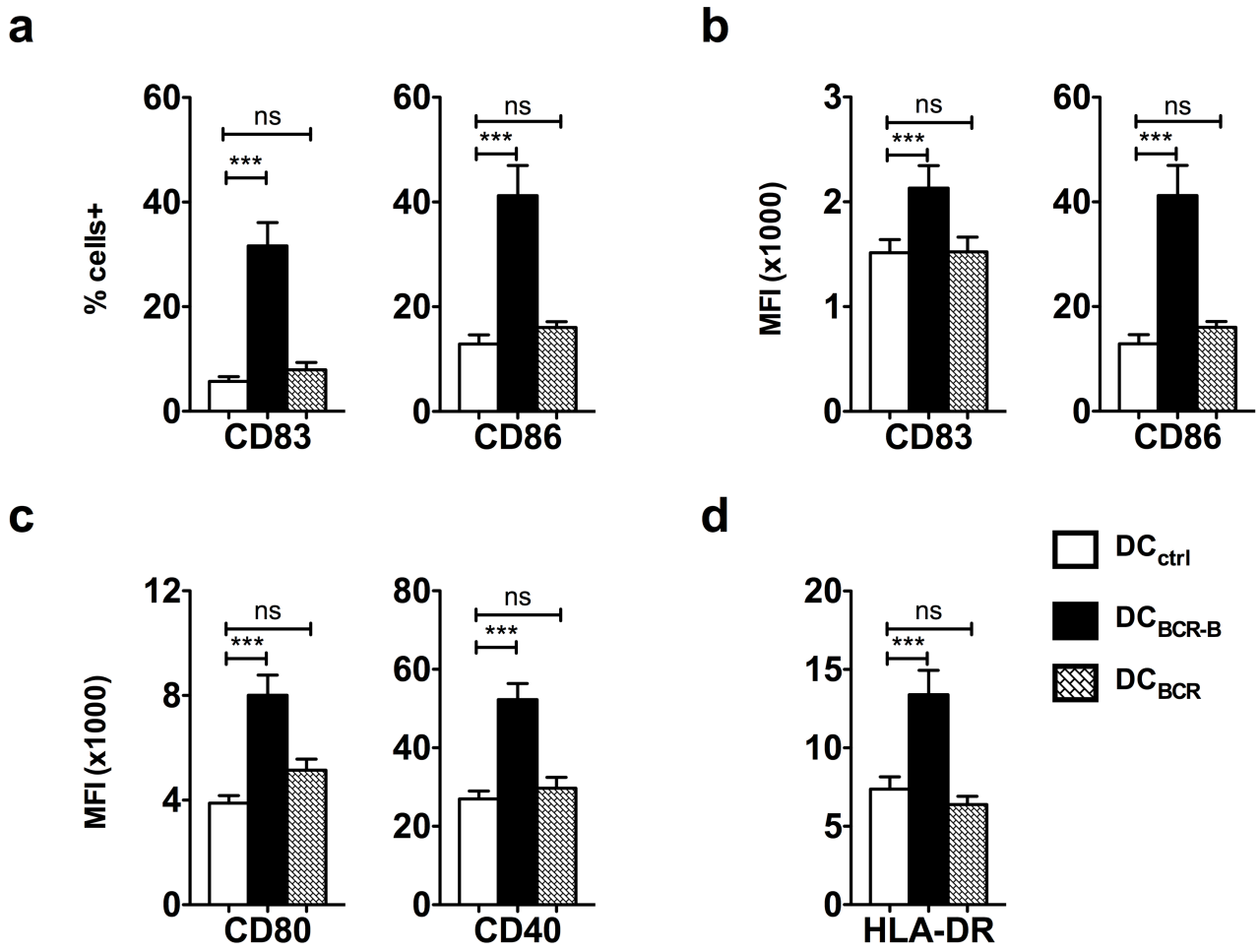
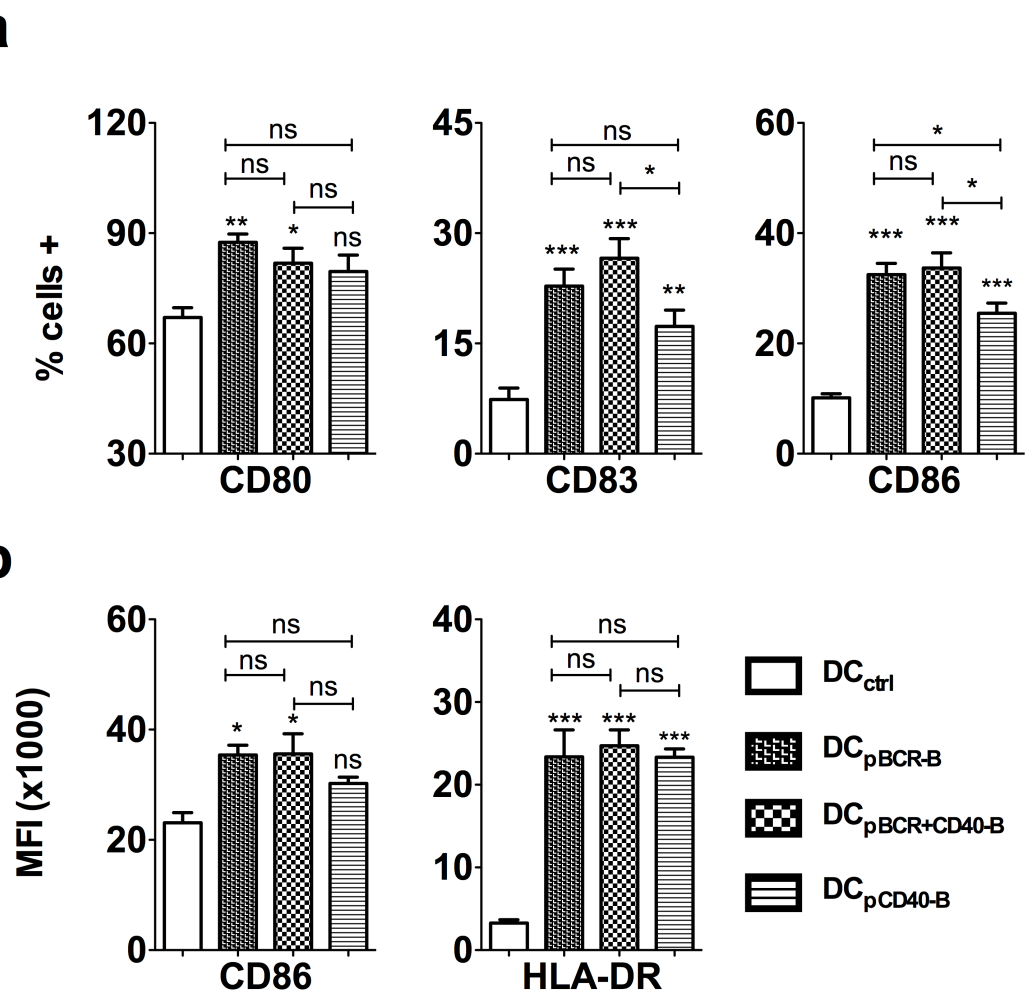
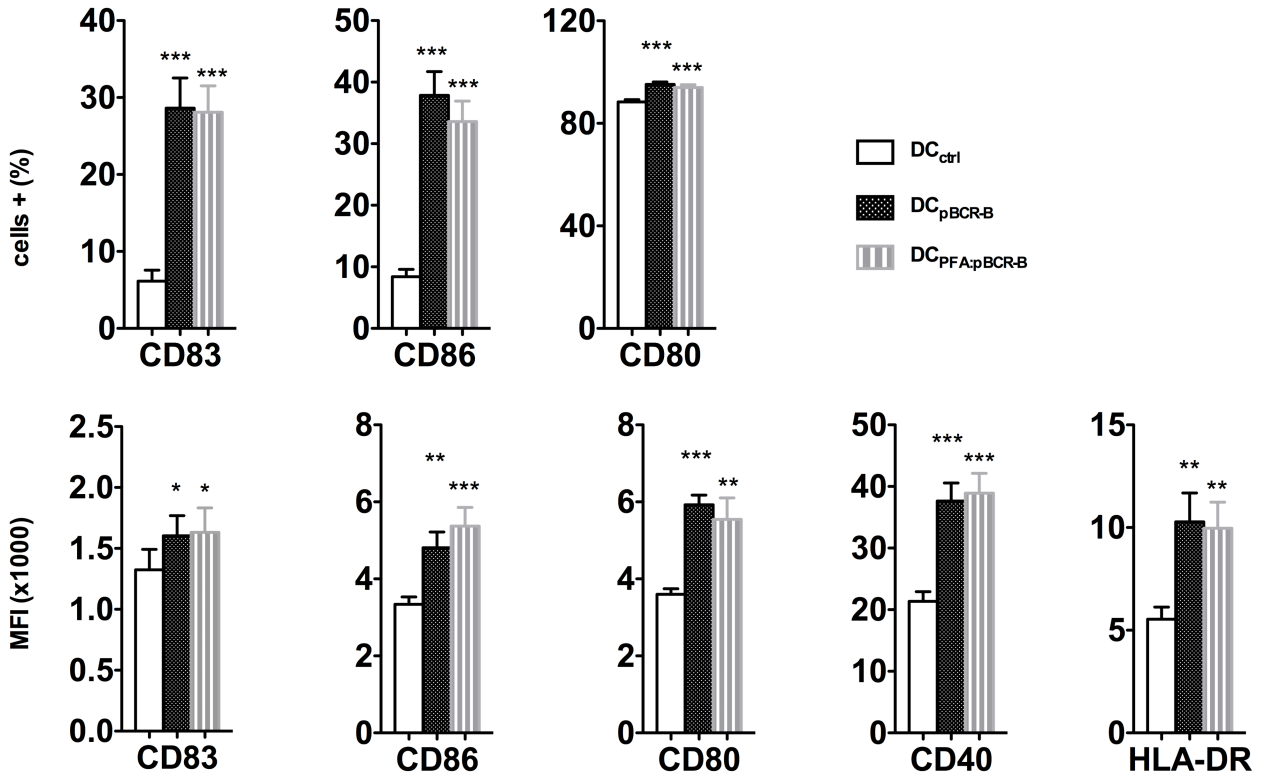


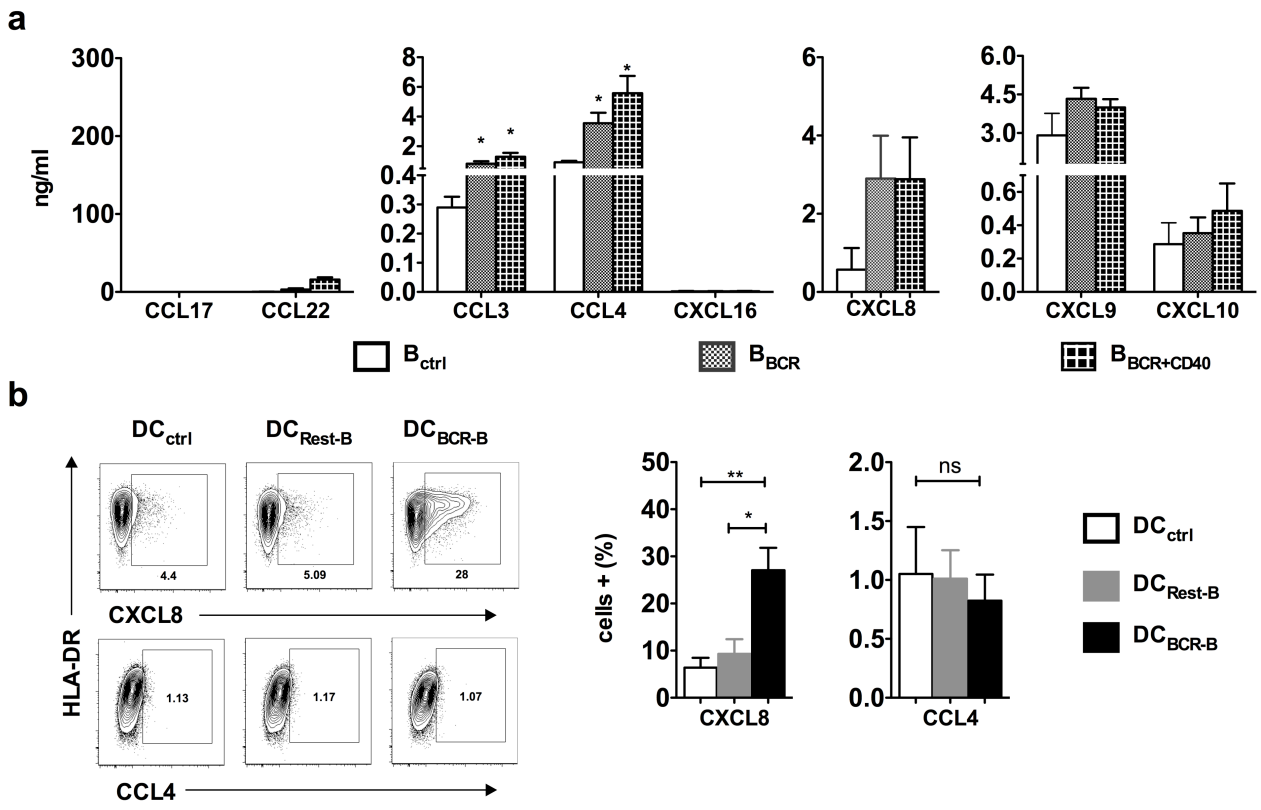
Supplementary Figure 1. F(ab')$_2$ fragments of anti-human IgM do not modulate DC phenotype. Immature DCs ($0.1x10^6$ cells/200 μl/well) with GM-CSF and IL-4 were cultured either alone (DC_{ctrl}) or F(ab')$_2$ of goat anti-human IgM (10 μg/ml) (DC_{BCR}) or co-cultured at 1:1 ratio with $CD19^+$ B cells that were directly activated in DC-B cell co-culture via BCR stimuli using F(ab')$_2$ of goat anti-human IgM (10 μg/ml) (DC_{BCR-B}) for 48 hours. **(a-d)** Phenotypic analysis (% positive cells and MFI) of CD20-negative cells was done by flow cytometry. Mean ± s.e.m of data from 10 donors. ****P <0.001*; ns, not significant by one way analysis of variance test.



