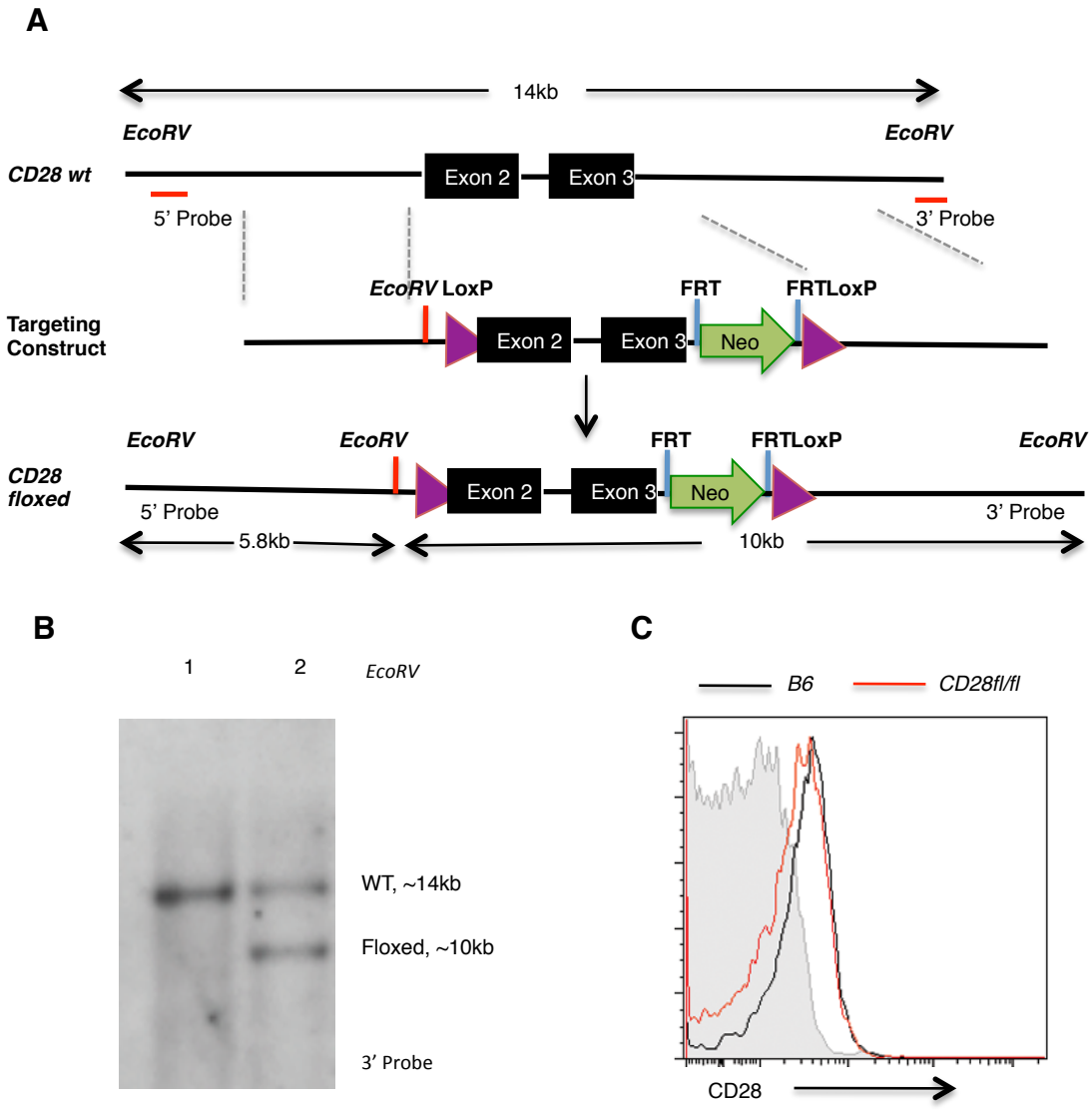


Figure S1: Generation and characterization of CD28 conditional knockout mice. **A**. Targeting construct designed to generate $CD28^{flox/flox}$ mice. **B**. Southern-blot to detect the floxed CD28 allele. Tail DNA was digested with EcoRV and electrophoresed on an agarose gel. The 3' probe shown in panel A was used to detect the floxed CD28 allele. Lane 1, B6 wild type mouse. Lane 2, $CD28^{flox/+}$ mouse. **C**. CD28 expression in $CD28^{flox/flox}$ mice. Gray line, $CD28^{-/-}$ mouse; black line, B6 wild type mouse; red line, $CD28^{flox/flox}$ mouse (all plots gated on $CD4^{+}$ cells).

