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Supplemental Data

Oncogenic Role of the Ec Peptide of the IGF-1Ec Isoform in Prostate Cancer

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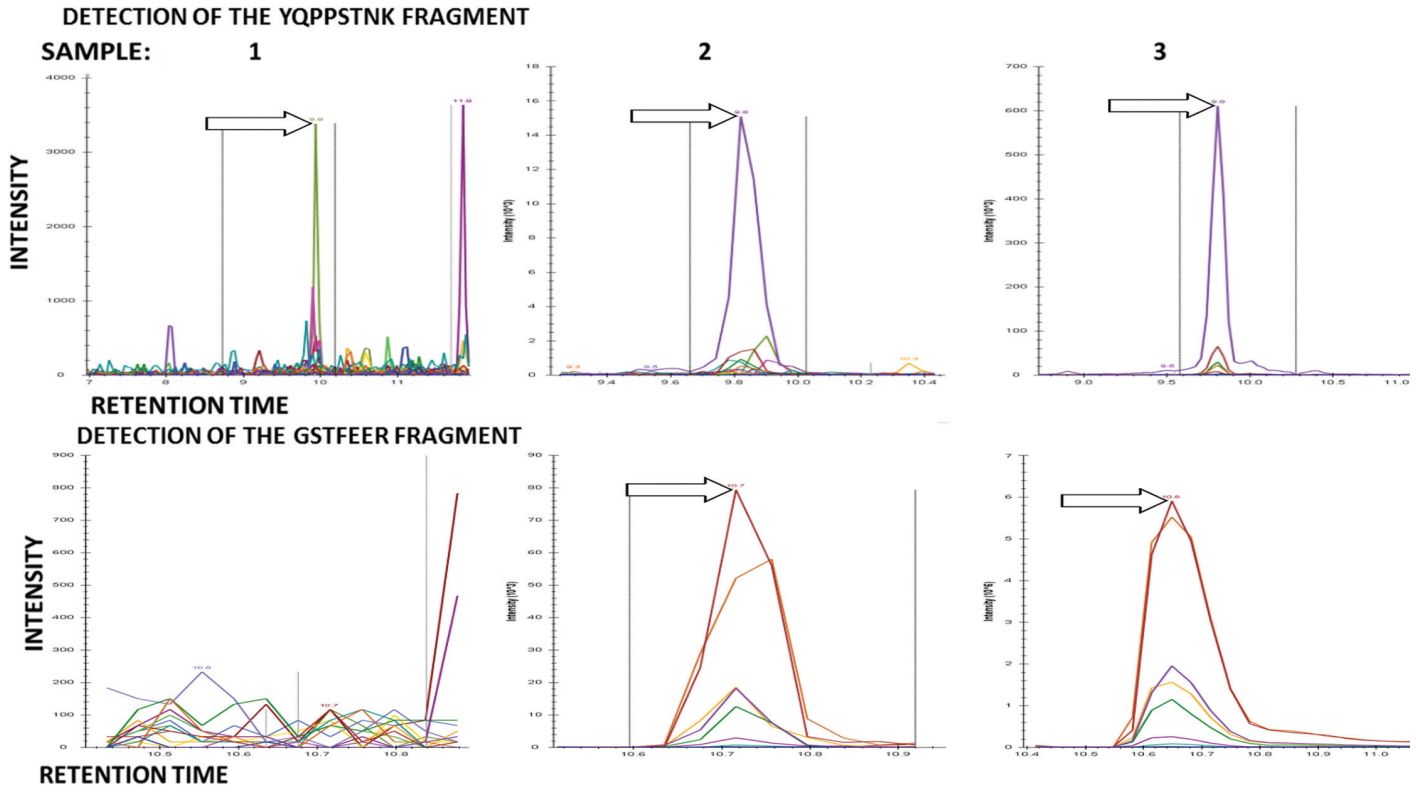
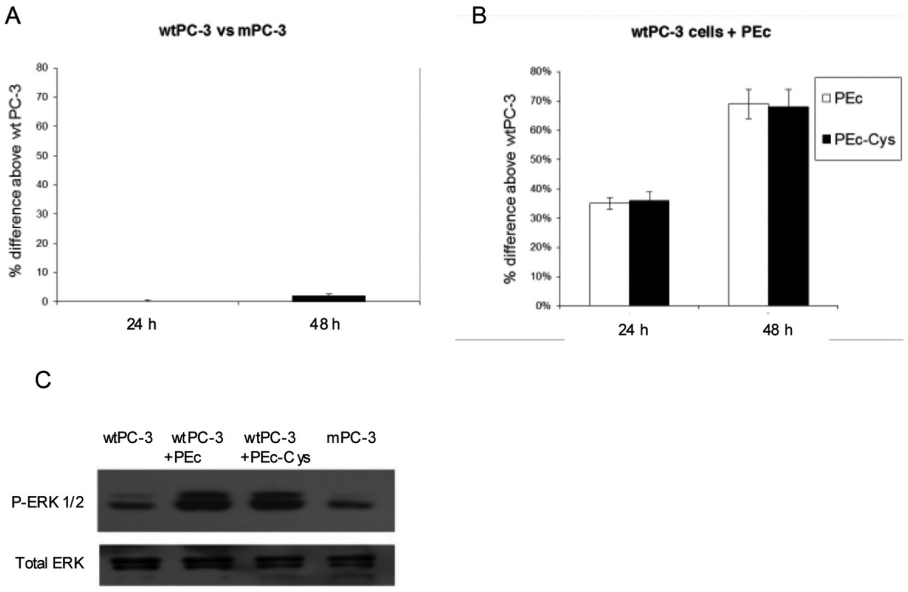
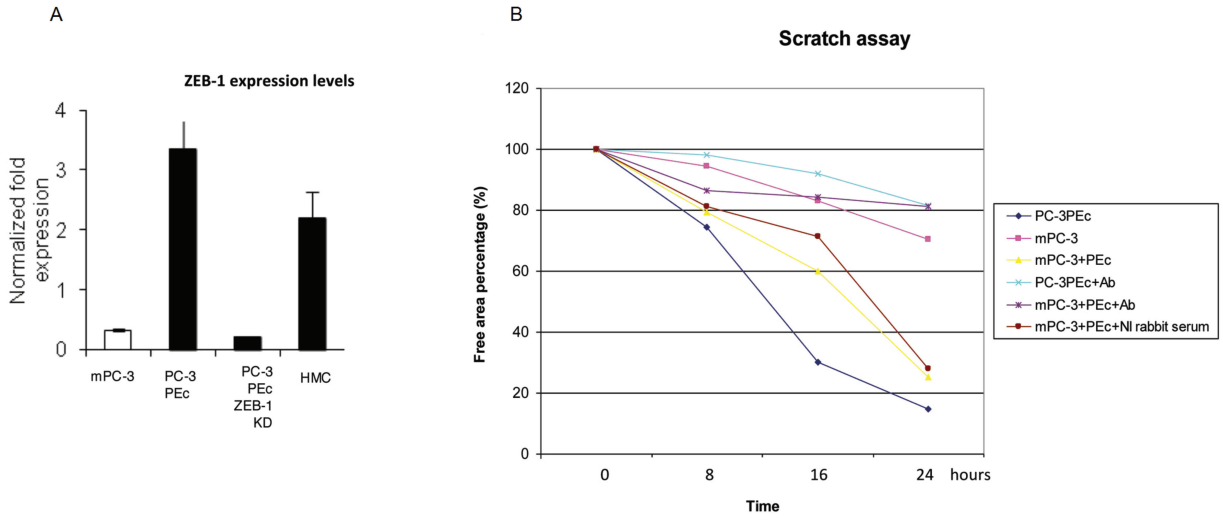


