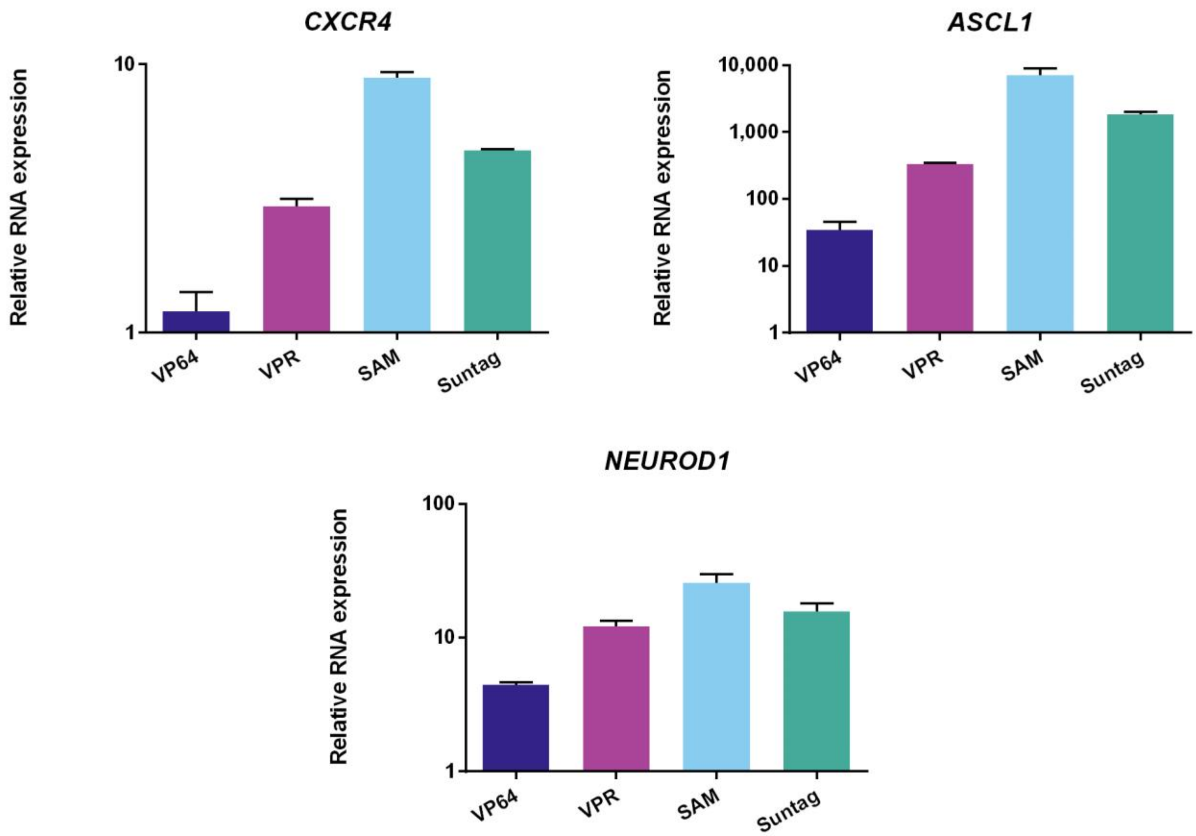
