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Supporting Online Material for

## A Synthetic Optogenetic Transcription Device Enhances Blood-Glucose Homeostasis in Mice

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## **Supporting Online Material**

# **A synthetic optogenetic transcription device enhances blood-glucose homeostasis in mice**

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## Materials and methods

**Vector construction.** pEYFP-C1 ( $P_{hCMV}$ -EYFP-pA<sub>SV40</sub>; Clontech, Mountain View, CA, USA), pSEAP2-Basic (MCS-SEAP-pA<sub>SV40</sub>; Clontech), pGL4.30 ( $P_{NFAT}$ -luc2P-pA<sub>SV40</sub>; Promega, Duebendorf, Switzerland), pTet-ON (P<sub>SV40</sub>-rTetR-VP16-pA<sub>SV40</sub>; Clontech) and pcDNA3.1 (Invitrogen, Basel, Switzerland) are commercially available. pIRES<sub>2</sub>-OPN<sub>4AI</sub> ( $P_{hCMV}$ -melanopsin-IRES<sub>PV</sub>-EGFP-pA<sub>SV40</sub>) containing human melanopsin has been described previously (38). The  $P_{NFAT}$ -inducible SEAP expression vector pHY30 ( $P_{NFAT}$ -SEAP-pA<sub>SV40</sub>) was cloned by excising SEAP from pSEAP2-Basic by *HindIII/MfeI* and inserting it into the corresponding sites (*HindIII/MfeI*) of pGL4.30. The  $P_{NFAT}$ -inducible EYFP expression vector pHY41 ( $P_{NFAT}$ -EYFP-pA<sub>SV40</sub>) was designed by inserting *HindIII/FseI*-restricted EYFP, PCR-amplified from pEYFP-C1 using oligonucleotides OHY82 (5'-gcgccgacaagcttATGGTGAGCAA GGGCGAG-3') and OHY83 (5'-cacgcacggccggccTTACTTGTACAGCTCGTC-3'), into the corresponding sites (*HindIII/FseI*) of pGL4.30. The melanopsin expression vector pHY42 ( $P_{hCMV}$ -melanopsin-pA<sub>SV40</sub>) was constructed by excising melanopsin from pIRES<sub>2</sub>-OPN<sub>4AI</sub> by *NheI/ApaI* and cloning it into the corresponding sites (*NheI/ApaI*) of pcDNA3.1. The gene encoding a synthetic secretion-engineered [N-terminal fusion to an Extendin-4 secretion signal and a furin cleavage site (39)], DPP-IV-resistant [harboring an alanine-to-glycine mutation (A8G) that confers resistance to endogenous dipeptidyl-peptidase IV (DPP-IV)] (39), short (7-37) variant of the human glucagon-like peptide 1 (shGLP-1) C-terminally fused to a mouse IgG-Fc (40) was synthesized (GeneScript, Piscataway, NJ, USA), restricted with *HindIII/FseI* and cloned into the corresponding sites (*HindIII/FseI*) of pGL4.30 to result in the  $P_{NFAT}$ -controlled shGLP-1 expression vector pHY57 ( $P_{NFAT}$ -shGLP-1-pA<sub>SV40</sub>). The light-inducible  $P_{NFAT}$ -driven Rip death domain (RipDD) expression vector pHY62 ( $P_{NFAT}$ -RipDD-pA<sub>SV40</sub>) was designed by inserting *AatII/BssHII*-restricted  $P_{NFAT}$ , PCR-amplified from pGL4.30 using oligonucleotides OHY123 (5'-gccccacgtcCCGCAATAAAATATCTTTA-3') and OHY124 (5'-cacgcacggcgccGGTG GCTTTACCAACAG-3'), into the corresponding sites (*AatII/BssHII*) of pWW326 ( $P_{hEF1-}$  RipDD-pA<sub>SV40</sub>) (23). The tetracycline-inducible RipDD expression vector pHY65 ( $P_{hCMV^{*-1}}$ -RipDD-pA<sub>SV40</sub>) was designed by inserting the *AatII/BssHII*-restricted tetracycline-responsive promoter ( $P_{hCMV^{*-1}}$ ), PCR-amplified from pMF111 ( $P_{hCMV^{*-1}}$ -SEAP-pA<sub>SV40</sub>) (23) using oligonucleotides OHY125 (5'-gccccacgtcCTCGAGTTACCACTCCCTATC-3') and

OHY126 (5'-cacgcacggcgccGGGGCCGCGAGGCTGGATC-3'), into the corresponding sites (*Aat*II/*Bss*HII) of pWW326.

