

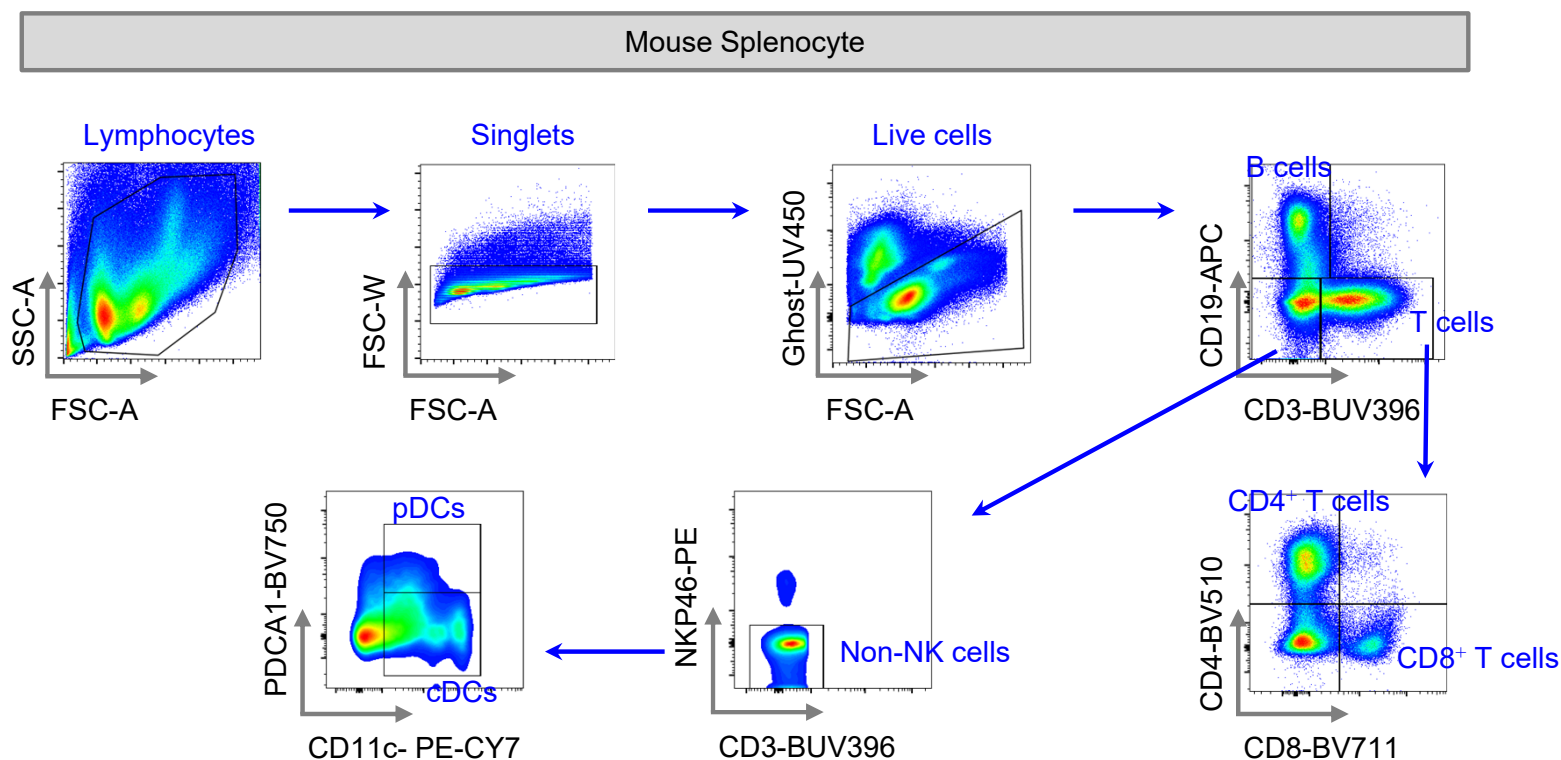
## **Supplementary Materials (Taghi Khani *et al.*)**

**Isoform-specific knockdown of long and intermediate prolactin receptors interferes with  
evolution of B-cell neoplasms**

### **Inventory**

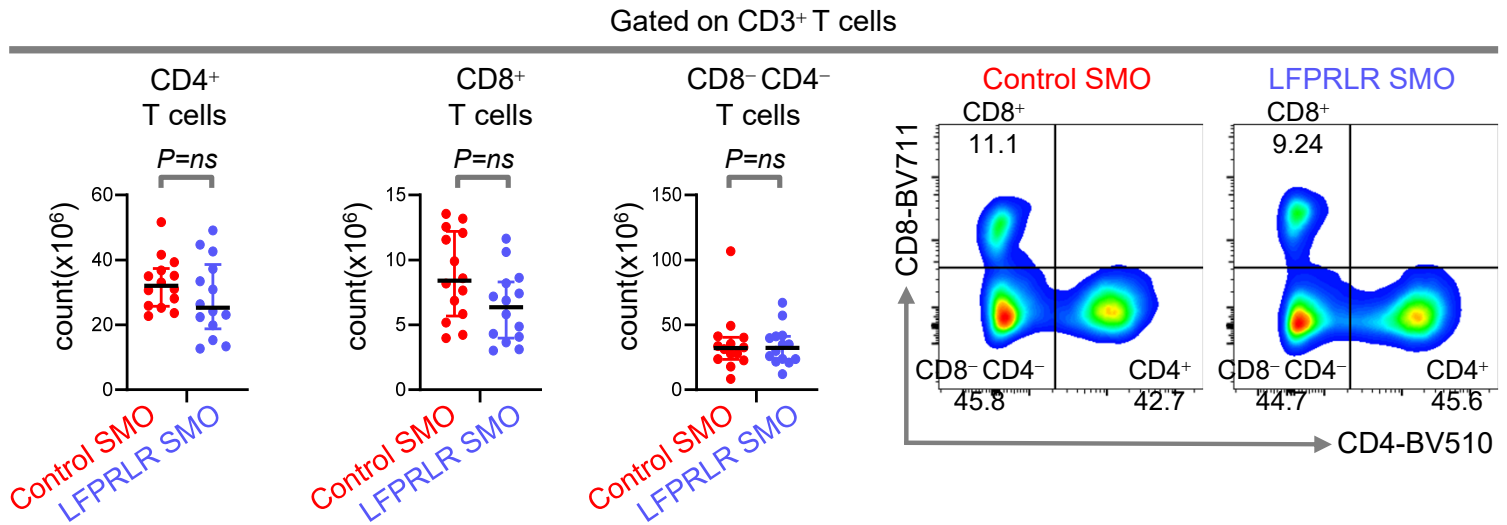
- Supplementary Figures 1-17
- Supplementary Tables 1 and 2

