Supplemental Information

Protein amounts of the MYC transcription factor determine germinal center B cell division capacity

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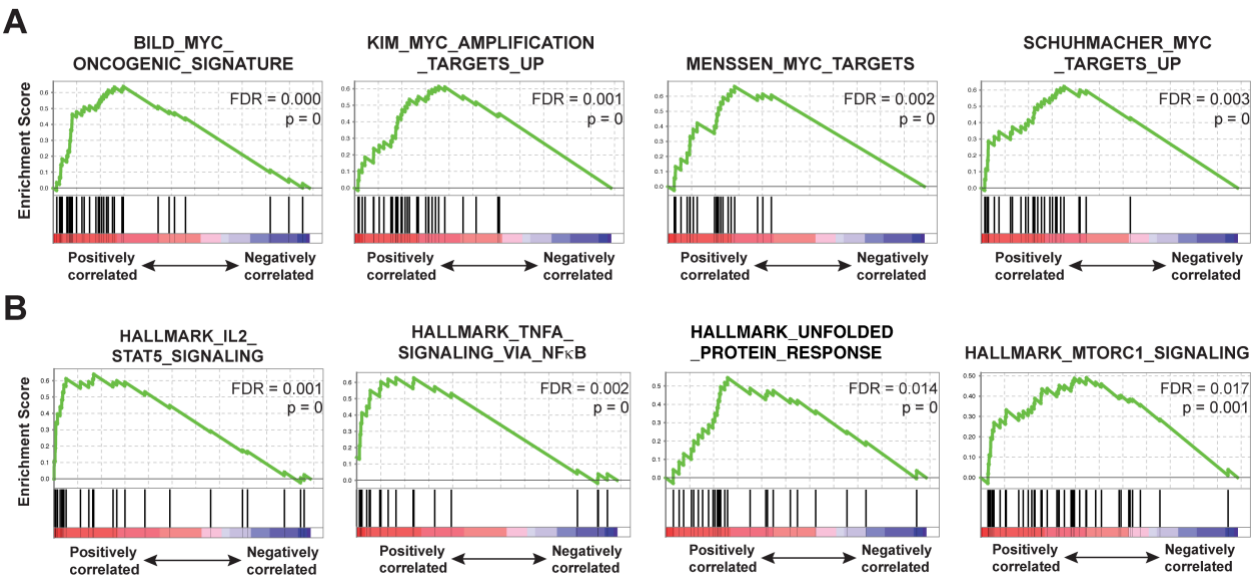

Figure S1. Gene Set Enrichment Analysis of LZ selected germinal center B cells. Related to Figure 1.

(A) Gene Set Enrichment Plots for MYC pathway activated genes in LZ *B1-8^{hi}DEC205$^{+/+}$*Fucci^{+} GC B cells following graded αDEC-OVA treatment. (B) Gene Set Enrichment Plots for other activated gene signatures in LZ *B1-8^{hi}DEC205$^{+/+}$*Fucci^{+} GC B cells following graded αDEC-OVA treatment using hallmark gene sets. FDR = False Discovery Rate. All p-values are indicated.

