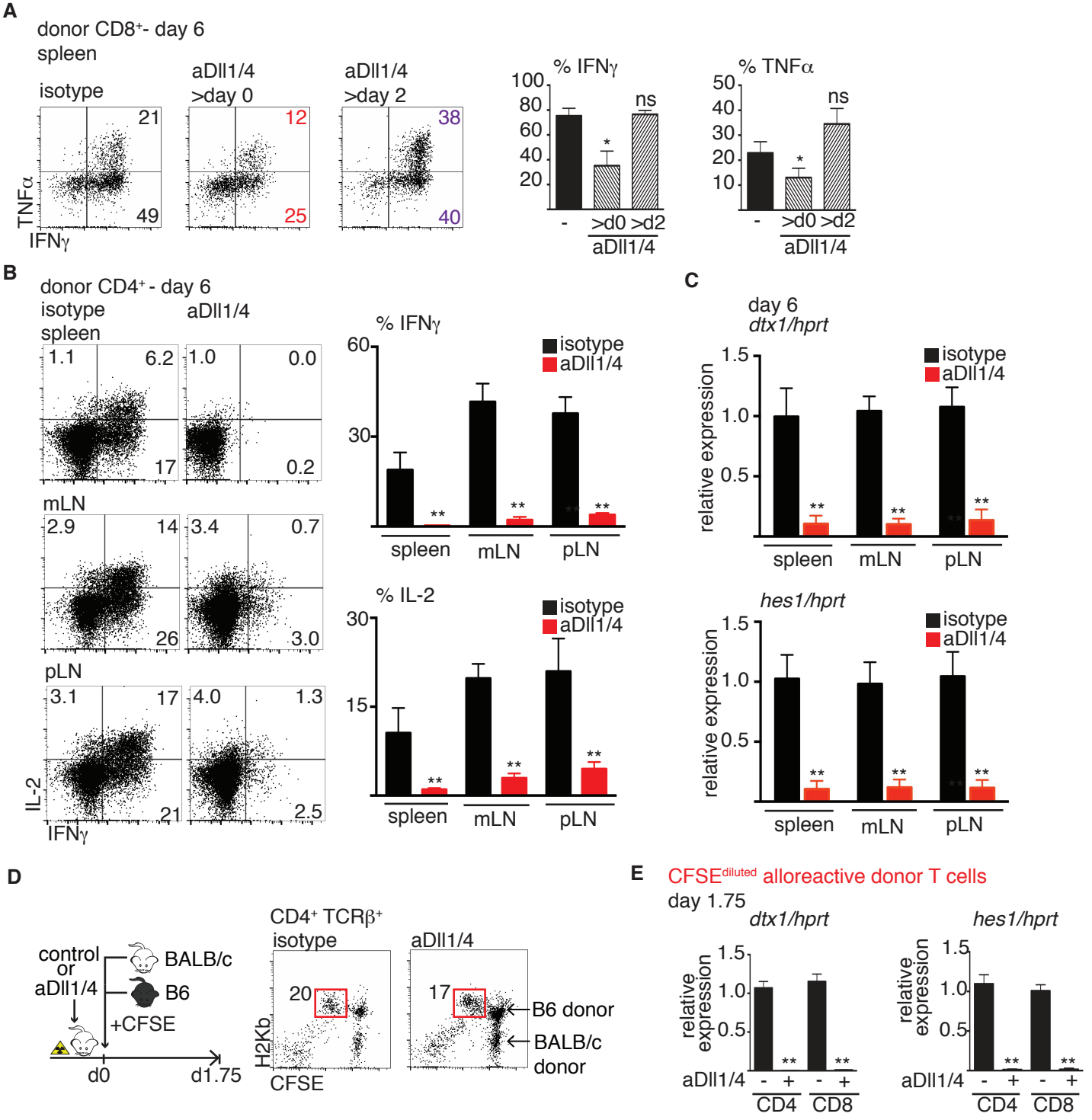
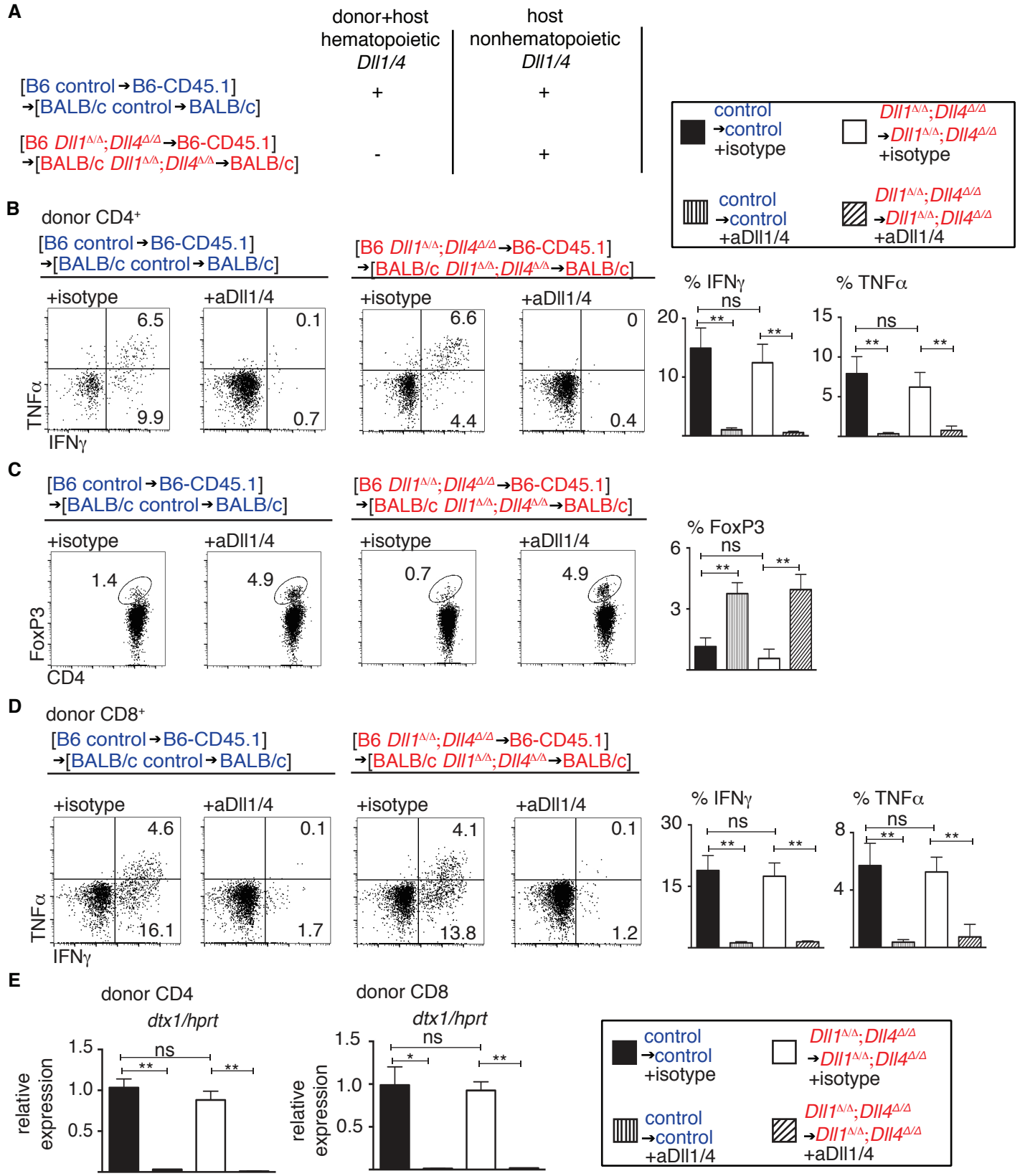
Supplemental Figure 1



