTGACGCA
SYVN1_844 ^{3,5*}	TCGTGCTGTGCAAAATAGCC

1: Guide RNA targeting EYFP reporter in the reporter screen assay

2: Guide RNA used in single gene targeting experiments

3: Guide RNA used in 'mixed gRNAs 1-16' group shown in Figure 2b and Supplementary Figure 8

4: Guide RNA used in 'mixed gRNAs 1-8' group shown in Figure 2b and Supplementary Figure 8

5: Guide RNA used in 'mixed gRNAs 8-16' group shown in Figure 2b and Supplementary Figure 8

6: Guide RNA used in 'mixed best 4 gRNAs' group shown in Figure 2b and Supplementary Figure 8

*: The numerical number indicates the position of spacer relative to transcription start site of the target gene.

Supplementary Table 10 Sequences of all qPCR primers in the studies.

Target gene	Forward qPCR primer sequence	Reverse qPCR primer sequence
NEAT1	GTGGCTGTTGGAGTCGGTAT	ATTCACTCCCCACCCTCTCT
CXCR4	ACTACACCGAGGAAATGGGCT	CCCACAATGCCAGTTAAGAAGA
SEL1L	GAGGGGGAAAGTGTCACAGA	GGTCAAAGCTGGTTTCCGTA
SYVN	ACCAGCATCCCTAGCTCAGA	TCCTCAGGCATCTCCTCTGT
ARPC2	CTGGAGGTGAACAACCGCAT	GACCCCATCGAAATCTGCAAA
ERK1	ATGTCATCGGCATCCGAGAC	GGATCTGGTAGAGGAAGTAGCA
BRCA1	CTCAAGGAACCAGGGATGAA	GCTGTAATGAGCTGGCATGA
BLM	CAGACTCCGAAGGAAGTTGTAT G	TTTGGGGTGGTGTAAACAATGA T
MET1	AGCAATGGGGAGTGTAAGAGG	CCCAGTCTTGTACTCAGCAAC
RHOA	GGAAAGCAGGTAGAGTTGGCT	GGCTGTCGATGGAAAAACACAT
CHK1	ATATGAAGCGTGCCGTAGACT	TGCCTATGTCTGGCTCTATTCTG
CHK2	GCGCCTGAAGTTCTTGTTTC	CGTAAACGTGCCTTTGGAT
CANX	GATCCAGACGCAGAGAAACC	CATCCAGGAGCTGACTCACA
TERC	CCCTAACTGAGAAGGGCGTA	GCTCTAGAATGAACGGTGGAA
XIST	AGGTCAGGCAGAGGAAGTCA	CTGCCTCCCGATACAACAAT
ACTB	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
CAS9	GAGTTGACGCCAAAGCAATC	TACCAAACAGGCCGTTCTTC

Supplementary Table 11 Sequence of sgRNA2-7SK template

sgRNA1 tail; 7SK promoter

gttttagagctatgctgaaaagcatagcaagttaaataaggcagtgattttaatccagtccgtacacaactgaaaaagtgcgcaccg
attcgggtgcttttttCCTACGCACCTCGTCGAACCCTCACTGCAGTATTTAGCATGCCCCACCCATCT
GCAAGGCATTCTGGATAGTGTCAAAACAGCCGGAAATCAAGTCCGTTTATCTCAAACTTTAG
CATTTTGGGAATAAATGATATTTGCTATGCTGGTTAAATTAGATTTTAGTTAAATTCCTGCTG
AAGCTCTAGTACGATAAGTAACTTGACCTAAGTGTAAGTTGAGATTCCTTCAGGTTTATAT
AGCTTGTGCGCCGCCTGGGTcaccg

Supplementary Table 12 Sequence of PCR primers for next generation sequencing

PCR 1 primers	Sequence
Forward primer (lethality screen)	CTTTCCTACACGACGCTCTTCCGATCT NNNNNNCCCTTGGAGAAAAGCCTTGTTT
Reverse primer (lethality screen)	GGAGTTCAGACGTGTGCTCTTCCGATCT TTGTACA AGAAAGCTGGGTCTAG
Forward primer (epistasis screen)	CTTTCCTACACGACGCTCTTCCGATCT NNNNNNCTTGTGGAAAGGACGAAACACC
Reverse primer (epistasis screen)	GGAGTTCAGACGTGTGCTCTTCCGATCT CATTG TCTCGAGGTCGAGAATTC

Sequences in bold are adaptor sequence for next generation sequencing.

