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Supplemental Information

Stearoyl-CoA Desaturase-Mediated Monounsaturated Fatty Acid Availability Supports Humoral Immunity

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Figure S1, related to Figure 1

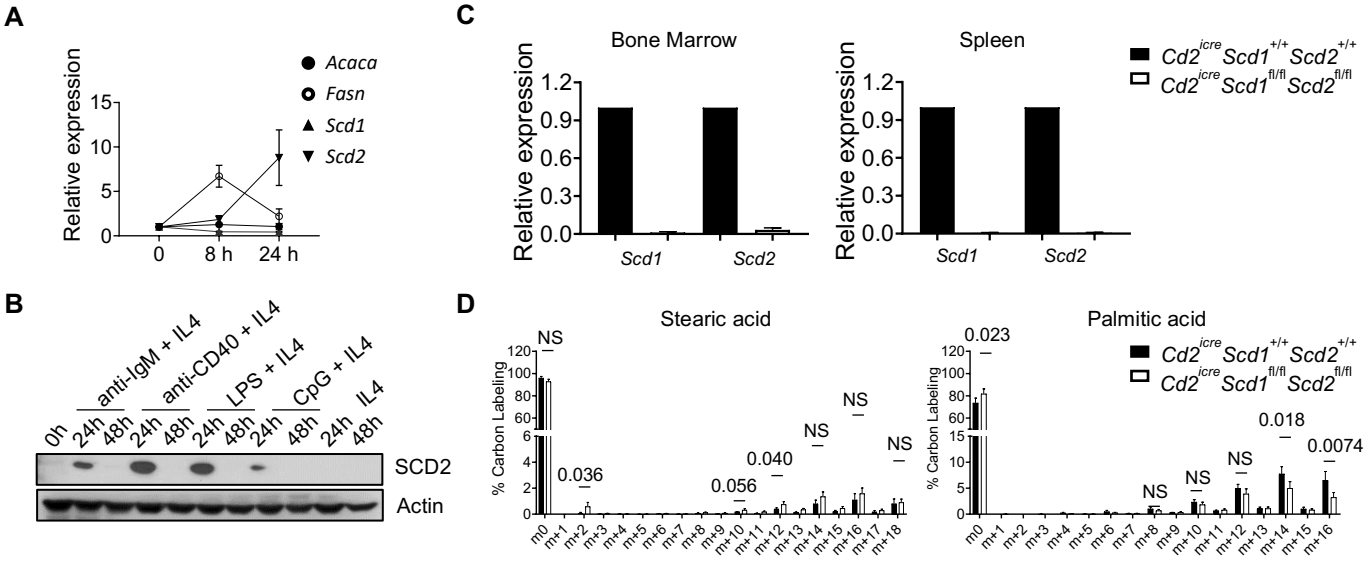

Figure S1. B cell activation induces SCD activity and increases MUFA content, related to Figure 1

(A) Reverse transcription polymerase chain reaction (RT-PCR) analysis of *Acaca*, *Fasn*, *Scd1* and *Scd2* expression in murine B cells at 0, 8 and 24 h with LPS/IL-4 activation. (B) Immunoblot analysis of SCD2 in fresh isolated murine B cells, and B cells activated with anti-IgM (10 μg/mL)/IL-4, anti-CD40 (10 μg/mL)/IL-4, LPS (10 μg/mL)/IL-4, CpG (2.5 μM)/IL-4, or IL-4 alone at 24 and 48 h by immunoblot. (C) RT-PCR analysis of *Scd1*, and *Scd2* expression in both BM CD19^{+} B and splenic total B cells isolated from WT or *Cd2*^{iCre}*Scd1*$^{fl/fl}$*Scd2*$^{fl/fl}$ mice. (D) Incorporation of ^{13}C labeled glucose into stearic acid (SA) and palmitic acid (PA) were measured by LC-MS/MS in activated B cell isolated from WT or *Cd2*^{iCre}*Scd1*$^{fl/fl}$*Scd2*$^{fl/fl}$ mice (n = 3). *p* was calculated with Student's *t* test (D). NS, not significant. Data were pooled from 5 (A) and represent 2 (D) independent experiments. Error bars represent SEM.

