Supplementary Materials for

Promoter keyholes enable specific and persistent multi-gene expression programs in primary T cells without genome modification

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Materials and Methods

T-Cell Culture

T-cells were sourced from Vitalant (https://www.vitalant.org/Home.aspx) as either TRIMA LRS (Leukocyte Reduction System) chambers or TRIMA Residuals from TRIMA Apheresis Collection Kits (TerumoBCT; https://www.terumobct.com/trima). CD3+ cells were isolated from donor blood by negative selection using the EasySep™ Human T Cell Isolation Kit (STEMCELL Technologies, 17951) and cryopreserved. Upon thawing, cells were activated with Dynabeads™ Human T-Activator CD3/CD28 for T Cell Expansion and Activation (Gibco, 1132D) at a ratio of 1:1 beads:cells and cultured in T cell media consisting of 10% Heat Inactivated Fetal Bovine Serum (HyClone, SH30071.03) and 1% PenStrep (Corning, 30-002-CI) in RPMI 1640 media (HyClone, SH30027.02) passed through a 0.22 um filter and supplemented with recombinant human IL-2 (Peprotech, 200-02) at a final concentration of 200U/mL. Cells were cultured in 37°C 5% CO2 humidified HeraCell incubators (Thermo Scientific). Cell counts were performed by 0.4% trypan blue dye exclusion (Invitrogen, T10282). T cell media supplemented with rhIL-2 was refreshed every 2-3 days and cell densities were maintained between ~500K-1.5M cells/mL.

Synthetic Repressor Design and Assembly

TAL monomers were cloned and assembled into full length TALs with modifications to established methods[36,37] into a pVAX-based plasmid and included an N-terminal 3x-FLAG tag and SV40 nuclear localization signal (sequences in table S1). Functional domains were selected by literature search for evidence of transcriptional repressive function and annotated DNA-binding domains removed *in silico* before synthesis and incorporation into TAL or heterodimer constructs. Functional domains were added by Infusion cloning (Takara Bio; catalog # 638909) onto the C-terminal end of the TAL. Functional domain constructs contained a 15 amino acid linker domain (GGGGGMDAKSLTAWS) and either an epigenetic-functional domain (e.g. – KRAB) or heterodimer protein (e.g. – ***1*** of the ***1:1'*** pair) (sequences in table S2).

Obligate heterodimers

Mutually orthogonal designed heterodimer pair***s*** were designed and synthesized as described in Chen et al. (*Science, in press*). Heterodimer sequences were appended to sequences encoding TAL-DBDs or effector domains via colinear placement in plasmids used for *in vitro* RNA transcription. Heterodimer epigenetic domain constructs for screening were designed with a T7 promoter, NLS (nuclear localization signal), heterodimer protein (e.g. – ***1'*** of the ***1:1'*** pair), the 15 amino acid linker (see above), and the functional domain (e.g. – KRAB); and generated as double-stranded DNA (Integrated DNA Technologies; gBlocks Gene Fragments).

In vitro Transcription of Synthetic Repressors

mRNA was generated by *in vitro* transcription (IVT) using the T7 mScript™ Standard mRNA Production System (CELLSCRIPT, C-MSC100625), including 5'-capping and poly-A addition reactions. RNA quality control was performed on a Fragment Analyzer Infinity (Agilent) using the standard RNA kit (DNF-471-1000). RNA was quantified based on absorbance at 260 nm. TALE plasmids were digested with Eag1 and column purified prior to IVT. Synthetic DNA was PCR amplified prior to IVT using the KAPA HiFi HotStart ReadyMix (Roche, KK2601) and column purified prior to IVT (Zymo, D4033).

Repressor Delivery to T Cells via Electroporation

Two days after thawing and activation (unless otherwise indicated), CD3+ T cells were spun down at 400xg for 5 minutes, then washed with PBS (Corning, 21-040-CM). Cells were resuspended in the appropriate volume of BTXpress High Performance Electroporation Solution (BTX, 45-0805) to give 250,000 cells per 100 uL electroporation. Cells were multi-channel pipetted into a PCR plate loaded with 1ug *in vitro* transcribed mRNA (for all repressors and heterodimer components) and mixed gently, then transferred to the MOS 96-Multi-Well Electroporation Plate_2mm (BTX, 45-0450). Electroporation was performed using the ECM 830 Square Wave Electroporation System with 96-well HT-200 plate handler at 250 V for 5 ms. Post-electroporation, cells were transferred to a 96-well deep well plate (Axygen, P-DW-11-C-S) with 800 uL of warm T cell media + rhIL-2 and electroporation plate wells were rinsed with media.

