Supplementary Materials for

IRE1α-mediated monounsaturated fatty acid synthesis drives B cell differentiation and lupus-like autoimmune disease

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Supplemental Material & Methods

Reagents. Murine recombinant IL-4 were purchased for Peprotech (Cat#: 214-14). LPS (Cat#: L4391), OA (Cat#: O3008), Sodium palmitate (Cat#: P9767), fatty acid-free BSA (Cat#: A8806) were purchased form Sigma-Aldrich. IRE1 endonuclease inhibitor BI09 (Cat#: No.6009) was from EOCRIS, anti-CD40 (Cat#: 16-0401-86) was from Thermo Fisher, SCDi (Cat#: Cay10012562) was purchased from Cayman.

Primary B cell isolation and culture. Primary B cells were negatively isolated from spleen of 4-6 weeks old *Ire1α*$^{fl/fl}$ and CD19-Cre/*Ire1α*$^{fl/fl}$/MRL.Fas^{lpr} mice using Dynabeads Mouse CD43 (ThermoFisher Scientific, Cat#: 11422D). Purified B cells were activated with LPS (1μg/ml; Sigma, Cat#: L4391), anti-CD40 (4μg/ml; eBioscience, Cat#: 16-0401-86), and IL-4 (40ng/ml; Peprotech, Cat#: 214-14) as indicated for 3-5d, then cell apoptosis was detected by Annexin V and PI staining according to the manufacturer's instructions.

Standard Flow Cytometry and Lipid Staining. Single-cell suspensions were incubated with fixable viability dye. Surface staining was performed in ice-cold PBS with 3% FBS in the presence of FcR blocking Ab CD16/32. All abs and reagents used for FACS are listed in the Supplemental supplementary Table 2. Intracellular lipid content was evaluated by flow cytometry using 4,4 Difluoro1,3,5,7,8-Pentamethyl-4-Bora-3a,4a-Diaza-s-Indacene (BODIPY493/503; ThermoFisher, Cat#: D3922) as previously described[1]. Following surface marker staining using antibodies, B cells were stained with 200 μl of BODIPY493/503 for 15 min at 37℃. BODIPY493/503 staining was detected in the FITC channel. Intracellular staining was performed using the Invitrogen Fixation/Permeabilization Concentrate and Diluent (ThermoFisher, Cat#: 00-5521-00) and Permeabilization buffers (ThermoFisher, Cat#: 00-8333-56). Cells were analyzed on a FACSCanto II instrument (BD Biosciences).

RNA isolation and quantitative real-time RT-PCR. Total RAN was extracted with RNAeasy Mini Kit (QIANGEN, Cat#: 74106), and cDNA was synthesized using the cDNA synthesis kit (qScript TM cDNA Synthesis Kit, Quanta, Cat#: 95047-500). SYBR Green fluorescent reagent (PerfeCTa SYBR Green FastMix for iQ, Quanta, Cat#: 95071-012) was used for quantitative real-time RT-PCR. The relative amount of mRNA was calculated by the comparative threshold cycle method with β-actin as an inter-control. All samples were run on a Bio-Rad MyiQ^{TM}2 instrument. Primer sequences are shown in the supplementary Table 1.

Genome-wide RNA-sequencing. Primary naïve B cells from spleen of 4-5 weeks old *Ire1a*$^{fl/fl}$ and CD19-Cre *Ire1a*$^{fl/fl}$ MRL.Fas^{lpr} mice were isolated by CD43^{-} untouched B cell beads. 1×10^{6} purified B cells were stimulated with LPS (1μg/ml). Then CD3^{-}B220^{int}CD138^{hi} plasma cells and CD3^{-}B220^{+}CD138^{-} activated B cells were sorted on a FACSMelody Cell Sorter (BD Biosciences) as described [2, 3]. RNA was extracted with RNAeasy Mini Kit (QIANGEN, cat#: 74106) following manufacturer's instructions and sent to NUseq Core (Northwestern University) for RNA-seq analysis. Differential expression was determined using DESeq2. The cutoff determining significantly differentially expressed genes was an FDR-adjusted p value less than 0.05.

