Supplementary Figure Legends

Supplementary Figure 1: p8 silencing provokes an increase of autophagy in cancer cells.

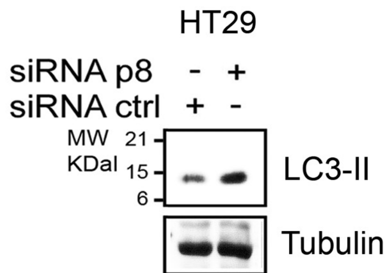
A) *Silencing of p8 causes LC3-II processing in HT29 cells.* Western blot analysis of total cellular lysates obtained from HT29 cells transfected with either *p8* or *crtl* oligonucleotides for 48 hours. Membranes were developed using anti LC3B, and Tubulin antibodies. **B)** *Silencing of p8 causes LC3-II processing in U87MG cells. Efficacy of siRNA mediated RNAi of U87MG cells.* Upper panel: Western blot analysis of total cellular lysates obtained from U87MG cells transfected with either *p8* or *crtl* oligonucleotides for 48 hours. Membranes were developed using anti LC3B, and actin antibodies as treatment and loading controls respectively. Lower panel: Cells were transfected either with oligos targeting p8 (*siRNA p8*) or with a mix of two non-targeting oligonucleotides as control (*siRNActrl*). After 24 hours total RNA was prepared and analyzed by RT-PCR, using *p8*-specific and *actin*-specific oligonucleotides. **C)** *p8 RNAi decreases cellular viability in response to autophagy stimuli.* U87MG cells were cultured for 48 hours following *p8* or *ctrl* siRNA. After this time the medium was changed and cell cultured for further 48 hours with (10%FBS) or without FBS (SF) or 1mM AICAR (AICAR) before cell viability assays. The values represent the mean $\pm$ standard deviation from the mean for two independent experiments made in triplicate. As indicated, samples were subjected to unpaired student's t-test to determine the significance of changes observed. #$p<0.05$.

Supplementary Figure 2: Characterization of stable cell lines and efficiency of knockdowns.

A) *Stable expression of GFP or GFPp8 in U2OS cells.* U2OS were transfected with pEGFP or pEGFp8 constructs using Fugene (Roche). The following day cells were replated and selected for one week with G418. Expression of the transgene and percentage of cells overexpressing the GFP was monitored by Western blot and immunofluorescence. Representative immunofluorescence is shown here. Cellular nuclei were labeled with Hoechst. **B)** *Stable knockdown of p8 in U2OS cells.* RT-PCR analysis of *p8* and *actin* expression using total RNA extracted from U2OS cells infected with either *p8* or control lentiviruses and selected for one week with 3µg/ml of puromycin. **C)** *Efficiency of atg5 and Bnip3 knockdown in U2OS cells.* U2OS were cultured for 48 hours in serum free after *ctrl*, *atg5, p8 and bnip3* alone or after combined *p8+atg5, p8+bnip3 and atg5+bnip3* RNAi. The western blot analysis of the total cellular lysates was performed using Atg5-12, Bnip3 and Tubulin antibodies (the latter membrane is the same shown in the Figure 4E).

Supplementary Figure 3: Cellular energy stress acts on p8 expression.

A) *Endogenous p8 is rapidly induced by AICAR in U2OS cells*. Western blot analysis of human U2OS cells treated for the indicated times with 1mM AICAR in 10% FBS. 10ng/ml of TNF was used as positive control for p8 up-regulation. Western blots were carried out with two different antibodies Ab1 and Ab2 for p8. P-JNK and actin antibodies were used as control. **B)** *Endogenous p8 is induced by energy and amino acid deprivation in rat cardiomycytes*. Western blot analysis of primary rat cardiomyocytes incubated for the indicated times in PBS containing 20mM D-Glucose or in DMEM without glucose but containing 20mM 2-deoxyGlucose (2DG). Immunoblots were carried out using anti p8, anti phospho S6 (P-S6) or SGK1 (SGK) antibodies.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HT29
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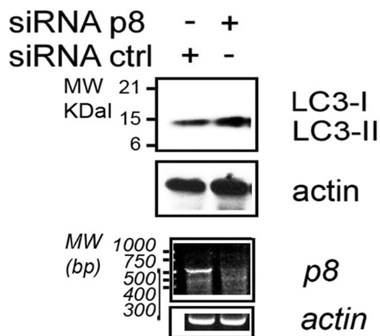
siRNA ctrl + -
MW KDal
21
15
6
LC3-II
Tubulin