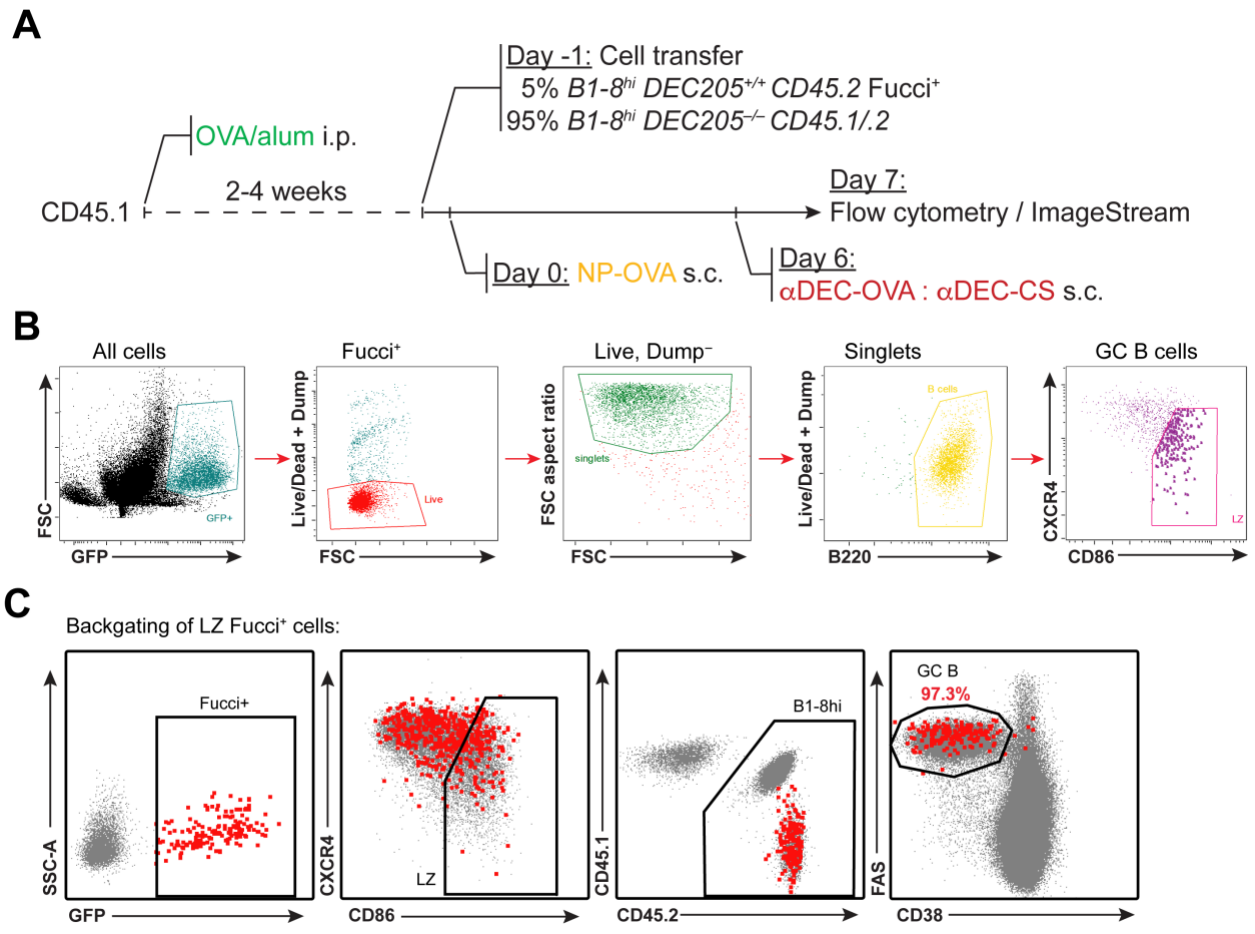

Figure S2. Proportional correlation between Tfh help and cell size of GC B cells *in vivo*. Related to Figure 2. (A) Schematic representation of the experimental protocol for Figure 2. i.p., intraperitoneally, s.c., subcutaneously. (B) Gating strategy for ImageStream experiment shown in Figure 2 (C-G). (C) Flow cytometry backgating of LZ *B1-8^{hi}DEC205$^{+/+}$Fucci^{+}* B cells used for ImageStream experiment showing that 97.3% of them are GC B cells.

