Additive hepatoprotective effects of semaglutide and resmetirom combination therapy in the GAN diet-induced obese and biopsy-confirmed mouse model of MASH

Authors
Kristoffer Voldum-Clausen[1], Jenny Norlin[2], Line Wendt Nilsson[1], Denise Oró[1], Grzegorz Maciag[1], Sanne Skovgård Veidal[2], Henrik H. Hansen[1], Michael Feigh[1].
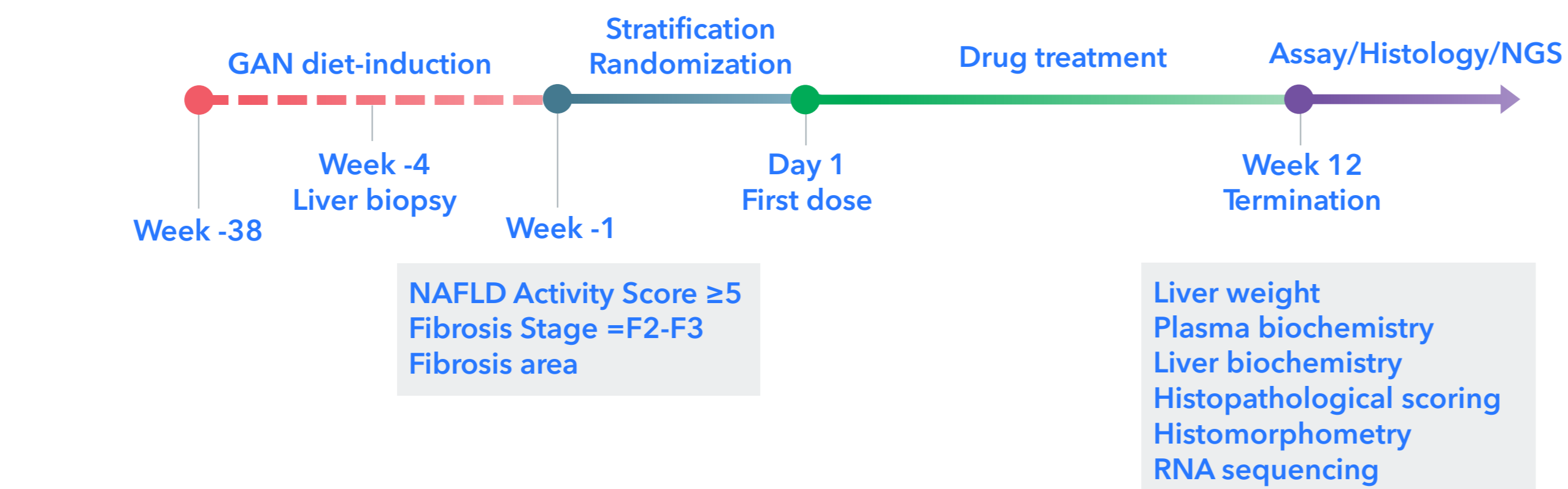
[1] Gubra, Hørsholm, Denmark
[2] Novo Nordisk, Måløv, Denmark

Corresponding author
Michael Feigh – mfe@gubra.dk

Background & Aim

Resmetirom (THR-β receptor agonist) has recently been approved for the treatment of metabolic dysfunction-associated steatohepatitis (MASH) and semaglutide (GLP-1R agonist) is in late-stage clinical development for treatment of MASH. The present study aimed to characterize metabolic, biochemical, histological and transcriptomic outcomes of resmetirom and semaglutide combination treatment in the translational GAN diet-induced obese (DIO) and biopsy-confirmed mouse model of MASH with liver fibrosis.

1 Study outline

Group	Animal	Gender	Number of animals	Treatment	Administration route	Dosing frequency	Dosing concentration
1	LEAN-CHOW	Male	10	Vehicle	SC	QD	NA
2	DIO-MASH	Male	18	Vehicle	SC	QD	NA
3	DIO-MASH	Male	18	Semaglutide	SC	QD	30 nmol/kg
4	DIO-MASH	Male	18	Resmetirom	PO	QD	3 mg/kg
5	DIO-MASH	Male	17	Semaglutide + Resmetirom	SC + PO	QD + QD	30 nmol/kg + 3 mg/kg

Figure 1. Study outline. PO; per oral, SC; subcutaneous, QD; once daily, NA; not applicable, GAN; Gubra Amylin NASH, NGS; next generation sequencing.

