

Supplementary data

Inactivation of folylpolyglutamate synthetase Met7 results in genome instability driven by an increased dUTP/dTTP ratio

Tobias T. Schmidt^{1,2}, Sushma Sharma³, Gloria X. Reyes¹, Anna Kolodziejczak^{1,2}, Tina Wagner⁴, Brian Luke^{4,5}, Anders Hofer³, Andrei Chabes^{3,6} and Hans Hombauer^{1,*}

¹DNA Repair Mechanisms and Cancer, German Cancer Research Center (DKFZ), Heidelberg, D-69120, Germany, ²Faculty of Bioscience, Heidelberg University, Heidelberg, D-69120, Germany,

³Department of Medical Biochemistry and Biophysics, Umeå University, Umeå, SE-901 87 Sweden,

⁴Institute of Developmental Biology and Neurobiology, Johannes Gutenberg Universität, 55128 Mainz, Germany, ⁵Institute of Molecular Biology (IMB), 55128 Mainz, Germany, ⁶Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå University, SE-901 87, Umeå, Sweden.

* To whom correspondence should be addressed. Tel: +49 6221 42 3239; Fax: +49 6221 42 3237;
Email: h.hombauer@dkfz.de

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Supplementary Experimental Procedures

Media, strains and plasmids. *S. cerevisiae* strains were grown at 30°C in yeast extract-peptone-dextrose (YPD) medium (1% Bacto yeast extract (Becton Dickinson), 2% Bacto peptone (Becton Dickinson) and 2% glucose) or in synthetic dropout (SD) medium (0.67% Difco yeast nitrogen base without amino acids (Becton Dickinson), 2% glucose and supplemented with the appropriate amino acid dropout mix). To identify mutations that may cause a *petite* phenotype, cells were grown in yeast extract-peptone-glycerol (YPG) medium (1% Bacto yeast extract (Becton Dickinson), 2% Bacto peptone (Becton Dickinson) and 3% glycerol). For the *CAN1* inactivation assay, plates were prepared in SD medium lacking arginine (Arg), supplemented with 60 mg/L canavanine (Sigma). For the GCR assays, plates were prepared using SD medium lacking Arg and uracil (Ura) supplemented with 60 mg/L canavanine, 1 g/L 5-FOA (US Biological) and 50 mg/L Ura (Sigma). 5-FOA plates were prepared in SD medium lacking uracil supplemented with 1 g/L 5-FOA and 50 mg/L Ura. Antibiotics were used at the following final concentrations: 200 µg/mL geneticin (Santa Cruz Biotechnology), 300 µg/mL hygromycin B (Thermo Fisher Scientific) and 100 µg/mL nourseothricin (clonNAT, Werner BioAgents). Drugs HU (H8627, Sigma) and phleomycin (P9564, Sigma) were used at the indicated concentrations to induce DNA replication stress or DNA damage, respectively.

To generate strains containing the *dut1-1* allele (at the chromosomal *DUT1* locus) we used the integrative vector pHHB1094 generated as following. The *DUT1* gene, including 1 kb upstream and 752 nt downstream of *DUT1* was amplified from genomic DNA with primers 5'-CAT GAT GGA TCC CAT GCC CCA TCT CCA CGC TC-3' and 5'-CAG ACC CTA TTA GGA GCC C-3'. The introduced BamHI site in combination with an Xhol site downstream of *DUT1* was used to clone the amplified fragment into pRS306 (40) to generate pRS306-*DUT1* (pHHB1093). The *dut1-G82S* mutation (24) was introduced into pHHB1093 by site-directed mutagenesis using primers 5'-GAA AAA CGG TAT CCA AAC CGG TGC TAG TGT TGT CGA CAG AGA TTA CAC CGG-3' and 5'-CCG GTG TAA TCT CTG TCG ACA ACA CTA GCA CCG GTT TGG ATA CCG TTT TTC-3' to generate pRS306-*dut1-1* (pHHB1094). Next, the pHHB1094 plasmid was linearized with HindIII (New England Biolabs) and transformed into RDKY3686 for integration at the chromosomal *DUT1* locus, selected in Ura-medium and then counter-selected on 5-FOA containing plates (pop-in/pop-out strategy) generating HHY6650. The presence of the *dut1-G82S* mutation, as well as the absence of unwanted mutations, was confirmed by sequencing. Next, the HHY6650 was crossed against HHY6451 to generate HHY6646.

Yeast cell lysates and immunoblotting.

S. cerevisiae whole-cell protein extracts were generated as previously described (38) and were analyzed on 7%, 8% or 10% SDS-PAGE, or 4-15% Mini-PROTEAN TGX precast gels (Bio-Rad) followed by immunoblotting. The following antibodies were used: rabbit polyclonal anti-Clb2 (1:1,000, sc-9071, Santa Cruz Biotechnology), mouse monoclonal anti-c-Myc (1:1,000, 9E10, Millipore), mouse monoclonal anti-Pfk1 (1:20,000, 22C5D8, Invitrogen), mouse monoclonal anti-Rad53 (1:1,000, EL7.E1, Abcam), rabbit polyclonal anti-Rnr1 (1:40,000, AS09576, Agrisera), rabbit polyclonal anti-Rnr2 (1:30,000, AS09575, Agrisera), rabbit polyclonal anti-Rnr3 (1:1,000, AS09574, Agrisera) and guinea pig polyclonal anti-Sic1 (1:10,000, this study, details below). The rat

monoclonal YL1/2 antibody (YL1/2, 1:40,000, Sigma) was used to probe against tubulin and Rnr4. Pgk1 and tubulin were used as loading controls. Horseradish peroxidase (HRP)-linked to rabbit anti-guinea pig IgG (A60-211P, Bethyl Laboratories), HRP-linked sheep to anti-mouse IgG (NA9310, GE Healthcare), HRP-linked donkey to anti-rabbit IgG (NA934, GE Healthcare) and HRP-linked to goat anti-rat IgG (401416, Calbiochem) were used as secondary antibodies at a dilution of 1:10,000. Western blots were developed using Immobilon Western Chemiluminescent HRP substrate (Millipore) and imaged using Super RX-N Fuji medical x-ray films (Fujifilm) and an Optimax TR X-ray film processor (Protec) or using Fusion Solo S (Vilber).

