Table S1. Clinical characteristics of patients at presentation

Patient	P1	P2	P3	P4	P5
Sex	Male	Female	Male	Female	Male
Age of onset of symptoms	9 mos.	11 mos.	9 mos.	5 mos.	6 mos.
Presenting symptom	Recurrent chest infections	Recurrent chest infections	Recurrent chest infections	Recurrent chest infections	Recurrent chest infections
Other manifestations	Hepatitis Bronchiectasis	Hepatitis. Bronchiectasis	Hepatitis Bronchiectasis	Hepatitis	Hepatitis
Infectious pathogens recovered from sputum	*H. Influenzae*, *S. pneumonia*, *Moraxella* species, Influenza A, Adenovirus.	*H. influenza*, *S. pneumonia*, *Moraxella* species, Influenza A, Parainfluenza 3, Rhinovirus, RSV.	*H. influenzae*, *S. pneumonia*, *Moraxella* species, Adenovirus, Rhinovirus.	Adenovirus	RSV
Infectious pathogens recovered from blood	CMV EBV	CMV	CMV	CMV EBV Parvovirus B19	CMV

CMV= Cytomegalovirus, EBV= Epstein-Barr virus, RSV=Respiratory syncytial virus

Table S2. Immunologic findings at presentation

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age at diagnosis	10 years	7 years	6 years	10 months	6 months
Serum Immunoglobulins, mg/dL					
IgG	1,120 (695-1,602)	**2,450** (695-1,602)	N/A	**740** (346-658)	500 (166-547)
IgA	**34** (51-223)	106 (34-181)	N/A	44 (13-75)	43 (6-47)
IgM	64 (39-167)	117 (42-181)	N/A	**231** (30-145)	**132** (18-98)
Lymphocyte Phenotyping, cells/μL					
$CD3^+$	**1,120** (1,200-2,660)	**532** (1,200-2,660)	**517** (1,400-3,700)	2,084 (1,900-5,900)	**1,030** (2,500-5,600)
$CD3^+CD4^+$	**71** (650-1,500)	**92** (650-1,500)	**107** (700-2,200)	**612** (1,400-4,300)	**514** (1,800-4,000)
$CD3^+CD8^+$	981 (370-1,100)	385 (370-1,100)	**376** (490-1,300)	1,418 (500-1,700)	**493** (590-1,600)
$CD19^+$	850 (270-860)	625 (270-860)	**217** (390-1,400)	1,122 (610-2,600)	**327** (430-3,000)
$CD16^+CD56^+$	**32** (100-480)	**19** (100-480)	266 (130-720)	**95** (160-950)	**17** (170-830)

Abnormal values are indicated in bold. Values of T, B, and NK cell markers shown between parentheses represent the normal range for age. N/A = Not available as P3 had been started on gamma globulin replacement therapy prior to evaluation.

Table S3. Acquired pathogens during co-housing with pet-store mice

Pathogen	SPF WT	SPF Mut	Pet-store	Co-housed WT	Co-housed Mut
Mouse adenovirus 1 & 2	0/1	0/1	4/4	3/6	5/5
Mouse hepatitis virus	0/1	0/1	4/4	3/6	2/5
Theiler's murine encephalomyelitis virus	0/1	0/1	4/4	6/6	4/5
Pasturella pneumotropica-Heyl	0/1	0/1	4/4	6/6	4/5
Pasturella pneumotropica-Jawetz	0/1	0/1	4/4	3/6	0/5
Cryptosporidium	0/1	0/1	4/4	6/6	5/5
Helicobacter ganmani	0/1	0/1	4/4	5/6	5/5
Helicobacter hepaticus	0/1	1/1	4/4	2/6	2/5
Helicobacter mastomyrinus	0/1	0/1	4/4	2/6	0/5
Helicobacter rodentium	0/1	0/1	1/4	3/6	1/5
Helicobacter typhlonius	1/1	1/1	0/4	2/6	2/5
Spironucleus muris	0/1	0/1	4/4	3/6	3/5
Tritrichomonas genus	0/1	0/1	4/4	4/6	3/5
Minute virus of mice	0/1	0/1	4/4	4/6	2/5
Mouse parvovirus 1	0/1	0/1	1/4	1/6	0/5
Mouse parvovirus 2	0/1	0/1	4/4	0/6	0/5
Mouse parvovirus 3	0/1	0/1	4/4	3/6	4/5
Mycoplasma pulmonis	0/1	0/1	4/4	1/6	1/5
Giardia duodenalis	0/1	0/1	1/4	0/6	1/5
Proteus mirabilis	0/1	0/1	3/4	1/6	0/5
Syphacia obvelata	0/1	0/1	4/4	2/6	2/5

Surveillance Plus PCR Rodent infectious agent panel performed by the Charles River Laboratories on the feces of mutant and WT mice on Day 22 of co-housing with pet-store mice.

Supplemental Figures

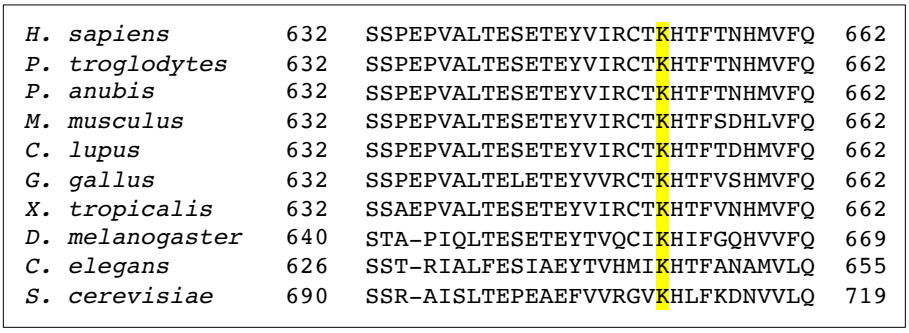

Figure S1. The K652 residue in *COPG1* is highly conserved among species.

Amino acids surrounding the *COPG1* K652 residue in humans and 9 other species. The conserved K652 residue is highlighted in yellow.

