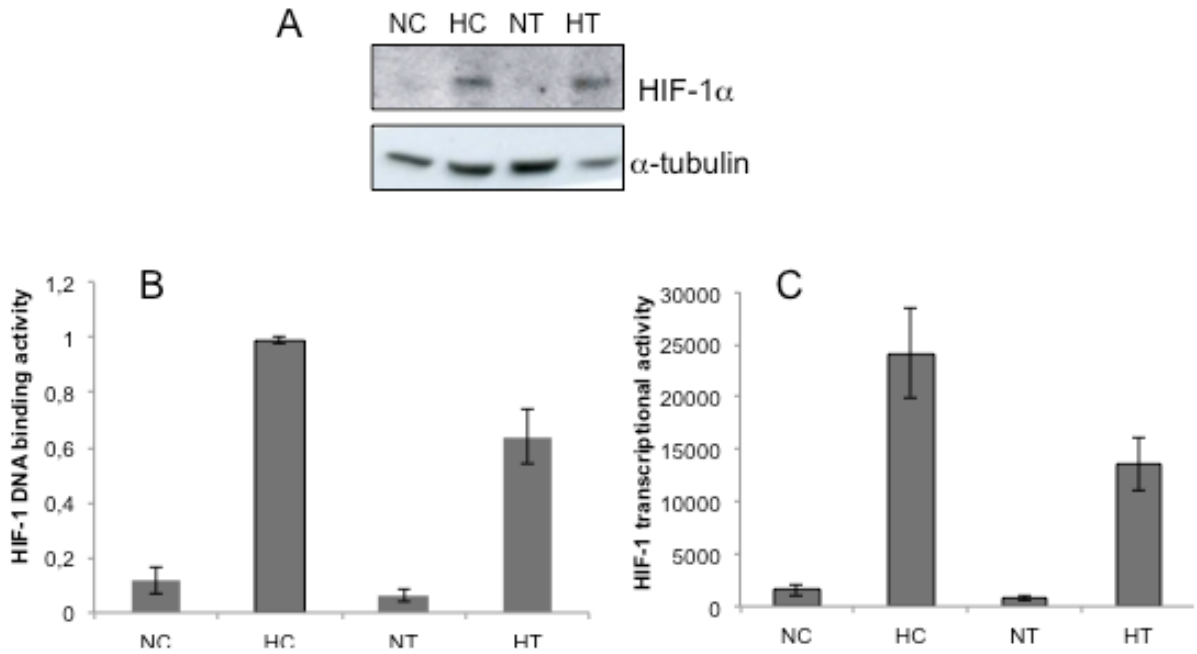
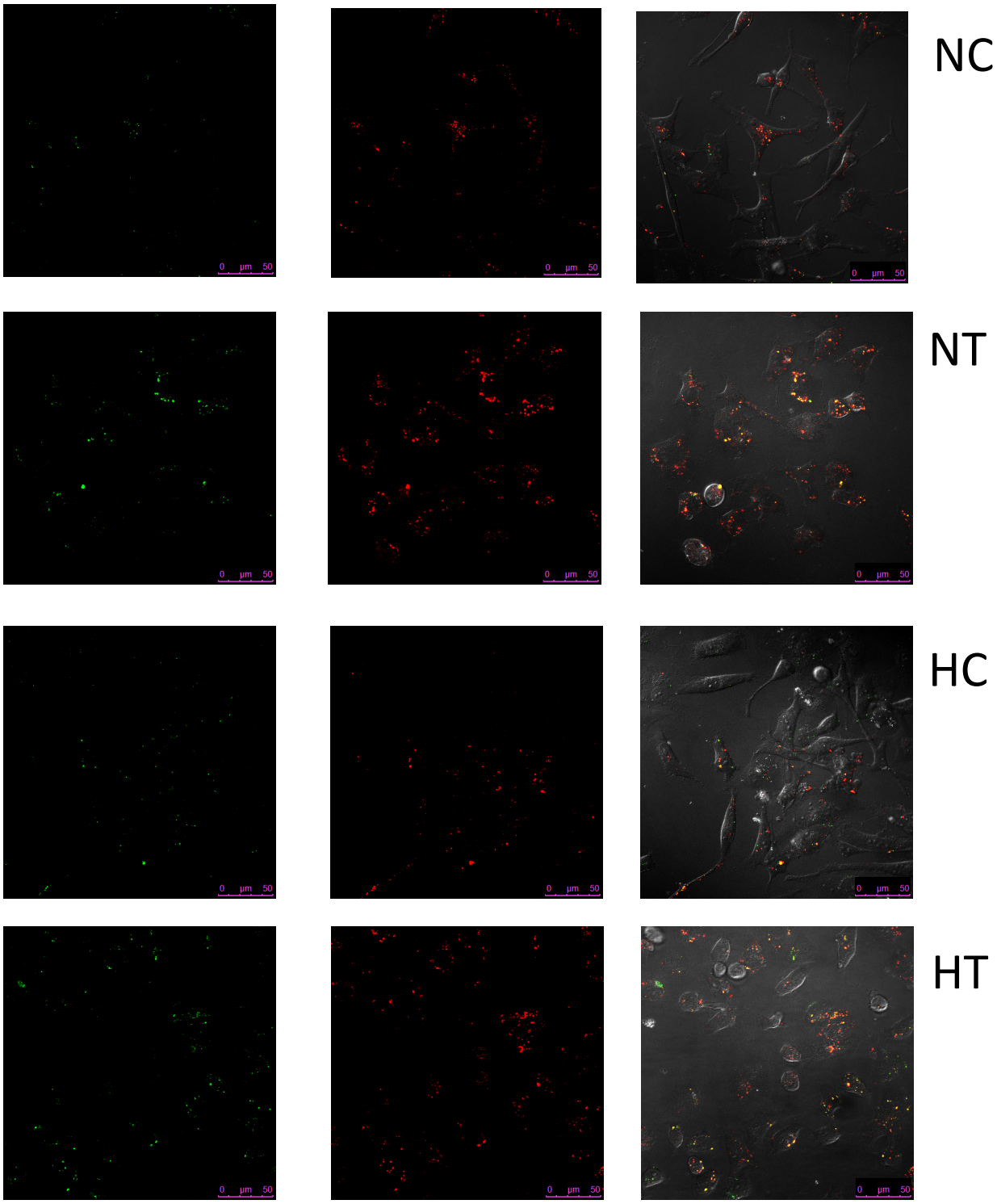


Supplementary data 1

Taxol slightly decreases HIF-1 activation under hypoxia.

