

## Supporting Information

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Effects of 3-HAA on HCC by Regulating the Heterogeneous Macrophages—A scRNA-Seq Analysis

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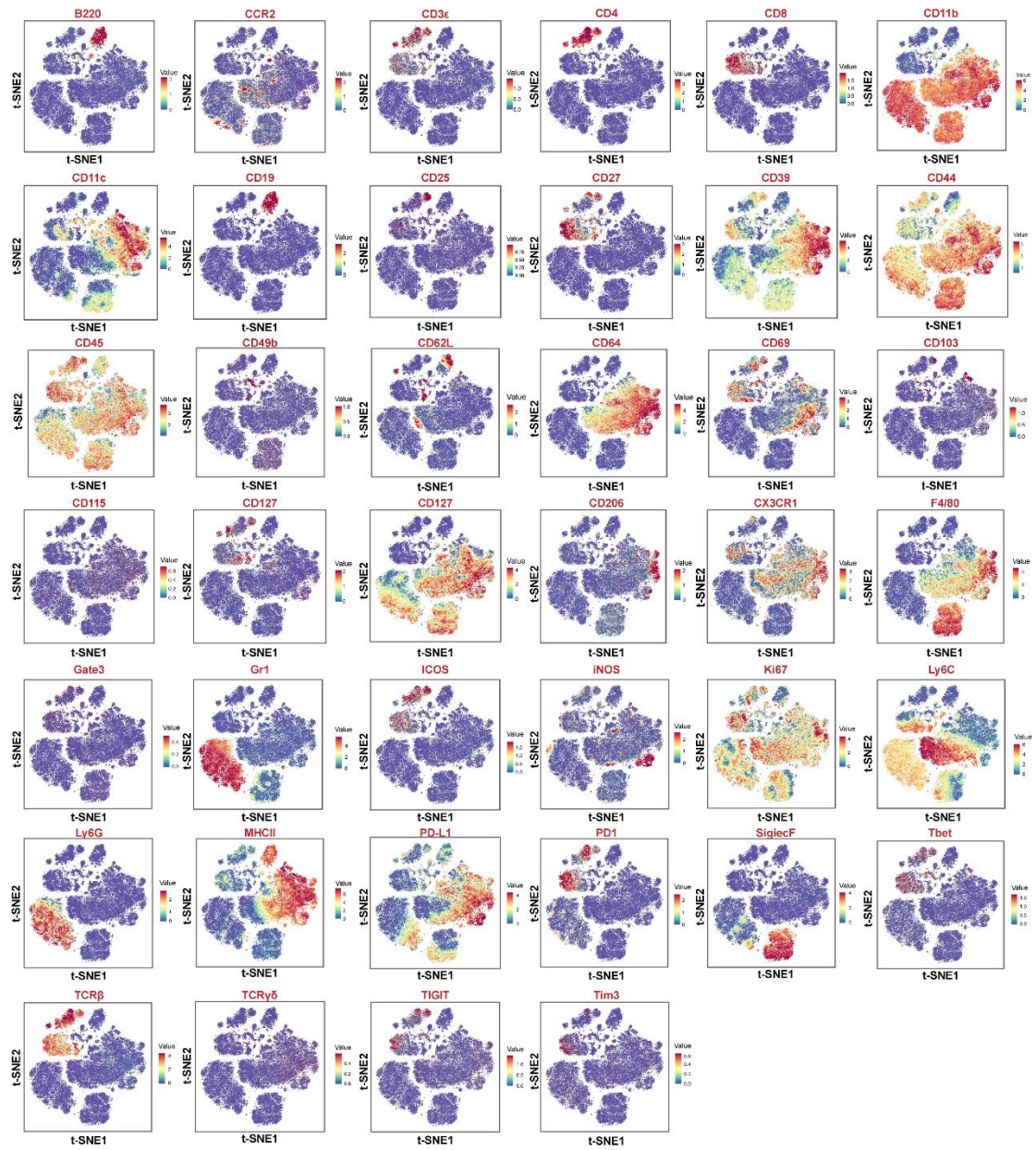
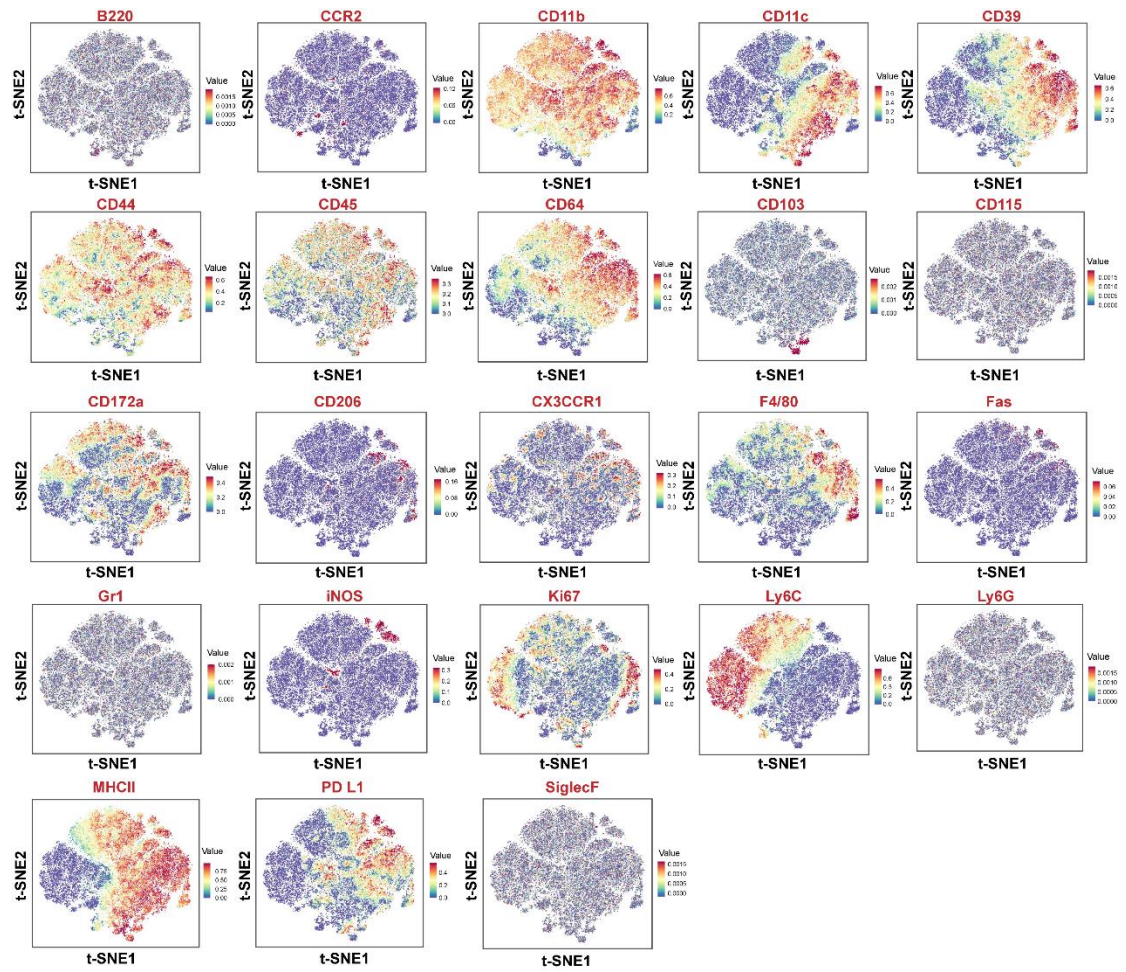
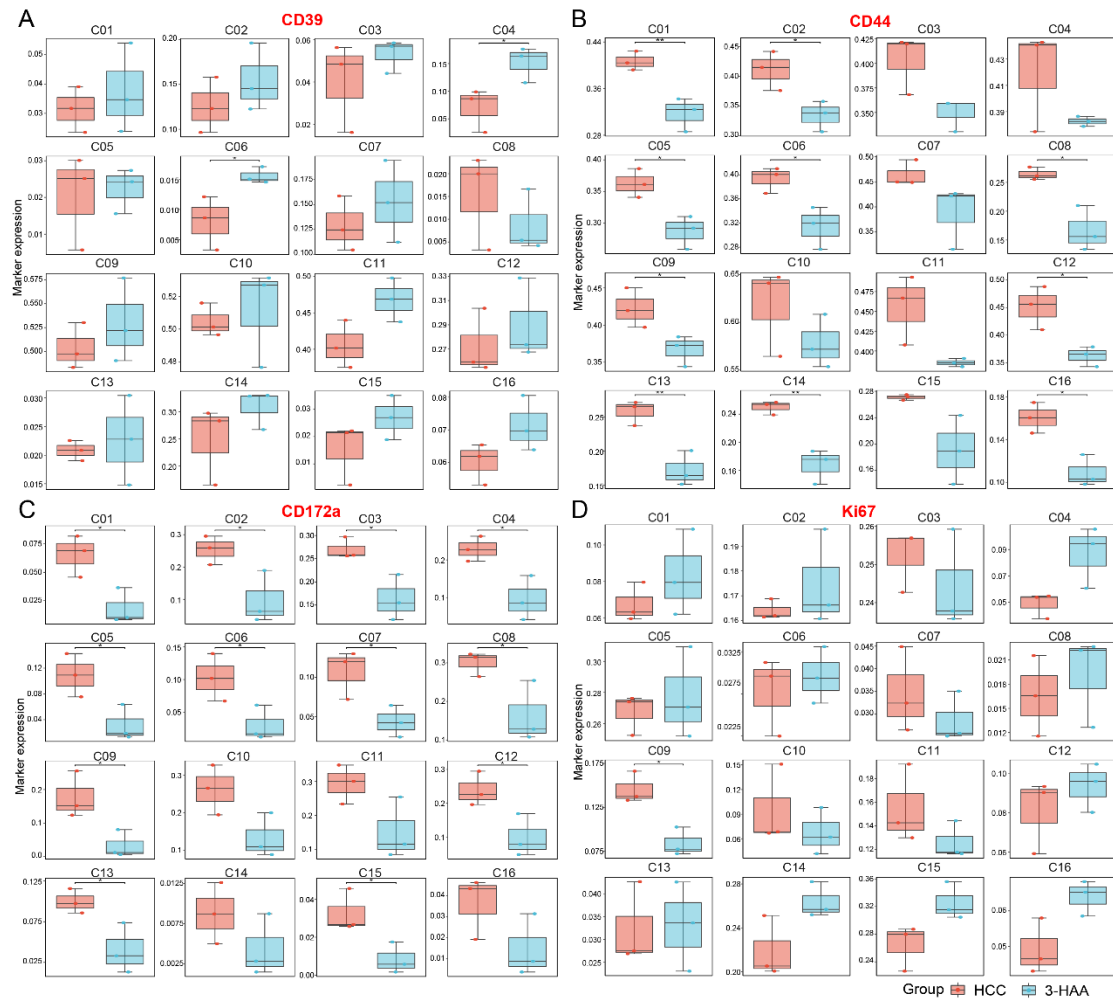


