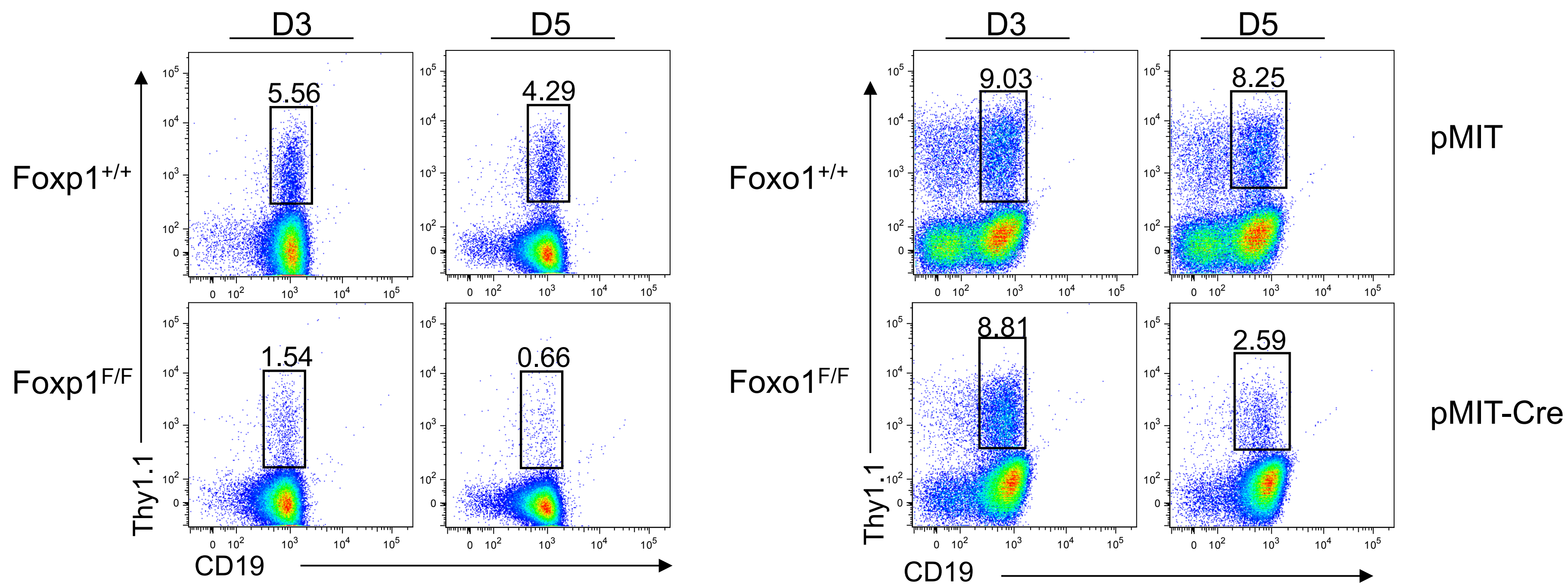
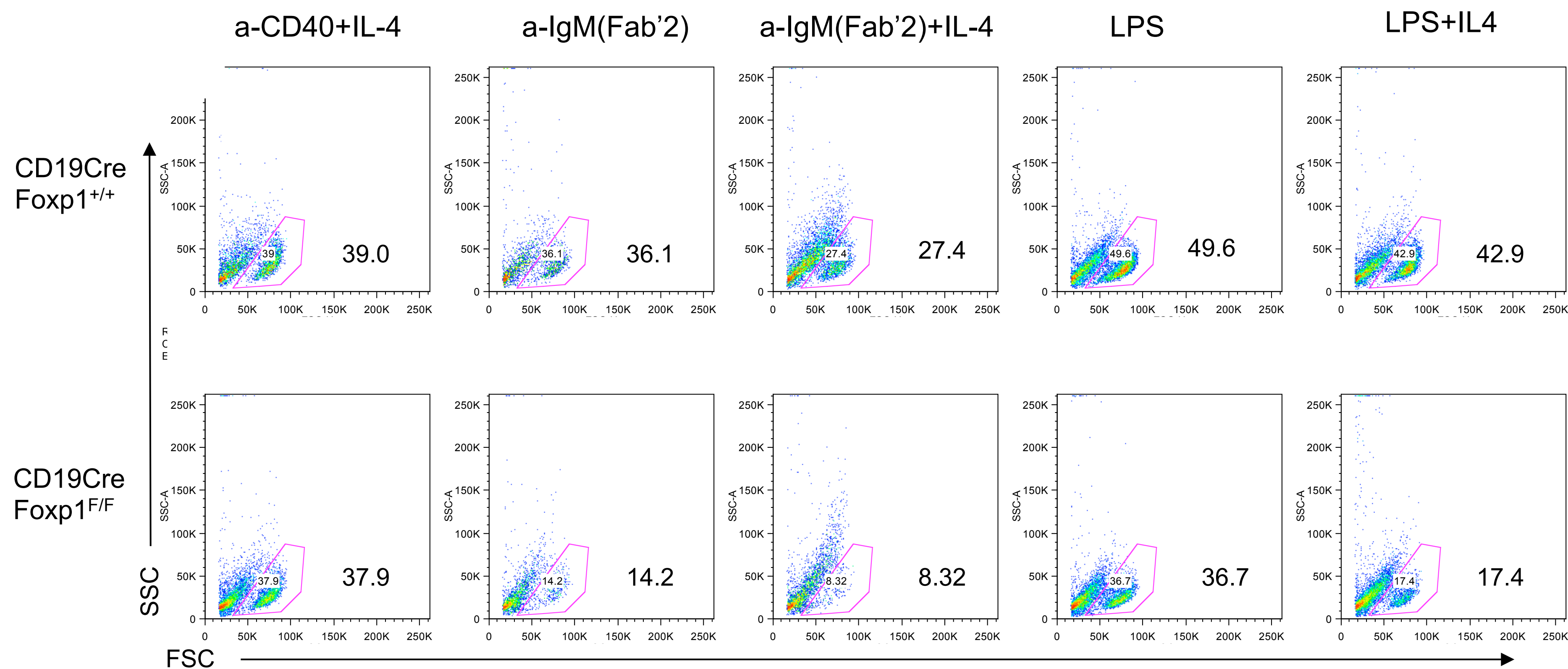
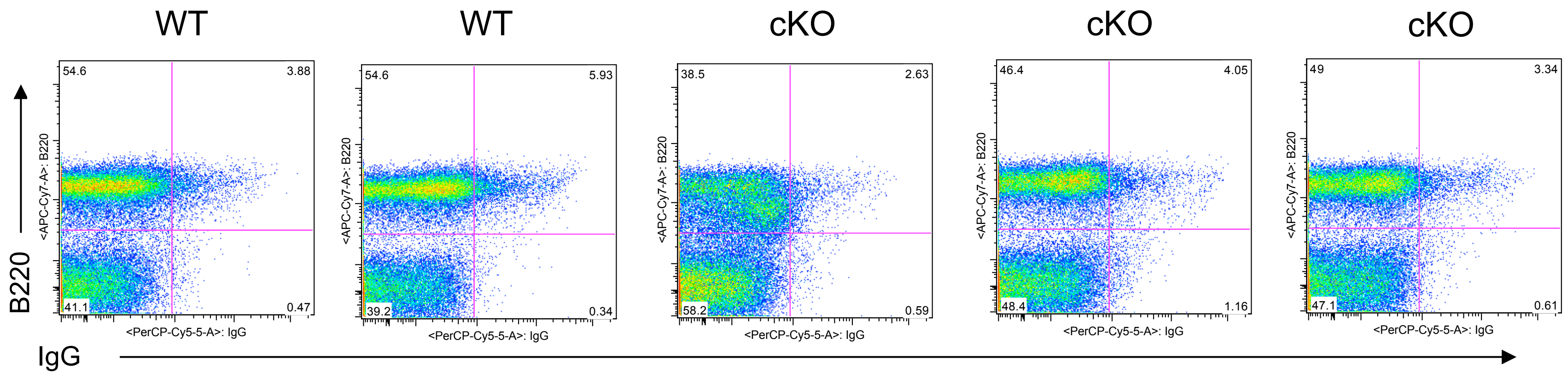


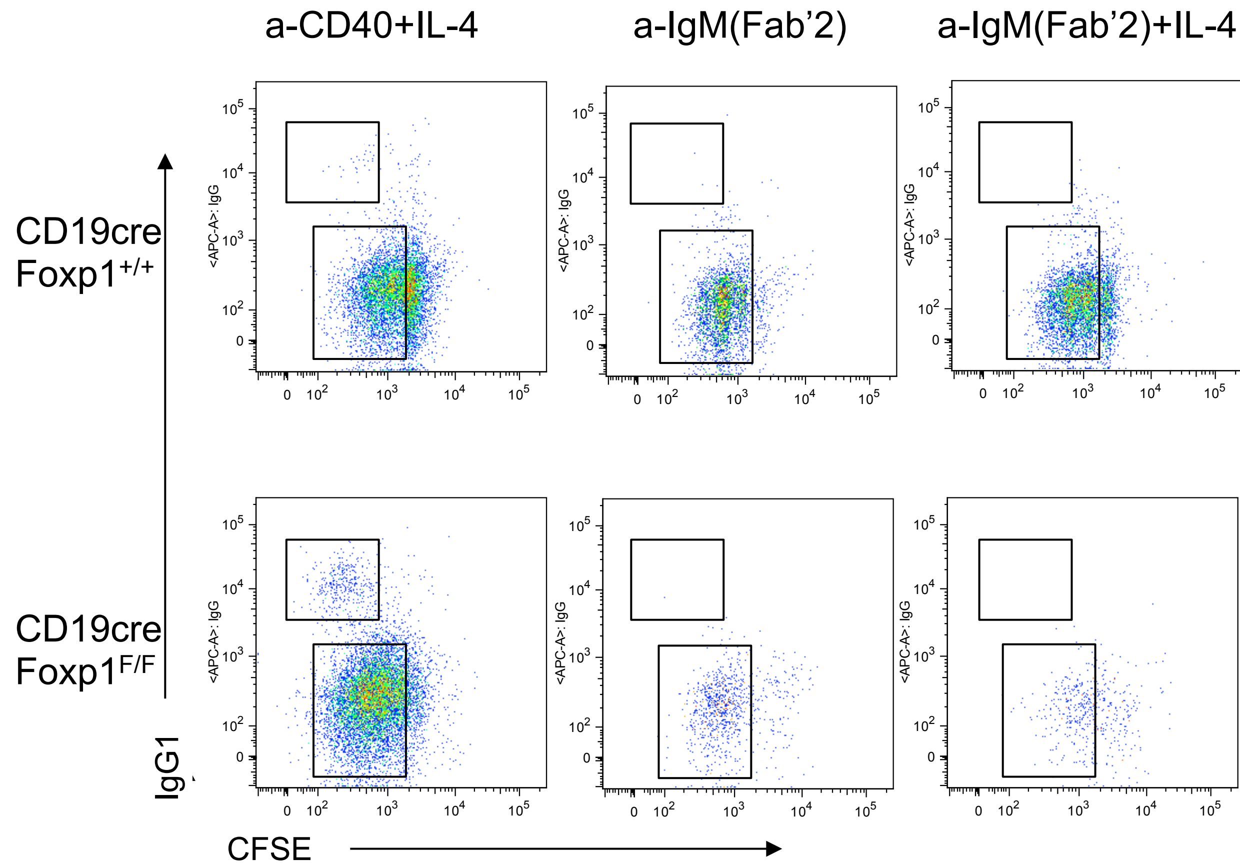
S-Figure 1. Efficient deletion of Foxo1 and Foxp1 floxed pro-B cells. B cells were positively selected from bone marrow with B220-labeled magnetic beads (Miltenyi) as described in Materials and Methods. Cells were treated with IL-7 (5 ng/ml), and following 2 days culture, were superinfected with either empty virus (MIT (mouse stem cell virus–internal ribosomal entry site–Thy-1.1)) or MIT-Cre. Cells were returned to culture with IL-7 and were analyzed 2d and 5d later by flow cytometry.. Both Foxp1 and Foxo1 were deleted efficiently from BM.



