

Supplementary Material

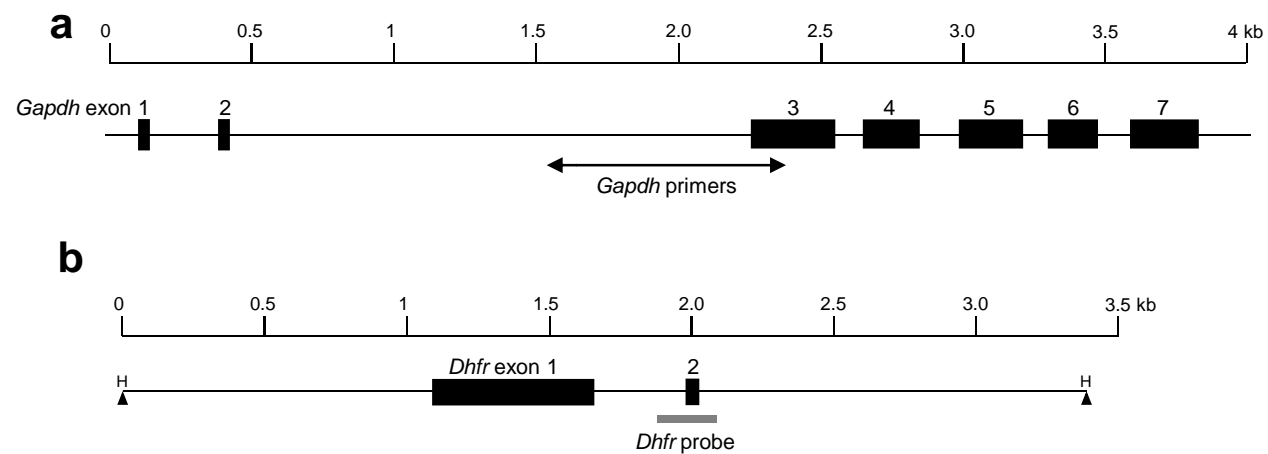
AID-dependent uracils in immunoglobulin gene variable and switch regions

Authors:

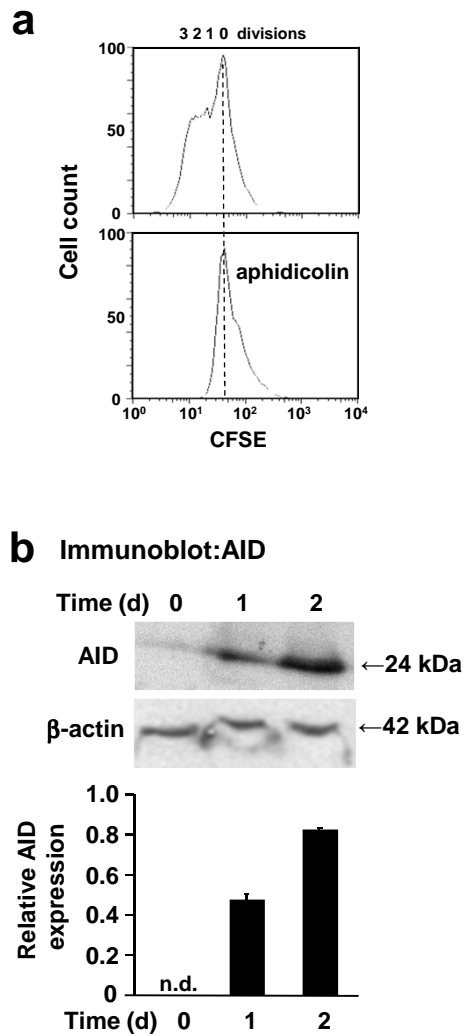
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Supplementary Figure 1. Map of primers and probes for controls. **(a)** *Gapdh* primers for qPCR amplify 837 bp. **(b)** *Dhfr* probe for Southern. The 200 bp probe spans exon 2 and detects a 3.4 kb HindIII (H) fragment.



Supplementary Figure 2. Expression of AID after aphidicolin treatment. **(a)** Aphidicolin blocks cell division. Cells were stained with CFSE and stimulated *ex vivo* in the absence or presence of aphidicolin, and examined after 72 h. **(b)** Aphidicolin does not prevent AID expression. Quantification is shown for 3 independent blots comparing the ratio of AID to β -actin with SEM.

a

1 TTCTTGTGTG ACACCAAGAA ∇ TTGGCATAAT ∇ GTCTGAGTTG \square CCCAAGGGTG ATCTT \square AGCTA

61 GACTCTGGGG TTTTGTGCGG ∇ GTACAGAGGA AAAACCCACT ATTGTGATTA CTATGCATATG ∇ J_H4

121 GACTACTGGG ∇ GTCAAGGAAC CTCAGTCACC ∇ GTCTCCTCAG ∇ GTAAGAATGG CCTCTCCAGG

181 TCTTTATTTT TAACCTTTGT TATGGAGTTT ∇ TCTGAGCAT \square GCAGACTAAT \triangle CTTGGATATT

241 \square TGCCCTGAGG \square GAGCCGGCTG AGAGAAGTTG GGAAATAAAT CTGTCTAGGG ∇ ATCTCAGAGC \triangle

