Supplementary Data

Supplementary Materials and Methods

Cloning of rapalog-regulated plasmid components

The Argent™ plasmids, transcription factor (pC_4N_2-R_HS3H/ZF3 and pC4N2-RhS/ZF3), and target gene (pZ_{12}I-PL-2) plasmid, were obtained from ARIAD Pharmaceuticals, Inc. The pZ_{12}I-PL-2 plasmid contains 12 binding sites for ZFHD1 and a minimal human interleukin-2 (IL2) promoter. For shRNA-expressing constructs, the PL-2 promoter was amplified from the pZ_{12}I-PL-2 plasmid with a 5′ primer containing ClaI and AscI sites and a 3′ primer containing an EcoRI site. The PCR product was inserted into pBluescript (pBS) using ClaI and EcoRI. This plasmid was named pBS-PL2. To generate the shFABP5 and shSCR expression cassettes under the control of this promoter, top- and bottom-strand oligos (Supplementary Table 1) were annealed and ligated into the EcoRI and BamHI site of plasmid pBS-PL2 to generate pBS-PL2-shRNA. A minimal poly adenylation signal sequence (Xia *et al.*, 2002) was added downstream from the shRNA using oligos with 5′ BamHI and 3′ AscI/NotI sites, generating plasmid pBS-PL2-shRNA-mpA (Supplementary Fig. 1). Plasmid pC_4N_2-R_HS3H/ZF3 (pDBD) was digested with MluI and XhoI and sticky ends were filled with Klenow. The 5.2-kb fragment containing the activation domain fusion and DNA domain fusion was cloned into BsaBI-linearized plasmid pRES3.2 (3.2-kb HindIII fragment from pC4HSU), resulting in pRES-DBD (Supplementary Fig. 1). Plasmid pBS-PL2-shRNA-mpA was digested with AscI and the fragment was cloned into AscI-linearized pRES-DBD, generating pRES-DBD/PL2-shRNA-mpA (Supplementary Fig. 1). This plasmid was digested with ClaI and EcoRV to release the expression cassette/s. Shuttle plasmid pSHL was digested with AscI. Competent *E. coli* BJ5183 cells were transformed with 100 ng of AscI-linearized pSHL and 300 ng of the fragment containing the expression cassette/s. The final expression construct, pSHL-DBD/PL2-shRNA-mpA, was generated by recombination between homologous regions (Supplementary Fig. 1) (Youil *et al.*, 2001; Witting *et al.*, 2008).

