

**Figure S10.** Positive and negative charge replacements within the Fis1p TA can generally support Fis1p function. (A) Normal mitochondrial morphology can be maintained even when charges are placed within the hydrophobic MAD of the Fis1p TA. fis1  $\Delta$  strain CDD741, in which mitochondria were visualized by expression of mitochondria-targeted GFP from plasmid pHS12, was transformed with vector pRS313, a plasmid expressing WT Fis1p (b239), or plasmids expressing Fis1p containing the indicated TA alterations (plasmids b240-251). Cells were treated with sodium azide to provoke Fis1p-dependent mitochondrial fragmentation, and representative images are shown. Scale bar, 5 μm. (B) Cells treated as in (A) were scored for the maintenance of a mitochondrial network (n=200 cells). (C) A genetic assay of Fis1p function demonstrates that charged residues within the TA allow Fis1p activity. fzo1 $\Delta$  fis1 $\Delta$  strain CDD688, carrying a CHX-counterselectable, FZO1-expressing plasmid, was transformed with the FIS1-expressing plasmids enumerated above. After allowing cells to lose the FZO1-encoding plasmid, serial dilutions were spotted to SLac-His medium containing 3 μg/mL CHX to counterselect against FZO1 expression ("lactate / no fusion") and incubated for 5 d to test for maintenance of mtDNA. Cell proliferation indicates a lack of Fis1p activity. To control for cell number spotted, cells from the same culture were placed on SMM-Trp-His medium ("glucose / fusion") and incubated for 2 d.