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Supplemental information

PD-L1-expressing tumor-associated macrophages are immunostimulatory and associate with good clinical outcome in human breast cancer

Lei Wang, Weihua Guo, Zhikun Guo, Jiangnan Yu, Jiayi Tan, Diana L. Simons, Ke Hu, Xinyu Liu, Qian Zhou, Yizi Zheng, Egelston A. Colt, John Yim, James Waisman, and Peter P. Lee

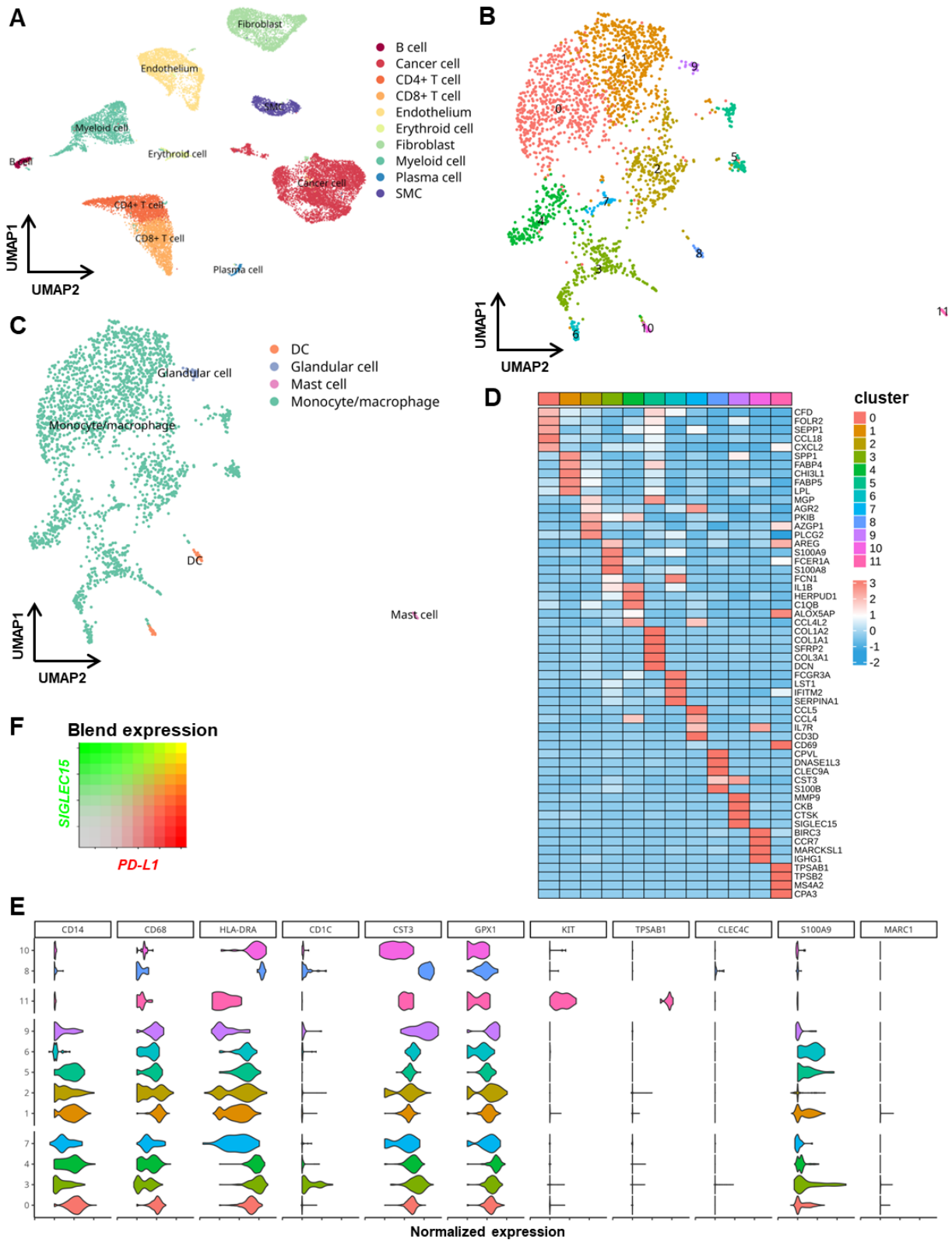
A
Fibroblast
Endothelium
SMC
Myeloid cell
B cell
Erythroid cell
Cancer cell
CD4+ T cell
CD8+ T cell
Plasma cell
UMAP1
UMAP2
B cell
Cancer cell
CD4+ T cell
CD8+ T cell
Endothelium
Erythroid cell
Fibroblast
Myeloid cell
Plasma cell
SMC
B
UMAP1
UMAP2
C
Glandular cell
Monocyte/macrophage
DC
Mast cell
UMAP1
UMAP2
DC
Glandular cell
Mast cell
Monocyte/macrophage
D
cluster
CFD
FOLR2
SEPP1
CCL18
CXCL2
SPP1
FABP4
CHI3L1
FABP5
LPL
MGP
AGR2
PKIB
AZGP1
PLCG2
AREG
S100A9
FCER1A
S100A8
FCN1
IL1B
HERPUD1
C1QB
ALOX5AP
CCL4L2
COL1A2
COL1A1
SFRP2
COL3A1
DCN
FCGR3A
LST1
IFITM2
SERPINA1
CCL5
CCL4
IL7R
CD3D
CD69
CPVL
DNASE1L3
CLEC9A
CST3
S100B
MMP9
CKB
CTSK
SIGLEC15
BIRC3
CCR7
MARCKSL1
IGHG1
TPSAB1
TPSB2
MS4A2
CPA3
F
Blend expression
SIGLEC15
PD-L1
E
CD14
CD68
HLA-DRA
CD1C
CST3
GPX1
KIT
TPSAB1
CLEC4C
S100A9
MARC1
Normalized expression

Figure S1. Single-cell analysis with blended expression analysis of PD-L1 and SIGLEC15 identified the PD-L1$^{+/hi}$ and PD-L1$^{-/lo}$ monocytes/macrophages, related to Figure 1. (A) 10 major cell types were identified and annotated in TME. **(B)** 12 clusters were identified in myeloid cells with the optimal clustering resolution (r = 0.5). **(C)** Monocytes/macrophages were identified and annotated in TME. **(D)** Average expression heatmap of the top 5 markers of each cluster. **(E)** Violin plot of the expression of key myeloid cell markers crossing all the myeloid cell clusters. **(F)** Blend expression of *PD-L1* and *SIGLEC15* on monocytes/macrophages.

