Supporting Information

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SI Materials and Methods

Ethics Statement. All animal experiments were conducted following the "Ministry of Health" national guidelines for housing and care of laboratory animals and performed in accordance with institutional regulations after review and approval by the Institutional Animal Care and Use Committee at National Institute of Biological Sciences.

Plasmids, Antibodies, and Reagents. Procaspase-1, pro- IL-1β, and ASC expression plasmids were described (1). AIM2 construct was a gift from Dr. Katherine A. Fitzgerald (University of Massachusetts Medical School, Worcester, MA). cDNA for SdhA was amplified from *L. pneumophila* genomic DNA. The truncation mutants of SdhA included SdhAΔGRIP (replacement of residues 1341 to the C terminus with SidC C-terminal 100 residues for Dot/Icm secretion), SdhA_A (Δ1–499 aa), SdhA_B (Δ501–809 aa), SdhA_C (Δ847–1104 aa). GFP-GRIP domain was constructed by inserting residue 1301–the C terminus of SdhA into pCS2-EGFP vector. All of the plasmids were verified by DNA sequencing.

Antibodies for caspase-1, Rab5, Rab7, and GFP were purchased from Santa Cruz. IL-1β antibody (3ZD) was obtained from Biological Resources Branch, National Cancer Institute-Frederick, MD. TGN38 antibody was a gift from Dr. Li Yu (Tsinghua University, Beijing, China). Cytochrome *c* antibody was a gift from Dr. Xiaodong Wang (National Institute of Biological Sciences, Beijing, China). Other antibodies used in this study include GM130 (BD), Careticulin (Calbiochem), ASC (Alexis), and Flag M2 (Sigma). Human and mouse IL-1β ELISA kits were purchased from Neobioscience Technology Company, Beijing, China.

Chemicals used in this research were LPS (Sigma), ATP (Sigma), poly(dA-dT) (Sigma), Bafilomycin A1 (Biomol), Brefeldin A (Sigma), Nocodazole (Sigma), colchicine (Calbiochem), Mitotracker (Invitrogen), Lysotracker (Invitrogen), 4',6-diamidino-2-phenylindole (DAPI) (Invitrogen), Trypan blue (Sigma), and YVAD (Tocris). Cell culture products were from Invitrogen, and all other chemicals were Sigma-Aldrich products unless noted.

Mice and Cell Culture. *Nlrp3*$^{-/-}$, *Nlrc4*$^{-/-}$, *Aim2*$^{-/-}$, and *Asc*$^{-/-}$ mice were kindly provided by Dr. Vishva Dixit (Genentech, South San Francisco, CA); *Irf3*$^{-/-}$ and *Irf7*$^{-/-}$ mice were from Dr. Tadatsugu Taniguchi (University of Tokyo, Tokyo, Japan). *Irf3*/7 double knockout mice were generated by regular cross. *Ifnar*$^{-/-}$ mice were from Dr. Daniel Portnoy (University of California, Berkeley, CA). All other mice including *Ifngr* knockout mice were obtained from The Jackson Laboratory.

293T and HeLa cells obtained from ATCC were grown in DMEM containing 10% (vol/vol) FBS and 2 mM L-glutamine at 37 °C in a 5% (vol/vol) CO_2 incubator. U937 cells also obtained from ATCC were grown in RPMI medium 1640 containing 10% (vol/vol) FBS and 2 mM L-glutamine at 37 °C. Fifty nanomolar PMA was used to differentiate U937 cells into macrophages for 48 h. Vigofect (Vigorous) was used for plasmid transfection in 293T and HeLa cells. Mouse BMMs were prepared as described (1). Immortalized macrophage line derived from C57BL/6 mice was kindly provided by Dr. Katherine A. Fitzgerald and cultured in DMEM containing 10% (vol/vol) FBS and 2 mM L-glutamine.

Immunofluorescence Microscopy. Cells were fixed by 4% paraformaldehyde (Electron Microscopy Sciences) and permeabilized by 0.5% Triton X-100. After blocking with 1% (wt/vol) BSA for 1 h, cells were stained with indicated antibodies for 1–2 h. After three times of wash with PBS, cells were then stained with Alexa Fluor 488/546-conjugated secondary antibodies (Invitrogen) for another hour. DAPI was used for nuclear staining. To stain Flag-tagged proteins, 10% (wt/vol) BSA was used for blocking and 3% (wt/vol) BSA was used to dilute the antibody (1:800). Mitotracker and Lysotracker were used by following the manufacturer's instruction. For Lysotracker staining, cells were imaged without fixation.

