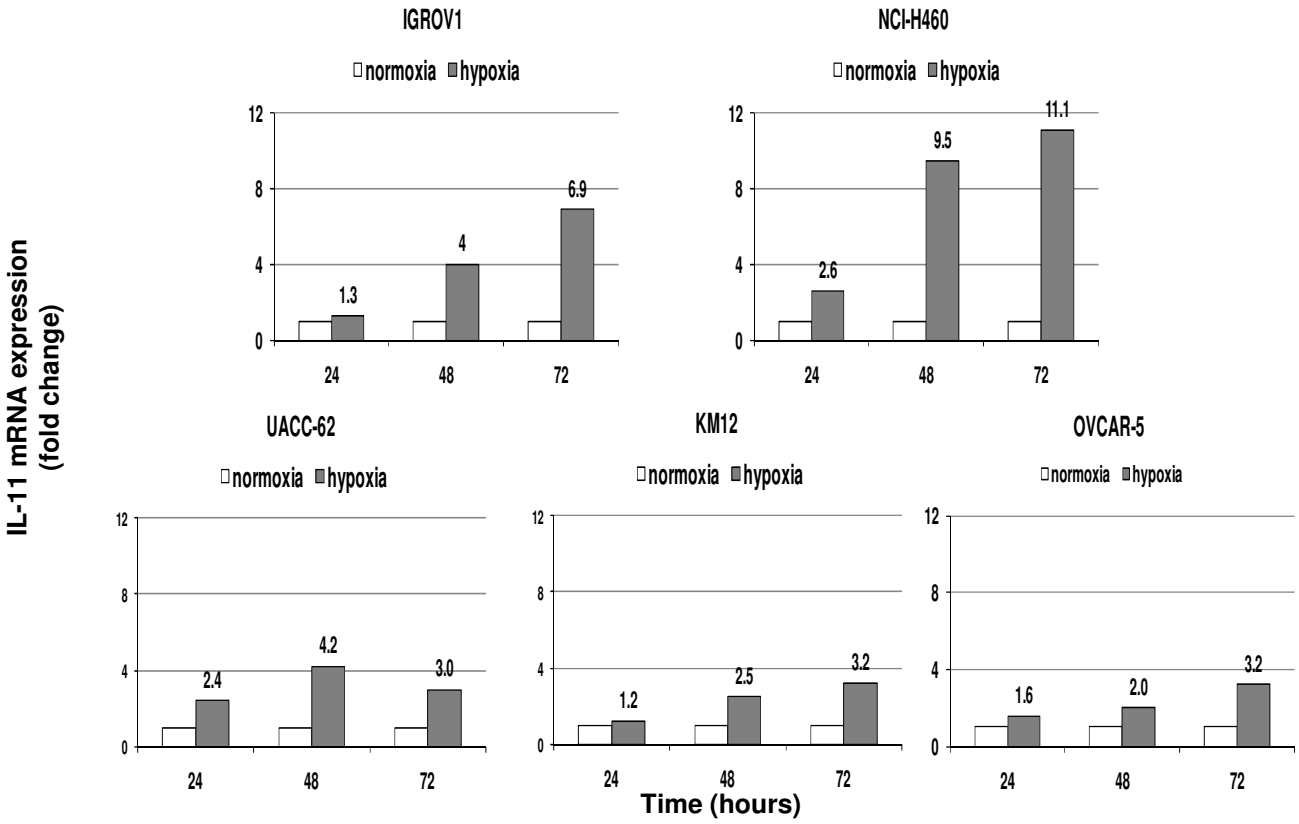
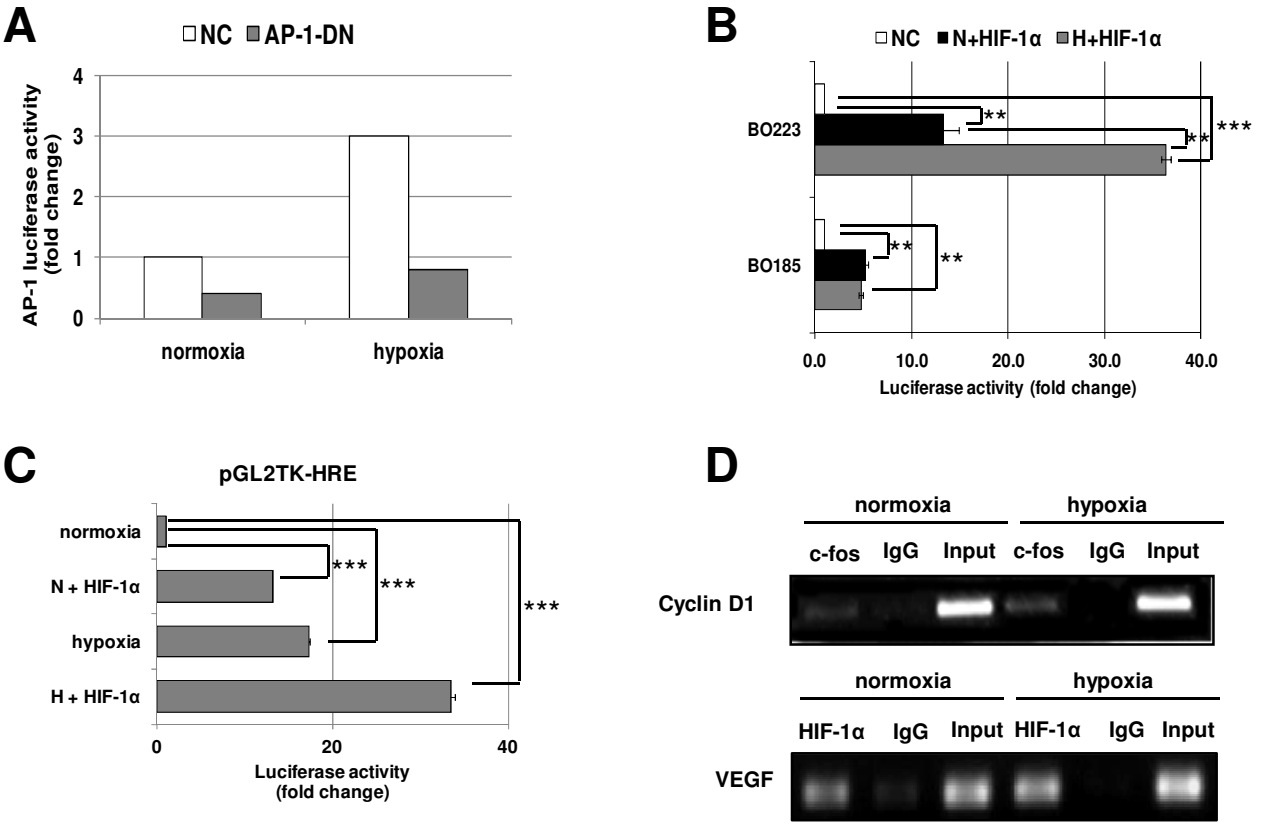


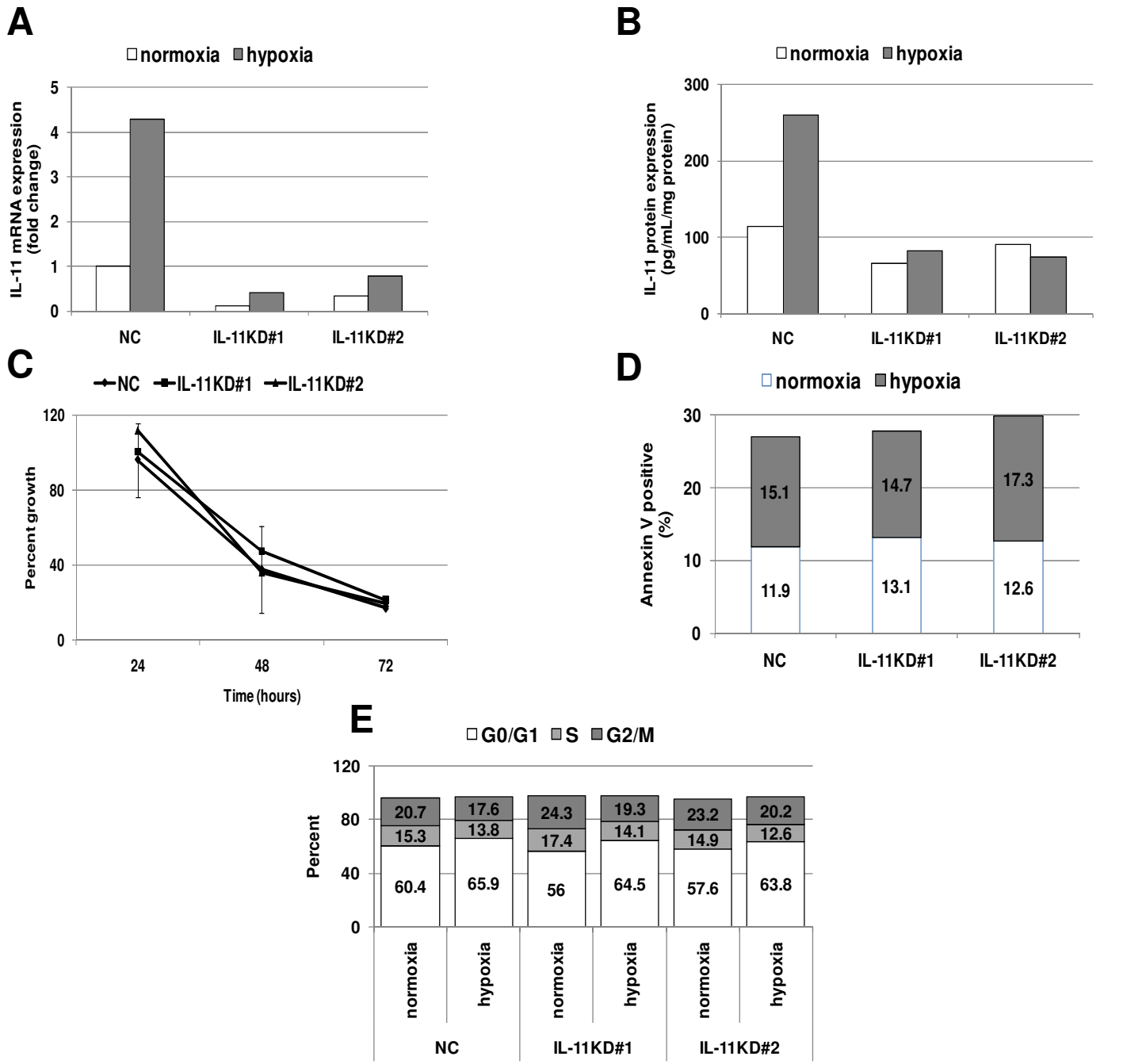
Suppl. Fig. 1. Prolonged hypoxia promotes a malignant phenotype in HCT-116. Cells were cultured under normoxic or hypoxic conditions for the indicated times and IL-11 mRNA levels were measured by QRT-PCR. The results are expressed as fold increase relative to normoxic controls.

