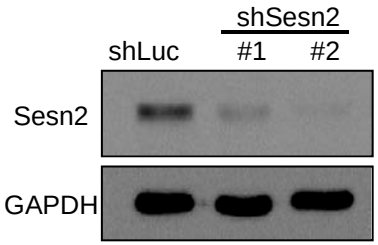
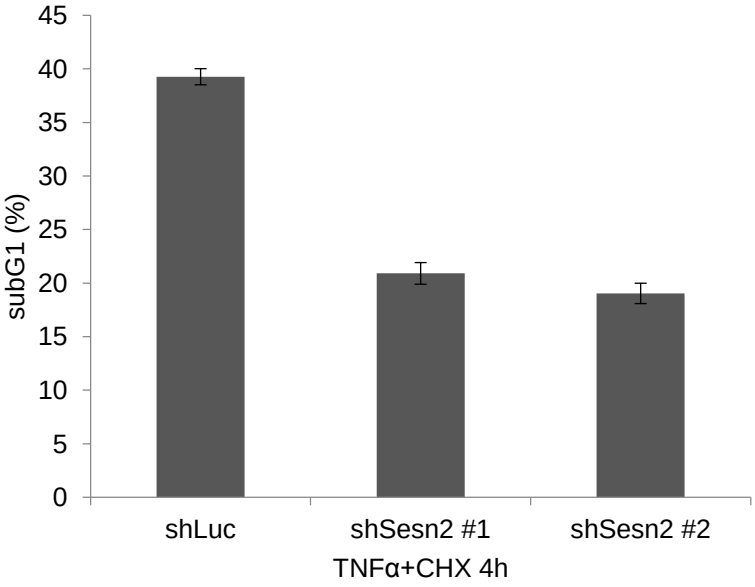
Fig. S1

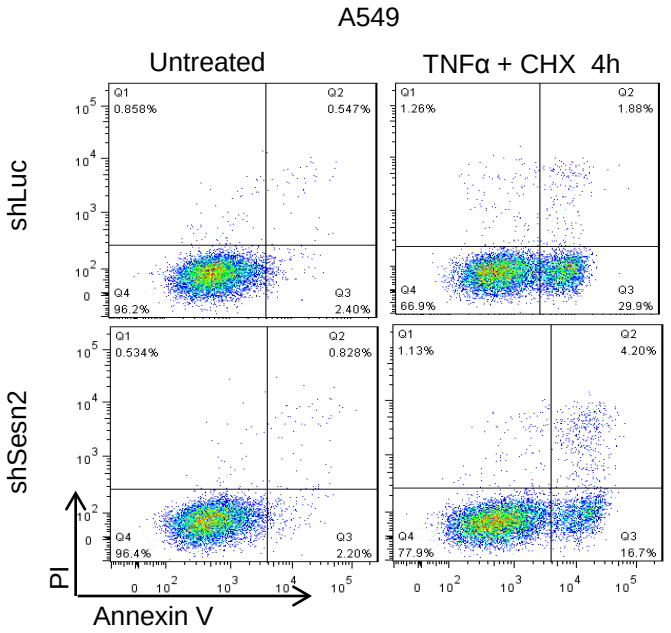
A


shSesn2
shLuc
#1
#2
Sesn2
GAPDH

B


subG1 (%)
45
40
35
30
25
20
15
10
5
0
shLuc
shSesn2 #1
shSesn2 #2
TNFα+CHX 4h

C


A549
Untreated
TNFα + CHX 4h
shLuc
shSesn2
Q1 0.858%
Q2 0.547%
Q4 96.2%
Q3 2.40%
Q1 1.26%
Q2 1.88%
Q4 66.9%
Q3 29.9%
Q1 0.534%
Q2 0.828%
Q4 96.4%
Q3 2.20%
Q1 1.13%
Q2 4.20%
Q4 77.9%
Q3 16.7%
PI
Annexin V

