

Figure S1

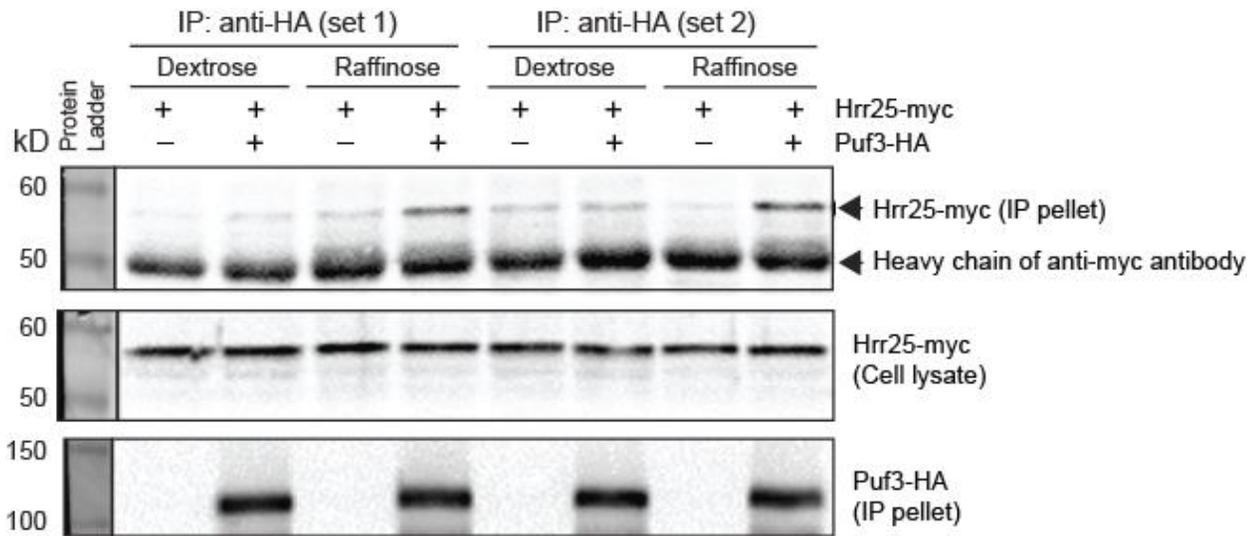


Figure S1. Hrr25 interacts with Puf3 in a co-immunoprecipitation assay. Yeast strains carrying plasmids encoding *PUF3-HA* (pZL1939) and *HRR25-myc* (pZL3338) as indicated were grown in YNBcas5D (Dextrose) and YNBcasR (Raffinose) medium to mid-logarithmic phase. Cell lysates were prepared, and Puf3-HA was immunoprecipitated with anti-HA antibody (clone 12CA5, Roche Life Science Products) and protein A agarose beads (Roche Life Science Products). Proteins in the immunoprecipitates (IP pellet) and from the total cell lysates (Cell lysate) were separated by SDS-PAGE and analyzed by immunoblotting.