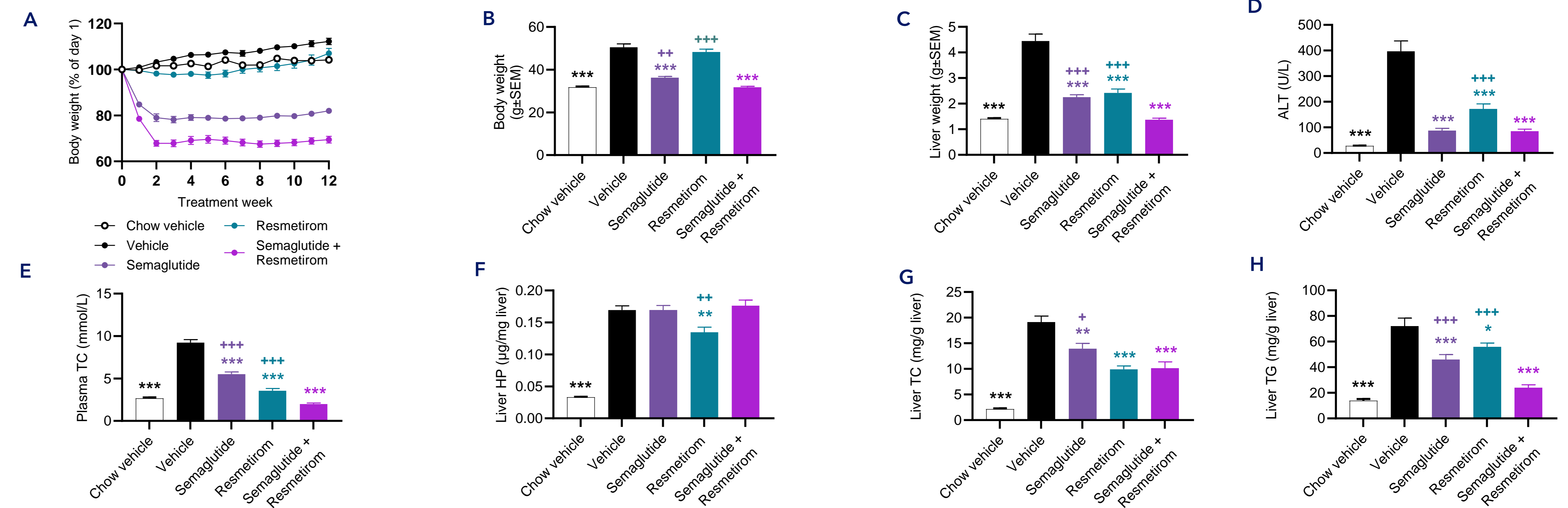## 2 Metabolic and biochemical parameters

Figure 2. Semaglutide + Resmetirom combo treatment reduced weight loss, liver weight and biochemical parameters superior to mono treatments. (A) Relative body weight during study period. (B) Terminal body weight. (C) Terminal liver weight. (D) Terminal plasma alanine aminotransferase (ALT). (E) Terminal plasma total cholesterol. (F) Liver hydroxyproline (HP). (G) Terminal liver total cholesterol (TC). (H) Liver triglycerides (TG).*p<0.05, **p<0.01, ***p<0.001 compared to Vehicle. +p<0.05, ++p<0.01, +++p<0.001 compared to semaglutide + resmetirom combo treatment (Dunnett's test one-factor linear model).

