E07-09-0868 Dunn

Supplemental Figure 1. Effects of sar1pT34N and sar1pH79G on cell growth. WDY64 (sar1pT34N) and WDY65 (sar1pH79G) were grown in minimal YND medium for 24 hours in the absence or presence of $CuSO_4$ and growth of the culture assessed by measuring the absorbance at 600nm. The effects of the Sar1p mutants on cell growth were minimal at five hours of copper induction. However, at longer times, growth was dramatically reduced compared to untreated cells.

Supplemental Figure 2. Cellular localization of Sec7p in cells expressing sar1pT34N and sar1pH79G. WDY64 (sar1pT34N) and WDY65 (sar1pH79G) expressing GFP-Sec7p were grown in minimal YND medium for 16 hours and an additional 3h in the absence and presence of $CuSO_4$. The distribution of GFP-Sec7p was visualized *in situ* by fluorescence microscopy. In the absence of copper, Sec7p was localized to the Golgi apparatus and appeared as small crescent-like structures. An increase in cytosolic localization of Sec7p was observed in the presence of sar1pT34N or sar1pH79G.

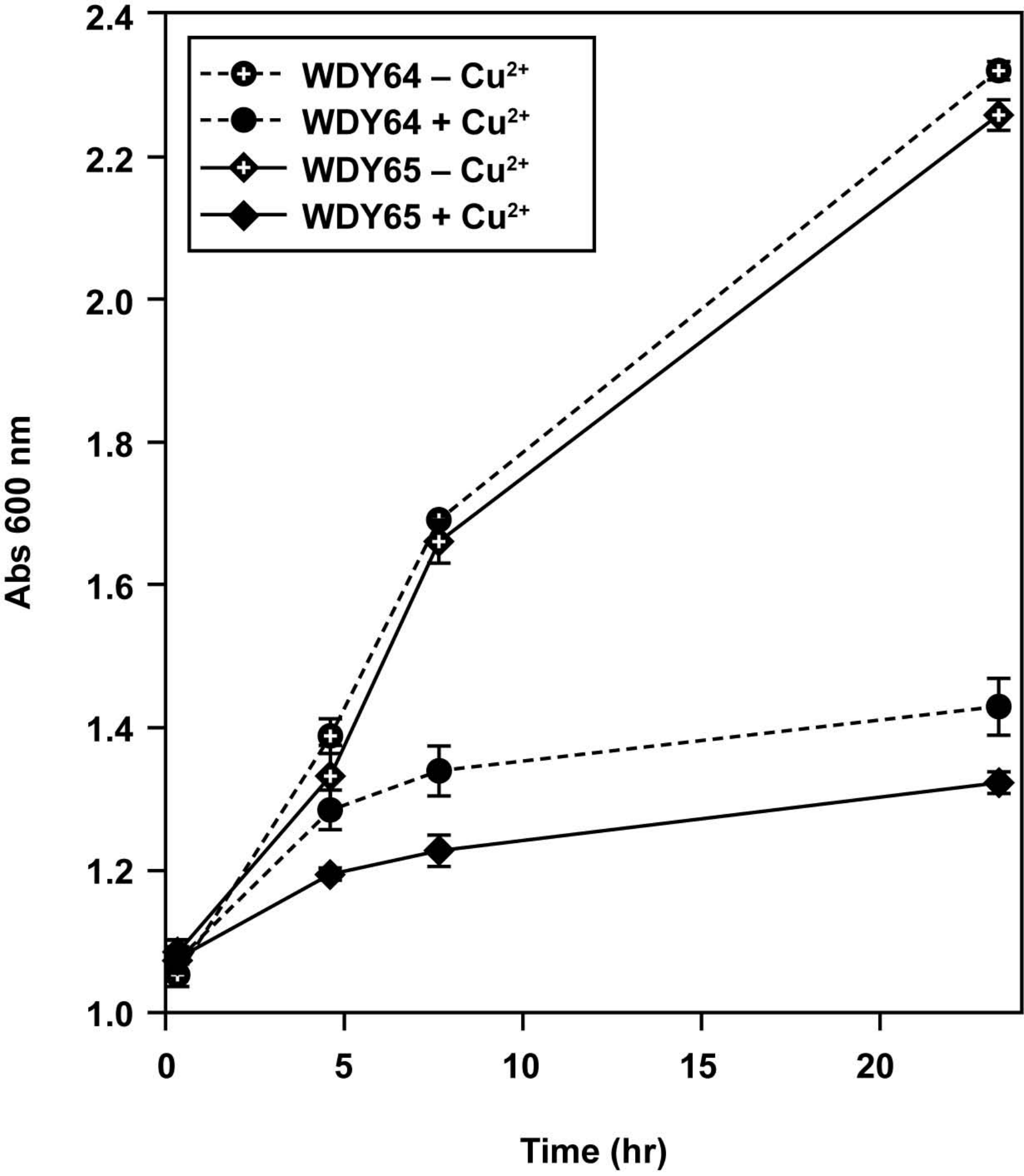
Supplemental Figure 3. Effects of sar1pT34N and sar1pH79G on starvation-induced autophagy. The degradation of cellular proteins during nitrogen starvation was performed as described previously (Tuttle and Dunn, 1995). Endogenous proteins of GS115, WDY64 (sar1pT34N), and WDY65 (sar1pH79G) cells were labeled with ^{14}C-valine for 16 hours in YND. The cells were then washed and switched to SD-N medium with and without $CuSO_4$ supplemented with 10 mM valine. Aliquots were removed at 2-24 hours of chase and precipitated with TCA. The rates of protein degradation based on the production of TCA-soluble radioactivity were expressed as a

