Supplementary Information

Self-adjusting synthetic gene circuit for correcting insulin resistance

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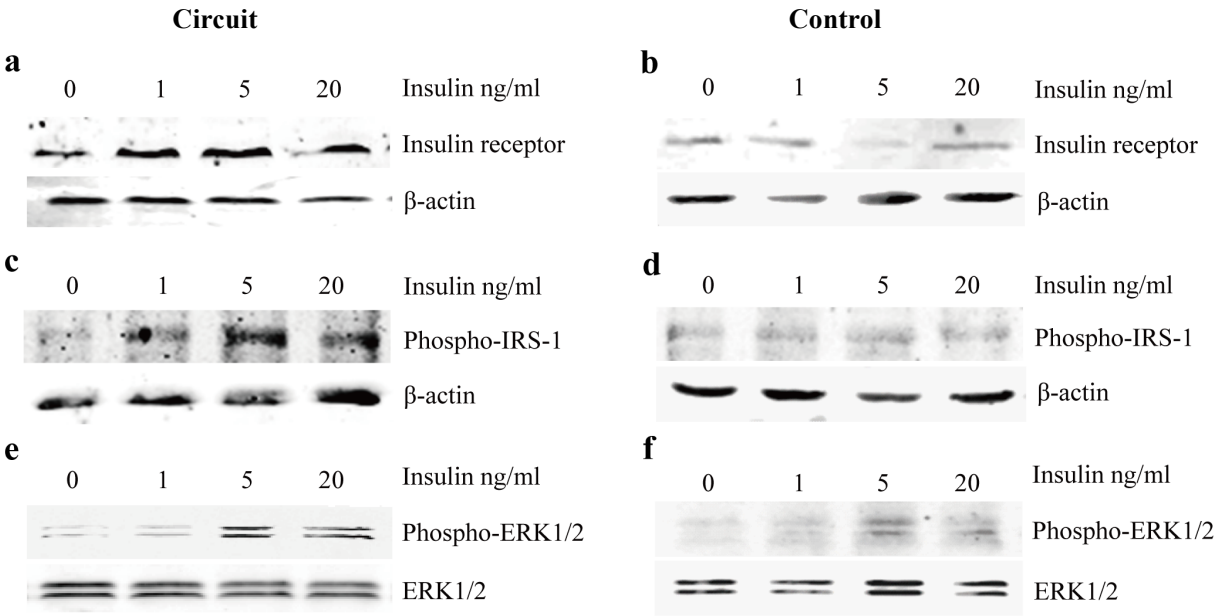
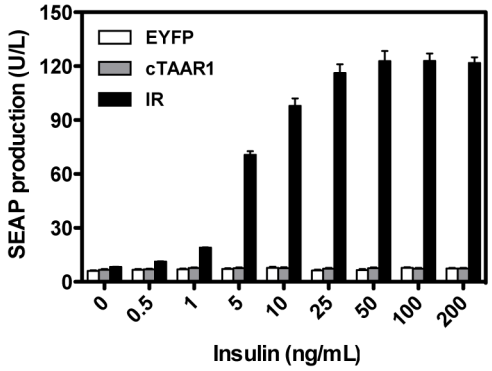
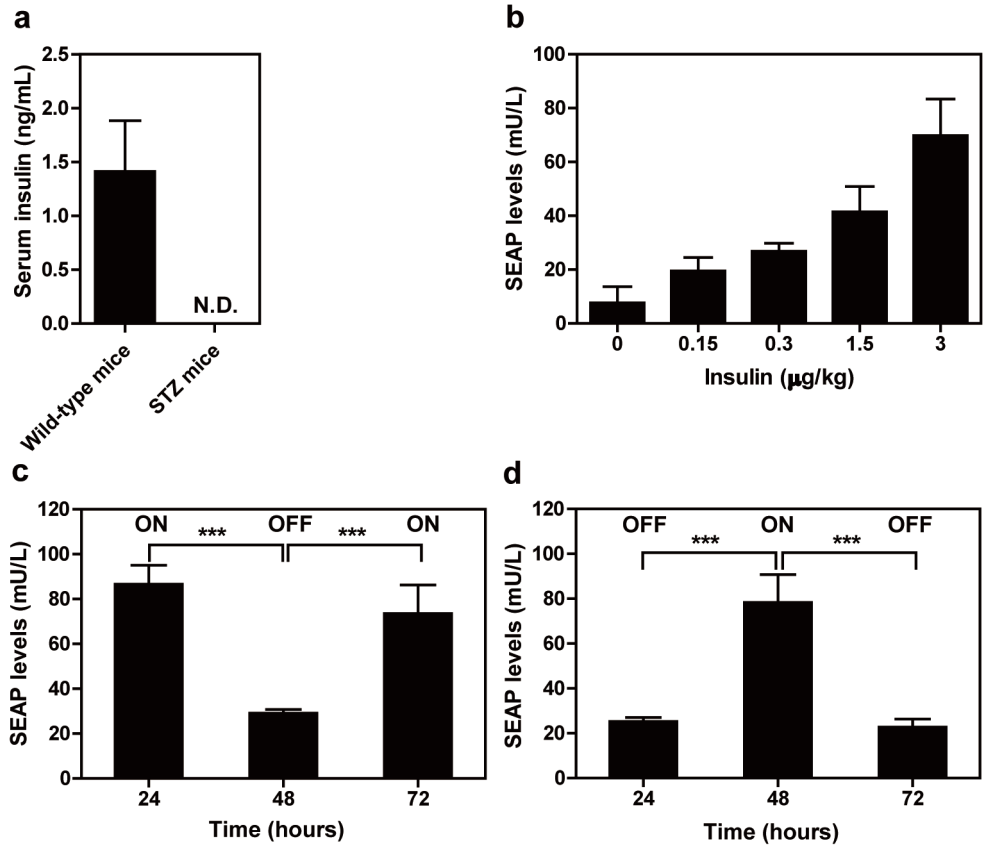


