

Humanizing Dyslipidemia in MASH: A PCSK9-Driven Dyslipidemia GAN DIO-MASH Model

Authors

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Background & Aim

Dyslipidemia, characterized by hypercholesterolemia and hypertriglyceridemia, is a central component of metabolic dysfunction-associated steatohepatitis (MASH) pathogenesis. While being highly instrumental in drug development, preclinical models of MASH often fail to recapitulate a human-like lipoprotein profile. To enhance translational relevance, the present study aimed to establish a humanized dyslipidemia profile in the GAN diet-induced obese (DIO) mouse model of MASH through AAV-mediated PCSK9 overexpression.

Methods

Male C57BL/6 mice were fed the GAN diet (high fat, fructose, and cholesterol) for 39 weeks prior to study start. Only mice with biopsy-confirmed MASH (NAFLD Activity Score (NAS) ≥ 5) and fibrosis (stage F2-F3) were included and stratified into treatment groups (n=15-18 pr. group). One week a single intravenous AAV-PCSK9 (murine PCSK9) injection, GAN DIO-MASH mice were administered subcutaneously with vehicle, Semaglutide (GLP-1 receptor agonist, 30 nmol/kg, once daily), Efruxifermin (FGF21 analogue; EFX, 1 mg/kg, bi-weekly), or Alirocumab (human PCSK9 inhibitor; 10 mg/kg, once daily) for 16 weeks. Lean chow fed mice served as healthy controls, while GAN fed mice administered the empty AAV-CAG (CAG) served as negative control. Endpoints included plasma lipid biochemistry, and liver histology.

