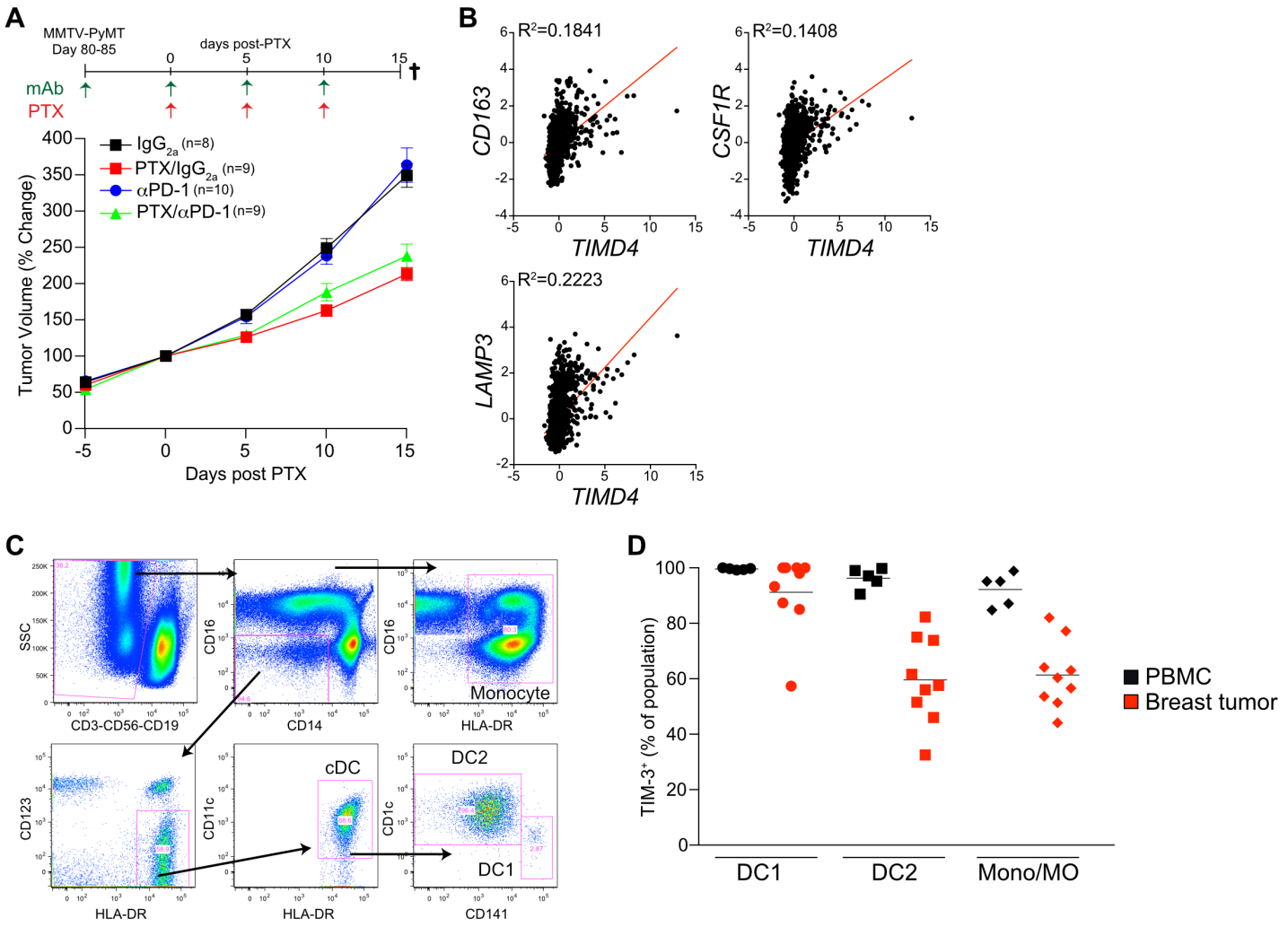


Figure S1, related to Figure 1

(A) Tumor volume shown as a relative change from the initiation of chemotherapy (day 0) in MMTV-PyMT animals. Mice were treated with an IgG_{2a} isotype control or αPD-1, alone or together with 10 mg/kg PTX as indicated. n=8-10 mice per group, pooled over 4 cohorts. Data are mean ± SEM. (B) Correlation between *TIMD4* expression and myeloid genes (*CD163*, *CSF1R*, *LAMP3*) in human breast cancer samples from the TCGA dataset (n=1161, R^2 values by linear regression). (C) Gating strategy for human DCs shown in a peripheral blood sample. (D) Percentage of TIM-3^+ cells within the $CD141^+$ cDC1, $CD1c^+$ cDC2 or $CD14^+$ monocyte/macrophage populations in the peripheral blood of healthy volunteers (n=5) or breast tumors (n=9). Horizontal bars represent the mean.