Generation of Sic1 antibodies. To raise antibodies against full-length yeast Sic1 protein we expressed 6xHis-Sic1 from pET28c-Sic1 (kind gift from Dr. Gislene Pereira) in *E. coli* BL21 (DE3) (Cat#200131, Agilent Technologies) followed by Ni-NTA affinity purification (30210, Qiagen). Two guinea pigs were immunized with purified 6XHis-Sic1 at the Antibody Unit of the DKFZ Genomics and Proteomics Core Facility. The specificity of serum was tested by immunoblotting.

α-factor arrest and release.

For α-factor arrest and release experiments, HHY6644 was grown overnight in YPD medium. The logarithmically growing control cultures were prepared as described earlier (38). To arrest cells in G1 with α-factor, 2 mL of the overnight culture was washed with sterile water twice and cells were incubated in 20 mL YPD medium containing 0.1 µg/mL α-factor (RP01002, GenScript) at 30 °C for 3 hours with shaking. Next, cells were washed with sterile water twice, released in 20 mL YPD medium containing 15 µg/mL nocodazole (T2802, TargetMol) and grown at 30 °C with shaking. Samples for DNA content analysis and cell lysates were taken every 10 min. Cell cycle progression was analyzed by DNA content using FACS and by SDS-PAGE/immunoblotting.

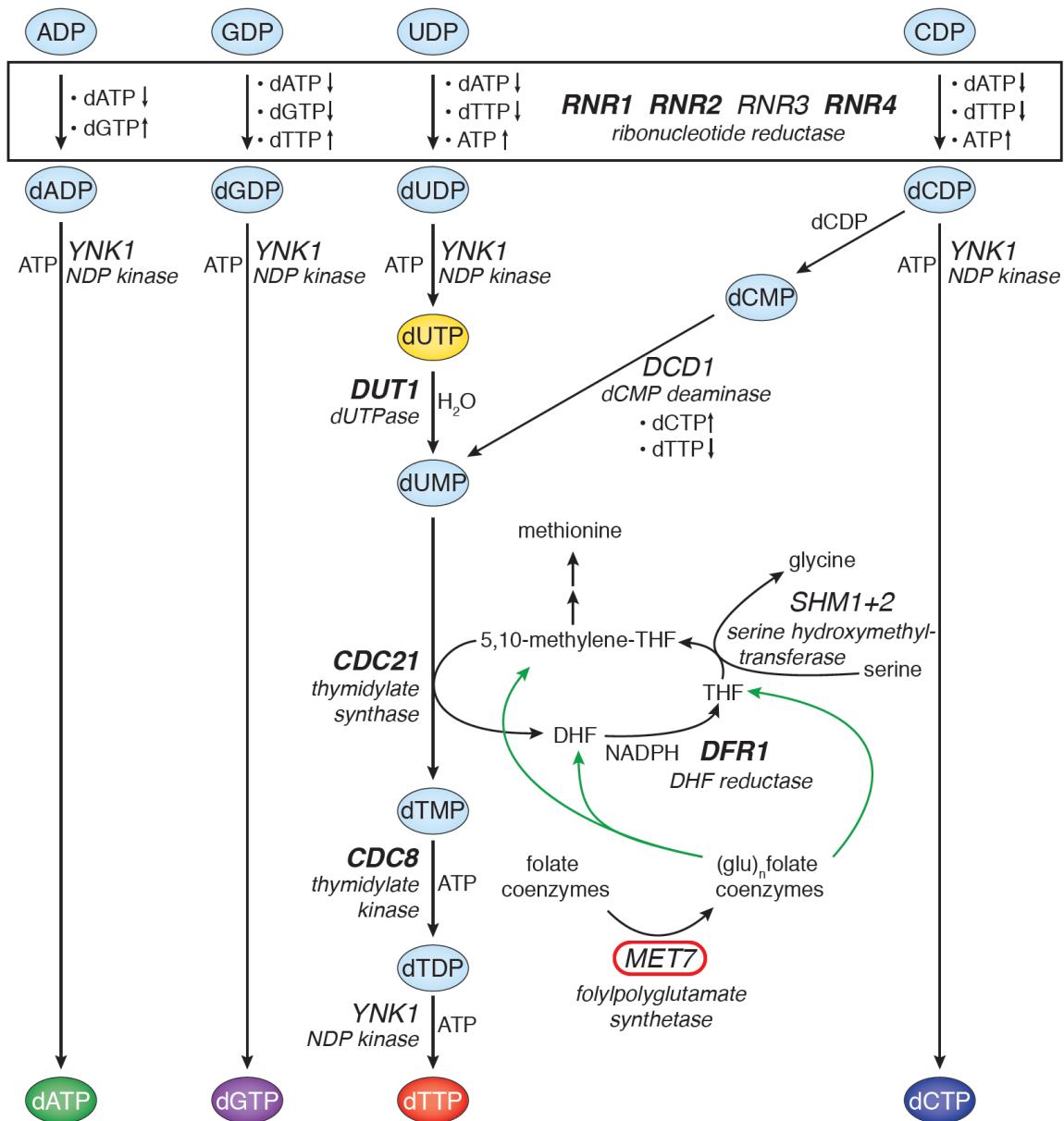
Live-cell imaging of Ddc2-GFP foci.

