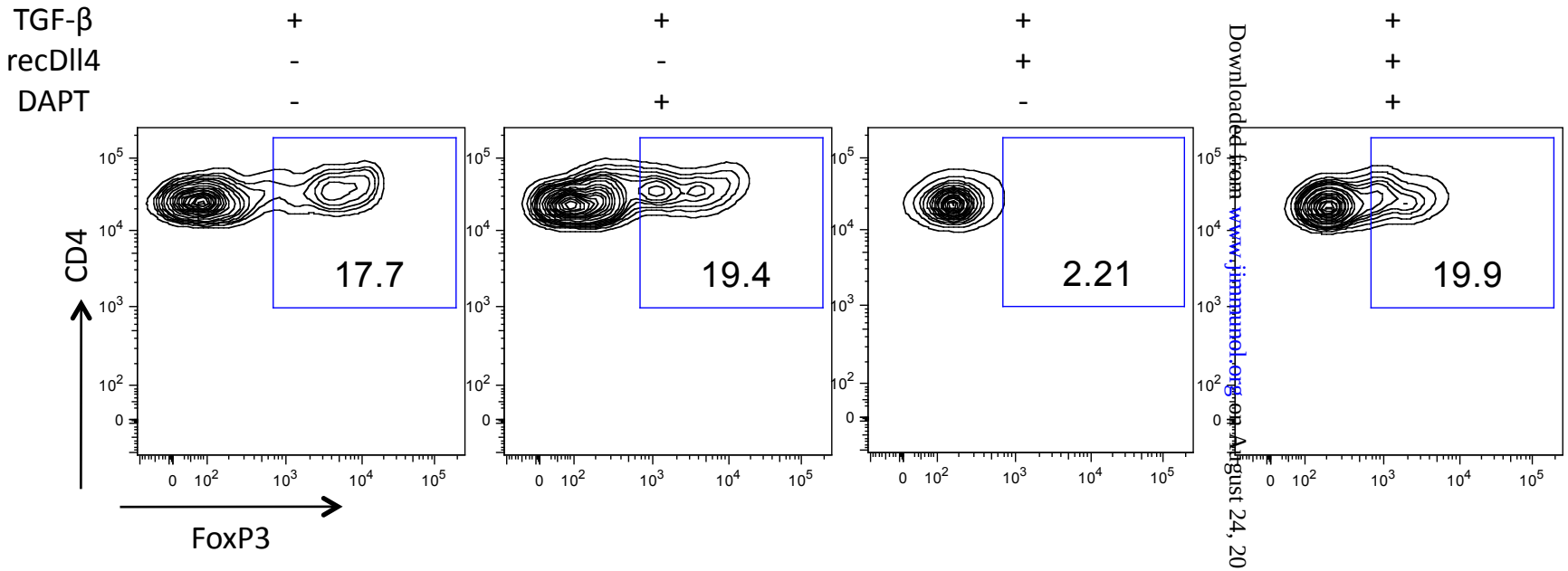

Supplemental Figure S1. Dll4 inhibition of Treg development is Notch dependent

Total CD4⁺ T cells were FACS sorted from naïve FoxP3.GFP.KI mice and stimulated *in vitro* with plate bound anti-CD3 and anti-CD28 (1 ug/ml) in the presence of plate bound recombinant Dll4 (2 ug/ml) and soluble TGF-ß (3 ng/ml). DAPT (1 uM), a gamma-secretase inhibitor that is also used to block notch signaling was added where indicated. IL-2 (20 ng/ml) was added to the culture medium on day two of culture. Flow cytometry analysis of CD4 and FoxP3 on day 4 of culture show that while treatment with recDll4 inhibits Treg expansion in vitro, DAPT abrogates this effect, suggesting that recDll4 inhibition of Treg development is Notch dependent. Results are representative of two independent experiments.

