

Figure W1. BCA2 knockdown in MCF-7 and T47D does not significantly affect the p21 mRNA levels. MCF-7 and T47D cells were transfected with Luc siRNA or BCA2 siRNA#A for 72 hours. Total RNAs were prepared from cultured cells using TRIzol reagents. RT of 1 μ g of total RNA was performed using the iScript cDNA Synthesis Kit. SYBR Select Master Mix (ABI, Catalog No. 4472908) was used for quantitative RT-PCR in an ABI-7900 HT machine. The primers are the same with the RT-PCR. Quantitative PCR on cDNA products was performed using the following parameters: predenaturation at 95°C for 2 minutes, 95°C for 15 seconds, annealing at 60°C for 1 minute, running 40 cycles. The mRNA levels of BCA2 and p21 were analyzed using the $\Delta\Delta C_t$ method. GAPDH was used as the loading control.

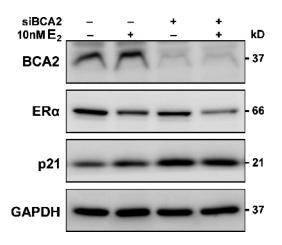


Figure W2. BCA2 regulates p21 protein levels independent of estradiol (E₂). MCF-7 cells were cultured in hormone-free media for 48 hours and transiently transfected with siLuc and siBCA2 siRNA, as indicated. Forty-eight hours later, the cells were treated with ethanol as control or 10 nM β -estradiol (E₂) for 24 hours and collected and subjected to WB analysis. Protein levels of p21, ER α , and BCA2 are shown.