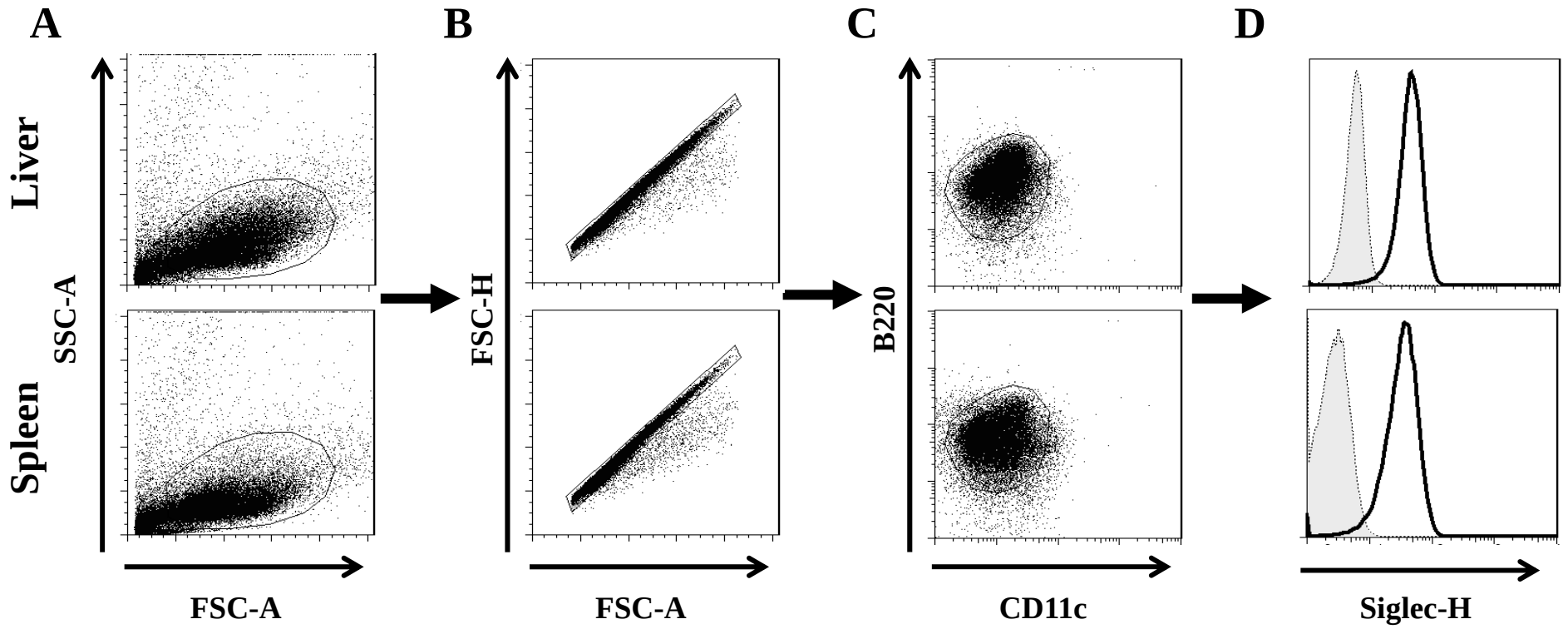
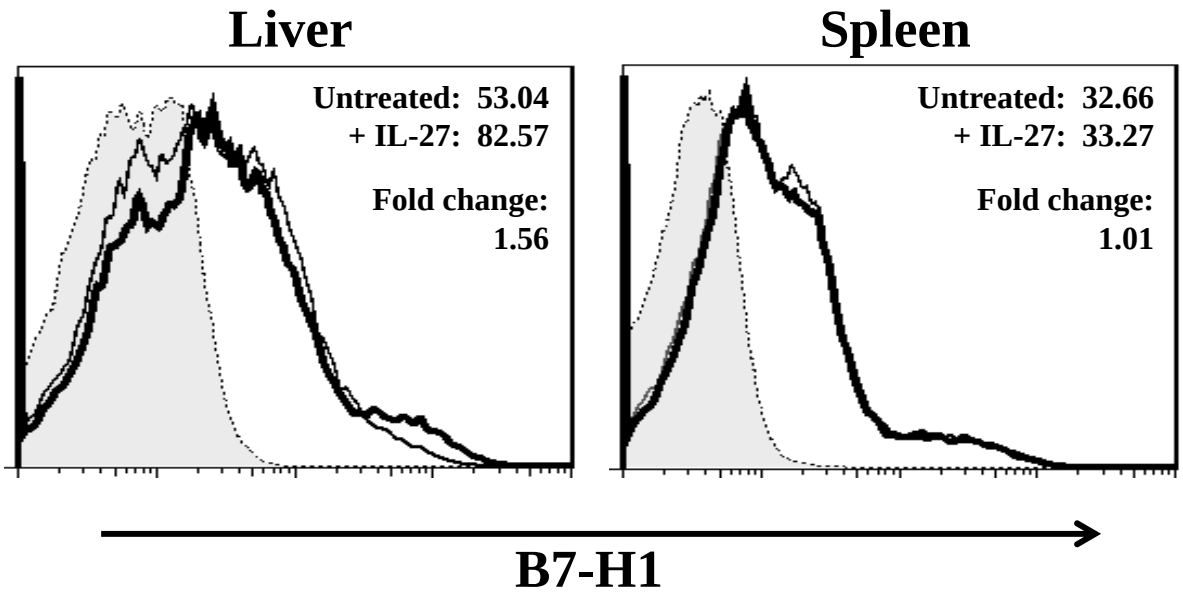
Suppl. Fig. 1



