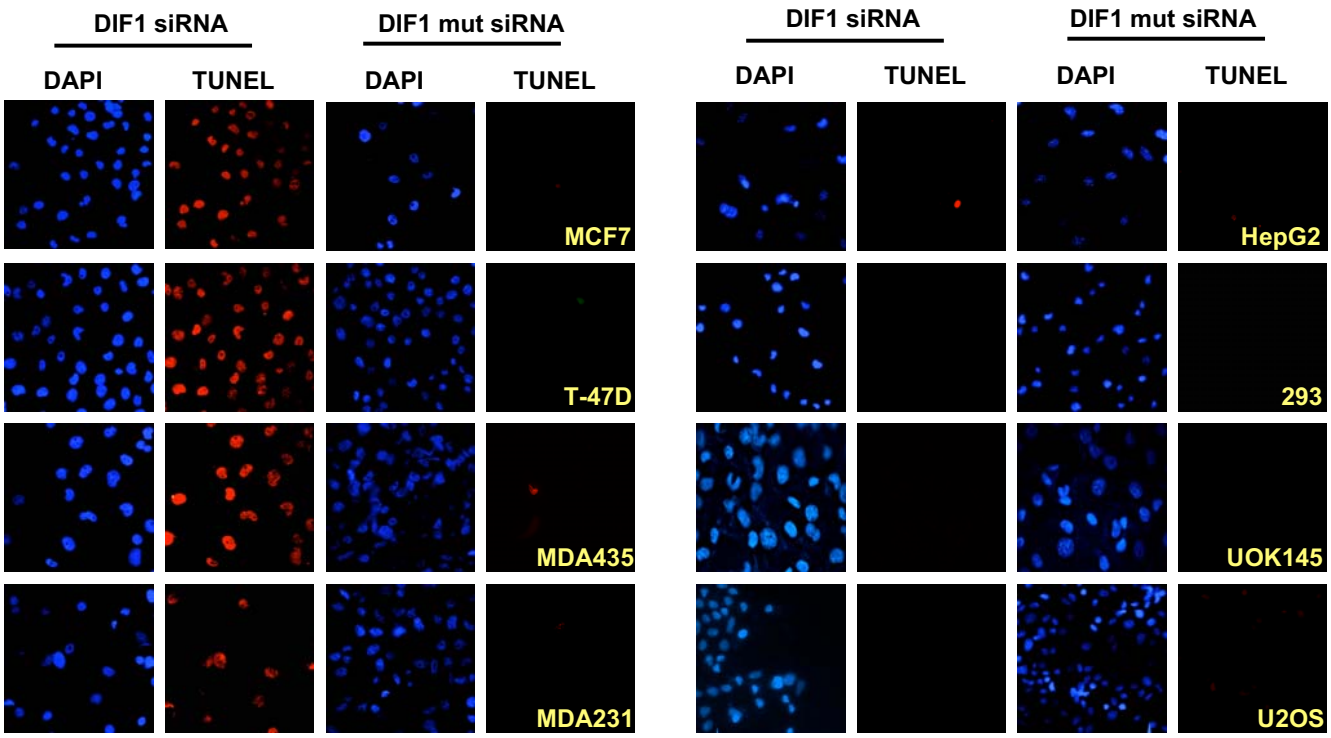
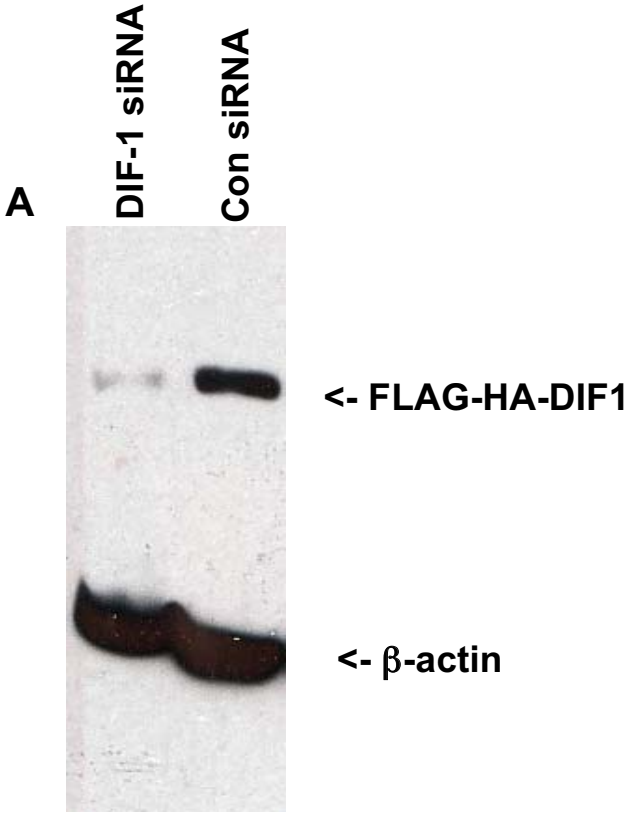
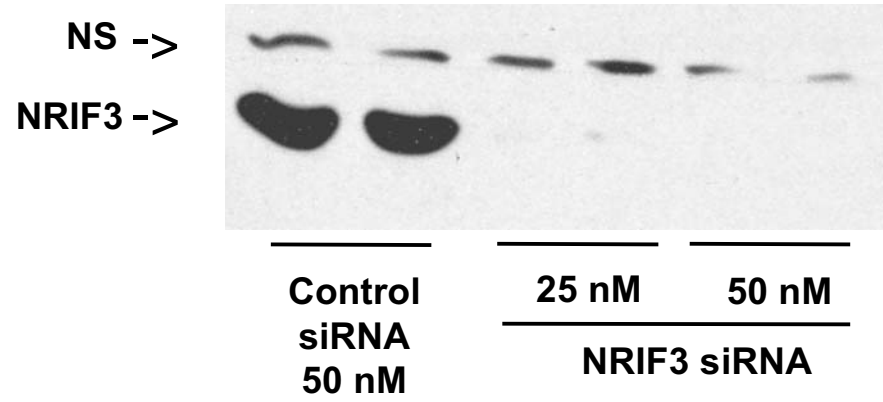
Supplemental Figure 1

