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Supplemental Material to:

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Reciprocal conversion of Gtr1 and Gtr2 nucleotide-binding states by Npr2-Npr3 inactivates TORC1 and induces autophagy

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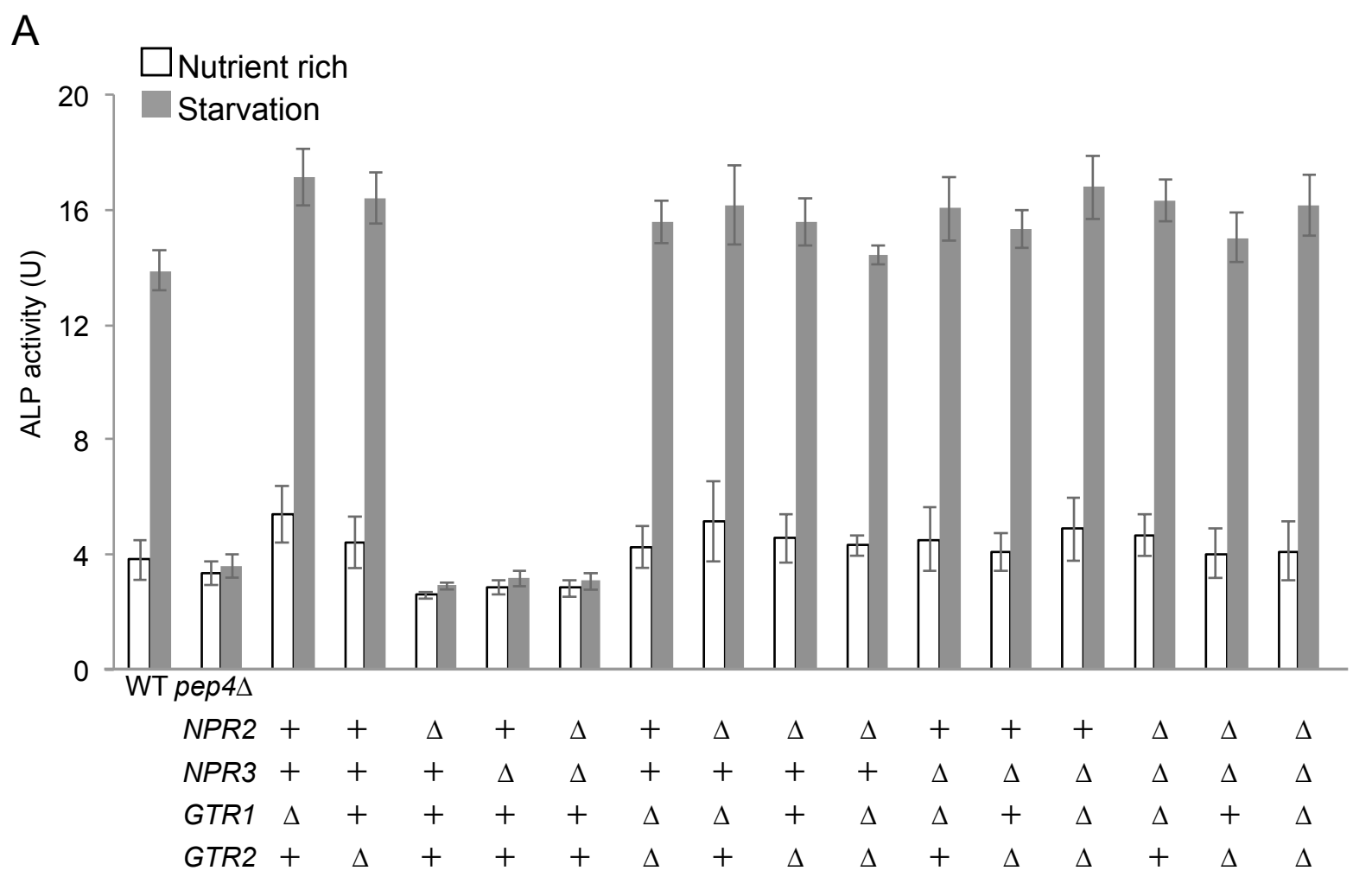
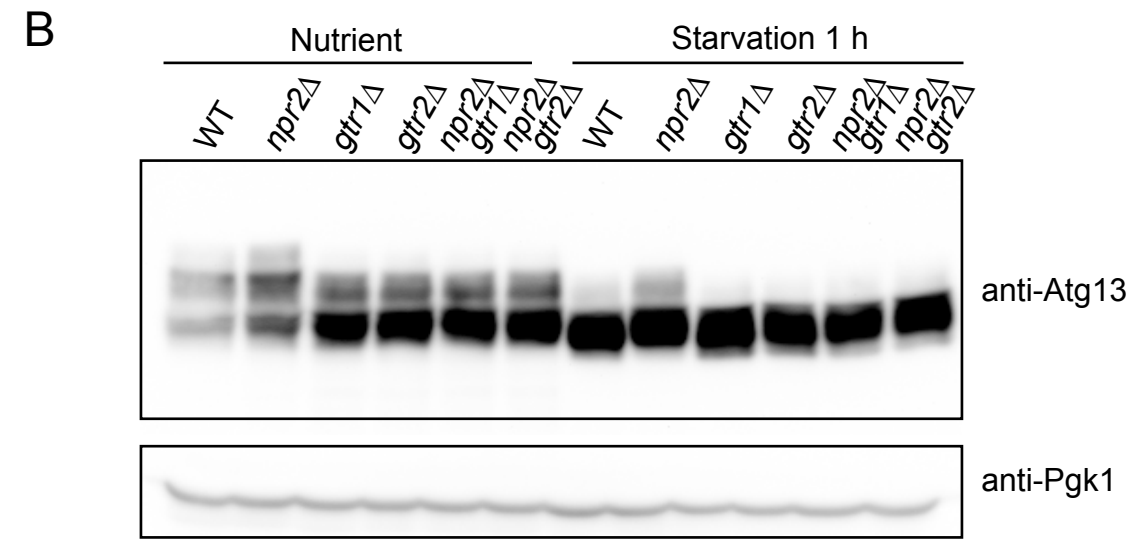
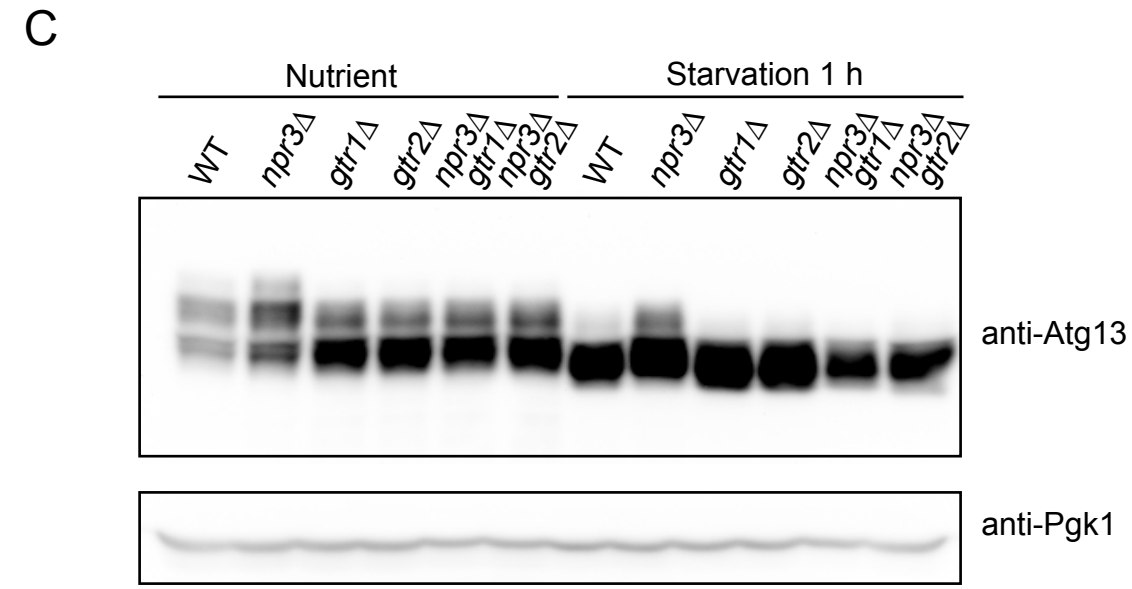
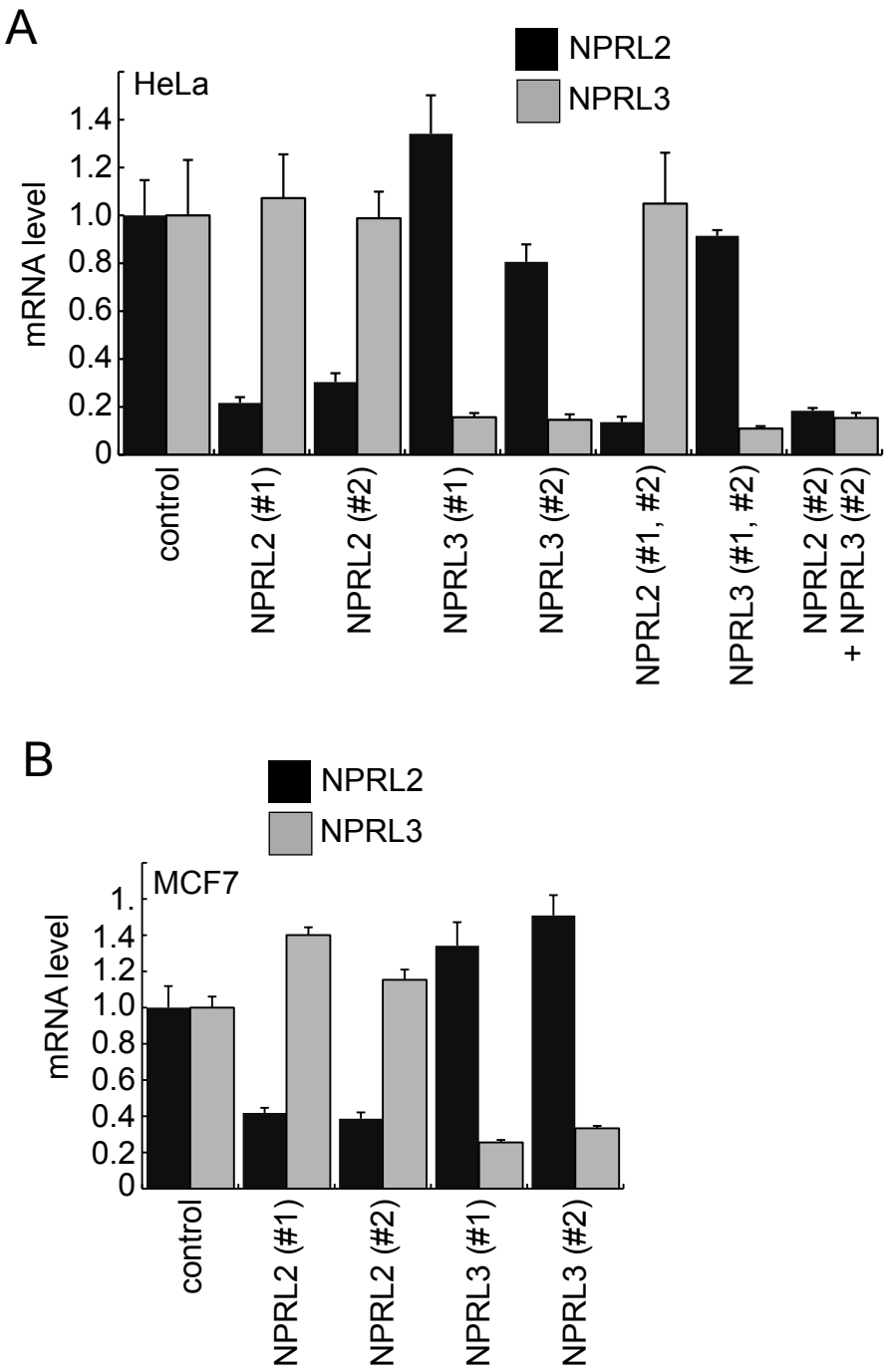


