Self-adjusting synthetic gene circuit for correcting insulin resistance

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Sophisticated genetic devices can be assembled to reprogram mammalian cell activities using tools from synthetic biology. Here, we demonstrate that a self-adjusting synthetic gene circuit can be designed to sense and reverse the insulin-resistance syndrome in different mouse models. By functionally rewiring the mitogen-activated protein kinase (MAPK) signalling pathway to produce MAPK-mediated activation of a hybrid transcription factor consisting of the tetracycline repressor, TetR, fused to the human ELK1-derived transactivation domain (TetR-ELK1), we assembled a synthetic insulin-sensitive transcription-control device that self-sufficiently distinguished between physiological and increased blood insulin levels and correspondingly fine-tuned the reversible expression of therapeutic transgenes from synthetic TetR-ELK1-specific promoters. In acute experimental hyperinsulaemia, the synthetic insulin-sensing designer circuit reversed the insulin-resistance syndrome by coordinating expression of the insulin-sensitizing compound adiponectin. Engineering synthetic gene circuits to sense pathologic markers and coordinate the expression of therapeutic transgenes may provide opportunities for future gene- and cell-based treatments of multifactorial metabolic disorders.

In recent decades, obesity has resulted in an epidemic prevalence of type 2 diabetes, affecting more than 400 million individuals worldwide1. Obesity-induced insulin resistance results from complex metabolic and inflammatory changes and is the key etiologic defect of the metabolic syndrome, a cluster of clinical findings including hypertension and dyslipidaemia that increase the incidence of cardiovascular disorders and type 2 diabetes2-3. The physiological response to insulin resistance and impaired glucose metabolism is the continuous stimulation of insulin secretion, which initiates the gradual decline of pancreatic beta cells, leading to progressive development of type 2 diabetes and its acute and chronic complications4. Current insulin-resistance therapies, which focus on the reduction of body weight or the administration of insulin-sensitizing drugs such as glitazones, are ineffective or associated with important side effects5-7. Insulin resistance is inversely correlated with blood levels of adiponectin8-10, the most abundant adipose tissue-derived hormone with insulin-sensitizing properties11. Therefore, the development of drugs that increase blood adiponectin levels is currently being pursued for the treatment of insulin resistance and the associated cardiovascular risk factors12.

Synthetic biology, the engineering science of reassembling standardized biological parts in a systematic, rational and predictable manner to program novel cellular behaviour, has enabled the design of synthetic gene networks that can synchronize early diagnosis of a pathologic situation with targeted therapeutic intervention in a closed-loop control manner13. For example, designer-cell implants with embedded therapeutic gene networks have been successfully used to diagnose, prevent and cure experimental diseases14. Therefore, synthetic biology-inspired therapeutic strategies15-18 may provide new treatment opportunities to mitigate insulin resistance.

We have designed and engineered a mammalian synthetic sensor-effector device that is exclusively activated by high insulin levels and triggers corresponding expression of adiponectin, which reverses insulin resistance and its related metabolic effects by restoring glucose and lipid homeostasis in different mouse models covering various stages of insulin resistance (Fig. 1a).

Insulin-sensor device design

The insulin-sensor device was designed by engineering mammalian cells for ectopic expression of the human insulin receptor19-20 and rewireing the native signalling cascade involving the insulin receptor substrate 1–Ras (a GTPase)–mitogen activated protein kinase (IRS-1–Ras–MAPK) pathway21 to the synthetic hybrid transcription factor, TetR-ELK1 (consisting of the doxycycline-responsive DNA-binding tetracycline repressor, TetR, fused to the human ELK1-derived transactivation domain (TetR-Elk1), which is driven by the constitutive human cytomegalovirus immediate early promoter (PscmV)22. The activation of the MAPK kinase phosphorylates TetR-ELK1, rendering it a potent transactivator that triggers transcription from synthetic promoters containing multiple TetR-ELK1-binding sites. In addition, the insulin-triggered transgene expression circuit is not only regulated by the phosphorylation status of the ELK1-domain; the operator-binding affinity of the TetR moiety can also be inhibited by administration of the antibiotic doxycycline. Thus, TetR-ELK1 represents a dual-input transcription factor which interfaces insulin levels with therapeutic transgene expression and provides a safety latch to modulate or switch off the device by a clinically licensed antibiotic (Fig. 1b). Western blot analyses, in which the tyrosine kinase family human insulin receptor (IR), phospho-IRS-1 and phospho-extracellular signal-regulated kinase (phospho-ERK) signals were probed, confirmed that human

