Expanded View Figures

Figure EV1. Characterization of the interaction of Stm1 with ribosomes.

A Heat map showing the fraction distribution of most abundant (top 80) ribosome-associated proteins and their response to rapamycin treatment.

B Immunoblots of Stm1, Rpl3, and Pab1 in fractions obtained from polysome profiles of yeast cells under control conditions, nitrogen starvation for 1 h, or rapamycin treatment for 1 h. The polysome profiles were analyzed with or without RNase I treatment for 10 min using 50 mM KCl containing 5–50% sucrose density gradients. Representative blots from a minimum of three independent experiments are shown.

C Immunoblot of Stm1 and Rpl3 in the polysome profile fractions of cells under control condition or nitrogen starved for 1 h, analyzed using sucrose density gradients containing 50, 150, 300, or 800 mM KCl, or from cell extracts treated with 50 mM EDTA (analyzed in 50 mM KCl sucrose density gradients).

D Immunoblot of Stm1 and Rpl3 in the polysome profile fractions of cells grown under nitrogen starved for 1 h with or without nitrogen restimulation for 30 min and analyzed using sucrose density gradients containing 150 mM KCl.

E Relative fraction distribution of Stm1 in 80S fraction. Minimum of three biological replicates are analyzed by multiple unpaired *t*-tests. Data are presented as the mean ± SD.

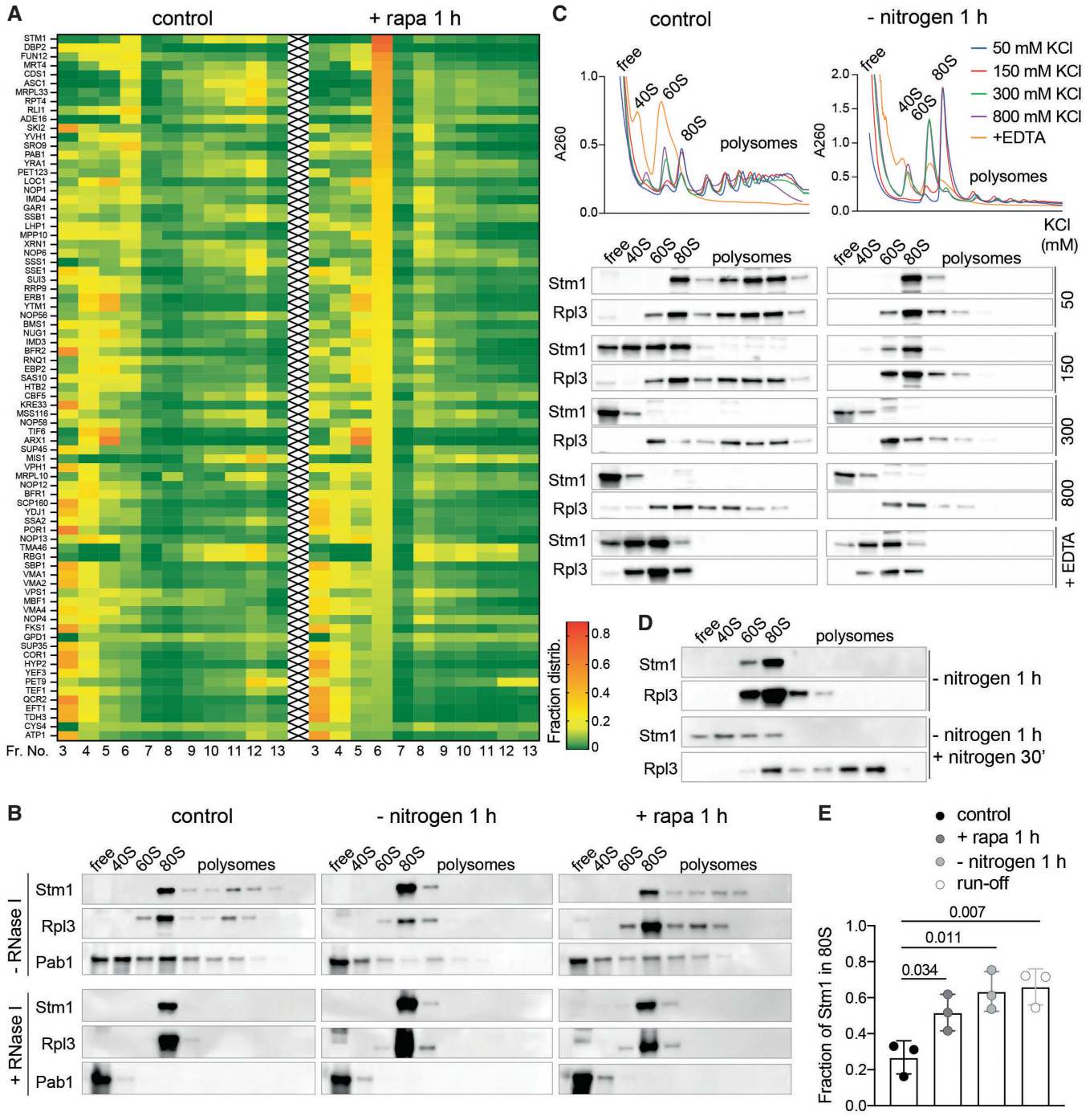
A
control
+ rapa 1 h
Fr. No. 3 4 5 6 7 8 9 10 11 12 13 3 4 5 6 7 8 9 10 11 12 13
Fraction distrib.
0.8
0.6
0.4
0.2
0
C
control
- nitrogen 1 h
50 mM KCl
150 mM KCl
300 mM KCl
800 mM KCl
+EDTA
A260
free
40S
60S
80S
polysomes
KCl (mM)
Stm1
Rpl3
50
150
300
800
+ EDTA
D
- nitrogen 1 h
- nitrogen 1 h
+ nitrogen 30'
B
control
- nitrogen 1 h
+ rapa 1 h
- RNase I
+ RNase I
Stm1
Rpl3
Pab1
E
control
+ rapa 1 h
- nitrogen 1 h
run-off
Fraction of Stm1 in 80S
0.007
0.011
0.034

