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Supplemental Information

Flow Dynamics and HSPC Homing in Bone Marrow Microvessels

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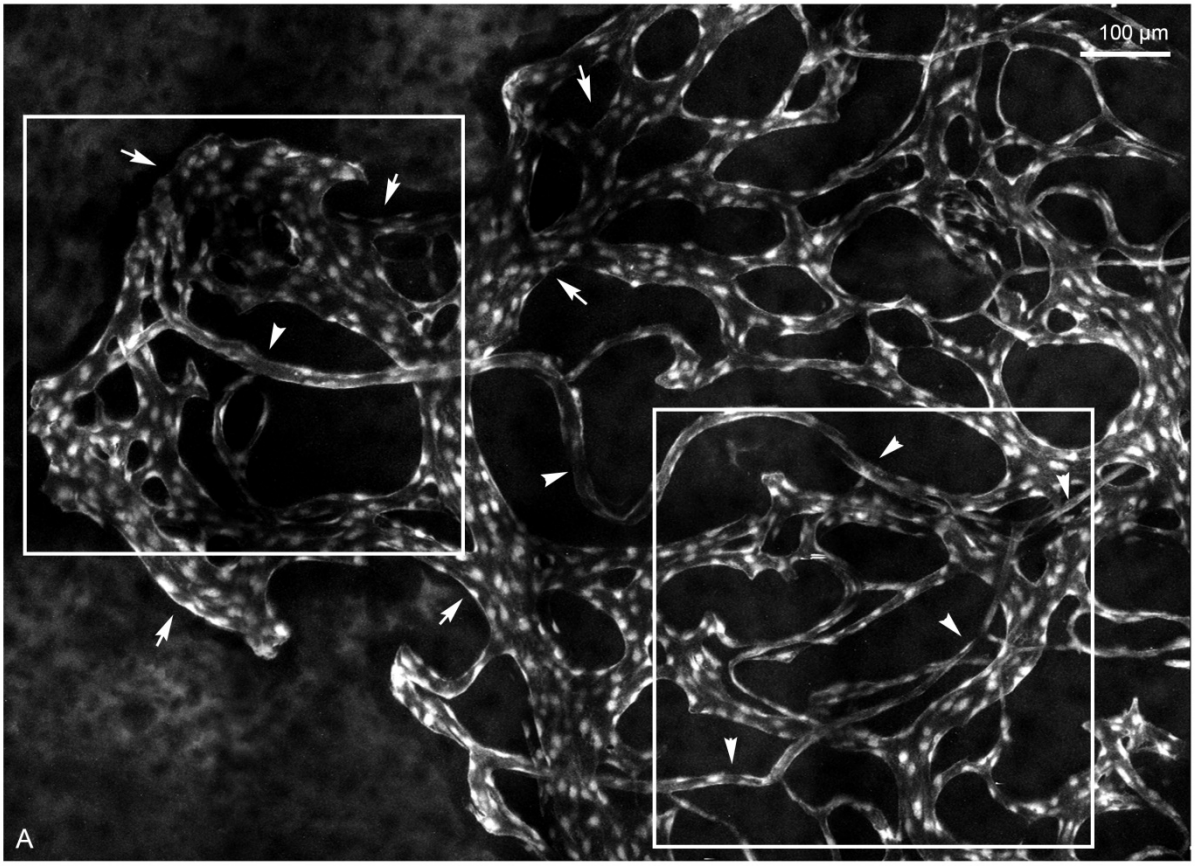
Supplementary Figures



