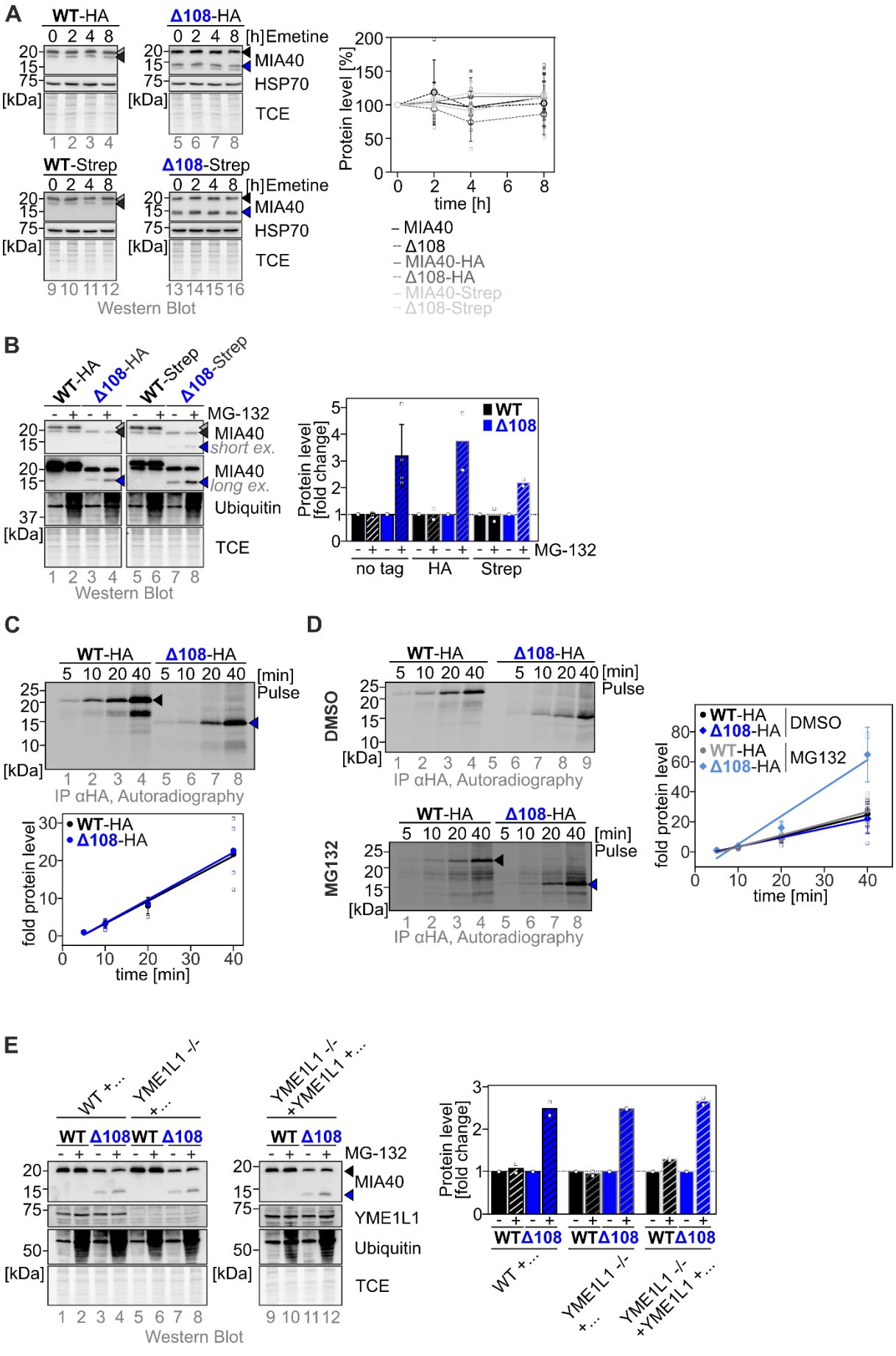
A
WT-HA
Δ108-HA
0 2 4 8 [h]Emetine
MIA40
HSP70
TCE
[kDa]
1 2 3 4
5 6 7 8
WT-Strep
Δ108-Strep
9 10 11 12
13 14 15 16
Western Blot
Protein level [%]
time [h]
– MIA40
-- Δ108
– MIA40-HA
-- Δ108-HA
– MIA40-Strep
-- Δ108-Strep
B
MG-132
MIA40 short ex.
MIA40 long ex.
Ubiquitin
Protein level [fold change]
WT
Δ108
no tag
HA
Strep
C
5 10 20 40 [min]
Pulse
IP αHA, Autoradiography
fold protein level
time [min]
D
DMSO
MG132
E
WT +…
YME1L1 -/- +…
YME1L1 -/- +YME1L1 +…
MG-132
MIA40
YME1L1
Ubiquitin
TCE

