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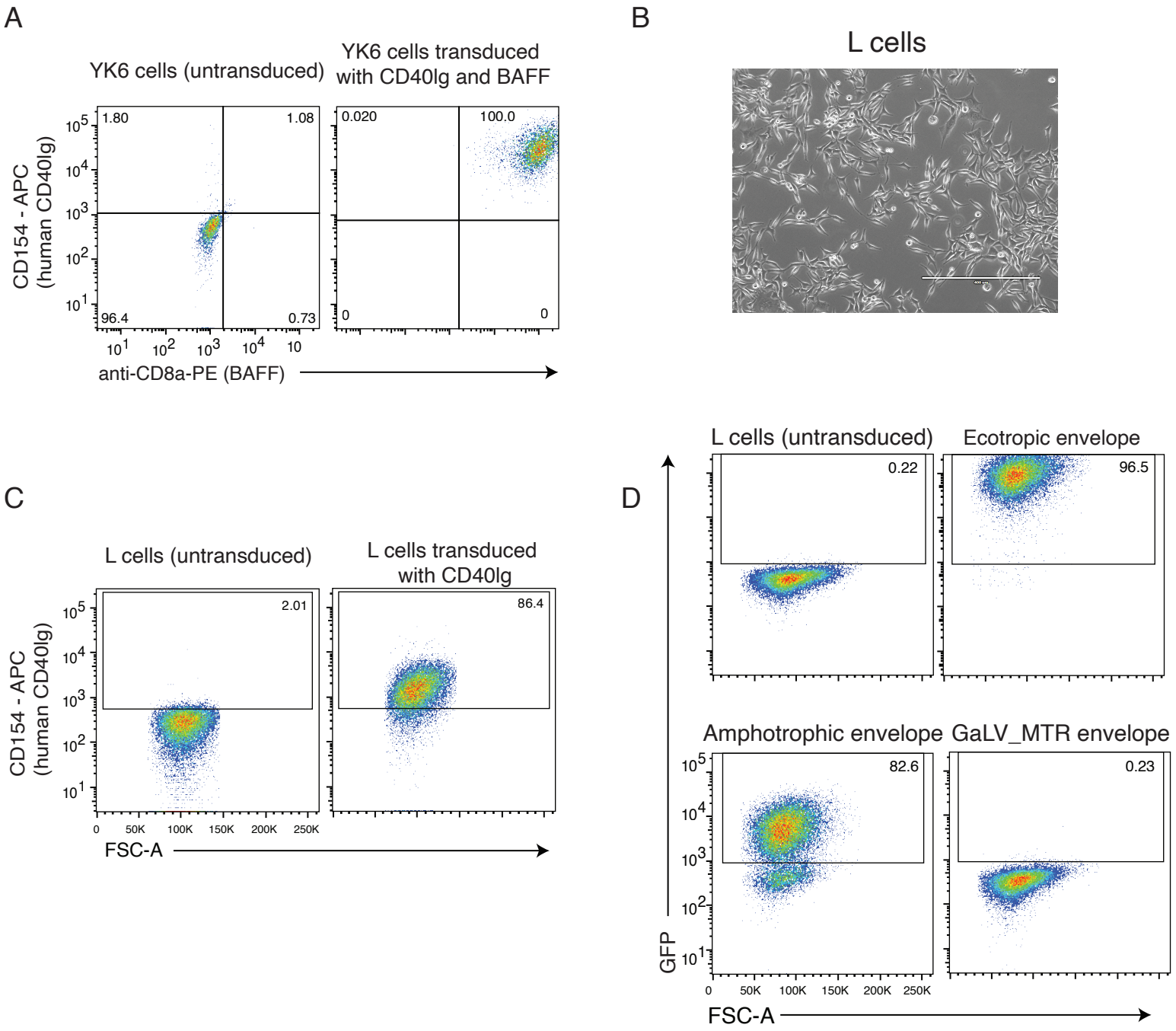
Supplementary information

Genetic manipulation and immortalized culture of ex vivo primary human germinal center B cells

In the format provided by the authors and unedited



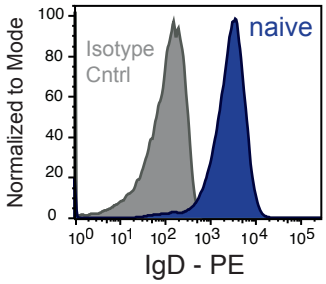
Supplementary Figure 1 Alternative feeder systems.

