**Supplemental Figure Legends**

**Fig. S1. PD-1$^{+}$Tim-3$^{high}$ CD8$^{+}$ TILs produce less cytokines but exhibit higher lytic capacities than PD-1$^{+}$Tim-3$^{-}$ and PD-1-Tim-3$^{-}$ CD8$^{+}$ TILs in metastatic melanoma.**

(A) Representative dot plots for one metastatic melanoma patient showing expression of PD-1 and Tim-3 on CD8$^{+}$ TILs (PD-1$^{+}$ Tim-3$^{high}$ CD8$^{+}$ T cells in red gate). (B) Summary data (n = 10) showing percentages of cytokine (INFγ, TNF, IL-2) and granzyme (A and B) producing PD-1$^{-}$Tim-3$^{-}$, PD-1$^{+}$Tim-3$^{-}$, and PD-1$^{+}$Tim-3$^{high}$ CD8$^{+}$TILs in metastatic melanoma.

(C) Dot plots from one representative metastatic melanoma showing frequencies of perforin, CD107a$^{+}$ and PS$^{+}$ CD8$^{+}$ TILs according to PD-1 and Tim-3 expression.

(D) Representative dot plots for one metastatic melanoma and summary data (n = 10) showing frequencies of CD209$^{+}$ CD8$^{+}$ TILs according to PD-1 and Tim-3 expression.

(E) Summary data showing frequencies of CD11c$^{+}$ and CD14$^{+}$ CD45$^{+}$CD3$^{-}$ cells in metastatic melanoma (n = 8). (F) Representative flow cytometry dot plots and summary data (n =10, left) evaluating the expression of active caspase-3 and PS by PD-1$^{+}$ Tim-3$^{+}$ CD8$^{+}$ TILs. P-values were obtained by one-way ANOVA followed by Holm-Šídák multiple comparison test and Friedman test followed by Dunn's multiple comparison test (B, F), Wilcoxon matched-pairs signed rank test (D). *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. Data indicate mean ± SD.

**Fig. S2. Identification of tumor-infiltrating myeloid cell clusters using scRNA-seq.**

(A) scRNA-seq data showing a UMAP projection of five myeloid clusters identified during the first clustering step. The bulk of myeloid cells clustered near each other on this

initial UMAP. (B) scRNA-seq data showing myeloid clusters, with cDC and monocyte cells identified and grouped. (C) Expression of common monocyte and cDC markers across UMAP projected cells.

**Fig. S3. Tim-3 blockade acts in APCs in a PS-mediated fashion and cooperates with PD-1 blockade to decrease trogocytosis of tumor antigen-specific CD8$^+$ T cells.**

(A) Summary data showing frequencies of PKH26$^+$ and PS$^+$ NY-ESO-1-specific clone 95/3 in the presence of HLA-A2$^+$ DCs pulsed with cognate peptide and indicated mAbs. Blocking mAbs were added to wells containing DCs before washing and co-incubation with CD8$^+$ T cell clones (DC blockade, left), or CD8$^+$ T cell clones before washing and co-incubation with HLA-A2$^+$ DCs (CD8 Blockade, right). Data are representative of three independent experiments. (B) Summary data showing frequencies of PKH26$^+$PD-1$^+$Tim-3$^+$ CD8$^+$ TILs co-incubated for 30 min with PKH26$^+$CD45$^+$CD3$^-$ cells isolated from metastatic melanoma in the presence of mAbs before flow cytometry (n = 5). (C) Representative flow cytometry dot plots (right) and summary data (left) evaluating PKH26 expression by CD8$^+$ T cell clone 95/3 after DC blockade (C, upper) or CD8 Blockade (C, lower). Data shown are representative of three independent experiments. (D) ELISPOT assay with DCs pulsed with proteins, peptides or melanoma cell lysates +/- aTim-3 or aPD-L1 mAbs in the presence of NY-ESO-1-specific CD8$^+$ T cell clone 95/3. Spots were developed and counted by computer-assisted video image analysis. Each bar represents the mean spot number of triplicates (± SD) with $1 \times 10^3$ CD8$^+$ T cells initially seeded per well. One representative experiment of at least three performed is depicted. P-values were calculated using paired t-tests (A, B and C). n.s. = non-significant (C).

**Fig. S4. Dual PD-1 and Tim-3 blockade impedes trogocytosis of CD8$^+$ TILs in mice with Yummer 1.7 or B16-OVA melanoma.** (A) Yeast epitope display (left) and cell-free PS binding assay (right) of aTim-3 RMT3-23. Epitope mapping of RMT3-23 on mouse Tim-3 (magenta) shows it binds to the PS binding site (orange sphere). Cell-free PS binding assay shows relative binding of PS liposomes to Tim-3 saturated with RMT3-23 or non-blocking control Ab. (B) Representative dot plots showing frequencies of PD-1$^+$Tim-3$^+$ CD8$^+$ TILs (upper) and PS$^+$PD-1$^+$Tim-3$^+$ CD8$^+$ TILs (lower) in B16-OVA tumors (day 21) treated with indicated mAbs. (C) Representative dot plots (left) and summary data (right, n = 5) showing frequenciesof Tcf1$^+$ and Slamf6$^+$ PD1$^+$Tim-3$^+$ CD8$^+$ TILs in B16-OVA tumors treated with indicated mAbs. (D) Representative dot plots and summary data (n = 5–8) showing frequencies of CD14$^+$PD-1$^+$Tim-3$^+$ CD8$^+$ T cells (left and right) and CD14$^+$ PD-1$^+$Tim-3$^-$ CD8$^+$ TILs (right) in mice implanted with YUMMER 1.7 (day 21) treated with indicated mAbs. (E) Representative flow cytometry dot plots and summary data (n=5, left) evaluating Tim-3 expression by *Havcr2$^{fl/fl}$* and *Havcr2$^{fl/fl}$Cd11c$^{cre}$* CD11c$^+$ CD45$^+$ CD3$^-$ TILs in YUMMER1.7 (day 21). Results shown are from one experiment, representative of two independent experiments. P-values were calculated using one-way ANOVA followed by Tukey's multiple comparison test (D). *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. Data indicate mean ± SD.

