Supplemental Data:

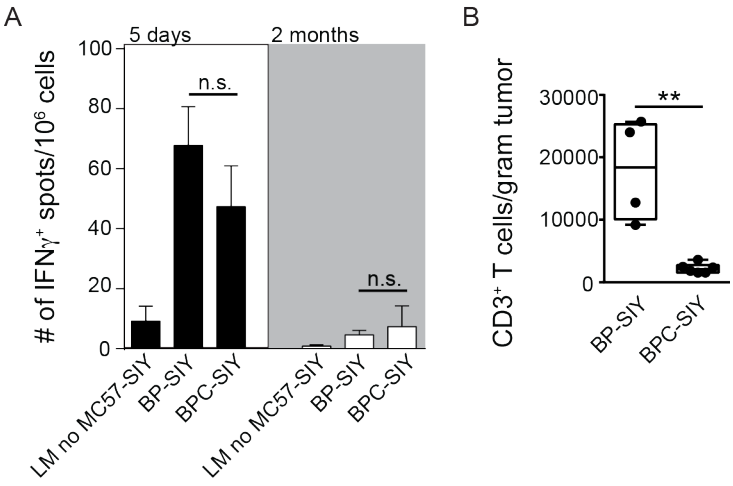

Figure S1. Related to Figure 2. T cell activation following immunization and tumor induction. (A) SIY-specific immune response was measured by IFN-γ ELISpot in BP-SIY and BPC-SIY mice inoculated with MC57.SIY, 5 days or 60 days prior to the assay. As a control, littermates not injected with MC57.SIY, were used (Mean ± SEM, n = 3, 4, 4, 5, 6, 5; left to right, pooled out of two independent experiments). (B) BP-SIY and BPC-SIY tumors were analyzed for the degree of T cell infiltration 6-8 weeks after tumor induction. Depicted is amount of $CD3^+$ infiltrated T cells per gram of tumor (n = 4 and 6 for BP-SIY and BPC-SIY, respectively, data are representative for 3 experiments). The tumor weight at time of analysis was 1.94 g ± 0.26 g for BP-SIY and 2.2 g ± 0.3 g for BPC-SIY (mean ± SD). Data are shown in mean with SEM; Box plots show median with 95^{th} percentile, maximal deviation shown by error bars. Significance was assumed with $p \leq 0.05$, with ** ≤ 0.01.