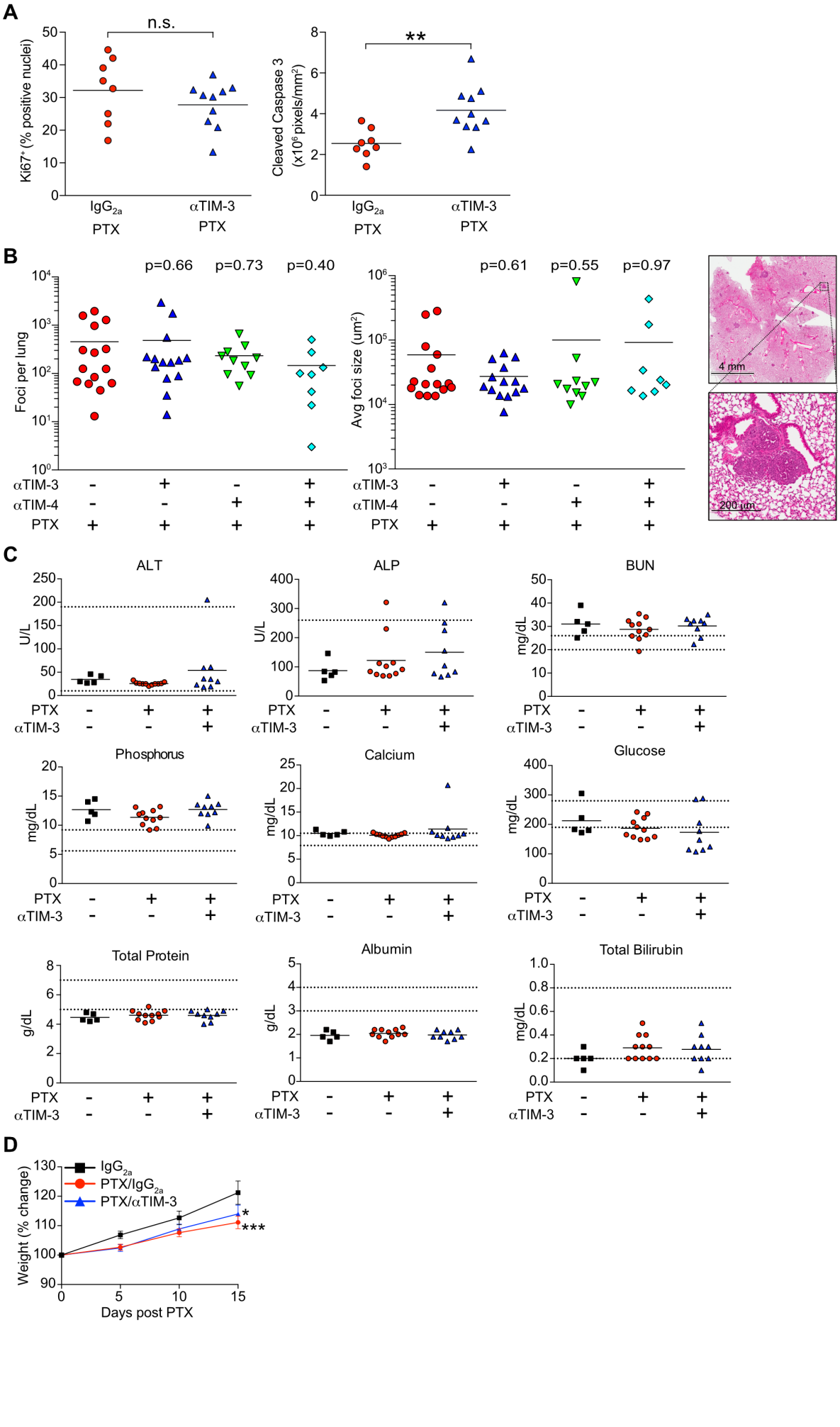

A
n.s.
**
Ki67+ (% positive nuclei)
Cleaved Caspase 3 (x10^6 pixels/mm^2)
IgG2a PTX
αTIM-3 PTX
B
p=0.66
p=0.73
p=0.40
p=0.61
p=0.55
p=0.97
Foci per lung
Avg foci size (um^2)
αTIM-3
αTIM-4
PTX
4 mm
200 um
C
ALT
ALP
BUN
Phosphorus
Calcium
Glucose
Total Protein
Albumin
Total Bilirubin
U/L
mg/dL
g/dL
D
IgG2a
PTX/IgG2a
PTX/αTIM-3
Weight (% change)
Days post PTX
*
***

Figure S2, related to Figure 2

(A) Automated analysis of Ki67 positive nuclei or cleaved caspase 3 immunohistochemistry staining in orthotopic PyMT tumors following treatment with IgG_{2a}/PTX or αTIM-3/PTX. n=8-10 per group, data pooled from 2 experiments. **p<0.01, significance determined by an unpaired t-test. (B) The frequency and size of metastatic foci in the lungs of MMTV-PyMT animals treated with αTIM-3, αTIM-4, or PTX. Representative images are shown to the right. n=8-16, pooled over 8 cohorts. p value shown compared to PTX alone, with significance determined by Mann-Whitney. (C) Metabolic function tests reflecting serum isolated from mice treated with IgG_{2a} alone, PTX/IgG_{2a}, or PTX/αTIM-3. Samples were taken by cardiac puncture at end-stage. Significance was determined by an unpaired t-test, with none found. The horizontal dotted lines indicate the expected range for healthy animals. ALT, alanine transaminase; ALP, alkaline phosphatase; BUN, blood urea nitrogen. (D) Weight of mice treated with IgG_{2a} alone, PTX/IgG_{2a}, or PTX/αTIM3. n=10 per group, pooled over 8 cohorts. Data are shown as the mean ± SEM; *p<0.05, ***p<0.001, with significance determined by 2-way ANOVA compared to IgG_{2a} control. Horizontal bars in A-C represent the mean.