Assessment of clinical disease. Survival was assessed in *Ire1a*$^{fl/fl}$ and CD19-Cre-*Ire1a*$^{fl/fl}$ MRL.Fas^{lpr} mice that died of disease spontaneously and those sacrificed due to general debility. Proteinuria and dermatitis were detected and scored as previously described [4]. For histopathology analysis, paraffin embedded, formalin-fixed tissue sections, stained with hematoxylin and eosin (H&E) as well as Periodic acid-Schiff (PAS), and were scored for both glomerular and interstitial nephtitis by a pathologist in a double-blinded manner. Formalin-fixed and paraffin-embedded sections from lung and liver, stained with H&E, were scored by cellular interstitial pneumonia and hepatitis from 0-6 (0, none; 6, severe) as described previously [4]. IgG1 immune deposits were determined by direct immunofluorescence on 7 μM OCT-embedded frozen kidney tissue sections using monoclonal rat anti- IgG1 FITC-conjugated (BD, Cat#: 553443) antibody with 1:100

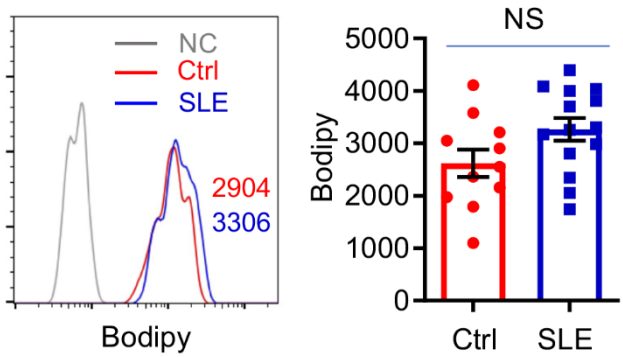
dilution as reported [5]. IgG immune complexes were measured by indirect immunofluorescence as previously described [4]. Images were captured and analyzed on a zeiss microscope with the ZEN software.

Immunoblot analysis. B cells were then lysed with RIPA lysis buffer (Millipore, Cat#: 20-188) added with protease inhibitor cocktail. Total cell extracts were separated in 10% SDS-PAGE gels and electro-transferred onto polyvinylidene difluoride membranes (Bio-Rad, Cat#: 1620177). Membranes were probed with the appropriate primary antibodies, including IRE1*a* (Abcam, Cat#: ab37073) and GAPDH (Abcam, Cat#: ab181602). After washing twice with TBST, membranes were incubated with HRP-conjugated secondary antibodies. Membranes were then washed and visualized with an ECL detection system (Bio-Rad).