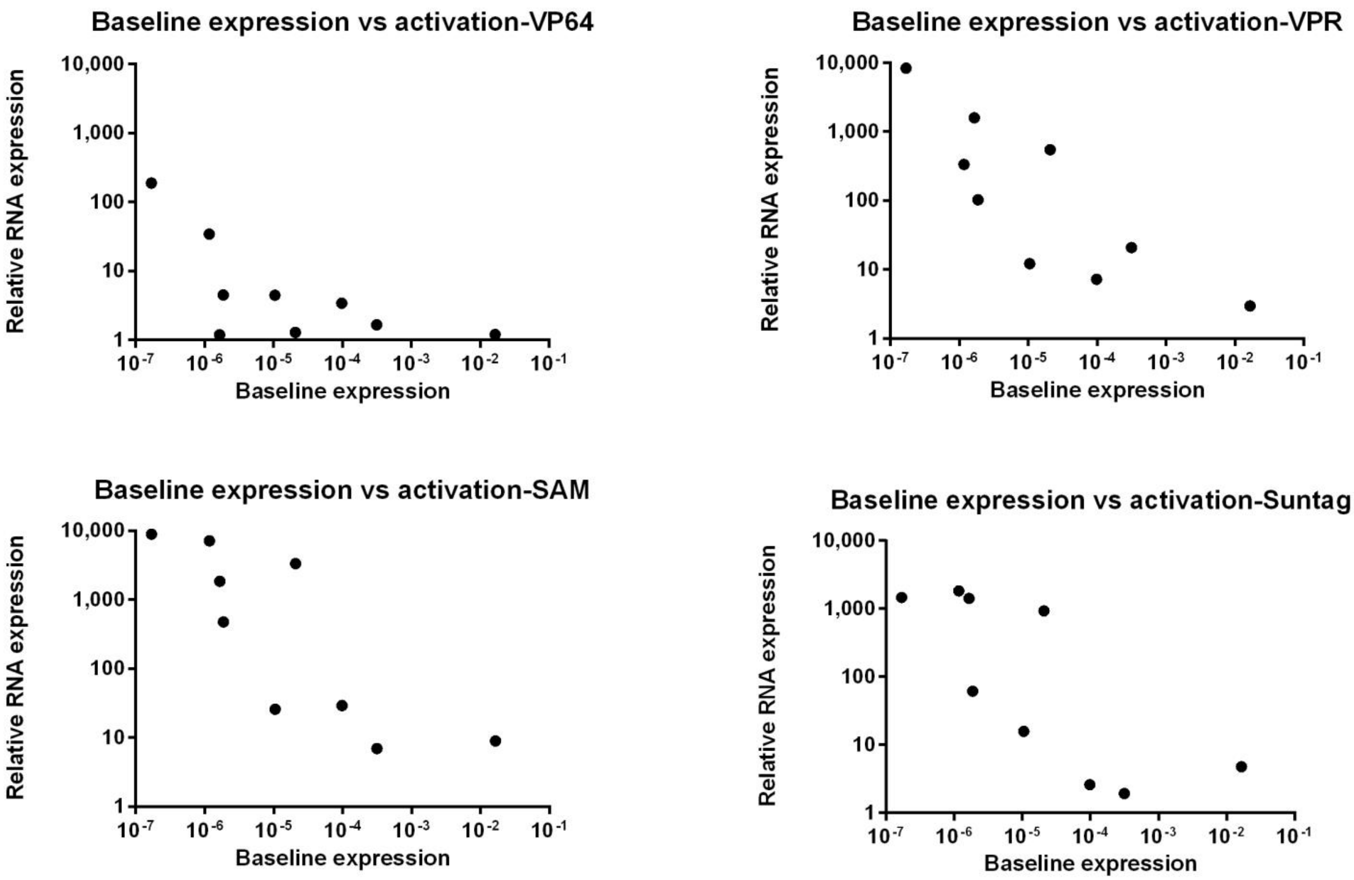