DNA Release Assay. At 4 h after infection, macrophages were harvested and washed with PBS twice. For each assay, 2.5×10^7 BMM cells or 1×10^8 U937 cells were used. Cytosolic extracts preparation was described as before (1). Briefly, cells were swelled in 3–4 volumes of ice-cold hypotonic buffer (20 mM Hepes at pH 7.5, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 0.1 mM PMSF, and the Roche protease inhibitor mixture). The cellular membrane was disrupted by 25 times of passage through a 22-gauge needle. pEGFP-N1 plasmid was then added into the cytosolic extracts at 10 ng/100 μL to serve as an internal control before plasmid DNA extraction by using a standard protocol. The extracted DNA was subjected to real-time PCR analysis. The primers (Set 1) for pJB908 were 5′-TCAGGAAGCACAAATGTCAATG-3′ (forward) and 5′-GGTCTACACCACCAAAATCACG-3′ (reverse); the primers for pJB908 (Set 2) were 5′-GCGAACCCGTAGAAAGCC-3′ (forward) and 5′-CGACCCGAGCAAACCCT-3′ (reverse). The primers for pEGFP-N1 were 5′-CCCTCGTGACCACCCTGAC-3′ (forward) and 5′-CGCTCCTGGACGTAGCCT-3′ (reverse). The extracted DNA was also transformed into competent *Escherichia coli* cells. The ratio of transformants growing on the ampicillin plate to those growing on the kanamycin plate was determined as DNA release fold and normalized to that from Lp03 infection.

Macrophage siRNA Transfection and SdhA Expression in *L. pneumophila*. BMMs were transfected with siRNA by using INTERFERin (Polyplus) as recently described (2). *L. pneumophila* infection was performed at 60–72 h after transfection. The scramble siRNA was 5′-UUCUCC GAACGUGUCACG UTT-3′, and the *Aim2*-targeting siRNA was 5′-CAU GUG GAA CAA UUG UGA ATT-3′. The siRNA was synthesized by GenePharma (China). The real-time PCR primers for *Aim2* were 5′-GAATGGGCTGTTTAAAGTCCAGAAG-3′ (forward) and 5′-CCTTCCTCGCACTTTGTTTTGC-3′ (reverse), and the ones for hypoxanthine-guanine phosphoribosyltransferase (as a control) were 5′-TGAAGAGCTACTGTAATGATCAGTCAAC-3′ (forward) and 5′-AGCAAGCTTGCAACCTTAACCA-3′ (reverse).

SdhA expression level in *L. pneumophila* was measured by real-time PCR analysis. The primers for *sdhA* were CGCTCAAAATCCGCAGAAG (forward) and TGGCAGCGGTAGAAAGACTAAA (reverse). Expression of *csrA* (carbon storage regulator CsrA) was used as an internal control. The primers for *csrA* were TTTGACTCGGCGTATAGGTG (forward) and AGCGAACTTGATTGCCTTTT (reverse).

DNA Conjugation Assay. Conjugation assay was carried out essentially as described (3) with slight modification. Briefly, mating was performed by mixing 1×10^9 *L. pneumophila* containing indicated plasmids with a 10-fold excess of a recipient bacteria strain *E. coli* ER1793. The mixed bacterial cultures were incubated for 2 h at 37 °C on prewarmed charcoal-yeast extract media with thymidine (CYET) plates. The plates containing donor and recipient strains were washed twice with sterile water, and the collected washing solutions were diluted and then plated on selective media for counting of donor and conjugants. Triplicate matings were performed for each donor/recipient pair assayed.

1. Gong YN, et al. (2010) Chemical probing reveals insights into the signaling mechanism of inflammasome activation. *Cell Res* 20:1289–1305.
2. Zhao Y, et al. (2011) The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. *Nature* 477:596–600.
3. Vogel JP, Andrews HL, Wong SK, Isberg RR (1998) Conjugative transfer by the virulence system of Legionella pneumophila. *Science* 279:873–876.

