SUPPLEMENTAL FIGURES

Fig. S1: **Effect of the various alterations of microtubule dynamics on the microtubule network and on tubulin acetylation in HeLa cells**
Left: Conditions of nocodazole treatment leading to labile (1µM, 15 min) or total (10µM 1.5h + cold) MT disassembly. Right: Conditions of MT stabilization using low and high Taxol concentrations (without and with MT bundling) or submicromolar nocodazole treatment. Note that both labile MT disassembly and MT stabilization using submicromolar nocodazole concentrations led to the persistence of nocodazole-resistant, highly acetylated MTs. Scale bar = 10 µm.

Fig. S2: **Cytoplasm extraction with saponin does not affect LC3 distribution in puncta**
HeLa cells transiently expressing GFP-LC3 and subjected to starvation were permeabilized with 0.02% saponin as indicated and time-lapse recorded. Time is indicated in s. Arrows show small puncta that had not changed in size or location after extraction. Scale bar = 10 µm.

Fig. S3: **The GFP channel of tandem-LC3 labels autophagosomes, not autolysosomes and allows mobility measurements identical to those performed with GFP-LC3**
(**A**) HeLa cells transiently expressing tandem-LC3 were subjected to the labelling of lysosomes using Lysotracker® (blue). As shown by the open arrowheads, the GFP signal was not detected in locations where the mRFP signal colocalized with the Lysotracker® signal. Conversely, GFP signals did not colocalize with lysosomes (arrows). Scale bar = 10 µm. (**B**) Percentage of mobile GFP-LC3 and Tandem-LC3 (GFP) puncta in starved cells (n = 30 and 20, respectively). (**C**) Following GFP-LC3 or the GFP signal from tandem-LC3 allow identical measurements of autophagosome velocities. Velocity spectra were drawn from 2395 kymographs (44 cells) and 1029 kymographs (7 cells) measured in starved cells transiently expressing GFP-LC3 and Tandem-LC3 respectively. Cumulative curves (bottom) allow a better comparison of the two spectra.

Fig. S4: **Expression of non-acetylatable tubulin does not alter the microtubule network**
HeLa cells cultured on glass coverslips were transiently transfected with plasmids encoding mCherry-tubulin (WT) or a non-acetylatable K40A mutant of the protein. Cells were observed 2 days after transfection to allow fluorescent tubulin incorporation into MTs. Scale bar = 10 µm.

Fig. S5: **Autophagosome mobility involves microtubules**
HeLa cells transiently expressing tandem-LC3 were subjected to total MT disassembly (10µM nocodazole, 37°C, 1h30 then 10 µM nocodazole 2h on ice), selective labile MT disassembly (0.1µM, 37°C, 2h), or MT stabilization (1 or 10 µM Taxol, 37°C, 1h) prior to autophagy induction. After 4h in EBSS, the percentage of mobile autophagosomes was counted from the GFP channel and revealed that only complete MT removal impaired autophagosome mobility. Note the ~50% drop in autophagosome mobility that occurred upon starvation. Data are the mean ± SEM from at least 500 autophagosomes in each condition (10 cells). *** means $p<0.001$, ns: non-significant.

