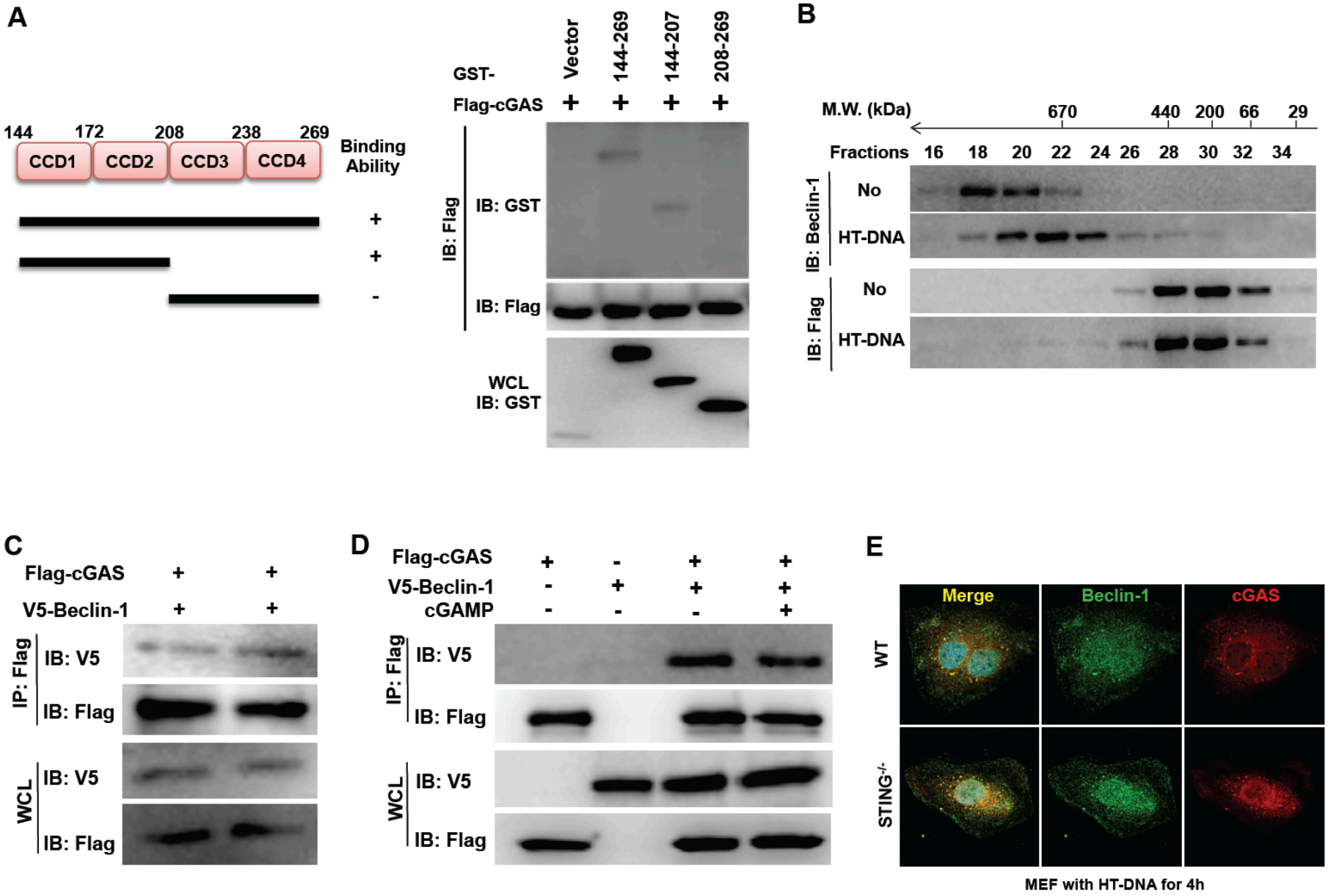
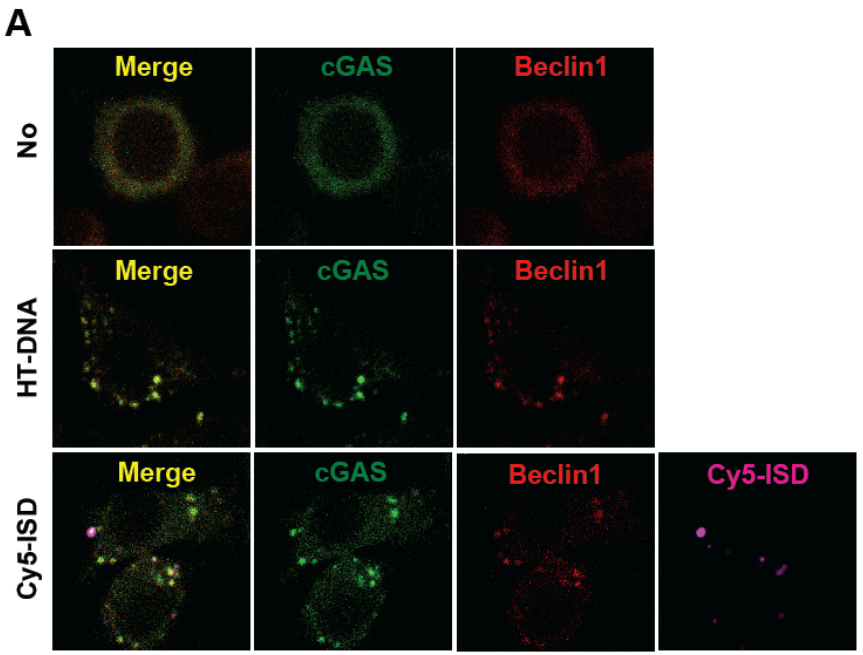
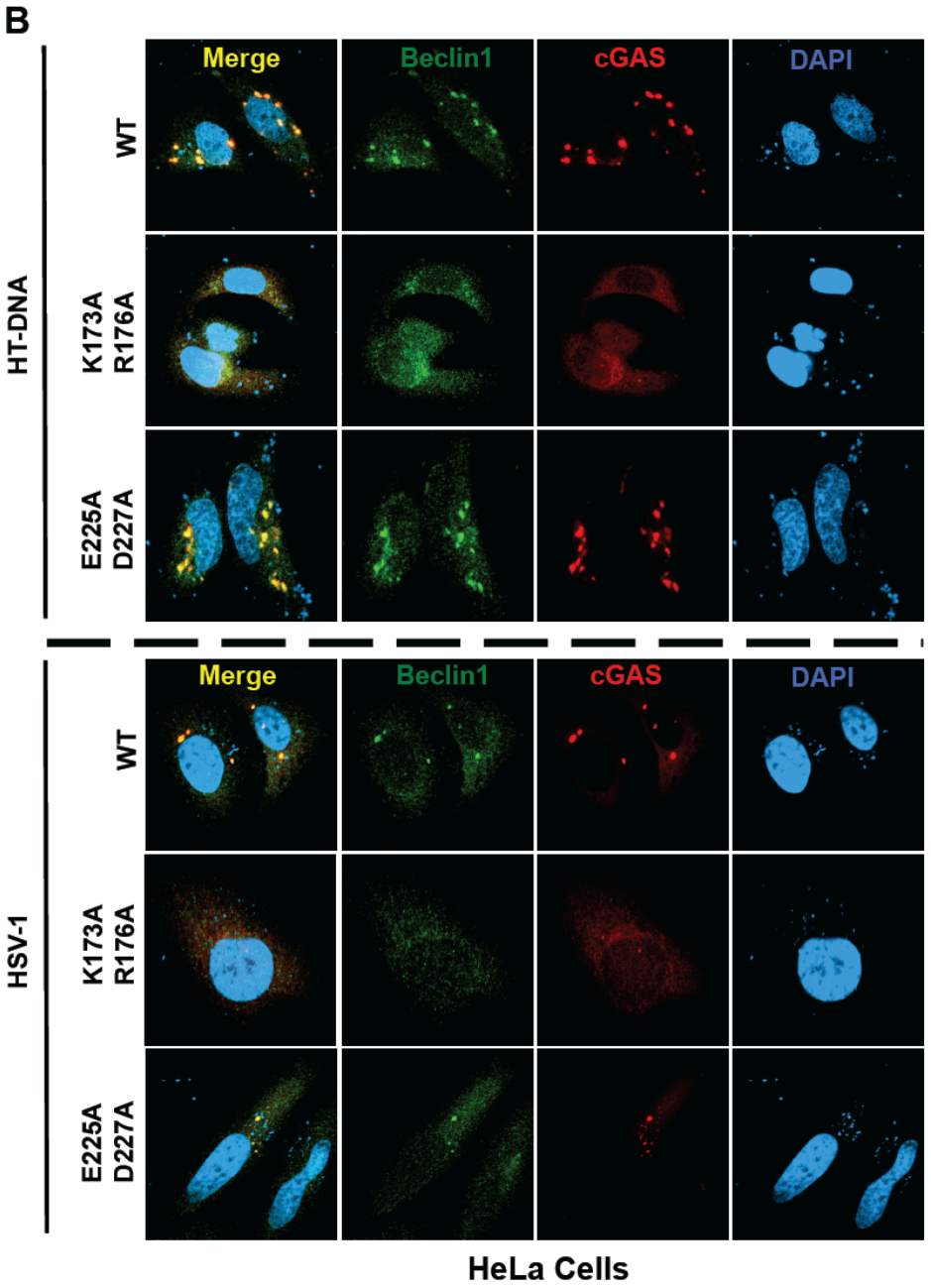
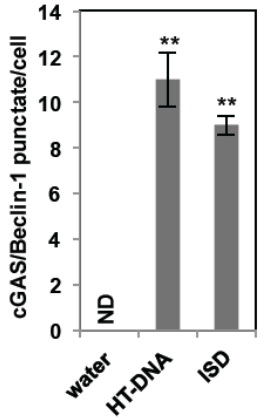
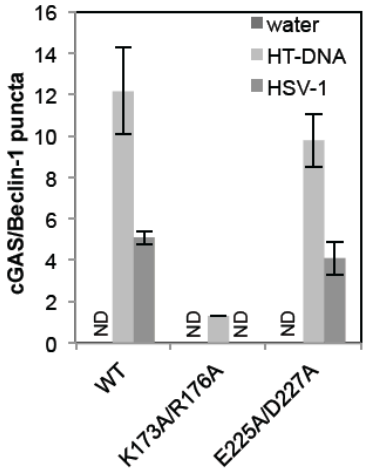


