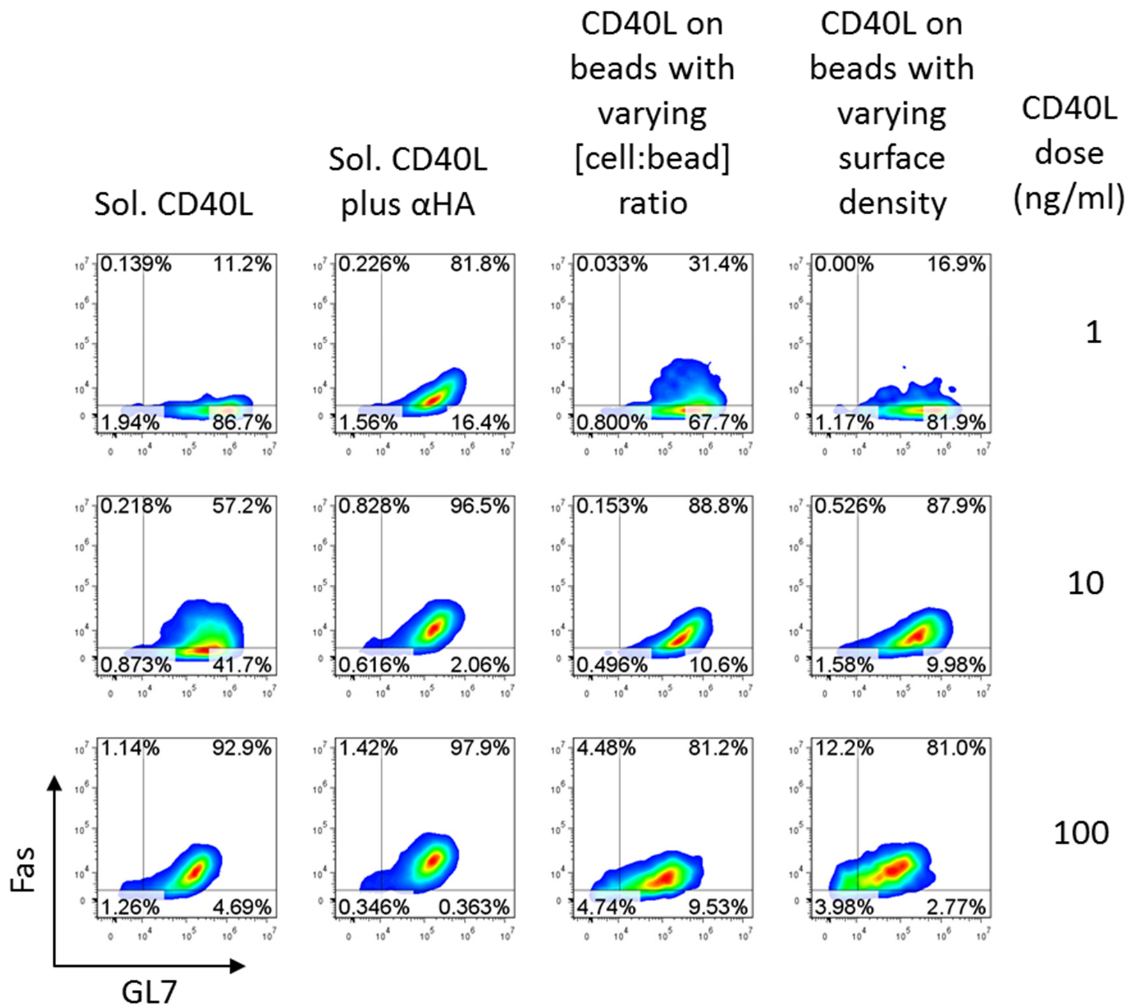
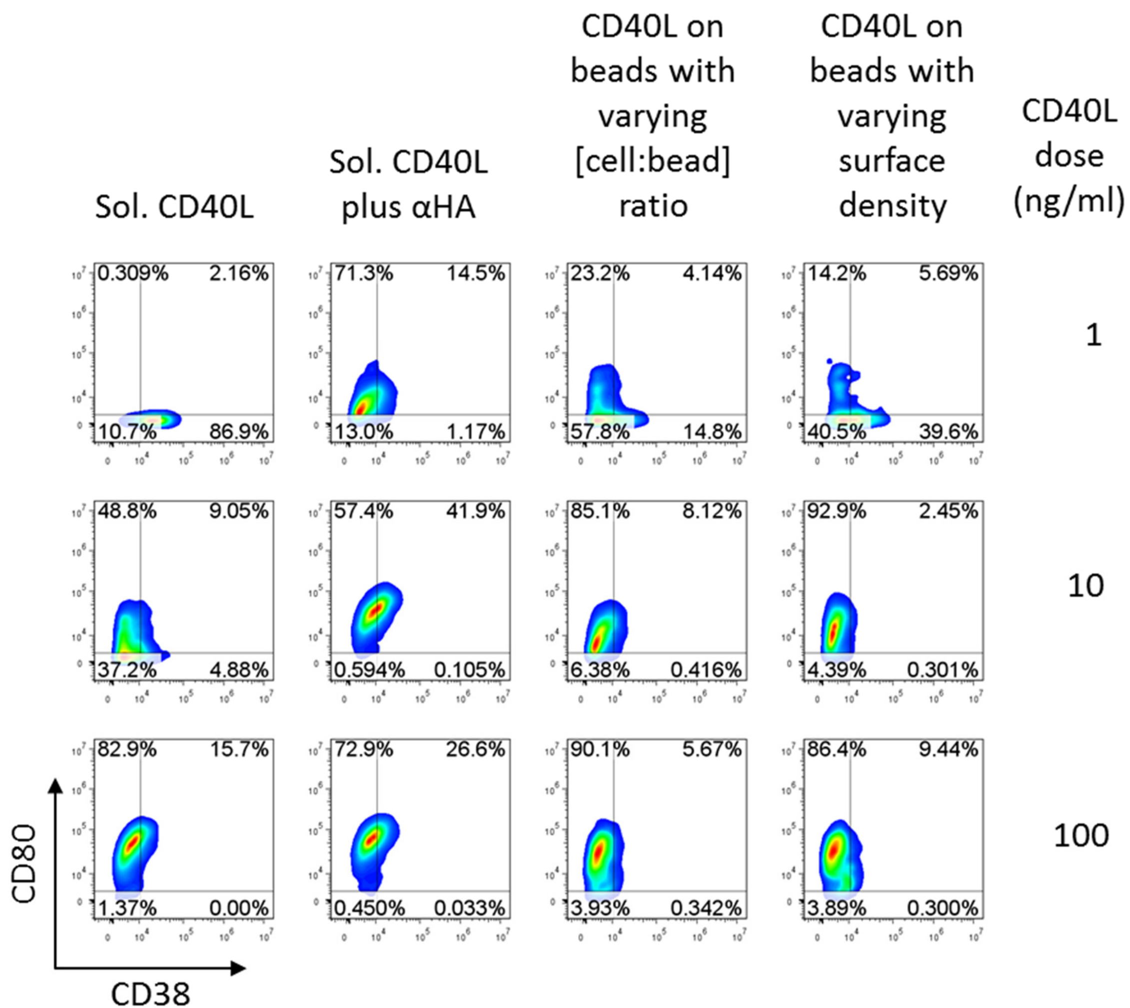
