

Expanded View Figures

Figure EV1. Short dsDNAs with ssDNA arms promote phase separation and activate cGAS.

- A Diagrams showing sequences of short dsDNAs with ssDNA arms and the short dsDNA controls.
- B Photographs of FL-hcGAS (0.294 mg/ml, 5.0 μ M) mixed with indicated DNAs (2.5 μ M) showing phase-separated droplets or condensates induced by short dsDNAs with ssDNA arms but not the short dsDNA controls ($n = 2$, biological replicates, data from one representative independent biological replicate are shown). The corresponding concentration in mg/ml were 0.009 for 6–6, 0.012 for 8–8, 0.014 for 10–10, 0.018 for 12–12, 0.024 for 16–16, 0.031 for 20–20, 0.043 for 48–6 and 48-6(m), 0.044 for 48–8 and 48-8(m), 0.046 for 48–10 and 48–10(m), 0.047 for 48–12 and 48-12(m), 0.05 for 48–16 and 48–16(m), 0.053 for 48–20 and 48–20(m), 0.015 for 20R, and 0.038 for 48F and 48F(m). The scale bar represents 20 μ m.
- C TLC analysis for cGAMP, which is indicative of cGAS activation. cGAMP was detected in samples containing short dsDNAs with ssDNA arms but not in samples containing the short dsDNA or ssDNA controls ($n = 2$, biological replicates, data from one representative independent biological replicate are shown). The FL-hcGAS and DNA concentrations were (0.294 mg/ml, 5.0 μ M) and 2.5 μ M, respectively. Samples were prepared in 20 mM HEPES at pH 7.5 and 150 mM NaCl and were incubated at room temperature for 1 h.