Figure S1

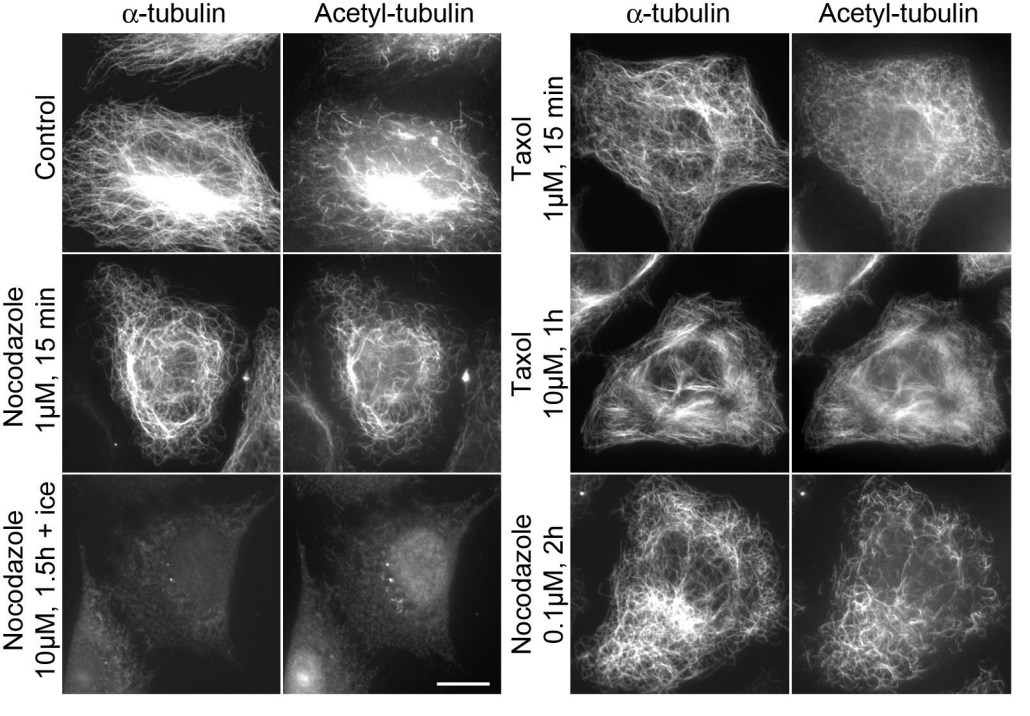

α-tubulin
Acetyl-tubulin
Control
Nocodazole 1μM, 15 min
Nocodazole 10μM, 1.5h + ice
α-tubulin
Acetyl-tubulin
Taxol 1μM, 15 min
Taxol 10μM, 1h
Nocodazole 0.1μM, 2h

