A synthetic biology-based device prevents liver injury in mice.

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Supplementary materials and methods

Analytical assays. Alanine aminotransferase. Serum levels of alanine aminotransferase (ALT) were determined by an ALT Activity Assay (Sigma-Aldrich; cat. no. MAK052, lot. no. B9G150752V). **Bile acids**. Serum bile acid levels were quantified using a mouse Total Bile Acids Assay Kit (Crystal Chem, Downers Grove, IL; cat. no. 80470, lot. no. MTB01140213). **Bilirubin**. Serum bilirubin levels were profiled using a Bilirubin Assay Kit (Sigma Aldrich; cat. no. MAK126-1KT, lot. no. BE12A01V). **cAMP.** Intracellular cAMP levels were quantified using the Cyclic AMP XP Assays Kit (Life Technologies, cat. no. 4339). In brief, $1x10^5$ cells were pretreated for 30min with FCS-free DMEM containing 0.5mM 3-isobutyl-1-methylxanthine (IBMX, Sigma-Aldrich, cat. no. I5879) and incubated for another 30min in FCS-free DMEM containing different concentrations of cholic acid before cAMP levels were determined in the cell lysates according to manufacturer's protocol. **Cell viability.** Viability was assayed using calcein AM dye (Affymetrix. Santa Clara, CA, USA; cat. no. 65-0853-39). In brief, 1×10^5 cells were detached using StemPro®Accutase®Cell Dissociation Reagent (Life Technologies; cat. no. A11105-01) and incubated for 15min with 20nM calcein AM dye at 22°C. The samples were washed twice with PBS and analysed by FACS using a Becton Dickinson LSRII Fortessa flow cytometer (Becton Dickinson, Allschwil, Switzerland) (488nm laser, 505nm long pass filter, 530/30 emission filter). **Human hepatocyte growth factor**. Human hepatocyte growth factor (HGF) levels were scored using a human HGF ELISA kit (RayBiotech Inc., Norcross CA; lot no. 613140201). **PKA inhibition.** $1x10^5$ cells were pretreated with 10μM H-89 (Sigma-Aldrich, cat. no. B1472) for 1h, incubated for 2h in cell culture medium in the presence of 100μM cholic acid and maintained for 24h in cell culture medium in the absence of cholic acid before SEAP levels were assessed in