Flow Cytometry

Cells were multi-channel pipetted into a 96-well V-bottom plate (Corning, 3894) and spun down (all spins at 500xg for 4 minutes). Cells were washed and spun once with 1X PBS (Corning, 21-040-CM) before staining with fluorophore-conjugated antibodies diluted 1:50 in 1X PBS for 30 minutes in the dark at room temperature. Following staining, FACS buffer (2% Heat-inactivated Fetal Bovine Serum (HyClone, SH30071.03), 1 mM EDTA (OmniPur, 4050) in 1X PBS, passed through a 0.22 um filter) was added to the samples and spun down. Cells were washed and spun down once more in FACS buffer before a final resuspension in FACS buffer for flow cytometry analysis. Antibodies used were: Brilliant Violet 421™ anti-human CD366 (Tim-3) Antibody (BioLegend, 345008); PE/Dazzle™ 594 anti-human CD223 (LAG-3) Antibody (BioLegend, 369332); APC anti-human CD279 (PD-1) Antibody (BioLegend, 329908); PE anti-EGFR Antibody (BioLegend 352904); AlexaFluor700 anti-human CD3 Antibody (BioLegend 300424). Flow analysis was performed on CytoFlex S (Beckman Coulter, B75442). Gating using unstained pooled cells or no mRNA controls.

RNA-seq data collection

RNA was collected from 5E5 to 1E6 cells 48-hours post electroporation, washed twice with PBS, pelleted at 500g for 5-minutes, resuspended in 350uL Buffer RLT (Qiagen), and frozen at -80C. RNA was isolated with Qiagen's RNeasy Micro kit (74004) with on column DNase treatment (RNase-Free Dnase Set #79254). RNA QC\quantification was performed on a Fragment Analyzer (Agilent) using kit # DNF-471-1000 (Standard RNA). RNA libraries were generated using Illumina's Truseq Stranded Total RNA-HT (with Ribo-Zero Gold) kit, #20020599, library QC\quantification was performed on our Fragment Analyzer (Agilent) using kit # DNF-474-1000 (High Sensitivity NGS). Sequencing was performed at 2x76bp on Illumina's Hiseq 4000.

RNA-seq data analysis

Raw sequencing reads were trimmed to remove adapter sequences and aligned to the human genome (hg38/GRCh38, no alts). RNA read mapping informatics was performed using RNA-STAR (2.3.1). RNA tag density tracks were generated from '.bam' files with RNA-STAR (2.4.2a) using '--outWigType bedGraph' and '--outWigStrand Unstranded' options for browser track viewing. Additional gene and transcript level quantification based on the GENCODE (v25) annotation was performed using featureCounts (http://bioinf.wehi.edu.au/featureCounts/) as contained in the Biocondutor Rsubread package (https://bioconductor.org/packages/release/bioc/html/Rsubread.html).

Normalization and differential gene expression analysis between samples was conducted with EdgeR (v3.8). Specifically, 'featureCounts' were used for analysis and a threshold of median > 1 was applied to remove genes that were lowly/not expressed across samples. Subsequently, the following EdgeR functions were applied, in order, to samples within an experiment: DGEList, model.matrix, estimatedGLMCommonDisp, estimatedGLMTrendedDisp, estimatedGLMTagwiseDisp, and glmFit. Subsequently, the glmLRT function was applied to pairwise comparisons of singly or multiplex cells versus control cells to generate gene expression change P-values. Volcano plots were generated by plotting the resulting -log10(P-value) versus the log2(gene expression fold-change) for each group of singly or multiplex repressed cells versus control cells (no repressor RNA treated).

CUT&RUN data collection and analysis

CUT&RUN was performed as describe[38]. Briefly, 4x10E5 CD3+ T cells were rinsed twice with wash buffer (20mM HEPES pH7.5, 150 mM NaCl, 0.5 mM spermidine) supplemented with proteinase inhibitor cocktail (Roche 4693159001) and incubated with 15 µl Concannavalin A-coated beads (Bangs Laboratories BP-531). The beads were set clear on a magnet and resuspended in 100 µl antibody binding buffer (20mM HEPES pH7.5, 150 mM NaCl, 0.5 mM spermidine, 0.02% digitonin, 2mM EDTA pH8) with a primary antibody and incubated overnight at 4C. The beads were set clear on a magnet and washed twice with digitonin-wash buffer (20mM HEPES pH7.5, 150 mM NaCl, 0.5 mM spermidine, 0.02% digitonin) followed by incubation with a secondary antibody (1:100 dilution in 100 µl digitonin-wash buffer) for an additional 1 hour at 4C and washed twice if the host of primary antibody is not rabbit. The beads were resuspended in 100 µl digitonin-wash buffer with 0.5 µl protein-MNase (final 14 µg/ml, kindly provide by Dr. Steven Henikoff from FredHutchinson Cancer Research Center) and incubated at 4C for an hour. The beads were set clear on a magnet and washed twice with digitoxin-wash buffer and then resuspended in 100 µl digitonin-wash buffer. 2 µl of 0.1 M CaCl2 were added to activate MNase and the digestion went on for 30 min at 0C. The digestion was stopped by adding 100 µl stop buffer (340 mM NaCl, 20 mM EDTA pH8, 4mM EGTA, 0.02% digitonin, 20 µg/ml glycogen, 50 µg/ml RNase A) and the reaction mixture was incubate at 37C for 10 min. The beads were spun at 16,000 g for 5 min at 4C and set clear on magnet. Supernatant was collected for proteinase K digestion (30C at 55C) and DNA were cleaned by phenol-chloroform-iso amyl alcohol extraction and ethanol precipitation. Antibodies used were obtained from following suppliers: FLAG (Sigma M2 1804), rabbit anti-mouse IgG (Jackson ImmunoResearch 31-005-048), H3K4me3 (Cell Signaling 9751), H3K9me3 (Abcam 8898). Sequencing libraries were generated with Takara's Thruplex DNA-Seq kit (R400677 +R400660), library QC\quantification was performed on our Fragment Analyzer (Agilent) using kit # DNF-474-1000 (High Sensitivity NGS). Sequencing was performed at 2x76bp on Illumina's Hiseq 4000. Raw sequencing reads were trimmed to remove adapter sequences and aligned to the human genome (hg38/GRCh38, no alts). Alignment was performed using bwa (version 0.7.12) with the following parameters: "-Y -l 32 -n 0.04" and "-n 10 -a 750" for alignment and mate-pairing (aln and sampe, respectively). Peak calls were generated with Hotspot (hotspot2; http://github.com/Altius/hotspot2). Read depth normalized signal, QC metrics were produced as described in https://www.encodeproject.org/pipelines/ENCPL202DNS/.

