

FIGURE S1. Incomplete CRC occupancy of the GC DZ. (A) TPLSM of intact Cxc/12-GFP pLN GC on day 10 post-immunization with SRBCs and 1 day after transfer of CFP+ naïve B cells (FOB) to mark the GC edge (white, dotted line) and treatment with PE-IC to label FDCs and TBMs. Due to the high laser power required, CFP+ cells appeared green in the deepest sections, but could be distinguished by their small, lymphocyte morphology. CRCs and FDCs are labeled and areas of undetectable DZ stroma indicated (arrowhead) in sections of the 203µm Z-stack shown as maximum intensity projections (x=19.9µm, y=19.9µm, z=70.0µm). (B and C) Confocal microscopy of CRCs and regions of undetectable stroma (arrowhead) in Cxcl12-GFP and CRC-like DZ stroma in UBI-GFP splenic GC DZs. UBI-GFP mice previously reconstituted with WT BM. Imaged on (B) day 15 p.i. with LCMV (3 Cxcl12-GFP mice, 5-14 GC views per mouse, and 2 UBI-GFP mice, 2-18 GC views per mouse) or (C) day 14 post-immunization with SRBCs (3 Cxcl12-GFP mice, 3-23 GC views per mouse, and 3 UBI-GFP mice, 9-13 GC views per mouse). GCs outlined with white, dotted line based on IgD stain. (D) TPLSM of intact Cxc/12-GFP pLN primary follicle treated and presented as in (A). Sections of the 256µm Z-stack are shown as maximum intensity projections (x=19.9µm, y=19.9µm, z=30.0µm) and the white, dotted line marks the follicle edge. Images (A and D) correspond to Movie S2 and are representative of 1-2 mice and 1-4 views per mouse. Scale bar is 50µm.



**FIGURE S2.** Phenotypic characterization of heterogeneous DZ stroma. Confocal microscopy of tissues from SRBC-immunized *Cxcl12*-GFP mice stained for (A) CD16/32 (representative of 1-2 mice and 4-9 GC views per tissue per mouse) and (B) Type IV collagen (3 mice and 1-7 GC views per spleen). White box indicates area enlarged in single channel images. (C) Additional examples of gp38 staining on *Cxcl12*-GFP pLN GCs on day 15 p.i. with LCMV (2 mice, 1-18 GC views per mouse). Examples of CRCs (arrowhead), FDCs (arrow) and gp38+ CD35- *Cxcl12*-GFP- stroma (^) are indicated. Scale bar is 50µm.



FIGURE S3. Loss of FDC surface markers and altered FDC morphology with blockade of LT and TNF signaling. GCs from UBI-GFP mice reconstituted with WT BM, immunized with SRBC and, on day 10, treated with 1mg/ml LT $\beta$ R-Fc and 1mg/ml TNFR-Fc or saline. Mice were analyzed on day 14. Dense, mesh structure of CD35+ FDCs indicated (#). For saline, duplicate images with select channels shown. Data are representative of 3 mice treated with saline (9-13 GC views per spleen, 3-7 GC views per MLN, 3-8 GC views per PP) and 3 mice treated with LT $\beta$ R-Fc + TNFR-Fc (10-17 GC views per spleen, 6-10 GC views per MLN, 2-6 GC views per PP). GCs outlined with white, dotted line based on BCL6+ GC B cell stain (inset only). Scale bar is 50µm.