SUPPLEMENTAL MATERIAL

DETAILED METHODS

Human Coronary Artery Disease Subjects

All human subjects were recruited for study through the Cardiac Catheterization laboratory at the University of Virginia. Patients were excluded if they had: any acute illness, type 1 diabetes, current acute coronary syndrome, autoimmune disease or on immunosuppressive therapy, prior organ transplantation, anemia, pregnancy, and HIV infection. No patient was on anticoagulation or had deep vein thrombosis or pulmonary embolism. All participants provided written informed consent before enrollment, and the study was approved by the Human Institutional Review Board. Peripheral blood was obtained from these participants prior to catherization.

Healthy Human Volunteers

Peripheral blood from healthy volunteers were obtained after providing with written informed consent. The study was approved by the Human Institutional Review Board at University of Virginia.

Mice

All animal protocols were approved by the Animal Care and Use Committee at the University of Virginia. $Id3^{fl/fl}$ mice were a generous gift from Dr. Yuan Zhuang at Duke University. $CD19^{Cre/+}$ mice were provided by Dr. Bender (University of Virginia). C57BL/6J mice were purchased from Jackson Laboratory. These mice were housed on coconut husk bedding, and were either fed a standard chow diet or a Western diet. All

the mice utilized in all the experiments were 8-10 weeks old female mice. The mice were healthy through the course of the study. Female mice were used rather than male mice as female mice have been historically underutilized to understand pathogenesis of atherosclerosis. The sample size of the mice used in this study was determined based on previously published data[10,14–16] from the related assays instead of power calculation as the data from the same exact assays were not available. To avoid differences arising from cage to cage variation in mouse experiments, each cage had at least one mouse from each experimental group, but otherwise, experimental groups were allocated randomly, and investigators were blinded to group allocation when performing all data collection.

Cell Preparations for Murine and Human flow cytometry

Peritoneal cavity cells were processed for flow cytometry or fluorescence activated cell (FAC) sorting as previously described[9]. Antibody titrations were performed to validate the antibodies and determine optimal concentrations. Fluorescence minus one (FMO) controls were utilized to distinguish target staining from background. Isolation of human PBMCs was performed as previously described[9]. Clone and fluorophore information as well as IgG isotype controls for flow cytometry antibodies were provided in supplementary table 3.

Samples preparation of Bulk RNA sequencing

Sort-purified peritoneal B1a and B1b obtained from Id3KO and Id3WT C57BL/6 mice were RNA extracted by using Qiagen RNeasy Plus kit. The purified RNAs were stored at -80°C and sent to a Novogene to perform sequencing.

Differentially expressed genes and pathway analysis

RNA sequences in raw FASTQ data files were obtained from Novogene and HISAT2/Stringtie/Ballgown pipeline was utilized for RNASeq analysis. Sequencing reads were aligned to reference genome (GRCm38/mm10) using HISAT2 (https://github.com/infphilo/hisat2). The annotated sequences were then quantified and assembled by using StringTie (https://github.com/gpertea/stringtie), and differentially expressed genes were analyzed using R Ballgown package (https://bioconductor.org/packages/release/bioc/html/ballgown.html). The codes to run the pipeline can be obtained from the previously published protocol[17]. Volcano plots of differentially expressed genes were visualized by using the python bioinfokit package. Ingenuity Pathway Analysis was performed on all the annotated RNA to analyze for differentially regulated cellular processes, canonical pathways and network analysis.

***In Vitro* and *In Vivo* Cell Proliferation Assay**

Peritoneal cavity cells were enriched for B cells using the EasySep Mouse Pan-B cell enrichment kit (STEMCELL, catolog #19844). $1x10^6$ B cells were then incubated with 5uM Celltrace-violet in 200uL B cell culture media (RPMI + 10%FBS + 10mM HEPES, 1mM Na-pyruvate + 10ug/mL Gentamycin + non-essential amino acid) for 20 minutes at

37°C, and then washed with FACS buffer (1% FBS, 0.1%NaN_3 in PBS). Labeled cells were cultured in B cell culture media for 3 days with 20ng/mL recombinant BAFF or PBS control to measure *in vitro* proliferation. For in vivo proliferation, sort-purified $2x10^5$ of p62gRNA-ATTO+ Celltrace-violet+ B1 cells were resuspended in 100uL DPBS and intraperitoneally transferred into C57BL/6 mice.