Immunofluorescence Imaging

T cells were debeaded, washed twice with PBS, and resuspended in 1x PBS for a final cell density of 1-2 million cells per ml. 30 ul of cell solution was then seeded into Poly-L-Lysine (PLL)-coated wells of a sterile 24-well plate (CellVis) and incubated for 20 minutes at RT. Cells were then fixed with 4% PFA (Polysciences Inc, #18814-10) in PBS for 10 minutes at room temperature. Fixed cells were washed 3x with 1x PBS, permeabilized with 0.5% Triton X-100 in PBS for 10 minutes at room temperature, blocked for 1 hour with 2% BSA (Jackson Immunoresearch, #001-000-161), and then incubated for 2 hours at room temperature with primary antibodies against FLAG (F1804, mouse, 1:1000 dilution) in 2% BSA/1x PBS. Subsequently, cells were washed 3x with 0.05% Tween-20 (Bio-rad, #161-0781) in PBS, and then incubated for 1 hr with donkey anti-mouse Cy5 (1:500 dilution, #711-166-152, Jackson Labs) secondary antibody in 2% BSA/1x PBS. Lastly, cells were counterstained with DAPI (100ng/mL in 1x PBS) for 10 minutes at room temperature and washed 3 times with 0.05% Tween in PBS prior to mounting on glass slides using Prolong Gold (Molecular Probes P36930). Each wash step lasted 3 minutes at room temperature and was performed on a shaker (Stovall Inc). Samples were imaged using an inverted Nikon Eclipse Ti widefield microscope equipped with an Andor Zyla 4.2CL10 CMOS camera with a 4.2-megapixel sensor and 6.5μm pixel size (18.8mm diagonal FOV). Focused 2D cell images were acquired using a 60x Nikon Plan Apo 1.4 NA oil objective. Acquired images were subject to 100 rounds of iterative blind deconvolution using Microvolution software (Microvolution, CA) to minimize the effect of out-of-focus blurring that is inherent to widefield microscopy optics. Deconvolved images were processed using in-house Matlab (version 2017B, Mathworks, Natick, MA) scripts to numerically estimate the FLAG protein content in every cell nucleus, and for downstream statistical analysis

CAR-T cells

CAR-T cells were manufactured using lentivirus delivery of a 3rd generation anti-CD19 CAR containing FMC63 scFv, CD28 and 4-1BB co-stimulatory domains, and a truncated EGFR tag (Lenti-EF1a-CD19-EGFRt-3rd-CAR Vector, Creative Biolabs). Primary human T cells were activated with Dynabeads as described above and transfected by electroporation with repressor mRNA at 48 hours post activation. Transfected cells along with no-mRNA transfected controls were allowed to recover for 24 hours after electroporation and then transduced with lentivirus encoding the CAR on RetroNectin (Takara Bio) according to manufacturers protocol at an MOI of 5 and in the absence of serum. At 24 hours post transduction beads and virus were removed and CAR-T cells were allowed to expand in media with rhIL-2 until day 11 post activation when they were washed with PBS and administered to mice. Prior to using in animals, CAR-T cells were analyzed by flow cytometry for CAR expression (via EGFR staining) and expression of immune checkpoint genes.

