Supplementary Information

Kinase Assay

For the kinase assay, a stationary phase culture of strain 774 (HA-HGC1 pMET3-cdc28-1as) was washed in distilled water and diluted 1:20 in 500mls pre-warmed YEPD, pH 7.0 plus 20% v/v fetal calf serum and incubated at 37°C for 120 minutes. In these conditions, Western blots using an anti-PSTAIRE monoclonal antibody (Roche) showed that Cdc28 was expressed physiological levels from the MET3 promoter (data not shown). Cells harvested as rapidly as possible by dividing the culture into 50ml Falcon tubes and spinning for one minute in an Accuspin 1^{TM} bench top centrifuge (Fisher Scientific, Loughborough, UK) at 3000 rpm. The pellet in each Falcon tube was resuspended in 1ml of ice-cold modified RIPA buffer and transferred to a pre-chilled microfuge tube. Modified RIPA buffer consists of 50mM Tris HCl pH 7.2, 0.1% sodium deoxycholate, 0.1% Triton X-100, 50mM sodium fluoride, 0.2 mM Sodium orthophosphate, 0.2mM β-glycerol phosphate and 2x complete protease inhibitor cocktail (Roche). The cells in each microfuge tube were centrifuged for 10 seconds in a microfuge and the pellet in each tube resuspended in 0.2 mls ice-cold modified RIPA buffer. The cell suspension was mixed with an equal volume of pre-chilled glass beads and the cells disrupted using a FastPrep beadbeater fitted with cryohead (MP Bio, Cambridge, UK). The lysate was cleared by centrifugation in a microfuge at 13,000 for 15 mins at 4°C and the supernatants from the 10 microfuge tubes combined. To purify the HA-Hgc1 fusion protein 20mgs of total cell protein in 2mls lysate was incubated with for 1h with 5µg of anti-HA monoclonal antibody or anti-GFP antibody (mock lysate) at 4°C, then 50µl of protein G sephadex beads were added and the incubation continued for a further hour. The beads were collected by centrifugation and washed 3x with ice-cold modified RIPA buffer + 500mM NaCl and 1x kinase reaction buffer (25mM Tris HCl pH 7.5, 5 mM β-glycerol phosphate, 15 mM magnesium Chloride, 100µM ATP). Finally, the beads were

resuspended in 50µl kinase reaction buffer to which 75µl glycerol was added and the beads stored at -80°C. To generate the peptides used as substrates for the kinase assay, DNA sequences were amplified by PCR from pFA plasmids carrying the wild type Sec2 sequence or the phosphomimetic allele at S584. These sequences were cloned into pGEX-6P-1 GST expression plasmid (GE Health Care) and the cloned insert sequenced. The GST-peptide fusion proteins were expressed and purified using the GST fusion kit (GE Health Care) according the manufacturer's instructions.

Cdc28 kinase activity was measured as follows. Immunoprecipitates were washed into 10 µl of EB buffer (80mM sodium β-glycerophosphate, 10mM $MgCl_2$ 5mM EGTA), and the reaction initiated by addition to an equal volume of reaction mixture containing 1-5µg of indicated substrate polypeptide in 40mM HEPES pH 7.7, 200µM ATP, 37kBq γ-labelled ^{32}P-ATP +/- 25 µM 1NM-PP1 inhibitor (or equal volume of DMSO) for 60 minutes at 30°C. Reactions were halted by addition of Laemmli buffer and reaction mixtures subjected to SDS-PAGE on 4-20% gradient gels (Thermo Fisher Scientific, MA, USA). Gels were Coomassie stained and dried down before exposure to Hyperfilm-MP (GE Healthcare, WI, USA). Autoradiographs images were acquired and image intensities quantified using ImageJ.

Figure S1. Confirmation that *cdc28Δ/cdc28-1as sec2Δ/Sec2* 584E strain carries only the analogue-sensitive F85G allele. After the experiments described in this paper were completed, the region surrounding the sequence that encodes the F85G allele was amplified by PCR (primers specified in supplementary table 2) and re-sequenced. The highlighted triplet GGT completely replaces TTT in the wild type sequence.

