Supplemental Materials

Molecular Biology of the Cell

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Supplementary Figure 1. (A) Wildtype and *ark1*Δ *prk1*-as cells were grown to log phase in YPD medium at 30ºC and 80 µM 1NA-PP1 was added to both strains. Cells were collected at the indicated time intervals and Ypk-pT662 and Ypk1 protein were assayed by western blot. Error bars represent the standard deviation of the mean of three biological replicates. *** indicates a p-value smaller than 0.005; ** indicates a p-value between 0.01 and 0.005 and * indicates a p-value between 0.05 and 0.01 when the indicated strain is compared to the wildtype. (B) Cells of the indicated genotypes were grown to log phase in YPD medium at 30ºC and Ypk-pT662 and Ypk1 protein were assayed by western blot.

Supplementary Figure 2. (A) Wildtype and *ypk1*-as *ypk2*Δ cells were grown to log phase in YPD medium at 25ºC and 50 µM 3MOB-PP1 was added to both strains. Cells were collected after one hour and Ark1 protein was detected by western blot. A fast migrating dephosphorylated form of Ark1 is marked with an asterisk. (B) Wildtype cells were grown to log phase in YPD medium at 30ºC in the presence or absence of 0.5 µg/ml myriocin. At the indicated time points, cells were collected and Ark1 protein was detected by western blot. Wildtype and *ypk1*-as *ypk2*Δ from panel A were loaded as a control.

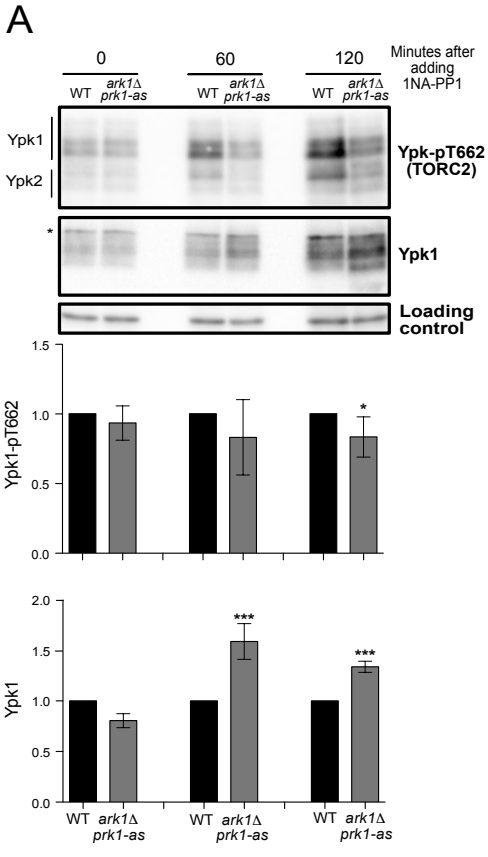
Supplementary Figure 3. (A) Wildtype and *rts1*Δ cells were grown to log phase in YPD medium at 30ºC and phosphorylation of Ark1:3xHA and Prk1:6XHA was analyzed by western blot. (B) Wildtype and *rts1*Δ mutant cells grown in YPD medium to early log phase and were then rapidly washed into YPG/E medium at 30ºC. Cells were collected at the indicated time intervals and phosphorylation of Ark1:3xHA and Prk1:6XHA was analyzed by western blot. An asterisk indicates a background band. (C) Same as (B), but phosphorylation was assayed by PhosTag western blot. On the left, wildtype and *rts1*Δ cells were directly compared 10 minutes after a shift to YPG/E. (D) Wildtype and *elm1*Δ cells were grown in YPD medium to early log phase and were then rapidly washed into YPG/E medium at 30ºC. Cells were collected at the indicated time intervals and phosphorylation of Ark1:3xHA and Prk1:6XHA was analyzed by western blot. An asterisk indicates a background band. (E) Same as D, but phosphorylation was assayed by PhosTag western blot.

Supplementary Figure 4. (A) A series of 10-fold dilutions of the indicated strains was grown in presence or absence of 15 µM auxin. (B) Wild type and *ent1*Δ *ent2-AID* cells were grown at 30ºC to log phase in YPD medium. 1 mM auxin was added and cells were collected and processed for western blotting with an anti-V5 tag antibody to detect the ent2-AID protein at the indicated time points.

