Supporting Information

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	Species	IDCL
Group I	*S. cerevisiae*	D I D A D F L K I E E L
	S. paradoxus	D I D A D F L K I E E L
	S. mikatae	D I D A D F L K I E E L
	S. bayanus	D I D A D F L K I E E L
	A. gossypii	E I D A D F L E I D Q I
	K. lactis	E I E T D F L E I S D M
	S. kluyveri	D I D A D F L E I E G M
	L. thermotolerans	D I D A D F L E I E G M
	K. waltii	D I D A D F L E I E G M
	P. pastoris	D I D A D L L D V G P M
	V. polyspora	D I D A D F L K I E E L
	Z. rouxii	D I D A D F L K I E E L
	C. glabrata	D I D A D F L K I E E L
	S. castellii	D I D A D F L K I E D L
	D. hansenii	D I D S E F L K I D D M
	C. guilliermondii	D I D S E F L K I D D M
	S. stipitis	N I D S E F L K I D D M
	L. elongisporus	N I D S E F L K I D D M
	C. parapsilosis	N I D S E F L K I D D M
	C. albicans	D I D S E F L Q I D D M
	C. tropicalis	D F D S E F L Q I E D M
	C. dubliniensis	D I D S E F L Q I D D M
	C. lusitaniae	D I D S E F L R I D D M
Group II	*Y. lipolytica*	T I D Q E H L G I P D T
	N. crassa	D I D Q E H L G I P D T
	M. oryzae	D I D Q E H L G I P D T
	S. sclerotiorum	D I D Q E H L G I P D T
	B. fuckeliana	D I D Q E H L G I P D T
	A. nidulans	D I D Q E H L A I P E T
	A. fumigates	D I D Q E H L A I P E T
	N. fischeri	D I D Q E H L A I P E T
	A. oryzae	D I D Q E H L A I P E T
	A. niger	D I D Q E H L A I P E T
	A. flavus	D I D Q E H L A I P E T
	A. clavatus	D I D Q E H L A I P E T
	P. chrysogenum	D I D Q E H L A I P E T
	C. immitis	D I D Q E H L A I P D T
	P. nodorum	D I D Q E H L G I P E T
	S. japonicas	D I D Q E H L G I P D I
	S. octosporus	D I D Q E H L G I P D I
	S. pombe	D I D Q E H L G I P D I
	C. neoformans	D I D Q E H L G I P D T
	L. bicolor	D I D S D T L G I P D T
	C. cinerea	E I D A D A L T I P D T
	U. maydis	D I D S E H L G I P D T
	M. globosa	D I D T E H L G I P D T
	T. stipitatus	D I D Q E H L A I P E T
	A. capsulatus	D I D Q E H L A I P E T
	A. terreus	D I D Q E H L A I P E T
	C. globosum	D I D Q E H L G I P D T
	P. anserine	D I D Q E H L G I P D T
	G. zeae	D I D Q E H L G I P D T
	P. marneffei	D I D Q E H L A I P D T

Fig. S1. IDCL sequence of PCNA from 53 fungal species retrieved from the fungal orthogroup database (http://www.yeastgenome.org/cgi-bin/blast-fungal.pl). Residues that are differentially conserved between group I and group II sequences (red and blue, respectively; Fig. 2) are colored according to Lesk (1). Small nonpolar residues (G, A, S, T) are highlighted in yellow, hydrophobic residues (C, V, I, L, P, F, Y, M, W) are highlighted in green, polar residues (N, Q, H) are highlighted in magenta, negatively charged residues (D, E) are highlighted in red, and positively charged residues (K, R) are highlighted in blue.

