**Appendix**

# Supplementary file 1a: Strains used in this study

The *S. cerevisiae* strains used in this study were all obtained by genetic manipulations from the BY4741 background described in Table S1. This strain corresponds to the wild-type of this study. For the sake of clarity, the BY4741 background was therefore not indicated in the genotype of each mutant strain.

|  |  |  |  |
| --- | --- | --- | --- |
| Strain | Genotype | Plasmid | Source |
| Wild-type (BY4741) | *MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* |  | Euroscarf |
| *pho4-yEGFP* | *pho4-link-yEGFP-CaURA3 hta2-mCherry*a |  | This study |
| *pho81-yEGFP* | *pho81-link-yEGFP-CaURA3* |  | This study |
| *pho81Δspx* | *pho81Δ200*a |  | This study |
| *pho81Δspx/pho4-yEGFP* | *pho81Δ200*a *pho4-link-yEGFP-CaURA3 hta2-mCherry*a |  | This study |
| *pho81Δspx-yEGFP* | *pho81Δ200*a *pho81-link-yEGFP-CaURA3* |  | This study |
| *pho81Δspx/prPho5-yEGFP* | *pho81Δ200*a  | pVC115 | This study |
| *vip1Δ* | *vip1Δ0*a |  | This study |
| *vip1Δ/pho4-yEGFP* | *vip1Δ0*a *pho4-link-yEGFP-CaURA3 hta2-mCherry*a |  | This study |
| *vip1Δ/pho81-yEGFP* | *vip1Δ0*a *pho81-link-yEGFP-CaURA3* |  | This study |
| *vip1Δ/prPho5-yEGFP* | *vip1Δ0*a | pVC115 | This study |
| *kcs1Δ* | *kcs1Δ0::kanMX4* |  | 1 |
| *kcs1Δ/pho4-yEGFP* | *kcs1Δ0::kanMX4 pho4-link-yEGFP-CaURA3 hta2-mCherry*a |  | This study |
| *kcs1Δ/pho81-yEGFP* | *kcs1Δ0::kanMX4 pho81-link-yEGFP-CaURA3* |  | This study |
| *kcs1Δ/prPho5-yEGFP* | *kcs1Δ0::kanMX4* | pVC115 | This study |
| *vip1Δ/kcs1Δ* | *vip1Δ0*a *kcs1Δ0::kanMX4* |  | This study |
| *vip1Δ/kcs1Δ/pho4-yEGFP* | *vip1Δ0*a *kcs1Δ0::kanMX4 pho4-link-yEGFP-CaURA3* *hta2-mCherry*a |  | This study |
| *vip1Δ/kcs1Δ/pho81-yEGFP* | *vip1Δ0*a *kcs1Δ0::kanMX4 pho81-link-yEGFP-CaURA3* |  | This study |
| *vip1Δ/kcs1Δ/prPho5-yEGFP* | *vip1Δ0*a *kcs1Δ0::kanMX4* | pVC115 | This study |
| *prGPD-vip1/kcs1Δ* | *kcs1Δ0::kanMX4* | pVC97 | This study |
| *prGPD-vip1/kcs1Δ/pho4-yEGFP* | *kcs1Δ0::kanMX4 pho4-link-yEGFP-CaURA3 hta2-mCherry*a | pVC97 | This study |
| *prGPD-vip1/kcs1Δ/pho81-yEGFP* | *kcs1Δ0::kanMX4 pho81-link-yEGFP-CaURA3* | pVC97 | This study |
| *prGPD-vip1/kcs1Δ/prPho5-yEGFP* | *kcs1Δ0::kanMX4* | pVC115, pVC114 | This study |
| *pho81K154A* | *pho81K154A a* |  | This study |
| *pho81K154A/pho4-yEGFP* | *pho81K154A a pho4-link-yEGFP-CaURA3 hta2-mCherry*a |  | This study |
| *pho81K154A/pho81-yEGFP* | *pho81K154A-link-yEGFP-CaURA3a* |  | This study |
| *pho81K154A/prPho5-yEGFP* | *pho81K154A a* | pVC115 | This study |
| *pho81K154A/vip1Δ/pho4-yEGFP* | *pho81K154A a vip1Δ0*a *pho4-link-yEGFP-CaURA3 hta2-mCherry*a |  | This study |
| *pho80R121K/pho81-yEGFP* | *pho80R121K a pho81-link-yEGFP-CaURA3* |  | This study |
| *pho80R121K/kcs1Δ/pho81-yEGFP* | *pho80R121K a kcs1Δ0::kanMX4 pho81-link-yEGFP-CaURA3* |  | This study |
| *pho80R121K/vip1Δ/pho81-yEGFP* | *pho80R121K a vip1Δ0*a *pho81-link-yEGFP-CaURA3* |  | This study |
| *pho80R121K/pho81K154A-yEGFP* | *pho80R121K a pho81K154A-link-yEGFP-CaURA3a* |  | This study |
| *pho80R121K/pho81Δspx-yEGFP* | *pho80R121K a pho81Δ200-link-yEGFP-CaURA3a* |  | This study |
| *pho80E154V/pho81-yEGFP* | *pho80E154V a pho81-link-yEGFP-CaURA3* |  | This study |
| *pho80E154V/kcs1Δ/pho81-yEGFP* | *pho80E154V a kcs1Δ0::kanMX4 pho81-link-yEGFP-CaURA3* |  | This study |
| *pho80E154V/vip1Δ/pho81-yEGFP* | *pho80E154V a vip1Δ0*a *pho81-link-yEGFP-CaURA3-hta2mCh* |  | This study |
| *pho80E154V/pho81K154A-yEGFP* | *pho80E154V a pho81K154A-link-yEGFP-CaURA3a* |  | This study |
| *pho80E154V/pho81Δspx-yEGFP* | *pho80E154V a pho81Δ200-link-yEGFP-CaURA3a* |  | This study |
| BY4742 | *MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0* |  | Euroscarf |
| *BY4742 pho80Δ* | *pho80Δ0::natNT2 (A3464)* |  | This study |

