SUPPLEMENTARY INFORMATION

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Supplementary Table 1. Data Sources

Data	Source
Yeast DNase I footprints[51]	http://downloads.yeastgenome.org/published_datasets/Hesselberth_2009_PMID_19305407/track_files/Hesselberth_2009_DNaseI_hypersensitive_sites_V64.bed
OF sequencing[17]	GEO:GSM835651, GEO:GSM835650[a] http://www.ncbi.nlm.nih.gov/geo/
Replication timing data[36]	http://www.sciencemag.org/site/feature/data/raghu1064351/SmoothedPooledHLData/smoothedpooledHLdata.html
Gene annotations[61]	http://hgdownload.soe.ucsc.edu/goldenPath/sacCer3/database/sgdGene.txt.gz
Origins of replication[50]	http://downloads.yeastgenome.org/published_datasets/Eaton_2010_PMID_20351051/track_files/Eaton_2010_ORC_ACS_V64.bed
Yeast ChIP-exo data (Reb1, Rap1)[55]	SRA:SRA044886[b]
TF binding motifs[57]	http://jaspar.genereg.net/html/DOWNLOAD/JASPAR_CORE/pfm/nonredundant/pfm_all.txt
Human DNase I[59]	ftp://ftp.ebi.ac.uk/pub/databases/ensembl/encode/supplementary/integration_data_jan2011/byDataType/footprints/jan2011/all.footprints.gz ftp://ftp.ebi.ac.uk/pub/databases/ensembl/encode/supplementary/integration_data_jan2011/byDataType/footprints/jan2011/combined.fps.gz
Yeast genomic alignments	http://hgdownload.soe.ucsc.edu/goldenPath/sacCer3/multiz7way/maf/
Yeast polymorphism data[53]	ftp://ftp.sanger.ac.uk/pub/users/dmc/yeast/latest/misc.tgz
Human ChIP-seq (CREB1, ETS1, MEF2A, NFIC, USF1)[62]	SRA:SRP008797[b]
Human ChIP-seq (ELK1, ELK4, IRF1, STAT1, YY1)	GEO:GSE31477[a], SRA:SRP007993[b]
Human ChIP-seq (CEBPA)[63]	SRA:SRP008215[b]
Human ChIP-seq (FOXA1)[64-66]	SRA:SRP003811, SRA:SRP007486, SRA:SRP006770[b]
Human ChIP-seq (FOXO3)[67]	SRA:SRP010705[b]
Human ChIP-seq (NFκB)[68]	SRA:SRP002125[b]
Human ChIP-seq (NFYA)[69]	SRA:SRP005174[b]
Human ChIP-seq (REST)[70,71]	SRA:SRP010385, SRA:SRP017090, SRA:SRP006944[b]
Human ChIP-exo (CTCF)[55]	SRA:SRX098245[b]
Mammalian GERP scores[41]	http://hgdownload.cse.ucsc.edu/gbdb/hg19/bbi/All_hg19_RS.bw

[a] GEO: http://www.ncbi.nlm.nih.gov/geo/

[b] SRA: http://www.ncbi.nlm.nih.gov/sra

Supplementary Table 2. Sequencing data

Run Name	Genotype[a]	Treatment[b]	Platform[c]	Mapped Reads
run184	*POL*	Nb.BtsI	318	3,701,465
run185	*pol1-L868M*	Nb.BtsI	318	4,657,409
run186	*pol1-L868M*	RNase H2	318	4,471,771
run190	*POL*	RNase H2	318	2,326,391
run192	*pol1-L868M*	RNase H2	318	3,513,472
run195	*POL*	RNase H2	318	3,914,015
run219	*pol3-L612M*	RNase H2	318	4,170,543
run220	*pol1-L868M*	RNase H2	318	2,991,737
run237	*POL*	RNase H2	318	5,657,073
run238	*pol1-L868M*	RNase H2	318	3,515,150
run254	*pol3-L612M*	RNase H2	318	2,644,442
run256	*pol2-M644G*	RNase H2	318	5,452,706
run258	*pol2-M644L*	RNase H2	318	5,309,315
run25	*pol3-L612M*	RNase H2	P1	51,728,218
run28	*pol2-M644G*	RNase H2	P1	58,479,674
run29	*pol2-M644L*	RNase H2	P1	58,669,607
run35	*POL*	RNase H2	P1	30,446,944
run36	*pol1-L868M*	RNase H2	P1	51,060,856
run37	*POL* (stat phase)	RNase H2	P1	52,949,855
run38	*pol1-L868M* (stat phase)	RNase H2	P1	57,880,810