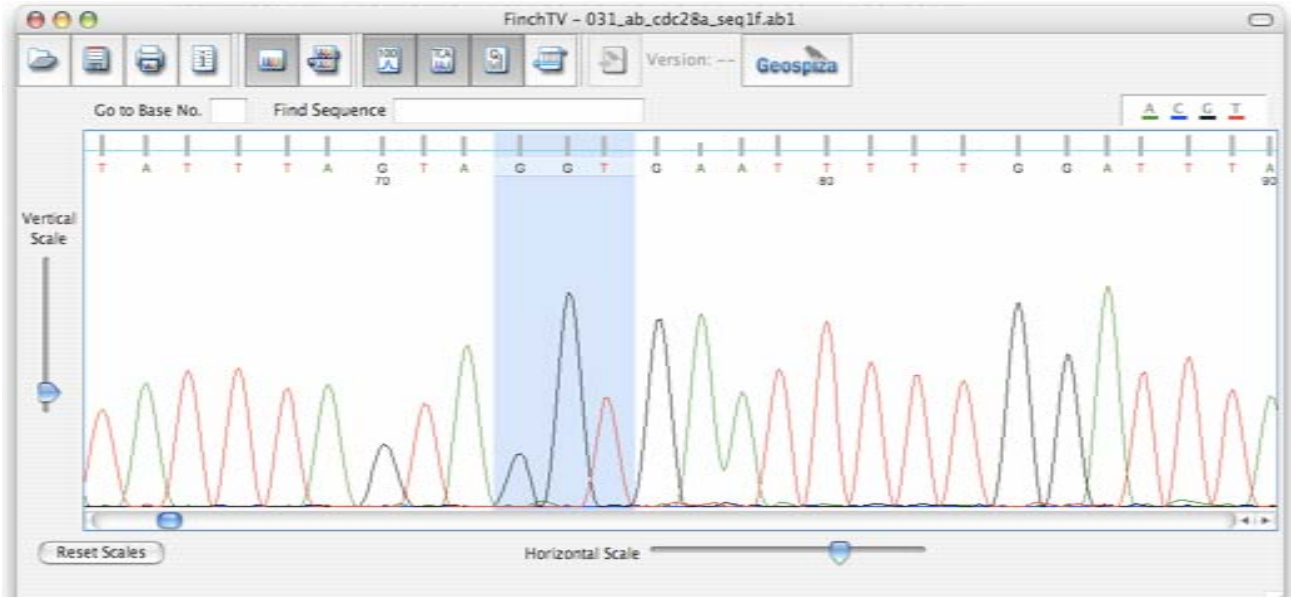
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Figure S1

Table S1. Strains used in this study

Sheffield Strain number	Relevant Genotype	Full Genotype	Source
305	BWP17	u*ra3::λimm434/ura3::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG*	Wilson *et al.*, 1999
301	CAI4	*ura3::λimm434/ura3::λimm434*	Fonzi and Irwin, 1993
1106	*CCN1-GFP/ SEC2-FLAG*	BWP17 *Sec2-FLAG-ARG4/ CCN1-GFP:URA4*	This Study
755	*hgc1*Δ/Δ	BWP17 *hgc1::HIS1/hgc1::ARG4*	Zheng *et al.*, (2004)
1107	*hgc1*Δ/Δ/ *SEC2-YFP*	BWP17 *hgc1::HIS1/hgc1::ARG4/ SEC2-YFP:URA3*	This Study
757	*ccn1*Δ/Δ	CA14 *ccn1::hisG/ccn1::hisG*	Loeb *et al.*, 1999
1109	*ccn1*Δ/Δ/ *SEC2-YFP*	*CAI4 ccn1::hisG/ccn1::hisG/ SEC2-YFP:URA3*	This Study
1110	*HGC1-GFP/ SEC2-FLAG*	BWP17 *HGC1-GFP-URA3/ SEC2-FLAG:ARG4*	This Study
1111	*HGC1-GFP*	BWP17 *HGC1-GFP:URA3*	This Study
1112	*cdc28-1as/*Δ/ *SEC2-S584E*	BWP17 *CDC28*-1as*:URA3/ cdc28::HIS1/ Sec2-S584E:ARG4*	This Study

