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Supplemental Information

An Essential Role for ECSIT in Mitochondrial Complex I Assembly and Mitophagy in Macrophages

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Supplemental Figures

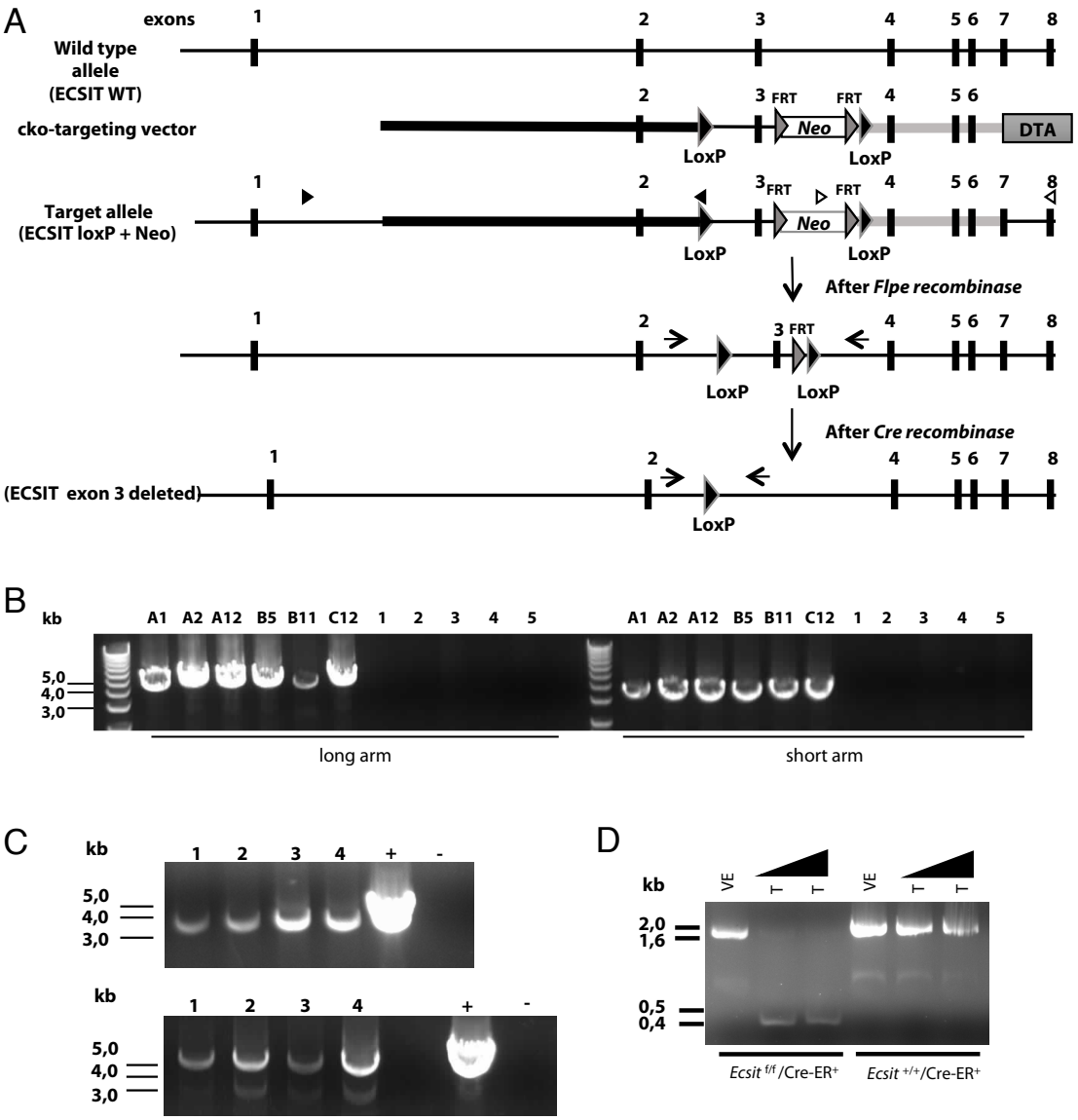

Figure S1. Construction of *Ecsit* conditional knockout mouse (cKO). ***Related to Figure 1.***
(A) Schematic representation of *Ecsit* cKO mouse generation. Exon 3 was flanked by 2 loxP sites and excised after conditional Cre expression. Thick black line: long arm; thick grey line: short arm. (B) Identification of correctly targeted embryonic stem (ES) cell clones by PCR. Correctly targeted ES cells (cKO allele) generated a 4.8 kb band (long arm recombination) and 3.7 kb band (short arm recombination). A1, A2, A12, B5, B11, and C12: ES clones. 1 to 5: PCR negative controls; templates used: 1- no DNA added; 2- cKO-targeting vector; 3- cKO-targeting vector in addition to WT black 6 mouse genomic DNA; 4- cKO-targeting vector in addition to WT black 6 ES cell lysate; 5- WT black 6 mouse genomic DNA. (C) Identification of *Ecsit* conditional allele in F1 line. Transmission through the chimeras germline to F1 was checked by PCR. DNA was extracted from the tails and used for amplification. Top, detection of the short arm (3.7 kb); Bottom, detection of the long arm (4.8 kb). 1-4: samples from 4 independent pups; + (Positive control): genomic DNA obtained from positive ES cell used as a template; - (Negative control): genomic DNA from WT black 6 mouse used as a template**.** (D) Detection of ECSIT deletion by PCR in Cre-ERT2^{+}/ECSIT$^{f/f}$ and E Cre-ERT2^{+}/ECSIT$^{+/+}$ mice. Bone marrow derived macrophages were treated for 7 days with 3 doses (days 1, 3 and 6 of differentiation) of 100 nM or 500 nM tamoxifen (T) or vehicle (VE). DNA was prepared at day 7 and tested by PCR. FRT: *DNA recognition site for Flpe* recombinase; DTA: diphtheria toxin A chain gene.

VE: vehicle; T: Tamoxifen treatment. Black arrow heads and open arrow heads: primers alignment positions for long arm and short arm amplifications by PCR, respectively. Arrows: primers to detect exon 3 deletion after Cre recombination.

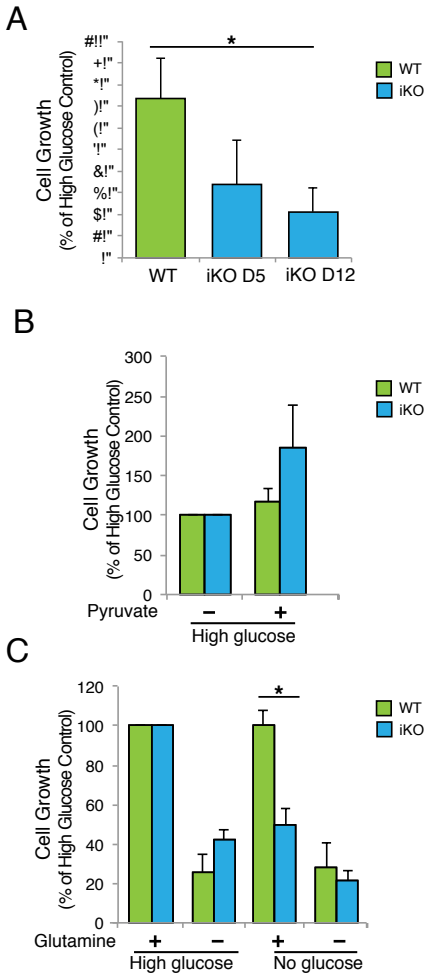

Figure S2. Metabolic adaptations in macrophage cells lacking ECSIT. ***Related to Figure 2.***
(A) Assessment of cell growth by crystal violet staining of IBMM 5 or 12 days after tamoxifen treatment. IBMM cultures were treated with 40 mM Sodium Oxamate for 24 h (n=3). (B, C) Cell growth by crystal violet staining of IBMM 12 days after Tamoxifen treatment initiation and maintained for 48 h in DMEM without or with 25 mM Glucose, 2 mM Sodium Pyruvate, 4 mM L-Glutamine (n=3). Means +/- SD of n experiments. *: $p<0.05$ in T-test.