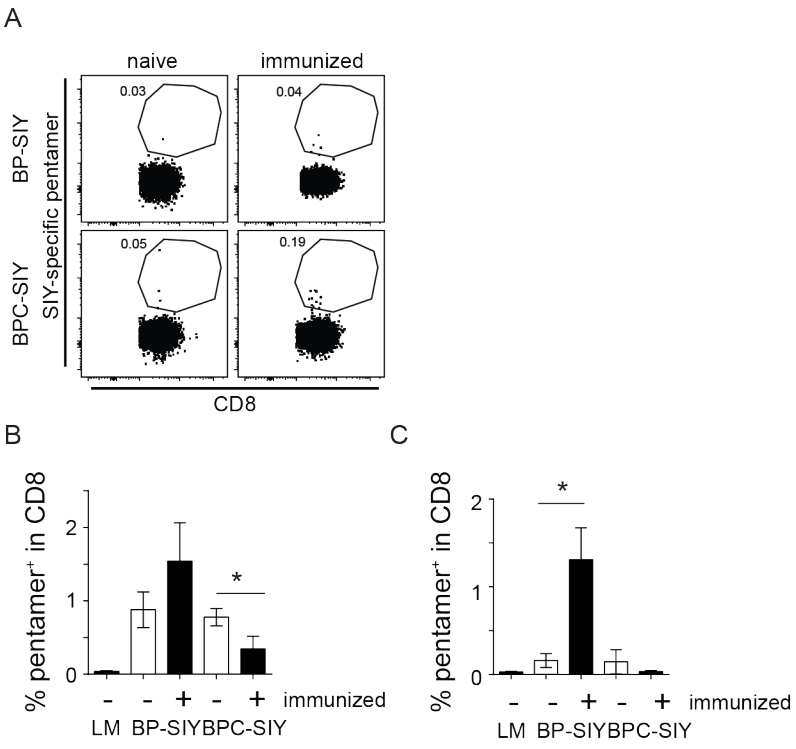

Figure S2. Related to Figure 3. T cell expansion following immunization and tumor induction. (A) Representative example of pentamer staining for SIY-specific T cells in the spleen of naive or immunized tumor-bearing BP-SIY and BPC-SIY mice, isolated at the end point of the experiment shown in Figure 2. (B-C) Statistical analysis of antigen-specific cells detectable within the spleen (B) and TdLN (C) assessed though SIY-specific pentamer staining at the end point of the experiment. Shown are mean with SEM and n numbers, corresponding to the experiment shown in Figure 3. Significance was assumed with $p \leq 0.05$, with * ≤ 0.05.

