Supplemental Data

The Vam6 GEF Controls TORC1 by Activating the EGO Complex

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Table S1. Strains Used in This Study

Strain	Genotype	Source	Figure
BY4741	*MAT***a**; *his3Δ1, leu2Δ0, ura3Δ0, met15Δ0*	Euroscarf	
BY4742	*MATα*; *his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0*	Euroscarf	3C
YL515	[BY4741/2] *MATα; his3Δ1, leu2Δ0, ura3Δ0*	This study	1A, B, D; 2; 3B; 4A, B; 5A, E, G-K ; 6B-D; S1; S2
YL516	[BY4741/2] *MAT***a;** *his3Δ1, leu2Δ0, ura3Δ0*	This study	3C, D; 5B-D, F; 6A
MB25	[YL515] *MATα*; *ego1Δ::HIS3*	This study	1A
MB26	[YL515] *MATα*; *ego3Δ::HIS3*	This study	1A
MB27	[YL515] *MATα*; *gtr1Δ::HIS3*	This study	1A, D; 2A
MB28	[YL515] *MATα*; *gtr2Δ::HIS3*	This study	1A; 2A
MB34	[YL515] *MATα*; *tco89Δ::HIS3*	This study	1A, D
MB32	[YL516] *MAT***a**; *gtr1Δ::kanMX4*	This study	1C; 3D; 5A-D; S1, 3
MB33	[YL516] *MAT***a**; *gtr2Δ::kanMX4*	This study	2B; 5B, C; S1
YJU531	[YL516] *MAT***a**; *tco89Δ::natMX*	This study	5D
MP07-1D	[YL516] *MAT***a**; *vam6Δ::kanMX4*	This study	5A-C, F, K; 6A, C, D
MP02-7B	[YL515] *MATα*; *gtr1Δ::kanMX4, tco89Δ::HIS3*	This study	1B, C
MP06-8B	[YL515] *MATα*; *gtr1Δ::kanMX4, gtr2Δ::kanMX4*	This study	3A
MB35	[YL515] *MATα; gln3Δ::HYGR*	This study	S1
MB36-2C	[YL516] *MAT***a**; *gtr1Δ::kanMX4, gln3Δ::HYGR*	This study	S1
MP01-4D	[YL516] *MAT***a**; *gtr2Δ::kanMX4, gln3Δ::HYGR*	This study	S1
YJU537	[YL516] *MAT***a**; *EGO1-GFP::kanMX6*	This study	6A, B
MP56	[YL516] *MAT***a**; *TCO89-GFP::kanMX6*	This study	6A, B
KT1960	*MAT***a**; *his3, leu2, ura3, trp1*	[1]	
FD34	[KT1960] *MAT***a**; *his3, leu2, ura3, trp1, KOG1-TAP::HIS3*	This study	4B, E
FD35	[KT1960] *MAT***a**; *his3, leu2, ura3, trp1, TCO89-TAP::HIS3*	This study	4A, D
MP52-2A	[YL516] *MAT***a**; *TOR1-D330-3xGFP*	This study	6A, B
Y07050	[BY4741] *MAT***a**; *gap1Δ::kanMX4*	Euroscarf	2C, D
Y00575	[BY4741] *MAT***a**; *ypt7Δ::kanMX4*	Euroscarf	5A, K; 6C, D
MB56	[BY4741] *MAT***a**; *GTR1-GFP::HIS3*	[2]	6A
NMY51	*MAT***a**; *his3Δ200, trp1-901, leu2-3,112, ade2, LYS2::(lexAop)4-HIS3, ura3::(lexAop)8- lacZ, ade2::(lexAop)8-ADE2, GAL4*	Dual-systems Biotech AG	4C