Mouse Studies

Animal experiments were conducted at the Fred Hutchinson Cancer Center, Comparative Medicine department (Seattle, WA) according to an approved IACCUC protocol. Female NOD SCID gamma (NSG) mice aged 6-8 weeks were implanted intravenously with $5x10^5$ NALM-6-luc-GFP tumor cells (human B-ALL cancer cells expressing CD19, GFP, and luciferase, provided generously by Stanley Riddell, Fred Hutch) and tumors were measured by total bioluminescent flux using a Xenogen Imaging System (Perkin Elmer). Each experimental arm contained 5 mice. At 4 days post tumor implantation mice were imaged and randomized into treatment arms based on baseline tumor burden. On day 5 post implantation mice were dosed intravenously

with $2.5x10^6$ or $2.5x10^5$ anti-CD19 CAR T cells either treated or untreated with synthetic repressor mRNA. Peripheral blood was collected via retroorbital bleeding at weekly intervals into EDTA-coated tubes at room temperature. Red blood cell lysis was performed using (1X RBC Lysis Buffer, eBiosciences Cat. # 333-57) according to manufacturer's protocol. Flow cytometry was performed as described above. For the rechallenge experiment, mice were re-injected intravenously with $5x10^5$ NALM-6-luc-GFP tumor cells at 3 weeks post CAR-T infusion and followed for an additional 3 weeks. When mice reached the study end point they were euthanized according to protocol.

References:

36. T. Cermak *et al.*, Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. *Nucleic Acids Res* **39**, e82 (2011).

37. T. Sakuma *et al.*, Efficient TALEN construction and evaluation methods for human cell and animal applications. *Genes Cells* **18**, 315-326 (2013).

38. P. J. Skene, J. G. Henikoff, S. Henikoff, Targeted in situ genome-wide profiling with high efficiency for low cell numbers. *Nat Protoc* **13**, 1006-1019 (2018).

Supplementary Figure Legends

Fig. S1

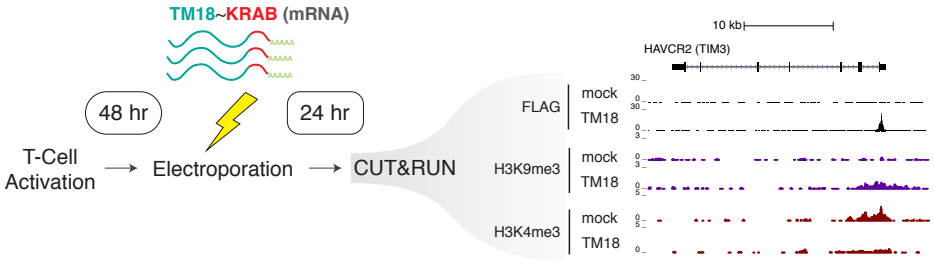

Fig. S1. Synthetic repressors trigger chromatin state modification at target sites. Activated T cells were electroporated with no RNA (mock) or the TIM3 repressor TM18, and CUT&RUN was performed 24 hours later. Chromatin binding by TM18 at the HAVCR2 (TIM3) locus was confirmed by CUT&RUN against the FLAG tag present on the TM18 repressor (right, black top panels showing sharp peak at the TIM3 promoter). Downstream chromatin modifications were determined by CUT&RUN, confirming localized deposition of the repressive mark H3K9me3 (purple), and removal of the activity-associated mark H3K4me3 (red).

