## SUPPLEMENTARY DATA

## MATERIAL AND METHODS

*Generation of IP118.* GLP-1 analogue G8,E22,G36-GLP-1 was genetically fused to the N-terminus of a human IgG4 Fc via a (G4S)3 flexible linker in a mammalian expression vector as previously described [Glaesner W, et. al., Engineering and characterization of the long-acting glucagon-like peptide-1 analogue LY2189265, an Fc fusion protein. Diabetes Metab Res Rev. 2010 May;26(4):287-96. doi: 10.1002/dmrr.1080]. The GLP-1 Fc-fusion protein was transiently expressed in Chinese hamster ovary cells under serum-free conditions. Cleared culture supernatant was loaded directly onto MapSelect SuRe column equilibrated with PBS (pH 7.2). GLP-1 Fc-fusion was eluted with a gradient (15 column volumes) 50 mM sodium acetate, pH 3.65. Pooled fractions were equilibrated to 25 mM Histidine, 7% Sucrose, 0.02% polysorbate 80, pH 6.0 by tangential flow filtration using a 30 kDa cut-off filter. The purified GLP-1 Fc-fusion contained at least 98.6% monomer when characterized by HPLC-SEC analysis. Intact mass of the constructs was confirmed by mass spectrometry and concentration assessed (absorption at 280 nm).

*Acute glucose tolerance test in diet induced obese (DIO) mice.* DIO mice (Charles River, Frederick, MD; n=8/group) were administered vehicle (PBS, s.c.) or IP118 (1 mg/kg, s.c.) 2 h prior to glucose bolus, whereas vehicle (10% DMSO in sterile water, p.o.) or OCA (30 mg/kg, p.o.) was administered to the respective mice 30 min prior to glucose challenge (1.5 mg/kg, i.p.). Vehicle or test agents were administered in a

volume of 10 mL/kg, thus all animals received both a s.c. and p.o. dose of vehicle and/or test agent, or both test agents in the IP118+OCA group. Blood glucose was measured via tail blood using a handheld glucometer (Ascensia Breeze 2, Bayer Healthcare LLC, Mishawaka, IN. *p<0.05 vs. vehicle, ^p<0.05 vs. OCA.

*Gene expression analysis.* Fragments of frozen liver or brown adipose tissue were homogenized using TRIzol Reagent (Ambion, Thermo Fisher Scientific, Carlsbad, CA) followed by RNA extraction using RNeasy Mini columns combined with DNase inactivation (Qiagen). One microgram of total RNA was reversed transcribed to cDNA using SuperScript III First Strand cDNA synthesis kit (Invitrogen, Thermo Fisher Scientific, Carlsbad, CA) according to manufacturer's instructions. Reaction mixes consisting of Applied Biosystems TaqMan Fast Universal PCR Master Mix and primer/probes (assays on demand), along with 2 µL cDNA, were made to 10 µL with nuclease-free water. All primer/probes targeted to genes of interest were labeled with FAM and performed using QuantStudi-7 Flex System (Applied Biosystems; Thermo Fisher Scientific, Foster City, CA). Relative gene expression calculated by the $^{\Delta\Delta}$CT method using the mean of *Ppia* and *Hprt* (liver), or *Ppia* alone (brown adipose tissue) expression for normalization. The following are Taqman primer sets used for genes encoding murine peptidylprolyl isomerase A (*Ppia*; Mm02342430_g1), hypoxanthine guanine phosphoribosyl transferase (*Hprt*; Mm03024075_m1), nuclear receptor subfamily 0, group B, member 2 (*Nr0b2*; Mm00442278_m1), nuclear receptor subfamily 1, group H, member 4 (*Nr1h4*; Mm00436425_m1), cytochrome P450, family 7, subfamily a, polypeptide 1 (*Cyp7a1*; Mm00484150_m1), cytochrome P450, family 8,