***Cell culture, transfections, reporter-protein profiling, conditional expression of highly toxic RipDD, apoptosis profiling.*** Baby hamster kidney cells (BHK-21, ATCC: CCL10), mouse fibroblasts (NIH/3T3, ATCC: CRL-1658), human embryonic kidney cells (HEK-293, ATCC: CRL-1573), human fibrosarcoma cells (HT-1080, ATCC: CCL-121), human cervical adenocarcinoma cells (HeLa, ATCC: CCL-2) and mouse Beta-TC-6 insulinoma cells (Beta-TC-6, ATCC: CRL-11506) were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Basel, Switzerland; cat. no. 52100-39) supplemented with 10% (15%, Beta-TC-6) fetal calf serum (FCS; cat. no. 3302, lot no. P231902, PAN Biotech GmbH, Aidenbach, Germany) and 1% penicillin/streptomycin solution (Sigma-Aldrich, Munich, Germany; cat. no. P4333). All cell types were cultivated at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. To assay shGLP-1 activity, 3x10<sup>5</sup> Beta-TC-6 were cultivated per well of a 24-well plate and glucose-/serum-starved for 2h by maintenance in Krebs buffer (118mM NaCl, 4.7mM KCl, 1.2mM KH<sub>2</sub>PO<sub>4</sub>, 1.2mM MgSO<sub>4</sub>, 4.2mM NaHCO<sub>3</sub>, 2mM CaCl<sub>2</sub>, 10mM HEPES and 0.1g/L BSA, pH7.4) before they were incubated with conditioned shGLP-1-containing medium and profiled for insulin production.

HEK-293 were (co)-transfected using an optimized CaHPO<sub>4</sub>-based protocol. In brief, 2.5x10<sup>5</sup> HEK-293 seeded per well of a 12-well plate were (co)-transfected with a total of 3µg DNA diluted in 50µL 0.5M CaCl<sub>2</sub> solution and subsequently mixed with 50µL 2×BES buffer (100mM N,N-bis [2-hydroxyethyl]-2-aminoethanesulfonic acid (BES), 280mM NaCl and 1.5mM Na<sub>2</sub>HPO<sub>4</sub>, pH6.95). The DNA-containing solution was added dropwise to the cells, incubated for 6h and then replaced by fresh medium. pHY30-/pHY42- and pHY42-/pHY57-transgenic HEK-293 cell lines were generated by clonal selection of co-transfected populations in medium containing 150µg/ml hygromycin (200µg/ml, Invitrogen, cat. no. 10687-010). HeLa and NIH/3T3 were transfected the same way except that the DNA-containing solution was incubated overnight. HT-1080 and BHK-21 were transfected with Lipofectamine LTX (Invitrogen; cat. no. 15338-500) according to the manufacturer's protocol. Transgene expression values were normalized for variations in transfection efficiency by parallel transfections using the constitutive EYFP-expression vector pEYFP-C1. Production of the human placental secreted alkaline phosphatase (SEAP) was quantified in cell culture supernatants (41) and mouse serum (42) as described previously. Luciferase was measured using the Tropix® luciferase assay kit according

to the manufacturer's instructions (Applied Biosystems, Bedford, MA, USA; cat. no. BC100L). Cell numbers were determined using a Casy<sup>®</sup> Cell Counter Model TTC; Roche Applied Science, Basel, Switzerland.

For conditional expression of the highly toxic protein RipDD, 2x10<sup>5</sup> HEK-293 were co-transfected with pHY42 and pHY62 (P<sub>NFAT</sub>-RipDD-pA<sub>SV40</sub>) or pTet-ON (P<sub>SV40</sub>-rTetR-VP16-pA<sub>SV40</sub>) and pHY65 (P<sub>hCMV\*-1</sub>-RipDD-pA<sub>SV40</sub>) and cultivated for 72h in the presence or absence of standard blue-light pulses (pHY42/pHY62) or 2μg/ml doxycycline (pTet-ON/pHY65; Sigma-Aldrich; cat. no. 44577). Mock- and pWW326- (P<sub>hEF1<sub>a</sub></sub>-RipDD-pA<sub>SV40</sub>) (23) transfected cells were used as controls. For quantification of apoptosis, harvested cells, stained using an annexin-V-FITC apoptosis detection kit (Bender MedSystems, Vienna, Austria; cat. no. ALX-850-020-KI02) according to the manufacturer's protocol, were analyzed by a Cytomics FC500 flow cytometer (Beckman Coulter International SA, Nyon, Switzerland) as described before (23).