**Supplementary Fig. 1: Gating strategy for flow cytometry analysis of mouse splenic WBCs.**



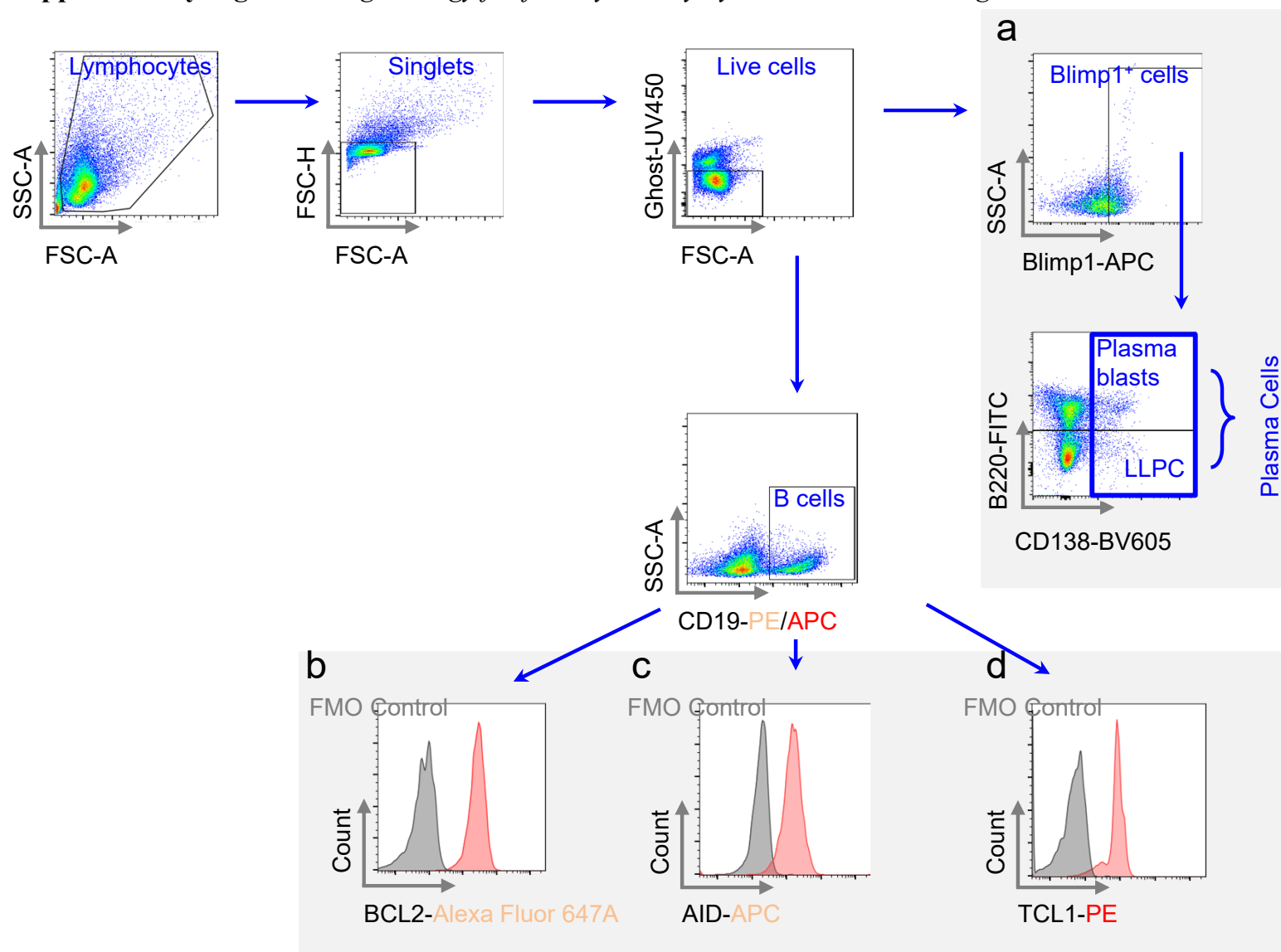
**Supplementary Fig. 1: Gating strategy for flow cytometry analysis of mouse splenic WBCs.** From the lymphocyte cluster; singlets were gated followed by selection of live (Ghost-UV450<sup>-</sup>) populations. B cells, T cells, and non-B non-T cells were then gated on CD19<sup>+</sup>, CD3<sup>+</sup>, and CD19<sup>-</sup> CD3<sup>-</sup>, respectively. T cells were gated on CD8<sup>+</sup>, CD4<sup>+</sup>, and CD4<sup>-</sup> CD8<sup>-</sup> T cells. pDCs and cDCs were gated on CD11c and PDCA1 after gating out NKP46<sup>+</sup> NK cells from the non-B non-T fraction.

**Supplementary Fig. 2:** *LPRLR* knockdown did not induce significant changes in numbers of T cell subsets in SLE prone mice.



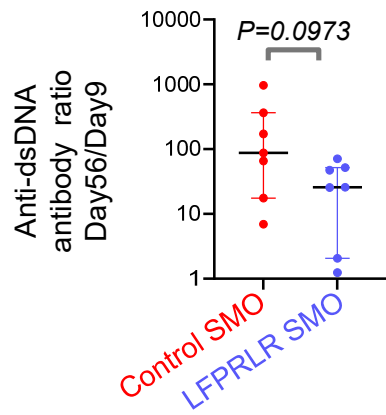
**Supplementary Fig. 2:** *LPRLR* knockdown did not induce significant changes in frequencies or numbers of T cell subsets in SLE-prone mice. Quantitation of numbers and representative flow cytometry plots of CD4<sup>+</sup>, CD8<sup>+</sup>, and CD4<sup>-</sup>CD8<sup>-</sup> T cells of *MRL-lpr* SLE prone mice treated with control SMO (n=14, red) or *LPRLR* SMO to knockdown *LPRLR* (n=14, blue). Graphs show median  $\pm$  interquartile range. Exact p-values were calculated using the Mann-Whitney U test. ns = non-significant.

**Supplementary Fig. 3: Gating strategy for flow cytometry by intracellular staining.**



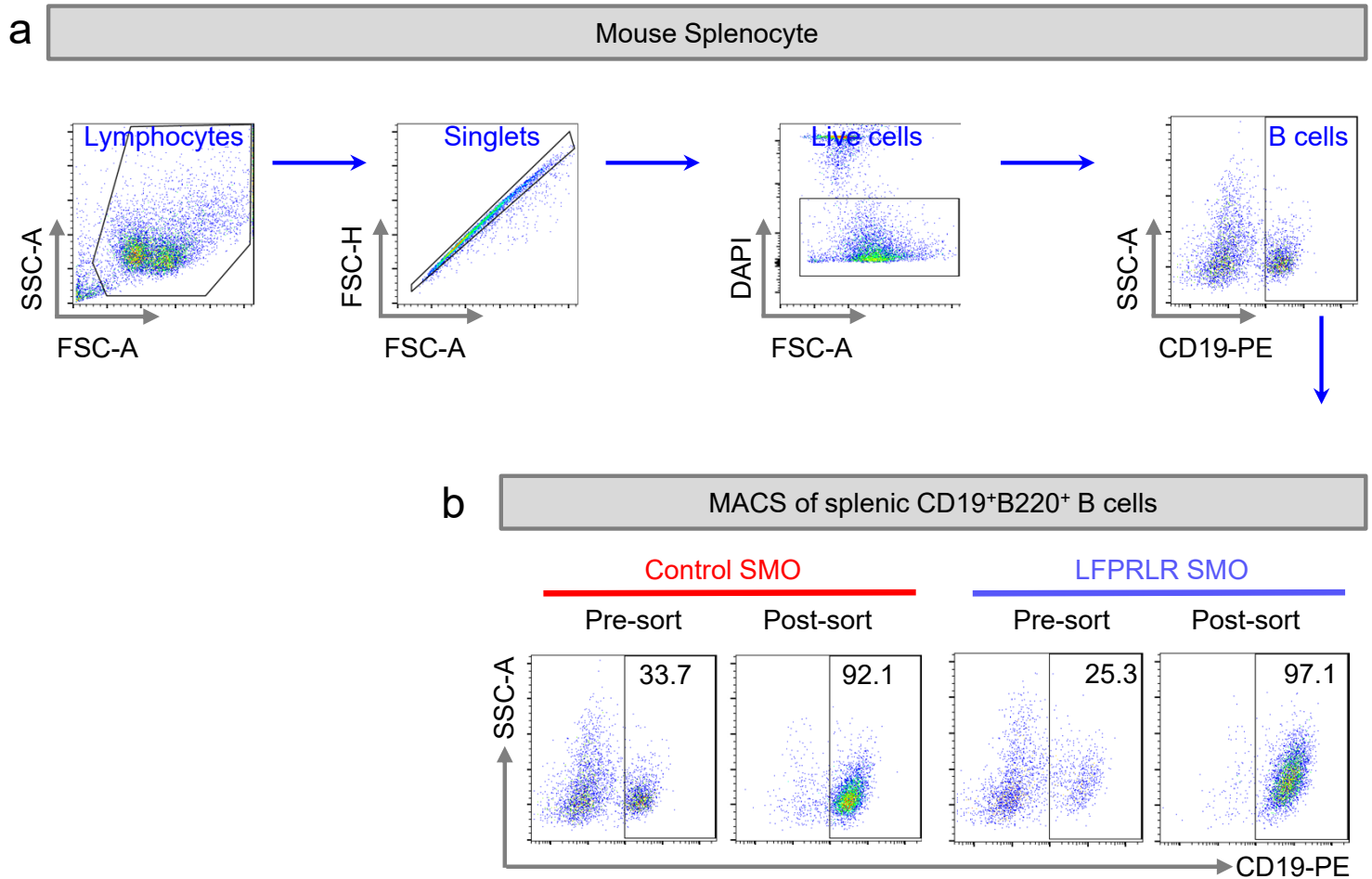
**Supplementary Fig. 3: Gating strategy for flow cytometry by intracellular staining.** From the lymphocyte cluster; singlets were gated followed by selection of live (Ghost-UV450<sup>-</sup>) populations. (a) Total CD138<sup>+</sup> plasma cells, CD138<sup>+</sup>B220<sup>+</sup> plasmablasts, and CD138<sup>+</sup>B220<sup>-</sup> LLPC were then measured by gating on Blimp1<sup>+</sup> cells. (b-c) Total CD19<sup>+</sup> B cells were then gated and BCL2 (b) AID (c) or TCL1 (d) was measured within these cells. Gates for all markers were set based on fluorescence minus one controls (FMO).

