

Semaglutide reverses clinically relevant biomarker alterations in the biopsy-confirmed GAN DIO-MASH mouse model

Authors
 Henrik H Hansen¹, Jacob Nøhr-Meldgaard¹, Jenny Norlin², Sanne S Veidal², Kristian Moss Bendtsen², Kristoffer Niss², Markus Latta², Susanne E Pors¹, Mathilde Teilmann Dalhoff¹, Kristoffer Voldum-Clausen¹

¹ Gubra, Hørsholm Kongevej 11B, Hørsholm, Denmark
² Research & Development, Novo Nordisk, Maaloev, Denmark

Corresponding author
 Henrik H Hansen - hbh@gubra.dk

Background & Aim

The long-acting glucagon-like peptide-1 (GLP-1) receptor agonist semaglutide was recently approved as the first GLP-1-based therapy for people with MASH and moderate-to-severe fibrosis. Clinical studies have demonstrated that semaglutide not only improves liver histology but also reverses key biomarker alterations associated with the disease. In this study, we characterized metabolic, histological, and biomarker responses to semaglutide in the Gubra Amylin NASH (GAN) diet-induced obese, biopsy-confirmed mouse model of MASH, and compared these findings with clinical observations.

Methods

Male C57BL/6 mice were fed the GAN diet, high in fat, fructose and cholesterol for 38 weeks prior to study start. A liver pre-biopsy was collected 4 weeks before treatment start. Only GAN DIO-MASH mice with NAFLD Activity Score (NAS ≥ 5) and fibrosis stage F2-F3 were included and stratified into treatment groups. Semaglutide (30 nmol/kg, n=16) or vehicle (n=15) was administered subcutaneously once daily for 12 weeks. Vehicle-dosed, chow-fed mice served as healthy controls (n=10). Endpoints included aptamer-based SomaScan plasma proteomics, blood/liver biochemistry, pre-to-post individual assessment of NAS and fibrosis stage, and quantitative histology.

Conclusion

- + 12-week treatment outcomes in the GAN DIO-MASH mouse closely mirror clinical findings, particularly for metabolic, hepatic steatosis and inflammation endpoints
- + The GAN DIO-MASH mouse model recapitulates many human MASH-associated changes in circulating proteins and semaglutide-responsive biomarkers