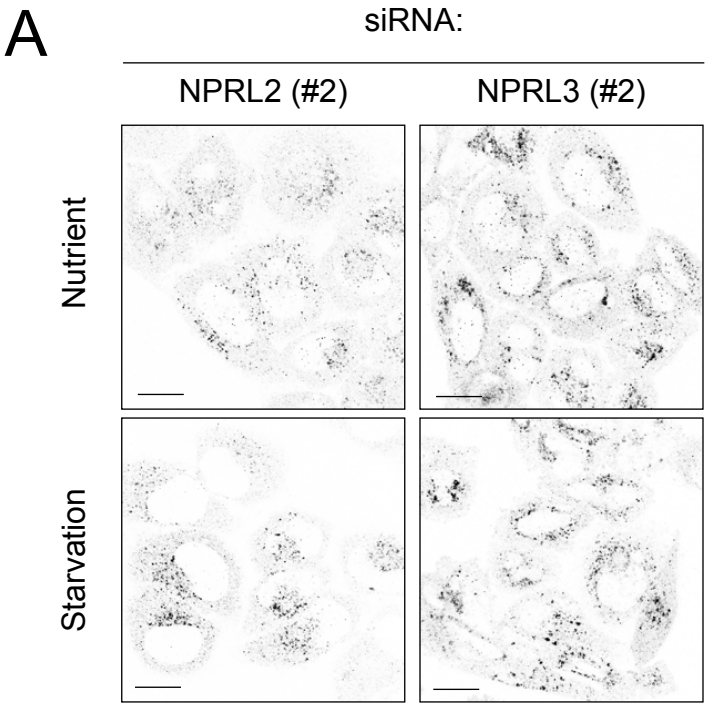


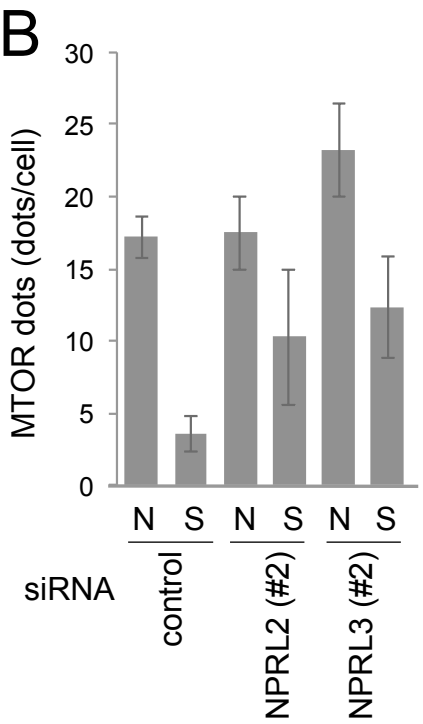


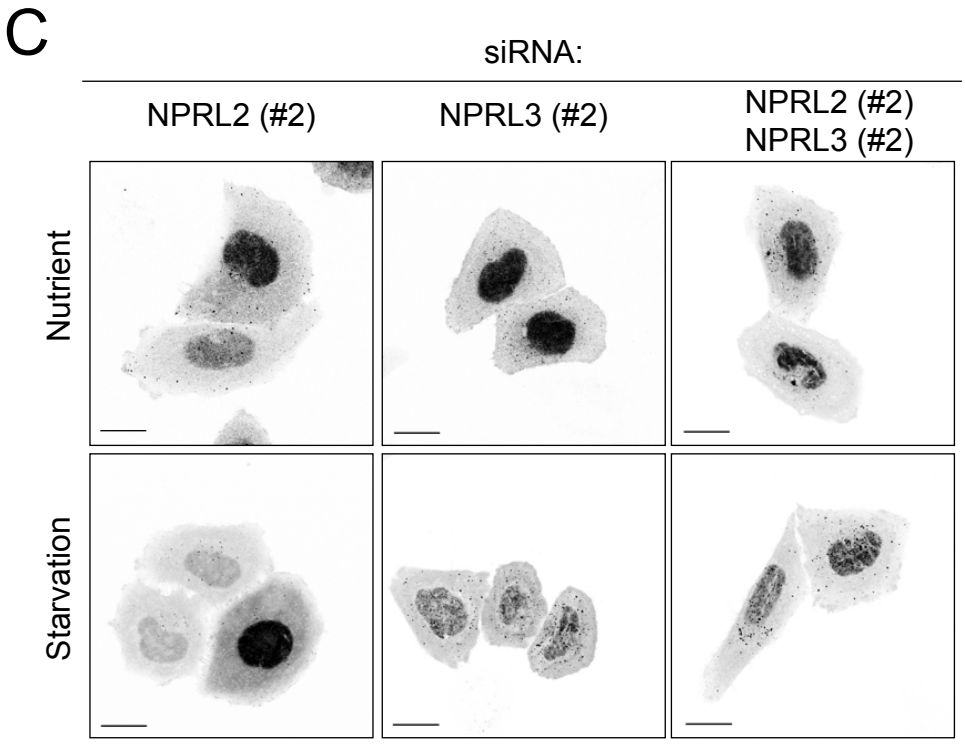
Supplemental Figure 1 Kira et al.

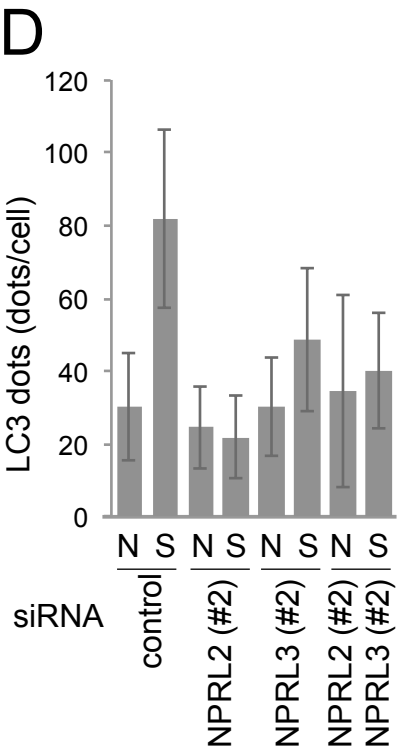


Supplemental Figure 2 Kira et al.

