

Table S1 Homologs of centromeric proteins

Protein	Synonyms	Human homolog	<i>S. cerevisiae</i> homolog
Cnp1	Sim2	CENP-A	CSE4
Mis17	–	CENP-U	AME1
Mis6	–	CENP-I	CTF3
Mis15	–	CENP-N	CHL4
Sim4	–	CENP-K	MCM22
Mal2	–	CENP-O	MCM21
Mis12	–	MIS12	MTW1
Mis14	–	NSL1	NSL1
Mis16	–	RBBP4, RBBP7	–
Mis18	–	MIS18A	–
Mis19	Eic1, Kis1	–	–
Mis20	Eic2	–	–

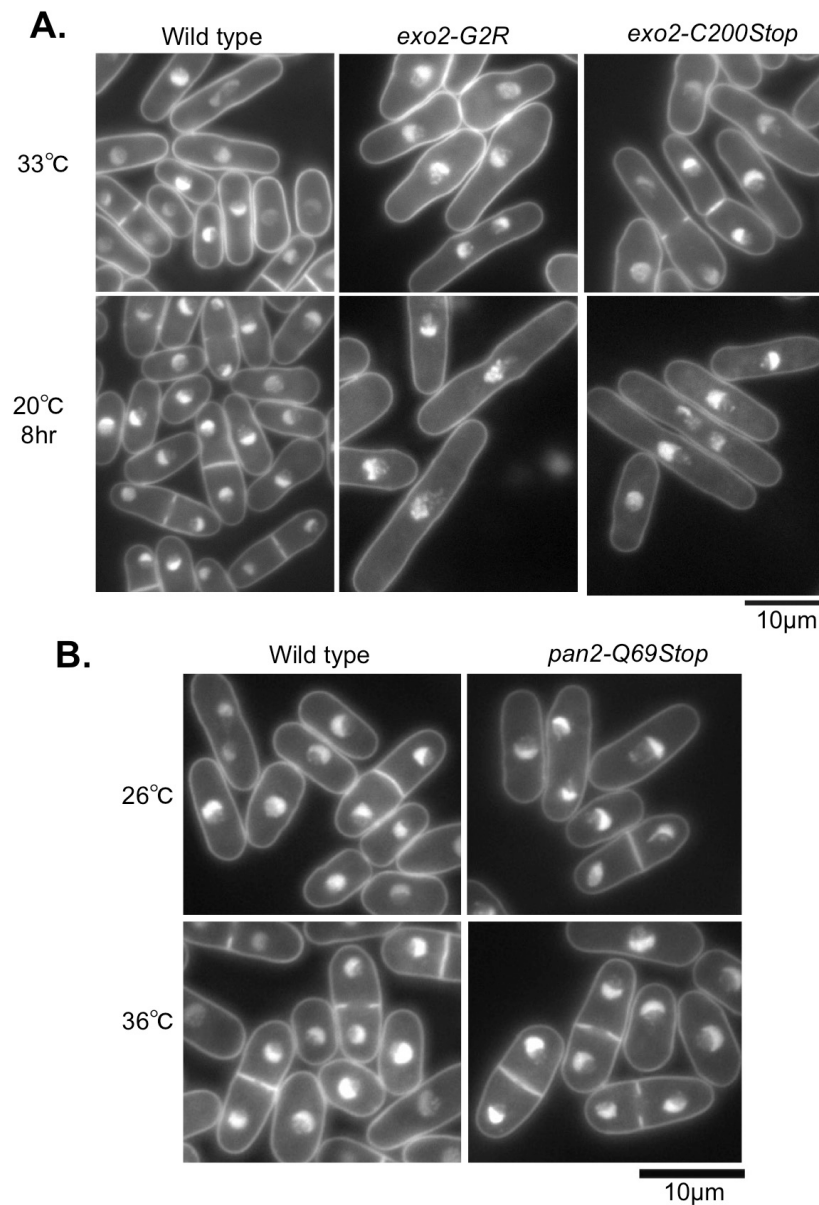


Figure S1 Micrographs of *exo2* and *pan2* mutants. (A) Micrographs of *exo2* mutants at the permissive (33°C) and restrictive (20°C for 8 hr) temperatures. *exo2-G2R* and *exo2-C200Stop* are cold sensitive (**Figure 1A**). (B) Micrographs of *pan2-Q69Stop* mutant at 26°C and 36°C. *pan2-Q69Stop* is neither ts nor cs (**Figure 1B**).