Lentiviral Production and p62 Overexpression on mouse B cells.

P62-eGFP lentivirus was generated using pLV-eGFP vector (purchased from Addgene) and mouse p62 was subcloned into the vector. The eGFP or P62-eGFP lentiviruses were generated using four plasmid system by co-transfecting pLV-eGFP or pLV-P62-eGFP plasmid along with pLP1(Gag-Pol vector), pLP2 (Rev vector), and pVSV-G (VSV-G vector) plasmids into 293T cells. The eGFP ctrl and P62-eGFP lentiviruses were used to transduce enriched peritoneal mouse B cells at multiplicity of infection (MOI) of 50 by using 5ug/mL polybrene and spinning at 1000g for 60 mins. Transduced murine B cells were harvested 48 hrs post transduction and FAC-sorted for GFP+ B1 cells to be used for adoptive transfer. Approximately 25-30% of B-1 cells were successfully transduced by this method.

P62 CRISPR/sgRNA Knockout and Pp62-GFP transfection on Mouse B cells.

crRNA molecule-targeting exon of p62 (UUGUAGCGGGUUCCUACCAC-PAM AGG) was purchased from IDT and conjugated with tracRNA-ATTO. Pp62-GFP plasmid was generated by subcloning 1000bp of p62 promoter to replace CMV promoter in CMV-GFP plasmid purchased from Addgene. Murine peritoneal cells were enriched for B

cells using the EasySep Mouse Pan-B cell enrichment kit (STEMCELL), cultured in B cell culture media, and stimulated with 50ng/mL LPS overnight. The stimulated B cells were then nucleotransfected with Cas9 ribonuclear protein and pre-conjugated p62 crRNA-tracRNA ATTO, Pp62-GFP, or CMV-GFP ctrl plasmid using the P3 Primary cells Nucleofection Kit purchased from Lonza. B cells were collected for analysis and sorting 24 hrs after nucleofection.

Nucleofection of Engineered Plasmids into Human B cells.

Cryopreserved PBMCs obtained from healthy donors were thawed and washed with warm complete media (RPMI supplemented with 5% FBS, 1 mM sodium pyruvate and Pen-Strep) with 95-98% cell viability. B cells were enriched using EasySep human pan-B cell enrichment kit (STEMCELL) with 10-15% of the total PBMCs. CMV-E12-Flag, CMV-E47-Flag were generated using CMV-Flag plasmid to subclone human E12 and E47 into. These plasmids were nucleotransfected into $2x10^6$ unstimulated enriched human B cells per each plasmid construct using the P3 Primary cells Nucleofection Kit purchased from Lonza. B cells were collected for analysis and sorting 24 hrs after nucleofection.

Quantification of markers colocalization by using imaging flow cytometry

Murine peritoneal cells obtained from Id3KO and Id3WT mice were enriched for B cells. The enriched B cells were incubated with 20ng/mL murine recombinant BAFF or PBS control for 12 hrs in B cell media. Prior to running on the Imagestream imaging flow cytometry machine, cells were stained with B220-BV421, CD5-PE, and CD19-PECF594

using previously developed surface staining protocol[9] following with permeabilization and intracellular staining of p62-AF488 and TRAF6-AF647 antibodies using FIX & PERM Cell Permeabilization Kit (Invitrogen). Images of 1000 Id3KO or Id3WT B1b cells from both unstimulated, BAFF stimulated conditions were collected, and quantitative co-localization analysis was performed using Amnis Imagestream colocalization software.