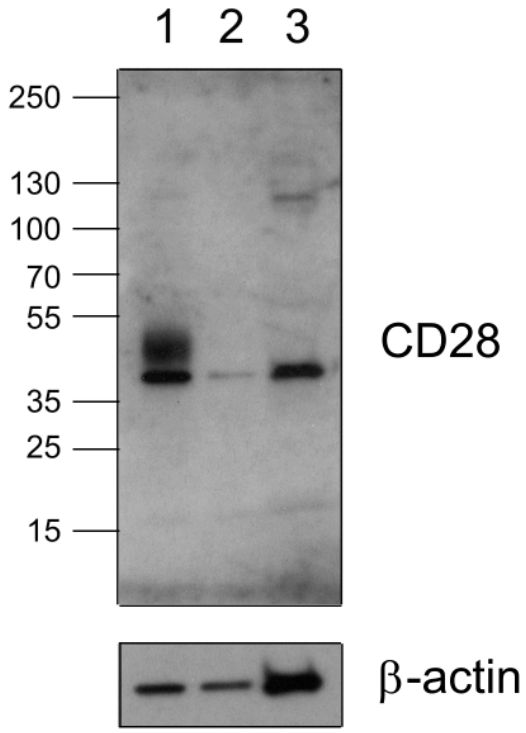
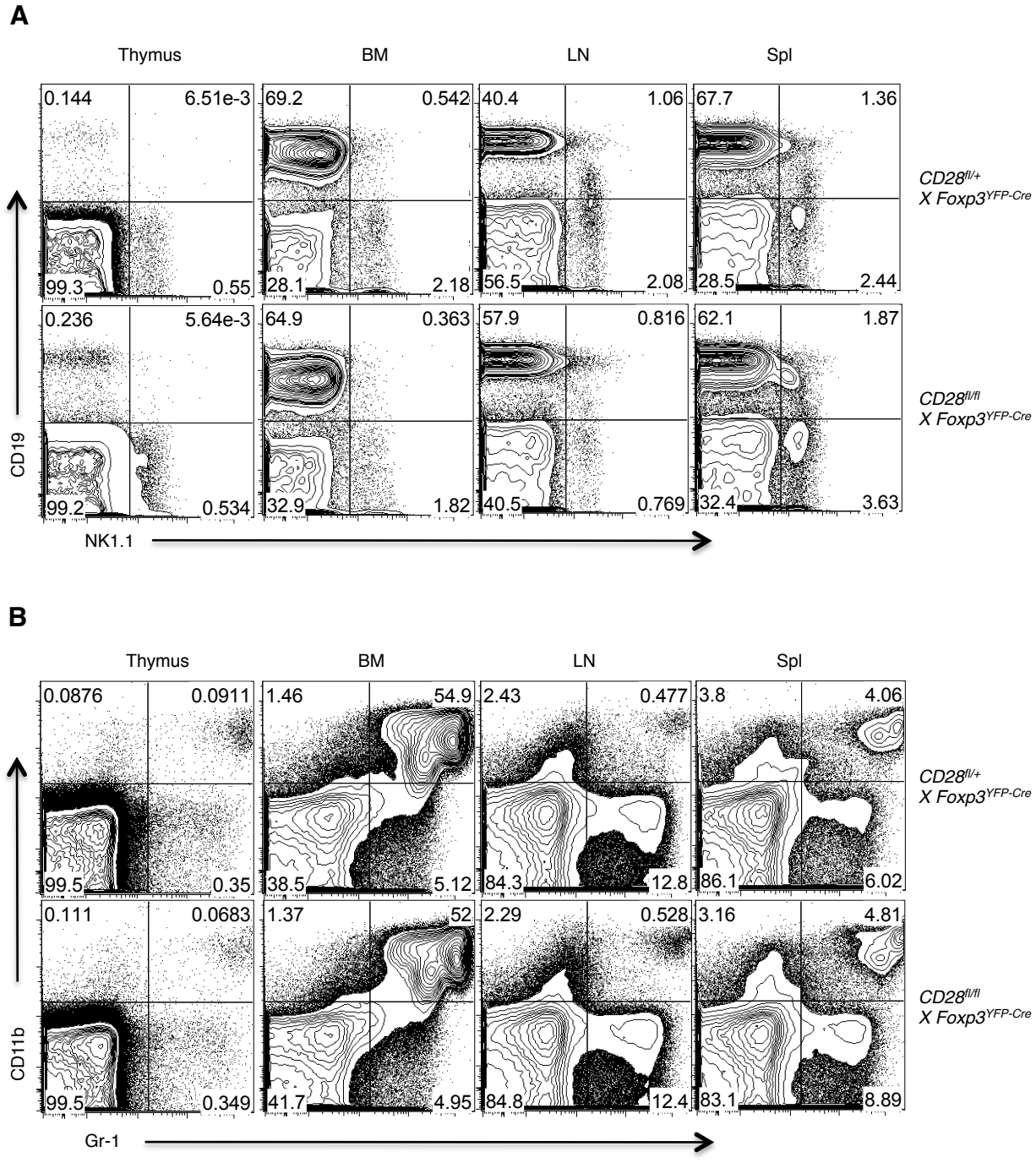


