

Table 3. Plasmids used in this study

Plasmid	Alias	Origin
pMVB100	N/A	(Versele <i>et al.</i> 2004)
pFA6a-V _C -His3MX6	G00019	(Sung and Huh 2007)
YCpHU-Cdc3-V _C	G00521	pMVB100 was co-transformed into M-1622 with a V _C -HisMX PCR product (primers CDC3-F2(BiFC) and CDC3-R1(BiFC), template pFA6a-V _C -His3MX6) flanked by sequences targeting recombination with the Cdc3 C terminus
YCpHU-Cdc3(G365R)-V _C	G00522	Created by site-directed mutagenesis from template plasmid YCpHU-Cdc3-V _C
pCMV-p53(R249S), Neo	G00637	Addgene Plasmid #16438 (Baker <i>et al.</i> 1990)
pEF1a-p53-mKate2-split_mVenus_C, Hyg	p663	(Gaglia and Lahav 2014)
pEF1a-p53(L344A)-mKate2-split_mVenus_C, Hyg	p666	(Gaglia and Lahav 2014)
pEF1a-p53(L344P)-mKate2-split_mVenus_C, Hyg	p668	(Gaglia and Lahav 2014)
pEF1a-p53-mKate2-split_mVenus_N, Neo	p662	(Gaglia and Lahav 2014)
pEF1a-p53(L344A)-mKate2-split_mVenus_N, Neo	p665	(Gaglia and Lahav 2014)
pEF1a-p53(L344P)-mKate2-split_mVenus_N, Neo	p667	(Gaglia and Lahav 2014)
pEF1a-p53-mKate2-mVenus, Neo	p675	(Gaglia and Lahav 2014)
pEF1a-p53(L344A)-mKate2-mVenus, Neo	p678	(Gaglia and Lahav 2014)
pEF1a-p53(L344P)-mKate2-mVenus, Neo	p679	(Gaglia and Lahav 2014)
Pgal-p53-mKate2-V _C , TRP1	H3.29	Cloned by homologous recombination of <i>Nco</i> I- and <i>Xma</i> I-digested RS314-H with PCR product of p663 (primers 5'TP53fw and Pgal-p53-Kate-Vx_re)
Pgal-p53(V272M)-mKate2-V _C , TRP1	pMAM67	Created by site-directed mutagenesis from template plasmid H3.29
Pgal-p53(R273H)-mKate2-V _C , TRP1	pMAM68	Created by site-directed mutagenesis from template plasmid H3.29
Pgal-p53-mKate2-V _C , LEU2	pMAM110	Marker swap (<i>TRP1</i> to <i>LEU2</i>) of H3.29
Pgal-p53(V272M)-mKate2-V _C , LEU2	pMAM111	Marker swap (<i>TRP1</i> to <i>LEU2</i>) of pMAM67
Pgal-p53(R273H)-mKate2-V _C , LEU2	pMAM112	Marker swap (<i>TRP1</i> to <i>LEU2</i>) of pMAM68

