

Supplementary Information

Rewiring of endogenous signaling pathways to genomic targets for therapeutic cell reprogramming

Krzysztof Krawczyk¹, Leo Scheller¹, Hyojin Kim¹ and Martin Fussenegger^{1,2,*}

¹Department of Biosystems Science and Engineering, ETH Zurich, Mattenstrasse 26, CH-4058 Basel, Switzerland.

²Faculty of Science, University of Basel, Mattenstrasse 26, CH-4058 Basel, Switzerland

*Corresponding author. E-mail: fussenegger@bsse.ethz.ch

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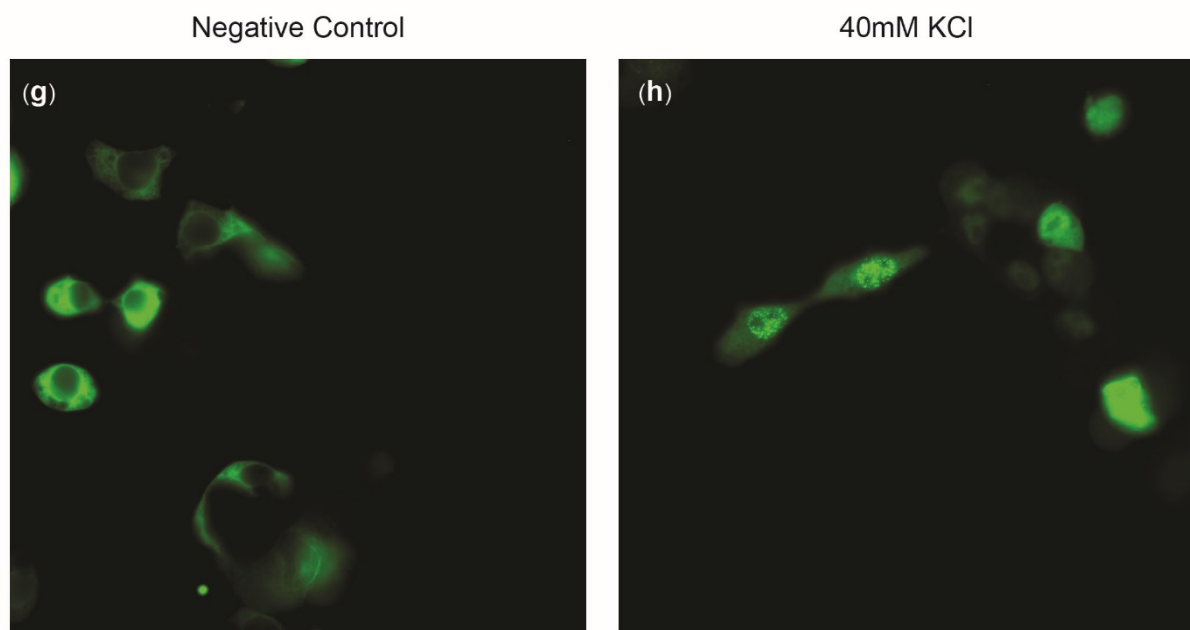
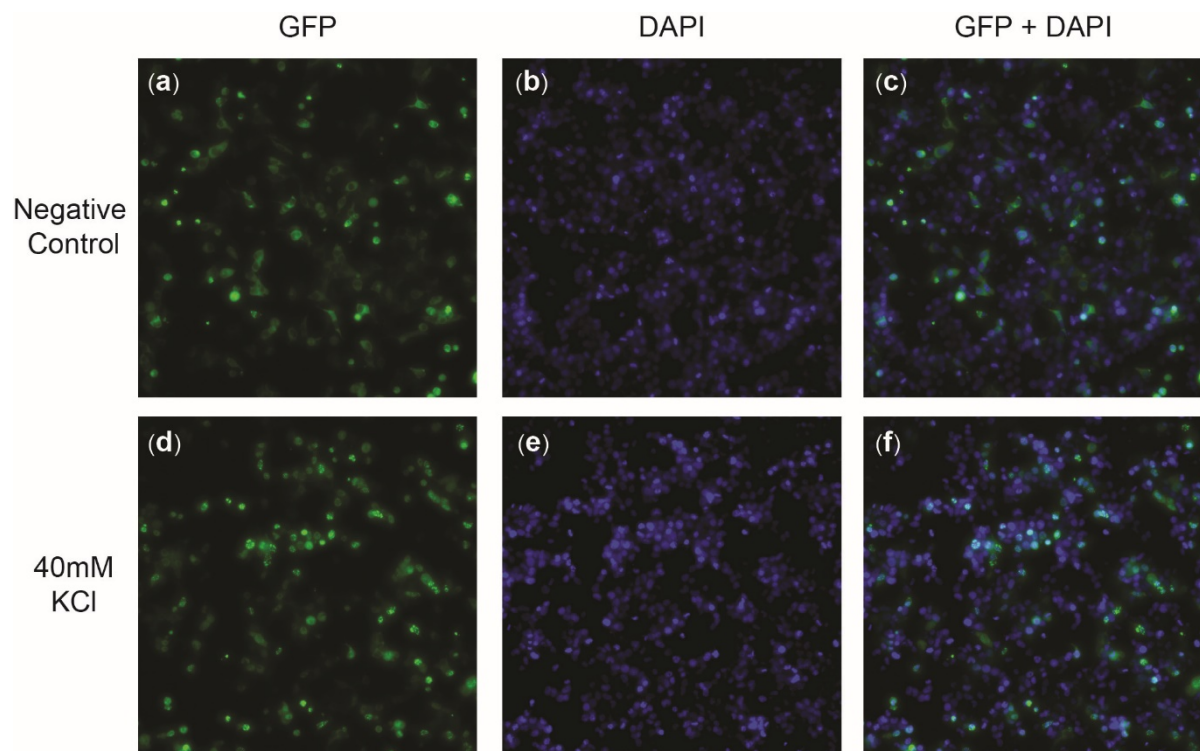
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Supplementary Table 2. Synthetic guide RNA sequences

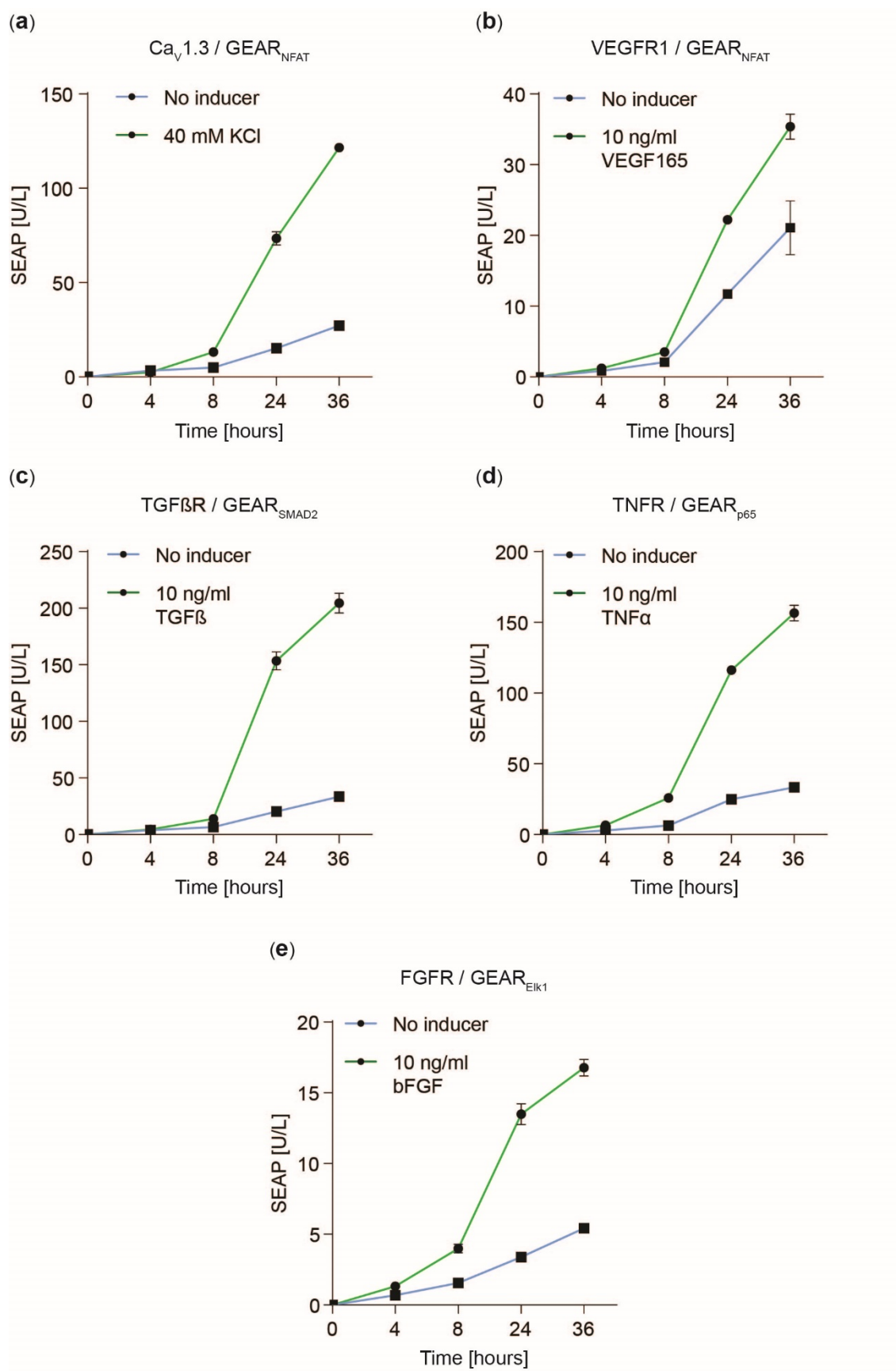
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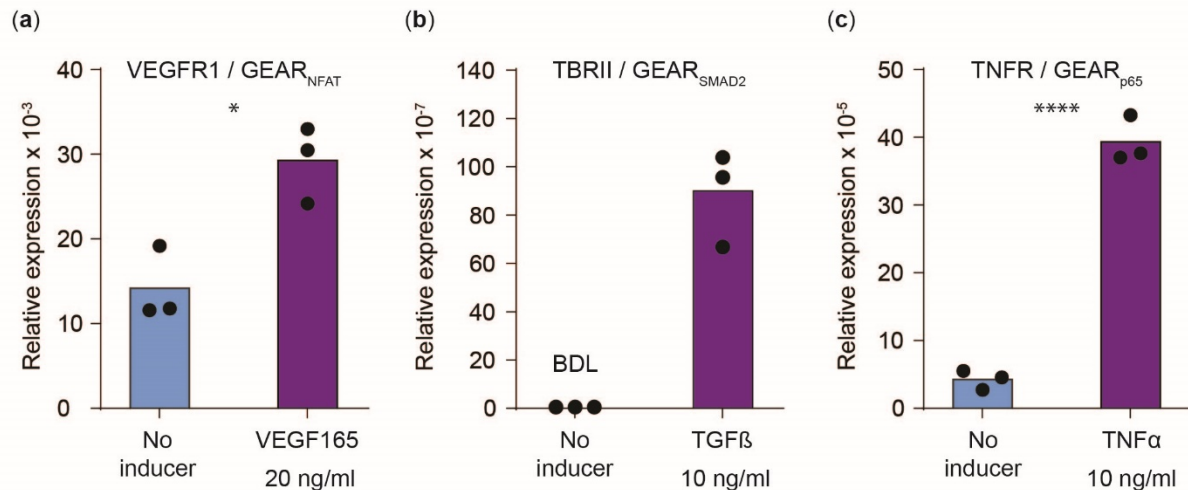


Supplementary Figure 1. Nuclear translocation of GEAR_{NFAT}. β -Mimetic cells (Cav1.3-transgenic HEK293T) transfected with plasmids encoding GEAR_{NFAT} were depolarized with 40 mM KCl for 4 hours. Images were captured with a wide-field fluorescence microscope. Panels **a**, **b**, **c** and **g** show non-induced cells (Negative Control). Panels **d**, **e**, **f**, and **h** show depolarized cells (40 mM KCl). Green fluorescence of GFP-tagged GEAR_{NFAT} is presented in panels **a**, **d**, **g**, and **h**, while panels **b** and **e** show blue fluorescence of DAPI-stained nuclei. Panels **c** and **f** present merged channels for non-induced and depolarized cells, respectively. Panels **a-f** were captured using a 10x magnification objective. For panels **g** and **h**, a 40x magnification objective was used.

