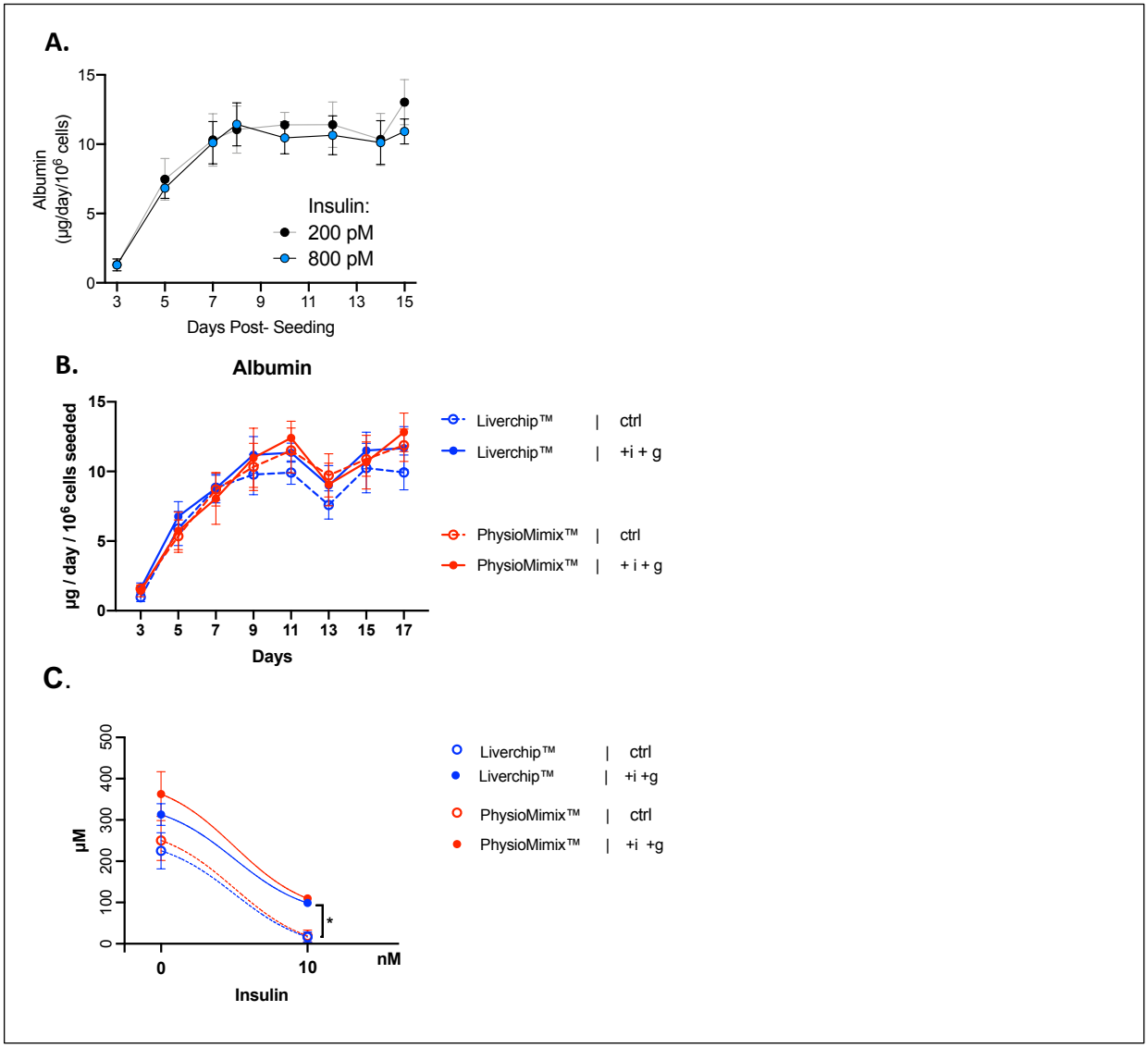
TITLE: A Microphysiological Model of Human MASLD Reveals Paradoxical Response to Resmetirom

