Supplemental material

JCB

Ping et al., http://www.jcb.org/cgi/content/full/jcb.201511021/DC1

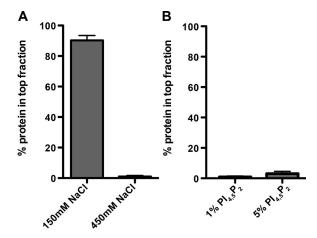


Figure S1. **Num1CC-phospholipid interactions.** (A and B) Num1CC was incubated with SoyCL liposomes in the presence of 150 or 450 mM NaCl (A) or liposomes composed of PC and the indicated mol% of $Pl_{4,5}P_2$ (B), and the reactions were subjected to liposome floatation and analysis as described in Fig. 3 A. Data are shown as the mean \pm SEM; n = 3 independent experiments.

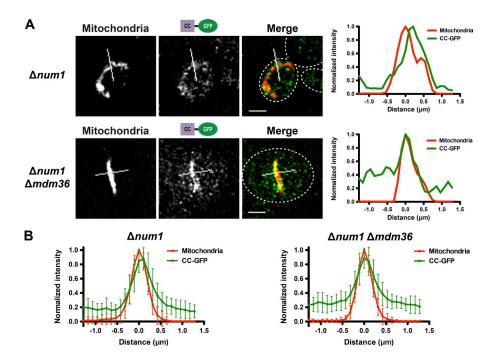


Figure S2. Num1CC associates with mitochondria in cells. (A) Δ num1 (top) and Δ num1 Δ mdm36 (bottom) cells expressing CC-GFP (as seen in the schematic) and mito-RED were analyzed by fluorescence microscopy. Single focal planes are shown. The cell cortex is outlined with a dashed white line. Bars, 2 μ m. The graphs show normalized fluorescence intensities of mito-RED (red) and CC-GFP (green) along a line drawn perpendicular to a mitochondrial tubule as indicated in the corresponding image (white line). The position of peak mito-RED intensity was set at 0 μ m. (B) Graphs of normalized fluorescence intensity profiles for lines analyzed as described in A. The mean intensities \pm SD are plotted; $n \ge 20$.

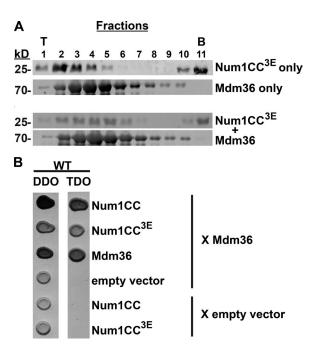


Figure S3. Num1^{3E} interacts with Mdm36. (A) Purified Num1CC^{3E} (5 μM) and Mdm36 (5 μM) alone and in combination were subjected to sucrose density gradient analysis. Gradients were manually fractionated from top (T) to bottom (B) and analyzed by SDS-PAGE and Western blot using an anti-T7 antibody. (B) Yeast two-hybrid analysis was used to assess the interaction of a Gal4BD-Mdm36 fusion with Gal4AD-Num1CC and Gal4AD-Num1CC^{3E} fusions. Protein–protein interactions were assessed by growth on triple-dropout (TDO) medium. TDO, SC-LEU-TRP-HIS; DDO, SC-LEU-TRP; WT, wild type.