Supplemental Figure 1. Impact of Notch blockade on proinflammatory cytokine production and Notch target gene expression in donor-derived T cells recovered from spleen and lymph nodes after transplantation.
A-B. Detection of intracellular cytokines in spleen-resident donor CD8+ T cells **(A)**, spleen-resident, mesenteric LN-resident (mLN), or peripheral LN-resident (pLN) donor CD4+ T cells **(B)** after anti-CD3/CD28 restimulation at day 6 post-transplantation (flow cytometry) (n = 5 mice/group). **C.** Abundance of *Dtx1* and Hes1 Notch target transcripts (qRT-PCR) in sort-purified CD4+ T cells from spleen, mLN, and pLN at day 6 post-transplantation. **D.** Experimental and flow cytometric strategy to isolate alloreactive donor CD4+ and CD8+ T cells at day 1.75 post-transplantation. Syngeneic BALB/c and allogeneic B6 splenocytes were simultaneously labeled with CFSE and co-injected into lethally irradiated (8.5 Gy) BALB/c mice. Divided CFSEdiluted B6 cells identified alloreactive T cells and were sort-purified. **E.** Abundance of *Dtx1* and *Hes1* Notch target gene transcripts (qRT-PCR) in sort-purified donor-derived CFSElow CD4+ and CD8+ T cells (n = 6 mice/group). *P<0.05, **P<0.01. ns=P>0.05 by unpaired two-tailed Student's t-test. Data are representative of at least 4 experiments, with error bars indicating SD.

