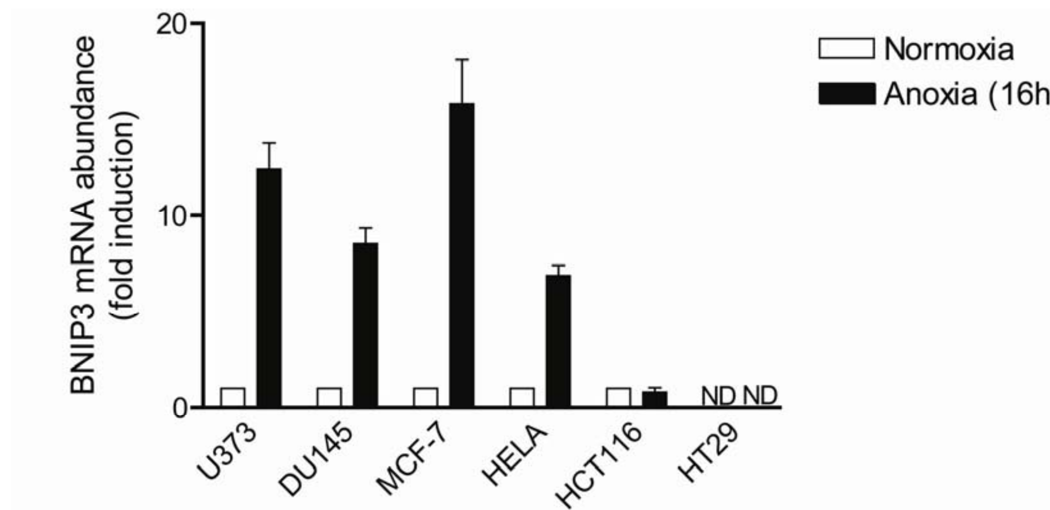
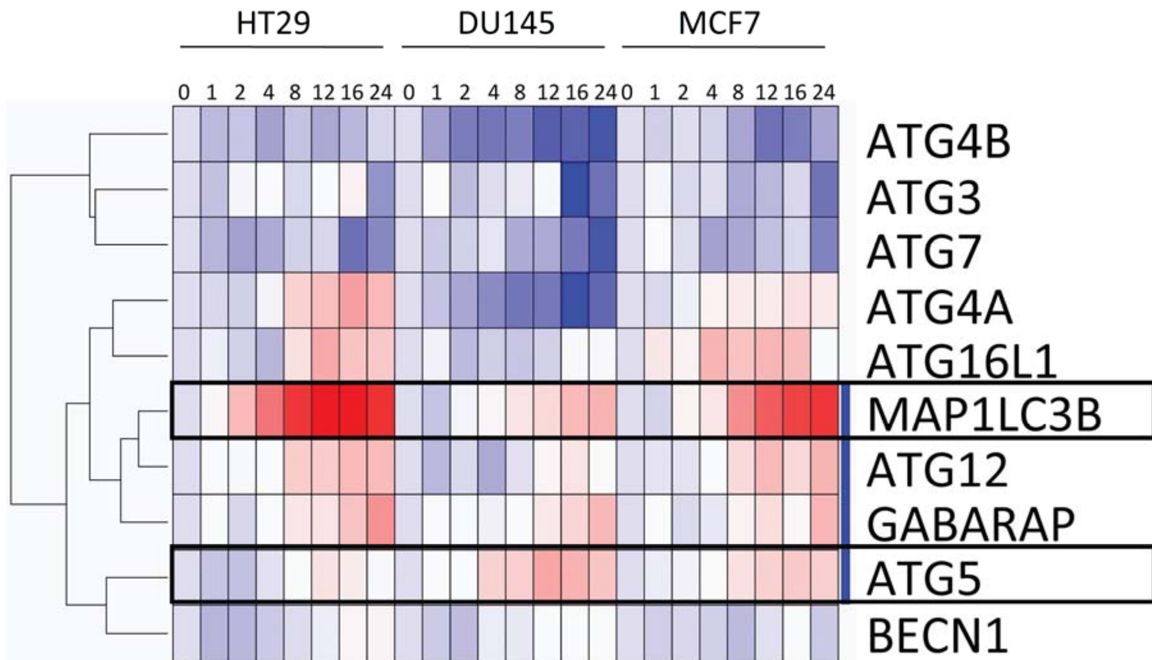


