

Supporting Information

An advanced comprehensive multi-cell-type-specific model for predicting anti-PD-1 therapeutic effect in melanoma

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Supplemental Figure

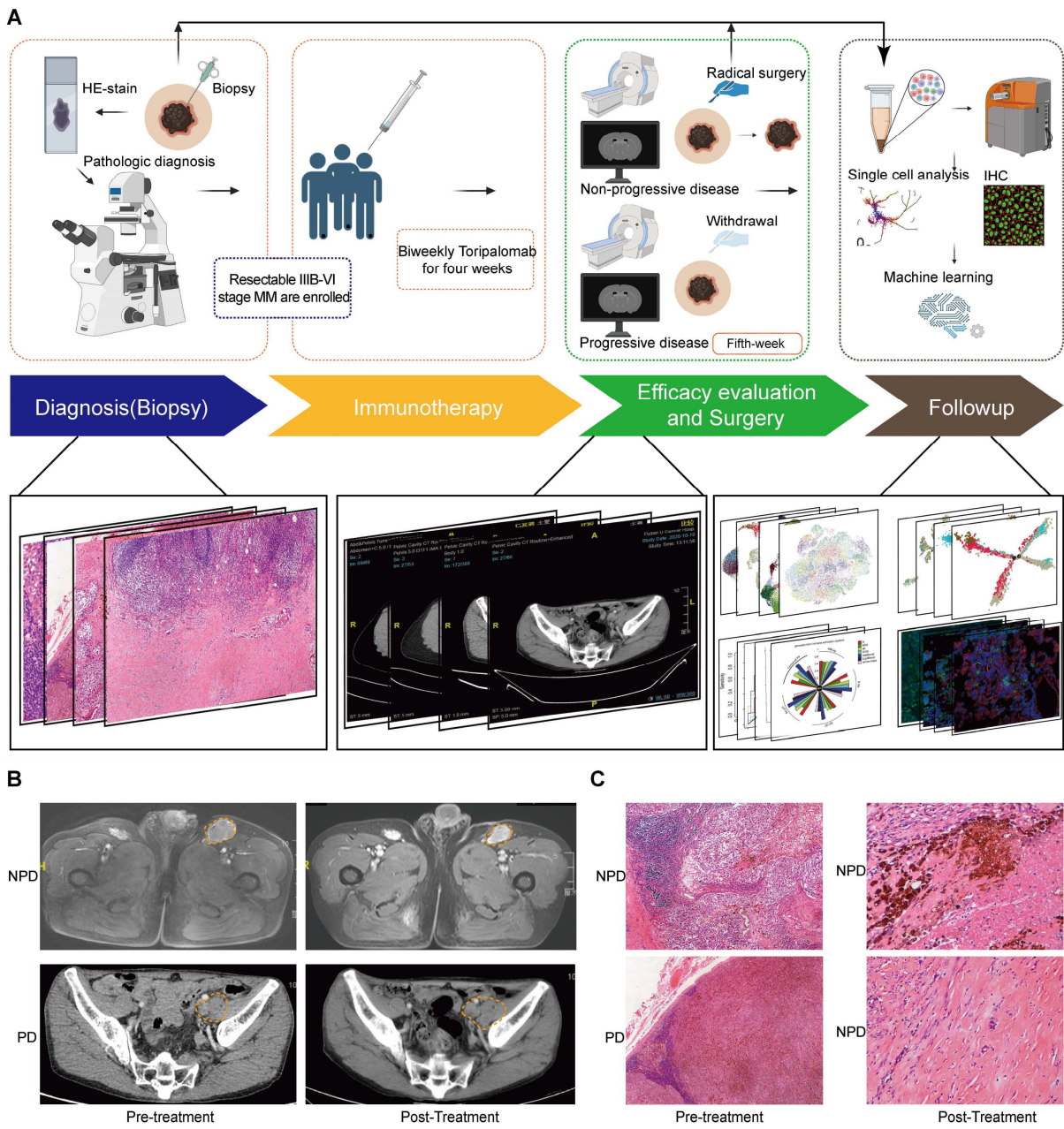


Figure S1. Schematic representation of the study A) A schematic diagram of the four stages of the study. B) Enhanced CT images of the enrolled patients for the evaluation of anti-PD-1 treatment response. C) Representative hematoxylin and eosin (H&E)-stained images of tumors in PD-pre/NPD-pre group (left) and NPD-post group (right); magnification: 40x.

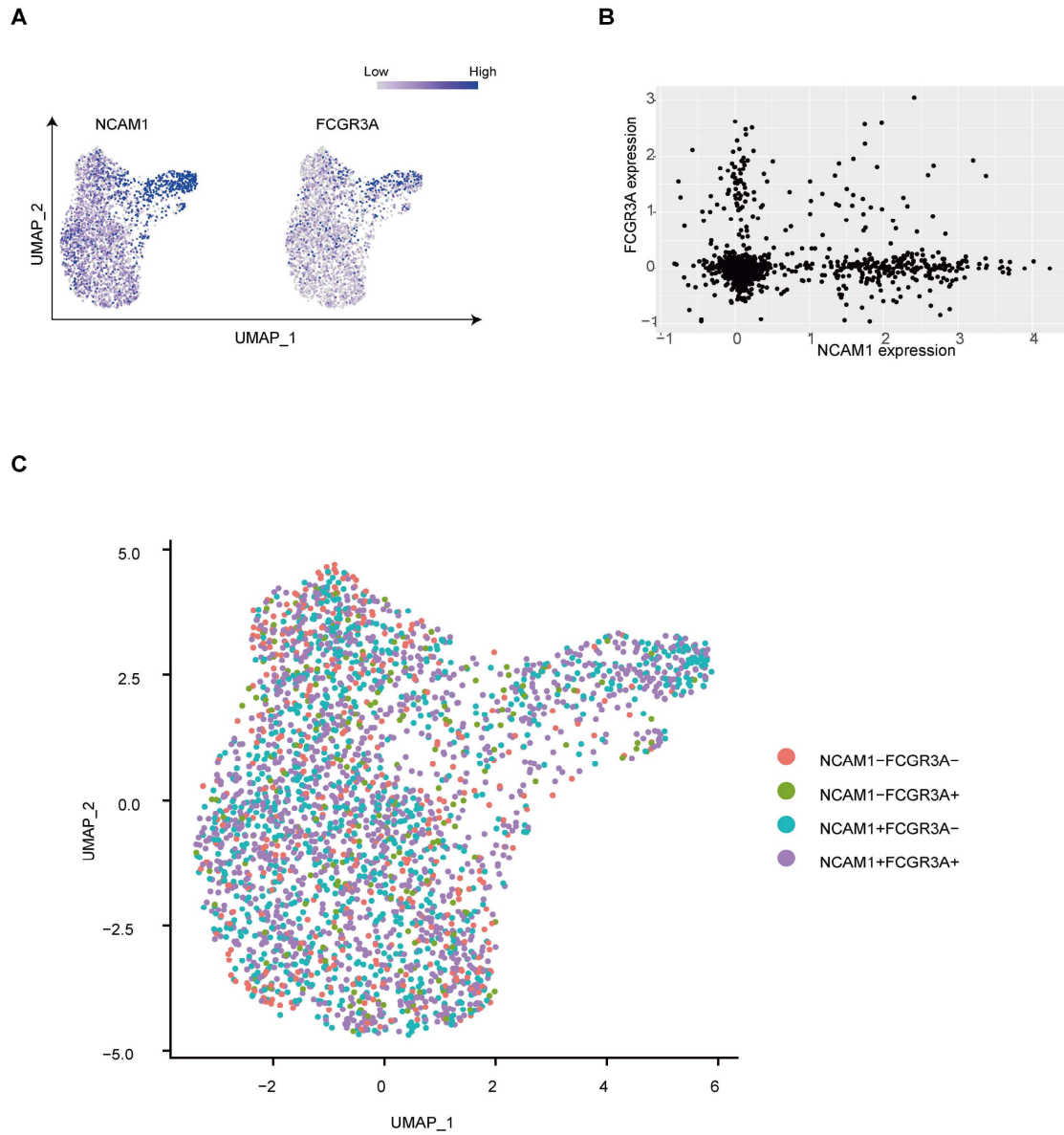


Figure S2. A. UMAP plot showing the distribution of NCAM1 and FCGR3A in NK/NKT cells **B.** Dot plot showing the distribution of NCAM1 and FCGR3A in NK cells **C.** The UMAP plot showing the distribution of NCAM1-positive and FCGR3A-positive NK/NKT cells

