Hepatoprotective effects of the oral fatty acid synthase inhibitor TVB-3664 in the GAN diet-induced obese and biopsy-confirmed mouse model of MASH

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Background & Aim

Fatty acid synthase (FASN) inhibitors is a promising drug class for the treatment of metabolic dysfunction-associated steatohepatitis (MASH). Accordingly, the oral FASN inhibitor denifanstat (TVB-2640) has recently been reported to improve NAFLD Activity Score (NAS) and fibrosis stage in a phase 2b clinical trial (FASCINATE-2) in MASH patients. The present study aimed to investigate the effect of TVB-3664, an oral FASN inhibitor, on metabolic, biochemical and histological endpoints in the translational Gubra Amylin NASH (GAN) diet-induced obese and biopsy-confirmed mouse model of MASH with liver fibrosis.
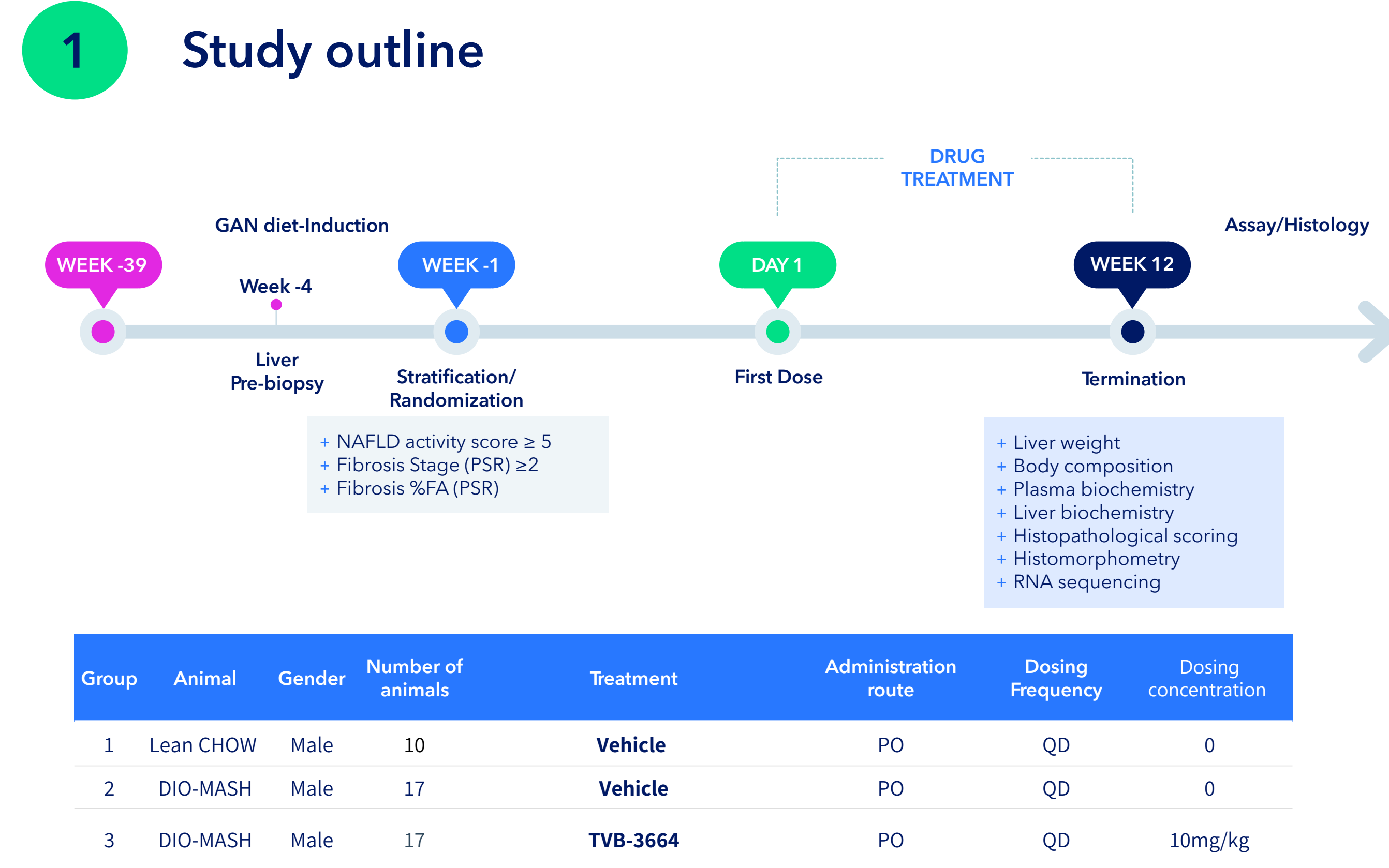
1 Study outline

