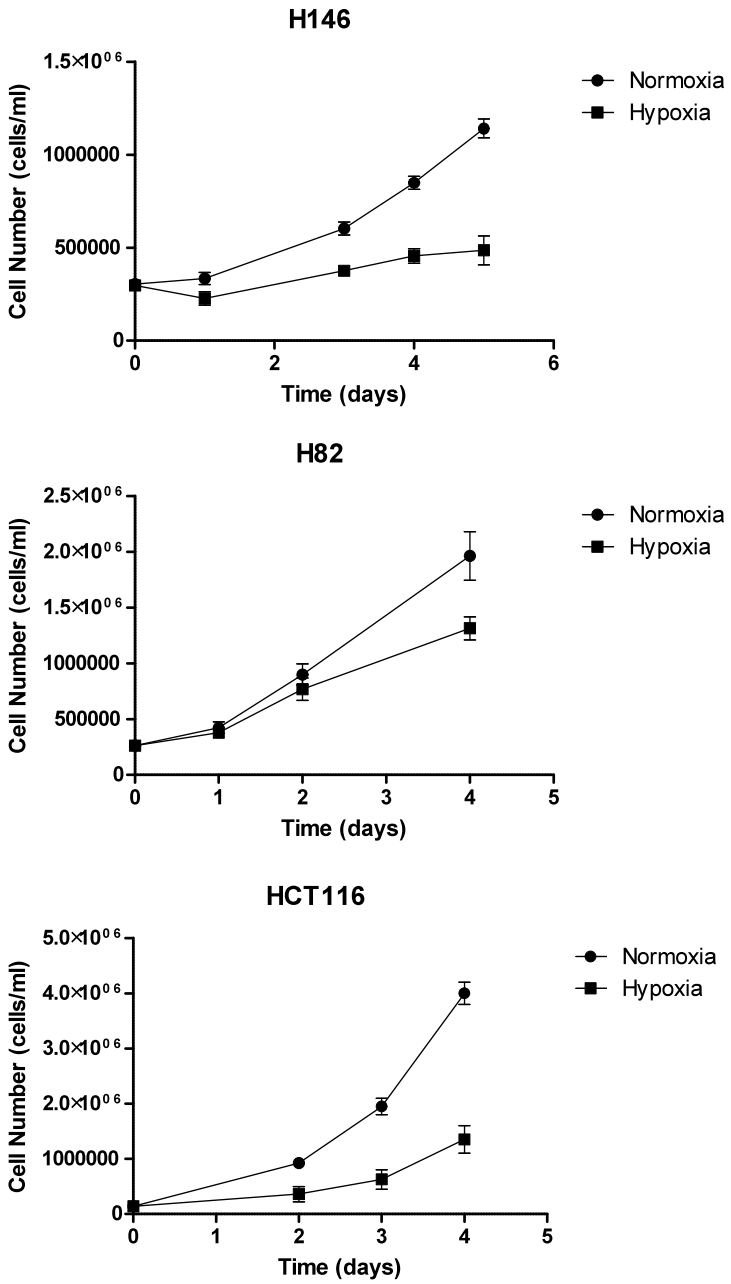
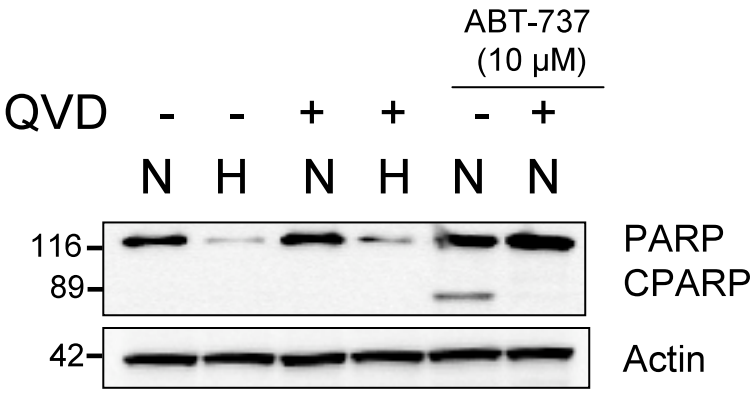


