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Supplemental Material to:

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Loss of a membrane trafficking protein αSNAP induces non-canonical autophagy in human epithelia

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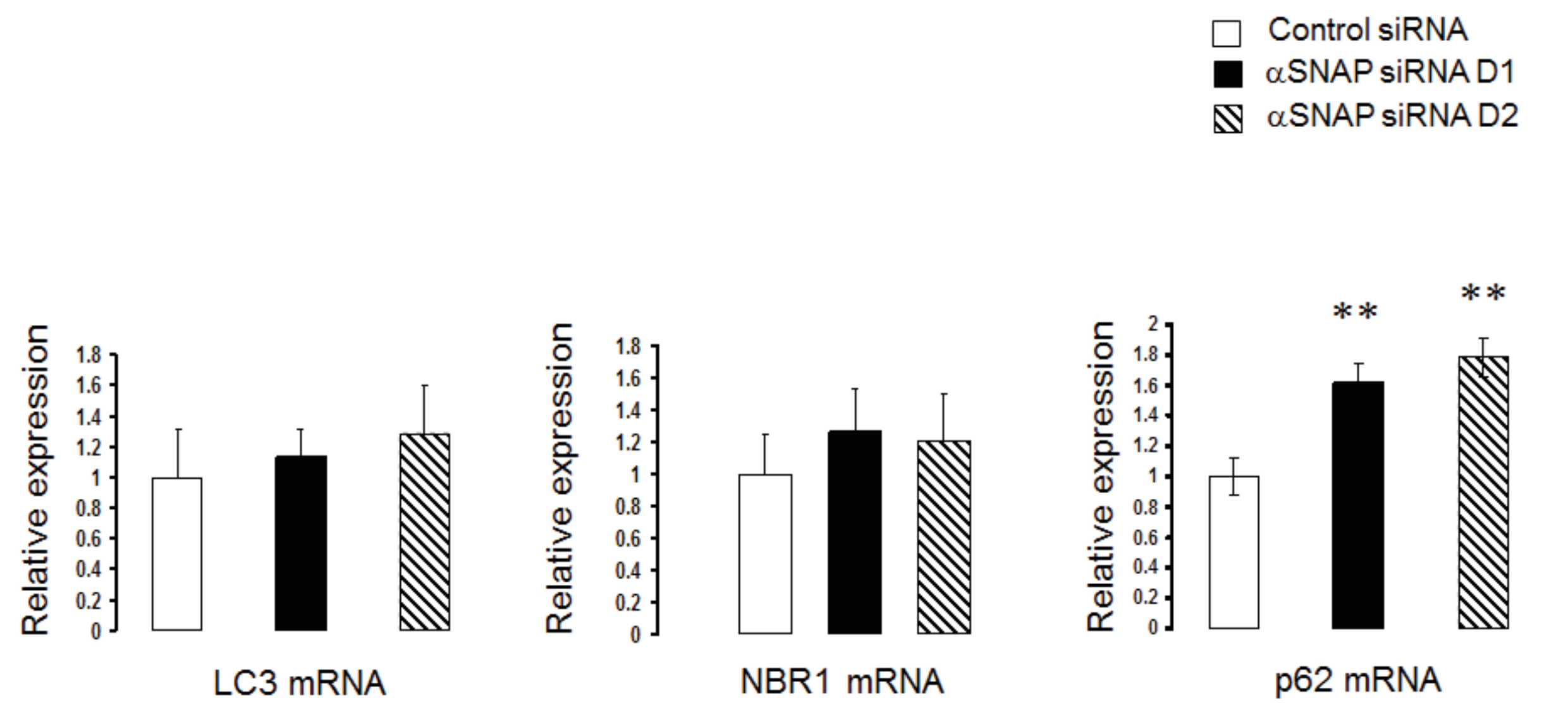
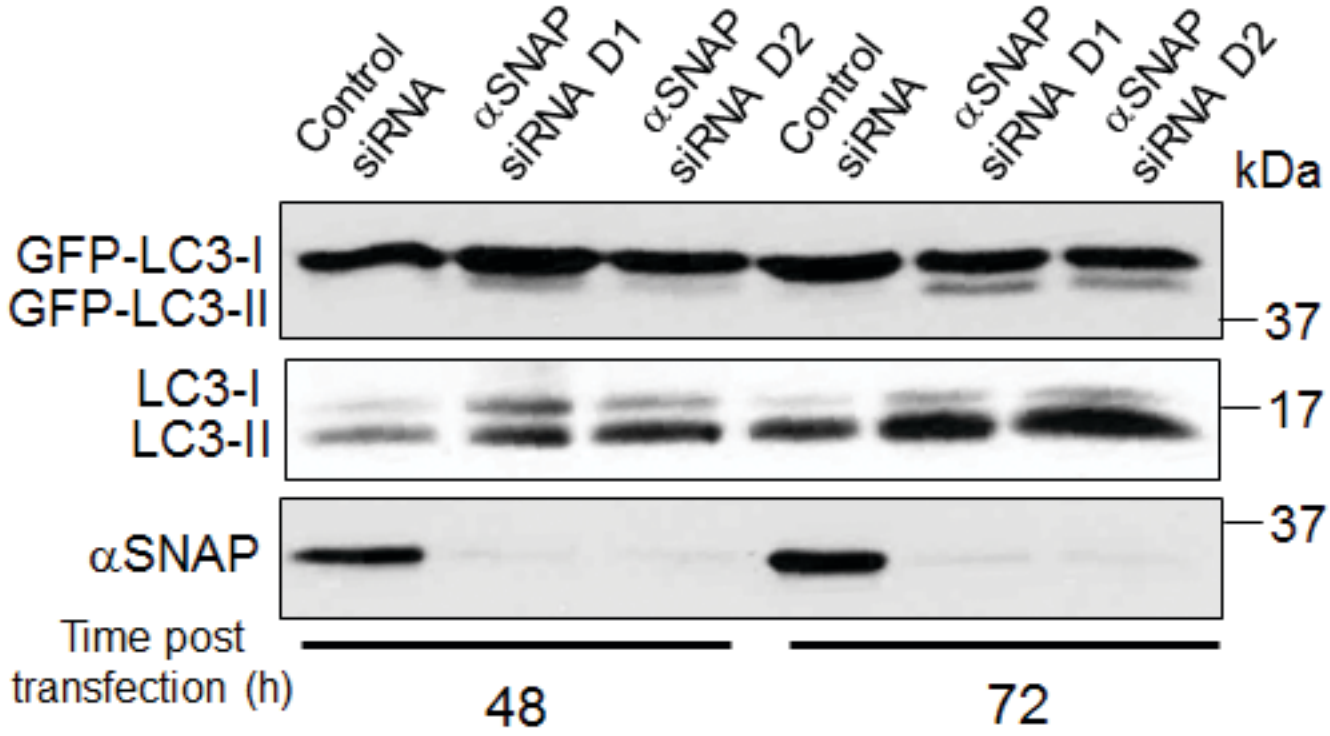
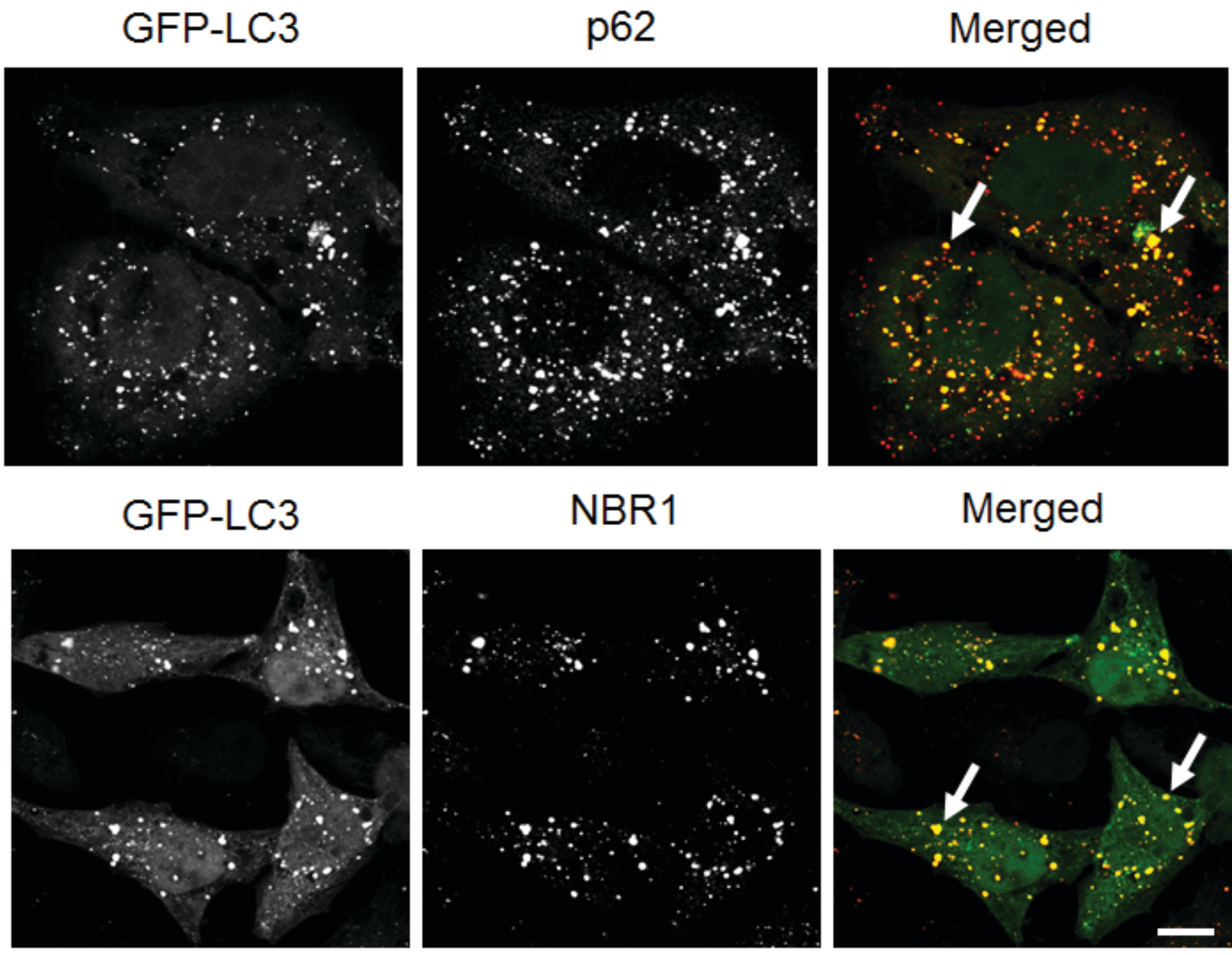