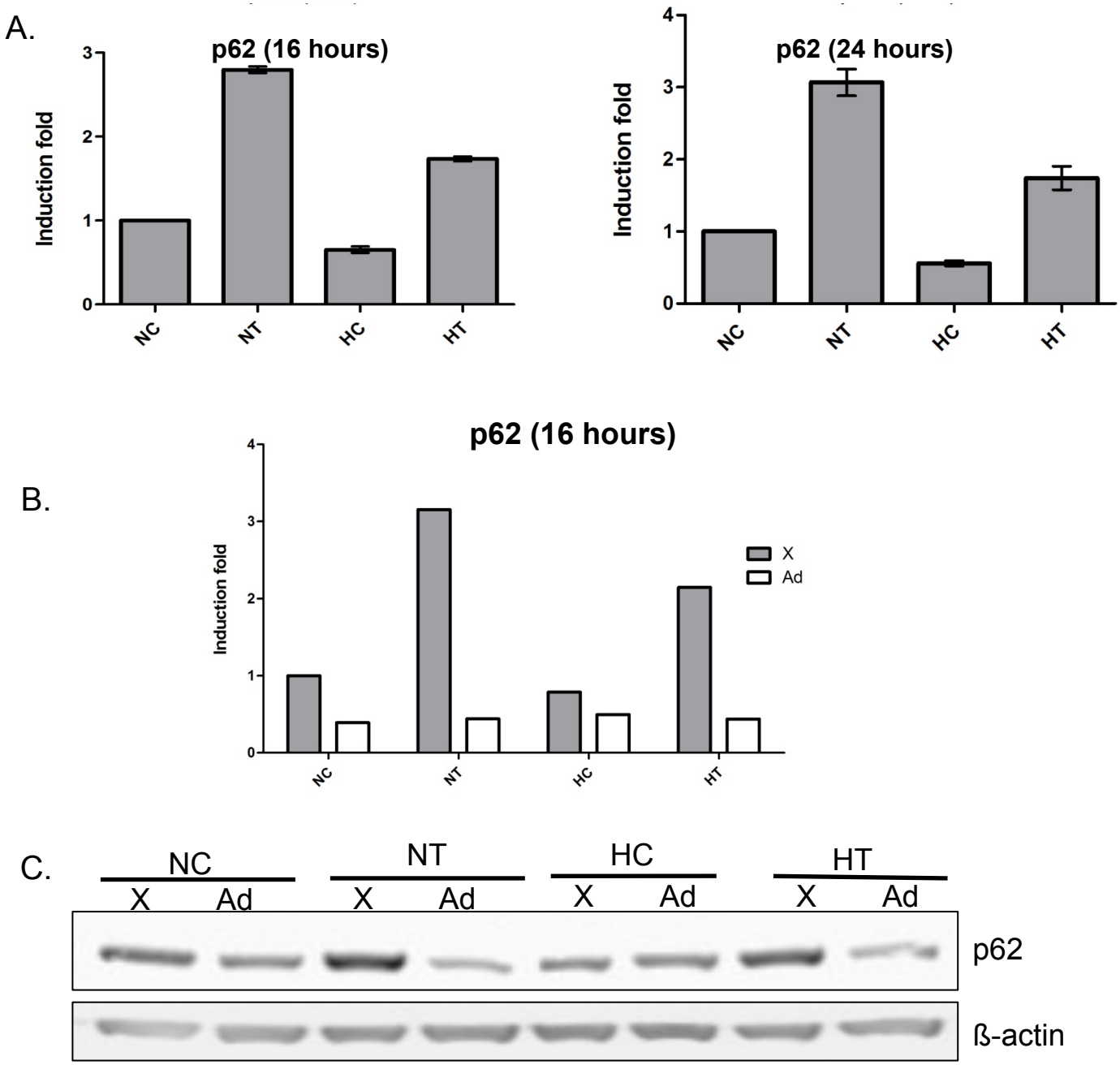
MDA-MB-231 cells were incubated under normoxia (N) or hypoxia (H) without (C) or with taxol (T) at 50 μM. (A) After 4 hours of incubation, HIF-1alpha was detected in total cell extracts by western blotting analysis, using specific antibodies. α-tubulin was used to assess the total amount of proteins loaded on the gel. (B) After 16 hours of incubation, nuclear extracts were recovered and HIF-1 DNA binding activity was assessed using a TransAM assay (means ± 1 SD, n=3). (C) Cells were transfected with the 6HRE-luc plasmid and the renilla-luc plasmid and then incubated as mentioned above. After 16 hours of incubation, luciferase activity was assayed. Results are expressed as the ratio between firefly luciferase and Renilla luciferase activities (means ± 1 SD, n=3).



Supplementary data 2

Degradation within autolysosomes still occurs after taxol exposure.

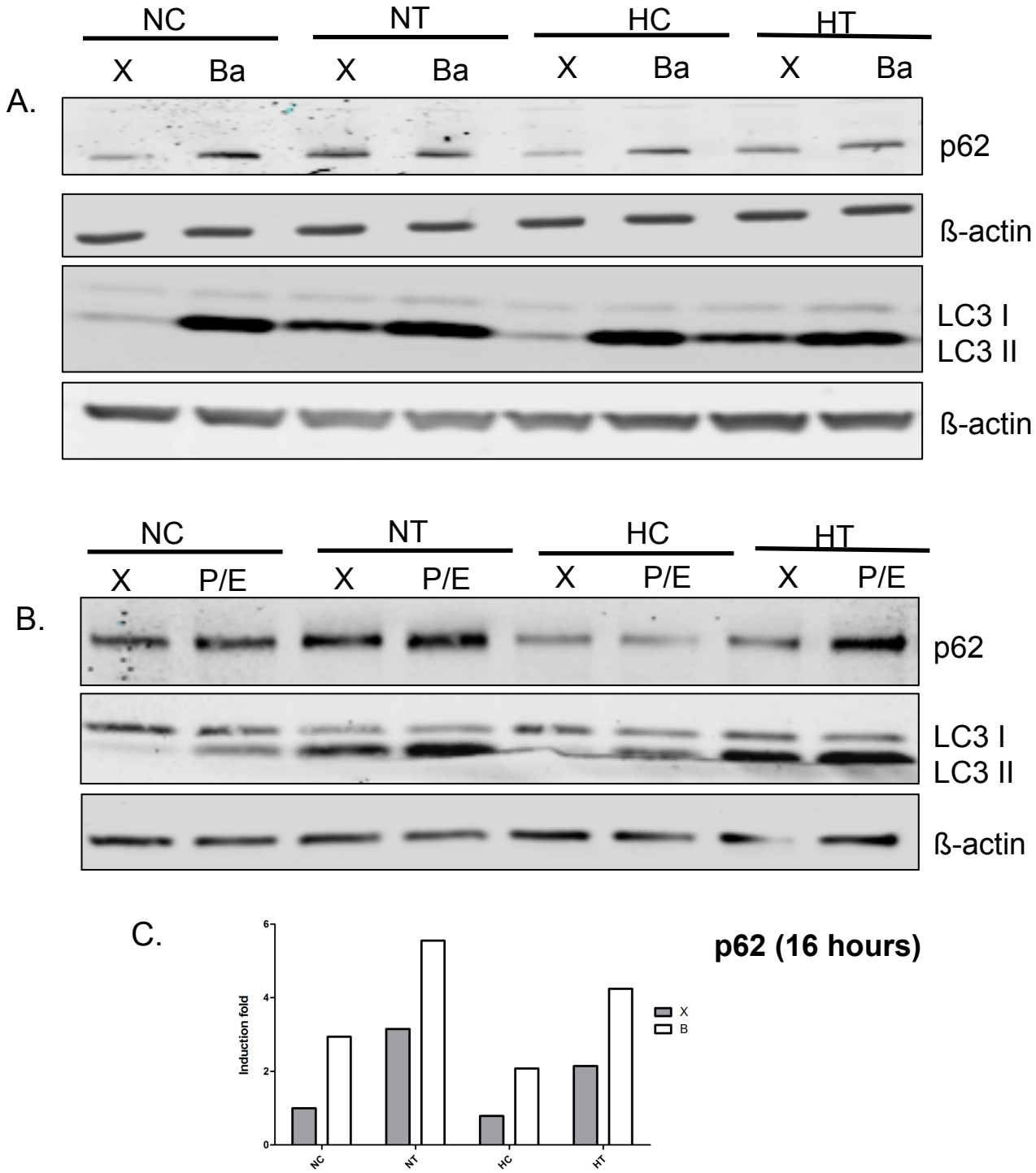
MDA-MB-231 cells were incubated under normoxia (N) or hypoxia (H) without (C) or with taxol (T) at 50 µM for 16 hours. After incubation, cells were incubated for two hours with the DQ-Green-BSA (50 µg/ml) and the lysotracker (100 nM) probes. Cells were then observed alive under confocal microscopy using constant photomultiplier.