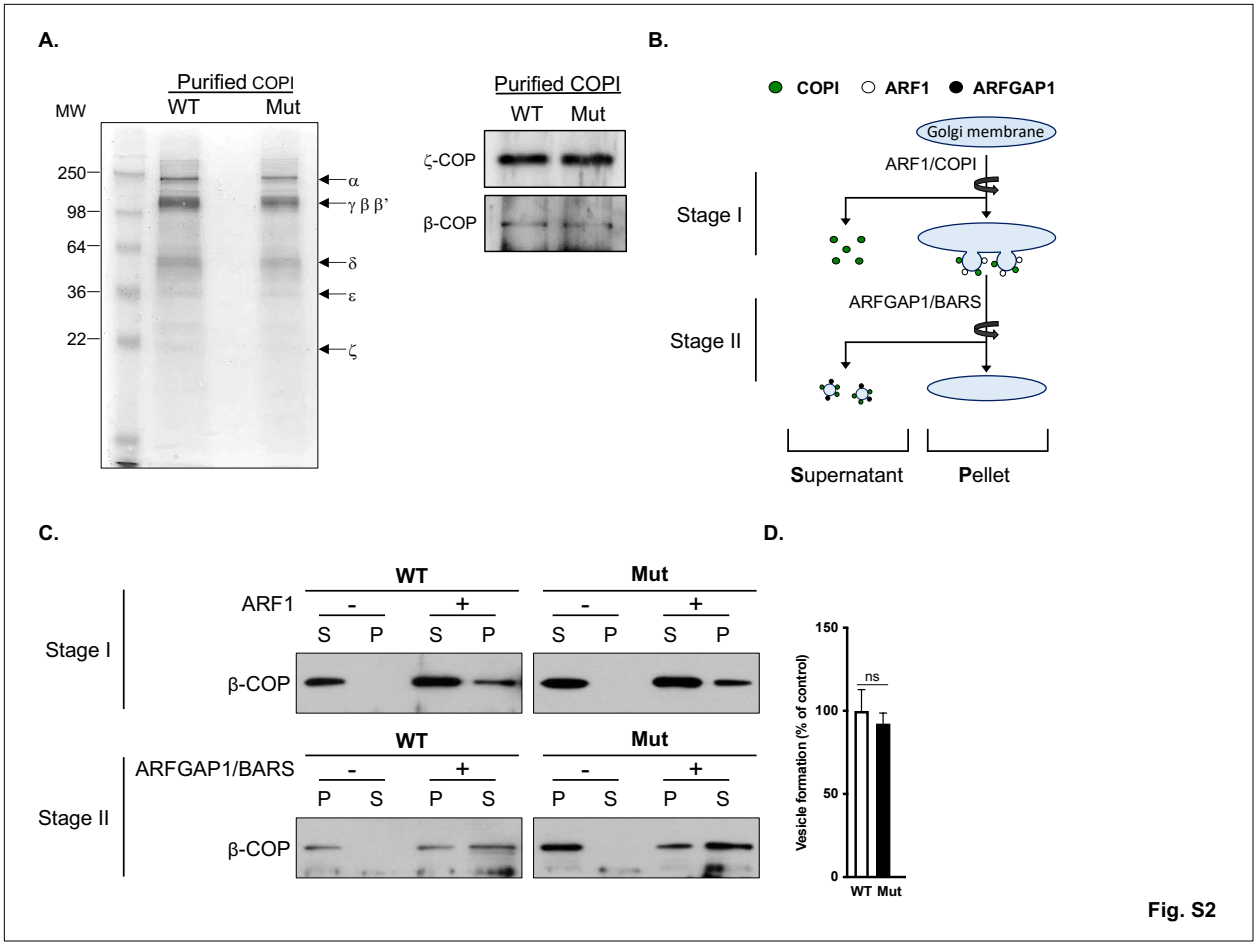

Figure S2. The *Copg1*^{K652E} mutation does not have a detectable effect on COPI composition or vesicle formation from Golgi membrane. A. The *Copg1*^{K652E} mutation does not affect coatomer integrity. Purified coatomers derived from liver of wild-type and mutant mice were analyzed by SDS-PAGE followed by silver staining (left) as well as by immunoblotting for ζ-COP using β-COP as control (right) because the band corresponding to ζ-COP was faint on SDS-PAGE. A representative result from two independent experiments is shown. A molecular weight (MW) ladder is shown in lane 1. **B.** Schematic of the COPI vesicle reconstitution system. The Golgi membrane is first incubated with purified ARF1 and coatomer for the ARF-dependent recruitment of coatomer onto Golgi membrane (Stage I). The Golgi membrane is then re-isolated and incubated with ARFGAP1 and BARS to generate COPI vesicles (Stage II). **C.** The

Copg1^{K652E} mutation does not affect COPI vesicle formation from Golgi membrane. Stage I was performed using conditions as indicated, followed by the detection of β-COP in the pellet (containing Golgi membrane) and supernatant (containing soluble protein) fractions. Stage II was then performed using conditions as indicated, followed by the detection of β-COP in the pellet (containing Golgi membrane) and supernatant (containing COPI vesicles) fractions. A representative result from three independent experiments is shown (left). **D.** Quantitation of coatomer redistribution after the Stage II incubation, which reflects COPI vesicles formed, mean ± SEM is shown, ns (not significant), two-tailed Student's t-test, n = 3 independent experiments.