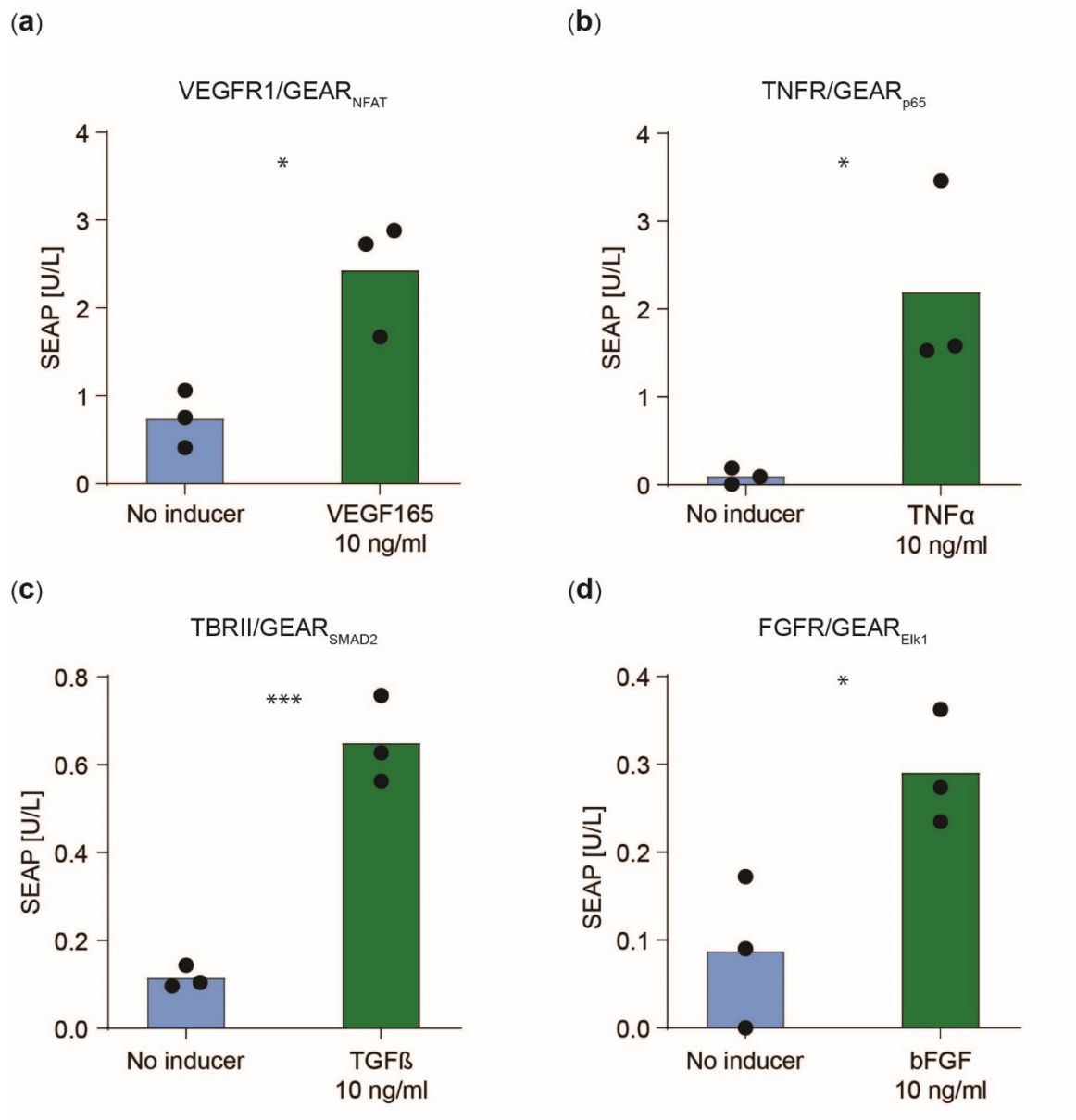


Supplementary Figure 2. Kinetics of GEAR-mediated transgene expression. Cells were transfected with plasmids encoding human insulin promoter-specific sgRNA (sgRNA_{INS}),

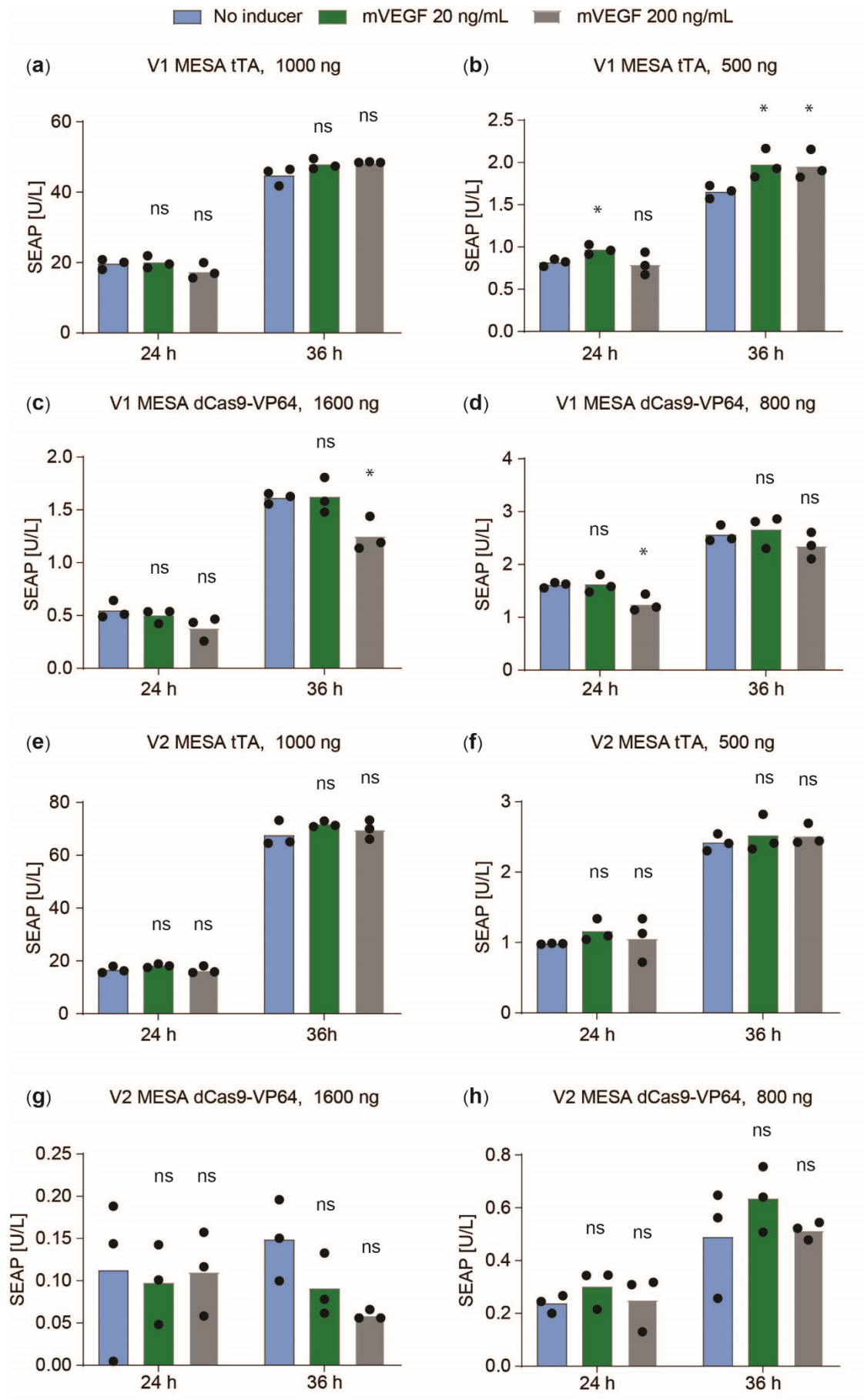
SEAP reporter plasmid controlled by human insulin promoter (P_{hINS} -SEAP), dCas9, an indicated GEAR and an indicated receptor. At 48 hours post-transfection, cells were stimulated with a compound indicated in the panel and SEAP was quantified from the supernatant after 4, 8, 24 and 36 hours. **(a)** Membrane depolarization (40 mM KCl) of β -mimetic cells ($Ca_v1.3$ -transgenic HEK293T) and $GEAR_{NFAT}$ activation. **(b)** 10 ng/ml VEGF165-mediated $GEAR_{NFAT}$ activation. **(c)** 10 ng/ml $TGF\beta$ -mediated $GEAR_{SMAD2}$ activation. **(d)** 10 ng/ml $TNF\alpha$ -mediated $GEAR_{p65}$ activation. **(e)** 10 ng/ml bFGF-mediated $GEAR_{EIk1}$ activation. Numbers above the data points indicate fold induction in the stimulated cells (green line) compared to the negative control (blue line). Black dots correspond to mean. Error bars show the standard error of the mean (SEM). $n = 3$ biological replicates. Source data are provided as a Source Data file.



