



Figure S5. Deletion of Msp1p or Dsk2p does not allow recovery of mitochondrial localization by poorly targeted mCherry-Fis1(TA) variants. (A) Deletion of the Msp1p extractase does not allow tail-anchored proteins mistargeted due to proline substitutions to return to mitochondria. Cells from *msp1Δ/msp1Δ* strain CDD1044 expressing pHS1 (signal not shown) and plasmids b109 (WT), b208 (V134P), b135 (L139P), b210 (A140P), or b211 (A144P) were examined to determine mCherry-Fis1(TA) location. (B) Deletion of ubiquilin ortholog Dsk2p does not allow mislocalized, proline-containing Fis1p TAs to target to mitochondria. Cells from *dsk2Δ/dsk2Δ* strain CDD1179 were transformed and analyzed as in (A). (C) Deletion of Msp1p does not allow mitochondrial localization of mistargeted Fis1p TAs carrying charge replacements. Strain CDD1044, deleted of Msp1p, was transformed with the following plasmids encoding mCherry fused to the mutant TAs: b192 (V132D), b196 (A140D), b197 (A140E), b198 (A140K), b199 (A140R), b200 (A144D), b201 (A144E), b134 (V145E), b204 (F148D), b205 (F148E). Transformants were examined as in (A). (D) Removal of Dsk2p fails to allow mutant TAs containing charge substitutions to re-localized to mitochondria. Strain CDD1179 was transformed with plasmids and analyzed as in (C). Scale bar, 5 μ m.