Supplementary Information

"A closed-loop synthetic gene circuit for the treatment of diet-induced obesity in mice"

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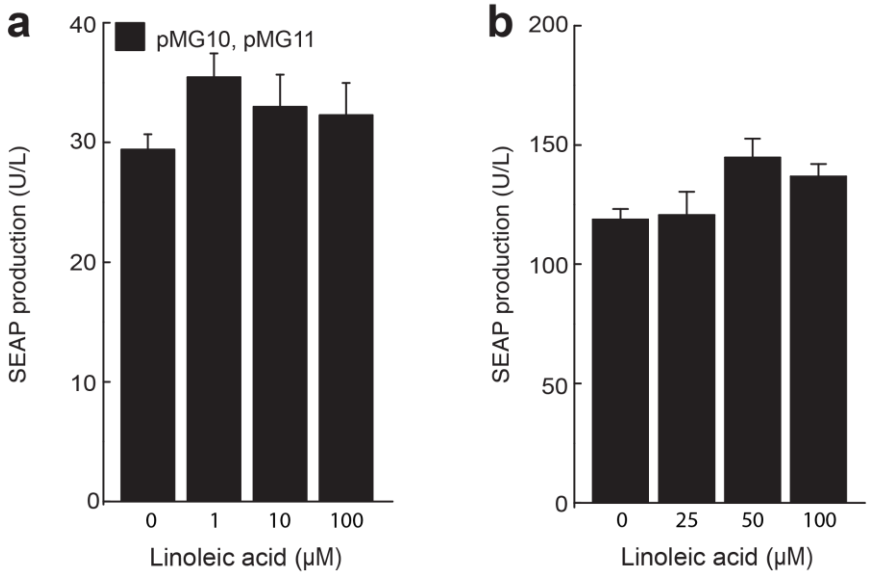
This pdf file includes:

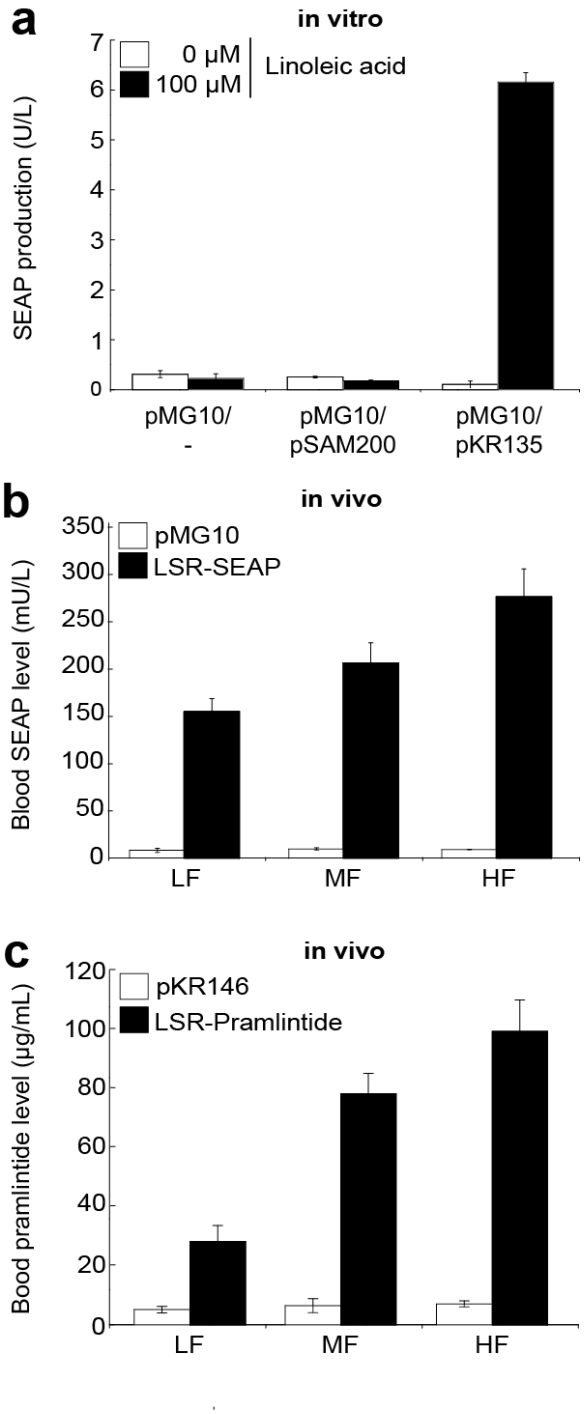
Supplementary Figures S1 to S5

Supplementary Table S1

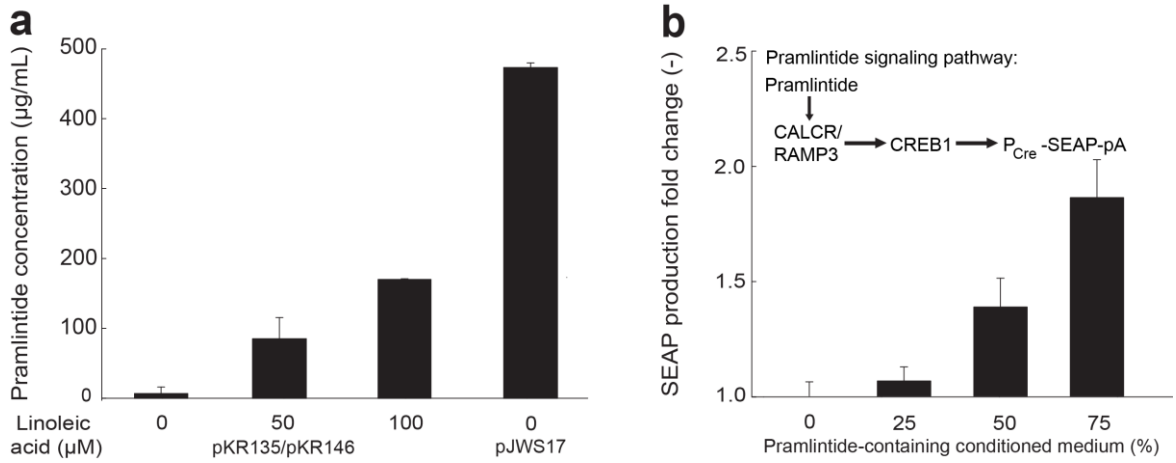
Supplementary References



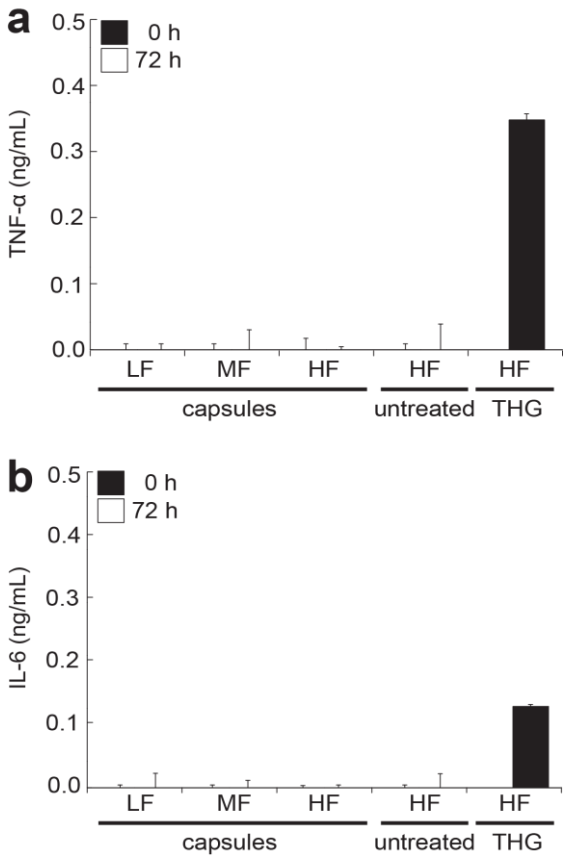
Supplementary Figure S1. Control specificity and viability impact of linoleic acid. (**a**) Insensitivity of the phloretin-responsive control element to linoleic acid. HT-1080 were cotransfected with the components encoding the phloretin-responsive gene switch (pMG10, (P_{TtgR1}-SEAP-pA) and pMG11, (P_{SV40}-$TtgA_1$-pA))[1] and grown for 48h in the presence of increasing linoleic acid concentrations before SEAP was profiled in the cell culture supernatant. (**b**) Viability of HT-1080 exposed to increasing concentrations of Linoleic acid. HT-1080 were transfected with pSEAP2-control (P_{SV40}-SEAP-pA) and exposed to increasing concentrations of linoleic acid. Any negative impact of excessive fatty acid on the metabolism or viability of the cells would impair their overall SEAP production capacity. The error bars indicate the s.d. (n = 3).