Group	Animal	Gender	Number of animals	Treatment	Administration route	Dosing Frequency	Dosing concentration
1	Lean CHOW	Male	10	**Vehicle**	PO	QD	0
2	DIO-MASH	Male	17	**Vehicle**	PO	QD	0
3	DIO-MASH	Male	17	**TVB-3664**	PO	QD	10mg/kg

Figure 1. Study outline. PO; per oral, QD; Once daily; GAN; Gubra Amylin NASH.
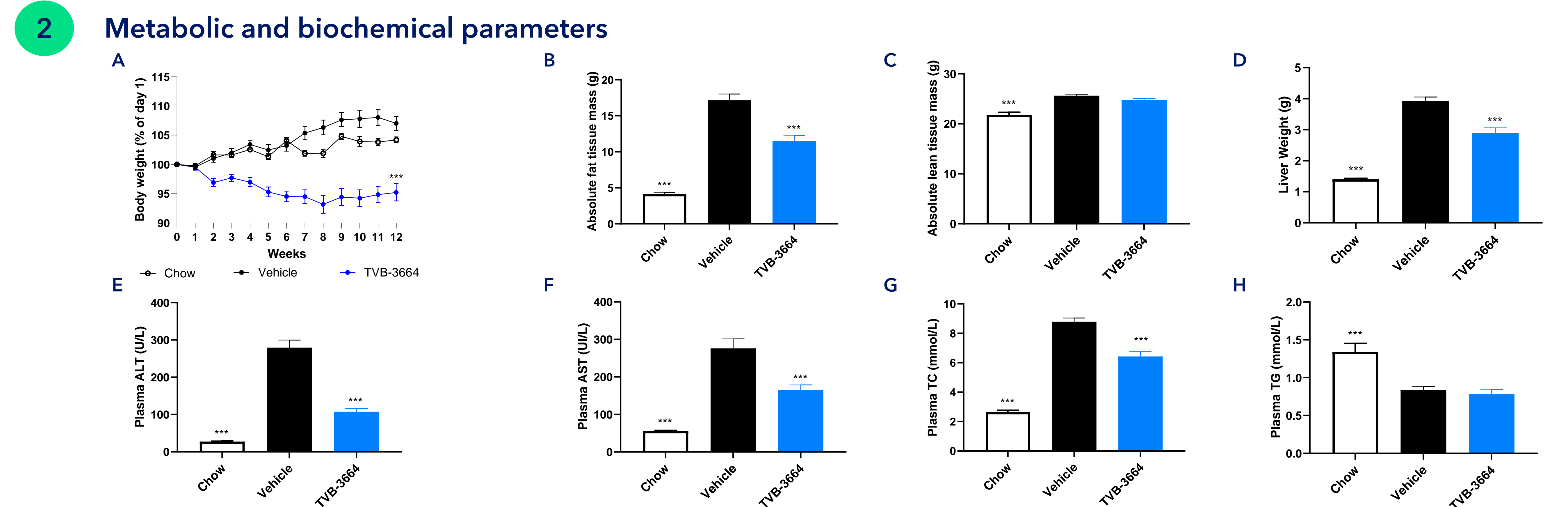
2 Metabolic and biochemical parameters

Figure 2. Metabolic and biochemical effects of TVB-3664. (A) Relative body weight during study period. **(B)** Terminal absolute fat tissue mass. **(C)** Terminal absolute lean tissue mass. **(D)** Terminal liver weight. **(E)** plasma alanine aminotransferase (ALT). **(F)** Terminal plasma aspartate aminotransferase (AST). **(G)** Terminal plasma total cholesterol (TC). **(H)** Terminal plasma triglycerides (TG).***p<0.001 compared to vehicle control (Dunnett's test one-factor linear model).

