Supplementary Information

Cdc28−Cln3 phosphorylation of Sla1 regulates actin patch dynamics in different modes of fungal growth

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Figure S1

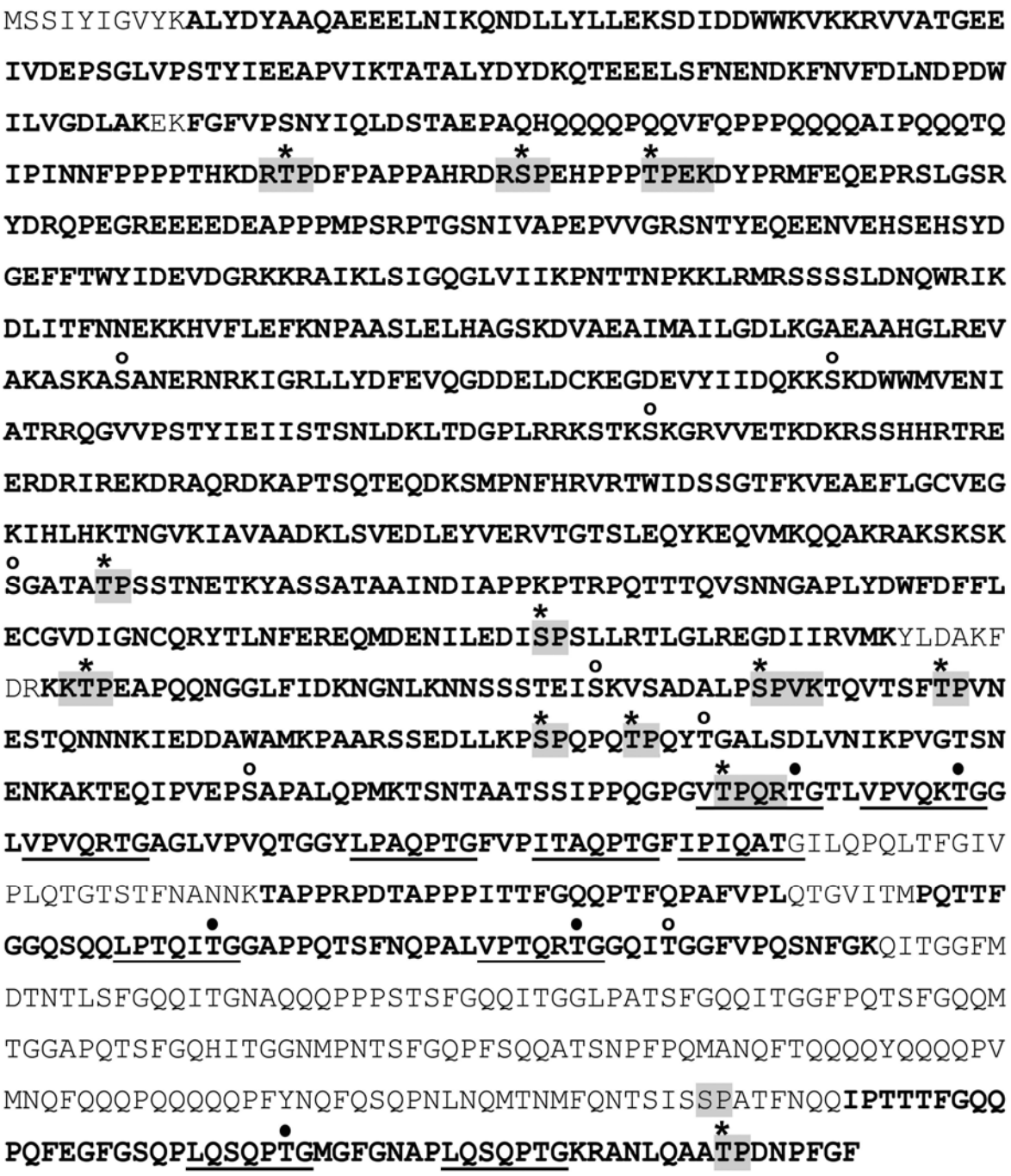

Figure S1. MS mapping of phosphorylation sites on Sla1. Amino acids covered by the mapping are highlighted in bold. CDK consensus sites are shaded in grey and putative Prk1 recognition motifs are underlined. Asterisks (*): phosphorylated Thr or Ser within CDK sites; Black dots (•): phosphorylated Thr within Prk1 recognition motifs; Open circles (o): Other phosphorylated Thr and Ser residues.