Supplement Figure 1

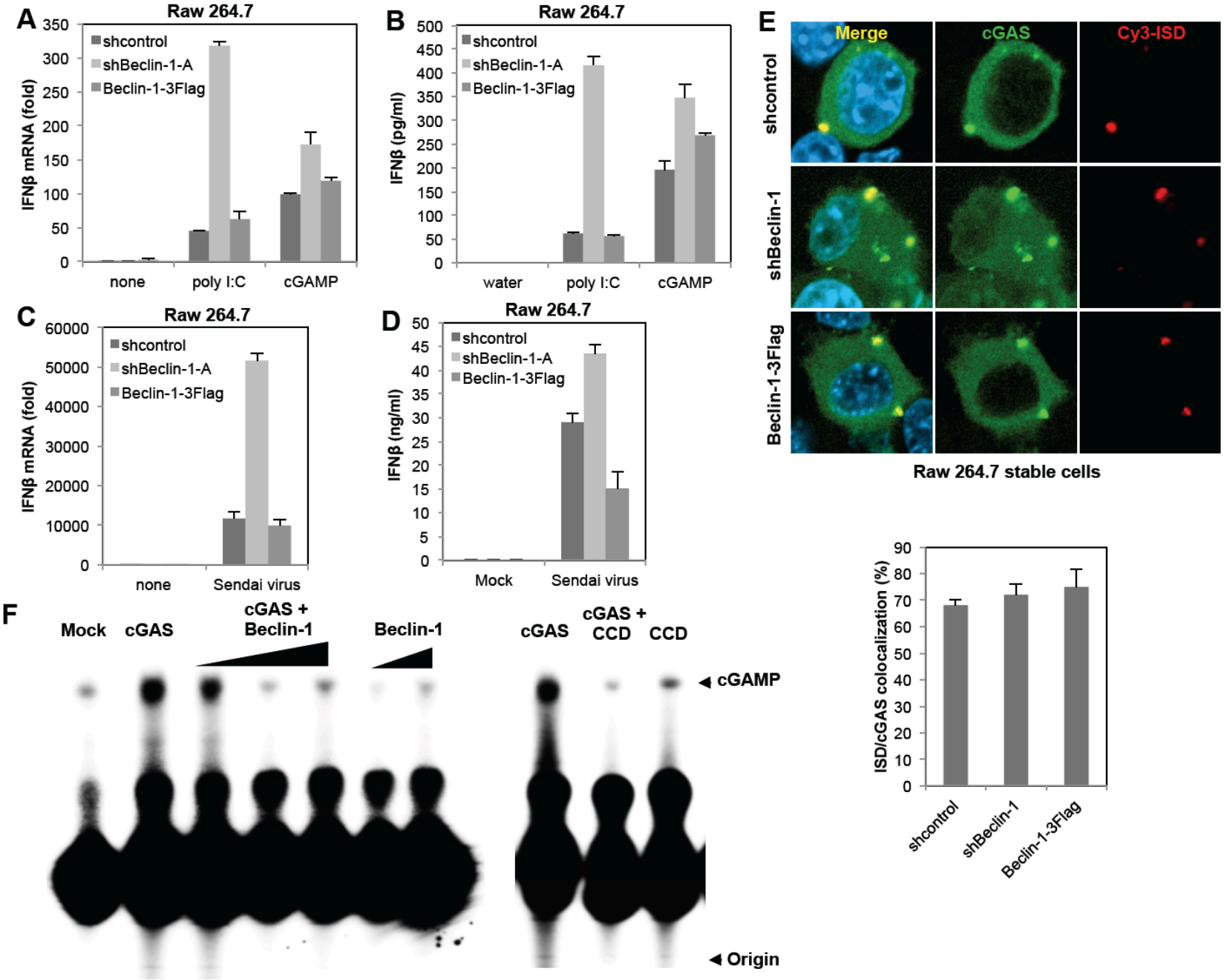




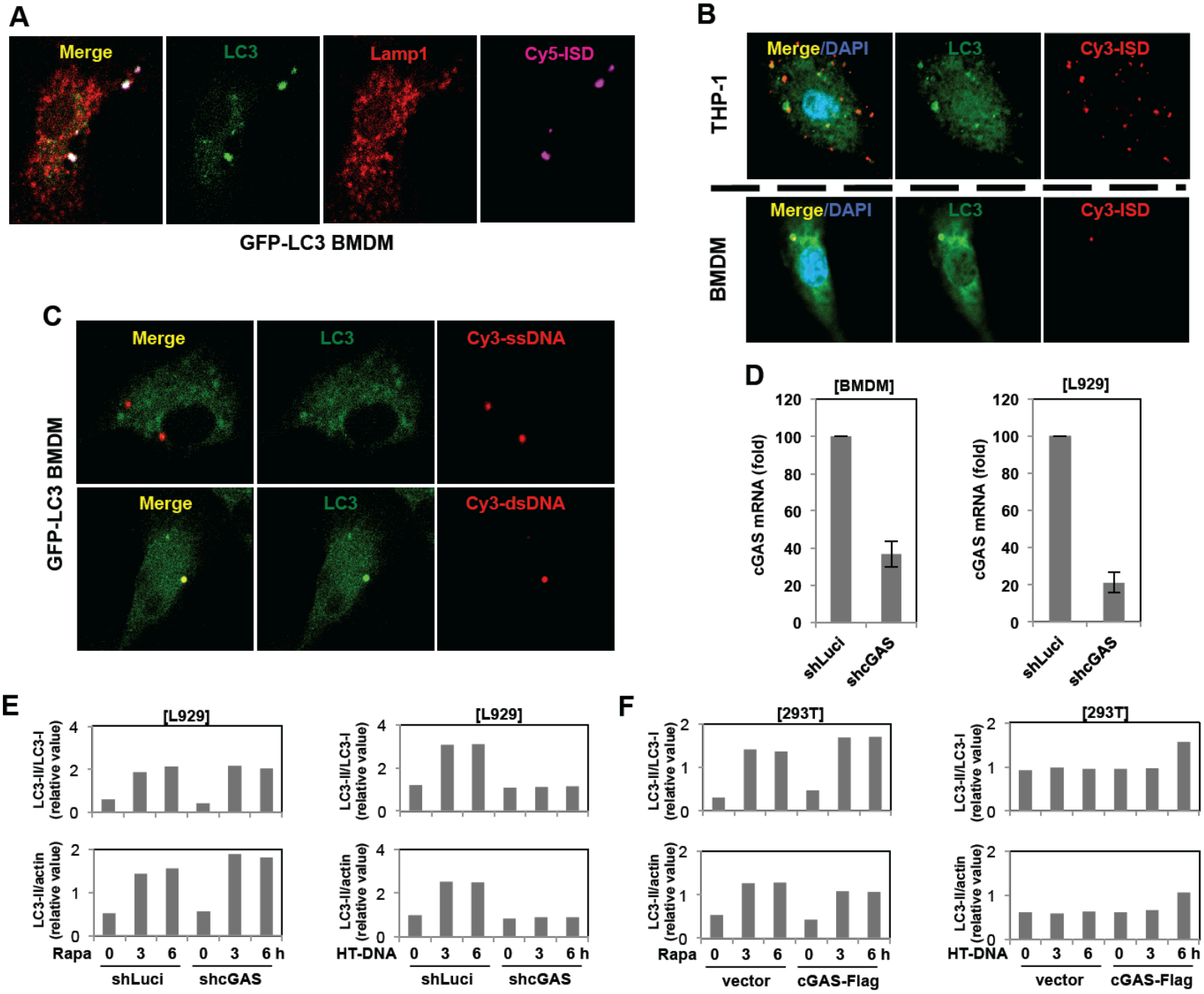




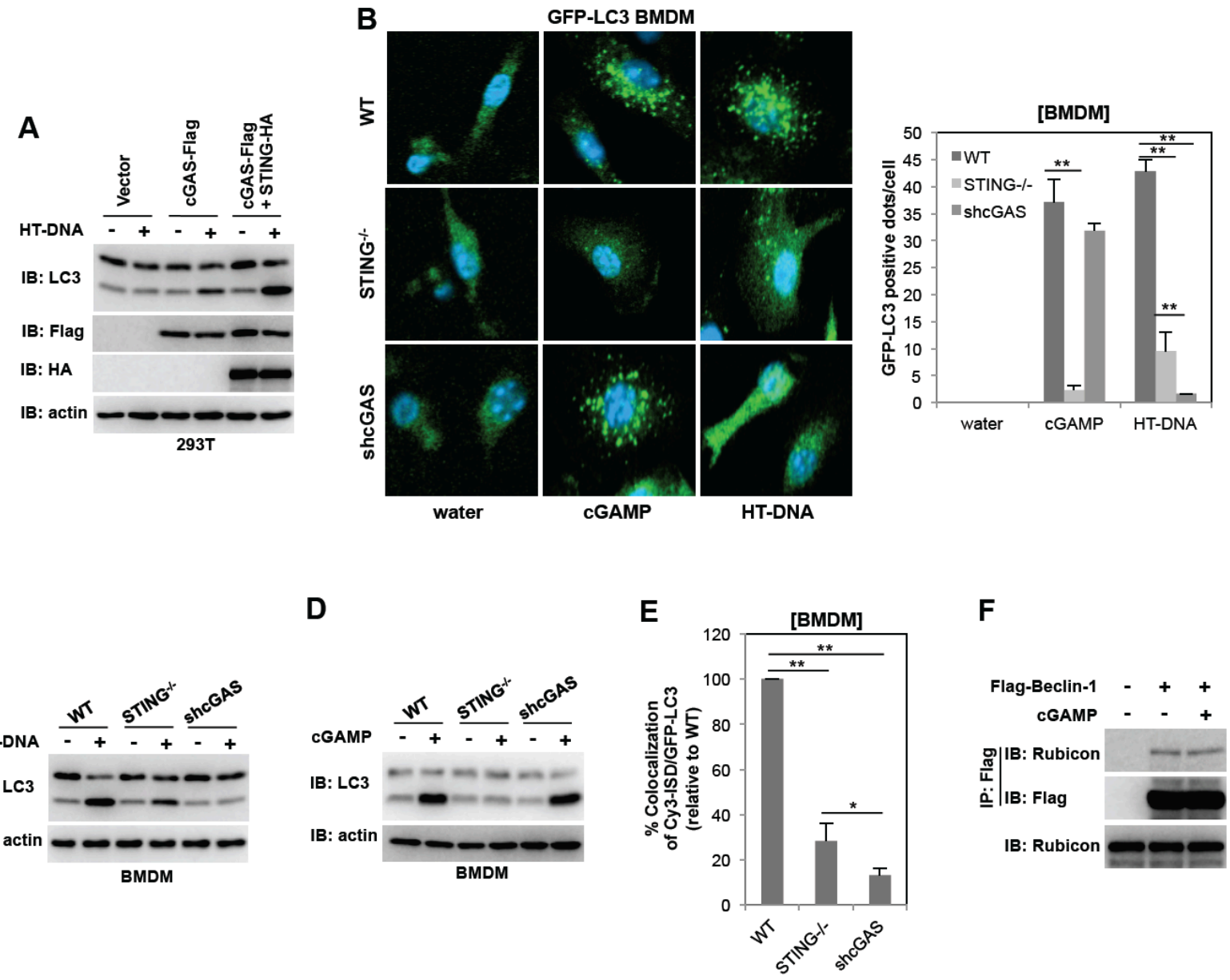
Supplement Figure 2



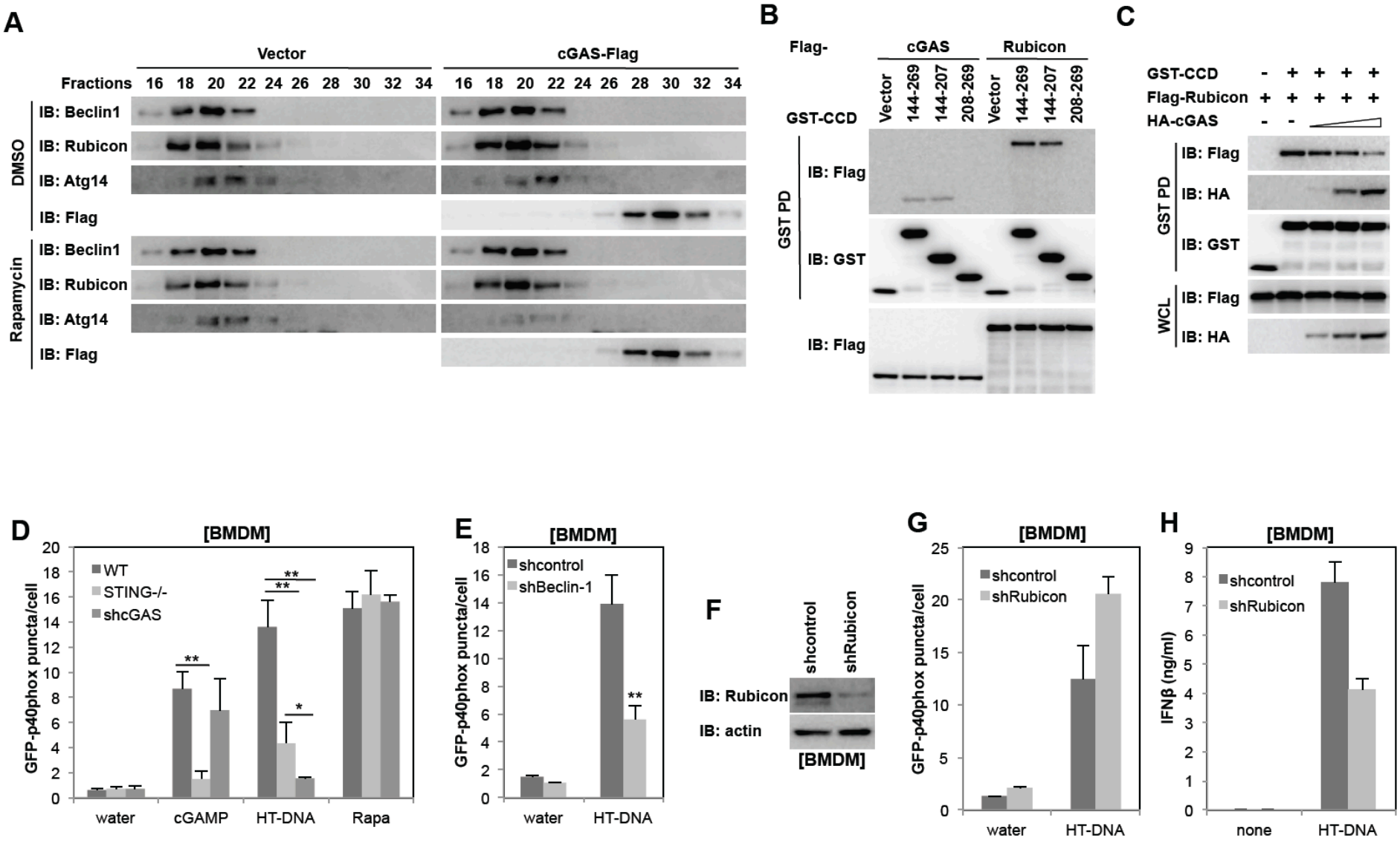
Supplement Figure 3



Supplement Figure 4



Supplement Figure 5



Supplement Figure 6

Supplement Information

Supplement Figure Legends

FIGURE S1. Roles of cGAMP and STING in the association between cGAS and Beclin-1, related to Figure 1. (A) Detailed mapping of Beclin-1 CCD domain for cGAS binding. 293T cells were co-transfected with Flag-cGAS and pEBG glutathione S transferase (GST) vector, pEBG-CCD or its truncated mutants. Cells were harvested at 48 h posttransfection and subjected to IP and IB with indicated antibody (right panel). The diagram indicates the 4 