Supplementary Figure Legends

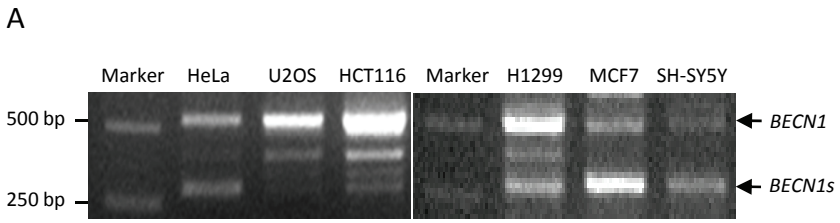
Figure S1. Expression and cellular localization of BECN1s. (**A**) RT-PCR was performed with total RNAs extracted from the indicated cell lines using primers P1 and P2. (**B**) U2OS cells expressing either GFP, GFP-BECN1 or GFP-BECN1s were stained with MitoTracker Red. The images were taken under a fluorescence microscope. (**C**) MEF cells expressing GFP, GFP-BECN1 or GFP-BECN1s were stained with MitoTracker Red. The images were taken under a fluorescence microscope.

Figure S2. Both BECN1 and BECN1s bind to ATG14 and BCL2. (**A**) HEK 293T cells were transfected with constructs encoding Flag-*BECN1*, Flag-*BECN1s* and GFP-*ATG14* in the indicated combinations. Twenty-four h later, cell lysates were immunoprecipitated with anti-Flag antibody, followed by immunoblotting with anti-Flag and anti-GFP antibodies. (**B**) HEK 293T cells were transfected with constructs encoding either Flag-*BECN1* or Flag-*BECN1s* as indicated. Twenty-four h later, cell lysates were immunoprecipitated with anti-Flag antibody, followed by immunoblotting with anti-BCL2 antibody.

Figure S3. Neither BECN1 nor BECN1s interacts with HSP90, FUNDC1 or BNIP3L. HEK 293T cells were transfected with the indicated plasmids. Twenty-four h after transfection, cell lysates were immunoprecipitated with anti-GFP antibody, followed by immunoblotting with anti-Flag and anti-GFP antibodies.

Figure S4. Effect of BECN1s on EBSS-induced autophagy. (**A**) The shRNA-mediated knockdown efficiency for *BECN1* and *BECN1s* was verified by real-time RT-PCR analysis for Figure 4A. (**B**) U2OS cells expressing the indicated shRNAs were treated with EBSS for the indicated periods of time. Cell lysates were then analyzed by western blot with the indicated antibodies. The shRNA-mediated knockdown efficiency for *BECN1* and *BECN1s* was verified by real-time RT-PCR analysis. (**C**) The shRNA-mediated knockdown efficiency for *BECN1s* was verified by real-time RT-PCR analysis for Figure 4C. (**D**) HCT116 cells transfected with the indicated shRNA were cultured in normal growth medium or treated with EBSS in the absence or presence of bafilomycin A_1 (BAF) treatment. Cell lysates were