**Supplementary Fig. 4:** *Anti-dsDNA autoantibody production trended towards a reduction after LFPRLR knockdown*



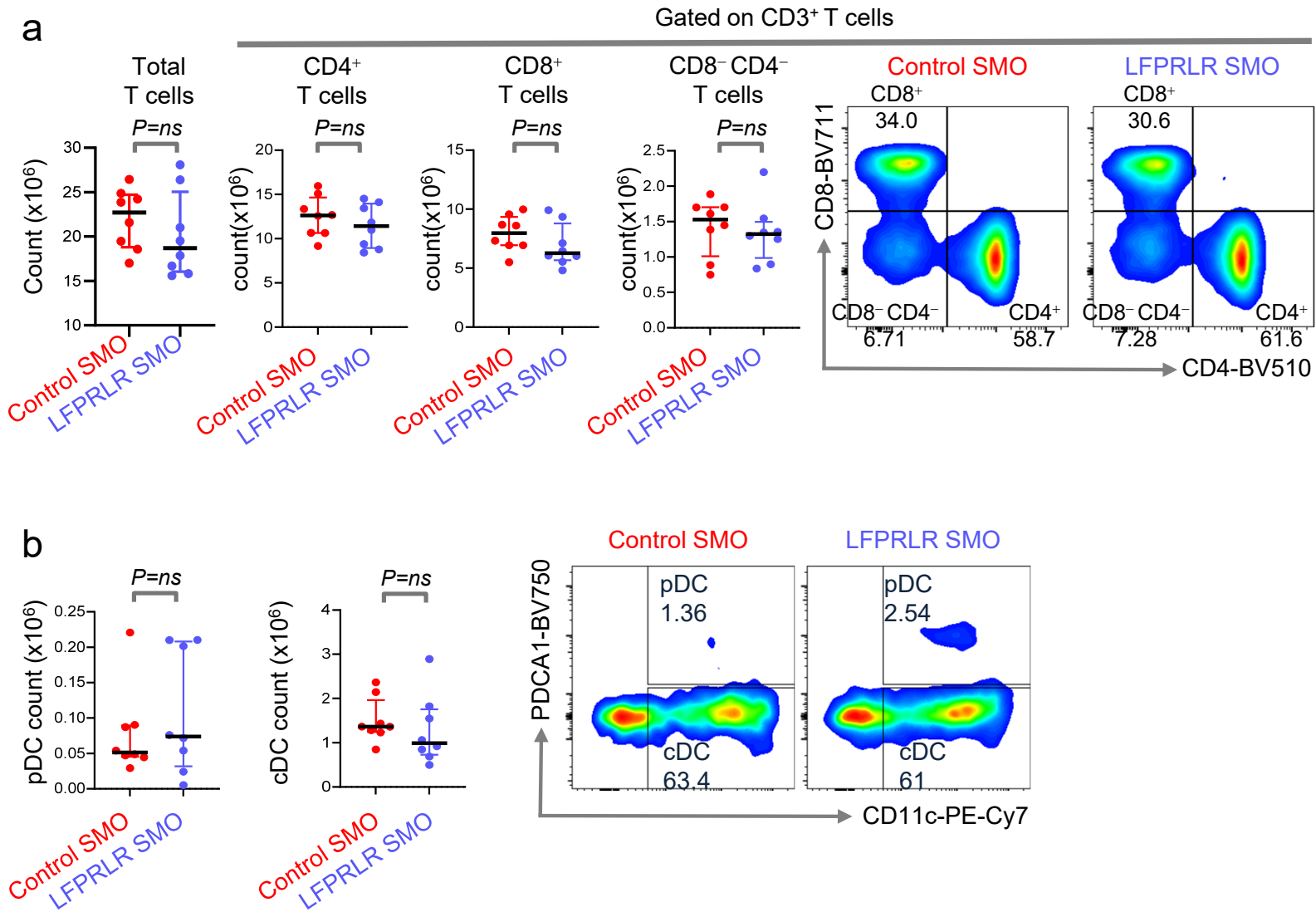
**Supplementary Fig. 4: Anti-dsDNA autoantibody production trended towards a reduction after LFPRLR knockdown.** Fold change/ increase in anti-dsDNA antibodies (day 56: day 9) in mice treated with either control SMO (n=7) or LFPRLR SMO (n=7). Exact p-values were calculated using the Mann-Whitney U test.

**Supplementary Fig. 5: Gating strategy for flow cytometry analysis and magnetic sorting of mouse splenic B cells**



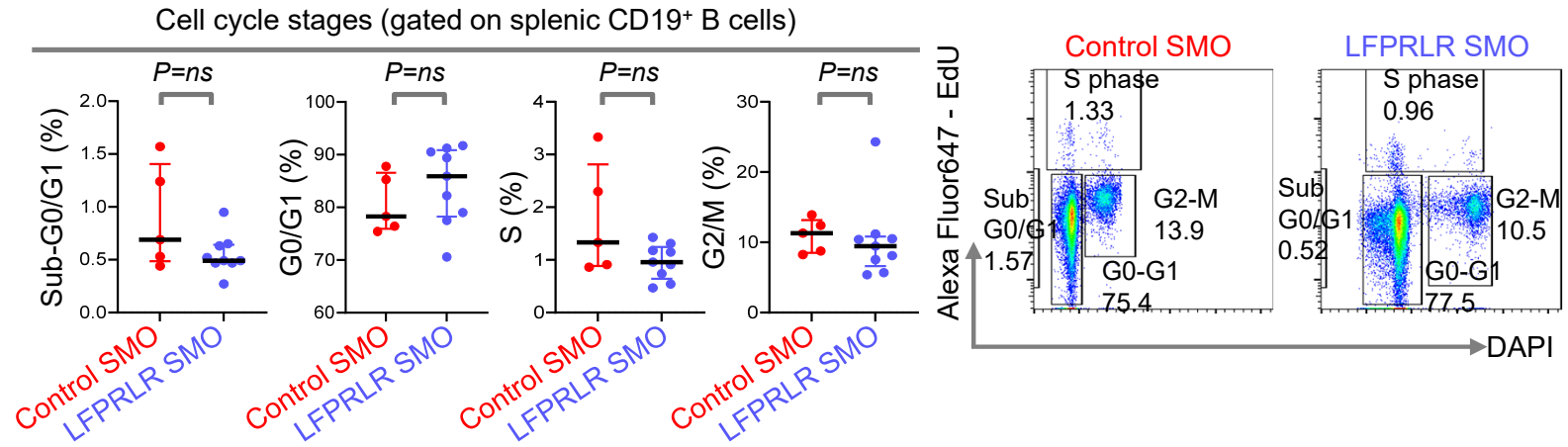
**Supplementary Fig. 5: Gating strategy for flow cytometry analysis and magnetic sorting of mouse splenic B cells.** (a) From the lymphocyte cluster; singlets were gated followed by selection of live (Ghost-UV450<sup>-</sup>) populations. B cells were then gated on CD19<sup>+</sup>. (b) Flow cytometry verification of splenic B cells sorted out magnetically using CD19 microbeads from one representative 14-week-old *MRL-lpr* SLE-prone mouse treated with either control SMO (n=14) or LFPRLR SMO (n=14) for 8 weeks.

**Supplementary Fig. 6: LFPRLR knockdown did not induce significant changes in numbers of T cell and DC subsets in DLBCL prone mice.**



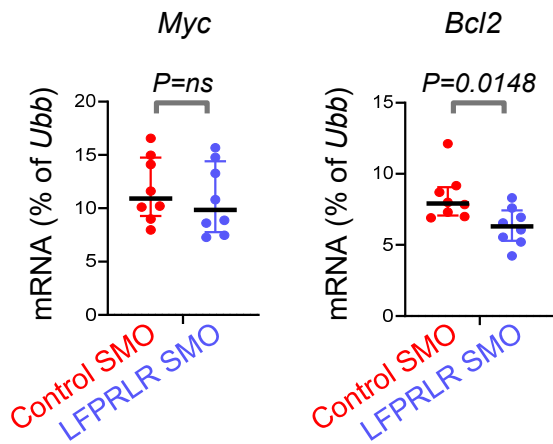
**Supplementary Fig. 6: LFPRLR knockdown did not induce significant changes in numbers of T cell and DC subsets in DLBCL-prone mice. (a,b)** Quantitation of numbers and representative flow cytometry plots of total, CD4<sup>+</sup>, CD8<sup>+</sup>, and CD4<sup>-</sup>CD8<sup>-</sup> T cells (a) and CD11c<sup>+</sup>PDCA1<sup>+</sup> pDCs and CD11c<sup>+</sup>PDCA1<sup>-</sup> cDCs (b) in *TCL1*-tg DLBCL-prone mice treated with control SMO (n=8, red) or LFPRLR SMO to knockdown LFPRLR (n=8, blue). Graphs show median  $\pm$  interquartile range. Exact p-values were calculated using the Mann-Whitney U test. ns = non-significant.