repetitive short CCD and their cGAS binding abilities: +, binding; -, no binding (left panel) (B) cGAS co-fractionates with Beclin-1 upon HT-DNA transfection. 293T-cGAS-Flag cells were transfected with mock or HT-DNA (2μg/ml) for 6 h and WCLs were harvested, fractionated by gel-filtration, and analyzed by IB with indicated antibodies. (C) cGAS binds to Beclin-1 in both wild-type and STING$^{-/-}$ MEFs. Wild-type or STING$^{-/-}$ MEFs were transfected with Flag-cGAS and V5-Beclin-1. Cells were harvested at 48 h posttransfection and subjected to IP and IB with indicated antibodies. (D) cGAMP does not affect the interaction between cGAS and Beclin-1. 293T cells were transfected with Flag-cGAS in the present or in the absence of V5-Beclin-1. Cells were treated with water or cGAMP for 6h, lysed, and subjected to IP and IB with indicated antibodies. (E) cGAS-Beclin-1 colocalization does not depend on STING expression. Wild-type or STING$^{-/-}$ MEFs were transfected with Flag-cGAS and V5-Beclin-1. At 36h posttransfection, MEFs were stimulated with HT-DNA (2μg/ml) for 4h, fixed, stained with anti-Flag (red) or anti-V5 (green) antibodies, and subjected to confocal microscopy analysis.

FIGURE S2. Colocalization between cGAS and Beclin-1 upon dsDNA or HSV-1 stimulation, related to Figure 1. (A) Beclin-1 colocalizes with cGAS upon dsDNA stimulation.

Raw 264.7 cells stably expressing GFP-cGAS (green) and mCherry-Beclin-1 (red) were stimulated with HT-DNA or Cy5-ISD for 6h. Cells were fixed and subjected to confocal microscope analysis. (B) The DNA binding activity of cGAS is