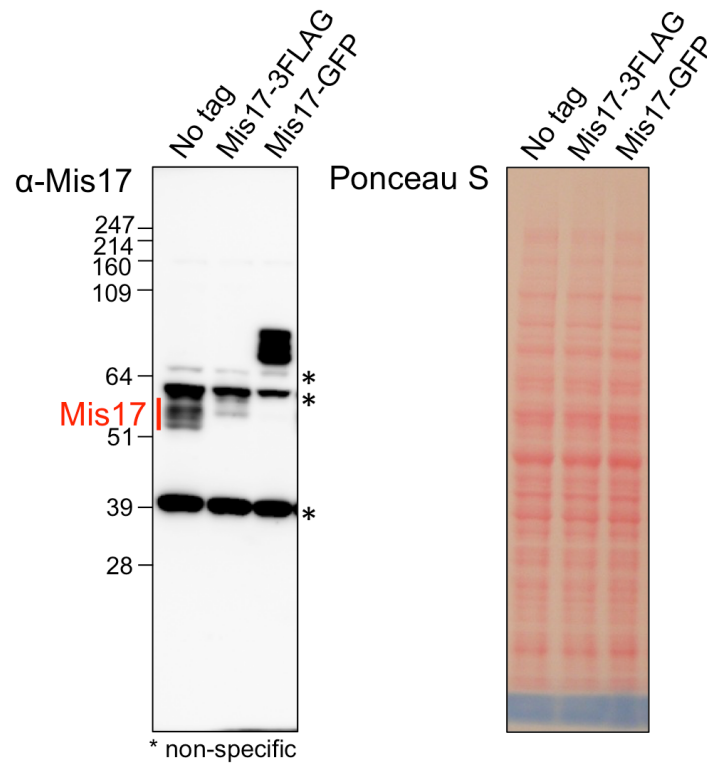


Figure S2 Validation of Mis17 protein bands detected by an anti-Mis17 antibody. Mis17 protein bands and non-specific bands were defined by comparing the pattern of protein bands of wild-type Mis17, Mis17-3FLAG, and Mis17-GFP, detected by the anti-Mis17 antibody.