Figure EV1.

Figure EV2. Stm1 forms vacant 80S ribosomes upon TORC1 inhibition.

A Immunoblot of Stm1 and Actin in *stm1*Δ cells harboring either vector alone, wild-type, or various deletion mutants of Stm1 in nutrient-sufficient conditions.
B Polysome profiles of *stm1*Δ cells containing various deletion mutants of Stm1 upon nitrogen starvation for 1 h.
C Immunoblot of Stm1 across the polysome profile fractions shown in Fig EV2B. Nine equal-volume fractions of each polysome profile were precipitated and analyzed by immunoblot for Stm1 and Rpl3. At least two biological replicates for each mutant were analyzed.
D Growth of *stm1*Δ cells expressing either vector alone, Stm1 to the wild-type level (YCp*STM1*, single-copy plasmid) or overexpression of Stm1 (YEp*STM1*, multi-copy plasmid) on YPD agar plates with or without rapamycin.
E Immunoblot of Stm1 in *stm1*Δ, wild-type, and Stm1-overexpressed cells.
F Growth of *stm1*Δ cells containing various deletion mutants of Stm1 on YPD agar plates with or without rapamycin. Plates were incubated for 36 h at 30°C.

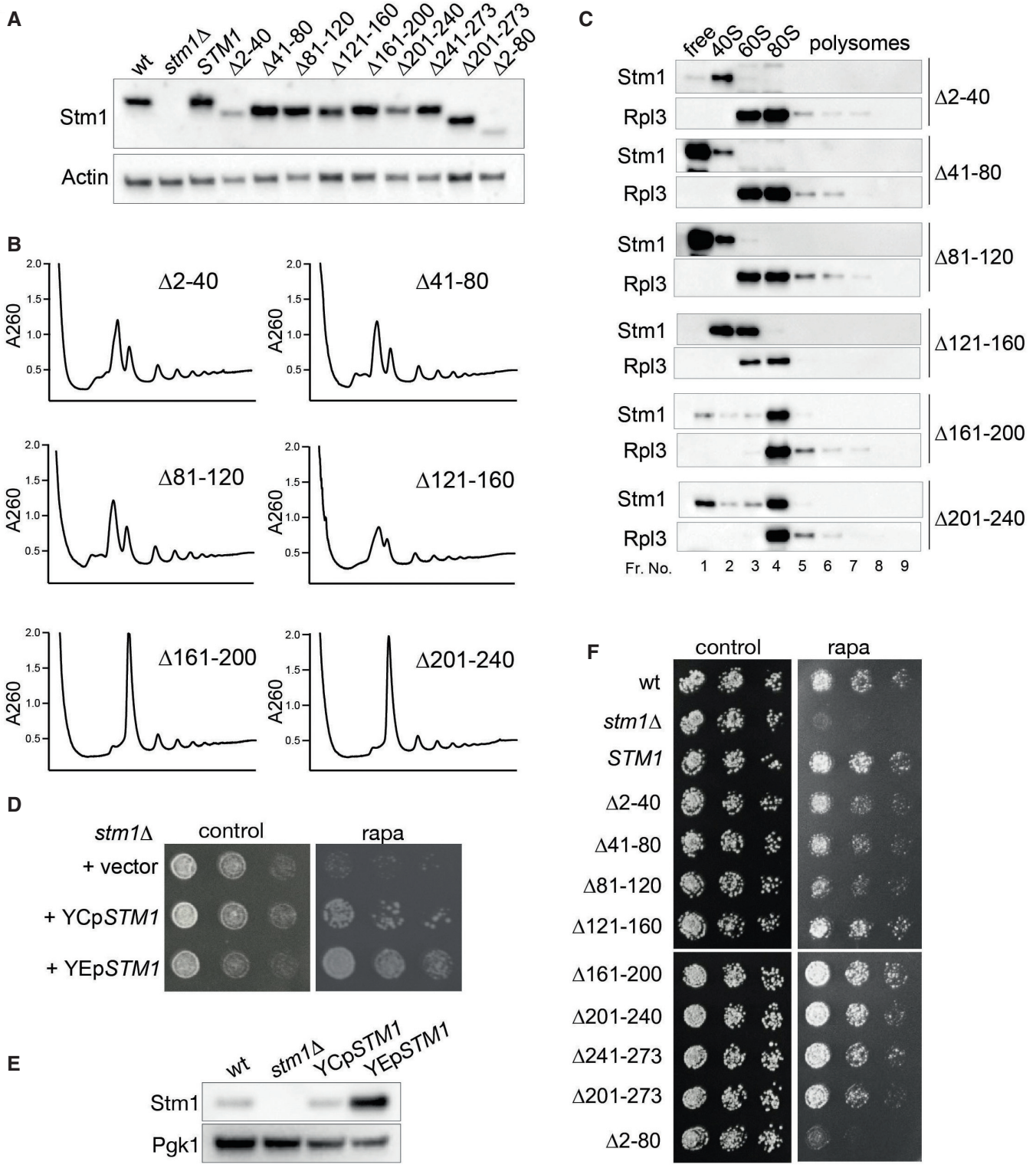

Figure EV2.

