SUPPLEMENTAL MATERIAL

Tsaalbi-Shtylik et al., http://www.jem.org/cgi/content/full/jem.201408017/DC1



Figure S1. **Msh2/Msh6-dependent suppression of the mutagenicity of UV-C.** (A) Mutant frequencies in isogenic ES cell lines after mock treatment or after low-dose UV-C (0.75 J/m^2). The Msh6^{-/-}Xpa^{-/-} line used here was line 30. Bars represent averages of three independent experiments. See Fig. 1 for results using an independently derived Msh6^{-/-}Xpa^{-/-} clone (line 4). (B) UV-C-induced mutagenesis in Msh6^{-/-} and in Msh6^{-/-}Xpa^{-/-} line 30 cells. (C) Msh2/Msh6-dependent suppression of induced mutagenesis in NER-proficient cells (WT; derived from Fig. 2 C) and in NER-deficient cells (X; Xpa^{-/-} and Msh6^{-/-}Xpa^{2/-} line 30).



Propidium Iodide

Figure S2. Accumulation of UV-C exposed ES cell lines at mitosis after nocodazole-treatment. ES cells were treated with 0.75 J/m2 UV-C or mock treated and replicating cells were pulse-labeled with BrdU, followed by the addition of Nocodazole. At 16 h after UV-C treatment, cells were fixed and stained for DNA content (using Propidium Iodide) and for BrdU and analyzed by bivariate FACS. A, sub-G1 fraction, G1, G1 phase, S, S phase, G2, and G2/M phases. 10,000 cells were analyzed per data point.



Figure S3. **Msh2/Msh6-dependent DNA damage signaling and induction of cell cycle checkpoints.** (A) Kinetics of phosphorylation of Chk1 in response to UV-C light (0.75 J/m²). bactin, loading control. X, Xpa^{-/-}, MX, Msh6^{-/-}Xpa^{-/-} line 4. Shown is a representative experiment of three independent experiments. See Fig. 4 A for one independent experiment. (B) Cell cycle responses of WT, Msh6^{-/-} (M6), and Msh2^{-/-} (M2) ES cell lines to UV-C exposure (2 J/m²). After exposure, cells were fixed at the indicated times and analyzed for DNA content (propidium iodide staining) by flow cytometry. Arrowheads indicate delayed progression of damaged WT cells, compared with the Msh2- or Msh6-deficient cells. 10,000 cells were analyzed per data point. Shown is a representative experiment of three independent experiments. (C) Bivariate cell cycle profiles after UV-C exposure (2 J/m²) of WT and Msh2^{-/-} (M2) ES cells. Replicating cells were pulse-labeled with BrdU immediately after exposure. Cells were fixed at the indicated times and analyzed for DNA and BrdU content (propidium iodide staining and immunostaining, respectively) by flow cytometry. Arrowheads indicate the increased reappearance of BrdU-positive Msh2^{-/-} ES cells at G1, compared with WT controls. 10,000 cells were analyzed per data point. Shown is a representative experiments (D) Quantification of WT and Msh2^{-/-} (M2) cells at G1, at 6 and 8 h after treatment with 2 J/m² UV, derived from C. (E) Maturation of nascent strands, 4 h after UV-C treatment (0.75 J/m²) of ES cells, cultured in the presence of the Atr inhibitor caffeine (5 mM). Circles, internal standards ([14C] label, representing intra-CPD fragments). Squares, nascent strands ([3H] label). (F) Frequencies of UV-C (2 J/m²)-induced Hprt mutants in WT (G) Suppression of induced mutations by post-TLS repair, cultured in the absence and in the presence of UCN201 after UV-C exposure, were derived from Fig. S3F.



Figure S4. **Msh2/Msh6-dependent induction of delayed apoptosis in response to low-dose UV-C.** Bivariate flow cytometry of $Xpa^{-/-}$ (X) and Msh62/ $2Xpa^{-/-}$ (MX) ES cells after exposure to 0.75 J/m² UV-C. Immediately after treatment, replicating cells were pulse labeled with BrdU. Cells were analyzed for DNA content (propidium iodide staining) and for levels of incorporated BrdU. A, sub-G1 fraction; G1, G1 phase; S, S phase, G2/M phase. 10,000 cells were analyzed per data point.