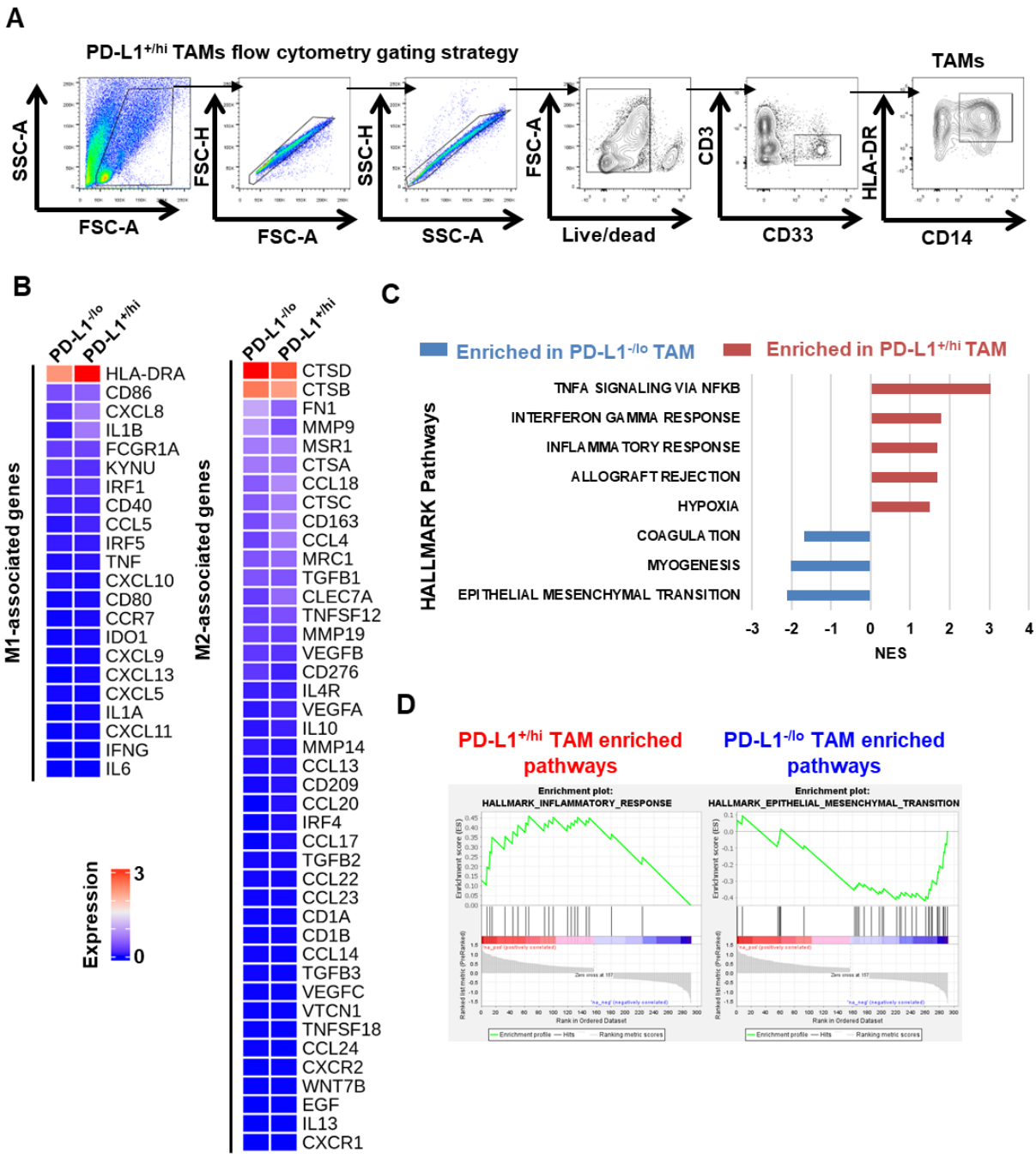
A
PD-L1+/hi TAMs flow cytometry gating strategy
TAMs
SSC-A
FSC-A
FSC-H
FSC-A
SSC-H
SSC-A
FSC-A
Live/dead
CD3
CD33
HLA-DR
CD14
B
PD-L1-/lo
PD-L1+/hi
M1-associated genes
HLA-DRA
CD86
CXCL8
IL1B
FCGR1A
KYNU
IRF1
CD40
CCL5
IRF5
TNF
CXCL10
CD80
CCR7
IDO1
CXCL9
CXCL13
CXCL5
IL1A
CXCL11
IFNG
IL6
M2-associated genes
CTSD
CTSB
FN1
MMP9
MSR1
CTSA
CCL18
CTSC
CD163
CCL4
MRC1
TGFB1
CLEC7A
TNFSF12
MMP19
VEGFB
CD276
IL4R
VEGFA
IL10
MMP14
CCL13
CD209
CCL20
IRF4
CCL17
TGFB2
CCL22
CCL23
CD1A
CD1B
CCL14
TGFB3
VEGFC
VTCN1
TNFSF18
CCL24
CXCR2
WNT7B
EGF
IL13
CXCR1
Expression
3
0
C
Enriched in PD-L1-/lo TAM
Enriched in PD-L1+/hi TAM
HALLMARK Pathways
TNFA SIGNALING VIA NFKB
INTERFERON GAMMA RESPONSE
INFLAMMATORY RESPONSE
ALLOGRAFT REJECTION
HYPOXIA
COAGULATION
MYOGENESIS
EPITHELIAL MESENCHYMAL TRANSITION
-3
-2
-1
0
1
2
3
4
NES
D
PD-L1+/hi TAM enriched pathways
PD-L1-/lo TAM enriched pathways
Enrichment plot:
HALLMARK_INFLAMMATORY_RESPONSE
Enrichment plot:
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION

Figure S2. PD-L1$^{+/hi}$ and PD-L1$^{-/lo}$ TAMs dichotomy confirmed by flow cytometry, related to Figure 1. (A) Representative flow plots showing the gating of TAMs from human breast tumor. **(B)** Expression levels of M1- or M2-associated genes in PD-L1^{+} and PD-L1^{-} TAMs. **(C)** All significantly enriched pathways from gene-set enrichment analysis of PD-L1^{+} and PD-L1^{-} TAMs based on the differential expressed genes (DEGs) identified by scRNA-seq (FDR q-value<0.1). **(D)** Enrichment plot of representative enriched pathways for PD-L1^{+} and PD-L1^{-}TAMs.

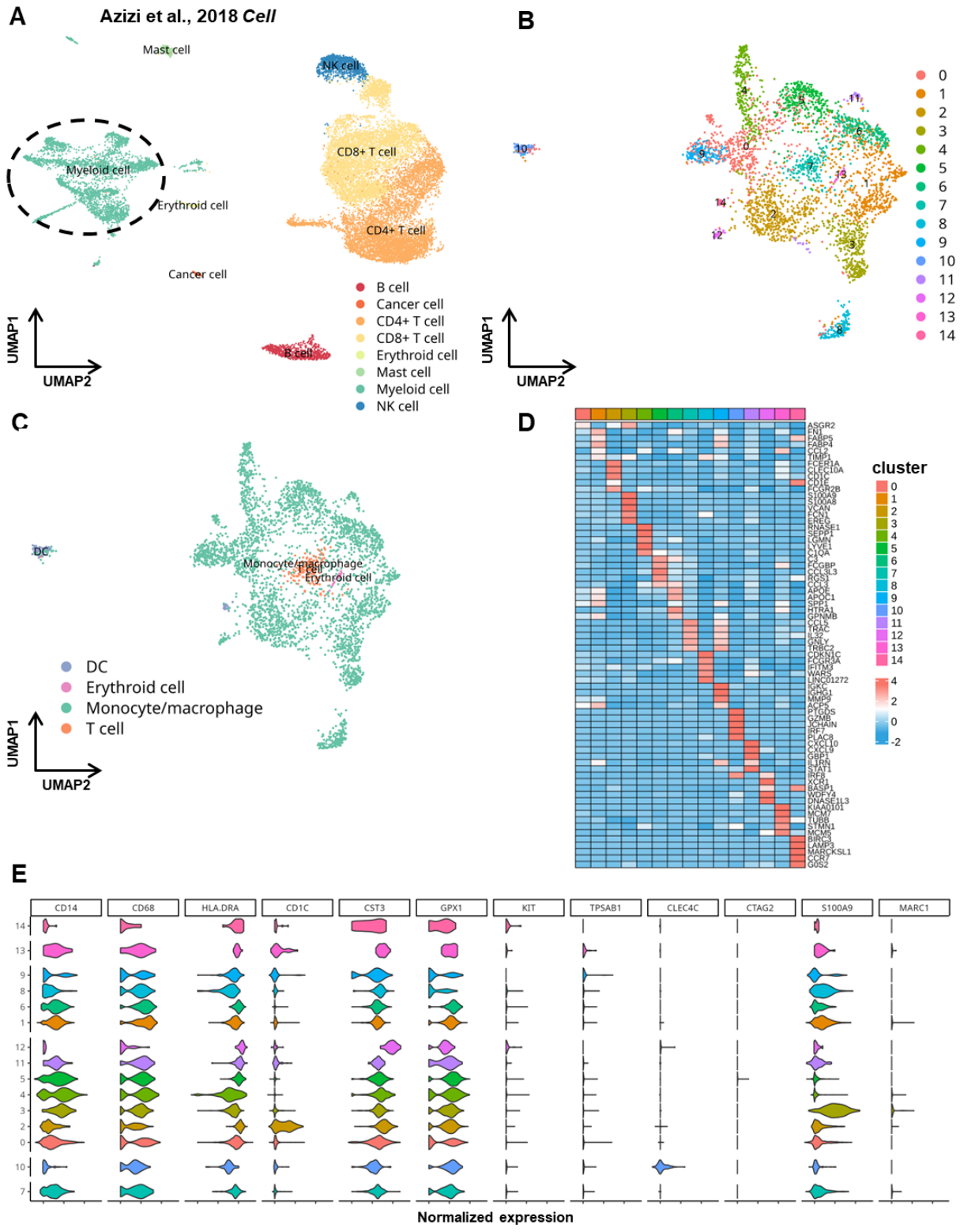
A
Azizi et al., 2018 Cell
Mast cell
NK cell
CD8+ T cell
Myeloid cell
Erythroid cell
CD4+ T cell
Cancer cell
B cell
B cell
Cancer cell
CD4+ T cell
CD8+ T cell
Erythroid cell
Mast cell
Myeloid cell
NK cell
UMAP1
UMAP2
B
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
UMAP1
UMAP2
C
DC
Monocyte/macrophage
B cell
Erythroid cell
DC
Erythroid cell
Monocyte/macrophage
T cell
UMAP1
UMAP2
D
cluster
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
4
2
0
-2
E
CD14
CD68
HLA.DRA
CD1C
CST3
GPX1
KIT
TPSAB1
CLEC4C
CTAG2
S100A9
MARC1
Normalized expression

Figure S3. Single-cell analysis identified the PD-L1$^{+/hi}$ and PD-L1$^{-/lo}$ monocytes/macrophages (Azizi et al., *Cell* 2018), related to Figure 1. (A) 8 major cell types were identified and annotated in TME. **(B)**15 clusters were identified in myeloid cells with the optimal clustering resolution (r = 0.6). **(C)** Monocytes/macrophages were identified and annotated in TME. **(D)** Average expression heatmap of the top 5 markers of each cluster. **(E)** Violin plot of the expression of key myeloid cell markers crossing all the myeloid cell clusters.