Figure S2, related to Figure 2

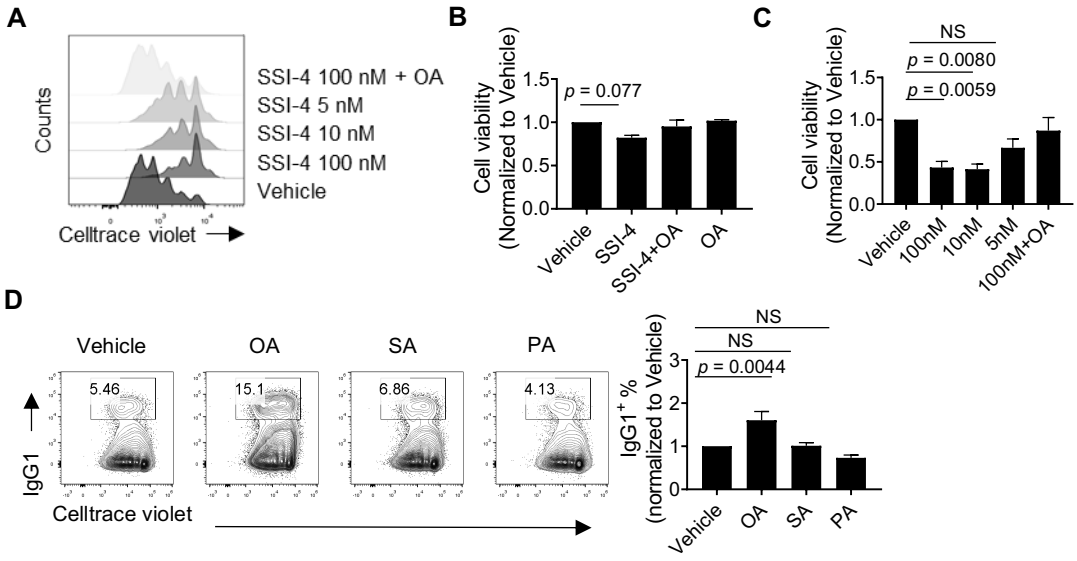

Figure S2. MUFAs promote B cell proliferation and survival in vitro, related to Figure 2

(A) Cell proliferation of murine B cells stimulated with LPS/IL-4/IL-5 in presence of 5, 10, and 100 nM SSI-4, or 100 nM SSI-4 plus OA. (B) The cell viability of murine B cells activated with LPS/IL-4 in the presence of vehicle, 100 nM SSI-4, SSI-4 plus OA, and OA alone for 48 h. (C) The cell viability of murine B cells activated with LPS/IL-4 in presence of different doses of SSI-4, or SSI-4 plus exogenous OA for 72 h. (D) Flow cytometry analysis of murine B cell proliferation and class switch when treated with OA, SA and PA. Right, the frequencies of $IgG1^+$ B cell activated with LPS/IL-4 in presence of OA, PA and SA. The percentages were normalized to vehicle (BSA) group. *p* value was calculated with one-way ANOVA (B-D). NS, not significant. Results were representative of 3 (A) and pooled from 4 (B-D) independent experiments. Error bars represent SEM.

