Supplementary Figure 1. Effect of IGF on prostate cancer cell lines. A Proliferation of prostate cancer cell lines in response to IGF-I. Proliferation of Du-145 cells was stimulated upon IGF treatment with a maximum at a concentration of 10 ng/ml. Higher IGF concentrations did not increase the proliferative response of these cells, suggesting saturation or a negative feedback regulation. The PTEN negative cell lines PC3 and LNCaP were unresponsive to IGF. For this experiment LNCaP cells were short-term serum depleted and Du145 and PC3 cells were cultured in serum-free medium with reduced insulin concentrations. Treatment with various concentrations of IGF-I was performed for 48h and new DNA synthesis was determined by measurement of $^3$H-thymidine incorporation. B, C The IGF signal is abrogated by an IGF IR blocking antibody (MAB 391) and an inhibitor of the PI3K-Akt pathway (LY294002). B MAB 391 and the PI3K-Akt pathway inhibitor are equally effective to inhibit cell proliferation stimulated by IGF-I in Du-145 cells. C IGF induced AKT phosphorylation can be reduced by MAB391 by about 70%, whereas it is completely blocked by LY294002 and not influenced by the MAP-kinase ERK inhibitor PD98059. Activity of the PI3k-Akt pathway was determined indirectly by quantification of Akt phosphorylation by Western Blot analysis after stimulation with IGF-I for 15 minutes, with or without preincubation with one of the inhibitors. This experiment confirms that the antibody used as a control in adhesion, motility and invasion experiments to block the IGF receptor abrogates IGF signaling. LY294002; chemical inhibitor of PI3 kinase and consequently of the AKT pathway; c 20µM. PD98059; chemical inhibitor of ERK used as control; c 20µM.