Pgal-p53(R273H), <i>TRP1</i>	RS314-H	Addgene Plasmid #17541 (Thiagalingam <i>et al.</i> 1995)
Padh1-p53-mKate2-Venus, <i>LEU2</i>	pMAM85	Cloned by homologous recombination of <i>Stul</i> -digested pLS76 and <i>Sspl</i> -digested PCR product of p675 (primers MidTP53fw and TP53-vc_re)
Padh1-p53(V272M)-mKate2-Venus, <i>LEU2</i>	pMAM86	Cloned by homologous recombination of <i>Stul</i> -digested pMAM69 and <i>Sspl</i> -digested PCR product of p675 (primers MidTP53fw and TP53-vc_re)
Padh1-p53(R273H)-mKate2-Venus, <i>LEU2</i>	pMAM87	Cloned by homologous recombination of <i>Stul</i> -digested pMAM79 and <i>Sspl</i> -digested PCR product of p675 (primers MidTP53fw and TP53-vc_re)
Padh1-p53(V272M R273H)-mKate2-Venus, <i>LEU2</i>	pMAM104	Cloned by homologous recombination of <i>Stul</i> -digested pMAM77 and <i>Sspl</i> -digested PCR product of p675 (primers MidTP53fw and TP53-vc_re)
Padh1-p53(L344A)-mKate2-Venus, <i>LEU2</i>	pMAM105	Cloned by homologous recombination of <i>Stul</i> -digested pLS76 and <i>Sspl</i> -digested PCR product of p678 (primers MidTP53fw and TP53re_Vc)
Padh1-p53(L344P)-mKate2-Venus, <i>LEU2</i>	pMAM106	Cloned by homologous recombination of <i>Stul</i> -digested pLS76 and <i>Sspl</i> -digested PCR product of p679 (primers MidTP53fw and TP53re_Vc)
Padh1-p53-mKate2-Vc, <i>LEU2</i>	pMAM82	Cloned by homologous recombination of <i>Stul</i> -digested pLS76 and <i>Sspl</i> -digested PCR product of p663 (primers MidTP53fw and TP53re_Vc)
Padh1-p53(V272M)-mKate2-Vc, <i>LEU2</i>	pMAM83	Cloned by homologous recombination of <i>Stul</i> -digested pMAM69 and <i>Sspl</i> -digested PCR product of p663 (primers MidTP53fw and TP53re_Vc)
Padh1-p53(R273H)-mKate2-Vc, <i>LEU2</i>	pMAM84	Cloned by homologous recombination of <i>Stul</i> -digested pMAM79 and <i>Sspl</i> -digested PCR product of p663 (primers MidTP53fw and TP53re_Vc)
Padh1-p53(L344A)-mKate2-Vc, <i>LEU2</i>	pMAM91	Cloned by homologous recombination of <i>Stul</i> -digested pLS76 and <i>Sspl</i> -digested PCR product of p666 (primers MidTP53fw and TP53re_Vc)
Padh1-p53(L344P)-mKate2-Vc, <i>LEU2</i>	pMAM93	Cloned by homologous recombination of <i>Stul</i> -digested pLS76 and <i>Sspl</i> -

Padh1-p53-mKate2-V _N , <i>LEU2</i>	pMAM90	digested PCR product of p668 (primers MidTP53fw and TP53re_Vc) Cloned by homologous recombination of <i>Stul</i> -digested pLS76 and <i>Sspl</i> -digested PCR product of p662 (primers MidTP53fw and TP53re_Vn)
Padh1-p53(L344A)-mKate2-V _N , <i>LEU2</i>	pMAM92	Cloned by homologous recombination of <i>Stul</i> -digested pLS76 and <i>Sspl</i> -digested PCR product of p665 (primers MidTP53fw and TP53re_Vn)
Padh1-p53(L344P)-mKate2-V _N , <i>LEU2</i>	pMAM94	Cloned by homologous recombination of <i>Stul</i> -digested pLS76 and <i>Sspl</i> -digested PCR product of p667 (primers MidTP53fw and TP53re_Vn)
Padh1-p53-mKate2-V _N , <i>TRP1</i>	pMAM98	Marker swap (<i>LEU2</i> to <i>TRP1</i>) of pMAM90
Padh1-p53(L344A)-mKate2-V _N , <i>TRP1</i>	pMAM99	Marker swap (<i>LEU2</i> to <i>TRP1</i>) of pMAM92
Padh1-p53(L344P)-mKate2-V _N , <i>TRP1</i>	pMAM100	Marker swap (<i>LEU2</i> to <i>TRP1</i>) of pMAM94
Padh1-p53, <i>TRP1</i>	pMAM78	Marker swap (<i>LEU2</i> to <i>TRP1</i>) of pLS76 (Ishioka <i>et al.</i> 1993)
Padh1-p53, <i>LEU2</i>	pLS76	
Padh1-p53(V272M), <i>LEU2</i>	pMAM69	Created by site-directed mutagenesis from template plasmid pLS76
Padh1-p53(R273H), <i>LEU2</i>	pMAM79	Cloned by gap repair of <i>NcoI</i> - and <i>Stul</i> -digested pLS76 with RS314-H
Padh1-p53(V272M R273H), <i>LEU2</i>	pMAM77	Created by site-directed mutagenesis from template plasmid pMAM69
Padh1-p53(L344A), <i>LEU2</i>	pMAM114	Cloned by gap repair of <i>NcoI</i> - and <i>Stul</i> -digested pMAM79 with pMAM99
Padh1-p53(L344P), <i>LEU2</i>	pMAM115	Cloned by gap repair of <i>NcoI</i> - and <i>Stul</i> -digested pMAM79 with pMAM100
Pgal-HSP82-TAP, <i>URA3</i>	G00613	Yeast ORF Collection (Gelperin <i>et al.</i> 2005)
Pgal-HSC82-TAP, <i>URA3</i>	G00791	Yeast ORF Collection (Gelperin <i>et al.</i> 2005)
Pgal-HSP104-TAP, <i>URA3</i>	G00614	Yeast ORF Collection (Gelperin <i>et al.</i> 2005)
Pgal-SSA2-TAP, <i>URA3</i>	G00622	Yeast ORF Collection (Gelperin <i>et al.</i> 2005)
Pgal-SSA4-TAP, <i>URA3</i>	G00790	Yeast ORF Collection (Gelperin <i>et al.</i> 2005)
Pgal-SSB2-TAP, <i>URA3</i>	G00621	Yeast ORF Collection (Gelperin <i>et al.</i> 2005)
Pgal-HSP12-TAP, <i>URA3</i>	G00617	Yeast ORF Collection (Gelperin <i>et al.</i> 2005)