1. Lesk A (2008) *Introduction to Bioinformatics* (Oxford Univ Press, Oxford).

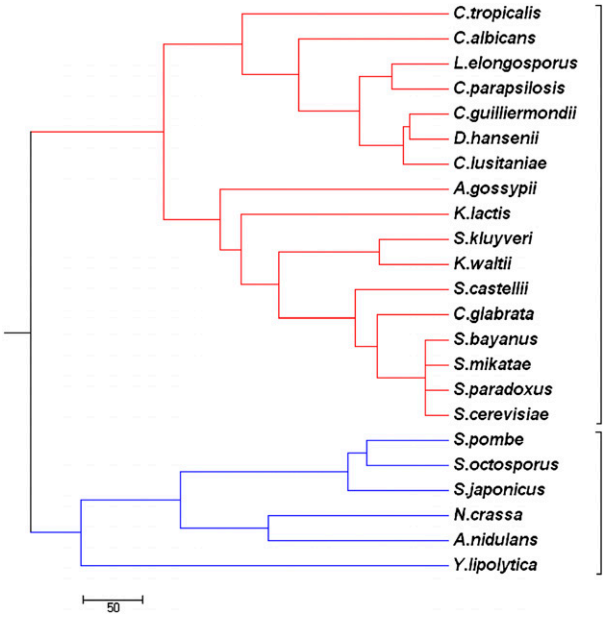

Fig. S2. PCNA protein phylogenetic tree indicating that PCNA sequences diverged into two main branches, separating those same species that are contained in group I and group II (Fig. 2*B*).

Species	C – terminal sequence of PCNA																												
S.cerevisiae	R	L	S	S	E	A	P	A	L	F	Q	F	D	L	K	-	S	G	F	L	Q	F	F	L	A	P	K	F	N	D	E	E
S.castelii	R	L	S	S	E	A	P	A	L	F	Q	F	D	L	S	-	S	G	F	L	Q	F	F	L	A	P	K	F	D	D	E	E
K.waltii	K	L	S	A	E	T	P	A	L	F	E	F	G	L	Q	-	S	G	Y	L	Q	F	F	L	A	P	K	F	N	E	E	E
K.lactis	K	L	S	E	E	A	P	A	L	F	Q	F	D	I	S	-	S	G	N	L	Q	F	Y	L	A	P	K	F	D	E	E	E
S.kluyveri	K	L	S	A	E	T	P	A	L	F	E	F	K	L	Q	-	S	G	Y	L	Q	F	F	L	A	P	K	F	N	E	E	E
A.gossypii	K	L	S	A	D	T	P	A	L	F	Q	F	N	L	D	G	A	G	H	L	Q	Y	F	L	A	P	K	F	N	E	E	E
C.lusitaniae	K	L	A	D	K	T	P	A	L	F	E	Y	K	L	D	A	G	G	Y	L	R	F	Y	L	A	P	K	F	D	E	D	D
D.hansenii	K	L	A	D	K	T	P	A	L	F	E	Y	K	L	D	A	G	G	Y	L	R	F	Y	L	A	P	K	F	D	E	D	D
C.tropicalis	K	M	A	D	K	T	P	A	L	F	E	F	K	M	E	S	G	G	Y	L	R	Y	Y	L	A	P	K	F	D	D	E	E
C.albicans	K	L	A	D	K	T	P	A	L	F	E	F	K	M	Q	S	G	G	Y	L	R	F	Y	L	A	P	K	F	D	D	D	E
C.parapsilosis	K	L	A	D	K	T	P	A	L	F	E	F	K	L	D	V	G	G	Y	L	R	F	Y	L	A	P	K	F	D	E	D	E
Y.lipolytica	G	M	S	S	E	V	P	I	M	V	E	Y	L	L	P	-	N	G	Y	L	R	F	Y	L	A	P	K	I	G	D	E	D
A.nidulans	S	L	S	Q	E	V	P	L	L	V	E	Y	G	L	G	-	S	G	H	L	R	F	Y	L	A	P	K	V	N	W	-	-
N.crassa	C	L	S	N	E	V	P	L	L	V	E	Y	N	I	S	A	S	S	Y	L	R	F	Y	L	A	P	K	I	G	D	E	E
S.pombe	S	M	S	N	D	V	P	L	L	V	E	Y	K	M	E	-	S	G	F	L	R	F	Y	L	A	P	K	I	g	E	E	D

Fig. S3. Sequence alignment of the C-terminal region of PCNA derived from species shown in Fig. 2 indicates changes mainly in five amino acids (highlighted in colors) that are differentially conserved between group I and group II sequences (red and blue, respectively). The correlated changes between the C-terminal and the IDCL regions can indicate coevolution between these regions of PCNA (Fig. 2). Fungal species were assigned to group I or group II according to their PCNA IDCL sequence (Fig. 1 and Fig. S1). Residues that are differentially conserved between the two groups are colored according to Lesk (1). Small nonpolar residues (G, A, S, T) are highlighted in yellow, hydrophobic residues (C, V, I, L, P, F, Y, M, W) are highlighted in green, polar residues (N, Q, H) are highlighted in magenta, negatively charged residues (D, E) are highlighted in red, and positively charged residues (K, R) are highlighted in blue.

