SUPPLEMENTAL MATERIAL

Masopust et al., http://www.jem.org/cgi/content/full/jem.20090858/DC1

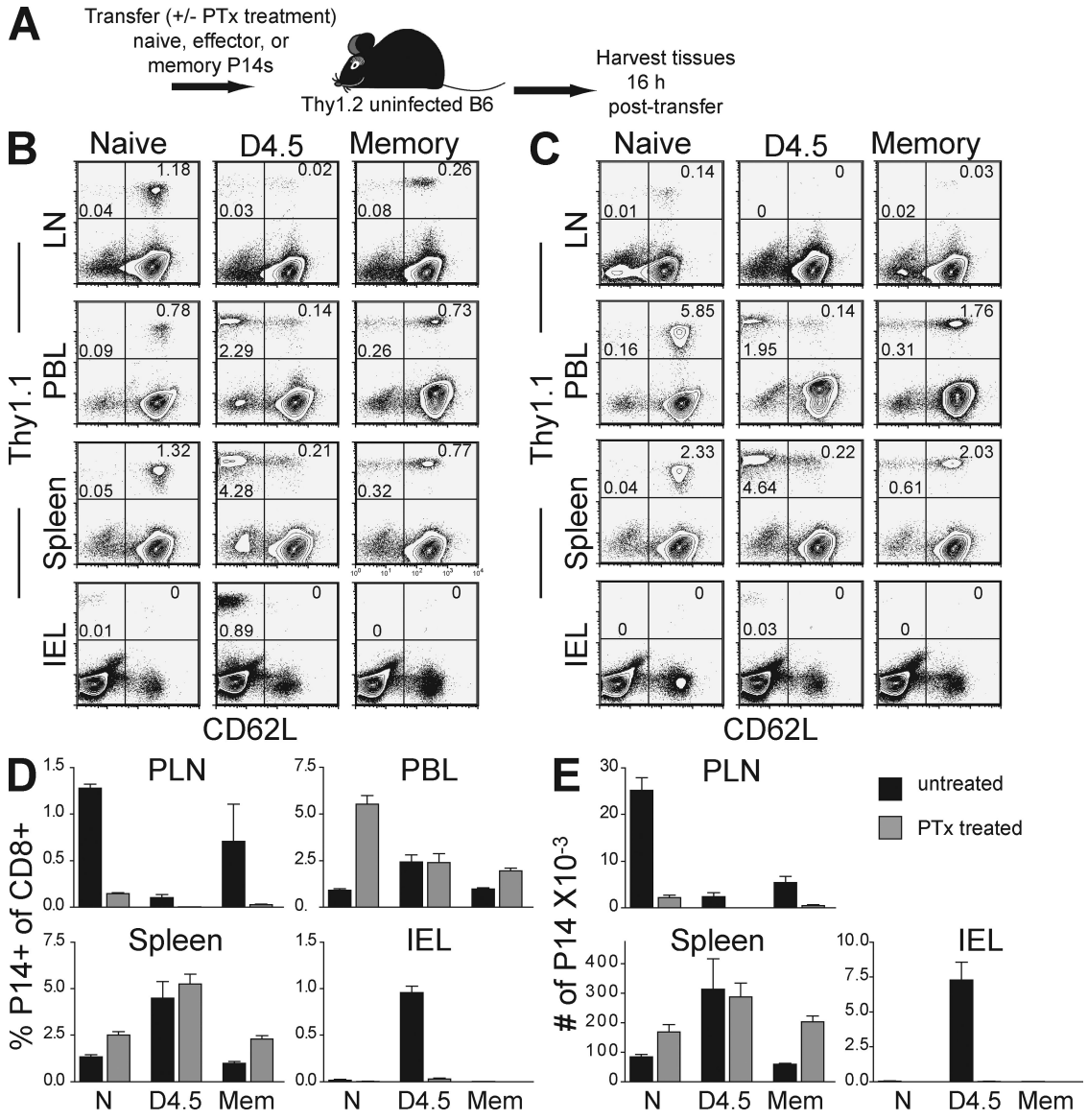

Figure S1. Migration of early effectors to intestinal epithelium is chemokine dependent. (A) Experimental design consists of transferring P14 at different stages of differentiation and harvesting tissues the next day. Cells were incubated for 1 h with or without pertussis toxin before transfer. (B) Representative flow cytometric data depicting recovery of naive early effectors (isolated 4.5 d after infection) and memory cells from recipient tissues. Donor cells are identified via the congenic marker Thy1.1 and all plots are gated on $CD8^+$ lymphocytes. (C) As in B, but donor cells were pretreated with pertussis toxin. (D and E) Percentage (D) and number (E) of P14 recovered after mock or pertussis toxin treatment, transfer, and recovery. Error bars indicate SEM. One of two experiments with similar results is depicted.

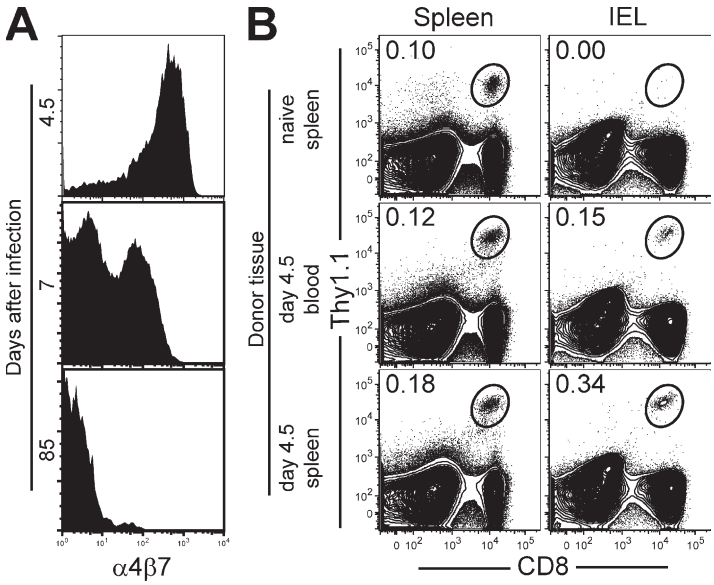