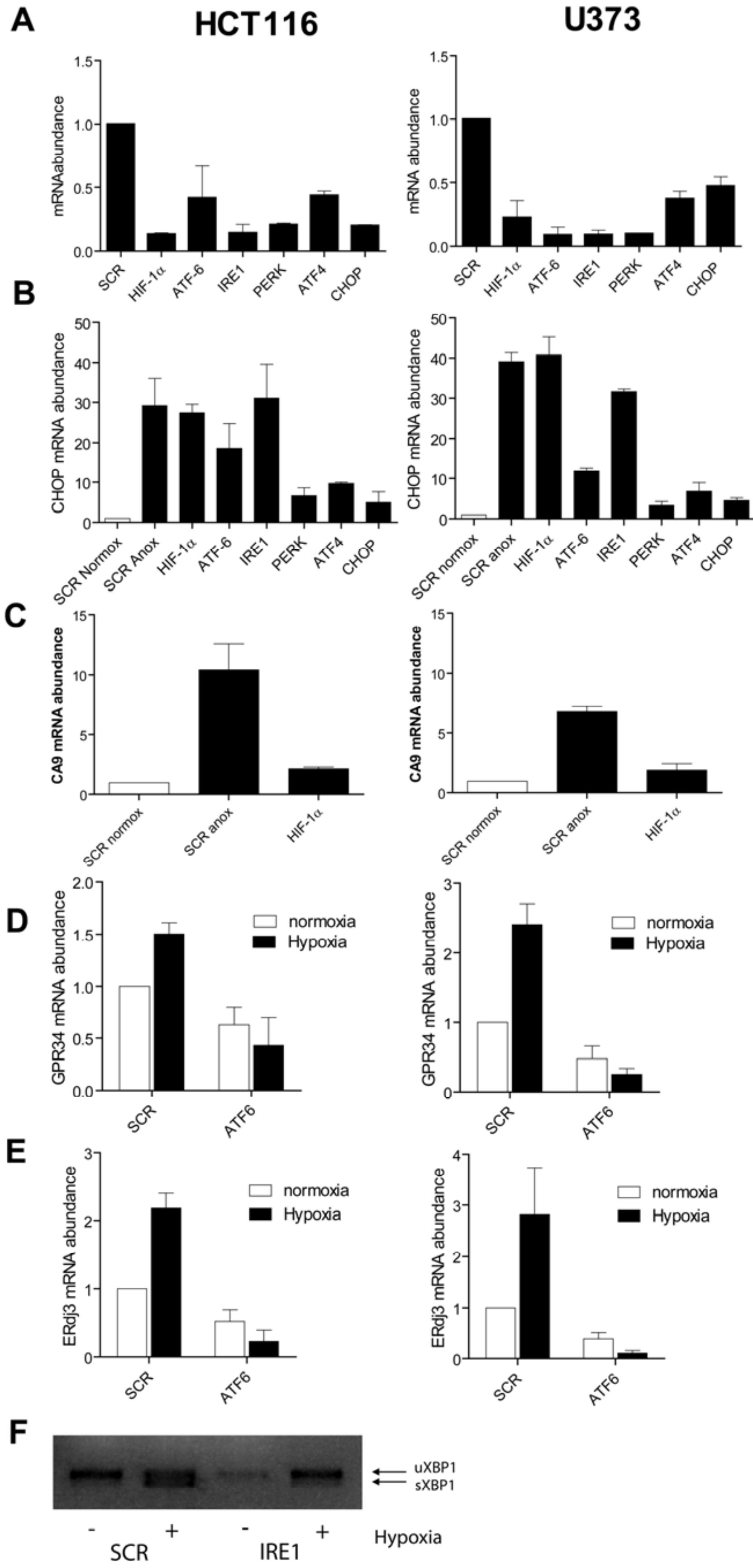

**Supplementary figure 1: BNIP3 mRNA induction in a panel of cell lines after anoxic exposure**

BNIP3 mRNA was analyzed using quantitative PCR after 16 hours anoxia. All samples were normalized to its own cell line after 16 hours normoxic exposure (100%). No BNIP3-transcripts could be detected in HT29 cells (ND). Data is presented as mean ± SEM, n=3.