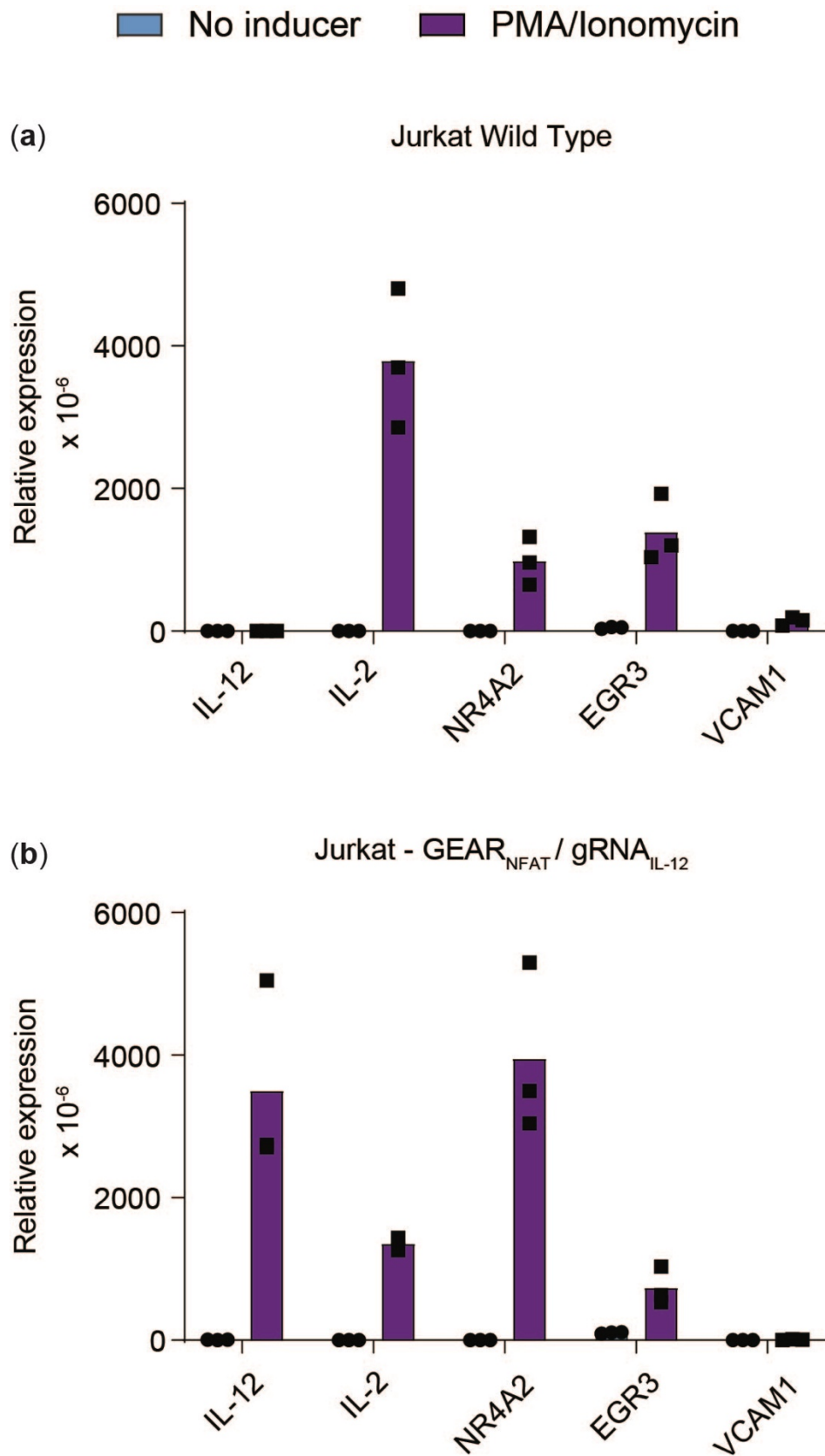
Supplementary Figure 3. Endogenous insulin expression. HEK293T cells expressing dCas9 and P_{hINS}-specific sgRNA (sgRNA_{INS}) were stimulated for 36 h with the indicated inducers. Insulin mRNA levels were quantified in relation to GAPDH. **(a)** VEGF165-induced GEAR_{NFAT}. **(b)** TGFβ-induced GEAR_{SMAD2}. **(c)** TNFα-induced GEAR_{p65}. Violet bars represent mRNA expression (relative to GAPDH) in stimulated cells (mean value). Blue bars represent mRNA expression in controls without inducer. Black dots correspond to individual data points of n = 3 biological replicates. BDL – below detection limit. * p<0.05, **** p<0.0001. Statistical significance was calculated using a two-tailed t-test. A detailed description of the statistical analysis is provided in Supplementary Table 5. Source data are provided as a Source Data file.



Supplementary Figure 4. GEAR-mediated transgene expression in immortalized human mesenchymal stem cells. hMSC-TERT cells containing a SEAP reporter plasmid under control of the human insulin promoter (P_{hINS} -SEAP) and expressing dCas9, as well as the indicated receptors and GEARs were stimulated for 36 hours with the indicated inducers. SEAP was quantified from the cell culture supernatant. **(a)** VEGF receptor 1 (VEGFR1) and GEAR_{NFAT}, **(b)** TNFα receptor (TNFR), GEAR_{p65} and IκB, **(c)** TGFβ receptor II (TBR11) and GEAR_{SMAD2}, **(d)** endogenous FGF receptor (FGFR) and GEAR_{Elk1}. Black dots correspond to individual data points of $n = 3$ biological replicates. * $p < 0.05$, *** $p < 0.001$. Statistical significance was calculated using a two-tailed t-test. A detailed description of the statistical analysis is provided in Supplementary Table 5. Source data are provided as a Source Data file.

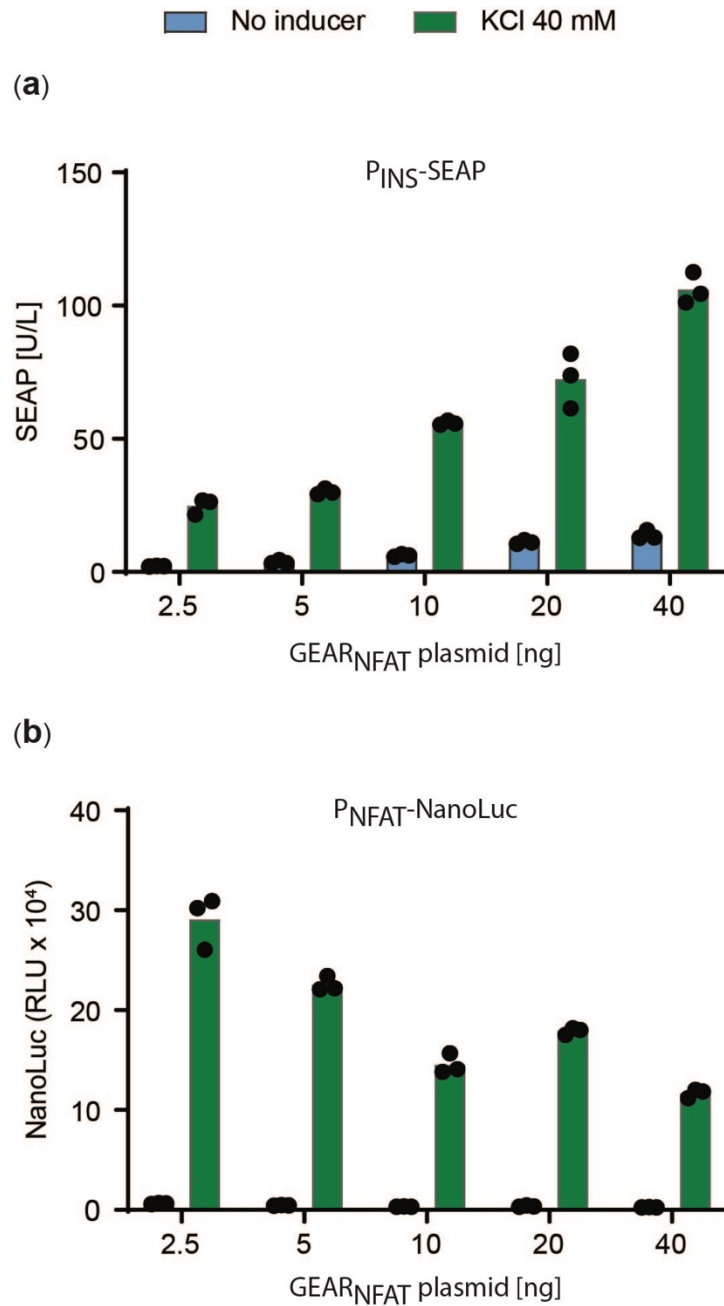


Supplementary Figure 5. Synthetic receptors, MESA. HEK293T cells were transfected with plasmids encoding a human insulin promoter-specific sgRNA (sgRNA_{INS}), and **(a,b)** a SEAP reporter plasmid controlled by tetracycline repressor binding sites (O_{TetR}-P_{hCMVmin}-SEAP), plasmids for V1 MESA tTA and V1 MESA TEV, **(c,d)** a SEAP reporter plasmid controlled by the human insulin promoter (P_{hINS}-SEAP), and plasmids for V1 MESA dCas9-VP64 and V1 MESA TEV, **(e,f)** a SEAP reporter plasmid controlled by tetracycline repressor binding sites (O_{TetR}-P_{hCMVmin}-SEAP), and plasmids for V2 MESA tTA and V2 MESA TEV, **(g,h)** a SEAP reporter plasmid controlled by human insulin promoter (P_{hINS}-SEAP), and plasmids for V2 MESA dCas9-VP64 and V2 MESA TEV. Cells were induced with the indicated concentrations of recombinant mouse VEGF-164 (mVEGF). At 36 hours after stimulation, SEAP was quantified in the supernatant. Values above the graphs indicate the total amount of two MESA-encoding plasmids per 1 well of a 24-well plate. These plasmids were transfected at a ratio of MESA-transactivator (tTA or dCas9) to MESA-TEV of 24:1, as described by Schwartz *et al.*¹ Black dots correspond to individual data points of n = 3 biological replicates. ns – nonsignificant, *p<0.05. Statistical significance was calculated using a two-tailed t-test. A detailed description of the statistical analysis is provided in Supplementary Table 5. Source data are provided as a Source Data file.



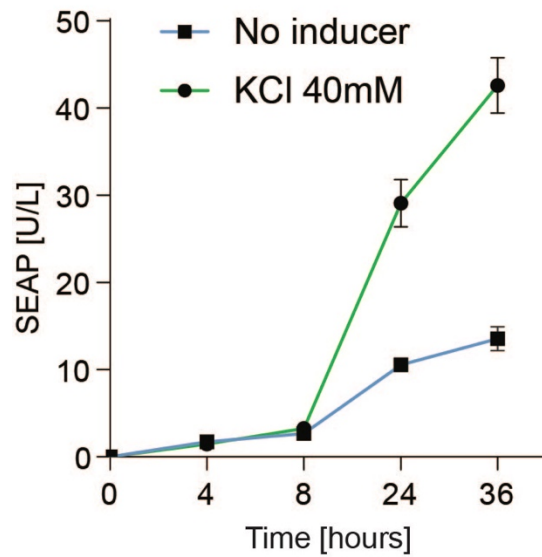
Supplementary Figure 6. Unspecific effect of GEAR_{NFAT} expression on endogenous gene transcription in Jurkat cells. (a) Wild-type Jurkat cells, or (b) Jurkat cells stably expressing

dCas9, GEAR_{NFAT}, and interleukin 12B promoter-specific sgRNA (sgRNA_{IL-12}) were stimulated for 8 hours with 0.5 μ g/mL ionomycin and 5 ng/mL phorbol 12-myristate 13-acetate (PMA). mRNA levels were quantified relative to GAPDH. Bars represent mean values for stimulated cells (violet bars) and control with no inducer (blue bars). Black dots correspond to individual data points of n = 3 biological replicates. Source data are provided as a Source Data file.