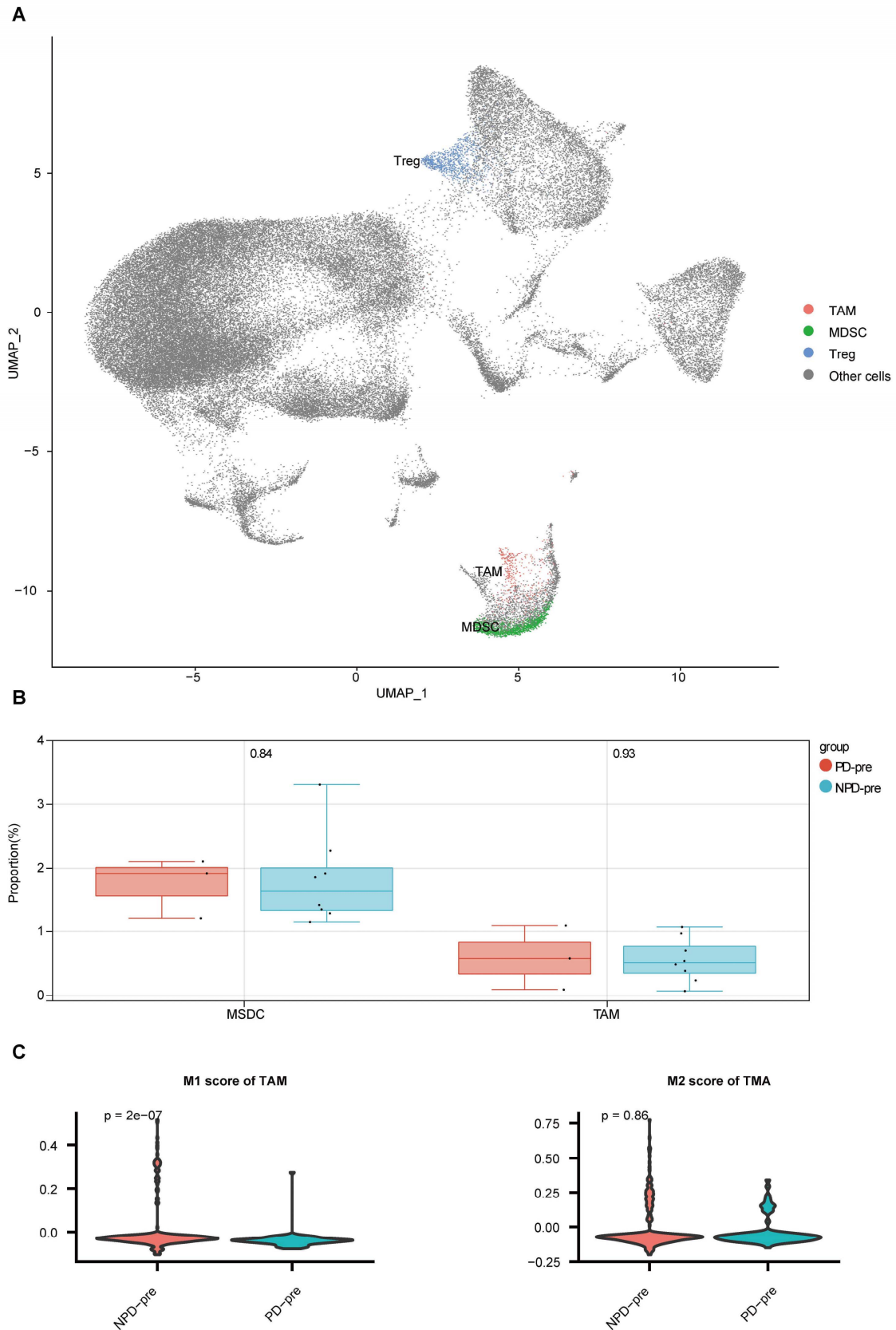


Figure S3. A. The UMAP plot showing the subgroups of Treg, TAM, and MDSC cells. **B.** Box plot showing the proportion of TAM, and MDSC between PD-pre and NPD-pre groups

(non-paired t test) C. Violin plots showing the differences in the M1 and M2 scores of TAM between the PD-pre and NPD-pre groups(non-paired t test).