**Supplementary Fig. 7: Knockdown of LFPRLR in DLBCL-prone mice does impact cycling of splenic B cells**



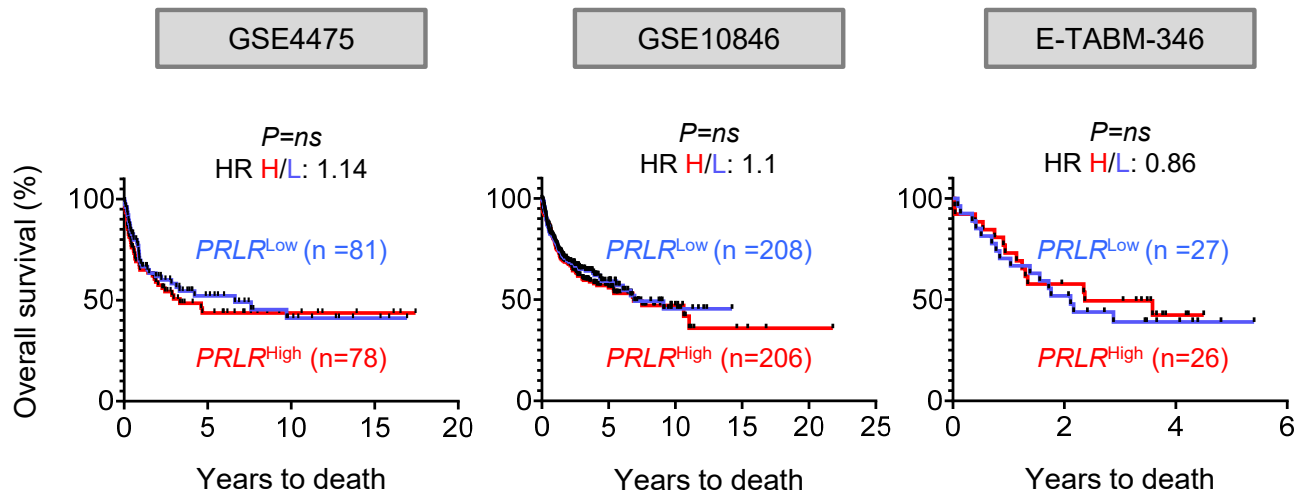
**Supplementary Fig. 7: Knockdown of LFPRLR in DLBCL-prone mice does not impact cycling of splenic B cells.** Percentages of B cells in sub G0/G1, G0/G1, S, G2/M phases and representative flow cytometry in spleens of *TCL1-tg* mice treated with control SMO (n=8, red) or LFPRLR SMO (n=8, blue). Graphs show median  $\pm$  interquartile range. Exact p-values were calculated using the Mann-Whitney U test. ns = non-significant.

**Supplementary Fig. 8:** Knockdown of LFPRLR reduced *Bcl2* expression but does not alter *Myc* expression in total splenic WBCs of DLBCL-prone mice



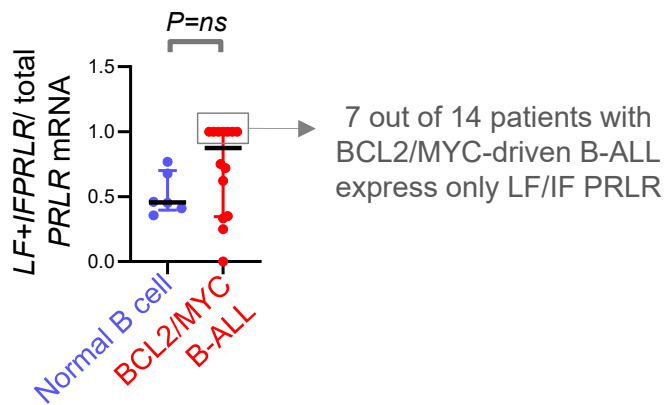
**Supplementary Fig. 8: Knockdown of LFPRLR reduced *Bcl2* expression but does not alter *Myc* expression in total splenic WBCs of DLBCL-prone mice.** Comparison of total splenocyte transcript levels of *Myc* and *Bcl2* by qPCR in *TCL1-tg* mice treated with control SMO (n=8, red) or LFPRLR SMO (n=8, blue). Graphs show median  $\pm$  interquartile range. Each dot in qPCR analyses of transcripts corresponds to the mean expression of that gene in one mouse calculated from 3 technical replicates. Exact p-values were calculated using the Mann-Whitney U test. ns = non-significant

**Supplementary Fig. 9: Total *PRLR* expression in diagnosis and treated DLBCL patients does not predict clinical outcome**



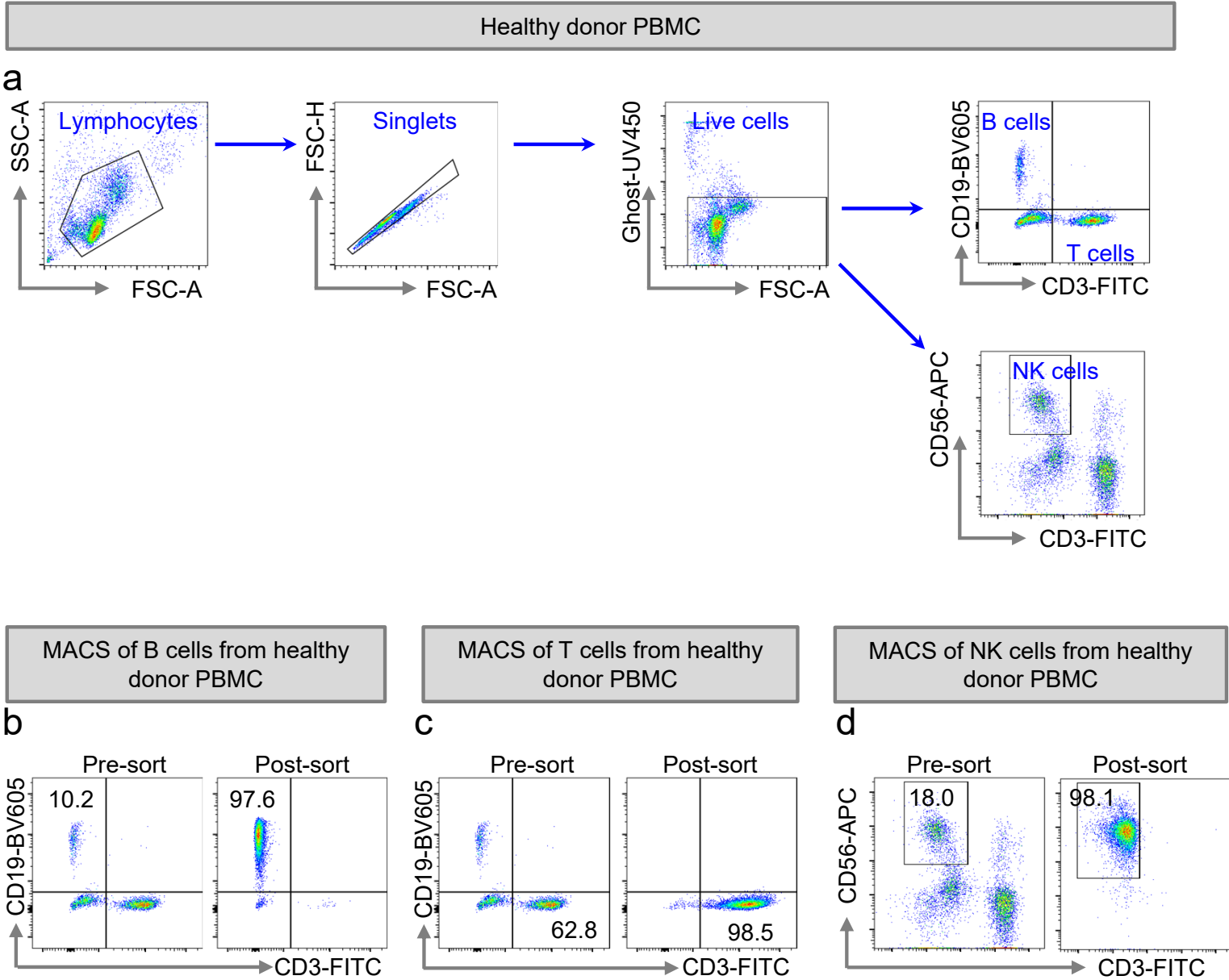
**Supplementary Fig. 9: Total *PRLR* expression in diagnosis and treated DLBCL patients does not predict clinical outcome.** Comparison of overall survival probabilities of patients with B cell lymphomas from 3 datasets (GSE4475, GSE10846, and E-TABM-346) divided into two groups as *PRLR*<sup>High</sup> and *PRLR*<sup>Low</sup> based on the median expression of total *PRLR* mRNA. GSE4475 includes 123 DLBCL and 36 BL patients at diagnosis. GSE10846 includes samples from 414 DLBCL patients collected after treatment with Rituximab-CHOP or CHOP and E-TABM-346 includes samples from 53 DLBCL patients collected after treatment with Rituximab-CHOP or CHOP. P values were measured by Log-rank (Mantel-Cox) test. ns=not significant. HR= Hazard Ratio.

