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Supplementary Information for

Genome-wide analysis of heat stress-stimulated transposon mobility in the human fungal pathogen *Cryptococcus deneoformans*

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Supplementary Information Text

Materials and Methods

Illumina sequencing and TE mapping

Pair-end reads from whole-genome sequencing were analyzed to identify discordant pairs mapping between a TE and the XL280α reference genome (similar to analysis presented in (1,2)). This was done by first identifying reads that align to the sequence of a TE (either the T1 or Tcn12 element) with BLAT – the BLAST like alignment tool (3) – using default settings. The mated pairs of these TE-associated reads that failed to align to the TE were then mapped (again using BLAT) to the XL280α genome. Reads mapping to the TE were labeled as supporting reads while their mates that mapped to the reference genome were denoted as anchor reads. Anchor reads were only considered if they mapped uniquely to the XL280α genome. Per chromosome, windows of 10 kb were used to quantify and identify the number and position of anchor reads throughout the genome. This analysis was conducted on both TA lines and the non-passaged XL280α strain.

Two methods of standardization were used to identify TE insertion and copy loss in TA lines, using the corresponding signal of anchor reads in non-passaged strains. For TE insertions in TA lines, the number of anchor reads (per 10 kb) was divided by the corresponding number of TE anchor reads (plus one) within the non-passaged strain. For TE copy loss, the anchor read signal of each non-passaged strain was divided by the negative of the anchor read count (plus one) of the given TA line. When plotted, this generates a positive or negative, genome-wide signal of anchor reads relative to the non-passaged strain for TE insertions or loss (respectively) in each TA line.

Loci that demonstrated a potential insertion or loss of a TE were identified per TA line by retaining 10 kb windows with greater than 10x (+ or -, respectively) anchor read coverage, removing regions that were identified in all TA lines, and regions with a position in a native TE site of the XL280α reference genome. The locations of predicted TE insertions and TE copy losses in passaged TA lines were confirmed by PCR and Sanger sequencing (see Table S5 for primers used).

Permutation Testing

A permutation strategy was implemented in python to test if the T1 insertion patterns are non-random within gene orientation categories; tandem, convergent, and divergent (Table S2). The underlying null-hypothesis: insertion sites are random and follow the distribution of gene orientation patterns. To generate a null-distribution, the portion of the summed intergenic distances between gene pairs, across the three orientation groups, was used to calculate the probability of a T1 insertion within that category (excluding non-positive, intergenic distances from overlapping gene pairs). The total number of identified T1 insertions (41), with these proportions was used to calculate the expected T1 insertion counts per gene orientation category. Across 10,000 permutations a multinomial distribution (built on the summed portion of intergenic distances per gene retention group) was used to randomly draw a random sample of T1 insertion counts across the three orientation groups. A chi-squared test was used to compare these randomly drawn T1 counts and their distribution across orientation patterns with the expected (Fig. S5*C*).

Variant Calling

Pair-end Illumina-sequenced reads for each sample were aligned to the Nanopore XL280α reference genome using bwa-mem (4) and its default settings. Duplicate reads were identified using samblaster (5). Duplicate marked alignments were filtered via the samtools view command (6) with the additional filtering options of -F 3852 and mapping quality threshold of ten (with -q 10). Read groups were added to each binary alignment map file using bamaddrg (7).

All filtered alignments were fed into samtools mpileup and bcftools to identify genetic variants segregating across the passaged isolates. The default parameters were used in the call to mpileup with the addition of the -d 100 flag to cap the maximum number of aligned reads considered in detecting variants (7). The default consensus calling strategy in bcftools, with a ploidy setting of one and the addition of the options “-vc” were used to detect variants.

Variant calls were filtered using bcftools and custom scripts in python. Specifically, using the bcftools view command, only variants with a quality score greater than 900 were considered. Invariant sites – loci with fixed genetic variants across all samples – were removed from analysis.

Per isolate, variant loci were considered only if they had greater than 6x coverage with 80% of reads suggesting a variant call. Per isolate, only variants detected and consistent across each technical replicate were retained for further consideration.

Using the Integrative Genomics Viewer (IGV), variant positions were visually inspected to remove those called within telomeric regions that displayed as artifacts, i.e. appearing with identical alignments across multiple, unrelated isolates in the passaging experiments (8). In total, 836 raw genetic variants were called via bcftools and after scripted filtering, 198 variants were examined in IGV, resulting in the identification and analysis of 142 unique variant sites. IGV was also used to examine inheritance patterns of genetic variants and estimate potential changes in amino acid sequences across isolates.

Nanopore sequencing and TE mapping

High molecular-weight DNA was isolated by a CTAB-DNA extraction method (9) and tested for its quality using NanoDrop. Samples were sequenced on the MinION system using the R9.4.1 Flow-Cell and SQK-LSK109 library preparation kit. For multiplexing of multiple samples in a single flow-cell, Native Barcoding Expansion 1-12 kit (EXP-NBD104) was used during the library preparation as instructed by the manufacturer's protocol. Nanopore sequencing was performed at the default voltage for 48 hours as per the MinION sequencing protocol provided by the manufacturer. MinION sequencing protocol and setup was controlled using the MinKNOW software. Base-calling was performed outside the MinKNOW software on the lab server using the standalone Guppy basecaller from Nanopore and the sequence reads thus obtained were used for genome assembly. In case of multiplexed runs, the reads were de-multiplexed using qcat or Guppy_barcoder before the genome assembly. Canu (10) was used to assemble the genome of most strains using reads that are longer than 2 kb (-minReadLength=2000) in length. The genome assembly was checked for integrity by mapping the reads back to the genome assembly using minimap2 (11), and duplicated small contigs were discarded. The contigs that were broken at centromeres were assembled manually after a synteny comparison and analysis using Geneious Prime 2020.1.1. All the chromosome-level contigs thus generated were then reoriented

to have the same configuration as the wildtype XL280α genome. Transposon locations were determined using BLASTn analysis and annotated using Geneious Prime 2020.1.1.

Detection of 5mC methylation

5mC DNA methylation was calculated using the scripts available with nanopolish, as previously described (12). Briefly, the XL280α nanopore reads were mapped to the genome assembly, and methylation status was identified using "call-methylation" followed by calculating methylation frequency. The frequency was then converted to bedGraph for visualization purposes in IGV and the final figures were generated in Adobe Illustrator.

Gene liftover and orientation analysis

Individual gene sequences were taken from the JEC21 reference genome (version 48, FungiDB) and mapped via BLAT to the assembled XL280α nanopore reference genome. After this mapping, those gene sequences with greater than 90% sequence identity were retained for analysis. Per chromosome, the distance between and orientation of pairs of genes were determined via python.

SI Appendix Figures

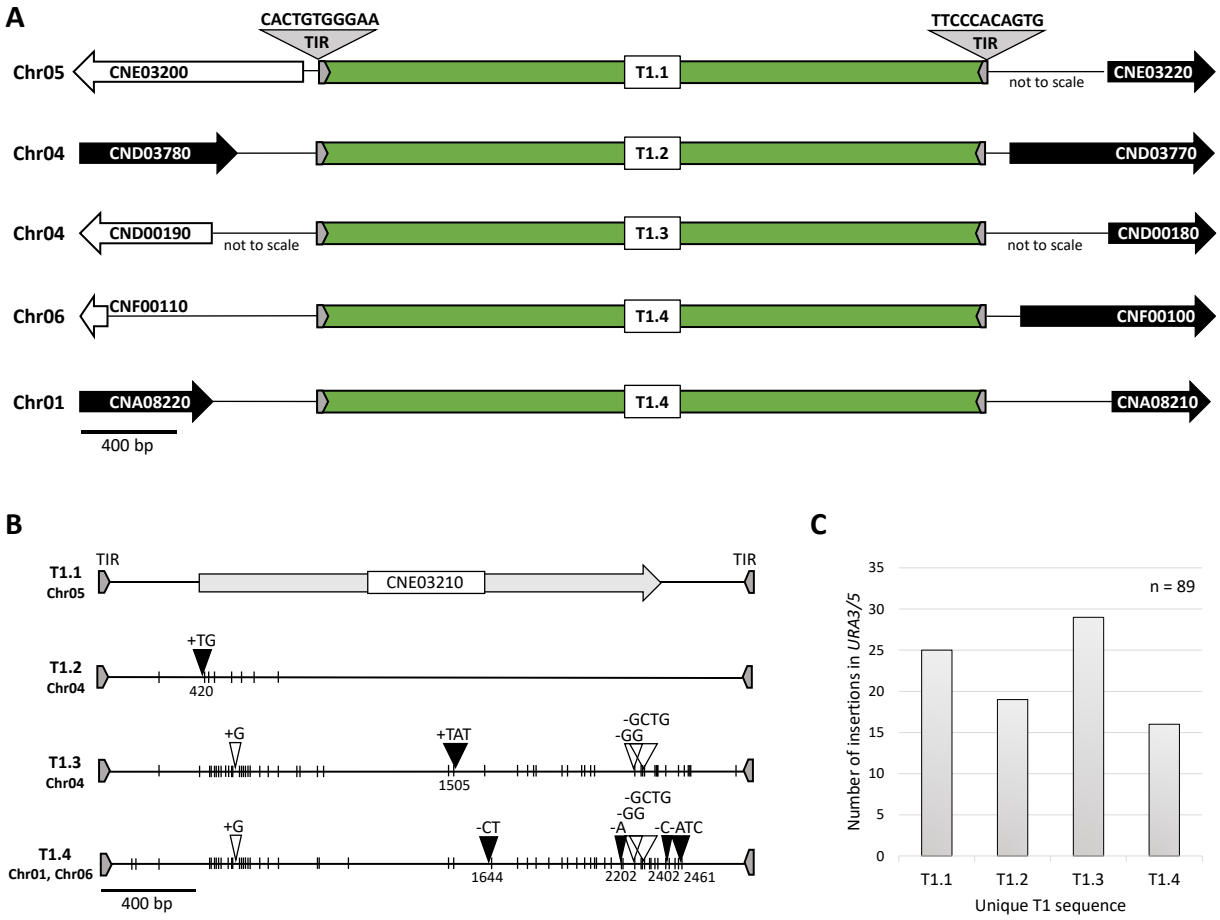

Figure S1. Locations of the four unique 2.7 kb T1 mobile elements in the XL280α genome. *(A)* Gene-level view of T1 copies and proximity to nearby genes. The sequence of the 11-bp terminal inverted repeats (TIRs) is indicated. *(B)* Sequence differences that distinguish each T1 element from T1.1. Single lines indicate SNPs, triangles indicate INDELs; black triangles indicate INDELs diagnostic for each element. *(C)* Number of *de novo* insertions of each T1 element in the *URA3/5* genes of 5FOA-resistant mutants (13).

