Supplemental Figure Legends

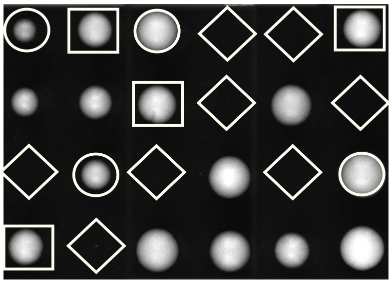
Figure S1: Synthetic-lethal interactions in DNA repair. (A) The indicated parental strains were mated, sporulated and the spores microdissected onto YPD plates. Spore clones were photographed after 7 days at 30C. Microscopic examination of the *tdp1*Δ *wss1*Δ spore clones indicated that the spores had germinated and formed microcolonies of up to 50 cells. (B) The indicated parental strains were treated as in (A).

Figure S2. Stability of Slx5 truncation mutants. The WT *SLX5* ORF, or fragments corresponding to the indicated N- and or C- terminal truncations, were cloned downstream of the *GAL10* promoter in vector pRS425 (*LEU2/2μm/CEN*) and transformed into PSY3884. Following growth in galactose for 6 hours, yeast extracts were prepared, resolved by 10% SDS-PAGE and immunoplotted for Slx5. The blot was later reprobed with antibody against Rfa1 as a loading control.

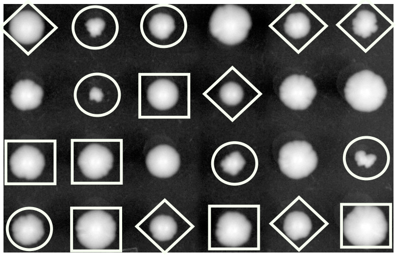
Fig. S3: Suppressor mutations and the nibbled colony phenotype A. PSY3497 (*tdp1*Δ *wss1*Δ *top1-Q668-STOP*) was crossed to NJY3292 (*tdp1*Δ *wss1*Δ + pNJ7478). Diploids were sporulated, micro-dissected vertically and allowed to grow for 4 days at 30˚C. The *top1-Q668-STOP* mutants are circled. Red color indicates the presence of pNJ7478. **B.** Strains PSY3791-PSY3794 containing the indicated *SLX5* allele integrated at *LEU2* were crossed to wild-type strain JMY3104 and treated as in (A). The respective *slx5* mutants are circled. **C.** Strain PSY3884 (*slx5*Δ) was transformed with single-copy *CEN/ARS* plasmids containing either no insert or the indicated alleles of *SLX5.* Transformation plates were photographed after 4 days incubation at 30˚C.

Figure S4. Amino acid sequence analysis of a variety of STUbLs from fungi and higher eukaryotes. Sequences are annotated as follows: lysine residues are represented by a blue **K** with yellow highlighting, and arginines are indicated by a red R. In each protein, the longest aa sequence uninterrupted by a lysine is shaded in gray, and its length is presented at the top of the sequence in parentheses along with its percentage of the total protein. RING domains are underlined. Putative SIMs are highlighted in green.