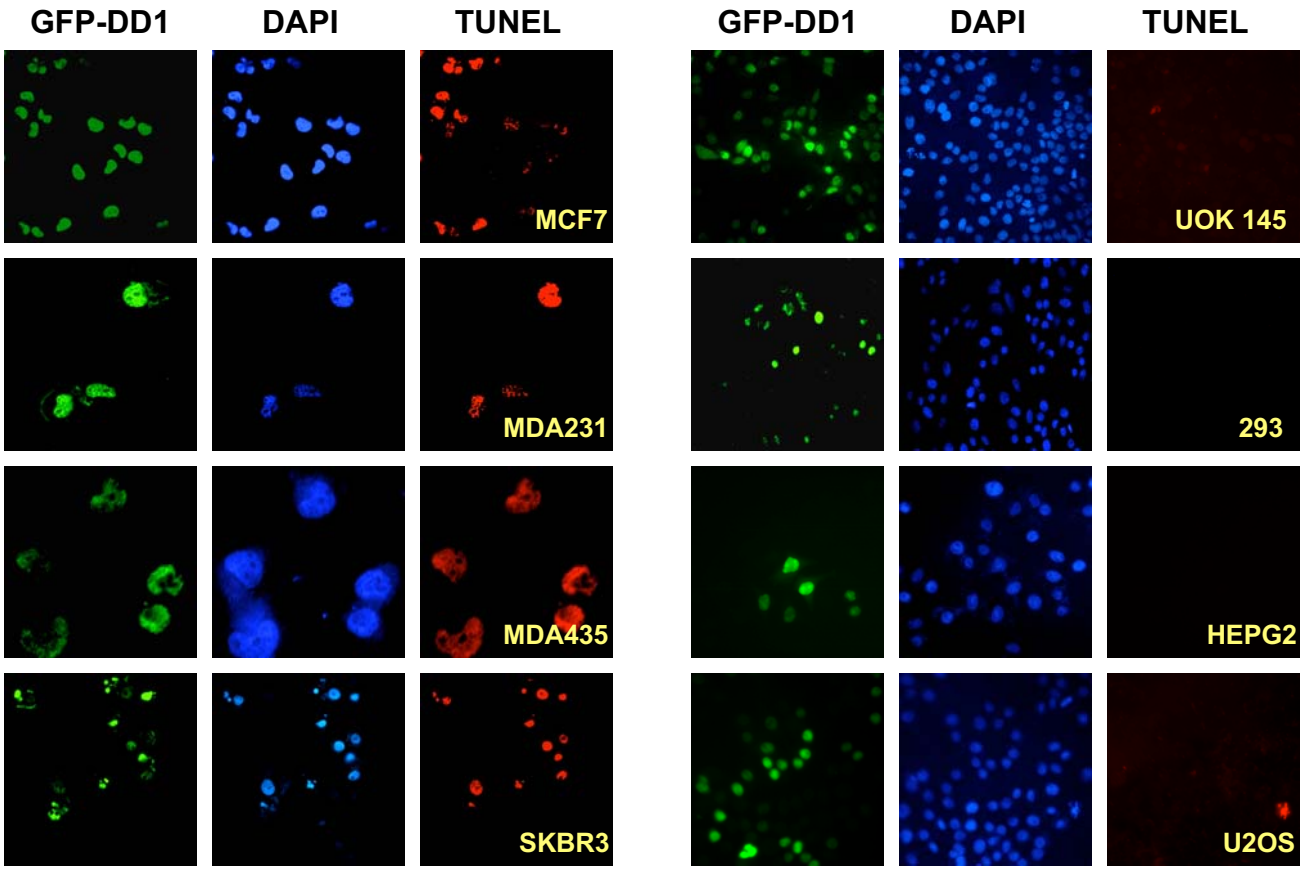


Supplemental Figure 2

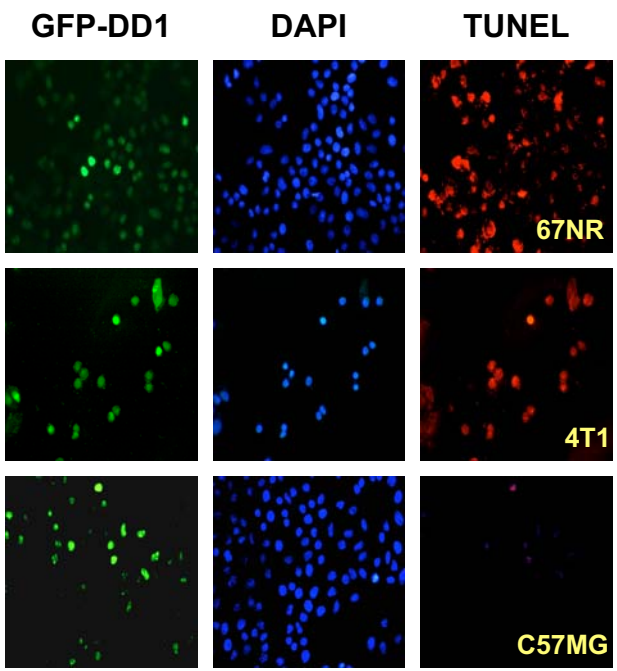