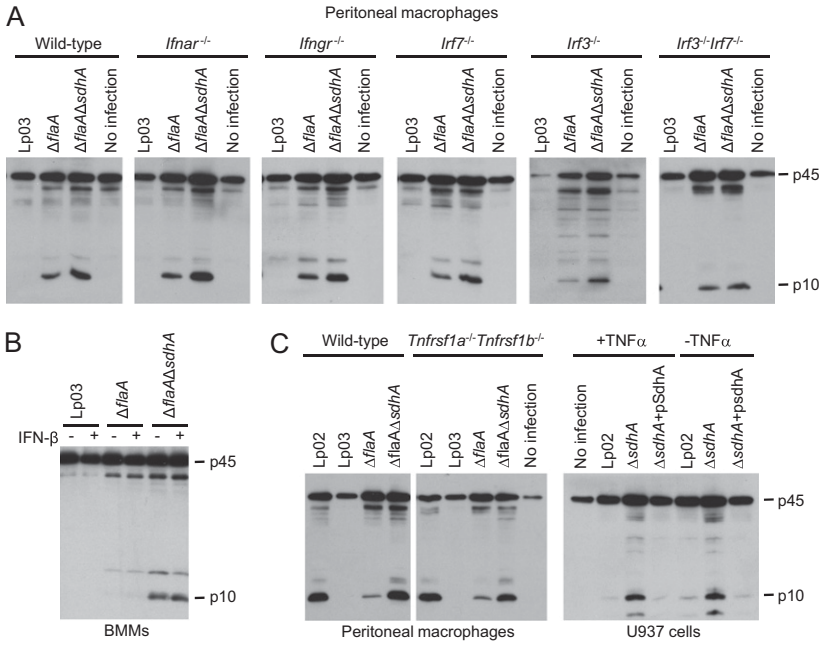

Fig. S1. The IFN and TNFα signalings are dispensable for Δ*sdhA*-induced caspase-1 activation. Primary peritoneal macrophages (*A* and *C Left*) or BMMs (*B*) were infected with indicated *L. pneumophila* strains. To analyze the genetic requirements of IFN and TNFα signaling, macrophages were derived from the corresponding knockout mice as indicated (*A* and *C Left*). IFN-β (250 U/mL) and TNFα (10 ng/mL) were used to treat wild-type BMM cells (*B*) and U937 cells (*C Right*), respectively, for 30 min before infection. Caspase-1 immunoblots of culture supernatant were shown.

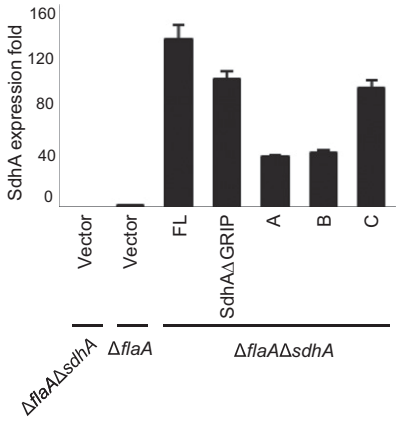

Fig. S2. qRT-PCR measurements of SdhA expression in *L. pneumophila*. SdhA [full-length (FL) or truncation mutants] or an empty vector was transformed into indicated *L. pneumophila* strains for complementation analyses. SdhAΔGRIP is described in Fig. 5; SdhA_A, SdhA_B, and SdhA_C are illustarted in Fig. 6. *L. pneumophila csrA* (carbon storage regulator CsrA) was used for normalization. Mean values ± SEM (error bars) from three independent experiments are shown.