Figure S3, related to Figure 3

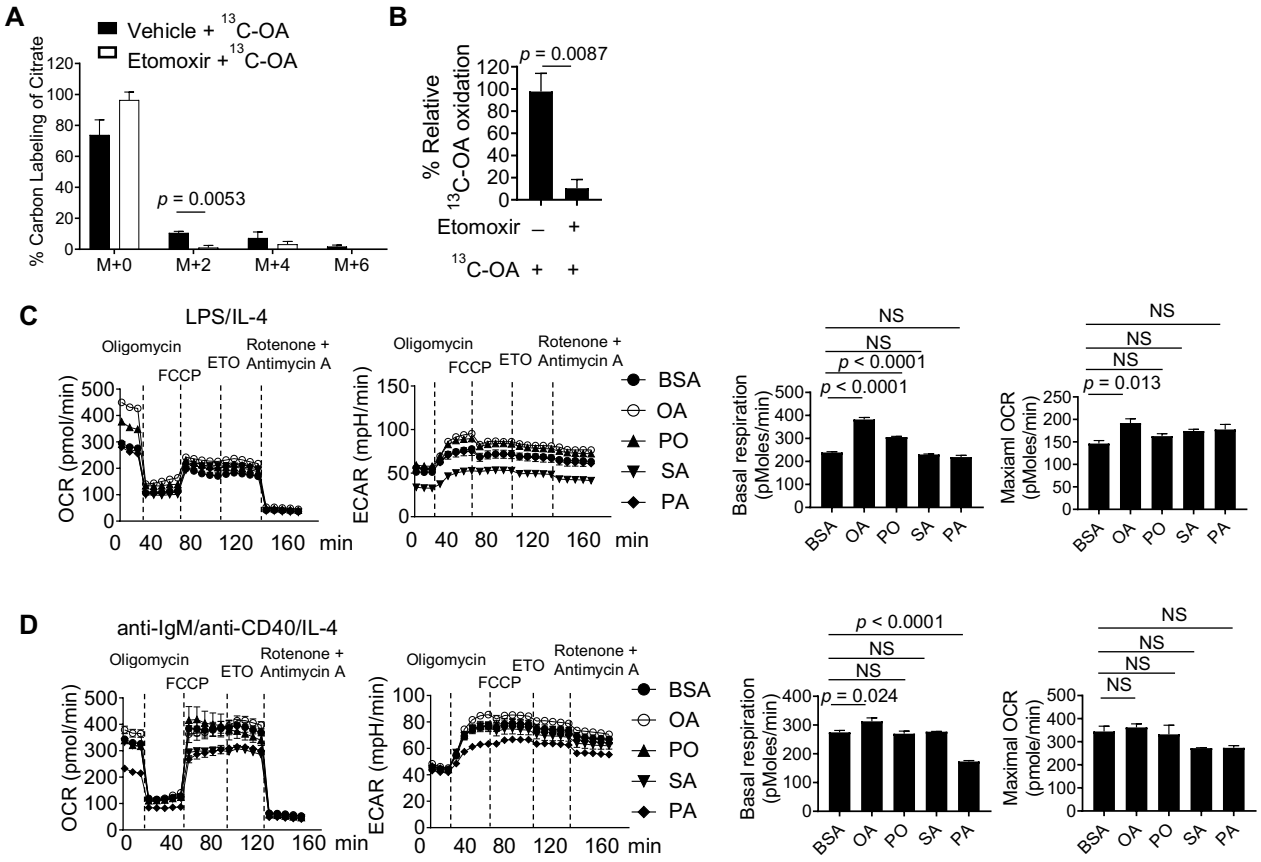

Figure S3. MUFAs maintains B cell metabolic fitness, related to Figure 3
(A, B) The U-^{13}C-OA tracing assay on murine activated B cells. (A) Isotopomer tracing of U-^{13}C-OA in activated B cells. (A) The conversion of U-^{13}C-OA to [M + 2, 4, 6]-^{13}C labeled citrate, with or without etomoxir treatment. (B) The summary of percentage of relative U-^{13}C-OA oxidation (normalized to etomoxir untreated sample). (C, D) Oxygen consumption rate (OCR) was measured with a Seahorse XFe96 analyzer using LPS/IL-4 (C) and anti-IgM/anti-CD40/IL-4 (D) activated murine B cells in the presence of BSA, OA, PO, SA and PA for 48 h with a MitoStress assay kit. Basal respiration and maximal OCR of either stimulation were summarized. *p* value was calculated with Student's *t* test (A, B) and one-way ANOVA (C, D). NS, not significant. Data were representative of 3 (C) and 2 (D) independent experiments. Error bars represent SEM.

