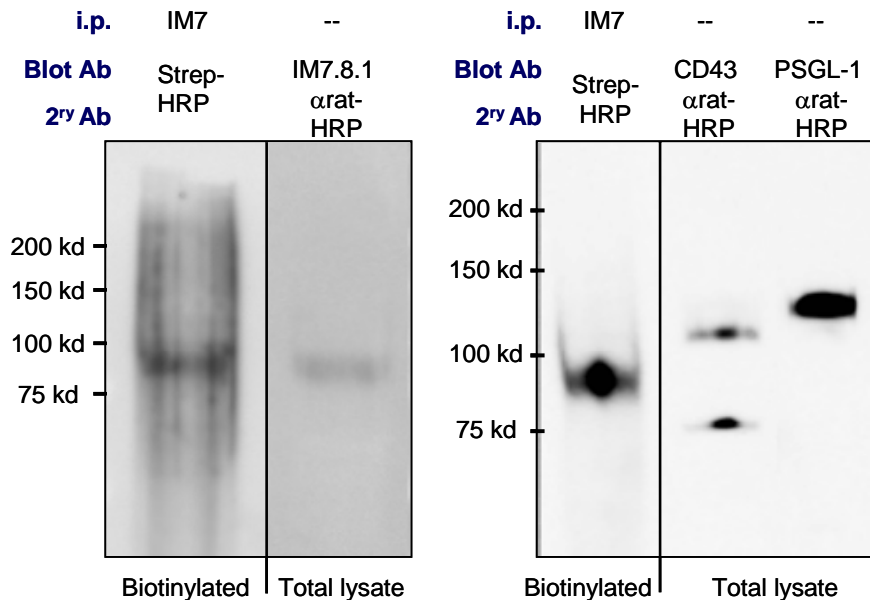


SUPPLEMENTAL MATERIALS

Nácher et al. **Physiological contribution of CD44 as a ligand for E-selectin during inflammatory T cell recruitment**



Supplemental Figure S1. Immuno-purification of CD44 from Th1 cells onto beads.

Wild-type Th1 cells were surface-biotinylated and lysed before incubation with beads pre-conjugated with an anti-CD44 antibody (clone IM7). After extensive washing, the immunoprecipitate was resolved by SDS-PAGE, transferred and membranes blotted with Streptavidin-peroxidase (Strep-HRP) to detect all bead-bound material (first lanes in both blots). In parallel, total lysates from non-biotinylated Th1 cells were resolved in the same gels, transferred and membranes blotted with antibodies against CD44 (clone IM7, left blot), CD43 (clone S7) or PSGL-1 (clone 2PH1), and developed with an anti-rat-HRP antibody. The single band immunoprecipitated with the beads matches the migration of CD44, while no bands with molecular weights matching CD43 or PSGL-1 were found (right plot). The fainter band for CD44 in total lysates (right lane in left blot) is due to the lower levels of this protein in the cell surface compared to CD43 or PSGL-1 or immunoprecipitated CD44 (left lane in left blot), and therefore this lane was contrasted independently from the i.p. lane next to it.