

Supplemental Fig. 1. PIC cannot be introduced to H9 cells without TR. **Top left**, H9 cells were treated with 50 $\mu\text{g}/\text{mL}$ PIC for 6 hrs. **Top middle**, 2 $\mu\text{g}/\text{mL}$ PIC can be delivered into H9 cells efficiently by transfection with Fugene HD. **Top right**, HeLa cells were treated with 50 $\mu\text{g}/\text{mL}$ PIC for 6 hrs. Note that direct delivery of PIC leads similar cytoplasmic dots in HeLa cells as transfection does, but this is not the case in H9 cells. **Bottom**, 2 $\mu\text{g}/\text{mL}$ or 50 $\mu\text{g}/\text{mL}$ PIC were directly added to HeLa and H9 cells for 6 hrs and IFN β responses were quantitatively measured by RT-QPCR and normalized to each endogenous *actin* mRNA. Experiments were at least duplicated and error bars were calculated from three biological repeats.

Supplemental Fig. 2. H9 cells on feeder cells do not respond to PIC. H9 cells were plated to the 8 days old irr-MEFs, and 4 days after plating, a variety of PIC treatments were performed to the H9 cells on irr-MEFs for 24 hrs. IFN β responses were quantitatively measured by RT-QPCR and normalized to each endogenous *actin* mRNA. Note that 12-day old irr-MEFs (the last column) show a weak IFN β response with high concentration (10 $\mu\text{g}/\text{mL}$) of PIC transfection. Experiments were duplicated and error bars were calculated from three biological repeats.

Supplemental Fig. 3. H14 cells grown on feeder-free conditions do not respond to PIC. H14 cells were transfected with 2 $\mu\text{g}/\text{mL}$ PIC for 6 hrs and 24 hrs. The IFN β responses were quantitatively measured by RT-QPCR and normalized to each endogenous *actin* mRNA. Experiments were duplicated and error bars were calculated from three biological repeats.

Supplemental Fig. 4. dsRNAs form cytoplasmic dots in a variety of human cell lines. Each cell line was transfected with 2 $\mu\text{g}/\text{mL}$ PIC for 6 hrs, and then stained with J2 antibody which was indicative of dsRNAs.

Supplemental Fig. 5. The fate of dsRNAs in human iPS cells. iPS (IMR90)-1 cells were transfected with 2 $\mu\text{g}/\text{mL}$ PIC for 24 hrs, and then stained with anti-dsRNA, J2 antibody and anti-DCP1 α (top row) or J2 and anti-TIA1 (bottom row). Note that dsRNA-associated cytoplasmic dots remain visible in iPS cells after 24 hrs. Also, iPS cells have large P-bodies (indicated by DCP1 α) and fewer stress granules (indicated by TIA1).

Figure S1

PIC cannot be introduced to H9 cells without transfection

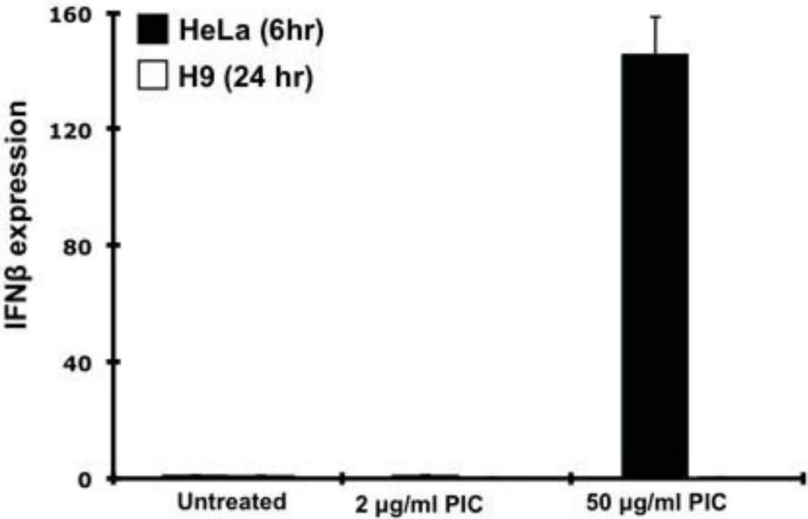
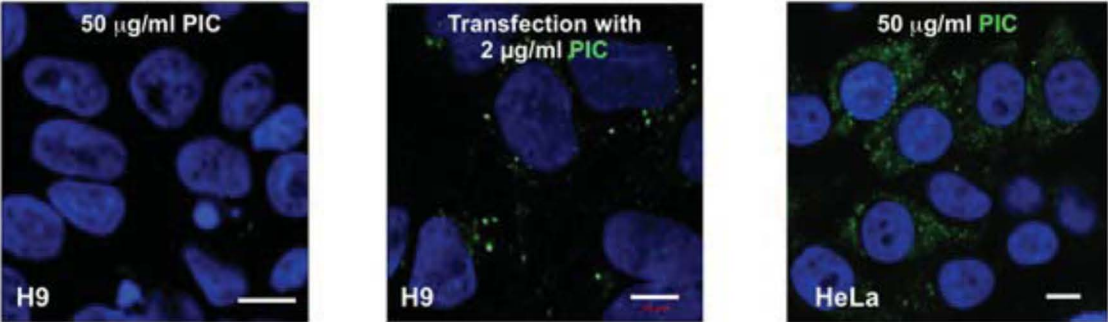