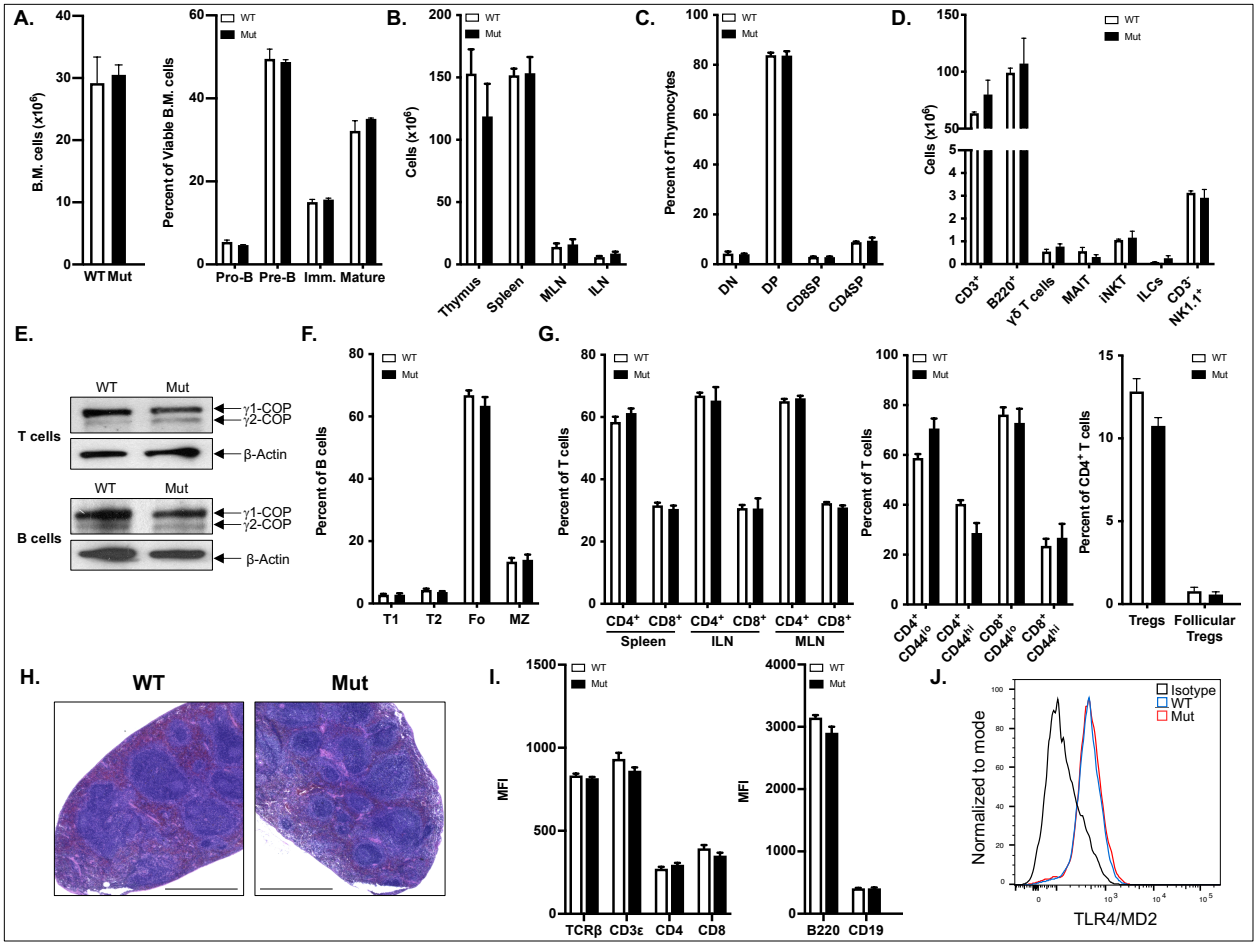

Figure S3. Homozygous *Copg1*^{K652E} mutant mice have normal lymphocyte development and expression of surface markers. A. Number of viable bone marrow (BM) cells (left) and percentages of pro-B cells ($B220^+CD43^+$), pre-B cells, ($B220^+CD43^-$), immature B cells ($B220^+CD43^-IgD^-IgM^+$), and mature B cells ($B220^+CD43^-IgD^+IgM^+$) in the BM (right) in mutant mice and WT controls. **B.** Number of viable thymocytes, spleen and lymph node (LNs) cells. **C.** Distribution of thymocyte subsets in mutant mice and WT controls. DN ($CD4^-CD8^-$), DP ($CD4^+CD8^+$), CD8SP ($CD4^-CD8^+$), and CD4SP ($CD4^+CD8^-$). **D**. Number of viable of splenic T ($CD3^+$) cells, B ($B220^+$) cells, γδ T cells MAIT ($TCR\beta^+MR\text{-}1^+$) cells, iNKT ($TCR\beta^+CD1d^+$) cells, innate lymphoid cells ($CD3^-Lin^-CD90.2^+$) and $CD3^-NK1.1^+$ NK cells in mutant mice and WT controls. **E.** γ1-COP

immunoblot of splenic T and B cells in mutant mice and WT controls. β–Actin is used as a loading control. **F.** Distribution of T1 ($CD93^+CD23^-IgM^+$), T2 ($CD93^+CD23^+IgM^+$), follicular (FO) ($CD93^-CD23^+CD21^{int}$) and marginal zone (MZ) ($CD93^-CD23^+CD21^+$) B cells within splenic $B220^+$ B cells in mutant mice and WT controls. **G.** Distribution of $CD4^+$ and $CD8^+$ T cells among $CD3^+$ cells in spleen, inguinal lymph nodes (ILN), and mesenteric lymph node (MLN), percentages of splenic naïve and memory $CD4^+$ cells and $CD8^+$ cells and percentages of $CD4^+FOXP3^+$ total Tregs and $CD4^+FOXP3^+CCXR5^+$ follicular Tregs in mutant mice and WT controls. **H.** Representative H&E stained sections of the spleen from unimmunized mutants and WT controls. Bar=650 μM. **I.** Mean fluorescence intensity (MFI) of expression of TCRβ, CD3ε, CD4 and CD8 by splenic T cells (left), B220 and CD19 by splenic B cells (right) of mutant mice and WT controls. **J.** Histogram of surface expression of the TLR4/MD2 complex by peritoneal macrophages in mutant mice and WT controls. Experiments in A-G and I were performed independently three times using 3 mice per group. The results in H and J are representative of two experiments each with one mouse per group. Columns and bars represent mean and SEM.

