

Supplementary Materials

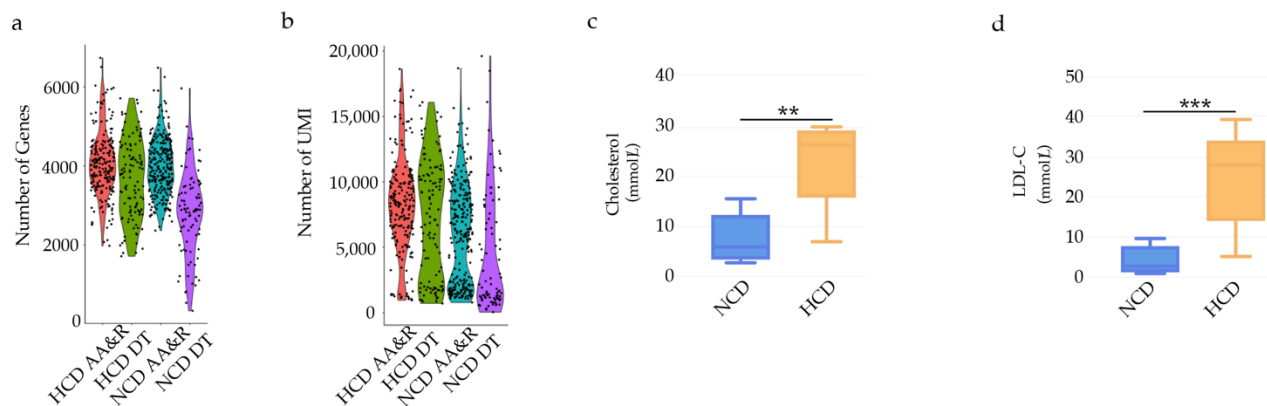


Figure S1. Quality control of the profiled scRNAseq transcriptomes of CD45⁺ cells derived from atherosclerosis-prone AA&R, and atherosclerosis-resistant DT aorta of *Apoe*^{-/-} mice on NCD and HCD and cholesterol levels. (a) Violin plots showing the quality control metrics of a number of genes per cell and (b) unique molecular identifiers (UMI) per cell, $n = 6$ mice. (c) Bar graphs represent the mean \pm SEM (c) cholesterol; (d) LDL-C, $n = 6$ mice/group, $**p < 0.01$ and $***p < 0.001$.