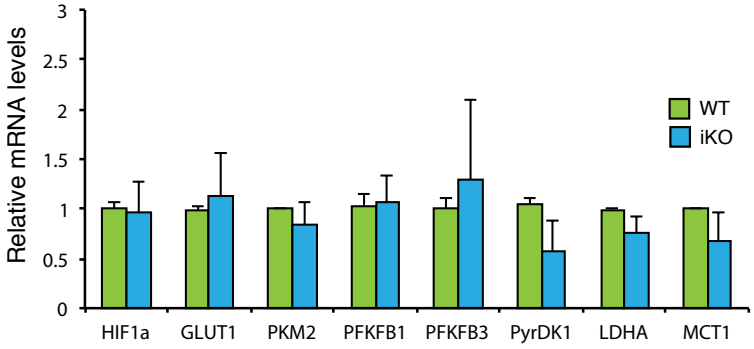

Figure S3. Metabolic shift in ECSIT-deleted macrophages is independent of transcriptional changes. ***Related to Figure 2***

Expression of transcripts relevant to the metabolic shift observed in ECSIT-deleted macrophages analyzed by RT-qPCR in WT and iKO IBMM 7 days after deletion induction by Tamoxifen, HPRT used as internal control.

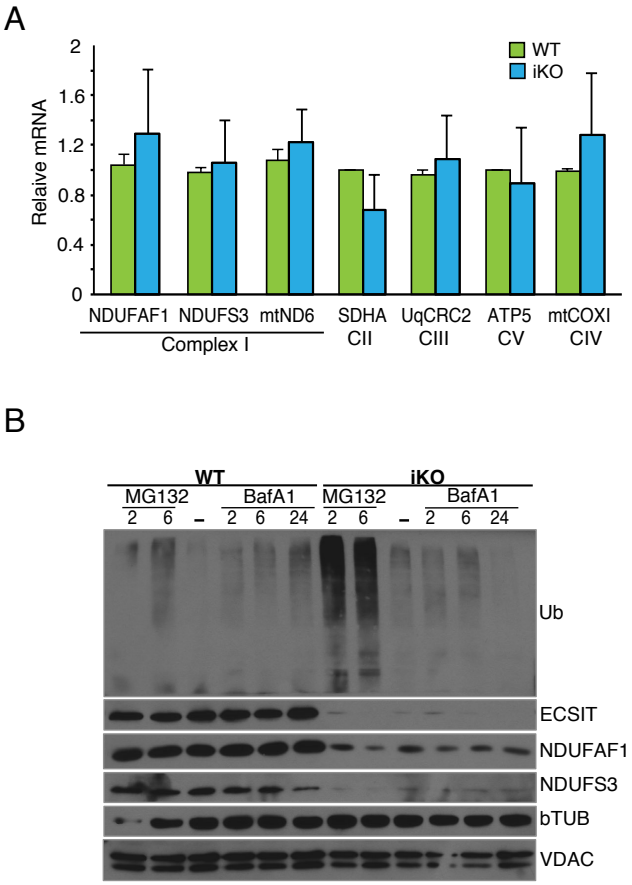

Figure S4. Loss of Complex I subunits in ECSIT-deleted macrophages is independent of transcriptional regulation and proteasomal and lysosomal degradation pathways. ***Related to Figure 4***

(A) Complex I subunits expression analyzed by RT-qPCR in WT and iKO IBMM 7 days after deletion induction, HPRT used as internal control. (B) WT or iKO macrophages treated with 10 µM MG132 or 10 nM Bafilomycin A1 (BafA1) for indicated time in hours. Total cell lysates were fractioned by SDS-PAGE and subjected to western blot analysis using antibodies against Ubiquitin (Ub), ECSIT, NDUFAF1, NDUFS3, beta Tubulin (bTUB) and VDAC1.

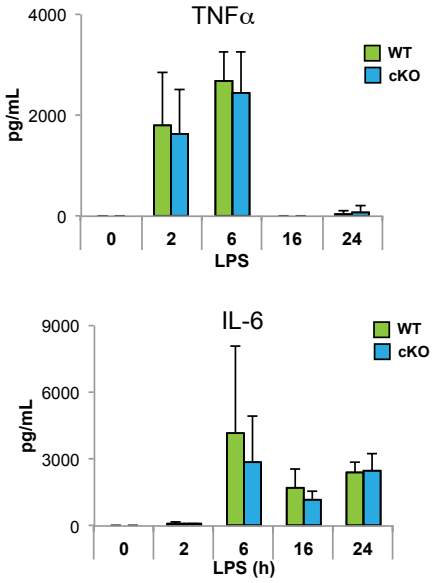

Figure S5. LPS response in macrophages lacking ECSIT. ***Related to Figure 5***
ELISA titration of IL-6 and TNFα release by cultured BMDM of the indicated genotype, following stimulation with 100 ng/mL LPS for the indicated times in hours.

Supplemental Experimental Procedures

Experimental Model Details

Mice

Animals used for experiments were age- (8-12 weeks old) and sex-matched and were bred and housed under standard conditions in accordance with Columbia University Institutional Animal Care and Use Committee policies. All mouse protocols were approved by Columbia University. The *Ecsit*$^{f/f}$ mouse was generated following the methods explained in corresponding Method Details section below and backcrossed into C57BL/6 background and then bred to Ecsit$^{+/-}$ (Xiao et al., 2003) and LysM-Cre (The Jackson Laboratory) or Rosa26Cre-ERT2 (from Dr. B. Reizis, made by Dr. T. Ludwig (unpublished)) mouse strains to produce Ecsit$^{f/-}$/LysM-Cre^{+} and Ecsit$^{+/+}$/LysM-Cre^{+} or Ecsit$^{f/f}$/Cre-ERT2^{+}, respectively.

Cell lines and primary cell cultures

L929 cells were obtained from American Type Culture Collection (ATCC CCL-1) and are of male origin. 293FT cells were obtained from ThermoScientific (R70007) and are suggested to be of female origin. ψCREJ2 cells (a kind gift of KA Fitzgerald) were used to produce J2 v-myc/v-raf transforming retrovirus (Xiao et al., 2012) (Blasi et al., 1987). Cell lines were plated in tissue culture dishes and maintained in standard culture media composed of DMEM (Gibco) medium supplemented with 10 % fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (100 µg/mL).

L929 conditioned media was prepared from the supernatant of a 7-day culture of L929 cells. Harvested supernatant was clarified by centrifugation at 1500 rpm for 5 min and filtered. Bone marrow derived macrophages (BMDM) were harvested from 8-12-week old ECSIT$^{f/-}$/LysM-Cre^{+} (cKO) and ECSIT$^{+/+}$/LysM-Cre^{+}(WT) or ECSIT$^{f/f}$/Cre-ERT2^{+} littermates, plated and cultured on 15 cm Petri dishes for a period of 7 days in DMEM containing 10 % FBS, penicillin (100 U/mL) and streptomycin (100 µg/mL) plus 30 % L929 conditioned media. On day 7, cells were lifted from plates by incubation in PBS containing 1 mM EDTA for 5 minutes at 4°C and replated in multi-well plates

depending on the experiment. BMDMs from ECSIT$^{f/f}$/Cre-ERT2^{+} mice were treated with 4OH-Tamoxifen (500 nm) or ethanol vehicle on days 1, 3 and 6 of differentiation and plated for experiments at day 7. Thioglycollate elicited peritoneal macrophages were prepared as previously described (Rao et al., 2010). In summary, 2 ml of 3 % thioglycollate was injected into the peritoneal cavity and, after 3 days, peritoneal exudates were collected in cold PBS. Cells were washed twice in PBS and suspended in RPMI 1640 10 % FBS with penicillin (100 U/ml) and streptomycin (100 µg/ml) and allowed to rest overnight.

