**Cell Reports, Volume *20***

## Supplemental Information

# Feedback Inhibition of the Rag GTPase GAP Complex Lst4-Lst7 Safeguards TORC1 from Hyperactivation by Amino Acid Signals

**Marie-Pierre Péli-Gulli, Serena Raucci, Zehan Hu, Jörn Dengjel, and Claudio De Virgilio**

# Supplemental Information

## Supplemental Figures

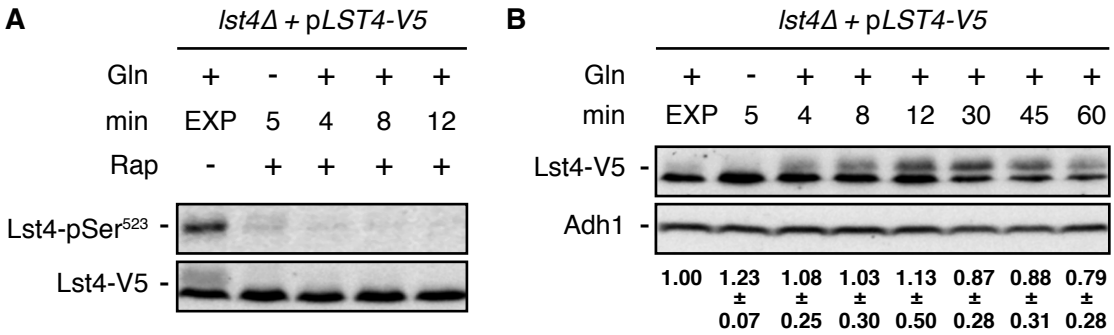


**Figure S1. Glutamine Refeeding Fails to Stimulate Lst4-Ser$^{523}$ Phosphorylation in the Presence of Rapamycin and Does Not Significantly Alter Lst4 Levels, Related to Fig. 2G**

(A) TORC1 inhibition prevents glutamine-stimulated phosphorylation of Ser$^{523}$ in Lst4. Cells (*lst4*Δ) expressing plasmid-encoded Lst4-V5 were treated as in Figure 2G, except that rapamycin (200 ng ml$^{-1}$) was added at the beginning of the amino acid starvation and maintained throughout the glutamine restimulation period.

(B) Amino acid starvation and glutamine refeeding do not significantly affect Lst4-V5 levels. Cells (as in [A]) were treated as in Figure 2G, including additional sampling time points up to 60 min following glutamine refeeding. Representative anti-V5 and anti-Adh1 immunoblots are shown together with respective values of the Lst4-V5 levels that were calculated as the mean ratio of Lst4-V5/Adh1 (n =3; ± SD) and normalized to the respective ratio in exponentially growing (EXP) cells (set to 1.0).

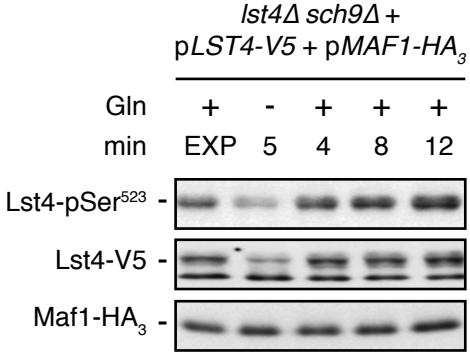


**Figure S2. Phosphorylation of Ser$^{523}$ in Lst4 Does Not Require Sch9, Related to Fig. 2H**

Exponentially growing *lst4*Δ *sch9*Δ cells expressing plasmid-encoded Lst4-V5 and Maf1-HA$_3$ were starved for amino acids and refed with glutamine and analyzed for the phosphorylation levels of Ser$^{523}$ in Lst4, for the total levels of Lst4-V5, and, in an additional control, for the extent of hyperphosphorylation of the Sch9 target Maf1-HA$_3$ (as in Figure 2H). As judged from the relative levels of the slowly migrating Lst4-V5 versus the respective faster migrating ones, and in line with the results in Figure 2H, loss of Sch9 appeared to result in Lst4 hyperphosphorylation in non-starved cells. Notably, while Maf1-HA$_3$ migrated in multiple phosphorylated isoforms in exponentially growing cells expressing functional Sch9 (Figure 2H), no such isoforms were detected in the absence of Sch9 (neither in exponentially growing nor in glutamine-refed *sch9*Δ cells).

