

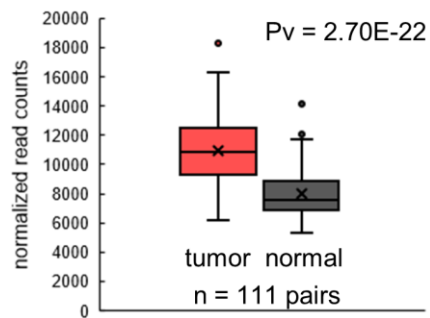
Appendix

Function of HNRNPC in breast cancer cells by controlling the dsRNA-induced interferon response

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Appendix Figures S1-S14.

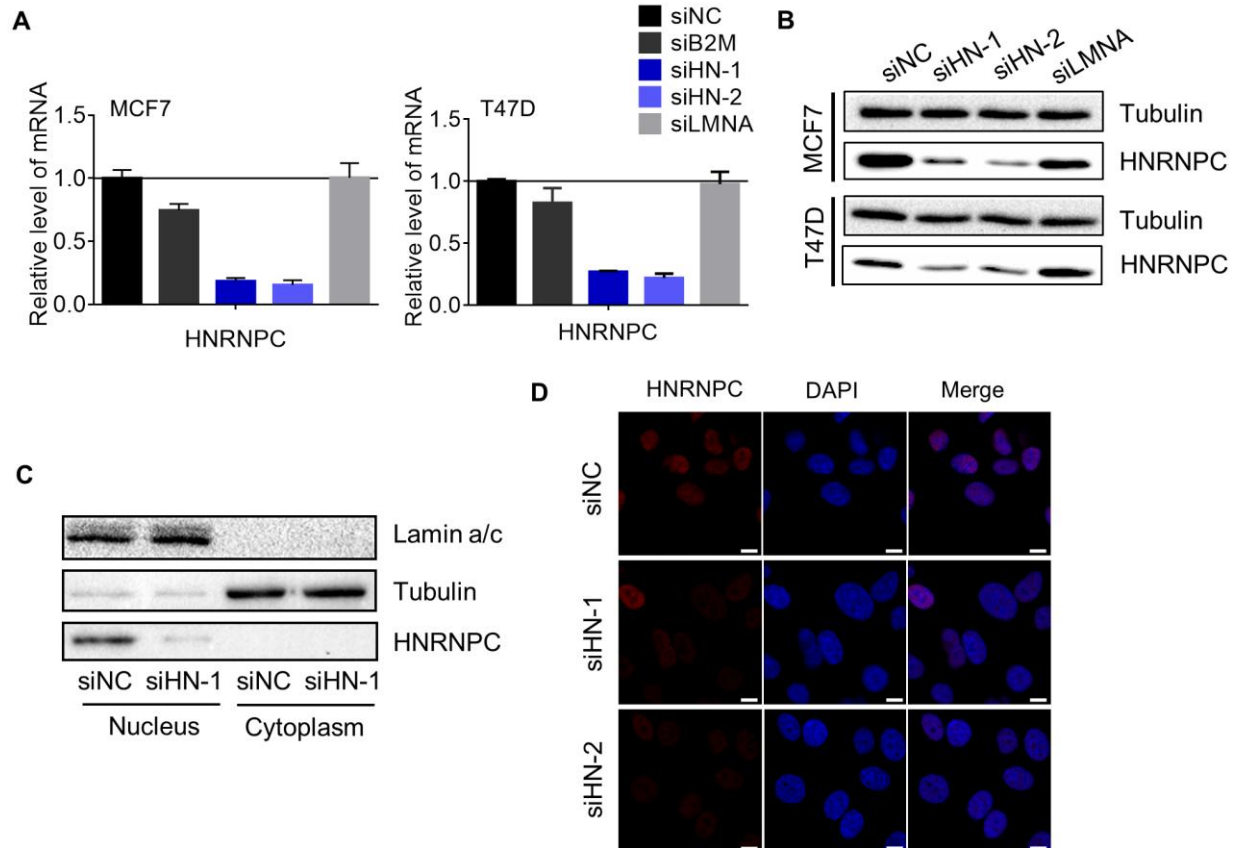
Appendix Figure S1



Appendix Figure S1. HNRNPC expression in breast cancer.

Box plots showing mRNA expression of HNRNPC in 111 breast cancer tumors and in their adjacent normal tissues. Data was obtained from TCGA. Paired t-test was performed to evaluate the statistical significance of higher HNRNPC expression in the tumors.