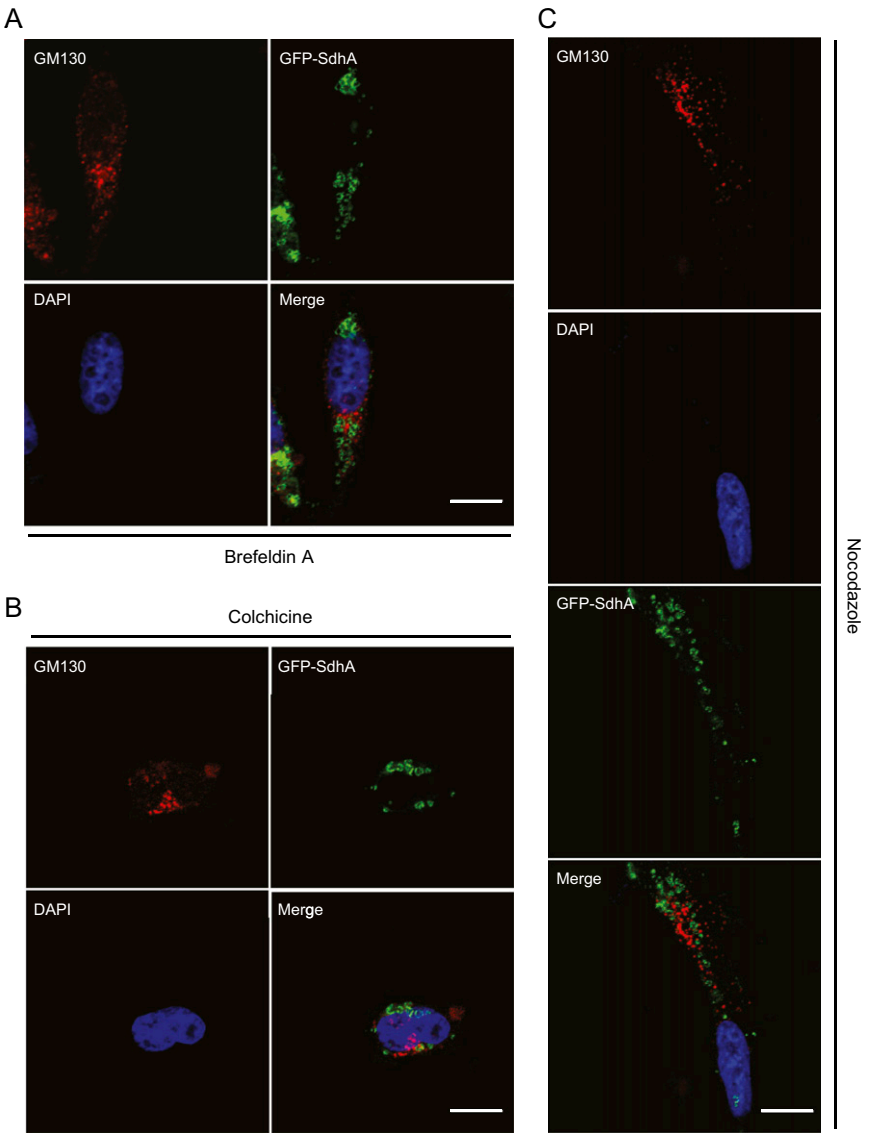

Fig. S3. Effects of Golgi disruption on SdhA ring structure. GFP-SdhA was expressed in HeLa cells. The Golgi apparatus was marked by anti-GM130 staining. Brefeldin A (10 μg/mL)(*A*), colchicine (20 μM)(*B*), and nocodazole (1 mM)(*C*) were used to disrupt the Golgi structure as shown. (Scale bars: 7.5 μm.)

