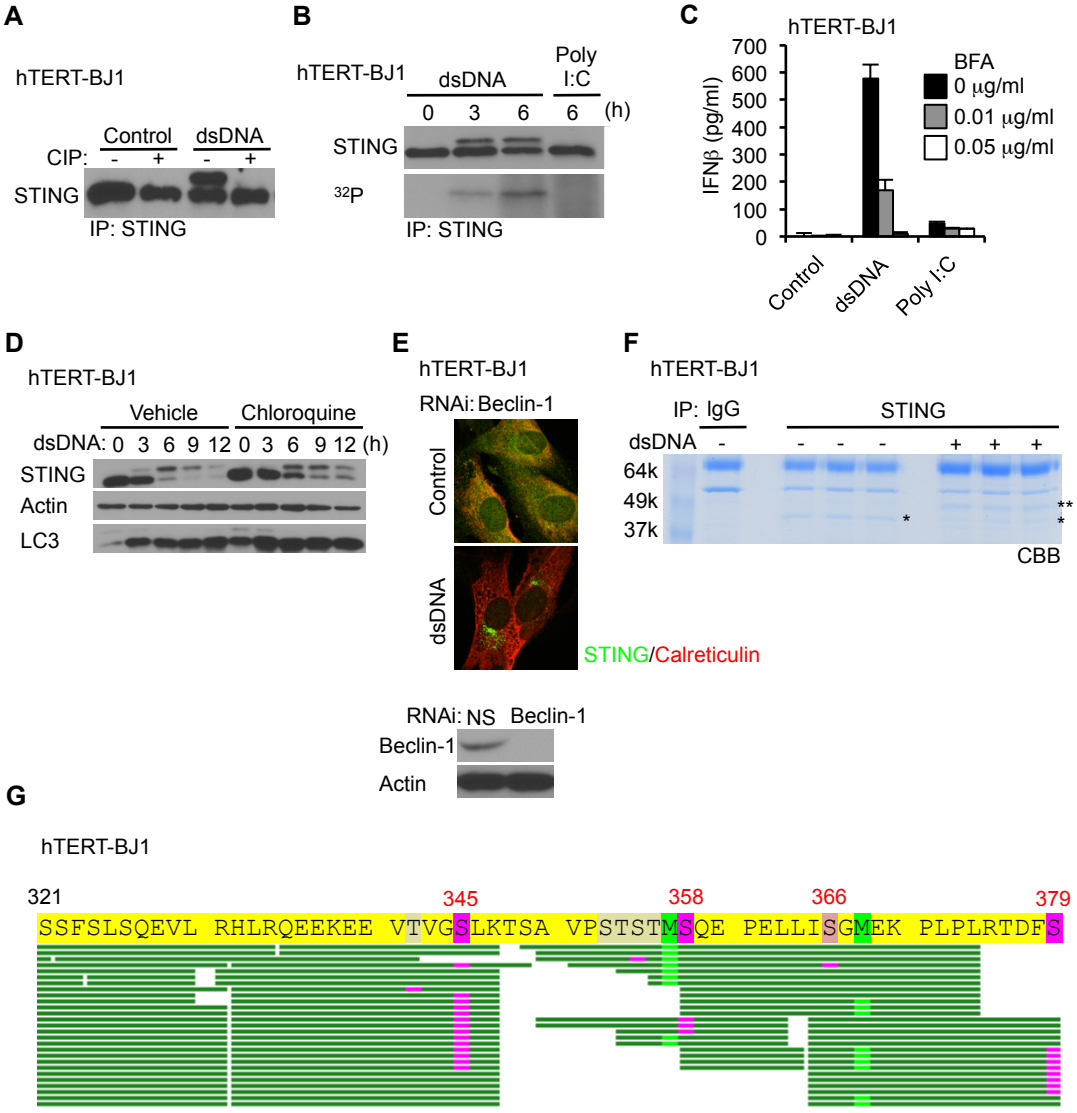

Figure S1. dsDNA Induces Phosphorylation of STING, Related to Figure 1

(A) hTERT-BJ1 cells were transfected with 4 μg/ml of dsDNA for the indicated times and then endogenous STING was precipitated, then treated with calf intestinal alkaline phosphatase (CIP).

(B) hTERT-BJ1 cells were cultured in phosphate-free DMEM for 2 hr and then cultured in presence of 0.2 mCi/ml of ^{32}P-labeled orthophosphate for 1 hr. After that the cells were transfected with dsDNA (4 μg/ml) or poly I:C (4 μg/ml) for the indicated time. Endogenous STING was pull-downed by anti-STING antibody and then autoradiography was performed.

