Supplementary Information

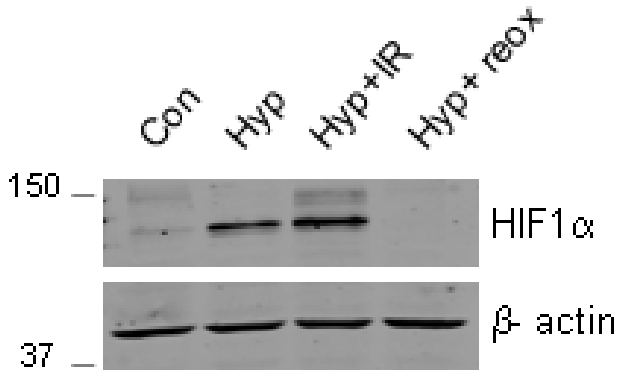

Figure S1 **Validation of methodology for irradiation in hypoxia.** RKO cells were exposed to either normoxia (Con) or hypoxia 2% O_2 for 16 hours and then treated as indicated: Con - cells harvested in normoxia; Hyp - cells harvested in hypoxia; Hyp + IR – cells placed in hypoxia box, transported to irradiator where they received a 4Gy dose and harvested 1 hour post irradiation; Hyp + Reox - Cells reoxygenated for one hour and then harvested. The sustained level of HIF-1α in the hypoxia/irradiated sample indicates that no reoxygenation took place during the irradiation process using the hypoxia box.