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embryonic kidney cells (HEK-293) transfected with the human insulin receptor-encoding expression vector pIR (P<sub>hCMV</sub>-IR-pA) and incubated with insulin activated the IRS-1–Ras–MAPK signalling pathway (Supplementary Fig. 1a,c,e). HEK-293 cells were cotransfected with a constitutive pIR; a constitutively expressed hybrid transcription factor TetR-ELK1 (pTetR-ELK1; P<sub>hCMV</sub>-TetR-ELK1-pA); and a phosphorylated TetR-ELK1-dependent, P<sub>hCMV*</sub>-1-driven human placental secreted alkaline phosphatase (SEAP) reporter construct, pMF111 (P<sub>hCMV*</sub>-1-SEAP-pA; P<sub>hCMV*</sub>-1 is a chimeric promoter containing a TetR-specific heptameric operator module (tetO<sub>7</sub>) linked to a minimal version of P<sub>hCMV</sub> (P<sub>hCMV</sub><sub>min</sub>)). As a result, although Western blot analyses showed the presence of endogenous IR and the associated signalling
Insulin-inducible transgene expression in different mammalian cell lines. Insulin-triggered SEAP expression by HeLa, CHO-K1, hMSC-TERT and HEK-293 cells 72 h after cotransfection with pIR, pTetR-ELK1 (pCMV-TetR-ELK1-pA) and pMF111 at a ratio of 1:1:1. The data represent the mean ± s.d., *n* = 4 independent experiments, **P < 0.001 versus control. B, The SEAP expression kinetics of HEK-293 cells cotransfected with pIR, pTetR-ELK1 and pMF111 at a ratio of 1:1:1 and cultivated for 24, 48 and 72 h in the presence or absence of different concentrations of insulin. For points with error bars comparable to the size of the symbols, only mean ± s.d. is displayed instead of individual points, *n* = 4 independent experiments. C, SEAP expression profiles of pIR-/pTetR-ELK1-/pMF111-cotransfected HEK-293 cells cultivated for different periods of time in the presence of 20 ng ml⁻¹ insulin. The data represent the mean ± s.d., *n* = 3 independent experiments. D, Fluorescence micrographs profiling EYFP expression by HEK-293 cells cotransfected with pIR, pTetR-ELK1 and pHY74 and cultivated for 72 h in the presence or absence of 20 ng ml⁻¹ insulin. E, Reversibility of insulin-triggered SEAP expression in HEK-293 cells. pIR-/pTetR-ELK1-/pMF111-transgenic HEK-293 cells were cultivated for 72 h while alternating the insulin status of the culture (20 ng ml⁻¹, on; 0 ng ml⁻¹, off) at 24 h and 48 h. The data represent the mean ± s.d., *n* = 3 independent experiments. F, The inhibition of insulin-triggered SEAP expression by doxycycline in HEK-293 cells. pIR-/pTetR-ELK1-/pMF111-transgenic HEK-293 cells were cultivated in the presence of 20 ng ml⁻¹ insulin and different concentrations of doxycycline. The data represent the mean ± s.d., *n* = 3 independent experiments. G, Reversibility of doxycycline-triggered SEAP expression in HEK-293 cells. pIR-/pTetR-ELK1-/pMF111-transgenic HEK-293 cells were cultivated in the presence of 20 ng ml⁻¹ insulin and 100 ng ml⁻¹ doxycycline for the first 24 h and in the presence of 20 ng ml⁻¹ insulin and absence of 100 ng ml⁻¹ doxycycline for the following 24 h. The data represent the mean ± s.d., *n* = 4 independent experiments.

