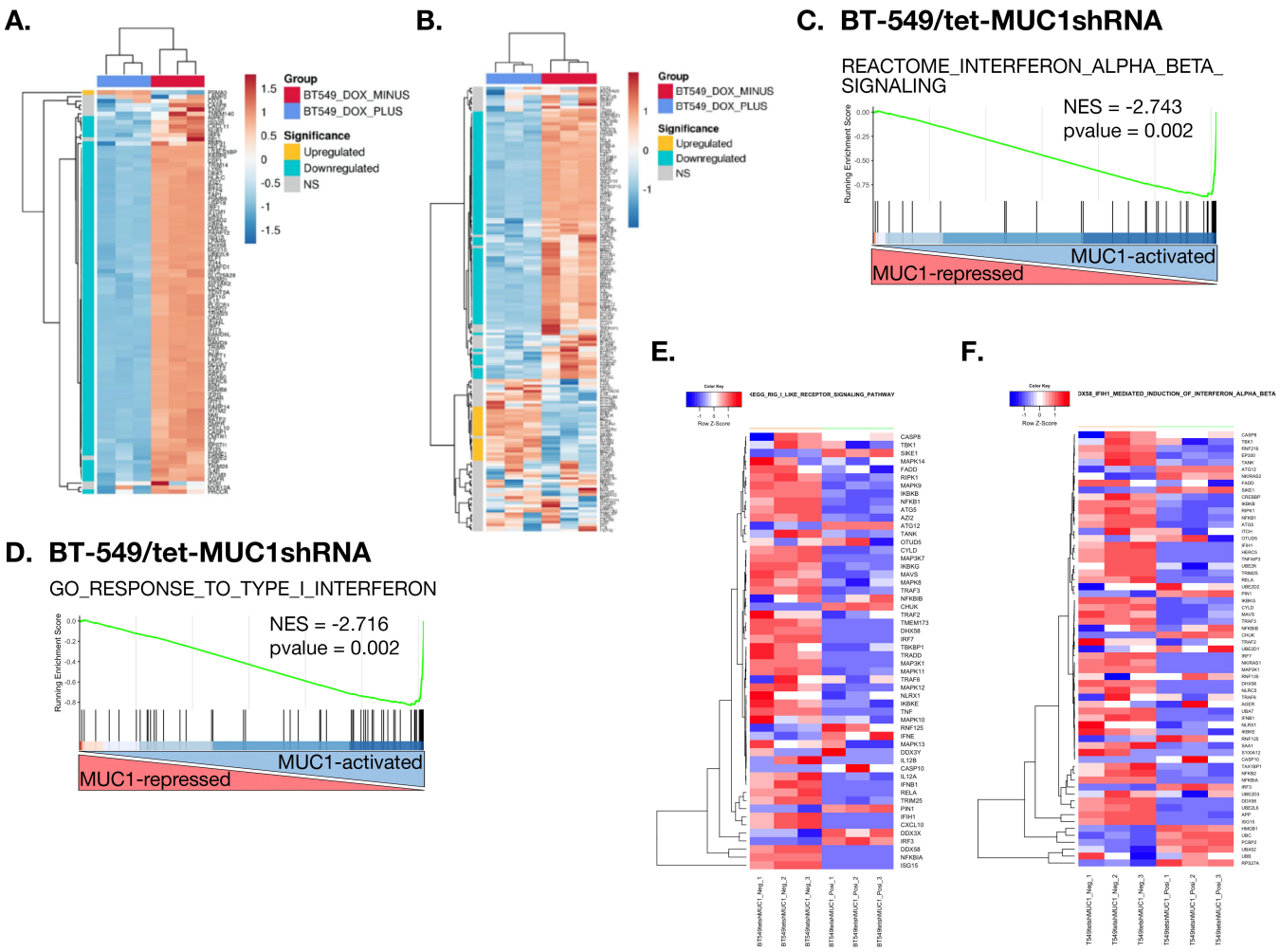
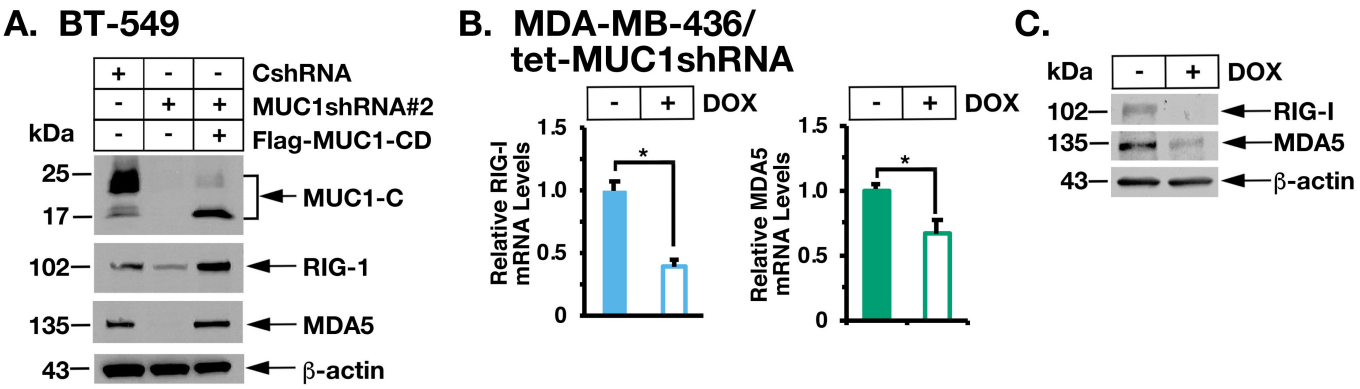
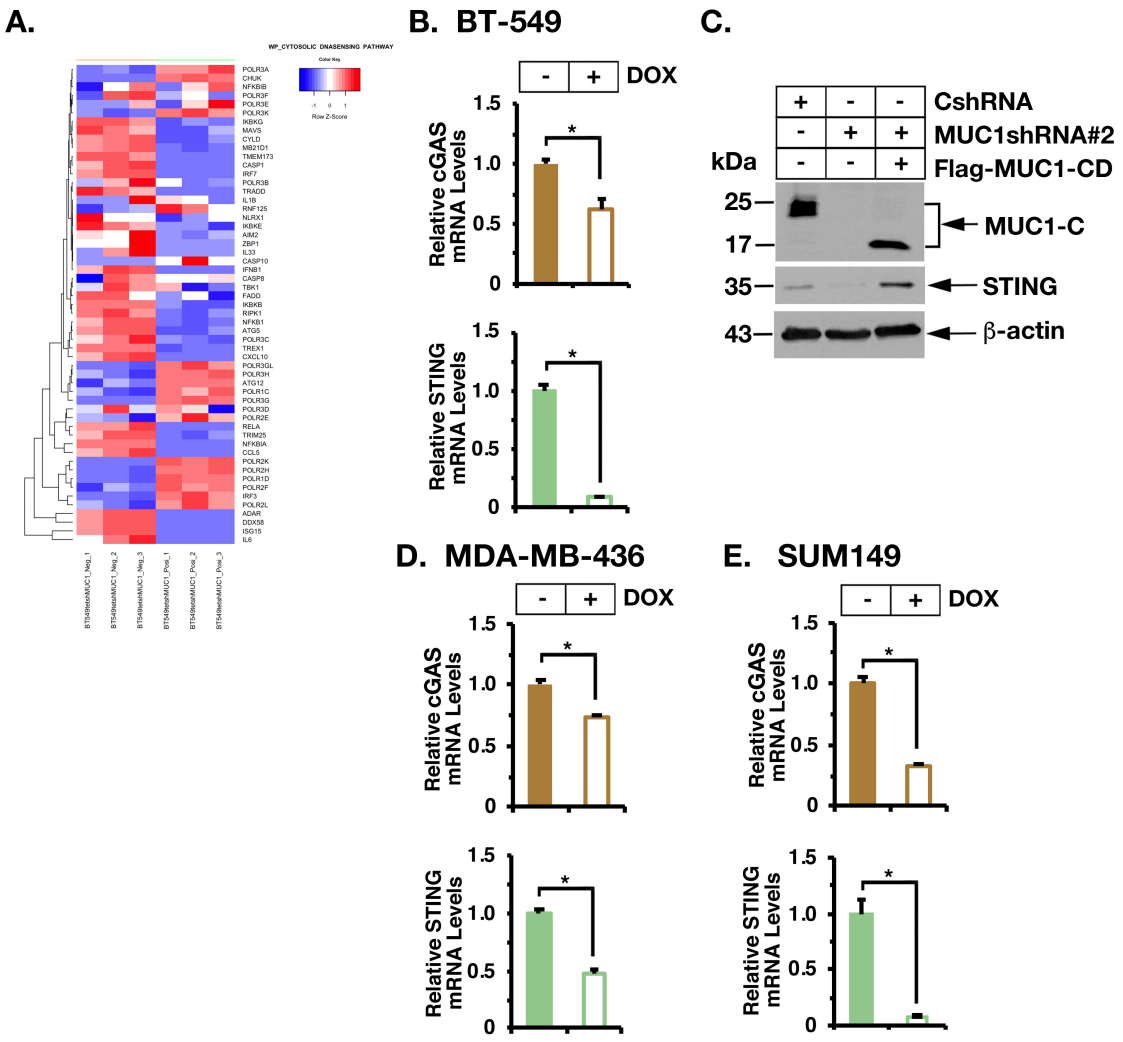


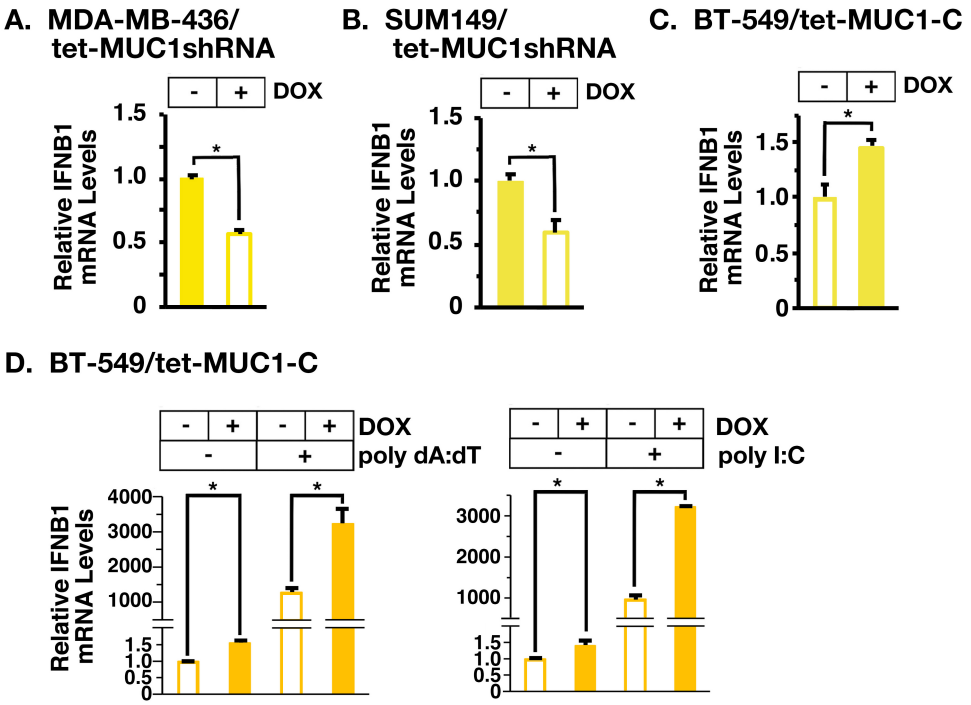
Supplemental Figure S1. Effects of silencing MUC1-C on expression type I IFN pathway genes. RNA-seq was performed in triplicate on BT-549/tet-MUC1shRNA cells treated with vehicle of DOX for 7 days. **A and B.** Heatmaps comparing the effects of MUC1 silencing on genes from the HALLMARK INTERFERON ALPHA RESPONSE (**A**) and HALLMARK INFLAMMATORY RESPONSE (**B**) pathways. **C and D.** Candidate pathway enrichment plots for the REACTOME INTERFERON ALPHA BETA SIGNALING (**C**) and GO RESPONSE TO TYPE I INTERFERON (**D**) pathways. **E and F.** Heatmaps comparing the effects of MUC1 silencing on genes from the KEGG RIG-I LIKE RECEPTOR SIGNALING REACTOME (**E**) and DDX58 IFIH1 MEDIATED INDUCTION OF INTERFERON ALPHA BETA (**F**) pathways.