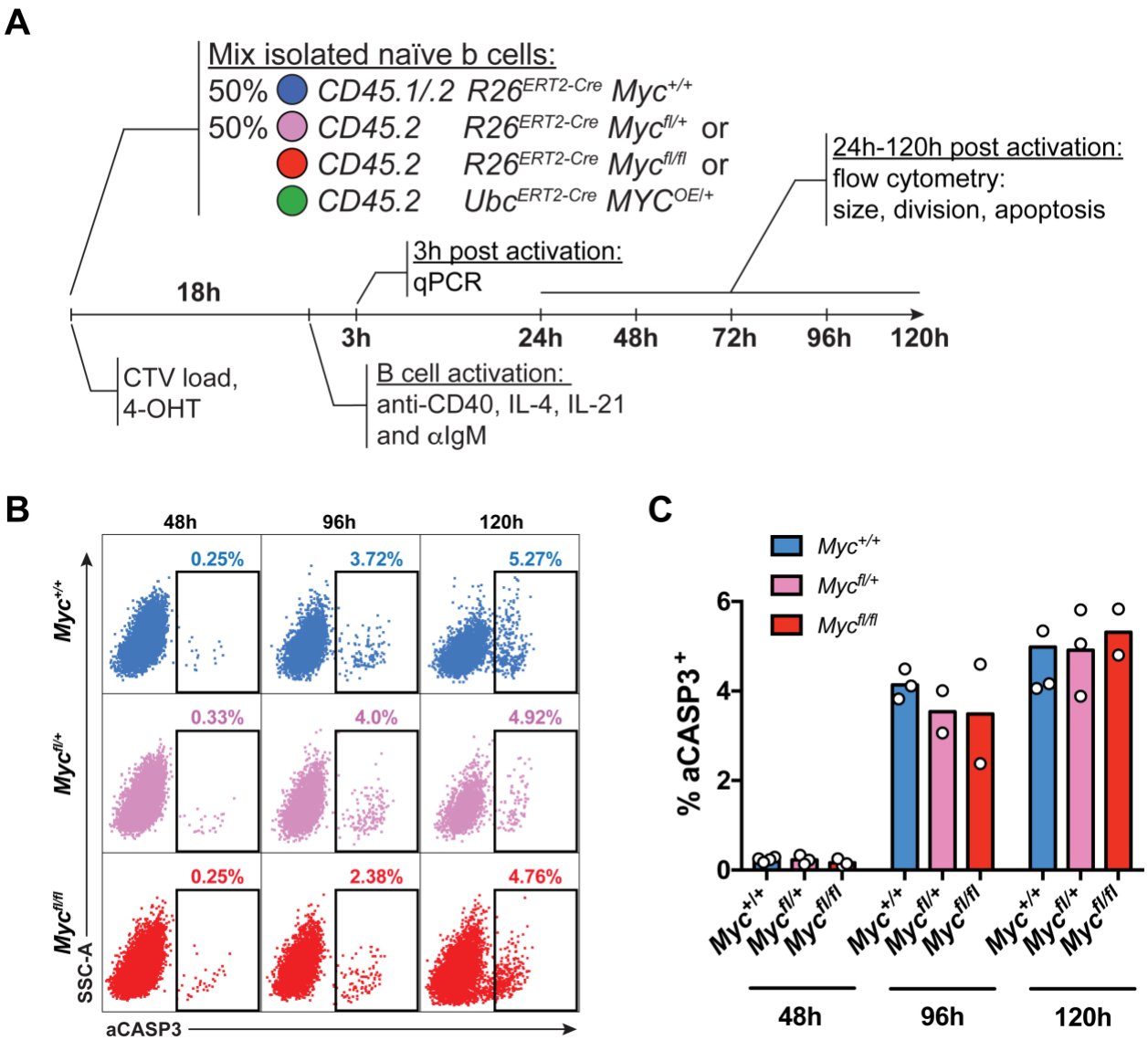

Figure S3. No significant change in cell death among B cells with varying amounts of MYC *in vitro*. Related to Figure 3. (A) Schematic representation of the experimental protocol for Figure 4 (E-H) and Figure S3 (B and C). (B and C) Representative flow cytometry (B) and quantification (C) of the percentage of aCASP3$^+$ B cells from the indicated mice analyzed 48, 96 or 120 hours after activation *in vitro*. Splenic B cells from the indicated mice, stained with CTV, co-cultured in the presence of 4-OHT and activated

with IL-4, IL-21, αCD40 and αIgM. B cells were analyzed for division and size by flow cytometry. Data represents two independent experiments with 2-3 technical repeats of each time point and condition.