1 Study design

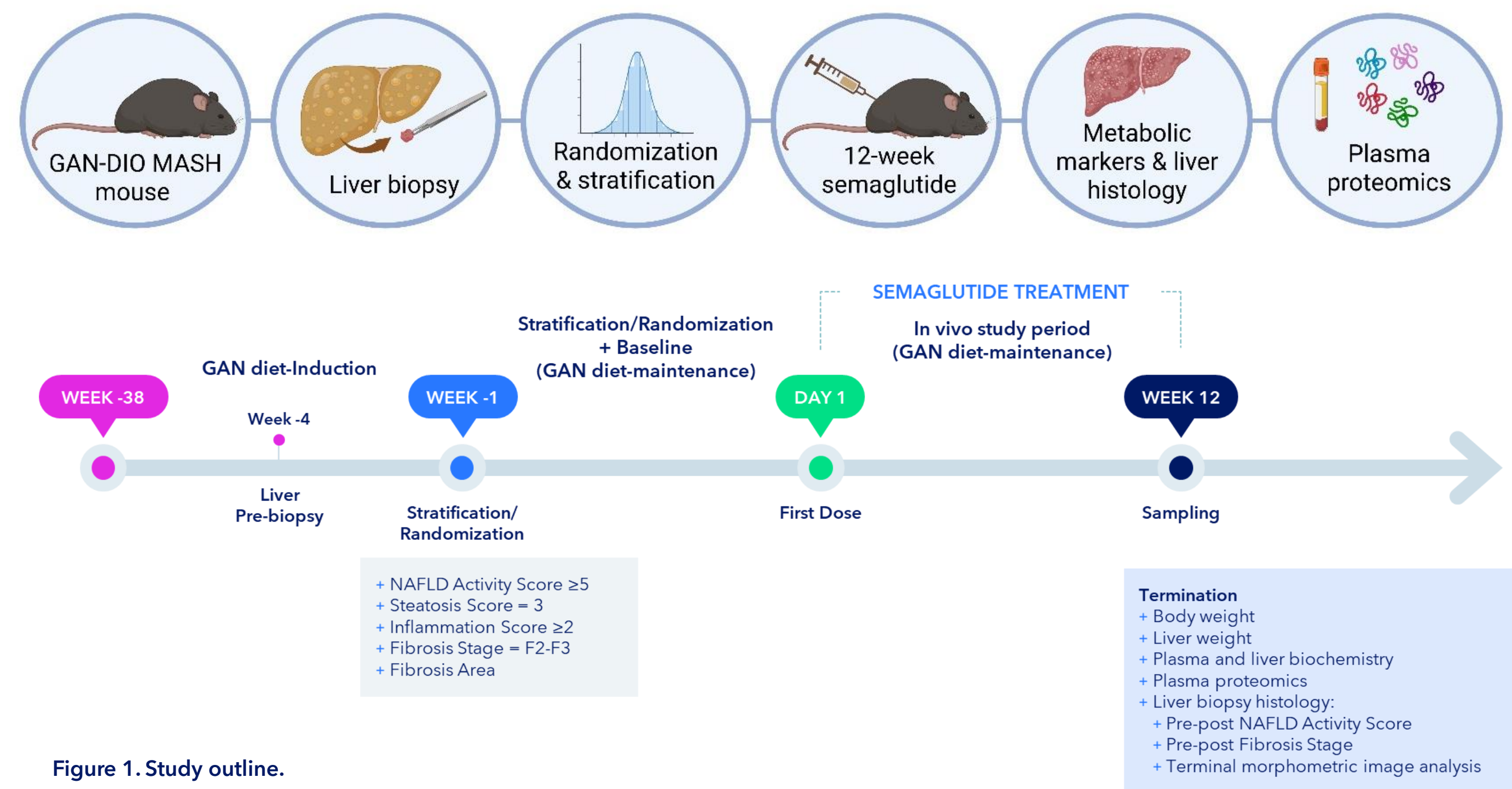


Figure 1. Study outline.

