**Supplementary File 6. Yeast strains used in this study.**

|  |  |  |  |
| --- | --- | --- | --- |
| Strain name  | Genotype  | Description  | Source  |
| yAAH0434  | MATɑ cup1Δ ura3 his3 trp1 lys2 ade2 leu2  | Cu2+ sensitive strain  | David Brow  |
| yAAH3353  | yAAH0434 + fyv6Δ::hphMX  | Cu2+ sensitive *fyv6Δ* strain  | Lipinski et al., 2023  |
| yAAH3399  | yAAH0434+upf1Δ::KanMX  | Cu2+ sensitive *upf1Δ* strain  | Lipinski et al., 2023  |
| yAAH3400  | yAAH3353+upf1Δ::KanMX  | Cu2+ sensitive *fyv6Δ* *upf1Δ* strain  | Lipinski et al., 2023  |
| yAAH3403  | yAAH0434 + dbr1Δ::NatR  | Cu2+ sensitive *dbr1Δ* strain  | This study  |
| yAAH3404  | yAAH3353 + dbr1Δ::NatR  | Cu2+ sensitive *fyv6Δ* *dbr1Δ* strain  | This study  |
| yAAH3388  | yAAH3353 + pAAH1555  | Cu2+ sensitive Fyv6 shuffle strain  | This study  |
| yAAH3393  | yAAH3353 + pAAH0135 | vector control, contains pRS414 | This study  |
| yAAH3419 | yAAH3353 + pAAH1572  | FLAG-Fyv6 (N-terminal tag) | This study  |
| yAAH3434  | yAAH3353 + pAAH1577  | FLAG-Fyv6-Δ1-16  | This study  |
| yAAH3435  | yAAH3353 + pAAH1578  | FLAG-Fyv6-Δ1-23  | This study  |
| yAAH3436  | yAAH3353 + pAAH1579  | FLAG-Fyv6-Δ1-51  | This study  |
| yAAH3437  | yAAH3353 + pAAH1580  | FLAG-Fyv6-Δ134-173  | This study  |
| yAAH3438  | yAAH3353 + pAAH1581  | FLAG-Fyv6-Δ103-173  | This study  |
| yAAH3418 | yAAH3353 + pAAH1573 | Fyv6-FLAG (C-terminal tag) | This study |
| yAAH3442 | yAAH3353 + pAAH1586 | Fyv6-Δ103-173 -FLAG | This study |
| yAAH3455  | yAAH0434 + pAAH1602  | Prp18WT merodiploid  | This study  |
| yAAH3456  | yAAH0434 + pAAH1603  | Prp18WT/Prp18V191A merodiploid  | This study  |
| yAAH3457  | yAAH0434 + pAAH1604  | Prp18WT /Prp18S162P merodiploid  | This study  |
| yAAH3458  | yAAH0434 + pAAH1605  | Prp18WT /Prp18S162P+V191A merodiploid  | This study  |
| yAAH3459  | yAAH3353 + pAAH1602  | Prp18WT merodiploid in *fyv6Δ* strain  | This study  |
| yAAH3460  | yAAH3353 + pAAH1603  | Prp18WT/Prp18V191A merodiploid in *fyv6Δ* strain  | This study  |
| yAAH3461  | yAAH3353 + pAAH1604  | Prp18WT /Prp18S162P merodiploid in *fyv6Δ* strain  | This study  |
| yAAH3462  | yAAH3353 + pAAH1605  | Prp18WT /Prp18S162P+V191A merodiploid in *fyv6Δ* strain  | This study  |
| yAAH3405  | yAAH3403 + pAAH0470  | *dbr1Δ* + WT ACT1-CUP1 (38 nt)  | This study  |
| yAAH3410  | yAAH3404 + pAAH0470  | *dbr1Δ* *fyv6Δ* + WT ACT1-CUP1 (38 nt)  | This study  |
| yAAH3498  | yAAH3403 + pAAH1632  | *dbr1Δ* + ACT1-CUP1 (9 nt)  | This study  |