Macrophage immortalization was performed by infection of BMDM from an ECSIT$^{f/f}$/Cre-ERT2^{+} male mouse with J2 recombinant retrovirus according to Blasi et al., 1989 (Blasi et al., 1989). Briefly, 0.45-micron-filtered supernatants from cultured ψCREJ2 cells were added to harvested BMDMs at Day 1 for 16-24 h. Cells were washed with PBS and allowed to rest for an additional 16-24 h. Then, a second round of infection was performed. Cells were then maintained in culture for a few weeks in DMEM containing 10% FBS, penicillin, streptomycin and 30 % of L929 conditioned media and monitored for growth. Immortalized bone marrow derived macrophages (IBMM) from ECSIT$^{f/f}$/Cre-ERT2^{+} mouse were treated with 500 nM of 4OH-tamoxifen (Sigma H6278) (iKO) or vehicle (100 % Ethanol) (WT) for 48 h, washed away and cultured for 5 days prior to testing. For cell growth assay, cells were analyzed 5 or 12 days after tamoxifen treatment initiation. IBMM transduced with Tet inducible vector were cultured with 10 % Tet System approved FBS (Takara) instead of standard FBS.
Relevance of the use of IBMM was tested in some assays performed in both BMDM and IBMM. When indicated, IBMM were cultured in restricted culture media as described in Method Details below.

All cells were cultured in a humidified incubator at 37°C in a 5 % CO_2 atmosphere.

Method Details

***Ecsit* conditional knockout mouse**
To examine the effect of ECSIT deficiency *in vivo*, we have generated *Ecsit* conditional knockout animals (cko or *Ecsit*$^{f/f}$) by flanking exon 3 with 2 *LoxP* sites, through a method for constructing cko-targeting vectors using *E. coli* recombineering (Liu et al., 2003) (Fig S1A). After conditional Cre expression, a 1.3 kb-fragment of the *Ecsit* gene that includes exon 3 was deleted. The junction of exon 2 and 4 generated a premature STOP codon such that only a truncated protein consisting of the first 32 amino acids of ECSIT should be produced upon Cre mediated deletion. The *E. coli* SW106 strain (Liu et al., 2003) was transformed with a BAC encompassing the *Ecsit* gene and used to perform all the homologous recombination steps to introduce the floxed Neomycin resistance (*NeoR*) cassette into *Ecsit* gene. This strain can efficiently carry out homologous recombination between short terminal homology regions on a linear PCR-derived DNA fragment and sequences on a recipient DNA. The first recombination step was performed using a linear DNA fragment generated by PCR containing short terminal homology regions (60 bp) introduced by the primers LNeoL-intron3F and LNeoL-intron3R (table below) and the vector pL452 (Liu et al., 2003) as a template. The *NeoR* gene was subsequently removed via Cre recombinase. The removal of the floxed *NeoR* gene leaved a single *loxP* site at the target locus. The following step was the introduction of the second *loxP* site into the BAC. We used the cassette from pL451 vector that contains a FRT site upstream of *NeoR*, and *FRT* and *loxP* sites downstream of *NeoR* (Liu et al., 2003) and the primers FNeoFL-intron4F and FNeoFL-intron4R (table below) to generate the linear DNA fragment by PCR. The last step before the electroporation of the modified *Ecsit* fragment into the ES cell was the generation of the final cko-targeting vector containing the two flanking homology arms (long arm and short arm) for homologous recombination into the ES genome. This vector was generated through homologous recombination between the PCR product using the primers Ret-left1-ECSIT and Ret-right1-ECSIT (table below) and the vector pMCS-DTA (gift from Dr. Kosuke Yusa, Wellcome Trust Sanger Institute, (Yusa et al., 2011)) as a template and the BAC containing the floxed *Ecsit* exon 3. The cko-targeting vector was subsequently linearized and electroporated into 129B6 hybrid ES cells (Transgenic mouse facility at Columbia HICCC). Homologous recombination between cko-targeting vector and the ES genome was checked by PCR (Fig S1A,B). The primers long-arm2F and L83loxP (table below) to check the long arm integration were designed to anneal into the first *loxP* site and outside of the recombination arm, generating a DNA fragment of 4.8 kb. The second pair of primers ARM2/F2 and short-arm1R, to check the short arm integration, targeted a region inside of the *NeoR* gene and outside of the recombination arm and amplify a DNA fragment of 3.7 kb (Fig S1A,B). Chimeras were mated to C57BL/6 to test for germline transmission. Germline transmission was checked by PCR using the same pairs of primers used to check the positive ES cells (Fig S1C). DNA located between the two *FRT* sites was

removed by breeding these mice to a mouse strain expressing *Flpe* (ACTB-FLPe, from Jackson Laboratory). In this case, single *FRT* and single *loxP* sites were left behind at the targeted locus. To verify the success of the deletion strategy, ECSIT cko mice were crossed with Cre-ERT2 mice, in which Cre recombinase activity can be induced by tamoxifen, to excise the entire DNA between the *loxP* sites located on either side of *Ecsit* exon 3. Exon 3 deletion was checked by PCR using primers ECSIT-intron3F and ECSIT-intro4R (table below) generating a 425 bp-fragment after deletion and 1800 bp-fragment for the non-deleted gene (Fig S1A,D). ECSIT expression was successfully abrogated following *in vitro* tamoxifen treatment of $ECSIT^{f/f}$/Cre-$ERT2^{+}$ bone marrow-derived macrophages (BMDM) (Figure 1A, Fig S1D). We also crossed $ECSIT^{f/f}$ mice with LysM-Cre mice to delete ECSIT in the myeloid lineage. For more efficient deletion in primary macrophages, we bred $ECSIT^{f/+}$/LysM-Cre^{+} mice with $ECSIT^{+/-}$ heterozygous mice (Xiao, 2003), generating $ECSIT^{f/-}$/LysM-Cre^{+}, containing one KO allele and one floxed allele. $ECSIT^{+/+}$/LysM-Cre^{+} and $ECSIT^{f/-}$/LysM-Cre^{+} cells will be represented as WT and cKO, respectively.

Phenotyping of myeloid cells in mice with ECSIT deleted in macrophages

8-12 weeks old $ECSIT^{+/+}$/LysM-Cre^{+} and $ECSIT^{f/-}$/LysM-Cre^{+} were sacrificed by CO_2 asphyxia. Peritoneal cells were harvested with 5 mL PBS. 2 brachial and 2 inguinal lymph nodes were harvested, cut and incubated in digestion solution (20 µg/mL DNase I and 10 µg/mL Collagenase (Sigma-Aldrich) in DMEM (Invitrogen)) for 30 min at 37°C. Spleens were infused with digestion solution and incubated for 30-45 min at 37°C. Remaining tissues were further dilacerated on a 70 µm strainer, rinsed with cold PBS, 5 mM EDTA, centrifuged at 1500 rpm for 6 min, resuspended in Red Blood Cell lysis buffer (Sigma-Aldrich) and incubated at 37°C for 2 min. Lungs were dissected, cut and incubated in digestion solution for 30-45 min at 37°C. Remaining tissues were further dilacerated on a 70 µm strainer, rinsed with cold PBS, 5 mM EDTA, centrifuged at 1,500 rpm for 6 min, resuspended in 36 % Percoll (Sigma-Aldrich) in PBS and centrifuged at 2,500 rpm for 15 min at 20°C with slow acceleration and deceleration. Pellet was collected and resuspended in PBS, 5 mM EDTA, centrifuged and red blood cells were lysed as described above. One tibia bone was dissected and bone marrow cells flushed out with 2 mL PBS 3 % FCS, passed through a 70 µm strainer, rinsed with PBS 3 % FCS and red blood cells were lysed as described above. Centrifuged isolated cells were resuspended in PBS 3 % FCS and counted for cellularity. For phenotyping, around 3.10^{6} cells per staining were incubated with Fc blocking antibody 2.4G2 (1/2000, Tonbo) in PBS 3 % FCS for 1 h at 4°C. Cells were centrifuged and incubated with surface antibody: F4/80-FITC (1/100, eBiosciences, BM8), CD11b-PE (1/400, 1/700 for peritoneal cavity, eBiosciences, M1/70), Ly6G-VF405 (1/200, Tonbo, 1A8), CD3-PerCPCy5.5 (1/100, Tonbo, 145-2C11), B220-APC780 (1/200, eBiosciences, RA3-6B2) for 1h at 4°C. Cells were washed and resuspended in PBS 3 % FCS with 1 µg/mL DAPI (Sigma-Aldrich), to exclude dead cells. Stained cells were analyzed by flow cytometry on an LSRII and data analysis was performed using FlowJo software. Macrophages were defined as CD11b+, F4/80+ and monocytes as CD11b+, F4/80- in lineage negative (CD3-, B220-, Ly6G-) cells.

