Supplementary information

Legends to supplementary figures

Supplementary Figure 1 Protein sequence alignment of Sld7 and its homologues in yeasts. The sequences were aligned using ClustalW. The conserved amino acids are indicated by white letters on black (100%) or by shaded letters (> 50%). Sc: *Saccharomyces cerevisiae* (NCBI: NP_014703.1); Zr: *Zygosaccharomyces rouxii* (EMB: CAR26697.1); Eg: *Eremothecium gossypii* (NCBI: NP_984299.1); Kl: *Kluyveromyces lactis* (NCBI: XP_455819.1); Ps: *Pichia stipitis* (NCBI: XP_001387485.2).

Supplementary Figure 2 Protein sequence alignment of Sld3 and its homologues in yeasts. The sequences were aligned using ClustalW. The conserved amino acids are indicated by white letters on black (100%) or by shaded letters (> 50%). Sc: *Saccharomyces cerevisiae* (NCBI: NP_011402.1); Zr: *Zygosaccharomyces rouxii* (EMB: CAR27344.1); Eg: *Eremothecium gossypii* (NCBI: NP_985529.1); Kl: *Kluyveromyces lactis* (NCBI: XP_455566.1); Ps: *Pichia stipitis* (NCBI: XP_001382642.2); Sp: *Schizosaccharomyces pombe* (NCBI: NP_592946.1); Nc: *Neurospora crassa* (NCBI: XP_964165.1).

Supplementary Figure 3 Coimmunoprecipitation of Sld3 and Sld7 from yeast cell extracts. YKK21 (*SLD3–9MYC*), YTT4 (*SLD7–3FLAG–HA*), and YTT5 (*SLD7–3FLAG–HA SLD3–9MYC*) cells were disrupted. The Flag-tagged and Myc-tagged proteins were precipitated using anti-Flag M2 beads (**A**) and anti-Myc antibodies (c-myc Ab-1 9E11) on Protein A beads (**B**), respectively, as shown in Figure 2. After precipitation, the supernatants (sup) and precipitates (IP) were subjected to western blotting, and probed with anti-Flag and anti-Myc antibodies. The asterisk indicates a nonspecific band.

Supplementary Figure 4 Cells bearing C-terminal tagged *SLD7* (*SLD7-3FLAG-1HA*: YTT4) grow as the wild-type cells (WT: YTT1). N-terminal deletion of Sld3 confers cell sensitivity to hydroxyurea (HU) and this sensitivity is not suppressed by high copy *SLD7*. YYK13 (*sld3*Δ*::LEU2*) cells that had lost YEp195SLD3 and carried pRS313 encoding the Sld3 (121-668) fragment (Figure 3A) and YEplac112-SLD7 (*SLD7*) or YEplac112 (V) were streaked onto YPD plates (**YPD**) or YPD plates containing 0.2 M HU (**0.2 M HU**) and incubated at 25 °C for four days.

Supplementary Figure 5 Levels of the Sld3 and Sld7 proteins in *sld3* mutant cells. The indicated *sld3* mutant cells were cultivated at 25 °C, disrupted, and subjected to SDS–polyacrylamide gel electrophoresis, followed by western blotting and probing with anti-Sld3 (**A**) or anti-Sld7 (**B**) antibodies. The asterisks indicate nonspecific bands.

Supplementary Figure 6 Specific interaction between Sld3 and Cdc45. (**A**) Reactions were carried out at 4 °C for 30 min, as described in the legend to Figure 7, with or without Cdc45–Flag on the beads. The Sld3 protein retained is estimated to be 0.06 (Sld3) and 0.03 (Sld3-Sld7) pmol. The Sld7 protein retained is 0.02 pmol. (**B**) The same reaction was carried out, except that Sld3 was replaced with purified GINS (1 pmol) (Muramatsu et al, 2010) or Flag–Sld2–P1 (1 pmol) (Tak et al, 2006). Sld5, a subunit of GINS, and Flag–Sld2–P1 were detected with anti-Sld5 and anti-Sld2 antibodies, respectively.