SUPPLEMENTAL MATERIAL

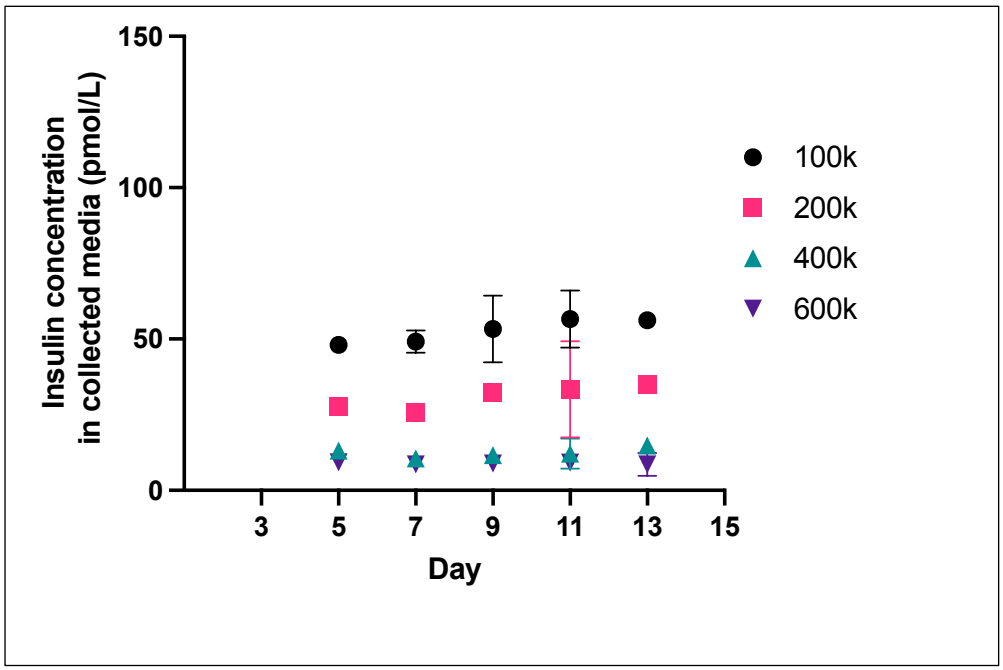
SUPPLEMENTAL FIGURES

Supplementary Figure 1



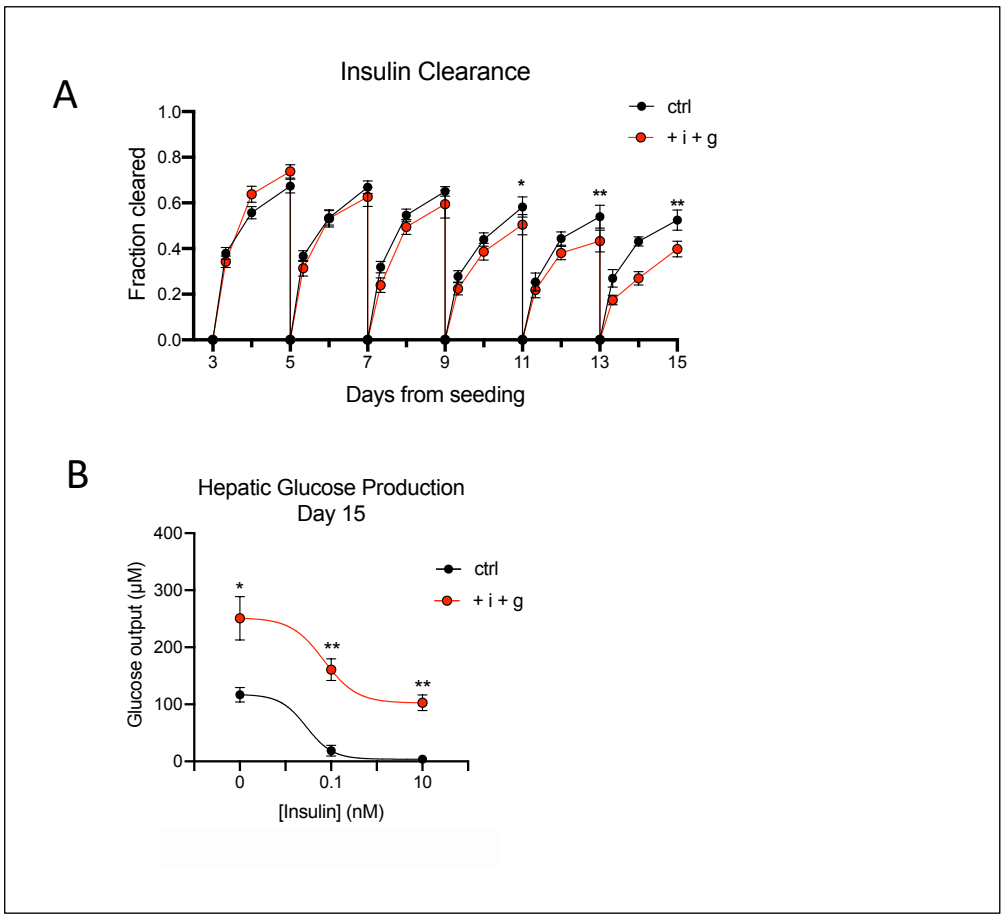
Supplementary Fig. 1. Albumin production rates stabilize after an initial transient increase and are independent of insulin and nutrient concentration or Bioreactor verson. A. 600,000 hepatocytes (donor SMC) were cultured in the Liverchip™ in either Condition 1 200 pM insulin medium (ctrl) or in Condition 1 + insulin (800 pM) medium. Media change was performed every 48h. The rates of albumin secretion are statistically identical. **B**. 600,000 hepatocytes (donor AQL) were cultured either the Liverchip™ or the PhysioMimix™ in either baseline 200 pM insulin (crtl) medium or in hi-ins (1000 pM) + hi-glucose (i + g) (11 mM glucose) medium. Media change was performed every 48h with the exception of 24h media change between D12 and D13. The rates of albumin secretion are statistically identical in both bioreactor types and both media formulations. (C) 600,000 hepatocytes (donor SMC) were cultured in two bioreactor formats (Liverchip™ vs PhysioMimix™) in two media condtions (ctrl: physiological | +i +g: hyperinsulinemia + hyperglycemia)(Two-way ANOVA * $p < 0.05$).

