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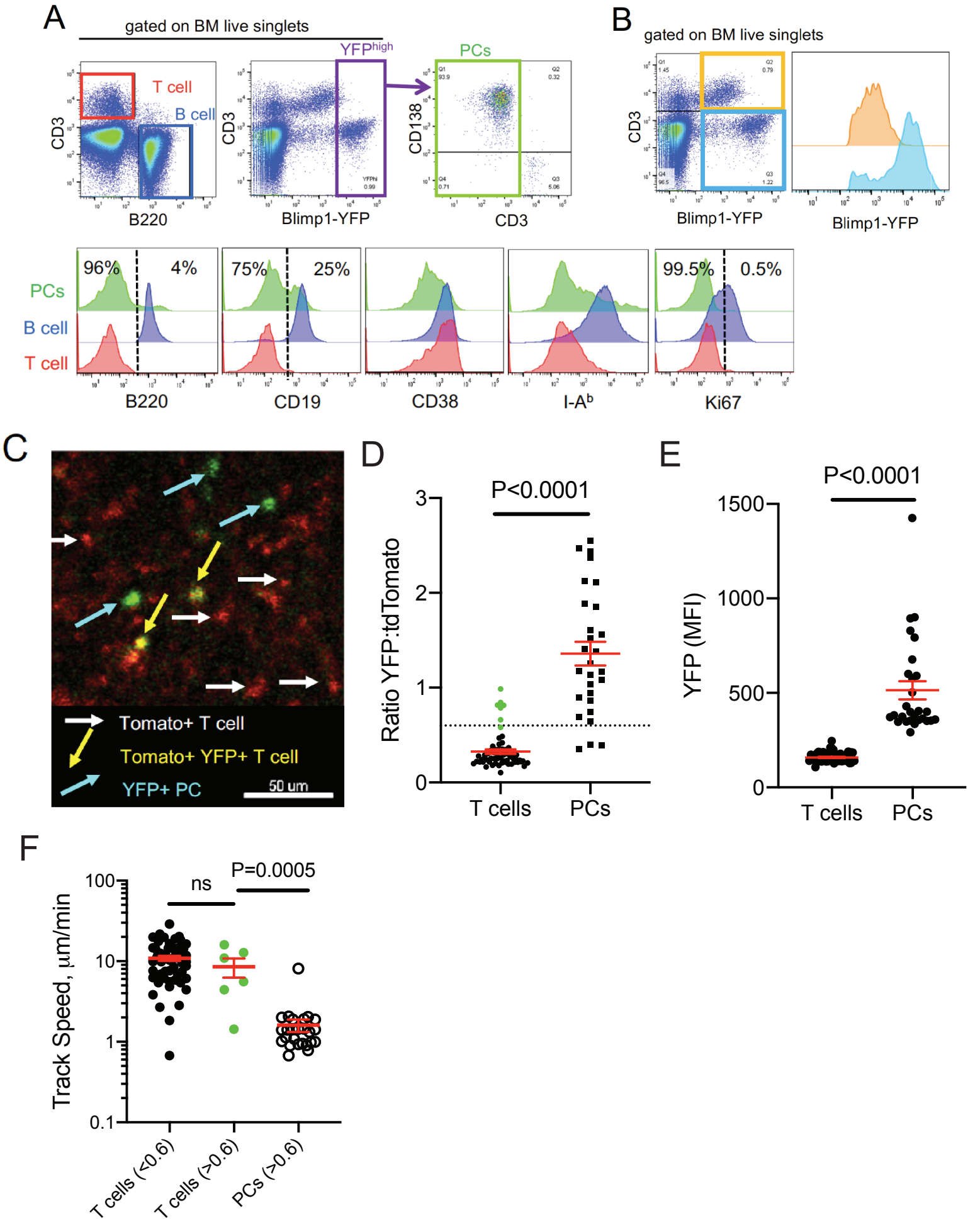
A
gated on BM live singlets
T cell
B cell
CD3
B220
YFPhigh
Blimp1-YFP
PCs
CD138
CD3
B
gated on BM live singlets
CD3
Blimp1-YFP
Blimp1-YFP
PCs
B cell
T cell
96%
4%
75%
25%
99.5%
0.5%
B220
CD19
CD38
I-Ab
Ki67
C
Tomato+ T cell
Tomato+ YFP+ T cell
YFP+ PC
50 um
D
P<0.0001
Ratio YFP:tdTomato
T cells
PCs
E
P<0.0001
YFP (MFI)
T cells
PCs
F
ns
P=0.0005
Track Speed, µm/min
T cells (<0.6)
T cells (>0.6)
PCs (>0.6)

Figure S1 related to Figure 1. Characterization of Blimp1-YFP expression and specificity

(A) Representative flow cytometric analysis of Blimp1-YFP-high purity (purple gate), indicating 94% are CD138+ plasma cells in the BM. To analyze their phenotype, PC cells (green gate) were compared to T cells (CD3+ red gate) and B cells in the BM ($B220^{high}$ blue gate) for surface expression of B220low (96%), $CD19^{low}$ (75%), $CD38^{low}$, I-A^{b}/MHC Class II^{low}, intracellular $Ki\text{-}67^{neg}$ (99%). (B) Blimp1-YFP expression in $CD3^{+}$ ($CD8^{+}$ effector) T cells is ~10-fold lower than YFP^{high} $CD3^{-}$ PCs. (C-F) Intra-vital BM imaging and analysis of PCs and T cells in Blimp-1-YFP^{+} CD4-Cre^{+} $Rosa^{LSL\text{-}tdTomato}$ mice. (C) Example image highlighting Tomato+ T cells (white arrows), Tomato+ YFP+ T cells (yellow arrows), YFP+ only PCs (cyan arrows). (D) Analysis of YFP/Tomato ratio of T cells and PCs. In