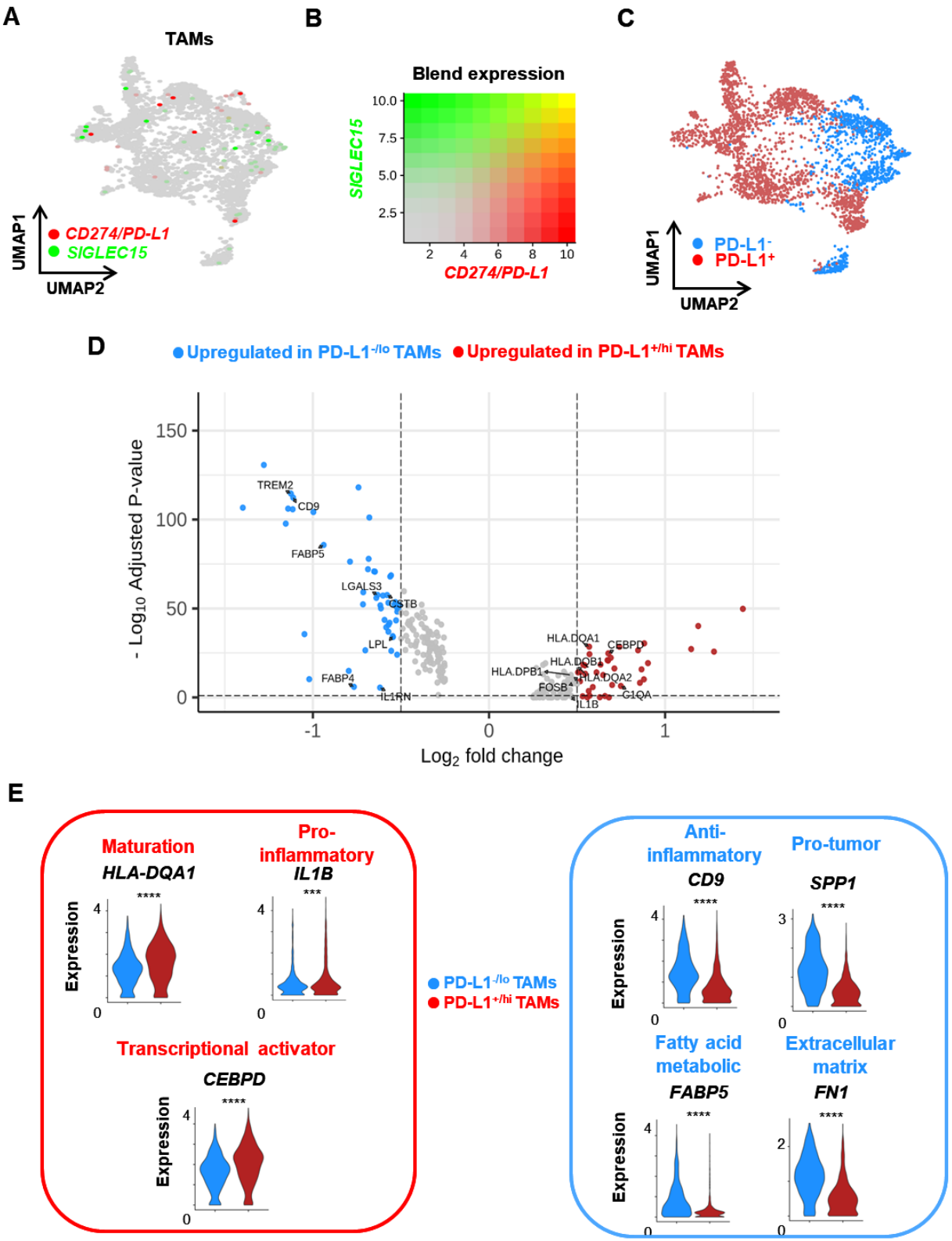
A
TAMs
UMAP1
UMAP2
CD274/PD-L1
SIGLEC15
B
Blend expression
SIGLEC15
CD274/PD-L1
10.0
7.5
5.0
2.5
2
4
6
8
10
C
UMAP1
UMAP2
PD-L1-
PD-L1+
D
Upregulated in PD-L1-/lo TAMs
Upregulated in PD-L1+/hi TAMs
- Log10 Adjusted P-value
Log2 fold change
150
100
50
0
-1
0
1
TREM2
CD9
FABP5
LGALS3
CSTB
LPL
FABP4
IL1RN
HLA.DQA1
CEBPD
HLA.DQB1
HLA.DPB1
HLA.DQA2
FOSB
C1QA
IL1B
E
Maturation
HLA-DQA1
Pro-inflammatory
IL1B
Expression
****
***
Transcriptional activator
CEBPD
PD-L1-/lo TAMs
PD-L1+/hi TAMs
Anti-inflammatory
CD9
Pro-tumor
SPP1
Fatty acid metabolic
FABP5
Extracellular matrix
FN1

Figure S4. Expression profile differences between PD-L1^{+} and PD-L1^{-} TAMs validated in public scRNA-seq data (Azizi et al., *Cell* 2018), related to Figure 1. (A) UMAP of TAMs (*CD14*$^{+}$*CD68*$^{+}$*HLA-DR*$^{+}$, n=3,130 cells) from the public scRNA-seq data. **(B)** Blend expression of *PD-L1* and *SIGLEC15*. **(C)** The dichotomization of the TAM clusters into *PD-L1*$^{+}$/*SIGLEC15*$^{-}$ and *PD-L1*$^{-}$/*SIGLEC15*$^{+}$ subpopulations. **(D)** Volcano plot showing differentially expressed genes (DEGs) between the subpopulation of PD-L1^{+} or PD-L1^{-} TAMs. **(E)** Expression distribution of selected genes involved in maturation, pro-inflammatory or transcriptional activator and anti-inflammatory, pro-tumor, fatty acid metabolic or extracellular matrix between PD-L1$^{+/hi}$ and PD-L1$^{-/lo}$ TAMs.