2 Metabolic markers

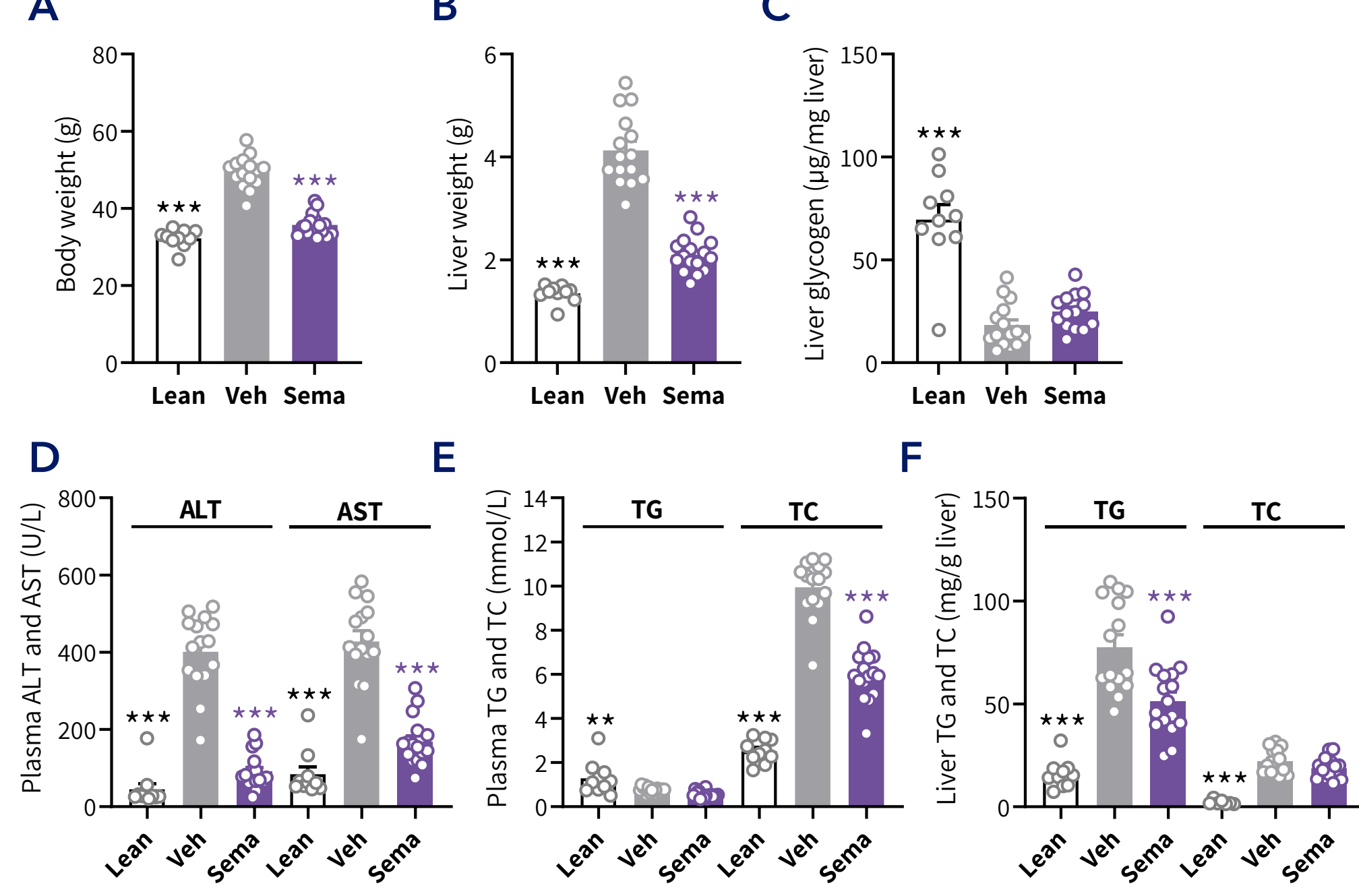


Figure 2. 12-week semaglutide improves markers in GAN DIO-MASH mice. (A) Terminal body weight. (B) Liver weight. (C) Liver glycogen. (D) Plasma alanine transaminase (ALT) and aspartate aminotransferase (AST). (E, F) Plasma and liver triglycerides and total cholesterol. Data are presented as mean $\pm$ SEM. ** p<0.01, ***p<0.001 vs. Vehicle DIO-GAN-MASH mice (one-way ANOVA with Tukey's multiple comparisons test).