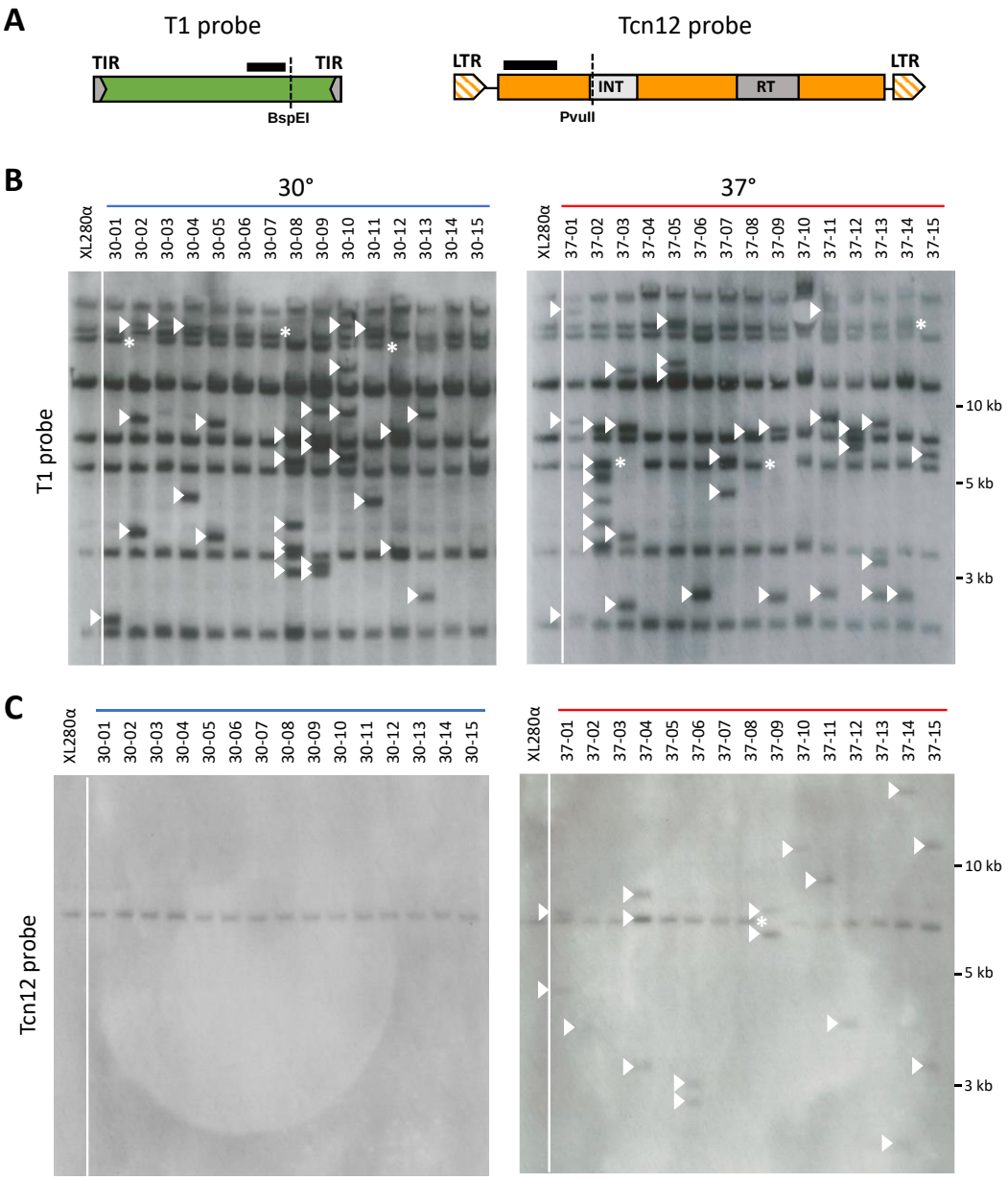

Figure S2. Southern analysis of TE movement in fifteen independent XL280α TA lines passaged ~800 generations on YPD at 30° or 37° as indicated. *(A)* Location of the restriction sites (dotted lines) and probes (black bars) used in Southern analysis. *(B)* Genomic DNA of non-passaged XL280α (left lane) and passaged isolates digested with BspEI, probed for T1. *(C)* DNA digested with PvuII, probed for Tcn12. Arrowheads indicate *de novo* TE insertions and asterisks indicate the loss of a native TE copy.

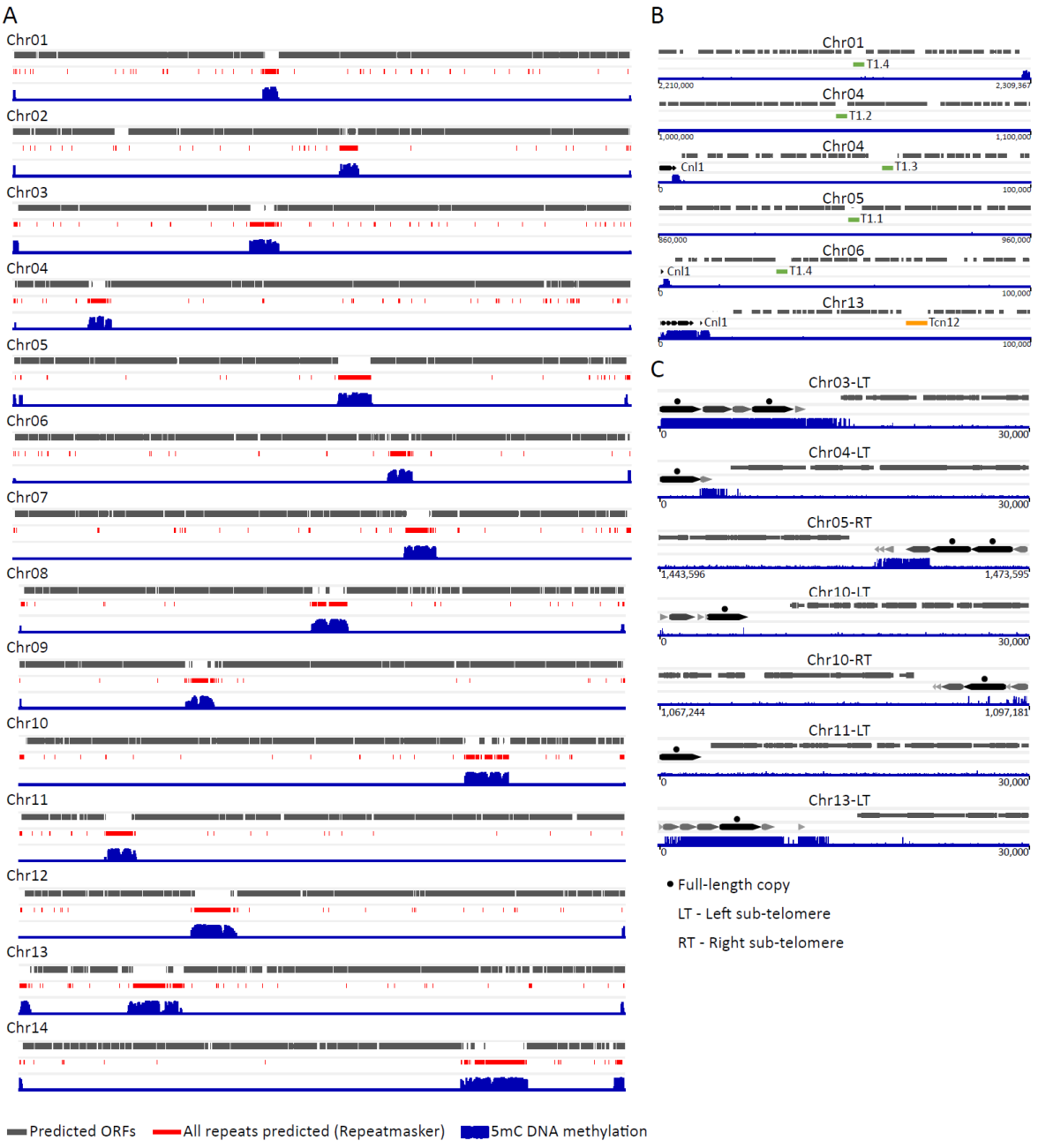

Figure S3. 5-methylcytosine (5mC) DNA methylation in XL280α as detected by Nanopore sequencing. *(A)* Genome-wide 5mC methylation pattern with predicted open reading frames (ORFs) and repetitive element sequences. 5mC methylation at regions of chromosomes encoding mobile T1 and Tcn12 sequences *(B)* and Cnl1 sequences, zoomed in to show full-length copies (black arrowheads) and truncated copies (gray arrowheads) in the left and right sub-telomeres *(C)*.

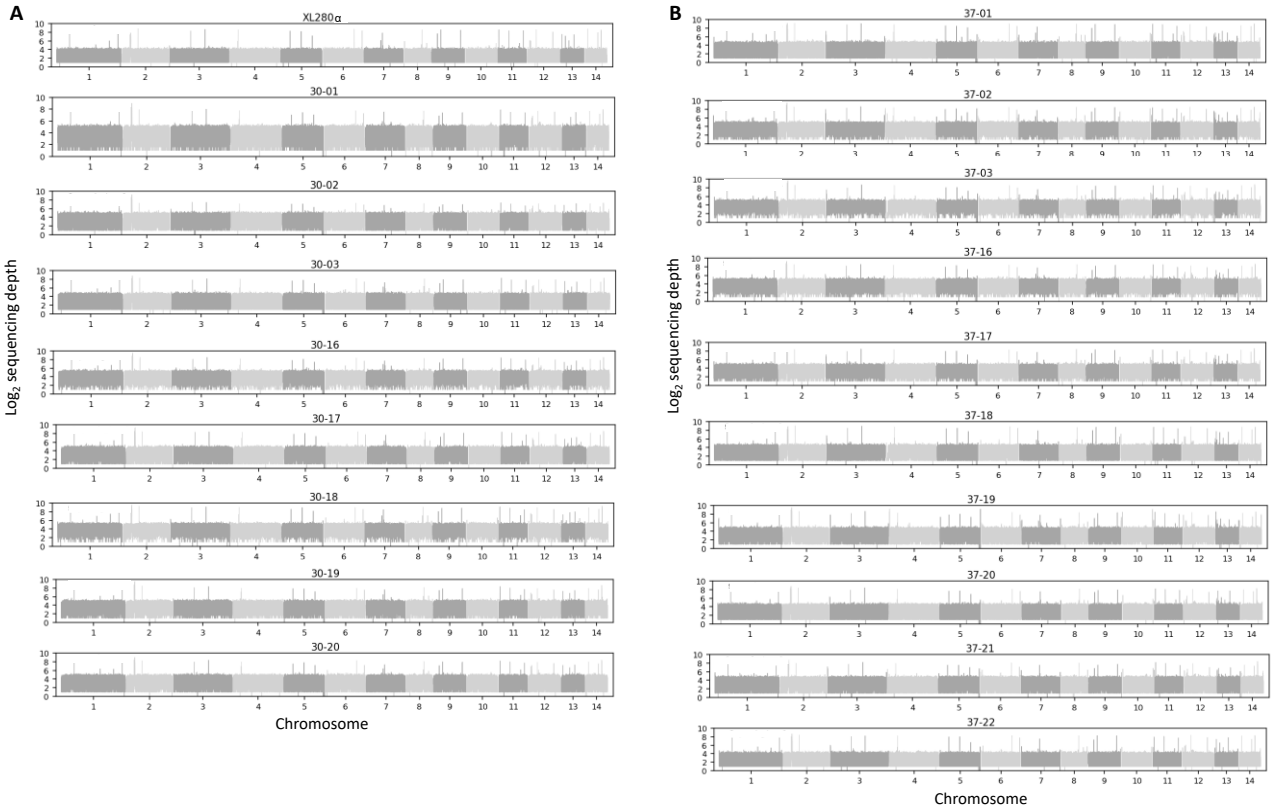

Figure S4. Sequencing depth of Illumina reads for the genomes of *(A)* XL280α and TA lines passaged at 30° and *(B)* TA lines passaged at 37°. Normalized read counts were transformed to the $\log_2$ scale for sequencing depth.