Figure S5. Normal frequency but increased mutagenicity of TLS at a defined (624)TT in Msh2/Msh6-deficient cells. X, $Xpa^{-/-}$, MX, $Msh6^{-/-}Xpa^{-/-}$. (A) Replicating plasmid pMTEX6 carrying a site-specific (624)TT photolesion (red triangle). Bold, disrupted EcoRV site. Red sequence, mismatched tetramer, enabling to distinguish both strands after replication. Py ori, mouse Polyoma virus replication origin. Py T antigen, mouse Polyoma virus large T antigen, required for replication origin function. Bsd, Blasticidin resistance cassette. (B) Relative efficiency of TLS at a site-specific (6–4)TT photolesion in MEF lines. X, MEFs deficient for the core NER gene Xpc, MX, MEFs deficient for Xpc and Msh2. Two Xpc^{-/-} lines (top) and one Msh6^{-/-}Xpc^{-/-} line (bottom) were used. (C) Calculation of frequencies of mutagenic and nonmutagenic TLS. (D) Quantification of mutations at, and adjacent to, the site-specific (624)TT photolesion in replicated plasmids rescued, from transfected MEFs. X, MEFs deficient for the key NER gene Xpc (two independent lines), MX, MEFs deficient for Xpc and Msh2 (one line, two independent experiments). The frequency of substitutions opposite the 39, T of the (6–4)TT photolesion was significantly (P = 0.0012) increased in the Msh2-deficient cells. Del, deletions of ≤ 4 nucleotides. The relatively high frequency of substitutions at the +1 position in both geno-types represents an artifact of the assay as these substitutions are not observed in the genomic mutation spectra (Table S1).

Table S1. Mutation spectra at genomic Hprt in ES cells

$Xpa^{-/-}$ ES cells (n = 127 substitutions, from in total two experiments), 0.75 J/m² UV-C

		. 11			<i>н</i> (, ,
position	mutation	strand	$5' \rightarrow 3'$ target	amino	# of mutants
				acid	
Transitions at dipyrimid	ine sites			change	
113	GC>AT	NTS	TTC/CITCA	Pro>Leu	1
145	GC>AT	NTS	AGA(C)IIG	leu>Phe	1
146	AT>GC	NTS	GACITIGC	leu>Pro	1
151	GC>AT	NTS	GCTICIGAG	Arasston	1
203	AT>GC	NTS	IGCIIICAA	leu>Pro	1
208	GC>AT	TS	AAGIGIGGG	Glv>Ara	4
209	GC>AT	TS	AGGIGIGGG	Glv>Glu	3
217	AT>GC	TS	TATIAIAGT	lys>Glu	1
325	GC>AT	NTS	GATICIAGT	Glasston	2
400	GC>AT	TS	GTT(G)AAG	Glusivs	13
/19	GC>AT	TS		GlysAsn	1
464	GC>AT	NTS	GCCICICAA	Prosleu	1
508	GC>AT	NTS	TCTICIGAA	Arasston	3
527	GC>AT	NTS	GGCICIAGA	Prosleu	1
538	GCSAT	TS	GITIGIGAT	GlysArg	1
539	GC>AT	TS		Gly>Gly	4 2
544	GC>AT	TS	TTT(G)AAA	Glustvs	7
545		TS	TTG(A)AAT	GlusGly	1
568	GCSAT	TS	GTTIGIGAT	GlusArg	1
560	GCSAT	TS		Gly>Gly	4
580	GCSAT	TS		Asp>Asp	4 2
580	GCSAT	TS		Glustve	1
500	GCSAT	TS		Ara>lys	5
601	GC>AT	TS		Arg>Lys	1
625	GC>AT	тс		Asp>Asi	1 2
033		15	CIOIOIAAA	0192010	2
Transversions at dipyrin	nidine sites				
96	GC>TA	TS	TTT(G)GAA	Leu>Phe	1
100	AT>TA	TS	GAA(A)AAG	Lys>stop	1
125	AT>TA	NTS	TGA(T)TAT	lle>Asn	1
133	AT>TA	TS	GAC(A)GGA	Arg>Trp	1
149	GC>TA	NTS	TTG(C)TCG	Ala>Asp	1
205	AT>TA	TS	CTC(A)AGG	Lys>stop	1
208	GC>CG	TS	AAG(G)GGG	Gly>Arg	1
271	AT>TA	TS	GAT(A)GAT	Arg>stop	1
475	AT>TA	TS	GTT(A)AGG	Lys>stop	1
486	GC>TA	NTS	AAG(C)TTG	Ser>Arg	1
548	AT>TA	NTS	AAA(T)TCC	Val>Asp	1
551	GC>TA	NTS	TTC(C)AGA	Pro>Gln	1
606	GC>TA	TS	TTT(G)AAT	Leu>Phe	1
623	AT>TA	NTS	TCA(T)TAG	lle>Asn	1
631	AT>TA	TS	GAA(A)CTG	Thr>Ser	1
635	GC>TA	TS	CTG(G)AAA	Gly>Val	1
640	GC>TA	TS	AAA(G)CCA	Ala>Ser	2
Tandem mutations					
118	GC>AT	TS	CAT(GG)ACT	Gly>Lys	5
119	GC>AT	TS		, ,	
207	CC AT	TC		Chadra	2
207		is TC	CAAIGGIGGG	Giy>Arg	۷
208	GC>AI	15			