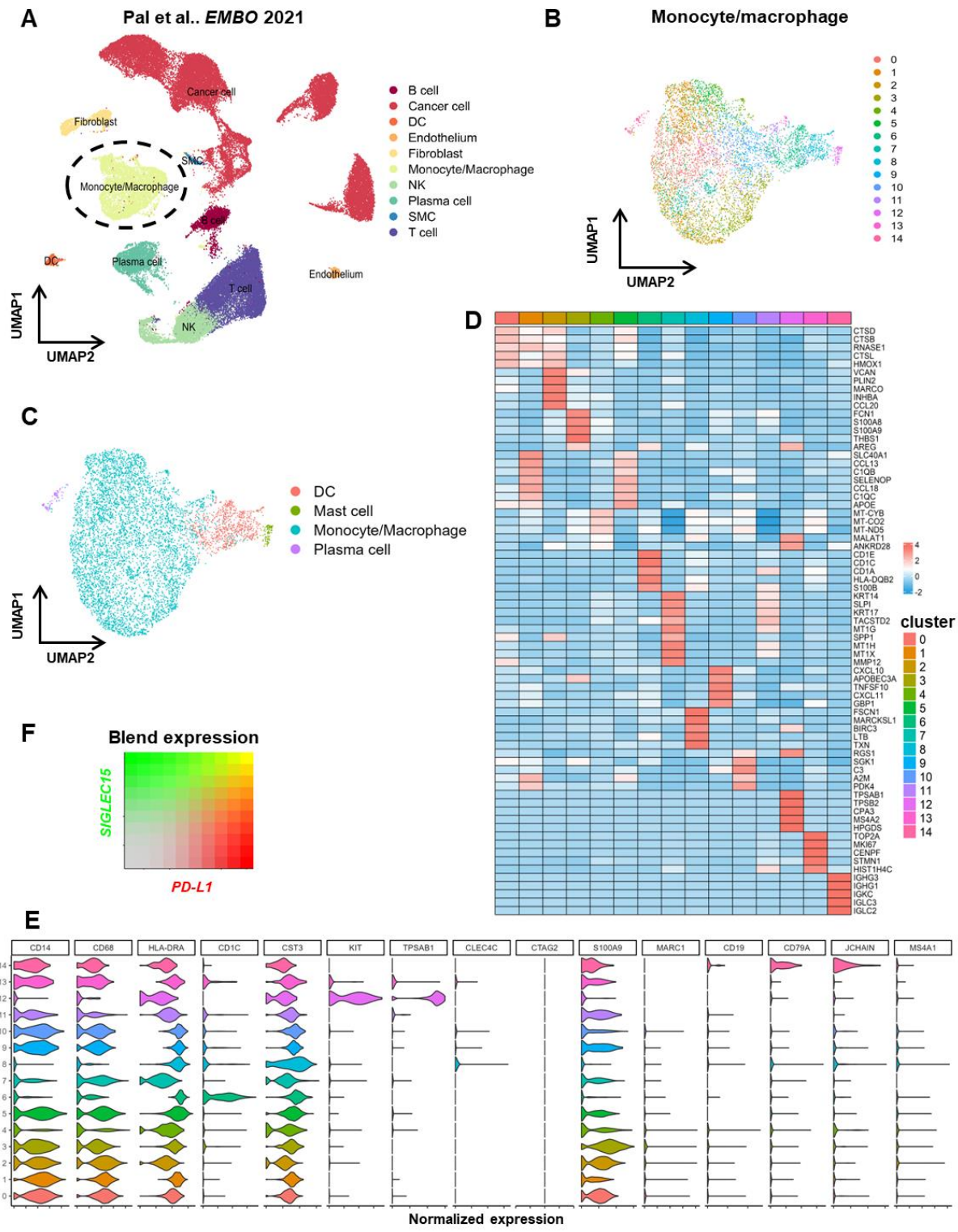
A
Pal et al.. EMBO 2021
Cancer cell
Fibroblast
SMC
Monocyte/Macrophage
B cell
DC
Plasma cell
T cell
NK
Endothelium
B cell
Cancer cell
DC
Endothelium
Fibroblast
Monocyte/Macrophage
NK
Plasma cell
SMC
T cell
UMAP1
UMAP2
B
Monocyte/macrophage
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
UMAP1
UMAP2
C
DC
Mast cell
Monocyte/Macrophage
Plasma cell
UMAP1
UMAP2
D
CTSD
CTSB
RNASE1
CTSL
HMOX1
VCAN
PLIN2
MARCO
INHBA
CCL20
FCN1
S100A8
S100A9
THBS1
AREG
SLC40A1
CCL13
C1QB
SELENOP
CCL18
C1QC
APOE
MT-CYB
MT-CO2
MT-ND5
MALAT1
ANKRD28
CD1E
CD1C
CD1A
HLA-DQB2
S100B
KRT14
SLPI
KRT17
TACSTD2
MT1G
SPP1
MT1H
MT1X
MMP12
CXCL10
APOBEC3A
TNFSF10
CXCL11
GBP1
FSCN1
MARCKSL1
BIRC3
LTB
TXN
RGS1
SGK1
C3
A2M
PDK4
TPSAB1
TPSB2
CPA3
MS4A2
HPGDS
TOP2A
MKI67
CENPF
STMN1
HIST1H4C
IGHG3
IGHG1
IGKC
IGLC3
IGLC2
4
2
0
-2
cluster
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
F
Blend expression
SIGLEC15
PD-L1
E
CD14
CD68
HLA-DRA
CD1C
CST3
KIT
TPSAB1
CLEC4C
CTAG2
S100A9
MARC1
CD19
CD79A
JCHAIN
MS4A1
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Normalized expression

Figure S5. Single-cell analysis identified the PD-L1$^{+/hi}$ and PD-L1$^{-/lo}$ monocytes/macrophages (Pal et al., *EMBO* 2021), related to Figure 2. (A) 10 major cell types were identified and annotated in TME. **(B)** 15 clusters were identified in myeloid cells with the optimal clustering resolution (r = 0.8). **(C)** Monocytes/macrophages were identified and annotated in TME. **(D)** Average expression heatmap of the top 5 markers of each cluster. **(E)** Violin plot of the expression of key myeloid cell markers crossing all the myeloid cell clusters. **(F)** Blend expression of *PD-L1* and *SIGLEC15* on monocytes/macrophages.

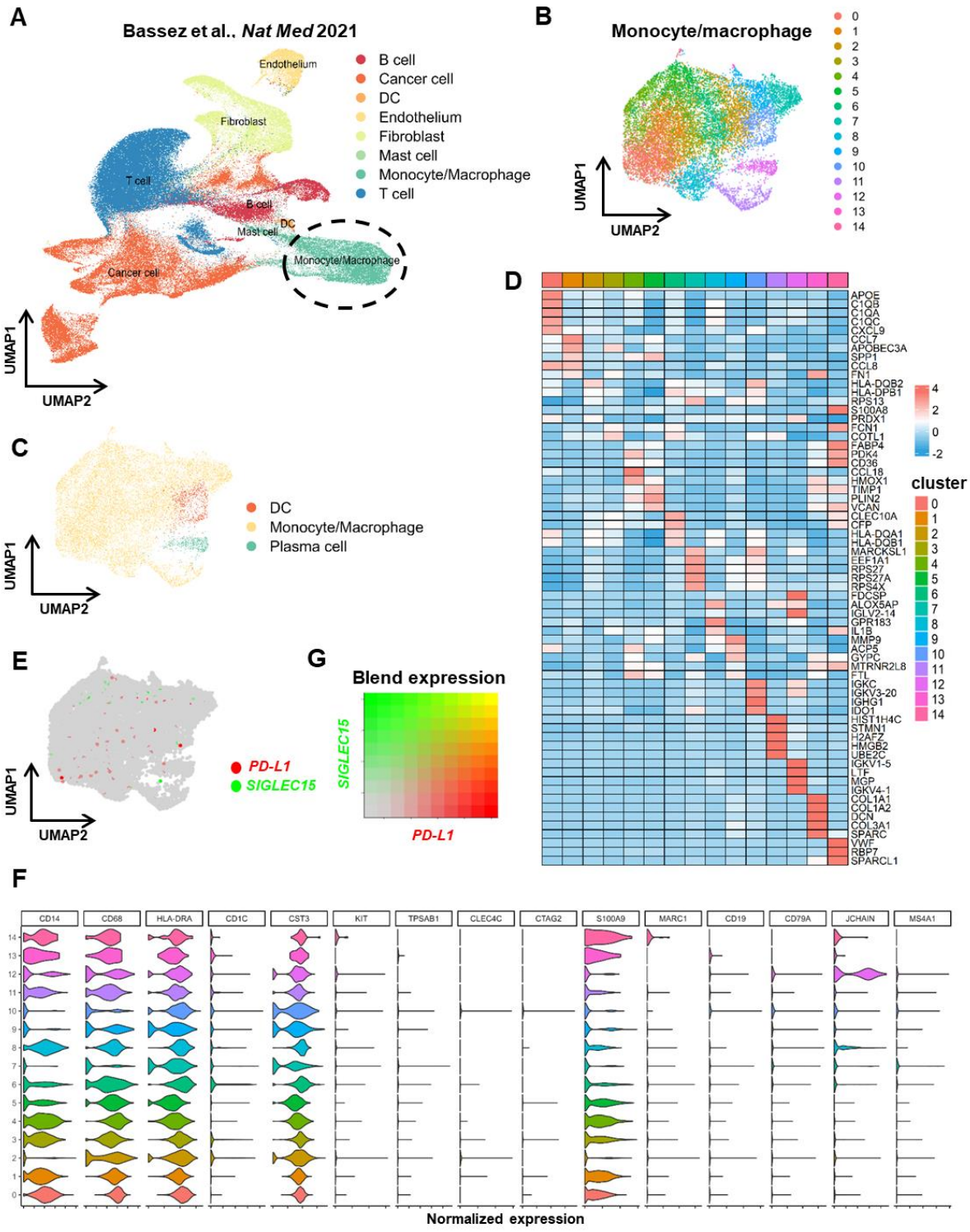
A
Bassez et al., Nat Med 2021
Endothelium
Fibroblast
T cell
B cell
DC
Mast cell
Cancer cell
Monocyte/Macrophage
B cell
Cancer cell
DC
Endothelium
Fibroblast
Mast cell
Monocyte/Macrophage
T cell
UMAP1
UMAP2
B
Monocyte/macrophage
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
C
DC
Monocyte/Macrophage
Plasma cell
D
APOE C1QB C1QA C1QC CXCL9 CCL7 APOBEC3A SPP1 CCL8 FN1 HLA-DQB2 HLA-DPB1 RPS13 S100A8 PRDX1 FCN1 COTL1 FABP4 PDK4 CD36 CCL18 HMOX1 TIMP1 PLIN2 VCAN CLEC10A CFP HLA-DQA1 HLA-DQB1 MARCKSL1 EEF1A1 RPS27 RPS27A RPS4X FDCSP ALOX5AP IGLV2-14 GPR183 IL1B MMP9 ACP5 GYPC MTRNR2L8 FTL IGKC IGKV3-20 IGHG1 IDO1 HIST1H4C STMN1 H2AFZ HMGB2 UBE2C IGKV1-5 LTF MGP IGKV4-1 COL1A1 COL1A2 DCN COL3A1 SPARC VWF RBP7 SPARCL1
4 2 0 -2
cluster
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
E
PD-L1
SIGLEC15
G
Blend expression
SIGLEC15
PD-L1
F
CD14 CD68 HLA-DRA CD1C CST3 KIT TPSAB1 CLEC4C CTAG2 S100A9 MARC1 CD19 CD79A JCHAIN MS4A1
Normalized expression