**Fig. S5. Dual PD-1 and Tim-3 blockade impedes tumor growth and prolongs survival of mice with YUMMER 1.7 melanoma.** (A) Growth curves of YUMMER 1.7 mouse tumors treated with aTim-3 (n = 13), aPD-1 (n = 14), or both mAbs, compared to isotype IgG control. Tumor growth curves are the summary of two independent experiments (n = 11–14). (B) Survival was monitored, and mice were euthanized when tumors reached 2000mm$^3$. Results

shown are from one experiment (n = 5-7), representative of two independent experiments. (D) Individual tumor growth curves of YUMMER 1.7 melanoma treated with indicated mAbs. *P*-values were obtained from Mann-Whitney U test. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. Data indicate mean ± SD.

**Fig. S6. Gating strategy of tetramer-positive cells in B16-OVA melanoma and evaluation of trogocytosed NY-ESO-1-specific CD8$^+$ T cell clone in the presence of HLA-A2$^+$ DCs pulsed with cognate antigen and Tim-3 blockade.** (A) Flow cytometry gating strategy for H-2kb/OVA257-264 tet$^+$ CD8$^+$ T cells in B16-OVA tumors. (B) Summary data showing frequencies of PKH67$^+$ PKH26$^-$ CD11c$^+$ and PKH67$^+$ PKH26$^-$ CD107a$^+$ NY-ESO-1-specific CD8$^+$ T cell clone 95/3. DCs obtained from HLA-A2$^+$ healthy donors were pulsed with either tumor lysate (T. Lysate) from an HLA-A2/NYESO-1$^+$ melanoma cell line MEL 285 or NY-ESO-1 157-165 peptide (NY-ESO-1) ± aTim-3 or IgG control mAbs, then washed and co-cultured with PKH26-labelled clone 95/3. PKH26$^+$ CD8$^+$ T cells were sorted and co-incubated with PKH67$^+$ clone 95/3 for 6 hours before flow cytometry (left). Data are representative of three independents experiments. (C) Representative ImageStream analysis showing cell surface expression of CD11c, CD107a, and PKH26 by PKH67$^+$CD8$^+$ T cell clone 95/3 with aTim-3 or IgG control mAbs. P-values were calculated using paired t-tests (B). *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

