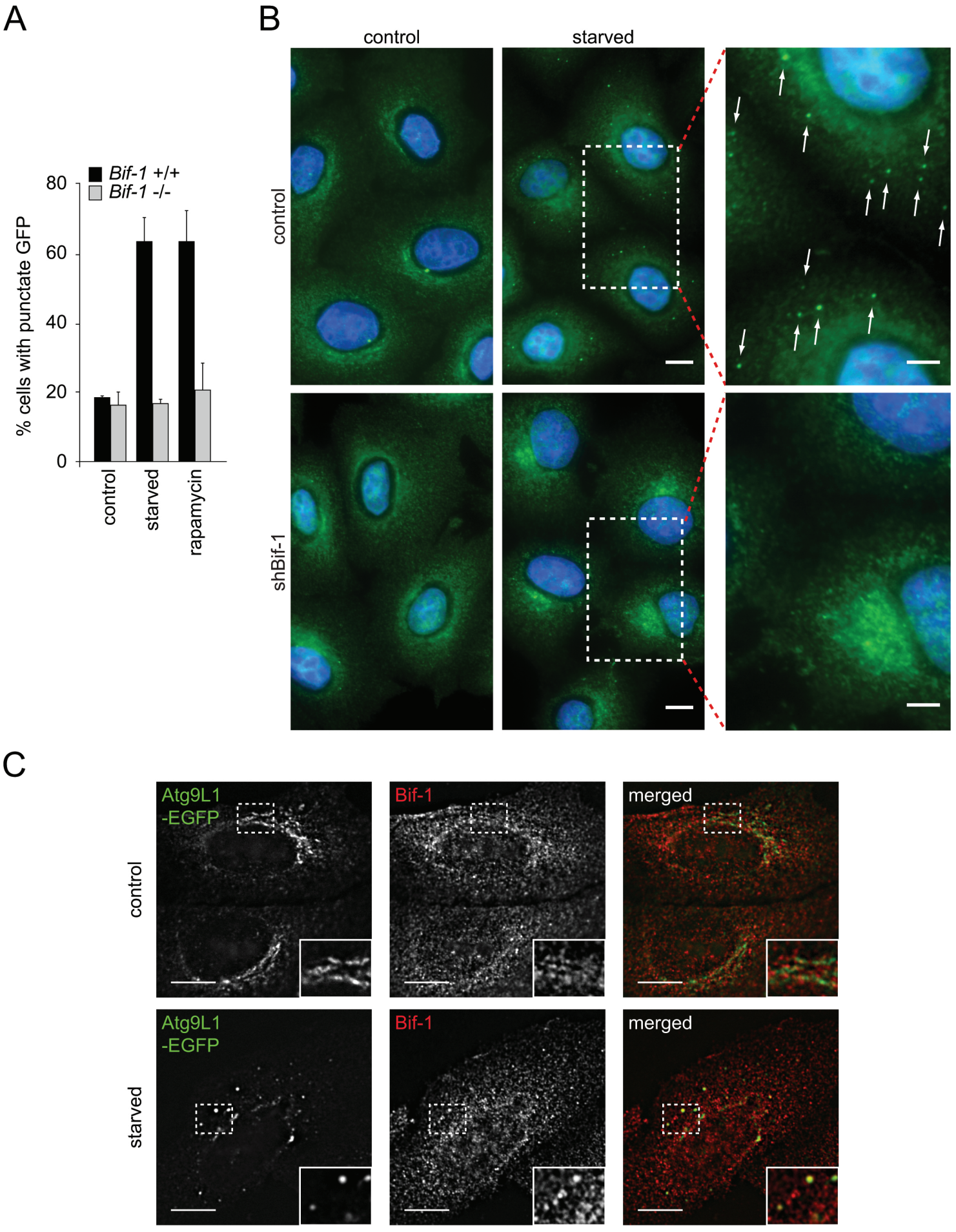
A
Bif-1 +/+
Bif-1 -/-
% cells with punctate GFP
80
60
40
20
0
control
starved
rapamycin
B
control
starved
control
shBif-1
C
Atg9L1
-EGFP
Bif-1
merged
control
starved