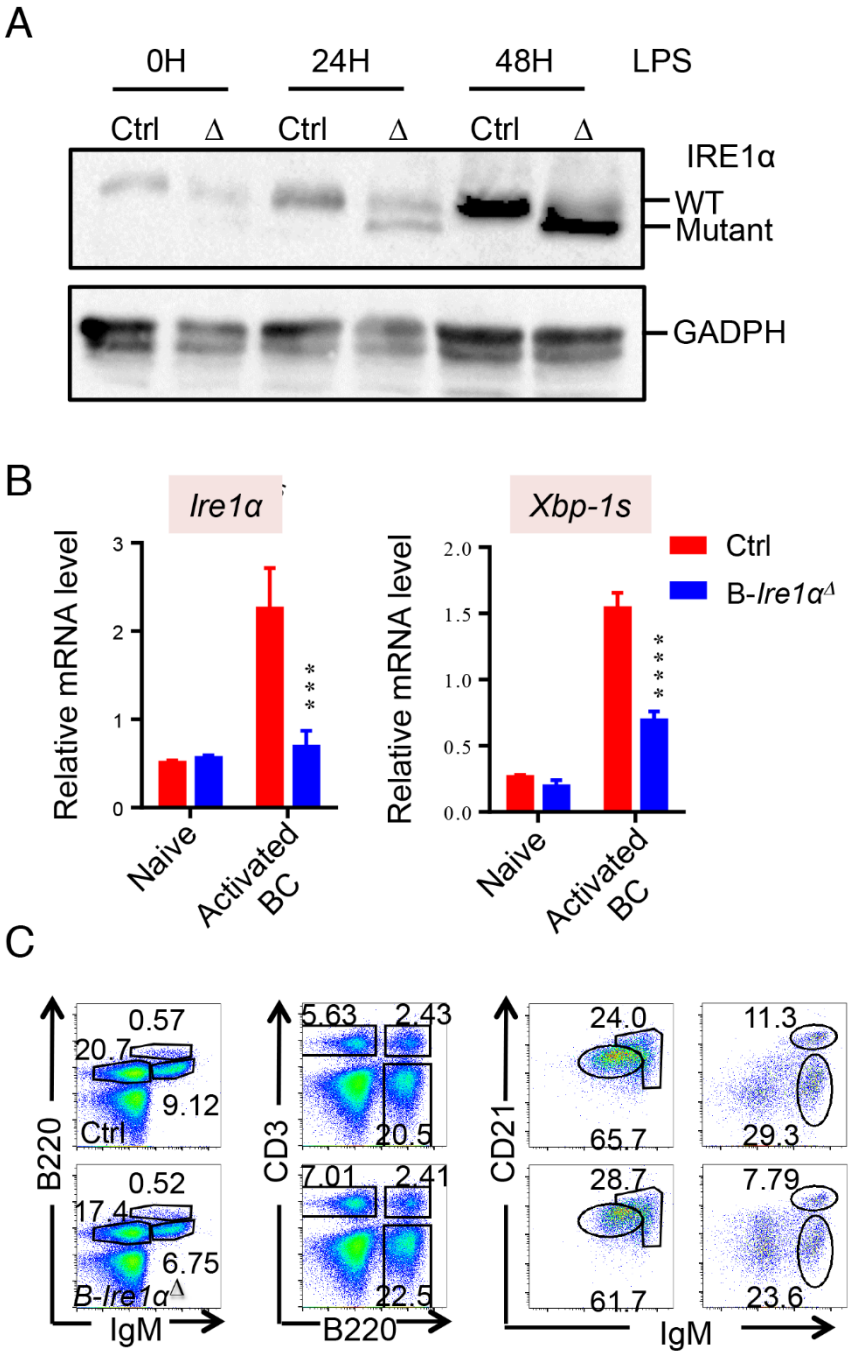
References

1. Qiu, B. and M.C. Simon, *BODIPY 493/503 Staining of Neutral Lipid Droplets for Microscopy and Quantification by Flow Cytometry.* Bio Protoc, 2016. **6**(17).
2. Kong, S., et al., *Deleted in Breast Cancer 1 Suppresses B Cell Activation through RelB and Is Regulated by IKKalpha Phosphorylation.* J Immunol, 2015. **195**(8): p. 3685-93.
3. Kong, S., et al., *DBC1 is a suppressor of B cell activation by negatively regulating alternative NF-kappaB transcriptional activity.* J Immunol, 2014. **193**(11): p. 5515-24.
4. Teichmann, L.L., et al., *Dendritic cells in lupus are not required for activation of T and B cells but promote their expansion, resulting in tissue damage.* Immunity, 2010. **33**(6): p. 967-78.
5. Zhang, J., et al., *The type III histone deacetylase Sirt1 is essential for maintenance of T cell tolerance in mice.* J Clin Invest, 2009. **119**(10): p. 3048-58.