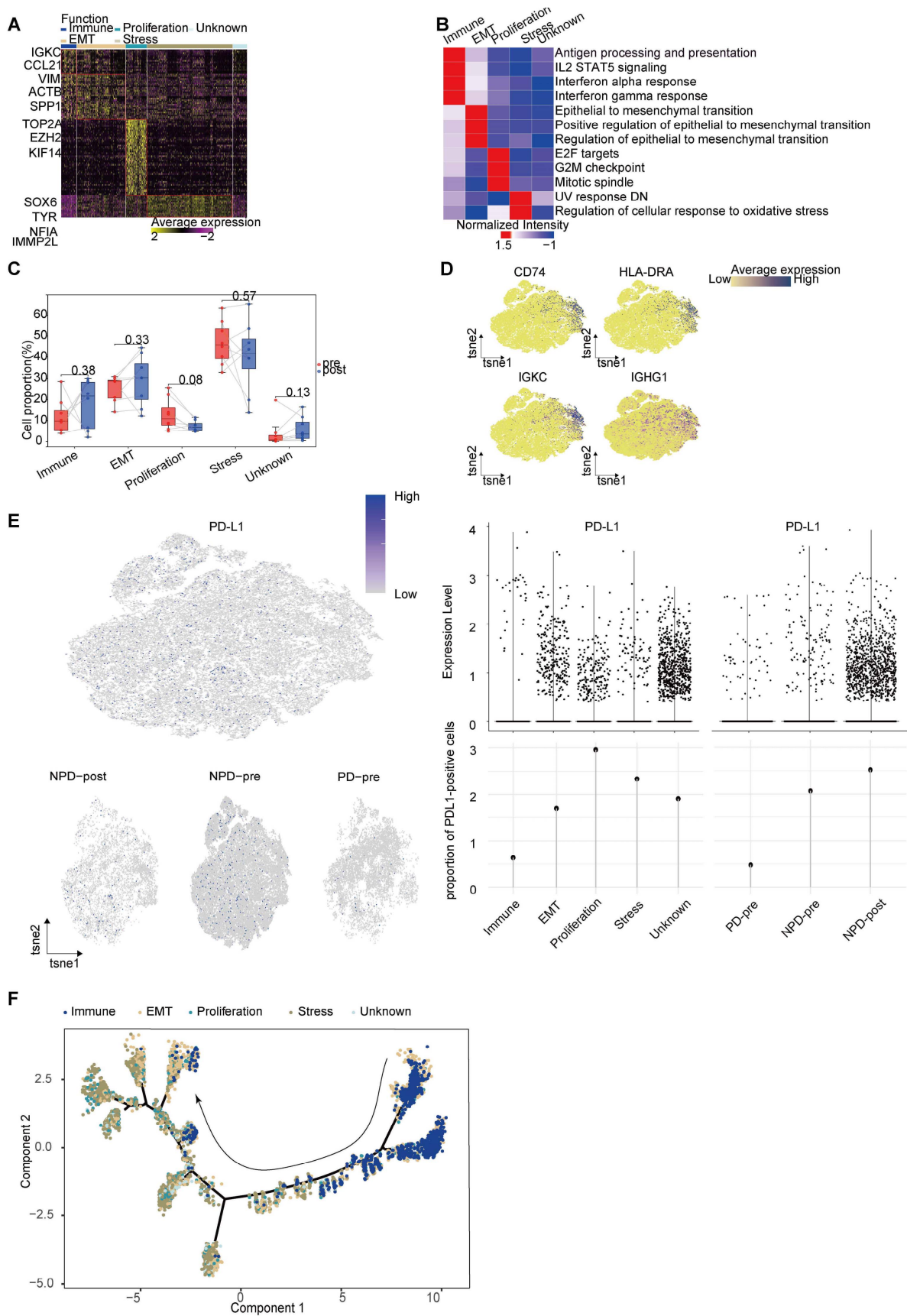


Figure S4. A. Heatmap showing the specifically highly expressed genes in each subgroup. **B.** GSEA of the GO and HALLMARK pathways in these subgroups. **C.** Box plot showing the proportion of annotated melanoma subgroups between NPD-pre and NPD-post groups (paired

t test). **D.** Feature plots showing immune related genes. **E.** The t-SNE plot and dot plot showing the distribution of PD-L1 in melanoma cells, the expression levels of PD-L1 and the proportion of PD-L1 positive cells in five melanoma subgroups. **F.** Pseudo-temporal trajectory of NPD-pre and NPD-post melanoma cells identified two distinct cell fates colored by groups.

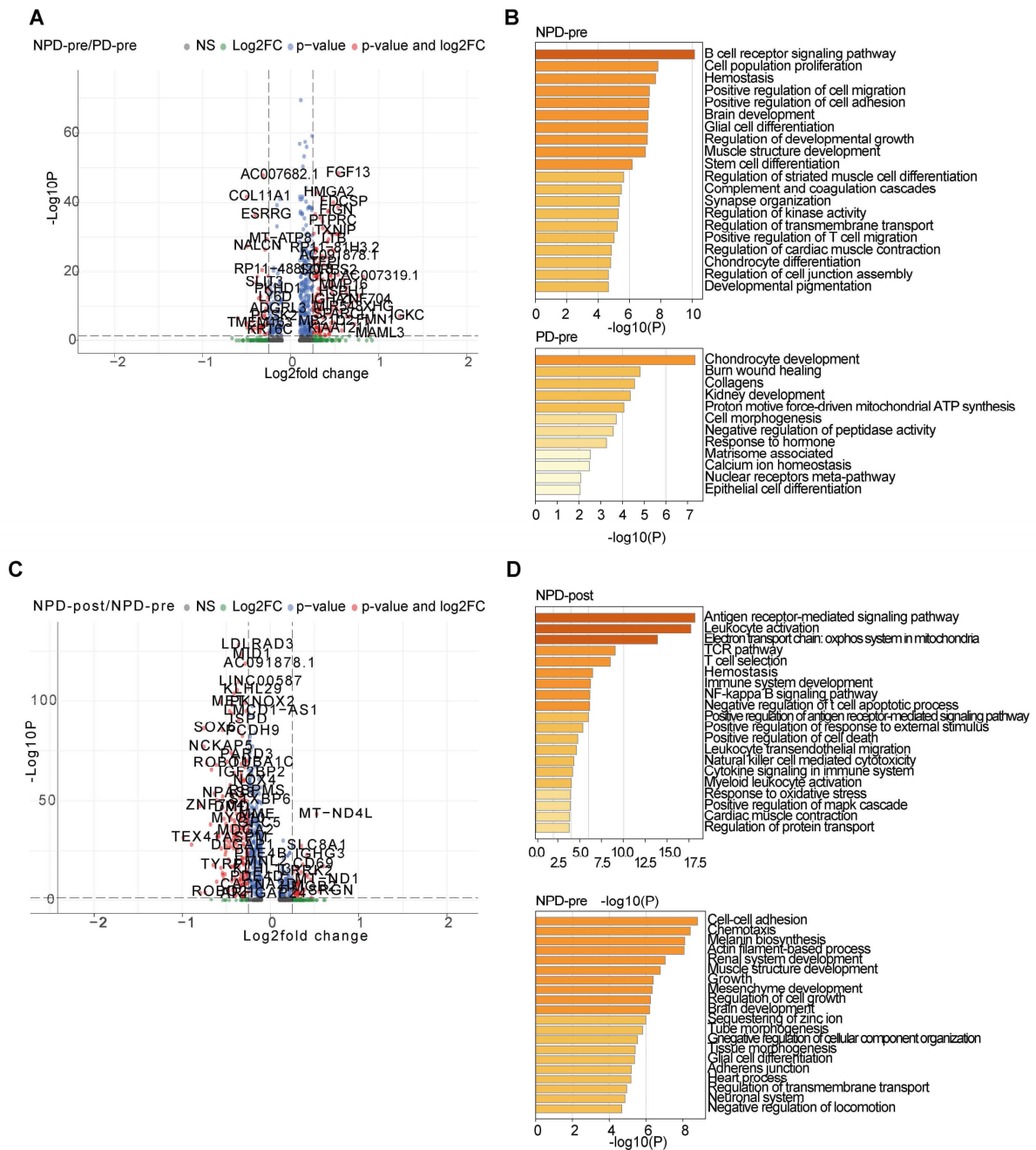


Figure S5. A. Volcano plot showing DEGs in MM_immune between PD-pre and NPD-pre groups. **B.** Bar plot showing the enriched functions for DEGs in A. **C.** Volcano plot showing DEGs in MM_immune between NPD-pre and NPD-post groups. **D.** bar plot showing the enriched functions for DEGs in C.