Pgal-HSP26-TAP, <i>URA3</i>	G00618	Yeast ORF Collection (Gelperin <i>et al.</i> 2005)
Pgal-HSP31-TAP, <i>URA3</i>	G00619	Yeast ORF Collection (Gelperin <i>et al.</i> 2005)
Pgal-SIS1-TAP, <i>URA3</i>	G00615	Yeast ORF Collection (Gelperin <i>et al.</i> 2005)
Pgal-YDJ1-TAP, <i>URA3</i>	G00353	Yeast ORF Collection (Gelperin <i>et al.</i> 2005)
Pgal-ZUO1-TAP, <i>URA3</i>	G00616	Yeast ORF Collection (Gelperin <i>et al.</i> 2005)
Pgal-CCT7-TAP, <i>URA3</i>	G00620	Yeast ORF Collection (Gelperin <i>et al.</i> 2005)
Pgal-HSP82-TAP, <i>TRP1</i>	G00630	Marker swap (<i>URA3</i> to <i>TRP1</i>) of G00613
Pgal-HSC82-TAP, <i>TRP1</i>	G00799	Marker swap (<i>URA3</i> to <i>TRP1</i>) of G00791
Pgal-HSP104-TAP, <i>TRP1</i>	G00631	Marker swap (<i>URA3</i> to <i>TRP1</i>) of G00614
Pgal-SSA2-TAP, <i>TRP1</i>	G00633	Marker swap (<i>URA3</i> to <i>TRP1</i>) of G00622
Pgal-SSA4-TAP, <i>TRP1</i>	G00789	Marker swap (<i>URA3</i> to <i>TRP1</i>) of G00790
Pgal-SSB2-TAP, <i>TRP1</i>	G00634	Marker swap (<i>URA3</i> to <i>TRP1</i>) of G00621
Pgal-HSP12-TAP, <i>TRP1</i>	G00627	Marker swap (<i>URA3</i> to <i>TRP1</i>) of G00617
Pgal-HSP26-TAP, <i>TRP1</i>	G00628	Marker swap (<i>URA3</i> to <i>TRP1</i>) of G00618
Pgal-HSP31-TAP, <i>TRP1</i>	G00629	Marker swap (<i>URA3</i> to <i>TRP1</i>) of G00619
Pgal-SIS1-TAP, <i>TRP1</i>	G00632	Marker swap (<i>URA3</i> to <i>TRP1</i>) of G00615
Pgal-YDJ1-TAP, <i>TRP1</i>	G00800	Marker swap (<i>URA3</i> to <i>TRP1</i>) of G00353
Pgal-ZUO1-TAP, <i>TRP1</i>	G00635	Marker swap (<i>URA3</i> to <i>TRP1</i>) of G00616
Pgal-CCT7-TAP, <i>TRP1</i>	G00626	Marker swap (<i>URA3</i> to <i>TRP1</i>) of G00620
HSF1 Δ N, <i>TRP1</i>	G00061	(Park <i>et al.</i> 2006)
pRS305, <i>LEU2</i>	N/A	(Sikorski and Hieter 1989)
pRS306, <i>URA3</i>	N/A	(Sikorski and Hieter 1989)
pRS314, <i>TRP1</i>	N/A	(Sikorski and Hieter 1989)
pRS316, <i>URA3</i>	N/A	(Sikorski and Hieter 1989)
pRS403, <i>TRP1</i>	N/A	(Brachmann <i>et al.</i> 1998)
pRS415, <i>LEU2</i>	N/A	(Brachmann <i>et al.</i> 1998)
pRS424, <i>TRP1</i>	N/A	(Christianson <i>et al.</i> 1992)