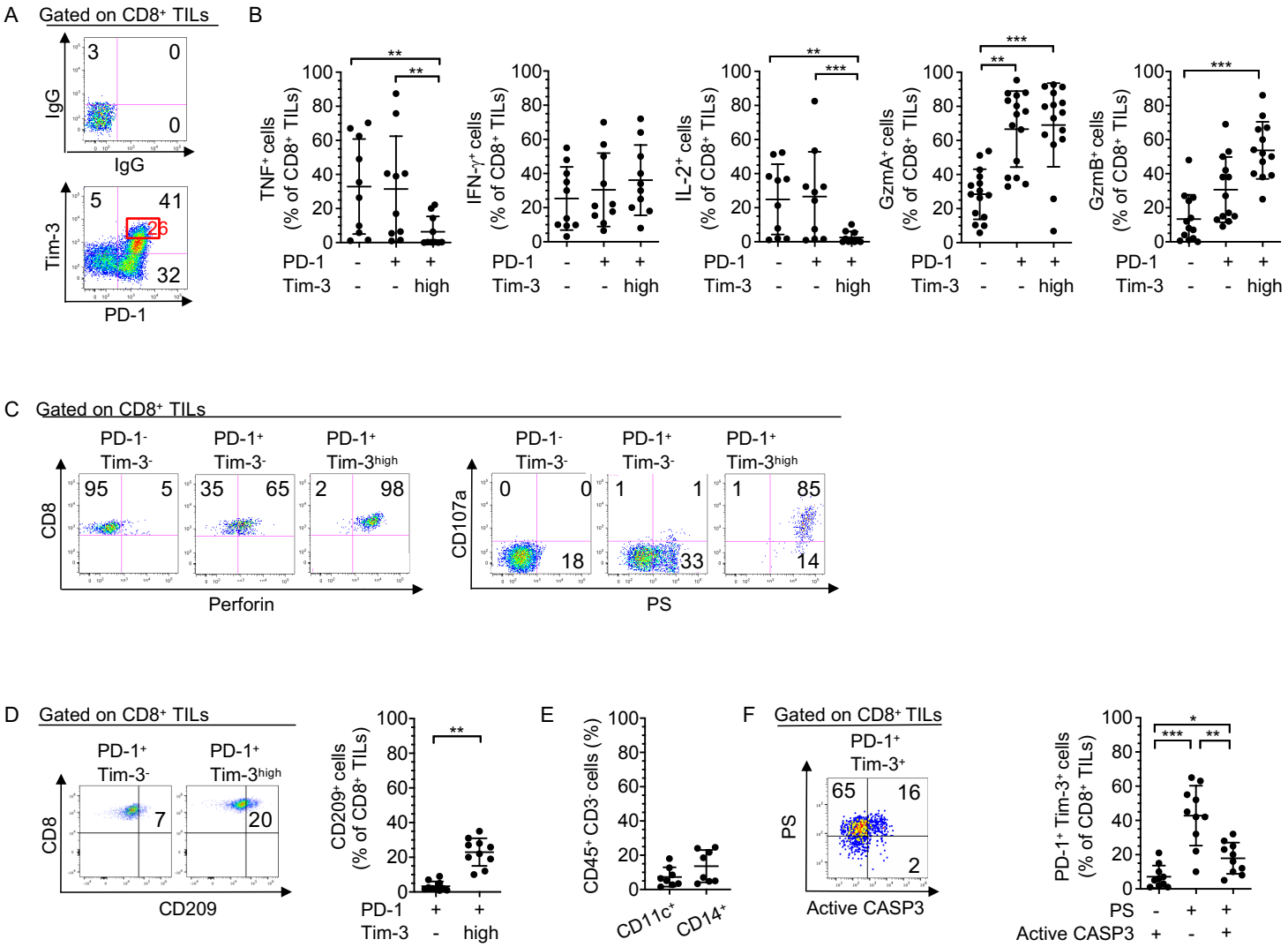

A
Gated on CD8⁺ TILs
IgG
IgG
Tim-3
PD-1
B
TNF⁺ cells (% of CD8⁺ TILs)
IFN-γ⁺ cells (% of CD8⁺ TILs)
IL-2⁺ cells (% of CD8⁺ TILs)
GzmA⁺ cells (% of CD8⁺ TILs)
GzmB⁺ cells (% of CD8⁺ TILs)
PD-1
Tim-3
high
C
Gated on CD8⁺ TILs
PD-1⁻ Tim-3⁻
PD-1⁺ Tim-3⁻
PD-1⁺ Tim-3high
CD8
Perforin
CD107a
PS
D
Gated on CD8⁺ TILs
CD8
CD209
CD209⁺ cells (% of CD8⁺ TILs)
E
CD45⁺ CD3⁻ cells (%)
CD11c⁺
CD14⁺
F
Gated on CD8⁺ TILs
PD-1⁺ Tim-3⁺
PS
Active CASP3
PD-1⁺ Tim-3⁺ cells (% of CD8⁺ TILs)

Figure S1

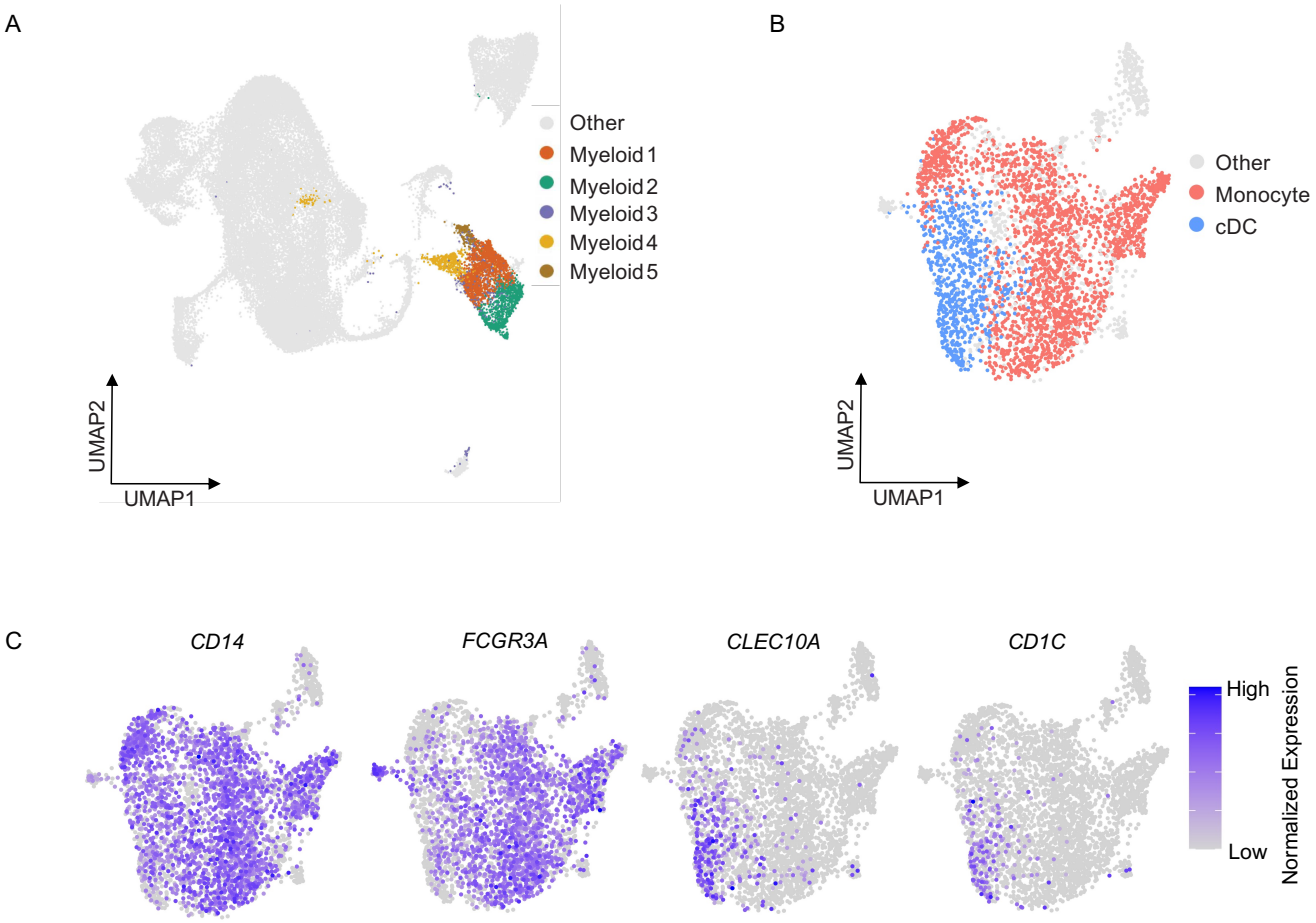

A
Other
Myeloid 1
Myeloid 2
Myeloid 3
Myeloid 4
Myeloid 5
UMAP2
UMAP1
B
Other
Monocyte
cDC
UMAP2
UMAP1
C
CD14
FCGR3A
CLEC10A
CD1C
High
Low
Normalized Expression