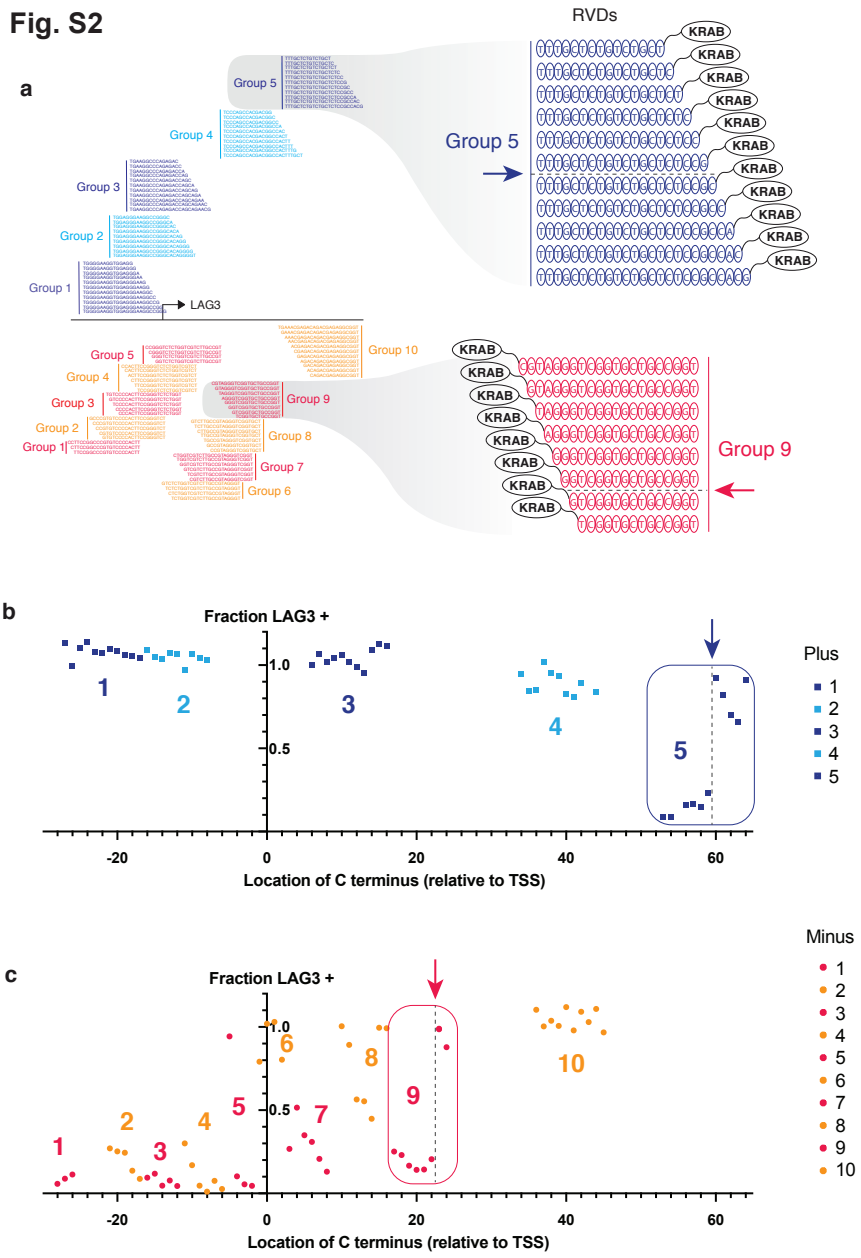

Fig. S2. Base-resolution triggering of LAG3 repression by synthetic repressors. (a) T-DBDs targeting seed sequences in the LAG3 promoter were sequentially extended by one repeat unit to produce groups of T-DBD-KRABs with different positioning of the C-terminal KRAB. Two example groups are shown, targeting either the plus or minus strand. Sequences are aligned to corresponding positions along the LAG3 promoter. (b) Fraction LAG3+ cells at 2 days post-transfection as measured by flow cytometry, normalized to no RNA controls, for plus strand targeted T-DBDs. X-axis indicates the location of the KRAB domain relative to the TIM3 TSS. Group 5 is highlighted to demonstrate loss/gain of repression activity when the KRAB domain was moved by one nucleotide. (c) Fraction LAG3+ cells as in (b) for minus strand targeted T-DBDs.

Group 9 is highlighted to demonstrate loss/gain of repression activity when the KRAB domain was moved by one nucleotide.

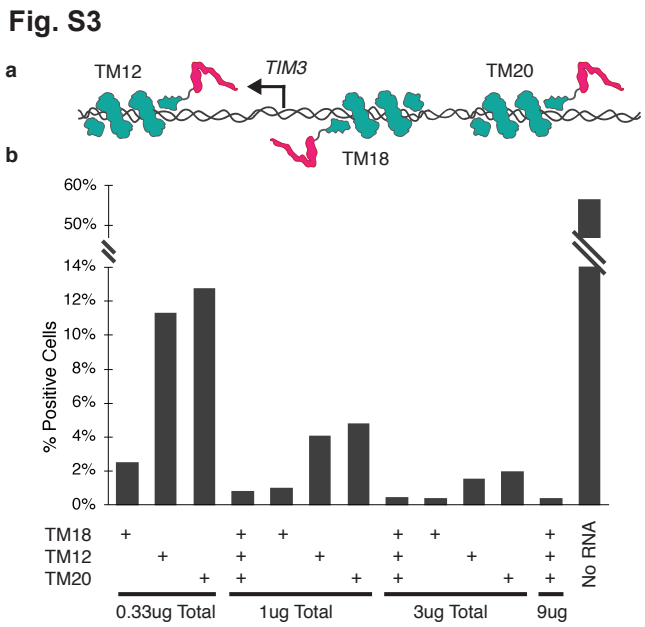

Fig. S3. Synthetic repressor activity is dose-dependent and can be multiplexed at an individual promoter to quantitatively modulate repression. (a) Experimental diagram showing the relative positions of three T-DBD-KRABs targeting TIM3 used to test dose dependence and effect of multiplexing at a single promoter. (b) Percent TIM3-positive cells at 48 hours post-transfection with the indicated amounts of the indicated T-DBD-KRAB combinations was measured by flow cytometry. Multiplexing repressors at a single promoter can achieve better than additive effects.

Fig. S4

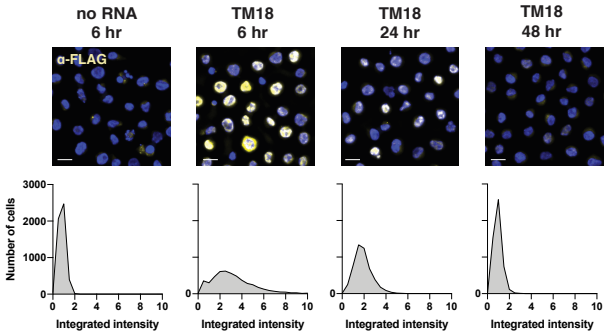

Fig. S4. Synthetic repressor protein lasts for less than two days post-electroporation in primary T cells. Cells were electroporated with either no RNA or the TIM3 repressor TM18 at time 0, and TM18 protein levels were determined by anti-FLAG immunofluorescence (yellow) for up to 48 hours. Nuclear DAPI staining is shown in blue. Scale bar indicates 10 μM. Histograms show integrated anti-FLAG fluorescence intensity per nucleus over a population of cells with a bin size of 0.5.