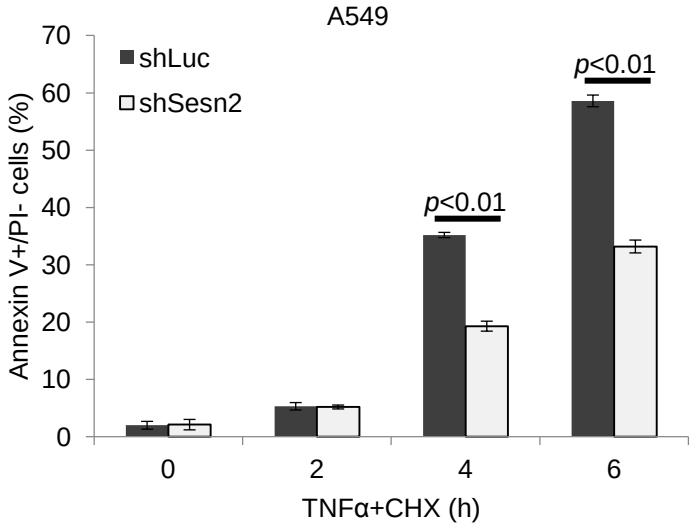
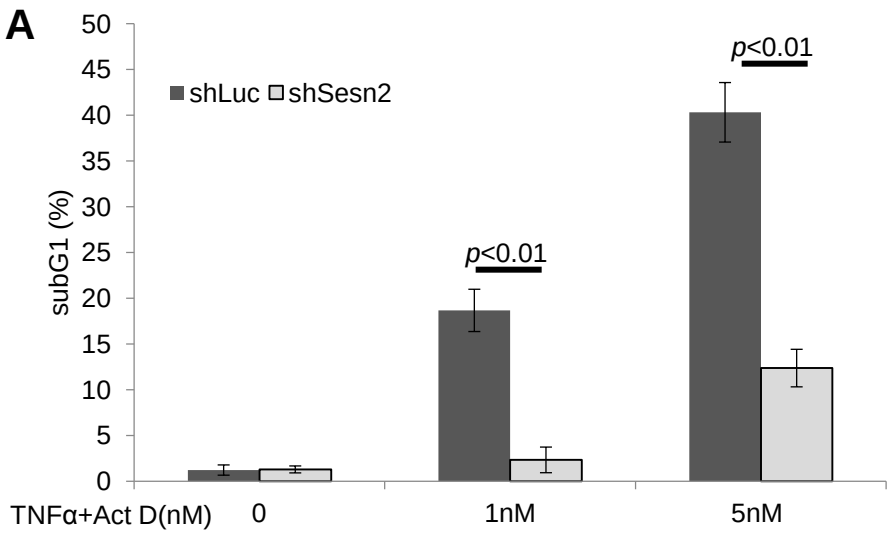
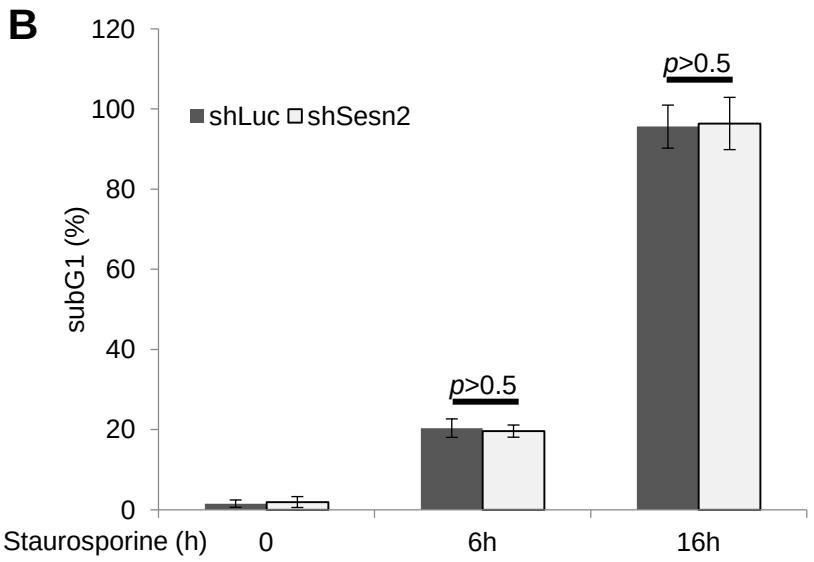