Figure S2. Migration of early effectors from blood to intestinal epithelium. (A) Dynamics of α4β7 expression among P14 in blood after LCMV infection. (B) Equal numbers of naive Thy1.1+ P14 isolated from spleen, or effector P14 isolated from spleen or blood 4.5 d after LCMV infection, were transferred to naive recipients. The next day, lymphocytes were harvested from spleen and small intestine epithelium. Plots gated on lymphocytes and numbers indicate percentage of CD8+ Thy1.1+ cells. Plots are representative of $n = 3–4$ per group.

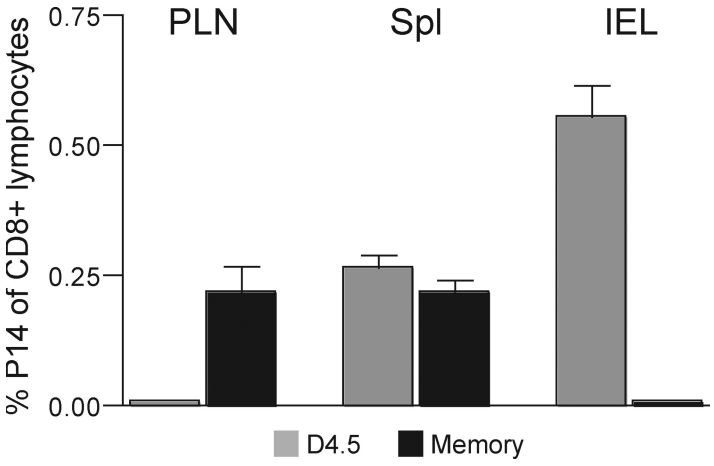

Figure S3. Effectors do not elicit bystander migration of memory cells into intestinal epithelium. Equal numbers of Thy1.1+ effector P14 isolated from spleen 4.5 d after LCMV infection and Ly5.1+ memory cells isolated from spleen 1 mo after infection were mixed and cotransferred into naive C57BL/6J mice. Recipient spleen and IEL were harvested the next day, and the proportion of CD8+ lymphocytes that were early effector versus memory P14 was determined by flow cytometry. $n = 3$ mice per group. One of two experiments with similar results is depicted. Error bars indicate SEM.