green, threshold for double positive Tomato+ YFP+ T cells. (E). Analysis of YFP fluorescence of T cells and PCs. (F). Tracking and track speed comparison of T cells and PCs. Each dot is a cell, pooled from 2 independent mice. Comparisons conducted by unpaired Mann-Whitney test. SEM error bars in red.

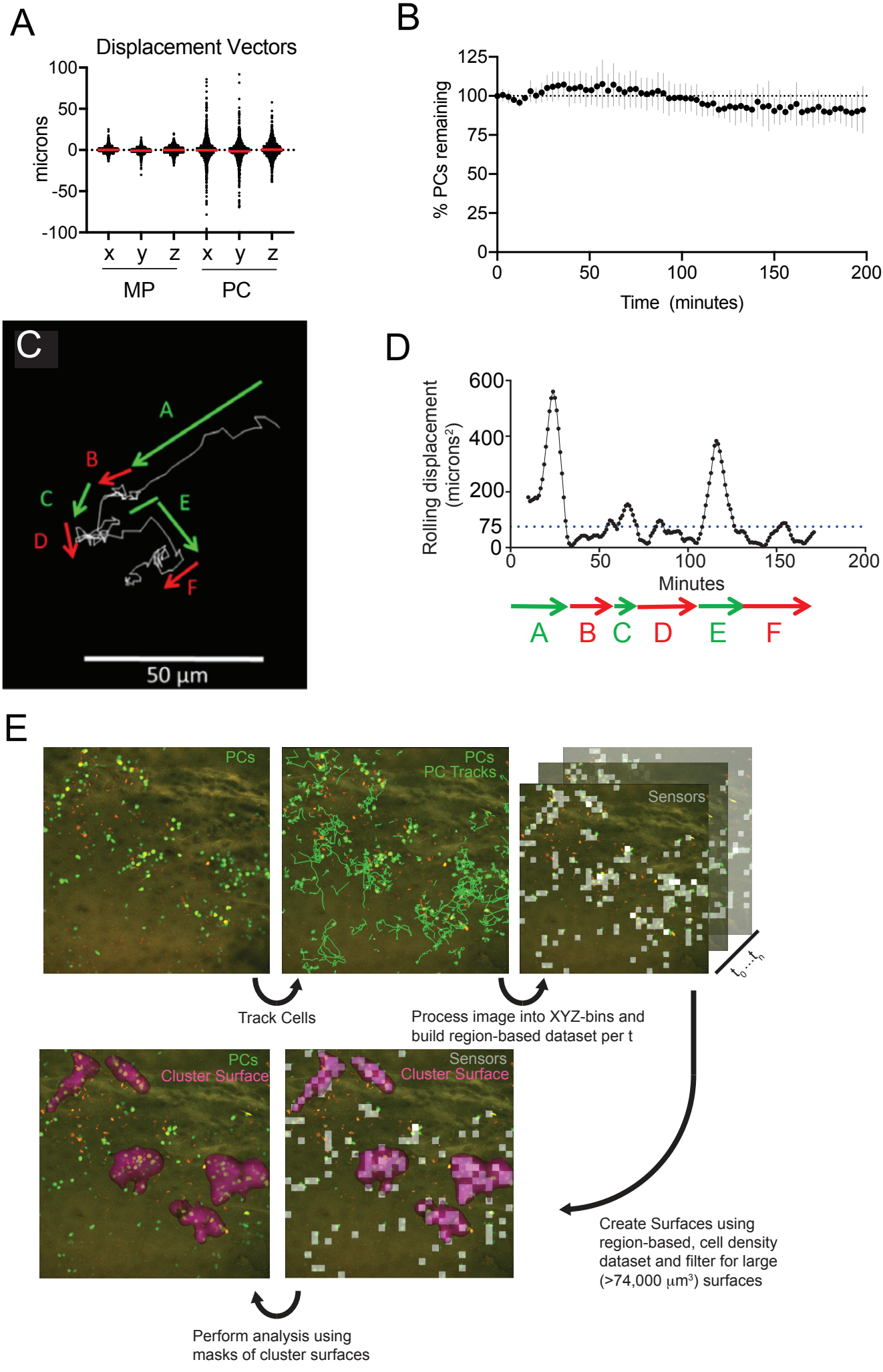
A
Displacement Vectors
microns
100
50
0
-50
-100
x y z x y z
MP PC
B
% PCs remaining
125
100
75
50
25
0
0 50 100 150 200
Time (minutes)
C
A
B
C
D
E
F
50 µm
D
Rolling displacement (microns²)
600
400
200
75
0
0 50 100 150 200
Minutes
A B C D E F
E
PCs
PCs
PC Tracks
Sensors
$t_0 \ldots t_n$
Track Cells
Process image into XYZ-bins and build region-based dataset per t
PCs
Cluster Surface
Sensors
Cluster Surface
Create Surfaces using region-based, cell density dataset and filter for large (>74,000 µm³) surfaces
Perform analysis using masks of cluster surfaces

Figure S2 related to Figures 2-3. Additional analyses of PC movements and clustering

Data in are pooled from 3 movies from three separate mice 16-36-weeks of age. (A) XYZ components of track displacements for macrophages (MPs) and plasma cells (PCs) to assess directionality, with mean in red. (B) Analysis of PC density in BM over time of intra-vital imaging, normalized to starting time PC number per movie, with SEM errors. (C). Sample BM PC track exhibiting periods of both high (green) and low (red) displacements. (D) Analysis of PC track in (C) using 30-minute rolling displacement over time, to segment of high and low MSD (based on a 75 micron^2 cutoff) used in Figure 1. (C) Analysis of all PC tracks with durations longer than 4 hours. (D) Comparison of high and low displacement periods. Data in (B-D) are pooled from movies used in Figure 1I-J. (E) PCs were tracked using Imaris Spot Tracking feature to generate spatial coordinates. Track data and the dimensions of the imaging field were then passed into a custom MATLAB script using ImarisXT. The imaging field was then subdivided into XYZ bins 15x15x15μm (3375 μm^3) in size, called sensors. 3375 μm^3 volumes were chosen because they best approximated a general outline of clusters but did not over fit nor exaggerate what could be discernable by eye. For each *t*, an image was generated wherein the intensity of the pixels within the sensor voxel were converted to the number of PCs present within that particular sensor, yielding a local density of PCs. The sensor image was then reconstructed in Imaris as a new channel. Using the Surface feature of Imaris and the newly generated sensor channel, region-based clusters could be generated based on their local density of PCs using consistent creation parameters (Surface Detail = 15 μm, Threshold = 0.03, Volume >= 74,000 μm^3). Using the generated cluster surface as a mask, PCs could then be segregated into those within and outside of clusters.