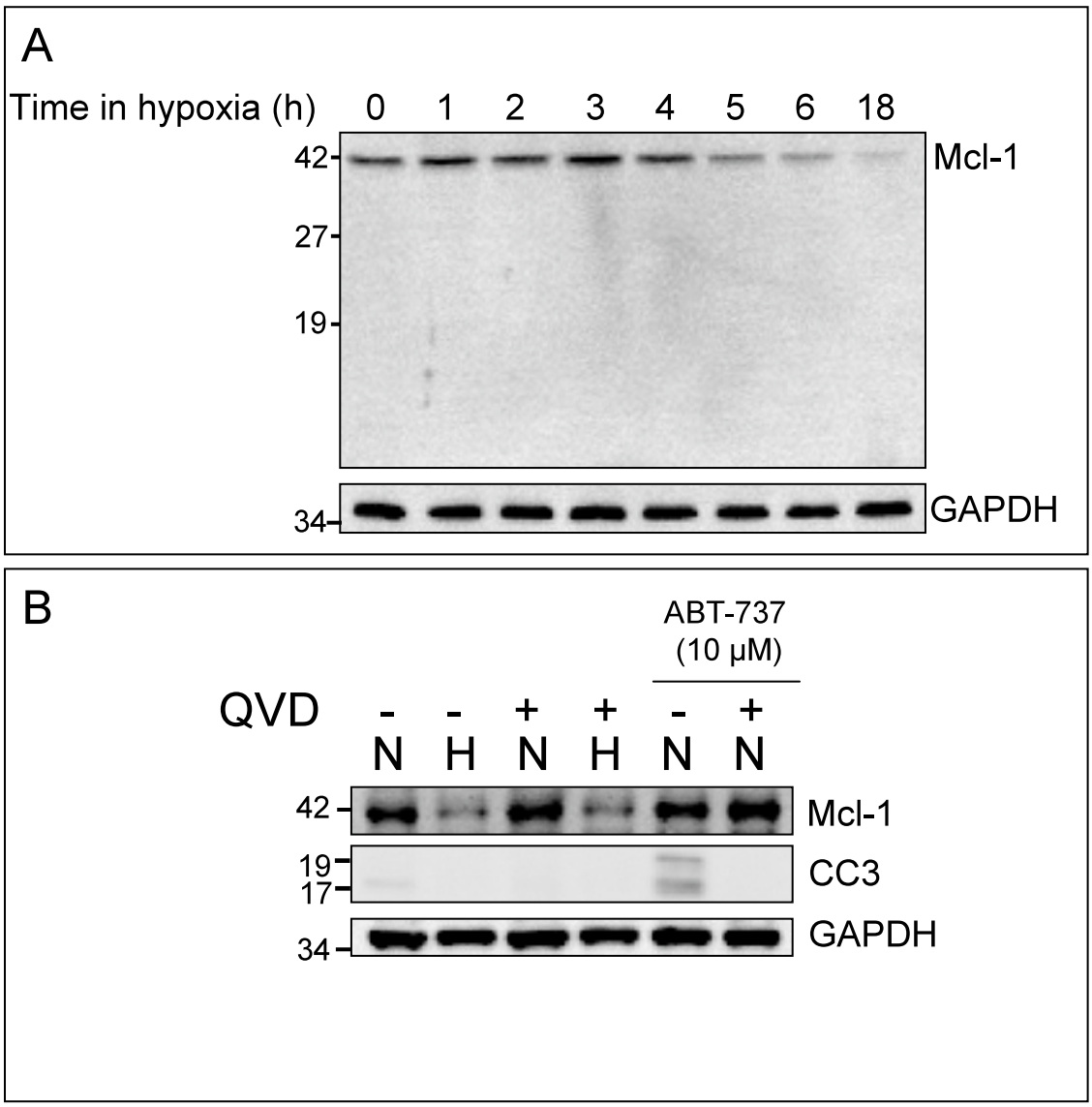
Supplementary Figure 1

H82, H146 and HCT116 cells were seeded into 6-well plates and grown in hypoxia or normoxia for up to 5 days. Cells were counted daily with a haemocytometer with trypan blue exclusion and plotted as a function of time. Data are the mean ± SEM from 3 independent experiments.