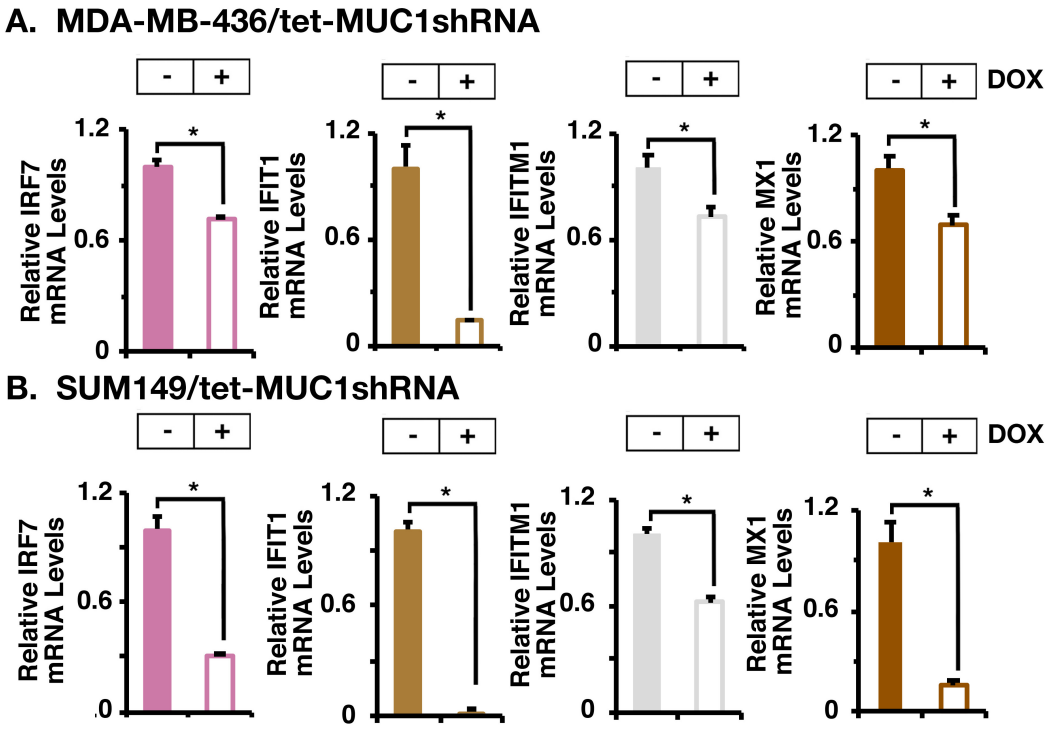
Supplemental Figure. S2. Silencing MUC1-C in TNBC cells suppresses RIG-I and MDA5 expression. A. Lysates from BT-549 cells expressing CshRNA, MUC1shRNA#2 and Flag-MUC1-CD were immunoblotted with antibodies against the indicated proteins. **B and C.** MDA-MB-436/tet-MUC1shRNA were treated with vehicle or DOX for 7 days. RIG-I and MDA5 mRNA levels were analyzed by qRT-PCR (**B**). The results (mean ± SD of 4 determinations) are expressed as relative mRNA levels compared to that obtained for vehicle-treated cells (assigned a value of 1). Lysates were immunoblotted with antibodies against the indicated proteins (**C**).