**Calcium imaging.** Engineered cells were washed once with Tyrode's salt solution (Sigma-Aldrich; cat. no. T2397) and loaded with the calcium indicator Fluo-4-AM (Invitrogen; cat. no. F-14201; 2.5μM, 37°C, 30min incubation in the dark). To improve loading and retention of Fluo-4-AM, 0.01% Probenecid (Invitrogen; cat. no. P36400) was also added. Treated cells were visualized by fluorescence microscopy using an inverted fluorescent microscope (DMI 6000B; Leica Microsystems, Heerbrugg, Switzerland) equipped with a DFC350FX R2 digital camera (Leica), a 20× objective, a 488 nm/509 nm (B/G/R) excitation and emission filter set and Leica Application Suite software (version V2.1.0R1). During a blue-light pulse (6 seconds, 3.6x10<sup>19</sup> photons·s<sup>-1</sup>·m<sup>-2</sup>, 488nm) using the excitation filter, fluorescence-based changes in intracellular Ca<sup>2+</sup> levels were recorded, analyzed using ImageJ software (<http://imagej.nih.gov/ij/index.html>) and quantified as post-stimulus change in fluorescence intensity above baseline divided by the baseline intensity (ΔF/F).

**Miscellaneous chemicals.** Ethylene glycol-bis(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) was purchased from Sigma-Aldrich (cat. no. E-0396). Ionomycin was purchased from Molecular Probes<sup>®</sup> (Invitrogen, ca. no. I24222) and diluted in DMSO. Lanthanum chloride (LaCl<sub>3</sub>) was obtained from Sigma-Aldrich (cat. no. 211605) and dissolved in ddH<sub>2</sub>O. Phorbol-12-myristate-13-acetate (PMA) was obtained from AppliChem (Darmstadt, Germany, ca. no. A0903,0005) and dissolved in DMSO.

**Light induction, optics and electronics.** Prior to illumination melanopsin-engineered cell cultures were supplemented with 100nM all-trans-retinal (Sigma-Aldrich; cat. no. R2500). For blue-light-based photostimulation of monolayer cultures, we designed custom-manufactured illumination arrays containing 6 (2x3) or 12 (3x4) blue LEDs (CREE Inc, Garching, Germany; cat. no. LC503FBL1-15P-A3-00001) which provide equal-intensity exposure of cells cultivated in 6- and 12-well plates, respectively (fig. S2A). For blue-light-based photostimulation of roller bottle cultures, we combined two custom-manufactured illumination arrays each containing 24 (4x6) LEDs (CREE Inc.), which provide equal-intensity exposure of the entire bottle profile (fig. S2B). For blue-light-controlled transgene expression in subcutaneous implants the treated mice were kept in standard cages equipped with two 24-LED arrays (4x6; CREE Inc.) as ceiling illumination (fig. S2D). The illumination arrays were plugged into a regulated DC power supply (Voltcraft, Conrad Electronic SE, Germany; cat. no. PS-1302D), which was connected to a computer for precise control of exposure times via Labview software (version 8.6, National Instruments, Austin, TX, USA).

To illuminate transgenic cell lines in hollow-fibre implants (see below) *ex vivo* as well as in mice we used a high-brightness LED ( $\lambda_{\text{max}} = 470 \text{ nm}$ ; Doric Lenses, Quebec, Canada; cat. no. LEDP\_HB01-B\_MM200-037) coupled to a multimode silica glass optical fibre with a core diameter of 200 $\mu\text{m}$  and a numerical aperture of 0.37 (Thorlabs, Newton, NJ, USA; cat. no. BFL37-200). The LED was connected to a computer and the light pulses were controlled using Labview software (version 8.6), modulating the voltage via a regulated DC power supply (Voltcraft) (fig. S2C). The blue-light pulses were set to 5s ON and 10s OFF and calibrated to reach a light intensity of  $1.5 \times 10^{18} \text{ photons}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$  ( $1.5 \times 10^{19} \text{ photons}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$  for transdermal control) at the cell or mouse surface controlled using a light meter (LI-COR<sup>®</sup> Biosciences, Lincoln, NB, USA; cat. no. LI-250A).