Supplementary Table 13 PCR cycling conditions to amplify libraries for next generation sequencing

PCR 1

Step	Temperature	Duration
Step 1	95 °C	8 min
Step 2	95 °C	30 sec
Step 3	60 °C	30 sec
Step 4	72 °C	30 sec [#] / 1 min [*]
Repeat step 2-4 for a total a 25-30 cycles		
Step 5	72 °C	2 min

extension time for lethality screen library; * extension time for epistasis screen library

PCR 2

Step	Temperature	Duration
Step 1	95	3 min
Step 2	95	10 sec
Step 3	55	20 sec
Step 4	72	30 sec
Repeat step 2-4 for a total a 5-10 cycles		

Supplementary Note 1 Interpretation of pi scores. A positive pi-score suggests that the fitness effect of the gene pair knockdown is less than expected from individual knockdowns (e.g., loss of two proteins in a pathway or complex), while a negative pi-score means that the fitness effect was more pronounced than expected based on the individual fitness effects from single-gene knockdowns (e.g., synthetic lethal effects).

Supplementary Note 2 This section describes the strategy used to identify and analyze genes with differential expression (DE) in our RNA-seq experiments. Related results are **Supplementary Figure 10-11** and **Supplementary Table 2**.

To analyze raw reads from RNA-sequencing experiments and profile whole transcriptome activity induced by dCas9, dCas9-KRAB and dCAS9-KRAB-MeCP2 repressors, we implemented edgeR quasi-likelihood (edgeR-quasi) pipeline for DE. EdgeR-quasi uses negative binomial generalized linear model¹ with F-tests², and holds advantages over other methods as it provides speed and reliable error rate control. For the DE analyses, we utilize edgeR-quasi and limma-voom pipelines for two independent biological replicates of dCas9, dCas9-KRAB and dCAS9-KRAB-MeCP2 repressors. The sample size of $n=2$ in each repressor group is reasonable due to the low biological variability characteristic of cell culture experiments. Our analysis involves importing of raw counts, filtering of lowly expressed counts, normalization due to library size bias, DE, and clustering testing.

First, we tested DE between each repressor group relative to a control group (delivered sgRNA only) using the edgeR QL functions set to *robust=TRUE* in *glmQLFit* to reduce the number of false positives from genes with extreme dispersions (very low and very high). In **Supplementary Figure 10a-c**, we plot these results on two axes - $\log_2$ fold change (FC) versus averaged $\log_2$ counts per million (CPM), where positive $\log_2$ FC indicates upregulated genes while negative $\log_2$ FC represent downregulated genes relative to the negative control. DE genes at FDR of 5% and corrected using Benjamini-Hochberg method are shown in grey, whereas genes with no significant fold change are shown in black. *glmQLFTest* function identifies all DE only based on statistical significance including genes with small fold changes. To remove such bias, we apply the TREAT method³ which leverages a negative binomial framework using the edgeR's *glmTreat* function, and simultaneously tests for significance and differential fold change at a cutoff of $\log_2$ FC > 1.5. This method is more stringent as it requires larger p-values for calling genes and leads to fewer detected genes. It therefore provides better specificity in recognizing genes with true biological function. The resulting genes are plotted in red on **Supplementary Figure 10a-c** and summarized in the table on **Supplementary Figure 10d**. The identity of these genes are listed in **Supplementary Table 2**. The application of log fold change cutoff of 1.5 results in no downregulated genes, and significantly reduces the number of upregulated genes.

We display expression patterns of transcriptional changes by plotting the top 35 genes with DE in the control, dCas9, dCas9-KRAB and dCas9-KRAB-MeCP2 groups. Clustering of genes with correlated expression provides insights into the biological effects of repressor's activity. To display relative changes in genes across the four groups, we performed scaling such that each gene has a mean of zero and standard deviation of 1. The displayed gene clusters are based on Euclidean distance, $(1-R)^2/2$ between each gene pair where R is the Pearson's correlation of the two genes. A scale bar key of normalized Log₂ CPM represents large negative (colored blue) and positive (colored red) correlations. Genes with large positive correlations correspond to small Euclidean distances and cluster together (**Supplementary Figure 11a**).

