**Supplementary File 1A. Strains used in this study.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Strain** | **Genotype** | **Source** | **Figure** |
| BY4741 | *MAT*a*;* *his3∆1, leu2∆0, met15∆0, ura3∆0* | Euroscarf |  |
| YL515 | [BY4741] *MATa; his3∆1, leu2∆0, ura3∆0* | ([Binda et al., 2009](#_ENREF_1)) | 1A; 1E; 1F; 6C; 6E; S1 |
| MC037 | [YL515] *MATa; snf11∆::HIS3MX6* | This study | 1A; 1F |
| MC012 | [YL515] *MATa; snf1as* | This study | 1A; 1C; 1F; 2A; 2C; 2E; 4F; 5D; 5F; 6A; 6D; 6E; S1; S2 |
| MC158 | [YL515] *MATa; reg1∆::kanMX* | This study | 1E; 1F |
| MB32 | [YL515] *MATa; gtr1∆::kanMX* | ([Binda *et al.*, 2009](#_ENREF_1)) | 1F |
| Snf1-TAP | [BY4741] *MAT*a*; SNF1-TAP:HIS3* | Open Biosystems([Powis et al., 2015](#_ENREF_3)) | 4D; 5C; S3 |
| MJ5682 | [YL515] *MATa;* *arg4∆::hisMX4 lys2∆::hphNT* | ([Hu et al., 2019](#_ENREF_2)) |  |
| NIC078 | [MJ5682] *MATa; snf1as* | This study | 3A |
| NIC103 | [BY4741] *MAT*a*; snf1T210A-TAP:HIS3* | This study | 4D; 5C; S3 |
| MC086 | [Snf1-TAP] *MAT*a*; SNF4-GFP:kanMX* | This study | 4E; 5E |
| MC013 | [MC012] *MATa; pib2∆::HIS3MX* | This study | 4F; 6E |
| MC058 | [MC012] *MATa; pib2SASA* | This study | 4F; 6A; 6D; 6E |
| MC059 | [MC012] *MATa; pib2SESE* | This study | 4F; 6A; 6D; 6E |
| MC145 | [MC012] *MATa; PIB2-myc13:kanMX* | This study | 4I |
| MC152 | [MC058] *MATa; pib2SASA-myc13:kanMX* | This study | 4I |
| MC153 | [MC059] *MATa; pib2SESE-myc13:kanMX* | This study | 4I |
| MC154 | [MC012] *MATa; KOG1-HA3:HIS3MX* | This study | 4H |
| MC155 | [MC145] *MATa; KOG1-HA3:HIS3MX* | This study | 4H |
| MC156 | [MC152] *MATa; KOG1-HA3:HIS3MX* | This study | 4H |
| MC157 | [MC153] *MATa; KOG1-HA3:HIS3MX* | This study | 4H |
| MC029 | [MC012] *MATa; sch9S288A* | This study | 5D; 5F; 6A; 6D; 6E |
| MC030 | [MC012] *MATa; sch9S288E* | This study | 5D; 5F; 6A; 6D; 6E |
| MC146 | [MC058] *MATa; sch9S288A* | This study | 6A; 6D; 6E |
| MC144 | [MC059] *MATa; sch9S288E* | This study | 6A; 6D; 6E |
| MC021 | [MC012] *MATa; lst4∆::HIS3MX* | This study | S2 |

**References**

Binda, M., Péli-Gulli, M.P., Bonfils, G., Panchaud, N., Urban, J., Sturgill, T.W., Loewith, R., and De Virgilio, C. (2009). The Vam6 GEF controls TORC1 by activating the EGO complex. Mol. Cell *35*, 563-573.

Hu, Z., Raucci, S., Jaquenoud, M., Hatakeyama, R., Stumpe, M., Rohr, R., Reggiori, F., De Virgilio, C., and Dengjel, J. (2019). Multilayered control of protein turnover by TORC1 and Atg1. Cell Rep. *28*, 3486-3496.

Powis, K., Zhang, T., Panchaud, N., Wang, R., De Virgilio, C., and Ding, J. (2015). Crystal structure of the Ego1-Ego2-Ego3 complex and its role in promoting Rag GTPase-dependent TORC1 signaling. Cell Res. *25*, 1043-1059.