Figure 2 | Synthetic insulin-inducible mammalian sensor circuit. A, Insulin-inducible transgene expression in different mammalian cell lines. Insulin-triggered SEAP expression by HeLa, CHO-K1, hMSC-TERT and HEK-293 cells 72 h after cotransfection with pIR, pTetR-ELK1 (pCMV-TetR-ELK1-pA) and pMF111 at a ratio of 1:1:1. The data represent the mean ± s.d., *n* = 4 independent experiments, **P < 0.001 versus control. B, The SEAP expression kinetics of HEK-293 cells cotransfected with pIR, pTetR-ELK1 and pMF111 at a ratio of 1:1:1 and cultivated for 24, 48 and 72 h in the presence or absence of different concentrations of insulin. For points with error bars comparable to the size of the symbols, only mean ± s.d. is displayed instead of individual points, *n* = 4 independent experiments. C, SEAP expression profiles of pIR-/pTetR-ELK1-/pMF111-cotransfected HEK-293 cells cultivated for different periods of time in the presence of 20 ng ml⁻¹ insulin. The data represent the mean ± s.d., *n* = 3 independent experiments. D, Fluorescence micrographs profiling EYFP expression by HEK-293 cells cotransfected with pIR, pTetR-ELK1 and pHY74 and cultivated for 72 h in the presence or absence of 20 ng ml⁻¹ insulin. E, Reversibility of insulin-triggered SEAP expression in HEK-293 cells. pIR-/pTetR-ELK1-/pMF111-transgenic HEK-293 cells were cultivated for 72 h while alternating the insulin status of the culture (20 ng ml⁻¹, on; 0 ng ml⁻¹, off) at 24 h and 48 h. The data represent the mean ± s.d., *n* = 3 independent experiments. F, The inhibition of insulin-triggered SEAP expression by doxycycline in HEK-293 cells. pIR-/pTetR-ELK1-/pMF111-transgenic HEK-293 cells were cultivated in the presence of 20 ng ml⁻¹ insulin and different concentrations of doxycycline. The data represent the mean ± s.d., *n* = 3 independent experiments. G, Reversibility of doxycycline-triggered SEAP expression in HEK-293 cells. pIR-/pTetR-ELK1-/pMF111-transgenic HEK-293 cells were cultivated in the presence of 20 ng ml⁻¹ insulin and 100 ng ml⁻¹ doxycycline for the first 24 h and in the presence of 20 ng ml⁻¹ insulin and absence of 100 ng ml⁻¹ doxycycline for the following 24 h. The data represent the mean ± s.d., *n* = 4 independent experiments.

insulin receptor-mediated input resulted in a wide range of induction profiles between different cell lines (Fig. 2a). Because HEK-293 cells exhibited the best insulin-triggered transgene expression profile, they were used in all of the following studies.
Insulin-sensor device validation

To evaluate the adjustability of the insulin-triggered transgene expression, pIR-/pTetR-ELK1-/pMF111-engineered HEK-293 cells were incubated with increasing insulin doses and the SEAP levels were profiled every 24 h for up to three days (Fig. 2b). The SEAP levels correlated precisely with increasing insulin doses up to 20 ng ml⁻¹ and exhibited reliable induction levels over the entire concentration range (Fig. 2b). When the engineered cells were exposed to high doses of insulin (20 ng ml⁻¹) for various time periods, the SEAP production kinetics could be precisely programmed (Fig. 2c). Similar insulin-triggered expression kinetics were also visualized by fluorescence microscopy in cells engineered with pHY79 (Fig. 2d). The product gene expression levels could be reliably switched on and off by alternating the presence and absence of insulin in the culture medium, demonstrating that this synthetic insulin-sensor device was fully reversible in vitro (Fig. 2e) and in vivo (Supplementary Fig. 3). Moreover, the insulin-triggered transgene expression levels could be dose- and time-dependently modulated by doxycycline (Fig. 2f) and the original levels could be fully recovered simply by the removal of doxycycline (Fig. 2g). Therefore, transgene expression by this insulin-sensor device was not only positively regulated by the addition of insulin, but could also be negatively adjusted by the administration of doxycycline, which functioned as a safety latch to flexibly terminate transgene expression during any undesired scenarios. More importantly, further control experiments showed that the engineered insulin-sensor device was specific for insulin (Supplementary Fig. 3) and insensitive to glucose (Supplementary Figs 4 and 5), which insulates the insulin-sensor device from insulin-controlled glucose metabolism.

