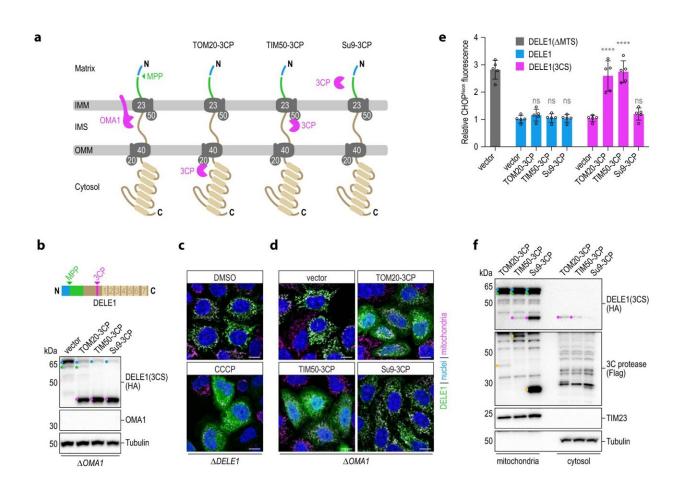


## **Cell biology: How mitochondria report stress**

## April 7 2022



Cleavage of DELE1 in the IMS is sufficient for its release to the cytosol and activation of the ISR. a Schematic depicting the localization of the proteases employed in this figure relative to the DELE1 protein during its import into mitochondria. b HeLa OMA1 knockout ( $\Delta OMA1$ ) cells were transiently transfected with DELE1(3CS) and empty vector control or the indicated 3C protease fused to TOM20, TIM50 or the sorting signal of Su9. Cleavage of the DELE1 protein was analyzed by immunoblotting. c, d HeLa cells were transiently transfected with DELE1(3CS) as in (b). Localization of the DELE1 protein was analyzed by confocal microscopy after a 2 h treatment with DMSO



or CCCP (c) or in the context of the co-transfected 3C proteases (d). Scale bars, 10 µm. Nuclei (DAPI, blue), mitochondria (MitoTrackerRed, pink), DELE1 (HA, green). e The induction of the ISR marker CHOP was measured in HAP1 CHOP<sup>Neon</sup> OMA1 knockout cells by flow cytometry upon transient transfection of the indicated constructs together with mCherry. Relative CHOP<sup>Neon</sup> fluorescence to empty vector transfected cells is shown. Graph depicts mean ± s.d. of n = 5 independent experiments. DELE1( $\Delta$ MTS) was used as positive control. Statistical significance within DELE1 constructs compared to the respective vector control was assessed by ordinary one-way ANOVA and Dunnett's multiple comparisons correction. DELE1: ns  $\geq$  0.4019; DELE1(3CS): \*\*\*\*\*P

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