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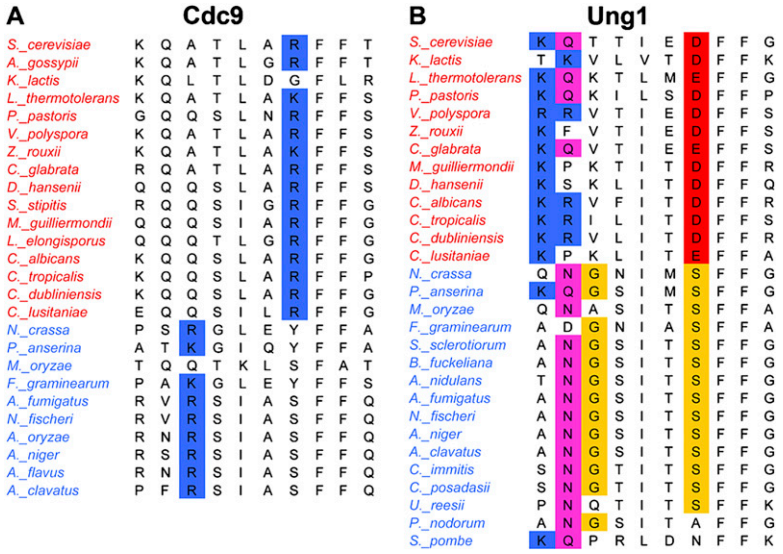

Fig. S4. PIP box sequence of Cdc9 (*A*) and Ung1 (*B*), PCNA partners from a variety of different fungal species. Sequences were retrieved from the KEGG orthology database (http://www.genome.jp/kegg/). Fungal species were assigned to group I (red) or group II (blue) according to their PCNA IDCL sequences (Fig. S1). Residues that are differentially conserved between the two groups are colored according to Lesk (1). Small nonpolar residues (G, A, S, T) are highlighted in yellow, hydrophobic residues (C, V, I, L, P, F, Y, M, W) are highlighted in green, polar residues (N, Q, H) are highlighted in magenta, negatively charged residues (D, E) are highlighted in red, and positively charged residues (K, R) are highlighted in blue.

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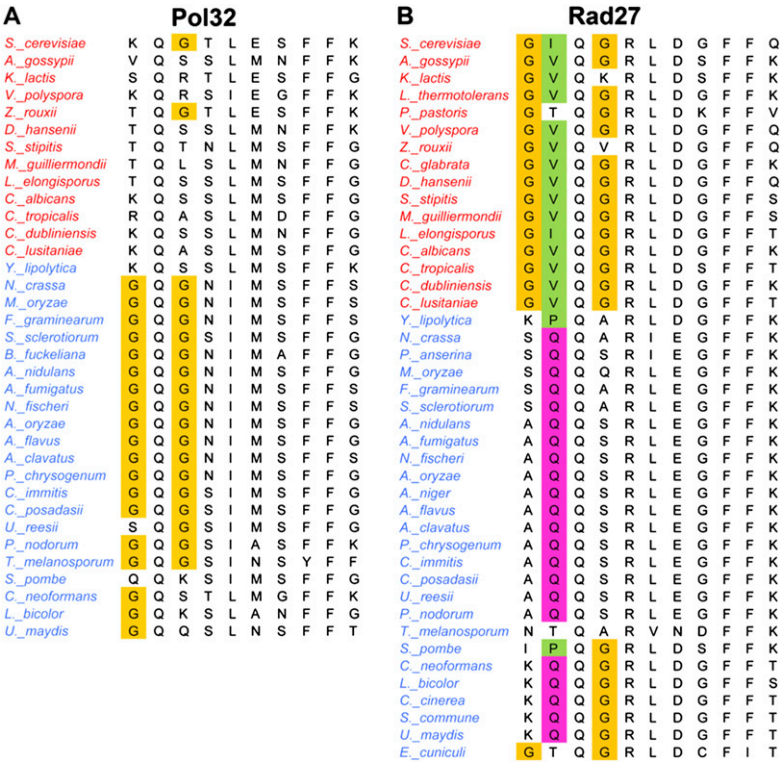