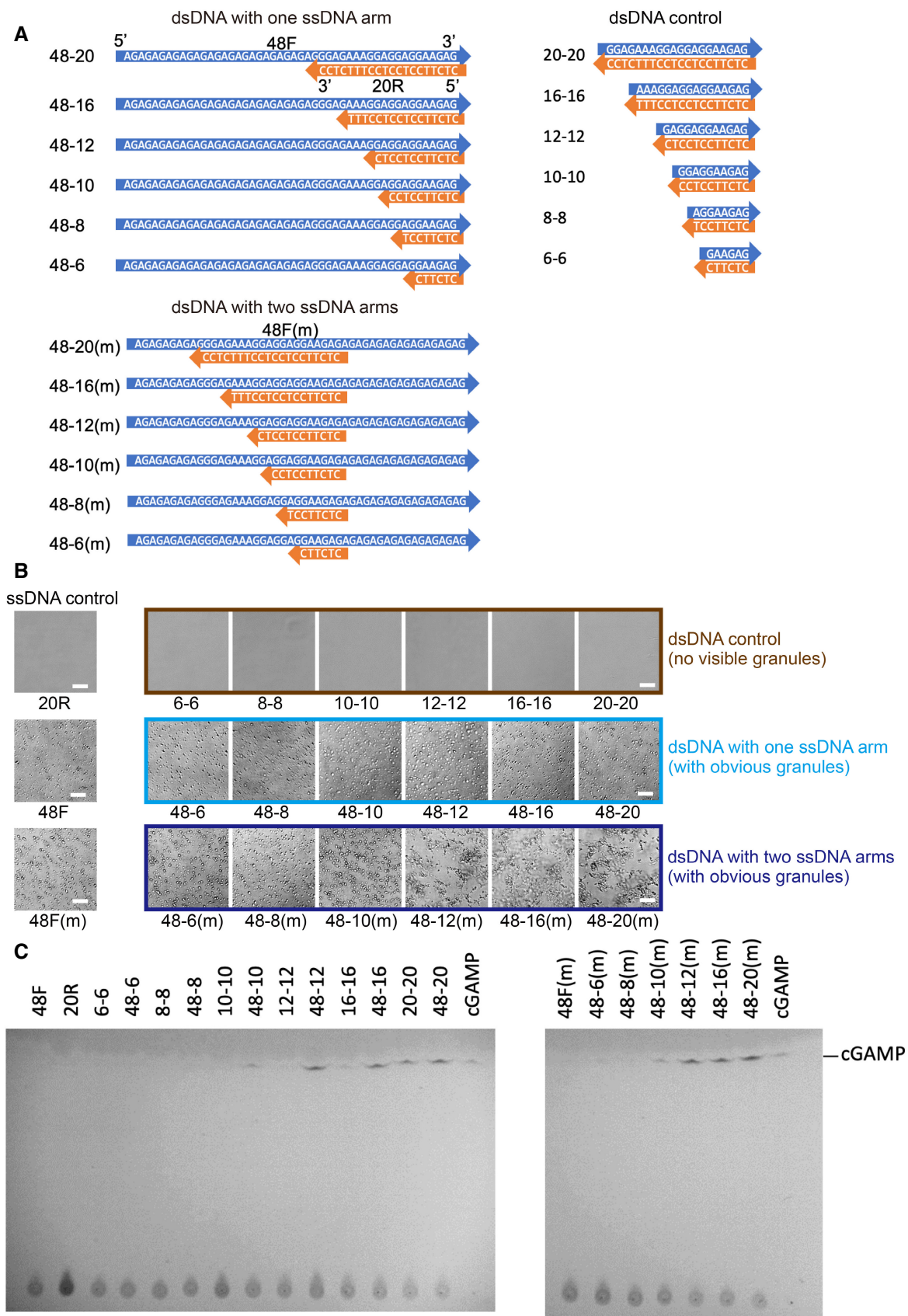


Figure EV1.