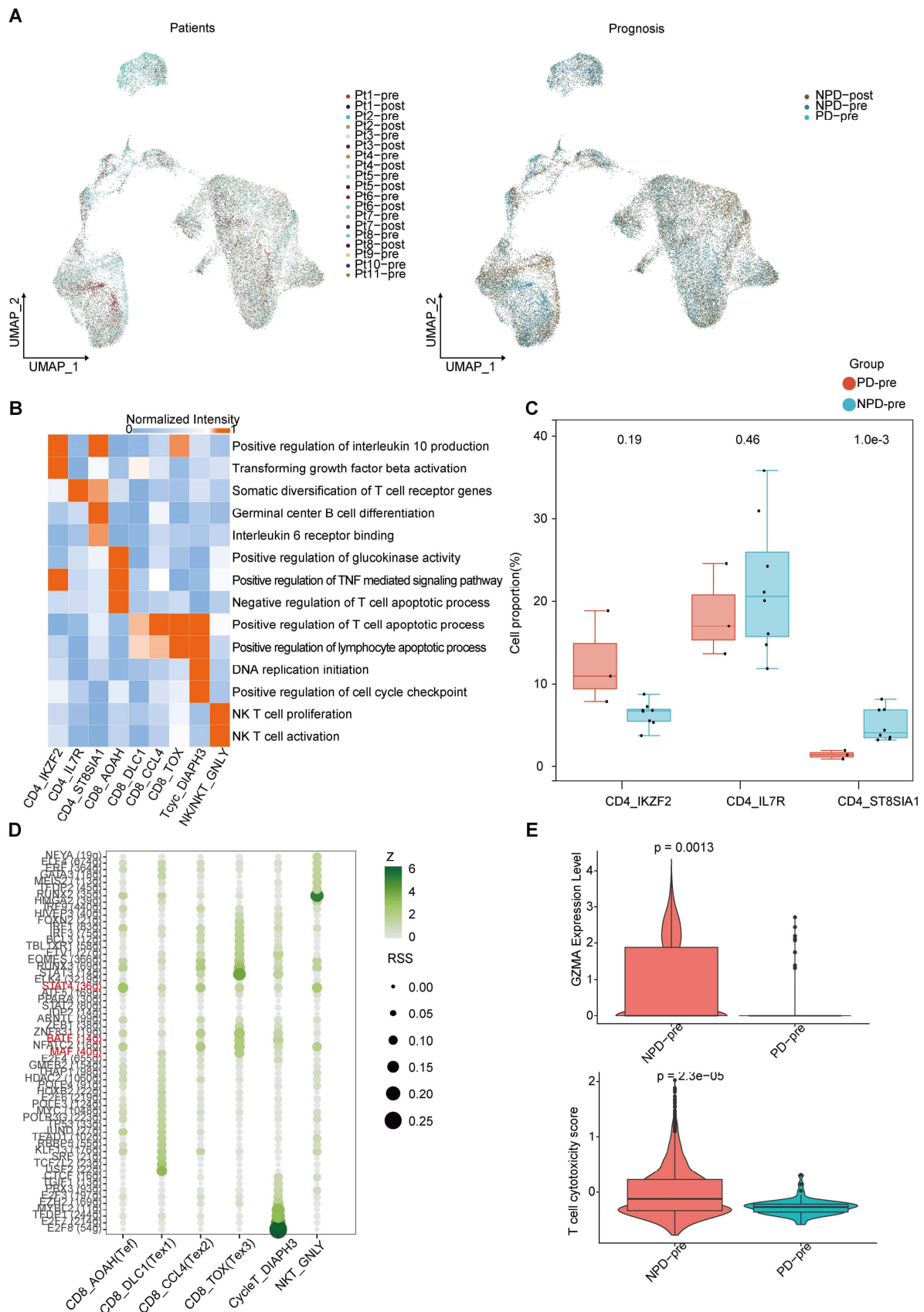


Figure S6. A. UMAP plot (left panel) of 27,766 lymphocytes, color-coded by patient origin; UMAP plot (right panel) of 27,766 lymphocytes, color-coded by groups. **B.** GSVA of the GO pathways in T cell (including NK/NKT) subgroups. **C.** Box plot showing the proportion of

annotated CD4⁺T subgroups between PD-pre and NPD-pre groups (non-paired t test) **D.** Dot plot of the t-values of AUC scores of expression regulation by transcription factors, as estimated using SCENIC, per subgroup of CD8⁺T and NK/NKT. **E.** Box plots and violin plots showing the differences in the expression levels of the GZMA gene and cytotoxicity scores of CD8⁺T between the PD-pre and NPD-pre groups(non-paired t test).

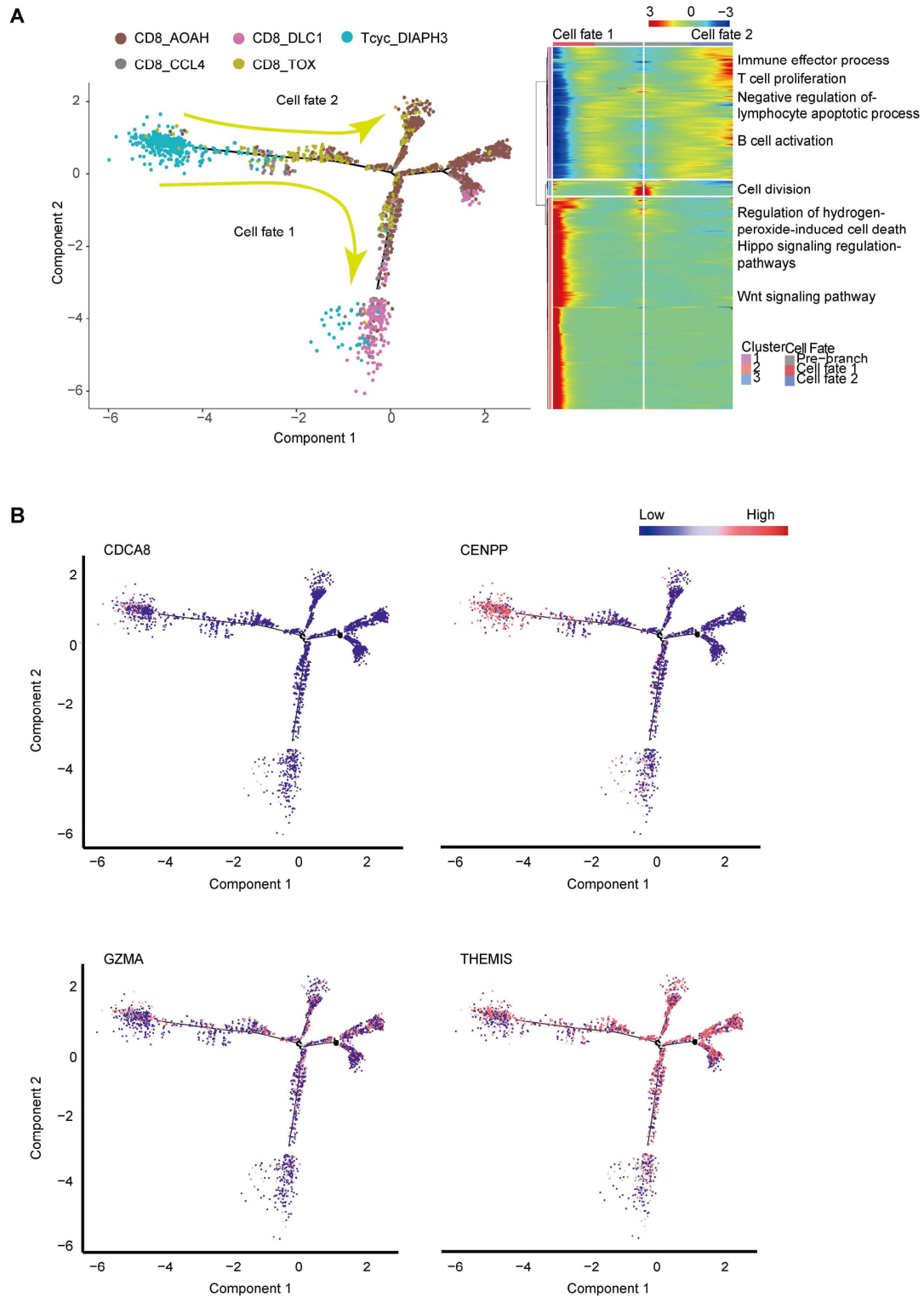


Figure S7. A. Pseudo-temporal trajectory (left panel) of CD8⁺T cells identified two distinct cell fates colored by subgroup; Heatmap (right panel) showing the enriched pathways in these three phases of the left panel. **B.** Pseudo-temporal trajectory of CD8⁺T cells colored by the expression of subgroup-specific genes.