**Bioreactor operation.** To assess light-inducible product-gene expression in bioreactors a 200ml suspension ( $2 \times 10^5 \text{ cells/ml}$ ,  $4 \times 10^7 \text{ cells}$ ) of HEK-293 transgenic for melanopsin (pHY42) and P<sub>NFAT</sub>-driven SEAP expression (pHY30) was seeded into 850cm<sup>2</sup> roller bottles with vented caps (Becton Dickinson, Le Pont De Claix, France; cat. no. 353007) which were placed on a CellRoll system (Vitaris, Baar, Switzerland; cat. no. 186001) set to 0.5rpm and operated in a standard CO<sub>2</sub> incubator to allow for attachment of the cells to the roller bottle surface overnight.

The cultures were then run at 1rpm and illuminated with LED-generated blue-light pulses for up to 10 days (fig. S2B).

**Animal experiments.** Intraperitoneal implants were produced by seeding  $1 \times 10^5$  pHY30-/pHY42-transgenic HEK-293 into 2cm CellMax® hollow fibre membranes (Spectrum Laboratories Inc., Rancho Dominguez, CA, USA; cat. no. M138615), inserting the optical fibre (see above) on one side and heat-sealing both ends using a Webster smooth needle holder (Harvard Apparatus, Holliston, MA, USA; cat. no. 512467). The implant was placed in the peritoneal cavity of anaesthetized female OF1 mice (oncins France souche 1, Charles River Laboratory, Lyon, France) through a small incision in the musculature of the dorsal abdominal wall, which was subsequently closed with skin staples. The optical fibres were connected to the LEDs as well as their controller units, and the implants were illuminated with pulsed light for 3h or 48h while those of the control mice received no light. Forty-eight hours after implantation the mice of all treatment groups were sacrificed, blood samples were collected and SEAP levels were quantified in the serum, which was isolated using microtainer SST tubes according to the manufacturer's instructions (Beckton Dickinson, Plymouth, UK; cat. no. 365968).

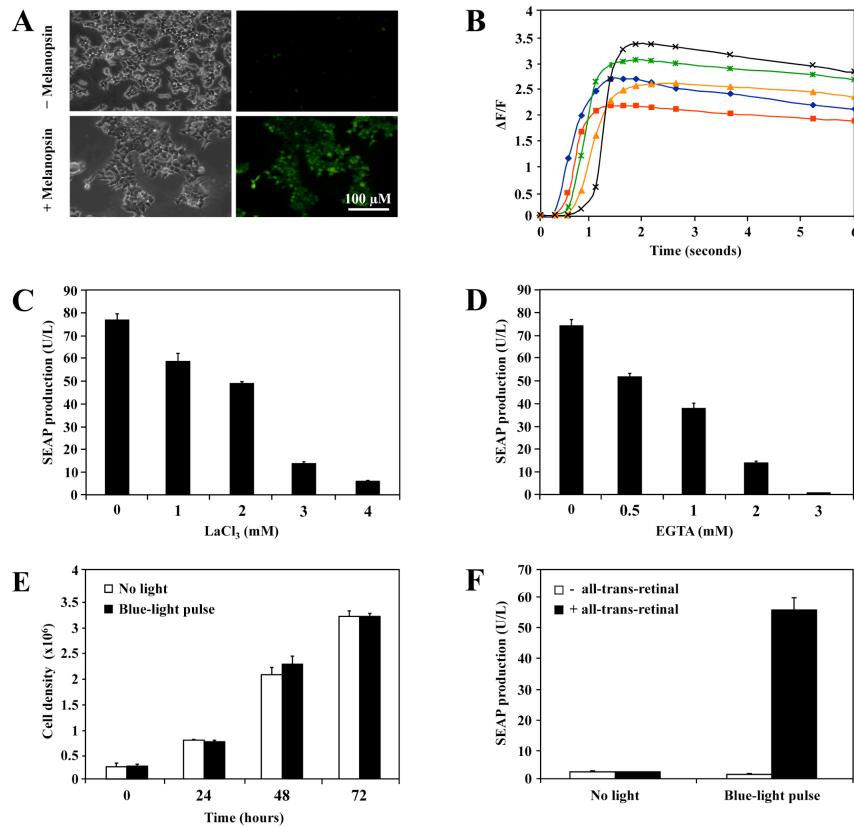
Subcutaneous implants were produced by encapsulating pHY30-/pHY42-transgenic HEK-293 into coherent alginate-poly-(L-lysine)-alginate beads (400μm; 200 cells/capsule) as described previously (43). The back of female OF1 mice was shaved to improve light exposure and 0.3ml DMEM containing  $1 \times 10^6$  encapsulated pHY30-/pHY42-transgenic HEK-293 cells were subcutaneously injected. The treated mice were directly illuminated with pulsed blue light for 3h or 48h while the control mice received no light. Two days after implantation, the mice were sacrificed, blood samples were collected and serum SEAP levels were quantified as described above.