301 CTTTAGGACA \triangle GATTATCTCC ACATCTTTGA \triangle AAAAC ∇ TAAGA ∇ ATCTGTGTGA TGGTGTGGT

361 ∇ GGAGTCCCTG ∇ GATGATGGG ∇ TAGGGACTTT ∇ GGAGGCTCAT TTGAAGAAGA \triangle \square TGCTAAAACA

421 ATCCTATGGC ∇ TGGAGGGATA \triangle GTTGGGGCTG \triangle TAGTTGGAGA TTTT ∇ CAGTTT ∇ TTAGAATAAA

481 AGTATTAGTT \triangle GTGGAATATA \triangle CTTCAGGACC \triangle ACCTCTGTGA \triangle \square CAGCATTTAT \triangle ACAGTATCCG

541 \square ATGCATAGGG ACAAAGAGTG ∇ GAGTGGGGCA ∇ CTTTCTTTAG ∇ ATTTGTGAGG ∇ AATGTTCCGC ∇

601 ∇ ACTAGATTGT \triangle TTAAAACTTC \triangle ATTTGTTGGA AGGAGAGCTG ∇ TCTTAGTGAT

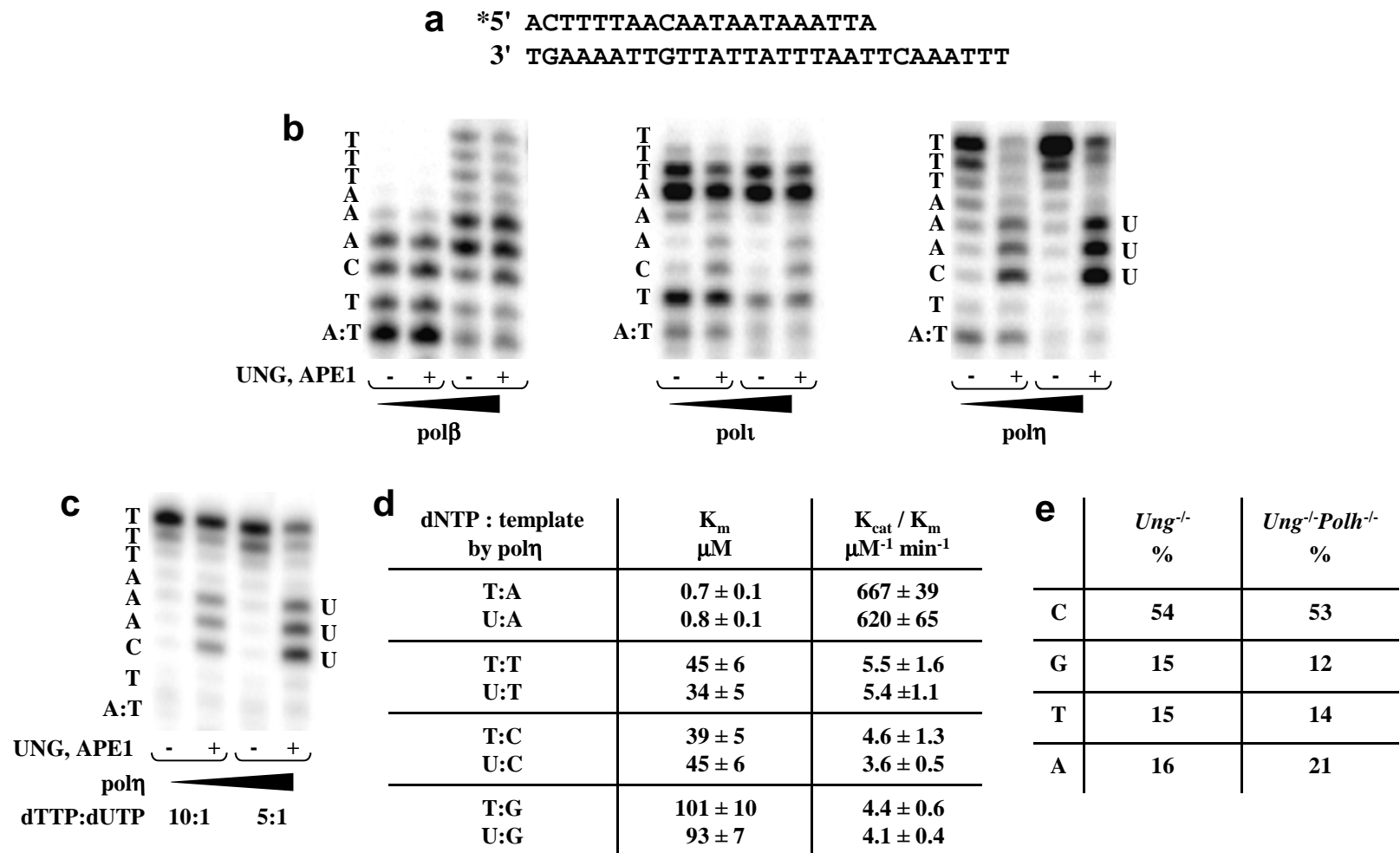
b

	C	G	T	A
<i>Ung</i> ^{-/-}	29	13	15	13
<i>Ung</i> ^{-/-} <i>Msh2</i> ^{-/-}	17	12	11	8

c

	#Linkers at C	#Linkers in WGC	%Linkers in WGC (%WGC in sequence)
<i>Ung</i> ^{-/-}	24	5	21 (11)
<i>Ung</i> ^{-/-} <i>Msh2</i> ^{-/-}	16	4	25 (11)

Supplementary Figure 3. Sequence context of LM-PCR linker sites. **(a)** Sequence of the J_H4 region. J_H4 gene is underlined. WGC hotspot motifs are highlighted with a box. Linker sites are designated by closed triangles for *Ung*^{-/-} samples, and open triangles for *Ung*^{-/-}*Msh2*^{-/-}. **(b)** Number of breaks analyzed. Several breaks occurred within V genes rearranged to J_H4 (5 from *Ung*^{-/-} and 1 from *Ung*^{-/-}*Msh2*^{-/-}), which were not utilized for the hotspot analysis. **(c)** Linkers found within the WGC hotspot motif. The % of linkers was compared to the total % of all C bases in the WGC motif (11%). Data derived from ~50 amplifications using genomic DNA from 6-9 mice from each strain.



Supplementary Figure 4. Incorporation of dUTP opposite template A by different polymerases during replication, described in **Supplementary Methods**. **(a)** Sequence of the primer/template used in replication assays. **(b)** Polymerases (pol) were examined for uracil incorporation in the presence of equal concentrations of dATP, dTTP, dCTP, dGTP, and dUTP. Increased cleavage products after UNG and APE1 digestion represent uracils. **(c)** Polη efficiently incorporates dU opposite template A even when dTTP is in excess of dUTP. **(d)** Kinetic analysis of dUTP incorporation by polη. **(e)** LM-PCR linker location in *Ung*^{-/-} and *Ung*^{-/-}*Polh*^{-/-} clones. Number of clones sequenced: *Ung*^{-/-}, 69; *Ung*^{-/-}*Polh*^{-/-}, 20.

