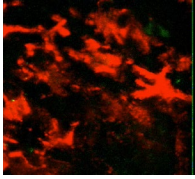
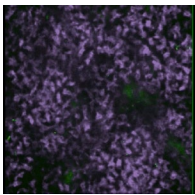


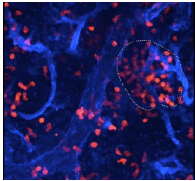
## SUPPLEMENTAL MATERIAL

Deniset et al., <https://doi.org/10.1084/jem.20161621>

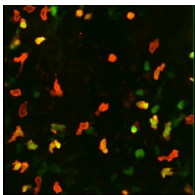
Video 1. ***S. pneumoniae* movement in splenic MZ.** MZ macrophages (CD209b; red) capture of i.v. injected *S. pneumoniae* ( $5 \times 10^6$  CFU D39-GFP) from the circulation in wild-type mice. Bacteria were injected 30 s before initiation of the 20-min time interval visualized by two-photon IVM. The playback rate is eight frames per second.



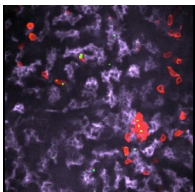
Video 2. ***S. pneumoniae* movement in splenic RP.** RP macrophages (F4/80; purple) capture of i.v. injected *S. pneumoniae* ( $5 \times 10^6$  CFU D39-GFP) from the circulation in wild-type mice. Bacteria were injected 1 min after initiation of the 20-min time interval visualized by SD-IVM. The playback rate is four frames per second.



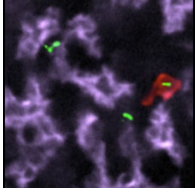
Video 3. **Splenic neutrophil behavior in the RP.** The behavior of neutrophils (Ly6G; red) in RP sinusoids (CD31; blue) of uninfected mice visualized by SD-IVM. The dotted line shows an immature neutrophil cluster. The playback rate is eight frames per second.



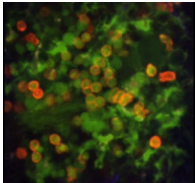
Video 4. **Splenic neutrophil population chimerism after parabiosis.** Parabolic partner contribution (single positive; Ly6G-red<sup>+</sup> only) to mobile (part 1) and immobilized (part 2) neutrophil populations in the splenic RP of a LysM-GFP mouse (double positive; Ly6G-red<sup>+</sup> and GFP<sup>+</sup>). The playback rate is 4.3 frames per second.



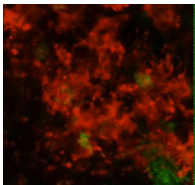
Video 5. **Splenic RP capture of *S. pneumoniae*.** RP macrophage (F4/80; purple) and neutrophil (Ly6G; red) ability to phagocytose i.v. injected *S. pneumoniae* ( $5 \times 10^6$  CFU D39-GFP) 30 min after infection in wild-type (part 1) or C3-deficient (part 2) mice. The 15-min time interval was visualized by SD-IVM. The playback rate is six frames per second.



Video 6. **Splenic neutrophil phagocytosis of *S. pneumoniae*.** Neutrophil (Ly6G; red) capture of *S. pneumoniae* (D39-GFP) after initial cell adhesion to RP macrophages (F4/80; purple). The 10-min time interval was visualized by SD-IVM (20× objective; cropped). The playback rate is six frames per second.



Video 7. **Progenitor neutrophil mobilization after pneumococcal infection.** Photoactivated progenitor neutrophil (double positive; GFP<sup>+</sup> Ly6G-red<sup>+</sup>) mobilization from their niche after saline (part 1) or *S. pneumoniae* injection (part 2). The 60-min time interval was visualized by SD-IVM. The playback rate is 17.5 frames per second.



Video 8. **Neutrophil behavior in the splenic MZ after *S. pneumoniae* infection.** The behavior of neutrophils (LysM-GFP; green) and MZ macrophages (CD209b; red) 24 h after saline (part 1) or *S. pneumoniae* (10<sup>4</sup> CFU; part 2) i.v. injection. The 10-min time interval was visualized by two-photon IVM (25× objective; 3× zoom). The playback rate is 4.2 frames per second.