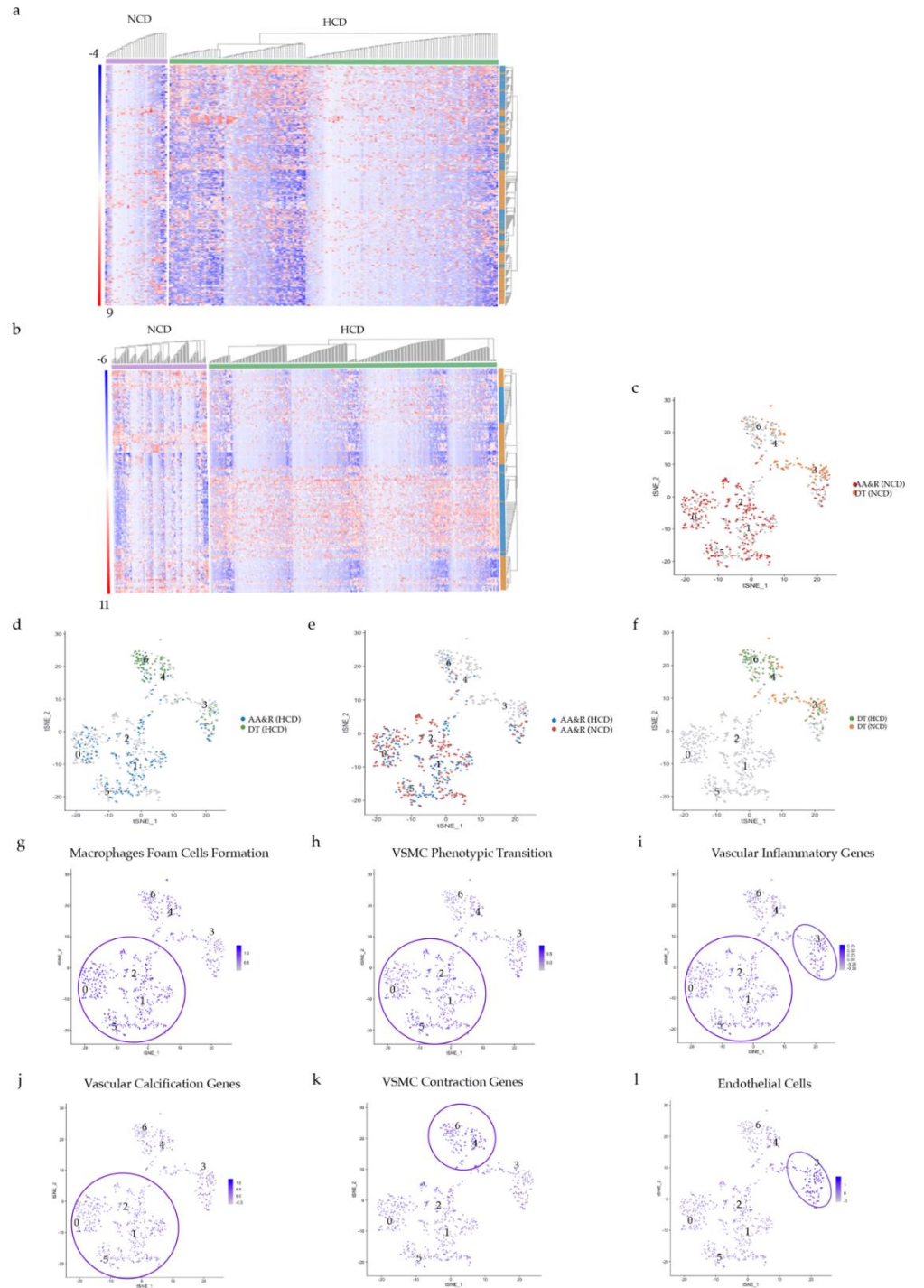


Figure S2. (a) Heatmap showing the expression of genes CD45⁺ cells derived from AA&R of *Apoe*^{-/-} on NCD versus HCD (HCD, red side strip or NCD, purple side strip) (p-Adj<0.05), *n*=6 mice/group. Rows represent genes and columns represent cells. (b) Heatmap showing the expression of genes CD45⁺ cells derived from DT of *Apoe*^{-/-} on NCD versus HCD (HCD, red side strip or NCD, purple side strip) (p-Adj<0.05), *n*=6 mice/group. Rows represent genes and columns represent cells. t-distributed stochastic neighbor embedding (tSNE) plot showing: (c) AA&R and DT aorta clusters of *Apoe*^{-/-} mice on NCD; (d) AA&R and DT aorta of *Apoe*^{-/-} mice on HCD; (e) AA&R clusters of *Apoe*^{-/-} mice on HCD and NCD; (f) DT clusters of *Apoe*^{-/-} mice on HCD and NCD. Predefined gene sets were overlaid on single cells on a tSNE plot to identify the cell identity of clusters with an enrichment of indicated gene sets (g) Macrophage foam cell formation; (h) VSMC phenotypic transition; (i) Vascular inflammation genes; (j) Vascular calcification gene; (k) VSMC contraction genes; (l) Endothelial cell.