Supplementary Table 1: Oligonucleotide primers and probes

Assay	Name	Oligonucleotide (5' → 3', 5' overhang in lower case)	
DT40 C _λ - Southern probe	ggIgλ external	Forward	GTGACACCTGGAGAGATGAGGG
		Reverse	ACAGAGGTGCATGTGTGTCTGTAC
	ggIgλ internal	Forward	AGGAGTCGACAGGCAGAGG
		Reverse	CAGCCATACATACGCGTGTGG
DT40 V _λ - qPCR	DT1F	Forward	CGCAGGGAGTTATTTGCAT
	DT2R	Reverse	GGAGAATCGTGAAGGGATGT
DT40 C _λ - qPCR	DTNR6F	Forward	ATACGCACACGTATAACATCCATG
	ggCλ 3RT	Reverse	GAGGAGTCGACAGGCAGAGGTG
Mouse S _μ - Southern probe	S7 external	Forward	GTGAGCTGAGCTAGGGTGA
		Reverse	TTTTCCCAGCTCATCCCGAA
	S7 internal	Forward	ggaattcGTGAGCTGAGCTGAGGTGAA
		Reverse	cgggatccCCAGGCCCTACTCAGCCTA
Mouse Dhfr - Southern probe	DHFR external	Forward	CACACCCACACCGGACTTGCACC
		Reverse	CAGAGATCCCAGATTCCCAGG
	DHFR internal	Forward	CGTCGCTGAATGGAGCCTGAGC
		Reverse	CGAGCTATATGCACTTGC
VDJ - qPCR	5'VDJ	Forward	AGCCTGACATCTGAGGACTCTGC
	3'Jh4	Reverse	GTCAGTTCTACACAAACAAGACCTCAAATTGATTGAC
Gapdh - qPCR	GAPDH 1439F	Forward	GAGACGGCCGCATCTTCTTGTG
	GAPDH 2276R	Reverse	GACTTCCACCCAGCCGAG
S _μ - qPCR	Ch5f49991	Forward	GAATCTATTCTGGCTCTTCTTAAGCAG
	Ch6r49133	Reverse	CCATCTCAGCTACTCCAGAGTATCTC
V _κ - qPCR	1 st 5'V _κ 4	Forward	TGGAGGCTGAAGATGCTGCC
	J _κ 2 3RT	Reverse	CCCTTACAAAACCTGGTCTCAGAAGCCT
C _κ - qPCR	C _κ 5RT	Forward	GTCCCATGTGGTTACAAACCATTAGACCAG
	C _κ 3RT	Reverse	CACTCATTCCTGTTGAAGCTCTTGACA
J _H 4 - LM-PCR	3'Jh3116	Forward	GAGCTCACTCCCATTCCTCGG
	3'Jh2906	Forward	GTGTTCCTTTGAAAGCTGGAC
	LL4	Reverse	ATGCACTACATACAGTCATCCGGAG
	3'Jh2826Bam	Forward	cacggatccTAGATGCCTTTCTCCCTTGACTCA
	LL2	Reverse	CAGTCATCCGGAGATCTGAATTC
	3'Jh2732	Hybridization Probe	GTTGGAAGGAGCTGTCTTAGTG
Gapdh - LM-PCR	GAPDH-F	Forward	TCCACCACCGTGTGCTGTAG
	GAPDH-R	Reverse	GACCACAGTCCATGCCATCACT
Linker - LM-PCR	Top	Linker	ATGCACTACATACAGTCATCCGGAGATCTGAATTC
	Bottom	Linker	GAATTCAGATCTCC