Lastly, we examine activated and repressed genes across all three repressor groups (all normalized to the negative control) by applying the limma-voom workflow. We performed linear modelling in limma, and used *lmFit* and *contrasts.fit* functions followed by empirical Bayes model, *eBayes*. This workflow removes variance-associated dependencies on the mean. In **Supplementary Figure 11b-c**, we plot genes on Venn diagrams for downregulation and upregulation where we define significance at 5% p-value with no log fold change cutoff as a less stringent method for examining transcriptome-wide transcriptional offsets for the different repressors. Based on these results, dCas9-KRAB repressor shows the largest clusters of activated genes followed by dCas9-KRAB-MeCP2, and dCas9.

Supplemental Note 3 This section describes the methods and bioinformatics analyses we used to interpret the repressor screens in HAP1, SH-SY5Y and 293T cell lines. Related results are **Supplementary Table 4-6** and **Supplementary Data 2-4**.

Alignment of sequencing reads to reference contig

The sequencing reads were aligned to a reference contig sequence (NNNNNNcccttgagaaaagccttggttNNNNNNNNNNNNNNNNNNNNgtttaagagctaga aatagcaagtttaaataaggctagtcggtatcaactgaaaaagtggcaccgagtcggtgcttttctagaccca gcttctgtacaaaaaaaagaattcctgcagccccgataaaataaaag) using the alignment software BWA-MEM (version 0.7.8)⁴. We used samtools (version 1.2) to extract aligned sequences that have a mapping quality score ≥ 30 ⁵. The 20-nucleotide variable sequence was then match to the 20-nucleotide sgRNA library with 683 guide sequences. Sequences that do not match any of the 683 guide sequences were discarded from the analysis.

Analysis of the repressor sgRNA library

We first sequenced the sgRNA library to determine the distribution of the guide sequences. A total 129,362 sequences map to a guide sequence within the library of 683 guides. However, 53 of the guides were severely underrepresented, i.e. less than 50 reads mapped to the guide ($< 0.04\%$) and

they were removed from all further analysis.

Comparing the guide sequences for all the conditions

For all the conditions, we compared the mapped guides against the corresponding control experiment. The control experiment for all the conditions is the experiment performed without any repressor, i.e. without dCas9, dCas9-KRAB or dCas9-KRAB-MeCP2. For each guide, we compared its frequency for the condition against its frequency for the control. This is done by calculating the odds-ratio (OR) for each guide using the formula,

$$OR_i = \frac{\frac{test_i}{testTotal}}{\frac{control_i}{controlTotal}}$$

where $test_i$ is the number of reads that map to guide i for the test condition and $control_i$ is the number of reads that map to guide i for the control while $testTotal$ and $controlTotal$ are the total number of reads for the test condition and control, respectively. If the guide is enriched in the test condition, the OR would be > 1 while if the guide is depleted, the OR would be < 1 (**Supplementary Data 2-4** list the OR and p-value of each tested guide).

Determining if the essential guides are depleted

Out of the 630 guides from the library, 370 target essential genes (essential guides) while the remaining 260 target non-essential genes. To determine if the essential guides were significantly depleted, we performed a 1-tailed Welch T-test between the $\log_2$ OR of the essential guides versus the $\log_2$ OR of the non-essential guides (**Supplementary Table 4-6**). Also, for the dCas9-KRAB-MeCP2 for HAP1 day 7 and day 14, there were a number of guides that were completely depleted, ie. they had 0 reads for the test condition. This presents a problem as the $\log_2$ OR for those guides would be $-\infty$. To allow for the T-test, we replaced the $\log_2$ OR for those guides to the minimal finite $\log_2$ OR for that condition, which is -7.61 for day 7 and -8.87 for day 14.

