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Supplemental information

A synthetic human 3D *in vitro* lymphoid model enhancing B-cell survival and functional differentiation

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Supplementary Figures

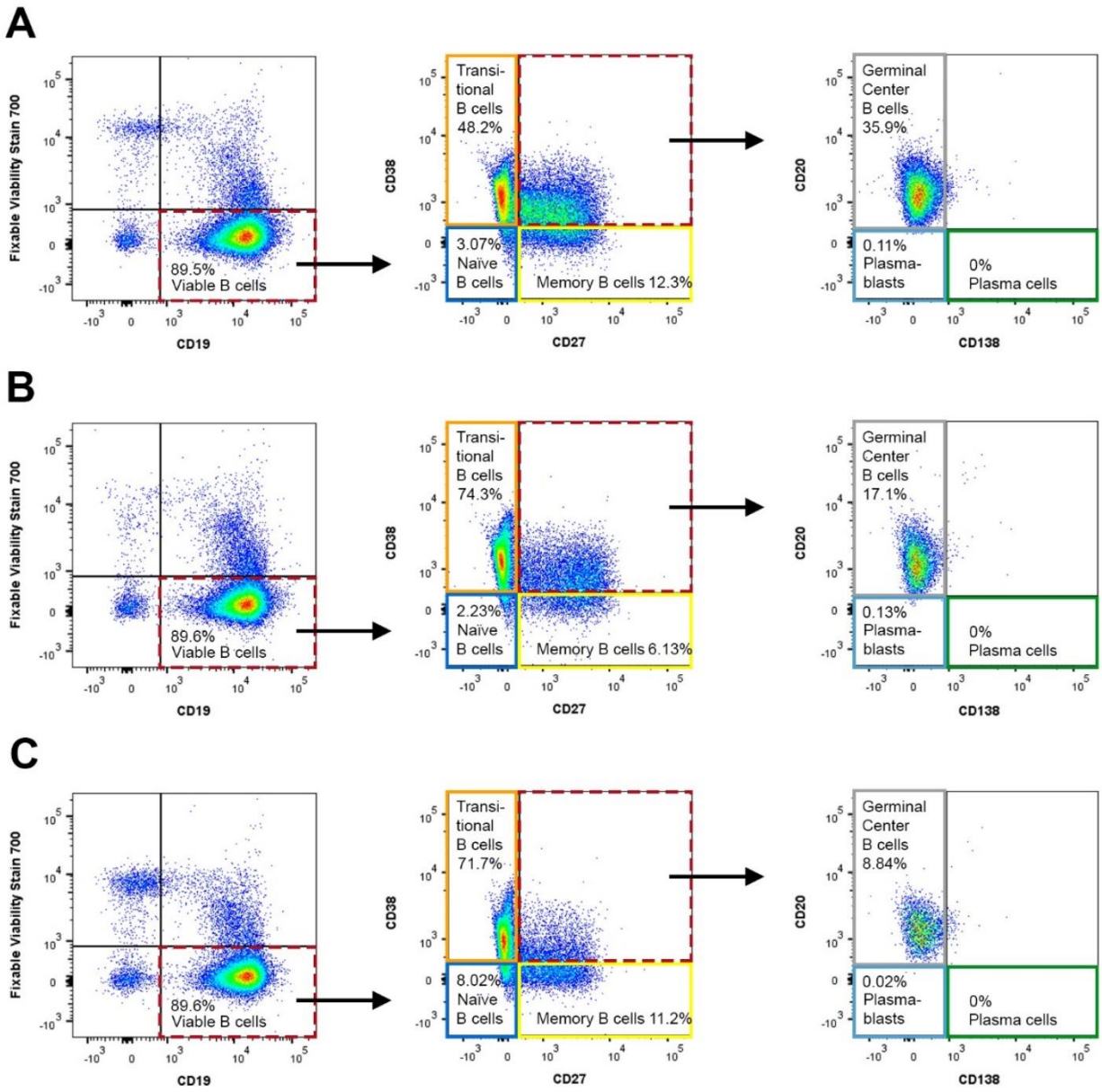

Figure S1 Characterization of the input B cells after CD19 positive selection, Related to Figure 1. Flowcytometry results of **(A)** Donor 1, **(B)** Donor 2, and **(C)** Donor 3 after CD19 positive selection, gating for viable fixable viability stain 700 negative, $CD19^{+}$ B-cells, naïve B-cells (CD19+CD20+CD27-CD38-CD138-), transitional B-cells (CD19+CD20+CD27-CD38+CD138-), germinal center B-cells (CD19+CD20+CD27+CD38+CD138-), memory B-cells (CD19+CD20+CD27+CD38-CD138-), plasmablasts (CD19+CD20-CD27+CD38+CD138-) and plasma cells (CD19+CD20-CD27+CD38+CD138+).

