SUPPLEMENTARY INFORMATION

METHODS

Plasmids and shRNAi constructions

Mouse OMA1 cDNA and its mutants were subcloned into pMSCV-puro vector with a C-terminal 3xFlag tag; in Retro-X Tet-On Advanced Inducible Expression System (Clontech), mouse OMA1 cDNA is subcloned into pRetroX-Tight-Pur vector with a C-terminal Flag tag; shRNAi against human OMA1, mouse Prohibitin 1 and 2 were performed, the oligonucleotides

5′-GATCCCCGAAGTGCTTTGTCATCTAATTCAAGAGATTAGATGACAAAGCACTTCTTTTTGGAAA-3′ and

5′-AGCTTTTCCAAAAAGAAGTGCTTTGTCATCTAATCTCTTGAATTAGATGACAAAGCACTTCGGG-3′ for human OMA1;

5′-GATCCCCGCGGCAACATTTGGGCTTATTCAAGAGATAAGCCCAAATGTTGCCGCTTTTTGGAAA-3′ and

5′-AGCTTTTCCAAAAAGCGGCAACATTTGGGCTTATCTCTTGAATAAGCCCAAATGTTGCCGCGGG-3′for mouse Prohibitin 1;

5′-GATCCCCGACCTGCAGATGGTGAACATTCAAGAGATGTTCACCATCTGCAGGTCTTTTTGGAAA-3′and5′-AGCTTTTCCAAAAAGACCTGCAGATGGTGAACATCTCTTGAATGTTCACCATCTGCAGGTCGGG-3′ for mouse Prohibitin 2.

Western Blot Analysis

Cell lysates were loaded onto the gel and separated by SDS-PAGE, and the resolved proteins were transferred to nitrocellulose membrane. After blocking with 5% nonfat milk in TBST (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% Tween 20) for overnight at 4 °C, the blot was incubated with primary antibody for 2 h at room temperature. The membrane was then washed three times with TBST and probed with HRP-conjugated secondary antibody for 1 h. The membrane was washed three times in TBST and developed by ECL according to the

manufacturer's protocol.

Co-immunoprecipitation

HeLa cells stably expressing OMA1-Flag or OMA1-E324Q were treated with or without CCCP, cells were lysed in a Triton X-100 based lysis buffer (1% Triton X-100, 10% glycerol, 150 mM NaCl, 20 mM Tris, pH7.4, 2 mM EDTA, protease inhibitor mixture) for 1h, and the nuclear and cellular debris was removed by centrifugation. Then the lysates were incubated with anti-FLAG M2 affinity gel (Sigma) for 4 h at 4 °C. The resin was washed 5 times with lysis buffer, and the protein extracts were analyzed on SDS-PAGE followed by Western blot analysis.

Confocal Microscopy and Image Processing

Confocal microscopy (FV1000) was perfomed with an Olympus IX81 microscope. MEFs were plated on cover slips and fixed with 10% formalin. The FV10-ASW 3.0 software was used for image processing and analysis. Mitochondria were identified by mito-GFP. To determine mitochondrial morphology, cells were randomly selected for quantitative analysis and visually scored into four classifications ('Highly connected', 'Tubular', 'Tubular and Fragmented' and 'Fragmented',).