SUPPLEMENTARY INFORMATION

STING activation by translocation from the ER associated with infection and autoinflammatory disease

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Figure S1-6

Movie 1

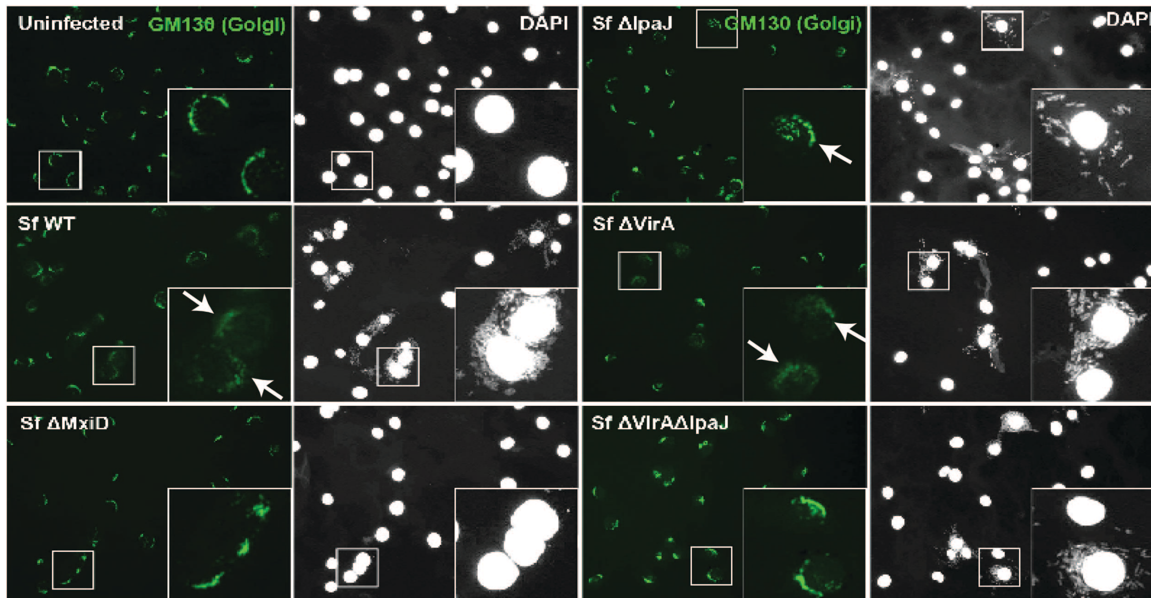
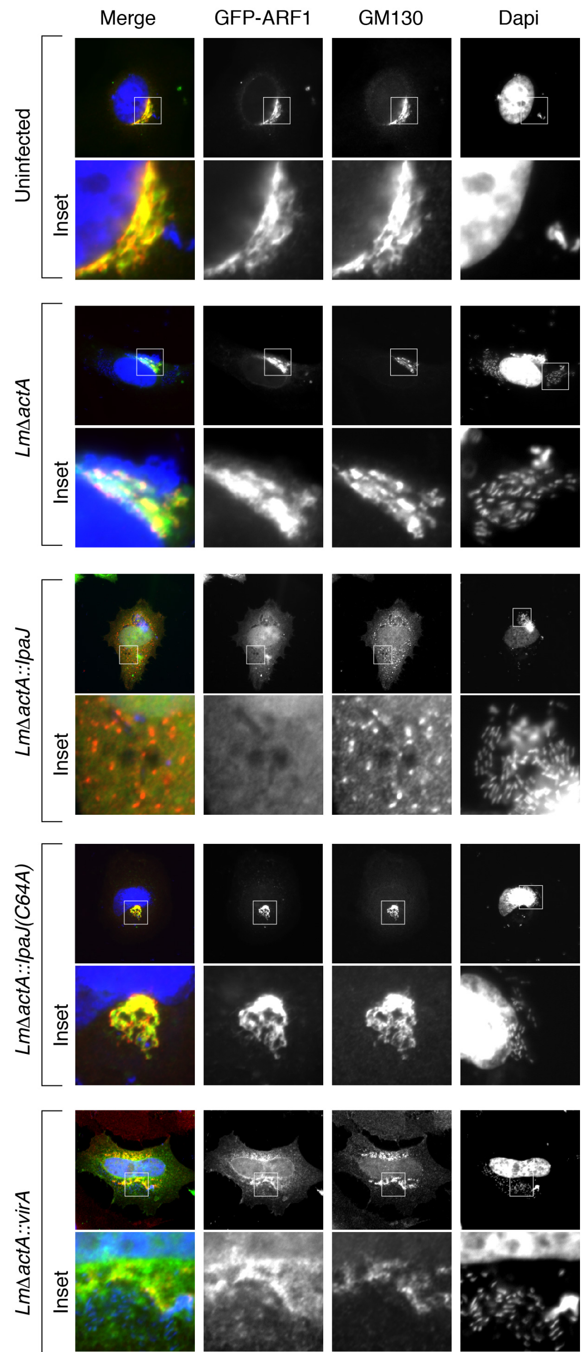
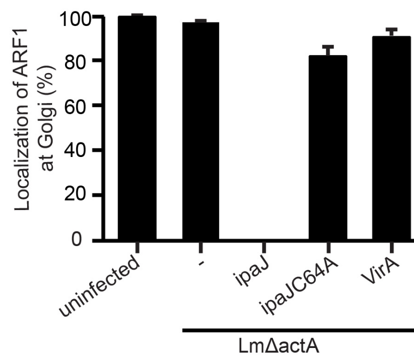