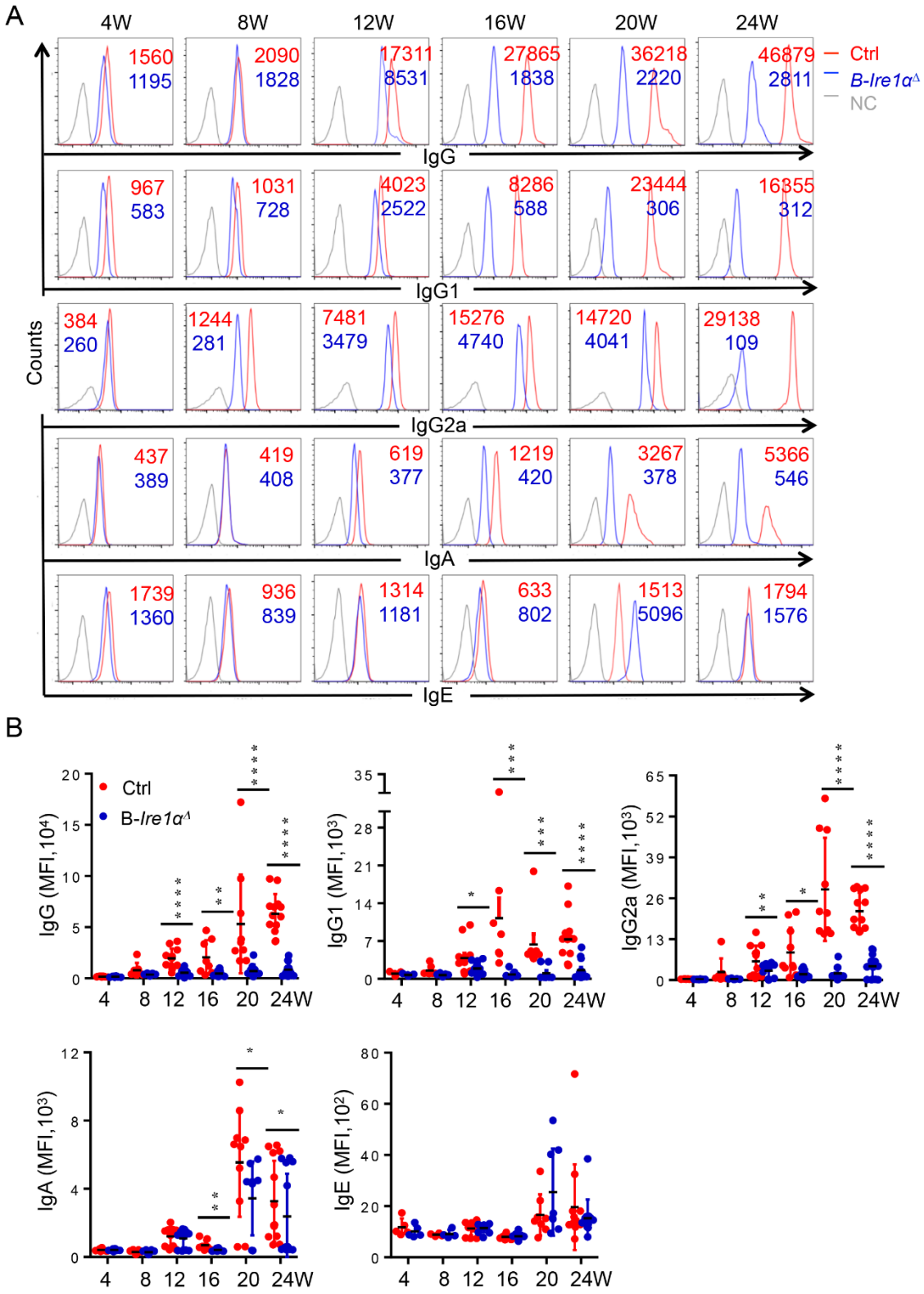
Supplemental Figures and legends



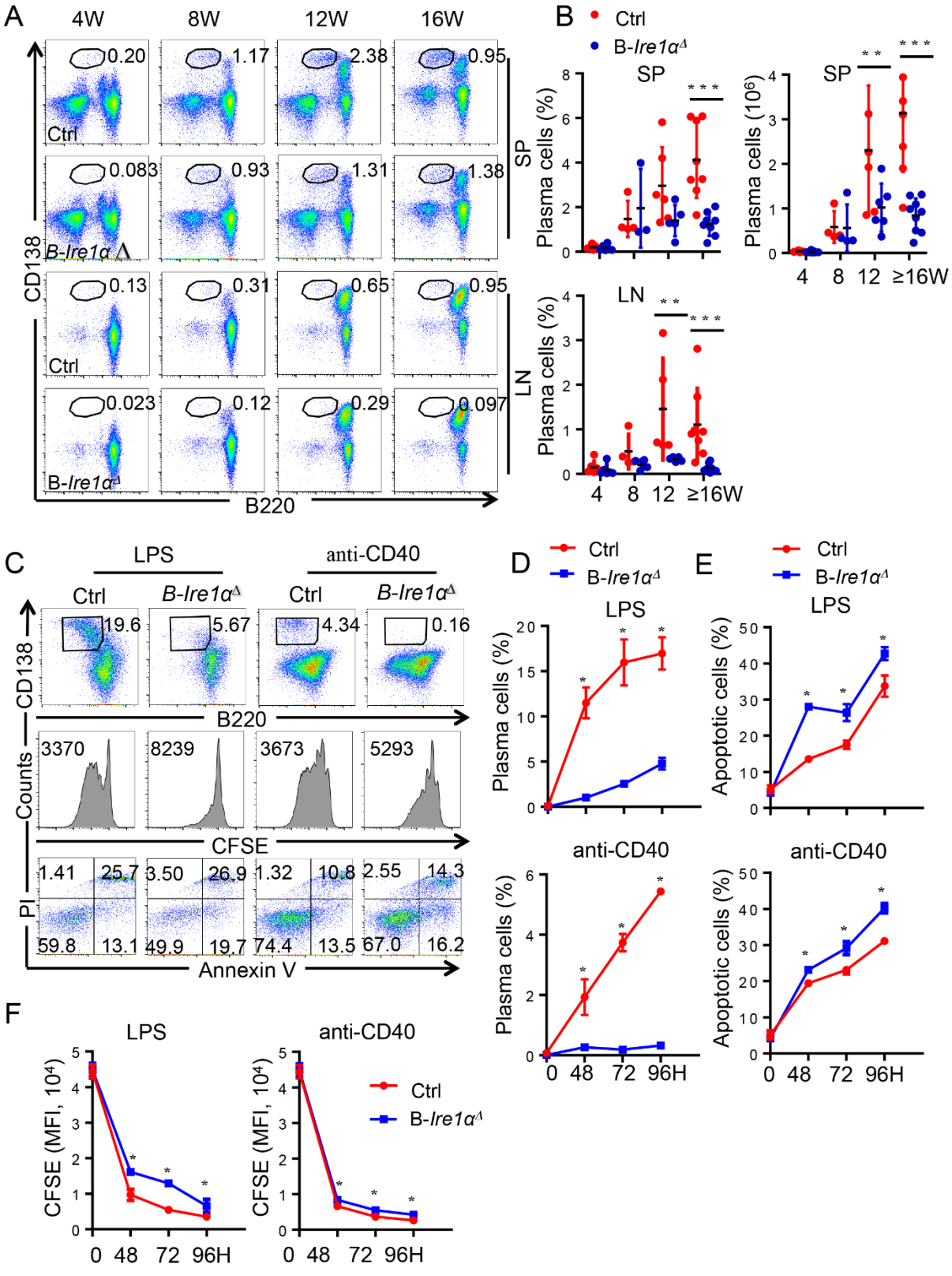
Supplemental Fig. 1. Analysis of lipid volume in T cells from lupus patients. Intracellular lipid content in CD3ε^{+} T cell populations. (Left) Representative flow cytometric profile of lipid staining for T cells from patients with active SLE and healthy controls. (Right) Lipid quantification expressed as mean fluorescence intensity (MFI) of Bodipy 493/503 staining. Data are shown as mean±SD. Student's *t* test (two-tailed) was used for statistical analysis. NS: not significant.



