SLC33A1/AT-1 REGULATES THE INDUCTION OF AUTOPHAGY DOWN-STREAM OF IRE1/XBP1

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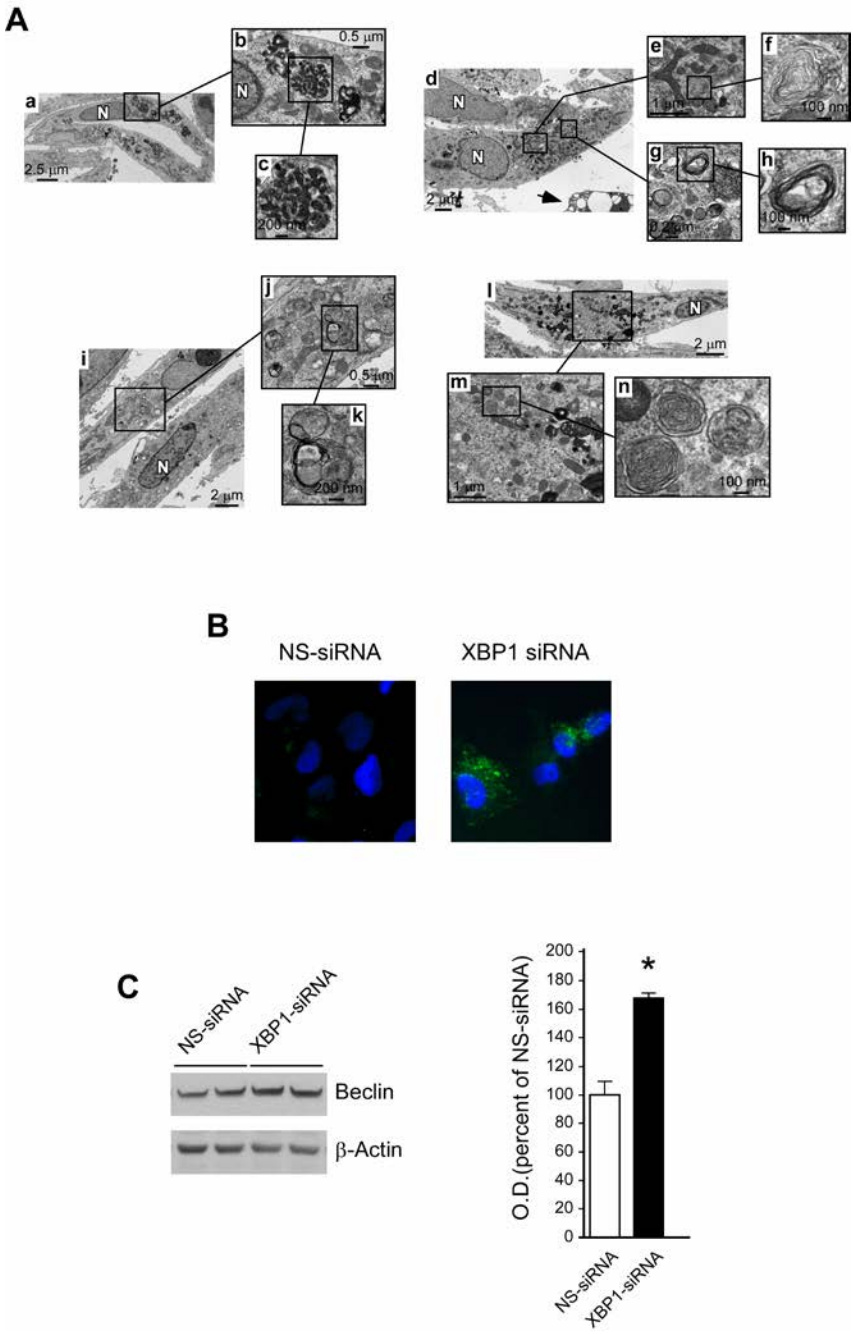
SUPPLEMENTAL DATA

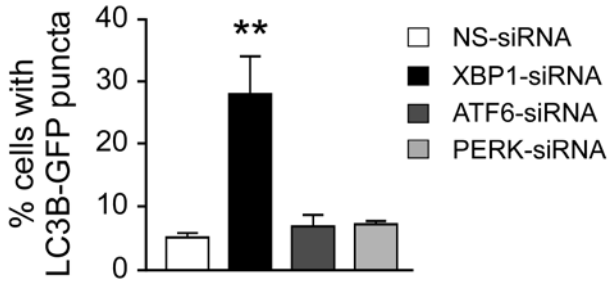
Supplemental Table S1. Primers used in real-time PCR experiments.

Primer pair	Sequence	Annealing temperature
AT-1	Forward: 5'-CAGGCGGTTGGGATGACAGT-3' Reverse: 5'-AAGATTTGCGACGACCGAAGTT-3'	55°C
ATF6	Forward: 5′-TCGGTTCAGATATTGCTGTGCTAAGG-3′ Reverse: 5'-CTCTTCGCTTTGGACTAGGGACTTTAAG-3′	59°C
GAPDH	Forward: 5'-TTTGTCAAGCTCATTTCCTGGTA-3' Reverse: 5'-TTCAAGGGGTCTACATGGCAACTG-3'	55°C
PERK	Forward: 5'-GGGACTATGGATGGCAATGATGAG-3' Reverse: 5'-GTGGTTGGTCTTGGAGGAGAAATAGAC-3'	55°C
Spliced XBP1*	Forward: 5'-CCGCAGCAGGTGCAGG-3' Reverse: 5'-GAGTCAATACCGCCAGAATCCA-3'	59°C
Total XBP1*	Forward: 5'-GCAAGCGACAGCGCCT-3' Reverse: 5'-TTTTCAGTTTCCTCCTCAGCG-3'	59°C