## Supplemental Tables

### Table S1. Strains Used in This Study

| Strain | Genotype | Source | Figure |
|---|---|---|---|
| KT1961 | *MATa*; *his3, leu2, ura3-52, trp1* | [1] | |
| KP09 | [KT1961] *MATa; lst4Δ::KanMX* | [2] | 2D; 2F-H; 4A, C; S1A, B |
| KP10 | [KT1961] *MATa; lst7Δ::KanMX* | [2] | 1C; 3B |
| MP372-2D | [KT1961] *MATa*; *LST7-GFP::HIS3MX, lst4Δ::KanMX* | [2] | 3C |
| MP412-1C | [KT1961] *MATa*; *lst4Δ::KanMX, lst7Δ::KanMX* | This study | 2F |
| MP4469 | [KT1961] *MATa*; *lst4Δ::KanMX, URA3::LST4p-LST4-ENVY* | This study | 1B; 2A, E; 3A; 4A-C |
| MP4509 | [KT1961] *MATa*; *lst4Δ::KanMX, URA3::LST4p-lst4$^{12A}$-ENVY* | This study | 3A; 4A-C |
| MP4510 | [KT1961] *MATa*; *lst4Δ::KanMX, URA3::LST4p-lst4$^{5D}$-ENVY* | This study | 3A; 4A-C |
| MP4569 | [KT1961] *MATa; URA3::CYC1p-lst4$^{loop}$-GFP* | This study | 1B; 1D, E |
| MP4570 | [KT1961] *MATa; lst4Δ::KanMX, URA3::CYC1p-lst4$^{loop}$-GFP* | This study | 1D |
| MP4571 | [KT1961] *MATa; lst7Δ::KanMX, URA3::CYC1p-lst4$^{loop}$-GFP* | This study | 1D |
| MP4572 | [KT1961] *MATa; lst4Δ::KanMX, lst7Δ::KanMX, URA3::CYC1p-lst4$^{loop}$-GFP* | This study | 1D |
| MP4573 | [KT1961] *MATa; gtr1Δ::natMX, gtr2Δ::natMX, URA3::CYC1p-lst4$^{loop}$-GFP* | This study | 1F |
| MP4638 | [KT1961] *MATa; lst4Δ::KanMX, sch9Δ::natMX, pRS414-SCH9$^{T492G}$ , pRS416-LST4p-LST4-V5-HIS$_6$* | This study | 2H |
| MP4680 | [KT1961] *MATa; lst4Δ::KanMX, lst7Δ::KanMX, URA3::LST4p-LST4-ENVY* | This study | 3B |
| MP4684 | [KT1961] *MATa; lst4Δ::KanMX, lst7Δ::KanMX, URA3::LST4p-lst4$^{12A}$-ENVY* | This study | 3B |
| MP4688 | [KT1961] *MATa; lst4Δ::KanMX, lst7Δ::KanMX, URA3::LST4p-lst4$^{5D}$-ENVY* | This study | 3B |
| MP268-2B | [KT1961] *MATa; gtr1Δ::NatMX, gtr2Δ::NatMX* | [2] | 3E |
| MP4704 | [MP268-2B] *MATa; lst4Δ::KanMX, URA3::LST4p-LST4-ENVY* | This study | 3E |
| MP4708 | [MP268-2B] *MATa; lst4Δ::KanMX, URA3::LST4p-lst4$^{12A}$-ENVY* | This study | 3E |
| MP4709 | [MP268-2B] *MATa; lst4Δ::KanMX, URA3::LST4p-lst4$^{5D}$-ENVY* | This study | 3E |
| MP4847 | [KT1961] *MATα; iml1Δ::KanMX, lst4Δ::KanMX, URA3::LST4p-LST4-ENVY* | This study | 4D, E |
| MP4849 | [KT1961] *MATα; iml1Δ::KanMX, lst4Δ::KanMX, URA3::LST4p-lst4$^{12A}$-ENVY* | This study | 4D, E |
| MP4642 | [KT1961] *MATa; lst4Δ::KanMX, sch9Δ::KanMX, pRS416-LST4p-LST4-V5-HIS$_6$* | This study | S2 |
| MP4693 | [KT1961] *MATa; lst4Δ::KanMX, lst7Δ::KanMX, URA3::LST4p-lst4$^{Δloop}$-ENVY* | This study | 1D |
| MP4697 | [KT1961] *MATa; URA3::LST4p-lst4$^{Δloop}$-ENVY* | This study | 1B; 1D, E |
| MP4698 | [KT1961] *MATa; lst4Δ::KanMX, URA3::LST4p-LST4-ENVY* | This study | 4D, E |
| MP4699 | [KT1961] *MATa; lst4Δ::KanMX, URA3::LST4p-lst4 $^{Δloop}$-ENVY* | This study | 1D |
| MP4700 | [KT1961] *MATa; lst4Δ::KanMX, URA3::LST4p-lst4$^{12A}$-ENVY* | This study | 4D, E |
| MP4510 | [KT1961] *MATa; lst4Δ::KanMX, URA3::LST4p-lst4$^{5D}$-ENVY* | This study | 4D, E |
| MP4703 | [KT1961] *MATa; lst7Δ::KanMX, URA3::LST4p- lst4 $^{Δloop}$-ENVY* | This study | 1D |
| TB50a | *MATa*; *trp1 his3 ura3 leu2 rme1* | [3] | |
| RL170-2C | [TB50a] *MATa*; *TCO89-TAP::TRP1* | [4] | 2B |