B
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siRNA ctrl + -
MW KDal
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15
6
LC3-I
LC3-II
actin
MW (bp)
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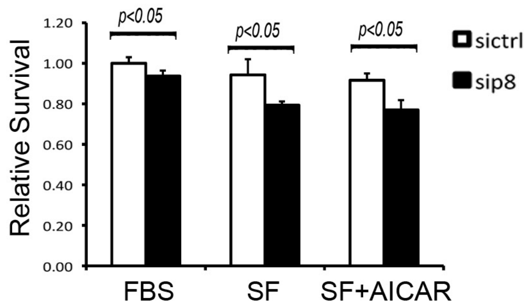
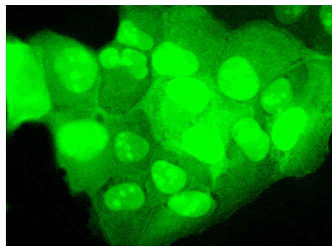
750
500
400
300
p8
actin

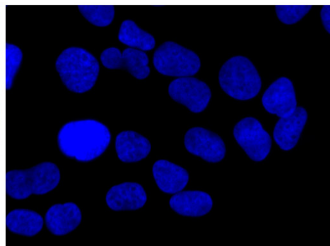

C
Relative Survival
1.20
1.00
0.80
0.60
0.40
0.20
0.00
p<0.05
p<0.05
p<0.05
sictrl
sip8
FBS
SF
SF+AICAR

A

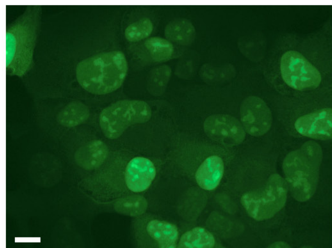
Hoechst

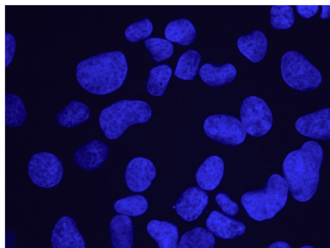
pEGFP



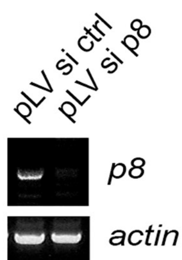


pEGFP p8



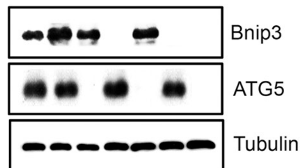


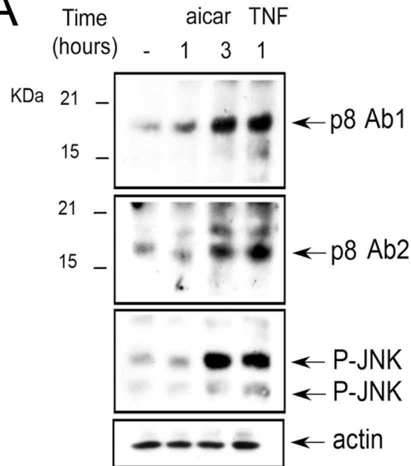
B



C

siRNA p8	-	+	-	-	+	+	-
siRNA ATG5	-	-	+	-	+	-	+
siRNA Bnip3	-	-	-	+	-	+	+
siRNA sictrl	+	-	-	-	-	-	-




A
Time
(hours)
aicar
TNF
-
1
3
1
KDa
21
15
p8 Ab1
21
15
p8 Ab2
P-JNK
P-JNK
actin
B
PBS+Glu
DMEM +2DG
Time
0
15
30
60
3h
15
30
60
3h
KDa
21
15
p8
P-S6
SGK