Supplemental Digital Content 1.

[Supplementary Materials and Methods]

**Cecal ligation and puncture (CLP)**

Briefly, mice were anesthetized with isoflurane (induction 5% and maintenance 2%). After cecum was exposed with a 1cm midline incision, cecum (1cm distance from the end) was ligated and added two punctures with 23G needle. Skin and peritoneum were closed separately with 3-0 silk brade. After the procedure, 1mL of 0.9% saline was administered subcutaneously. No antibiotics and analgesics were given. Mice were free access to only water.

**Surface marker staining**

In brief, centrifuged pellet of lymphocytes were stained with the following surface antigen markers for 15 minutes on ice. Then, washed with PBS, and resuspended in flowcytometry buffer (10%NaN3, fetal calf serum, PBS). Surface marker antibodies were purchased from the following manufactures: CD4/PE (BD Biosciences, San Jose, CA, USA), CD4/APC (eBioscience. San Diego, CA, USA), CD8/APC (Invitrogen, Carlsbad, CA, USA), B220/APC (BD Biosciences), B220/PE (BD Biosciences).

**Apoptosis assay**

For apoptosis assay, cells were stained with Annexin V/FITC detection kit (Bender Medsystems, Vienna, Austria) and Propidium Iodide (Bender Medsystems) according to the manufacturer’s protocol. Briefly, lymphocytes were stained with Annexin V for 15 minutes in room temperature. Then, they were diluted in binding buffer.

**Intracellular p62 staining**

Intracellular p62 staining was performed using Foxp3/Transcription Factor Staining Buffer Set (eBioscience) to permeabilize cells after surface staining. After washing, cells were incubated with p62 antibodies (MBL, Nagoya, Japan) on ice for 15min, followed by secondary antibodies (Anti-rabbit FITC antibody, Jackson immunoresearch, West Grove, PA, USA) on ice for 15 min.

**Mitochondrial and lysosomal staining**

Splenocytes were suspended in RPMI1640 medium (Sigma Aldrich) and stained at 37°C for 30 min with Mitotracker FM green (Invitrogen) or Mitotracker CMGR to assess mitochondrial mass or membrane potential (MMP) respectively. For lysosomal staining, splenocytes were stained at 37°C for 60 min with LysoTracker DND-99. Then, the samples were stained with CD4-PE, CD8-APC, or B220-APC. The samples were analyzed with the FACS caliber (BD Biosciences).

**Western blotting**

Total proteins were extracted from the sorted CD4+ T cells. Sample preparation and western blotting were performed as previously described [1]. The following antibodies were used: rabbit polyclonal anti-LC3B (Sigma-Aldrich, 1:1000), mouse monoclonal anti-GAPDH (Abcam, Cambridge, UK, 1:1000), rabbit polyclonal anti-p62 (MBL, Nagoya, Japan, 1:1000), goat anti-rabbit IgG (Jackson Immuno Research,; 1:5000), goat anti-mouse IgG (Jackson Immuno Research; 1:5000). Band images were scanned and densitometric analysis was performed by NIH Image software (Bethesda, MD, USA).

**Genomic DNA polymerase chain reaction (PCR)**

Total DNA was extracted from sorted CD4+ T cells, CD8+ T cells, and B cells. Amplification of genomic DNA was decided using the following primers: *ATG5 flox,*5'- GAATATGAAGGCACACCCCTGAAATG-3', 5'- ACAACGTCGAGCACAGCTGCGCAAGG-3', and 5'- GTACTGCATAATGGTTTAACTCTTGC-3'; *ATG5 deleted allele,* 5'- CAGGGAATGGTGTCTCCCAC -3'and5'-

GTACTGCATAATGGTTTAACTCTTGC-3'; *CD4-Cre,* 5'- TCGACCAGTTTAGTTACCC -3'and5'-

AGGTTCGTTCACTCATGGA -3';

**Real-time quantitative reverse transcription polymerase chain reaction (PCR)**

Total RNA was extracted from sorted CD4+ T cells using RNeasy Mini Kit (Qiagen Inc., Valencia, CA, USA), and single-stranded cDNA was synthesized with SuperScript VILO cDNA Synthesis Kit (Life Technologies, Grand Island, NY, USA). The expression of several genes which are related to apoptosis and cytokine production was determined by quantitative real-time PCR with the cDNA, using a KOD SYBR PCR Master Mix (TOYOBO, Osaka, Japan) and the following specific primers: *BIM,*5'- CGACAGTCTCAGGAGGAACC-3' and 5'- CCTTCTCCATACCAGACGGA-3'; *BCL2,* 5'- CTCGTCGCTACCGTCGTGACTTCG -3'and5'-

CAGATGCCGGTTCAGGTACTCAGTC-3'; *PDCD1,* 5*'*-CGTCCCTCAGTCAAGAGGAG-3*'* and 5'- GTCCCTAGAAGTGCCCAACA-3'; *IL10,* 5'-CCAGTTTTACCTGGTAGAAGTGATG-3' and 5'-TGTCTAGGTCCTGGAGTCCAGCAGACTC-3'; *IL2,* 5'- GGAGCAGCTTTGATGGACCTAC -3' and 5'- AATCCAGAACATGCCGCAGAG -3'; *IFNγ,* 5'-TCAAGTGGCATAGATGTGGAAGAA-3' and 5'-TGGCTCTGCAGGATTTTCATG-3'; *GAPDH*, 5'- TGTGTCCGTCGTGGATCTGA-3' and 5'- TTGCTGTTGAAGTGCAGGAG -3'. The reaction was run on the StepOne Real-Time PCR System (Life Technologies). The mRNA levels were measured by the relative standard method.