**Supplementary Fig. 10:** Normal B cells always express both *SFPRLR* and *LF/IF PRLR* but 50% of *BCL2/MYC*-driven B-ALL express only *LF/IF PRLR*



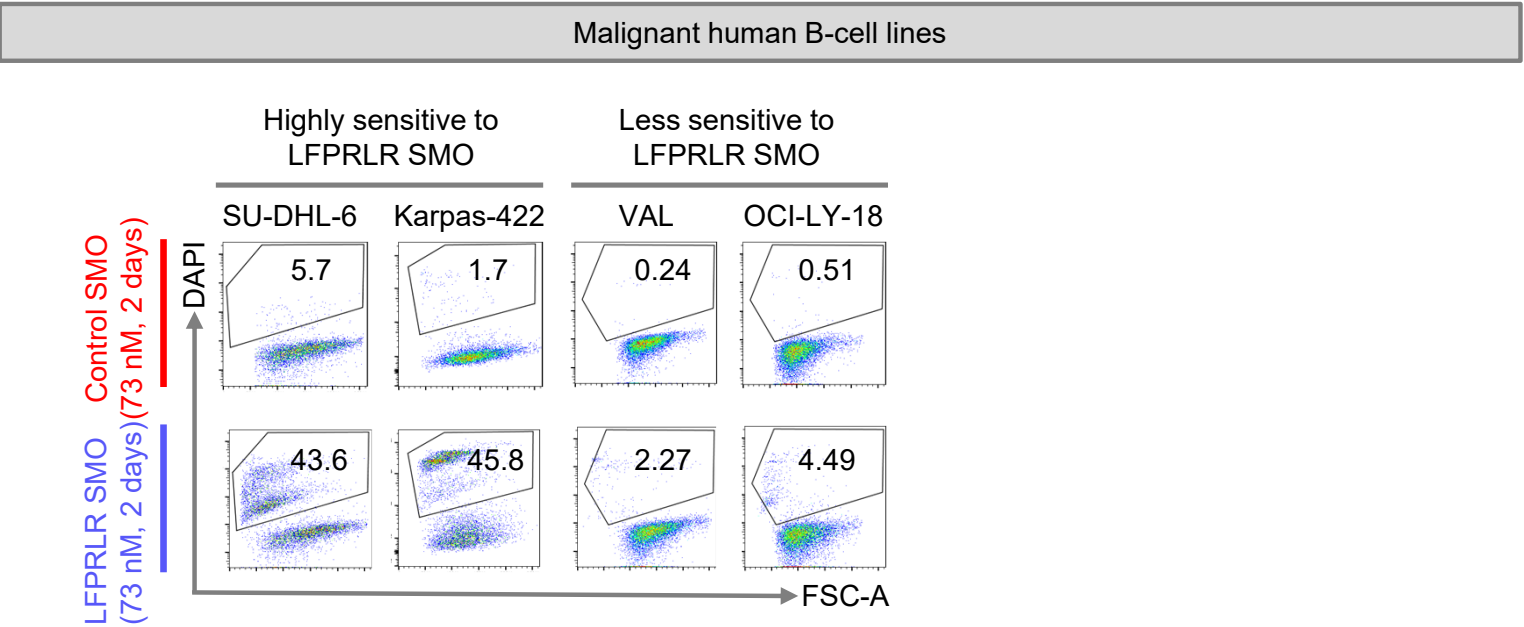
**Supplementary Fig. 10:** Normal B cells always express both *SFPRLR* and *LF/IF PRLR* but 50% of *BCL2/MYC*-driven B-ALL express only *LF/IF PRLR*. Comparison of expression of ratio of *LF+IF*: total *PRLR* mRNA between normal B cells and patients with *BCL2/MYC*-driven B-ALL from the St. Jude RNA sequencing cohort EGAS00001003266. Graph shows median  $\pm$  interquartile range. Exact p-value was calculated using the Mann-Whitney U test. ns = non-significant.

**Supplementary Fig. 11:** *Gating strategy for flow cytometry analysis and magnetic sorting of human peripheral blood B ,T and NK cells*



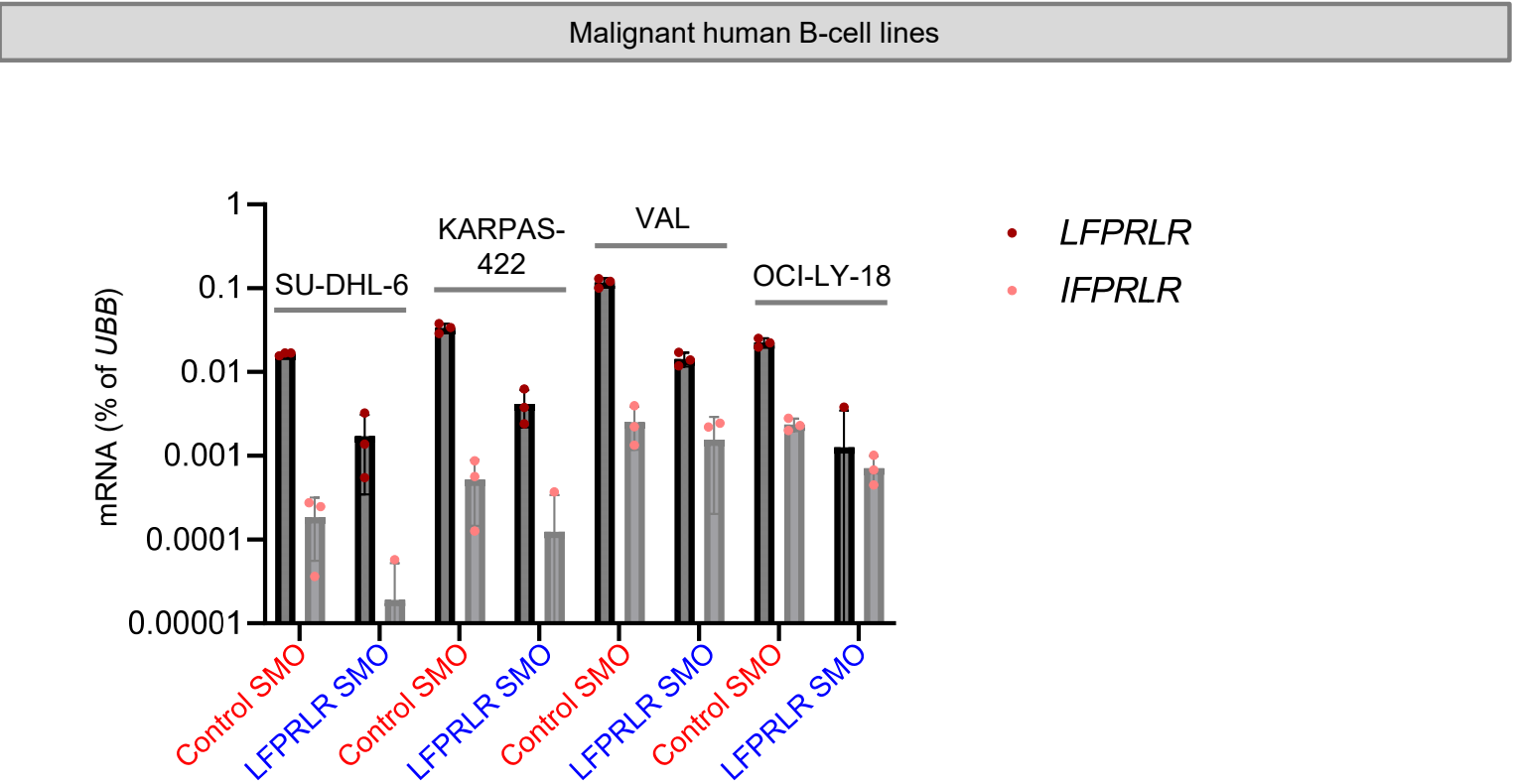
**Supplementary Fig. 11: Gating strategy for flow cytometry analysis and magnetic sorting of human peripheral blood B, T and NK cells.** (a) From the lymphocyte cluster; singlets were gated followed by selection of live (Ghost-UV450<sup>-</sup>) populations. B cells were then gated on CD3<sup>-</sup> CD19<sup>+</sup>, T cells were gated on CD3<sup>+</sup> CD19<sup>-</sup> and NK cells were then gated on CD3<sup>-</sup> CD56<sup>+</sup>. (b-d) Verification of post-sort purity of magnetically sorted B (b), T (c) and NK (d) cells from PBMC of healthy donors by flow cytometry.