Fig. S1

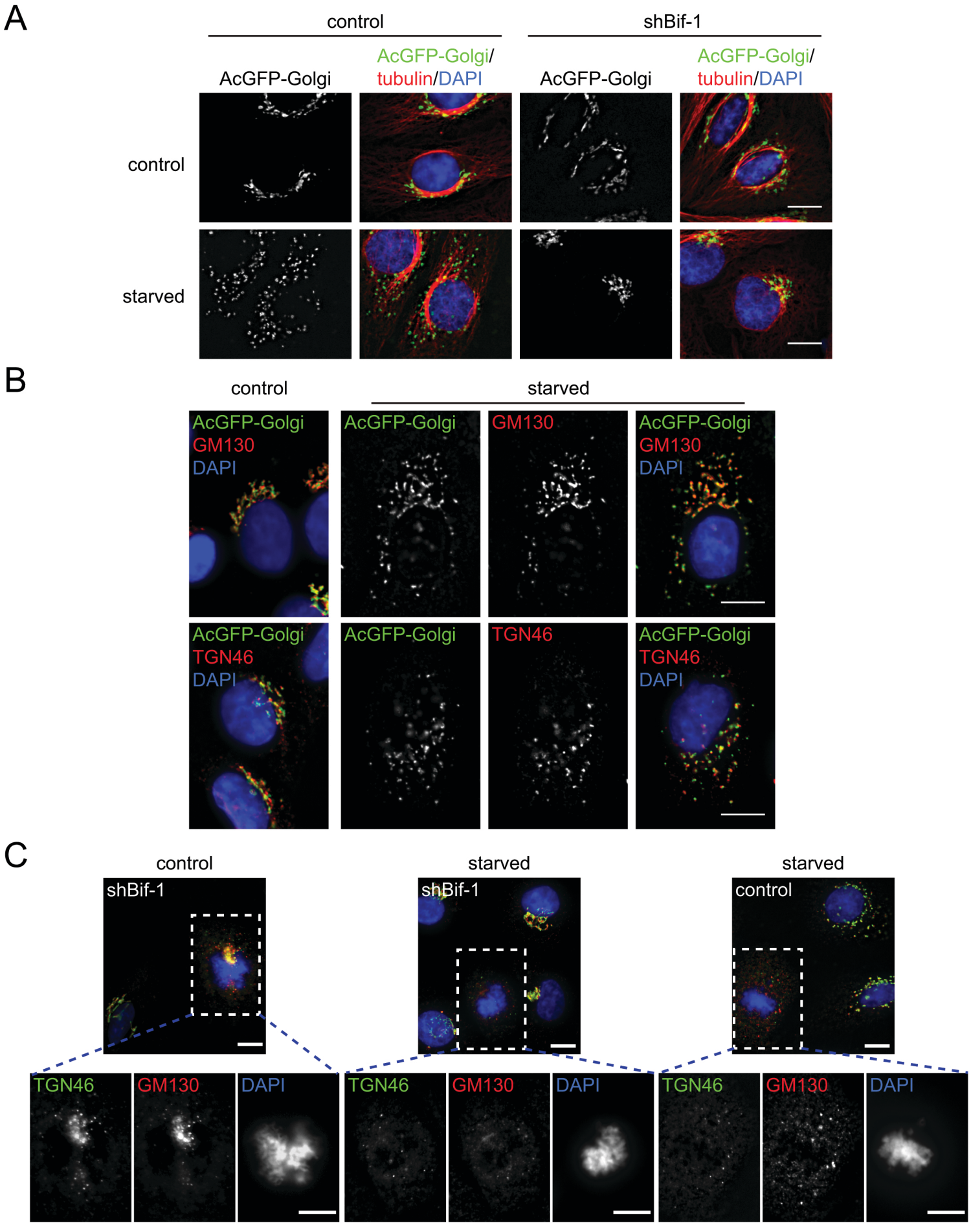
A
control
shBif-1
AcGFP-Golgi
AcGFP-Golgi/
tubulin/DAPI
AcGFP-Golgi
AcGFP-Golgi/
tubulin/DAPI
control
starved
B
control
starved
AcGFP-Golgi
GM130
DAPI
AcGFP-Golgi
GM130
AcGFP-Golgi
GM130
DAPI
AcGFP-Golgi
TGN46
DAPI
AcGFP-Golgi
TGN46
AcGFP-Golgi
TGN46
DAPI
C
control
starved
starved
shBif-1
shBif-1
control
TGN46
GM130
DAPI
TGN46
GM130
DAPI
TGN46
GM130
DAPI