Marker swapping was accomplished by PCR with 5_pRS and 3_pRS of the relevant marker gene from pRS305 for *LEU2*, pRS306 for *URA3*, or pRS404 for *TRP1*, followed by co-transformation of the new-marker PCR product and original-marker plasmid into yeast harboring a deletion of the new marker (BY4741 for *LEU2* or *URA3*, Y0134 for *TRP1*). Selection for the new marker was followed by yeast DNA extraction and rescue of the marker-swapped plasmid in DH5 α .

Literature cited

- Baker S. J., S. Markowitz, E. R. Fearon, J. K. Willson, and B. Vogelstein, 1990
Suppression of human colorectal carcinoma cell growth by wild-type p53.
Science 249: 912–915. <https://doi.org/10.1126/science.2144057>
- Brachmann C. B., A. Davies, G. J. Cost, E. Caputo, J. Li, *et al.*, 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. [https://doi.org/10.1002/\(SICI\)1097-0061\(19980130\)14:2<115::AID-YEA204>3.0.CO;2-2](https://doi.org/10.1002/(SICI)1097-0061(19980130)14:2<115::AID-YEA204>3.0.CO;2-2)
- Christianson T. W., R. S. Sikorski, M. Dante, J. H. Shero, and P. Hieter, 1992
Multifunctional yeast high-copy-number shuttle vectors. *Gene* 110: 119–122.
[https://doi.org/10.1016/0378-1119\(92\)90454-w](https://doi.org/10.1016/0378-1119(92)90454-w)
- Gaglia G., and G. Lahav, 2014 Constant rate of p53 tetramerization in response to DNA damage controls the p53 response. *Molecular Systems Biology* 10: 753.
<https://doi.org/10.15252/msb.20145168>
- Gelperin D. M., M. A. White, M. L. Wilkinson, Y. Kon, L. A. Kung, *et al.*, 2005
Biochemical and genetic analysis of the yeast proteome with a movable ORF collection. *Genes Dev* 19: 2816–2826. <https://doi.org/10.1101/gad.1362105>
- Ishioka C., T. Frebourg, Y. X. Yan, M. Vidal, S. H. Friend, *et al.*, 1993 Screening patients for heterozygous p53 mutations using a functional assay in yeast. *Nat Genet* 5: 124–129. <https://doi.org/10.1038/ng1093-124>
- Park K.-W., J.-S. Hahn, Q. Fan, D. J. Thiele, and L. Li, 2006 De novo appearance and “strain” formation of yeast prion [PSI⁺] are regulated by the heat-shock transcription factor. *Genetics* 173: 35–47.
<https://doi.org/10.1534/genetics.105.054221>
- Sikorski R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* 122: 19–27.
- Sung M.-K., and W.-K. Huh, 2007 Bimolecular fluorescence complementation analysis system for in vivo detection of protein-protein interaction in *Saccharomyces cerevisiae*. *Yeast* 24: 767–775. <https://doi.org/10.1002/yea.1504>

Thiagalingam S., K. W. Kinzler, and B. Vogelstein, 1995 PAK1, a gene that can regulate p53 activity in yeast. *Proc Natl Acad Sci U S A* 92: 6062–6066.
<https://doi.org/10.1073/pnas.92.13.6062>

Versele M., B. Gullbrand, M. J. Shulewitz, V. J. Cid, S. Bahmanyar, *et al.*, 2004 Protein-protein interactions governing septin heteropentamer assembly and septin filament organization in *Saccharomyces cerevisiae*. *Mol Biol Cell* 15: 4568–83.
<https://doi.org/10.1091/mbc.E04-04-0330>