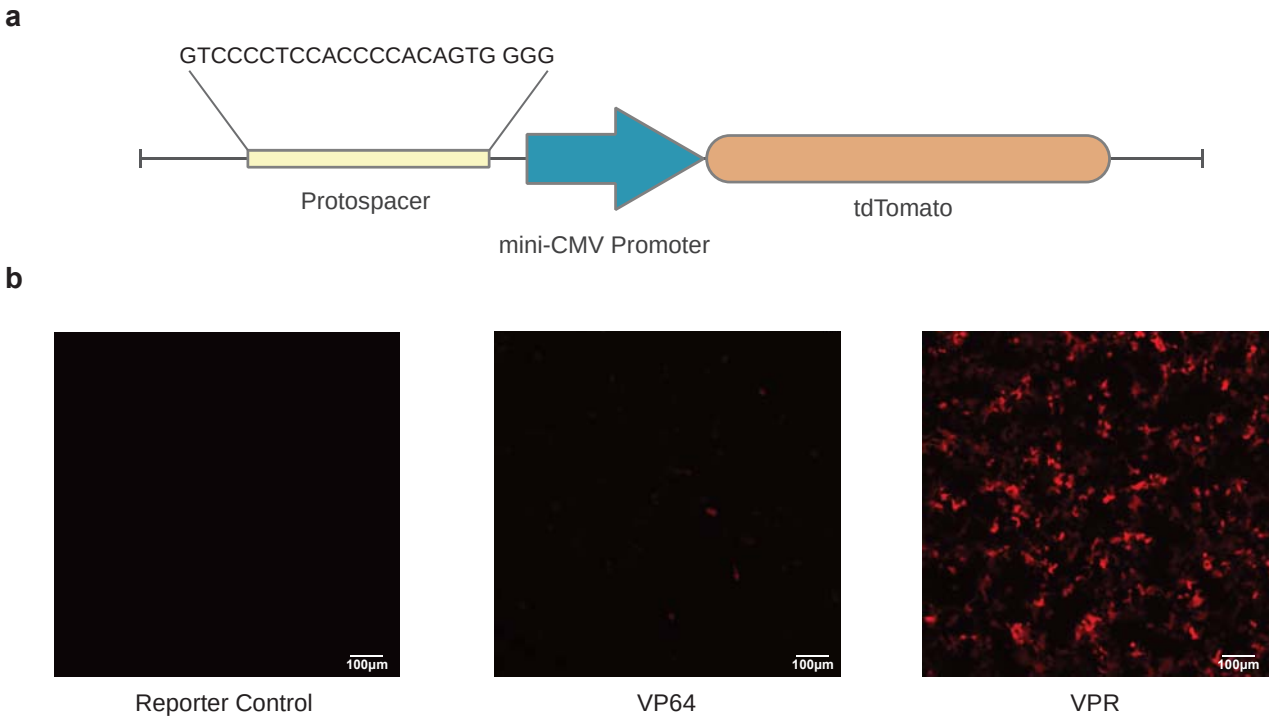
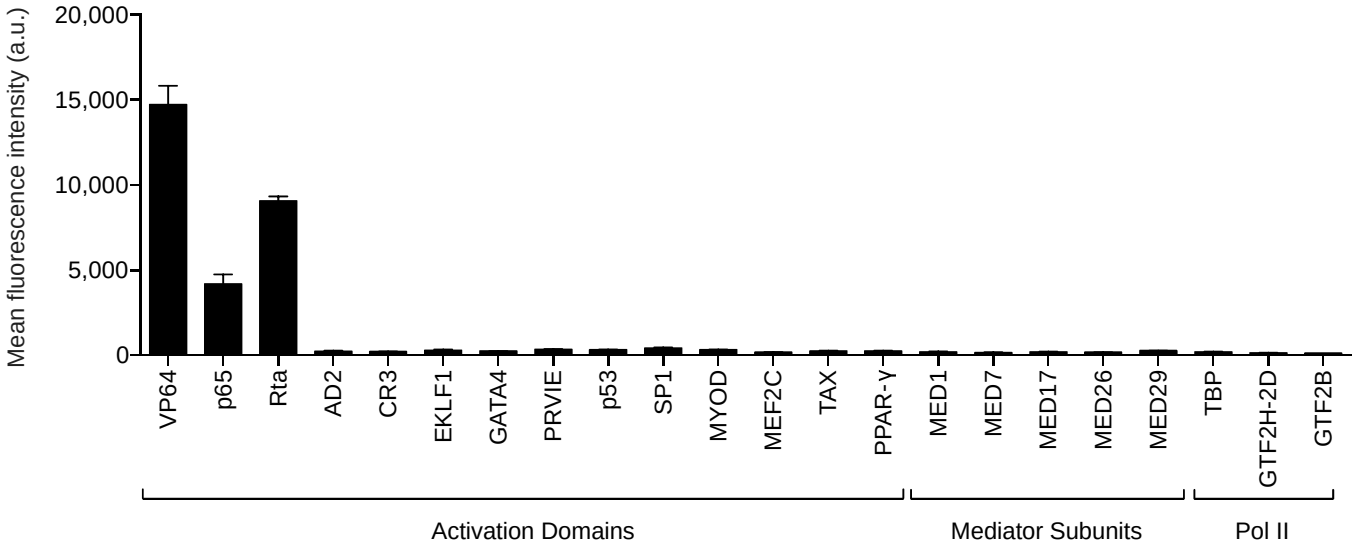


Supplementary Figure 1

dCas9 transcriptional reporter design.