ELISA for Quantification of Total and Anti-OSE IgM or IgG Isotypes in Mice and Humans

Total IgM subtypes in mouse plasma were measured using colorimetric ELISA as described previously[9]. Levels of IgM and IgG against the MDA mimotope[18], a peptide mimotope of MDA-LDL (peptide sequence: HSWTNSWMATFL), in human plasma were measured by chemiluminescent ELISA as previously described[18]. For the detection of antibodies, plasma was diluted at 1:100 dilution.

Adoptive Transfer

Celltrace violet+ or eGFP+ (eGFP-ctrl or p62-eGFP) B1 (CD3-CD19+B220-) cells were sort-purified. After sorting, $2x10^5$ of these cells were adoptively transferred into C57BL/6 host mice intraperitoneally. Mice adoptively transferred with Celltrace-violet+ cells were fed a chow diet for 2 weeks. Mice adoptively transferred with eGFP-ctrl or p62-eGFP B1 cells were fed with Western diet for 8 weeks. After 2 weeks or 8 weeks, mice were sacrificed and cells from the peritoneal cavity were processed for flow cytometry as previously described[9]. For western diet fed mice, aortas were harvested and stained using Sudan IV as previously described[9]. Flow cytometry was used to quantify for

number of B1a and B1b cells as well as degradation of Celltrace-violet signal of harvested cells obtained from C57BL/6 mice.

Analysis of Atherosclerotic Lesions

Hearts and aortas were harvested as previously described[10]. Briefly, hearts were embedded in optimal cutting temperature compound (Tissue-Tek) and snap frozen. Serial 5-μm sections were cut by Cryostat (Leica Biosystems) from the beginning of the 3 aortic leaflets to the aortic arch and stained using Oil Red O (Sigma) as previously described[10]. Aortas were fixed in 4% paraformaldehyde then opened longitudinally, pinned, and stained using Sudan IV (Sigma) as previously described[10]. Plaque areas were assessed using Image-Pro Plus software (Media Cybernetics). The analysis was done by the lab specialist without knowing the group allocation.

Statistics

Statistics were performed using GraphPad Prism Version 7.0a (GraphPad Software, Inc), Python 3.0, R 3.6.1, or SAS 9.4. Results from all replicated experiments are displayed, and bar graphs display mean±SEM. Nonparametric test with two-sided p values, Mann-Whitney test, was used to determine statistical significance for comparing between two groups. For comparing more than two groups of data, one-way ANOVA for non-parametric data, Kruskal-Wallis test was used. Following Kruskal-Wallis test, post hoc Dunn was run to correct for multiple comparisons. Spearman was used to determine correlation coefficient, and false discovery rate (FDR) method was used to perform p-value corrections for DEG analysis. Normality and equal variance were not assessed as a precondition for the analyses. Data were represented as mean $\pm$ SD. P

values and the specific statistical methods used were specified in figures and figure legends.

