Figure S1. Reverse transcription-quantitative PCR of ATF4, HIF1α and HIF2α KD validations. Negative controls for these experiments were PANC-1 cells exposed to 0.2% oxygen treated with transfection reagent with no KD siRNAs. Knockdowns of these three genes using siRNA confirm efficient KD of genes in hypoxia compared with the hypoxic negative controls. ATF4 mRNA expression after KD is comparable to ATF4 mRNA levels in normoxia because there is lower ATF4 expression in normoxia. HIF1α and HIF2α siRNA KD show no cross-reactivity between the two genes despite high sequence homology of ~50%, and exhibit high specificity. **P<0.01 and ****P<0.0001. ATF4, activating transcription factor 4; HIF, hypoxia-inducible factor; siRNA, small interfering RNA; KD, knockdown.

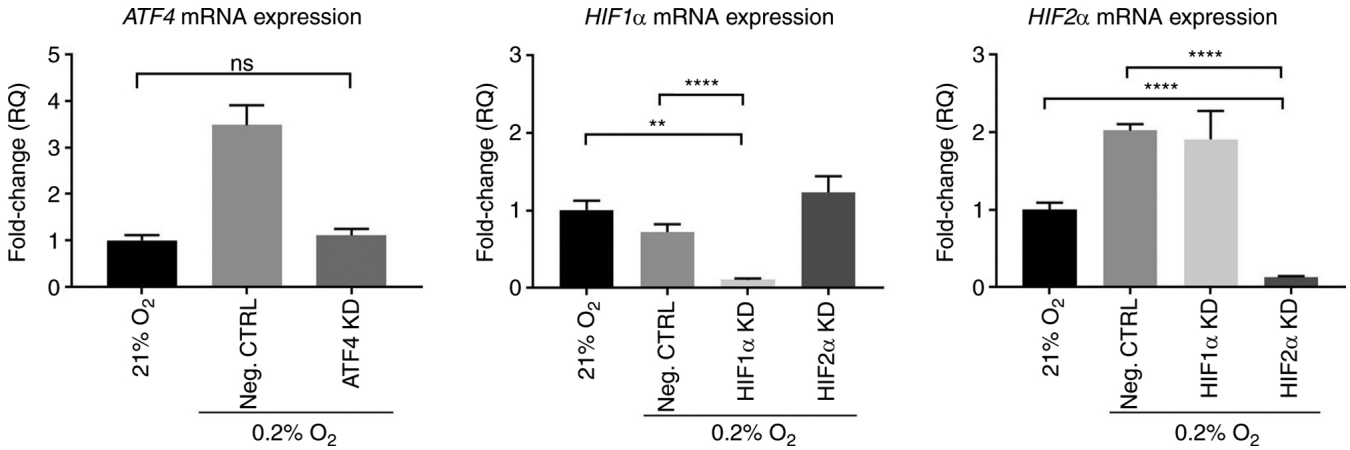

Figure S2. ATF4 and HIF1α inhibition in acute or chronic hypoxia does not affect cell viability compared with its normoxic controls. The negative control were PANC-1 cells exposed to 0.2% oxygen with transfection reagent but no KD small interfering RNA. (A and B) PANC-1 cells were transiently transfected and seeded into 96-well plates and incubated in hypoxia or normoxia for (A) 16 or (B) 48 h. Cell viability was measured by CellTiter Glo luminescence. Error bars represent the mean ± SD for n=5. The RLU was analyzed by two-way ANOVA followed by Bonferroni statistical hypothesis test. $^{*}P<0.05$ and $^{**}P\leq0.0052$. ATF4, activating transcription factor 4; HIF, hypoxia-inducible factor; KD, knockdown; RLU, relative luminescence unit.

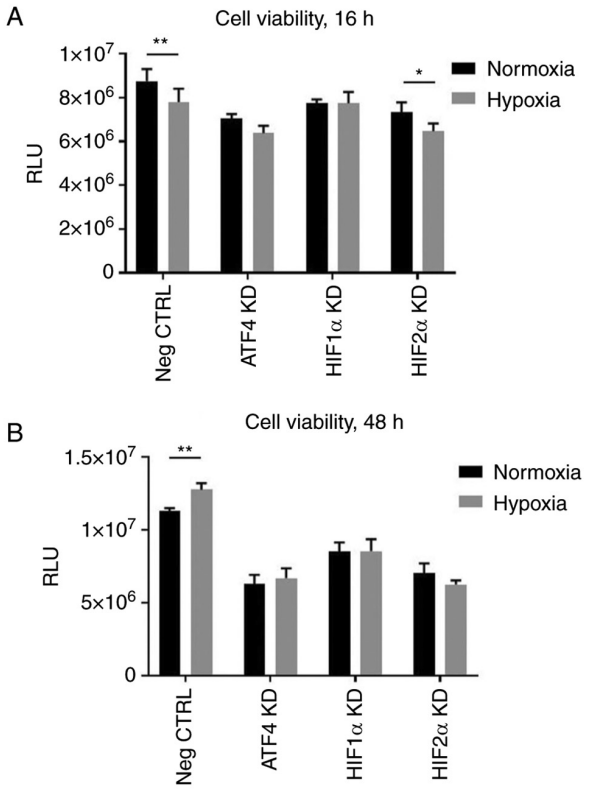

Figure S3. HIF1α and HIF2α KD ablate colony formation abilities in PANC-1 cells in normoxia, acute and chronic hypoxia. Colony formation assays were performed by transiently transfecting PANC-1 cells and seeded into plates. The plates were placed in either normoxia or hypoxia for 16 or 48 h. The cells were fixed, stained and colonies were counted. HIF, hypoxia-inducible factor; KD, knockdown.

