

**Supplementary Materials for
Novel Molecular Subgroups of Intrahepatic Cholangiocarcinoma
Discovered by Single-Cell RNA Sequencing-Assisted Multi-Omics
Analysis**

Xuanwen Bao¹, Qiong Li¹, Jinzhang Chen², Diyu Chen³, Chanqi Ye¹, Xiaomeng Dai¹, Yanfang Wang⁴, Xin Li⁵, Xiaoxiang Rong², Fei Cheng⁶, Ming Jiang⁷, Zheng Zhu⁸, Yongfeng Ding¹, Rui Sun^{9,10}, Lingling Huang¹¹, Wei Wu¹, Yixuan Guo¹, Jiangchao Wu¹², Wenguang Fu¹³, Vincent Tano¹⁴, Weijia Fang¹, Tiannan Guo^{9,10*}, Jianpeng Sheng^{12*}, Peng Zhao^{1*}, Jian Ruan^{1*}

These authors contributed equally to this work: Xuanwen Bao, Qiong Li, Jinzhang Chen, Diyu Chen, Chanqi Ye.

*Corresponding authors:

Jian Ruan, E-mail: software233@zju.edu.cn.

Peng Zhao, E-mail: zhaop@zju.edu.cn.

Jianpeng Sheng, E-mail: shengjp@zju.edu.cn.

Tiannan Guo, E-mail: guotiannan@westlake.edu.cn.