Appendix Figure S2



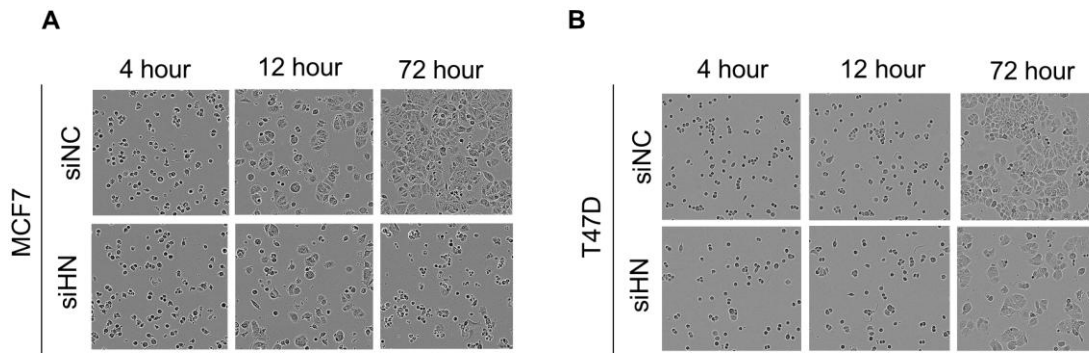
Appendix Figure S2. Silencing efficiency of HNRNPC by siRNA.

(A, B) mRNA (A) and protein (B) expression of HNRNPC upon siRNA-mediated gene knock-down in MCF7 and T47D cells. Error bars represent mean $\pm$ SD.

(C) Protein expression of HNRNPC in nucleus and cytoplasm upon siRNA-mediated gene knock-down in MCF7 cells. Error bars represent mean $\pm$ SD.

(D) Immunofluorescence images showing intranuclear locations of HNRNPC (red) and its reduction upon siRNA-mediated gene knock-down.

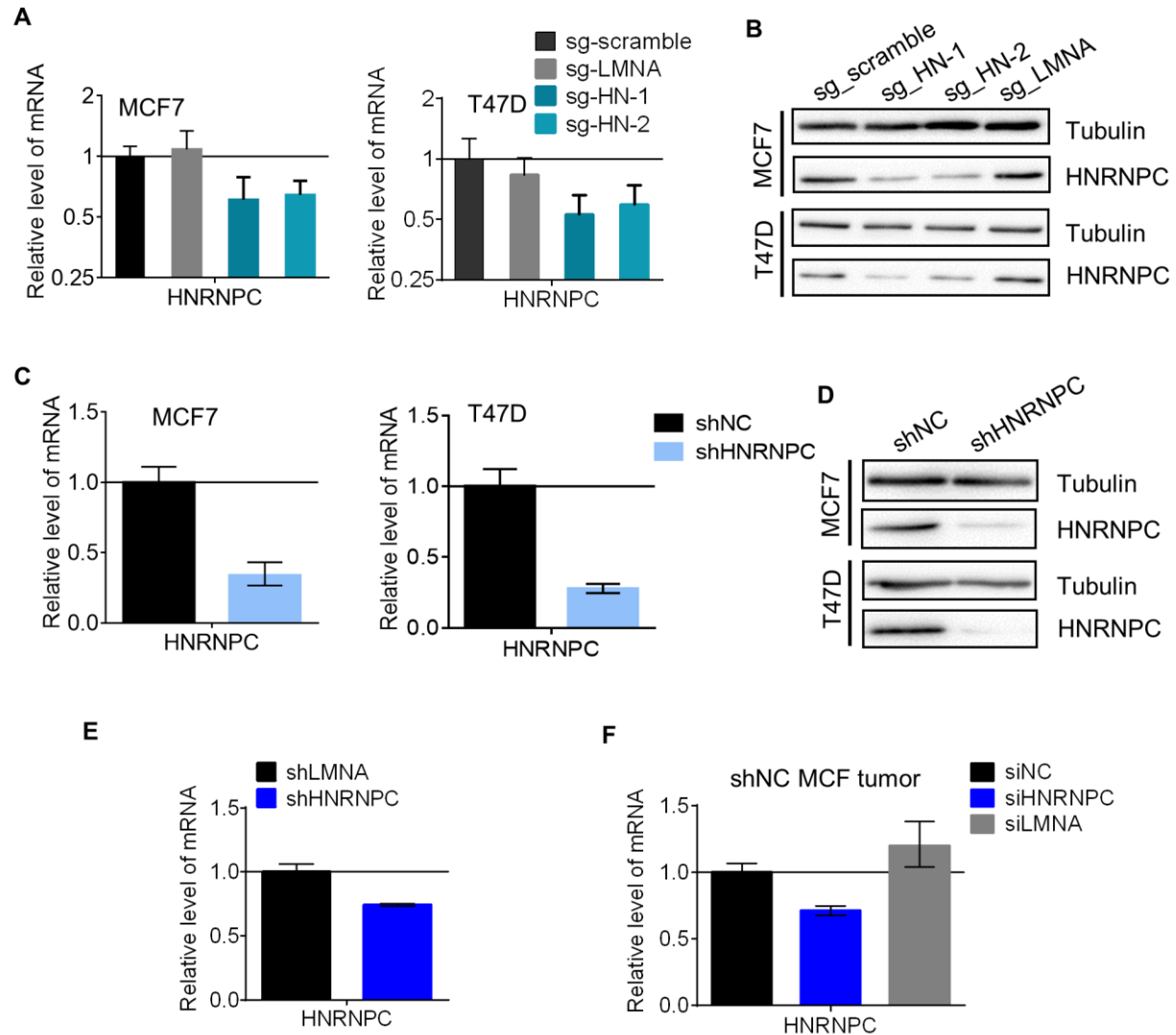
Appendix Figure S3



Appendix Figure S3. Representative images of the cells after knock-down of HNRNPC.