**Electron microscopic analysis**

Separated CD4+ T cells with the above cell sorting procedure were fixed with 4% paraformaldehyde (PFA), 2.5% glutaraldehyde in sodium phosphate buffer (0.1M, pH 7.2) overnight at 4°C, and post-fixed with 1% OsO4 in distilled water for 1hr at room temperature. Prior to the dehydration session, lymphocytes were embedded in 2% ager for collection. After dehydration through graded ethanol, the samples were embedded in Epon 812. Ultrathin sections were cut with an ultramicrotome (Ultracut E; Reihert-Jung, Vienna, Austria), stained with aquenous uranyl acetate and lead citrate, and observed in a transmission electron microscope (JEOL 1200 EX, Tokyo, Japan) at an acceleration voltage of 80kV. Images for lymphocytes in CLP and sham operated mice were randomly selected.

Autophagosomes were defined as double membrane structures that enclosed cytoplasm with damaged organelles in various stages of degradation. Autolysosomes were also defined as single membrane vesicles with cytoplasmic or organellar debris in various stages of degradation.

**Cytokine secretion assay**

CD4+ T cells were separated from splenocytes in sham and CLP-operated mice with the above procedure. Then, sorted CD4+ T cells were incubated with anti-CD3 antibody (Cedarlane, Ontario, Canada) and anti-CD28 antibody (southern Biotech, Birmingham, AL, USA) in 24-wells for 24 hours. Supernatants were collected from each well, and then measured IL-2, IFN-γ, and IL-10 concentrations by enzyme linked immune sorbent assay (ELISA) according to the manufacturers’ protocol (Mouse Quantikine ELISA Kit, R&D Systems, Minneapolis, MN, USA). iMARK (Bio Rad) and accompanying Microplate manager software6 were used for data analysis.

[Supplementary Figure Legends]

Supplemental Figure 1: Electron microscopic examination for autophagic structures in CLP treated CD4+ T cells. Harvested splenocytes from12 mice in the CLP group were sorted with magnetic beads. Selected lymphocytes in CLP operated mice were documented and observed. Representative figures are shown. *Upper Left:* a single double-membrane bound structure with membrane/organellar debris within, adjacent to a less well defined vesicle (a black arrow). Remarkable mitochondrial damage was observed (a black arrow head). *Lower left:* There were two vesicles (one incomplete) with internal membrane debris - a possible autophagosome (a black arrow). *Upper Right:* Two structures consistent with heterolysosomes/autolysosomes (white arrows), with electron dense material, but no residual organellar structure. Remarkable mitochondrial damage was observed (a white arrow head). *Lower Right:* Prominent dilation of endoplasmic reticulum and a possible autophagosome (a white arrow) adjacent to the nucleus were recognized.

Supplemental Figure 2: MFI ratio between Mitotracker Red/ Mitotracker FM green. The MFI of Mitotracker Red was normalized with mitochondrial mass (i.e. Mitotracker FM green). Raw data are plotted in the Figure. Since mitochondrial mass was different in each sample, we normalized with the amount of mitochondrial mass as previously described [2]. Data are expressed as mean and SD; n = 6-8 mice in each group; # p<0.05 was significance analyzed by student-t test (CLP-operated Atg5f/f miceversus CLP-operated CD4-Cre/Atg5f/f mice).

Supplemental Figure 3: A: IL-4 secretion of murine splenic lymphocytes. After lymphocytes were harvested from spleen, they were stimulated with anti- CD3antibody and anti-CD28 for 24 hours. Then, we measured IL-4 concentrations in the supernatant fluid of incubated lymphocytes by ELISA. Data are expressed as mean and SD; n = 8-9 mice in each group; # p<0.05 was regarded as significance analyzed by two-way ANOVA and student-t test. B: Relative RNA expression for IL-4 gene in sham-, CLP-operated Atg5f/fandsham-, CLP-operated CD4-Cre/Atg5f/f mice. Total RNA in CD4 + lymphocytes was extracted from experimental mice at 24hr after the procedure, and then relative RNA expression of *IL4* were analyzed. Data are expressed as mean and SD; n = 8-10 mice in each group; # p<0.05 was regarded as significance analyzed by two-way ANOVA and student-t test.

[REFERENCES]

1. Takahashi W, Watanabe E, Fujimura L, Watanabe-Takano H, Yoshidome H, Swanson PE, Tokuhisa T, Oda S, Hatano M: **Kinetics and protective role of autophagy in a mouse cecal ligation and puncture-induced sepsis**. *Crit Care* 2013, **17**:R160.

2. Weiss SL, Selak MA, Tuluc F, Perales Villarroel J, Nadkarni VM, Deutschman CS, Becker LB: **Mitochondrial dysfunction in peripheral blood mononuclear cells in pediatric septic shock**. *Pediatr Crit Care Med* 2015, **16**:e4-e12.