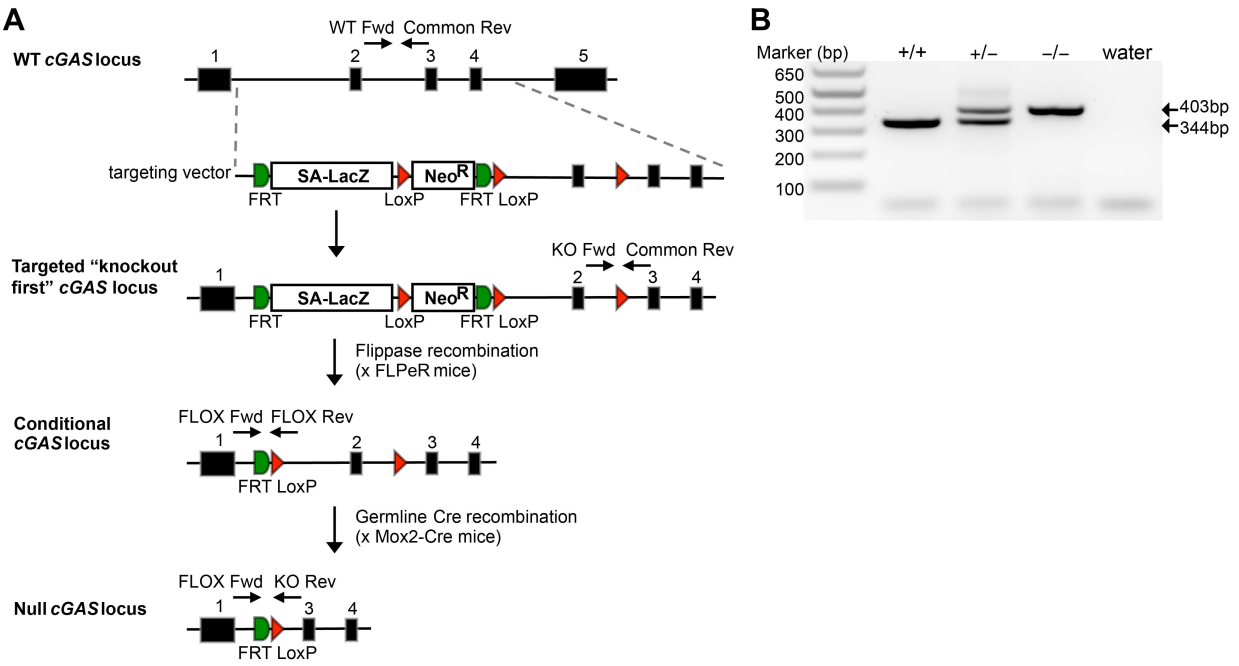
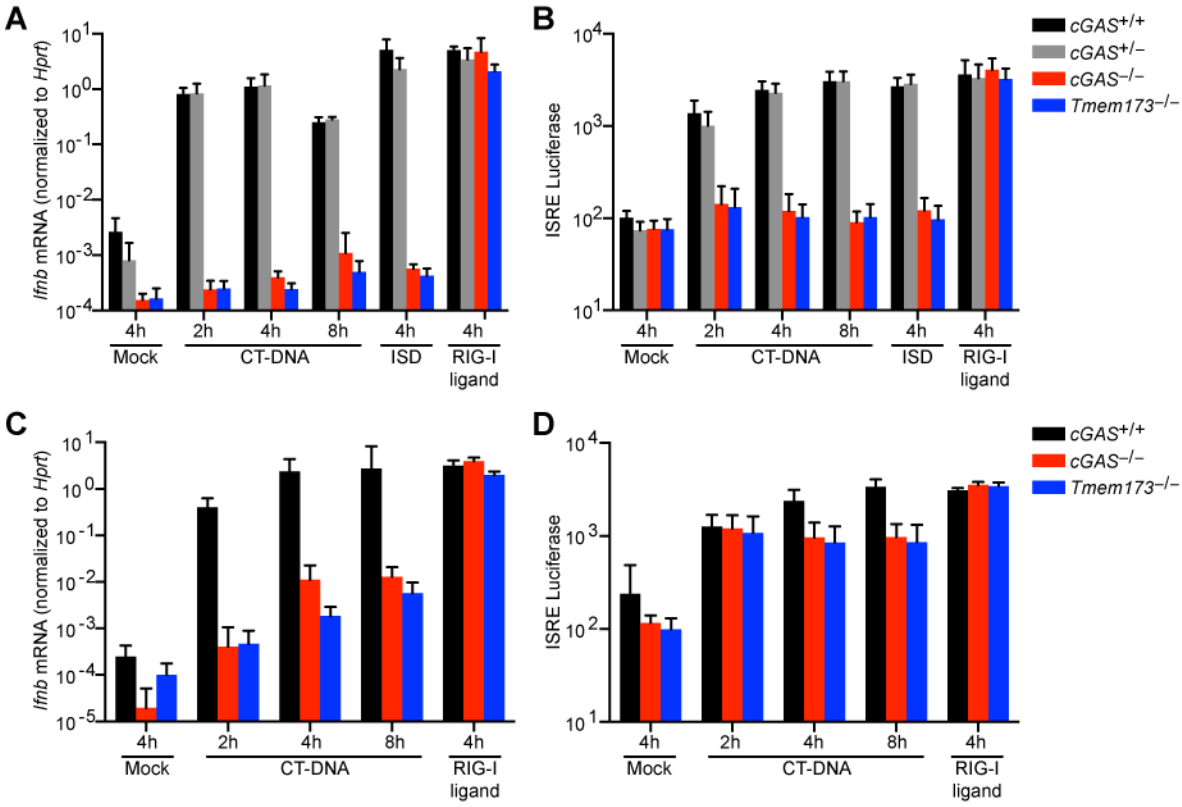