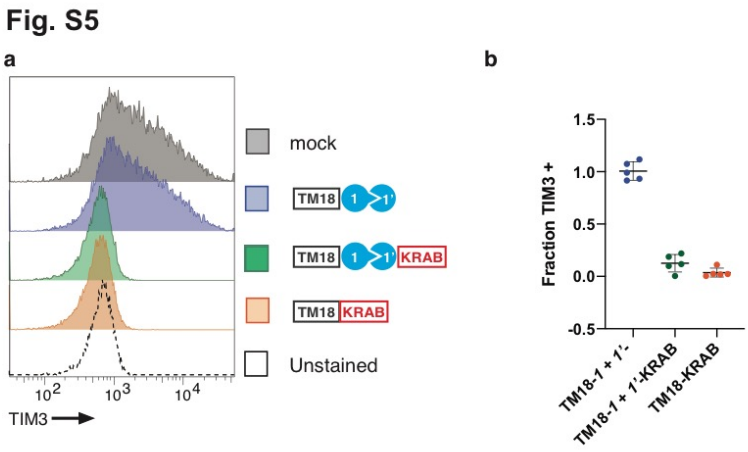

Fig. S5. Split synthetic repressors are equipotent with single chain repressors. (a) Flow cytometry plots at 48 hours post-transfection showing expression of TIM3 in primary T cells separately treated with the indicated construct(s), diagrammed to the right. From top, no mRNA (mock transfection), TM18-DBD fused to the *1* heterodimer co-administered with the *1'* heterodimer lacking an epi-domain, TM18-DBD fused to *1* co-administered with KRAB fused to *1'*, and the covalently fused TM18-DBD-KRAB repressor. (b) Quantification of aggregated results from 5 independent experiments showing the fraction of T cells expressing TIM3, normalized to mock transfection, at 48 hours post-transfection with the indicated construct.

Fig. S6

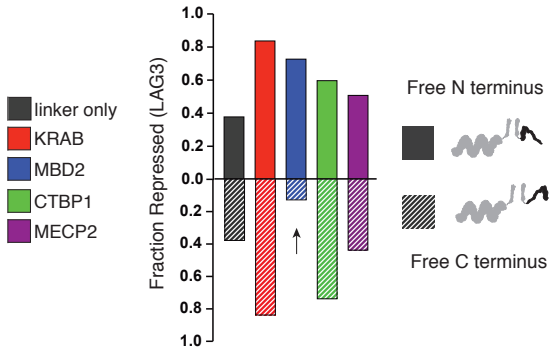

Fig. S6. Repressor domains may exhibit orientation-specific potency. The effect of translation order on epigenetic domain efficacy was tested by reversing the order of domain-heterodimer translation. Mirror plot shows fraction of cells repressed for LAG3 normalized to mock (no RNA) condition. Solid colors indicate translation order of ***1′***-linker-epigenetic effector domain; dashed colors indicate the reverse order (effector domain-linker-***1′***). Values indicate average of two electroporations in the same experiment. Epigenetic domain efficacy can differ depending on whether the N-terminus or C-terminus is freely presented and available to endogenous binding partners.

