Supporting information

Recognition of acrolein-specific epitopes by B cell receptors triggers an innate immune response

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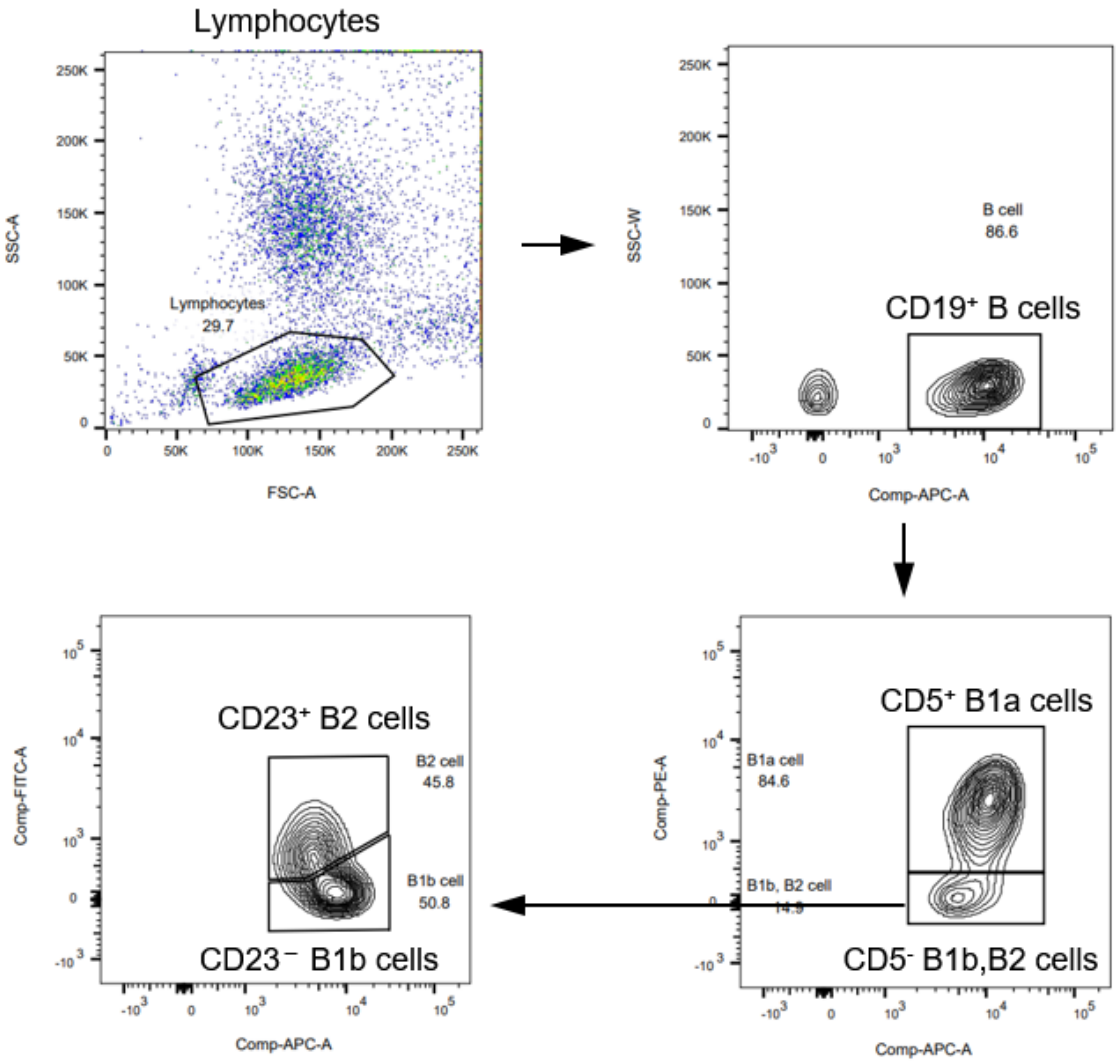

Figure S1. Sorting of B cell subsets.

B cell populations were defined as the total B cells ($CD19^+$), B-2 cells ($CD19^+CD5^-CD23^+$), B-1a cells ($CD19^+CD5^+$), and B-1b cells ($CD19^+CD5^-CD23^-$).

