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Supplemental information

Global phosphoproteomics pinpoints uncharted Gcn2-mediated mechanisms of translational control

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Supplemental Information

Supplemental Figures

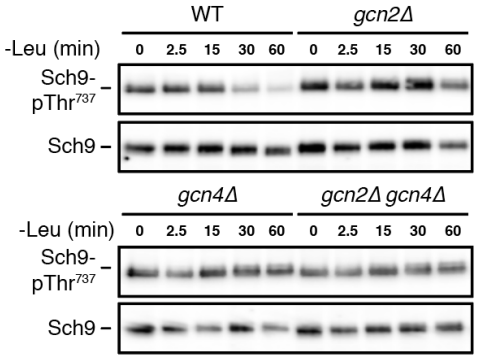

Figure S1. Gcn2 and Gcn4 are Required for Proper TORC1 Inactivation in Leucine-Starved Cells, Related to Figure 1
Wild-type (WT), *gcn2*Δ, *gcn4*Δ, and *gcn2*Δ *gcn4*Δ cells were grown exponentially (time point 0) and starved for leucine (-Leu) for the indicated time periods. Phosphorylation of the TORC1 target residue Thr^{737} in Sch9 was monitored by immunoblotting with anti-Sch9-pThr^{737} and anti-Sch9 antibodies.

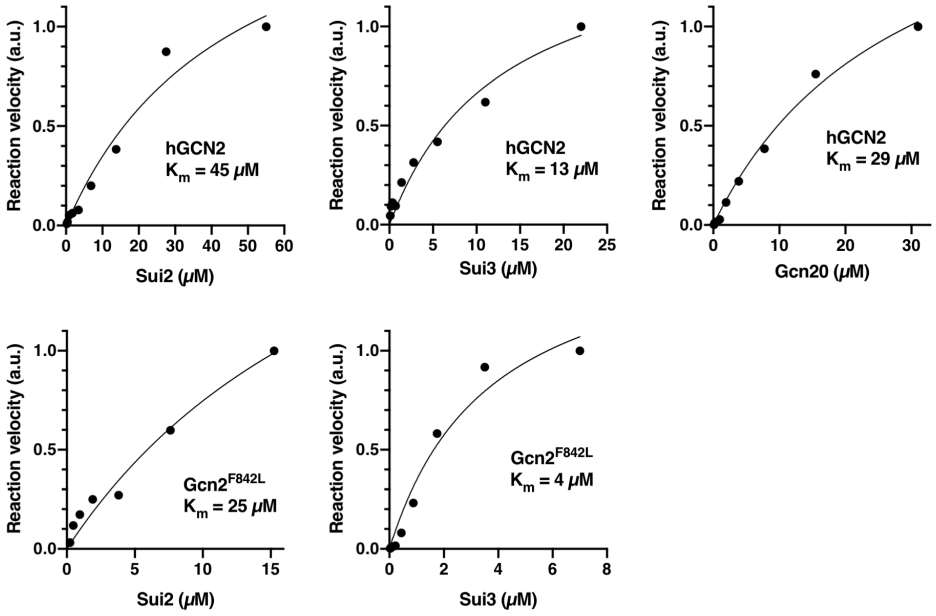

Figure S2. Gcn2 Targets Sui3 and Gcn20 *in Vitro*, Related to Figures 2A and 2B
Kinetic analyses of human GCN2 and yeast Gcn2^{F842L} phosphorylation of yeast Sui2, Sui3, and/or Gcn20. Recombinant substrates were purified from bacteria and examined for phosphorylation by human GCN2 and/or yeast Gcn2^{F842L}. The V_{max} values for the hGCN2-Sui3 and hGCN2-Gcn20 reactions were 67% and 60%, respectively, when compared to the one of the hGCN2-Sui2 reaction (set to 100%). Gcn20 was not subjected to phosphorylation by Gcn2^{F842L} because endogenous Gcn20 co-immunoprecipitating with Gcn2^{F842L} may compete in this assay with the recombinant Gcn20 substrate. Because Gcn2 had very low activity towards Sui2 (control), the kinetic *in vitro* analyses we performed with hyperactive Gcn2^{F842L}. Data are expressed in arbitrary units (a.u.).

