Prophylactic and therapeutic hepatoprotective effects of lanifibranor in the CDAA-HFD mouse model of advanced NASH with progressive fibrosis

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Background & Aim

The pan peroxisome proliferator-activated receptor (PPAR-α/δ/ɣ) agonist has recently been reported to improve liver histological outcomes in patients with non-alcoholic steatohepatitis (NASH) and fibrosis (NATIVE study; Francque et al, NEJM, 2021). Lanifibranor is currently in phase-3 clinical trial (NATiV3) for the treatment of NASH. The present study aimed to evaluate prophylactic vs. therapeutic intervention with lanifibranor in the non-obese choline-deficient L-amino-acid defined high-fat diet (CDAA-HFD) mouse model of advanced NASH with progressive fibrosis.

Methods

C57BL/6JRj mice were fed chow or CDAA-HFD (45 kcal% fat, 0.1% methionine, 1% cholesterol, 28 kcal% fructose) for 3 or 6 weeks prior to treatment start (i.e. before or after onset of fibrosis, respectively). Animals were randomized into treatment groups based on body weight. A baseline group (n=12) was terminated at study start (3 and 6 weeks). CDAA-HFD fed mice (n=12 per group) received treatment (PO) with vehicle or lanifibranor (30 mg/kg) for 9 weeks (prophylactic, 12w on diet) or 8 weeks (therapeutic, 14w on diet). Chow-fed mice (n=8) served as normal controls. Terminal endpoints included plasma biomarkers [alanine/aspartate aminotransferase (ALT/AST), liver biochemistry, NAFLD Activity Score (NAS), fibrosis stage, quantitative liver histology and liver RNA sequencing.

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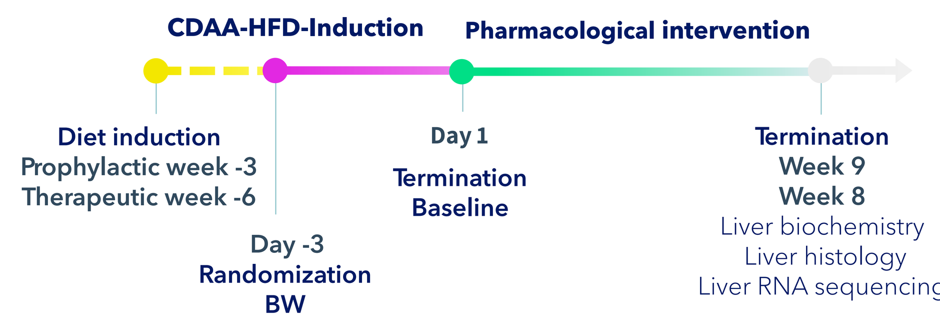
1 Study Outline



Group no.	Group	Name	Number of animals	Administration route	Dosing frequency	Dosing concentration
1	Chow	**Chow**	8	PO	QD	-
2	Baseline CDAA-HFD 3w	**Baseline 3w**	12	-	-	-
3	Vehicle CDAA-HFD 12w	**Vehicle 12w**	12	PO	QD	-
4	Lanifibranor CDAA-HFD 12w	**Lanifibranor 12w**	12	PO	QD	30mg/kg
5	Baseline CDAA-HFD 6w	**Baseline 6w**	12	-	-	-
6	Vehicle CDAA-HFD 14w	**Vehicle 14w**	12	PO	QD	-
7	Lanifibranor CDAA-HFD 14w	**Lanifibranor 14w**	12	PO	QD	30mg/kg