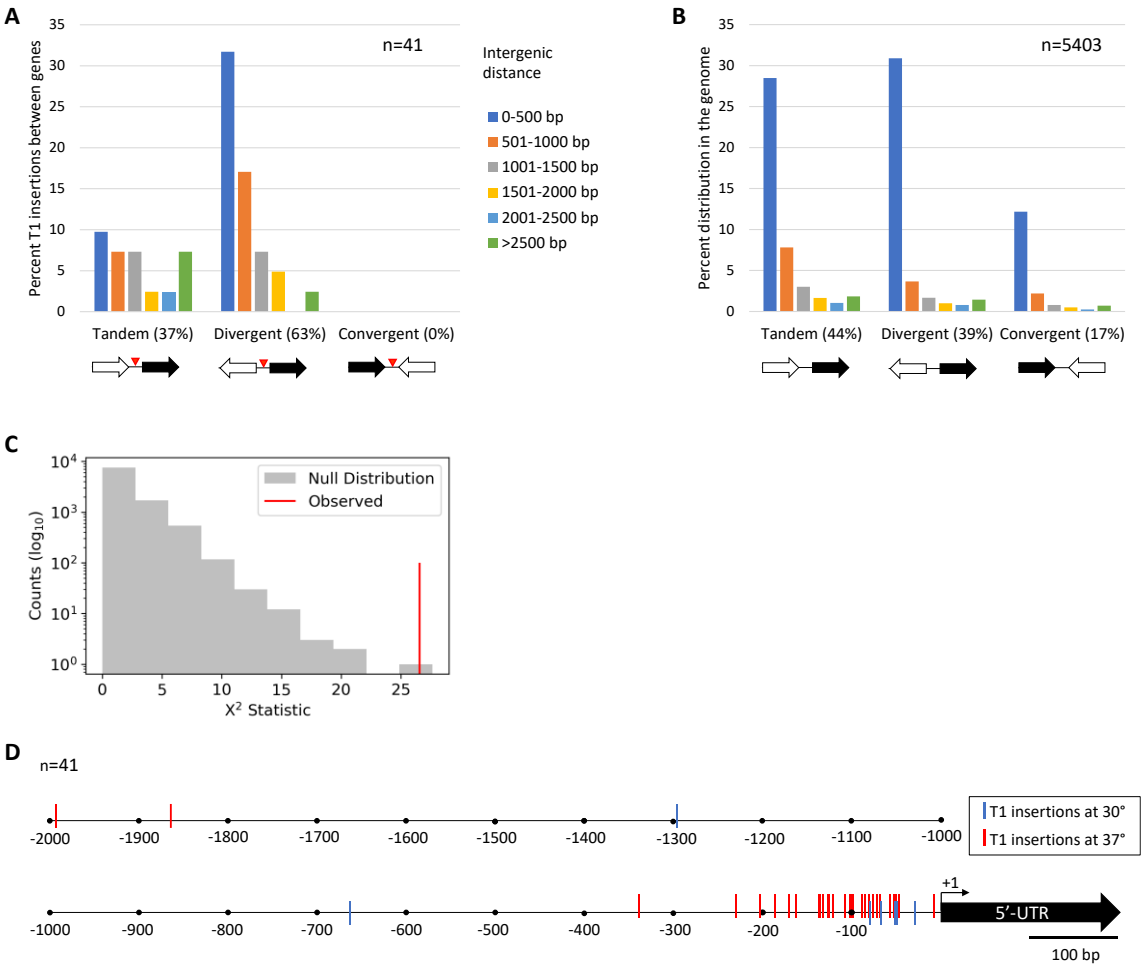

Figure S5. Analysis of *de novo* T1 insertions between genes in XL280α TA lines. *(A)* Percentage of mapped T1 insertions (red arrowheads) between genes in tandem, divergent or convergent orientations. *(B)* Distribution of tandem, divergent and convergent gene orientations in the XL280α genome. Overlapping genes in all orientations were excluded. *(C)* A histogram displaying the null-distribution of chi-squared statistics (grey, x-axis) and observed statistic (red, vertical line) testing the non-random distribution of T1 insertions within gene orientation patterns (see Table S2). A randomization strategy using the portion of summed, intergenic distances between genes across orientation groups (tandem, divergent, and convergent) was used to generate the null-distribution. The x-axis delineates the value of the chi-squared statistic while the y-axis shows the counts on a log10 scale. *(D)* Distance of mapped T1 insertions upstream of the nearest +1 start of transcription (as annotated for the *C. deneoformans* JEC21 reference genome, EnsemblFungi).

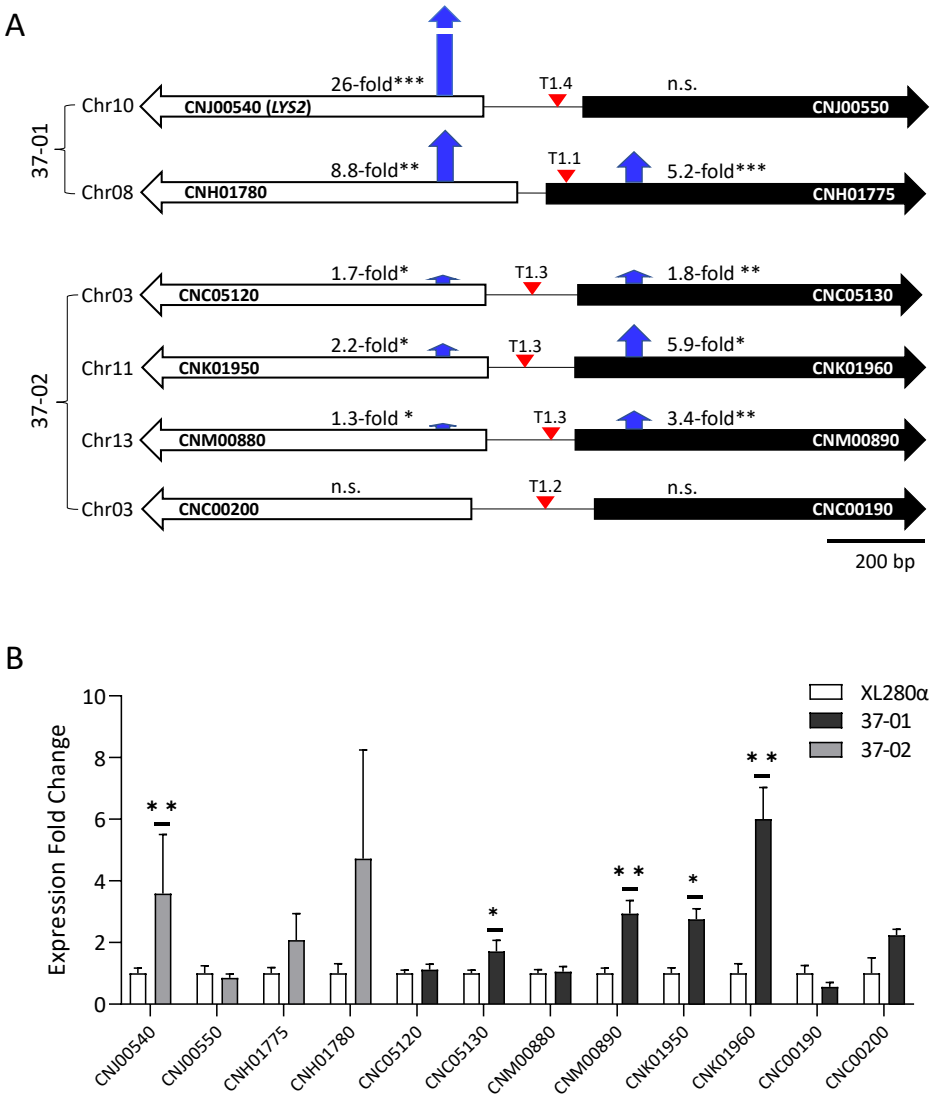

Figure S6. T1 insertions and changes in gene expression in 37°-passaged lines 37-01 and 37-02. *(A)* Locations of *de novo* T1 insertions (red arrowheads) relative to predicted genes and fold changes in expression compared to the non-passaged XL280α strain are shown. Black or white shading represents forward or reverse gene orientation, respectively, in the JEC21 reference genome (EnsemblFungi). Blue arrows indicate the relative fold-changes in transcript compared to the non-passaged XL280α strain measured by qRT-PCR. *(B)* Fold change in expression of control genes in 37°-passaged lines 37-01 and 37-02 relative to the XL280α strain. These control genes are not proximal to the *de novo* T1 insertions in 37-01 and 37-02 shown in *(A).* Gene expression levels were normalized to the endogenous reference gene *GPD1* using the comparative $\Delta\Delta C_T$ method. For each target gene and each sample, technical triplicate and biological triplicate qRT-PCR reactions were performed. Error bars represent standard error of the means (SEM) for three biological replicates. Statistical analyses were performed using the GraphPad Prism 9 software. A Welch's unpaired t test (two-tailed) was performed for each pairwise comparison using the mean ΔC_T values for three biological replicates (ns, not significant; * indicates $0.01<p\leq0.05$; and ** indicates $0.001<p\leq0.01$).

