SUPPLEMENTARY MATERIAL AND METHODS

Additional primer sequences:

qPCR sequences

PHD2

For-GAAAGCCATGGTTGCTTGTT

Rev-TGTCCTTCTGGAAAAATTCG

BNIP3

For-GCCCACCTCGCTCGCAGACAC

Rev-CAATCCGATGGCCAGCAAATGAGA

Immunofluorescence microscopy

For immunofluorescence, cells were grown on coverslips and treated as indicated prior to fixation by incubation in 3.7% formaldehyde/PBS (pH 6.8) for 15 minutes. Cells were permeabilized in PBS-0.1% Triton X-100 for 15 minutes and then blocked in PBS-0.05% Tween-20 supplemented with 1% normal donkey serum for 30 minutes. The dilutions of the antibodies used were: rabbit anti-RelA 1:200 and goat anti-LC3B 1:50. Secondary antibody labelled with X-Red and FITC were purchased from Jackson Immunoresearch and used at 1:500. Cells were stained with primary antibodies without secondary antibodies to control for auto-florescence or stained with secondary antibodies alone to control for background staining. Cells were analysed and images were acquired using a DeltaVision microscope. Images were deconvolved and analysed using OMERO client software (Open Microscopy Environment).

Supplementary Figure Legends

Sup. Figure 1. Hypoxia represses APC in a HIF-1α dependent manner. (A) U2OS cells were exposed to 1% O_2 for the indicated periods of time prior to lysis. Whole cell lysates were analysed by immunoblotting using the depicted antibodies. (B) U20S, HCT-116 and HAβ18 cells were transfected with control and HIF-1α siRNA oligonucleotides prior to exposure to 1% O_2 for 24h and total RNA extraction. RT-qPCR for APC and HIF-1α was performed with Actin as a reference gene. Graph depicts the mean plus SD of a minimum of 3 independent experiments performed in duplicate. Anova student t-test were performed and * indicates the level of significance. *p<0.05, **p<0.01, ***p<0.005.

Sup. Figure 2. Hypoxia reduced the levels of Acetylated H3 at the APC promoter. U2OS cells were exposed to 1% O_2 for 24h prior to fixation and lysis. Acetylated H3 bound DNA was amplified with specific primers spanning the annotated HRE region on the CA9 and the putative HRE at the APC promoter. Rabbit IgG was used as a control for the immunoprecipitation. Levels of Acetylated H3 were quantified using ImageJ software and graph depicts the results of 3 independent experiments. Anova student t-test were performed and * indicates the level of significance. *p<0.05, **p<0.01, ***p<0.005.

Sup. Figure 3. APC depletion increases HIF-1 levels and activity. (A) U2OS cells were transfected with control and APC siRNA oligonucleotides and treated with 50μM MG132 for 3h prior to harvest. Whole cell lysates were analysed by Western blot to detect the levels of HIF-1α and Actin. (B) U2OS cells were transfected with control and

APC siRNA oligonucleotides for 48h prior to total RNA extraction. Where indicated, cells were exposed to 1% O_2 for 24h. Following cDNA synthesis, qPCR was performed for the HIF-1α target genes ADM and PHD2. Graph depicts the mean plus SD of a minimum of 3 independent experiments performed in duplicate. Anova student t-test were performed and * indicates the level of significance. *p<0.05, **p<0.01, ***p<0.005.

Sup. Figure 4. Increased HIF-1α activity following APC depletion requires wildtype β-catenin. HCT-116 (parental), HAβ85 (wildtype β-catenin) and HAβ18 (mutant β-catenin) were transfected with control or APC siRNA oligonucleotides for 48h prior to RNA extraction and exposed or not to 1% O_2 for 24h. RT-qPCR was performed for the HIF-1α target genes indicated. Graph depicts mean plus SD of a minimum of 3 independent experiments performed in duplicate. Anova student t-test were performed and * indicate the level of significance. *p<0.05, **p<0.01, ***p<0.005.

