Expanded View Figures

Figure EV1. Derepression of sexual differentiation in *hmt* mutants.

A Sporulation of *hmt* mutants. Cells of the wild-type (WT, JY450), *tor2-ts6* (JV303), *nrs1/hmt1* (JS159), *prs1/hmt2* (JS160), *tad3/hmt3* (JS161), *rpc34/hmt4* (JS162), *sfc4/hmt5* (JS163), *cts1/hmt6* (JS164), and *pcm1/hmt7* (JS165) strains were grown on nutrient-rich YE medium at 30°C for 3 days and then exposed to iodine vapor, which stains sporulated cells dark brown.

B TORC1 activity in *hmt* mutants. Cells of wild-type, *tor2-ts6*, *nrs1/hmt1*, *prs1/hmt2*, *tad3/hmt3*, *rpc34/hmt4*, *sfc4/hmt5*, *cts1/hmt6*, and *pcm1/hmt7* strains were grown in liquid YE medium at 25°C and subsequently shifted to 30°C for 4 h. Cell extracts were subjected to Western blot analysis using anti-Atg13 antibody and anti-phospho-S6 kinase antibody. γ-tubulin is shown as a loading control.

C TORC1 activity in *ths1* (JS167) and *lrs1* (JS168) mutants under the same conditions as in (B).

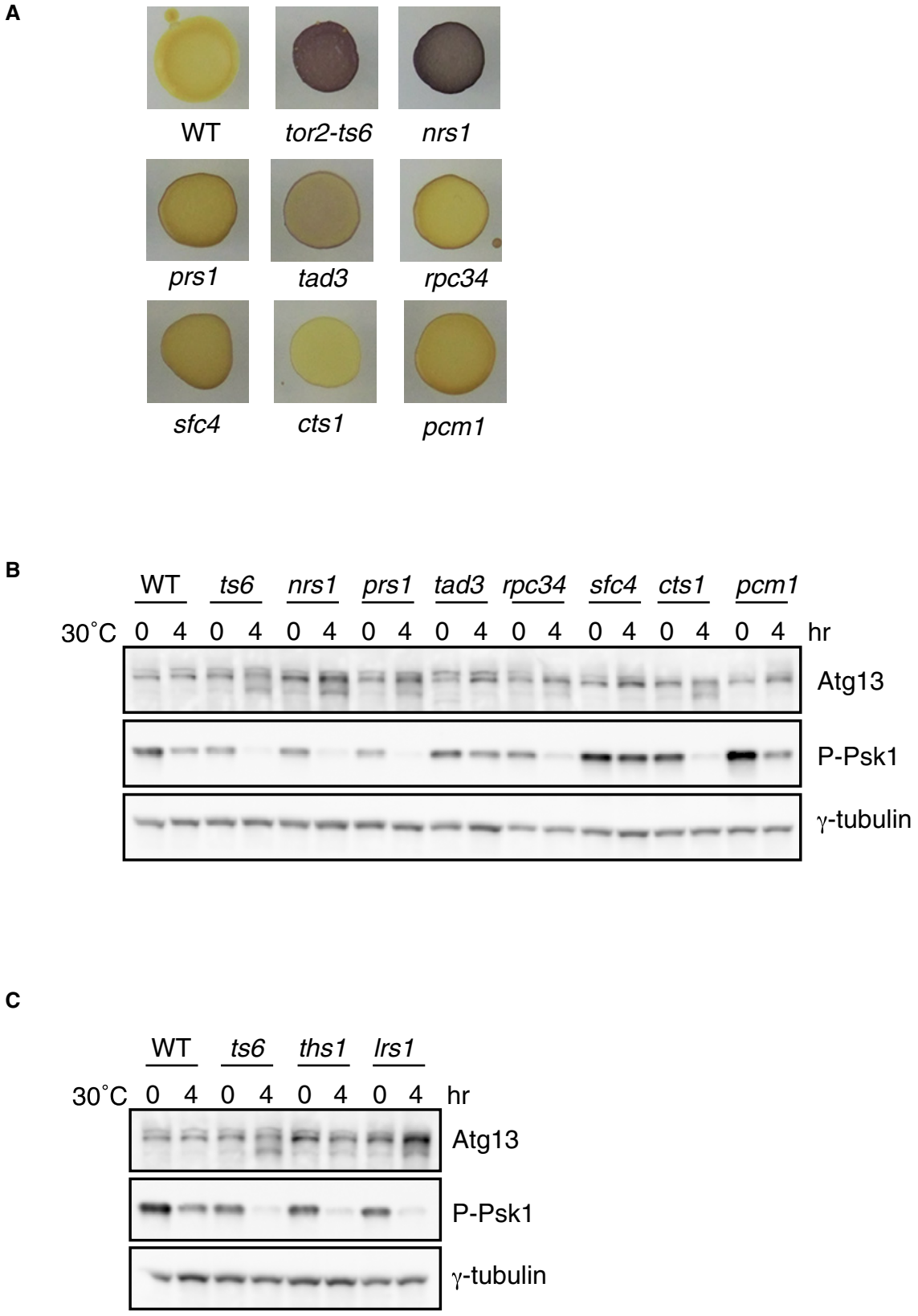

Figure EV1.