**Supplementary Fig. 12:** *Knockdown of LFPRLR in malignant human B cells reduces their viability*



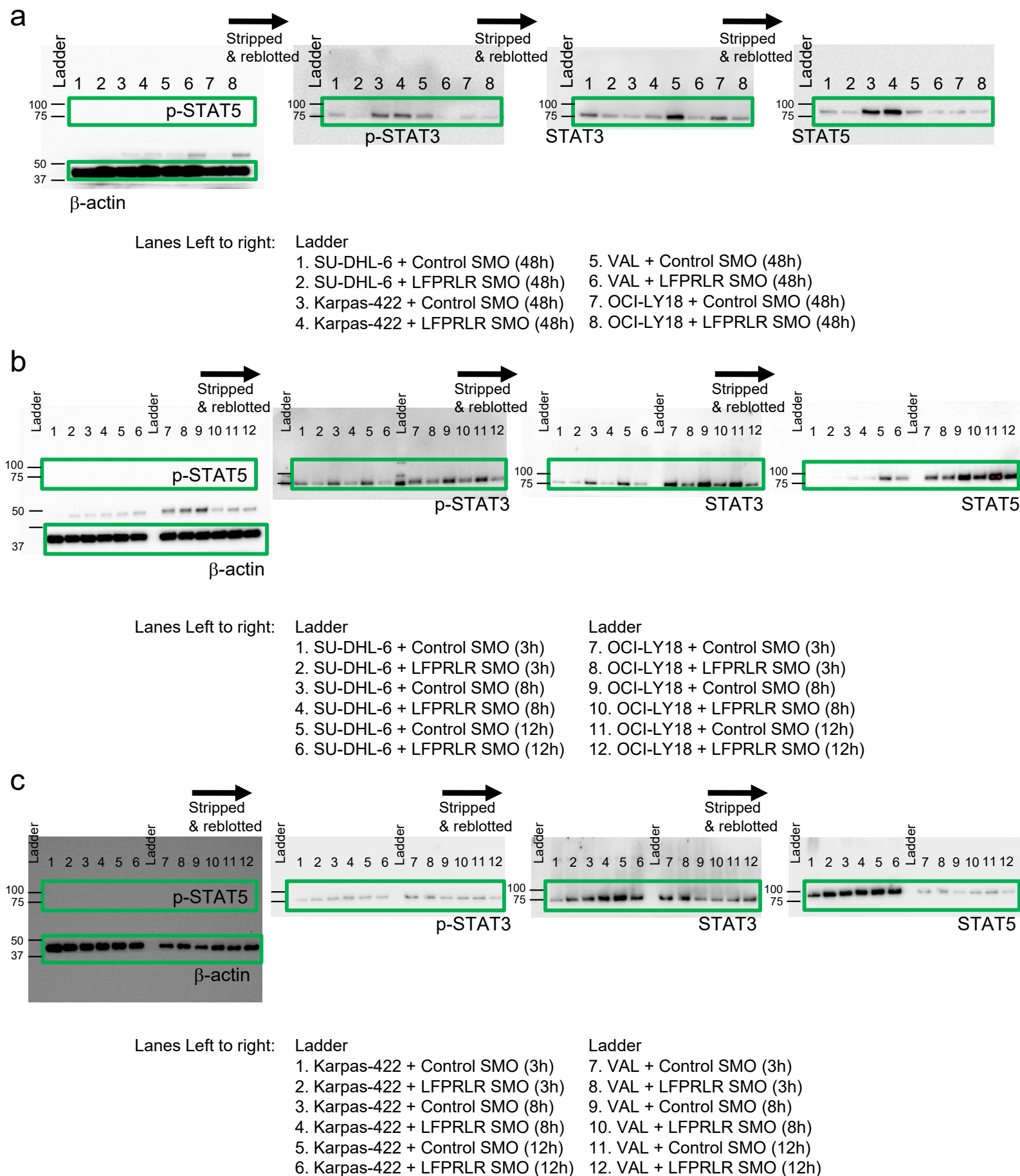
**Supplementary Fig. 12: Knockdown of LFPRLR in malignant human B cells reduces their viability.** Dead cells were assessed by measuring DAPI+ cells by flow cytometry in malignant human B-cell lines after treatment with 73nM control SMO or LFPRLR SMO for 48h.

**Supplementary Fig. 13:** *Expression of LF and IF PRLR in malignant human B cells*



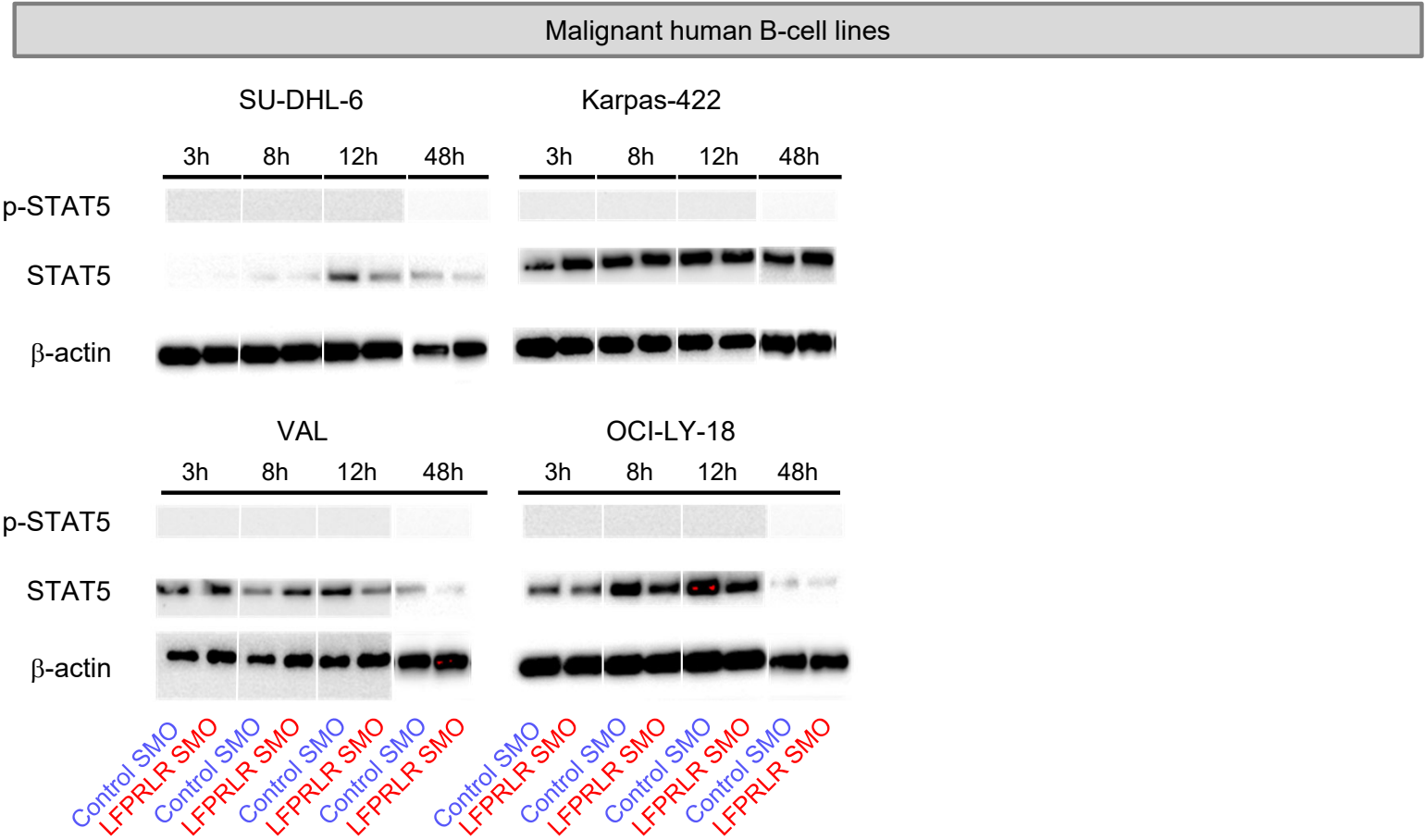
**Supplementary Fig. 13:** *Expression of LF and IF PRLR in malignant human B cells.* *LFPRLR* (red dots) and *IFPRLR* (pink dots) mRNA levels in malignant human B-cell lines (SU-DHL-6, Karpas-422, VAL and OCI-LY18) treated with their IC50 concentration of control SMO or LFPRLR SMO for 48h. Each qPCR sample was run in 3 technical replicates. For bars where <3 dots are shown, the remaining values are zero and could not be plotted on log scale. *UBB* was used as the house keeping gene in qPCR.

