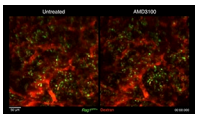
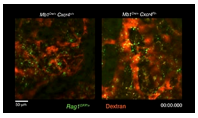
SUPPLEMENTAL MATERIAL

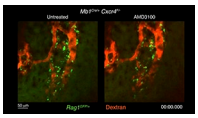
Beck et al., http://www.jem.org/cgi/content/full/jem.20140457/



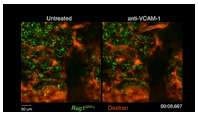
Video 1. Intravital imaging of developing B cell migration before and after treatment with AMD3100 (25 min). Time-lapse image sequences (39-µm-thick z stack) of BM calvaria showing developing B cells (GFP+, green). BM vasculature (red) is labeled using tetramethylrhodamine-conjugated dextran (molecular mass 2,000 kD) injected i.v. (left) Parenchymal immature B cells move in an amoeboid random walk fashion. (right) ∼5 min after treatment with 80 µg AMD3100, acute arrests of random walk of parenchymal immature B cells as well as a cell shape change from an amoeboid morphology to rounder cell morphology are shown. White boxes indicate regions of interest selected for higher magnification visualizations. Elapsed time is shown as hh:mm:ss. Data are representative of more than five independent experiments.



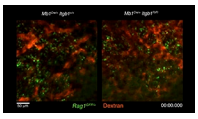
Video 2. Intravital imaging of *Cxcr4*–deficient developing B cell migration (30 min). Time-lapse image sequences (left, 39-µm; right, 51-µm z stack) of BM calvaria showing control developing B cells (GFP+, green). BM vasculature (red) is labeled using tetramethylrhodamine-conjugated dextran injected i.v. (left) WT developing B cells move in parenchyma in an amoeboid random walk fashion. (right) *Cxcr4*-deficient developing B cell random walk is slowed down and proximal to sinusoids, and cells have a more rounded morphology than control developing B cells. White boxes indicate regions of interest selected for higher magnification visualizations. Elapsed time is shown as hh:mm:ss. Data are representative of three independent experiments.



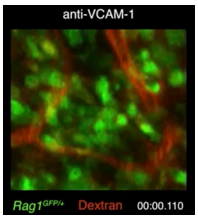
Video 3. Intravital imaging of *Cxcr4*–deficient developing B cell migration before and after treatment with AMD3100 (30 min). Time-lapse image sequences (51-µm-thick z stack) of BM calvaria showing *Cxcr4*-deficient developing B cells (GFP+, green) before (left) and after (right) treatment with i.v. injection of 80 µg AMD3100. BM vasculature (red) is labeled using tetramethylrhodamine-conjugated dextran injected i.v. (right) ∼5 min after treatment with AMD3100, *Cxcr4*-deficient parenchymal immature B cell random walk is further arrested. White boxes indicate regions of interest selected for higher magnification visualizations. Elapsed time is shown as hh:mm:ss. Data are representative of three independent experiments.



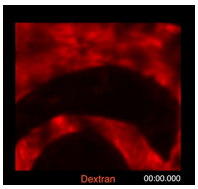
Video 4. Intravital imaging of developing B cell migration before and after treatment with anti–VCAM-1 (30 min). Time-lapse image sequences (51-µm-thick z stack) of BM calvaria showing developing B cells (GFP+, green). BM vasculature (red) is labeled using tetramethylrhodamine-conjugated dextran injected i.v. (left) Parenchymal developing B cells move in an amoeboid random walk fashion. (right) 30 min after treatment with anti–VCAM-1 (M17/4 200 µg), developing B cell random walk is arrested and a cell shape change from an amoeboid morphology to rounder cell morphology is seen. White boxes indicate regions of interest selected for higher magnification visualizations. Elapsed time is shown as hh:mm:ss. Data are representative of three independent experiments.



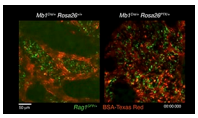
Video 5. Intravital imaging of *Itgb1*–deficient developing B cell migration (30 min). Time-lapse (30 min) image sequences (left, 39-µm; right, 42-µm z stack) of BM calvaria showing developing B cells (GFP+, green). BM vasculature (red) is labeled using tetramethylrhodamine-conjugated dextran injected i.v. (left) WT parenchymal immature B cells move in amoeboid random walk fashion. (right) *Itgb1*-deficient parenchymal B lineage cell random walk is slowed down and has rounder cell morphology than control parenchymal B lineage cells. White boxes indicate regions of interest selected for higher magnification visualizations. Elapsed time is shown as hh:mm:ss. Data are representative of three independent experiments.



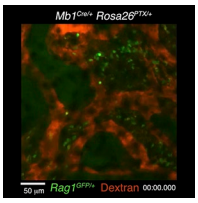
Video 6. Intravital imaging of developing B cell migration before and after treatment with anti–VCAM-1 (30 min). Time-lapse image sequences (51-µm-thick z stack) of BM calvaria showing developing B cells (GFP+, green). BM vasculature (red) is labeled using tetramethylrhodamine-conjugated dextran injected i.v. After treatment with anti–VCAM-1 (M17/4), we zoomed in on an example of a rounded, nonamoeboid, developing B cell moving in the parenchyma for a period of 5 min, as noted by the white track. Elapsed time is shown as hh:mm:ss.



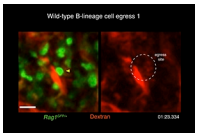
Video 7. Intravital imaging of FITC-conjugated dextran perfusion into the BM parenchyma (29 min). Time-lapse image sequence (42-µm-thick z stack) of BM calvaria showing perfusion of FITC-conjugated dextran (molecular mass 500 kDa) during a period of 29 min into the parenchyma. Elapsed time is shown as hh:mm:ss.



Video 8. Intravital imaging of PTX-expressing B lineage cell migration in BM (30 min). Time-lapse image sequences (39-µm-thick z stack) of BM calvaria showing developing B cells (GFP+, green). BM vasculature (red) is labeled using BSA–Texas red injected i.v. (left) WT parenchymal developing B cells move in an amoeboid random walk fashion. (right) PTX parenchymal developing B cell random walk is arrested and has rounder cell morphology than control parenchymal developing B cells. White boxes indicate regions of interest selected for higher magnification visualizations. Elapsed time is shown as hh:mm:ss. Data are representative of four independent experiments.



Video 9. Intravital imaging of motile PTX-expressing B lineage cell migration in BM (30 min). Time-lapse image sequence (39-µm-thick z stack) of BM calvaria showing developing B cells (GFP+, green). BM vasculature (red) is labeled using tetramethylrhodamine-conjugated dextran injected i.v. Video is representative of 2 out of 13 areas imaged from four independent mice in which we observed a small fraction of motile developing B cells. Elapsed time is shown as hh:mm:ss.



Video 10. Intravital imaging of WT and PTX-expressing B lineage cell egress from BM. Time-lapse image sequences of BM calvaria of *Rag1*$^{GFP/+}$ mice (first two examples) and of *Mb1*$^{Cre/+}$ *Rosa26*$^{PTX/+}$ mice showing developing B cells exiting into BM sinusoids (GFP+, green). BM vasculature (red) is labeled using tetramethylrhodamine-conjugated dextran injected i.v. The yellow arrowhead indicates a cell of interest. Elapsed time is shown as mm:ss:ms. Bar, 15 µm.