Supplementary data 3

Taxol leads to an increase in p62 mRNA expression under normoxia which is lower under hypoxia.

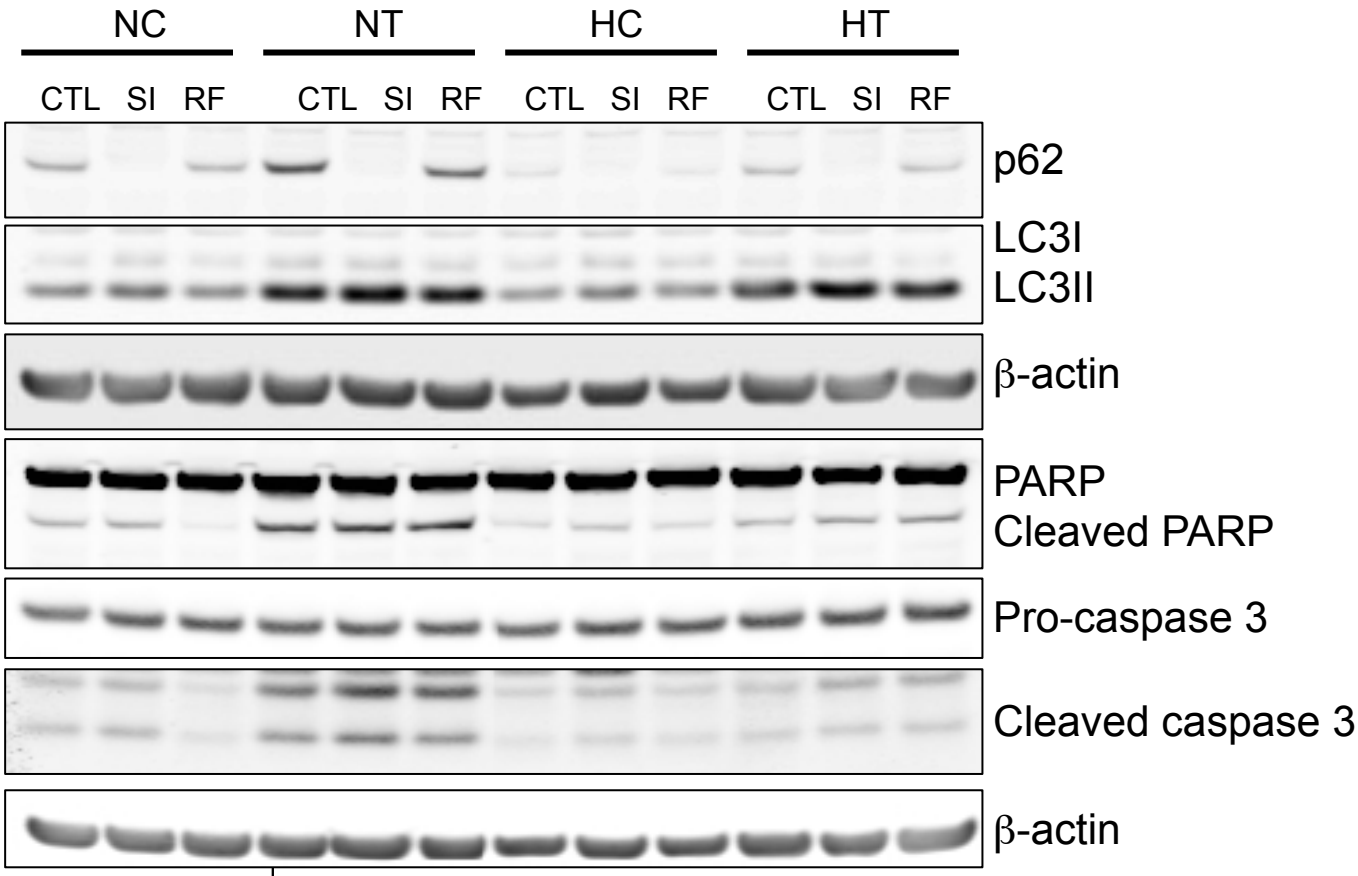
(A) MDA-MB-231 cells were incubated under normoxia (N) or hypoxia (H) without (C) or with taxol (T) at 50 µM. After 16 and 24 hours of incubation, total RNA was extracted, submitted to reverse transcription, and then to real time PCR in the presence of SYBR Green and specific primers for p62 (n=3). (B) MDA-MB-231 cells were incubated under normoxia (N) or hypoxia (H) without (C) or with taxol (T) at 50 µM and without (X) or with actinomycin D (Ad). After 16 hours of incubation, total RNA was extracted, submitted to reverse transcription, and then to real time PCR in the presence of SYBR Green and specific primers for p62 (n=1). RPL13A was used as the housekeeping gene for data normalization. (C) After 16 hours of incubation, LC3 and p62 were detected in total cell extracts by western blotting analysis, using specific antibodies. ß-actin was used to assess the total amount of proteins loaded on the gel.