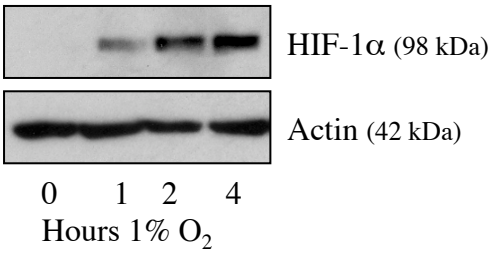
Sup. Figure 5 NF-κB activation following TNF-α is altered by different levels of β-catenin. (A) HCT-116 (parental), HAβ85 (wildtype β-catenin) and HAβ18 (mutant β-catenin) were treated with 10ng/mL TNF-α for 30m prior to cellular fractionation. Nuclear extracts were analysed by Western blot for the level of RelA nuclear translocation. Chk1 was used as a loading control. (B) HCT-116 (parental), HAβ85 (Wildtype β-catenin) and HAβ18 (Mutant β-catenin) were treated with 10ng/mL TNF-α for 30m prior to fixation and immunostaining with RelA antibody. Cells were imaged using a DeltaVision microscope, deconvolved and analysed using OMERO open source

software. (C) Cells were processed as in A, with cytoplasmic extracts analysed by Western blot for the levels of phosphorylated and total IκB-α. Actin was used as a loading control.

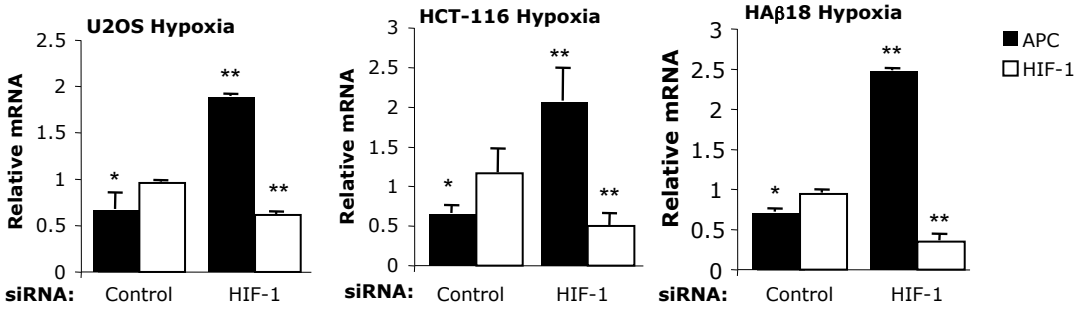
Sup. Figure 6. Increased HIF-1α following APC depletion requires NF-κB. (A) U2OS were transfected with the indicated siRNA oligonucleotides for 48h prior to RNA extraction. qPCR was performed for the levels of HIF-1α, RelA and APC, with Actin used as normalising gene. Graph depicts the mean plus SD of a minimum of 3 independent experiments performed in duplicate. (B) U2OS cells were treated as in A but whole cell lysates were performed. Levels of APC and RelA were analysed by Western blot. Tubulin was used as loading control. Anova student t-test were performed and * indicate the level of significance. *$p<0.05$, **$p<0.01$, ***$p<0.005$.

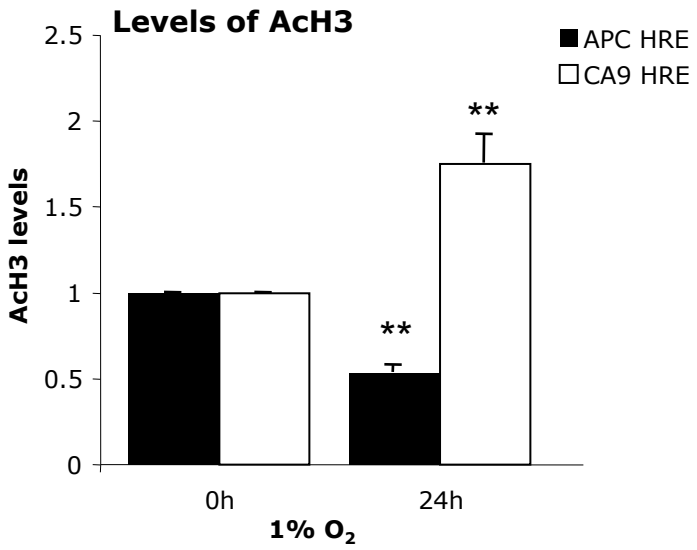
Sup. Figure 7. Hypoxia induced LC3B puncta is HIF-1α dependent. HAβ85 (wildtype β-catenin) and HAβ18 (mutant β-catenin) were transfected with control, APC or HIF-1α siRNA oligonucleotides prior to exposure to 1% O_2 for 24h. Cells were fixed and immunostained with LC3B antibody. Cells were imaged using a DeltaVision microscope, deconvolved and analysed using OMERO open source software.

