Supplemental information

Expansion of macrophage and liver sinusoidal endothelial cell subpopulations during non-alcoholic steatohepatitis progression

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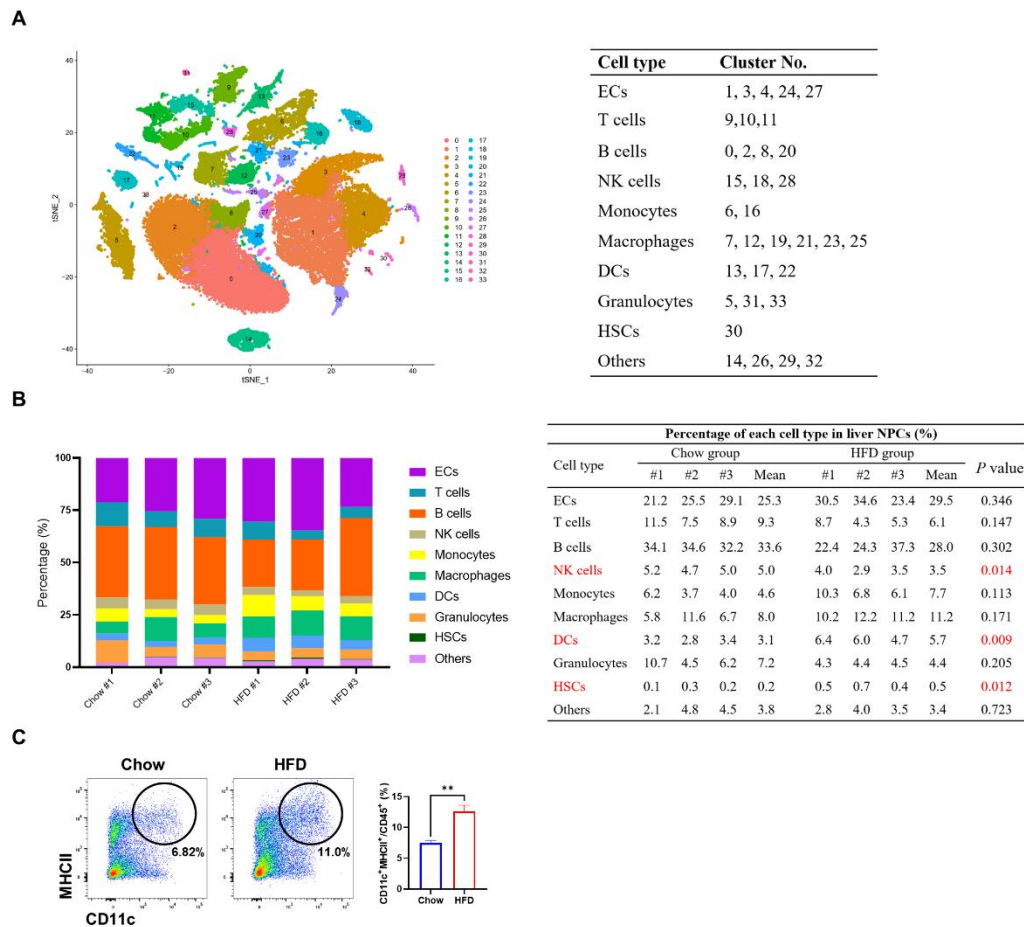
A

Cell type	Cluster No.
ECs	1, 3, 4, 24, 27
T cells	9,10,11
B cells	0, 2, 8, 20
NK cells	15, 18, 28
Monocytes	6, 16
Macrophages	7, 12, 19, 21, 23, 25
DCs	13, 17, 22
Granulocytes	5, 31, 33
HSCs	30
Others	14, 26, 29, 32

B

Percentage of each cell type in liver NPCs (%)

Cell type	Chow group				HFD group				P value
	#1	#2	#3	Mean	#1	#2	#3	Mean	
ECs	21.2	25.5	29.1	25.3	30.5	34.6	23.4	29.5	0.346
T cells	11.5	7.5	8.9	9.3	8.7	4.3	5.3	6.1	0.147
B cells	34.1	34.6	32.2	33.6	22.4	24.3	37.3	28.0	0.302
NK cells	5.2	4.7	5.0	5.0	4.0	2.9	3.5	3.5	0.014
Monocytes	6.2	3.7	4.0	4.6	10.3	6.8	6.1	7.7	0.113
Macrophages	5.8	11.6	6.7	8.0	10.2	12.2	11.2	11.2	0.171
DCs	3.2	2.8	3.4	3.1	6.4	6.0	4.7	5.7	0.009
Granulocytes	10.7	4.5	6.2	7.2	4.3	4.4	4.5	4.4	0.205
HSCs	0.1	0.3	0.2	0.2	0.5	0.7	0.4	0.5	0.012
Others	2.1	4.8	4.5	3.8	2.8	4.0	3.5	3.4	0.723