Control siRNA
αSNAP siRNA D1
αSNAP siRNA D2
Relative expression
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
**
**
LC3 mRNA
NBR1 mRNA
p62 mRNA

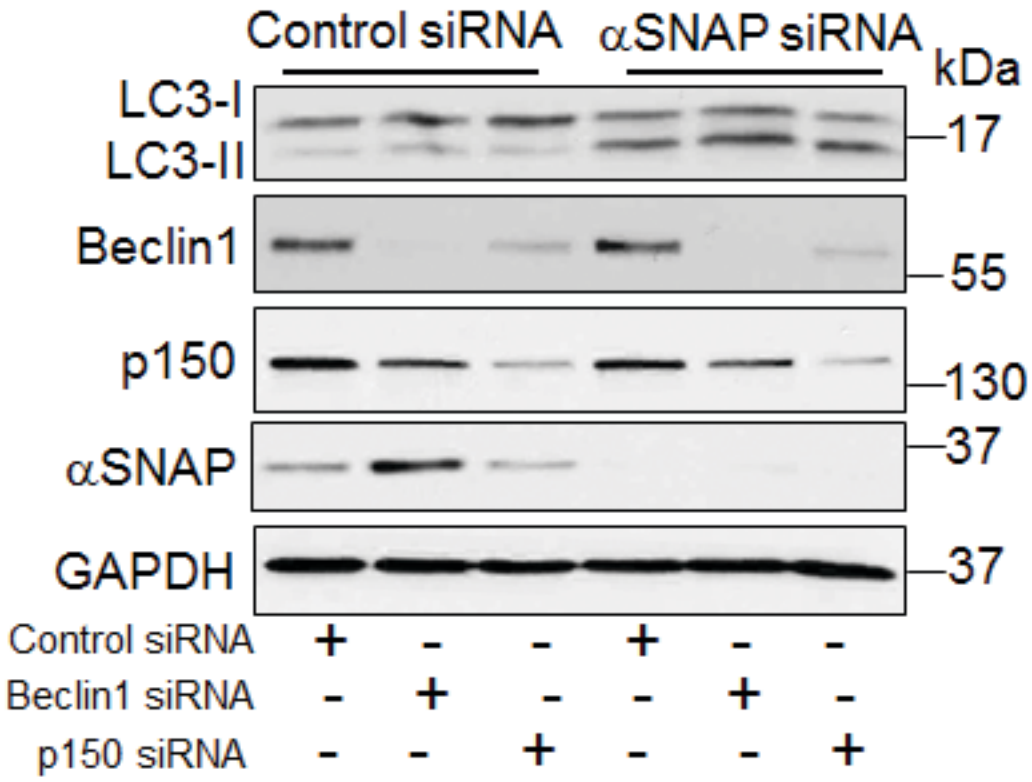
A


Control siRNA
αSNAP siRNA D1
αSNAP siRNA D2
Control siRNA
αSNAP siRNA D1
αSNAP siRNA D2
kDa
GFP-LC3-I
GFP-LC3-II
37
LC3-I
LC3-II
17
αSNAP
37
Time post transfection (h)
48
72