[a] All genotypes are *Δrnh201* with the relevant polymerase mutations indicated

[b] Treatments indicated refer to the endonuclease nicking step in the library preparation

[c] Ion-semiconductor sequencing was performed using Ion PGM 318 chips or Ion Proton PI chips (Life Technologies)

Supplementary Table 3. ***Saccharomyces cerevisiae*** **strains**

Name	Strain	Relevant genotype[a]	Reference
wt	SNM8	*POL1 POL2 POL3 RNH201*	[27]
Δrnh201	SNM106	*rnh201::hphMX4*	[27]
pol1-L868M	SNM15	*pol1-L868M*	[b]
pol1-L868M Δrnh201	YJW13	*pol1-L868M rnh201::hphMX4*	[b]
pol2-M644G	SNM70	*pol2-M644G*	[27]
pol2-M644G Δrnh201	SNM120	*pol2-M644G rnh201::hphMX4*	[27]
pol2-M644L	SNM82	*pol2-M644L*	[27]
pol2-M644L Δrnh201	SNM132	*pol2-M644L rnh201::hphMX4*	[27]
pol3-L612M	SNM11	*pol3-M644L*	[30]
pol3-L612M Δrnh201	YJW11	*pol3-M644L rnh201::hphMX4*	[30]
Δrnh201 + ycplac111	MRY46	*rnh201::hphMX4 [ycplac111]* [c]	This work, [39]
Δrnh201 + pRNH201-wt	MRY47	*rnh201::hphMX4 [pRNH201-wt]* [c]	This work, [39]
Δrnh201 + pRNH201-sf	MRY48	*rnh201::hphMX4 [pRNH201-sf]* [c]	This work, [39]
pol1-L868M Δrnh201 + ycplac111	MRY49	*pol1-L868M rnh201::hphMX4 [ycplac111]* [d]	This work, [39]
pol1-L868M Δrnh201 + pRNH201-wt	MRY50	*pol1-L868M rnh201::hphMX4 [pRNH201-wt]* [d]	This work, [39]
pol1-L868M Δrnh201 + pRNH201-sf	MRY51	*pol1-L868M rnh201::hphMX4 [pRNH201-sf]* [d]	This work, [39]

[a] All strains are isogenic derivatives of strain Δ|(-2)|-7B-YUN1300 (MATα CAN1 his7-2 leu2-Δ::kanMX ura3-Δ trp1-289 ade2-1 lys2-ΔΔGG2899-2900) and contain a *URA3* reporter knock in (*agp1::URA3-OR1*)[27,30]. All SNM and YJW strains were kindly provided by JS Williams and TA Kunkel (NIEHS).

[b] JS Williams, AR Clausen and TA Kunkel, unpublished

[c] ycplac111 (empty vector), pRNH201-wt and pRNH201-sf (ycNPH2-FL2 expressing FLAG-tagged wild type Rnh201p and Rnh201p-P45D-Y219A separation of function mutant respectively)[39] were kindly provided by SM Cerritelli and RJ Crouch (NICHD), and transformed into SNM106 and maintained on SD-Leu medium

[d] Vectors were transformed into YJW13 and maintained on SD-Leu medium

Supplementary References

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