percentage relative to untreated controls. The statistical differences relative to wild-type were determined by Student t-test (*, $p = 0.015$; **, $p = 0.033$)

Supplemental Figure 4. Stages of glucose-induced micropexophagy. Cells expressing BFP-SKL were adapted from YNM to YND for 2h. Cells at all stages of micropexophagy were visualized by fluorescence microscopy. During signaling, the peroxisomes labeled with BFP-SKL were situated next to a round vacuole visualized by FM4-64. Upon initiation, the sequestering membranes that arise by segmentation from the vacuole begin to extend around the peroxisomes. The expansion stage was characterized by sequestering membranes extending 50 to 90% around the peroxisomes. The completion stage was characterized by peroxisomes fully surrounded by sequestering membranes that appear segmented and not fused. Finally, the degradative stage was characterized by continuous FM4-64 labeled membranes enclosing peroxisomes that were not clearly delineated.

Supplemental Figure 5. Cellular localization of Atg18p and Atg17p during glucose-induced micropexophagy in cells expressing sar1pT34N or sar1pH79G. WDY64 (sar1pT34N) and WDY65 (sar1pH79G) cells expressing GFP-Atg18p or GFP-Atg17p were adapted from YNM to YND ($\pm$ $CuSO_4$). At 2h of glucose adaptation, the cells were visualized by fluorescence microscopy. Regardless of the presence of the Sar1p mutants, GFP-Atg18p trafficked normally to the vacuole and sequestering membranes. In untreated cells, GFP-Atg17p was visualized at 1 or 2 foci (PAS) near the vacuole (white arrowhead). In cells expressing sar1pT34N or sar1pH79G, GFP-Atg17p localized to multiple foci that at times appeared to coalesce (yellow arrowhead).

Supplemental Figure 6. Pexophagosomes form in the presence of sar1pH79G. WDY64 (sar1pT34N) and WDY65 (sar1pH79G) cell lines were adapted from YNM to YNE in the presence of $CuSO_4$. At 2h of ethanol adaptation, the cells were fixed and prepared for viewing by electron microscopy. When sar1pT34N was expressed, membranes were occasionally observed near peroxisomes, but not engulfing them (arrow). However, in the presence of sar1pH79G, individual peroxisomes were observed surrounded by the multiple membranes of the pexophagosome (arrowheads). V, vacuole; P, peroxisome.