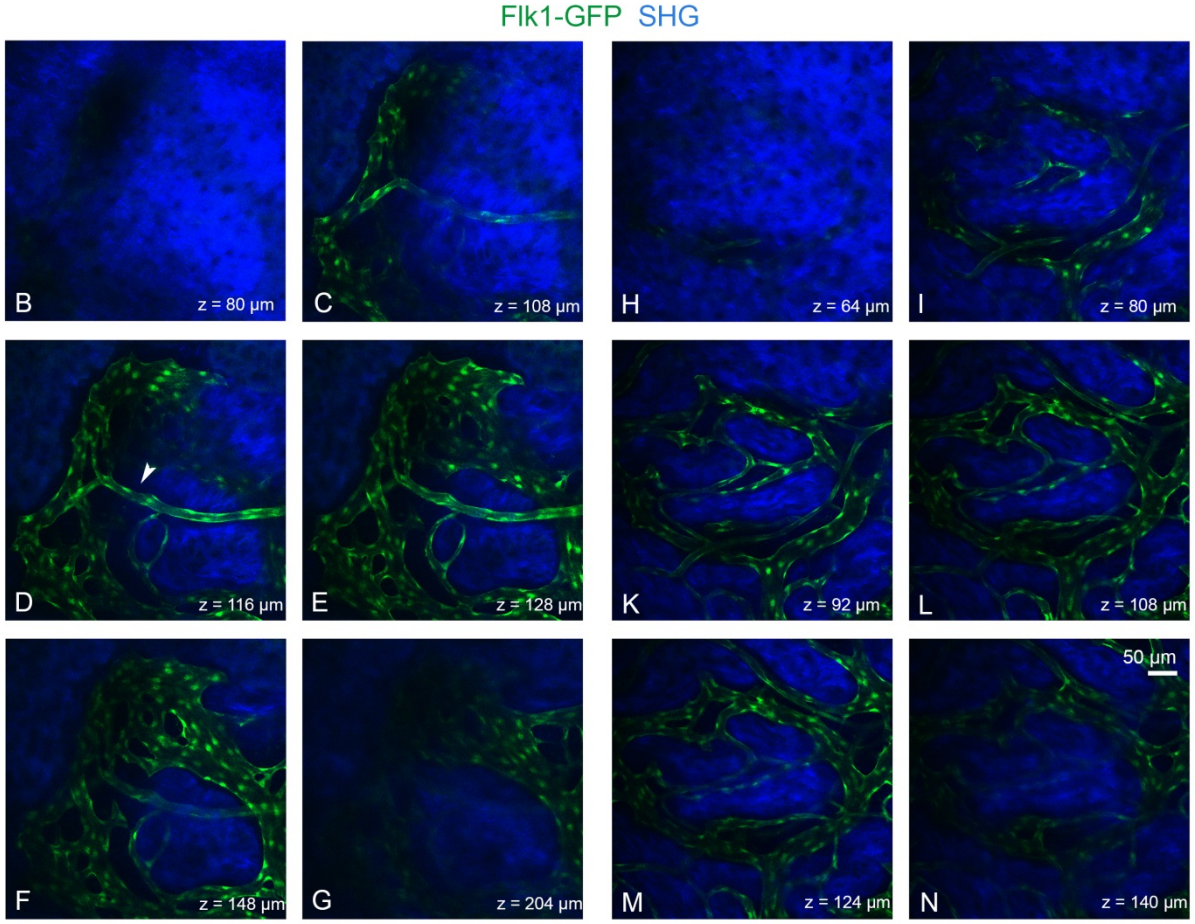


Figure S1. Microvascular architecture of the BM compartment, related to Figure 1 and 2.

(**A**) Maximum intensity projection of tile scans showing the microarchitecture of GFP^+ (white) arterial vessels (arrow heads) connecting to a network of irregularly shaped GFP^+ sinusoids (arrows) in *Flk1-GFP* transgenic mouse calvarium (frontoparietal bone). (**B-G, H-N**) Two representative areas of **A** (boxed regions) with individual z-planes showing GFP^+ (green) blood vessels and SHG^+ (blue) bone tissue. GFP^+ blood vessels are in close proximity to the endosteal surface. Note the interconnection of an arterial vessel (arrowhead) to a terminal network of sinusoidal capillaries (**D**).