 $^1\text{TS},$ transcribed DNA strand; NTS, non-transcribed DNA strand; NA, not applicable.

Table S1.	Mutation spectra	at aenomic Hprt	in ES cells (Cont	inued)
	monument specific	al gononne ripri		moody

$X_{pa^{-/-}}$ ES cells (n = 1	27 substitutions,	from in total two ex	periments), 0.75 J/m² UV-C			
208	GC>AT	TS	AAG(GG)GGG	Glv>Lvs	2	
209	GC>AT	TS		0.1/ 2/0	-	
207	0077					
209	GC>AT	TS	AGG(GG)GGC	Gly>Glu	1	
210	GC>AT	TS			·	
210	00774	10				
211	GC>TA	TS	GGG(GG)CTA	Gly>Tyr	1	
212	GC>AT	TS	000(00)00		·	
212	00774	10				
418	GC>AT	TS	ACT(GG)TAA	Glv>Asn	2	
419	GC>AT	TS		01/771011	-	
	00774	10				
495	GC>TA	TS	GGT(GA)AAA	lys>stop	1	
496	AT>TA	TS		-7 - · · · · · · · · · · · · · · · · · ·		
499	AT>TA	TS	AAA(AG)GAC	Ara>stop	1	
500	GC>AT	TS				
538	GC>AT	TS	GTT(GG)ATT	Val>Lvs	1	
539	GC>AT	TS				
568	GC>AT	TS	GTT(GG)ATA	Gly>Lys	2	
569	GC>AT	TS		, ,		
600	GC>AT	TS	CAG(GG)ATT	Asp>Asn	4	
601	GC>AT	TS				
600	GC>CG	TS	CAG(GGA)TTT	Asp>lle	1	
601	GC>AT	TS				
602	AT>TA	TS				
Multiple independent	mutations					
34	GC>TA	TS	AGC(G)(+C)ATG		1	
	+C					
113	GC>AT	NTS	TTC(C)T(-C)ATG		1	
115	-C					
202	GC>AT	NTS	GTG(C)T(C)AAG	Leu>Phe	2	
204	GC>AT	NTS				
					_	
380	GC>AT	TS	CTG(G)A(A)AGA	Gly>Glu	1	
382	AI>GC	IS		Lys>Glu		
(00	00.47	TO				
403	GC>AI		GAA(G)A(I)AIA	Asp>Lys	I	
405	AI>IA	NI5				
500		тс	CTTICLAICITAT	A I	1	
500	GC>AI	I J NITC	CHIGIAICITAT	Asp>Lys	I	
302	GC>IA	1413				
580	GCNTA	TS	A ATIGI A IGITAC	Glusston	1	
501		TS		010/3100	I	
Insertions		10				
511	т		GAALTIGTG		1	
625	 +T				1	
-20					'	
Deletions						
47	-G		CAGI-GITTA		1	
					-	
15, transcribed DNA	strand; NIS, not	n-transcribed DNA s	trana; INA, not applicable.			