Exponentially growing cells were washed, resuspended and placed on agar pads, covered with a coverslip and sealed with valap (a 1:1:1 mixture of Vaseline, lanolin and paraffin by weight). Cells were imaged on a Leica SP5 confocal microscope, using an Argon laser, a 63x1.4NA objective, and a high resonance scanner @8kHz frequency. Ten 0.4 µm z sections were acquired and image processing, such as maximum intensity projections, were performed using ImageJ. The data represent the percentage of cells with foci. Three independent biological replicates were analyzed per genotype. Data is presented in box-plots with whiskers (showing the 25th and 75th percentile) and dots represent outliers. The line inside the box represents the median of cells with foci. Mann-Whitney test was used to compare Ddc2-GFP foci abundance in WT and *met7Δ*.

CAN1 mutation spectra analysis. The CAN1 gene of independent Can^R colonies was amplified from genomic DNA by PCR and sequenced (GATC) as described in (26). Sequences were analyzed with Lasergene 12 (DNASTAR). Mutation spectra and mutational hotspots were compared by Fisher's exact test in R. Values of $p \leq 0.05$ were defined as significantly different. Full CAN1 mutation spectra for the reported strains are available upon request.

Phleomycin sensitivity and *petite* phenotype. To test for phleomycin sensitivity yeast cultures were grown overnight in YPD medium. Next day, cultures were 10-fold serially diluted and spotted on YPD agar plates either containing or not 2.5 μ g/ml phleomycin. To assess for a *petite* phenotype, strains were grown and serially diluted as before, but were spotted on YPD and YPG agar plates for comparison. Spotted plates were incubated at 30 °C for 4-5 days and imaged using the GelDoc system (Bio-Rad).

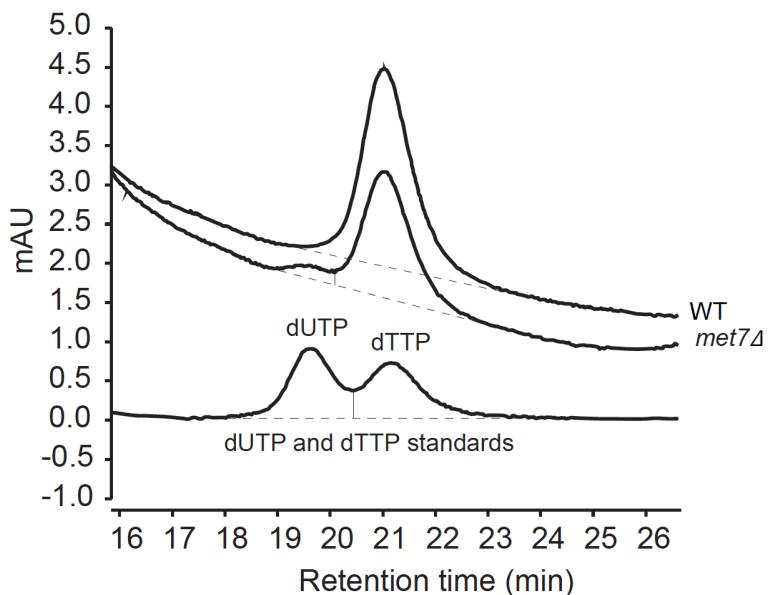
Supplementary Figure S1



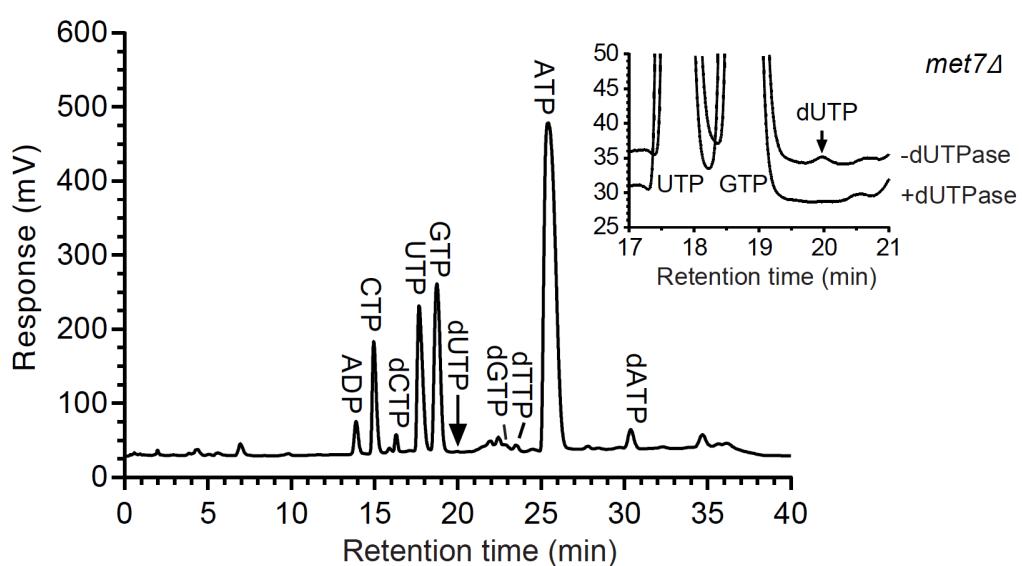
Supplementary Figure S1. Pathways of the *de novo* dNTP biosynthesis in *S. cerevisiae* (adapted from (26, 85)). Essential genes are shown in bold (*RNR1*, *RNR2*, and *RNR4* are non-essential genes in certain yeast genetic backgrounds). Met7 (encircled in red) catalyzes the polyglutamylation of folate coenzymes (indicated by green arrows).