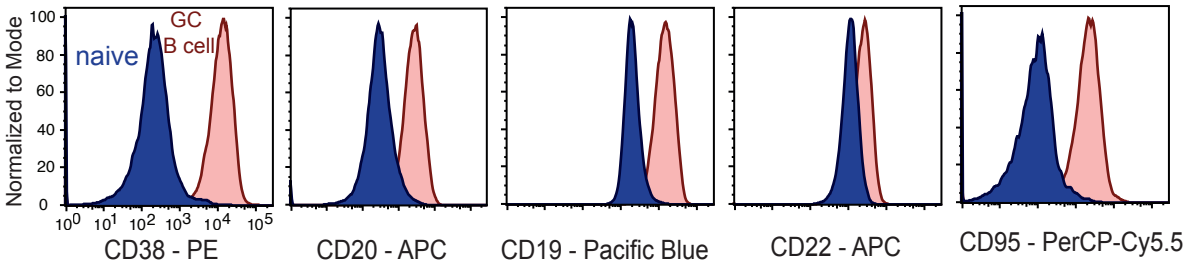
(A) Flow cytometry analysis for the expression of CD40Lg (CD154) and BAFF (transduction marker CD8a) on immortalized FDC-like cells (YK6) engineered to express CD40Lg and BAFF. FSC, forward scatter.

(B) Image showing the appearance of the murine feeder cell line (L-cells) at the desired confluence. Scale bar represents 400µm.

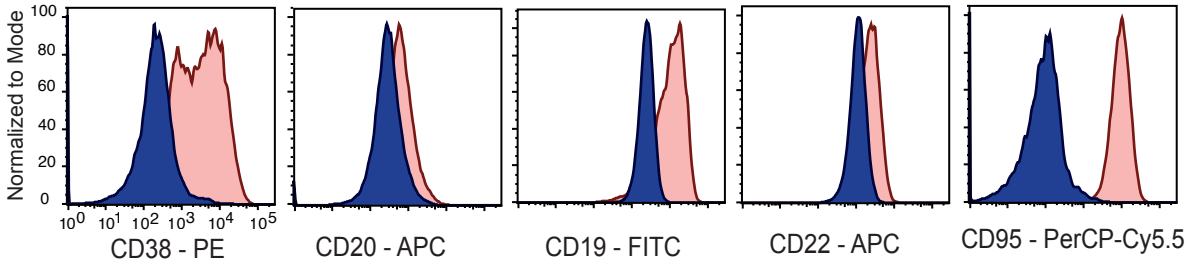
(C) Flow cytometry analysis for the expression of CD40Lg (CD154) on L-cells engineered to express CD40Lg. FSC, forward scatter.

(D) L-cells were transduced with a GFP construct using an amphotrophic, ecotropic or GaLV_MTR fusion construct. Transduction efficiency was determined by expression of GFP two days after transduction. FSC, forward scatter.