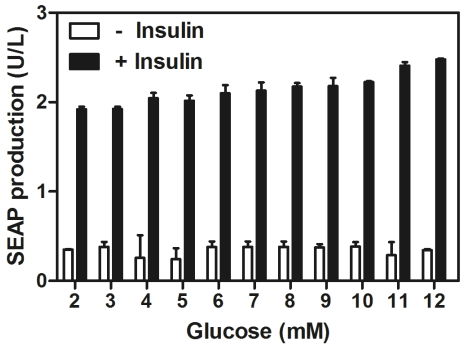
Supplementary Figure 1 | Western blot analysis of insulin-dependent activation of the IRS-1-Ras-MAPK signalling cascade in HEK-293 cells. HEK-293 cells co-transfected with the circuit components pIR (P_{hCMV}-IR-pA), pTetR-ELK1 (P_{hCMV}-TetR-ELK1-pA) and pMF111 ($P_{hCMV*-1}$-SEAP-pA) (**a, c, e; Circuit**) were stimulated with different concentrations of insulin, and the cells were harvested and probed for IR, phospho-IRS-1 and phospho-Erk1/2. HEK-293 cells co-transfected with pKZY73, pTetR-ELK1 and pMF111 (**b, d, f; Control**) were used as a control. Total ERK1/2 and β-actin were used as loading controls.



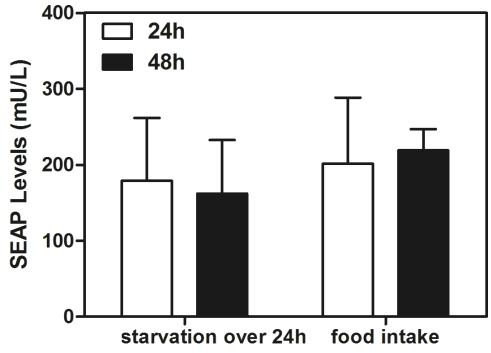
Supplementary Figure 2 | Insulin-triggered SEAP expression in human insulin receptor (IR)-transfected HEK-293 cells. HEK-293 cells were co-transfected with pIR (P_{hCMV}-IR-pA), pTetR-ELK1 (P_{hCMV}-TetR-ELK1-pA) and pMF111 ($P_{hCMV*-1}$-SEAP-pA) at a ratio of 1:1:1 and cultivated for 72 hours in the presence or absence of different concentrations of insulin. Control cells were co-transfected with pEYFP-C1 (P_{hCMV}-EYFP-pA), pTetR-ELK1 and pMF111 or pKZY73 (P_{SV40}-cTAAR1-pA), pTetR-ELK1 and pMF111. The data represent the mean ± SD; n=3 independent experiments.