Figure S1. t-SNE maps of 40 selected panel markers.



**Figure S2. t-SNE maps of 23 special markers representing myeloid populations.**



**Figure S3. Bar plots of differently expressed functional markers in myeloid populations.**

**Table S1. The details of all immune cell clusters, cell types, and subtypes were analyzed by CyTOF**

Cell types	Clusters	Marker expression
CD4 <sup>+</sup> TN	C01	CD3e <sup>+</sup> TCRβ <sup>+</sup> CD27 <sup>+</sup> CD62L <sup>+</sup> CD127 <sup>+</sup> CD4 <sup>+</sup>
CD8 <sup>+</sup> TN	C02	CD103 <sup>+</sup> TCRβ <sup>+</sup> CD27 <sup>+</sup> CD62L <sup>+</sup> CD127 <sup>+</sup> CD8 <sup>+</sup>
CD4 <sup>+</sup> TE	C03	TCRβ <sup>+</sup> CD127 <sup>+</sup> CD4 <sup>+</sup>
CD4 <sup>+</sup> Treg	C04	CD3e <sup>+</sup> ICOS <sup>+</sup> Ki67 <sup>+</sup> CD39 <sup>+</sup> TIGIT <sup>+</sup> TCRβ <sup>+</sup> CD69 <sup>+</sup> CD25 <sup>+</sup> CD27 <sup>+</sup> MHCII <sup>+</sup> CD4 <sup>+</sup>
CD4 <sup>+</sup> TE	C05	CD3e <sup>+</sup> PD1 <sup>+</sup> CX3CR1 <sup>+</sup> ICOS <sup>+</sup> Ki67 <sup>+</sup> CD39 <sup>+</sup> TIGIT <sup>+</sup> TCRβ <sup>+</sup> CD69 <sup>+</sup> CD27 <sup>+</sup> MHCII <sup>+</sup> CD4 <sup>+</sup>
CD4 <sup>+</sup> TE	C06	PD1 <sup>+</sup> ICOS <sup>+</sup> CD39 <sup>+</sup> TCRβ <sup>+</sup> CD69 <sup>+</sup> CD4 <sup>+</sup>
B cells	C07	CD19 <sup>+</sup> B220 <sup>+</sup> CD62L <sup>+</sup> MHCII <sup>+</sup>
B cells	C08	CD19 <sup>+</sup> B220 <sup>+</sup> MHCII <sup>+</sup>
B cells	C09	CD19 <sup>+</sup> B220 <sup>+</sup> MHCII <sup>+</sup>
DC	C10	Ki67 <sup>+</sup> CD103 <sup>+</sup> CD39 <sup>+</sup> CD11c <sup>+</sup> PD-L1 <sup>+</sup> MHCII <sup>+</sup>
B cells	C11	Ly6C <sup>+</sup> Fas <sup>+</sup> Ki67 <sup>+</sup> CD39 <sup>+</sup> TIGIT <sup>+</sup> CD69 <sup>+</sup> PD-L1 <sup>+</sup> MHCII <sup>+</sup> CD11b <sup>+</sup>
Macrophage	C12	CX3CR1 <sup>+</sup> CD39 <sup>+</sup> F4/80 <sup>+</sup> CD64 <sup>+</sup> CD206 <sup>+</sup> PD-L1 <sup>+</sup> MHCII <sup>+</sup> CD11b <sup>+</sup>
Macrophage	C13	Fas <sup>+</sup> CX3CR1 <sup>+</sup> CD39 <sup>+</sup> F4/80 <sup>+</sup> iNOS <sup>+</sup> CD11c <sup>+</sup> CD64 <sup>+</sup> PD-L1 <sup>+</sup> CD172a <sup>+</sup> MHCII <sup>+</sup> CD11b <sup>+</sup>
Macrophage	C14	Ly6C <sup>+</sup> CX3CR1 <sup>+</sup> Ki67 <sup>+</sup> CD39 <sup>+</sup> F4/80 <sup>+</sup> CD69 <sup>+</sup> CD11c <sup>+</sup> CD64 <sup>+</sup> PD-L1 <sup>+</sup> CD172a <sup>+</sup> MHCII <sup>+</sup> CD11b <sup>+</sup>
NK	C15	CD49b <sup>+</sup> Ki67 <sup>+</sup> PD-L1 <sup>+</sup> CD11b <sup>+</sup>

