PARL is an intramembrane-protease residing in the inner membrane of mammalian mitochondria. It belongs to the protein superfamily of rhomboid proteases. Deletion of the *Parl* gene in mice causes multisystemic atrophy of Thymus, Spleen and skeletal muscle resulting in premature death 6 to 8 weeks after birth. Increased rates of apoptosis were shown to be causative for the observed atrophies. *In vitro* analyses of mouse embryonic fibroblasts (MEFs) also revealed an increased sensitivity towards apoptotic stimuli in these cells. Although a processing and release defect of the mitochondrial fusion protein OPA1 was postulated to cause the observed phenotypes, the role of PARL remained elusive. Therefore, in this study new interactors and substrates of the rhomboid protease PARL were sought to be identified in order to gain new insights into the mechanistic role of PARL in mitochondria. Biochemical analyses including affinity purification, immunoprecipitations and size-exclusion chromatography led to the identification of a multi-protein complex in the inner mitochondrial membrane consisting of PARL, the ATP-dependent *i*-AAA protease and the SPFH protein SLP2. With respect to its composition the complex was termed SPY-complex (SLP2-PARL-YME1L-complex). Further characterization of this complex led to the hypothesis that the SPY-complex likely generates a specific microdomain in the inner mitochondrial membrane to regulate the proteolytic activities of PARL as well as YME1L. This study describes that the activity of YME1L depends on the enrichment of the mitochondria specific phospholipid cardiolipin within the microdomain of the SPY-complex. Proteolytic processing of long OPA1 isoforms by YME1L is critical for the maintenance of the dynamic mitochondrial network. This means the SPY-complex is associated with mitochondrial dynamics. Moreover, experiments revealed that the increased apoptotic sensitivity in *Parl* depleted MEFs can be complemented by RNAi mediated depletion of YME1L or SLP2. Taken together, this study describes the identification of the SPY-complex as well as its initial characterization as key regulator of mitochondrial functions in MEFs.