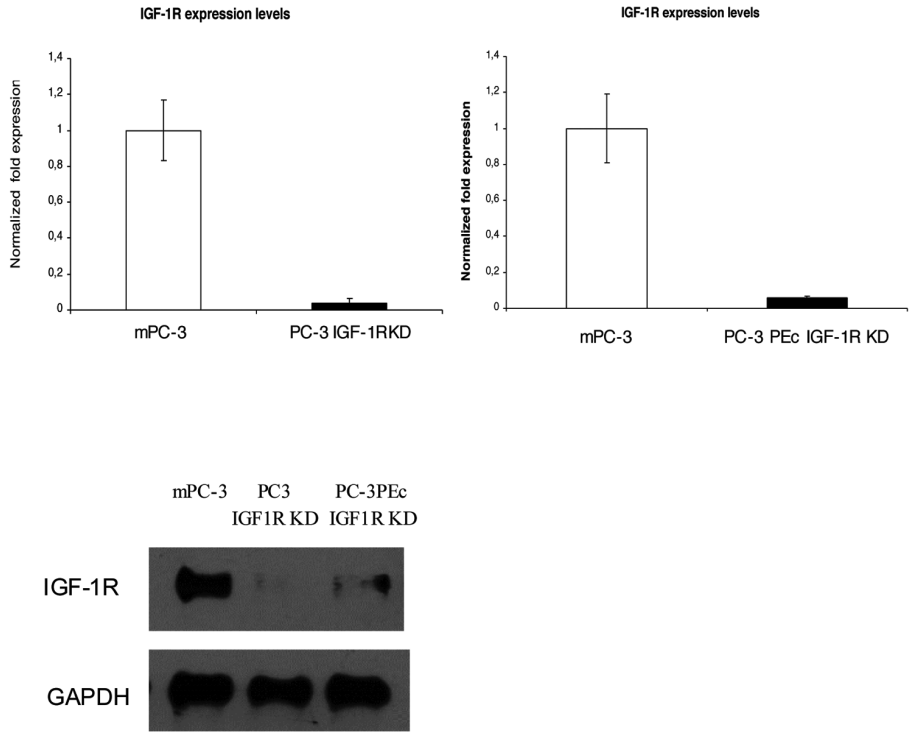
Supplementary Figure S1. Protein analysis of the mPC-3 cells and PC-3PEc cells extracts using Multiple Reaction Monitoring (MRM). Very low levels of the PEc specific tryptic peptides YQPPSTNK and GSTFEER, were detected in the mPC-3 protein samples (sample 1). Significant amounts of these tryptic products were detected in the PC-3PEc samples (sample 2). These products were similar to those obtained after tryptic digest of the control synthetic PEc (sample 3); (retention time vs relative intensity of the detected transitions). (n=3).