Fig. S5. PIP box sequence of Pol32 (*A*) and Rad27 (*B*) partners from a variety of different fungal species. Sequences were retrieved from the KEGG orthology database (http://www.genome.jp/kegg/). Fungal species were assigned to group I (red) or group II (blue) according to their PCNA IDCL sequence (Fig. S1). Residues that are differentially conserved between the two groups are colored according to Lesk (1). Small nonpolar residues (G, A, S, T) are highlighted in yellow, hydrophobic residues (C, V, I, L, P, F, Y, M, W) are highlighted in green, polar residues (N, Q, H) are highlighted in magenta, negatively charged residues (D, E) are highlighted in red, and positively charged residues (K, R) are highlighted in blue.

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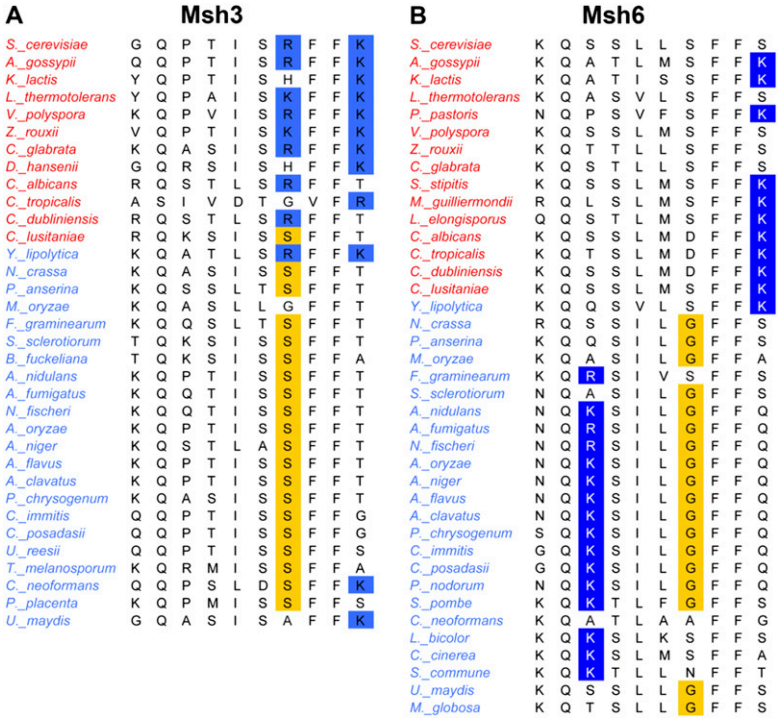

Fig. S6. PIP box sequence of Msh3 (*A*) and Msh6 (*B*) partners from a variety of different fungal species. Sequences were retrieved from the KEGG orthology database (http://www.genome.jp/kegg/). Fungal species were assigned to group I (red) or group II (blue) according to their PCNA IDCL sequence (Fig. S1). Residues that are differentially conserved between the two groups are colored according to Lesk (1). Small nonpolar residues (G, A, S, T) are highlighted in yellow, hydrophobic residues (C, V, I, L, P, F, Y, M, W) are highlighted in green, polar residues (N, Q, H) are highlighted in magenta, negatively charged residues (D, E) are highlighted in red, and positively charged residues (K, R) are highlighted in blue.