Figure S2

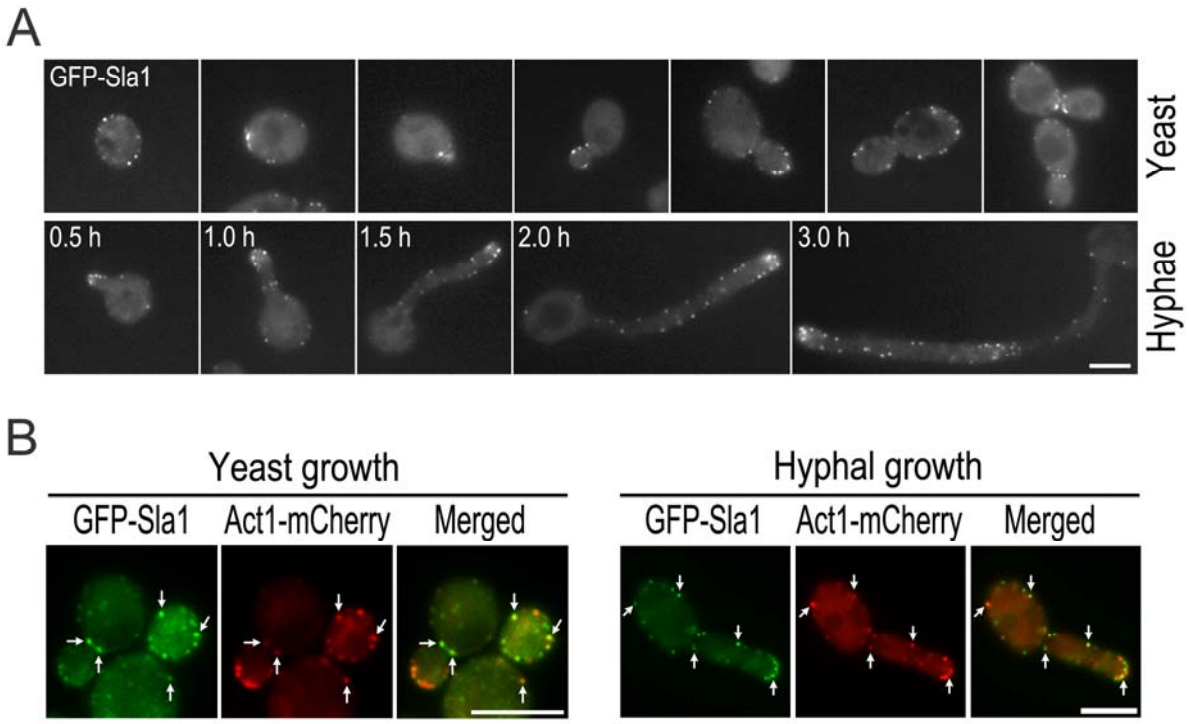

Figure S2. Subcellular localization of Sla1 during yeast and hyphal growth. (**A**) Visualization of GFP-Sla1 in yeast and hyphal cells of GZY707 (*sla1Δ/P$_{MET3}$-GFP-SLA1*). (**B**) Visualization of GFP-Sla1 and Act1-mCherry in yeast and hyphal cells of GZY752 (*sla1Δ/P$_{MET3}$-GFP-SLA1 ACT/ACT1-mCherry*). Cells were either grown in GMM at 30°C, or induced for hyphal formation by incubating with 20% FBS at 37°C for several hours. Samples were taken at the indicated time points and cells were fixed with Stopmix before microscopic visualization. Note that Act1-mCherry is not fully functional in vivo and results in abnormal hyphal morphology.

Figure S3

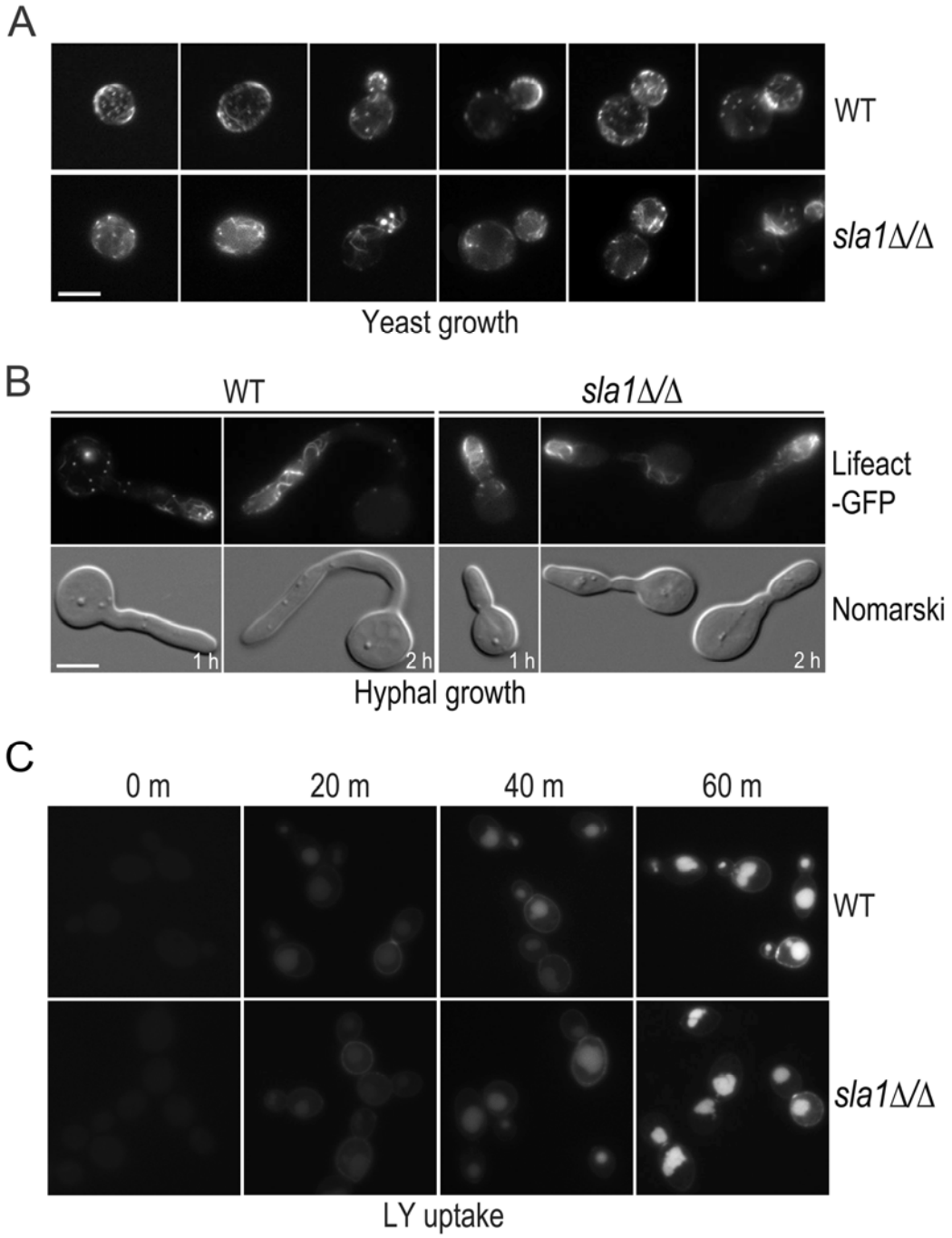

Figure S3. Actin cytoskeleton organization and LY uptake in *sla1*Δ/Δ cells. (**A** and **B**) Direct visualization of actin cytoskeleton in the *sla1*Δ/Δ cells during yeast (A) and hyphal growth (B). WT (GZY730) and *sla1*Δ/Δ (GZY733) cells expressing Lifeact-GFP were either grown in GMM at 30°C or induced for hyphal growth with 20% FBS at 37°C for 1 to 2 h. Cells were fixed with Stopmix for microscopy. (**C**) LY uptake of the *sla1*Δ/Δ cells. WT (BWP17) and *sla1*Δ/Δ (GZY602) cells were grown in GMM at 30°C and incubated with LY for 20, 40, and 60 min, respectively. Cells were washed with PBS and examined for LY uptake.

