Supplemental material

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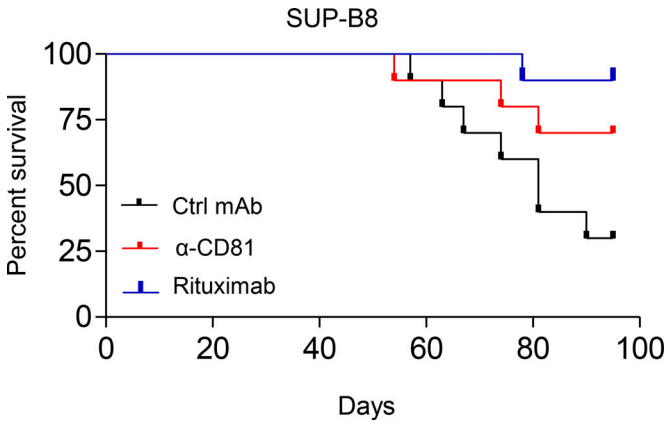

Figure S1. **5A6, an anti-human CD81 mAb, improves survival of SUP-B8–bearing mice.** SUP-B8-luc cells (10^6) were injected i.v. into 6–8-wk-old female SCID-beigemice. Mice were treated with 100 µg of 5A6, rituximab, or control MsIgG1 beginning on day 2 after tumor injection and every other day thereafter for two more doses. Mice survival was monitored up to 95 d (n = 10 mice in each group). Ctrl, control.