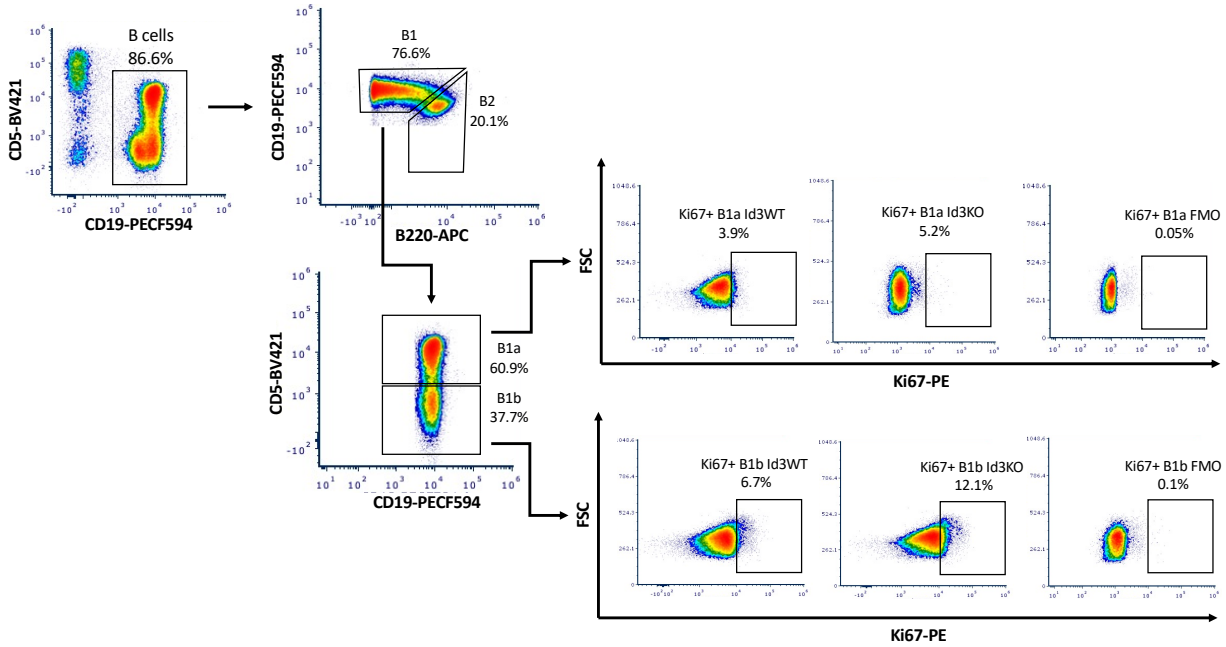

Figure S1: Gating strategy of B1a and B1b as well as Ki67+ population.

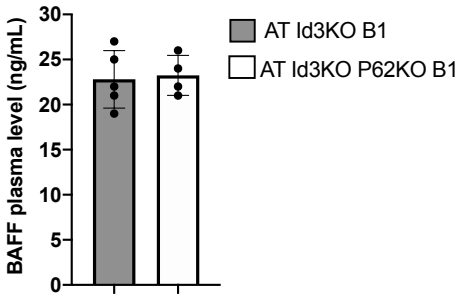

Figure S2: BAFF level in the plasma of mice receiving Id3KO B1 cells (n = 5) or Id3KO p62KO B1 cells (n = 4) 2 weeks post adoptive transfer