Figure S4

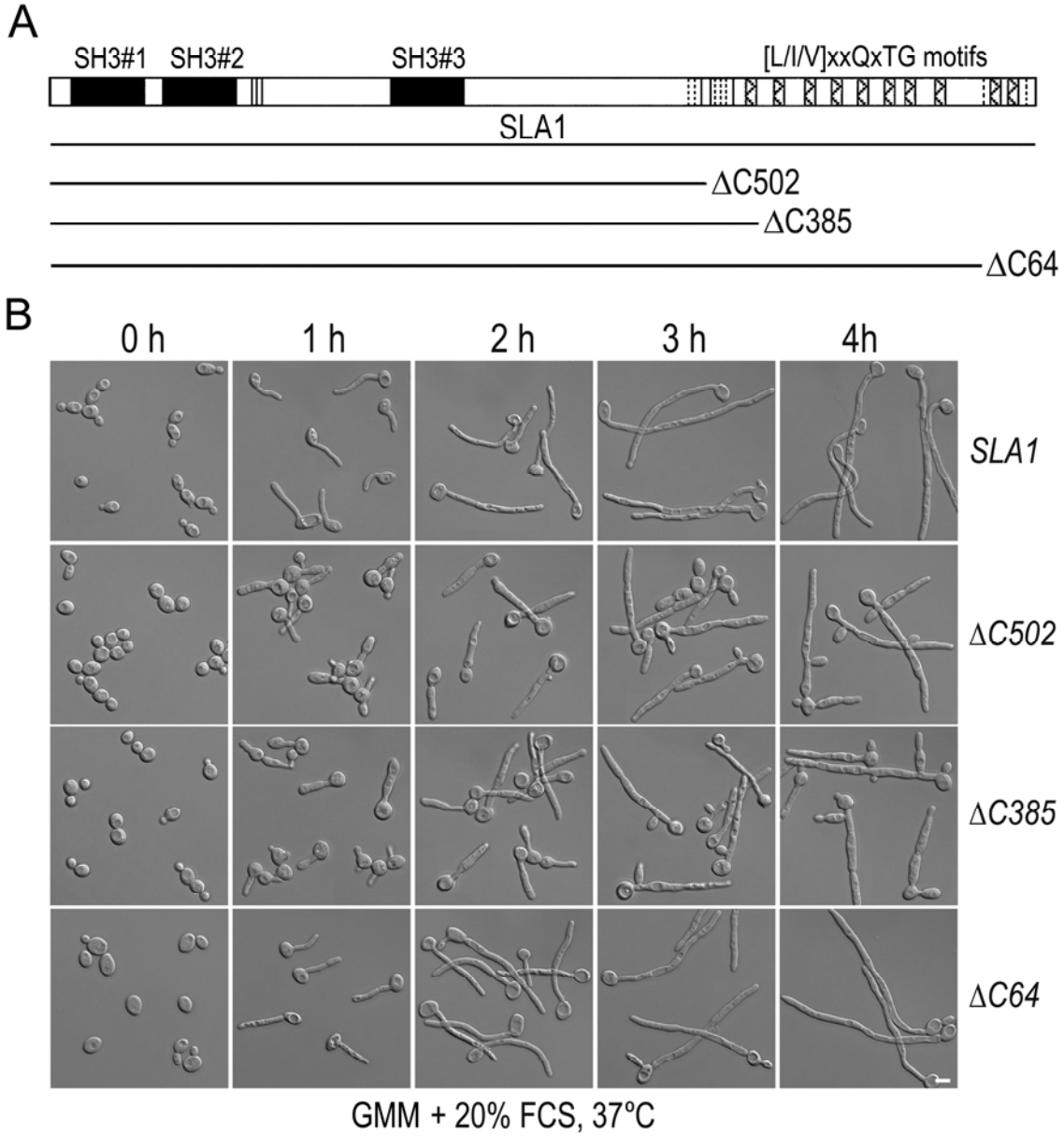

Figure S4. Defective hyphal formation of *sla1* C-terminal truncated mutants. (**A**) Schematic diagram to indicate the regions absent in different *sla1* mutants. (**B**) Hyphal morphology of the *sla1* C-terminal truncated mutants. Cells of *SLA1-WT* (GZY652), *sla1-ΔC502* (GZY625), *sla1-ΔC385* (GZY629), and *sla1-ΔC64* (GZY639) were grown in YPD at 30°C and induced for hyphal growth by incubation with 20% of FBS at 37°C. Samples were taken at 1-h intervals upon hyphal induction for morphological examination.

Movie 01. Live cell imaging of Lifeact-GFP in BWP17 (GZY730) yeast cells.

Movie 02. Live cell imaging of Lifeact-GFP in *sla1*Δ/Δ (GZY733) yeast cells.

Movie 03. Live cell imaging of Lifeact-GFP in BWP17 (GZY730) hyphal cells.

Movie 04. Live cell imaging of Lifeact-GFP in *sla1*Δ/Δ (GZY733) hyphal cells.

References

Murad, A.M., Lee, P.R., Broadbent, I.D., Barelle, C.J., and Brown, A.J. (2000). CIp10, an efficient and convenient integrating vector for Candida albicans. Yeast *16*, 325-327.

Sinha, I., Wang, Y.M., Philp, R., Li, C.R., Yap, W.H., and Wang, Y. (2007). Cyclin-dependent kinases control septin phosphorylation in Candida albicans hyphal development. Dev Cell *13*, 421-432.

Wilson, R.B., Davis, D., and Mitchell, A.P. (1999). Rapid hypothesis testing with Candida albicans through gene disruption with short homology regions. J Bacteriol *181*, 1868-1874.

Zheng, X., Wang, Y.M., and Wang, Y. (2004). Hgc1, a novel hypha-specific G1 cyclin-related protein regulates Candida albicans hyphal morphogenesis. EMBO J *23*, 1845-1856.