A549
shLuc
shSesn2
Annexin V+/PI- cells (%)
70
60
50
40
30
20
10
0
p<0.01
p<0.01
0
2
4
6
TNFα+CHX (h)

Fig. S2


A
50
45
40
35
30
25
20
15
10
5
0
subG1 (%)
shLuc
shSesn2
p<0.01
p<0.01
TNFα+Act D(nM)
0
1nM
5nM


B
120
100
80
60
40
20
0
subG1 (%)
shLuc
shSesn2
p>0.5
p>0.5
Staurosporine (h)
0
6h
16h

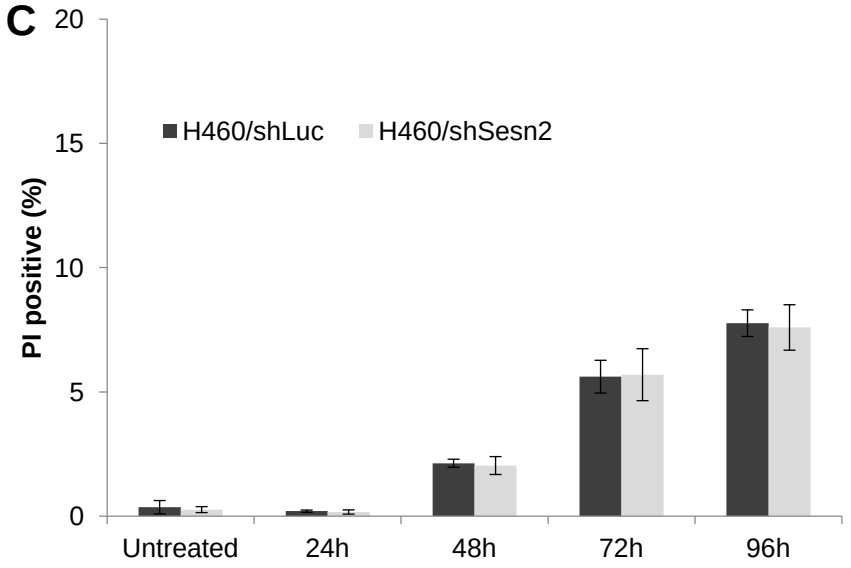

C
20
15
10
5
0