| yAAH3499  | yAAH3403 + pAAH1633  | *dbr1Δ* + ACT1-CUP1 (12 nt)  | This study  |
| yAAH3500  | yAAH3403 + pAAH1634  | *dbr1Δ* + ACT1-CUP1 (21 nt)  | This study  |
| yAAH3501  | yAAH3403 + pAAH1635  | *dbr1Δ* + ACT1-CUP1 (27 nt)  | This study  |
| yAAH3503  | yAAH3404 + pAAH1633  | *dbr1Δ* *fyv6Δ* + ACT1-CUP1 (9 nt)  | This study  |
| yAAH3504  | yAAH3404 + pAAH1634  | *dbr1Δ* *fyv6Δ* + ACT1-CUP1 (12 nt)  | This study  |
| yAAH3505  | yAAH3404 + pAAH1635  | *dbr1Δ* *fyv6Δ* + ACT1-CUP1 (21 nt)  | This study  |
| yAAH3506  | yAAH3404 + pAAH1632  | *dbr1Δ* *fyv6Δ* + ACT1-CUP1 (27 nt)  | This study  |
| yAAH3509  | yAAH3403 + pAAH1636  | *dbr1Δ* + ACT1-CUP1 (15 nt)  | This study  |
| yAAH3510  | yAAH3403 + pAAH1637  | *dbr1Δ* + ACT1-CUP1 (42 nt)  | This study  |
| yAAH3511  | yAAH3403 + pAAH1638  | *dbr1Δ* + ACT1-CUP1 (46 nt)  | This study  |
| yAAH3512  | yAAH3403 + pAAH1639  | *dbr1Δ* + ACT1-CUP1 (50 nt)  | This study  |
| yAAH3513  | yAAH3404 + pAAH1636  | *dbr1Δ* *fyv6Δ* + ACT1-CUP1 (15 nt)  | This study  |
| yAAH3514  | yAAH3404 + pAAH1637  | *dbr1Δ* *fyv6Δ*+ ACT1-CUP1 (42 nt)  | This study  |
| yAAH3515  | yAAH3404 + pAAH1638  | *dbr1Δ* *fyv6Δ*+ ACT1-CUP1 (46 nt)  | This study  |
| yAAH3516  | yAAH3404 + pAAH1639  | *dbr1Δ* *fyv6Δ*+ ACT1-CUP1 (50 nt)  | This study  |
| yAAH3517 | yAAH0434+syf1Δ::KanMX + pAAH1624 | Cu2+ sensitive Syf1 shuffle strain | This study |
| yAAH3518 | yAAH3517+ fyv6Δ::HygR | Cu2+ sensitive Syf1 shuffle strain with *fyv6Δ* | This study |
| yAAH3519 | yAAH0434+syf1Δ::KanMX + pAAH1625 | WT Syf1 | This study |
| yAAH3577 | yAAH0434+syf1Δ::KanMX + +pAAH1666 | Syf1 Δ817-859 | This study |
| yAAH3578 | yAAH0434+syf1Δ::KanMX + fyv6Δ::HygR + pAAH1666 | *fyv6Δ* + Syf1 Δ817-859 | This study |
| yAAH3579 | yAAH0434+syf1Δ::KanMX + +pAAH1667 | Syf1 Δ778-859 | This study |
| yAAH3580 | yAAH0434+syf1Δ::KanMX + fyv6Δ::HygR + pAAH1667 | *fyv6Δ* + Syf1 Δ778-859 | This study |
| yAAH3581 | yAAH0434+syf1Δ::KanMX + +pAAH1668 | Syf1 Δ634-859 | This study |
| yAAH3582 | yAAH0434+syf1Δ::KanMX + fyv6Δ::HygR + pAAH1668 | *fyv6Δ* + Syf1 Δ634-859 | This study |
| yAAH3583 | yAAH0434+syf1Δ::KanMX + fyv6Δ::HygR + pAAH1625 (WT Syf1) | *fyv6Δ* + WT Syf1 | This study |
| yAAH3593 | yAAH0434 + cef1Δ::KanMX + pAAH1658 | Cu2+ sensitive Cef1 shuffle strain | This study |
| yAAH3594 | yAAH3353 + cef1Δ::KanMX + pAAH1658 | Cu2+ sensitive Cef1 shuffle strain with fyv6Δ | This study |
| yAAH3634 | yAAH0434 + cef1Δ::KanMX + pAAH1611 | Cef1WT | This study |
| yAAH3636 | yAAH0434 + cef1Δ::KanMX + pAAH1642 | Cef1M175I | This study |