(A-B) After siRNA transfection into the MCF7 (A) and T47D (B) cells, images of the cells growing on 12-well plates were obtained by the IncuCyte live-cell imaging and analysis system.

Appendix Figure S4



Appendix Figure S4. Silencing efficiencies of HNRNPC in different experiments.

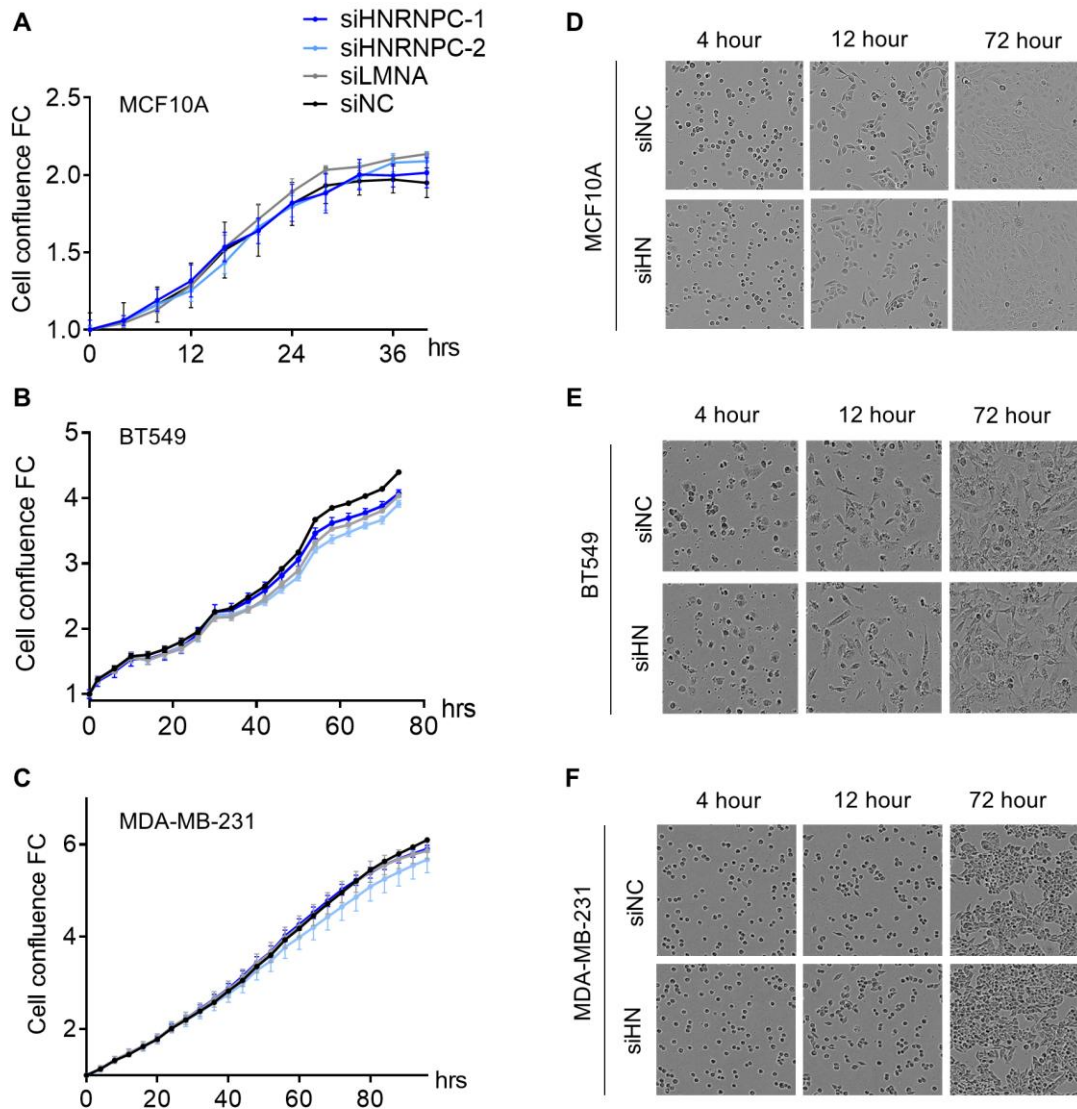
(A, B) mRNA and protein expression of HNRNPC upon gene knock-down with CRISPR-Cas9 in MCF7 (left) and T47D (right) cells. Error bars represent mean $\pm$ SD.

(C, D) Silencing efficiency of shRNA-mediated stable knock-down in MCF7 (left) and T47D (right) cells. Error bars represent mean $\pm$ SD.