Cell types	Clusters	Marker expression
NK	C16	CD49b <sup>+</sup> CD69 <sup>+</sup> CD11c <sup>+</sup> CD27 <sup>+</sup> CD62L <sup>+</sup> CD11b <sup>+</sup>
Others	C17	Ly6C <sup>+</sup> Ki67 <sup>+</sup> PD-L1 <sup>+</sup>
Neutrophils	C18	Ly6C <sup>+</sup> Gr1 <sup>+</sup> Ly6G <sup>+</sup> Ki67 <sup>+</sup> CD39 <sup>+</sup> CD172a <sup>+</sup> CD11b <sup>+</sup>
Eosinophils	C19	Ly6C <sup>+</sup> Ki67 <sup>+</sup> CD39 <sup>+</sup> F4/80 <sup>+</sup> CD172a <sup>+</sup> SiglecF <sup>+</sup> CD11b <sup>+</sup>
Eosinophils	C20	CD39 <sup>+</sup> F4/80 <sup>+</sup> CD11c <sup>+</sup> PD-L1 <sup>+</sup> CD172a <sup>+</sup> SiglecF <sup>+</sup> CD11b <sup>+</sup>
Eosinophils	C21	Ly6C <sup>+</sup> CD39 <sup>+</sup> F4/80 <sup>+</sup> CD11c <sup>+</sup> PD-L1 <sup>+</sup> CD172a <sup>+</sup> SiglecF <sup>+</sup> CD11b <sup>+</sup>
Macrophage	C22	Ly6C <sup>+</sup> CX3CR1 <sup>+</sup> Ki67 <sup>+</sup> CD39 <sup>+</sup> F4/80 <sup>+</sup> CD64 <sup>+</sup> CD172a <sup>+</sup> MHCI I <sup>+</sup> CD11b <sup>+</sup>
Macrophage	C23	Ly6C <sup>+</sup> F4/80 <sup>+</sup> CD64 <sup>+</sup> CD172a <sup>+</sup> CD11b <sup>+</sup>
Macrophage	C24	Ly6C <sup>+</sup> CX3CR1 <sup>+</sup> F4/80 <sup>+</sup> CD64 <sup>+</sup> CD172a <sup>+</sup> CD11b <sup>+</sup>
NK	C25	CD39 <sup>+</sup> CD69 <sup>+</sup> CD11c <sup>+</sup>
NK	C26	Ly6C <sup>+</sup> Tbet <sup>+</sup> CX3CR1 <sup>+</sup> CD49b <sup>+</sup> CD11c <sup>+</sup> CD11b <sup>+</sup>
pDC	C27	Ly6C <sup>+</sup> CD39 <sup>+</sup> B220 <sup>+</sup> CD11c <sup>+</sup> CD172a <sup>+</sup> MHCII <sup>+</sup> CD4 <sup>+</sup>
Others	C28	Ly6C <sup>+</sup> Ki67 <sup>+</sup> CD11c <sup>+</sup>
$\gamma\delta$ T	C29	Ly6C <sup>+</sup> TCR $\gamma\delta$ <sup>+</sup> CD27 <sup>+</sup> CD62L <sup>+</sup> CD127 <sup>+</sup>
CD8 <sup>+</sup> T	C30	Ly6C <sup>+</sup> TCR $\beta$ <sup>+</sup> CD27 <sup>+</sup> CD62L <sup>+</sup> CD127 <sup>+</sup>
CD8 <sup>+</sup> TN	C31	Ly6C <sup>+</sup> PD1 <sup>+</sup> CX3CR1 <sup>+</sup> Ki67 <sup>+</sup> CD39 <sup>+</sup> TCR $\beta$ <sup>+</sup> CD69 <sup>+</sup> CD27 <sup>+</sup> MHCII <sup>+</sup> CD8 <sup>+</sup>
CD8 <sup>+</sup> TN	C32	Ly6C <sup>+</sup> CD39 <sup>+</sup> TCR $\beta$ <sup>+</sup> CD69 <sup>+</sup> CD11c <sup>+</sup>



**Table S2. The details of all myeloid cell clusters, cell types, and subtypes were analyzed by CyTOF**

Cell types	Subtypes	Clusters	Marker expression
macrophage		C01	Ly6C <sup>+</sup> MHCII <sup>+</sup> CD11b <sup>+</sup> CD44 <sup>+</sup>
macrophage		C02	Ly6C <sup>+</sup> MHCII <sup>+</sup> CD11b <sup>+</sup> CD64 <sup>+</sup> CD44 <sup>+</sup>
macrophage		C03	Ly6C <sup>+</sup> MHCII-Ki67 <sup>+</sup> CD11b <sup>+</sup> CD64 <sup>+</sup> CD172a <sup>+</sup> CD44 <sup>+</sup>
macrophage		C04	Ly6C <sup>+</sup> MHCII-Ki67 <sup>-</sup> CD11b <sup>+</sup> CD64 <sup>+</sup> CD172a <sup>+</sup> CD44 <sup>+</sup>
macrophage		C05	Ly6C <sup>+</sup> MHCII-Ki67 <sup>+</sup> CD11b <sup>+</sup> CD44 <sup>+</sup>
macrophage		C06	Ly6C <sup>+</sup> MHCII-CD11b <sup>+</sup> CD44 <sup>+</sup>
macrophage		C07	CD11b <sup>+</sup> CD64 <sup>+</sup> CD44 <sup>+</sup>
macrophage		C08	CD11c <sup>+</sup> CD11b <sup>+</sup> CD172a <sup>+</sup>
macrophage	M2	C09	F4/80 <sup>+</sup> CD64 <sup>+</sup> CD39 <sup>+</sup> CD44 <sup>+</sup>
macrophage	M1	C10	F4/80 <sup>+</sup> CD64 <sup>+</sup> CD39 <sup>+</sup> CD44 <sup>+</sup> CD11b <sup>+</sup> iNOS <sup>+</sup> PD-L1 <sup>+</sup>
macrophage		C11	F4/80 <sup>+</sup> CD64 <sup>+</sup> CD39 <sup>+</sup> CD44 <sup>+</sup> CD11b <sup>+</sup> CD11c <sup>+</sup> PD-L1 <sup>+</sup>
macrophage		C12	CD64 <sup>+</sup> CD39 <sup>+</sup> CD44 <sup>+</sup> CD11b <sup>+</sup> CD11c <sup>+</sup>
pDC		C13	CD11c <sup>+</sup> MHCII <sup>+</sup> Ly6C <sup>+</sup>
cDC		C14	CD11c <sup>+</sup> MHCII <sup>+</sup> Ki67 <sup>+</sup> CD39 <sup>+</sup> CD103 <sup>+</sup>
cDC		C15	CD11c <sup>+</sup> MHCII <sup>+</sup> Ki67 <sup>+</sup>
cDC		C16	CD11c <sup>+</sup> MHCII <sup>+</sup> PD-L1 <sup>+</sup>