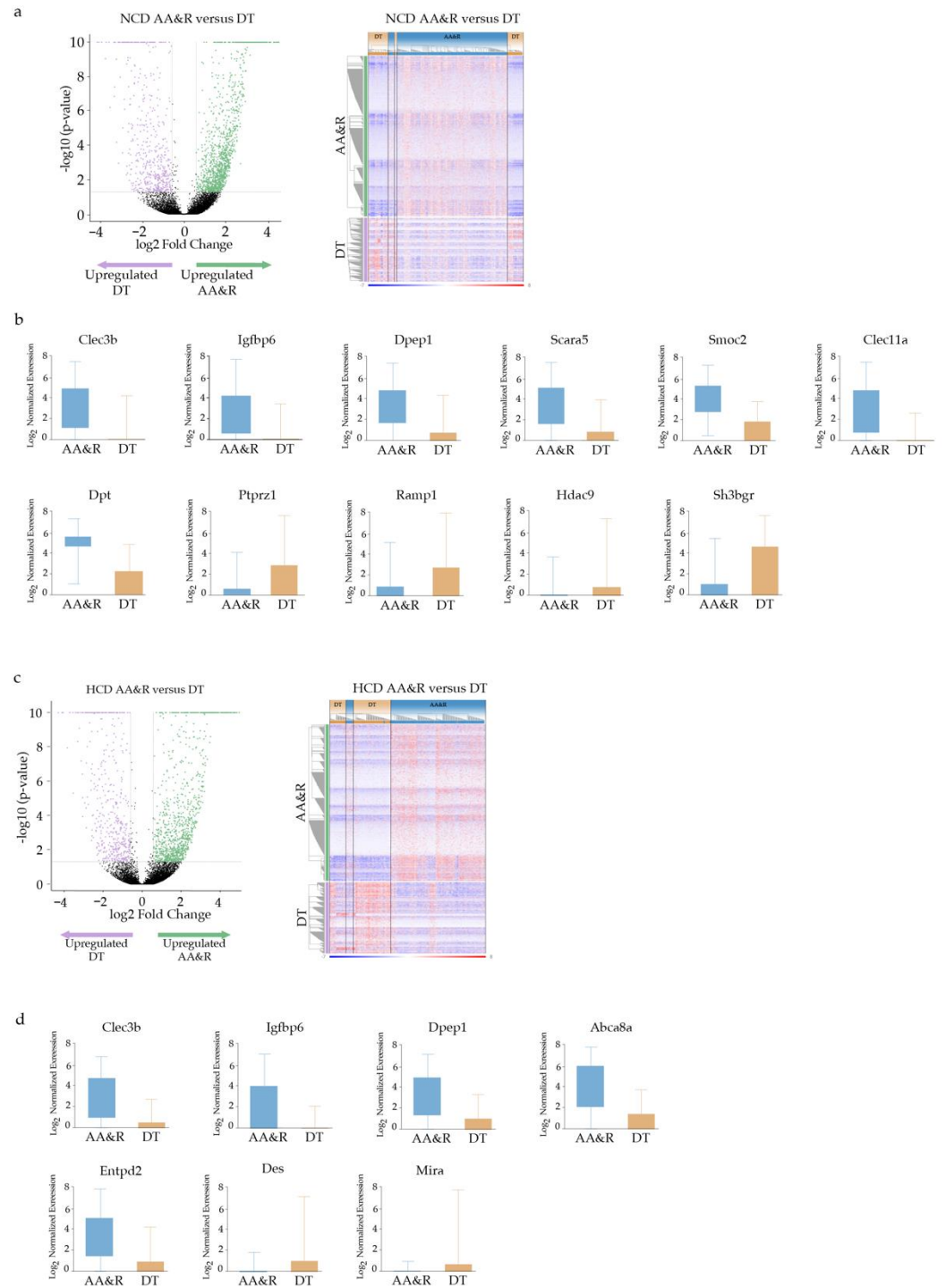


Figure S3. (a) Volcano plot showing significance ($-\log_{10} p\text{-value}$) versus relative gene expression of CD45⁺ cells derived from AA&R of $\text{Apoe}^{-/-}$ versus DT derived from $\text{Apoe}^{-/-}$ on NCD. Each dot represents a gene within the performed comparison ($p\text{-Adj}<0.05$, $\text{Log}_2>\pm 4$), $n=6$ mice. Rows represent genes and columns represent cells. Heatmap showing the expression of genes (HCD, red side strip or NCD, purple side strip) ($p\text{-Adj}<0.05$). **(b)** Genes of interest with significant differences in expression of CD45⁺ cells derived from AA&R of $\text{Apoe}^{-/-}$ versus DT derived from $\text{Apoe}^{-/-}$ on NCD are labelled and presented as graphs, ($p\text{-Adj}<0.05$), $n=6$ mice. **(c)** Volcano plot showing significance ($-\log_{10} p\text{-value}$) versus relative gene expression of CD45⁺ cells derived from AA&R of $\text{Apoe}^{-/-}$ versus DT derived from $\text{Apoe}^{-/-}$ on HCD. Each dot represents a gene within the performed comparison ($p\text{-Adj}<0.05$, $\text{Log}_2>\pm 4$), $n=6$ mice. Rows represent genes and columns represent cells. Heatmap showing the expression of genes ($p\text{-Adj}<0.05$). **(d)** Genes of interest with highly significant differences in expression of CD45⁺ cells derived from AA&R of $\text{Apoe}^{-/-}$ versus DT derived from $\text{Apoe}^{-/-}$ on HCD are labelled and presented as graphs, ($p\text{-Adj}<0.05$), $n=6$ mice.