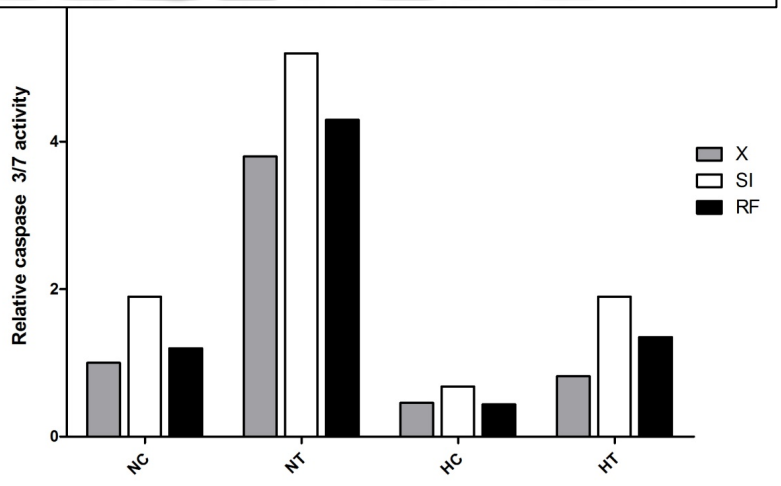


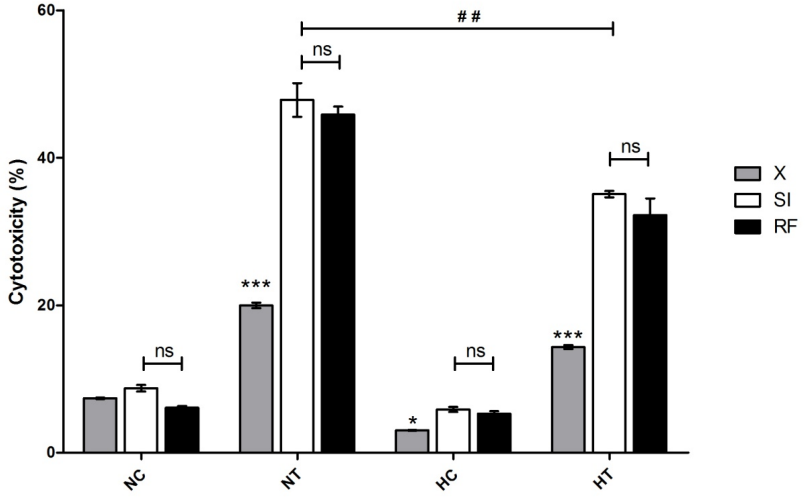
Supplementary data 4

Autophagic degradation is saturated under normoxia but not under hypoxia.

MDA-MB-231 cells were incubated under normoxia (N) or hypoxia (H) without (C) or with taxol (T) at 50 µM and without (X) or with bafilomycin (Ba) at 10 µM or pepstatinA + E64D (P/E) each at 10 µg/ml. (A and B) After 16 hours of incubation, LC3 and p62 were detected in total cell extracts by western blotting analysis, using specific antibodies. ß-actin was used to assess the total amount of proteins loaded on the gel. (C) After 16 hours of incubation, total RNA was extracted, submitted to reverse transcription, and then to real time PCR in the presence of SYBR Green and specific primers for p62. RPL13A was used as the housekeeping gene for data normalization (n=1).


NC
NT
HC
HT
CTL SI RF CTL SI RF CTL SI RF CTL SI RF
p62
LC3I
LC3II
β-actin
PARP
Cleaved PARP
Pro-caspase 3
Cleaved caspase 3
β-actin

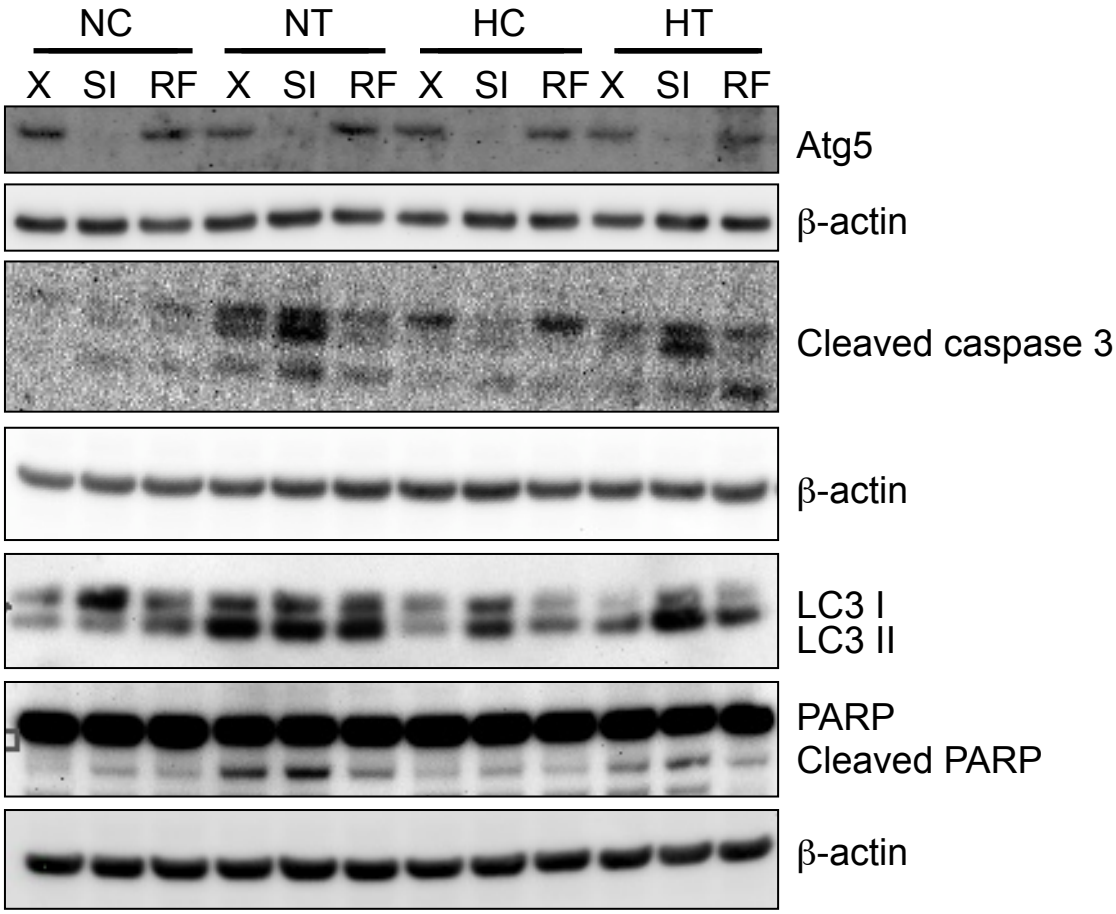

Relative caspase 3/7 activity
0
2
4
NC
NT
HC
HT
X
SI
RF


Cytotoxicity (%)
0
20
40
60
##
ns
ns
ns
ns
***
***
*
NC
NT
HC
HT
X
SI
RF

Supplementary data 5

P62 accumulation is not involved in cell death activation.