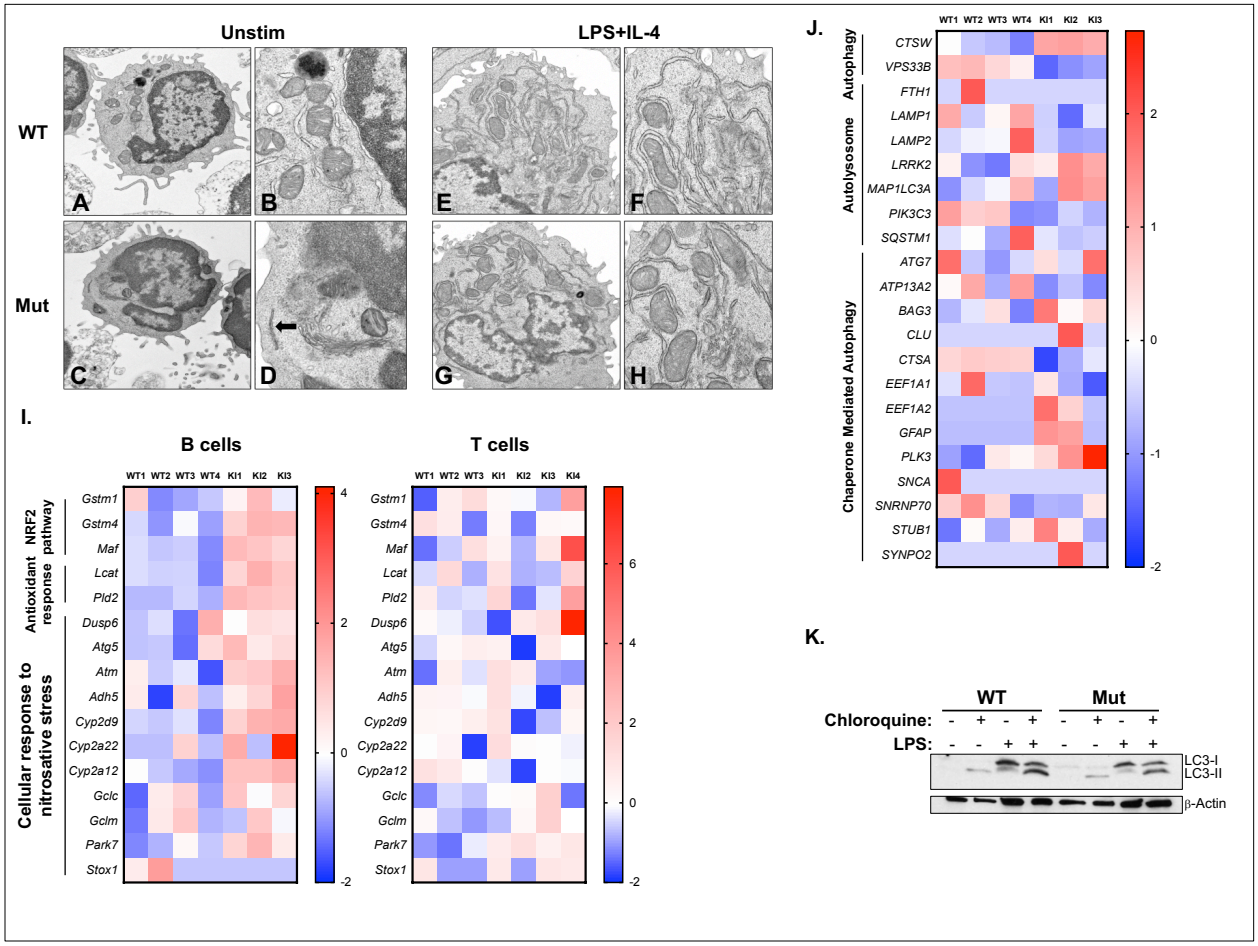

Figure S4. Decreased ER development, but normal antioxidant response and autophagy in unstimulated and LPS+IL4 activated mutant B cells. A-H. Electron micrographs of WT (upper panel) or mutant (lower panel) B cells unstimulated (A-D) or stimulated with LPS+IL4 for 3 days (E-H). Representative cells are shown. The ER patterns are highlighted in the corresponding enlarged images. Original magnification 4000x (A,C,E,G), or 15,000x (B,D,F,H). The arrow points to a short strand of ER near the Golgi apparatus. **I, J.** Transcriptomic analysis of 16 genes important for the antioxidant response (I) and 22 genes important for autophagy in LPS+IL-4 stimulated WT and mutant B cells and anti-CD3+anti-CD28 stimulated T cells (I). **K.** Immunoblotting for the

autophagsome marker LC3II in resting and LPS activated mutant and WT B cells cultured with or without chloroquine.

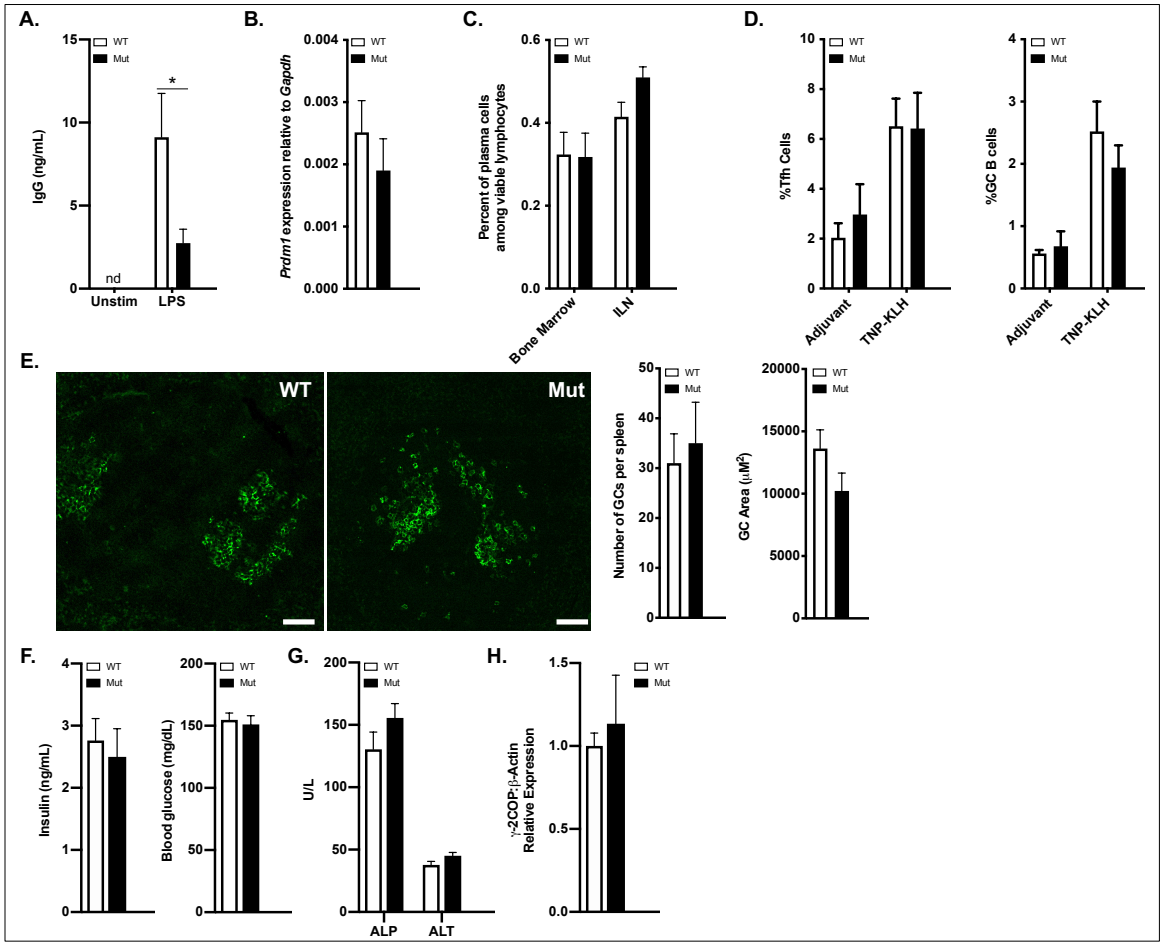

Figure S5. *Copg1*^{K652E} mutant mice have decreased LPS-driven IgG secretion by B cells, normal proportions of Tfh and GC B cells following TNP-KLH immunization, normal serum levels of glucose, insulin and liver enzymes and normal γ2-COP expression in the liver. A. IgG secretion of mutant and WT B cells after 3 days of treatment with media alone or LPS. **B.** *Prdm1* expression relative to *Gapdh* in resting WT and mutant B cells **C.** Percent of plasma cells in the bone marrow and inguinal lymph nodes of WT and mutant mice **D.** Percentages of T follicular helper cells ($CD4^+CXCR5^+PD1^+$) (left) and germinal center B cells ($B220^+FAS^+GL7^+$) (right) in

