Expanded View Figures

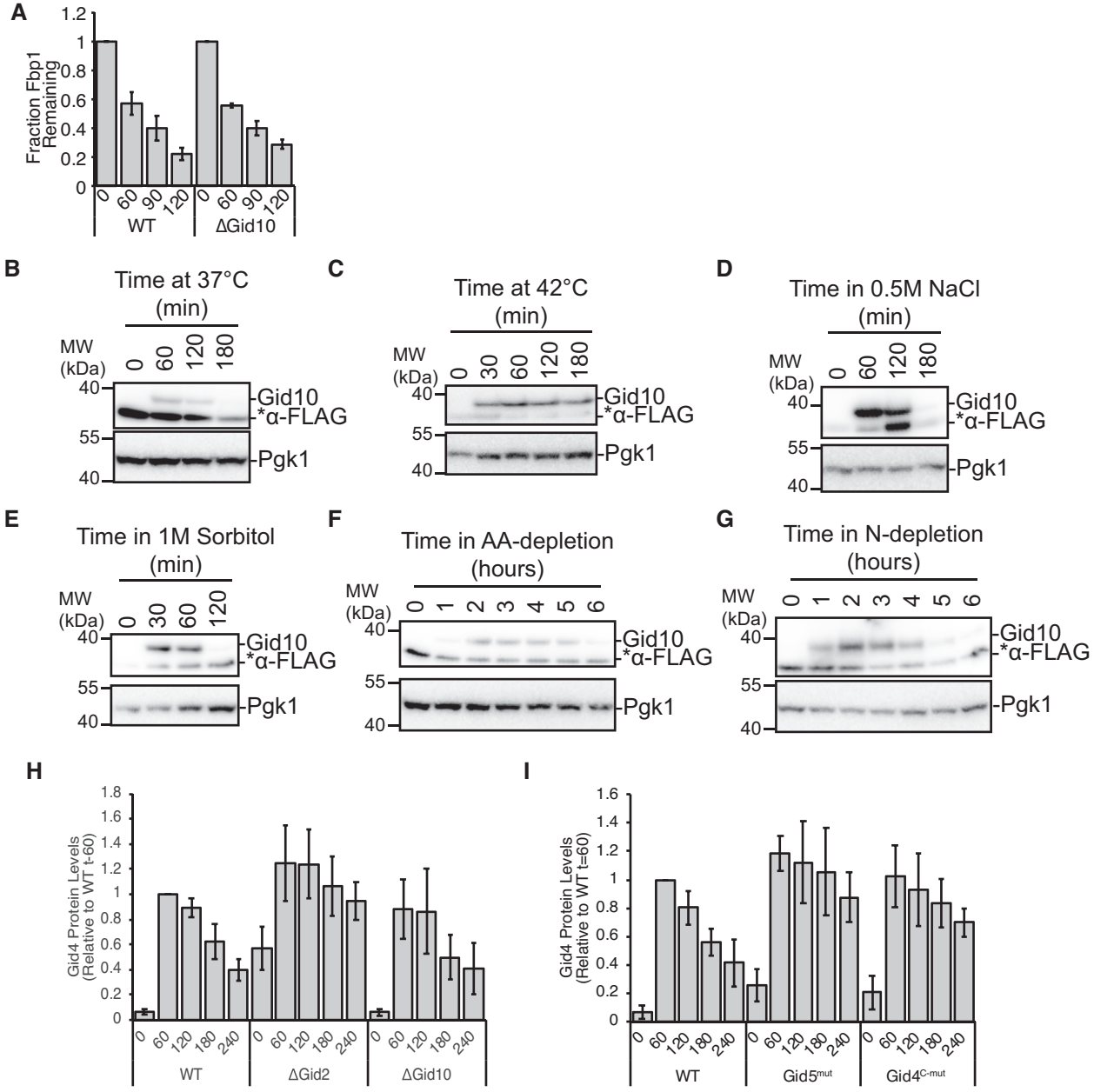


Figure EV1.

Figure EV1. Regulation of Gid10 and Gid4 expression.

A Tetracycline reference-based chase performed during transition from ethanol to glucose media with wildtype and ΔGid10 strains. Bars represent mean, error bars represent standard deviation ($n > 3$ biological replicates).