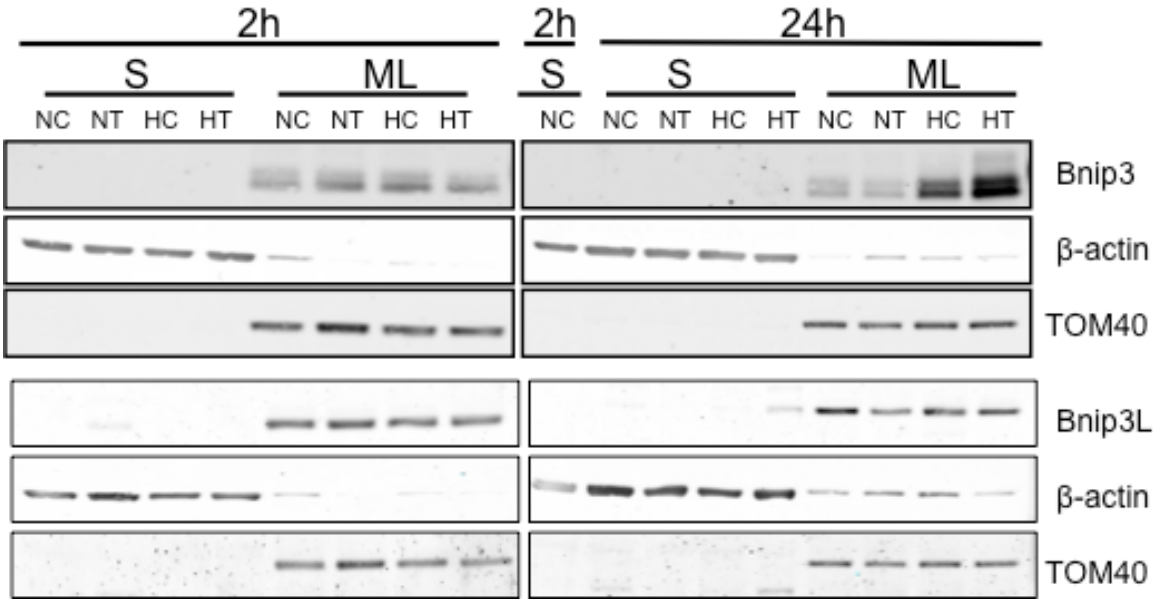
MDA-MB-231 cells were untransfected (X) or transfected with p62 siRNA (SI) or negative control Risc free siRNA (RF) at 50 nM for 24 hours. The transfection media were removed and replaced by culture media for 24 hours. Cells were then incubated under normoxia (N) or hypoxia (H) for 16 hours, without (C) or with taxol (T) at 50 µM. (A) p62, PARP, cleaved PARP, caspase 3 and LC3 were detected in total cell extracts by western blotting analysis, using specific antibodies. ß-actin was used to assess the total amount of proteins loaded on the gel. (B) After 16 hours of incubation, caspase 3 and 7 activity was assayed by measuring fluorescence intensity associated to free AFC released from the cleavage of caspase 3 and 7 substrate Ac-DEVD-AFC. Results are expressed in relative caspase 3/7 activity normalized by fluorescence intensity of the control cells (NC) (n=1). (C) After 40 hours of incubation, LDH release was assayed. Results are expressed as means ± 1 SD (n=3). Statistical analysis was carried out with the two way ANOVA test followed by a Bonferonni post-test.*: significantly different from NC X ($0.05 > p > 0.01$); ***: significantly different from NC X ($p < 0.001$); ##: significant difference between NT X and HT X ($0.01 > p > 0.001$); ns: no significant difference between NC SI and NC RF, HC SI and HC RF, HT SI and HT RF, NT SI and NT RF.



Supplementary data 6

Autophagy promotes cell survival after taxol incubation under normoxia and hypoxia.

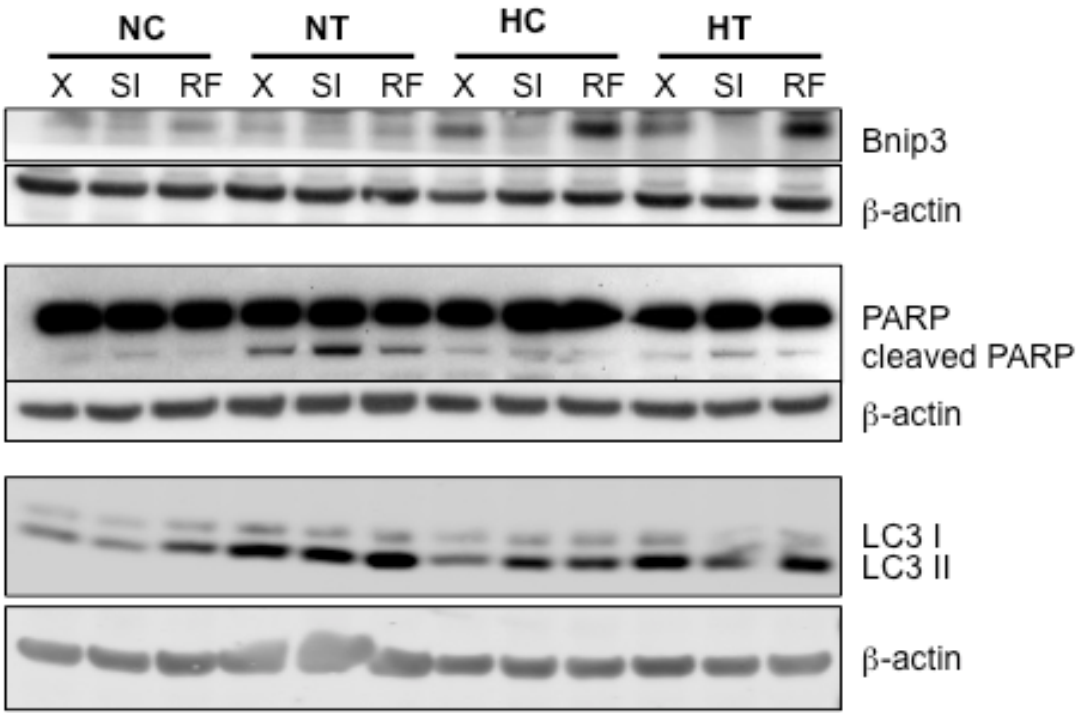
MDA-MB-231 cells were untransfected (X) or transfected with Atg5 siRNA (SI) or negative control Risc free siRNA (RF) at 50 nM for 24 hours. The transfection media were removed and replaced by culture media for 24 hours. Cells were then incubated under normoxia (N) or hypoxia (H) for 16 hours, without (C) or with taxol (T) at 50 µM. Atg5, PARP, cleaved PARP, cleaved caspase 3 and LC3 were detected in total cell extracts by western blotting analysis, using specific antibodies. ß-actin was used to assess the total amount of proteins loaded on the gel.



