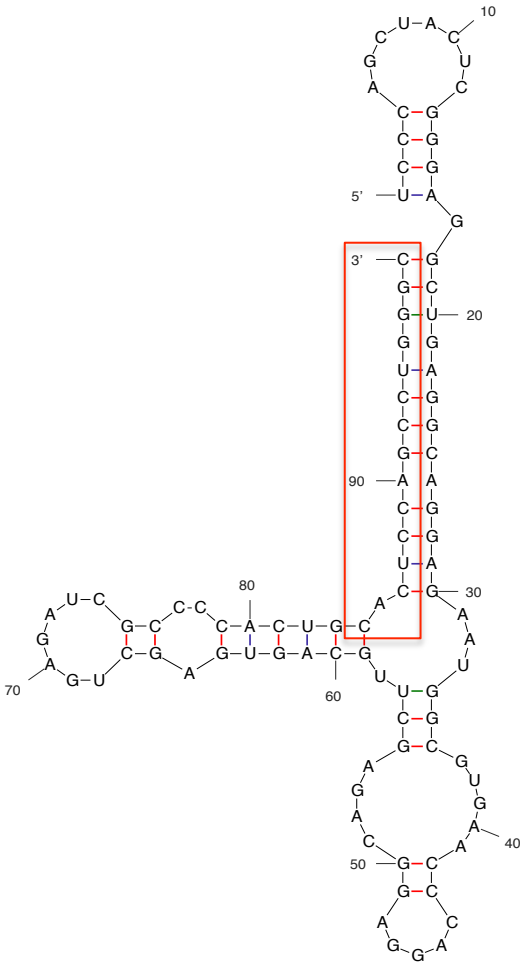
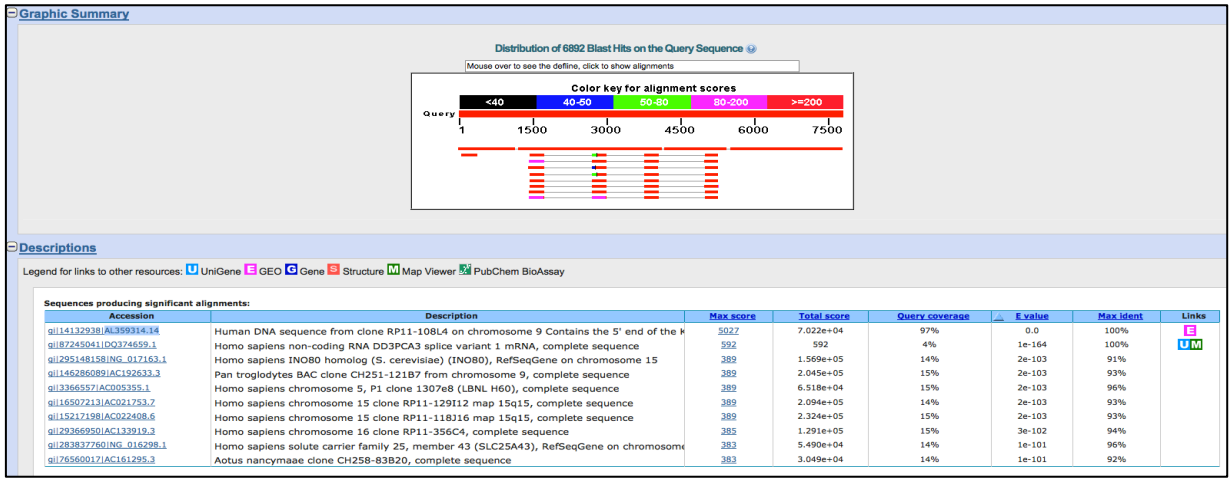
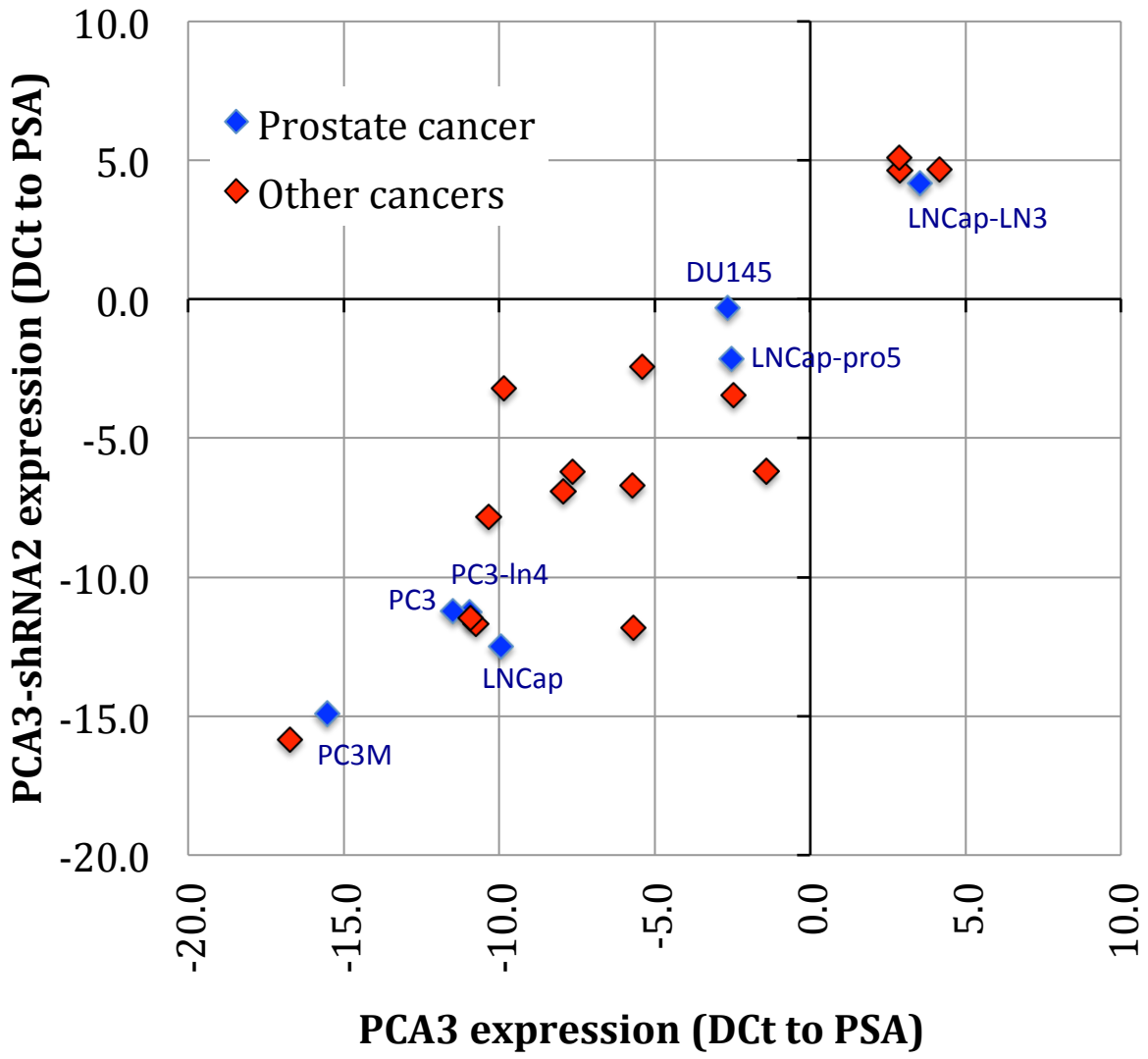
(a).