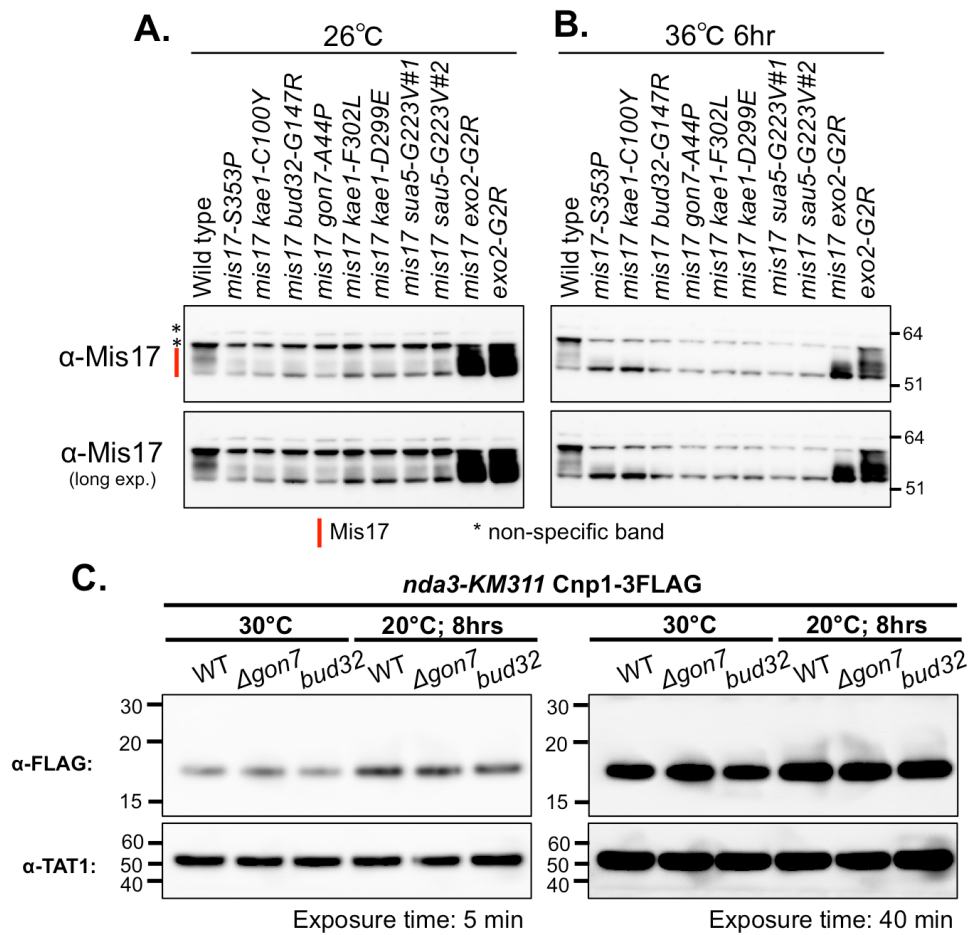


Figure S3 Mis17 and Cnp1/spCENP-A protein levels in EKC/KEOPS complex mutants. (A) Immunoblot results showing that Mis17 protein levels were not recovered in double mutant of *mis17-S353P* with EKC/KEOPS complex mutants at the permissive temperature (26°C). (B) Immunoblot results showing that Mis17 protein levels in strains used in (A) at restrictive temperature (36°C for 5 hr). (C) Cnp1/spCENP-A protein level in wild type, *Δgon7*, and *bud32-G147R* (a suppressor mutant of *mis17-S353P*). Asynchronous (30°C) and mitotic (20°C, 8 hr) extracts were prepared from cold-sensitive *nda3-KM311* Cnp1-3FLAG cells and were subjected to SDS-PAGE.

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Bud32_Sp      1  -----MSEKPLRQR-CSDIYRE--IKEKKLTIVVKQGAEAITIKTEFY-----
Bud32_Sc      1  -----MT-----QE-FIDKVSSYLTPDVDIAPISQGAEAIVFTTTHPYLPR
TP53RK_Human  1  MAAARATTPADGEEPAPAEALAAARERS--RFLSGLEIVKQGAEARVFRGR-F-----
Trp53rk_Mouse 1  -----MAGVSSEAEALAAARERSR--LFLSGLEIVKQGAEARVFRGR-F-----
CG10673_Dm    1  -----MSLETIKQGAEGRLYLGD-F-----

Bud32_Sp      41  ---PGEVCLLKORPAKRWRHPTLDQILSRKRCLEEARLLAKCH--YVGIKCPMLYFIDANR
Bud32_Sc      42  AKDSHQKYLIKYPKPKRYRHPOIDQALTKHRTLNE SRLLAkLYLIPGLCVPTIACDPYN
TP53RK_Human  53  ---QGRAAVIKHRFPKGYRHPALEARLGRRTVQEARALLRCR-RAGISAPVVFVVDYAS
Trp53rk_Mouse 44  ---QGRAAVVKHRFPKSYRHPLEARLGRRTVQEARALLRCR-RAGIAAPVVFVVDYAS
CG10673_Dm    20  ---KGEACLKERFVKRYRHPLELNTQITRQRMKAEEKASGRCL-AAGILAPKLLHSDLNT

Bud32_Sp      97  GQLYMEWIDGP-CVRDYIR---EICECEI---EKKLIPIMKRIGSEVAKMHRNDIVHGD
Bud32_Sc      102  GFIWLEFTGEDIPGGHGFSNLKNFTWMHDQDPYSDLVATTLRKVGRIQLLHWNDYCHGD
TP53RK_Human  109  NCLYMEETEGSVTVRDYIQ---STMETE---KTPQGLSNLAKTIGQVILARMHDEDLIHGD
Trp53rk_Mouse 100  NCLYMEETEDSVTVRDYIQ---STMETE---KDPQCLLDLARRMGQVILAGMHDDLIHGD
CG10673_Dm    76  HKLYMEVFDAAKTAKQTIQ---ETVSTQTEDEAKKCLLEFCTRIGEIIQKMHSNHLIHGD