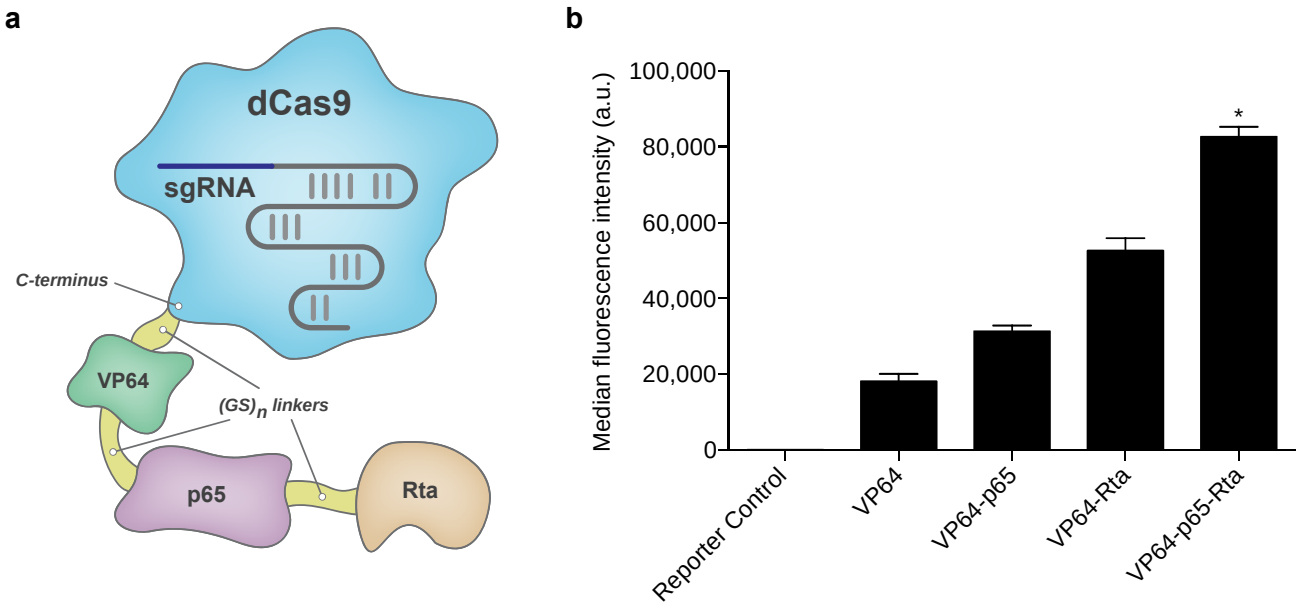
(**a**) Fluorescent reporter construct consists of a tdTomato or sfGFP reporter downstream of a minimal CMV promoter, with an upstream dCas9 binding site (sequence of protospacer and PAM (GGG) are shown, separated by a space), tdTomato version of the reporter is illustrated. (**b**) Fluorescence microscopy images of HEK 293T cells all transfected with dCas9 reporter and the corresponding guide RNA along with the indicated dCas9 activator. Scale bar represents 100 µm.



Supplementary Figure 2

Targeted screen to identify activation domains that function with dCas9.

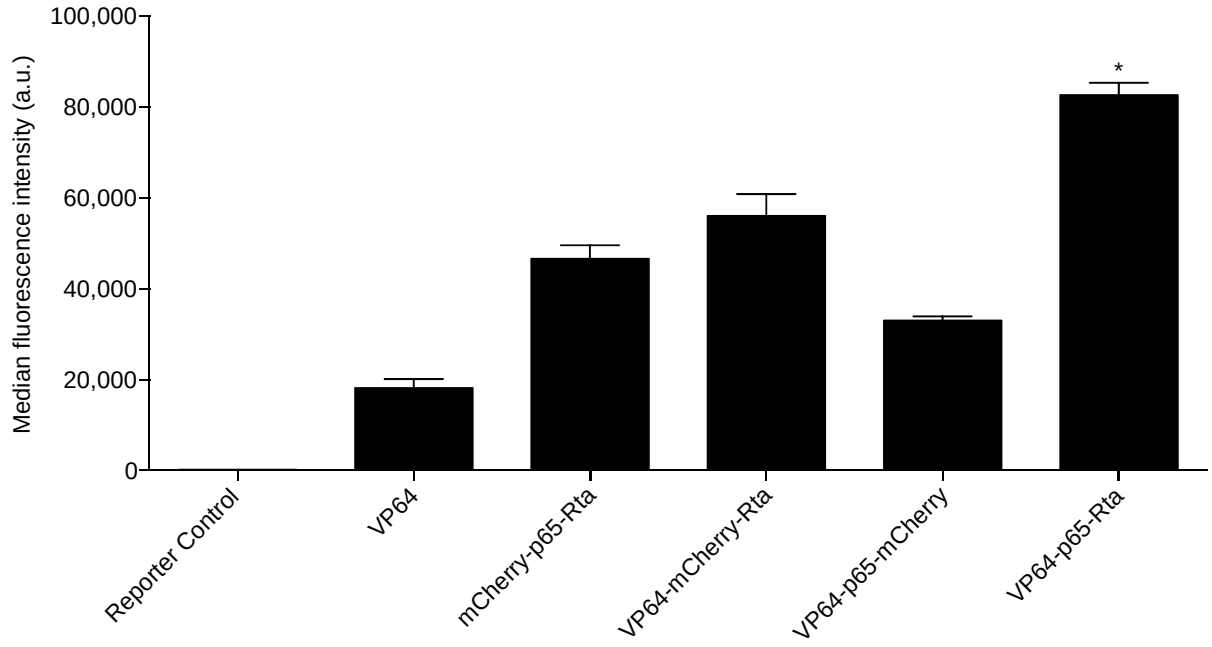
Fluorescent reporter assay quantifying the amount of transcriptional activation for the various dCas9 fusion proteins. The particular activation domain, mediator complex member or RNA polymerase subunit fused to the C terminus of dCas9 is listed. The tested activation domains represent minimal activation domains. Mediator and RNA polymerase members fused to dCas9 were full length cDNAs. Data are shown as mean fluorescence ± s.e.m., n = 2 independent transfections.



Supplementary Figure 3

Serial fusion of activation domains to dCas9.