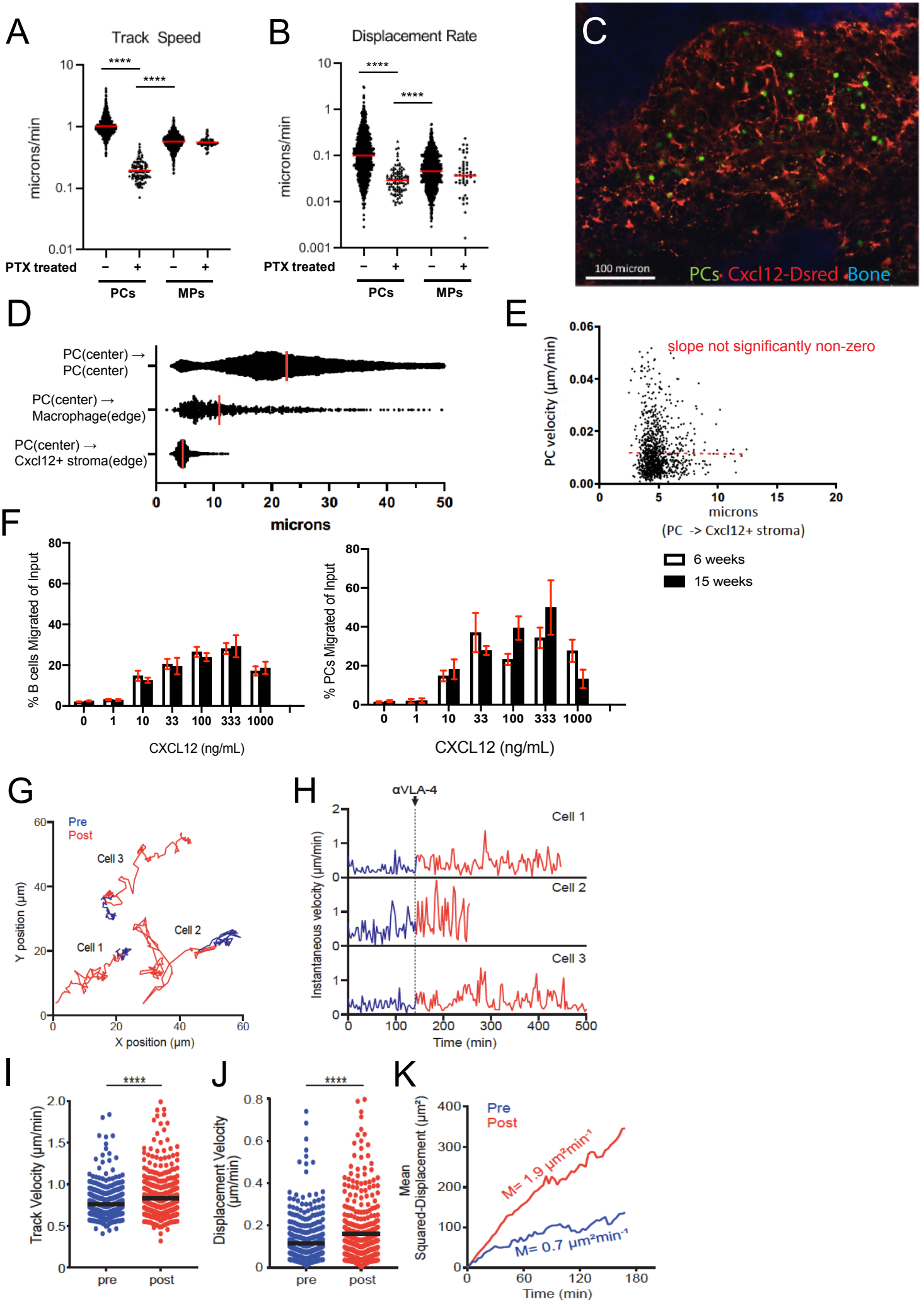
A
Track Speed
microns/min
PTX treated
PCs
MPs
B
Displacement Rate
microns/min
PTX treated
PCs
MPs
C
100 micron
PCs Cxcl12-Dsred Bone
D
PC(center) → PC(center)
PC(center) → Macrophage(edge)
PC(center) → Cxcl12+ stroma(edge)
microns
E
slope not significantly non-zero
PC velocity (µm/min)
microns
(PC -> Cxcl12+ stroma)
6 weeks
15 weeks
F
% B cells Migrated of Input
CXCL12 (ng/mL)
% PCs Migrated of Input
CXCL12 (ng/mL)
G
Pre
Post
Cell 1
Cell 2
Cell 3
Y position (µm)
X position (µm)
H
αVLA-4
Cell 1
Cell 2
Cell 3
Instantaneous velocity (µm/min)
Time (min)
I
Track Velocity (µm/min)
pre
post
J
Displacement Velocity (µm/min)
pre
post
K
Pre
Post
M= 1.9 µm²min⁻¹
M= 0.7 µm²min⁻¹
Mean Squared-Displacement (µm²)
Time (min)

Figure S3, related to Figures 4-6. Additional data on the role of chemokines and integrins on PC motility in the BM

(A-B) Blimp1-YFP reporter mice were treated 16 hrs with pertussis toxin (PTX, 2.5μg i.v.) and imaged PCs and macrophage motility in the BM. (A) Track velocity analyzed. (B) Displacement velocity analyzed. (C-E) Analysis of PC motility vs CXCL12 expressing cells using Blimp1-YFP Cxcl12-dsred reporter mice. (C) Sample image of Blimp1-YFP+ PCs (green) in close contact with dense Cxcl12-dsred+ network. (D) Analysis of PC