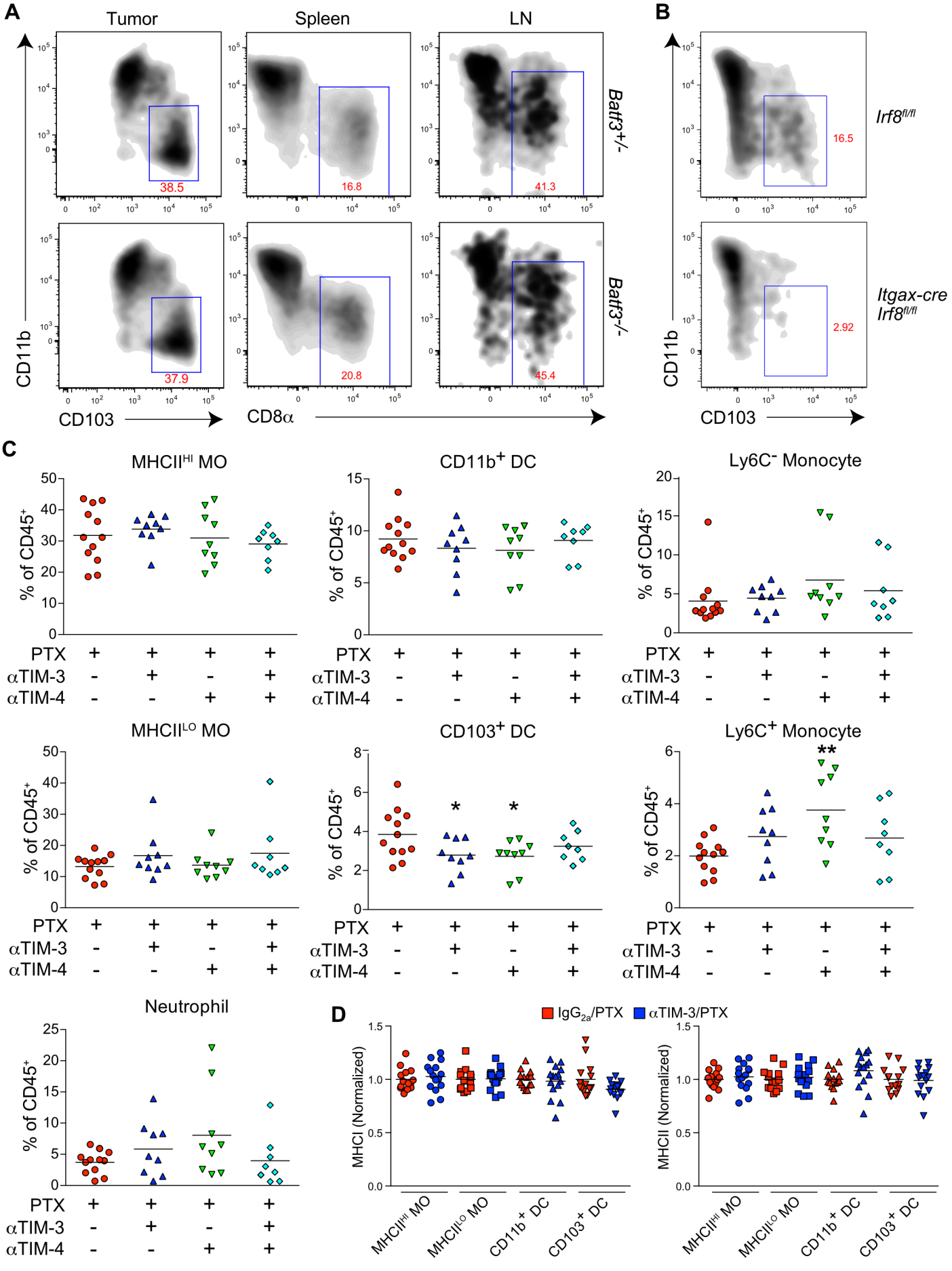

A
Tumor
Spleen
LN
Batf3+/-
Batf3-/-
38.5
16.8
41.3
37.9
20.8
45.4
CD11b
CD103
CD8α
B
Irf8fl/fl
Itgax-cre Irf8fl/fl
16.5
2.92
CD11b
CD103
C
MHCIIHI MO
CD11b+ DC
Ly6C- Monocyte
MHCIILO MO
CD103+ DC
Ly6C+ Monocyte
Neutrophil
% of CD45+
PTX
αTIM-3
αTIM-4
D
IgG2a/PTX
αTIM-3/PTX
MHCI (Normalized)
MHCII (Normalized)
MHCIIHI MO
MHCIILO MO
CD11b+ DC
CD103+ DC

Figure S3, related to Figure 3

(A) cDC subsets (gated on $CD45^+CD11c^+MHCII^+F4/80^-$) within MMTV-PyMT tumors from animals either proficient (+/-) or deficient (-/-) in *Batf3*, or from the spleen or lymph nodes (LN) of wild type FVBN/J animals either proficient or deficient in *Batf3*. One of two representative experiments shown. (B) cDC subsets (gated on $CD45^+CD11c^+MHCII^+F4/80^-$) within PyMT tumors implanted into chimeric C57BL/6J animals reconstituted with either *Itgax-cre;Irf8*$^{fl/fl}$ or *Irf8*$^{fl/fl}$ bone marrow. (C) Frequency of myeloid subsets as a percentage of total $CD45^+$ cells within MMTV-PyMT tumors of mice treated for 15 days with PTX and a combination of αTIM-3 and/or αTIM-4 antibodies. n=8-12 per group, data pooled over 7 cohorts. *$p<0.05$, **$p<0.01$, with significance determined by unpaired t-test with Welch's correction compared to PTX alone group. (D) Surface expression of MHCI and MHCII on macrophages and cDCs from MMTV-PyMT animals treated with PTX for 7 days in conjunction with either IgG_{2a} or αTIM-3. n=13-15 per group with data pooled over 3 experiments by normalizing to 1. Horizontal bars in C, D represent the mean.

