Supplemental Figure 1

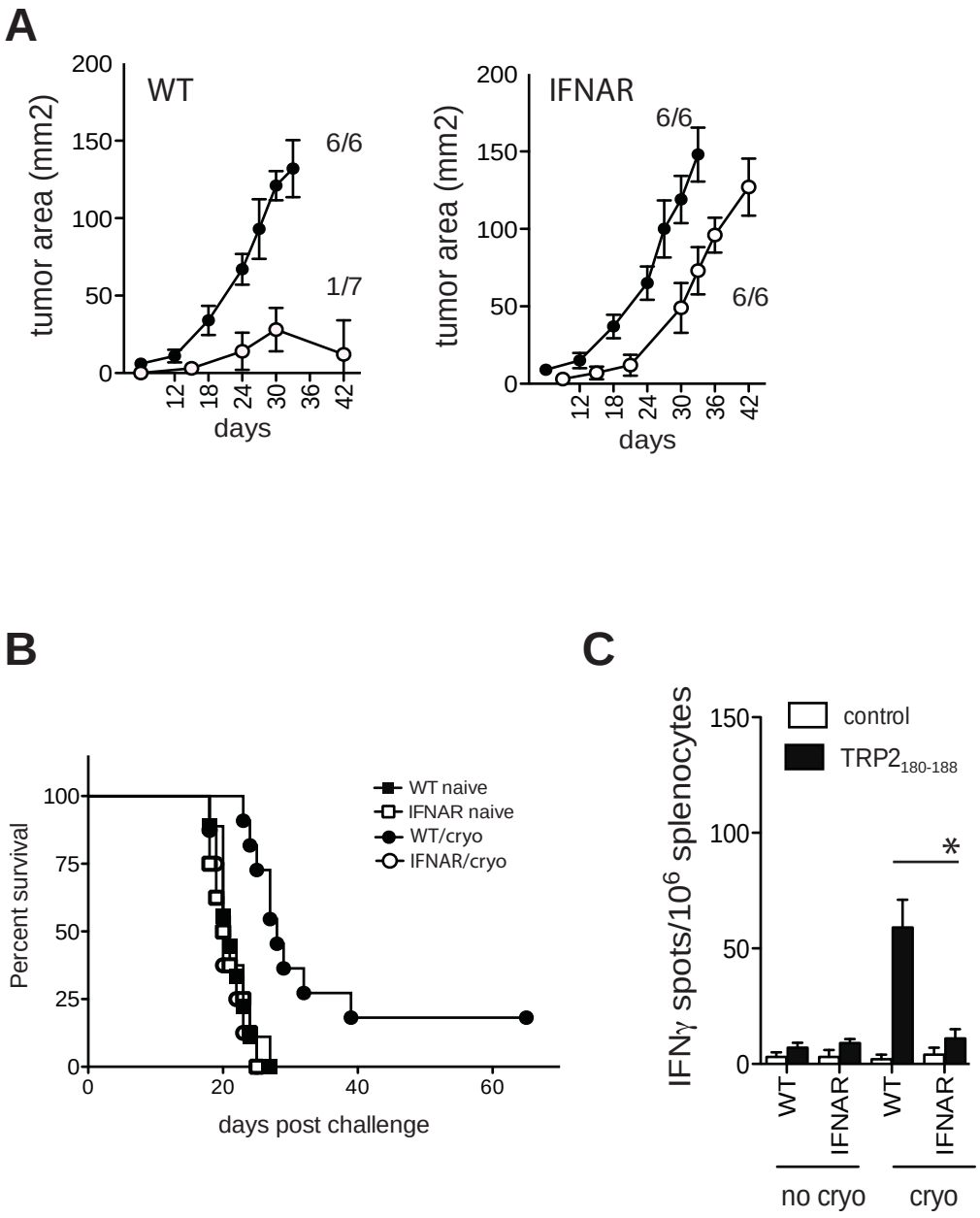

Figure S1. Compromised CD8+ T cell priming to cell-associated antigens in IFNAR-/- mice. **A.** WT and IFNAR-/- mice were immunized with irradiated OVA-Kb-/- splenocytes (open circles) or irradiated Kb-/- splenocytes (black circles). Forty days after immunization mice were s.c. challenged with EL-4-mOVA and tumor growth was monitored. Data are expressed as mean± s.e.m with indicated N/group. The X-axis depicts the days after tumor challenge.
B. WT and IFNAR-/- mice were s.c. inoculated with B16/F10. After 10 days tumors were cryo-ablated and mice were challenged 40 days later with B16/F10 cells s.c. (n=11-14/group). Survival curve of WT and IFNAR-/- mice upon secondary challenge with B16/F10. **C.** Frequency of TRP-2180-188 specific splenic T cells as determined by ELISPOT 7 days after cryoablation. Representative data of one experiment (of 3) are shown (mean± s.e.m, n=8-12).