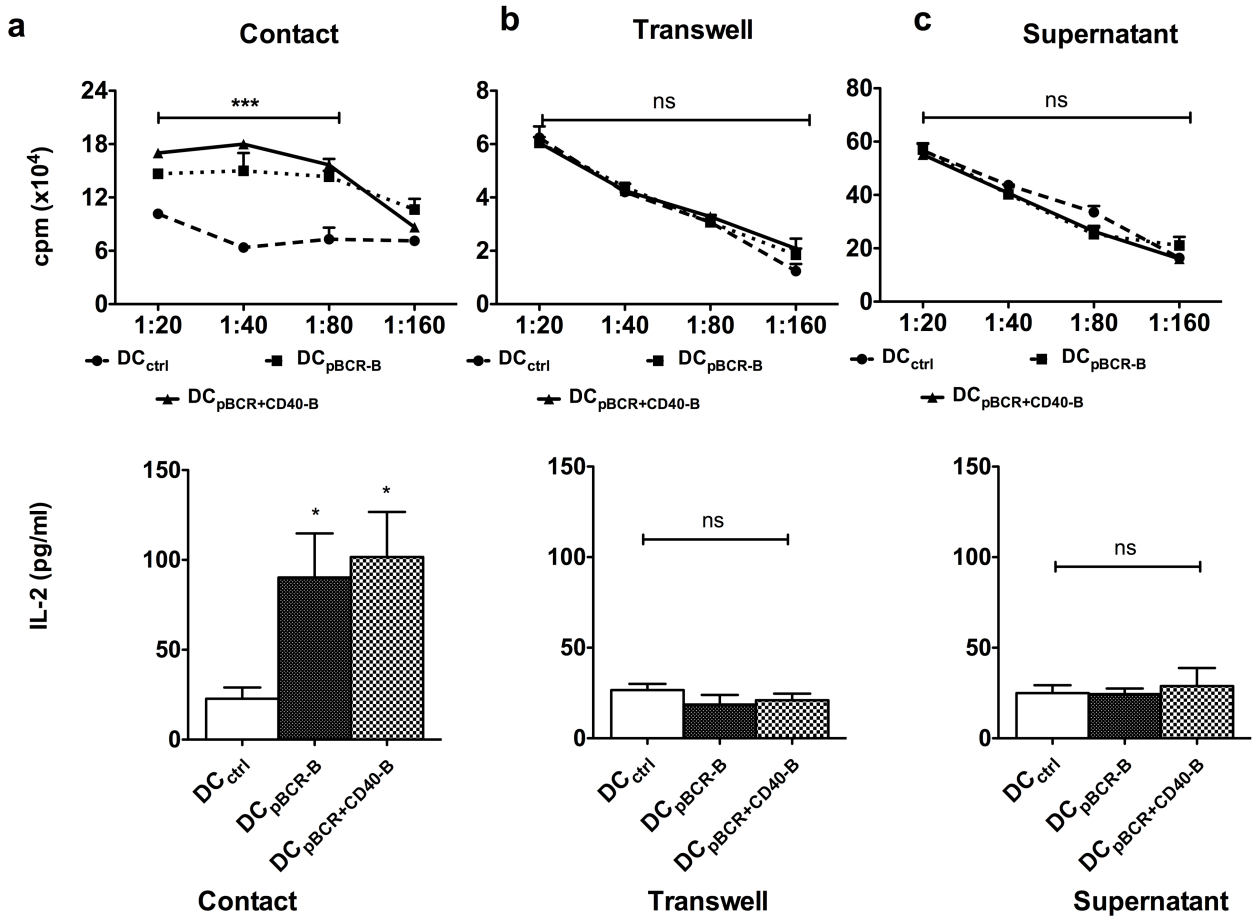
Supplementary Figure 2. Comparison of DC activation capacity of B cells stimulated with various stimuli. Immature DCs were cultured in medium containing GM-CSF and IL-4 for 48 hours either alone (DC_{ctrl}) or co-cultured with $CD19^+$ B cells that were pre-activated by BCR ($DC_{pBCR\text{-}B}$) or CD40 ($DC_{pCD40\text{-}B}$) or BCR+CD40 ($DC_{pBCR+CD40\text{-}B}$) stimulation. **(a and b)** Phenotypic analysis (% positive cells and MFI) of DCs that were gated negative for CD20. Mean ± s.e.m of data from 5 donors. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant by one way analysis of variance test.



