

Supplementary Figure 1. Anti-CD45RB enhances HP and survival of Treg.

- (a) Proliferation and replication indexes based on CFSE dilution profiles of adoptively transferred Foxp3+CD4+Thy1.1+ cells recovered from spleen 10 days after transfer of highly purified Foxp3+CD4+Thy1.1+ cells and treatment with control IgG or anti-CD45RB (n = 8 mice / group, from 4 independent experiments). Each square represents an individual value and black horizontal bars show averages.
- (b) % of annexin⁺ cells amongst CD4⁺Foxp3⁻ or CD4⁺Foxp3⁺ cells in spleen 10 days after treatment with control IgG or anti-CD45RB (n = 6 mice / group, from 2 independent experiments).
- (c) Number of transferred Foxp3⁺CD4⁺ that retained or lost TGFβRII expression in spleen on day 10 of mice treated with tamoxifen + anti-CD45RB, relative to tamoxifen + control IgG. CD4⁺CD25⁺ T cells from TGFβRII^{fl/flx}UBC-Cre/ERT2 mice were adoptively transferred into congenic hosts (d-6), treated with tamoxifen (days -6 to -2), and then treated with control IgG or anti-CD45RB (days 0, 1, 6). Each bar represent average ± s.e.m. n = 4-6 mice / group, from 3 independent experiments.



Supplementary Figure 2. Anti-CD45RB does not promote IL-2R signaling in Tregs.

- (a) Representative flow cytometry plots of pSTAT-5 in CD4+Foxp3⁻ or CD4+Foxp3⁺: splenocytes of mice 7 days after receiving control IgG or anti-CD45RB were treated with or without 50 U/ml rmIL-2 for 15 min followed by intracellular staining for pSTAT5.
- (b) % of cells in (a) with detectable pSTAT-5. Duplicate samples from 3 pooled mice in each of 2 independent experiments.

Supplementary Movie 1. Anti-CD45RB specifically promotes Treg:DC interactions *in vivo* (related to Figure 6). Representative time-lapse images by 2PIM of spleen red pulp on day 1 showing the tracks (last 20 positions) of Foxp3⁻ or Foxp3⁺CD4⁺ T cells previously exposed to either control IgG (green) or anti-CD45RB (red) for 4 days *in vivo* and co-transferred into naïve CD11c-YFP mice (DCs shown in blue). Acquisition at 30-sec/time frame over 30 minutes for each imaging volume.