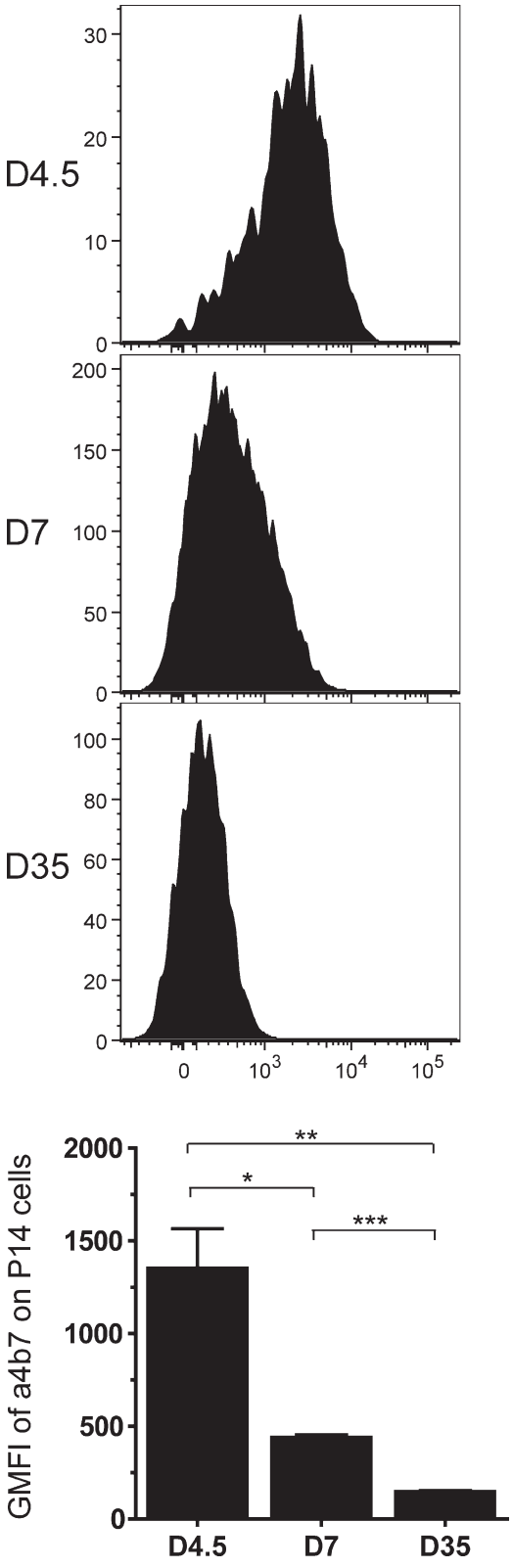

Figure S4. CD8 T cells gradually lose α4β7 expression after entry into intestinal epithelium. Naive Thy1.1+ P14 was transferred to naive mice. Recipients were challenged with LCMV Arm the next day. 4.5, 7, and 35 d after infection, P14 was isolated from small intestine IEL and examined for α4β7 expression. Infections were staggered and cells from each time point were isolated on the same day. $n = 3$. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, unpaired Student's *t* test. One of two experiments with similar results is shown. Error bars indicate SEM.

