Supplemental Digital Content - ***Mannose reduces fructose metabolism and reverses MASH in human liver slices and murine models in vivo***, John G. Hong

SDC, Supplemental Methods:

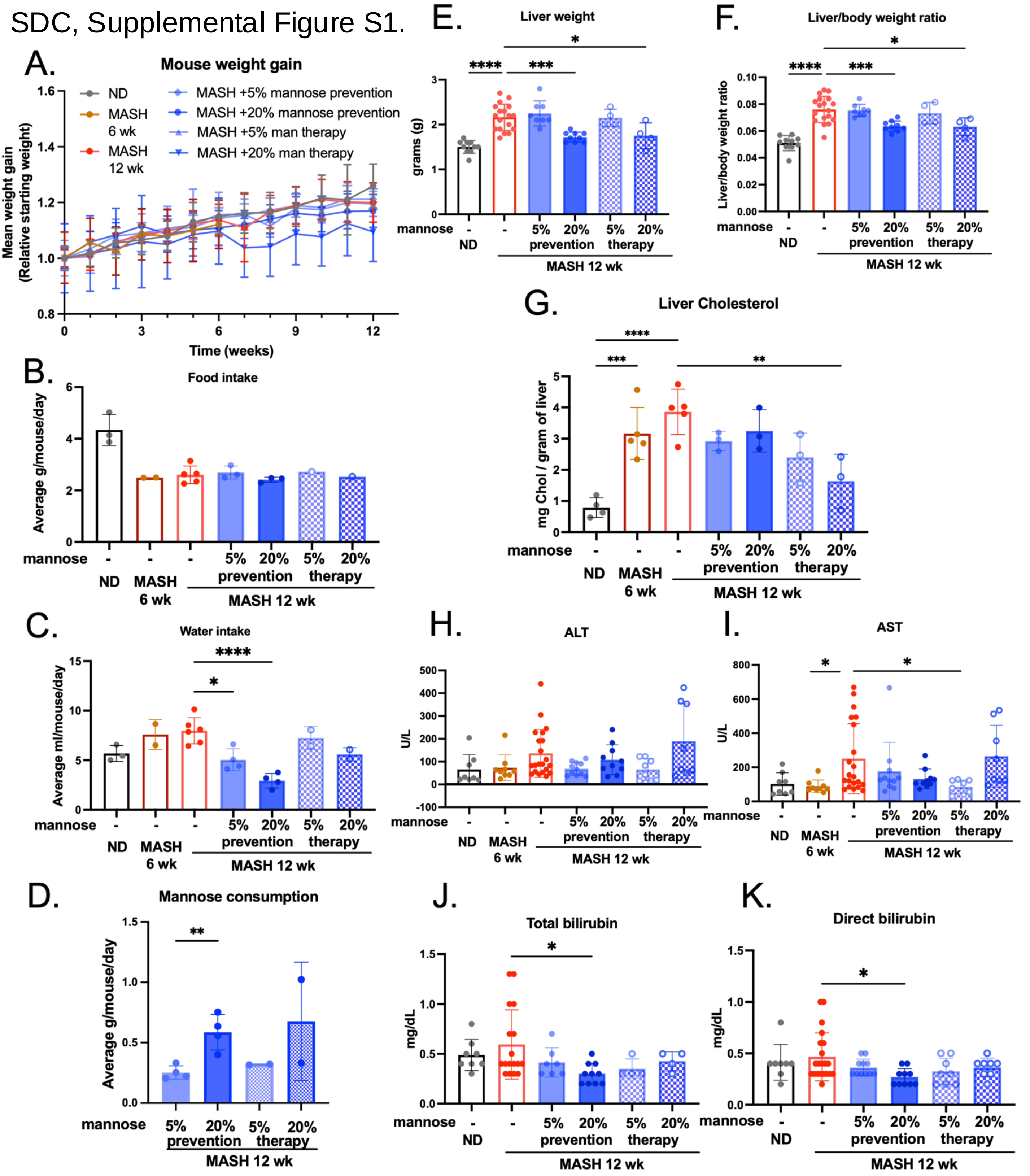
Gene Set Enrichment Analysis (GSEA):

GSEA (Broad Institute, UC San Diego)[1] was performed using the mouse hallmark signature gene sets. Analysis was run as described in the GSEA-MSigDB documentation, using total size-factor-normalized counts (DESeq2) as input, and 1000 permutations, weighted enrichment statistic, and Signal2Noise metric for ranking genes as enrichment parameters. Normalized enrichment scores were compiled and plotted for heatmap visualization using R heatmap package.

SDC, Supplemental reference:

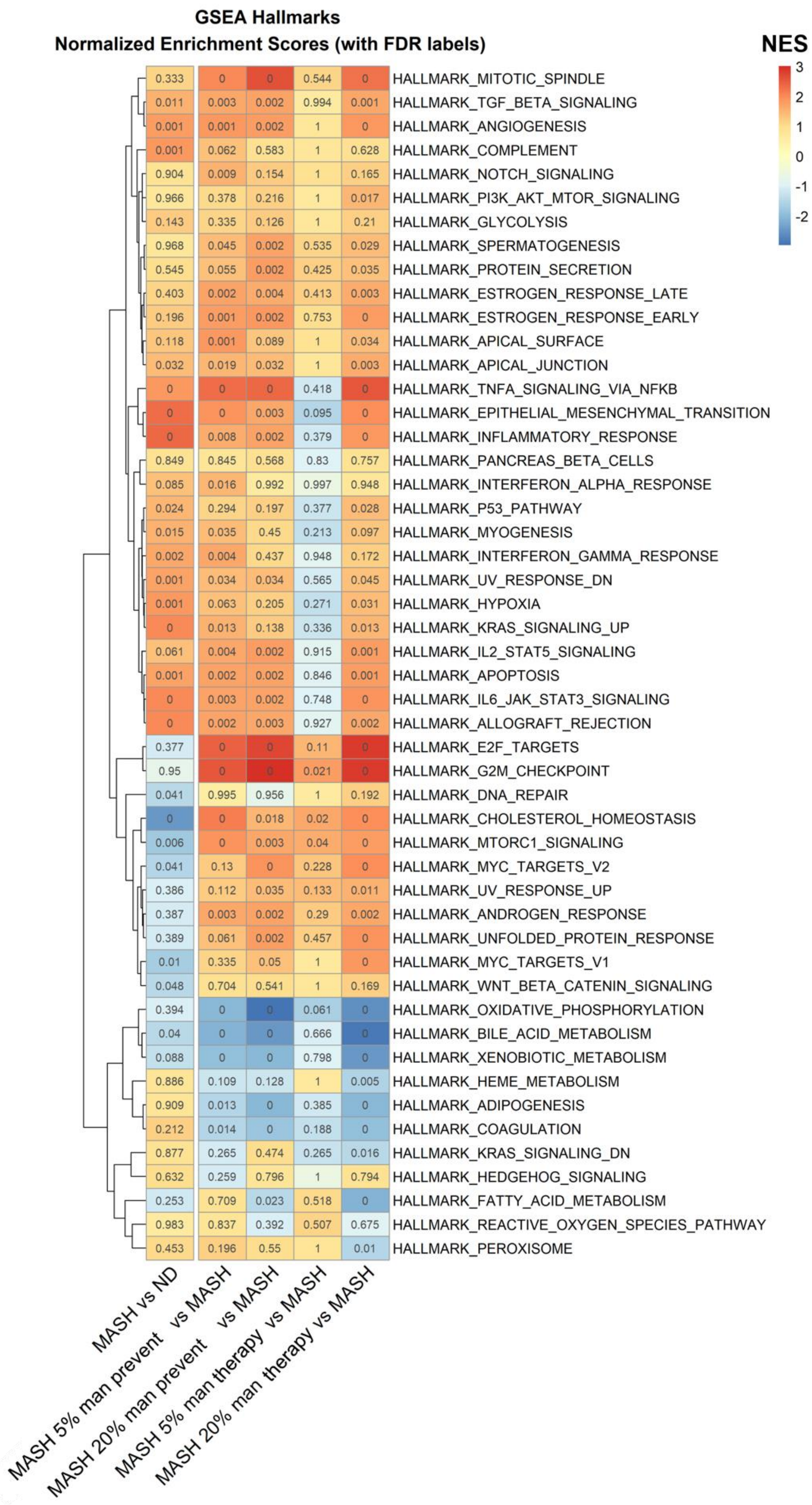
1. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A*. Oct 25 2005;102(43):15545-50. doi:10.1073/pnas.0506580102

SDC, Supplemental Figure S1.



Supplemental Figure S1. Metabolic profile of FAT-MASH mice supplemented with mannose. Mouse total body weight measurements (A), average food intake (B), average water intake (C), and average mannose consumption (D) during course of MASH diet and mannose treatment regimens; n = 1-6 cage per group with 2-5 mice per cage. Liver weight (E), liver to body weight ratio (F), cholesterol levels (G) in whole liver tissue (n=3-5), serum ALT (H), AST (I), total bilirubin (J), and direct bilirubin (K) measured at 12 weeks; n= 4-18 mice per group. Results are expressed as mean ± SD and were compared by Student's t-test or one-way ANOVA (*p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001). ND, normal diet; MASH, FAT-MASH diet; man, mannose.

SDC, Supplemental Figure S2.