C

Figure S1. ScRNA-seq analysis of liver NPCs in healthy and NASH mice, related to Figures 1-3. (A) t-SNE visualization of liver cell clusters based on 48,272 single cell transcriptomes derived from Chow and HFD mice (left panel). Each cell type consisted of several clusters (right panel). (B) Percentage of each cell type in liver NPCs of Chow and HFD mice. (C) Representative flow-cytometry plot showing the percentage of $CD11c^+MHCII^+$ double-positive DCs in $CD45^+$ cells isolated from Chow and HFD mice liver respectively. The statistics were shown on the right. n = 3 per group. The data are expressed as mean ± SEM. Statistical significance is shown as ** for $p <$ 0.01, as evaluated by Student's t-test.
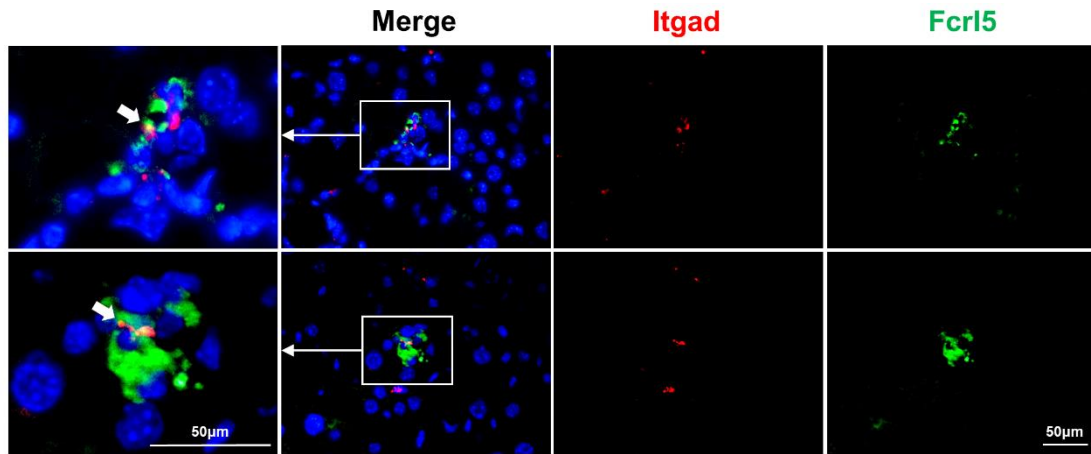
Figure S2. Clusters of macrophages in mice liver, related to Figure 2. (A) Illustration of Kupffer cells (KCs, blue), monocyte-derived macrophages (MDMs, red) and dividing cells (green) in hepatic macrophages. (B) Percentage of Cluster 21 and Cluster 23 macrophages in liver NPCs of Chow and HFD group. The percentage of Cluster 23 was extremely low in Chow group, and increased dramatically in two out of three mice in HFD group. Statistical significance between two groups was observed in the case of Cluster 21, but not achieved in the case of Cluster 23. n = 3 per group. The data are expressed as mean ± SEM. Statistical significance is shown as * for $p < 0.05$, as evaluated by Student's t-test.