A

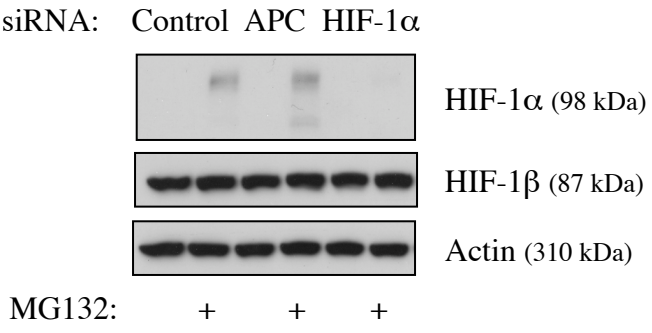

HIF-1α (98 kDa)
Actin (42 kDa)
0 1 2 4
Hours 1% O2

B

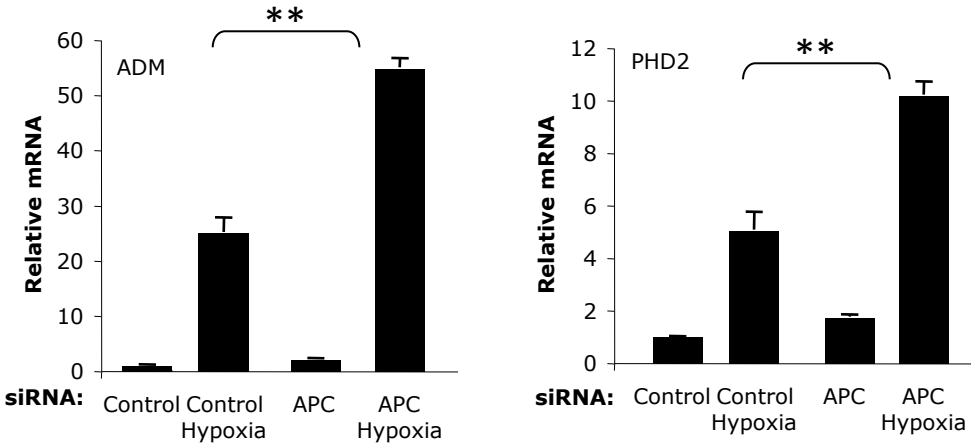

U2OS Hypoxia
HCT-116 Hypoxia
HAβ18 Hypoxia
Relative mRNA
siRNA: Control HIF-1
APC
HIF-1