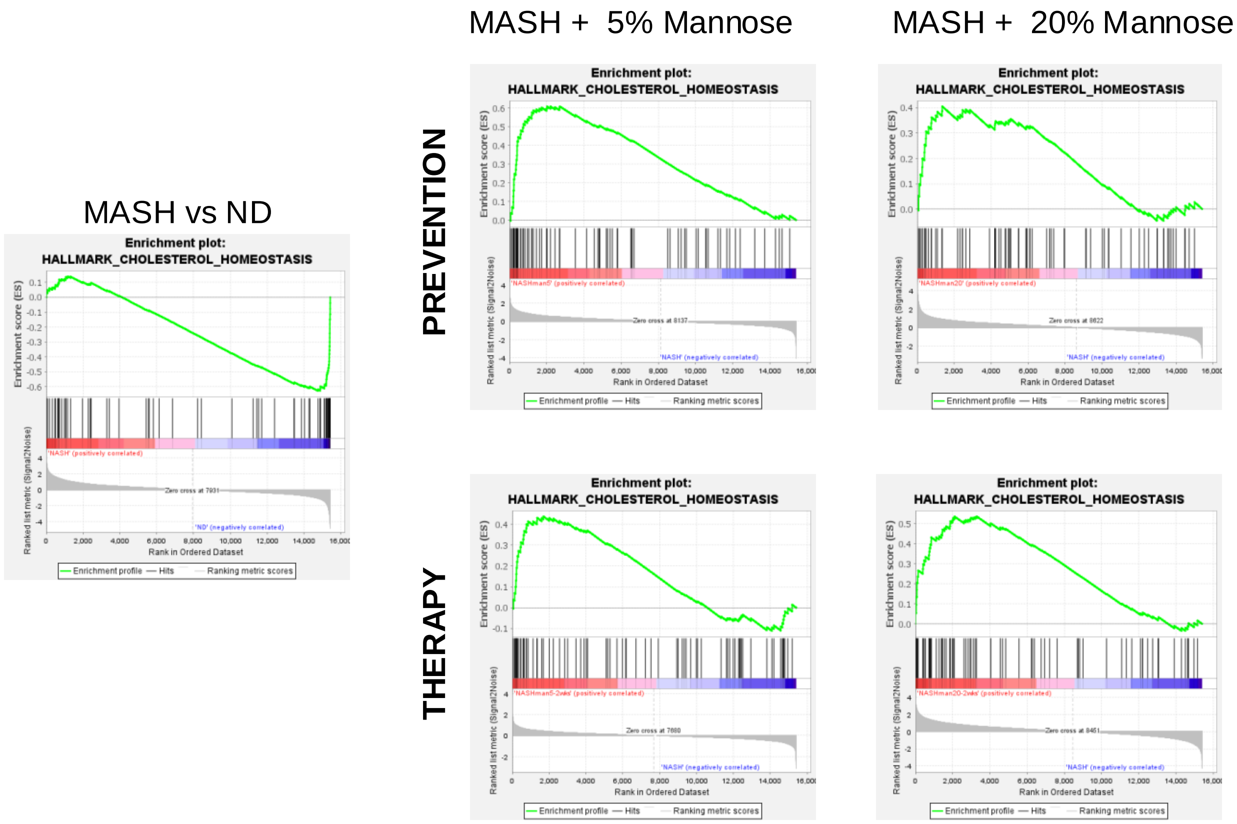


MASH vs ND	MASH 5% man prevent vs MASH	MASH 20% man prevent vs MASH	MASH 5% man therapy vs MASH	MASH 20% man therapy vs MASH	
0.333	0	0	0.544	0	HALLMARK_MITOTIC_SPINDLE
0.011	0.003	0.002	0.994	0.001	HALLMARK_TGF_BETA_SIGNALING
0.001	0.001	0.002	1	0	HALLMARK_ANGIOGENESIS
0.001	0.062	0.583	1	0.628	HALLMARK_COMPLEMENT
0.904	0.009	0.154	1	0.165	HALLMARK_NOTCH_SIGNALING
0.966	0.378	0.216	1	0.017	HALLMARK_PI3K_AKT_MTOR_SIGNALING
0.143	0.335	0.126	1	0.21	HALLMARK_GLYCOLYSIS
0.968	0.045	0.002	0.535	0.029	HALLMARK_SPERMATOGENESIS
0.545	0.055	0.002	0.425	0.035	HALLMARK_PROTEIN_SECRETION
0.403	0.002	0.004	0.413	0.003	HALLMARK_ESTROGEN_RESPONSE_LATE
0.196	0.001	0.002	0.753	0	HALLMARK_ESTROGEN_RESPONSE_EARLY
0.118	0.001	0.089	1	0.034	HALLMARK_APICAL_SURFACE
0.032	0.019	0.032	1	0.003	HALLMARK_APICAL_JUNCTION
0	0	0	0.418	0	HALLMARK_TNFA_SIGNALING_VIA_NFKB
0	0	0.003	0.095	0	HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
0	0.008	0.002	0.379	0	HALLMARK_INFLAMMATORY_RESPONSE
0.849	0.845	0.568	0.83	0.757	HALLMARK_PANCREAS_BETA_CELLS
0.085	0.016	0.992	0.997	0.948	HALLMARK_INTERFERON_ALPHA_RESPONSE
0.024	0.294	0.197	0.377	0.028	HALLMARK_P53_PATHWAY
0.015	0.035	0.45	0.213	0.097	HALLMARK_MYOGENESIS
0.002	0.004	0.437	0.948	0.172	HALLMARK_INTERFERON_GAMMA_RESPONSE
0.001	0.034	0.034	0.565	0.045	HALLMARK_UV_RESPONSE_DN
0.001	0.063	0.205	0.271	0.031	HALLMARK_HYPOXIA
0	0.013	0.138	0.336	0.013	HALLMARK_KRAS_SIGNALING_UP
0.061	0.004	0.002	0.915	0.001	HALLMARK_IL2_STAT5_SIGNALING
0.001	0.002	0.002	0.846	0.001	HALLMARK_APOPTOSIS
0	0.003	0.002	0.748	0	HALLMARK_IL6_JAK_STAT3_SIGNALING
0	0.002	0.003	0.927	0.002	HALLMARK_ALLOGRAFT_REJECTION
0.377	0	0	0.11	0	HALLMARK_E2F_TARGETS
0.95	0	0	0.021	0	HALLMARK_G2M_CHECKPOINT
0.041	0.995	0.956	1	0.192	HALLMARK_DNA_REPAIR
0	0	0.018	0.02	0	HALLMARK_CHOLESTEROL_HOMEOSTASIS
0.006	0	0.003	0.04	0	HALLMARK_MTORC1_SIGNALING
0.041	0.13	0	0.228	0	HALLMARK_MYC_TARGETS_V2
0.386	0.112	0.035	0.133	0.011	HALLMARK_UV_RESPONSE_UP
0.387	0.003	0.002	0.29	0.002	HALLMARK_ANDROGEN_RESPONSE
0.389	0.061	0.002	0.457	0	HALLMARK_UNFOLDED_PROTEIN_RESPONSE
0.01	0.335	0.05	1	0	HALLMARK_MYC_TARGETS_V1
0.048	0.704	0.541	1	0.169	HALLMARK_WNT_BETA_CATENIN_SIGNALING
0.394	0	0	0.061	0	HALLMARK_OXIDATIVE_PHOSPHORYLATION
0.04	0	0	0.666	0	HALLMARK_BILE_ACID_METABOLISM
0.088	0	0	0.798	0	HALLMARK_XENOBIOTIC_METABOLISM
0.886	0.109	0.128	1	0.005	HALLMARK_HEME_METABOLISM
0.909	0.013	0	0.385	0	HALLMARK_ADIPOGENESIS
0.212	0.014	0	0.188	0	HALLMARK_COAGULATION
0.877	0.265	0.474	0.265	0.016	HALLMARK_KRAS_SIGNALING_DN
0.632	0.259	0.796	1	0.794	HALLMARK_HEDGEHOG_SIGNALING
0.253	0.709	0.023	0.518	0	HALLMARK_FATTY_ACID_METABOLISM
0.983	0.837	0.392	0.507	0.675	HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY
0.453	0.196	0.55	1	0.01	HALLMARK_PEROXISOME