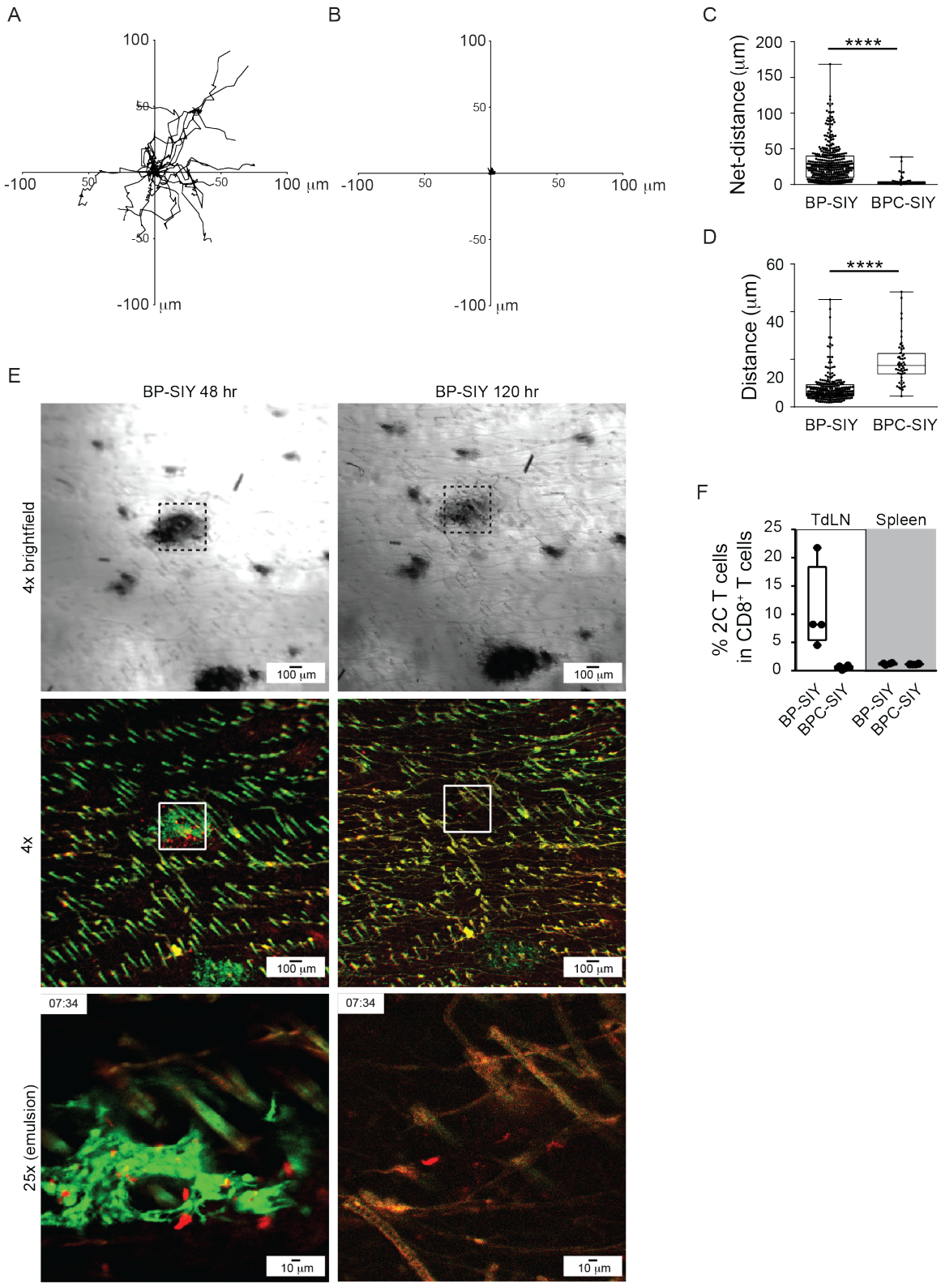

Figure S3. Related to Figure 4. Behavior of adoptively transferred effector T β-catenin-negative and -positive tumors. (A, B) Representative example of T cell

motility analyses in (A) a BP-SIY tumor lesion and (B) a BPC-SIY tumor lesion; start position was normalized to 0. Spider plots are derived from the example Movie 1 and Movie 2. (C) Net-displacement of adoptively transferred T cell in β-catenin-negative and positive tumors. Net displacement was calculated from start and end points obtained from the total displacement/distance analysis (Figure 4 G) and displays the most linear distance traveled by a given T cell (n= 357 (BP-SIY) and 55 (BPC-SIY). (D) The individual distances between tumor cell and T cell used to calculate the mean distance (n= 126 (BP-SIY) and 96 (BPC-SIY), pooled out of 20 images obtained from 3 independent experiments). We only assessed events with a distance of 50 μm or less. (E) Representative example of eradicated tumor lesion in BP-SIY tumor model after transfer of effector 2C T cells. Left, tumor lesion on day 2 post-transfer, bright-field, 4x and 25x magnification (top to bottom); right, same area on day 5 post- transfer. These images correspond to Movie S3. (F) Number of effector T cells present in spleen and TdLN three days post-adoptive transfer of $1x10^6$ *in vitro* activated 2C T cells. Left panel shows percent within $CD8^+$ T cells and right panel depicts the total number of 2C T cells per gram tumor. Shown are mean with 95^{th} percentile, n = 4 and 6 for BP-SIY and BPC-SIY, respectively. Data are shown in mean with SEM, box plots show median with 95^{th} percentile, maximal deviation shown by error bars; significance was assumed with $p \leq 0.05$, with **** ≤ 0.0001.

Movie S1. Related to Figure 4. Representative time-lapse of effector T cells migrating through BP-SIY (left) and BPC-SIY (right) tumor microenvironments acquired using a 25x emulsion lens (scale bar 10 μm). YFP-positive tumor cells are pseudo-colored in green and effector T cells in red. Movies were acquired at a speed of 4.128 s/frame (14.5 frames/min) and are shown as 20 frames/s.

Movie S2. Related to Figure 4. MTrackJ analysis of T cell motility in Movie S1. Each line is tracking one T cell moving across the tumor (BP-SIY (left) and BPC-SIY (right); scale bar 10 μm); YFP-positive tumor cells are pseudo-colored in green and effector T cells in red. Movies were acquired at a speed of 4.128 s/frame (14.5 frames/min) and are shown as 20 frames/s).

Movie S3. Related to Figure 4. Representative time-lapse of effector T cell migrating through BP-SIY at 48 hr (left) and 120 hr (right) after adoptive transfer (acquired using a 25x emulsion lens; scale bar 10 μm; YFP-positive tumor cells are

pseudo-colored in green and effector T cells in red). Movies were acquired at a speed of 4.128 s/ frame (14.5 frames/min) and are shown as 20 frames/s. At 48 hr T cells engage close contact with tumor while at the 120 hr time-point the tumor was eradicated and T cell are patrolling.

