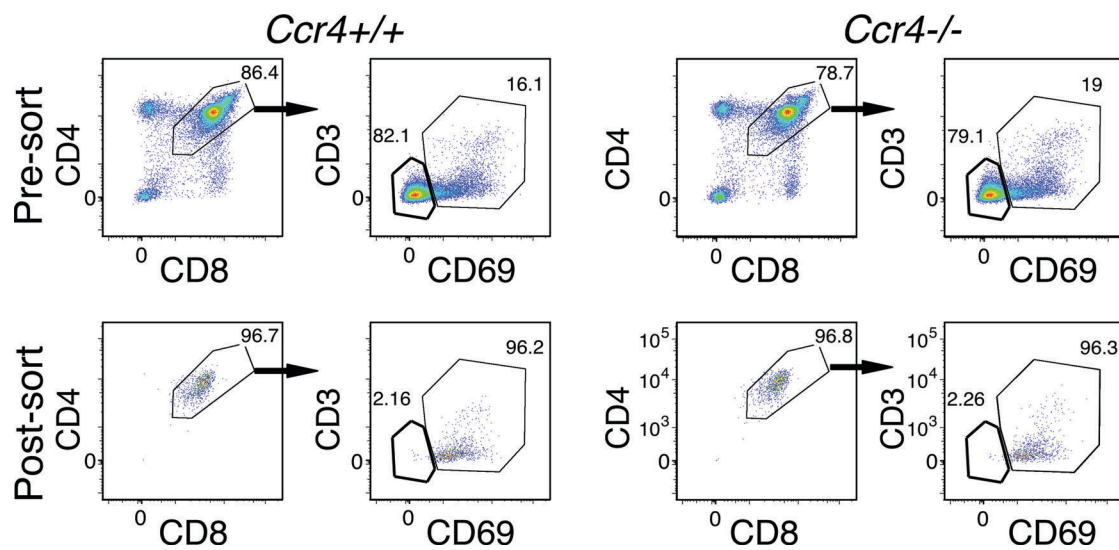
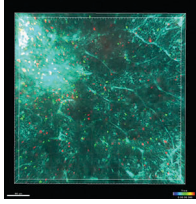


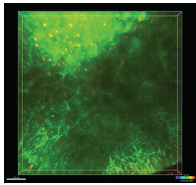
## SUPPLEMENTAL MATERIAL

Hu et al., <http://www.jem.org/cgi/content/full/jem.20150178/DC1>

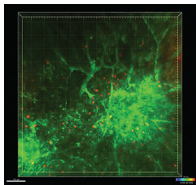
**Figure S1. Post-sort analysis of DP CD69<sup>+</sup> thymocytes.** Representative flow cytometric plots display the purity of DP CD69<sup>+</sup> cells before and after FACS sorting. Sorted cells were used for two-photon imaging on thymic slices, as in Fig. 8 D. Plots are pregated on live PI<sup>-</sup> cells. Arrows indicate gating hierarchy.



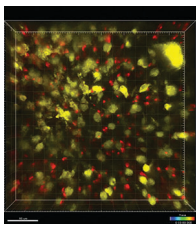
**Video 1. CCR4 is required for efficient medullary accumulation of CD4SP thymocytes.** Two-photon time-lapse video microscopy of *Ccr4*<sup>+/+</sup> (red) and *Ccr4*<sup>-/-</sup> (green) CD4SP thymocytes migrating in a live thymic slice from a pCX-EGFP (blue) transgenic mouse. Frames were acquired at 15-s intervals. This video correlates with Fig. 8 A.



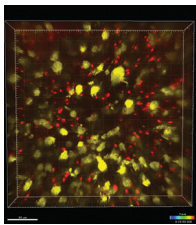
**Video 2. CCR4 is required for efficient medullary accumulation of DP CD69<sup>+</sup> thymocytes.** Two-photon time-lapse video microscopy of *Ccr4*<sup>+/+</sup> DP CD69<sup>+</sup> thymocytes (red) migrating in a pCX-EGFP live thymic slice (green). Frames were acquired at 15-s intervals. This video correlates with Fig. 8 D.



**Video 3. CCR4 is required for efficient medullary accumulation of DP CD69<sup>+</sup> thymocytes.** Two-photon time-lapse video microscopy of *Ccr4*<sup>-/-</sup> DP CD69<sup>+</sup> thymocytes (red) migrating in a pCX-EGFP live thymic slice (green). Frames were acquired at 15-s intervals. This video correlates with Fig. 8 D.



**Video 4. CCR4 is required for efficient interactions between CD4SP thymocytes and medullary DCs.** Two-photon time-lapse video microscopy of *Ccr4*<sup>+/+</sup> CD4SP cells (red) migrating among medullary DCs (yellow) in a live CD11c-EYFP thymic slice. Frames were acquired at 15-s intervals. This video correlates with Fig. 9 B.



**Video 5. CCR4 is required for efficient interactions between CD4SP thymocytes and medullary DCs.** Two-photon time-lapse video microscopy of *Ccr4*<sup>-/-</sup> CD4SP cells (red) migrating among medullary DCs (yellow) in a live CD11c-EYFP thymic slice. Frames were acquired at 15-s intervals. This video correlates with Fig. 9 B.