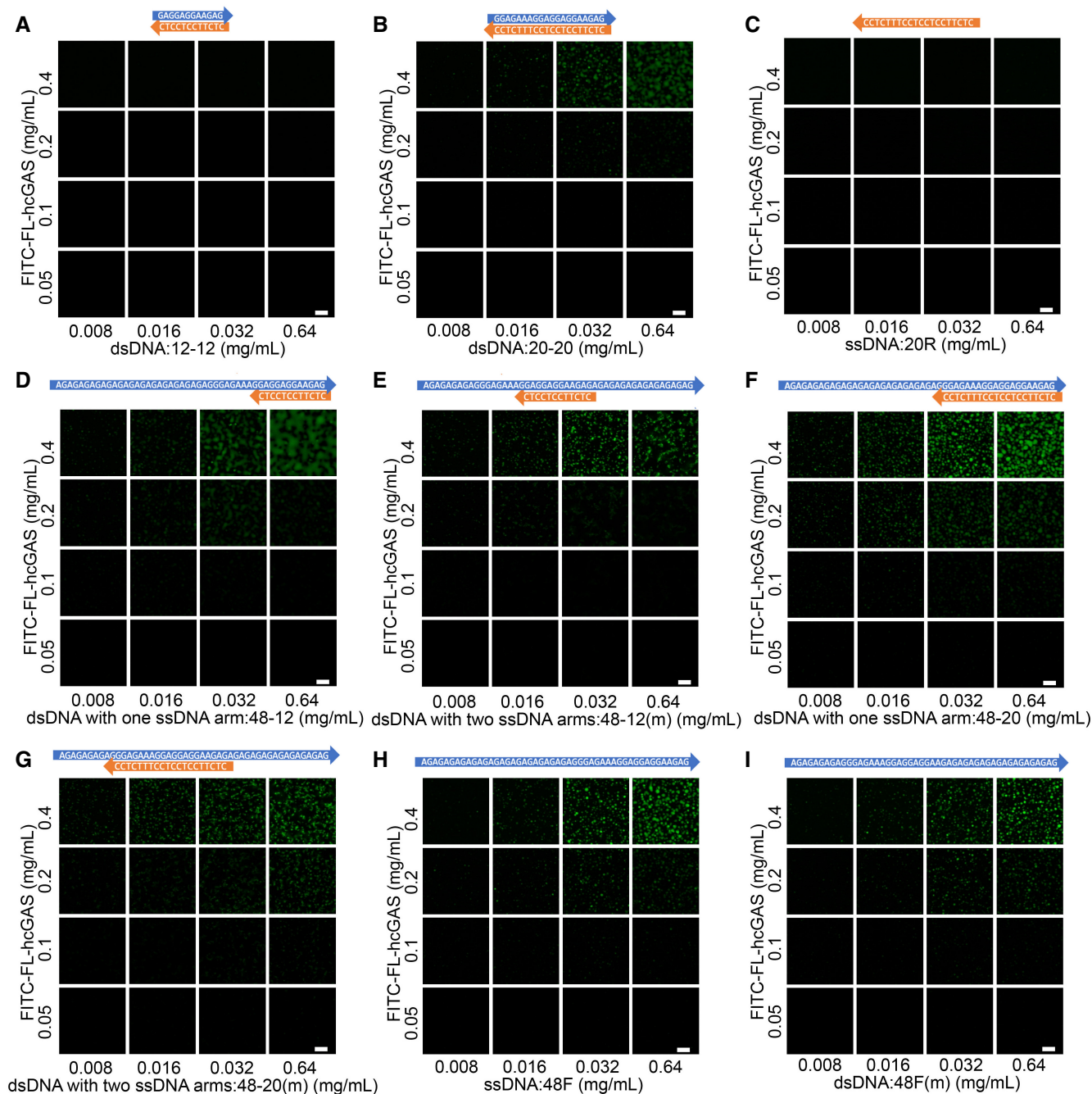


Figure EV2. Phase diagrams showing phase separations of FITC-labeled cGAS induced by short dsDNAs with one or two ssDNA arms.

- A Phase diagram of cGAS and the 12-bp dsDNA (12–12) as indicated in Fig EV1A. The scale bar represents 20 μ m.
- B Phase diagram of cGAS and the 20-bp dsDNA (20–20) as indicated in Fig EV1A. The scale bar represents 20 μ m.
- C Phase diagram of cGAS and the 20 nt ssDNA (20R) as indicated in Fig EV1A. The scale bar represents 20 μ m.
- D Phase diagram of cGAS and a 12-bp dsDNA with one ssDNA arms (48–12) as indicated in Fig EV1A. The scale bar represents 20 μ m.
- E Phase diagram of cGAS and a 12-bp dsDNA with two ssDNA arms (48–12(m)) as indicated in Fig EV1A. The scale bar represents 20 μ m.
- F Phase diagram of cGAS and a 20-bp dsDNA with one ssDNA arms (48–20) as indicated in Fig EV1A. The scale bar represents 20 μ m.
- G Phase diagram of cGAS and the 20-bp dsDNA with two ssDNA arm (48–20(m)) as indicated in Fig EV1A. The scale bar represents 20 μ m.
- H Phase diagram of cGAS and a 48 nt ssDNA (48F) that has the same sequence as the ssDNA arm strand in “D” and “F.” The scale bar represents 20 μ m.
- I Phase diagram of cGAS and a 48 nt ssDNA (48F(m)) that has the same sequence as the ssDNA arm strand in “E” and “G.” The scale bar represents 20 μ m.