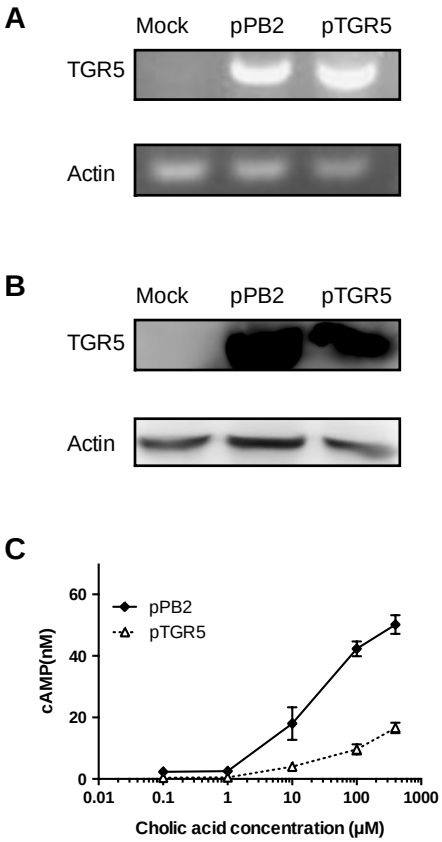
the cell culture supernatant. **RNA isolation and RT-PCR.** Total RNA was extracted from mammalian cells using the ZR RNA MiniPrep™ kit (Zymo Research, Irvine, CA, USA; cat. no. R1064) and TURBO™ DNase (Life Technologies, CA, USA; cat. no. AM2238). RT-PCR was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA; cat. no. 4368814) according to the manufacturer's instructions. Primers specific for human TGR5 (forward, 5'-TCGTCTACTTGGCTCCCAAC-3'; reverse, 5'-GCTCATAGGCCAGGACTGAG-3') were used to detect TGR5 transcripts in HEK-293 cells. Actin mRNA (forward, 5'-AGATGTGGATCAGCAAGCAG-3'; reverse: 5'-GCGCAAGTTAGGTTTTGTCA-3') was used as control. **SEAP**. Production of human placental secreted alkaline phosphatase (SEAP) was quantified in the culture supernatant using a p-nitrophenylphosphate-based light-absorbance time course [1]. In brief, 120µL of substrate solution (100µL of 2x SEAP assay buffer [20mM homoarginine, 1mM $MgCl_2$, 21% diethanolamine, pH9.8] and 20µL substrate solution [120mM *p*-nitrophenylphosphate]) was added to 80µL heat-inactivated (65°C, 30min) cell culture supernatant and the light absorbance was recorded at 405nm (37°C) for 30min using a GeniosPro multi-well reader (Tecan, Maennedorf, Switzerland). The SEAP levels in serum of mice were quantified using a chemiluminescence-based assay (Roche Diagnostics GmbH, Mannheim, Germany; cat. no. 11779842001) [2]. **shGLP1.** GLP-1-mIgG immunofusin levels were scored using a mouse IgG ELISA kit (Immunology Consultants Laboratory, Inc., Portland, OR; cat. no. E-90G). **Western Blot Analysis.** For immunohistochemical detection of TGR5 expression, $5x10^6$ HEK-293 were collected 48h after transfection of pPB2 or pTGR5 and protein extracts were prepared as described before [3]. Twenty micrograms of protein were resolved on a 12% SDS polyacrylamide gel and electroblotted onto a polyvinylidene fluoride

membrane (Millipore, Billerica, MA, USA; cat. no. IPVH00010). TGR5 was visualized using a primary rabbit polyclonal anti-TGR5 antibody (Sigma-Aldrich; cat. no: HPA062890; lot no. R88059; 1:500 dilution) and a secondary horseradish-peroxidase–coupled anti-rabbit IgG antibody (AbD Serotec, Kidlington, UK; cat. no. STAR54; lot no. 050614; 1:1000 dilution). ECL Prime Western blot detection reagents (Amersham, Little Chalfont, UK; cat. no. RPN2232) were used for chemiluminescence-based signal detection by a Chemilux CCD camera (ImageQuant LAS 400 mini; GE Healthcare, Little Chalfont, UK). Actin was used as control (primary rabbit polyclonal anti-actin IgG, Sigma-Aldrich, cat. no. A2066; lot no. 103M4826V; 1:1000 dilution).