Supplementary Figure 2



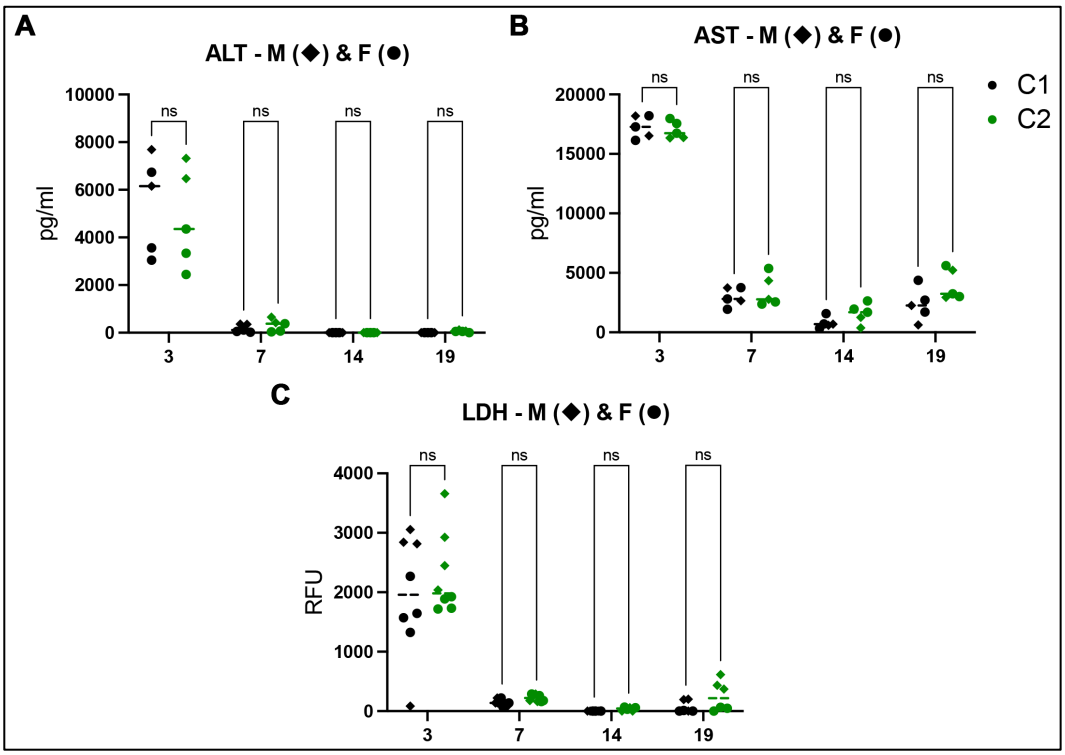
Supplementary Fig. 2. Insulin depletion from initial 200 pM concentration depends on cell number seeded in PhysioMimix™. A. Hepatocytes (donor HU2098, ThermoFisher) were cultured in the PhysioMimix™ in baseline 200 pM insulin medium, which was changed every 48 hr and insulin was measured in the collected culture supernate.