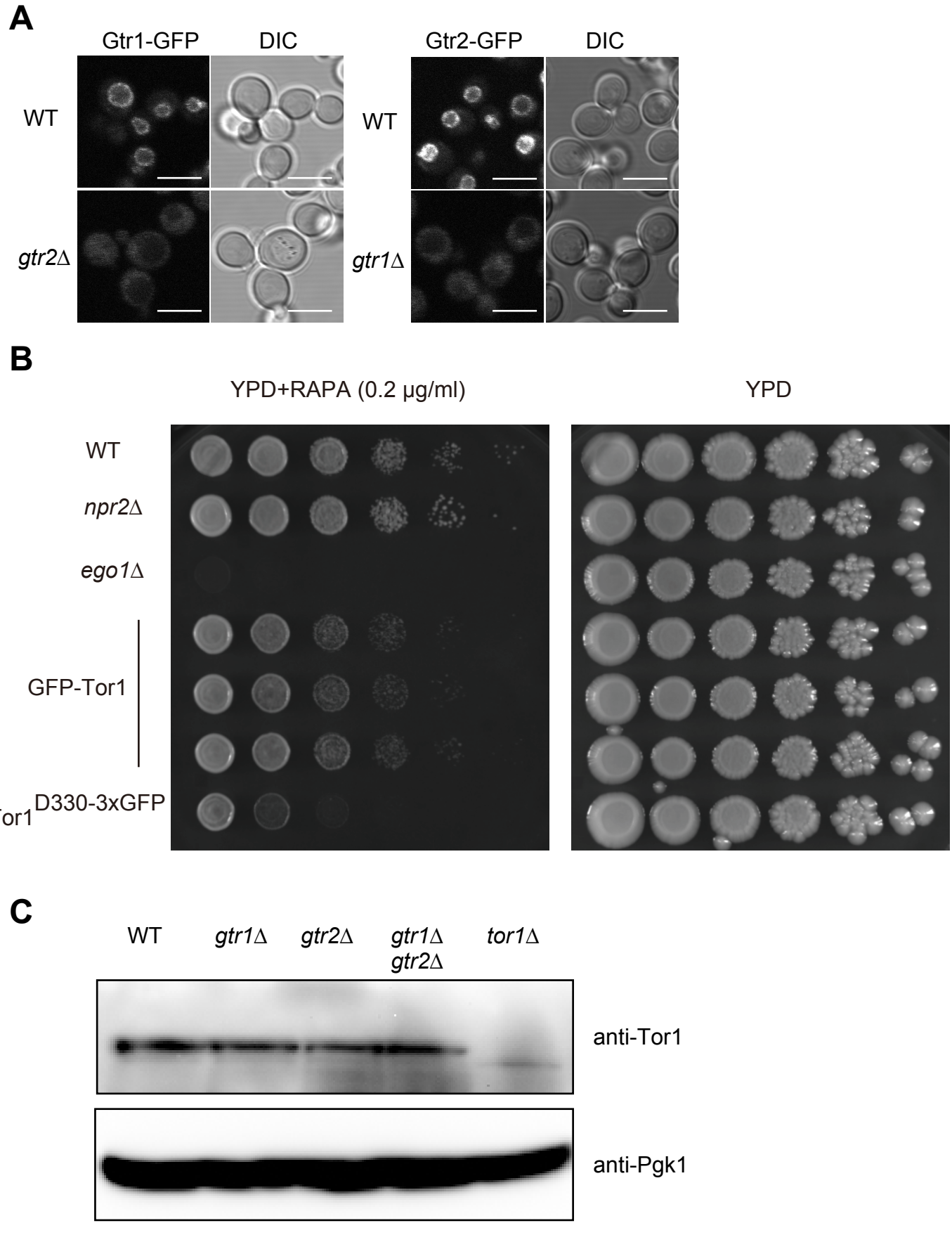






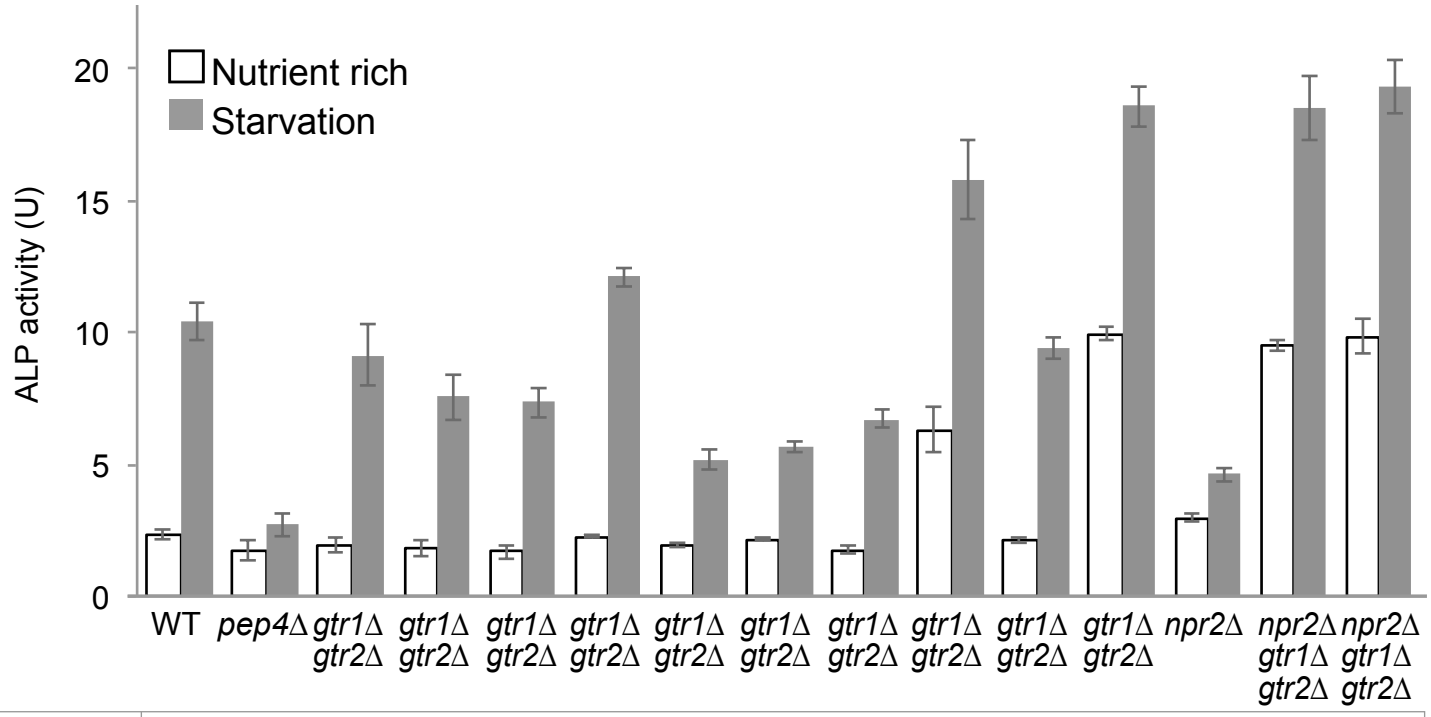


Supplemental Figure 3 Kira et al.



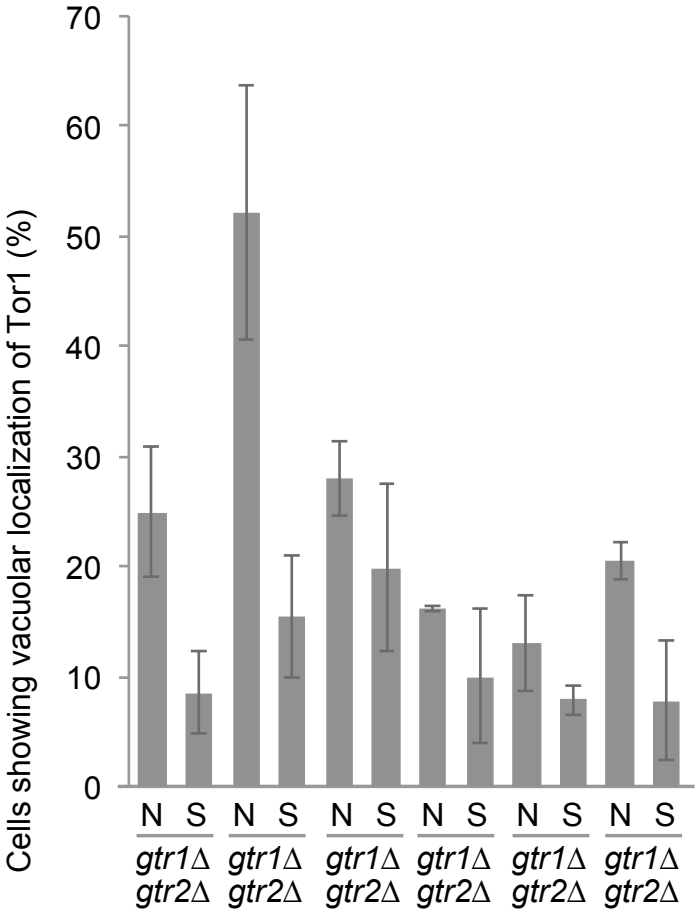
Supplemental Figure 4 Kira et al.

A



Gtr1 in plasmid	-	-	-	WT	WT	WT	GTP	GTP	GTP	GDP	GDP	GDP	-	GDP	GDP
Gtr2 in plasmid	-	-	-	WT	GDP	GTP	WT	GDP	GTP	WT	GDP	GTP	-	GTP	WT