1117	*sec2Δ/ SEC2-S601A-(1-607)-YFP*	BWP17 *sec2::HIS1/ SEC2-(1-607)-S601A-YFP:URA3*	This Study
1124	*SEC2-FLAG*	BWP17 *SEC2-FLAG:ARG4*	This Study
1127	*cbk1Δ/ CBK1-S570A/ SEC2-YFP*	BWP17 *cbk1::HIS1/ CBK1-S570A:ARG4/ SEC2YFP:URA3*	This Study
1134	*hgc1Δ/Δ/ SEC2-S584E*	BWP17 *hgc1::ARG4/ hgc1::HIS1/ SEC2-S584E:URA3*	This Study
1138	*ccn1Δ/Δ/ SEC2-S584E*	BWP17 *ccn1::ARG4/ ccn1::HIS1/ SEC2-S584E:URA3*	This Study
1143	*CDC28*-1as*/Δ/ SEC2YFP*	BWP17 *CDC28*-1as*:URA3/ cdc28::HIS1/ SEC2-YFP:HIS*	This Study
774	*6HA-HGC1 CDC28*-1as*/Δ*	*Cdc28Δ::ARG4/pMET3-cdc28as-URA3 HGC1/6HA-HGC1-HIS1*	Zheng., et al 2007
1144	*efg1Δ/Δ/ cph1Δ/Δ*	*CAI4 efg1::hisG/ efg1::hisG/ cph1::hisG/ cph1::hisG*	Lo *et al*., 1997
1153	*efg1Δ/Δ/ cph1Δ/Δ/ SEC2-YFP*	*CAI4 efg1::hisG/ efg1::hisG/ cph1::hisG/ cph1::hisG/ Sec2-YFP:URA3*	This Study
1154	*pMET3-YFP-SEC2-S584E/ sec2Δ*	BWP17 *URA3:pMET3-YFP-SEC2-S584E:ARG4/ sec2::HIS1*	This Study
1156	*pMET3-YFP-SEC2-S645E/ sec2Δ*	BWP17 *URA3:pMET3-YFP-SEC2-S645E:ARG4/ sec2::HIS1*	This Study

1158	*pMET3-YFP-SEC2-T493A/ sec2*Δ	BWP17 *URA3:pMET3-YFP-SEC2-T493A:ARG4/ sec2::HIS1*	This Study
810	*sec2*Δ	BWP17 *SEC2/sec2::HIS1*	This Study
813	*SEC2-YFP/sec2*Δ	BWP17 *SEC2-YFP:URA3/sec2::HIS1*	This Study
820	$SEC2_{(1-450)}$*-YFP*	BWP17 *SEC2/*$SEC2_{(1-450)}$*-YFP:URA3*	This Study
845	$SEC2_{(1-492)}$*-YFP*	BWP17 *SEC2/*$SEC2_{(1-492)}$*-YFP :URA3*	This Study
818	$SEC2_{(1-508)}$*-YFP*	BWP17 *SEC2/*$SEC2_{(1-508)}$*-YFP:URA3*	This Study
821	$SEC2_{(1-541)}$*-YFP*	BWP17 *SEC2/*$SEC2_{(1-541)}$*-YFP:URA3*	This Study
826	$SEC2_{(1-550)}$*-YFP/sec2*Δ	BWP17 $SEC2_{(1-550)}$*-YFP:URA3/sec2::HIS1*	This Study
837	$SEC2_{(1-583)}$*-YFP/sec2*Δ	BWP17 $SEC2_{(1-583)}$*-YFP:URA3/sec2::HIS1*	This Study
1132	$SEC2_{(1-591)}$*-YFP/sec2*Δ	BWP17 $SEC2_{(1-591)}$*-YFP:URA3/sec2::HIS1*	This Study
1163	$SEC2_{(1-597)}$*-YFP/sec2*Δ	BWP17 $SEC2_{(1-597)}$*-YFP :URA3/sec2 ::HIS1*	This Study
1133	$SEC2_{(1-607)}$*-YFP/sec2*Δ	BWP17 $SEC2_{(1-607)}$*-YFP:URA3/sec2::HIS1*	This Study
1139	$SEC2_{(1-625)}$*-YFP/sec2*Δ	BWP17 $SEC2_{(1-625)}$*-YFP/sec2::HIS1*	This Study
1113	*sec2*Δ*/*$SEC2^{584E}$	BWP17 *sec2::HIS1/*$SEC2^{584E}$*:ARG4*	This Study
855	*sec2*Δ*/*$SEC2^{588A}$	BWP17 *sec2::HIS1/*$SEC2^{588A}$*:ARG4*	This Study
850	*sec2*Δ*/*$SEC2^{645A}$	BWP17 *sec2::HIS1/*$SEC2^{645A}$*:ARG4*	This Study
861	*sec2*Δ*/*$SEC2^{645E}$	BWP17 *sec2::HIS1/*$SEC2^{645E}$*:ARG4*	This Study