Supplementary data 7

Hypoxia and taxol increase BNIP3 protein level.

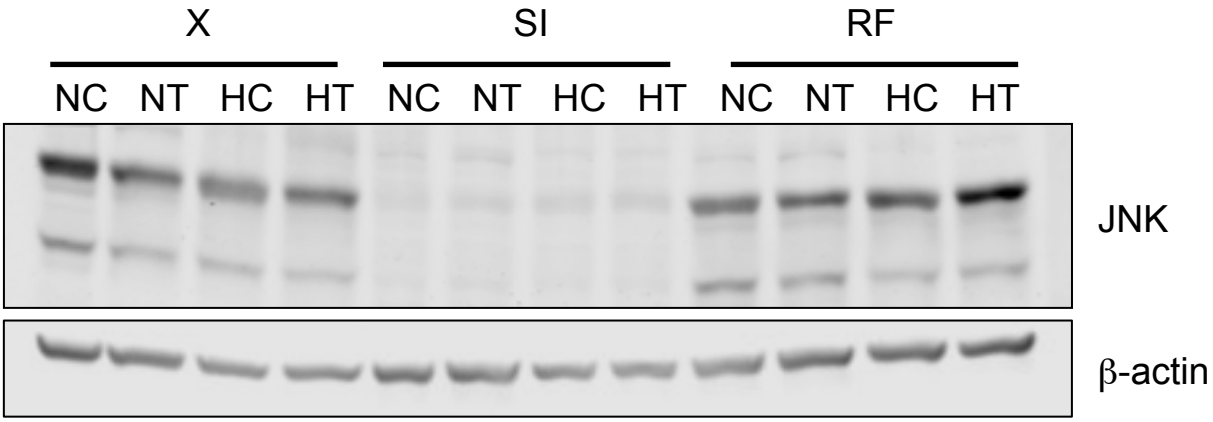
MDA-MB-231 cells were incubated under normoxia (N) or hypoxia (H) without (C) or with taxol (T) at 50 μM. (A) After 2 and 24 hours of incubation, subcellular fractionation was performed and BNIP3 and BNIP3L were detected in the mitochondria containing fraction (ML) and the cytosolic fraction (S) by western blotting analysis, using specific antibodies. β-actin was used to assess the total amount of proteins loaded on the gel for the S fraction and TOM40 for the ML fraction.



Supplementary data 8

BNIP3 silencing does not influence autophagy and apoptosis induction by taxol

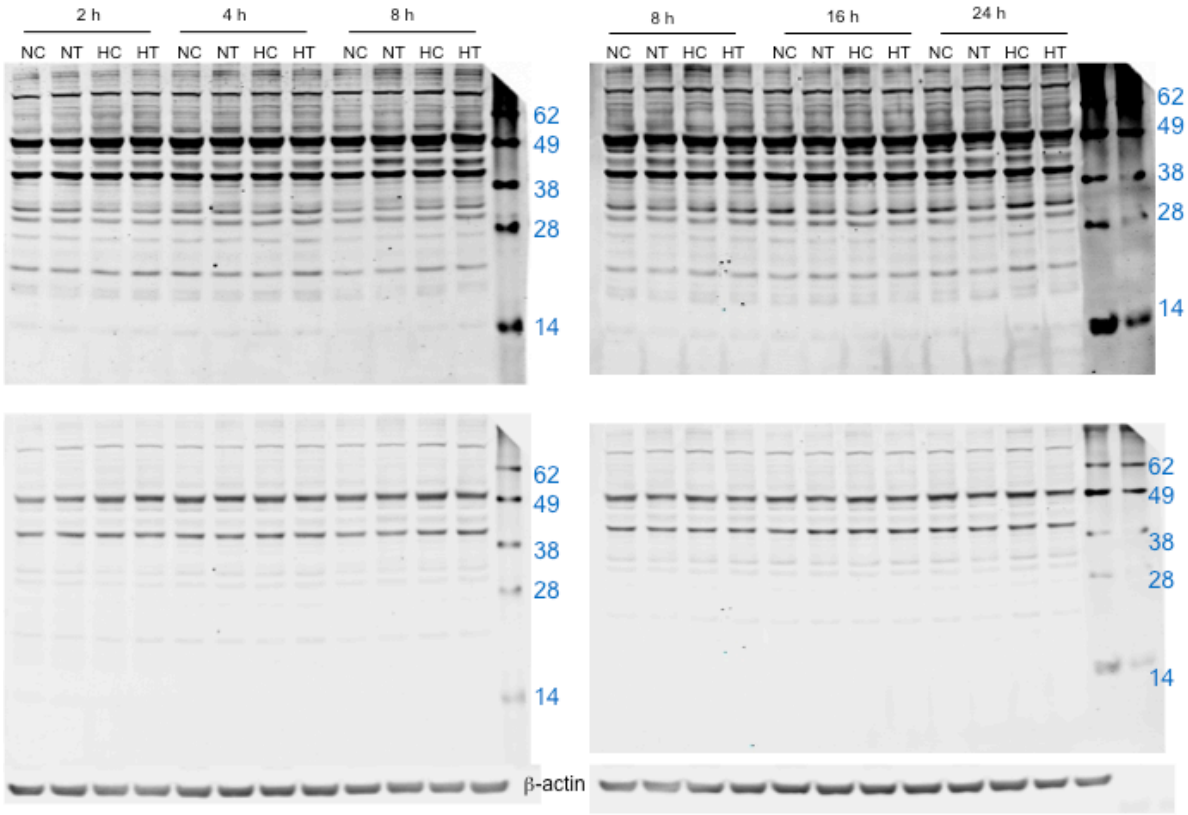
MDA-MB-231 cells were untransfected (X) or transfected with BNIP3 siRNA (SI) or negative control Risc free siRNA (RF) at 50 nM for 24 hours. The transfection media were removed and replaced by culture media for 24 hours. Cells were then incubated under normoxia (N) or hypoxia (H) for 16 hours, without (C) or with taxol (T) at 50 μM. BNIP3, PARP, cleaved PARP and LC3 were detected in total cell extracts by western blotting analysis, using specific antibodies. β-actin was used to assess the total amount of proteins loaded on the gel.