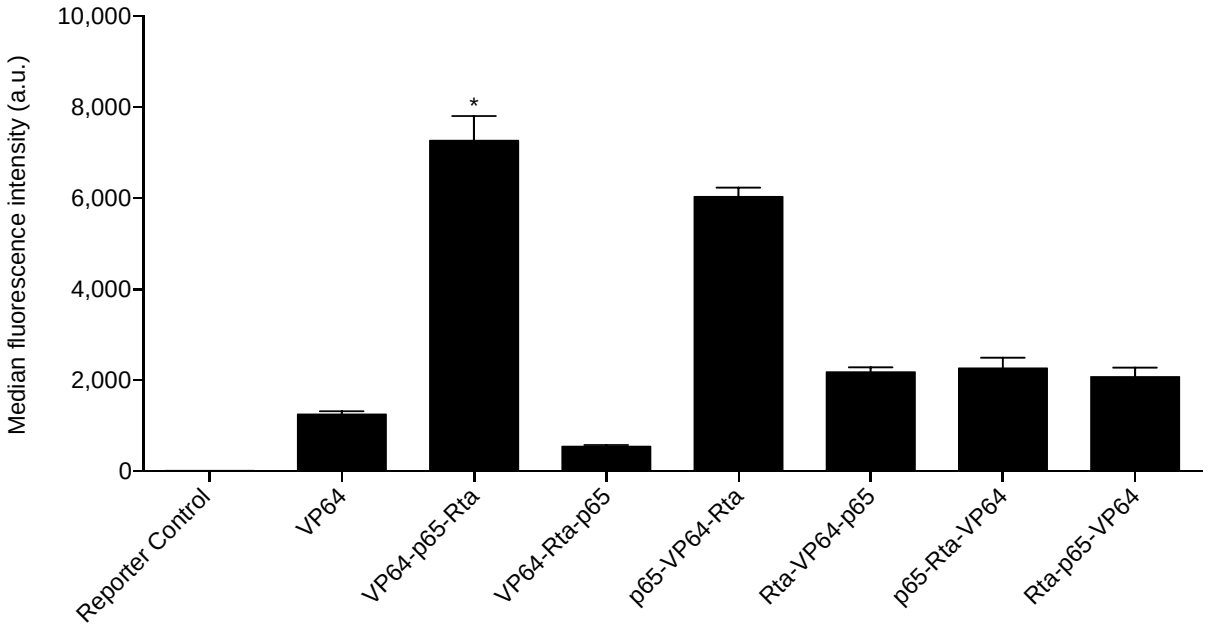
(**a**) Transcriptional activation via Cas9 was performed by fusing activation domains to the C terminus of a nuclease-null dCas9 protein. The tripartite VPR activator consisting of VP64-p65-Rta activation domains fused in tandem to dCas9, is illustrated. (**b**) Fluorescent reporter assay quantifying the amount of activation from the various dCas9 domain assemblies. Data are shown as median fluorescence ± s.e.m. *n* = 5 independent transfections. * denotes significance of dCas9-VP64-p65-Rta over all constructs including Reporter Control, *P* = <0.0001.



Supplementary Figure 4

Determining the essentiality of all VPR components.

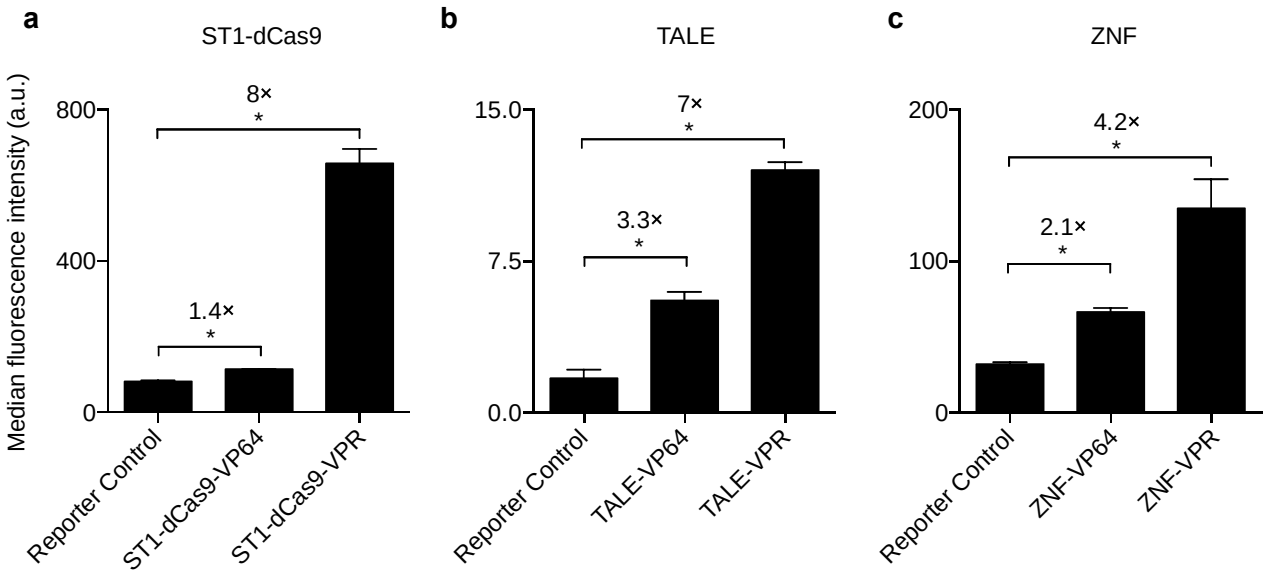
Fluorescent reporter assay quantifying the effects of substituting each member of the VPR complex with the mCherry fluorescent protein compared to VP64 and the intact VPR complex. Data are shown as median fluorescence ± s.e.m. *n* = 5 independent transfections. * denotes significance of dCas9-VP64-p65-RTA over all constructs including Reporter Control, *P* = <0.0001.



Supplementary Figure 5

Testing the effects of VP64, p65 and Rta order on activation.