B Lysates from a yeast strain expressing endogenously tagged 3xFLAG-Gid10 that was grown in YPD at 37°C for the indicated timepoints were run on an SDS-PAGE gel and immunoblotted with αFLAG and αPGK.

C Lysates from a yeast strain expressing endogenously tagged 3xFLAG-Gid10 that was grown in YPD at 42°C for the indicated timepoints were run on an SDS-PAGE gel and immunoblotted with αFLAG and αPGK.

D Lysates from a yeast strain expressing endogenously tagged 3xFLAG-Gid10 that was grown in YPD supplemented with 0.5 M NaCl for the indicated timepoints were run on an SDS-PAGE gel and immunoblotted with αFLAG and αPGK.

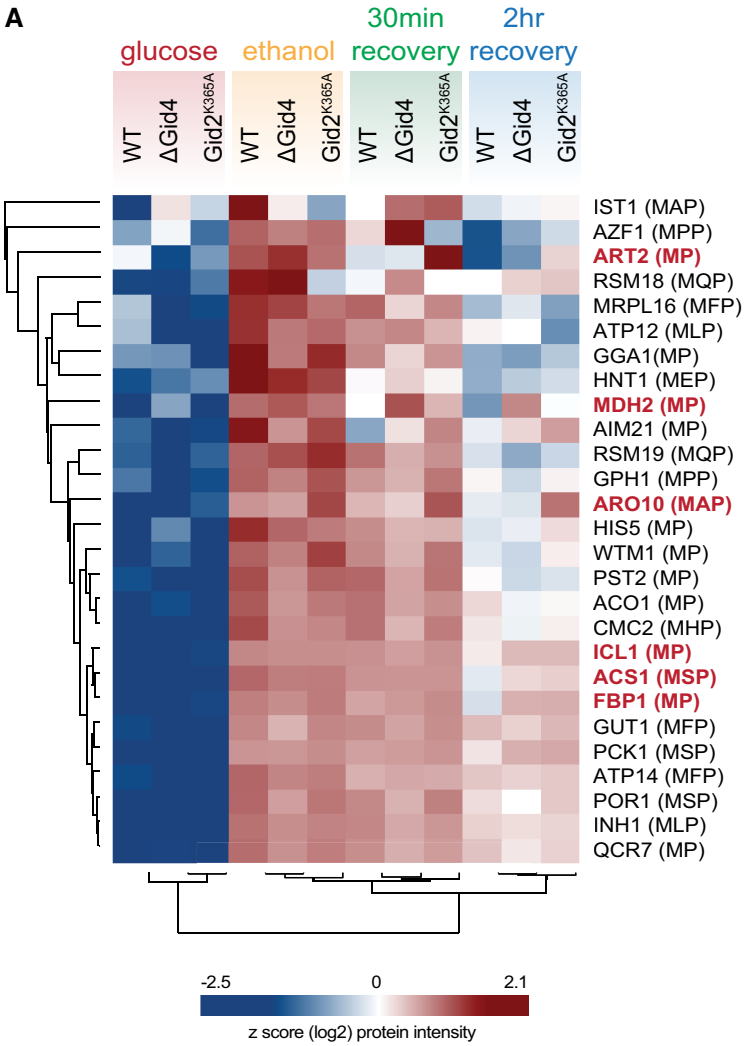
E Lysates from a yeast strain expressing endogenously tagged 3xFLAG-Gid10 that was grown in SD complete supplemented with 1 M Sorbitol for the indicated timepoints were run on an SDS-PAGE gel and immunoblotted with αFLAG and αPGK.

F Lysates from a yeast strain expressing endogenously tagged 3xFLAG-Gid10 that was grown in SD-AA for the indicated timepoints were run on an SDS-PAGE gel and immunoblotted with αFLAG and αPGK.

G Lysates from a yeast strain expressing endogenously tagged 3xFLAG-Gid10 that was grown in SD-N for the indicated timepoints were run on an SDS-PAGE gel and immunoblotted with αFLAG and αPGK.

H Wildtype, ΔGid2 and ΔGid10 yeast strains expressing endogenously tagged 3xFLAG-Gid4 were grown in YPE for 19 h and then shifted to YPD for the indicated timepoints. Lysates were run on an SDS-PAGE gel and immunoblotted with αFLAG and αPGK (as a reference control). Bars represent mean, error bars represent standard deviation ($n > 3$ biological replicates).