ADDITIONAL FILE 2: Figure S2. In intact cells, C-terminal truncated MIA40 variants can be stabilized by proteasomal inhibition.

(A) Emetine chase analyses of truncated MIA40 variants. As **Figure 2B** except that tagged MIA40 variants were expressed (1 µg ml^{-1} doxycyclin for 24 h). Mature MIA40$^{\Delta 108}$ is equally stable as MIA40^{WT} independently of the presence of a tag. Quantification using Image Lab. Data from at least 2 experiments (HA tagged: n= 3, Strep tagged: n=2) were combined and standard deviations are presented if n>2. Black arrowhead, endogenous MIA40; gray arrowhead, MIA40-HA; blue arrowhead, signal of MIA40$^{\Delta 108}$

(B) Steady state levels of MIA40$^{\Delta 108}$ and MIA40^{WT} upon proteasomal inhibition. As **Figure 2C** except that tagged MIA40 variants were expressed (1 µg ml^{-1} doxycyclin and 1 µM MG132 for 16 h). MIA40$^{\Delta 108}$ is present at strongly decreased levels compared to MIA40^{WT} but can be partially stabilized by proteasomal inhibition independently of the presence of a tag. Quantification using Image Lab. Data from (HA tagged: n= 2, Strep tagged: n=2) experiments were combined and standard deviations are presented if n>2. Black arrowhead, endogenous MIA40; gray arrowhead, MIA40-HA; blue arrowhead, signal of MIA40$^{\Delta 108}$

(C) Pulse analysis of MIA40 variant synthesis. HEK293 cells stably and inducibly expressing MIA40^{WT}-HA and MIA40$^{\Delta 108}$-HA were pulse-labeled with ^{35}S-methionine for different times. Cells were lysed and MIA40 variants isolated by immunoprecipitation against the HA tag. Eluates were analyzed by SDS-PAGE and autoradiography. Synthesis of both variants followed similar kinetics although absolute levels were higher for MIA40^{WT} indicating degradation of MIA40$^{\Delta 108}$ during the radioactive pulse.. Quantification using ImageQuantTL. Data from 2-3 experiments were combinedand standard deviations are presented if n>2. Black arrowhead, wildtype MIA40; blue arrowhead, signal of MIA40$^{\Delta 108}$

(D) Pulse analysis of MIA40 variant synthesis upon proteasomal inhibition. Experiment was performed as in **(C)**, except that cells were treated with MG132 or DMSO. The wildtype is not stabilized by MG132 treatment. MIA40$^{\Delta 108}$ became stabilized upon MG132 treatment indicating that already during the radioactive pulse degradation takes place. Quantification using Image Lab. Data from 2-3 experiments were combined and standard deviations are presented if n>2. Black arrowhead, wildtype MIA40; blue arrowhead, signal of MIA40$^{\Delta 108}$

(E) Steady state levels of MIA40 truncation variants in HEK293-based YME1L deletion cells upon proteasomal inhibition. The experiment was performed as in **Figure 2A** except that cells were treated with MG132 or DMSO (1 µg ml^{-1} doxycyclin and 1 µM MG132 for 16 h). MIA40$^{\Delta 108}$ is present at decreased levels compared to MIA40^{WT}. It is stabilized by MG132 treatment but not by loss of YME1L. Combination of both MG132 and loss of YME1L did not further increase MIA40 levels. Quantification using Image Lab. Data from 2 experiments were combined. Black arrowhead, wildtype MIA40; blue arrowhead, signal of MIA40$^{\Delta 108}$