Insulin-sensor device sensitivity

We evaluated whether the synthetic insulin-sensor device could sense high insulin concentrations and trigger transgene expression both in vitro and in vivo. To demonstrate the diagnostic capacity of the synthetic insulin-sensor device in vitro, pIR-/pTetR-ELK1-/pMF111-engineered HEK-293 cells were incubated with 10% serum from three different mouse models of insulin resistance (insulin-resistant diabetes due to leptin (ob/ob) or leptin-receptor (db/db) deficiency and diet induced obese, DIO). The SEAP levels were profiled after 72 h (Supplementary Fig. 6a) and confirmed that the serum containing high levels of insulin (Supplementary Fig. 6b) from insulin-resistant mice could trigger transgene expression in insulin-sensor cells. Similarly, serum from human patients who developed obesity-induced insulin resistance could also trigger SEAP expression (Supplementary Fig. 6c,d). To validate insulin-triggered transgene...
expression in vivo, $2 \times 10^6$ microencapsulated pIR-/pTetR-ELK1-/pMF111-transgenic HEK-293 cells were intraperitoneally implanted into the three different mouse models (ob/ob, db/db and DIO) and all of these developed hyperinsulinaemia. Control animals were implanted with pKZY73 (pCMV-Fc-adiponectin-pA)-/pTetR-ELK1-/pMF111-transgenic HEK-293 cells. The corresponding serum SEAP levels of the treated mice were profiled after 48 h (Supplementary Fig. 7a–c) and confirmed that the synthetic insulin-sensor circuit could sense high insulin concentrations (5–20 ng ml$^{-1}$) in insulin-resistant ob/ob, db/db and DIO mouse models.

**Closed-loop adiponectin expression in ob/ob mice**

To convert the diagnostic insulin-sensor device into a self-sensing therapeutic cell implant that automatically detects and treats insulin resistance, we linked the hyperinsulinaemia input to the expression of adiponectin (Supplementary Fig. 8) and implanted microencapsulated pIR-/pTetR-ELK1-/pHY79 (Fc-adiponectin-pA)-transgenic HEK-293 cells into the peritoneum of insulin-resistant ob/ob mice (Fc-adiponectin is a human IgG-Fc-tagged single-chain globular adiponectin derivative consisting of three tandem adiponectin modules fused to the IgG1-derived Fc fragment). Adiponectin can reverse obesity-induced insulin resistance by improving insulin sensitivity and decreasing the lipid content in the body$^{21,22}$ and the Fc-adiponectin derivative was selected on the basis of improved half-life and therapeutic efficacy$^{23}$. After 48 h of implantation, the ob/ob mice containing transgenic cell implants exhibited significantly increased serum adiponectin levels (Fig. 3a). Within 48 h, the increased adiponectin levels significantly lowered free fatty acid, cholesterol and insulin levels in the insulin-resistant ob/ob mice (Fig. 3b–d). Within 24 h, increased adiponectin levels (1.56 ± 0.23 nM) attenuated glycaemic excursions in response to intraperitoneal glucose tolerance tests (Fig. 3e) and increased insulin sensitivity in response to intraperitoneal insulin tolerance tests (Fig. 3f). Homeostatic model assessment-estimated insulin resistance (HOMA-IR$^{24,25}$) revealed that insulin resistance significantly decreased during the treatment (Fig. 3g). Additionally, decreased food intake (Fig. 3h) and body weight (Fig. 3i) was observed three days after implantation. These results suggest that the synthetic insulin-sensor circuit was sufficiently sensitive to alleviate the insulin-resistance syndrome and may be suitable for the treatment of obesity-induced insulin resistance.

**Closed-loop adiponectin expression in DIO mice**

The synthetic insulin-sensor circuit was also evaluated for the treatment of DIO mice, which develop prediabetic obesity. As above, we

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**Figure 4 | Self-sufficient insulin-sensor-based control of adiponectin expression in insulin-resistant DIO mice.** Mice fed for 10 weeks with normal caloric food (10 kcal% fat; wild-type mice) or high-fat food (60 kcal% fat; DIO mice) were intraperitoneally implanted with $2 \times 10^6$ microencapsulated pIR-/pTetR-ELK1-/pHY79-transgenic HEK-293 cells (200 cells per capsule). Control mice were implanted with $2 \times 10^6$ microencapsulated pKZY73-/pTetR-ELK1-/pHY79-transgenic HEK-293 cells. a–d. Serum adiponectin (a), free fatty acid (b), cholesterol (c) and insulin (d) levels were profiled after 48 h of implantation. e,f. Glucose tolerance (e) and insulin tolerance (f) tests were performed 24 h after implantation (serum adiponectin levels: 1.38 ± 0.18 nM). g. HOMA-IR levels of DIO mice were determined 48 h after implantation. h,i Food intake (h) and body-weight change (i) were quantified 72 h after implantation. The data represent the mean ± s.e.m., n = 8 mice per group. *P < 0.05, **P < 0.01, ***P < 0.001 versus control.
implanted with $2 \times 10^6$ encapsulated HEK_{IR-Adipo} cells (200 cells per capsule). Control mice received $2 \times 10^6$ encapsulated pIR/pTetR-ELK1/pHY79-transgenic HEK-293 cells. a, e, Serum adiponectin (a), insulin (b), free fatty acid (c) and cholesterol (d) levels as well as HOMA-IR values (e) were determined for 20 days. f, g, Food intake (f) and body-weight change (g) were quantified on days 3 and 20. The data represent the mean ± s.e.m., n = 8 mice per group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus control.