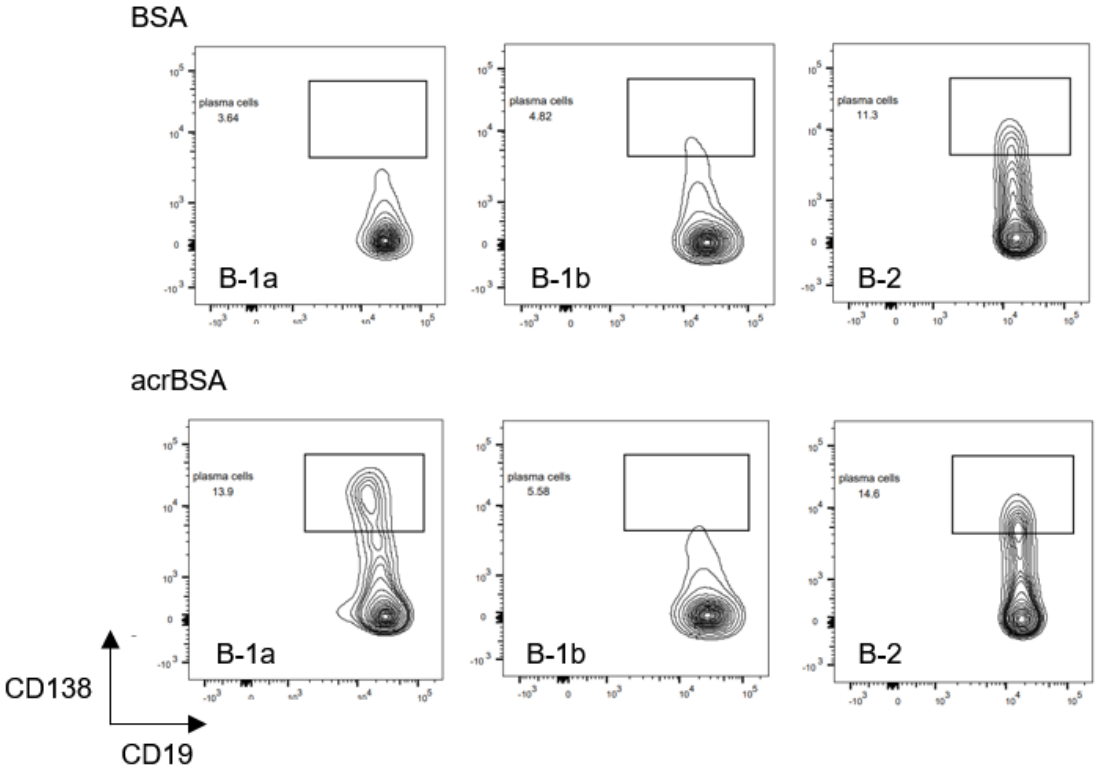

Figure S2. Representative flow-cytometry plots of the PerC B cells treated with biotin-BSA or biotin-acrBSA.

The PerC cells that had been treated with biotin-BSA (*upper three panels*) or biotin-acrBSA (*lower three panels*) for 72 h were sorted into the B cell subsets (B-1a, B-1b, and B-2 cells) and the changes in the number of plasma cells were measured. The gated B cell populations were stained for CD19 (anti-mouse CD19-APC) versus CD138 (anti-mouse CD138-FITC-PECy7).

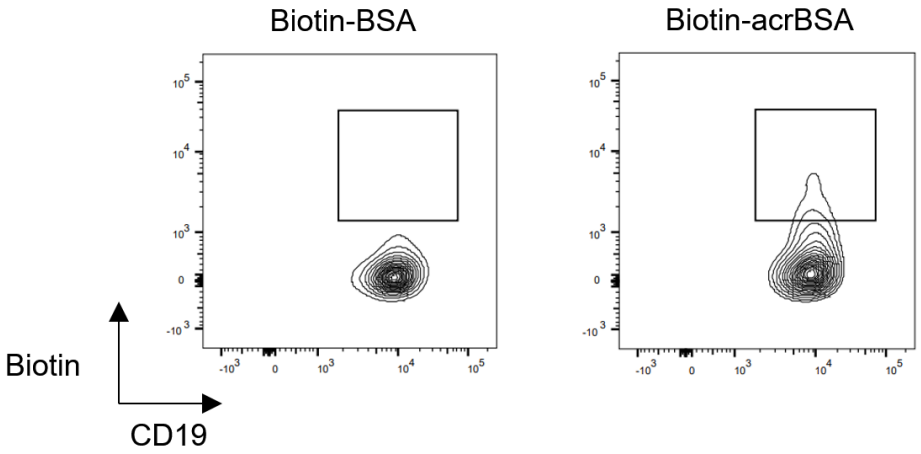

Figure S3. Representative flow-cytometry plots of PerC B-1a cells treated with biotin-BSA or biotin-acrBSA.

The PerC cells treated with biotin-BSA (*left*) or biotin-acrBSA (*right*) for 72 h were sorted into the B-1a cells and the changes in the number of antigen-binding cells were measured. The gated B-1a cell populations were stained for CD19 (anti-mouse CD19-APC) versus biotin (anti-mouse SA-PECy7).

VH: base sequence

GAGGTGCAGCTGGTGGAGTCTGGGGGAGACTTAGTGAAGCCTGGAGGGTC

CCTGAAACTCTCCTGTGCAGCCTCTGGATTCACTTTCAGTAGCTATGGCA

TGTCTTGGGTTCGCCAGACTCCAGACAAGAGGCTGGAGTGGGTCGCAACC

ATTAGTAGTGGTGGTAGTTACACCTACTATCCAGACAGTGTGAAGGGGCG

ATTCACCATCTCCAGAGACAATGCCAAGAACACCCTGTACCTGCAAATGA

GCAGTCTGAAGTCTGAGGACACAGCCATGTATTACTGTGCAAGACATGAT

TACGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCAG

VH: amino acid sequence

CDR1

EVQLVESGGDLVKPGGSLKLSCAASGFTFSSYGMSWVRQTPDKRLEWVAT

CDR2 CDR3

ISSGGSYTYYPDSVKGRFTISRDNAKNTLYLQMSSLKSEDTAMYYCARHD

YDYWGQGTTLTVSS

Figure S4. DNA and amino acid sequences of VH in IgM mAb RE-G25. The CDRs are highlighted.