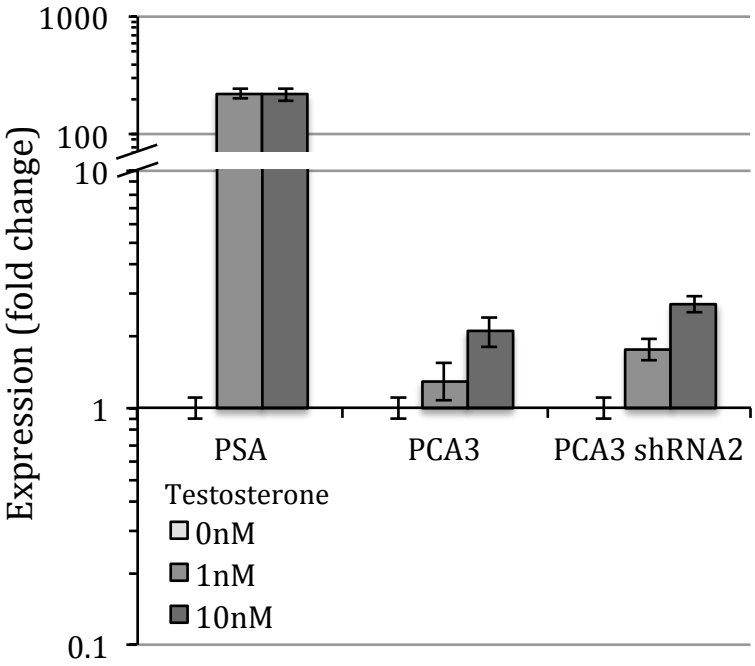
(b).



