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**Supplemental Information**

**Feedback Inhibition of the Rag GTPase**

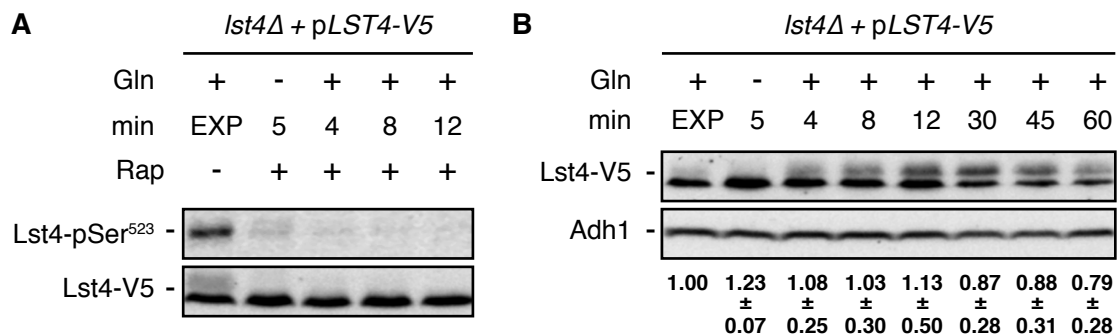
**GAP Complex Lst4-Lst7 Safeguards TORC1**

**from Hyperactivation by Amino Acid Signals**

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## Supplemental Information

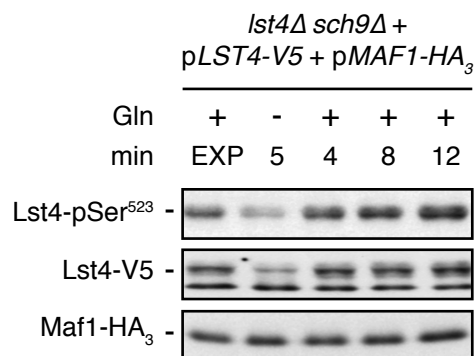
### Supplemental Figures



**Figure S1. Glutamine Refeeding Fails to Stimulate Lst4-Ser<sup>523</sup> 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<sup>523</sup> in Lst4. Cells (*lst4Δ*) expressing plasmid-encoded Lst4-V5 were treated as in Figure 2G, except that rapamycin (200 ng ml<sup>-1</sup>) 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<sup>523</sup> in Lst4 Does Not Require Sch9, Related to Fig. 2H**

Exponentially growing *lst4Δ sch9Δ* cells expressing plasmid-encoded Lst4-V5 and Maf1-HA<sub>3</sub> were starved for amino acids and re-fed with glutamine and analyzed for the phosphorylation levels of Ser<sup>523</sup> 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<sub>3</sub> (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<sub>3</sub> 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 *sch9Δ* cells).

