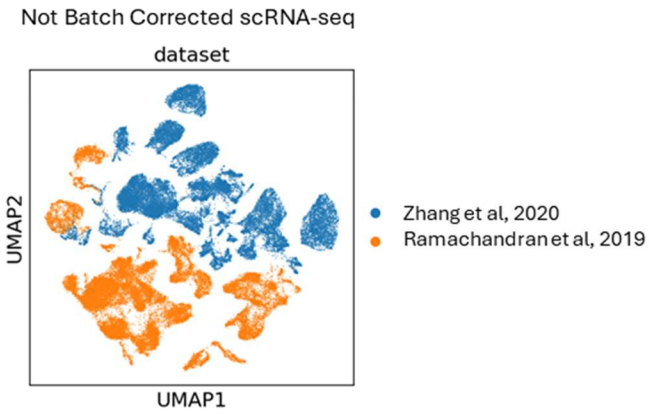
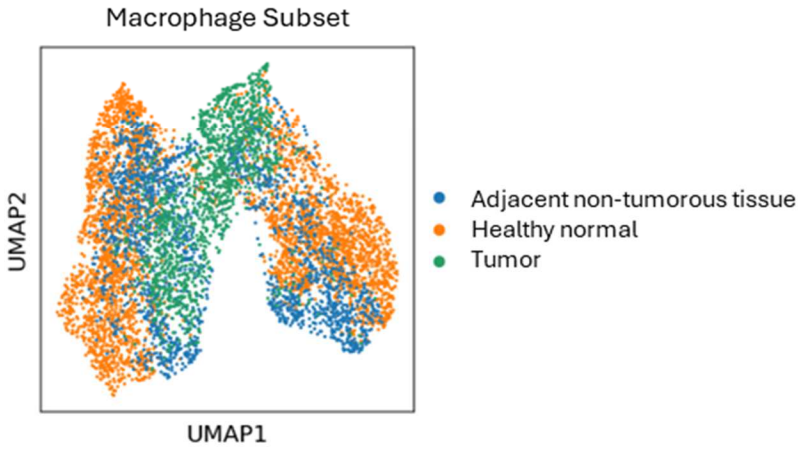
SUPPLEMENTARY FIGURE 1



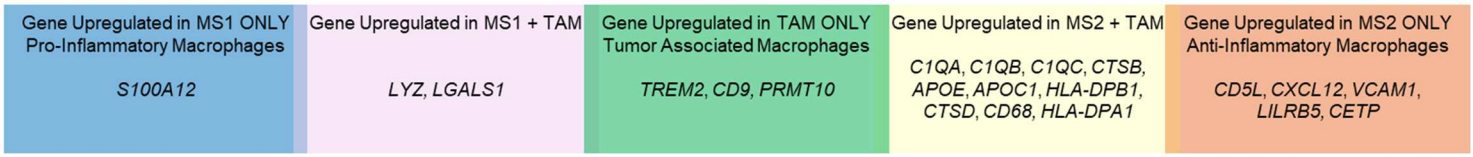
Supplementary Figure S1: UMAP plot before batch correction. The merged Uniform Manifold Approximation and Projection (UMAP) prior to batch correction for the 5 ICC and 3 adjacent non-tumorous tissue samples (Zhang et al, 2020), and 4 healthy liver samples (Ramachandran et al, 2019).

SUPPLEMENTARY FIGURE 2



Supplementary Figure S2: UMAP plot illustrating macrophage cell subsets. The plot highlights three groups: adjacent non-tumorous tissue (blue, n = 3), and healthy normal (orange, n = 4), and tumor (green, n = 5).

SUPPLEMENTARY FIGURE 3



Supplementary Figure S3: Gene upregulation in MS1, MS2, TAM. Genes upregulated exclusively in MS1, MS2, TAM or in both MS1 + TAM and MS2 + TAM.

