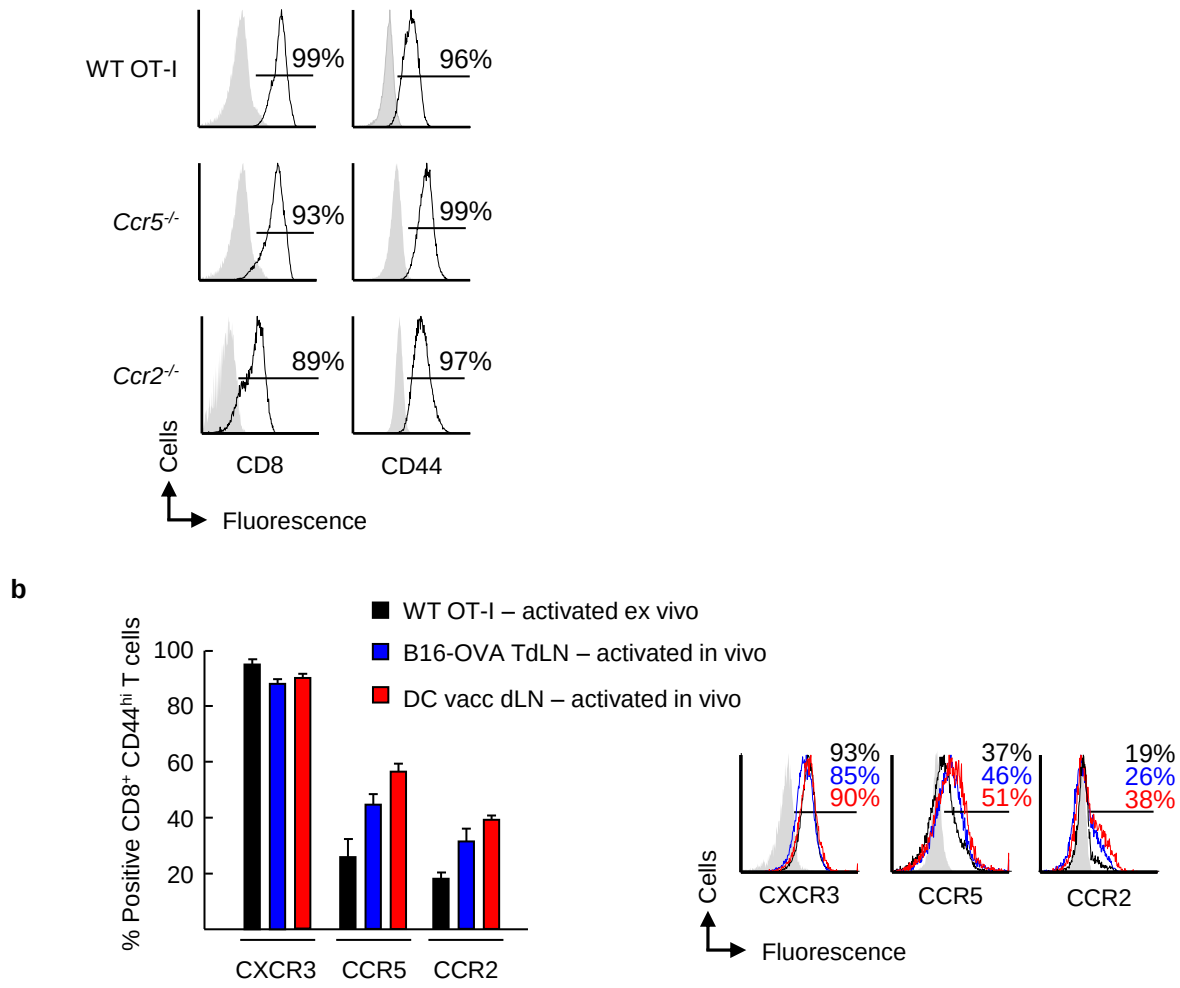