Figure S1, related to Figure 1. *Shigella* IpaJ and VirA disrupt Golgi apparatus in mouse embryonic fibroblasts (MEFs). WT MEFs were infected with indicated *Shigella* strains for 8 hours. Cells were then fixed and stained with anti-GM130 antibody (green) to visualize Golgi apparatus integrity and DAPI to visualize intracellular bacteria. Sf, *Shigella flexneri*. Arrows indicate disrupted Golgi apparatus. Images are representative of three independent experiments.



Merge
GFP-ARF1
GM130
Dapi
Uninfected
Inset
LmΔactA
Inset
LmΔactA::IpaJ
Inset
LmΔactA::IpaJ(C64A)
Inset
LmΔactA::virA
Inset



Localization of ARF1 at Golgi (%)
100
80
60
40
20
0
uninfected
-
ipaJ
ipaJC64A
VirA
LmΔactA

Figure S2, related to Figure 2. IpaJ expressed from *ListeriaΔactA::ipaJ* disrupt ARF1 association with the Golgi. ARF1-GFP expressing HeLa cells were uninfected or infected with *Listeria* strain indicated on the left. ARF1-GFP localization to the Golgi (GM130) was shown by microscopy and quantified below. Data a representative of at least two independent experiments.

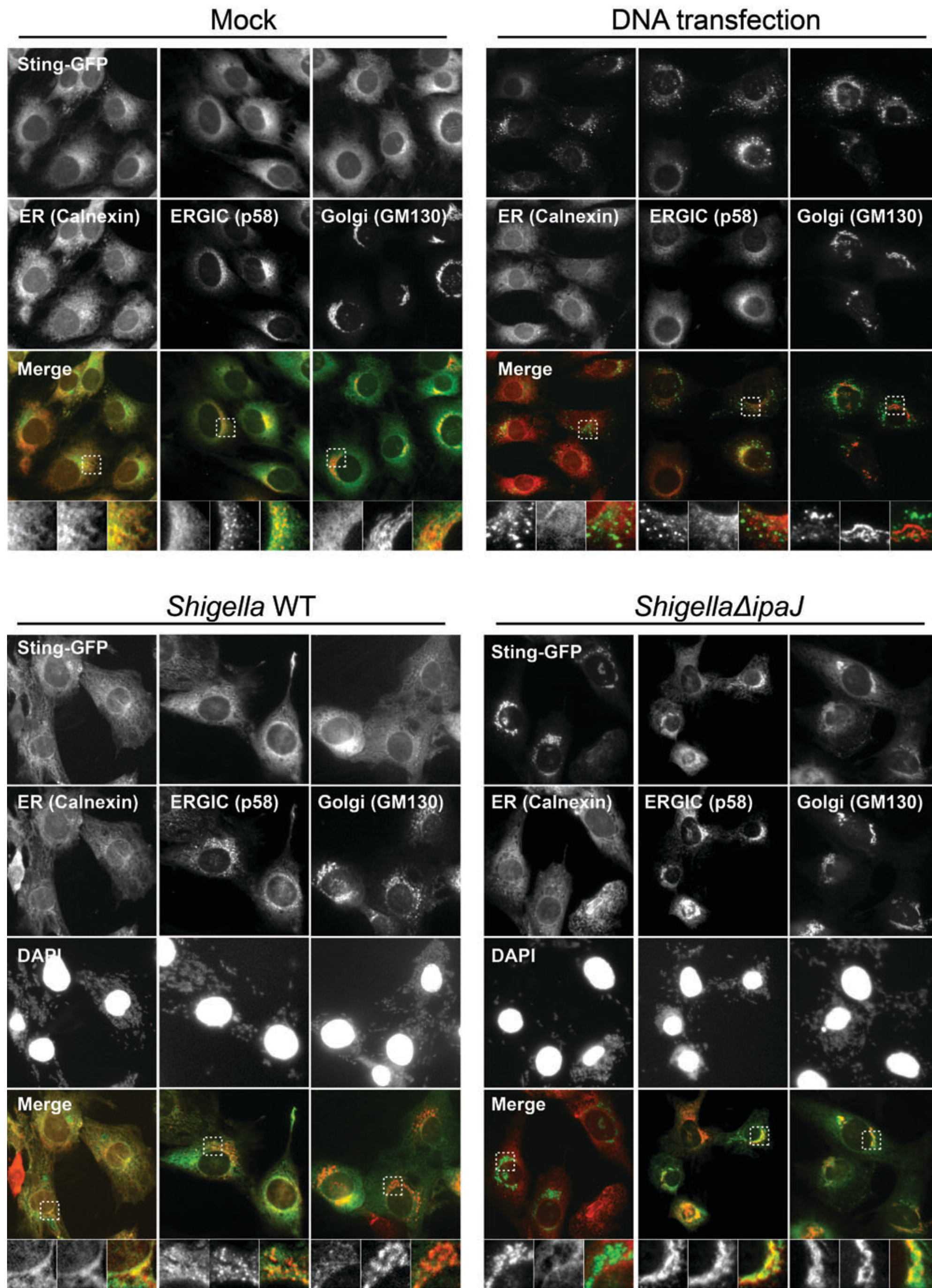
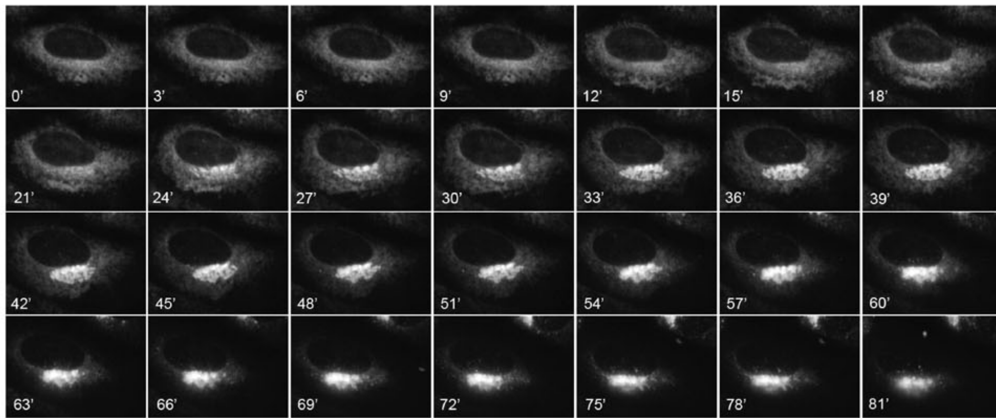
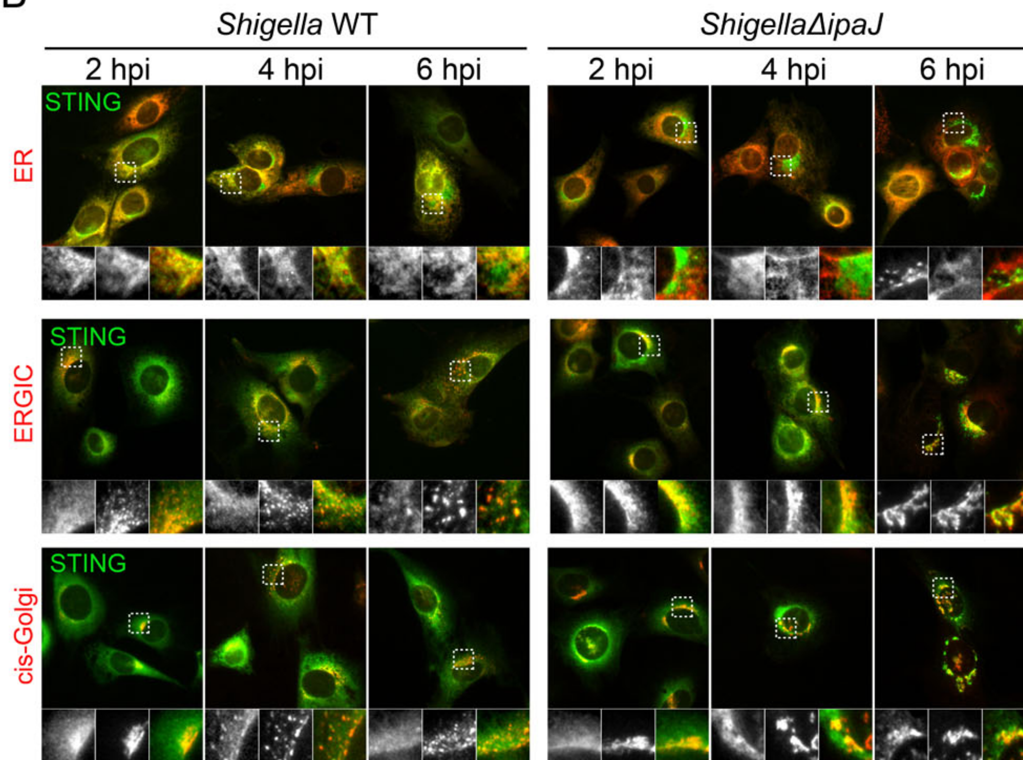