SUPPLEMENTARY TABLES

Supplementary Table S1 Patient characteristics						
Intrahepatic Cholangiocarcinoma Dataset (GEO GSE107943)						
Patient ID	Sex	Tumor Grade	AJCC Stage	Virus Infection	Pathology	Sample GEO ID
1T	M	Moderately differentiated	I	HBV+	ICC tumor	GSM2883928
2T	M	--	I	HBV+	ICC tumor	GSM2883929
3T	M	Poorly differentiated	IVA	HBV-	ICC tumor	GSM2883930
4T	M	Moderately differentiated	II	HBV-	ICC tumor	GSM2883931
5T	M	Moderately differentiated	I	HBV-	ICC tumor	GSM2883932
6T	M	Moderately differentiated	I	HBV-	ICC tumor	GSM2883933
7T	M	Moderately differentiated	IVB	HBV-	ICC tumor	GSM2883934
8T	M	Moderately differentiated	I	HBV-	ICC tumor	GSM2883935
10T	M	Moderately differentiated	II	HBV-	ICC tumor	GSM2883936
12T	M	Moderately differentiated	IVA	HBV-	ICC tumor	GSM2883938
13T	M	Moderately differentiated	I	HBV-	ICC tumor	GSM2883939
14T	F	Poorly differentiated	I	HBV-	ICC tumor	GSM2883940
15T	M	Moderately differentiated	IVA	HBV-	ICC tumor	GSM2883941
16T	M	Poorly differentiated	II	HBV-	ICC tumor	GSM2883942
17T	M	Moderately differentiated	II	HBV-	ICC tumor	GSM2883943
18T	M	Poorly differentiated	IVA	HBV+	ICC tumor	GSM2883944
19T	M	Moderately differentiated	I	HBV-	ICC tumor	GSM2883945
22T	F	Moderately differentiated	I	HBV+	ICC tumor	GSM2883948
23T	M	Moderately differentiated	I	HBV-	ICC tumor	GSM2883949
24T	F	Poorly differentiated	I	HBV-	ICC tumor	GSM2883950
25T	M	Moderately differentiated	IVA	HBV-	ICC tumor	GSM2883951
26T	M	Moderately differentiated	I	HBV-	ICC tumor	GSM2883952
27T	F	Poorly differentiated	III	HBV-	ICC tumor	GSM2883953
28T	M	Moderately differentiated	II	HBV-	ICC tumor	GSM2883954
29T	F	Moderately differentiated	I	HBV-	ICC tumor	GSM2883955
30T	M	Poorly differentiated	IVB	HBV-	ICC tumor	GSM2883956
31T	M	NA	I	HBV-	ICC tumor	GSM2883957
1N	M	Moderately differentiated	I	HBV+	ICC adjacent	GSM2883958
2N	M	--	I	HBV+	ICC adjacent	GSM2883960
3N	M	Poorly differentiated	IVA	HBV-	ICC adjacent	GSM2883961
4N	M	Moderately differentiated	II	HBV-	ICC adjacent	GSM2883962
5N	M	Moderately differentiated	I	HBV-	ICC adjacent	GSM2883963
6N	M	Moderately differentiated	I	HBV-	ICC adjacent	GSM2883964
7N	M	Moderately differentiated	IVB	HBV-	ICC adjacent	GSM2883965
8N	M	Moderately differentiated	I	HBV-	ICC adjacent	GSM2883966

10N	M	Moderately differentiated	II	HBV-	ICC adjacent	GSM2883967
12N	M	Moderately differentiated	IVA	HBV-	ICC adjacent	GSM2883968
13N	M	Moderately differentiated	I	HBV-	ICC adjacent	GSM2883969
14N	F	Poorly differentiated	I	HBV-	ICC adjacent	GSM2883970
15N	M	Moderately differentiated	IVA	HBV-	ICC adjacent	GSM2883971
16N	M	Poorly differentiated	II	HBV-	ICC adjacent	GSM2883972
17N	M	Moderately differentiated	II	HBV-	ICC adjacent	GSM2883973
18N	M	Poorly differentiated	IVA	HBV+	ICC tumor	GSM2883974
19N	M	Moderately differentiated	I	HBV-	ICC tumor	GSM2883975
22N	F	Moderately differentiated	I	HBV+	ICC tumor	GSM2883976
23N	M	Moderately differentiated	I	HBV-	ICC tumor	GSM2883977
24N	F	Poorly differentiated	I	HBV-	ICC tumor	GSM2883978
25N	M	Moderately differentiated	IVA	HBV-	ICC tumor	GSM2883979
26N	M	Moderately differentiated	I	HBV-	ICC tumor	GSM2883980
27N	F	Poorly differentiated	III	HBV-	ICC tumor	GSM2883981
28N	M	Moderately differentiated	II	HBV-	ICC tumor	GSM2883982
29N	F	Moderately differentiated	I	HBV-	ICC tumor	GSM2883983
30N	M	Poorly differentiated	IVB	HBV-	ICC tumor	GSM2883984
31N	M	NA	I	HBV-	ICC tumor	GSM2883959

Abbreviations: AJCC, American Joint Committee on Cancer; M, Male; F, Female; HBV+, Hepatitis B positive; HBV-, Hepatitis B negative; ICC, Intrahepatic Cholangiocarcinoma.