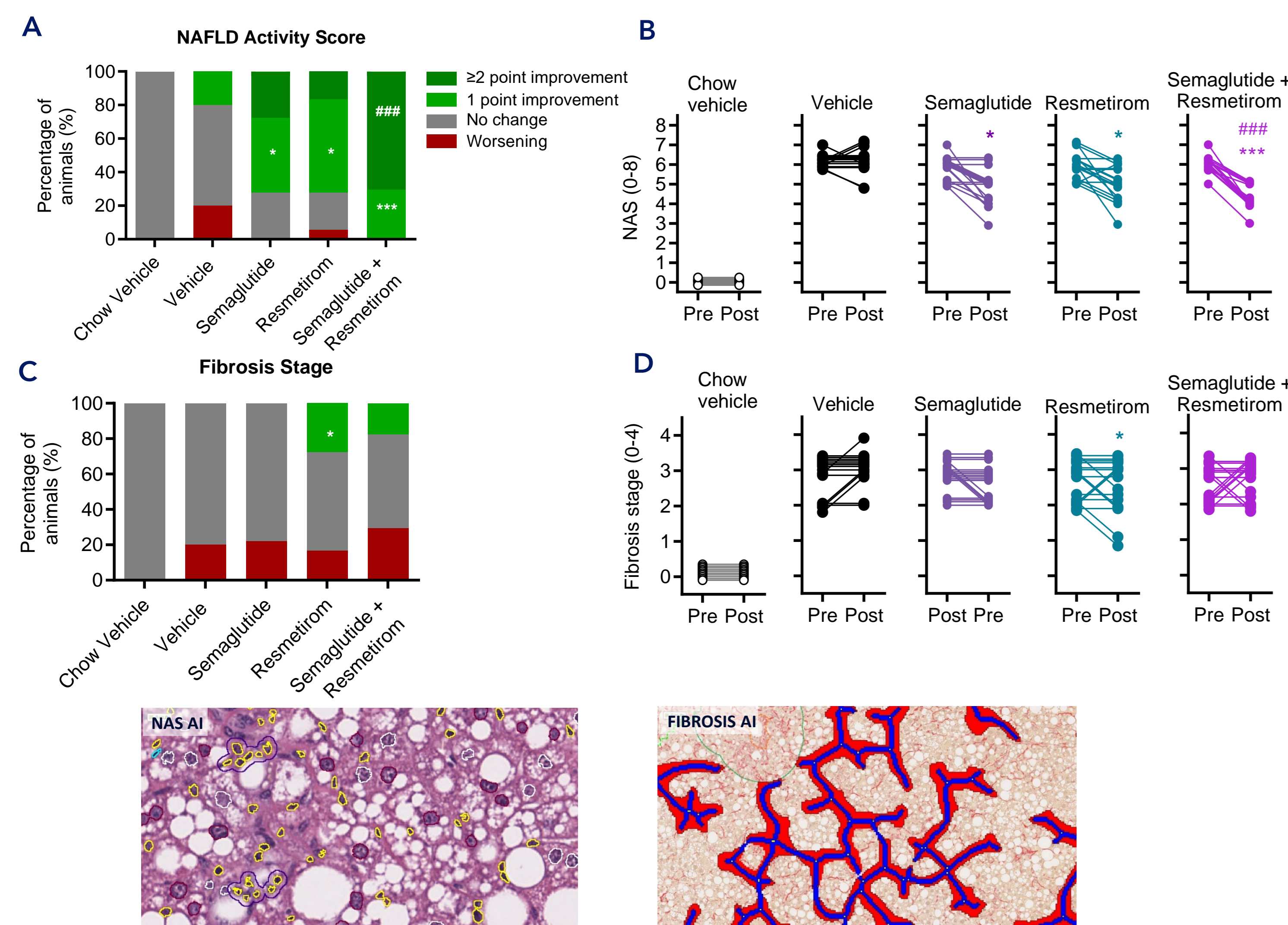## 3 NAFLD Activity Score and Fibrosis Stage

NAS AI

FIBROSIS AI

Figure 3. Semaglutide + Resmetirom combo treatment improved NAFLD Activity Score superior to mono treatments. Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. (A) NAFLD Activity Score (NAS). (B) Individual pre-post NAS. (C) Fibrosis stage. (D) Individual pre-post fibrosis stage. *p<0.05, ***p<0.001, and ### p<0.001 compared to vehicle (One-sided Fisher's exact test).