Supplementary Figure 1. Identification of the PCA3-shRNA2 hairpin. (a). Predicted hairpin of PCA3 RNA2. The bases in red are those identified within the prostate RNA transcriptome. (b). BLAST results of the 98bp fragment derived from PCA3-shRNA2 using 3'RACE, indicates that the sequence is found within PCA3 intron 1.

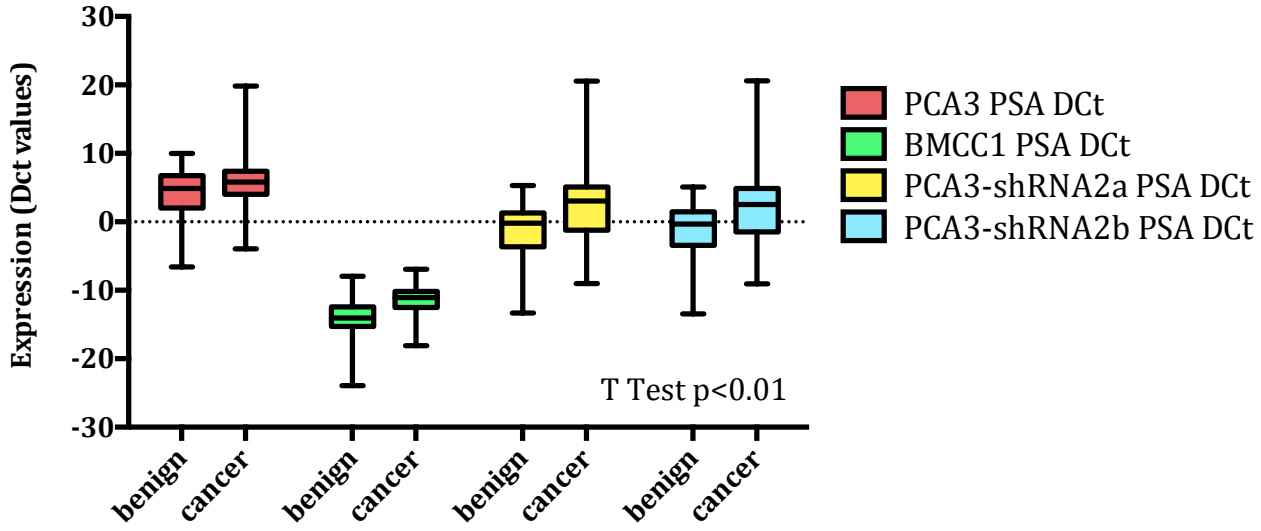


Supplementary figure 2. Expression of PCA3 and PCA3-shRNA2 in cell lines representing prostate cancer (blue and labeled) and other malignancies (red). The expression of the two PCA3 RNAs is shown (normalised to PSA mRNA expression) for each of 22 cell lines. The non-prostate cancer cell lines are not labeled for clarity. IN order of PCA3-shRNA2 expression these are (from HCT-116 (PCA3-shRNA2 DCt=-15.85), HEK 293, A549, NCI-H460, WM793, RT112, T47D, MRC5, AN3CA, RT4, SKOV-3, EJ, MCF-7, Jurkat and HeLa (PCA3-shRNA2 DCt=5.08)).