Table S1. Yeast strains used in this study

Strain	Relevant genotype*
BWP17	*ura3::imm434/ura3::imm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG* (Wilson *et al.*, 1999)
IS82	*ccn1Δ::URA3-FLP/ccn1Δ::ARG4* (Sinha *et al.*, 2007)
IS89	*cdc28^{F85G}-ARG4/cdc28Δ::HIS1* (Sinha *et al.*, 2007)
WYZ12	*hgc1Δ::ARG4/hgc1Δ::HIS1* (Zheng *et al.*, 2004)
GZY559	*CLN3/CLN3-Myc-URA3*
GZY584	*SLA1/SLA1-HA-ARG4*
GZY585	*CLN3/CLN3-Myc-URA3 SLA1/SLA1-HA-ARG4*
GZY601	*SLA1/sla1Δ::FRT*
GZY602	*sla1Δ::FRT/sla1Δ::ARG4*
GZY603	*cln3Δ::FRT/P_{MET3}-Myc-CLN3-ARG4*
GZY622	*cln3Δ::FRT/P_{MET3}-Myc-CLN3-ARG4 SLA1/SLA1-HA-URA3*
GZY624	*hgc1Δ::ARG4/hgc1Δ::HIS1 SLA1/SLA1-HA-URA3*
GZY625	*sla1Δ::FRT/sla1Δ::ARG4 sla1-ΔC502-HA-URA3*
GZY629	*sla1Δ::FRT/sla1Δ::ARG4 sla1-ΔC385-HA-URA3*
GZY631	*SLA1/P_{GAL1}-HA-SLA1-URA3*
GZY639	*sla1Δ::FRT/sla1Δ::ARG4 sla1-ΔC64-HA-URA3*
GZY641	*cdc28^{F85G}-ARG4/cdc28Δ::HIS1 CLN3/CLN3-Myc-URA3*
GZY652	*sla1Δ::FRT/sla1Δ::ARG4 SLA1-WT-HA-URA3*
GZY658	*sla1Δ::FRT/sla1Δ::ARG4 sla1-13A-HA-URA3*
GZY670	*sla1Δ::FRT/sla1Δ::ARG4 sla1-13E-HA-URA3*
GZY707	*sla1Δ::FRT/P_{MET3}-GFP-SLA1-URA3*
GZY718	*sla1Δ::FRT/sla1Δ::ARG4 SLA1-WT-HA-URA3 PAN1/PAN1-Myc-HIS1*
GZY719	*sla1Δ::FRT/sla1Δ::ARG4 sla1-13A-HA-URA3 PAN1/PAN1-Myc-HIS1*
GZY720	*sla1Δ::FRT/sla1Δ::ARG4 sla1-13E-HA-URA3 PAN1/PAN1-Myc-HIS1*
GZY727	*sla1Δ::FRT/sla1Δ::ARG4 sla1-23E-HA-URA3*
GZY730	*TEF1/tef1::P_{TEF1}-LifeAct-GFP-HIS1*
GZY733	*sla1Δ::URA3-FLP/sla1Δ::ARG TEF1/tef1::P_{TEF1}-Lifeact-GFP-HIS1*
GZY739	*sla1Δ::FRT/sla1Δ::ARG4 SLA1-WT-HA-URA3 TEF1/tef1:: P_{TEF1}-LifeAct-GFP-HIS1*
GZY740	*sla1Δ::FRT/sla1Δ::ARG4 sla1-13A-HA-URA3 TEF1/tef1:: P_{TEF1}-LifeAct-GFP-HIS1*
GZY741	*sla1Δ::FRT/sla1Δ::ARG4 sla1-13E-HA-URA3 TEF1/tef1:: P_{TEF1}-LifeAct-GFP-HIS1*

GZY742	*ccn1Δ::FRT/ccn1Δ::ARG4*
GZY744	*ccn1Δ::FRT/ccn1Δ::ARG4 SLA1/SLA1-HA-URA3*
GZY745	*prk1Δ::ARG4/prk1Δ::HIS1 SLA1/sla1Δ::FRT*
GZY746	*MET3/MET3::P_{MET3}-ARN1-GFP-URA3*
GZY747	*sla1Δ::FRT/sla1Δ::ARG4 MET3/MET3::P_{MET3}-ARN1-GFP-URA3*
GZY750	*prk1Δ::ARG4/prk1Δ::HIS1 sla1Δ::FRT/SLA1-HA-URA3*
GZY752	*SLA1/P_{MET3}-GFP-SLA1-HIS1 ACT1/ACT1-mCherry-TADH1-URA3*
GZY761	*sla1Δ::FRT/sla1Δ::ARG4 SLA1-WT-HA-URA3 MET3/MET3::P_{MET3}-ARN1-GFP-HIS1*
GZY762	*sla1Δ::FRT/sla1Δ::ARG4 sla1-13A-HA-URA3 MET3/MET3::P_{MET3}-ARN1-GFP-HIS1*
GZY763	*sla1Δ::FRT/sla1Δ::ARG4 sla1-13E-HA-URA3 MET3/MET3::P_{MET3}-ARN1-GFP-HIS1*

* Strains used in this study are of derivatives of BWP17.

