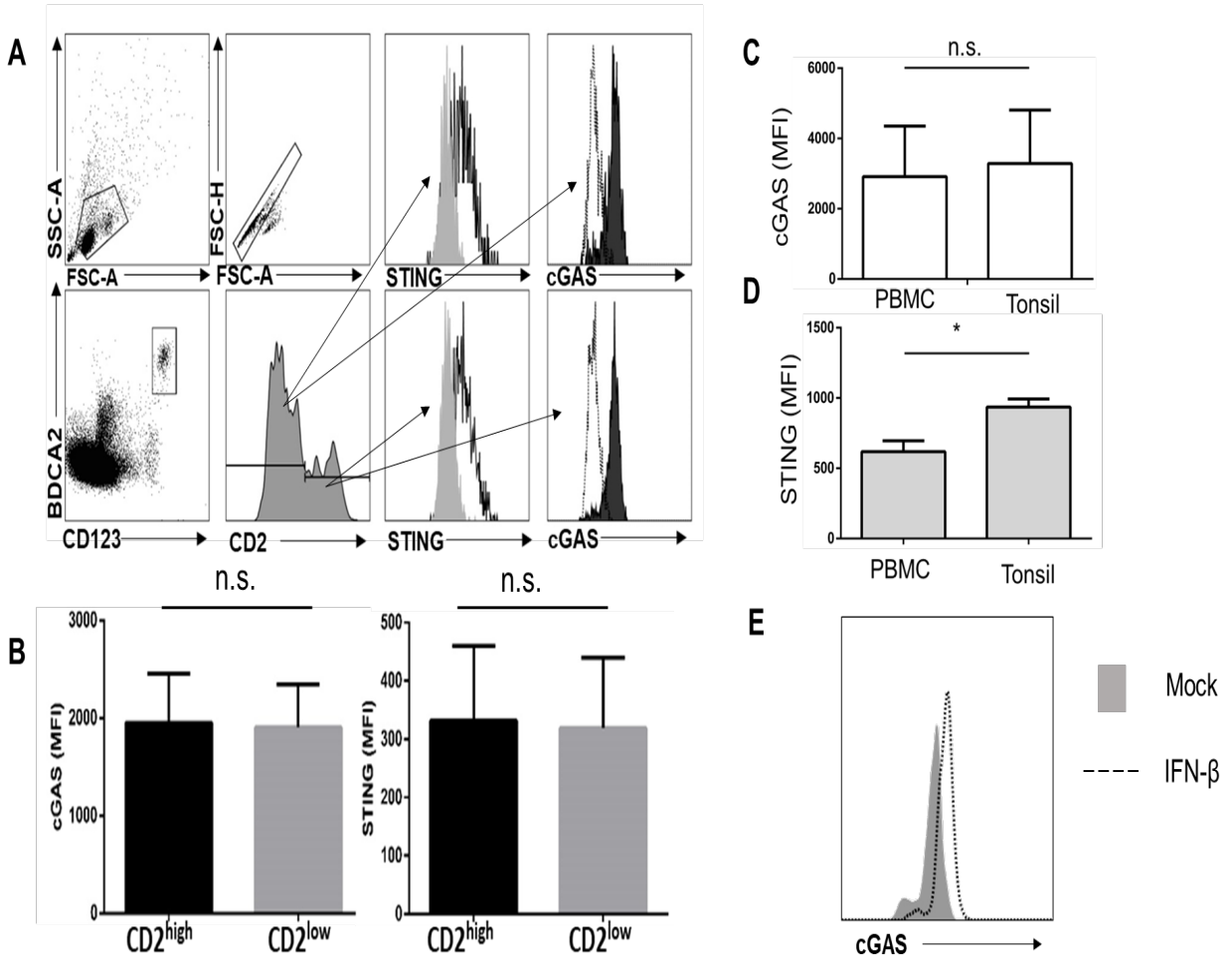
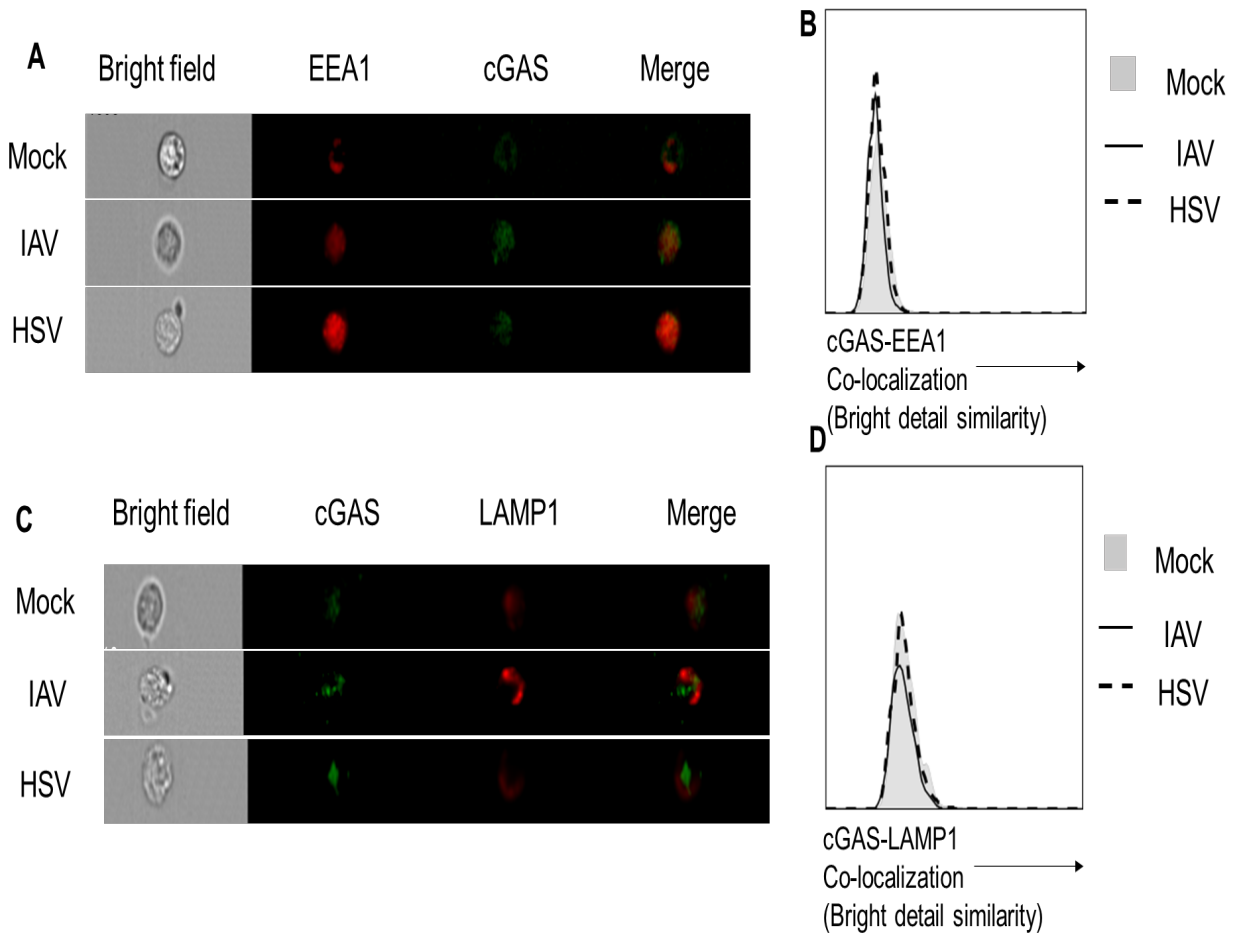
Supplemental Figure 1



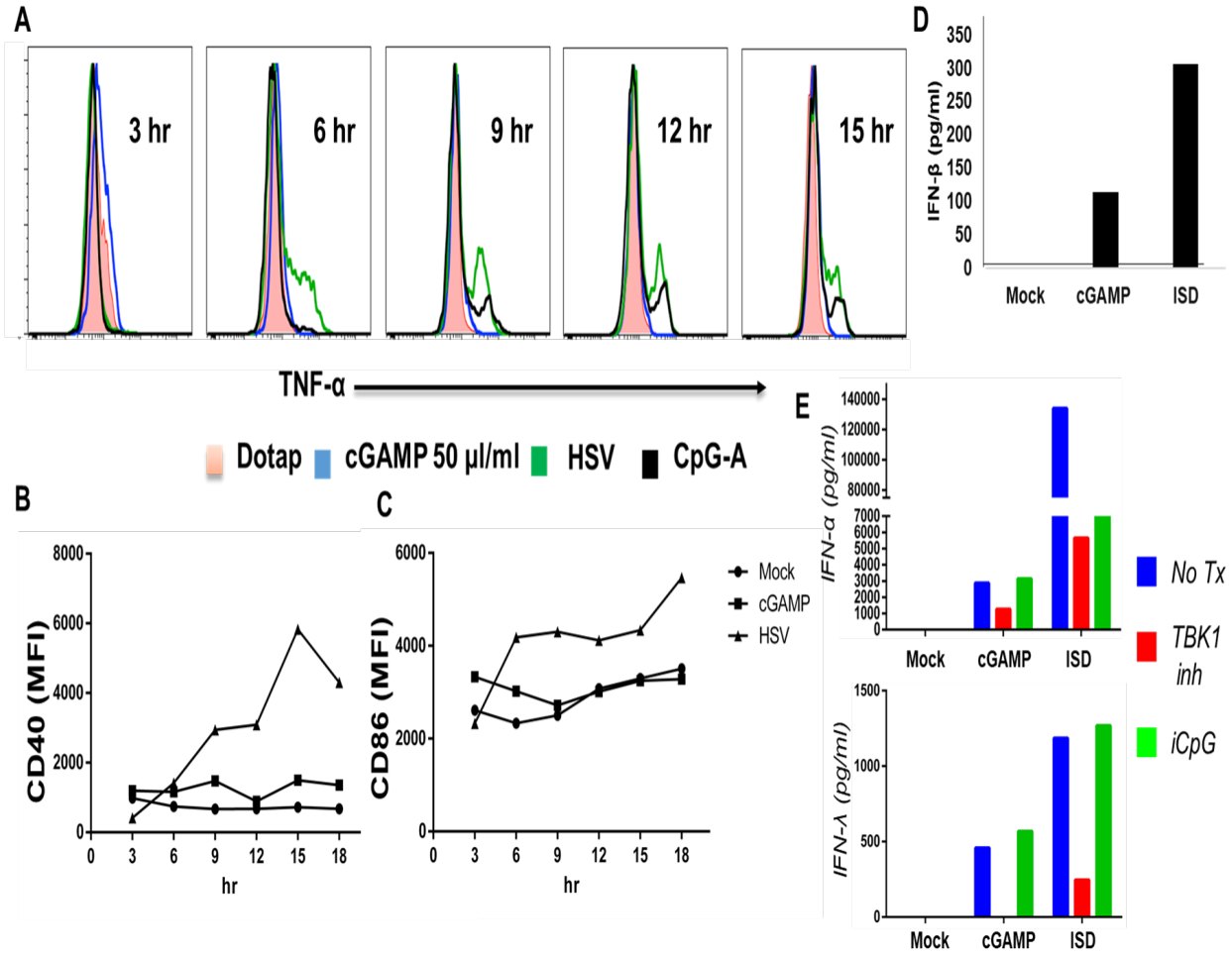
Supplemental Fig. 1: CD2^{high} and CD2^{low} subsets of pDCs express similar levels of cGAS and STING, as do tonsil-derived and peripheral blood-derived pDCs. PBMCs were stained for pDC-markers and CD2, then permeabilized and stained for cGAS and STING. **(A)** Gating strategy to identify CD2^{high} and CD2^{low} subsets of pDCs. **(B)** Expression of cGAS and STING by CD2^{high} and CD2^{low} subsets of pDCs (*n* = *3*). PBMCs and tonsil derived mononuclear cells were stained for pDC markers, permeabilized and stained for cGAS and STING. Expression of **(C)** cGAS and **(D)** STING in pDCs derived from PBMCs and tonsils (*n* =*3*). **(E)** Expression of cGAS in pDCs with IFN-β stimulation. PBMCs from healthy donors were stimulated with recombinant human IFN-β for 8 hours, stained for pDC markers, permeabilized and stained for cGAS and acquired. Data represents one of 3 independent experiments.

