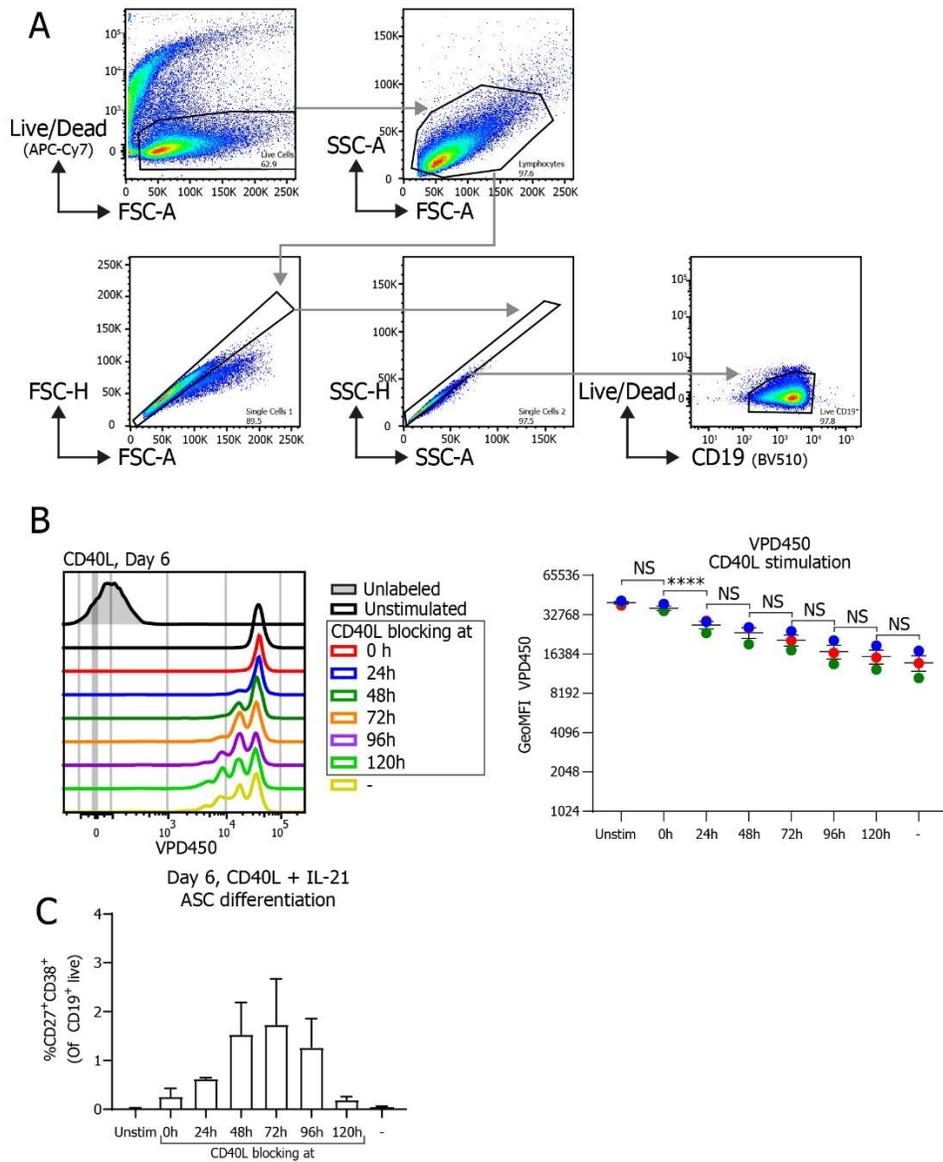


Supplementary Figure 1

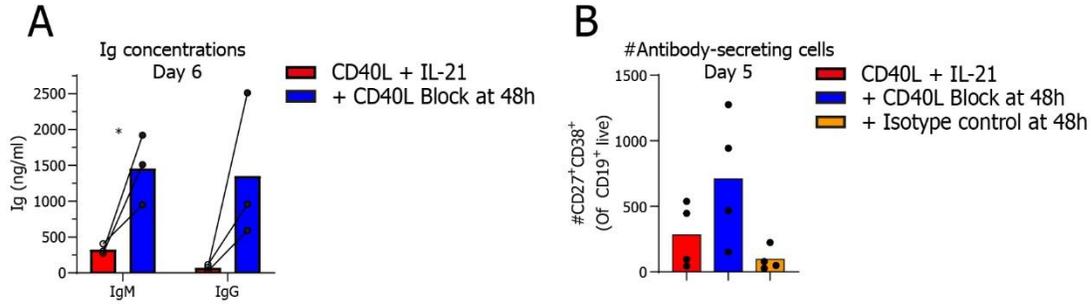


Suppl Fig 1

CD40L blocking antibodies efficiently inhibit CD40L induced proliferation and promote differentiation after CD40L and IL-21 stimulation