Table S2. Plasmid constructs used in this study

Construct	Description
CIP10U	Yeast integration vector generated by removing the *RP10* gene from Clp10 (Murad *et al.*, 2000).
pYGS829	CLN3c-Myc-UTR/CIP10U; DNA fragment encoding Cln3 (268-465 aa) was cloned in frame with a C-terminal 6x*Myc* epitope (followed by *UTR,* the 3' untranslated region of *CaGAL4*) into CIP10U. The plasmid was linearized by *BamH*I within *CLN3* for integration to generate *CLN3-Myc-URA3*.
pYGS837	GST-CLN3; DNA fragment encoding Cln3 (1-465 aa) was cloned into pGEX-4T-1.
pYGS859	SLA1c-HA-UTR/CIP10U; DNA fragment encoding Sla1 (658-1257 aa) was cloned in frame with a C-terminal 2x*HA* epitope (followed by *UTR*) into CIP10U. The plasmid was linearized by *Xcm*I within *SLA1* for integration to generate *SLA1-HA-URA3*.
pYGS861	CLN3ΔURA3-FLP/pBKS; *CLN3* promoter region (-556 to -1 bp) and terminator region (1399 to 1773 bp) were cloned to flank the *CaURA3* flipper in pBKS vector. The knock-out cassette was released by *Kpn*I and *Sac*II for transformation to generate *cln3*Δ*::FRT*.
pYGS868	SLA1ΔURA3-FLP/pBKS; *SLA1* promoter region (-509 to -1 bp) and terminator region (3775 to 4240 bp) were cloned to flank the *CaURA3* flipper in pBKS vector. The knock-out cassette was released by *Kpn*I and *Sac*II for transformation to generate *sla1*Δ*::URA3-FLP* and *sla1*Δ*::FRT*.
pYGS870	SLA1ΔARG4/pBKS; *CaURA3* flipper in pYGS868 was replaced with *CaARG4*. The knock-out cassette was released by *Kpn*I and *Sac*II for transformation to generate *sla1*Δ*::ARG4.*
pYGS877	CLN3ΔARG4-P_{MET3}-Myc/pTEZ; *CLN3* promoter region (-444 to -1 bp) and coding region (1 to 500 bp) were cloned to flank *ARG4-*P_{MET3}*-Myc* (*CaARG4* followed by 6x*Myc* epitope under *CaMET3* promoter control) in pTEZ vector. The knock-in cassette was released by *Kpn*I and *Sac*II for transformation to generate P_{MET3}*-Myc-CLN3-ARG4*.
pYGS893	SLA1c-HA-UTR/CIP10A; *CaURA3* in pYGS859 was replaced with *CaARG4*. The plasmid was linearized by *Xcm*I within *SLA1* for integration to generate *SLA1-HA-ARG4*.
pYGS894	SLA1ΔC502-HA-UTR/CIP10U; DNA fragment encoding Sla1 (457-755 aa) was cloned in frame with a C-terminal 2x*HA* epitope (followed by *UTR*) into CIP10U. The plasmid was linearized by *Sal*I within *SLA1* for integration to generate *SLA1*Δ*C502-HA-URA3*.
pYGS895	P_{GAL1}-HA-SLA1n/CIP10U; DNA fragment encoding Sla1 (1-304 aa) was cloned in frame with an N-terminal 2x*HA* epitope (under *CaGAL1* promoter control) into CIP10U. The plasmid was linearized by *BsaB*I within *SLA1* for integration to generate P_{GAL1}*-HA-SLA1-URA3*.
pYGS896	SLA1ΔC385-HA-UTR/CIP10U; DNA fragment encoding Sla1 (457-872 aa) was cloned in frame with a C-terminal 2x*HA* epitope (followed by *UTR*) into CIP10U. The plasmid was linearized by *Sal*I within *SLA1* for integration to generate *SLA1*Δ*C64-HA-URA3*.
pYGS913	SLA1ΔC64-HA-UTR/CIP10U; DNA fragment encoding Sla1 (658-1193 aa) was cloned in frame with a C-terminal 2x*HA* epitope (followed by *UTR*) into CIP10U. The plasmid was linearized by *Xcm*I within *SLA1* for integration to generate *SLA1*Δ*C385-HA-URA3*.
pYGS920	Pro-SLA1-WT-HA-UTR/CIP10U; DNA fragment containing *SLA1* promoter and coding region (-1000 to 3771 bp) was cloned in frame with a C-terminal 2x*HA* epitope (followed by *UTR*) into CIP10U. The plasmid was linearized by *Pme*I (generated by mutagenesis at promoter region -550 bp) for integration to generate *SLA1-WT-HA-URA3*.

pYGS924 Pro-sla1-13A-HA-UTR/CIP10U; pYGS920 was used as the template for mutagenesis to introduce 13 mutations (T181A, S194A, T201A, T611A, S690A, T720A, S757A, T767A, S800A, T805A, T865A, S1194A, T1250A) on Sla1. The plasmid was linearized by *Pme*I for integration to generate *sla1-13A-HA-URA3*.

pYGS929 Pro-sla1-13E-HA-UTR/CIP10U; pYGS920 was used as the template for mutagenesis to introduce 13 mutations (T181E, S194E, T201E, T611E, S690E, T720E, S757E, T767E, S800E, T805E, T865E, S1194E, T1250E) on Sla1. The plasmid was linearized by *Pme*I for integration to generate *sla1-13E-HA-URA3*.

pYGS964 P_{MET3}-GFP-SLA1n/CIP10U; DNA fragment encoding Sla1 (1-304 aa) was cloned in frame with an N-terminal *GFP* epitope (under *CaMET3* promoter control) into CIP10U. The plasmid was linearized by *BsaB*I within *SLA1* for integration to generate P_{MET3}-*GFP-SLA1-URA3*.

pYGS965 P_{MET3}-GFP-SLA1n/CIP10H; *CaURA3* in pYGS964 was replaced with *CaHIS1*. The plasmid was linearized by *BsaB*I within *SLA1* for integration to generate P_{MET3}-*GFP-SLA1-HIS1*.

pYGS968 PAN1c-Myc-UTR/CIP10U; DNA fragment encoding Pan1 (800-1397 aa) was cloned in frame with a C-terminal 6x*Myc* epitope (followed by *UTR*) into CIP10U.

pYGS970 PAN1c-Myc-UTR/CIP10H; Ca*URA3* in pYGS968 was replaced with Ca*HIS1*. The plasmid was linearized by *Hind*III within *PAN1* for integration to generate *PAN1-Myc-HIS1*.

pYGS971 Pro-SLA1^{23E}-HA-UTR/CIP10U; pYGS929 was used as the template for mutagenesis to introduce 10 mutations (T869E, T878E, T887E, T905E, T915E, T923E, T1002E, T1022E, T1226E, T1240E) on Sla1-13E. The plasmid was linearized by *Pme*I for integration to generate *SLA1^{23E}-HA-URA3*.

pYGS972 ACT1c-mCherry-TADH1/CIP10U; DNA fragment encoding Act1 (4-376 aa, including 367 bp intron region) was cloned in frame with a C-terminal mCherry epitope (followed by *CaADH1* terminator) into CIP10U. The plasmid was linearized by *Cla*I within *ACT1* for integration to generate *ACT1-mCherry-TADH1-URA3*.

pYGS974 TEF1ΔLifeAct-GFP-HIS1/pJET; DNA fragment amplified from pFA-GFP-CaHIS1 with the primer pairs Lifeact S1 (5'-atcttgatttttacttcttcttcttcttcttttttcctttcttcacacattatagtcataa tcaatcatgggtg tcgcagatttgatcaagaaattcgaaagcatctcaaaggaagaaggtgctggcgcaggtgcttc-3') and LifeAct S2 (5'-taagaaaataaactaaataaatgaatacaaaataaaaactctaacagatcttacataatatt caactagc*tctgatatcatcgatgaattcgag*-3') was cloned into pJET vector. The knock-in cassette was released by *Not*I and *Xba*I for transformation to generate P_{TEF1}-*LifeAct-GFP-HIS1*.

pYGS983 PRK1ΔHIS1/pTEZ; *PRK1* promoter region (-499 to -1 bp) and terminator region (2073 to 2472 bp) were cloned to flank *CaHIS1* in pTEZ vector. The knock-out cassette was released by *Not*I for transformation to generate *prk1Δ::HIS1*.

pYGS985 PRK1ΔARG4/pTEZ; Ca*HIS1* in pYGS983 was replaced with Ca*ARG4*. The knock-out cassette was released by *Not*I for transformation to generate *prk1Δ::ARG4*.

pYGS987 P_{MET3}-ARN1-GFP-UTR/CIP10U; DNA fragment encoding Arn1 (1-604 aa) was placed under *CaMET3* promoter control and cloned in frame with a C-terminal *GFP* epitope (followed by *UTR*) into CIP10U. The plasmid was linearized by *Nsi*I within P_{MET3} for integration to generate P_{MET3}-*ARN1-GFP-URA3*.

pYGS994 P_{MET3}-ARN1-GFP-UTR/CIP10H; *CaURA3* in pYGS987 was replaced with *CaHIS1*. The plasmid was linearized by *Nsi*I within P_{MET3} for integration to generate P_{MET3}-*ARN1-GFP-URA3*.