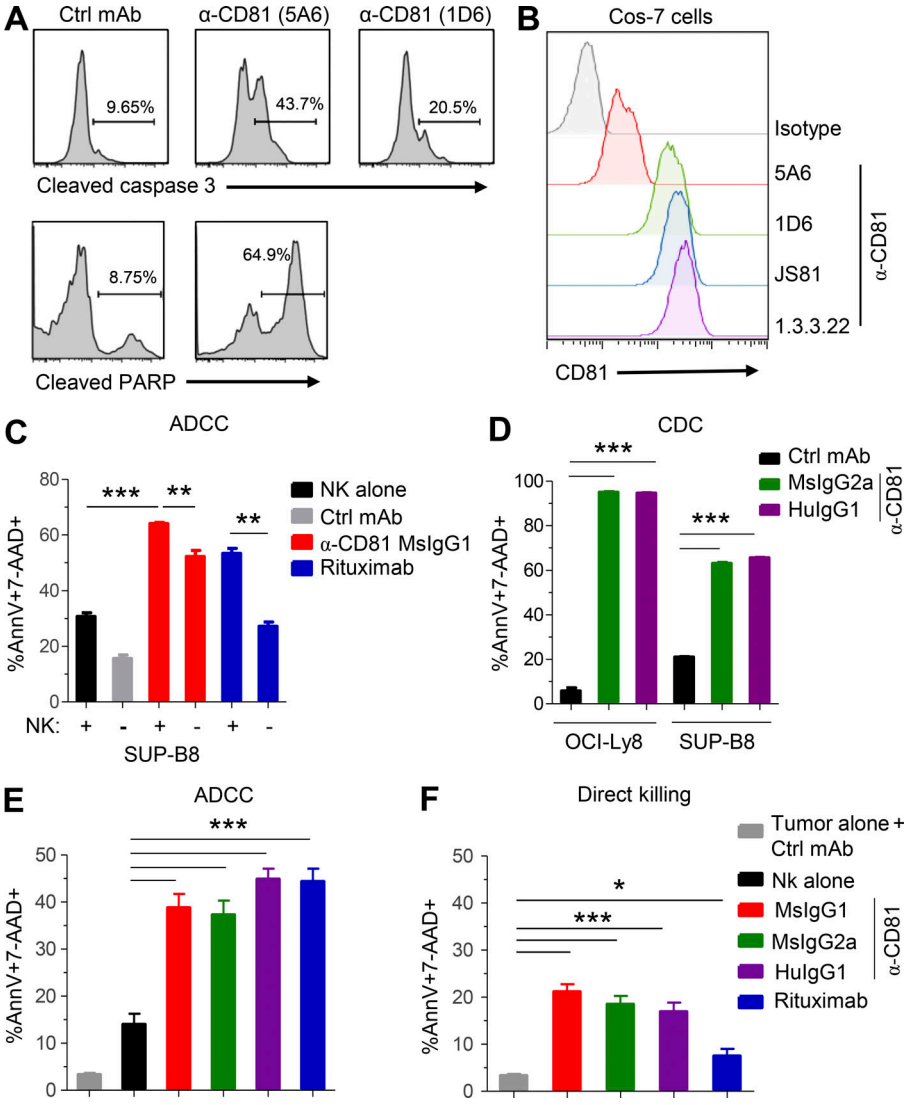

Figure S2. **Engagement of CD81 by 5A6 induces diverse killing mechanisms. (A)** Anti-CD81, 5A6 induces direct killing by activating caspase-3 and PARP. OCI-ly8 cells were incubated for 1 h with 5A6, 1D6, or isotype control (all MsIgG1). Cells then were fixed and permeabilized and stained with anti-cleaved caspase-3 or anti-cleaved PARP and analyzed by flow cytometry. Data represented here were done in triplicate in three independent experiments. Ctrl, control. **(B)** Binding of anti-CD81 mAbs to monkey cells. CV-1 in origin with SV40 gene (COS) cells, of African green monkey origin, were stained with the indicated anti-CD81 (MsIgG1) mAbs for 15 min, followed by staining with polyclonal goat anti-mouse IgG antibody for 15 min. Cells were washed and fixed with 1% para-formaldehyde, followed by flow cytometry analysis. **(C–F)** In vitro sensitivity of B lymphoma cell lines to direct and indirect cytotoxicity induced by 5A6 mAbs. **(C)** SUP-B8 cells were incubated overnight with 1 µg/ml of 5A6 (MsIgG1), rituximab (HuIgG1), or control MsIgG1 in the presence or absence of purified human NK cells (5:1). **(D)** OCI-Ly8 or SUP-B8 cells were incubated for 1.5 h with the indicated 5A6-based mAbs in the presence of fresh pooled human serum. **(E and F)** 10^6 Raji cells were incubated overnight with 1 µg of the indicated mAbs in the presence (E) or absence (F) of purified human NK cells (5:1 ratio). **(C–F)** Cell death was measured by Annexin-V and 7-AAD staining. Data represented here were done in triplicate in at least three independent experiments. Error bars represent mean ± SEM. *, P < 0.0054; **, P < 0.0036; ***, P < 0.0001, Student's *t* test (C–F).