Supplemental Figure 2



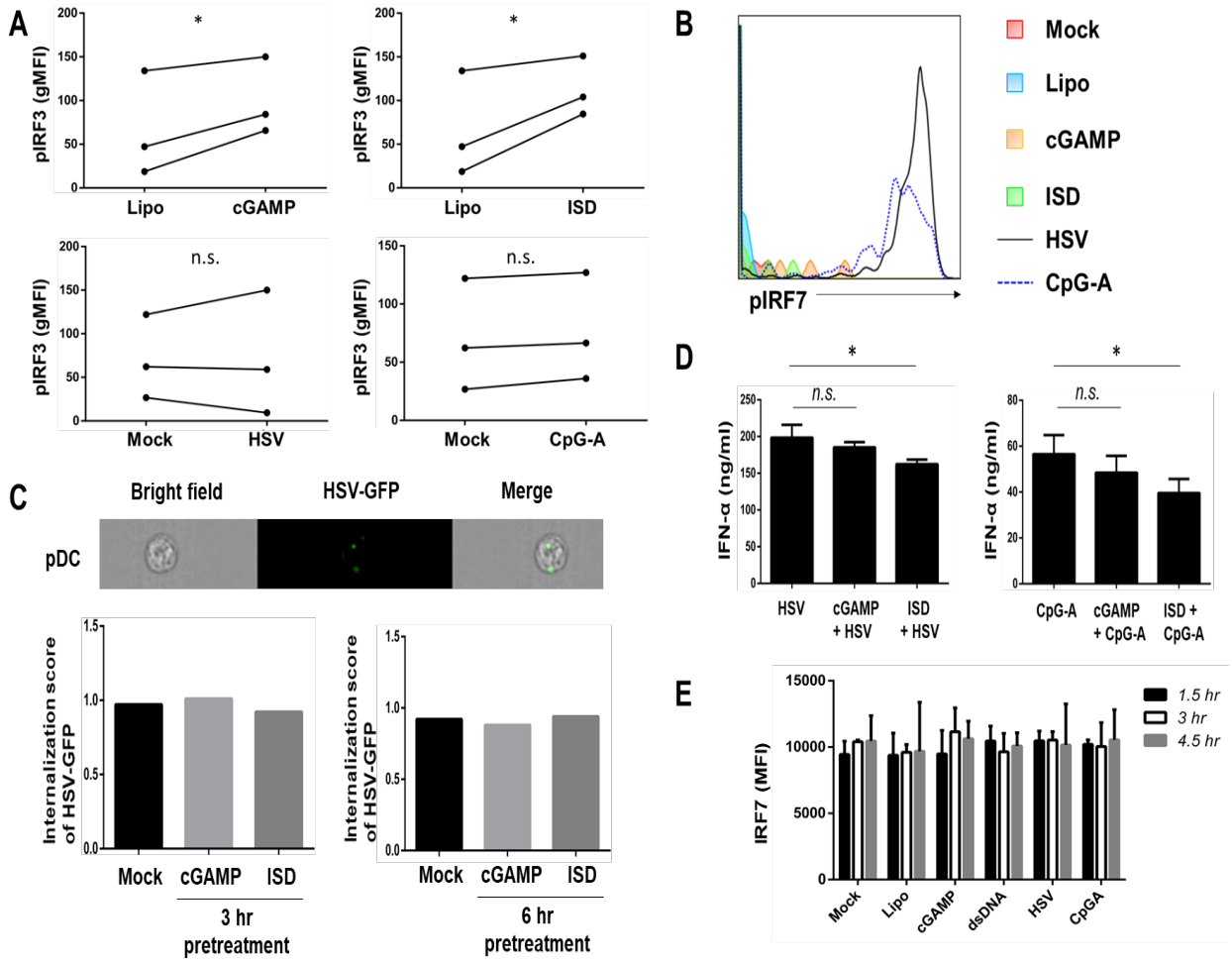
Supplemental Fig. 2: cGAS does not co-localize with EEA1 or LAMP1 in pDCs with or without stimulation. PBMCs were stimulated with HSV or IAV for 8 hours, stained for pDC markers, permeabilized, and stained for EEA1 and cGAS. **(A)** Expression of EEA1 and cGAS in pDCs with or without stimulation. **(B)** Co-localization of cGAS and EEA1. PBMCs were stimulated with HSV or IAV for 8 hours, stained for pDC markers, permeabilized, and stained for LAMP1 and cGAS. **(C)** Expression of LAMP1 and cGAS in pDCs with or without stimulation **(D)** Co-localization of cGAS and LAMP1.

Supplemental Figure 3



Supplemental Fig. 3: cGAMP do not induce TNF-α, CD40, or CD86 expression in pDCs. Purified pDCs are stimulated with cGAMP, HSV, and CpG-A for 3, 6, 9, 12, and 15 hr, fixed, permeabilized and stained for TNF-α. BFA was added 2 hours before each time point. **(A)** Expression of TNF-α pDCs with or without stimulation. **(B,C)** Purified pDCs were stimulated with or without cGAMP and HSV and stained for CD40 and CD86. Data represents one of two representative experiments with similar results. **(D)** IFN-β yield by THP1 cells upon lipofection with cGAMP (50 µg/ml) and ISD (5 µg/ml). Data represents one of two independent experiments. **(E)** IFN-α and IFN-λ yield of pDCs upon 1 hour pretreatment with TBK1 inhibitor BX795 and overnight stimulation with cGAMP and ISD. Data represents one of two independent experiments.

