

Supplementary Material

Differentially activated B cells develop regulatory phenotype and show varying immunosuppressive features: a comparative study

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Figure S1. Flow cytometry gating strategies for the assessment of cell ratio and proliferation rates of Breg subsets and effector cells. **Right green area**: Identification of the ratio and cell division rates of mBreg (CD24^{hi}CD27⁺) and tBreg (CD24^{hi}CD38^{hi}) subpopulations in live CD19⁺ cells isolated by magnetic separation. **Left orange area**: Identification of the ratio and proliferation rates of effector lymphocytes: CD3⁺ T cells (CD8⁺ T killers, CD4⁺ T helpers and CD4⁺CD25^{hi} regulatory T cells) and CD3⁻CD16⁺ NK cells in CD19⁺-depleted PBMCs.



Figure S2. (A) The assessment of $CD4^+CD25^{hi}$ (regulatory T cells) ratio of $CD3^+$ T cells after 5 days of co-incubation of $CD19^+$ -depleted PBMCs with activated (CD40L + CpG + IL21) and untreated B cells. (B) Verification of $CD4^+CD25^{hi}$ T cells for low expression of CD127, which is characteristic of Tregs.



Figure S3. Distribution of CD4⁺ (T helpers) and CD8⁺ (T killers) T cell subsets after 5 days of coincubation of CD19⁺-depleted PBMCs with activated (CD40L + CpG + IL21) and untreated B cells.



Figure S4. Distribution of CD27⁻CD38^{int} (naïve B cells), CD27⁺CD38^{+/-} (memory B cells), and CD27^{hi}CD38^{hi} (plasmablasts) B cell subsets after 5 days of stimulation with CD40L + CpG + IL21 or without any activation.