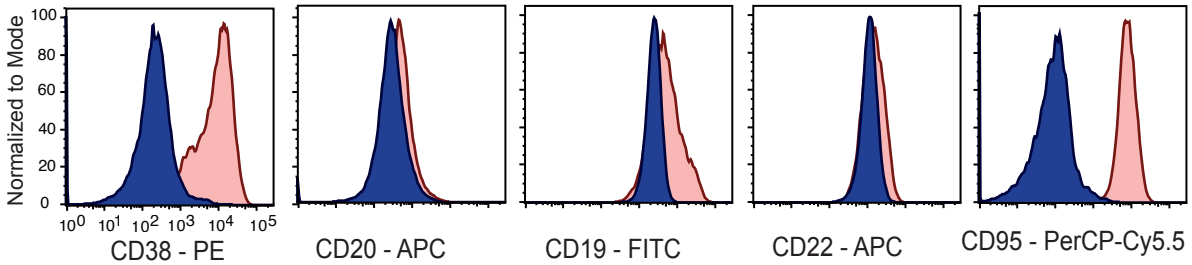




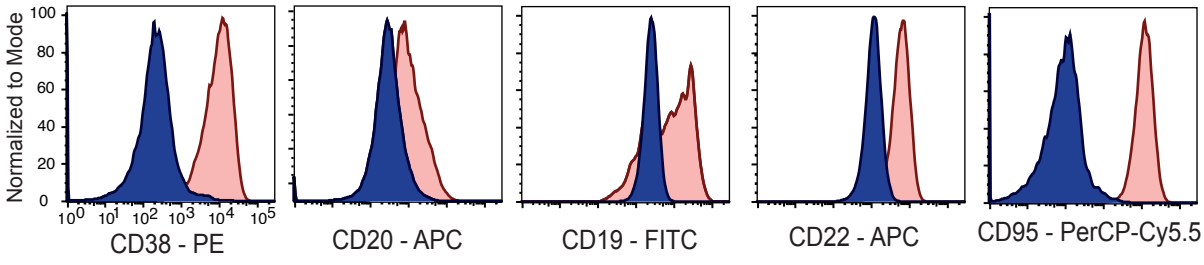
Day of isolation



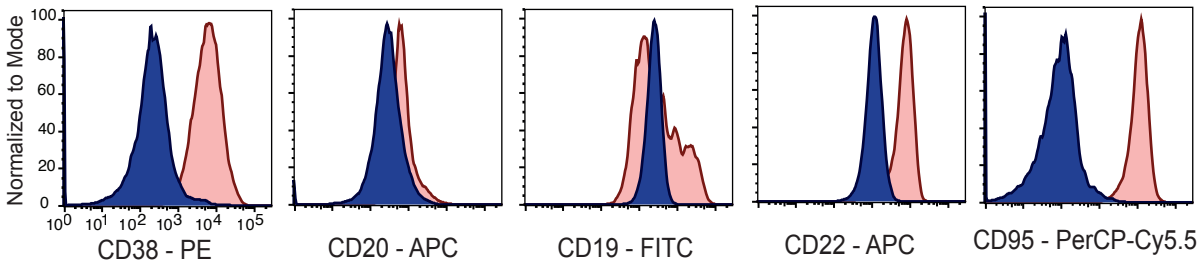
Day 5 after transduction with BCL2 and BCL6