Figure S3, related to Figure 3. IpaJ blocks STING ER exit. Fluorescent micrographs show STING-GFP localization in MEFs after DNA transfection or *Shigella* infection. Expanded panels are shown here (as in **Figure 3B**).

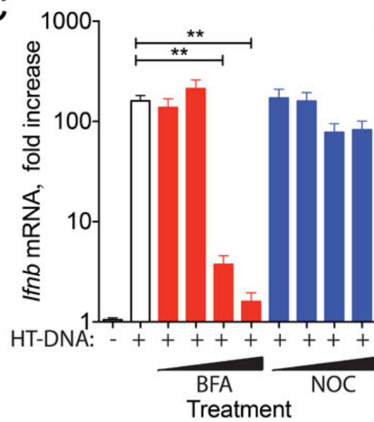
A



B



C



D

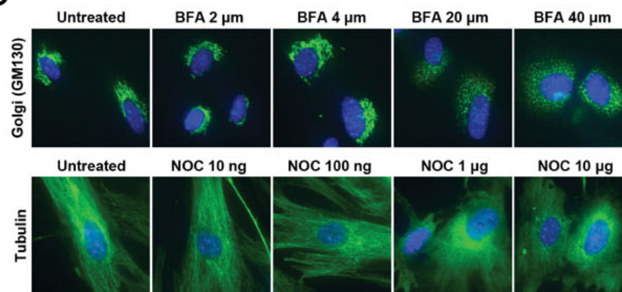


Figure S4, related to Figure 4. Kinetics of STING translocation. (A) Live cell time lapse microscopy of STING-GFP MEFs transfected with HT-DNA. The movie starts at 20 mins after DNA transfection (time 0) and the recording lasts 90 minutes. Still shots were taken from the movie and arrayed here. Numbers indicate minutes in the movie. (B) Fluorescent micrographs show STING-GFP localization in MEFs after *Shigella* infection at indicated time (2, 4 and 6 h post infection). These microscopy experiments were done as in **Figure 3B** where 8 h post infection images were presented. (C, D) Brefeldin A (BFA), but not Nocodazole (NOC), inhibits STING signaling. WT MEFs were pretreated with BFA or NOC at indicated dose (2, 4, 20 and 40 uM for BFA; 0.01, 0.1, 1 and 10 ug/mL for NOC) for 1 hour. Cells were then either transfected with HT-DNA and *Ifnb* mRNA was measured by qRT-PCR 6 h later (C), or fixed and stained for Golgi or Tubulin to examine the effectiveness of each drug at various doses (D). BFA at 20 uM clearly disrupted Golgi apparatus. NOC at 1 ug/mL clearly disrupted microtubules. Data are representative of at least three independent experiments. Error bars, SEM. Unpaired t-test (C).

