Supplementary materials to

Mitochondrial Genomic Dysfunction Causes Dephosphorylation of Sch9 in the Yeast *Saccharomyces cerevisiae*

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Supplementary Table S1.

Supplementary Fig. S1.

Supplementary Table S1. Yeast strains used in this study.

Strains	Genotypes	Sources
TB50α	MATα *trp1 his3 ura3 leu2 rme1* [ρ^+]	Lab. stock
RL170-2b	TB50α *TCO89-TAP[KlTrp1]* [ρ^+]	Lab. stock
YGSK226	TB50α *TCO89-TAP[KlTrp1]* pJU676 pJU450 [ρ^+]	This study
YGSK232	TB50α *TCO89-TAP[KlTrp1]* [ρ^0]	This study
YGSK242	TB50α *TCO89-TAP[KlTrp1]* pJU676 pJU450 [ρ^0]	This study
YGSK269	TB50α *TCO89-TAP[KlTrp1]* pJU675 pJU450 [ρ^+]	This study
YGSK270	TB50α *TCO89-TAP[KlTrp1]* pJU841 pJU450 [ρ^+]	This study
YGSK271	TB50α *TCO89-TAP[KlTrp1]* pJU675 pJU450 [ρ^0]	This study
YGSK272	TB50α *TCO89-TAP[KlTrp1]* pJU841 pJU450 [ρ^0]	This study
TB50a	MATa *trp1 his3 ura3 leu2 rme1* [ρ^+]	Lab. stock
RL276-5D	TB50a *HIS3 LEU2* [ρ^+]	Lab. stock
YGSK239 [a]	TB50a *HIS3 LEU2* [ρ^0]	This study
YGSK197 [a]	TB50a *TCO89-TAP[KlTrp1] kog1Δ::kanMX6 snf1Δ::hph* YCplac111::*KOG1* [ρ^+]	This study
BY4742	MATα *leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1* [ρ^+]	Euroscarf
YGSK229	BY4742 pJU676 pJU450 [ρ^+]	This study
YGSK233 [a]	BY4742 [ρ^0]	This study
YGSK243	BY4742 pJU676 pJU450 [ρ^0]	This study
BY4741	MATa *leu2Δ0 met15Δ0 ura3Δ0 his3Δ1* [ρ^+]	Euroscarf
YGSK238 [a]	BY4741 [ρ^0]	This study

YGSK221	BY4741 *atp2Δ::kanMX4* [ρ^+]	Euroscarf
YGSK247	BY4741 *atp2Δ::kanMX4* pJU676 pJU450 [ρ^+]	This study
YGSK219	BY4741 *cox6Δ::kanMX4* [ρ^+]	Euroscarf
YGSK248 [a]	BY4741 *cox6Δ::kanMX4* pJU676 pJU450 [ρ^+]	This study
YGSK222	BY4741 *mrpl16Δ::kanMX4* [ρ^+]	Euroscarf
YGSK249	BY4741 *mrpl16Δ::kanMX4* pJU676 pJU450 [ρ^+]	This study
MK3306	BY4742 pRS315 pJU676 pRS413 [ρ^+]	This study
MK3307	BY4742 pRS315 pJU676 pRS413 [ρ^0]	This study
MK3309 [b]	BY4742 pPL132 (pRS315::*TOR1*) pRS413 pJU450 [ρ^0]	This study
MK3311 [b]	BY4742 pPL156 (pRS315::*TOR1* I1954V) pRS413 pJU450 [ρ^0]	This study
MK3313 [b]	BY4742 pPL158 (pRS315::*TOR1* I1954V W2176R) pRS413 pJU450 [ρ^0]	This study
MK3354 [c]	BY4742 *gtr1Δ::kanMX4* YCplac33::pTetON-GTR1-Q65L pJU679 pRS413 [ρ^+]	This study
MK3355	BY4742 *gtr1Δ::kanMX4* YCplac33 pJU679 pRS413 [ρ^+]	This study
MK3356 [c]	BY4742 *gtr1Δ::kanMX4* YCplac33::pTetON-GTR1-Q65L pJU679 pRS413 [ρ^0]	This study
MK3357	BY4742 *gtr1Δ::kanMX4* YCplac33 pJU679 pRS413 [ρ^0]	This study

[a] These strains were used for checking ρ^+ or ρ^0 phenotype.

[b] Plasmids (pPL132, 156, and 158) were kindly gifted from Dr. Ted Powers (3).

[c] Plasmid (YCplac33::pTetON-GTR1-Q65L) is described in ref (2).