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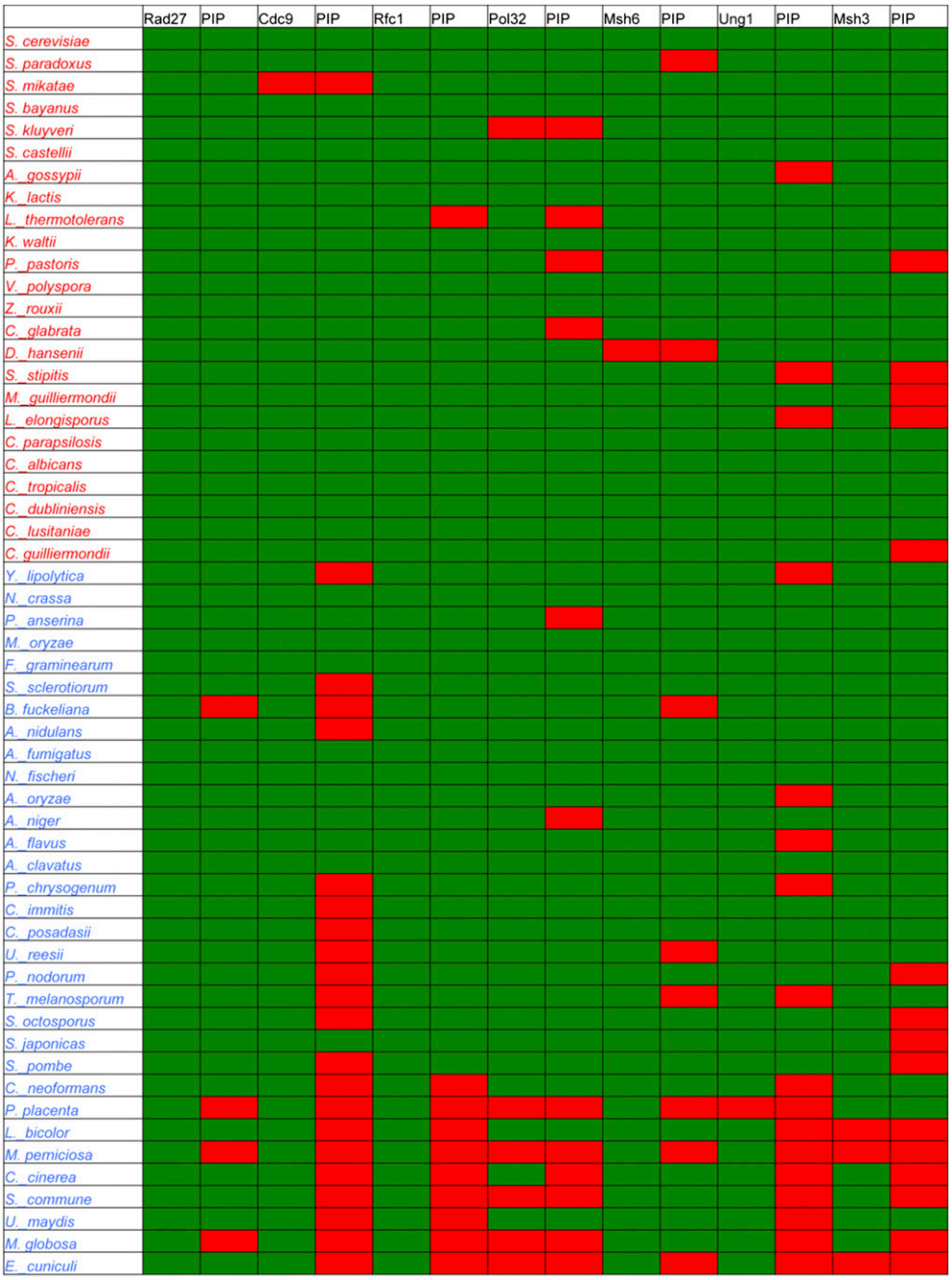

Fig. S7. Analysis of PCNA partners containing a PIP box that facilitates interaction with the IDCL region of PCNA. The data are presented as a heat map, where the presence of a partner or conserved PIP box is depicted in green and its absence is presented in bright red. Fungal species were assigned to group I (red) or group II (blue) according to their PCNA IDCL sequence (Fig. S1).