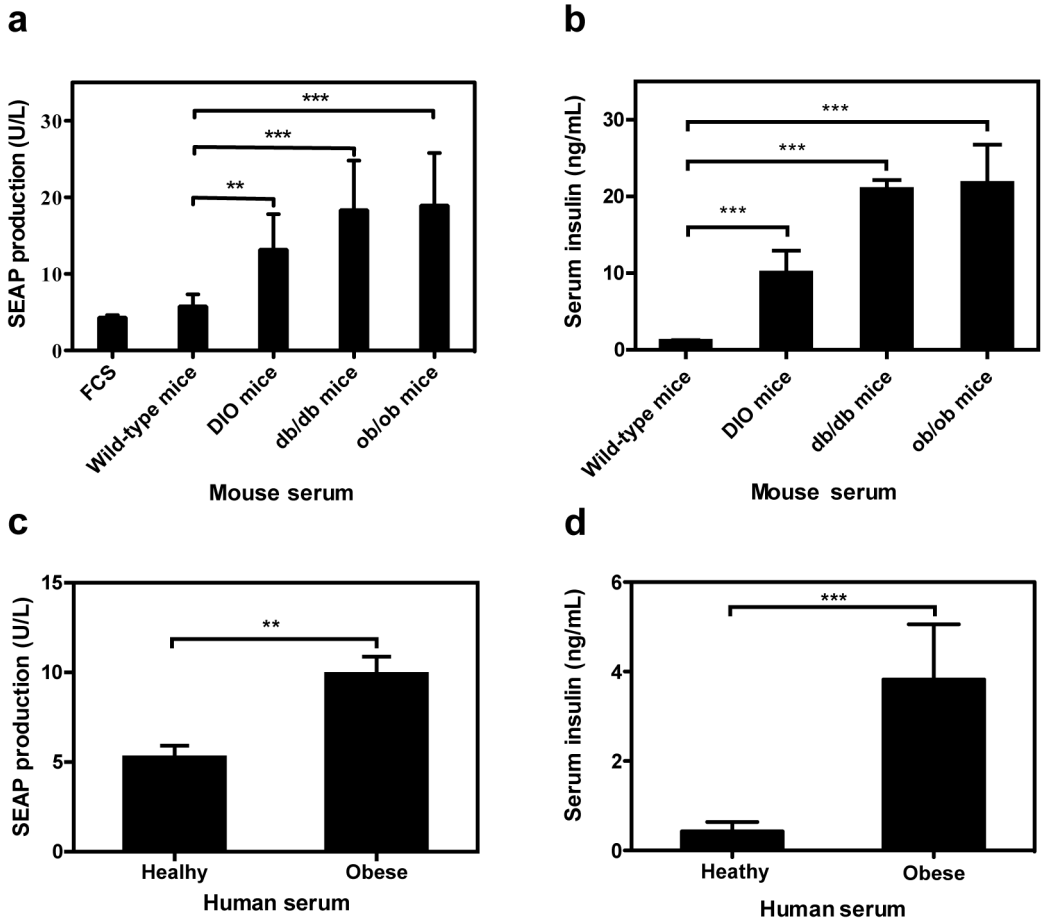
Supplementary Figure 3 | Adjustability and reversibility of the synthetic insulin-sensor device in an insulin-deficient mouse model. (**a**) Insulin ELISA was performed 7 days after STZ injection. (**b**) Animals were intraperitoneally implanted with $2x10^6$ encapsulated pIR-/pTetR-ELK1-/pMF111-transgenic HEK-293 cells (200 cells/capsule) and received daily injection of insulin. Serum SEAP levels were profiled of treated animals after 48 h. (**c, d**) Reversibility of insulin-triggered SEAP expression in mice. Mice implanted with $2x10^6$ encapsulated pIR-/pTetR-ELK1-/pMF111-transgenic HEK-293 cells were injected with 3µg/kg insulin at 0 and 48 h (ON-OFF-ON) or only at 24 h (OFF-ON-OFF) after implantation. SEAP levels in the serum were profiled of treated animals after 24, 48, and 72h. The data represent the mean ± SEM, statistical analysis using a two-tailed Student's *t*-test, n=6 mice per group. **P* < 0.05, ***P*<0.01, ****P* < 0.001 vs. control. N.D., not detectable (Detection limit: $\leq$0.2 ng/mL).