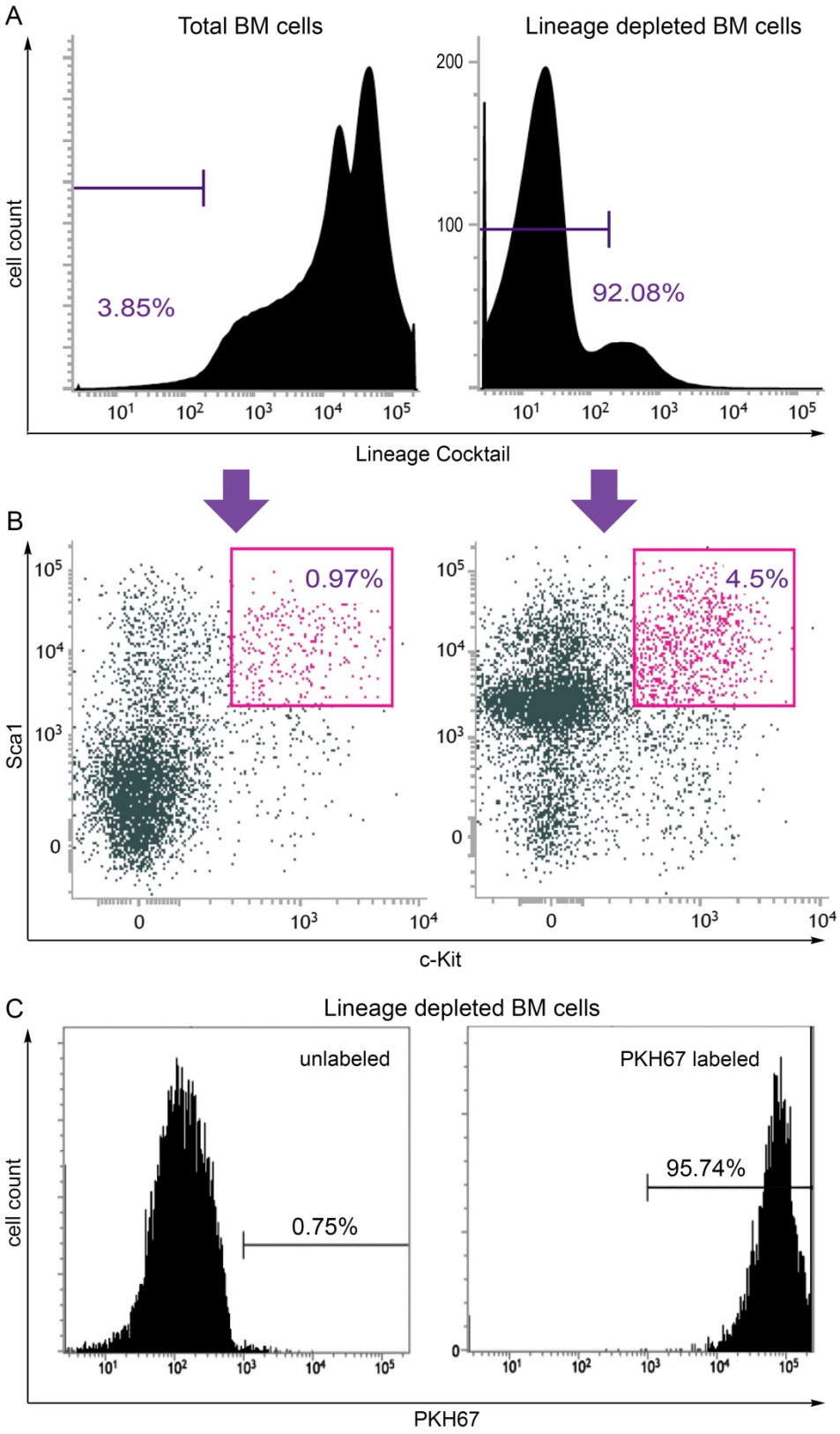


Figure S2. **PKH67-labeled lineage-depleted HSPCs enriched from total BM cells, related to Figure 6.**

(**A**) Representative histogram plots showing flow cytometric quantitation of BM cells before (left) and after (right) lineage depletion using MACS based cell sorting and staining with lineage antibodies.
(**B**) Representative dot plots showing flow cytometric quantitation of c-Kit^{+}/Sca1^{+} HSPCs before (left) and after (right) lineage depletion of BM cells. Note the 4.5-fold enrichment of HSPCs after lineage depletion.
(**C**) Lineage depleted BM cells before (left) and after (right) PKH67-labeling.