centroid distances to nearest stromal cell edge (~5 micron), macrophage edge (~10 micron), or nearest PC centroid (~20 micron). (E) Analysis of PC speeds as a function of distance to stromal cell shows no correlation of speed and distance. (F) Titration of CXCL12 concentration, for both B cells and PCs from input BM from 6- and 15-week mice. Experiment is pooled from 2 independent experiments, 6 mice per group pooled. (G-K) Blimp1-YFP mice were injected i.v. with 300 μg anti-alpha4 (clone PS/2) . Cell movement was tracked by intravital imaging of tibia for ~2 hours before (blue) treatment and for ~6 hours immediately after (red) treatment and analyzed pre and post treatment. (G) Examples of movement of 3 sample individual PCs shown by colored trajectories before (blue) and after (red) antibody treatment (H) Instantaneous velocities of Cells in (G) shown pre and post treatment. (I) Track velocity of PCs. (J) Displacement velocity of PCs. (K) Mean square displacement of PCs over time. Slopes indicate the average mean motility coefficient of 3 individual experiments. Data are pooled from 3 individual experiments. All quantification plots show mean. *, $P< 0.05$; **, $P< 0.01$; ***, $P< 0.001$; ****, $P< 0.0001$ by unpaired Mann-Whitney U test. Bars reflect mean, and errors are SEM.