TGTTGGAGAGGAAAGCCTAAAACTGGGATTGTCCTCTGGGGCAATAGGTGTGTACCTTCTAGCCACCAGGGAAATGGCAGTCA
CACCTGTGTGGCAAATCACTGTCCATTGCTGAGTTTTCCTAAAATTACAGATTTTTCTGCTCTCACAAAACCAATTCTGAGAGAG
GTCCAGGGAAGACATGATAACTGGGAGAGGGGCCCTAGGAAGCTGGTGTGGAGACCCTGCCTCAGGGAGACGACTTGAGCTC
CAATTCTGCCCTGCATGTCTTCTCCCATGACTTTGACTCAGCAGGTCTAGTCCTATGGAAAGGACCATACCTAGAAGGCCTTACA
GGGTGACACCCTTCTCAGCCTCTAGCCTTCCCAGGACTGGGTTCCTGCTAAGAGCTCATTTTACTTTATTCATTTAATTTGTGGTT
TGTTTTTATTTTTCCCTGAGACAGGGTTGCTCTGTGTAGCCCTGGCTGTCCTGGAACTCACTCTGTAGACCAGGCTGGCCTTGAA
CTCAGAAATCCACCTGCCTCTGCCTCCCAAGTTCTGGGGTTAAAGGCATGTGCCACCACTGCCCTGCTTAATTTGTTTTTTTTTG
AGAGTAGGACTTGCTATGCAAGCCTGGCCGAGCCTTGAATTAGAATTCCTCTTGACTCAGCTTCCCAAGGGCTGGGGTTACAGA
TGTGTGTCATCATGCTAGCTAACAGGTCACTCTGGCCACAGAAAGCAGCATTTCCTGGTTTTGGCGTTTGTATTGGGGGTTGAA
CCTAAGATGTAACACATACTAGGCAGGTGCTCTGTCACTGAGCTATATCCCTATGCACCAAATTCCCATTAATGATATCTCCCGG
GTATATCCCTACTAAGCTAAATCCTCATGACCTGTATCCCTACTAAGCTAAATCTCTATAAGCTGTATCCCTACTAAGCTAAATCT
CCTGTATCCTTACTAAACTGTATAGCCAGATTCTTTATTCCCGCCCCCCAGCACCCAGACTTTTTTTCTTTCTTAGAAGCCTCATGC
AGCCAAGGCTGGCTTTGAACTCTGGAACCTCTGACCTCCCAAGTACTAGGATCACTTTCTATTTTGAAACAGTTTCACCAAGTTG
CTCAAGCAGAATATCTTGAACTTGGTCTTTTCCTGCCTCAGCACCTCAAACAGCTGGAATAACCAACCTGTGGCACAGGACTGG
CTATAGCTTGTATTTTGGTTCCTAGATGGAGGGCAGGTGGTCTGATCCCTTTGTGCTTTGAAGCCACTAAGGCCTGAGATACAT
GTACCTTCCGAGAGAGAGAGAGAGAGAGAAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAATATGAATGTGTGTGTA
TGTGTGTGTGTGTCTGGGCTGCAGTGAGGAATCTCTGGTTGCAATGAACAGGTTGTGACTACTGCTGCCTCTGGAGCCCTGGCT
ACCCCTCTTACAGGGTGGGTTTGTGAGAAGTCACCAGTGCTTGTCCAACACCTCTCAACCTGATCTTTGTTAAATTGGGTGAGGC
CTAAGATGGTGGGTGATGTTCCTTTATTCAGTGAGGGGCTGGTCATGGTGGATGCCTGGAAGGGGGTGGGAGTGGGGGCTAC
TACCGGGGACACTATGTTTAGGAGGGTCGGAATAGCATCCTGTGAGACAGCCTGTTTGGCTAAAAGAATGGCTACCTGACAGT
AGACATCTGTGTCCTCCTAGCTTCTGGGCCGTACATCCCCCCTGGCTTGGGGACGACACTGCTGAGTCATAGGCAGGCCCAAGC
CTGGGAGGTACCTAGGCTCGATACTTACGGGTCCTTTTCCCAACGACTTGAGGCTGAGGCTGGTCTCTTGTGGTCACCCATGTA
TTCGGTGGGAAAGAAAAGAGTAGGCAGTGGGGTCGTCTCGACCCCGTACCTTTCAAGGGTCCCATCAAGGGGGCTCCAGCGG
CCGCCCCAGCCTAGGGGAGGGGCGGGGCTCATAGAGGCTCCGCCCCCACGAGGCGGGG

Figure S3: E-box sequences (CANNTG) located in p62 promoter region (yellow).