Fig. S7

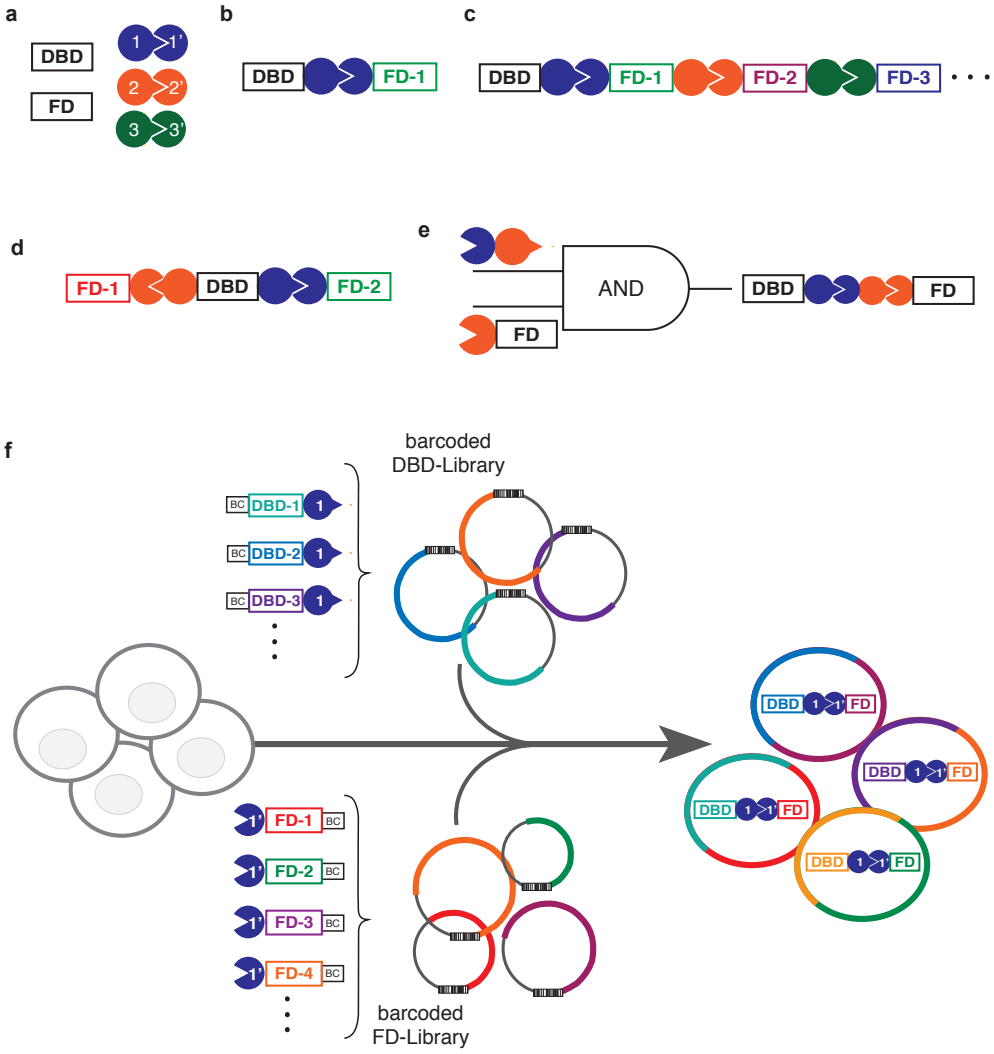

Fig. S7. Obligate heterodimers enable complex combinatorial engineering and deployment of cell programming effectors. (a) Building blocks for epigenetic engineering system. DBD: DNA-Binding Domain; FD: Functional Domain; ***1*:*1′*-*3*:*3′***: pairs of obligate heterodimers. (b) DNA-binding domain complexed with an functional domain. (c) Chain of alternating heterodimer pairs and functional domains can generate molecules of arbitrary chain length. (d) Bifunctional molecule with 2 functional domains flanking a single DNA-binding domain. (e) Logic gates can be generated using heterodimer pairs and inputs. (f) Conceptual diagram of DNA-binding domain library versus functional domain library screening. Cells are transduced with vectors encoding barcoded constructs.

Table S1: Synthetic repressor target sequences

Name	**Target sequence (5'-3')**	**RVD sequence (N-C)**
TM18	TGGCAGTGTTACTATAA	NH-NH-HD-NI-NH-NG-NH-NG-NG-NI-HD-NG-NI-NG-NI-NI
PD02	TGGTGGGGCTGCTCC	NH-NH-NG-NH-NH-NH-NH-HD-NG-NH-HD-NG-HD-HD
LG09	TGCCGTTCTGCTGGTCT	NH-HD-HD-NH-NG-NG-HD-NG-NH-HD-NG-NH-NH-NG-HD-NG
TM12	TGGCAATCAGACACCCGGGTG	NH-NH-HD-NI-NI-NG-HD-NI-NH-HI-HD-NI-HD-HD-HD-NH-NH-NH-NG-NH
TM20	TGCCACACTACACACAT	NH-HD-HD-NI-HD-NI-HD-NG-NI-HD-NI-HD-NI-HD-NI-NG