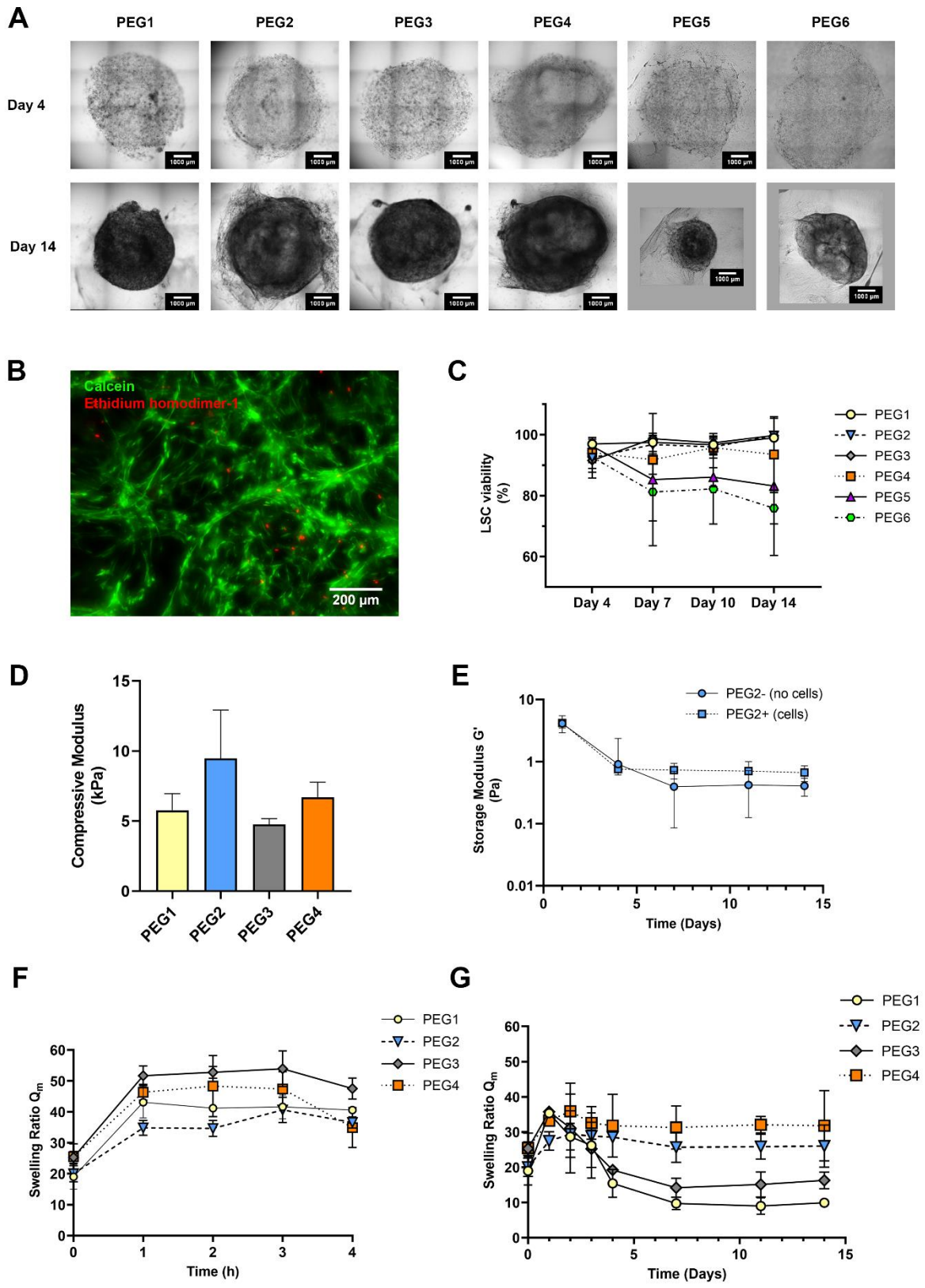

Figure S2 Optimization of functionalized hydrogels for 3D in vitro B cell co-cultures, Related to Figure 3. **PEG1**: 4% (w/v) PEG-4MAL, 2.0 mM RGD, 4.9 mM GPQ-W, **PEG2**: 5% (w/v) PEG-4MAL, 2.0 mM RGD, 6.6 mM GPQ-W,

PEG3: 4% (w/v) PEG-4MAL, 1.0 mM RGD, 1.0 mM REDV, 4.9 mM GPQ-W, **PEG4**: 5% (w/v) PEG-4MAL, 1.0 mM RGD, 1.0 mM REDV, 6.6 mM GPQ-W, **PEG5**: 4% (w/v) PEG-4MAL, 2.0 mM REDV, 4.9 mM GPQ-W, **PEG6**: 5% (w/v) PEG-4MAL, 2.0 mM REDV, 6.6 mM GPQ-W. **(A)** Brightfield images of 40 µL crosslinked 3D co-cultures at day 4 and day 14. Scalebars present 1000 µm. **(B)** Live/dead image of a PEG2 3D coculture at day 14 (green = calcein, staining living cells. Red = ethidium homodimer-1, staining dead cells). **(C)** Lymphoid stromal cell viability (n=3) over time in all 6 PEG conditions, measured using flowcytometry. **(D)** Compressive modulus (kPa) of PEG-4MAL gels (PEG1 – PEG4) without cells at day 1 **(E)** Storage modulus (G') of PEG2 over time, with and without cells, as an indirect measure of hydrogel degradation. Data shown was collected at 4 rad/s. **(F)** Swelling ratio (Q_m) of PEG-4MAL gels (PEG1 – PEG4) without cells during the first hours after crosslinking and subsequent medium addition, **(G)** and during 14 days of culture. **(D-G)** Data showing the mean ± SD (n=3).

Table S1 Culture conditions (per well) of CD19$^+$ B cells co-cultured with CD40LCs and LSCs in either 2D or 3D, Related to Figure 4.

Culture components (per well, 48 well plate)	2D cultures	3D cultures
CD19$^+$ B cells	5,000 cells	5,000 cells
LSCs	12,500 cells	12,500 cells
CD40LCs	12,500 cells	12,500 cells
Hydrogel	-	20 µL 5% (w/v) PEG-4MAL 2.0 mM RGD, 6.6 mM GPQ-W
Culture medium	250 µL CK2 medium	250 µL CK2 medium
48 well plate	Tissue culture treated	Non tissue culture treated

