Supporting Information

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Fig. S1. Analysis of polyclonal and OT-II CD4⁺ T-cell (ie, CD4⁺ T cells that express a transgenic receptor specific for the antigen ovalbumin) dynamics. (A and B) Individual mean velocity plotted to the corresponding confinement index or arrest coefficient. Each symbol represents an individual cell. (A) Polyclonal CD4⁺ T cells. (B) OT-II T cells.



Fig. S2. *Shigella* is captured by resident macrophages and dendritic cells in the subcapsular sinus (SCS) area. (*A* and *B*) Two-photon microscopy (2PM) images of lymph node (LN) sinus macrophages (Mqs) stained in vivo with CD169-specific antibodies injected s.c. 2 h post inoculation. (*A*) Maximum intensity projections of an uninfected LN (*Left*) or LNs infected for 4 h with DsRed-expressing WT *Shigella flexneri* showing the distribution of bacteria in the SCS (*Center*) and contacts with sinus Mqs (*Right*). (*B*) Orthogonal views of regions marked with dashed squares in *A* (*Right*) showing CD169⁺ Mqs infected with WT *S. flexneri*. (*C* and *D*) Two-photon microscopy of LNs taken from CD11c-YPF reporter mice. (*C*) Maximum-intensity projection showing interactions of WT *S. flexneri* with dendritic cells. (*D*) Orthogonal view of infected dendritic cells marked with a dashed square on *C*.



Fig. S3. Intravital 2PM images of a popliteal LN from LysM-GFP reporter mice infected with WT *Shigella* showing neutrophil extravasation and engulfment of bacteria 30 min (*A*) and 3 h (*B*) post inoculation. Intracellular bacteria (arrowheads) and extravasating neutrophils, some with torpedo-like morphology (arrows), are indicated.



Movie S1. Polyclonal CD4⁺ T-cell motility in uninfected LNs. Dynamic 2PM analysis of carboxyfluorescein succinimidyl ester (CFSE)-labeled CD4⁺ T cells in the SCS region of uninfected intact LNs.

Movie S1



Movie S2. Polyclonal CD4⁺ T-cell motility in T3SS⁻ S. flexneri-infected LNs. Dynamic 2PM analysis of CFSE-labeled CD4⁺ T cells in the subcaspsular sinus region of intact LNs infected with T3SS⁻ S. flexneri 4 h post s.c. inoculation.

Movie S2



Movie S3. Polyclonal CD4⁺ T-cell motility in WT 5. flexneri-infected LNs. Dynamic 2PM analysis of CFSE-labeled CD4⁺ T cells in the SCS region of intact LNs infected with WT 5. flexneri 4 h post s.c. inoculation.

Movie S3



Movie S4. OT-II T-cell motility in uninfected LNs. Dynamic 2PM analysis of CFSE-labeled CD4⁺ T cells in the subcaspsular sinus region of uninfected intact LNs.

Movie S4



Movie S5. OT-II T-cell motility in T3SS⁻ S. flexneri-infected LNs. Dynamic 2PM analysis of CFSE-labeled CD4⁺ T cells in the SCS region of intact LNs infected with T3SS⁻ S. flexneri 4 h post s.c. inoculation.

Movie S5



Movie S6. OT-II T-cell motility in WT S. flexneri-infected LNs. Dynamic 2PM analysis of CFSE-labeled CD4⁺ T cells in the SCS region of intact LNs infected with WT S. flexneri 4 h post s.c. inoculation.

Movie S6