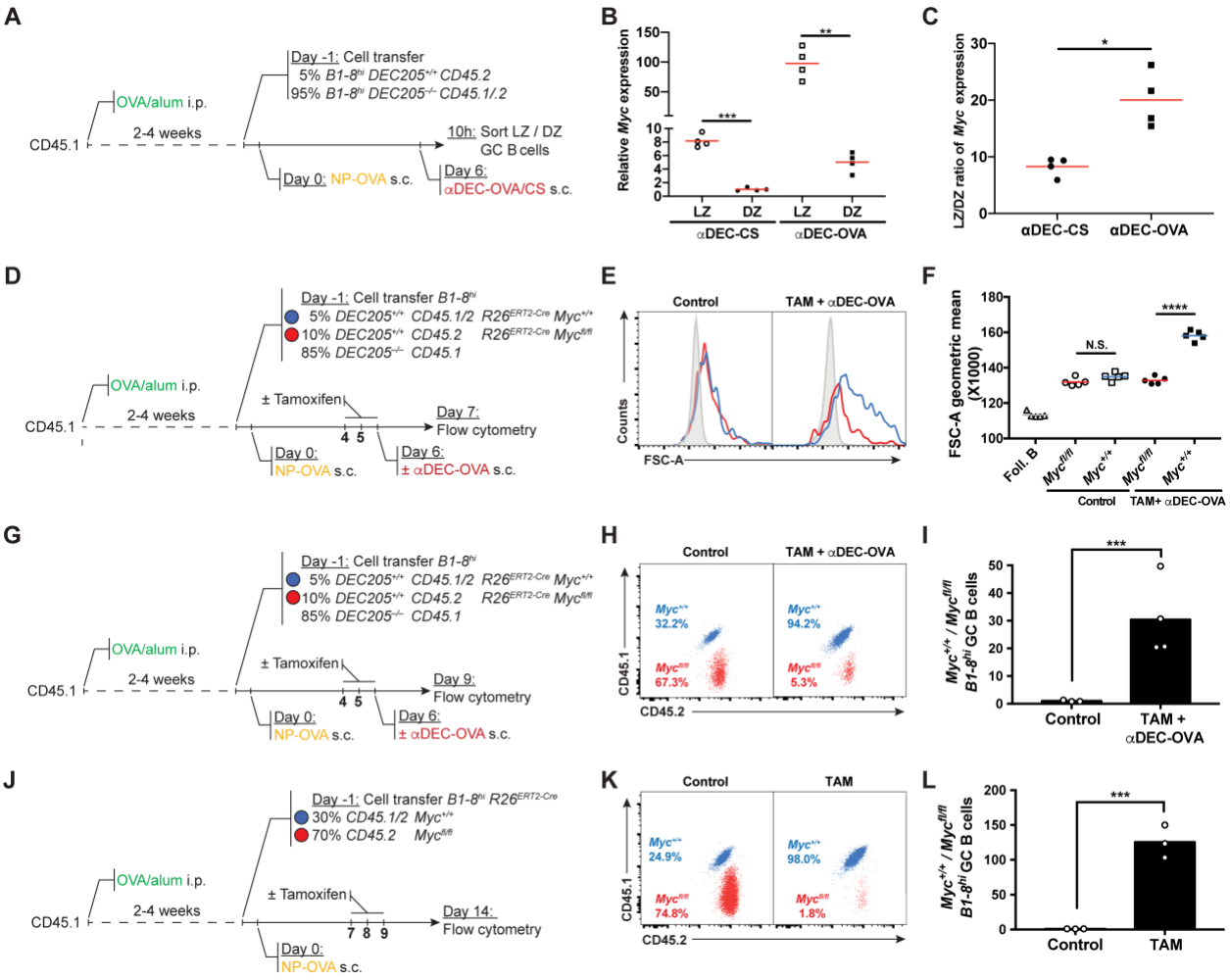

Figure S4. MYC proportionally regulates GC B cell size and expansion. Related to Figure 4. (A) Schematic representation of the experimental protocol for (B) and (C). (B) Relative Myc expression in LZ or DZ GC B 10 hours after DEC-CS or DEC-OVA administration. (C) LZ to DZ ratio of Myc expression shown in (B). Summary of results from 4 mice in two independent experiments. (D) Schematic representation of the experimental protocol for (E) and (F). (E) Representative histograms showing forward scatter of *B1-8^{hi}R26*$^{ERT2\text{-}Cre}$*DEC205$^{+/+}$* GC B cells from *Myc$^{+/+}$* (blue) and *Myc$^{fl/fl}$* (red) mice without (left panel) or with (right panel) tamoxifen and 30 hours after αDEC-OVA administration. Solid grey represents follicular B cells. (F) Quantification of (E). Each