I Wildtype, Gid5$^{W606A,\ Y613A,\ Q649A}$ (mut), and Gid4^{F359A,F361A} (c-mut) yeast strains expressing endogenously tagged 3xFLAG-Gid4 were grown in YPE for 19 h and then shifted to YPD for the indicated timepoints. Lysates were run on an SDS-PAGE gel and immunoblotted with αFLAG and αPGK (as a reference control). Bars represent mean, error bars represent standard deviation ($n > 3$ biological replicates).



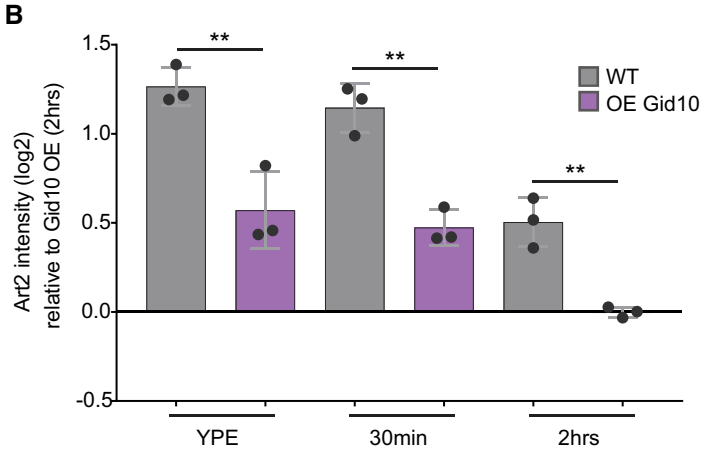


Figure EV2. Protein expression during recovery from ethanol starvation.

A Heat map of z-scored abundances (log2) of the proteins which have the following criteria: (i) significantly upregulated in ethanol compared to glucose, (ii) significantly upregulated in ethanol compared to 2 h recovery, and (iii) contains a proline in position 2 or 3.

B Art2 protein abundance in wildtype and Gid10 overexpressing yeast strains grown in ethanol medium (YPE) for 19 h, and following 30 min and 2 h glucose recovery. Bars represent mean, error bars represent standard deviation, significance was determined by one-way ANOVA (unpaired) followed by Tukey's multiple comparison test,** indicates $P < 0.01$ ($n = 3$ biological replicates).