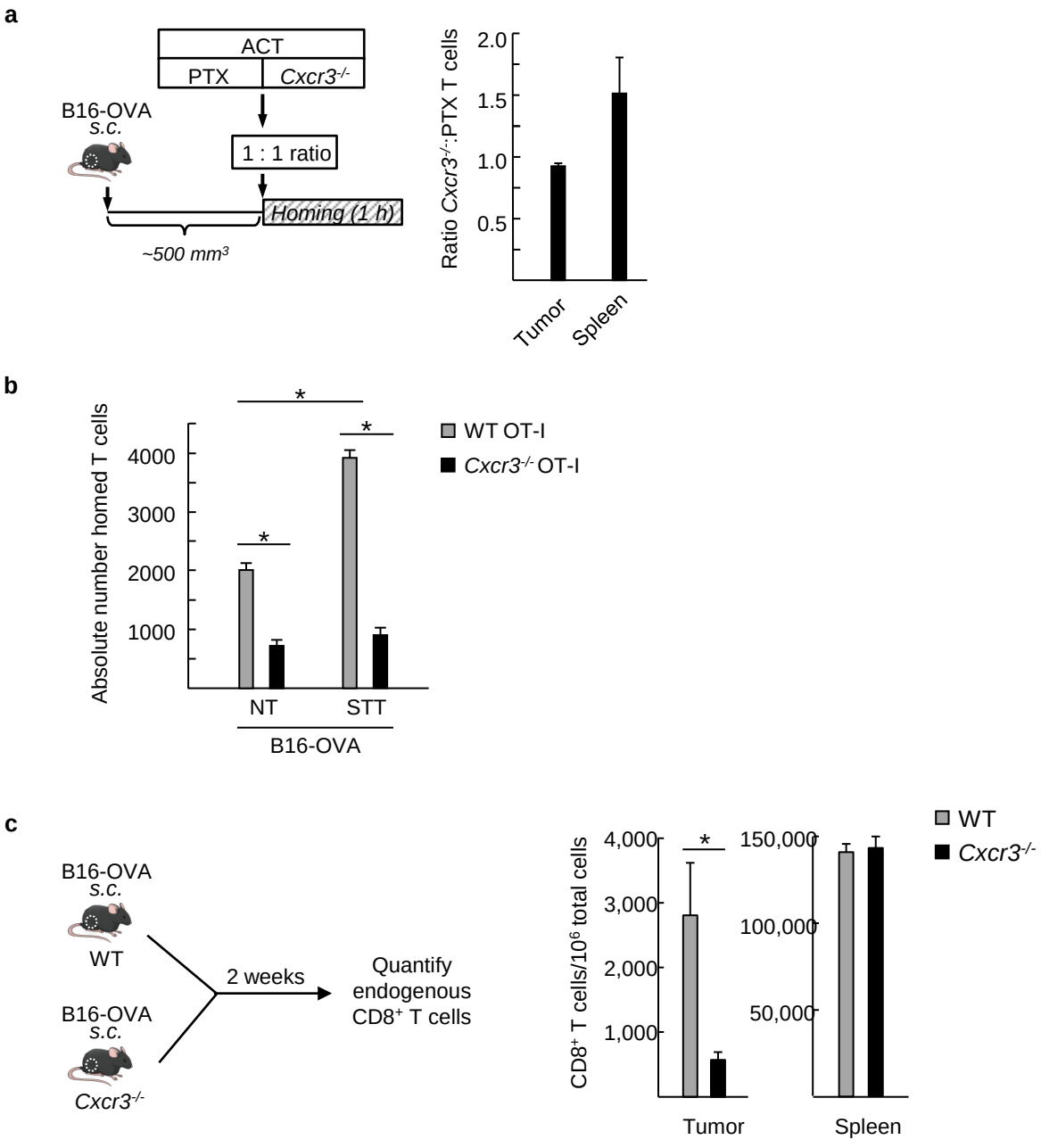