Supplemental Figure 2

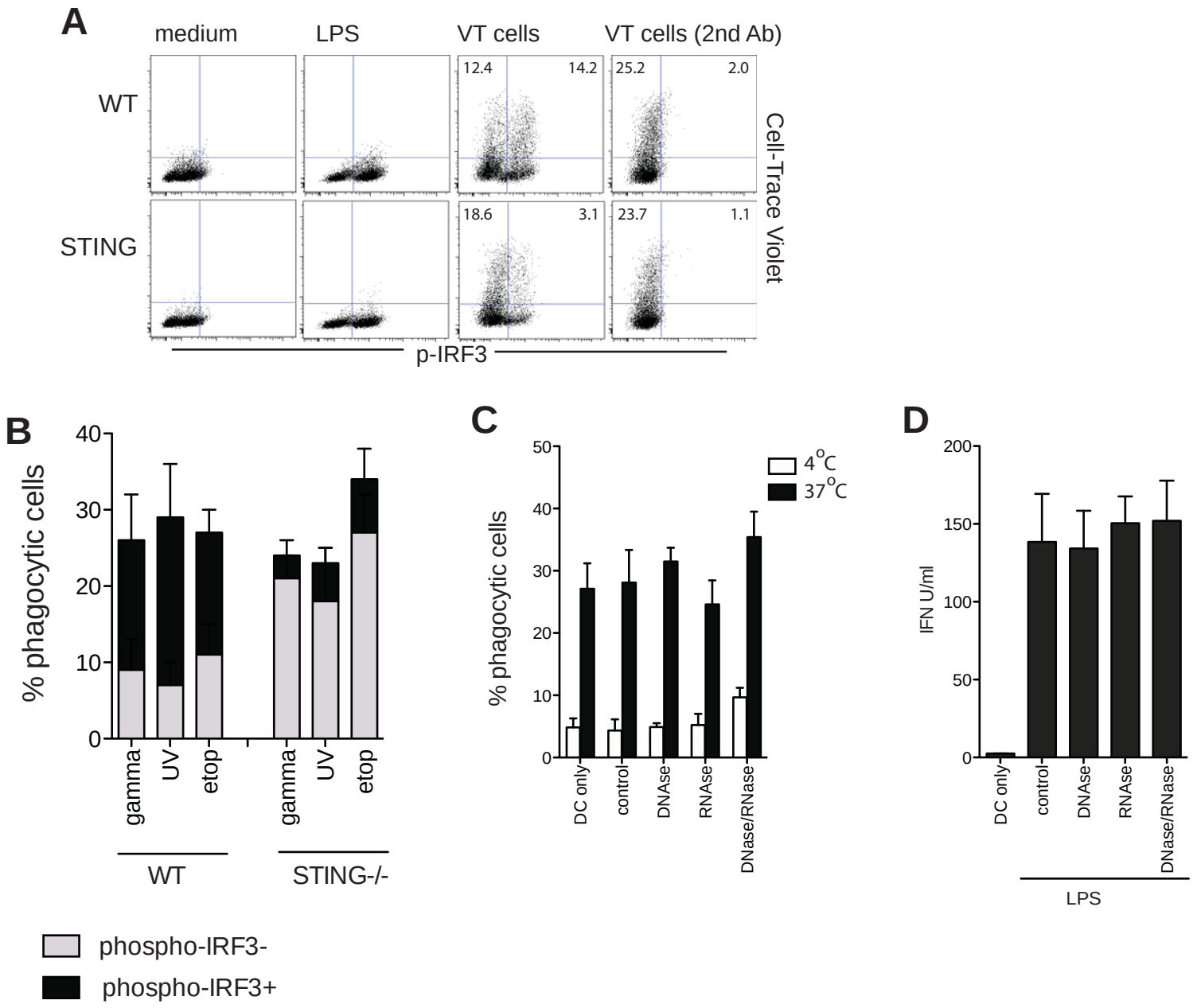

Figure S2. Phagocytosis, IRF3 phosophorylation and type I IFN induction by WT and STING-/- DCs. **A.** Flow cytometric analysis of phagocytosis and p-IRF3 in WT and STING-/- DCs. Purified WT and STING-/- DCs were cultured with CellTrace Violet-labeled irradiated IRF3-/- cells for 8 hr and phagocytosis and IRF3 phosphorylation was determined by flow cytometry. A representative set of one (out of 4 mice) is shown. **B.** Similar as in A, frequency of WT and STING-/- DCs with p-IRF3 staining within the DCs population that has phagocytosed CellTrace Violet material. CellTrace Violet cells were treated with gamma irradiation, UV irradiation or etoposide (5mM). **C**. Purified WT DCs were cultured at 4°C (white bars) and 37°C (black bars) with CellTrace Violet-labeled irradiated splenocytes in the presence or absence of RNAses and DNAses. Uptake of CellTrace Violet materials was determined 6 hr later by flow cytometry. Data is expressed as percentage of DCs that contain CellTrace Violet. **D.