3 Liver histopathology

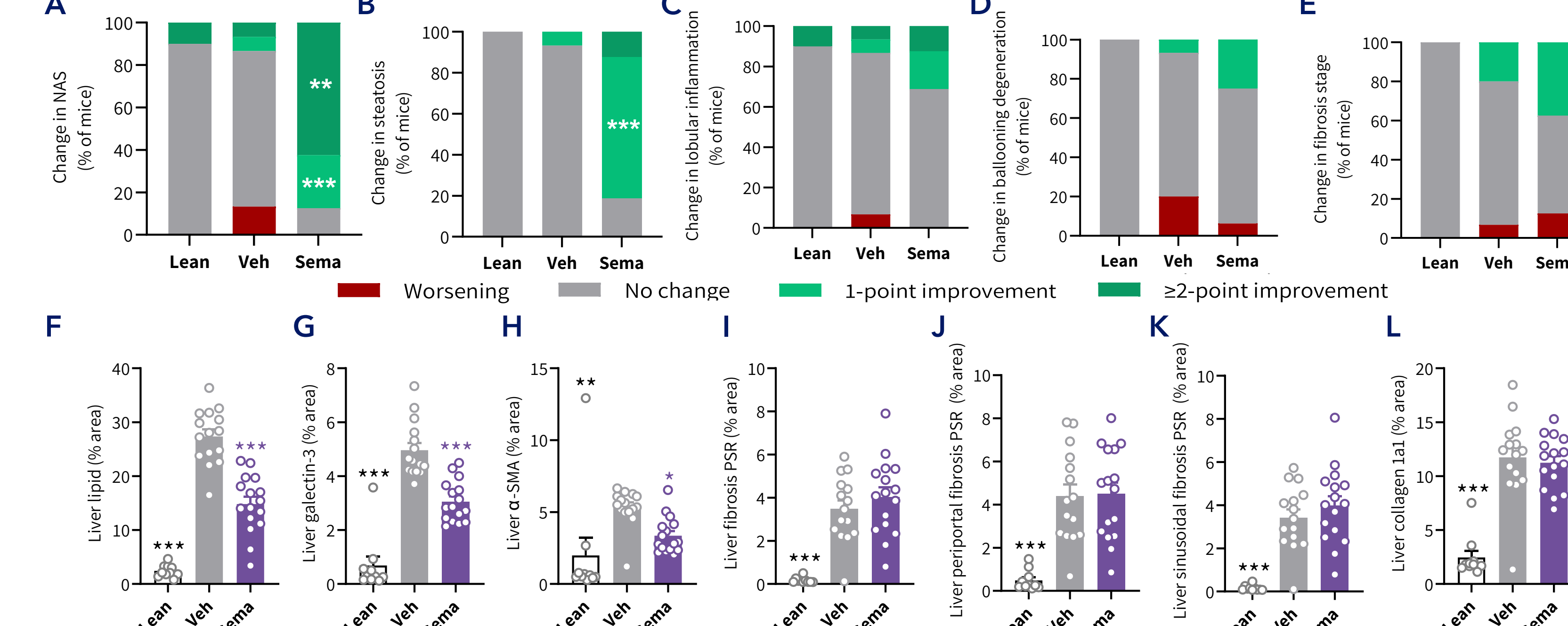


Figure 3. 12-week semaglutide improves MASH histological markers in GAN DIO-MASH mice. (A-E) Histopathological scores. (A) NAFLD Activity Score (NAS). (B) Steatosis score. (C) Lobular inflammation score. (D) Ballooning degeneration score. (E) Fibrosis stage. **p<0.01, ***p<0.001 vs. Vehicle GAN-DIO-MASH mice (Fisher's exact test). (F-L) Quantitative histology (%-area of staining) determined by AI-assisted (GHOST) and conventional image analysis. Data were adjusted for %-area of steatosis (HE-staining). (F) Liver lipid (HE). (G) Galectin-3. (H) Alpha-smooth muscle actin (α -SMA). (I) Picro-Sirius red (whole-section PSR). (J) Periportal fibrosis (PSR). (K) Sinusoidal fibrosis (PSR). (L) Collagen-1a1. Data are presented as mean $\pm$ SEM. *p<0.05, **p<0.01, ***p<0.001 vs. Vehicle GAN-DIO-MASH mice (one-way ANOVA with Tukey's multiple comparisons test).

