Supporting Information

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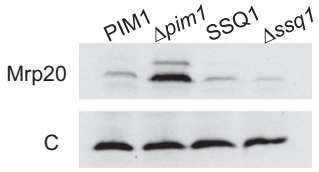

Fig. S1. Levels of Mrp20, a substrate of Pim1. Because Mrp20 is unstable only in the absence of functional mtDNA, indicated strains were rendered ρ-negative by culturing in rich media containing 2 μg/mL of ethidium bromide for 4 d. Respiratory incompetent cells were then cultured in fresh media. Mitochondrial lysates were prepared and subjected to immunoblot analysis with Mrp20-specific antibodies or, as a loading control, Mge1-specific antibodies (c).

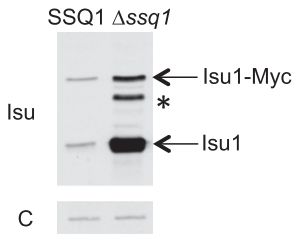

Fig. S2. Isu1-Myc migrates more slowly than Isu in SDS/PAGE. Cell lysates of *Δisu1Δisu2 ISU1-Myc* with either *a deletion of SSQ1* (*Δssq1*) or a WT copy of the gene (*SSQ1*) and harboring *ISU1* on a plasmid were prepared. Proteins were separated by SDS/PAGE and subjected to immunoblot analysis with Isu-specific antibodies or, as a loading control, Mge1-specific antibodies (c). The asterisk indicates a likely partial degradation product of Isu1-Myc.

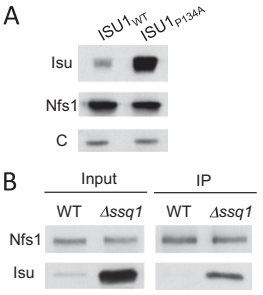

Fig. S3. Nfs1 levels. (*A*) Cell lysates of *Δisu1 Δisu2* cells expressing WT Isu1 or $Isu1_{P134A}$ were subjected to immunoblot analysis using Isu, Nfs1, and, as a control, Mge1-specific antibodies. (*B*) Lysates of mitochondria prepared from WT or *Δssq1* cells were subjected to immunoprecipitation (IP) using Nfs1-specific antibodies. Precipitated Nfs1 and Isu1 (IP) were detected by immunoblot analysis using specific antibodies, with 4% of mitochondrial lysate used as a loading control (Input). More complex was observed in *Δssq1* lysates, consistent with the presence of more Nfs1 than Isu.