Figure S6. Single-cell analysis identified the PD-L1$^{+/hi}$ and PD-L1$^{-/lo}$ monocytes/macrophages (Bassez et al., *Nat Med* 2021), related to Figure 2. (A) 8 major cell types were identified and annotated in TME. **(B)** 15 clusters were identified in myeloid cells with the optimal clustering resolution (r = 0.8). **(C)** Monocytes/macrophages were identified and annotated in TME. **(D)** Average expression heatmap of the top 5 markers of each cluster. **(E)** Mutually exclusive expression of *PD-L1* and *SIGLEC15* in myeloid cells. **(F)** Violin plot of the expression of key myeloid cell markers crossing all the myeloid cell clusters. **(G)** Blend expression of *PD-L1* and *SIGLEC15* on monocytes/macrophages.

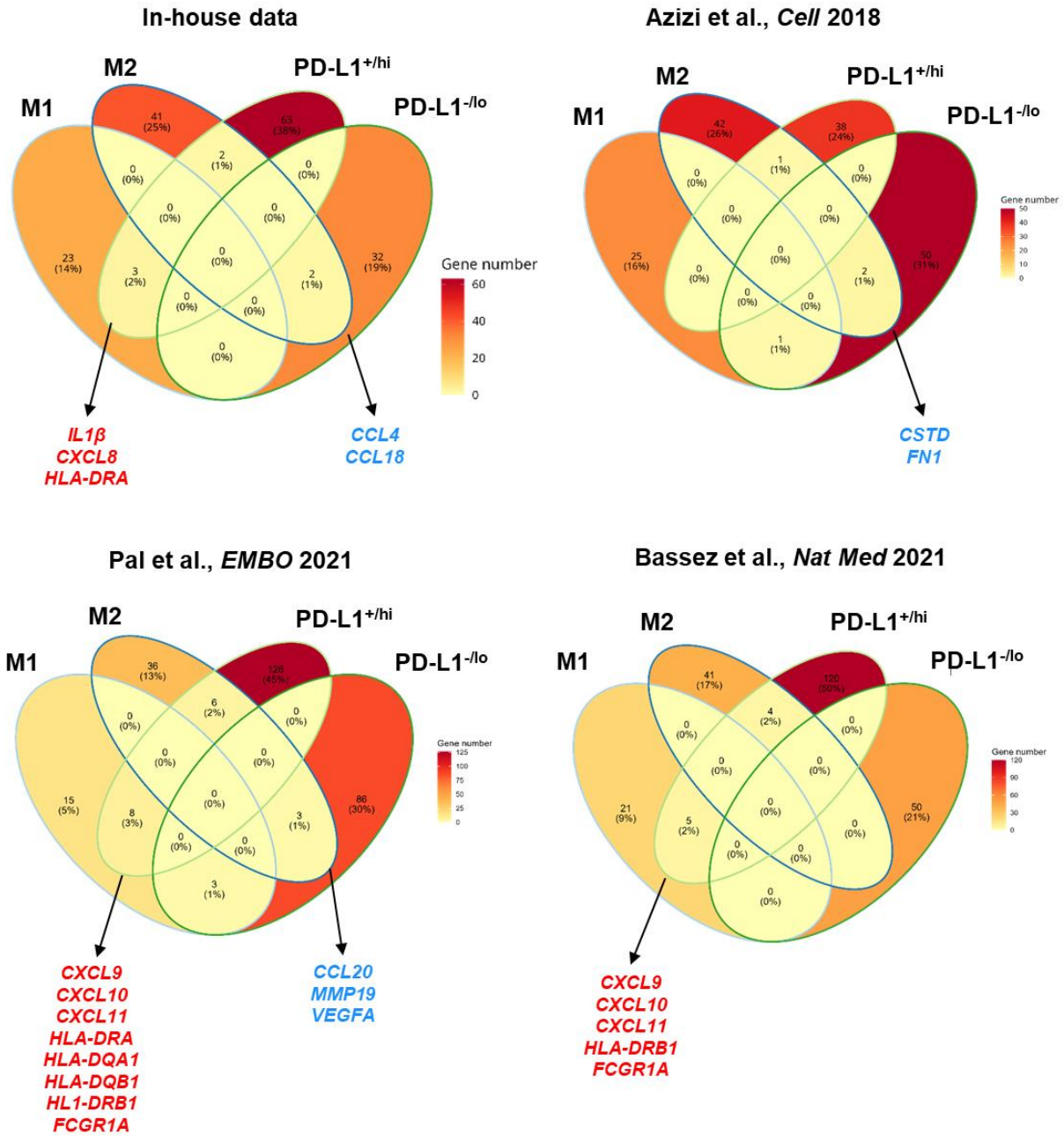

Figure S7. Venn diagram analysis of the overlap genes between PD-L1$^{+/hi}$ or PD-L1$^{-/lo}$ DEGs with M1 or M2 marker genes using in-house and public scRNA-seq data, related to Figure 3.