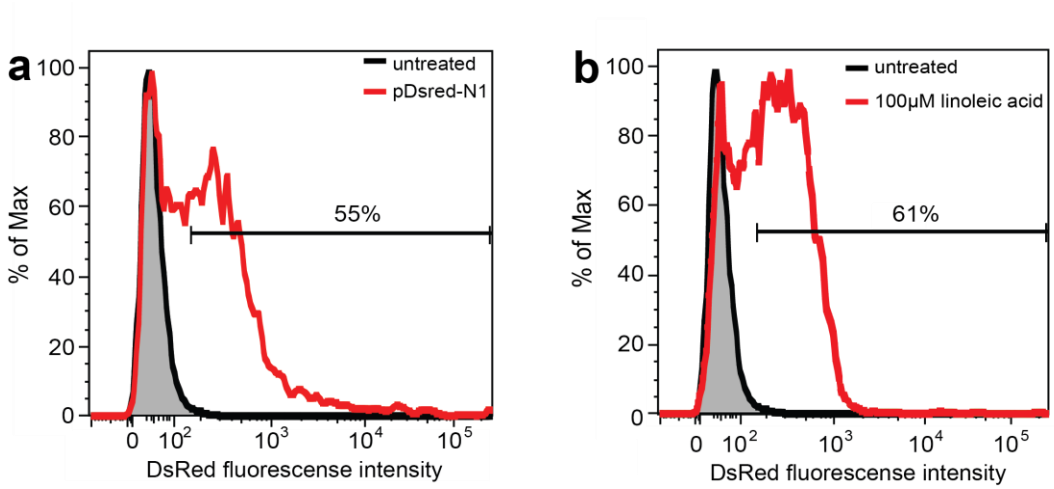
Supplementary Figure S2. Specificity and leakiness of LSR components. (**a**) HT-1080 cells were (co-)transfected with pMG10 (P_{TtgR1}-SEAP-pA), pSAM200 (P_{hCMV}-tTA-pA) and pMG10 or pKR135 (P_{hCMV}-LSR-pA) and pMG10 and cultivated for 48h in the presence and absence of linoleic acid before SEAP production was profiled in the culture supernatant. (**b, c**) Wild-type mice kept on a standard low-fat diet were implanted with microencapsulated pMG10-, pKR135-/pMG10$^{\#}$- (**b**), pKR146 (P_{TtgR1}-Pram-pA)- or pKR135/pKR146$^{\#\#}$- (**c**) transgenic HT-1080 ($2x10^6$ cells/mouse) and put on standard low fat (LF, 5kcal% fat), medium fat (MF, 10kcal% fat) and high fat (HF, 60kcal% fat) diets. $^{\#,\#\#}$ For comparison and clarity, data from Figs. 3d ($^{\#}$) and 4a ($^{\#\#}$) of the main text were replicated, respectively. The error bars indicate the s.d. (n = 4).



Supplememtary Figure S3. Characterization of pramlintide expression. (**a**) Comparative analysis of linoleic acid (LA)-induced LSR-driven (pKR135/pKR146) and constitutive (pJWS17, P_{hCMV}-Pram-pA) pramlintide expression by HT-1080 (co-)transfected with indicated vectors and cultivated for 48h. (**b**) Validation of functional pramlintide production. The culture supernatant of pKR135/pKR146-transgenic pramlintide-producing HT-1080 cultivated for 48h in the presence of 100µM linoleic acids was mixed at different percentages with DMEM and used to cultivate HEK-293 (co-)transfected with expression vectors encoding the pramlintide-specific receptors pCALCR, pRAMP3 and the corresponding reporter unit (pCK53; P_{CRE}-SEAP-pA[2]). Pramlintide-triggered SEAP expression was profiled after 48h. The error bars indicate the s.d. (n = 3).