Figure S1.
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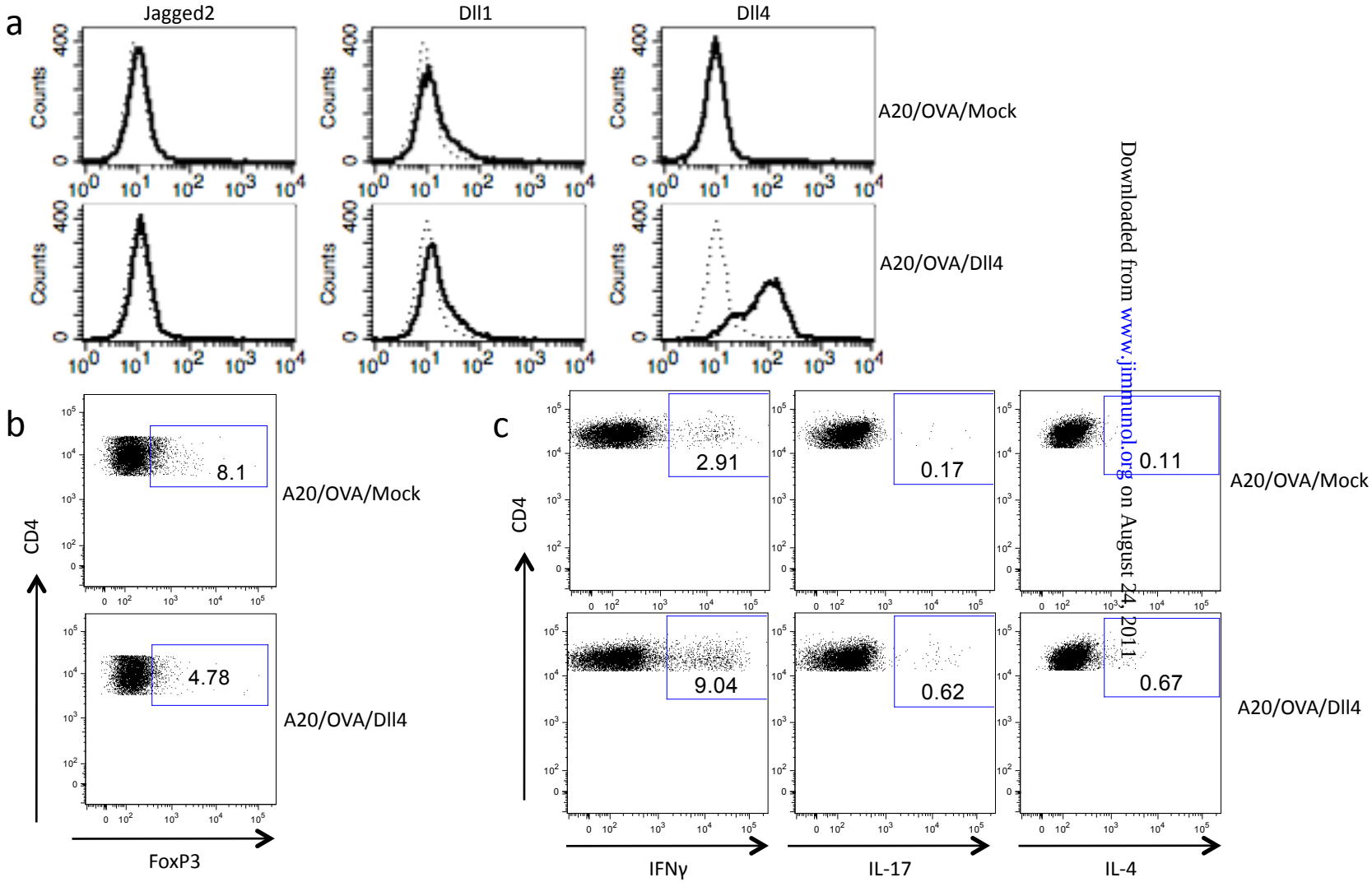

Supplemental Figure S2. Dll4 expression on A20/OVA Dll4

(**a**) Dll4 over-expressing A20/OVA B cells or Mock A20/OVA B cells were stained for their surface expression of Jagged2, Dll1 and Dll4 showing the high specificity of the surface Dll4 expression. (**b,c**) Naïve OVA-specific T cells were isolated by MACS sorting from splenocytes of naïve OVA-TCR^{tg} (DO11.10) mice and incubated with irradiated Dll4 over-expressing A20/OVA B cells or Mock A20/OVA B cells (1:2 ratio) in complete culture medium in the presence of OVA(323-339) peptide (0.2ug/ml). No recombinant cytokines were added. After one week of culture, cells were re-stimulated with PMA/ionomycin in the presence of monensin for 4 hours. Flow cytometry analysis of IFN-γ, IL-17, IL-4 and FoxP3 producing OVA specific T cells shows that under neutral condition Dll4 promotes expansion of IFN-γ producing cells (**c**) while suppressing Treg expansion (**b**).

Figure S2.
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