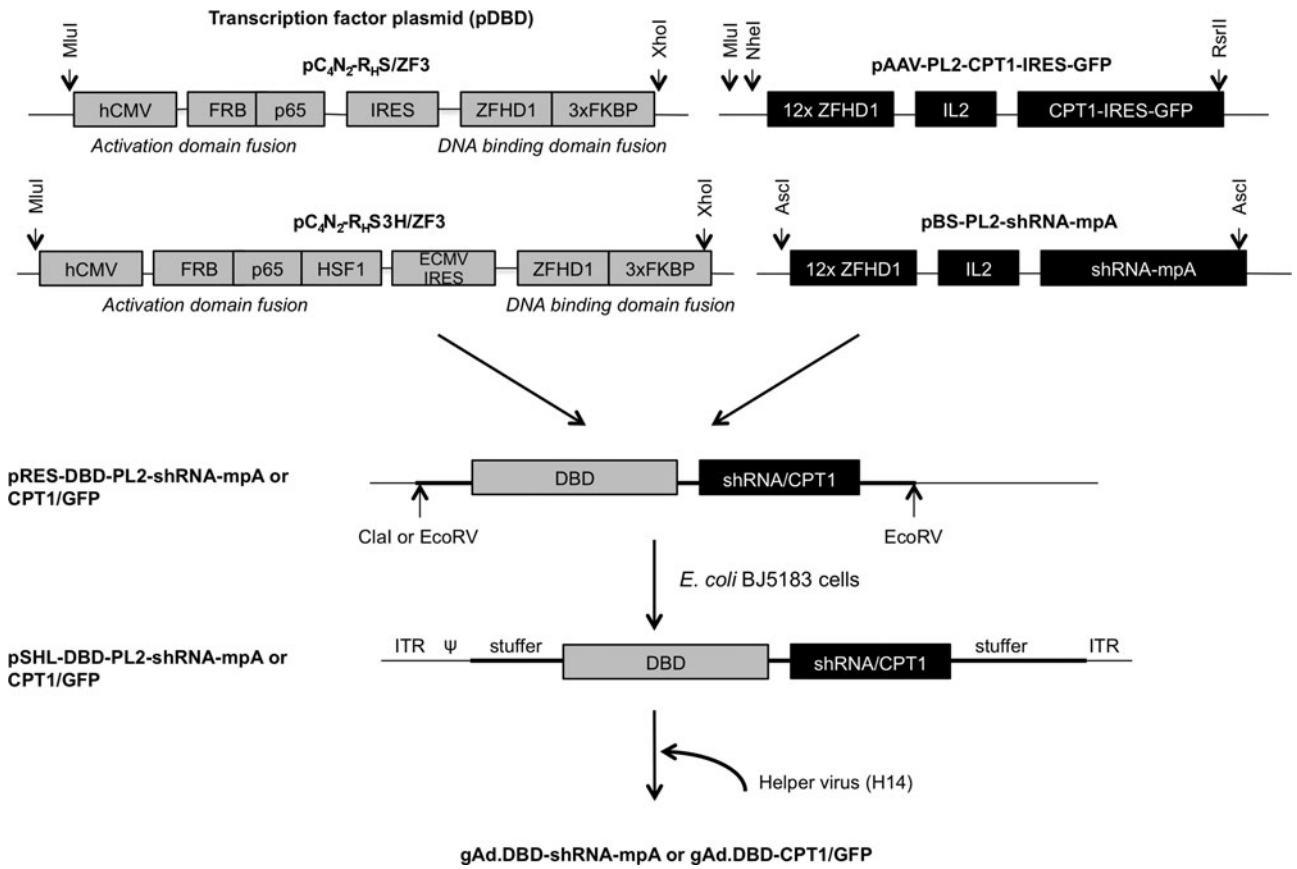
Plasmids containing either a wild-type rat CPT1A cDNA (GenBank number NM_031559; denoted CPT1AWT) or a missense mutation (denoted CPT1E3A) were a kind gift of Dr. Victor Zammit (Warwick Medical School, University of Warwick). In order to generate constructs under the control of the rapalog system, each of the cDNAs was initially subcloned into the pAAV-IRES-hrGFP vector (Agilent Technologies, Stratagene Products Division) using EcoRI and XhoI. These molecules, denoted as either pAAV-CPT1AWT or -E3A, contain a bicistronic cDNA consisting of: 1) the respective CPT1A sequence driven by the CMV promoter and an in-frame 3xFLAG tag sequence; and 2) an ECMV-IRES, followed by a humanized recombinant green fluorescence protein (GFP) coding sequence and an HGH (human growth hormone) poly-adenylation signal. The PL-2 promoter from pZ_{12}I-PL-2 was removed by ClaI digestion, filling with Klenow and then cutting with MluI. After double digestion of pAAV-CPT1A-WT or -E3A with MluI and BsaBI to remove the CMV promoter, the gel purified PL-2 promoter fragment was cloned into the plasmid. The resulting intermediates were denoted pAAV-PL2-CPT1AWT or -E3A. These first intermediates were then double digested with MluI/RsrII and the sticky ends were filled with Klenow. The gel-purified fragments containing the respective PL-2 promoter-driven CPT1A and GFP sequences were then subcloned into the blunted XhoI site of plasmid pC_4N_2-R_HS/ZF3 (pDBD) in opposite orientation to the activation and DNA-binding fusion domains. These second intermediates were denoted pDBD-CPT1AWT or -E3A. Finally, in order to subclone the fragment containing all the elements of the dimerizer system and respective CPT1A-IRES-GFP expression cassettes of either pDBD-CPT1AWT or -E3A into pRES3.2, additional EcoRV, MluI, and NheI sites were first created in the plasmid pRES3.2 utilizing the QuickChange Multi Site-Directed Mutagenesis Kit, according to the manufacturer's instructions (Agilent Technologies, Stratagene Products Division). Both pDBD-CPT1AWT or -E3A and the modified pRES3.2 plasmids were then double digested with MluI/NheI, allowing for directional subcloning of the 9,928 bp fragment to generate pRES-DBD-CPT1AWT or -E3A (Supplementary Fig. 1). At this stage, multiple clones for both plasmid constructs were grown, purified, and sequenced in both directions to ensure sequence fidelity and proper orientation of all elements. These plasmids were digested with EcoRV and cloning into pSHL was carried out as described above for shRNA-expressing constructs.