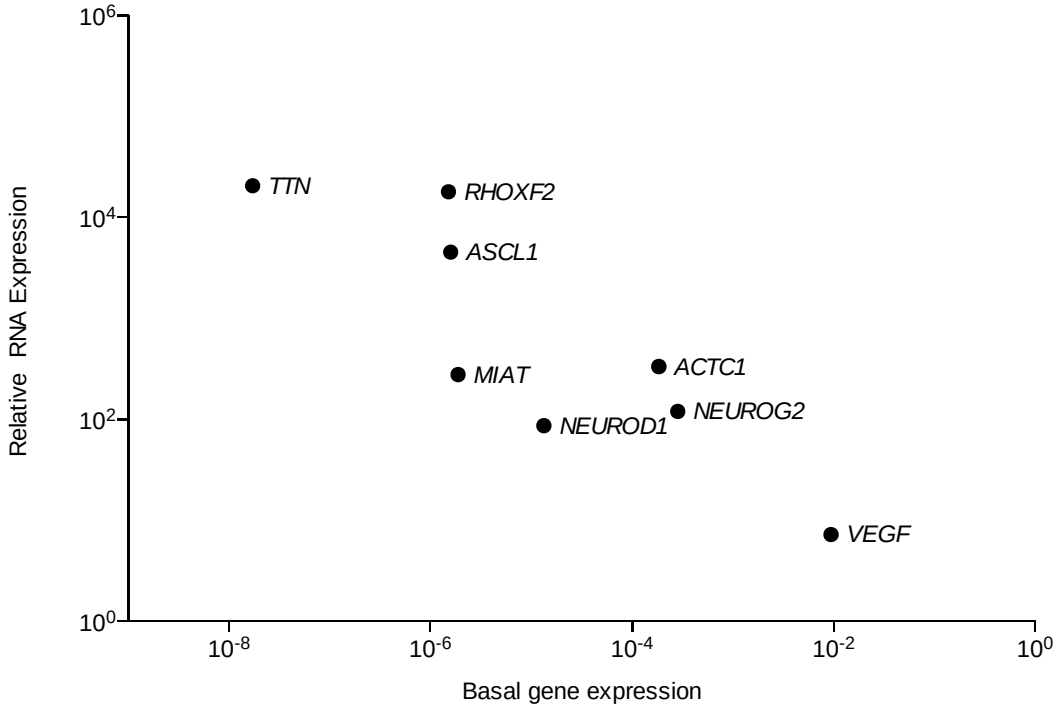
Fluorescent reporter assay quantifying the activation potential of each of the different non-repeating combinations between VP64, p65 and Rta. The activation domain fused to dCas9 is listed. Data are shown as median fluorescence ± s.e.m. n = 5 independent transfections. * denotes significance of dCas9-VP64-p65-Rta over all constructs including Reporter Control, P = <0.005.



Supplementary Figure 6

Testing VPR activity when fused to other programmable DNA binding proteins.

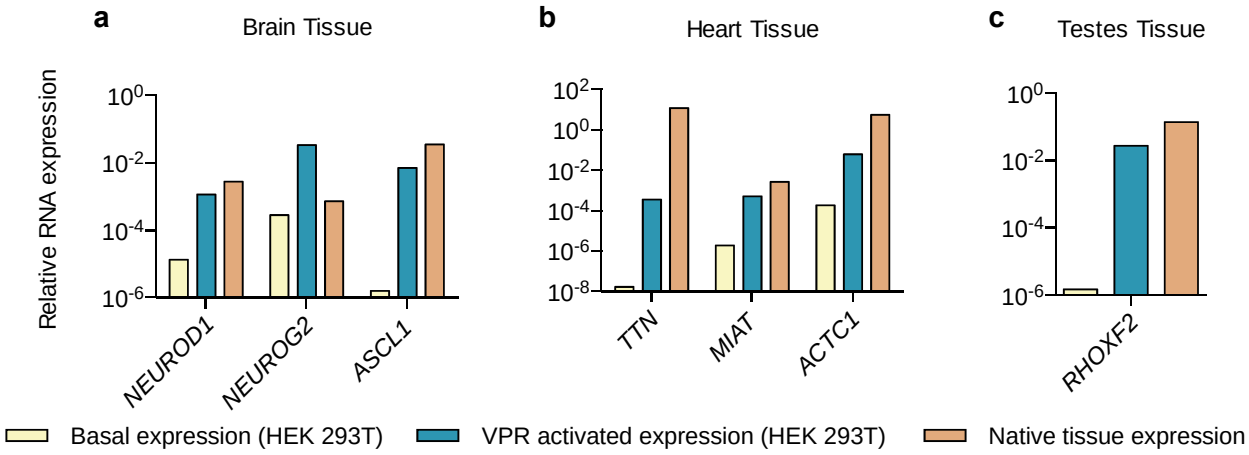
(**a**) Fluorescent reporter assay quantifying the level of transcriptional activation from *Streptococcus thermophilus*, ST1-dCas9-VP64 and ST1-dCas9-VPR proteins. Data are shown as median fluorescence ± s.e.m. n = 3 independent transfections. For *, P = <0.001. Difference between ST1-dCas9-VP64 vs. ST-dCas9-VPR is significant, P = <0.0001 (**b**) Fluorescent reporter assay quantifying transcriptional activation for designer transcription activator like effector, TALE-VP64 and TALE-VPR proteins. Data are shown as median fluorescence ± s.e.m. n = 3 independent transfections. For *, P = <0.005. Difference between TALE-VP64 vs. TALE-VPR is significant, P = <0.0005 (**c**) Fluorescent reporter assay quantifying the level of transcriptional activation for zinc-finger protein (ZNF) fused to either VP64 or VPR. Data are shown as median fluorescence ± s.e.m. n = 3 independent transfections. For *, P = <0.01. Difference between ZNF-VP64 vs. ZNF-VPR is significant, P = <0.05.



Supplementary Figure 7

Efficiency of VPR mediated activation as a function of basal expression.