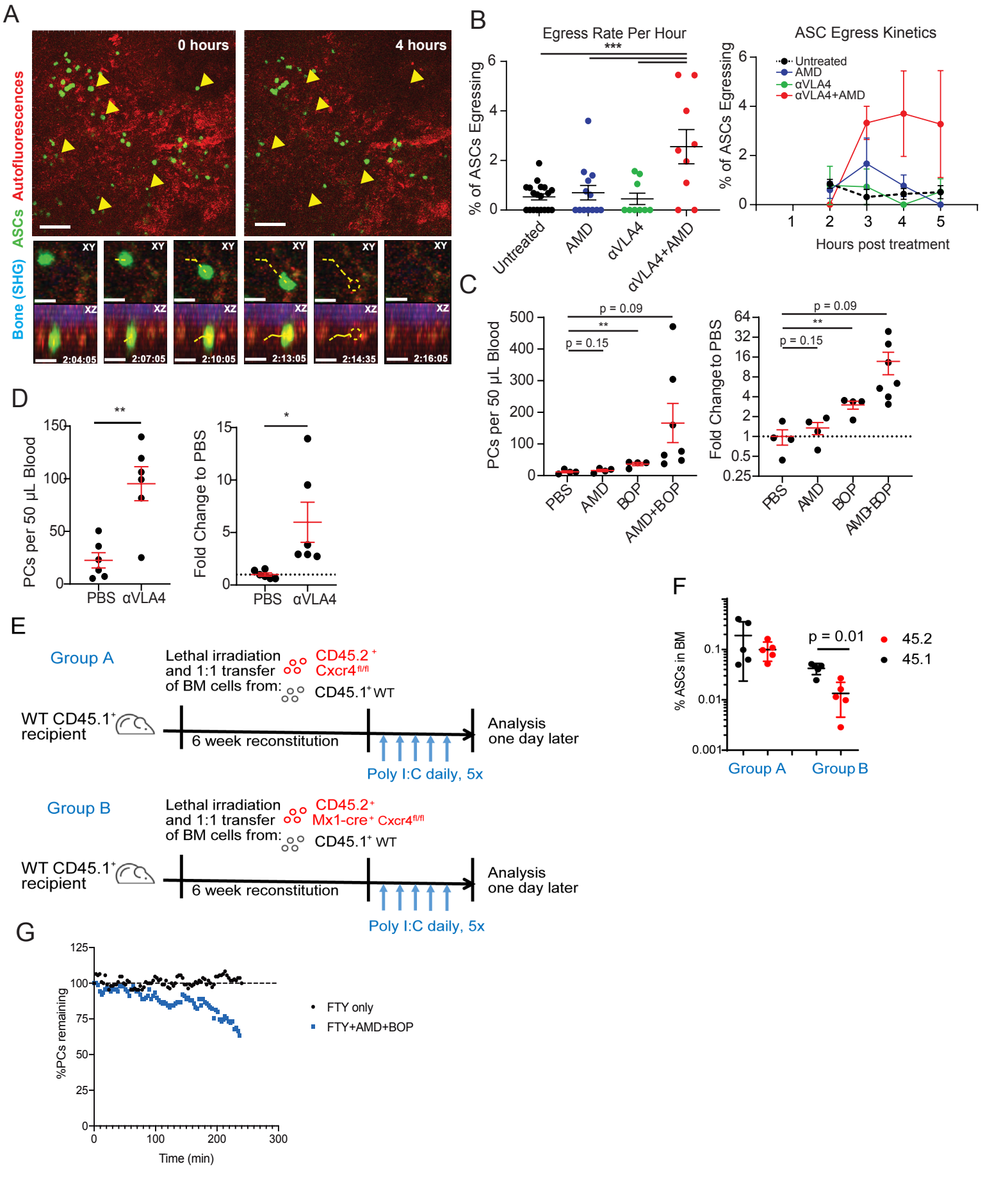
A
0 hours
4 hours
Autofluorescences
ASCs
Bone (SHG)
XY
XZ
2:04:05
2:07:05
2:10:05
2:13:05
2:14:35
2:16:05
B
Egress Rate Per Hour
***
% of ASCs Egressing
Untreated
AMD
αVLA4
αVLA4+AMD
ASC Egress Kinetics
Hours post treatment
C
p = 0.09
**
p = 0.15
PCs per 50 µL Blood
Fold Change to PBS
PBS
AMD
BOP
AMD+BOP
D
PCs per 50 µL Blood
Fold Change to PBS
PBS
αVLA4
E
Group A
Lethal irradiation and 1:1 transfer of BM cells from:
CD45.2+ Cxcr4fl/fl
CD45.1+ WT
WT CD45.1+ recipient
6 week reconstitution
Analysis one day later
Poly I:C daily, 5x
Group B
Lethal irradiation and 1:1 transfer of BM cells from:
CD45.2+ Mx1-cre+ Cxcr4fl/fl
CD45.1+ WT
WT CD45.1+ recipient
6 week reconstitution
Analysis one day later
Poly I:C daily, 5x
F
p = 0.01
% ASCs in BM
45.2
45.1
Group A
Group B
G
%PCs remaining
Time (min)
FTY only
FTY+AMD+BOP

Figure S4. Additional data analyzing of BM PC mobilization related to Figure 7

(A) Representative 3D image volume at the start and 4 hours post treatment with egressed cells labeled (yellow arrows) (top, scale bar = 50 μm) and representative images in XY and XZ planes of an egressing cells (bottom, scale bar = 10 μm). (B) Percent of total PCs egressing per hour (left) and kinetics of PC egress following treatment (right) (2-4 mice per treatment, 9-19 total hours per