Unbiased Screenings Illuminate Regulators of Mitochondria-Endoplasmic Reticulum Contact Sites

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Mitochondria and endoplasmic reticulum (ER) are physically linked and appropriately spaced at specific points known as Mitochondria-Endoplasmic Reticulum (ER) contacts (MERCs) through partially understood protein bridges. To establish a comprehensive molecular atlas of MERCs, we conducted a genome-wide screening using short hairpin RNA (shRNA) and combined it with high-content, ratiometric, quantitative microscopy of a FRET ER-mitochondria proximity probe (FEMP) and iBAQ proteomic analysis of MERCs. Through automated image analysis, statistical evaluations, and iterative screening, we identified 107 gene candidates classified as tethers (genes whose removal increases the distance between ER and mitochondria), which included well-known mammalian tethers like Mfn2. Additionally, we identified 97 Spacer genes (genes whose removal decreases the distance between ER and mitochondria). These gene candidates were found to be enriched in calcium signaling, lipid biosynthesis, and metabolism processes known to localize at this interface. By cross-referencing the gene list with the proteome of MERCs determined by iBAQ analysis, we refined our findings to 25 Spacers and 18 Tethers. Orthogonal assays validating mitochondria-ER juxtaposition further highlighted the effectiveness of our screening approach in identifying and functionally characterizing MERCs components.