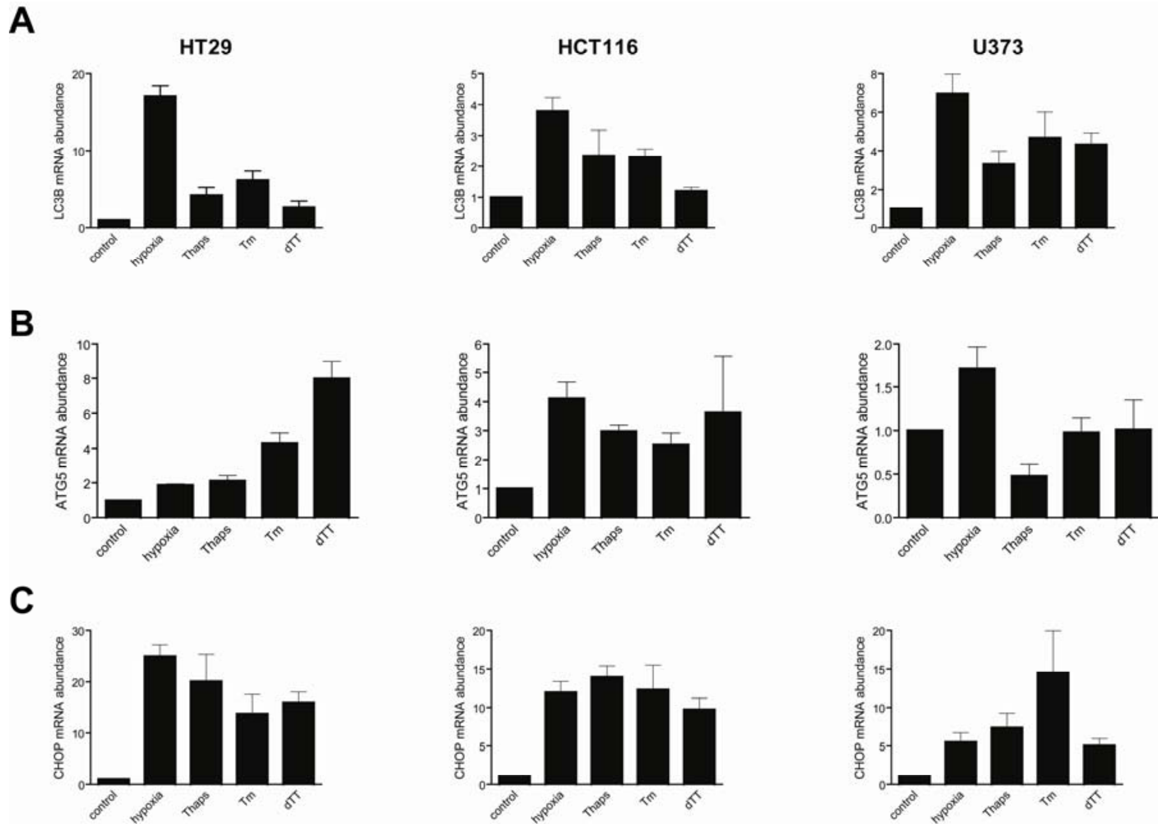

**Supplementary figure 2: Expression of autophagy genes during hypoxia**

Changes in expression of autophagy genes were detected by Affymetrix microarray analysis in HT29, MCF7 and DU145 cells. Probes were filtered for expression, those with a Log(2) signal >7 were averaged. Normalized values were clustered and visualized using GENEPATTERN for up- (red) and down-(blue) regulation compared to the control. Of the 4 genes demonstrating induction during hypoxia in the 3 cell lines we validated MAP1LC3B and ATG5 using quantitative RT-PCR.


A
HCT116
U373
mRNAabundance
mRNA abundance
SCR
HIF-1α
ATF-6
IRE1
PERK
ATF4
CHOP
B
CHOP mRNA abundance
SCR Normox
SCR Anox
SCR normox
SCR anox
C
CA9 mRNA abundance
D
GPR34 mRNA abundance
normoxia
Hypoxia
ATF6
E
ERdj3 mRNA abundance
F
uXBP1
sXBP1
Hypoxia
IRE1

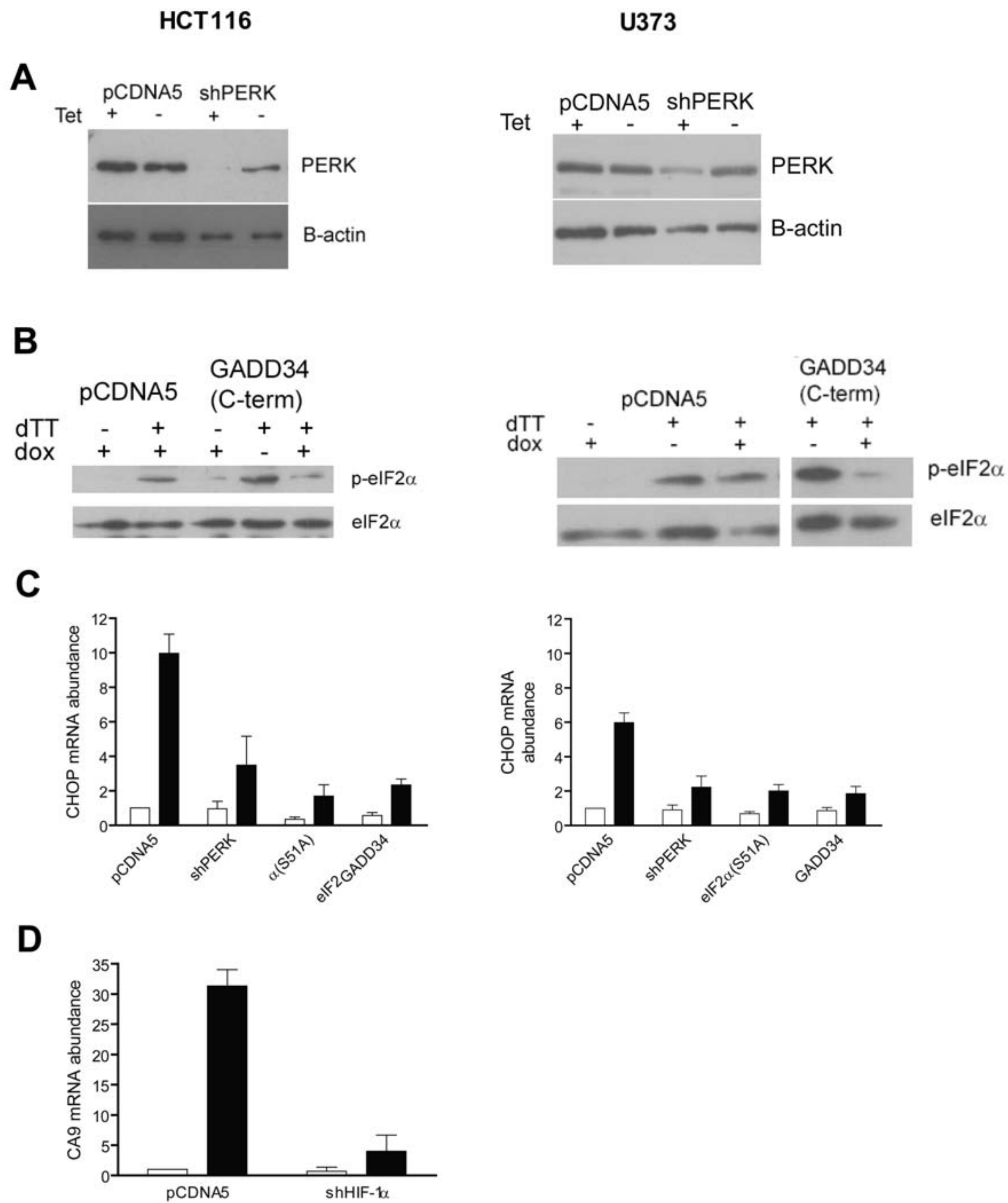
**Supplementary figure 3: Knockdown of HIF-1α, ATF-6, IRE1, PERK, ATF4, and CHOP after transfection with the according siRNA in HCT116 and U373 cells.**