Bud32_Sp      149  LTTSNMMLSESHNNPV-PFIIDFGLGSVSESEEDKAVDLYVLERALLSTLPESESIFHH--
Bud32_Sc      162  LTTSNIVLVRDGARWTPHLIDFGLGSVSNLEVEDKGVDLYVLERALLSTHSHKHAEKYNAWI
TP53RK_Human  163  LTTSNMMLKPPLEQLNIVLIDFGLSEFISALPEDKGVDLYVLEKAFSLSTHPNLETVFEA--
Trp53rk_Mouse 154  LTTSNMMLRRPLAQIHIVLIDFGLSEVSGLEPEDKGVDLYVLEKAFSLSTHPNLETAFEA--
CG10673_Dm    133  LTTSNILLNPKGGDYDVILIDFGLSHYNQATEDKGVDLYVLERALLSTHSEQPYIFEH--

Bud32_Sp      206  -----VLDSYAQSW-KQSKATLRRFEEVRRGRKRITMG
Bud32_Sc      222  MEGFEEVYREQAKGAKKLKEVTKRFEEVRLRGRKRSMIG
TP53RK_Human  221  -----FLKSYSTSS-KKARPVLKKLEEVRLRGRKRSMVG
Trp53rk_Mouse 212  -----FLKSYEASS-KKSSPVLKKLEEVRLRGRKRSMVG
CG10673_Dm    191  -----VLAAYRTACGRDEQAVLTKEFEVRRGRKRITMG

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Figure S4 Sequence alignment of Bud32. G147 is indicated by a red arrowhead.