Fig. S2

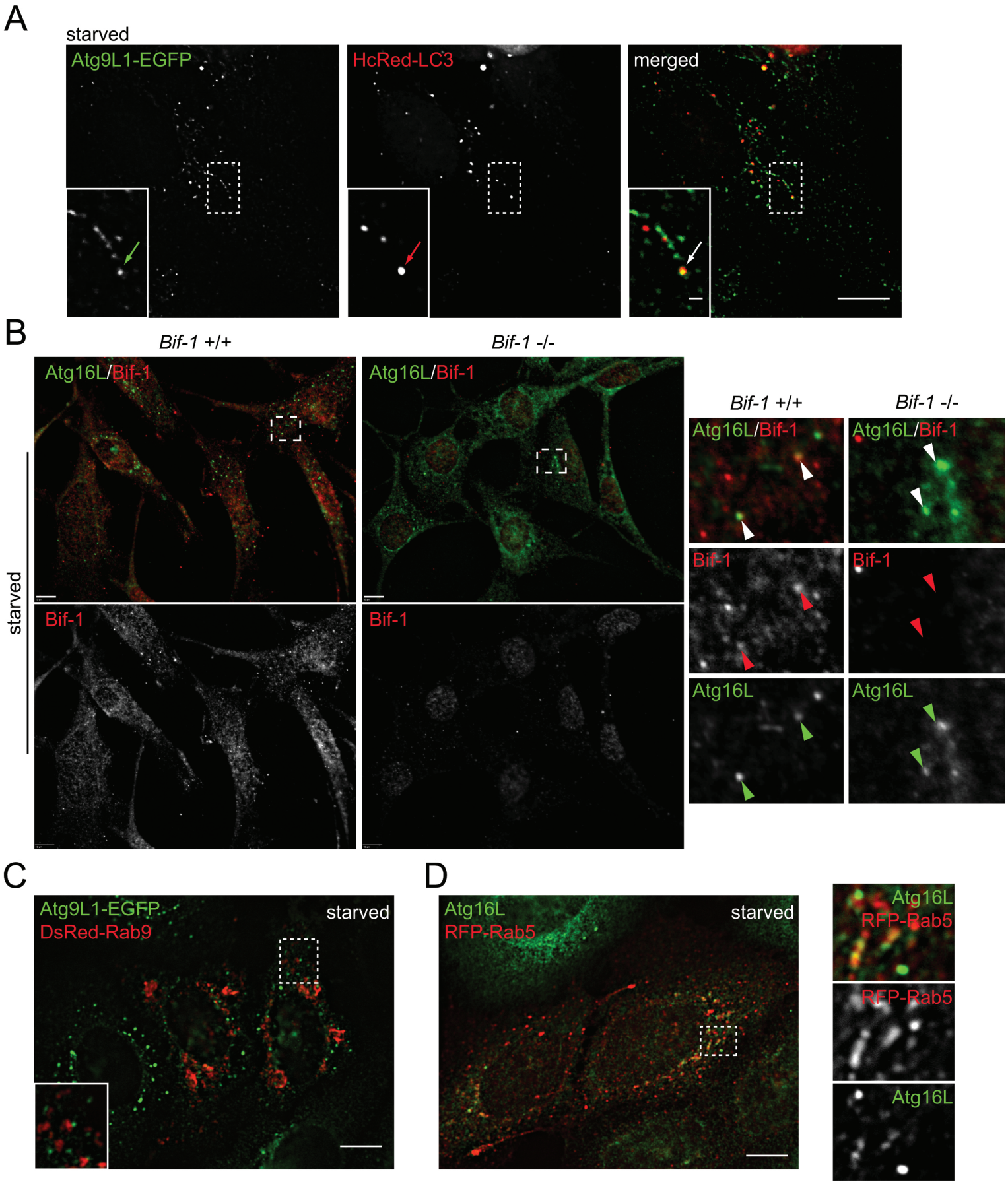
A
starved
Atg9L1-EGFP
HcRed-LC3
merged
B
Bif-1 +/+
Bif-1 -/-
Atg16L/Bif-1
Atg16L/Bif-1
starved
Bif-1
Bif-1
Bif-1 +/+
Bif-1 -/-
Atg16L/Bif-1
Atg16L/Bif-1
Bif-1
Bif-1
Atg16L
Atg16L
C
Atg9L1-EGFP
DsRed-Rab9
starved
D
Atg16L
RFP-Rab5
starved
Atg16L
RFP-Rab5
RFP-Rab5
Atg16L