PI positive (%)
H460/shLuc
H460/shSesn2
Untreated
24h
48h
72h
96h

Fig. S3

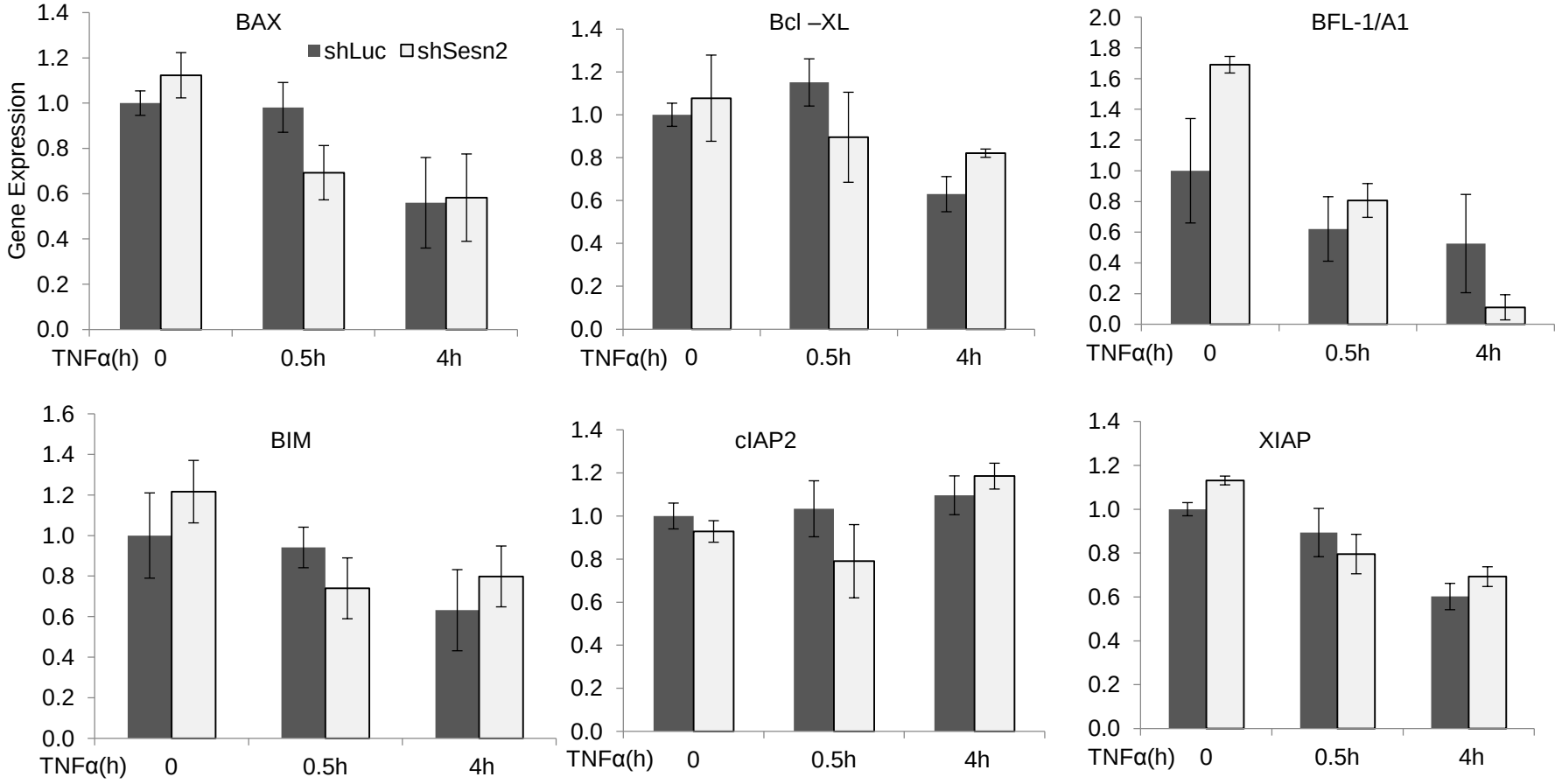

BAX
shLuc
shSesn2
Gene Expression
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
TNFα(h) 0
0.5h
4h
Bcl –XL
BFL-1/A1
2.0
1.8
1.6
BIM
cIAP2
XIAP

Fig. S4

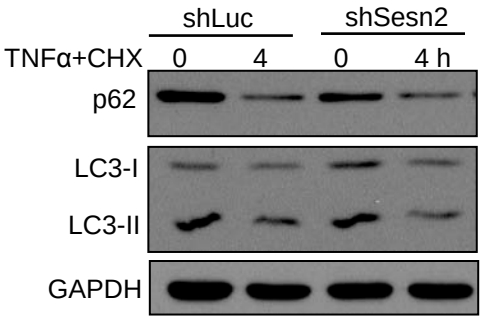
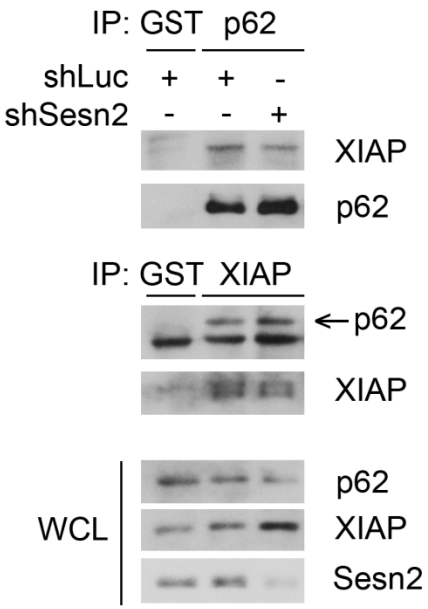