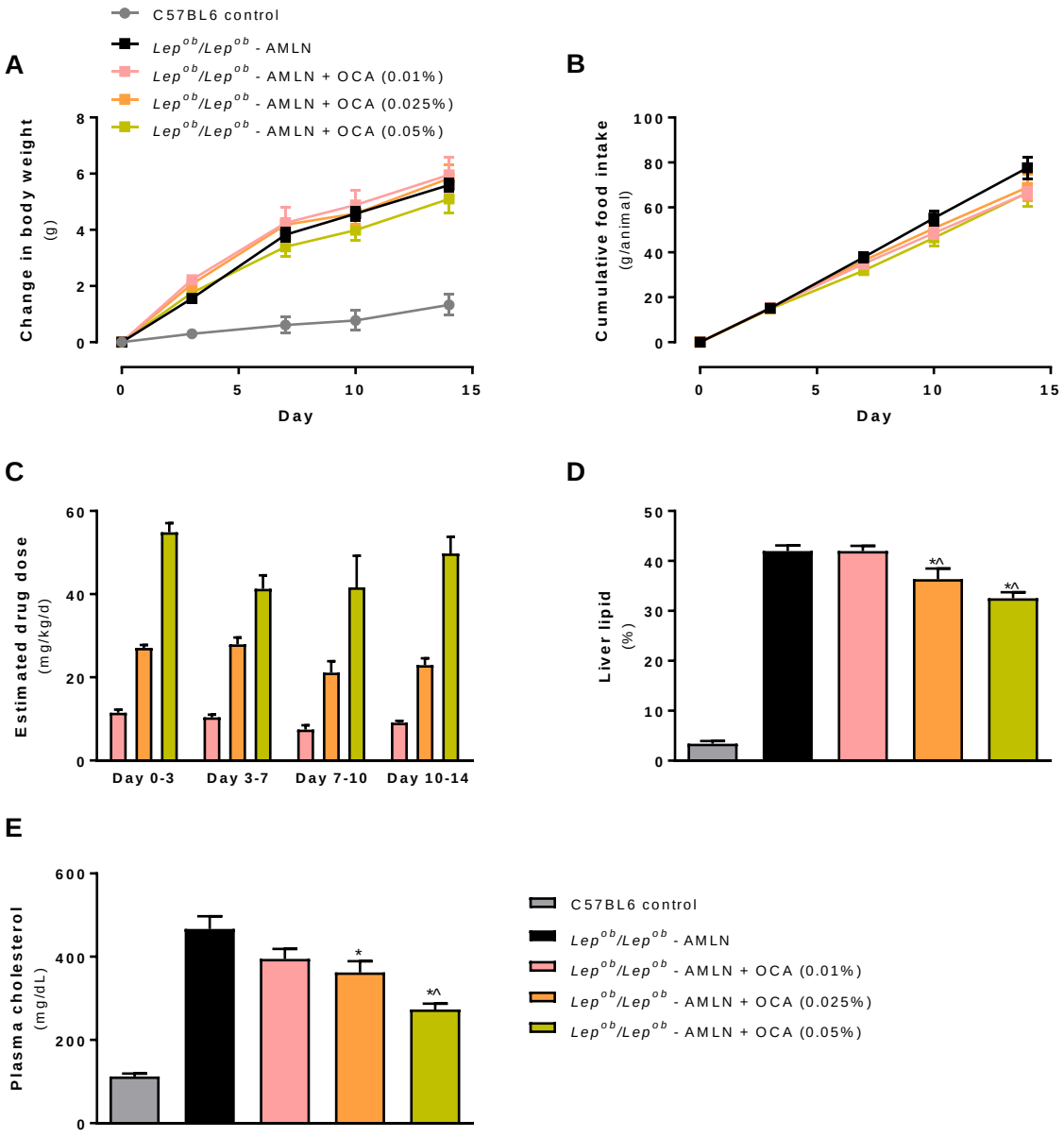
subfamily b, polypeptide 1 (*Cyp8b1*; Mm00501637_s1), mitofusin 1 (*Mfn1*; Mm00612599_m1), transcription factor A, mitochondrial (*Tfam*; Mm00447485_m1), sirtuin 1 (*Sirt1*; Mm01168521_m1), carnitine palmitoyltransferase 1a (*Cpt1a*; Mm01231183_m1), peroxisome proliferative activated receptor, gamma, coactivator 1 alpha (*Ppargc1a;* Mm01208835_m1), uncoupling protein 1 (*Ucp1*; Mm01244861_m1), solute carrier family 51, beta subunit (*Slc51b*; Mm01175040_m1), fatty acid binding protein 6, ileal (*Fabp6*; Mm00434315_m1).

