## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### *Antibodies and reagents*

Rabbit α-phospho-AKT-S473 (p-AKT), α-phospho-STAT-5-Y694 (p-STAT-5), α-phospho-STAT-3-Y705 (p-STAT-3), α-phospho-STAT-1-Y701 (p-STAT-1), α-phospho-ERK1/2-T202/Y204 (p-ERK1/2), α-phospho-SFK-Y418 (p-SFK), α-STAT-5, α-STAT-1, α-PPAR-γ, α-JAK-2, α-SCD1, α-FasN, α-SHP1 and mouse α-STAT-3, and α-AKT (pan) were from Cell Signaling Technology (Beverly, MA). Rabbit α-SHP2, α-SREBP1, α-ERK2, α-JAK-1 (sc-7228), α-JAK-2 (sc-294), goat α-PDK4 and mouse IRβ and β-actin (sc-1616) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit α-phospho-IRβ-Y1162/Y1163 (p-IRβ) and α-phospho-JAK-1-Y1022/Y1023 (p-JAK-1) were from Invitrogen (Carlsbad, CA), mouse α-PTP1B from BD Bioscience (San Jose, CA), α-DEP-1 (143-41) from R&D Systems (Minneapolis, MN), rabbit α-Gpx1 and rabbit α-CD45 from Abcam (San Francisco, CA), mouse α-phospho-tyrosine (p-Tyr) and rabbit α-phospho-JAK-2-Y1007/Y008 (p-JAK-2) from Millipore (Billerica, MA), α-tubulin from Sigma-Aldrich (St Louis, MO) and α-actin (pan) from Neomarkers (Fremont, CA). α-Ptpn2 (6F3) was from Medimabs (Quebec, Canada) and mouse oxidised PTP active site (PTPox) antibody from R&D systems (Minneapolis, MN). CMP6 (JAK Inhibitor I) was from Calbiochem (San Diego, CA), sodium palmitate, bovine and human insulin and recombinant human GH from Sigma-Aldrich (St Louis, MO), recombinant human IL-6 and mouse IFN-γ from PeproTech (Rocky Hill, NJ) and JI-38 from Phoenix Pharmaceuticals (Burlingame, CA). The m/rIG1 ELISA kit was from Mediagnost (Reutlinger, Germany) and the m/r Insulin and m/r GH ELISA kits were from Millipore (Billerica, MA). MitoTempol from Enzo Life Sciences (Farmingdale, NY) and the mitochondrial-localised peptide SS31 (Bendavia, active ingredient is MTP-131) was provided by Stealth Peptides Incorporated (Newton Center, MA)

### *Mice*

We maintained mice on a 12 h light-dark cycle in a temperature-controlled high barrier facility (Monash ARL) with free access to food and water. Aged- and sex-matched mice were used for all experiments. *Ptpn2*$^{lox/lox}$ (C57BL/6) described previously (Loh et al., 2012; Wiede et al., 2011) were mated with *Alb*-Cre (C57BL/6) mice (JAX, Bar Harbor, Maine) for the postnatal deletion of *Ptpn2* specifically in hepatocytes (Postic and Magnuson, 2000). *Stat5*$^{lox/lox}$ (C57BL/6) mice described previously (Cui et al., 2007) and provided by Lothar Henighausen (The National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD) were mated with *Alb*-Cre;*Ptpn2*$^{lox/lox}$ mice to generate *Ptpn2*$^{lox/+}$;*Stat5*$^{lox/+}$ and *Alb*-Cre;*Ptpn2*$^{lox/+}$;*Stat5*$^{lox/+}$ mice which were thereon bred with *Ptpn2*$^{lox/lox}$ to generate experimental mice. *Gpx1–/–* (C57BL6) mice have been described previously (Loh et al., 2009). Mice were fed a standard chow (20% protein, 6% fat and 3.2% crude fibre; Barastoc, Australia) or a high fat diet (23% fat; 45% of total energy from fat; SF04-027; Specialty Feeds) as indicated. All experiments were approved by the Monash University School of Biomedical Sciences Animal Ethics Committee and performed in accordance with the NHMRC Australian Code of Practice for the Care and Use of Animals.