**Supplementary Fig. 14: Full scan of blot shown in Figure 5j**



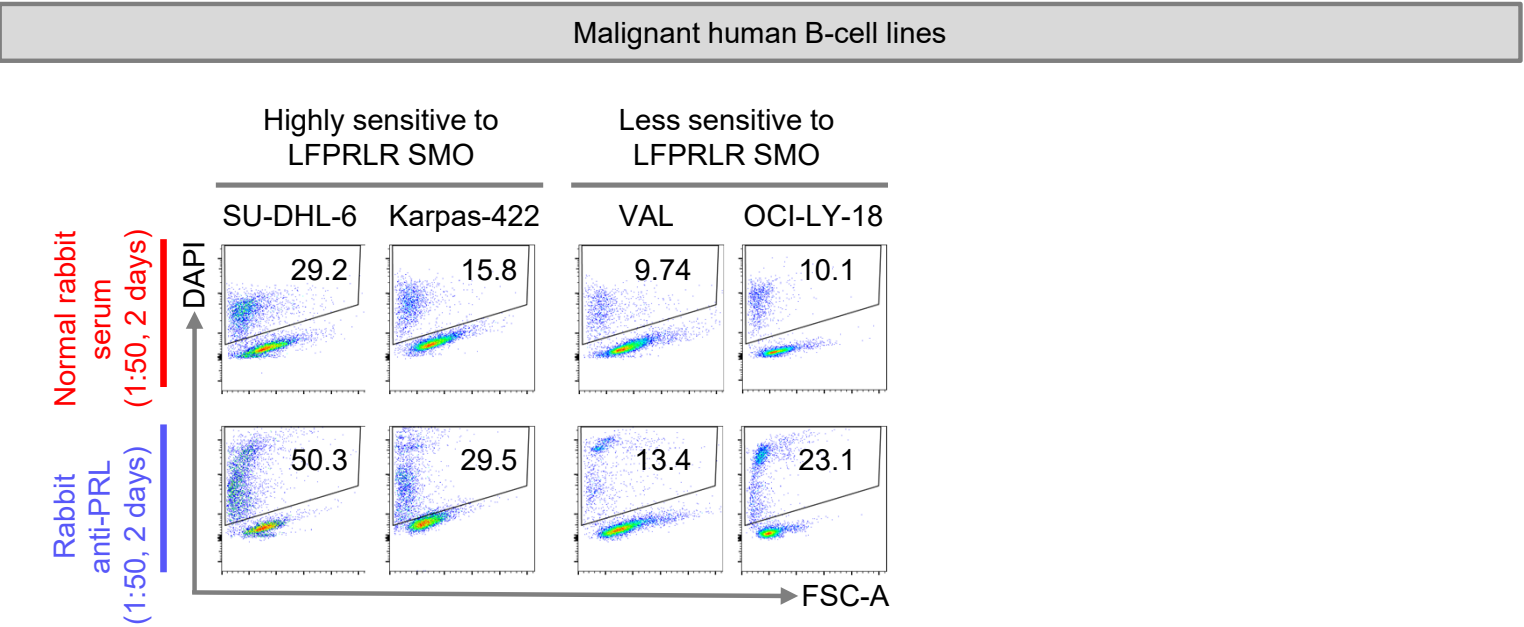
**Supplementary Fig. 14: Full scan of blot shown in Figure 5j.** Blot was developed in ChemiDoc MP imaging system (BioRad). Membrane was re-blotted for similar molecular weight proteins using stripping buffer.

**Supplementary Fig. 15:** *Changes in STAT5 activation and level after LFPRLR knockdown in malignant B cells*



**Supplementary Fig. 15:** *Changes in STAT5 activation and level after LFPRLR knockdown in malignant B cells.* Immunoblotting showing reduction in global STAT5 levels in three out of four malignant B cell lines treated at their IC50 concentrations of LFPRLR SMO for 12 hours. β-actin was used as the loading control in immunoblotting.

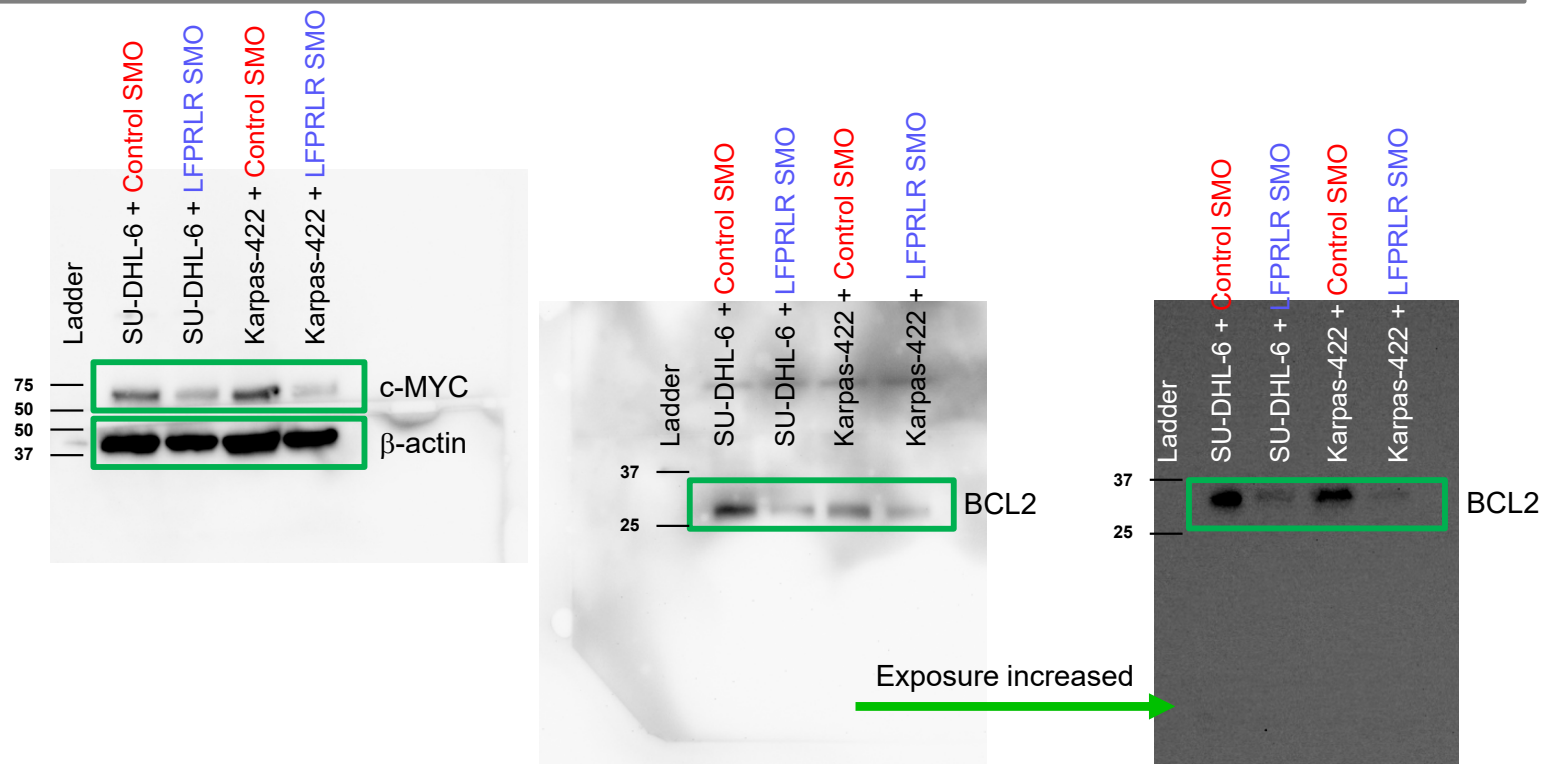
**Supplementary Fig. 16:** *Neutralization of PRL secreted by malignant human B cells reduces their viability*



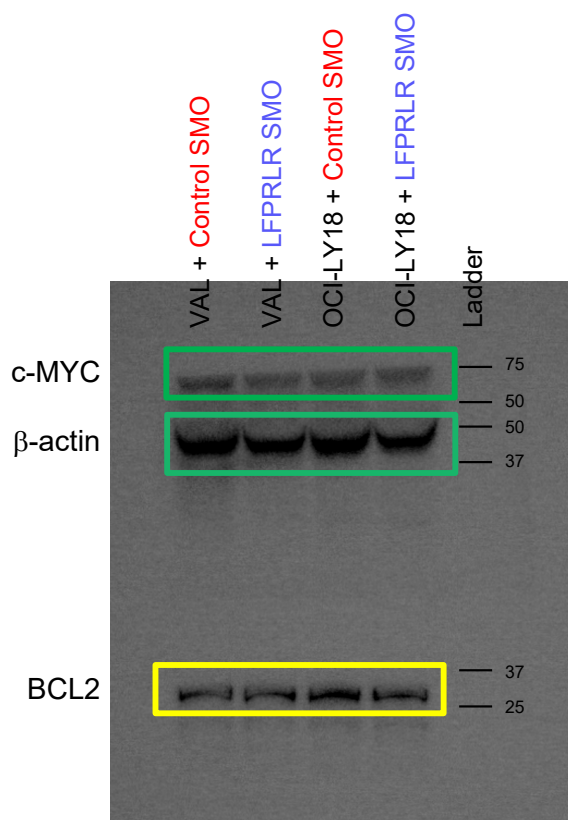
**Supplementary Fig. 16: Neutralization of PRL secreted by malignant human B cells reduces their viability.** DAPI<sup>+</sup> dead cells were assessed by flow cytometry in malignant human B-cell lines after treatment with 1:50 dilution of normal rabbit serum or rabbit anti-human PRL for 48h.