A



tdp1Δ x *wss1Δ*

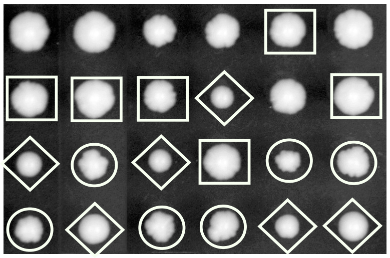


□ *tdp1Δ*

○ *slxΔ* or *wss1Δ*

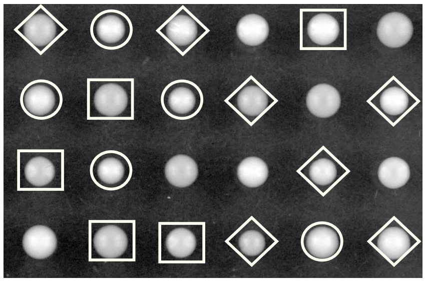
◇ *slxΔ tdp1Δ*
or *wss1Δ tdp1Δ*

tdp1Δ x *slx5Δ*



tdp1Δ x *slx8Δ*

B

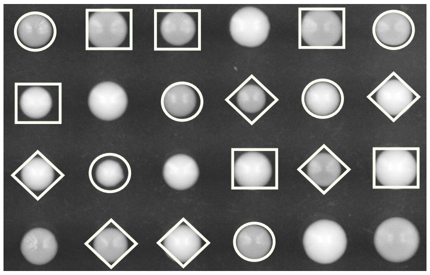


□ *tdp1Δ*

○ *esc2Δ*

◇ *esc2Δ tdp1Δ*

esc2Δ x *tdp1Δ*



□ *tdp1Δ*

○ *mms21*

◇ *mms21 tdp1Δ*

mms21-11 x *tdp1Δ*

Figure S1

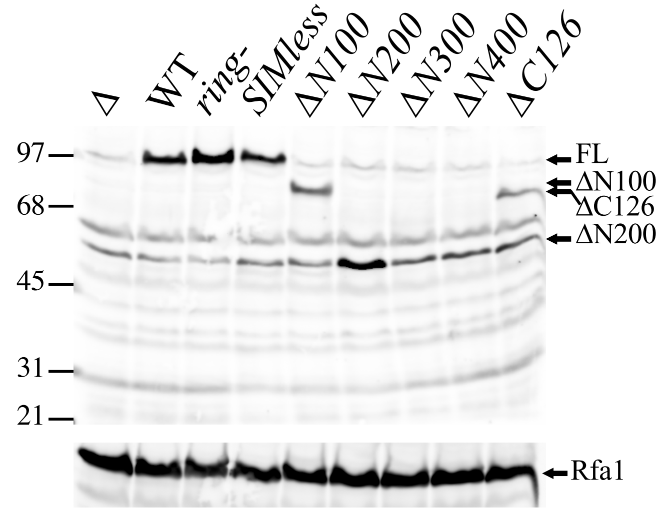
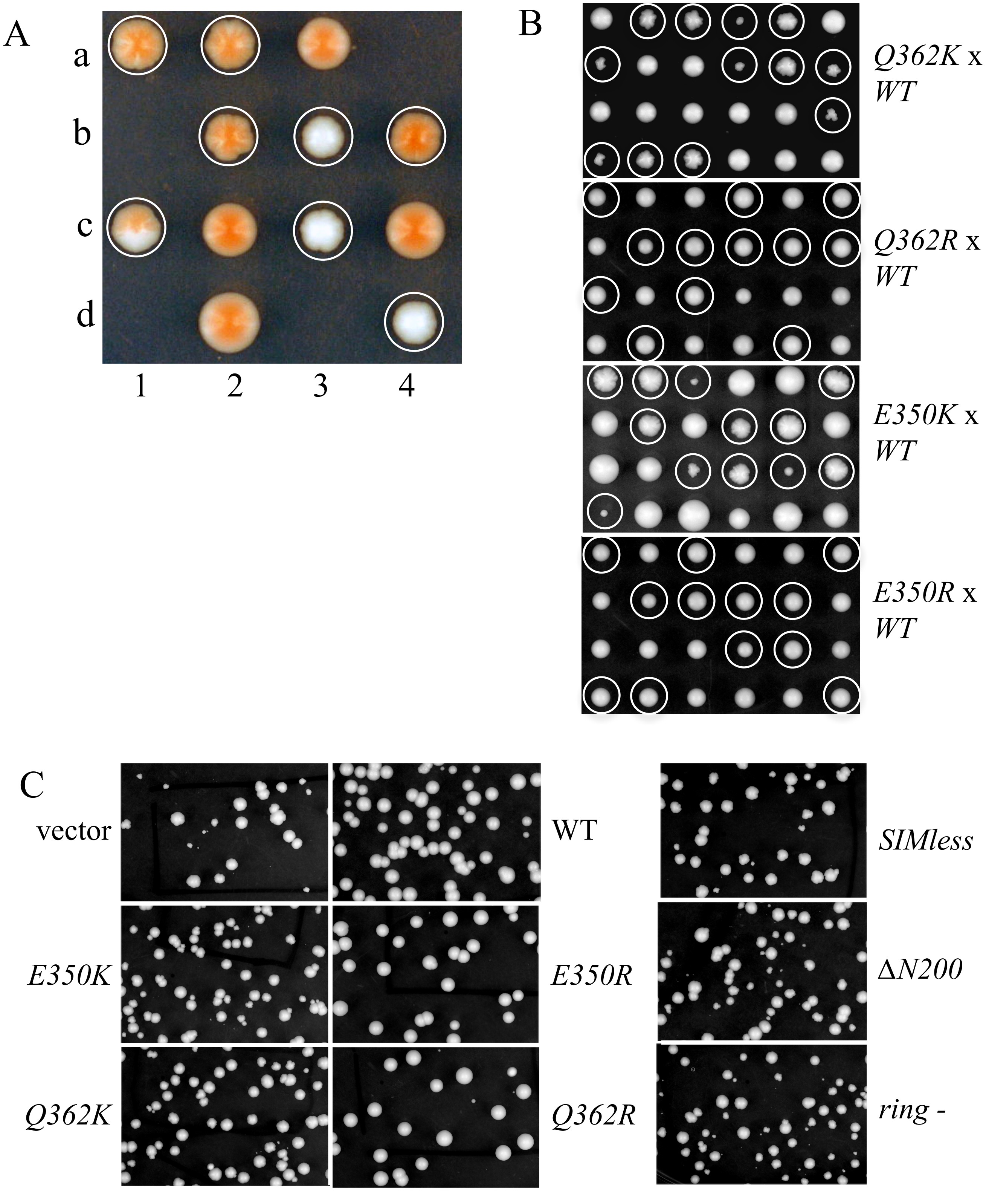