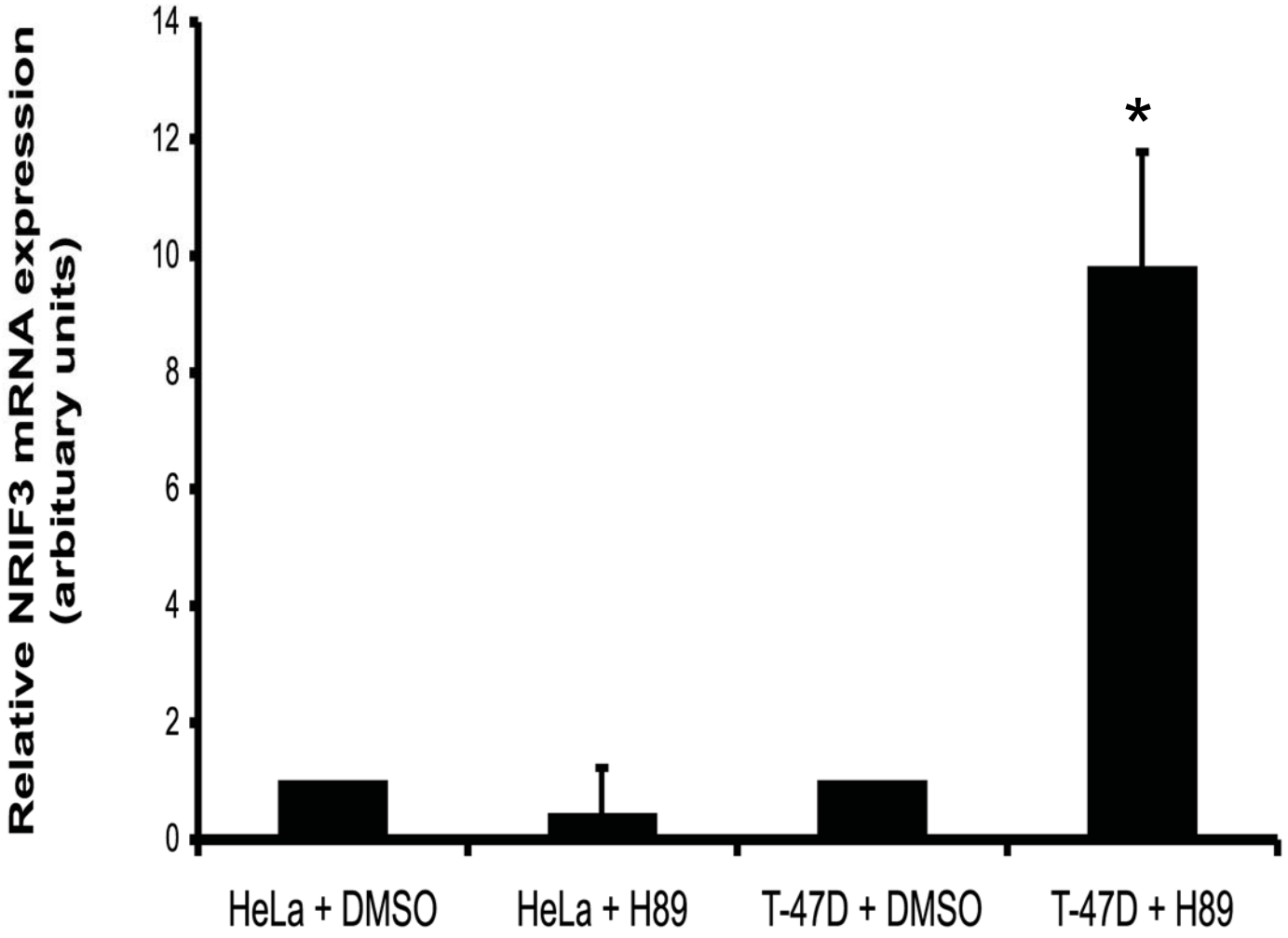
Supplemental Figure 3



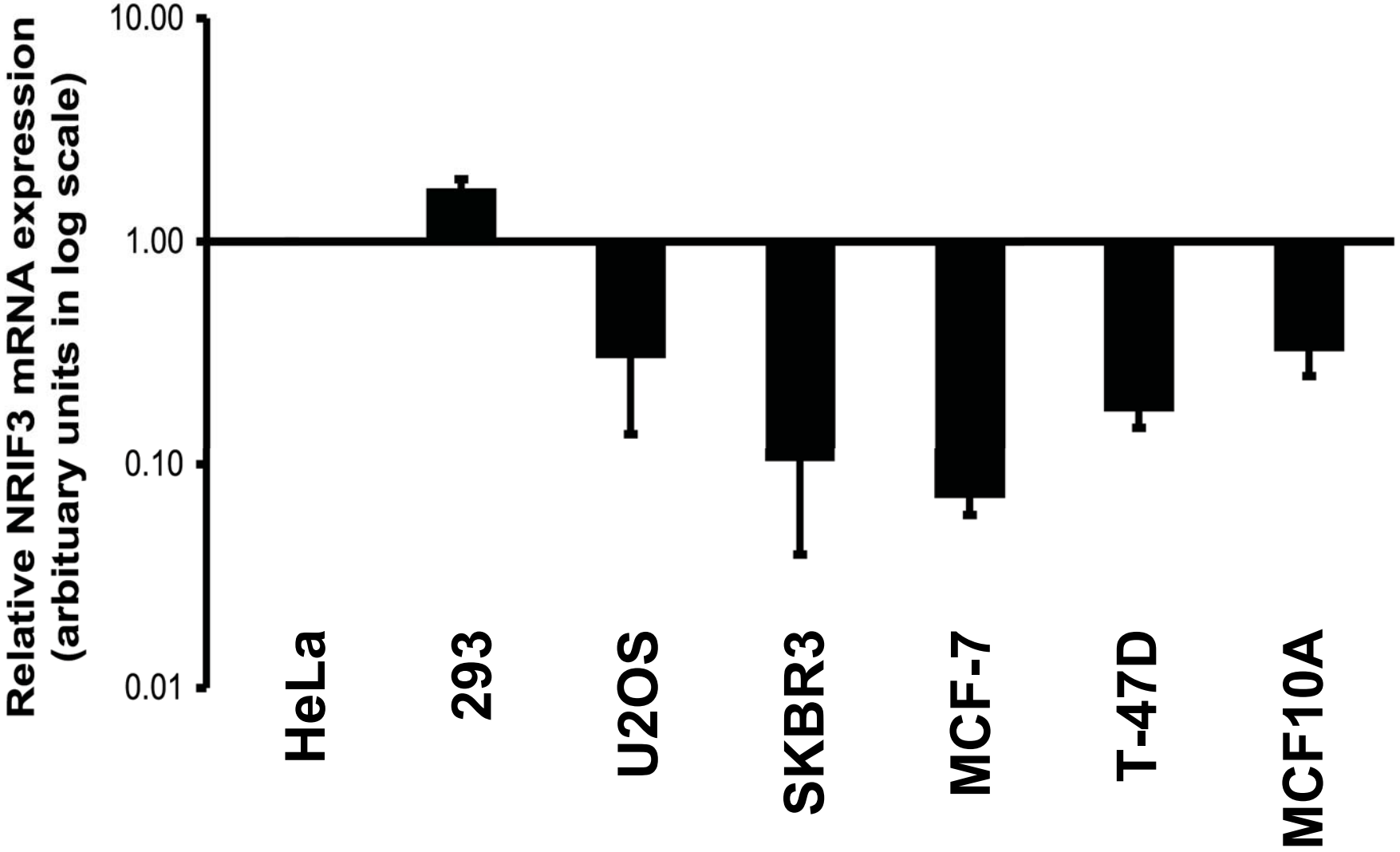
Supplemental Figure 4



Supplemental Figure 5.



Supplemental Figure 6.



Supplemental Figure 7.