Naïve B cells were sorted and 25,000 cells were stimulated with a human-CD40L expressing 3T3 feeder layer with or without recombinant IL-21 (50 ng/ml). After the indicated culture duration CD40L blocking antibodies (13 µg/ml) were added and cells were analyzed at day 6 by flow cytometry. **(A)** Representative gating strategy for the gating of Live CD19⁺ B cells as depicted. First, dead cells were excluded using a live/dead fixable viability stain. FSC/SSC gate is set for lymphocyte population that contains the CD19⁺ B cells. Doublets were excluded by setting two single cell gates on both the FSC-A/FSC-H and SSC-A/SSC-H parameters. Finally, Live CD19⁺ cells were gated on. **(B)** Naïve B cells were labelled with proliferation dye VPD450 prior to culture. Representative histogram overlay (left) and quantification of VPD450 GeoMFI after 6 days of culture under indicated conditions (n=3). Data from a single independent experiment with the mean of 3 individual donors marked as red, green or blue dots with triplicate measurements (n=3), scatter plots show mean +/- SD **(C)** Quantification of relative percentages of CD27⁺CD38⁺ ASC at day 6 under indicated conditions (n=3). Data from a single independent experiment with 3 individual donors, bar indicate mean +/- SD P-values were calculated using one-way ANOVA with Tukey's multiple comparison test. **** p ≤ 0.0001

Supplementary Figure 2

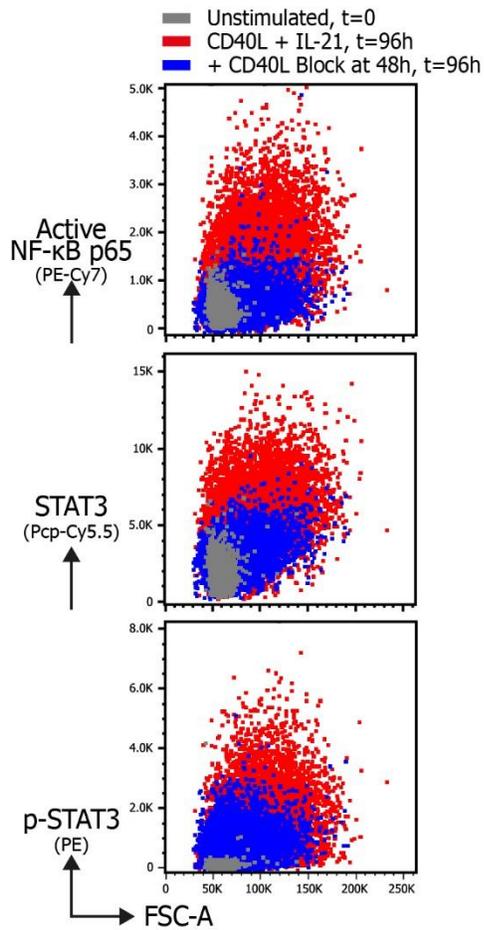


Suppl Fig 2

Termination of CD40L stimulation promotes secretion of immunoglobulins.

Naïve B cells were sorted and 25,000 cells were stimulated with a human-CD40L expressing 3T3 feeder layer and recombinant IL-21 (50 ng/ml). **(A)** After 48 hours CD40L blocking antibodies (13 ug/ml) were added or not. Quantification of secreted IgM and IgG measured by ELISA in day 6 culture supernatants. Combined data from 2 independent experiments performed with 1-2 donors. Symbols represent the mean of an individual with triplicate measurements, bars indicate mean. P-values were calculated by student t-test. * $P \leq 0.05$. **(B)** After 48 hours CD40L blocking antibodies (13 ug/ml) or isotype control antibodies were added not. Quantification of absolute counts of CD27⁺CD38⁺ ASC at day 5. Data from a single independent experiment with 4 individual donors, bar indicate mean and dots represent individual donors.

Supplementary Figure 3



Suppl Fig 3

Relationship between cell size and reduced phospho-signaling in CD40L blocked cultures

Naïve B cells were sorted and 25,000 cells were stimulated with a human-CD40L expressing 3T3 feeder layer and recombinant IL-21 (50 ng/ml). After 48 hours CD40L blocking antibodies (13 ug/ml) were added or not. Representative dot plot overlays of unstimulated B cells (Grey) and stimulated (Red) and CD40L blocked B cells (Blue) after 96 hours. The FSC-A parameter is plotted against measured phospho-signaling proteins.