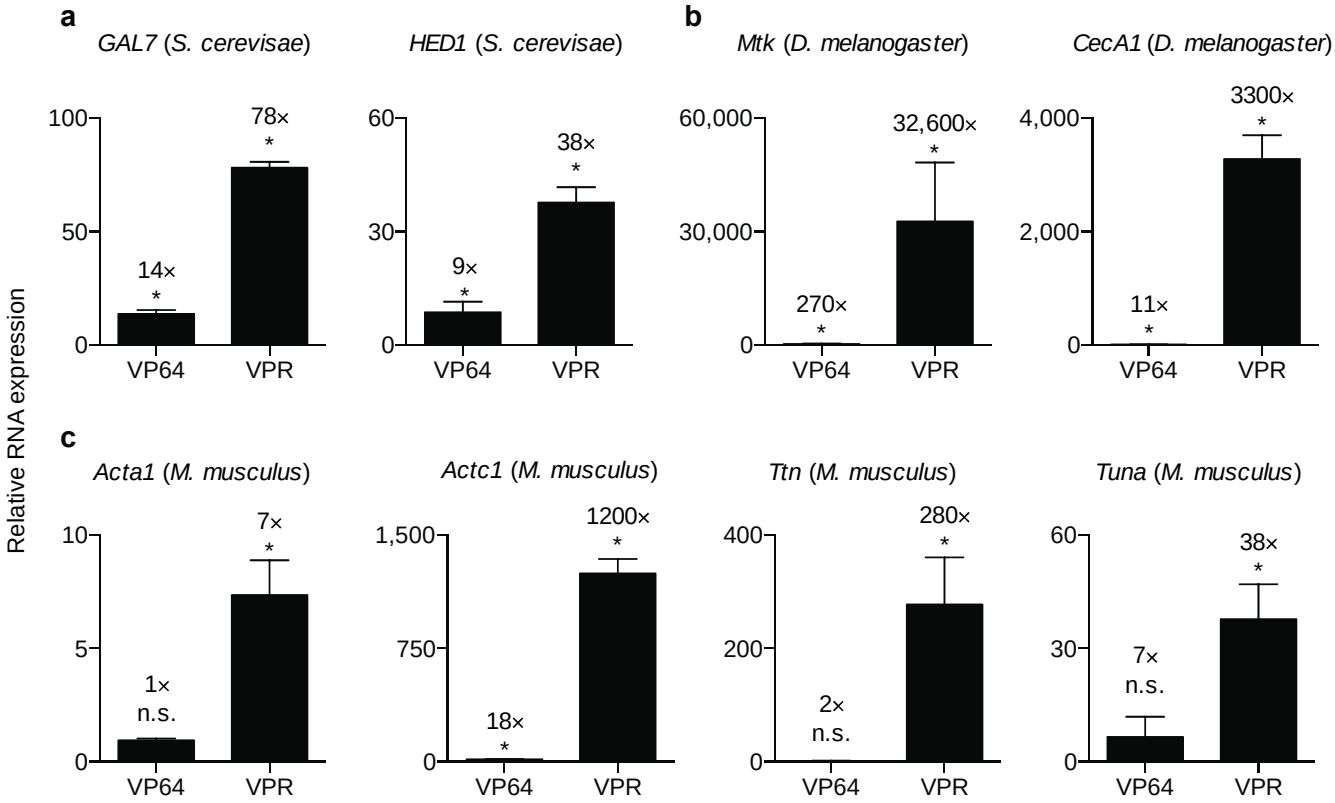
Fold activation (y-axis) is calculated by measuring the level of target expression above background, when indicated gene is activated with dCas9-VPR. Basal expression level (x-axis) is calculated by measuring basal target gene expression relative to ß-actin. For all data points *n* = 3 independent transfections.



Supplementary Figure 8

Comparison of VPR activated gene expression to that of native tissue.

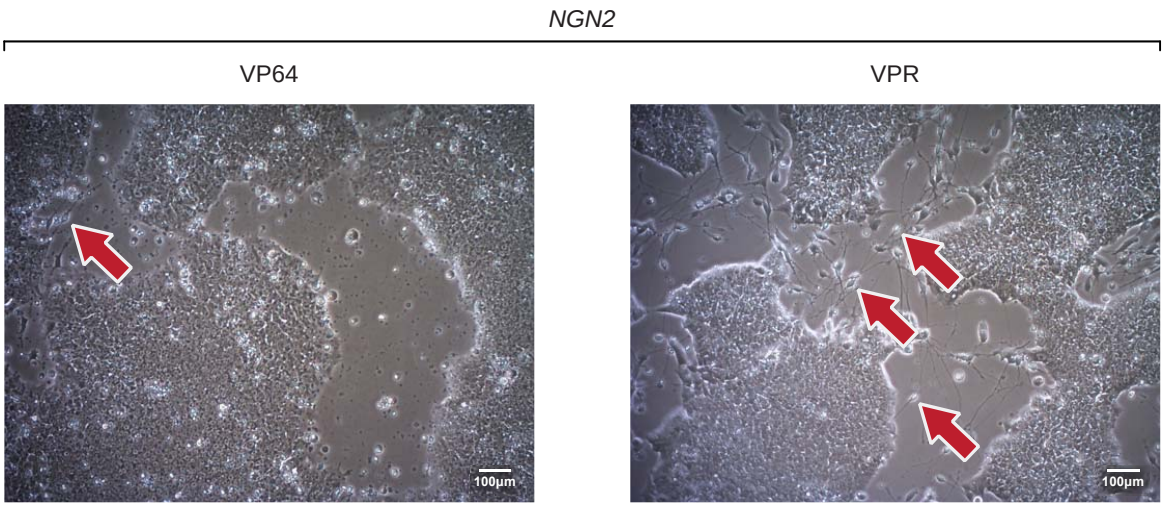
(a) Levels of RNA expression for the neuronal targets *NEUROD1*, *NEUROG2*, and *ASCL1* in non-activated HEK 293Ts, VPR activated HEK 293Ts, and human brain tissue – target expression is calculated relative to ß-actin level within each sample (b) Levels of RNA expression for the cardiac targets *TTN*, *ACTC1*, and *MIAT* in non-activated HEK 293Ts, VPR activated HEK 293Ts, and human heart tissue – all relative to ß-actin level within each sample. (c) Relative levels of *RHOXF2* transcript expressed in non-activated HEK 293Ts, VPR activated HEK 293Ts, and human testes tissue – all relative to ß-actin level within each sample. For all 293T data, *n* = 3 independent transfections, for human tissue samples *n* = 1 total RNA extract.



Supplementary Figure 9

dCas9-VPR activity within various model organisms.