Kael_Sp	1	-----MGKPLIALGLEGSANKLGVGII LHDT-----NGSAKILANVRH
Kael_P.abysssi	1	-----MIALGLEGTAHTLGTIGVSE-----DKVLANVFD
Kael_T.Acidophi	1	-----MIVLGLEGTAHTLSCGIIDE-----SRILAMESS
Kael_Sc	1	MVNLNTIPPKNGRDYYIALGLEGSANKLGVGIVKHPLLPKHANSDLSDYDCEAEMLSNIRD
CG4933_Dm	1	-----MVCAIGLEGSANKIGTIGIIRD-----GKVLANVRR
OSGEP_Human	1	-----MPAVLGEFGSANKIGVGIVRD-----GKVLANPRR
Osgep_Mouse	1	-----MPAVLGEFGSANKIGVGIVRD-----CTVLANPRR
Kael_Sp	39	TYITPPGGFLPSSDTAKHHRAMIPLIKQAFAEAKISF--KDIDCICFTKGPGLGAPLNS
Kael_P.abysssi	30	TLT-TEKGGTHPKAAAEHHRALMKPLLRKALSEAGVSL--DDIDVIAFSQGPGLGAPLRV
Kael_T.Acidophi	30	MYR-PKTGGIRPLDAAVHHSEVIDTVISRALKAKISII--HDIDLIGFSMGPGGLAPSLRV
Kael_Sc	61	TYVTTPPGEGLPRDTAKHHRNWCIRLIKQALAEADIKSPTLDIDVICFTKGPGMGAPLHS
CG4933_Dm	31	TYITPPGEGFLPKETAKHHRERAILGLVESSLKEAQLKS--SDIDVICFTKGPGMGAPPLLV
OSGEP_Human	31	TYVTTPPGTGFLPGDTAKHHRAMIIDLQEQALTESGLTS--QDIDCIAYTKGPGMGAPLVS
Osgep_Mouse	31	TYVTAPGTGFLPGDTAKHHRAMIIDLQEQALTEAGLTS--KDIDCIAFTKGPGMGAPLVS
Kael_Sp	97	VALCARMLSLIHKKPLVAVNHCIGHIEMGREITGAONPVVLYVSGGNTQVIAYSEKKYRI
Kael_P.abysssi	87	VATAARALAKYRKPTVGVNHCIAHVEITK-MFGVKDPVGLYVSGGNTQVLALEGGRYRV
Kael_T.Acidophi	87	TATAARTISVLTGKPIIGVNHILGHIEIGRRVTGALDPVLYVSGGNTQVIAYHVNGRYRV
Kael_Sc	121	VVTAARTCSLLWDVPLGVNHCIGHIEMGREITKAONPVVLYVSGGNTQVIAYSEKKYRI
CG4933_Dm	89	GATVARTISLLWNIPLGVNHCIGHIEMGRLLITGAONPTVLYVSGGNTQVIAYSNNRYRI
OSGEP_Human	89	VAVVARTVAQLWNKPLGVNHCIGHIEMGRLLITGATSPVLYVSGGNTQVIAYSEHRYRI
Osgep_Mouse	89	VAVVARTVAQLWNKPLGVNHCIGHIEMGRLLITGAVNPTVLYVSGGNTQVIAYSEHRYRI
Kael_Sp	157	FGETLDIAIGNCLDRFARITGLSNAPSPGYNIMOEAQKG---KFIELPYTVKGMDCSFS
Kael_P.abysssi	146	FGETLDIGIGNALDVFARELGLGFPGG--PKVEKLAQKG---EKYIELPYAVKGMDSLFS
Kael_T.Acidophi	147	LGETLDIGIGNMIDKFAREAGIPFPGG--PETEKLAQKG---TKLLDLPYVKGMDTAFS
Kael_Sc	181	FGETLDIAIGNCLDRFARTLKIPNEPSPGYNIEQLAKKAPHKENLVELPYTVKGMDLSMS
CG4933_Dm	149	FGETLDIAIGNCLDRFARILKLSNDPSPGYNIEQLAKSS---NRYTKLPYVKGMDVSFS
OSGEP_Human	149	FGETLDIAIGNCLDRFARVLKISNDPSPGYNIEQMAKRG---KKLVELPYTVKGMDVSFS
Osgep_Mouse	149	FGETLDIAIGNCLDRFARVLKISNDPSPGYNIEQMAKRG---KKLVELPYTVKGMDVSFS
Kael_Sp	214	GILSGVEAAATELDPK-----NPSSVTKQDLCSLOETGFAMLVEITERAMAH
Kael_P.abysssi	201	GILTEAIRK-----YRSGKYRVEDLAYSFQETAFALVEVTERAAH
Kael_T.Acidophi	202	GILTAALQY-----LKTG-QAIEDISYSIQETAFAMLVEVLERAYV
Kael_Sc	241	GILASTDLLAKDLPFGNKKNKILFDKTTGEQKVTVEDLCYSLOENLIFAMLVEITERAMAH
CG4933_Dm	206	GILSYIEDLAEPGKRQNRKKRPQ---EEEVNNYSQADLCYSLOETIFAMLVEITERAMAH
OSGEP_Human	206	GILSFIEDVAHRLA-----TGECTPEDLCSLOETVFAMLVEITERAMAH
Osgep_Mouse	206	GILSFIEDAAQRILA-----TGECTPEDLCSLOETVFAMLVEITERAMAH
Kael_Sp	263	IRADSVLIVGGVGCNERLQOMMAEMSSDRG-ADVFSIDERFCIDNGIMIAQAGLLAYKTG
Kael_P.abysssi	243	TEKDEVVIVGGVIANNRILREMLRIMTEDRG-IKFFVPPYDLCRDNGAMIAYTGLRMYKAG
Kael_T.Acidophi	243	SKDELLMAGGVVLRRLRDMVTNMAEAG-IRSYLTDRFYCDNGIMIAQAALLMYKSG
Kael_Sc	301	VNSNOVLIVGGVGCNVRLOEMMAQMKDRANGQVHATDNRFCIDNGIMIAQAGLLAYRMC
CG4933_Dm	263	CGSNEVLIVGGVGCNERLQEMMRIMCEERG-GKLFATDERFYCIDNGIMIAHAGAMFRSG
OSGEP_Human	252	CGSQEALIVGGVGCNVRLOEMMATMCQERG-ARLFATDERFCIDNGAMIAQAGWEMFRAG
Osgep_Mouse	252	CGSKEALIVGGVGCNLRLOEMMGTMCQERG-AQLFATDERFCDNGAMIAQAGWEMFQAG
Kael_Sp	322	DR-CAVAESTITQRYRTDDVYISWRD----
Kael_P.abysssi	302	IS-FRLEETIVKQKFRTDEVEIVVHHHHHH
Kael_T.Acidophi	302	VR-MSVEETAVNPRFRITDEVLA PWITDAS-
Kael_Sc	361	GIVKDFSEIVVTKQKFRTDEVYAAWRD----
CG4933_Dm	322	TR-MPFEESYVTQRFRTDEVLVSWRDD---
OSGEP_Human	311	HR-TPISDSGVTRQRYRTDEVVTRD----
Osgep_Mouse	311	HR-TPIKDSAITQRYRTDEVVTRD----