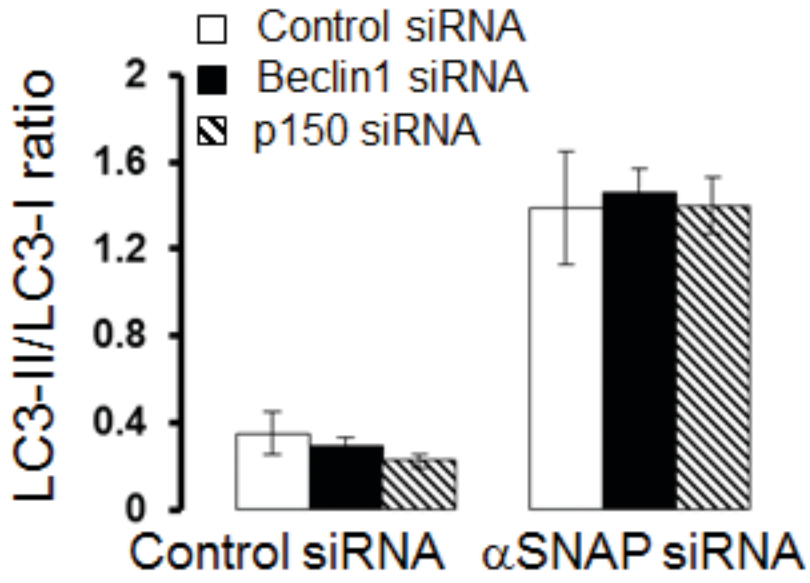
B


GFP-LC3
p62
Merged
GFP-LC3
NBR1
Merged

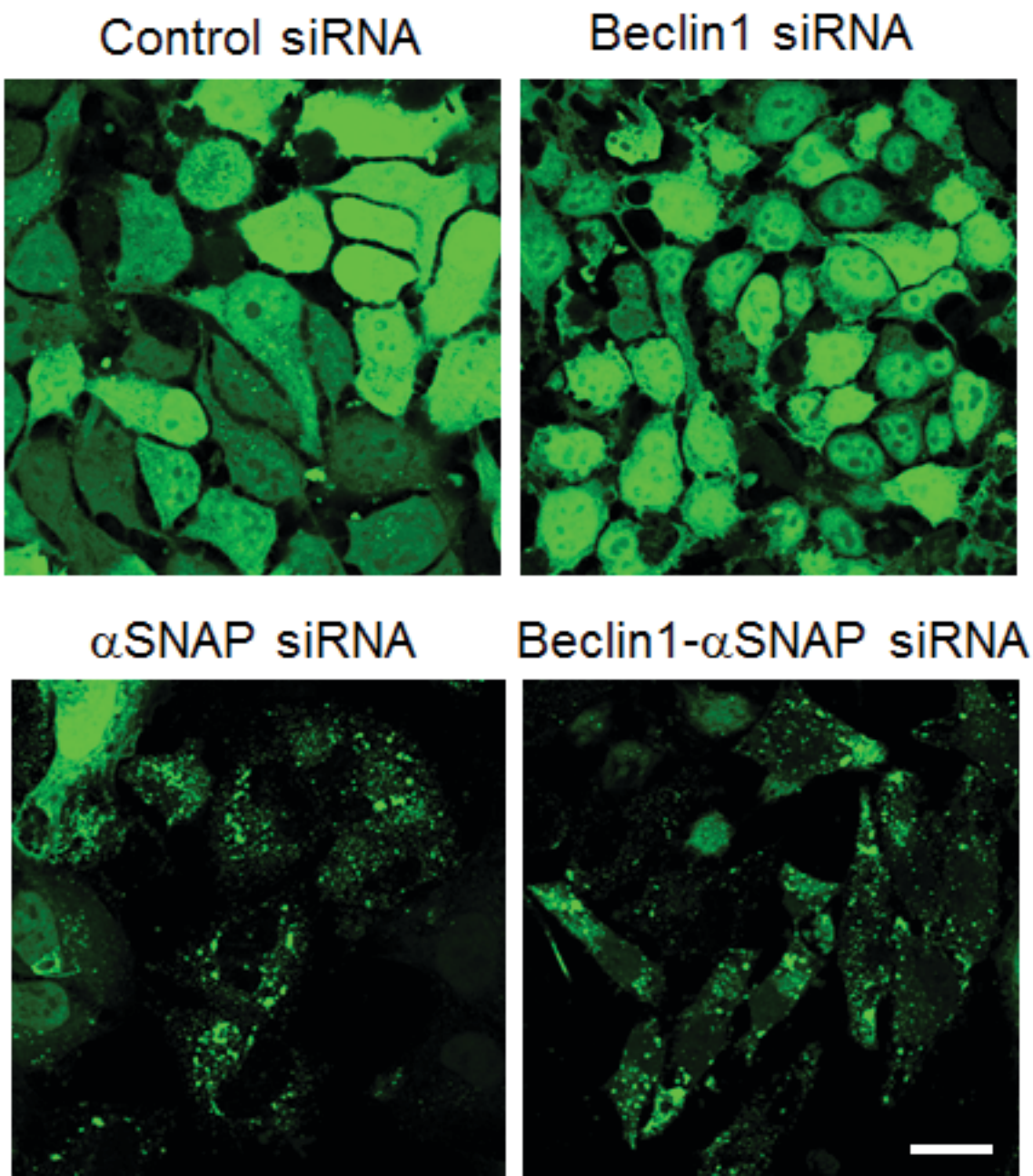
A


Control siRNA
αSNAP siRNA
kDa
LC3-I
LC3-II
17
Beclin1
55
p150
130
αSNAP
37
GAPDH
37
Control siRNA + - - + - -
Beclin1 siRNA - + - - + -
p150 siRNA - - + - - +

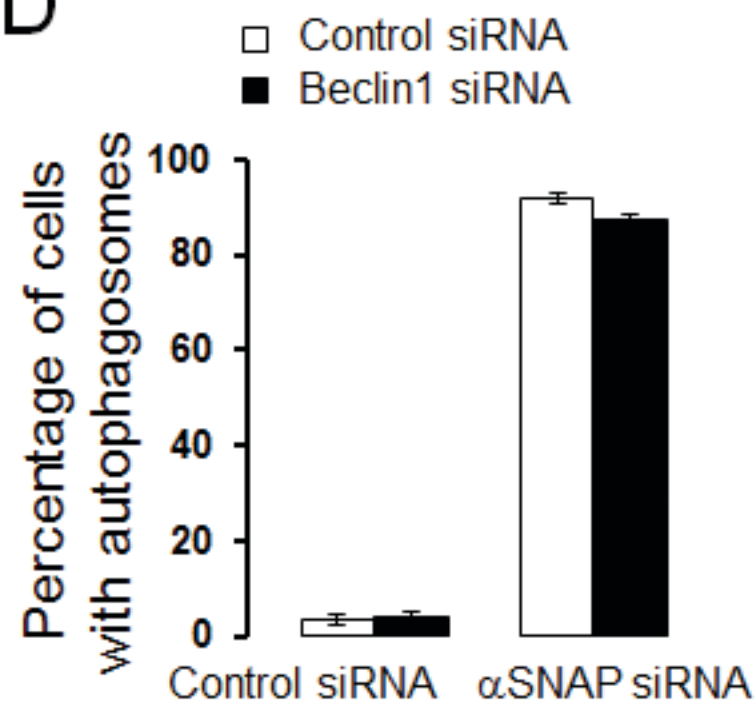
B


Control siRNA
Beclin1 siRNA
p150 siRNA
LC3-II/LC3-I ratio
2
1.6
1.2
0.8
0.4
0
Control siRNA
αSNAP siRNA