popliteal LNs 9 days after TNP-KLH immunization in the hock of 8-12 weeks old mutant mice and WT controls. **E.** Representative immunofluorescence staining of spleen sections for GL7 (left), numbers and size of GCs (right) in spleens from mutant mice and WT controls 21 days after TNP-KLH immunization. Bar=50 μM. **F,G.** Serum levels of insulin and glucose (F) and of ALP and ALT (G) in 8-12 weeks old mutant mice and WT controls. **H.** Expression of γ2-COP in the liver of 8-12 weeks old mutant mice and WT controls. Results are expressed as the ratio of γ2-COP to β-actin relative to WT control, as determined by scanning densitometry of liver lysate immunoblots. All experiments were performed independently three times using 3 mice per group. Columns and bars represent mean and SEM. *, $p \leq 0.05$, ns= not significant by two-tailed Student's t-test.

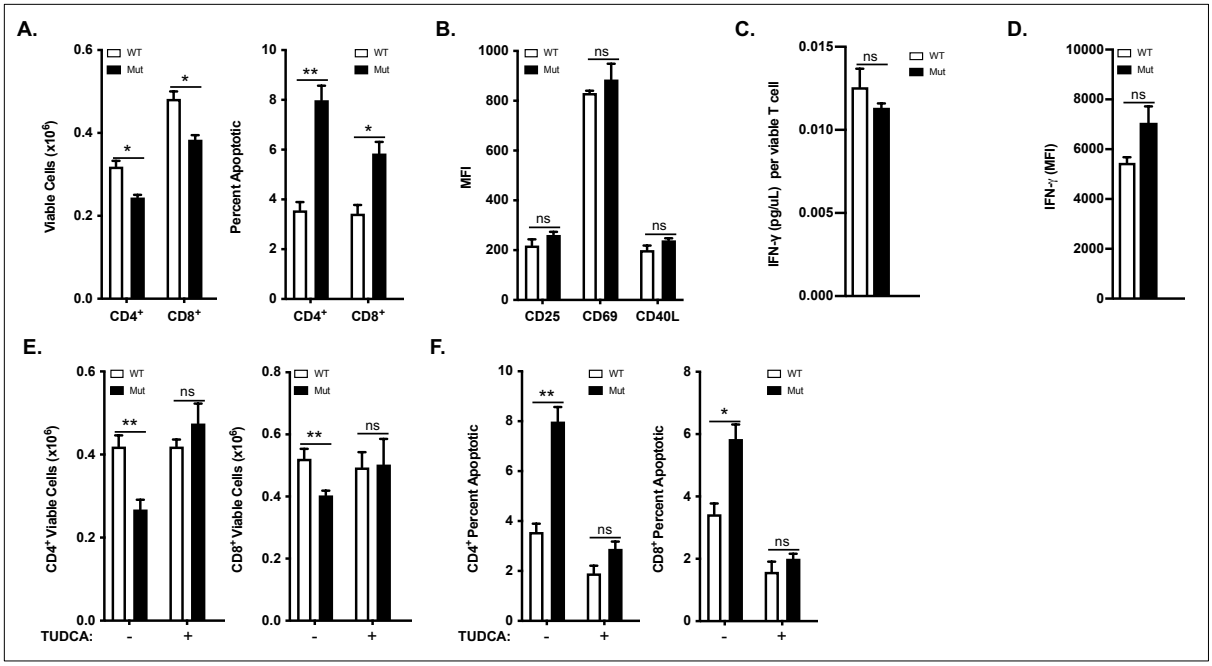

Figure S6. Decreased viability and increased apoptosis of activated CD4⁺ and CD8⁺ T cells from *Copg1*^{K652E} mutant mice are reversed by TUDCA. A. Viable numbers of CD4⁺ and CD8⁺ T cells (left) and percentage FVD⁻ Annexin⁺ apoptotic cells (right) after αCD3+αCD28 stimulation of purified splenic T cells from mutant mice and WT controls. **B.** Quantitation of the mean fluorescence intensity (MFI) of the T cell activation surface markers CD25, CD69, and CD40L in CD3⁺ T cells after anti-CD3+ anti-CD28 stimulation of purified splenic T cells from mutant mice and WT controls. **C**. IFN-γ secretion per viable cell in cultures of anti-CD3+anti-CD28 stimulated T cells from mutant mice and WT controls. **D**. MFI of IFN-γ expression by viable anti-CD3+ anti-CD28 stimulated T cells from mutant mice and WT controls. **E, F.** Effect of TUDCA addition on CD4⁺ and CD8⁺ viability (E) and apoptosis (F) in anti-CD3+ anti-CD28 stimulated T cells from mutant mice and WT controls. All experiments were performed independently three times using 3 mice per group. Columns and bars represent mean

and SEM. *, $p \leq 0.05$, **, $p \leq 0.01$, ns= not significant by two-tailed Student's t-test in A-D, and by Holm-Šídák test to control for multiple comparisons in E and F.