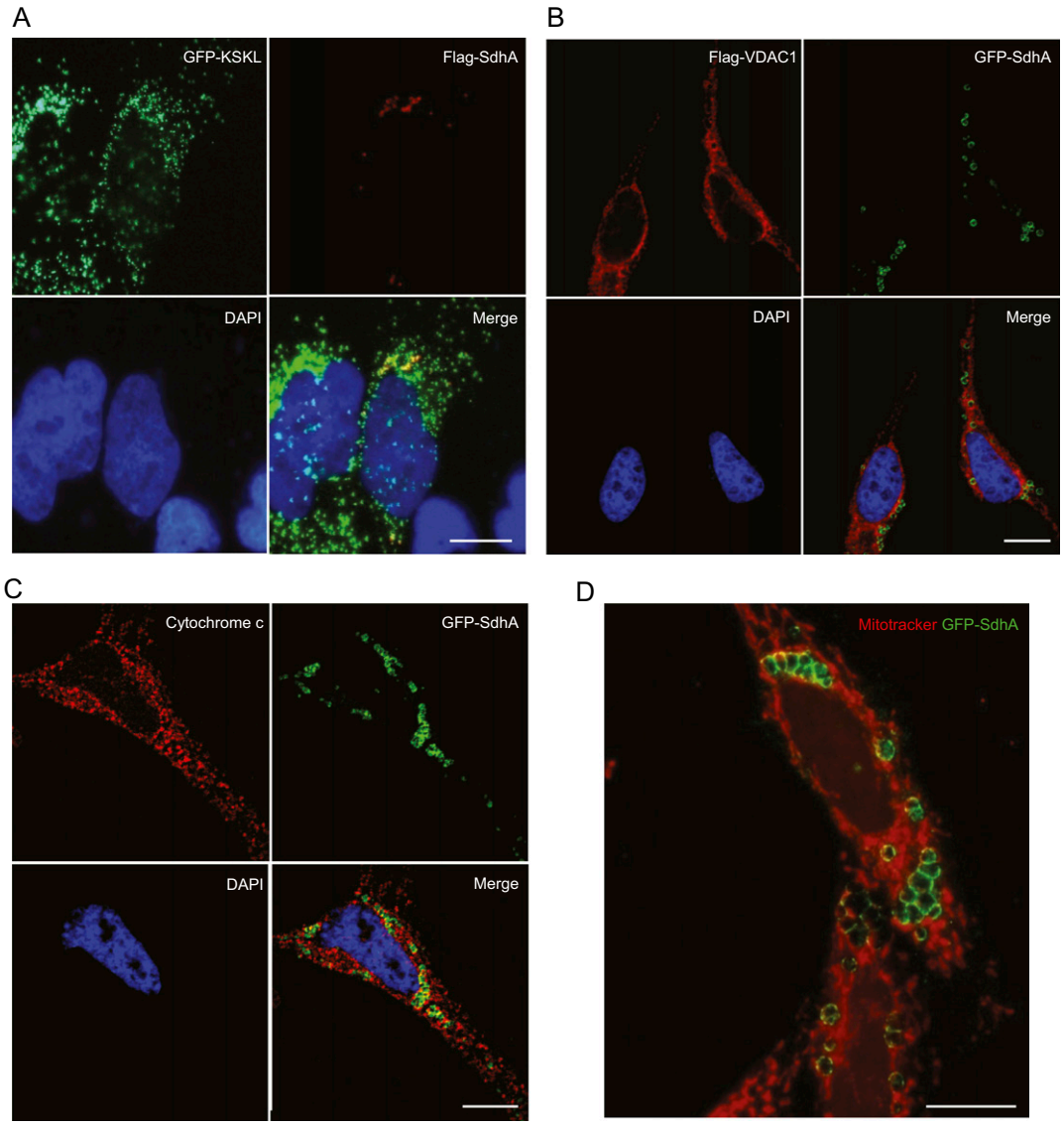

Fig. S4. Colocalization of SdhA ring structure with peroxisome and mitochondria markers. Flag or GFP-tagged SdhA was expressed in HeLa cells. Transfected GFP-KSKL (the KSKL sequence was fused to the C terminus of GFP) labels the peroxisome (*A*). Mitochondria were visualized by Flag-VDAC1 (*B*), cytochrome *c* (*C*), and Mitotracker staining (*D*). (Scale bars: 7.5 μm.)