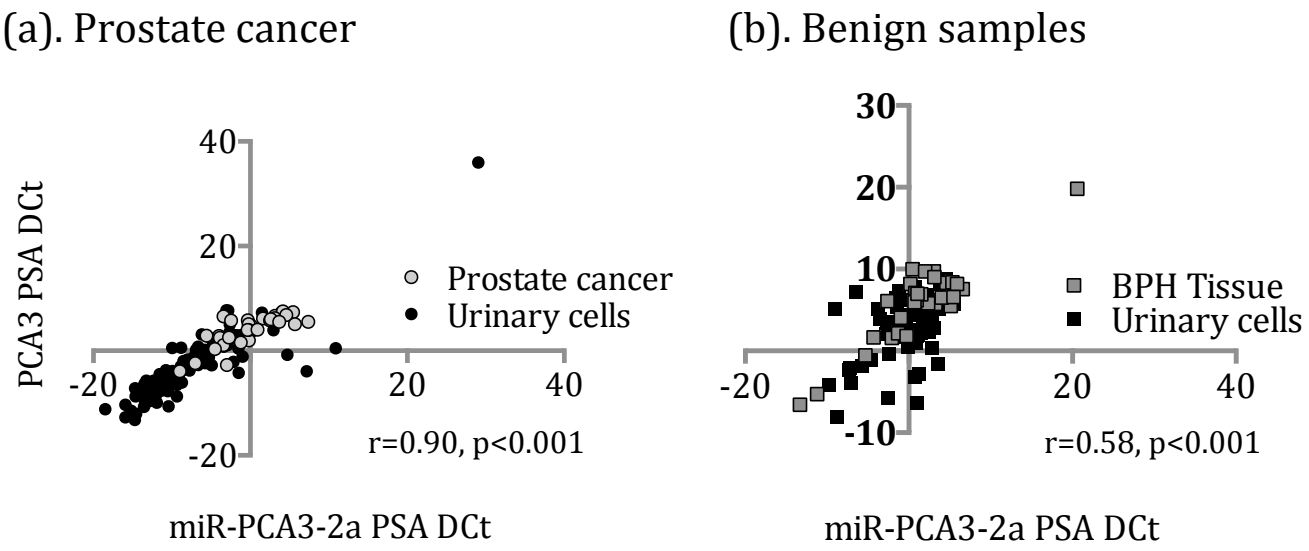


Supplementary figure 3. Androgen regulated expression of PSA, PCA3 and PCA3 shRNA2. Expression (fold change) was determined using qrtPCR and normalized to a non-androgen regulated U1 RNA. PCA3 and PCA3-shRNA expression was directly related to testosterone concentration within growth media (0, 1nM and 10nM)

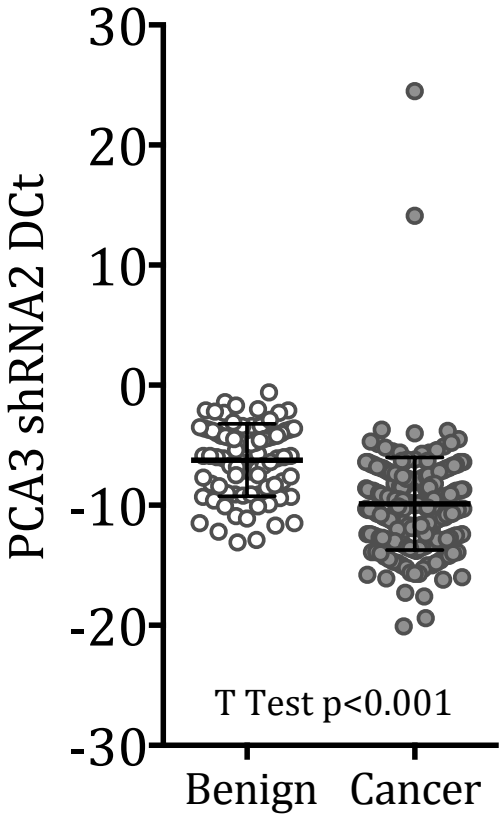
(a).