*Primary hepatocyte isolation.* Murine primary hepatocytes were isolated using a modified two-step non-recirculating perfusion method. The portal vein was cannulated and approximately 50 mL of Hank's Balanced Salt Solution (HBSS with 5 mM glucose, 0.5 mM EGTA, 25 mM HEPES pH 7.4) was perfused through the liver. Digestion medium (low-glucose DMEM (Gibco), supplemented with 15 mM HEPES, 100 U/mL penicillin and 0.1 mg/mL streptomycin (Pen/Strep), and 100 U/mL type IV collagenase (B6 mice) or type I collagenase (*Lep$^{ob}$/Lep$^{ob}$* mice) (Worthington Biochemical, Lakewood, NJ) was then perfused at 8 mL/min for approximately 8 min or until the liver was fully digested. The digested liver was excised, placed in culture medium and gently ripped and shaken to release the cells. The cells were filtered and washed with ice-cold isolation medium (high glucose DMEM; Gibco) supplemented with 1 mM sodium lactate, 2 mM L-glutamine, 15 mM HEPES, 0.1 µM dexamethasone, 1x Pen/Strep, and 10% fetal bovine serum). The hepatocytes were plated on collagen-coated plates after assessing yield and viability (over >90%). All assays were carried out within 18-24 hours post plating.

*Citrate synthase activity.* Citrate synthase activity (CSA) of primary hepatocytes was measured to assess mitochondrial content according to the manufacturer's instructions (BioVision, Milipitas, CA). Activities were normalized to total protein content.

*Oxygen consumption.* Mitochondrial oxygen consumption was measured using the Seahorse Xfe96 analyzer (Agilent; Santa Clara, CA). Primary hepatocytes (7500 cells per well) were incubated with 10 µM OCA (Selleckchem), 100 nM liraglutide (Novo Nordisk, Denmark via Blue Door pharmacy, Rockville, MD), the combination of 10 µM OCA and 100 nM liraglutide, or 500 µM AICAR (Sigma-Aldrich, St Louis, MO) for 4 h. The medium was exchanged (DMEM containing 5 mM glucose, 4 mM L-glutamine, 2 mM sodium pyruvate, pH 7.4), and the plate was placed in a $CO_2$-free incubator for 30 min prior to being placed in the analyzer. The following compounds were used in the mitochondrial stress test: 1 µM oligomycin (Sigma-Aldrich), 0.5 µM FCCP (Sigma-Aldrich), and 5 µM antimycin A (Sigma-Aldrich)/ 1 µM rotenone (Sigma-Aldrich). The data represent the average of two independent experiments each with a minimum of 8 replicates per group.

**Supplementary Figure S1.** Pilot study to investigate optimal OCA concentration in diet admixture. *p<0.05 vs. *Lep*$^{ob}$/*Lep*$^{ob}$ – AMLN diet, ^p<0.05 vs. *Lep*$^{ob}$/*Lep*$^{ob}$ – AMLN + OCA (0.01%).



