

## Supplemental Tables

**Supplemental Table S1.** List of oligonucleotides used in this study.

| Name     | Sequence  | Purpose                                   |
|----------|---|---|
| oli 2078 | AATACTCGAGTTAGTTGTCTTTGCAGTTTC                                      | Rvs. ALA2 stop<br>( <i>XhoI</i> )         |
| oli 2953 | GTAGAGACTGCAGCTTTAGATGGTCAAAGTCTCAA<br>ACAAGAGTG                    | Fwd ala2E159Q                             |
| oli 2954 | CACTCTTGTGTTTGAGATCAGTTTGACCATCTAAAGCTGC<br>AGTCTCTAC               | Rvs ala2E159Q                             |
| oli 2390 | ATTGCATGCATGTACCCATACGATGTTCCAGATTACGCT<br>GAATTTTCTTCCGAAGAAGTGTGG | Fwd ALA2 start<br>( <i>SphI</i> , HA-tag) |
| oli3422  | GAATTCTTATGAAGCGTTTTGTGTACATTAACG                                   | Fwd on ALA2 start<br>( <i>BamHI</i> )     |
| oli3423  | GAGCTCTTAGTTGTCTTTGCAGTTTCTGG                                       | Rvs ALA2 stop<br>( <i>SacI</i> )          |

**Supplemental Table S2.** List of plasmids used in this study.

| Plasmid    | Description   | Source or Reference <sup>#</sup> |
|------------|---|----------------------------------|
| pRS423-GAL | <i>HIS3</i> -based <i>S. cerevisiae</i> expression vector   | [1]                              |
| pRS426-GAL | <i>URA3</i> -based <i>S. cerevisiae</i> expression vector   | [1]                              |
| pMP3157    | Modified pRS423-GAL containing an RGS10-thrombin cleavage-3gly motif on the <i>EcoRI</i> side of the polylinker | [2]                              |
| pMP3395    | ALA2 in pMP3157   | [3]                              |
| pMP2766    | HA-ala2D381N in pRS423-GAL  | [4]                              |
| pMP3836    | FLAG-ALIS5 in pRS426-GAL  | This study                       |
| pMP3681    | Modified pRS423-GAL containing RGS10-ALA2 and FLAG-ALIS5  | This study                       |
| pMP3464    | HA-ala2E159Q in pRS423-GAL  | This study                       |
| pMP4131    | FLAG-ala2E159Q in pRS423-GAL  | This study                       |

### <sup>#</sup>Reference list to Supplemental Table S2:

- Burgers, P. M. J. (1999) Overexpression of Multisubunit Replication Factors in Yeast. *Methods* **18**, 349–355.
- Costa, S. R. R., Marek, M., Axelsen, K. B., Theorin, L., Pomorski, T. G. and Lopez-Marques, R. L. (2016) Role of post-translational modifications at the -subunit ectodomain in complex association with a promiscuous plant P4-ATPase. *Biochem. J.* **473**, 1605–1615.
- Poulsen, L. R., López-Marqués, R. L., Pedas, P. R., McDowell, S. C., Brown, E., Kunze, R., Harper, J. F., Pomorski, T. G. and Palmgren, M. (2015) A phospholipid uptake system in the model plant *Arabidopsis thaliana*. *Nat. Commun.* **6**, 7649.
- López-Marqués, R. L., Poulsen, L. R., Hanisch, S., Meffert, K., Buch-pedersen, M. J., Jakobsen, M. K., Pomorski, T. G. and Palmgren, M. G. (2010) Intracellular targeting signals and lipid specificity determinants of the ALA/ALIS P4-ATPase complex reside in the catalytic ALA alpha-subunit. *Mol. Biol. Cell* **21**, 791–801.

**Supplemental Table S3.** Proteins which co-purify with the ALA2-ALIS5 complex identified by mass spectrometry.

| Protein      | Digitonin <sup>1</sup> |                       | DDM <sup>2</sup>    |                       |
|--------------|------------------------|-----------------------|---------------------|-----------------------|
|              | Peptides identified    | Sequence coverage [%] | Peptides identified | Sequence coverage [%] |
| ALA2         | 93                     | 73.2                  | 62                  | 55.6                  |
| ALIS5        | 34                     | 66.9                  | 17                  | 40.9                  |
| Ssa1p        | 60                     | 83.3                  | 42                  | 65.7                  |
| Tom40p       | 30                     | 95.3                  | 12                  | 53.2                  |
| Fet4p        | 29                     | 46.7                  | 22                  | 33.9                  |
| Tom22p       | 13                     | 72.4                  | 1                   | 11.2                  |
| Kar2p        | 56                     | 60.4                  | 43                  | 48.2                  |
| Rps31p/Ubi4p | 11                     | 60.5                  | 6                   | 35.5                  |
| Rpl2ap       | 30                     | 79.5                  | 16                  | 64.6                  |
| Rpp0p        | 18                     | 52.6                  | 9                   | 30.4                  |
| Hsc82p       | 52                     | 70.4                  | 29                  | 38.3                  |
| Rpl4ap       | 27                     | 75.4                  | 18                  | 50.3                  |
| Ssb1p        | 39                     | 52.5                  | 28                  | 50.2                  |
| Rpl16bp      | 38                     | 72.8                  | 12                  | 45.7                  |
| Por1p        | 24                     | 68.2                  | 14                  | 62.5                  |
| Rpl8bp       | 22                     | 94.0                  | 21                  | 64.1                  |
| Pma1p        | 28                     | 73.4                  | 28                  | 32.8                  |
| Yos9p        | 23                     | 46.7                  | 9                   | 18.5                  |
| Pdr12p       | 61                     | 50.0                  | 31                  | 24.2                  |

<sup>1</sup>Yeast membranes co-expressing FLAG-ALIS5 and His<sub>10</sub>-tagged ALA2 were solubilized with 1% (w/v) digitonin and subjected to anti-FLAG affinity chromatography followed by ESI mass spectrometry analysis.

<sup>2</sup>Yeast membranes co-expressing FLAG-ALIS5 and His<sub>10</sub>-tagged ALA2 were solubilized with 0.8% (w/v) DDM and subjected to anti-FLAG affinity chromatography followed by ESI mass spectrometry analysis.