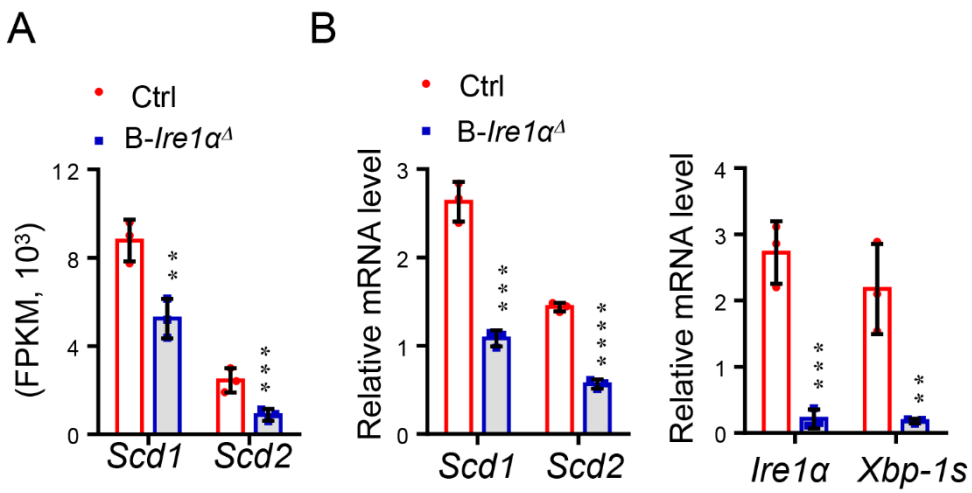
Supplemental Fig. 2. Characterization of *Ire1α* gene deletion in B cells. (A-B) Naïve B cells ($B220^{+}CD43^{-}$) from the spleens of *Ire1α* conditional knockout (*Cd19-Ire1α*Δ) mice and their littermate controls were sorted, and stimulated with LPS (1μg/ml) for indicated time points *in vitro*. (A) IRE1α protein expression levels were analyzed by western blotting (top panel) using GADPH as a loading control (bottom panel). (B) The mRNA levels of *Ire1α* and *Xbp-1s* in B cell from MRL.Fas^{lpr} SLE mice and *Cd19-Ire1α*Δ mice were determined by RT-qPCR. **(C)** Bone marrow isolated from MRL.Fas^{lpr} SLE mice and *Cd19-Ire1α*Δ mice was analyzed. Representative FACS profiles of $B220^{lo}IgM^{-}$pro-B cells and pre-B, $B220^{lo}IgM^{+}$ immature-B cells, and $B220^{hi}IgM^{+}$ mature-B cells were shown. **(D)** $B220^{+}$ cells from spleen of MRL.Fas^{lpr} SLE mice and *Cd19-Ire1α*Δ mice were analyzed for $CD23^{+}CD21^{int}IgM^{int}$ mature B cells and $CD23^{+}CD21^{hi}IgM^{hi}$ T2 B cells and $CD23^{-}CD21^{lo}IgM^{+}$ T1 and $CD23^{-}CD21^{hi}IgM^{+}$ marginal zone (MZ) B cells. **(E)** Splenocytes from *Cd19-Ire1α*Δ mice and their littermate controls were analyzed for $CD3\varepsilon^{+}$ T cells and $B220^{+}$ B cells. Statistical differences were tested using an unpaired Student's *t* -test (two-tailed). *** $p<0.001$, **** $p<0.0001$. Xbp-1s, spliced Xbp-1.