Supplemental Figure 4



Supplemental Fig. 4: cGAS-STING pathway selectively phosphorylate IRF3, while TLR9-mediated stimulation phosphorylates IRF7. Purified pDCs were stimulated with cGAMP and ISD for 2 hours, and HSV and CpG-A for 4 hours, fixed, permeabilized, and stained for pIRF3 or pIRF7. **(A)** Expression of pIRF3 in lipofectamine-2000 (Lipo) vs. cGAMP, lipofectamine-2000 (Lipo) vs. ISD, Mock vs. HSV, and Mock vs. CpG-A. Each connecting line represent one independent experiment. **(B)** Overlay histogram showing expression of pIRF7 in Mock, Lipo, cGAMP, ISD, HSV, and CpG-A. Data represents one of two independent experiments. **(C)** Internalization of HSV-GFP in pDCs with or without cGAMP and ISD pretreatment for 3 hours and 6 hours. Data represents one of two independent experiments. **(D)** Interferon yield upon simultaneous stimulation with cGAMP + HSV or ISD + HSV for 6 hours (n = 3 independent experiments). **(E)** Purified pDCs were stimulated with cGAMP, ISD, HSV, and CpG-A for 1.5, 3, and 4.5 hours, fixed, permeabilized, and stained for total IRF7 expression. Data expressed as the median fluorescent intensity (MFI) of IRF7 in pDCs with each bar representing mean ± s.d. (n = 3).