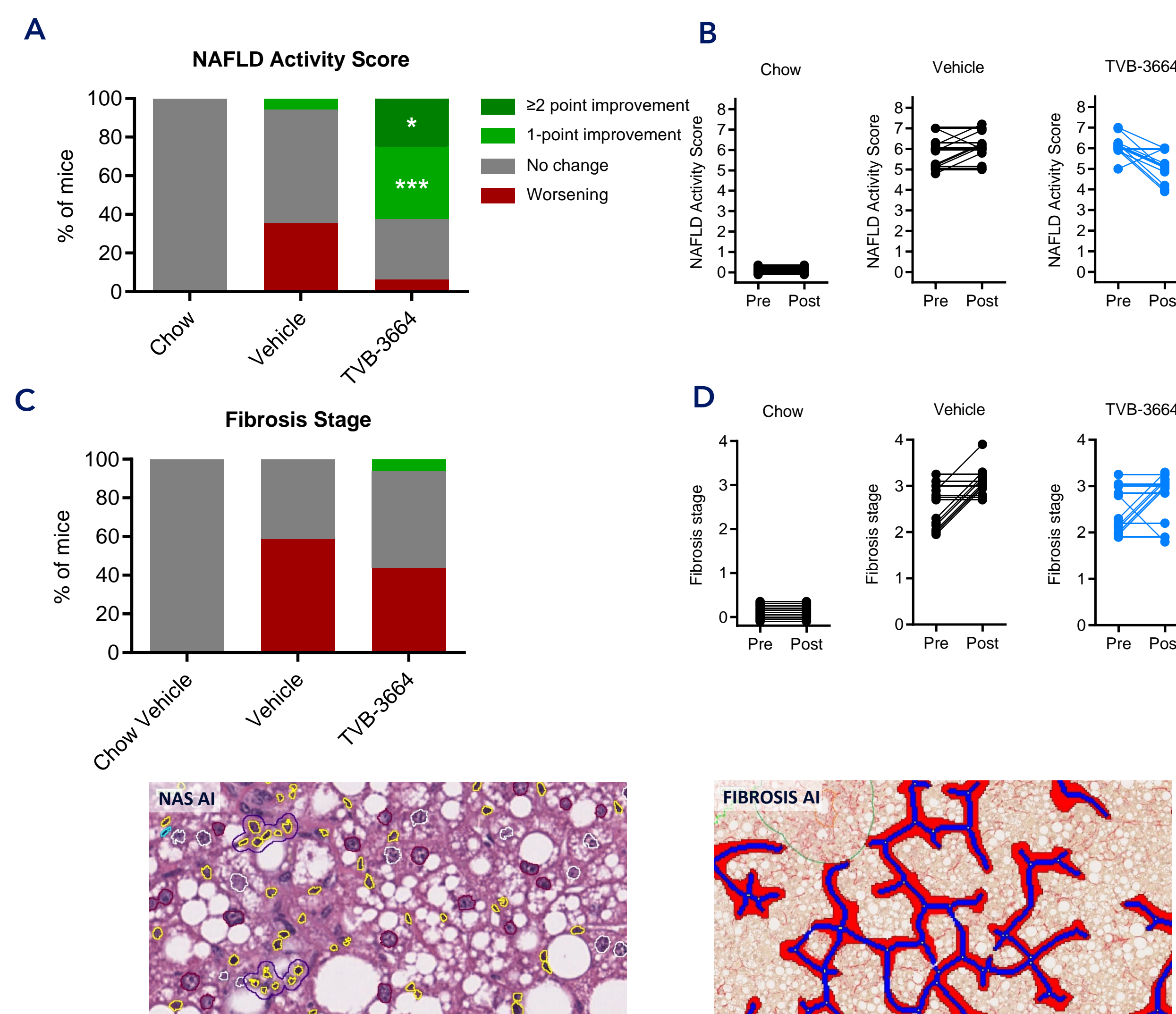## 3 NAFLD Activity Score and Fibrosis Stage

Figure 3. TVB-3664 improves NAFLD Activity Score in GAN DIO-MASH mice. 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 compared to vehicle control (one-sided Fisher's exact test).