1183	*SEC2^{598A}$_{(1-607)}$-YFP/sec2Δ*	BWP17 *SEC2^{598A}$_{(1-607)}$-YFP:URA3/sec2::HIS1*	This Study
1182	*SEC2^{600E}$_{(1-607)}$-YFP/sec2Δ*	BWP17 *SEC2^{600E}$_{(1-607)}$-YFP:URA3/sec2::HIS1*	This Study
1161	*SEC2^{601A}$_{(1-607)}$-YFP/sec2Δ*	BWP17 *SEC2^{601A}$_{(1-607)}$-YFP:URA3/sec2::HIS1*	This Study
1178	*SEC2-GFP/ EXO70-YFP*	BWP17 *SEC2-GFP:ARG4/ EXO70-YFP:URA3*	This Study

Table S2. Oligonucleotides used in this study

Primer	Description	Sequence
SEC2 YUF	5' primer for C-terminal tagging of *SEC2*	ACAAACTTTAGAGATGTTAGCCGAAAATATTGATTTTGATGAGAGTAGTAATGGTAATGGTAATGGTAATGGTGGTGGTTCTAAAGGTGAAGAATTATT
SEC2 YUR	3' primer for C-terminal tagging of *SEC2*	TATTGATTTGTACTTGTACCAATCACATTTGACTTGT GTATTATATAATTCAACTCAAATCACTAATCTGTCTAGAAGGACCACCTTTGATTG
SEC2 TRUNC F CHECK	Internal checking primer in 3' region of *SEC2*	TCTTTACCTGCTACCACGAC
SEC2(1-450) YUF	5' primer for C terminal truncation via *YFP* tag	GATTTCTTTGCCAAAATCAGATTGATAAGATCCAAT ATTTTTAAATTAAAACAAAATGATTCTTTTGATGAA GGTGGTGGTTCTAAAGGTGAAGAATTATT

SEC2(1-492) YUF	5’ primer for C terminal truncation via *YFP* tag	AATAGAAATGGGACGTATTCTTCATCGTCAACATC A TCATCCTCGACTTCTTCGGTATCTTCATCGTCTGCT GGTGGTGGTTCTAAAGGTGAAGAATTATT
SEC2(1-508) YUF	5’ primer for C terminal truncation via *YFP* tag	TCTTCGGTATCTTCATCGTCTGCTACAACTGCAAAT GGTGAATCATTAAACAGTACAACTCATAACATTCA A GGTGGTGGTTCTAAAGGTGAAGAATTATT
SEC2(1-541) YUF	5’ primer for C terminal truncation via *YFP* tag	AAGTTATATATAATGATGTTATTGATTCGATCAAA G ATTTTTTGGAGTAAATTAGGATTTTGGGATACT GGTGGTGGTTCTAAAGGTGAAGAATTATT
SEC2(1-550) YUF	5’ primer for C terminal truncation via *YFP* tag	ATTCGATCAAAGATTTTTTGGAGTAAATTAGGATT T TGGGATACTATTGATCAAATTAATGAGATTAATTT A GGTGGTGGTTCTAAAGGTGAAGAATTATT

