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Mitochondria are remarkably dynamic organelles in eukaryotic cells that undergo continuous cycles of homotypic fusion and fission events. Maintaining proper mitochondrial morphology is essential not only for accurate organelle inheritance but also for optimizing physiologic functions, such as signal transduction, metabolic activity, and quality control (Wai and Langer, 2016; Ng et al, 2021; Picard and Shirihai, 2022; Tábara et al, 2025). Recent studies in an in vivo model revealed that genetic ablation of individual components involved in mitochondrial dynamics impairs their organ functions and whole-body metabolism (Chen et al, 2003; Ishihara et al, 2009; Wakabayashi et al, 2009; Wai et al, 2015; Tezze et al, 2017). Abnormal mitochondrial dynamics are also associated with some human diseases or disorders, such as Charcot-Marie-Tooth disease type 2 A (Züchner et al, 2004), autosomal dominant optic atrophy (Delettre et al, 2000), neuromuscular defects (Shamseldin et al, 2012), and a lethal developmental disorder (Waterham et al, 2007). In addition to these inherited disorders, an imbalance in mitochondrial fusion and fission can suppress innate immune responses to viral infection (Castanier et al, 2010; Koshiba et al, 2011; Hanada et al, 2020; Yasukawa et al, 2020), highlighting the intimate link between mitochondrial dynamics and the host defense system.

Defects in mitochondrial fusion significantly enhance the heterogeneity and dysfunction of the mitochondrial population within the cell (Chen et al, 2005), leading to impaired stress responses and loss of cellular homeostasis (MacVicar and Langer, 2016). In mammals, three conserved guanosine triphosphatases (GTPases) of the dynamin family coordinate the fusion process: Mitofusins (Mfn1 and Mfn2), which are localized to the mitochondrial outer membrane (MOM), and optic atrophy 1 (OPA1), which is localized to the mitochondrial inner membrane (MIM). In humans, OPA1 has eight isoforms that derive from alternative splicing variants with further post-translational processing by mitochondrial proteases (Wai et al, 2015; Chan, 2020). Overlapping with the m-AAA protease 1 homolog (OMA1) is a stress-activated MIM peptidase that belongs to the M48 family of zinc metallopeptidases (Käser et al, 2003; López-Pelegrín et al, 2013) and constitutively processes OPA1 under normal conditions (Ishihara et al, 2006; Song et al, 2007; Ehses et al, 2009; Head et al, 2009; Quirós et al, 2012). General cellular stresses (e.g., depolarization, oxidization, or heat shock), however, lead to OMA1 activation and complete processing of long-form OPA1 (L-OPA1) isoforms in mitochondria (Ehses et al, 2009; Head et al, 2009; Quirós et al, 2012; Anand et al, 2014; Baker et al, 2014; Murata et al, 2020). Various studies of OMA1 gene ablation in mice revealed the physiologic role of OMA1-regulated pathways. Knockout of OMA1 generally produces relatively mild background-dependent phenotypes characterized by increased body weight and defective thermogenesis (Quirós et al, 2012). In certain genetic backgrounds, however, such as mouse models of mitochondrial cardiomyopathy caused by oxidative phosphorylation (OXPHOS) deficiency (Ahola et al, 2022) or by simultaneous loss of Parkin (Yamada et al, 2025), OMA1 deficiency results in more severe mitochondrial dysfunction and disrupted dynamics. In contrast, however, OMA1 depletion is protective in mouse models of neurodegeneration (Korwitz et al, 2016), ischemic kidney injury (Xiao et al, 2014), or heart

failure (Wai et al, 2015; Acin-Perez et al, 2018), supporting the pro-apoptotic function of OMA1 by preventing OPA1 cleavage under stress conditions. Thus, OMA1 regulates cell survival in a cell- and tissue-specific manner, although the precise mechanisms remain unknown. Recent studies have further implicated OMA1 in another mitochondrial signaling pathway that activates the integrated stress response (ISR) via DAP3-binding cell death enhancer 1 (DELE1), a protein kinase activator (Fessler et al, 2020, 2022; Guo et al, 2020). In this pathway, stress-activated OMA1 cleaves DELE1 during import, generating a short form (S-DELE1) that is released from the mitochondria into the cytosol, where it ultimately triggers the ISR. Therefore, OMA1 also regulates the dynamic properties of mitochondrial morphology and cellular stress signaling to ensure mitochondrial integrity and cellular homeostasis. Despite its role as a central pivotal protease involved in a wide range of mitochondrial homeostatic processes, only a limited number of OMA1 substrates have been functionally characterized.

In the present study, using a proteome-wide approach combined with in vitro and in vivo biochemical analysis, we revealed that the intermembrane space (IMS) protein apoptosis-inducing factor mitochondria-associated 1 (AIFM1) is an OMA1 substrate under mitochondrial stress. AIFM1 is a mitochondrial flavoprotein initially identified as a trigger of caspase-independent apoptosis under certain conditions (Susin et al, 1999). Besides its role in cell death, AIFM1 contributes to cellular energy metabolism and redox homeostasis (Vahsen et al, 2004; Pospisilik et al, 2007; Hangen et al, 2015; Meyer et al, 2015; Salscheider et al, 2022; Rothemann et al, 2025). Here, we demonstrate at the molecular level, that OMA1-mediated processing of AIFM1 induces its release from the MIM and reduces its association with OXPHOS machinery subunits, ultimately impairing respiration and cell growth. Interestingly, stress-induced proteolysis of AIFM1 in mitochondria is also observed in the lungs of influenza virus-infected mice and in the hearts of mice with targeted deletion of Cox10 (Ahola et al, 2022), an assembly factor for cytochrome c oxidase. Together, these findings reveal a previously unrecognized physiologic role for OMA1 as a key integrator of mitochondrial stress and cellular energetics by altering the topology of one of its substrates.