**Table S3. The details of all myeloid cell clusters, cell types, and subtypes were analyzed by scRNA-seq**

<b>Clusters</b>	<b>Cell types</b>	<b>Subtypes</b>	<b>Key Marker</b>
Cluster0	M2-Macrophages	Fn1 <sup>+</sup> M2	Arg1 <sup>+</sup> Cd14 <sup>+</sup> Lyz2 <sup>+</sup>
Cluster01	M1-Macrophages	Cxcl9 <sup>+</sup> M1	H2-Dmb1 <sup>+</sup> Lyz2 <sup>+</sup>
Cluster02	M2-Macrophages	Arg1 <sup>+</sup> M2	Arg1 <sup>+</sup> Lyz2 <sup>+</sup>
Cluster03	Monocytes	Chil3 <sup>+</sup> Monocytes	Chil3 <sup>+</sup> Ly6c2 <sup>+</sup> Lyz2 <sup>+</sup>
Cluster04	Resident Macrophages	CD81 <sup>+</sup> Macrophages	Cd81 <sup>+</sup> C1qa <sup>+</sup> Lyz2 <sup>+</sup>
Cluster05	cDC	CD209a <sup>+</sup> DC	Cd209a <sup>+</sup> H2-Aa <sup>+</sup> Clec10a <sup>+</sup>
Cluster06	Macrophages	Ace <sup>+</sup> Macrophages	Cd300a <sup>+</sup> Lyz2 <sup>+</sup>
Cluster07	Proliferating Macrophages	Mki67 <sup>+</sup> Macrophages	Mki67 <sup>+</sup> Lyz2 <sup>+</sup>
Cluster08	Migratory cDC	Ccr7 <sup>+</sup> Ccl5 <sup>+</sup> DC	Ccr7 <sup>+</sup> Ccl5 <sup>+</sup> Tbc1d4 <sup>+</sup> Cst3 <sup>+</sup>
Cluster09	Monocytes	Ace <sup>+</sup> Monocytes	Ace <sup>+</sup> Ly6c2 <sup>+</sup>
Cluster10	Kuffer cells	Clec4f <sup>+</sup> Kuffer cells	Clec4f <sup>+</sup>
Cluster11	pDC	Siglech <sup>+</sup> pDC	Siglecf <sup>+</sup>
Cluster12	Proliferating Macrophages	Mcm3 <sup>+</sup> Macrophages	Mcm3 <sup>+</sup> Mcm6 <sup>+</sup> C1qa <sup>+</sup>
Cluster13	cDC	Xcr1 <sup>+</sup> cDC	Xcr1 <sup>+</sup> Cst3 <sup>+</sup> Clec9a <sup>+</sup>
Cluster14	M2-Macrophages	Arg1 <sup>+</sup> Saa3 <sup>+</sup> M2	Arg1 <sup>+</sup> C1qa <sup>+</sup>
Cluster15	Proliferating cDC	Mcm3 <sup>+</sup> cDC	Mcm3 <sup>+</sup> Mcm6 <sup>+</sup> CST3 <sup>+</sup>
Cluster16	pDC	CD209d <sup>+</sup> pDC	Cd209d <sup>+</sup> Cd209a <sup>+</sup> Siglech <sup>+</sup>