Figure S4, related to Figure 4

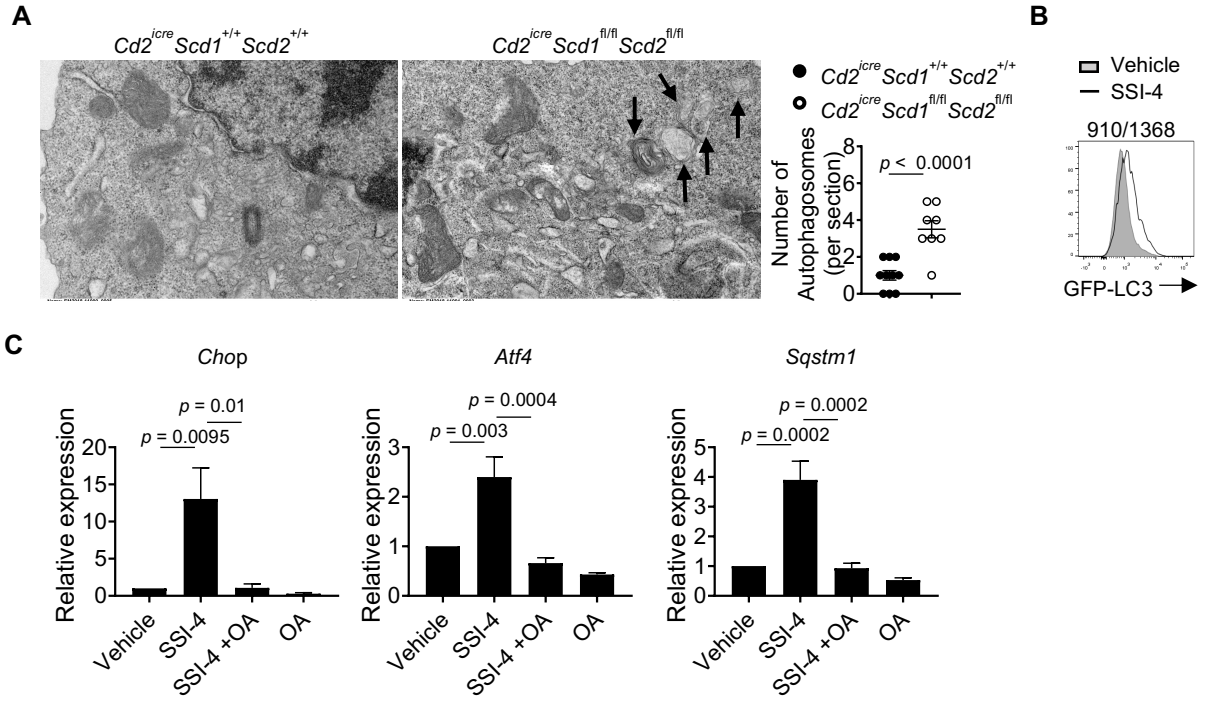

Figure S4. MUFAs prevent overactivation of autophagy and ER stress, related to Figure 4
(A) B cells isolated from *Cd2*^{iCre}*Scd1*$^{+/+}$*Scd2*$^{+/+}$ and *Cd2*^{iCre}*Scd1*$^{fl/fl}$*Scd2*$^{fl/fl}$ mice were activated with LPS/IL-4 for 48 h. They were imaged on transmission electron microscope. Right, numbers of autophagosomes (per section) found in B cells from *Cd2*^{iCre}*Scd1*$^{+/+}$*Scd2*$^{+/+}$ mouse (n = 10) and those from *Cd2*^{iCre}*Scd1*$^{fl/fl}$*Scd2*$^{fl/fl}$ mouse (n = 8). (B) Flow cytometry analysis of GFP-LC3 expression in activated B cells isolated from GFP-LC3 reporter mouse treated with vehicle, and SSI-4. Numbers indicate the mean fluorescence intensity (MFI) of GFP. (C) RT-PCR analysis of *Chop*, *Atf4*, and *Sqstm1* in activated B cells in the presence of vehicle, SSI-4, SSI-4 plus OA, or OA alone. *p* value was calculated with Student *t*-test (A) and one-way ANOVA (C). Data were representative of 2 (A, B) experiments, or were pooled from 3 (C) independent experiments. Error bars represent SEM.