Supplementary Table S2 Functional Characteristics of Gene Products				
Gene Name	MS1	TAM	MS2	Relevant Features
S100A12	+	-	-	Induces a pro-inflammatory innate immune response[37]
LYZ	+	+	-	Encodes lysozyme, upregulation correlates with poor tumor prognosis[38]
LGALS1	+	+	-	Induces TAM phenotype, promotes macrophage reprogramming and reduces inflammation[39,40]
TREM2	-	+	-	A surface lipid receptor generally expressed on scar-associated macrophages drives immunosuppression and regulates anti-inflammatory responses[40-42]
CD9	-	+	-	It is associated with scar-associated macrophages and mediates anti-inflammatory responses[43,44].
PRMT10	-	+	-	Part of the protein arginine methyltransferase family is a driving factor in cancer tumor growth and metastasis[45]
C1QA	-	+	+	Complement component C1q is expressed in healthy and tumor macrophages and contributes to immunosuppression[46,47]
C1QB	-	+	+	
C1QC	-	+	+	
CTSB	-	+	+	When located in lysosomes, it can promote autophagy and immune responses in macrophages and when secreted by TAMs drives pro-metastatic function[48,49]
APOE	-	+	+	A pivotal molecule in lipid metabolism is often expressed in M2 macrophages. In a tumor setting it influences macrophage polarization, tumor immune responses, antioxidation, tissue repair, and cellular proliferation[50,51].
APOC1	-	+	+	It is mainly expressed in macrophages, regulating macrophage polarization and TAM formation. It can also promote metastasis of tumor cells[52].
HLA-DPB1	-	+	+	A study showcased the correlation of *HLA-DPB1* expression with M2 macrophages[53].
CTSD	-	+	+	A lysosomal proteinase promotes polarization of TAMs and metastasis[54,55]
CD68	-	+	+	CD68 is overexpressed in TAMS are associated with higher tumor grade, large tumor size, and tumor progression[56]
HLA-DPA1	-	+	+	Regulates immune responses by processing and presenting protein antigens[57]
CD5L	-	-	+	Also known as apoptosis inhibitor expressed by macrophages, promotes M2 macrophage polarization and an anti-inflammatory cytokine profile[58,59]
CXCL12	-	-	+	Primarily homeostatic chemokine promotes polarization of macrophages to the M2 type[60,61]
VCAM1	-	-	+	Induces macrophage migration and alters inflammatory gene expression in macrophages[62]
LILRB5	-	-	+	Leukocyte immunoglobulin like receptor can initiate an inflammation resolution program[63]
CETP	-	-	+	CETP expressing macrophages diminished expression of pro-inflammatory genes and phagocytic activity[64]