Figure S2

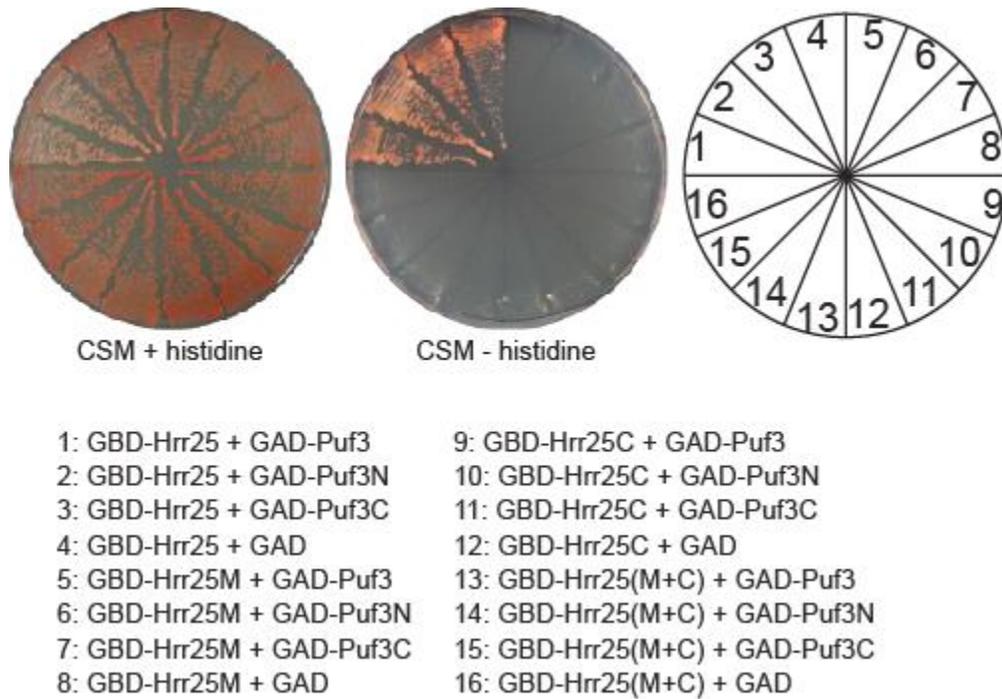


Figure S2. The middle and C-terminal regions of Hrr25 do not interact with full-length Puf3 or the N-terminal domain of Puf3 (Puf3N) in a yeast two-hybrid assay. A yeast two-hybrid analysis of the interaction between full-length Hrr25, the middle region of Hrr25 (Hrr25M), the C-terminal region of Hrr25 (Hrr25C), or the middle region plus the C-terminal region of Hrr25 (Hrr25(M+C)) and full-length Puf3, the N-terminal domain of Puf3 (Puf3N), or the C-terminal region of Puf3 (Puf3C) was conducted as described in Materials and Methods. AH109 cells carrying plasmids encoding GBD-Hrr25 (pZL3557), GBD-Hrr25M (pMB218), GBD-Hrr25C (pMB220), or GBD-Hrr25(M+C) (pZL3805) and Y187 cells carrying plasmids encoding the Gal4 transcriptional activation domain, GAD (pZL3539), GAD-Puf3 (pMB212), GAD-Puf3N (pMB214), or GAD-Puf3C (pMB216) were crossed and the resulting diploid cells were selected and streaked onto CSM dropout medium with histidine (+ histidine) or without histidine (- histidine).