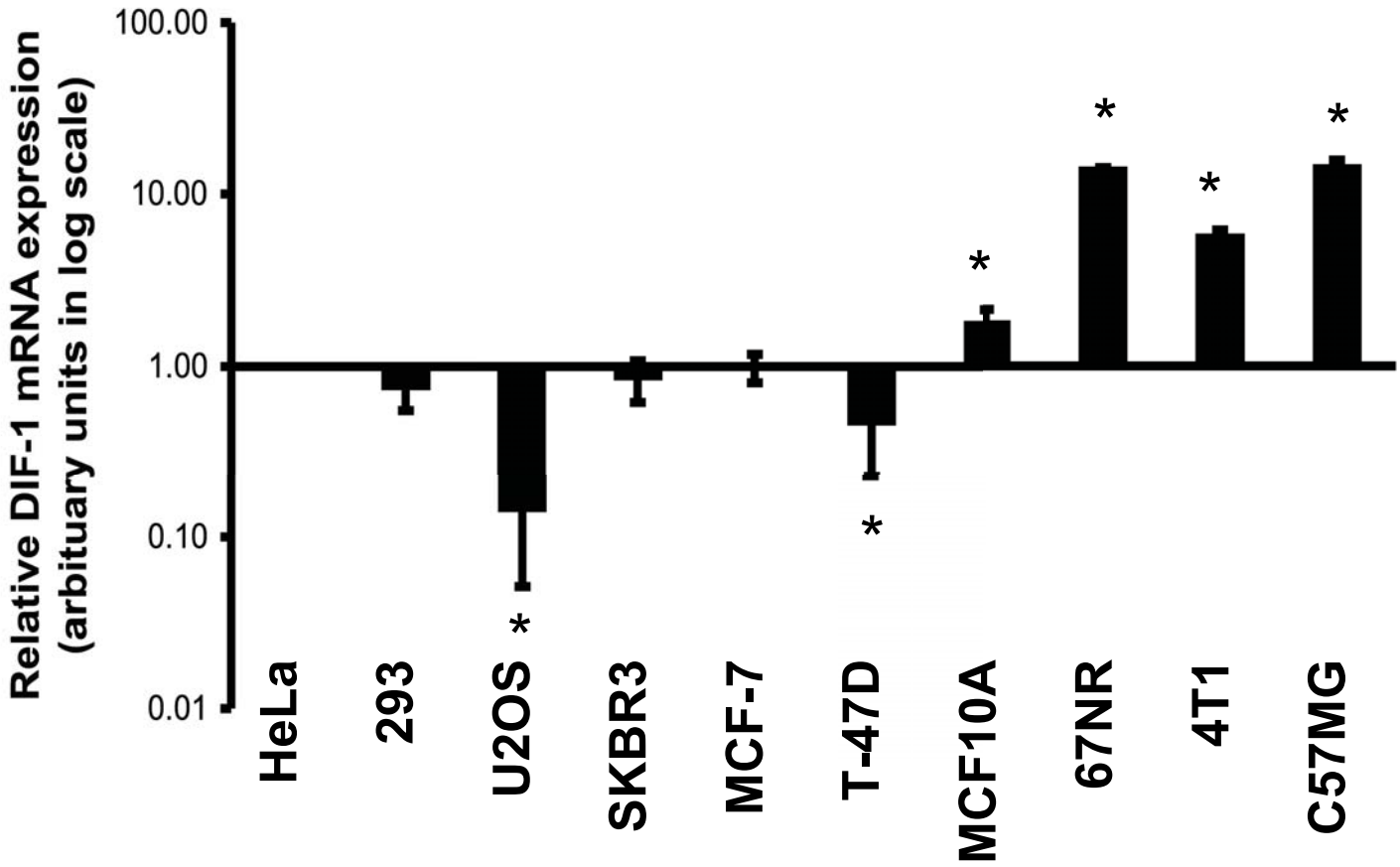


Figure Legends - Supplemental

Supplemental Figure 1. Knockdown of DIF-1 selectively leads to apoptosis of breast cancer cells. siRNA (25 nM) was used to knockdown DIF-1 expression in five different breast cancer cell lines (SKBR3, MCF-7, T-47D, MDA-435, and MDA-231) or cells of other origin (U2OS, human osteosarcoma; 293, human kidney epithelium; UOK-145, kidney carcinoma; HepG2, human hepatoma, and HeLa, human cervical epithelium). The results for SKBR3 cells and HeLa cells are shown in Fig. 4 in the manuscript. We used an siRNA (25 nM) containing 4 base changes as a control (mut siRNA). Thirty h later the cells were examined for apoptosis by TUNEL assay. All the breast cancer cell lines exhibited apoptosis while the cells of other origin did not exhibit apoptosis.