Figure S2

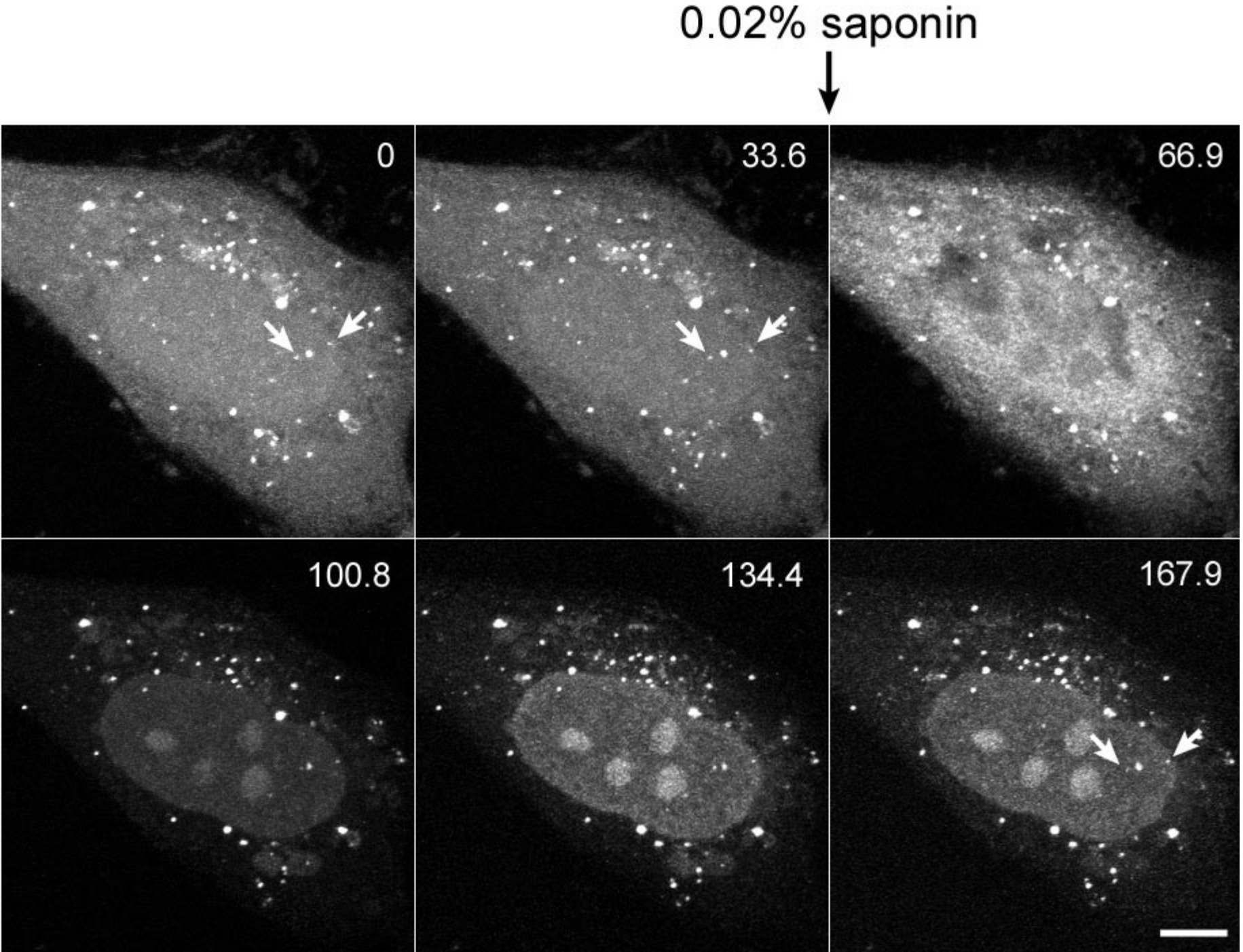


Figure S3

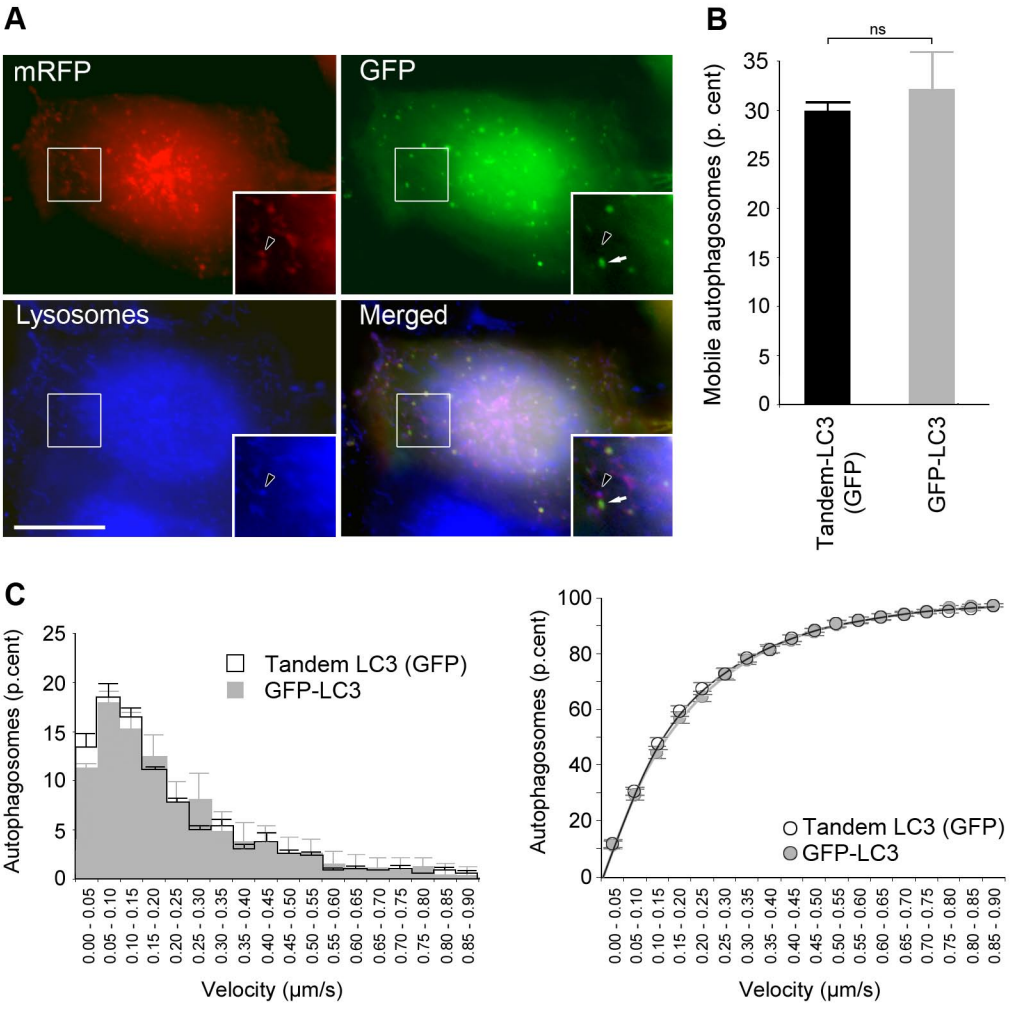

A
mRFP
GFP
Lysosomes
Merged
B
ns
Mobile autophagosomes (p. cent)
35
30
25
20
15
10
5
0
Tandem-LC3 (GFP)
GFP-LC3
C
Autophagosomes (p.cent)
25
20
15
10
5
0
Tandem LC3 (GFP)
GFP-LC3
0.00 - 0.05
0.05 - 0.10
0.10 - 0.15
0.15 - 0.20
0.20 - 0.25
0.25 - 0.30
0.30 - 0.35
0.35 - 0.40
0.40 - 0.45
0.45 - 0.50
0.50 - 0.55
0.55 - 0.60
0.60 - 0.65
0.65 - 0.70
0.70 - 0.75
0.75 - 0.80
0.80 - 0.85
0.85 - 0.90
Velocity (µm/s)
Autophagosomes (p.cent)
100
80
60
40
20
0
Tandem LC3 (GFP)
GFP-LC3
0.00 - 0.05
0.05 - 0.10
0.10 - 0.15
0.15 - 0.20
0.20 - 0.25
0.25 - 0.30
0.30 - 0.35
0.35 - 0.40
0.40 - 0.45
0.45 - 0.50
0.50 - 0.55
0.55 - 0.60
0.60 - 0.65
0.65 - 0.70
0.70 - 0.75
0.75 - 0.80
0.80 - 0.85
0.85 - 0.90
Velocity (µm/s)

