

**miR-331-3p REGULATES *ERBB-2* EXPRESSION AND ANDROGEN RECEPTOR SIGNALING
IN PROSTATE CANCER – SUPPLEMENTARY SECTION**

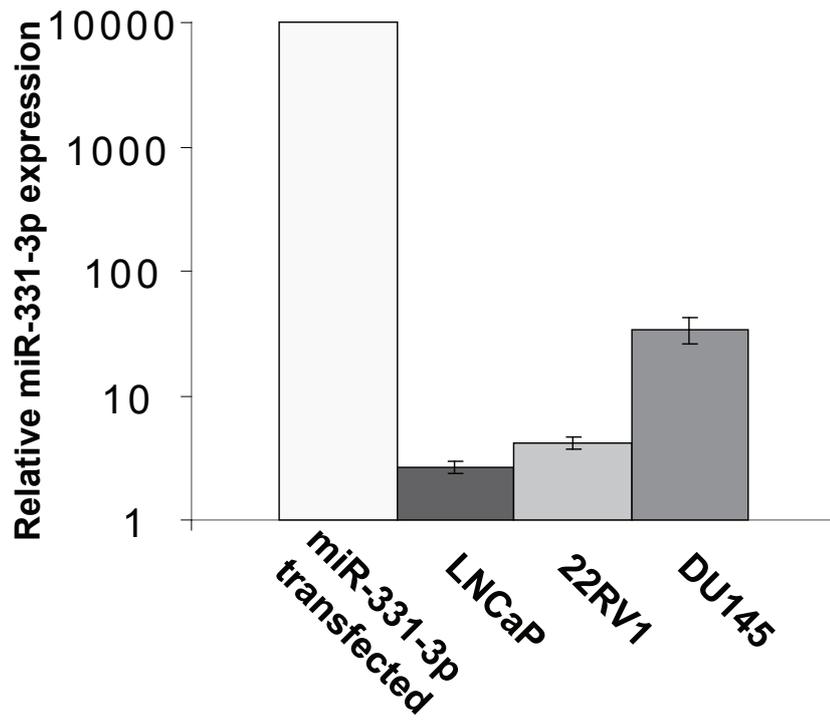
Supplementary Fig. 1. miR-331-3p expression is inversely correlated with *ERBB-2* mRNA expression in PCa cell lines. *A.* qRT-PCR analysis of miR-331-3p expression between miR-331-3p transfected 22RV1 cells (30 nM) and PCa cell lines. Total RNA was reverse-transcribed and miR-331-3p expression determined by TaqMan miRNA qRT-PCR assay. miR-331-3p expression was normalized to U44 and U6 snRNA expression and relative miR-331-3p levels in PCa cell lines were expressed relative to miR-331-3p transfected 22RV1. *B.* qRT-PCR analysis of *ERBB-2* expression between PCa cell lines LNCaP, 22RV1 and DU145. 125 ng of total RNA was reverse-transcribed and *ERBB-2* and *GAPDH* expression determined by qRT-PCR. Data was normalized to *GAPDH* RNA expression and *ERBB-2* expression levels between the PCa cell lines was calculated relative to LNCaP *ERBB-2* RNA expression. Error bars as described in Figure 2.

Supplementary Fig. 2. Sites A and B within the *ERBB-2* mRNA 3'-UTR are specific targets for miR-331-3p. 22RV1 cells were co-transfected with pmiR-REPORT *ERBB-2* 3'-UTR miR-331-3p target site constructs and CMV-*Renilla*, and miR-NC, miR-331-3p or unrelated miRNA (hsa-miR-183). Relative luciferase expression (firefly normalized to *Renilla*) values were expressed as a ratio to each miR-NC-transfected reporter (\pm SD).

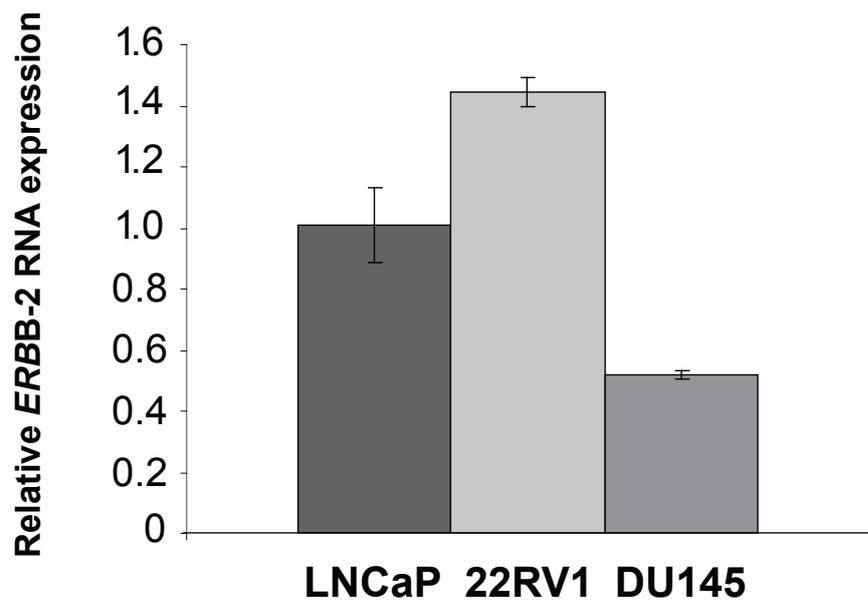
Supplementary Fig. 3. miR-331-3p blocks *ERBB-2* expression and signaling in 22RV1 and DU145 PCa cells. 22RV1 (*A.*) or DU145 (*B.*) cells were transfected with miR-NC or miR-331-3p (30 nM) for 48 h and serum starved for 24 h thereafter, followed by stimulation +/- heregulin (HRG; 50 ng/mL) for 20 min. Cell lysates were analysed for total *ERBB-2*, phospho-*ERBB-2*, total AKT, phospho-AKT and β -actin expression by immunoblotting.