FIGURE S1

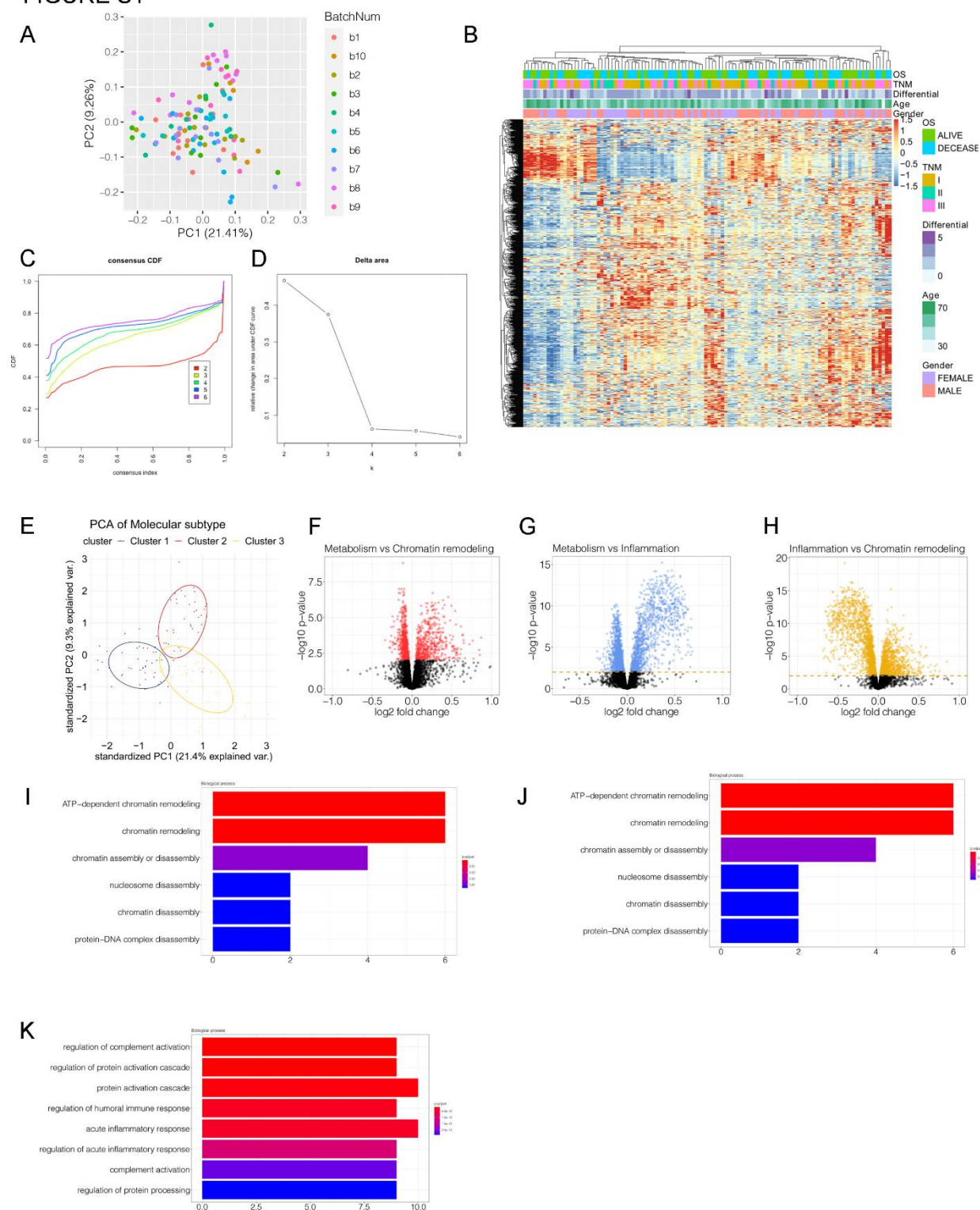
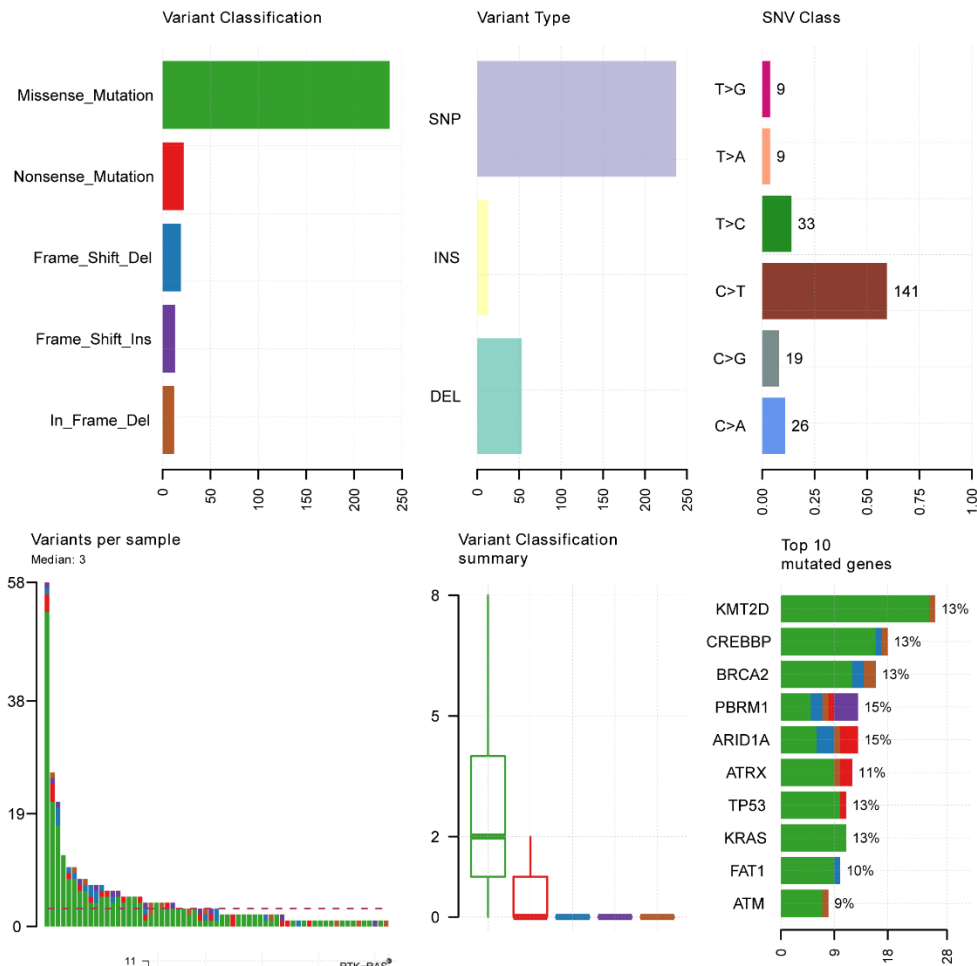


Fig. S1.