Δ
WT
ring-
SIMless
ΔN100
ΔN200
ΔN300
ΔN400
ΔC126
97
68
45
31
21
FL
ΔN100
ΔC126
ΔN200
Rfa1

Figure S2



A
a
b
c
d
1
2
3
4
B
Q362K x *WT*
Q362R x *WT*
E350K x *WT*
E350R x *WT*
C
vector
WT
SIMless
E350K
E350R
Δ*N200*
Q362K
Q362R
ring -

Figure S3

Figure S4

A. Fungal Slx5 homologs.

>SceSLX5.619 (S. cerevisiae) (398=64%)
MHSDTNGRTKSNNSPSDNNPNETVILIDSDKEEDASIREANLPVRLYPDRRVGRRRDALNRFVRSDSRSRNSQRTHIT
ASSERPDFQANNDDITIIREVGRFFGDDGPIDPSAHYVDLDQEPGSETLETPRTIQVDNTNGYLNDNGNNNESDDGLTIV
EERTTRPRVTLNLPGGERLEVTATTTDIPIRRSFEFQEDLGASRRQLLRRSATRARNLFVDRSDENDEDWTDDTHNLPE
AIQRARRESRMRMSRRIAERQRRVQQQRVSSDENISTSIRLQSIRERIQSYTPDIRSAFHRAESLHEFRSILQNVAPITLQ
ECEEELMALFTEFRNQLLQNWAIDRVRNTQEEALRLHREALERQERTAGRVFHRGTLRESITNYLNFNGEDGFLSRLW
SGPALSDADEERHTQNIIDMIQEREERERDVVMKNLMNKTRAQQEEFEARAASLPEGYSASFDTTPKMKLDITKNGKE
ETIIVTDDDLAKTLEDIPVCCLCGAELGVGIPDDFTGISQKDRGVSFEGLVSKYKFHCPYQTLARPSMLDRDLSKRTFIAS
CGHAFCGRCFARIDNAKKKSKMPKKKLAQLKGSAHPDNYGPKLCPADSCKKLIRSRGRLKEVYF

>CalSlx5.602 (Candida albicans) (430=71%)
MNSDNNPTREPGFKRRKIEPEIRNNNSPQQATSNPGSNDQVIEILSDEETAPPLQSTNTVVHLDTESEDSSLSDIDYRD
DDNNGDANNDVQIVSVNTANPTPLSTPSSNLNHSGVPTNTNTNNNNNNNNRNGEDRDEDDDDLQILGVRNIYDPNNG
RLSTPVGDIPFVREDNDYTIVEDAPRRNLNPAIARAIRRILDMERRYPLPSQEQIRQILMENRPWMQVSQSEFRDRRVTF
DNSAGGGGEEGGGGGGESQPRPQNYHEALQESARRALAELAAMREAAERAERENSSRQGENSFGFGTPRRSYVEL
TENETSQPVAVPLPRQRSVHFTEEALRRGNGGPTFSESRDGNIIRRERRQPVRYTDSPQFLHNLPFSGAVVHRVPHEL
RFLGNTFHDVGFALDNLMDDSDTPRGFNSAIERSIMQRIEDDTNRMIDRRIAQETNYNKKTKNEIEKKIANQDERHTSNI
KSNENLVCELCNIVLGEGAPDDFKGDIRYNEKFSKYCETYNCQAPWFCVYPFTEVDIELSKRIFVAKCGHLFCGRCVK
NIGNRPKSRKSKTSNANSSTAEISILNPKFYAPSTCPELQCQKKFSSKSFTEV
YF