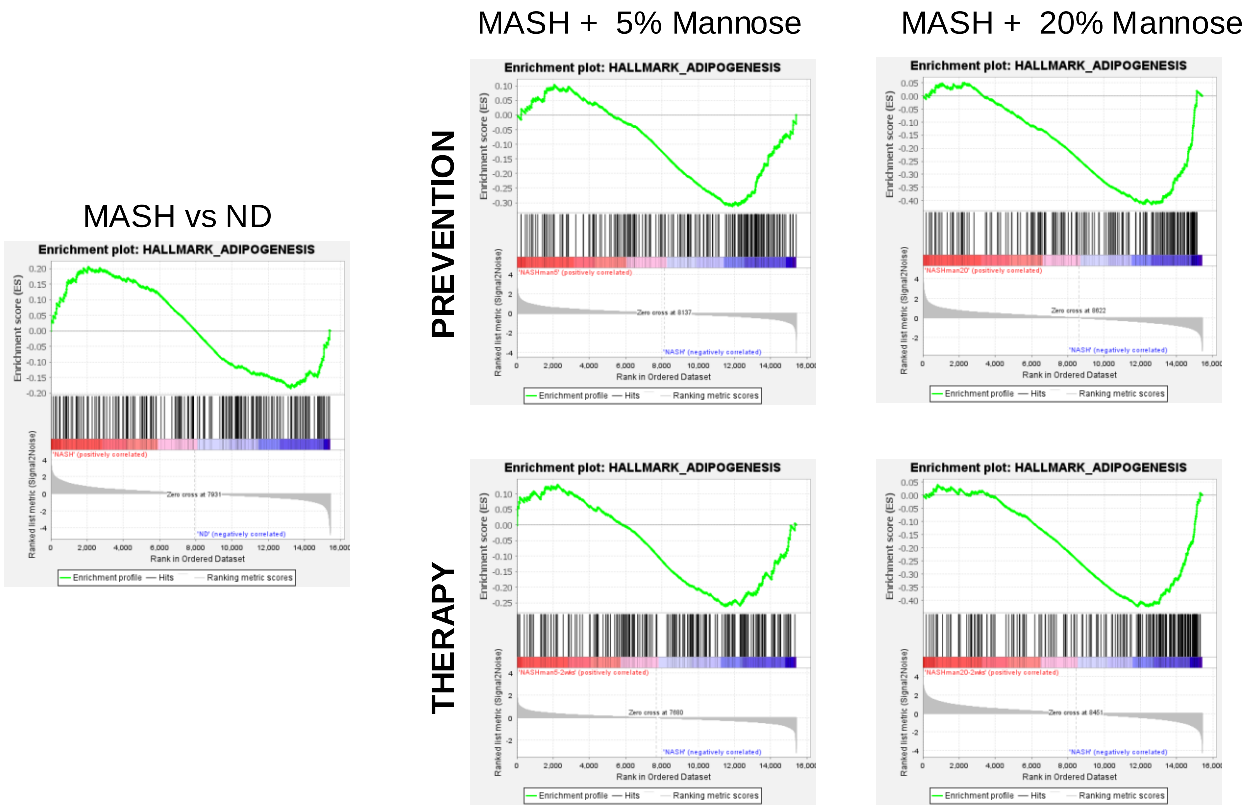
Supplemental Figure S2. Gene Set Enrichment Analysis (GSEA) for Hallmark pathways. Heatmap of compiled results of GSEA from comparative gene expression patterns for different treatment groups of ND, MASH, and MASH + mannose. Hallmark gene set signatures were used for pathway identification. Normalized enrichment scores (NES) are indicated by heatmap color, and false discovery rate (FDR) are labeled within each cell.

SDC, Supplemental Figure S3.

GSEA: Hallmark Cholesterol homeostasis



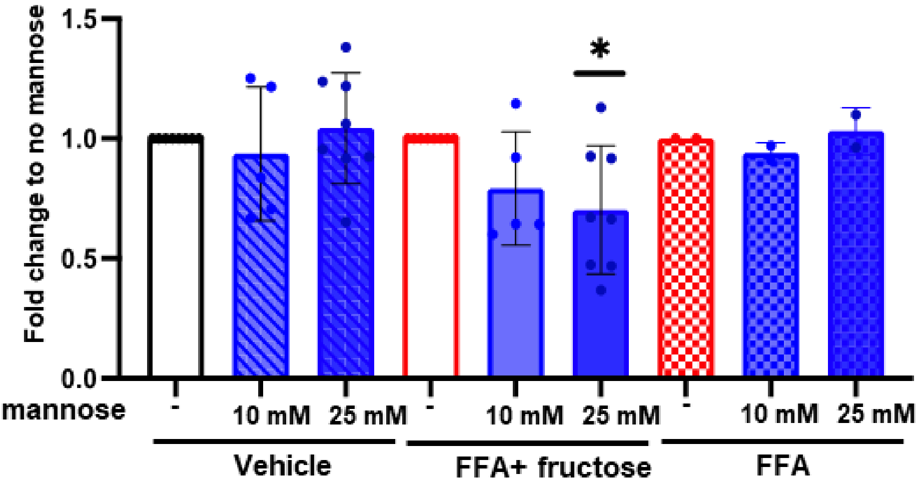
GSEA: Hallmark Adipogenesis



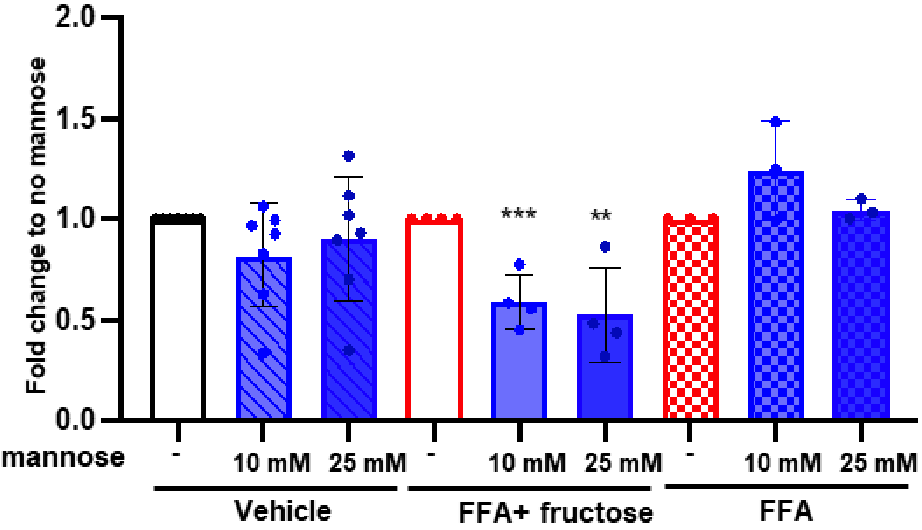
Supplemental Figure S3. Select differentially regulated GSEA Hallmark pathways. Enrichment plots for select hallmark gene sets significantly enriched between indicated treatment groups.