1 Study outline

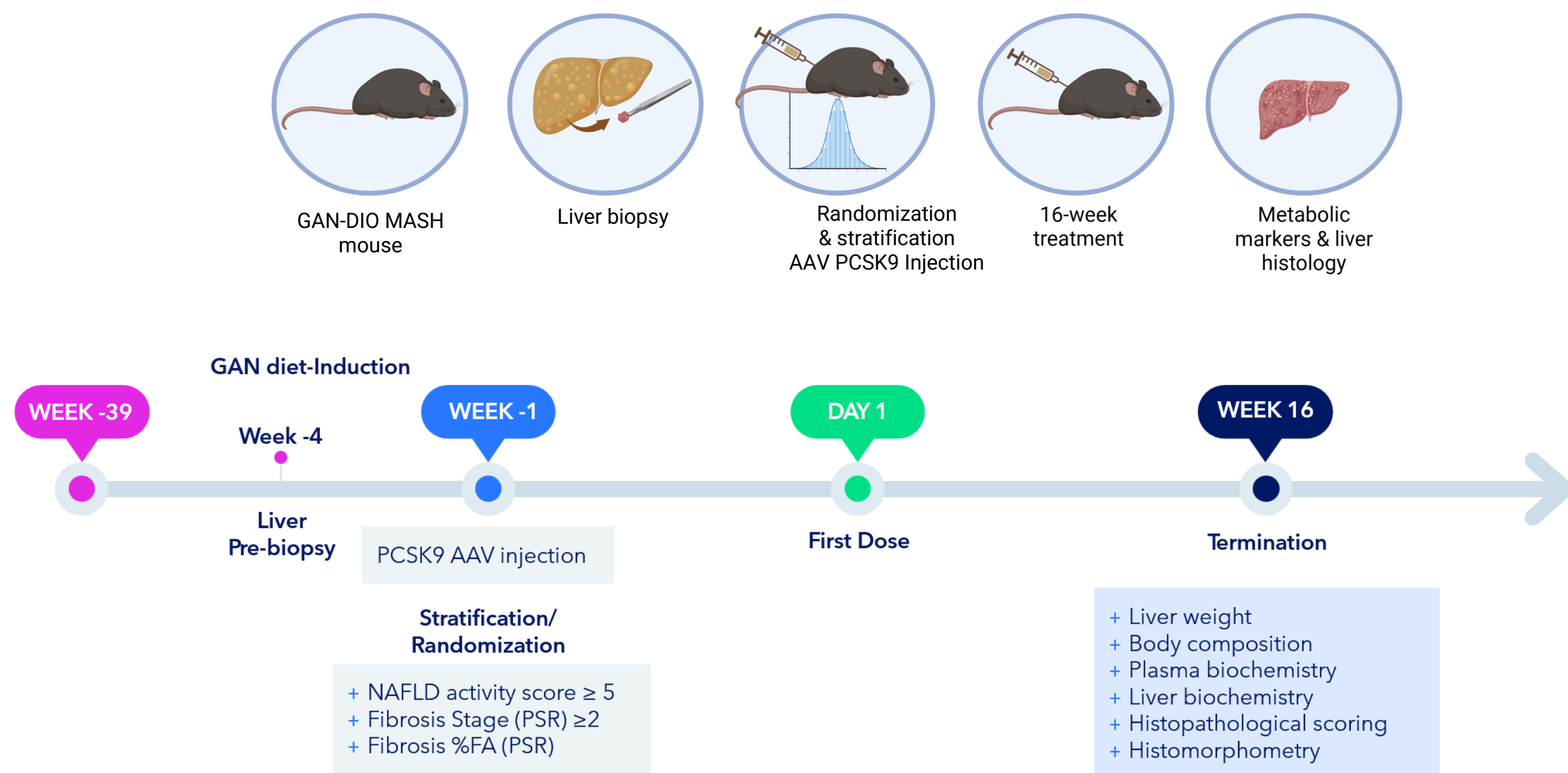


Figure 1. Study outline. %FA, % fractional area; GAN diet; Gubra Amylin NASH diet; NAFLD activity score, nonalcoholic fatty liver disease activity score (NAS); PCSK9, proprotein convertase subtilisin/kexin type 9; PSR, picrosirius red staining.

2 Metabolic and biochemical parameters

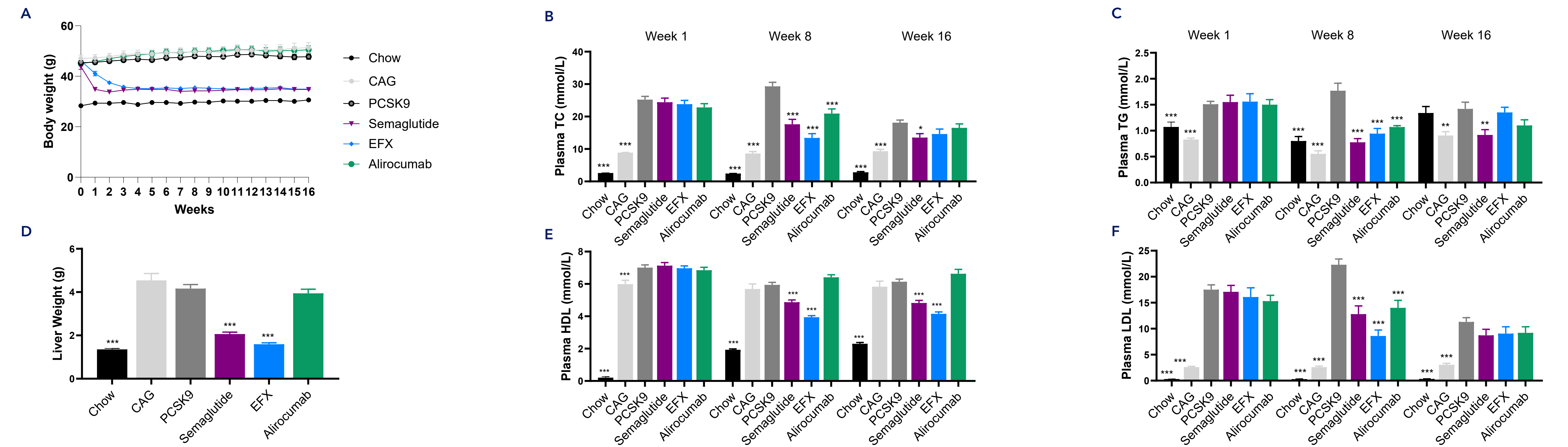


Figure 2. Metabolic and biochemical effects of PCSK9 AAV, Semaglutide, EFX and Alirocumab. (A) Body weight during study period. (B) Plasma total cholesterol (TC) at study week 1, 8, and 16 (terminal). (C) Terminal plasma triglycerides (TG), at study week 1, 8, and 16 (terminal). (D) Terminal liver weight. (E) Terminal plasma high density lipoprotein (HDL), at study week 1, 8, and 16 (terminal). (F) Terminal plasma high density lipoprotein (HDL), at study week 1, 8, and 16 (terminal). *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ compared to DIO-MASH PCSK9 (Dunnett's test one-factor linear model).