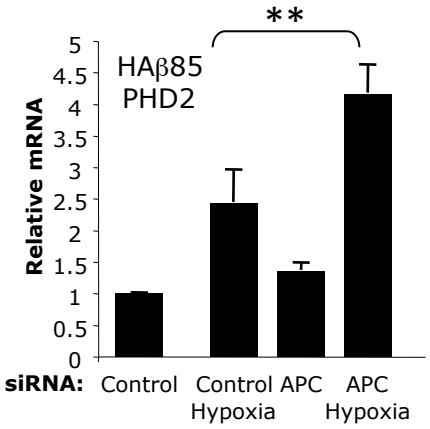

Levels of AcH3
APC HRE
CA9 HRE
AcH3 levels
2.5
2
1.5
1
0.5
0
**
**
0h
24h
1% O2

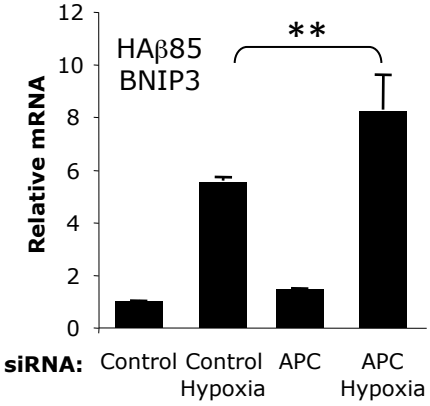
A

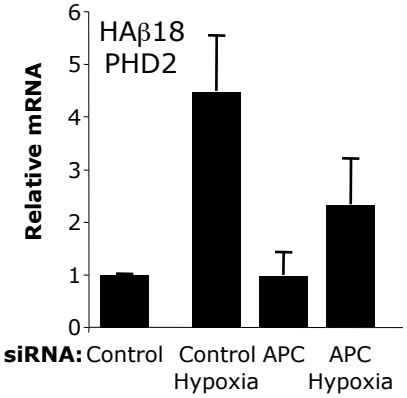

siRNA: Control APC HIF-1α
HIF-1α (98 kDa)
HIF-1β (87 kDa)
Actin (310 kDa)
MG132: + + +

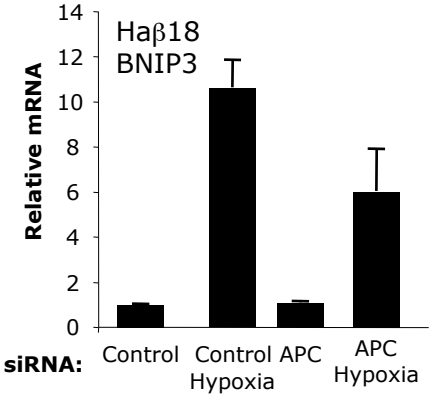
B

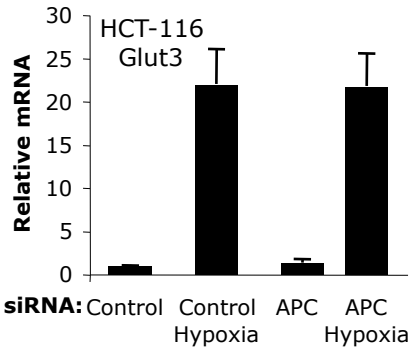

ADM
Relative mRNA
60
50
40
30
20
10
0
**
siRNA:
Control
Control Hypoxia
APC
APC Hypoxia
PHD2
Relative mRNA
12
10
8
6
4
2
0
**
siRNA:
Control
Control Hypoxia
APC
APC Hypoxia

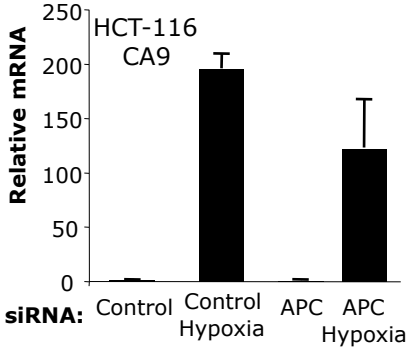

**
HAβ85
PHD2
Relative mRNA
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
siRNA: Control Control Hypoxia APC APC Hypoxia