Fig. S3

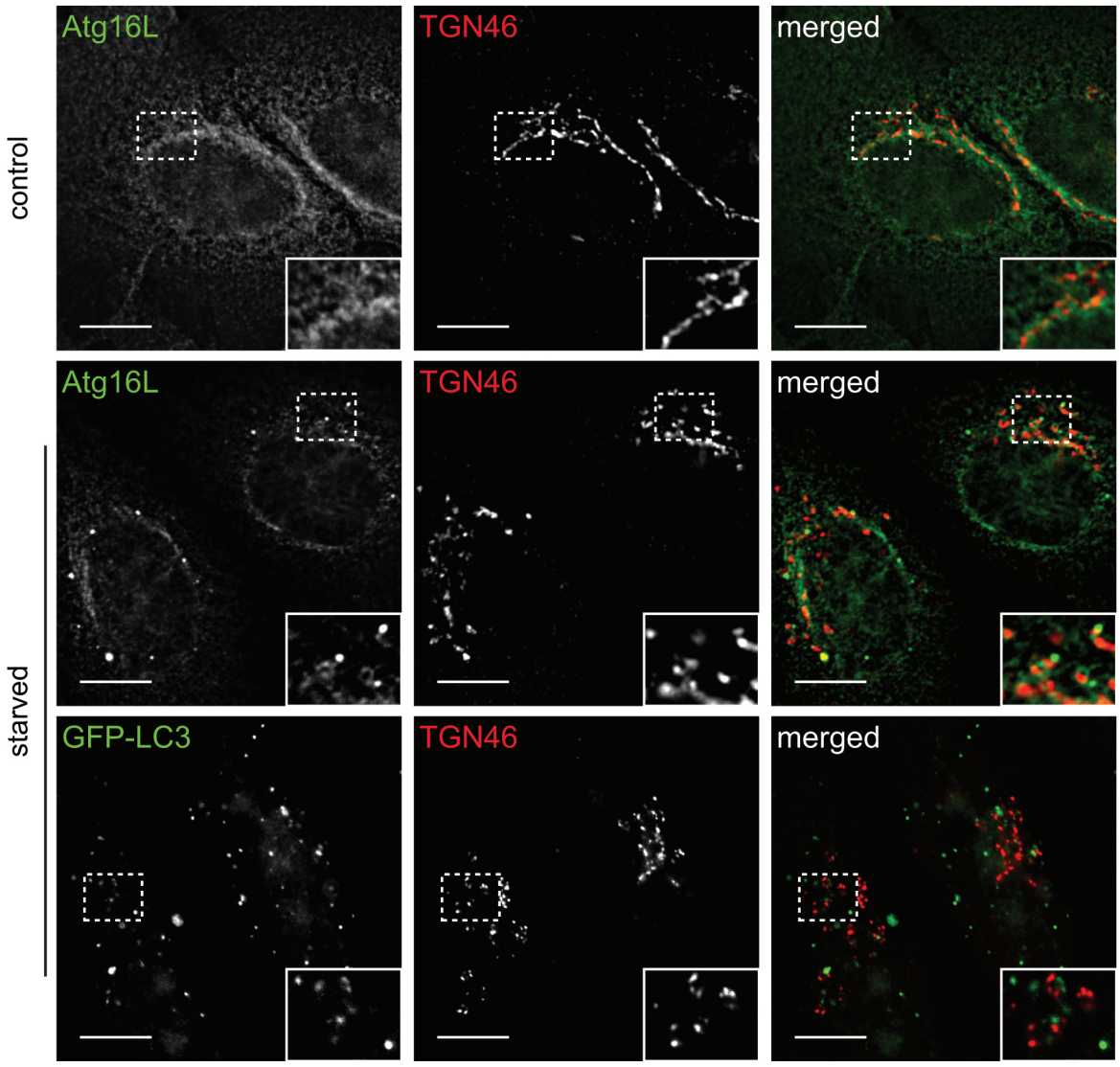
Atg16L
TGN46
merged
control
Atg16L
TGN46
merged
starved
GFP-LC3
TGN46
merged

Fig. S4

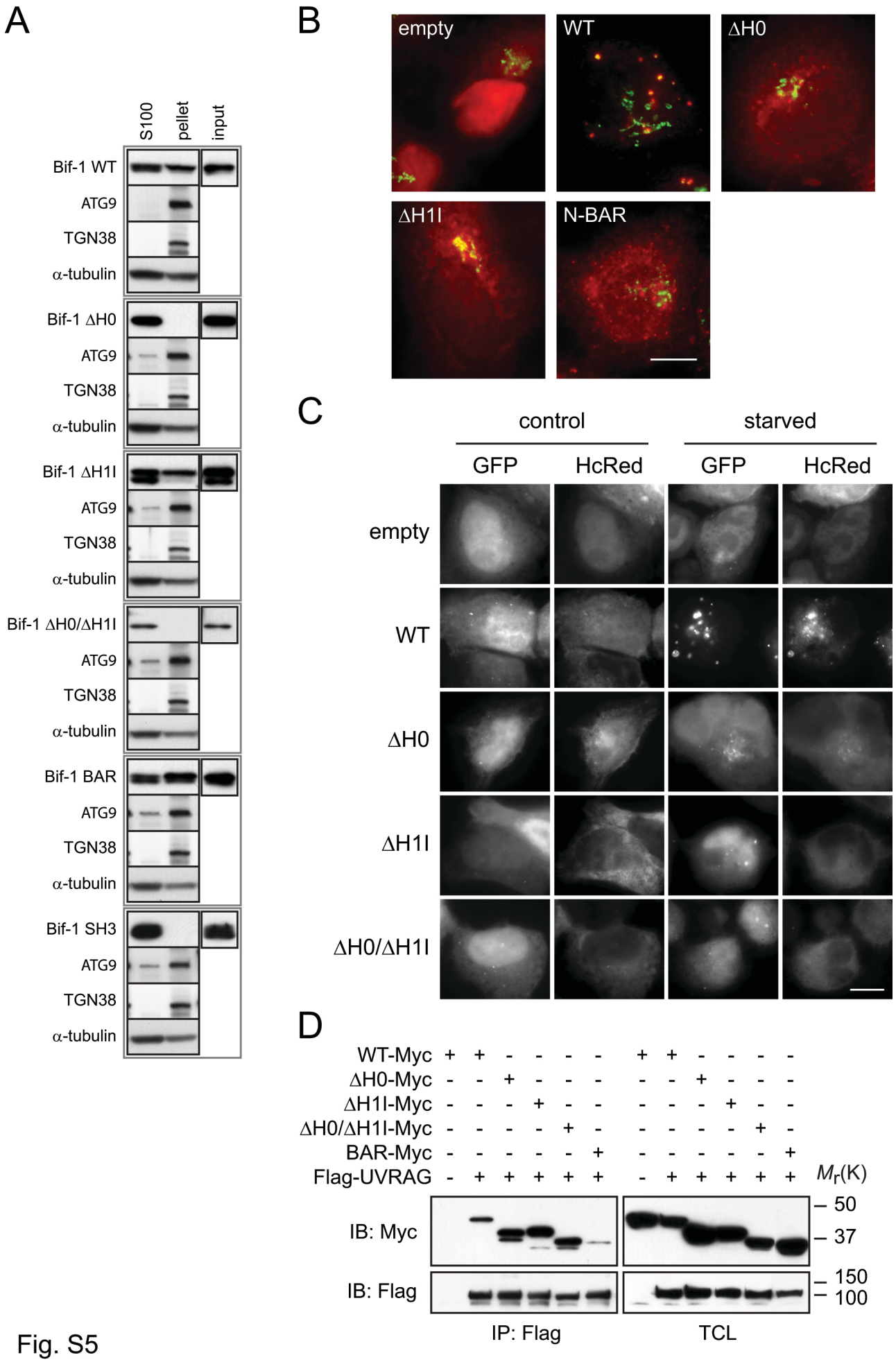
A
S100
pellet
input
Bif-1 WT
ATG9
TGN38
α-tubulin
Bif-1 ΔH0
ATG9
TGN38
α-tubulin
Bif-1 ΔH1I
ATG9
TGN38
α-tubulin
Bif-1 ΔH0/ΔH1I
ATG9
TGN38
α-tubulin
Bif-1 BAR
ATG9
TGN38
α-tubulin
Bif-1 SH3
ATG9
TGN38
α-tubulin
B
empty
WT
ΔH0
ΔH1I
N-BAR
C
control
starved
GFP
HcRed
GFP
HcRed
empty
WT
ΔH0
ΔH1I
ΔH0/ΔH1I
D
WT-Myc + + - - - - + + - - - -
ΔH0-Myc - - + - - - - - + - - -
ΔH1I-Myc - - - + - - - - - + - -
ΔH0/ΔH1I-Myc - - - - + - - - - - + -
BAR-Myc - - - - - + - - - - - +
Flag-UVRAG - + + + + + - + + + + +
Mr(K)
IB: Myc
IB: Flag
50
37
150
100
IP: Flag
TCL