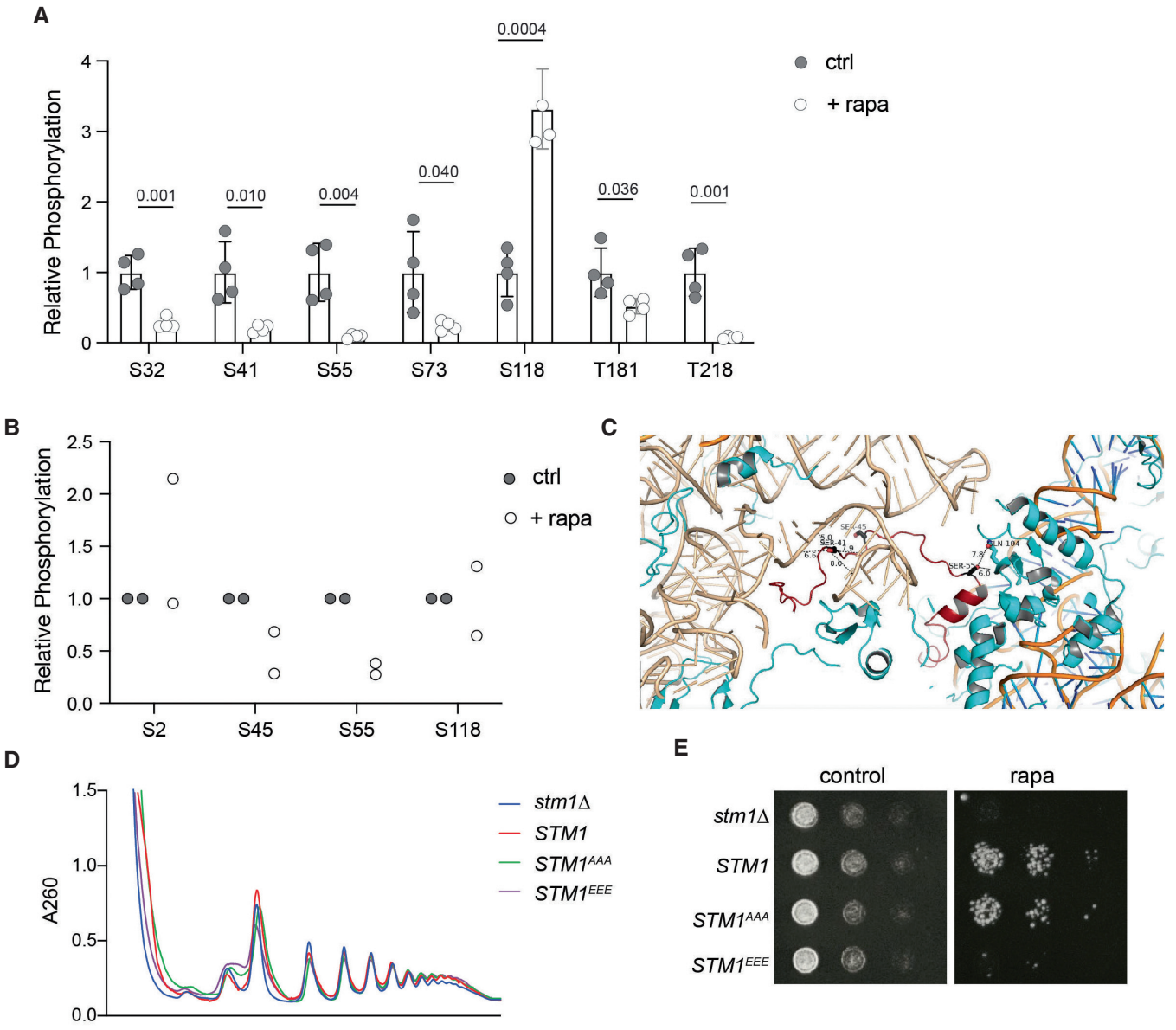

Figure EV3. TORC1 phosphorylates Stm1.

A Relative phosphorylation of Stm1 identified from phosphoproteomic analysis of yeast cells treated with or without rapamycin for 2 h. Four biological replicates are used and analyzed by multiple unpaired *t*-tests. Data are presented as the mean ± SD.

B Relative phosphorylation of Stm1 identified by mass spectrometry. Stm1-GFP was pulled down from yeast cells treated with or without rapamycin for 1 h and subjected to mass spectrometric analysis. Two biological replicates were used.

C Analysis of the structure of Stm1 bound to 80S ribosome. The distance (in Å) between serine 41 of Stm1 with its surrounding ribosomal component and serine 55 of Stm1 with its surrounding ribosomal component is shown in dashed line. Stm1 is shown in red, three serines are shown as sticks, 18S rRNA is shown in orange, 25S rRNA is shown in wheat color and ribosomal proteins in cyan.

D Polysome profiles of *stm1Δ*, wild-type, $STM1^{AAA}$, and $STM1^{EEE}$ cells under control conditions.

E Growth of *stm1Δ*, wild-type, $STM1^{AAA}$, and $STM1^{EEE}$ cells on YPD agar plates with or without rapamycin. Plates were incubated for 48 h at 30°C.

Figure EV4. Stm1 preserves ribosomes under TORC1-inhibited conditions.

A, B Ribosomal subunit profiles of *stm1Δ* cells containing no (+vector), wild-type (+YCp*STM1*), or overexpressed (+YEp*STM1*) levels of Stm1 without (A) or with (B) rapamycin treatment for 24 h. For the ribosomal subunit profile analyses, the cell extracts were treated with 50 mM EDTA and separated on 5–25% sucrose density gradients.