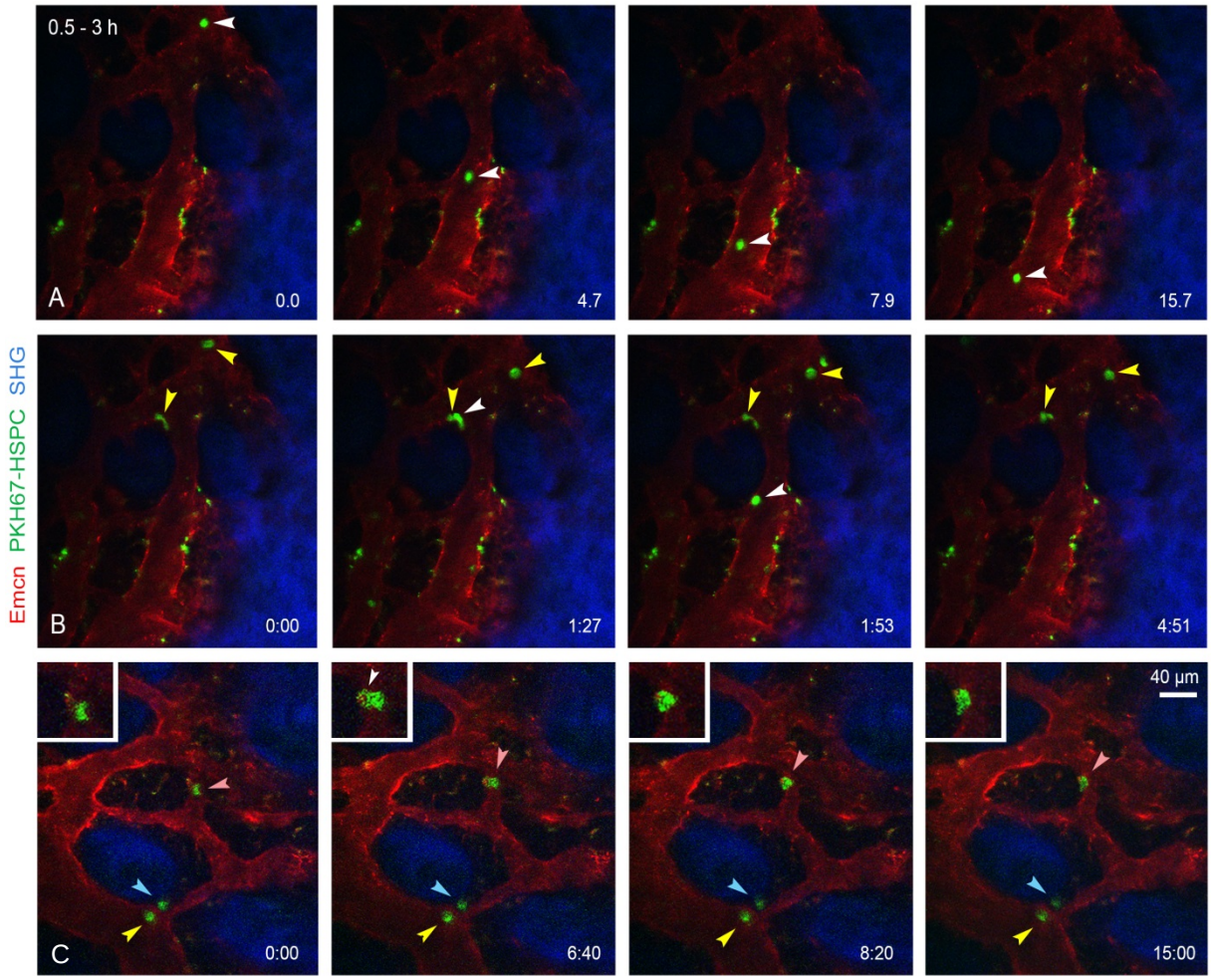


Figure S3. HSPC rolling and adhesion in sinusoidal capillaries, related to Figure 6.

(**A**) Fluorescently labeled BM lineage-depleted HSPCs (green) were intravenously injected and their homing behavior in Enmc^{+} BM sinusoids (red) was analyzed using in vivo two-photon imaging. SHG signals (blue) indicate calcified bone tissue. Representative HSPC showing rolling and transient adhesion in Enmc^{+} BM sinusoidal capillaries, time in sec (**Movie S6**). Arrow heads follow a rolling HSPC which is found at early time points (preferentially after 0.5 - 1.5 hr). (**B**) HSPCs adhere at later time points (preferentially after 1 -3 hr) and show a slow migration behavior, time in min. Yellow arrow heads follow adhering and slowly migrating HSPCs. (**Movie S8**).White arrow heads show a HSPC rolling on Enmc^{+} sinusoids. (**C**) Luminal adhering HSPC (red arrow head) forming a protrusion (white arrow head) across the endothelium (from a lower imaging plane reaching to a higher plane to the left) and translocating its cell body through the vessel wall of the sinusoid. Blue arrow heads point to an already transmigrated HSPC close to a sinusoid. Yellow arrow heads show a luminal adhering HSPC.