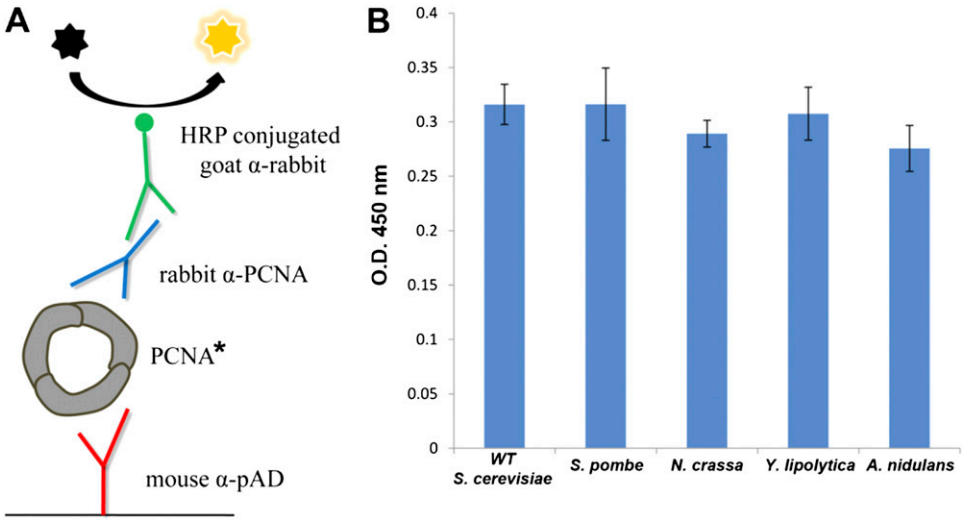

Fig. S8. ELISA used for the detection of the expression levels of chimeric *S. cerevisiae* PCNA containing the group II IDCL region. (*A*) Schematic illustration of the ELISA used to detect the relative expression levels of the different strains. PCNA* is PCNA that is fused to the AD. (*B*) ELISA signal for WT ScPCNA and the chimeric PCNA variants containing *S. pombe*, *N. crassa*, *Y. lipolytica*, or *A. nidulans* IDCL region. Signals at 450 nm are presented following subtraction of the background signal from cells expressing only the AD under identical conditions (a detailed description is provided in *Methods*). The values shown indicate similar expression levels as WT ScPCNA, with deviations of up to 15%, and represent averages of at least three independent repeats.

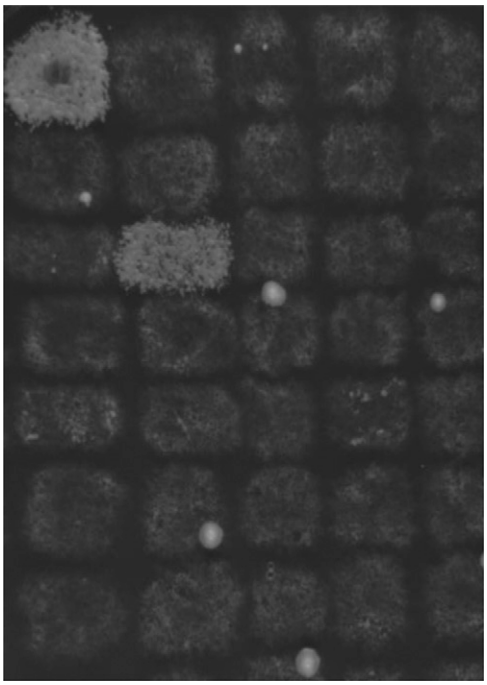

Fig. S9. Screening of a *Ylpcna* random mutant library for mutants that can complement the *ScPCNA (POL30)* deletion in *S. cerevisiae*. A typical agar plate containing 5-FOA was used to examine the function of the *Ylpcna* mutants as the sole source of PCNA in *S. cerevisiae*. Two mutants that can complement the *pol30* mutation and facilitate growth of *S. cerevisiae* are clearly observed on the plate. Using this screening approach, 2,500 mutants were examined to identify variants that can function in *S. cerevisiae*.