SEC2(1-583) YUF	5’ primer for C terminal truncation via *YFP* tag	TATTTAATACCTAAACCAACCCAACAACAACAGCAA CAACAAGGAGATATACGTAGCCAATCAAATTTCAAT GGTGGTGGTTCTAAAGGTGAAGAATTATT
SEC2(1-591) YUF	5’ primer for C terminal truncation via *YFP* tag	ACCCAACAACAACAGCAACAACAAGGAGATATACGTAGC CAATCAAATTTCAATTCACCCAGACAACTGGTAGAT GGTGGTGGTGGTTCTAAAGGTGAAGAATTATT
SEC2(1-597) YUF	5’ primer for C terminal truncation via *YFP* tag	GGAGATATACGTAGCCAATCAAATTTCAATTCACCCAGA CAACTGGTAGATGGTAGATCAGTGCTAAGCGGTGGTGGT GGTTCTAAAGGTGAAGAATTATT

SEC2(1-607) YUF	5’ primer for C terminal truncation via *YFP* tag	AGCCAATCAAATTTCAATTCACCCAGACAACTGGT AGAT GGTAGATCAGTGCTAAGCGGTAGTATTAGTTCTGG TGGT GGTTCTAAAGGTGAAGAATTATT
SEC2(1-625) YUF	5’ primer for C terminal truncation via *YFP* tag	CAACCAAATCTCGACAAACAACAAAAGGACTCTA TT GTGGATGAGACAGTAGCACAATTACAAAGAGGT GGTGGTTCTAAAGGTGAAGAATTATT
SEC2 C-TERMINUS F CHECK	Internal C-terminal f checking primer	ACAAGGAGATATACGTAGCC
SEC2 C-TERMINUS R CHECK	Internal C-terminal r check	CCATTACCATTACTACTCTC
SEC2::HIS1 F	5’ primer for disruption of *SEC2*	ATATATCAACTGATTCACAAAGTACTGATCAGTTG T TGTTTTTTGAGTGATTATTATATATTTGATTTGG

		GTTTTCCCAGTCACGACGTT
SEC2::HIS R	3'primer for disruption of *SEC2*	TATTGATTTGTACTTGTACCAATCACATTTGACTTGT GTATTATATAATTCAACTCAAATCACTAATCTG TGTGGAATTGTGAGCGGATA
SEC2::HIS1 F CHECK	5' primer for checking disruption of *SEC2*	ATTTGCTCCAACAGACATCCA
SEC2 F1	5' primer for cloning *SEC2* into pfa arg4 for mutagenesis	GGATCCGTCGTTCCTGGTAGTTTTGG
SEC2 R1	3' primer for cloning *SEC2* into pfa arg4 for mutagenesis	CCTAGGCCAATCACATTTGACTTGTG
*SEC2*F2 (T493A)	5' mutagenesis primer	GTATCTTCATCGTCTGCTGCAACTGCAAATGGTGAATCA
*SEC2*R2 (T493A)	3' mutagenesis primer	GATTCACCATTTGCAGCTGTAGCAGACGATGAAGAATACG
*SEC2*F2 (T494A)	5' mutagenesis primer	CGTATTCTTCATCGTCTGCTACAGCTGCAAATGGTGAATC