analyzed by western blot with anti-SQSTM1 and anti-LC3 antibodies. The knockdown efficiency for *BECN1s* is also shown. (**E**) The shRNA-mediated knockdown efficiency for *BECN1s* was verified by real-time RT-PCR analysis for Figure 4D.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U2OS
HCT116
Marker
H1299
MCF7
SH-SY5Y
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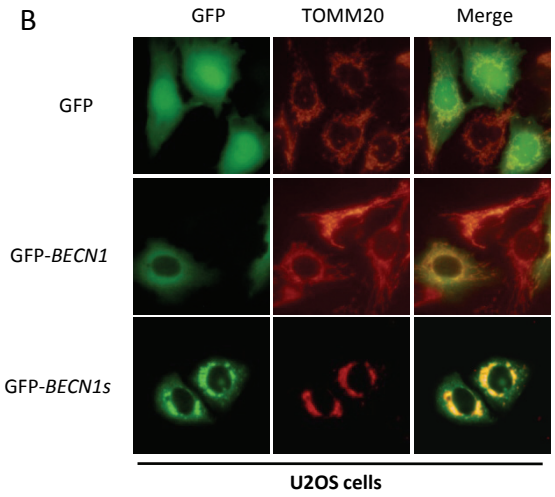
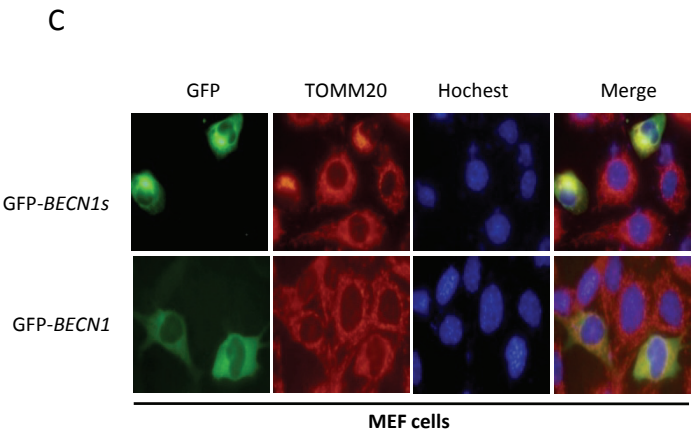
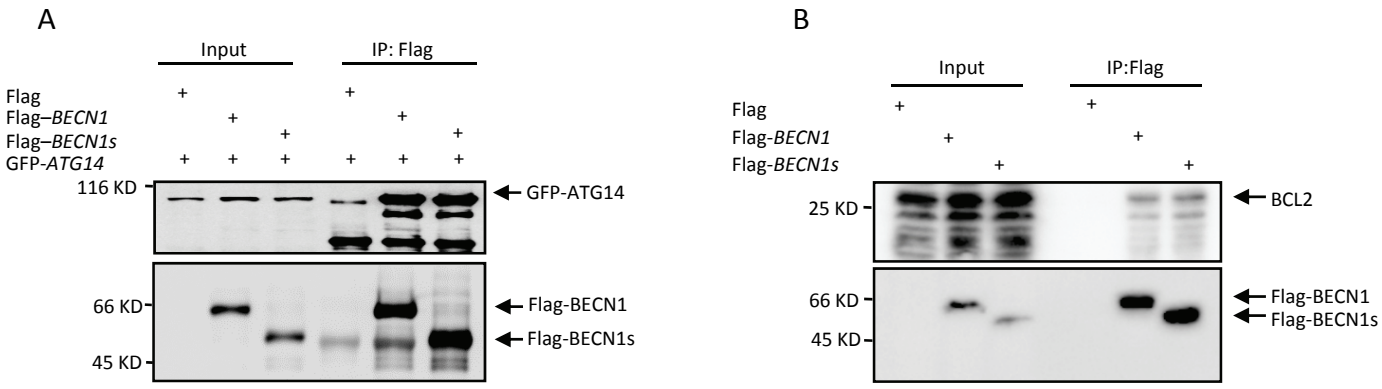
250 bp
BECN1
BECN1s