Figure S2

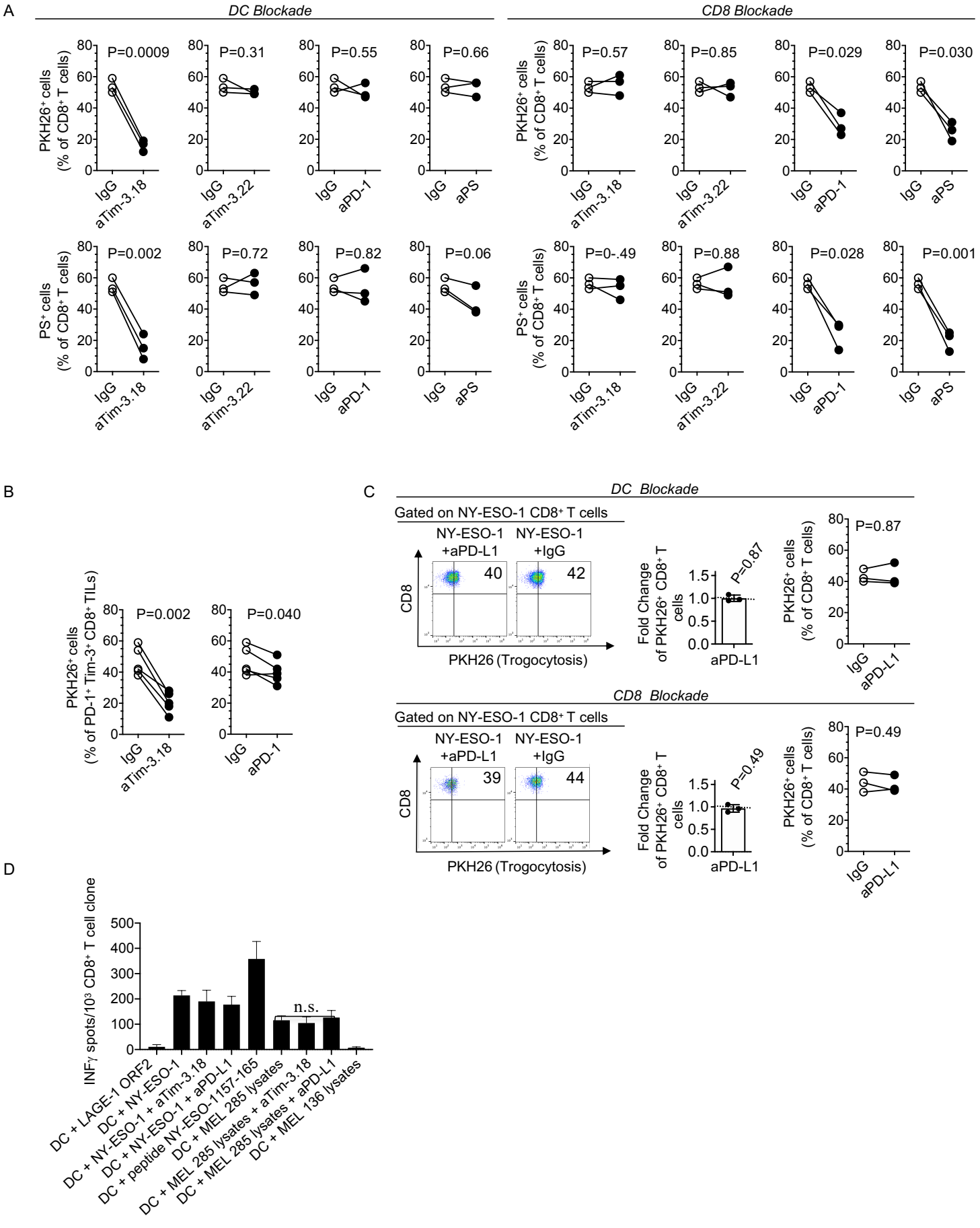

A
DC Blockade
CD8 Blockade
PKH26+ cells (% of CD8+ T cells)
PS+ cells (% of CD8+ T cells)
P=0.0009
P=0.31
P=0.55
P=0.66
P=0.57
P=0.85
P=0.029
P=0.030
P=0.002
P=0.72
P=0.82
P=0.06
P=0-.49
P=0.88
P=0.028
P=0.001
IgG
aTim-3.18
aTim-3.22
aPD-1
aPS
B
PKH26+ cells (% of PD-1+ Tim-3+ CD8+ TILs)
P=0.002
P=0.040
C
DC Blockade
Gated on NY-ESO-1 CD8+ T cells
NY-ESO-1 +aPD-L1
NY-ESO-1 +IgG
CD8
PKH26 (Trogocytosis)
40
42
Fold Change of PKH26+ CD8+ T cells
P=0.87
aPD-L1
PKH26+ cells (% of CD8+ T cells)
CD8 Blockade
39
44
P=0.49
D
INFγ spots/10³ CD8+ T cell clone
n.s.
DC + LAGE-1 ORF2
DC + NY-ESO-1
DC + NY-ESO-1 + aTim-3.18
DC + NY-ESO-1 + aPD-L1
DC + peptide NY-ESO-1157-165
DC + MEL 285 lysates
DC + MEL 285 lysates + aTim-3.18
DC + MEL 285 lysates + aPD-L1
DC + MEL 136 lysates