required for the co-localization with Beclin-1. At 36h posttransfection with Flag-cGAS WT, DNA-binding mutant (K173A/R176A), or enzymatic dead mutant (E225A/D227A), Hela cells were stimulated with HT-DNA or HSV-1 (MOI=5) for 4h, fixed, stained with anti-Flag (red) and anti-Beclin-1 (green) antibodies, and subjected to confocal microscopy analysis. The bottom two panels show the colocalization index (# of puncta/cell) between cGAS and Beclin-1. (**p<0.005)

FIGURE S3. Beclin-1 does not affect the colocalization between cGAS and dsDNA, but blocks cGAS enzymatic activity, related to Figure 2 and Figure 3. (A-D) The role of Beclin-1 in polyI:C or cGAMP-induced IFN responses. Raw-shcontrol, Raw-shBeclin-1 and Raw-Beclin-1-3Flag cell lines were stimulated with poly (I:C), cGAMP (A and B) or sendai virus (C and D). IFNβ mRNA levels were measured by qPCR at 6h post-stimulation (A and C) and IFNβ production was measured by ELISA at 12h post-stimulation (B and D). (E) Beclin-1 does not affect the colocalization between cGAS and dsDNA. Raw-shcontrol, Raw-shBeclin-1 and Raw-Beclin-1-3Flag cell lines were transfected with GFP-cGAS (green). At 36h posttransfection, cells were stimulated with Cy3-ISD (red) for 4h, fixed, and subjected to confocal microscopy analysis. (F) *In vitro* enzymatic assays were performed in the presence of P^{32}-α-ATP and GTP with cGAS (141-507aa) and full-length Beclin-1 or CCD domain purified from *E. coli*. cGAMP production was analyzed by TLC and autoradiograph. The bottom arrow shows the spotted origin and the top arrow shows the migrated cGAMP.