a obtained by the CRISPR-Cas9 method

# Supplementary file 1b: Plasmids

|  |  |  |
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| Plasmid | Description | Source |
| pVC115 | pCEV-G1-LEU- *prPHO5-yegfp prTEF-mcherry leu2* | This study |
| pVC97 | pRS415 *prGPD-vip1 leu2* | This study |
| pVC114 | pRS413 *prGPD-vip1 his3* | This study |
| pED109 | *pGTL-mcherry* | Serge Pelet |
| pSP473 | pRS423 *prGPD-cas9 his3* | Serge Pelet |
| pSP475 | pRS425 *prGPD-cas9 leu2* | Serge Pelet |
| pSP476 | pRS426 *prGPD-cas9 ura3* | Serge Pelet |
| pSP478 | pRS425 *prGPD-cas9\_sgRNA\_hta2 leu2* | Serge Pelet |
| pVC24 | pRS425 *prGPD-cas9\_sgRNA\_pho81 leu2* | This study |
| pVC43 | pRS425 *prGPD-cas9\_sgRNA\_kcs1 leu2* | This study |
| pVC44 | pRS423 *prGPD-cas9\_sgRNA\_vip1 his3* | This study |
| pVC110 | pRS425 *prGPD-cas9\_sgRNA\_vip1 leu2* | This study |
| pVC72 | pRS423 *prGPD-cas9\_sgRNA\_pho80\_1 his3* | This study |
| pVC73 | pRS423 *prGPD-cas9\_sgRNA\_pho80\_2 his3* | This study |
| pVC108 | pRS425 *prGPD-cas9\_sgRNA\_pho80\_1 leu2* | This study |
| pVC109 | pRS425 *prGPD-cas9\_sgRNA\_pho80\_2 leu2* | This study |
| pVC119 | pRS315 *hta2-mCherry* | This study |
| pGK1 | pCEV-G1-LEU- *prPHO84-yegfp prTEF-mcherry leu2* | This study |
| pMJK1 | pRS416 *prADH1\_Pho81-SPX1-394-yEGFP ura3* | This study |