SDC, Supplemental Figure S4.

A.

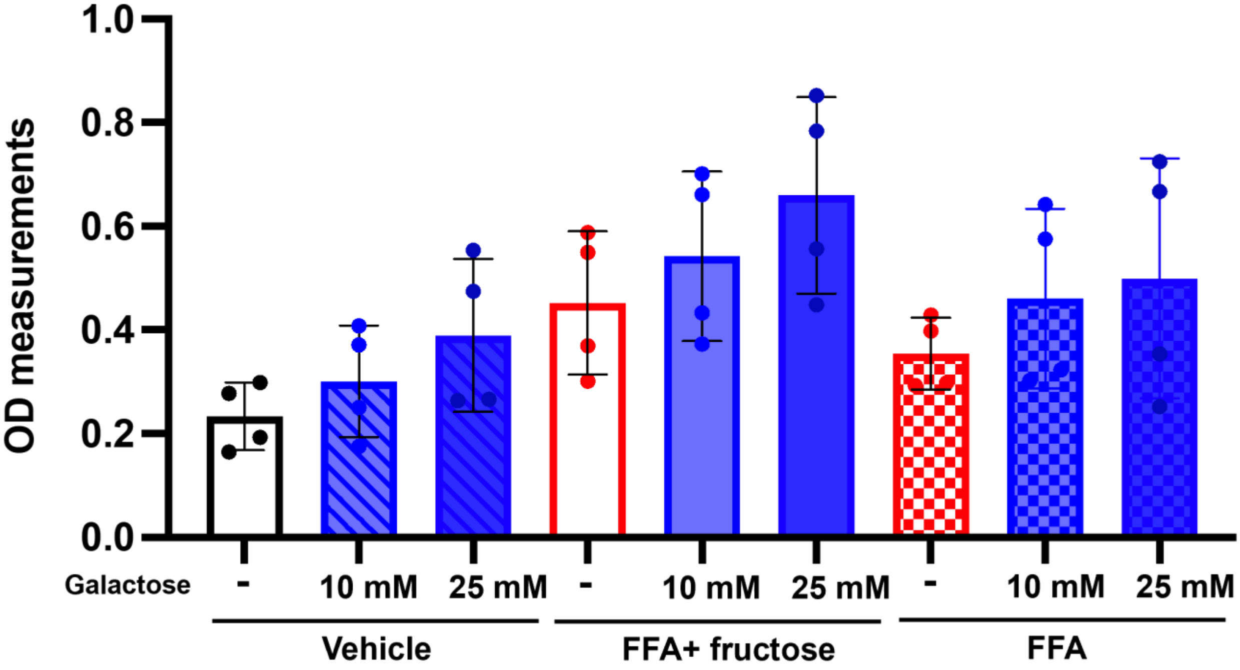


B.



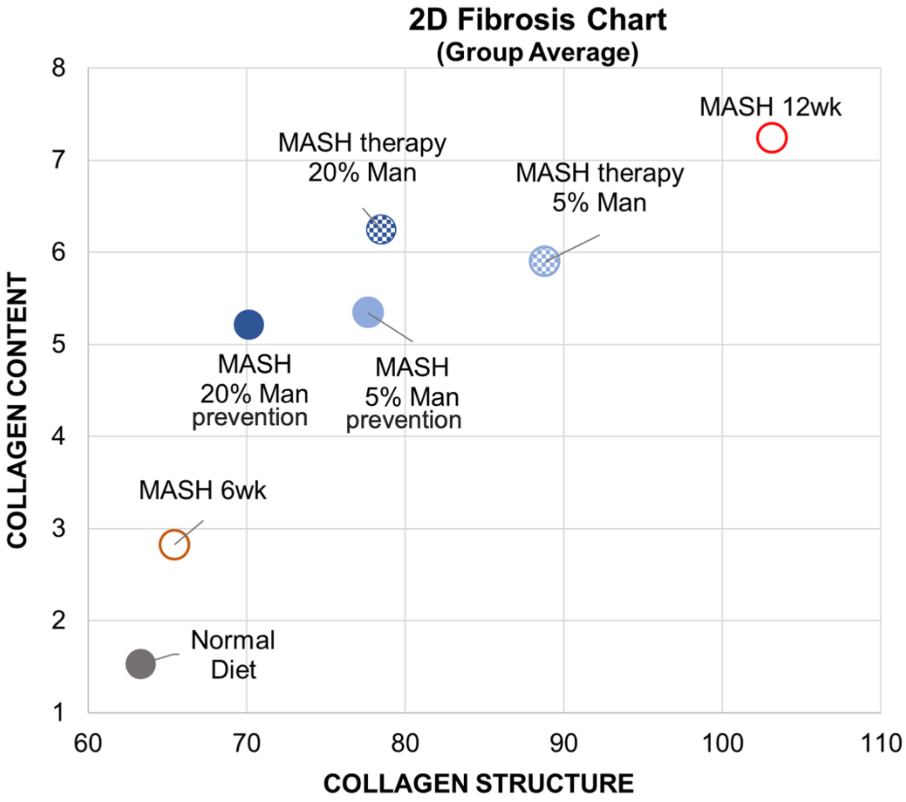
Supplemental Figure S4. *In vitro* Oil Red O quantification. Oil Red O quantifications in primary mouse hepatocytes (A) and THLE-5B (B). Results are expressed as fold change to no mannose treatments in each condition and analyzed with one sample t-test (*p<0.05, **p<0.01, ***p<0.001).

SDC, Supplemental Figure S5.