Supplementary Methods

DNA polymerases and reaction template. Human DNA pol β was a generous gift of S. Wilson (NIEHS, NIH). GST-tagged human pol ι and His-tagged human pol η were purified from baculovirus infected insect cells as previously described^{1,2}. The replication template was constructed by annealing the template with ³²P-labeled primer at a 1.5:1 molar ratio. Hybridization was achieved by heating the required mixture of oligonucleotides in an annealing buffer (50 mM Tris-HCl-pH 8, 50 μ g/ml BSA, 1.42 mM 2-mercaptoethanol) for 10 min at 100 °C followed by slow cooling to 21 °C over a period of about 2 h. Annealing efficiencies were >95%, as evidenced by the different mobility of the ³²P-labeled primers before and after hybridization to the template on non-denaturing polyacrylamide gels.

Primer extension assays. 100 fmol of primer-template (expressed as primer termini) was incubated with pols β , ι , or η at 37 °C for 5 min in 10- μ l reactions containing 100 μ M of all four dNTPs with or without dUTP, 40 mM Tris-HCl pH 8.0, 1 mM MgCl₂, 10 mM dithiothreitol, 250 μ g/ml bovine serum albumin, and 2.5% glycerol; 100 mM NaCl was added in reactions with pol β and pol η . Enzymes were used at two concentrations: 0.02 and 0.2 nM for pol β , 100 and 200 nM for pol ι , and 1 and 2 nM for pol η . Reactions were terminated by mixing with 1 volume of formamide loading dye solution containing 500 mM EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue in 90% formamide. Before loading onto the gel, the reactions were denatured by heating at 100 °C for 10 min and immediately transferred into ice for 2 min. Products were resolved by denaturing polyacrylamide gel electrophoresis (8 M urea, 20% acrylamide, 4 h at 2000 V) and then visualized using a Fuji PhosphorImager.

Kinetic analysis of replication products. Steady-state kinetic parameters K_m and V_{max} for dTTP and dUTP incorporation were measured in standing-start reactions as described previously¹. Initial time-course studies were performed to ensure that the reactions were in the linear range. The efficiencies of nucleotide incorporation were determined in 5 min reactions. 100 fmol of DNA substrates were replicated at 37 °C in 10 μ l reaction mixtures containing 1 fmol of pol η and variable concentrations of nucleotides. Reaction products were separated in a 20% polyacrylamide gel containing 8 M urea, and then visualized and quantified using a Fuji PhosphorImager and Image Gauge software. The velocity of dNTP incorporation was determined by dividing the percent of product generated by the respective time of the reaction. The apparent V_{max} and K_m were determined from a Hanes-Woolf plot by linear least squares fit using the Sigma Plot software. The efficiency of nucleotide insertion by a polymerase was calculated as k_{cat}/K_m .

1. Tissier, A., McDonald, J.P., Frank, E.G. & Woodgate, R. pol ι , a remarkably error-prone human DNA polymerase. *Genes Dev* **14**, 1642-1650 (2000).
2. Masutani, C., Kusumoto, R., Iwai, S. & Hanaoka, F. Mechanisms of accurate translesion synthesis by human DNA polymerase η . *EMBO J* **19**, 3100-3109 (2000).