2 Metabolic and biochemical parameters

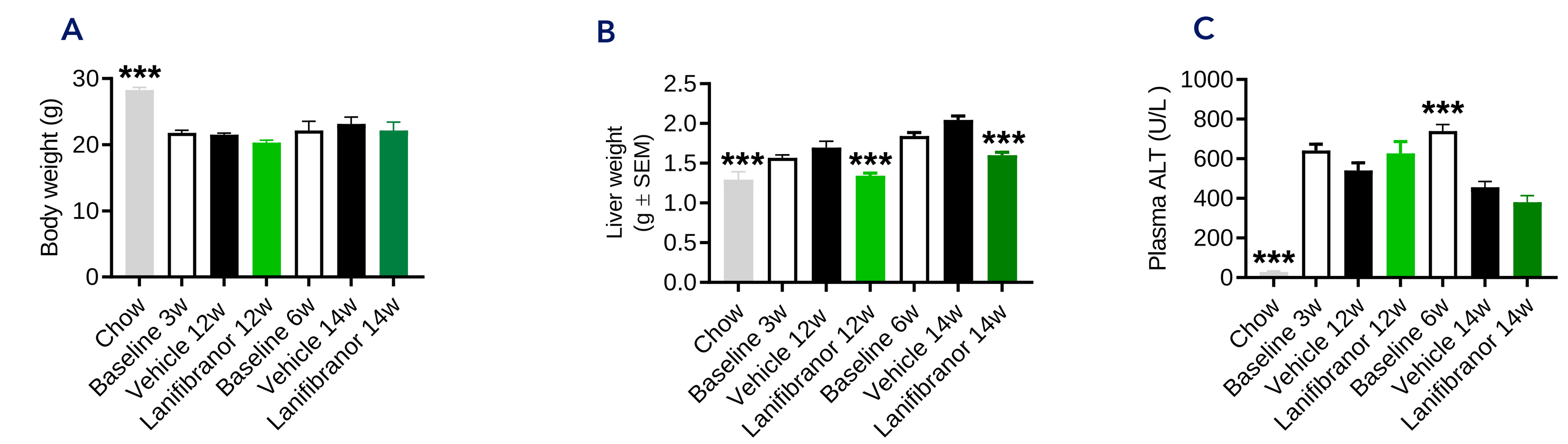
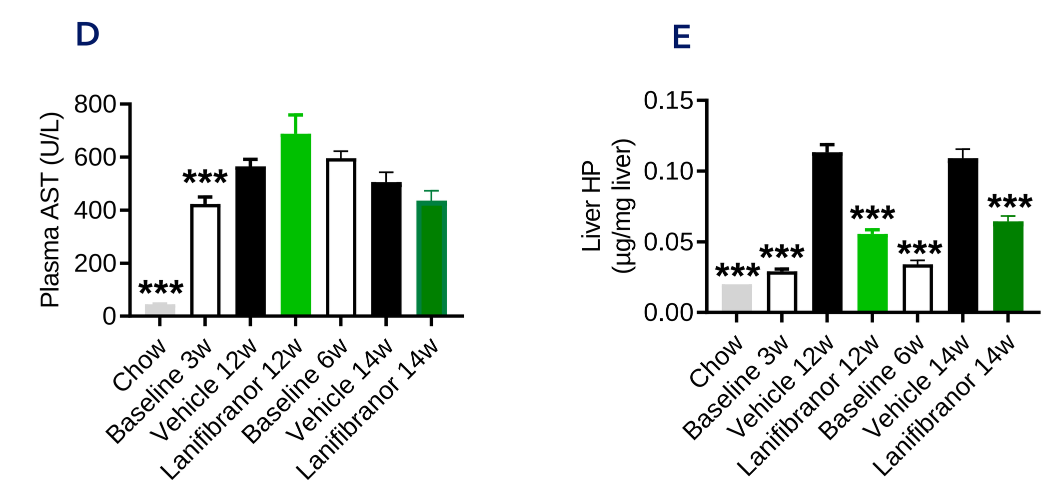


Figure 1. Prophylactic and therapeutic lanifibranor intervention improves hepatomegaly and liver hydroxyproline levels in CDAA-HFD mice. (A) Terminal body weight **(g) (B)** Terminal liver weight (g). **(C)** Terminal plasma alanine aminotransferase (ALT, U/L). **(D)** Terminal plasma aspartate aminotransferase (AST, U/L). **(E)** Terminal liver hydroxyproline (HP, µg/mg). ***p<0.001 compared to corresponding CDAA-HFD vehicle group (Dunnett's test one-factor linear model).