Supplementary Note 4 This section describes detailed methods used to analyze the repressor-dual gRNA screen. The analysis was performed using an adapted version of a published workflow for computing genetic interactions using a combinatorial CRISPR-Cas9 knockouts⁶ as follows.

Read alignment

Pair-end reads were first aligned to the sequence immediately upstream and downstream of the 20 bp protospacers, thus allowing us to extract protospacer sequences from each read. The protospacer sequences were then aligned to expected sequences, allowing for 3 mismatches ($3 < \frac{1}{2} * \min(\text{hamming}(g_i, g_j))$ for all i, j). To ensure the robustness of the mapping, constructs with fewer than 5

reads mapped will be excluded from downstream analysis. Only when both reads in a pair were matched with a designed construct sequence was the pair considered for downstream scoring.

Quantification of fitness and gRNA fitness and gRNA–gRNA interactions

We modeled the cell population change over the 14 day duration of the screen using an assumption of exponential growth⁶. For each synthesized construct, we estimated the relative abundance (x_c) of the sub-population of cells harboring the construct. We did this by using the count of the reads that aligned to the designed construct.

$$x_c(t) = a_c + f_c t - \log_2 \sum_c 2^{a_c + f_c t}$$

In which a_c denotes the initial abundance of the construct at day 0, f_c is the fitness of the cells with the construct. Since each gene is targeted by at least 3 gRNAs, gene-gene fitness $f_{g_i g_j}$ are calculated by collapsing f_c from all dual gRNA constructs targeting the same gene pairs.

We quantified genetic interactions as the difference between summation of single gene fitnesses and the double gene fitness, as follows:

$$f_{g_i g_j} = f_{g_i} + f_{g_j} + \pi_{g_i g_j}$$

Single gene fitnesses f_{g_i} , f_{g_j} were obtained by fitting of above equations to the screen data. The residual of the fit, $\pi_{g_i g_j}$, denotes the genetic interaction score (i.e., pi-score) for a gene pair $g_i g_j$. Note that theoretically f_{g_i} , f_{g_j} can be more easily obtained by measuring the fitness of the constructs that contain a negative control gene (lacZ, GFP and luc). We noticed that the negative controls lacZ and luc seemed neutral to cell fitness when a regression model was used to estimate the single gene fitnesses f_{g_i} from the fitness of all constructs $f_{g_i g_j}$ in which f_{g_i} participates, as previously done⁶. Interestingly, GFP gRNAs, expected to serve as a third negative control, showed positive fitness, suggesting that the used GFP targeting guide may be hitting an off-target sequence within the mammalian genome that when mutated causes a fitness benefit. **Supplementary Data 7** lists the genetic interactions uncovered in our screen.

Validation of genetic interaction screens by examining the topology of protein complex network and genetic interactions

We first filtered out gene-pairs that show zero interaction ($\{g_i g_j \mid \pi_{ij} > 0\}$), and the shortest path was computed for the remaining gene-pairs. The shortest paths between gene pairs were computed based on a network of experimentally characterized protein complexes⁷. The number of intermediate genes that connect the gene pair as determined by the protein complex network is treated as the distance between the two genes ($d_{g_i g_j}$). $d_{g_i g_j}$ values are subsequently multiplied by the genetic interaction score π_{ij} derived from the 3 repression screens for gene-pair $g_i g_j$ to get the enrichment score $ES_{ij} = d_{g_i g_j} \cdot \pi_{ij}$. The overall enrichment score can then be calculated by the summing all enrichment

scores ($ES_{\text{overall}} = \sum_{i,j} ES_{ij}$). Permutation tests are performed by shuffling the genetic interaction scores and repeating the above steps for a total of 10000 permutations. As valid screen should have a smaller ES_{overall} compared to a non-valid one, left-tailed p values are reported.

Supplementary Note 5 This section describes detailed materials and methods used to perform circuit experiments.