Figure S3

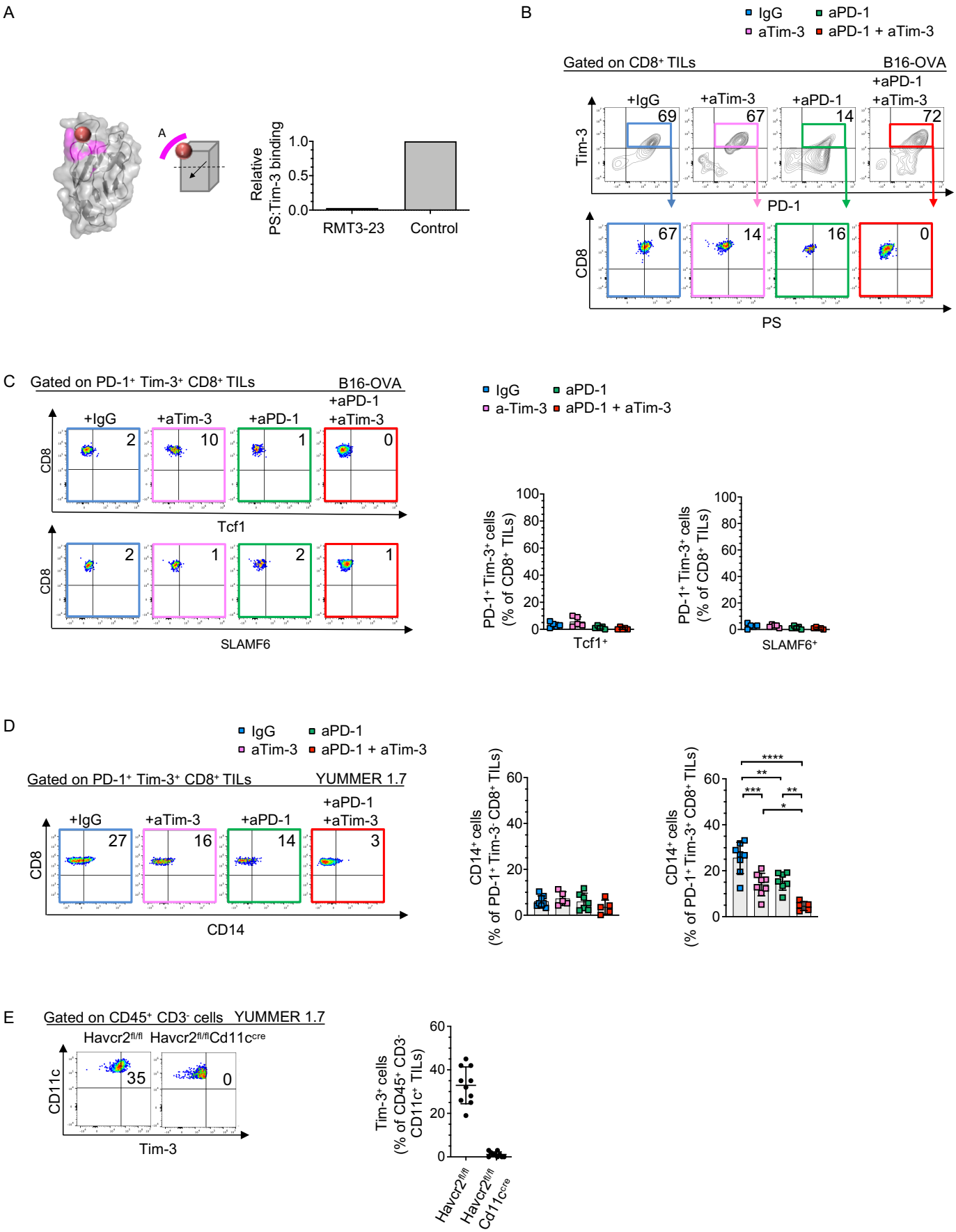

A
A
Relative PS:Tim-3 binding
1.0
0.5
0.0
RMT3-23
Control
B
IgG
aPD-1
aTim-3
aPD-1 + aTim-3
Gated on CD8⁺ TILs
B16-OVA
+IgG
+aTim-3
+aPD-1
+aPD-1 +aTim-3
Tim-3
PD-1
CD8
PS
69
67
14
72
67
14
16
0
C
Gated on PD-1⁺ Tim-3⁺ CD8⁺ TILs
B16-OVA
IgG
aPD-1
a-Tim-3
aPD-1 + aTim-3
CD8
Tcf1
SLAMF6
2
10
1
0
2
1
2
1
PD-1⁺ Tim-3⁺ cells (% of CD8⁺ TILs)
Tcf1⁺
SLAMF6⁺
D
IgG
aPD-1
aTim-3
aPD-1 + aTim-3
Gated on PD-1⁺ Tim-3⁺ CD8⁺ TILs
YUMMER 1.7
CD8
CD14
27
16
14
3
CD14⁺ cells (% of PD-1⁺ Tim-3⁺ CD8⁺ TILs)
****
**
***
**
*
E
Gated on CD45⁺ CD3⁻ cells
YUMMER 1.7
Havcr2fl/fl
Havcr2fl/flCd11ccre
CD11c
Tim-3
35
0
Tim-3⁺ cells (% of CD45⁺ CD3⁻ CD11c⁺ TILs)
Havcr2fl/fl
Havcr2fl/fl Cd11ccre