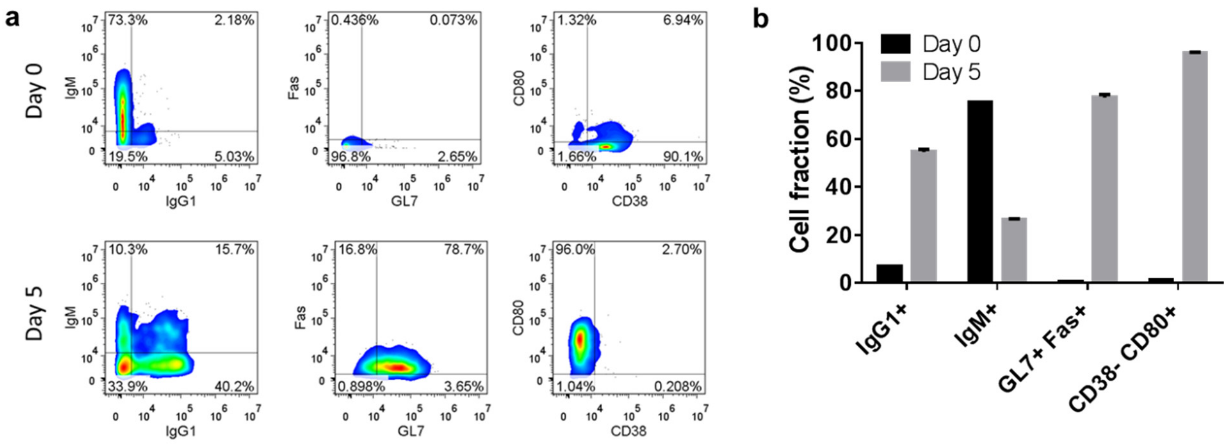