Supplementary Figure 2

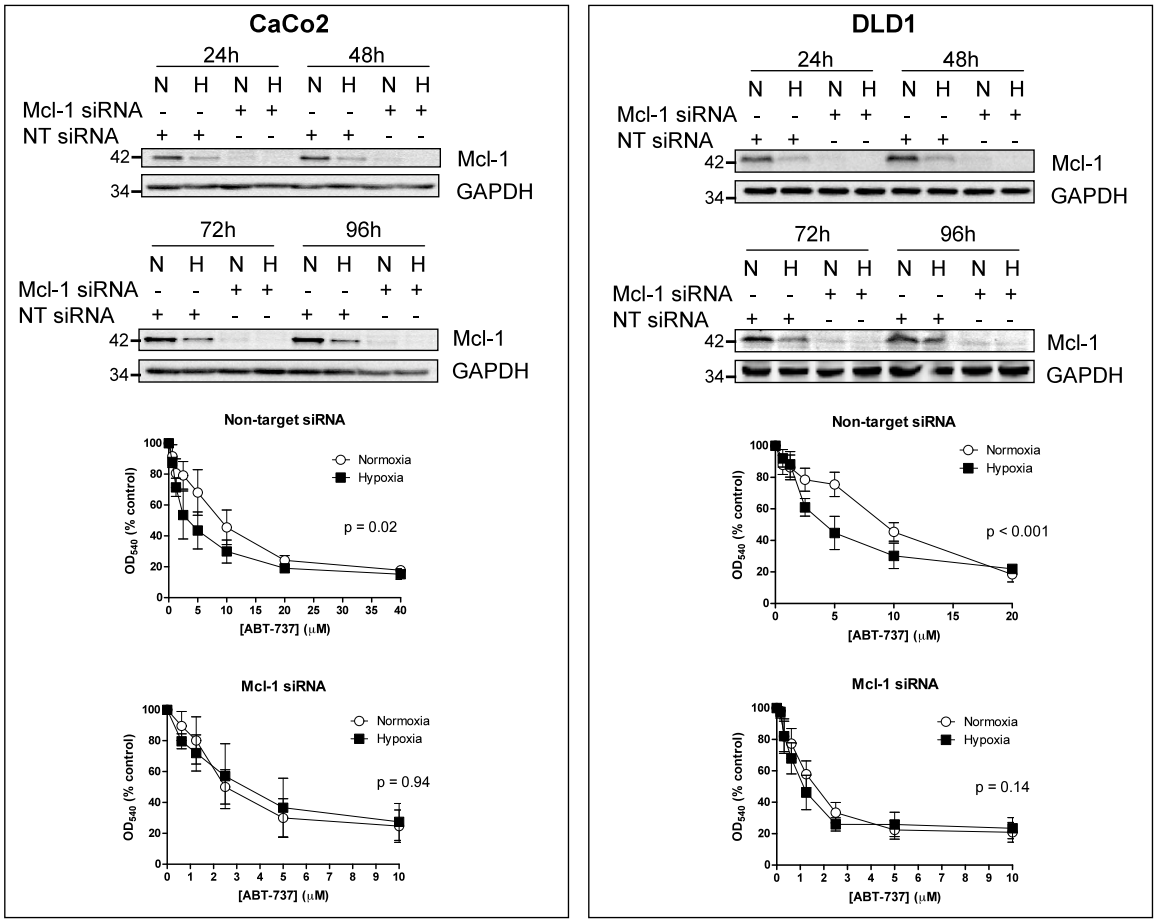
HCT116 cells were incubated in normoxia or hypoxia in the absence and presence of the pan-caspase inhibitor, QVD (20 μM), for 24 hours. As a control for QVD efficacy, cells were treated with 10 μM ABT-737 for 24 h with and without QVD. After treatments, cells were harvested and levels of Mcl-1, PARP/cleaved PARP and actin were determined by western blot.



Supplementary Figure 3

(A) HCT116 cells were incubated in hypoxia for between 0 and 18 hours. At the indicated time points, cells were harvested and levels of Mcl-1 and GAPDH were determined by western blot. Blots show lack of any potential cleaved products of Mcl-1.