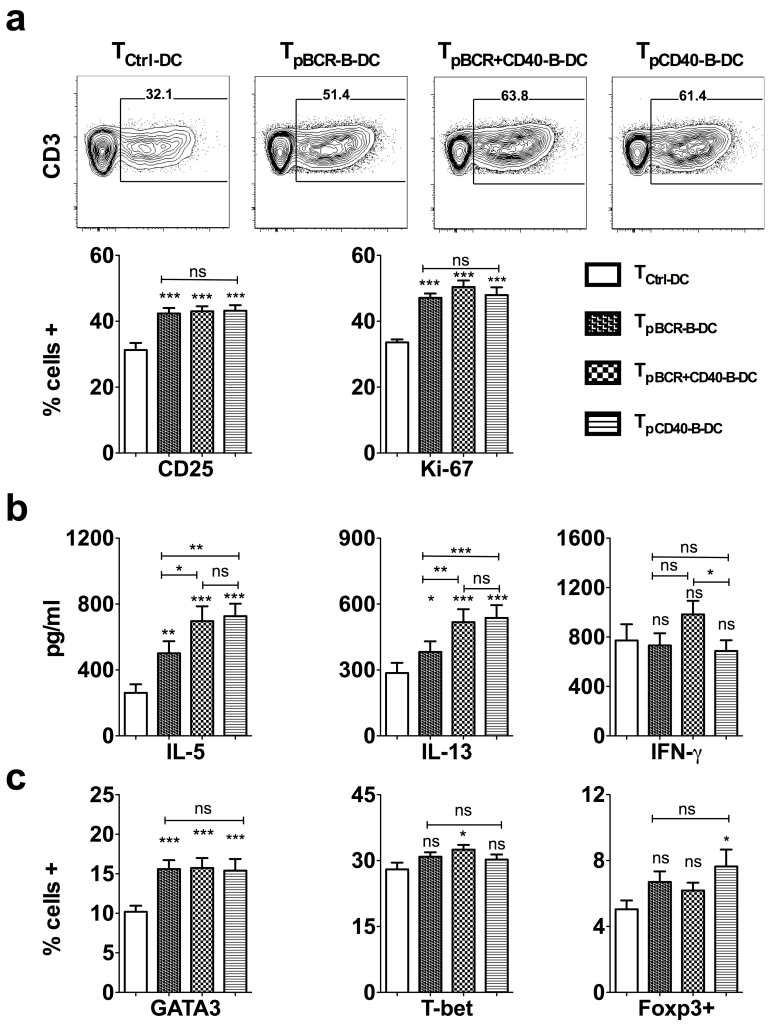
Supplementary Figure 3. Pre-activated live and paraformaldehyde-fixed B cells are capable of triggering maturation of DCs. Immature DCs were cultured in medium containing GM-CSF and IL-4 for 48 hours either alone (DC_{ctrl}) or co-cultured with live $CD19^{+}$ B cells pre-activated by BCR (DC_{pBCR-B}) or paraformaldehyde-fixed BCR-activated B cells ($DC_{PFA:pBCR-B}$). Phenotypic analysis of DCs that were gated negative for CD20 was done by flow cytometry. Mean ± s.e.m of data from 6 different donors. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ by one way analysis of variance test.



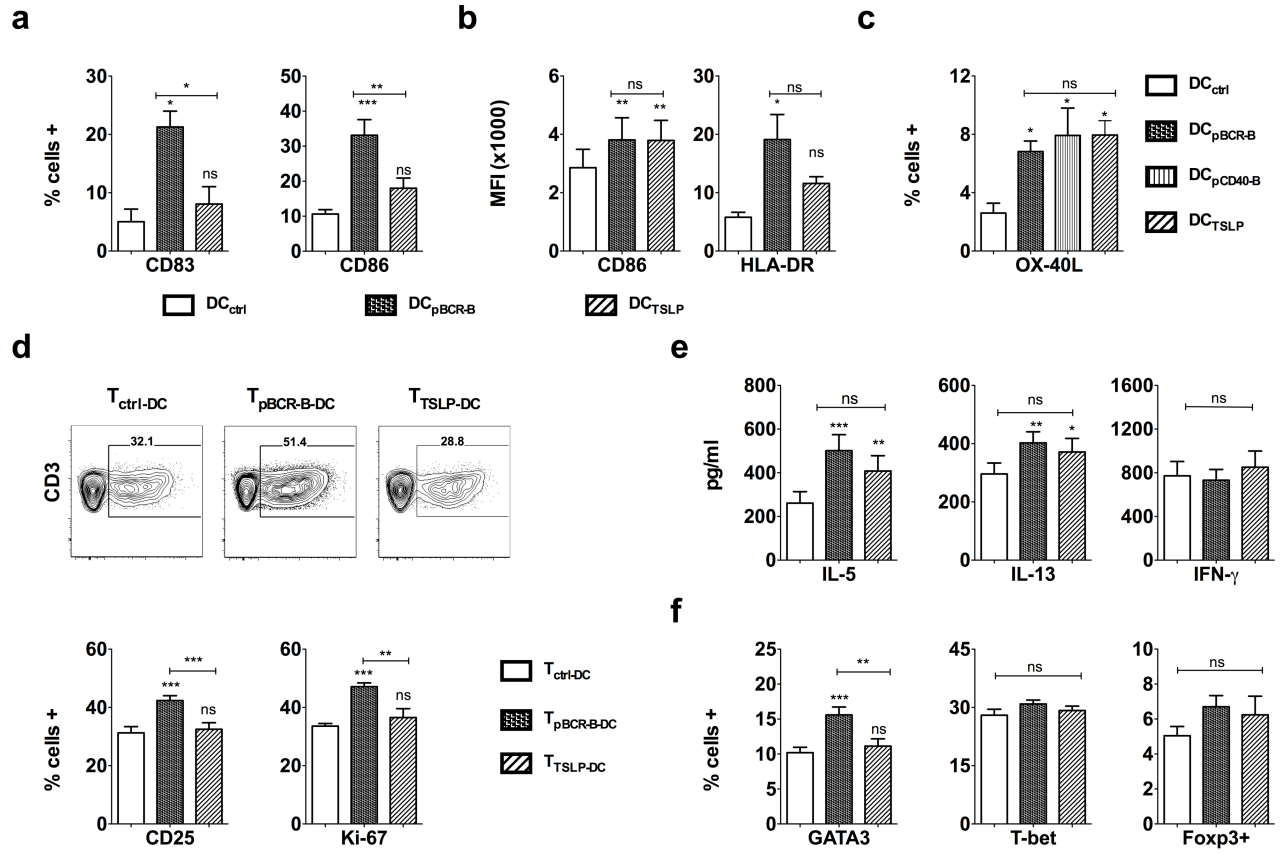
Supplementary Figure 4. Secretion of chemokines by B cells and DCs. (a) $CD19^+$ B cells were either cultured alone (B_{ctrl}) or in the presence of BCR (B_{BCR}) or BCR+CD40 ($B_{BCR+CD40}$) stimulation for 48 hours. Supernatants were analyzed for various chemokines. Mean±SEM of data from 5 donors. *, P<0.05 by one way-ANOVA test. **(b)** Immature DCs were cultured for 48 hours in the medium containing GM-CSF and IL-4 alone (DC_{ctrl}) or co-cultured at 1:1 ratio with $CD19^+$ B cells that were either in resting phase (DC_{Rest-B}) or directly activated in DC-B cell co-culture via BCR stimuli (DC_{BCR-B}). CD20-negative DCs from B cell-DC co-culture were analyzed for the intracellular expression of chemokines, CXCL8 and CCL4 by flow cytometry. Representative plot and mean ± s.e.m of data from 4 donors. **P<0.05*; ***P <0.01*; ns, not significant by one way analysis of variance test.