Column:	*1 2*	*3 4*	*5 6*	*7 8*	*9 10*	*11 12*	*13 14*	*15 16*	*17 18*	*19 20*	*21 22*	*23 24*	*25 26*	*27 28*	*29 30*

B



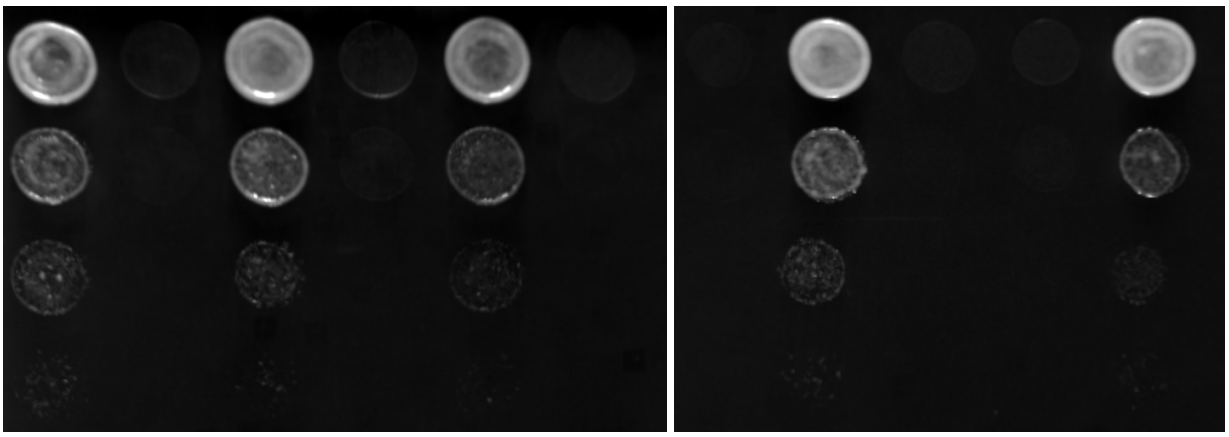
Gtr1 in plasmid	-	WT	WT	GDP	GDP	GDP
Gtr2 in plasmid	-	GDP	GTP	WT	GDP	GTP
Column:	*1 2*	*3 4*	*5 6*	*7 8*	*9 10*	*11 12*

