SUPPLEMENTAL MATERIAL

Bruno et al., http://www.jem.org/cgi/content/full/jem.20090226/DC1

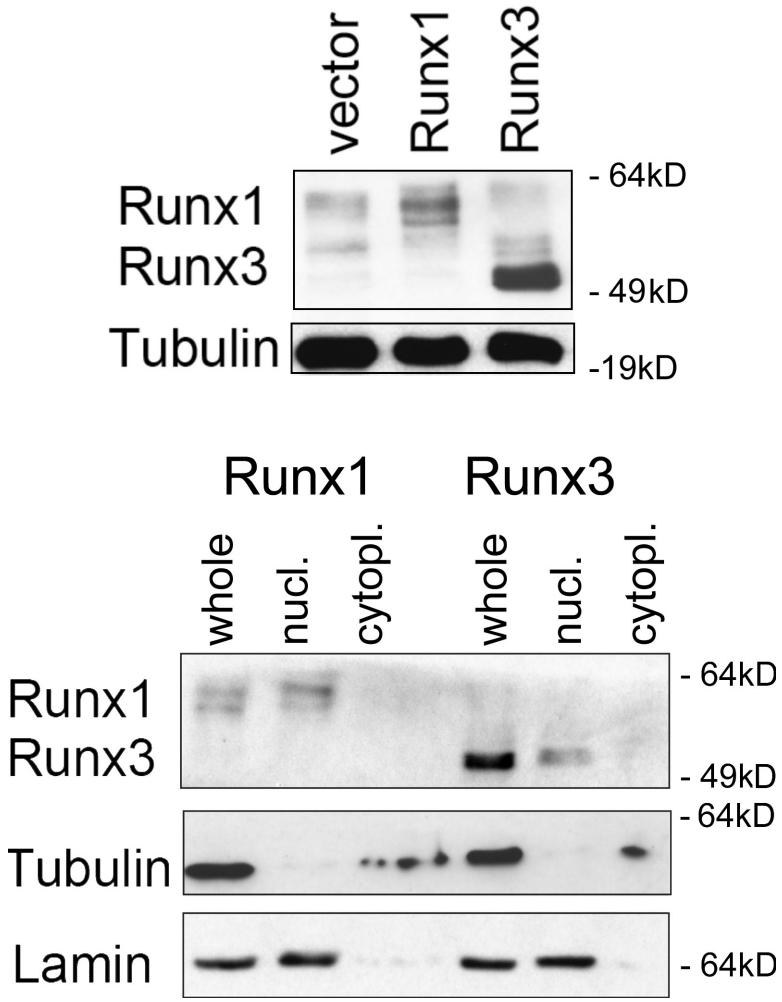

Figure S1. Overexpression of Runx1 and Runx3 after retroviral transduction of conventional CD4 T cells. (Top) Naive CD4 T cells were activated overnight and transduced with MSCV IRES-GFP (vector), Runx1-IRES-GFP (Runx1), or Runx3-IRES-GFP (Runx3). GFP^+ cells were sorted 48 h later, and whole cell extracts were prepared and subjected to Western blotting with anti-Runx1/3. Tubulin was used as a loading control. Runx3 appears to be more highly expressed than Runx1. (Bottom) Whole cell (whole), nuclear (nucl.), and cytoplasmic (cytopl.) extracts from cells prepared as in the experiments shown in the top of the figure were subjected to Western blotting with anti-Runx1/3. Tubulin and lamin were used as cytoplasmic and nuclear loading controls. Runx1 and Runx3 are mainly nuclear. Data are representative of two independent experiments.