B
GFP
TOMM20
Merge
GFP
GFP-BECN1
GFP-BECN1s
U2OS cells


C
GFP
TOMM20
Hochest
Merge
GFP-BECN1s
GFP-BECN1
MEF cells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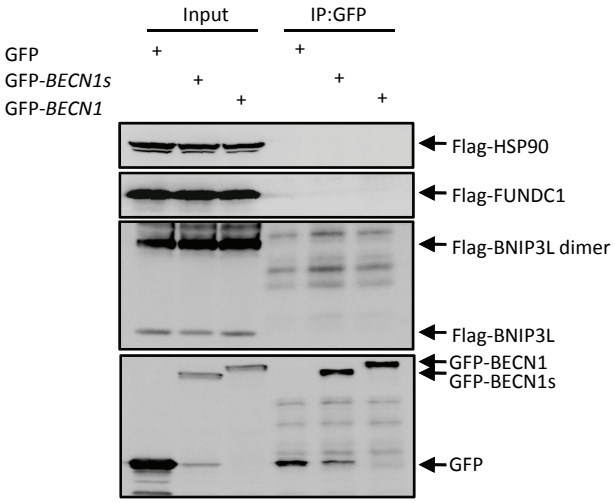
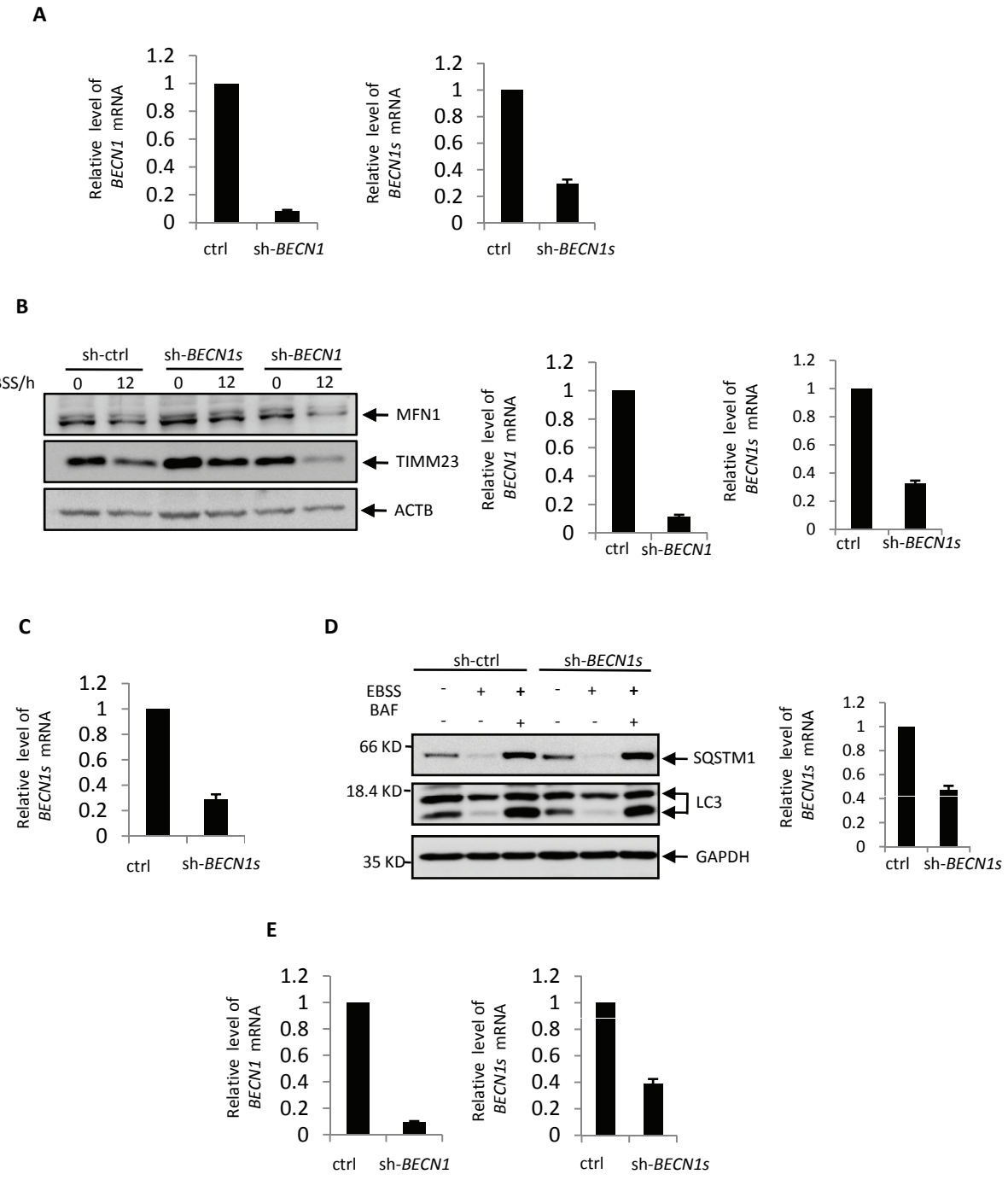
Flag–BECN1s
GFP-ATG14
116 KD
66 KD
45 KD
GFP-ATG14
Flag-BECN1
Flag-BECN1s
B
Input
IP:Flag
Flag
Flag-BECN1
Flag-BECN1s
25 KD
66 KD
45 KD
BCL2
Flag-BECN1
Flag-BECN1s

Supplementary Figure S3




A
Relative level of BECN1 mRNA
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0.8
0.6
0.4
0.2
0
ctrl
sh-BECN1
Relative level of BECN1s mRNA
1.2
1
0.8
0.6
0.4
0.2
0
ctrl
sh-BECN1s
B
sh-ctrl
sh-BECN1s
sh-BECN1
EBSS/h
0
12
0
12
0
12
MFN1
TIMM23
ACTB
Relative level of BECN1 mRNA
1.2
1
0.8
0.6
0.4
0.2
0
ctrl
sh-BECN1
Relative level of BECN1s mRNA
1.2
1
0.8
0.6
0.4
0.2
0
ctrl
sh-BECN1s
C
Relative level of BECN1s mRNA
1.2
1
0.8
0.6
0.4
0.2
0
ctrl
sh-BECN1s
D
sh-ctrl
sh-BECN1s
EBSS
BAF
66 KD
18.4 KD
35 KD
SQSTM1
LC3
GAPDH
Relative level of BECN1s mRNA
1.2
1
0.8
0.6
0.4
0.2
0
ctrl
sh-BECN1s
E
Relative level of BECN1 mRNA
1.2
1
0.8
0.6
0.4
0.2
0
ctrl
sh-BECN1
Relative level of BECN1s mRNA
1.2
1
0.8
0.6
0.4
0.2
0
ctrl
sh-BECN1s