Figure S4

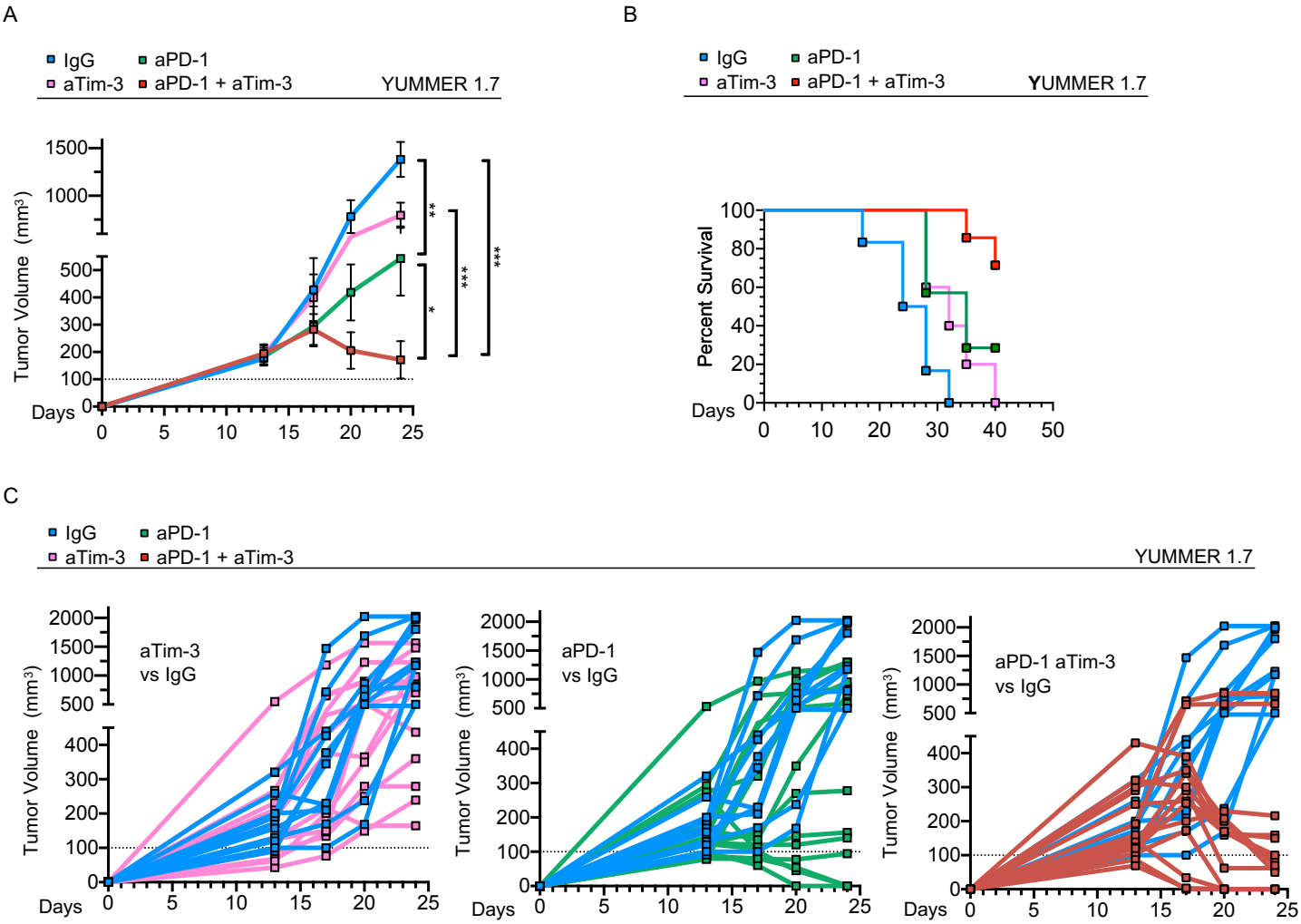
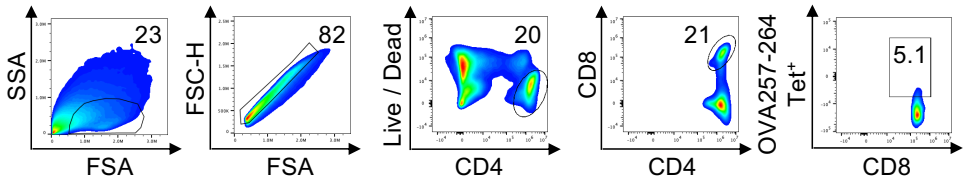
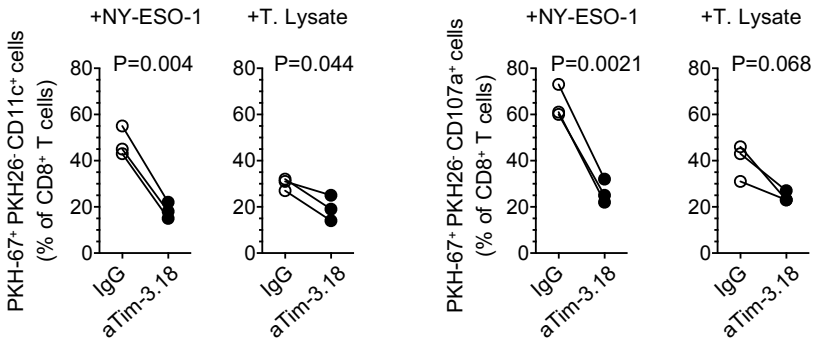

A
IgG
aPD-1
aTim-3
aPD-1 + aTim-3
YUMMER 1.7
Tumor Volume (mm³)
Days
**
*
***
***
B
IgG
aPD-1
aTim-3
aPD-1 + aTim-3
YUMMER 1.7
Percent Survival
Days
C
IgG
aPD-1
aTim-3
aPD-1 + aTim-3
YUMMER 1.7
aTim-3
vs IgG
aPD-1
vs IgG
aPD-1 aTim-3
vs IgG
Tumor Volume (mm³)
Days

Figure S5

A B16-OVA tumors




B




C

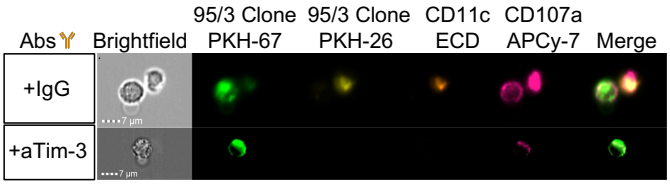



Figure S6

**Supplemental Table 1. Top 20 differentially expressed genes identified for the 5 myeloid clusters during the first clustering step.**

| Myeloid 1 | | | Myeloid 2 | | |
|---|---|---|---|---|---|
| gene | avg_logFC | p_val_adj | gene | avg_logFC | p_val_adj |
| APOE | 3.187285681 | 0 | S100A8 | 3.699699 | 0 |
| C1QC | 2.9105797 | 0 | LYZ | 3.346131 | 0 |
| SPP1 | 2.851410092 | 0 | S100A9 | 3.307772 | 0 |
| APOC1 | 2.824481654 | 0 | CST3 | 2.842015 | 0 |
| C1QB | 2.768863398 | 0 | IFI30 | 2.58107 | 0 |
| IL1B | 2.586938364 | 0 | FCN1 | 2.400923 | 0 |
| TIMP1 | 2.575994449 | 0 | TIMP1 | 2.330417 | 0 |
| C1QA | 2.549302238 | 0 | PLAUR | 2.217533 | 0 |
| IFI30 | 2.547962418 | 0 | TYROBP | 2.193984 | 0 |
| CXCL8 | 2.516595807 | 0 | FCER1G | 2.09211 | 0 |
| RNASE1 | 2.464094773 | 0 | IFITM3 | 2.06748 | 0 |
| CCL2 | 2.367808879 | 0 | SERPINA1 | 2.028154 | 0 |
| TYROBP | 2.340880518 | 0 | AIF1 | 2.025192 | 0 |
| CCL3 | 2.327496789 | 0 | LST1 | 1.94864 | 0 |
| CTSB | 2.287319137 | 0 | IL1B | 1.92112 | 0 |
| CST3 | 2.287223474 | 0 | G0S2 | 1.860119 | 0 |
| FTL | 2.259877145 | 0 | VCAN | 1.77685 | 0 |
| FCER1G | 2.221359572 | 0 | CXCL8 | 1.740869 | 0 |
| IER3 | 2.220358691 | 0 | C15orf48 | 1.704201 | 0 |
| CTSL | 2.182859352 | 0 | CD14 | 1.691724 | 0 |
| **Myeloid 3** | | | **Myeloid 4** | | |
| gene | avg_logFC | p_val_adj | gene | avg_logFC | p_val_adj |
| CST3 | 1.526552266 | 0 | IL1B | 2.994295 | 0 |
| GPNMB | 1.428882141 | 0 | RNASE1 | 2.903717 | 0 |
| LYZ | 1.160848958 | 0 | CCL3 | 2.84662 | 0 |
| CCL2 | 2.766580853 | 1.6E-301 | APOE | 2.721637 | 0 |
| APOC1 | 2.099036564 | 2E-300 | APOC1 | 2.67413 | 0 |
| RNASE1 | 1.63982232 | 1E-290 | SPP1 | 2.668884 | 0 |
| TGFBI | 1.641456982 | 1.3E-290 | CXCL8 | 2.612032 | 0 |
| CYP27A1 | 0.681956822 | 1.4E-285 | C1QB | 2.503179 | 0 |
| APOE | 1.84231219 | 2.7E-284 | IER3 | 2.480689 | 0 |
| SPP1 | 2.120742591 | 8.6E-269 | CCL3L1 | 2.464352 | 0 |
| CXCL8 | 1.673091791 | 5.6E-262 | C1QC | 2.399699 | 0 |
| CD68 | 1.575989253 | 3.8E-258 | C1QA | 2.322705 | 0 |
| ADM | 0.979155433 | 1.5E-254 | C3 | 2.17995 | 0 |
| CD163 | 0.943145339 | 2.5E-243 | SGK1 | 2.1793 | 0 |
| SMIM25 | 0.884269662 | 5.4E-226 | TNFAIP2 | 2.136878 | 0 |