*SEC2*R2 (T494A)	3’ mutagenesis primer	GATTCACCATTTGCAGCTGTAGCAGACGATGAAGA ATACG
*SEC2*F2 (T494E)	5’ mutagenesis primer	CTTCTTCGGTATCTTCATCGTCTGCTGAGACTGCAA ATGGTGAATCATTAAAC
*SEC2*R2 (T494E)	3’ mutagenesis primer	GAAGAAGCCATAGAAGTAGCAGACGACTCTGACG TTTACCACTTAGTAATTTG
*SEC2*F2 (S515A)	5’ mutagenesis primer	CTCATAACATTCAATTAGAAAGAACAGAAGAGGC TAAATTGATCAAGTTATATATAATGATGTTAT
*SEC2*R2 (S515A)	3’ mutagenesis primer	ATAACATCATTATATATAACTTGATCAATTTAGCCT CTTCTGTTCTTTCTAATTGAATGTTATGAG
*SEC2*F2 *SEC2*(Y521A)	5’ mutagenesis primer	AAGAACAGAAGAGAGTAAATTGATCAAGTTAGCT ATAATGATGTTATTGATTCGATCAAAGATT
*SEC2*R2 (Y521A)	3’ mutagenesis primer	AATCTTTGATCGAATCAATAACATCATTATAGCTA ACTTGATCAATTTACTCTCTTCTGTTCTT
*SEC2*F2 (S534A)	5’ mutagenesis primer	TGTTATTGATTCGATCAAAGATTTTTTGGGCTAAAT TAGGATTTTGGGATACTATTGATC

*SEC2*R2 (S534A)	3’ mutagenesis primer	GATCAATAGTATCCCAAAATCCTAATTTAGCCCAAAAAATCTTTGATCGAATCAATAACA
SEC2 (S484A) F	5’ mutagenesis primer	CATCATCATCCTCGACTTCTGCGGTATCTTCATCGT
SEC2 (S484A) R	3’ mutagenesis primer	ACGATGAAGATACCGCAGAAGTCGAGGATGATGATG
SEC2 (S484E) F	5’ mutagenesis primer	AACATCATCATCCTCGACTTCTGAGGTATCTTCATCGTCTGC
SEC2 (S484E) R	3’ mutagenesis primer	GCAGACGATGAAGATACCTCAGAAGTCGAGGATGATGATGTT
SEC2 (S584A) F	5’ mutagenesis primer	AGATATACGTAGCCAATCAAATTTCAATGCACCCAGACAACTGG
SEC2 (S584A) R	3’ mutagenesis primer	CCAGTTGTCTGGGTGCATTGAAATTTGATTGGCTACGTATATCT

SEC2 (S584E) F	5’ mutagenesis primer	CAAGGAGATATACGTAGCCAATCAAATTTCAATGA GCCCAGACAACTGGTAGA
SEC2 (S584E) R	3’ mutagenesis primer	TCTACCAGTTGTCTGGGCTCATTGAAATTTGATTGG CTACGT ATATCTCCTTG
SEC2 (S588A) F	5’ mutagenesis primer	CAAATTTCAATTCACCCAGACAAGCGGTAGATGGT AGATCAGTGC
SEC2 (S588A) R	3’ mutagenesis primer	GCACTGATCTACCATCTACCGCTTGTCTGGGTGAA TTGAAATTTG
SEC2 (S588E) F	5’ mutagenesis primer	CAAATTTCAATTCACCCAGACAAGAGGTAGATGGT AGATCAGTGC
SEC2 (S588E) R	3’ mutagenesis primer	GCACTGATCTACCATCTACCTCTTGTCTGGGTGAAT TGAAATTTG
SEC2 (S588+584A) F	5’ mutagenesis primer	CAAGGAGATATACGTAGCCAATCAAATTTCAATGA GCCCAGACAAGAGGTAGATGGTAGATCAGTGC