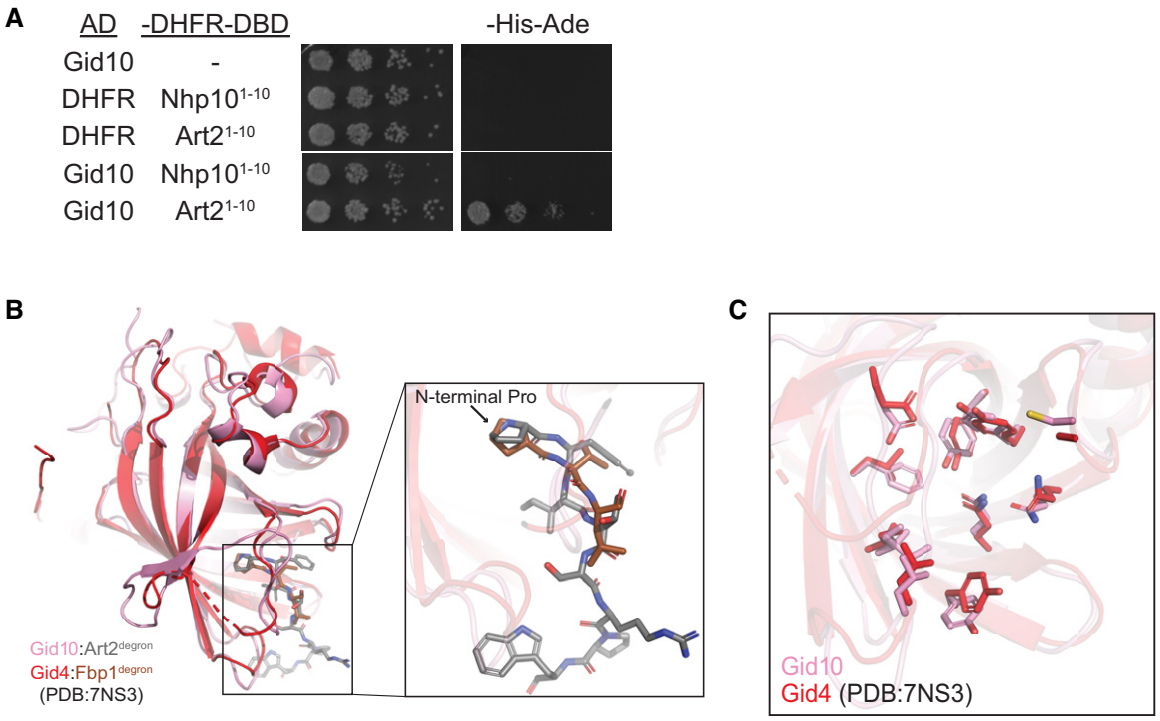


Figure EV3. Gid10 interacts with the Art2 N-terminus.

A Yeast two-hybrid between SR-Gal4 activation domain (AD) and substrate degrons fused to DHFR-DNA binding domain (-DHFR-DBD). Growth on -His-Ade is indicative of an interaction between the two test proteins. Spots represent 1:5 serial dilutions.

B Overlay of Gid10 (pink): Art2$^{2-8}$ (gray) and Gid4 (red): Fbp1$^{2-4}$ (brown) (extracted from PDB:7NS3) showing overall similarity of their substrate-binding domains as well as the trajectory of the bound degrons.

C Overlay of Gid10 (pink) and Gid4 (red, extracted from PDB:7NS3) highlighting the residues inside their substrate-binding pockets (shown as sticks).