Supplememtary Figure S4. Profiling of the inflammatory response after implantation of microencapsulated circuit-transgenic HT-1080. Wild-type mice kept on a standard diet were implanted with circuit-transgenic (pKR135/pKR146) HT-1080 ($2x10^6$ cells/mouse), non-treated (negative control) or injected with thioglycollate (THG; positive control) and put on different caloric diets (low fat, LF = 5kcal% fat; medium fat, MF = 10kcal% fat and high fat, HF = 60kcal% fat) before circulating TNF-α (**a**) as well as IL-6 (**b**) levels were profiled after 72h. The error bars indicate the s.d. (*n* = 3).



Supplememtary Figure S5. FACS-based analysis of HT-1080's circuit transfection efficiency. (**a**) Constitutive expression of DsRed (red) in HT-1080 48h after transfection compared to untreated viable cells (black). (**b**) DsRed-based expression of the LSR circuit in pKR135/pKR153 cotransfected HT-1080 48h after transfection and cultivation with 100µM linoleic acid (green) compared to untreated viable cells (black).

Supplementary Table S1. Plasmids used and designed in this study

Plasmid	Description and Cloning Strategy	Reference or Source
pcDNA3.1	Mammalian expression vector (P_{hCMV}-MCS-pA).	Life Technologies, Carlsbad, CA, USA
pSEAP2-control	Constitutive mammalian SEAP expression vector (P_{SV40}-SEAP-pA).	Clontech, Mountain View, CA,USA
pDsRed-N1	Constitutive mammalian DsRed expression vector (P_{hCMV}-DsRed-pA).	Clontech, Mountain View, CA, USA
pCALCR	Constitutive pcDNA3.1-derived CALCR expression vector (P_{hCMV}-CALCR-pA).	www.cdna.org
pRAMP3	Constitutive pcDNA3.1-derived RAMP3 expression vector (P_{hCMV}-RAMP3-pA).	www.cdna.org
pUC57-Pram	Custom-designed pUC57-derived vector containing pramlintide.	GenScript Inc. Piscataway, NJ, USA
pCK53	Vector encoding a P_{CRE}-driven SEAP expression unit (P_{CRE}-SEAP-pA).	55
pMG10	Vector encoding a P_{TtgR1}-driven SEAP expression unit (P_{TtgR1}-SEAP-pA).	30
pMG11	Constitutive mammalian $TtgA_1$ expression vector (P_{SV40}-$TtgA_1$-pA; $TtgA_1$, TtgR-VP16).	30
pSAM200	Constitutive tTA expression vector (P_{SV40}-tTA-pA, tTA, TetR-VP16).	56
pWW29	Constitutive mammalian MphR(A) expression vector ($P_{hEF1\alpha}$-MphR(A)-pA).	33
pJWS17	Constitutive pcDNA3.1-derived pramlintide expression vector (P_{hCMV}-Pram-pA). Pram was excised from pUC57-Pram with *Xba*I/*Apa*I and cloned into the corresponding sites (*Xba*I/*Apa*I) of pcDNA3.1.	This work
pKR124	Constitutive mammalian LSR expression vector (P_{SV40}-LSR-pA; LSR, TtgR-PPARα). PPARα was PCR-ampified from human genomic cDNA using oligonucleotides OKR189 (5'-gtacagccgcgcgcATCTCAA ATCTCTGGCCAAGAG-3') and OKR190 (5'-ctatcccggatccTTAGTAC ATGTCCCTGTAGATC-3'), digested with *BSS*HII/*Bam*HI and cloned into the corresponding sites (*BSS*HII/*Bam*HI) of pMG11.	This work
pKR135	Constitutive mammalian LSR expression vector (P_{hCMV}-LSR-pA). P_{hCMV} was exised from pcDNA3.1 with *Mlu*I/*Eco*RI and cloned into the corresponding sites (*Mlu*I/*Eco*RI) of pKR124.	This work
pKR136	Constitutive mammalian LSR expression vector ($P_{hEF1\alpha}$-LSR-pA). $P_{hEF1\alpha}$ was excised from pWW29 with *Ssp*I/*Kpn*I and cloned into the corresponding sites (*Ssp*I/*Kpn*I) of pKR135.	This work
pKR146	P_{TtgR1}-driven pramlintide expression vector (P_{TtgR1}-Pram-pA). Pram was excised from pUC57-Pram with *Eco*RI/*Hin*dIII and cloned into the corresponding sites (*Eco*RI/*Hin*dIII) of pMG10.	This work
pKR151	Constitutive mammalian HA-tagged LSR expression vector (P_{hCMV}-LSR-HA-pA). TtgR-PPARα–HA was PCR-amplified from pKR135 using oligonucleotides OKR237 (5'- gggaattcaagcttccaccATGGTCCGT CGAACCAAAGAAG-3') and OKR239 (5'-catacctctagaTTAGGTG GATCCGAGCTCGGTACCGGCATAGTCAGGAACATCGTATGGG TACATGTACATGTCCCTGTAGATCTC-3'), digested with	This work