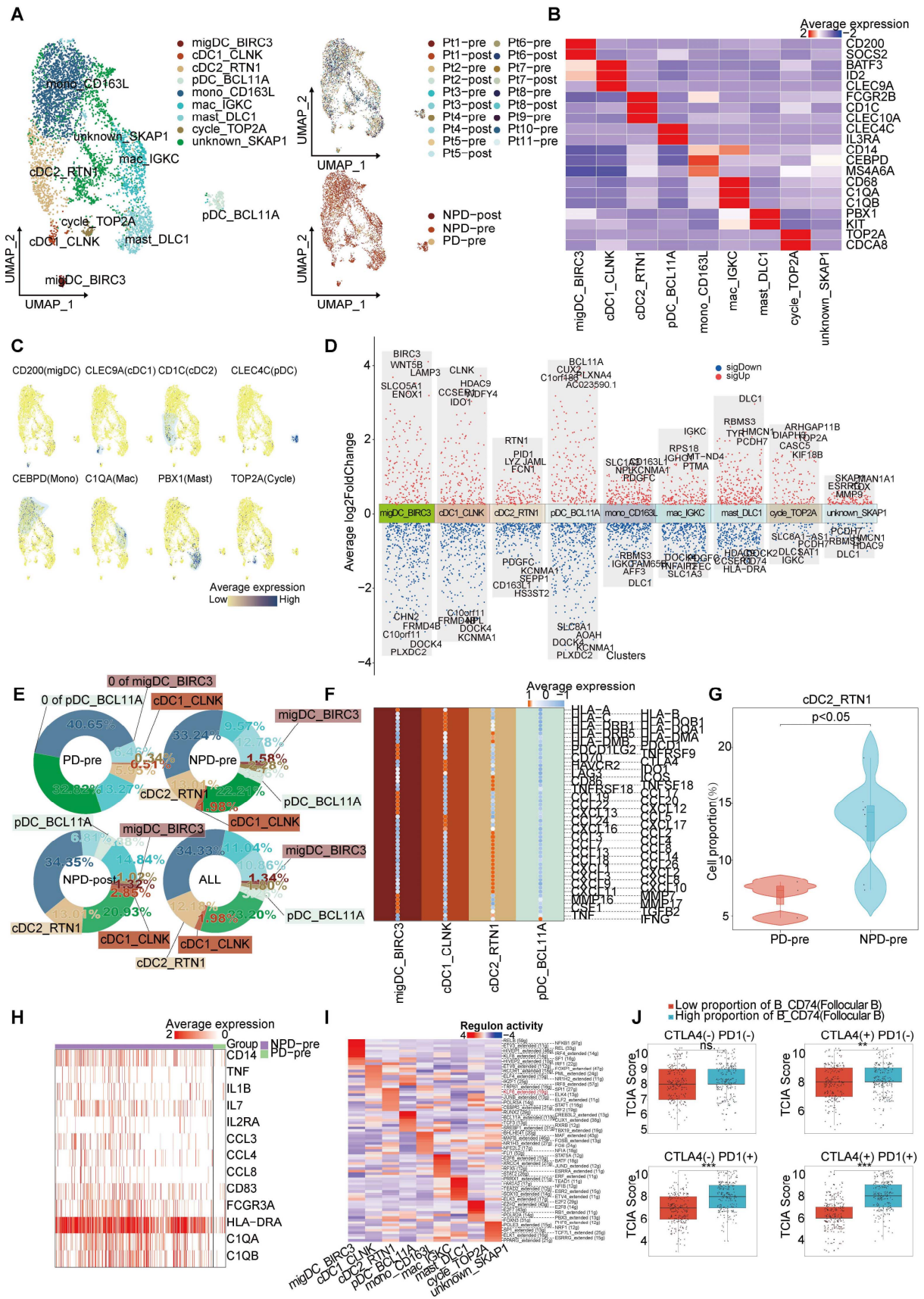


Figure S8. cDC2 shows the strongest immune activation function in myeloid cells. A. UMAP plot (left panel) of 4,999 myeloid cells, color-coded by their associated cluster; UMAP plot (upper right panel) myeloid cells, color-coded by patient origin; UMAP plot

(lower right panel) myeloid cells, color-coded by groups. **B.** Heatmap showing the specifically highly expressed genes in each myeloid cell subgroup. **C.** Heatmap the specifically highly expressed genes in each myeloid cell subgroup. **D.** Volcano plot showing the differentially expressed genes in the nine annotated subgroups. **E.** The proportion of nine annotated subgroups between groups. **F.** Expression of MHC molecule, immune checkpoint, immune regulation factor, chemokine and migration-related gene of four DC subgroups. **G.** Violin plot showing the proportion of cDC2_RTN1 between PD-pre and NPD-pre groups.(non-paired t test) **H.** Heatmap showing the expression of immune-related gene of cDC2_RTN1 between PD-pre and NPD-pre groups. **I.** Heatmap of the t-values of AUC scores of expression regulation by transcription factors, as estimated using SCENIC, per subgroup of myeloid cells. **J.** Box plots of TCIA score of TCGA patients categorized into high and low groups based on their score of the percentage of the cDC2_RTN1 subgroup.

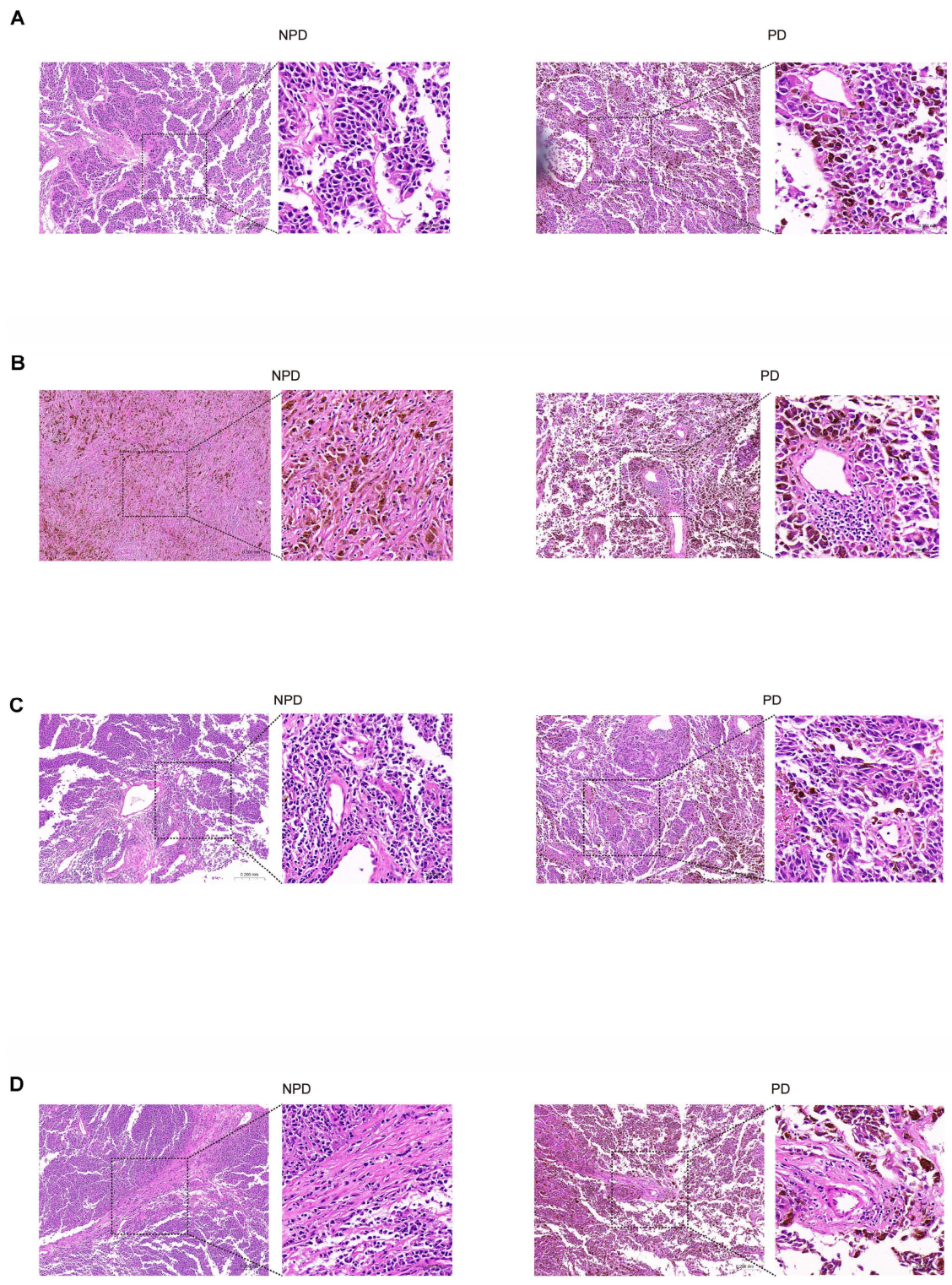


Fig. S9. A-D. Representative corresponding hematoxylin and eosin (H&E)-stained images for Fig. 6, scale bar: 200 μm (left); 50 μm (right);