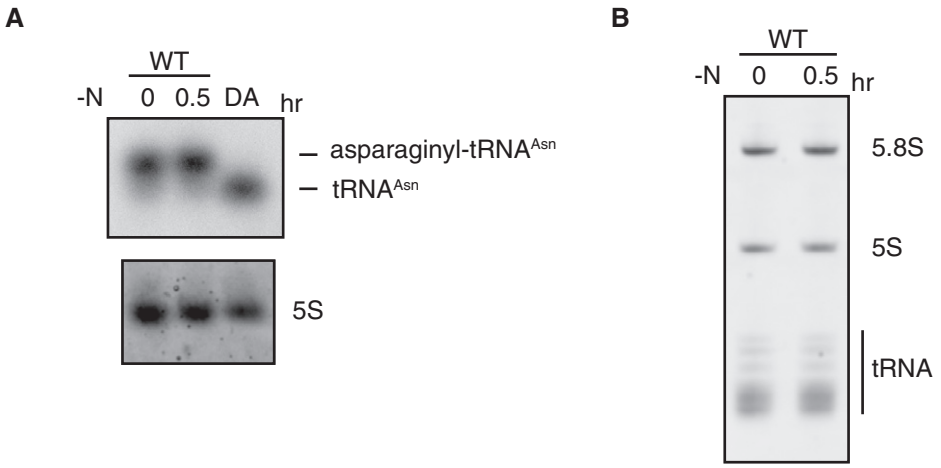

Figure EV2. The extent of aminoacylation and amount of total tRNA do not change under nitrogen starvation.

A Aminoacylation of tRNA-Asn under nitrogen starvation. Wild-type (JY3) cells were cultured in minimal medium, MM, and shifted to nitrogen-deprived MM for 30 min. RNA extracted in acidic conditions was analyzed by northern blot analysis with probes for tRNA-Asn. The rightmost lane is deacylated wild-type RNA (DA). 5S rRNA is shown as a loading control.

B Amount of total tRNA under nitrogen starvation. Total RNA was separated, followed by ethidium bromide staining.

Figure EV3. Overexpression of pre-tRNA-Asn and pre-tRNA-Pro.

A Schematic diagram of pre-tRNAMetAsn-RZ. A thiamine-repressible promoter and a terminator of the *nmt1* gene are represented by P*nmt1* and T*nmt1*, respectively.

B Overexpression of pre-tRNA-Asn. Wild-type cells (JY450) carrying pREP1, pREP1-*sla1*, or pREP1-pre-tRNAMetAsn-RZ were grown in minimal medium (MM) and subsequently shifted to nitrogen-deprived MM for 30 min. Total RNA was analyzed using northern blot analysis with a probe for pre-tRNA-Asn. 5.8S rRNA is shown as a loading control.

C TORC1 activity in cells overexpressing pre-tRNA-Asn. TORC1 activity was analyzed by Western blot analysis using the anti-Atg13 antibody and anti-phospho-S6 kinase antibody under the same conditions as in (B). γ-tubulin is shown as a loading control.

D Schematic diagram of pre-tRNA-Pro-intr-RZ.

E Mating efficiency of cells overexpressing pre-tRNA-Pro. Wild-type cells carrying pREP1 or pREP1-pre-tRNA-Pro-intr-RZ were incubated on SSA medium at 30°C for 2 days, and mating frequency was measured. Mean ± SD values of three independent measurements are shown (total $n > 300$). *$P < 0.05$ (Student's *t*-test).