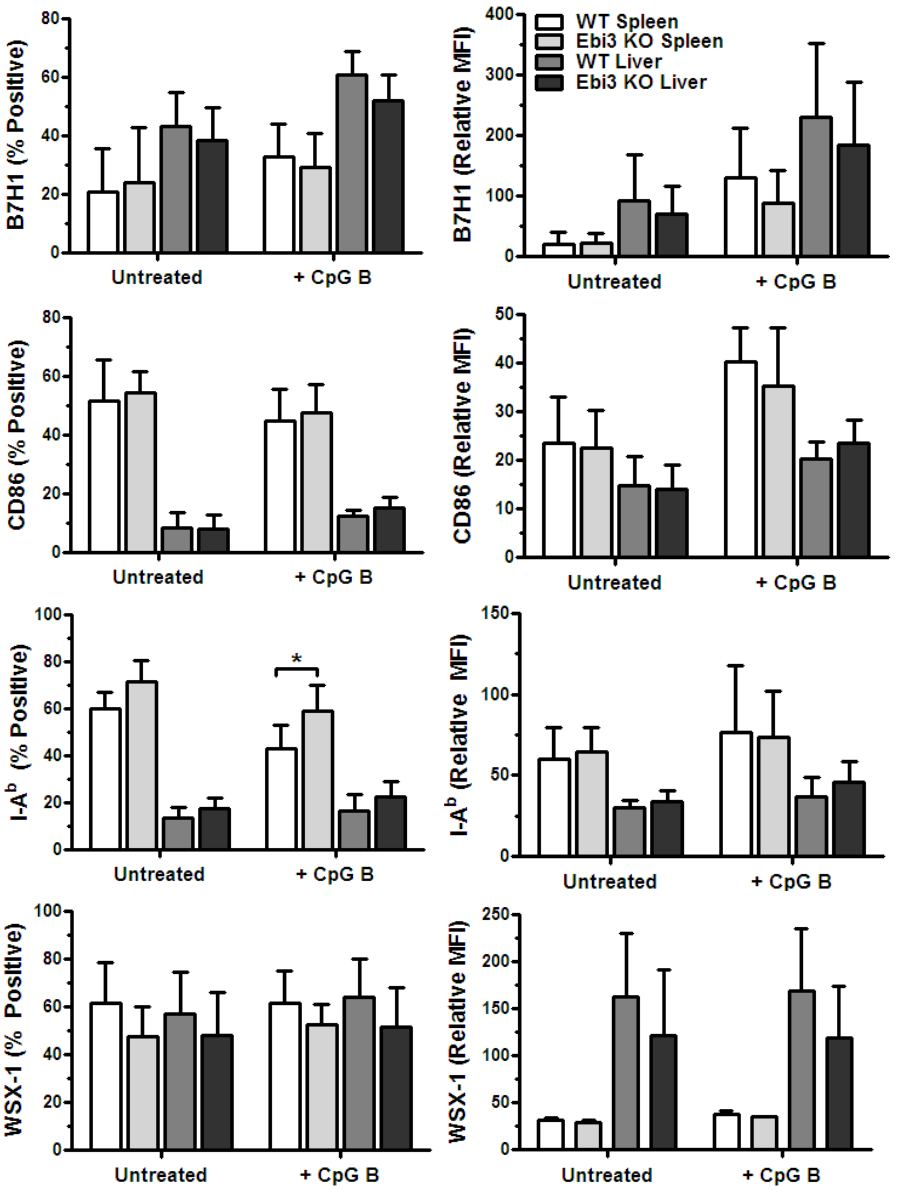
SUPPLEMENTAL FIGURE 1. Gating strategy for flow cytometric analysis of PDCA-1^{+} immunobead-purified liver and spleen pDC. For identification of pDC, cells were stained using antibodies against B220 and CD11c. *A*, First, a live cell gate was made using forward (FSC) versus side (SSC) scatter plot. Cell doublets were excluded using (*B*) FSC-Height (H) versus FSC-Area (A). Analysis of surface molecule expression was analyzed on B220^{+}CD11c^{low} cells (*C*), including the pDC-specific marker, Siglec-H (*D*).