Supplementary Figure 1

Additional tests of activators on endogenous genes in HEK293T cells.

Data represents the mean + s.e.m. (*n* = 2 independent transfections).

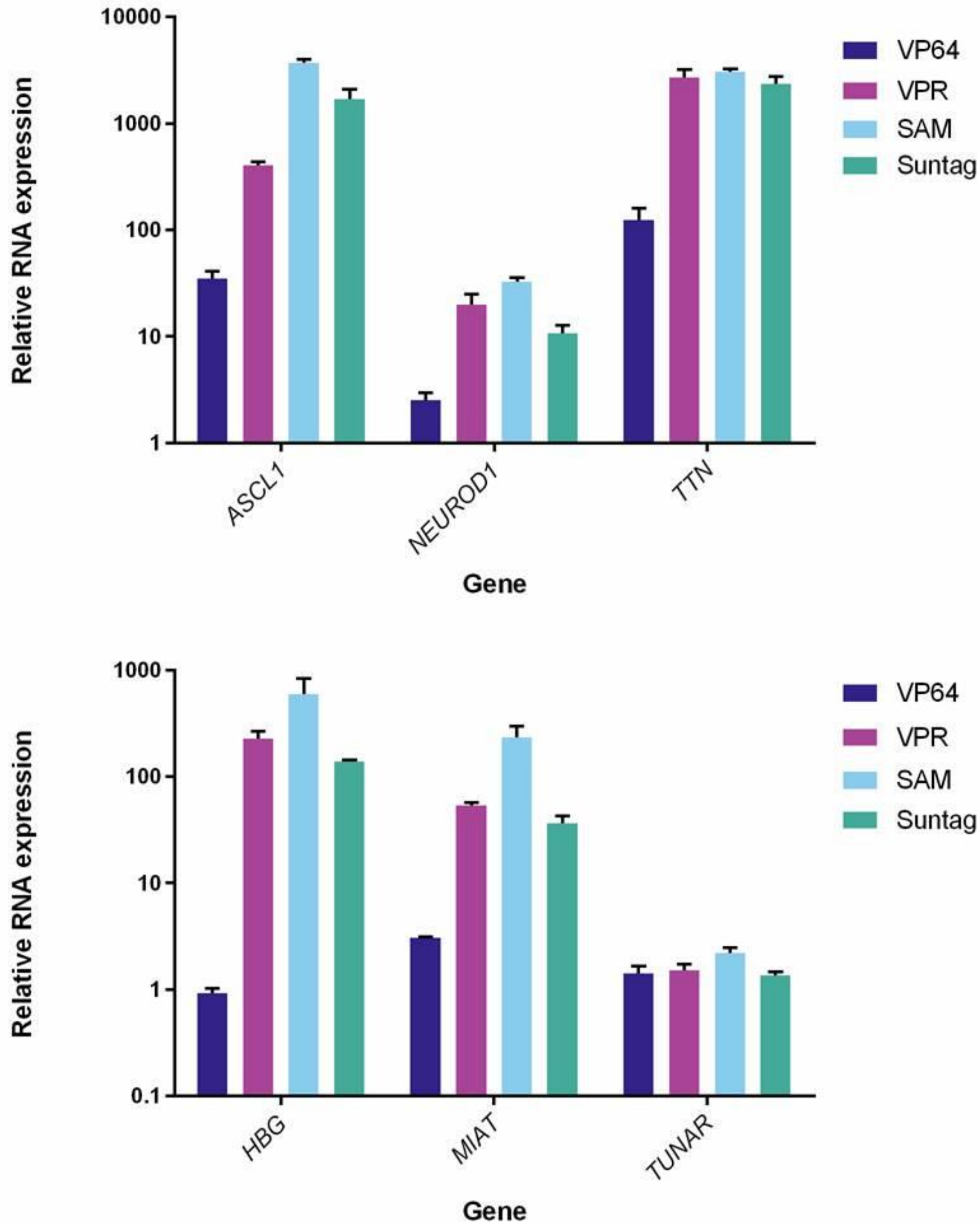


Supplementary Figure 2

Effect of Baseline Level of Expression on Activation.

Data represents the mean activation from **Fig. 2b** and **Supplementary Fig. 1.** Baseline expression data calculated from the Puc19 control sample for each experiment.