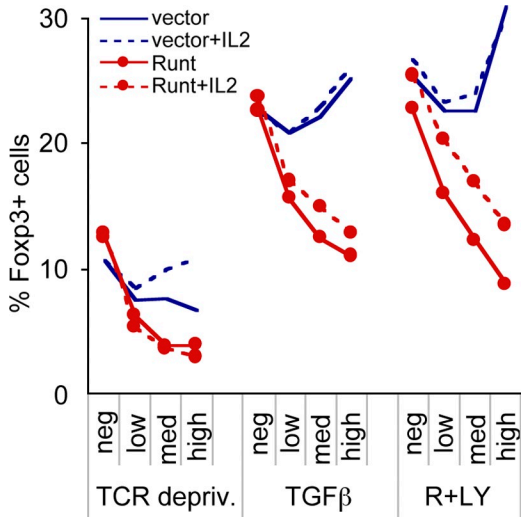

Figure S2. Exogenous IL2 does not relieve inhibition of Foxp3 induction by the isolated Runt DNA binding domain. The role of IL2 in the block of Foxp3 induction by Runt was assessed in naive CD4 T cells that were activated and transduced with control IRES-GFP (vector; blue) or Runt-IRES-GFP (Runt; red) as in Fig. 1 B. TGF-β or rapamycin and LY294002 (R+LY) were added as indicated and the cells were cultured for 48 h with (dashed line) or without exogenous IL2 and Foxp3 expression was assessed by intracellular staining, gating on the level of GFP expression (negative, low, medium, or high). Data are representative of two experiments.

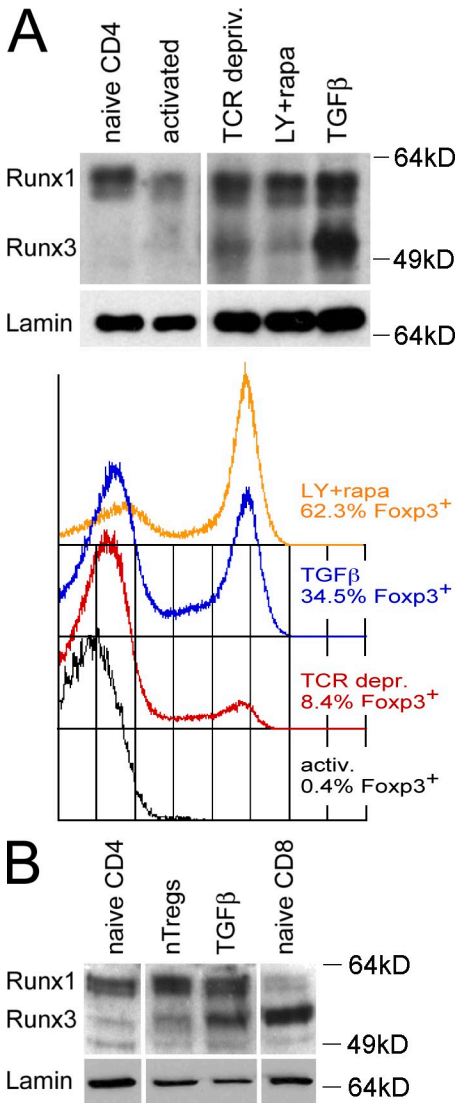

Figure S3. Runx expression in induced and nT reg cells compared with naive CD4 and CD8 T cells. (A, top) Immunoblot analysis of Runx proteins in freshly isolated naive CD4 T cells (naive), 18-h activated CD4 cells (activ.), or 18-h activated CD4 cells cultured for 24 h without anti-TCR (TCR depr.), with LY294002 and rapamycin (LY+rapa) or with TGF-β to induce Foxp3 expression. The position of the indicated bands was determined based on extracts of 293T cells transduced with complementary DNAs for Runx1 or Runx3 (not depicted). Lamin was used as a loading control. Data are representative of two independent experiments. (A, bottom) Histogram overlays of Foxp3 expression by activated CD4 cells (activ.) or 18-h activated CD4 cells cultured for 24 h without anti-TCR (TCR depr.), with TGF-β, or with LY294002 and rapamycin (LY+rapa). Foxp3 expression was determined by intracellular staining and flow cytometry in >10 independent experiments (Sauer et al., 2008). (B) Immunoblot analysis of Runx protein expression in ex vivo nT reg cells and in vitro TGF-β–iT reg cells was compared with ex vivo naive CD4 and CD8 T cells. nT reg cells mainly express Runx1, wheras conditions that trigger Foxp3 induction result in an up-regulation of Runx1 and Runx3 (Runx isoforms may not be coexpressed in individual cells). One of two independent experiments is shown.