Table S1. Mutation spectra at genomic Hprt in ES cells (Continued)

$Xpa^{-/-}$ ES cells (n = 127 substitutions, from in total two experiments), 0.75 J/m² UV-C

Transversions at r	non-dipyrimidine sites			
1	AT>TA	(A)TGC	Met>Leu	3
2	AT>GC	A(T)GCC	Met>Thr	1
84	AT>CG	TTA(T)GCC	Tyr>stop	1
397	GC>TA	ATT(G)TTG	Val>Phe	1
Multiple independ	dent mutations			
199	GC>TA	TGT(G)T(G)CTC	Val>Phe	1
201	GC>TA			
Insertions				
631	+T	AAA(+T)CTG		1
Deletions				
533-553	21bp			1

 $Msh6^{-/-}Xpa^{-/-}$ ES cell line 4 (n = 141 substitutions, from in total two experiments) 0.75 J/m² UV-C

position	mutation	strand	$5' \rightarrow 3'$ target	amino acid change	# of mutants
Transitions at dip	yrimidine sites				
40	GC>AT	TS	GAT(G)AAC	Glu>Lys	1
113	GC>AT	NTS	TTC(C)TCA	Pro>Leu	5
118	GC>AT	TS	CAT(G)GAC	Gly>Arg	2
122	AT>GC	NTS	GAC(T)GAT	Leu>Pro	1
139	GC>AT	TS	ACT(G)AAA	Glu>Lys	3
149	GC>AT	NTS	TTG(C)TCG	Ala>Val	1
196	AT>GC	NTS	CTC(T)GTG	Cys>Arg	1
209	GC>AT	TS	AGG(G)GGG	Gly>Glu	1
212	GC>AT	TS	GGG(G)CTA	Gly>Asp	6
233	AT>GC	NTS	ACC(T)GCT	Leu>Pro	1
254	AT>GC	NTS	CAC(T)GAA	Leu>Pro	1
374	AT>GC	NTS	CTT(T)AAC	Leu>Ser	1
400	GC>AT	TS	GTT(G)AAG	Glu>Lys	9
403	GC>AT	TS	GAA(G)ATA	Asp>Asn	1
418	GC>AT	TS	ACT(G)GTA	Gly>Ser	2
508	GC>AT	NTS	TCT(C)GAA	Arg>stop	1
526	GC>AT	NTS	AGG(C)CAG	Pro>Ser	1
533	AT>GC	NTS	ACT(T)TGT	Phe>Ser	1
538	GC>AT	TS	GTT(G)GAT	Gly>Arg	1
539	GC>AT	TS	TTG(G)ATT	Gly>Glu	4
541	AT>GC	NTS	GGA(T)TTG	Phe>Leu	1
544	GC>AT	TS	TTT(G)AAA	Glu>Lyss	7
550	GC>AT	NTS	ATT(C)CAG	Pro>Ser	1
551	GC>AT	NTS	TTC(C)AGA	Pro>Leu	2
568	GC>AT	TS	GTT(G)GAT	Gly>Arg	1
580	GC>AT	TS	CTT(G)ACT	Asp>Asn	1
589	GC>AT	TS	AAT(G)AGT	Glu>Lys	10
599	GC>AT	TS	TCA(G)GGA	Arg>Lys	5
601	GC>AT	TS	AGG(G)ATT	Asp>Asn	2
605	AT>GC	NTS	ATT(T)GAA	Leu>Ser	1
635	GC>AT	TS	CTG(G)AAA	Gly>Glu	3
655	AT>GC	NTS	GCC(T)AAG	stop>Gln	1

Transversions at dipyrimidine sites

 $^1\text{TS},$ transcribed DNA strand; NTS, non-transcribed DNA strand; NA, not applicable.