Knockdown of the respective genes was assessed 48 hours after transfection, when experiments with these cells (fig 3) were conducted. A) mRNA expression of the respective genes was assessed by quantitative PCR. The mRNA content was compared to scrambled siRNA (SCR) transfected cells. B) Assessment of the expression of CHOP mRNA after 24h exposure to anoxia (black bars) compared to SCR control during normoxia (gray bar). C) Assessment of CA9 mRNA induction after 24h exposure to hypoxia (black bars) compared to SCR control during normoxia in SCR and HIF-1α knockdown cells. D) GPR34 and E) ERdj3 mRNA abundance was measured after 24 hours hypoxia in SCR controls and ATF6 siRNA treated cells. Data are presented as mean ± SEM, n=3. F) Representative example of XBP1-splicing in U373 cells transfected with SCR or IRE1 siRNA after 24 hours hypoxia. uXP1 (unspliced) and sXBP1 (spliced) are indicated by arrows.



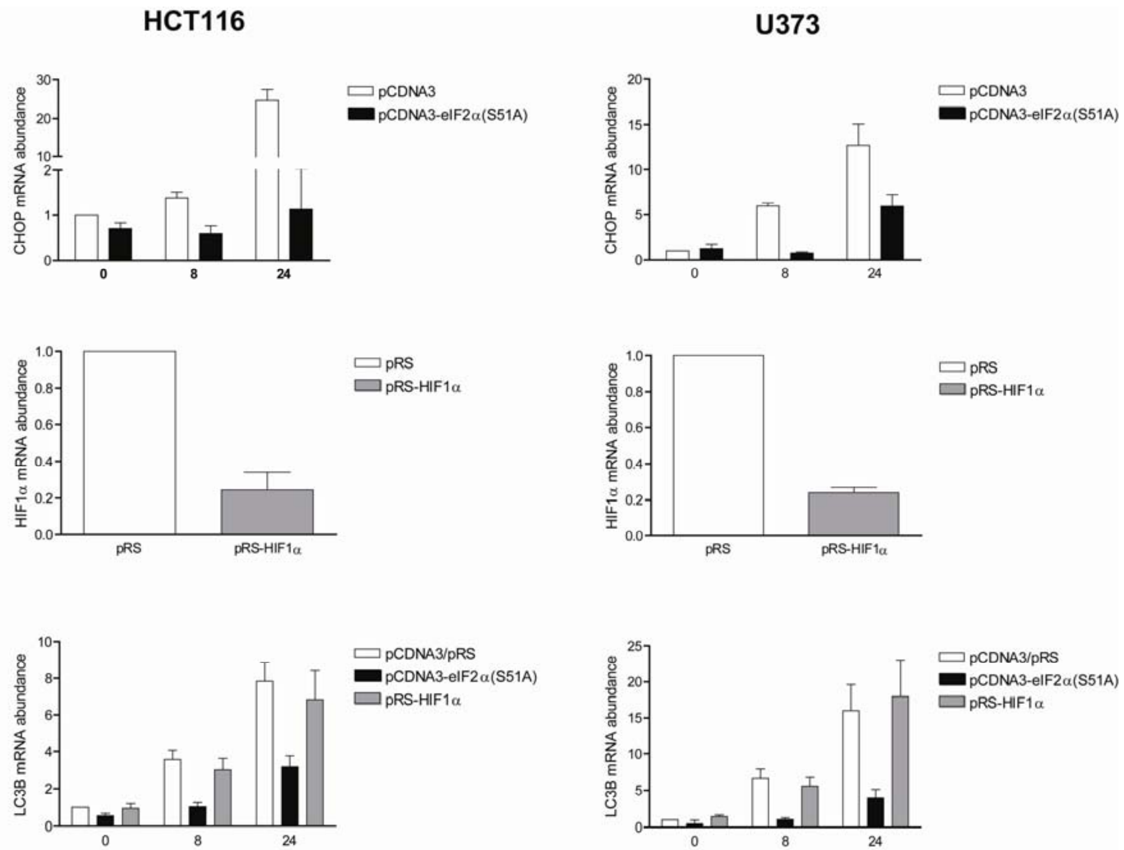

**Supplementary figure 4: Transcriptional induction of LC3b and ATG5 by UPR inducing agents.**

As shown, UPR-signaling controls transcription of LC3b mRNA. Activation of UPR-signaling by addition of thapsigargin (1µM, Sigma), tunicamycin (2.5µg/ml) or dTT (2mM) for 8 hours induced mRNA transcription of LC3B (A) and ATG5 (B). Induction of UPR is comparable between samples as assessed by CHOP (C) determination. Data is presented as mean ± SEM, n=3