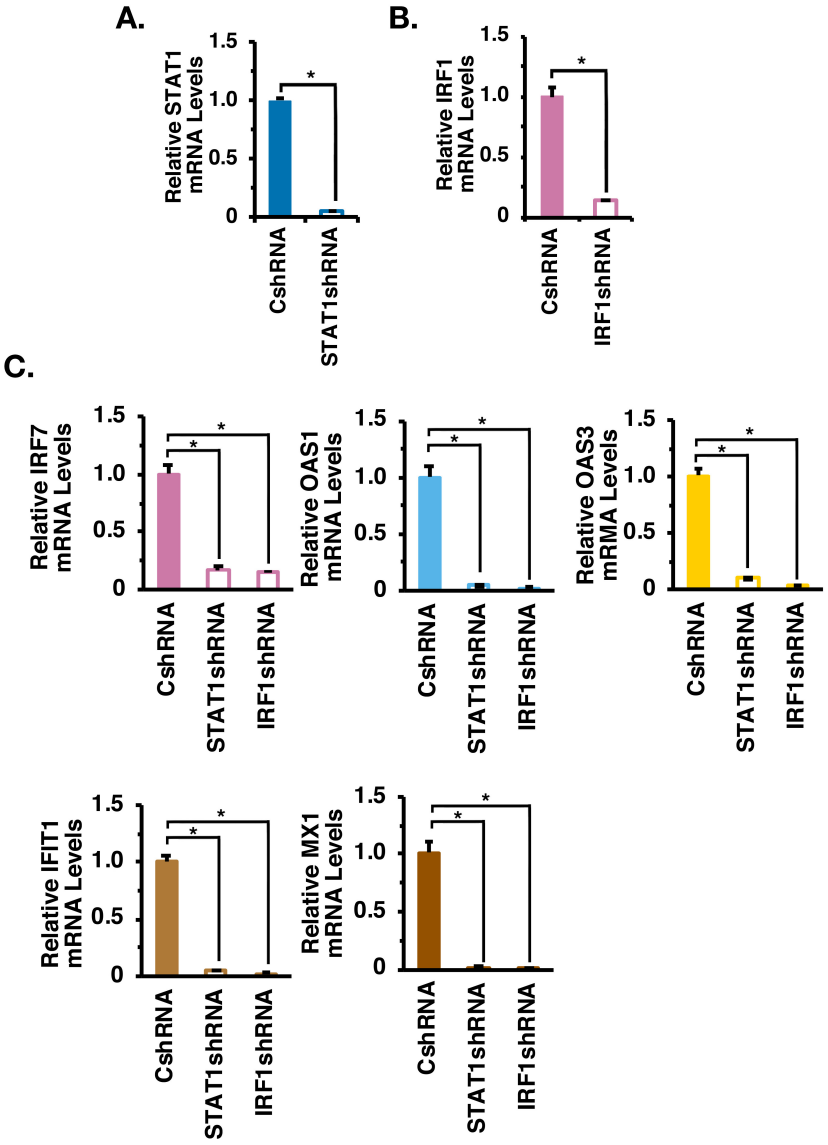
Supplemental Figure S3. MUC1-C induces cGAS and STING expression in BT-549, MDA-MB-436 and SUM149 TNBC cells. A. Heatmap comparing the effects of MUC1 silencing in BT-549 cells on genes from the WP CYTOSOLIC DNA SENSING PATHWAY signature. **B.** BT-549/tet-MUC1shRNA cells treated with vehicle or DOX for 7 days were analyzed for cGAS and STING mRNA levels by qRT-PCR. The results (mean±SD of 4 determinations) are expressed as relative mRNA levels compared to that obtained for vehicle-treated cells (assigned a value of 1). **C.** Lysates from BT-549 cells expressing CshRNA, MUC1shRNA#2 and Flag-MUC1-CD were immunoblotted with antibodies against the indicated proteins. **D and E.** MDA-MD-436/tet-MUC1shRNA (**D**) and SUM149/tet-MUC1shRNA (**E**) cells treated with vehicle or DOX for 7 days were analyzed for cGAS and STING mRNA levels by qRT-PCR. The results (mean±SD of 4 determinations) are expressed as relative mRNA levels compared to that obtained for vehicle-treated cells (assigned a value of 1).



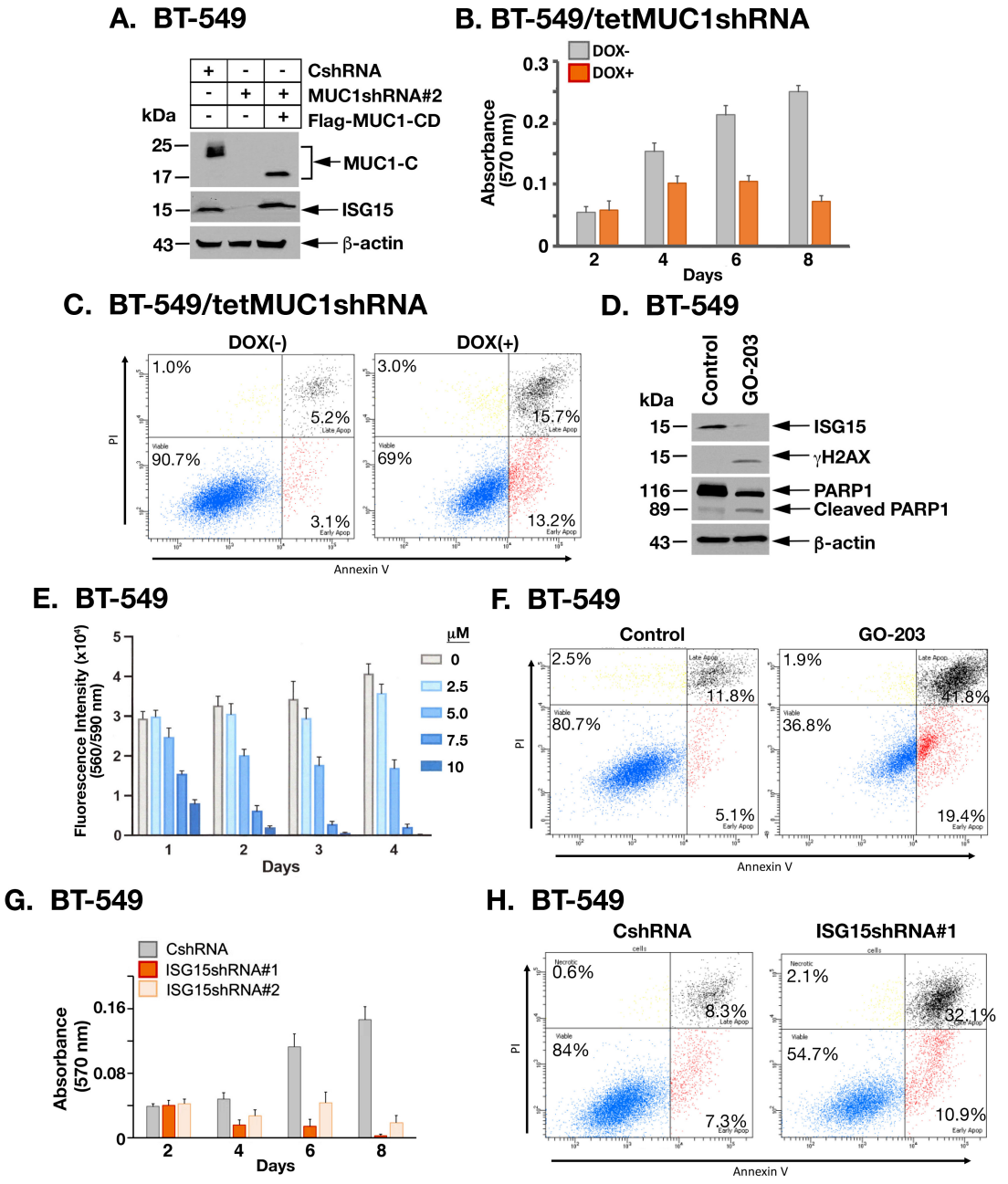
Supplemental Figure S4. MUC1-C drives IFNB1 expression in TNBC cells. A and B. MDA-MB-436/tet-MUC1shRNA (**A**) and SUM149/tet-MUC1shRNA (**B**) cells treated with vehicle or DOX for 7 days were analyzed for IFNB1 mRNA levels by qRT-PCR. **C.