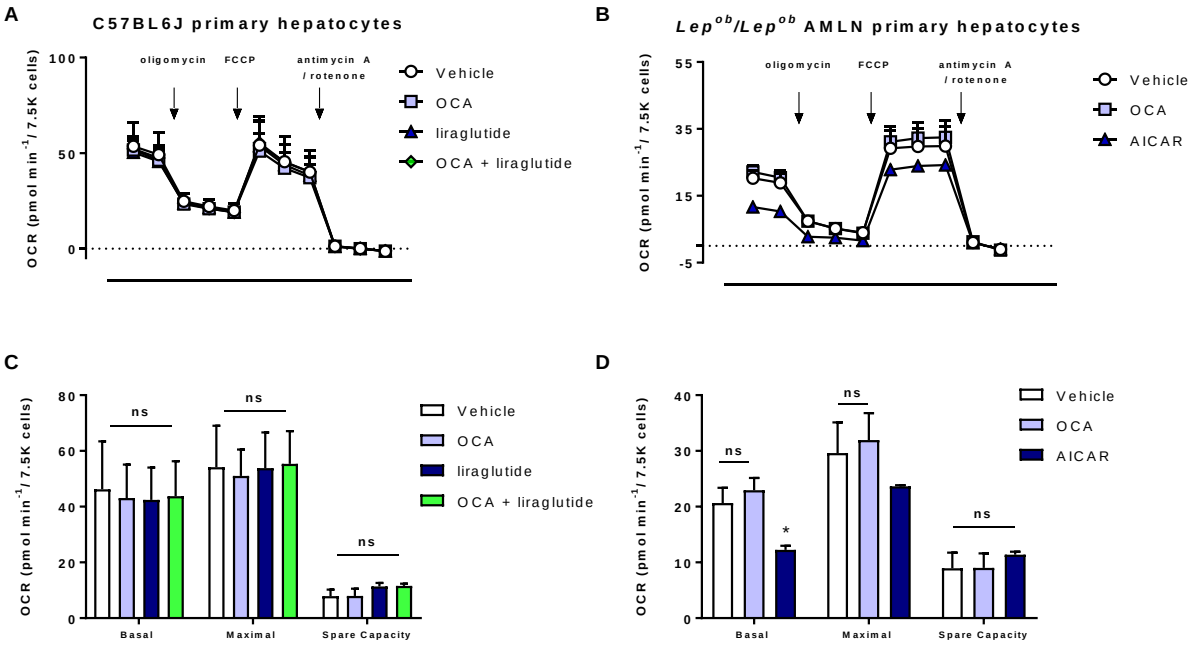
**Supplementary Table S2.** Expression of FXR target genes in liver and jejunum of *Lep$^{ob}$/Lep$^{ob}$* mice after two weeks on AMLN diet containing various concentration of OCA. *p<0.05 vs. C57BL6 controls, ^p<0.05 vs. *Lep$^{ob}$/Lep$^{ob}$* AMLN.

| **Tissue** | **Gene** | **C5BL6J controls** | ***Lep$^{ob}$/Lep$^{ob}$* – AMLN** | ***Lep$^{ob}$/Lep$^{ob}$* – AMLN + OCA (0.01%)** | ***Lep$^{ob}$/Lep$^{ob}$* – AMLN + OCA (0.025%)** | ***Lep$^{ob}$/Lep$^{ob}$* – AMLN + OCA (0.05%)** |
|---|---|---|---|---|---|---|
| **Liver** | ***Nr0b2* (Shp)** | 1.0 ± 0.1 | 2.6 ± 0.7 | 3.1 ± 0.7 | 3.6 ± 0.8 | 4.2 ± 0.7* |
| | ***Nr1h4* (FXR)** | 1.0 ± 0.2 | 4.4 ± 0.9* | 3.4 ± 0.7* | 2.5 ± 0.4 | 2.3 ± 0.3 |
| | ***Slc51b* (Ostb)** | 1.0 ± 0.4 | 14.3 ± 3.8 | 25.5 ± 6.0 | 36.9 ± 7.0* | 51.8 ± 14.5*^ |
| **Jejunum** | ***Nr0b2* (Shp)** | 1.0 ± 0.3 | 0.6 ± 0.1 | 0.9 ± 0.2 | 0.7 ± 0.1 | 0.8 ± 0.2 |
| | ***Nr1h4* (FXR)** | 1.0 ± 0.1 | 1.1 ± 0.2 | 2.1 ± 0.7 | 1.4 ± 0.2 | 1.2 ± 0.2 |
| | ***Fabp6* (I-BABP)** | 1.0 ± 0.6 | 0.2 ± 0.2 | 12.9 ± 6.8 | 3.6 ± 1.5 | 8.6 ± 2.9 |