** Type I IFN production by purified WT DCs upon overnight stimulation with LPS in the presence of DNAses/RNAses. Data are expressed as mean± s.e.m with n=4.

Supplemental Figure 3

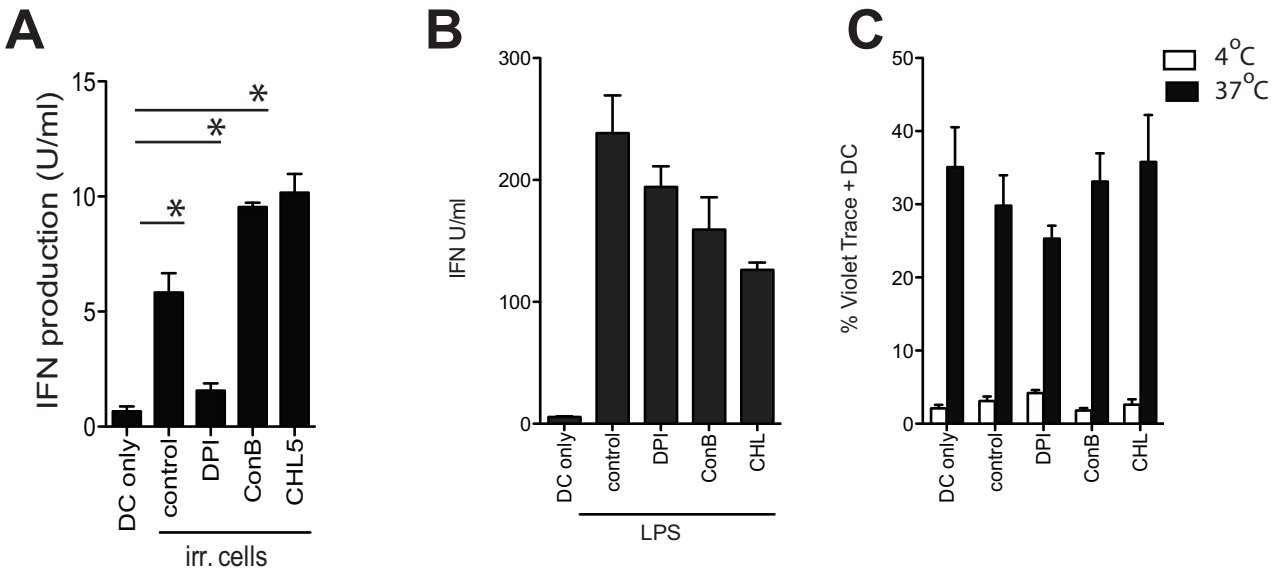

Figure S3. Lysosomal acidification rate determines IFN production. **A**. Type I IFN production by purified WT DCs upon stimulation with irradiated cells in the presence of diphenyliodonium (DPI; acceleration of phagosomal acidification) or inhibitors of lysosomal acidification (Chloroquine and ConB). **B.** Type I IFN production by purified WT DCs upon overnight stimulation with LPS in the presence of indicated agents. **C**. Purified WT DCs were cultured at 4°C (white bars) and 37°C (black bars) with CellTrace Violet-labeled irradiated splenocytes in the presence or absence of indicated agents. Uptake of CellTrace Violet materials was determined 6 hr later by flow cytometry. Data are expressed as mean± s.e.m with n=4.