Supplemental Figure Legend

**Supplemental Figure S1:** Flow charts demonstrating the patient selection. (A) for the study and (B) for the validation cohort.

**Supplemental Figure S2:** Scatter- and Box blots show the expression of miR-221 in the study- and validation cohort in relation to clinical/histopathological parameters. Relative miR-221 expression was analysed in 134 (cohort1) and 89 (cohort2) prostate cancer samples by the ΔCt method using qRT-PCR. (A) The scatter plot of the miR-221 expression distribution by prostate specific antigen serum levels showed no correlation (p=0.51 for cohort1 and p=0.26 for cohort2). Samples were divided into subgroups of tumor severity based on the Gleason score (B) and the pathological tumor stage (C). No significant differences in the mean expression levels (black bars) between groups were observed (p>0.05). P-values were calculated with the Pearson’s chi-squared test (PSA), Mann-Whitney test and ANOVA (Gleason score, pathological stage). Specifics of the different subgroups for both cohorts are summarized in supplementary Table 1.

**Supplemental Figure S3:**
MiR-221 down-regulation predicts clinical failure (CF) in high-risk prostate cancer. (A) Relative MiR-221 expression levels (ΔCt) of PCa samples of both cohorts were analysed by qRT-PCR and subsequently divided into risk groups based on CF. Significant reductions in the median expression levels were identified between the two groups in both cohorts and indicated by * p <0.001 using two-sided Mann-Whitney test. The cutoff level defined in the ROC analysis is indicated by the horizontal black line. (B) Receiver operating characteristic (ROC) analysis for predicting CF by miR-221 expression. The prognostic value of miR-221 for CF was evident from areas under curve (AUC) of 0.922 (learning cohort). An optimal
ROC-derived threshold value to dichotomize the patients by normalized miR-221 expression was -0.375. (C) Kaplan-Meier analysis of patients with high-risk prostate cancer. Patients were grouped by miR-221 expression cutoffs defined in the ROC analysis; survival curves are shown for both groups. Low miR-221 expression is associated with earlier CF (log-rank p <0.0001 in both groups). (D) Cox proportional hazards regression analysis for time to CF revealed that dichotomised miR-221 expression level predicted CF.

**Supplemental Figure S4**

Upregulation of miR-221 in pre miR-221 transfected cell lines. (A) Indicated cell lines were transfected with pre-miR-221 or pre miR precursor negative control (ctrl) in 6 well plates. At day 2 post transfection total RNA was isolated and expression of miR-221 and RNU6B was analyzed by qRT-PCR. Results are shown as relative miR-221 expression ($\Delta$Ct levels) normalized by RNU6B. MiR-221 is strongly upregulated in all three cell lines after pre miR-221 transfection (p< 0.001, Wilcoxon rank sum test). Presented data are mean values +/- SD from four independent experiments. B and C) For technical validation of array data we analyzed relative expression of selected genes, that were shown to be up- (B) or down-(C) regulated on the array, using qRT-PCR analysis. We analyzed RNA from two independently performed transfections (pre miR-221 A and pre miR-221 B, respectively ctrl A and ctrl B (see Fig.3)) that were also used in the array experiment. Normalized qRT-PCR results from miR-221 transfected cells are calculated as x-time expression changes in comparison to PC-3 cells transfected with pre miR-precursor negative control. The median expression level of both control transfected PC-3 cells was arbitrarily set as 1.0. We analyzed the expression of RNA by the $\Delta\Delta$Ct relative quantification method using $\beta$-Actin as internal control. Significant differences (P< 0.01) between expression in control and miR-221 transfected cells were indicated by asterisk (*). P values were calculated by student’s t-test.
Supplemental Figure S5

Interferon signaling pathway and Enriched pathways based on lists of down-regulated or up-regulated genes. A) Red color indicates a log fold change < (-1) and p-val < 0.05 of a gene. The pathway is generated through the use of IPA (Ingenuity Systems)

B) Enriched pathways based on lists of down-regulated or up-regulated genes. Red bars show the number of observed genes in the dataset, blue bars show the statistically expected number of genes, given the result to be random.

Supplemental Figure S6

Stable miR-221 expression in PC-3 clones mediates growth inhibition and activates genes involved in the interferon signaling pathway. A) PC-3 cells and PC-3/miR-221 clones #A1, #A3 and #B3 were tested for induced expression of miR-221 after doxycycline treatment (0.5 μg/ml). Quantitative real time PCR confirmed stable and significant miR-221 overexpression in all three PC-3/miR-221 clones after doxycycline treatment for 48 hours. The expression levels were relative to that in corresponding un-induced PC-3 /miR-221 clones, which arbitrarily were set as 1.0. B) Growth inhibition of PC-3/miR-221 clones #A1 and #B3 after induction of miR-221 expression. MTS assay for the growth of PC-3/miR-221 clones #A1 and #B3 after doxycycline induced miR-221 overexpression. MTS assay showed significant differences in growth of doxycycline treated (red lines and symbols) and untreated (black lines and symbols) PC-3 /miR-221 clones. Growth of doxycycline treated and untreated PC-3 cells were used as controls and showed no significant difference. Experiments were performed as triplicates. Each point represent the mean +/- SD from five independent experiments. C) MiR-221 mediated overexpression of genes involved in the interferon signaling pathway. Expression of indicated genes was significantly overexpressed in miR-221 induced PC-3/miR-221 clones #A1 (black bars) and #B3 (white bars). Normalized qRT-PCR
results from doxycycline treated (48 hours) PC-3/miR-221 clones are calculated as x-time expression changes in comparison to un-induced PC-3/miR-221 clones. Expression differences in doxycycline treated PC-3 cells (grey bars) were used as negative control. Data represent mean values +/- SD of five independent experiments. A, B and C) Significant differences (P< 0.05) were indicated by asterisk (*). P values were calculated by two-sided Mann-Whitney test and Student’s t-test.

**Supplemental Figure S7**

Mir-221 expression decrease mRNA expression levels of SOCS3 or IRF2. PC-3 cells were transfected with negative control or pre miR-221 for 48 hr. at 6 well plates. qRT-PCR were performed to analyse the relative mRNA expression levels of SOCS-3 and IRF2 using GAPDH and β-Actin as loading control. Results are displayed on fold differences to control cells (value of control transfected cells are arbitrarily set as 1). The results are presented from four independent experiments (p<0.05, Wilcoxon rank sum test).