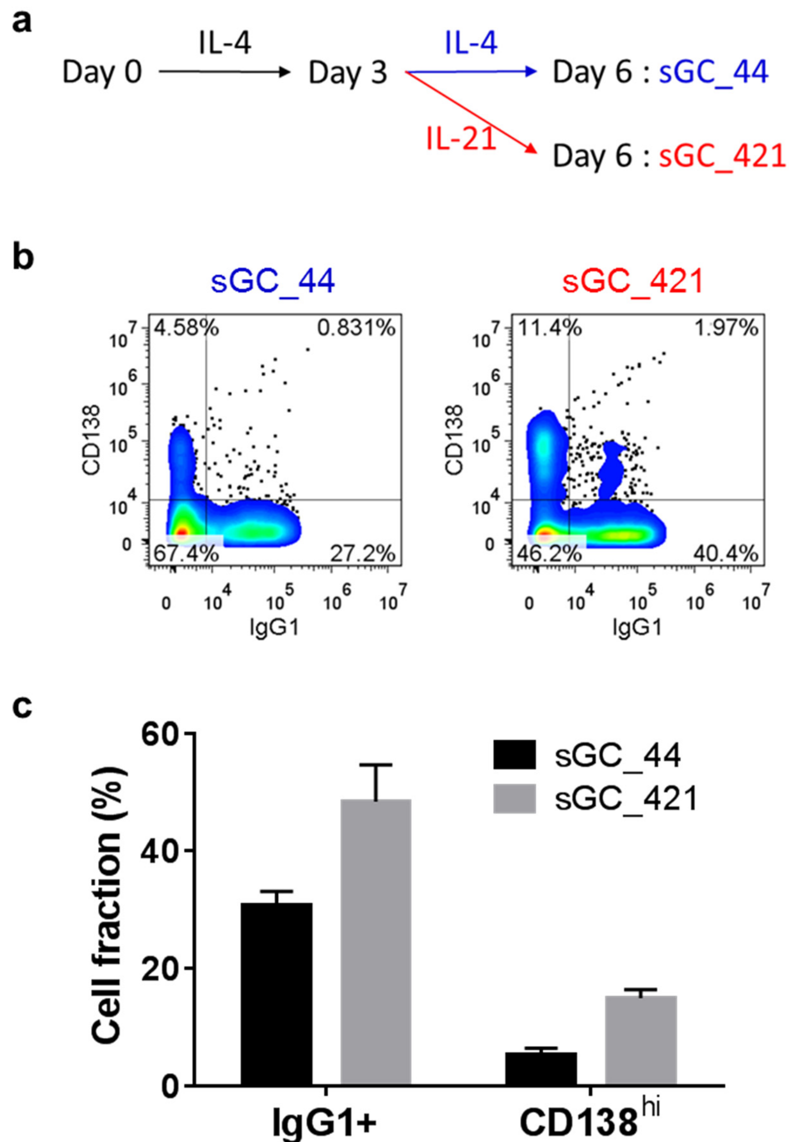
Supplementary Figure 1. Flow cytometry analysis to examine expression level of GL7 and Fas genes on B cells after 6-day culture in each indicated condition. Numbers indicate the percentages of cells in the respective quadrants. The GL7+Fas+ B cell populations are regarded as GC-like B cells, and their percentages were plotted in **Fig. 2g**.



