

## **SUPPLEMENTARY EXPERIMENTAL PROCEDURES**

### **Antibodies and Reagents.**

Recombinant mouse IFN- $\gamma$  was from PeproTech, recombinant human IFN- $\beta$  was from PBL Biomedical, recombinant mouse IFN- $\beta$  was from R&D Systems and recombinant human Flt-3L was from Origene Inc. Anti-CD32a (clone IV.3) was from Stemcell Technologies, Alexa-Fluor-647 conjugated anti-LAMP1 (clone eBio1D4B) was from eBioscience, APC-conjugated anti-B220 (clone R3-6B2) was from Biolegend, PE-conjugated anti-CD11c (clone HL3) was from BD Pharmingen and anti-TNF- $\alpha$  (clone MP6-XT22) was from eBioscience. Mouse IgG, Fc and F(ab)<sup>2</sup> were from Jackson Immunoresearch. Wortmannin (W1628) was from Sigma, Chloroquine diphosphate (L10382), Lipofectamine 2000 and LysoTracker Red DND-99 were from Invitrogen. ODN1585, ODN1826, Biotin ODN2216, ODN2216, TLR9 inhibitory ODN (ODN TTAGGG ), ODN TTAGGG control and Imiquimod were from Invivogen.

### **Differentiation of pDCs from Mouse Bone Marrow and Fetal Liver Cells.**

Bone marrow cells from the femur and tibia of adult C57BL/6 mice and fetal livers from pups at day E14 were harvested and differentiated into pDCs as previously described (Brawand et al., 2002; Zhang et al., 2000). Briefly, harvested cells were resuspended in blood cell lysis buffer (Sigma) to remove RBCs. Cells were then re-suspended in RPMI 1640 supplemented with 10% (v/v) FBS, 100U/ml penicillin, 100  $\mu$ g/ml streptomycin and 2-Mercaptoethanol (2-ME, 1x) at a concentration of 1-1.5 x 10<sup>6</sup> cell/ml. Cells were then kept in culture for 7 days in the presence of 100 ng/ml of recombinant human Flt-3L. Cells were harvested on day 8 and PDCA1<sup>+</sup> B220<sup>+</sup> CD11c<sup>+</sup> cells (pDCs) were

sorted. The purity of PDCA1<sup>+</sup> B220<sup>+</sup> CD11c<sup>+</sup> cells was consistently above 90%. Transient expression of PH-TRAF3 and TLR9-GFP in mouse pDCs was performed using Lipofectamine 2000 according to manufacturer's protocol.

### **Constructs.**

PX-GFP was obtained by cloning the PX domain of mouse p40 (PHOX) to the N-terminus of eGFP using a 6 amino acid linker. The p40PX domain was cloned from RAW 264.7 cDNA by standard RT-PCR. The primers used to amplify the p40PX domain-eGfp fusion were 5'-TTAATTGCGGCCGCCTAGGTTGAAACCATGGCCCTGGCCAG-3' and 5'-TTAATTCTCGAGATCTGAATTCTTACTTGTACAGCTCGTCCATGCCG 3'. The PCR product was digested with AvrII/XhoI and cloned into the NheI/XhoI sites of pMSCVNeo. A murine TLR9-eGfp fusion construct (muTLR Genbank#NM\_031178.2; 6 amino acid linker between genes) was codon optimized and synthesized by GeneArt. This cassette was cloned into the pMSCVPuro retroviral backbone (Clontech). Mouse UNC93B-mCherry-wtpMSCVNeo and UNC93B-Gfp-wtpMSCVNeo plasmids were generated as previously described (Kim et al., 2008). TLR9a-mCherry was obtained by fusing hTLR9a, from pUNO1-hTLR09a (InvivoGen) to the N-terminus of mCherry, using a 6 amino acid linker. Both hTLR9a and mCherry were PCR amplified, from their respective parental plasmids, and subsequently fused using SOEing PCR. The primers, 5'-TTAATTGCTAGCACCATGGGTTTCTGCCGCAGCGC-3', and 5'-TTAATTCTCGAGTTACTTGTACAGCTCGTCCA-3' were used to amplify, and add NheI/XhoI sites to hTLR9a-mCherry. This PCR product was cloned into the AvrII/XhoI