SEC2 (S588+584A) R	3’ mutagenesis primer	GCACTGATCTACCATCTACCTCTTGTCTGGGCTCAT TGAAATTTGATTGGCTACGTATATCTCCTTG
PFA-ARG4-REV	3’ primer for checking integration of arg4	TACACGACCCACAGTTAGTC
SEC2-DNS-REV	3’ primer for checking mutagenesis of *SEC2*	TAGTAGATACGTATCATAT
URA3-MET3-YFP-SEC2-F	5’ primer for n terminal *YFP* tag	TTAGAATACTTTTTCAGAAACTTTTCAGATTAATCA ATTTAATTCAGTTATTGCTCCAACCAACATAAATCT AGAAGGACCACCTTTGATTG
URA3-MET3-YFP-SEC2-R	5’ primer for n terminal *YFP* tag	TGTAACTAATCGTGTCGATAAACTGCCAACTTCTT CTGCCAATCTTTTATCATAATCGGCTTGTGATGATT TGTACAATTCATCCATAC
RC8	5’ primer to confirm integration of ura3-met3-*YFP*	TATGCGATTGTGGCTACTAGTAACG
SEC2(1-607, S601A)YUF	5’ primer for C terminal truncation	AGACAACTGGTAGATGGTAGATCAGTGCTAAGCG GTAGTATTAGTGCTCCAAGACAACCAAATCTCGGT

	and mutagenesis via *YFP* tag	GGTGGTTCTAAAGGTGAAGAATTATT
SEC2(1- 607, S598A,S600A YUF	5’ primer for C terminal truncation and mutagenesis via *YFP* tag	AGACAACTGGTAGATGGTAGATCAGTGCTAAGCG GTGCAATTGCATCTCCAAGACAACCAAATCTCGGT GGTGGTTCTAAAGGTGAAGAATTATT
C28FCL	5’ primer for cloning *CDC28* into pfa-ura3 for mutagenesis	CCCAAGCTTACCAAGCACAATAATAGAGT
C28RCL	3’ primer for cloning *CDC28* into pfa-ura3 for mutagenesis	GAACTGCAGGGTTATTATGTTCATATGCC
C28DF	5’ deletion primer	ATGTTTACTAACCAACTATAGAACACACACATCCC AAGCCAAGACCAACACTTATTGCAAGTTTTCCCAG TCACGACGTT
C28DR	3’deletion primer	TTGATTTTTTTTCCGTTTTTTCTTTTCGATGTCGATA TTTTATTGAGGAGGCGACAAGATTGTGGAATTGTG AGCGGATA
CDC28 UF	5’ integration primer	ACCAAGCACAATAATAGAGTTGTTGCATTAAAGAA AATTCGATTAGAATCAGAAGATGAAGGTGTACCTA GTACCGCCATTAGA

CDC28 UR	3’ integration primer	GATAATATTCATCTTTTCCATTATAAGAAGAGTATGAACCATACCCTTCTGAACCGTTTGTCTGATATCATCGATGAATTCGAG
CDC28 F85GF	5’ mutagenesis primer	TAAATTATATTTAGTAGGTGAATTTTTGGATTTAG
CDC28 F85GR	3’ mutagenesis primer	CTAAATCCAAAAATTCACCTACTAAATATAATTTA
CDC28-EF	5’ primer for amplification of *CDC28*	AGTGGTAGTGGAAGTGGTTG
CDC28-SEQ2R	3’ primer for amplification of *CDC28*	CGATATTTTATTGAGGAGGC
CDC28-SEQ3R	5’ primer for sequencing *CDC28*	AGATTTCGGGAGCTCGATAC
CDC28-SEQ1F	3’ primer for sequencing *CDC28*	ACCTAGTACCGCCATTAGAG
SEC2-FLAG-F	5’ primer for C-terminal tagging of *SEC2*	ATGTTAGCCGAAAATATTGATTTTGATGAGAGTAGTAATGGTAATGGTAATGGTAATGCAGGCGGAGATTATAAAGATGACGATGACAAATAAGGTTTCCCAGTCACGACGTT