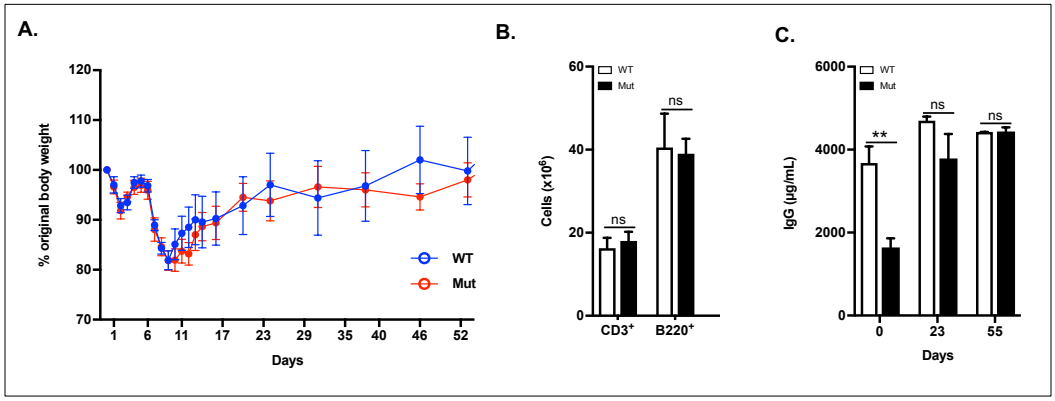

Figure S7. Response of *Copg1*^{K652E} mice to chronic LCMV infection A. Weight of *Copg1*^{K652E} mutant mice and WT controls following *i.v.* injection of 4x10^6 PFU LCMV clone 13. **B.** Splenic T and B cell counts on Day 55 post-infection. **C.** Serum IgG levels on Days 0, 23 and 55 post-infection. The experiments were performed independently twice each using 5 mice per group. Columns and bars represent mean and SEM. **, $p \leq 0.01$, ns= not significant by two-tailed Student's t-test.

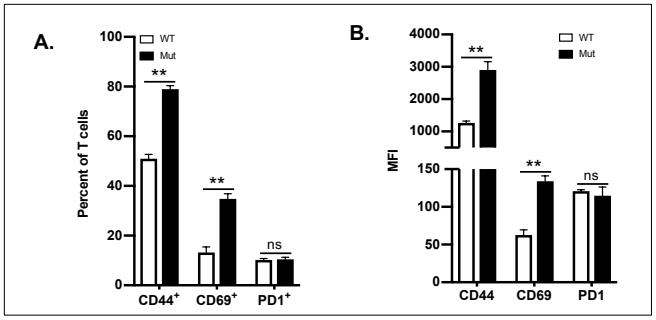

Figure S8. Expression of activation markers by splenic T cells from *Copg1*^{K652E} mice co-housed with pet store mice. A, B. Percentages of CD44^{+}, CD69^{+} and PD1^{+} cells (A), and MFI of expression of CD44, CD69 and PD1 (B) by splenic T cells from *Copg1*^{K652E} mice co-housed with pet store mice for 22 days. The experiments were performed independently twice using 4 mice per group. Columns and bars represent mean and SEM.**, $p \leq 0.01$, ns= not significant by two-tailed Student's t-test.

Supplemental Methods

Flow cytometry. Standard flow cytometric methods were used for the staining of cell-surface and intra-cellular proteins. Anti-mouse mAbs with the appropriate isotype-matched controls were used for staining. All flow cytometry data was collected with an LSR Fortessa (BD Biosciences) cell analyzer and analyzed with FlowJo software (Tree Star).

Reagents and monoclonal antibodies for flow cytometry FACS were used as described by the manufacturer: Zombie Aqua Fixable Viability Dye (Biolegend: 77143), CellTrace Violet (ThermoFisher: C34557), AnnexinV (Biolegend: 640912), B220/CD45R (ThermoFisher: RA3-6B2), CD138 (Biolegend: 281-2), ER-Tracker Green (ThermoFisher: E34251), CD3 (Biolegend: 17A2), CD4 (Biolegend: GK1.5), CD8 (ThermoFisher: 53-6.7), NK1.1 (ThermoFisher: PK136), Ly51 (Biolegend: 6C3), CD24 (Biolegend: M1/69), CD43 (BD Biosciences: S7), IgD (ThermoFisher: 11-26c), IgM (ThermoFisher: II/41), CD93 (ThermoFisher: AA4.1), CD23 (ThermoFisher: B3B4), CD21 (ThermoFisher: 4E3), CD19 (ThermoFisher: 1D3), GL7 (ThermoFisher: GL7), FAS/CD95 (ThermoFisher: 15A7), PD1/CD279 (ThermoFisher: J43), CXCR5/CD185 (ThermoFisher: SPRCL5), Streptavidin (ThermoFisher: 12-4317-87), F4/80 (ThermoFisher: BM8), Ly6G/GR-1 (Biolegend: RB6-8C5), IgD (Biolegend: 11-26c.2a), CD79a (Biolegend: F11-172), CD25 (ThermoFisher: PC61), CD69 (Biolegend: H1.2F3), CD40L (BD Biosciences: MR1), TCRβ (H57-597), TLR/MD2 (ThermoFisher: UT41), MR-1 (NIH Tetramer Facility: 5-OP-RU), CD1d (NIH Tetramer Facility: PBS-57), CD11b (ThermoFisher: M1/70), CD11c (ThermoFisher: N418), FceR1 (ThermoFisher: MAR-1),

CD90.2 (ThermoFisher: 53-2.1), FOXP3 (ThermoFisher: FJK-16s), CD44 (Biolegend: IM7).

Gene expression analysis. mRNA was extracted from B and T cells using RNeasy Mini kit (QIAGEN) and was reverse-transcribed with the iScript cDNA synthesis kit (Bio-Rad Laboratories). Gene expression was measured using quantitative PCR (qPCR) in Power SYBR Green Master Mix (Thermo Fisher Scientific) or TaqMan Fast Advanced Master Mix (Applied Biosystems). Experiments were run on a QuantStudio 3 Real-Time PCR System (Applied Biosystems). The expression of genes was analyzed using the $2^{-\Delta\Delta CT}$ method in comparison to the housekeeping gene *Gapdh*. The following primers were used: *Xbp1-* *(*Fp: 5′- TCCGCAGCACTCAGACTATG -3′, Rp: 5′- ACTTGTCCAGAATGCCCAAA -3′), *sXbp1-* *(*Fp: 5′- CTGAGTCCGCAGCAGGTG -3′, Rp: 5′- ACTTGTCCAGAATGCCCAAA -3′), *Ddit3-* *(*Fp: 5′- CTGCCTTTCACCTTGGAGAC -3′, Rp: 5′- CGTTTCCTGGGGATGAGATA -3′), *Hspa5-* *(*Fp: 5′- CATGGTTCTCACTAAAATGAAAGG -3′, Rp: 5′- GCTGGTACAGTAACAACTG -3′), Germline IgG1- *(*Fp: 5′- CTCTGGCCCTGCTTATTGTTG -3′, Rp: 5′ GGCCCTTCCAGATCTTTGAG -3′), Mature IgG1- *(*Fp: 5′- CTCTGGCCCTGCTTATTGTTG -3′, Rp: 5′ GGATCCAGAGTTCCAGGTCAC -3′), *Prdm1-* *(*Fp: 5′-CAGCAAAGAGGTTATTGGCGT -3′, Rp: 5′ - GTTGCTTTCCGTTTGTGTGAGA -3′), *Ccta-* (Fp: 5′- GATGAGCTAACGCACAACTTCAA -3′, Rp: 5′- GTGCTGCACGGCGTCATA -3′), *Gapdh-* *(*Fp: 5′- TCACCACCATGGAGAAGGC -3′, Rp: 5′- TGCACCACCAACTGCTTAGC -3′). *Ifng-* (Mm01168134_m), *Dock8-* (Mm00613802_m1), *Gapdh-* (Mm99999915_g1).

