



**Figure S4. Memory CD8 T cell motility is influenced by bystander inflammation and the motility of tolerant CD8 T cells is not affected by antigen, related to Figure 4.** (A-C) Memory P14 mice received OT-I CD8 T cells and 1 day later were orally infected with Yptb-OVA. Graphs depict average values per mouse of (A) mean track speed, (B) median turning angle and (C) confinement ratio for P14 memory T cells at steady state (filled green circles) or in the presence of Yptb-OVA infection (open green circles). (D-F) Mice with memory P14 T cells were given LPS to mimic endotoxemia. Graphs depict average values per mouse of (D) mean track speed, (E) median turning angle and (F) confinement ratio for P14 memory cells at steady state (filled green circles) or in the presence of LPS (filled purple circles). A Mann-Whitney test was performed to evaluate statistical significance.  $*p < 0.03$ . (G-O) Naïve OT-I/CD45.1 CD8 T cells were transferred to mice expressing OVA in mature enterocytes of the SI (iFABP-OVA) and evaluated at day 5 and day 10 after transfer. (G) Flow cytometric analysis of OT-I IEL (top) and LPL (bottom) in iFABP-OVA mice. Plots are gated on lymphocytes. (H) Interferon- $\gamma$  production after *ex vivo* SIINFEKL peptide stimulation of OT-I IEL and LPL. Plots are gated on CD8 T cells. (I) Enumeration of OT-I cells from (G). (J) Percentage of CD45.1+ OT-I T cells in the epithelium and LP producing IFN- $\gamma$  (K) PD-1 expression on OT-I IEL and LPL at day 5 (magenta) and day 10 (blue) after transfer to iFABP-OVA mice. Histograms gated on OT-I CD8 T cells or CD44<sup>lo</sup> splenocytes (grey). (L) OT-I-GFP CD8 T cells (cyan) were transferred to iFABP-OVA mice and evaluated in the villi using TPLSM at day 5 and 10 after transfer. Anti-K<sup>b</sup>-SIINFEKL antibody was administered to iFABP-OVA mice at both time points at least 1 hour prior to imaging. Cell tracks are in orange. Scale bars are 30 $\mu\text{m}$ . (M) Mean track speed, (N) median turning angle and (O) confinement ratio of day 10 (blue, filled circles), and day 10+K<sup>b</sup>-SIINFEKL blockade (blue, open circles) of OT-I-GFP CD8 T cells in iFABP-OVA mice. Graphs of TPLSM are combined data from  $n=2$  mice with and without Ab blocking. A Mann-Whitney test was performed to evaluate statistical significance. Error bars are SEM.