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Working

Proteomic mapping of ER-PM junctions in living HEK293 cells

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ABSTRACT

Specialized junctional sites that connect the plasma membrane(PM) and endoplasmic reticulum(ER) are intimately involved in controlling lipid metabolism and calcium signaling in mammalian cells. Store operated calcium entry mediated by dynamic STIM1-ORAI1 coupling constitutes one of the most well-established molecular events occurring at ER-PM junctions, but the protein composition at this particular subcellular compartment remain poorly defined. Using an in situ spatially-restricted biotin-labeling coupled with mass spectrometry, we mapped the proteome of intact ER-PM junctions in living cells without disrupting their architectural integrity. Our approaches lead to the discovery of >70 candidate proteins at ER-PM junctions, with the majority falling into the categories of ER/PM-resident proteins, cytoskeletal components, and proteins functioning in intracellular membrane trafficking or post-translational modifications. Although the current protocol is limited to ER-PM junctions, the methods described herein can be readily extended to study other types of inter-membrane appositions in various types of cells.

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