# Supplementary file 1c: Oligonucleotides

|  |  |
| --- | --- |
| Oligonucleotide | Sequence |
| 645\_fw | ACTTGTTGCCAAAGAAGTCTGCCAAGACTGCCAAAGCTTCTCAAGAACTGGCGGCCGCTCTAGAACTA |
| 1435\_rev | TACTATACACTGTCTTTAATAAAACGACGCTATTTATAAATTATTTAGAAGTGGCGCGCC |
| CRISPR\_sg\_F | GGTCAAACGCTGTAGAAGTG |
| Pho81\_K150A\_F2 | TATGTTGAGTTAAATAAAACGGGATTTTCAGCAGCTCTGAAGAAATGGGACAAGAGATCTCAATCTCACGATAAAGATTTTTATCTTGCTACTGTTGTTTCCATTCAACCAATT |
| Pho81\_K150A\_R2 | AATTGGTTGAATGGAAACAACAGTAGCAAGATAAAAATCTTTATCGTGAGATTGAGATCTCTTGTCCCATTTCTTCAGAGCTGCTGAAAATCCCGTTTTATTTAACTCAACATA |
| Pho81\_K154A\_F2 | AATAAAACGGGATTTTCAAAAGCTCTGAAGGCATGGGACAAGAGATCTCAATCTCACGATAAAGATTTTTATCTTGCTACTGTTGTTTCCATTCAACCAATT |
| Pho81\_K154A\_R2 | AATTGGTTGAATGGAAACAACAGTAGCAAGATAAAAATCTTTATCGTGAGATTGAGATCTCTTGTCCCATGCCTTCAGAGCTTTTGAAAATCCCGTTTTATT |
| Pho81\_sgRNA\_F | TTCAAAAGCTCTGAAGAAATGTTTTAGAG |
| Pho81\_sgRNA\_R | CTAGCTCTAAAACATTTCTTCAGAGCTTTTGAAACGT |
| Vip1\_sgRNA\_F | TCGTAGCATATTAATATATTGCAGAAGGTCTGATCACACCCAATTTTTAATTTAGTAACC |
| Vip1\_sgRNA\_R | GGTTACTAAATTAAAAATTGGGTGTGATCAGACCTTCTGCAATATATTAATATGCTACGA |
| Kcs1\_sgRNA\_F | TTGTATATATAAAACTAAAGCTAAAAGACTAAAGAAAGGATAGAACTAATGAATATTCTT |
| Kcs1\_sgRNA\_R | AAGAATATTCATTAGTTCTATCCTTTCTTTAGTCTTTTAGCTTTAGTTTTATATATACAA |
| Pho80\_sgRNA\_F1 | TTAATTCGTTGACTGCCCATGTTTTAGAG |
| Pho80\_sgRNA\_R1 | CTAGCTCTAAAACATGGGCAGTCAACGAATTAAACGT |
| Pho80\_sgRNA\_F2 | ATGTCACGAATTGAATATACGTTTTAGAG |
| Pho80\_sgRNA\_R2 | CTAGCTCTAAAACGTATATTCAATTCGTGACATACGT |
| Pho80\_R121K\_F | ATTTTACGCTTAATTCGTTGACTGCCCATAAATTTTTATTAACAGCCACCACAGTCGCAA |
| Pho80\_R121K\_R | TTGCGACTGTGGTGGCTGTTAATAAAAATTTATGGGCAGTCAACGAATTAAGCGTAAAAT |
| Pho80\_E154V\_F | ATGCAAAAGTTGGAGGAGTACGATGTCACGTTTTGAATATACTTGAGAACGATTTTTTAAAGAGAGTAAACTAC |
| Pho80\_E154V\_R | GTAGTTTACTCTCTTTAAAAAATCGTTCTCAAGTATATTCAAAACGTGACATCGTACTCCTCCAACTTTTGCAT |
| Pho81\_yEGFP\_F | CTACGCTTGTGAGTTGCTTTTTGAGAATAATATTGATATGGGTGACGGTGCTGGTTTA  |