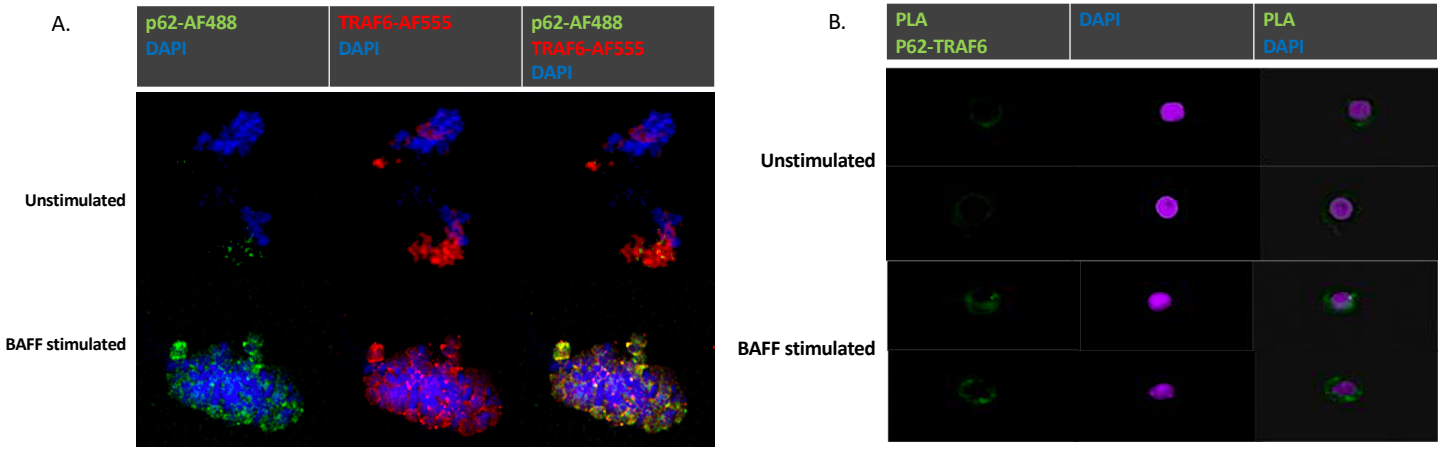

Figure S4: A,Confocal imaging of p62 and TRAF6 colocalization. **B,** Visualization of interaction p62 and TRAF6 through PLA assay with and without BAFF stimulation.

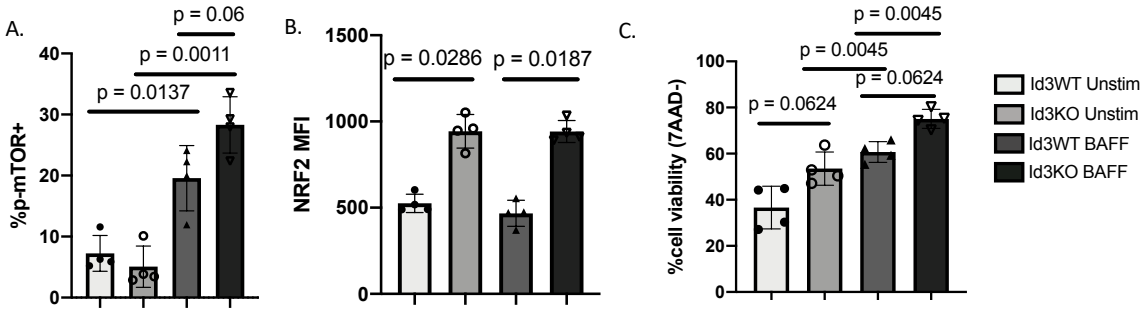

Figure S5: A-C, Flow cytometry quantifying percentage of p-mTOR+ population **(A),** MFI of NRF2 **(B),** and percentage of cell viability measured as 7AAD- population **(C)** in Id3WT and Id3KO B1b cells with and without BAFF stimulation (n = 4 per group).

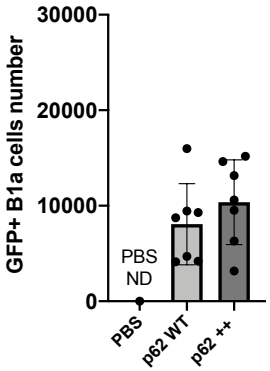

Figure S6: Number of recovered GFP+ B1a cells (n = 4 for PBS group; n = 7 for p62WT group; n = 6 for p62++ group) 8 weeks post adoptive transfer