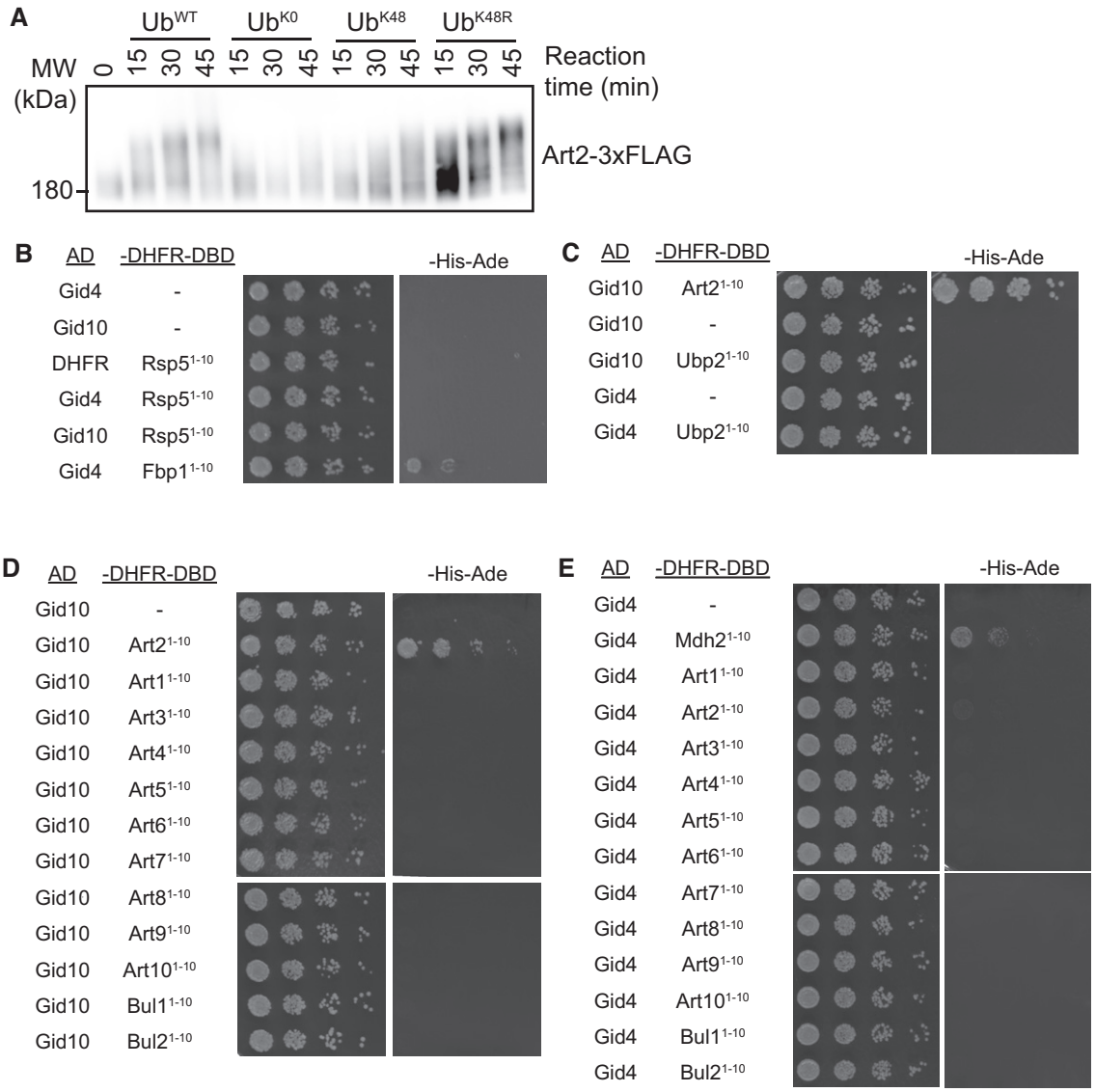


Figure EV4. Gid10 and Gid4 do not interact broadly with arrestin degrons.

A Art2-3xFLAG was immunocaptured from yeast cells grown in YPD and incubated with Gid^{SR10} and either wildtype ubiqtuin, lysine-less ubiquitin (Ub^{KO}), lysine-less ubiqtuin containing only K48 (Ub^{K48}), or ubiquitin containing a K48R point mutation (Ub^{K48R}) for the indicated timepoints. Progress of the reaction was followed by αFLAG immunoblot.

B Yeast two-hybrid between SR-Gal4 activation domain (AD) and Rsp5 degron fused to DHFR-DNA binding domain (-DHFR-DBD). Interaction between Gid4-AD and Fbp1-DBD is shown as a control. Growth on -His-Ade is indicative of an interaction between the two test proteins. Spots represent 1:5 serial dilutions.

C Yeast two-hybrid between SR-Gal4 activation domain (AD) and Ubp2 degron fused to DHFR-DNA binding domain (-DHFR-DBD). Interaction between Gid10-AD and Art2-DBD is shown as a control. Growth on -His-Ade is indicative of an interaction between the two test proteins. Spots represent 1:5 serial dilutions.

D Yeast two-hybrid between Gid10-Gal4 activation domain (AD) and arrestin degrons fused to DHFR-DNA binding domain (-DHFR-DBD). Growth on -His-Ade is indicative of an interaction between the two test proteins. Spots represent 1:5 serial dilutions.

E Yeast two-hybrid between Gid4-Gal4 activation domain (AD) and arrestin degrons fused to DHFR-DNA binding domain (-DHFR-DBD). Interaction between Gid4-AD and Mdh2-DBD is shown as a control. Growth on -His-Ade is indicative of an interaction between the two test proteins. Spots represent 1:5 serial dilutions.

Figure EV5. Regulation of amino acid receptors during heat shock.

A Wildtype, ΔGid2 and ΔGid5 yeast strains expressing endogenously tagged Lyp1-GFP were grown at 42°C for the indicated timepoints. Lysates were run on an SDS-PAGE gel and immunoblotted with αGFP and αPGK.

B Growth assay of wildtype, ΔArt2, ΔArt1, ΔArt2ΔArt1, and ΔArt1 overexpressing Art2 yeast strains on SD-Lys (−) and SD-Lys containing 1.0 μg/ml thialysine (+). Spots represent 1:5 serial dilutions.

C Wildtype, ΔArt2, ΔArt1, and ΔArt2ΔArt1 yeast strains expressing endogenously tagged Lyp1-GFP were grown at 42°C for the indicated timepoints. Lysates were run on an SDS-PAGE gel and immunoblotted with αGFP and αPGK.

D ΔArt1 strains containing *GID2* or *GID5* deletions and expressing endogenously tagged Lyp1-GFP were grown at 42°C for the indicated timepoints. Lysates were run on an SDS-PAGE gel and immunoblotted for αGFP and αPGK.

E Growth assay of wildtype and ΔArt2 strains containing *GID2* or *GID5* deletions on SD-Lys (−) and SD-Lys containing 1.5 μg/ml thialysine (+). Spots represent 1:2.6 serial dilutions.