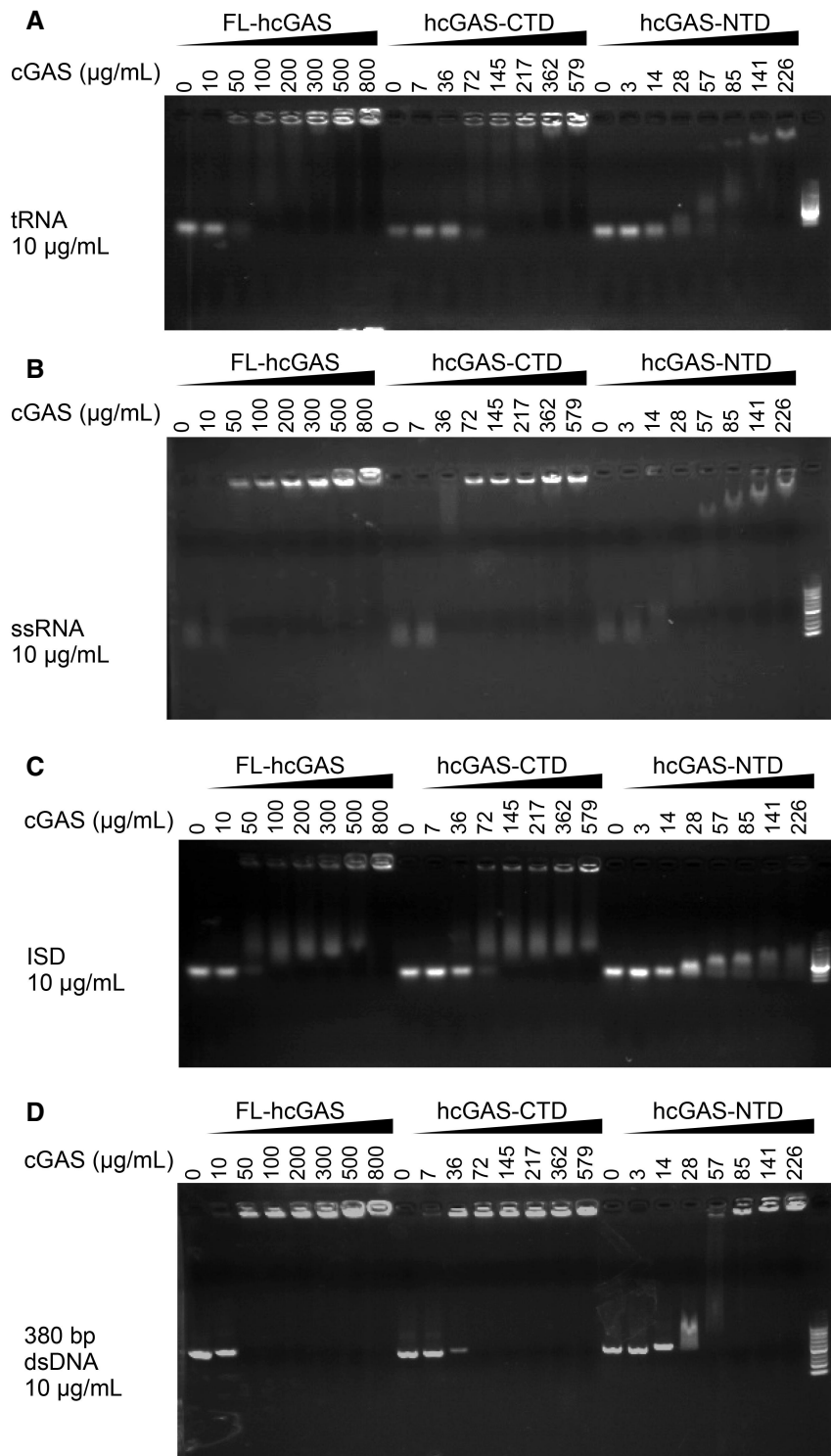


Figure EV3. Electrophoretic mobility shift analysis of interactions of RNA or DNA with cGAS.

A–D Electrophoretic mobility shift analysis (EMSA) of (A) yeast tRNA, (B) ssRNA, (C) ISD, and (D) 380-bp dsDNA in presence of FL-hcGAS ($n = 2$, biological replicates, data from one representative independent biological replicate are shown). The nucleic acid concentration was 10 μg/ml. The FL-hcGAS concentrations were 0, 10, 50, 100, 200, 300, 500, and 800 μg/ml. The hcGAS-CTD concentrations were 0, 7, 36, 72, 145, 217, 362, and 579 μg/ml. The hcGAS-NTD concentrations were 0, 3, 14, 28, 57, 85, 141, and 226 μg/ml.

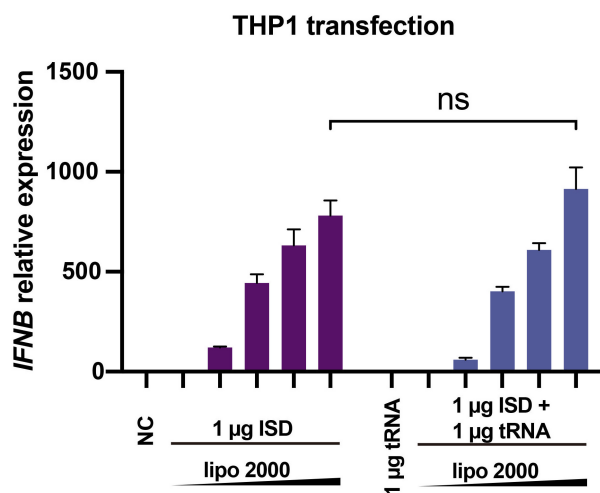


Figure EV4. RNAs regulate the dsDNA-induced expression of *IFNB* in cells.

Expression of *IFNB* mRNA in THP1 cells transfected with 1 µg dsDNA (ISD) and 1 µg tRNA by using different amount of liposomes ($n = 4$, biological replicates). Data are presented as mean \pm SEM, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and ns = not significant (two-way ANOVA).