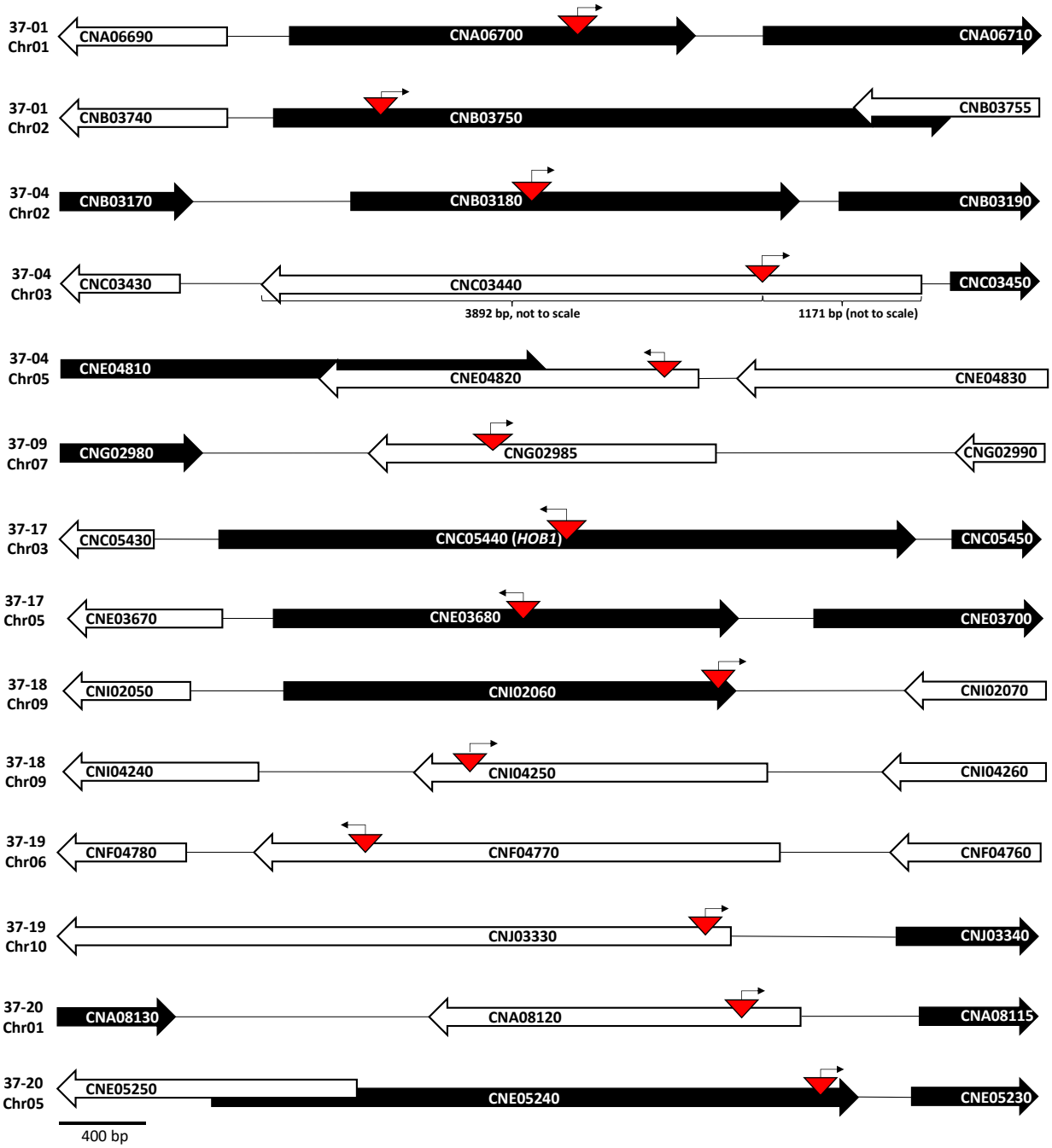

Figure S7. Analysis of *de novo* Tcn12 integration sites in TA lines passaged at 37°. Gene-level view of the 14 novel Tcn12 insertions (triangles) within gene-coding regions. Arrows indicate the forward or reverse orientation of Tcn12 with respect to the *gag-pol* polyprotein encoded within the element.

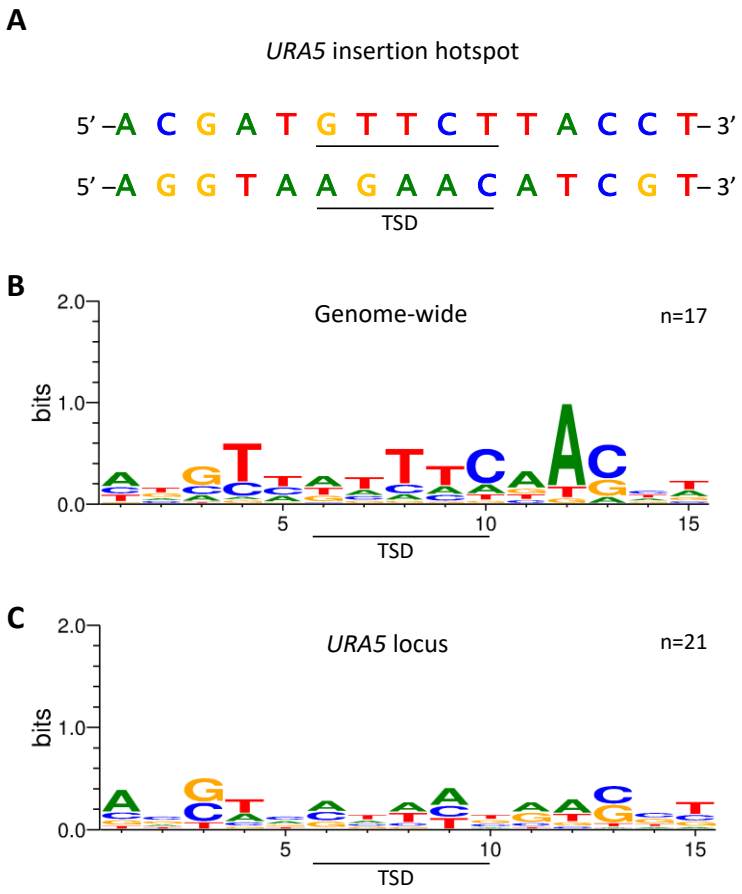

Figure S8. Alignment of *de novo* Tcn12 integration sites with respect to the forward orientation of the element. *(A) URA5* insertion hotspot for Tcn12, centered at the TSD [6]. The 15-bp 5' to 3' *URA5* sequence is shown for both DNA strands. *(B)* Sequence logo (WebLogo 3.7.4) generated by aligning 17 *de novo* Tcn12 integration sites (Table S3) in 37° TA lines. *(C)* Sequence logo generated by aligning unique Tcn12 integration sites in the *URA5* locus of 5FOA-resistant XL280α mutants recovered from mouse organs (13).

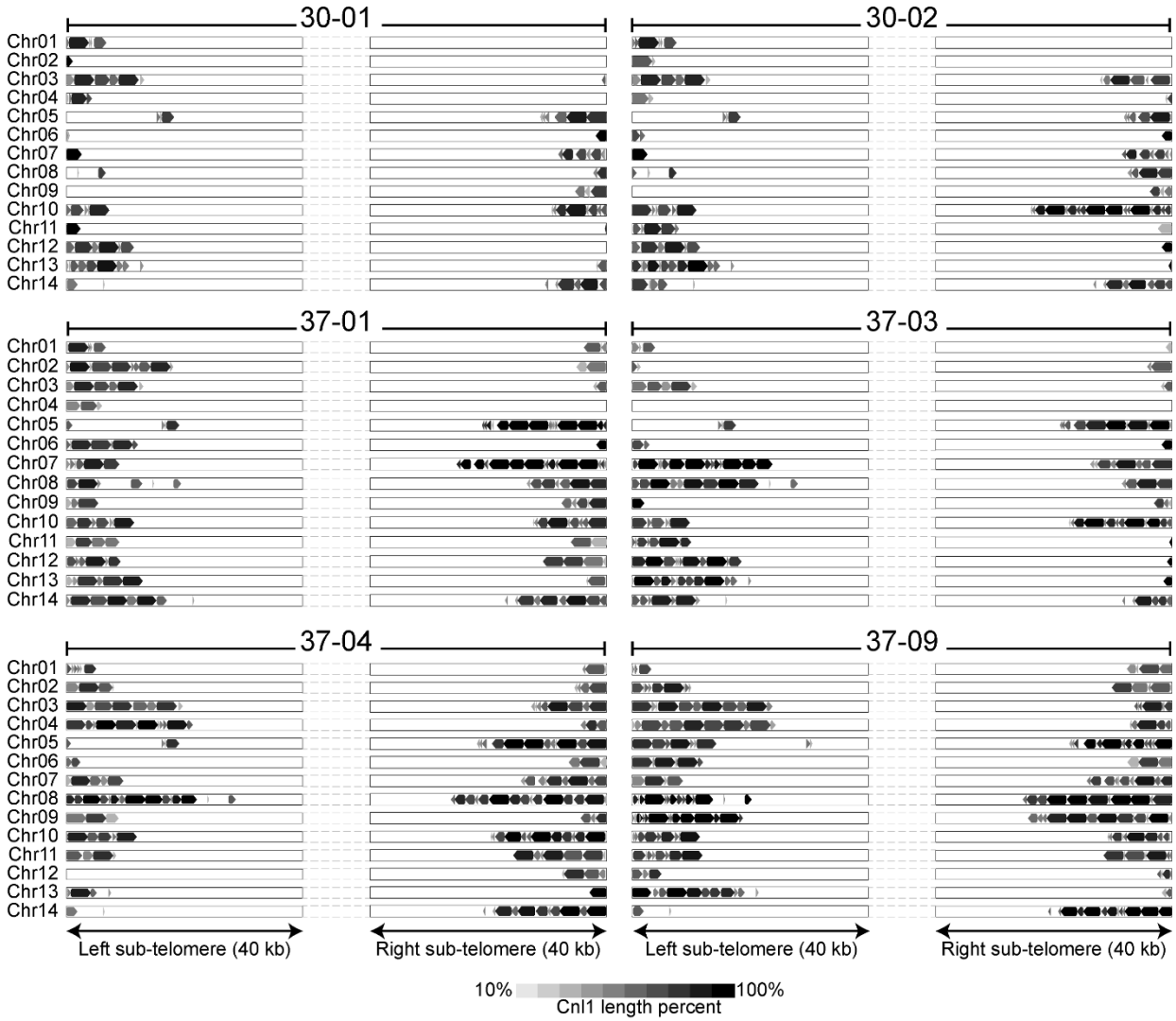

Figure S9. Full-length and truncated copies of the non-LTR Cnl1 retrotransposon at the sub-telomeres of chromosomes of TA lines passaged at 30° (30-01, 30-02) and 37° (remaining isolates), sequenced by Nanopore. Left and right panels for each isolate indicate the left or right sub-telomeric region as indicated. The size of each block arrows approximates the percent length of the 3.4 kb Cnl1 element with the darkest color (black) indicating full-length copies and lighter shades of gray indicating truncated copies of the element. The distribution of Cnl1 on chromosomes for the non-passaged XL280α strain is shown in Fig. 5A.

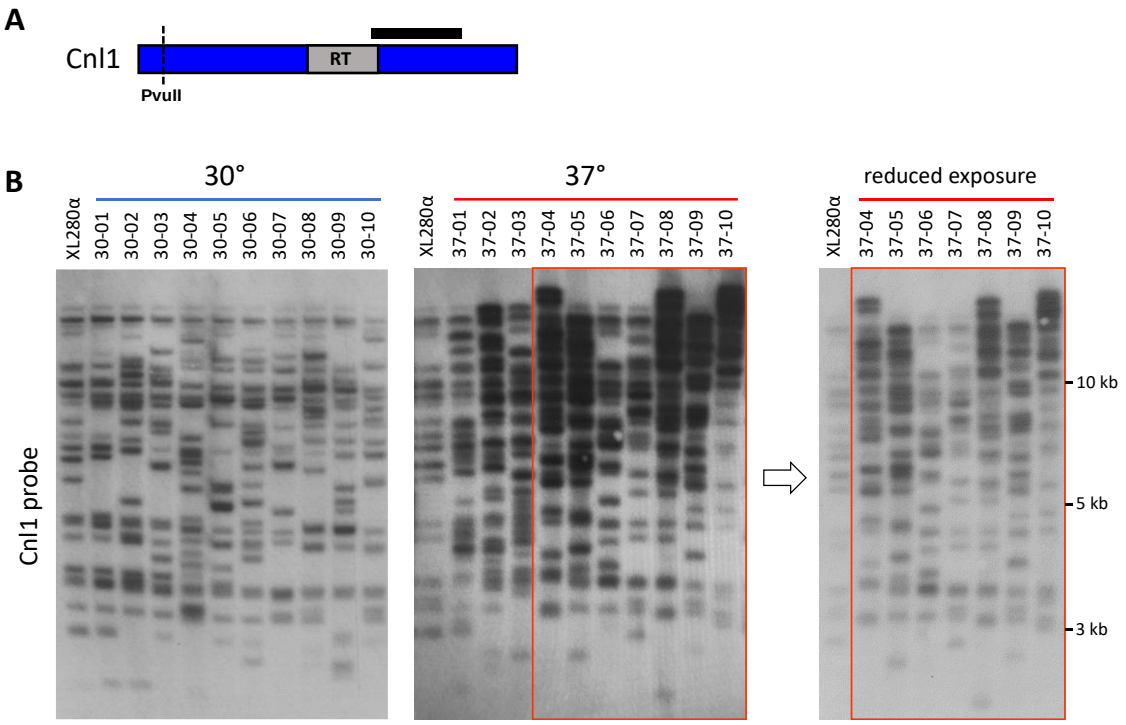

Figure S10. Southern analysis probing for the Cnl1 retrotransposon in the genomes of XL280α TA lines passaged at 30° and 37°. *(A)* Location of the PvuII restriction site (dotted line) and probe (black bar) used to identify Cnl1 copies. *(B)* Southern analysis of Cnl1 copies in XL280α and TA lines passaged at 30° (left) or 37° (right). The far-right panel shows a reduced exposure of the 37° passaged lines indicated in the red box for better resolution of larger bands.