Day 5 after transduction with BCL2 and MYC

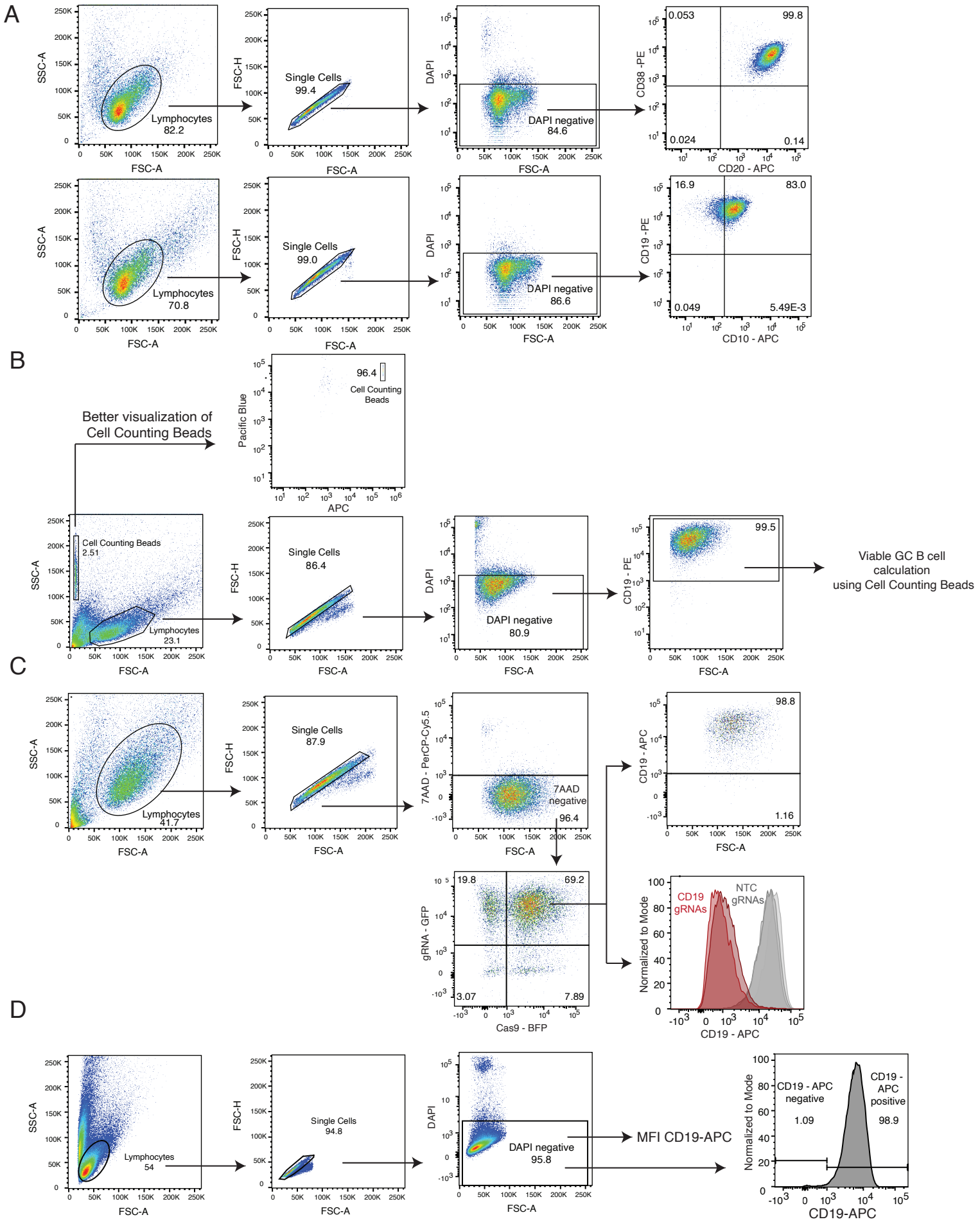


Long term culture with BCL2 and BCL6



Long term culture with BCL2 and MYC

Supplementary Figure 2 Phenotype of immortalised B cells.
Flow cytometry analysis for the expression of the GC B cell markers CD38, CD20, CD19, CD22 and CD95 at day of isolation, Day 5 after transduction, and long term culture with either BCL2 and BCL6 or BCL2 and MYC. Red histograms show GC B cells compared to primary human naïve B cells (blue). Day of isolation and Day 5 after transduction represents a different donor to long-term culture.



Supplementary Figure 3 Flow cytometry gating strategy.
Gating strategy for (A) live, CD38, CD20, CD10, CD19 positive cells as seen in Figure 3.
(B) live, CD19 positive cells and the indicated Cell Counting Beads were used to calculate cell number as seen in Figure 4.
(C) live, BFP+GFP+ (Cas9/gRNA) expressing cells with knockdown of CD19 or CD38 or control as seen in Figure 5.
(D) live cells with knockdown of CD19 as seen in Figure 6.