>KlaSlx5.607 (Kluvermyces lactis) (417=69%)
MTEQGCVIEIDSDPELDAQIREEYAPRRLIPDRRRNRPRDHLSRFVSNAEDRNVAPIAPATDLPVSTDGEDLEITAEVSL
RPDGPINDDAEYINLDNYRASNNGTVVVDHREGDDDLMLVEERIQESGRPVLLQLPAGQTLQVDASWNELPVGRSFQ
NQVYRNPNQLRRLRRHIDHATAMFMGGYNQSGTGDSDFSDNDADYIPPDIVNERNQYTMQTRMREQRERQNQMQR
LQRAADETIDSLDPDLRSLFFESQSLSQFREGLARFNFPPARLEQLLGQYRDFRARAMQSWAEGRRTRSMSYQNRNS
LRPQRTRSGTQQNHSRGSNGDSRDNALYQQMLFGMPDDDENMDGNNFTGTTQSLQRIISNTMNRRFGNLPFGVGPE
FGFYEDGDDAANTELIIRMIQEREERDASTRTKKLNEVTKSQQQKYIDKANSLPEGYSASFNTAPLMSMTMENEGREE
TVMVTDDVAAKTYIDVPVCVLCGVELGVGIPDDWKGVSEEDKGVSFEALQSHYKFHCPYQTLARPTLVDRDLSRRTYV
SSCGHTFCGRCQVRIKNARDISAKEKNKLKNSQGPSHPDNYGPKKCPAEGCNSNLRKKGIMREVYF

>AgoSlx5.557 (Ashbya gossypii) (366=66%)
MTDPNAARNAVIEIESDPEEDQRIINQYRQTRLVPDRRLNRPRDAANRFVSVSGRVAAAAGEGDDEVEIVNEVYLDDEG
PIDSTADFVDLDEQQGPSSSMVINHDGGGDDDVAIIEERTTHPTFLLNLPNGQTLRISGSVHDRPLRRSFETQRTARAN
MLRRAARSAQRLFMNENDGEPSEREASDREYLPQSVLRQRQQALMQERLQRQREHARQHEHIADPQVDALAPELRSI
FYHAETLHEVRTMLNASGLGTSAAAMQDLLQLYMQFRSRQINNWARQRAQEFRHGSAREADHAQDDSASRARLRNP
ALNAQRRNSFTSYVLARSLGGFGVWPNDFFGDDDEVTQNIIDIIQAREERDLDSRKRKYMEDTKSQQQAFVARAQRLP
EGYYASFDPTPKMKMTLEKNGKTEEVVVADDLAAKSYVEVPVCCLCGVELGLGIPDDFAGISAVDRGVSFESLVSKYD
FHCPYQALAHPSIADRDLSRKTFVAHCGHTFCGRCFARINNAKRFSKISKKRLAELHGPSHPDNYGPRVCPAEGCRGQ
LRTRGKMREVFF

>YliSlx5.332 (Yarrowia lipolytica) (179=54%)
MTPQRECDPITVLDDETEVVGSTDCIPEPASEDPFIVISSDEEDNTNTDTGNYRSRTPEIIDISDTERPDEPEEEEEDPRL
NMERIFMNSFQQDQHEYLNNGFGFFRQPHYPVRAERNPGQPSFSPRQFAHYHHFISRLQGIPGLGFGGPNMREDPAF
EEDLQRAMRASMEDSLSNFGPKKVSHPEPPTTTTEGYTRSVRKRQVVCCALCRSDLGVGIPPETKKPSKSSSPKKRK
RFSRFSDLTDVERTLSKRMFFTKCGHVYCGLCVQFIKRRRTSRQKKEDKSNINVDCITDFKGRYVDTPFSLIDHCVVPG
CKEKLPRSRGKFFKEMFT