Cellular fractionation, mitochondrial isolation

iBMM and BMDM from 2-3 15 cm-Petri dishes were washed twice in PBS and detached in TEN buffer (40 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA) for 5 min at 4°C. Cells were centrifuged and cell pellets were re-suspended in ten pellet volumes of RSB buffer (10 mM NaCl, 1.5 mM $CaCl_2$, 10 mM Tris-HCl, pH 7.5), swelled on ice for 10 min, homogenized with a dounce homogenizer, then 2.5× MS buffer (525 mM mannitol, 175 mM sucrose, 12.5 mM Tris-HCl, pH 7.5, 12.5 mM EDTA) was added to 1× final buffer concentration. The homogenate was centrifuged three times at 980*g* for 10 min to pellet nuclei. The supernatant was transferred to a fresh tube, an aliquot taken for total lysate sample and remaining supernatant spun at 17,000*g* for 30 min to pellet mitochondria. Supernatant consisted of cytosol fraction. The mitochondrial pellet was washed three times with 1× MS buffer, by centrifugation at 13,000 rpm for 10 min.

Purified mitochondria proteins were then solubilized with specific detergents according to the different purposes or resuspended in 1× MS with 10 % glycerol, flash frozen in liquid nitrogen and stored at -80°C. For SDS-PAGE, mitochondrial pellets were re-suspended in SDS lysis buffer (20 mM Tris-HCl, 1% SDS, pH 7.5). Protein concentrations were determined using the microBCA protein kit (Thermo Scientific).

For complex I assay, mitochondrial protein extraction was performed according the manufacturer's instructions. For blue native gel, mitochondrial complexes were prepared as previously described using DDM detergent (dodecylmaltoside) (Wittig et al., 2006). Briefly, 400 µg of purified mitochondria were solubilized in 40 µL of 50 mM NaCl, 50 mM bis-tris HCl pH 7.0, 2 mM aminohexanoic acid, 1.0 mM EDTA, 2 % DDM, incubated on ice for 5 min and centrifuged for 20 min at 20,000g. Supernatants were collected and protein concentration determined. Samples were prepared by adding 4× native gel sample buffer (0.1 % Ponceau S, 50 % glycerol, (wt/vol)) to final 1×

concentration and 2.5 µL of additive (5 % (wt/vol) Coomassie blue G-250 (Serva) in 500 mM 6-aminohexanoic acid).

Blue Native Gel Electrophoresis and transfer and in-gel complex I activity assay
100-150 µg solubilized mitochondrial complexes and 5 µL Native marker (Invitrogen) were loaded on a NativePAGE 4-16 % Bis-Tris Protein gel (Invitrogen) and gels were run for 30 min at 30 V and then overnight at 80 V at 4°C according to Nijtmans et al (Nijtmans et al., 2002) and following the manufacturer's instructions. Anode buffer (50 mM Bis-Tris, 50 mM Glycine pH 7) and dark blue cathode buffer (anode buffer with 0.02% Coomassie Blue G-250) were replaced by light blue cathode buffer (anode buffer with 0.002% Coomassie Blue G-250) when sample buffer reached 1/3 of the gel. Gels were either stained for proteins using Imperial Protein Stain (ThermoScientific) for 1 h and destained overnight, or transferred for immunoblotting, or processed for in-gel activity. For immunoblotting, complexes were transferred to PVDF according to Diaz et al (Diaz et al., 2009). Gels were incubated in anode buffer with 0.1 % SDS for 15 min. Wet transfer was performed in transfer buffer (143 mM glycine, 19 mM Tris Base) with 10% Methanol and 0.05 % SDS overnight at 30 V at 4°C. Membranes were dried and destained quickly with methanol before immunoblotting. In-gel Complex I activity was performed according to Diaz et al (Diaz et al., 2009). Gel was washed 3 times in 0.1 M Tris pH 7.4 and then incubated in 0.1 M Tris, 1 mg/mL NBT (nitro blue tetrazolium), 0.14 mM NADH for 2 h. Gel was then destained in 25 % Methanol, 10% Acetic Acid in water overnight.

Lactate, NADH, Complex I and ATP assays
L-lactate was measured at indicated time points on cellular supernatants from macrophage cultures non stimulated or stimulated with E. *coli* LPS (100 ng/mL) using L-lactate assay Kit I (Eton Bioscience). For complex I NADH dehydrogenase activity, WT and ECSIT-deleted iBMM were treated with vehicle (70 % Ethanol) or chloramphenicol (Sigma-Aldrich) for 5 days at a final concentration of 20 µg/mL before harvesting cells. Crude mitochondrial extracts were then assessed using a Complex I Enzyme Activity Microplate Assay kit (Abcam). Total cell lysate was analyzed for NADH/NAD^+ ratio using NAD^+/NADH quantification colorimetric kit (BioVision). ATP levels were determined using the ATPlite™ Luminescence Assay System (Perkin Elmer). When indicated, cells were pretreated for 4 h with 50 mM 2-DG (2-deoxyglucose). ATP levels were normalized to cell numbers. All the measurements were performed according to the manufacturer's instructions.

ROS, mitochondrial mass and mitochondrial potential measurements
Cells were plated at 300,000 cells/well in non-treated 6-well plates and treated with E. *coli* LPS (100 ng/mL), Rotenone (1 µM), Antimycin A1 (5 µM) or vehicle (DMSO) for 20 min (Sigma-Aldrich). Culture medium was removed, cells washed with PBS, then incubated with MitoSOX (Invitrogen) to measure mROS superoxide or chloromethyl derivative of 2',7'-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) to measure total ROS at 2.5 µM final concentration in serum-free OPTIMEM (Invitrogen) for 15 to 30 min at 37°C. Cells were washed with warm PBS, removed from plates with TEN buffer (40 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA) by pipeting, pelleted and resuspended in cold PBS containing 1% FBS for FACS analysis. To analyze the mitochondrial potential and mitochondrial mass, IBMM and BMDM were seeded at 150,000 cells/well in a non-treated 12-well plate, treated with CCCP for 15mn (30 µM) and stained with 50 nM TMRM (Tetramethylrhodamine, Methyl Ester, Perchlorate, Invitrogen) or Mitotracker Green FM (Invitrogen) for 45 min at 37°C. For each condition, unstained controls were treated similarly, except that dyes were replaced by vehicle (DMSO). Mitochondrial ROS, potential and mass were analyzed by flow cytometry on a Fortessa or LSRII cytometer (BD). Data analysis was performed using FlowJo software and fluorescence from unstained controls was subtracted from respective stained samples. MFI: mean fluorescence intensity.