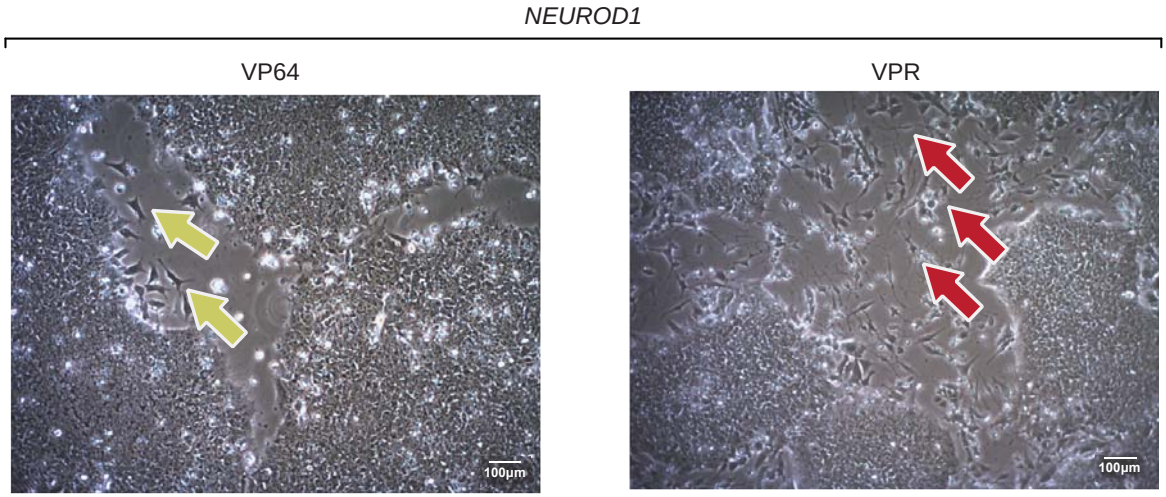
(**a**) RNA expression of individual targets within *S. cerevisiae* containing the indicated activator along with a gRNA against either *GAL7*, *HED1* or a control guide with no genomic target. Data are shown as the mean ± s.e.m (*n* = 3 independent colonies for *GAL7* and *n* = 4 independent colonies for *HED1*). For *, *P* = <0.01. (**b**) RNA expression of individual targets in *D. melanogaster* S2R+ cells, transfected with the indicated dCas9 activator and guide RNAs against the fly genes *Metchnikowin* (*Mtk*) or *Cecropin-A1* (*CecA1*). Data are shown as the mean ± s.e.m (*n* = 3 independent transfections). For *, *P* = <0.05. (**c**) RNA expression of individual targets within *M. musculus* Neuro-2A cells transfected with the indicated dCas9 activator along with gRNAs targeting either *Acta1, Actc1*, *Ttn*, or *Tuna*. Data are shown as the mean ± s.e.m (*n* = 3 independent transfections). For *, *P* = <0.01 (n.s. = not significant). Comparisons between VP64 and VPR within all panels are significant, *P* = <0.05.



Supplementary Figure 10

Generation of iNeurons by *NGN2* activation.

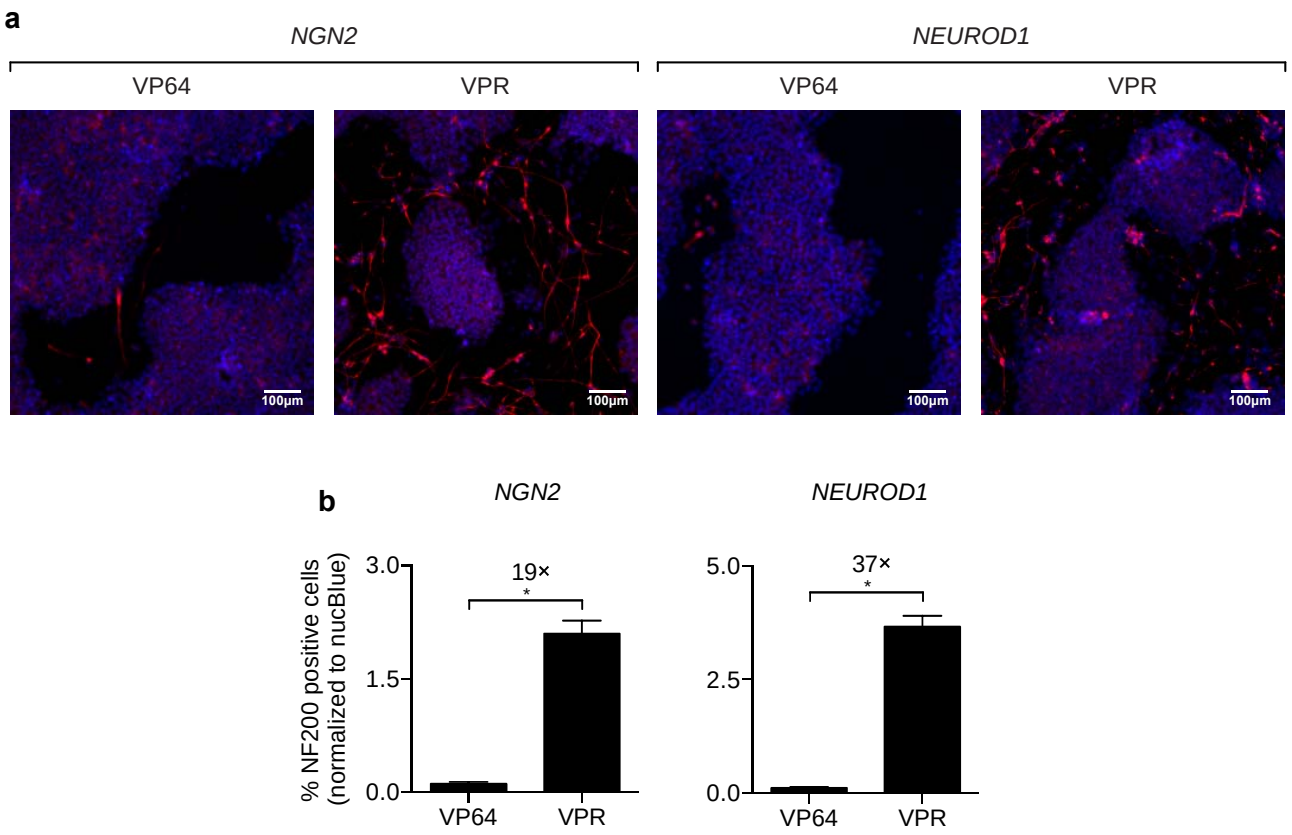
Bright field images of dCas9-AD NGN2 mediated iNeurons, four days after doxycycline addition. Red arrows point towards the cell body of iNeurons. Scale bar represents 100 µm.