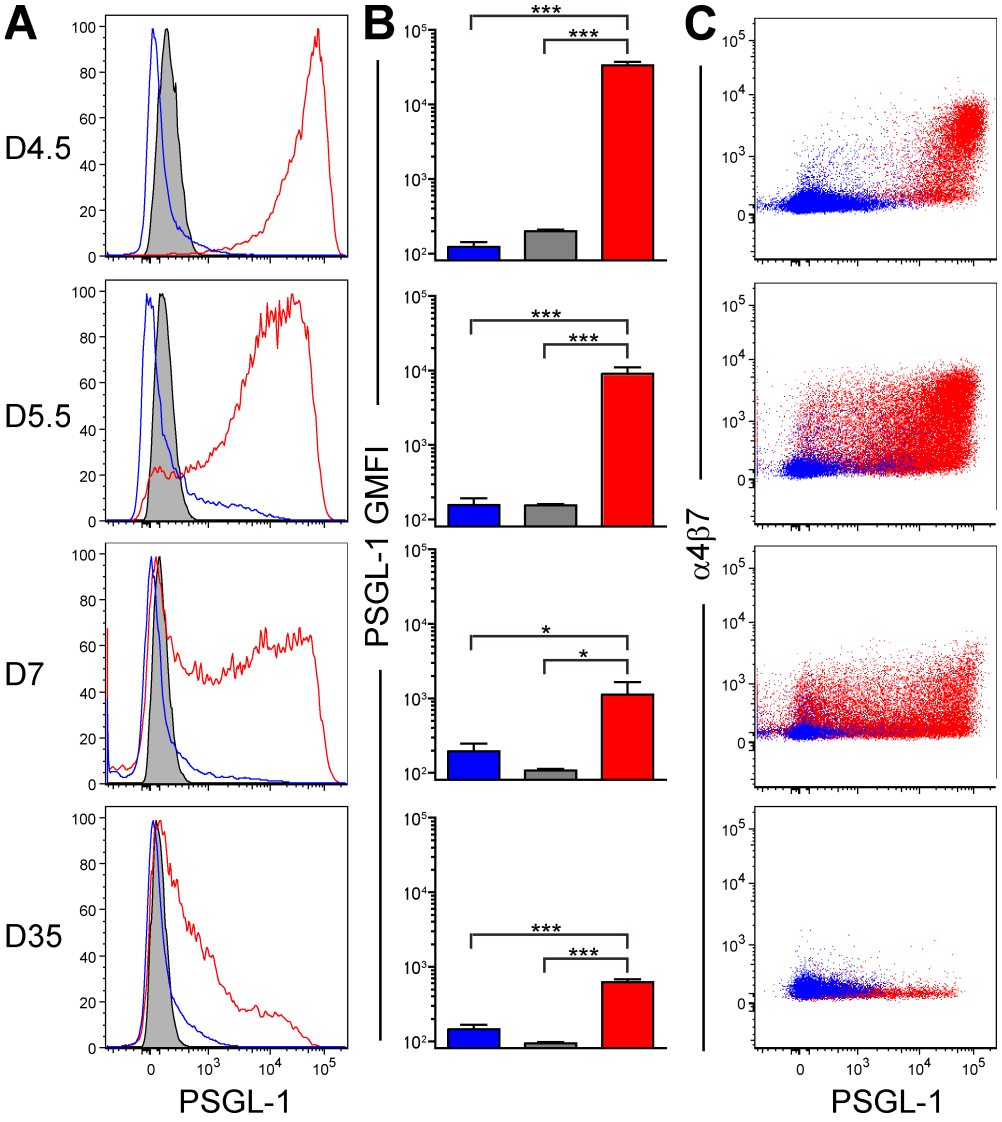

Figure S5. Transient coexpression of functional P-selectin binding ligand and α4β7 among effector splenocytes. Naive Thy1.1+ P14 cells were transferred to naive mice. Recipients were challenged with LCMV Arm the next day. 4.5, 5.5, 7, and 48 d after infection, P14 cells were isolated from spleen and examined for PSGL-1 binding and α4β7 expression. Infections were staggered and cells from each time point were isolated on the same day. Blue lines and bars are gated on naive ($CD44^{lo}$) CD8 T cells, gray are gated on P14 stained with secondary antibody alone, and red are gated on P14 stained with PSGL-1 and secondary antibody. $n = 5$. *, $P < 0.05$; ***, $P < 0.001$, unpaired Student's t test. One of two experiments with similar results is shown. Error bars indicate SEM.