(C) hTERT-BJ1 cells were treated with BFA for 1 hr before transfection of dsDNA (4 μg/ml) or poly I:C (4 μg/ml). At 16 hr after the transfection, the supernatant was collected and IFNβ level was measured by ELISA.

(D) hTERT-BJ1 cells were treated with chloroquine (50 μM) for 1 hr prior to dsDNA transfection. Western blot was performed using STING and LC3 antibodies.

(E) hTERT-BJ1 cells were treated with siRNA for Beclin-1 as described in Figure 1E and transfected with dsDNA (4 μg/ml) for 6 hr. Immunostaining was performed with STING antibody and also knockdown efficiency was confirmed by western blot.

(F and G) Endogenous STING protein was precipitated from hTERT-BJ1 cells transfected with dsDNA for 6 hr. Precipitated STING was visualized in the gel with CBB (F) and all the bands including STING were analyzed by mass spectrometry (G). * and ** mean native STING and phosphorylated STING, respectively. Untreated cells showed no phosphorylated STING. Highlighted amino acids with pink mean identified phosphorylation sites of STING by mass spectrometry. No phosphorylation sites were identified in native STING.

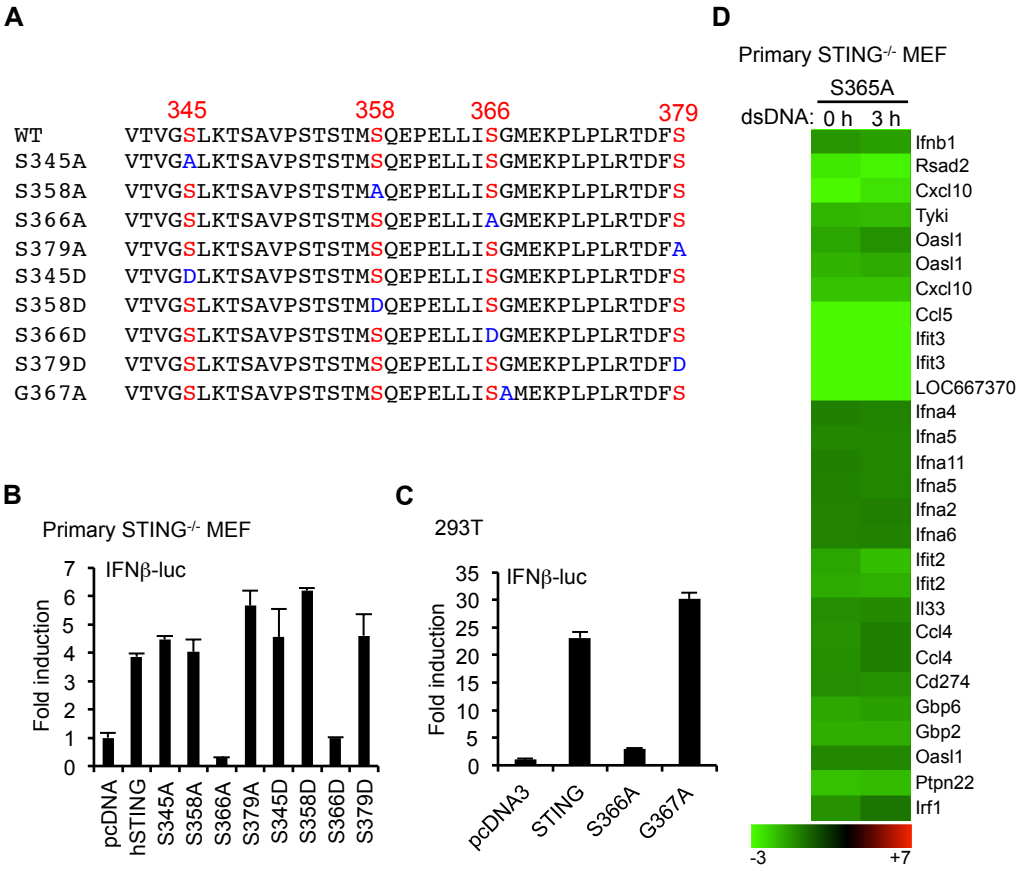

Figure S2. Phosphorylation of STING on S366 Negatively Regulates IFN Production, Related to Figure 2
(A) STING variants used in this study.
(B and C) pcDNA3-hSTING plasmid with the indicated mutation was transfected into primary STING $^{-/-}$ MEF cells (B) or HEK293T (C) cells with plasmid encoding luciferase gene driven under IFNβ promoter. After 36 hr, luciferase activity was measured.
(D) Reconstituted STING$^{-/-}$ MEF cells with mSTING variant (S365A) were transfected with dsDNA (4 mg/ml) for 3 hr. Purified RNA was examined for gene expression as described in Figure 2E.