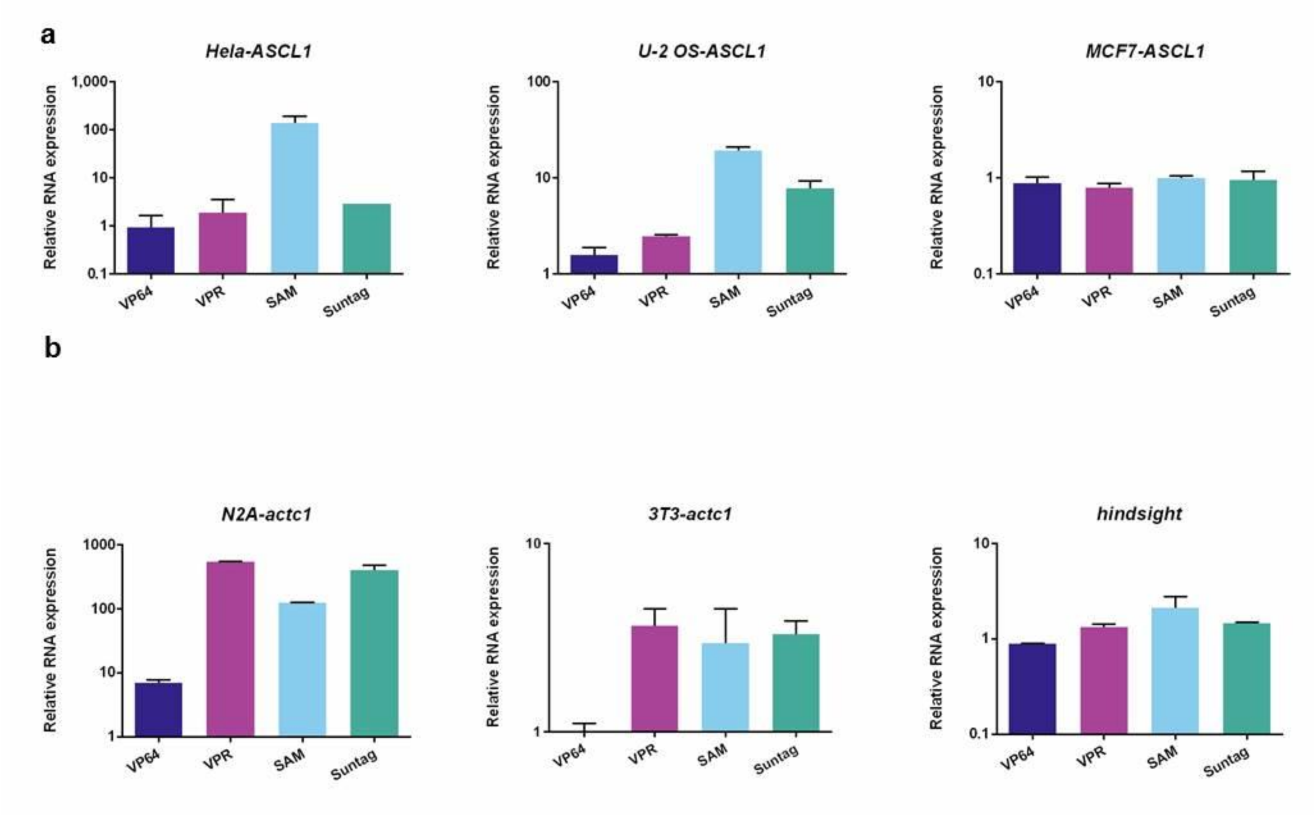
Nature Methods: doi:10.1038/nmeth.3871



Supplementary Figure 3

Multiplexed activation of two sets of three endogenous human genes.

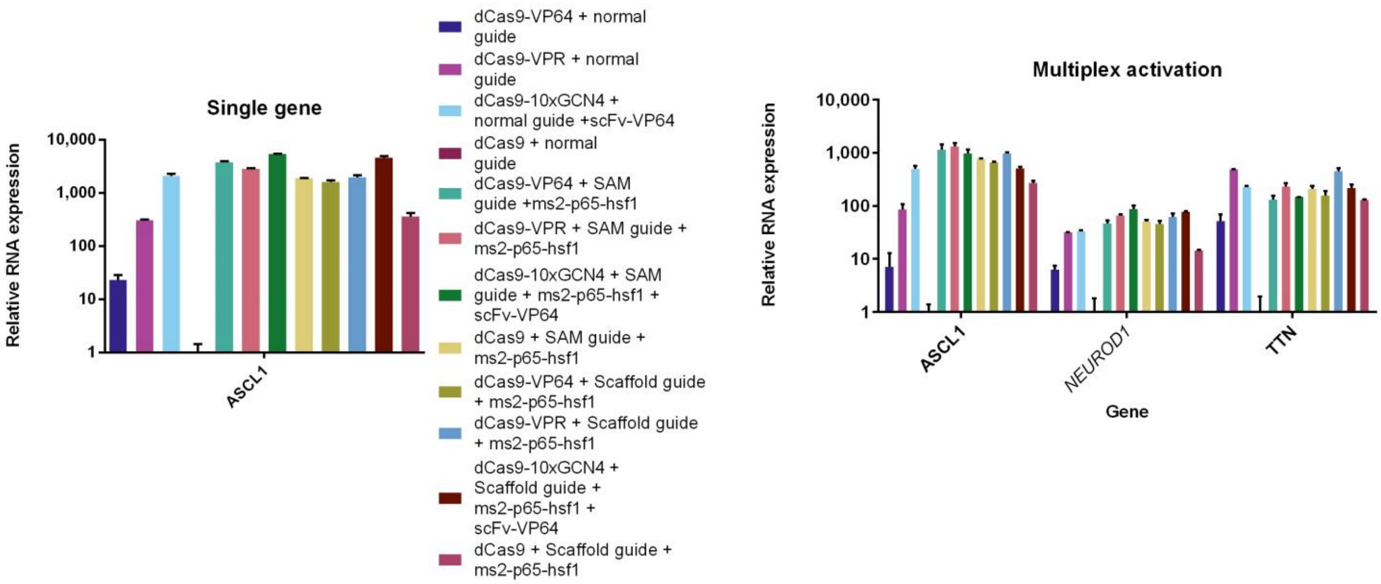
Data indicate the mean + s.e.m (*n* = 2 independent transfections).