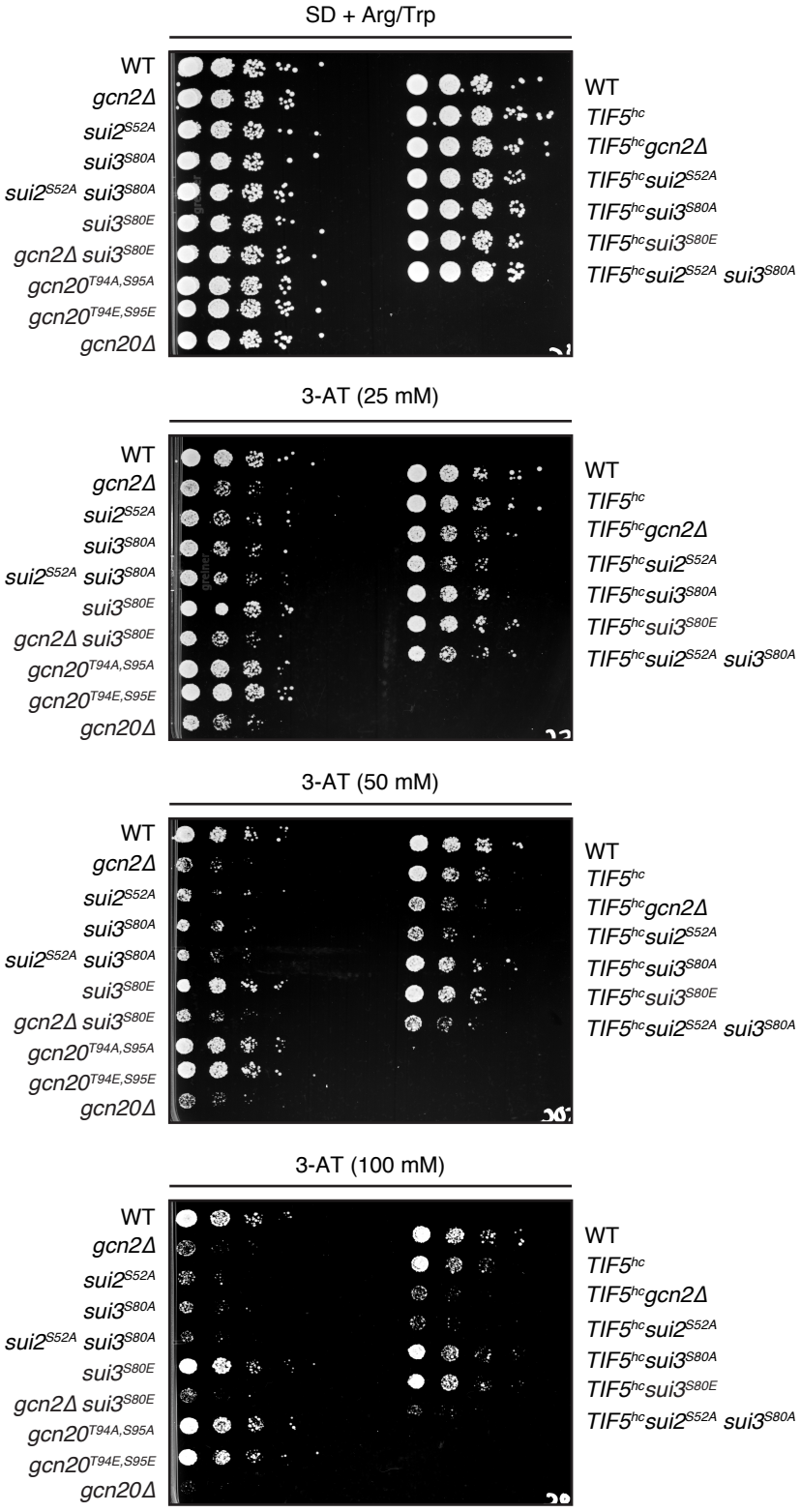

Figure S3. 3-AT Sensitivity of *sui2*, *sui3*, *gcn20*, and *gcn2*Δ Mutants Overexpressing, or Not, Tif5 from a High-copy Plasmid (*TIF5*^{hc}), Related to Figures 2D and 3E

Exponentially growing strains with the indicated genotypes were spotted (10-fold serial dilutions) and grown for 3 days at 30°C on SD + Arg/Trp or on plates containing the indicated concentrations of 3-AT. The pictures of the control plate (SD + Arg/Trp) and the one with 100 mM 3-AT were used to assemble Fig. 2D and 3E.

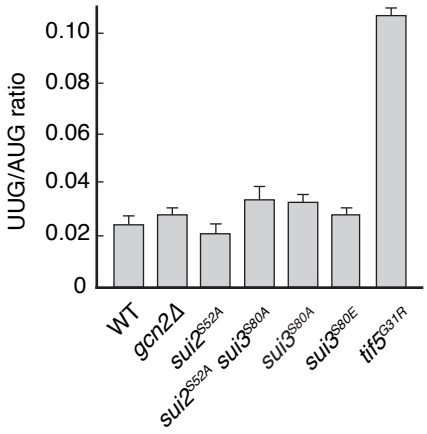

Figure S4. Mutation of Ser^{80} in Sui3 Has Little Impact on Near Cognate Start Codon Recognition on the *HIS4-lacZ* Reporter, Related to Figure 3

Strains with the indicated genotypes expressing either *his4-UUG-LacZ* or *HIS4-AUG-LacZ* reporters from plasmids were assayed for β-galactosidase activities. *tif5*^{G31R} (control) was expressed from a plasmid over the wild-type allele. The respective values were used to calculate the mean UUG/AUG ratio (n = 3; + SD).