Cell culture

Transfections were performed on HEK293ft cells using polyethylenimine (PEI). Cells were cultured in Debulcco's Modified Eagle Medium (DMEM), with 10% Fetal Bovine Serum (FBS), non-essential amino acids (NEAA), glutamine, sodium pyruvate, and penicillin/streptomycin. The day prior to transfection, cells were passaged and split into 24-well plates, then allowed to grow to 70-90% confluence. Mixes of DNA were used with a 2:1 PEI:DNA ratio to transfect the 90% confluent cells using standard transfection protocols. All conditions were transfected in quadruplicate. For inducible circuits, doxycycline was added to cultures to a concentration of 2000ng/uL. Media and inducers were changed daily post-transfection until flow cytometry was performed 72 hours later. In wells designated for control experiments, corresponding plasmid DNA under study was replaced by equal amount of empty DNA plasmid. 72 hours post-transfection, cells were collected for flow cytometry assay.

Flow Cytometry and Data analysis

72 hours post-transfection, cells were trypsinized, washed with Hank's Balanced Salt Solution (HBSS) with 2% FBS, then resuspended in 200uL HBSS+FBS. Then flow cytometry was performed using a BD FACSCelesta flow cytometer. 200,000 events were collected, measuring forward scatter (FSC), side scatter (SSC), EBFP expression (BV421), and EYFP expression (BB515). Data were analyzed using FlowJo (FlowJo, LLC). For analysis, all data were compensated using single color and non-transfected controls. Cells were then gated by FSC and SSC to separate healthy, living cells from dead cells and debris. Living cells were further gated by EBFP; laser voltage was set in such a way as to make non-fluorescing cells express at 10^2 or lower, so the BV421 gate was set at 10^2 .

Cells with above 10^2 a.u of EBFP expression were considered transfected and were further analyzed by taking the geometric mean of the population's EYFP (BB515) expression. The geometric means of all samples were exported and further analyzed in Excel (Microsoft) or Prism (GraphPad Software). For single repression circuits, fold repression was calculated by dividing the average EYFP expression of the unrepressed samples by the EYFP of each of the four replicates. These four measures were then used to find average fold repression and standard error of the mean (s.e.m.).

For layered transcriptional repression circuits, fraction of maximum expression was calculated by dividing the average EYFP expression of the

unrepressed samples by the EYFP of each of the four replicates. These four measures were then used to find average fraction of maximum and standard deviation. For inducible layered transcriptional repression circuits, fold derepression was calculated by dividing the EYFP expression of each sample in the +Dox condition by the average EYFP expression of their No Dox counterpart. These four measures were then used to find the average fold derepression and s.e.m.

For transfections involving CXCR4 as the output, after supernatant was removed, cells in each well were labeled with 5 μ l of CD184 (CXCR4) monoclonal antibody (eBioscience™) conjugated to PE and diluted (1:40) in HBSS without calcium and magnesium supplemented with 2% FBS and incubated at 4°C for 30 minutes. Cells were then centrifuged and supernatant was removed. They were then resuspended in 7-AAD solution to exclude dead cells and subjected to FACS. Flow cytometry measurements were performed using BD FACSCelesta with the following settings: EBFP measured with 405 nm laser and a 450/40 filter, EYFP measured with 488 nm laser and a 530/30 filter, 7-AAD measured with a 488 nm laser and a 695/40 filter and CXCR4 measured with 561 nm laser and a 586/15 filter. At least 300,000 events were gathered from each well. Appropriate compensation controls were dedicated for each experiment. Untransfected and unstained cells were used as negative control. Similarly, untransfected cells stained only with CD184-PE antibody were used for CXCR4 control, cells transfected only with EBFP were used as BFP controls. Finally, a mixture of live and dead cells from untransfected wells were used as 7-AAD controls. Data from flow cytometry was analyzed using FlowJo software. Briefly, live cells (7-AAD negative population) were selected and then gated for EBFP expression $>10^3$ A.U. Geometric mean of PE fluorescence level was then calculated in this population.

Statistical analysis

Statistical comparison was performed using one-tailed Student's T-test with a p -value < 0.05 as the threshold for significance. In all synthetic reporter gene circuits, sample size (n) of 4 biologically independent samples (cell cultures) was used for statistical test. In the endogenous gene circuit, n of 3 biologically independent samples (cell cultures) was used for statistical test.