Supplementary Figure 1

Phenotype of murine effector CD8⁺ T cells activated ex vivo and in vivo. (**a**) Flow cytometric analysis of CD8 and CD44 expression on murine T cells post-activation ex vivo; data are representative of transferred populations used in homing assays in 3 independent experiments (n ≥ 2 mice per group). (**b**) Chemokine receptor expression was compared between $CD8^+$ $CD44^{hi}$ T cells activated either ex vivo (WT OT-I) or in vivo (i.e., T cell populations in tumor draining lymph nodes [TdLN] from mice 7 days after implantation of B16-OVA tumors or in dLN 7 days post DC vaccination in hind footpad). Data shown in bar graph (mean ± s.e.m.) are from n = 3 mice per group with representative histograms shown. Gray-filled histograms represent isotype control Ab staining.

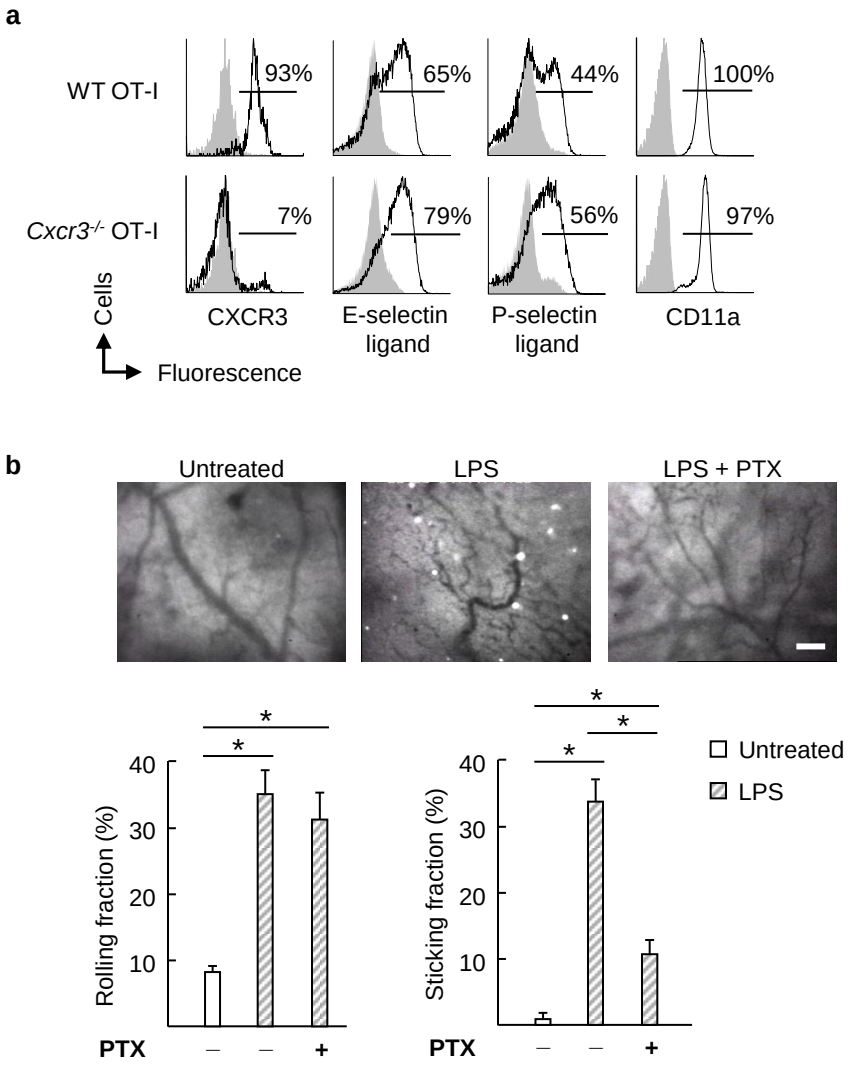


Supplementary Figure 2

Murine effector CD8⁺ T cell trafficking to tumors under homeostatic and elevated trafficking conditions. (**a**) Schematic and quantification for competitive homing studies between WT cells pretreated with PTX and *Cxcr3*$^{-/-}$ cells in the same recipient mice. Data represent ratio of adoptively transferred *Cxcr3*$^{-/-}$:PTX treated T cells determined by flow cytometry in tumor and spleen. Data showing that the 1:1