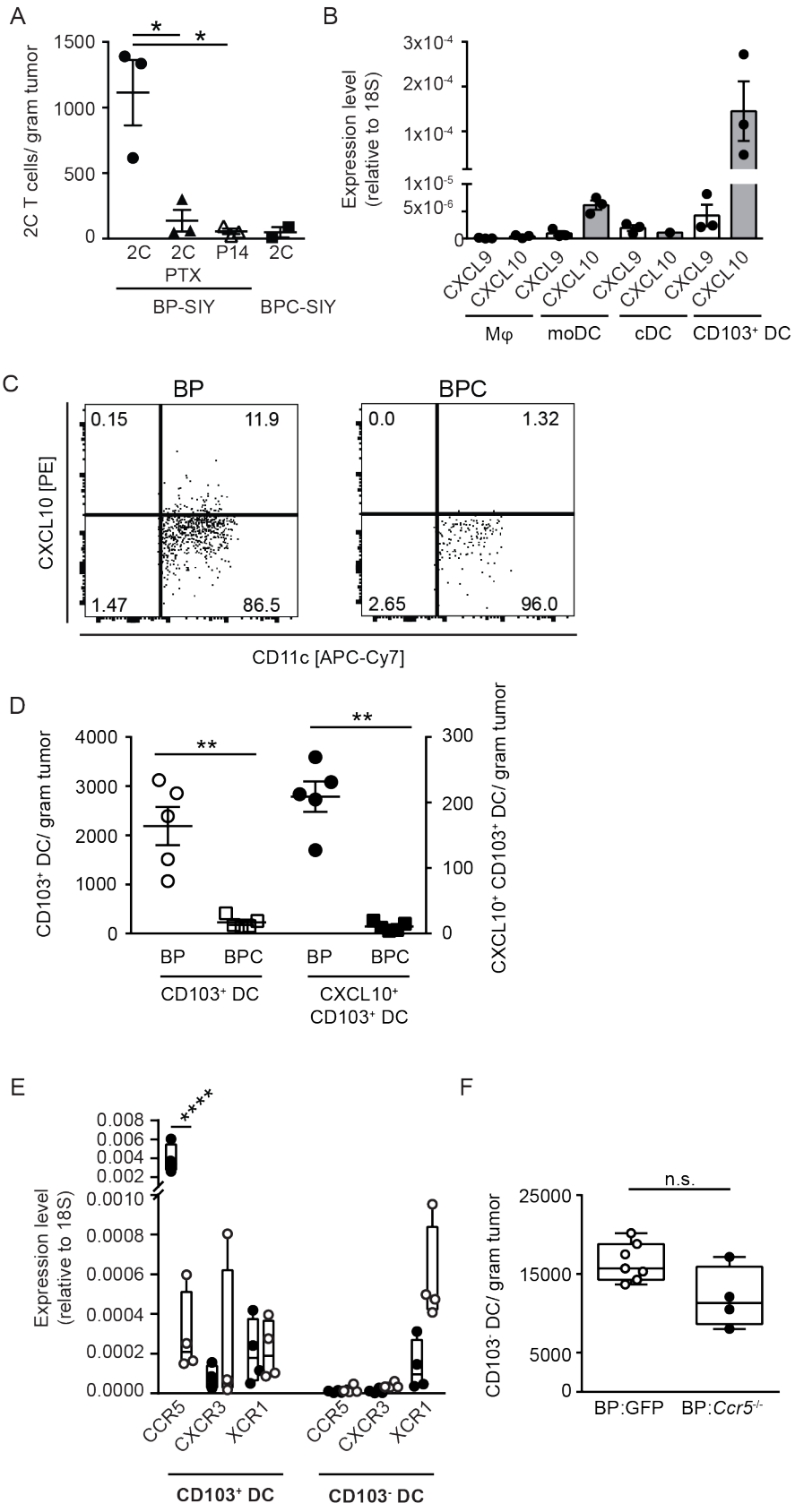
A
2C T cells/ gram tumor
1500
1000
500
0
*
*
2C
2C
P14
2C
PTX
BP-SIY
BPC-SIY
B
Expression level (relative to 18S)
3x10-4
2x10-4
1x10-4
1x10-5
5x10-6
0
CXCL9
CXCL10
Mφ
moDC
cDC
CD103+ DC
C
BP
BPC
0.15
11.9
1.47
86.5
0.0
1.32
2.65
96.0
CXCL10 [PE]
CD11c [APC-Cy7]
D
CD103+ DC/ gram tumor
4000
3000
2000
1000
0
**
**
300
200
100
0
CXCL10+ CD103+ DC/ gram tumor
BP
BPC
CD103+ DC
CXCL10+ CD103+ DC
E
Expression level (relative to 18S)
0.008
0.006
0.004
0.002
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
****
CCR5
CXCR3
XCR1
CD103+ DC
CD103- DC
F
CD103- DC/ gram tumor
25000
15000
5000
0
n.s.
BP:GFP
BP:Ccr5-/-