Figure S4

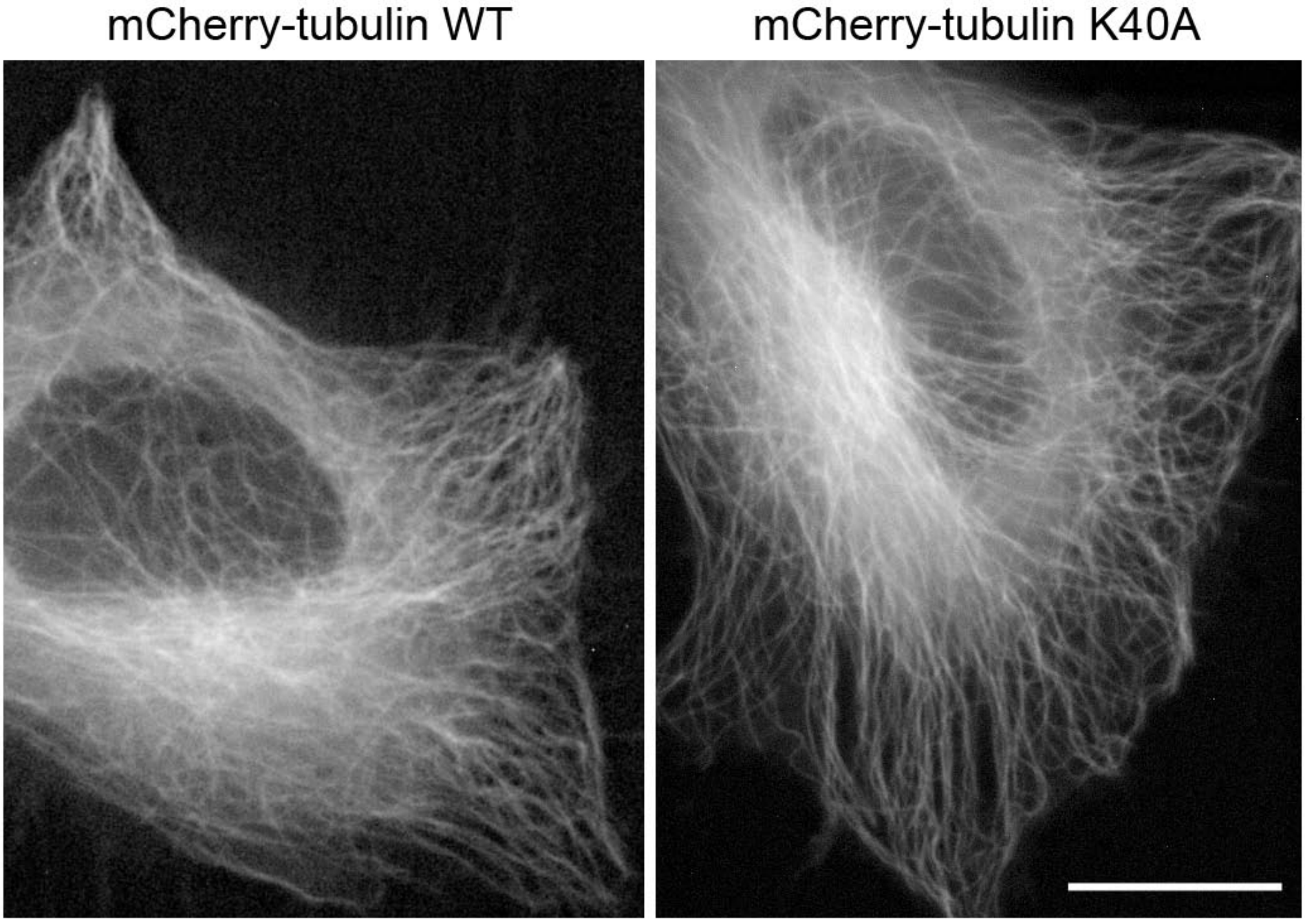
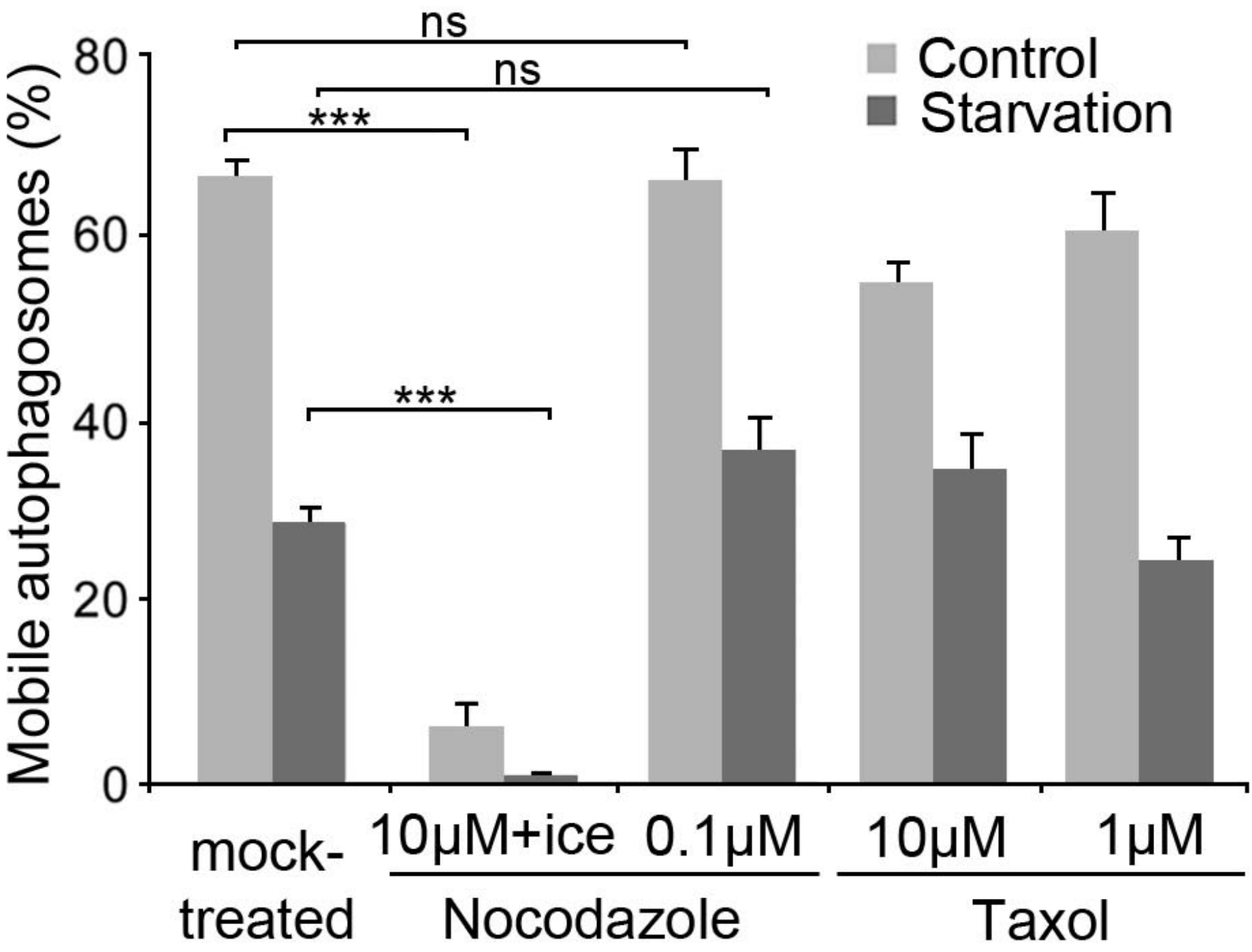


Figure S5


ns
ns
***
***
Control
Starvation
Mobile autophagosomes (%)
80
60
40
20
0
mock-treated
10μM+ice
0.1μM
10μM
1μM
Nocodazole
Taxol