(E) mRNA expression of HNRNPC in the xenograft tumor models (Fig 1G) derived from the stable MCF7 cells expressing the shRNA. Error bars represent mean $\pm$ SD.

(F) Silencing efficiency of periodic siRNA injections into the MCF7-derived xenograft tumors shown in Fig 1H. Error bars represent mean $\pm$ SD.

Appendix Figure S5

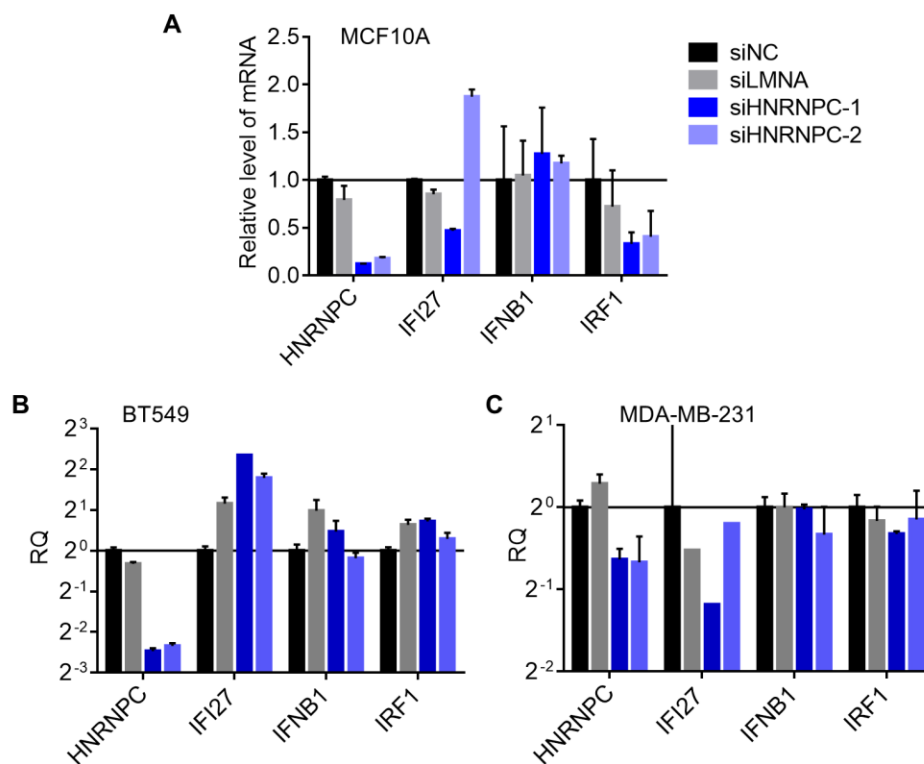


Appendix Figure S5. Silencing of HNRNPC had no effect on the proliferation rate of MCF10A, BT549, and MDA-MB-231.

(A-C) Growth curves of the MCF10A (A), BT549 (B), and MDA-MB-231 (C) cells upon gene silencing with siRNAs.

(D-F) After siRNA transfection into the MCF10A (D), BT549 (E), and MDA-MB-231 (F) cells, images of the cells growing on 12-well plates were obtained by the IncuCyte live-cell imaging and analysis system.