symbol represents one mouse. Summary of results from 3-4 mice in two independent experiments. (G) Schematic representation of the experimental protocol for (H) and (I). (H and I) Percentage (H) and ratio (I, fold change over input ratio) of GC B cells from the indicated mice in untreated control (left) or tamoxifen and αDEC-OVA treated mice 3 days after αDEC-OVA treatment (right). (J) Schematic representation of the experimental protocol for (K) and (L). (K and L) Percentage (K) and ratio (L, fold change over input) of GC B cells from the indicated mice 7 days after the first tamoxifen treatment. Each symbol represents one mouse. Summary of results from 3-6 mice in two independent experiments. Unpaired two-tailed student's *t* test. N.S.: $p > 0.05$ (not statistically significant); *$p < 0.05$, **$p < 0.01$,***$p < 0.001$, ****$p < 0.0001$.

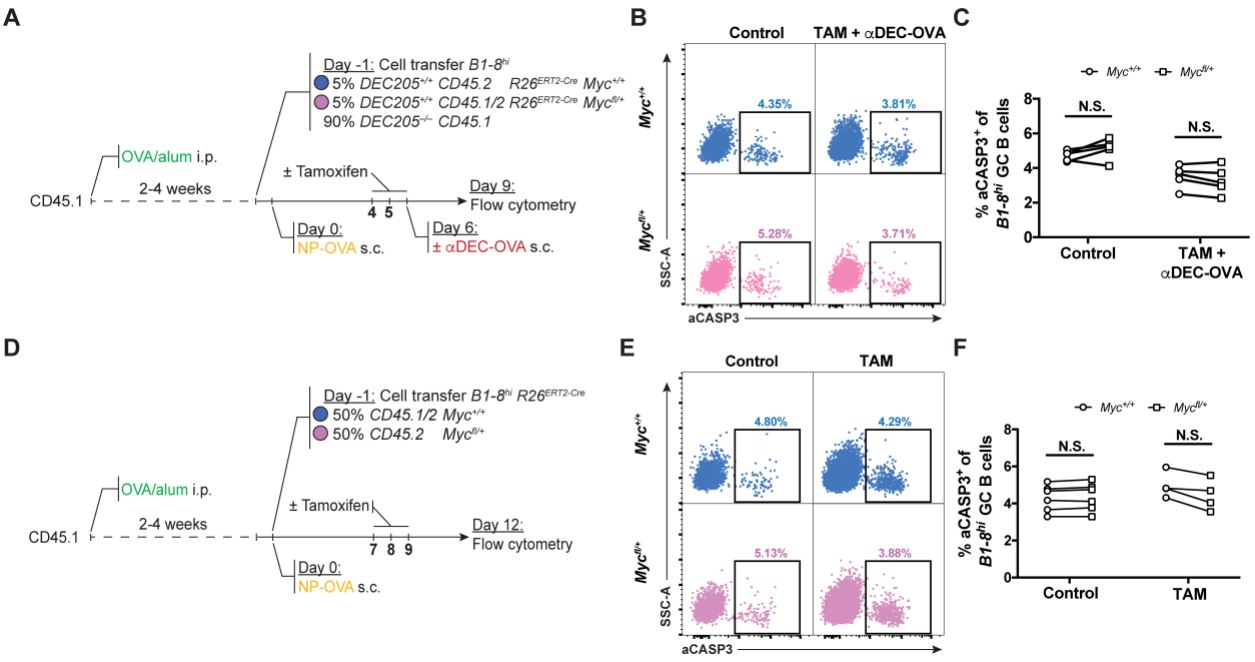

Figure S5. **No significant change in cell death among GC B cells with reduced amounts of MYC *in vivo*. Related to Figure 4.** (A) Schematic representation of the experimental protocol for (B) and (C). (B and C) Representative flow cytometry (B) and quantification (C) of the percentage of aCASP3$^+$ B cells from the indicated mice analyzed 3 days after αDEC–OVA injection in untreated control (left) or tamoxifen and αDEC-OVA treated (right) mice of the indicated genotype. (D) Schematic representation of the experimental protocol for (E) and (F). (E and F) Representative flow cytometry (E) and quantification (F) of the percentage of aCASP3$^+$ B cells from the indicated mice analyzed 12 days after cell transfer in untreated control (left) or tamoxifen-treated (right) mice of the indicated genotype. Each symbol represents one mouse. Summary of results from 4-6 mice in two independent experiments. Unpaired two-tailed student's *t* test. N.S.: $p > 0.05$ (not statistically significant).