Supplementary Figure S2

A

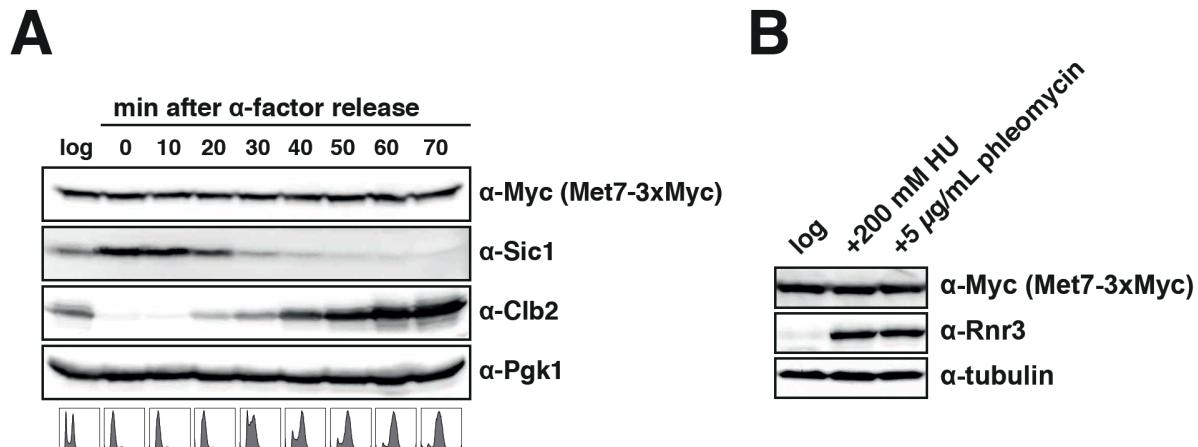


B



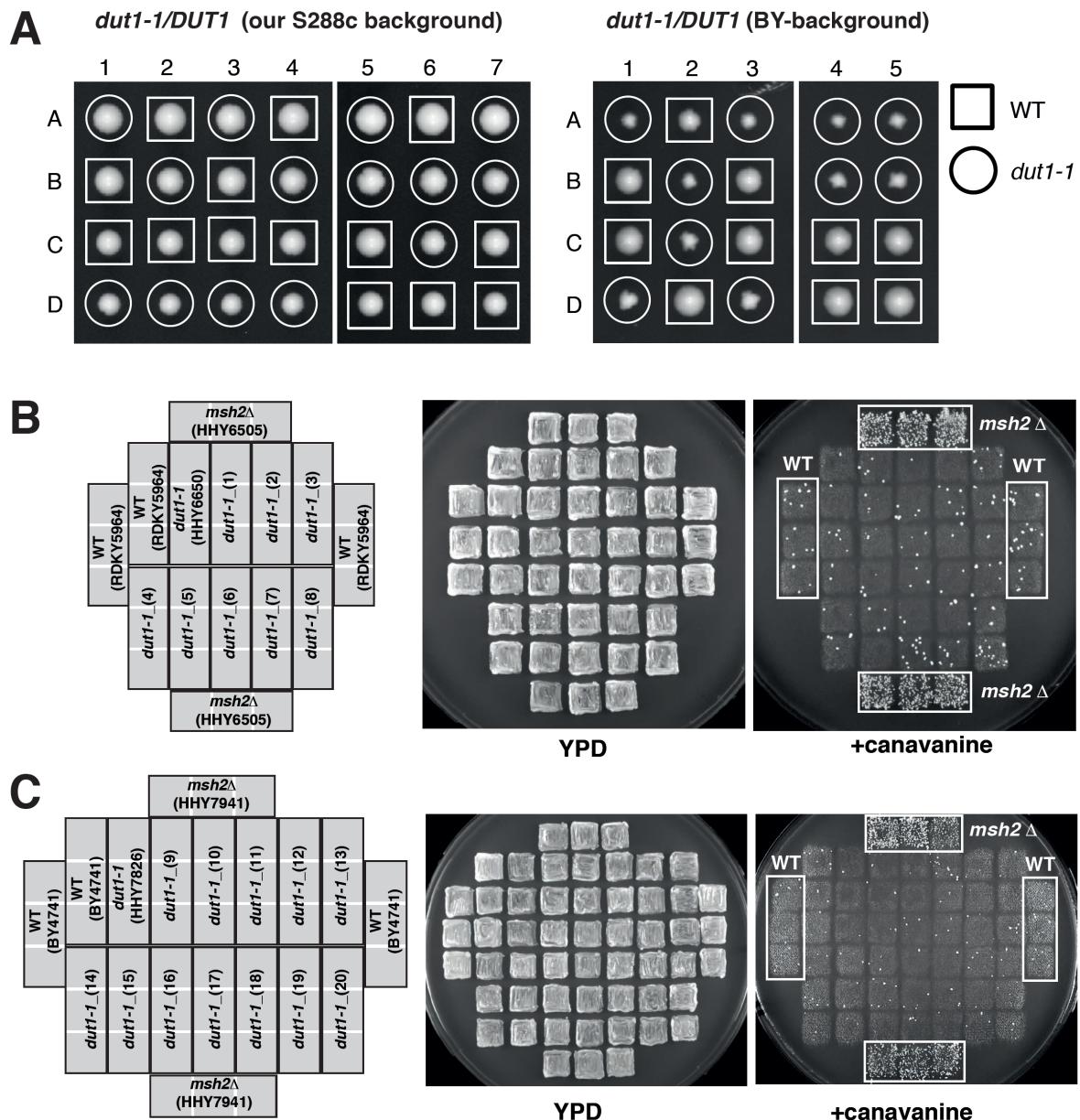
Supplementary Figure S2. Quantification of dUTP levels in yeast extracts. (A) HPLC-SAX chromatography showing that dUTP is only partially separated from dTTP. As shown in the figure, dUTP was measurable in the *met7Δ* mutant but was masked by dTTP at lower levels (WT). (B) RP chromatography procedure used for the quantification of NTPs, ADP, dCTP and dUTP. The figure shows a chromatogram from the *met7Δ* extract. The dUTP peak is magnified in the inset with a control sample treated with recombinant dUTPase shown below. The two chromatograms in the inset are plotted 5 mV apart to visualize the disappearance of the dUTP peak. Due to small interfering peaks from co-purifying metabolites, the dGTP, dTTP and dATP pools were not quantified by the RP method.