4 Histological markers of steatosis, inflammation and fibrosis
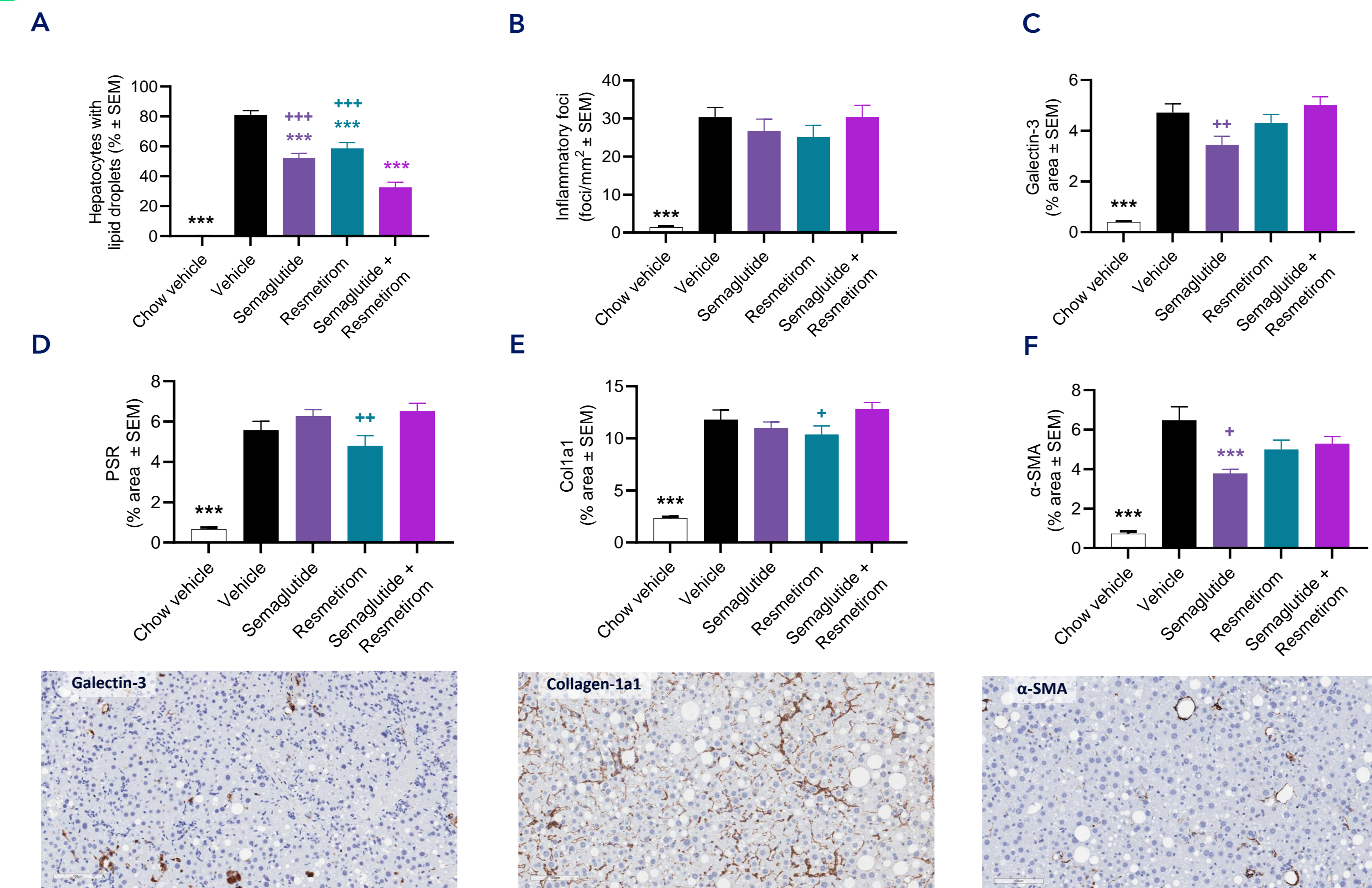

Galectin-3

Collagen-1a1

α-SMA

Figure 4. Semaglutide + Resmetirom combo treatment improved quantitative histological marker of steatosis superior to mono treatments. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables (panels A-B) and conventional IHC image analysis (panels C-F). (A) % hepatocytes with lipid droplets. (B) Number of inflammatory foci. (C) % area of galectin-3. (D) % area of PSR. (E) % area of collagen-1a1 (Col1a1). (F) % area of alpha-smooth muscle actin (α-SMA, marker of stellate cell activation). Mean ± SEM. *p<0.05, ***p<0.001 compared to vehicle. +p<0.05, ++p<0.01, +++p<0.001 compared to semaglutide + resmetirom combo treatment (Dunnett's test one-factor linear model). Bottom panels: Representative photomicrographs of galectin-3, α-SMA and Col1a1 (scale bar, 100 μm).