| yAAH3637 | yAAH0434 + cef1Δ::KanMX + pAAH1612 | Cef1A37P | This study |
| yAAH3638 | yAAH0434 + cef1Δ::KanMX + pAAH1641 | Cef1A37V | This study |
| yAAH3639 | yAAH0434 + cef1Δ::KanMX + pAAH1614 | Cef1V36R | This study |
| yAAH3640 | yAAH0434 + cef1Δ::KanMX + pAAH1613 | Cef1S48R | This study |
| yAAH3641 | yAAH0434 + cef1Δ::KanMX + pAAH1643 | Cef1Q193P | This study |
| yAAH3655 | yAAH3353 + cef1Δ::KanMX + pAAH1612 | *fyv6Δ* Cef1A37P | This study |
| yAAH3656 | yAAH3353 + cef1Δ::KanMX + pAAH1642 | *fyv6Δ* Cef1M175I | This study |
| yAAH3657 | yAAH3353 + cef1Δ::KanMX + pAAH1641 | *fyv6Δ* Cef1A37V | This study |
| yAAH3658 | yAAH3353 + cef1Δ::KanMX + pAAH1614 | *fyv6Δ* Cef1V36R | This study |
| yAAH3659 | yAAH3353 + cef1Δ::KanMX + pAAH1613 | *fyv6Δ* Cef1S48R | This study |
| yAAH3660 | yAAH3353 + cef1Δ::KanMX + pAAH1643 | *fyv6Δ* Cef1Q193P | This study |
| yAAH3661 | yAAH3353 + cef1Δ::KanMX + pAAH1658 | *fyv6Δ* Cef1WT | This study |
| yAAH0117 | ade2, cup1Δ:ura3 his3 leu2 lys2 prp8Δ:lys2 trp1 pJU169:PRP8(URA) | Cu2+ sensitive Prp8 shuffle strain | Christine Guthrie |
| yAAH3352 | yAAH117 + fyv6Δ::HygR | Cu2+ sensitive *fyv6Δ* Prp8 shuffle strain | Lipinski et al., 2023 |
| yAAH3093 | ade2, cup1Δ:ura3 his3 leu2 lys2 prp8Δ:lys2 trp1 +pAAH1440 | Prp8WT | This study |
| yAAH3683 | ade2, cup1Δ:ura3 his3 leu2 lys2 prp8Δ:lys2 trp1 +pAAH1659 | Prp8S1584Y | This study |
| yAAH3684 | ade2, cup1Δ:ura3 his3 leu2 lys2 prp8Δ:lys2 trp1 +pAAH1660 | Prp8S1584F | This study |
| yAAH3685 | ade2, cup1Δ:ura3 his3 leu2 lys2 prp8Δ:lys2 trp1 +pAAH1661 | Prp8V1862L | This study |
| yAAH3686 | ade2, cup1Δ:ura3 his3 leu2 lys2 prp8Δ:lys2 trp1 +pAAH1662 | Prp8G1868R | This study |
| yAAH3687 | ade2, cup1Δ:ura3 his3 leu2 lys2 prp8Δ:lys2 trp1 +pAAH1663 | Prp8T1982S | This study |
| yAAH3688 | ade2, cup1Δ:ura3 his3 leu2 lys2 prp8Δ:lys2 trp1 fyv6Δ::HygR +pAAH1659 | *fyv6Δ* Prp8S1584Y | This study |
| yAAH3689 | ade2, cup1Δ:ura3 his3 leu2 lys2 prp8Δ:lys2 trp1 fyv6Δ::HygR +pAAH1660 | *fyv6Δ* Prp8S1584F | This study |
| yAAH3690 | ade2, cup1Δ:ura3 his3 leu2 lys2 prp8Δ:lys2 trp1 fyv6Δ::HygR +pAAH1661 | *fyv6Δ* Prp8V1862L | This study |
| yAAH3691 | ade2, cup1Δ:ura3 his3 leu2 lys2 prp8Δ:lys2 trp1 fyv6Δ::HygR +pAAH1662 | *fyv6Δ* Prp8G1868R | This study |
| yAAH3692 | ade2, cup1Δ:ura3 his3 leu2 lys2 prp8Δ:lys2 trp1 fyv6Δ::HygR +pAAH1663 | *fyv6Δ* Prp8T1982S | This study |
| yAAH3693 | ade2, cup1Δ:ura3 his3 leu2 lys2 prp8Δ:lys2 trp1 fyv6Δ::HygR +pAAH1440 | *fyv6Δ* Prp8WT | This study |