| Pho81\_yEGFP\_R | TAATGTATAAGATTTCAAAACTACATATTACAGAACTTTATCGATGAATTCGAGCTCG  |
| Pho81Δspx\_F | GAGGCAACATAGATAGATAAACGTGCAATGGATATTGACAACAACAATAGAAGGGCAGAC |
| Pho81Δspx\_R | GTCTGCCCTTCTATTGTTGTTGTCAATATCCATTGCACGTTTATCTATCTATGTTGCCTC |
| Vip1\_F | GGGGATCCATGAGTGGGATAAAGAAGGAACC |
| Vip1\_R | CCCGTCGACCTAATCTAATGTCTTGTTAACGG |
| Vip1\_F2 | AATAACCTCACCGGTCACGG |
| Vip1\_R2 | GTAAGACGTTATTTCCCACC |
| pCEV-G1\_Leu\_F1 | CGCCGGGTCACCCGGCCAGCCTGTGCGGTATTTCACACCG |
| pCEV-G1\_Leu\_R1 | CGGTGTGAAATACCGCACAGGCTGGCCGGGTGACCCGGCG |
| pCEV-G1\_Leu\_F2 | GTGCACTCTCAGTACAATCTCTGTCGATTCGATACTAACG |
| pCEV-G1\_Leu\_R2 | CGTTAGTATCGAATCGACAGAGATTGTACTGAGAGTGCAC |
| pPho5\_-1500\_F\_Nae1 | TATGCCGGCCTACCAACATTTGGACCCGACAG |
| pPho5\_-1\_R\_Xba1 | TGCTCTAGATGGTAATCTCGAATTTGCTTGCTCTATTTG |
| yegfp\_F\_Hind3 | CCCAAGCTTATGTCTAAAGGTGAAGAATTATTCACTGGTGT |
| yefgp\_R\_SacII | TCCCCGCGGTTATTTGTACAATTCATCCATACCATGGGTAATACC |
| mcherry\_F\_NotI | ATAAGAATGCGGCCGCATGGTTAGCAAAGGCGAGGAAGATAA |
| mcherry\_R\_PacI | ACCTTAATTAATTACTTGTACAGTTCATCCATACCACCTGTA |
| pPho84\_P1F | CATTTTGAAGCTATGGTGTGTGCGGCCGGCAGAACAAAAATTTTGGCGTCGGGAGAGTAC |
| pPho84\_P1R | GTACTCTCCCGACGCCAAAATTTTTGTTCTGCCGGCCGCACACACCATAGCTTCAAAATG |
| pPho84\_P2F | AACAAACAAAACTCCACGAATACAATCCAATCTAGAACTAGTGGATCCCCCGGGCTGCAG |
| pPho84\_P2R | CTGCAGCCCGGGGGATCCACTAGTTCTAGATTGGATTGTATTCGTGGAGTTTTGTTTGTT |
| Pho81\_SPX-GFP\_P1F | TTTCAAGCTATACCAAGCATACAATCAACTATGAAATTCGGCAAGTATTTGGAAGCCAGG |
| Pho81\_SPX-GFP\_P1R | CCTGGCTTCCAAATACTTGCCGAATTTCATAGTTGATTGTATGCTTGGTATAGCTTGAAA |
| Pho81\_SPX-GFP\_P2F | GGCTGCAAGTTGTCCAGAAAAATCAGGTGGTGGTGGTGGTTCAATGTCTAAAGGTGAAGAATTATTCACTGG |
| Pho81\_SPX-GFP\_P2R | CCAGTGAATAATTCTTCACCTTTAGACATTGAACCACCACCACCACCTGATTTTTCTGGACAACTTGCAGCC |
| Pho81\_SPX-GFP\_P3F | CCCATGGTATGGATGAATTGTACAAATAATCATGTAATTAGTTATGTCACGCTTACATTC |
| Pho81\_SPX-GFP\_P3R | GAATGTAAGCGTGACATAACTAATTACATGATTATTTGTACAATTCATCCATACCATGGG |