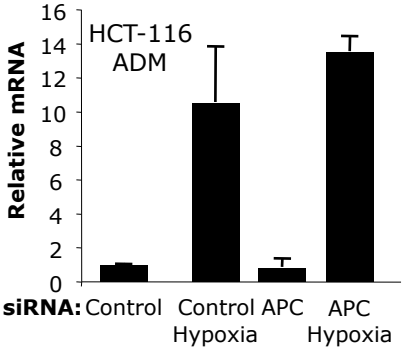

**
HAβ85
BNIP3
Relative mRNA
12
10
8
6
4
2
0
siRNA: Control Control Hypoxia APC APC Hypoxia

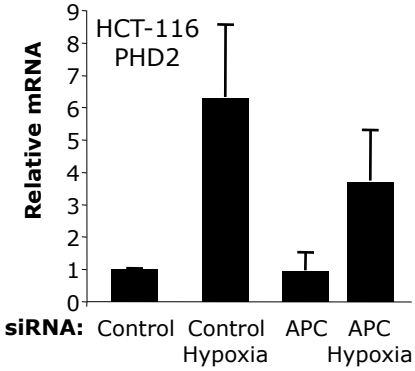

HAβ18
PHD2
Relative mRNA
6
5
4
3
2
1
0
siRNA: Control Control Hypoxia APC APC Hypoxia

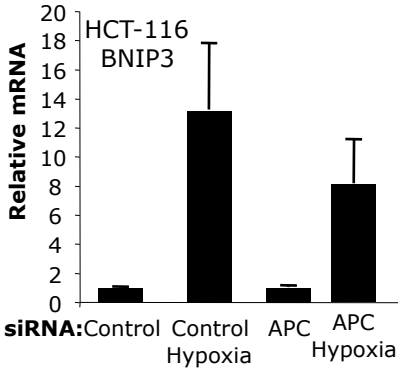

Haβ18
BNIP3
Relative mRNA
14
12
10
8
6
4
2
0
siRNA: Control Control Hypoxia APC APC Hypoxia


HCT-116
Glut3
Relative mRNA
30
25
20
15
10
5
0
siRNA: Control Control Hypoxia APC APC Hypoxia

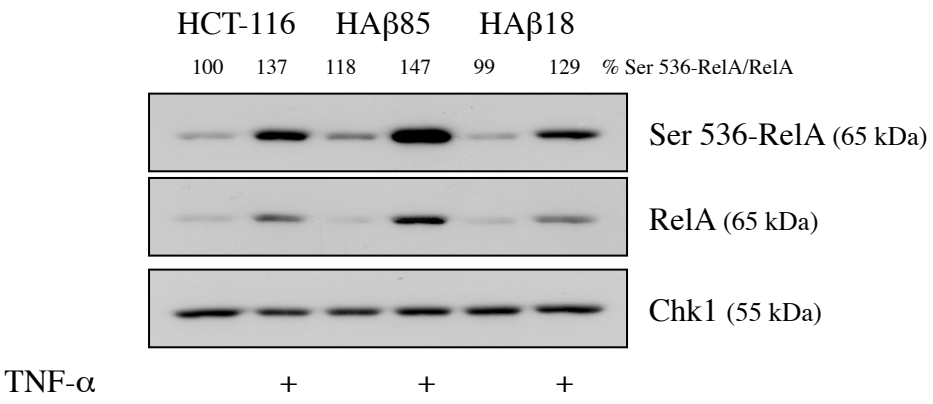

HCT-116
CA9
Relative mRNA
250
200
150
100
50
0
siRNA: Control Control Hypoxia APC APC Hypoxia


HCT-116
ADM
Relative mRNA
16
14
12
10
8
6
4
2
0
siRNA: Control Control Hypoxia APC APC Hypoxia

