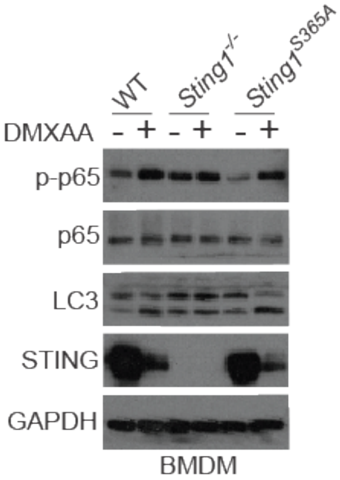

Figure S1. Sting-S365A mutation does not block STING-mediated NFκB signalling and autophagy in BMDMs. Related to Figure 1.

WT, *Sting1*$^{-/-}$ or *Sting1*$^{S365A/S365A}$ (*Sting1*^{S365A}) BMDMs were treated with mock or DMXAA (10 μg/ml) for 6 h. NFκB pathway and autophagy activation were measured by Western blot with indicated antibodies.

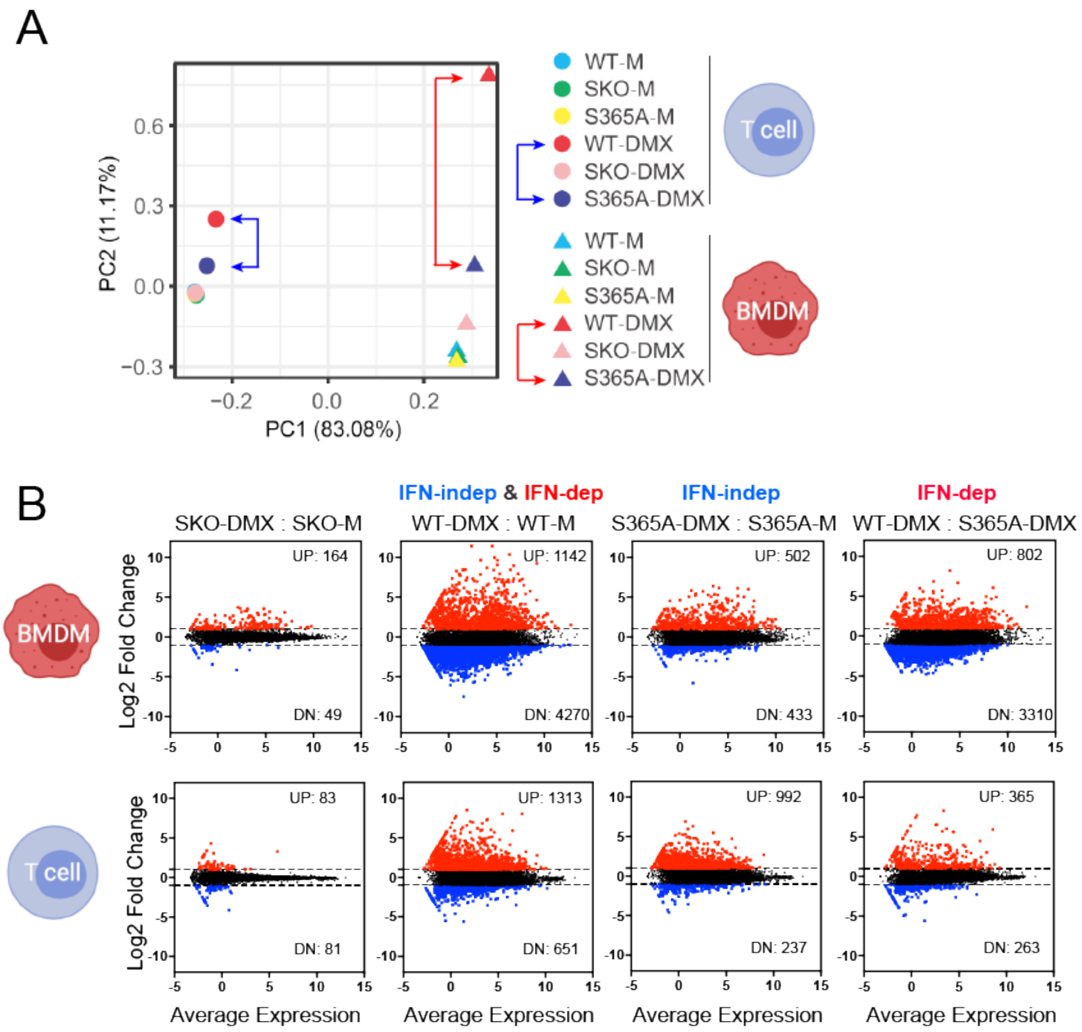

Figure S2. **Transcriptomic discovery of IFN-dependent and IFN-independent activities of STING. Related to Figure 2.**

(A) Principal component analysis (PCA) showing transcriptomic landscape of WT, *Sting1*$^{-/-}$ (SKO), and *Sting1*^{S365A} (S365A) BMDMs or T cells after mock or DMXAA stimulation.

(B) MA plots of differentially express genes (DEGs) in paired groups. The plot visualizes the differences between measurements taken in a pair of samples, by transforming the data onto M (log ratio) and A (mean average) scales, then plotting these values. Sample pair names are showing on top. UP, up-regulated genes; DN, Down-regulated gene. See **STAR Method** for more details.