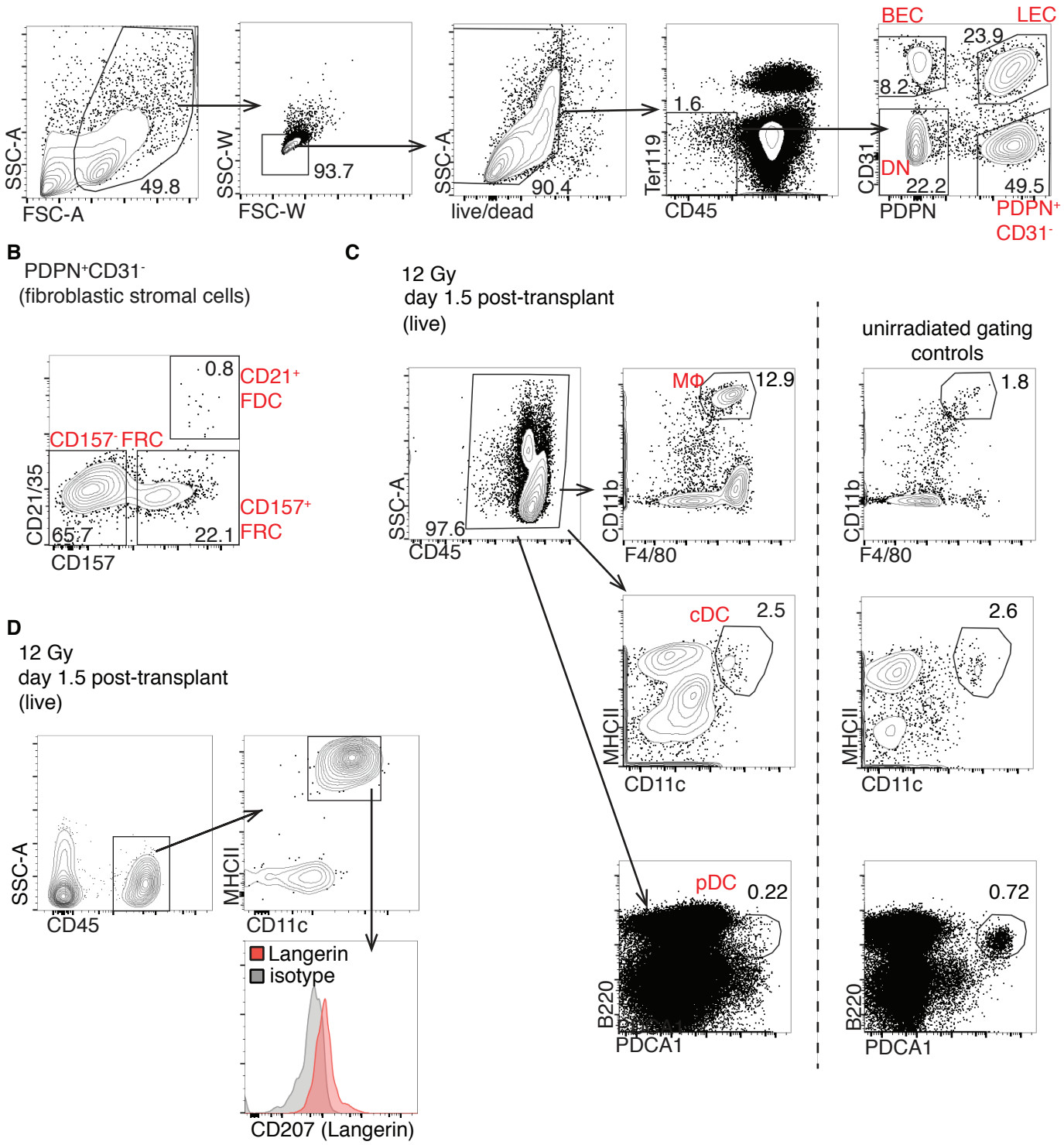
Supplemental Figure 2



Supplemental Figure 2. Both donor and host hematopoietic cells are dispensable as cellular sources of Delta-like Notch ligands in acute GVHD.