C Immunoblot of Rpl3 in *stm1Δ* cells containing no (+vector), wild-type (+YCp*STM1*), or overexpressed (+YEp*STM1*) levels of Stm1 along with or without rapamycin treatment for 24 h.

D Ribosome content in *stm1Δ*, wild-type, $STM1^{AAA}$, and $STM1^{EEE}$ cells starved for nitrogen for 24 h, measured from ribosomal subunit profiles. Minimum of five biological replicates are used for multiple unpaired *t*-tests.

E, F Immunoblot (E) and the quantification (F) of Rpl3 in *stm1Δ*, wild-type, $STM1^{AAA}$, and $STM1^{EEE}$ cells under nitrogen starvation for 24 h. At least six biological replicates are subjected to statistical analysis using multiple unpaired *t*-tests.

G, H Ribosomal subunit profiles (G) and quantification of relative ribosome content (H) of *stm1Δ*, wild-type, $STM1^{AAA}$, and $STM1^{EEE}$ cells under nutrient-sufficient conditions. Minimum three biological replicates are used for multiple unpaired *t*-tests.

I Quantification of Rpl3 in *stm1Δ*, wild-type, $STM1^{AAA}$, and $STM1^{EEE}$ cells under nutrient-sufficient conditions. Minimum five biological replicates are used for multiple unpaired *t*-tests.

J–L Immunoblot of Rpl24-GFP and free GFP (J), quantification of total GFP relative to Pgk1 (K), and free GFP relative to Rpl24-GFP (L) in wild-type and *stm1Δ* cells treated with rapamycin for 16 and 24 h. At least three replicates are used for multiple unpaired *t*-tests.

M Immunoblots for ubiquitylation in the supernatant and ribosomal pellet obtained from wild-type and *stm1Δ* cells starved for nitrogen (24 h) along with or without puromycin treatment. Immunoblots for Stm1, Rpl3, Pgk1, and puromycin are also shown.

N, O Immunoblot for ubiquitin (N) and its quantification (O) after pull-down of Rpl24-GFP in *stm1Δ*, wild-type, $STM1^{AAA}$, and $STM1^{EEE}$ cells treated with rapamycin for 24 h along with or without bortezomib for 8 h (in *pdr5Δ* background). Minimum of four replicates are quantified and analyzed using multiple unpaired *t*-tests.

Data information: Data are presented as the mean ± SD.