Table S2: Functional domain and heterodimer sequences

Domain	Sequence
KRAB	RTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSL GYQLTKPDVILRLEKGEEP
MBD2	MRAHPGGGRCCPEQEEGESAAGGSGAGGDSAIEQGGQGSALA PSPVSGVRREGARGGGRGRGRWKQAGRGGGVCGRGRGRGRG RGRGRGRGRGRGRPPSGGSGLGGDGGGCGGGGSGGGGAPRRE PVPFPSGSAGPGPRGPRATESGKRMSKLQKNKQRLRNDPLNQN KGKPDLNTTLPIRQTASIFKQPVTKVTNHPSNKVKSDPQRMNE QPRQLFWEKRLQGLSASDVTEQIIKTMELPKGLQGVGPGSNDET LLSAVASALHTSSAPITGQVSAAVEKNPAVWLNTSQPLCKAFIV TDEDIRKQEERVQQVRKKLEEALMADILSRAADTEEMDIEMDS GDEA
MeCP2	MASSPKKKRKVEASVQVKRVLEKSPGKLLVKMPFQASPGGKGE GGGATTSAQVMVIKRPGRKRKAEADPQAIPKKRGRKPGSVVA AAAAEAKKKAVKESSIRSVQETVLPIKKRKTRETVSIEVKEVVKP LLVSTLGEKSGKGLKTCKSPGRKSKESSPKGRSSSASSPPKKEHH HHHHHAESPKAPMPLLPPPPPPEPQSSEDPISPPEPQDLSSSICKE EKMPRAGSLESDGCPKEPAKTQPMVAAAATTTTTTTTTVAEKY KHRGEGERKDIVSSSMPRPNREEPVDSRTPVTERVSEF
CtBP1	MGSSHLLNKGLPLGVRPPIMNGPLHPRPLVALLDGRDCTVEMP ILKDVATVAFCDAQSTQEIHEKVLNEAVGALMYHTITLTREDLE KFKALRIIVRIGSGFDNIDIKSAGDLGIAVCNVPAASVEETADSTL CHILNLYRRATWLHQALREGTRVQSVEQIREVASGAARIRGETL GIIGLGRVGQAVALRAKAFGFNVLFYDPYLSDGVERALGLQRVS TLQDLLFHSDCVTLHCGLNEHNHHLINDFTVKQMRQGAFLVN TARGGLVDEKALAQALKEGRIRGAALDVHESEPFSFSQGPLKD APNLICTPHAAWYSEQASIEMREEAAREIRRAITGRIPDSLKNCV NKDHLTAATHWASMDPAVVHPELNGAAYRYPPGVVGVAPTG IPAAVEGIVPSAMSLSHGLPPVAHPPHAPSPGQTVKPEADRDH ASDQL
MBD3	MRVRYDSSNQVKGKPDLNTALPVRQTASIFKQPVTKITNHPSN KVKSDPQKAVDQPRQLFWEKKLSGLNAFDIAEELVKTMDLPKG LQGVGPGCTDETLLSAIASALHTSTMPITGQLSAAVEKNPGVWL NTTQPLCKAFMVTDEDIRKQEELVQQVRKRLEEALMADMLAH VEELARDGEAPLDKACAEDDDEEDEEEEEEPDPDPEMEHV
ZNF45	MTKSKEAVTFKDVAVVFSEEELQLLDLAQRKLYRDVMLENFRN VVSVGHQSTPDGLPQLEREEKLWMMKMATQRDNSSGAKNLK EMETLQEVGLRYLPHEELFCSQIWQQITRELIKYQDSVVNIQRTG CQLEKRDDLHYKDEGFSNQSSHLQVHRVHTGEKP
ZNF133	MAFRDVAVDFTQDEWRLLSPAQRTLYREVMLENYSNLVSLGIS FSKPELITQLEQGKETWREEKKCSPATCPDPEPELYLDPFCPPGFS SQKFPMQHVLCNHPPWIFTCLCAEGNIQPGDPGPGDQEKQQQ ASEGRPWSDQAEGPEGEGAMPLFGRTKKRTLGAFSRPPQRQPV SSRNGLRGVELEASPAQSGNPEETDKLLKRIEVLGFGTV
ZNF140	MSQGSVTFRDVAIDFSQEEWKWLQPAQRDLYRCVMLENYGHL VSLGLSISKPDVVSLLEQGKEPWLGKREVKRDLFSVSESSGEIKDF

	SPKNVIYDDSSQYLIMERILSQGPVYSSFKGGWKCKDHTEMLQE NQGCIRKVTVSHQEALAQHMNISTVERP
ZNF283+b	MESRSVAQAGVQWCDLGSLQAPPPGFTLFSCLSLLSSWDYSSGF SGFCASPIEESHGALISSCNSRTMTDGLVTFRDVAIDFSQEEWEC LDPAQRDLYVDVMLENYSNLVSLGYQLTKPDVILRLEKGEEPIF RNNWKCKSIFEGLKGHQEGYFSQMIISYEKIPSYRKSKSLTPHQRI HNTE
1 heterodimer	MDSDEHLKKLKTFLENLRRHLDRLDKHIKQLRDILSENPEDERV KDVIDLSERSVRIVKTVIKIFEDSVRKKE
1′ heterodimer	MDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKV VETYVELLKRHEKAVKELLEIAKTHAKKVE
9 heterodimer	GTKEDILERQRKIIERAQEIHRRQQEILEELERIIRKPGSSEEAMKR MLKLLEESLRLLKELLELSEESAQLLYEQR
9′ heterodimer	GTEKRLLEEAERAHREQKEIIKKAQELHRRLEEIVRQSGSSEEAK KEAKKILEEIRELSKRSLELLREILYLSQEQKGSLVPR
1′-9	GSDDKELDKLLDTLEKILQTATKIIDDANKLLEKLRRSERKDPKV VETYVELLKRHEKAVKELLEIAKTHAKKVEGSEGSGSEGSGTKE DILERQRKIIERAQEIHRRQQEILEELERIIRKPGSSEEAMKRMLKL LEESLRLLKELLELSEESAQLLYEQR
linker	GGGGGMDAKSLTAWS