Supplementary Figure 3



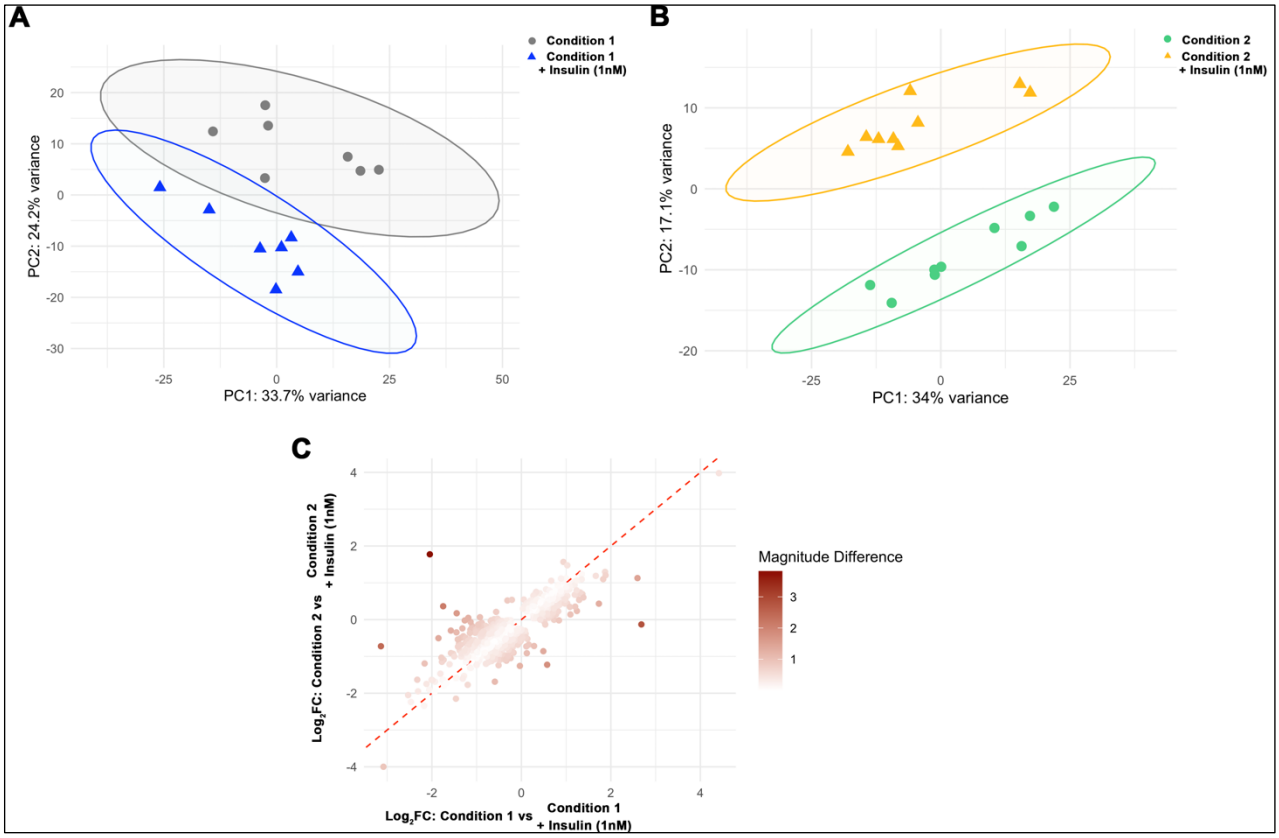
Supplementary Fig. 3. Hyperinsulinemia and hyperglycemia induce insulin resistance in the 3D perfused liver MPS with donor AQL and alternate bioreactor. (A) Cells cultured in hi-ins (1000 pM) + hi-glucose (11 mM) conditions (legend: +i +g) gradually lose the ability to clear insulin over 15 days in culture compared to 200 pM insulin baseline medium (legend: ctrl). Two-way ANOVA (* $p < 0.05$, ** $p < 0.01$, $n = 6$). (B) Cells maintained in hi-ins + hi-glucose show decreased suppression of HGP output upon 24 hour insulin stimulation (interaction term $p < 0.01$, post-hoc Sidak, ** $p < 0.01$, * $p < 0.05$, $n = 2$). (C) 600,000 hepatocytes (donor SMC) were cultured in two bioreactor formats (Liverchip™ vs PhysioMimix™) in two media condtions (ctrl: physiological | +i +g: hyperinsulinemia + hyperglycemia)(Two-way ANOVA * $p < 0.05$).