SEC2-FLAG-R	3’ primer for C-terminal tagging of *SEC2*	TACTTGTACCAATCACATTTGACTTGTGTATTATAT AATTCAACTCAAATCACTAATCTGTGGAATTGT GAGCGGATA
CCN1-GFP-S1	5’ primer for C-terminal tagging of ccn1	AATGAATCAACAACAACAACAACAAGTGACCCAA TCATCATTATATCAACATCATCATCAATATCATCA Aggtgctggcgcaggtgcttc
CCN1-GFP-S2	3’ primer for C-terminal tagging of ccn1	ATTATCAAATTAATCAAATCAAGCAAATAAACAAA CAAACAAAGCATTATAAATTAAAAGTGTATGGTTA tctgatatcatcgatgaattcgag
CCN1GFP-CHECK-F	internal C-terminal f checking primer	GTATTGGTAGTAATAGTAGT
HGC1-GFP-S1	5’ primer for C-terminal tagging of hgc1	TAGTGGTACACCTATTAGTGAAAATGATTCTCCTA TTTATACTAAAACTCGATTATGTAATATGATTCATG GTGCTGGCGCAGGTGCTTC
HGC1-GFP-S2	3’ primer for C-terminal tagging of hgc1	TAAACTAATAAATGGGGGATATGATGGATAATATA AGTTATTAATTGAATGTAGGTAGGTAGGTGTAGAA TCTGATATCATCGATGAATTCGAG
HGC1-	internal C-terminal	GTATTGCTTCATCAATCTCA

CHECK-F	f checking primer	
SEC2-GFP-S1	5’ primer for C-terminal tagging of *SEC2*	ACAAACTTTAGAGATGTTAGCCGAAAATATTGATTTTGATGAGAGTAGTAATGGTAATGGTAATGGTAATGGTGCTGGCGCAGGTGCTTC
SEC2-GFP-S2	3’ primer for C-terminal tagging of *SEC2*	TATTGATTTGTACTTGTACCAATCACATTTGACTTGTGTATTATATAATTCAACTCAAATCACTAATCTGTCTGATATCATCGATGAATTCGAG
XFP-REV	internal *YFP* and *GFP* reverse checking primer	CCATGTGGTCTCTCTTTTCG
PRS ARG4 F CHECKING PRIMER	internal checking primer arg4	CATCAATGGATCAGTGGCAC
PRS ARG4 R CHECK	internal reverse checking primer	CATCAATGGATCAGTGGCAC
SEC2-S598A-607-YUF	5’ primer for C terminal truncation via *YFP* tag including point	AGACAACTGGTAGATGGTAGATCAGTGCTAAGCGGTGCAATTAGTTCTCCAAGACAACCAAATCTCGGTGGTGGTTCTAAAGGTGAAGAATTATT

	mutation	
SEC2-S600E-607-YUF	5’ primer for C terminal truncation via *YFP* tag including point mutation	AGACAACTGGTAGATGGTAGATCAGTGCTAAGCGGTAGTATTGAGTCTCCAAGACAACCAAATCTCGGTGGTGGTTCTAAAGGTGAAGAATTATT
SEC2-S601A-607-YUF	5’ primer for C terminal truncation via *YFP* tag including point mutation	AGACAACTGGTAGATGGTAGATCAGTGCTAAGCGGTAGTATTAGTGCTCCAAGACAACCAAATCTCGGTGGTGGTTCTAAAGGTGAAGAATTATT
EXO70 YUR	3’ primer for C terminal tagging of *EXO70*	AATACTTAAGCCTATTTGTGTGTGTTACAACATTATGTGTATTTGTGACTTGTCGTCAAGAATTGACCTCTAGAAGGACCACCTTTGATTG
EXO70 YUF	5’ primer for C-terminal tagging of *EXO70*	CACGAAAAATAAATCAAAATACGTTAAGTATGATAAATTGAATTTTGAAAAGTTGTTGAACGAGAGGTTAGGTGGTGGTTCTAAAGGTGAAGAATTATT
EXO70 CHECK	5’ checking primer for *EXO70*	GGGACTGTTACGAATTTGTC