

## Cell Article

## **Supplemental figures**

A CD169<sup>Tom</sup> mice



CD3 CD169<sup>Tom</sup> CD169

B CX3CR1<sup>Gfp</sup> mice





C CD68<sup>Gfp</sup> mice











Figure S1. Reporter systems for tracking TBMs in vivo, related to Figure 1

<sup>(</sup>A) Mosaic-tiled image of a representative lymph node section from a CD169<sup>Tom</sup> mouse stained with CD3 (green) and CD169 (blue). IF, interfollicular zone. (B) GFP expression by GC TBMs in CX3CR1<sup>Gfp</sup> mice co-stained with IgD (red), CD68 (antibody, magenta), and CD21/35 (yellow). Confocal analysis of inguinal lymph nodes on day 7 post CGG/AddaSO3 s.c. immunization.

<sup>(</sup>C) GFP expression by GC TBMs in CD68<sup>Gfp</sup> mice co-stained with IgD (red) and CD21/35 (yellow). Confocal analysis of inguinal lymph nodes on day 7 post CGG/ AddaSO3 s.c. immunization.

<sup>(</sup>D) FACS analysis of CD169<sup>Tom</sup> mice for expression of MERTK, CD169, CD11b, F4/80, and CX3CR1.

<sup>(</sup>E) Confocal analysis of a CD169<sup>Tom</sup> lymph node on day 9 post immunization with HEL-OVA showing TOM+ cells inside GC contain CD68<sup>+</sup> vacuoles.

<sup>(</sup>F) Numbers of GFP + TBMs in GCs from CX3CR1<sup>Gfp/wt</sup> (heterozygous reporter) and CX3CR1<sup>Gfp/Gfp</sup> (homozygous knock-out) mice. Quantitation from immunofluorescence microscopy of inguinal lymph nodes. Data from one experiment, three mice per group.

<sup>(</sup>G) Volume of fragments from intravital microscopy and image analysis using the surfaces feature in Imaris.







## Figure S2. Isolation of CD169-lineage cells for CITE-seq, related to Figure 4

δµm

(A) FACS sorting gates for CITE-seq experiment. CD169<sup>Tom</sup>CD11b<sup>+</sup> DAPI<sup>-</sup> cells were sorted (red gate) from both resting and immunized mice.

(B) Cophenetic correlation coefficient scores from 50 iterations of NMF at ranks 2 through to 14.
(C) FACS analysis of day 12 immunized lymph nodes from CD169<sup>Tom</sup> mice for CD206 and CX3CR1 expression on TOM<sup>+</sup>CD11b<sup>+</sup>CD169<sup>low</sup> and

(C) FACS analysis of day 12 immunized lymph nodes from CD169<sup>1011</sup> mice for CD206 and CX3CR1 expression on TOM\*CD11b\*CD169<sup>1011</sup> and TOM\*CD11b\*CD169<sup>1011</sup> mice for CD206 and CX3CR1 expression on TOM\*CD11b\*CD169<sup>1011</sup> and

EdU

Neg. example 3

Pos. example 4

(D) Confocal microscopy showing expression of CD206 in TOM+ cells in medulla and GC of  $CD169^{Tom}$  mice.

(E) Confocal images of a GC and zoomed TBMs, where TBM nuclei were marked as H2B-mCherry+ in CX3CR1-CreERT2, Rosa\_LSL\_H2b-mCherry, CD68-Gfp mice. EdU treatments were provided continuously from day 1 post immunization with GCC/AddSO3, with harvest on day 11.







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Figure S3. Mathematical modeling of TBM and apoptotic cell fragment dynamics, related to Figure 3

(A) Histogram (top-left), Q-Q plot (top-right), CDF plot (bottom-left), and P-P plot (bottom-right) of observed fragment speeds compared to lognormal fit (red, mu = 1.17, delta = 0.335).

(B) CDF plot of observed fragment speeds compared to simulated fragment speeds.