For the intraperitoneal glucose tolerance tests and the assessment of insulin levels following transdermal light-triggered shGLP-1 expression in subcutaneous implants, wild-type CD-1® (4-week-old female, Charles River Laboratory, Lyon, France) and diabetic *db/db* mice (8-week-old female, The Jackson Laboratory, Bar Harbor, ME, USA) were shaved, subcutaneously injected with 2ml DMEM containing  $1 \times 10^7$  encapsulated pHY42-/pHY57-transgenic HEK-293 and illuminated with blue-light pulses for 48h. Thirty hours after implantation, the mice were fasted for 16h before they received an intraperitoneal glucose (1.5g/kg) injection. Plasma glucose levels were monitored in tail-vein blood samples 0, 15, 30, 60, 90 and 120min after glucose

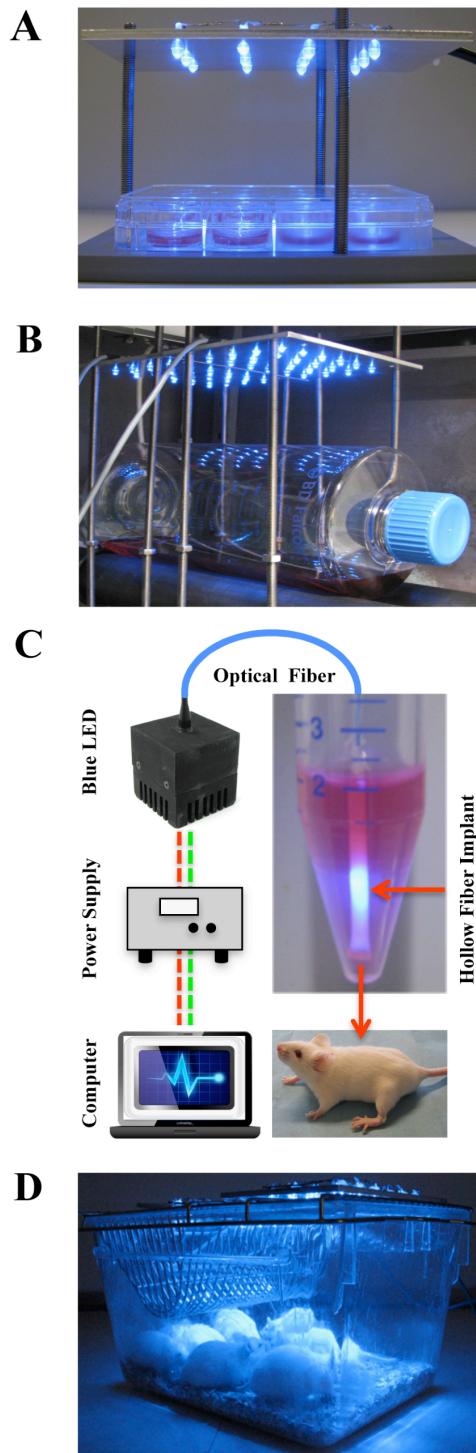
administration using a glucometer (Contour, Bayer HealthCare, Zurich, Switzerland). Fourty-eight hours post-implantation, the mice were sacrificed, their serum samples prepared as described above and shGLP-1 and insulin levels were quantified by ELISA (insulin: Mercodia, Uppsala, Sweden; cat. no. 10-1113-01; shGLP-1: Millipore, Zug, Switzerland; cat. no. EGLP-35k). For detection of shGLP-1's Fc part the kit was used in combination with an IgG-Fc-alkaline phosphatase antibody (Sigma-Aldrich, cat. no. A1418).