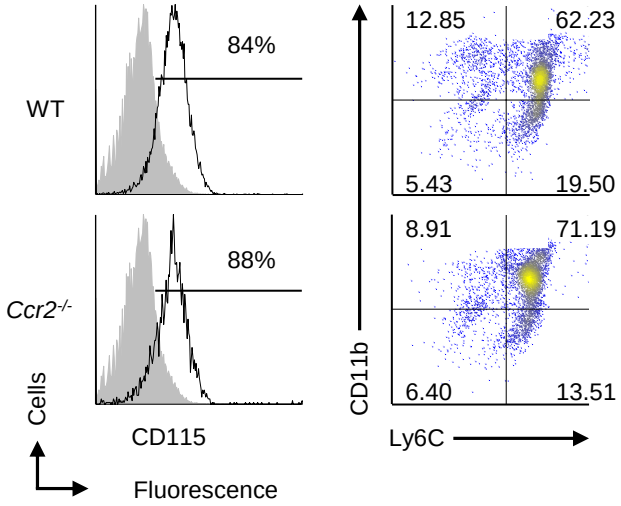
Supplementary Figure 2 *(continued)*

ratio in cells recovered from tumors was equivalent to the 1:1 input ratio indicate that *Cxcr3*$^{-/-}$ cells and PTX-treated cells homed to the same extent. Data (mean ± s.e.m.) are from 3 independent experiments (n ≥ 2 mice per group). (**b**) Absolute number of WT OT-I and *Cxcr3*$^{-/-}$ OT-I T cells homed to B16-OVA tumors under baseline normothermic (NT) conditions or in mice administered preconditioning systemic thermal therapy (STT). Data (mean ± s.e.m.) are from n ≥ 2 mice per group and are representative of 3 independent experiments. (**c**) Schematic for comparison of endogenous CD8^{+} T cell infiltration in WT and *Cxcr3*$^{-/-}$ mice at 2 weeks after implantation of B16-OVA tumors (tumor volume was 475 ± 266 and 638 ± 306 mm^3 for WT and *Cxcr3*$^{-/-}$ recipients, respectively). Data represent number of endogenous CD8^{+} T cells determined by flow cytometry per 10^6 total cells in tumor or spleen. Data (mean ± s.e.m.) are from n ≥ 3 mice per group. (**b, c**) ***** $P < 0.05$, unpaired two-tailed Student's *t*-test.