Supplementary Figure 5. (A) Wildtype and *ent1*Δ *ent2-AID* cells were grown at 30ºC to log phase in YPD medium in the presence of 10 µM auxin. As a control, the *ent1*Δ *ent2-AID* cells were also grown in the presence of the solvent for auxin (ethanol). Cells were collected and Ypk-pT662 and Ypk1 protein were analyzed by western blot. (B) A series of 10-fold dilutions of the indicated strains were grown at 30ºC on YPD in the presence or absence of 0.25 µg/ml Myriocin. (C and D) A series of 10-fold dilutions of the indicated strains were grown at 30ºC on YPD or YPG/E in the absence or presence 10 µM (C) or 500 µM (D) auxin. (E) A series of 10-fold dilutions of the indicated strains were grown at 30ºC on YPD or YPG/E.

Supplementary Figure 6. Protein extracts were made from strains of the indicated genotypes and were used for Western blotting with an anti-Ypk1 antibody. Background bands are marked with an asterisk.

Figure S1


A
0
60
120
Minutes after adding 1NA-PP1
WT
ark1Δ prk1-as
Ypk1
Ypk2
Ypk-pT662 (TORC2)
Ypk1
Loading control
Ypk1-pT662
Ypk1
1.5
1.0
0.5
0.0
2.0
1.5
1.0
0.5
0.0
*
***
***

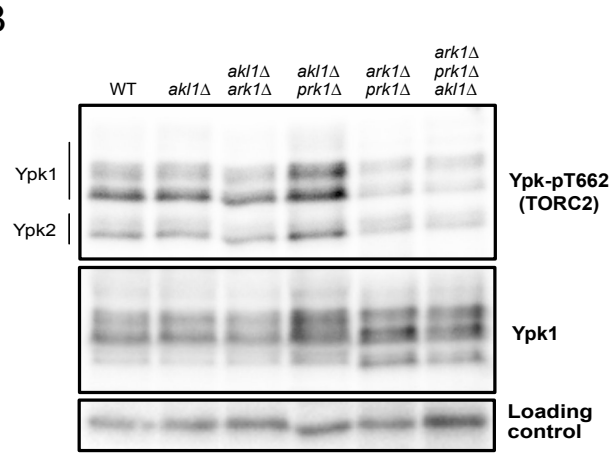

B
WT
akl1Δ
akl1Δ ark1Δ
akl1Δ prk1Δ
ark1Δ prk1Δ
ark1Δ prk1Δ akl1Δ
Ypk1
Ypk2
Ypk-pT662 (TORC2)
Ypk1
Loading control

Figure S2

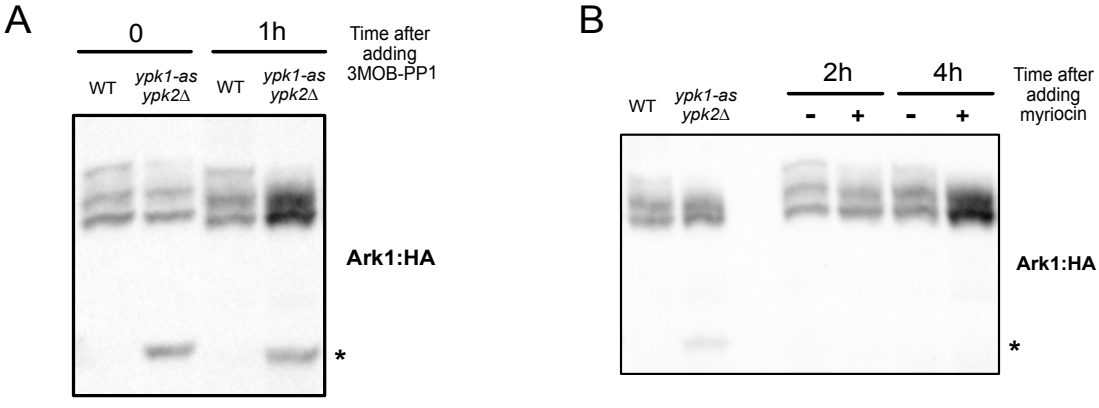

A
0
1h
Time after adding 3MOB-PP1
WT
ypk1-as ypk2Δ
WT
ypk1-as ypk2Δ
Ark1:HA
*
B
2h
4h
Time after adding myriocin
WT
ypk1-as ypk2Δ
-
+
-
+
Ark1:HA
*