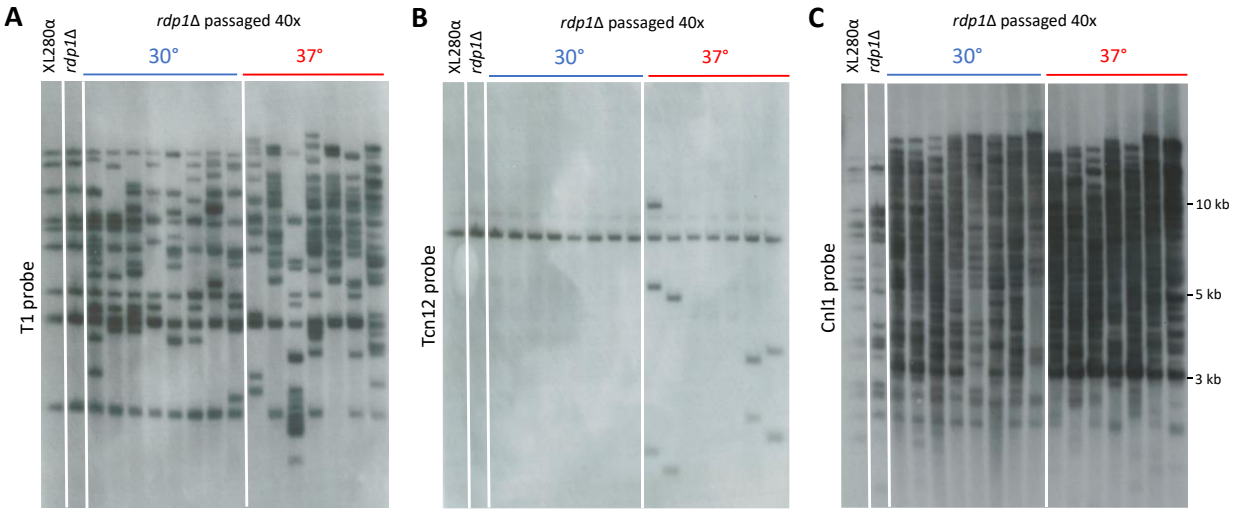

Figure S11. Southern analysis of TE movements in *Cryptococcus* XL280α *rdp1*Δ mutants passaged 40 times (~800 generations) at 30° and 37°. Genomic DNA from the wildtype XL280α strain, *rdp1*Δ mutant strain and passaged *rdp1*Δ TA lines was digested with PvuII and probed for *(A)* T1, *(B)* Tcn12, and *(C)* Cnl1.

Table S1. Novel T1 and Tcn12 insertions mapped in lines passaged 40 times at 30° or 37°. Genomic locations of novel TEs are defined as upstream (US) or downstream (DS) of predicted transcription start sites for proximal genes, as annotated for the *C. deneoformans* JEC21 reference genome (EnsemblFungi and FungiDB).

Isolate	TE	Chr	Category	Location
30-01	T1	8	intergenic - divergent	51 bp US CNH02260, 109 bp US CNH02250
30-02	T1	2	intergenic - divergent	50 bp US CNB02630, 959 bp US CNB02620
30-02	T1	3	intragenic	4484 bp into CNC05220
30-02	T1	11	intergenic - divergent	50 bp US CNK03170, 1014 bp US CNK03180
30-02	T1	13	intergenic - divergent	665 bp US CNM02240, 1137 bp US CNM02230
30-03	T1	7	intergenic - divergent	66 bp US CNG03170, 115 bp US CNG03180
30-16	T1	3	intergenic - tandem	1294 bp US CNC01660, 640 bp DS CNC01670
30-16	T1	6	intragenic	3115 bp into CNF02870
30-17	T1	6	intergenic - divergent	80 bp US CNF00080, 93 bp US CNF00090
30-20	T1	10	intergenic - divergent	28 bp US CNJ02860, 501 bp US CNJ02870
37-01	T1	7	intergenic - divergent	340 bp US CNG02000, 972 bp US CNG01990
37-01	T1	8	intragenic	40 bp into CNH01775 (5'-UTR), 99 bp US CNH01780
37-01	T1	10	intergenic - divergent	52 bp US CNJ00550, 146 bp US CNJ00540 (*LYS2*)
37-01	T1	10	intragenic	195 bp into CNJ02670 (5'-UTR), 1526 bp DS CNJ02660
37-01	Tcn12	1	intragenic	1300 bp into CNA06700
37-01	Tcn12	2	intragenic	480 bp into CNB03750
37-01	Tcn12	8	intragenic	within Cnl1 left arm; subtelomere
37-02	T1	1	intergenic - divergent	99 bp US CNA06060, 491 bp US CNA06050
37-02	T1	1	intergenic - tandem	1866 bp US CNA07370, 274 bp DS CNA07360
37-02	T1	2	intergenic - tandem	170 bp US CNB02680, 3813 bp DS CNB02690
37-02	T1	3	intergenic - divergent	102 bp US CNC00190, 144 bp US CNC00200
37-02	T1	3	intergenic - divergent	89 bp US CNC05130, 90 bp US CNC05120
37-02	T1	4	intergenic - tandem	232 bp US CND03560, 790 bp DS CND03550
37-02	T1	4	intergenic - tandem	within a tRNA (CND05420); 203 bp US CND05430, 118 bp DS CND05410
37-02	T1	11	intergenic - divergent	76 bp US CNK01950, 105 bp US CNK01960
37-02	T1	13	intergenic - divergent	50 bp US CNM00890, 132 bp US CNM00880
37-02	Tcn12	2	other	centromere, near CNB02910
37-03	T1	2	intragenic	1212 bp into CNB01810, 106 bp US CNB01800 (*FKBP12*)
37-03	T1	2	intergenic - divergent	135 bp US CNB05060, 682 bp US CNB05080
37-03	T1	3	intergenic - tandem	71 bp US CNC00160, 584 bp DS CNC00170
37-03	T1	4	intergenic - tandem	132 bp US CND05850, 272 bp DS CND05860
37-03	T1	6	intergenic - divergent	directly US of native T1.4 copy; 866 bp DS of CNF00110
37-03	T1	11	intergenic - divergent	106 bp US CNK01520, 110 bp US CNK01510
37-04	Tcn12	2	intragenic	830 bp into CNB03180
37-04	Tcn12	3	intragenic	3892 bp into CNC03440
37-04	Tcn12	5	intragenic	155 bp into CNE04820
37-04	Tcn12	12	other	telomere region
37-09	T1	6	intergenic - divergent	66 bp US of CNF00100, 60 bp DS of native T1.4 copy
37-09	T1	10	intergenic - divergent	100 bp US CNJ00250, 552 bp US CNJ00260
37-09	Tcn12	7	intragenic	1032 bp into CNG02985
37-09	Tcn12	13	other	non-coding region near CNM00220
37-16	T1	7	intergenic - divergent	84 bp US CNG00580, 1504 bp US CNG00590
37-17	Tcn12	3	intragenic	1569 bp into CNC05440
37-17	Tcn12	5	intragenic	1170 bp into CNE03680
37-18	T1	2	intergenic - tandem	1995 bp US of CNB02910, 647 bp DS CNB02900
37-18	T1	14	intergenic - tandem	188 bp US CNN00920, 68 bp DS CNN00910
37-18	Tcn12	9	intragenic	1374 bp into CNI04250
37-18	Tcn12	9	intragenic	1982 bp into CNI02060
37-19	T1	1	intergenic - tandem	162 bp US CNA01370, 709 bp DS CNA01360
37-19	T1	1	intergenic - tandem	52 bp US CNA08100, 384 bp DS CNA08090
37-19	T1	6	intergenic - tandem	46 bp US CNF01870, 476 bp DS CNF01880
37-19	T1	13	intergenic - divergent	47 bp US CNM02270, 448 bp US CNM02280
37-19	Tcn12	6	intragenic	1917 bp into CNF04770
37-19	Tcn12	10	intragenic	191 bp into CNJ03330
37-20	T1	1	intergenic - tandem	122 bp US CNA03220, 3027 bp DS CNA03240
37-20	Tcn12	1	intragenic	267 bp into CNA08120
37-20	Tcn12	5	intragenic	2791 bp into CNE05240
37-21	T1	4	intergenic - divergent	57 bp US CND00300, 3545 bp US CND00310
37-21	T1	4	intergenic - divergent	80 bp US CND03930, 396 bp US CND03920
37-21	T1	9	intergenic - divergent	135 bp US CNI01880, 144 bp US CNI01890
37-21	Tcn12	4	intergenic	410 bp US CND04840
37-22	T1	1	intergenic - tandem	6 bp US CNA00670, 1407 bp DS CNA00660
37-22	T1	2	intergenic - tandem	76 bp US CNB02550, 1081 bp DS CNB02545
37-22	T1	3	intergenic - divergent	68 bp US CNC04820, 187 bp US CNC04810
37-22	T1	5	intergenic - divergent	82 bp US CNE03320, 532 bp US CNE03310

Table S2. Distribution of genes in tandem, divergent and convergent orientation in the XL280α genome compared to the distribution of novel T1 insertions between genes in each orientation. Gene predictions based on those annotated in the JEC21 reference genome (EnsemblFungi).

	XL280α gene orientations		
Intergenic distance	Tandem	Divergent	Convergent
Overlapping	77	98	1335
0 - 501 bp	1539	1669	658
501 - 1,001 bp	423	198	119
1,001 - 1,501 bp	163	91	43
1,501 - 2,001 bp	89	54	27
2,001 - 2,500 bp	57	43	14
>2,500 bp	100	78	38
TOTAL	2448	2231	2234
Number of genes (excluding overlapping)	2371	2133	899
Proportion of genes	0.44	0.39	0.17
	T1 insertions between genes in each orientation		
	Tandem	Divergent	Convergent
Number of genes	15	26	0
Proportion of genes	0.37	0.63	0

Table S3. Location and alignment of novel Tcn12 insertion sites centered around the target site duplication (TSD) sequence (red letters).