**Table S2. Plasmids Used in This Study**

| Plasmid | Genotype | Source | Figure |
|---|---|---|---|
| pRS413 | CEN, ARS, *HIS3* | [5] | 1B-F; 2A; 2D, E; 2H; 3A, B, E; 4A; 4D; S2 |
| pRS414 | CEN, ARS, *TRP1* | [5] | 1B; 1D-F; 2A; 2D-H; 3A; 3D; 4A-D, S1A, B; S2 |
| pRS415 | CEN, ARS, *LEU2* | [5] | 1B-F; 2A; 2D-H; 3A, B; 3D; 4A-D; S1A, B |
| pRS416 | CEN, ARS, *URA3* | [5] | 1C; 2D; 2F-H; 3B; 4A; 4C; S1A, B |
| pMP3008 | [pRS413] *LST4p-LST4-V5-HIS$_6$* | This study | 2D; 2F, G; S1A, B |
| pMP3055 | [pRS413] *LST4p-lst4$^{S523A}$-V5-HIS$_6$* | This study | 2D |
| pMP2576 | [pRS414] *LST7p-LST7-HA$_3$* | This study | 1C; 3B |
| pAH145 | [pRS414] *sch9$^{T492G}$* | [6] | 2H |
| pPL155 | [pRS415] *HA$_3$-TOR1$^{A1957V}$* | [7] | 1F |
| p1392 | [pRS415] *MAF1-HA$_3$* | [6] | 2H; S2 |
| pMP2780 | [pRS416] *LST4p-LST4-V5-HIS$_6$* | This study | 1C; 2H; 3C; S2 |
| pMP3143 | [pRS416] *CYC1p-lst4$^{loop}$-V5-HIS$_6$* | This study | 1C |
| pMP3147 | [pRS416] *LST4p-lst4$^{\Delta loop}$-V5-HIS$_6$* | This study | 1C |
| pMP3149 | [pRS416] *LST4p-lst4$^{5D}$-V5-HIS$_6$* | This study | 3C |
| pMP3165 | [pRS416] *LST4p-lst4$^{12A}$-V5-HIS$_6$* | This study | 3C |
| pRS306 | integrative, *URA3* | [8] | |
| pMP3042 | [pRS306] *LST4p-LST4-ENVY* | This study | 1B; 2A, E; 3A; 4A-C |
| pMP3062 | [pRS306] *LST4p-lst4$^{12A}$-ENVY* | This study | 3A; 4A-C |
| pMP3064 | [pRS306] *LST4p-lst4$^{5D}$-ENVY* | This study | 3A; 4A-C |
| pMP3077 | [pRS306] *CYC1p-lst4$^{loop}$-GFP* | This study | 1B; 1D-F |
| pSIVu | integrative, *URA3* | [9] | |
| pMP3166 | [pSIVu] *LST4p-LST4-ENVY* | This study | 2A; 2E; 3A, B; 4A-D |
| pMP3167 | [pSIVu] *LST4p-lst4$^{\Delta loop}$-ENVY* | This study | 1B; 1D, E |
| pMP3168 | [pSIVu] *LST4p-lst4$^{12A}$-ENVY* | This study | 3A, B; 4A-D |
| pMP3169 | [pSIVu] *LST4p-lst4$^{5D}$-ENVY* | This study | 3A, B; 4A-D |
| pRS423 | 2μ, *HIS3* | [10] | |
| pRH2953 | [pRS423] *VAC8p-vhhGFP4-PHO8N* | R. Hatakeyama | 4B, C |
| pAS2570 | [pET28b$^+$] *HIS$_6$-LST4* | [2] | 2B; 3D |
| pAS2571 | [pET15b$^+$] *HIS$_6$-LST7* | [2] | 2B; 3D |
| pMP3057 | [pET28b$^+$] *HIS$_6$-lst4$^{12A}$* | This study | 2B; 3D |
| pMP3058 | [pET28b$^+$] *HIS$_6$-lst4$^{5D}$* | This study | 2B; 3D |
| pNP2038 | [pET-24d] *GST-TEV-GTR2* | [11] | 3D |
| pJU1046 | [pGEX-6P] *GST-TEV-gtr1$^{Q65L}$-HIS$_6$* | R. Loewith | 3D |
| p3285 | pYEGFP-GAC111-*RPL25* | D. Kressler | 3E |
| pMP2789 | [pRS415] *GTR1p-GTR1-HA$_3$* | This study | 3E |
| pMP2337 | [pRS416] *GTR1p-GTR1-HA$_3$* | [2] | 3E |
| pMP2782 | [pRS414] *GTR2p-gtr2$^{Q66L}$-V5-HIS$_6$* | [2] | 3E |