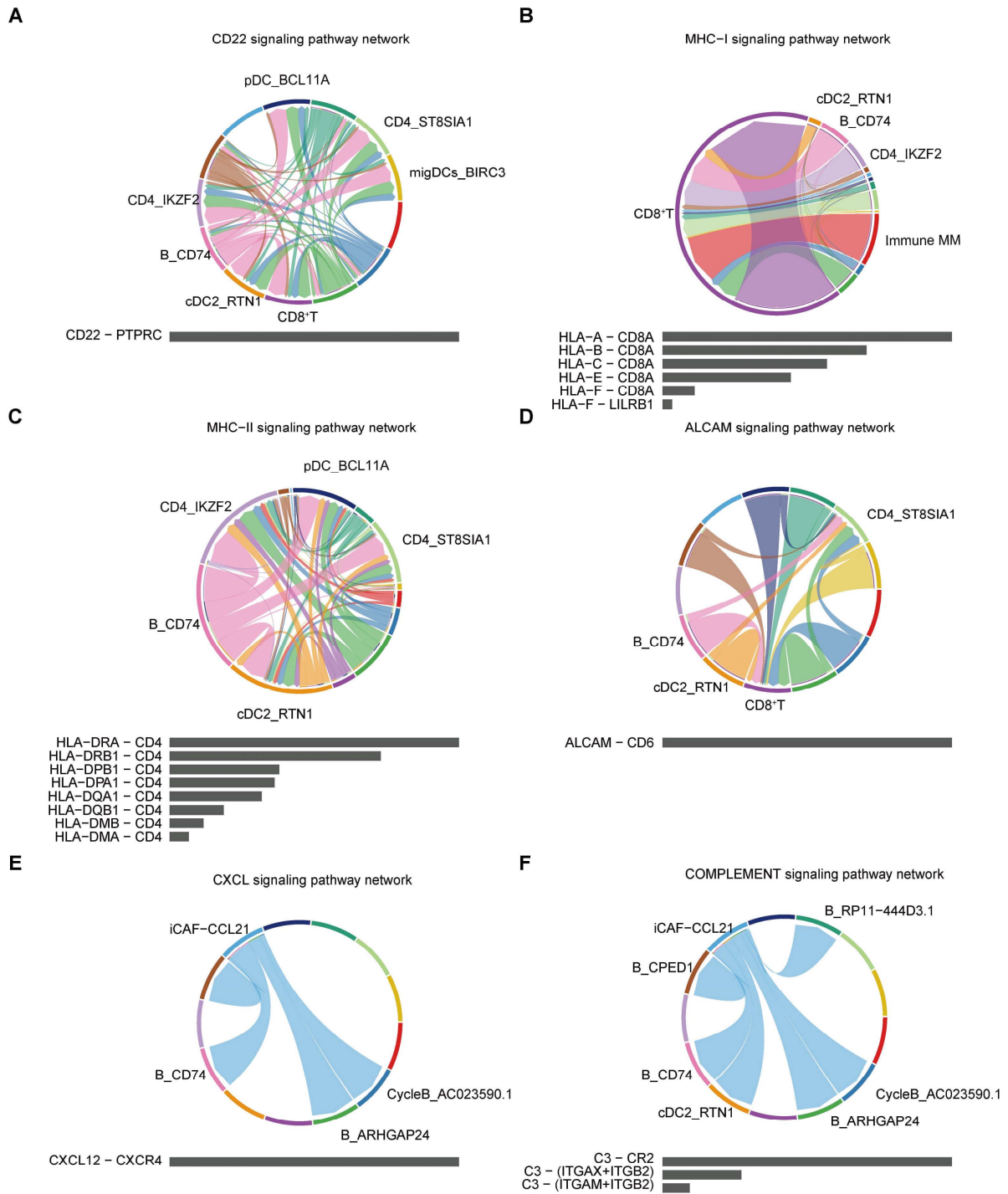


Figure S10. A-F. Chord plots showing the networks of CD22 (A), MHC-I (B), MHC-II (C), ALCAM(D), CXCL (E) and Complement (F) signaling pathways in the ecosystem of NPD-pre group.

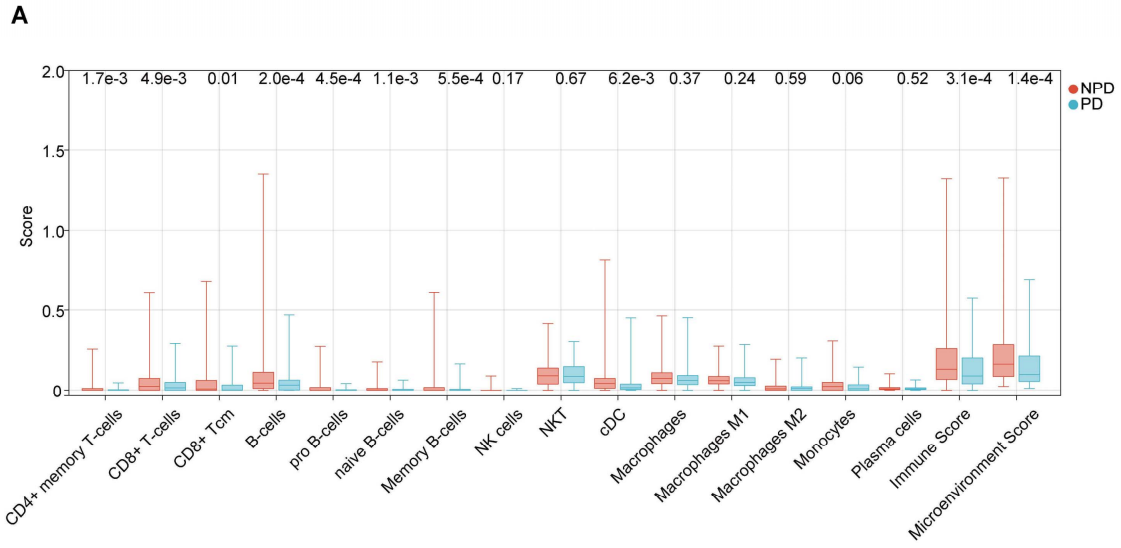


Figure S11. A. Boxplot showing the proportions of immune cells predicted by xCell for the "PD-predicted" and "NPD-predicted" groups (non-paired t-test).