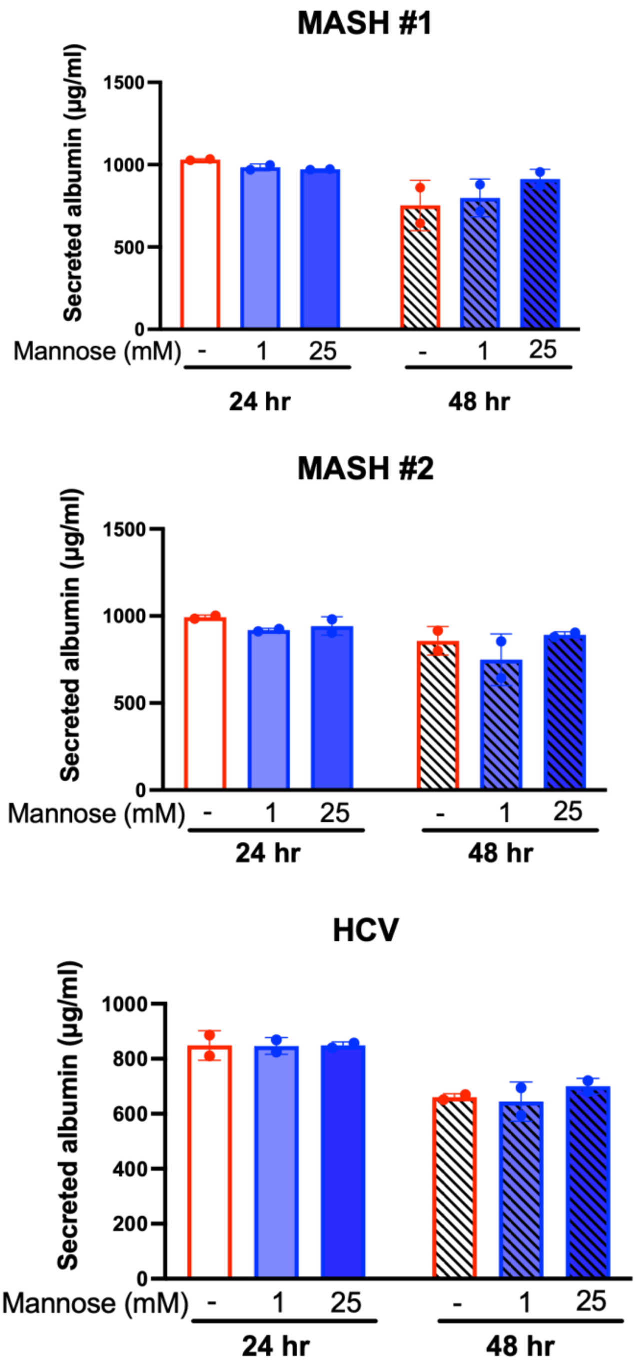
Supplemental Figure S5. ***In vitro* galactose treatment.** Bar plot showing THLE-5B hepatocytes conditioned with vehicle, FFA + fructose, or FFA alone, without or with 10- or 25-mM galactose for 72 hours (n=4).

SDC, Supplemental Figure S6.



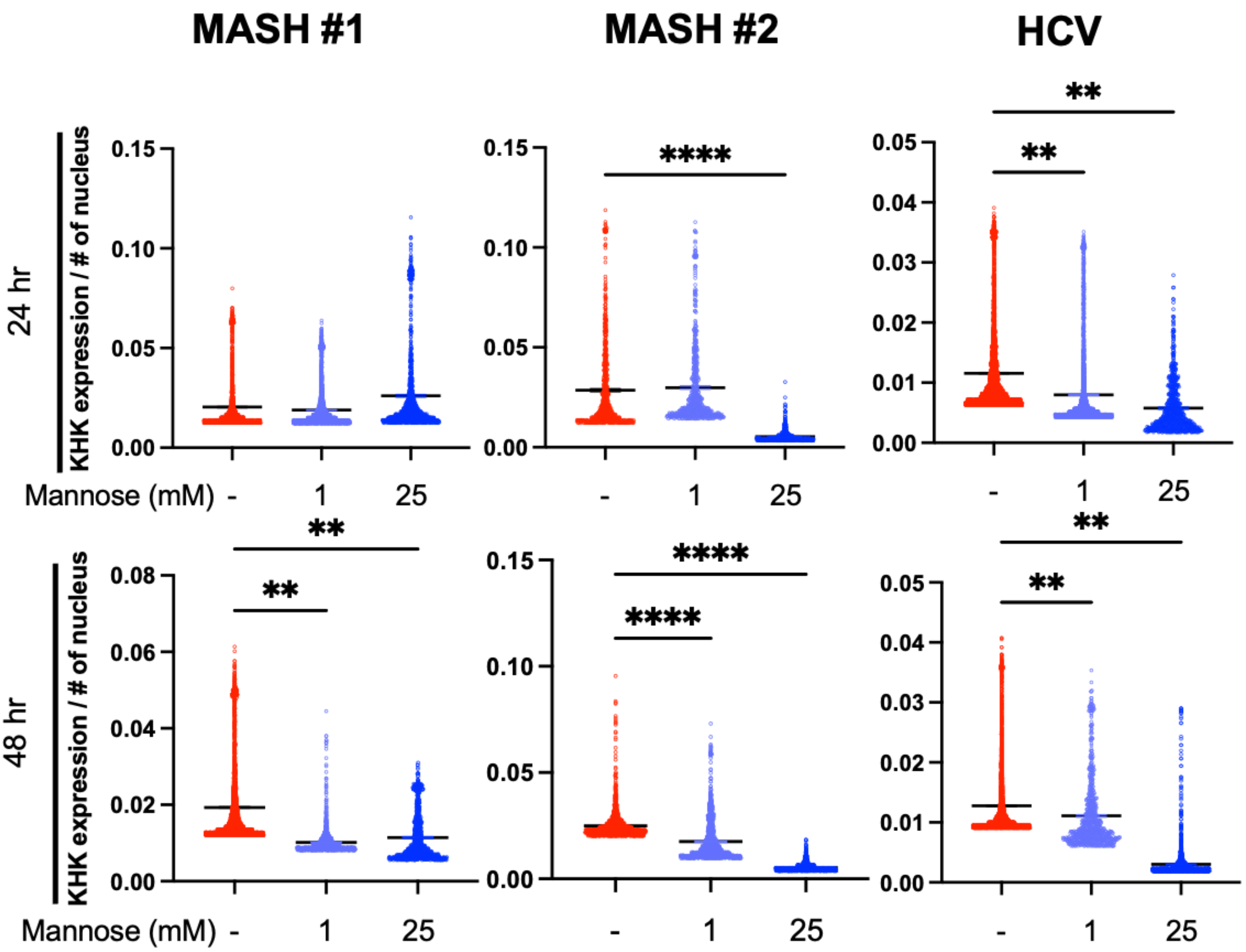
Supplemental Figure S6. Fibrosis profile in FAT-MASH mice. 2D Fibrosis Chart shows the relationship between the collagen deposition and its structure. n=4-9 mice per group.