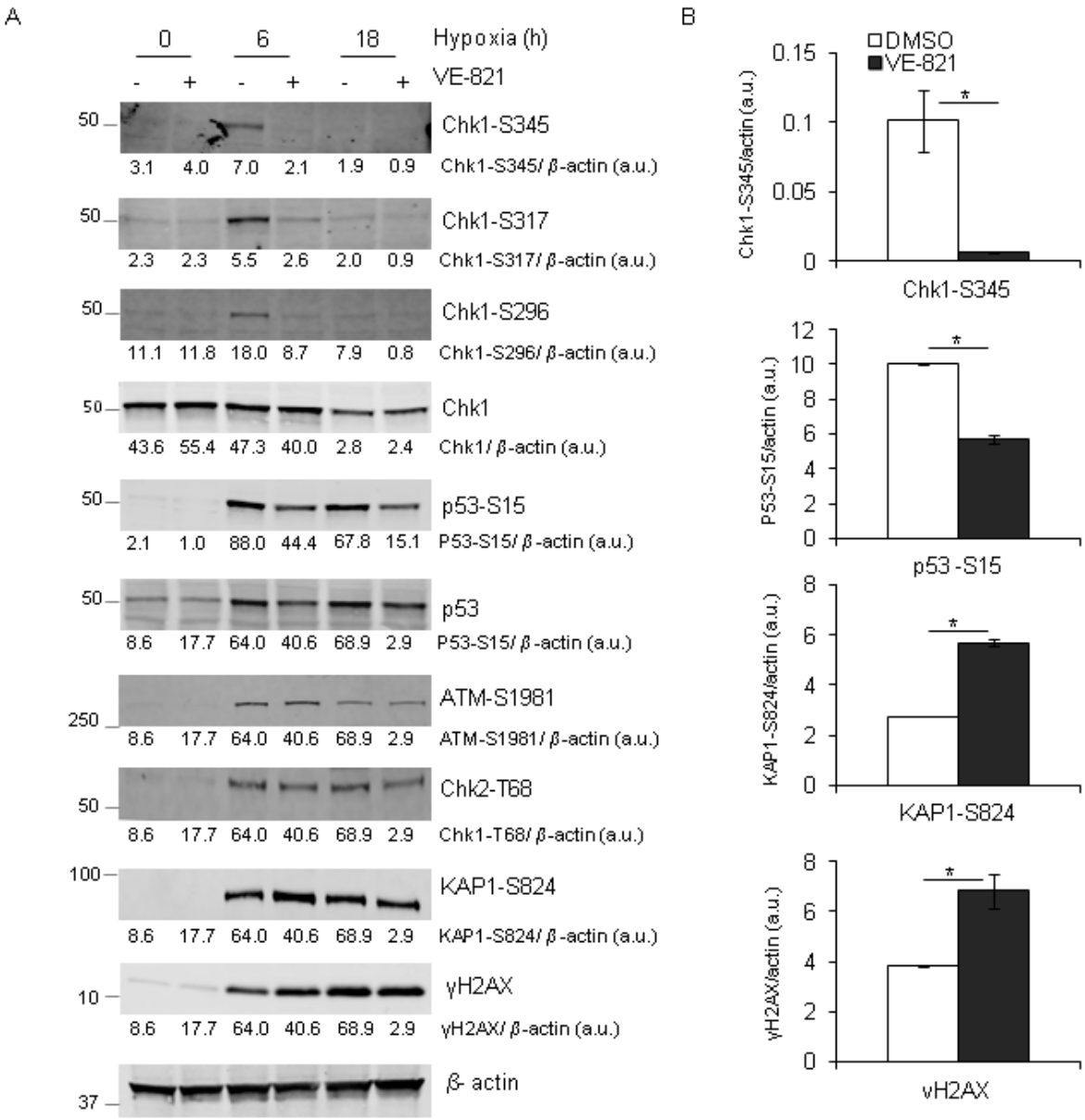

Figure S2 **Quantification of the effects of ATR inhibition of the severe hypoxic DDR kinetics.** RKO cells were exposed to ≤0.02% O_2 for the times indicated in the presence of DMSO/1 μM VE-821. (A) Semi-quantitative analysis of Western blots from Fig.3A (a.u. = arbitrary units of fold increase relative to β-actin). Representative blots of n=3 experiments are shown. (B) Statistical analysis of the difference between the means of Chk1-S345, p53-S15, KAP1-S824 and γH2AX for DMSO vs. VE-821. Significance values: * $p<0.005$.

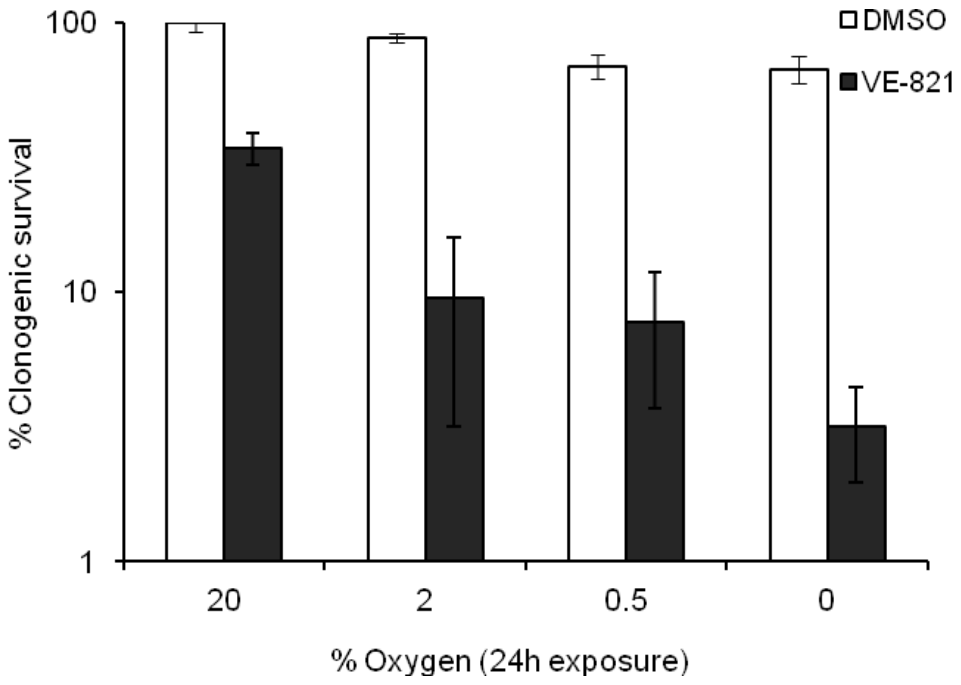

Figure S3 **VE-821 sensitises MDA-MB-231 cells to hypoxia.** Clonogenic assays were carried out using MDA-MB-231 cells in the presence of DMSO/1 μM VE-821. Cells were exposed to 20%, 2% 0.5% and ≤0.02% O_2 for 24h and grown for 10 days. Graph depicts the sensitisation effect observed normalised to control (20% DMSO).