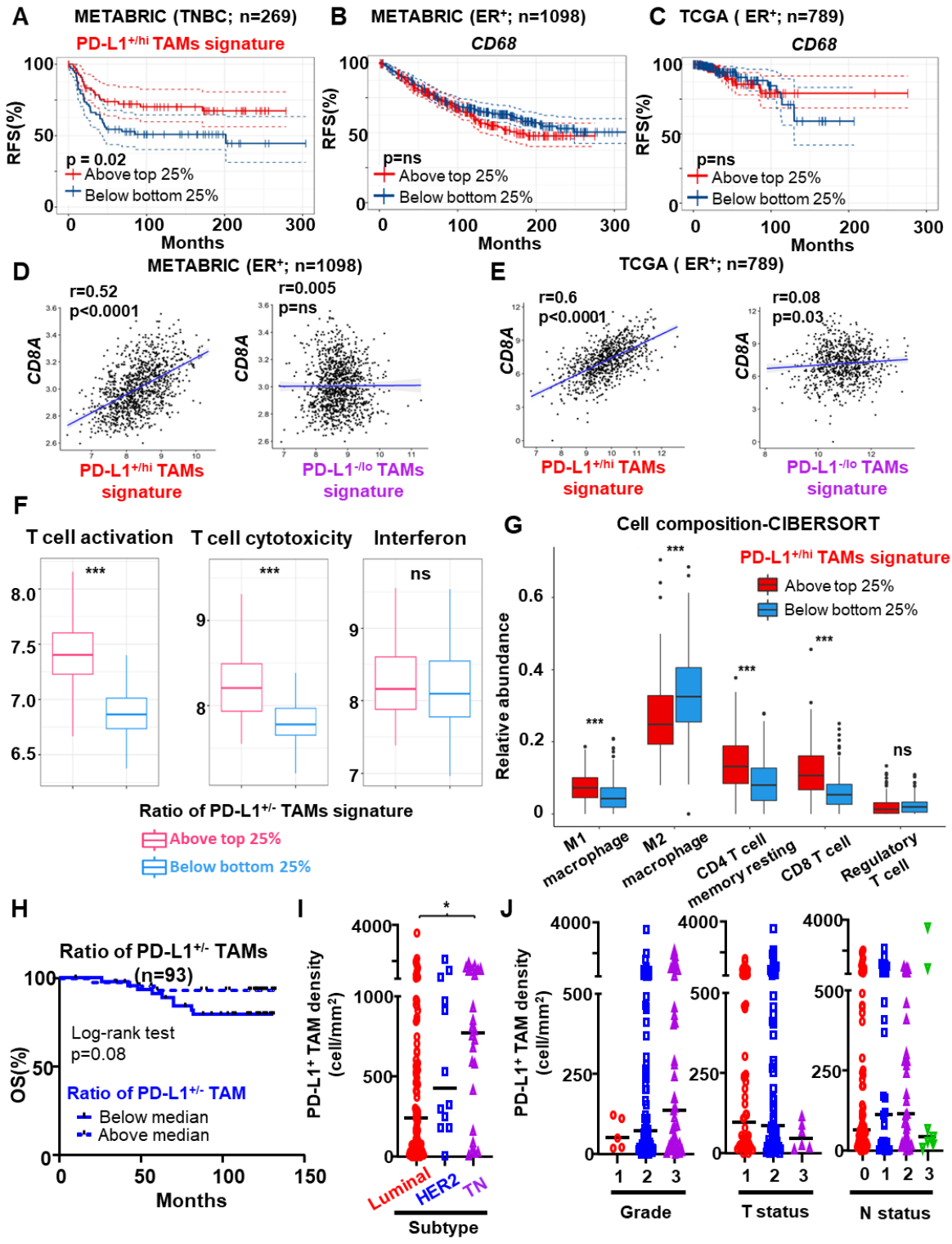
A
METABRIC (TNBC; n=269)
PD-L1+/hi TAMs signature
RFS(%)
p = 0.02
Above top 25%
Below bottom 25%
Months
B
METABRIC (ER+; n=1098)
CD68
p=ns
C
TCGA (ER+; n=789)
D
r=0.52
p<0.0001
r=0.005
p=ns
CD8A
PD-L1+/hi TAMs signature
PD-L1-/lo TAMs signature
E
r=0.6
p<0.0001
r=0.08
p=0.03
F
T cell activation
T cell cytotoxicity
Interferon
Ratio of PD-L1+/- TAMs signature
G
Cell composition-CIBERSORT
Relative abundance
M1 macrophage
M2 macrophage
CD4 T cell memory resting
CD8 T cell
Regulatory T cell
H
Ratio of PD-L1+/- TAMs (n=93)
OS(%)
Log-rank test
p=0.08
Ratio of PD-L1+/- TAM
Below median
Above median
I
PD-L1+ TAM density (cell/mm2)
Luminal
HER2
TN
Subtype
J
Grade
T status
N status

Figure S8. Analysis of PD-L1^{+} and PD-L1^{-} TAMs gene signature in public bulk-tumor transcriptomic datasets, related to Figure 3. (A) Kaplan-Meier relapse-free survival (RFS) curves and log-rank test generated for the gene signature of PD-L1$^{+/hi}$ TAMs $^{-}$ in the TNBC cohort of METABRIC (n=269) dataset. **(B-C)** Kaplan-Meier RFS curves and log-rank test generated for the gene expression of *CD68* in the luminal BC cohorts of METABRIC (n=1098) **(B)** and TCGA (n=789) **(C)** datasets. **(D-E)** The association between *CD8A* expression and PD-L1^{+} or PD-L1^{-} TAMs gene signature in METABRIC **(D)** and TCGA **(E)** datasets. Correlation coefficient test. **(F)** Gene signatures of T cell activation, T cell cytotoxicity or interferon were compared between patients with high vs. low gene signature ratio of PD-L1^{+}/PD-L1^{-} TAMs in the luminal BC cohort of METABRIC (n=1098). **(G)** Cell composition differences determined by CIBERSORT deconvolution method between tumors with high- and low-expressing gene signature of PD-L1$^{+/hi}$ TAMs. Patients were divided into high- and low-expressing groups based on a 25% cut-off of the gene signature or *CD68* expression. ****p<0.0001. **(H)** Kaplan-Meier overall survival (OS) curves and log-rank test generated for above or below median density ratio of PD-L1^{+}/PD-L1^{-} TAMs in cohort #2 (n=93). **(I)** The density of PD-L1^{+} TAMs in BC patients with luminal, HER2 or TN subtype (n=129). **(J)** The density of PD-L1^{+} TAMs in combined cohort #1 and 2 (n=142) with various tumor grade, T status and N status. *p<0.05.

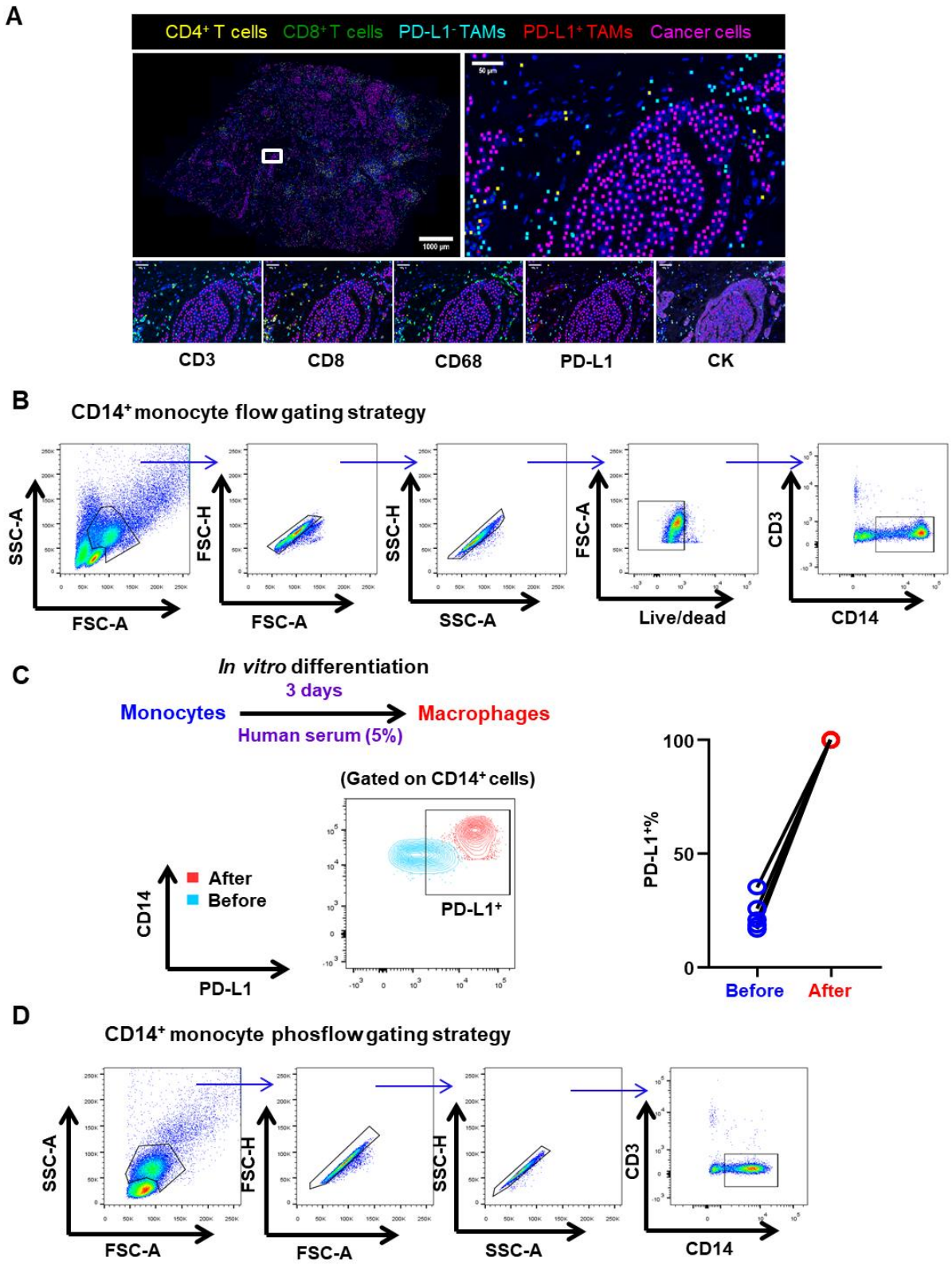
A
CD4+ T cells
CD8+ T cells
PD-L1- TAMs
PD-L1+ TAMs
Cancer cells
50 μm
1000 μm
CD3
CD8
CD68
PD-L1
CK
B
CD14+ monocyte flow gating strategy
SSC-A
FSC-A
FSC-H
FSC-A
SSC-H
SSC-A
FSC-A
Live/dead
CD3
CD14
C
In vitro differentiation
3 days
Monocytes
Macrophages
Human serum (5%)
(Gated on CD14+ cells)
After
Before
CD14
PD-L1
PD-L1+
PD-L1+%
100
50
0
Before
After
D
CD14+ monocyte phosflow gating strategy
SSC-A
FSC-A
FSC-H
FSC-A
SSC-H
SSC-A
CD3
CD14