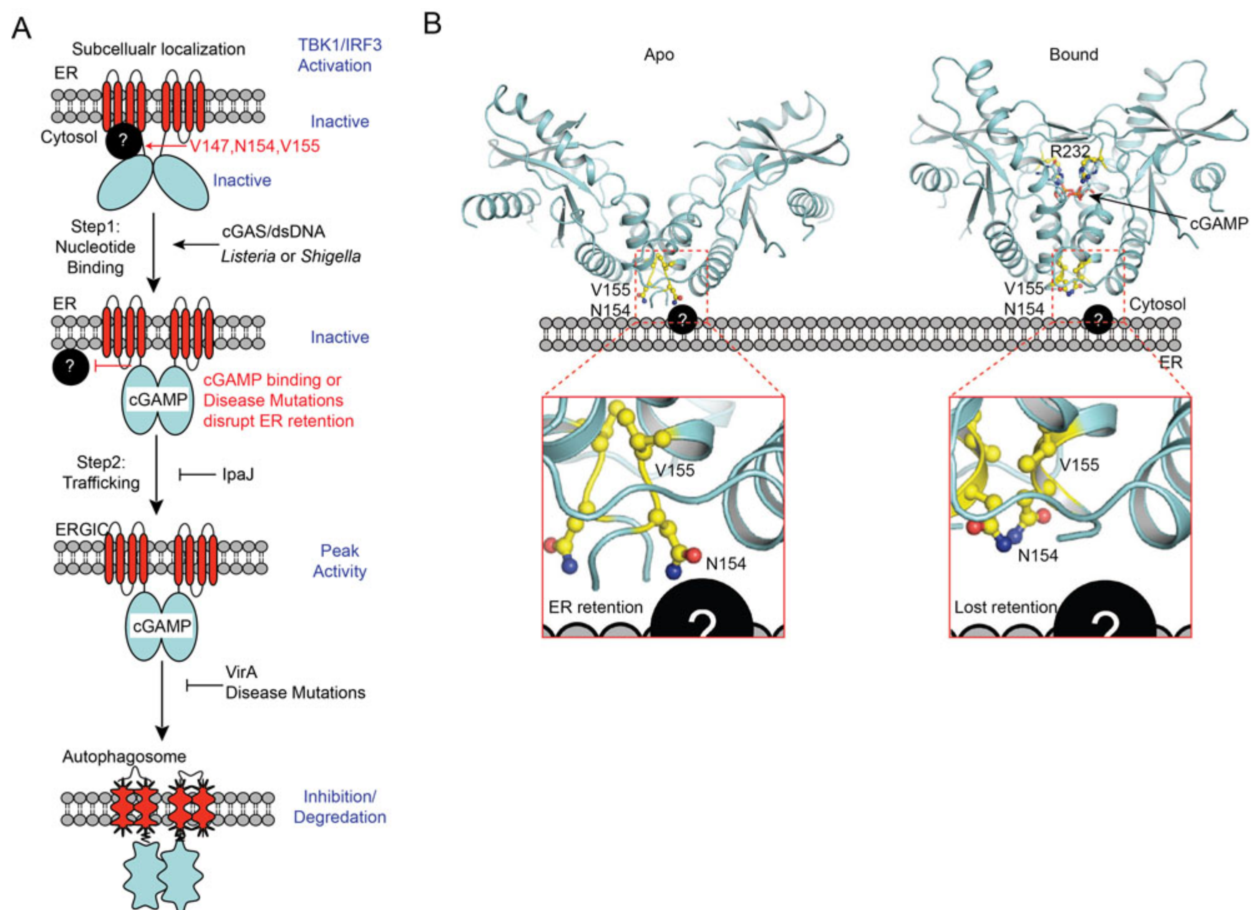


Figure S5, related to Figure 5. A model for STING activation. (A) A schematic model of two-step STING activation. (B) STING structure modeling illustrating how cGAMP binding causes a 'up-lifting' motion of the STING dimer that may break ER retention. Apo (4EF5) and cGAMP-bound (4KSY) structures were published previously (Zhang et al., 2013).