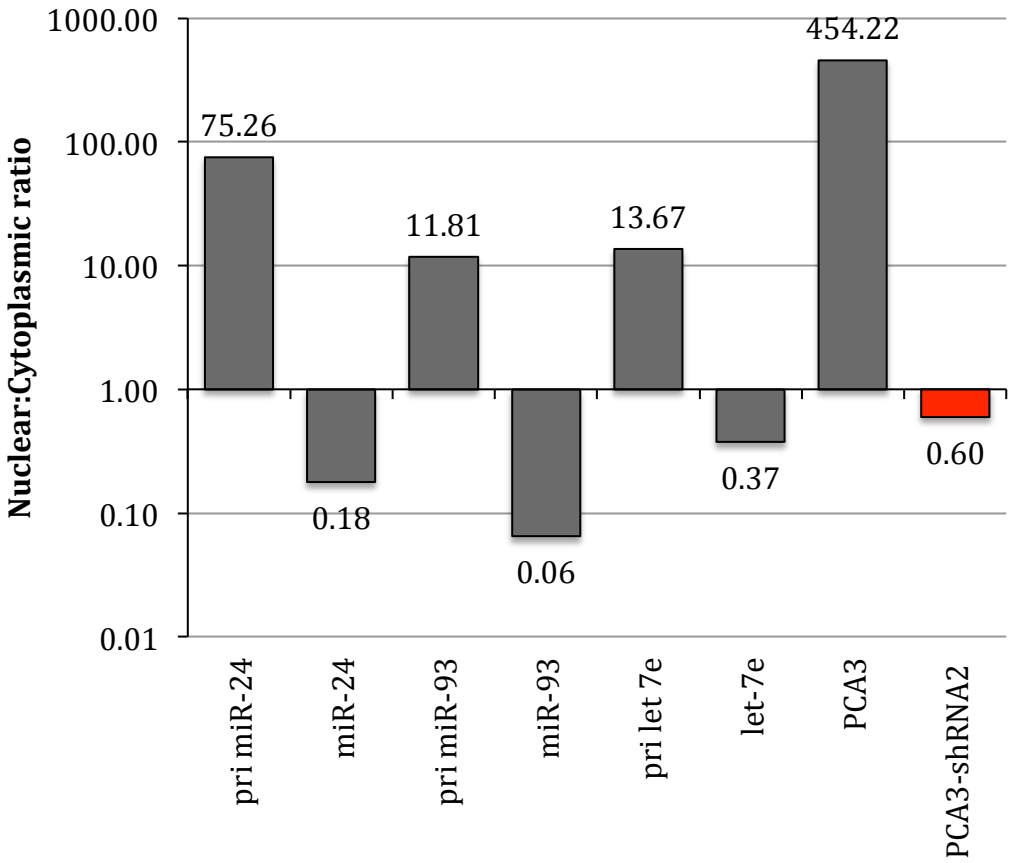
Supplementary figure 4. Expression of PCA3, BMCC1, PCA3-shRNA2a and PCA3-shRNA2b in malignant and benign prostatic tissue. Significantly higher expression is seen in malignant tissues, when compared to benign tissues, for BMCC1 (DCt: -11.5±2 vs. -14.1±3, T Test $p<0.01$ for malignant vs. benign) and PCA3-shRNA2 (e.g. PCA3-shRNA2a DCt: 2.15±5 vs. -1.17±4).



