Supplementary Figure 6. Mitochondrial TA proteins are poor substrates of yeast Msp1.

(A) The wild-type strain harboring the N-terminally GFP tagged mitochondrial TA proteins (Tom5, Tom6, Tom7, Tom22, Fis1, Gem1 and Fmp32) expressed from the GAL1 promoter and empty vector (-), Msp1-His6-HA3 or Msp1E193Q-His6-HA3 fusion were cultured in galactose medium for 6 hours and subjected to co-immunoprecipitation. Both 1% whole-cell lysates and anti-HA antibody-precipitated final eluates were analyzed and immunoblotted with anti-HA, GFP and Por1 antibodies.

(B) Both the wild-type (WT) and msp1Δ mutant strains expressing the indicated GFP-tagged mitochondrial TA proteins from the GAL1 promoter were cultured in galactose medium for 6 hours to induce protein expression and switched back to glucose medium. Whole-cell lysates were harvested every hour post-induction and analyzed by immunoblot. Pgk1 is a cytoplasmic protein used a loading control.

(C) The indicated strains were five-fold diluted, spotted on glucose or galactose plates and incubated at 30°C.