Suppl. Fig. 2



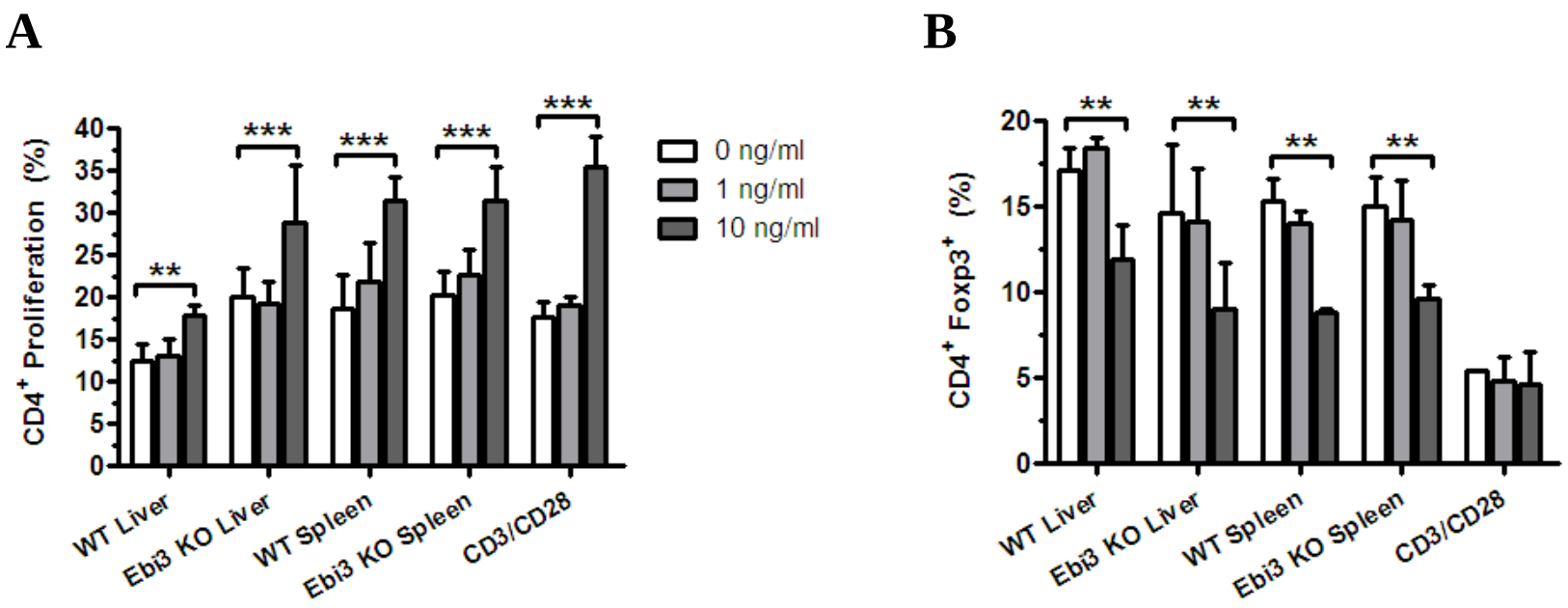
SUPPLEMENTAL FIGURE 2. Histograms depicting B7-H1 expression at baseline and following exposure to IL-27. Freshly-isolated liver and spleen PDCA-1^{+} pDC from Flt3L-mobilized, C57BL/6J WT mice were cultured in the presence of 25 μg/ml CpG B ODN for 18 h, collected and analyzed by 5-color flow cytometry. Surface expression of B7-H1 was analyzed on B220^{+}CD11c^{low} gated cells as previously described in Materials in Methods. Relative MFI of untreated (thin line) and IL-27-conditioned (bold line) liver and spleen pDC is shown, along with the fold change in B7-H1 MFI following exposure to IL-27.

Suppl. Fig. 3



SUPPLEMENTAL FIGURE 3. Ebi3$^{-/-}$ pDC are phenotypically similar to WT pDC in the steady state and following TLR9 stimulation. Freshly-isolated liver and spleen PDCA-1^{+} pDC from Flt3L-mobilized, WT or Ebi3$^{-/-}$ mice were cultured in the presence of 1 μg/ml CpG B ODN for 18 h, collected and analyzed by 5-color flow cytometry. Surface protein expression was analyzed on B220^{+}CD11c^{low} gated cells (Suppl. Fig. 1) as previously described in Materials in Methods. Relative MFI and percent positive cells were average from 3 independent experiments, * $p < 0.05$.

Suppl. Fig. 4



SUPPLEMENTAL FIGURE 4. Exogenous IL-27 directly enhances T cell proliferation and reduces Foxp3 expression when added directly to MLR. Freshly-isolated liver and spleen PDCA-1^{+} pDC from Flt3L-mobilized, C57BL/6J WT or Ebi3$^{-/-}$ mice were cultured with allogeneic BALB/c splenic T cells in the absence (white bars) or presence of 1 (light gray bars) or 10 (dark gray bars) ng/ml IL-27 for 3 d. Cultures were harvested and analyzed by flow cytometry for proliferation by CFSE dilution (*A*) and Foxp3 expression (*B).* T cells were also stimulated with plate-bound anti-CD3 and soluble CD28 (*A-B*). Results are an average of *n* =2 independent experiments, ** $p < 0.01$, *** $p < 0.001$.