Table S3. List of GST-Cln3 affinity purified proteins identified by MS

Protein ID	Matched Peptides (Bold)
URA2	1 MAQLSVPITP PMESTGDVLM TLETQDGIAL QGYSFGAAKP AAGEVVFQTG MVGYPESITD PSYEGQILVI TYPLVGNYGV PDR**ELLDEDY EPALPK**YFES 101 NKIHIAGLVV AHYTEEYSHW LAKSSLGKWL QEQGIPAIYG VDTRSLTKRL REKGSTLGRL AIQNSDYKSE EIISQSKSNP QNWKK**FFNVP EFDDPNVK**NL 201 VAKVSTDKPI LYTPKKTNEN IKLGKNGKPI RILAVDVGMK YNQIRCFVRR GVELLVVPWD YDFTTEEYDG LFISNGPGDP AVMDKTVERL QKILKEGKTP 301 VFGICLGHQL LARATGASTL KLKFGNRGHN IPCTSTISGR CYITSQNHGY AVDTATLSNG WKELFVNAND GSNEGIYHES KPFFSVQFHP ESTPGPRDTE 401 FLFDVFIK**SV VDFNQSGGVY K**QVEFPGGKL AENRAAHPKV DVKKVLVLGS GGLSIGQAGE FDYSGSQAIK ALKEEGIYTV LINPNIATIQ TSKGLADK**VY** 501 **FLPVTPEFVR** KVIKHERPDG IYCTFGGQTA LSVGIALKDE FEGLGVKVLG TQIDTVITTE DRELFASAMA EINEKCARSE ACNTVK**EAVD AANAIGYPLI** 601 **VR**AAYALGGL GSGFADNEEE LVALCNK**AFA TSPQVLVER**S MKGWKEVEYE VVRDAFDNCI TVCNMENFDP LGIHTGDSIV VAPSQTLSDE DYNMLRTTAV 701 NVIRHLGVVG ECNIQYALNP FSKEYCIIEV NARLSRSSAL ASKATGYPLA YTAAK**LGLNI PLNEIK**NSVT KSTSACFEPS LDYCVVKIPR WDLKKFTRVS 801 ALLSSSMKSV GEVMAIGRTF EEAIQKAIRS TDYHNLGFNK TAALMSIDID QELQTPSDQR LFAIANALSD GYSVDKVWKL TNIDKWFLNK LDGLIKFGNK 901 IASYGAKEDV PMSILRQAKQ LGFEDRQIAK FLGSNEVAIR RLRKDAGIIP FVKQIDTVAA EFPAFTNYLY VTYNADSSDV KFDDNGVIVL GSGVYRIGSS 1001 VEFDWCAVRA IRTLRENNVK