Figure S5 Sequence alignment of Kae1. Amino acids, involved in suppression of *mis17-S353P* once mutated, are indicated by red arrowheads.

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Sua5_Sp      1  -----METKIQTVDTRLISFEKPSNDSEHPFEHTRVSTPPS-----
Sua5_Sc      1  MYLGRHFLAMTSK-----ALFDTKILKVNPLSIFSPD-----AHIDGSLPTITDP-----
CG33786_Dm   1  -----MRRLLQT-----SLYRLLRAHHTSSRMQHQASELRTP-----
YRDC_Human   1  MSPARRCRGMRAAVAASVGLSEGP-AGSR-SGRLEFRPPSPAPAA-PGARLLRLPLSGSNAVQ
Yrdc_Mouse   1  MSTARPCAGLRRAAVAAGMGLSDGP-ASSGRGCRLLRPEPAPAL-PGARLLRLPLSEPEVE

Sua5_Sp      37  -----ETRSALENANILRNTDYPVAFPTETVYGLCADARRTEAQLSTYMAKNRPADN
Sua5_Sc      47  -----ETEAALVEAARIIRDDETVAEPPTETVYGLGGSALNDNSVLSIYRAKNRPASN
CG33786_Dm   32  ---VCAVGDEAALQ-LARQCLLGGOVIALPTDTVYGLACDANNETAQQLYEIKGRDEHK
YRDC_Human   58  AASPERACWTEALR-AAVAELRAGAVVAVPTDTLYGLACAASCSAALRAVYRLKGRSEAK
Yrdc_Mouse   59  AASPERSGWTEALR-AAVAELRAGAVVAVPTDTLYGLACSASSAALSCVYRLKGRSEAK

Sua5_Sp      90  PLIVHVFSLDOLRRLLLSAYPKAKSEVKNAHDSEEIIPKVYLPLKKFWPGPLSILLPV
Sua5_Sc      100 PLITHVSSIDOLNRKVFNQPHLS-----GTSLFDNIPSTYRPLSSSLWPGPLTILLPV
CG33786_Dm   88  PLVATCVHNIDALRRFGQ-----AAHLSDELLTRLPLPGPLTVIER
YRDC_Human   117 PLAVCLGRVADVYRYCR-----V-RVPEGLKDLLPGPVTIVMER
Yrdc_Mouse   118 PLAVCLGRVADVYRYCQ-----V-RVPRELLEDLPGPVTIVMER

Sua5_Sp      150 VDEANPPVSPITAGQKTFVVRMPQHPVALALISIS-LSPLAAPSANASTRPSPTLAKHV
Sua5_Sc      153 PSSEHSALSKLTTADQPTFAVRIPANPVARALIALS-DTPLAAPSANASTRPSPTLASHV
CG33786_Dm   128 TSQLS---NRELNPSTSKIGRIPDENFMRLCAVWQEKPLALTSANRSSAPSS-LQVSE
YRDC_Human   156 SEEL---NKDLNPFITPLVGIRIPDHAFMODLAQMF-EGPLALTSANLSSQASS-LNVEE
Yrdc_Mouse   157 SEEL---NKDLNPFITRLVGIRIPDHAFMLDLAQMF-GGPLALTSANLSSQASS-LSVEE