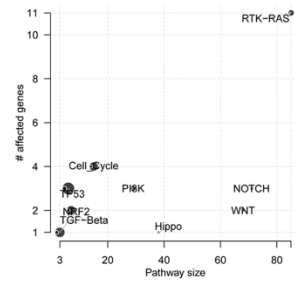
Comparison between the three molecular subtypes. (A) Heatmap of the proteomic data. **(B)** Relationship between CDF and consensus index. **(C)** Relationship between the clusters numbers and the area under the CDF curve. **(D)** The PCA analysis suggests a clear distribution of samples within each molecular class. **(E-G)** Volcano plot showing the DEGs between two molecular subtypes.

FIGURE S2

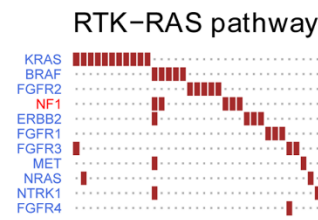
A



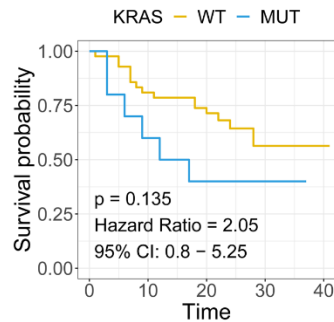
B



C



D



E

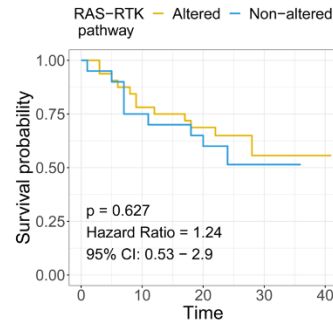


Fig. S2.

Mutation landscape and KRAS mutation. (A) Summary of the mutation landscape. (B) RTK-RAS pathway was enriched in ICC. (C) Mutation frequency of the genes involved in the RTK-RAS pathway. (D) Kaplan-Meier curve of KRAS mutations and OS. (E) Kaplan-Meier curve of RAS-RTK pathway alterations and OS.