sites of a modified pMSCV-Neo vector. GFP-LC3B was gene optimized, synthesized, and sequenced by GeneArt. The fusion gene was cloned into the BglII/AvrII sites of the pMSCV-Neo vector to generate pMSCV-Gfp-LC3B-Neo plasmid. mCherry-LC3B was generated by fusing mCherry to the N-terminus of the LC3B of the pMA-Gfp-LC3B-RQ plasmid. Both mCherry and LC3B were PCR amplified, from their respective parental plasmids, and subsequently fused using SOEing PCR. The primers, 5'-TTAATTGCTAGCACCATGGTGAGCAAGGGCGAGGA-3', and 5'-TTAATTCTCGAGTCACACGCTCAGCTTCATGC-3' were used to amplify, and add NheI/XhoI sites to mCherry-LC3B. Next, the fusion gene was digested with appropriate enzymes and cloned into AvrII/XhoI sites of pMSCV-Neo vector to generate pMSCV-mCherry-LC3B-Neo plasmid. PH-TRAF3 construct was generated as previously described (Ikonomov et al., 2006; Sasai et al., 2010).

The fusion plasmids were used to transfect the pT67 packaging cell line (Clontech), or the Gryphon amphotropic packaging cells (Allele Biotech), using the standard transfection protocol. Amphotropic retroviral particles encoding the desired fusion protein were produced from the selected cell populations, and these viral particles were collected and filtered prior to their use for transductions.

### **ELISA Cytokine Measurements.**

Human PBMCs: DNA-immune complexes used to stimulate human PBMCs were generated by combining human anti-histone polyclonal antibody (clone MD-14-0357, RayBiotech) or human polyclonal isotype control antibody (clone 7112H, Meridian Life Science) with Jurkat cell lysate. Antibodies were used at a 1:50 (v/v) final dilution unless

otherwise indicated in the figure legend. Antibodies were mixed with 1.25% Jurkat cell lysates for 30 min. Jurkat cells lysates were prepared by pelleting cells and resuspending in 100 $\mu$ L sterile dH<sub>2</sub>O per 40 x 10<sup>6</sup> cells. Cells were sonicated using a 550 Sonic Dismembrator sonicator (Fisher Scientific), cell debris were removed and supernatant volume was adjusted to 1mL RPMI with 10% (v/v) FBS. Human PBMCs were pre-treated with 500IU/mL of recombinant IFN- $\beta$  for 30 min prior to stimulation. Following IFN- $\beta$  pre-incubation, immune complexes were added to the cells and incubated for 20-22 hours at 37°C/5% CO<sub>2</sub>. For pharmacological inhibition of IFN- $\alpha$  and TNF- $\alpha$  production, serial dilutions of pharmacological inhibitors were prepared in RPMI 1640 supplemented with 10% (v/v) FBS and stored at 4 °C until use. Heat aggregated human IgGs were generated by heating 12 mg/mL of human IgG (Talecris Biotherapeutics) for 30 minutes at 63°C. IFN- $\beta$  pre-treated human PBMCs were incubated with the inhibitors for 20 minutes at 37°C/5% CO<sub>2</sub>. DNA Immune complexes were then added for 20-22 hours at 37°C/5% CO<sub>2</sub>. IFN- $\alpha$  and TNF- $\alpha$  levels in harvested supernatants were evaluated with a multisubtype IFN- $\alpha$  ELISA kit (PBL Biomedical) or TNF- $\alpha$  human ELISA kit (Invitrogen) according to the manufacturer's protocol. Mouse pDCs: CG50 plasmid was made by cloning a previously described sequence that contains 50 optimal CpG motifs (Viglianti et al., 2003) into a pMK-RQ vector. The DNA antibody clone E11 was generated by phage library screening. Clone E11 showed high affinity binding to plate-bound DNA. Unless mentioned otherwise in the figure legend, DNA-immune complexes (DNA-IgGs) used to stimulate mouse pDCs were generated by combining 50  $\mu$ g/ml of E11 DNA antibody and 0.5  $\mu$ g/ml of CG50 plasmid DNA. Formation of DNA-immune complexes was achieved by incubating both components for 30 min on a plate

shaker. Flt-3L-differentiated pDCs from bone marrow or fetal livers were resuspended at a concentration of  $5 \times 10^6$ /ml in fresh RPMI 1640 supplemented with 10% (v/v) FBS, 100U/ml penicillin, 100  $\mu$ g/ml streptomycin and 2-Mercaptoethanol (2-ME, 1x). Recombinant mouse IFN- $\beta$  (100 u/ml) was added to the cells 1 h prior to stimulation. CpG-ODN, E11 antibody and CG50 DNA were added to the cells at the indicated concentrations for 24 h and IFN- $\alpha$  and TNF- $\alpha$  levels were measured in supernatant by ELISA (R&D Systems).