**Discussion**

Insulin resistance is the hallmark of metabolic syndrome, which has a complex multifactorial aetiology; it is primarily induced by visceral obesity and can trigger the initiation of type 2 diabetes and atherosclerotic vascular disease[27–29]. Decreased adiponectin signalling appears to play a central role in the process and represents an attractive treatment target[30–31]. Recently, synthetic biology has substantially advanced the design of biomedical circuits, providing new therapeutic strategies[32–35]. Synthetic-biology-inspired gene- and cell-based therapies may provide new opportunities in the treatment of insulin-resistance-related metabolic disorders.

By functionally assembling a human insulin receptor and control modules (TetR-ELK1, P_{mPGK-1} with native intracellular machineries (the IRS-1–Ras–MAPK signalling cascade), we rewired the insulin-receptor-mediated tyrosine-kinase-coupled-receptor signalling pathway to a synthetic hybrid TetR-ELK1 transcription factor, utilizing their common intracellular MAPK-based signalling system as the interface. We designed the insulin-sensor-controlled expression of the half-life-optimized version of adiponectin[36] that enabled: (1) the constant monitoring of insulin levels in the blood; (2) the automatic activation of the sensor device in response to high insulin levels; (3) the coordinated production of adiponectin, which promptly reduced blood glucose, free fatty acid, cholesterol and HOMA-IR levels; (4) the coordinated production of adiponectin, which promptly reduced blood glucose, free fatty acid, cholesterol and HOMA-IR levels; (5) the coordinated production of adiponectin, which promptly reduced blood glucose, free fatty acid, cholesterol and HOMA-IR levels; (6) the coordinated production of adiponectin, which promptly reduced blood glucose, free fatty acid, cholesterol and HOMA-IR levels; (7) the coordinated production of adiponectin, which promptly reduced blood glucose, free fatty acid, cholesterol and HOMA-IR levels. The synthetic biology- and cell-based therapies may provide new opportunities in the treatment of insulin-resistance-related metabolic disorders.

Adiponectin acts on various peripheral tissues as well as the central nervous system and has been associated with a wide variety of therapeutic effects, including anti-inflammatory activities, insulin sensitization, anti-atherogenesis as well as reduction of blood-fat levels, food intake and body weight[37–39]. This multifaceted role of adiponectin makes a precise prediction of its therapeutic impact and potential side effects challenging. Therefore, dosing of adiponectin may be of particular importance as long-term high-dose administration of adiponectin could cause some adverse effects.
effects. The closed-loop topology of the insulin-sensor device managing self-adjusting adiponectin production in response to the state of insulin resistance is expected to prevent adiponectin overdosing.

The synthetic signalling cascade of the insulin-sensor device contains the chimeric transcription factor TetR-ELK1, which provides a non-limiting example for an additional layer of control using the clinically licensed antibiotic doxycycline. Although antibiotic-triggered interventions were not required to tune the insulin-sensor device to correct experimental insulin resistance, orthogonal remote control may become important in future clinical applications to tune, adapt and adjust adiponectin expression to the optimal patient-specific therapeutic window and provide emergency interventions to inactivate the device in case of complications.

The current insulin-sensor device was engineered into HEK-293 cells, but future clinical applications may require autologous parental cells such as the patients’ own mesenchymal stem cells, which have already been clinically validated. No matter which cell type will prevail in future therapies, the designated treatment strategy remains identical: (1) large-scale manufacturing of patient-specific designer cells; (2) frozen storage of the designer cells, either before or after encapsulation inside a vascularizing immunoprotective container; and (3) implantation of the encapsulated designer cells, preferably subcutaneously where they can easily be replaced at regular intervals by minimal ambulant intervention in the event of fibrosis.