Supplementary figure 1



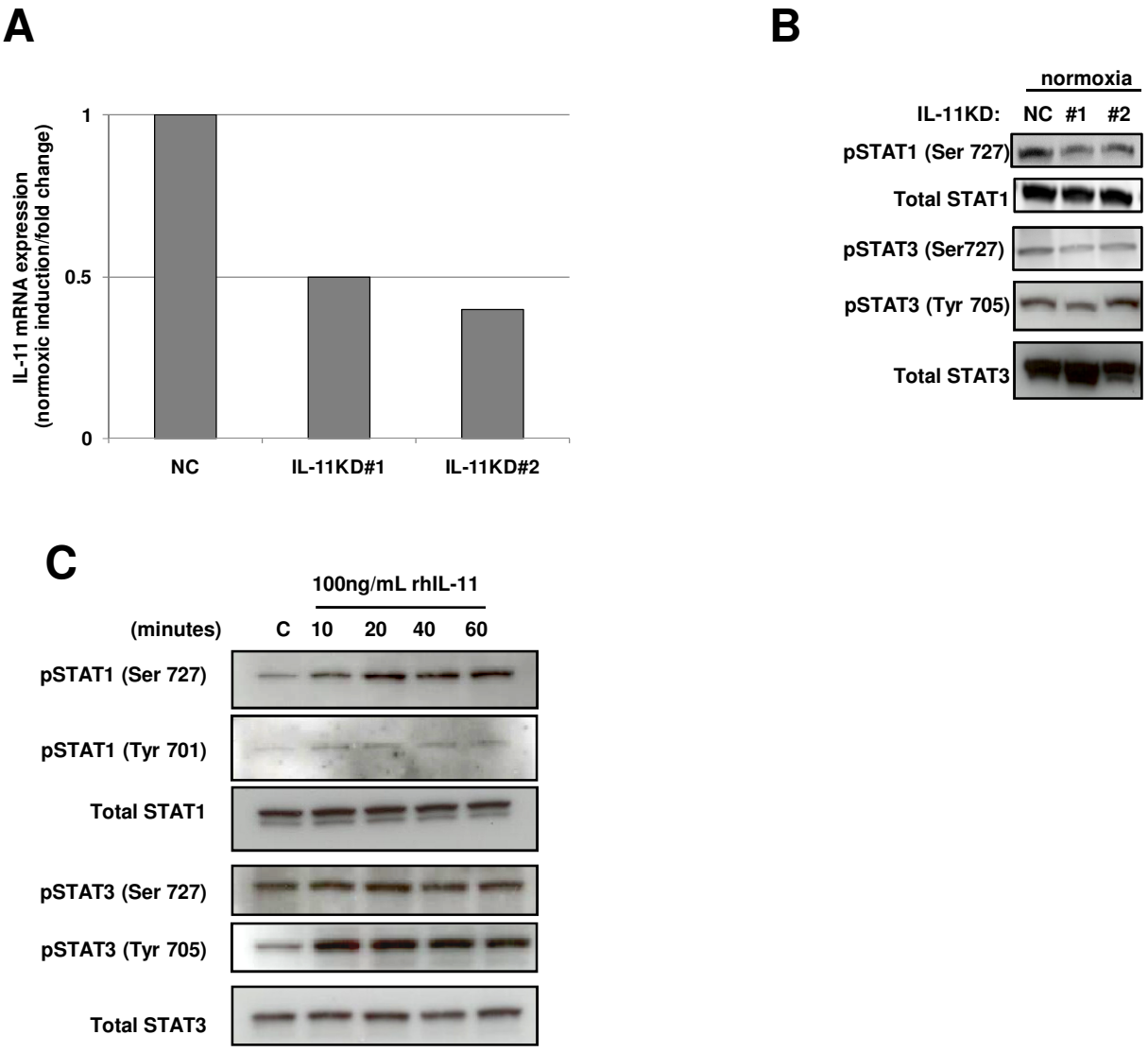
Suppl. Fig. 2. Activation of IL-11 promoter by HIF-1α co-transfection. A. PC3 cells were transfected with either an irrelevant plasmid DNA (NC) or with an AP-1-DN expression vector. Twenty-four hours after transfection cells were cultured under normoxic or hypoxic conditions for an additional 24 hours and then harvested to assess luciferase activity. Results are expressed as hypoxic induction relative to normoxic PC3 cells. **B.** PC3 cells were transfected with either the BO223 IL-11 promoter construct or the BO185 and co-transfected with irrelevant plasmid DNA (NC) or with a constitutively active form of HIF-1α .Twenty-four hours after transfection cells were cultured under normoxic or hypoxic conditions for an additional 24 hours and then harvested to assess luciferase activity. Results are expressed as a fold increase relative to the corresponding normoxic control (mean ±SE of three independent experiments; t-Test, ** indicates $p<0.001$, *** indicates $p<0.0001$). **C.** PC3 cells were transfected with pGL2TK-HRE plasmid, which contains three copies of a canonical hypoxia responsive element upstream or the luciferase reporter gene and co-transfected with either irrelevant plasmid DNA or with a constitutively active form of HIF-1α .Twenty-four hours after transfection cells were cultured , harvested and assessed as described in panel B (mean ±SE of three independent experiments; t-Test, *** indicates $p<0.0001$). **D.** PC3 cells were cultured for 24 hours in normoxia or hypoxia and ChIP assay was conducted as described in *Materials and Methods* using an anti-HIF-1α, anti-fos or isotype-matched control antibody (IgG). Primer sequences for cyclin D1 and VEGF are listed in table 3 and PCR fragments of 163bp from cyclin D1 promoter and 135bp from VEGF promoter are shown (n=2).