Fig. S5

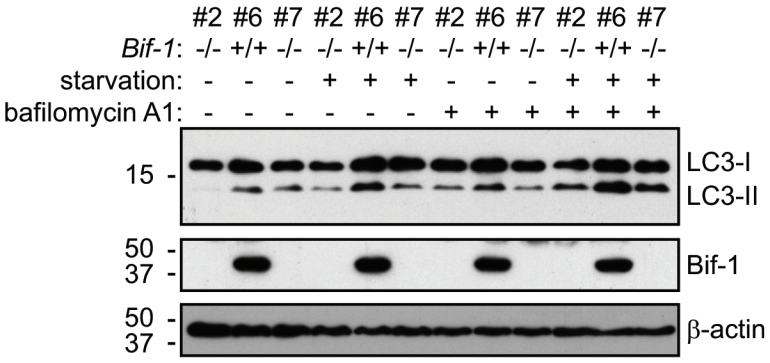
#2 #6 #7 #2 #6 #7 #2 #6 #7 #2 #6 #7
Bif-1: -/- +/+ -/- -/- +/+ -/- -/- +/+ -/- -/- +/+ -/-
starvation: - - - + + + - - - + + +
bafilomycin A1: - - - - - - + + + + + +
15
LC3-I
LC3-II
50
37
Bif-1
50
37
β-actin