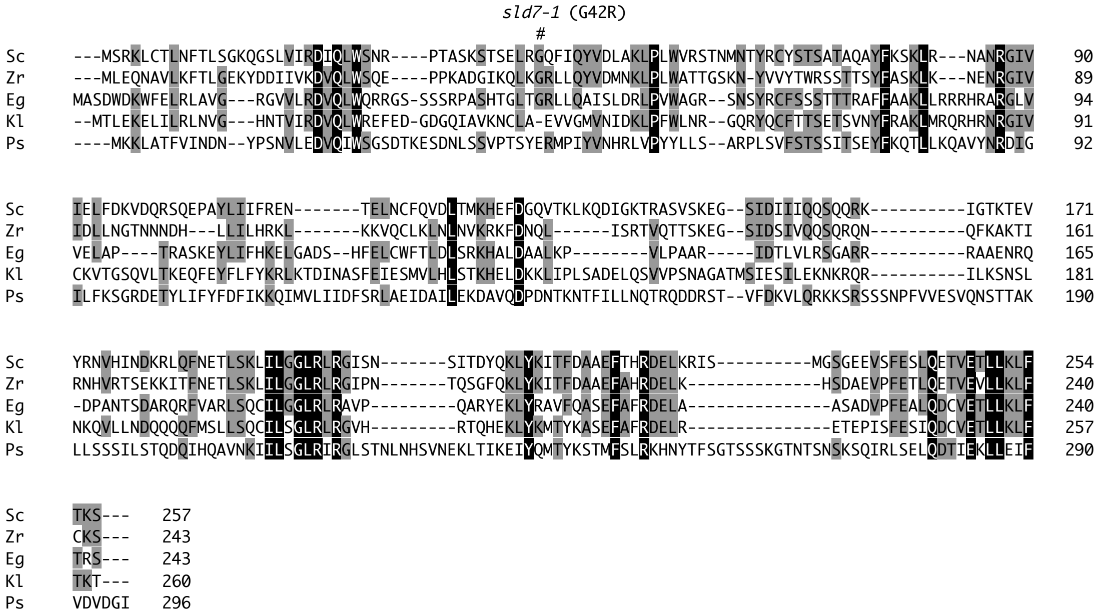
Supplementary Table 1. Yeast strains used in this study

Strain name	Genotype	Source & Reference*
W303-1A	*MAT**a** ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	R. Rotstein
YYK9	*MAT**a** ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 bar1Δ*	Kamimura et al. (2001)
YTT1	YYK9, *sld7Δ::LEU2*	This study
YTT4	YYK9, *SLD7-3FLAG-1HA*	This study
YTT5	YYK9, *SLD7-3FLAG-1HA SLD3-9MYC*	This study
YTT6	YYK9, *5FLAG-SLD3*	This study
YTT7	YYK9, *5FLAG-SLD3 sld7Δ::LEU2*	This study
YTT9	YYK9, *6FLAG-PSF1 sld7Δ::LEU2*	This study
YYT2	YYK9, *6FLAG-PSF1*	Takayama et al. (2003)
YYK14	W303-1A, *sld3-4*	Kamimura et al. (2001)
YYK15	W303-1A, *sld3-5*	Kamimura et al. (2001)
YYK16	W303-1A, *sld3-6*	Kamimura et al. (2001)
YYK17	W303-1A, *sld3-7*	Kamimura et al. (2001)
YYK18	W303-1A, *sld3-8*	Kamimura et al. (2001)
YYK19	YYK9, *sld3-5*	Kamimura et al. (2001)
YYK21	YYK9, *SLD3-9MYC*	Kamimura et al. (2001)
YYK13	*MAT**a** ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 sld3Δ::LEU2* [YEp195SLD3]	Kamimura et al. (2001)
L40	*MAT**a** ade2-1 his3-Δ200 leu2-3,112 trp1-901 ura3-1::LexA-LacZ Lys2::LexA-HIS3*	Bartel and Fields (1995)
BJ2168	*MAT**a** pep4-3 prc1-407 prb1-1122 gal2 leu2 trp1 ura3-52*	Laboratory stock

*Bartel PL, Fields S (1995) Analyzing protein-protein interactions using two-hybrid system. *Methods Enzymol* **254**: 241-263.