Supplemental Figure 5 Kira et al.

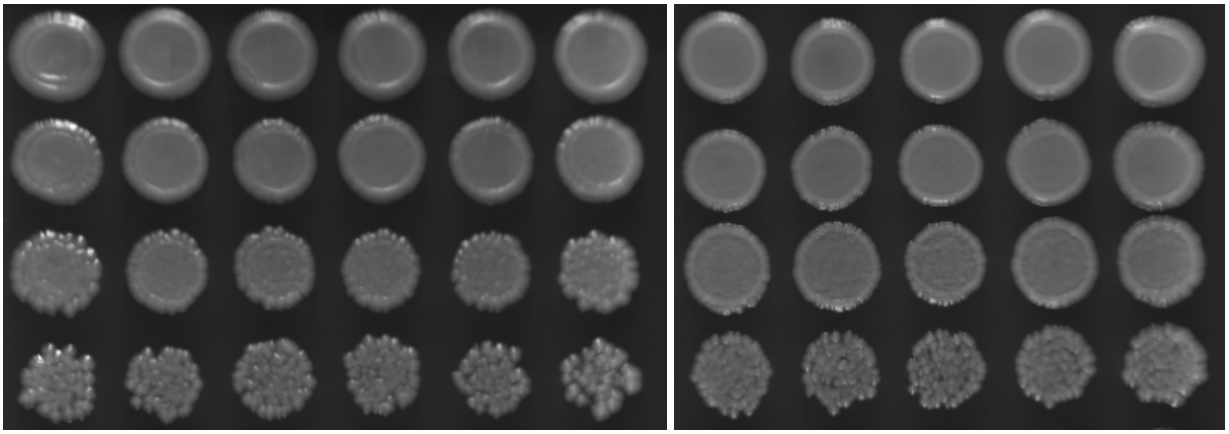
	WT	*gtr1*Δ *gtr2*Δ	*gtr1*Δ *gtr2*Δ	*gtr1*Δ *gtr2*Δ	*gtr1*Δ *gtr2*Δ	*gtr1*Δ *gtr2*Δ	*gtr1*Δ *gtr2*Δ	*gtr1*Δ *gtr2*Δ	*gtr1*Δ *gtr2*Δ	*gtr1*Δ *gtr2*Δ	*gtr1*Δ *gtr2*Δ
Gtr1 in plasmid	-	-	WT	GTP	GTP	GDP	GDP	GTP	GDP	WT	WT
Gtr2 in plasmid	-	-	WT	GTP	GDP	GTP	GDP	WT	WT	GTP	GDP
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*

YPD

+RAPA (0.2 μg/ml)



YPD



Supplemental Figure 6 Kira et al.

Figure S1. Effect of multiple knockouts of the *GTR1*, *GTR2*, *NPR2*, and *NPR3* genes. (**A**) Wild-type (SKY084), *pep4*Δ (SKY100), *gtr1*Δ (SKY244), *gtr2*Δ (SKY246), *npr2*Δ (SKY091), *npr3*Δ (SKY131), *npr2*Δ *npr3*Δ (SKY127), *gtr1*Δ *gtr2*Δ (SKY167), *gtr1*Δ *npr2*Δ (SKY245), *gtr2*Δ *npr2*Δ (SKY247), *gtr1*Δ *gtr2*Δ *npr2*Δ (SKY186), *gtr1*Δ *npr3*Δ (SKY270), *gtr2*Δ *npr3*Δ (SKY298), *gtr1*Δ *gtr2*Δ *npr3*Δ (SKY308), *gtr1*Δ *npr2*Δ *npr3*Δ (SKY271), *gtr2*Δ *npr2*Δ *npr3*Δ (SKY291), and *gtr1*Δ *gtr2*Δ *npr2*Δ *npr3*Δ (SKY309) yeast cells were either grown in YPD or starved for 3 h, and subjected to ALP assays. +: wild-type, Δ: deletion mutant of each gene. Data represent means ± standard deviation from 3 independent experiments. Figure 1D is extracted from part of this figure. (**B and C**) Strains. (**B**) Wild-type (SKY084), *npr2*Δ (SKY091), *gtr1*Δ (SKY244), *gtr2*Δ (SKY246), *gtr1*Δ *npr2*Δ (SKY245), *gtr2*Δ *npr2*Δ (SKY247), (**C**) Wild-type (SKY084), *npr3*Δ (SKY131), *gtr1*Δ (SKY244), *gtr2*Δ (SKY246), *gtr1*Δ *npr3*Δ (SKY270), and *gtr2*Δ *npr3*Δ (SKY298)) harboring pRS426/Atg13 (pSK048) were cultured in SD medium supplemented with 0.5% casamino acids or starved of nitrogen for 1 h, and then subjected to western-blot analysis. Immunoreactive bands were detected with anti-Atg13 or anti-Pgk1 antibodies.