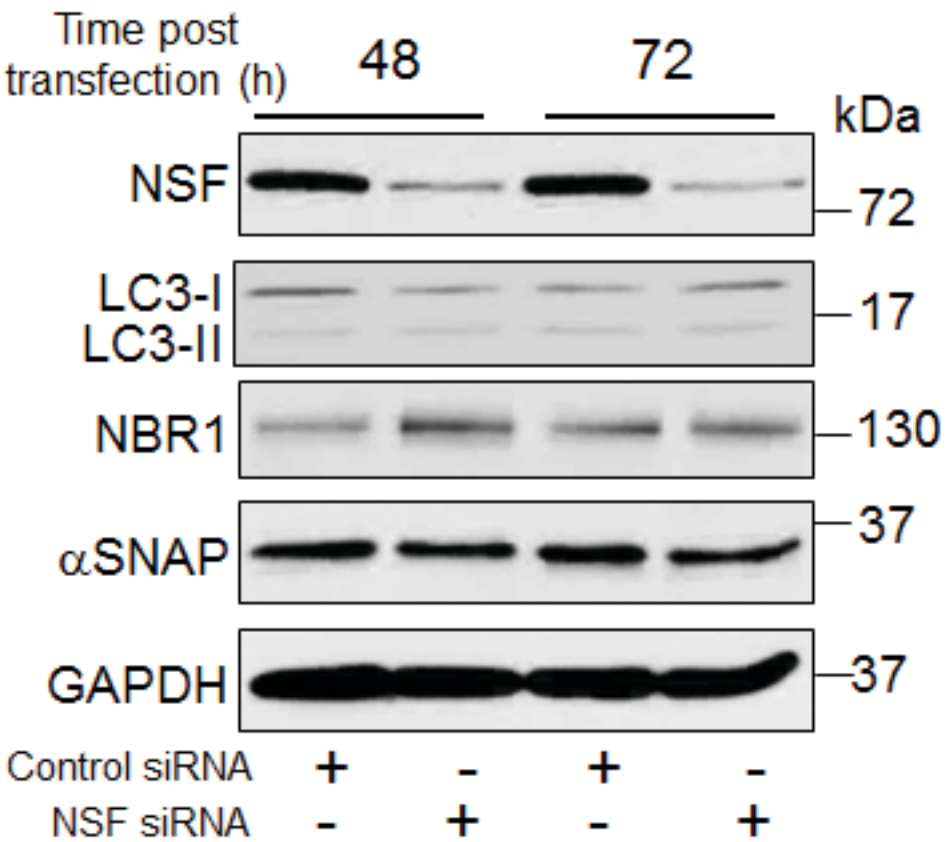
C


Control siRNA
Beclin1 siRNA
αSNAP siRNA
Beclin1-αSNAP siRNA

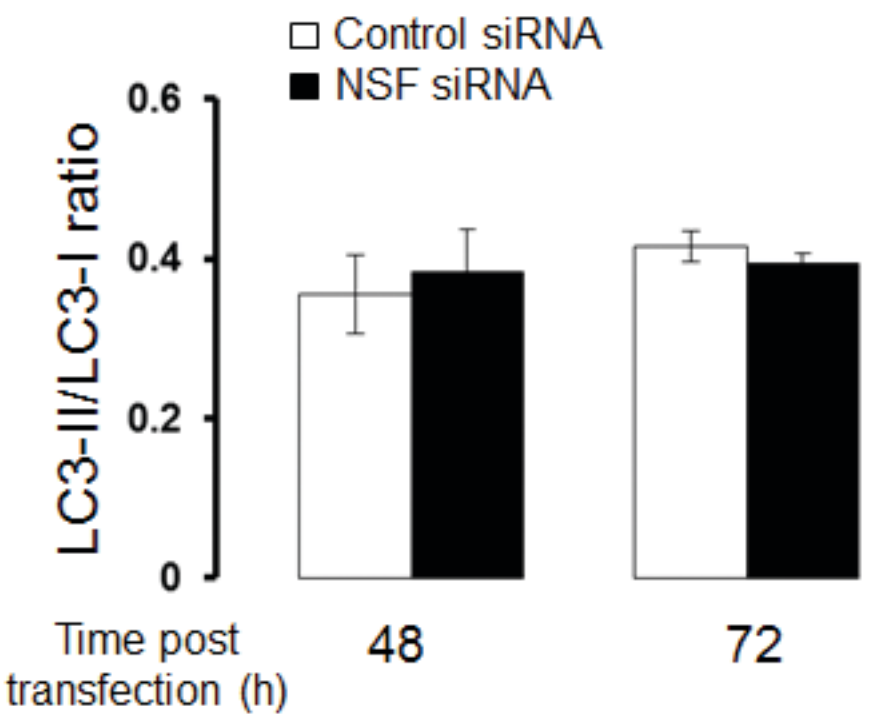
D


Control siRNA
Beclin1 siRNA
Percentage of cells with autophagosomes
100
80
60
40
20
0
Control siRNA
αSNAP siRNA