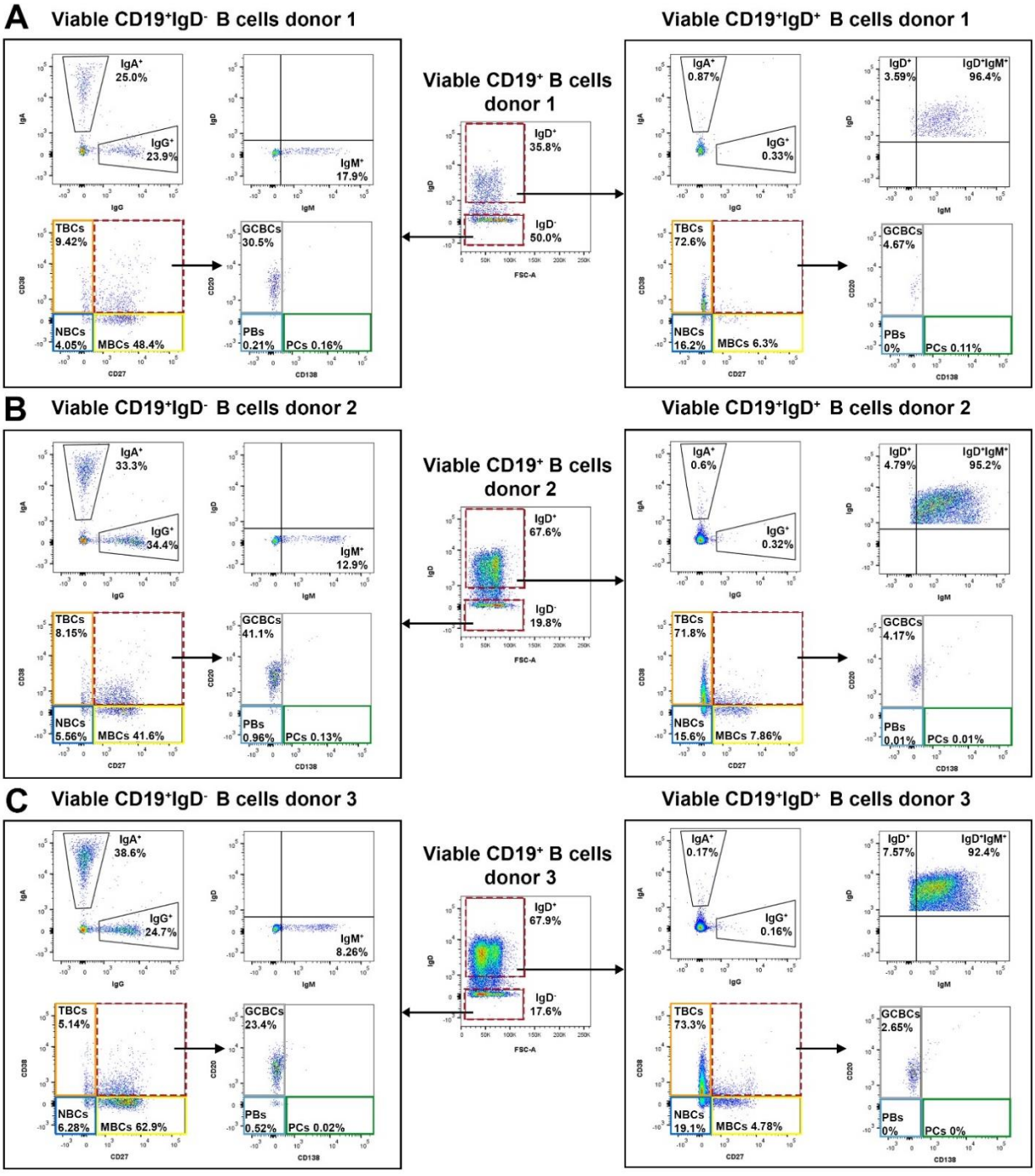

Figure S3 Characterization of the input B cells after CD19 positive selection and subsequent CD19+IgD+7AAD- and CD19+IgD-7AAD- sorting, Related to Figure 5. Flowcytometry results of **(A)** Donor 1, **(B)** Donor 2, and **(C)** Donor 3 after the sorting of IgD^+ and IgD^- populations. Each IgD FSC-A plot shows the set gates used for sorting. Subsequent flowcytometry results show the frequency of IgA+, IgG+, IgM+, IgD+ and IgD+IgM+ B cells and naïve B cells (NBCs), transitional B cells (TBCs), memory B cells (MBCs), germinal center B cells (GCBCs), plasmablasts (PBs) and plasma cells (PCs) within either the $CD19^+IgD^+7AAD^-$ or $CD19^+IgD^-7AAD^-$ population.