ELISA
Cells were plated at 300,000 cells/well in non-treated 6-well plates and treated with E. *coli* LPS (100 ng/mL) for indicated times. Supernatant were harvested and clarified and cytokine levels were assessed with mouse IL-6 and TNFα ELISA kits (BD Biosciences), following the manufacturer's protocol.

Cell growth
8,000 cells were plated in flat bottom 96-well plates in DMEM without glucose (Invitrogen) supplemented with 10 % FBS, 20 ng/mL M-CSF (R&D) and 25 mM glucose (Sigma, High Glucose condition) or 10 mM galactose (Sigma). 40 mM Sodium Oxamate (Sigma) was added to inhibit LDH. In other conditions, DMEM without glucose,

nor glutamine (Invitrogen) was used with addition of 25 mM glucose (Sigma, High Glucose condition), 2 mM Sodium Pyruvate (Invitrogen) or 4 mM L-Glutamine (Invitrogen).
24 or 48 hours later, cells were washed once with PBS, fixed in 4% PFA in PBS for 15 min, washed once with water and stained with 0.1 % crystal violet for 20 min. Cells were washed three times with water and allowed to dry. Retained crystal violet was solubilized in 100 % methanol and OD read at 595 nm.

RT-PCR and qPCR
Total RNA was prepared using Rneasy minikit (Qiagen) according to manufacturer's protocol and RNA purity and quantity were analyzed by photometry (Gen5 BioTek). For cDNA synthesis, 2 µg total RNA were reverse transcribed in cDNA using Superscript III enzyme and oligo dT (Invitrogen). Quantitative PCR (qPCR) was performed using SYBR Green (Quanta) and primers as listed in table below. Differences in cDNA inputs were corrected by normalization to HPRT cDNA levels. Relative quantitation of target cDNA was determined by the formula $2^{-\Delta CT}$, with ΔCT denoting fold increases above WT.
For mitochondrial mass determination, genomic DNA from 1-2. 10^6 cells was extracted using the DNeasy Blood and Tissue Kit (Qiagen), following the manufacturer's instructions. DNA concentrations were determined by photometry (Gen5 BioTek) and 15 ng, 7.5 ng and 3.75 ng DNA were used to perform qPCR for mitochondrial gene *mtCOI* and nuclear gene *ndufv1*. Ratios of 2^{-CT} for *mtCOI* over *ndufv1* for the different DNA concentrations were averaged and fold of WT is shown.

Respiration and glycolysis
For real-time analysis of ECAR and OCR, IBMM were analyzed with a XF-24 Extracellular Flux Analyzer following the manufacturer's instructions (Seahorse Bioscience). Briefly, cells were seeded at 60,000 cells/well 24 h before the assay. For OCR measurements, 1 h before analysis, media was changed to XF base medium (Seahorse Bioscience) containing 25 mM glucose (Sigma), 2 mM L-glutamine and 1 mM sodium pyruvate (Invitrogen). Five consecutive measurements were obtained under basal conditions. Mitochondrial respiration was further characterized after the sequential addition of 1 µg/mL oligomycin A1 (Santa Cruz Biotechnologies), to inhibit mitochondrial ATP synthase; 3 µM FCCP (fluoro-carbonyl cyanide phenylhydrazone) (Santa Cruz Biotechnologies), a protonophore that uncouples ATP synthesis from oxygen consumption by the electron-transport chain, and 100 nM rotenone (Sigma-Aldrich), which inhibits the electron transport chain. Three consecutive measurements were made after each sequential treatment. Instrumental background was measured in separate control wells using the same conditions without biological material. Metabolic rates were normalized to cell numbers.
In this assay, basal oxygen consumption can be established by measurement of OCR in the absence of drugs. A decrease in OCR after the addition of oligomycin and rotenone is expected and indicates that cells are consuming oxygen for mitochondrial oxidative phosphorylation. In particular, the difference between basal OCR and after oligomycin treatment reveals OCR used for ATP production by the ATP synthetase. OCR at the end of the sequence corresponds to non-mitochondrial OCR. Proton leak, dependent on the proton gradient across the mitochondrial inner membrane, is the difference between non-mitochondrial OCR and OCR when the electron-transport chain is blocked by oligomycin. Maximal OCR occurs after the addition of FCCP, which dissipates the proton gradient, leading to oxygen reduction independently of ATP production. SRC is calculated as the difference between basal OCR and maximal OCR after the addition of FCCP.

Similarly, for glycolysis characteristics analysis, media was changed to XF base medium (Seahorse Bioscience) containing 2 mM L-glutamine (Invitrogen) 1 h prior analysis. ECAR was measured after the sequential addition of 10 mM glucose to induce glycolysis, 1 µg/mL oligomycin to force cells to increase glycolysis to the maximal rate (glycolytic capacity), and 100 mM glycolysis inhibitor 2-DG. Glycolytic reserve is the difference between glycolytic capacity and glycolysis.

Analysis of protein degradation pathways
10^6 IBMM cells (6 days after with tamoxifen treatment initiation) were plated in 6-cm dish and 24 h later, were incubated with 10 µM MG132 (Sigma) or 10 nM Bafilomycin A1 (Sigma) for 2, 6 and 24 h. Cells were washed twice with PBS, lysed in Triton lysis buffer (1 % Triton X-100, 150 mM NaCl, 50 mM HEPES pH 7.5, 5 mM EDTA) with 1 mM DTT and protease inhibitors (1 mM PMSF, 1 µg/mL aprotinin, 10 µg/mL pepstatin and 1 µg/mL leupeptin) and incubated for 20 min on ice. Lysates were then clarified at 15,000 rpm for 15 min at 4°C. Total cell lysates were fractionated by SDS-PAGE and analyzed by western blot.

Lentivirus generation and IBMM transduction

FUW-TetON vector was modified from FUW-TetON-hOCT4, a gift from Rudolf Jaenisch (Addgene plasmid # 20726, Hockemeyer, 2008), to include AgeI and NheI sites. NDUFAF1 cDNA was amplified from mouse macrophages total cDNA using forward primer 5' ataaccggtgccaccatggcaatgtcttccattcac 3' and reverse primer 5' atagctagcctatctgaagagtcttgggttaag and cloned into FUW-TetON between AgeI and NheI sites. For inducible expression upon Doxycyclin treatment, plasmid FUW-M2rtTA, a gift from Rudolf Jaenisch (Addgene plasmid # 20342, (Hockemeyer et al., 2008)), was used. Viral stocks were produced by calcium phosphate transfection of 293FT cells with FUW-TetON, FUW-TetON-NDUFAF1 or FUW-M2rtTA in combination with packaging vectors psPAX2, a gift from Didier Trono (Addgene plasmid # 12260), and envelope pCMV-VSV-G (Addgene plasmid #8454). Medium of 70 % confluent 293FT in 75 cm^2 flasks was changed 2 h before transfection. Calcium phosphate precipitates were prepared by mixing 12.5 µg FUW plasmid with 12.5 µg psPAX2 and 5 ug pCMV-VSV-G in water for a final volume of 875 uL. 125 uL 2 M $CaCl_2$ and 1 mL HBS 2X (50 mM HEPES, 10 mM KCl, 280mM NaCl, 1.5 mM Na_2HPO_4, pH 7.05) were sequentially added dropwise in slowly vortexed solution. Solutions were incubated at RT for 20 min and mixed gently to 293FT supernatant. Medium was replaced by 7 mL of culture medium 24 h later. Supernatants were collected, centrifuged at 1,500 rpm for 5 min and filtered. 500,000 IBMM were transduced with 1mL FUW-TetON or FUW-TetON-NDUFAF1 in conjunction with 0.5 mL FUW-M2rtTA lentiviral vectors, 6 ug/mL polybrene (Sigma) and 10 mM HEPES (Invitrogen) by spinfection at 1,800 rpm 27°C for 1h 30min in non-treated 12-well plate. 1mL fresh medium was added and spinfection was repeated the following day. iBMM were subsequently cultured in the presence of 10 % Tet System approved FBS (Takara #631107) instead of standard FBS. 48 h later, transduced iBMM were selected with 300 ug/ml zeocin (Invivogen) for 4 days and maintained in 100 ug/mL zeocin after selection.