Figure S3

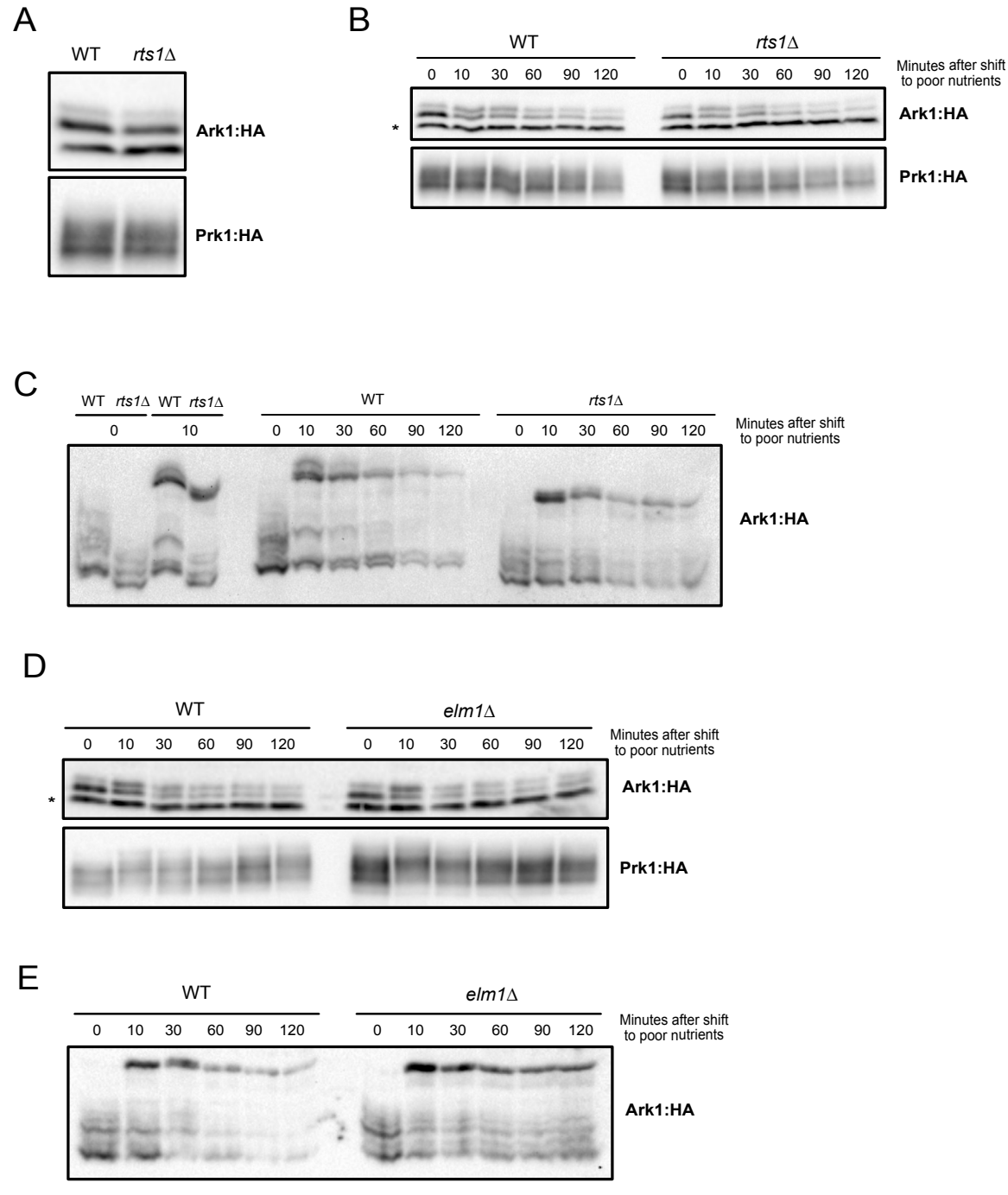
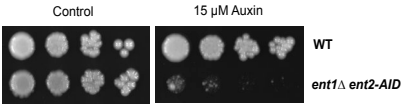

A
WT
rts1Δ
Ark1:HA
Prk1:HA
B
WT
rts1Δ
0 10 30 60 90 120
0 10 30 60 90 120
Minutes after shift to poor nutrients
*
Ark1:HA
Prk1:HA
C
WT rts1Δ WT rts1Δ
0
10
WT
rts1Δ
0 10 30 60 90 120
0 10 30 60 90 120
Minutes after shift to poor nutrients
Ark1:HA
D
WT
elm1Δ
0 10 30 60 90 120
0 10 30 60 90 120
Minutes after shift to poor nutrients
*
Ark1:HA
Prk1:HA
E
WT
elm1Δ
0 10 30 60 90 120
0 10 30 60 90 120
Minutes after shift to poor nutrients
Ark1:HA