SDC, Supplemental Figure S7.



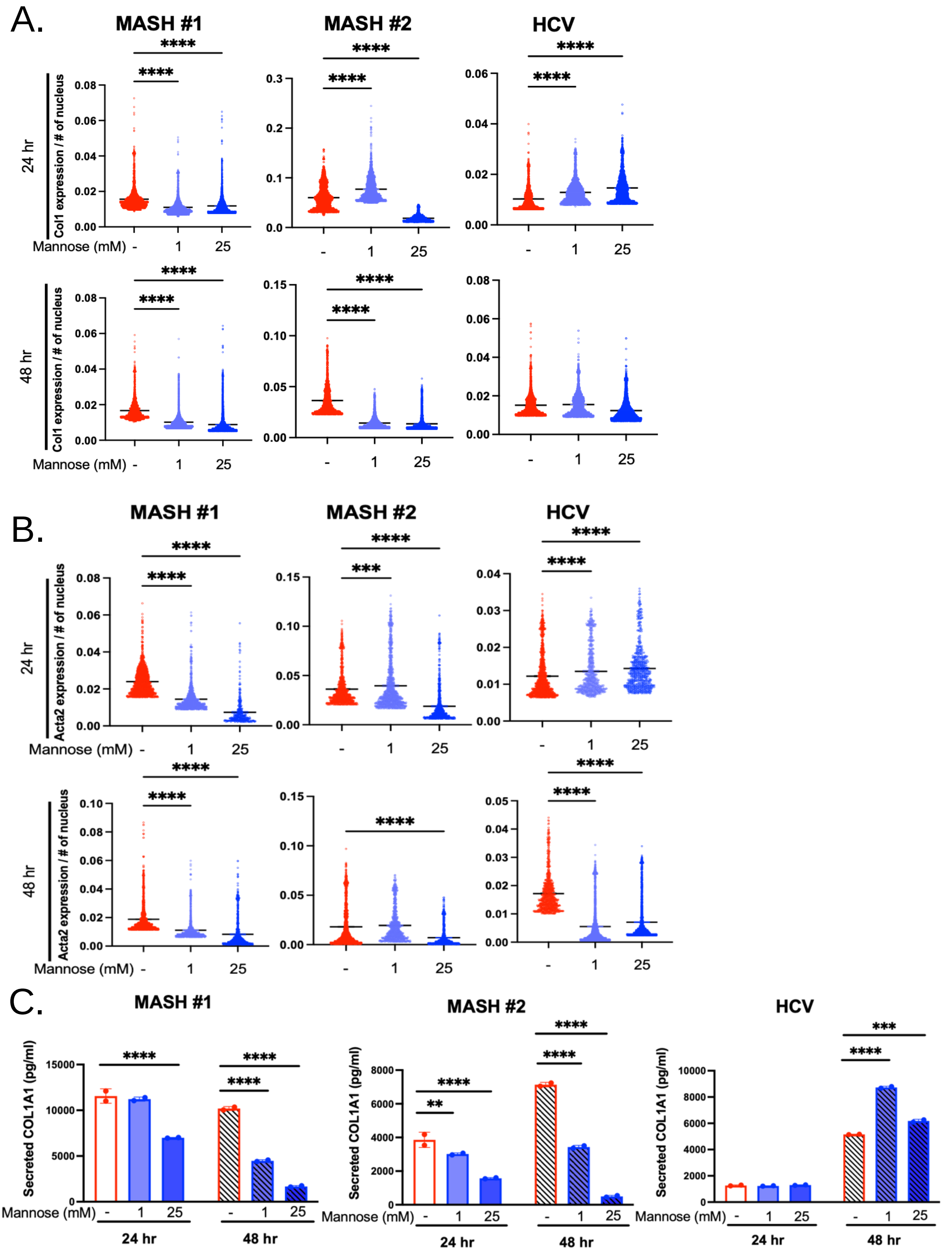
Supplemental Figure S7. Viability profile of human MASH PCLS with mannose treatment. Bar plots showing secreted albumin in human MASH PCLS.

SDC, Supplemental Figure S8.



Supplemental Figure S8. KHK expression in human MASH PCLS with mannose treatment. Quantifications of KHK immunofluorescence, normalized by number of nuclei. Statistical comparisons are by one sample t-test and two-way ANOVA with Dunnett's post hoc test for multiple comparisons (**p<0.01, ****p<0.0001).

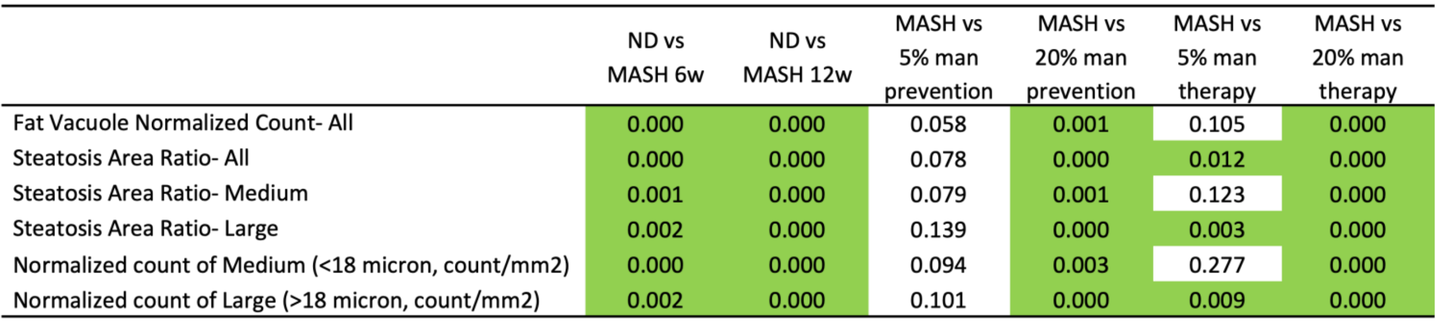
SDC, Supplemental Figure S9.