Figure S4. Related to Figure 6. Adoptively transferred 2C T cells migrate into the TME in a chemokine-dependent manner. (A) Amount (number/gram tumor) of 2C T cells detectable after 72 h post transfer into BP-SIY (circle, n = 3) and BPC-SIY (square, n = 2) tumor or after transfer of 2C T cells, pretreated with pertussis toxin, into BP-SIY tumors (triangle, n = 3) or after transfer of P14 effector T cells into BP-SIY tumors (open triangle, n = 3). The tumor weight at time of analysis was 1.5 g ± 0.2 g for BP-SIY + 2C, 1.8 g ± 0.18 g for BP-SIY + 2C-PTX, 1.4 g ± 0.12 g for BP-SIY + P14 and 1.2 g ± 0.08 g for BPC-SIY + 2C (mean ± SD). (B) Expression level of CXCL9 and CXCL10 in macrophages (MΦ; $CD45^+$, $MHCII^+$, $CD11c^-$, $CD11b^+$), monocyte-derived DC (moDC; $CD45^+$, $MHCII^+$, $CD11c^+$, $CD11b^{high}$), conventional DC (cDC; $CD45^+$, $MHCII^+$, $CD11c^+$, $CD11b^{dim}$, CD103/$CD8a^-$) and $CD103^+$ DC ($CD45^+$, $MHCII^+$, $CD11c^+$, $CD11b^{dim}$, CD103/$CD8a^+$) assessed using qRT-PCR (n=3, cDC CXCL10 two samples were below detection level). (C-D) Protein expression of CXCL10 in $CD103^+$ DC ($CD45^+$, $MHCII^+$, $CD11c^+$, $CD11b^{dim}$, CD103/$CD8a^+$) assessed using intracellular staining. (C) Depicts a representative staining in BP and BPC tumor-derived DC (pre-gated $CD45^+$, $MHCII^+$, $CD11c^+$, $CD11b^{dim}$, CD103/$CD8a^+$) and (D) depicts statistical analysis (n=5). The tumor weight at time of analysis was 1.2 g ± 0.15 g for BP and 1.1 g ± 0.2 g for BPC (mean ± SD). (E) Chemokine expression profiling on $CD103^+$ and $CD103^-$ DC assessed from sorted DC via quantitative PCR analysis (n =4). The tumor weight at time of analysis across all experiments was 1.4 g ± 0.1 g for BP and 2 g ± 0.14 g for BPC (mean ± SD). (F) Amount of conventional $CD103^-$ DC found in tumors with control (GFP, grey circles) or *Ccr5*$^{-/-}$ (black circles) bone marrow (n = 7 and 4, corresponding data to Figure 6 D and E). The tumor weight at time of analysis was 0.7 g ± 0.08 g for control chimeras and 0.8 g ± 0.2 g for *Ccr5*$^{-/-}$ chimeras (mean ± SD). Data are shown in mean with SEM, box plots show median with 95th percentile, maximal deviation shown by error bars; significance was assumed with $p \leq 0.05$, with * ≤ 0.05; ** ≤ 0.01; **** ≤ 0.0001.