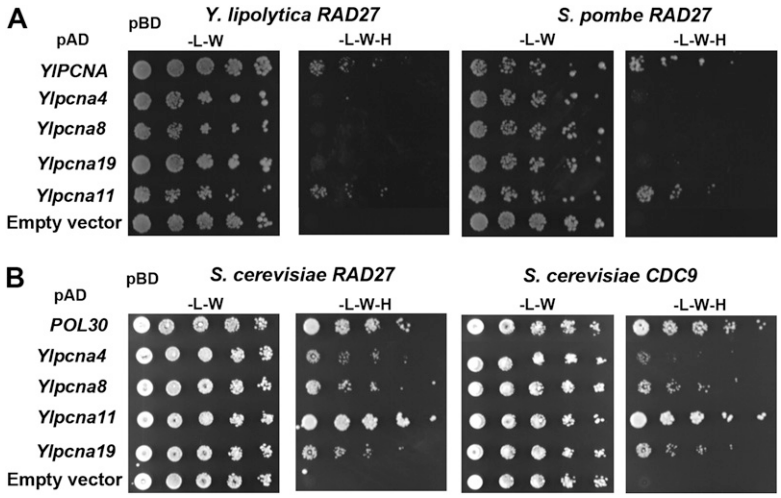

Fig. S10. Yeast two-hybrid analysis of the *Ylpcna* mutants from the directed evolution experiment with *RAD27* orthologs from *S. pombe* and *Y. lipolytica* (*A*) and *RAD27* and *CDC9* from *S. cerevisiae* (*B*). Interactions with all partners are detected only in *YlPCNA* and *Ylpcna11* that combine sequence motifs from *YlPCNA* and *ScPCNA* (Fig. 6).

Table S1. Y2H analysis of *SpPCNA (PCN1)* and *YlPCNA* interactions with *S. cerevisiae* PCNA partners

	S. cerevisiae PCNA partners*						
Chimeric PCNA	Pol32	Rad27	Rad30	Cdc9	Msh6	Ung1	Rrm3
ScPCNA (POL30)	++++	+++	+++	++++	+++	++++	+
SpPCNA (PCN1)	++++	+++	+++	++++	+++++	++++	++++
YlPCNA	++++	+++	+++	++++	+++	++++	++
pcn1-POL30-IDCL	++++	+++	+++	++++	+++	++++	+
Ylpcna-POL30-IDCL	++++	+++	+++	++++	+	++++	+

*WT or PCNA mutants were fused to the pAD, and the different partners were fused to the pBD. Each Y2H strain was spotted in five dilutions (e.g., Fig. 5), and the interactions were scored according to the growth of the different dilutions on selective plates lacking histidine (–, no growth; +, growth observed at first serial dilution; ++, growth observed at second serial dilution; +++, growth observed at third serial dilution; ++++, growth observed at fourth serial dilution).

Table S2. Y2H analysis of evolved *Ylpcna* mutants

	S. cerevisiae PCNA partners				*S. pombe* PCNA partners				*Y. lipolytica* PCNA partners		
	Pol32	Rad27	Cdc9	Ung1	Pol32	Rad27	Cdc9	Ung1	Pol32	Rad27	Ung1
POL30	++++	++++	++++	++++	–	–	–	–	–	–	–
*Ylpcna*4	–	+++	+	+++	–	–	–	–	–	–	–
*Ylpcna*8	+++	+++	+++	+++	–	–	–	–	–	–	–
*Ylpcna*11	++++	++++	++++	++++	+	+	+	+	+	+	ND
*Ylpcna*19	+++	+++	+++	+++	–	–	–	–	–	–	–

POL30 or *Ylpcna* mutants were fused to the pAD, and the different partners were fused to the pBD. Each Y2H strain was spotted in five dilutions (e.g., Fig. 6), and the interactions were scored according to the growth of the different dilutions on selective plates lacking histidine (–, no growth; +, growth observed at first serial dilution; ++, growth observed at second serial dilution; +++, growth observed at third serial dilution; ++++, growth observed at fourth serial dilution). ND, not determined.