VL: base sequence

GACATCCAGATGAACCAGTCTCCATCCAGTCTGTCTGCATCCCTTGGAGA

CACAATTACCATCACTTGCCATGCCAGTCAGAACATTAATGTTTGGTTAA

GCTGGTACCAGCAGAAACCAGGAAATATTCCTAAACTATTGATCTATAAG

GCTTCCAACTTGCACACAGGCGTCCCATCAAGGTTTAGTGGCAGTGGATC

TGGAACAGGTTTCACATTAACCATCAGCAGCCTGCAGCCTGAAGACATTG

CCACTTACTACTGTCAACAGGGTCAAAGTTATCCTCTCACGTTCGGTGCT

GGGACCAAGCTGGAAATAAAAC

VL: amino acid sequence

CDR1 CDR2

DIQMNQSPSSLSASLGDTITITCHASQNINVWLSWYQQKPGNIPKLLIYK

CDR3

ASNLHTGVPSRFSGSGSGTGFTLTISSLQPEDIATYYCQQGQSYPLTFGA

GTKLEIK

Figure S5. DNA and amino acid sequences of VL in IgM mAb RE-G25. The CDRs are highlighted.

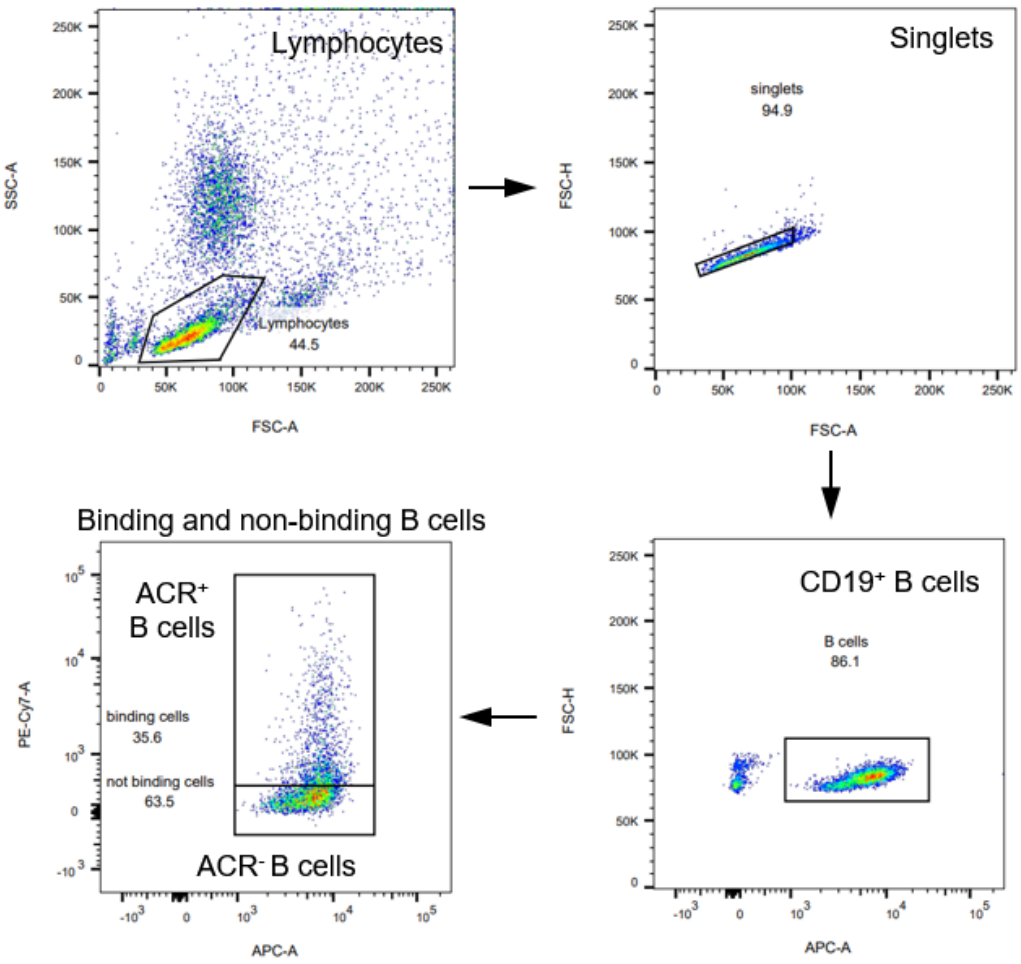

Figure S6. Sorting of acrolein-binding (ACR^+) and acrolein-non-binding (ACR^-) B cell populations from the PerC of BALB/c mice.