Due to dietary intake of vitamin A or synthesis from  $\beta$ -carotene it was not necessary to administer any type of retinal to the animals (44, 45). All experiments involving animals were performed according to the directive of the European Community Council (86/609/EEC), approved by the French Republic (No. 69266310) and carried out by M.D.E at the Institut Universitaire de Technologie, IUTA, F-69622 Villeurbanne Cedex, France.

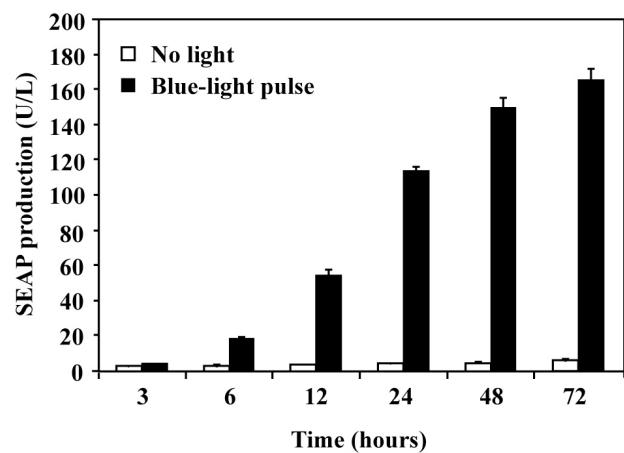
## Supplementary figures



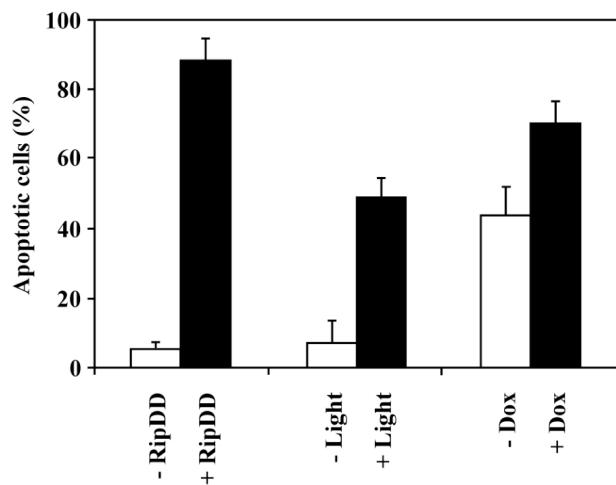
**Figure S1:** Correlation of intracellular  $\text{Ca}^{2+}$  levels and target gene expression following blue-light illumination of engineered HEK-293. **(A)** Fluorescence microscopy-based visualization of intracellular  $\text{Ca}^{2+}$  levels following blue-light illumination of fluo-4-AM-loaded HEK-293 engineered for constitutive melanopsin expression by transfection with pHY42 ( $P_{hCMV}\text{-melanopsin-pASV40}$ ). **(B)** Single-cell trajectories of intracellular  $\text{Ca}^{2+}$  levels of five different melanopsin-expressing fluo-4-AM-loaded HEK-293 cells **(C, D)**  $2.5 \times 10^5$  HEK-293 cultures co-transfected with pHY30 ( $P_{NFAT}\text{-SEAP-pASV40}$ ) and pHY42 were supplemented with different concentrations of either **(C)** lanthanum chloride ( $\text{LaCl}_3$ ) or **(D)** ethylene glycol tetraacetic acid (EGTA) and illuminated with blue-light pulses for 24h before SEAP levels were quantified in the culture supernatant. **(E)** Impact of blue-light illumination on the viability of mammalian cells.  $1 \times 10^5$  native HEK-293 were illuminated with standard blue-light pulses (5s ON, 10s OFF) for 72h and their proliferation was profiled for 3 days. **(F)** SEAP production profiles of blue-light-illuminated pHY30-/pHY42-co-transfected HEK-293 cultivated for 24h in the presence (+) or absence (-) of 100nM all-trans-retinal. Data are mean +/- SD; N=4.