Table S3. Sequences of oligonucleotides used in this study

	DNA oligonucleotide sequence 5′-3′
fr-pRS-PCNA	TTTCACTCACAGCAACAAGCAGCAAGCACTAAGTACGCAGTCAAAAGAGAGAAAAAATGTT AGAAGCAAAATTTGAAGAAGCATC
rev-pRS-PCNA	GTTTTTTTTTTGTTTATTATTTTTAGTATACAACTATATAGATAATTTACATTTTTATTC TTCG TCATTAAATTTAGGAGCC
fr-waltii-IDCL	GATATTGATGCGGATTTTCTGGATATTGAAGGCATGCAGTACGACTCCACCCTGTCATTG
rev-waltii-IDCL	TACCCGCTTTAACTAGACAAACTACGCCTATAACTACATCAATTTCAGAGAGTATTCCGCTATAC
fr-gossypii-IDCL	GAAATTTATGCGGATTTTCTGGAAATTGATCAGATTCAGTACGACTCCACCCTGTCATTG
rev-gossypii-IDCL	AATCTGATCAATTTCCAGAAAATCCGCATAAATTTCCATCAATTTCAGAGAGTATTCGGCTATAC
fr-hansenii-IDCL	GATATTGATAGCGAATTTCTGAAAATTGATGATATGCAGTACGACTCCACCCTGTCATTG
rev-hansenii-IDCL	CATATCATCAATTTTCAGAAATTCGCTATCAATATCCATCAATTTCAGAGAGTATTCGGCTATAC
fr-albicans-IDCL	GATATTGATAGCGAATTTCTGCAGATTGATGATATGCAGTACGACTCCACCCTGTCATTG
rev-albicans-IDCL	TACCTACTATAAGTCGACAAACTTTCGCTATAACTACATCAATTTCAGAGAGTATTCCGCTATAC
fr-crassa-IDCL	GATATTGATCAGGAACATCTGGGCATTCCGGATACCCAGTACGACTCCACCCTGTCATTG
rev-crassa-IDCL	GGTATCCGGAATGCCCAGATGTTCCTGATCAATATCCATCAATTTCAGAGAGTATTCGGCTATAC
fr-nidulans-IDCL	GATATTGATCAAGAACATCTTGCTATTCCTGAAACTCAGTACGACTCCACCCTGTCATTG
rev-nidulans-IDCL	AGTTTCAGGAATAGCAAGATGTTCTTGATCAATATCCATCAATTTCAGAGAGTATTCGGCTATAC
fr-lipolytica-IDCL	ACCATTGATCAGGAACATCTGGGCATTCCGGATACCCAGTACGACTCCACCCTGTCATTG
rev-lipolytica-IDCL	GGTATCCGGAATGCCCAGATGTTCCTGATCAATGGTCATCAATTTCAGAGAGTATTCGGCTATAC
fr-pombe-IDCL	GACATTGATCAAGAACACTTGGGTATACCAGATATCCAGTACGACTCCACCCTGTCATTG
rev-pombe-IDCL	GATATCTGGTATACCCAAGTGTTCTTGATCAATGTCCATCAATTTCAGAGAGTATTCGGCTATAC
fr-pAD-PCNA	CCAAACCCAAAAAAAGAGATCGAATTAATGTTAGAAGCAAAATTTGAAGAAGCATC
rev-pAD-PCNA	CACTATAGGGCTCTAGAGTCGACTAATACTCTCGAGTTATTCTTCGTCATTAAATTTAGGAGCC
fr-pcn1-pRS	TTCACTCACAGCAACAAGCAGCAAGCACTAAGTACGCAGTCAAAAGAGAGAAAAAATGCTTGAAGCTAGATTTCAGCAG
rev-pcn1-pRS	GTTTTTTTTTTGTTTATTATTTTTAGTATACAACTATATAGATAATTTACATCTACTCCTCATCCTCCTCACC
fr-pcn1-pol30-IDCL	GATATCGATGCTGATTTCTTAAAGATTGAAGAATTAGAATACGATGCTACTATTACTATGCCTG
rev-pcn1-pol30-IDCL	TAATTCTTCAATCTTTAAGAAATCAGCATCGATATCGTCCATTAATTTAACATCATAATCAGAGATCC
fr-pombe-pol32-pBD	AGTTGACTGTATCGCCGGAATTCGCCCGGGCCTCGAGCCCGATGGATGGAGGAATGGAGAAACTTCTTAGATATT
fr-pcn1-pRS	TTCACTCACAGCAACAAGCAGCAAGCACTAAGTACGCAGTCAAAAGAGAGAAAAAATGCTTGAAGCTAGATTTCAGCAG