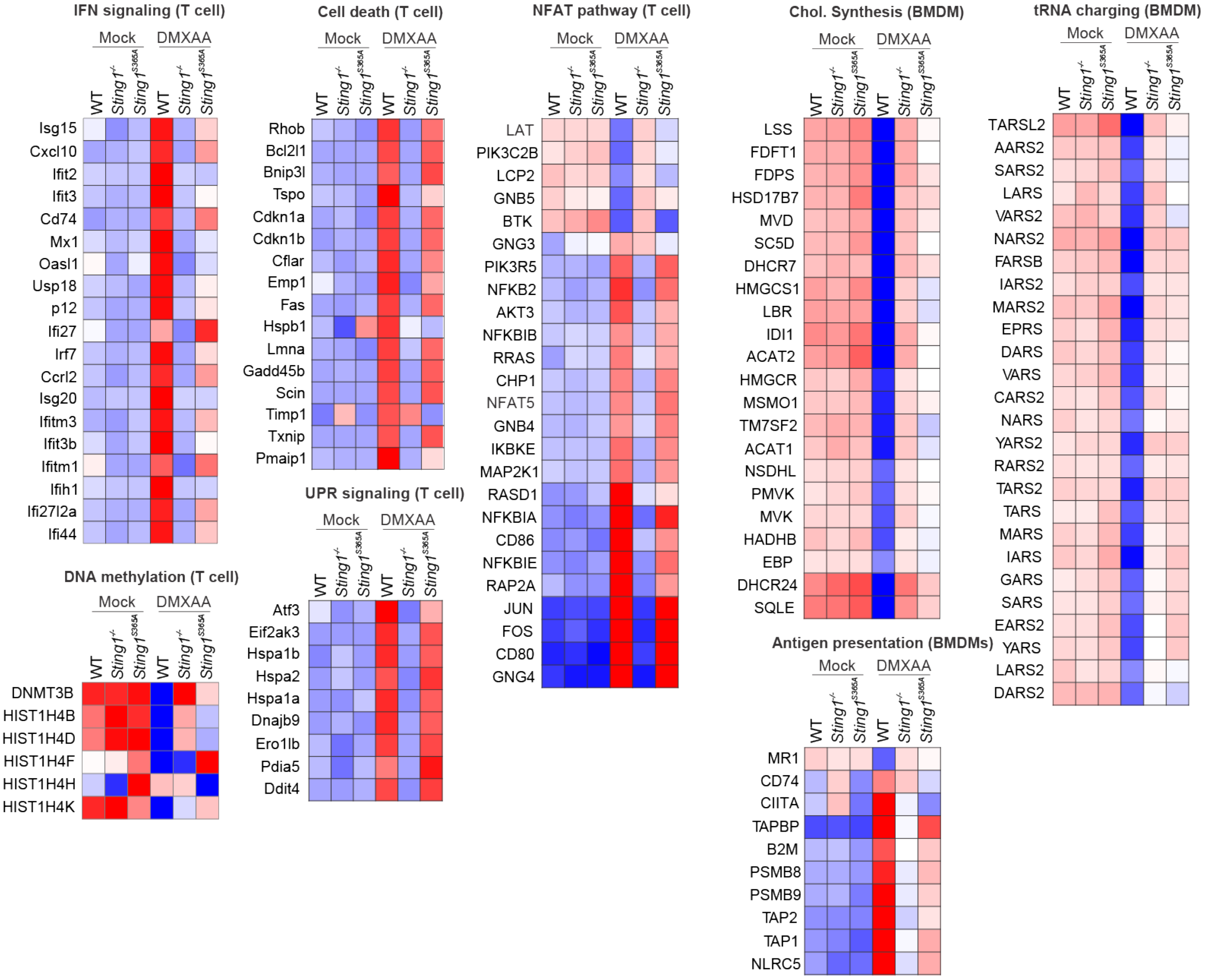

Figure S3. Selected IFN-dependent and IFN-independent pathways altered by STING activation. Related to Figure 2. Heatmaps showing previously known and unknown pathways altered by STING activation in BMDMs or T cells. Data from RNA-seq and pathway analysis in **Figure 2**.