| yAAH1930  | MATa ade2 cup1Δ::ura3 his3 leu2 lys2 trp1 ura3 GAL+ prp22Δ::loxP pPrp22 (URA3) | Prp22 Shuffle Strain  | Charles Query  |
| yAAH1931  | MATa ade2 cup1Δ::ura3 his3 leu2 lys2 trp1 ura3 GAL+ prp22Δ::loxP +pAAH1042 | Prp22WT  | Charles Query  |
| yAAH3377  | MATa ade2 cup1Δ::ura3 his3 leu2 lys2 trp1 ura3 GAL+ prp22Δ::loxP fyv6Δ::hygR pPrp22 (URA3) | f*yv6Δ* Prp22 Shuffle Strain  | Lipinski et al., 2023  |
| yAAH3379  | MATa ade2 cup1Δ::ura3 his3 leu2 lys2 trp1 ura3 GAL+ prp22Δ::loxP fyv6Δ::hygR + pAAH1042  | *fyv6Δ* Prp22WT  | Lipinski et al., 2023  |
| yAAH3558  | MATa ade2 cup1Δ::ura3 his3 leu2 lys2 trp1 ura3 GAL+ prp22Δ::loxP +pAAH1648 | Prp22I1133R  | This study  |
| yAAH3359  | MATa ade2 cup1Δ::ura3 his3 leu2 lys2 trp1 ura3 GAL+ prp22Δ::loxP fyv6Δ::hygR +pAAH1648 | *fyv6Δ* Prp22I1133R | This study  |
| yAAH3606 | MATa ade2 cup1Δ::ura3 his3 leu2 lys2 trp1 ura3 GAL+ prp22Δ::loxP +pAAH1665 | Prp22R805A | This study  |
| yAAH3607 | MATa ade2 cup1Δ::ura3 his3 leu2 lys2 trp1 ura3 GAL+ prp22Δ::loxP +pAAH1664 | Prp22G810A | This study  |
| yAAH3608 | MATa ade2 cup1Δ::ura3 his3 leu2 lys2 trp1 ura3 GAL+ prp22Δ::loxP fyv6Δ::hygR +pAAH1664 | *fyv6Δ* Prp22G810A | This study  |
| yAAH3612 | yAAH1930 + pAAH1675 | pre-5-FOA selection Prp22R805A+I1133R | This study  |
| yAAH3613 | yAAH1930 + pAAH1674 | pre-5-FOA selection Prp22G810A+I1133R | This study  |
| yAAH3614 | yAAH3377 + pAAH1665 | pre-5-FOA selection *fyv6Δ* Prp22R805A | This study  |
| yAAH3615 | yAAH3377 + pAAH1675 | pre-5-FOA selection *fyv6Δ* Prp22R805A+I1133R | This study  |
| yAAH3616 | yAAH3377 + pAAH1674 | pre-5-FOA selection *fyv6Δ* Prp22G810A+I1133R | This study  |
| yAAH3632 | MATa ade2 cup1Δ::ura3 his3 leu2 lys2 trp1 ura3 GAL+ prp22Δ::loxP fyv6Δ::hygR +pAAH1675 | *fyv6Δ* Prp22R805A+I1133R | This study  |
| yAAH3633 | MATa ade2 cup1Δ::ura3 his3 leu2 lys2 trp1 ura3 GAL+ prp22Δ::loxP fyv6Δ::hygR +pAAH1674 | *fyv6Δ* Prp22G810A+I1133R | This study  |
| yAAH3635 | MATa ade2 cup1Δ::ura3 his3 leu2 lys2 trp1 ura3 GAL+ prp22Δ::loxP +pAAH1674 | Prp22G810A+I1133R | This study  |
| yAAH3662 | MATa ade2 cup1Δ::ura3 his3 leu2 lys2 trp1 ura3 GAL+ prp22Δ::loxP +pAAH1675 | Prp22R805A+I1133R | This study  |
| yAAH3663 | yAAH1931 + pAAH0470 | Prp22WT + WT ACT1-CUP1 | This study |
| yAAH3664 | yAAH1931 + pAAH0526 | Prp22WT + ACT1-CUP1 U301G | This study  |
| yAAH3665 | yAAH1931 + pAAH0527 | Prp22WT + ACT1-CUP1 A302U | This study |
| yAAH3666 | yAAH3607 + pAAH0470 | Prp22G810A + WT ACT1-CUP1 | This study |
| yAAH3667 | yAAH3607 + pAAH0527 | Prp22G810A + ACT1-CUP1 A302U | This study  |