Supplementary Table

Table S1. Hemodynamic parameters of a terminal BM compartment, related to Figure 5.

segment #	diameter [µm]	velocity [mm/s]	volume flux [pl/s]	RBC density [%]	RBC flux [pL/s]	viscosity [cP]	shear rate [1/s]	shear stress [dyn/cm^2]
1	6.9	1.29 ± 0.19	24.17 ± 3.52	68	16.44 ± 2.39	3.2	1496 ± 220	48.5 ± 7.2
2	7.6	1.53 ± 0.14	34.70 ± 3.13	72	24.98 ± 2.25	3.4	1611 ± 147	54.1 ± 5.0
3	9.4	0.79 ± 0.14	27.48 ± 4.75	78	21.98 ± 3.70	3.5	672 ± 119	23.9 ± 4.2
4	10.9	0.44 ± 0.07	20.60 ± 3.22	74	15.24 ± 2.38	3.4	323 ± 51	11.0 ± 1.8
5	19.3	0.12 ± 0.01	17.66 ± 1.19	64	11.30 ± 0.76	3.1	50 ± 4	1.56 ± 0.13
6	39.4	0.07 ± 0.01	41.13 ± 3.79	56	23.03 ± 2.12	2.9	14 ± 2	0.41 ± 0.06
7	31.5	0.07 ± 0.00	25.68 ± 1.39	52	13.35 ± 0.72	2.8	18 ± 1	0.50 ± 0.03
8	20.0	0.26 ± 0.02	41.58 ± 3.85	70	29.11 ± 2.69	3.3	104 ± 8	3.44 ± 0.26
9	19.3	0.2 ± 0.01	29.48 ± 2.15	53	15.62 ± 1.14	2.8	83 ± 4	2.32 ± 0.12
10	11.7	0.08 ± 0.02	4.36 ± 0.99	13	0.57 ± 0.13	1.6	55 ± 14	0.88 ± 0.22
11	11.6	0.12 ± 0.03	6.38 ± 1.32	15	0.96 ± 0.20	1.7	83 ± 21	1.37 ± 0.34
12	12.6	0.08 ± 0.01	4.91 ± 0.80	53	2.6 ± 0.43	2.8	51 ± 6	1.42 ± 0.18
13	19.1	0.13 ± 0.01	19.05 ± 4.71	18	3.43 ± 0.29	1.7	54 ± 4	0.95 ± 0.07
14	9.3	0.14 ± 0.01	4.71 ± 0.48	55	2.59 ± 0.27	2.9	120 ± 9	3.43 ± 0.25
15	16.3	0.16 ± 0.02	16.61 ± 2.04	38	6.31 ± 0.77	2.3	79 ± 10	1.84 ± 0.23
16	8.5	0.25 ± 0.05	6.99 ± 1.36	15	1.05 ± 0.20	1.7	235 ± 47	3.88 ± 0.78

Representative blood flow velocities, vessel dimensions, RBC densities, shear rates and wall shear stress of an arterial BM vessel, post-arterial and intermediate capillaries and downstream sinusoids shown in Figure 5. Bars represent mean values ± SD from one representative animal, 11-16 vessels segments per animal, n=4 animals.

Supplementary Movies

Movie S1. Microarchitecture of bone and BM vessels, related to Figure 1.

3D representation of bone vessels in the outer compact bone, sinusoidal vessels in the bone marrow cavities with trabecular bone and inner compact bone with few bone vessels. GFP^+, vasculature; SHG, bone tissue; step size z=4 µm.

Movie S2. Blood flow dynamics through an arterial vessel, related to Figure 3.

Blood flowing through an arterial vessel in the BM compartment visualized by two-photon intravital imaging. The blood serum (red) was labeled by intravenous injection with TexasRed-dextran (**Figure 3B**). Note that tightly packed erythrocyte staples move regularly through the vessel segment. Occasionally the packing is less dense leaving a small gap between neighboring staples.

Movie S3. Blood flow dynamics through an early sinusoidal capillary, related to Figure 3.

Blood flow through an early sinusoidal capillary in the BM compartment visualized by two-photon intravital imaging. The blood serum (red) was labeled by intravenous injection with TexasRed-dextran (**Figure 3D**). Note the irregular flow pattern of erythrocytes in the curved vessels segment.

Movie S4. Blood flow dynamics through a small sinusoidal capillary, related to Figure 3.