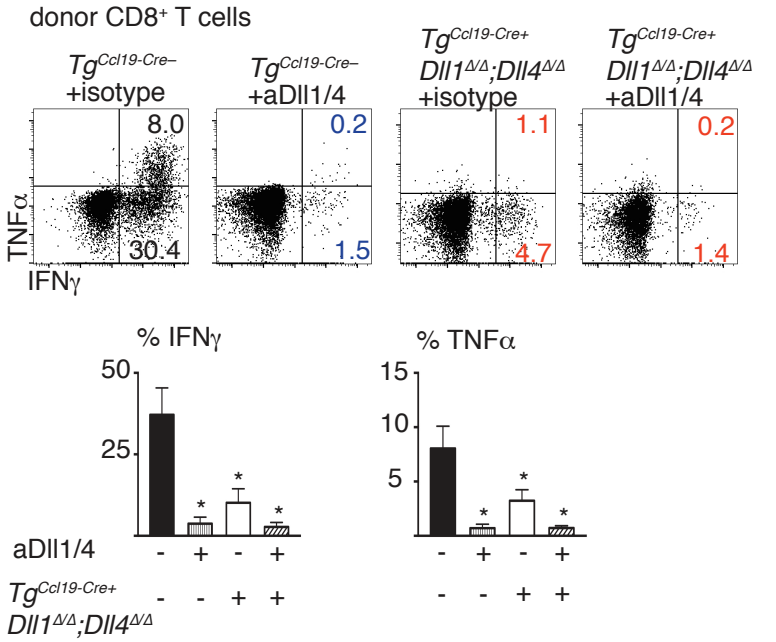
A. Experimental strategy. BALB/c bone marrow (BM) chimeras were generated by syngeneic transplantation of BALB/c poly(I:C)-induced control or *Tg*$^{Mx1\text{-}Cre+}$;*Dll1*$^{\Delta/\Delta}$;*Dll4*$^{\Delta/\Delta}$ T cell-depleted (TCD) BM into irradiated BALB/c recipients (with T cell depletion performed to remove preexisting mature T cells that may escape *Mx1-Cre*-mediated target gene excision). B6 BM chimeras were generated by syngeneic transplantation of B6 poly(I:C)-induced control or *Tg*$^{Mx1\text{-}Cre+}$;*Dll1*$^{\Delta/\Delta}$;*Dll4*$^{\Delta/\Delta}$ BM into irradiated B6-CD45.1 recipients. 5x10^6 TCD BM + 5x10^6 allogeneic splenocytes from B6 BM chimeras were transplanted into lethally irradiated (8.5 Gy) BALB/c BM chimeras. Recipient BALB/c BM chimeras were injected i.p. with isotype control or anti-Dll1/4 antibodies. **B.** Intracellular cytokines in donor CD4+ after anti-CD3/anti-CD28 stimulation at day 7 post-transplantation (n = 5 mice/group). **C.** Intracellular FoxP3 in donor CD4+ T cells at day 7 post-transplantation (n = 5 mice/group). **D.** Intracellular cytokines in donor CD8+ T cells after anti-CD3/anti-CD28 stimulation at day 7 post-transplantation (n = 5 mice/group). **E.** Abundance of *Dtx1* Notch target gene transcripts (qRT-PCR) in sort-purified donor CD4+ T cells or donor CD8+ T cells at day 7 post-transplantation (n = 5 mice/group). *P<0.05, **P<0.01, ns=P>0.05 by unpaired two-tailed Student's t-test with Sidak correction for multiple comparisons. Data are representative of at least 3 experiments, with error bars indicating SD.

Supplemental Figure 3



Supplemental Figure 3. Gating strategy for flow cytometric analysis of lymph node stromal cells and hematopoietic antigen-presenting cells post-irradiation.
Peripheral LNs (cervical, brachial, axial, inguinal) from lethally irradiated (12 Gy) mice receiving allogeneic BALB/c splenocytes were enzymatically digested into a single cell suspension (see **Materials and Methods**) and stained for flow cytometric analysis at day 1.5 post transplantation. **A.** Fibroblastic stromal cells were identified as CD45–Ter119–PDPN+CD31–, lymphatic endothelial cells (LECs) as CD45–Ter119–PDPN+CD31+, and blood endothelial cells (BECs) as CD45–Ter119–PDPN–CD31+ cells. **B.** Fibroblastic stromal cells were further subfractionated as CD157–CD21/35– fibroblastic reticular cells (FRCs), CD157+CD21/35– FRCs, and CD157+CD21/35+ follicular dendritic cells (FDCs). Macrophages (MΦ) were identified as F4/80+CD11b+, conventional dendritic cells (cDCs) as CD45+CD11c+MHCIIhi, and plasmacytoid dendritic cells (pDCs) as CD45+PDCA1+B220int. Data are representative of at least 4 experiments. **D.** Epidermal sheaths harvested from the ear of irradiated mice were collected at day 1.5 post transplantation and cultured for 2 days in GM-CSF and TNFα to mobilize hematopoietic cells. Langerhans cells were subsequently identified as CD45+CD11c+MHCIIhi displaying CD207 (Langerin) expression.