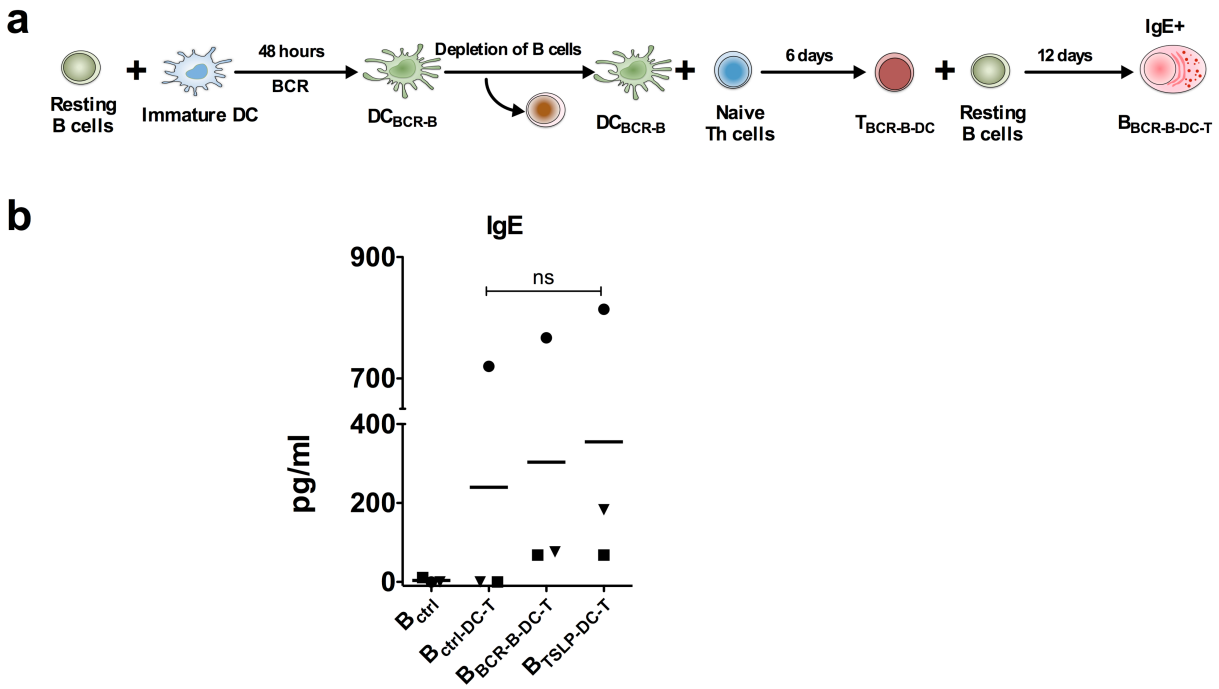
Supplementary Figure 5. DCs gain ability to induce proliferation of CD4$^+$ cells and secretion of T cell cytokines only when they receive activation signals from B cells that are in direct contact with DCs. (a-c) Immature DCs were cultured in medium containing GM-CSF and IL-4 for 48 hours either alone (DC_{ctrl}) or with CD19$^+$ B cells pre-activated by BCR ($DC_{BCR\text{-}B}$) or BCR+CD40 ($DC_{pBCR+CD40\text{-}B}$) stimulation. (**a**) Co-culture was done either in U-bottomed 96 well plates to allow direct contact between DCs and B cells (Contact) or (**b**) in trans-well plate to separate the B cells from DCs (Transwell). (**c**) In addition, immature DCs were cultured in the supernatants from activated B cells (Supernatant). DCs were purified from B cell-DC co-culture by depleting B cells. CD4$^+$ T cells were co-cultured with DCs at various ratios for 4 days. T cell proliferation (upper panels) was determined by [^{3}H] incorporation assay and values are expressed as counts per minute (cpm). Values are mean±SEM of data from quadruplicate wells and representative of 3 independent experiments. Supernatants from DC-T cell co-cultures at 1:20 ratio were analyzed for IL-2 (lower panels). Mean ± s.e.m of data from 4 donors. *$P < 0.05$; ***$P < 0.001$; ns, not significant by one way analysis of variance test.