Supplementary data 9

JNK silencing by siRNA.

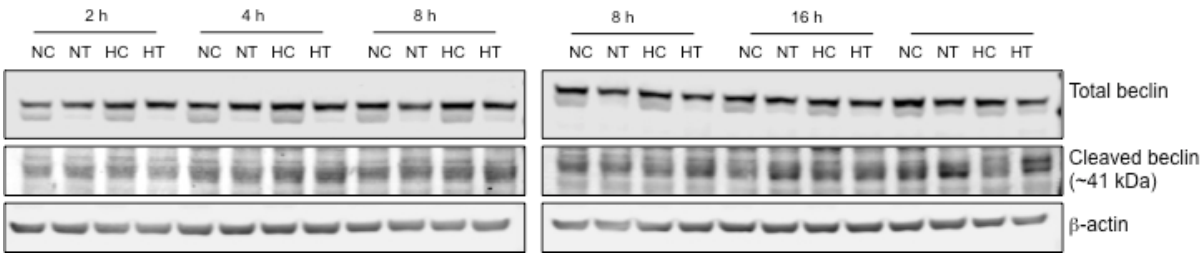
MDA-MB-231 cells were untransfected (X) or transfected with 25 nM of JNK1 and 25 nM of JNK2 siRNA (SI) or negative control Risc free siRNA (RF) at 50 nM for 24 hours. The transfection media were removed and replaced by culture media for 24 hours. Cells were then incubated under normoxia (N) or hypoxia (H) for 16 hours, without (C) or with taxol (T) at 50 µM. JNK was detected in total cell extracts by western blotting analysis, using specific antibodies. ß-actin was used to assess the total amount of proteins loaded on the gel.



Supplementary data 10

Analysis of Atg5 cleavage.

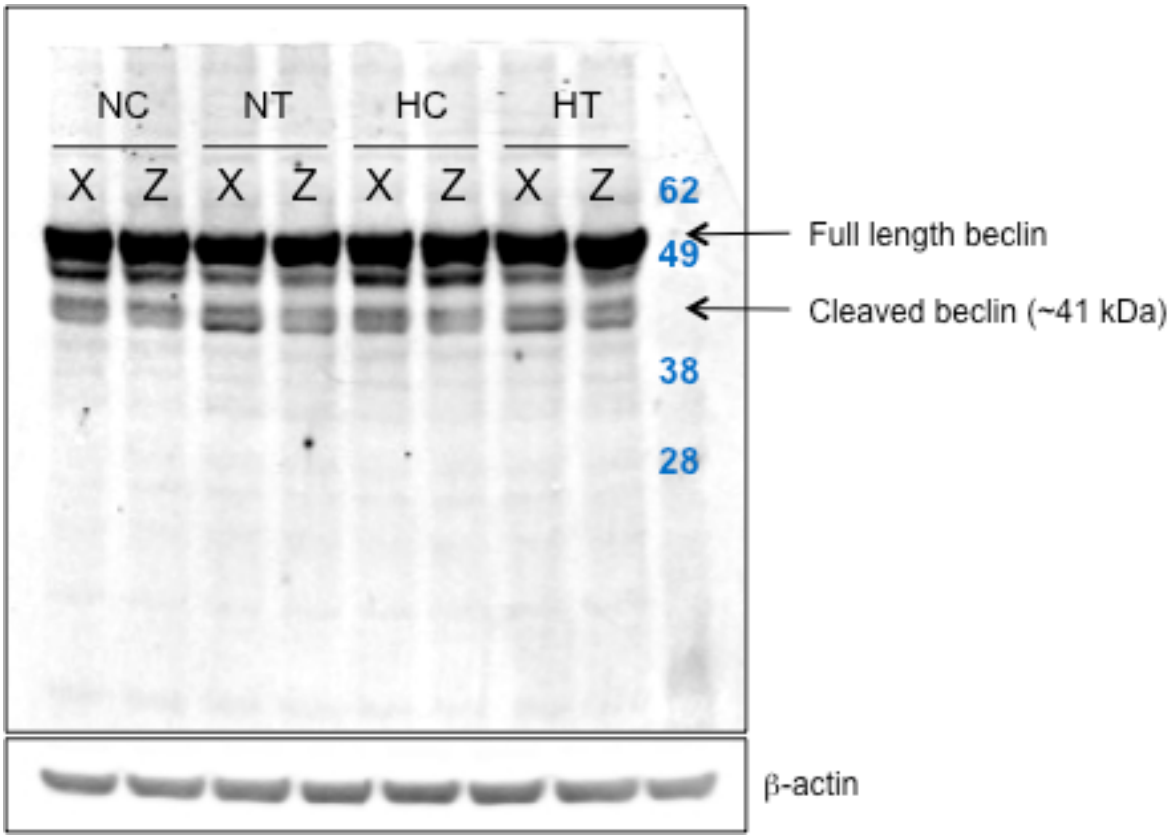
MDA-MB-231 cells were incubated under normoxia (N) or hypoxia (H) without (C) or with taxol (T) at 50 µM. (A) After 2, 4, 8, 16 and 24 hours of incubation, Atg5 was detected in total cell extracts by western blotting analysis, using specific antibodies. β-actin was used to assess the total amount of proteins loaded on the gel. Atg5 apparent molecular weight is 33 kDa, apparent molecular weight for conjugated Atg5-Atg12 is 55 kDa and apparent molecular weight for cleaved Atg5 is 25 kDa.



Supplementary data 11

Analysis of Beclin cleavage.