Supplemental Figure 2. Knockdown of DIF-1 and NRIF3 by siRNA. (A) We generated a stable T-47D cell line expressing FLAG-HA-tagged DIF-1 (Yeung and Samuels) and (B) a stable HeLa cell line expressing FLAG-HA-tagged NRIF3 (Tinnikov and Samuels). T-47D cells were transfected with 25 nM siRNA using HiPerfect while the HeLa cells were transfected with 25 nM and 50 nM siRNA as described in Materials and Methods in the manuscript. Twenty-four h later the expression of DIF-1 was assessed by Western blotting with HA antibody (A) and the expression of NRIF3 by Western blotting with anti-NRIF3 antibody (B). Loading controls for DIF-1 was assessed by Western blotting using β-actin antibody. NRIF3 antibody frequently recognizes a non-specific (NS) protein other than NRIF3 which acts to serve as a loading control.

Supplemental Figure 3. Expression of DD1 leads to apoptosis of breast cancer cells but not cells of other origin. GFP-DD1 was expressed in five different breast cancer cell lines (SKBR3, MCF-7, T-47D, MDA-435, and MDA-231) or cells of other origin (U2OS, 293, UOK-145, HepG2, and HeLa). Results for T-47D and HeLa have been previously published. The figure shows the results for the other cell lines.

Supplemental Figure 4. DD1 mediates apoptosis in two mouse breast cancer cell lines (4T1 and 67NR) but not in a cell line derived from normal mouse breast epithelium (C57MG). Cells

were transfected to express GFP-DD1 and assayed for apoptosis using a TUNEL assay as described in Materials and Methods in the manuscript.

Supplemental Figure 5. H89 stimulates NRIF3 mRNA levels in T-47D cells but not HeLa cells. HeLa or T-47D cells were treated with DMSO or 200 nM H89 for 16 h. T-47D cells were also incubated with zVDVAD-fmk to block apoptosis. RNA was then isolated and quantitated by RT-PCR as described in the Supplemental Materials and Methods Section. Fold difference in NRIF3 mRNA expression in cells treated with H89 or DMSO (control) cells, is graphed on a log scale. *$p<0.001$; statistically significant difference between indicated data points and control calculated by Student's t-test.

Supplemental Figure 6. NRIF3 mRNA expression profile in breast and non-breast cancer cell lines – HeLa, 293, U2OS, SKBR3, MCF-7, T-47D, and MCF10A cells. RNA was isolated and relative NRIF3 mRNA levels were assessed by quantitative RT-PCR as described in the Supplemental Materials and Methods Section. Fold difference in NRIF3 mRNA expression in each cell line, as compared to that in HeLa cells, is graphed on a log scale. Data points for all cell lines are statistically significantly different from that of HeLa ($p<0.05$) calculated Student's t-test.

Supplemental Figure 7. DIF-1 mRNA expression profile in breast and non-breast cancer cell lines – HeLa, 293, U2OS, SKBR3, MCF-7, T-47D, MCF10A, 67NR, 4T1, and C57MG cells. RNA was isolated and relative DIF-1 mRNA levels were assessed by quantitative RT-PCR as described in the Supplemental Materials and Methods Section. Fold difference in DIF-1 mRNA expression in each cell line, as compared to that in HeLa cells, is graphed on a log scale. *$p<0.05$; statistically significant difference between indicated data points and HeLa calculated by Student's t-test.