Supplementary Figure 4

Additional tests of activators on endogenous genes in Hela, U-2 OS, MCF7, N2A, NIH-3T3, and S2R+ cells.

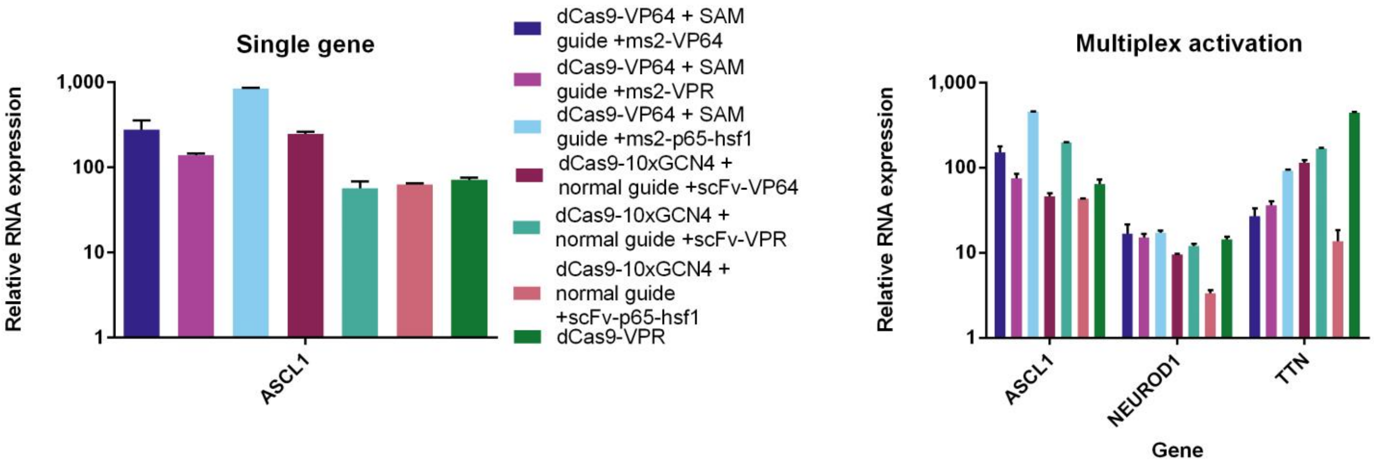
(**a**) Each human cell line was transfected with the indicated activators and guides. Data indicate the mean + s.e.m (n = 2 independent transfections) (**b**) Activation of endogenous genes in mouse and fly. Data indicate the mean + s.e.m (n = 2 independent transfections).



Supplementary Figure 5

Combinations of different activator components.