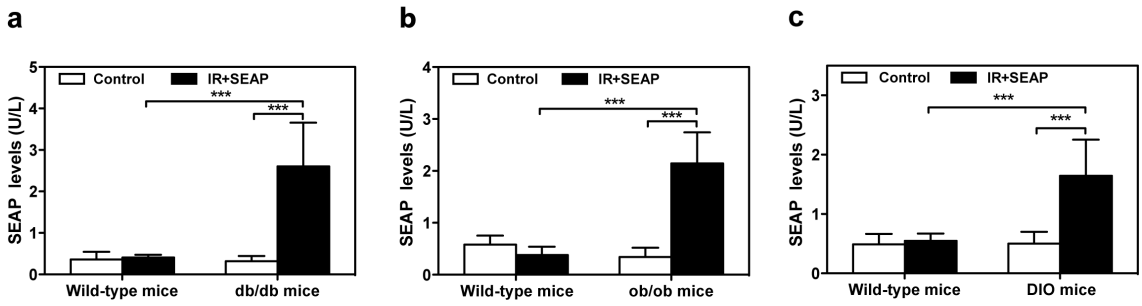
Supplementary Figure 4 | The impact of glucose levels on insulin-triggered SEAP expression *in vitro*. HEK-293 cells were co-transfected with pIR, pTetR-ELK1 and pMF111 at a ratio of 1:1:1 and cultivated for 48 hours in the presence of different concentrations of glucose and in the presence or absence of human insulin (1 ng/mL) before SEAP levels were profiled in the culture supernatant. The data represent the mean ± SD; n=3 independent experiments.



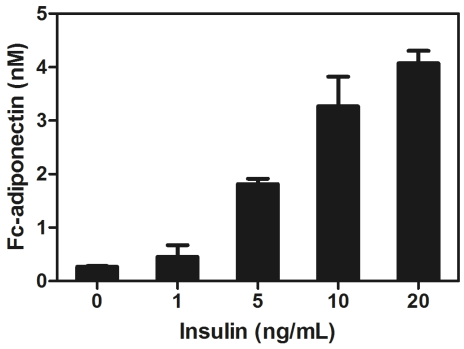
Supplementary Figure 5 | Insensitivity of the insulin-sensor circuit to feed-fasting cycles mice. Wild-type mice were intraperitoneally implanted with $2x10^6$ encapsulated pIR-/pTetR-ELK1-/pMF111-transgenic HEK-293 cells (200 cells/capsule) and either subjected to an initial starvation phase of 24 hours (average glycaemia of starved mice prior to food exposure: 4.9±0.6mM) or normal access to food over 48 hours (average glycaemia of non-fasted mice: 9.5±0.9mM). SEAP levels in the animals' sera were quantified at 24 and 48 hours after implantation. The data represent the mean ± SEM, n=8 mice per group.



Supplementary Figure 6 | Serum containing high levels of insulin triggered transgene expression in HEK-293 cells. (a, c) Cell-based assay. Insulin-triggered SEAP expression of pIR-/pTetR-ELK1-/pMF111-co-transfected HEK-293 cells cultivated for 72 hours in the presence of (**a**) 10% mouse serum or (**c**) 10% human serum. **(b, d)** ELISA of undiluted serum used in (a) and (c). Corresponding insulin levels in the (**b**) mouse and (**d**) human sera were quantified by an insulin-specific ELISA. The data represent the mean ± SD, statistical analysis by a two-tailed Student's *t*-test, n=8, $^{**}P<0.01$, $^{***}P < 0.001$ vs. control.



Supplementary Figure 7 | Self-sufficient insulin-sensor-based control of SEAP expression in insulin-resistant (a) db/db, (b) ob/ob and (c) DIO mouse models. Mice were intraperitoneally implanted with $2x10^6$ encapsulated pIR-/pTetR-ELK1-/pMF111-transgenic HEK-293 cells (200 cells/capsule). Control mice were intraperitoneally implanted with $2x10^6$ encapsulated pKZY73-/pTetR-ELK1-/pMF111-transgenic HEK-293 cells (200 cells/capsule). After 48 hours of implantation, SEAP levels in the serum were profiled. The data represent the mean ± SEM, statistical analysis by a two-tailed Student's *t*-test, n=8 mice per group. ***$P < 0.001$ vs. control.



