SUPPLEMENTAL METHODS

Table 1. Yeast and E. coli strains

E. coli

CJ236	*FΔ(HindIII)::cat (Tra+ Pil+ CamR)/ ung-1 relA1 dut-1 thi-1 spoT1 mcrA*

Yeast

YJH513	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 trp1Δ bar1Δ::LEU2*
YJH602	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 bar1Δ::LEU2*
YJH516	*MATa leu2-3-112 trp1-289 his7-2 ura3-52 lys1-1 ade2Δ dut1-1 ung1Δ::URA3 bar1Δ::kanMX hENT::LEU*
YJH506	*MATalpha can1Δ::STE2pr-Sp_his5 lyp1Δ ura3Δ0 leu2Δ0 his3Δ1 met15Δ0 ung1Δ::natMX*
YJH532	YJH506 *dut1-1::URA3*

Supplemental table 1: *E. coli* and *S. cerevisiae* strains used for this study.

Table 2. Oligonucleotides

PCR primers

JH0801	CAAGCAGAAGACGGCATACGAGCTCTTCCGATCT
JH1140	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
JH1141	CAAGCAGAAGACGGCATACGAGATCGGTATCACGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
JH1147	CAAGCAGAAGACGGCATACGAGATCGGTCGATGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC
JH1151	CAAGCAGAAGACGGCATACGAGATCGGTGCCAATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATC

Adaptor sequences

JH0805	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGATC*

JH0806	/5Phos/GATCAGATCGGAAGAGCGGTTCAGCAGGAATGCCGAG
JH0807	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACTG*T
JH0808	/5Phos/CAGTAGATCGGAAGAGCGGTTCAGCAGGAATGCCGAG
JH0813	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTCA*T
JH0814	/5Phos/TGACAGATCGGAAGAGCGGTTCAGCAGGAATGCCGAG
JH0815	ACACTCTTTCCCTACACGACGCTCTTCCGATCTATCG*T
JH0816	/5Phos/CGATAGATCGGAAGAGCGGTTCAGCAGGAATGCCGAG
JH0804	ACACTCTTTCCCTACACGACGCTCTTCCGATC*T
JH1139	/5Phos/GATCGGAAGAGCACACGTCT

A "*" denotes a phosphothioate bond in the adaptor.

Generation of Excision-seq libraries to map uracil

Pre-digestion libraries. *E. coli (*CJ236) were grown to log phase at 37 ˚C in Luria Broth and collected by centrifugation. *S. cerevisiae* YJH516 or YJH532 were grown to mid-log phase (OD_{600} = 0.6), collected by centrifugation and resuspended in 5 mL of Buffer Z (1 M Sorbitol, 50 mM Tris pH 8.0, 10 mM 2-mercaptoethanol) and spheroplasted with 0.35 mg of zymolyase (20T, MP Biomedical) for 30 min at 30˚C. Spheroplasts were centrifuged and resuspended in 400 µL of lysis buffer (1 M Sorbitol, 1% SDS, 20 mM EDTA, 1 unit Proteinase K (New England Biolabs) and incubated overnight at 55 ˚C. High molecular weight genomic DNA was isolated by gentle phenol:chloroform extraction and ethanol precipitation. High molecular weight DNA was digested with 5 units of UDG and 10 units of T4 Endonuclease IV (New England Biolabs) for 2 hours at 37˚C. Following confirmation of digestion by agarose gel electrophoresis, DNA fragment ends were blunted using T4 DNA polymerase (150 units), 50 units T4 PNK (New England Biolabs) and 1 mM dNTPs for 30 minutes at 20˚C, A-tailed with Klenow fragment (15 units, Enzymatics) and dATP for 30 minutes at 37˚C, and ligated to pre-annealed adapters for 15 minutes at 15˚C with 3000 units of T4 Rapid DNA ligase (Enzymatics). *E. coli* sheared libraries used adaptors JH0813 and JH0814; E*. coli*

Excision-seq libraries used adaptors JH0815 and JH0816; pre-digestion Excision-seq libraries from *S. cerevisiae* used adaptors JH0807 and JH0808 for library preparation. Libraries were size selected 2% agarose gels (Size-select E-gel, Invitrogen) and 200-400 bp fragments were collected. Libraries were PCR amplified with primers JH0801 and JH1140 using Platinum Taq polymerase (Invitrogen), purified using AMPure XP beads (Beckman Coulter), and pooled for analysis by Illumina sequencing. Selection of other size ranges (400-600 bp) had no effect on the observed pattern of uracil content variation.