Supplementary Figure 11

Generation of iNeurons by *NEUROD1* activation.

Bright field images of dCas9-AD NEUROD1 mediated iNeurons, four days after doxycycline addition. Yellow arrows point towards the cell body of partially differentiated iPSCs. Red arrows point towards the cell body of iNeurons. Scale bar represents 100 µm.



Supplementary Figure 12

dCas9 mediated differentiation as determined by neurofilament 200 (NF200) staining.

(**a**) Pseudocolored immunofluorescence images for NucBlue (blue, total cells) and neurofilament 200 (red, iNeurons). Images were taken 4 days after doxycycline induction and are representative of biological triplicates (separately seeded wells). Scale bar represents 100 µm. (**b**) Immunofluorescence quantification and comparison of iNeurons generated by either dCas9-VP64 or dCas9-VPR. Data are shown as the mean ± s.e.m. (*n* = 3 independent platings of stable cell lines, with each replicate being an average of 24 separate images). For *, *P* = < 0.001.

#

Highly efficient Cas9-mediated transcriptional programming

Alejandro Chavez, Jonathan Scheiman, Suhani Vora, Benjamin W. Pruitt, Marcelle Tuttle, Eswar Prasad R. Iyer, Shuailiang Lin, Samira Kiani, Christopher D. Guzman, Daniel J. Wiegand, Dmitry Ter-Ovanesyan, Jonathan L. Braff, Noah Davidsohn, Benjamin E. Housden, Norbert Perrimon, Ron Weiss, John Aach, James J. Collins, George M. Church

Supplementary Table 1: List of qPCR primers used in study

Human qPCR Primers

Target	Forward primer	Reverse primer
ß-actin	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
NGN2	TGGGTCTGGTACACGATTGC	GGGTCTCGATCTTGGTGAGC
NEUROD1	GGATGACGATCAAAAGCCCAA	GCGTCTTAGAATAGCAAGGCA
MIAT	TGGCTGGGGTTTGAACCTTT	AGGAAGCTGTTCCAGACTGC
ASCL1	CGCGGCCAACAAGAAGATG	CGACGAGTAGGATGAGACCG
RHOXF2	GGAGATTTAGGAAGTATGGGGTTAGTG	AAAACCTCCTCTCTTACTTTTCTACTTC
ACTC1	ATGTGTGACGACGAGGAGAC	CACGATGGACGGGAAGAC
TTN	TGTTGCCACTGGTGCTAAAG	ACAGCAGTCTTCTCCGCTTC
VEGF	GGGCAGAATCATCACGAAGT	TGGTGATGTTGGACTCCTCA

***S. Cerevisiae* qPCR Primers**

Target	Forward primer	Reverse primer
FBA	GACTTGTACACCAAGCCAGA	GATGTCACCAGCGTACAAAC
HED1	AAGAGCTTGTGCACCGAAGT	TGGCACGAAGTTGTTGTTTT
GAL7	GCCATTCCCATAGACGTTACA	GCTTGTAAGCAGCCTCCTGT

***Drosophila* qPCR Primers**

Target	Forward primer	Reverse primer
RpL32	CGGTTACGGATCGAACAAG	TCTGCATGAGCAGGACCTC
Mtk	ATGCAACTTAATCTTGGAGCGA	GACGGCCTCGTATCGAAAATG
CecA1	AAGCTGGGTGGCTGAAGAAA	TGTTGAGCGATTCCCAGTCC

***M. musculus* qPCR Primers**

Target	Forward primer	Reverse primer
ß-actin	GAGTCCTACGACATCATCGCT	CGTCCGACATAGTTTGGGAAA
Acta1	CCCAAAGCTAACCGGGAGAAG	GACAGCACCGCCTGGATAG
Actc1	ATGTGTGACGACGAGGAGAC	CGGACAATTTCACGTTCAGCA
Ttn	GACACCACAAGGTGCAAAGTC	CCCACTGTTCTTGACCGTATCT
Tuna	TTCGGGGATGACTATTTTGG	CTTTGTTCCTCACCCCTTGA

Supplementary Data:

gRNA sequences used in study:
All sequences are listed in the 5'-3' direction. Spacers are listed by distance from the transcriptional start site to the last base of the N20, immediately prior to the PAM sequence.

Human Guide RNA sequences
NEUROD1
-52: AGGGGAGCGGTTGTCGGAGG
-144: ACCTGCCCATTTGTATGCCG
-240: AGGTCCGCGGAGTCTCTAAC
-596: TAGAGGGGCCGACGGAGATT

ASCL1
-200: CGGGAGAAAGGAACGGGAGG
-422: AAGAACTTGAAGCAAAGCGC
-576: TCCAATTTCTAGGGTCACCG
-857: GTTGTGAGCCGTCCTGTAGG

ACTC1
-229: TGGCGCCCTGCCCTCTGCTG
-331: ACCGCAGCAGCACATCTGAG
-410: AATGGCTTTACTCAGAGAGC

TTN
-169: CCTTGGTGAAGTCTCCTTTG
-252: ATGTTAAAATCCGAAAATGC
-326: GGGCACAGTCCTCAGGTTTG
-480: ATGAGCTCTCTTCAACGTTA

MIAT
-7: GCGCCCATGAAATTTTAATG
-219: ATGCGGGAGGCTGAGCGCAC
-282: CATTAGGCCGCAGAGAGCTC
-333: GCTTCTGCGCCCCTGGTCCG

RHOXF2
-44: ACGCGTGCTCTCCCTCATC
-43: CGCGTGCTCTCCCTCATCC
-10,-627: CTGTGGGTTGGGCCTGCTG

NGN2
-71: GGCGGTGGCGGGGGAGGAGG
-139: CAATGAAAAGAATAAGCCAG
-183: GGGAAAGGCGGTGAAGAAAG
-320: CGGAGCTGGCGAAGCCGCAG

VEGF
-439: GTTGGAGCGGGGAGAAGGCC
-472: GGTGAGTGAGTGTGTGCGTG
-512: GAGCAGCGTCTTCGAGAGTG
-574: GTGTGCAGACGGCAGTCACT

S. Cerevisiae Guide RNA Sequences
HED1
-112: ACGGCTTTAATTAGCGTACG

GAL7
-167: AACTGTTGACCGTGATCCGA

Control
CGAGACGATTAATGCGTCTCG

D. melanogaster Guide RNA Sequences
Mtk
-39: TTTCGTGGGAGGTGGAGA
-86: TCATTCATTCGGCTGCTTAT
-735: AAAGATATCGGCACACGGAC
-193: TTTAGTCTAGGCTGATAATC
-335: CAACCACGGCAGCCATTCAA