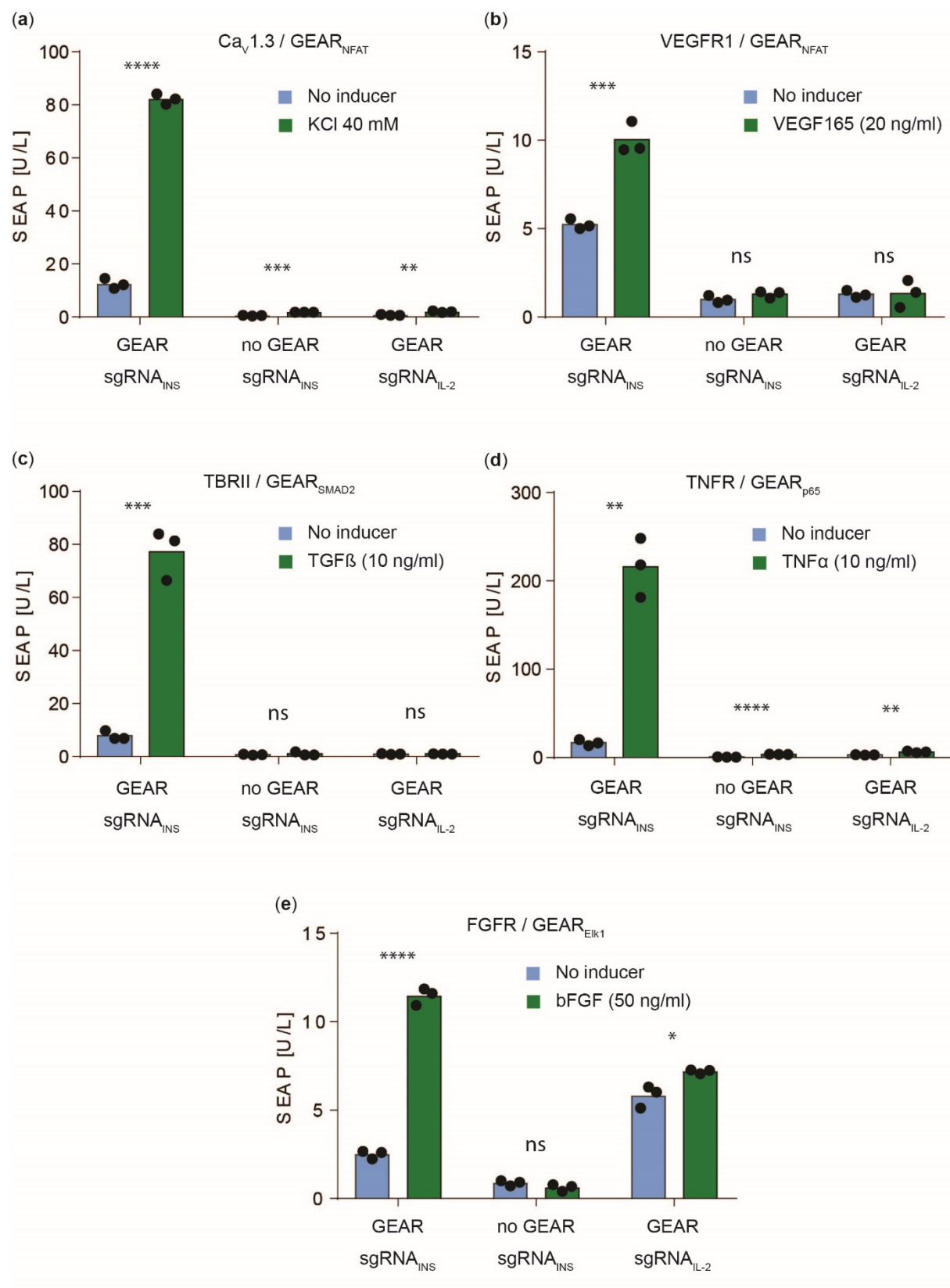


Supplementary Figure 7. GEAR_{NFAT} plasmid titration. β -Mimetic cells (Cav1.3-transgenic HEK293T) were transfected with plasmids encoding dCas9 and the indicated amounts of GEAR_{NFAT}, human insulin promoter-specific sgRNA (sgRNA_{INS}), as well as the SEAP reporter plasmid controlled by human insulin promoter (P_{hINS}-SEAP), and the NanoLuc reporter plasmid controlled by an NFAT-dependent promoter (P_{NFAT}-NanoLuc). Cells were depolarized with 40 mM KCl. **(a)** SEAP and **(b)** NanoLuc were quantified from the cell culture supernatant after 48 hours. Black dots correspond to individual data points of n = 3 biological replicates. Source data are provided as a Source Data file.

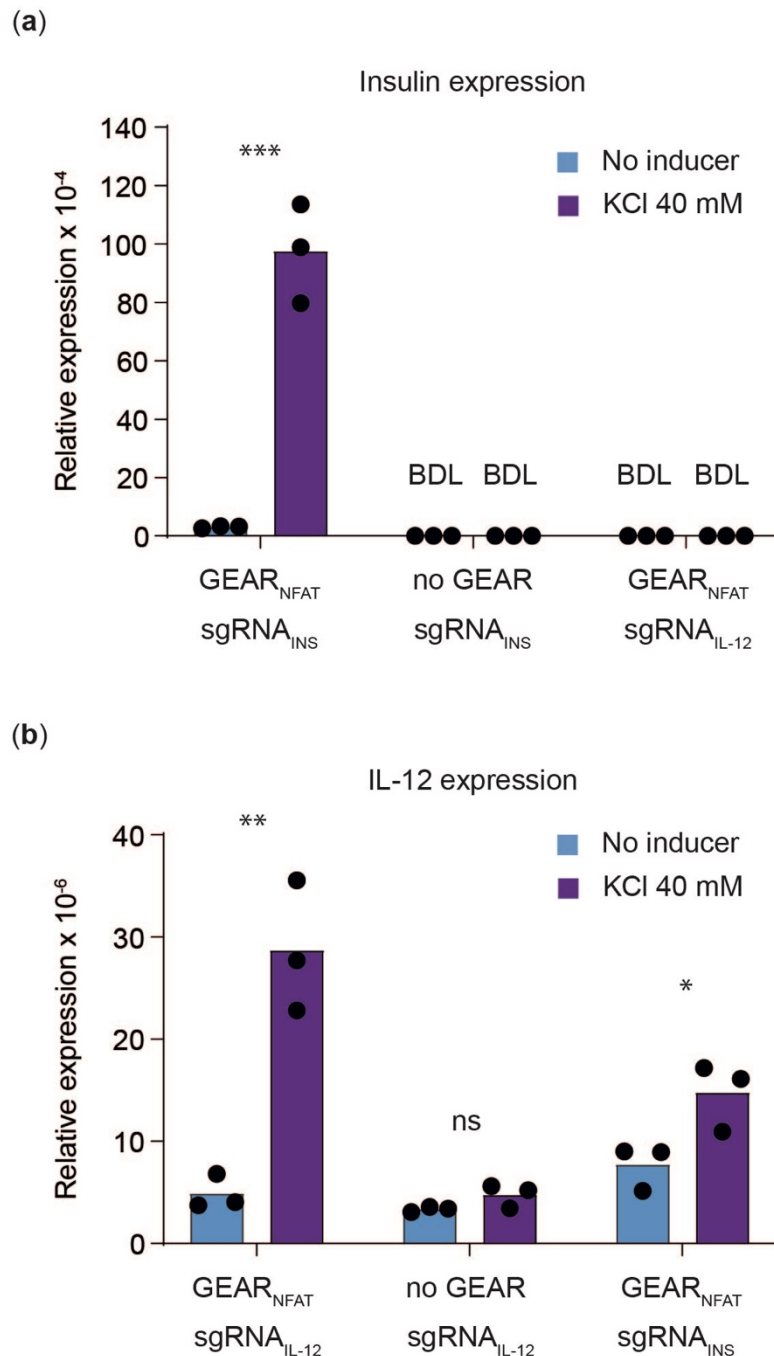
HEK293T / Ca_v1.3 / CaRROT



Supplementary Figure 8. Calcium-inducible dCas9-based nuclear translocation protein “CaRROT”. (a) β -Mimetic cells (Ca_v1.3-transgenic HEK293T) were transfected with plasmids encoding a human insulin promoter-specific sgRNA (sgRNA_{INS}), as well as the SEAP reporter plasmid controlled by the human insulin promoter (P_{hINS}-SEAP) and CaRROT. At 48 hours post-transfection cells were depolarized with 40 mM KCl and SEAP was quantified from supernatant samples after 4, 8, 24 and 36 hours. Black dots correspond to mean. Error bars show standard error of the mean (SEM). n = 3 biological replicates. Source data are provided as a Source Data file.



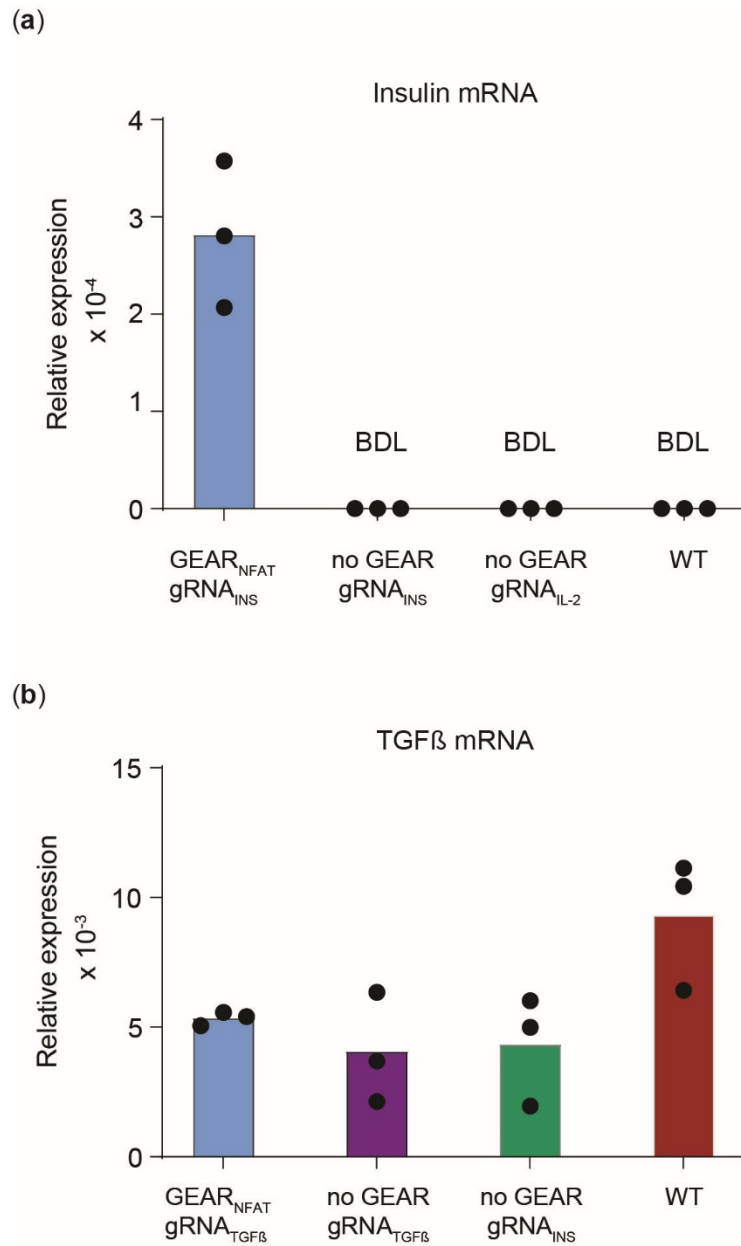
Supplementary Figure 9. Negative control experiments – transgene expression. At 24 hours after the beginning of transfection, HEK293T cells containing a SEAP reporter plasmid for human insulin promoter (P_{hINS} -SEAP) and expressing dCas9 were stimulated for 36 hours with the indicated inducers in the presence or absence of GEARS, as well as in the presence or absence of P_{hINS} -specific sgRNA (sgRNA_{INS}). SEAP was quantified from cell culture supernatant. In No-GEAR control cells, GEARS were replaced by an equal amount of a plasmid expressing MCP-YPet under control of the identical promoter (YPet is a yellow fluorescent protein). sgRNA-negative control cells were transfected with a plasmid encoding a sgRNA targeting human interleukin 2 promoter (sgRNA_{IL-2}). Green bars represent SEAP concentrations measured in the supernatant of stimulated cells (mean values). Blue bars represent controls without inducer. Black dots correspond to individual data points of $n = 3$ biologically independent samples. **(a)** Membrane depolarization-induced GEAR_{NFAT}. **(b)** VEGF165-induced GEAR_{NFAT}. **(c)** TGF β -induced GEAR_{SMAD2}. **(d)** TNF α -induced GEAR_{p65}. **(e)** bFGF-induced GEAR_{EIK1}. ns – nonsignificant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Statistical significance was calculated using a two-tailed t-test. A detailed description of the statistical analysis is provided in Supplementary Table 5. Source data are provided as a Source Data file.