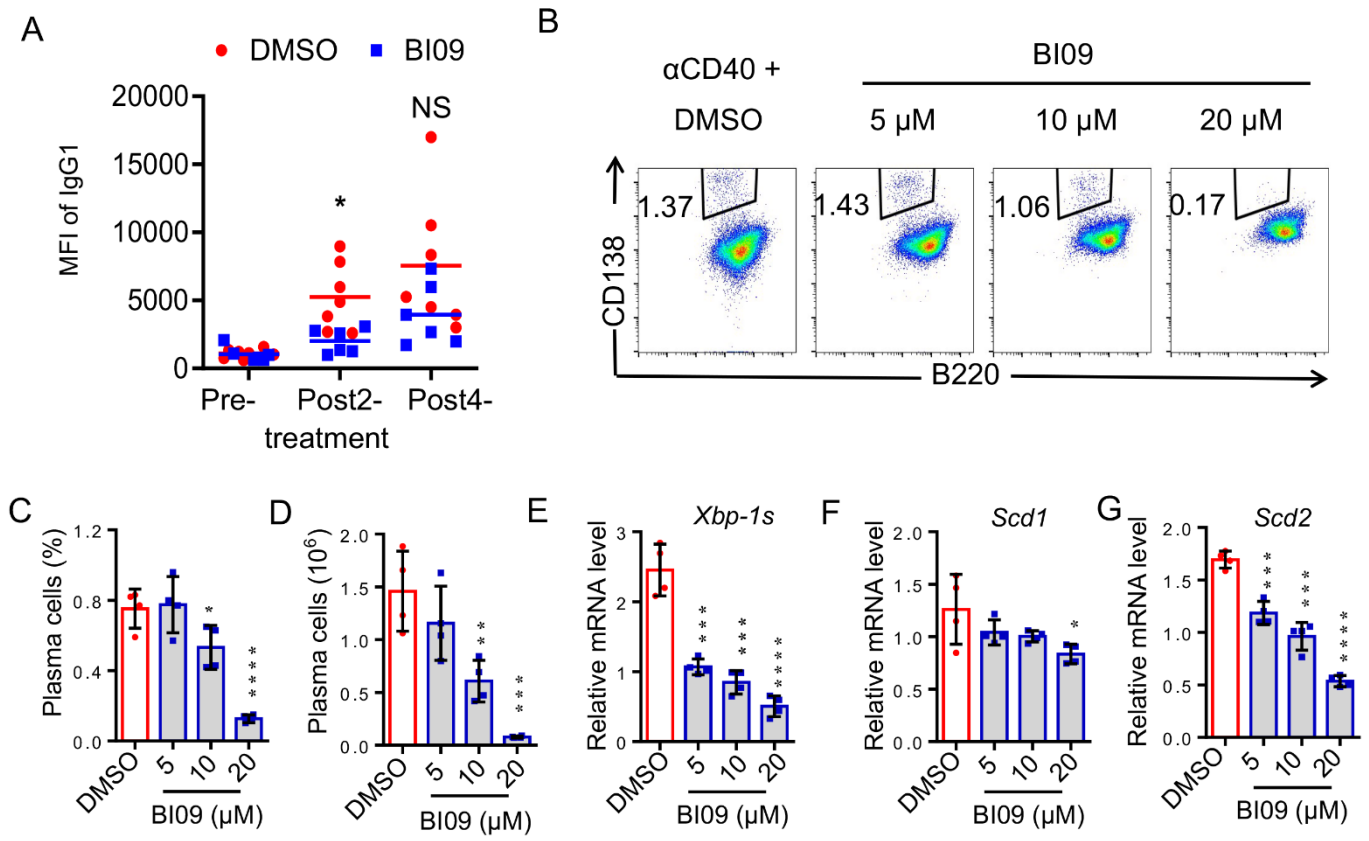
Supplemental Fig. 3. Auto-Ab generation is largely impaired in IRE1α -null B cells from fas mutant lupus mice. **(A)** Representative flow cytometric profiles of different subclasses of Auto-Ab from the sera of indicated age- *Cd19- Ire1α*$^{\Delta}$ lupus mice and their littermate control mice using EL4 murine T lymphoma cell line. **(B)** Intracellular Auto-Ab quantification expressed as mean fluorescence intensity (MFI) of IgG (1:32000), IgG1(1:100), IgG2a (1:1600), IgA (1:1600), or IgE (1:100). Data are shown as mean ± SD. Statistical differences in were tested using an unpaired Student's *t* -test (two-tailed). * *p<0.05,* ** *p<0.01,* *** *p<0.001, and* **** *p<0.0001.*



