Supplementary Material

**Optimized protocols for in vitro T cell-dependent and T cell-independent activation for B cell differentiation studies using limited cells**

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**Supplemental Figure 1. CD27 and CD38 expression of stimulated primary human B cells**

**(A)** Representative FACS plot (left panel) show gating strategy of CD27/CD38 subpopulations and quantification of the relative percentages of CD27 and CD38 subpopulations in the total CD19+ B cell population between 6 and 9 days of culture (n = 4). **(B)** The frequency of activated B cells (CD27-CD38+). Each data point represents the mean of an individual donor with duplicate culture measurements. Mean values are represented by bars and the error bars depict SEM. P values were calculated using two-way ANOVA with Tukey’s multiple comparison test. \*\*\* P ≤ 0.001, \*\*\*\* P ≤ 0.0001.

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**Supplemental Figure 2. Proliferation, differentiation and antibody production of primary human CD19+ B cells after T cell dependent in vitro stimulation and culturing of PBMCs.**

**(A)** Schematic overview of the T cell dependent (TD) culture system to induce B cell differentiation. A total of 250, 2500 or 25000 CD19+ human B cells in PBMCs (n = 3) were stimulated with a human-CD40L-expressing 3T3 feeder layer and recombinant IL-21 (50 ng/mL) enabling condition I.2 (dark blue), II.2 (cobalt blue) and III.2 (light blue). Cells were analyzed at day 6 and day 9 by flow cytometry to evaluate plasmablast and plasma cell generation. The supernatant was collected at day 6 and day 9 to evaluate IgG, IgA and IgM production by ELISA. **(B)** The number of live CD19+ events was analyzed using flow cytometry. A cut off of 1000 events was used to proceed with further analysis. **(C)** Representative histograms of CTY dilution (left panel) and quantification (right panel) on day 6 compared to their unstimulated condition. **(D)** The frequency of CD27+CD38+ B cells and **(E)** CD27+CD38+CD138+ B cells was analyzed by using flow cytometry. **(F)** IgG, IgA and IgM production in culture supernatants was evaluated by ELISA after 6 and 9 days (n = 3). Each data point represents the mean of an individual donor with duplicate culture measurements. Mean values are represented by bars and the error bars depict SEM. P values were calculated using two-way ANOVA with Sidak’s multiple comparison test. \* P ≤ 0.05, \*\* P ≤ 0.01, \*\*\* P ≤ 0.001, \*\*\*\* P ≤ 0.0001.

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**Supplemental Figure 3. Anti-IgA/G/M F(ab’)2fragments interfere with ELISA readouts**

Interference of F(ab’)2 fragment Goat Anti-Human IgA/G/M in **(A)** IgG, **(B)** IgA and **(C)** IgM ELISA. Serial dilutions of F(ab’)2 fragments (5, 2.5, 1.25 and 0.625 μg/mL) were added to the standard curve dilutions as indicated. Black lines indicate no F(ab’)2 fragments added.

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**Supplemental Figure 4. Proliferation, differentiation and antibody production primary human CD19+ B cells after T cell independent in vitro stimulation and culturing of PBMCs**

**(A)** Schematic overview of the T cell independent (TI) culture system to induce B cell differentiation. A total of 250, 2500 or 25000 CD19+ human B cells and PBMCs (n = 3) were stimulated with CpG (1 µM) and IL-2 (50 ng/ml) enabling condition IV.2 (dark blue), V.2 (cobalt blue) and VI.2 (light blue). Cells were analyzed at day 6 and day 9 by flow cytometry to evaluate plasmablast and plasma cell generation. The supernatant was collected at day 6 and day 9 to evaluate IgG, IgA and IgM production by ELISA. **(B)** The number of live CD19+ events was analyzed using flow cytometry. A cut off of 1000 events was used to proceed with further analysis. **(C)** Representative histogram of CTY dilution (left panel) and quantification (right panel) of condition IV.2 and V.2 on day 6 compared to their unstimulated condition. **(D)** Analysis of proliferation by CTY dilution of CD3+ T cells in condition IV.2 and V.2 on day 6. **(E)** The frequency of CD27+CD38+ B cells and **(F)** CD27+CD38+CD138+ B cells. **(G)** IgG, IgA and IgM production in culture supernatants was evaluated by ELISA after 6 and 9 days (n = 4). Each data point represents the mean of an individual donor with duplicate culture measurements. Mean values are represented by bars and the error bars depict SEM. P values were calculated using two-way ANOVA with Sidak’s multiple comparison test. \* P ≤ 0.05, \*\* P ≤ 0.01, \*\*\* P ≤ 0.001, \*\*\*\* P ≤ 0.0001.

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**Supplemental Figure 5. Cryopreserved and freshly isolated B cells produce similar amounts of antibodies in T cell dependent and independent assays**

Comparison of IgA and IgM production of B cells isolated freshly fromPBMCs or from cryopreserved PBMCs obtained from the same healthy donor (n=4). Total human B cells were isolated fromfresh PBMCs (indicated in white) or frozen PBMCs (indicated in gray) and cultured for 6 and 9 days. **(A-B)** Using T cell dependent (TD) stimuli (CD40L and IL-21 with/without IL-4) 2500 B cells (fresh and frozen) were cultured under conditions described previously **(A)** without PBMCs (condition II) and **(B)** with PBMCs (condition II.2). IgA (left panel) and IgM production (right panel) on day 6 (upper graphs) and day 9 (lower graphs) are shown. **(C-D)** Using T cell independent (TI) stimuli (CpG and IL-2 with/without BAFF) 25000 B cells (fresh and frozen) were cultured under conditions described previously **(C)** without PBMCs (condition IV) and **(D)** with PBMCs (condition IV.2). IgA (left panel) and IgM production (right panel) on day 6 (upper graphs) and day 9 (lower graphs) are shown. Each data point represents the mean of an individual donor with duplicate culture measurements. Mean values are represented by bars and the error bars depict SEM. P values were calculated using two-way ANOVA with Sidak’s multiple comparison test. \* P ≤ 0.05, \*\* P ≤ 0.01.