treatment). (C) Number of PCs (left) in the blood of C57BL/6 mice treated with PBS, 80 μg AMD3100, 250μg BOP, or both AMD3100 and BOP for 24 hours and their fold change normalized to PBS (right). (N = 4-7 mice/group, 1 experiment). (D) Number of PCs (left) in the blood of C57BL/6 mice treated with PBS or 300 μg of anti-VLA4 antibody for 24 hours and their fold change normalized to PBS (right) (N = 3 mice/group, 2 experiments). (E) Experimental schema. Mixed chimeric mice (Group B) were generated by lethally irradiating WT (CD45.1) mice and reconstituting with 50:50 mix of BM cells from w Mx1-cre+ $Cxcr4^{fl/fl}$ CD45.2 mice and control CD45.1 WT mice. As a control for treatment, a second cohort (Group A) of mice was made with $Cxcr4^{fl/fl}$ CD45.2 and control CD45.1 WT mice. To trigger cre-recombination in Mx1-cre+ cells, mice were treated with PolyI:C for 5 days and then composition of BM was analyzed one day later. (F) Cxcr4-deleted PCs (CD45.2 group A) were reduced 4-fold following treatment compared to controls. Data is representative from one of two independent experiments. (G) Analysis of number of PCs in field over time by intra-vital imaging, normalized to start prior to treatment with FTY720, or AMD+BOP+FTY720 as in Figure 7B. All bars represent mean and error is SEM. *, $P < 0.05$. **, $P < 0.005$. ***, $P < 0.001$ using Mann-Whitney U test.