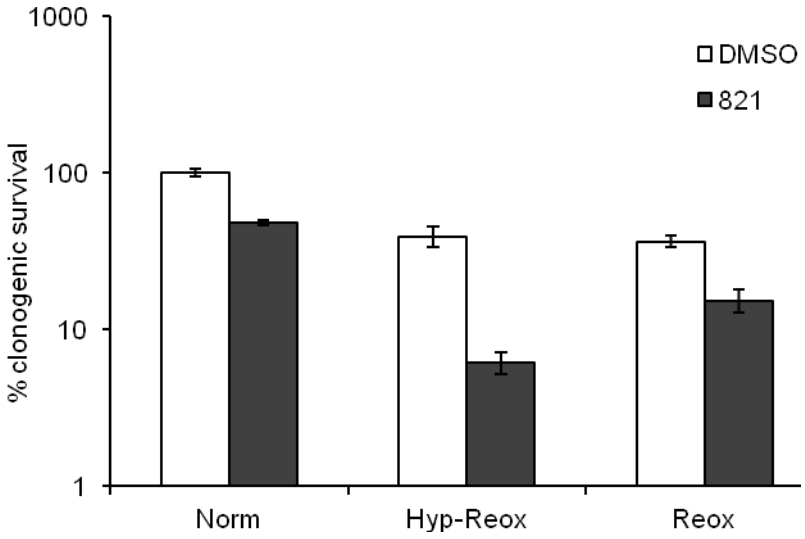

Figure S4 **Contribution of reoxygenation to the VE-821 dependent sensitisation to moderate hypoxia/reoxygenation.** Clonogenic assays were carried out using RKO cells in the presence of DMSO/1 μM VE-821. DMSO/VE-821 were present in the media throughout the experiment in normoxia (Norm), in hypoxia/reoxygenation (Hyp-Reox - 24h at 2% O_2 followed by reoxygenation) or added just after reoxygenation (Reox). Colonies were then grown for 10 days.

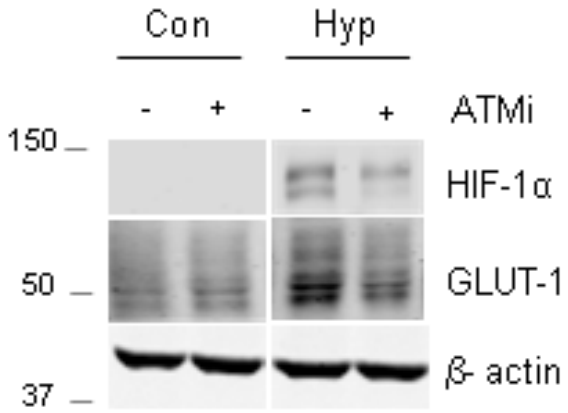

Figure S5 **ATM inhibition leads to reduced HIF1 levels.** RKO were treated with DMSO or 10 μM ATMi and exposed to normoxia (Con) or 2% O_2 (Hyp) for 6h. Western blotting was carried out with the antibodies indicated. The levels of β−actin are shown as a loading control.