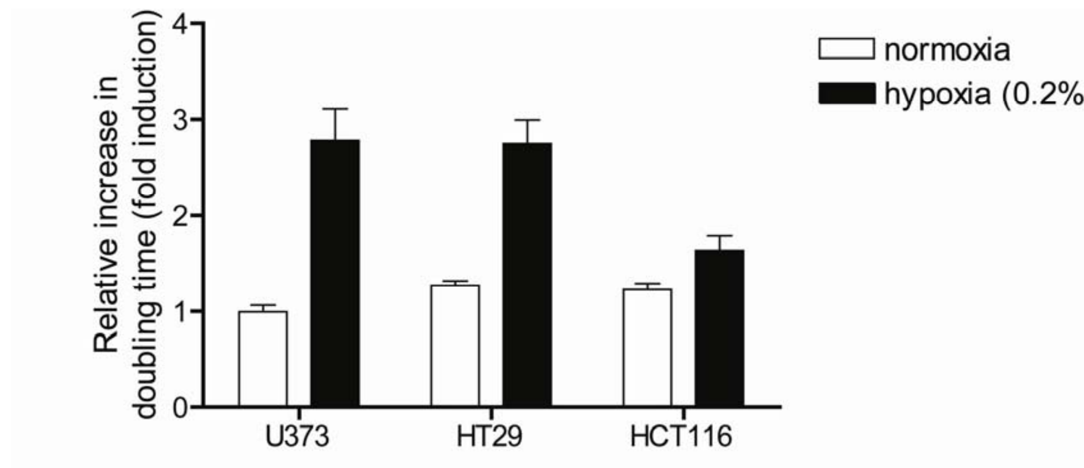



**Supplementary figure 5: Validation of the stable, inducible, isogenic cells used**
HCT116 an U373 cells inducibly defective in UPR-signaling were (if applicable) incubated with tetracycline (tet) or doxycycline (dox) (1μg/ml) for 48 hours for induction. A) PERK expression was assessed by imunoblotting. B) eIF2α phosphorylation was assessed in pCDNA5 and constitutively active GADD34-expressing cells after dTT stimulation (1mM) by immunoblotting. C) Cells pre-exposed to doxycycline were maintained in normoxia (white bars) or exposed to anoxia for 8 hours (black bars). UPR defectiveness was validated by means of CHOP mRNA abundance measurements. Data is presented as mean ± SEM, n=3. D) HIF-1α inducible knockdowns were incubated with

doxycycline (1µg/ml) for 48 hours, maintained in normoxia (white bars) or exposed to anoxia for 24 hours (black bars). HIF-signaling deficiency was determined by CA9 mRNA abundance measurements. Data is presented as mean ± SEM, n=3.