**Table S3. TORC1-Controlled Phosphorylation Sites in Lst4[a]**

| No. | Position | PEP | Score | Phospho (ST) Probabilities |
|---|---|---|---|---|
| P1 | 484 | 1.00E-02 | 303.5 | NSNTSVSVSSSESLAEVIQPS(0.044)**S**(0.956)FK |
| P2 | 498 | 1.00E-02 | 173.0 | SGSSSLHYLS(0.009)S(0.039)**S**(0.901)IS(0.047)SQPGSYGSWFNK |
| P3 | 523 | 5.30E-57 | 126.8 | RPTISQFFQP**S**(0.997)PS(0.003)LK |
| P3 | 525 | 8.75E-29 | 101.9 | RPTISQFFQPS(0.166)P**S**(0.834)LK |
| P4 | 547 | 8.12E-08 | 74.4 | TS(0.003)**S**(0.983)S(0.013)SSLQQATSR |
| P4 | 549 | 2.21E-108 | 162.4 | TSSS(0.003)**S**(0.752)S(0.244)LQQATSR |
| P4 | 550 | 2.27E-56 | 131.4 | TSSSS(0.029)**S**(0.969)LQQATSR |

[a] Peptides are numbered according to Figure 2. The position of the most likely phosphorylated amino acid residue as identified by MS-analysis is indicated (see also respective phosphosite localization probabilities). Sites marked in red (*i.e.* probability > 0.001) were exchanged to alanine and sites marked in blue (*i.e.* probability > 0.9) were exchanged to alanine and/or phospho-mimetic aspartate. In addition, we also included the conserved $Thr^{545}$, which is adjacent to a serine cluster, in these analyses. PEP: posterior error probability; Score: Andromeda score.

## Supplemental References


1 Pedruzzi I, Dubouloz F, Cameroni E *et al.* TOR and PKA signaling pathways converge on the protein kinase Rim15 to control entry into $G_0$. *Mol Cell* 2003; **12**:1607-1613.

2 Péli-Gulli MP, Sardu A, Panchaud N, Raucci S, De Virgilio C. Amino Acids Stimulate TORC1 through Lst4-Lst7, a GTPase-Activating Protein Complex for the Rag Family GTPase Gtr2. *Cell Rep* 2015; **13**:1-7.

3 Beck T, Hall MN. The TOR signalling pathway controls nuclear localization of nutrient-regulated transcription factors. *Nature* 1999; **402**:689-692.

4 Shimada K, Filipuzzi I, Stahl M *et al.* TORC2 signaling pathway guarantees genome stability in the face of DNA strand breaks. *Mol Cell* 2013; **51**:829-839.

5 Brachmann CB, Davies A, Cost GJ *et al.* Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 1998; **14**:115-132.

6 Huber A, Bodenmiller B, Uotila A *et al.* Characterization of the rapamycin-sensitive phosphoproteome reveals that Sch9 is a central coordinator of protein synthesis. *Genes Dev* 2009; **23**:1929-1943.

7 Reinke A, Chen JC, Aronova S, Powers T. Caffeine targets TOR complex I and provides evidence for a regulatory link between the FRB and kinase domains of Tor1p. *J Biol Chem* 2006; **281**:31616-31626.

8 Sikorski RS, Hieter P. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. *Genetics* 1989; **122**:19-27.

9 Wosika V, Durandau E, Varidel C, Aymoz D, Schmitt M, Pelet S. New families of single integration vectors and gene tagging plasmids for genetic manipulations in budding yeast. *Molecular genetics and genomics : MGG* 2016; **291**:2231-2240.

10 Christianson TW, Sikorski RS, Dante M, Shero JH, Hieter P. Multifunctional yeast high-copy-number shuttle vectors. *Gene* 1992; **110**:119-122.

11 Panchaud N, Péli-Gulli MP, De Virgilio C. Amino acid deprivation inhibits TORC1 through a GTPase-activating protein complex for the Rag family GTPase Gtr1. *Sci Signal* 2013; **6**:ra42.