Supplemental Table

Table S1. Prognostic information of patients

Patient name	subject name	Accession number	Residual tumor % (total viable tumor/total surface area of cross-sectional tumor bed assessed)	Tumor
CXF	Pt1	2020-40530	pNR : 88%	Acral melanoma
FZD	Pt2	2020-49338	pPR:43%	Cutaneous melanoma
LYH	Pt3	2020-40591	pNR : 93%	Cutaneous melanoma
LYZ	Pt4	2022-12575	pPR:36%	Cutaneous melanoma
QRJ	Pt5	2020-42505	pNR:72%	Acral melanoma
XGS	Pt6	2021-61640	pPR:37%	Cutaneous melanoma
YZY	Pt7	2020-42038	pNR : 73%	Acral melanoma
ZYC	Pt8	2020-52864	pPR:25%	Acral melanoma
LSX	Pt9	2020-33375	PD: >100%	Acral melanoma
YYX	Pt10	2021-30008	PD: >100%	Cutaneous melanoma
ZWX	Pt11	2022-02343	PD: >100%	Cutaneous melanoma

Table S2. Information about the patients

submitter_id .samples	age_at_init ial_pathologic diagnosis	breslow w_level	breslow _depth_ value(mm)	melanoma ulceration indicator	neoadjuvant _medicine	melanoma _c lark_level	pathologic _M
Pt1	50	3	3.1	yes	Toripalimab	4	x
Pt2	69	5	6	yes	Toripalimab	5	x
Pt4	44	5	>4.0	no	Toripalimab	NA	x
Pt5	72	NA	NA	NA	Toripalimab	NA	x
Pt6	72	NA	NA	NA	Toripalimab	NA	x
Pt7	68	5	13	yes	Toripalimab	4	x
Pt8	59	3	2.2	yes	Toripalimab	3	x
Pt9	64	4	3.7	no	Toripalimab	4	x
Pt10	68	5	>4.0	yes	Toripalimab	4	x
Pt11	56	5	5	yes	Toripalimab	5	x
submitter_id .samples	pathologic _N	pathologic _T	radiation _therapy	submitted _tumor_ location	tissue_ source_ site	withdrawn	year_of_ initial_ pathologic_ diagnosis
Pt1	0	3b	no	left popliteal fossa	lymph node	no	2017/5/1
Pt2	3b	4b	no	left groin	lymph node	no	2020/11/25
Pt4	3b	4a	no	right axilla	lymph node	no	2020/7/27
Pt5	3b	x	no	right groin	lymph node	no	2020/1/1
Pt6	1a	x	no	left axilla	lymph node	no	2020/3/6
Pt7	3b	4b	no	right groin	lymph node	no	2020/9/9
Pt8	3b	3b	no	right popliteal fossa	lymph node	no	2019/10/15
Pt9	1a	3b	no	left groin	lymph node	yes	2020/8/1

Pt10	0	4b	no	right axilla	lymph node	yes	2020/4/20
Pt11	3b	4b	no	right groin	lymph node	yes	2022/1/29
submitter_id	gender.demographic	vital_status.demographic	year_of_death.demographic	age_at_diagnosis	prior_malignancy.diagnoses	tumor_stage.diagnoses	year_of_diagnosis
Pt1	female	alive	NA	50	melanoma	2b	2017/5/1
Pt2	male	dead	2022/3/1	69	melanoma	3d	2020/11/25
Pt4	female	alive	NA	44	melanoma	3d	2020/8/13
Pt5	female	dead	2021/11/2	72	melanoma	x	2020/1/1
Pt6	male	alive	NA	72	melanoma	x	2020/3/6
Pt7	female	alive	NA	68	melanoma	3d	2020/9/9
Pt8	male	alive	NA	59	melanoma	3c	2019/10/15
Pt9	male	dead	2020/10/9	64	melanoma	3c	2020/9/11
Pt10	male	alive	NA	68	melanoma	2c	2020/5/22
Pt11	male	alive	NA	56	melanoma	3d	2022/1/29
submitter_id	disease_type	nerve_invasion	Vascular_invasion	Number_of_(+)SLN	total_number_of_sentinel_lymph_nodes	Number_of_(+)NSLN	OS/month
Pt1	acral melanoma	no	yes	NA	NA	5	15
Pt2	acral melanoma	yes	yes	1	1	5	63
Pt4	cutaneous melanoma	NA	NA	1	1	1	36
Pt5	acral melanoma	NA	NA	2	2	10	22

Pt6	cutaneous melanoma	no	yes	1	1	8	35
Pt7	acral melanoma	no	no	1	1	NA	2
Pt8	acral melanoma	NA	NA	0	13	1	39
Pt9	acral melanoma	NA	NA	NA	NA	5	1
Pt10	cutaneous melanoma	NA	NA	1	1	0	40
Pt11	acral melanoma	NA	NA	4	4	1	45

Table S3. Markers for the MM_Immune, B_CD74, cDC2_RTN1, and iCAF-CCL21 subgroups

Subgroup	Marker
MM_Immune	S100A11
MM_Immune	FTL
MM_Immune	RPS21
MM_Immune	UBC
MM_Immune	TMSB10
MM_Immune	PTMA
MM_Immune	S100A6
MM_Immune	H2AFZ
MM_Immune	VIM
MM_Immune	IGKC
MM_Immune	FTH1
MM_Immune	LGALS1
MM_Immune	MT-ND5
MM_Immune	CRYAB
MM_Immune	MT-ND2
MM_Immune	S100A1
MM_Immune	RPS18
MM_Immune	MT2A
MM_Immune	MT-CO3
MM_Immune	ATP5E
MM_Immune	MT-ND4L
MM_Immune	MT-ND4
MM_Immune	MT-ND3
MM_Immune	MT-ND1
MM_Immune	ACTB
MM_Immune	MT-ATP6
MM_Immune	MT-CO1
MM_Immune	S100A10
MM_Immune	MT-CYB
MM_Immune	MT-CO2
MM_Immune	HSPB1
MM_Immune	SPARC
MM_Immune	APOC1
MM_Immune	CSTB
MM_Immune	IGHG4
MM_Immune	SPP1
MM_Immune	IGHG3
MM_Immune	IGFBP2
MM_Immune	IGLC2
MM_Immune	IGHG1
MM_Immune	IGFBP7
MM_Immune	SERPINE2
MM_Immune	S100A4
MM_Immune	ANXA1

MM_Immune	RP11-191L9.4
MM_Immune	CCND1
MM_Immune	MT1E
MM_Immune	IGLC3
MM_Immune	ARL6IP1
MM_Immune	PTPRZ1
MM_Immune	MT-ATP8
MM_Immune	C11orf96
MM_Immune	FABP5
MM_Immune	PMEL
MM_Immune	EDNRB
MM_Immune	CTSB
MM_Immune	MGP
MM_Immune	ABCB5
MM_Immune	RP11-30J20.1
MM_Immune	KRT14
MM_Immune	TYRP1
MM_Immune	GDF15
MM_Immune	LYZ
MM_Immune	C1QB
MM_Immune	AC007319.1
MM_Immune	NELL1
MM_Immune	RP11-317N12.1
MM_Immune	C1QA
MM_Immune	TGFBI
MM_Immune	S100A9
MM_Immune	MT-ND6
MM_Immune	JCHAIN
MM_Immune	HIST1H4C
MM_Immune	S100A8
MM_Immune	CCL21
MM_Immune	SPARCL1
MM_Immune	APP
MM_Immune	CLU
MM_Immune	PTGDS
MM_Immune	CXCL10
MM_Immune	FRMD5
MM_Immune	RP11-734I18.1
MM_Immune	WWTR1
MM_Immune	HLA-DRA
MM_Immune	RP11-608O21.1
MM_Immune	LINGO2
MM_Immune	MIR548XHG
MM_Immune	IGHGP
MM_Immune	RHOBTB3