5 Hepatic transcriptomic profile
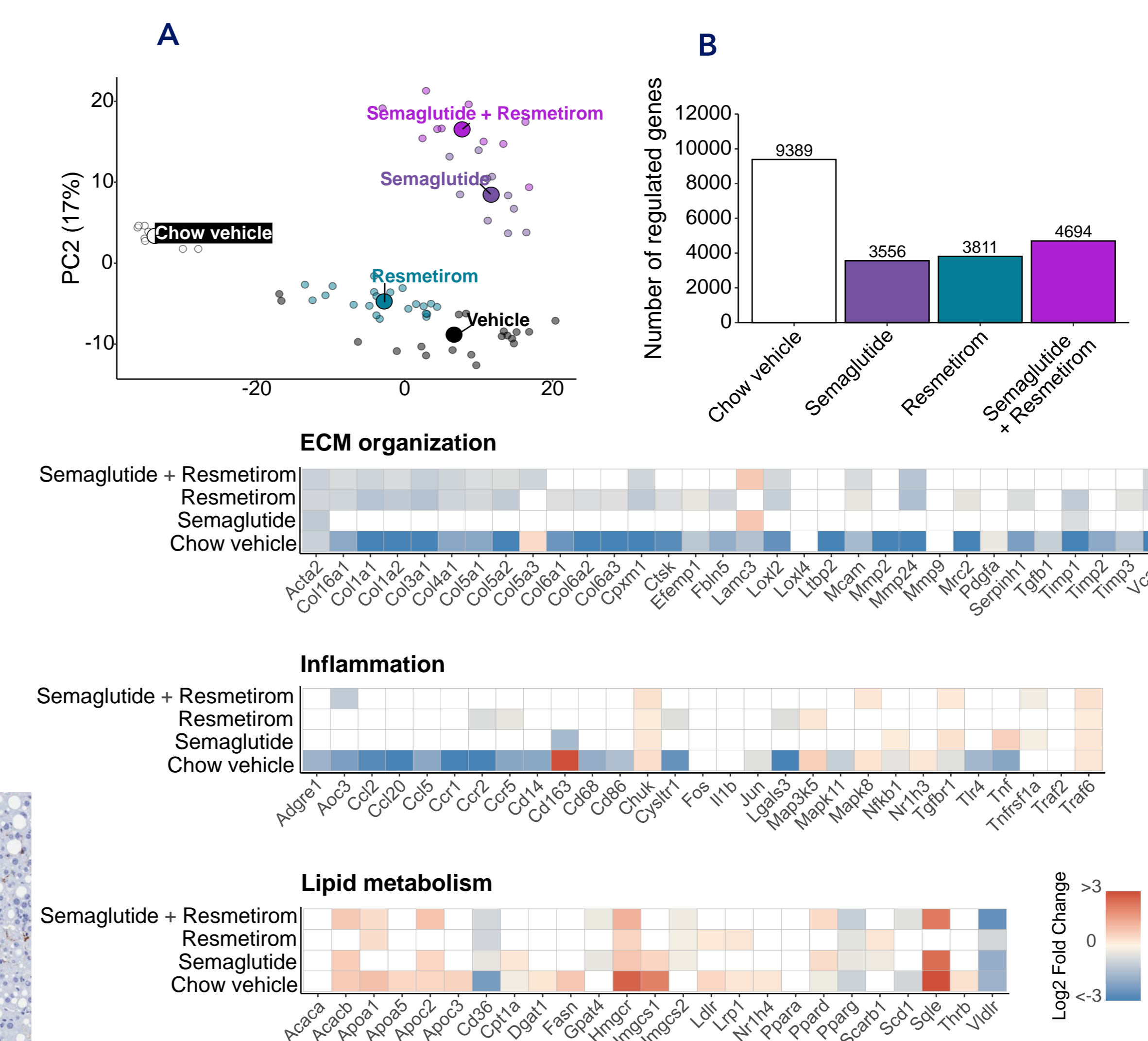

ECM organization

Inflammation

Lipid metabolism

Figure 5. Semaglutide + Resmetirom combo treatment increased number of differentially expressed genes (A) Principal component analysis (PCA) of samples based on top 500 most variable gene expression levels. (B) Differentially expressed genes. (C) Regulation of hepatic extracellular matrix (ECM), inflammation and lipid metabolism candidate genes (log2-fold change compared to corresponding DIO-NASH vehicle control mice). Blue and red colour gradients indicate significantly (p<0.05) down-regulated and up-regulated gene expression, respectively. White boxes indicate genes not significantly regulated (p>0.05).

Conclusion

+ Semaglutide + Resmetirom combo treatment promote superior weight loss to mono treatments.

+ Semaglutide + Resmetirom combo treatment improved hepatomegaly and plasma/liver lipids superior to mono treatments.

+ Semaglutide + Resmetirom combo treatment improved NAS (2-point) superior to mono treatments.

+ Semaglutide + Resmetirom combo treatment reduces steatosis superior to mono treatments.

+ Only resmetirom mono treatment reduces markers of fibrosis and improves fibrosis stage.

+ Semaglutide + Resmetirom combo treatment amplified hepatic transcriptomic profile.

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