3 NAFLD Activity Score and Fibrosis Stage

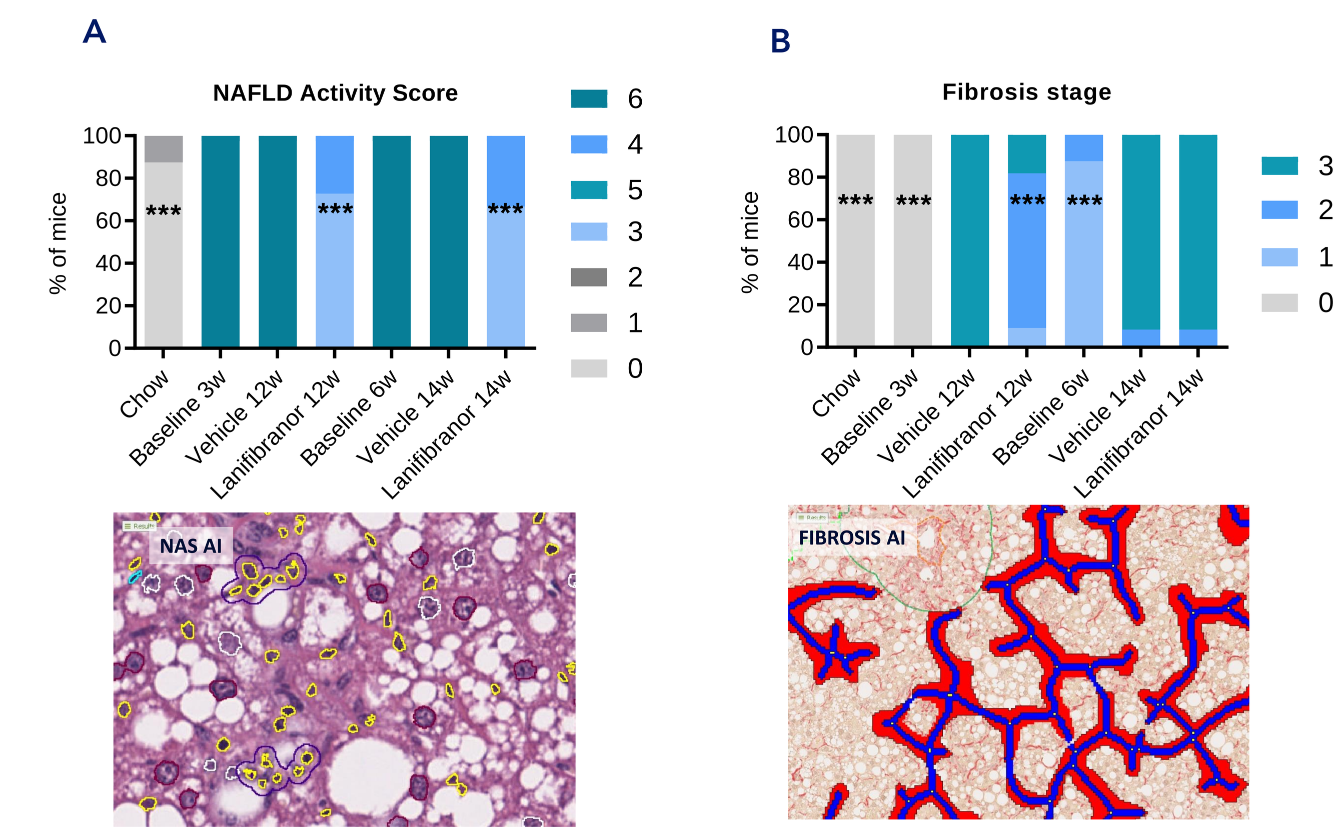


Figure 2. Prophylactic and therapeutic lanifibranor intervention improves NAFLD activity score and fibrosis stage (early-stage), in CDAA-HFD mice. Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A) NAFLD Activity Score (NAS). (B)** Fibrosis Stage. ***p<0.001 compared to corresponding CDAA-HFD vehicle group (One-sided Fisher's exact test with Bonferroni correction). Bottom panels: Representative HE and PSR photomicrographs used for GHOST evaluation.