Supplementary Figure 2. Flow cytometry analysis to examine expression level of CD38 and CD80 genes on B cells after 6-day culture in each indicated condition. Numbers indicate the percentages of cells in the respective quadrants. The CD38-CD80+ B cell populations are regarded as GC-like B cells, and their percentages were plotted in **Fig. 2h**.

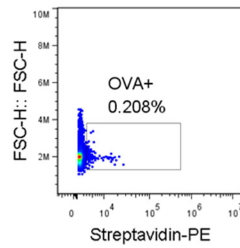


Supplementary Figure 3. Outcome of 5-day sGC culture applied to PBMC isolated B cells. (**a**) Flow cytometry analysis to determine class switching recombination (CSR) and expression of GC B cell phenotypes by examining the expression levels of IgG1 and IgM (left), GL7 and Fas (middle), and CD38 and CD80 (right), for before (Day 0, upper) and after (Day 5, lower) the sGC culture. (**b**) Percentage of indicated populations were plotted with the means and s.d. acquired from triplicates.

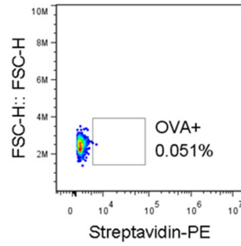


Supplementary Figure 4. Effects of switching cytokines from IL-4 to IL-21 during sGC cell culture. (**a**) Schematic diagram depicting two sGC culture conditions: In sGC_44 condition, IL-4 (20 ng ml^{-1}) is maintained for the whole period of 6-day sGC culture. In sGC_421 condition, IL-4 (20 ng ml^{-1}) is used for the initial 3 days, and switched to IL-21 (10 ng ml^{-1}) on Day 3. (**b**) Flow cytometry analysis for the B cells after 6-day culture of sGC_44 (left) and sGC_421 (right). Expression levels of IgG1 and CD138 were tested and numbers indicate the percentages of cells in the respective quadrants. (**c**) Cell percentages of indicated gates were plotted with mean and s.d. acquired from triplicates.