Supplemental Fig. 4. B cell-specific IRE1α expression is responsible for plasma cell differentiation. **(A-B)** Representative flow cytometric profiles (A) and data plots (B) show frequencies and absolute number of $CD3^{-}B220^{low}CD138^{+}$ PCs from splenocytes and lymphocytes from *Cd19- Ire1α*$^{\Delta}$ lupus mice and their littermate control with indicated ages. **(C-F)** Naïve ($B220^{+}CD43^{-}$) B cells from the splenocytes of *Cd19-Ire1α*Δ mice and their littermate controls were sorted, and stimulated with LPS (1μg/ml) or anti-CD40 (4 μg/ml) at indicated time points *in vitro*. Representative FACS analysis (C) and data plots (D-F) of differentiation, proliferation, and apoptosis of B cells were analyzed. Significance determined by an unpaired Student's *t* - test (two-tailed). * $p<0.05$, ** $p<0.01$, *** $p<0.001$.



Supplemental Fig. 5. mRNA expression levels of Scd1 and Scd2 in plasma cells were down-regulated in *Ire1α* – null B cells. Naïve ($B220^{+}CD43^{-}$) B cells from the splenocytes of *Cd19-Ire1α*Δ mice and their littermate controls were isolated, and stimulated with LPS (1μg/ml) for 4 days *in vitro*. **(A)** Sorted $B220^{+}CD138^{-}$ activated B cells were analyzed by RNA sequencing. mRNA expression levels of Scd1 and Scd2 in B cells from the indicated mice. **(B)** RT-qPCR analysis of expression levels of Scd1, Scd2, Ire1α and Xbp-1s in Ire1α-deficient- and sufficient- B cells. Statistical differences were tested using an unpaired unpaired Student's *t* -test (two-tailed). ** *p<0.01, *** p<0.001, and **** p<0.0001*. Scd1, Stearoyl-CoA desaturase-1; Scd2, Stearoyl-CoA desaturase-2; Xbp-1s, spliced Xbp-1.



Supplemental Fig. 6. Effect of BI09 on humoral autoimmunity in vivo and in vitro. (A) The self-reactive IgG1 levels in sera from MRL/Lpr mice 2 and 4 weeks after the termination of BI09 treatment were analyzed. **(B-G)** Primary naïve ($B220^{+}CD43^{-}$) B cell from splenocytes were treated with anti-CD40 (4μg/ml) plus different concentrations of BI09 at indicated time points *in vitro*. Representative flow cytometric profile (B) and data plots of frequencies (C) and cell numbers (D) of plasma cells were shown. Expression levels of XBP-1s (E), SCD1 (F), and SCD2 (G) transcripts were evaluated using RT-qPCR. Significance were tested using an unpaired Student's *t* - test (two-tailed). * $p<0.05$, *** $p<0.001$, **** $p<0.0001$. Scd1, Stearoyl-CoA desaturase-1; Scd2, Stearoyl-CoA desaturase-2; Xbp-1s, spliced Xbp-1.

Supplemental Table 1: Primers used for this study.