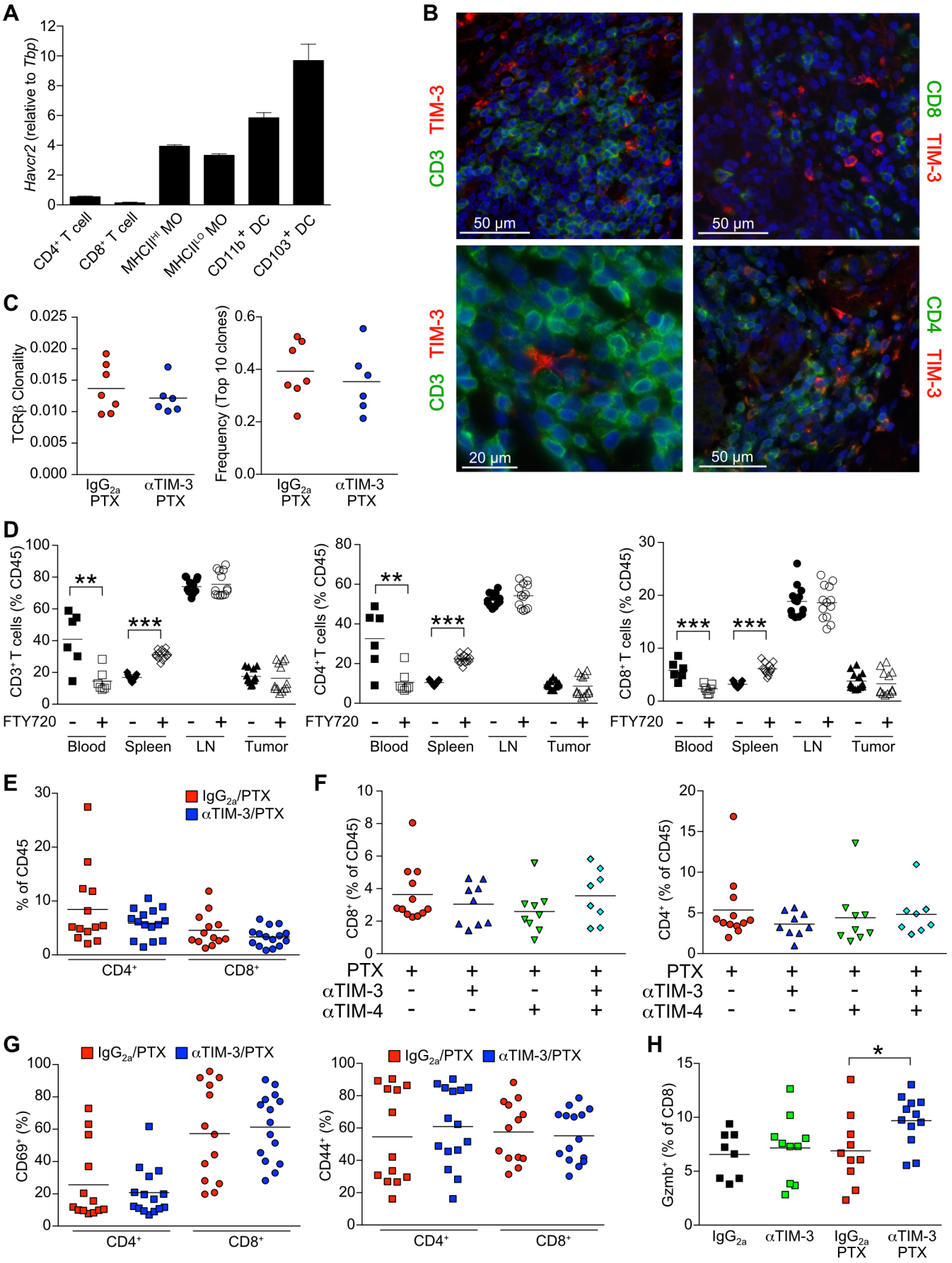

A
Havcr2 (relative to Tbp)
CD4+ T cell
CD8+ T cell
MHCIIHI MO
MHCIILO MO
CD11b+ DC
CD103+ DC
B
CD3 TIM-3
CD8 TIM-3
CD3 TIM-3
CD4 TIM-3
50 μm
20 μm
C
TCRβ Clonality
Frequency (Top 10 clones)
IgG2a PTX
αTIM-3 PTX
D
CD3+ T cells (% CD45)
CD4+ T cells (% CD45)
CD8+ T cells (% CD45)
FTY720
Blood
Spleen
LN
Tumor
E
IgG2a/PTX
αTIM-3/PTX
% of CD45
CD4+
CD8+
F
CD8+ (% of CD45)
CD4+ (% of CD45)
PTX
αTIM-3
αTIM-4
G
CD69+ (%)
CD44+ (%)
H
Gzmb+ (% of CD8)
IgG2a
αTIM-3

