

TABLE S1. Primer Sequences Used in Quantitative Real-Time PCR Detection Assays.

Primer sequences used to monitor endogenous mRNA levels for a particular gene of interest in the breast cancer cell lines via quantitative real-time and end-point PCR assays. All primers generated were optimized to have a T_m of 60 – 55 °C, a GC content between 45 – 55%, and an annealing region that spans exon-exon junctions of the corresponding transcript (assisted by Primer 3 on-line software and NCBI database of annotated gene sequences).

FIGURE S1. Loss of Argoanute-2 Abrogates miR-206 Activity in ER α ⁻ Breast Cancer Cell Lines.

(A) Western blot analyses of Ago2 protein expression in MDA-MB-231 and MDA-MB-435 cell lines \pm pSM2-Ago2, a construct that generates a shRNA towards Ago2, from Open Biosystems (Huntsville, AL). During these time-course studies (0 – 32 h) each cell line underwent a mock transfection for 32 h as a control (M), while the non-specific band (N.S.B) generated from the Ago2 primary antibody was used as a loading control for this analysis. The western blots depicted here are representative images of three independent experiments. **(B)** Luciferase assays were performed to determine if miR-206 activity was ablated in pSM2-Ago2 containing cells. Luciferase activity was generated from 400 ng of various pIS-ER α 1 constructs 36 h-post transfection in the cell lines noted above \pm 800 ng of the pSM2-Ago2 construct. For each pIS-ER α 1 construct, Firefly luciferase activity was normalized to 200 ng Renilla luciferase, and values obtained from pSM2-Ago2 containing cells (pSM2-Ago2 +) were set relative to mock transfected cells (pSM2-Ago2 -). The values reported are indicative of three independent experiments with replicates performed in duplicate \pm SEM. **(C)** The effects of Ago2 loss on luciferase mRNA levels as assayed by end-point RT-PCR. The cells mentioned above were transfected for 24 h with 200 ng β -gal, 400 ng of the indicated pIS-ER α 1 luciferase constructs, plus or minus pSM2-Ago2. After 23 cycles, PCR products from the various pIS-ER α 1 constructs, β -gal, and Ago2 were run on a 1% agarose gel. Each series of gels are from the same sample and represents three independent experiments.