4 Plasma biomarkers upregulated by disease and reversed by semaglutide

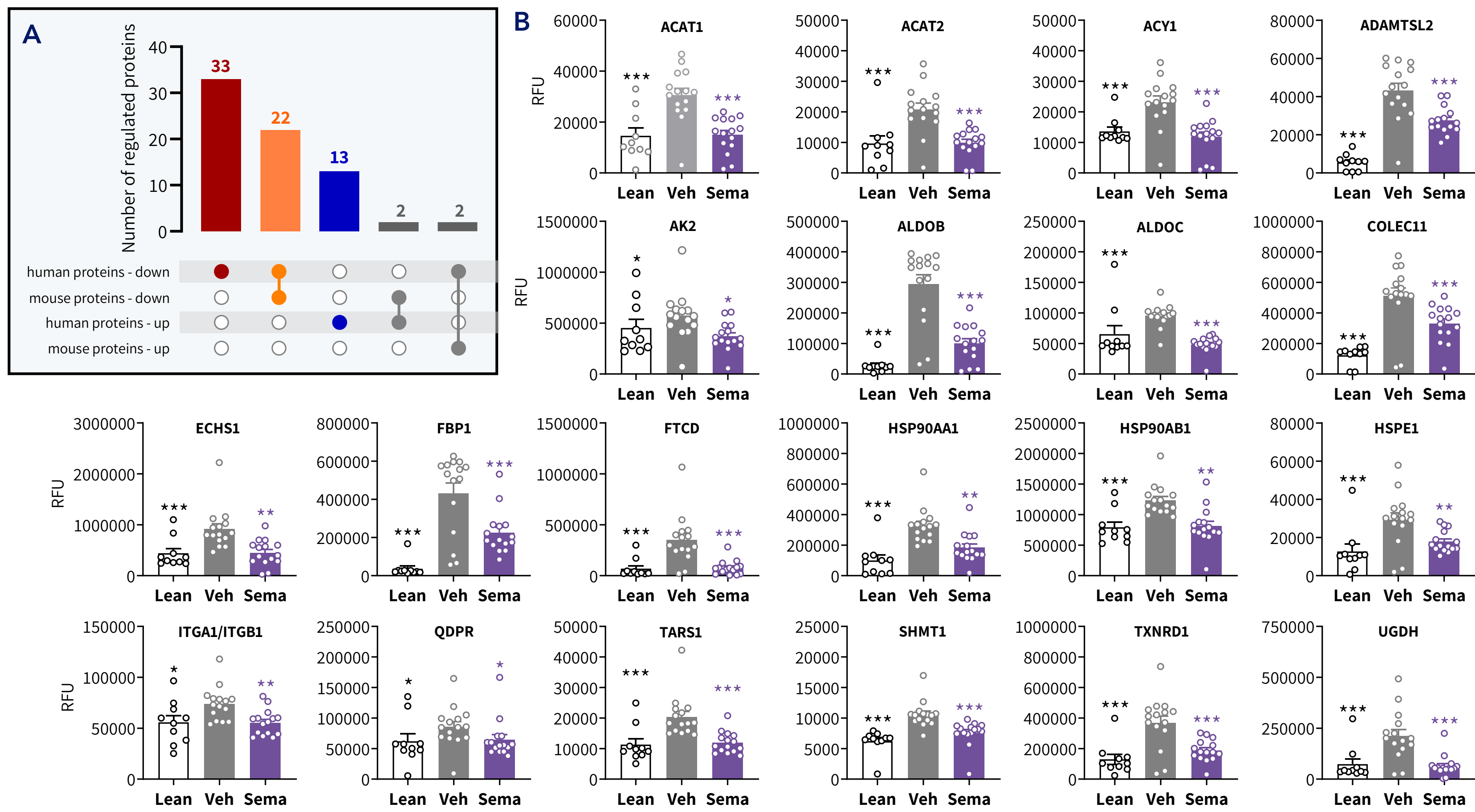


Figure 4. Clinically translatable circulating biomarker signature of semaglutide in male GAN DIO-MASH mice. (A) Upset plot depicting overlapping and distinct regulations in the mediating 72-protein panel between semaglutide-treated people with MASH (vs. placebo controls) and GAN DIO-MASH mice (vs. vehicle-dosed GAN DIO-MASH mice). 67 of the 72 human mediating proteins had identifiable mouse homologues (B) 20 out of these circulating proteins showed similar profile in GAN DIO-MASH mice and people with MASH, i.e. upregulated by disease and reversed by semaglutide treatment. Expression levels (relative fluorescence units, RFU) of the 20 plasma protein classifiers in GAN DIO-MASH mice and lean controls. *p<0.05, **p<0.01, ***p<0.001 vs. vehicle-dosed GAN DIO-MASH mice (FDR-correction for multiple comparisons; correction applied across all proteins assessed in the SomaScan analysis). Protein abbreviations are listed in Figure 5.

5 Plasma biomarker roles in MASH pathogenesis

Functional classification			Disease association	
Biological role	Protein	Protein Name	Associated with MASLD/MASH	Associated with Cirrhosis
Carbohydrate and Lipid Metabolism	ACAT1	Acetyl-CoA acetyltransferase, mitochondrial	YES	YES
	ACAT2	Acetyl-CoA acetyltransferase, cytosolic	YES	
	ALDOB	Aldolase B, fructose-bisphosphate	YES	YES
	ALDOC	Fructose-bisphosphate aldolase C	YES	YES
	ECHS1	Enoyl-CoA hydratase, mitochondrial	YES	YES
	FBP1	Fructose-1,6-bisphosphatase 1	YES	YES
	FTCD	Formimidoyltransferasecyclodeaminase	YES	YES
	TARS1	Threonine-tRNA ligase, cytoplasmic		YES
Redox and One-Carbon Metabolism	TXNRD1	Thioredoxin reductase 1, cytoplasmic	YES	
	SHMT1	Serine hydroxymethyltransferase, cytosolic	YES	
Chaperones and Protein Folding	HSP90AA1	Heat shock protein HSP 90-alpha	YES	
	HSP90AB1	Heat shock protein HSP 90-beta	YES	
	HSP90B1	10-kDa heat shock protein, mitochondrial	YES	YES
	HSP90B1	10-kDa heat shock protein, mitochondrial	YES	YES
ECM Remodelling and Fibrosis	ADAMTSL2	ADAMTS-like protein 2	YES	YES
	UGDH	UDP-glucose 6-dehydrogenase	YES	YES
Cell Adhesion and Signaling	ITGA1/ITGB1	Integrin alpha-1: beta-1 complex	YES	YES
Amino Acid Metabolism and Signaling	ACY1	Aminoacylase 1	YES	YES
	AK2	Adenylate kinase 2, mitochondrial	YES	
	QDPR	Dihydropteridine reductase	YES	
Innate Immunity and Complement	COLEC11	Collectin-11	YES	YES

Figure 5. Biological roles of the 20 plasma protein classifiers upregulated by disease and reversed by semaglutide in GAN DIO-MASH mice and people with MASH. These proteins have previously been associated with MASLD/MASH or cirrhosis and are implicated in pathways related to metabolism, inflammation, fibrosis and cellular stress responses.