Cloning of elongation factor 1 α (EF1α) and albumin promoter-driven expression cassettes

Mouse *Fabp5* cDNA (GenBank number NM_010634) was generated from total liver RNA and cloned into a plasmid containing the elongation factor 1α promoter and

Supplementary Table 1. Oligonucleotides Used to Generate shRNA Constructs

PL2	5′-AACTCAATCGATGGCGCGCCCCCGGGAGGTACCGAGCTCT-3′ 3′-TAACGTAGAGAACAAGTTCTCTTAAGACTCAA-5′
shFABP5	5′-AATTCGGAGAGAAGTTTGATGAAACGCGAACGTTTCATCAAACTTCTCTCCG-3′ 3′-GCCTCTCTTCAAACTACTTTGCGCTTGCAAAGTAGTTTGAAGAGAGGCCTAG-5′
shSCR	5′-AATTCGAGAGTATAAGGAGGTCAAGTCGAAACTTGACCTCCTTATACTCTCG-3′ 3′-GCTCTCATATTCCTCCAGTTCAGCTTTGAACTGGAGGAATATGAGAGCCTAG-5′
mpA	5′-GATCCAATAAAGGATCTTTTATTTTCATTGGATCTGTGTGTTGGTTTTTTGTATGGCGCGCCGC-3′ 3′-GTTATTTCCTAGAAAATAAAAGTAACCTAGACACACAACCAAAAAACATACCGCGCGGCGCCGG-5′



SUPPLEMENTARY FIG. 1. Schematic structure of plasmids. Plasmids pC_4N_2-R_HS/ZF3 and pC_4N_2-R_HS3H/ZF3 encode the transcription domain and DNA binding domain fusion proteins (Pollock *et al.*, 2000). They are identical except that the activation domain of pC_4N_2-R_HS3H/ZF3 has a larger portion of the p65 subunit of human NFκB fused to the activation domain of heat shock factor 1, which results in a more potent transcription activator. Plasmid pBS-PL2 contains 12 binding sites for the DNA binding domain fusion protein (ZFHD1), followed by a minimal human interleukin-2 (IL2) promoter. Transgenes (shRNA or CPT1) were cloned downstream from the basal IL2 promoter (generating pBS-PL2-shRNA-mpA or CPT1-IRES-GFP). The CPT1 expression cassette is bicistronic and translation of GFP takes place from an internal ribosome entry site (IRES). The MluI/XhoI fragment of pDBD was transferred to pRES, together with the transgene cassette (pRES-DBD/PL2-shRNA-mpA or CPT1-IRES-GFP). This plasmid was digested with EcoRV and ClaI and cloned into pSHL (Table 1) by recombination in *E. coli* BJ5183 cells. The pSHL plasmids were used to rescue helper-dependent adenoviral vectors.

bovine growth hormone polyadenylation signal as described previously (Witting *et al.*, 2008). The resulting plasmid, pEF1α-FABP5, was cut with XbaI and SalI to release the expression cassette, blunted by Klenow treatment, and cloned into the AscI site of pRES3.2. This plasmid was then digested with XbaI to transfer the expression cassette to pSHL through recombination in *E. coli* BJ5183 cells, as described above.

The EF1αLacZ expression cassette was generated by digestion of plasmid pSV-βgal (Promega) with HindIII and SalI (releasing the LacZ gene plus small T antigen polyA) and cloned into plasmid pXCJL.1 containing the EF1α promoter. This construct was digested with XbaI and NruI to release the expression cassette, which was inserted into the AscI site of pRES3.2, after blunt-ending plasmid and insert with Klenow. Plasmid pRES-EF1αLacZ was digested with ApaI and SacII to be transferred to pSHL, as described above.

The plasmid pAlb, containing the albumin promoter/enhancer, was a gift from Dr. Richard Palmiter (Howard Hughes Medical Institute, University of Washington, Seattle, WA) (Pinkert *et al.*, 1987). pRES-Alb was created in two steps. First, pRES3.2 was cut with XbaI and AscI and the 1,500-bp fragment was ligated into the NotI site of pAlb after blunting reactions with Klenow to create pAlb-5′. In a second reaction, pRES3.2 was cut with XhoI and AscI, blunt-ended, and the 800-bp fragment was ligated into the XhoI site of pAlb-5′ to create construct pRES-Alb. pRES-AlbLacZ was created by cutting out the LacZ cDNA from pSVβ-gal (Promega) with StuI and SalI and ligating into the EcoRV and SalI sites of pRES-Alb. The resulting plasmid, pRES-AlbLacZ, was digested with ApaI and SacII to be transferred to pSHL, as described above.

Supplementary References

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