Fig. S6

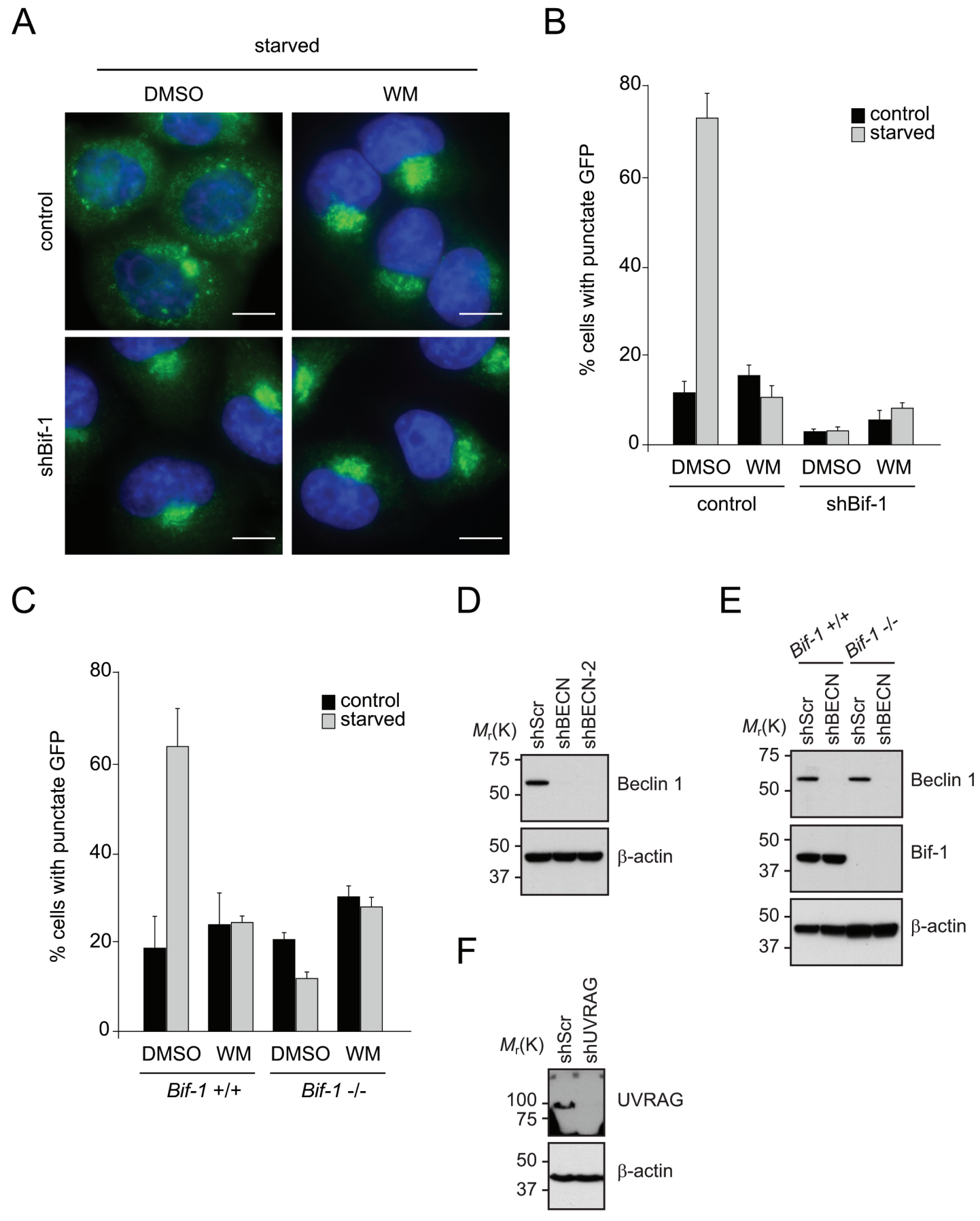
A
starved
DMSO
WM
control
shBif-1
B
% cells with punctate GFP
control
starved
DMSO
WM
DMSO
WM
control
shBif-1
C
% cells with punctate GFP
control
starved
DMSO
WM
DMSO
WM
Bif-1 +/+
Bif-1 -/-
D
Mr(K)
shScr
shBECN
shBECN-2
Beclin 1
β-actin
E
Bif-1 +/+
Bif-1 -/-
Mr(K)
shScr
shBECN
shScr
shBECN
Beclin 1
Bif-1
β-actin
F
Mr(K)
shScr
shUVRAG
UVRAG
β-actin

