SUPPLEMENTARY FIGURE LEGENDS

Figure S1 related to Figure 1. DNA-IgG-mediated production of IFN-α requires both FcγR and TLR9 in human PBMCs.

(A) Human PBMCs were challenged for 18 hours with ODN 2216 (CpG-ODN) or with serial dilutions of anti-histone antibodies in the presence of Jurkat cell lysate to form DNA-containing immune complexes (DNA-IgG), as described in experimental procedures. IFN-α production in supernatant was measured by ELISA.

(B-E) Human PBMCs were stimulated with the Jurkat lysates and a 1:50 dilution of anti-histone antibodies for 18 hours. Cells were pretreated for 30 min. with serial dilutions of antagonistic anti-FcγRIIa (B), heat aggregated human IgG (C), the TLR9 antagonist ODN TTAGGG (inhibitory ODN) and control ODN TTAGGG (D) or chloroquine (E). Supernatants were collected and IFN-α was quantitated by ELISA. Data are representative of three independent experiments. Data are presented as mean ± s.d.

Figure S2 related to Figure 2. DNA-IC-mediated enhancement of TLR9 and UNC93B trafficking to phagosomes depends on FcγR.

(A-C) Mouse macrophages expressing TLR9-GFP and UNC93B-mCherry were stimulated for 60 min with either 3.0 μM of free biotin-ODN 2216 (Free CpG-ODN)) or biotin-ODN 2216 immobilized on beads (Immobilized CpG-ODN). Cells were then fixed, permeabilized and incubated with Alexa Fluor 647-labeled streptavidin. Intracellular localization of TLR9-GFP, UNC93B-mCherry and biotin-ODN 2216 (CpG-ODN) was assessed by confocal microscopy. Yellow arrows point to internalized particles. Representative images and signal intensity profiles for TLR9 (green), UNC93B

(red) and CpG-ODN (blue) are shown (A). The percentage of cells positive for TLR9/CpG-ODN colocalization (B) and UNC93B/CpG-ODN colocalization (C) were quantified for three independent experiments ($n \geq 75$ / group). Data are presented as mean ± s.d. Scale bar, 5μm.

(D) Mouse macrophages were fed with 3.0 μM of free or immobilized CpG-ODN, as in (A), for 4 hours. Cellular mRNA levels for IFN-β were assessed by quantitative RT-PCR. Data are presented as mean ± s.d. of three independent experiments.

(E) Immunoblot analysis of phagosome proteins. Raw 264.7 cells expressing TLR9-GFP were incubated with CG50 plasmid DNA-coated magnetic beads (DNA) or magnetic beads coated with a combination of both CG50 plasmid DNA and DNA antibody (DNA-IgG) for 0, 1, 2 or 4 hours. Phagosomes were purified using magnet as described in experimental procedures. Phagosome proteins or control whole cell lysates were solubilized in SDS-PAGE and TLR9-GFP was detected using anti-GFP antibodies by immunoblotting. As previously described, both full length and the cleaved form of TLR9 were detected in whole cell lysates, while only the cleaved form was detected in the phagosome fraction (Ewald et al., 2008). The results presented are representative of three independent experiments.

(F-H) Mouse macrophages expressing TLR9-GFP and UNC93B-mCherry were incubated with uncoated beads (Control) or beads covered with mouse $F(ab)^2$, Fc or IgG. Engulfed particles were visualized by time lapse confocal acquisition. Representative frames acquired 30 min after particle internalization are shown (F). Yellow arrows point to internalized particles. The percentage of particles recruiting TLR9-GFP (G) and

UNC93B-mCherry (H) were quantified for three independent experiments (n ≥ 75 / group). Data are presented as mean ± s.d. Scale bar, 5μm.

(I-K) Mouse macrophages expressing TLR9-GFP and UNC93B-mCherry were treated with siRNA oligonucleotides targeting FcRγ or control siRNA. FcRγ protein expression in cells was assessed by immunoblotting 48 hours after siRNA oligonucleotide treatment (I). siRNA-treated cells were then fed beads coated with a combination of CG50 plasmid DNA and DNA antibody and recruitment of TLR9-GFP and UNC93B-mCherry to the phagosomes was visualized by confocal microscopy. Representative images taken 60 min after bead internalization are shown (J). Yellow arrows point to internalized particles. The percentage of beads recruiting TLR9 and UNC93B-mCherry were quantified for three independent experiments (n ≥ 75 / group) (K). Data are presented as mean ± s.d. Scale bar, 5μm.