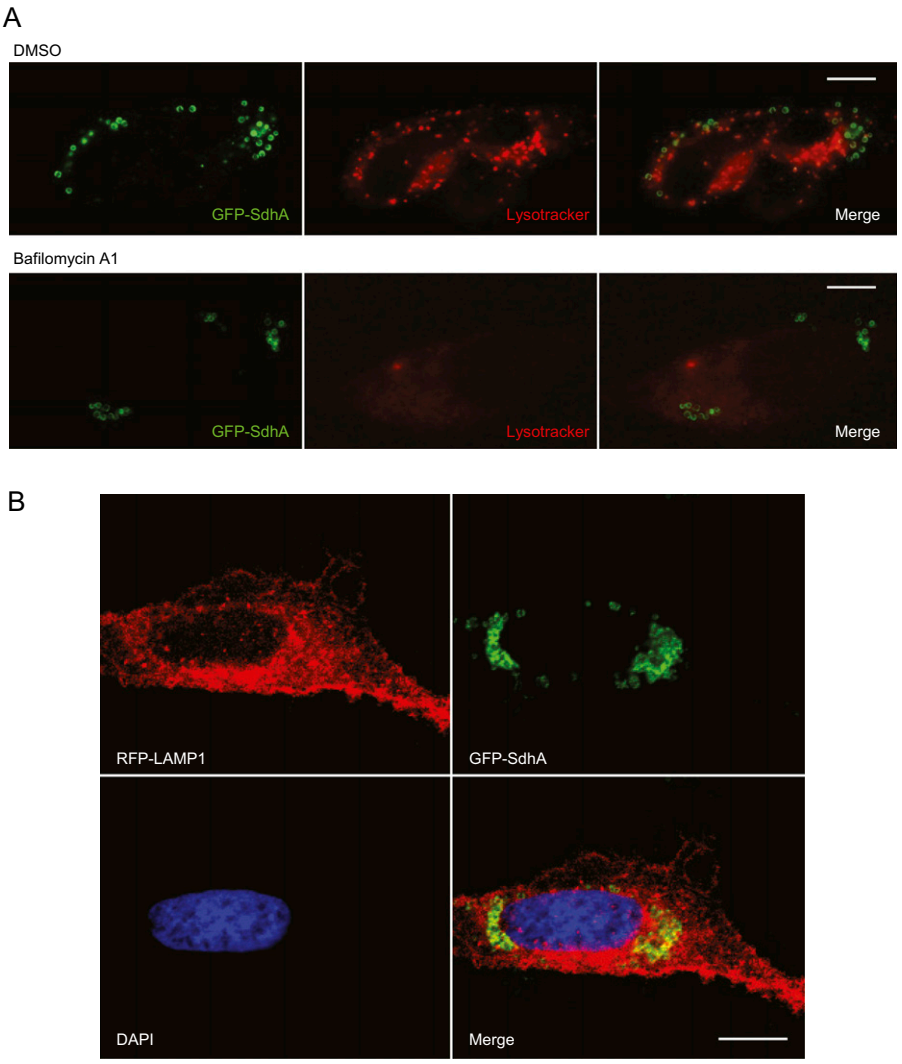

Fig. S5. Colocalization of SdhA ring structure with lysosome markers and effects of lysosome disruption. GFP-SdhA was expressed in HeLa cells. Lysosome was stained with Lysotracker in *A* and RFP-LAMP1 in *B*. Cells were also treated with bafilomycin A1 (400 nM) to block lysosome biogenesis in *A*. (Scale bars: 7.5 μm.)