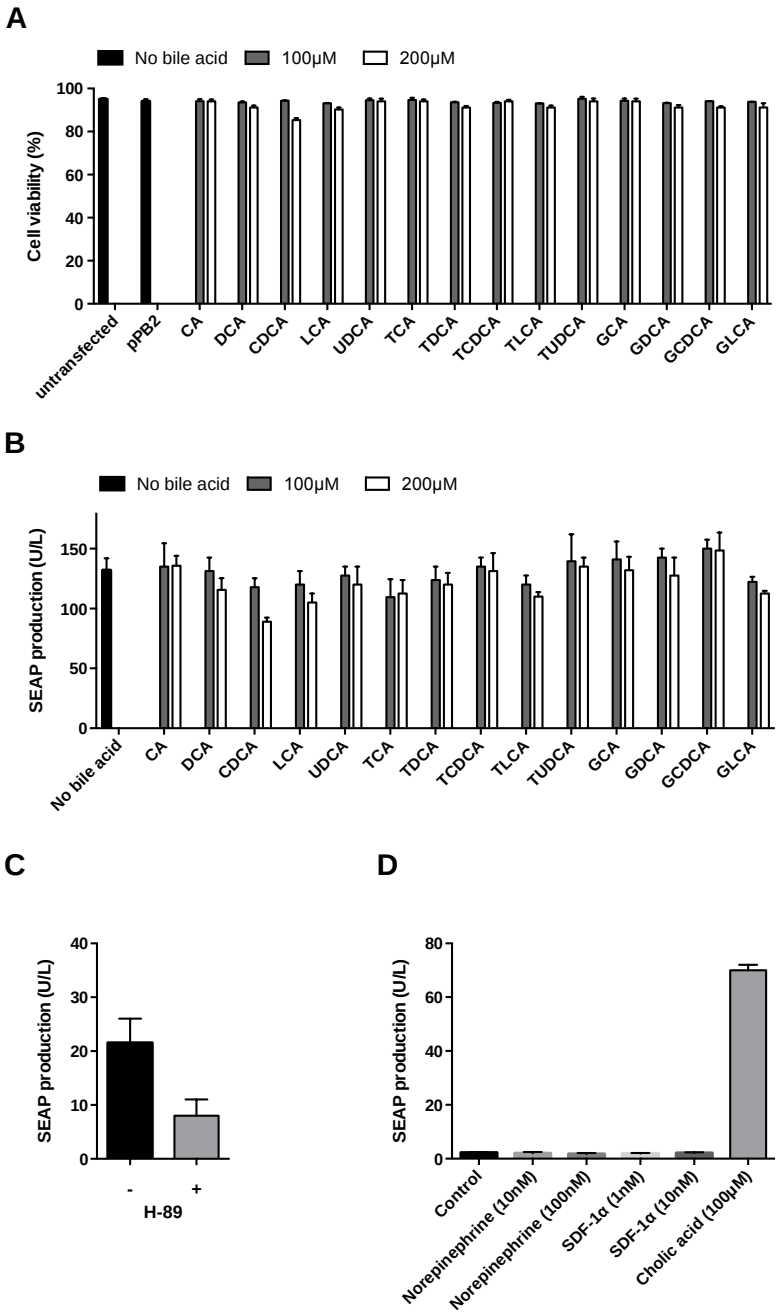
Chemical compounds. Cholic acid (10mM stock solution in ddH_2O; Sigma-Aldrich; cat. no. C9282), taurocholic acid (10mM stock solution in ddH_2O; Sigma-Aldrich; cat. no. T4009), glycocholic acid (10mM stock solution in ddH_2O; Sigma-Aldrich; cat. no. G7132), chenodeoxycholic acid (10mM stock solution in ddH_2O ; Sigma-Aldrich; cat. no. C8261), taurochenodeoxycholic acid (10mM stock solution in ddH_2O; Sigma-Aldrich; cat. no.T6260), glycochenodeoxycholic acid (10mM stock solution in ddH_2O; Sigma-Aldrich; cat. no. G0759), lithocholic acid (100mM stock solution in DMSO; Sigma-Aldrich; cat. no. L6250), taurolithocholic acid (100mM stock solution in DMSO; Sigma-Aldrich; cat. no. T7517), glycolithocholic acid (100mM stock solution in DMSO; Santa Cruz Biotechnology; cat. no. sc-396741), ursodeoxycholic acid (100mM stock solution in DMSO; Sigma-Aldrich; Switzerland; cat. no. U5127), tauroursodeoxycholic acid (10mM stock solution in ddH_2O; Merck, Schaffhausen, Switzerland; cat. no. 580549), deoxycholic acid (10mM stock solution in ddH_2O; Acros Organics, Geel, Belgium; cat. no. 21859), taurodeoxycholic acid (10mM stock solution in ddH_2O; Sigma-Aldrich; cat. no. T0875), glycodeoxycholic

acid (10mM stock solution in ddH_2O; Sigma-Aldrich; cat. no. G9910), *L*-Norepinephrine hydrochloride (10mM stock solution in ddH_2O; Sigma-Aldrich; cat. no. 74480), SDF-1α (1µM stock solution in PBS; R&D Systems, cat. no. 350-NS), 1-Naphthyl isothiocyanate (ANIT, Sigma-Aldrich; cat. no. N4525), carbon tetrachloride (CCl_4, Sigma-Aldrich; cat. no. 87031). ANIT (12.5g/L stock solution) and CCl_4 (12.5% (v/v) stock solution) were dissolved in olive oil (Sigma-Aldrich, cat. no. O1514).