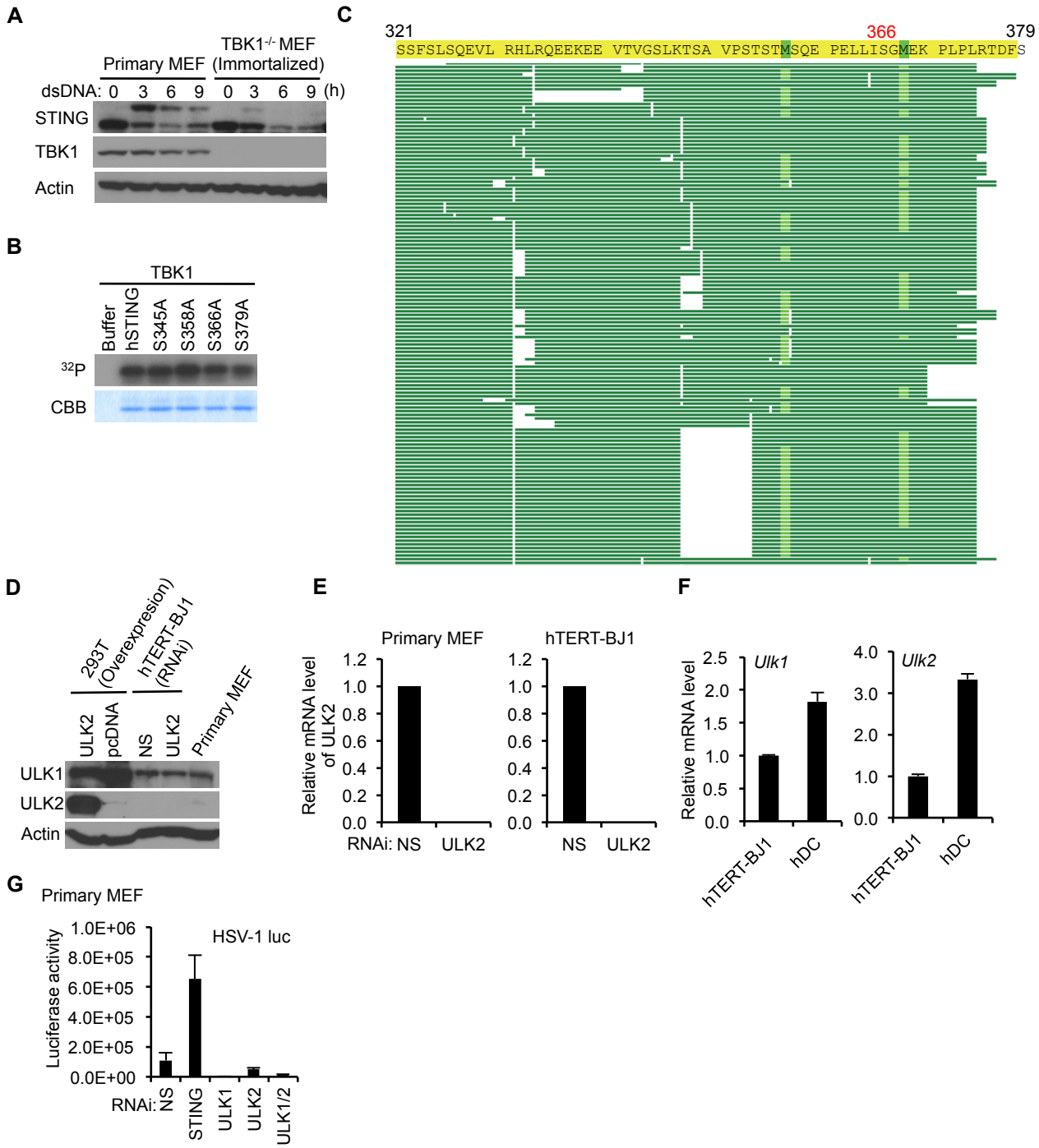

Figure S3. STING is Likely not a Substrate for TBK1, Related to Figure 3

(A) Primary MEF cells and immortalized TBK1$^{-/-}$ MEF cells were transfected with dsDNA (4 μg/ml) for the indicated times. Western blot was performed using STING and TBK1 antibodies.

