

Supplementary information, Fig. S2 Identification of USP29 as a cGAS-interacting protein.

a Luciferase reporter assays analyzing IFN- β promoter activity (top) and immunoblot analysis (bottom) of FLAG-tagged USPs, HA-STING, HA-cGAS and β -Actin of HEK293 cells transfected with the indicated plasmids for 24 h. **b** Luciferase reporter assays analyzing IFN- β promoter activity (top) and immunoblot assay (bottom) of FLAG-tagged USP29, HA-STING, HA-cGAS and β -Actin of HEK293 cells transfected with the indicated plasmids for 24 h. **c** Immunoblot assay of USP29, cGAS and GAPDH in various mouse organs, primary mouse cells and human cell lines. **d** Immunoprecipitation (with anti-FLAG) and immunoblot analysis (with anti-FLAG or anti-HA) of HEK293 cells transfected with plasmids encoding FLAG-USP29 and HA-tagged cGAS, MITA, RIG-I, MAVS, TBK1 or IRF3 for 24 hours. **e-f** Immunoprecipitation (with control IgG or anti-cGAS) and immunoblot assay (with anti-USP29, anti-USP25, anti-cGAS, anti-GAPDH or anti- β -Actin) of MEFs or MLFs infected with HSV-1 for 0-8 h. **g** FLAG-cGAS was purified by anti-FLAG agarose from FLAG-cGAS-transfected HEK293 cell lysates. GST, GST-tagged USP29(1-210) or USP29(211-600) were purified by GST affinity chromatography from BL21. GST, GST-tagged USP29(1-210) or USP29(211-600) was incubated with FLAG-cGAS in PBS overnight, followed by coomassie blue staining and immunoblot analysis (with anti-FLAG). **h** Immunoprecipitation (with control IgG, anti-TRIM38 or anti-USP29) and immunoblot assay (with anti-TRIM38, anti-USP29, anti-cGAS, or anti-GAPDH) of MEFs infected with HSV-1 for 0-8 h. **i** qRT-PCR analysis of *USP29, IFNB, ISG56* mRNA in THP-1 cells infected with HSV-1, SeV, transfected with ISD45 or treated with IFN- β for 6 h. ***P<0.001 (analysis of two-way ANOVA followed by Bonferroni post-test). Data are representative of two (**c-h**) or three (**a, b, i**) independent experiments (mean \pm S.D. in **a, b, i**).