CecA1
-57: TGACAGATAAGGCATGCACG
-124: AAGCACACATCTGCACATCT
-178: CAATTTGTAATTTACATATT
-256: GATCAATTCAAAATGCCAAA
-339: ACATAAACGCATCCAATTTG

M. musculus Guide RNA Sequences
Acta1
-53: ACCCAAATATGGCTTGGGAA
-97: CCAGGCTGAGAACCAGCCGA
-136: TGCACTGACCAAAGAAGGAG
-188: TCTCCATATACGGCCTGGTC

Actc1
-43: ACGGGGTCAGTTGGAGCAGC
-135: GGCTCCAAGAATGGCCTCAG
-184: CTCCCAGACCATGTAAGGAA
-244: GGGAGGGGCAGGCCAGCAAG

Ttn
-52: GAGCCGGGCTGTAAGGATGT
-97: TGATGGGAGAGGACCTATTT
-143: AATTTAGCACTGCCAATCAG
-188: AAAATACAATTCTATTTCCT

Tuna
-106: GGCAGAGGCCACGTCTTCCC
-147: GGCTCCTGGGTGAGGGTGGA
-195: CTGCTGTAACCCCAGCACAG
-246: TCTGCATTCCTGTGAGCGGC

Guide library pool used for *NEUROD1* targeting in iPS cells
-51: GGGGAGCGGTTGTCGGAGGA

-52: AGGGGAGCGGTTGTCGGAGG
-55: GTGAGGGGAGCGGTTGTCGG
-108: CCATATGGCGCATGCCGGGG
-111: AGACCATATGGCGCATGCCG
-113: GAAGACCATATGGCGCATGC
-123: GCTGGACCGGGAAGACCATA
-144: ACCTGCCCATTTGTATGCCG
-159: TGCCGCGGAGCGCTCCATTC
-172: AGCGCTCCGCGGCATACAAA
-195: TGGCCACAAAGGGGCCGGAA
-229: GTCTCTAACTGGCGACAGAT
-230: AGTCTCTAACTGGCGACAGA
-240: AGGTCCGCGGAGTCTCTAAC
-280: GAAGGGACGGGGATAGAGGG
-365: CCTGCTTTCGCGCCGGAAGT
-387: CCTACTTCCGGCGCGAAAGC
-461: ACAAGAAATCGAAAGGAGCG
-596: TAGAGGGGCCGACGGAGATT
-685: AGAGGACGATCCGGTTAGGG
-688: GGGAGAGGACGATCCGGTTA
-689: TGGGAGAGGACGATCCGGTT
-834: AAAGCGAGCTAGTTCTCGCG
-837: GCGAGCTAGTTCTCGCGAGG
-1752: AAGCGTGGCGTGAATCGTTG
-1753: AGCGTGGCGTGAATCGTTGT
-1768: CGATTCACGCCACGCTTCGG
-1771: CAACGATTCACGCCACGCTT
-1901: GGGCTAAAACTCGAGGGCGT
-1948: CACAGCCCGACGTTTGCGGC

Guide library pool used for *NGN2* targeting in iPS cells
-48: TTTTCTTGGTGGTATATAAG
-320: CGGAGCTGGCGAAGCCGCAG
-387: TGTGATTGGTGGCTCGCGCT
-430: ATTAATGAATGGAGGTCGCG
-457: GCTGGCCAATCAGGGCGCCC
-489: GGCCTGCGAGCCACGCGCAC
-601: AGCGAGGACGAAGGCGGGGG
-604: GGCAGCGAGGACGAAGGCGG
-605: CGGCAGCGAGGACGAAGGCG
-607: GGCGGCAGCGAGGACGAAGG
-660: CCTCCTAACTCCCGGGTGAT
-870: TGTTTGGGGTCCGTCGAAAC
-875: GGGGTCCGTCGAAACTGGCG
-876: GGGTCCGTCGAAACTGGCGT
-877: CCCAAACACACTTGTTCACG
-902: CTCTCCCACGCCAGTTTCGA
-942: TGGGAGGGGAGGTCGGATAG
-1134: GTGGGTGGCAGCGAACCGAG
-1135: TGGGTGGCAGCGAACCGAGC
-1201: AGCGCAGCGCATTTGCTTGC
-1210: TGTGACCTCTGCTCCGCGCT

-1212: TGACCTCTGCTCCGCGCTGG
-1294: TGAAGGGCTACTGGACCTCG
-1295: GAAGGGCTACTGGACCTCGG
-1367: TGCCCACCCTCTTGTCGACA
-1391: CCCCATGTCGACAAGAGGGT
-1395: CCATCCCCATGTCGACAAGA
-1396: TCCATCCCCATGTCGACAAG
-1399: CGGACTTCAGTAGACCGGAG
-1435: GATCTCAAGGGACGCCACTC

Plasmids:

Sequences prefaced with "[hCas9-m4]" are fused to the 3' end of the SP-hCas9-m4-SV40 NLS sequence derived from the hCas9m4 plasmid (Addgene plasmid #47316). Sequences prefaced with "[hCas9-ST1-m4-SV40 NLS-VP64]" are fused to the 3' end of the ST1-hCas9-m4-SV40 NLS-VP64 sequence derived from the M-ST1n-VP64 plasmid (Addgene plasmid #48675).

>[hCas9-m4]-VP64

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CTCGGCAGTGACGCCCTTGATGATTTCGACCTGGACATGCTGATTAACTCTAGATAG

>[hCas9-m4]-VP64-SV40-P65-RTA

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>[hCas9-m4]-VP64-SV40-P65-mCherry

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AGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGC
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>[hCas9-m4]-mCherry-SV40-P65-RTA

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