TVMINYNPET VSTDYDEADR **LYFEPINLER** VLDIYDLEQS SGVIISMGGQ TSNNIALPLY RQNVKILGTS PEMIDSAENR 1101 YKFSRMLDRI GVDQPAWKEL TSIDEAEDFA EKVGYPVLVR PSYVLSGAAM NTVYSK**DDLA SYLGQAVDVS PDYPVVITK**Y IENAKEIEMD AVAKDGKMIM 1201 HVVSEHVENA GVHSGDATLI VPPQDLDPET VRRIVEATAK IGKALDITGP YNIQFIAKDN DIKVIECNVR ASRSFPFVSK VVGVDLIEMA TKAMLDLPIV 1301 PYPGERLPED YCAVKVPQFS FSRLAGADPV LGVEMASTGE VATFGKNKYE AYLKSLISTG FKLPKKNILF SIGSFKEKQE LLPSIAKLHE LGYKIFATAG 1401 TADFIKEHGI PVHYLEVLSK DEDQKSEYNL SQHLANNLID LYVNLPSANR FRRPASYMSK GYESRRMAVD YAVPLVTNVK NAKLLVEAIA RNISLEVSNR 1501 DAQTSHGTAI LPGLINITSF ATSFDDFEQT TKESLAAGFT FNAFLPHTQA GASVTDYRSL IDAIDAVGAS AYTDYSVSIA ASETNSQSVS DAADNAGSLF 1601 LPFNDFANSK VSALTAQFSS WPNNKPIITD AKTTDLASVL LLASLYNRSI HITGVSSKED LDLIKMAKEK TLQVTCDVAV HSLFLSK**DEL DYPFLPNK**QD 1701 QEYLWENLKD VDCFSIGVLP YLIAKASGTE IAPGMGIKEA VPLLLTAVKA GKLTIGDIVS KFHDNPAKIF NLPKQDAQVV LDLDR**FATVE PVYPEFSK**LR 1801 LRGAVERVSF HNETVVLDGS VLSQIALGKN EVVPRSRFGS TAGIPESPRL GNKRVSFSSD MRRPSLAPET PEPQPAIFEK **LGSELVSQPI AGSLGEIAAL** 1901 **SDYIR**HNNTF LRNNIISVKD ITRSDLHSLF TVAQEMRLAV ERQGVLDLLQ GRVLATMFYE PSTRTSTSFD AAMQRLGGRV VAVDHGSSSV KKGETLQDTI 2001 RTLSCYSDAI VLRHPSEESA DIAAK**YSPVP IINAGNGTK**E HPTQALLDLF TIREELGTVN GITVTFMGDL KYGRPVHSLC HLLRHYQVRV QLVAPKELQI 2101 PAEIRQQLID NNMLIAESEE LTKEILARSD VLYCTRVQEE RFADKEQYQR LKDTYIVDNK ILSNAKQHMC VMHPLPRTNE IREEVDFDQR AAYFRQMRHG 2201 LFIRMALLAM VIGVDF