Figure S2: Sorted WT or CD28-DTregs were lysed and CD28 was detected with an antibody to the c-terminal portion of CD28. $CD4^+$ T cells from a $CD28^{-/-}$ mouse were used as control. Full length CD28 protein is the upper band which runs at 44kD and is only present in lane 1. The lower band in each lane is non-specific.


A
Thymus
BM
LN
Spl
0.144
6.51e-3
99.3
0.55
69.2
0.542
28.1
2.18
40.4
1.06
56.5
2.08
67.7
1.36
28.5
2.44
CD28fl/+
X Foxp3YFP-Cre
0.236
5.64e-3
99.2
0.534
64.9
0.363
32.9
1.82
57.9
0.816
40.5
0.769
62.1
1.87
32.4
3.63
CD28fl/fl
X Foxp3YFP-Cre
CD19
NK1.1
B
Thymus
BM
LN
Spl
0.0876
0.0911
99.5
0.35
1.46
54.9
38.5
5.12
2.43
0.477
84.3
12.8
3.8
4.06
86.1
6.02
CD28fl/+
X Foxp3YFP-Cre
0.111
0.0683
99.5
0.349
1.37
52
41.7
4.95
2.29
0.528
84.8
12.4
3.16
4.81
83.1
8.89
CD28fl/fl
X Foxp3YFP-Cre
CD11b
Gr-1

C

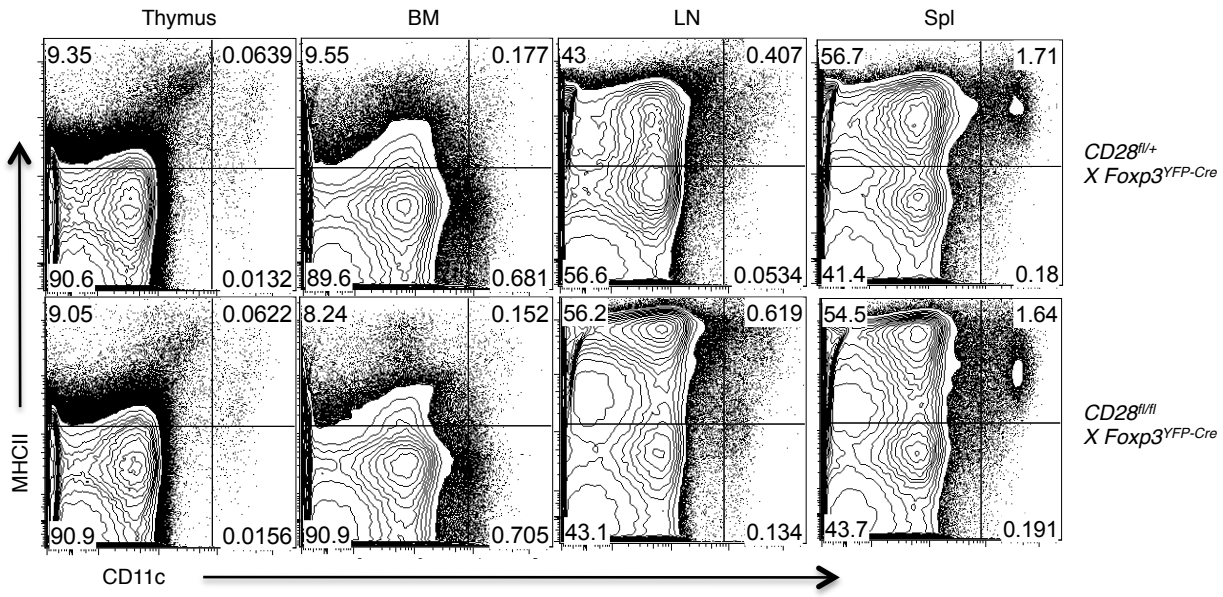


Figure S3: Characterization of B cells, NK cells, myeloid cells and dendritic cells in CD28-DTreg mice. Thymus, BM, LN and spleen from 5 week old mice and littermate controls were stained with markers for B and NK cells (**A**), myeloid cells (**B**) and dendritic cells (**C**). Data are representative of 3 independent experiments.