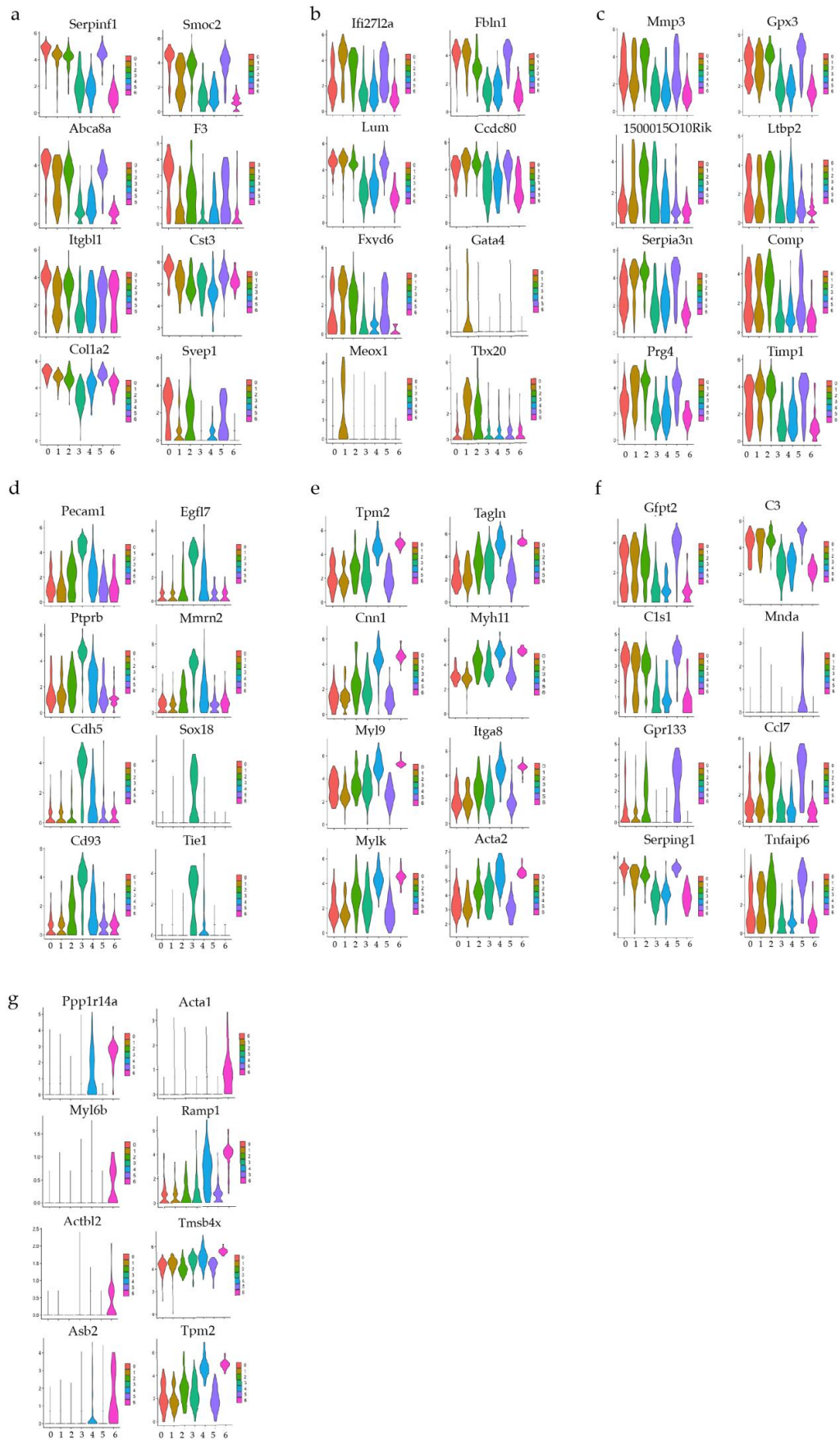
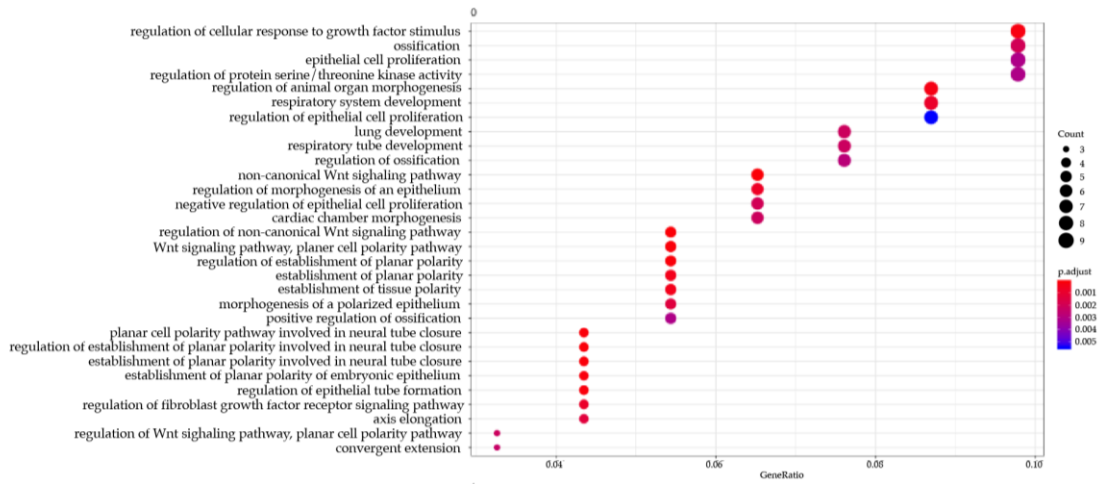
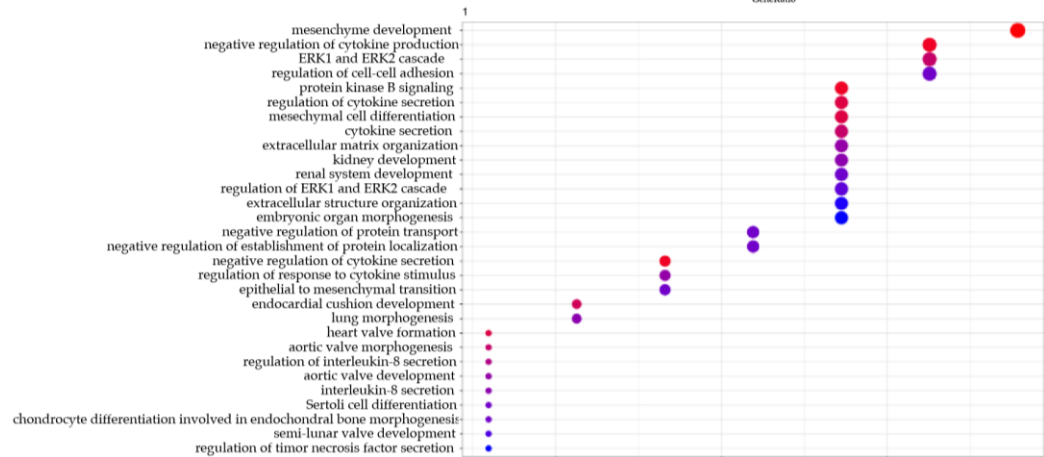


Figure S4. Top eight expressed genes per cluster: (a) cluster 0; (b) cluster 1; (c) cluster 2; (d) cluster 3; (e) cluster 4; (f) cluster 5; (g) cluster 6.