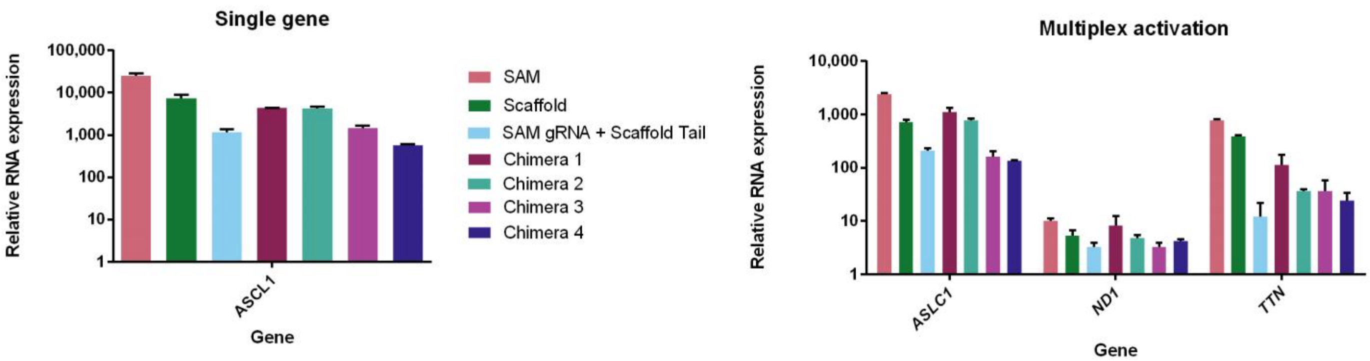
Samples were tested on both a single gene and a panel of multiplexed genes. Data represents the mean + s.e.m. (*n* = 2 independent transfections) See **Supplementary Note 1** for more explanation on the canonical activator components. For the purposes of this figure, dCas9-10xGCN4 + normal guide + scFV-VP64 represents the canonical Suntag activator and dCas9-VP64 + SAM guide + ms2-p65-hsf1 represents the canonical SAM activator.



Supplementary Figure 6

Recruitment of different activation domains to SAM and Suntag.

dCas9-VP64 denotes the SAM version of VP64. Data represents the mean + s.e.m. (*n* = 2 independent transfections). See **Supplementary Note 1** for more explanation on the canonical activator components. For the purposes of this figure, dCas9-VP64 + SAM guide + ms2-p65-hsf1 represents the canonical SAM activator.



Supplementary Figure 7

SAM and Scaffold gRNA chimeras.

All samples contain dCas9 recruiting MCP-p65-hsf1 via different hairpin designs. All chimeras represent the SAM gRNA with the Scaffold tail appended on it with various parts disabled by either point mutation or deletion. Chimera 1 has the first MS2 extension of the SAM gRNA deleted. Chimera 2 has the second MS2 extension of the SAM gRNA deleted. Chimera 3 has the MS2 loop of the scaffold gRNA disabled via point mutation while Chimera 4 has the F6 loop of the scaffold gRNA disabled via point mutation. For full chimera gRNA tail sequences, refer to the Plasmids section of the supplement. Addition of the Scaffold tail to the end of the SAM gRNA resulted in worse activation than each system alone and there was no method of disabling any part of the hybrid hairpin which led greater activation. Data represents the mean + s.e.m. (*n* = 2 independent transfections).

Supplementary Table 1 gRNA			
Organism	Gene	Location	Sequence
Homo sapiens	*ASCL1*	-181	CGGGAGAAAGGAACGGGAGG
Homo sapiens	*NEUROD1*	-221	AGGTCCGCGGAGTCTCTAAC
Homo sapiens	*TTN*	-169	CCTTGGTGAAGTCTCCTTTG
Homo sapiens	*HBG*	-100	CTTGACCAATAGCCTTGACA
Homo sapiens	*MIAT*	-219	ATGCGGGAGGCTGAGCGCAC
Homo sapiens	*TUNAR*	-275	GGCGGCGTCGGGGTCCCTAC
Homo sapiens	*CXCR4*	-116	GCAGACGCGAGGAAGGAGGGCGC
Homo sapiens	*RHOXF2*	-44	ACGCGTGCTCTCCCTCATC
Homo sapiens	*ACTC1*	-229	TGGCGCCCTGCCCTCTGCTG
Homo sapiens	*ASCL1*	-442	TCCAATTTCTAGGGTCACCG
Homo sapiens	*ASCL1*	-557	AAGAACTTGAAGCAAAGCGC
Homo sapiens	*NEUROD1*	-164	ACCTGCCCATTTGTATGCCG
Homo sapiens	*NEUROD1*	-33	AGGGGAGCGGTTGTCGGAGG
Homo sapiens	*CXCR4*	-162	CCGACCACCCGCAAACAGCA
Homo sapiens	*CXCR4*	-193	GCCTCTGGGAGGTCCTGTCCGGCTC
Drosophila melanogaster	*wingless*	-337	GGAAATGGAAAAACTCTGCCCGG
Drosophila melanogaster	*twist*	-156	GCATCGGCAGGTATGACGTCAGG
Drosophila melanogaster	*hindsight*	-180	ATTTGAAACGAAGAATGAGAAGG
Mus musculus	*ttn*	-143	AATTTAGCACTGCCAATCAG
Mus musculus	*hbb-bh1*	-108	AGAGAGTCTGGGCAAGACAG
Mus musculus	*actc1*	-204	CTCCCAGACCATGTAAGGAA