Figure S4, related to Figure 4

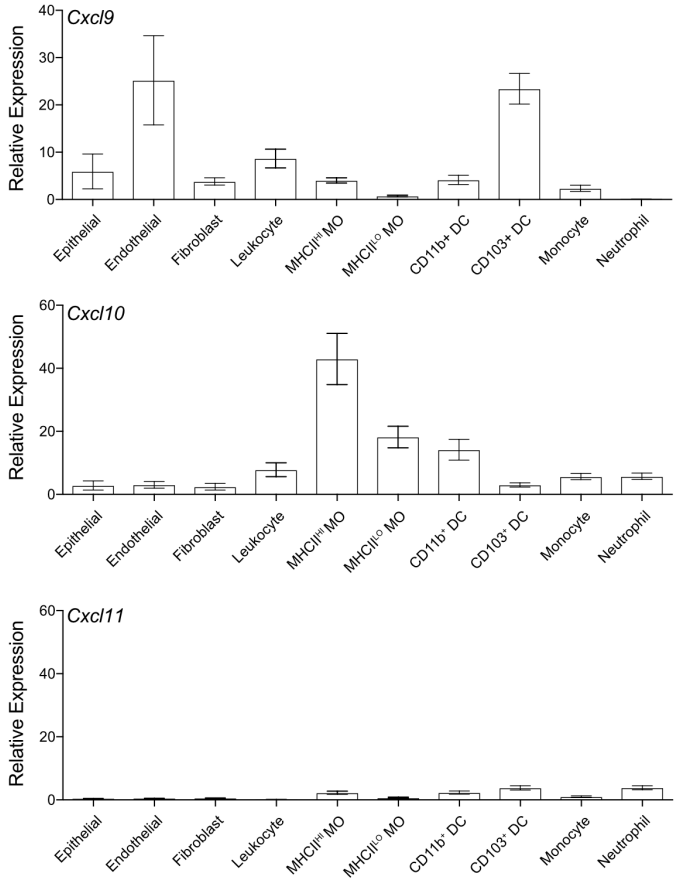
(A) *Havcr2* mRNA expression levels in leukocyte populations isolated from MMTV-PyMT mice treated with PTX, as determined by real time PCR. Data are normalized to *Tbp* expression and displayed as mean ± SEM with n=6-8 per cell type. (B) Immunofluorescent staining for TIM-3 (red) and CD3, CD4 or CD8 (green) in human breast cancer. DNA was visualized with Hoechst 33342 (blue). 3 patient samples were analyzed for each combination. (C) Clonality of T cell receptor β (TCRβ) in the peripheral blood of MMTV-PyMT mice treated with αTIM-3 and PTX (day 15), as determined by immunoSEQ. n=6-7 per group, data pooled over 2 cohorts. (D) Percentage of $CD3^+$, $CD3^+CD4^+$ or $CD3^+CD8^+$ T cells within the lymph nodes (LN), spleens, and tumors of animals bearing implanted tumors treated with DMSO or FTY720 for 7 days. Data merged from 3 experiments. (E) Frequency of $CD8^+$ and $CD4^+$ T cells within MMTV-PyMT tumors as a percent of total $CD45^+$ cells 2 days following the 2nd dose of PTX (day 7). n=13-15 mice per group pooled over 3 cohorts. (F) Frequency of $CD8^+$ and $CD4^+$ T cells within MMTV-PyMT tumors as a percent of total $CD45^+$ cells (day 15). n=8-12 per group, data pooled over 7 cohorts (G) Percent of T cells expressing CD69 (left) or CD44 (right) within MMTV-PyMT tumors, 2 days post PTX (day 7). n=13-15 mice per group pooled over 3 cohorts. (H) $CD8^+Gzmb^+$ T cells shown as percentage of total $CD8^+$ T cells. Data are from mice bearing PyMT implantable tumors treated with IgG_{2a} isotype control, αTIM-3, or PTX. Tumors were analyzed 2 days post PTX (day 7). n=8-12 per group, data pooled from 2 experiments. Horizontal bars in C-H represent the mean; *$p<0.05$, **$p<0.01$, ***$p<0.001$ by an unpaired t-test.