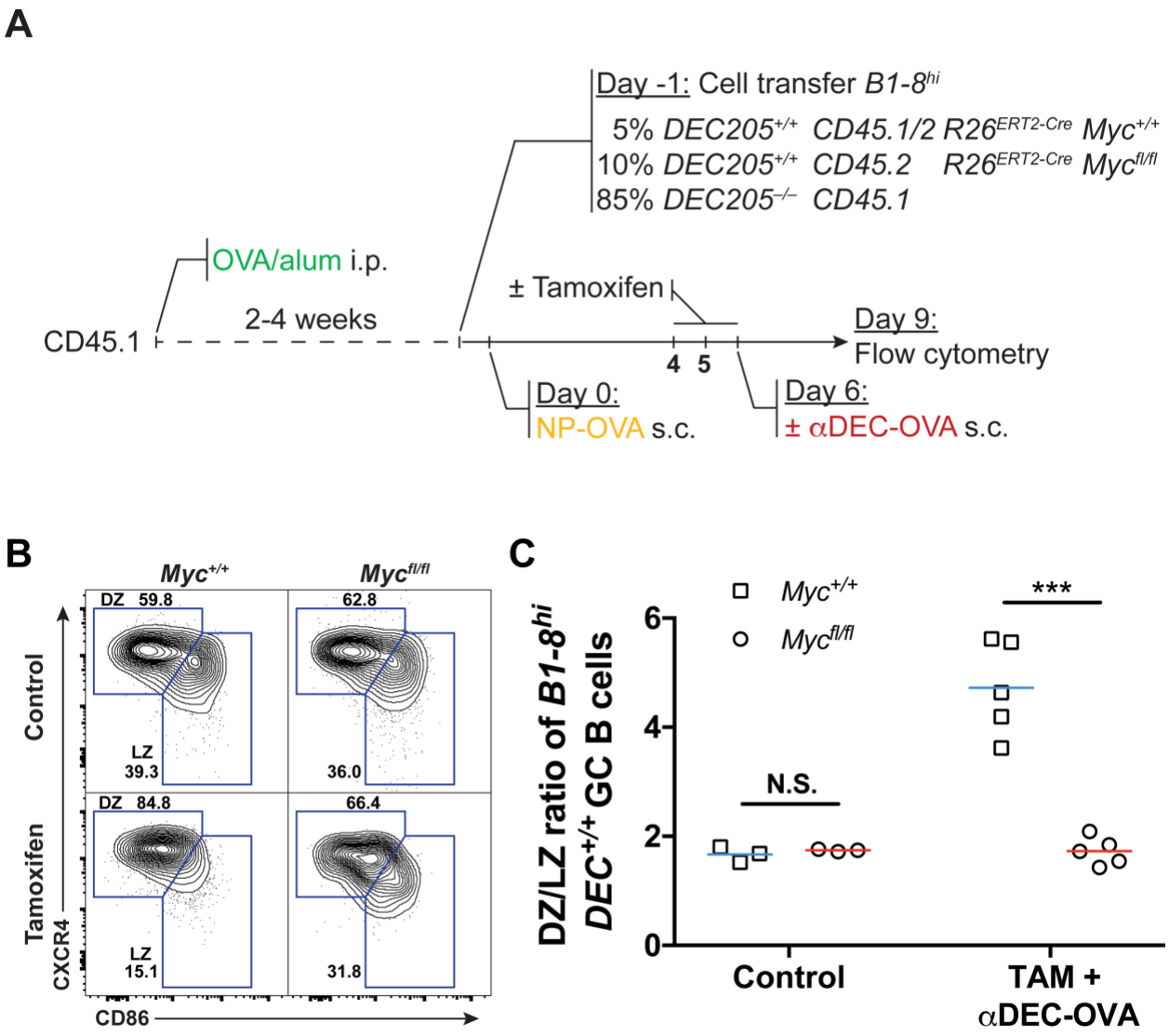

Figure S6. MYC expression plays a role in LZ to DZ transition of selected GC B cells. Related to Figure 5. (A) Schematic representation of the experimental protocol for (B) and (C). (B and C) Representative flow cytometry (B) and quantification (C) of DZ/LZ distribution of *Myc*$^{+/+}$ (top panel) and *Myc*$^{fl/fl}$ (bottom panel) *B1-8*^{hi}*R26*$^{ERT2\text{-}Cre}$*DEC205*$^{+/+}$ GC B cells. Mice were injected with corn oil (control, left panels) or tamoxifen and αDEC-OVA (right panels). Unpaired two-tailed student's *t* test. N.S.: $p > 0.05$ (not statistically