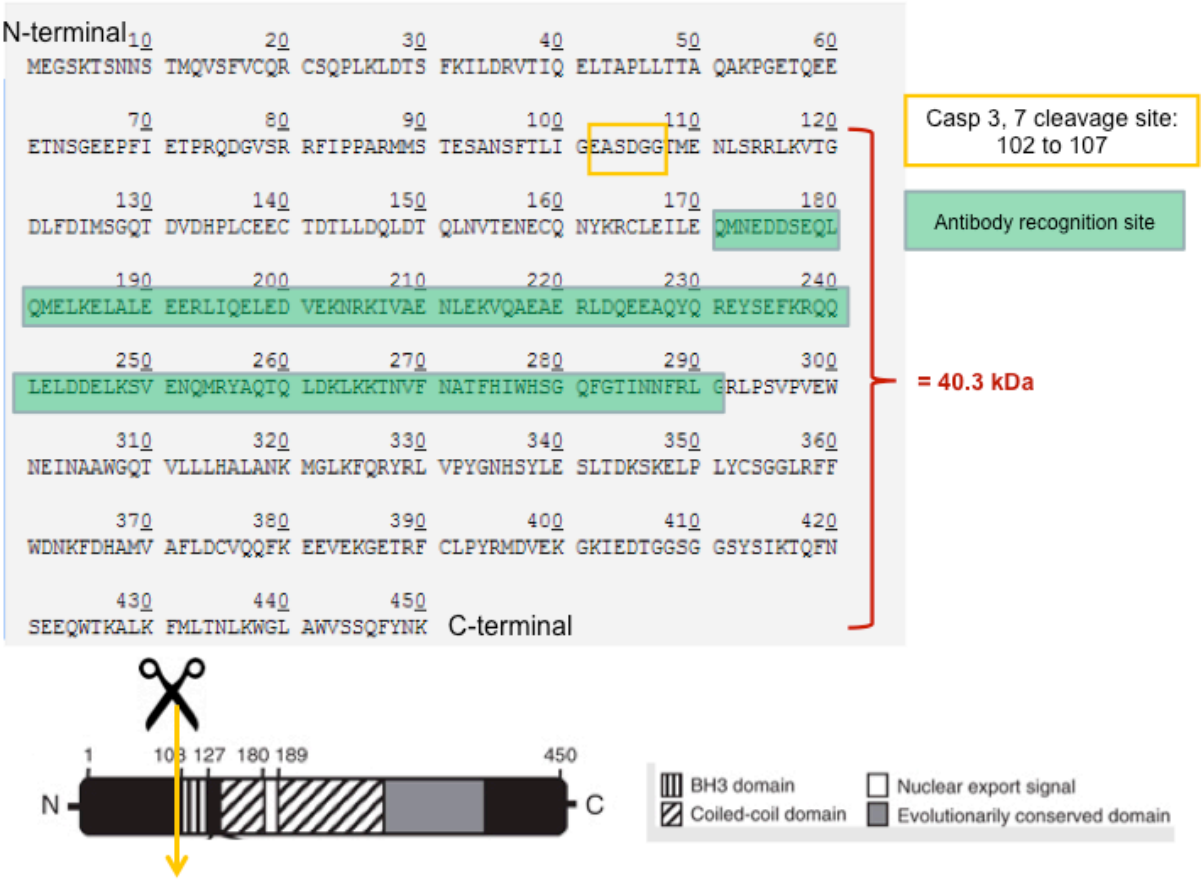
MDA-MB-231 cells were incubated under normoxia (N) or hypoxia (H) without (C) or with taxol (T) at 50 μM. (A) After 2, 4, 8, 16 and 24 hours of incubation, Beclin was detected in total cell extracts by western blotting analysis, using specific antibodies. β-actin was used to assess the total amount of proteins loaded on the gel. Beclin apparent molecular weight is 60 kDa, and apparent molecular weight for cleaved Beclin 1 is described to be 35 and 37 kDa according to Wirawan et al (Cell Death & Disease. 2010;1:e18. Epub 2010/01/01).



Supplementary data 12

Effect of caspase inhibition on Beclin cleavage.

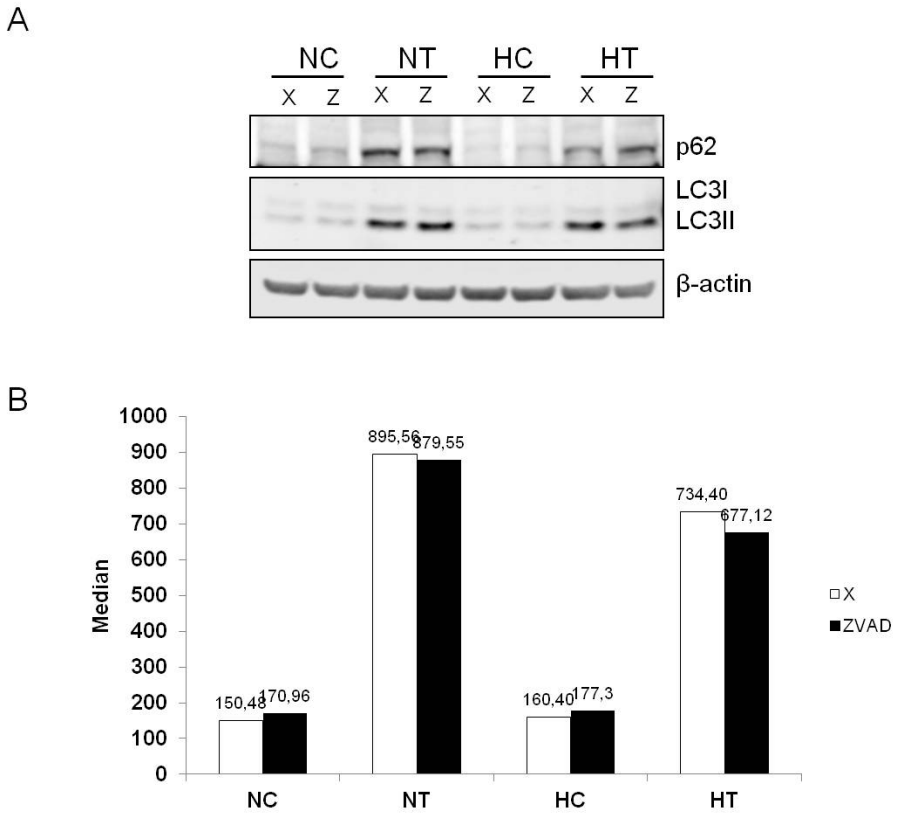
MDA-MB-231 cells were incubated under normoxia (N) or hypoxia (H) without (C) or with taxol (T) at 50 μM and without (X) or with Z-VAD-fmk (Z) at 20 μM. After 16 hours of incubation, Beclin was detected in total cell extracts by western blotting analysis, using specific antibodies. β-actin was used to assess the total amount of proteins loaded on the gel.



Supplementary data 13

Beclin caspase cleavage sites.

Schematic representation of the beclin 1 amino acid sequence showing the potential cleavage site for caspase 3 and caspase 7 proteases in yellow determined by in silico analysis with the siteprediction website (http://www.dmbr.ugent.be/prx/bioit2-public/SitePrediction/). The antibody recognition site is highlighted in green showing that the 40.3 kDa fragment can still be recognized by the antibody. Below, the figure shows that the cleavage after EASD105occurs just upstream from the BH3 only domain.



Supplementary data 14

Effect of caspase inhibition on autophagy.

MDA-MB-231 cells were incubated under normoxia (N) or hypoxia (H) without (C) or with taxol (T) at 50 µM and without (X) or with Z-VAD-fmk (Z) at 20 µM. (A) After 16 hours of incubation, p62 and LC3 abundance was detected in total cell extracts by western blotting analysis, using specific antibodies. ß-actin was used to assess the total amount of proteins loaded on the gel. (B) Cells were incubated in the presence of the DQ-Green-BSA fluorescent dye (unlabelled cells serve as a negative control). After 16 hours of incubation, cells were harvested and analyzed by flow cytometry.