A

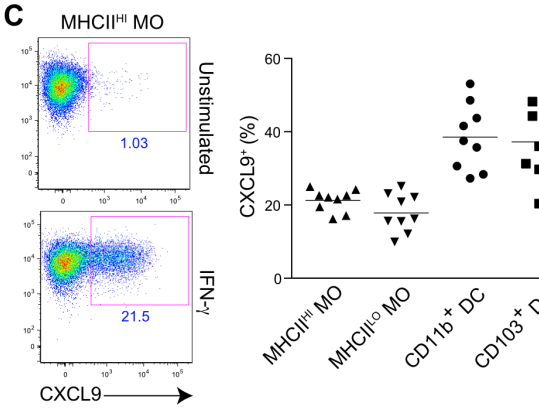
MHCII^{HI} MOs		
Gene	Fold	p value
Ccl7	1.7976	0.0015
Hcst	1.7330	0.0481
Irf7	0.6562	0.0069
Tlr5	0.6477	0.0102
Cxcl1	0.6376	0.0045
Cd34	0.6036	0.0366
Ifitm1	0.5872	0.0106
Il1r2	0.3914	0.0010
CD11b^{+} DCs		
Gene	Fold	p value
Traf2	2.6839	0.0022
Cxcl9	2.5956	0.0141
Cd22	2.5538	0.0029
Cxcl11	1.9942	0.0237
Cx3cr1	1.8214	0.0071
Pdgfb	1.8168	0.0008
Fcgr4	1.7617	0.0355
Cxcl10	1.6686	0.0311
Ccl3	1.6685	0.0305
Vcam1	1.6580	0.0356
Tnfsf13b	1.6482	0.0425
Tlr1	1.6399	0.0371
Cd69	1.6384	0.0144
Tgfbr1	1.5793	0.0087
Cmklr1	1.5758	0.0142
Irf8	1.5616	0.0120
Trem2	1.5530	0.0474
Tnfrsf14	1.5506	0.0430
Ccl12	1.5366	0.0186
C1qa	1.5204	0.0300
Cd40	1.5066	0.0051
Tlr5	0.6409	0.0344
Fkbp5	0.6153	0.0040

MHCII^{LO} MOs		
Gene	Fold	p value
Cd3eap	2.5285	0.0425
Gata3	2.2161	0.0025
Ltf	2.2066	0.0087
Zbtb7b	2.0216	0.0496
Bcl2	1.9110	0.0079
Rorc	1.8199	0.0012
Smad5	1.7975	0.0076
Casp2	1.7947	0.0148
Ptk2	1.7739	0.0471
Abcb10	1.7576	0.0047
Il15ra	1.7369	0.0257
S100a8	1.7351	0.0302
Muc1	1.6901	0.0346
Cd24a	1.6558	0.0047
C3	1.6094	0.0000
Rela	1.6025	0.0441
Il6st	1.5794	0.0066
Psmd7	1.5782	0.0405
Stat5a	1.5377	0.0112
Pdcd2	1.5259	0.0193
Ccl12	0.6253	0.0435
Ifngr2	0.6061	0.0143
Cxcl1	0.6008	0.0393
Clec4e	0.5897	0.0451
Msr1	0.5672	0.0453
Ifit2	0.5576	0.0155
Crlf2	0.5559	0.0281
Fcgr2b	0.4848	0.0386
Trem1	0.4236	0.0350
Ifitm1	0.4209	0.0078
CD103^{+} DCs		
Gene	Fold	p value
Cxcl11	2.5306	0.0037
Cxcl10	1.9417	0.0201
Cxcl9	1.6854	0.0440
Tagap	1.5823	0.0181
Cd40	1.5252	0.0243

B



C



D

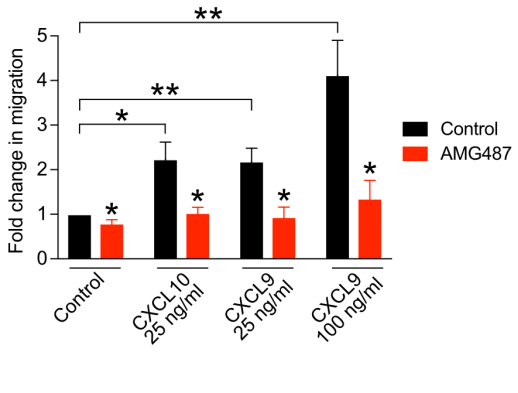


Figure S5, related to Figure 5

(A) Fold change in mRNA expression levels in tumor macrophages and cDCs isolated from mice bearing orthotopically implanted PyMT tumors 2 days following second dose of PTX (day 7). Expression was determined by NanoString. Significance was determined by an unpaired t-test, with data pooled from 2 experiments. (B) mRNA expression of *Cxcl9, Cxcl10*, or *Cxcl11* by epithelial and stromal cells in untreated MMTV-PyMT tumors. Data are normalized to *Tbp* expression and displayed as mean ± SEM with n=8 per cell type. (C) Percentage of MMTV-PyMT tumor macrophages and cDCs expressing CXCL9 following ex vivo stimulation with 40 ng/ml IFN-γ for 4 hr in the presence of brefeldin A. Representative intracellular staining is shown to the left. Horizontal bars represent the mean (n=9), with data pooled from two experiments. (D) Transwell migration of activated $CD8^+$ splenic T cells in response to recombinant murine CXCL9 or CXCL10, either in the presence or absence of 100 nM of the CXCR3 inhibitor (±)-AMG 487. Normalized data are displayed as mean ± SEM of biological replicates compiled from three independent experiments. Significance was determined by an unpaired t-test, with *$p<0.05$, **$p<0.01$.