significant); ***$p < 0.001$. Two independent experiments with 3 to 5 mice total for each experiment.

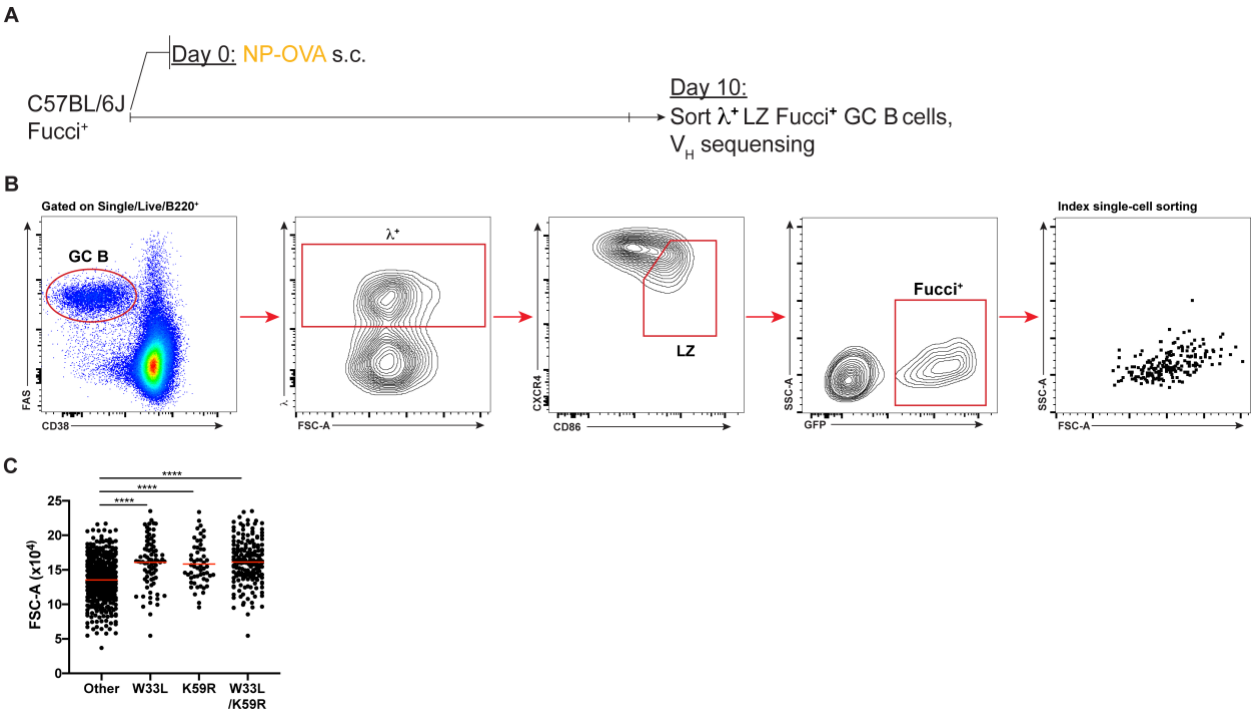

Figure S7. Correlation between affinity and size of selected LZ B cells in polyclonal GCs. Related to Figure 7. (A) Schematic representation of the experimental protocol for (B) and (C). (B) Gating strategy for the experiment shown in Figure 7. (C) Forward scatter of individual λ^+ LZ Fucci$^+$ GC B cells with the affinity-enhancing mutations V_H186.2 W33L$^+$ or K59R$^+$ or all other successfully sequenced V_H clones sorted 10 day after immunization with NP-OVA combined from all three mice shown in figure 7A. Red bars indicate mean. ****p < 0.0001, Unpaired two-tailed student's *t* test.