Figure S9. PD-L1 is upregulated during the monocyte-macrophage differentiation, related to Figure 5. (A) Representative multiple immunofluorescence staining of breast tumor tissue section for PD-L1^{+} TAMs (CD68^{+}PD-L1^{+}), PD-L1^{-} TAMs (CD68^{+}PD-L1^{-}), CD8^{+} T cells (CD8^{+}), CD4+ T cells (CD3^{+}CD8^{-}) and cancer cells (CK^{+}). **(B)** Representative flow plots showing the gating strategy of peripheral blood monocytes from patients with BC. **(C)** Schematic and the representative flow plot of PD-L1 expression after in vitro macrophage differentiation. **(D)** Representative flow plots showing the gating strategy of peripheral monocytes in phosflow cytometry.

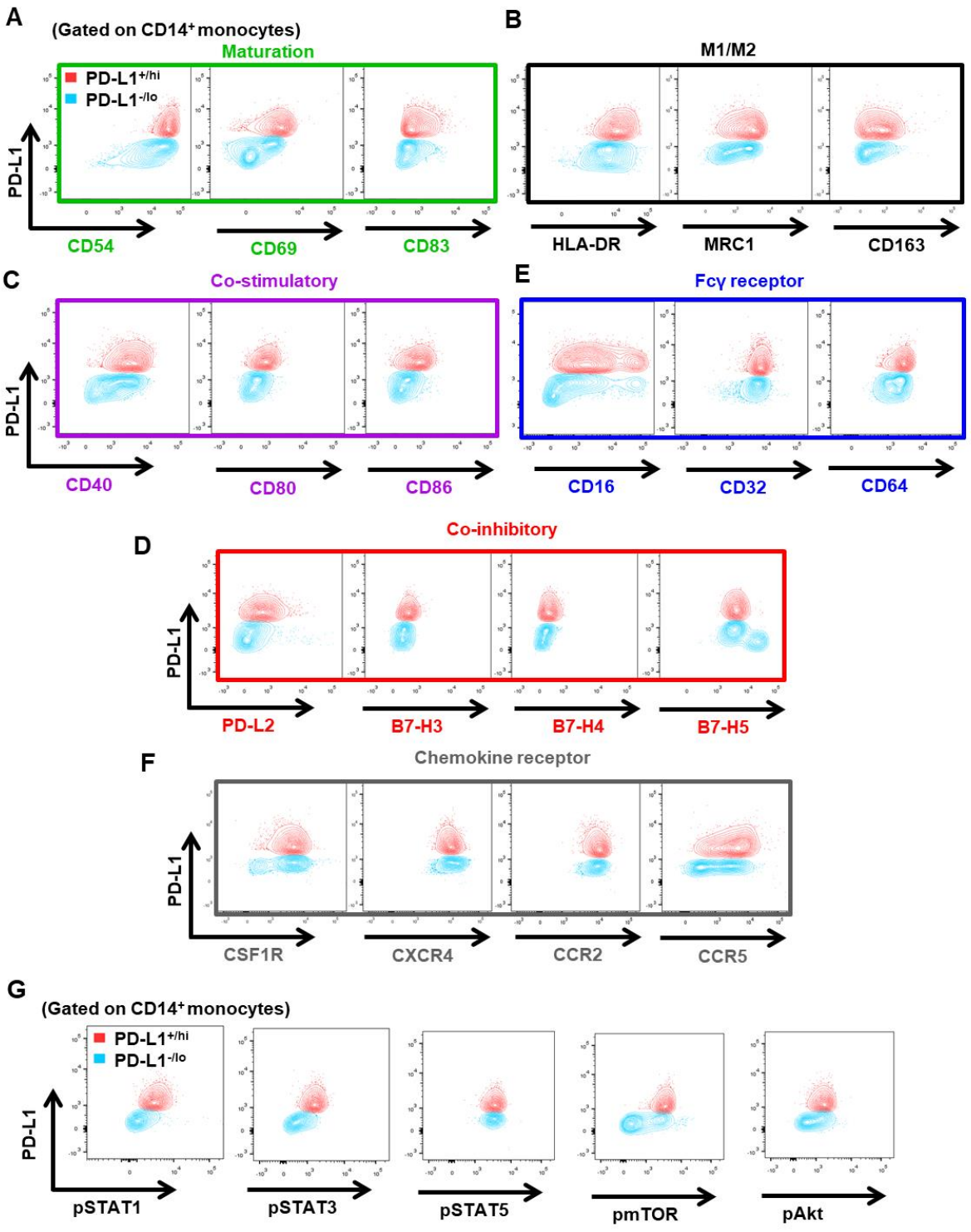
A
(Gated on CD14+ monocytes)
Maturation
PD-L1+/hi
PD-L1-/lo
PD-L1
CD54
CD69
CD83
B
M1/M2
HLA-DR
MRC1
CD163
C
Co-stimulatory
CD40
CD80
CD86
E
Fcγ receptor
CD16
CD32
CD64
D
Co-inhibitory
PD-L2
B7-H3
B7-H4
B7-H5
F
Chemokine receptor
CSF1R
CXCR4
CCR2
CCR5
G
(Gated on CD14+ monocytes)
pSTAT1
pSTAT3
pSTAT5
pmTOR
pAkt

Figure S10. Protein expression profiles of PD-L1^{+} and PD-L1^{-} monocytes, related to Figure 5. (A-F) Representative flow plots showing the expression of surface proteins of maturation **(A)**, M1/M2 marker **(B)**, co-stimulatory ligands **(C)**, co-inhibitory ligands **(D)**, Fcγ receptors **(E)** and chemokine receptors **(F)**. **(G)** Representative flow plots showing the levels of phosphorylated signal transduction proteins.

