**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 MIA40Δ108 is equally stable as MIA40WT 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 MIA40Δ108

(B) Steady state levels of MIA40Δ108 and MIA40WT 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). MIA40Δ108 is present at strongly decreased levels compared to MIA40WT 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 MIA40Δ108

(C) Pulse analysis of MIA40 variant synthesis. HEK293 cells stably and inducibly expressing MIA40WT-HA and MIA40Δ108-HA were pulse-labeled with ³⁵S-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 MIA40WT indicating degradation of MIA40Δ108 during the radioactive pulse.. Quantification using ImageQuantTL. Data from 2-3 experiments were combined and standard deviations are presented if n>2. Black arrowhead, wildtype MIA40; blue arrowhead, signal of MIA40Δ108

(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. MIA40Δ108 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 MIA40Δ108

(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). MIA40Δ108 is present at decreased levels compared to MIA40WT. 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 MIA40Δ108