Post-digestion libraries. *S. cerevisiae* YJH516 or YJH532 were grown to log phase, collected by centrifugation, and spheroplasted as described above. High molecular weight DNA was mechanically sheared using a Diagenode bioruptor for 20 minutes (30 second intervals on/off at maximum intensity). DNA shearing was confirmed using agarose gel electrophoresis, and library preparation was performed as described above. Post-digestion Excision-seq libraries from *S. cerevisiae* used adaptors JH0804 and JH1139. After size selection, post-digestion libraries were digested with 5 units of UDG and 10 units of T4 Endonuclease IV (New England Biolabs) for 2 hours at 37˚C. Libraries were PCR amplified with primers JH1140 and JH1147 using Platinum Taq polymerase (Invitrogen), purified using AMPure XP beads (Beckman Coulter), and pooled for analysis by Illumina sequencing.

Analysis of uracil Excision-seq data

Sequences were analyzed by alignment to a reference genome (*sacCer1*) using Bowtie 2 (Langmead et al. 2009) and SAMtools (Li et al. 2009), processed to bedGraph format using BEDTools (Quinlan and Hall 2010), and visualized in the UCSC Genome Browser (Karolchik et al. 2011). Coverage at each position was normalized by the number of reads aligned in the library (*i.e.* reads per million, RPM). Using this method, the level of coverage at a specific site or region in the genome represents the relative quantity of uracil at that position.

Pharmacological TTP depletion via 5-fluorouracil

Yeast cells were grown to logarithmic phase in YEPD, diluted to 5×10^6 cells/mL and cultured for 6 hours at 30°C with 150 µM 5-fluorouracil (Sigma) (Seiple et al. 2006). Cells were collected by centrifugation and DNA was prepared as described above. For these libraries, pre-annealed oligonucleotides JH0804 and JH1139 were ligated to double stranded DNA fragments and amplified with primers JH1140 and JH1151.

Generation of Excision-seq libraries to map pyrimidine dimers

Yeast were grown to an OD_{600} of ~.4 and arrested with alpha factor. Cells were resuspended in Buffer Z (1 M sorbitol, 50 mM Tris-HCl pH 8.0, 10 mM 2-mercaptoethanol). Because these cells have a light-dependent photolyase repair enzyme (*PHR1*) subsequent steps were performed in the dark. Cells were irradiated in a petri dish with 256 nm light (Stratalinker) until a specified dose was obtained, as measured by a dosimeter (UVP). Following irradiation cells were harvested as in predigestion library. For initial preparations, UV damaged DNA (10 µg) was digested with 10 µg of UVDE (Trevigen, Inc.) for 2 hours at 30 ˚C and checked for shearing by agarose gel electrophoresis. Samples were treated with *Vibrio cholera* CPD photolyase

(Sancar and Sancar 2006) (50mM Tris-HCl pH 7.0, 50 mM NaCl, 5mM DTT, 1 mM EDTA pH 8.0, 20% glycerol) or *X. laevis* 6-4 photolyase (Kim et al. 1996) (50 mM Tris-HCl pH 7.5, 100 mM NaCl, 10 mM DTT, 10% (v/v) glycerol) for 2 hours at room temperature with illumination by 365 nm light (~280 kJ/m^2). In subsequent library preps, UV damaged DNA (10 μg) was digested with 10 μg of UVDEΔ288 (Avery et al. 1999) for 2 hours at 30 ˚C and checked for shearing by agarose gel electrophoresis. Samples were treated with *E. coli* CPD photolyase (Liu et al. 2011) (50mM Tris-HCl pH 7.0, 50 mM NaCl, 5mM DTT, 1 mM EDTA pH 8.0, 20% glycerol) or *A. thaliana* 6-4 photolyase (Li et al. 2010) (50 mM Tris-HCl pH 7.5, 100 mM NaCl, 10 mM DTT, 10% (v/v) glycerol) for 2 hours at room temperature with illumination by UVA light (~280 kJ/m^2). DNA fragments were made into a library as above and ligated to pre-annealed adapters (JH0804 and JH1139) for 15 minutes at 15˚C with T4 Rapid DNA Ligase (3000 units, Enzymatics). The sample was size fractionated (Size-select gels; Invitrogen) and 400 bp fragments were collected and purified using AMPure XP beads (Beckmann Coulter). Libraries were amplified with Phusion polymerase (NEB) using primers JH1140 and JH1141 for CPD samples and JH1140 and JH1147 for 6-4pp samples. Indexed libraries were pooled and sequenced on an Illumina HiSeq.

Analysis of dipyrimidine Excision-seq data

Sequences were aligned to the *S. cerevisiae* genome using Bowtie 2 (Langmead and Salzberg 2012) and SAMtools (Li et al. 2009), separated by strand, and dinucleotide counts for the 5’ ends of the reads were determined. The percentages of each dinucleotide combination were normalized to the mononucleotide and dinucleotide frequencies found in *S. cerevisiae* genomic DNA to account for A:T bias in the genome.