Table S2. Plasmids Used in This Study

Plasmid	Description	Source	Figure
pRS413	CEN, *HIS3*	[3]	1; 2A, B; 3B, C; 5B-E; 6A, C; S1
pRS415	CEN, *LEU2*	[3]	1; 2; 3A-C; 5B-D; 6B-D; S1-3
pRS416	CEN, *URA3*	[3]	1D; 2B-D; 3A, B; 5B; 6; S2
YCplac33	CEN, *URA3*	[4]	1C; 3D
pJU660	[pRS415] *GTR1*	This study	1B; 3A
pJU656	[pRS415] $GTR1^{S20L}$	This study	1B; 3A
pMB1483	[pRS415] $GTR1^{Q65L}$	This study	1B; 3A
pJU651	[pRS416] *GTR2*	This study	3A
pJU654	[pRS416] $GTR2^{S23L}$	This study	3A
pJU655	[pRS416] $GTR2^{Q66L}$	This study	3A
pMB1393	[YCplac33] Tet_{ON}-*GTR1*	This study	1C; 3D; S3
pMB1394	[YCplac33] Tet_{ON}-$GTR1^{Q65L}$	This study	1C; 3D; S3
pMB1395	[YCplac33] Tet_{ON}-$GTR1^{S20L}$	This study	1C; 3D
pMB1344	[YCplac33] *GTR1-TAP*	This study	5I, K
pMB1371	[YCplac33] $GTR1^{S20L}$-*TAP*	This study	5I
pMB1372	[YCplac33] $GTR1^{Q65L}$-*TAP*	This study	5I
YCpIF2	CEN, *LEU2, GAL1-GST*	[5]	
pMB1348	[YCpIF2] *GAL1-GST-GTR1*	This study	4A, B,
pMB1579	[YCpIF2] *GAL1-GST-*$GTR1^{Q65L}$	This study	4A, B
pMB1580	[YCpIF2] *GAL1-GST-*$GTR1^{S20L}$	This study	4A, B
pMB1667	[YCpIF2] *GAL1-GST-GTR1, leu2::URA3*	This study	4D, E
pMB1668	[YCpIF2] *GAL1-GST -*$GTR1^{Q65L}$*, leu2::URA3*	This study	4D, E
pVW1146	[YIpIF2] *GAL1-GTR1*	This study	5A, E
pVW1148	[YIpIF2] *GAL1-*$GTR1^{S20L}$	This study	5A, E
pJU1064	[pRS413] $SCH9^{T570A}$-HA_5	[6]	2C, D; 3D; 6D; S2; S3
pJU1030	[pRS416] $SCH9^{T570A}$-HA_5	[6]	1A,B; 3C; 5C; S1
pCDV1084	[YCpIF2] *ADH1-GST-EGO1*	[7]	5I, K
pMPG1574	2μ, Tet_{ON}-HIS_6-HA_3, *URA3*	This study	5D, E, H
pMPG1576	[pMPG1574] Tet_{ON}-HIS_6-HA_3-*VAM6*	This study	5D, E, G, H
pPL132	[pRS315] HA_3-*TOR1*	[8]	1D; 5F
pPL155	[pRS315] HA_3-$TOR1^{A1957V}$	[8]	1D; 5F
pPL156	[pRS315] HA_3-$TOR1^{I1954V}$	[8]	1D; 5F
pPL157	[pRS315] HA_3-$TOR1^{W2176R}$	[8]	1D
pMB1650	[pGEX3X] *RAS2*	This study	5G, H
pMB1651	[pGEX3X] *YPT7*	This study	5G, H
pJU1032	[pGEX6P] *GTR1-*HIS_6	This study	5G, H
pJU1015	[pET28a] *GTR2*	This study	5G
pJU793	[pRS416] *GFP-SCH9*	[6]	6A
pJOD10	CEN, *URA3, GAL1-GAP1-GFP*	[9]	2A
pMB1730	[pRS413] *ADH1-RFP-VAM6*	This study	6B
pMB1379	[YCplac33] *MET15*	This study	2C, D; 6C, D
pMPG1728	[pRS413] *CYC1-GFP-VAM6*	This study	6A
pDL2-Alg5	2μ, *ADH1-HA-NUBG, TRP1*	Dualsystems	4C
pCabWT	CEN, *CYC1-CUB-LEXA, LEU2*	Dualsystems	4C
pNP1711	[pCabWT] *CYC1-TCO89-CUB-LEXA*	This study	4C
pNP1712	[pCabWT] *CYC1-EGO1-CUB-LEXA*	This study	4C
pPR3-N	2μ, *CYC1-NUBG-HA, TRP1*	Dualsystems	
pNP1689	[pPR3-N] *CYC1-NUBG-HA-GTR1*	This study	4C
pNP1690	[pPR3-N] *CYC1-NUBG-HA-*$GTR1^{Q65L}$	This study	4C
pNP1691	[pPR3-N] *CYC1-NUBG-HA-*$GTR1^{S20L}$	This study	4C