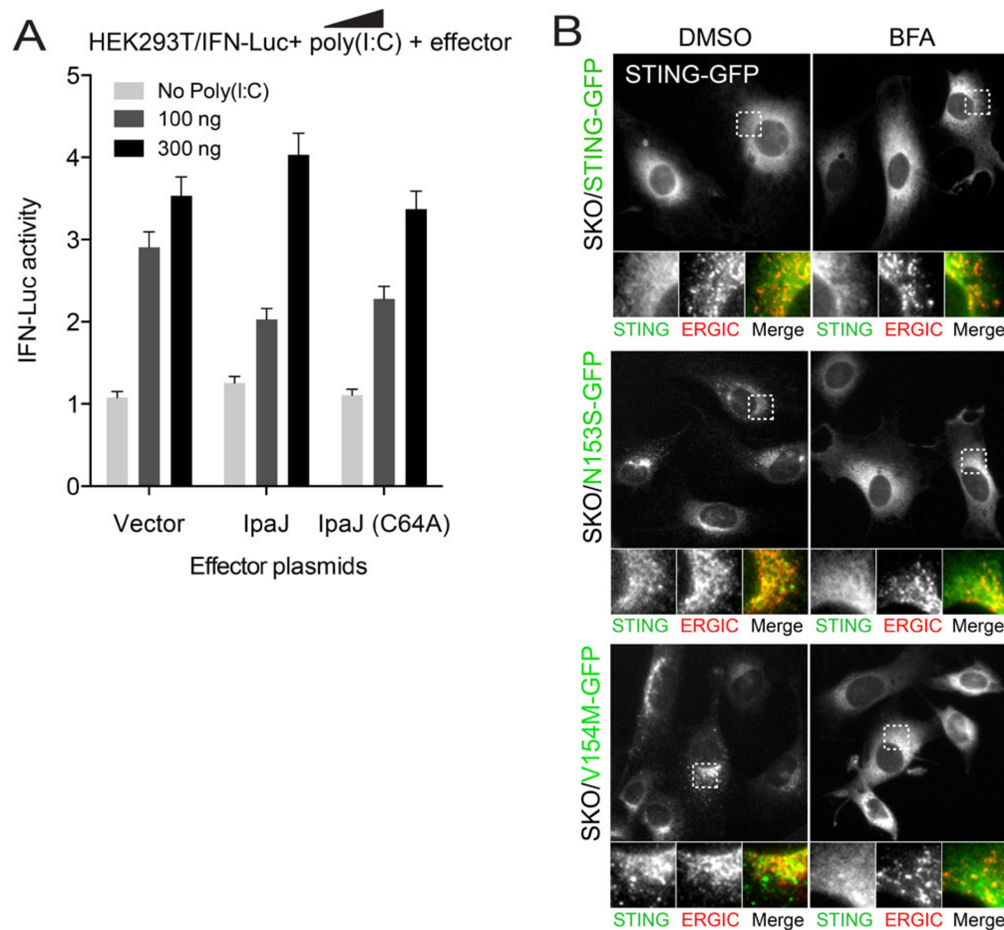


Figure S6, related to Figure 6. IpaJ does not block RNA-mediated IFN response, and BFA treatment blocks ER-exit of STING disease mutants. (A) IFNβ-Luc reporter assay. HEK293T cells were transfected with increasing amount of poly(I:C) to stimulate RNA-mediated IFN response. Fixed amount of Vector, IpaJ or IpaJ(C64A) plasmids were co-transfected as indicated on the bottom. (B) *Sting*-/- MEFs reconstituted with STING-GFP WT or disease mutants were treated with 20 uM BFA for 1 hour. Cells were then fixed and co-stained with an ERGIC marker (P58).

Movie 1, relates to Figure 4. Live cell time lapse imaging of DNA-stimulated STING translocation. STING-GFP MEFs were transfected with HT-DNA. Recording started at 20 minutes after adding DNA/lipofectmine complex, and lasted 90 minutes with 3 minutes per frame.

Supplemental references:

Zhang, X., Shi, H., Wu, J., Zhang, X., Sun, L., Chen, C., and Chen, Z.J. (2013). Cyclic GMP-AMP Containing Mixed Phosphodiester Linkages Is An Endogenous High-Affinity Ligand for STING. Molecular cell.