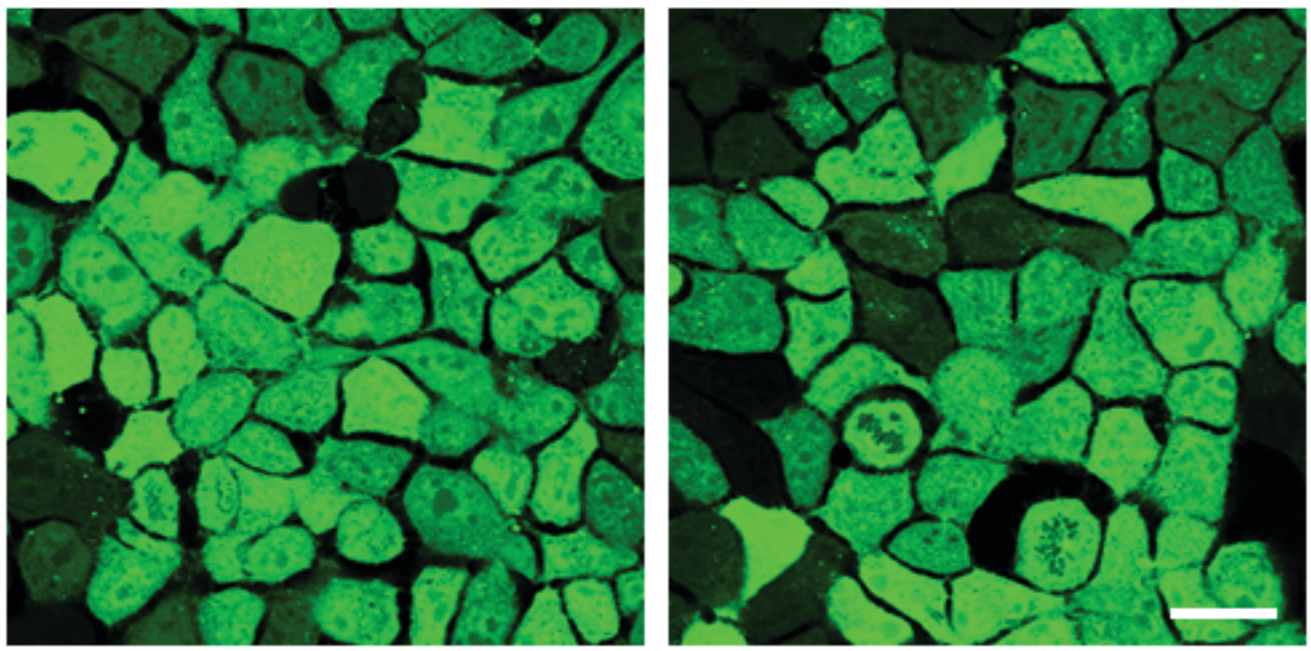
A


Time post transfection (h)
48
72
kDa
NSF
72
LC3-I
LC3-II
17
NBR1
130
αSNAP
37
GAPDH
37
Control siRNA + - + -
NSF siRNA - + - +

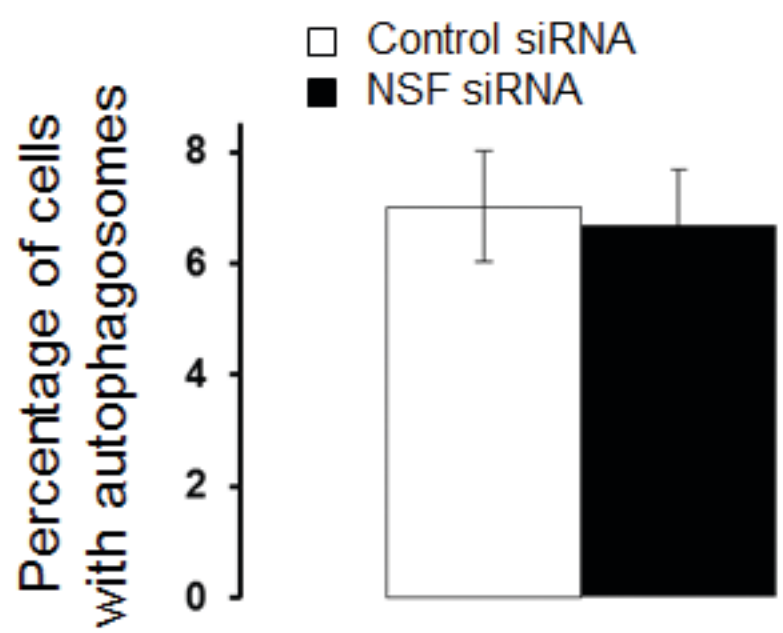
B


Control siRNA
NSF siRNA
LC3-II/LC3-I ratio
0.6
0.4
0.2
0
Time post transfection (h)
48
72