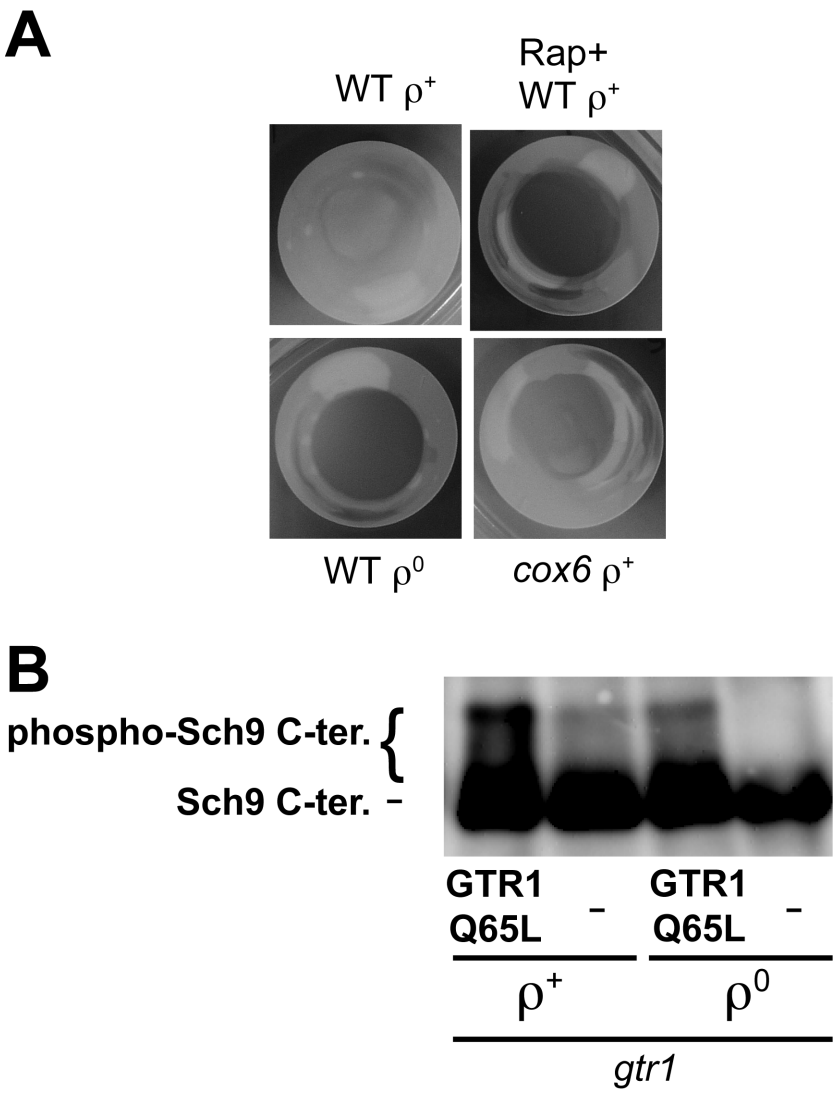
A
WT ρ+
Rap+
WT ρ+
WT ρ0
cox6 ρ+
B
phospho-Sch9 C-ter.
Sch9 C-ter.
GTR1
Q65L
-
GTR1
Q65L
-
ρ+
ρ0
gtr1

Fig. S1

Figure legend of Supplementary Fig. S1

FIG. S1 (A) Accumulation of glycogen. YGSK229 (WT ρ^+), YGSK243 (WT ρ^0), and YGSK248 (*cox6* ρ^+) (*cox6* ρ^+) cells were diluted to an A_{600} of 0.4 in fresh SD+Asn medium (containing: 2 % glucose, 10 mM Asn, 0.17 % yeast nitrogen base without ammonium sulfate [pH 5.6] plus 50 μg/ml Lys for YGSK229 and YGSK243, 20 μg/ml Met for YGSK248) and cultivated for approximately 4.5 h. YGSK229 cells were also cultivated in another flask as above for 3 h, and further cultivated for 1.5 h in the presence of 200 ng/ml rapamycin. 7.5 A_{600} equivalents of cells were filtered and treated with iodine vapour to stain glycogen as described (1). Dark staining indicates that glycogen was accumulated in; YGSK243 (WT ρ^0) and rapamycin-treated YGSK229 (Rap+ WT ρ^+) cells, but not in YGSK229 (WT ρ^+) and YGSK248 (*cox6* ρ^+) cells. (B) Sch9 is dephosphorylated independently of GTR1 in ρ^0 cells. MK3354 (*gtr1* YCplac33::pTetON-GTR1-Q65L ρ^+), MK3355 (*gtr1* YCplac33 ρ^+), MK3356 (*gtr1* YCplac33::pTetON-GTR1-Q65L ρ^0), and MK3357 (*gtr1* YCplac33 ρ^0) cells were cultured in SD+Asn medium containing 50 μg/ml Lys and 5 μg/ml doxycycline, diluted to an OD_{600} of 0.4 in the same medium, and further cultivated for 5.5 h. Sch9-phopshorylations were monitored as described in the text. Prior to SDS-PAGE / anti-HA blot, protein extracts were treated with NTCB to cleave proteins.

REFERENCES

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