>SpRFP1.254 (S. pombe) (Schizosaccharomycetes) (163=80%)
MQFNGSNGIDESSVIDLTRSPSPPVETSISSTNIIDLDAIPDDSFPSSPVLSPRRRRMNRRRNERSRNFPSNHLSYLEDMI
YLGPQVSTRRSSSRRDLMGMIARTFPEFSSVNSLSPSLFQLIVNRMRFDAIHPEWTNGSDDEYFSNHFEESYDDFTSSL
ENIKQSYKPPGPPKSGFTRSFNNDTLMVCPRCQEPLGTSKSKEKSALWATKCGHVYCGSCAKVLKTSKRSQSKCLV
NDCGRYLNTKNAMWELFY

>SpRFP2.205 (S. pombe) (Schizosaccharomycetes) (115=56%)
MNLHGLELPGRDQRLSPEVIDLTEDIEDDGADVSEVTLLDLTRIPEFQPRRRIRTSRNHLDANLSNVPTINSIPSPVTRPP
VAVGGGIFYGARRTRNRSQTQRRTLLENGFRNSRKKAQDSSNSIAERVSPPPGFCYDVHPHNNIACAKCGNELVSDE
KKSIFAAKCGHLFCSTCAKELRKKTVPCPVQHCRKRITKKFIFPLYL

>UmaGI71004004.641 (U. maydis) (Ustilaginomycetes) (254=40%)
MTVYGYNRKQGYRNARFGRALRHFCSAAAQVWTEIQPRIELHGSLCQIGLIFSIYLPIPSQCGGVALREASGVAQNPTF
NWPAKPGASPCVQALFESIINSKLVLFMNSDHPLPRPTVVAGSRPSSRSSSRGRSIASSGHTSRSASPAQRTNSIPRNR
SAGSSNRTPLRMSRSASQEAGASTTFDTVANSRSEGQTDVHSATNSSDLIVISSDSSDGDQEDDDEFVAQWPEHPVV
RAPAPPSDFLEVSVVRRAQPLSSTPRAELFSPPPSAGRPAEPIRLSSASEDRRVGVSADPHTGDFLVRPMPIVRPTDST
AYSDSDGSFSIVSAQLAPSHRPRSDHPITEQRLRALNLAMPTKSPPRSKPPPIEHPLLSTYTCPICFDAPTNLSVTPCGH
FFCGECLFQALKSQAVQRGAMEEEATLYSFGGLFASLVPGATFGAALGGVGAQTASGQVYSGGGPAGSAGGAGRGR
GGRGGGGGRGGGSGSRGGSVGGGGGRNKPDPLAGQCPVCRAKIKGAFNGRDKNGIVGLRLTMGTPVNDPRKENG
QMKSDSVEKNVDLSASDETYDDDEVIFPTSKQAEATAKTDSFESSVADEKSGSPRGPSPVNNASGSTISTSSPMQGR
CVRTRKRPRATSDTSAHQTE

B. Fungal Slx8 homologs.

>SceSlx8.274 (134=49%)
MARRPDNQNPEGENLRIKRVRLESVRQNDEEEENEVSRTQNIVTDNRHDSPEAVVEIIGERALENTSEEDGDDDLSLF
RALEEDPGSDHNTSNNDSGNHDRETMHTEEPEASSGNNITLTNNVEELHTMDVLSQTANTPSASPMLDAAPPTTKPG
TNSKEQTVDLTADAIDLDAEEQQVLQISDDDFQEETKEAPKEYGAAKDYRCPICFEPPETALMTLCGHVFCCPCLFQM
VNSSRTCRQFGHCALCRSKVYLKDVRLIILRKKQVKKKVKS

>AgoSlx8.241 (117=49%)
MAGERGGAALGAPLGGDEADGAQAEEWNTDGGTQESGASSESEAPARKRRRVGRNRQMYDSDDSDYDGSPPQVA
VGDSAAGRAAAATARADESGDGSQSSVELADEGSAGGGEEPDIVYLEARGPVSGSVVDVEAEARLQQVVEIPDEEVG
EEQTRLGGDRPVECKKAMDYKCPICFDPPEAALMTPCGHIYCTVCLFQMVNSSRGYRRNGQCALCRKDVKLKEVGLV
VLRKKRVRKQSAE