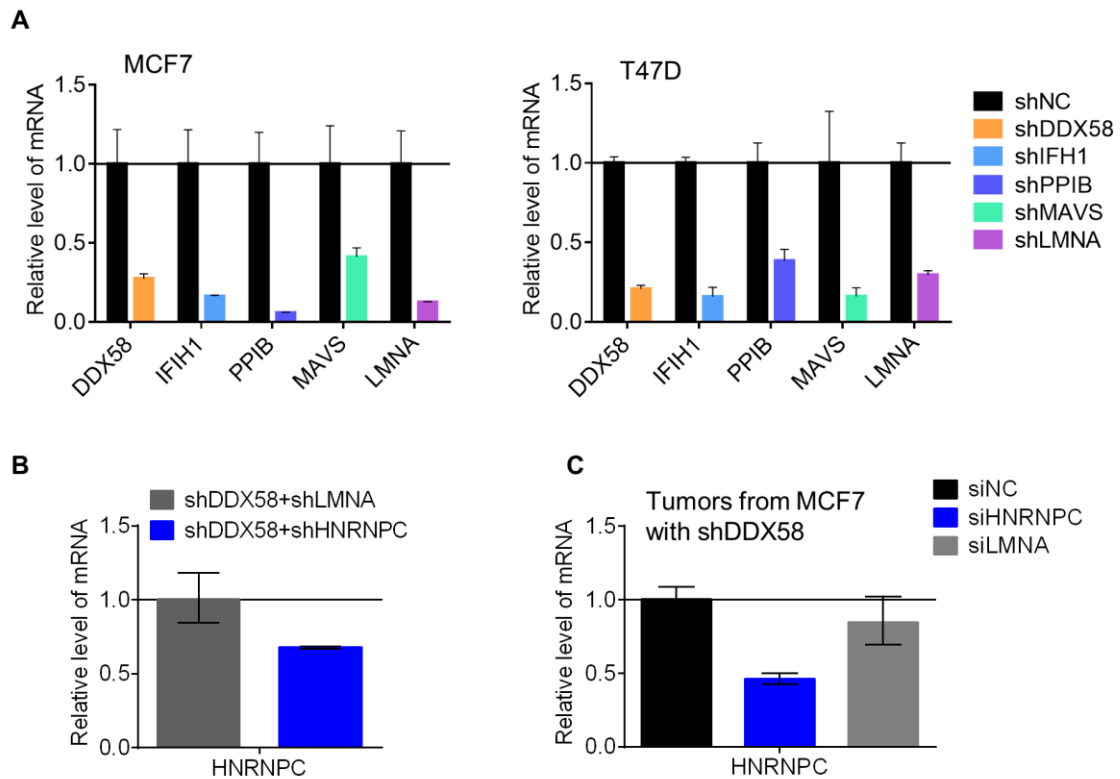
Appendix Figure S6



Appendix Figure S6. Silencing of HNRNPC had no effect on expression of the ISGs in MCF10A, BT549 and MDA-MB-231.

The expression changes of ISGs were measured in MCF10A (A), BT549 (B) and MDA-MB-231 (C), upon knock-down of HNRNPC using siRNA. Error bars represent mean $\pm$ SD.

Appendix Figure S7



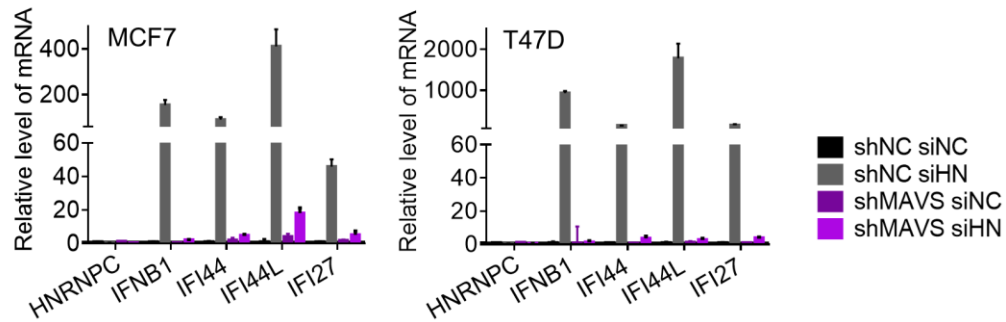
Appendix Figure S7. Gene silencing efficiencies for Fig 5.

(A) Gene silencing efficiencies in the MCF7 (left) and T47D (right) cells subjected to lentiviral shRNA-mediated stable gene knock-down. Error bars represent mean $\pm$ SD.

(B) mRNA expression of HNRNPC in the xenograft tumor models (Fig 5E) derived from the MCF7 cells with stable knock-down of DDX58 and HNRNPC. Error bars represent mean $\pm$ SD.

(C) Silencing efficiency of periodic siRNA injections into the xenograft tumors (Fig 5F) derived from the MCF7 cells with stable DDX58 knock-down. Error bars represent mean $\pm$ SD.