Supplemental Figure S9. Fibrogenic gene expression in human MASH PCLS with mannose treatment. Quantifications of (A) COL1 and (B) ACTA2 immunofluorescence, normalized by number of nuclei. (C) Bar graph showing secreted COL1A1 measurements. Statistical comparisons are by one sample t-test and two-way ANOVA with Dunnett's post hoc test for multiple comparisons (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

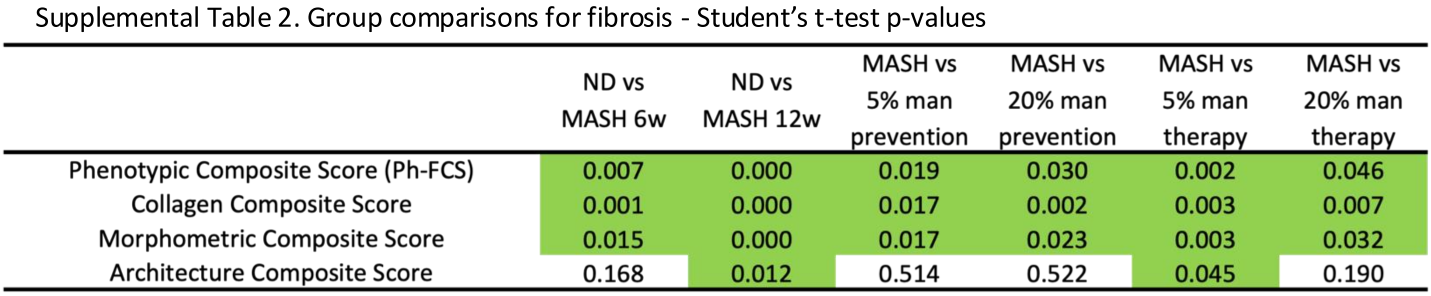
SDC, Supplemental Table 1.

Supplemental Table 1. Group comparisons for steatosis - Student’s t-test p-values



	ND vs MASH 6w	ND vs MASH 12w	MASH vs 5% man prevention	MASH vs 20% man prevention	MASH vs 5% man therapy	MASH vs 20% man therapy
Fat Vacuole Normalized Count- All	0.000	0.000	0.058	0.001	0.105	0.000
Steatosis Area Ratio- All	0.000	0.000	0.078	0.000	0.012	0.000
Steatosis Area Ratio- Medium	0.001	0.000	0.079	0.001	0.123	0.000
Steatosis Area Ratio- Large	0.002	0.000	0.139	0.000	0.003	0.000
Normalized count of Medium (<18 micron, count/mm2)	0.000	0.000	0.094	0.003	0.277	0.000
Normalized count of Large (>18 micron, count/mm2)	0.002	0.000	0.101	0.000	0.009	0.000

SDC, Supplemental Table 2.



Supplemental Table 2. Group comparisons for fibrosis - Student’s t-test p-values

	ND vs MASH 6w	ND vs MASH 12w	MASH vs 5% man prevention	MASH vs 20% man prevention	MASH vs 5% man therapy	MASH vs 20% man therapy
Phenotypic Composite Score (Ph-FCS)	0.007	0.000	0.019	0.030	0.002	0.046
Collagen Composite Score	0.001	0.000	0.017	0.002	0.003	0.007
Morphometric Composite Score	0.015	0.000	0.017	0.023	0.003	0.032
Architecture Composite Score	0.168	0.012	0.514	0.522	0.045	0.190

SDC, Supplemental Table 3.

Supplemental Table 3. Patient characteristics for hPCLS

Patient ID	Age (years)	Sex	Disease background	Pathological diagnosis	METAVIR Fibrosis stage	Ischemia time (hours)
MASH #1	59	Male	MASH-HCC	Cirrhosis with thin acellular fibrous septa and mild steatohepatitis (Grade 0)	F4	3
MASH #2	58	Male	MASH	Dense periportal fibrosis with hyperplastic hepatocyte nodules with glycogenated nuclei	F4	4
HCV	69	Male	HCV-HCC	Chronic HCV infected cirrhosis without inflammation	F4	4

SDC, Supplemental Table 4.

Supplemental Table 4. Primer sequences for qPCR

Gene	**Species**	**Primers**
ACTA2	Human	Forward: AGATCAAGATCATTGCCCC Reverse: TTCATCGTATTCCTGTTTGC
COL1A1	Human	Forward: GGCTTCCCTGGTCTTCCTGG Reverse: CCAGGGGGTCCAGCCAAT
Col1a1	Mouse	Forward: GTCCCTGAAGTCAGCTGCATA Reverse: TGGGACAGTCCAGTTCTTCAT
KHK	Human	Forward: GGGGCTTGTATGGTCGTGTGAG Reverse: CCACCTGGCACCCGAATCTC
Khk	Mouse	Forward: ATTCTGCACGCCTACAGCTTC Reverse: TACGGGAGCCATTGGAGTTG
PPIA	Human	Forward: ATGGTTCCCAGTTTTTCATC Reverse: CTCCACAATATTCATGCCTTC
Ywhaz	Mouse	Forward: GAAAAGTTCTTGATCCCCAATGC Reverse: TGTGACTGGTCCACAATTCCTT

SDC, Supplemental Table 5.

Supplemental Table 5. Antibody information

Antibody	Dilution	Supplier	Catalog number
Western blot			
Anti-KHK	1:1,000 (2% milk/TBST)	ThermoFisher	PA5-29004
Anti-Collagen-1	1:1,000 (2% milk/TBST)	Bioss	bs-10423R
Anti-β-Actin	1:2,000 (2% milk/TBST)	GeneTex	GTX629630
Anti-α-Tubulin	1:2,000 (2% milk/TBST)	DSHB	12G10
Immunofluorescence			
Anti-KHK	1:100 (1% BSA/PBS)	ThermoFisher	PA5-29004
AlexaFluor-488	1:2,000 (1% BSA/PBS)	ThermoFisher	A-11008