6 Plasma biomarker-histology correlations

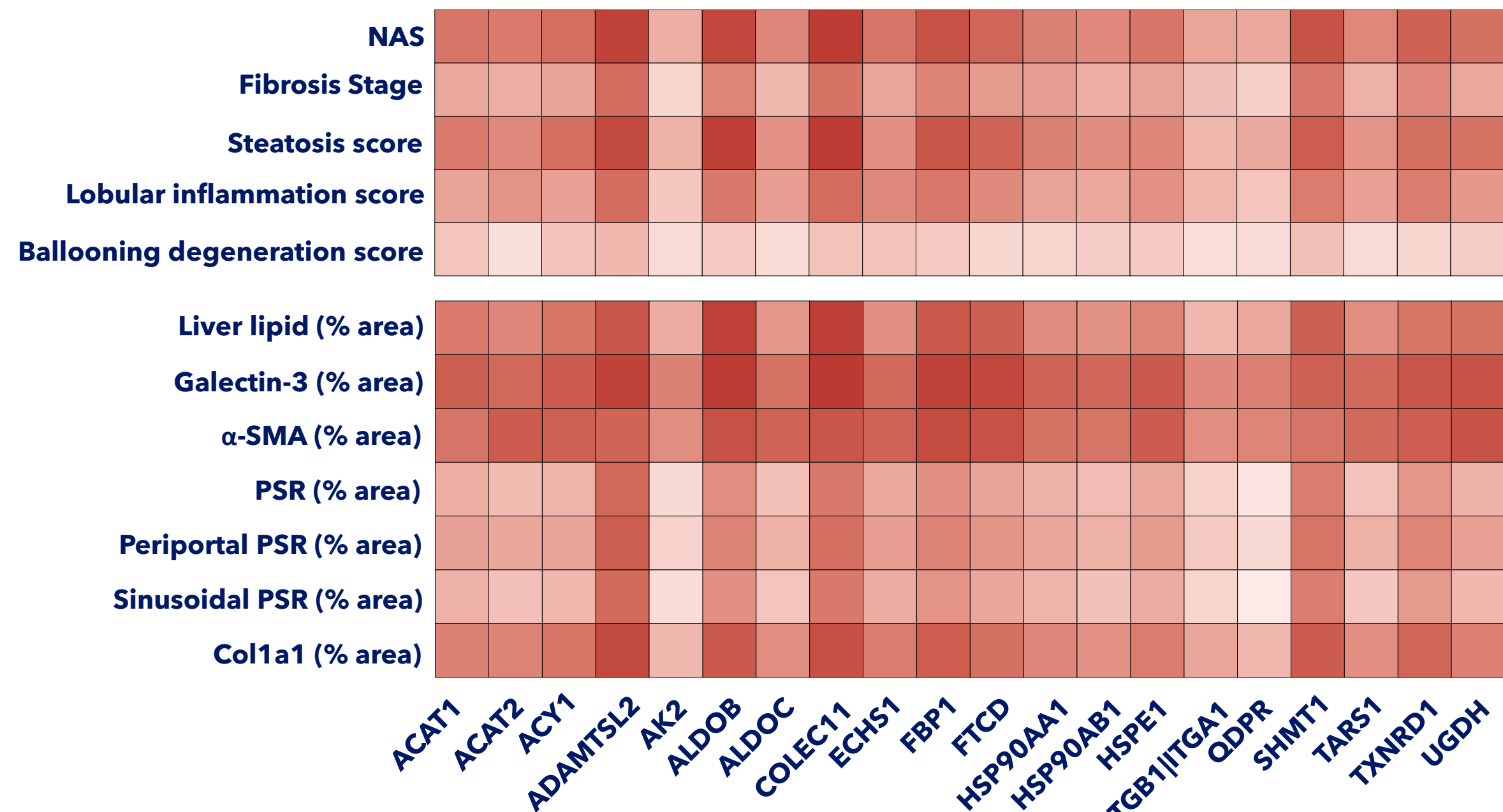


Figure 6. Correlation and functional classification of clinically translatable circulating biomarker signature of semaglutide in male GAN DIO-MASH mice. (A) Correlation between terminal plasma protein levels and histopathological scores (upper panel) as well as quantitative histological endpoints (lower panel). Heatmap colors denote the direction and magnitude of correlations, where red indicates positive and blue indicates inverse (negative) correlations.

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