Fig. S7

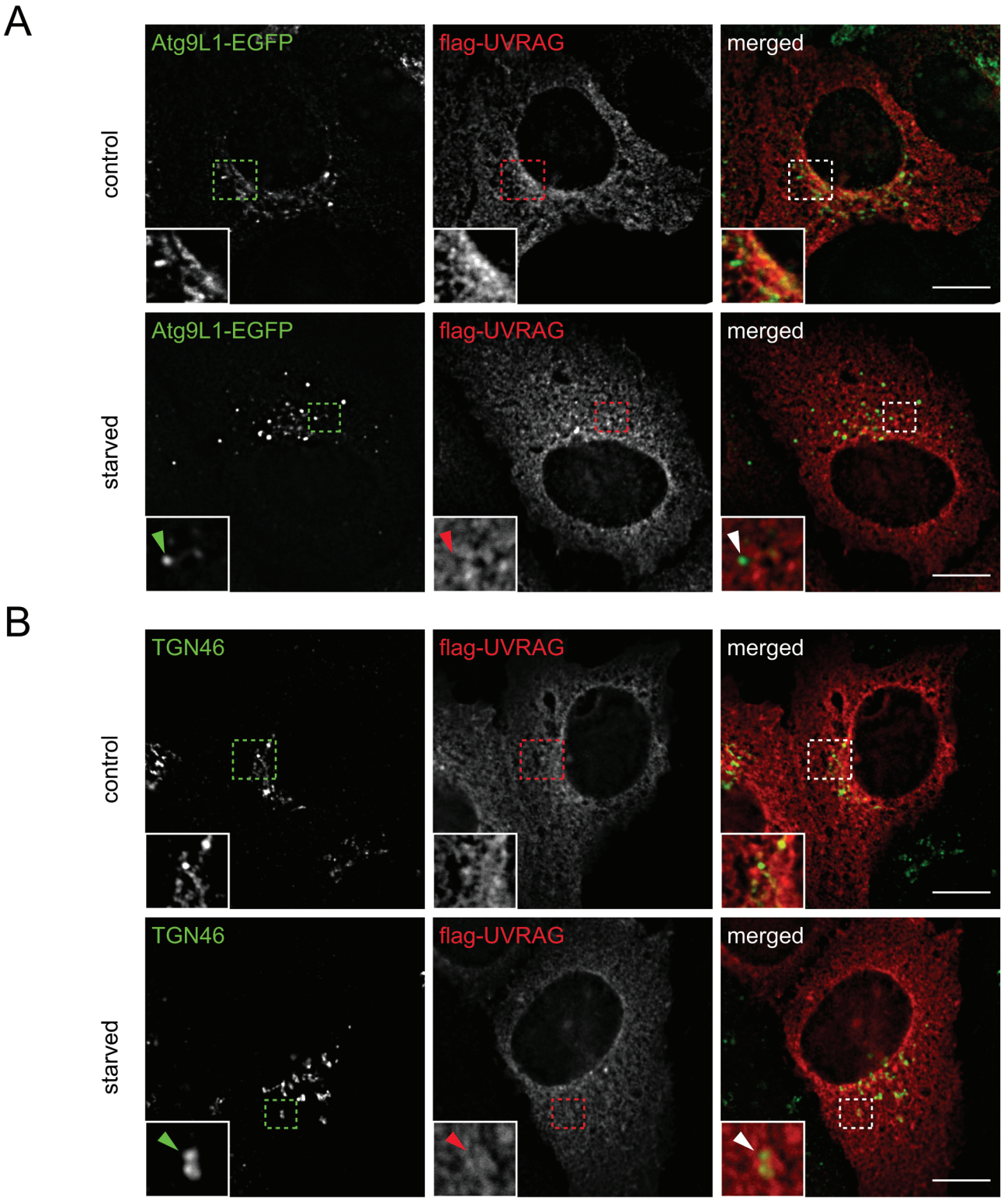
A
Atg9L1-EGFP
flag-UVRAG
merged
control
Atg9L1-EGFP
flag-UVRAG
merged
starved
B
TGN46
flag-UVRAG
merged
control
TGN46
flag-UVRAG
merged
starved

Fig. S8

Supplementary Figure Legends

Figure S1. Bif-1 regulates Atg9 foci formation during starvation or rapamycin treatment. (A) *Bif-1* +/+ and *Bif-1* -/- MEFs were transfected with Atg9L1-EGFP for 24 h, then cultured in complete or starvation medium for 1.5 h or treated with 100 nM rapamycin for 4 h. The cells were subjected to fluorescent microscopic analysis and the percentage of cells with cytoplasmic Atg9 foci was calculated (mean ± s.d.; n = 3 x 86). (B) Control or Bif-1 knockdown HeLa cells were cultured in complete or starvation medium for 1.5 h and subjected to immunofluorescent staining for endogenous Atg9. (C) HeLa cells stably expressing Atg9L1-EGFP were incubated in complete or starvation medium for 1.5 h and subjected to immunofluorescent staining for endogenous Bif-1. Magnified images are shown as the insets. Fluorescent images were obtained using a deconvolution microscope. Magnified images are shown in the right panel. Arrows indicate representative Atg9 dots. The scale bars represent 10 μm (5 μm in magnified images).

Figure S2. Bif-1 is required for starvation-induced Golgi fragmentation. (A) Control or Bif-1 shRNA-expressing HeLa cells were stably transfected with AcGFP-Golgi and incubated in complete or starvation medium for 1.5 h. Microbutubles were labeled with anti-α-tubulin monoclonal antibodies. (B) HeLa cells stably transfected with AcGFP-Golgi were cultured in complete or starvation medium for 1.5 h and immunostained for GM130 or TGN46. Notably, AcGFP-Golgi signals were positive for or adjacent to the signals of GM130 and TGN46 in both normal and starvation conditions, indicating that AcGFP-Golgi specifically localizes at both *cis*- and *trans*- regions of the Golgi complex. (C) Control or Bif-1 shRNA-expressing HeLa cells were starved for 1.5 h and immunostained for TGN46 (green) and GM130 (red). Magnified images are shown in the lower panels. All images were obtained using a deconvolution microscope. The scale bars represent 10 μm.