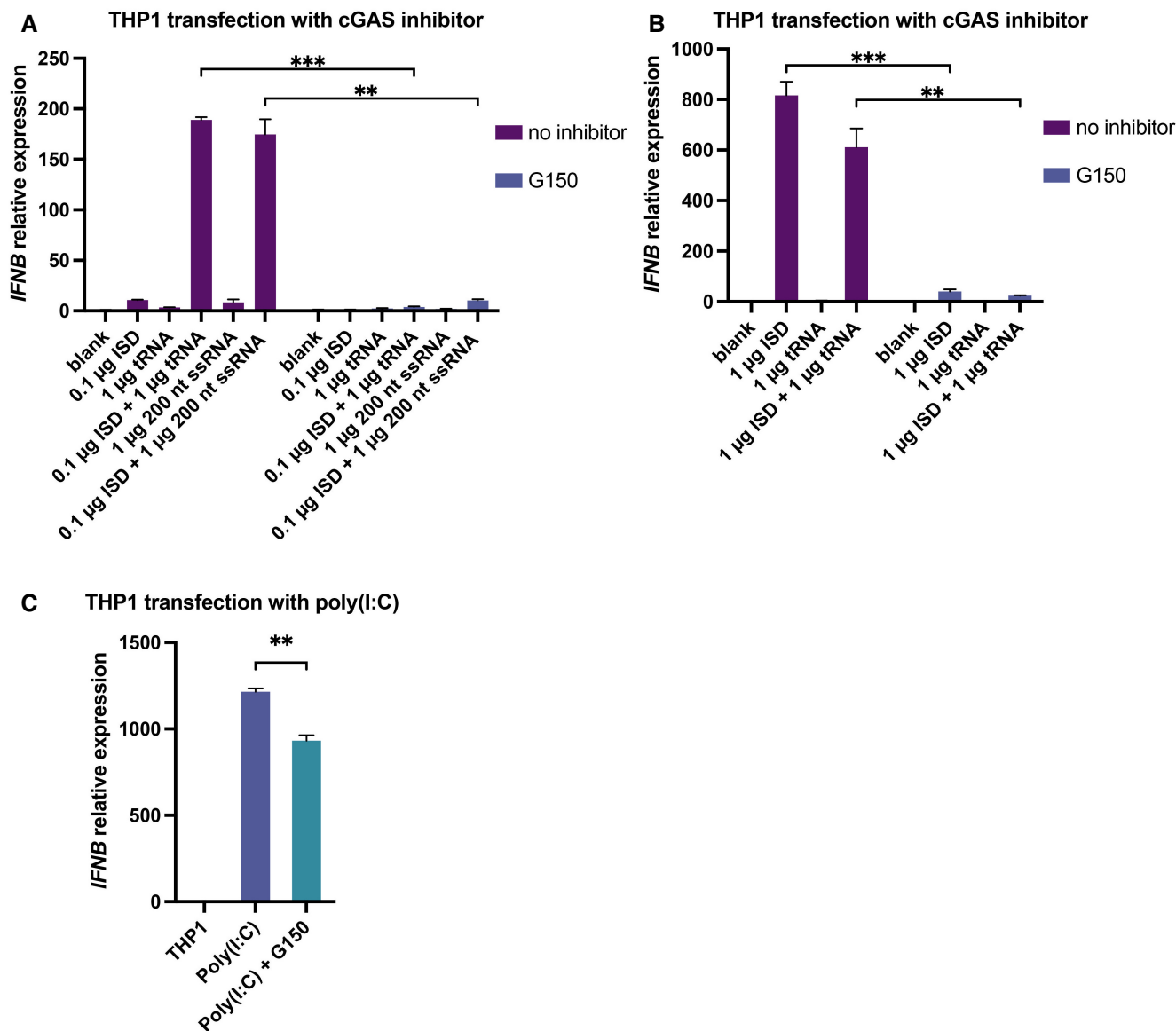


Figure EV5. cGAS-specific inhibitor G150 blocks the RNA-dsDNA mediated expression of *IFNB* in cells.

- A Expression of *IFNB* mRNA in THP1 cells transfected with 0.1 µg dsDNA (ISD) and 1 µg tRNA or 200 nt ssRNA with or without the cGAS-specific inhibitor G150 ($n = 4$, biological replicates).
- B Expression of *IFNB* mRNA in THP1 cells transfected with 1 µg dsDNA (ISD) and 1 µg tRNA with or without the cGAS-specific inhibitor G150 ($n = 4$, biological replicates).
- C Expression of *IFNB* mRNA in THP1 cells transfected with 1 µg poly(I:C) with or without cGAS-specific inhibitor G150 ($n = 3$, biological replicates).

Data information: The concentration of G150 used was 10 µM. The transfection reagent used was Lipofectamine 2000 and the amount of transfection reagent used per well in a 6-well plate was 6 µl. Data are presented as mean \pm SEM, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and ns = not significant (two-way ANOVA for A and B, unpaired two-tailed t -test for C).