	*Hin*dIII/*Xba*I and cloned into the corresponding sites (*Hin*dIII/*Xba*I) of pKR135.	

Supplementary Table S1. Continued

Plasmid	Description and Cloning Strategy	Reference or Source
pKR153	Vector encoding a P_{TtgR1}-driven DsRed expression unit (P_{TtgR1}-DsRed-pA). DsRed was PCR-amplified from pDsRed-N1 using oligonucleotides OMM50 (5'-cgggatccgtcgacgaattcaccATGGCCTCC TCCGAGAACGTC-3') and OMM51 (5'- ccatcgataagctttctagaCTACA GGAACAGGTGGTG-3'), digested with *Eco*RI/*Hin*dIII and cloned into the corresponding sites (*Eco*RI/*Hin*dIII) of pMG10.	This work

Restriction endonuclease-specific sites are underlined in oligonucleotide sequences. Annealing base pairs contained in oligonucelotide sequences are shown in capital letters.

Abbreviations: **CALCR**, human calcitonin receptor; **DsRed,** *Discosoma sp*. red fluorescent protein; **EGFP**, enhanced green fluorescent protein; **LSR**, lipid-sensing receptor (TtgR-PPARα); **MCS**, multiple cloning site; **MphR(A)**, repressor of the *Escherichia coli* 2'-phosphotransferase I; **pA**, SV40-derived polyadenylation site; $\mathbf{P_{hCMV}}$, human cytomegalovirus immediate early promoter; $\mathbf{P_{hEF1\alpha}}$, human elongation factor 1α promoter; $\mathbf{P_{SV40}}$, simian virus 40 promoter; $\mathbf{P_{TtgR1}}$, phloretin-responsive promoter; $\mathbf{P_{CRE}}$, synthetic mammalian promoter containing a cAMP-response element; **PPARα**, human peroxisome proliferator-activated receptor alpha; **Pram**, pramlintide, a stabilized variant of human peptide hormone amylin; **RAMP3**, human calcitonin receptor-like receptor activity modifying protein 3; **SEAP**, human placental secreted alkaline phosphatase; **TetR**, Tetracycline repressor from *Escherichia coli*; **tTA**, Tetracycline-dependent transactivator; $\mathbf{TtgA_1}$, phloretin-dependent transactivator; **TtgR**, repressor of the *Pseudomonas putida* DOT-T1E ABC multi-drug efflux pump; **VP16**, *Herpes simplex*-derived transactivation domain.

Supplementary References

55. Kemmer, C. *et al.* A designer network coordinating bovine artificial insemination by ovulation-triggered release of implanted sperms. *J. Control. Release* 150, 23-29 (2011).
56. Fussenegger, M., Moser, S., Mazur, X., Bailey, J.E. 1997. Autoregulated multi- cistronic expression vectors provide one-step cloning of regulated product gene expression in mammalian cells. *Biotechnol. Prog.* **13**, 733–740 (1997).