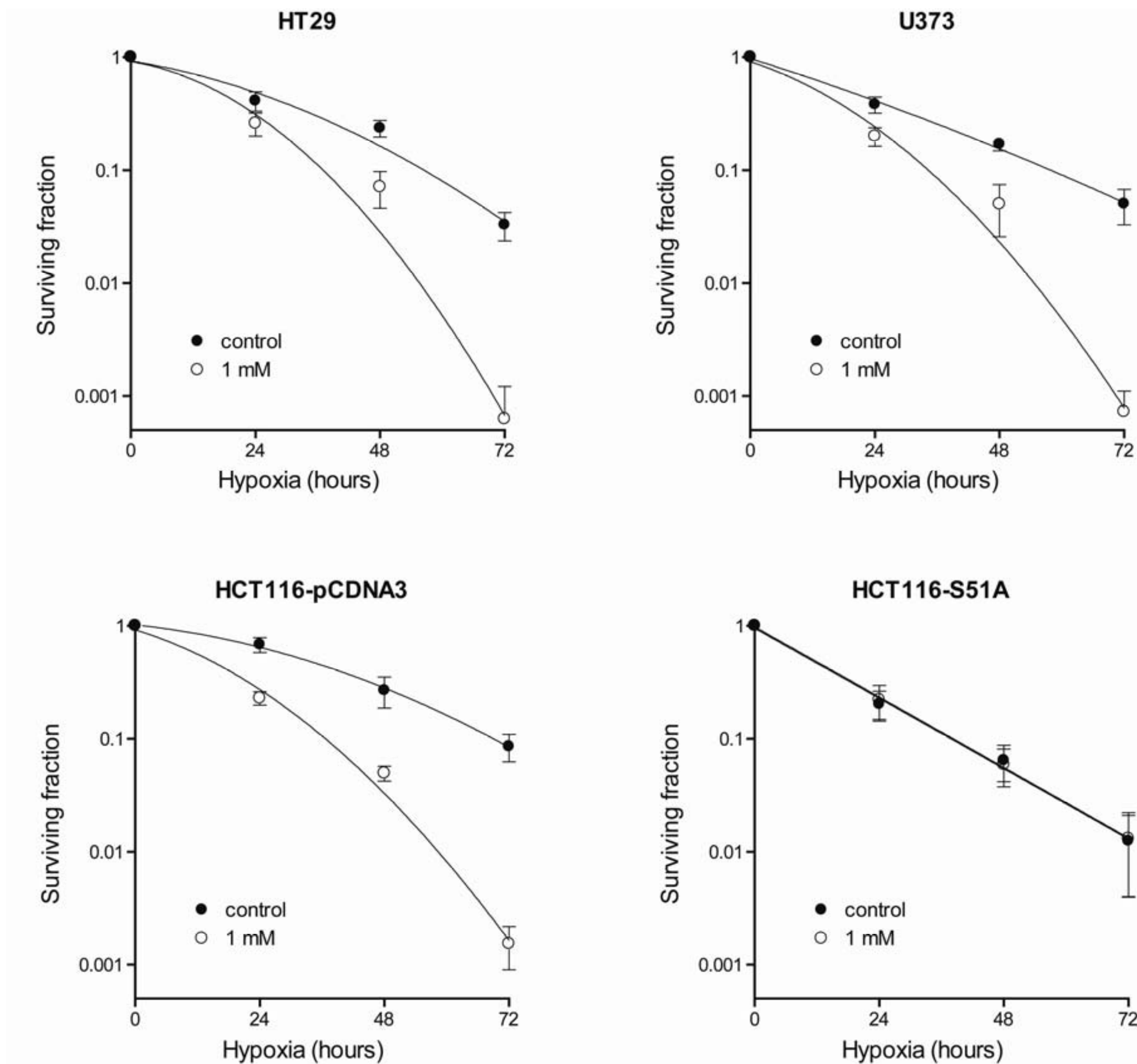



**Supplementary figure 6: characterization of cells used in xenograft experiments**
HCT116 and U373 cells expressing a non-phosphorylatable form of eIF2α, eIF2α(S51A) were inhibited in UPR signaling, as shown by a delayed CHOP mRNA-induction (left panels). CHOP levels were determined by quantitative PCR. Knockdown of HIF-1α was determined using quantitative PCR (middle panels). The lc3b-response in these cells was determined after anoxic exposure (8 and 24 hours). Control cells, pCDNA3 and pRS behaved similarly and are therefore pooled. As expected lc3b mRNA induction was inhibited in ISR-hampered cells (right panels). Data is presented as mean ± SEM, n=3