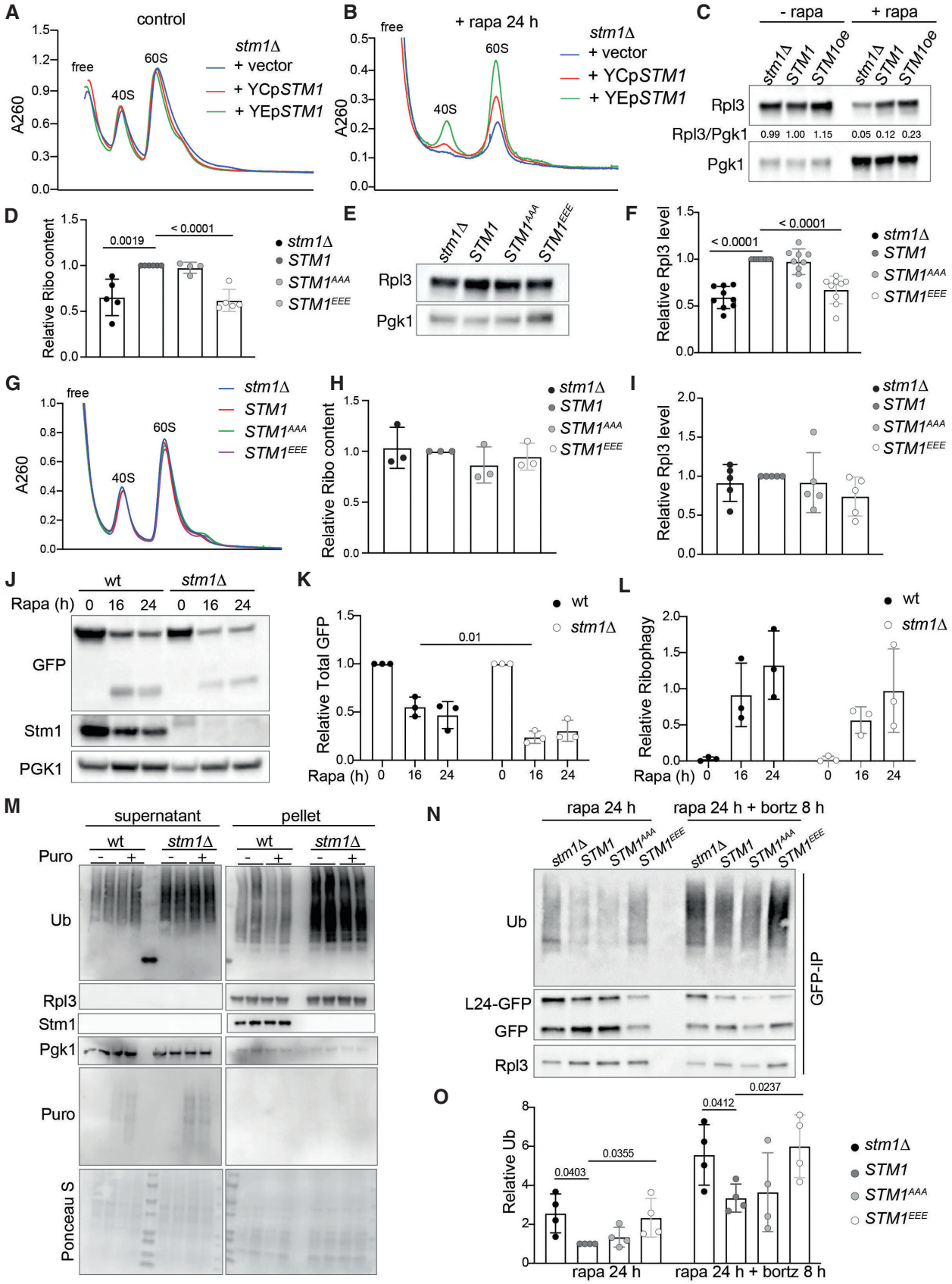

Figure EV4.

Figure EV5. Stm1 is required for translation recovery.

A Puromycin incorporation in wild-type and *stm1Δ* cells subjected to nitrogen starvation for 24 h and restimulated with amino acids for 30 min. Immunoblots for phospho-Rps6, Stm1, and Pgk1 are shown. Three biological replicates are shown for each condition.

B, C Puromycin incorporation (B) and its quantification (C) in *stm1Δ*, wild-type, $STM1^{AAA}$, and $STM1^{EEE}$ cells under nutrient-sufficient conditions. Immunoblots for puromycin, Stm1, and Pgk1 are shown. Six biological replicates are analyzed by multiple unpaired *t*-tests.

D, E Growth of germinated spores (D) and its quantification (E) upon dissection of tetrads obtained from diploid wild-type and *stm1Δ*/*stm1Δ* strains on YPD agar media after 48 h of incubation. At least 24 replicates are analyzed by unpaired *t*-test. (Each row shows four spores from individual tetrads of wild-type and *stm1Δ*, which are labeled as t1, t2, etc.).

F Immunoblots for Rpl3, Stm1, and Pgk1 in diploid wild-type (TB50a), heterozygous *stm1Δ*, and homozygous *stm1Δ* strains grown in sporulation media.

G, H Growth of germinated spores (G) and its quantification (H) upon dissection of tetrads obtained from diploid control (*RPL24-GFP*) and *stm1Δ RPL24-GFP* cells on YPD agar media after 30 h of incubation. At least 28 replicates are analyzed by unpaired *t*-test.

I, J Immunoblots of SERBP1, RPS6, and RPL7a across the polysome fractions of HEK293T cells treated with DMSO or INK128 for 1 h. Polysome profiles were analyzed on 150 mM KCl (I) or 50 mM KCl (J) containing sucrose density gradients.

K Immunoblots of SERBP1, phospho-RPS6 (S240/244), and RPS6 in HEK293T cells treated with control- or *SERBP1*-siRNA, with or without INK128 for 1 h.

L Quantification of ribosome subunit analysis of HEK293T cells treated with control sgRNA (sgCtrl) or *SERBP1* sgRNA (sg*SERBP1*) under normal conditions (Related to Fig 6F). Minimum of four biological replicates are analyzed for each condition by unpaired *t*-test.