Figure S2. Quantification of mRNA and protein levels in cells treated with siRNA targeting *NPRL2* and *NPRL3*. (**A and B**) Relative *NPRL2* and *NPRL3* mRNA levels in HeLa cells (**A**) and MCF7 cells (**B**) were analyzed by qPCR and normalized to *ACTB* (actin, beta). Data represent means ± standard deviation from 3 independent experiments.

Figure S3. Effect of knocking down NPRL2 and NPRL3 (related to Figure2) (**A, B**) Effect of siRNA knockdown of *NPRL2* or *NPRL3* on MTOR localization, using the #2 clones for both genes (these are distinct from the clones used in Figure 2A and B; see Figure S1). (**C, D**) Effect of *NPRL2* and *NPRL3* siRNA (#2 clones) on LC3 dot formation. These are the remaining results not already shown in Figures 2D and 2E.

Figure S4. Evaluation of Tor1 integrity in various mutants. (**A**) The following strains were grown in SD medium containing 0.5% casamino acids and subjected to fluorescence microscopy: wild-type (SKY112) and *gtr2*Δ (SKY249) endogenously expressing Gtr1-GFP; and wild-type (SKY259) and *gtr1*Δ (SKY260) endogenously expressing Gtr2-GFP. Scale bars: 5 μm. (**B**) Two OD_{600} units of cells were serially 10-fold diluted and inoculated, using a 48-pin replicator, onto YPD plates with or without 0.2 μg/ml rapamycin. Images shown were collected after 3 days of culture at 30 °C. The strains used were wild-type (BY4741); *npr2*Δ (SKY032); *ego1*Δ (NKY002); 3 independent clones of GFP-Tor1, an N-terminally GFP-tagged allele of Tor1 (including SKY222); and $Tor1^{D330\text{-}3\times GFP}$, an internally 3×GFP-tagged allele of Tor1 (SKY173). (**C**) Wild-type (BY4741), *gtr1*Δ (SKY037), *gtr2*Δ (SKY038), *gtr1*Δ *gtr2*Δ (SKY086), and *tor1*Δ (from the knockout library) were subjected to western blot analysis with anti-Tor1 antibody.

Figure S5. Autophagic phenotypes of all combinations of WT, GTP mutants, and GDP mutants of Gtr1 and Gtr2 in *gtr1*Δ *gtr2*Δ cells. (**A**) Wild-type (SKY084), *pep4*Δ (SKY100), and *gtr1*Δ *gtr2*Δ double-mutant cells (SKY167) harboring empty vector (-),

and SKY167 cells harboring vector encoding WT, GTP mutants, and GDP mutants of Gtr1 and Gtr2 as indicated (pSK122-130), were either grown in SD medium supplemented with 0.5% casamino acids or starved of nitrogen for 3 h, and then subjected to ALP assays. Data represent means ± standard deviation from 3 independent experiments. (**B**) Wild-type (SKY084), *pep4*Δ (SKY100) and *npr2*Δ (SKY091) cells harboring empty vector (-), and *gtr1*Δ *gtr2*Δ (SKY167) and *gtr1*Δ *gtr2*Δ *npr2*Δ (SKY186) cells expressing GDP-bound mutant Gtr1 (top row, GDP) along with wild-type (bottom row, WT) or GTP-bound mutant Gtr2 (bottom row, GTP) were either grown in SD medium supplemented with 0.5% casamino acids or starved of nitrogen for 3 h, and then subjected to ALP assays. Data represent means ± standard deviation from 3 independent experiments. (**C**) Quantification of vacuolar localization of Tor1 expressing Gtr1 and Gtr2 mutants (remaining combinations not already shown in Figure 4C).

Figure S6. Cells expressing GTP-bound Gtr2 are rapamycin-sensitive. Wild-type (SKY084) and *gtr1*Δ *gtr2*Δ double-mutant cells (SKY167) harboring empty vector (-), and *gtr1*Δ *gtr2*Δ (SKY167) expressing WT, GTP mutants, or GDP mutants of Gtr1 and Gtr2 in the indicated combinations (pSK122-130), were grown in SD medium supplemented with 0.5% casamino acids. The logarithmically growing cells were serially 10-fold diluted, and then inoculated onto YPD plates with or without 0.2 μg/ml rapamycin using a 48-pin replicator. Images shown were collected after 3 days of culture at 30 °C.