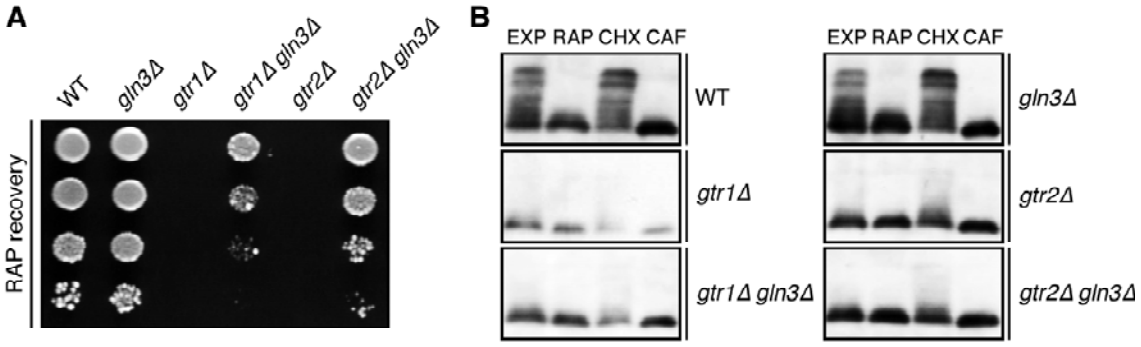

Figure S1. Loss of the TORC1 Downstream Effector Gln3 Suppresses the Defect in Recovery following Rapamycin Treatment, but Not the TORC1 Activity Defect, of EGO Complex Mutants

(A) Indicated strains were assayed (as in Figure 1A) for their ability to recover from a rapamycin treatment.

(B) TORC1 activity was assayed in exponentially growing wild-type, *gln3*Δ, *gtr1*Δ, *gtr2*Δ, *gtr1*Δ *gln3*Δ, and *gtr2*Δ *gln3*Δ strains, which were treated, or not, with the indicated drugs as in Figure 1A.

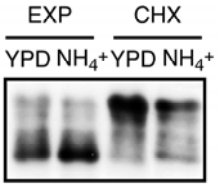

Figure S2. TORC1 Activation following Cycloheximide Treatment Does Not Depend on Uptake of Extracellular Amino Acids

Prototroph wild-type cells were either grown on YPD (+ 0.2% glutamine) or on SD (75 mM NH_4^+; without amino acids) and analysed for TORC1 activity prior to (EXP) and following a 30-min cycloheximide (CHX) treatment. For details see Figure 1A.

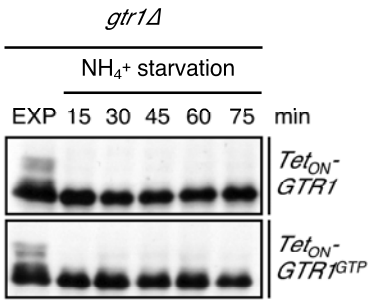

Figure S3. TORC1 Inactivation following NH_4^+ Starvation Is Not Reversed by Overproduction of $Gtr1^{GTP}$

Prototroph *gtr1*Δ cells carrying either a vector that expresses *GTR1* or $GTR1^{GTP}$ from the doxycycline-inducible (Tet_{ON}) promoter were grown exponentially on NH_4^+-rich medium (EXP), transferred to NH_4^+-free medium (at time point 0), and assayed for TORC1 activity (as in Figure 2C) at the times indicated.

References

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