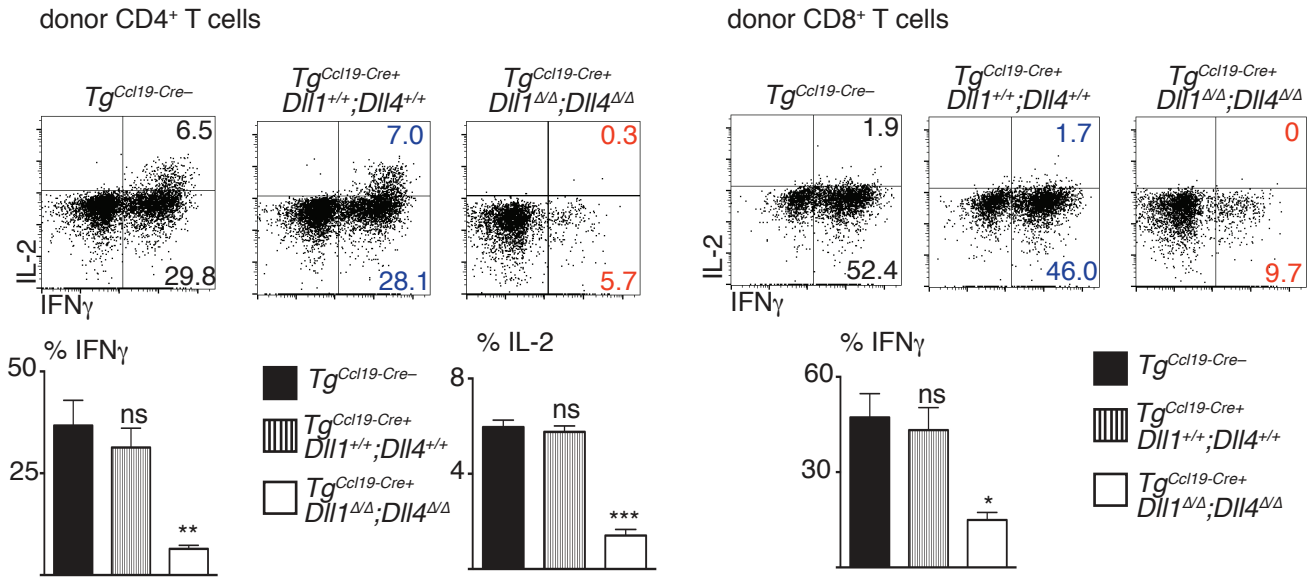
Supplemental Figure 4



Supplemental Figure 4. Impact of stromal cell-specific inactivation of Dll1/4 Notch ligand genes on proinflammatory cytokine production by CD8+ donor-derived T cells.

Detection of intracellular cytokines in donor CD8+ T cells after anti-CD3/anti-CD28 restimulation (day 6 post-transplantation, flow cytometry) (n = 5 mice/group). Control $Tg^{Ccl19\text{-}Cre–}$ recipient mice treated with isotype control antibodies were compared to $Tg^{Ccl19\text{-}Cre–}$ mice receiving anti-Dll1/4 antibodies vs. $Tg^{Ccl19\text{-}Cre+}$;$Dll1^{Δ/Δ}$;$Dll4^{Δ/Δ}$ mice treated with isotype control or anti-Dll1/4 antibodies. *P<0.05 by unpaired two-tailed Student's t-test with Sidak correction for multiple comparisons. Data are representative of at least 3 experiments, with error bars indicating SD.