MM_Immune	A2M
MM_Immune	DCT
MM_Immune	ROBO2
MM_Immune	NOV
MM_Immune	IL1RAPL1
MM_Immune	KPNA2
MM_Immune	ITGA11
MM_Immune	LRRTM4
MM_Immune	XBP1
MM_Immune	CD14
MM_Immune	POLR2F
MM_Immune	POSTN
MM_Immune	HES1
MM_Immune	KRT6A
MM_Immune	CACNA2D3
iCAF-CCL21	FDCSP
iCAF-CCL21	XKR4
iCAF-CCL21	CCL21
iCAF-CCL21	LIFR
iCAF-CCL21	ADAM12
iCAF-CCL21	CLU
iCAF-CCL21	ZFPM2
iCAF-CCL21	SDK1
iCAF-CCL21	TENM3
iCAF-CCL21	ABI3BP
iCAF-CCL21	ROBO1
iCAF-CCL21	ANKRD29
iCAF-CCL21	SLC22A3
iCAF-CCL21	C7
iCAF-CCL21	LPAR1
iCAF-CCL21	C3
iCAF-CCL21	ABCC3
iCAF-CCL21	VCAM1
iCAF-CCL21	GRIN2B
iCAF-CCL21	SEMA5A
iCAF-CCL21	LAMA3
iCAF-CCL21	TMEM178B
iCAF-CCL21	OSMR
iCAF-CCL21	SYNPO2
iCAF-CCL21	CDH11
iCAF-CCL21	RNF150
iCAF-CCL21	NR2F2-AS1
iCAF-CCL21	HS3ST3A1
iCAF-CCL21	SNAP91
iCAF-CCL21	NCEH1
iCAF-CCL21	NOVA1
iCAF-CCL21	RORA
iCAF-CCL21	ST6GALNA
iCAF-CCL21	C5

iCAF-CCL21	SLCO3A1
iCAF-CCL21	ADAMTS9-AS2
iCAF-CCL21	GNA14
iCAF-CCL21	FAM155A
iCAF-CCL21	PARD3B
iCAF-CCL21	CCL19
iCAF-CCL21	CACNA1C
iCAF-CCL21	CR2
iCAF-CCL21	PRRX1
iCAF-CCL21	RP1-84O15.2
iCAF-CCL21	PAPPA
iCAF-CCL21	NRXN3
iCAF-CCL21	KIAA1217
iCAF-CCL21	CXCL14
iCAF-CCL21	SVIL
iCAF-CCL21	BICC1
iCAF-CCL21	VEGFC
iCAF-CCL21	SLC9A9
iCAF-CCL21	CCDC102B
iCAF-CCL21	ALPK2
iCAF-CCL21	CLMP
iCAF-CCL21	DLG2
iCAF-CCL21	ZNF804B
iCAF-CCL21	FLRT2
iCAF-CCL21	MAGI1
iCAF-CCL21	NOL4
iCAF-CCL21	EPB41L2
iCAF-CCL21	NFASC
iCAF-CCL21	NFIB
iCAF-CCL21	SLIT3
iCAF-CCL21	SVEP1
iCAF-CCL21	NRG1
iCAF-CCL21	IGFBP3
iCAF-CCL21	ARHGAP29
iCAF-CCL21	TNFAIP3
iCAF-CCL21	LINC01197
iCAF-CCL21	NAV2
iCAF-CCL21	PTGDS
iCAF-CCL21	ADGRL2
iCAF-CCL21	SLIT2
iCAF-CCL21	NKAIN2
iCAF-CCL21	RARB
iCAF-CCL21	ROBO2
iCAF-CCL21	TENM4
iCAF-CCL21	IL15
iCAF-CCL21	COL4A4
iCAF-CCL21	PRUNE2
iCAF-CCL21	SEMA3A

iCAF-CCL21	PLPP3
iCAF-CCL21	PALLD
iCAF-CCL21	ANK2
iCAF-CCL21	EGFR
iCAF-CCL21	PLD5
iCAF-CCL21	LURAP1L
iCAF-CCL21	PLEKHG1
iCAF-CCL21	PCDH11X
iCAF-CCL21	COL12A1
iCAF-CCL21	PTPRK
iCAF-CCL21	LINC00693
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iCAF-CCL21	HEG1
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iCAF-CCL21	SULF1
iCAF-CCL21	GLIS3
iCAF-CCL21	ITIH5
iCAF-CCL21	USP53
iCAF-CCL21	ADAMTS3
iCAF-CCL21	DCLK1
iCAF-CCL21	MYLK
iCAF-CCL21	FBXL7
iCAF-CCL21	SLCO2B1
iCAF-CCL21	CFH
iCAF-CCL21	APBB2
iCAF-CCL21	TCF4
iCAF-CCL21	CSMD1
iCAF-CCL21	FGD6
iCAF-CCL21	CXCL12
iCAF-CCL21	TBX15
iCAF-CCL21	PLA2G4C
iCAF-CCL21	PTPRM
iCAF-CCL21	MUSK
iCAF-CCL21	LTBP1
iCAF-CCL21	LARGE
iCAF-CCL21	TNFRSF11B
iCAF-CCL21	C1R
iCAF-CCL21	IRAK3
iCAF-CCL21	CR1
iCAF-CCL21	GHR
iCAF-CCL21	SLCO1A2
iCAF-CCL21	DSE
iCAF-CCL21	SDC2
iCAF-CCL21	ARHGAP24
iCAF-CCL21	CNTN1
iCAF-CCL21	RP11-266O8.1
iCAF-CCL21	DDR2
iCAF-CCL21	NR2F2

iCAF-CCL21	LRRC4C
iCAF-CCL21	ARNTL2
iCAF-CCL21	FBN1
iCAF-CCL21	EPAS1
iCAF-CCL21	RAMP3
iCAF-CCL21	RP11-81H14.2
iCAF-CCL21	RFTN1
iCAF-CCL21	COL5A2
iCAF-CCL21	NR2F1-AS1
iCAF-CCL21	THRB
iCAF-CCL21	RBMS3
iCAF-CCL21	RAPGEF5
iCAF-CCL21	MIR4435-2HG
iCAF-CCL21	PBX1
iCAF-CCL21	MROH9
iCAF-CCL21	GUCY1A3
iCAF-CCL21	PLXNA4
iCAF-CCL21	FNDC1
iCAF-CCL21	COL4A2
iCAF-CCL21	COL6A2
iCAF-CCL21	CDK14
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iCAF-CCL21	MEOX2
iCAF-CCL21	TCF7L2
iCAF-CCL21	RP11-739G5.1
iCAF-CCL21	C10orf11
iCAF-CCL21	RIN2
iCAF-CCL21	ITPR2
iCAF-CCL21	ANKS1B
iCAF-CCL21	PCOLCE2
iCAF-CCL21	NNMT
iCAF-CCL21	SRGAP1
iCAF-CCL21	ADAMTS12
iCAF-CCL21	ADAMTS9
iCAF-CCL21	COL6A1
iCAF-CCL21	TFPI
iCAF-CCL21	FAM20A
iCAF-CCL21	RUNX1T1
iCAF-CCL21	ZNF804A
iCAF-CCL21	UNC5C
iCAF-CCL21	LHFP
iCAF-CCL21	ANTXR1
iCAF-CCL21	FIGN
iCAF-CCL21	SMOC2

iCAF-CCL21	CLSTN2
iCAF-CCL21	DEPTOR
iCAF-CCL21	ABTB2
iCAF-CCL21	COL1A2
iCAF-CCL21	NRG2
iCAF-CCL21	NEGR1
iCAF-CCL21	PDGFRB
iCAF-CCL21	GRIA4
iCAF-CCL21	CPE
iCAF-CCL21	ZNF385B
iCAF-CCL21	DPYSL3
iCAF-CCL21	PKP2
iCAF-CCL21	ZNF521
iCAF-CCL21	OCA2
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iCAF-CCL21	SORCS1
iCAF-CCL21	FNDC3B
iCAF-CCL21	GREM1
iCAF-CCL21	RSPO3
iCAF-CCL21	AFAP1
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iCAF-CCL21	LINC01239
iCAF-CCL21	NLGN4Y
iCAF-CCL21	CDH1
iCAF-CCL21	PIEZO2
iCAF-CCL21	DOK6
iCAF-CCL21	RP11-759A24.3
iCAF-CCL21	COL1A1
iCAF-CCL21	DCBLD2
iCAF-CCL21	GPC6
iCAF-CCL21	NPHS1
iCAF-CCL21	TIMP1
iCAF-CCL21	PAK3

iCAF-CCL21	PGM5
iCAF-CCL21	RND3
iCAF-CCL21	STK32B
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