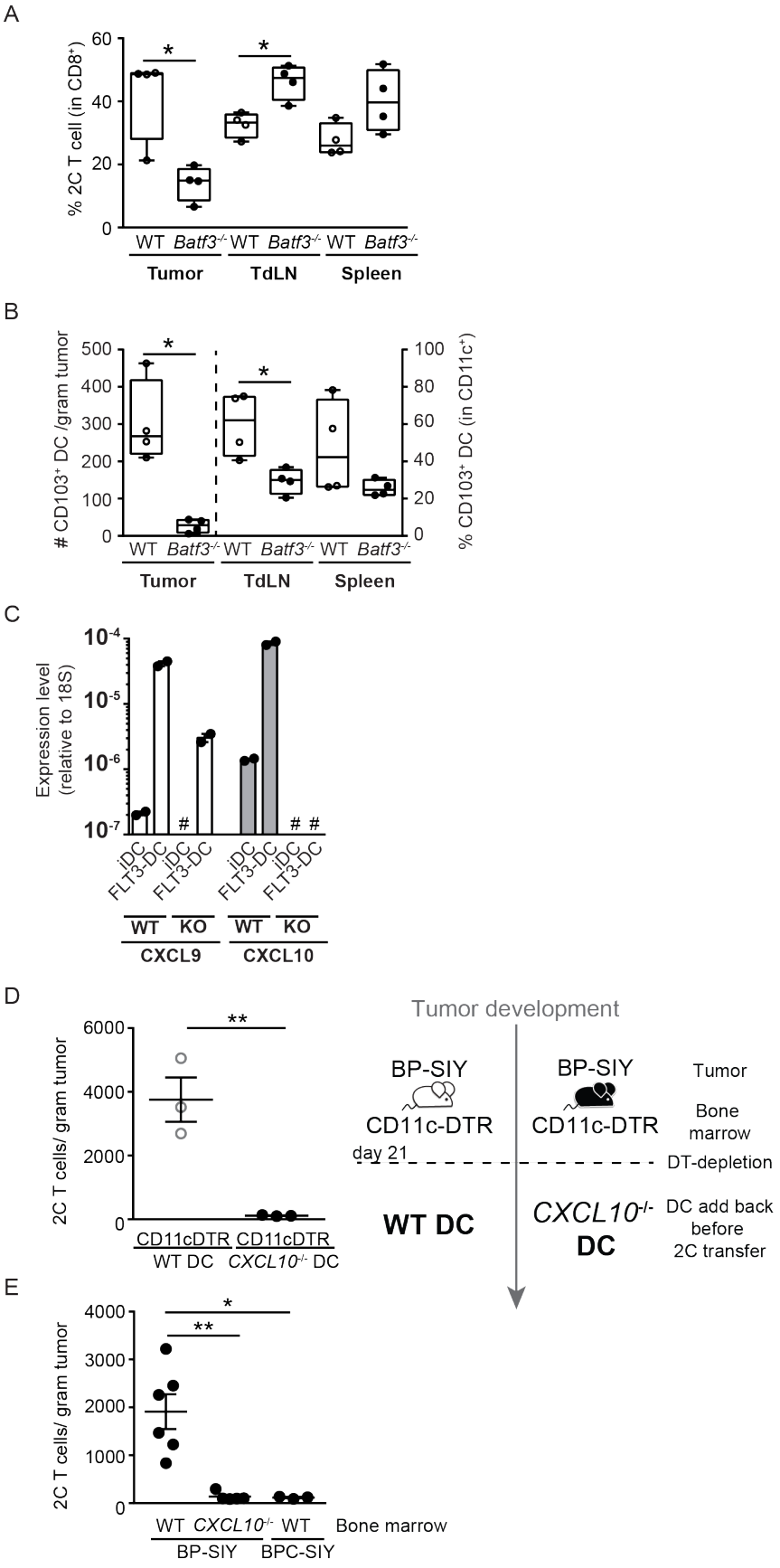
A
% 2C T cell (in CD8+)
60
40
20
0
*
*
WT
Batf3-/-
WT
Batf3-/-
WT
Batf3-/-
Tumor
TdLN
Spleen
B
CD103+ DC /gram tumor
500
400
300
200
100
0
% CD103+ DC (in CD11c+)
100
80
60
40
20
0
*
*
WT
Batf3-/-
WT
Batf3-/-
WT
Batf3-/-
Tumor
TdLN
Spleen
C
Expression level (relative to 18S)
10-4
10-5
10-6
10-7
#
# #
iDC
FLT3-DC
iDC
FLT3-DC
iDC
FLT3-DC
iDC
FLT3-DC
WT
KO
WT
KO
CXCL9
CXCL10
D
2C T cells/ gram tumor
6000
4000
2000
0
**
CD11cDTR
CD11cDTR
WT DC
CXCL10-/- DC
Tumor development
BP-SIY
CD11c-DTR
BP-SIY
CD11c-DTR
Tumor
Bone marrow
day 21
DT-depletion
WT DC
CXCL10-/- DC
DC add back before 2C transfer
E
2C T cells/ gram tumor
4000
3000
2000
1000
0
*
**
WT
CXCL10-/-
WT
Bone marrow
BP-SIY
BPC-SIY