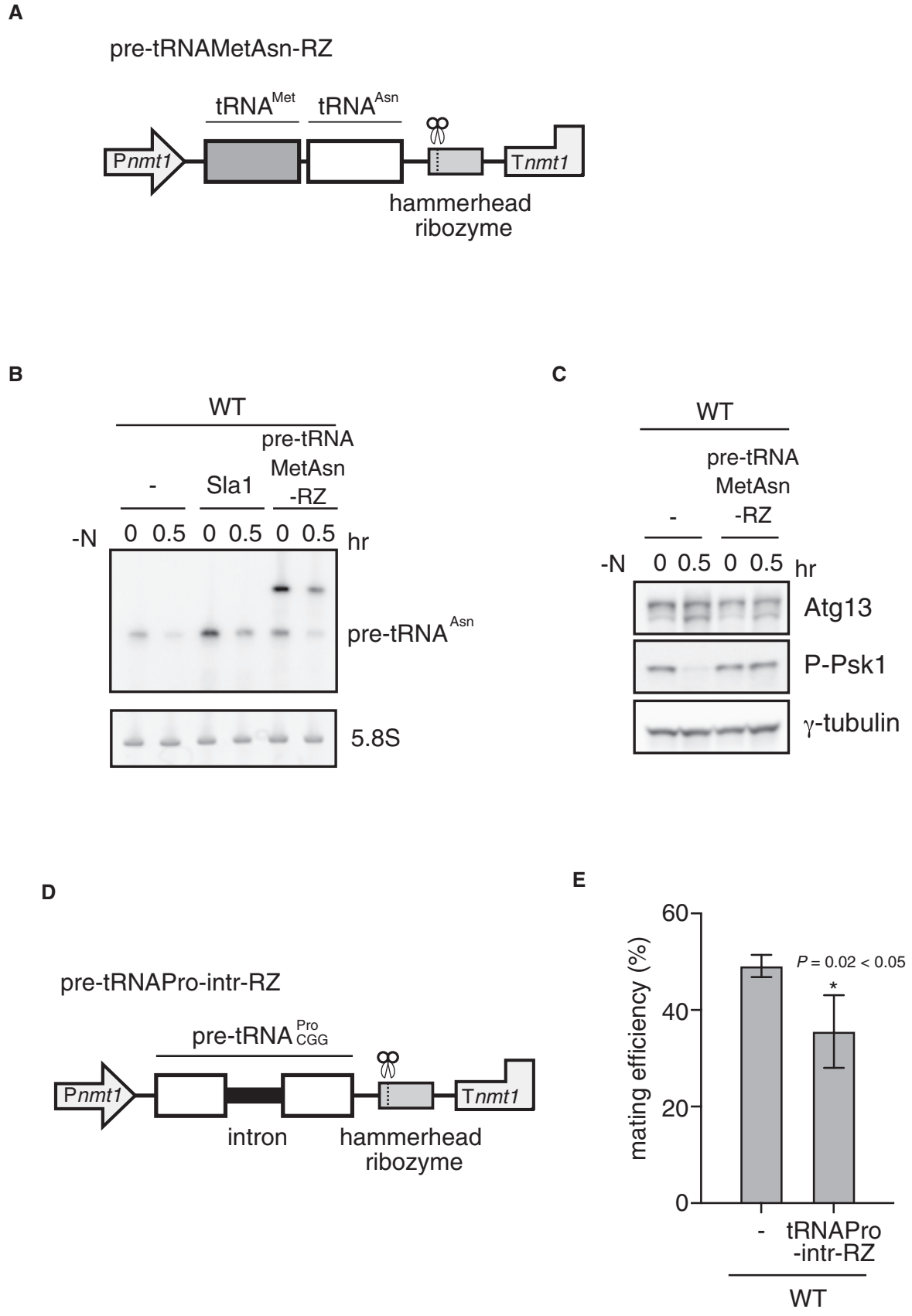

Figure EV3.

Figure EV4. Genetic analysis of overexpression of pre-tRNA.

A Sla1 overexpression in *tor2-ts6* mutant cells. *tor2-ts6* cells (JV303) carrying pREP1 or pREP1-Sla1 were incubated on MM medium at 25°C for 4 days.

B Mating efficiency of cells overexpressing constitutive active TORC1 mutant (Tor2-s69). *tor2-ts6* (JV303), *nrs1/hmt1* (JS159), *prs1/hmt2* (JS160), *tad3/hmt3* (JS161), *rpc34/hmt4* (JS162), *sfc4/hmt5* (JS163), *cts1/hmt6* (JS164), *pcm1/hmt7* (JS165), *ths1* (JS167), and *lrs1* (JS168) cells carrying pREP1 or pREP1-*tor2-s69* were incubated on MM medium at 30°C for 2 days, and mating frequency was measured. Mean ± SD values of three independent measurements are shown (total $n > 300$).

C–F Mating efficiency of cells overexpressing pre-tRNA in *prs1*, *ths1*, *rpc34*, and *pcm1* mutant cells. *prs1*, *ths1*, *rpc34*, and *pcm1* cells carrying pREP1 or pREP1-pre-tRNA-Leu-intr-RZ were incubated on MM medium at 30°C for 2 days (C and D) or 3 days (E and F), and mating frequency was measured. Mean ± SD values of three independent measurements are shown (total $n > 300$). *$P < 0.05$; **$P < 0.01$ (Student's *t*-test).