Supplementary Table 2 qPCR			

Primers			
Organism	Gene	Forward	Reverse
Homo sapiens	*ACTB*	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
Homo sapiens	*ASCL1*	CGCGGCCAACAAGAAGATG	CGACGAGTAGGATGAGACCG
Homo sapiens	*NEUROD1*	GGATGACGATCAAAAGCCCAA	GCGTCTTAGAATAGCAAGGCA
Homo sapiens	*TTN*	TGTTGCCACTGGTGCTAAAG	ACAGCAGTCTTCTCCGCTTC
Homo sapiens	*HBG1*	AGATGCCACAAAGCACCTG	CTGCAGTCACCATCTTCTGC
Homo sapiens	*MIAT*	TGGCTGGGGTTTGAACCTTT	AGGAAGCTGTTCCAGACTGC
Homo sapiens	*TUNAR*	AGAACAAGGGGGAAAGCTCG	ATACCCCACCCGCTTTTGAG
Homo sapiens	*CXCR4*	ACTACACCGAGGAAATGGGCT	CCCACAATGCCAGTTAAGAAGA
Homo sapiens	*RHOXF2*	GGCAAGAAGCATGAATGTGA	TGTCTCCTCCATTTGGCTCT
Homo sapiens	*ACTC1*	ATGTGTGACGACGAGGAGAC	CGGACAATTTCACGTTCAGCA
Drosophila melanogaster	*wingless*	CCAAGTCGAGGGCAAACAGAA	TGGATCGCTGGGTCCATGTA
Drosophila melanogaster	*twist*	AAGTCCCTGCAGCAGATCAT	CGGCACAGGAAGTCAATGTA
Drosophila melanogaster	*hindsight*	ACATCCGGTGCCACAATTA	AGGGATGAAGCCGAGGATAGC
Mus musculus	*ttn*	GACACCACAAGGTGCAAAGTC	CCCACTGTTCTTGACCGTATCT
Mus musculus	*hbb-bh1*	CTGGGAAGGCTCCTGATTGT	GTTCTTAACCCCCAAGCCCA
Mus musculus	*actc1*	ATGTGTGACGACGAGGAGAC	CGGACAATTTCACGTTCAGCA

Supplementary Note 1. Description of main activators.

dCas9-VP64 consists of dCas9 with four copies of the VP16 activation domain fused the C-terminus of dCas9. dCas9-VPR consists of dCas9 fused to the activation domains VP64, p65, and rta with each activation domain separated by a short amino acid linker. This results in six activation domains fused to the C-terminus of dCas9. SAM (synergistic activation mediator) consists of dCas9-VP64 with a modified gRNA which recruits the construct, MCP-p65-hsf1. The modified gRNA within the SAM system contains two MS2 hairpins which protrude from the gRNA at the tetraloop and stem loop 2 and are what are used to recruit MCP-p65-hsf1. MCP-p65-hsf1 binds these MS2 hairpin sequences as a dimer, resulting in four sets of the activation domains p65 and hsf1 being recruited. Along with the VP64 component, this means that 12 domains are theoretically recruited within the SAM system. Suntag consists of a dCas9 component with a chain of 10 peptide epitopes called GCN4 fused repetitively to the C-terminus of dCas9. Along with this epitope containing dCas9 protein a single chain antibodies with specificity to the GCN4 epitope fused to VP64 is also expressed in trans. This results in the theoretical recruitment of 10 VP64 molecules or 40 activation domains recruited to one locus. Scaffold consists of a dCas9 component with a modified gRNA similar to the SAM gRNA except only three copies of MCP-VP64 are recruited[14]. The gRNA has one normal MS2 hairpin which recruits a dimer of MCP-VP64 and an F6 aptamer which recruits two MCP-VP64 protein resulting in a theoretical total of sixteen activation domains being recruited by Scaffold. P300 is the catalytic core of the human acetyltransferase p300 protein directly fused to dCas9[18]. VP160 consists of 10 repeats of VP16 fused to the C-terminus of dCas9 instead of the usual four repeats used in dCas9-VP64[19]. VP64-dCas9-BFP-VP64 is dCas9 with VP64 fused to the N-terminus and BFP-VP64 fused to the C-terminus resulting in eight activation domains driving transcription[17].