Supplementary figure 2



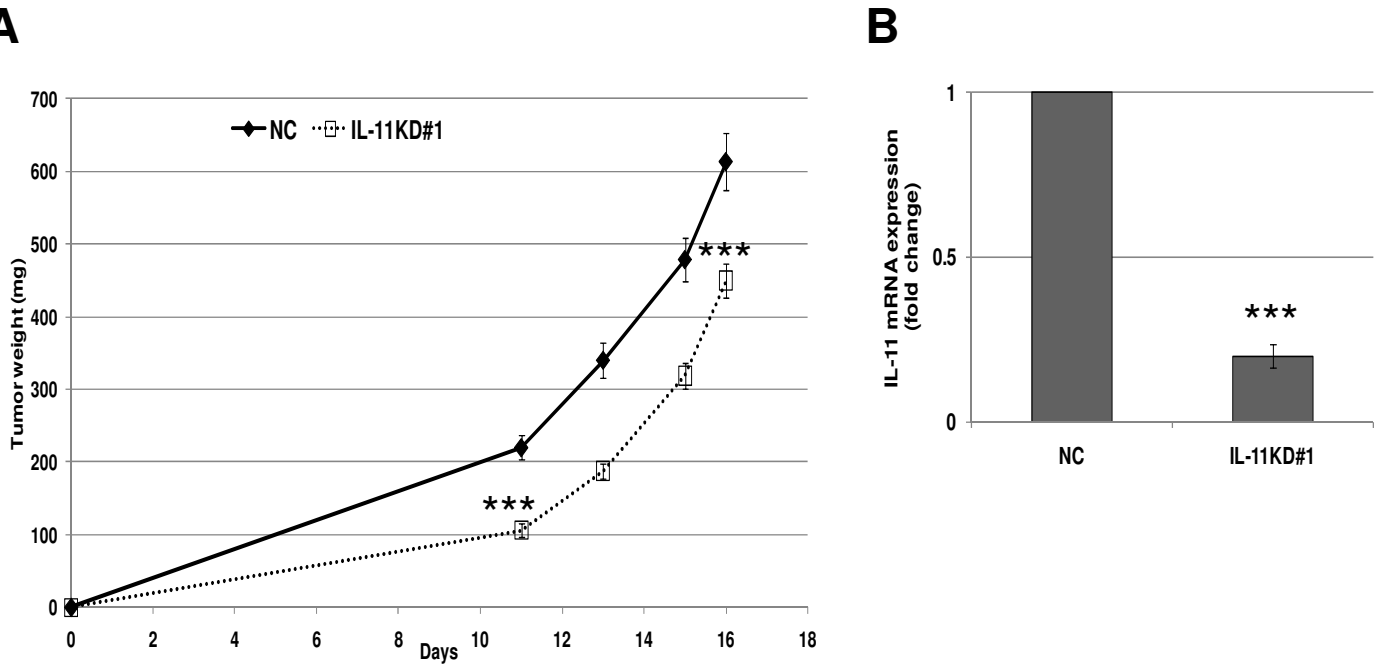
Suppl. Fig. 3. Hypoxic induction of IL-11 does not affect cell proliferation, apoptosis or cell cycle *in vitro*. **A.** PC3-NC, IL-11KD#1 and IL-11KD#2 were generated using lentiviral vectors expressing the corresponding shRNA. Levels of IL-11 mRNA expression was tested by QRT-PCR in cells cultured under normoxic or hypoxic conditions for 24 hours. Results are expressed as fold increase relative to normoxic PC3-NC and are representative of three independent experiments performed. **B.** PC3 cells were cultured under normoxic or hypoxic conditions for 48 hours and levels of IL-11 protein were measured in supernatants using a commercially available ELISA kit. Results are presented as fold increase relative to IL-11 protein levels detected in PC3-NC cells and were normalized for total protein content (n=2). **C.** PC3-NC, IL-11KD#1 and IL-11KD#2 cells were cultured in hypoxia for the indicated times and cellular proliferation was assessed as described in *Materials and Methods*. Hypoxic samples were compared to PC3-NC normoxic samples and expressed as an average percent growth value ±SE of three independent experiments. **D.** PC3 cells were cultured under normoxic or hypoxic conditions for 24 hours and stained with Annexin V to evaluate the percentage of apoptotic cells with flow cytometric analysis. The percentage of Annexin V positive cells reported in the graph are relative to the gated population. **E.** PC3 cells were cultured as described in panel C and then stained as indicated in *Material and Methods* to evaluate the cell cycle distribution of a gated population.