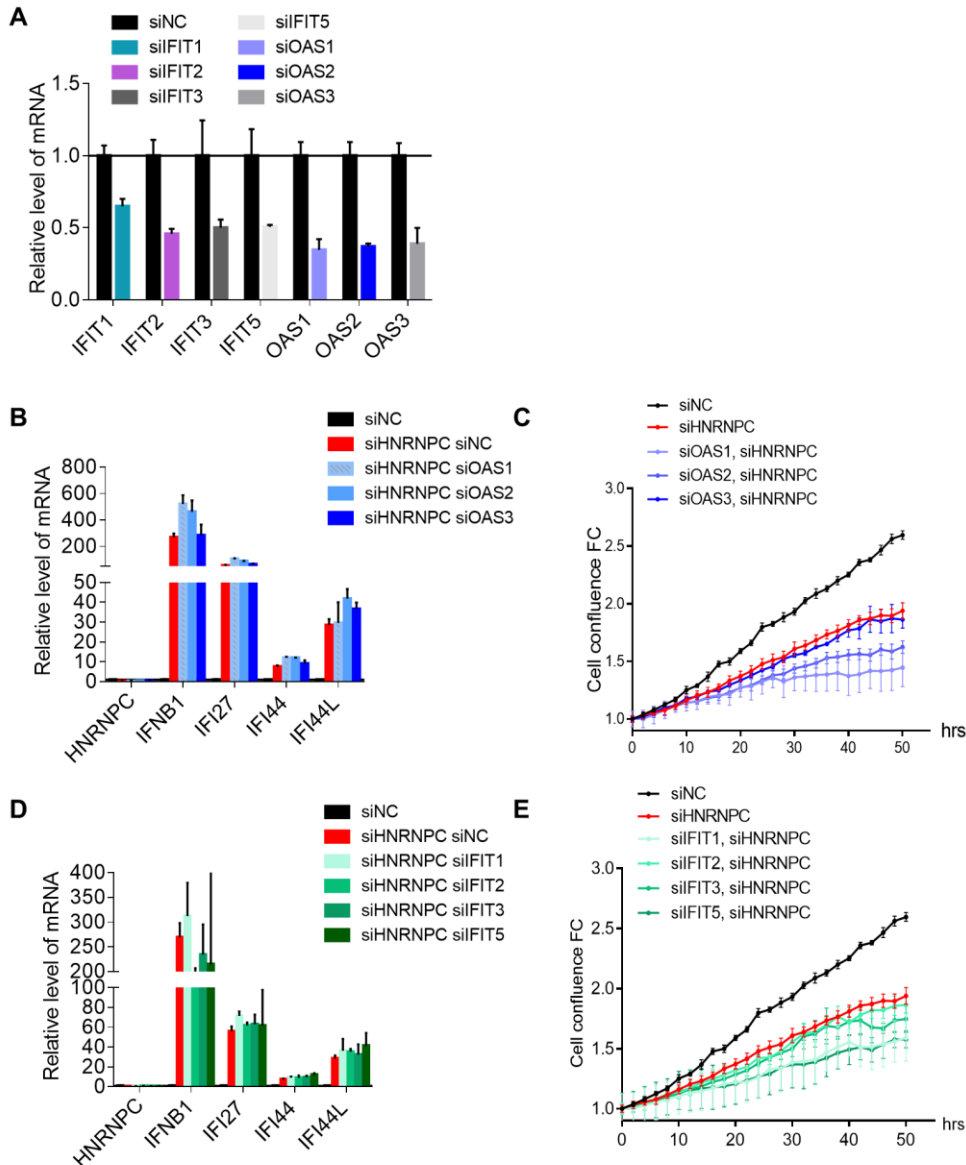
Appendix Figure S8



Appendix Figure S8. Involvement of MAVS in mediating the effects of HNRNPC repression on ISG expressions

The expressions of ISGs were measured by qPCR in MAVS-deficient MCF7 (left) and T47D (right) cells upon HNRNPC knock-down. Error bars represent mean $\pm$ SD.

Appendix Figure S9



Appendix Figure S9. Involvements of OAS1-3 and IFIT1-5 in interferon response upon HNRNPC KD.

(A) mRNA silencing efficiency of OAS1-3 and IFIT1-5 siRNA. Error bars represent mean $\pm$ SD.

(B) Expression levels of ISGs upon double KD of HNRNPC and OAS1-3. Error bars represent mean $\pm$ SD.

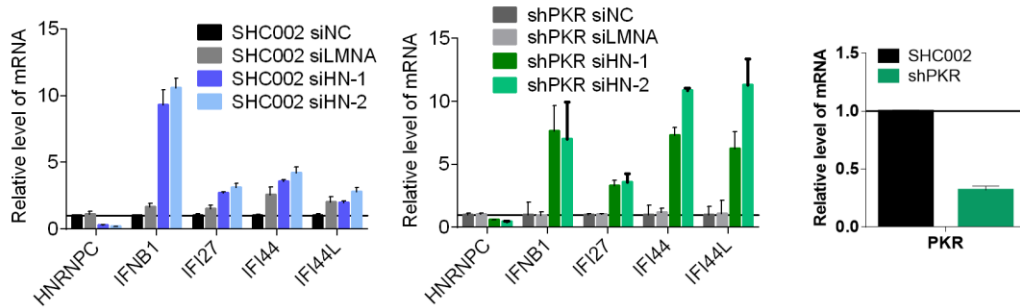
(C) Cell proliferation upon single KD of HNRNPC or double KD of HNRNPC with OAS1-3 in MCF7.

(D) Expression levels of ISGs upon double KD of HNRNPC and IFIT1-5. Error bars represent mean $\pm$ SD.

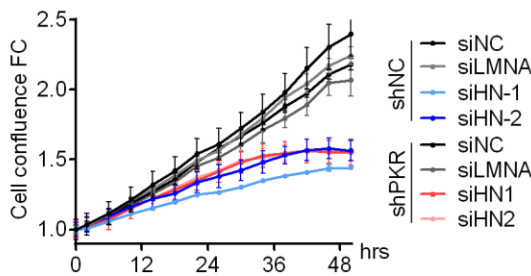
(E) Cell proliferation upon single KD of HNRNPC or double KD of HNRNPC with IFIT1-5 in MCF7.