TubeSpin® Bioreactor operation. Freestyle™ 293-F suspension cells were cultivated in TubeSpin® bioreactor 50 (TPP, Trasadingen, Switzerland) containing 10mL FreeStyle™ 293 Expression Medium supplemented with 1% penicillin/streptomycin solution in an 5% CO_2 incubator. The bioreactors were placed on an orbital shaker (IKA KS 260 basic; IKA-Werke GmbH, Staufen im Breisgau, Germany; cat. no. 0002980200) set to 200rpm. Samples for assessment of GLP-1/IgG-Fc production were centrifuged (1min, 14000×g) and the supernatant was stored at -20°C until analysis.

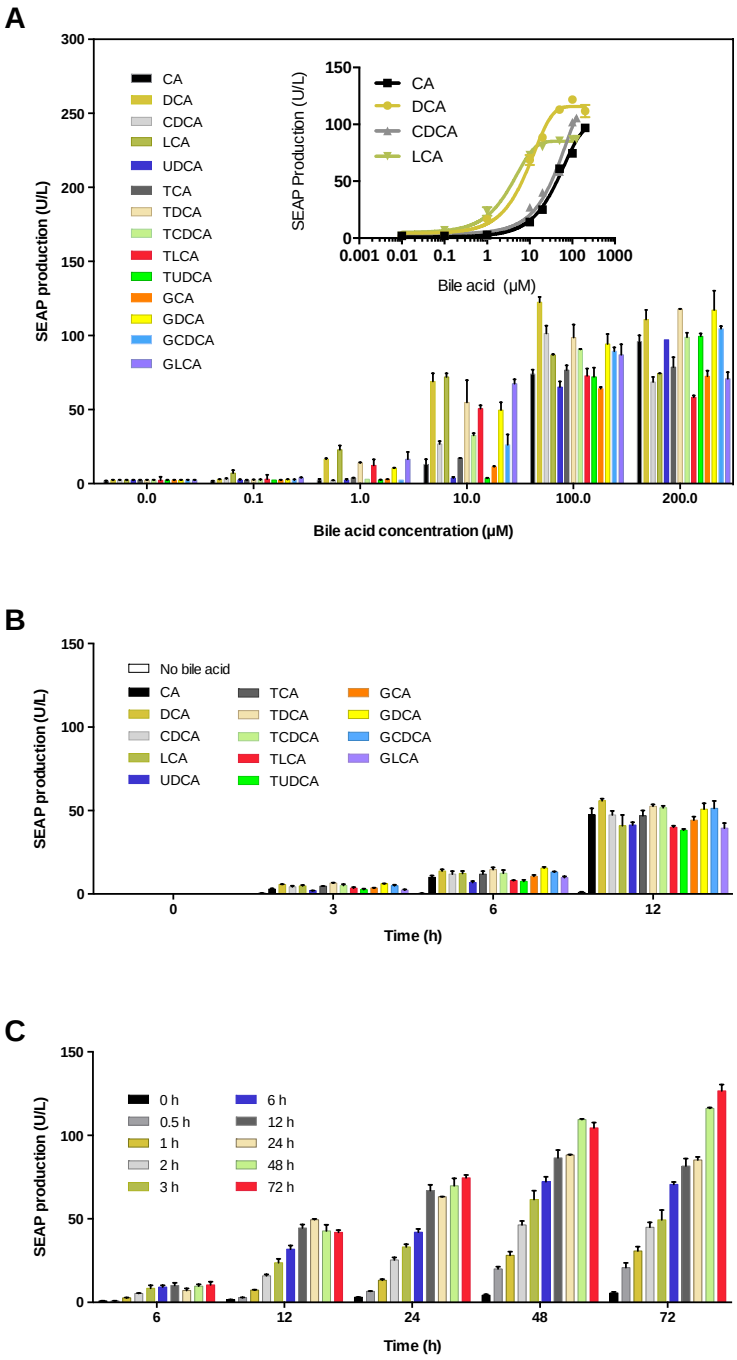


Supplementary Fig. 1. Comparative analysis of different TGR5 expression vectors. (A, B) TGR5 expression levels. HEK-293 cells were transfected with either pTGR5 (P_{hCMV}-TGR5-pA) or pPB2 ($P_{hCMV*-1}$-TGR5-pA) and TGR5 transcript levels (**A**) and (**B**) protein expression