

## ABSTRACT

The small open reading frame (sORF)-encoded peptides (SEPs) are the uncharted portion of the proteome remain to be discovered. Mitochondria, the powerhouse of the cell, are enriched with SEPs indicating their potential as the source of finding novel SEPs. A multi-pronged prediction combining mitochondrial gene expression signature, motifs prediction, and proteomics has yielded the identification of 16 novel mito-SEPs. Here, we adapted the split-GFP system to assess the sub-mitochondrial localization of mitochondrial proteins. Our effort has explored the potential of imaging-based identification of mito-SEPs intra-mitochondrial localization and topology. Native protein association study revealed the interaction of 6 newly identified mito-SEPs with large mitochondrial complexes, suggesting their essential function in mitochondria. Our findings validated the existence of mito-SEPs and implied their significance in mitochondrial respiration.