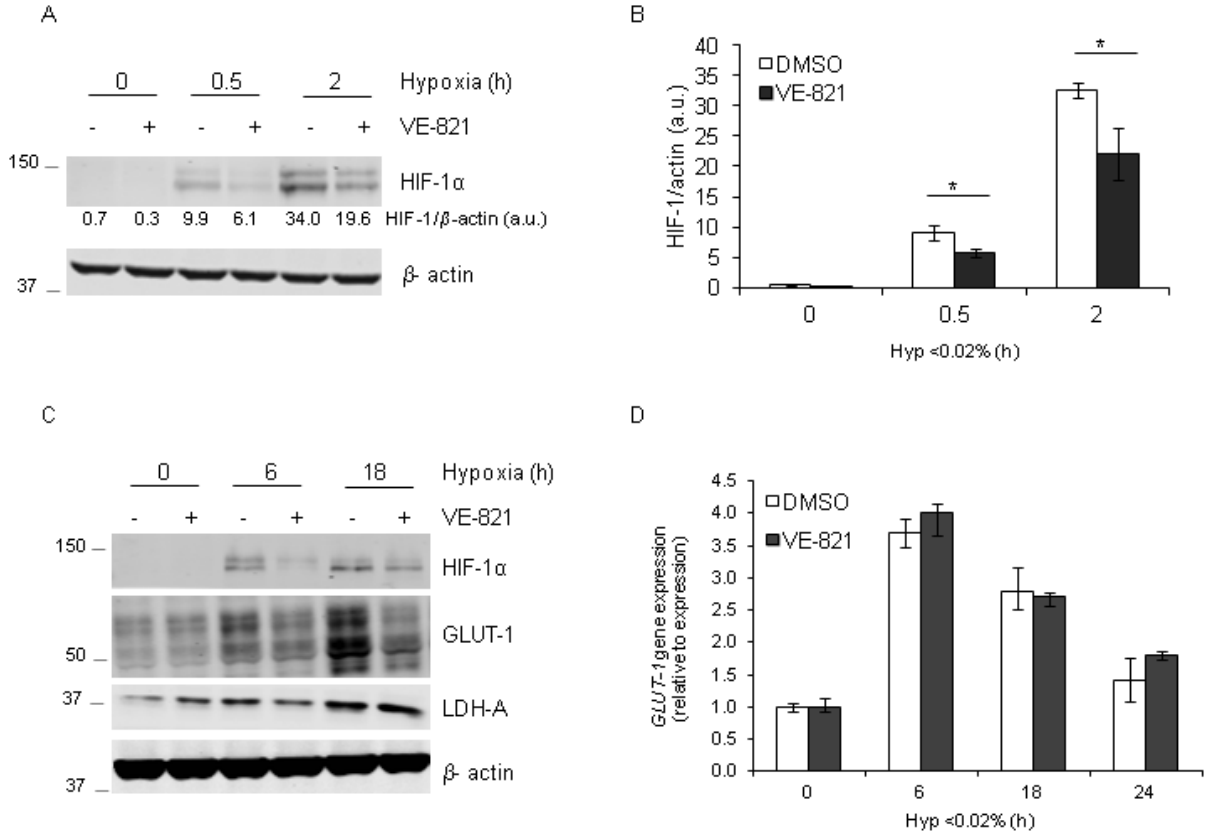

Figure S6 **VE-821 inhibition of HIF signalling is transient at the transcript level but not at the protein level.** RKO cells were exposed to ≤0.02% O_2 for the times indicated in the presence of DMSO/1 μM VE-821. (A) Semi-quantitative analysis of Western blots from Fig.6A (a.u. = arbitrary units of fold increase relative to β-actin). (B) Statistical analysis of the difference between the means of HIF1-α for DMSO vs. VE-821 at the indicated times. Significance values: * $p<0.05$. (C) Western blotting was carried out for HIF-1α, GLUT-1, LDH-A and β-actin (loading control). Representative blots of n=3 experiments are shown. (D) The levels of *GLUT-1* mRNA were determined by qRT-PCR. *18S* was used as the control.

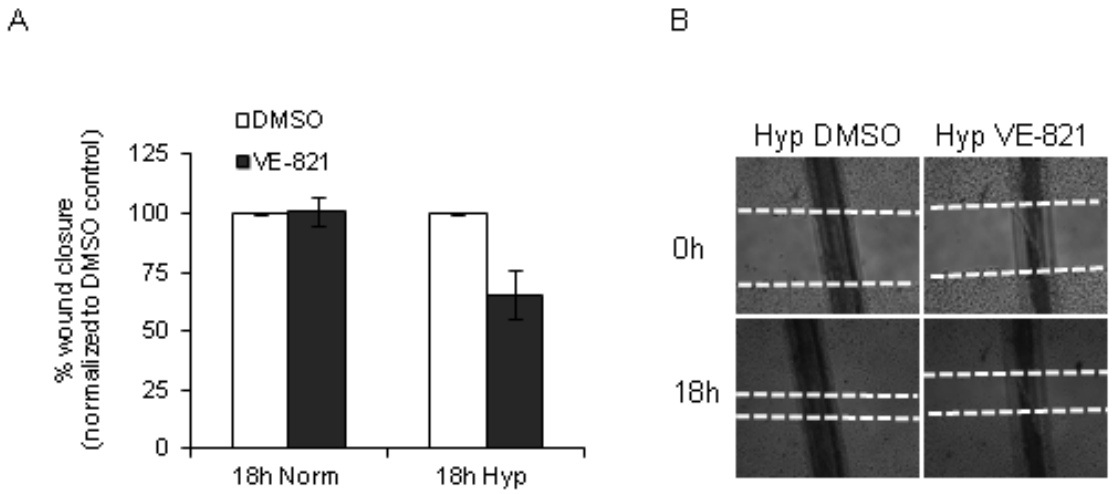

Figure S7 **Effect of VE-821 on cell motility in normoxic vs. hypoxic conditions.** MDA-MB-231 cells were treated with DMSO/1 μM VE-821 and scratch would assays were performed. (A) The graph represents the percentage of wound closure after 18h normoxia/hypoxia (≤0.02% O_2). (B) Representative scratch wounds.