5 days after tamoxifen treatment initiation, 250-500,000 transduced iBMM were plated in 6-well plates and after 24 hrs treated with 300 ng/mL doxycycline (Sigma) for 24 hrs, washed twice in PBS, lysed in Triton lysis buffer (1 % Triton X-100, 150 mM NaCl, 50 mM HEPES pH 7.5, 5 mM EDTA) with 1 mM DTT and protease inhibitors (1 mM PMSF, 1ug/mL aprotinin, 10ug/mL pepstatin and 1 ug/mL leupeptin), incubated 20 min on ice, clarified at 15,000 rpm for 15 min at 4°C, and supernatants collected. Total cell lysates were fractionated by SDS-PAGE and analyzed by western blotting.

ECSIT expression plasmids

To build ECSIT-3CFLAG plasmid, murine ECSIT2 was cloned from ECSIT-FLAG plasmid (West et al., 2011) into p3XFLAG-CMV14 (Sigma-Aldrich) between KpnI and XbaI sites using forward primer: 5'-ATAGGTACCGCCACCATGGGCAGCTGG-3' and reverse primer 5'-ATATCTAGAACTTTGCCCCTGCTGCTGC-3'. ΔMLS-3CFLAG was built similarly by cloning ECSIT2 amino acids 49 to 435 (without the mitochondrial localization sequence (MLS)) using forward primer: 5'-ATAGGTACCGCCACCATGGGCAAGGATG-3' and the same reverse primer as above.

Coimmunoprecipitation (coIP)

For ECSIT/PINK1 and ECSIT/LC3 coIPs, 1-2.10^6 293FT cells were plated in 6-cm dishes and transfected with 3 µL lipofectamine 2000 (Invitrogen) following the manufacturer's protocol. ECSIT/PINK1 coIP was performed using 0.25 µg ECSIT-3CFLAG, 1 µg pCMVTNT PINK1 C-myc (Beilina et al., 2005), 1 µg pMXs-IP HA-Parkin (Yoshii et al., 2011), and corresponding empty vector controls. For ECSIT/LC3 coIP, 0.5 µg ECSIT-3CFLAG, 2 µg YFP-LC3 (a king gift from the laboratory of T. Meila), and corresponding empty vector controls were used. The following day, cells were treated with 30 µM CCCP as indicated **(**carbonyl cyanide m-chlorophenyl hydrazine, Sigma-Aldrich). 24 h after transfection, cells were washed twice with PBS, lysed in 500 µL coIP buffer (150 mM NaCl, 25 mM Hepes, 0.2% NP40, 10% Glycerol) with 1 mM DTT and protease inhibitors (1 mM PMSF, 1 µg/mL aprotinin, 10 µg/mL pepstatin and 1 µg/mL leupeptin), incubated for 20 min on ice and, clarified at 15,000 rpm for 15 min at 4°C. A sample was aliquoted for input and remaining lysate was incubated for 6 h at 4°C with 0.5 µg anti-FLAG antibody (Sigma-Aldrich, clone M2) or anti-Myc-Tag antibody (Cell Signaling, clone 9B11) and overnight with 10 µL of washed Protein G sepharose 4B beads (Invitrogen). Beads were washed in coIP buffer and resuspended in 2X Laemmli buffer. Samples were fractionated by SDS-PAGE and analyzed by western blotting.

Ubiquitination Assay

2.10^6 293FT cells were plated in 6-cm dishes and transfected with 3 µL lipofectamine 2000 (Invitrogen) and 0.25 µg ECSIT-3CFLAG, 0.1 µg ΔMLS-3CFLAG, 1 µg pCMVTNT PINK1 C-myc (Beilina et al., 2005), 1 µg pMXs-IP HA-Parkin (Yoshii et al., 2011), 1 µg pcDNA3 VSV-Ubiquitin (Chastagner et al.) and corresponding empty vector controls, following the manufacturer's protocol. 24 h after transfection, cells were washed twice with PBS,

solubilized in 200 µL boiling SDS lysis buffer (20 mM Tris-HCl, 1% SDS, pH 7.5) and boiled for an additional 10 min. After shearing DNA, the samples were diluted to 0.4 % SDS with water, then an equal volume of 2X TNT buffer (50 mM Tris-HCl pH 7.4, 300 mM NaCl, 2% Triton X-100, 20% glycerol, 2 mM EDTA, 2X protease inhibitors) was added. An aliquot was taken for input and remaining samples were immunoprecipitated with 1 µg anti-VSV-G antibody (Sigma-Aldrich, clone P5D4) for 6 h at 4°C and overnight with 10 µL of washed Protein G sepharose 4B beads (Invitrogen). Beads were washed in TBS (50mM Tris HCl pH7.4, 150mM NaCl) and resuspended in 2X Laemmli buffer. Samples were fractionated by SDS-PAGE and analyzed by western blotting.

Analysis of mitochondrial damage induction in macrophages

WT IBMM and BMDM were plated in 10 cm tissue culture dish (3.10^6 cells/well) and, the following day, treated with 100 ng/mL LPS, 10 µM CCCP and 10 nM Bafilomycin A1 or DMSO control (Sigma-Aldrich) for 1h. Cells were lysed in coimmunoprecipitation buffer and processed as described above using anti-mouse ECSIT2 antibody (West et al). For time course of treatment, cells were treated with 100 ng/mL LPS, 30 µM CCCP or DMSO control for 15, 30 and 60 min, washed twice in PBS, lysed in 1% SDS lysis buffer as above (Ubiquitination assays).
For coimmunoprecipitation, cells were treated with 10 nM Bafilomycin A1 (Ba1) for 5h and/or 20 µM of CCCP (CC) for 1h. Cells were washed twice with PBS, lysed in 500 µL coIP buffer (150 mM NaCl, 25 mM Hepes, 0.2% NP40, 10% Glycerol) with 1 mM DTT and protease inhibitors (1 mM PMSF, 1 µg/mL aprotinin, 10 µg/mL pepstatin and 1 µg/mL leupeptin), incubated for 20 min on ice and, clarified at 15,000 rpm for 15 min at 4°C. A sample was aliquoted for input and remaining lysate was incubated for 6 h at 4°C with anti-ECSIT2 antibody (West et al, 2011) or IgG control (Cell Signaling, #2729) and overnight with 30 µL of washed Protein G sepharose 4B beads (Invitrogen). Beads were washed in coIP buffer and resuspended in 2X Laemmli buffer. Samples were fractionated by SDS-PAGE and analyzed by western blotting.

SDS-PAGE and Western blotting

Protein concentrations were determined using the microBCA protein kit (Thermo Scientific). 20-40 µg total cell lysate or 10-20 µg solubilized mitochondrial proteins from BMDM or IBMM were separated by SDS-PAGE and transferred onto a PVDF membrane (Immobilon-P, Millipore).
Membranes for SDS-PAGE and Blue Native PAGE were blocked in 5 % non-fat milk and incubated with primary and horseradish peroxidase (HRP)–conjugated secondary antibodies. We used Super Signal West Pico chemiluminescent substrate (Thermo Fisher Scientific) or Luminata Forte (Millipore) and BioExcell autoradiographic films (WWMP) for image development. Primary antibodies were: anti-VDAC1 (Abcam, clone 20B12AF2), ubiquitin (Santa Cruz, clone P4D1), NDUFS3 (Abcam, clone 3F9DD2), ND6 (Molecular Probe, clone 20E9), SDHA (Abcam, clone EPR9043), UQCRC2 (Abcam, clone 13G12AF12BB11), ATP synthase F1 alpha and beta subunits (kindly provided by Dr. *Alexander Tzagoloff*, Columbia University), NDUFAF1 (Origene), TOM20 (Sigma, clone 4F3), GAPDH (Fitzgerald), beta-Tubulin (Sigma, clone TUB2.1), HDAC1 (Santa Cruz), Parkin (Cell Signaling, Prk8), PINK1 (Novus Biologicals), LC3b (Sigma-Aldrich), Myc (Cell Signaling, clone 9B11), HA (Sigma-Aldrich, clone HA-7), FLAG (Sigma-Aldrich, clone M2), and ECSIT (rabbit polyclonal antibody against ECSIT was previously described (West et al, 2011)).