Kamimura Y, Tak Y-S, Sugino A, Araki H (2001) Sld3, which interacts with Cdc45 (Sld4), functions for chromosomal DNA replication in *Saccharomyces cerevisiae*. *EMBO J* **20:** 2097-2107.

Takayama Y, Kamimura Y, Okawa M, Muramatsu S, Sugino A, Araki H (2003) GINS, a novel multiprotein complex required for chromosomal DNA replication in budding yeast. *Genes Dev* **17:** 1153-1165.



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                                         #
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Zr     ---MLEQNAVLKFTLGEKYDDIIVKDVQLWSQE----PPKADGIKQLKGRLLQYVDMNKLPLWATTGSKN-YVVYTWRSSTTSYFASKLK---NENRGIV    89
Eg     MASDWDKWFELRLAVG---RGVVLRDVQLWQRRGS-SSSRPASHTGLTGRLLQAISLDRLPVWAGR--SNSYRCFSSSTTTRAFFAAKLLRRRHRARGLV    94
Kl     --MTLEKELILRLNVG---HNTVIRDVQLWREFED-GDGQIAVKNCLA-EVVGMVNIDKLPFWLNR--GQRYQCFTTSETSVNYFRAKLMRQRHRNRGIV    91
Ps     ----MKKLATFVINDN--YPSNVLEDVQIWSGSDTKESDNLSSVPTSYERMPIYVNHRLVPYYLLS--ARPLSVFSTSSITSEYFKQTLLKQAVYNRDIG    92

Sc     IELFDKVDQRSQEPAYLIIFREN-------TELNCFQVDLTMKHEFDGQVTKLKQDIGKTRASVSKEG--SIDIIIQQSQQRK----------IGTKTEV   171
Zr     IDLLNGTNNNDH---LLILHRKL-------KKVQCLKLNLNVKRKFDNQL------ISRTVQTTSKEG--SIDSIVQQSQRQN----------QFKAKTI   161
Eg     VELAP----TRASKEYLIFHKELGADS--HFELCWFTLDLSRKHALDAALKP--------VLPAAR-----IDTLVLRSGARR----------RAAENRQ   165
Kl     CKVTGSQVLTKEQFEYFLFYKRLKTDINASFEIESMVLHLSTKHELDKKLIPLSADELQSVVPSNAGATMSIESILEKNKRQR----------ILKSNSL   181
Ps     ILFKSGRDETYLIFYFDFIKKQIMVLIIDFSRLAEIDAILEKDAVQDPDNTKNTFILLNQTRQDDRST--VFDKVLQRKKSRSSSNPFVVESVQNSTTAK   190

Sc     YRNVHINDKRLQFNETLSKLILGGLRLRGISN-------SITDYQKLYKITFDAAEFTHRDELKRIS----------MGSGEEVSFESLQETVETLLKLF   254
Zr     RNHVRTSEKKITFNETLSKLILGGLRLRGIPN-------TQSGFQKLYKITFDAAEFAHRDELK--------------HSDAEVPFETLQETVEVLLKLF   240
Eg     -DPANTSDARQRFVARLSQCILGGLRLRAVP---------QARYEKLYRAVFQASEFAFRDELA---------------ASADVPFEALQDCVETLLKLF   240
Kl     NKQVLLNDQQQQFMSLLSQCILSGLRLRGVH---------RTQHEKLYKMTYKASEFAFRDELR---------------ETEPISFESIQDCVETLLKLF   257
Ps     LLSSSILSTQDQIHQAVNKIILSGLRIRGLSTNLNHSVNEKLTIKEIYQMTYKSTMFSLRKHNYTFSGTSSSKGTNTSNSKSQIRLSELQDTIEKLLEIF   290

