

## Descriptions of additional supplementary Data files

### **Supplementary movie 1: Movement of B<sub>EM</sub> between GC and SCS and inside the SCS.**

Intravital microscopy. Overview of a Cy1Cre mTmG ACKR4<sup>+/+</sup> drLN day 8 after foot immunization.

Cy1Cre-dependent expression of eGFP (green) shows GC and B<sub>EM</sub>. CD169 (blue) indicates location of SCS with SCS macrophages. Red: mTomato expressing stroma. Grey: second harmonic.

### **Supplementary movie 2: Tracking GFP<sup>+</sup> B<sub>EM</sub> recycling between GC and SCS**

Intravital microscopy of three drLN data of Cy1Cre mTmG ACKR4<sup>+/+</sup> mice. eGFP-labelled B<sub>EM</sub> (green), mTomato-labelled stroma (red) and CD169 labelled SCS macrophages (blue). B<sub>EM</sub> were manually tracked moving from the GC towards the SCS (pink tracks) or recycling from the SCS to the GC (blue tracks).

### **Supplementary movie 3: Location of B<sub>EM</sub> in relation to SCS macrophages and SCS lumen in wt and ACKR4<sup>ko</sup> drLN.**

Intravital microscopy. 3D still image of SCS of a Cy1Cre mTmG Ackr4<sup>+/+</sup> and Ackr4<sup>-/-</sup> drLN 8 d after foot immunization. Cy1Cre-dependent expression of eGFP (green) indicating B<sub>EM</sub>. CD169 (blue) SCS macrophages lining the SCS floor endothelium. Red: mTomato expressing stroma. Grey: second harmonic indicating the LN capsule. B<sub>EM</sub> can be seen inside the LN parenchyma and having entered the SCS.

### **Supplementary movie 4: B<sub>EM</sub> moving along the SCS in ACKR4<sup>-/-</sup> drLN**

Intravital microscopy of SCS of a Cy1Cre mTmG ACKR4<sup>-/-</sup> drLN 8 d after foot immunization. Cy1Cre-dependent expression of eGFP positive B cells (green and white). CD169 (blue) SCS macrophages lining the SCS floor endothelium. Red: mTomato expressing stroma. Tracked cells (white) can be seen moving inside the SCS, but not reentering the LN parenchyma.

### **Supplementary movie 5: Prolonged interaction of B<sub>EM</sub> with SCS macrophage**

Intravital microscopy of a Cy1Cre mTmG drLN 8 d after foot immunization. Cy1Cre-dependent expression of eGFP (green) indicating B<sub>EM</sub>. Red: mTomato expressing stroma. Blue: CD169 on SCS macrophages.

### **Supplementary movie 6: Colocalization of B<sub>EM</sub> with increased Ca<sup>2+</sup> and SCS macrophages**

Animation merging images from Fig. 4B, showing surface rendering of CD169<sup>+ve</sup> macrophages (purple), FDC staining outlining a GC (orange), and eGFP<sup>+</sup> B cells (green) in one frame, and the FRET intensity of B cells in the second frame. The B cell FRET intensity is color coded from purple (low) to orange (high).

Orange B cells with Ca<sup>2+</sup> FRET signal are seen in close contact with CD169<sup>+</sup> macrophages in the SCS at top edge of the lymph node section, and inside the GC.

**Supplementary movie 7: B<sub>EM</sub> acquiring CD169<sup>+</sup> material from SCS macrophage**

Intravital microscopy of a Cγ1Cre mTmG drLN 8 d after foot immunization. Cγ1Cre-dependent expression of eGFP (green) indicating B<sub>EM</sub>. CD169<sup>+</sup> material (blue) can be seen at the trailing edge of the migrating B<sub>EM</sub>.

**Supplementary movie 8: 360-rotation showing B<sub>EM</sub> trafficking between the GC and the SCS**

Light sheet microscopy shows tdTomato<sup>+</sup> B<sub>EM</sub> from a S1PR1<sup>CreERT2</sup> Ai14 mouse of a, 8 d after NP-CGG foot immunization. dTomato<sup>+</sup> BEM in red, CD169<sup>+</sup> macrophages indicating the SCS floor in blue.

**Supplementary movie 9: 360-rotation showing B<sub>EM</sub> interacting with IC in SCS**

Light sheet microscopy of tdTomato<sup>+</sup> B<sub>EM</sub> (red) in a drLN of a S1PR1<sup>CreERT2</sup> Ai14 mouse, 10 min after injection with Alexa488 labelled immune complex (IC) into the foot. IC is shown in green. B<sub>EM</sub> are seen in the GC, between the GC and interacting with IC in the SCS floor.

**Supplementary movie 10: B<sub>EM</sub> moving between GC and SCS**

Light sheet microscopy shows tdTomato<sup>+</sup> B<sub>EM</sub> (red) in a drLN of a S1PR1<sup>CreERT2</sup> Ai14 mouse, 10 min after injection with Alexa488 labelled immune complex (IC) into the foot. IC is shown in green. B<sub>EM</sub> are seen travelling between the GC (left) and IC in the SCS (bottom left), and in close contact with IC in the SCS floor.