Supplementary Figure 4



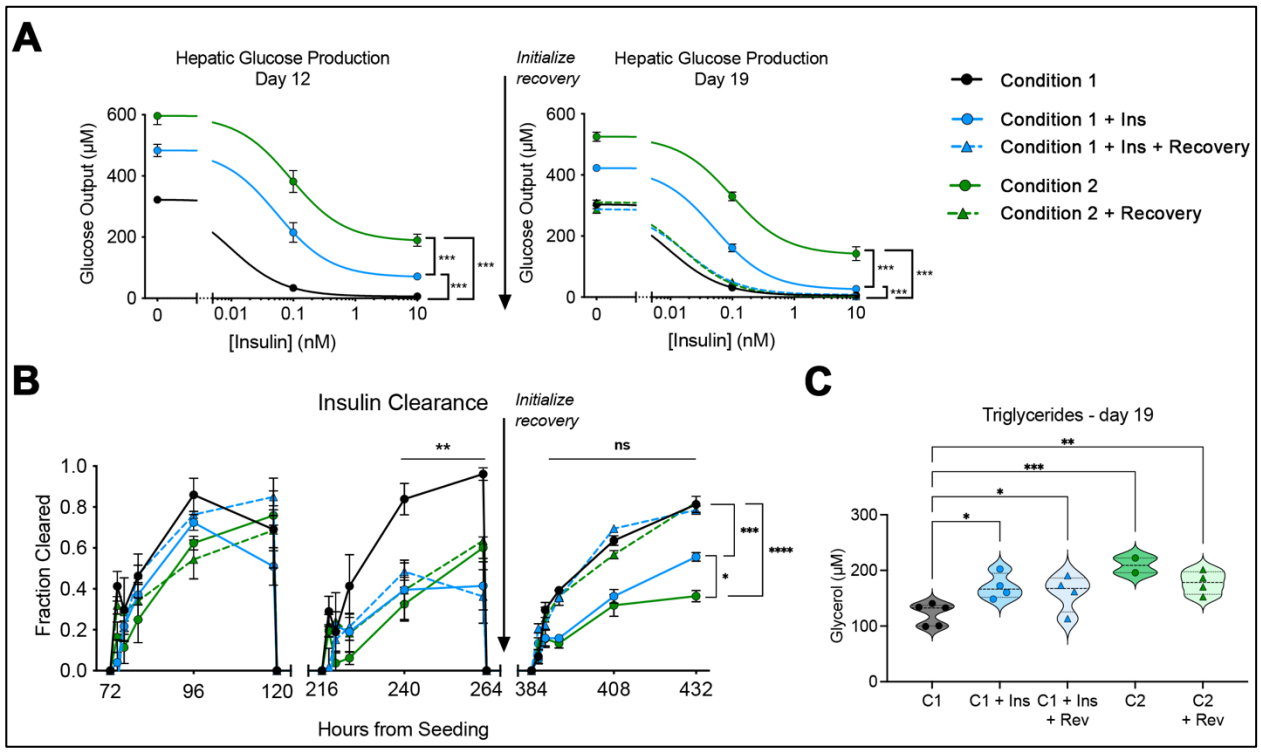
Supplementary Fig. 4. Measurement of ALT, AST, and LDH in C1 & C2 hepatocytes. (A) Measurement of ALT, (B) AST, and (C) LDH on days 3, 7, 14, and 19 in hepatocytes incubated with either C1 or C2 media. Two-way ANOVA; ns = non-significant; $n \geq 5$ for each condition; M = male (♦)(donor SMC), F = female (●)(donor HU8406).

Supplementary Figure 5



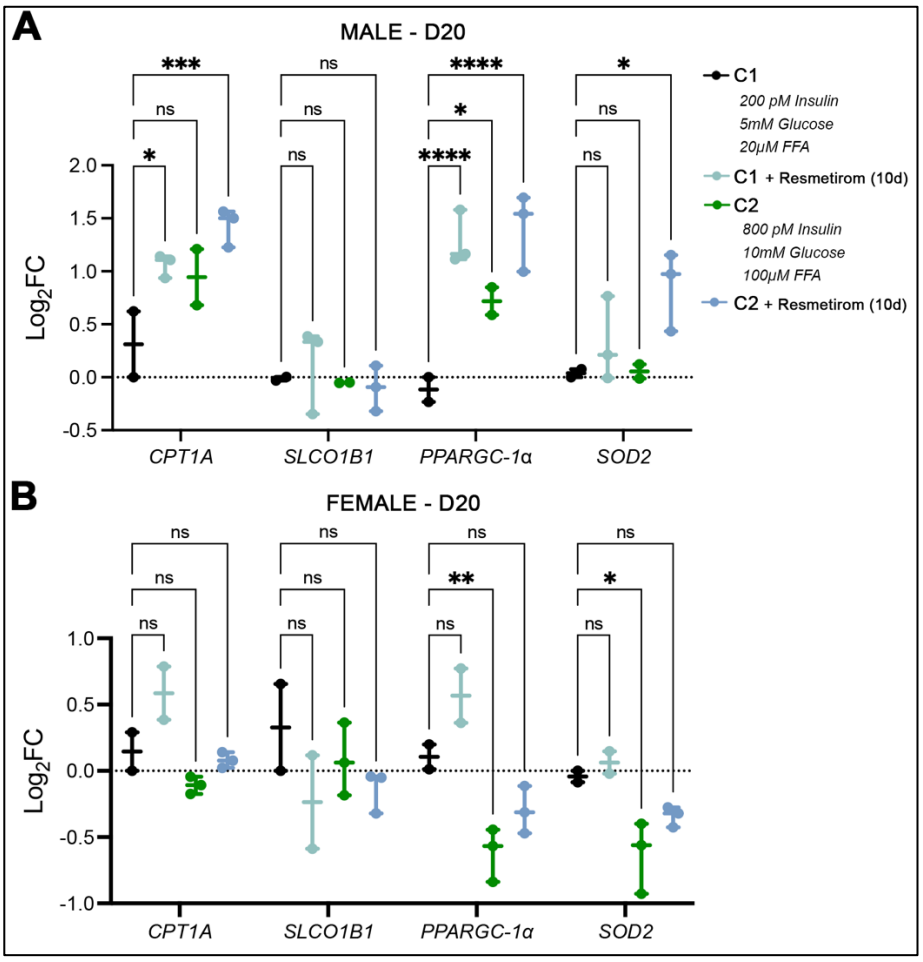
Supplementary Fig. 5. Dimensionality reduction and magnitude difference of insulin-responsive baseline and disease state samples. (A,B) Principal component analysis of Condition 1 (left) and Condition 2 (right) samples before and after exposure to 1nM insulin. (C) Magnitude plot of DEGs identified between Condition 1 and Condition 2 samples before and after exposure to 1nM insulin. All data acquired from male SMC donor.