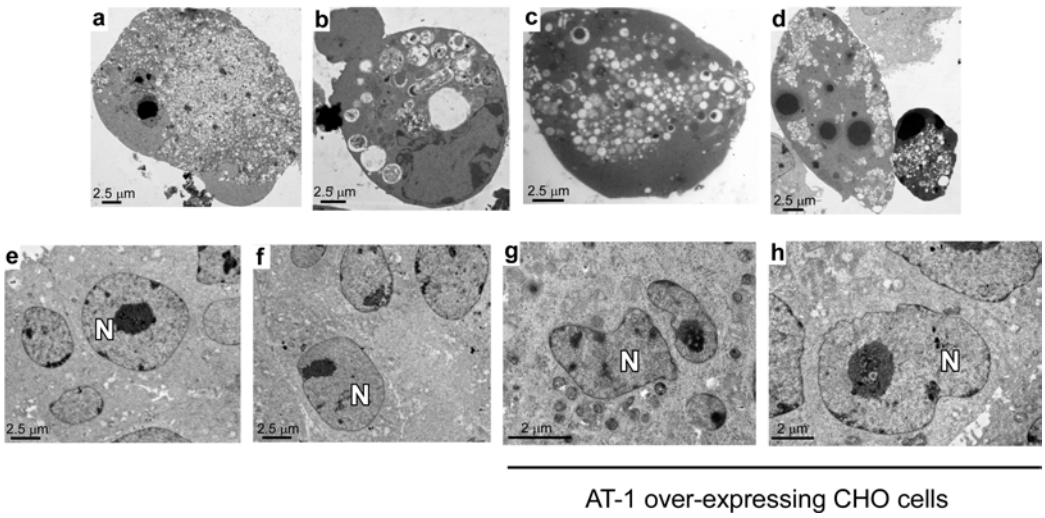
* Primers for total and spliced XBP1 were described in Back, S. H., Schroder, M., Lee, K., Zhang, K., and Kaufman, R. J. (2005) *Methods* **35**, 395-416.



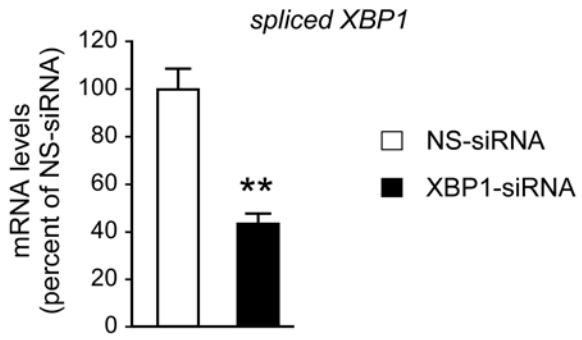
Supplemental Figure S1. Down-regulation of XBP1 induces autophagy.
A, H4 cells were treated with the siRNA against XBP1 for 4 days prior to transmission electron microscopy. Different cellular features that are typical of the early stages of autophagy are shown. Nuclei are labeled with N. Mitochondria appear normal. Arrow in *d* points to a cell with advanced autophagic features. *B*, H4 cells were treated with non-silencing (NS-siRNA) or XBP1-specific (XBP1-siRNA) siRNAs for 3 days and then transduced with BacMan GFP-LC3B. The induction of autophagy, as assessed by the redistribution of LC3B was evaluated 24 hours later. Nuclei were counterstained with DAPI (blue). *C*, Western blot assessment of beclin levels following 3 days of XBP1-siRNA treatment. Representative images are shown in the left panel while quantitative data is shown in the right panel. *, $P<0.05$.



Supplemental Figure S2. Down-regulation of ATF6 or PERK does not cause redistribution of LC3B to autophagosomes.
H4 cells were treated with non-silencing (NS-siRNA) or the indicated siRNAs for 3 days prior to BacMan GFP-LC3B transduction. The induction of autophagy, as assessed by the redistribution of LC3B was evaluated 24 hours later. Results are expressed as percent of non-silencing siRNA and are the average (n = 3) $\pm$ S.E.M.; **, p<0.005.



Supplemental Figure S3. Over-expression of AT-1 prevents autophagic cell death in Chinese Hamster Ovary (CHO) cells.
CHO cells were treated with siRNA against XBP1 for 4 days prior to transmission electron microscopy. Panels *a-f* show untransfected CHO cells whereas panels *g-h* show CHO cells over-expressing transgenic AT-1. Panels *a-d*, XBP1-siRNA treated cells; panel *e*, control (untreated) cells; panel *f*, non silencing-siRNA (NS-siRNA) treated cells; panels *g* and *h*, XBP1-siRNA treatment of CHO cells over-expressing transgenic AT-1. Cells in panels *a-d* display features that are typical of autophagic cell death. Cells in *g* and *h* display normal features and no evidence of cell death.



Supplemental Figure S4. Successful down-regulation of XBP1 in AT-1 over-expressing cells.
H4 cells over-expressing CMV-regulated transgenic AT-1 were treated with the siRNA against XBP1 for 3 days prior to quantitative real-time assessment of sXBP1 mRNA levels. Results are expressed as percent of non-silencing siRNA (NS-siRNA) and are the average (n=3) $\pm$ SEM **, P<0.005.