G Mating efficiency of cells overexpressing Gtr1QL in *nrs1*, *prs1*, *rpc34*, *ths1*, and *lrs1* mutant cells. Wild-type (WT), *nrs1*, *prs1*, *rpc34*, *ths1*, and *lrs1* cells carrying pREP1 or pREP1-Gtr1Q61L were incubated on MM medium at 30°C for 2 days, and mating frequency was measured. Mean ± SD values of three independent measurements are shown (total $n > 300$).

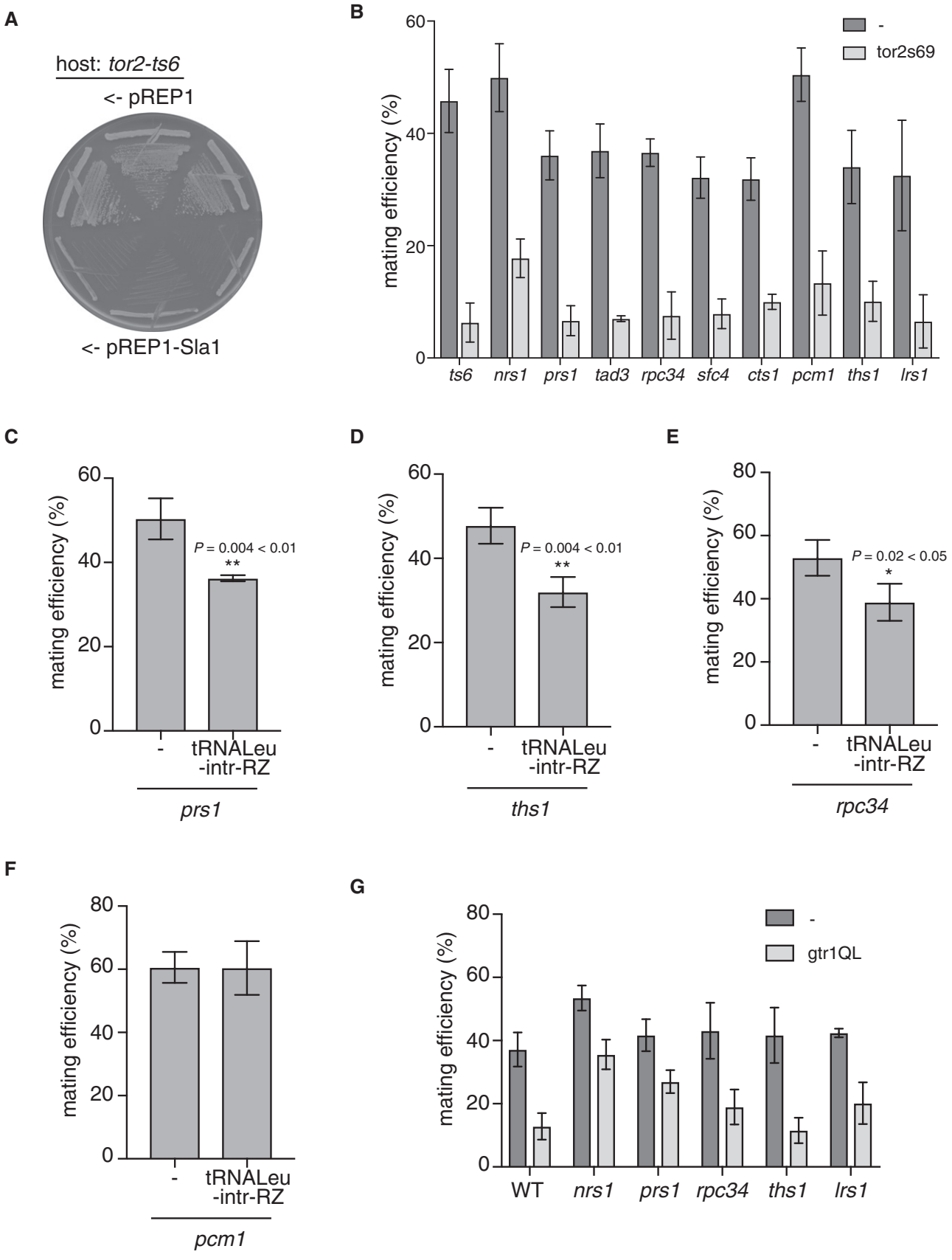

Figure EV4.