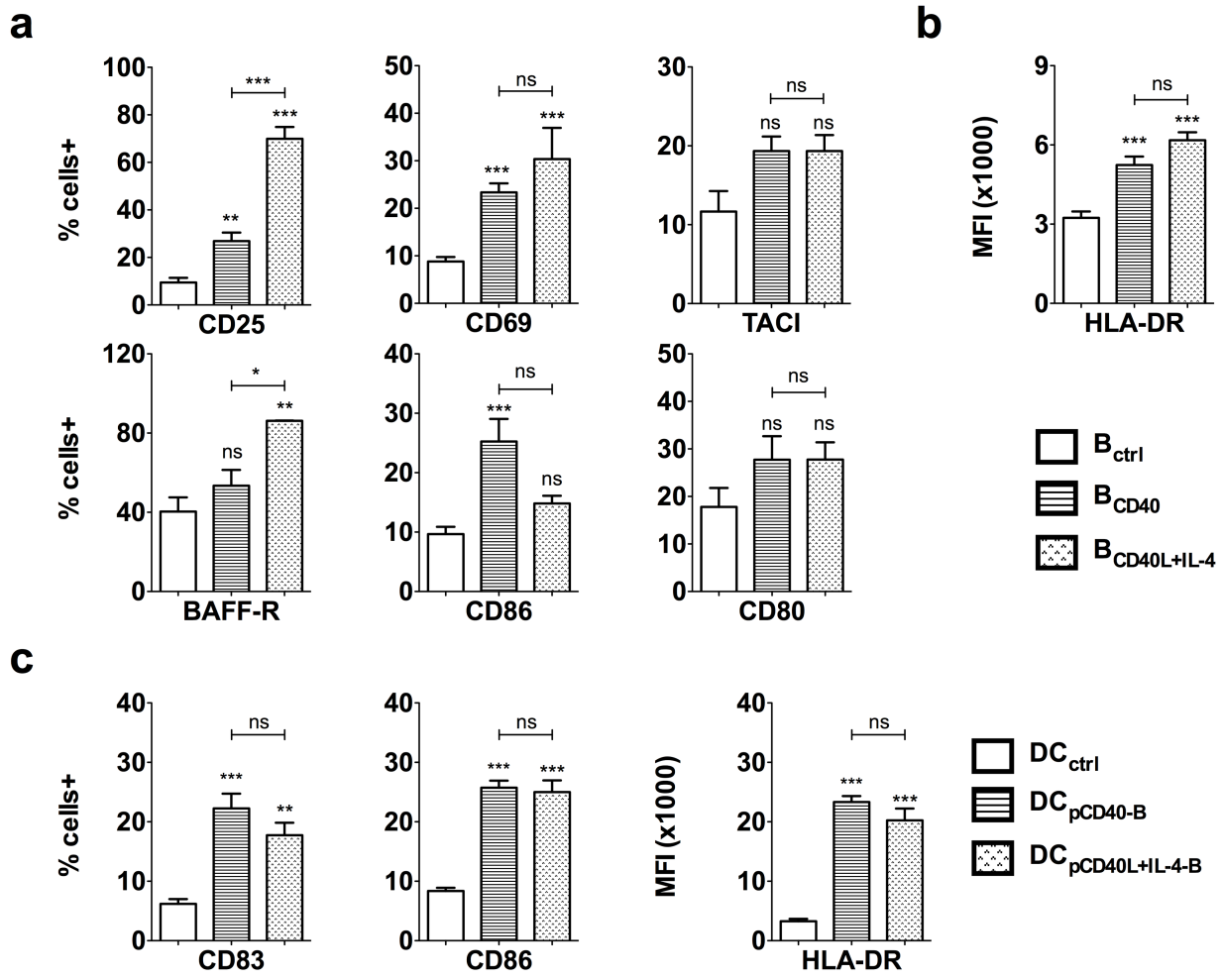
Supplementary Figure 6. DCs activated by B cells stimulated with various stimuli favor polarization of naive CD4$^+$ T cells to Th2 cells. Immature DCs were cultured in medium containing GM-CSF and IL-4 for 48 hours either alone (DC_{ctrl}) or co-cultured at 1:1 ratio with CD19$^+$ B cells that were pre-activated by BCR (DC_{pBCR-B}) or CD40 ($DC_{pCD40-B}$) or BCR+CD40 ($DC_{pBCR+CD40-B}$) stimulation. DCs were purified from B cell-DC co-culture by depleting B cells. CD4$^+$CD45RO$^-$ naive Th cells were co-cultured with control DCs ($T_{ctrl-DC}$) or B cell-matured DCs ($T_{pBCR-B-DC}$ or $T_{pBCR+CD40-B-DC}$ or $T_{pCD40-B-DC}$) at the ratio of 20:1 for 6 days. **(a)** CD3$^+$ T cells were analyzed for the surface phenotype and intracellular expression of Ki-67 by flow cytometry. **(b)** After 6 days, supernatants from DC-T cell co-cultures were analyzed for T cell cytokines. **(c)** Flow cytometric analysis of transcription factors expressed by CD3$^+$ T cells co-cultured with DCs. Representative plot and mean ± s.e.m of data from 6 donors. *P <0.05; **P <0.01; ***P <0.001; ns, not significant by one way analysis of variance test.