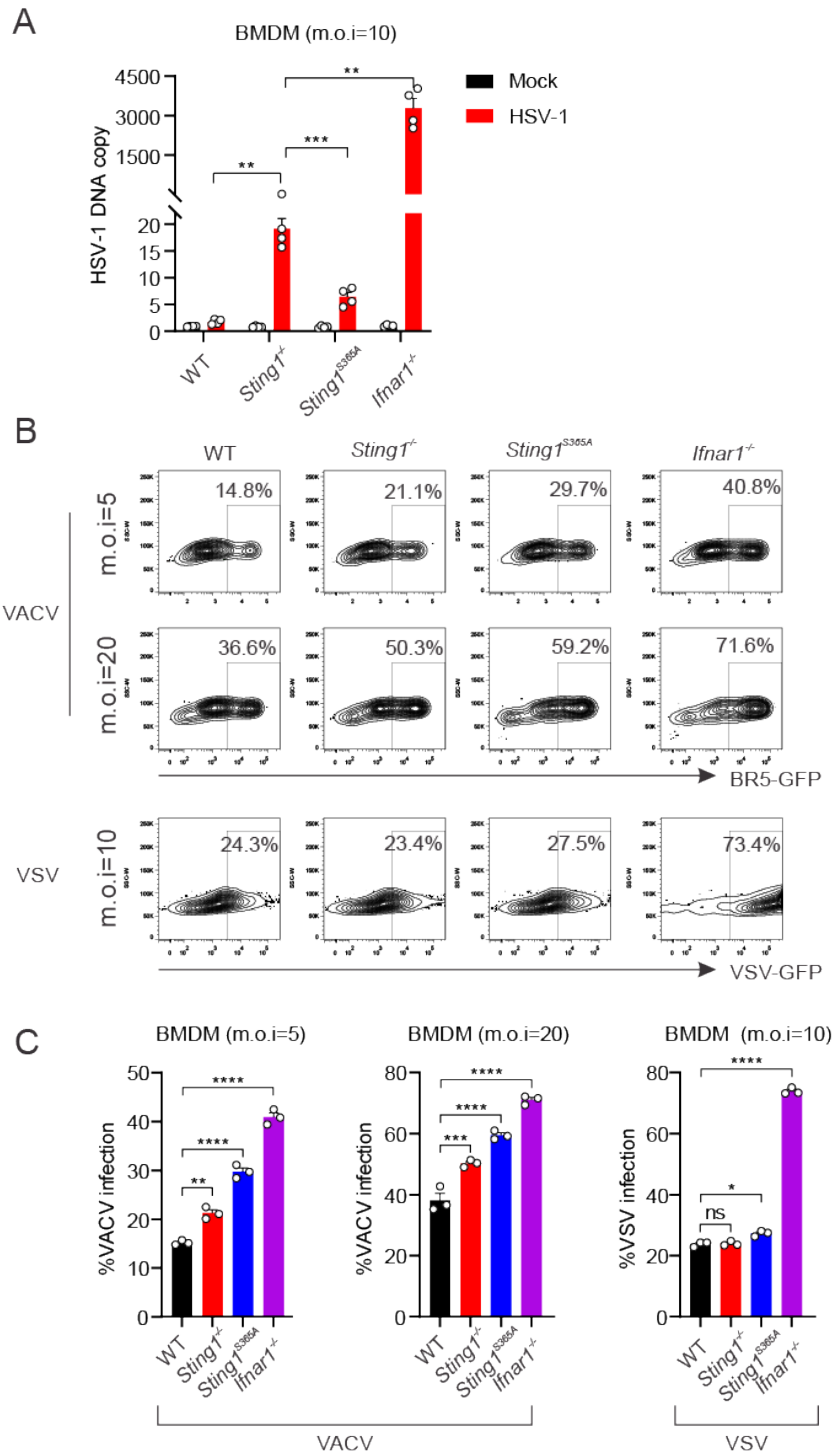

Figure S4. *Sting1*$^{S365A/S365A}$ restricts HSV-1 but not Vaccinia virus infection in BMDMs. Related to Figure 3.

(A) HSV-1 infection of BMDMs. WT, *Sting1*$^{-/-}$, *Sting1*$^{S365A/S365A}$ (*Sting1*^{S365A}) or *Ifnar1*$^{-/-}$ BMDMs were infected with HSV-1 (m.o.i=10) for 24 hours. HSV-1 DNA copy was quantified by qPCR.

(**B-C**) Vaccinia virus (VACV) and Vesicular stomatitis virus (VSV) infection of BMDMs. WT, *Sting1*$^{-/-}$, *Sting1*$^{S365A/S365A}$ (*Sting1*^{S365A}) or *Ifnar1*$^{-/-}$ BMDMs were infected with VACV or VSV at indicated m.o.i. for 12 h. Then, cells were fixed and analysed by FACS. Representative FACS plots are shown in **B** and quantitation of viral infection are shown in **C**.
Data are representative from at least two independent experiments.