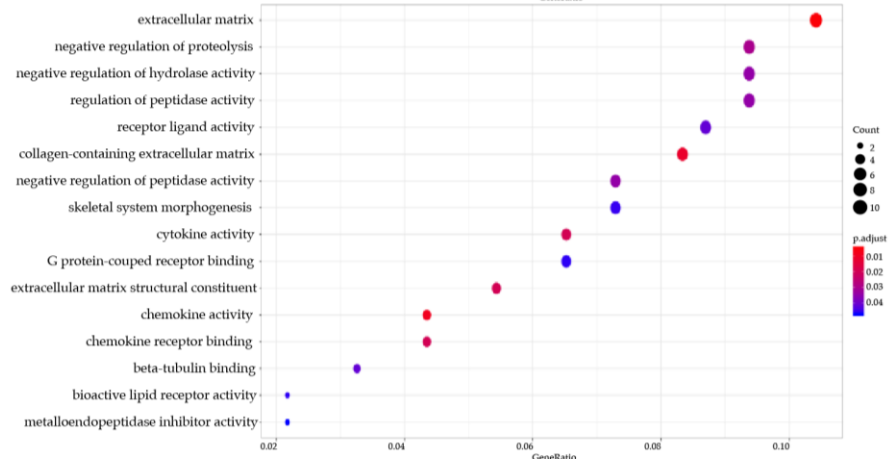
a



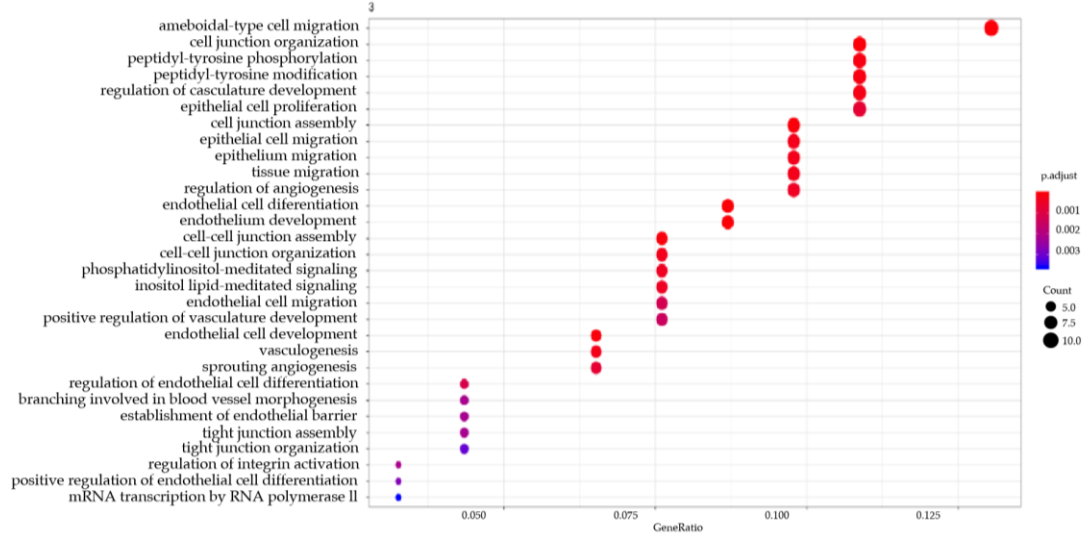
b



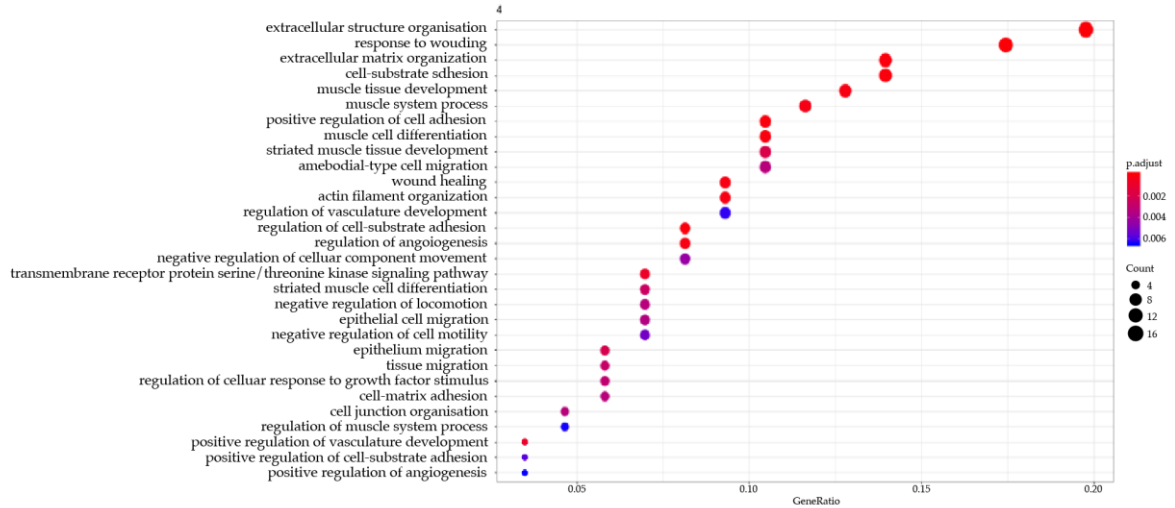
c



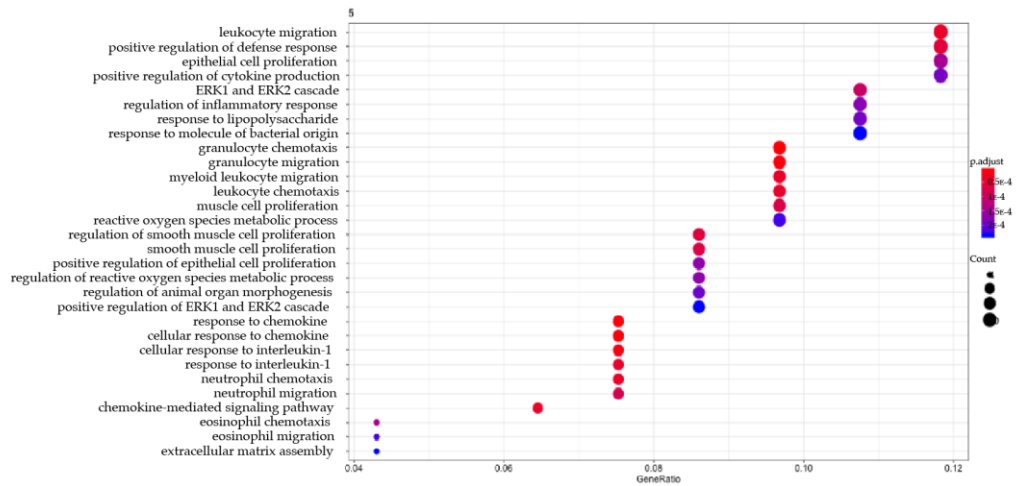
d



e



f



g

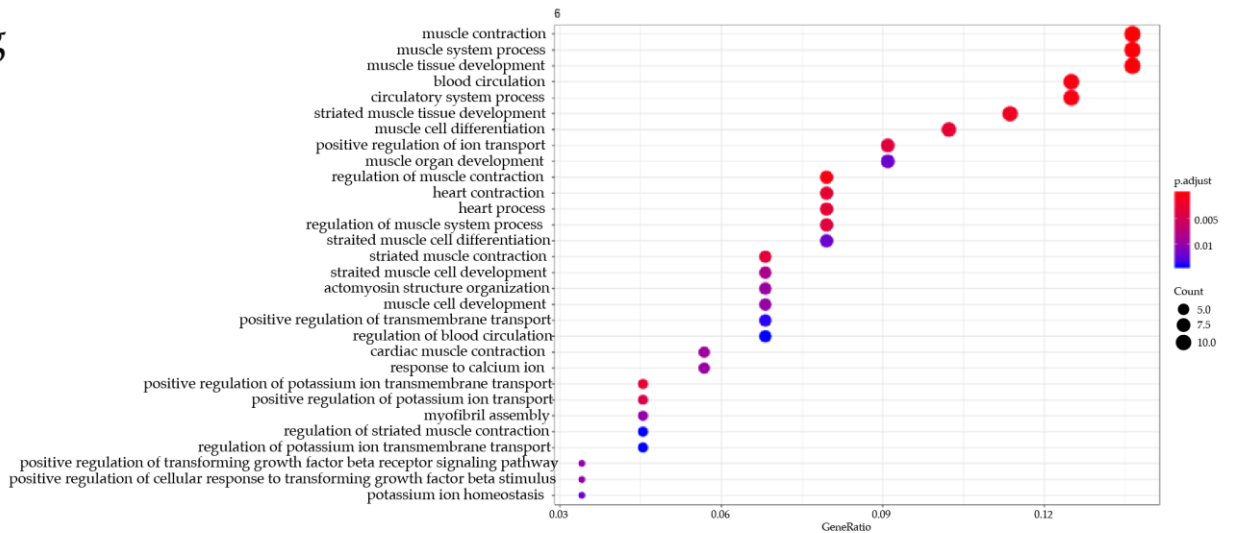


Figure S5. Bubble plot of GO term enriched analysis of cluster: (a) cluster 0; (b) cluster 1; (c) cluster 2; (d) cluster 3; (e) cluster 4; (f) cluster 5; (g) cluster 6. Dot size is proportional to the number of genes overlapping with each GO term and the adjusted p -value is colour-coded from red to blue, $n = 6$ mice/group.