WDY64 – Cu2+
WDY64 + Cu2+
WDY65 – Cu2+
WDY65 + Cu2+
Abs 600 nm
2.4
2.2
2.0
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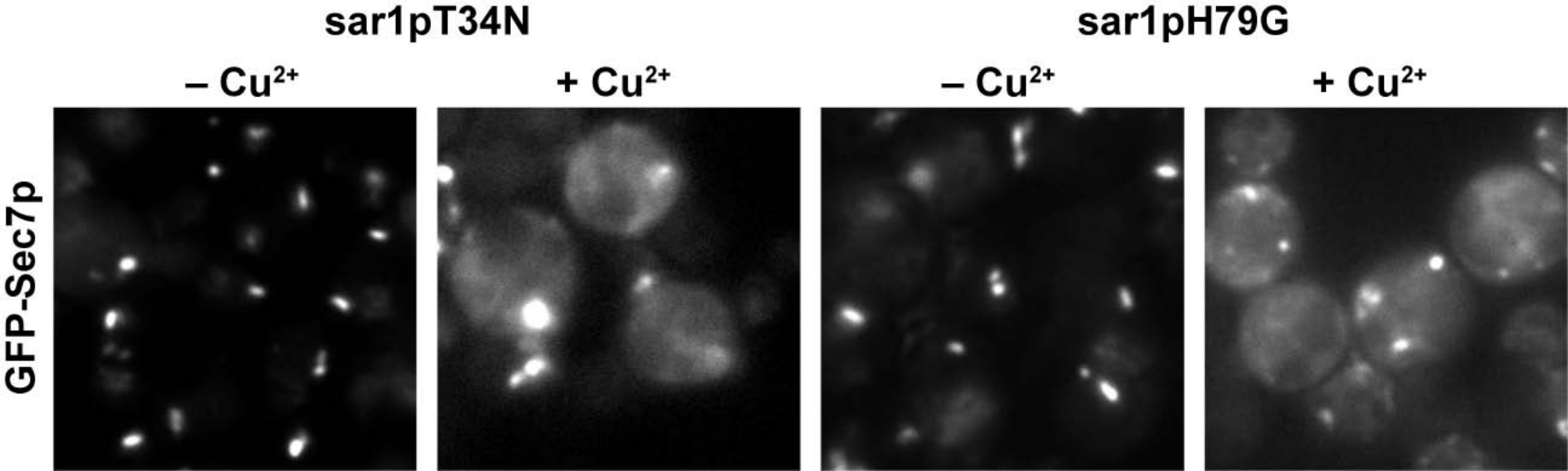
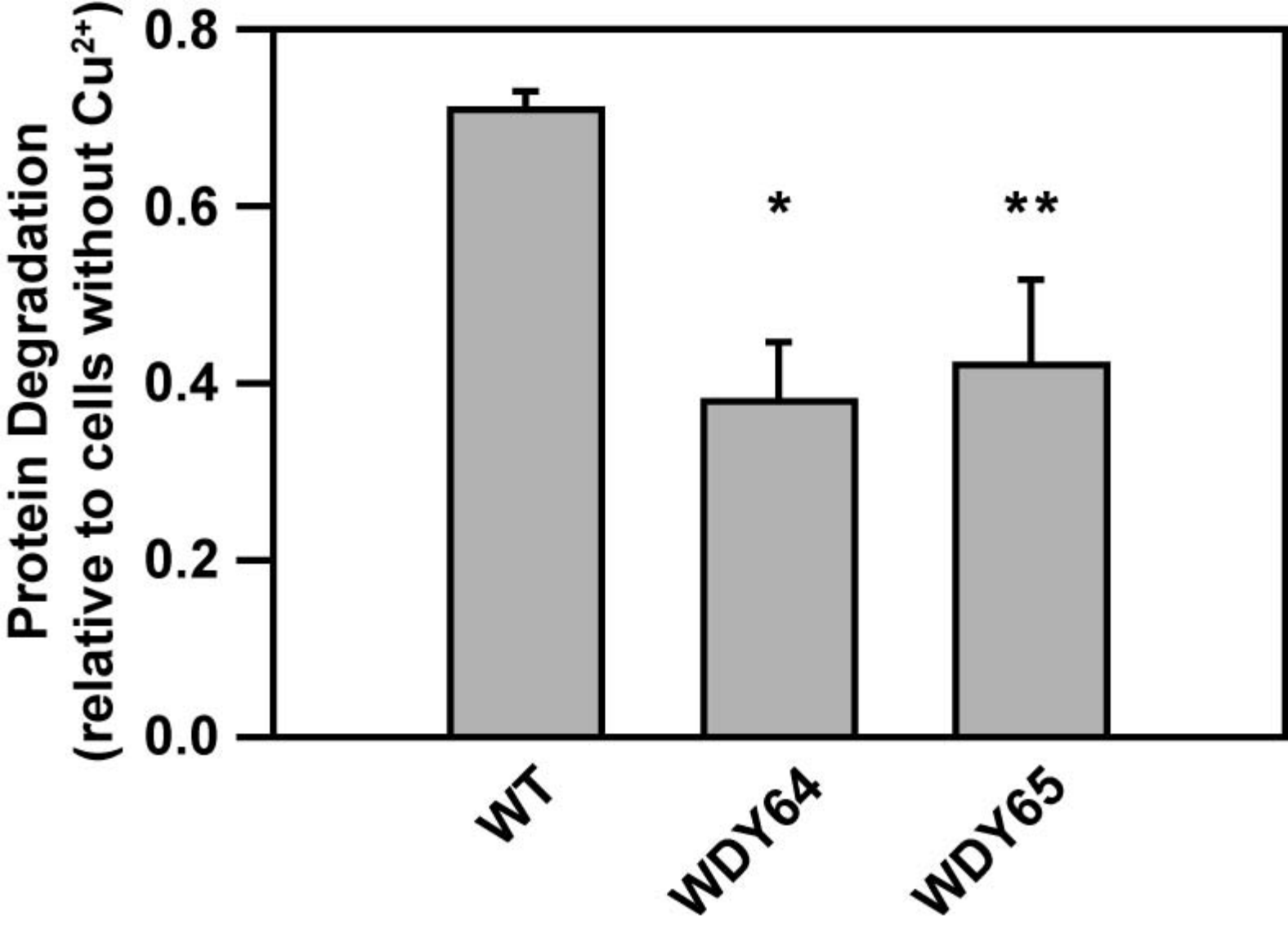