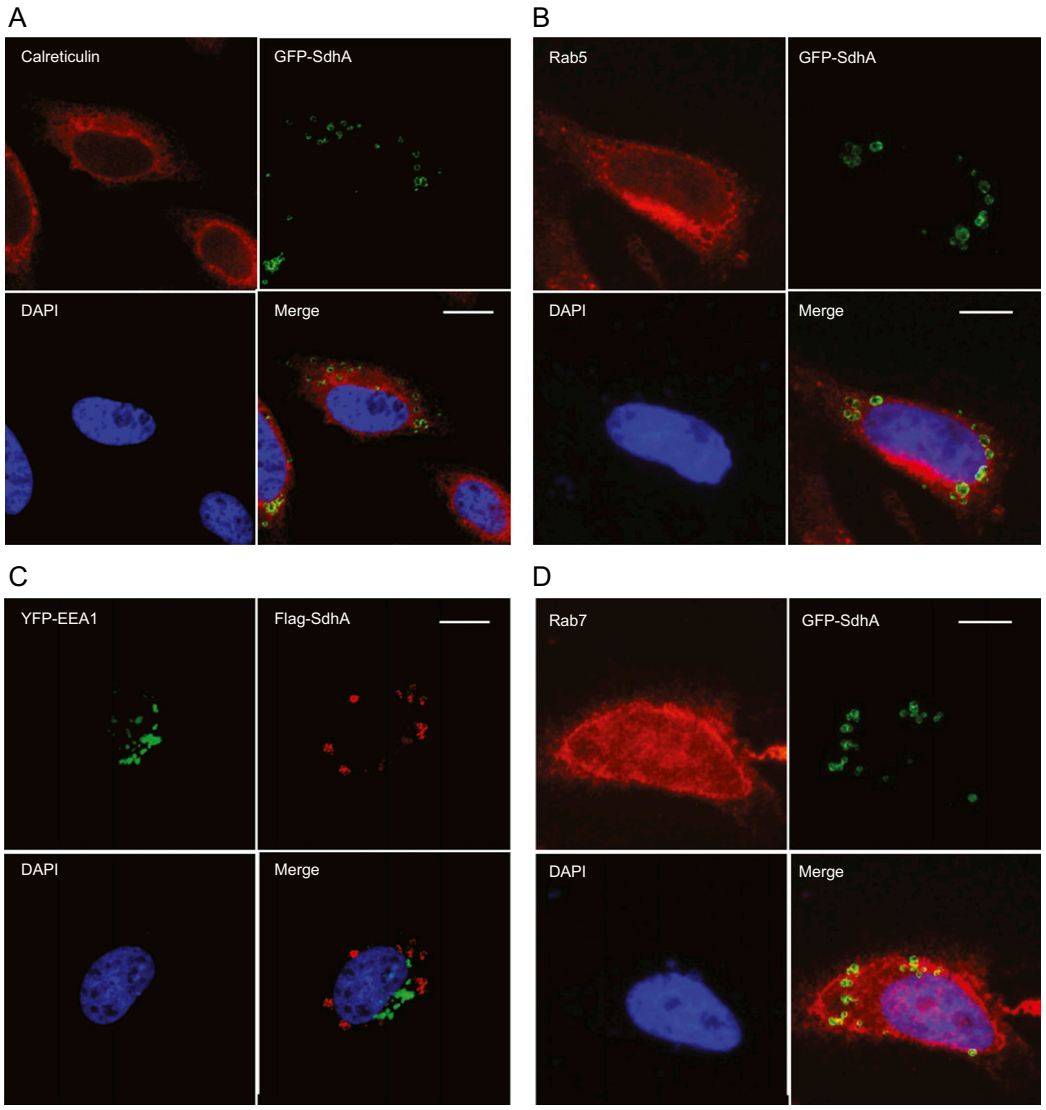

Fig. S6. Colocalization of SdhA ring structure with endoplasmic reticulum and early endosome markers. GFP-SdhA was expressed in HeLa cells. Calreticulin stains endoplasmic reticulum (*A*); Rab5 and YFP-EEA1 staining mark the early endosome (*B* and *C*); and Rab7 is a later endosome marker (*D*). (Scale bars: 7.5 μm.)