### **siRNA Gene Silencing.**

Silencing of mouse ATG5, FIP200, ATG13, and FcR $\gamma$  chain was achieved using ON-TARGET plus siRNA oligonucleotides from Dharmacon. siRNA oligonucleotides were delivered in cells using lipofectamine RNAi Max (Invitrogen) following manufacturer's recommendations.

### **Quantitative RT-PCR.**

Mouse macrophages: Total RNA was isolated from macrophages using RNeasy Plus Mini Kit (Qiagen) according to the manufacturer's recommendations. First strand cDNA synthesis was completed using the SuperScript III First Strand Synthesis (Invitrogen) and RT-PCR was completed with TaqMan Fast Universal PCR mix in a 7900HT thermocycler (Applied Biosystems) using the following PCR parameters: 40 cycles of 95°C for 21 seconds and 60°C for 20 seconds. mRNA expression was normalized against murine 18s allowing for comparison of mRNA levels. The following Applied Biosystems primers were utilized: mouse 18s (Mm03928990\_g1), mouse TNF $\alpha$ .

(Mm00443260\_g1), mouse IL-6 (Mm00446190\_m1), and mouse IFN $\beta$  (Mm00439552\_s1). Mouse pDCs: Total RNA was isolated from pDC cells using TRIzol (Gibco) according to the manufacturer's instructions. First strand synthesis was performed using M-MLV reverse transcriptase (Invitrogen). Real time PCR<sup>TM</sup> was performed using SYBR GREEN PCR master mix (Applied Biosystems), in a Applied Biosystems 7900HT thermocycler using SyBr Green detection protocol as outlined by the manufacturer using the following PCR conditions: 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min. mRNA expression was normalized against L32, allowing comparison of mRNA levels. The following primers were used: mouse TLR9 (forward primer: 5- GTACCCTGCCTGCCTTCCTAC-3; reverse primer: 5- GAACAGCCAATTGCAGTCCA-3), mouse MyD88 (forward primer: 5- CATGGTGGTGGTTGTTTCTGAC-3; reverse primer: 5- TGGAGACAGGCTGAGTGCAA- 3), mouse IRF7 (forward primer: 5- CACCCCATCTTCGACTTCA-3; reverse primer: 5- CCAAACCCAGGTAGATGGTGTA-3), and mouse L32 (forward primer: 5- GAAACTGGCGGAAACCCA-3; reverse primer: 5-GGATCTGGCCCTTGAACCTT-3). mRNA expression was normalized against murine actin allowing for comparison of mRNA levels.

### **Cell Lysis and Immunoblotting.**

Cells were lysed in RIPA buffer for 30 min on ice (50 mM Tris, pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.5% DOC, 0.1% SDS, protease inhibitor tablet (Roche), 1 mM NaF, 1 mM Na<sub>3</sub>VO<sub>4</sub>, and 1 mM PMSF). After centrifugation (13.2k rpm, 15 minutes, 4°C),

supernatants were analyzed by SDS-PAGE. Anti-TLR9 antibody was from Abcam, anti-MyD88 antibody was from Cell Signaling, anti-actin antibody (clone C4) was from MP Biomedicals and anti-ATG7, anti-LC3B, and anti-Beclin1 antibodies were from Cell Signaling. Anti-VAMP3 was from Synaptic Solutions. Anti-LAMP2 was from eBioscience. Anti-GFP antibodies were from Santa Cruz and Clontech.

### **Quantification of Phagocytosis and Fusion-Protein Translocation.**

The percentage of cells positive for phagocytosis was calculated by dividing the number of cells that had fully engulfed a bead by the total number of cells in the field of vision. Calculation is an average of four independent time-lapse movies, with more than 50 total cells counted per frame.

Quantification of fusion-protein translocation to the bead-containing phagosome was performed by acquiring 2-18 hour time-lapse movies and counting the number of fusion-protein-positive bead-containing phagosomes out of the total number of engulfed beads for that period. For each condition, three independent experiments were performed, with at least 25 phagosomes counted per experiment. At the end of each experiment, we confirmed that particles were completely internalized by differential focusing.