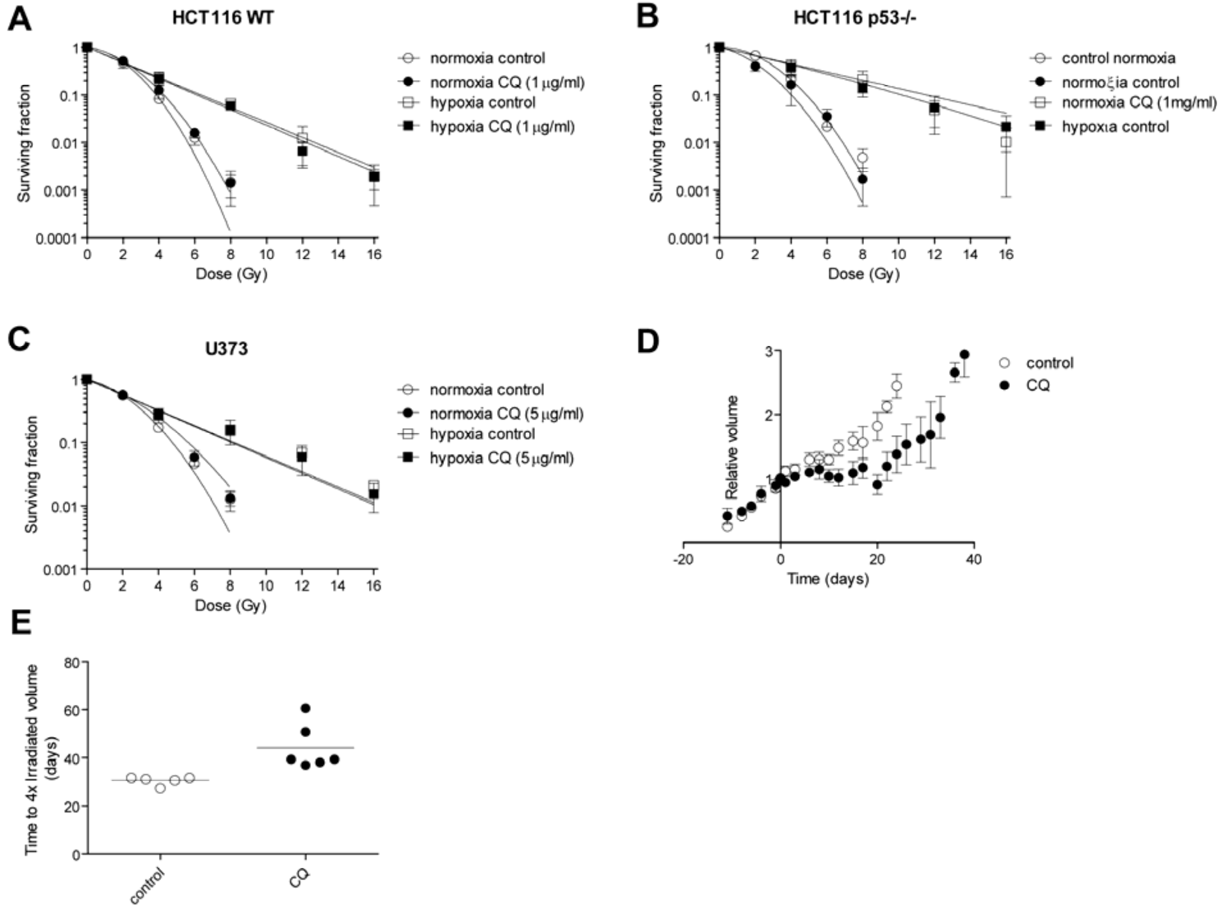

**Supplementary figure 7: Autophagy inhibition by 3-Ma increases doubling time under hypoxia.**

Growth curves under normoxic and moderate hypoxic (0.2% $O_2$) were performed as in figure 7. Increase in the 3-Ma-mediated inhibition of cell proliferation during normoxic and hypoxic condition was assessed, data is showing the relative increase in doubling time after 3-Ma-addition. n=3 mean ± SEM.



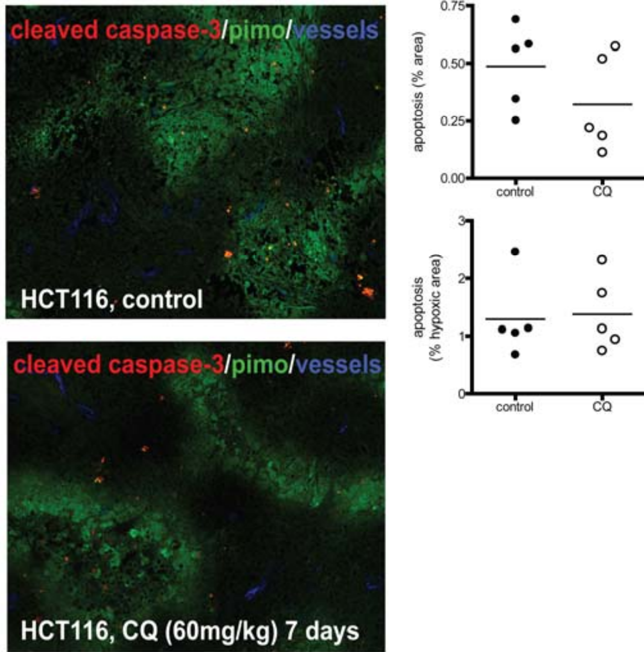

**Supplementary figure 8: Autophagy inhibition, by 3-Ma addition, sensitizes cells to hypoxia.**

Clonogenic survival after anoxic exposure with or without addition of 3-Ma, revealing increased sensitivity to hypoxia after 3-Ma addition in the cell lines tested. HCT116-S51A shows no increased sensitivity towards 3-Ma. mean ± SEM, n=3.

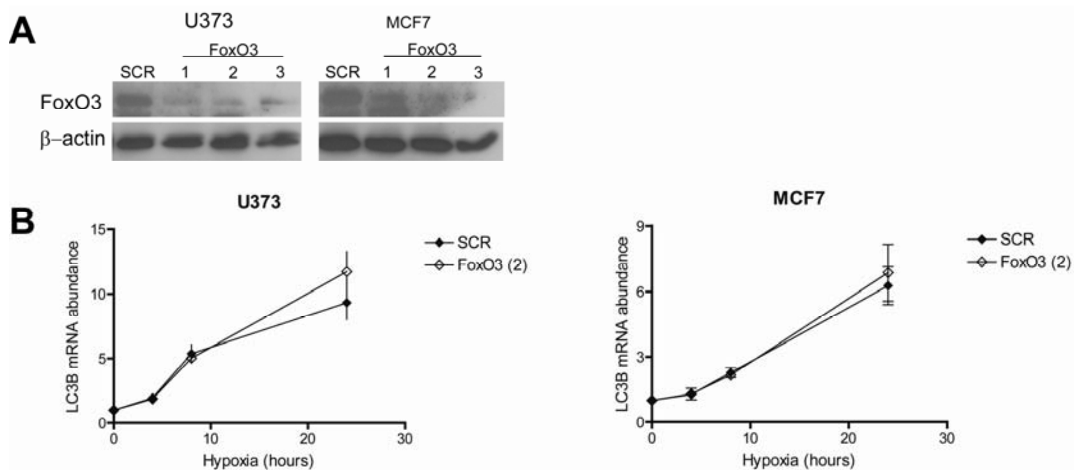



**Supplementary figure 9: CQ-treatment sensitizes p53-mutant tumors to radiation**

CQ-treatment does not sensitize (A) p53 wild-type HCT116, (B) p53 knockout HCT116 or (C) p53-mutant U373 cells to irradiation under normal or hypoxic (O2<0.02 for 4 hours) conditions as determined by clonogenic survival. (D) U373 xenografts untreated (saline, n=5) or treated with CQ (60 mg/kg)(n=6), (day -4 to +3)) were irradiated at t=0 with a single, tumor specific dose (10Gy, 16 MeV), outgrowth after treatment was determined. (E) time to reach 4x irradiated tumor volume was calculated and depicted in the graph.