| yAAH3694 | yAAH3607 + pAAH0526 | Prp22G810A + ACT1-CUP1 U301G | This study |
| yAAH3695 | yAAH3379 + pAAH0470 | *fyv6Δ* Prp22WT + WT ACT1-CUP1 | This study |
| yAAH3696 | yAAH3379 + pAAH0526 | *fyv6Δ* Prp22WT + ACT1-CUP1 U301G (gAG) | This study |
| yAAH3697 | yAAH3379 + pAAH0527 | *fyv6Δ* Prp22WT + ACT1-CUP1 A302U (UuG) | This study |
| yAAH3698 | yAAH3608 + pAAH0470 | *fyv6Δ* Prp22WT + WT ACT1-CUP1 | This study |
| yAAH3699 | yAAH3608 + pAAH0526 | *fyv6Δ* Prp22WT + ACT1-CUP1 U301G (gAG) | This study |
| yAAH3700 | yAAH3608 + pAAH0527 | *fyv6Δ* Prp22WT + ACT1-CUP1 A302U (UuG) | This study |
| yAAH3701 | yAAH1931 + pAAH0880 | Prp22WT + ACT1-CUP1 BSG | This study |
| yAAH3702 | yAAH3607 + pAAH0880 | Prp22G810A + ACT1-CUP1 BSG | This study |
| yAAH3703 | yAAH3379 + pAAH0880 | *fyv6Δ* Prp22WT + ACT1-CUP1 BSG | This study |
| yAAH3704 | yAAH3608 + pAAH0880 | *fyv6Δ* Prp22G810A + ACT1-CUP1 BSG | This study |
| yAAH3748 | yAAH3559 + pAAH0470 | *fyv6Δ* Prp22I1133R  + WT ACT1-CUP1 | This study |
| yAAH3749 | yAAH3559 + pAAH0526 | *fyv6Δ* Prp22I1133R  + ACT1-CUP1 U301G (gAG) | This study |
| yAAH3750 | yAAH3559 + pAAH0527 | *fyv6Δ* Prp22I1133R  + ACT1-CUP1 A302U (UuG) | This study |
| yAAH3751 | yAAH3559 + pAAH0880 | *fyv6Δ* Prp22I1133R  + ACT1-CUP1 BSG | This study |
| yAAH3771 | yAAH3635 + pAAH0470 | Prp22G810A+I1133R + WT ACT1-CUP1 | This study |
| yAAH3772 | yAAH3635 + pAAH0526 | Prp22G810A+I1133R + ACT1-CUP1 U301G | This study |
| yAAH3773 | yAAH3635 + pAAH0527 | Prp22G810A+I1133R + ACT1-CUP1 A302U | This study |
| yAAH3774 | yAAH3635 + pAAH0880 | Prp22G810A+I1133R + ACT1-CUP1 BSG | This study |
| yAAH3779 | yAAH3558 + pAAH0470 | Prp22I1133R  + WT ACT1-CUP1 | This study |
| yAAH3780 | yAAH3558 + pAAH0526 | Prp22I1133R  + ACT1-CUP1 U301G | This study |
| yAAH3781 | yAAH3558 + pAAH0527 | Prp22I1133R  + ACT1-CUP1 A302U | This study |
| yAAH3782 | yAAH3558 + pAAH0880 | Prp22I1133R  + ACT1-CUP1 BSG | This study |
| yAAH3783 | yAAH3633 + pAAH0470 | *fyv6Δ* Prp22G810A+I1133R + WT ACT1-CUP1 | This study |
| yAAH3784 | yAAH3633 + pAAH0526 | *fyv6Δ* Prp22G810A+I1133R + ACT1-CUP1 U301G | This study |
| yAAH3785 | yAAH3633 + pAAH0527 | *fyv6Δ* Prp22G810A+I1133R + ACT1-CUP1 A302U | This study |
| yAAH3786 | yAAH3633 + pAAH0880 | *fyv6Δ* Prp22G810A+I1133R + ACT1-CUP1 BSG | This study |
| BCY123  | MATa pep4::HIS3 prb1::LEU2 bar1::HIS6 lys2::GAL1/10-GAL4 can1 ade2 trp1 ura3 his3 leu2-3,112  | Protease-deficient yeast protein expression strain  | Galej et al., 2013 |