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Eg     TRS---    243
Kl     TKT---    260
Ps     VDVDGI    296
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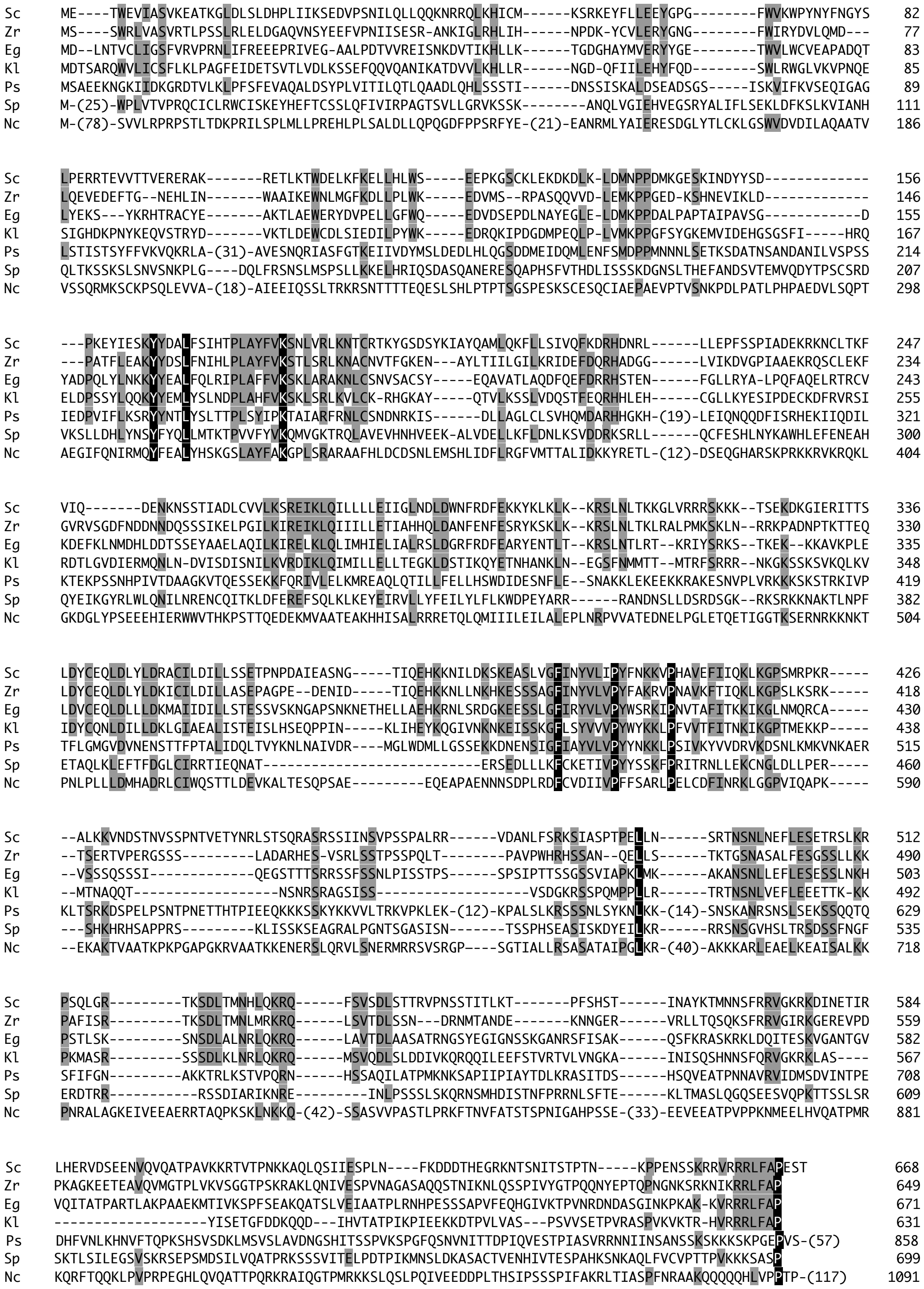
Supplementary Figure 1