Supplementary Figure 7. Comparison of DC phenotype and Th2 polarizing capacity of B cell-matured DCs versus TSLP-treated DCs. Immature DCs were cultured in medium containing GM-CSF and IL-4 for 48 hours either alone (DC_{ctrl}) or co-cultured at 1:1 ratio with $CD19^+$ B cells that were pre-activated by BCR (DC_{pBCR-B}) or CD40 ($DC_{pCD40-B}$) or treated with TSLP (20 ng/ml) (DC_{TSLP}). B cell-matured DCs were purified from the DC-B cell co-cultures by depleting B cells. **(a-c)** Expression of various markers (% positive cells and MFI) and of OX-40L (% positive cells) on DCs that were gated negative for CD20. **(d)** $CD4^+CD45RO^-$ naive Th cells were co-cultured at the ratio of 20:1 for 6 days with control DCs ($T_{ctrl-DC}$) or B cell-matured DCs ($T_{pBCR-B-DC}$) or with TSLP-DC ($T_{TSLP-DC}$). $CD3^+$ T cells were analyzed for the surface phenotype and intracellular expression of Ki-67 and transcription factors by flow cytometry. **(e)** After 6 days, supernatants from DC-T cell co-cultures were analyzed for T cell cytokines. **(f)** Flow cytometric analysis of transcription factors expressed by $CD3^+$ T cells co-cultured with DCs. Representative plot and mean ± s.e.m of data from 4 donors. **P <0.05*; ***P <0.01*; ****P <0.001*; ns, not significant by one way analysis of variance test.



Supplementary Figure 8. Th2-mediated IgE production in B cells induced by B cell-matured DC and TSLP-DC. Immature DCs were cultured in medium containing GM-CSF and IL-4 for 48 hours either alone (DC_{ctrl}) or co-cultured at 1:1 ratio with $CD19^+$ B cells that were directly activated in DC-B cell co-culture via BCR stimuli (DC_{BCR-B}) or treated with TSLP (20 ng/ml) (DC_{TSLP}). **(a)** Experimental design for Th2-mediated IgE production in B cells. B cell-matured DCs were purified from the DC-B cell co-cultures by depleting B cells. $CD4^+CD45RO^-$ naive Th cells were co-cultured at the ratio of 20:1 for 6 days with control DC ($T_{ctrl-DC}$) or B cell-matured DC ($T_{BCR-B-DC}$) or with TSLP-DC ($T_{TSLP-DC}$). $CD3^+$ T cells separated by DC-T cell co-culture were cultured with fresh $CD19^+$ B cells at the ratio of 2.5:1 for 12 days. **(b)** Supernatants from B cells that were cultured alone (B_{ctrl}) or co-cultured with T cells that were differentiated by control DC ($B_{ctrl-DC-T}$) or B cell-matured DC ($B_{BCR-B-DC-T}$) or TSLP-treated DC ($B_{TSLP-DC-T}$) were analyzed for the amounts of secreted IgE by ELISA. Mean values are presented by horizontal lines. Data are from 3 donors. ns, not significant by one way analysis of variance test.



Supplementary Figure 9. Comparison of B cell stimulation by CD40 and CD40L+IL-4 and subsequent DC maturation. (a, b) $CD19^+$ B cells were either cultured alone (B_{ctrl}) or in the presence of agonistic CD40 antibody (B_{CD40}) alone or CD40L+IL-4 ($B_{CD40L+IL-4}$) for 2 days. Expression of surface activation markers was analyzed by flow cytometry. Mean ± s.e.m of data from 5 donors. **(c)** Immature DCs were cultured in medium containing GM-CSF and IL-4 for 48 hours either alone (DC_{ctrl}) or co-cultured with $CD19^+$ B cells that were pre-activated by agonistic CD40 antibody ($DC_{pCD40-B}$) or CD40L+IL-4 ($DC_{pCD40L+IL-4-B}$) stimulation. Expression of surface activation markers on DCs was analyzed by flow cytometry. Mean ± s.e.m of data from 4 donors. **P <0.05; **P <0.01*; ****P <0.001*; ns, not significant by one way analysis of variance test.