**Materials and methods**

**Generation and cloning of MIC26 mutant**

The MIC26 mutation was generated in pcDNA3.1\_MIC26 38 plasmid using site-directed mutagenesis (stratagene protocol) using the following primer pairs: CGAGGATATATAGTCATATAAGATTTGTGGAAGGAGAAC (forward) and GTTCTCCTTCCACAAATCTTATATGACTATATATCCTCG (reverse). The MIC26WT and MIC26MUT transgene from pcDNA3.1\_MIC26 were subsequently re-cloned in pEGFP-N1 plasmid using Kpn1 (forward) and Age1 (reverse) restriction site for the MIC26WT and Kpn1 (forward) and EcoRV (reverse) for MIC26MUT respectively using following primers: GCGCGCGGTACCATGTTCAAGGTAATTCAGAGGTCC (forward for MIC26WT and MIC26MUT), GCGCGCACCGGTCCCGATCCCTTAGTTCCAGGTGAATTCTTCACATT (reverse, for MIC26WT) and GCGCGCACCGGTCCCGATCCTATGACTATATATCCTCGTAAACCCCA (reverse, for MIC26MUT). pMSCVpuro-MIC26WT plasmid, which was used for making stable cell lines using retroviral transfection, was previously generated 40. The mutant pMSCVpuro-MIC26MUT was generated using Q5®site-directed mutagenesis kit (NEB) according to manufacturer’s protocol.

**Cell culture**

HAP1 WT, *MIC26* KO were obtained and custom-made by Horizon (UK) using Crispr-Cas method as described earlier in 40. The cells were cultured in IMDM media that contained 20% fetal bovine serum and 1% penicillin and streptomycin. The stable cells lines overexpressing pMSCVpuro-MIC26WT and pMSCVpuro-MIC26MUT in HAP1 *MIC26* KO were generated using retroviral transduction method as described earlier in 40. The incorporation of the MIC26WT and MIC26MUT transgene in the stable cell lines was confirmed by PCR (data not shown). The transient transfection was performed using Novagen® Genejuice® transfection reagent according to manufacturer’s protocol.

**Western blotting**

The cells were scraped in PBS and lysed using RIPA lysis buffer for protein extraction. The protein amount in each lysate was determined by lowry method and equal amounts of protein were loaded onto a 15% SDS electrophoresis gel. Proteins were blotted onto nitrocellulose membrane and probed with following antibodies: MIC13 (custom-made by Pineda; against human MIC13 peptide CKAREYSKEGWEYVKARTK), MIC27 (HPA000612; Atlas Antibodies), MIC26 (MA5-15493; Thermo Fisher Scientific), MIC60 (custom-made, Pineda; against human MIC60 using the peptide CTDHPEIGEGKPTPALSEEAS), MIC10 (ab84969; Abcam), MIC25 (20639-1-AP; Proteintech), β-tubulin (Cell Signaling Technology), and MIC19 (25625-1-AP; Proteintech). The chemiluminescent images were acquired using VIBER LOURMAT Fusion SL (Peqlab).

**Microscopy for mitochondrial morphology**

Cells were transfected with MitoGFP (matrix-targeted GFP) using Novagen® Genejuice® transfection reagent according to manufacturer’s protocol. Cells were subsequently imaged using PerkinElmer spinning disk confocal microscope equipped with Hamamatsu C9100 camera and 60x oil objective (NA = 1.49). For quantification, each cell was categorized as tubular, intermediate or fragmented, based on the majority of mitochondria present in a cell belonging to one of the categories.

**Coimmunoprecipitation**

HAP1 *MIC26* KO cells were transiently transfected with either EGFP or MIC26WTGFP or MIC26MUTGFP containing plasmids using Novagen® Genejuice® transfection reagent according to manufacturer’s protocol. Mitochondria were isolated from each condition after 72 hours of transfection using the protocol described in 40. 250 µg of mitochondrial pellet was resuspended in an isotonic buffer (150 mM NaCl, 10 mM Tris/HCl (pH 7.5), 5 mM EDTA, protease inhibitor cocktail). For homogenization, 5µl of 10% Digitonin was added to obtain a detergent to protein ratio of 2 g/g and incubated for 10 mins on ice. Centrifugation at 21,000 x g was used to clear the reaction and supernatant was incubated with anti-GFP nanobody, ChromoTek GFP-Trap® (proteintech). Elution was performed using Laemmli buffer and eluted proteins were subjected to SDS electrophoresis and western blotting.

**Electron microscopy**

EM was performed as described earlier 40. Briefly, cells were fixed using 3% glutaraldehyde buffered with 0.1 M sodium cacodylate buffer, pH 7.2 and subsequently stained using 1% osmium tetroxide for 50 mins at room temperature (RT). After washing two times with 0.1 M sodium cacodylate buffer and one time with 70% ethanol, cells were stained with 1% uranyl acetate (Merck) / 1% phosphotungstic acid (Merck) in 70% ethanol for 1 hour. Samples were dehydrated and embedded in Spurr resin for polymerization at 70°C for atleast 48 hours. The ultrathin sections were prepared using the microtome. Images were acquired using H600, Hitachi equipped with Bioscan model 792 camera (Gatan).

**Blue native gel electrophoresis**

100 µg of mitochondria were solubilized for 15 min on ice using 2 g/g of digitonin/protein ratio. Solubilized complexes were supplemented with loading buffer (50% glycerol, 8 g/g coomassie/detergent ratio) and immediately loaded on a gradient gel (3–13%). Blue native gel electrophoresis was proceeded according to the method described earlier (Anand et al, 2016). For identification the membrane was decorated with OXPHOS antibody cocktail (Abcam ab110412, 1:1000) followed by Goat IgG anti-Mouse IgG (Abcam ab97023, 1:10000). The chemiluminescent signals were obtained using Pierce™ SuperSignal™ West Pico PLUS Chemiluminescent Substrate reagent (Thermo Scientific) and VILBER LOURMAT Fusion SL equipment (Peqlab).