Supplementary figure 3



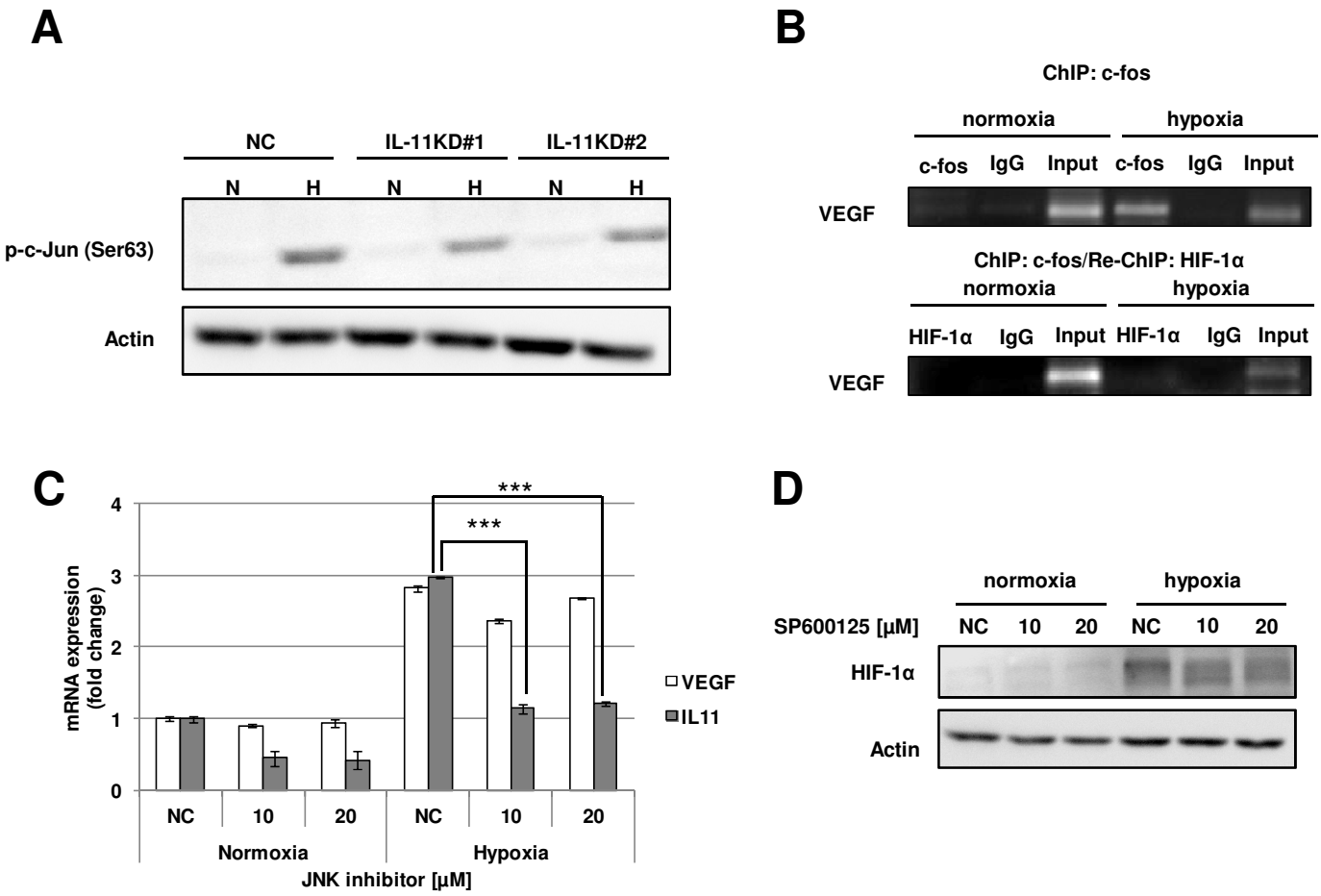
Suppl. Fig. 4. Phenotype and signaling effects in IL-11 knockdown RCC4 cells. A RCC4-NC, IL-11KD#1 and IL-11KD#2 were generated using lentiviral vectors expressing the corresponding shRNA. Levels of IL-11 mRNA expression were tested by QRT-PCR in cells cultured under normoxic or hypoxic conditions for 24 hours. Results are expressed as fold increase relative to normoxic RCC4-NC. **B.** RCC4-NC, IL-11KD#1 and IL-11KD#2 were serum starved for 24 hours and cultured for an additional 48 hours under normoxic or hypoxic conditions. Levels of phosphorylated STAT1 (Ser727) and STAT3 (Ser 727 and Tyr705) were assessed by Western blot. Total STAT1 and STAT3 were assessed as an internal control. **C.** RCC4 cells were serum starved for 24 hours and then incubated in normoxia for the indicated times in the presence of 100ng/mL of human recombinant IL-11. Levels of phosphorylated STAT1 (Ser727) and STAT3 (Ser 727 and Tyr705) were assessed by Western blot. This is a representative of two independent experiments performed.

Supplementary figure 4



Suppl. Fig. 5. IL-11 knockdown impairs the growth of PC3 xenografts. A. PC3-NC and IL-11KD#1 were injected s.c. into nude mice (26 mice/group) and tumor weight was measured as described in *Materials and Methods*. A statistically significant difference of tumor weight in mice injected with IL-11KD#1 compared to PC3-NC was observed (t-Test, *** indicates $p < 0.0001$). **B.** IL-11 mRNA levels were measured by QRT-PCR in tumor tissue harvested on day 16. Results are presented as mean ± SE (10 mice/group), *** represent a statistical difference relative to PC3-NC tumors ($p<0.0001$).