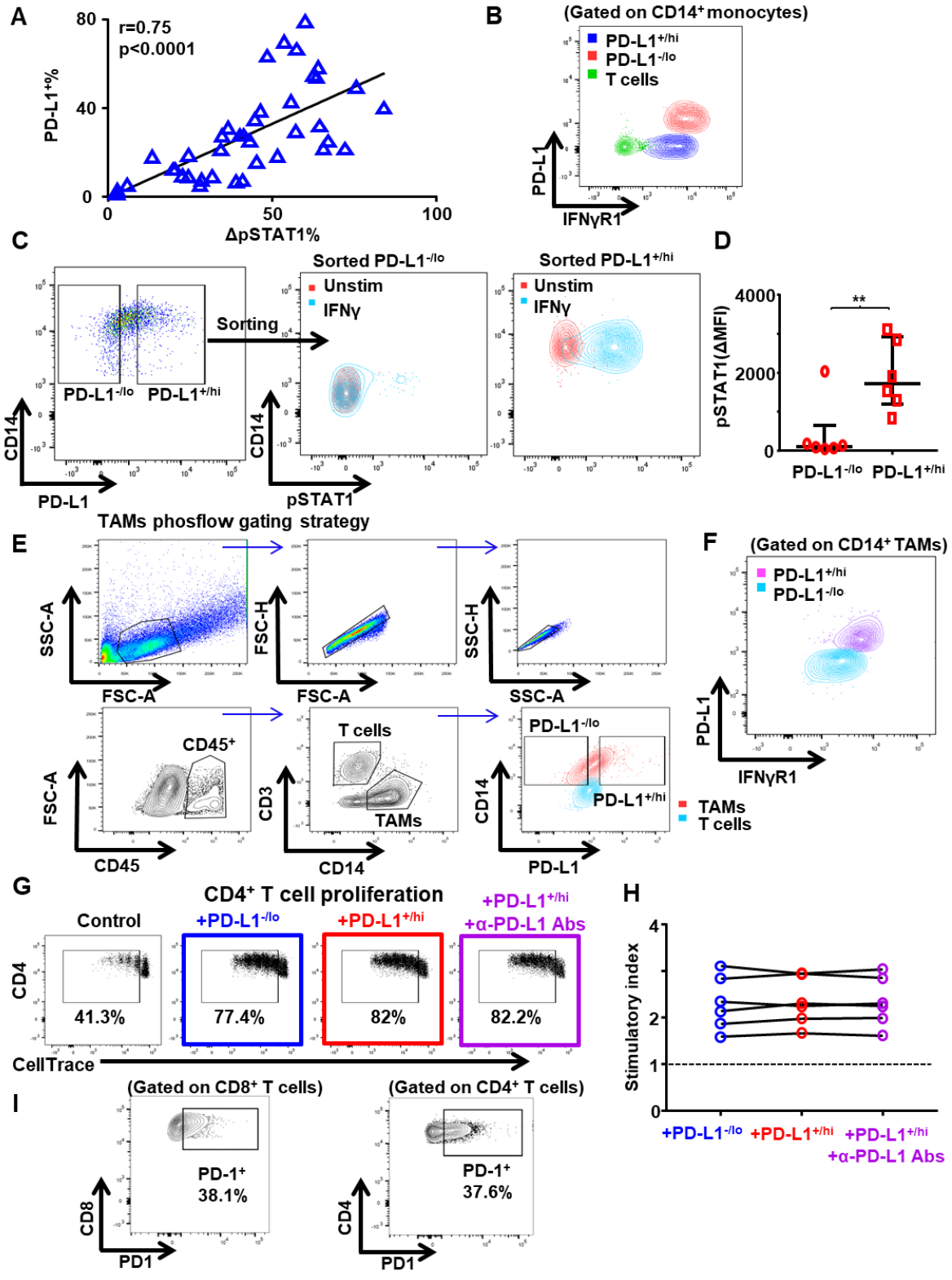
A
r=0.75
p<0.0001
PD-L1+%
ΔpSTAT1%
B
(Gated on CD14+ monocytes)
PD-L1+/hi
PD-L1-/lo
T cells
PD-L1
IFNγR1
C
Sorting
Sorted PD-L1-/lo
Sorted PD-L1+/hi
Unstim
IFNγ
PD-L1-/lo
PD-L1+/hi
CD14
PD-L1
pSTAT1
D
pSTAT1(ΔMFI)
**
PD-L1-/lo
PD-L1+/hi
TAMs phosflow gating strategy
E
SSC-A
FSC-A
FSC-H
SSC-H
CD45+
CD45
T cells
CD3
TAMs
CD14
PD-L1-/lo
PD-L1+/hi
PD-L1
F
(Gated on CD14+ TAMs)
TAMs
T cells
G
CD4+ T cell proliferation
Control
+PD-L1-/lo
+PD-L1+/hi
+PD-L1+/hi
+α-PD-L1 Abs
CD4
41.3%
77.4%
82%
82.2%
CellTrace
H
Stimulatory index
+PD-L1-/lo
+PD-L1+/hi
+PD-L1+/hi
+α-PD-L1 Abs
I
(Gated on CD8+ T cells)
(Gated on CD4+ T cells)
PD-1+
38.1%
PD-1+
37.6%
CD8
CD4
PD1

Figure S11. PD-L1$^+$ TAMs are primed for IFNγ stimulation, related to Figure 6. (A) The association between PD-L1$^+$% and IFNγ-induced STAT1 phosphorylation (ΔpSTAT1%) in peripheral monocytes from patients with BC (n=40). Pearson's correlation coefficient test. **(B)** Representative flow plots showing the levels of IFNγR1 in PD-L1$^+$ and PD-L1$^-$ monocytes. **(C-D)** IFNγ-induced STAT1 phosphorylation were shown in representative flow plots **(C)** and were compared between flow sorted PD-L1$^{+/hi}$ and PD-L1$^{-/lo}$ peripheral monocytes **(D)**. **p<0.01. Paired t test. **(E)** Representative flow plots showing the gating strategy of TAMs from breast tumors in phosflow cytometry. **(F)** Representative flow plots showing the levels of IFNγR1 in PD-L1$^+$ and PD-L1$^-$ TAMs. **(G-H)** CellTrace Violet dilution by CD4$^+$ T cells determined after 4 days of TCR-stimulated coculture with autologous PD-L1$^+$ or PD-L1$^-$ monocytes/macrophages from patients with BC at a 1 to 1 ratio (n=6). **(G)** Representative flow plots showing percentage of proliferated CD8$^+$ T cells. **(H)** The proliferation stimulation activity was measured by the cell number ratio of (CD8/CD4+CD14)/(CD8/CD4) as the stimulatory index. **(I)** Representative flow plots showing the expression of PD1 on CD8$^+$ and CD4$^+$ T cells during the CellTrace proliferation assays.

Table S1. M1 vs. M2 gene signature, related to Figure 1 and 3.

M1	M2
FCGR1A	*MRC1*
CD40	*CD163*
CD80	*CD209*
CD86	*CD1A*
HLA-DRA	*CD1B*
HLA-DRB1	*CXCR1*
HLA-DQA1	*CXCR2*
HLA-DQB1	*IL4R*
IRF1	*EGF*
IRF5	*CTSA*
IDO1	*CTSB*
KYNU	*CTSC*
CCR7	*CTSD*
IFNG	*CLEC7A*
TNF	*WNT7B*
IL1A	*FASLG*
IL1B	*TNFSF12*
IL6	*TNFSF18*
CXCL8	*CD276*
IL12B	*VTCN1*
IL23A	*MSR1*
CXCL9	*FN1*
CXCL10	*IRF4*
CXCL11	*VEGFA*
CXCL13	*VEGFB*
CCL5	*VEGFC*
	VEGFD
	TGFB1
	TGFB2
	TGFB3
	MMP9
	MMP14
	MMP19
	IL4
	IL10
	IL13
	CCL4
	CCL13
	CCL14
	CCL17
	CCL18
	CCL20
	CCL22
	CCL23
	CCL24

Table S2. Gene signature of PD-L1$^{+/-}$TAMs generated from scRNA-seq, related to Figure 3.

PD-L1^{+} TAM			PD-L1^{-} TAM		
Gene	**Log_2 FC**	**Related-function**	**Gene**	**Log_2 FC**	**Related-function**
IL1B	1.40	Pro-inflammatory	*SPP1*	-1.61	Pro-tumor
HLA-DQA1	1.12	Maturation	*FABP5*	-1.06	Metabolism
HLA-DPB1	1.10	Maturation	*FN1*	-0.75	ECM organization
CEBPD	1.06	Activation	*IL1RN*	-0.71	Anti-inflammatory
FCER1A	1.02	Pro-inflammatory	*CSTB*	-0.68	Anti-inflammatory
SEPP1	0.99	Anti-tumor	*LDHA*	-0.51	Metabolism
HLA-DQB1	0.94	Maturation			
FOSB	0.81	Activation			

Note: The METABRIC analysis used expression levels of the gene signature;
The TCGA analysis used expression levels of the gene signature normalized to *CD68*.

Table S3. The characteristics of patients with luminal breast cancer, related to Figure 3.

	Cohort #1 Whole-slide	Cohort #2 TMA
Characteristics	N=49 (%)	N=93 (%)
Age—yr		
Median	51	55
Range	27-93	29-87
Tumor stage— no.(%)		
DCIS	1 (2)	0 (0)
T1	20 (41)	34 (37)
T2	23 (47)	58 (62)
T3	5 (10)	1 (1)
Grade— no.(%)		
G1	5 (10)	0 (0)
G2	28 (57)	68 (73)
G3	16 (33)	25 (27)
Nodal status— no.(%)		
N0	25 (51)	48 (52)
N1-3	22 (45)	45 (48)
Unknown	2 (4)	0 (0)