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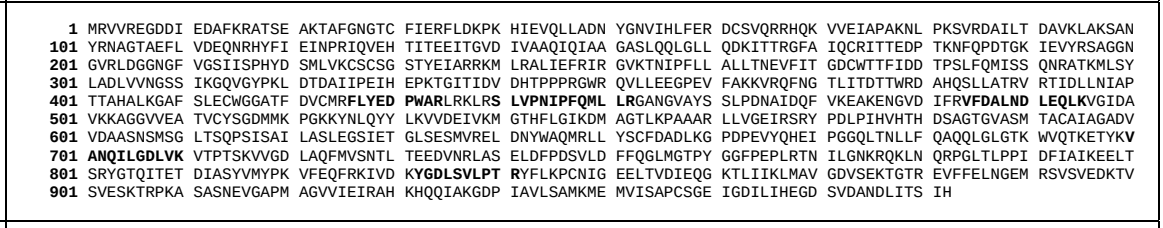
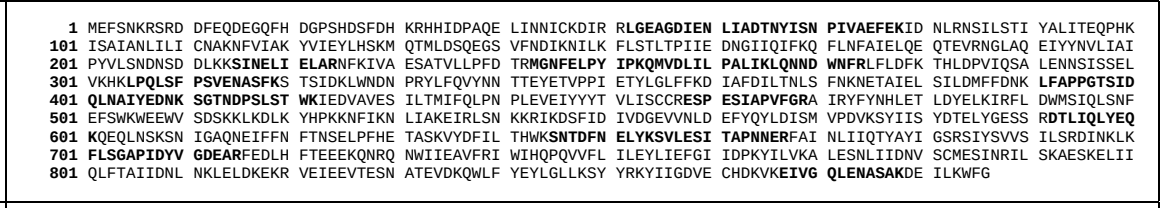
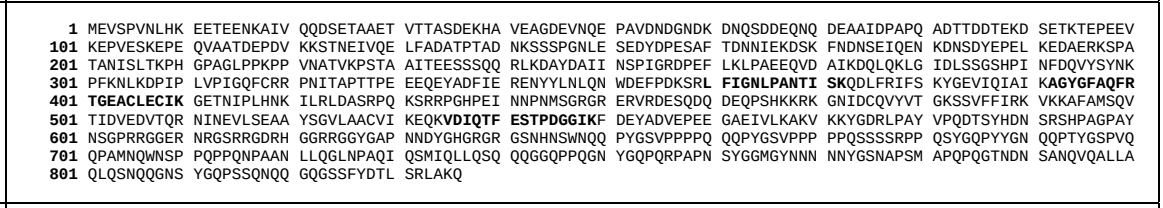
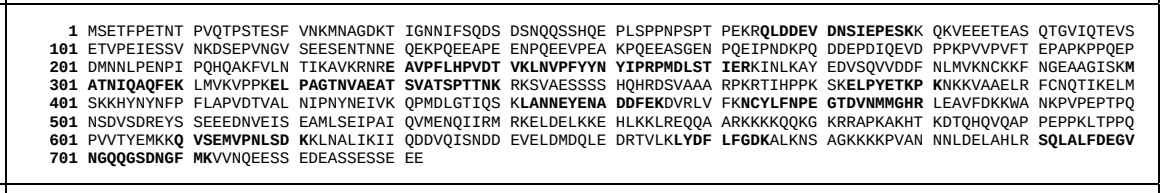
PYC2	
GCR3	
NAB3	
BDF1	
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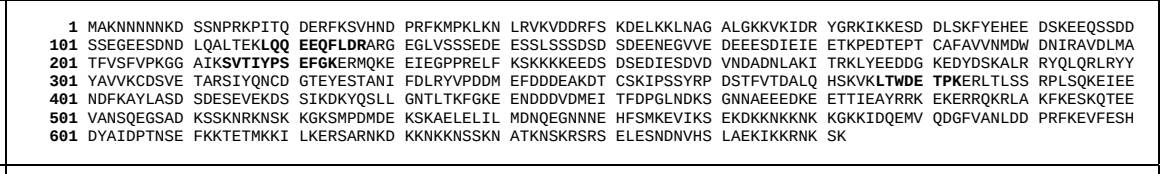
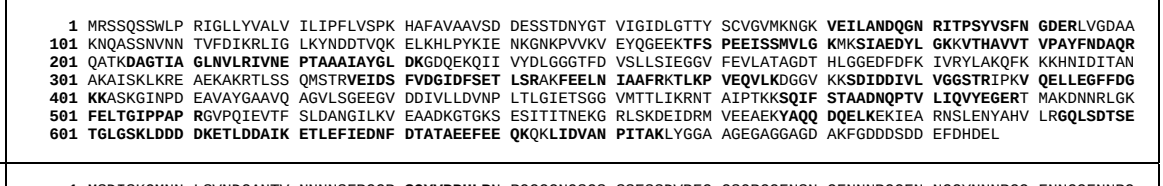
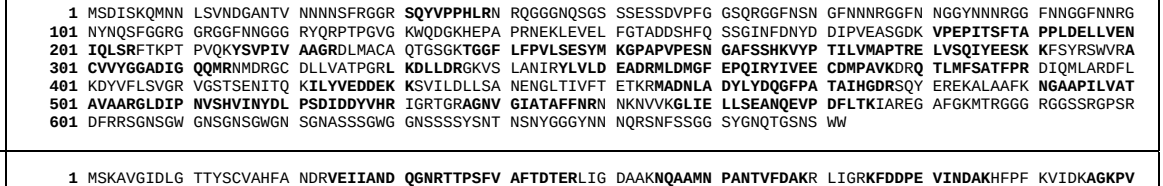
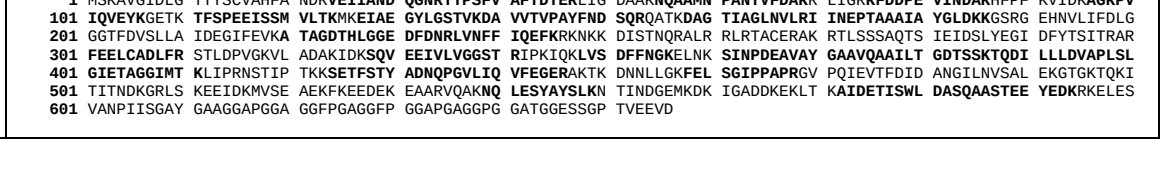
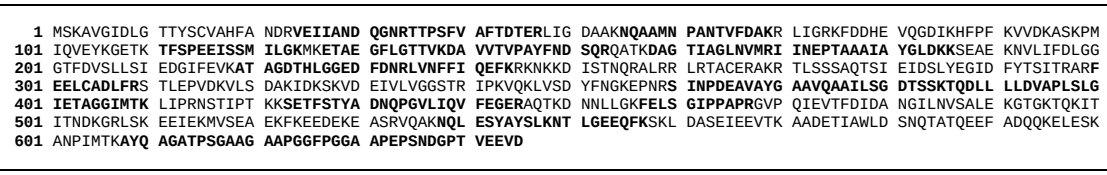
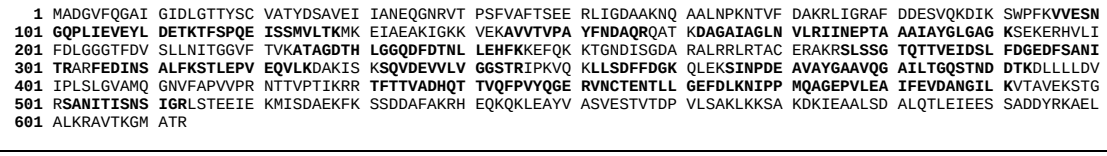
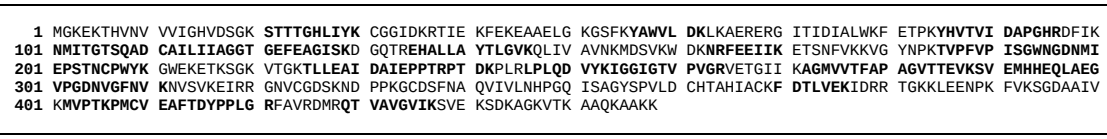
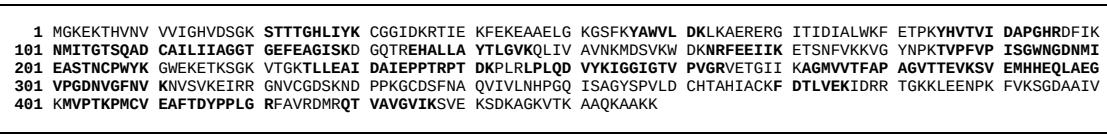
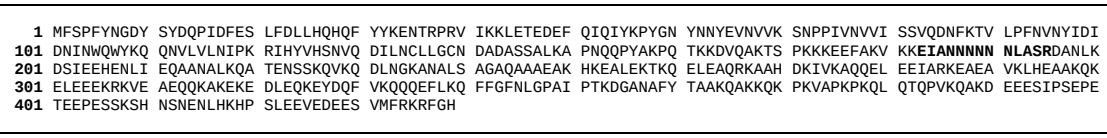
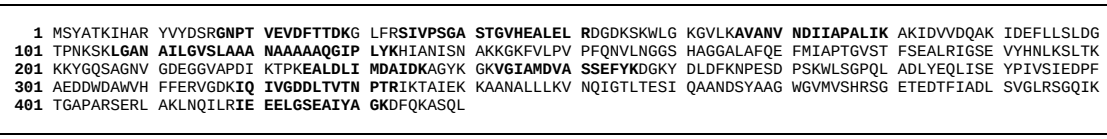
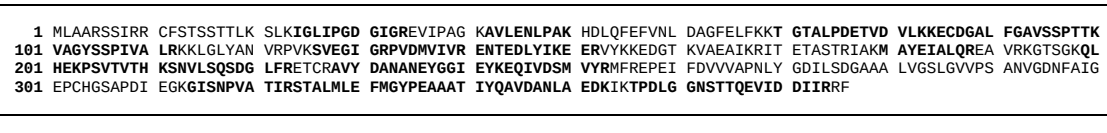
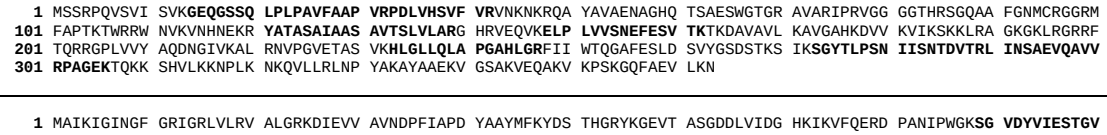
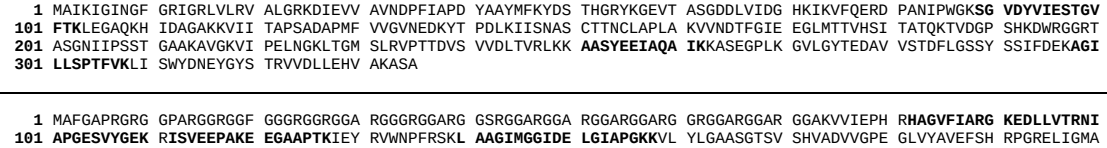
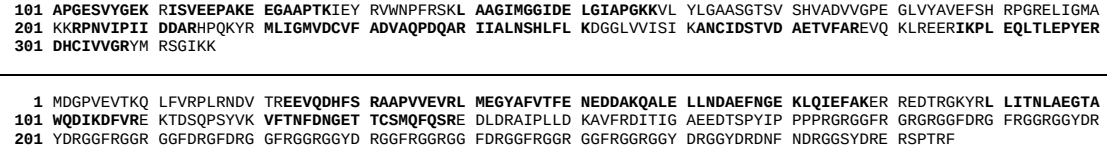
ESF1	
KAR2	
DED1	
HSP70	

SSA1	
SSB1	
TEF1	
TEF2	
ORF 19.7085	
ENO1	
LYS12	
RPL4B	
TDH3	
NOP1	
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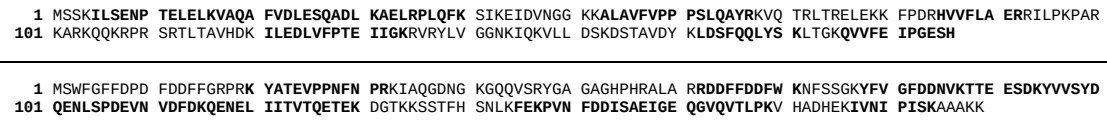
RPL8B	
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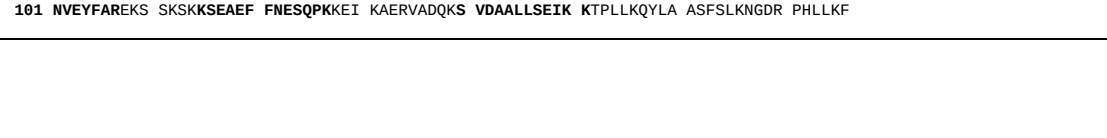
ORF 19.8442	
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