** BT-549 cells expressing a tet-inducible MUC1-C vector treated with vehicle or DOX for 7 days were analyzed for IFNB1 mRNA levels by qRT-PCR. **D.** BT-549 cells expressing a tet-inducible MUC1-C vector treated with vehicle or DOX for 7 days were transfected with poly I:C (left) or poly dA:dT (right) for 4 hours and analyzed for IFNB1 mRNA levels by qRT-PCR. The results (mean±SD of 4 determinations) are expressed as relative mRNA levels compared to that obtained for vehicle-treated cells (assigned a value of 1).



Supplemental Figure S5. Effects of silencing MUC1-C on gene expression. A and B. MDA-MB-436/tet-MUC1shRNA (**A**) and SUM149/tet-MUC1shRNA (**B**) cells treated with vehicle of DOX for 7 days were analyzed for the indicated mRNA levels by qRT-PCR. The results (mean±SD of 4 determinations) are expressed as relative mRNA levels compared to that obtained for vehicle-treated cells (assigned a value of 1).



Supplemental Figure S6. Effects of silencing STAT1 and IRF1 on IRDS gene expression. A and B. BT-549 cells expressing a control CshRNA, STAT1shRNA (**A**) or IRF1shRNA (**B**) were analyzed for the indicated mRNA levels by qRT-PCR. **C.** BT-549 cells expressing a CshRNA, STAT1shRNA or IRF1shRNA were analyzed for the indicated mRNA levels by qRT-PCR. The results (mean±SD of 4 determinations) are expressed as relative mRNA levels compared to that obtained for vehicle-treated cells (assigned a value of 1).