Supplementary Figure S3



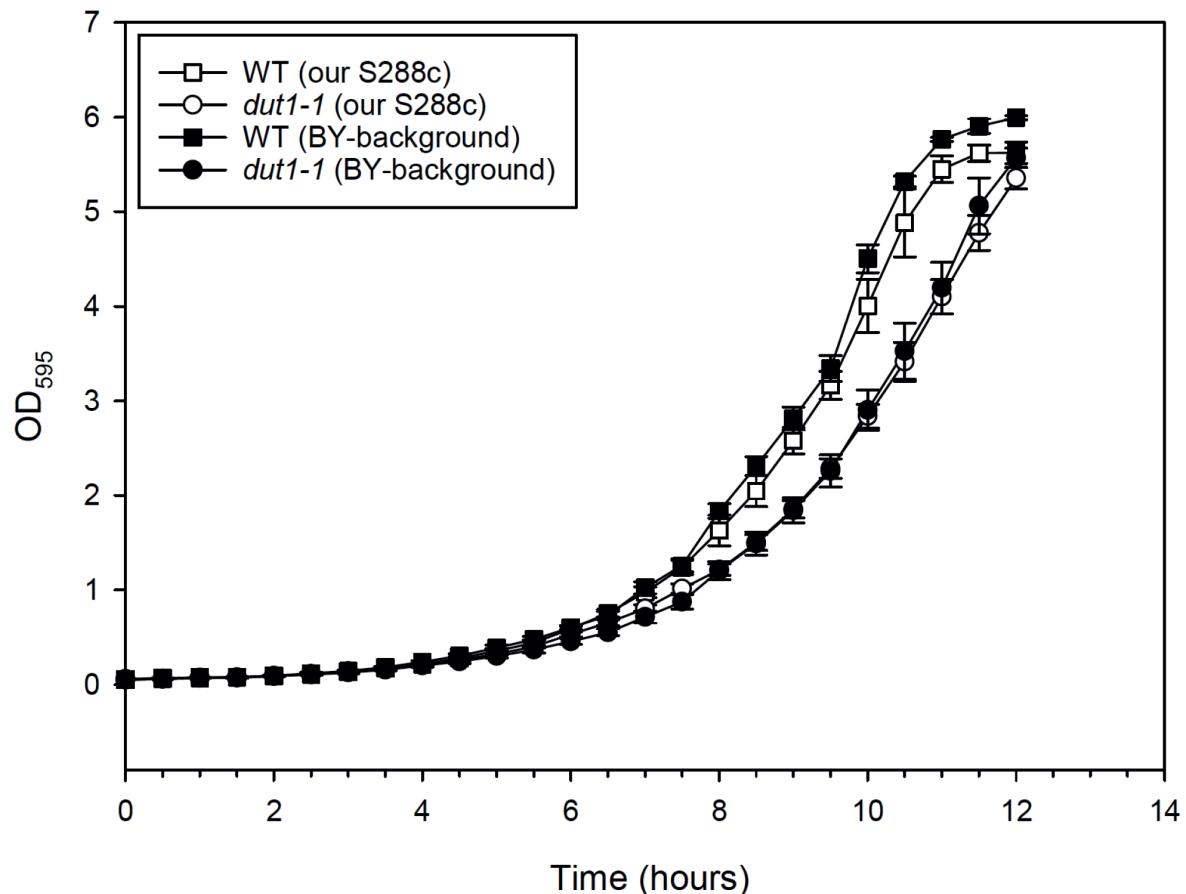
Supplementary Figure S3. Met7 expression is constant throughout the cell cycle and it is not affected by DNA damage or replication stress. (A) Cells expressing Met7-3Myc (C-terminal tagged at the endogenous locus) were arrested in G1 phase with α-factor and released into YPD containing 15 µg/mL nocodazole. Samples were collected at 10 minutes intervals for DNA content analysis and whole cell lysates. Lysates were analyzed by SDS-PAGE and immunoblotting with antibodies against Myc (for Met7-3xMyc), Sic1 (used as G1 phase marker), Clb2 (as G2 phase marker) and Pgk1 (loading control). (B) Met7 expression is not induced upon DNA replication stress or DNA damage. Cells expressing Met7-3Myc were either grown in YPD (log) or in YPD containing either 200 mM HU or 5 µg/mL phleomycin for 3 hours. Whole cell lysates were analyzed by SDS-PAGE and immunoblotting with antibodies anti-Myc (for detection of Met7-3xMyc), anti-Rnr3 (as DNA damage-inducible control) and anti-tubulin (loading control).