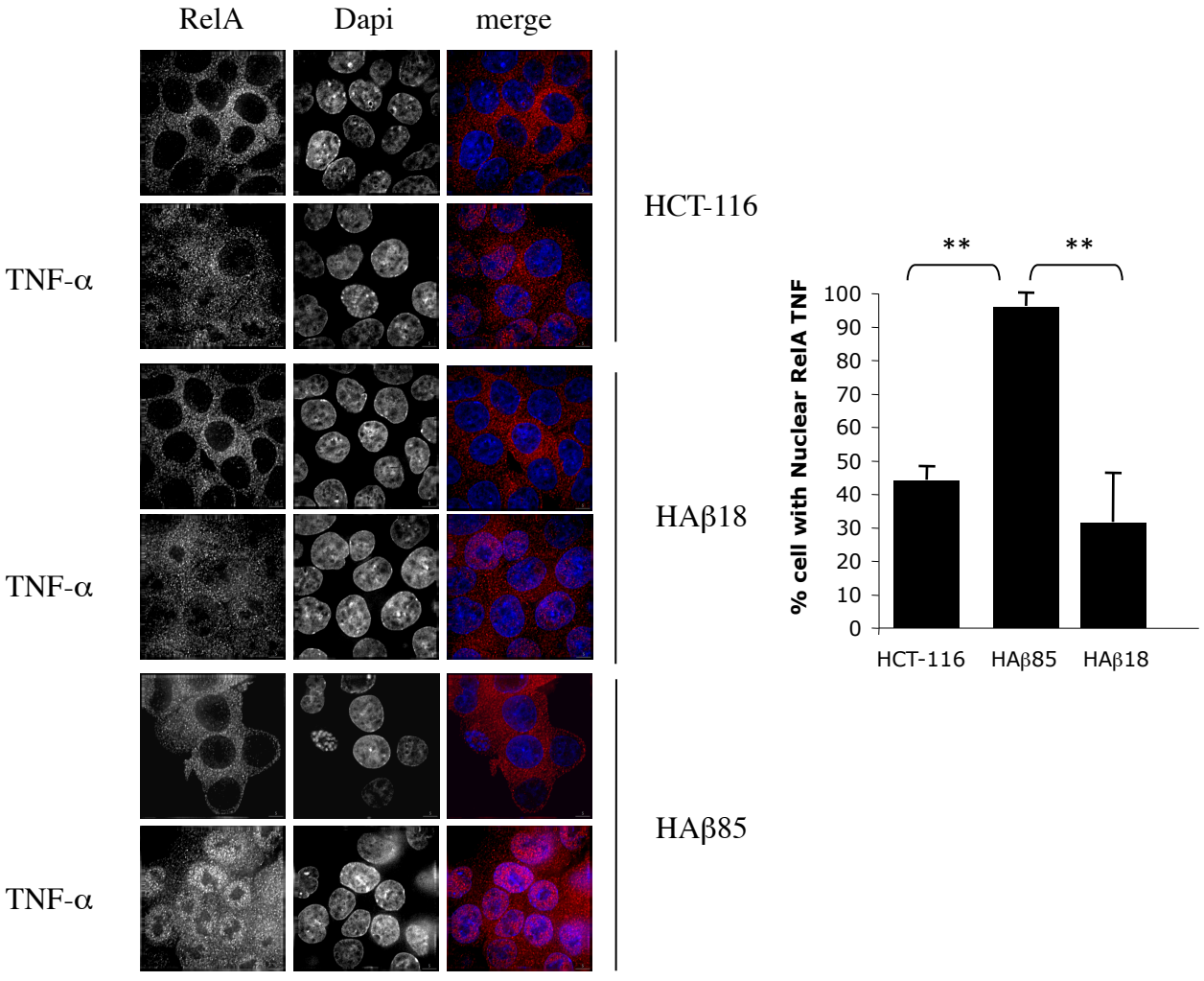

HCT-116
PHD2
Relative mRNA
9
8
7
6
5
4
3
2
1
0
siRNA: Control Control Hypoxia APC APC Hypoxia


HCT-116
BNIP3
Relative mRNA
20
18
16
14
12
10
8
6
4
2
0
siRNA: Control Control Hypoxia APC APC Hypoxia