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Eg     MD--LNTVCLIGSFVRVPRNLIFREEEPRIVEG-AALPDTVVREISNKDVTIKHLLK------TGDGHAYMVERYYGE--------TWVLWCVEAPADQT    83
Kl     MDTSARQWVLICSFLKLPAGFEIDETSVTLVDLKSSEFQQVQANIKATDVVLKHLLR------NGD-QFIILEHYFQD--------SWLRWGLVKVPNQE    85
Ps     MSAEEKNGKIIDKGRDTVLKLPFSFEVAQALDSYPLVITILQTLQAADLQHLSSSTI------DNSSISKALDSEADSGS-----ISKVIFKVSEQIGAG    89
Sp     M-(25)-WPLVTVPRQCICLRWCISKEYHEFTCSSLQFIVIRPAGTSVLLGRVKSSK--------ANQLVGIEHVEGSRYALIFLSEKLDFKSLKVIANH   111
Nc     M-(78)-SVVLRPRPSTLTDKPRILSPLMLLPREHLPLSALDLLQPQGDFPPSRFYE-(21)-EANRMLYAIERESDGLYTLCKLGSWVDVDILAQAATV   186

Sc     LPERRTEVVTTVERERAK-------RETLKTWDELKFKELLHLWS-----EEPKGSCKLEKDKDLK-LDMNPPDMKGESKINDYYSD------------   156
Zr     LQEVEDEFTG--NEHLIN-------WAAIKEWNLMGFKDLLPLWK-----EDVMS--RPASQQVVD-LEMKPPGED-KSHNEVIKLD------------   146
Eg     LYEKS---YKRHTRACYE-------AKTLAEWERYDVPELLGFWQ-----EDVDSEPDLNAYEGLE-LDMKPPDALPAPTAIPAVSG-----------D   155
Kl     SIGHDKPNYKEQVSTRYD-------VKTLDEWCDLSIEDILPYWK-----EDRQKIPDGDMPEQLP-LVMKPPGFSYGKEMVIDEHGSGSFI-----HRQ   167
Ps     LSTISTSYFFVKVQKRLA-(31)-AVESNQRIASFGTKEIIVDYMSLDEDLHLQGSDDMEIDQMLENFSMDPPMNNNLSETKSDATNSANDANILVSPSS   214
Sp     QLTKSSKSLSNVSNKPLG----DQLFRSNSLMSPSLLKKELHRIQSDASQANERESQAPHSFVTHDLISSSKDGNSLTHEFANDSVTEMVQDYTPSCSRD   207
Nc     VSSQRMKSCKPSQLEVVA-(18)-AIEEIQSSLTRKRSNTTTTEQESLSHLPTPTSGSPESKSCESQCIAEPAEVPTVSNKPDLPATLPHPAEDVLSQPT   298

Sc     ---PKEYIESKYYDALFSIHTPLAYFVKSNLVRLKNTCRTKYGSDSYKIAYQAMLQKFLLSIVQFKDRHDNRL------LLEPFSSPIADEKRKNCLTKF   247
Zr     ---PATFLEAKYYDSLFNIHLPLAYFVKSTLSRLKNACNVTFGKEN---AYLTIILGILKRIDEFDQRHADGG------LVIKDVGPIAAEKRQSCLEKF   234
Eg     YADPQLYLNKKYYEALFQLRIPLAFFVKSKLARAKNLCSNVSACSY-----EQAVATLAQDFQEFDRRHSTEN------FGLLRYA-LPQFAQELRTRCV   243
Kl     ELDPSSYLQQKYYEMLYSLNDPLAHFVKSKLSRLKVLCK-RHGKAY-----QTVLKSSLVDQSTFEQRHHLEH------CGLLKYESIPDECKDFRVRSI   255
Ps     IEDPVIFLKSRYYNTLYSLTTPLSYIPKTAIARFRNLCSNDNRKIS------DLLAGLCLSVHQMDARHHGKH-(19)-LEIQNQQDFISRHEKIIQDIL   321
Sp     VKSLLDHLYNSYFYQLLMTKTPVVFYVKQMVGKTRQLAVEVHNHVEEK-ALVDELLKFLDNLKSVDDRKSRLL------QCFESHLNYKAWHLEFENEAH   300
Nc     AEGIFQNIRMQYFEALYHSKGSLAYFAKGPLSRARAAFHLDCDSNLEMSHLIDFLRGFVMTTALIDKKYRETL-(12)-DSEQGHARSKPRKKRVKRQKL   404