Appendix Figure S10

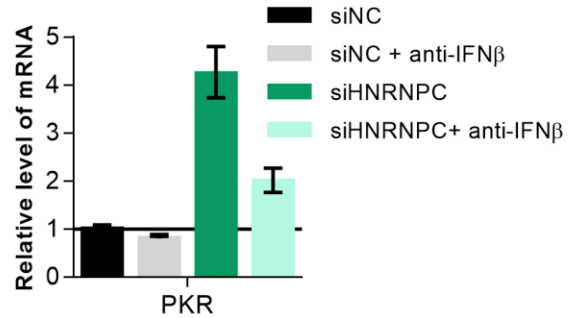
A



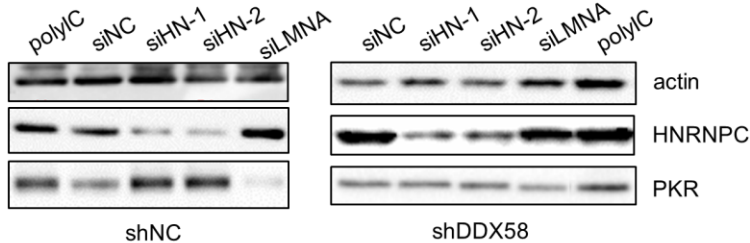
B



C



D



Appendix Figure S10. The responses of PKR upon HNRNPC silencing.

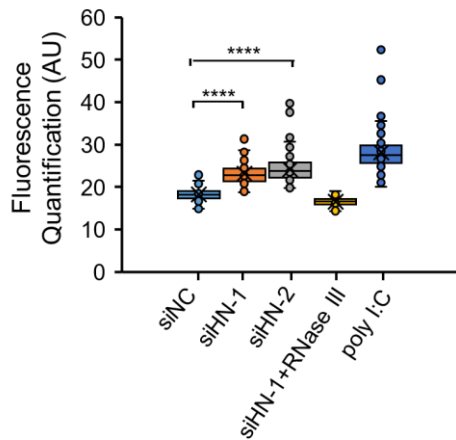
(A) The expressions of ISGs were measured by qPCR in PKR-deficient MCF7 upon HNRNPC knock-down. Error bars represent mean $\pm$ SD.

(B) Growth curves of the PKR-deficient MCF7 cells upon HNRNPC knock-down. Error bars represent mean $\pm$ SD.

(C) The mRNA expression of PKR, measured by qPCR, upon silencing of HNRNPC in MCF7, with or without neutralization of IFN β by its antibody (60U/ml) in the culturing media. Error bars represent mean $\pm$ SD.

(D) Western blots showing the protein levels of HNRNPC and PKR upon silencing of HNRNPC in the regular MCF7 cells (left) or in the MCF7 cells with stable knock-down of DDX58 (right).

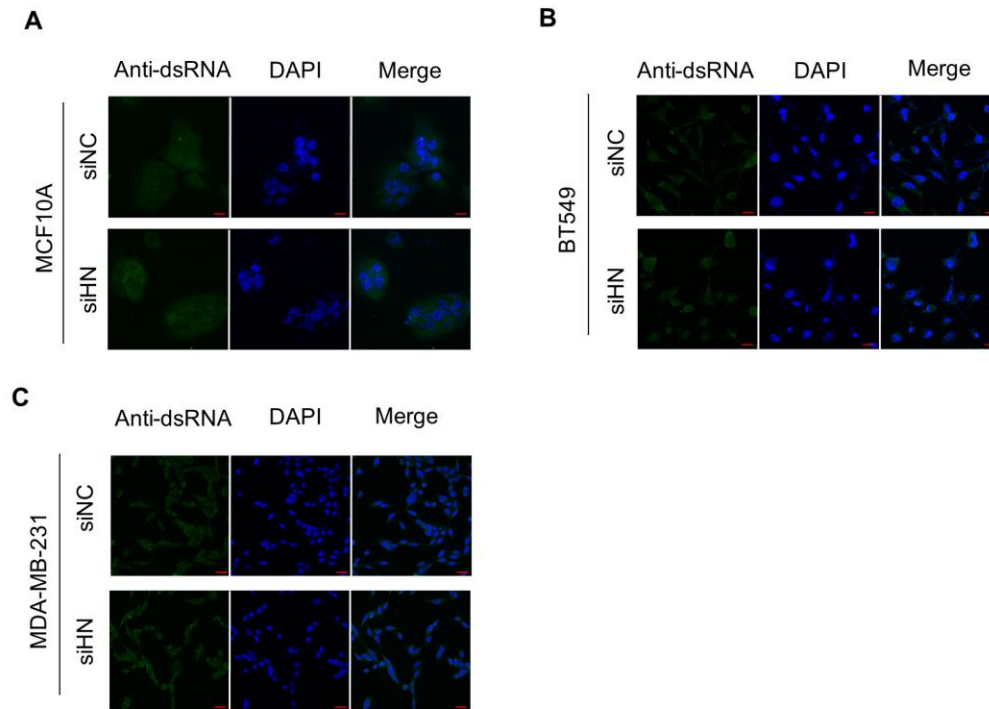
Appendix Figure S11



Appendix Figure S11. Quantifications of dsRNA immunofluorescence signals.

Box plots showing dsRNA immunofluorescence signals with J2 antibody under different conditions. Each box summarizes fluorescence signal strength (artificial unit) from more than 100 randomly selected areas in the IF images.