## Supplemental Tables

**Table S1. Strains Used in This Study**

Strain	Genotype	Source	Figure
KT1961	<i>MATa; his3, leu2, ura3-52, trp1</i>	[1]	
KP09	[KT1961] <i>MATa; lst4Δ::KanMX</i>	[2]	2D; 2F-H; 4A, C; S1A, B
KP10	[KT1961] <i>MATa; lst7Δ::KanMX</i>	[2]	1C; 3B
MP372-2D	[KT1961] <i>MATa; LST7-GFP::HIS3MX, lst4Δ::KanMX</i>	[2]	3C
MP412-1C	[KT1961] <i>MATa; lst4Δ::KanMX, lst7Δ::KanMX</i>	This study	2F
MP4469	[KT1961] <i>MATa; lst4Δ::KanMX, URA3::LST4p-LST4-ENVY</i>	This study	1B; 2A, E; 3A; 4A-C
MP4509	[KT1961] <i>MATa; lst4Δ::KanMX, URA3::LST4p-lst4<sup>12A</sup>-ENVY</i>	This study	3A; 4A-C
MP4510	[KT1961] <i>MATa; lst4Δ::KanMX, URA3::LST4p-lst4<sup>5D</sup>-ENVY</i>	This study	3A; 4A-C
MP4569	[KT1961] <i>MATa; URA3::CYC1p-lst4<sup>loop</sup>-GFP</i>	This study	1B; 1D, E
MP4570	[KT1961] <i>MATa; lst4Δ::KanMX, URA3::CYC1p-lst4<sup>loop</sup>-GFP</i>	This study	1D
MP4571	[KT1961] <i>MATa; lst7Δ::KanMX, URA3::CYC1p-lst4<sup>loop</sup>-GFP</i>	This study	1D
MP4572	[KT1961] <i>MATa; lst4Δ::KanMX, lst7Δ::KanMX, URA3::CYC1p-lst4<sup>loop</sup>-GFP</i>	This study	1D
MP4573	[KT1961] <i>MATa; gtr1Δ::natMX, gtr2Δ::natMX, URA3::CYC1p-lst4<sup>loop</sup>-GFP</i>	This study	1F
MP4638	[KT1961] <i>MATa; lst4Δ::KanMX, sch9Δ::natMX, pRS414-SCH9<sup>T492G</sup>, pRS416-LST4p-LST4-V5-HIS<sub>6</sub></i>	This study	2H
MP4680	[KT1961] <i>MATa; lst4Δ::KanMX, lst7Δ::KanMX, URA3::LST4p-LST4-ENVY</i>	This study	3B
MP4684	[KT1961] <i>MATa; lst4Δ::KanMX, lst7Δ::KanMX, URA3::LST4p-lst4<sup>12A</sup>-ENVY</i>	This study	3B
MP4688	[KT1961] <i>MATa; lst4Δ::KanMX, lst7Δ::KanMX, URA3::LST4p-lst4<sup>5D</sup>-ENVY</i>	This study	3B
MP268-2B	[KT1961] <i>MATa; gtr1Δ::NatMX, gtr2Δ::NatMX</i>	[2]	3E
MP4704	[MP268-2B] <i>MATa; lst4Δ::KanMX, URA3::LST4p-LST4-ENVY</i>	This study	3E
MP4708	[MP268-2B] <i>MATa; lst4Δ::KanMX, URA3::LST4p-lst4<sup>12A</sup>-ENVY</i>	This study	3E
MP4709	[MP268-2B] <i>MATa; lst4Δ::KanMX, URA3::LST4p-lst4<sup>5D</sup>-ENVY</i>	This study	3E
MP4847	[KT1961] <i>MATα; iml1Δ::KanMX, lst4Δ::KanMX, URA3::LST4p-LST4-ENVY</i>	This study	4D, E
MP4849	[KT1961] <i>MATα; iml1Δ::KanMX, lst4Δ::KanMX, URA3::LST4p-lst4<sup>12A</sup>-ENVY</i>	This study	4D, E
MP4642	[KT1961] <i>MATa; lst4Δ::KanMX, sch9Δ::KanMX, pRS416-LST4p-LST4-V5-HIS<sub>6</sub></i>	This study	S2
MP4693	[KT1961] <i>MATa; lst4Δ::KanMX, lst7Δ::KanMX, URA3::LST4p-lst4<sup>loop</sup>-ENVY</i>	This study	1D
MP4697	[KT1961] <i>MATa; URA3::LST4p-lst4<sup>loop</sup>-ENVY</i>	This study	1B; 1D, E
MP4698	[KT1961] <i>MATa; lst4Δ::KanMX, URA3::LST4p-LST4-ENVY</i>	This study	4D, E
MP4699	[KT1961] <i>MATa; lst4Δ::KanMX, URA3::LST4p-lst4<sup>loop</sup>-ENVY</i>	This study	1D
MP4700	[KT1961] <i>MATa; lst4Δ::KanMX, URA3::LST4p-lst4<sup>12A</sup>-ENVY</i>	This study	4D, E
MP4510	[KT1961] <i>MATa; lst4Δ::KanMX, URA3::LST4p-lst4<sup>5D</sup>-ENVY</i>	This study	4D, E
MP4703	[KT1961] <i>MATa; lst7Δ::KanMX, URA3::LST4p-lst4<sup>loop</sup>-ENVY</i>	This study	1D
TB50a	<i>MATa; trp1 his3 ura3 leu2 rme1</i>	[3]	
RL170-2C	[TB50a] <i>MATa; TCO89-TAP::TRP1</i>	[4]	2B