DNA sequences at novel Tcn12 insertion sites in 40x passaged isolates (37°)			
Passaged Isolate	**Chr**	**Gene**	**5'-> 3' sequence with respect to forward orientation of Tcn12**
37-01	1	CNA06700	**GACGGTAACATCAAATTCG**
37-01	2	CNB03750	**GACTGTTACTTTTACTACC**
37-01	8	Cnl1	**TCTCGCATTTCCAACCCGA**
37-02	2	CNB02910	**CCAAGATGTTTCAACAAAG**
37-04	2	CNB03180	**TTAACTCATTACAAGTCAC**
37-04	3	CNC03440	**CAACCCAAAATCAAACTAT**
37-04	5	CNE04820	**GTATGTCTTCTAAACAACC**
37-09	7	CNG02985	**AGATCTTGTTCCCAGGTAC**
37-09	13	non-coding	**GATGGTTATTTCGGGGGCA**
37-17	3	CNC05440	**ACTATCTAACACTACCTAG**
37-17	5	CNE03680	**TCTTGTGACTTTTTCCATA**
37-18	9	CNI04250	**TTACCTCTTTCCATCTTCT**
37-18	9	CNI02060	**CGAGAGTGGTAAGAGATTG**
37-19	6	CNF04770	**GACGGTCCAGAAGACTACC**
37-19	10	CNJ03330	**TTGGGTCTTCGCAAGCGGT**
37-20	1	CNA08120	**ATCTACATATTCAACAGTA**
37-20	5	CNE05240	**ACATATTGGTTGCACGTCT**

Table S4. Sequence variations detected in Illumina-sequenced TA lines.

TA line	Type	Chromosome	Position	Reference Allele	Alternative Allele	Location	Amino Acid Change
30-01	SNP	XL280_Chr05	15362	G	C	Start of CNE05200	
30-01	SNP	XL280_Chr09	239663	G	A	Third exon of CNI00980	synonymous
30-01	SNP	XL280_Chr12	1064289	T	C	intergenic, upstream CNL06555	
30-01	SNP	XL280_Chr03	1823885	A	G	intergenic	
30-01	SNP	XL280_Chr14	288677	G	A	Third exon of CNN00890	R → Q
30-02	SNP	XL280_Chr01	10750	G	A	Last exon of CNA00030	G → S
30-02	SNP	XL280_Chr01	219916	C	G	Last exon of CNA00760	P → A
30-02	SNP	XL280_Chr04	1685043	C	A	Exon of CND06090	S → N
30-02	SNP	XL280_Chr08	497081	A	T	Start of CNH01460 and CNH01480	
30-02	SNP	XL280_Chr10	566700	C	G	First exon on CNJ01930	G → D
30-02	SNP	XL280_Chr13	722802	T	A	intergenic	
30-02	ins	XL280_Chr02	930641	AATA	AATATA	intergenic	
30-02	ins	XL280_Chr06	57476	CTATTA	CTATTATTA	Downstream of CNC02090	
30-03	SNP	XL280_Chr03	1712429	C	T	intergenic, upstream of CNC05750	
30-03	SNP	XL280_Chr11	862569	T	C	intronic, between the third and fourth exons of CNK02850	
30-03	del	XL280_Chr02	930643	TAC	T	intergenic	
30-16	SNP	XL280_Chr02	1112	C	T	End of First exon in CNB00010	H → Y
30-16	SNP	XL280_Chr04	556359	C	T	Sixth exon of CND02040	Q → STOP
30-16	SNP	XL280_Chr05	1151182	C	T	Within CNE04070 (hypothetical protein)	
30-16	SNP	XL280_Chr08	168992	C	T	intronic in CNH02510, between exons four and five	
30-16	SNP	XL280_Chr08	573639	G	A	First exon of CNH01250	G → S
30-16	SNP	XL280_Chr11	191418	T	C	intergenic	
30-16	SNP	XL280_Chr12	422229	G	A	Middle of seventh exon of gene CNL04260	synonymous
30-16	ins	XL280_Chr02	976982	CATATATATAT	CATATATATATAT	5' UTR CNB03180	
30-16	ins	XL280_Chr08	442476	ATT	ATTTT	intergenic	
30-16	del	XL280_Chr11	39688	GAAAAAAAAAA	GAAAAAAAAA	3' UTR CNK00120 and upstream of CNK00110	
30-17	SNP	XL280_Chr01	92717	G	A	5' UTR CNA00290	
30-17	SNP	XL280_Chr01	802397	C	G	Fourth exon of CNA03070	synonymous
30-17	SNP	XL280_Chr05	170156	G	A	Start of third exon of CNE00630	A → V
30-17	SNP	XL280_Chr09	878884	A	C	intergenic	
30-17	SNP	XL280_Chr09	1148096	T	G	Middle of CNI04310	
30-17	SNP	XL280_Chr10	489241	T	C	intergenic, between CNJ01650 and CNJ01660	
30-17	ins	XL280_Chr12	959798	ATT	ATTT	intergenic	
30-18	SNP	XL280_Chr01	2295566	G	A	Fifth exon of CNA08320	A → V
30-18	SNP	XL280_Chr04	1184333	C	T	intergenic	
30-18	SNP	XL280_Chr07	520652	G	C	5' UTR CNG01820	
30-18	SNP	XL280_Chr08	643616	G	A	intergenic	
30-18	SNP	XL280_Chr11	8490	T	A	intergenic, upstreamof CNK00020	
30-18	SNP	XL280_Chr14	77727	C	T	3' UTR CNN00175	
30-18	ins	XL280_Chr01	535657	CA	CAA	intergenic	
30-18	del	XL280_Chr12	1060876	AG	A	3' UTR CNL06555	
30-18	ins	XL280_Chr02	930640	TAA	TAAA	intergenic	
30-19	SNP	XL280_Chr03	211100	C	T	5' UTR CNC00705	
30-19	SNP	XL280_Chr03	1353827	A	T	5' UTR CNC04440	
30-19	SNP	XL280_Chr05	529530	G	A	Intronic, between the 12th and 13th exons of CNE01970	
30-19	SNP	XL280_Chr05	529532	T	A	Intronic, between the 12th and 13th exons of CNE01970	
30-19	SNP	XL280_Chr05	529533	A	T	Intronic, between the 12th and 13th exons of CNE01970	
30-19	SNP	XL280_Chr06	270095	C	G	3' UTR CNF00810	
30-19	SNP	XL280_Chr09	993670	A	G	First exon of CNI03680	F → S
30-19	SNP	XL280_Chr12	672917	A	G	intergenic, downstream of CNL05120	
30-19	del	XL280_Chr01	1701439	AATATAT	AATAT	3' UTR CNA06220	
30-19	ins	XL280_Chr12	984440	TAA	TAAA	5' UTR CNL06320	
30-20	SNP	XL280_Chr01	296665	G	T	Third exon of CNA01100	A → D
30-20	SNP	XL280_Chr01	1894226	C	A	intergenic, downstream of CNA06940	
30-20	SNP	XL280_Chr01	1894227	A	T	intergenic, downstream of CNA06940	
30-20	SNP	XL280_Chr02	773886	T	C	Intronic, between the 14th and 15th exons of CNB02580	
30-20	SNP	XL280_Chr03	361184	T	G	Intronic, between the eighth and ninth exons of CNC01260	
30-20	SNP	XL280_Chr03	818514	A	T	intergenic	
30-20	SNP	XL280_Chr04	69985	C	T	intergenic, upstream of CND00210	
30-20	SNP	XL280_Chr05	1321956	T	A	3' UTR CNE04710	
30-20	SNP	XL280_Chr07	1266968	G	A	Second exon of CNG04300	D → N
30-20	SNP	XL280_Chr08	461733	C	T	intergenic	
30-20	SNP	XL280_Chr14	45058	A	G	First exon of CNN00120	synonymous
30-20	del	XL280_Chr12	959796	AC	A	intergenic	
30-20	SNP	XL280_Chr01	1012449	G	C	3' UTR CNA03770 or 2nd to last exon of CNA03780	R→ G
30-20	ins	XL280_Chr07	675124	TAA	TAAA	intergenic	

Table S4 cont. Sequence variations detected in Illumina-sequenced TA lines.