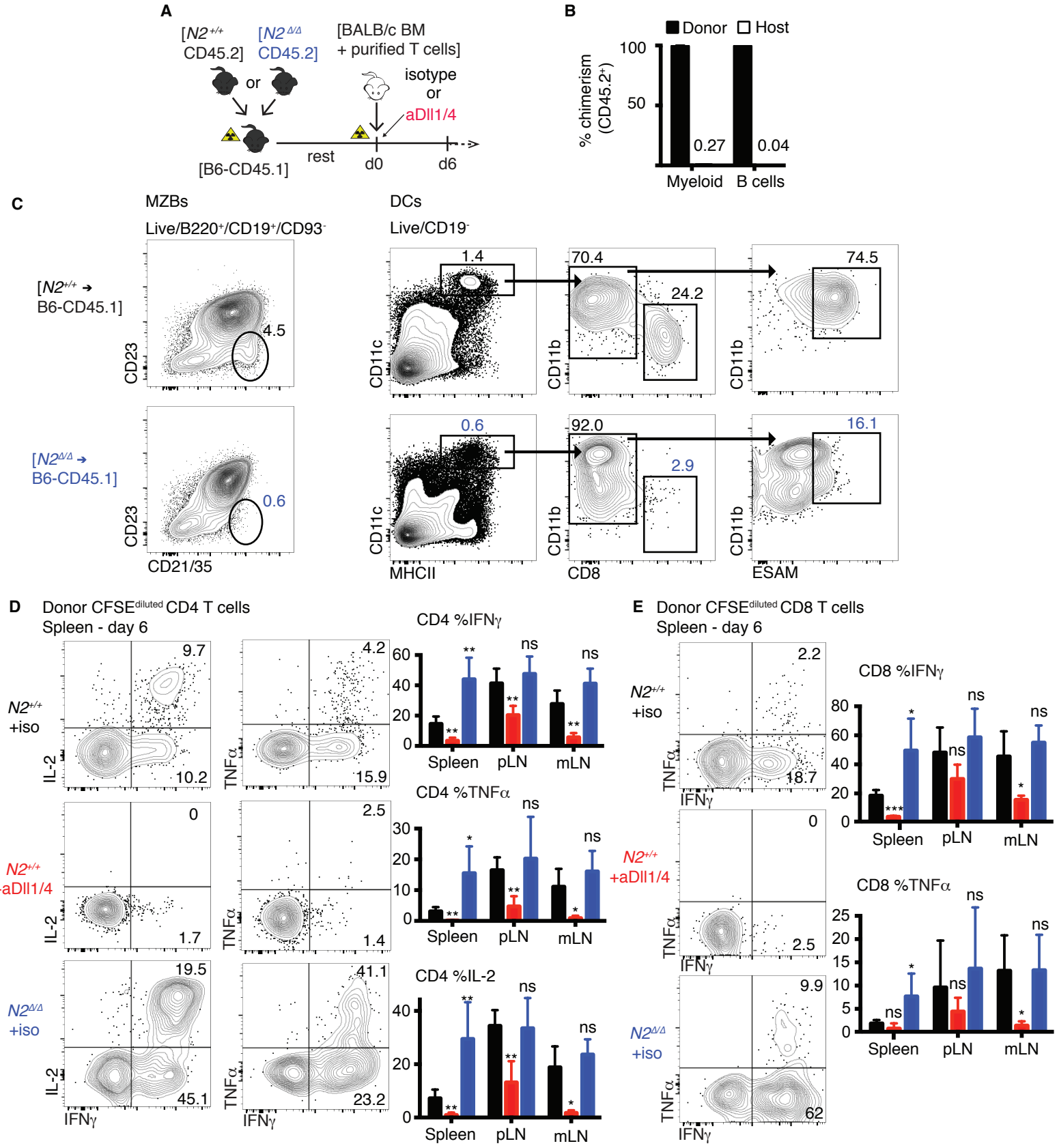
Supplemental Figure 5



Supplemental Figure 5. ***Ccl19-Cre*** **expression by itself has no impact on T cell alloreactivity after allogeneic bone marrow transplantation.**

10x10^6 TCD BM + 20x10^6 allogeneic BALB/c splenocytes were transplanted into lethally irradiated (12 Gy) B6 control *Tg$^{Ccl19\text{-}Cre–}$*, *Tg$^{Ccl19\text{-}Cre+}$;Dll1$^{+/+}$;Dll4$^{+/+}$* or *Tg$^{Ccl19\text{-}Cre+}$;Dll1$^{Δ/Δ}$;Dll4$^{Δ/Δ}$* mice. Detection of intracellular cytokines in donor CD4+ T cells and CD8+ T cells after anti-CD3/anti-CD28 stimulation at day 6 post-transplantation (flow cytometry) (n = 5 mice/group). *P<0.05, **P<0.01, ***P<0.001, ns=P>0.05 by unpaired two-tailed Student's t-test with Sidak correction for multiple comparisons. Data are representative of 2 experiments, with error bars indicating SD