FIGURE S3

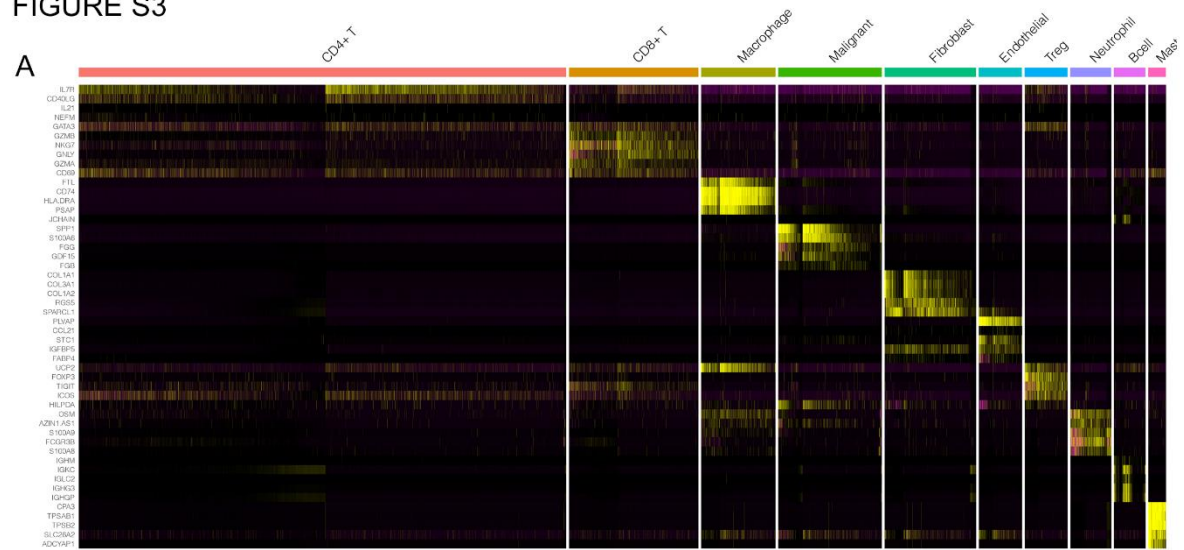


Fig. S3.

Panel sequencing for the KMT2Dmut tissues (A) and heatmap of the feature genes in each cell type (B).