**Supplementary File 1B. Plasmids used in this study.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Plasmid** | **Genotype** | **Source** | **Figure** |
| pRS413 | *CEN, ARS, ampR, HIS3* | ([Brachmann et al., 1998](#_ENREF_1)) | 1A; 1C; 2A; 2C; 2E; 3A; 4F; 4H; 4I; 5D; 5F; 6A; 6C; 6D; 6E; S1; S2 |
| pRS415 | *CEN, ARS, ampR, LEU2* | ([Brachmann et al., 1998](#_ENREF_1)) | 1A; 1C; 2A; 2C; 2E; 3A; 4F; 4H; 4I; 5D; 5F; 6A; 6C; 6D; 6E; S1; S2 |
| pRS416 | *CEN, ARS, ampR, URA3* | ([Brachmann et al., 1998](#_ENREF_1)) | 1A; 1C; 2A; 2C; 2E; 3A; 4F; 4H; 4I; 5D; 5F; 6A; 6C; 6D; 6E; S1; S2 |
| pET-24d | *kanR, T7p, lacO* | Novagen |  |
| p3138 | [pET-24d] *His6-PIB2221-635* | This study | 4D; 4E |
| pMC030 | [pET-24d] *His6-pib2221-635,S268A* | This study | 4D |
| pMC031 | [pET-24d] *His6-pib2221-635,S309A* | This study | 4D |
| pMC032 | [pET-24d] *His6-pib2221-635,S268,S309A* | This study | 4D |
| YEplac195 | 2µ, *ampR, URA3* | ([Gietz and Sugino, 1988](#_ENREF_4)) |  |
| pMC013 | [YEplac195] *GAL1p-SCH91-394-TAP* | This study | 5C; 5E; S3 |
| pMC016 | [YEplac195] *GAL1p-sch91-394,S288A-TAP* | This study | 5C; S3 |
| pMC017 | [YEplac195] *GAL1p-sch9K441A-TAP* | This study | S3 |
| *pYX242-ACC1* | 2µ, *ampR, LEU2, TPI1p-ACC1-GFP-HA* | ([Deroover et al., 2016](#_ENREF_2))  | S1 |
| pRCC-K | 2µ, *ampR, kanMX, ROX3p-CAS9, SNR52p*  | ([Generoso et al., 2016](#_ENREF_3)) |  |
| pNIC012 | [pRCC-K] *SNR52p-SNF1I132* (gRNA) | This study | 1A; 1C; 1F; 2A; 2C; 2E; 3A; 4F; 5D; 5F; 6A; 6D; 6E; S1; S2 |
| pNIC015 | [pRCC-K] *SNR52p-SNF1T210* (gRNA) | This study | 4D; 5C; S3 |
| pMC005 | [pRCC-K] *SNR52p-SCH9S288* (gRNA) | This study | 5D; 5F; 6A; 6D; 6E |
| pMC008 | [pRCC-K] *SNR52p-PIB2S268* (gRNA) | This study | 4F; 6A; 6D; 6E |
| pMC009 | [pRCC-K] *SNR52p-PIB2S309* (gRNA) | This study | 4F; 6A; 6D; 6E |

**References**

Brachmann, C.B., Davies, A., Cost, G.J., Caputo, E., Li, J., Hieter, P., and Boeke, J.D. (1998). Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast *14*, 115-132.

Deroover, S., Ghillebert, R., Broeckx, T., Winderickx, J., and Rolland, F. (2016). Trehalose-6-phosphate synthesis controls yeast gluconeogenesis downstream and independent of SNF1. FEMS Yeast Res. *16*, fow036.

Generoso, W.C., Gottardi, M., Oreb, M., and Boles, E. (2016). Simplified CRISPR-Cas genome editing for *Saccharomyces cerevisiae*. J. Microbiol. Methods *127*, 203-205.

Gietz, R.D., and Sugino, A. (1988). New yeast-*Escherichia coli* shuttle vectors constructed with *in vitro* mutagenized yeast genes lacking six-base pair restriction sites. Gene *74*, 527-534.

**Supplementary File 1C. Oligonucleotides used in this study.**

|  |  |  |
| --- | --- | --- |
| **Name** | **Orientation** | **Sequence** |
| *snf1*I132 Proto-F | Forward | GAAATCATTATGGTTATAGAGTACGCCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG |
| *snf1*I132 Proto-R | Reverse | GGCGTACTCTATAACCATAATGATTTCGATCATTTATCTTTCACTGCGGAG |
| *snf1*I132G Donor | Forward | TGATGTTATCAAATCCAAAGATGAAATCATTATGGTTGGAGAGTACGCCGGAAACGAATTGTTTGACTATATTGTTCAGA |
| *snf1*T210 Proto-F | Forward | GGTAATTTCTTAAAGACTTCTTGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG |
| *snf1*T210 Proto-R | Reverse | GAAGAAGTCTTTAAGAAATTACCGATCATTTATCTTTCACTGCGGAG |
| *sch9*S288 Proto-F | Forward | GAAGATGATCTGTGTGTATAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG |
| *sch9*S288 Proto-R | Reverse | TTATACACACAGATCATCTTCGATCATTTATCTTTCACTGCGGAG |
| *sch9*S288A Donor | Reverse | TACTGAAGAGCAAGAGTTTAGCTGATCTAATTGGGAAGATGCTCTGTGTGTATAAAGAGGTTTTTTCTTCAAGTGCTCTT |
| *sch9*S288E Donor | Reverse | TACTGAAGAGCAAGAGTTTAGCTGATCTAATTGGGAAGATTCTCTGTGTGTATAAAGAGGTTTTTTCTTCAAGTGCTCTT |
| *pib2*S268 Proto-F | Forward | GAATTCTAGCTCGATGTCCCAACTGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG |
| *pib2*S268 Proto-R | Reverse | CAGTTGGGACATCGAGCTAGAATTCGATCATTTATCTTTCACTGCGGAG |
| *pib2*S268A Donor | Forward | GAAAATATTGTCGACAAGCTGACTACAACGAATTCTAGCGCGATGTCCCAACTGCGATTTGGCAACACGAACGTCATTAT |
| *pib2*S268E Donor | Forward | GAAAATATTGTCGACAAGCTGACTACAACGAATTCTAGCGAGATGTCCCAACTGCGATTTGGCAACACGAACGTCATTAT |
| *pib2*S309 Proto-F | Forward | GAATTAAAATTCAGATTAGTGCTCGAAGCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG |
| *pib2*S309 Proto-R | Reverse | GCTTCGAGCACTAATCTGAATTTTAATTCGATCATTTATCTTTCACTGCGGAG |
| *pib2*S309A Donor | Reverse | GATTTATGTTTATTGGAATTAAAATTCAGATTAGTGCTCTCAGCAGGCTGCGGTAAAAATTCCAGCGAGGGTTTCCTTAG |
| *pib2*S309E Donor | Reverse | GATTTATGTTTATTGGAATTAAAATTCAGATTAGTGCTCGAAGCAGGCTGCGGTAAAAATTCCAGCGAGGGTTTCCTTAG |