4 Quantitative histological markers of steatosis, inflammation and fibrogenesis

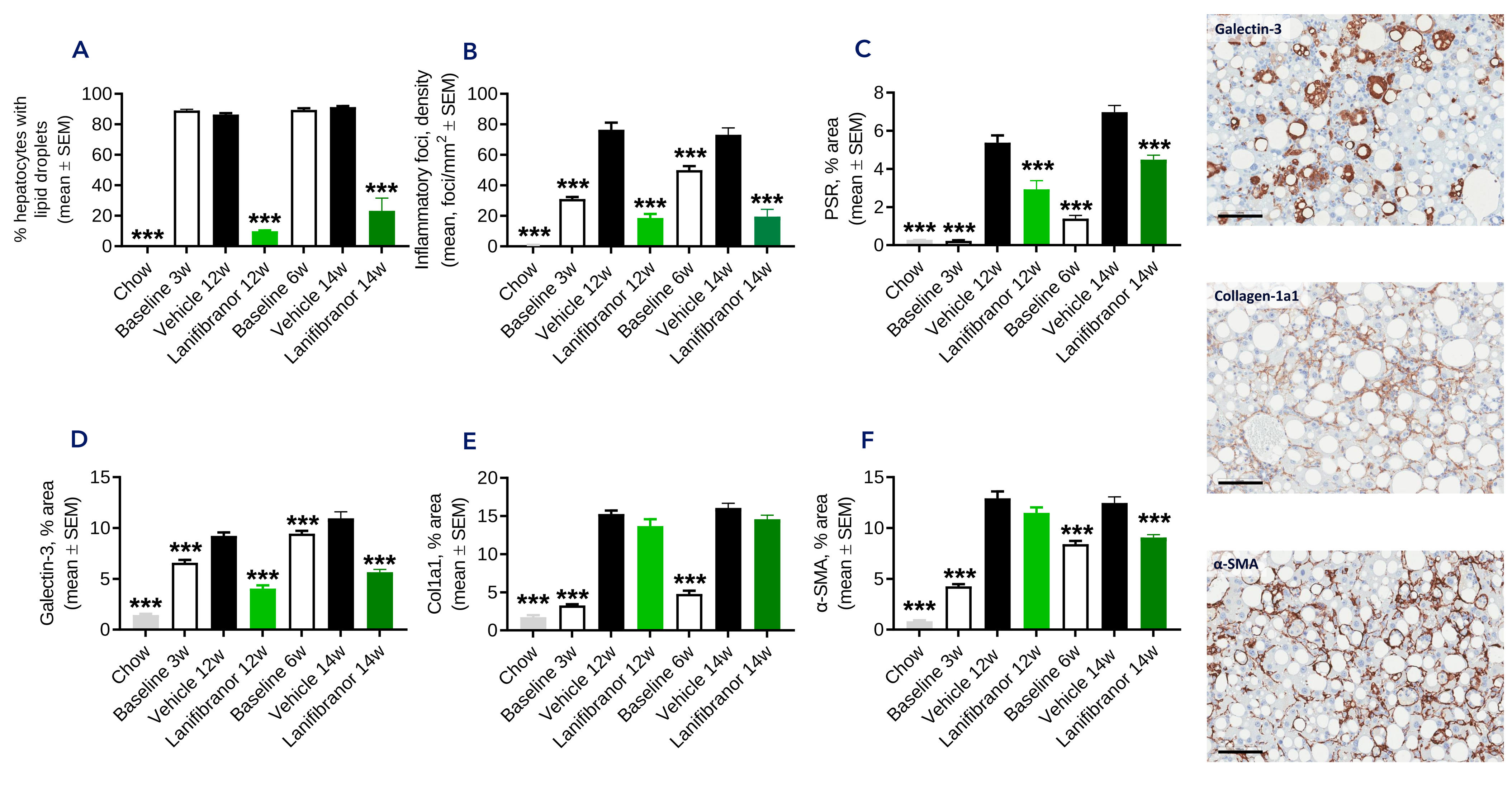


Figure 3. Prophylactic and therapeutic intervention improves quantitative histological markers of steatosis, inflammation and fibrogenesis in CDAA-HFD mice. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables and conventional IHC image analysis **(A)** % hepatocytes with lipid droplets. **(B)** Number of inflammatory foci. **(C)** % area of PSR. **(D)** % area of galectin-3. **(E)** % area of collagen-1a1. **(F)** % area of alpha-smooth muscle actin (α-SMA). Mean ± SEM. ***p<0.001 compared to corresponding CDAA-HFD vehicle group (Dunnett's test one-factor linear model). Right panels: Representative galectin-3, collagen 1a1 and α-SMA photomicrographs (scale bar, 100 µm).