Table S1. Differentially expressed gene HCD compared with NCD specifically of AA&R-derived cells of Apoe^{-/-} mice.

DIFFERENTIAL EXPRESSION		
Arch Root HCD vs Arch Root NCD		
149 Genes UP		
Z210407C18RIK	ACKR1	PERP
SREK1IP1	FOSL1	LAYN
GLIPR1	ANGPTL4	TMPPE
MMD	EMCN	RAB13
TAPT1	GBE1	EPB41
SLC35B4	FAM20A	ABCA3
PTGES3L	ZFP598	SH3BGR
FAM63A	MIF4GD	1700021K19RIK
RRAS2	LY96	MED25
TESK1	EXOC2	OTUB1
POLE3	TMEM214	TMED8
TMEM173	PELP1	NOL10
SLC30A9	CACNB3	NEK6
B3GALT1	LDLRAD4	FOXJ3
STX3	GPATCH11	TMEM53
GNE	TNFRSF18	HSPB6
1190005I06RIK	RGS2	DNAJC30
ADSL	CEP164	SRD5A3
SLC1A4	ANO10	EIF1AD
EIF4E	TSPAN11	FABP4
PGM5	PLOD1	TLN2
SF3A1	YTHDF2	MRPL3
SDF2L1	LSMEM1	MAP2K1
COX19	RPAIN	INAFM1
RNF220	CISD1	TUG1
SPP1	IFT46	BCL10
FIGF	Z210016L21RIK	REL8
GIGYF2	MVB12A	MYDGF
AURKAIP1	RGS7BP	ARL6IP5
PDLIM1	HTRA4	LOXL3
FBXO33	CCDC25	SYAP1
TLK1	RAMP1	AARS
RICTOR	ESYT1	GRPEL1
ENAH	RAB8A	GM6402
RIOK3	NDUF83	HIGD1A
0610037L13RIK	OXR1	FUNDG2
KCNMB1	MRPS21	ZC3H15
SNRPE	XPO1	CDK11B
PA2G4	MICAL2	TPM2
CFLAR	LRP10	PRDX6
CRELD2	CNN1	ECH1
RPS7	TBCA	NPNT
RPL7	NDUF89	TOMM7
MCFD2	NARS	ITGAV
RPSA	BC048507	AKAP2
GSTT1	MUSTN1	UQCRB
FAT1	CSNK1A1	COX7A2
SRSF2	PSMC3	Z410006H16R...
PDLIM7	RPS16	ATP5O
TBRG1	GPC6	
28 Genes DOWN		
ZFP623	CHAD	SOLE
CIPC	CXCL14	LPAR6
PACS1	RARB	GM20199
DCLRE1A	PF4	LMTK2
ACY1	COL2A1	DCAF17
TMEM88	MAPKBP1	AIS07597
VLDLR	HHIP	SELE
LAMC3	PJA1	TSPAN5
PLTP	RBP4	SMOC1
NR4A1		

Table S2. Differentially expressed gene HCD compared with NCD specifically of DT aorta of *Apoe*^{-/-} mice.