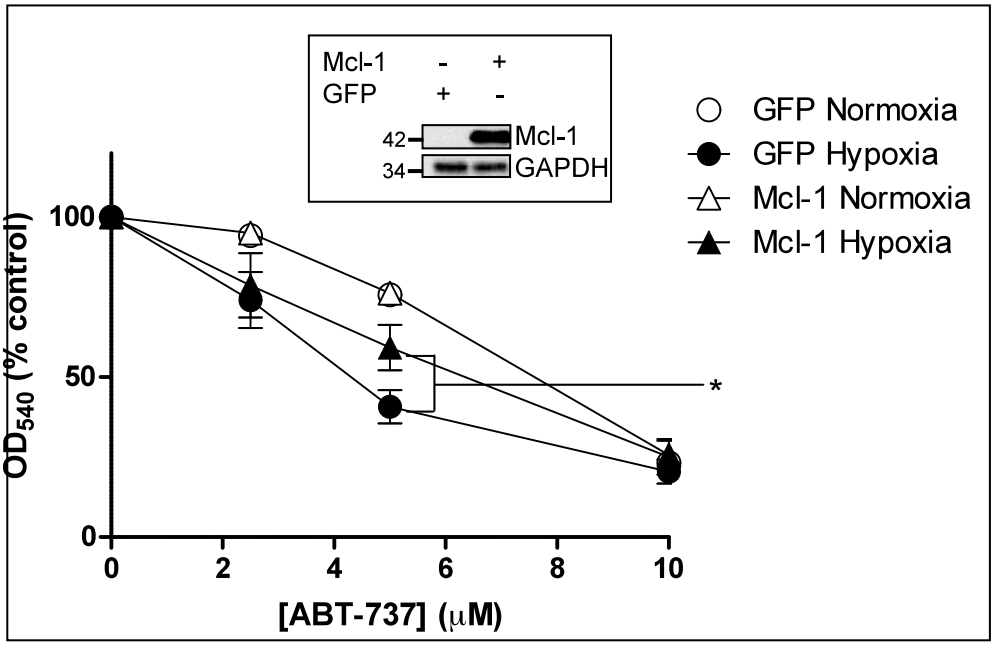
(B) HCT116 cells were incubated in normoxia or hypoxia in the absence and presence of the pan-caspase inhibitor, QVD (20 μM), for 24 hours. As a control for QVD efficacy, cells were treated with 10 μM ABT-737 for 24 h with and without QVD. After treatments, cells were harvested and levels of Mcl-1, cleaved caspase 3 and actin were determined by western blot.



Supplementary Figure 4

CaCo2 or DLD1 cells were transfected with 100nM Mcl-1 targeted siRNA or Non-targeting (NT) control for 24 hours after which siRNA was removed and replaced with full growth media. Cells were then incubated in normoxia or hypoxia (1% O_2) for 24, 48, 72 or 96 hours and cells were harvested for western blot analysis of Mcl-1 and actin levels.

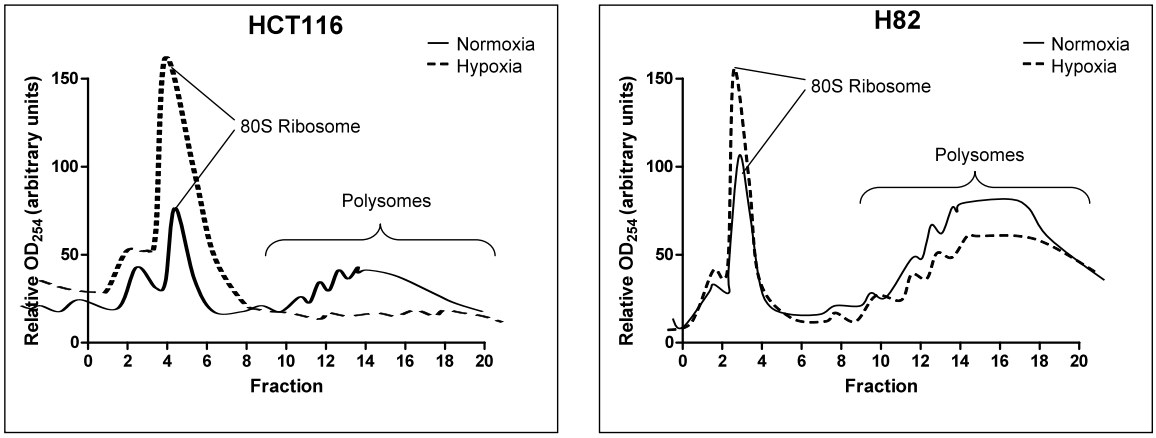
CaCo2 or DLD1 cells were treated with Mcl-1 or NT siRNA as described above and then incubated in normixia or hypoxia (1% O_2) for 18 hours after which they were exposed to a range of ABT-737 concentrations under continuous normoxia (white circles) or hypoxia (black squares) for 72h prior to determination of IC_{50} values using the resazurin assay. Data are the mean ± SEM from 3 independent experiments.