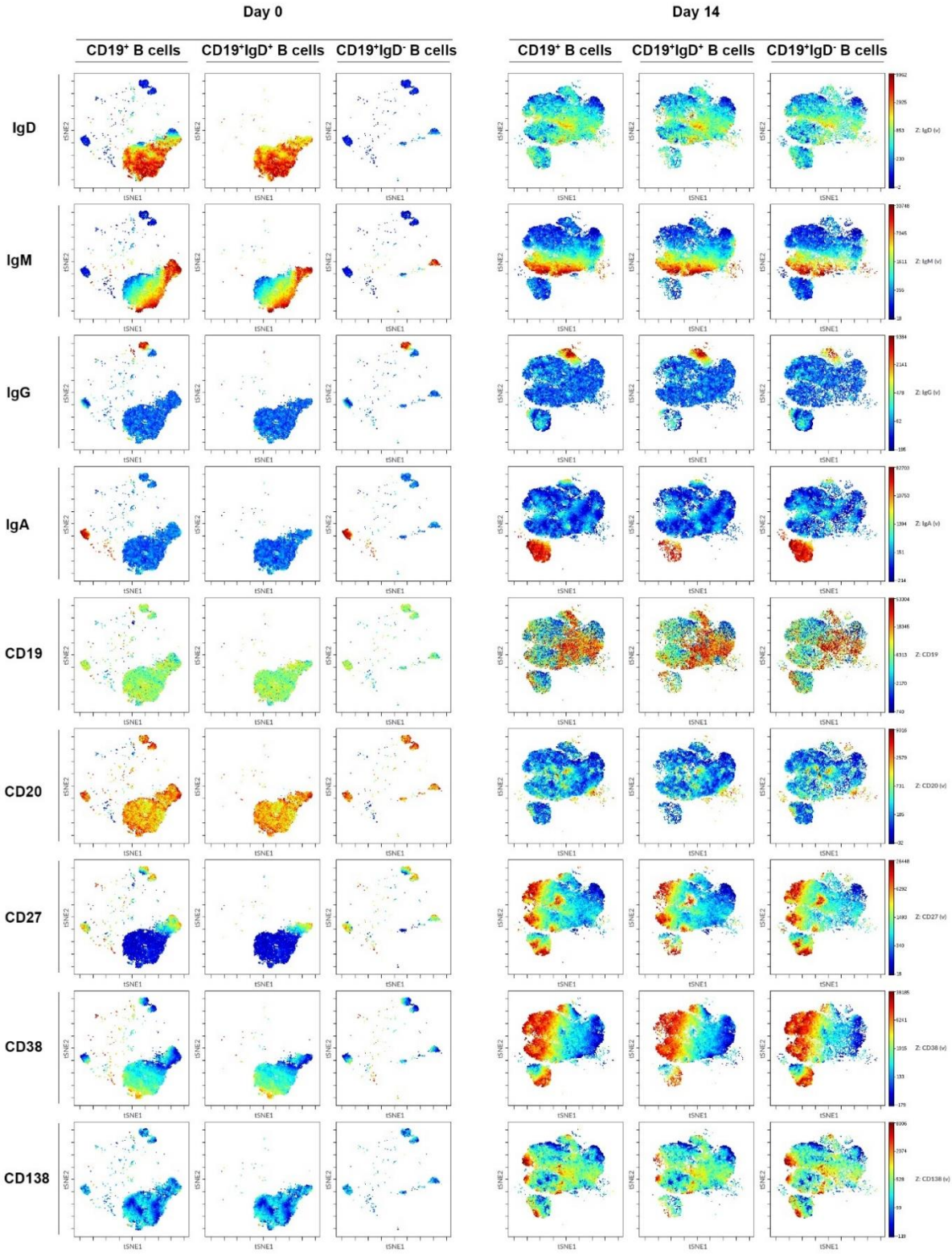

Figure S4 Surface marker and membrane-bound immunoglobulin expression of total CD19⁺ and sorted CD19⁺IgD⁺ CD19+IgD- B cells, Related to Figure 5. viSNE dot plots of the concatenated (n=3) data at day 0 versus 14. Blue indicates low expression, yellow intermediate expression and red high expression.