Supplementary Figure 6



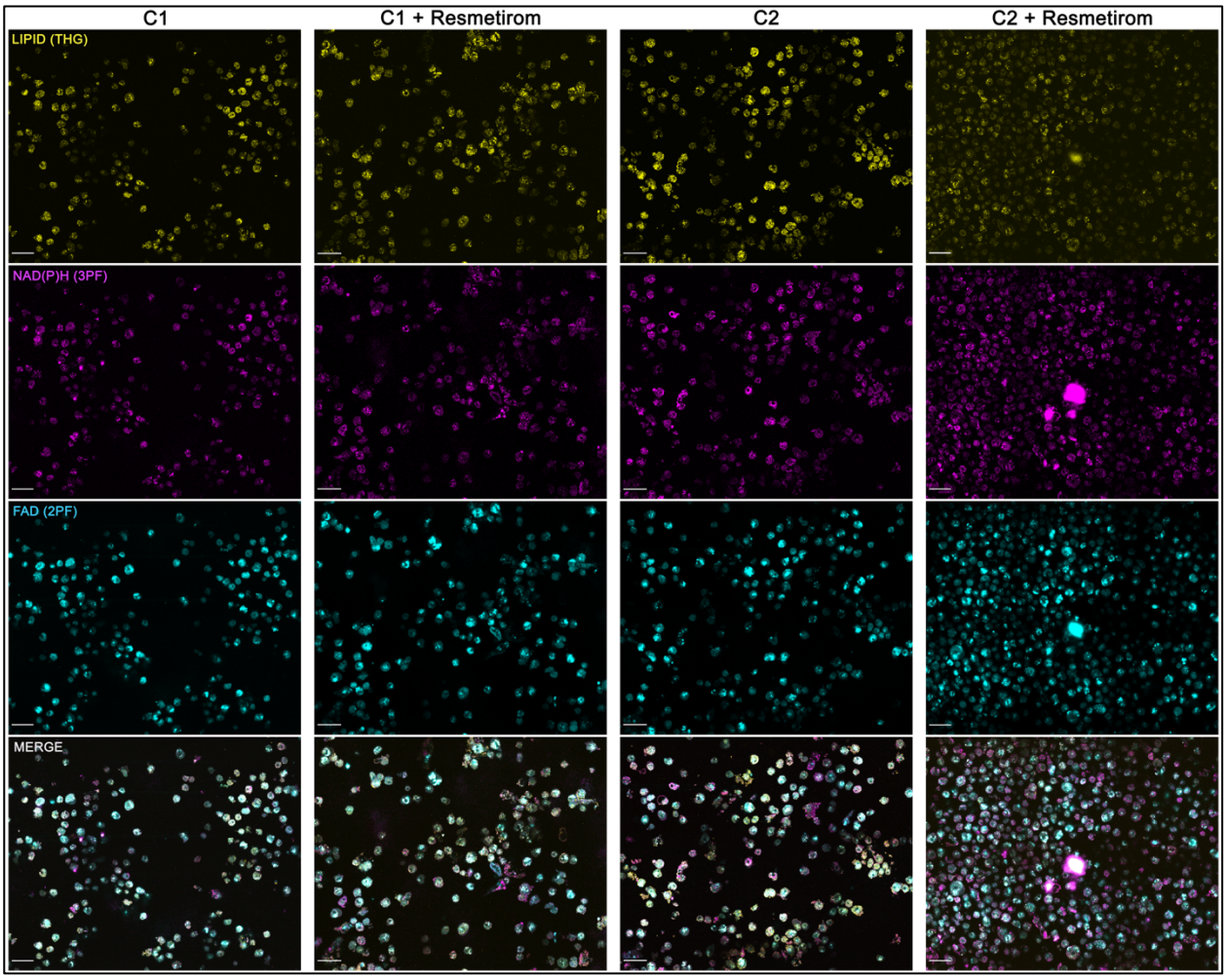
Supplementary Fig. 6. Partial recovery of insulin sensitivity after return to Condition 1 media. (A) Hepatic glucose output is significantly different between all groups at day 12 (*** p<0.001 Tukey test main column effect). At day 19, cells returned to Condition 1 from days 12-19 show no significant difference in glucose output, while cells maintained in high insulin (Condition 1 + Ins) or high insulin with nutrient overload (Condition 2) are still significantly different than all other groups and each other (*** p<0.001 Tukey test main column effect). (B) Insulin clearance is diminished in Condition 1 + Ins and Condition 2 by day 12 (hour 240-264) in culture (** p<0.01, Condition 1 vs all other groups). Upon return to healthy conditions, the recovery groups clear insulin similar to the healthy control (no significance; *ns*), while the chronic high insulin (Condition 1 + Ins) and Condition 2 hepatocytes continue to exhibit decreased insulin clearance by 18 days. (C) Intracellular triglyceride content at day 19 is significantly higher than the control in cells exposed at any time to Condition 2 media, regardless of whether the cells were returned to healthy media. * p<0.05, ** p<0.01, *** p<0.001 via two-way ANOVA. All data acquired from male SMC donor.

Supplementary Figure 7



Supplementary Fig. 7. Upregulation of resmetirom target and mitochondrial stress genes in male Condition 2 hepatocytes. Healthy and *MASLD* hepatocytes were established following 10 days of culture in Condition 1 or 2 media, respectively, with 2 μM resmetirom supplementation every 48 hours for an additional 10 days and then assayed via RT-qPCR. (A) RT-qPCR of relevant resmetirom target, transporter, and mitochondrial stress genes in male (donor SMC) and (B) female (donor HU8406) hepatocytes. *n* = 2-3 per group. * p<0.05, ** p<0.01, **** p<0.0001 via two-way ANOVA.

Supplementary Figure 8



Supplementary Fig. 8. Multiphoton label-free images reveal metabolic response to resmetirom. Condition 1 (C1), Condition 2 (C2), Condition 1 + resmetirom and Condition 2 + resmetirom were imaged using multiphoton label-free microscopy. Third harmonic generation (THG; Lipids), three-photon autofluorescence (3PAF; NAD(P)H), and two-photon autofluorescence (2PAF; FAD). Scale bar: 50 μm. All data acquired from male SMC donor.