Supplementary Figure 5

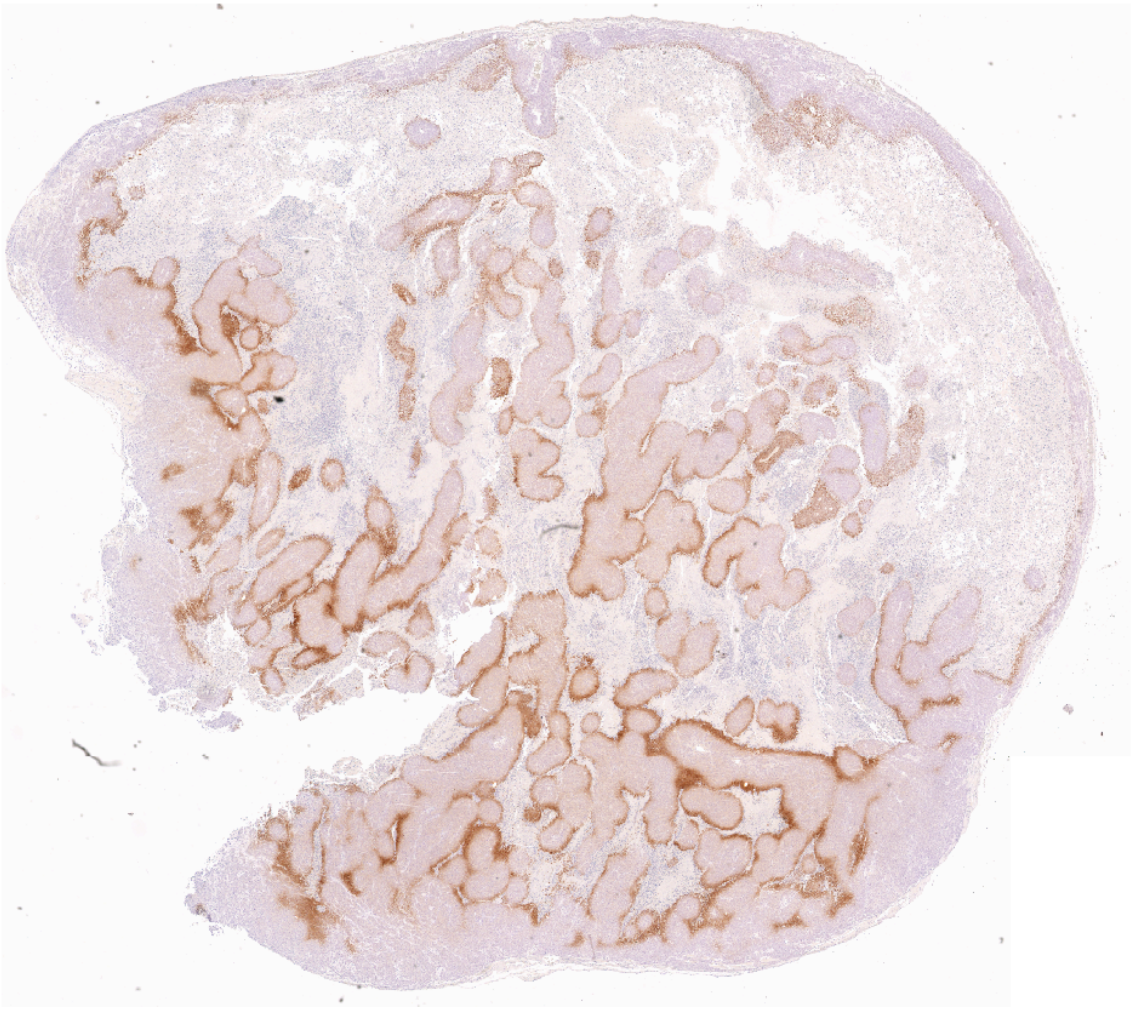
HCT116 cells were transfected with *GFP* and *Mcl-1* (triangles) or *GFP* alone (circles), after which they seeded into 96-well plates and their sensitivity to ABT-737 in normoxia (white circles/triangles) or hypoxia (black circles/triangles) was determined by SRB assay. The inserted western blot shows the maintained over-expression of Mcl-1 in transfected cells in both normoxia and hypoxia.