levels were profiled by semi-quantitative RT-PCR and Western blot analysis after 48h, respectively. Actin was used as control. **(C)** Cholic acid-responsive TGR5-mediated cAMP surge. HEK-293 cells transfected with either pTGR5 or pPB2 were scored for cAMP levels in response to various concentrations of cholic acid. Data presented are mean ± SD; n=3.



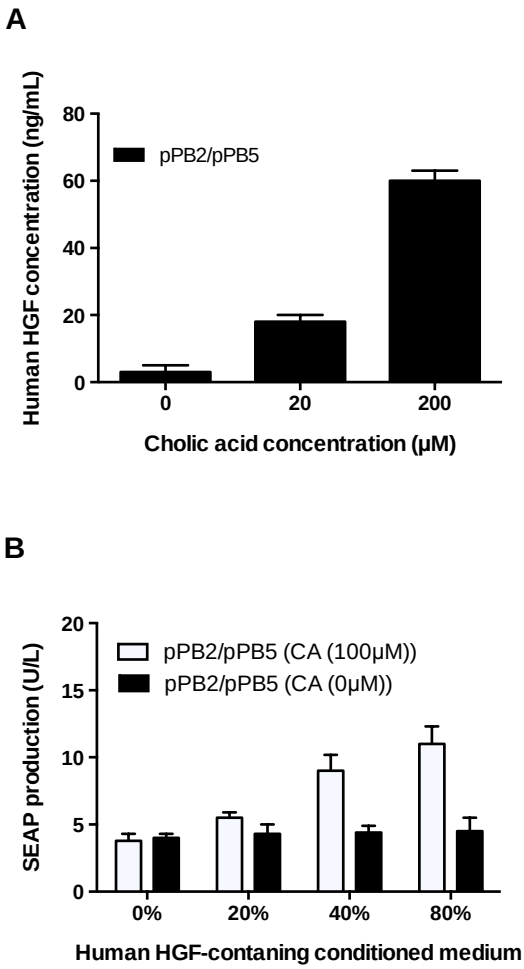
Supplementary Fig. 2. Bile acid sensor control experiments. (A) Impact of constitutive TGR5 expression and different bile acid derivatives on cell viability. HEK-293 cells were transfected with pPB2 and grown for 24h in cell culture medium containing different bile acid derivatives (CA, cholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; LCA, lithocholic acid; UDCA, ursodeoxycholic acid; TCA, taurocholic acid; TDCA, taurodeoxycholic acid; TCDCA, taurochenodeoxycholic