Table S1. Mutation	spectra at genomi	c Hprt in ES cells	(Continued)

(pa ^{-/-} ES cell	s (n = 127 substitutions,	trom in total two expe	eriments), 0.75 J/m ² UV-C			
01	AT>TA	TS	AAA(A)AGT	Lys>lle	1	
18	GC>TA	TS	CAT(G)GAC	Gly>stop	1	
25	AT>CG	NTS	tga(t)tat	lle>Ser	3	
40	AT>TA	TS	CTG(A)AAG	Glu>Val	1	
22	GC>TA	NTS	GTT(C)TTT	Phe>Leu	1	
26	AT>CG	NTS	ATTITITAT	Phe>Cvs	1	
) () D 1		NITS	GICITIICA	Loux Mot	1	
		NITC			1	
70				lie>Asn	1	
78	AI>IA	15	GAA(A)AGG	Lys>Asn		
44	GC>TA	TS	TTT(G)AAA	Glu>stop	1	
48	AT>CG	NTS	AAA(T)TCC	lle>Ser	1	
31	AT>CG	TS	TTG(A)CTA	Asp>Ala	1	
33	AT>TA	NTS	GAC(T)ATA	Tyr>Asn	2	
74	GC>CG	NTS	GTAICITTC	, Tvr>stop	1	
7.5	GC>TA	TS 2T	TTTIGIAAT	Leu>Phe	1	
	0021/1	10			'	
ndem mutati	ons	тс		Char Ass	0	
)	GC>AI	15	CCA(GG)TIA	Gly>Asn	Z	
,	GC>AT	ſS				
)9	AT>GC	no dimer	TTT(AT)TCC	lle>Glv	1	
10	AT>CG	NTS	V / · · -	/		
		-				
2	GC>AT	NTS	ATT(CC)TCA	Pro>Phe	1	
3	GC>AT	NTS				
1.9		TS		Glasha	3	
10		10 TC	CAILOGIACI	GIY>LYS	5	
19	GC>AI	15				
24	AT>TA	TS	CTG(AT)TAT	lle>Tyr	1	
25	AT>TA	NTS	· · /	1		
07	GC>AT	TS	CAA(GG)GGG	Gly>Arg	1	
08	GC>AT	TS				
	00.17	TO			0	
08	GC>AT	TS	AAG(GG)GGG	Gly>Lys	3	
)9	GC>AT	TS				
90		۶T		Glysolu	1	
10		TC	700,000	017-010	-	
	GC>AI	13				
00	GC>AT	TS	GTT(GA)AGA	Glu>Ara	1	
01	AT>GC	TS	· · · · · · · · · · · ·			
		. –				
18	GC>AT	TS	ACT(GG)TAA	Gly>Asn	2	
19	GC>AT	TS				
53	GC>TA	NIS	AGC(CC)CAA	Pro>lle	I	
54	GC>AT	NTS				
88	GC>AT	TS	GTT(GG)ATT	Glusius	2	
30	CC_AT	TS		017-275	-	
17	GC>AI	15				
58	GC>AT	TS	GTT(GG)ATA	Glv>Lvs	2	
59	GC>AT	TS	· /· ·/ ····	- / / -		
	AT OC	тс	TTCIACICCA	ArasGlu	1	

Table S1. Mutation	spectra at genom	nic Hprt in ES cells	(Continued)
--------------------	------------------	----------------------	-------------