DIFFERENTIAL EXPRESSION

Thalamic HCD vs Thalamoc NCD

337 Genes

111 Genes

DOWN

TPP3	DDX54	TMEM261	ATXN10	RAD23B	ANO10	SLC4A6	OLFR1033	TMEM110				KLK8	AHSG	BACE2								
SLC25A39	MFAP3	CREB3	ABCE1	SLC25A24	SPP1	LSM14A	FBIN	ANAPC4				KLK9	AHSG	BACE2								
CD44	QARD01	P1GX	LRPPRC	GALNT16	GPAA1	NAT6	RAPH1	LYST				ADH1	G0S2	KANK4								
ACAMTSL5	TARS	ARRB1	FAM98A	HNRNPPLL	SOHC	NF1	THBS2	ANAPC7				1500015010R..	IFT3	ABCG2								
SEPT6	FABP4	TMC6	FBXW7	1810062J23BK	PIK3R1	AMMEER1L	QRICH1	PRKAR2A				FZD4	PAGR1A	FAM74B								
FZD1	ELOF1	NUS1	FUNCDC1	SLC25A46	CERS2	MUSTN1	NBL1	E03001319RK				ARL5B	RNF31	HS6ST1								
SYNRG	ITIH4	OPTN	MAP2K7	GNAI1	ZCHC124	ARFGEF1	SCARB2	TNKG				RBBP5	S100B	GOLM1								
KCNH2	TCAP	KCNMA1	ABHD14A	PRDM5	GNAO1	HSPB6	2310022B05RK	ARL8B				RRS1	SPPT20	NDOUAF5								
PRPS2	LAS1L	PHYH	TUBB6	TMEM389	ZFP771	FKBP10	EP300	MMP23				TMEM183A	COCH	ALOX5AP								
SSSCA1	AARS01	AGAP1	MRPL41	RNF181	DESIZ	ZBTB38	RERE	BICD2				MFNG	ZFP9	ILVLB								
NUDT9	MARF1	OXSR1	TMEM253	UBE2D2A	VP54A	DIK15	1810058J24RK	MTFN				ACKR3	LDLRAP1	ALDH1A1								
CACNA1C	MOIRF4L2	119000506/RK	RHOG	SPSB1	PRKAB2	TMEM65	EFEMP2	PPP2R5A				SLC02A1	MICN	NR1P1								
PAWR	SREBF1	RPRD1B	ALDH7A1	SEMA5A	ATRB81	AGRN	MTUS2	UNC5C				MBO1	MB1	FLNB7								
FAM210A	PROHSD1	IFT46	FAM89B	CAN01	JAK1	DAG1	RAN	FKBP8				ZFP669	CDK12	SIPA1L1								
GM15417	MROH1	RAD23A	INF2	SEC24C	TRAPPC2L	FKBP3	MRPL33	RIGF				XIRP1	INT56	APOD								
NCPB1	TESK1	EDEM1	SMARCD3	MYO1E	ADCY7	SORBS2	PDHA1	2310036022R..				ZFP280G	CNNM3	KL4								
LZTR1	WBSCR22	GLO1	ZFP68	FNIP1	CSNK2A1	MSRB1	HSPG1	IMMT				GJ44	2310035C23RK	WSB1								
ACP2	MPS56	RPS56	PAIP1	GLUPR1	KANSL3	DNAJC5	MYOCD	ARF1				CERK	ATG9A	SOS1								
NEK9	COL8A1	SLAN2	SHARP2	SDHA72	MALSU1	EC031181	CHCHD1	ZCH313				EGLF7	TMEM9	PLCE1								
MLEC	CLMP	FAT4	WDK6	ESD	EXOC68	S100A4	PCP4L1	NES				ELP2	PER1	FAM168Z								
ABDC5	NFATC3	WFS1	SOD4	OPT1A	SCFD1	ALCAM	GLG1	EEF1B2				SFRP1	CYTL1	AD03								
MRC2	KLHL28	TBPL1	HXCD1	ARHGAP23	IGSF3	HMCN3	SEMA3C	ANP32E				KDM6B	EPH4Z	NR4A2								
SEC61A1	DAPK2	ITSN1	EFHD1	TMEM167	CRAT	BRD4	MDA	MSRB3				MA2P9K3	2900060814RK	IFNGR1								
HBS1L	SLC20A2	COL4A6	POF5	GATAD2A	CUEDC1	QUCY1B3	PP2YCA	ATP1B3				EFEMP1	METTL7A1	BMX								
MAN2A2	SAMHD1	PPP1R15B	POF5	GATAD2A	CUEDC1	QUCY1B3	PP2YCA	ATP1B3				NR4A1	FNRC1	EIF5								
BAG5	CSNK1G2	CTCF	RFBMS2	HSPB2	OSGPE	STS	ITGAV	NDRG2				CH102	BMP4	MZP2K3								
ARHGEF4	MMP14	ZMYM4	SEC61G	DYNLT3	OLFM12B	SAMD4	LRP1	HMGN1				ENTPD2	NP-904339.1	FAP36L1								
F70	LYPLAL1	NOMY01	CACXYB	AGL	MAN1A2	PNP	HNRNPCC	CENPB				FOSB	SMAD7	KCNQ10T1								
COA5	UBXN7	DYNLC1L1	UPF38	B3GNT1	DOX1	MYEOV2	FBXO30	ATP6V1A				POLR2A	ABLUM1	NUAK1								
AHSA1	STAUI1	GXY10L1	CRYAB	INTS3	CTDSBP2	DEGSI	PPP1CB	MERIP				CC4C2EP3	ADAMTSL1	NFKBIZ								
SEPT8	DDP3	110009WE24RK	MTSPS1	TMEM873	DNAJC13	GSTP1	PLEC	PI5				KLIF9	PECAM1	FAMC3								
ARHGAP44	MARCK3	SURF1	SBCP5	M8T59	PPP1R9B	BBF3	RARRS2	POLIM7				GAT6A	PTPRB	MALAT1								
CAV3	SLC21	KOLRL3	MNTO3	WZC05	DLG1	BLOC1S1	SOST	HSPB1				FBLN1	IER3	CFH								
DHX29	RCAN3	DECRI	EXOC5	OSR1	NUP153	COL3A1	VGQRIC1	RP2S1				RBM39	FOS	ID3								
0610009L18RK	EBP	MED14	EMC8	MPND	OSBP1L1A	ACTN1	HSPB1	UCR10				HE51	DDX5	RPS24								
WOFY1	IGF1B3	SEHL1	PTK2	DTNA	PPPIA1	LGALS1	CX3A5A1	OGN				UBC	RPS4X	DCN								
ATG5	IFB35	SNAP23	PTPN2	NEO1	PPIC	OSPL14P51																
MAPK6	KIFAP3	A430105I19RK																				