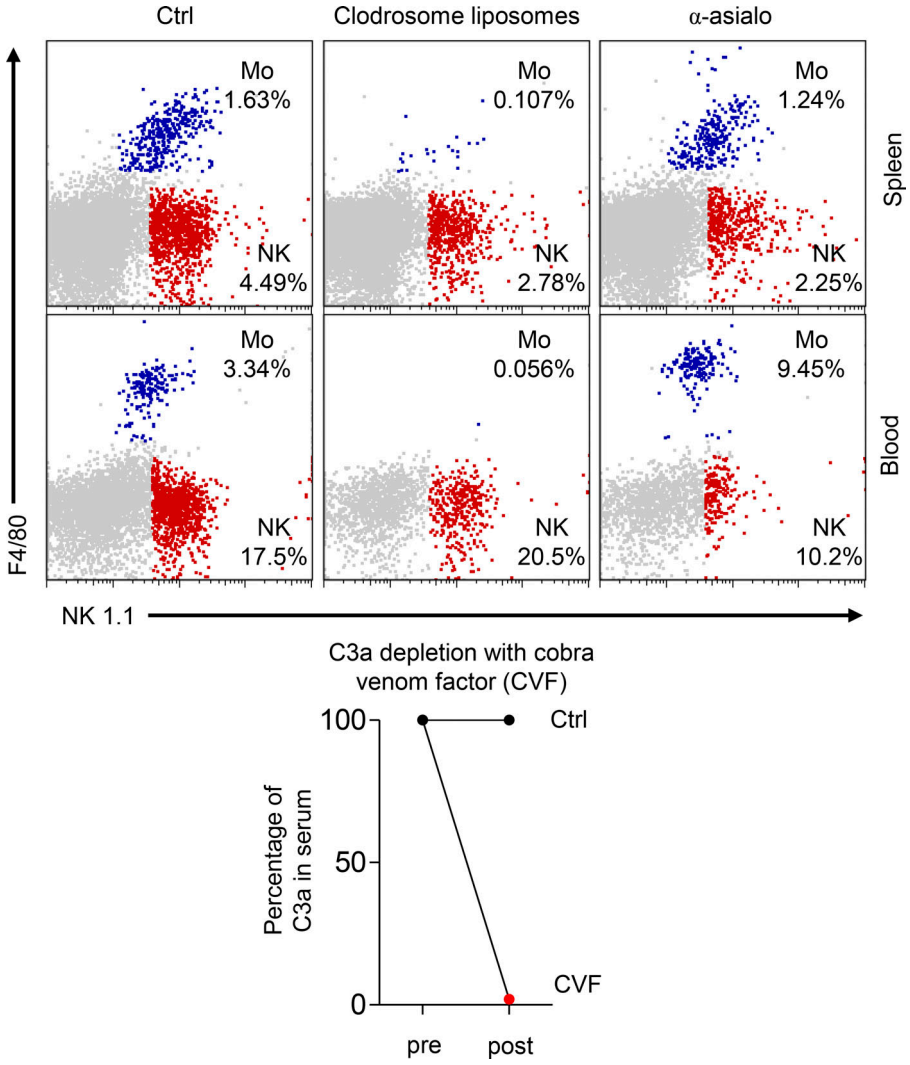

Figure S3. **Depletion of macrophages (Mo), NK cells, and complement.** Macrophages from SCID-Beige mice were depleted with 200 µl (5 mg/ml) of clodrosome liposomes i.p. 4 h after tumor challenge and on day 5. NK cells were depleted by i.p. injection of 50 µg of anti-asialo GM1 on days −1 and 0 of tumor challenge and every 5 d thereafter for 3 wk. Complement was depleted by i.p. injection of 25 U CVF on the day of tumor challenge and on days 3, 6, and 9 after challenge. Depletion was confirmed on blood and spleen on day 5 after the second dose of clodrosome and on day 4 after the third dose of anti-asialo by flow cytometry using F4/80 for macrophages and NK1.1 for NK cells (top; dot plots show F4/80 staining in the y axis and NK1.1 in the x axis in spleen and blood), and serum was collected before CVF injection (pre) and on day 8 after CVF injection (post). C3a was measured by ELISA (bottom; ELISA shows C3a serum concentration; n = 5 mice in each depletion group). Ctrl, control.