Sc     VIQ-------DENKNSSTIADLCVVLKSREIKLQILLLLEIIGLNDLDWNFRDFEKKYKLKLK--KRSLNLTKKGLVRRRSKKK--TSEKDKGIERITTS   336
Zr     GVRVSGDFNDDNNDQSSSIKELPGILKIREIKLQIIILLETIAHHQLDANFENFESRYKSKLK--KRSLNLTKLRALPMKSKLN--RRKPADNPTKTTEQ   330
Eg     KDEFKLNMDHLDDTSSEYAAELAQILKIRELKLQLIMHIELIALRSLDGRFRDFEARYENTLT--KRSLNTLRT--KRIYSRKS--TKEK--KKAVKPLE   335
Kl     RDTLGVDIERMQNLN-DVISDISNILKVRDIKLQIMILLELLTEGKLDSTIKQYETNHANKLN--EGSFNMMTT--MTRFSRRR--NKGKSSKSVKQLKV   348
Ps     KTEKPSSNHPIVTDAAGKVTQESSEKKFQRIVLELKMREAQLQTILLFELLHSWDIDESNFLE--SNAKKLEKEEKKRAKESNVPLVRKKKSKSTRKIVP   419
Sp     QYEIKGYRLWLQNILNRENCQITKLDFEREFSQLKLKEYEIRVLLYFEILYLFLKWDPEYARR------RANDNSLLDSRDSGK--RKSRKKNAKTLNPF   382
Nc     GKDGLYPSEEEHIERWWVTHKPSTTQEDEKMVAATEAKHHISALRRRETQLQMIIILEILALEPLNRPVVATEDNELPGLETQETIGGTKSERNRKKNKT   504

Sc     LDYCEQLDLYLDRACILDILLSSETPNPDAIEASNG-----TIQEHKKNILDKSKEASLVGFINYVLIPYFNKKVPHAVEFIIQKLKGPSMRPKR-----   426
Zr     LDYCEQLDLYLDKICILDILLASEPAGPE--DENID-----TIQEHKKNLLNKHKESSSAGFINYVLVPYFAKRVPNAVKFTIQKLKGPSLKSRK-----   418
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Kl     IDYCQNLDILLDKLGIAEALISTEISLHSEQPPIN-----KLIHEYKQGIVNKNKEISSKGFLSYVVVPYWYKKLPFVVTFITNKIKGPTMEKKP-----   438
Ps     TFLGMGVDVNENSTTFPTALIDQLTVYKNLNAIVDR----MGLWDMLLGSSEKKDNENSIGFIAYVLVPYYNKKLPSIVKYVVDRVKDSNLKMKVNKAER   515
Sp     ETAQLKLEFTFDGLCIRRTIEQNAT---------------------------ERSEDLLLKFCKETIVPYYSSKFPRITRNLLEKCNGLDLLPER-----   460
Nc     PNLPLLLDMHADRLCIWQSTTLDEVKALTESQPSAE---------EQEAPAENNNSDPLRDFCVDIIVPFFSARLPELCDFINRKLGGPVIQAPK-----   590

Sc     --ALKKVNDSTNVSSPNTVETYNRLSTSQRASRSSIINSVPSSPALRR------VDANLFSRKSIASPTPELLN------SRTNSNLNEFLESETRSLKR   512
Zr     --TSERTVPERGSSS---------LADARHES-VSRLSSTPSSPQLT--------PAVPWHRHSSAN--QELLS------TKTGSNASALFESGSSLLKK   490
Eg     --VSSSQSSSI-------------QEGSTTTSRRSSFSSNLPISSTPS------SPSIPTTSSGSSVIAPKLMK------AKANSNLLEFLESESSLNKH   503
Kl     --MTNAQQT------------------NSNRSRAGSISS-------------------VSDGKRSSPQMPPLLR------TRTNSNLVEFLEEETTK-KK   492
Ps     KLTSRKDSPELPSNTPNETTHTPIEEQKKKSSKYKKVVLTRKVPKLEK-(12)-KPALSLKRSSSNLSYKNLKK-(14)-SNSKANRSNSLSEKSSQQTQ   629
Sp     ---SHKHRHSAPPRS---------KLISSKSEAGRALPGNTSGASISN-------TSSPHSEASISKDYEILKR------RRSNSGVHSLTRSDSSFNGF   535
Nc     --EKAKTVAATKPKPGAPGKRVAATKKENERSLQRVLSNERMRRSVSRGP----SGTIALLRSASATAIPGLKR-(40)-AKKKARLEAELKEAISALKK   718