S-Figure 2. FOXP1-deficient B cells expanded normally to proliferative stimuli with the exception of CD40+IL4. Cd19Cre/Foxp1F/F cKO and control FO B cells were purified by depletion of Cd43+ cells from spleens of control and cKO mice. Cells were analyzed 4 days after culture with 10 ug/mL of anti-IgM (Fab'2) or LPS alone or in combination with 10 ng/mL of IL-4 or with a-Cd40+IL4. Proliferating cells were gated based on FSC vs. SSC in which an increase in size indicates blasting and cell expansion.



S-Figure 3. Prior to immunization, Cd19Cre/Foxp1F/F mutants expressed equivalent, modest levels of total IgG (IgG1, 2A, and 2B) on their surfaces. FO splenic B cells, purified as detailed in S-Fig.2 from Foxp1F/F;Cd19-Cre CKO (CKO lanes 2, 3 and 5) and matched controls (CT1 and CT4) were stained with allophycocyanin-conjugated anti-Cd19 (ID3; BD Pharmingen) and biotinylated anti-IgG2a–IgG2b (R2-40) (BD Pharmingen) and then were assessed by FACS for expression.



S-Figure 4. In vitro Cd19Cre/Foxp1F/F CKO mutant FO B cells proliferated normally and expressed higher surface levels of IgG1 than controls. Cd43+ FO B cells from spleens of control and Foxp1F/F;CD19-Cre CKO mutant mice were labeled with CFSE for analysis of cell proliferation 4 days after culture with 10 µg/mL of anti-IgM (Fab'2), with LPS alone or in combination with 10 ng/mL of IL-4 or with anti-Cd40+IL4. Cultured cells were harvested and cell proliferation was tracked by CFSE dilution (bottom gate) and on the induction of IgG1 class switch as determined by staining with anti–mouse IgG1 (top gate, A85-1; BD Pharmingen).