Figure S3

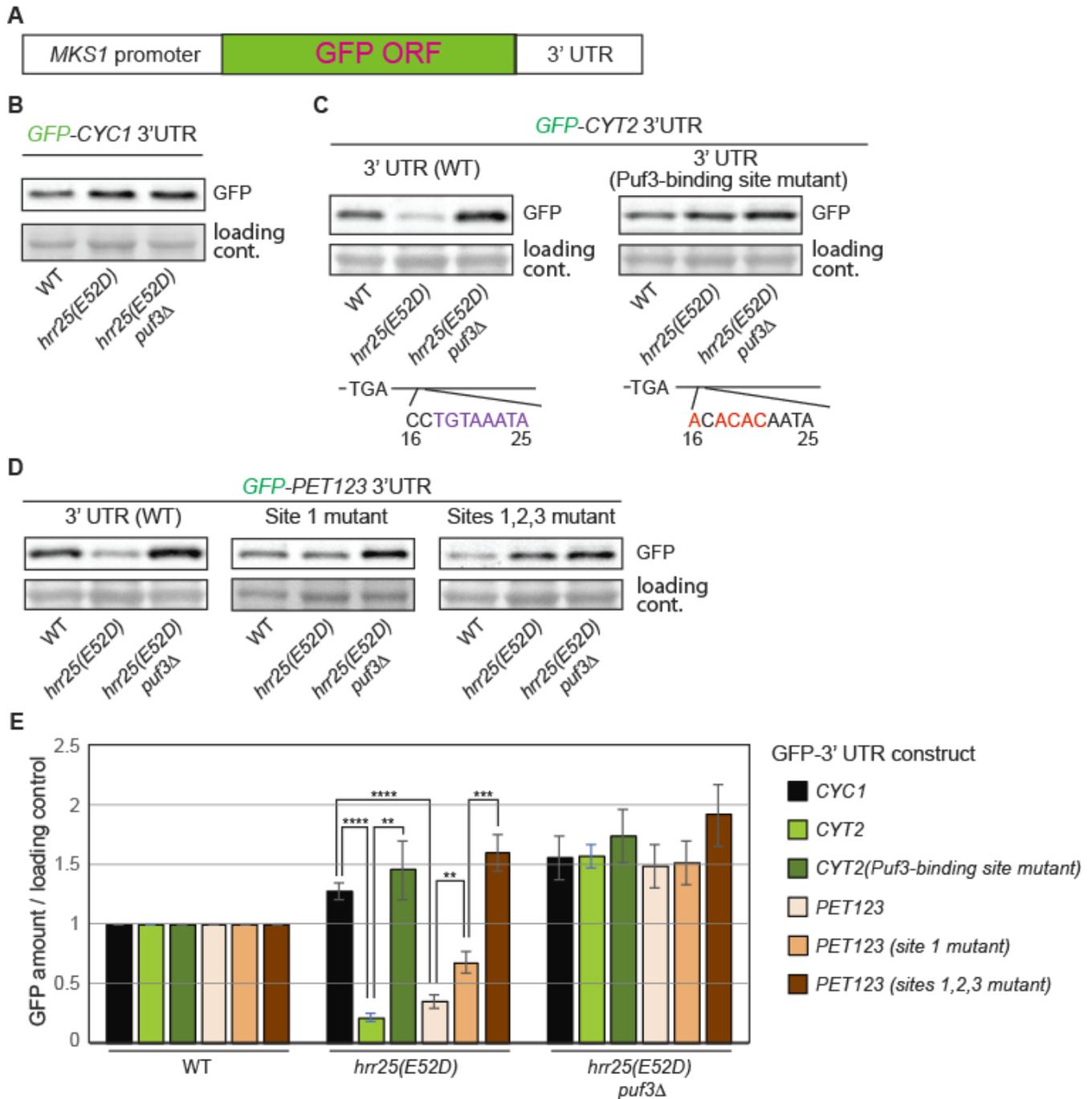


Figure S3. An *hrr25* mutation reduces the expression of GFP reporter genes carrying 3' UTRs targeted by Puf3. (A) A diagrammatic representation of GFP reporter constructs used in this study. (B) A

Western blot analysis of GFP expression from the *MKS1p-GFP-CYC1* 3' UTR reporter construct (pZL4248) in wild type (WT, BY4741), *hrr25(E52D)* (ZLY4467) and *hrr25(E52D) puf3Δ* (ZLY4578) mutant cells. The *CYC1* 3' UTR is not a target of Puf3. The loading control (loading cont.) was a ~33 kD band from Ponceau S stained membrane. The result was representative of four independent experiments.

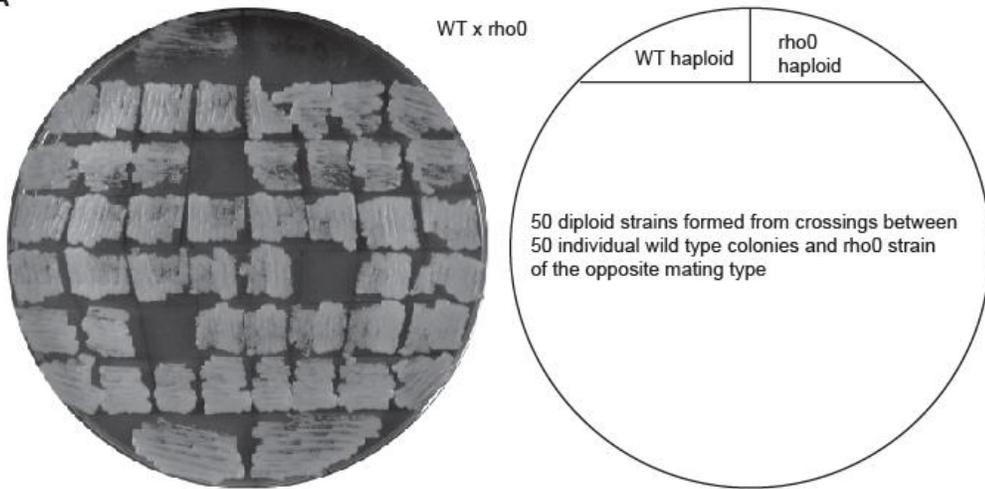
(C) A Western blot analysis of GFP expression from *MKS1p-GFP* constructs carrying the wild type 3' UTR of *CYT2* (pZL4250) or a Puf3-binding site mutant allele (pZL4252). The result was representative of four independent experiments. The DNA sequence downstream of the stop codon of GFP from wild type and Puf3-binding site mutant constructs was given beneath the gel picture. The numbers indicate the positions of nucleotides downstream of the stop codon.