Supplementary Figure S4



Supplementary Figure S4. Characterization of *dut1-1* mutant strains. (A) Tetrad dissection analysis of *dut1-1/DUT1* diploid strain (obtained after introducing the *dut1-1* mutation into the WT-diploid strain HHY5311 (which derives from RDKY3686/5964) (left panel) or after backcrossing a *dut1-1* x WT in the BY-4741/4742 background (right panel). Tetrad were dissected on YPD plates and grown at 30 °C for 4 days. Spores containing the *dut1-1* mutation were identified by sequencing. (B) and (C) Qualitative mutator analysis (CAN1 gene inactivation) of *dut1-1* mutants and few additional WT and parental controls. *dut1-1* mutants (clones 1-8) obtained after tetrad dissection in HHY5311 are shown in (B), and *dut1-1* clones (9-20) in the BY-background are shown in (C). Three independent colonies for each strain were grown as patches on YPD and then replica-plated on canavanine-containing plates. Plates were incubated at 30 °C for 4-5 days. The increased abundance of canavanine-resistant colonies (relative to the WT) is indicative of a mutator phenotype. Mismatch repair deficient strains (*msh2 Δ*), in their respective genetic backgrounds, were used as reference (mutator controls).

Supplementary Figure S5



Supplementary Figure S5. Growth rates analysis of WT and *dut1-1* mutants. Yeast cultures of WT and *dut1-1* mutant strains (in our S288c or in the BY-background) were diluted to an optical density (OD) OD₅₉₅=0.05 and grown at 30 °C in YPD for 12 hours. OD₅₉₅ measurements were taken every 30min. Doubling times mentioned in the main text were calculated based on the exponential phase of each growth curve.

Supplementary Table S1. List of single gene deletions resulting in increased mutator phenotypes.

Gene	<i>lys2-10A</i>	<i>CAN1</i>	Biological function	Reference
<i>CCS1</i>		X	Copper chaperone, oxidative stress response	27, 28
<i>CSM2</i>		X	Component of Shu complex, error-free DNA repair	27, 28
<i>ELG1</i>	X	X	Subunit of RFC1-like complex, DNA replication and genome integrity	27, 28
<i>EXO1</i>	X	X	5'-3' exonuclease and flap endonuclease, DSB repair, error-free PRR and MMR	56
<i>MET7</i>		X	Folylpolyglutamate synthetase	This study
<i>MLH1</i>	X	X	MMR	27, 28
<i>MLH3</i>	X		MMR, meiotic recombination	57
<i>MMS2</i>		X	Ubiquitin-conjugating enzyme, error-free PRR	27, 28
<i>MPH1</i>		X	DNA helicase	28
<i>MRE11</i>		X	Nuclease subunit of MRX complex in DSB	27, 28
<i>MSH2</i>	X	X	MMR	27, 28
<i>MSH3</i>	X	X	MMR	27, 28
<i>MSH6</i>	X	X	MMR	27, 28
<i>OGG1</i>		X	8-oxoguanine DNA glycosylase, BER	27, 28
<i>PIF1</i>		X	DNA helicase	27, 28
<i>PMS1</i>	X	X	MMR	27, 28
<i>PSY3</i>		X	Component of Shu complex, error-free DNA repair	27
<i>RAD1</i>		X	Single-stranded DNA endonuclease, NER, DSB	28
<i>RAD4</i>		X	NER	55
<i>RAD5</i>		X	DNA helicase, PRR	27, 28
<i>RAD10</i>		X	Single-stranded DNA endonuclease, NER, DSB	58
<i>RAD14</i>		X	NER	59
<i>RAD17</i>		X	DNA damage checkpoint	60
<i>RAD18</i>		X	E3 ubiquitin ligase, PRR	27, 28
<i>RAD27</i>		X	5' to 3' exonuclease, 5' flap endonuclease, DNA replication and repair	27, 28
<i>RAD50</i>		X	Subunit of MRX complex, DSB repair	27
<i>RAD51</i>		X	DSB repair	27
<i>RAD52</i>		X	DSB repair	27, 28
<i>RAD54</i>		X	DSB repair	27
<i>RAD55</i>		X	DSB repair	27, 28
<i>RAD57</i>		X	DSB repair	27, 28
<i>RNH203</i>		X	Ribonucleotide H2 subunit, ribonucleotide excision repair	27
<i>SHU1</i>		X	Component of Shu complex, error-free DNA repair	27, 28
<i>SHU2</i>		X	Component of Shu complex, error-free DNA repair	27
<i>TSA1</i>		X	Thioredoxin peroxidase, oxidative stress response	27, 28
<i>UBC13</i>		X	E2 ubiquitin-conjugating enzyme, error-free PRR	61
<i>UNG1</i>		X	Uracil-DNA glycosylase, BER	27, 28
<i>XRS2</i>		X	Subunit of MRX complex, DSB repair	27
<i>YAP1</i>		X	Transcription factor, oxidative stress response	27, 28

The following mutants including: *sli15Δ*, *ygr050cΔ*, *yhl005cΔ*, *yml083c*, *ymr166cΔ*, and *zwf1Δ* were identified as false positives. Abbreviations: base excision repair (BER), double-strand break (DSB), mismatch repair (MMR), nucleotide excision repair (NER), post-replicative repair (PRR).

Supplementary Table S2. NTP and dNTP concentrations in cell extracts of the indicated yeast strains measured by SAX-HPLC and RP-HPLC.