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

Plasmid	Genotype	Source	Figure
pRS413	CEN, ARS, <i>HIS3</i>	[5]	1B-F; 2A; 2D, E; 2H; 3A, B, E; 4A; 4D; S2
pRS414	CEN, ARS, <i>TRP1</i>	[5]	1B; 1D-F; 2A; 2D-H; 3A; 3D; 4A-D, S1A, B; S2
pRS415	CEN, ARS, <i>LEU2</i>	[5]	1B-F; 2A; 2D-H; 3A, B; 3D; 4A-D; S1A, B
pRS416	CEN, ARS, <i>URA3</i>	[5]	1C; 2D; 2F-H; 3B; 4A; 4C; S1A, B
pMP3008	[pRS413] <i>LST4p-LST4-V5-HIS<sub>6</sub></i>	This study	2D; 2F, G; S1A, B
pMP3055	[pRS413] <i>LST4p-lst4<sup>S523A</sup>-V5-HIS<sub>6</sub></i>	This study	2D
pMP2576	[pRS414] <i>LST7p-LST7-HA<sub>3</sub></i>	This study	1C; 3B
pAH145	[pRS414] <i>sch9<sup>T492G</sup></i>	[6]	2H
pPL155	[pRS415] <i>HA<sub>3</sub>-TOR1<sup>A1957V</sup></i>	[7]	1F
p1392	[pRS415] <i>MAF1-HA<sub>3</sub></i>	[6]	2H; S2
pMP2780	[pRS416] <i>LST4p-LST4-V5-HIS<sub>6</sub></i>	This study	1C; 2H; 3C; S2
pMP3143	[pRS416] <i>CYC1p-lst4<sup>loop</sup>-V5-HIS<sub>6</sub></i>	This study	1C
pMP3147	[pRS416] <i>LST4p-lst4<sup>Alloop</sup>-V5-HIS<sub>6</sub></i>	This study	1C
pMP3149	[pRS416] <i>LST4p-lst4<sup>5D</sup>-V5-HIS<sub>6</sub></i>	This study	3C
pMP3165	[pRS416] <i>LST4p-lst4<sup>I2A</sup>-V5-HIS<sub>6</sub></i>	This study	3C
pRS306	integrative, <i>URA3</i>	[8]	
pMP3042	[pRS306] <i>LST4p-LST4-ENVY</i>	This study	1B; 2A, E; 3A; 4A-C
pMP3062	[pRS306] <i>LST4p-lst4<sup>I2A</sup>-ENVY</i>	This study	3A; 4A-C
pMP3064	[pRS306] <i>LST4p-lst4<sup>5D</sup>-ENVY</i>	This study	3A; 4A-C
pMP3077	[pRS306] <i>CYC1p-lst4<sup>loop</sup>-GFP</i>	This study	1B; 1D-F
pSIVu	integrative, <i>URA3</i>	[9]	
pMP3166	[pSIVu] <i>LST4p-LST4-ENVY</i>	This study	2A; 2E; 3A, B; 4A-D
pMP3167	[pSIVu] <i>LST4p-lst4<sup>Alloop</sup>-ENVY</i>	This study	1B; 1D, E
pMP3168	[pSIVu] <i>LST4p-lst4<sup>I2A</sup>-ENVY</i>	This study	3A, B; 4A-D
pMP3169	[pSIVu] <i>LST4p-lst4<sup>5D</sup>-ENVY</i>	This study	3A, B; 4A-D
pRS423	2μ, <i>HIS3</i>	[10]	
pRH2953	[pRS423] <i>VAC8p-vhhGFP4-PHO8N</i>	R. Hatakeyama	4B, C
pAS2570	[pET28b <sup>+</sup> ] <i>HIS<sub>6</sub>-LST4</i>	[2]	2B; 3D
pAS2571	[pET15b <sup>+</sup> ] <i>HIS<sub>6</sub>-LST7</i>	[2]	2B; 3D
pMP3057	[pET28b <sup>+</sup> ] <i>HIS<sub>6</sub>-lst4<sup>I2A</sup></i>	This study	2B; 3D
pMP3058	[pET28b <sup>+</sup> ] <i>HIS<sub>6</sub>-lst4<sup>5D</sup></i>	This study	2B; 3D
pNP2038	[pET-24d] <i>GST-TEV-GTR2</i>	[11]	3D
pJU1046	[pGEX-6P] <i>GST-TEV-gtr1<sup>Q65L</sup>-HIS<sub>6</sub></i>	R. Loewith	3D
p3285	pYEGFP-GAC111- <i>RPL25</i>	D. Kressler	3E
pMP2789	[pRS415] <i>GTR1p-GTR1-HA<sub>3</sub></i>	This study	3E
pMP2337	[pRS416] <i>GTR1p-GTR1-HA<sub>3</sub></i>	[2]	3E
pMP2782	[pRS414] <i>GTR2p-gtr2<sup>Q66L</sup>-V5-HIS<sub>6</sub></i>	[2]	3E

**Table S3. TORC1-Controlled Phosphorylation Sites in Lst4<sup>a</sup>**

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	RPTISQFFQPS(0.997)PS(0.003)LK
P3	525	8.75E-29	101.9	RPTISQFFQPS(0.166)PS(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	TSSS(0.029)S(0.969)LQQATSR

<sup>a</sup> 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<sup>545</sup>, which is adjacent to a serine cluster, in these analyses. PEP: posterior error probability; Score: Andromeda score.

## Supplemental References

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