3 NAFLD Activity Score and Fibrosis Stage

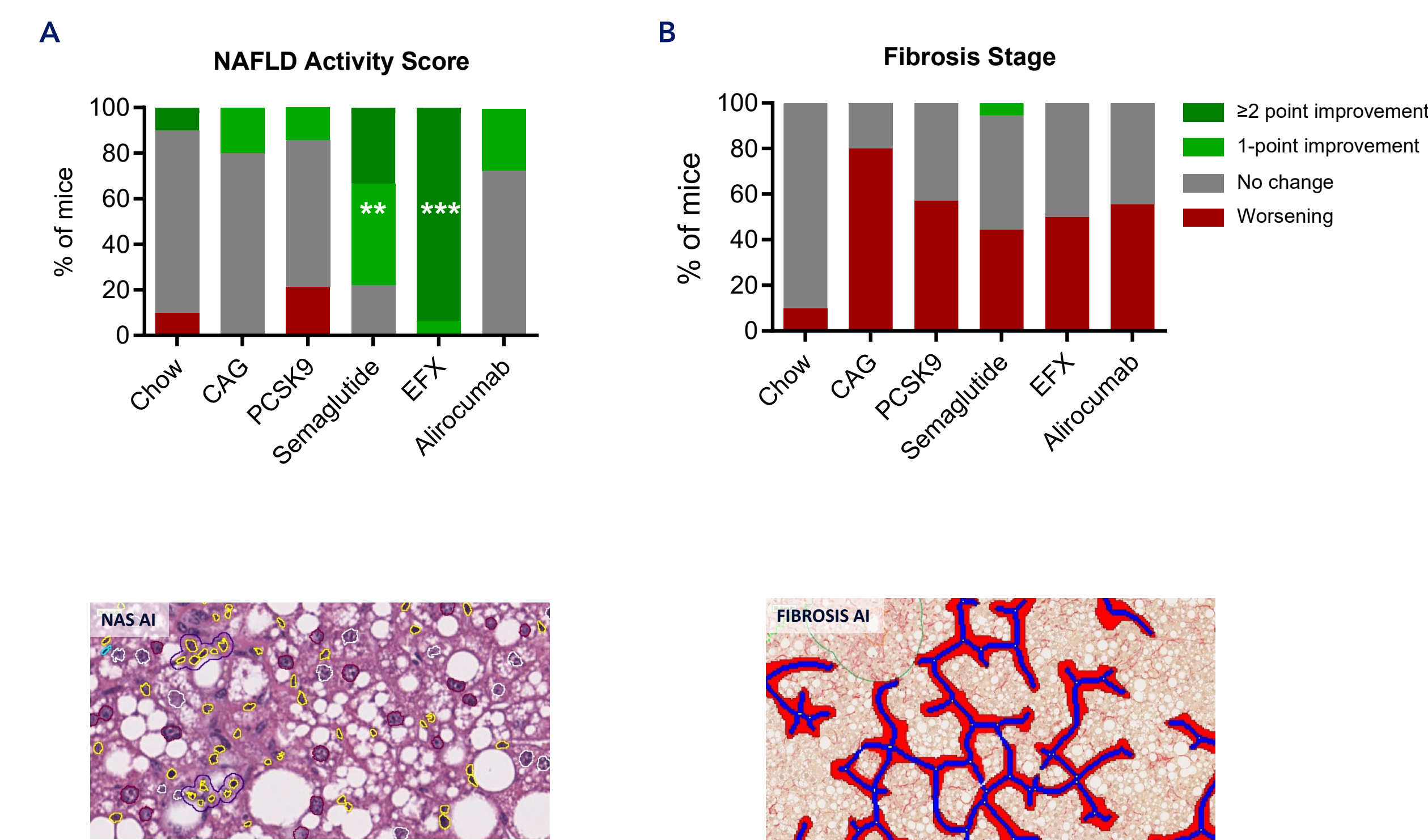


Figure 3. Semaglutide and Efruxifermin improves NAFLD Activity Score in PCSK9 GAN DIO-MASH mice. Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. (A) NAFLD Activity Score (NAS). (B) Fibrosis stage. ** $p < 0.01$, *** $p < 0.001$ compared to PCSK9 vehicle (one-sided Fisher's exact test).