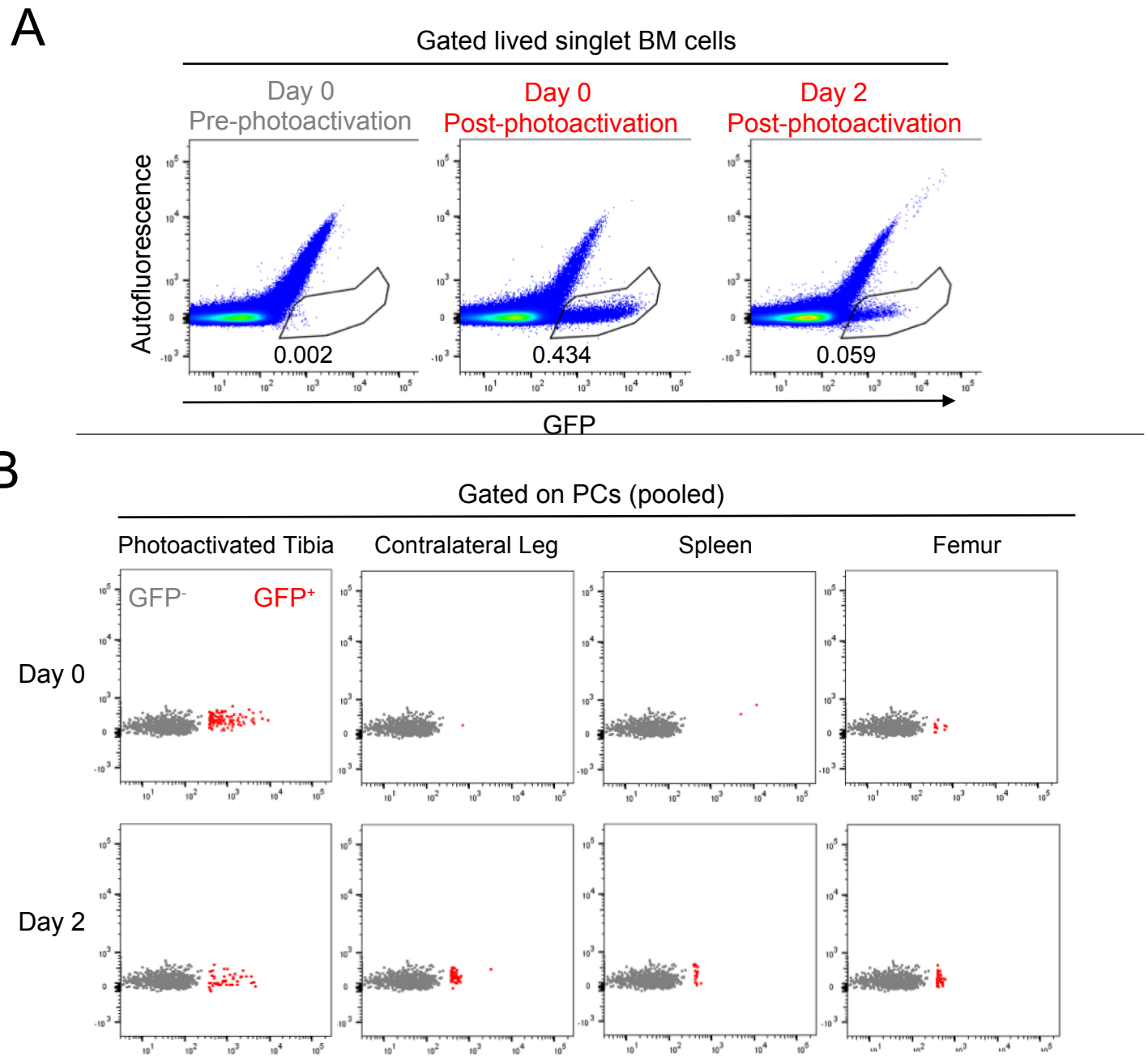
A
Gated lived singlet BM cells
Day 0
Pre-photoactivation
Day 0
Post-photoactivation
Day 2
Post-photoactivation
Autofluorescence
0.002
0.434
0.059
GFP
B
Gated on PCs (pooled)
Photoactivated Tibia
Contralateral Leg
Spleen
Femur
GFP-
GFP+
Day 0
Day 2
C
GFP+ PCs (cell counts)
300
200
100
0
p=0.15
**
p=0.22
p=0.11
0 2 0 2 0 2 0 2
days post photoactivation
Photoactivated Tibia
Contralateral leg
Femur
Spleen

Figure S5. Intra-tibial Photoactivation and tracking PC recirculation related to Figure 7

PA-GFP mice were exposed for BM imaging, photoactivated with 840nm light, and then analyzed immediately or sutured and analyzed 48hours later for GFP+ PC distribution. (A) FACS analysis of BM cells prior to or immediately following photoactivation, with GFP+ (activated) gate shown with frequencies. (B) Dot plots of PCs (CD138high B220low) cells on day 0 or day 2 pooled from 3 mice per group. GFP- PCs gate shown grey for reference, and GFP+ PCs (red) shown in photoactivated tibia, contralateral leg bones (femur+tibia), spleen, and ipsilateral femur. (C). Analysis and comparison of total GFP+ PCs per tissue on day 0 and day 2, each dot reflects 1 mouse, pooled from 2 independent experiments. Analyzed by Mann-Whitney U-test. All bars represent mean and error is SEM.