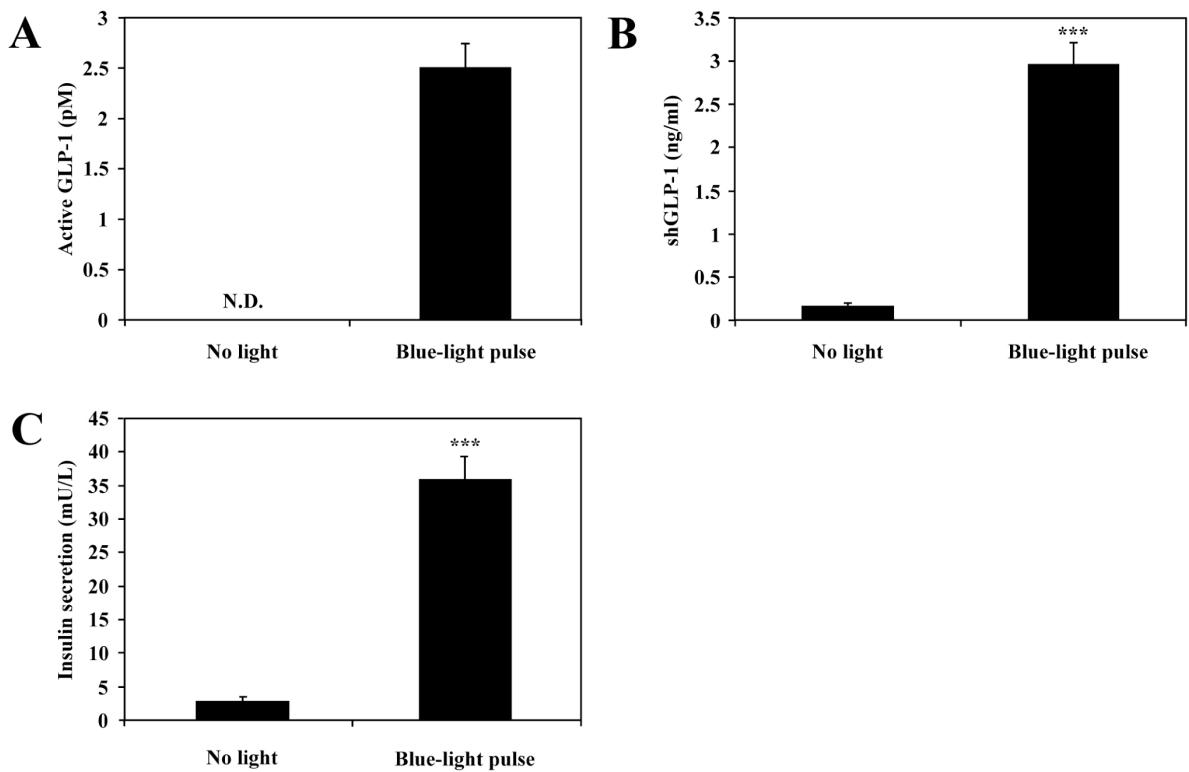
**Figure S2:** Custom-designed LED arrays and LED-coupled optical fibre devices used for blue light-triggered transgene expression in mammalian cells grown in (A) monolayer cultures (B) roller-bottle bioreactors or implanted (C) intraperitoneally or (D) subcutaneously into mice.



**Figure S3:** SEAP expression kinetics of HEK-293 co-transfected with pHY30 and pHY42 and cultivated for 72h in the presence or absence of blue-light pulses. Data are mean +/- SD; N=4.



**Figure S4:** Apoptosis of HEK-293 expressing the highly toxic RipDD gene product. HEK-293 co-transfected with pHY42/pHY62 or pTet-ON/pHY65 were cultivated for 72h in the presence or absence of standard blue-light pulses (pHY42/pHY62; +light, -light) or 2 $\mu$ g/ml doxycycline (pTet-ON/pHY65; +Dox, -Dox). Mock- (-RipDD) and pWW326- (+RipDD) transfected cells were used as controls. Data are mean +/- SD; N=4.



**Figure S5:** Light-inducible shGLP-1 production of  $2.5 \times 10^5$  HEK- 293 cells cultivated per well of a 12-well plate and co-transfected with pHY42 and pHY57 ( $P_{NFAT}$ -shGLP-1-pASV40) was quantified after 48h-blue-light illumination using (A) active GLP-1- and (B) mouse IgG-Fc-specific ELISAs. (C) The conditioned medium of this culture was used to cultivate starved Beta-TC-6 cells ( $3 \times 10^5$  cells/well of a 24-well plate) for 2h before insulin secretion was profiled. Data are mean +/- SD;  $N=4$ . \*\*\* $P<0.0001$ .

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