(B) Purified TBK1 protein was mixed with purified STING protein or STING variant proteins in presence of [γ-^{32}P] ATP. After the reaction samples were boiled in SDS-sample buffer and then analyzed by gel electrophoresis followed by autoradiography.

(C) Purified TBK1 protein was mixed with purified STING protein and then analyzed by mass spectrometry as described in Figure 3D.

(D) HEK293T cells were transfected with plasmid encoding human ULK2 for 36 hr as a positive control for western blot. hTERT-BJ1 cells were treated with siRNA, NS (non-specific) or ULK2 for three days. Western blot was performed using ULK1 and ULK2 antibodies.

(E) Primary MEF cells or hTERT-BJ1 cells were treated with siRNA, NS or ULK2 for three days and then total RNA was extracted. After cDNA synthesis, qPCR was performed using murine or human ULK2 probe. GAPDH was used for normalization. These results indicate that siRNA for ULK2 used in this study can suppress ULK2 expression.

(F) cDNA from hTERT-BJ1 cells or primary human DC (hDC) was examined by qPCR to determine ULK1 and ULK2 expression.

(G) Primary MEF was treated with NS, STING, ULK1, ULK2 siRNA, then infected with HSV-1 luc (MOI = 1) and luciferase activity was measured.

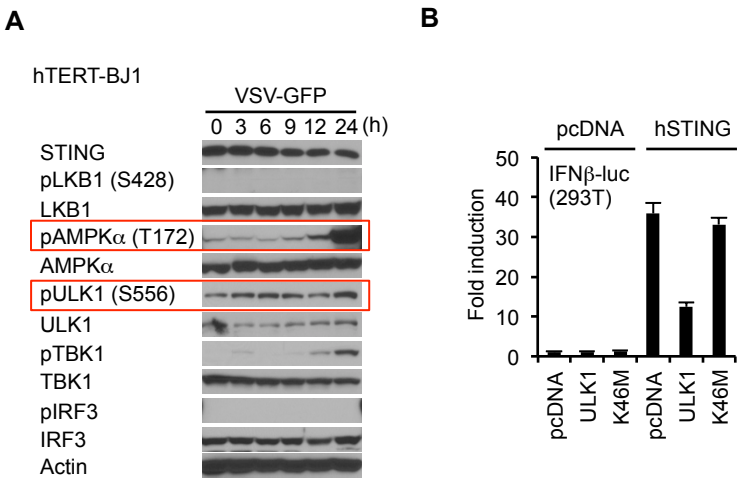

Figure S4. VSV (RNA virus) does not Induce the Dephosphorylation of AMPK T172 and ULK1 S556, Related to Figure 4

(A**)** hTERT-BJ1 cells were infected with VSV-GFP (MOI = 1) for the indicated times. Western blot was performed with the indicated antibodies.

(B) pcDNA3-hSTING plasmid was transfected into HEK293T cells with pcDNA-hULK1 or variant (K46M) as described in Figure S2C. After 36 hr, luciferase activity was measured.

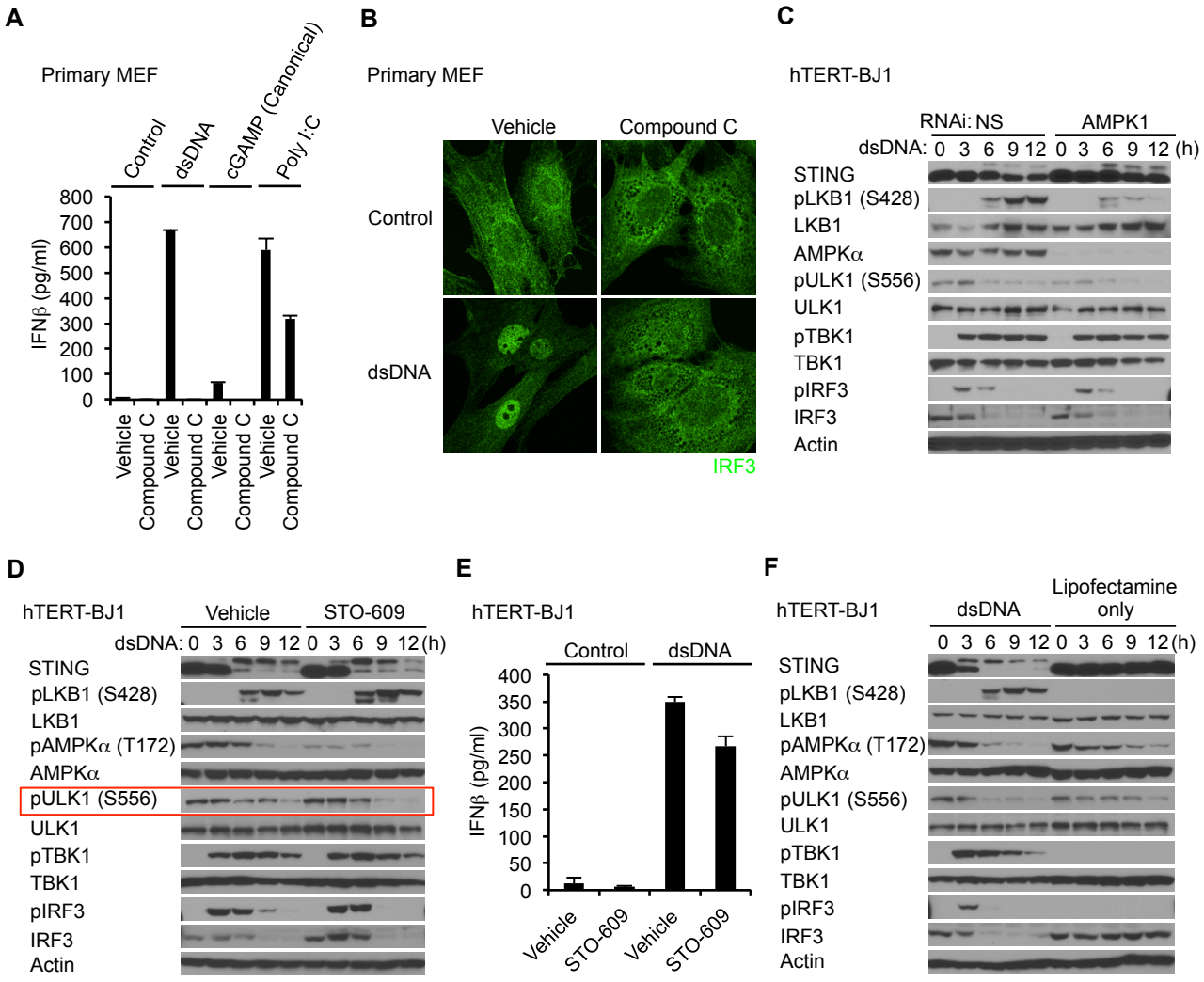

Figure S5. Compund C Inhibits IRF3 Translocation Induced by dsDNA, Related to Figure 5

(A and B) Primary MEF cells were treated with compound C as described in Figure 5A and then transfected with dsDNA (4 μg/ml) for 16 hr (A) or 3 hr (B). In addition, canonical cGAMP (8 μg/ml) and poly I:C (4 μg/ml) treated primary MEF cells were used to measure IFNβ (A). IFNβ level was measured by ELISA (A) or IRF3 translocation was observed by confocal microscope using IRF3 antibody (B).

(C) hTERT-BJ1 cells were treated with siRNA for AMPK1 for three days and then transfected with dsDNA (4 μg/ml) for the indicated times. Western blot was performed with the indicated antibodies.

(D) hTERT-BJ1 cells were treated with DMSO (Vehicle) or STO-609 (10 μM) for 1 hr prior to transfection of dsDNA (4 μg/ml) for the indicated times. Western blot was performed with the indicated antibodies.

(E) hTERT-BJ1 cells were treated with STO-609 as described in Figure S5D and then transfected with dsDNA (4 μg/ml) for 16 hr. IFNβ level was measured by ELISA.

(F) hTERT-BJ1 cells were transfected with dsDNA (4 μg/ml) using lipofectamine 2000 or treated with lipofectamine 2000 only for the indicated times. Western blot was performed with the indicated antibodies.

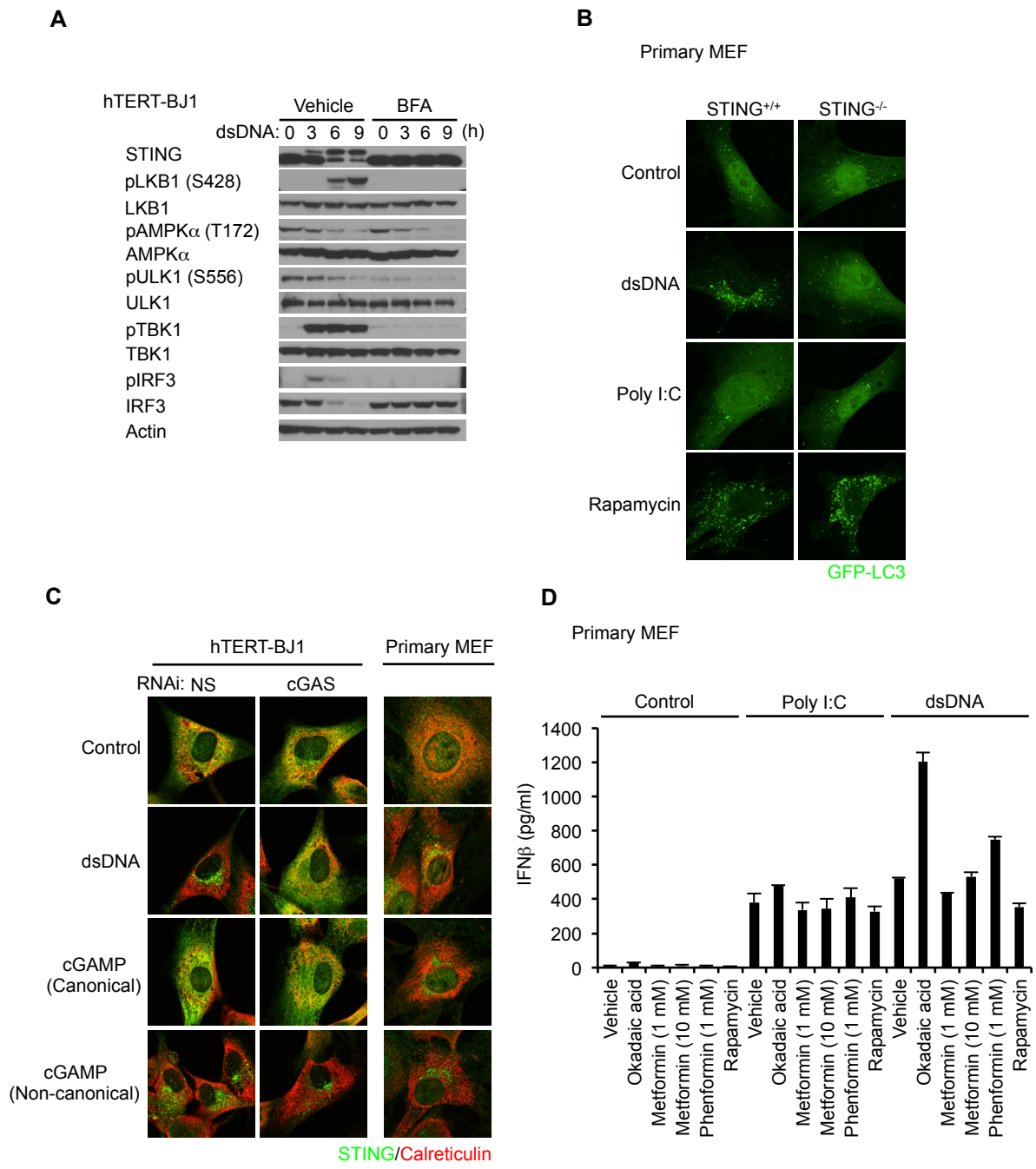

Figure S6. STING Mediated Autophagy is Specific to dsDNA, Related Figure 6 and 7

(A**)** hTERT-BJ1 cells were treated with BFA as described in Figure 1C and then transfected with dsDNA (4 μg/ml) for the indicated times. Western blot was performed with the indicated antibodies.

(B) Primary STING$^{+/+}$ and STING$^{-/-}$ MEF cells were transduced with retrovirus vector encoding GFP-LC3. These cells were transfected with dsDNA (4 μg/ml) or poly I:C (4 μg/ml) for 3 hr, or treated with rapamycin (0.5 μM) for 3 hr. LC3 puncture structure was observed by confocal microscope.

(C) siRNA treated hTERT-BJ1 cells or primary MEF cells were transfected with dsDNA or cGAMP as described in Figure 7 and then immnostaining was performed with the indicated antibodies.

(D) Primary MEF cells were treated with the indicated drugs (Okadaic acid: 50 nM, Metformin: 1 mM or 10 mM, Phenformin: 1 mM, Rapamycin: 0.5 μM) for 1 hr prior to dsDNA (4 μg/ml) or poly I:C (4 μg/ml) transfection. After 16 hr, IFNβ level was measured by ELISA.