TA line	Type	Chromosome	Position	Reference Allele	Alternative Allele	Location	Amino Acid Change
37-01	SNP	XL280_Chr03	1153796	C	T	Start of second exon of CNC03640	synonymous
37-01	SNP	XL280_Chr03	1258899	C	A	Start of the first exon of CNC04030	A → S
37-01	SNP	XL280_Chr05	750241	C	T	First exon of CNE02840	H → Y
37-01	SNP	XL280_Chr08	812409	T	A	5′ UTR CNH00440	
37-01	SNP	XL280_Chr08	812416	T	A	5′ UTR CNH00440	
37-01	SNP	XL280_Chr11	31047	T	C	First exon of CNK00090	synonymous
37-01	SNP	XL280_Chr11	192953	C	T	intergenic	
37-01	SNP	XL280_Chr13	588580	G	C	Intronic, between the second and third exons of CNM01790	
37-01	ins	XL280_Chr06	550147	GGTCG	GGTCGTCG	intergenic	
37-01	del	XL280_Chr09	83065	TAAA	TAA	5′UTR CNI00350	
37-02	SNP	XL280_Chr01	2187372	C	T	End of second exon of CNA07900	synonymous
37-02	SNP	XL280_Chr02	146365	G	C	intergenic, downstream of CNB00500	
37-02	SNP	XL280_Chr03	984134	C	G	intergenic	
37-02	SNP	XL280_Chr05	884767	A	T	intergenic	
37-02	SNP	XL280_Chr12	547270	A	G	3′ UTR CNL04680	
37-02	ins	XL280_Chr02	56981	CAAAAAA	CAAAAAAA	Right side of unkown gene CNB00180	
37-02	ins	XL280_Chr04	740203	ACAG	ACAGCAG	3′ UTR CND02690	
37-02	ins	XL280_Chr07	984812	ATAG	ATAGTAG	intergenic	
37-03	SNP	XL280_Chr01	1258883	C	T	End of second exon of CNA04760	A → S
37-03	SNP	XL280_Chr02	1516464	C	G	second exon of CNB05310	synonymous
37-03	SNP	XL280_Chr03	1429233	C	T	5′ UTR CNC04720	
37-03	SNP	XL280_Chr04	1209022	G	T	intronic, between the second and third exons of CND04340	
37-03	SNP	XL280_Chr07	59136	G	A	intergenic	
37-03	SNP	XL280_Chr12	414271	C	T	End of first exon of CNL04230	E → K
37-03	SNP	XL280_Chr13	814001	C	G	Middle of second exon of CNM02550	synonymous
37-16	SNP	XL280_Chr01	372508	C	G	Last exon of CNA01360	A → G
37-16	SNP	XL280_Chr01	1995534	T	C	3′ UTR CNA07270	
37-16	SNP	XL280_Chr01	2105474	C	T	Start of seventh exon of CNA07650	synonymous
37-16	SNP	XL280_Chr01	2135960	A	C	intergenic	
37-16	SNP	XL280_Chr03	1918108	T	G	Sixth exon of CNC06530	V → G
37-16	SNP	XL280_Chr03	1918109	T	G	Sixth exon of CNC06530	V → G
37-16	SNP	XL280_Chr03	1918110	C	G	Sixth exon of CNC06530	R → G
37-16	SNP	XL280_Chr04	924944	C	T	Last exon of CND03390	A → V
37-16	SNP	XL280_Chr06	1245185	T	C	Third exon of CNF04340	synonymous
37-16	SNP	XL280_Chr07	499876	C	T	Start of last exon of CNG01730	L → F
37-16	SNP	XL280_Chr07	627715	G	A	intergenic	
37-16	SNP	XL280_Chr07	820480	C	T	5′ UTR CNG02950	
37-16	SNP	XL280_Chr09	554445	G	A	Third exon of CNI02020	S → N
37-16	SNP	XL280_Chr11	717431	A	T	intergenic	
37-16	SNP	XL280_Chr12	61390	T	G	Start of CNH03240	
37-16	SNP	XL280_Chr12	432012	C	T	Fifteenth exon of CNL04300	S → L
37-16	SNP	XL280_Chr13	293825	A	G	End of third exon of CNM00850	synonymous
37-16	del	XL280_Chr01	1741511	GCGCTCGAAT	G	Three amino acid deletion in first exon of CNA06390	Non-synonymous
37-17	SNP	XL280_Chr02	1554542	C	T	End of the fourth exon of CNB05450	T → I
37-17	SNP	XL280_Chr04	1429507	C	T	intronic, between the third and fourth exons of CND05210	
37-17	SNP	XL280_Chr09	249373	A	C	intronic, betweeen the second and thrid exon of CNI01010	
37-17	SNP	XL280_Chr10	192884	T	A	5′ UTR CNJ00650	
37-17	del	XL280_Chr02	818765	CA	C	intergenic	
37-18	SNP	XL280_Chr03	655336	C	T	Twelfth exon of CNC02260	synonymous
37-18	SNP	XL280_Chr04	1532615	C	T	5′ UTR CND05660	
37-18	SNP	XL280_Chr06	1387880	G	A	Intronic, between the third and fourth exons of CNF04830	
37-18	SNP	XL280_Chr09	190234	A	C	Second exon of CNI00790	I → L
37-18	ins	XL280_Chr02	930637	AAATAATA	AAATAATAATA	intergenic	
37-19	SNP	XL280_Chr02	1025015	G	A	First exon of CNB03350	R → C
37-19	SNP	XL280_Chr04	891040	G	C	Middle of the last exon of CND03290	E → D
37-19	SNP	XL280_Chr05	1468894	G	A	intergenic	
37-19	SNP	XL280_Chr12	243336	T	G	Intergenic, downstream of CNL03840	
37-19	SNP	XL280_Chr13	105634	C	T	Middle of last exon of CNM00300	synonymous
37-19	del	XL280_Chr05	1465624	TTAGGGG	T	intergenic	
37-19	del	XL280_Chr10	1095668	TTAGGGG	T	intergenic	
37-19	ins	XL280_Chr11	3415	AAC	AACAC	intergenic	
37-19	ins	XL280_Chr12	984442	AT	ATT	5′ UTR CNL06320	
37-19	SNP	XL280_Chr08	942065	C	A	intergenic, near telomeric end of chromosome	
37-19	SNP	XL280_Chr08	942141	T	C	intergenic, near telomeric end of chromosome	
37-20	SNP	XL280_Chr04	1500543	G	T	Middle of first exon of CND05510	P → Q
37-20	SNP	XL280_Chr05	928064	C	T	Third exon of CNE03250	P → S
37-20	SNP	XL280_Chr06	402347	T	G	Start of the first exon of CNF01360	Q → H
37-20	SNP	XL280_Chr08	330959	C	T	Middle of the last exon of CNH01920	G → W
37-20	SNP	XL280_Chr09	176732	T	C	Fourth exon of CNI00720	D → G
37-20	del	XL280_Chr02	976993	TA	T	5′ UTR CNB03180	
37-21	SNP	XL280_Chr01	613931	G	A	Eighth exon of CNA02350	E → K
37-21	SNP	XL280_Chr11	194687	T	A	intergenic	
37-21	del	XL280_Chr03	464160	TAA	TA	intergenic	
37-22	SNP	XL280_Chr07	122589	G	A	Last exon of CNG00470	synonymous
37-22	SNP	XL280_Chr09	899517	T	A	intergenic, upstream of CNI03320	
37-22	SNP	XL280_Chr12	1046929	C	T	5′ UTR CNL06500	

Table S5. Primers used in this study.

ID	5'-> 3' DNA Sequence	Target region	Chr	TA line	TE
ES90	AAGTAGCTTTCTCCTCTATCGTCCCTCCC				
ES91	CCTCTGTTCTTTGTGTTCGGCAATTCTCG	CNE03200/CNE03210	5	XL280α	T1.1 (native)
ES92	CAGGTGGACATCATCATCCAGAGGTATGC				
ES93	TCTTTAAAGGCATCTTGTTCCCGCTTTGC	CND03770/CND03780	4	XL280α	T1.2 (native)
ES94	GAGATGAACGTAGCGCGCTTAGTAGTAGG				
ES95	GGCTGTAAAGTAGCGAAGAAACAAAGCCC	CND00180/CND00190	4	XL280α	T1.3 (native)
ES96	AGTGTCGTGAGAGTAGTGACAATCAAGCG				
ES97	TGGGATTGAATGTGGCAGAATAGGGATCG	CNF00100/CNF00110	6	XL280α	T1.4 (native)
ES102	TGCCATTTTGAAGAAGAGCTGAATGGTGC				
ES103	AGCTCTAAGCACAGATATGTCCGACATGC	CNA08210/CNA08220	1	XL280α	T1.4 (native)
ES62	GTGGACGATGGTAAAACGCATTTGGTAGC				
ES63	CTCCGTATCCCATAGACTGGTTTTTGGGG	CNH02250/CNH02260	8	30-01	T1
ES203	ATGAGAAAGCGTCCCGGGGACAAG				
ES204	TGGTAAATAAATGGAGCGAAGAGAGC	CNB02620/CNB02630	2	30-02	T1
ES134	AAGACTGCGATGACGATGAACGCG				
ES135	TGGTCGACGTTACCCCATCCCATC	CNC05220	3	30-02	T1
ES136	TCGATGATCAGAAGGGGCTCTGGC				
ES137	ATGGTGGTGGTCAAAGCTGTGCAC	CNK03170/CNK03180	11	30-02	T1
ES138	CAATTTGATCCCGTGGTGGAGGGC				
ES139	TCCAAACCCAGCACCCAGAGACTC	CNM02230/CNM02240	13	30-02	T1
ES146	CGCTACAGCCTTGCAGCATCTCTG				
ES147	CAACTGGCTGGACACAGAACTGCC	CNG03170/CNG03180	7	30-03	T1
ES140	TCCATCCTTCCGGGCCAGTACAAG				
ES141	CCCGCCCAATCACAGCAATAGACG	CNC01660/CNC01670	3	30-16	T1
ES142	GAGTCGGTGTTGACAGCAAGCTGG				
ES143	TTGGAGAGGGGGTCATCTTGCTCG	CNF02870/CNF02875	6	30-16	T1
ES144	TATTCGGAGAGCTGGGTGCGGTAC				
ES145	AGGGTCTGATCGGGCTGGATCAAC	CNF00090/CNF00080	6	30-17	T1
ES199	ATCTGGGTGCTGCACACAAATCCG				
ES200	GTGATAGTGGAGCTTGCGGCAGTG	CNJ02860/CNJ02870	10	30-20	T1
ES66	AATTCGAGAAGAGCGGGGTCATGAATACC				
ES67	AAATAAGGTGACAGAAAGCTGAGGAGGGC	CNG01990/CNG02000	7	37-01	T1
ES68	TTACTTCCCGTTGCACTGTCATCTTCTCC				
ES69	ACTATAGGCTGACTGAGAGAGTAGAGCGC	CNH01775/CNH01780	8	37-01	T1
ES64	CTGAATGTTAAGTGCAGCGTAATGAGCCG				
ES65	TCAATTCCAGTGGATAGCGTACGTTACCG	CNJ00540/CNJ00550	10	37-01	T1
ES70	CTGGACAACCTCCACCTTTGTTTCTTTGC				
ES71	TCTGCTTCTTGTCCATCCATTTCTTCCCC	CNJ02660/CNJ02670	10	37-01	T1
ES116	ATTAGAAGCTACCCTTGTTGTCGTCGTCG				
ES117	TGTGAGCAATTCGACATTGGGCATAAACG	CNA06700	1	37-01	Tcn12
ES114	TAAACGAATTGTCACTAGGTCGTGGAGCC				
ES115	TGGCAATATTTGGTCCCGCTATCTGATCC	CNB03750	2	37-01	Tcn12
ES74	ACTTACTACTGCACTCTCAATGGTGTGGC				
ES75	ACCGAATTTAGGGATGAGGATCTTGGTGC	CNA06050/CNA06060	1	37-02	T1
ES72	CCTGCTTTTGCTTATGCCCAATCATACCC				
ES73	GTCTCTTCCCACTCTCCTTCTGTCTTTGC	CNA07360/CNA07370	1	37-02	T1
ES118	TAGCCAACTGCGGATTGTTTGTTACAAGC				
ES119	ATTGCTGTTTCTCCGACTCATACAGTCCC	CNA07360/CNA07370	1	37-02	T1
ES76	GACGTTGGCTGCCGTAATAATCTTTGTCC				
ES77	TCGATGGAATGGAAGGAAAGACACTGACG	CNB02690/CNB02680	2	37-02	T1

Table S5 cont. Primers used in this study.

ES211	GTCGCAGGTGCAGTTACCCACTTG				
ES212	TTCGATCACTTCCCTCCCATCCCC	CNC05440	3	37-17	Tcn12
ES213	CAAGTCGAAGTGGGCTTGACCGTG				
ES214	AGGTGGTATCTGATGGGCGTCGTC	CNE03680	5	37-17	Tcn12
ES201	ATGTGAGCCGCGTGATCCAAAAGG				
ES202	CGGTTGAAACACGGCAACAAGCAC	CNB02900/CNB02910	2	37-18	T1
ES150	TTCTGAGCTCTGGGCATCCATCCC				
ES151	GTCCGCAAGAGCATGAGCACTACG	CNN00910/CNN00920	14	37-18	T1
ES215	TTTGGTCTTGAGAGACGGCGGAGG				
ES216	GTACCCGAAAACCGCTCGATGTCG	CNI04250	9	37-18	Tcn12
ES217	GCCGCCTCTCTTCATGTCGGTTTC				
ES218	ACCATGTGTGTACGGGATTGGTGC	CNI02060	9	37-18	Tcn12
ES154	CGTACGTTCTCGTCGTGCTTGACC				
ES155	TGGGATCAGGCGGATGAAGTCGAG	CNA01360/CNA01370	1	37-19	T1
ES152	CGCTTATGCAAGACCAGCCTCGAC				
ES153	GCTGACTAGCGCGAGAAACAGGTG	CNA08090/CNA08100	1	37-19	T1
ES156	ATTGCGCAGAGGAGGTAGGAGACG				
ES157	AGACGATGGTTCGCGCACATAAGC	CNF01870/CNF01880	6	37-19	T1
ES158	CGCTCAAAGGCTCGAAGTATCGCC				
ES159	ACTGGTGGAGGTGATTCCGGTGTC	CNM02270/CNM02280	13	37-19	T1
ES219	AAACGAGCGCACGTACCAACATCG				
ES220	GGGTGGGGTATCACTTGCCATTGC	CNF04770	6	37-19	Tcn12
ES221	CACAGTTGGTGTGGCCAGATCAGC				
ES222	CGGCAAGCTGATCTCAGGAACGTC	CNJ03330	10	37-19	Tcn12
ES160	CGCTCAGTGCCGGTGTCAGTATTG				
ES161	GCGTCTGGTCCAACGATATTGGCC	CNA03220/CNA03230	1	37-20	T1
ES223	AGAGAGCGTCGGAAGATTCGGGTG				
ES224	TGGAGCACAGGCAAATTGTCGAGG	CNA08120	1	37-20	Tcn12
ES225	ACGCCAGCATTTATGACGACCACG				
ES226	AAGAACCTCATCCTGCGGCGATTG	CNE05240	5	37-20	Tcn12
ES164	GAGAGCAAGCTCGTCAACGTCGTC				
ES165	TTGCTTGGTGGCATTGTCTGACGG	CND00300/CND00310	4	37-21	T1
ES166	AGAAGCCTACATACCGGGCGTCAG				
ES167	ACTCGTGCCCAGGTTCCCTAAAGG	CND03920/CND03930	4	37-21	T1
ES168	CGTGAAGCGCATGGGAACTGTAGG				
ES169	GGAGCATCGAACCATCCGTCGTTG	CNI01880/CNI01890	9	37-21	T1
ES227	CAAAGGAGTGCCAGTCCGAGTTGC				
ES228	CGAGTGTTGCGCAGGTCTACCATG	CND04840	4	37-21	Tcn12
ES170	ATCACTCACTCCTGGCCCAAGAGC				
ES171	CTCAAAAGGAGGCGGGTTGACACG	CNA00660/CNA00670	1	37-22	T1
ES172	CTTCAACCCTTGCCACGTCTGCTC				
ES173	TGCAGAAACAGTGACTGTGCGTCG	CNB02545/CNB02550	2	37-22	T1
ES174	GACTCGCAACCGATCCAGAAGTGC				
ES175	AGAAGCGGAACCTGCGTGTGAAAC	CNC04810/CNC04820	3	37-22	T1
ES176	GCTTGTCCCAAGCTGCTCGTATGG				
ES177	CCGTACCATTCTCGCACTCGCATG	CNE03310/CNE03320	5	37-22	T1