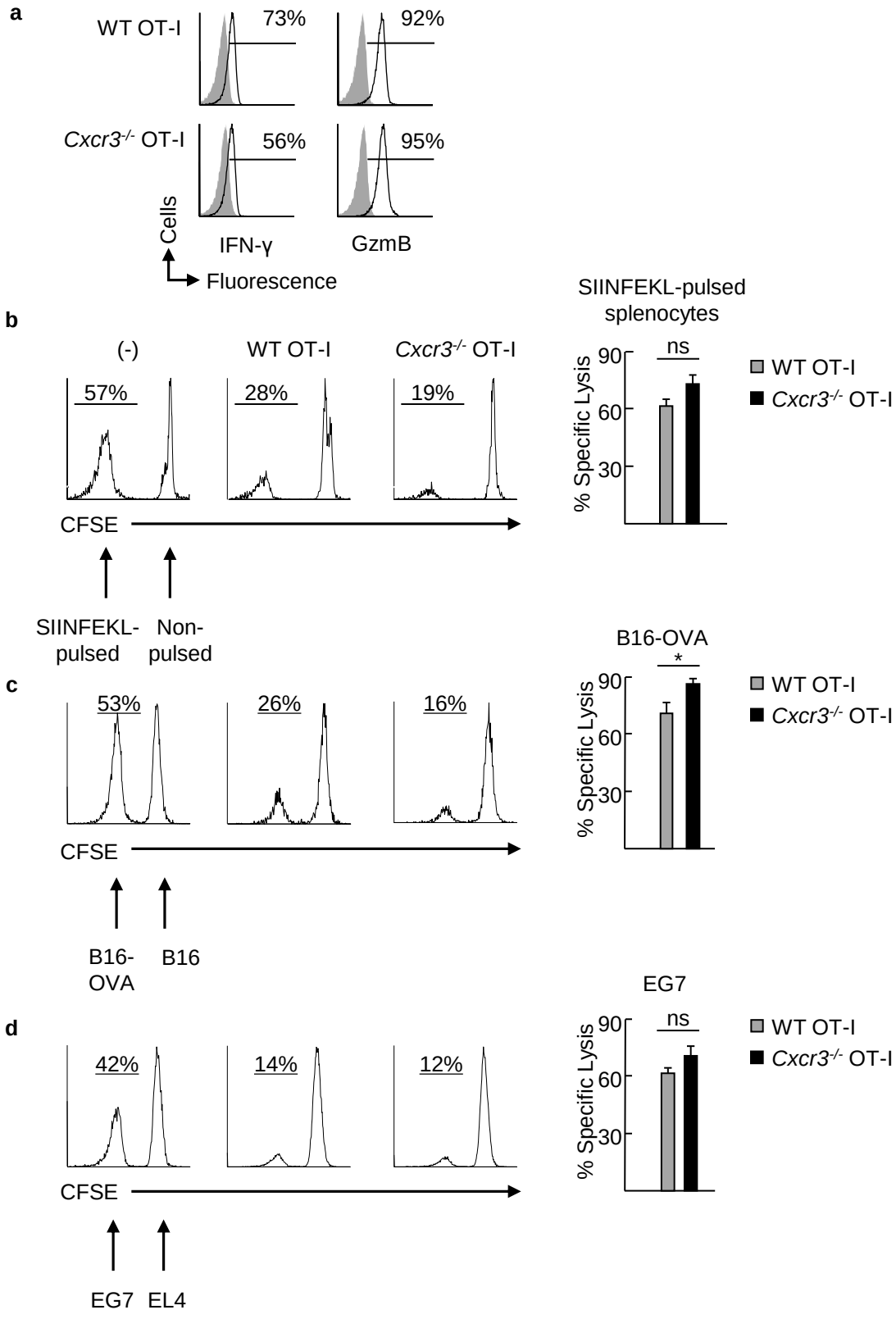
Supplementary Figure 3

Effector T cells require $G_{\alpha i}$ signaling in LPS-inflamed vessels. (**a**) Phenotype of WT OT-I and *Cxcr3*$^{-/-}$ OT-I T cells for prototypical trafficking molecules post-activation ex vivo; this phenotype is representative of populations used for intravital imaging studies in tumor vessels and normal skin. Gray-filled histograms represent isotype control Ab staining. (**b**) Representative photomicrographs depict stable interactions of fluorescently-tagged WT OT-I cells or PTX-treated WT OT-I in normal skin vessels in untreated mice or mice pre-treated with LPS. Data (mean ± s.e.m.) for rolling fractions and sticking fractions are from ≥ 3 independent experiments (n ≥ 2 mice per group). ***** $P < 0.002$, unpaired two-tailed Student's *t*-test. Scale bar, 100µM.



Supplementary Figure 4

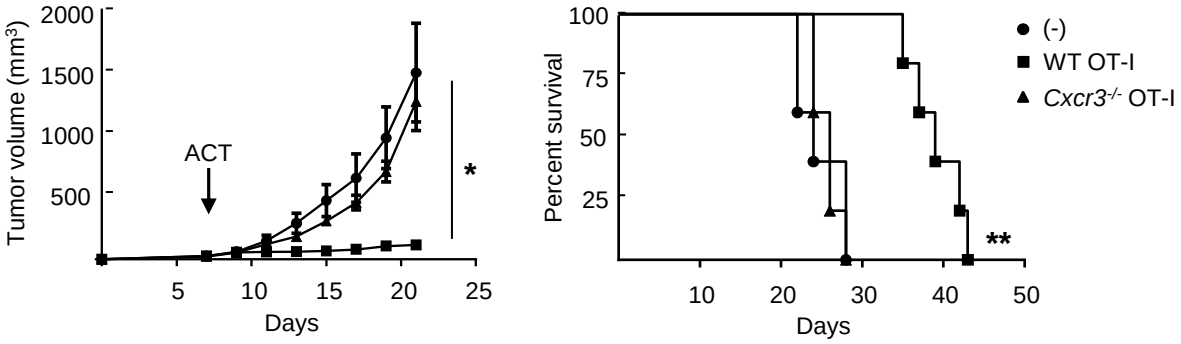
Phenotype of inflammatory monocytes used for trafficking studies. Representative flow cytometric analysis of adoptively transferred inflammatory monocytes after CD115^{+} selection by AutoMacs. Purity of transferred WT or *Ccr2*$^{-/-}$ monocytes (left) and expression of inflammatory markers (right). Gray-filled histograms represent isotype control Ab staining; numbers denote percent positively stained cells. Data are representative of ≥ 3 independent experiments.


a
WT OT-I
73%
92%
Cxcr3-/- OT-I
56%
95%
Cells
IFN-γ
GzmB
Fluorescence
b
(-)
WT OT-I
Cxcr3-/- OT-I
57%
28%
19%
CFSE
SIINFEKL-pulsed
Non-pulsed
SIINFEKL-pulsed splenocytes
ns
% Specific Lysis
90
60
30
WT OT-I
Cxcr3-/- OT-I
c
53%
26%
16%
CFSE
B16-OVA
B16
B16-OVA
*
% Specific Lysis
90
60
30
WT OT-I
Cxcr3-/- OT-I
d
42%
14%
12%
CFSE
EG7
EL4
EG7
ns
% Specific Lysis
90
60
30
WT OT-I
Cxcr3-/- OT-I