acid; TLCA, taurolithocholic acid; TUDCA, Tauroursodeoxycholic acid; GCA, glycocholic acid; GDCA, glycodeoxycholic acid; GCDCA, glycolchenodeoxycholic acid; GLCA, glycolithocholic acid) before cell viability was profiled. **(B)** Impact of constitutive TGR5 expression and different bile acid derivatives on constitutive SEAP expression of HEK-293. HEK-293 cells were co-transfected with pPB2 and pSEAP2-Control and grown for 24h in cell culture medium containing different bile acid derivatives (CA, cholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; LCA, lithocholic acid; UDCA, ursodeoxycholic acid; TCA, taurocholic acid; TDCA, taurodeoxycholic acid; TCDCA, taurochenodeoxycholic acid; TLCA, taurolithocholic acid; TUDCA, Tauroursodeoxycholic acid; GCA, glycocholic acid; GDCA, glycodeoxycholic acid; GCDCA, glycolchenodeoxycholic acid; GLCA, glycolithocholic acid) before SEAP levels were profiled in the culture supernatant. **(C)** Protein kinase A (PKA)-dependent bile acid signaling. HEK-293 were co-transfected with pPB2 and pSP16, treated for 1h with the PKA inhibitor H-89 (-, 0µM; +, 10µM) and grown for 2h in the presence of 100µM cholic acid before the culture medium was exchanged and SEAP levels profiled after 24h. **(D)** Bile acid-specificity of the TGR5-mediated cAMP-dependent signalling cascade in HEK-293 cells. HEK-293 express different endogenous GPCRs such as adrenoceptor alpha 1B (ADRA1B) and chemokine (C-X-C motif) receptor 4 (CXCR4) which also use cAMP as second messenger and whose activation by norepinephrine and stromal cell-derived factor 1 alpha (SDF-1α) may bias bile-acid control, respectively. pPB2/pSP16-cotransfected HEK-293 cells were cultivated for 24h in culture medium supplemented with physiologic concentrations of norepinephrine or SDF-1α before SEAP levels were quantified in the culture supernatant. Data presented are mean ± SD; n=3.

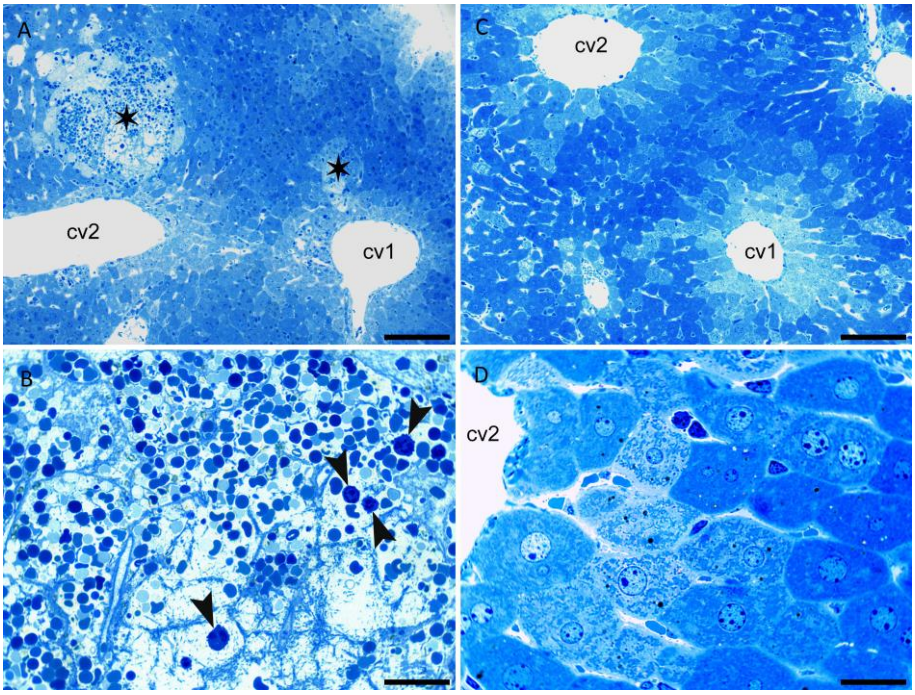


Supplementary Fig. 3. Induction of SEAP expression by different bile acid derivatives. (A) SEAP expression control. HEK-293 cells were co-transfected with pPB2 ($P_{hCMV*-1}$-TGR5-pA) and pSP16 (P_{CREm}-SEAP-pA) and cultivated for 24h in the presence of different concentrations (0-200µM) of various bile acid derivatives (DCA deoxycholic acid; CDCA, chenodeoxycholic acid; LCA, lithocholic acid; UDCA, ursodeoxycholic acid; TCA, taurocholic acid; TDCA, taurodeoxycholic acid; TCDCA,