>KlaSlx8.279 (96=34%)
MVNEDDSLRVVSSSDTTDQLQLVPGLSMGQSTVRTGEDEDERIVTGRRRHELQESETDIEEEEEELRRKSRRISRTSIV
SIDDADILSETQHVTEIGDTTEQSNLATAPPQEVSDNEEPAIISTADYGVAYQNVQDQSAEAQDQEEEEEEEELHEAVG
EGDDDDIKVLTEEEQIKAQQVIEIDDEDETEGHKSGKDMSETPLETKKAADYVCPICMEPPEAALVTKCGHVFCTTCLY
GMVNSSKGNGRRNGLCALCRENVKLQDLRLIVMRKNRIRKPN

>YliSlx8.254 (89=35%)
MSSTILFSSRFTRRLSLLTISHTPNQVSPVLKPKQSPFFSPTKHHTKLQTVHRQQVQRQAMSGTPLSVHSSASTPESR
MLTINDTDTDNSSQPLYIDSGDEETHNVIDLDASDASDYDSNDDDGLDLSAPPPDQHSKLREVKCPICLEPPDRLCVTE
CGHLYCGDCVFKALSSGVRASDSVGECSICRKSVVYKNVVFLETRMGPLLKDDDDEYETDDSIEDDRNVKVQKMHES
LTYDLDEVDTLDLTPSLPDA

>CalSlx8.129 (57=44%)
MSTPNVTNISSDDEDDEIEVLEFRKLTQDLIDNPTQPEERRITKKLSDVECPICFDEVRRAITTSCGHVFCLECIERSISSS
HARGQVRSSQRGRGLCPLCRKQVVFKETIPLKMKKAEKIDKPDLPPK

>NcaSlx8.263 (160=61%)
MGKLRSDGPSKDEPEPREKESVKRRRIDNDHHNEATTGVNDEEESIDVLDNDDDSVEIVHENAAPASPEDLLIRALEE
GLAETPSMEDNLHETNATATPALNTDNTQGQTAENIDESVVINSQPVTSNTRTPTGVSAASSVVTPRVLDTSGFDPLIEA
IDLEAAEQQVIQIPDDEIEDEIQRGKKQETYRPVTEYRCPICMDPPETALIAPCGHVFCCDCLFQMVNSSRTYRKDGHC
ALCRKEVRLRDVKLIILRKKRIKKVA

>SpoSlx8.269 (161=60%)
MPPAHKRDTNVRNLSAPYNIPSQSARVAAGNAAINRRRSSPVENSPGNGFPVSEDATDYPSGTTSENESLPLNRAPRS
LREVASELAQEETLPVETSDLNIDVESEVFDLEDINFQNDADDINQRFTYNNHPASVENSLTNVNSIHAQPTTISDMIDLT
DETSYDPRKQKFEQGKNPSTTNAEIEKEEPSKKQVVPSSQRLADYKCVICLDSPENLSCTPCGHIFCNFCILSALGTTA
ATQKCPVCRRKVHPNKVICLEMMLGSQKKKS

>NcrSlx8.428 (281=66%)
MDDDFPLSDAAYDDIFADLDLDALPPPLSSVAHPNPGVPVPQSLPSLAPLLQQYPAPNPNSNSDNSQIHGQRQQHPHN
IPGQTTNILHSLSIQPQAPSPANAYITPRTSLPCPHPQARAEPGRHLDNRQSQAAYLPPASRALPPPRPLPPALSFLLSG
SSSTPDSRGTSFDNNADPALNTNLATGSRAPGLFVTPHRSDREPNSDDFLNELASRDFSSPSLPPQTPRHLGDLLSPS
RNSCNNTMPSRRDESAAEGSRRRSRGTSHNIVSLPTLPPQSSGAPKRKREDGLEIRAHKRKAHVLLSSDDEKDPFGD
DNFMDVVDLADTEEVPESMRAKPRPKNEIKLSAFQCVICMDNVTGLTVTHCGHLFCSECLHSALTIDPTKRTCPVCRQ
KIDKAPIGGKWTAKAKGYYPLELKLVTKKSLGKRAAQ