Plasmids

The plasmids used in each circuit, along with a brief description of their function, are as follows:

Circuit		1	2	3	4	5
Background Plasmids and dCas9	ND220	20	20	20	20	25
	114	20	20	20	20	20
	Csy4			50	50	
	dCas9 Variant	70	70	70	70	70
gRNA repression devices	3475	100	200			500
	LR2002			100	200	
	U6/Tal4-sgRNA-CXCR4					50
TALE repressor	473		50		50	50
Reporter	1341	20		20		
	ND252		20		20	
	Midi129					

- dCas9 Variants: CMV promoter driving dCas9, dCas9-KRAB, or dCas9-KRAB-MeCP2 expression.
 - Background plasmids:
 1. ND220: CAG promoter driving EBFP expression. EBFP expression was used to gate transfected cells.
 2. 114: Hef1a promoter driving Gal4-VP16 and rtTA expression; the protein products are separated by a T2A amino acid sequence. The Gal4-VP16 activator binds upstream of the CRP promoter, driving its expression in the absence of a repressor. rtTA, when combined with Dox, binds upstream of the TRE promoter, driving its expression.
 3. Csy4: PGK promoter driving Csy4 expression. The Csy4 protein was used to cleave gRNAs in an mRNA transcript into functional gRNAs.
 - Regulators in single repression circuits:
 1. 3475: U6 promoter driving gRNA expression.
 2. LR2002: TRE promoter driving gRNA expression 3' to iRFP flanked by Csy4 target sites.
 3. U6/Tal14_CXCR4 gRNA: gRNA targeting endogenous CXCR4 locus under U6 promoter (repressed by TALER).
 - Regulators in layered transcriptional repression circuits:
 1. 473: CRP promoter driving TALER expression.
 - Reporters:
 1. 1341: CRP promoter driving EYFP expression.
 2. Midi129: CRP promoter driving EYFP expression, containing two gRNA target sites flanking the mini CMV.
- ND252: pTal promoter driving EYFP expression.

Supplementary References:

1. McCarthy, D. J., Chen, Y, and Smyth, G. K. Differential Expression Analysis of Multifactor RNA-Seq Experiments with Respect to Biological Variation. *Nucleic Acids Research* **40**, 4288–97 (2012).
2. Lund, S. P., Nettleton, D, McCarthy, D. J., and Smyth, G. K. Detecting Differential Expression in RNA-Sequence Data Using Quasi-Likelihood with Shrunken Dispersion Estimates. *Statistical Applications in Genetics and Molecular Biology* **11**, Article 8 (2012).
3. McCarthy, D. J., and Smyth, G. K. Testing Significance Relative to a Fold-Change Threshold Is a TREAT. *Bioinformatics* **25**, 765–71 (2009).
4. Li, H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *ArXiv13033997 Q-Bio* (2013).
5. Li, H. *et al.* The Sequence Alignment/Map format and SAMtools. *Bioinforma. Oxf. Engl.* **25**, 2078–2079 (2009).
6. Shen, JP *et al.* Combinatorial CRISPR-Cas9 screens for de novo mapping of genetic interactions. *Nat Methods.* **14**, 573-576 (2017).
7. Ruepp, A *et al.* CORUM: the comprehensive resource of mammalian protein complexes--2009. *Nucleic Acids Res.* **38** (Database issue), D497-501 (2010).

Supplementary Data 1 A list of differentially expressed genes considered significant at FDR < 0.05 in the RNA-seq experiment.

Supplementary Data 2 A list of all sgRNA sequences in single guide RNA library and their log2 odd ratios in HAP1 lethality screen.

Supplementary Data 3 A list of all sgRNA sequences in single guide RNA library and their log2 odd ratios in SH-SY5Y lethality screen.

Supplementary Data 4 A list of all sgRNA sequences in single guide RNA library and their log2 odd ratios in 293T lethality screen.

Supplementary Data 5 A list of non-essential gene-targeting sgRNAs that showed depletion in lethality screens.

Supplementary Data 6 Summary of rank-ordered genes identified from sgRNA enrichment analysis performed using MAGeCK software.

Supplementary Data 7 Genetic interactions captured through repressor screens.

Supplementary Data 8 DNA sequences and species origins of all protein domains used to construct the different repressors in this study.