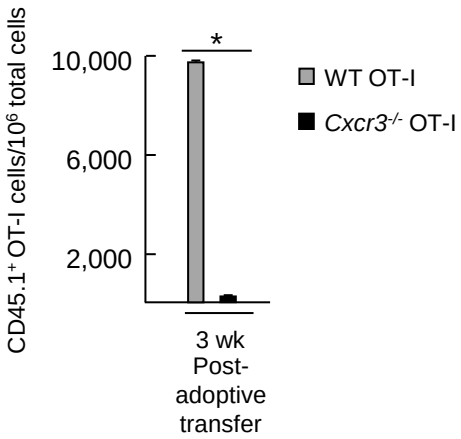
Supplementary Figure 5

WT and *Cxcr3*$^{-/-}$ OT-I exhibit equivalent cytotoxicity in vitro. (**a**) Intracellular staining of WT effector OT-I and *Cxcr3*$^{-/-}$ OT-I for IFN-γ and granzyme B (GzmB). Gray-filled histograms represent isotype control Ab staining. (**b**) In vitro cytotoxicity of SIINFEKL-pulsed target splenocytes, (**c**) B16-OVA, and (**d**) EG7 target cells. The proportion of live fluorescent OVA-specific and non-specific tumor targets was evaluated in the absence of T cells (-), or after incubation with effector WT OT-I or with *Cxcr3*$^{-/-}$ OT-I T cells for 24 hrs. Left, representative flow plots of target cells. Right, percent specific lysis of OVA-expressing targets. (**b-d**) Data (mean ± s.e.m.) are from 2 independent experiments performed in triplicate. ***** $P < 0.05$; ns, not significant; unpaired two-tailed Student's *t*-test.