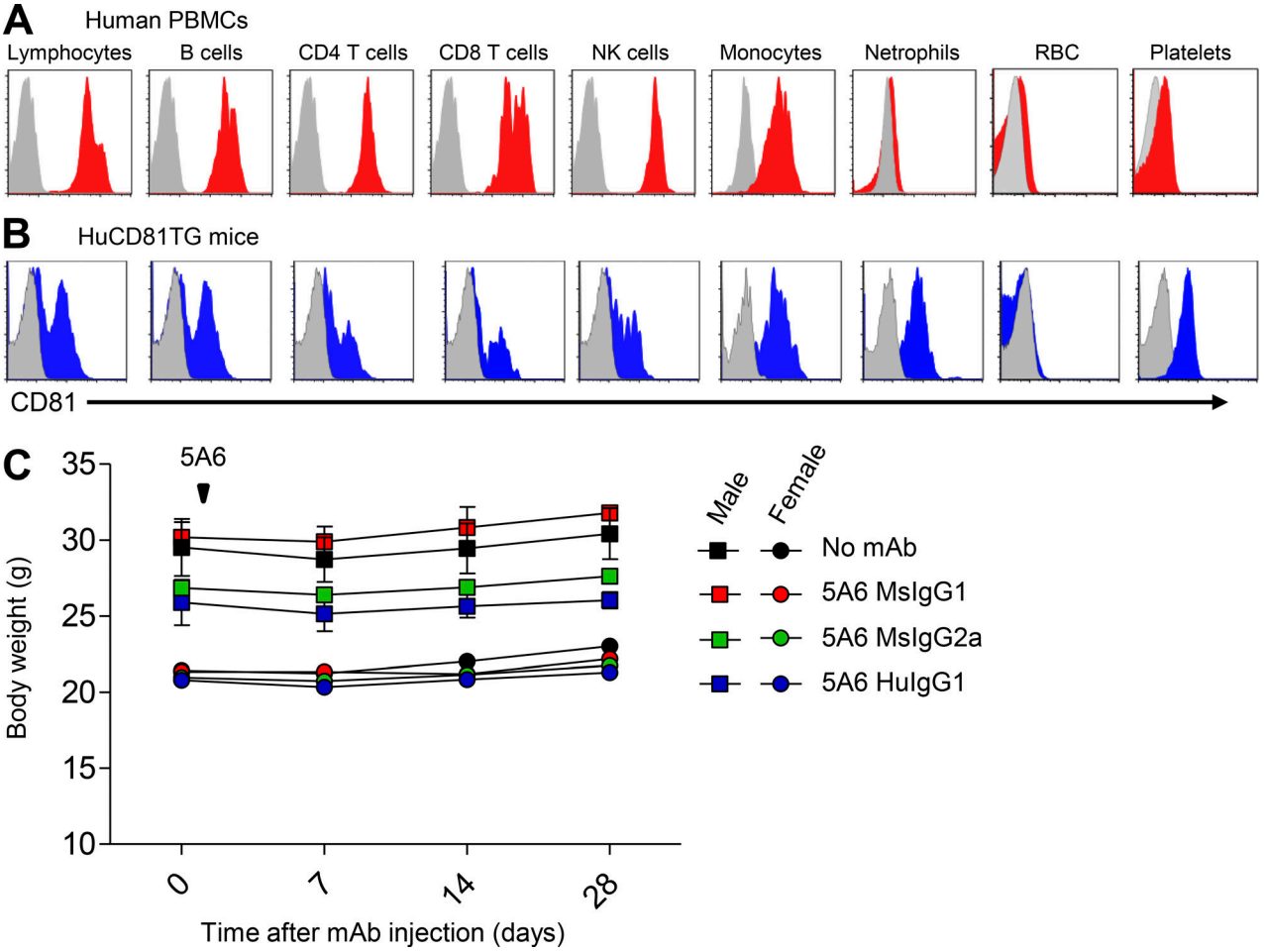

Figure S4. **Expression of human CD81 in human and human CD81 transgenic mice PBMCs. (A and B)** Binding of anti-human CD81 mAb to human PBMCs (A) and transgenic mouse PBMCs (B). The indicated subpopulations of human or mouse PBMCs were stained with a combination of lineage specific markers and anti-human CD81 (JS81), followed by flow cytometry (red and blue filled histograms show CD81 staining; gray-filled histograms show isotype control). **(C)** Weight measurements of female and male mice injected with 500 µg of 5A6-based mAbs mouse IgG1, IgG2a, and chimeric HuIgG1 (*n* = 6 mice in each group). Error bar represent mean ± SEM.