* = $p < 0.05$ (Student's t-test). Data are the mean ± SEM from 3 independent experiments.



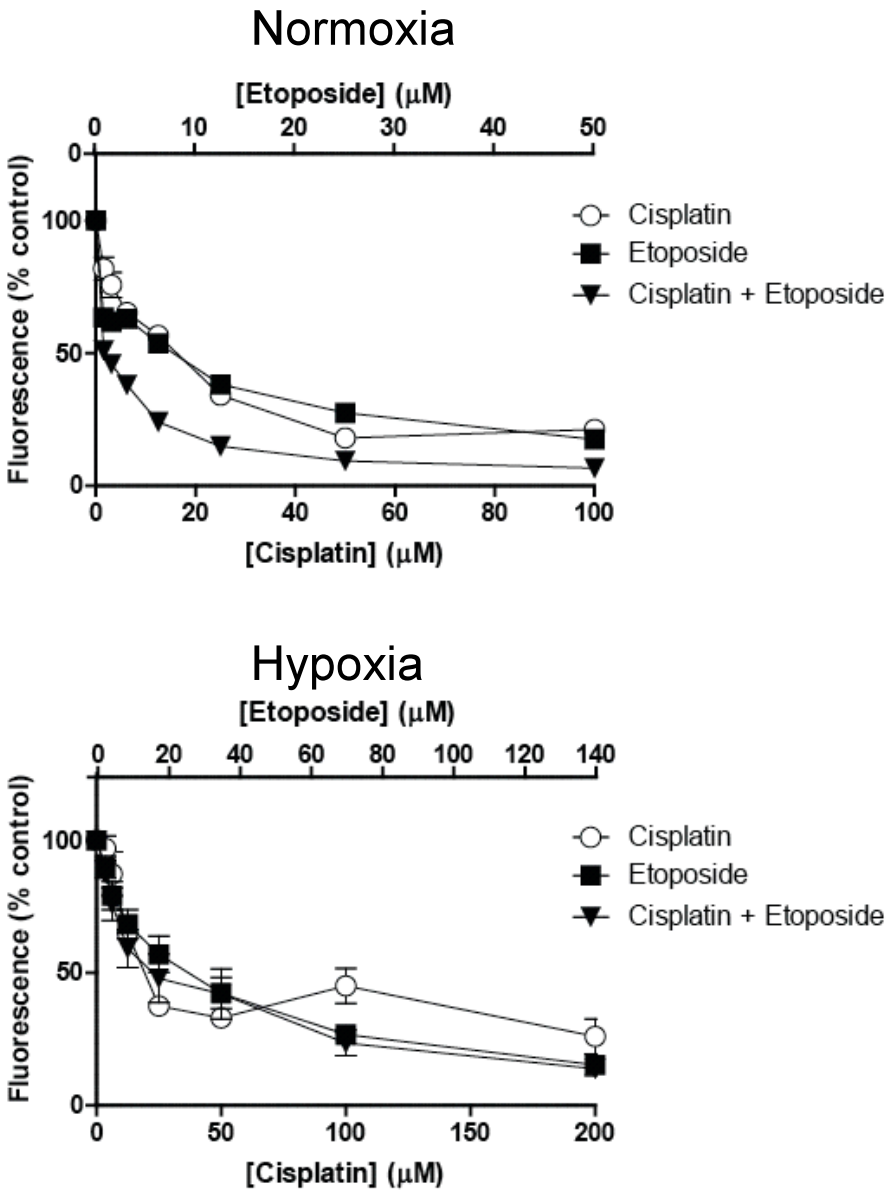
Supplementary Figure 6

Lysates from hypoxic and normoxic HCT116 or H82 cells (24 hour incubation) were separated by density on a 10-60% sucrose gradient before being fractionated into non-polysomal (factions 1-9) or polysomal (fractions 10-18) fractions and OD_{254} measured.