Supplementary Figure 8 | Insulin-triggered adiponectin expression in HEK-293 cells. The adiponectin expression kinetics of HEK-293 cells co-transfected with pIR, pTetR-ELK1, and pHY79 ($P_{hCMV*-1}$-Fc-adiponectin-pA) at a ratio of 1:1:1 and cultivated for 72 h in the presence or absence of different concentrations of human insulin. The Fc-adiponectin expression levels in the culture medium were quantified using a human IgG1-specific ELISA kit. The data represent the mean ± SD; n=3 independent experiments.

Table S1. Plasmids designed and used in this study.

Plasmid	Description	Reference
pcDNA3.1(+)	Mammalian expression vector (P_{hCMV}-MCS-pA).	Invitrogen, CA
pEYFP-C1	Constitutive EYFP expression vector (P_{hCMV}-EYFP-pA).	Clontech, CA
pSEAP2-Control	Constitutive SEAP expression vector (P_{SV40}-SEAP-pA).	Clontech, CA
pIR	Constitutive human IR expression vector (P_{hCMV}-IR-pA) (Addgene no. 24049).	(19)
pCMV-T7-SB100	Constitutive SB100X expression vector (P_{hCMV}-SB100X-pA) (Addgene no. 34879).	(38)
pSBtet-GP	SB100X-specific transposon containing a tetracycline-responsive luciferase expression unit and a constitutive expression unit for EGFP, rtTA and PuroR. ($P_{hCMV*-1}$-Luc-pA:P_{RPBSA}-EGFP-P2A-rtTA-P2A-PuroR-pA) (Addgene no. 60495).	(43)
pH107	Lentiviral vector containing constitutive expression units for EGFP-3FLAG and ZeoR (LTR-P_{hCMV}-EGFP-3FLAG-pA:P_{mPGK}-ZeoR-pA-LTR).	ObiO, Shanghai
pTetR-ELK1	Constitutive TetR-ELK1 expression vector (P_{hCMV}-TetR-ELK1-pA).	(44)
pMF111	Tetracycline-responsive SEAP expression vector ($P_{hCMV*-1}$-SEAP-pA).	(33)
pKZY73	Constitutive cTAAR1 expression vector (P_{SV40}-cTAAR1-pA).	(16)
pXS39	Mammalian expression vector containing constitutive expression units for SEAP and ZeoR (P_{hCMV}-SEAP-pA:P_{mPGK}-ZeoR-pA). A linearized vector (Fragment 1) was PCR-amplified from pcDNA3.1(+) using OXS79 (5'-GTTTAAACCCGCTGATCAGCCTCG-3') and OXS80 (5'-CTAGCCAGCTTGGGTCTCCCTATAG-3'), SEAP-pA (Fragment 2) was PCR-amplified from pSEAP2-Control using OXS81 (5'-AGGGAGACCCAAGCTGGCTAGGCCCACCATGCTGCTGCTGCTGCTGC-3') and OXS82 (5'-AGCGCCTCCCCTACCCGGTAGGCTCCATCGTTCAGATCCTTATCG-3'), P_{mPGK}-ZeoR (Fragment 3) was PCR-amplified from pH107 using OXS83 (5'-CTACCGGGTAGGGGAGGCGCTTTTC-3') and OXS84 (5'-GGCTGATCAGCGGGTTTAAACCCGCTCAGTCCTGCTCCTCGGCCAC-3'), and all fragments were assembled by homologous recombination using the GeneArt® Seamless Cloning and Assembly Kit.	This work