Figure S5, related to Figure 5

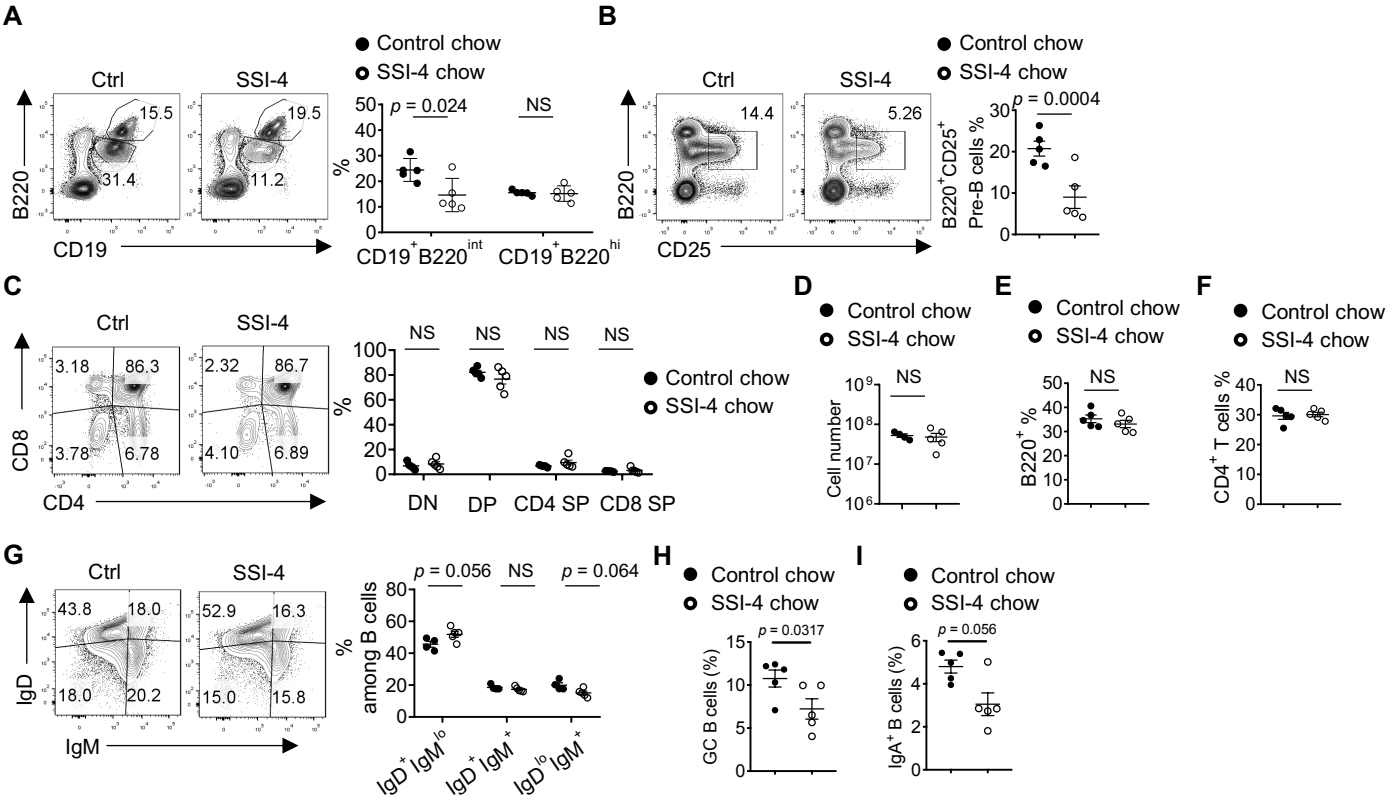

Figure S5. SCD activity supports B cell development and immunization induced humoral immune response, related to Figure 5

(A) Flow cytometry analysis of B cell precursors ($B220^{int}CD19^{+}$) and circulating mature B cells ($B220^{hi}CD19^{+}$) in bone marrow from mice fed with control chow or SSI-4 chow. Right, frequencies of B cell precursors and circulating mature B cells. (B) Flow cytometry of pre-B cell ($B220^{+}CD25^{+}$) in bone marrow from mice fed with control chow or SSI-4 chow. Right, frequencies of pre-B cells. (C) Flow cytometry analysis of thymic $CD4^{-}CD8^{-}$ (DN), $CD4^{+}CD8^{+}$ (DP), $CD4^{+}$ single positive (SP) and $CD8^{+}$ SP T cells from mice fed with control chow or SSI-4 chow. Right, the frequencies of DP, DN, $CD4^{+}$ and $CD8^{+}$ SP T cells. (D-F) Mice were fed with control chow or SSI-4 chow for 2 weeks. The total splenic cellularity (D), and percentages of splenic $B220^{+}$ B cells (E), and $CD4^{+}$ T cells (F). (G) Flow cytometry analysis of $IgM^{lo}IgD^{+}$ (mature B), $IgM^{+}IgD^{+}$, and $IgM^{+}IgD^{lo}$ B cells among splenic $CD19^{+}$ B cells. Right, the frequencies of each B cell subsets. (H, I) The frequencies of GC B cells (H) and IgA expression (I) among total B cells in Peyer's patches from mice fed with control or SSI-4 chow. *p* value was calculated with Student *t*-test (A-I). Data were representative of 2 (A-I) independent experiments. Error bars represent SEM.