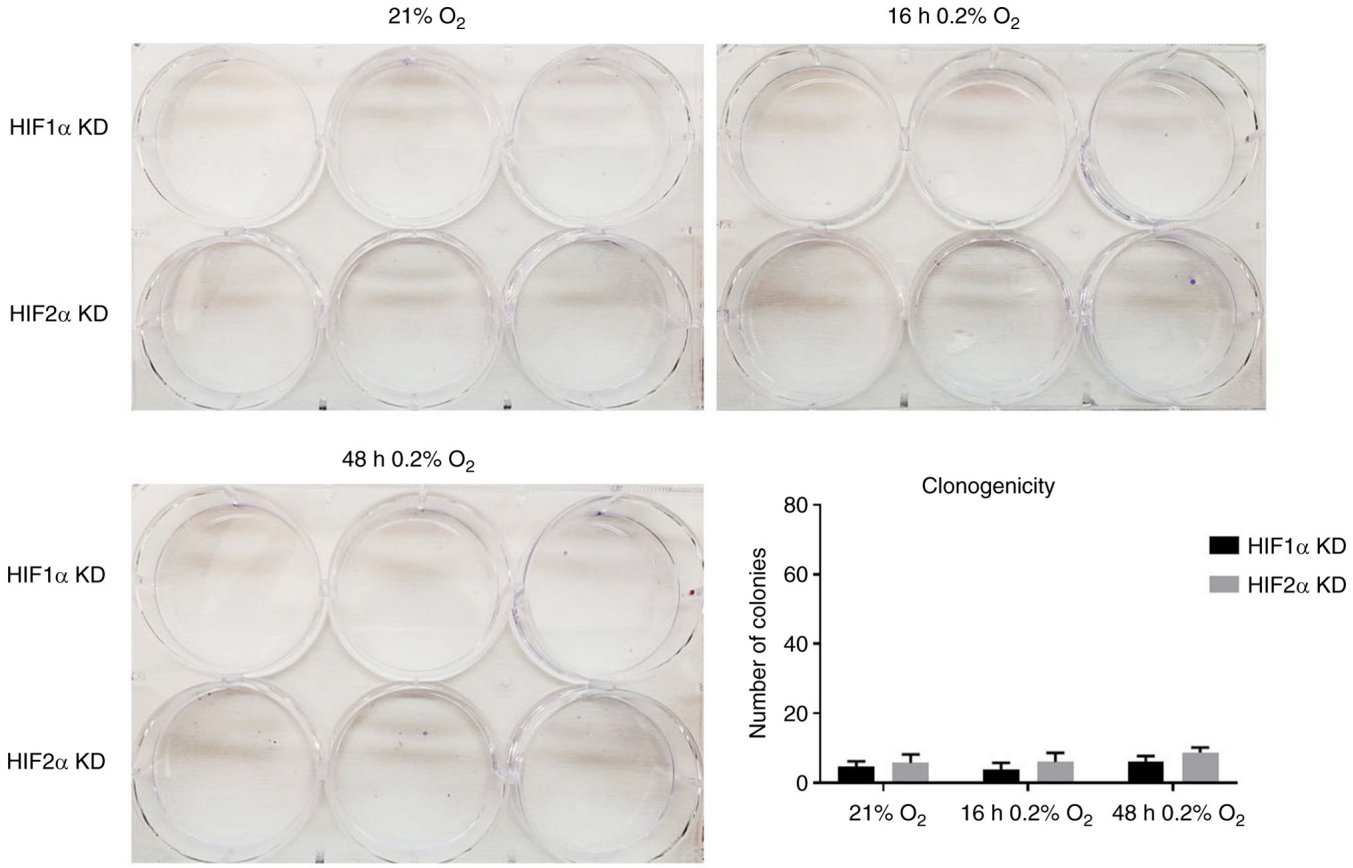

Figure S4. TGF-β increases ATF4 expression and improves colony formation in acute hypoxia but not chronic hypoxia. PANC-1 cells were transiently transfected with either vehicle or ATF4 siRNA and incubated for 24 h in normoxia. The cells were supplemented with 10 ng/ml TGF-β and placed in hypoxia (0.2% oxygen) or normoxia (21% oxygen) for 16 or 48 h. (A) Western blot analysis was conducted to determine the protein expression levels of HIF1α after 16 or 48 h of hypoxia. (B) For colony formation assay, the PANC-1 cells were transiently transfected and incubated for 24 h. TGF-β was added and the plates were incubated in normoxia, 16 or 48 h of hypoxia. ****P<0.0001. ATF4, activating transcription factor 4; KD, knockdown.