Supplementary Note 2: Sequences for original constructs
ms2-VPR
SV40-NLS + MS2 + VP64 + SV40-NLS + p65 + rta
CCTAAGAAAAAGAGGAAGGTGGCGGCCGCTGACTACAAGGATGACGACGATAAATCTA
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CTGTCGCCCCAAGCAACTTCGCTAACGGGATCGCTGAATGGATCAGCTCTAACTCGCGT
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CACCATCAAAGTCGAGGTGCCTAAAGGCGCCTGGCGTTCGTACTTAAATATGGAACTAA
CCATTCCAATTTTCGCCACGAATTCCGACTGCGAGCTTATTGTTAAGGCAATGCAAGGTC
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AGCCTGAGGCCGGCTCCGCTATTAGTGACGTGTTTGAGGGCCGCGAGGTGTGCCAGCCA
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ATCTGAACCTGGACTCACCCCTGACCCCGGAATTGAACGAGATTCTGGATACCTTCCTGA
ACGACGAGTGCCTCTTGCATGCCATGCATATCAGCACAGGACTGTCCATCTTCGACACAT
CTCTGTTT**

All scFv proteins are downstream of an SV40 promoter and upstream of a GB1 sequence and a Rex NLS

scFv-VPR

scFv + sfGFP + VP64 + SV40-NLS + p65 + rta

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CACAGGACTGTCCATCTTCGACACATCTCTGTTTGGAGGAGGATCTCGGACCGAA

scFv-p65-hsf1

scFv + sfGFP + p65 + hsf1

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GGCTTCGCCGAGGACCCCACCATCTCCCTGCTGACAGGCTCGGAGCCTCCCAAAGCCAA
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Scaffold Variants
All Scaffold variants start after guide sequence and end before the terminator

SAM gRNA + Scaffold Tail with point mutations disabling the second half of the tail
**GTTTTAGAGCTAGGCCAACATGAGGATCACCCATGTCTGCAGGGCCTAGCAAGTTAAAA
TAAGGCTAGTCCGTTATCAACTTGGCCAACATGAGGATCACCCATGTCTGCAGGGCCAA
GTGGCACCGAGTCGGTGCGGGAGCACATGAGGATCACCCATGTGCGACTCCGAGAGTA
ACTGGGGAGTCTTCCC**

SAM gRNA + Scaffold Tail with point mutations disabling the first half of the tail
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SAM gRNA + Scaffold Tail
GTTTTAGAGCTAGGCCAACATGAGGATCACCCATGTCTGCAGGGCCTAGCAAGTTAAAA TAAGGCTAGTCCGTTATCAACTTGGCCAACATGAGGATCACCCATGTCTGCAGGGCCAA GTGGCACCGAGTCGGTGCGGGAGCACATGAGGATCACCCATGTGCGACTCCCACAGTC ACTGGGGAGTCTTCCC

SAM gRNA with first ms2 hairpin extension deleted + Scaffold Tail
GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGGCCAACA TGAGGATCACCCATGTCTGCAGGGCCAAGTGGCACCGAGTCGGTGCGGGAGCACATGA GGATCACCCATGTGCGACTCCCACAGTCACTGGGGAGTCTTCCC

SAM gRNA with second ms2 hairpin extension deleted + Scaffold Tail
GTTTTAGAGCTAGGCCAACATGAGGATCACCCATGTCTGCAGGGCCTAGCAAGTTAAAA TAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCGGGAGCACATGA GGATCACCCATGTGCGACTCCCACAGTCACTGGGGAGTCTTCCC

Supplementary References:

41. Nishimasu, H. *et al.* Crystal structure of Cas9 in complex with guide RNA and target DNA. *Cell* **156,** 935–949 (2014).