Outlook

The insulin-sensor–effector device was able to substantially improve insulin resistance and the associated metabolic dysfunctions in different mouse models exhibiting various features of the metabolic syndrome similar to that observed in humans. This designer circuit performed as expected and has the potential as a therapeutic tool for treatment of early stages of diabetes mellitus in future clinical applications. Self-sufficient synthetic gene circuits could become of clinical relevance in the not-too-distant future for the treatment of multifactorial metabolic disorders in which the presence of specific disease markers can be rapidly and specifically detected and for which the expression of a suitable therapeutic protein can be immediately coordinated.

Therapeutic gene circuits designed in this manner most efficiently coordinate disease-marker monitoring with disease-modulating molecular interventions for a particular disorder. Therefore, developing and optimizing biologically safe implants that harbour designer cells transgenic for those rationally designed circuits would be an important breakthrough and may represent a new era in modern molecular personalized medicine.

Methods

Vector design. Comprehensive design and construction details for all expression vectors are listed in Supplementary Table 1. Some expression vectors were constructed by Gibson assembly using the GeneArt Seamless Assembly Cloning Kit (Ohio Technology, cat. no. BACR(G)2014001). All constructs were confirmed by sequencing (Genewiz).

Cell culture and transfection. HEK-293 cells (ATCC: CRL-11268), HeLa (ATCC: CCL-2) and hMSC-TERT were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen) supplemented with 10% (vol/vol) fetal calf serum (FCS; BioConcept, cat. no. 201F10, lot no. PE01026P) and 1% (vol/vol) penicillin/streptomycin solution (Biowest, cat. no. L0022-100, lot no. S0996SLO022). Chinese hamster ovary cells (CHO-K1, ATCC: CCL-61) were cultured in Chomaster high-throughput screening medium (Cell Culture Technologies) supplemented with 5% (vol/vol) FCS and 1% (vol/vol) penicillin/streptomycin solution. All of the cell types were cultivated at 37°C in a humidified atmosphere containing 5% CO2 and were regularly tested for the absence of Mycoplasma and bacterial contamination.

HEK-293, HeLa, hMSC-TERT and CHO-K1 cells were (co-)transfected using an optimized polyethylenimine (PEI)-based protocol. In brief, 5 × 106 cells were seeded per well of a 24-well plate and (co-)transfected with a total of 0.6 µg of plasmid DNA mixture (for the cotransfections, a 1:1:1 receptor/processor/reporter plasmid ratio was used) diluted in 25 µl of FCS-free DMEM solution and subsequently mixed with 25 µl PEI solution containing 1.8 µl of PEI (1 mg/ml−1 in ddH2O; Polysciences, cat. no. 24765-2) diluted in 23.2 µl FCS-free DMEM. The DNA–PEI mixture solution was incubated for 15 min at 22°C and added dropwise to the cells. After a 6 h (co-)transfection, the medium was replaced. For the mouse
experiment, 7×10² HEK-293 cells were seeded into a 15-cm cell culture dish and (co-)transfected with a total of 30 μg of plasmid DNA mixture (a 1:1:1 receptor:reporter plasmid ratio was used) diluted in 1,500 μl of FCS-free DMEM solution. The cells were subsequently mixed with 1,500 μl PEI solution containing 90 μg of PEI (1 mg/ml in ddH₂O) diluted in 1,410 μl FCS-free DMEM. After 15 min of incubation at 22°C, the DNA–PEI mixture was added dropwise to the cells and the cells were harvested for encapsulation after 6 h of transfection.