4 Histological markers of steatosis, inflammation and fibrosis

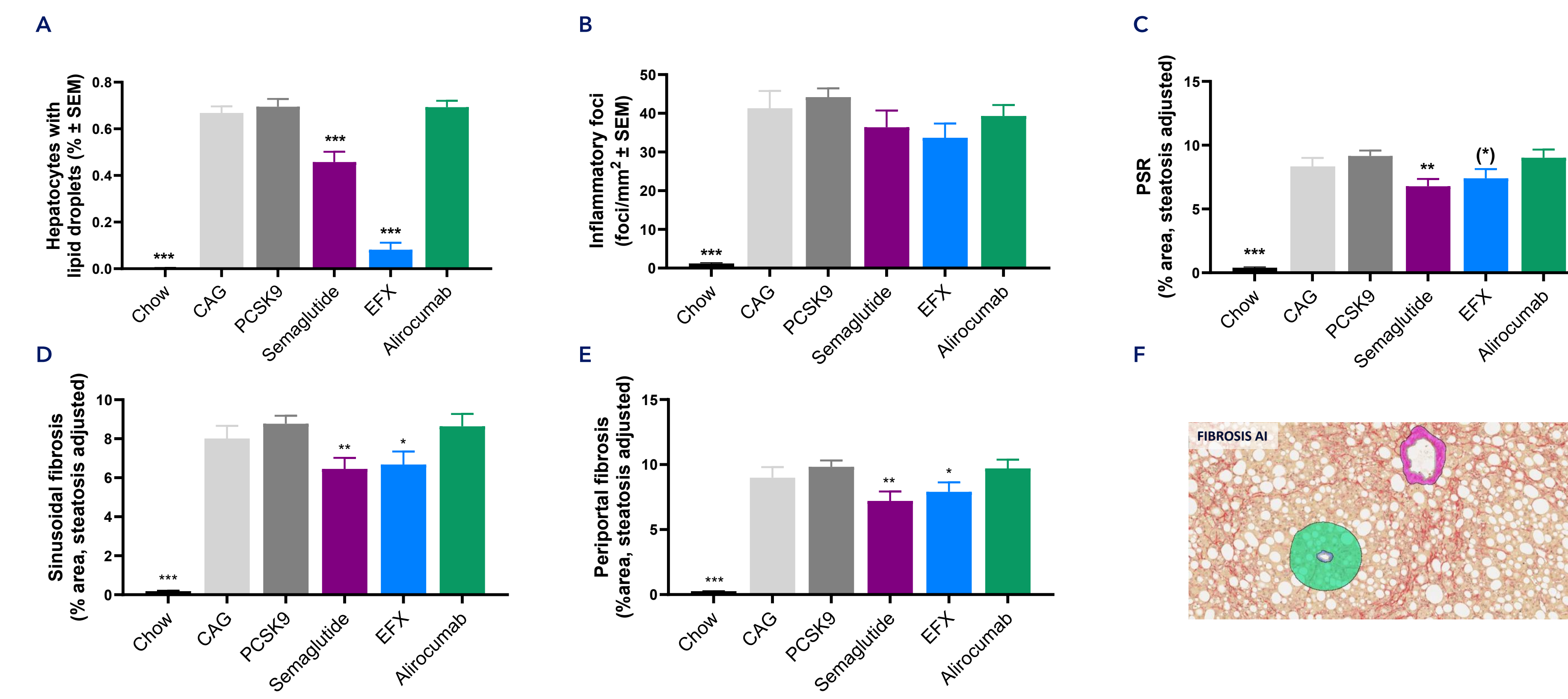


Figure 4. Semaglutide and Efruxifermin improves quantitative histological endpoints in PCSK9 DIO-MASH mice. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables (panels A-B) and conventional IHC image analysis (panels C-F). (A) % hepatocytes with lipid droplets. (B) Number of inflammatory foci. (C) % area of PSR (steatosis-adjusted). (D) % area of sinusoidal fibrosis, PSR (steatosis-adjusted). (E) % area of periportal fibrosis, PSR (steatosis-adjusted). (F) AI-assisted image analysis of fibrosis localization. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, and (*) $p < 0.1$ compared to PCSK9 vehicle. (Dunnett's test one-factor linear model).

Conclusion

- + The PCSK9 GAN DIO-MASH mouse combines advanced MASH pathology with a transient humanized dyslipidemic phenotype
- + PCSK9 overexpression does not change the histological phenotype of GAN DIO-MASH mice
- + Semaglutide, EFX, and alirocumab most consistently attenuated LDL-hypercholesterolemia and hypertriglyceridemia at week 8
- + Semaglutide and EFX, but not Alirocumab, improved NAFLD Activity Score as well as quantitative markers of steatosis, sinusoidal and periportal fibrosis
- + The PCSK9 GAN DIO-MASH mouse model provides a translational platform for evaluating both lipid-lowering and MASH-directed therapies within a single preclinical study setting