Quantification and Statistical Analysis

Statistical analysis was conducted using GraphPad Prism 6 software. All data were tested for normal distribution of variables. All normally distributed data were displayed as means ± standard deviation (SD) unless otherwise noted. Measurements between two groups were performed with an unpaired Student's t test if normally distributed, or Mann-Whitney U test if otherwise. Groups of three or more were analyzed by one-way analysis of variance (ANOVA) or the Kruskal-Wallis test. Values of n for each experiment are reported in the figures and figure legends. $p < 0.05$ was considered significant. Statistical parameters for each experiment can be found within the corresponding figure legends.

Primer name	Sequence (5'-3')
LNeoL_intron3F	CTGCCAGATTAGCTCATGGTACTTCATCACAGTAACAGGGAAGCACTAGTACAGAAGTTGCCGATCATATTCAATAACCCTTAAT
LNeoL_intron3R	ACACACGCATACACACACGCGAAGAAAACATCAAGAAGCATTGCCAGGACCACATTGTTGCTAGTGGATCCCCTCGAGGGACCTA
FNeoFL_intron4F	GTTGCTGGGATTTGAACTTTGGACCTTCTGAAGAACAGTCGGGTGCTCTTACCCACTGAGATCGATAAGCTTGATATCGAATTCC
FNeoFL_intron4R	GTACCTATCTCCAGACACACAAGAATAGGACATCAGATCCCATTATGGATGGTTGTGAACGCTCTAGAACTAGTGGATCCACCTA
Ret_Left1_ECSIT	CTGAGTTTAAGGCCAGCTTGGTCTACACAGCAACTTCCAGATTAGCCGGCGCGCCAGTGTGGTTTTCAAGAGGA
Ret_Right1_ECSIT	CCCATTGCGAGAGAATTTTATAGTCATATTCAAGAGGCACTAGTGTGCAGCGGCCGCAATTCGCCCTATAGTGAGT
longarm2F	CATGTTCTCTGTGGCCTAGATAC
L83 loxp	CCTCGAGGGACCTAATAACTTCGTA
Short_arm1R	GCCAGGCGGAATACCACTGG
ARM2/F2	CGTTGGCTACCCGTGATATT

List of primers used for qPCR

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
Hprt	GTTAAGCAGTACAGCCCCAAA	AGGGCATATCCAACAACAAACTT
HIF1a	GGGGAGGACGATGAACATCAA	GGGTGGTTTCTTGTACCCACA
GLUT1	AGCCCTGCTACAGTGTAT	AGGTCTCGGGTCACATC
PKM2	GCCGCCTGGACATTGACTC	CCATGAGAGAAATTCAGCCGAG
PFKFB1	ATGAGCTGCCCTATCTCAAGT	GTCCCGGTGTGTGTTCACAG
PFKFB3	CAACTCCCCAACCGTGATTGT	GAGGTAGCGAGTCAGCTTCTT
LDHA	AGGCTCCCCAGAACAAGATT	TCTCGCCCTTGAGTTTGTCT
MCT1	TGTTAGTCGGAGCCTTCATTTC	CACTGGTCGTTGCACTGAATA
bTub	CCCTCAGCTTTCTCCAACTG	CACCATTTACCCCCAATGAG
mtND6	GGTTAGCATTAAAGCCTTCACCT	CATCAACCAATCTCCCAAACCAT
NDUFS3	TGGCAGCACGTAAGAAGGG	CTTGGGTAAGATTTCAGCCACAT
NDUFAF1	TGTCCTTGTAAGCCCTCTTCAG	TTTGTGGGTAGTCACTGTGTAGA
Uqcrc2	AAAGTTGCCCCGAAGGTTAAA	GAGCATAGTTTTCCAGAGAAGCA
SDHA	GGAACACTCCAAAAACAGACCT	CCACCACTGGGTATTGAGTAGAA

ATP5a1	TCTCCATGCCTCTAACACTCG	CCAGGTCAACAGACGTGTCAG
mtCOI	TGCTAGCCGCAGGCATTAC	GGGTGCCCAAAGAATCAGAAC
Ndufv1	CTTCCCCACTGGCCTCAAG	CCAAAACCCAGTGATCCAGC

Other Resources Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-mouse ECSIT2, rabbit polyclonal	(Kopp et al., 1999)	N/A
Anti-VDAC1, mouse monoclonal (20B12AF2)	Abcam	Cat#ab14734, RRID:AB_443084
Anti-ubiquitin, mouse monoclonal (P4D1)	Santa Cruz Biotechnology	Cat#sc-8017, RRID:AB_628423
Anti-NDUFS3, mouse monoclonal (3F9DD2)	Abcam	Cat#ab110246, RRID:AB_10861972
Anti-ND6, mouse monoclonal (20E9)	Dr Malgorzata Rak/ Thermo Fischer	Cat#A31857, RRID:AB_1501823
Anti-SDHA, rabbit monoclonal (EPR9043)	Abcam	Cat#ab137040
Anti-Uqcrc2, mouse monoclonal (13G12AF12BB11)	Abcam	Cat#ab14745, RRID:AB_2213640
Anti-ATP synthase F1 alpha subunit, rabbit polyclonal	Dr. Alexander Tzagoloff, Columbia University	(Rak et al., 2011)
Anti-ATP synthase F1 beta subunit, rabbit polyclonal	Dr. Alexander Tzagoloff, Columbia University	(Rak et al., 2011)
Anti-NDUFAF1, rabbit polyclonal	OriGene	Cat#TA30792
Anti-TOM20, mouse monoclonal (4F3)	Sigma-Aldrich	Cat#WH0009804M1, RRID:AB_1843992
Anti-LC3b, rabbit polyclonal	Sigma-Aldrich	Cat#L7543, RRID: AB_796155
Anti-Parkin, mouse monoclonal	Cell Signaling	Cat#4211S, RRID:AB_10694914
Anti-PINK1, rabbit polyclonal	Novus Biologicals	Cat#BC100-494, RRID:AB_10127658
Anti-HDAC1, rabbit polyclonal	Santa Cruz Biotechnology	Cat#sc7872, RRID:AB_2279709
Anti-GAPDH, mouse monoclonal (6C5)	Fitzgerald	Cat#10R-G109a, RRID:AB_1285808
Anti-beta-Tubulin, mouse monoclonal (TUB2.1)	Sigma-Aldrich	Cat#T4026, RRID:AB_477577
Anti-FLAG, mouse monoclonal (M2)	Sigma-Aldrich	Cat#F1804, RRID:AB_262044
Anti-VSV-G, mouse monoclonal (P5D4)	Sigma-Aldrich	Cat#V5507, RRID:AB_261877
Anti-Myc-Tag, mouse monoclonal (9B11)	Cell Signaling	Cat#2276S, RRID:AB_331783
Anti-HA, mouse monoclonal (HA-7)	Sigma-Aldrich	Cat#9658, RRID:AB_260092