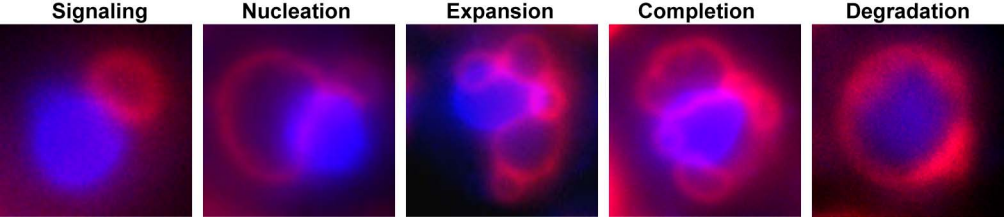
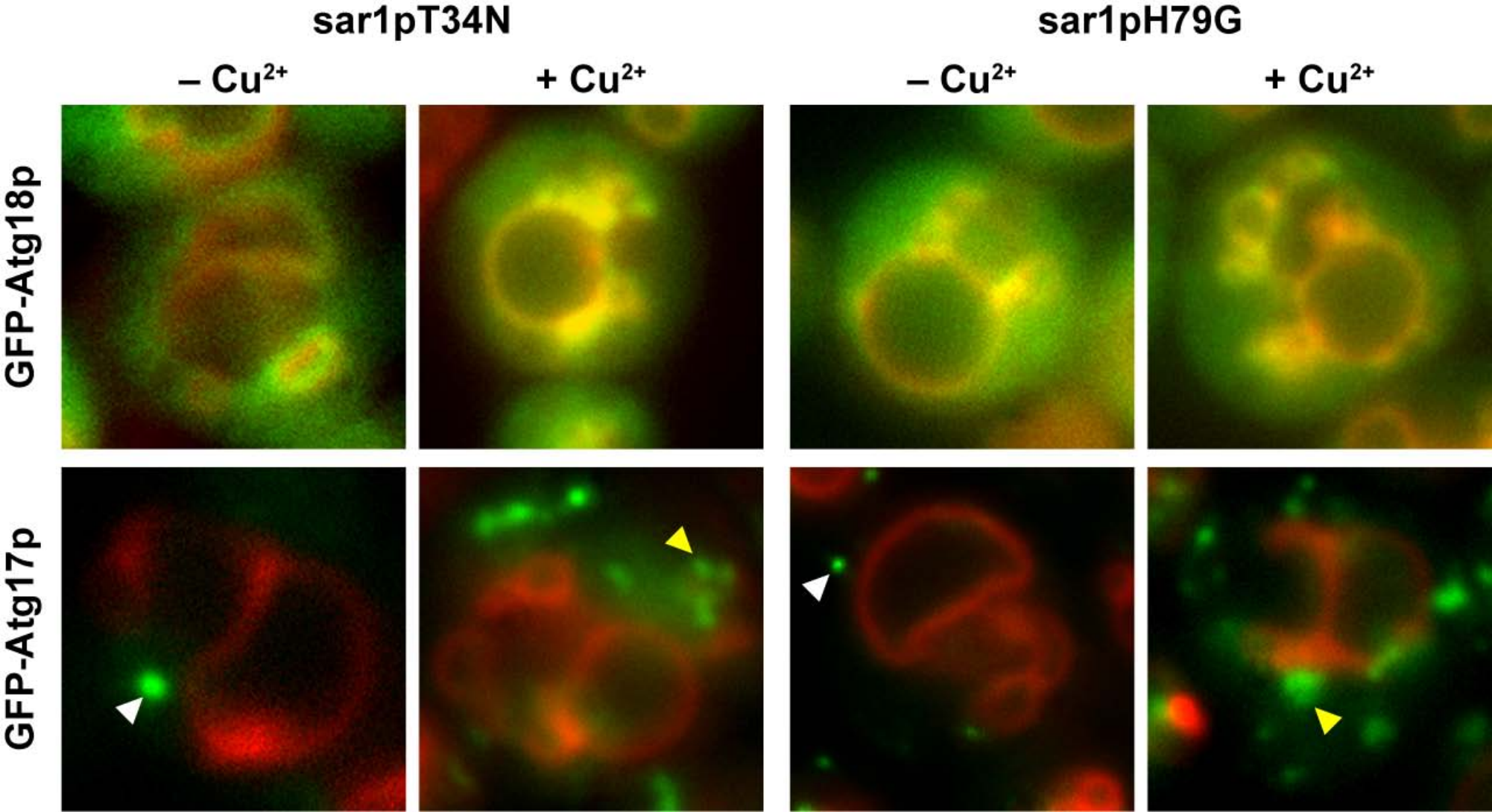
1.6
1.4
1.2
1.0
0
5
10
15
20
Time (hr)


sar1pT34N
sar1pH79G
– Cu2+
+ Cu2+
– Cu2+
+ Cu2+
GFP-Sec7p


Protein Degradation
(relative to cells without Cu2+)
0.8
0.6
0.4
0.2
0.0
*
**
WT
WDY64
WDY65


Signaling
Nucleation
Expansion
Completion
Degradation


sar1pT34N
sar1pH79G
– Cu2+
+ Cu2+
– Cu2+
+ Cu2+
GFP-Atg18p
GFP-Atg17p

sar1pT34N

sar1pH79G