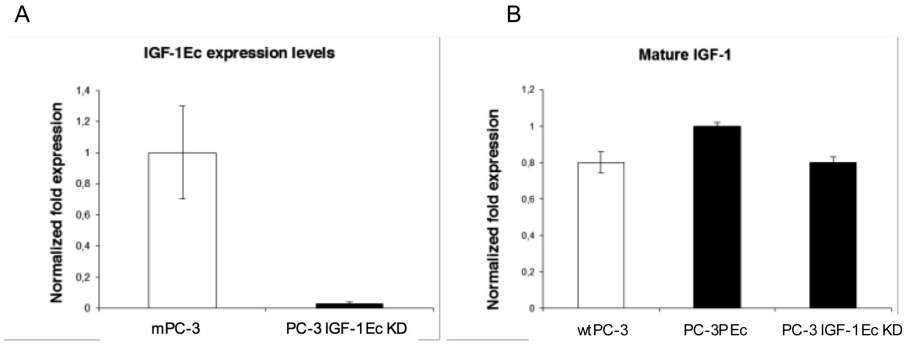
Supplementary Figure S2. Characterization of the mock cell lines. (A) Cell proliferation analysis of wtPC-3 cells vs mock PC-3 cells (mPC-3) by trypan blue exclusion assay. No statistically significant difference was observed in respect to proliferation of these two cell lines at 24 and 48 h. (Student *t* test, n =4). (B) Analysis of the effects of the synthetic Ec peptide and Ec-Cys peptide (17nM in each case) on wtPC-3 cellular proliferation by trypan blue exclusion assay. Both peptides induced similarly the proliferation of wtPC-3 cells. (C) Analysis of the effects of the synthetic Ec peptide and Ec-Cys peptide (17nM in each case) on wtPC-3 in ERK 1/2 phosphorylation by western blot analysis. No statistically significant difference was observed on the peptides' effects on wtPC-3 between the two peptides examined (Student *t* test, n=4). The ERK 1/2 phosphorylation was also examined by western blot analysis in the PC-3 cells after stable transfection with the mock vector (mPC-3). We did not observe a significant difference in ERK 1/2 phoshorylation between wtPC-3 and mPC-3 cells.



Supplementary Figure S3. (A) ZEB-1 expression in PC-3PEc cells and silencing of ZEB-1 expression. PC-3PEc cells presented significantly elevated ZEB-1 mRNA levels compared to that produced by mPC-3 cells. A significant decrease of ZEB-1 expression was observed in PC-3PEc ZEB-1KD cells compared to the PC-3PEc cells as assessed by qRT-PCR (Student *t* test, $P < 0.001$, n=3). The human mesenchymal cells (HMC) were used as positive control. (B) Wound healing assay analysis suggested that the PC-3PEc cells and mPC-3 cells when exogenously treated with PEc present significant difference in migration at 24 h when compared with the untreated mPC-3 or when the anti-PEc antibody was introduced either in PC-3PEc cells or in addition to PEc in mPC-3 cells, ($p<0.005$ in every case, n=3). The analysis was obtained by the Tscratch programme (CSF, Laboratory, Swiss Federal Institute of Technology, Zurich, Switzerland).



Supplementary Figure S4. Determination of the efficiency of the silencing of the IGF-1R expression by qRT-PCR and western blot analysis, in PC-3PEc and in wtPC-3 cells respectively. In both cases the silencing efficiency of the IGF-1R was >90%.



Supplementary Figure S5. (A) Determination of the efficiency of the IGF-1Ec silencing in the PC-3 IGF-1Ec KD mutants by qRT-PCR. The silencing of the IGF-1Ec isoform was >90%. (B) Characterization of the expression of the mature IGF-1 by qRT-PCR in the PC-3PEc and PC-3 IGF-1Ec KD cells using qRT-PCR in comparison to the wtPC3. IGF-1Ec silencing and PEc overexpression in the PC-3 cell lines did not alter the IGF-1 mRNA expression. No statistically significant difference was observed in the expression of the mature IGF-1 between the cell lines examined.