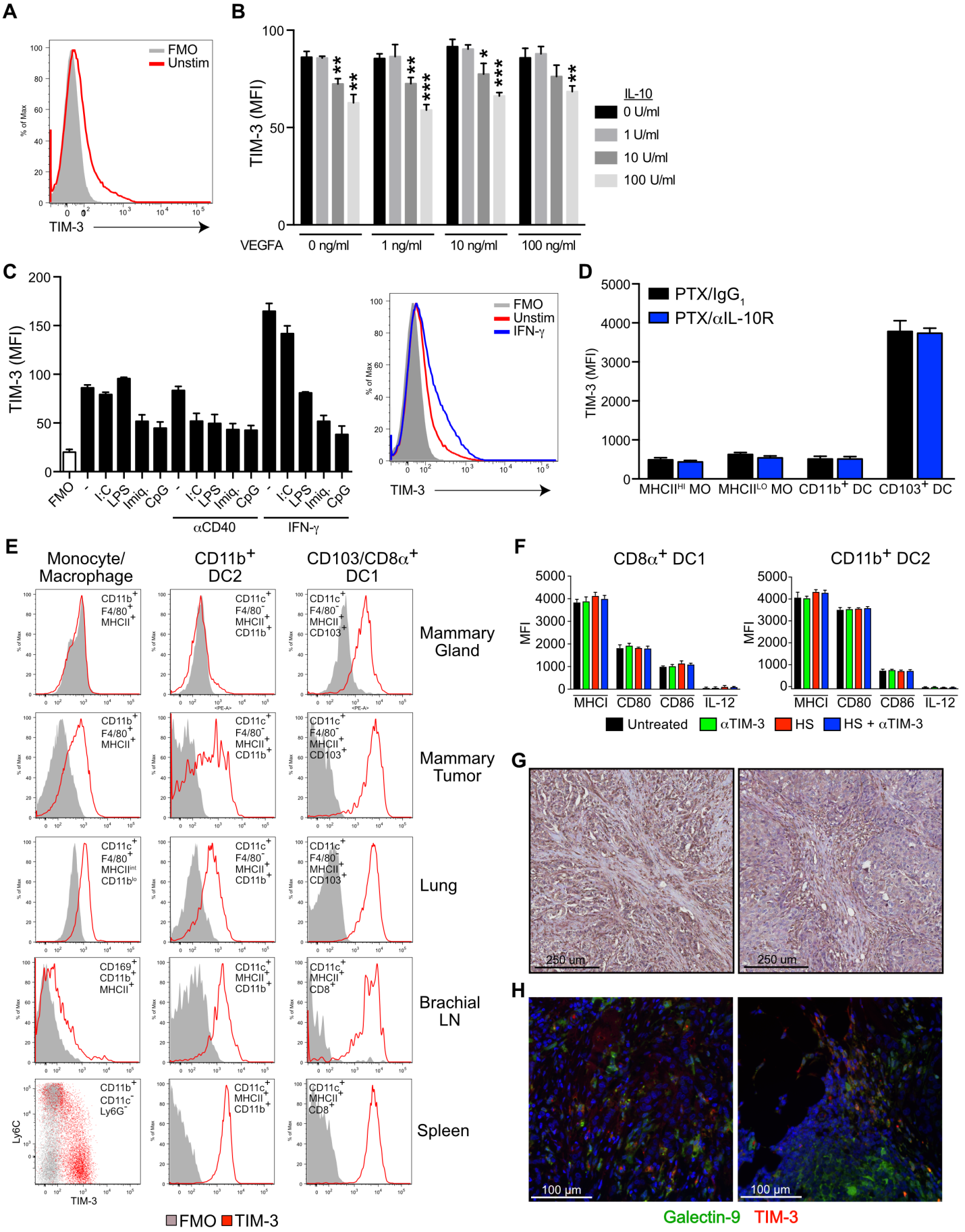

A
FMO
Unstim
% of Max
TIM-3
B
TIM-3 (MFI)
IL-10
0 U/ml
1 U/ml
10 U/ml
100 U/ml
VEGFA
0 ng/ml
1 ng/ml
10 ng/ml
100 ng/ml
C
TIM-3 (MFI)
FMO
I:C
LPS
Imiq.
CpG
αCD40
IFN-γ
FMO
Unstim
IFN-γ
TIM-3
D
PTX/IgG1
PTX/αIL-10R
TIM-3 (MFI)
MHCIIHI MO
MHCIILO MO
CD11b+ DC
CD103+ DC
E
Monocyte/ Macrophage
CD11b+ DC2
CD103/CD8α+ DC1
Mammary Gland
Mammary Tumor
Lung
Brachial LN
Spleen
Ly6C
TIM-3
FMO
TIM-3
F
CD8α+ DC1
CD11b+ DC2
MFI
MHCI
CD80
CD86
IL-12
Untreated
αTIM-3
HS
HS + αTIM-3
G
250 um
H
100 um
Galectin-9
TIM-3

Figure S6, related to Figure 6

(A-C) Surface expression of TIM-3 on FLT-3L-induced BMDCs as measured by flow cytometry, either unstimulated or stimulated with the indicated conditions for 24 hr. (A) Histogram displaying TIM-3 surface expression relative to fluorescence minus one (FMO) control. (B) TIM-3 surface mean fluorescence intensity (MFI) following incubation with IL-10 or VEGFA. (C) TIM-3 surface MFI following stimulation with Poly(I:C), LPS, Imiquimod, CpG, αCD40, or IFN-γ. A representative histogram is shown on the right. Data for B-C are displayed as mean ± SD of technical replicates; *$p<0.05$, **$p<0.01$, ***$p<0.001$, significance determined by an unpaired t-test. One of two representative experiments is shown. (D) TIM-3 surface MFI on macrophage and cDC subsets from tumors of MMTV-PyMT mice treated with PTX and either IgG_1 isotype control or αIL-10R antibody. n=7 per group, data shown as mean ± SEM. (E) Surface expression of TIM-3 in monocytes, macrophages, $CD11b^+$ cDCs, and $CD103^+/CD8^+$ cDCs in the mammary gland, lung, spleen and lymph nodes (LNs) of non-tumor bearing animals. n=4, one of two representative experiments shown. (F) Expression of activation markers by splenic cDCs following incubation with αTIM-3 and/or tumor cell debris generated by heat shock (HS). One of two representative experiments is shown, with data displayed as mean ± SD of 4 technical replicates. (G) Representative galectin-9 immunohistochemistry in human breast cancer samples (n=5). (H) Representative immunofluorescent staining for TIM-3 (red) and galectin-9 (green) in human breast cancer. DNA was visualized with Hoechst 33342 (blue). 3 patient samples were analyzed for each combination.