a

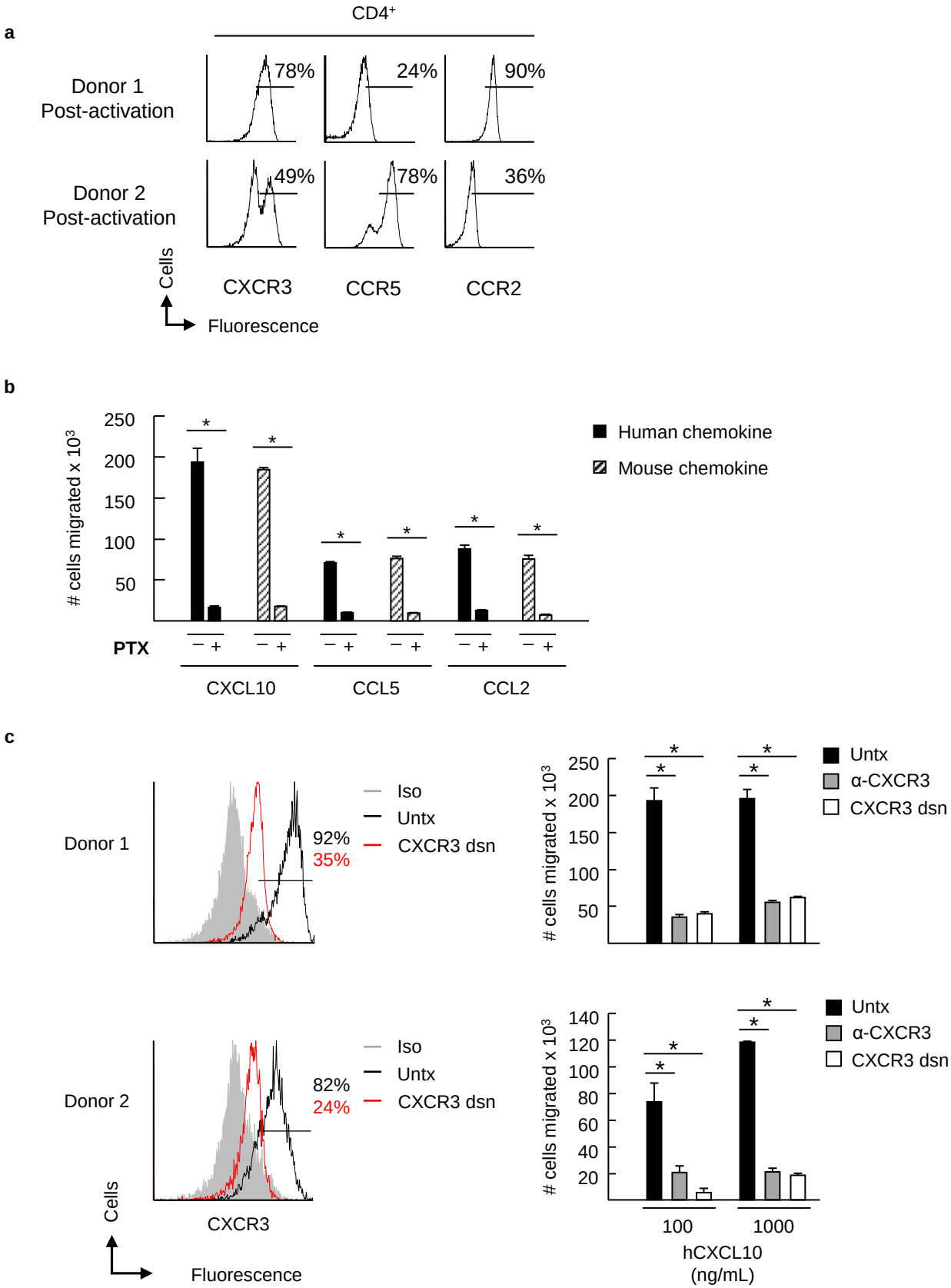


b



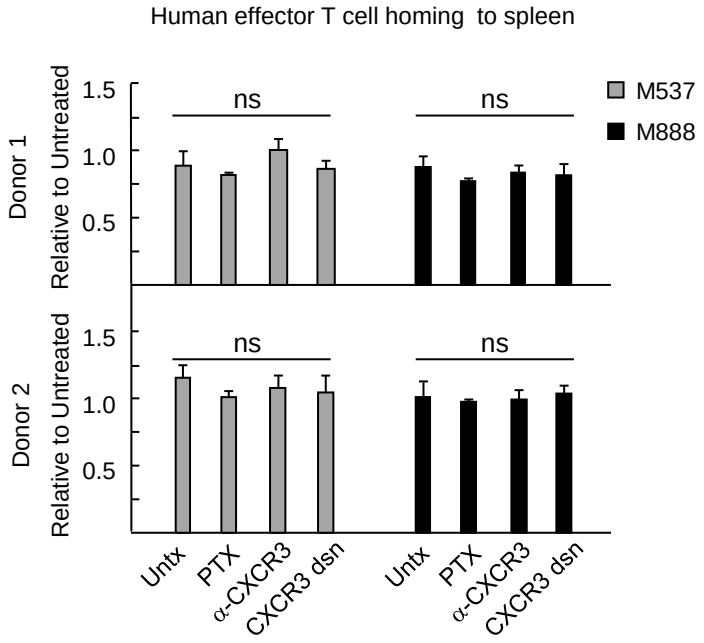
Supplementary Figure 6

WT and *Cxcr3*$^{-/-}$ OT-I accumulation and control of tumor growth in vivo. (**a**) No T cells (-), effector WT OT-I, or *Cxcr3*$^{-/-}$ OT-I T cells were adoptively transferred (ACT) into mice bearing B16-OVA tumors; time of ACT administration is denoted by the arrow. Tumor growth (left) and survival (right) were monitored over time. Data (mean ± s.e.m.) are representative of 3 independent experiments (n = 5 mice per group). ***** $P < 0.02$, ****** $P < 0.005$, WT OT-I compared to no adoptive transfer or transfer of *Cxcr3*$^{-/-}$ OT-I; statistical significance of tumor growth, two-way ANOVA for repeated measures; survival, Kaplan-Meier log rank tests. (**b**) Tumor-infiltrating CD45.1^{+} WT or *Cxcr3*$^{-/-}$ CD45.1^{+} OT-I T cells were tracked 3 weeks after ACT in CD45.2^{+} congenically mismatched mice bearing B16-OVA tumors. Data (mean ± s.e.m.) are from 1 experiment (n ≥ 5 mice per group). ***** $P < 0.05$; unpaired two-tailed Student's *t*-test.


a
CD4+
Donor 1
Post-activation
78%
24%
90%
Donor 2
Post-activation
49%
78%
36%
Cells
CXCR3
CCR5
CCR2
Fluorescence
b
cells migrated x 10³
250
200
150
100
50
Human chemokine
Mouse chemokine
PTX
− +
CXCL10
CCL5
CCL2
c
Donor 1
Iso
Untx
CXCR3 dsn
92%
35%
Donor 2
82%
24%
Cells
CXCR3
Fluorescence
Untx
α-CXCR3
CXCR3 dsn
140
120
100
80
60
40
20
100
1000
hCXCL10
(ng/mL)