FIGURE S4. Colocalization between LC3 and dsDNA in a cGAS-dependent manner, related to Figure 4. (A) Colocalization among GFP-LC3 (green), LAMP1 (red) and Cy5-ISD (pink) in GFP-LC3 BMDMs. GFP-LC3 BMDMs were stimulated with Cy5-ISD for 4h, fixed,

stained with anti-LAMP1 antibody, and subjected to confocal microscope analysis. (B) Colocalization between Cy3-ISD (red) and endogenous LC3 (green) in THP-1 or BMDM cells. THP-1 cells or BMDMs were stimulated with Cy3-ISD for 4h, fixed, stained with anti-LC3 antibody, and subjected to confocal microscope analysis. (C) dsDNA, but not ssDNA, colocalizes with LC3 in GFP-LC3 BMDM cells. GFP-LC3 BMDMs were stimulated with Cy3-labeled dsDNA or ssDNA for 4h, fixed, and subjected to confocal microscope analysis. (D) qPCR analysis of cGAS mRNA levels in L929 cells or BMDMs after lentivirus-shRNA-mediated gene depletion. (E) Ratios of LC3-II/LC3-I and LC3-II to actin in Figure 4C. (F) Ratios of LC3-II/LC3-I and LC3-II to actin in Figure 4D.

FIGURE S5. The role of STING in dsDNA or cGAMP-induced autophagy, related to Figure 4. (A) 293T-vector, 293T-cGAS, or 293T-cGAS/STING cells were stimulated with HT-DNA for 6h, lysed, and subjected to IB with indicated antibodies. (B) Wild-type, STING$^{-/-}$ or cGAS knockdown BMDMs were infected with lentivirus carrying GFP-LC3. At 48h postinfection, cells were stimulated with cGAMP or HT-DNA for 6h, fixed, and subjected to confocal microscopy analysis. (C and D) Wild-type, STING$^{-/-}$ or cGAS knockdown BMDMs were stimulated with HT-DNA (C) or cGAMP (D) for 6h, lysed, and subjected to IB with indicated antibodies. (E) Wild-type, STING$^{-/-}$ or cGAS knockdown BMDMs were infected with lentivirus carrying GFP-LC3. At 48h postinfection, cells were stimulated with Cy3-ISD for 6h, fixed, and subjected to confocal microscopy analysis. (F) cGAMP does not cause the dissociation of Rubicon from the Beclin-1 complex. 293T cells were transfected with Flag-Beclin-1. At 48h posttransfection, cells were stimulated with cGAMP for 6h, lysed, and subjected to IP and IB with indicated antibodies. (*p<0.05, **p<0.005)

FIGURE S6. The role of Rubicon in dsDNA-induced autophagy and IFN responses, related to Figure 6. (A) Gel filtration of Beclin-1 complexes upon rapamycin stimulation. 293T-

vector or 293T-cGAS-Flag cells were stimulated with rapamycin for 6h and WCLs were harvested, fractionated, and analyzed by IB with indicated antibodies. (B) Both cGAS and Rubicon binds to the first two CCD of Beclin-1 (aa144-207). 293T cells were co-transfected with Flag-cGAS or Flag-Rubicon and pEBG glutathione S transferase (GST) vector, pEBG-CCD or its truncated mutants. Cells were harvested at 48 h posttransfection and subjected to GST pull down and IB with indicated antibodies. (C) cGAS competes with Rubicon for Beclin-1 CCD binding. 293T cells were transfected with GST-CCD and Flag-Rubicon with an increasing amount HA-cGAS. Cells were harvested at 48 h posttransfection and subjected to GST pull down and IB with indicated antibodies. (D) Wild-type, $STING^{-/-}$ or cGAS knockdown BMDMs were transfected with GFP-p40phox-PX. At 48h postinfection, cells were stimulated with cGAMP, HT-DNA or rapamycin for 6h, fixed, and subjected to confocal microscopy analysis. (E) BMDM-shcontrol and BMDM-shBeclin-1 were transfected with GFP-p40phox-PX. At 48h postinfection, cells were stimulated with HT-DNA for 6h, fixed, and subjected to confocal microscopy analysis. (F) Lentivirus-shRNA-mediated Rubicon knockdown in BMDMs. (G) BMDM-shcontrol and BMDM-shRubicon were transfected with GFP-p40phox-PX. At 48h postinfection, cells were stimulated with HT-DNA for 6h, fixed, and subjected to confocal microscopy analysis. (H) BMDM-shcontrol and BMDM-shRubicon were stimulated with HT-DNA. IFNβ production was measured by ELISA at 12h post-stimulation. (*p<0.05, **p<0.005)