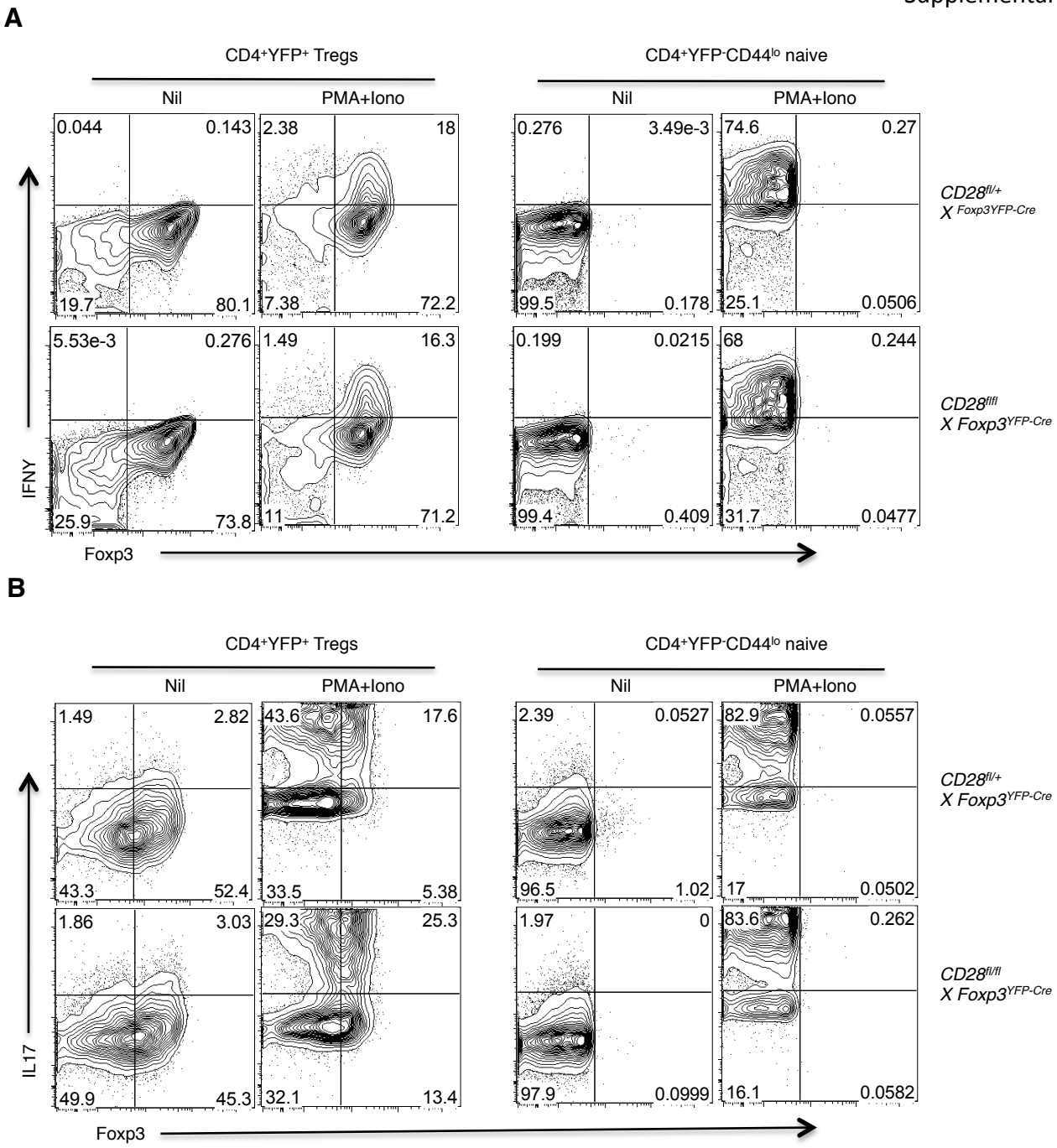


Figure S4: CD28-DTregs demonstrate stability in vitro. **A & B** Sorted CD28-sufficient (top panels) or CD28-DTregs (bottom panels) from 4-5 week old mice were cultured for 4 days under Th1 (**B**) or Th17 (**C**) differentiation conditions and then restimulated for 5 hours with PMA plus ionomycin. Naïve cells were included as positive controls. Data are representative of 3 experiments.