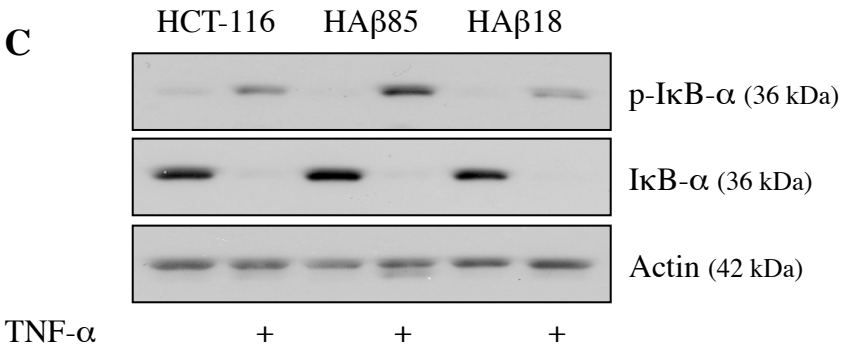
A


HCT-116
HAβ85
HAβ18
100
137
118
147
99
129
% Ser 536-RelA/RelA
Ser 536-RelA (65 kDa)
RelA (65 kDa)
Chk1 (55 kDa)
TNF-α
+
+
+

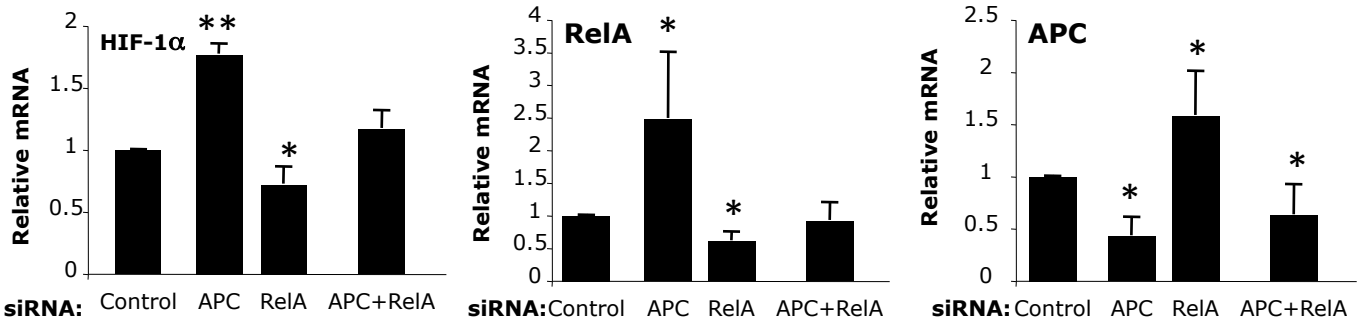
B


RelA
Dapi
merge
HCT-116
TNF-α
HAβ18
TNF-α
HAβ85
TNF-α
**
**
% cell with Nuclear RelA TNF
100
90
80
70
60
50
40
30
20
10
0
HCT-116
HAβ85
HAβ18