Anti-CD16/CD32 (2.4G2)	Tonbo Biosciences	Cat#70-0161, RRID:AB_2621487
Anti-F4/80-FITC (BM8)	eBiosciences	Cat#11-4801-81, RRID:AB_465226
CD11b-PE (M1/70)	eBiosciences	Cat#12-0112-81, RRID:AB_465546
Ly6G-VF405	Tonbo Biosciences	Cat#75-1276, RRID:AB_2621955
CD3-PerCPCy5.5 (145-2C11)	Tonbo Biosciences	Cat#65-0031, RRID:AB_2621872
B220-APC780 (RA3-6B2)	eBiosciences	Cat#47-0452-82, RRID:AB_1518810
Bacterial and Virus Strains		
E. coli SW106	Liu et al., 2003	(Liu et al., 2003)
Chemicals, Peptides, and Recombinant Proteins		
4OH-Tamoxifen	Sigma-Aldrich	Cat#H6278, CAS 68392-35-8
DDM (Dodecylmaltoside)	Sigma-Aldrich	Cat#D4641, CAS 69227-93-6
Aminohexanoic acid	Sigma-Aldrich	Cat#07260, CAS 60-32-2
Coomassie Blue G-250	AmericanBIO	Cat#AB00325, CAS 6104-58-1
Sodium Pyruvate	Thermo Fisher	Cat#11360070
L-Glutamine	Thermo Fisher	Cat#25030081
MitoSOX Red Mitochondrial Superoxide Indicator	Thermo Fisher	Cat#M36008
Tetramethylrhodamine, Methyl Ester, Perchlorate (TMRM)	Thermo Fisher	Cat#T668
Mitotracker Green FM	Thermo Fisher	Cat#M7514
CM-H_2DCFDA chloromethyl derivative of 2',7'-dichlorodihydrofluorescein diacetate	Thermo Fisher	Cat#C6827
Imperial Protein Stain	Thermo Fisher	Cat#24615
Nitro blue tetrazolium (NBT)	Research Products International	Cat#66000-0.1, CAS 298-83-9
Chloramphenicol	Sigma-Aldrich	Cat#C0378, CAS 56-75-7
2-Deoxyglucose (2-DG)	Sigma-Aldrich	Cat#D6134, CAS 154-17-6
D-(+)-Glucose	Sigma-Aldrich	Cat#G8270, CAS 50-99-7
D-(+)-Galactose	Sigma-Aldrich	Cat#G0625, CAS 59-23-4
Sodium oxamate	Sigma-Aldrich	Cat#O2751, CAS 565-73-1
LPS from E. *coli* 026:B6	Sigma-Aldrich	Cat#8274
Recombinant mouse M-CSF	R&D Systems	Cat#416-ML
Oligomycin	Santa Cruz Biotechnology	Cat#sc-203342, CAS 1404-19-9

FCCP	Santa Cruz Biotechnology	Cat# sc-203578, CAS 370-86-5
Rotenone	Sigma-Aldrich	Cat#8875, CAS 83-79-4
Antimycin	Sigma-Aldrich	Cat#A8674, CAS 1397-94-0
MG132	Calbiochem/Millipore	Cat#474790, CAS 133407-82-6
Bafilomycin A1	Sigma-Aldrich	Cat#B1793, CAS 88899-55-2
Zeocin	Invivogen	Cat#ant-zn-1, CAS 11006-33-0
CCCP	Sigma-Aldrich	Cat#2759, CAS 555-60-2
Doxycyclin	Sigma-Aldrich	Cat#3447, CAS 10592-13-9
Dnase I	Sigma-Aldrich	Cat#D4513, CAS 9003-98-9
Collagenase	Sigma-Aldrich	Cat#C5138, CAS 9001-12-1
Critical Commercial Assays		
L-lactate assay Kit I	Eton Bioscience	Cat#120001100P
Complex I Enzyme Activity Assay Kit	Abcam	Cat# ab109721
NAD/NADH Quantitation Colorimetric Kit	BioVision	Cat#K337
ATPlite Luminescence Assay System	Perkin Elmer	Cat#6016943
RNeasy Mini Kit	QIAGEN	Cat#74104
DNeasy Blood and Tissue Kit	QIAGEN	Cat#69504
PerfeCTa SYBR Green FastMix	QantaBio	Cat#95072
Seahorse FluxPak Mini	Seahorse Bioscience/Agilent	Cat#100867-100
Seahorse XF Base medium	Seahorse Bioscience/Agilent	Cat#102353100
Mouse IL-6 ELISA Set	BD Biosciences	Cat#555240
Mouse TNF (Mono/Mono) ELISA Set	BD Biosciences	Cat#555268
Experimental Models: Cell Lines		
Mouse: L929	ATCC	CCL-1
Human: 293FT	ThermoScientific	R70007
Mouse: ψCREJ2	Laboratory of KA Fitzgerald	(Hornung et al., 2008)
Mouse: iBMM ECSIT$^{f/f}$/Cre-ERT2^{+}	This paper	N/A
Experimental Models: Organisms/Strains		
Mouse: ECSIT CKO : ECSIT$^{f/f}$	This paper	N/A
Mouse: ECSIT KO : ECSIT$^{+/-}$	(Xiao et al., 2003)	(Xiao et al., 2003)
Mouse: LysM-Cre : B6.129P2-*Lyz2*$^{tm1(cre)Ifo}$/J	The Jackson Laboratory	Stock 004781
Mouse: Cre-ERT2 : Rosa26Cre-ERT2	Laboratory of B. Reizis (Thomas Ludwig)	Unpublished
Mouse : C57BL/6	The Jackson Laboratory	Stock 000664

Oligonucleotides		
Primers for *Escit* conditional knockout mouse generation, See Table S1	This paper	N/A
Primers for qPCR, See Table S2	This paper	N/A
Primer NDUFAF1 cloning forward: ataaccggtgccaccatggcaatgtcttccattcac	This paper	N/A
Primer NDUFAF1 cloning reverse: atagctagcctatctgaagagtcttgggttaag	This paper	N/A
Primer ECSIT-3CFLAG cloning forward: ataggtaccgccaccatgggcagctgg	This paper	N/A
Primer ECSIT-3CFLAG cloning reverse: atatctagaactttgcccctgctgctgc	This paper	N/A
Primer ΔMLS-3CFLAG cloning forward: ataggtaccgccaccatgggcaaggatg	This paper	N/A
Recombinant DNA		
pL452	Frederick National Laboratory NCI	(Liu et al., 2003)
pL451	Frederick National Laboratory NCI	(Liu et al., 2003)
pMCS-DTA	Kosuke Yusa, Wellcome Trust Sanger Institute	(Yusa et al., 2011)
FUW-TetON-hOCT4	(Hockemeyer et al., 2008)	Addgene #20726
FUW-TetON	This paper	N/A
FUW-M2rtTA	(Hockemeyer et al., 2008)	Addgene #20342
FUW-TetON-NDUFAF1	This paper	N/A
psPAX2	Laboratory of D. Trono	Addgene #12260
pCMV-VSV-G	(Stewart et al., 2003)	Addgene #8454
ECSIT-FLAG	(West et al., 2011)	N/A
p3XFLAG-CMV14	Sigma-Aldrich	Cat#E7908
pCMVTNT PINK1 C-myc	(Beilina et al., 2005)	Addgene # 13314
pMXs-IP HA-Parkin	(Yoshii et al., 2011)	Addgene # 38248
pcDNA3 VSV-G Ubiquitin	(Chastagner et al., 2006)	N/A
YFP-LC3	Laboratory of T. Meila	N/A
Software and Algorithms		
GraphPad Prism 6	GraphPad	https://www.graphpad.com/scientific-software/prism/
FlowJo	FlowJo, LLC	https://www.flowjo.com/solutions/flowjo
Other		
Tet System approved FBS	Takara	Cat#631107
DMEM, no glucose	Thermo Fisher	Cat#11966025
DMEM, no glucose, no glutamine	Thermo Fisher	Cat#A1443001
NativePAGE 4-16 % Bis-Tris Protein gel	Thermo Fisher	Cat#BN1002BOX

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