(C) Histogram (top-left), Q-Q plot (top-right), CDF plot (bottom-left), and P-P plot (bottom-right) of observed turn angles compared to a beta fit (red, alpha = 2.02, beta = 1.707).

(D) Histogram (top-left), Q-Q plot (top-right), CDF plot (bottom-left), and P-P plot (bottom-right) of observed meandering index (MI) compared to a lognormal fit (red, mu = -3, delta = 1.5).

(E) CDF plot of observed fragment MI compared to simulated MI.



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## Figure S4. Origin of TBMs, related to Figure 5

(A) FACS plot of sham and photoconverted CD169<sup>Kik</sup> inguinal resting lymph nodes showing unphotoconverted KikumeGreen vs. photoconverted KikumeRed for CD11b<sup>+</sup> single cells.

(B) Quantification of the number of TBMs per GC that were photoconverted or not photoconverted at day 7 post-HEL-OVA immunization. n = number of GCs; data are representative of 3 independent experiments.

Whole-body irradiation experiment

(C) Wildtype non-fluorescent mice were lethally irradiated and reconstituted with CD68<sup>Gfp</sup> bone marrow. Mice were immunized s.c. with CGG/AddaSO3 >8 weeks later. Confocal image of a GC, showing follicular mantle (IgD, blue), follicular dendritic cell network (CD21/35, gray), macrophages (CD68 antibody, red), and donor-derived GFP (green).

(D) Quantitation of (C) the proportion of CD68<sup>+</sup> (antibody) GC TBMs that were CD68-GFP+. Data points are individual GCs from 11 mice and 2 independent experiments. Mean (+/- S.E.M.).

Stable labeling of tissue-resident macrophages

(E) Schematic for fate mapping the contribution to GC TBMs of cells that were stably expressing CX3CR1 prior to immunization. Immunization, CGG/AddaSO3. (F) Quantification of blood monocytes and inflammatory blood monocytes (Ly6C+) at the indicated time points post-tamoxifen.

(G) Confocal image of a non-immunized naive follicle (Day 20) and post-immunization GC (Day 27), showing follicular mantle (IgD, blue), follicular dendritic cell network (CD21/35, mustard), macrophages (CD68 antibody, green), and tdTomato (red).

Contribution of circulating monocytes

(H) Related to Figure 5E. Quantification of blood monocytes and inflammatory blood monocytes (Ly6C+) at the indicated time-points post-tamoxifen. Shielded irradiation experiment

(I) CD68<sup>Gfp</sup> mice received lethal whole-body X-ray irradiation. Individual inguinal lymph nodes from opposite body sides were left exposed (non-shielded) or lead shielded. Mice were subsequently reconstituted with WT non-fluorescent bone marrow. 8–10 weeks later, mice were immunized s.c. with GCC/AddaS03, with analysis on day 8. GC TBMs were identified by antibody staining for CD68 (magenta), IgD (red, follicular mantle), and CD21/35 (mustard, FDCs). GFP expression by TBMs was assessed.

(J) Quantitation of the frequencies of CD68<sup>+</sup> (antibody) GC TBMs that were GFP+ in non-shielded or shielded lymph nodes. Data points are individual GCs, pooled from 5 mice and 2 independent experiments. INSERT NEW LINE

CSF1R blockade

(K) Schematic for anti-CSF1R experiment to deplete resident SSMs and MSMs prior to and during HEL-OVA immunization.

(L) Quantification of CD169<sup>Tom</sup>CD11b<sup>+</sup>CD169<sup>+</sup>F4/80<sup>-</sup> SSM (left) and CD169<sup>Tom</sup>CD11b<sup>+</sup>CD169<sup>+</sup>F4/80<sup>+</sup> MSMs (right) from mice treated with IgG2a or anti-CSF1R m-Ab. n = number of individual mice.

(M) Quantification of number of TBMs per GC in day 8 response lymph nodes of CD169<sup>Tom</sup> mice treated with IgG2a isotype control antibody (left) or anti-CSF1R blocking antibody (right). n = number of GCs; data are representative of 3 independent experiments.