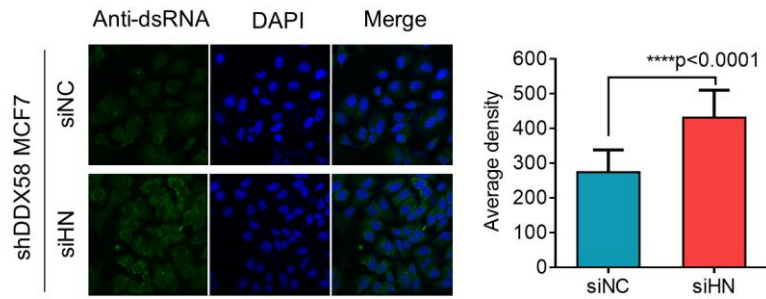
Appendix Figure S12



Appendix Figure S12. Staining of dsRNA in MCF10A, BT549, and MDA-MB-231 in response to knock-down of HNRNPC.

Immunofluorescence analysis of the dsRNA in the MCF10A (A), BT549 (B) and MDA-MB-231 (C) cells after knock-down of HNRNPC, with 4',6-diamidino-2-phenylindole (DAPI) staining (blue) and anti-dsRNA antibody J2 (green).

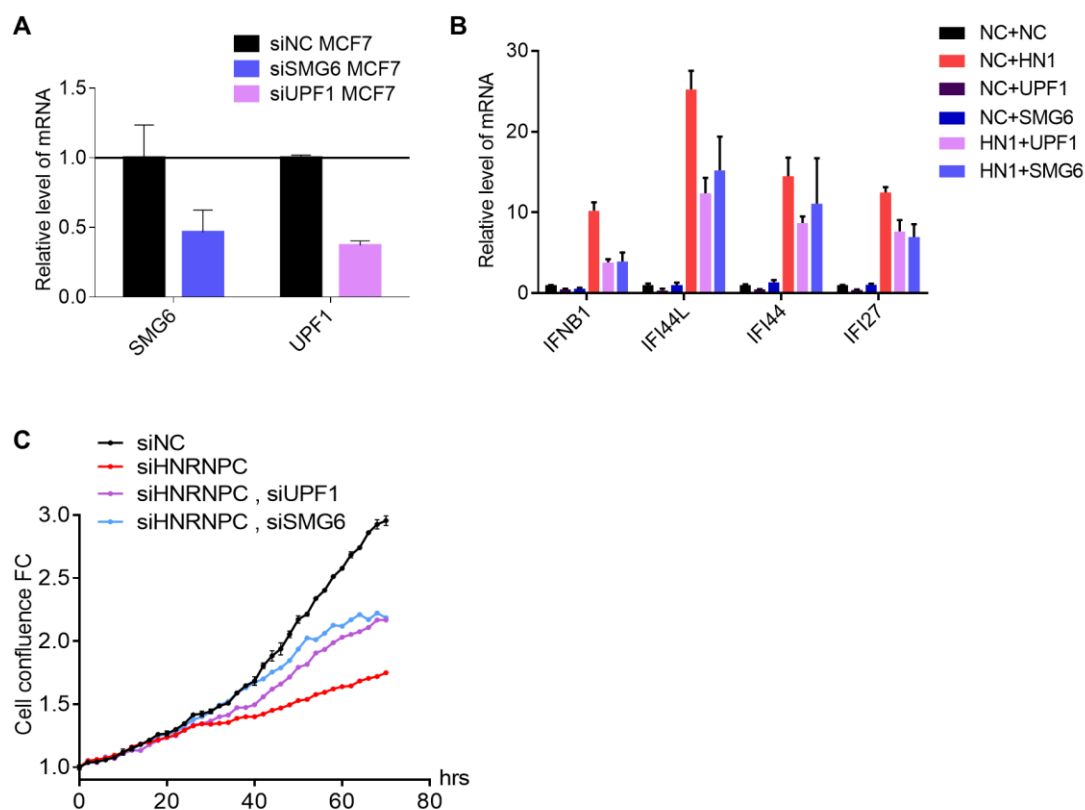
Appendix Figure S13



Appendix Figure S13. Repression of HNRNPC induced increase of endogenous dsRNA in DDX58-deficient MCF7 cells.

Immunofluorescence analysis of the dsRNA in DDX58-deficient MCF7 cells after knock-down of HNRNPC, with 4',6-diamidino-2-phenylindole (DAPI) staining (blue) and anti-dsRNA antibody J2 (green).

Appendix Figure S14



Appendix Figure S14. Effects of SMG6 in interferon response upon HNRNPC KD.

(A) mRNA silencing efficiency of SMG6 and UPF1 siRNA. Error bars represent mean $\pm$ SD.

(B) Expression levels of ISGs upon HNRNPC, SMG6, or UPF1 silencing in MCF7 cells. Error bars represent mean $\pm$ SD.

(C) Cell proliferation upon single knock-down of HNRNPC or double knock-down of HNRNPC with UPF1 or SMG6 in MCF7.