Figure S5. Related to Figure 7. Batf3-dendritic cell-derived CXCL10 is required for the recruitment of effector T cells into the tumor microenvironment. (A) Percentage of adoptively transferred 2C T cells in total CD8 T cells detected in tumor, TdLN and spleen of BP-SIY mice engrafted with CD11c-DTR/WT (grey circles) or CD11c-DTR/*Batf3*$^{-/-}$ (black circles) bone marrow and treated with diphtheria toxin. (B) Number (tumor, left) or percentage (TdLN and spleen, right) of CD103^{+} DC detectable after diphtheria toxin-depletion. Mice depicted in C and D are corresponding to mice shown in Figure 5F (n = 4). The tumor weight at time of analysis was 0.8 g ± 0.1 g for WT chimeras and 0.6 g ± 0.1 g for Batf3$^{-/-}$ chimeras (mean ± SD). (C) Amount of adoptively transferred 2C effector T cells migrated into the tumor following intratumoral injection of WT (open) or *Cxcl10*$^{-/-}$ (filled) FLT3-L derived DC into DT-depleted CD11c-DTR bone marrow chimeras on the BP-SIY background (n = 3). The tumor weight at time of analysis was 0.4 g ± 0.05 g for WT DC and 0.38 g ± 0.06 g for *Cxcl10*$^{-/-}$ DC (mean ± SD). (D) Expression level of CXCL9 and CXCL10 in immature (iDC) and FLT3-L matured DC (FLT3-DC) of WT and *Cxcl10*$^{-/-}$ (KO) bone-marrow derived DC, used in the experiment depicted in (C). # indicates samples with expression below detection limits. (E) Number of detected 2C effector T cell per gram of tumor in BP-SIY bone marrow chimeras reconstituted with WT or *Cxcl10*$^{-/-}$ bone marrow (n = 6 /5). BPC-SIY mice engrafted with WT bone marrow were used as control (n = 3). The tumor weight at time of analysis was 0.35 g ± 0.05 g for BP-SIY WT, 0.5 g ± 0.1 g for BP-SIY *Cxcl10*$^{-/-}$ and 0.6g ± 0.05 g for BPC-SIY WT (mean ± SD). Data are shown in mean with SEM, box plots show median with 95th percentile, maximal deviation shown by error bars; significance was assumed with $p \leq 0.05$, with * ≤ 0.05; ** ≤ 0.01.