Cell stimulation. Splenic B and T cells were isolated using the CD43 (Ly48) or Pan T cell microbeads (Miltenyi), respectively then cultured in RPMI supplemented with 10% fetal bovine serum, 50,000 IU penicillin, 50,000 µg streptomycin, 10 µM HEPES, 2 mM Glutamine, and 2-ME. B cells were seeded at 0.5 x 10^6 cells/mL in 5 mL culture tubes and stimulated at 37°C for 72 h with LPS (InvivoGen, 10 µg/mL), and rec. muIL-4 (Miltenyi, 50 ng/mL). Supernatants and cells were collected and stored at −80°C for future analysis or stained for FACS. T cells or splenocytes were seeded at 1x10^6 cells/mL in 5 mL culture tubes and were then stimulated at 37°C for 72 h with anti-CD3+ anti-CD28 Dynabeads (ThermoFisher, bead-to-cell ratio of 1:1), or with 200 µg/mL OVA, respectively. Supernatants and cells were collected and stored at −80°C for future analysis or stained for FACS. Respective cultures were supplemented with 0.1 mM TUDCA (Millipore).

ELISA assays for immunoglobulins and cytokines. Supernatants from stimulated B cells were analyzed for IgG and IgG1 secretion (Southern Biotech). Serum samples or supernatants from mice were analyzed for levels of total IgG, IgG1 IgA, and IgM (Southern Biotech). T cell supernatants were analyzed for IFN-γ secretion (Biolegend). Serum samples from mice which were immunized with either TNP-KLH or TNP-Ficoll, were analyzed for TNP-specific antibodies. Serum samples were added to plates coated with either 10 µg/mL of TNP(25)-BSA or NP(8)-BSA (BioSearch Technology). Serum samples from mice which were co-housed with pet-store mice were analyzed for IFN-γ, IL-6, and TNF-α (Biolegend).

Confocal microscopy. Colocalization studies were performed using the Zeiss LSM800 system, which is equipped with the Zeiss Axio Observer Z1 Inverted

Microscope having a Plan-Apochromat 63x objective, the Airyscan confocal package with Zeiss URGB (488 and 561 nm) laser lines, and Zen 2.3 blue edition confocal acquisition software.

For quantitation of colocalization, ten fields of cells were examined, with each field typically containing about 5 cells. Images were imported into the NIH Image J version 1.50e software, and then analyzed through a plugin software (https://imagej.net/Coloc_2). Colocalization values were then calculated by the software and expressed as the fraction of protein of interest colocalized with an organelle marker.

For B cell confocal, 200,000 cells were fixed with 4% formaldehyde diluted in warm PBS for 15 min at room temperature. Samples were then blocked for 60 min at room temperature in 1x PBS/5% normal serum/0.3% Triton™ X-100 (Sigma). Primary antibodies were diluted in 1x PBS/1% BSA/0.3% Triton™ X-100 and were incubated with samples overnight at 4°C. Samples were then incubated with fluorescent secondary antibodies for 2 hours at room temperature and applied to slides by Cytospin before imaging.

Immunoblotting. Cells were lysed in PBS that contains 30mM Tris-HCl pH 7.5, 120mM NaCl, 2mM KCl, 1% Triton X-100 and 2mM EDTA supplemented with a protease inhibitor (Roche). Proteins were separated by electrophoresis on 4-15% precast polyacrylamide gels (Bio-Rad) and were transferred to 0.45 μm nitrocellulose membrane (Bio-Rad). Membranes were blocked in a 1x solution Tris-Buffered Saline/Tween 20 (TBST) with 5% nonfat dry milk for one hour at room temperature and then incubated overnight at 4°C with the specified primary antibody. Antigen-antibody complexes were visualized with peroxidase-conjugated secondary antibodies (GE

Healthcare) and ECL Western blotting substrate (Pierce). Densitometry of immunoblots was done using the ImageJ analyzer software (1.48v).

Primary antibodies for immunoblot used were as follows: γ-COP (Santa Cruz, sc-30092), β-actin (Cell Signaling, 3700), β-COP (Abcam, 2899), BiP (Cell Signaling, 3177), DOCK8 (Santa Cruz, H-159), Calnexin (MA3-027), Calreticulin (D3E6), PDI (C81H6), GM130 (BD Biosciences, #610822). Mouse antibodies against β-COP (M3A5), coatomer (CM1A10), and the Myc epitope (9E10), and rabbit antibodies against giantin and the Myc epitope have been described previously (1, 2). Rabbit antibody against ARFGAP1 was obtained (Abcam #71759). All secondary antibodies were obtained from Jackson ImmunoResearch Laboratory.

Sanger sequencing. Sanger sequencing was used to validate the missense mutation in the *COPG1* gene identified by WES in the affected patients. Amplification and sequencing primers were made to amplify the nucleotide sequence surrounding the mutated base pairs in the *COPG1* gene (Fp: 5′- CATATCTATCCATCTAAGGTAGG -3′, Rp: 5′- CACCTCTTTCCTATTGACCAAG -3′, SFp: 5′- CTGTGGATTTCAGAGCAGTTG -3′, SRP: 5′- TTCTGTCATCAAGTTGGGCTC -3′).