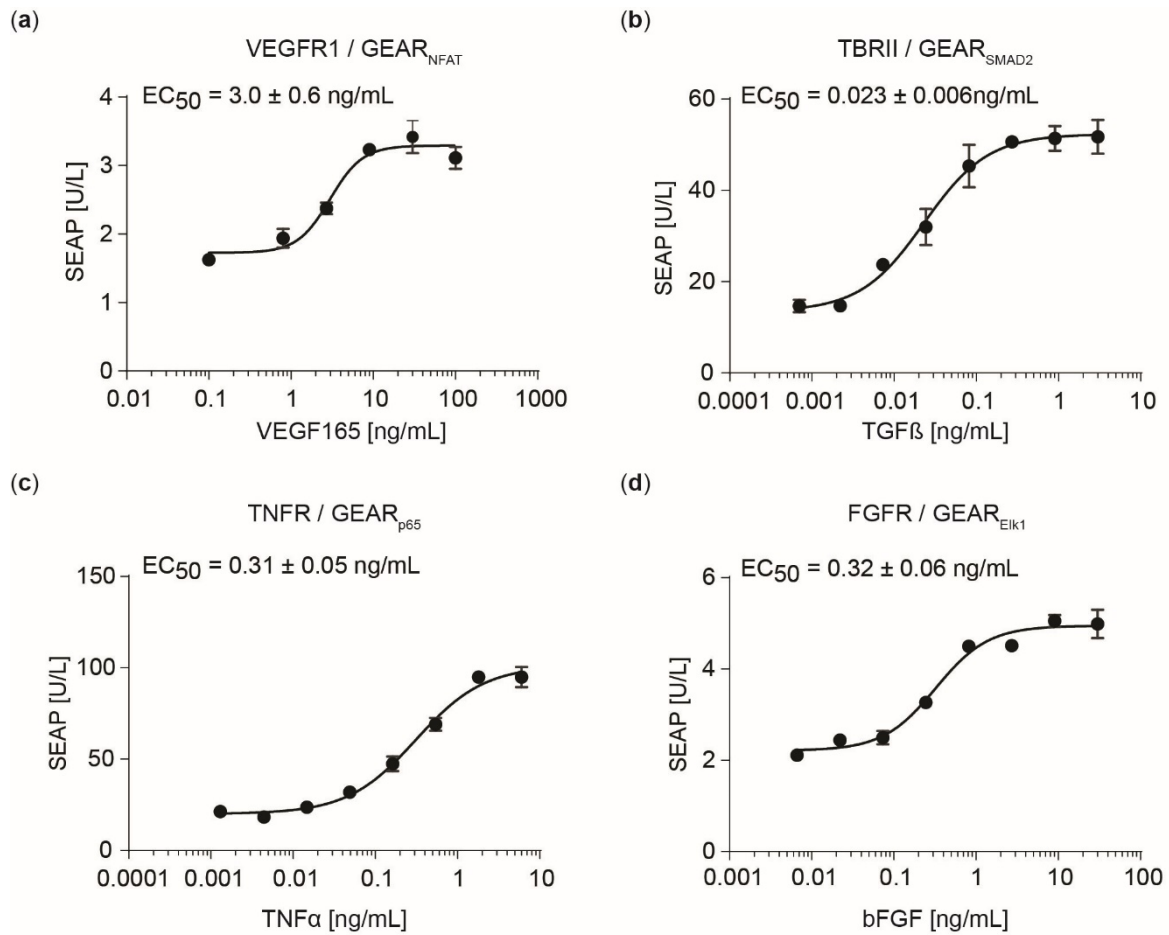
Supplementary Figure 10. Negative control experiments – endogenous gene expression.

At 24 hours after the beginning of transfection, HEK293T cells expressing dCas9 were stimulated for 36 hours with the indicated inducers in the presence or absence of GEARS, as well as in the presence or absence of specific sgRNAs. mRNA levels were quantified in relation to GAPDH. In No-GEAR control cells, GEARS were replaced by an equal amount of a plasmid expressing MCP-YPet under control of the identical promoter. Blue bars represent relative gene expression for controls without inducer (mean values). Violet bars represent relative gene expression for stimulated cells (mean values). Black dots correspond to individual data points

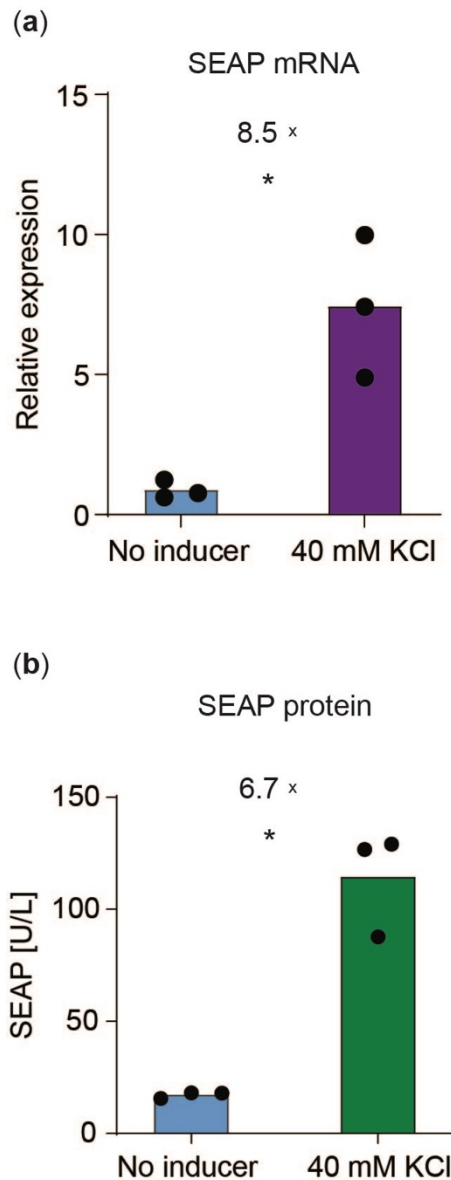
of $n = 3$ biologically independent samples. **(a)** Endogenous insulin expression. **(b)** Endogenous interleukin 12 (IL-12) expression. BDL – below detection limit. ns – nonsignificant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Statistical significance was calculated using a two-tailed t-test. A detailed description of the statistical analysis is provided in Supplementary Table 5. Source data are provided as a Source Data file.



Supplementary Figure 11. Effect of GEAR expression on basal transcription of endogenous genes. HEK293T cells transfected with plasmids encoding dCas9, the indicated sgRNAs, the indicated GEARS, or not transfected (WT). RNA was isolated and (a) insulin mRNA, or (b) TGFβ mRNA was quantified. Plasmid encoding a fusion protein MCP-YPet (P_{hCMV}-MCP-YPet) was used for the “no GEAR” controls. BDL – below detection limit. Black dots correspond to individual data points of n = 3 biological replicates. Source data are provided as a Source Data file.



Supplementary Figure 12. Dose-response relationships. HEK293T cells containing a reporter plasmid for human insulin promoter (P_{hINS} -SEAP) and expressing dCas9, human insulin promoter specific sgRNA (sgRNA_{INS}), as well as the indicated receptors and GEARs: **(a)** VEGF receptor 1 (VEGFR1) and GEAR_{NFAT}, **(b)** TGF β receptor type II (TBR2) and GEAR_{SMAD2}, **(c)** TNF α receptor (TNFR), GEAR_{p65} and I κ B, **(d)** endogenous FGF receptor (FGFR) and GEAR_{Elk1}. Cells were stimulated for 24 hours with the indicated concentration of the inducer, and the reporter protein SEAP was quantified from the cell culture supernatant. Black dots represent mean. Error bars show standard error of the mean (SEM). $n = 3$ biological replicates. Solid lines represent four-parameter nonlinear fitted dose-response curves. EC_{50} – half maximal effective concentration $\pm$ 95% confidence range. Source data are provided as a Source Data file.



Supplementary Figure 13. SEAP assay and qPCR comparability. β -Mimetic cells expressing dCas9, GEAR_{NEFAT}, a human insulin promoter-specific sgRNA (sgRNA_{INS}), as well as a SEAP reporter plasmid controlled by the human insulin promoter (P_{hINS}-SEAP) were depolarized with 40 mM KCl. 24 hours later (a) SEAP mRNA was quantified in the cell lysate and (b) SEAP protein was quantified in supernatant samples. 8.5 × and 6.7 × correspond to the fold induction values in stimulated cells (40 mM KCl) compared with noninduced cells. Black dots correspond to individual data points of n = 3 biological replicates. * p<0.05. Statistical significance was calculated using a two-tailed t-test. A detailed description of the statistical analysis is provided in Supplementary Table 5. Source data are provided as a Source Data file.

Supplementary Table 1. Plasmids used and designed in this study

Plasmid	Description and cloning strategy	Reference
pCaRROT-V5	Constitutive, P _{hCMV} -driven vector expressing calcium-inducible dCas9-based nuclear translocation protein “CaRROT” (P _{hCMV} -CaRROT-V5-pA).	2
pCaV1.3	Constitutive α_1D subunit of the murine L-type voltage-gated calcium channel Cav1.3 expression vector (P _{hCMV} - α_1D -pA) (Addgene no. 26576).	3
pCaVb3	Constitutive β_3 subunit of the murine L-type voltage-gated calcium channel expression vector (P _{hCMV} - β_3 -pA) (Addgene no. 26574).	Lipscombe Lab (unpublished)
pCaV $\alpha_2\delta_1$	Constitutive α_2/δ_1 subunits the murine L-type voltage-gated calcium channel expression vector (P _{hCMV} - α_2/δ_1 -pA) (Addgene no. 26575).	4
pcDNA3.1(+)	Constitutive P _{hCMV} -driven mammalian expression vector (P _{hCMV} -MCS-pA)	Thermo Fisher Scientific, CA
pCEP4YPet-MAMM	Constitutive YPet expression vector (P _{hCMV} -YPet-pA) (Addgene no. 14032).	5
pCGN-ELK-1	Constitutive human Elk-1 expression vector (P _{hCMV} -Elk1-pA) (Addgene no. 27156).	6
pCMV-T7-SB100	Constitutive SB100X expression vector (P _{hCMV} -SB100X-pA). (Addgene no. 34879).	7

Plasmid	Description and cloning strategy	Reference
pCOLADuet-1	Prokaryotic expression vector used as a filler plasmid for cotransfection of mammalian cells.	Merck, Germany
pdCas9-humanized	Catalytically dead, human codon-optimized Cas9 under the control of Murine Stem Cell retroVirus LTR promoter (Addgene no. 44246).	8
pFOX8	Constitutive YPet expression vector (P _{hCMV} -YPet-pA).	Unpublished
pGM47	Constitutive <i>Streptococcus pyogenes</i> catalytically dead CRISPR associated protein 9 (dCas9) expression vector (P _{hCMV} -dCas9 -pA). dCas9 was PCR-amplified from pdCas9-humanized using OMM223 (5'- <i>gcgaattcaccatgactagtGACAAGAAGTATTCTATCGGAC</i> -3') and OMM224 (5'- <i>aagctttctagacaccggtggatccgtagcAGCTCCCTCATCCCCTCCGAGCTG</i> -3'), digested with EcoRI/BamHI, and cloned into corresponding sites of pMM1 (GenBank accession code MN811115).	This work
pGM70	P _{hU6} -driven sgRNA complementary to a sequence in the human insulin promoter (P _{hU6} -sgRNA _{hINS}). OGM99 (5'- <i>CACCGCGGCAGATGGCTGGGGGCTG</i> -3') and OGM100 (5'- <i>AAACCAGCCCCCAGCCATCTGCCGC</i> -3') were annealed and cloned into BbsI-digested psgRNA(MS2) (GenBank accession code MN811116).	This work
pGM74	Constitutive <i>Streptococcus pyogenes</i> catalytically dead CRISPR associated protein 9 (dCas9) expression vector (P _{hCMV} -dCas9-NLS-pA). dCas9 was cloned from pGM47 (EcoRI/NheI) into pMM587 (EcoRI/SpeI) (GenBank accession code MN811114).	This work