Supplementary Table S3 Top DEGs in MS1, MS2 and TAM

Group	Gene	Score	Adjusted P-value	Mean Expression
M1	FCN1	101.95	0.00E+00	1.32
M1	S100A9	77.85	0.00E+00	2.26
M1	S100A8	72.75	0.00E+00	1.82
M1	LYZ	64.85	0.00E+00	2.97
M1	CSTA	59.66	0.00E+00	1.05
M1	VCAN	55.16	0.00E+00	0.82
M1	LGALS2	51.26	0.00E+00	0.71
M1	S100A12	50.43	0.00E+00	0.66
M1	LGALS1	49.67	0.00E+00	1.97
M1	MNDA	48.21	0.00E+00	0.85
M1	RP11-1143G9.4	46.66	0.00E+00	1.07
M1	SERPINA1	42.26	0.00E+00	1.32
M1	DIO3	41.65	0.00E+00	0
M1	TMC5	35.29	1.16E-248	0
M1	MAL2	35.1	7.16E-246	0.01
M1	MGST1	34.95	2.84E-240	0.2
M1	FAM83A	33.19	4.13E-222	0
M1	SERPINB3	33.13	7.94E-222	0
M1	CRIP1	32.88	2.03E-213	0.56
M1	SFN	32.64	1.36E-215	0.02
M1	AGR3	32.54	2.28E-213	0
M1	AKR1C2	32.5	1.65E-213	0.01
M1	TSPAN1	32.32	2.10E-211	0.01
M1	AKR1B10	32.27	5.88E-211	0.01
M1	S100A14	32.21	1.30E-210	0.01
M1	APOBEC3A	32.1	2.23E-199	0.28
M1	LST1	32.01	7.16E-208	2.31
M1	ALDH3A1	31.58	7.87E-202	0.01
M1	ELF3	31.52	7.55E-203	0.04
M1	KRT18	30.92	1.56E-195	0.08
M2	SEPP1	165.52	0.00E+00	1.5
M2	SLC40A1	119.4	0.00E+00	1.04
M2	CD5L	118.81	0.00E+00	1.05
M2	C1QA	107.58	0.00E+00	2.44
M2	C1QB	105.18	0.00E+00	2.33
M2	CD163	103.92	0.00E+00	1.11
M2	C1QC	99.21	0.00E+00	1.96
M2	LIPA	98.13	0.00E+00	1.44
M2	CXCL12	98.05	0.00E+00	0.66
M2	LGMN	89.32	0.00E+00	1.09
M2	MS4A7	88.49	0.00E+00	1.87
M2	CTSB	73.05	0.00E+00	2.23

M2	SDC3	72.17	0.00E+00	0.58
M2	AXL	71.81	0.00E+00	0.61
M2	FOLR2	71.04	0.00E+00	0.74
M2	VCAM1	69.26	0.00E+00	0.56
M2	MARCO	68.88	0.00E+00	1.09
M2	MS4A6A	68.1	0.00E+00	1.91
M2	LILRB5	66.9	0.00E+00	0.43
M2	CCL3	64.98	0.00E+00	2.06
M2	CETP	61.85	0.00E+00	0.53
M2	APOE	60.92	0.00E+00	1.69
M2	PLD3	60.2	0.00E+00	0.96
M2	FCGR3A	59.11	0.00E+00	1.44
M2	MAF	56.44	0.00E+00	0.53
M2	MS4A4A	53.92	0.00E+00	0.85
M2	CCL4L2	53.92	0.00E+00	0.88
M2	MSR1	53.75	0.00E+00	0.77
M2	C1orf54	53.42	0.00E+00	0.56
M2	VMO1	52.83	0.00E+00	0.54
TAM	TREM2	34.72	4.66E-186	0.22
TAM	GPR183	32.61	3.10E-179	0.79
TAM	OLR1	27.24	3.56E-131	0.38
TAM	APOC1	26.76	3.13E-127	1.19
TAM	TMEM176B	26.53	1.65E-129	0.9
TAM	ACP5	24.99	2.25E-113	0.42
TAM	SDS	24.72	4.75E-111	0.35
TAM	CREM	24.66	2.25E-113	0.88
TAM	ZNF331	24.39	2.97E-111	0.71
TAM	PLAUR	23.55	4.99E-106	1.4
TAM	SLC6A19	22.85	8.39E-100	0
TAM	C15orf48	22.63	1.62E-97	0.6
TAM	CH17-360D5.2	22.62	6.32E-98	0
TAM	CD9	21.11	1.05E-84	0.26
TAM	PRMT10	20.48	3.32E-80	0.15
TAM	LGALS3	19.97	2.17E-78	1.11
TAM	IL8	19.89	1.93E-77	0.8
TAM	IGLL5	19.64	1.24E-74	0.31
TAM	APOE	19.15	2.44E-72	1.69
TAM	CXCL2	18.82	2.52E-70	0.8
TAM	C1QC	18.53	7.58E-70	1.96
TAM	HLA-DPB1	18.04	6.52E-67	2.86
TAM	CTSD	17.96	5.28E-65	1.86
TAM	PLIN2	17.92	1.45E-64	0.91
TAM	CD68	17.42	2.57E-62	1.64
TAM	CXCL3	17.07	1.45E-58	0.58

TAM	HLA-DQB1	17.04	3.02E-59	1.57
TAM	LGALS1	16.99	1.23E-59	1.97
TAM	RGCC	16.8	5.74E-57	0.37
TAM	HLA-DPA1	16.73	2.62E-58	2.72