**Table S4. The Quantibody Mouse Inflammation Array Kit contains information of 40 cytokines**

Name	Average expression		Fold change	logFC	P-value	Adjust P-value
	3-HAA group	HCC group				
TIMP-1	12.75564	10.710364	4.12752	2.045275	1.43E-11	5.73E-10
TNFa	4.377788	6.538326	0.223673	-2.16054	7.33E-11	1.47E-09
BLC	11.71714	9.799796	3.777275	1.917346	8.98E-07	1.20E-05
GM-CSF	2.810518	4.086877	0.412836	-1.27636	3.72E-05	0.000305109
MCP-5	5.612818	4.620736	1.989053	0.992081	3.81E-05	0.000305109
IL-5	5.363004	6.441348	0.473572	-1.07834	0.000116	0.000775834
RANTES	6.002099	4.510055	2.812873	1.492044	0.000259	0.001480086
IL-6	7.206499	5.501432	3.260441	1.705067	0.000511	0.002553632
Leptin	10.37657	9.113039	2.400821	1.263528	0.001701	0.007558416
IL-2	3.152177	4.078661	0.526139	-0.92648	0.00256	0.010056094
MIG	9.073243	7.526394	2.921782	1.546848	0.002765	0.010056094
TNF RII	6.544429	7.504444	0.514052	-0.96002	0.011813	0.039375828
MCP-1	5.062386	6.435346	0.386098	-1.37296	0.015084	0.046413391
PF4	11.29392	11.895787	0.6589	-0.60187	0.01782	0.050914471
MCSF	3.313862	2.305273	2.011943	1.00859	0.021437	0.057164209
IL-3	3.171101	2.189877	1.97414	0.981224	0.02646	0.066151083
IL-21	7.125611	6.458344	1.588062	0.667267	0.032672	0.076875037
MIP-1g	8.509542	8.723811	0.861983	-0.21427	0.056067	0.124593099

Name	Average expression		Fold change	logFC	P-value	Adjust P-value
	3-HAA group	HCC group				
Eotaxin-2	3.78015	3.143810	1.55438	0.63634	0.072737	0.148316922
IL-4	2.607128	3.039943	0.740815	-0.43282	0.080715	0.148316922
IL-17	3.903029	3.142582	1.694015	0.760447	0.081574	0.148316922
TARC	4.876337	4.293003	1.498308	0.583334	0.076627	0.148316922
Eotaxin	6.139995	5.851608	1.221274	0.288387	0.08905	0.148416743
KC	5.609233	5.038121	1.485668	0.571112	0.085922	0.148416743
IL-12p70	4.566027	3.846635	1.646489	0.719393	0.109385	0.175015778
IL-13	6.870799	6.282661	1.503306	0.588139	0.187424	0.288344496
G-CSF	7.973006	8.645769	0.627304	-0.67276	0.211464	0.313280535
IL-1a	2.885475	2.655678	1.17267	0.229797	0.300054	0.428648632
IL-7	8.934043	8.484258	1.365837	0.449785	0.341399	0.470895216
IL-10	5.228037	5.511335	0.821711	-0.2833	0.365852	0.487802505
CD30L	2.459745	2.074961	1.305665	0.384785	0.40115	0.517612996
Fas L	6.232679	5.596861	1.553818	0.635818	0.457481	0.570968336
IL-1b	4.044307	4.401684	0.780583	-0.35738	0.471049	0.570968336
TCA-3	3.535392	3.689248	0.898845	-0.15386	0.565541	0.665341843
LIX	10.47742	10.176661	1.231789	0.300755	0.665115	0.739863335
MIP-1a	2.936758	2.707100	1.172557	0.229658	0.665877	0.739863335
IL-15	10.84046	11.229492	0.763641	-0.38903	0.756719	0.818075035

Name	Average expression		Fold change	logFC	P-value	Adjust P-value
	3-HAA group	HCC group				
ICAM-1	9.81125	9.767351	1.030897	0.0439	0.782412	0.823591896
IFNg	5.576423	5.536569	1.02801	0.039854	0.898118	0.921147128
TNF RI	7.087993	7.098878	0.992484	-0.01088	0.937442	0.937442013