Plasmid	Description and cloning strategy	Reference
pHA-NFAT1(4-460)-GFP	Constitutive expression of the regulatory domain of mouse NFAT1 (amino acids 4-460) (P _{hCMV} -HA-NFAT1(4-460)-GFP-pA) (Addgene no. 11107).	9
pHFLT1	Constitutive expression of human VEGFR1 (P _{hCMV} -VEGFR1-pA) (Addgene no. 83435).	10
pHY57	P _{NFAT} -driven Fc-stabilized shGLP1 expression vector (P _{NFAT} -shGLP1-Fc-pA).	11
pKK05	Constitutive Kir2.1 expression vector (PhCMV-Kir2.1-pA). Kir2.1 was excised from pGEMTEZ-Kir2.1 using EcoRI and cloned into the corresponding site (EcoRI) of pcDNA3.1(+).	Unpublished
pKK110	Constitutive, P _{hCMV} -driven expression of MCP-YPet fusion protein (P _{hCMV} -MCP-YPet-pA). Ypet was cloned from pFOX8 (SpeI/BamHI) into pMM565 (NheI/BamHI) (GenBank accession code MN811103).	This work
pKK112	Constitutive, P _{SV40} -driven expression of a MCP-YPet fusion protein (P _{SV40} -MCP-YPet-pA). MCP-Ypet was cleaved from pKK110 using SpeI/BamHI and cloned into corresponding sites of pTS1016 (GenBank accession code MN811104).	This work
pKK122	Constitutive, P _{SV40} -driven GEAR _{p65} expression (P _{SV40} -GEAR _{p65} -pA). Transcription factor p65 was PCR-amplified from HEK293T cDNA using OKK254 (5'-attaactagtgtggtggaGACGAAGTGTTCCTCCCTC-3') and OKK255 (5'- attaggatccGCTGATCTGACTCAGCAGGG-3'), digested with SpeI/BamHI and cloned into NheI/BamHI-digested pKK112 (GenBank accession code MN811102).	This work
pKK126	P _{hU6} -driven sgRNA complementary to a sequence in the human interleukin 2 promoter. OKK248	This work

Plasmid	Description and cloning strategy	Reference
	(5'- <i>cacc</i> GAGGTAATGTTTTTTCAGAC-3') and OKK249 (5'- <i>aaac</i> GTCTGAAAAAACATTACCT-3') were annealed together and cloned into the BbsI sites of the plasmid psgRNA(MS2) (GenBank accession code MN811109).	
pKK130	Constitutive, P _{SV40} -driven IκB expression vector (P _{SV40} -IκB-pA). Human IκB was PCR-amplified from HEK293T cDNA using OKK266 (5'- <i>attaactagt</i> TTCCAGGCGGCCGAG-3') and OKK267 (5'- <i>attagatccgctagc</i> TAACGTCAGACGCTGGCCTC-3'), digested with SpeI/BamHI, and cloned into the corresponding sites of pKK112 (GenBank accession code MN811112).	This work
pKK136	Constitutive, P _{hCMV} -driven GEAR _{SMAD2} expression vector (P _{hCMV} -GEAR _{SMAD2} -pA). Human SMAD2 was PCR-amplified using OKK256 (5'- <i>attaactagtgg</i> tggtTCGTCCATCTTGCCATTC-3') and OKK315 (5'- <i>attatctagacta</i> TGACATGCTTGAGCAACG-3'), digested with SpeI/XbaI, and cloned into NheI/XbaI-digested pKK110 (GenBank accession code MN811108).	This work
pKK158	P _{hU6} -driven sgRNA complementary to a sequence in the human IL-12B promoter. OKK312 (5'- <i>caccg</i> AGTTTAAGTTTCCATCAGAA-3') and OKK313 (5'- <i>aaac</i> TTCTGATGGAACTTAAACTc-3') were annealed and cloned into BbsI-digested psgRNA(MS2) (GenBank accession code MN811110).	This work
pKK160	P _{hU6} -driven sgRNA complementary to a sequence in the human TGFβ promoter. OKK329 (5'- <i>caccg</i> CCGCCCACGCGAGATGAGGA-3') and OKK330 (5'- <i>aaac</i> TCCTCATCTCGCGTGGGCGGC-3') were annealed and cloned into BbsI-digested psgRNA(MS2) (GenBank accession code MN811111).	This work

Plasmid	Description and cloning strategy	Reference
pKK171	ITR-containing vector for SB100X-specific transposon mediated stable genomic integrations, containing a constitutive dTomato and BlastR expression unit, and a constitutive expression unit for <i>Streptococcus pyogenes</i> catalytically dead CRISPR associated protein 9 (dCas9) (ITR-P _{hEF1α} -dCas9-NLS-pA:PRPBSA-dTomato-P2A-BlastR-pA-ITR). dCas9-NLS was PCR-amplified from pGM74 using OKK183 (5'-attaggcctgacaggccTTAGGGGTCCTCCACCTTGCGCTTCTTCTTGGGATCCCCTCCGAGCTGTG-3') and OKK184 (5'-attaggcctctgaggccACCATGACTAGTGACAAGAAGTATTCTATCGG-3'), cleaved with SfiI and cloned into the corresponding site of pSBbi-RB (GenBank accession code MN811107).	This work
pKK172	ITR-containing vector for SB100X-specific transposon mediated stable genomic integrations, containing a constitutive dTomato and BlastR expression unit, and a constitutive expression unit for GEAR _{NFAT} (ITR-P _{hEF1α} -GEAR _{NFAT} -pA:PRPBSA-dTomato-P2A-BlastR-pA-ITR). GEAR _{NFAT} was PCR-amplified from pKK50 using OKK350 (5'-attaggcctctgaggccACCATGGCTTCAAACCTTTACTCAGTTCG-3') and OKK351 (5'-attaggcctgacaggccTTAAACGGGCCCTCTAGACTC-3'), cleaved with SfiI and cloned into corresponding site of pSBbi-RB (GenBank accession code MN811106).	This work
pKK44	Constitutive expression of a fusion protein that consists of MCP, the transactivation domain of transcription factor p65 (p65 _{TA}), and the transactivation domain of heat shock factor 1 (HSF1 _{TA}), without nuclear localization signal (P _{hCMV} -MCP-p65 _{TA} -HSF1 _{TA} -pA). MCP was PCR-amplified with OKK84 (5'-attaagccttCGTACGGCCACCATGG-3') and OKK85 (5'-CGCTACCTCCTCCTCCGCTTC-3') from pMS2-P65-HSF1_Hygro. p65 _{TA} -HSF1 _{TA} was PCR-amplified with OKK86 (5'-AAGCGGAGGAGGAGGTAGCGGACCTTCAGGGCAGATCAGC-3') and OKK87 (5'-	This work

Plasmid	Description and cloning strategy	Reference
	attagaattcTGTACAGGAGACAGTGGGGTC-3'). The resulting fragments were assembled with a PCR reaction, amplified using OKK84 and OKK87, digested with HindIII/EcoRI, and cloned into corresponding sites of pcDNA3.1(+) (GenBank accession code MN811105).	
pKK50	Constitutive GEAR _{NFAT} expression vector (P _{hCMV} -GEAR _{NFAT} -pA). MCP-p65 _{TA} -HSF1 _{TA} was PCR-amplified from pKK44 using OKK84 (5'-attaaagcttCGTACGGCCACCATGG-3') and OKK96 (5'-CGCTGCCTCCTGAACCGCCGCTTCCGCCTGTACAGGAGACAGTGGGGTC-3'). NFAT _{reg} -GFP was PCR-amplified from pHA-NFAT1(4-460)-GFP using OKK97 (5'-GCGGTTTCAGGAGGCAGCGGTGGATCAGGCTCCACCATGATCTTTTACCC-3') and OKK93 (5'-attagaattcCTTGTACAGCTCGTCCATGC-3'). The resulting fragments were assembled by PCR to create MCP-p65 _{TA} -HSF1 _{TA} -NFAT _{reg} -GFP, were amplified using OKK84 and OKK93, and were cloned into pcDNA3.1(+) using HindIII/EcoRI (GenBank accession code MN811100).	This work
pKK82	Constitutive GEAR _{Elk} expression vector. (P _{hCMV} -GEAR _{Elk1} -pA) MCP was PCR-amplified with OKK155 (5'-attaacgcgtaagcttCGTACGGCCACCATG-3') and OKK85 (5'-CGCTACCTCCTCCTCCGCTTC-3') from pKK44. Elk1 was PCR-amplified from pCGN-ELK-1 using OKK128 (5'-gaggaagcggaggaggtagcgaccGATCTCCCAGCCGCAG-3') and OKK129 (5'-ctagaaggcacagtcgaggctgatctagaTCATGGCTTCTGGGG-3'). The resulting fragments were assembled by PCR to create MCP-Elk1, were amplified using OKK155 and OKK129, and were cloned using HindIII/XbaI into pcDNA3.1(+) (GenBank accession code MN811101).	This work

Plasmid	Description and cloning strategy	Reference
pKK89	P_{NFAT}-driven secreted stabilized NanoLuc expression vector (P_{NFAT}-NanoLuc-Fc-pA). NanoLuc was PCR-amplified from pProinsulin-NanoLuc using OKK193 (5'-attagaattcgccaccATGGGCGTCAAGGTGCTGTTCCGCCCTCATTTGTATAGCTGTCGCTGAGGCGgtcttcacactcgaagatttcg-3') and OKK194 (5'-CCACTTCCACCGCCTCCCGCCAGAATGCGTTTCG-3'). Fc fragment of a murine IgG was PCR-amplified from pHY57 using OKK191 (5'-GGAGGCGGTGGAAGTGGTGGTTGTAAGCCTTGCATATGTAC-3') and OKK180 (5'-attagtcgacCCACATTTGTAGAGGTTTTACTTGC-3'). The resulting fragments were assembled by PCR using OKK193 and OKK180, cleaved with EcoRI/SalI and cloned into corresponding sites of pMX57 (GenBank accession code MN811113).	This work
pLeo1207	ITR containing vector for SB100X-specific transposon mediated stable genomic integrations, containing a constitutive dTomato, PuroR expression unit and a constitutive expression unit for sgRNA complementary to a sequence in the human insulin promoter (ITR-P_{hU6}-sgRNA_{hINS}:P_{RPBSA}-BFP-P2A-PuroR-pA-ITR). P_{hU6}-sgRNA_{hINS} was PCR-amplified from pGM70 using OKK356 (5'-attaaccggtCGAGGGCCTATTTCCCATGATTCCTTC-3') and OKK357 (5'-attatctagaCAGATGCGTAAGGAGAAAATACCGCATC-3'), cleaved with AgeI and XbaI and cloned into corresponding sites of pSBbi-BP (GenBank accession code MN811118).	This work
pLeo1209	ITR containing vector for SB100X-specific transposon mediated stable genomic integrations, containing a constitutive dTomato, PuroR expression unit and a constitutive expression unit for sgRNA complementary to a sequence in the human interleukin 12B promoter (ITR-P_{hU6}-sgRNA_{hIL-12B}:P_{RPBSA}-BFP-P2A-PuroR-pA-	This work

Plasmid	Description and cloning strategy	Reference
	ITR). P_{hU6} -sgRNA _{hIL-12B} was PCR-amplified from pKK158 using OKK356 (5'- <i>attaaccggt</i> CGAGGGCCTATTTCCCATGATTCCTTC-3') and OKK357 (5'- <i>attatctaga</i> CAGATGCGTAAGGAGAAAATACCGCATC-3'), cleaved with AgeI and XbaI and cloned into corresponding sites of pSBbi-BP (GenBank accession code MN811119).	
pLeo628	Constitutive expression of MAPK-GEMS _{RR120} (P_{SV40} -MAPK-GEMS _{RR120} -pA)	12
pMF111	Mammalian reporter plasmid for TetR-Elk1 induced SEAP expression (O_{TetR} - $P_{hCMVmin}$ -SEAP-pA)	13
pMM1	Cloning vector for mammalian gene expression (P_{hCMV} -MCS-pA).	14
pMM565	Constitutive expression of MCP (P_{hCMV} -MCP-pA).	15
pMM587	Constitutive expression of an NLS peptide (P_{hCMV} -NLS-pA).	Unpublished
pMS2-P65-HSF1_Hygro	Lentiviral vector containing a fusion protein consisting of MCP, a nuclear localization signal, the transactivation domain of transcription factor p65 (p65 _{TA}), and the transactivation domain of heat shock factor 1 (HSF1 _{TA}) ($P_{EF1\alpha}$ -MCP-NLS-p65 _{TA} -HSF1 _{TA} -pA) (Addgene no. 61426).	16
pMX57	P_{NFAT} -driven SEAP expression vector (P_{NFAT} -SEAP-pA)	17
pProinsulin-NanoLuc	Lentiviral vector for constitutive expression of Proinsulin-NanoLuc (P_{hCMV} -Proinsulin-NanoLuc-pA). (Addgene no. 62057).	18