Supplemental Figure S7. Effects of silencing MUC1-C and ISG15 on induction of DNA damage and apoptosis. A. Lysates from BT-549 cells expressing CshRNA, MUC1shRNA#2 and Flag-MUC1-CD were immunoblotted with antibodies against the indicated proteins. **B and C.** BT-549/tet-MUC1shRNA cells treated with vehicle or DOX were monitored at the indicated times after seeding for cell proliferation as determined by the AlamarBlue assay (**B**). Cells were analyzed by flow cytometry for Annexin V and propidium iodide staining at 5 days (**C**). The results are representative of 3 determinations. **D.** Lysates from BT-549 cells left untreated or treated with 10 μM GO-203 for 24 hours were immunoblotted with antibodies against the indicated proteins. **E and F.** BT-549 cells left untreated or treated with the indicated concentrations of GO-203 were monitored at the indicated times for cell proliferation as determined by the AlamarBlue assay (**E**). Cells were analyzed by flow cytometry for Annexin V and propidium iodide staining at 2 days (**F**). The results are representative of 3 determinations. **G and H.** BT-549 cells expressing a CshRNA, ISG15shRNA#1 or ISG15shRNA#2 were monitored at the indicated times after seeding for cell proliferation as determined by the AlamarBlue assay (**G**). Cells were analyzed by flow - cytometry for Annexin V and propidium iodide staining at 3.5 days (**H**). The results are representative of 3 determinations.

Table S1. Primers used for qRT-PCR.

Primer	FWD	REV
MUC1-C	AGACGTCAGCGTGAGTGATG	GCCAAGGCAATGAGATAGAC
STAT1	GGAACTTGATGGCCCTAAAGGA	ACAGAGCCCACTATCCGAGACA
STAT2	GCAGCACAATTTGCGGAA	ACAGGTGTTTCGAGAACTGGC
IRF1	CATGGCTGGGACATCAACAA	TTGTATCGGCCTGTGTGAATG
IRF7	GCAGCGTGAGGGTGTGTCTT	GCTCCATAAGGAAGCACTCGAT
IRF9	CCCGACCTCACCGATGAC	TCTCGCGAAGCTGGATGTC
RIG-I	CTGGACCCTACCTACATCCTG	GGCATCCAAAAAGCCACGG
MDA5	CCATGGAGAAGGCTGGGG	CAAAGTTGTCATGGATGACC
CGAS	TAACCCTGGCTTTGGAATCAAAA	TGGGTACAAGGTAAAATGGCTTT
STING	AGCATTACAACAACCTGCTACG	GTTGGGGTCAGCCATACTCAG
IFNB1	ATGACCAACAAGTGTCTCCTCC	GGAATCCAAGCAAGTTGTAGCTC
OAS1	TGAGGTCCAGGCTCCACGCT	GCAGGTCGGTGCACTCCTCG
OAS3	CCGAACTGTCCTGGGCCTGATCC	CCCATTCCCCAGGTCCCATGTGG
IFIT1	TTGCAGGAAACACCCACTTCT	GCAAAGCCCTATCTGGTGATG
MX1	CTTTCCAGTCCAGCTCGGCA	AGCTGCTGGCCGTACGTCTG
IFITM1	GGATTTCGGCTTGTCCCGAG	CCATGTGGAAGGGAGGGCTC
ISG15	CGCAGATCACCCAGAAGATCG	TTCGTCGCATTTGTCCACCA
β-actin	GATGAGATTGGCATGGCTTT	CACCTTCACCGTTCCAGTTT
GAPDH	CCATGGAGAAGGCTGGGG	CAAAGTTGTCATGGATGACC

Table S2. Primers used for ChIP-qPCR.

Primer	FWD	REV
pRIG1	GGAGGGAAACGAAACTAGCC	TTAAAGCCGGGTAGGAGGAG
pMDA5	CTTTGTAAACGTAATCTGCCTGG	GCTTTCCTTTTCTGTTTCCCG
pSTING	GTGCAGCCTGAAAATGAGATG	TTCCTACCTCCCTTCCTGAG
pISG15	CGGTTTTGTTTCTTCCGCTCA	AGCACCGGCCCTATTATAAGC