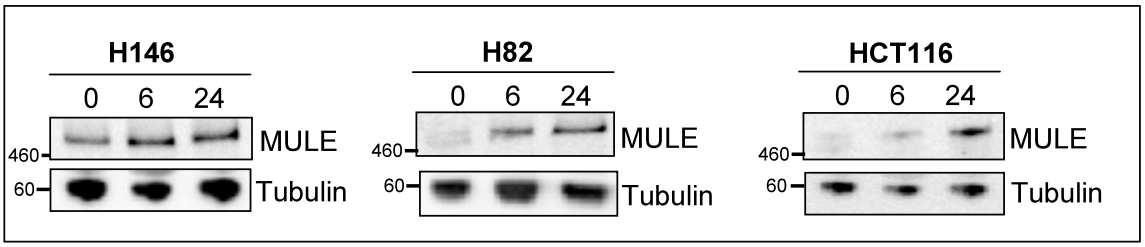
Supplementary Figure 7

Representative of image of H526 xenograft stained by IHC for pimonidazole to show amount of hypoxia in a typical tumour xenograft.



Supplementary Figure 8

H146 cells were treated with either cisplatin (open circles) or etoposide (black squares) or the combination (black triangles) continuously for 72 hours in normoxia or hypoxia (1% O_2) after which the effect on population growth was examined by resazurin assay. Data are the mean ± SEM from 3 independent repeat experiments.



Supplementary Figure 9

HCT116, H82 or H146 cells were incubated in hypoxia for 0, 6 or 24 hours after which cells were harvested and levels of MULE and tubulin were determined by western blot.

Supplementary Table 1

Cell line	Drug	IC_{50} Normoxia (μM)	IC_{50} Hypoxia (μM)
H146	Etoposide	9	31
H146	Cisplatin	25	50
H82	Etoposide	3	15
H82	Cisplatin	8	21
HCT116	Oxaliplatin	32	107
HCT116	5FU	6	183
HCT116	SN38	5	362

IC_{50} values for conventionally used cytotoxic drugs in normoxia and hypoxia. Cells were incubated in normoxia or hypoxia for 18h after which they were exposed to a range drug concentrations under continuous normoxia or hypoxia for 72h prior to determination of IC_{50} values.

Supplementary Table 2

Cell line	IC_{50} Normoxia	IC_{50} Hypoxia	Fold Change
H146	82.1nM	17.8nM	4.6
H82	9.5μM	5.3μM	1.7
H526	5.6μM	0.26μM	21.5
H1048	1.3μM	0.23μM	5.7
H345	0.85μM	0.049μM	17.3
HCT116	8.6μM	4.3μM	2.0
HT29	9.2μM	2.9μM	3.2
DLD-1	8.0μM	2.4μM	3.3
CaCo2	8.5μM	2.7μM	3.1

IC_{50} values for ABT-737 in normoxia and hypoxia.

Cells were incubated in normoxia or hypoxia for 18h after which they were exposed to a range of ABT-737 concentrations under continuous normoxia or hypoxia for 72h prior to determination of IC_{50} values.

Supplementary Table 3

Cell line	Cytotoxic drug	Combination Index with ABT-737	
		Normoxia	Hypoxia
H146	Cisplatin	0.4	0.5
	Etoposide	0.3	0.5
H82	Cisplatin	0.6	0.03
	Etoposide	0.6	0.02
HCT116	Oxaliplatin (1h)	1.5	0.4
	5FU	0.9	0.2
	SN38	0.7	0.2

Combination Indices (CI) for ABT-737 with clinically relevant conventional cytotoxic drugs in normoxia and hypoxia

Cells were also treated with a range of concentrations of ABT-737 alone, the named cytotoxic drug alone or both drugs in combination for 72h (unless specified) under normoxia and hypoxia after which the effect on cell population growth was determined. CI <1.0 indicates synergy and >1.0 indicates antagonism. Data were generated using the resazurin assay and are the average of 3 independent experiments.