Supplementary Figure 1: Generation of cGAS-deficient mice
(A) Schematic of the wild-type and targeted *cGAS* (*Mb21d1*) locus. Exons are indicated with solid black squares and primers used for genotyping PCR are indicated with arrows. The targeted "knockout first" locus contains a strong splice acceptor (SA) in the LacZ cassette, resulting in a truncated *cGAS* transcript in which exon 1 is spliced into the LacZ cassette. The majority of $cGAS^{-/-}$ mice used in this manuscript carry this "knockout first" null allele of cGAS, including all mice crossed to $Trex1^{-/-}$ mice. The $cGAS^{-/-}Trex1^{+/+}$ mice used as controls in Figures 2 and 3 carry the final null *cGAS* allele that was generated as follows: mice with a conditional $cGAS^{FLOX}$ allele were generated by crossing to FLPeR mice (1) to remove the FRT-flanked LacZ and Neo casettes; mice with the final null *cGAS* allele were generated by crossing $cGAS^{FLOX}$ mice to Mox2-Cre mice (2) for germline excision of the LoxP-flanked exon 2. The $cGAS^{FLOX}$ and final *cGAS* alleles were detected with the following primers: FLOX Fwd, AAGGCGCATAACGATACCACG; FLOX Rev, GCTGGGTCTAGATATCTCGAC; and KO Rev, AAGGGCACTGAGCCTCTGAG. **(B)** Genotyping PCR to distinguish $cGAS^{+/+}$, $cGAS^{+/-}$, and $cGAS^{-/-}$ mice carrying the "knockout first" *cGAS* allele.



Supplementary Figure 2: Characterization of the IFN response in cGAS-deficient cells
(A) Quantification of *Ifnb* mRNA induction in bone marrow-derived macrophages from *cGAS*$^{+/+}$, *cGAS*$^{+/-}$, *cGAS*$^{-/-}$, or *Tmem173*$^{-/-}$ (STING-deficient) mice transfected with calf-thymus DNA (CT-DNA), ISD 100-mer oligonucleotides (3), or RIG-I ligand (4) for 2h, 4h, or 8h as indicated. **(B)** Quantification of type I IFN production (right panel) using an IFN bioassay with supernatents from bone marrow-derived macrophages stimulated with the indicated ligands as in (A). **(C)** Quantification of *Ifnb* mRNA induction in mouse embryonic fibroblasts (MEFs) from *cGAS*$^{+/+}$, *cGAS*$^{-/-}$, or *Tmem173*$^{-/-}$ (STING-deficient) embryos stimulated as in (A). **(D)** Quantification of type I IFN production (right panel) using an IFN bioassay with supernatents from MEFs stimulated with the indicated ligands as in (C). Data are representative of two experiments.

Supplementary References

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