

**Supplemental figure 1.** AMD3100 specifically inhibits human thymocyte migration in response to both human and mouse CXCL12. Relative migration of total human thymocytes in response to mouse and human chemokines in the presence or absence of AMD3100 as assessed by transwell assay. Migration normalized to no chemokine controls as indicated by dashed red line. Experiment was performed twice with similar results, and a representative experiment is shown.

## Supplemental video legends

Supplemental video 1. Human thymocyte migration on human and mouse thymic slices. 2-photon laser scanning microscopy of human thymocytes (red) on mouse (top panels) and human (bottom panels) thymic slices corresponding to Fig. 2. CD11c-YFP dendritic cell (yellow) density allowed distinction between cortex and medulla in mouse thymic slices (top). Examples of representative cell tracks are in turquoise and are 17-20min in duration. Maximum intensity projection of 20min movies acquired every 30 seconds to a total depth of 60μm. Scale bar represents 40μm.

Supplemental video 2. Effect of PTX-treatment on CD8<sup>+</sup> human thymocyte migration. 2-photon laser scanning microscopy of human thymocytes (red) untreated (top panels) versus PTX-treated (bottom panels) with representative tracks (aqua, right) on mouse thymic slices corresponding to Fig. 4C. Representative tracks are 6-8min in duration. CD11c-YFP dendritic cell (yellow) density allowed identification of medulla. Scale bar represents 40μm in the xy plane. Maximum intensity projection of 20min movies acquired every 30 seconds to a total depth of 96μm.