| | | | | | |
|---|---|---|---|---|---|
| TYROBP | 1.438477984 | 5.3E-222 | STAB1 | 2.111032 | 0 |
| MAFB | 1.061970899 | 8.9E-211 | PLAU | 1.963226 | 0 |
| PLPP3 | 0.416776092 | 6.4E-210 | NUPR1 | 1.936259 | 0 |
| FCER1G | 1.419444896 | 4E-209 | CXCL2 | 1.864898 | 0 |
| CTSL | 1.99445162 | 2.5E-208 | TYROBP | 1.838547 | 0 |
| **Myeloid 5** | | | | | |
| **gene** | **avg_logFC** | **p_val_adj** | | | |
| APOD | 3.554955161 | 0 | | | |
| SERPINE2 | 2.550065669 | 0 | | | |
| S100A1 | 2.49425411 | 0 | | | |
| S100B | 2.344634122 | 0 | | | |
| IFI27 | 2.211303121 | 0 | | | |
| CTSK | 2.056528372 | 0 | | | |
| S100A13 | 1.92989759 | 0 | | | |
| MIA | 1.870078081 | 0 | | | |
| PMEL | 1.864447283 | 0 | | | |
| CRYAB | 1.673817698 | 0 | | | |
| RAB13 | 1.530600079 | 0 | | | |
| SPARC | 1.449488699 | 0 | | | |
| MLANA | 1.367992397 | 0 | | | |
| VGF | 1.228123184 | 0 | | | |
| CCN3 | 1.214773447 | 0 | | | |
| MITF | 1.20955852 | 0 | | | |
| BACE2 | 1.182131031 | 0 | | | |
| PLP1 | 1.177594891 | 0 | | | |
| CAPN3 | 1.169496941 | 0 | | | |
| CAV1 | 1.115816276 | 0 | | | |

**Supplemental Table 2. Top 20 differentially expressed genes between the monocyte and cDC clusters.**

| Monocyte | | | cDC | | |
|---|---|---|---|---|---|
| **gene** | **avg_logFC** | **p_val_adj** | **gene** | **avg_logFC** | **p_val_adj** |
| CTSD | 1.130807774 | 1.45999E-89 | CLEC10A | 1.03065792 | 5.6232E-118 |
| FTL | 0.685211687 | 2.83936E-80 | PPA1 | 0.840551508 | 1.89031E-96 |
| RNASE1 | 1.85560444 | 1.11829E-78 | CD1C | 0.702203859 | 1.57518E-87 |
| APOE | 1.314189697 | 2.61665E-76 | HLA-DRB5 | 1.160919322 | 6.25523E-84 |
| SPP1 | 1.718812346 | 1.30997E-66 | CPVL | 0.688056533 | 4.76053E-79 |
| CCL3 | 1.241232341 | 1.21779E-65 | PLAC8 | 0.713944029 | 1.33584E-75 |
| APOC1 | 1.392923509 | 1.21592E-64 | FCER1A | 0.881101659 | 1.53815E-73 |
| CXCL8 | 1.095901872 | 1.70083E-63 | PSME2 | 0.700576865 | 1.78576E-71 |
| CCL4 | 1.373733 | 5.99083E-60 | LIMD2 | 0.527905119 | 6.86712E-71 |
| NUPR1 | 1.547678246 | 1.1007E-57 | PSMB9 | 0.594811918 | 4.67969E-67 |
| C1QB | 0.833088563 | 7.71366E-45 | HLA-DQA1 | 0.864073323 | 1.28825E-66 |
| CTSB | 0.626626907 | 1.57963E-43 | CRIP1 | 0.653559691 | 2.96479E-66 |
| STAB1 | 1.126478567 | 3.00372E-43 | RPS23 | 0.461145762 | 4.2604E-66 |
| C1QA | 0.801926554 | 1.72589E-41 | JAML | 0.491021092 | 7.77405E-65 |
| GPNMB | 0.893821952 | 3.23863E-41 | WARS | 0.76376329 | 1.43655E-64 |
| C1QC | 0.743782383 | 4.0114E-41 | EEF1A1 | 0.391400803 | 1.89465E-64 |
| IL1B | 0.81129734 | 2.39961E-39 | HLA-DQB1 | 0.736702246 | 2.94173E-62 |
| NEAT1 | 0.643174976 | 2.49539E-39 | LAP3 | 0.535145028 | 6.33087E-61 |
| CCL4L2 | 1.212316962 | 6.59087E-39 | IFITM1 | 0.910726568 | 4.36001E-60 |
| A2M | 0.991070547 | 1.82457E-36 | RPL28 | 0.319764678 | 5.85742E-60 |