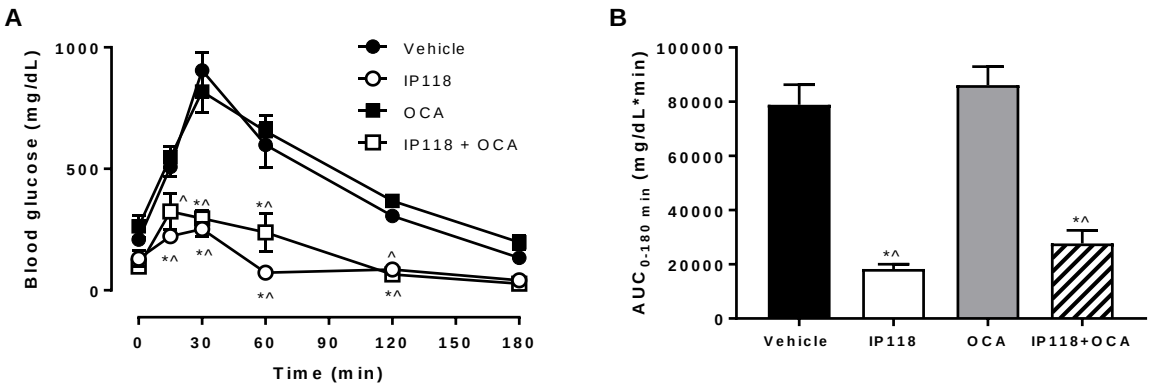
**Supplementary Table S3.** Liver gene expression analysis of key mitochondrial and FXR target genes of *Lep$^{ob}$/Lep$^{ob}$* mice on LFD or AMLN diet treated with OCA, IP118 or OCA + IP118 for 4 weeks. *p<0.05 vs. *Lep$^{ob}$/Lep$^{ob}$* LFD, $^{\#}$p<0.05 vs. *Lep$^{ob}$/Lep$^{ob}$* – AMLN, ^p<0.05 vs. *Lep$^{ob}$/Lep$^{ob}$* – AMLN + OCA, $^{+}$p<0.05 vs. *Lep$^{ob}$/Lep$^{ob}$* – AMLN + IP118.

| **Gene** | ***Lep$^{ob}$/Lep$^{ob}$* – LFD** | ***Lep$^{ob}$/Lep$^{ob}$* – AMLN** | ***Lep$^{ob}$/Lep$^{ob}$* – AMLN + OCA** | ***Lep$^{ob}$/Lep$^{ob}$* – AMLN + IP118** | ***Lep$^{ob}$/Lep$^{ob}$* – AMLN + OCA + IP118** |
|---|---|---|---|---|---|
| ***Nr0b2*** | 1.0 ± 0.13 | 1.42 ± 0.14 | 2.53 ± 0.16*$^{\#}$ | 1.50 ± 0.17^ | 2.23 ± 0.10*$^{\#+}$ |
| ***Cyp7a1*** | 1.0 ± 0.16 | 1.74 ± 0.34 | 0.49 ± 0.08$^{\#}$ | 1.64 ± 0.37^ | 0.26 ± 0.05$^{\#+}$ |
| ***Cyp8b1*** | 1.0 ± 0.11 | 0.38 ± 0.02* | 0.08 ± 0.02*$^{\#}$ | 0.28 ± 0.02*^ | 0.08 ± 0.01*$^{\#+}$ |
| ***Mfn1*** | 1.0 ± 0.07 | 0.92 ± 0.03 | 1.14 ± 0.11 | 1.24 ± 0.11 | 1.09 ± 0.05 |
| ***Tfam*** | 1.0 ± 0.02 | 1.10 ± 0.03 | 1.22 ± 0.06* | 1.30 ± 0.04*$^{\#}$ | 1.24 ± 0.03* |
| ***Sirt1*** | 1.0 ± 0.03 | 1.15 ± 0.03 | 1.44 ± 0.12* | 1.50 ± 0.08*$^{\#}$ | 1.45 ± 0.07* |
| ***Cpt1a*** | 1.0 ± 0.07 | 1.05 ± 0.05 | 1.33 ± 0.16 | 1.33 ± 0.14 | 1.31 ± 0.10 |