FIGURE S4

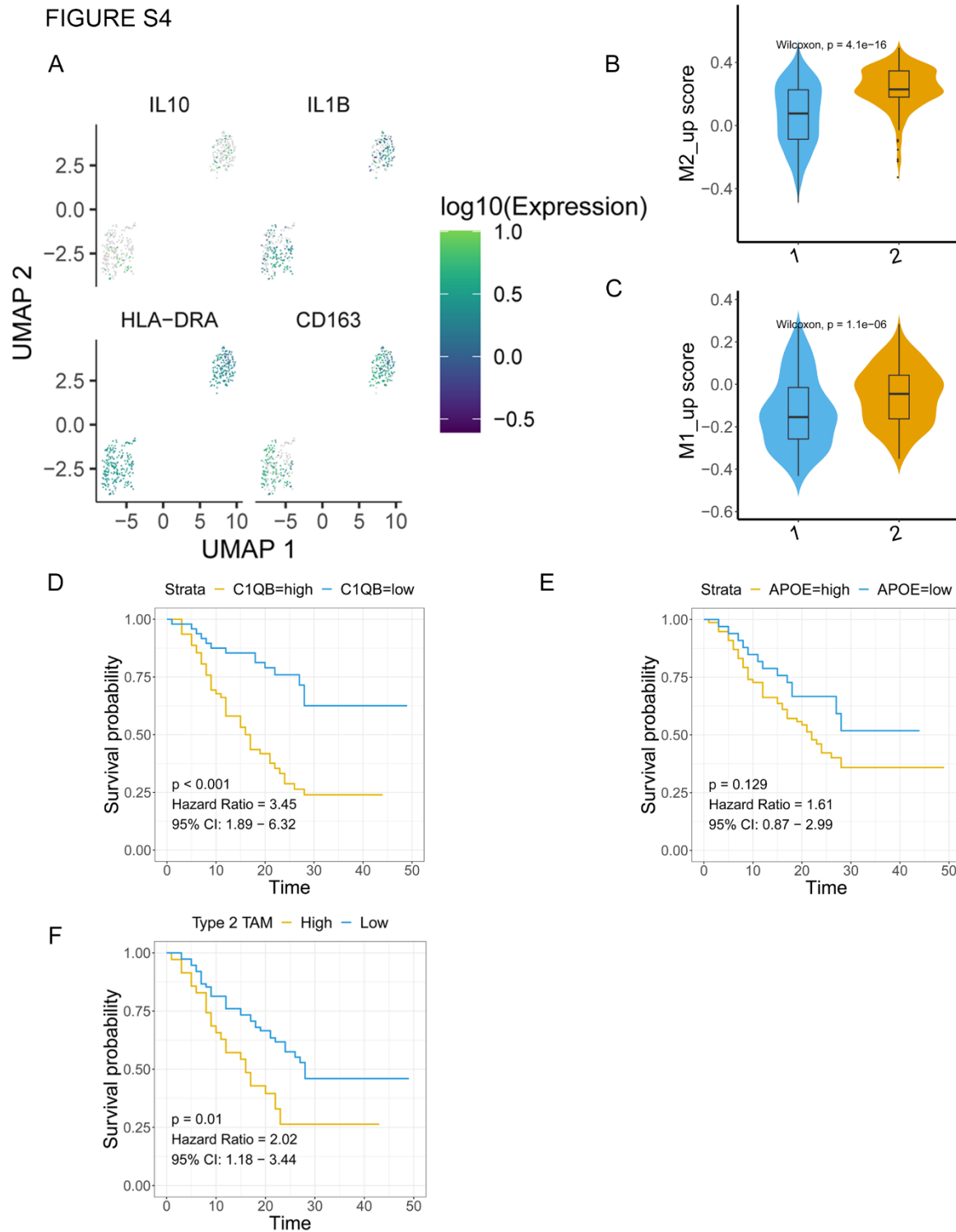


Fig. S4.

(A) UMAP plot of macrophages with their gene expression level. **(B)** M2 score of type 1 and type 2 TAMs. **(C)** M1 score of type 1 and type 2 TAMs. **(D)** Kaplan-Meier curve of the C1QB

expression and OS (n=110). **(E)** Kaplan-Meier curve of the APOE expression and OS (n=110). **(F)** APOE⁺C1QB⁺ TAM signature scores stratified patients with poor prognoses.

Table S1.**Patient demographics**

Characteristics		Number of patients (n = 110)
Gender		
	Male	61 (55.5%)
	Female	49 (45.5%)
Median age (years)		60 (54-66)
Differential level		
	0	2 (1.8%)
	1	23 (20.9%)
	2	33 (30.0 %)
	3	42 (38.2%)
	4	6 (5.5%)
	5	4 (3.6%)
HBV		
	Negative	17 (15.5%)
	Positive	93 (84.5%)
Stage		
	I	50 (45.5%)
	II	18 (16.3%)
	III	42 (38.2%)
OS (months)		20 (12-27)
Status		
	Alive	52 (47.3%)
	Deceased	48 (52.7%)

Data are median (IQR) or n (%). HBV=hepatitis B virus. OS=overall survival

Table S2.**Hub-gene list**

Hub-gene list
<i>LUZP1</i>
<i>NIPSNAP1</i>
<i>ATP5F1A</i>
<i>CRYZ</i>
<i>XPNPEP3</i>
<i>AHCY</i>
<i>CARHSP1</i>
<i>ATP5PO</i>
<i>GLO1</i>
<i>NUDT5</i>
<i>CNDP2</i>
<i>MAT2A</i>
<i>PALM3</i>
<i>KLC1</i>
<i>HDDC3</i>
<i>TRAP1</i>
<i>PSMA7</i>
<i>THOP1</i>
<i>SLCO2B1</i>
<i>SH3PXD2B</i>
<i>UROD</i>
<i>PHLDB1</i>
<i>ZYX</i>
<i>ARHGAP18</i>
<i>PSMA1</i>
<i>ATP5PD</i>
<i>MYO5A</i>
<i>ABCB11</i>
<i>CAPG</i>
<i>MYG1</i>
<i>SCAMP2</i>
<i>C1R</i>
<i>VPS13A</i>
<i>TK2</i>
<i>HSPA12B</i>
<i>C2</i>
<i>MTA2</i>

PLXNB1
CEPT1
GSPT2
INTS10
FUBP1
C8A
SMARCE1
PROS1
ALG11
CFH
RO60
SMARCC2
HDAC1
C6
RPN2
C1S
SLC30A1
A1BG
ACTL6A
SPAG7
KNG1
SLC9A3R1
MRPL18
SLC27A4
BUB3
ITIH4
RALY
ATIC
SEC61B
L1CAM
PDLIM2
HDGF
ATXN10
LBR
NMT1
SMARCA5
PSPC1
PPM1G
C3
SERPIND1

C5

ANP32A

SON

SUB1

DDX5

NCOA5

SYTL4

SAE1

Table S3.