	Tetracycline-responsive EYFP expression vector ($P_{hCMV*-1}$-EYFP-pA).	
pHY74	EYFP was PCR-amplified from pEYFP-C1 using OHY163 (5'-gcgccgacgaattcGCCACCATGGTGAG CAAGGGCGAGGAGCTGTTCACC-3') and OHY164 (5'-cacgcacgaagcttTTACTTGTACAGCTCGT CCATGCC-3'), restricted with *Eco*RI/*Hin*dIII and cloned into the corresponding sites of pMF111.	This work
pHY79	Tetracycline-responsive Fc-adiponectin expression vector ($P_{hCMV*-1}$-Fc-adiponectin-pA). Custom-designed Fc-adiponectin[7] was restricted with *Eco*RI/*Hin*dIII and cloned into the corresponding sites of pMF111.	This work
pHY112	Mammalian expression vector containing a constitutive bicistronic expression unit for SEAP and EGFP (P_{SV40}-SEAP-P2A-EGFP-pA). A linearized vector (Fragment 1) was PCR-amplified from pSEAP2-Control using OHY205 (5'-CTCGGCATGGACGAGCTGTACAAGGGAGAATGGGCGGAACTGGGCGGAG-3') and OHY206 (5'-GGCTGAAGTTAGTAGCTCCGCTTCCTGTCTGCTCGAAGCGGCCGGCCG-3'), P2A-EGFP (Fragment 2) was PCR-amplified from pSBtet-GP using OHY207 (5'-GGAAGCGGAGCT ACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTTCCGGA GTGAGCAAGGGCGAGGAGCTGTTC-3') and OHY208 (5'-CTTGTACAGCTCGTCCATGCCGAG -3'), and both fragments were assembled by homologous recombination using the GeneArt® Seamless Cloning and Assembly Kit.	This work
pHY113	SB100X-specific transposon containing a constitutive bicistronic expression IR and PuroR unit (ITR-P_{hCMV}-IR-P2A-PuroR-pA-ITR). A linearized vector (Fragment 1) was PCR-amplified from pSBtet-GP using OHY209 (5'-GGGTCCGG CGCTACTAACTTCAGCC-3') and OHY210 (5'-CTAGATAGCGGACCCCTTACCGAAAC-3'), P_{hCMV}-IR (Fragment 2) was PCR-amplified from pIR using OHY211 (5'-CGGTAAGGGGTCCGCTATCTAGGCGCTGCTTCGCGATGTAC GGGCCAG-3') and OHY212 (5'- GAAGTTAGTAGCGCCGGACCCGGAAGGATTGGACCGAGGCAAGGTCAG-3'), and both fragments were assembled by homologous recombination using the GeneArt® Seamless Cloning and Assembly Kit.	This work

pHY115	Tetracycline-responsive bicistronic Fc-adiponectin and EGFP expression vector ($P_{hCMV*-1}$-Fc-adiponectin-P2A-EGFP-pA). Fc-adiponectin (Fragment 1) was PCR-amplified from pHY79 using OHY213 (5'-CTACCATGACACCAACactagtCCTGGAGAAGGTGCCTATGTATAC-3') and OHY214 (5'-GTTAGTAGCTCCGCTTCCGTTGGTGTCATGGTAGAGAAGAAAG-3') and P2A-EGFP (Fragment 2) was PCR-amplified from pHY112 using OHY215 (5'-CTCTACCATGACACCAACGGAAGCGGAGCTACTAACTTCAGC-3') and OHY216 (5'-CATGTCTGGATCGAAgctagcTTACTTGTACAGCTCGTCCATGCCG-3'). Both fragments were assembled by homologous recombination using the GeneArt® Seamless Cloning and Assembly Kit and cloned into pHY79 (*Spe*I/*Nhe*I).	This work
pHY117	SB100X derivative containing a constitutive PuroR expression unit (ITR -P_{mPGK}-PuroR-pA-ITR). A linearized vector (Fragment 1) was PCR-amplified from pSBtet-GP using OHY217 (5'-ATGACCGAGTACAAGCCCACGGTGC-3') and OHY218 (5'-CTAGATAGCGGACCCCTTACCGAAAC-3'), P_{mPGK} (Fragment 2) was PCR-amplified from pH107 using OHY219 (5'-GGTAAGGGGTCCGCTATCTAGCCGGGTAGGGGAGGCGCTTTTCCC-3') and OHY220 (5'-CGTGGGCTTGTACTCGGTCATGGTAAGCTTGGGCTGCAGGTCGAAAG-3'), and both fragments were assembled by homologous recombination using the GeneArt® Seamless Cloning and Assembly Kit.	This work
pHY118	SB100X derivative containing a tetracycline-responsive bicistronic Fc-adiponectin and EGFP expression unit and a constitutive PuroR expression unit. (ITR-$P_{hCMV*-1}$-Fc-adiponectin-P2A-EGFP-pA: P_{mPGK}-PuroR-pA-ITR). A linearized vector (Fragment 1) was PCR-amplified from pHY117 using OHY221 (5'-CCGGGTAGGGGAGGCGCTTTTCCC-3') and OHY222 (5'-CTAGATAGCGGACCCCTTACCGAAAC-3'), $P_{hCMV*-1}$-Fc-adiponectin-P2A-EGFP-pA (Fragment 2) was PCR-amplified from pHY115 using oligonucleotides OHY223 (5'-GGTAAGGGGTCCGCTATCTAGGTGCCACCTGACGTCTAAGAAACC-3') and OHY224 (5'-GAAAAGCGCCTCCCCTACCCGGG CAGGATCATAATCAGCCATACCAC-3'), and both fragments were assembled by homologous recombination using the GeneArt® Seamless Cloning and Assembly Kit.	This work