Figure S3. Starvation promotes translocation of Atg9, Bif-1 or Rab5 to autophagosomal membranes. (A) HeLa cells stably expressing Atg9L1-EGFP were transfected with HcRed-LC3 for 24 h, then cultured in starvation medium for 2 h. Magnified images are shown as insets. Arrows indicate co-localization of HcRed-LC3 with Atg9L1-EGFP. (B) *Bif-1* +/+ and -/- MEFs were incubated in complete or starvation medium for 1.5 h and immunostained for Atg16L (green) and Bif-1 (red). Magnified images are shown in the right panels. (C) HeLa cells stably expressing Atg9L1-EGFP were transiently transfected with DsRed-Rab9 for 24 h, starved for 1.5 h and analyzed by deconvolution fluorescent microscopy. Magnified images are shown as insets. (D) HeLa cells were transfected with RFP-Rab5 for 24 h, starved for 1.5 h and immunostained for Atg16L. Magnified images are shown in the right panels. All images were obtained using a deconvolution microscope. The scale bars represent 10 μm in (A-D) and 1 μm in magnified images in (A).

Figure S4. Fragmented TGN46-positive membranes locate adjacent to Atg16L-positive foci. HeLa cells or HeLa cells stably expressing GFP-LC3 were incubated in complete or starvation medium for 1.5 h and immunostained for Atg16L (green) and TGN46 (red) or TGN46 (red), respectively. The images were obtained using a deconvolution microscope. Magnified images are shown as insets. The scale bars represent 10 μm.

Figure S5. The H0 domain is required for Bif-1 binding to membranes, but not for Bif-1 interaction with UVRAG. (A) Total cell extracts prepared from *Bif-1* -/- MEFs were incubated with recombinant WT Bif-1 or mutant (ΔH0, ΔH1I, ΔH0/ΔH1I, N-BAR or SH3) proteins, fractionated and subjected to immunoblot analysis with the indicated antibodies. (B) Bif-1 knockdown HeLa cells were transfected with Bif-1 shRNA-resistant WT or mutant Bif-1-HcRed or empty HcRed vector for 24 h. After incubation in complete or starvation medium for 1.5 h, the cells were stained with anti-TGN46 sheep polyclonal antibodies followed by secondary antibody conjugated with Alexa Fluor 468. The

images were obtained using a fluorescent microscope. (C) HeLa cells stably expressing Bif-1 shRNA were co-transfected with GFP-LC3 and Bif-1 shRNA-resistant WT or mutant Bif-1-HcRed or empty HcRed1 vector for 24 h, then cultured in complete or starvation medium for 2 h. The images were obtained using a fluorescent microscope. (D) 293T cells were co-transfected with Flag-UVARG and WT or mutant Bif-1-Myc plasmids. Thirty hours after transfection, the cells were subjected to immunoprecipitation with anti-Flag monoclonal antibodies. The resultant immune complexes and total cell lysates (TCL) were analyzed by immunoblotting with anti-Myc and anti-Flag polyclonal antibodies. The scale bars represent 10 μm.

Figure S6. Loss of Bif-1 suppresses starvation-induced LC3-I lipidation. SV40 large T antigen-immortalized *Bif-1* +/+ and -/- MEFs were cultured in complete or starvation medium in the presence or absence of 100 nM bafilomycin A1 for 3 h and subjected to immunoblot analyses using the indicated antibodies.

Figure S7. The PI3KC3 complex II plays a key role in the regulation of starvation-induced Atg9 redistribution. (A) Control or Bif-1 shRNA-expressing HeLa cells were stably transfected with Atg9L1-EGFP. The cells were then incubated in complete or starvation medium in the presence of wortmannin (WM) or control DMSO for 1.5 h. (B) The percentage of cells with GFP foci in (A) was calculated (mean ± s.d.; n = 3 x 88). (C) *Bif-1* +/+ and *Bif-1* -/- MEFs were transfected with Atg9L1-EGFP for 24 h, cultured in complete or starvation medium in the presence of 0.2 μM wortmannin or control DMSO for 1.5 h. The cells were subjected to fluorescent microscopic analysis and the percentage of cells with cytoplasmic Atg9 foci was quantified (mean ± s.d.; n = 3 x 64). Knockdown of Beclin 1 in HeLa (D), *Bif-1* +/+ and *Bif-1* -/- MEFs (E) or UVRAG in HeLa cells (F) was confirmed by immunoblot analyses using the indicated antibodies. The scale bars represent 10 μm.

Figure S8. UVRAG is absent from starvation-induced Atg9 punctate structures. HeLa cells stably expressing Atg9L1-EGFP (A) or parental cells (B) were transiently transfected with flag-UVRAG for 24 h. The cells were incubated in complete or starvation medium for 1.5 h and immunostained for flag (red) (A) or flag (red) and TGN46 (green) (B). The images were obtained using a fluorescence deconvolution microscope. Magnified images are shown in the insets. The scale bars represent 10 μm.

Movies 1 and 2. Atg9-positive Golgi membranes undergo continuous tubulation and fragmentation in a Bif-1-dependent manner during starvation. Control (Movie 1) or Bif-1 knockdown (Movie 2) HeLa cells stably expressing Atg9L1-EGFP were nutrient starved for 45 min and then analyzed by time-lapse fluorescent microscopy at 5-sec intervals.