4 Histological markers of steatosis, inflammation and fibrosis
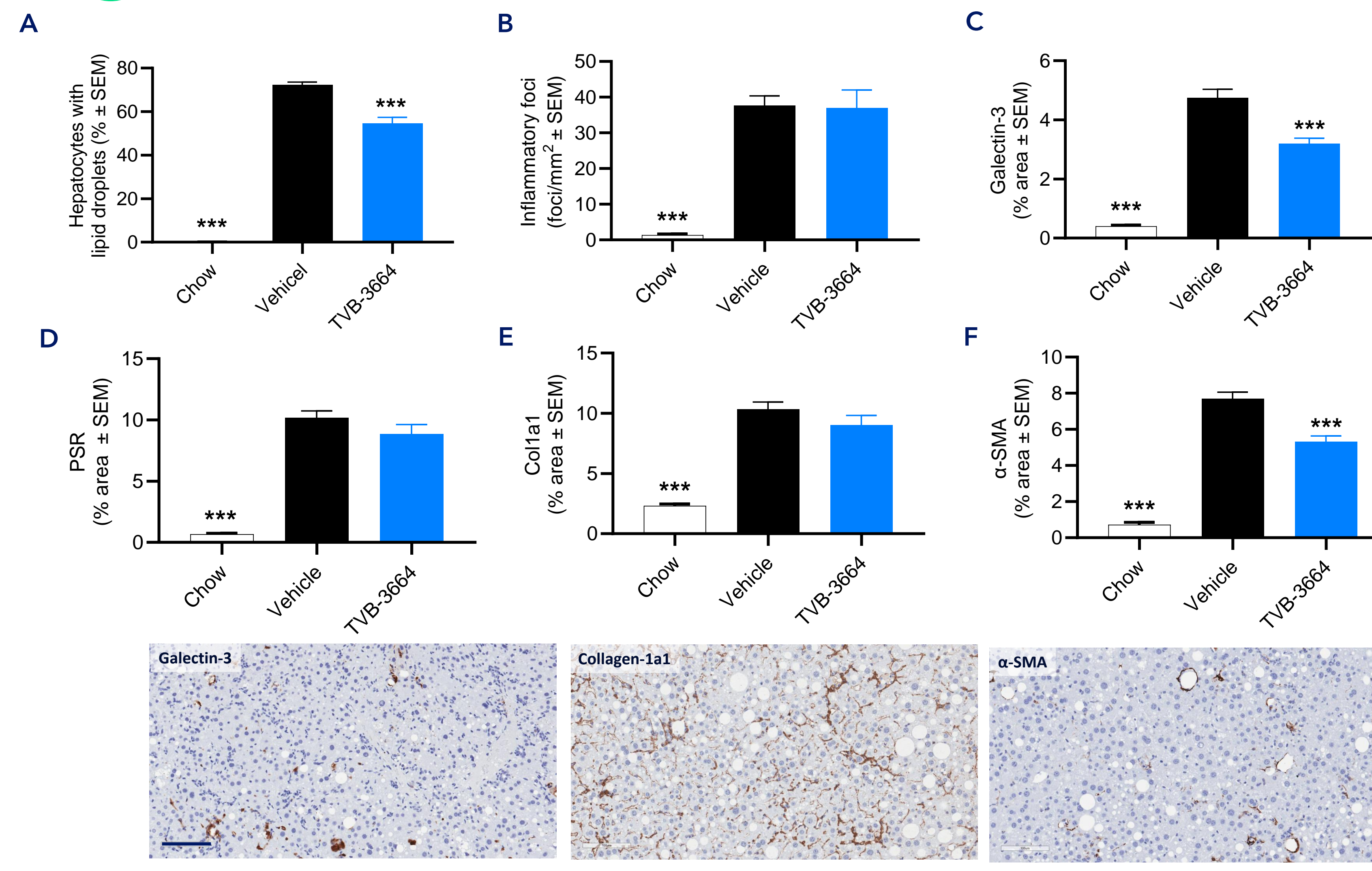

Figure 4. TVB-3664 improves quantitative histological endpoints. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables (panels A-B) and conventional IHC image analysis (panels C-F). **(A)** % hepatocytes with lipid droplets. **(B)** Number of inflammatory foci. **(C)** % area of galectin-3 (steatosis-adjusted). **(D)** % area of PSR (steatosis-adjusted). **(E)** % area of collagen-1a1 (Col1a1, steatosis-adjusted) **(F)** % area of alpha-smooth muscle actin (α-SMA, marker of stellate cell activation; steatosis-adjusted). ***p<0.001 compared to vehicle control (Dunnett's test one-factor linear model). Bottom panels: Representative photomicrographs of galectin-3, Col1a1 and α-SMA (scale bar, 100 μm).
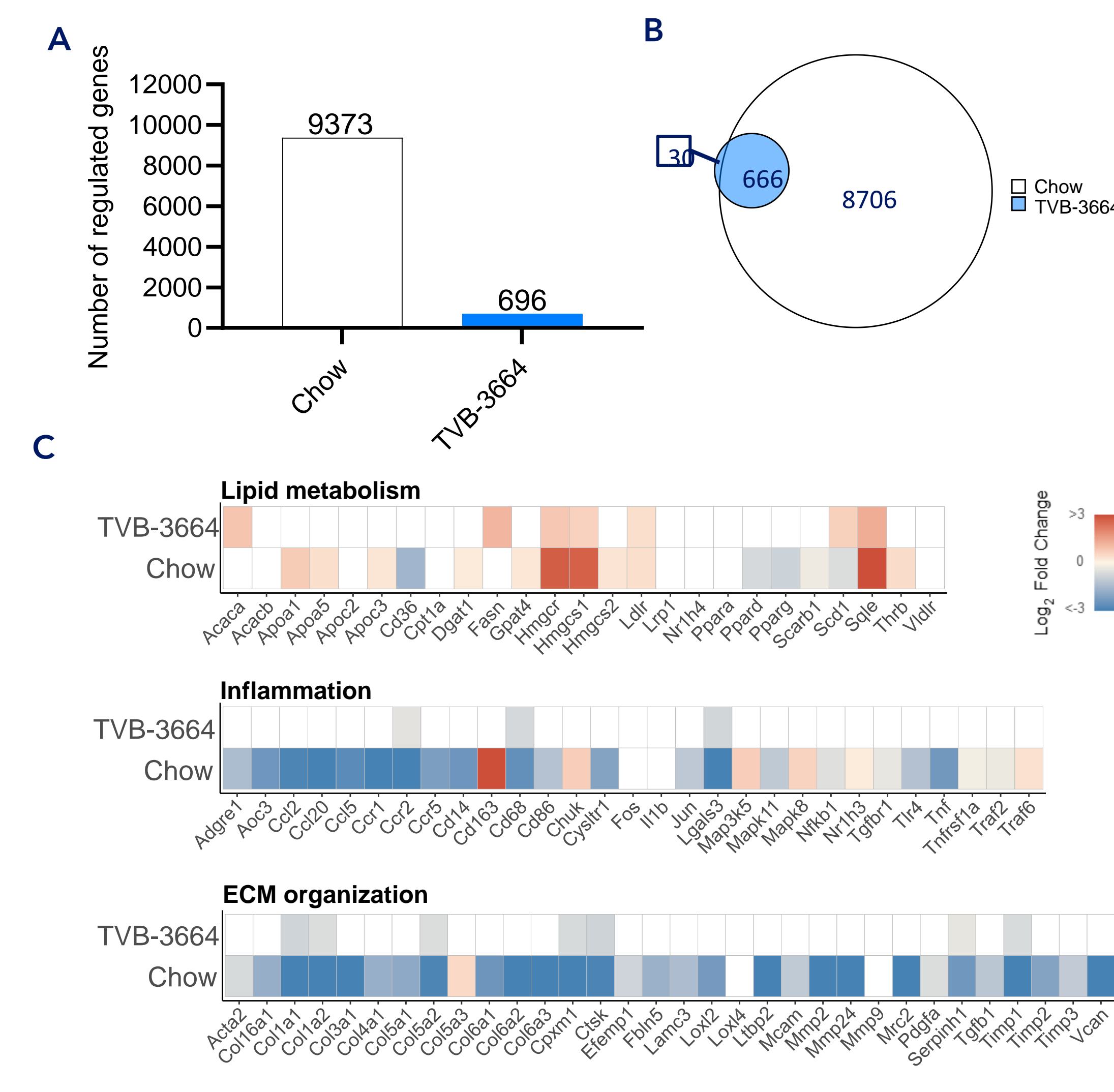
5 Liver transcriptome analysis

Figure 5. Hepatic transcriptome signatures following TVB-3664 therapy. (A) Total number of differentially expressed genes. **(B)** Venn diagram on overlapping gene expression signatures. **(C)** Regulation of gene expression markers of hepatic lipid metabolism. Log2 fold change is indicated for significantly regulated genes (p_adj < 0.05 after correcting for multiple testing). Red and blue colours indicate up- and down-regulation, respectively, compared to Vehicle.

Conclusion

+ TVB-3664 improves body weight and adiposity, while conserving lean tissue mass.

+ TVB-3664 improves hepatomegaly, plasma liver enzymes and cholesterol levels.

+ TVB-3664 improves NAFLD Activity Score, but not Fibrosis Stage.

+ Benefits on NAS are supported by quantitative histological markers for steatosis and inflammation.

+ TVB-3664 reduces quantitative histological marker for fibrogenesis.

+ TVB-3664 minimally regulates hepatic transcriptomic profile, predominantly improving gene expression markers of hepatic lipid metabolism and fibrogenesis.

+ Longer treatment intervention might be needed for demonstrating anti-fibrotic action for TVB-3664 in GAN DIO-MASH mouse model.