pHY121	SB100X derivative containing a constitutive bicistronic IR and TetR-Elk1 expression unit and a constitutive ZeoR expression unit (ITR-P_{hCMV}-IR-P2A-TetR-Elk1-pA:P_{mPGK}-ZeoR-pA-ITR). A linearized vector (Fragment 1) was PCR-amplified from pHY113 using OHY231 (5'-TTCGAAGGCCTGTCGTGAAGCTTGG-3') and OHY232 (5'-GGGACCAGGATTCTCCTCGACGTC-3'), TetR-Elk1 (Fragment 2) was PCR-amplified from pTetR-ELK1 using OHY233 (5'-GTCGAGGAGAATCCTGGTCCCATGTCTAGACTGGACAAGAGCAAAG-3') and OHY234 (5'-GTCTGGATCGAAGCTTAGTTACCCGGGACCGGTTCATGGC-3'), pA-P_{mPGK}-ZeoR (Fragment 3) was PCR-amplified from pXS39 using OHY235 (5'-GAACCGGTCCCGGGTAACTAAGCTTCGATCCAGACATGATAAGATAC-3') and OHY236 (5'-GCTTCACGACAGGCCTTCGAATCAGTCCTGCTCCTCGGCCACGAAG-3'), and all fragments were assembled by homologous recombination using the GeneArt® Seamless Cloning and Assembly Kit.	This work

Oligonucleotides: Restriction endonuclease-specific sites are underlined in lowercase letters, annealing base pairs are indicated in capital letters, the homologous recombination sequences are underlined in capital letters.

Abbreviations: **3FLAG**, a polypeptide epitope containing three DYKDDDDK[45] repeats; **cTAAR1**, chimeric trace-amine-associated receptor 1; **EGFP,** enhanced green fluorescent protein; **ELK1,** human ETS domain-containing transcription factor Elk1; **EYFP,** enhanced yellow fluorescent protein; **Fc-adiponectin**, synthetic secretion-engineered adiponectin[23] containing a 5' Kozak sequence, an interleukin-2 secretion signal and the Fc sequence of human IgG1 fused N'-terminally to three human globular adiponectin modules; **ITR**, inverted terminal repeats of SB100X; **IR**, insulin receptor; **Luc,** firefly luciferase; **LTR**, lentiviral long terminal repeat; **MCS**, multiple cloning site; **pA**, polyadenylation signal; **PCR**, polymerase chain reaction; **P_{hCMV}**, human cytomegalovirus immediate early promoter; **$P_{hCMVmin}$**, minimal version of P_{hCMV}; **$P_{hCMV*-1}$**, tetracycline-responsive promoter ($tetO_7$-$P_{hCMVmin}$); **P_{mPGK}**, mouse phosphoglycerate kinase gene promoter; **P_{RPBSA},** synthetic constitutive promoter[3]; **P_{SV40}**, simian virus 40 promoter; **P2A**, picornavirus-derived self-cleaving peptide engineered for bicistronic gene

expression in mammalian cells; **PuroR**, gene conferring puromycin resistance; **rtTA**, reverse tetracycline-dependent transactivator; **SEAP**, human placental secreted alkaline phosphatase; **SB100X**, Sleeping Beauty transposase; **TetR**, *Escherichia coli Tn*10-derived tetracycline-dependent repressor of the tetracycline resistance gene; **tetO$_7$**, TetR-specific heptameric operator sequence; **ZeoR**, gene conferring zeocin resistance.

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