	Tumor_Sample_ Barcode	Start_Pos ition	End_Pos ition	Reference_ Allele	Tumor_Seq_ Allele2	Transcri pt	Protein_Ch ange	Variant_Classi fication
117 60	180555107T1	4943118 1	4943118 1	C	T	NM_00 3482	p.A3320T	Missense_Mut ation
132 02	180555111T1	4942676 6	4942677 1	GTTGCA	-	NM_00 3482	p.3906_39 08del	In_Frame_Del
200 26	180558018T1	4943527 0	4943527 0	G	A	NM_00 3482	p.R2095C	Missense_Mut ation
200 27	180558018T1	4943858 9	4943858 9	G	A	NM_00 3482	p.A1634V	Missense_Mut ation
200 28	180558018T1	4944600 3	4944600 3	G	A	NM_00 3482	p.P488L	Missense_Mut ation
279 90	180558022T1	4941560 7	4941560 7	G	A	NM_00 3482	p.P5524S	Missense_Mut ation
279 91	180558022T1	4942416 8	4942416 8	G	A	NM_00 3482	p.P4632S	Missense_Mut ation
279 92	180558022T1	4943481 3	4943481 3	G	A	NM_00 3482	p.P2247L	Missense_Mut ation
279 93	180558022T1	4943854 6	4943854 6	A	C	NM_00 3482	p.D1648E	Missense_Mut ation
389 08	180558031T1	4943433 7	4943433 7	C	T	NM_00 3482	p.D2406N	Missense_Mut ation
466 31	180558035T1	4942553 0	4942553 0	G	A	NM_00 3482	p.P4320S	Missense_Mut ation
516 30	180558037T1	4943478 1	4943478 1	G	A	NM_00 3482	p.P2258S	Missense_Mut ation
650 01	180558041T1	4942077 7	4942077 7	C	T	NM_00 3482	p.R4991Q	Missense_Mut ation
674 49	180558042T1	4943588 7	4943588 7	T	G	NM_00 3482	p.K2032Q	Missense_Mut ation
806 61	180558043T1	4942081 7	4942081 7	G	A	NM_00 3482	p.R4978C	Missense_Mut ation
806 62	180558043T1	4942697 6	4942697 6	G	A	NM_00 3482	p.P3838S	Missense_Mut ation
806 63	180558043T1	4943141 4	4943141 4	G	A	NM_00 3482	p.A3242V	Missense_Mut ation
806 64	180558043T1	4943237 1	4943237 1	C	T	NM_00 3482	p.G2923D	Missense_Mut ation
806 65	180558043T1	4943306 6	4943306 6	C	T	NM_00 3482	p.D2769N	Missense_Mut ation
806 66	180558043T1	4943521 2	4943521 2	C	T	NM_00 3482	p.G2114D	Missense_Mut ation
806 68	180558043T1	4944418 0	4944418 0	A	G	NM_00 3482	p.V1064A	Missense_Mut ation
806 69	180558043T1	4944487 8	4944487 8	C	T	NM_00 3482	p.R863Q	Missense_Mut ation
806 70	180558043T1	4944499 3	4944499 3	A	G	NM_00 3482	p.S825P	Missense_Mut ation
806 71	180558043T1	4944537 0	4944537 0	G	A	NM_00 3482	p.S699F	Missense_Mut ation
934 58	180558044T1	4942769 9	4942769 9	T	C	NM_00 3482	p.N3597D	Missense_Mut ation
934 60	180558044T1	4943187 4	4943187 4	C	T	NM_00 3482	p.V3089M	Missense_Mut ation

Table S4.**APOE⁺C1QB⁺ macrophage signature**

Gene signature
<i>LIPA</i>
<i>APOE</i>
<i>C1QB</i>
<i>CTSD</i>
<i>CPM</i>
<i>APOC1</i>
<i>IGSF6</i>
<i>ACP5</i>
<i>CTSL</i>
<i>LGMN</i>
<i>HLA-DQA1</i>
<i>PDK4</i>
<i>PLA2G7</i>
<i>KLF2</i>
<i>APOC1P1</i>
<i>ALDH1A1</i>
<i>TXNIP</i>
<i>SCD</i>
<i>PLIN2</i>
<i>EGR1</i>