Table S5 cont. Primers used in this study.

ID	Full Name	5'-3' DNA Sequence	Probe size (bp)	Source
ES130	RetroF Cnl1	AGCAGCACATCATCAAGCTC	844	Janbon G. et al., *Fungal Genet Biol.* 2010.
ES131	RetroR Cnl1	TAAATGACGCGGTTGATGGC		
ES180	Tcn12 Probe B F	TGAGAAAGCCATTCATTCGGCCGG	537	This study
ES181	Tcn12 Probe B R	ATCCACTGGCGAGCCCTTCTACAC		
JW83	T1 Probe F	GTCGACGAGAGGAAATCTCATACTGTAC	388	This study
JW84	T1 Probe R	CAGAGAGGACATCGGCAGCGG		

ID	Target	5'-3' DNA Sequence	Source
AG34	GPD1	GTCTCCACTGATTTCATTGGCTCTAC	JOHE44120; Fu et al. PLoS Genetics 2019
AG35	GPD1	GTAACCATACTCATTGTCATACCAGCT	JOHE44121; Fu et al. PLoS Genetics 2019
AG36	GPD1	GTAACCATACTCATTGTCATACTAAAAG	This study
AG37	CNH01775	CAGAAATTACAAGACGAACAGGACG	
AG38	CNH01775	GTGAAGCAACCGCTGGACTATC	
AG41	CNH01780	GCACTTATCCAAGGCCCTCC	
AG42	CNH01780	AGATCGGTACCCTCCGTTCAG	
AG45	CNJ00540	ATCAAGCCAGTCCCAGCATC	
AG46	CNJ00540	ATTGGCTTGGGTCTCAGCTC	
AG63	CNJ00550	GGCGTTCACCAAACCAACTG	
AG64	CNJ00550	TCCAAGAACGAATAGGGGCCA	
AG71	CNC00200	CTGGTGCAAAGATTCCCCCG	
AG72	CNC00200	CTCGAACCCCACACCTTCCTC	
AG75	CNC00190	ATGCGGCCACTTTTACCGAG	
AG76	CNC00190	GGTCGGCCATTGTTCTGAAGG	
AG81	CNC05120	TCAAGGCAAGCCAATGCTGG	
AG82	CNC05120	GCCGTCCGCTGAAGTTTGG	
AG87	CNC05130	GCTTCAATCCGCAAGCAAACT	
AG88	CNC05130	TTCCAGTCGATCTCGAAACGC	
AG91	CNK01950	AGGCCTTGGACTTACAGGAAAAC	
AG92	CNK01950	CTGGGAGTTGCCTGACAAGG	
AG97	CNK01960	AGGAGGAATGCCCATGGTTG	
AG98	CNK01960	AACATCCGCAACGGTCTTGG	
AG105	CNM00880	CGAGGACGAACTTCAGGACG	
AG106	CNM00880	TGGCTCGCTTATTGGCCTTG	
AG109	CNM00890	ACAGGACCAATGTCGCACAA	
AG110	CNM00890	TGATCGGCCCACACATTCC	
AG143	5'-end Cnl1	GGCTCTTCCCTAGTCGCTTG	
AG144	5'-end Cnl1	CAGTATTGGAGGGAGGGCAG	

Table S6. Whole-genome sequencing data for the progenitor and evolved lines in this study.

Strain	Genotype	Type	Sequencing	Library ID	Contigs	Number of bases	Npairs, Number of Reads	Mapped reads	Mapped percent	N50 Nanopore reads (kb)	Coverage	GC (%)	SRA Accessions
XL280α.1	XL280α	Progenitor	Illumina	JW-S21		526938248	3466699	5826445	84%		23X	46.7	SRR17430304
XL280α.2	XL280α	Progenitor	Illumina	JW-S22		471931880	3104815	4339438	70%		17X	44.4	SRR17430303
XL280α.3	XL280α	Progenitor	Illumina	JW-S23		505179144	3323547	5599854	84%		22X	47.0	SRR17430302
30-01	XL280α	Evolved	Illumina	JW-S1		1004562224	6608962	11838035	90%		43X	49.2	SRR17430309
30-02	XL280α	Evolved	Illumina	JW-S3		1083684456	7129503	12593297	88%		46X	49.4	SRR17430301
30-03	XL280α	Evolved	Illumina	JW-S7		835705880	5498065	9697873	88%		37X	48.8	SRR17430296
30-16	XL280α	Evolved	Illumina	JW-S4		1491342832	9811466	17446347	89%		65X	49.0	SRR17430300
30-17	XL280α	Evolved	Illumina	JW-S6		1038913616	6834958	12100455	89%		45X	48.9	SRR17430298
30-18	XL280α	Evolved	Illumina	JW-S8		1452042168	9552909	16751488	88%		63X	48.6	SRR17430295
30-19	XL280α	Evolved	Illumina	JW-S9		1141589160	7510455	13296008	89%		50X	49.2	SRR17430294
30-20	XL280α	Evolved	Illumina	JW-S10		1009917488	6644194	11837423	89%		44X	49.0	SRR17430308
37-01	XL280α	Evolved	Illumina	JW-S11		867208032	5705316	9608368	84%		37X	48.6	SRR17430297
37-02	XL280α	Evolved	Illumina	JW-S12		1122254608	7383254	12824509	87%		50X	48.9	SRR17430293
37-03	XL280α	Evolved	Illumina	JW-S15		1122254608	7383254	14476049	88%		56X	48.9	SRR17430293
37-16	XL280α	Evolved	Illumina	JW-S13		1245138552	8191701	11814801	88%		56X	49.4	SRR17430292
37-17	XL280α	Evolved	Illumina	JW-S14		1019033992	6704171	14064636	87%		47X	49.0	SRR17430291
37-18	XL280α	Evolved	Illumina	JW-S16		841991384	5539417	9420983	85%		37X	48.0	SRR17430289
37-19	XL280α	Evolved	Illumina	JW-S17		971206736	6389518	10808290	85%		42X	49.1	SRR17430288
37-20	XL280α	Evolved	Illumina	JW-S18		789026376	5190963	8845862	85%		35X	48.7	SRR17430287
37-21	XL280α	Evolved	Illumina	JW-S19		903086416	5941358	10495289	88%		41X	49.2	SRR17430307
37-22	XL280α	Evolved	Illumina	JW-S20		586909696	3861248	6553746	85%		26X	48.0	SRR17430305
30-01	XL280α	Evolved	Nanopore	barcode01	16	1910087201	178799			19.1	95X	44.5	SRR17722990
30-02	XL280α	Evolved	Nanopore	barcode05	17	1810680829	169584			19.6	90X	45.2	SRR17722989
37-01	XL280α	Evolved	Nanopore	barcode03	27	1856254210	215193			14.8	92X	40.0	SRR17722988
37-02	XL280α	Evolved	Nanopore	barcode04	17	1667488478	148974			20.1	83X	45.2	SRR17722987
37-03	XL280α	Evolved	Nanopore	barcode06	15	1920025405	168187			22.1	96X	45.5	SRR17722986
37-04	XL280α	Evolved	Nanopore	barcode07	16	2033781623	199905			18.5	102X	44.8	SRR17722985
37-09	XL280α	Evolved	Nanopore	barcode08	17	1692920250	180541			17	84X	44.7	SRR17722984

SI References

1. J. Chen, T. R. Wrightsman, S. R. Wessler, J. E. Stajich, RelocaTE2: a high resolution transposable element insertion site mapping tool for population resequencing. *PeerJ* **5**, e2942 (2017).
2. S. Han *et al.*, Transposable element profiles reveal cell line identity and loss of heterozygosity in Drosophila cell culture, *Genetics* 219.2 (2021).
3. W. J. Kent, BLAT--the BLAST-like alignment tool. *Genome Res* **12**, 656-664 (2002).
4. H. Li, R. Durbin, Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**, 1754-1760 (2009).
5. G. G. Faust, I. M. Hall, SAMBLASTER: fast duplicate marking and structural variant read extraction. *Bioinformatics* **30**, 2503-2505 (2014).
6. P. Danecek *et al.*, Twelve years of SAMtools and BCFtools. *Gigascience* **10** (2021).
7. E. P. Garrison, G. T. Marth, Haplotype-based variant detection from short-read sequencing. *arXiv: Genomics* (2012).
8. H. Thorvaldsdóttir, J. T. Robinson, J. P. Mesirov, Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Brief Bioinform* **14**, 178-192 (2013).
9. J. W. Pitkin, D. G. Panaccione, J. D. Walton, A putative cyclic peptide efflux pump encoded by the TOXA gene of the plant-pathogenic fungus Cochliobolus carbonum. *Microbiology* **142**, 1557-1565 (1996).
10. S. Koren *et al.*, Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. *Genome Res* **27**, 722-736 (2017).
11. H. Li, Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics* **34**, 3094-3100 (2018).
12. K. Schotanus, V. Yadav, J. Heitman, Epigenetic dynamics of centromeres and neocentromeres in *Cryptococcus deuterogattii*. *PLoS Genet* **17**, e1009743 (2021).
13. A. Gusa *et al.*, Transposon mobilization in the human fungal pathogen *Cryptococcus* is mutagenic during infection and promotes drug resistance in vitro. *Proc Nat Acad Sci USA* **117**, 9973 (2020).