Species	Primer name	Sequence (5' to 3')	Purpose
HUMAN	IRE1α - F	TAG TCA GTT CTG CGT CCG CT	RT-qPCR
	IRE1α - R	TTC CAA AAA TCC CGA GGC CG	
HUMAN	XBP-1S - F	GCT GAG TCC GCA GCA GGT	RT-qPCR
	XBP-1S - R	ACT GGG TCC AAG TTG TCC AG	
HUMAN	XBP-1t - F	GAG TTA AGA CAG CGC TTG GG	RT-qPCR
	XBP-1t - R	ACT GGG TCC AAG TTG TCC AG	
HUMAN	SCD1 - F	TTC CCG ACG TGG CTT TTT CT	RT-qPCR
	SCD1 - R	AGC CAG GTT TGT AGT ACC TCC	
HUMAN	SCD2 - F	CCC TGG TAC ATC TGG GGA GA	RT-qPCR
	SCD2 - R	GAA GCC TTC ACC AAT GGC AC	
HUMAN	CHOP - F	CTG CCT TTC ACC TTG GAG AC	RT-qPCR
	CHOP - R	CGT TTC CTG GGG ATG AGA TA	
HUMAN	β-ACTIN - F	CTG AGC GTG GCT ACT CCT TC	RT-qPCR
	β-ACTIN - R	GGC ATA CAG GTC CTT CCT GA	
Mouse	β-Actin - F	GCT CTG GCT CCT AGC ACC AT	RT-qPCR
	β-Actin - R	GCC ACC GAT CCA CAC AGA GT	
Mouse	Ire1α - F	CCC ATC ACC CTG CTT CAT CA	RT-qPCR
	Ire1α - R	TGC AGA GGC CAA AGT CAG AG	
Mouse	Xbp-1s - F	GAG TCC GCA GCA GGT G	RT-qPCR
	Xbp-1s - R	GTG TCA GAG TCC ATG GGA	
Mouse	Scd1 - F	TTC TTG CGA TAC ACT CTG GTG C	RT-qPCR
	Scd1 - R	CGG GAT TGA ATG TTC TTG TCG T	
Mouse	Scd2 - F	GCA TTT GGG AGC CTT GTA CG	RT-qPCR
	Scd2 - R	AGC CGT GCC TTG TAT GTT CTG	
Mouse	Ire1α - F	CAG AGA TGC TGA GTG AAG AC	Ire1α floxed mice genotyping
	Ire1α - R	ACA GTG GTT CCT GTG AAG GT	
Mouse	Cre - F	GCG GTC TGG CAG TAA AAA CTA TC	CD4-Cre TG mice genotyping
	Cre - R	GTG AAA CAG CAT TGC TGT CAC TT	

Supplemental Table 2: Antibodies and reagents used for the flow cytometry analysis.

Species	Fluorescence	Antigen	Company	Cat. #
Mouse/ Human	FITC	Annexin V	Biolegend	640906
Mouse/ Human	PE	Annexin V	Biolegend	640908
Mouse/ Human	APC	Annexin V	Biolegend	640920
Mouse/ Human	FITC	B220 (RA3-6B2)	Biolegend	103206
Mouse/ Human	APC	B220 (RA3-6B2)	Biolegend	103212
Mouse/ Human	Alexa Fluor 488	B220 (RA3-6B2)	Biolegend	103225
Mouse	PE	CD3ε (145-2C11)	Biolegend	100308
Mouse	PE/Cyanine7	CD3ε (145-2C11)	Biolegend	100320
Mouse	PerCP/Cy5.5	CD4 (GK1.5)	Biolegend	100433
Mouse	Brilliant Violet 510	CD8a (53-6.7)	Biolegend	100752
Mouse	Purified	CD16/32 (93)	Biolegend	101302
Mouse	PE	CD21/CD35 (7E9)	Biolegend	123409
Mouse	PerCP/Cy5.5	CD21/CD35 (7E9)	Biolegend	123415
Mouse	Brilliant Violet 510	CD23 (B3B4)	Biolegend	101623
Mouse	Brilliant Violet 421	CD138 (281-2)	Biolegend	142508
Mouse	PE	IgM (RMM-1)	Biolegend	406508
Mouse/ Human	APC	CFSE	eBioscience	65-0850
Mouse	APC	CD80 (16-10A1)	Biolegend	104714
Mouse	PE	CD86 (GL-1)	Biolegend	105008
Mouse	PE	IgG (Poly4053)	Biolegend	405307
Mouse	FITC	IgG1	BD	553443
Mouse	APC	IgG2a (RMG2a-62)	Biolegend	407109
Mouse	FITC	IgA	BD	559354
Mouse	PE	IgE (RME-1)	Biolegend	406907
Mouse	PE	MHCII (M5/114.15.2)	Biolegend	107608
Mouse	Brilliant Violet 510	MHCII (M5/114.15.2)	Biolegend	107635
Mouse/ Human	eFluor450	Live and Dead	Invitrogen	65-0863-14
Mouse/ Human	eFluor506	Live and Dead	Invitrogen	65-0866-14
HUMAN	PE	CD19 (4G7)	Biolegend	392503
HUMAN	APC	CD138(DL-101)	Biolegend	352307
HUMAN	PE/Cyanine7	CD3ε (HIT3a)	Biolegend	300316
		Fixation/Permeabilization	ThermoFisher	00-5521-00