**Supplementary Fig. 17: Full scan of blot shown in Figs. 7e-f**

Full scan of blot shown in Figure 7e



Full scan of blot shown in Figure 7f



**Supplementary Fig. 17: Full scan of blot shown in Figs. 7e-f.** Blot was developed in ChemiDoc MP imaging system (BioRad)

**Supplementary Table 1:** *List of oligonucleotide sequences used in the study***a. Splice modulating oligomer (SMO)**

| <b>SMO</b>  | <b>Sequence</b>                 |
|-------------|---------------------------------|
| Control SMO | 5'-AGACGAGATTCGATCGGAGTA-3'     |
| mLFPRLR SMO | 5'-GCCCTTCTATTGAAACACAGATACA-3' |
| hLFPRLR SMO | 5'-GCCCTTCTATTAAACACAGACACA-3'  |

**b. Quantitative RT-PCR (m= mouse, h= human)**

| <b>Primer</b> | <b>Sequence</b>                      |
|---------------|--------------------------------------|
| mUbb- F       | 5'-AGCCCAGTGTTACCACCAAG-3'           |
| mUbb- R       | 5'-ACCCAAGAACAAGCACAAGG-3'           |
| mLF Prlr- F   | 5'-ATAAAAGGATTTGATACTCATCTGCTAGAG-3' |
| mLF Prlr- R   | 5'-TGTCATCCACTTCCAAGAACTCC-3'        |
| mSF1 Prlr- F  | 5'-AAGCCAGACCATGGATACTGGAG-3'        |
| mSF1 Prlr- R  | 5'-AACTGGAGAATAGAACACCAGAG-3'        |
| mSF2 Prlr- F  | 5'-TGCATCTTTCCACCAGTTCCGGGGC-3'      |
| mSF2 Prlr- R  | 5'-TCAAGTTGCTCTTTGTTGTCAAC-3'        |
| mSF3 Prlr- F  | 5'-TGCATCTTTCCACCAGTTCCGGGGC-3'      |
| mSF3 Prlr- R  | 5'-TTGTATTTGCTTGGAGAGCCAGT-3'        |
| mMyc- F       | 5'-TCGCTGCTGTCCTCCGAGTCC-3'          |
| mMyc- R       | 5'-GGTTTGCCTCTTCTCCACAGAC-3'         |
| mBcl2- F      | 5'-CCTGTGGATGACTGAGTACCTG-3'         |
| mBcl2- R      | 5'-AGCCAGGAGAAATCAAACAGAGG-3'        |
| mAicda- F     | 5'-AAATGTCCGCTGGGCCAA-3'             |
| mAicda- R     | 5'-CATCGACTTCGTACAAGGG-3'            |
| hUBB- F       | 5'-GCCGCACTCTTTCTGACTACAAC-3'        |
| hUBB- R       | 5'-ACCTCCAGAGTGATGGTCTTGC-3'         |
| hLFPRLR- F    | 5'-TCCAGGTATGTGGGTTTCAT-3'           |
| hLFPRLR- R    | 5'-GATTTGATGCTCATCTGTTGGA-3'         |
| hIFPRLR-F     | 5'-CTGTTCTCTCTCTTGA CT TGGG-3'       |
| hIFPRLR-R     | 5'-GATTTGATGCTCATCTGTTGGA-3'         |
| hSF1aPRLR- F  | 5'-TGGACTGTGGTCAATGTTGC-3'           |
| hSF1aPRLR- R  | 5'-GATAGTGAGGACCAGCATCTAATG-3'       |
| hSF1bPRLR- F  | 5'-CAACATCAAGGGGTACCTC-3'            |
| hSF1bPRLR- R  | 5'-CATGAATGATACAACCGTGTGG-3'         |
| hMYC- F       | 5'-CCTGGTGCTCCATGAGGAGAC-3'          |
| hMYC- R       | 5'-CAGACTCTGACCTTTTGCCAGG-3'         |
| hPRL- F       | 5'-GAGGAGCAAACCAAACGGCTTC-3'         |
| hPRL- R       | 5'-AAGGCGAGACTCTTCATCAGCC-3'         |
| hBCL2- F      | 5'-ATCGCCCTGTGGATGACTGAGT-3'         |
| hBCL2- R      | 5'-GCCAGGAGAAATCAAACAGAGGC-3'        |

**c. mIGH sequencing**

| <b>Primer</b>       | <b>Sequence</b>             |
|---------------------|-----------------------------|
| V <sub>H</sub> 1- F | 5'-AAGGCCCACTGACTGTAGAC-3'  |
| C $\mu$ - R         | 5'-TGGCCACCAGATTCTTATCAG-3' |

**Supplementary Table 2:** *List of antibodies used in the study***a. Flow cytometry**

| <b>Antibodies</b>       | <b>Clone ID</b> | <b>Specificity</b> | <b>Dilution</b> | <b>Source</b> |
|-------------------------|-----------------|--------------------|-----------------|---------------|
| Anti-CD19               | 1D3/CD19        | Mouse              | 7:1000          | Biolegend     |
| Anti-CD45R/B220         | RA3-6B2         | Mouse/Human        | 5:1000          | Biolegend     |
| Anti-CD138 (Syndecan-1) | 281-2           | Mouse              | 1:100           | Biolegend     |
| Anti-Blimp-1            | 5E7             | Mouse              | 1:100           | Biolegend     |
| Anti-AID                | mAID-2          | Mouse/Human        | 1:100           | eBioscience   |
| Anti-BCL2               | BCL/10C4        | Mouse/Rat          | 1:100           | Biolegend     |
| Anti-CD3                | UCHT1           | Human              | 1:100           | Biolegend     |
| Anti-CD56               | 5.1H11          | Human              | 1:100           | Biolegend     |
| Anti-CD19               | HIB19           | Human              | 1:100           | Biolegend     |
| Anti-CD4                | RM4-5           | Mouse              | 1:100           | Biolegend     |
| Anti-CD19               | 1D3             | Mouse              | 1:100           | BD            |
| Anti-CD11c              | N418            | Mouse              | 1:100           | Biolegend     |
| Anti-CD8                | 5306.7          | Mouse              | 1:100           | Biolegend     |
| Anti-CD3                | 17A2            | Mouse              | 1:100           | BD            |
| Anti-PDCA1              | 927             | Mouse              | 1:100           | BD            |
| Anti-NKP46              | 29A1.4          | Mouse              | 1:100           | BD            |
| Ghost Dye™ UV 450       |                 |                    | 1:100           | Tonbo         |
| Anti-TCL1               | 1-21            | Human              | 1:100           | Biolegend     |

**b. Immunoblotting**

| <b>Antibodies</b>              | <b>Clone ID</b> | <b>Specificity</b> | <b>Dilution</b> | <b>Source</b>             |
|--------------------------------|-----------------|--------------------|-----------------|---------------------------|
| Anti-cMYC                      | D84C12          | Mouse/Human        | 1:1000          | Cell Signaling Technology |
| Anti-β-Actin                   | 8H10D10         | Mouse/Human        | 1:500           | Cell Signaling Technology |
| Anti-BCL2                      | D17C4           | Mouse/Human        | 1:1000          | Cell Signaling Technology |
| Anti-STAT5                     | D2O6Y           | Mouse/Human/Rat    | 1:1000          | Cell Signaling Technology |
| Anti-STAT3                     | 124H6           | Mouse/Human/Rat    | 1:1000          | Cell Signaling Technology |
| Anti-phospho<br>STAT3 (Tyr705) |                 | Mouse/Human/Rat    | 1:1000          | Cell Signaling Technology |
| Anti-phospho<br>STAT5 (Tyr694) | 14H2            | Mouse/Human        | 1:1000          | Cell Signaling Technology |