Figure S2

H9 cells on irr-MEFs do not respond to PIC

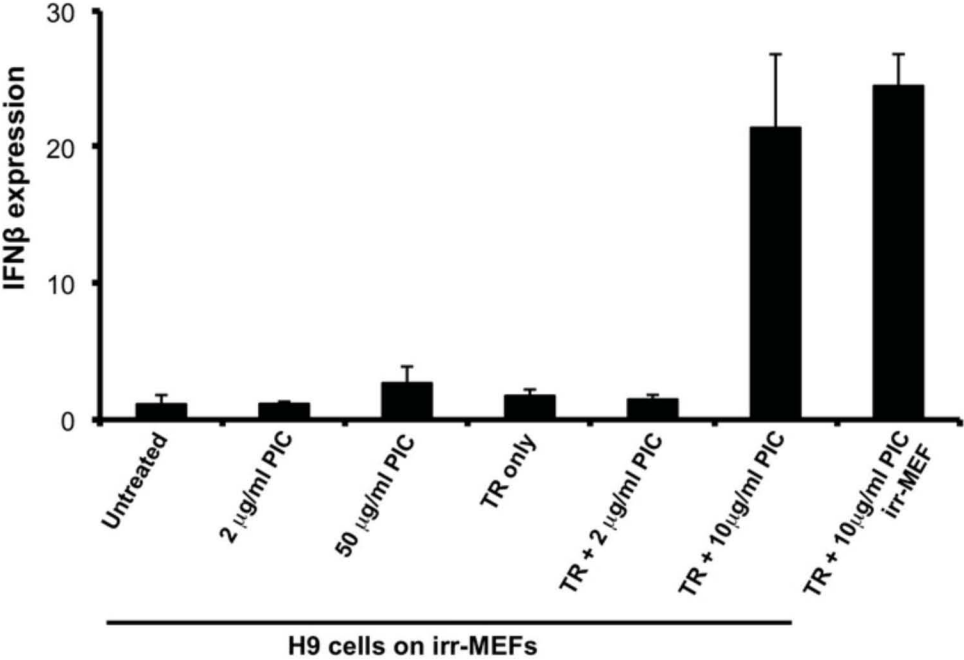


Figure S3

H14 cells have weak IFN β response to PIC

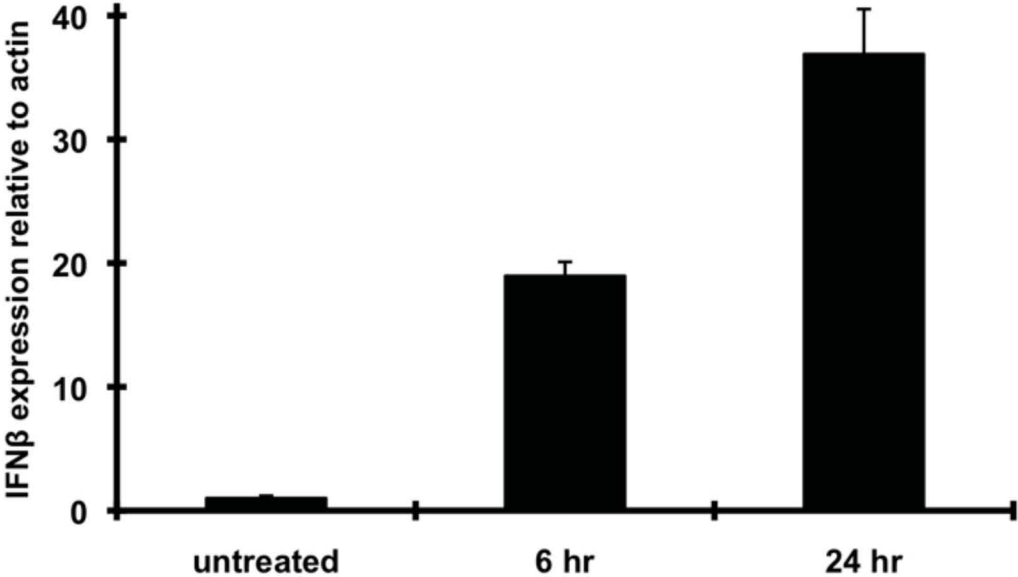


Figure S4

Cytoplasmic uptake of dsRNAs from a variety of cell lines

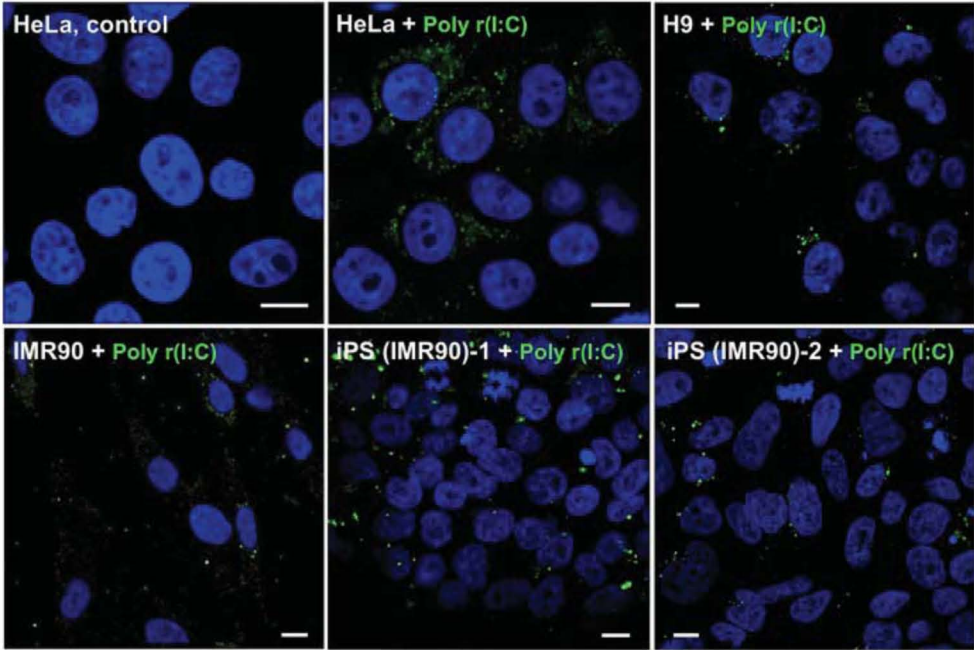
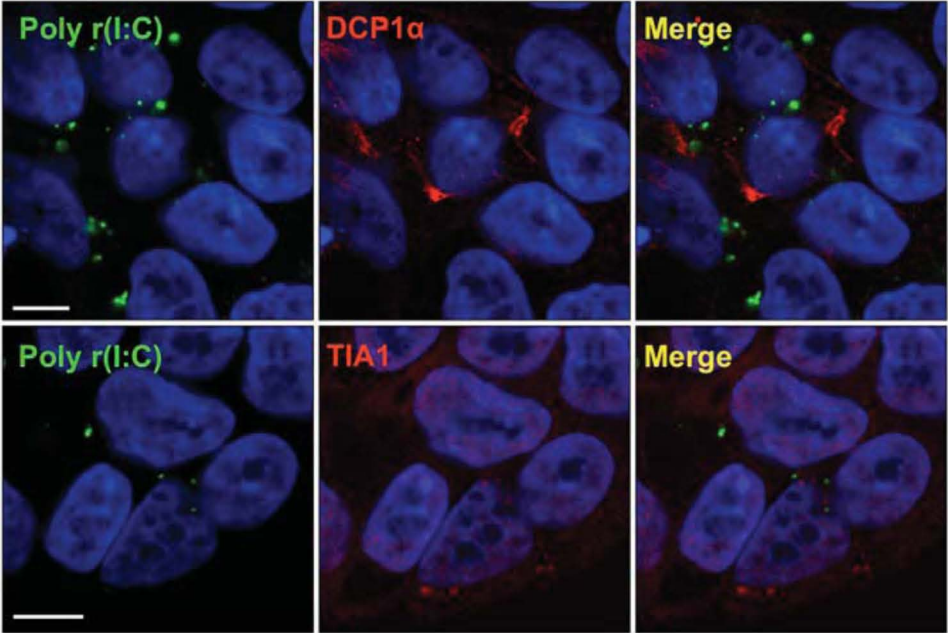


Figure S5

**iPS (IMR90)-1 cells
(24 hr after PIC transfection)**



Supplemental Table 1
Relative mRNA expression of RIG-I regulators
(reads per thousand bases of exon, per million reads)

Gene	HeLa	H9	Function	
RNF135	5.1	3.1	E3 ligase (1)	Positive
TRIM25	23.6	9.4	E3 ligase (2)	
LGP2	0.06	0.8	Binds RNA; competes with MDA5, RIG-I (3)	Negative
RNF125	1.1	2.2	RIG-I degradation (4)	
A20	6.9	15.5	Inhibitor of RIG-I mediated IRF3 phosphorylation (5)	
NLRX1	3.4	6.2	Inhibits RIG-I/IPS-1 interaction (6)	
CYLD	1.4	0.7	Deubiquitinase (7)	
DUBA	30.2	15.7	Deubiquitinase (8)	

References:

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