Sc     PSQLGR---------TKSDLTMNHLQKRQ------FSVSDLSTTRVPNSSTITLKT-------PFSHST------INAYKTMNNSFRRVGKRKDINETIR   584
Zr     PAFISR---------TKSDLTMNLMRKRQ------LSVTDLSSN---DRNMTANDE-------KNNGER------VRLLTQSQKSFRRVGIRKGEREVPD   559
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Ps     SFIFGN---------AKKTRLKSTVPQRN------HSSAQILATPMKNKSAPIIPIAYTDLKRASITDS------HSQVEATPNNAVRVIDMSDVINTPE   708
Sp     ERDTRR-----------RSSDIARIKNRE---------INLPSSSLSKQRNSMHDISTNFPRRNLSFTE------KLTMASLQGQSEESVQPKTTSSLSR   609
Nc     PNRALAGKEIVEEAERRTAQPKSKLNKKQ-(42)-SSASVVPASTLPRKFTNVFATSTSPNIGAHPSSE-(33)-EEVEEATPVPPKNMEELHVQATPMR   881

Sc     LHERVDSEENVQVQATPAVKKRTVTPNKKAQLQSIIESPLN----FKDDDTHEGRKNTSNITSTPTN-----KPPENSSKRRVRRRLFAPEST       668
Zr     PKAGKEETEAVQVMGTPLVKVSGGTPSKRAKLQNIVESPVNAGASAQQSTNIKNLQSSPIVYGTPQQNYEPTQPNGNKSRKNIKRRLFAP          649