M Relative phosphorylation of SERBP1 identified from phosphoproteomic analysis of HEK293 cells treated with or without INK128 for 2 h. Data were analyzed by multiple unpaired *t*-tests.

Data information: Data are presented as the mean ± SD.

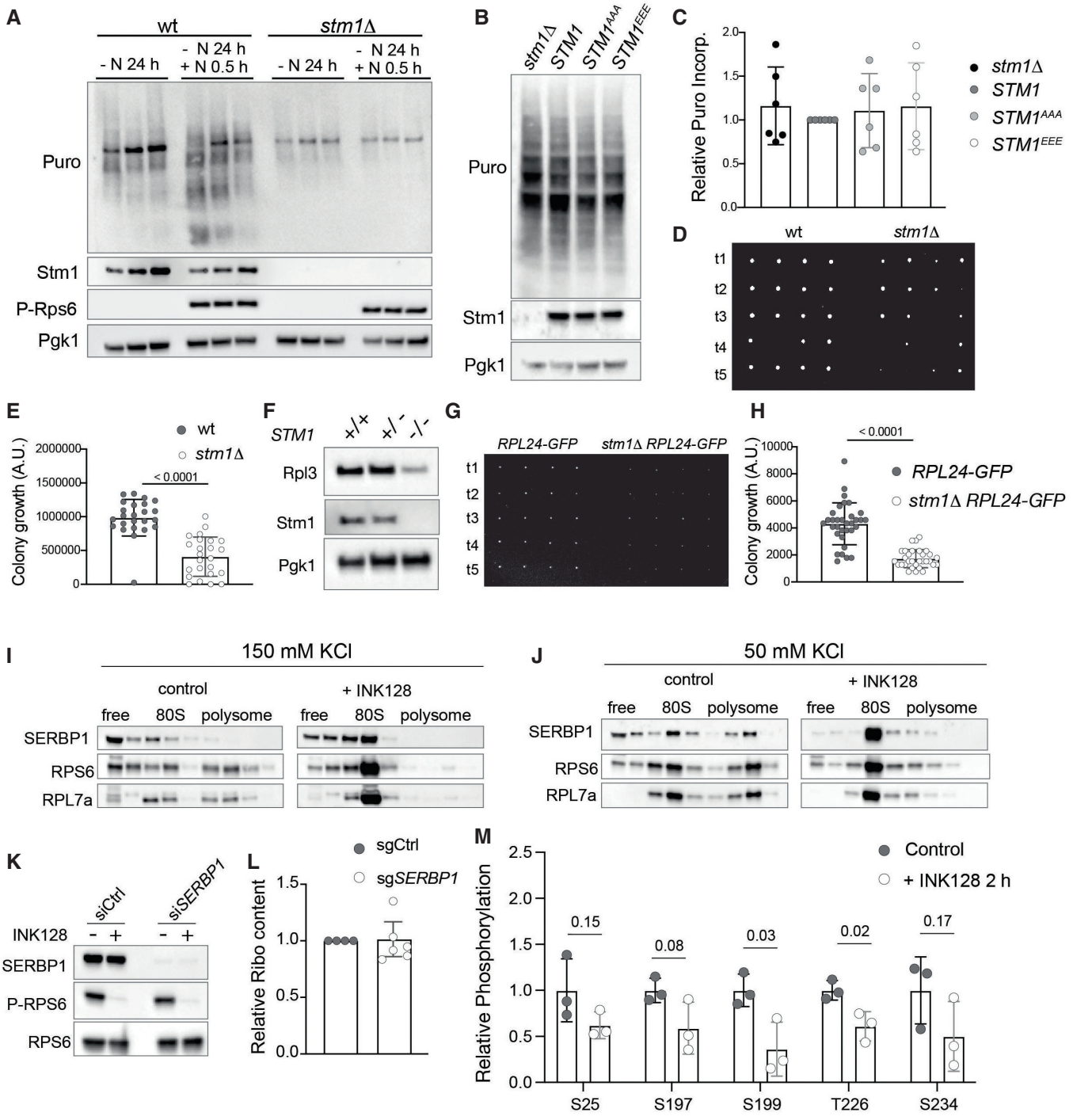

Figure EV5.