taurochenodeoxycholic acid; TLCA, taurolithocholic acid; TUDCA, Tauroursodeoxycholic acid; GCA, glycocholic acid; GDCA, glycodeoxycholic acid; GCDCA, glycol-chenodeoxycholic acid; GLCA, glycolithocholic acid) before SEAP levels were profiled in the culture supernatant. Inset: log-representation of dose-dependent SEAP expression levels triggered by CA, DCA, CDCA and LCA. Data presented are mean ± SD, n≥3. **(B)** Induction kinetics. HEK-293 cells were co-transfected with pPB2 and pSP16 and cultivated in the presence of various bile acid derivatives (CA, cholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; LCA, lithocholic acid; UDCA, ursodeoxycholic acid; TCA, taurocholic acid; TDCA, taurodeoxycholic acid; TCDCA, taurochenodeoxycholic acid; TLCA, taurolithocholic acid; TUDCA, Tauroursodeoxycholic acid; GCA, glycocholic acid; GDCA, glycodeoxycholic acid; GCDCA, glycol-chenodeoxycholic acid; GLCA, glycolithocholic acid; all at 100µM) and SEAP levels were profiled in the culture supernatant after 3, 6, and 12h. Data presented are mean ± SD, n≥3. **(C)** Exposure-time-dependent SEAP expression kinetics. pPB2/pSP16-transgenic HEK-293 cells cultivated for different periods of time in cholic acid (100µM)-containing medium, before the culture was switched to cholic acid-free medium and SEAP production kinetics was profiled for 72h. Data presented are mean ± SD, n≥3.



Supplementary Fig. 4. Characterization of HGF expression *in vitro*. (A) Cholic acid-triggered HGF expression. HEK-293 cells were co-transfected with pPB2 ($P_{hCMV*-1}$-TGR5-pA) and pPB5 (P_{CREm}-HGF-pA) and cultivated for 24h in the presence of different cholic acid concentrations (0-200µM) before HGF levels were profiled in the culture supernatant. **(B)** Validation of functional HGF production. hMSC cells were co-transfected with MK_p37 (P_{hCMV}-TetR-ELK1-pA) and pMF111 (P_{hCMV*1}-SEAP-pA) and grown in medium containing different amounts of culture supernatant of cholic acid-induced (100µM cholic acid, 24h) pPB2/pPB5-transgenic HEK-293 cells. HGF-triggered SEAP production was measured after 24h. Data presented are mean ± SD, n≥3.



Supplementary Fig. 5. Histological analysis of liver damage in control animals (toluidine blue staining). (**A, B**) ANIT-treated animals containing placebo implants exhibit central veins (cv1, cv2) with necrotic and hemorrhagic lesions (asterisk) containing immune cell infiltrations (arrowhead) (**B,** larger magnification of **A**; scale bars: **A**, 100µm; **B**, 20µm). (**C, D**) Control mice implanted with the liver-protection device and treated with the diluent olive oil show normal liver structures (cv1, cv2) without any pathological changes. (**D,** larger magnification of **C**; scale bars: **C**, 100µm; **D**, 20µm).

References

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[2] Weber W, Rimann M, Spielmann M, Keller B, Daoud-El Baba M, Aubel D, et al. Gas-inducible transgene expression in mammalian cells and mice. Nat Biotechnol 2004;22:1440-1444.

[3] Peng R-W, Guetg C, Tigges M, Fussenegger M. The vesicle-trafficking protein munc18b increases the secretory capacity of mammalian cells. Metabolic Engineering 2010;12:18-25.