A

Relevant genotype	CTP	UTP	ATP	GTP
WT	2374 ± 16 (1.0)	5605 ± 32 (1.0)	11339 ± 48 (1.0)	3987 ± 67 (1.0)
<i>met7Δ</i>	2206 ± 5 (0.9)	4109 ± 60 (0.7)	13697 ± 153 (1.2)	4514 ± 3 (1.1)
<i>met7Δ pGPD-DUT1</i>	2242 ± 9 (0.9)	3915 ± 13 (0.7)	13676 ± 57 (1.2)	4601 ± 15 (1.2)
Relevant genotype	dCTP	dTTP	dATP	dGTP
WT	114 ± 4 (1.0)	203 ± 6 (1.0)	125 ± 4 (1.0)	82 ± 4 (1.0)
WT + hDut1	104 ± 1 (0.9)	203 ± 7 (1.0)	123 ± 4 (1.0)	81 ± 1 (1.0)
<i>met7Δ</i>	302 ± 7 (2.7)	125 ± 5 (0.6)	497 ± 1 (4.0)	49 ± 4 (0.6)
<i>met7Δ</i> + hDut1	282 ± 3 (2.5)	125 ± 6 (0.6)	490 ± 9 (3.9)	49 ± 6 (0.6)
<i>met7Δ pGPD-DUT1</i>	314 ± 1 (2.8)	95 ± 2 (0.5)	463 ± 1 (3.7)	38 ± 2 (0.5)
<i>met7Δ pGPD-DUT1</i> + hDut1	284 ± 1 (2.5)	95 ± 1 (0.5)	444 ± 5 (3.6)	40 ± 2 (0.5)

B

Relevant genotype	CTP	UTP	ATP	GTP
WT	2425 ± 216 (1.0)	5839 ± 312 (1.0)	11978 ± 928 (1.0)	3654 ± 356 (1.0)
<i>met7Δ</i>	2160 ± 218 (0.9)	3846 ± 255 (0.7)	13664 ± 1468 (1.2)	4152 ± 626 (1.2)
<i>met7Δ</i> + hDut1	2122 ± 396	3753 ± 568	13305 ± 2609	4126 ± 1009
Relevant genotype	dCTP	dUTP	ADP	ATP/ADP
WT	102 ± 15 (1.0)	≤ 1	692 ± 11 (1.0)	17
<i>met7Δ</i>	306 ± 44 (3.0)	11 ± 2	566 ± 45 (0.8)	24
<i>met7Δ</i> + hDut1	298 ± 49 (2.9)	≤ 1	752 ± 4 (1.1)	18

NTP and dNTP concentrations determined by SAX-HPLC (A) or RP-HPLC (B). Concentrations are indicated as pmol/10⁸ cells and correspond to the average of two biological replicates ± standard deviation with the fold increase over WT in brackets. Extracts treated with recombinant hDut1 (+ hDut1 samples) prior the measurement are indicated. The dUTP peak was partially overlapping with dTTP with the SAX method (shown in A). "nd" means "not detectable" or below our detection limit (≤ 3 pmol dUTP). The dUTP pools were instead determined more accurately with the RP-HPLC method. According to this method, extracts of *met7Δ* strains contained about 10 times more dUTP than WT extracts. The RP-HPLC method also allowed to measure ADP, and the high ATP/ADP ratio in the samples indicate that there is no disturbance of the general energy metabolism in the cells. Measurements of dGTP, and to some extent dTTP and dATP, were disturbed by interfering peaks in the RP method and because of that not included in the data set.

Supplementary Table S3. NTP and dNTP concentrations in several mutant strains.

A

Relevant genotype	CTP	UTP	ATP	GTP
WT	2256 ± 63 (1.0)	5092 ± 79 (1.0)	11151 ± 219 (1.0)	3584 ± 80 (1.0)
<i>met7Δ</i>	2137 ± 7 (0.9)	3563 ± 27 (0.7)	13356 ± 199 (1.2)	4107 ± 71 (1.1)
<i>dut1-1</i>	2215 ± 28 (1.0)	5213 ± 339 (1.0)	11247 ± 335 (1.0)	3532 ± 82 (1.0)
<i>dcd1Δ</i>	2009 ± 12 (0.9)	5688 ± 109 (1.1)	11392 ± 179 (1.0)	3476 ± 36 (1.0)
<i>dut1-1 dcd1Δ</i>	2269 ± 48 (1.0)	4870 ± 65 (1.0)	11820 ± 152 (1.1)	3501 ± 19 (1.0)

B

Relevant genotype	dCTP	dTTP	dATP	dGTP
WT	79 ± 9 (1.0)	178 ± 22 (1.0)	107 ± 9 (1.0)	69 ± 5 (1.0)
<i>met7Δ</i>	278 ± 2 (3.5)	110 ± 5 (0.6)	472 ± 31 (4.4)	36 ± 3 (0.5)
<i>dut1-1</i>	114 ± 3 (1.4)	262 ± 2 (1.5)	161 ± 7 (1.5)	88 ± 1 (1.3)
<i>dcd1Δ</i>	1908 ± 90 (24.2)	90 ± 3 (0.5)	124 ± 3 (1.2)	48 ± 2 (0.7)
<i>dut1-1 dcd1Δ</i>	1543 ± 0 (19.6)	165 ± 2 (0.9)	191 ± 4 (1.8)	55 ± 1 (0.8)

NTP (A) and dNTP (B) concentrations (pmol per 10^8 cells) determined by SAX-HPLC correspond to the average of two biological replicates ± standard deviation with the fold increase over WT in parentheses.