Supplementary figure 5



Suppl. Fig. 6. Hypoxic activation of c-Jun pathway. A. PC3-NC, IL-11KD#1 and IL-11KD#2 cells were serum starved for 24 hours and then cultured under normoxic (N) or hypoxic (H) conditions for an additional 48 hours. Total cell lysates were prepared as described in *Materials and Methods* and immunoblotting was conducted using a polyclonal phosphorylated c-Jun antibody. Actin was assessed as an internal control. **B.** PC3 cells were cultured for 24 hours in normoxia or hypoxia and ChIP assay was conducted as described in *Materials and Methods* using an anti-HIF-1α, anti-fos or isotype-matched control antibody (IgG). Re-ChIP protocol was conducted on anti-fos samples using anti-HIF-1α antibody. Primer sequences for VEGF containing AP1 site are in table 3 (n=2). **C.** PC3 were pretreated with 10μM or 20μM SP600125, a JNK inhibitor and then cultured under normoxic or hypoxic conditions for 24 hours. IL-11 and VEGF mRNA levels were measured by QRT-PCR. The results are expressed as fold increase relative to normoxic control (SE of three data points; t-Test, *** indicates $p < 0.0001$). **D.** PC3 cells were treated as described above with SP600125 for 24 hours. Total cell lysates were prepared as described in *Materials and Methods* and immunoblotting was conducted using a HIF-1α antibody. Actin was assessed as an internal control.

Supplementary figure 6