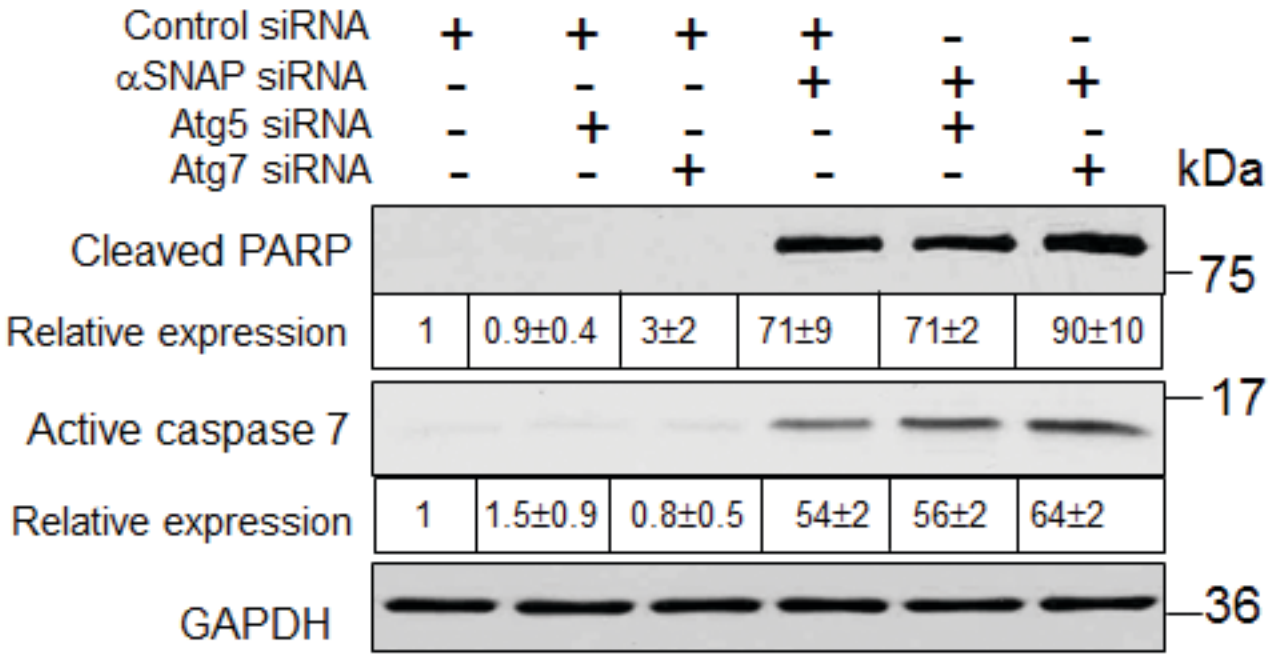
C


Control siRNA
NSF siRNA

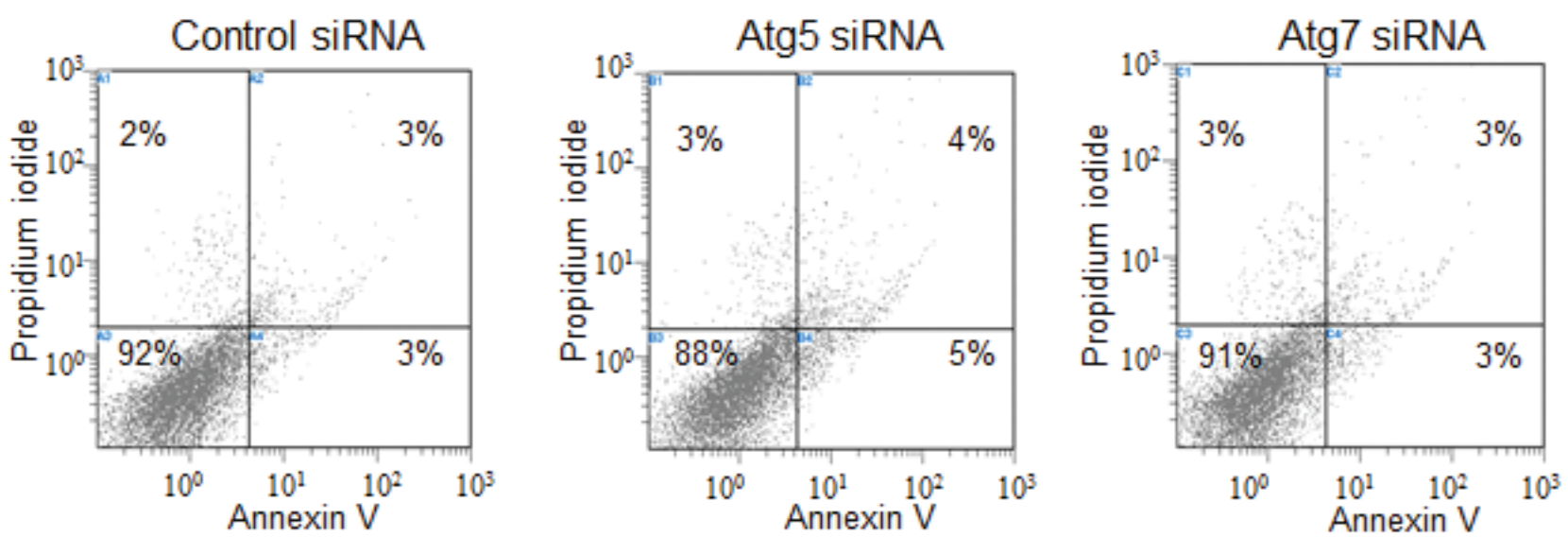
D

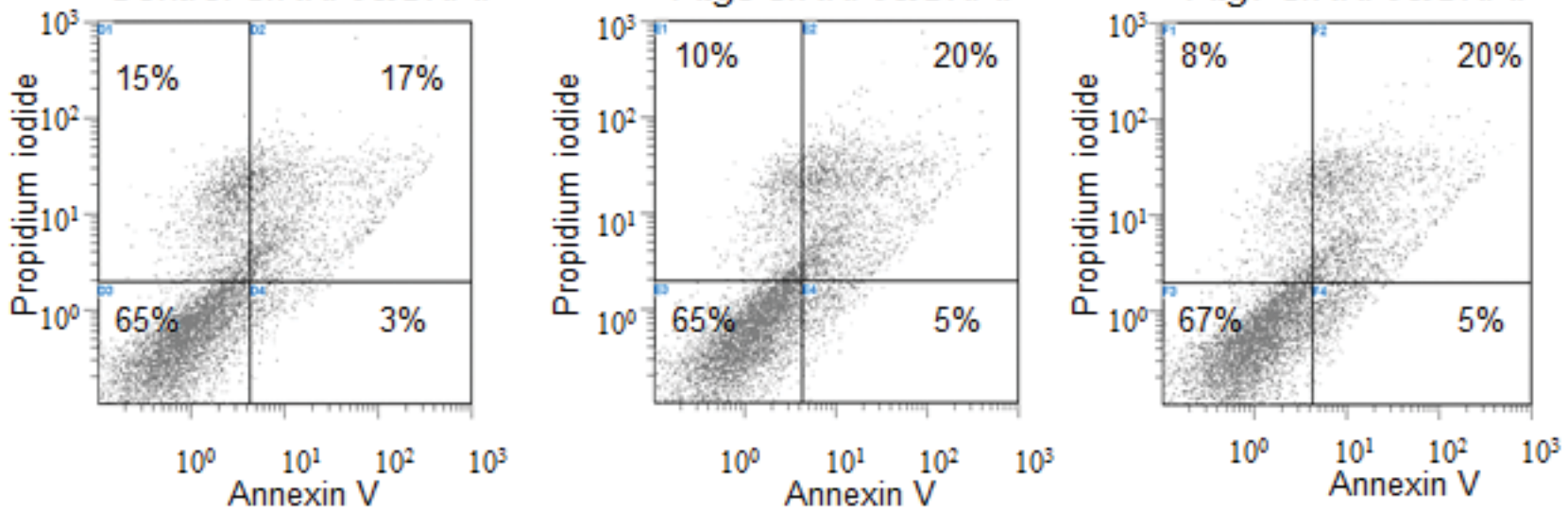

Control siRNA
NSF siRNA
Percentage of cells with autophagosomes
8
6
4
2
0

A


Control siRNA + + + + - -
αSNAP siRNA - - - + + +
Atg5 siRNA - + - - + -
Atg7 siRNA - - + - - +
kDa
Cleaved PARP
75
Relative expression
1
0.9±0.4
3±2
71±9
71±2
90±10
Active caspase 7
17
Relative expression
1
1.5±0.9
0.8±0.5
54±2
56±2
64±2
GAPDH
36