Fig. S5


IP: GST p62
shLuc + + -
shSesn2 - - +
XIAP
p62
IP: GST XIAP
p62
XIAP
WCL
p62
XIAP
Sesn2

Supplemental Information

Sestrin2 facilitates death receptor-induced apoptosis in lung adenocarcinoma cells through regulation of XIAP degradation

Boxiao Ding, Anita Parmigiani, Yang Chen, and Andrei V. Budanov

Supplementary Figures:

Figure S1: Sesn2 supports TNFα-induced cell death. (A) shSesn2-1 and shSesn2-2 have similar effect on Sesn2 silencing. H460 cells were infected with shSesn2-1 and shSesn2-2 lentiviral vectors and selected with puromycin (1 μg/ml) for 2 weeks. (B) Sesn2 silencing with either shSesn2-1 or shSesn2-2 (from A) has similar effect on the sensitivity to TNFα+CHX treatment. Cells were treated with TNFα+CHX for 4 hrs and analyzed by PI staining followed by flow cytometry. (C) Sesn2 silencing inhibits TNFα+CHX induced apoptosis in A549 cells. shSesn2-silenced or control A549 cells were treated with TNFα+CHX for 4 hrs and the levels of apoptotic and necrotic cell death were analyzed by Annexin V-PI staining.

Figure S2: Sesn2 supports TNFα-induced apoptosis but not staurosporine-induced cell death or TNFα-induced necroptosis. (A) Sesn2-silencing protects H460 cells from TNFα+Actinomycin D induced cell death. Sesn2-silenced or control H460 cells were treated with TNFα+Actinomycin D for 5 hrs and cell death was evaluated by number of cells with sub-G1 DNA content assessed by flow cytometry (B) Sesn2 does not play any role in the regulation of staurosporine-induced apoptosis. Sesn2-silenced or control H460 cells were treated with staurosporine for the indicated time intervals and analyzed by flow cytometry as in (A). (C) Sesn2 does not contribute to TNFα-induced necroptosis. Sesn2-silenced or control H460 cells were treated with TNFa+CHX in the presence of pan-caspase inhibitor ZVAD-FMK (50 μM) and analyzed 24, 48, 72 and 96 hrs after treatment by PI staining followed by flow cytometry.

Figure S3: Sesn2 does not affect expression of NF-κB regulated genes. Sesn2-silenced or control H460 cells were treated with TNFα and the expression of the corresponding NF-κB-inducible genes was analyzed by qPCR.

Figure S4: Sesn2 does not play an important role in regulation of general autophagy in response to TNFα+CHX treatment. Sesn2-silenced and control H460 cells were treated with TNFα+CHX for 4 hrs and expression of p62 and LC3 proteins were determined by immunoblot analysis.

Figure S5: Sesn2 is not involved in regulation of XIAP-p62 interactions. Sesn2-silenced or control H460 cells were lysed and either XIAP or p62 proteins were immunoprecipitated and analyzed by immunoblotting using the indicated antibodies.

Supplemental Experimental Procedures

Constructs

The sequence for shSesn2 is 5'-GAAGACCCTACTTTCGGAT-3', shSesn2-2: 5'-GAGATGGAGAGCCGCTTT-3'. The primers used for qPCR were:: BAX: 5'-TTCCTTACGTGTCTGATCAATCC-3' and 5'- GGGCAGAAGGCACTAATCAA-3'; Bcl-XL: 5'- TCAGGCTGCTTGGG TAAAG -3' and 5'-AGGCTTCTGGAGGACATTTG-3'; BFL-1/A1: 5'-CACGAAAGTGACTAGGAGGAAG-3' and 5'-CTCACTGAGCTTGACTGAGTTAT-3'; BIM: 5'-CTGCTGGACACACACATACA-3' and 5'-GGGCTGAGGAAACAGAGTAAA-3'; cIAP2 5'- TGCTCGTGCTGGTTTCTATT-3' and 5'-TCAGTAGGACTGTCTCCTCTTT-3'.