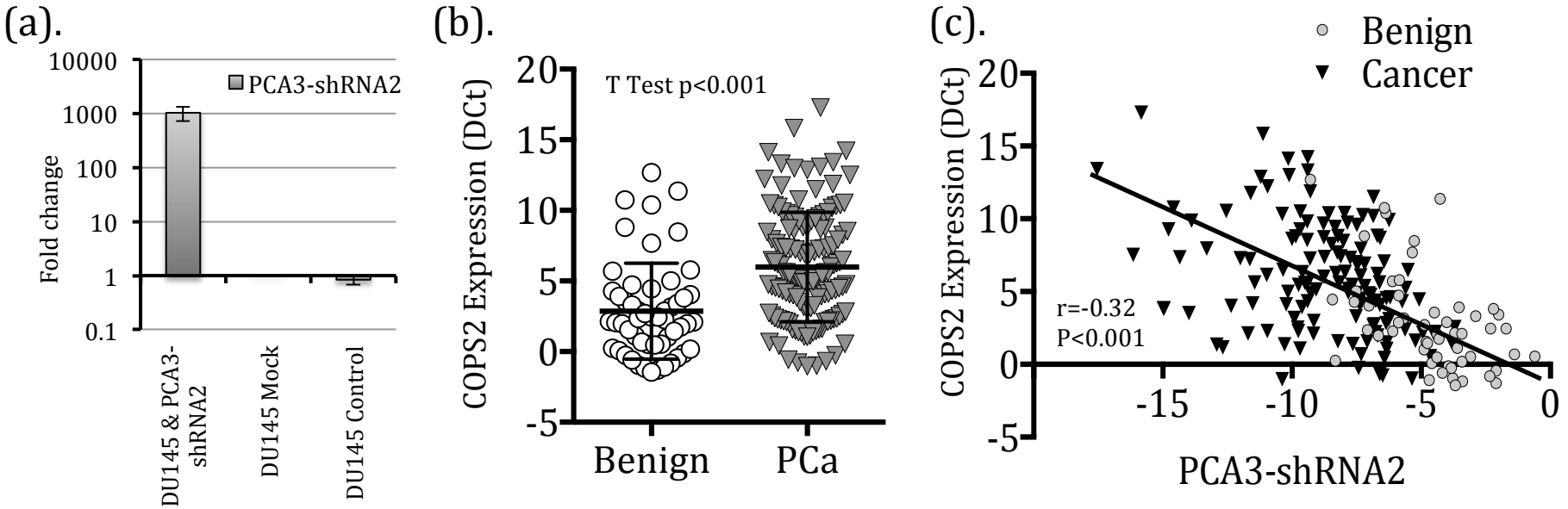
Supplementary figure 5. Correlation of PCA3 and PCA3-shRNA2 in urinary and frozen tissue according to pathology. Expression of the two RNAs was closer correlation in (a). malignant than in (b). benign samples.



Supplementary figure 6. Expression of PCA3-shRNA2 in the validation cohort of men, stratified by the presence of prostate cancer and benign prostatic hyperplasia.



Supplementary figure 7. Expression of PCA3-shRNA2 and primary/mature microRNAs according to nuclear and cytoplasmic localization. The ratio between nuclear and cytoplasmic RNA normalized expression is shown for PCA3-shRNA3 in red and various primary and mature microRNAs for comparison. For each mature short RNA (including PCA3-shRNA2), the majority of the transcript is expressed within the cytoplasm, in contrast to the primary miR hairpin transcript. The majority of the PCA3 mRNA is within the nucleus (nuclear:cytoplasmic ratio 454.22:1)



Supplementary figure 8. PCA3-shRNA2 in DU145 and SOX11 expression. (a). Expression of PCA3-shRNA2 RNA in cells transfected with the correct sequence, mock transfection with a scrambled RNA sequence and untransfected cells. Bars represent the mean of three independent repeats and standard deviation (error bars). (b). Expression of COPS2 is lower in the urinary cells of patients with prostate cancer when compared to controls (BPH) and is (c). correlated to that of PCA3-shRNA2 .