Supplementary Figure 7

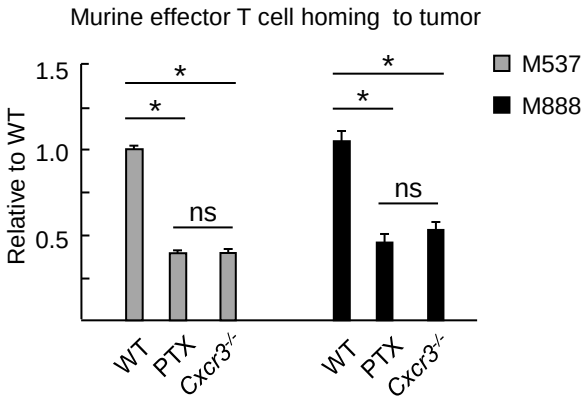
Ex vivo activated human T cells express functional CXCR3, CCR5, and CCR2 and respond to both human and murine chemokines in vitro. (**a**) Chemokine receptor phenotype on $CD4^+$ population in ex vivo activated human T cells. (**b**) Chemotaxis assay demonstrating migration of human T cells (donor 1) to both human and murine recombinant chemokines in vitro; similar results were detected in independent experiments for activated T cells from donor 2. (**c**) Flow cytometric analysis of CXCR3 surface expression on activated human PBL after desensitization with human CXCL10 (left). Chemotaxis assays for CXCR3-dependent migration after CXCR3 desensitization (CXCR3 dsn) or antibody blockade (right). Gray-filled histograms represent isotype control Ab staining. (**b, c**) Data (mean ± s.e.m.) are representative of 2 independent experiments. ***** $P < 0.05$, unpaired two-tailed Student's *t*-test. α-CXCR3, CXCR3 blocking Ab.



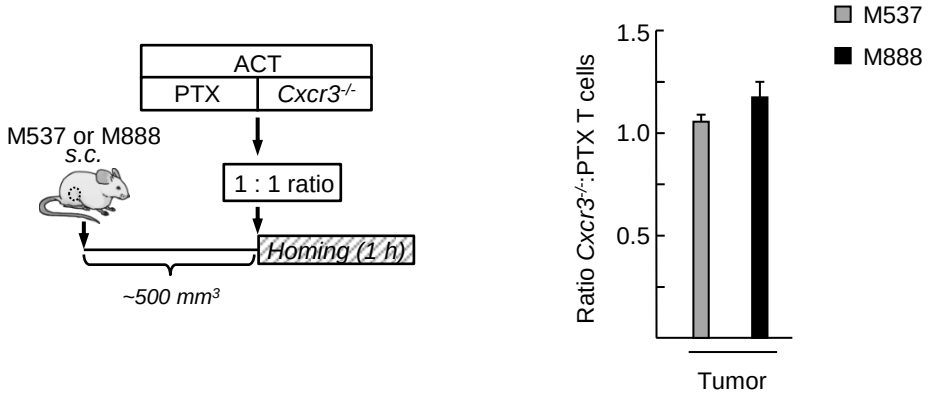
Supplementary Figure 8

CXCR3 does not alterhuman effector T cell localization in the spleens of SCID mice bearing human melanoma xenografts. Short-term (1 h) competitive homing studies using adoptive cell transfer (ACT) of untreated (Untx) human effector T cells from donor 1 or donor 2 that were comixed at a 1:1 ratio with untreated cells, PTX-pretreated cells, α-CXCR3 Ab-pretreated cells, or cells where CXCR3 was desensitized by exposure to recombinant CXCL10 prior to transfer into mice (CXCR3 dsn). Ratio of adoptively transferred T cells relative to untreated cells in spleens of M537 or M888 tumor-bearing SCID mice following short-term competitive homing assays is shown. PTX, pertussis toxin; α-CXCR3, CXCR3 blocking Ab. Data (mean ± s.e.m.) are from ≥ 2 independent experiments (n = 2 mice per group); ns, not significant; unpaired two-tailed Student's *t*-test.

a



b



Supplementary Figure 9

Murine effector T cells require CXCR3 during trafficking to human melanoma xenografts. (**a**) Murine WT effector cells were comixed at a 1:1 ratio with WT cells, PTX-pretreated WT cells, or *Cxcr3*$^{-/-}$ cells (all T cells on a C57BL/6 background). Ratio of adoptively transferred T cells relative to WT in M537 or M888 melanoma tumor-bearing SCID mice following short-term (1 h) competitive homing assays is shown. ***** $P < 0.001$, compared to WT; ns, not significant; unpaired two-tailed Student's *t*-test. (**b**) Schematic and quantification for competitive homing studies between PTX-pretreated WT and *Cxcr3*$^{-/-}$ murine cells following adoptive T cell transfer (ACT) in M537 and M888 human melanoma xenografts. (**a, b**) Data (mean ± s.e.m.) are from ≥ 3 independent experiments (n ≥ 2 mice per group).