Figure S6, related to Figure 7

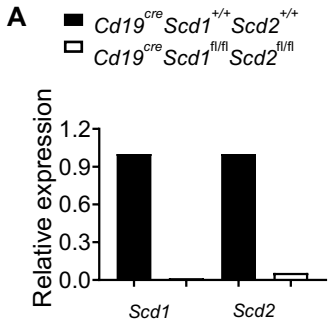

Figure S6. B cell intrinsic SCD activity is dispensable for humoral immunity *in vivo*, related to Figure 7

(A) RT-PCR analysis of *Scd1*, and *Scd2* expression in murine splenic B cells isolated from WT and $Cd19^{Cre}Scd1^{fl/fl}Scd2^{fl/fl}$ mice.

Table S1. The concentrations of non-esterified free fatty acids (NEFA) were measured in 8 weeks old male (n = 5) and female (n = 8) mice sera, related to Figure 2

Non-esterified free fatty acids (NEFA)	Concentration (μM, Mean ± SD)	
	Male (n = 5)	Female (n = 8)
EPA	3.08 ± 0.83	4.44 ± 0.56
Linolenic acid	26.3 ± 3.74	44.6 ± 12.7
DHA	18.6 ± 2.94	23 ± 3.5
Myristic acid	5.69 ± 1.2	7.55 ± 2.17
Palmitoleic acid	32.8 ± 2.543	31.7 ± 5.53
Arachidonic acid	10.1 ± 0.77	14.8 ± 1.24
Linoleic acid	221 ± 20.1	304 ± 74
Palmitic acid	222 ± 5.97	239 ± 26.1
Oleic acid	162 ± 18.2	184 ± 37.9
Stearic acid	44.7 ± 5.01	53.8 ± 8.39

Table S2. Primers for RT-PCR are listed below, related to STAR Methods

Gene names	Species	Sequences
Scd1	Murine	F 5'-TTCTTGCGATACACTCTGGTGC-3' R 5'-CGGGATTGAATGTTCTTGTCGT-3'
Scd2	Murine	F 5'-GCATTTGGGAGCCTTGTACG-3' R 5'-AGCCGTGCCTTGTATGTTCTG-3'
Fasn	Murine	F 5'-GGAGGTGGTGATAGCCGGTAT-3' R 5'-TGGGTAATCCATAGAGCCCAG-3'
Acaca	Murine	F 5'-GTCCCCAGGGATGAACCAATA-3' R 5'-GCCATGCTCAACCAAAGTAGC-3'
Chop	Murine	F 5'-CTGGAAGCCTGGTATGAGGAT-3' R 5'-CAGGGTCAAGAGTAGTGAAGGT-3'
Atf4	Murine	F 5'-GGGTTCTGTCTTCCACTCCA-3' R 5'-AAGCAGCAGAGTCAGGCTT-3'
Sqstm1	Murine	F 5'-AGGATGGGGACTTGGTTGC-3' R 5'-TCACAGATCACATTGGGGTGC-3'
SCD	Human	F 5'-AGTTCTACACCTGGCTTTGG-3' R 5'-GTTGGCAATGATCAGAAAGAGC-3'
ACACA	Human	F 5'-GATATCCCAGAGATGTTTCGGC-3' R 5'-GTCAGCATGTCAGAAGGCAGAG-3'
FASN	Human	F 5'-AGAACTTGCAGGAGTTCTGGGACA-3' R 5'-TCCGAAGAAGGAGGCATCAAACCT-3'