Blood flow through a sinusoidal capillary in the BM compartment visualized by two-photon intravital imaging. The blood serum (red) was labeled by intravenous injection with TexasRed-dextran (**Figure 3E**). Note that only occasionally individual erythrocytes flow through this vessel segment.

Movie S5. Global blood flow dynamics in the BM compartment, related to Figure 3.

Blood flow was animated simultaneously in the microvasculature of the BM compartment showing an arterial vessel (in the lower middle of the screen) with rapid blood flow connecting to post-arterial vessels into a network of interconnecting sinusoids of wider vessel lumen, slower blood flow and turbulent flow pattern. The blood serum (red) was labeled by intravenous injection with TexasRed-dextran. Calcified bone was visualized by SHG (blue).

Movie S6. HSPC rolling and transient adhesion in sinusoidal capillaries, related to Figure 6 and S3.

Two-photon intravital imaging of fluorescently labeled BM lineage-depleted HSPC (green) rolling and transiently adhering on $Enmc^+$ labeled BM sinusoidal capillaries (red). HSPC were intravenously injected and imaged for their homing in different types of BM vessels after 0.5-3 hr. Rolling velocities of representative HSPC are in the range 40-80 µm/sec. SHG signal (blue) indicates bone tissue.

Movie S7. HSPC slow rolling and adhesion in sinusoidal vessels, related to Figure 6.

Two-photon intravital imaging of fluorescently labeled BM lineage-depleted HSPC (green) rolling slowly and subsequently adhering to $Enmc^+$ labeled BM sinusoids (red). HSPC were intravenously injected and imaged for their homing in different types of BM vessels after 0.5-3 hr. Rolling velocities of representative HSPC are in the range 5-15 µm/sec. SHG signal (blue) indicates bone tissue. Note that slow rolling is frequently followed by adhesion of HSPC on $Enmc^+$ BM sinusoids.

Movie S8. HSPC adhesion and slow migration in sinusoidal vessels, related to Figure 6 and S3.

Two-photon intravital imaging of fluorescently labeled BM lineage-depleted HSPC (green) caught at a branch point of $Enmc^+$ labeled BM sinusoidal vessels (red) starts slowly migrating on the luminal vessel surface. HSPC were intravenously injected and imaged for their homing in different types of BM vessels after 0.5-3 h. SHG signal (blue) indicates bone tissue. Note a second HSPC is transiently adhering on the same site, but continues rolling in a different vessel segment.

Movie S9. HSPC transmigration through the BM endothelium, related to Figure 6 and S3.

Two-photon intravital imaging of fluorescently labeled BM lineage-depleted HSPC (green) in Enmc^{+} labeled BM sinusoidal capillaries (red). Note the slowly rolling HSPC that adheres in close proximity to an already adhering HSPC. After a short adhesion the newly arrived HSPC forms a protrusion that projects through the endothelial layer of the sinusoidal capillary. Within ~1.5 min the HSPC transmigrates and remains in close proximity to the vessel wall. The perivascular located cell barely migrates, but shows active cell protrusions with the environment including the endothelial lining. SHG signal (blue) indicates bone tissue.

Movie S10. HSPC dynamic behavior in the BM cavity, related to Figure 6.

Two-photon intravital imaging of fluorescently labeled BM lineage-depleted HSPC (green) 24 hr after intravenous injection. HSPC left the Enmc^{+} labeled BM sinusoidal capillaries (red) and reside in proximity of to the sinusoids in the BM cavity. The majority of cells hardly migrate (mean migration speed: 63±34 µm/h), but show active protrusions indicating lively cell interactions with the environment. SHG signal (blue) indicates bone tissue.

Supplementary Experimental Procedures

FACS immunostaining

To analyze HSPC frequency before and after lineage depletion, BM cells were isolated from femurs and lineage depleted as described above. Undepleted and lineage depleted BM cells were stained with the following antibodies: biotin-labeled lineage markers CD5, CD11b, CD45R, Gr-1 and Ter119 (Mouse lineage panel biotin; 59971; BD Pharmingen), c-Kit (553356; BD Pharmingen), Sca1 (MSCA18; Invitrogen). Cells were first incubated with lineage markers for 45 min. Then, cells were washes twice and incubated with PE-Cy5-conjugated streptavidin antibody, APC-conjugated c-Kit and PE-Cy7-conjugated Sca1 antibody for 45 min. Subsequently, cells were washed twice and protected from light. Then, BM cells were acquired using a BD FACSVerse and analyzed with BD FACSuite software.