***Metabolic measures***

Insulin and pyruvate or glucose tolerance tests were performed on 4 h and 6 h fasted mice, respectively, by injecting human insulin (0.5 mU/g body weight), D-glucose (2 mg/g body weight), or pyruvate (2 mg/g body weight) intraperitoneally and measuring glucose in tail blood with an Accu-chek performa glucose meter (Roche Diagnostics, Germany) as described previously (Fukushima et al., 2010; Loh et al., 2009). For an assessment of hepatic insulin signaling, mice were fasted for 4 h, injected intraperitoneally with the indicated concentrations of insulin and after 10 min the livers excised rapidly and frozen in liquid N2 for subsequent biochemical analyses. For the determination of fed and fasted blood glucose and corresponding plasma insulin levels, blood was collected by retro-orbital bleeding after a 6 h fast. Plasma insulin levels were determined using a rat insulin RIA kit (Linco Research, St. Charles, MO) according to the manufacturer's

instructions, or with an in-house ELISA. For metabolic measures mice were acclimated for 24 h and monitored for 48 h in an environmentally controlled Comprehensive Lab Animal Monitoring System (CLAMS; Columbus Instruments, Columbus. OH) fitted with indirect open circuit calorimetry and food consumption and activity monitors to measure activity, food intake and energy expenditure. Energy expenditure and the respiratory exchange ratio (RER = $V_{CO2}/V_{O2}$) were calculated from the gas exchange data. Body composition was measured by DEXA (Lunar PIXImus2; GE Healthcare) and analysed using PIXImus2 software; the head region was excluded from analyses.

Hyperinsulinaemic euglycaemic clamps (without tracer) were performed on overnight fasted and anaesthetized mice as described previously (Loh et al., 2009). Briefly 20 week-old mice were fasted overnight (16 h) and anesthetized on the morning of the experiment with an intraperitoneal injection of sodium pentobarbitone (100 mg/kg). Two catheters were inserted, one in the right jugular vein for insulin and glucose infusion and the other in the left carotid artery for sampling. A tracheostomy was also performed to prevent upper respiratory tract obstruction and body temperature maintained using a heat lamp. Insulin (60 mU/ml) was infused at a rate of 20-40 uL/min during 4 h. Euglycemia was maintained by the co-infusion of a 5% (w/v) glucose solution. Immediately following the collection of the last blood sample at 4 h, animals were sacrificed and livers removed rapidly, frozen in liquid nitrogen and stored at -70$^{o}$C for subsequent analyses.

***Cell culture and RNA interference***

Hepatocytes from were isolated by a two-step collagenase A (0.05% w/v; Roche Diagnostics, Germany) perfusion method as described previously (Fukushima et al., 2010). Hepatocytes were cultured in M199 medium (Invitrogen, Carlsbad, CA) containing 10% (v/v) heat-inactivated FBS, 100 units/ml penicillin, 100 μg/ml streptomycin and 20 ng/ml EGF (R&D Systems, Minneapolis, MN). After 24 h cells were serum starved in M199 medium for 2-4 h and then stimulated with bovine insulin, GH, IFN-γ or IL-6 as indicated. Alternatively, cells were

treated with 0.5 mM sodium palmitate in the presence of 1% w/v fatty acid free BSA overnight (16 h) and then serum starved (4 h) and stimulated as indicated. AML12 hepatocytes were cultured as recommended (ATCC) and treated with 0.5 mM sodium palmitate in the presence of 1% w/v fatty acid free BSA overnight for subsequent analysis of $H_2O$ production or PTP oxidation.

*Ptpn2 or Jak-2* were knocked down transiently in primary murine hepatocytes using *Ptpn2* (TCPTP#1: AAGCCCATAUGAUCACAGUCG; TCPTP#2: AAGAUUGACAGACACCUAAAU; Dharmacon Thermo Scientific, Waltham, MA) or *Jak2*-specific siRNAs (sc-39100; Santa Cruz Biotechnology, Santa Cruz, CA); enhanced green fluorescent protein (*GFP*; CAAGCUGACCCUGAAGUUCdTdT; Dharmacon Thermo Scientific, Waltham, MA) siRNA was used as a control. Primary hepatocytes were transfected 24 h after isolation with 40 nM siRNA using Lipofectamine® RNAiMAX (Invitrogen, Carlsbad, CA) as described previously (Galic et al., 2005). After overnight incubation the medium was replaced and cells allowed to recover for 24 h before further treatment.

***ROS determinations***

For monitoring $H_2O_2$, hepatocytes were cultured in 96 well white ViewPlates (Perkin Elmer, Waltham, MA) and $H_2O_2$ levels determined in live hepatocytes using the Amplex® Red hydrogen peroxide assay kit (Invitrogen, Carlsbad, CA). Amplex Red fluorescence was measured on a BMG Fluorostar fluorescent plate reader (Hamilton, Reno, NV) at excitation 545 nm and emission 590 nm and normalised to the corresponding protein content. Total (GSH) and oxidised (GSSG) glutathione levels in the clarified supernatants or in whole blood were measured using a BIOXYTECH GSH/GSSG-412 assay kit (Oxis International, Inc., Foster City, CA), and GSH to GSSG ratios determined as per the manufacturer's instructions.