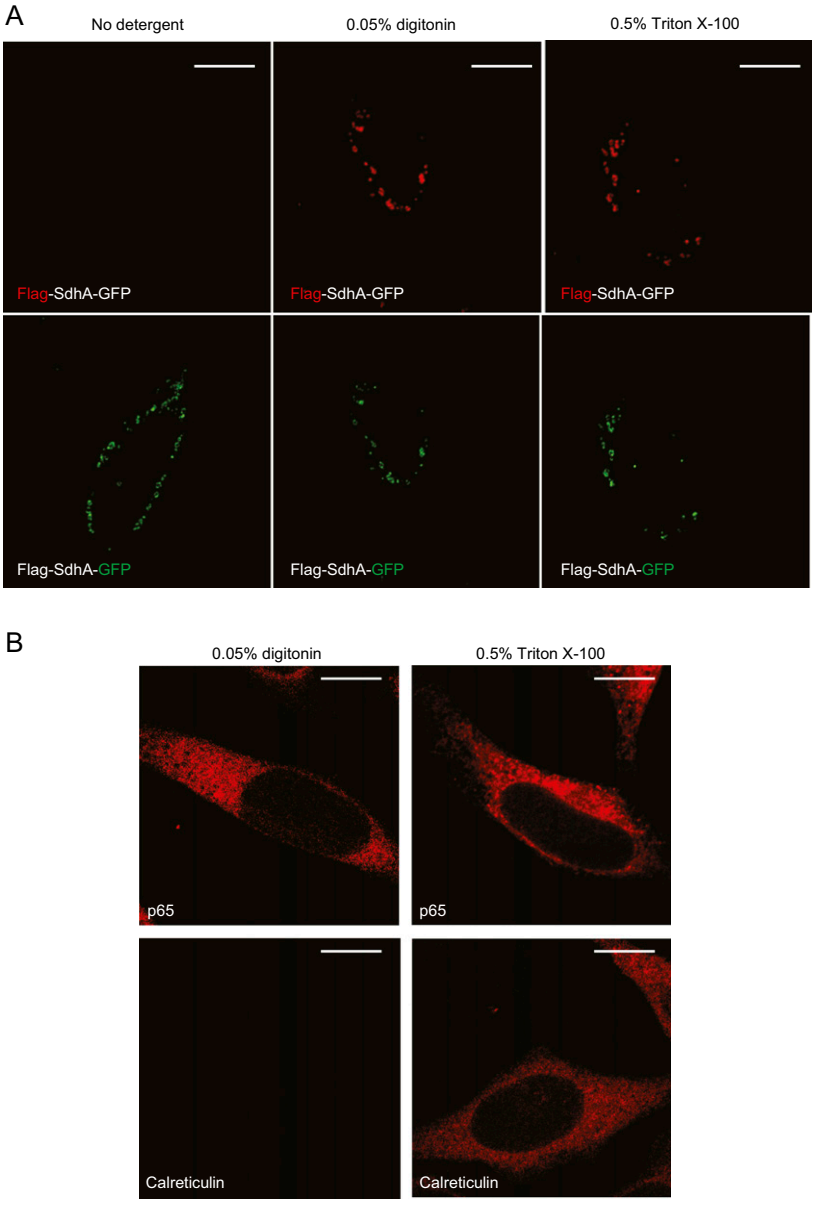

Fig. S7. Effects of different permeabilization detergents on anti-Flag immunostaining of SdhA ring structure formed by Flag-SdhA-GFP (*A*). (*A*) SdhA tagged with a Flag epitope at the N terminus and GFP at the C terminus was transfected into HeLa cells. Fixed cells were imaged directly or permeabilized with digitonin or Triton X-100 before fluorescence imaging. *Upper* and *Lower* show anti-Flag and GFP staining, respectively. (*B*) Fixed HeLa cells were permeabilized with digitonin or Triton X-100 as in *A*. Immunostaining was performed by using anti-p65 antibody (*Upper*) and anti-calreticulin antibody (*Lower*), respectively. (Scale bars: 7.5 μm.)

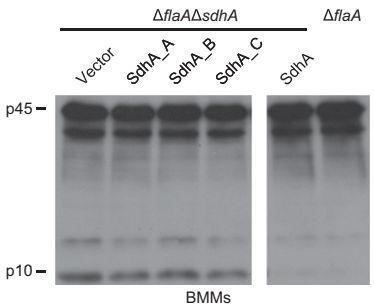

Fig. S8. Complementation assays of SdhA truncation mutants. BMMs derived from 129S mice were infected with *L. pneumophila* Δ*flaA* or Δ*flaA*Δ*sdhA* strain transformed with a plasmid expressing full-length SdhA or the indicated truncation mutants. Caspase-1 immunoblots of culture supernatant were shown.

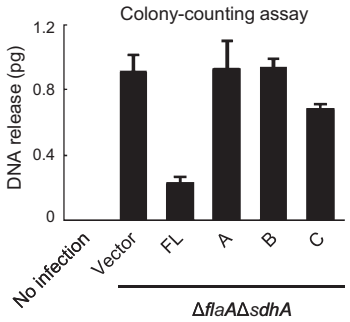

Fig. S9. Assay of bacterial DNA release from SdhA complementation strains into macrophage cytosol. PMA-differentiated U937 cells were infected with *L. pneumophila* Δ*flaA*Δ*sdhA* strains transformed with a plasmid expressing full-length SdhA or the indicated truncation mutants. DNA fractions extracted from the cytosol of infected macrophages were subjected to colony-counting assay for measuring DNA release.

Table S1. Effects of *sdhA* deletion on Dot/Icm-mediated DNA conjugative transfer

Donor *L. pneumophila* strain	Donor vector	Mating media	Recipient	Selection media	No. of conjugants per donor
Lp03	pJB908(w/o oriT)	CYET	*E.coli* ER1793	LB+Amp	$<7.5 \times 10^{-9}$
Δ*flaA*	pJB908(w/o oriT)	CYET	*E.coli* ER1793	LB+Amp	$<7.5 \times 10^{-9}$
Δ*flaA*Δ*sdhA*	pJB908(w/o oriT)	CYET	*E.coli* ER1793	LB+Amp	$<7.5 \times 10^{-9}$
Lp03	pJB2819(w/ oriT)	CYET	*E.coli* ER1793	LB+Kan	$<6.4 \times 10^{-8}$
Δ*flaA*	pJB2819(w/ oriT)	CYET	*E.coli* ER1793	LB+Kan	3.2×10^{-6}
Δ*flaA*Δ*sdhA*	pJB2819(w/ oriT)	CYET	*E.coli* ER1793	LB+Kan	8.7×10^{-6}