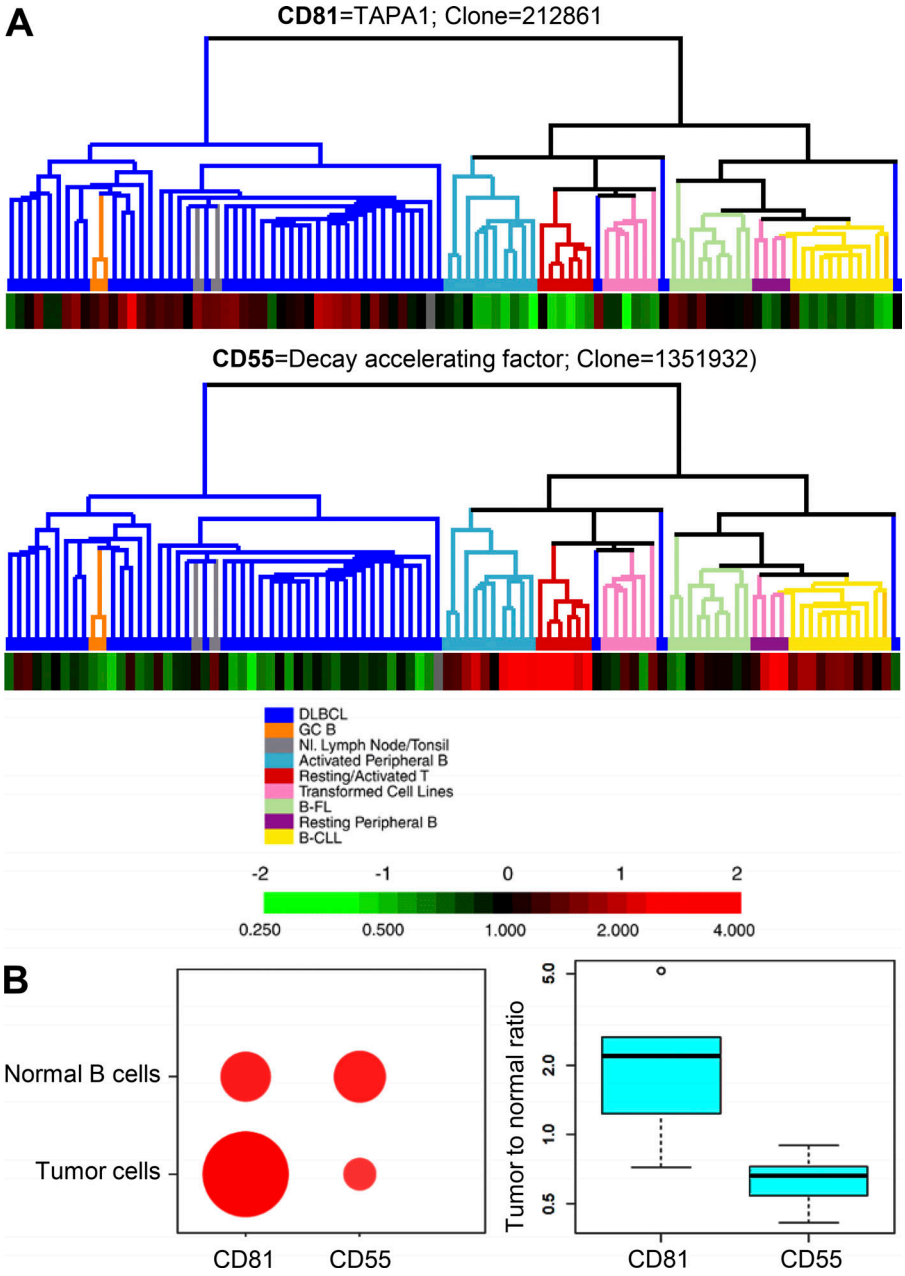

Figure S5. **mRNA CD81 and CD55 expression in B cell lymphomas and in normal B cells. (A)** Reanalysis of CD81 and CD55 mRNA expression in the web supplement to Alizadeh et al., 2000 (https://llmpp.nih.gov/lymphoma/). **(B)** Reanalysis of CD81 and CD55 single-cell mRNA expression in tumor cells versus imbedded normal B cells (Andor et al., 2019). DLBCL, diffuse large B cell lymphoma. GC, germinal center; Nl, normal. Error bar represents mean ± SD.

References

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