F ΔArt1 strains containing a *GID5* deletion or a *GID5ART2* double deletion and expressing endogenously tagged Lyp1-GFP were grown at 42°C for the indicated timepoints. Lysates were run on an SDS-PAGE gel and immunoblotted for αGFP and αPGK.

G ΔArt1 strains containing an *ART2* deletion or a *GID5ART2* double deletion and expressing endogenously tagged Lyp1-GFP were grown at 42°C for the indicated timepoints. Lysates were run on an SDS-PAGE gel and immunoblotted for αGFP and αPGK.

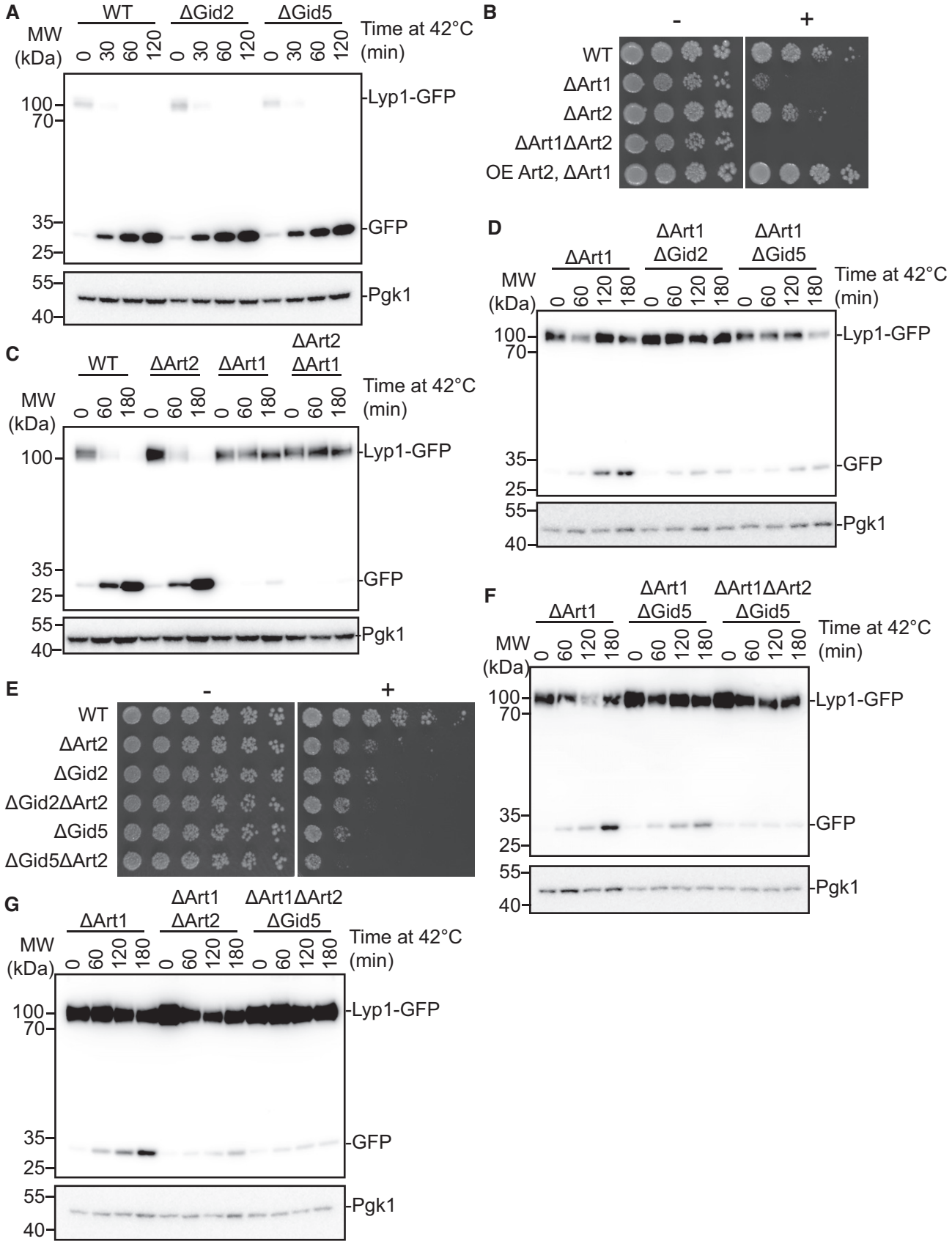


Figure EV5.