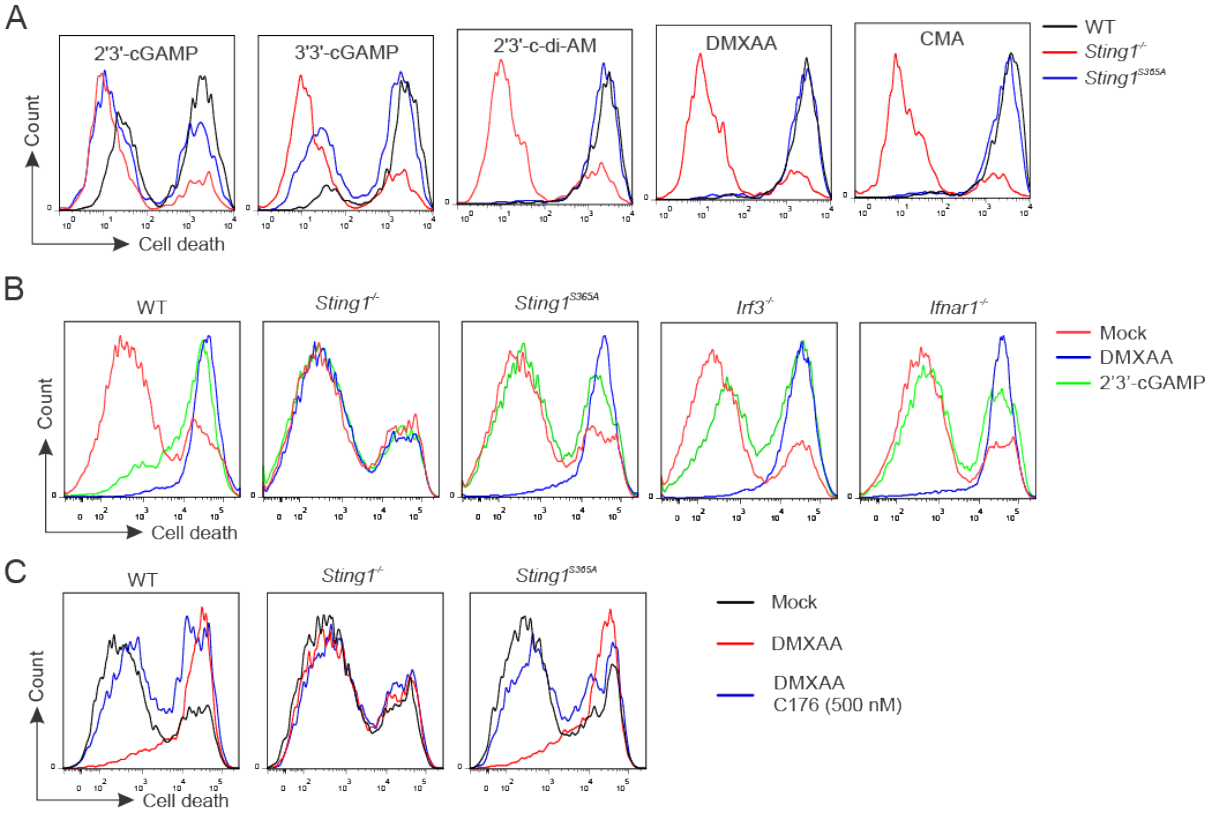

Figure S5. STING agonists show differential dependency on S365/IFN in inducing T cell death. Related to Figure 4.

Representative FACS plots corresponding to data in **Figure 4**, **A** for **Figure 4B-F**; **B** for **Figure 4G**; **C** for **Figure 4H**.