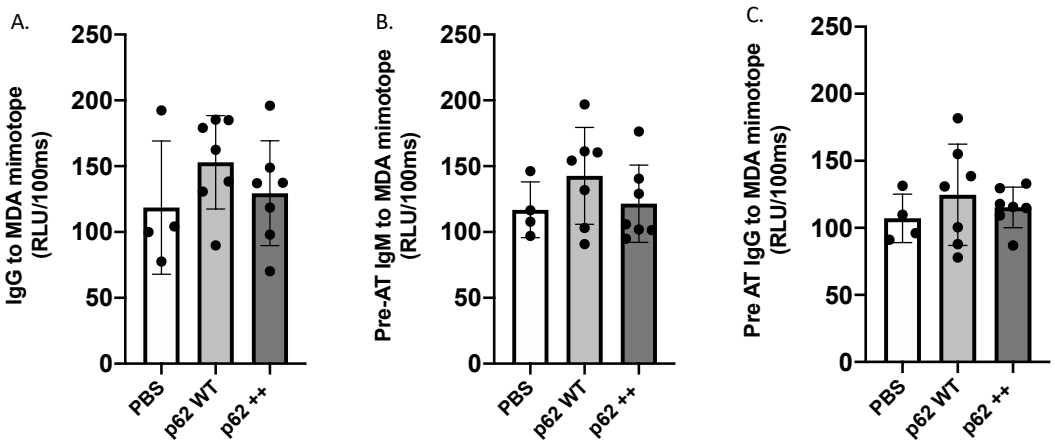

Figure S7: A-C, Plasma IgG to MDA-mimotope level (A) 8 wks post transfer and WD fed, pre-adoptive transfer plasma IgM to MDA-mimotope (B), and pre-adoptive transfer IgG to MDA-mimotope (C) measured by ELISA obtained from PBS (n =4), p62WT (n = 7) and p62++ (n =6) AT groups.

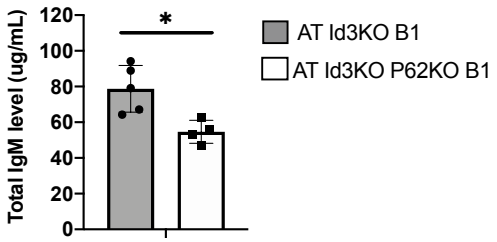

Figure S8: Plasma total IgM level 2 wks post transfer measured by ELISA obtained from AT of Id3KO (grey, n = 5) and Id3KO p62KO (white, n = 4) B1 cells into C57BL/6 host mice.

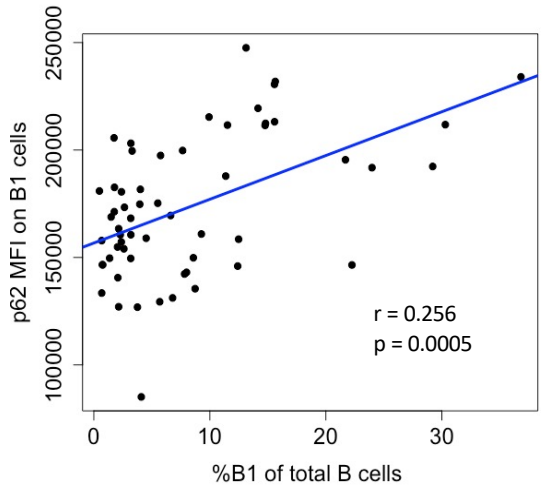

Figure S9: correlations between human B1 frequency and p62 expression in B1 cells (n = 58)

Table S3: Flow cytometry antibodies used for sorting, analyzing B cells from *in vitro* culture and tissue harvest.

Fluorophore	Clone	Manufacturer	Catalog#	Antigen
PE-CF594	1D3	BD Bioscience	562291	CD19
AF700	O323	Biolegend	152316	CD3
BV421	53-7.3	BD Bioscience	562739	CD5
PE	53-7.3	BD Bioscience	553022	CD5
APC	RA3-6B2	Invitrogen	14-0452-82	B220
BV421	RA3-6B2	Invitrogen	562922	B220
AF488	EPR4844	Abcam	ab185015	p62
AF647	EP591Y	Abcam	ab214941	TRAF6
PE	MFRDTRK	Invitrogen	12-9036-41	IKBa
AF555	Y69	Abcam	ab32072	c-myc
PE	7H22-E16	Biolegend	316906	BAFFR
PE	16A8	Biolegend	652404	Ki-67
PE	MRRBY	Thermo Fisher	12-9718-42	p-mTOR
PE	D1Z9C	Cell Signaling	14409S	NRF2
Unconjugated	SP137	Invitrogen	MA5-16384	IgG Isotype control