Figure S3. Representative images of Itgad and Fcrl5 immunofluorescence staining in murine liver tissues, related to Figure 2. Scale bar, 50 μm.
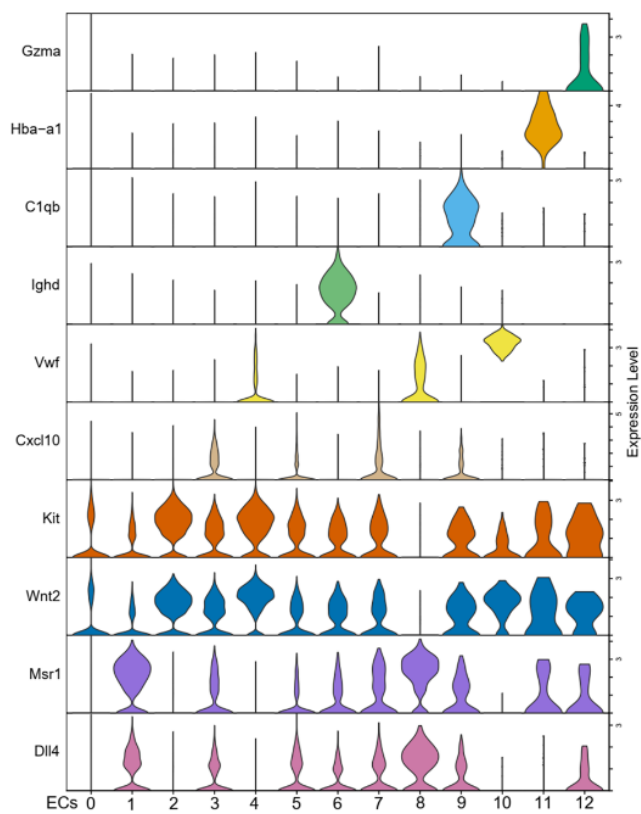
Figure S4. Representative marker gene expression for each cluster of ECs after re-clustering, related to Figure 3.
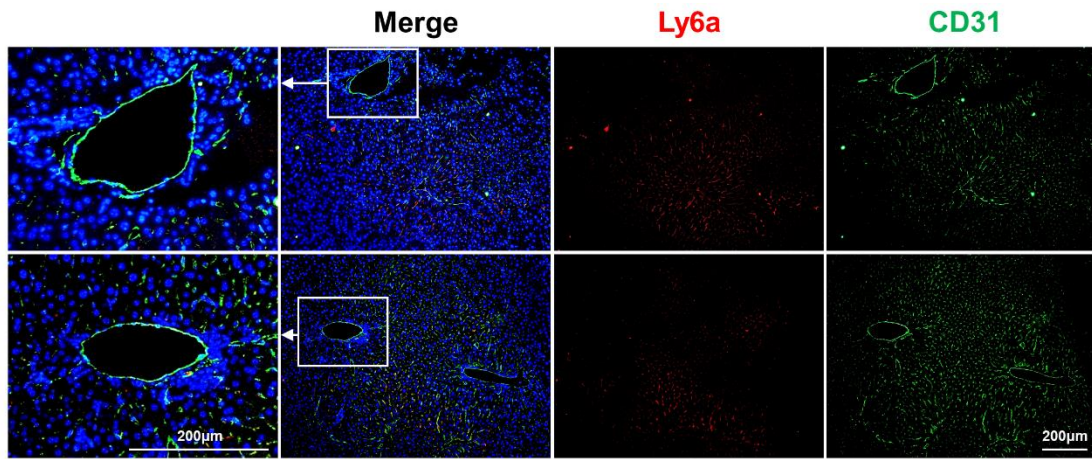
Figure S5. Representative images of Ly6a and CD31 immunofluorescence staining in murine liver tissues, related to Figure 5. Scale bar, 200 μm.
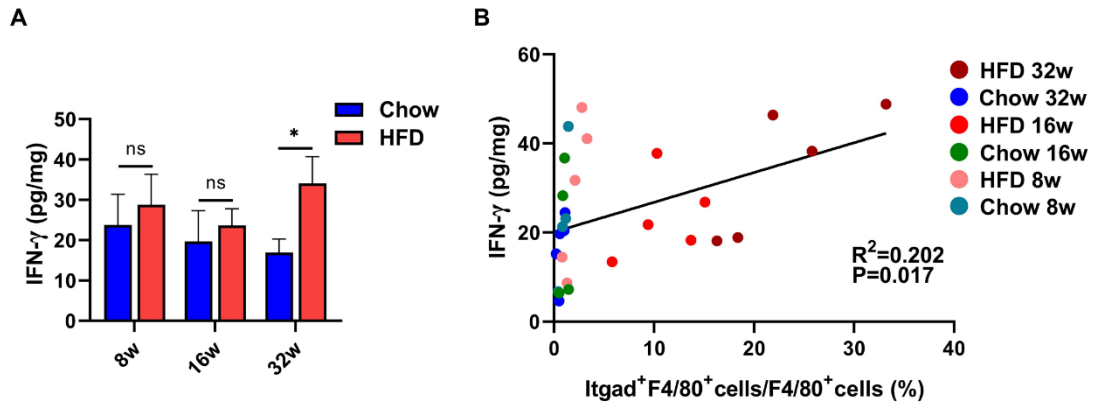
Figure S6. Hepatic expression of IFN-γ and its relationship with the amount of Itgad+ macrophages in Chow and HFD (week 8, 16 and 32) mice liver, related to Figure 5. (A) The expression of IFN-γ in liver tissues of Chow and HFD (week 8, 16 and 32) mice. (B) Correlation of IFN-γ expression and the amount of Itgad+ macrophages in Chow and HFD (week 8, 16 and 32) mice liver. The data are presented as mean ± SEM. Statistical significance is shown as * for $p < 0.05$, as evaluated by Student's t-test.

Table S3. Primer sequences for qPCR, related to STAR Methods.

Gene	Sequence (5'->3')	
	Forward primer	Reverse primer
Trem2	CTACCAGTGTCAGAGTCTCCGA	CCTCGAAACTCGATGACTCCTC
Gpnmb	GGCTACTTCAGAGCCACCATCA	CTTTGCAGGTCACAGTGAAGTCC
Itgad	GCTTAGGAGTCTGCCTTTGCTG	GTCAGGTGAACCTTTGCGGACA
Fcrl5	TTTCTTCAGAAACCTCCAGCTTC	CCTTGCACTGGTATCTCTTTGACTC
Egr1	TGAGCACCTGACCACAGAGTC	TGAAAGGGGGTTCAGGCCAC
Plk2	GATAACCCAGCAGCCTAGCA	CTGTCTTCAAGGCATTCGCTG
Ly6a	GGACTGGAGTGTTACCAGTGCTAT	TGTTTGAGAATCCACAATAACTGC
Ifit1	AAAGGTCTAAAAGTGGAAGAGAAGTC	AAGATGAACATTCTGACAAACACG
Ifit3	GCTCAGGCTTACGTTGACAAGG	CTTTAGGCGTGTCCATCCTTCC
Isg15	CATCCTGGTGAGGAACGAAAGG	CTCAGCCAGAACTGGTCTTCGT
Col1a1	CCTCAGGGTATTGCTGGACAAC	TTGATCCAGAAGGACCTTGTTTG
Gapdh	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTCAG