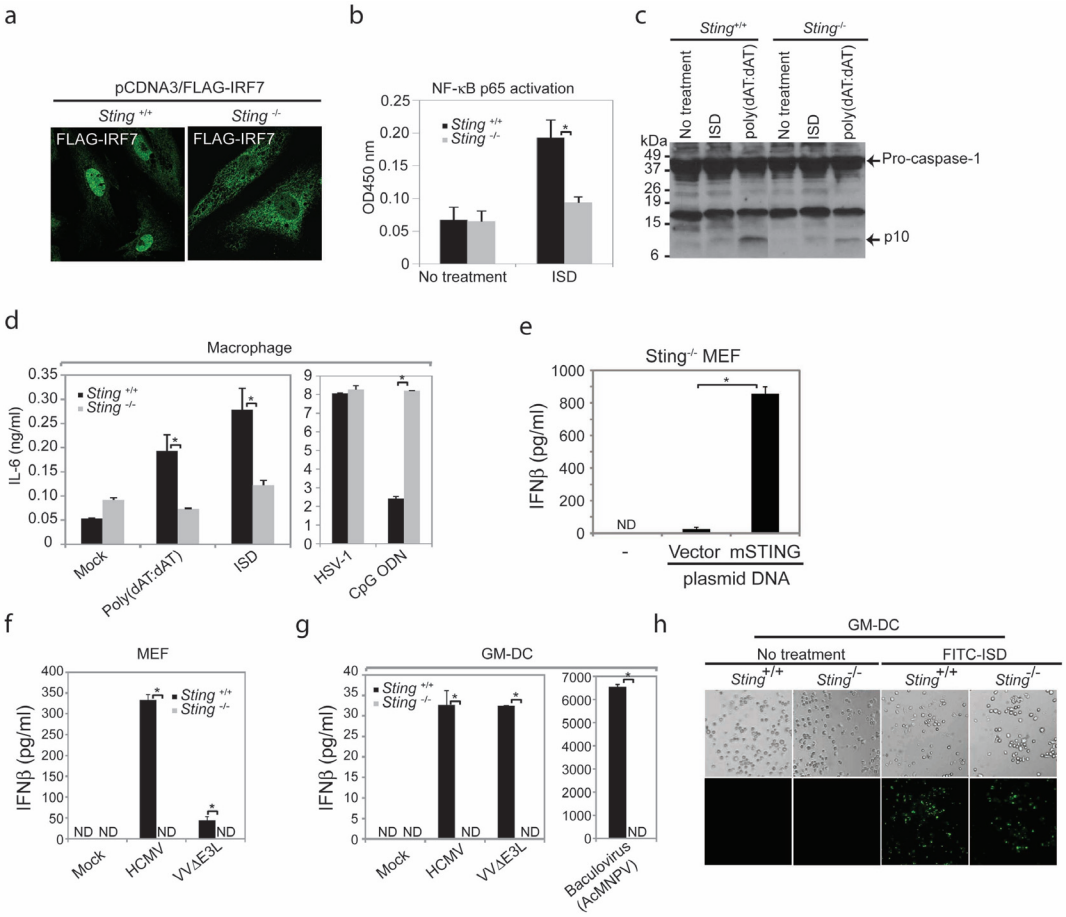
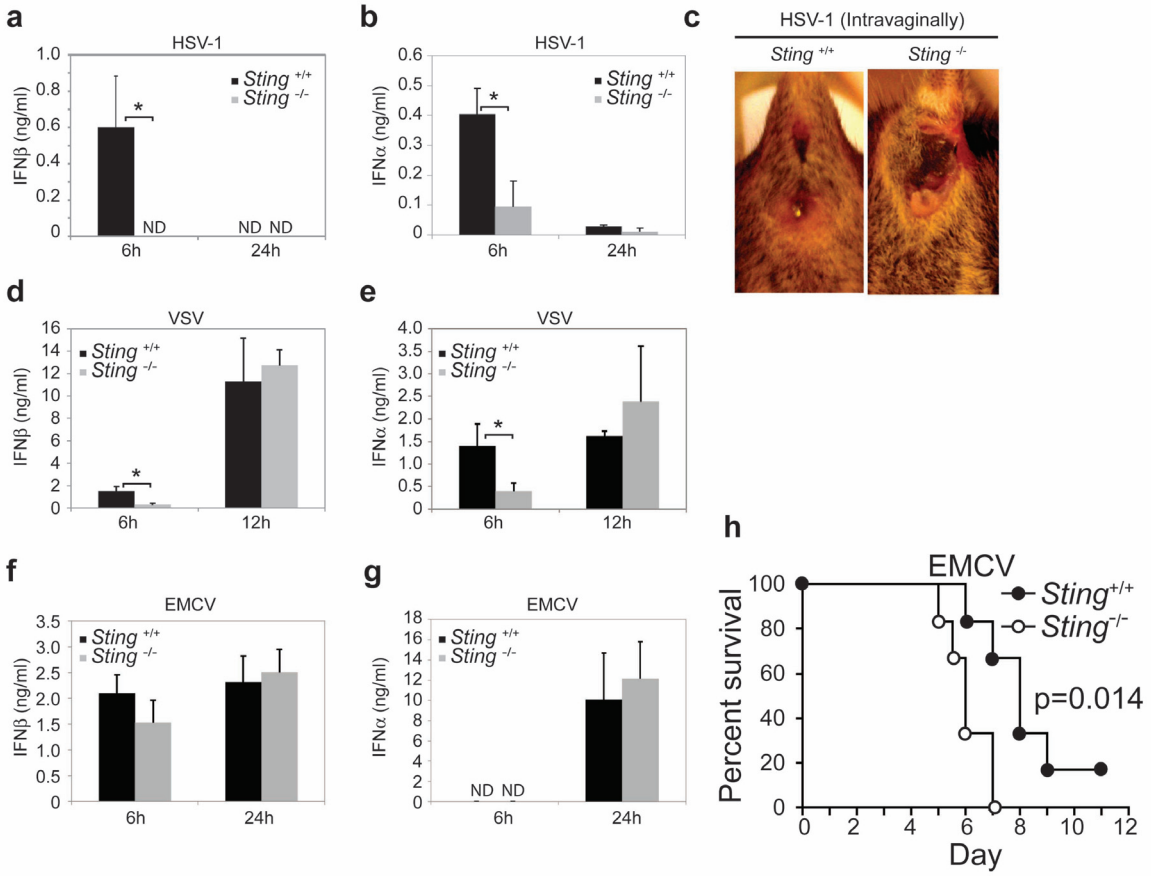
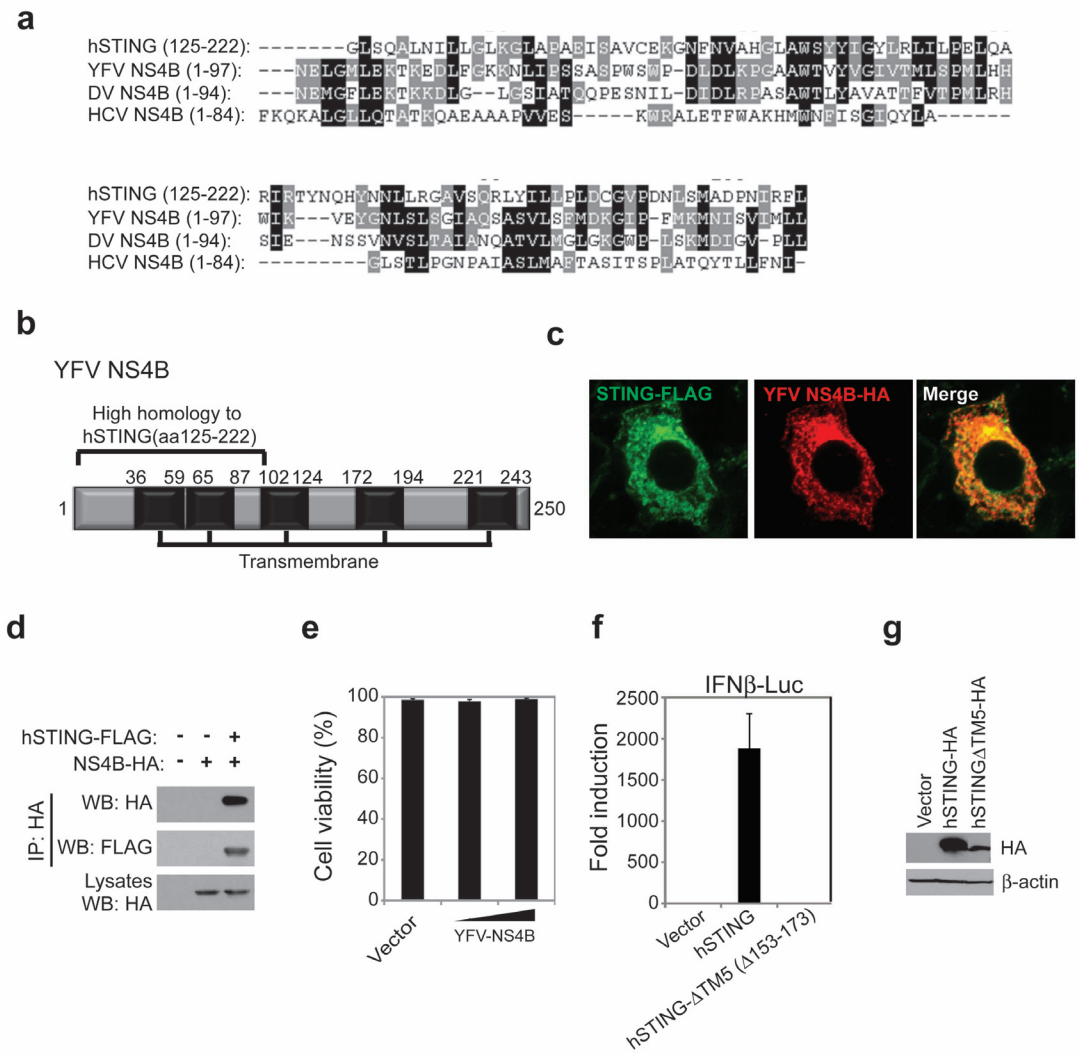
SUPPLEMENTARY INFORMATION



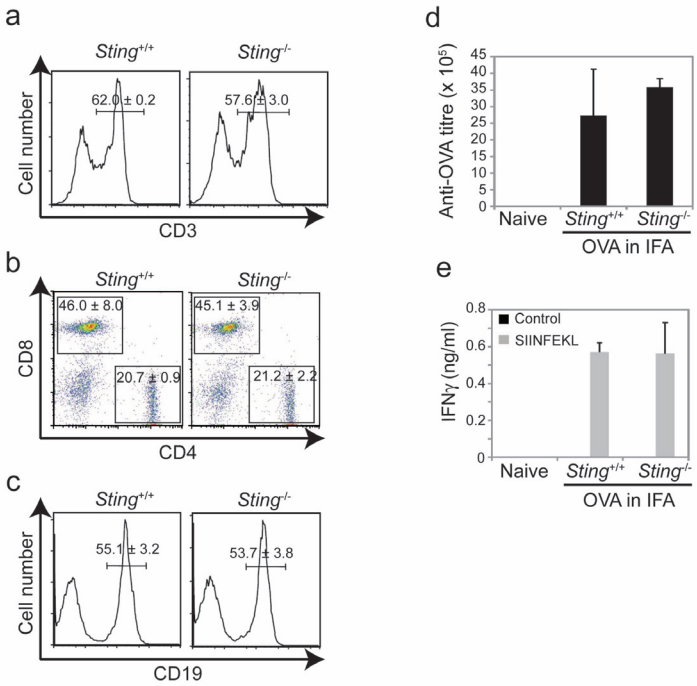
Supplemental Fig 1. STING is required for intracellular DNA-mediated type I IFN type production. a. *Sting* $^{+/+}$ or $^{-/-}$ MEFS were transfected with FLAG-IRF7 for 24 hours and immunostained using anti-FLAG antibody. Plasmid transfection stimulates the STING pathway, activating IRF7 and nuclear localization. This does not occur in the absence of STING. **b**. *Sting* $^{+/+}$ or $^{-/-}$ MEFS were treated with ISD for 16 hours and cell lysates analyzed for NF-κB p65 activity using ELISA. **c**. *Sting* $^{+/+}$ or $^{-/-}$ macrophage were treated with ISD or poly (dAT:dAT) for 16 hours and lysates analyzed for caspase-1 p10 fragment using antibody. **d**. Macrophages were transfected with 1ug/ml of DNA ligands or infected for 16 hours with HSV-1 (moi 10). IL-6 was measured by ELISA. IL-6 production in the absence of STING is defective in response to poly(dAT:dAT) and ISD, but not HSV-1 or CpG ODN. **e**. *Sting* $^{-/-}$ MEFS were re-constituted with STING and IFNβ production rescued as measured by ELISA. **f** and **g**. *Sting* $^{+/+}$ or $^{-/-}$ MEFS were infected (moi 10) with human cytomegalovirus (HCMV) or vaccinia virus (VVΔE3L) or baculovirus and IFNβ production measured by ELISA after 16 hours.



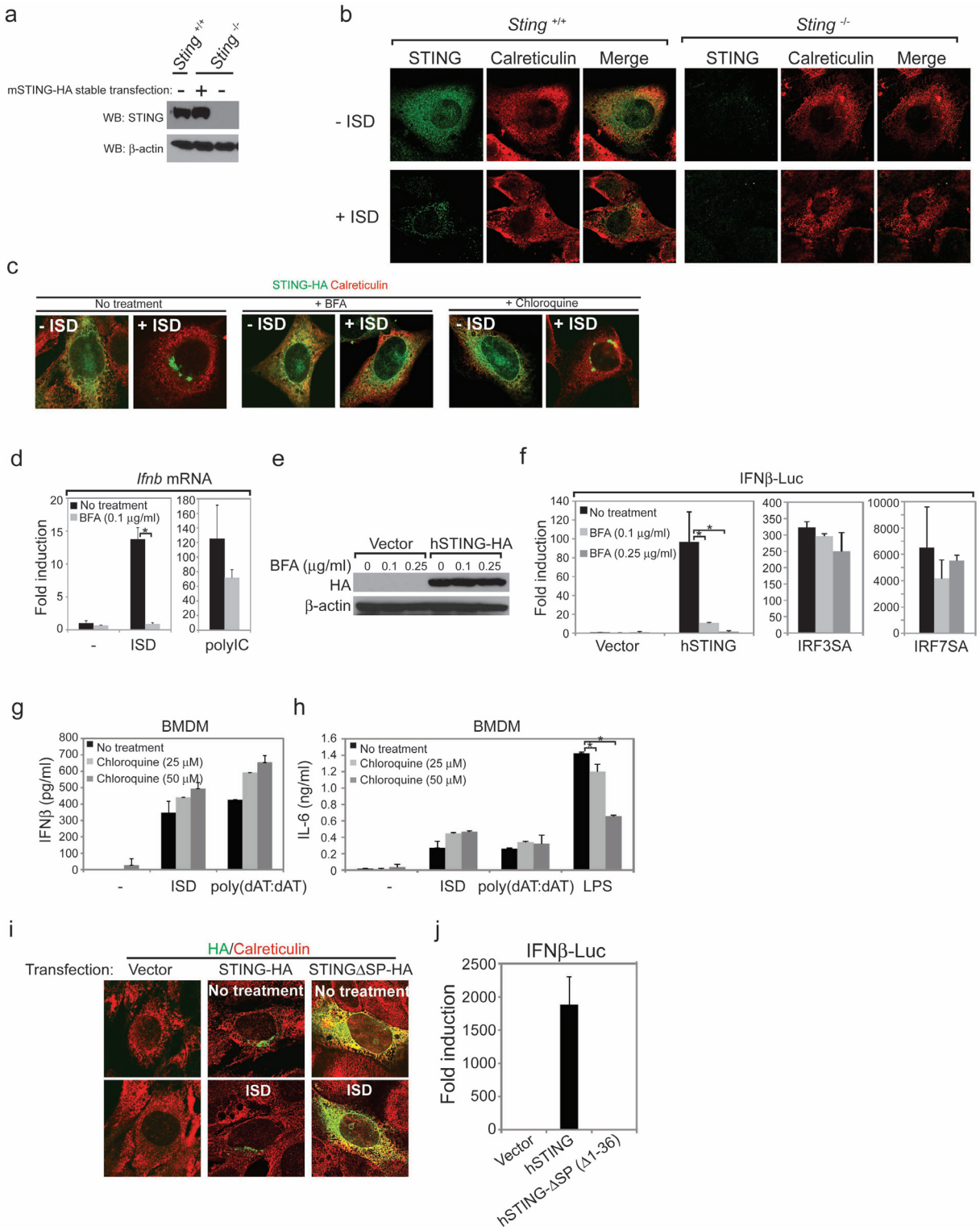
Supplemental Fig 2. Defective type I IFN production in viral-infected STING knockout mice. a,b *Sting* $^{-/-}$ mice or litter mate controls $^{+/+}$ [n=3; approximately 8 weeks of age] were infected intravenously (i.v.) with 1 x 10^7 HSV and survival monitored. Serum from animals were analyzed for IFNβ or IFNα production after 24 hours). **c** *Sting* $^{-/-}$ mice or litter mate controls $^{+/+}$ [n=7; approximately 8 weeks of age] were infected intravaginally with 1 x 10^6 HSV and disease intensity measured (*Zhang et al., 2009 Mucosal Immunology 2, p129*). By 14 day severe inflammation was observed in STING -/- animals (3/7), but not in any control animals (0/7). **d,e** STING deficient animals or controls (n=3) were infected I.V. with vesicular stomatitis virus (VSV, 5 x 10^7) and IFNβ or IFNα measured after 12 hours). **f, g** STING $^{-/-}$ mice or controls (n=3) were infected intraperitoneally (i.p) with encephalomyocarditis virus (EMCV, 1 x 10^5). Serum from animals was analyzed for IFNβ or IFNα production after 6 and 24 hours). **h.** *Sting* $^{-/-}$ mice or litter mate controls $^{+/+}$ [n=6; approximately 8 weeks of age] were infected intraperitoneally (i.p.) with EMCV (10^2) and survival monitored. Asterisks indicate significant difference ($P<0.05$) as determined by Student's t-test. Error bars indicate s.d.



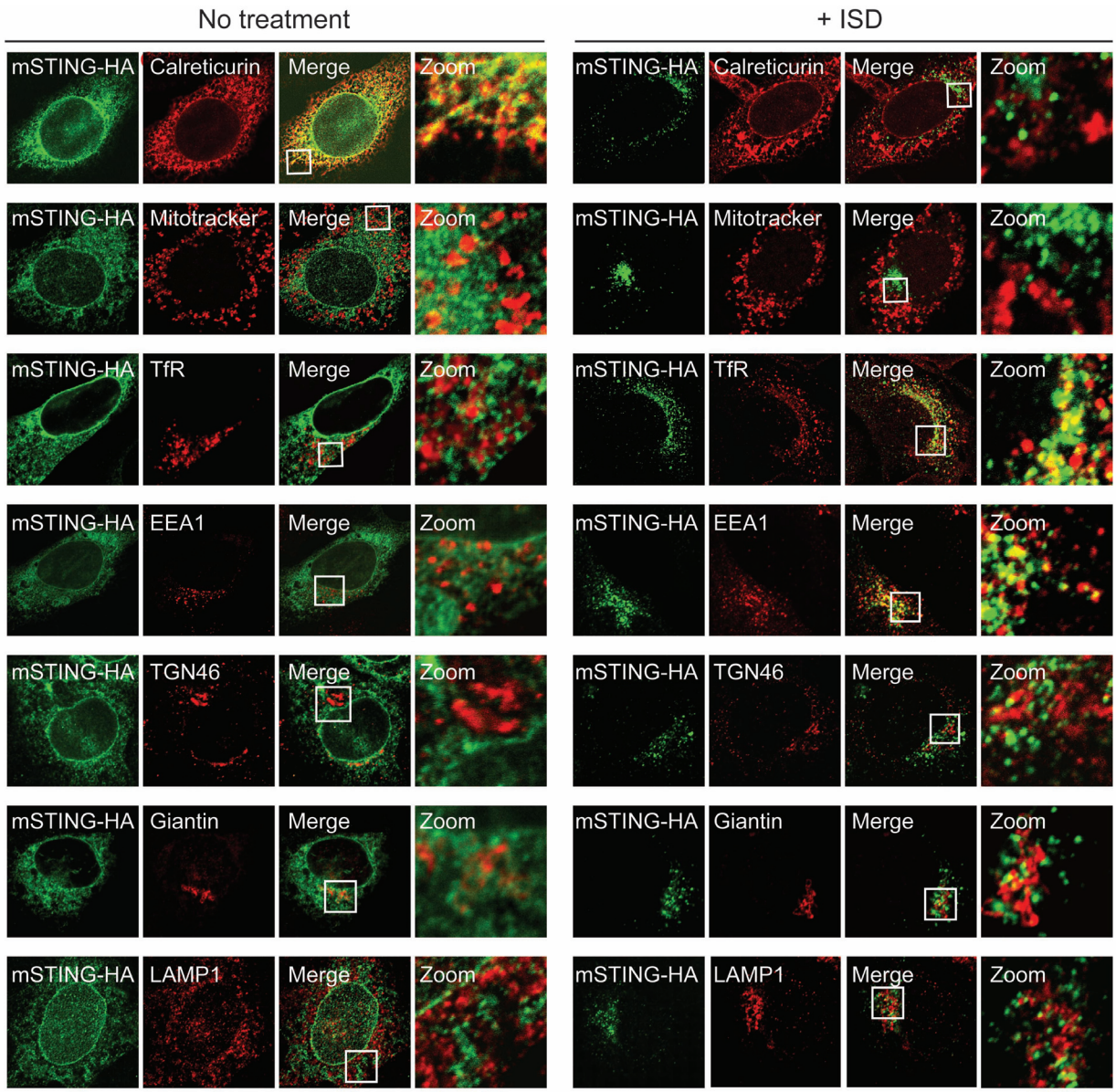
Supplemental Fig 3. Human STING (hSTING, amino acids 125-222) exhibits significant homology to flavivirus NS4B proteins. a, Homology between Yellow Fever Virus (YFV), Dengue Virus (DV) and hepatitis C virus (HCV) NS4b are shown. **b** schematic of YFV NS4b homology versus STING. **c**. 293T cells were co-transfected with FLAG-tagged STING and HA tagged YFV-NS4B for 36 hours and observed by confocal microscopy. **d**. 293T cells were co-transfected with FLAG-tagged STING and HA tagged YFV-NS4B for 36 hours and lysates immunoprecipitated using anti-HA antibody and immunoblotted using anti-FLAG antibody. **e**. 293T cells were transfected with FLAG- HA tagged YFV-NS4B for 36 hours and cell viability measured using Trypan blue assay. **f**. 293T cells were co-transfected with FLAG-tagged full length STING or STING mutant (ΔTM5, lacking amino acids 153-173) with IFN promoter driving luciferase, and luciferase measured. **g**. Immunoblot (anti-HA antibody) of transfected STING molecules analyzed in (**f**) to ensure expression. Asterisks indicate significant difference ($P<0.05$) as determined by Student's t-test. Error bars indicate s.d.



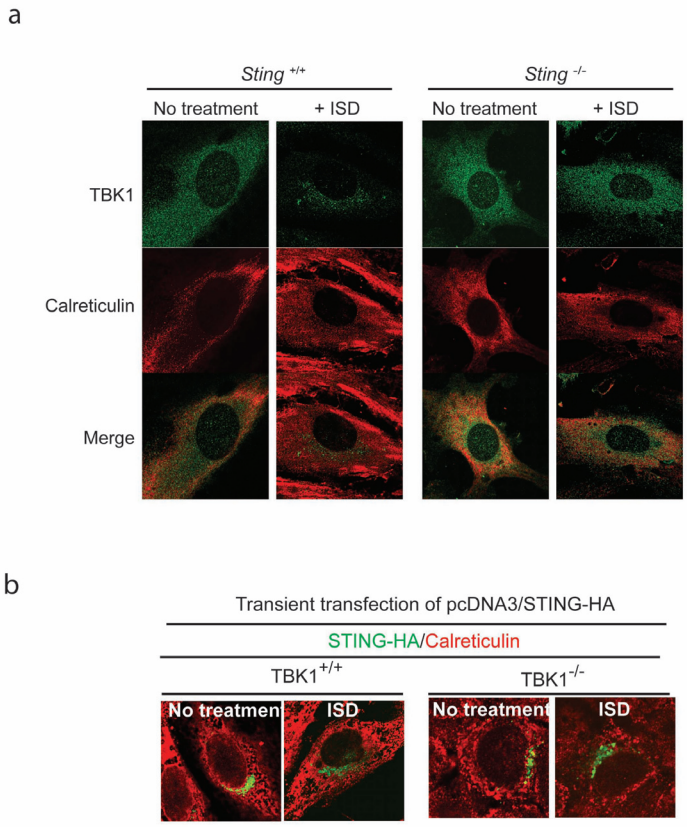
Supplemental Fig 4: Normal T and B cell repertoire in STING -/- animals under non-stimulatory conditions a-c Splenocytes from STING $^{+/+}$ or $^{-/-}$ mice were analyzed by facs using anti-CD3, anti- CD4 or CD8 (for T cell) or anti-CD19 (for B cells) under non-stimulated conditions. **d**. Ovalbumin (10 ug) in incomplete Freunds adjuvant (IFA) was inoclulated i.p. into STING $^{+/+}$ or $^{-/-}$ mice (n =4). Animals were boosted after 14 days. Antibody production to OVA was measured 14 days after the last boost. **e**. Splenocytes from (**d**) were stimulated with anti-OVA peptide (SIINFEKL) and after 4 days IFNγ was measured by ELISA. Asterisks indicate significant difference ($P<0.05$) as determined by Student's t-test. Error bars indicate s.d.



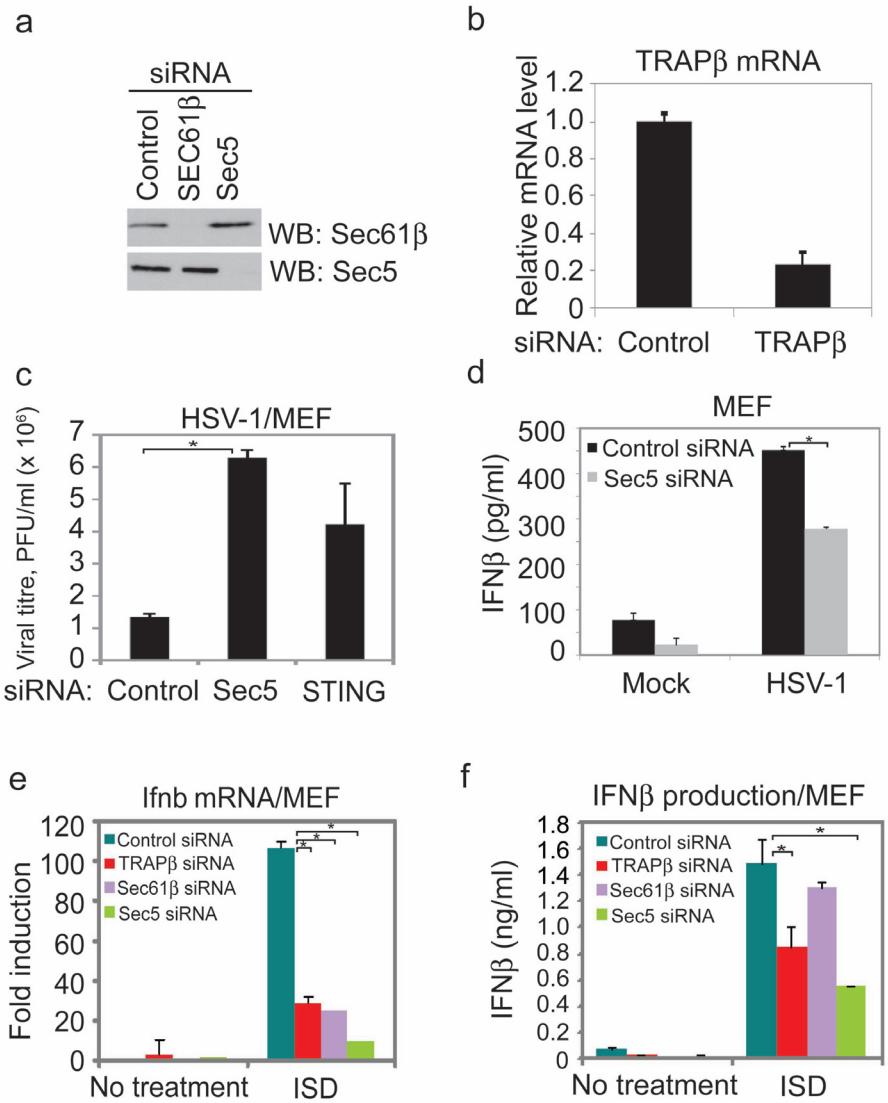
Supplemental Fig 5: Brefeldin A blocks STING trafficking. a. Immunoblot analysis of STING expression in *Sting* $^{-/-}$ MEFs stably transfected with HA-STING, to demonstrate comparable amounts of STING in both cell types. **b.** STING $^{+/+}$ or $^{-/-}$ MEFs were treated with ISD (1 ug/ml with Lipofactamine 2000) and after 3 hours endogenous STING localization was analyzed using anti-STING-C antibody (green) or anti-calreticulin (red). c. MEFs stably transfected with HA-STING were treated with Brefeldin A (0.1 ug/ml) or chloroquine (0.1 mM) for 1 hour prior to ISD stimulation (3 hours 1 ug/ml in Lipofectamine 2000). Green = anti-HA and red = anti-calreticulin. **d.** Wild type MEFs were treated with Brefeldin A as above and IFNβ mRNA measured. **e, f.** 293T cells were treated with various concentrations of Brefeldin A as above were co-transfected with STING, constitutively active IRF3 or IRF7 and IFN-luciferase reporter plasmid. Luciferase was measured after 24 hours. **g,h.** Macrophages treated with various chloroquine amounts for one hour were treated with ISD (1 ug/ml), poly (dAT-dAT) [1 ug/ml] or LPS (1ug/ml) and after 16 hours IFNβ or IL6 measured by ELISA. **i.** STING $^{-/-}$ MEFS were transfected with HA-STING or HA-STINGΔSP (that lacks the first 36 amino acids representing a potential signal peptide motif) for 24 hours prior to treatment with ISD (1 ug/ml). After 3 hours STING localization was measured using immunofluorescence and an anti-HA antibody (green) or anti calreticulin antibody (red). **j.** 293T cells were co-transfected with HA-STING or HA-STINGΔSP and an IFN-Luciferase plasmid and after 24 hours luciferase was measured. STINGΔSP does not traffic in response to ISD and cannot activate type I IFN. Asterisks indicate significant difference ($P<0.05$) as determined by Student's t-test. Error bars indicate s.d.



Supplemental Fig 6. STING translocates to endosomes following exposure to ISD. STING $^{-/-}$ MEFs, stably reconstituted with HA tagged STING (mSTING-HA) were treated with or without transfected ISD (4 hours) and stained using HA antibody (green) and either calreticulin, ER; mitotracker, mitochondria; TfR, recycled endosome; EEA1, early endosome; TGN46, trans-golgi; Giantin, cis-golgi; LAMP1, lysosome (red).



Supplemental Fig 7. STING is essential for TBK-1 trafficking in response to intracellular DNA. a. STING $^{+/+}$ or $^{-/-}$ MEFs were treated with ISD (1 ug/ml with Lipofactamine 2000) and after 3 hours endogenous TBK1 localization was analyzed using anti-TBK antibody (green) or anti-calreticulin (red). **b.** TBK1$^{+/+}$ or $^{-/-}$ MEFs were transfected with HA-STING and after 24 hours cells were treated with ISD for 3 hours and STING localization analyzed by immunofluorescence using an anti HA antibody (green) or anti-calreticulin (red). STING localizes in the absence of TBK1.



Supplemental Fig 8: Sec5 is required for efficient intracellular DNA-mediated signaling. a. Immunoblot of RNAi (to Sec5 or Sec61β) treated cells indicating knockdown of Sec5 and Sec61β. **b**, RT-PCR measurements of RNAi (Trapβ) treated cells indicating knockdown of Trapβ. **c.** MEFs treated with RNAi to Sec5 or STING for 3 days were infected with HSV1 (moi 1) for 24 hours and viral titers measured. **d**. Same as in (c) except that endogenous IFNβ was measured. **e.** MEFs treated with RNAi to Trapβ, Sec61β or Sec5 for 3 days were treated with ISD for 3hours and Ifnb mRNA measured by RT-PCR. **f.** Same as in (e) except that endogenous IFNβ was measured.