**Supplemental Table 3. Differentially expressed genes between $TIM3^{+}$ and $TIM3^{-}$ myeloid cells.**

| gene | p_val | avg_logFC | pct.1 | pct.2 | p_val_adj | log10Padj | higher_in |
|---|---|---|---|---|---|---|---|
| CTSL | 3.05783E-27 | 0.59422444 | 0.75 | 0.524 | 6.71103E-23 | 22.17321113 | TIM3+ |
| HLA-DQB1 | 4.16535E-25 | 0.327872767 | 0.907 | 0.705 | 9.14169E-21 | 20.03897347 | TIM3+ |
| TPM4 | 1.60027E-23 | 0.269815111 | 0.703 | 0.488 | 3.51212E-19 | 18.45443047 | TIM3+ |
| LITAF | 4.62381E-22 | 0.254061138 | 0.872 | 0.662 | 1.01479E-17 | 16.99362448 | TIM3+ |
| CD63 | 6.00604E-22 | 0.257672314 | 0.858 | 0.647 | 1.31814E-17 | 16.88003695 | TIM3+ |
| CTSB | 1.07887E-21 | 0.360495463 | 0.939 | 0.819 | 2.36781E-17 | 16.62565382 | TIM3+ |
| CD163 | 5.06066E-19 | 0.27453049 | 0.403 | 0.23 | 1.11066E-14 | 13.95441759 | TIM3+ |
| PLIN2 | 1.30534E-18 | 0.27190201 | 0.535 | 0.343 | 2.86484E-14 | 13.54289976 | TIM3+ |
| LGMN | 2.4385E-18 | 0.293714808 | 0.61 | 0.41 | 5.35177E-14 | 13.2715025 | TIM3+ |
| C15orf48 | 3.29022E-18 | 0.337644065 | 0.561 | 0.372 | 7.22106E-14 | 13.14139925 | TIM3+ |
| FABP5 | 2.73296E-16 | 0.45108647 | 0.485 | 0.316 | 5.99804E-12 | 11.22199094 | TIM3+ |
| IFI30 | 4.2012E-16 | 0.284007598 | 0.981 | 0.91 | 9.22038E-12 | 11.03525126 | TIM3+ |
| TIMP1 | 7.21191E-16 | 0.411295502 | 0.699 | 0.536 | 1.5828E-11 | 10.80057475 | TIM3+ |
| INSIG1 | 2.12225E-15 | 0.292953727 | 0.577 | 0.393 | 4.6577E-11 | 10.33182856 | TIM3+ |
| SDS | 1.02503E-14 | 0.255631941 | 0.465 | 0.301 | 2.24963E-10 | 9.647889031 | TIM3+ |
| CTSD | 1.61888E-14 | 0.310702097 | 0.858 | 0.746 | 3.55296E-10 | 9.449410087 | TIM3+ |
| IFI6 | 2.20659E-14 | 0.32531417 | 0.537 | 0.368 | 4.8428E-10 | 9.314903827 | TIM3+ |
| IFITM3 | 1.92996E-12 | 0.277972806 | 0.755 | 0.571 | 4.23567E-08 | 7.37307752 | TIM3+ |
| LGALS3 | 3.27129E-12 | 0.332920679 | 0.843 | 0.694 | 7.17949E-08 | 7.14390616 | TIM3+ |
| CCL2 | 3.44491E-12 | 0.397027542 | 0.455 | 0.316 | 7.56054E-08 | 7.121447084 | TIM3+ |
| CCL7 | 9.05403E-11 | 0.485152806 | 0.101 | 0.04 | 1.98709E-06 | 5.701782921 | TIM3+ |
| ISG15 | 1.57643E-10 | 0.382714232 | 0.439 | 0.304 | 3.4598E-06 | 5.460949416 | TIM3+ |
| APOBEC3A | 2.11962E-10 | 0.559624249 | 0.246 | 0.151 | 4.65193E-06 | 5.332367227 | TIM3+ |

## Supplemental Table 4. Data collection and refinement statistics.

| Wavelength | Tim3.18 Fab-hTim3 complex |
|---|---|
| Resolution range | 48.56 - 1.49 (1.543 -1.49) |
| Space group | P 1 21 1 |
| Unit cell | 66.105 63.856 66.407 90 94.254 90 |
| Total reflections | 284783 (29411) |
| Unique reflections | 88986 (8955) |
| Multiplicity | 3.2 (3.3) |
| Completeness (%) | 98.74 (99.62) |
| Mean I/sigma(I) | 13.01 (2.33) |
| Wilson B-factor | 18.75 |
| R-merge | 0.05611 (0.4747) |
| R-meas | 0.06776 (0.5676) |
| R-pim | 0.03752 (0.3076) |
| CC1/2 | 0.99 (0.884) |
| CC* | 0.997 (0.969) |
| Reflections used in refinement | 88901 (8932) |
| Reflections used for R-free | 4367 (463) |
| R-work | 0.1770 (0.2564) |
| R-free | 0.2013 (0.2955) |
| CC(work) | 0.963 (0.908) |
| CC(free) | 0.965 (0.844) |
| Number of non-hydrogen atoms | 4890 |
| macromolecules | 4222 |
| ligands | 24 |
| solvent | 644 |
| Protein residues | 544 |
| RMS(bonds) | 0.009 |
| RMS(angles) | 1.34 |
| Ramachandran favored (%) | 97.39 |
| Ramachandran allowed (%) | 2.61 |
| Ramachandran outliers (%) | 0 |
| Rotamer outliers (%) | 0 |
| Clashscore | 5.01 |

| | |
|---|---|
| **Average B-factor** | 29.73 |
| **macromolecules** | 28.21 |
| **ligands** | 40.71 |
| **solvent** | 39.33 |
| **Number of TLS groups** | 20 |

Statistics for the highest-resolution shell are shown in parentheses.

**Supplemental Table 5. Determination of aTim-3 antibody binding kinetics and affinities via surface plasmon resonance.**

| Antibody | Analyte | ka (1/Ms) | kd (1/s) | KD (M) |
|---|---|---|---|---|
| RMT3-23 | mTim3 | 9.80E+05 | 2.70E-02 | 2.80E-08 |
| Tim 3.18 | hTim3 | 2.60E+06 | 4.60E-03 | 1.80E-09 |
| Tim 3.22 | hTim3 | 2.80E+06 | 1.40E-03 | 5.10E-10 |