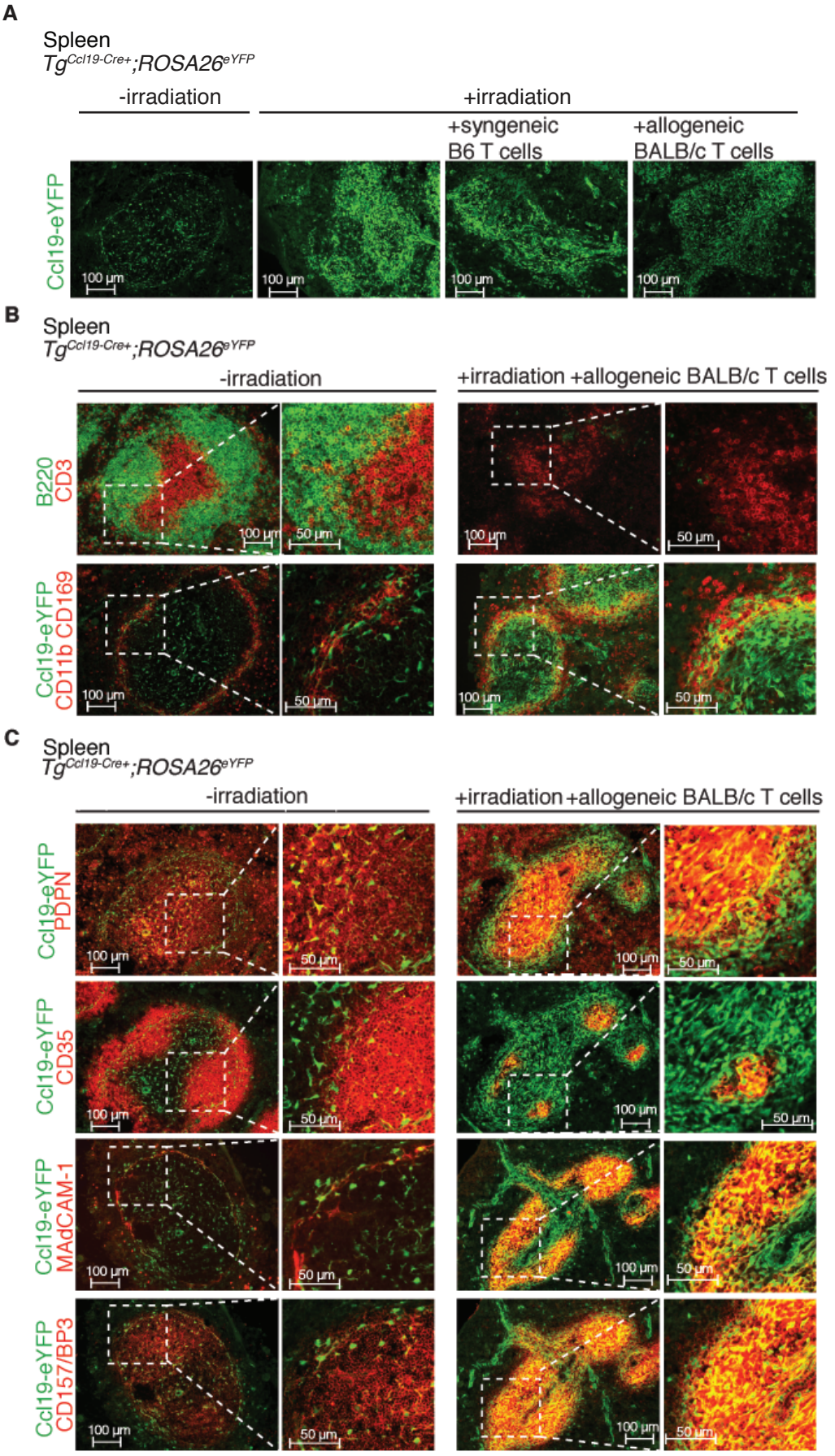
Supplemental Figure 6



Supplemental Figure 6. Loss of Notch2-dependent host hematopoietic subsets does not explain the decreased cytokine production of alloreactive T cells with Delta-like1/4 blockade.

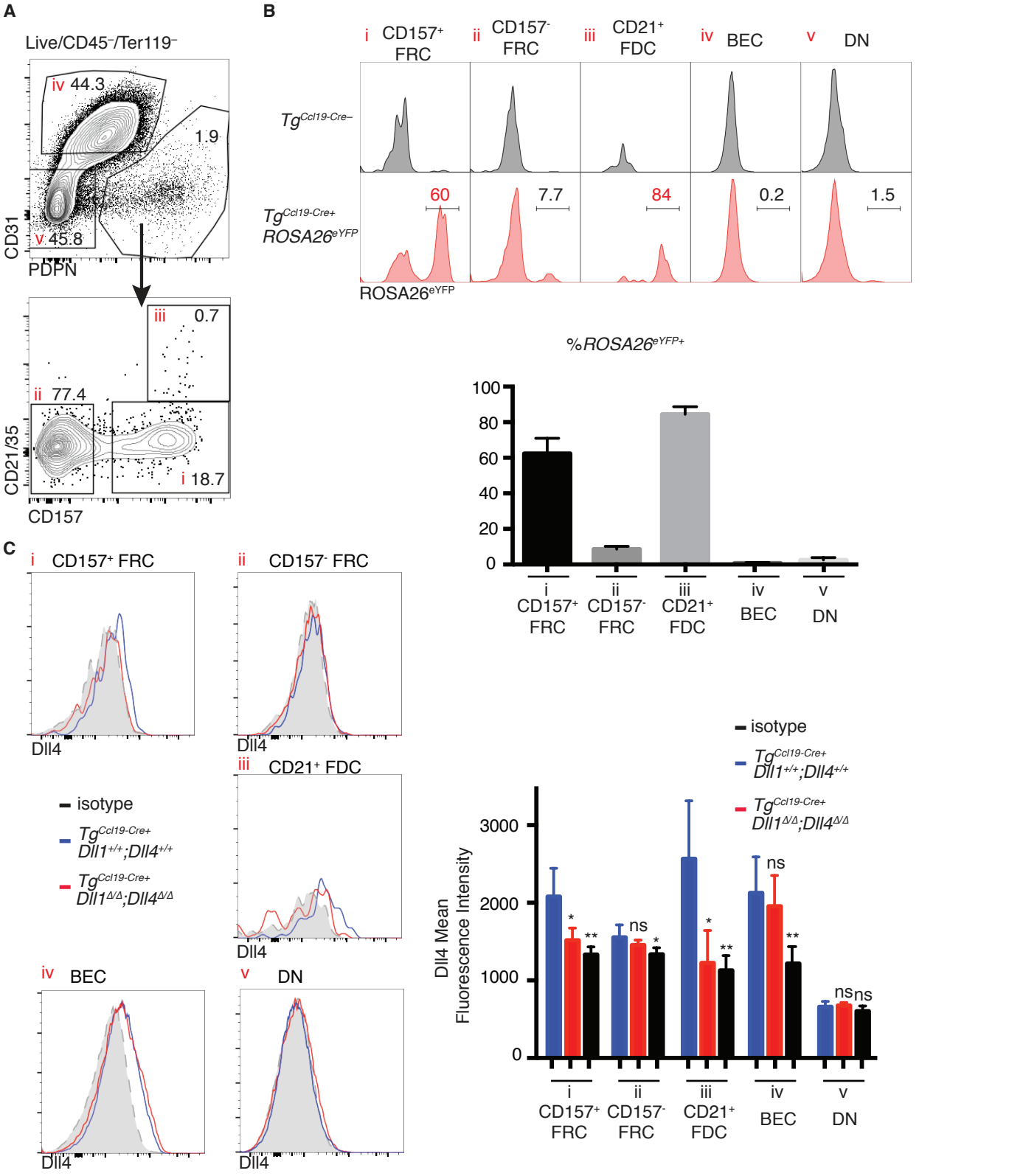
A. Experimental strategy. Bone marrow (BM) chimeras were generated via transplantation of syngeneic B6-CD45.2+ poly(I:C)-induced $Tg^{Mx1\text{-}Cre-};N2^{f/f}$ littermate control or $Tg^{Mx1\text{-}Cre+};N2^{\Delta/\Delta}$ BM into irradiated B6-CD45.1 recipients to generate $N2^{+/+}$→B6-CD45.1 and $N2^{\Delta/\Delta}$→B6-CD45.1 chimeras, respectively. After reestablishment of steady-state hematopoiesis (28 weeks post-transplantation), BM chimeras were subjected to a second allogeneic transplant with BALB/c bone marrow and purified T cells, with or without systemic Dll1/4 blockade. **B.** Donor chimerism (frequency of CD45.2+ donor cells) of experimental mice is indicated in blood populations >12 weeks after transplantation. **C.** Analysis of Notch2-dependent splenic populations in $N2^{+/+}$→B6-CD45.1 and $N2^{\Delta/\Delta}$→B6-CD45.1 chimeras. MZBs = marginal zone B cells. Data are representative of 3 mice per group transplanted with the same BM as mice used experimentally in **(D)**. **D.** Intracellular cytokine production by donor CD4+ T cells harvested from spleen, mesenteric lymph node (mLN), or peripheral lymph nodes (pLN) after anti-CD3/anti-CD28 restimulation at day 6 post-transplantation (n = 5 mice/group). **E.** Intracellular cytokine production by donor CD8+ T cells as in **(D)** (n = 5 mice/group). *P<0.05, **P<0.01, ***P<0.001, ns=P>0.05 by unpaired two-tailed Student's t-test with Sidak correction for multiple comparisons. Errors bars represent SD.