**Supplementary figure 10: CQ administration does not increase tumor cell apoptosis.**
Left panels, stainings for apoptosis (cleaved caspase-3) and pimo after 7 days of CQ-treatment (n=5). Graphs are depicted as single data points, horizontal line shows the calculated mean.




**Supplementary figure 11: FoxO3 does not contribute to lc3b mRNA upregulation during hypoxia.**

U373 and MCF7 cells were transfected using 3 unique siRNAs directed against FoxO3 and analyzed for FoxO3-expression by western-blotting. Transfection with siRNA 2 gave the best knockdown (A). Cells transfected with siRNA 2 were exposed to anoxia and analyzed for lc3b mRNA by quantitative PCR (B). No differences were observed after knockdown of FoxO3. Data is presented as mean ± SEM, n=3.

**Supplementary table 1**: siRNA use

| Gene | Ambion® siRNA ID |
| --- | --- |
| | |
| ATF4 | 122372 |
| ATF6 | 115889 |
| CHOP | 146321 |
| GADD34 | 5762 |
| HIF | 42840 |
| IRE1 | 289308 |
| PERK | 594 |
| SCR | AM4611 |

**Supplementary table 2.** Q-PCR primers and probe sets

| Gene | Forward | Reverse |
| --- | --- | --- |
| 18S | 5'-AGTCCCTGCCCTTTGTACACA-3' | 5'-GATCCGAGGGCCTCACTAAAC-3' |
| ATF4 | 5'-TGGCCAAGCACTTCAAACCT-3' | 5'-GTTGTTGGAGGGACTGACCAA-3' |
| ATF-6 | 5'-AGACTGAAGAGCAGGTGAGCAAA-3' | 5'-GATGATGAAAAATGGAGCAGCTT-3' |
| ATG5 | 5'-GCAAGCCAGACAGGAAAAAG-3' | 5'-GACCTTCAGTGGTCCGGTAA-3' |
| bnip3 | 5'-ACCCTCAGCATGAGGAACAC-3' | 5'-ATCAAAAGGTGCTGGTGGAG-3' |
| CHOP | 5'-GGAGCATCAGTCCCCCACTT-3' | 5'-TGTGGGATTGAGGGTCACATC-3' |
| GADD34 | 5'-CCCAGAAACCCCTACTCATGATC-3' | 5'-GCCCAGACAGCCAGGAAAT-3' |
| IRE1 | 5'-CGAAACTTCCTTTTACCATCCC-3' | 5'-CGATGACAAAGTCTGCTGCTT-3' |
| MAP1Lc3b | 5'-AACGGGCTGTGTGAGAAAAC-3' | 5'-AGTGAGGACTTTGGGTGTGG-3' |
| PERK | 5'-CTGATTTTGAGCCAATTC-3' | 5'-CCGGTACTCGCGTCGCTG-3' |
| | | |
| **Probe** | | |
| 18S | HS99999901_s1 | |
| HIF1α | HS00153153_m1 | |

**Supplementary table 3.** Q-PCR primers used to detect promoter-enrichment in ChIP

| Gene | Forward | Reverse | Location Transcript (bp) |
| --- | --- | --- | --- |
| ATG5 | 5'-CACCATTTCAGCAATGAAGC-3' | 5'-TGGCAATTACTAGGGCCATC-3' | -583 to -472 |
| ATG5 | 5'-GCCCTCTGAATACTCAAATGATT-3' | 5'-TGGCATTTAGAGCAAAACCA-3' | -1357 to -1107 |
| CHOP | 5'-GGGCCAAGAAATATGGGAGT-3' | 5'-TAGTCGGTCGTGAGCCTCTT-3' | -3651 to -3557 |
| CHOP | 5'-AGCCAAAATCAGAGCTGGAA-3' | 5'-ACAAGTTGGCAAGCTGGTCT-3' | -311 to -19 |
| GADD34 | 5'-GACGTTGCAAAAGCTGGAAT-3' | 5'-CACAATGGCCACTAGGCTTT-3' | -152 to -7 |
| GADD34 | 5'-AAAAAGACCTGCCTGGTCAA-3' | 5'-TCGGCTTCCCAAAGTGTTAG-3' | -1415 to -1148 |
| MAP1lc3b | 5'-GCAGCACCACCAAGTCTCTC-3' | 5'-ACTCTTGAGGGAGGGGTCAG-3' | -458 to -376 |
| MAP1lc3b | 5'-CCCTGAGAGCTGGGTTTACA-3' | 5'-CCTCGGCCTCCTTAAGAGTT-3' | -1482 to -1216 |