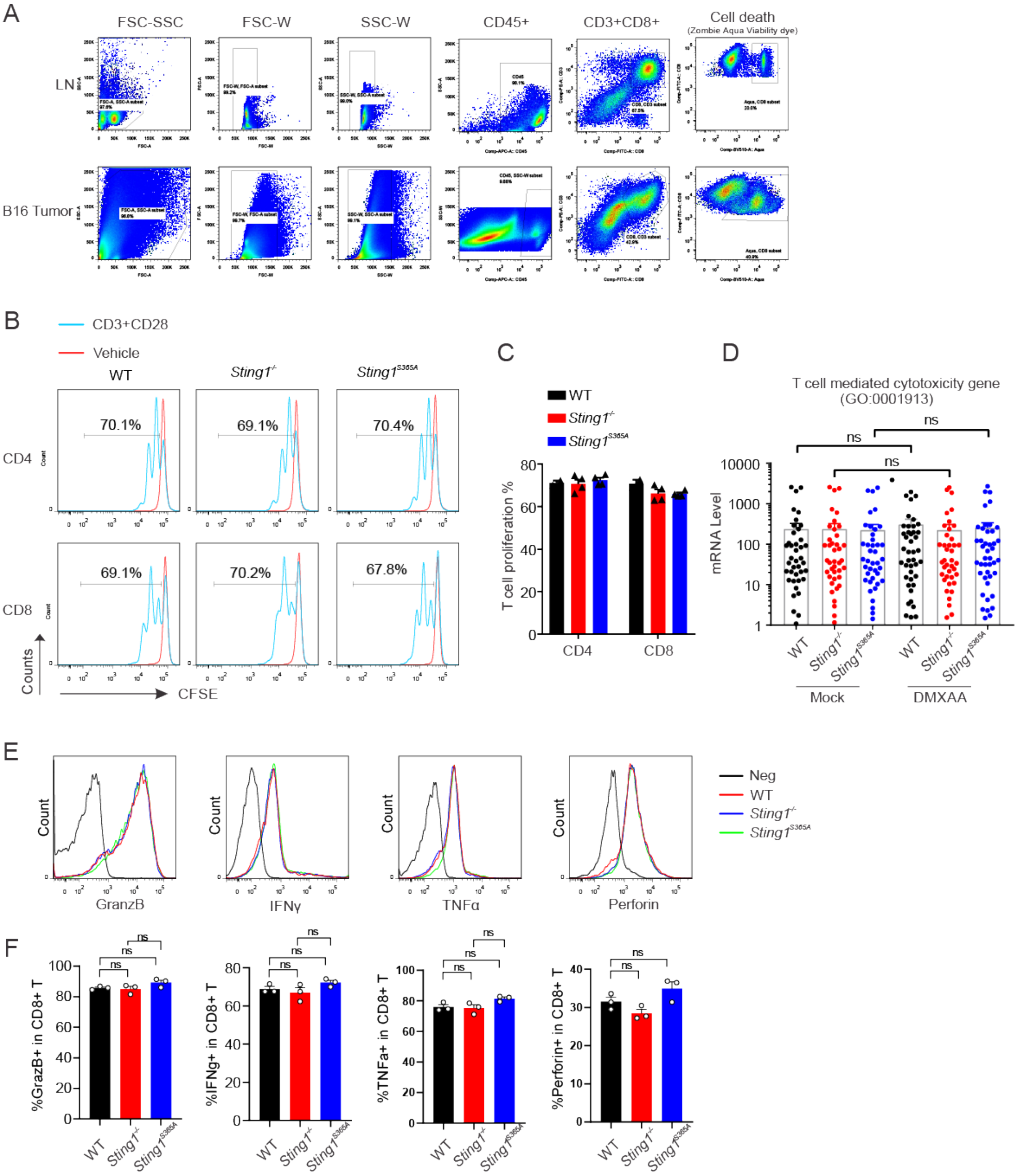

Figure S6. WT, *Sting1*$^{-/-}$ and *Sting1*$^{S365A/S365A}$ T cells show similar proliferation and cytotoxicity in vitro. Related to Figure 5.

(**A**) Gating strategy of T cell death FACS analysis in B16 tumour and draining lymph node.

(**B,C**) Proliferation analysis of WT, *Sting1*$^{-/-}$ and *Sting1*$^{S365A/S365A}$ CD4+ and CD8+ T cells. Splenic T cells were labelled with CFSE and treated with vehicle or α-CD3/CD28 antibodies for 3 days. Cell proliferation was analysed by FACS and quantified by CFSE dilution. Representative FACS plots (**B**) and quantitation (**C**) are shown.

(**D**) T cell-mediated cytotoxicity (GO term: 0001913) mRNA expression. Data from RNA-seq dataset. Each dot represents a different gene. ns, not significant. Two-way ANOVA test.

(**E,F**) Cytotoxicity of primary CD8+ T cells. CD8+ T cell were isolated from spleen and stimulated with α-CD3/CD28 antibodies for 2 days followed by intracellular staining of Granzym B (GranzB), IFNγ, TNFα and Perforin. Representative FACS plots (**E**) and quantitation (**F**) are shown.
Data are reprehensive of at least two independent experiments.