Figure S3 related to Figure 4. Wortmannin blocks the recruitment of LC3, TLR9 and UNC93B to the phagosome.

(A) RAW 264.7 cells expressing LC3-mCherry, TLR9-mCherry or UNC93B-mCherry were incubated with IgG-opsonized RBC particles in the presence or absence of 1.0 μM PI3K inhibitor Wortmannin (Wort). Representative images taken 15 to 30 min after particle internalization are shown. The percentage of particles recruiting LC3, TLR9 and UNC93B-mCherry were quantified for three independent experiments (n ≥ 75 / group). Yellow arrows point to internalized particles. Data are presented as mean ± s.d. Scale bar, 5μm.

(B) Freshly isolated human pDCs were stimulated with immune complexes made by combining CG50 plasmid DNA and DNA antibody, in presence or absence of 1.0 μM Wort. IFN-α and TNF-α in supernatant were assessed by ELISA after 16 hours. Data are presented as mean ± s.d. of two independent experiments.

Figure S4 related to Figure 6. ATG7 and ATG5 are not required for TLR9 expression and DNA-IC-mediated mobilization to the phagosome.

(A-F) Fetal liver-derived pDCs from WT, *Atg7*$^{-/-}$, *Tlr9*$^{-/-}$ and *Myd88*$^{-/-}$ mice were prepared as described in experimental procedures.

(A-C) Protein expression of ATG7 (A), TLR9 (B) and MyD88 (C) was assessed in pDCs by immunoblotting. Data are representative of at least two independent experiments.

(D) TLR9, MyD88 and IRF7 mRNA transcript expression in pDCs was determined by quantitative RT-PCR. Data are presented as mean ± s.d.

(E) Percentage of CD11c^{+} B220^{+} cells in fetal-liver-derived preparations from WT and *Atg7*$^{-/-}$ mice was assessed by flow cytometry Pre and Post-sort. PDCA1 expression in CD11c^{+} B220^{+} was also assessed.

(F) Sorted CD11c^{+} B220^{+} pDCs were stimulated with a combination of CG50 plasmid DNA and DNA antibody (DNA-IgG) as described in experimental procedures or with imiquimod for 16 hours. Expression of PDCA1 and intracellular production of TNF-α was assessed by flow cytometry. Data presented are representative of at least two independent experiments.

(G-H) Mouse macrophages expressing both TLR9-GFP and UNC93B-mCherry (G) or RAW 264.7 cells expressing LC3-GFP (H) were treated with non-targeting control or

ATG5 siRNA oligonucleotides and then incubated with uncoated beads or mouse IgG-coated beads. Engulfed particles were followed by time lapse confocal microscopy and representative frames taken between 15 to 30 min after particle internalization are shown. Yellow arrows point to internalized particles. The percentages of phagosomes that were positive for TLR9 and UNC93B (G) and for LC3 (H), upon particle ingestion, were quantified for three independent experiments ($n \geq 75$ phagosomes per group). Data are presented as mean ± s.d. Scale bar, 5µm.

Figure S5 related to Figure 7. **Phagosome acidification is compromised in *Atg7*$^{-/-}$ pDCs.**

(A) WT and *Myd88*$^{-/-}$ fetal liver-derived pDCs were fed with uncoated beads (Control) or beads coated with a combination of CG50 plasmid DNA and DNA antibody (DNA-IgG) for 4 hours ($n \geq 50$ phagosomes per group). Cells were then fixed and LAMP1 was detected by immunofluorescence. Confocal images were obtained and representative images shown. Scale bar, 5µm.

(B) Fetal liver-derived pDCs from WT and *Atg7*$^{-/-}$ mice expressing LC3-GFP (green) were allowed to phagocytose DNA-IgG-coated beads, as in (A), for the indicated time. Phagosome acidification was visualized with Lysotracker (red). Representative images are shown. Yellow arrows point to internalized particles. The percentage of particles recruiting Lysotracker (red) were quantified for three independent experiments ($n \geq 75$ per / group). Data are presented as mean ± s.d. ($^*p < 0.05$). Scale bar, 10µm.