B


Control siRNA
Atg5 siRNA
Atg7 siRNA
Propidium iodide
Annexin V
2%
3%
92%
3%
3%
4%
88%
5%
3%
3%
91%
3%


Control siRNA/αSNAP
Atg5 siRNA/αSNAP
Atg7 siRNA/αSNAP
Propidium iodide
Annexin V
15%
17%
65%
3%
10%
20%
65%
5%
8%
20%
67%
5%

C

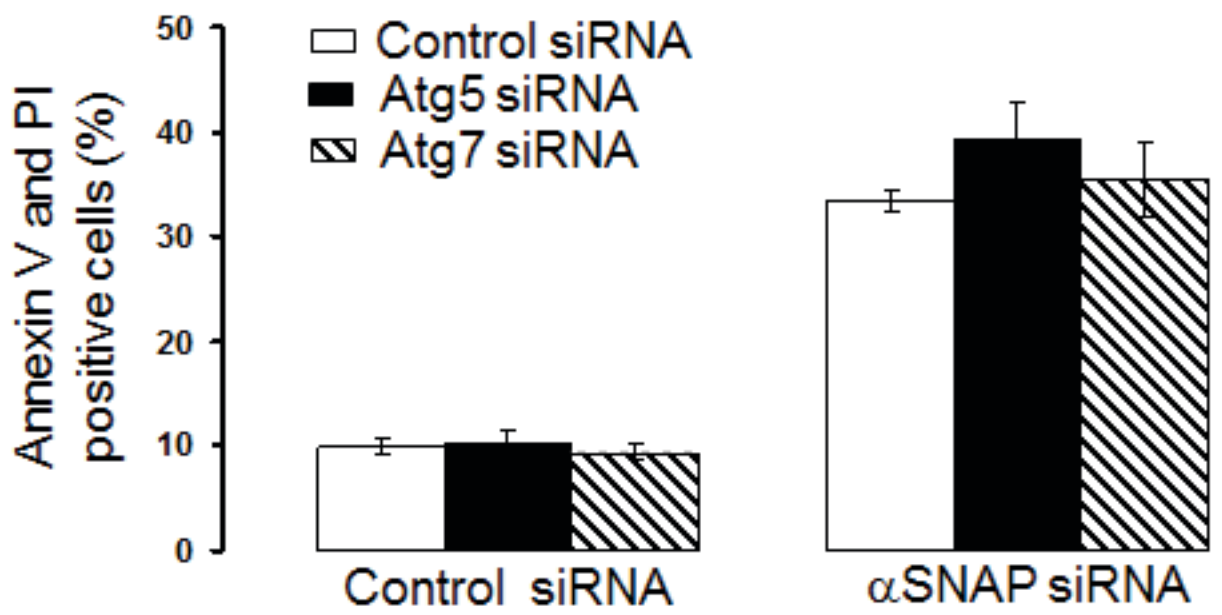

Annexin V and PI positive cells (%)
50
40
30
20
10
0
Control siRNA
Atg5 siRNA
Atg7 siRNA
Control siRNA
αSNAP siRNA

Figure S1. Loss of αSNAP selectively increases mRNA transcription of LC3. SK-CO15 cells were transfected with either control or two different αSNAP-specific siRNAs. mRNA levels of LC3, NBR1 and p62 were examined by a quantitative real-time RT-PCR at 72 h post-transfection. ** $p < 0.05$ compared to control αSNAP siRNA-transfected cells

Figure S2. Loss of αSNAP increases LC3 conjugation and triggers formation of p62 and NBR1-positive autophagosomes in HeLa-GFP-LC3 cells. (A) Representative immunoblots show induction of autophagic markers in HeLa-GFP-LC3 cells at different times after αSNAP depletion. (B) Confocal microscopy images demonstrate accumulation of NBR1 and p62-positive in autophagosomes (arrows) in these cells following αSNAP knockdown. Scale bar, 10 μm.

Figure S3. Enhanced autophagy in αSNAP-depleted epithelial cells is not mediated by beclin-1. (A, B) SK-CO15 cells were subjected to sequential transfections with one of the following siRNA pairs: control-control, control-beclin-1, control-p150, control-αSNAP, beclin-1-αSNAP and p150-αSNAP. Expression of LC3, αSNAP, beclin-1 and p150 was determined by immunoblotting 48 h after the second transfection. (C, D) HeLa-GFP-LC3 cells were sequentially transfected with control-control, control-beclin-1, control-αSNAP and beclin-1-αSNAP siRNA combinations and formation of autophagosomes was analyzed by fluorescence spectroscopy 72 h after the second transfection. Scale bar, 20 μm.

Figure S4. Downregulation of N-ethylmaleimide sensitive factor (NSF) does not stimulate epithelial cell autophagy. (A, B) SK-CO15 cells were transfected with either control or NSF-specific siRNAs and expression of NSF, αSNAP and autophagic markers were analyzed by immunoblotting at different times post-transfection. (C, D) HeLa-GFP-LC3 cells were transfected with either control or NSF-specific siRNAs and formation of autophagosomes was analyzed by fluorescence microscopy at 72 h post-transfection. Scale bar, 20 μm.

Figure S5. Induction of autophagy does not affect apoptosis in αSNAP-deficient epithelial cells. SK-CO15 cells were subjected to sequential transfections with combinations of control, Atg5, Atg7 and αSNAP siRNAs as described in the Figure 4 legend. Induction of apoptotic markers was determined by immunoblotting (A) and flow cytometry (B, C).