**Generation of stable cell lines.** The HEK₂₉₃ cell line, transgenic for insulin-triggered Fc-adiponectin and enhanced green fluorescent protein (EGFP) expression, was constructed by cotransfecting 5×10⁴ HEK-293 cells with 135 ng of pHY118 (ITR-Fcγ(IgG1)-Fc-adiponectin-P2A-EGFP-P2A-YPKα-Rho-PTx-pTat) and 135 ng of pHY121 (ITR-Fcγ(IgG1)-IR-pTat-EK1-P1-AcAdo-ZeoR-pTat-IR) and 30 mg Sleeping Beauty transposase expression vector⁸ pCMV-T7-B100 (PhCMV- SB100X-pA). After cultivation with 1 μg/ml pTat-EK1 and 10 μg/ml mycinin and 10 μg/ml zeocin for two weeks, the HEK₂₉₃ cell line was selected on the basis of green-fluorescence intensity by fluorescence-activated cell sorting (FACS)-mediated cell sorting using a Becton Dickinson FACSAria Cell Sorter (Becton Dickinson). The stable cell line HEK₂₉₃ was further confirmed by polymerase chain reaction (PCR) and was regularly tested for the absence of Mycoplasma and bacterial contamination.

**Reporter gene assays.** The production of human placental SEAP was quantified in cell culture supernatants and mouse serum as described previously. EYFP expression was visualized using a LEICA DMI-600 microscope (Leica Microsystems) equipped with a DFC390FX R2 digital camera (Leica Microsystems), a x10 objective, a 488 nm/509 nm (B/G/R) excitation/emission filter set and Leica Application Suite software (V2.1.0.1).

**Western blot analysis.** To analyse the IRS-1→Ras→MAPK signaling cascade activation through the human insulin receptor, HEK-293 cells were cotransfected with pIR-, pTetR-ELK1 and pMF111. After 48 h of transfection, the cells were starved for 6 h and then 0–20 μg/ml insulin was added to the cells. After 1 h of insulin stimulation, 2×10⁶ HEK-293 cells were collected. Protein extracts were prepared as described previously⁴,5. The proteins were resolved on a 12% SDS polyacrylamide gel and electroblotted onto a polyvinylidine fluoride (PVDF) membrane (Immobilon-P Millipore). Phospho-Erk1/2 was visualized using a primary rabbit polyclonal anti-phospho-p44/p42 MAPK (Erk1/2) (1:2000 dilution) antibody (Cell Signaling, BioConcept, cat. no. 4370, lot no. 14) and a secondary DyLight 800-labelled anti-rabbit IgG (KPL, cat. no. 07-02-17-06, lot no. 103061). ERK1/2 was visualized using a primary rabbit p44/p42 MAPK (ERK1/2) antibody (Cell Signaling, cat. no. 4695, lot no. 14) and the secondary DyLight 800-labelled goat anti-rabbit IgG. IR was visualized using an insulin receptor-specific primary rabbit monoclonal antibody (Abcam, cat. no. ab131238, lot no. GR219882-1) and the DyLight 800-labelled goat anti-rabbit IgG. Phospho-IRS-1 was visualized using a phospho-IRS-1-specific primary rabbit monoclonal antibody (Abcam, cat. no. abE6680, lot no. GR98847-1) and the secondary DyLight 800-labelled goat anti-rabbit IgG. β-actin was visualized using a β-actin-specific primary mouse monocular antibody (Sigma-Aldrich, cat. no. A5441, lot no. 122m4782) and a secondary DyLight 800 labelled goat anti-mouse IgG (Thermo Fisher, cat. no. SA-5-10176, lot no. 103061). All proteins were detected using the Odyssey system (LI-COR Biosciences).

**Animal experiments.** Intraperitoneal implants were produced by encapsulating transgenic HEK-293 cells into coherent alginate-poly-[(ethylene oxide)-co-(propylene oxide)] copolymers to form implants that can be loaded in 1,500 μl of FCS-free DMEM solution. The cells were subsequently mixed with 1,500 μl PEI solution containing 90 μg of PEI (1 mg/ml in ddH₂O) diluted in 1,410 μl FCS-free DMEM. After 15 min of incubation at 22°C, the DNA–PEI mixture was added dropwise to the cells and the cells were harvested for encapsulation after 6 h of transfection.

**References.** All data supporting the findings of this study are available within the paper and the Supplementary Information. Sequence information of key components is available from GenBank (accession numbers: human insulin receptor hIR, BC171727; human Fc-adiponectin, KY033479; and custom-designed Fc-adiponectin, KY033479).

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Author contributions

H.Y., M.Z. and M.F. designed the project, analysed the results and wrote the manuscript. H.Y., M.Z., G.H.E., S.X. and J.Y. performed the experimental work.

Additional information

Supplementary information is available for this paper.

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Competing interests

The authors declare no competing financial interests.