Plasmid	Description and cloning strategy	Reference
pSBbi-BP	ITR-containing vector for SB100X-specific transposon-mediated stable genomic integrations, containing a constitutive BFP and PuroR expression unit, and a constitutive expression unit (ITR-P _{hEF1α} -MCS-pA:P _{RPBSA} -BFP-P2A-PuroR-pA-ITR). (Addgene no. 60512)	⁷
pSBbi-RB	ITR-containing vector for SB100X-specific transposon-mediated stable genomic integrations, containing a constitutive dTomato and BlastR expression unit, and a constitutive expression unit (ITR-P _{hEF1α} -MCS-pA:P _{RPBSA} -dTomato-P2A-BlastR-pA-ITR). (Addgene no. 60512)	⁷
psgRNA(MS2)	sgRNA cloning vector with MCP-binding loops at tetraloop and stemloop 2 (Addgene no. 61424).	¹⁶
pSP20	P _{hINS} -driven SEAP expression vector (P _{hINS} -SEAP-pA) (GenBank accession code MN811117).	This work
pTBRII	Constitutive TBRII expression vector (P _{hCMV} -TBRII-pA) (Addgene no. 16622).	¹⁹
pTS1016	Constitutive, P _{SV40} -driven SEAP expression (P _{SV40} -SEAP-pA).	Unpublished
pV1-MESA-35F-M-tTA	Constitutive, P _{hCMV} -driven vector expressing MESA target chain with V2-MESA ectodomain, 35 extracellular linkers, a flag tag, the methionine-containing cleavage sequence, and tTA (P _{hCMV} -MESA V2 tTA-pA). (Addgene no. 84502).	¹
pV1-MESA-45F-M-dCas9	Constitutive, P _{hCMV} -driven vector expressing MESA target chain with V1-MESA ectodomain, 45 extracellular linkers, a flag tag, the methionine-containing cleavage sequence, and dCas9-VP64 (P _{hCMV} -MESA V1 dCas9-VP64-pA). (Addgene no. 84504).	¹

Plasmid	Description and cloning strategy	Reference
pV1-MESA-45F-M-tTA	Constitutive, P _{hCMV} -driven vector expressing MESA target chain with V1-MESA ectodomain, 45 extracellular linkers, a flag tag, the methionine-containing cleavage sequence, and tTA (P _{hCMV} -MESA V1 tTA-pA). (Addgene no. 84500).	1
pV1-MESA-45F-Tev	Constitutive, P _{hCMV} -driven vector expressing MESA protease chain with V1-MESA ectodomain, 45 extracellular linkers, a flag tag, and Tev protease (P _{hCMV} -MESA V1 TEV-pA). (Addgene no. 84501).	1
pV2-MESA-35F-M-dCas9	Constitutive, P _{hCMV} -driven vector expressing MESA target chain with V2-MESA ectodomain, 35 extracellular linkers, a flag tag, the methionine-containing cleavage sequence, and dCas9-VP64 (P _{hCMV} -MESA V2 dCas9-VP64-pA). (Addgene no. 84506).	1
pV2-MESA-35F-Tev	Constitutive, P _{hCMV} -driven vector expressing MESA protease chain with V2-MESA ectodomain, 35 extracellular linkers, a flag tag, and Tev protease (P _{hCMV} -MESA V2 TEV-pA). (Addgene no. 84503).	1

Oligonucleotides: Restriction endonuclease-specific sites are shown in *italics* and annealing sequences are shown in capital letters.

Abbreviations: **α_1D** , α_1D subunit of the murine L-type voltage-gated calcium channel Ca_v1.3; **α_2/δ_1** , α_2 and δ_1 subunits of the murine L-type voltage-gated calcium channel; **β_3** , β_3 subunit of the murine L-type voltage-gated calcium channel; **BFP**, blue fluorescent protein; **BlastR**, blasticidin resistance gene; **Ca_v1.3**, member 3 of the Ca_v1 family of L-type voltage-gated calcium channels; **CaRROT**, calcium-responsive transcriptional reprogramming tool; **CRISPR**, clustered regularly interspaced short palindromic repeats; **dCas9**, catalytically inactive *Streptococcus pyogenes* CRISPR associated protein 9; **dTomato**, dimeric red fluorescent protein variant; Fc – Fc fragment of murine IgG antibody; **HEK293T**, human endothelial kidney 293 cell line with stably incorporated Simian virus large T antigen; **HSF1**, human heat shock factor 1; **HSF1_{TA}**, human heat shock factor 1 transactivation domain; **GEAR**, generalized engineered activation regulator; **GEAR_{Elk}**, GEAR containing the transactivation domain of human Elk1; **GEAR_{NFAT}**, GEAR

containing the regulatory domain of murine NFAT1; **GEAR_{p65}**, GEAR containing the human transcription factor p65; **GEAR_{SMAD2}**, GEAR containing the human SMAD2; MAPK-GEMS_{RR120}, generalized extracellular molecule sensor activating MAPK pathway specific to RR120; **GFP**, green fluorescent protein; **HygroR**, hygromycin resistance gene; **ITR**, inverted terminal repeats of SB100X; **IκB**, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor; **MAPK**, mitogen-activated protein kinase; **MCP**, MS2 bacteriophage coat protein; **MCS**, multiple cloning site; **MESA**, modular extracellular sensor; **NanoLuc**, *Oplophorus gracilirostris luciferase*; **NLS**, nuclear localization signal; **NFAT**, nuclear factor of activated T-cells; **NFAT_{reg}**, nuclear factor of activated T-cells regulatory domain; O_{TetR}, TetR-binding operator sequence; **p65**, human transcription factor p65; **p65_{TA}**, human transcription factor p65 transactivation domain; **pA**, polyadenylation signal; **PCR**, polymerase chain reaction; **P_{hCMV}**, human cytomegalovirus immediate early promoter; **P_{hEF1α}**, human elongation factor 1 alpha promoter; **P_{hINS}**, human insulin promoter; **P_{NFAT}**, synthetic mammalian promoter containing five tandem repeats of a human IL-4 NFAT-binding site; **P_{RPBSA}**, constitutive synthetic mammalian promoter; **P_{SV40}**, simian virus 40 promoter; **PuroR**, puromycin resistance gene; **SB100X**, optimized Sleeping Beauty transposase; **SEAP**, human placental secreted alkaline phosphatase; **sgRNA**, synthetic guide RNA; **shGLP1**, short human glucagon-like peptide 1; **SMAD2**, mothers against decapentaplegic homolog 2; **TBR11**, transforming growth factor β type II receptor; **TEV**, tobacco etch virus nuclear-inclusion-a endopeptidase; **tTA**, tetracycline-controlled transactivator; **VEGFR1**, vascular endothelial growth factor receptor, type 1; **YPet**, yellow fluorescent protein variant.

Supplementary Table 2. Synthetic guide RNA target sequences

Plasmid	Target	Complementary sequence	Reference
pGM70, pLeo1207	Human insulin promoter	5'-CGGCAGATGGCTGGGGGCTG-3'	This work
pKK126	Human interleukin 2 (IL-2) promoter	5'-AGGTAATGTTTTTTCAGAC-3'	²⁰
pKK158, pLeo1209	Human interleukin 12 B (IL-12B) promoter	5'-AGTTTAAGTTTCCATCAGAA-3'	This work
pKK160	Human TGF β promoter	5'-CCGCCCACGCGAGATGAGGA-3'	This work

Supplementary Table 3. Detailed transfection protocols used for Figures 2, 3, 5 and Supplementary Figures 3 and 9.

The amount of DNA was calculated per 1 well of a 24-well plate.

Application	GEAR	sgRNA	Other plasmids	Total DNA
Membrane depolarization-induced transgene activation	pKK50 10 ng	pGM70 25 ng	pGM74 – 25 ng	300 ng
			pKK05 – 15 ng	
			pSP20 – 150 ng	
			pCaV1.3 – 30 ng	
			pCaV α 2 δ 1 – 30 ng	
Membrane depolarization-induced endogenous insulin activation	pKK50 10 ng	pGM70 25 ng	pCaVb3 – 10 ng	300 ng
			pGM74 – 50 ng	
			pKK05 – 15 ng	
			pCOLADuet-1 – 130 ng	
			pCaV1.3 – 30 ng	
			pCaV α 2 δ 1 – 30 ng	
			pCaVb3 – 10 ng	

Application	GEAR	sgRNA	Other plasmids	Total DNA
VEGF165-induced transgene activation	pKK50 2.5 ng	pGM70 25 ng	pGM74 – 50 ng pSP20 – 150 ng phFLT1 – 25 ng pCOLADuet-1 – 47.5 ng	300 ng
VEGF165-induced endogenous gene activation	pKK50 6.25 ng	pGM70 25 ng	pGM74 – 50 ng phFLT1 – 25 ng pCOLADuet-1 – 193.75 ng	300 ng
TGFβ-induced transgene activation	pKK136 25 ng	pGM70 25 ng	pGM74 – 50 ng pSP20 – 150 ng pTBRII – 25 ng pCOLADuet-1 – 25 g	300 ng
TGFβ-induced endogenous gene activation	pKK136 25 ng	pGM70 or pKK158 25 ng	pGM74 – 50 ng pTBRII – 25 ng	300 ng

Application	GEAR	sgRNA	Other plasmids	Total DNA
			pCOLADuet-1 – 175 ng	
TNF α -induced transgene activation	pKK122 150 ng	pGM70 25 ng	pGM74 – 25 ng pSP20 – 150 ng pKK130 – 150 ng	500 ng
TNF α -induced endogenous gene activation	pKK122 200 ng	pGM70 or pKK158 25 ng	pGM74 – 50 ng pKK130 – 200 ng	475 ng
bFGF-induced transgene activation	pKK82 25 ng	pGM70 25 ng	pGM74 – 50 ng pSP20 – 150 ng pCOLADuet-1 – 50 ng	300 ng
RR120-induced transgene activation	pKK82 25 ng	pGM70 25 ng	pGM74 – 25 ng pSP20 – 150 ng pLeo628 – 275 ng	500 ng

Supplementary Table 4. qPCR primer pairs

Target	Sequence	Reference
Human insulin	5'-ATCAGAAGAGGCCATCAAGCA-3' 5'-TAGAGAGCTTCCACCAGGTGTGA-3'	21
Human GAPDH	5'-GTCTCCTCTGACTTCAACAGCG-3' 5'-ACCACCCTGTTGCTGTAGCCAA-3'	Origene Technologies (Cat. No. HP205798)
Human interleukin 12B (IL-12B)	5'-GACATTCTGCGTTCAGGTCCAG-3' 5'-CATTTTTGCGGCAGATGACCGTG-3'	Origene Technologies (Cat. no. HP205923)
SEAP	5'-ACAAACTGGGGCCTGAGATACC-3' 5'-CTGCACTCAAGCCAATGGTCTG-3'	This work
Human TGF β	5'-TACCTGAACCCGTGTTGCTCTC-'3 5'-GTTGCTGAGGTATCGCCAGGAA-3'	Origene Technologies (Cat. no. HP200609)
Human interleukin 2 (IL-2)	5'-AGAACTCAAACCTCTGGAGGAAG-'3 5'-GCTGTCTCATCAGCATATTCACAC-'3	Origene Technologies (Cat. no. HP200553)
Human nuclear receptor related-1 protein (NR4A2)	5'-AAACTGCCCAGTGGACAAGCGT-'3 5'-GCTCTTCGGTTTCGAGGGCAAA-'3	Origene Technologies (Cat. no. HP209171)