5 Liver transcriptome profile

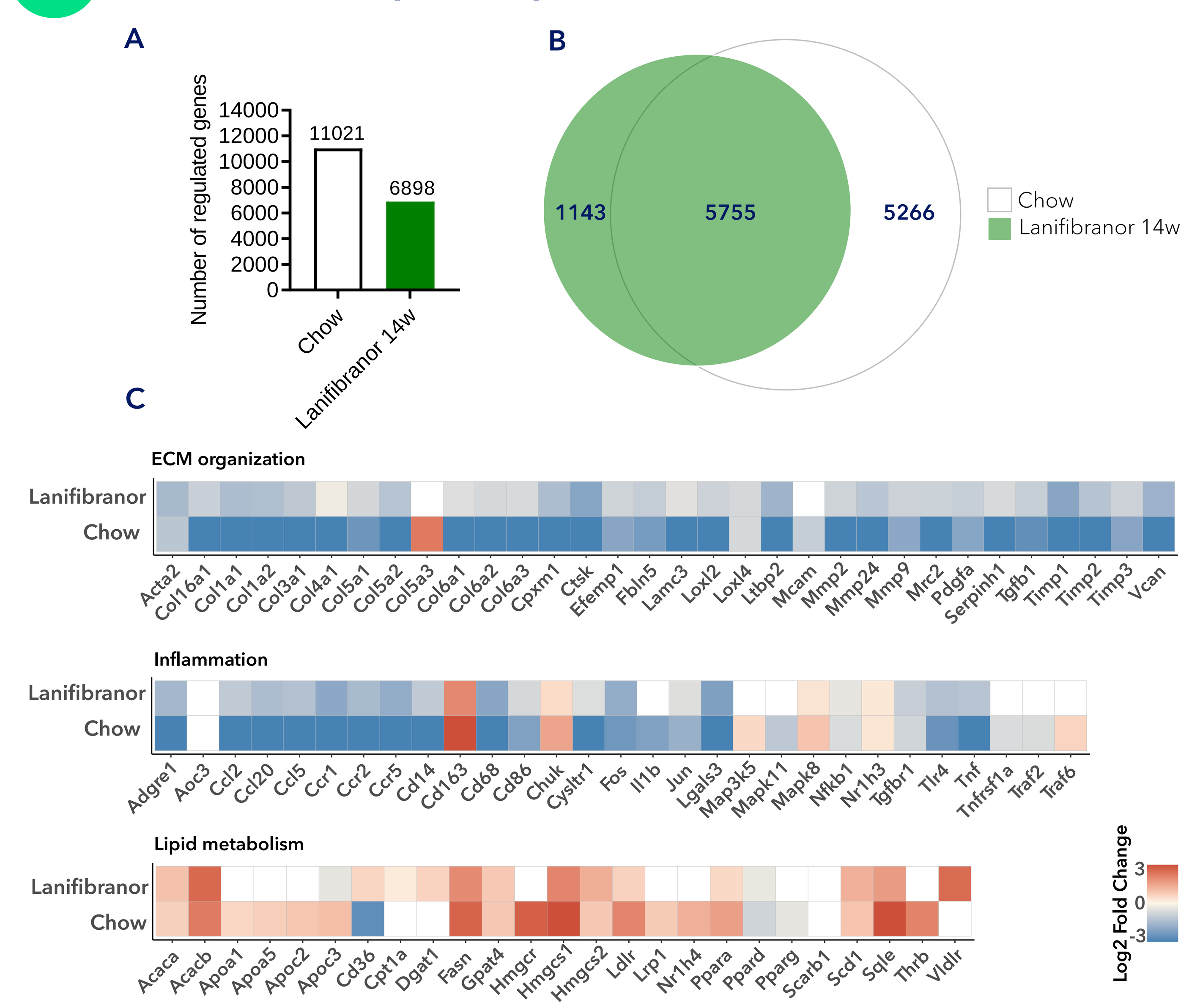


Figure 4. Therapeutic lanifibranor intervention robustly suppresses candidate gene expression markers of liver fibrosis, inflammation and lipid metabolism in CDAA-HFD mice. (A) Total number of differentially expressed genes compared to CDAA-HFD vehicle controls.**(B)** Visualization of the number of genes regulated by combinations of compounds compared to CDAA-HFD **(C)** Regulation of candidate genes associated with extracellular matrix (ECM organization), inflammation and lipid handling (log2-fold change compared to CDAA-HFD vehicle group). Color gradients indicate significantly (p<0.05) upregulated (red) and downregulated (blue) genes. White boxes indicate genes not significantly regulated (p>0.05).

Conclusion

Lanifibranor treatment outcomes in CDAA-HFD mice:

+ Prophylactic and therapeutic intervention reduces hepatomegaly, liver hydroxyproline levels, improves NAS and quantitative histological markers of NASH and fibrosis
+ Only prophylactic intervention also improves fibrosis score
+ Histological benefits are supported by transcriptome signatures of improved liver metabolism with reduced inflammation and fibrogenesis

These findings are in good agreement with clinical trial outcomes in NASH patients, highlighting the suitability of the CDAA-HFD mouse model for profiling novel drug therapies targeting advanced NASH with progressive fibrosis.