C. RNF4 homologs.

>HsaRNF4.190 (H. sapiens)(149=78%)
MSTRKRRGGAINSRQAQKRTREATSTPEISLEAEPIELVETAGDEIVDLTCESLEPVVVDLTHNDSVVIVDERRRPRRNA
RRLPQDHADSCVVSSDDEELSRDRDVYVTTHTPRNARDEGATGLRPSGTVSCPICMDGYSEIVQNGRLIVSTECGHVF
CSQCLRDSLKNANTCPTCRKKINHKRYHPIYI

>RnoRNF4.194 (R. norvegicus)(83=43%)
MSTRNPQRKRRGGAVNSRQTQKRTRETTSTPEISLEAEPIELVETVGDEIVDLTCESLEPVVVDLTHNDSVVIVEERRR
PRRNGRRLRQDHADSCVVSSDDEELSKDKDVYVTTHTPRSTKDEGTTGLRPSGTVSCPICMDGYSEIVQNGRLIVSTE
CGHVFCSQCLRDSLKNANTCPTCRKKINHKRYHPIYI

>MmuRNF4.194 (M. musculus)(85=44%)
MSTRNPQRKRRGGTVNSRQTQKRTRETTSTPEVSLETEPIELVETVGDEIVDLTCESLEPVVVDLTHNDSVVIVEERRR
PRRNGRRLRQDHADSCVVSSDDEELSRDKDVYVTTHTPRSTKDDGATGPRPSGTVSCPICMDGYSEIVQNGRLIVST
ECGHVFCSQCLRDSLKNANTCPTCRKKINHKRYHPIYI

>GgaRNF4.194 (G. gallus) (92=47%)
MSTTPRKRRGGSANSRQAQKRSRLIASTTEMASEGEPIELEESAGEEVVDLTCESSDPVVVDLTHNDSIVIVEENQQRR
RNLRLRGQRQSDSCVLSSDDEDETRDNDVYVTDKVSRELGPLEDETASSKPSGTVSCPICMDGYSEIVQSGRLIVSTK
CGHVFCSQCLRGSLRNANSCPTCRKKLTHRQYHPIYI

>BtaRNF4.186 (B. taurus)(85=46%)
MSTRKRRGGTVNSRQAQKRTRESTSPPEMALEAEPVELVESAGDEIVDLTCESLEPVVVDLTHNDSVVIVEERRRPRR
NGRRLRQDHADSCVVSSDEEELARDKDVYVTTHSPRSAATGFRPSGTVSCPICMDGYSEIVQNGRLIVSTECGHIFCS
QCLRDSLKNANTCPTCRKKMSHKRYHPIYI

>XtrRNF4.190 (X. tropicalis)(92=48%)
MSMAQRKRKGAEPGHSKSSKRRAPGSTAAMTAATEPIELESGEEVVDLTCESTEPVVVDLTNNDLSINDSVVIVEDTP
RQRRALSRPSQQTTSCVLSSDDEDSRHADHFAANKDISSQAYGSSRSSSGKVSCPICMDSYSEIVQSGRLIVSTKCGH
IFCSQCLRDALKNAPSCPTCRKKLNHKQYHPIYV

>DreRNF4.184 (D. rerio) (123=67%)
MSTTVTQRKRRTSTTCSRRGNSKRNRAQMSQTVMETIDVLENDRTNSEDVVDLTCEGSEPAVVDLTNNDSIVVVEDG
VQRRVGPCTESYVLSSDEEEESSLRLSPGLLSSLRDSSRARSTPGAISCPVCMDVYSEIMDSGRLMVSTKCGHLFCSQ
CIRDSLSRAHSCPTCRKKLTHKQYHPIYI

>DmeDegringoladeA.319 (D. melanogaster) (248=78%)
MSDSTIFRFNSSILDQSADSNVSSSSSNSSVSDSSVDSHSSIESNSGHNTSVESQSSAESDSSADSHSSLERPSPVERD
LSVESDSSSATNTSESSVGEASNQLQSPQSNNPLNMANLSVSTEDAHSQIRSLTQDVIQMEAELDRMNRYCEEVVAQI
DTFATRTSTPTTRRIRRRTSPVEVIDLSHLDRAPPVRSARNRDPDAFIDLCTPEGPRSRTVNLHSNDSLTILPRRSAEND
PVVDLDVASPPKRVNRDIDESQKEELYKCPICMDSVSKREPVSTKCGHVFCRECIETAIRATHKCPICNKKLTARQFFRI
YL