Supplemental Figure 7



Supplemental Figure 7. Impact of allogeneic bone marrow transplantation on spleen architecture.
A-C. Immunofluorescence microscopy of spleen cryosections from *Tg*$^{Ccl19\text{-}Cre+}$*;ROSA26*^{eYFP} reporter mice stained for GFP only **(A)**, B220 and CD3 **(B, top panel)**, GFP, CD11b, and CD169 **(B, bottom panel)**, GFP and podoplanin/gp38 **(C, first panel)**, GFP and CD35 **(C, second panel)**, GFP and MAdCAM1 **(C, third panel)**, or GFP and CD157/BP3 **(C, fourth panel)**. Cryosections were prepared from unirradiated or lethally irradiated (12 Gy) mice receiving allogeneic BALB/c CD4+ T cells at day 1.5 post-transplantation. The high intensity of CD35 staining in the absence of irradiation is due to expression of CD21/35 by B cells. After irradiation and depletion of radiation-sensitive B cells, CD35 staining highlighted stromal cells in the B cell follicles consistent with follicular dendritic cells. Data are representative of 2 experiments.

Supplemental Figure 8



Supplemental Figure 8. *Ccl19-Cre+* lineage-traced stromal cells in the spleen express Dll4 during acute GVHD.
A. Splenic stromal cell subsets. $Tg^{Ccl19\text{-}Cre+};ROSA26^{eYFP}$ or $Tg^{Ccl19\text{-}Cre-}$ mice were lethally irradiated and transplanted with $10x10^6$ BALB/c bone marrow cells plus $20x10^6$ allogeneic BALB/c splenocytes. Recipient spleens were collected at 12 hours post-transplantation and enzymatically digested (see **Materials and Methods**) to be analyzed by flow cytometry as in **Figure 3**. Plots represent concatenated data from 5 $Tg^{Ccl19\text{-}Cre+};ROSA26^{eYFP}$ mice. **B.** eYFP expression in spleen-resident stromal subsets. Roman numerals refer to the populations identified in **(A)**. Summary data represent these mice as individual replicates (n=5 mice/group). **C.** Surface Dll4 expression in spleen resident stromal cell subsets from control $Tg^{Ccl19\text{-}Cre+};Dll1^{+/+};Dll4^{+/+}$ and $Tg^{Ccl19\text{-}Cre+};Dll1^{\Delta/\Delta};Dll4^{\Delta/\Delta}$ mice. Histograms represent concatenated data from n=5 $Tg^{Ccl19\text{-}Cre+};Dll1^{+/+};Dll4^{+/+}$ and n=4 $Tg^{Ccl19\text{-}Cre+};Dll1^{\Delta/\Delta};Dll4^{\Delta/\Delta}$ mice. Bar graphs represent these mice as individual replicates. Mean fluorescence intensity of Dll4 expression in stromal subsets were compared between $Tg^{Ccl19\text{-}Cre+};Dll1^{+/+};Dll4^{+/+}$ and $Tg^{Ccl19\text{-}Cre+};Dll1^{\Delta/\Delta};Dll4^{\Delta/\Delta}$, as well as isotype staining controls by unpaired two-tailed Student's t-test with Sidak correction for multiple comparisons. *P<0.05, **P<0.01, ns=P>0.05. Errors bars indicate SD.