Supplementary Table S4. *CAN1* mutation spectrum of WT and *met7Δ*.

genotype	insertions / deletions		base substitutions		complex*
	mutation	occurrence	mutation	occurrence	occurrence
WT[†]	ΔA	A6 → A5	1 (1)	A-T → G-C	6 (7)
	ΔT	T6 → T5	2 (2)	G-C → A-T	18 (20)
		T2 → T1	2 (2)	G-C → T-A	29 (32)
	ΔC	C2 → C1	1 (1)	A-T → C-G	3 (3)
		C1 → C0	2 (2)	A-T → T-A	7 (8)
	+T	T6 → T7	3 (3)	C-G → G-C	6 (7)
		T2 → T3	3 (3)		
	+G	G2 → G3	1 (1)		
			15 (16)	69 (75)	8 (9)
<i>met7Δ</i>	ΔA	A3 → A2	1 (1)	A-T → G-C	5 (3)
	ΔT	T6 → T5	4 (2)	G-C → A-T	54 (29)
		T4 → T3	4 (2)	G-C → T-A	40 (22)
		T3 → T2	2 (1)	A-T → C-G	11 (6)
		T1 → T0	3 (2)	A-T → T-A	14 (8)
	ΔG	G4 → G3	2 (1)	C-G → G-C	19 (10)
		G2 → G1	2 (1)		
		G1 → G0	1 (1)		
	ΔC	C3 → C2	2 (1)		
		C2 → C1	1 (1)		
	+T	T6 → T7	1 (1)		
			23 (12)	143 (77)	19 (10)

Mutation spectra analysis based on DNA sequencing of the *CAN1* gene in independent Can^R mutants of WT (n=91) and *met7Δ* (n=185), shown as the number of clones containing the indicated mutations, and in parenthesis as the percentage relative to the total.

* includes: multiple mutations within 10 nucleotides, insertions or deletions of more than 1 nucleotide and duplication events.

[†] In few cases (about 1-2% of the sequenced clones) two simultaneous *CAN1* mutations (more than 100 bp apart) were found. These mutations were included in the analysis and considered as independent mutational events. *CAN1* spectrum of WT strain was taken from (26).

Supplementary Table S5. *S. cerevisiae* strains used in this study.

Name	Relevant genotype ^a	Reference
RDKY5964	<i>MATa ura3-52 leu2Δ1 trp1Δ63 hom3-10 his3Δ200 lys2-10A</i>	(38)
HHY5298	RDKY5964 <i>cyh2 Q38K hom3-10.HIS3 pMFA1-kLEU2.hphNT1.lys2-10A</i> <i>MLH2.klURA3 POL1.natNT2</i>	(26)
HHY5311	<i>MATa/a ura3-52/ura3-52 leu2Δ1/ leu2Δ1 trp1Δ63/ trp1Δ63 hom3-10/ hom3-10 his3Δ200/ his3Δ200 lys2-10A/ lys2-10A</i>	This study
HHY6370	RDKY5964 <i>met7::kanMX4</i>	This study
HHY6505	RDKY5964 <i>msh2::HIS3</i>	This study
HHY7735	RDKY5964 <i>kanMX4.pGPD-DUT1</i>	This study
HHY6441	RDKY5964 <i>kanMX4.pGPD-DUT1 met7::klTRP1</i>	This study
HHY7737	RDKY5964 <i>ung1::hphNT1</i>	This study
HHY7739	RDKY5964 <i>ung1::hphNT1 met7::kl-TRP1</i>	This study
HHY1602	RDKY5964 <i>rev3:: kanMX4</i>	This study
HHY6636	RDKY5964 <i>rev3::natNT2 met7::klTRP1</i>	This study
HHY6707	RDKY5964 <i>dut1-G82S</i>	This study
HHY7733	RDKY5964 <i>dcd1::kanMX4</i>	This study
HHY7564	RDKY5964 <i>dut1-G82S dcd1::kanMX4</i>	This study
RDKY3686	<i>MATa ura3-52 leu2Δ1 trp1Δ63 hom3-10 his3Δ200 lys2-10A</i>	(37)
HHY2883	RDKY3686 <i>ddc2-GFP.hphNT1</i>	This study
HHY6479	RDKY3686 <i>ddc2-GFP.hphNT1 met7::klTRP1</i>	This study
HHY6650	RDKY3686 <i>dut1-G82S</i>	This study
HHY6443	RDKY5964 <i>iYEL072W::hph can1::hisG yel072w::CAN1/URA3</i> <i>bar1::loxP.kLEU2.loxP</i>	(26)
HHY6445	HHY6443 <i>met7::kanMX4</i>	This study
HHY6638	HHY6443 <i>natNT2.pGPD-DUT1</i>	This study
HHY6447	HHY6443 <i>natNT2.pGPD-DUT1 met7::klTRP1</i>	This study
HHY6451	HHY6443 <i>ung1::kanMX4</i>	This study
HHY6449	HHY6443 <i>ung1::kanMX4 met7::klTRP1</i>	This study
HHY6640	HHY6443 <i>natNT2.pGPD-DUT1 met7::klTRP1 ung1::kanMX4</i>	This study
HHY6644	HHY6443 <i>met7-3xMYC::klTRP1</i>	This study
HHY6646	HHY6443 <i>dut1-G82S</i>	This study
HHY6716	HHY6443 <i>dcd1::natNT2</i>	This study
HHY6648	HHY6443 <i>dut1-G82S ung1::kanMX4</i>	This study
HHY6713	HHY6443 <i>dut1-G82S dcd1::natNT2</i>	This study
RDKY3615	<i>MATa ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys2ΔBgl hom3-10 ade2Δ1 ade8</i> <i>yel069c::URA3</i>	(39)
HHY6477	RDKY3615 <i>met7::HIS3</i>	This study
BY4741	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Euroscarf
BY4742	<i>MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	Euroscarf
HHY7826	BY4741 <i>dut1-1</i>	This study
HHY7941	BY4742 <i>msh2::kanMX4</i>	Euroscarf

^a All strains derived from S288c. The genotype corresponds to the listed strain with the indicated modifications.