(D) A Western blot analysis of GFP expression from *MKS1p-GFP* constructs carrying the wild type 3' UTR of *PET123* (pZL4253), a single site 1 mutant allele (pZL4255), or a triple Puf3-binding site mutant allele (pZL4256). The result was representative of four independent experiments.

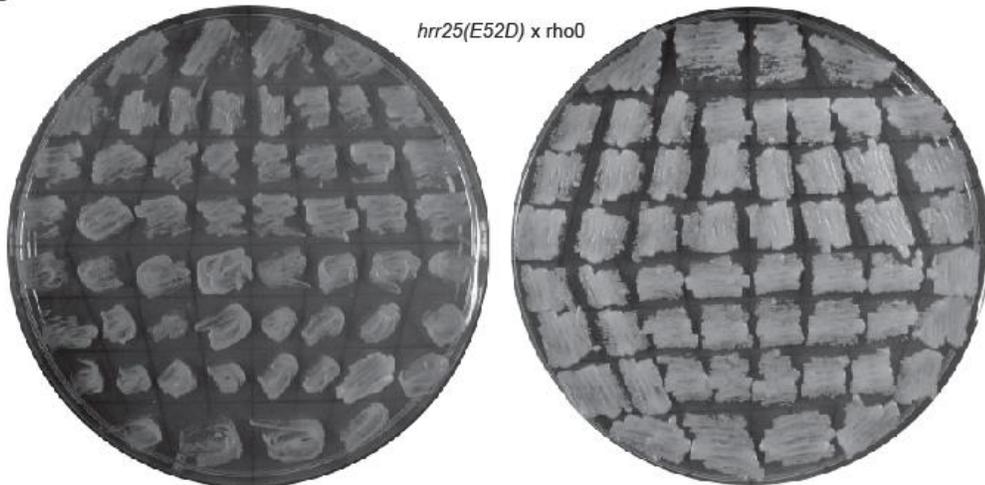
(E) Quantification of the ratio of the GFP signals to the loading control on Western blots. The ratio of GFP/loading control was set as 1 in wild-type cells. The data were presented as mean \pm standard deviation, n=4. The means of the results were compared by *t*-test, and asterisks indicate significant difference in the means of two groups of data (*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$).

Figure S4

A



B



C

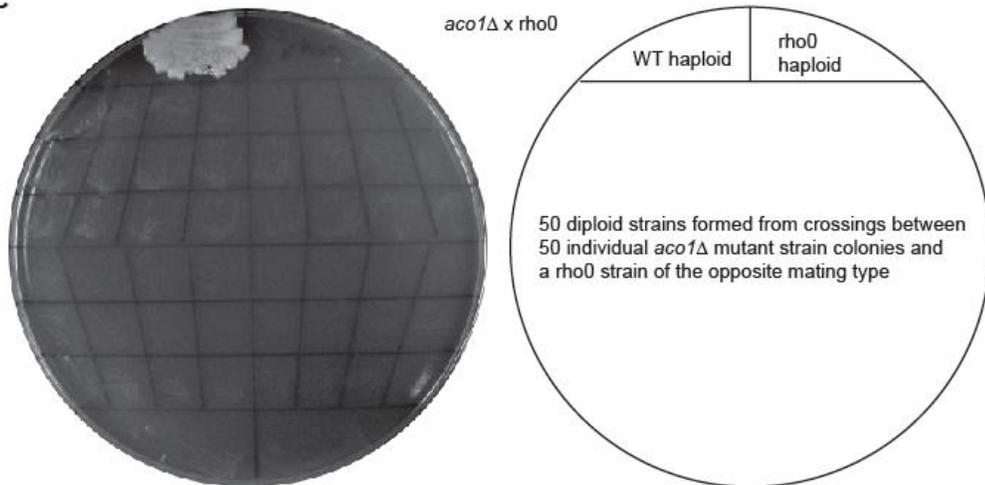


Figure S4. An *hrr25(E52D)* mutation does not lead to an increased formation of mitochondrial petite mutants. Diploid strains formed between a rho0 strain and individual colonies of wild type (WT, BY4741) (panel A), an *hrr25(E52D)* mutant (ZLY4467) (panel B), and an *aco1Δ* mutant (ZLY2630) (panel C) were streaked on YPEG plate with ethanol and glycerol as the carbon source.