Figure S4

A


Control
15 μM Auxin
WT
ent1Δ ent2-AID

B

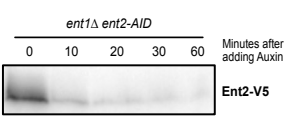

ent1Δ ent2-AID
0
10
20
30
60
Minutes after adding Auxin
Ent2-V5

Figure S5

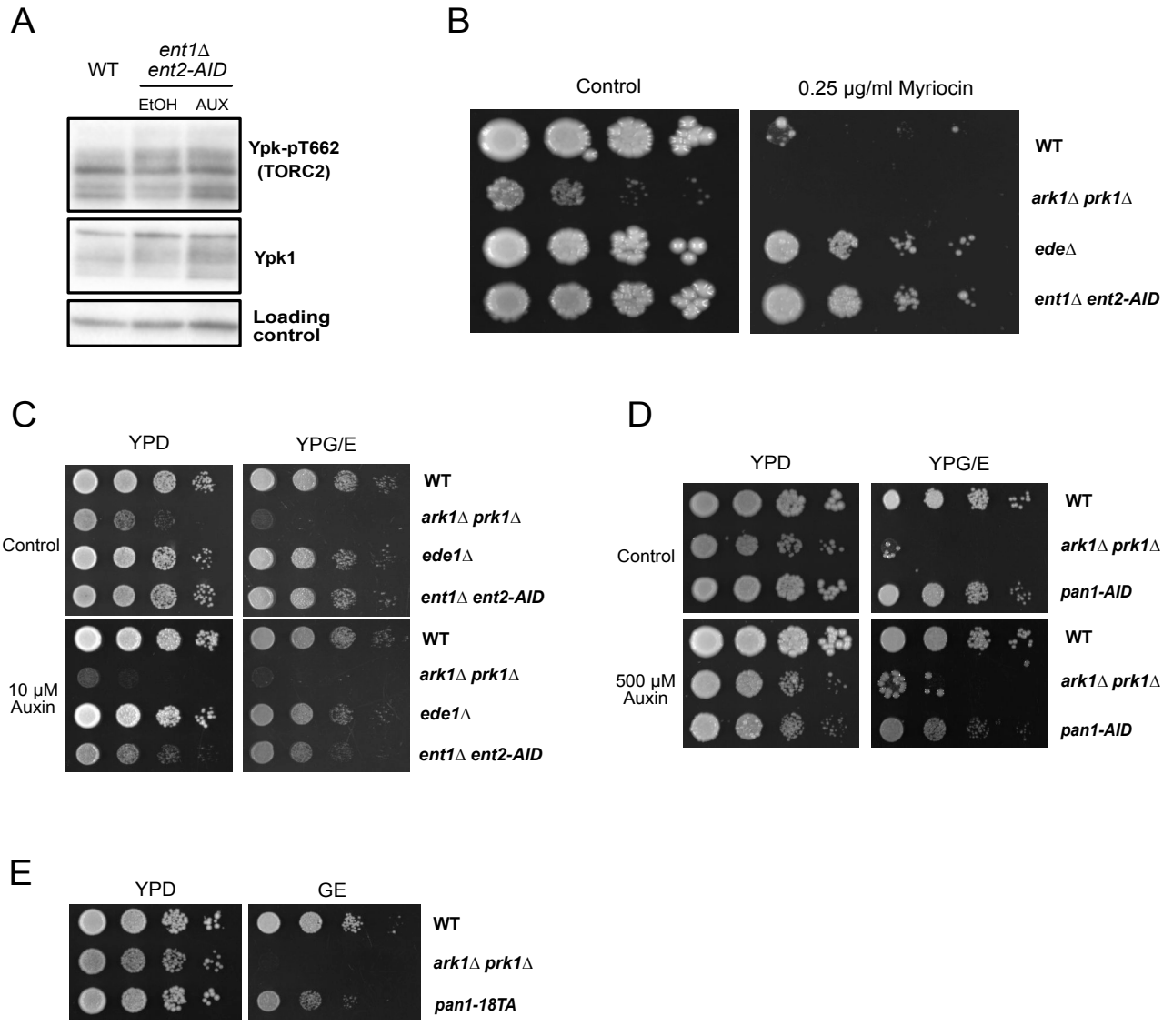

A
WT
ent1Δ ent2-AID
EtOH
AUX
Ypk-pT662 (TORC2)
Ypk1
Loading control
B
Control
0.25 µg/ml Myriocin
WT
ark1Δ prk1Δ
edeΔ
ent1Δ ent2-AID
C
YPD
YPG/E
Control
10 µM Auxin
WT
ark1Δ prk1Δ
ede1Δ
ent1Δ ent2-AID
D
YPD
YPG/E
Control
500 µM Auxin
WT
ark1Δ prk1Δ
pan1-AID
E
YPD
GE
WT
ark1Δ prk1Δ
pan1-18TA

Figure S6