



**Figure S1. CD8 T cell migration in the SI is dynamic during infection, related to Figure 1.** Naïve Thy1.1 P14-GFP CD8 T cells were transferred to B6 mice and infected with LCMV 1 day later. **(A)** Experimental setup. **(B)** Intraepithelial lymphocytes (IEL, left) and lamina propria lymphocytes (LPL, right) were isolated from the small intestine (SI) at the indicated time points. The numbers reflect the percent P14-GFP CD8 T cells within the indicated gate. Plots are gated on CD8 T cells. **(C)** 3D schematic of the SI (left) with a cross section indicating the area being imaged. Prior to imaging, B6 mice were injected with Hoechst stain (dark blue) to visualize intestinal architecture. P14-GFP CD8 T cells are cyan-colored within the tissue. **(D-F)** Several positions within the tissue were analyzed for each mouse and averaged to obtain a single data point per mouse for **(D)** mean track speed, **(E)** median turning angle and **(F)** confinement ratio at day 5 (magenta), day 8 (blue), day 14 (purple) and day 30 (green) after LCMV. Data are representative of 2 or more experiments totaling  $n=4$  for day 5,  $n=7$  for day 8,  $n=5$  for day 14, and  $n=8$  for day 30. A Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison test was performed to evaluate statistical significance. \*\*\* $p=0.0010$  and \* $p<0.05$ . Error bars are SEM.