Supplementary Figure 1

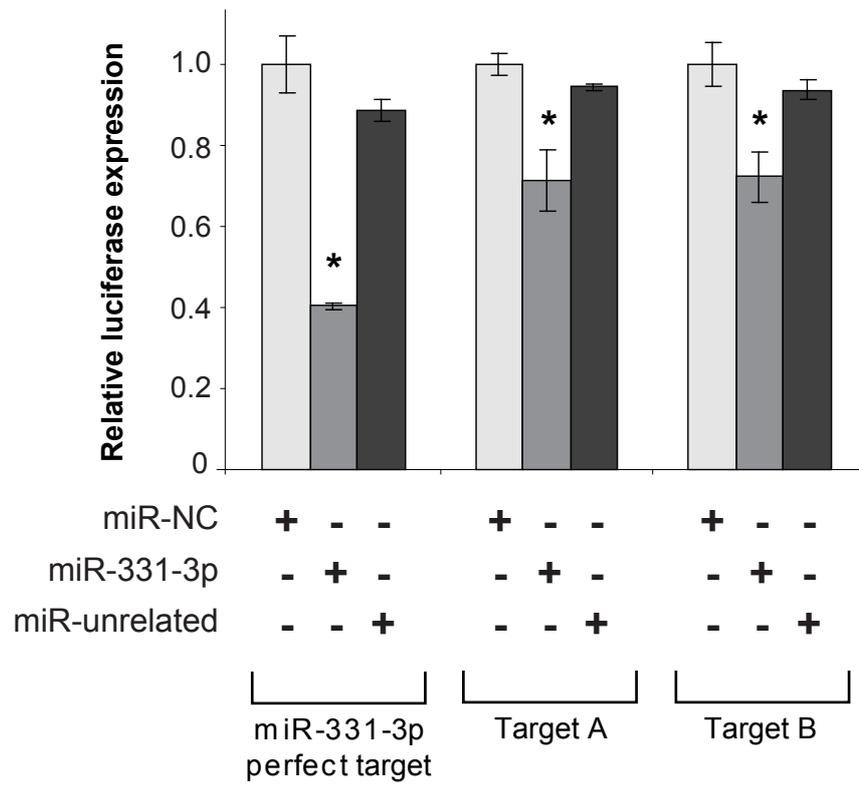
A



B

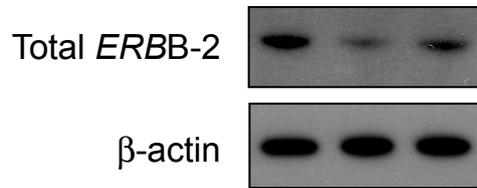


Supplementary Figure 2

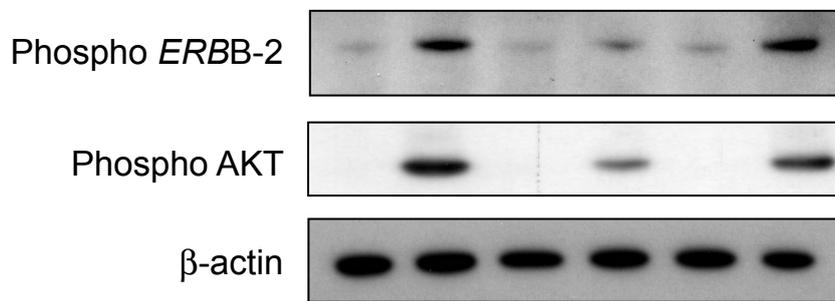


A

22RV1



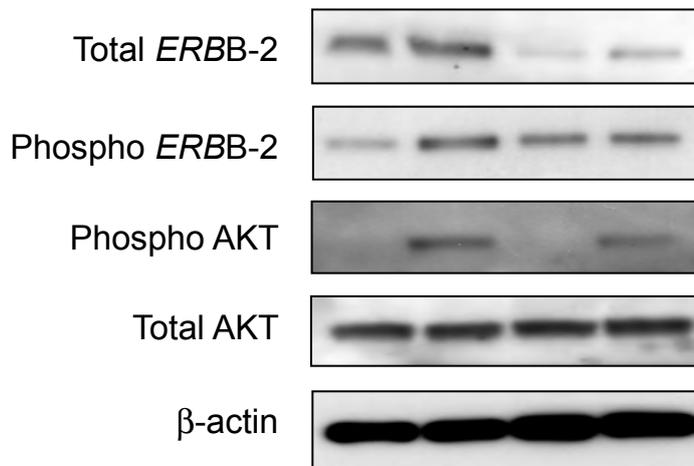
miR-331-3p	-	+	-
miR-NC	-	-	+



miR-331-3p	-	-	+	+	-	-
miR-NC	-	-	-	-	+	+
HRG	-	+	-	+	-	+

B

DU145



miR-331-3p	-	-	+	+
miR-NC	+	+	-	-
HRG	-	+	-	+