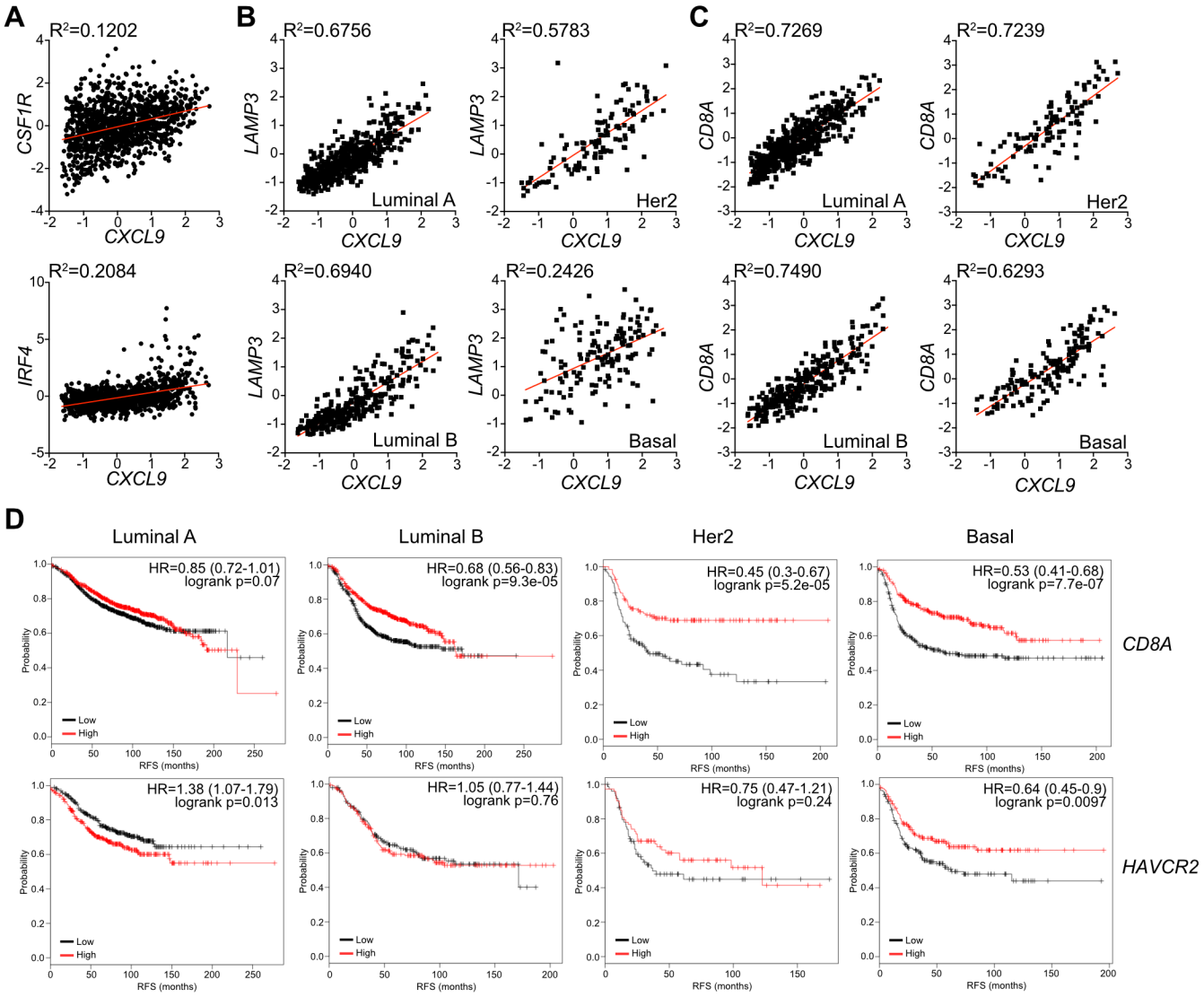


Figure S7, related to Figure 7

(A) Linear regression analysis between *CXCL9* expression and *CSF1R* or *IRF4* in human breast cancer samples from the TCGA dataset (n=1161). (B-C) Linear regression analysis between *CXCL9* expression and (B) *LAMP3* or (C) *CD8A* in samples from intrinsic luminal A (n=513), luminal B (n=286), Her2 (n=112) and basal (n=145) molecular subtypes. (D) Recurrence free survival (RFS) based upon median expression of *CD8A* or *HAVCR2* in breast tumor tissue. Data are shown for intrinsic luminal A, luminal B, Her2 and basal molecular subtypes (n=1933, 1149, 251, 618 for *CD8A*; n=841, 407, 156, 360 for *HAVCR2*). Hazard ratio (HR) and logrank p values are shown in the upper right of each Kaplan-Meier Plot.