Sua5_Sp      209 YNDLQGGKIPLIIDGGACGCV-----ESTVVNGLCDP-PVILRPGGISLEBIIQSSGG-A
Sua5_Sc      212 YHDLKDKIPLIIDGGACKVGV-----ESTVVVGLCNP-PTILRPGGFTYEIVKLGGEA
CG33786_Dm   184 FRSLWPQLGAVFDAGRIGT--EERRLASTVIDLATPGYYEIVRAGV-ALKPTLSL----
YRDC_Human   210 FQDLWPQLSLVIDGGQIGDSQSPGRLGSTVVVDSVPCKFGIIRPGC-ALESTTAI----
Yrdc_Mouse   211 FQDLWPQLSLVIDGGPIGDSQSPGRLGSTVVVDSVPCKFGIIRPGC-ALENTTSL----

Sua5_Sp      261 WERTKVFAKKSDMETDEIPQTPGMKYRHYSPTAKVLLFVNYTESDAYGVF-----EK
Sua5_Sc      265 WSLCKVE--NKKTVKGEKVRTPGMKYRHYSPTAKVLLVPHCEGDGILKGVDRMERLKR
CG33786_Dm   237 -----ME-EFGIRELKMM-----
YRDC_Human   265 -----LQQKYGLPSHASYL-----
Yrdc_Mouse   266 -----LQQKYGLPSQGSCS-----

Sua5_Sp      314 YLS-EQGITKEKQKIGV-----LCSKRWNEESFSPSHCFVFLHMGDRGHEITKNL
Sua5_Sc      323 LIETELKANSNIKKIAILTSKLRLSDLSKIFNEPDFSS-KTFIIERLGQSAGEEIQTNL
CG33786_Dm   -----
YRDC_Human   -----
Yrdc_Mouse   -----

Sua5_Sp      363 FAQLRDLDL-QGVDFVLVEGVSENEGLAIMNRLGKAASVVFEGPSH
Sua5_Sc      382 FAALRKVDENDKVDLIFVEGINEEGEGLAVMNRLRKAANNCIQF--
CG33786_Dm   -----
YRDC_Human   -----
Yrdc_Mouse   -----

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Figure S6 Sequence alignment of Sua5. G223 is indicated by a red arrowhead.

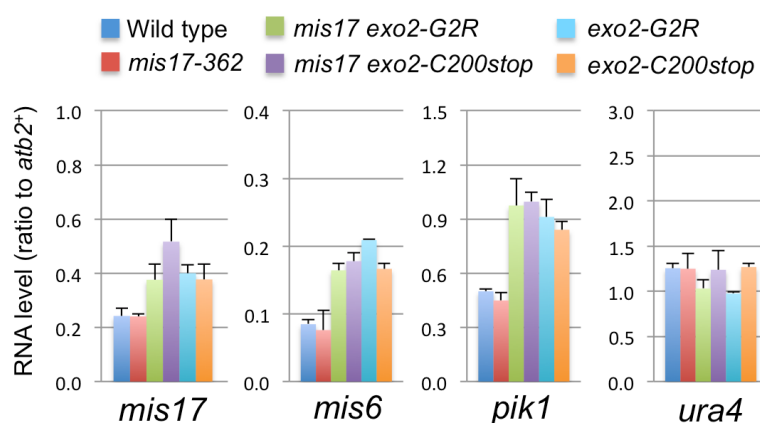


Figure S7 mRNA measurement of *mis17* and *mis6* genes in wild type and mutants. The mRNA levels of *mis17* and *mis6* genes relative to that of *atb2* (α -tubulin gene) were measured by reverse transcription-quantitative PCR (RT-qPCR), along with *pik1* and *ura4* genes. The *pik1* gene is transcriptionally activated in *exo2* mutant cells, while the *ura4* gene is not (Malecki *et al.* 2013). The mRNA levels were quantified with error bars showing the standard deviation ($n = 3$). The mRNA level of *mis17* gene is partly increased in *exo2* mutant background, however, it is possible that protein stabilization of Mis17 is not solely explained by mRNA stabilization of *mis17* gene.

Literature Cited

Malecki, M., S. C. Viegas, T. Carneiro, P. Golik, C. Dressaire *et al.*, 2013 The exoribonuclease Dis3L2 defines a novel eukaryotic RNA degradation pathway. EMBO J 32: 1842-1854.