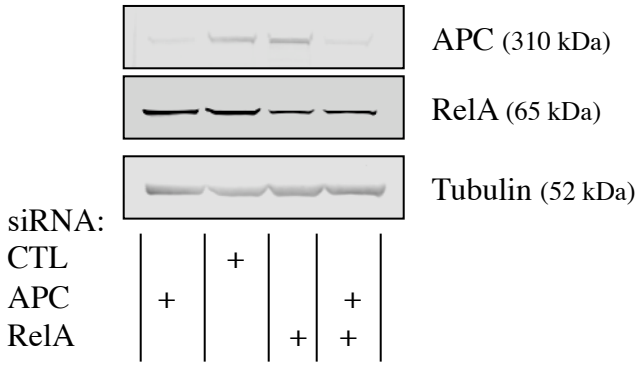
C

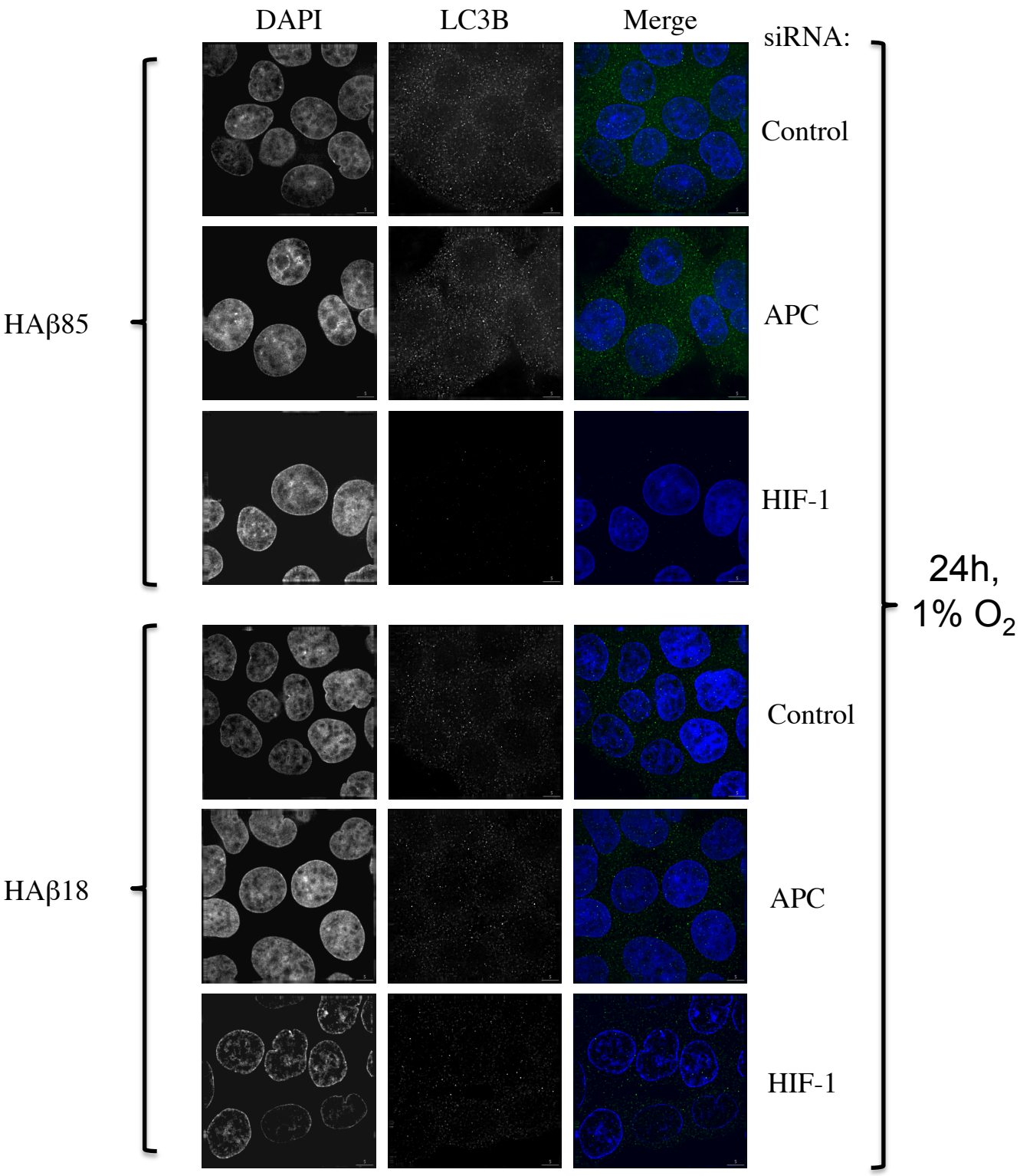

HCT-116
HAβ85
HAβ18
p-IκB-α (36 kDa)
IκB-α (36 kDa)
Actin (42 kDa)
TNF-α
+
+
+

A


HIF-1α
Relative mRNA
2
1.5
1
0.5
0
**
*
siRNA: Control APC RelA APC+RelA
RelA
Relative mRNA
4
3.5
3
2.5
2
1.5
1
0.5
0
*
*
siRNA: Control APC RelA APC+RelA
APC
Relative mRNA
2.5
2
1.5
1
0.5
0
*
*
*
siRNA: Control APC RelA APC+RelA

B


APC (310 kDa)
RelA (65 kDa)
Tubulin (52 kDa)
siRNA:
CTL + +
APC + +
RelA + +


DAPI
LC3B
Merge
siRNA:
Control
APC
HIF-1
HAβ85
Control
APC
HIF-1
HAβ18
24h,
1% O2