T cell cytotoxicity. A primary one-way mixed lymphocyte reaction (MLR) was performed by mixing responder C57BL/6 (H-2^b) WT or *Copg1*^{K652E} mutant splenocytes and mitomycin-C treated (50 μg/mL for 20 minutes at 37°C) BALB/c (H-2^d) splenocytes. Responders and stimulators were cultured at 5 x 10^6 or 2.5 x 10^6, respectively, in 2 mL cultures in complete mouse media in 24-well tissue culture plates for 5 days at 37°C. Following the primary MLR, T cells were used in a 4 h allocytotoxicity reaction based on cytofluoremetry. P815 (H-2^d) and EL4 (H-2^b) cells were loaded with the intracellular dye,

CellTrace Violet (CTV) (ThermoFisher) at a low (0.1 μM) or high (5 μM) concentration, respectively. 10,000 of each CTV stained P815 and EL4 cells were added to effectors at varying effector:target ratios in a total of 200 μL in a 96-well V-bottom cell culture plate at 37°C for 4 h. Cytotoxicity was determined by FACS. $CD8^{-}B220^{-}CTV^{+}$ cells were gated on CTV and FVD and divided into four quadrants (Q1= Specific Dead, Q2= Bystander Dead, Q3= Bystander Live, Q4= Specific Live). Specific lysis was calculated as previously described[53] by the following equation: % specific lysis= 100 x [Q1/(Q1+Q4)] – [Q2/(Q2+Q3)].

NK cell cytotoxicity. NK-mediated cytotoxic lysis of YAC-1target cell was performed by a standard ^{51}Cr release assay(3). All mice were treated with 100 μg of poly I:C via *i.p.* injection 16-24h before sacrifice.

COPI vesicle reconstitution system. The two-stage incubation system has been described previously (1, 2). Briefly, Golgi membrane (100 μg) was washed with 3M KCl at 4°C for 5 minutes to release endogenous level of ARF1 and coatomer on membrane. Washed membrane was then resuspended with reaction buffer (25 mM HEPES-KOH, pH7.2, 50 mM KCl, 2.5 mM magnesium acetate, 1 mg/mL soybean trypsin inhibitor, 1 mg/mL BSA, and 200 mM sucrose). For Stage I, Golgi membrane was incubated with ARF1, coatomer, and GTP at 37ºC for 15 minutes. Reaction was stopped on ice for 5 minutes, followed by centrifugation at 12,000 x g for 10 minutes to pellet the Golgi membrane. The pelleted Golgi membrane was then resuspended in reaction buffer. Stage II was then performed by incubating the resuspended Golgi membrane with ARFGAP1 and BARS at 37ºC for 20 minutes. Reaction was then stopped on ice for 5 minutes, followed by centrifugation at 12,000 x g for 10 minutes to

pellet the Golgi membrane. The fate of coatomer after each stage of incubation was tracked by immunoblotting for β-COP after each centrifugation.

Tissue histology. Spleen specimens were fixed in 4% PFA and embedded in paraffin. H&E staining was performed in 4 μM sections.

Tissue immunofluorescence. Spleens from mice immunized with TNP-KLH were fixed in 4% PFA and embedded in paraffin. 4 μM sections were deparaffinized with xylene and rehydrated using decreasing concentrations of ethanol in water. Antigen unmasking was done by incubating the slides in boiling 10 mM citric acid (pH 6.0). Samples were blocked with 10% goat serum, then incubated with a fluorochrome-conjugated antibody against GL7 (GL7) from BioLegend was used. After washing 3 times with PBS, stained sections were mounted with ProLong Diamond Antifade Mountant (Thermo Fisher Scientific). Images were acquired on a Zeiss LSM800 confocal microscope.

Electron microscopy. A pellet of cells was fixed in 2.5% Glutaraldehyde 1.25% Paraformaldehyde and 0.03% picric acid in 0.1 M sodium cacodylate buffer (pH 7.4) for 2 hours at room temperature, washed in 0.1M cacodylate buffer and postfixed with 1% Osmiumtetroxide (OsO_4)/1.5% Potassiumferrocyanide($KFeCN_6$) for 1 hour, washed 2x in water, 1x Maleate buffer (MB) 1x and incubated in 1% uranyl acetate in MB for 1 hour followed by 2 washes in water and subsequent dehydration in grades of alcohol (10min each; 50%, 70%, 90%, 2x10min 100%). The samples were then put in propyleneoxide for 1 hour and infiltrated overnight in a 1:1 mixture of propyleneoxide and TAAB (TAAB Laboratories Equipment Ltd, https://taab.co.uk). The following day the samples were embedded in TAAB Epon and polymerized at 60°C for 48 hours. Ultrathin sections

(about 60nm) were cut on a Reichert Ultracut-S microtome, picked up on to copper grids stained with lead citrate and examined in a JEOL 1200EX Transmission electron microscope or a TecnaiG² Spirit BioTWIN and images were recorded with an AMT 2k CCD camera.

Transcriptome analysis. RNA was purified from PBMCs using the RNeasy Mini Kit (Qiagen). cDNA was then synthesized from 10 ng of total RNA using SuperScript™ VILO™ cDNA Synthesis Kit (ThermoFisher Scientific). Barcoded libraries were prepared using the Ion AmpliSeq Transcriptome Human Gene Expression Kit as per the manufacturer's protocol and sequenced using an Ion S5^{TM} system. Differential gene expression analysis was performed using the AmpliSeqRNA plugin (ThermoFisher). Pathway analysis was done using Ingenuity Pathway Analysis (Qiagen) on genes with at least 1.5-fold difference between.

LC3 immunoblot. B cells purified from the spleens of WT and mutant mice as described in the main text were cultured with or without LPS (20ug/mL) or chloroquine (100uM) for 3 days at 37°C. The cells were lysed and immunoblotted for LC3A/B (Cell Signaling), as described in the Methods section of the main text.

Blood chemistry analysis. Serum insulin was measured by ELISA (ThermoFisher). Blood glucose levels were measured using a standard glucometer. ALP and ALT levels in the blood were measured using the Vetscan VS2 (Abaxis).

LCMV infection. 8-12 -week-old mice were injected *i.v.* with $4x10^6$ PFU of LCMV clone 13 (a gift from John Wherry, University of Pennsylvania) once on Day 0 of the experiment. Mice were sacrificed 55 days post-infection.

References

1. Yang JS, Lee SY, Gao M, Bourgoin S, Randazzo PA, Premont RT, et al. ARFGAP1 promotes the formation of COPI vesicles, suggesting function as a component of the coat. *J Cell Biol.* 2002;159(1):69-78.
2. Yang JS, Lee SY, Spano S, Gad H, Zhang L, Nie Z, et al. A role for BARS at the fission step of COPI vesicle formation from Golgi membrane. *EMBO J.* 2005;24(23):4133-43.
3. Wonderlich J, Shearer G, Livingstone A, Brooks A, Soloski MJ, and Presby MM. Induction and Measurement of Cytotoxic T Lymphocyte Activity. *Curr Protoc Immunol.* 2018;120:3 11 1-3 29.