Target	Sequence	Reference
Human early growth response 3 (EGR3)	5'-GACTCGGTAGTCCATTACAATCAG-'3	Origene Technologies
	5'-AGTAGGTCACGGTCTTGTTGCC-'3	(Cat. no. HP207741)
Human vascular cell adhesion molecule 1 (VCAM1)	5'-GATTCTGTGCCCACAGTAAGGC-'3	Origene Technologies
	5'-TGGTCACAGAGCCACCTTCTTG-'3	(Cat. no. HP230503)

Supplementary Table 5. Detailed statistics

Confidence level = 95%

Figure	Description	Mean 1 (control)	Mean 2 (induced)	Fold induction	F test to compare variances (p-value)	Welch's correction applied (yes/no)	Two-tailed t-test (p-value)
2b	Cav1.3, GEAR _{NFAT} ;	3.71	33.5	9.0	0.0092	yes	0.0063
2d	VEGFR, GEAR _{NFAT}	6.54	13.27	2.0	0.090	no	0.0035
2f	TBR1, GEAR _{SMAD2}	7.71	50.76	6.6	0.33	no	0.00014
2h	TNFR, GEAR _{p65}	4.55	89.28	19.6	0.0067	yes	0.0085
2j	FGFR, GEAR _{Elk1}	2.10	9.54	4.5	0.69	no	0.00000037
2l	GEMS-MAPK (RR120), GEAR _{Elk1}	1.40	14.41	10.3	0.067	no	0.0000057
3b	Cav1.3, GEAR _{NFAT} – Insulin mRNA	3.02	97.42	32.3	0.0010	yes	0.011

Figure	Description	Mean 1 (control)	Mean 2 (induced)	Fold induction	F test to compare variances (p-value)	Welch's correction applied (yes/no)	Two-tailed t-test (p-value)
3c	Cav1.3, GEAR _{NFAT} - Insulin protein	0	0.26	N/A	N/A	yes	0.0004
3d	Insulin	6.77	234.5	34.6	0.0097	yes	0.0027
	IL-12	11.3	81.26	7.2	0.017	yes	0.021
4b	gRNA _{IL-12}	12.34	8546	692.5	0.00000065	yes	0.032
	gRNA _{INS}	2.87	3.17	1.1	0.16	no	0.7314
4c	2 h	18.37	25.7	1.4	0.21	no	0.17
	4 h	23.92	322.3	13.5	0.037	yes	0.0008
	8 h	20.93	2112	100.9	0.74	no	0.00000000004
5b	TBR1, GEAR _{SMAD2}	8.83	14.51	1.6	0.48	no	0.0080
5d	TNFR, GEAR _{p65}	20.04	193.8	9.7	0.044	yes	0.020
S2	8 h	0.84	3.002	3.6	0.020	yes	0.0397

Figure	Description	Mean 1 (control)	Mean 2 (induced)	Fold induction	F test to compare variances (p-value)	Welch's correction applied (yes/no)	Two-tailed t-test (p-value)
	24 h	6.80	30.16	4.4	0.08	no	0.000006
	36 h	9.731	49.26	5.1	0.0045	yes	0.0019
	48 h	48.2	117.8	2.4	0.8037	no	0.0003
S3a	VEGFR, GEAR _{NFAT}	14.2	29.23	2.1	0.95	no	0.014
S3b	TBRIL, GEAR _{SMAD2}	0	87.31	N/A	N/A	yes	0.018
S3c	TNFR, GEAR _{p65}	4.30	39.3	9.2	0.29	no	0.000082
S4a	VEGFR/GEAR _{NFAT}	0.74	2.42	3.3	0.39	no	0.0164
S4b	TNFR/ GEAR _{p65}	0.098	2.19	22.4	0.014	yes	0.0305
S4c	TBRIL/ GEAR _{SMAD2}	0.11	0.65	5.7	0.12	no	0.0008
S4d	FGFR/GEAR _{Elk1}	0.087	0.29	3.3	0.73	no	0.0314
S5a	MESA V1 TetR 24 h 20 ng	19.65	20	1.0	0.82	no	0.825
	MESA V1 TetR 36 h 20 ng	44.72	47.85	1.1	0.498	no	0.073

Figure	Description	Mean 1 (control)	Mean 2 (induced)	Fold induction	F test to compare variances (p-value)	Welch's correction applied (yes/no)	Two-tailed t-test (p-value)
	MESA V1 TetR 24 h 200 ng	19.65	17.51	0.9	0.59	no	0.237
	MESA V1 TetR 36 h 200 ng	44.72	48.5	1.1	0.0045	no	0.063
S5b	MESA V1 TetR 24 h 20 ng	0.82	0.97	1.2	0.72	no	0.107
	MESA V1 TetR 36 h 20 ng	1.65	1.98	1.2	0.33	no	0.005
	MESA V1 TetR 24 h 200 ng	0.82	0.80	1.0	0.19	no	0.839
	MESA V1 TetR 36 h 200 ng	1.65	1.96	1.2	0.34	no	0.013
S5c	MESA V1 dCas9 24 h 20 ng	0.55	0.50	0.9	0.79	no	0.579
	MESA V1 dCas9 36 h 20 ng	1.61	1.62	1.0	0.17	no	0.902
	MESA V1 dCas9 24 h 200 ng	0.55	0.39	0.7	0.70	no	0.115
	MESA V1 dCas9 36 h 200 ng	1.61	1.26	0.8	0.18	no	0.021
S5d	MESA V1 dCas9 24 h 20 ng	1.61	1.62	1.0	0.17	no	0.948
	MESA V1 dCas9 36 h 20 ng	2.56	2.66	1.0	0.43	no	0.569

Figure	Description	Mean 1 (control)	Mean 2 (induced)	Fold induction	F test to compare variances (p-value)	Welch's correction applied (yes/no)	Two-tailed t-test (p-value)
	MESA V1 dCas9 24 h 200 ng	1.61	1.26	0.8	0.18	no	0.021
	MESA V1 dCas9 36 h 200 ng	2.56	2.36	0.9	0.58	no	0.299
S5e	MESA V2 TetR 24 h 20 ng	16.6	18.14	1.1	0.45	no	0.133
	MESA V2 TetR 36 h 20 ng	67.64	71.71	1.1	0.11	no	0.232
	MESA V2 TetR 24 h 200 ng	16.6	16.49	1.0	0.89	no	0.924
	MESA V2 TetR 36 h 200 ng	67.64	69.8	1.0	0.70	no	0.569
S5f	MESA V2 TetR 24 h 20 ng	0.98	1.16	1.2	0.0032	yes	0.128
	MESA V2 TetR 36 h 20 ng	2.42	2.52	1.0	0.34	no	0.570
	MESA V2 TetR 24 h 200 ng	0.98	1.06	1.1	0.0008	no	0.681
	MESA V2 TetR 36 h 200 ng	2.42	2.52	1.0	0.79	no	0.405
S5g	MESA V2 dCas9 24 h 20 ng	0.11	0.097	0.9	0.39	no	0.819
	MESA V2 dCas9 36 h 20 ng	0.15	0.091	0.6	0.75	no	0.175

Figure	Description	Mean 1 (control)	Mean 2 (induced)	Fold induction	F test to compare variances (p-value)	Welch's correction applied (yes/no)	Two-tailed t-test (p-value)
	MESA V2 dCas9 24 h 200 ng	0.11	0.11	1.0	0.43	no	0.981
	MESA V2 dCas9 36 h 200 ng	0.15	0.059	0.4	0.028	yes	0.033
S5h	MESA V2 dCas9 24 h 20 ng	0.24	0.30	1.3	0.34	no	0.250
	MESA V2 dCas9 36 h 20 ng	0.49	0.63	1.3	0.54	no	0.353
	MESA V2 dCas9 24 h 200 ng	0.24	0.25	1.1	0.19	no	0.831
	MESA V2 dCas9 36 h 200 ng	0.49	0.52	1.1	0.05	no	0.839
S6a	IL-12	0.00	1.47	N/A	N/A	yes	0.50
	IL-2	1301.47	3948.24	3.0	0.46	no	0.0033
	NR4A2	0.83	642.07	770.7	0.32	no	0.00000006
	EGR3	85.77	1072.99	12.5	0.063	no	0.00092
	FOSb	0.87	2.75	3.2	0.24	no	0.48
	ATF3	401.20	322.81	0.8	0.51	no	0.30

Figure	Description	Mean 1 (control)	Mean 2 (induced)	Fold induction	F test to compare variances (p-value)	Welch's correction applied (yes/no)	Two-tailed t-test (p-value)
	VCAM1	0.00	342.29	N/A	N/A	yes	0.037
S6b	IL-12	3.76	226.73	60.3	0.0042	yes	0.0073
	IL-2	593.28	1012.55	1.7	0.18	no	0.11
	NR4A2	2.52	331.47	131.4	9.15E-05	yes	0.027
	EGR3	52.70	358.27	6.8	0.020	yes	0.0007
	FOSb	2.07	13.18	6.4	0.041	yes	0.091
	ATF3	203.23	456.59	2.2	0.066	no	0.020
	VCAM1	0.00	16.63	N/A	N/A	yes	0.082
S8a	GEAR _{NFAT}	1.389	23.63	11.0	0.018	yes	0.0018
	CaRROT	1.61	5.55	3.4	0.82	no	0.00002
S8b	24 h	1.36	2.25	1.7	0.050	no	0.0021
	48 h	2.19	5.31	2.4	0.058	no	0.0010
S9a	GEAR _{NFAT}	12.47	82.2	6.6	0.98	no	0.0000015
	No GEAR	0.52	1.80	3.5	0.077	no	0.00014

Figure	Description	Mean 1 (control)	Mean 2 (induced)	Fold induction	F test to compare variances (p-value)	Welch's correction applied (yes/no)	Two-tailed t-test (p-value)
	control						
	No gRNA	0.73	1.98	2.7	0.67	no	0.0032
	control						
S9b	GEAR _{NFAT}	5.235	10.03	1.9	0.18	no	0.0010
	No GEAR	1.00	1.29	1.3	0.92	no	0.14
	control						
	No gRNA	1.29	1.34	1.0	0.13	no	0.93
	control						
S9c	GEAR _{SMAD2}	7.87	77.25	9.8	0.063	no	0.00023
	No GEAR	0.71	1.02	1.4	0.11	no	0.50
	control						
	No gRNA	0.94	1.00	1.1	0.15	no	0.58
	control						

Figure	Description	Mean 1 (control)	Mean 2 (induced)	Fold induction	F test to compare variances (p-value)	Welch's correction applied (yes/no)	Two-tailed t-test (p-value)
S9d	GEAR _{p65}	16.89	215.8	12.8	0.021	yes	0.0087
	No GEAR control	0.80	3.74	4.6	0.75	no	0.000036
	No gRNA control	3.16	6.42	2.0	0.22	no	0.0049
S9e	GEAR _{Elk1}	2.51	11.47	4.6	0.37	no	0.0000082
	No GEAR control	0.88	0.62	0.7	0.77	no	0.14
	No gRNA control	5.82	7.19	1.2	0.064	no	0.019
S10a	GEAR _{NFAT}	0.61	117.58	192.74	0.000050	yes	0.00059
	No GEAR control	N/A	N/A	N/A	N/A	N/A	N/A
	No gRNA	N/A	N/A	N/A	N/A	N/A	N/A

Figure	Description	Mean 1 (control)	Mean 2 (induced)	Fold induction	F test to compare variances (p-value)	Welch's correction applied (yes/no)	Two-tailed t-test (p-value)
	control						
	GEAR _{NFAT}	4.88	26.68	5.87	0.13	no	0.0034
	No GEAR						
S10b	control	3.36	4.76	1.41	0.08	no	0.11
	No gRNA						
	control	7.70	14.75	1.92	0.61	no	0.038
S13a	SEAP mRNA	0.88	7.43	8.5	0.0316	yes	0.045
S13b	SEAP protein	17.16	114.5	6.7	0.0070	yes	0.018

N/A – not applicable

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