**Supplementary Figure S4.** No direct effect of OCA on mitochondrial function in primary hepatocytes isolated from lean CB7BL6J or *Lep*$^{ob}$/*Lep*$^{ob}$ mice on AMLN diet. Oxygen consumption rate (OCR) of hepatocytes are shown in response to oligomycin, the uncoupling agent FCCP or antimycin A/rotenone in the presence of OCA (10 μM), liraglutide (100 nM) or the combination of OCA and liraglutide for C57BL6J hepatocytes (A), or in the presence of OCA (10 μM) or AICAR (500 μM) in NASH hepatocytes (B) for 4 h. Basal, maximal and spare respiratory capacity were calculated for C57BL6J (C) and NASH hepatocytes (D).



**Supplementary Figure S5.** Glucose tolerance test following single dose of vehicle, IP118, OCA or IP118 + OCA in DIO mice. Vehicle (PBS, s.c.) or IP118 (1 mg/kg, s.c.) was administered 2 h prior to glucose bolus, whereas vehicle (10% DMSO in sterile water, p.o.) or OCA (30 mg/kg, p.o.) was administered 30 min prior to glucose challenge (1.5 mg/kg, i.p.). Mice were fasted a total of 6 h prior to glucose administration. Blood glucose (A) was measured via tail blood and (B) area under the curve calculated. *p<0.05 vs. vehicle, ^p<0.05 vs. OCA.



**Supplementary Table S6.** Gene expression analysis of liver and brown adipose tissue (BAT) from DIO mice treated with vehicle, IP118, OCA or IP118 + OCA for 21 days. ND, not detected; *p<0.05 vs. vehicle, #p<0.05 vs. IP118, ^p<0.05 vs. OCA.

| Tissue | Gene | Vehicle | IP118 | OCA | IP118 + OCA |
|---|---|---|---|---|---|
| **Liver** | ***Nr0b2*** | 1.0 ± 0.15 | 1.42 ± 0.16 | 1.35 ± 0.12 | 2.56 ± 0.27*#^ |
| | ***Cyp7a1*** | 1.0 ± 0.12 | 0.69 ± 0.29 | 0.21 ± 0.05* | 0.22 ± 0.07* |
| | ***Cyp8b1*** | 1.0 ± 0.09 | 0.56 ± 0.12* | ND | ND |
| | ***Mfn1*** | 1.0 ± 0.08 | 1.22 ± 0.14 | 0.99 ± 0.07 | 1.22 ± 0.14 |
| | ***Tfam*** | 1.0 ± 0.11 | 1.17 ± 0.14 | 0.80 ± 0.05 | 1.02 ± 0.12 |
| | ***Sirt1*** | 1.0 ± 0.09 | 0.93 ± 0.08 | 0.78 ± 0.05 | 1.01 ± 0.10 |
| | ***Cpt1a*** | 1.0 ± 0.08 | 1.24 ± 0.14 | 0.72 ± 0.05# | 0.93 ± 0.11 |
| **BAT** | ***Mfn1*** | 1.0 ± 0.03 | 0.94 ± 0.04 | 0.90 ± 0.03 | 0.88 ± 0.03* |
| | ***Tfam*** | 1.0 ± 0.07 | 1.09 ± 0.04 | 0.94 ± 0.04 | 1.32 ± 0.06*#^ |
| | ***Sirt1*** | 1.0 ± 0.02 | 0.98 ± 0.04 | 0.92 ± 0.03 | 1.03 ± 0.03 |
| | ***Cpt1a*** | 1.0 ± 0.05 | 0.80 ± 0.05* | 0.80 ± 0.05* | 0.80 ± 0.02* |
| | ***Ppargc1a*** | 1.0 ± 0.07 | 0.90 ± 0.05 | 0.99 ± 0.05 | 0.91 ± 0.06 |
| | ***Ucp1*** | 1.0 ± 0.03 | 1.27 ± 0.10 | 0.92 ± 0.06# | 1.66 ± 0.09*#^ |