Eg     VQITATPARTLAKPAAEKMTIVKSPFSEAKQATSLVEIAATPLRNHPESSSAPVFEQHGIVKTPVNRDNDASGINKPKAK-KVRRRLFAP          671
Kl     -------------------YISETGFDDKQQD---IHVTATPIKPIEEKKDTPVLVAS---PSVVSETPVRASPVKVKTR-HVRRRLFAP          631
Ps     DHFVNLKHNVFTQPKSHSVSDKLMSVSLAVDNGSHITSSPVKSPGFQSNVNITTDPIQVESTPIASVRRNNIINSANSSKSKKKSKPGEPVS-(57)     858
Sp     SKTLSILEGSVSKRSEPSMDSILVQATPRKSSSVITELPDTPIKMNSLDKASACTVENHIVTESPAHKSNKAQLFVCVPTTPVKKKSASP          699
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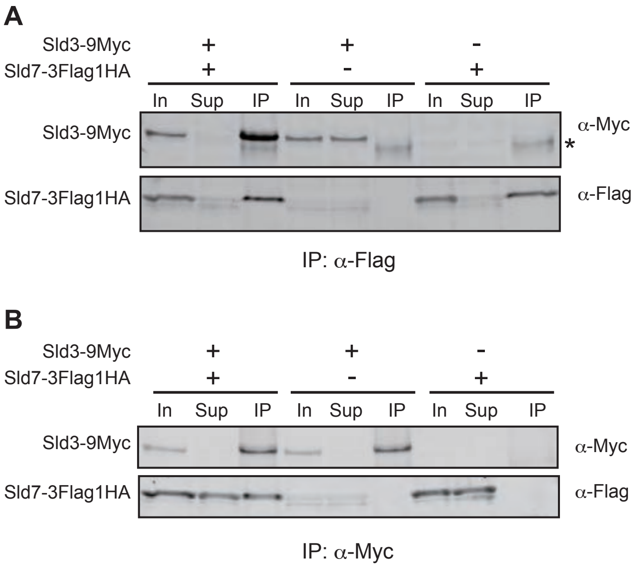
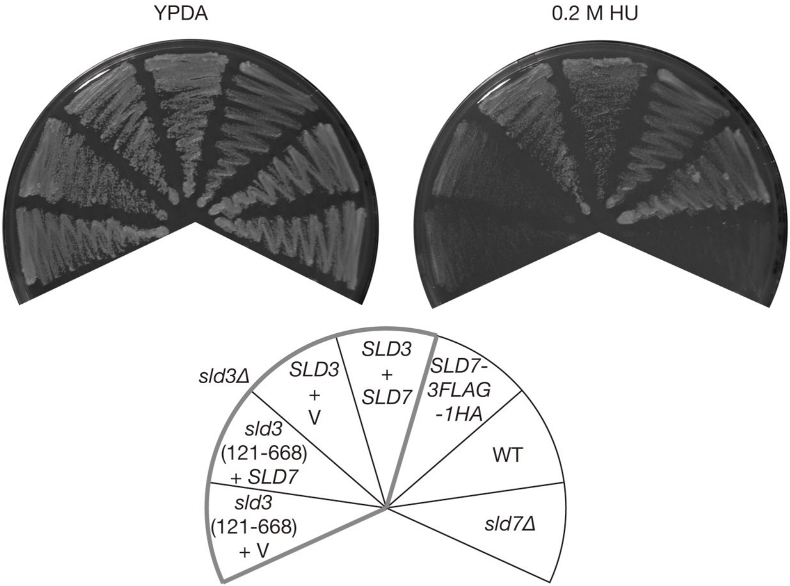
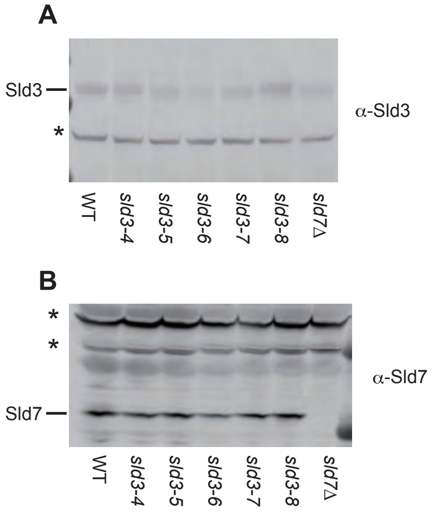
Supplementary Figure 2



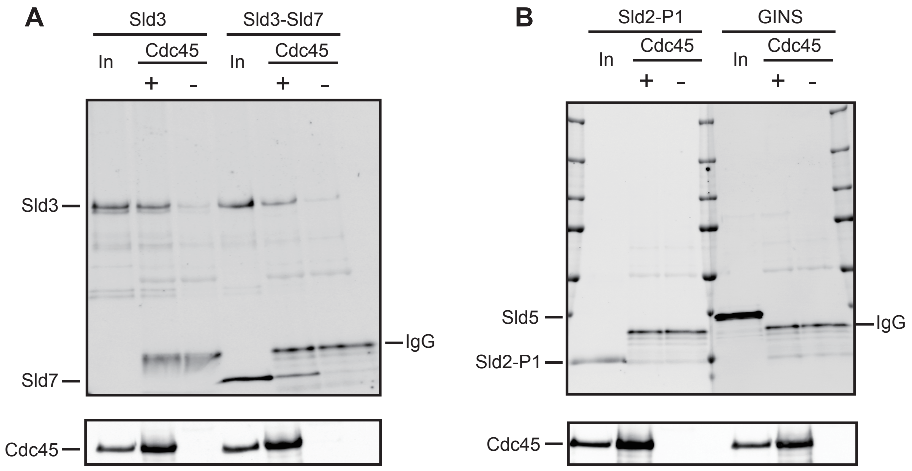
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6