***Biochemical Analysis***

Mouse tissues were excised rapidly and snap-frozen in liquid N2. Frozen tissues were homogenised using a mechanical homogeniser in 10-20 volumes of ice cold RIPA lysis buffer (50 mM HEPES [pH 7.4], 1% (vol/vol) Triton X-100, 1% (vol/vol) sodium deoxycholate, 0.1% (vol/vol) SDS, 150 mM NaCl, 10% (vol/vol) glycerol, 1.5 mM MgCl2, 1 mM EGTA, 50 mM sodium fluoride, leupeptin (5 µg/ml), pepstatin A (1 µg/ml), 5 µg/ml aprotinin, 1 mM benzamadine, 1 mM phenylmethysulfonyl fluoride, 1 mM sodium vanadate) and clarified by centrifugation at 50, 000 g for 20 min at 4°C as described previously (Loh et al., 2009). Cells were lysed in RIPA buffer and clarified by centrifugation (16,000 g, 5 min, 4°C). Supernatants were resolved by SDS-PAGE and immunoblotted as described previously (Tiganis et al., 1998).

***PTP oxidation***

Total (reversible and irreversible) PTP oxidation was assessed essentially as described previously (Karisch et al., 2011) with some modifications. Briefly, frozen liver tissue or freshly isolated hepatocytes were homogenised under anaerobic conditions in de-gassed, ice-cold PTPox lysis buffer [50 mM Hepes, pH 6.5, 150 mM NaCl, 10 % (vol/vol) Glycerol, 1 % (w/v) NP40, 20 mM NaF, 1 µg/ml pepstatin A, 5 µg/ml leupeptin, and 5 µg/ml aprotinin, 1 mM benzamadine, 1 mM phenylmethysulfonyl fluoride] containing 10 mM N-ethylmaleimide to prevent post-lysis oxidation and to alkylate all reduced and active PTPs, and incubated for 1 h at 4°C. Cell lysates and liver homogenates were clarified by centrifugation at 16,000 g and 50,000 g respectively for 20 min and the buffer exchanged (NAP$^{TM}$-5 columns, GE Healthcare) to 20 mM HEPES containing 10 mM DTT to reduce oxidised PTPs. Reduced PTPs were then hyperoxidised to their sulfonic (-$SO_3H$) state by exchanging the buffer to 20 mM HEPES containing 100 µM pervanadate and resolved by SDS-PAGE and immunoblotted with PTPox antibody. Alternatively pervanadate-treated samples were made to 9 M urea and 4.5 mM DTT, incubated at 60°C for 30 min and then treated with 10 mM iodoacetamide before being diluted to a final concentration of 2 M urea for digestion with

TPCK-trypsin (Thermo Scientific) and processing for PTPox immunoprecipitation and analysis by mass spectrometry as described previously (Karisch et al., 2011).

***Lipid analyses and hepatic lipogenesis***

TAGs , diglycerides and ceramide were extracted and quantified as described previously (Loh et al., 2009). Blood TAGs and were quantified using a commercial enzymatic colorimetric assay (GPO-PAP reagent, Roche Diagnostics, Germany) and FFAs assessed as described previously (Loh et al., 2009). Hepatic lipogenesis was assessed by measuring the incorporation of $^{14}$C-glucose (Glucose, D-[$^{14}$C(U)]-, 100 μCi/ml, Perkin Elmer) into TAG. Briefly, liver slices (~20 mg) were incubated for 2 h in DMEM (5 mM glucose, 1 μCi/ml $^{14}$C-glucose). Tissues were washed with warm PBS and the intracellular lipids were extracted in 1.8 mL chloroform:methanol (2:1 v:v); 600 ul 4 mM $MgCl_2$ was added, the mixture mixed vigorously and centrifuged at 1,000 x g for 10 min. The organic phase containing lipids was subjected to thin layer chromatography to separate the TAGs, and incorporated radioactivity counted in a beta-counter.

***Immunohistochemistry***

Livers were frozen in OCT or fixed with formalin and embedded in paraffin. Liver sections were stained with Oil red-O (OCT fixed) or hematoxylin and eosin (formalin fixed) counterstained before being analyzed using a BX51 Olympus slide system and OlyVIA imaging software (Olympus, Tokyo, Japan).

***Real time PCR.***

RNA from frozen liver or hepatocytes was extracted using Trizol reagent (Invitrogen, Carlsbad, CA). mRNA was reverse transcribed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) and quantitative real time PCR performed TaqMan™ Universal PCR Master Mix and TaqMan™ Gene Expression Assays (Applied Biosystems, Foster

City, CA) fo*r G6pc, Pck1, Pdk4, Igf-1* and *CD36*; *Gapdh* (TaqMan™ Endogenous Controls Mouse GAPDH) was used as an internal control. Reactions were performed in triplicate and relative quantification achieved using the ΔΔCt method.

***Statistical Analysis***

Results shown are means ± standard error of mean (SEM) for the indicated number of mice or experimental repeats. Statistical significance was determined by a two-tailed paired Student's t-test or ANOVA with Bonferroni correction. $P$ values $< 0.05$ were considered statistically significant.

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