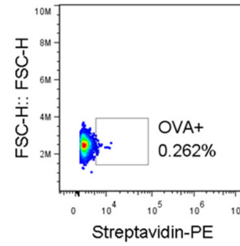
Day 0



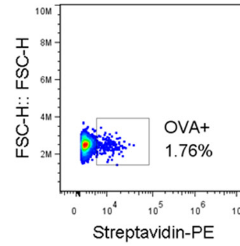
Day 6



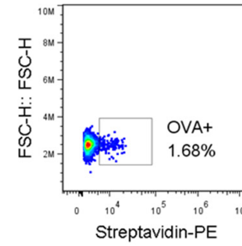
Only streptavidin staining control



No OVA in the culture

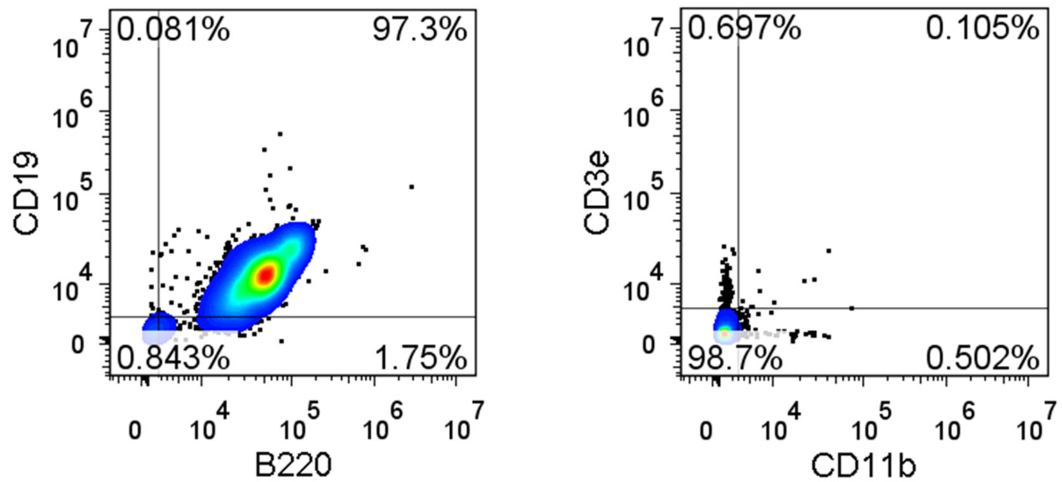


Monomer OVA 2 μg/ml in the culture

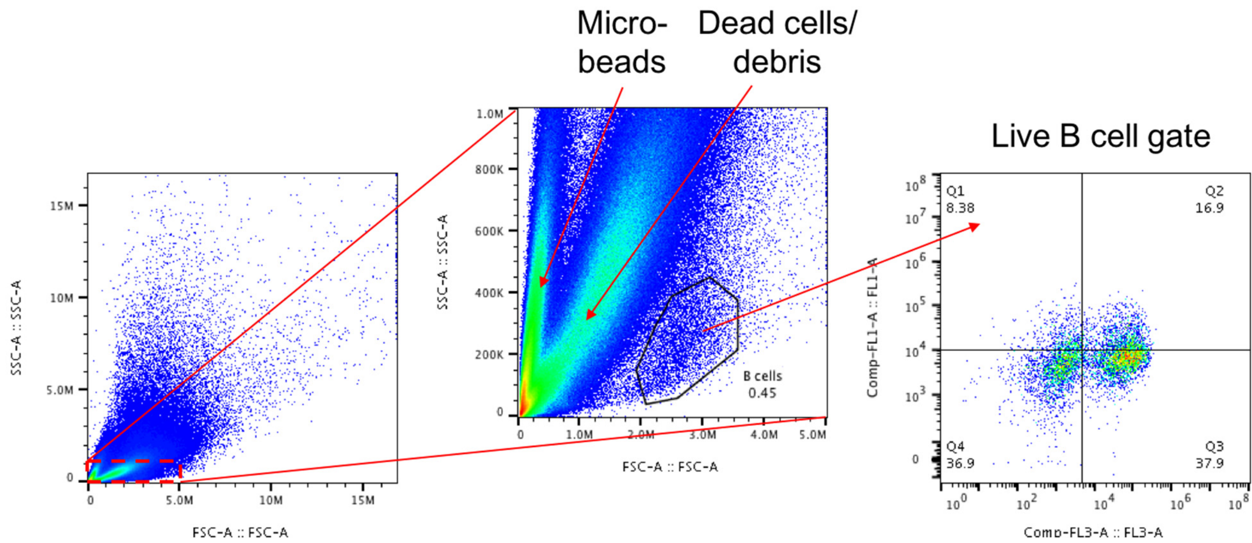


Tetramer OVA 2 μg/ml in the culture

Supplementary Figure 5. Size of OVA-specific B cell populations before (Day 0) and after (Day 6) sGC cultures with or without the soluble OVA antigens (2 μg ml^{-1}). The OVA-specific B cell populations were detected by flow cytometry analysis followed by staining with PE-conjugated OVA-tetramer. The indicated numbers are the percentages of gated populations for the positively stained B cells.



Supplementary Figure 6. Composition of the initial cell populations after MACS B cell isolation applied for RBC-depleted splenocytes. Shown are the representative flow cytometry data from at least 5 independent experiments. Majority (>97%) is B cells (CD19+B220+) with a minimal contamination (<1%) with T cells (CD3e) and CD11b+ cells.



Supplementary Figure 7. The flow cytometry gating strategy for live B cells following sGC culture. The live B cell population after 6-day sGC culture using microbead-bound CD40L (100 ng ml^{-1}), soluble IL-4 (20 ng ml^{-1}), and soluble BAFF (50 ng ml^{-1}) is <1% out of the whole detectable events. The FSC-SSC plot (left) should be zoomed-in (middle) to precisely gate the live B cell population that is larger (i.e., higher in FSC) than both the microbeads and the majority of the dead cell debris. This live B cell gated population is analyzed for the fluorescent channels (right, in this example with FL1 and FL3 channels for FITC-anti-IgM and PE-CY7-anti-IgG1, respectively).

1st PCR	Primer Name	Full Primer Seqeunce (5'->3')
	5'MsVHE	GGGAATTCGAGGTGCAGCTGCAGGAGTCTGG
	3′ Cμ outer	AGGGGGCTCTCGCAGGAGACGAGG
	3′ Cγ1 outer	GGAAGGTGTGCACACCGCTGGAC

2nd PCR	Primer Name	454 Adaptor	MID	Template Specific Primer	Modifications
	5'MsVHE-1	CGTATCGCCTCCCTCGCGCCATCAG	ACGAGTGCGT	GGGAATTCGAGGTGCAGCTGCAGGAGTCTGG	
	5'MsVHE-2	CGTATCGCCTCCCTCGCGCCATCAG	ACGCTCGACA	GGGAATTCGAGGTGCAGCTGCAGGAGTCTGG	
	3′ Cμ inner-1	CTATGCGCCTTGCCAGCCCGCTCAG	ACGAGTGCGT	AGGGGGAAGACATTTGGGAAGGAC	5' biotinylation
	3′ Cμ inner-2	CTATGCGCCTTGCCAGCCCGCTCAG	ACGCTCGACA	AGGGGGAAGACATTTGGGAAGGAC	5' biotinylation
	3′ Cγ1 inner-1	CTATGCGCCTTGCCAGCCCGCTCAG	ACGAGTGCGT	GCTCAGGGAAATAGCCCTTGAC	5' biotinylation
	3′ Cγ1 inner-2	CTATGCGCCTTGCCAGCCCGCTCAG	ACGCTCGACA	GCTCAGGGAAATAGCCCTTGAC	5' biotinylation

Supplementary Figure 8. The list of primer designs employed for two rounds of semi-nested PCR for amplification of mouse Ig heavy chain V region gene transcripts. For the 2nd round of PCR, adaptor sequences used in 454 Sequencing System (blue) and multiplex identifiers (red) were added onto the template specific primer sequences (yellow).