SUPPLEMENTAL METHODS

Animal experiments (C57BL/6)

Two days old neonatal *C57BL/6N* mice were injected intravenously in the retro-orbital sinus with 1E11 vector genome of rAAV8 targeting *Pck1* or saline in a total volume of 20 µL. At 6 weeks of age, mice were fasted for 24 hours and sacrificed by cardiac puncture under anaesthesia using syringes conditioned with EDTA. Liver sections were snap frozen and fixed in 4% PFA for downstream applications. Plasma was stored at -80ºC for further biochemical analyses.

Plasma triglycerides and NEFAs quantification

Plasma metabolites were assayed in duplicate using the following commercial kits; triglycerides (Thermo Fisher Scientific, TR22421), NEFAs (Wako, 999-34691, 995-34791, 991-34891, 993-35191, 276-76491).

Hepatic triglyceride content extraction and measurement

Hepatic triglycerides extraction was previously described in detail (Caron et al. 2017). Briefly, liver sections (25-50 mg) were homogenized in a 2:1 chloroform:methanol mixture, combined with methanol, and centrifuged for 15 min at 3,000 rpm. 825 µl of supernatant was transferred to a new glass tube, chloroform and 0.73% NaCl was added, and the resulting mixture was centrifuged at 5,000 rpm for 3 min. The upper phase was discarded and the lower phase was washed 3 times with a 3/48/47 mixture of chloroform:methanol:NaCl (0.58%). The lower phase was then evaporated and resuspended in 1 ml of fresh isopropanol. Triglyceride levels were determined with a standard assay kit (Thermo Fisher Scientific, TR22421) according to the manufacturer's instructions.

Hepatic glycogen content extraction and measurement

Glycogen was measured in liver samples as described (Lo et al. 1970). Briefly, glycogen was extracted in 30% KOH saturated with Na_2SO_4, precipitated in 95% ethanol, and re-suspended in distilled H_2O. Absorbance at 490 nm was measured in triplicates, after addition of phenol and H_2SO_4.

Transcription reporter system

K562 cells were transfected with M_ST1n_VPR (0.25 µg) (aka dSt1Cas9-VPR) (Chavez et al. 2015) (Addgene plasmid #63799, a gift from George Church), M-tdTom-ST1 (0.25 µg) (Addgene #48678, a gift from George Church), and the indicated amounts of M-ST1-sgRNA (v0) (Addgene #48672, a gift from George Church) (Esvelt et al. 2013). Where indicated, the sgRNA (v0) vector was exchanged for sgRNA (v1) (St1Cas9_LMD-9_sgRNA_pUC19; Addgene plasmid #110627) containing the same guide. Empty pUC19 vectors were used to normalize DNA concentration in all transfections. Fluorescence microscopy images were taken with an EVOS FL Cell Imaging System 3 days post-transfection. The intensity and the frequency of cells expressing tdTomato were assessed with a BD LSR II flow cytometer 3 days post-transfection.

SUPPLEMENTAL REFERENCES

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Esvelt KM, Mali P, Braff JL, Moosburner M, Yaung SJ, Church GM. 2013. Orthogonal Cas9 proteins for RNA-guided gene regulation and editing. *Nat Methods* **10**: 1116-1121.

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