$Xpa^{-/-}$ ES cells ($n = 12$	27 substitutions, from	in total two experime	ents), 0.75 J/m ² UV-C		
599	GC>AT	TS			
599	GC>AT	TS	TCA(GG)GAT	Arg>Lys	1
600	GC>AT	TS			
600	GC>AT	TS	CAG(GG)ATT	Asp>Asn	1
601	GC>AT	TS			
Multiple Independent r	nutations				
69	AT>TA	no dimer	TTG(T)ATA	Cvs>stop	1
79	GC>AT	NTS	AAT(C)ATT	His>Tyr	
				,	
103	GC>TA	TS	AAA(G)TGT	Val>Leu	1
112	GC>AT	NTS	ATT(C)CTC	Pro>Ser	
170	AT. TA	na diman		Him Law	1
1/9			GCCIAITICIACA		I
181	GC>AI	N12	GCC[A]T[C]ACA	His>lyr	
209	insertion		GGG(+A)GGG		1
213	GC>AT	NTS	GGG(C)T(A)TAA		
215	AT>TA	no dimer	GGG(C)T(A)TAA		
400	GC>AT	TS	GTT(G)A(A)GAT	Glu>Lys	1
402	AT>GC	TS	GTT(G)A(A)GAT		
471	CC AT	тс		Mote lle	1
47 1			TCICIAAC		I
482	GC>CG	no aimer	TIGICIAAG	Ald>Gly	
553	insertion		CCA(+T)GAC		1
554	AT>CG	TS	CAG(AC)AAG		
555	GC>TA	no dimer	CAG(AC)AAG		
558	GC>TA	TS	CAA(G)TTT		
la continue d					
	0	то			1
207-212	+G	15	GGGGGG		I
Transitions at non-dipy	rimidine sites				
3	GC>AT		AT(G)CCG	Met>lle	1
197	GC>AT		ICI(G)IGI	Cvs>Tvr	1
521	AT>GC		GAT(A)CAG	Tyr>stop	1
				, ,	
Transversions at non-d	ipyrimidine sites				
109	AT>TA		TTT(A)TTC	lle>Phe	2
197	GC>CG		TCT(G)TGT	Cys>Tyr	1
572	AT>CG		GAT(A)TGC	Tyr>Ser	1
Tandem mutations					
571	ΑΤ>ΤΑ		GGA(TA)TGC	Tvr>Ser	1
572	ATSGC			1912001	
572	AIVOC				
Spontaneous substitutio	ons in Msh6 ^{-/-} Xpa ^{-/-}	ES cell line 4, trom	in total two experiments $(n = 30)$		#af mutanta
position	INVICIION	sirand	J → S laiger	amino	#OF INUIGINS
				acia	
Transitions at dispurimit	dinas sitas			change	
		NIA	HEICHEE	Alas Val	2
147					10
∠ I ∠ 222				Giy>Asp	1
200	AI>GC	INA		LGD>LIO	I
'TS, transcribed DNA	strand; NTS, non-tran	scribed DNA strand;	NA, not applicable.		

Table 51. Mutation spectra at genomic ripri in ES cells (Continuea)	Table S1.	. Mutation spectra at genomic Hprt in ES cells (Continued)	
---	-----------	--	--

$c_{pa^{-/-}}$ ES cells (<i>n</i> = 127 substitutions, from in total two experiments), 0.75 J/m ² UV-C										
440	AT>GC	NA	TGC(T)TTC	Leu>Pro	1					
491	AT>GC	NA	TGC(T)GGT	Leu>Pro	1					
526	GC>AT	NA	AGG(C)CAG	Pro>Ser	1					
563	AT>GC	NA	TTG(T)TGT	Val>Ala	1					
589	GC>AT	NA	AAT(G)AGT	Glu>Lys	3					
Transversions of	at dipyrimidines sites									
125	AT>CG	NA	tga(t)tat	lle>Ser	1					
223	AT>CG	NA	TTC(T)TTG	Phe>Val	2					
293	AT>TA	NA	TAG(A)TTT	Asp>Val	1					
Transitions at r	on-dipyrimidine sites									
148	GC>AT	NA	CTT(G)CTC	Ala>Thr	1					
617	GC>AT	NA	TTT(G)TGT	Cys>Tyr	2					

Table S2. Mutations induced at a site-specific (6-4)TT embedded within a replicating plasmid.

				Хрс (lin	e 1, exp. 1)	Хрс (lin	e 2, exp. 2)	Msh2X _l	oc (exp. 1)	Msh2Xp	oc (exp. 2)
TLS events				87	98.9%	93	98.9%	88	100%	89	100.0%
Correct TLS											
5′- <u>∏</u>	\rightarrow		5'-TT	36	40.9%	45	47.9%	46	52.3%	42	47.2%
Mutagenic TLS				50	56.8%	46	48.9%	40	45.5%	39	43.8%
5′- <u>TT</u>	\rightarrow		5'-TA	0	0.0%	5	5.3%	5	5.7%	15	16.9%
5′- <u>TT</u>	\rightarrow		5'-TC	4	4.5%	2	2.1%	5	5.7%	4	4.5%
5′- <u>TT</u>	\rightarrow		5'-TG	0	0.0%	0	0.0%	1	1.1%	1	1.1%
5′- <u>∏</u>	\rightarrow		5'-AT	0	0.0%	4	4.3%	1	1.1%	2	2.2%
5′- <u>∏</u>	\rightarrow		5'-CT	0	0.0%	0	0.0%	0	0.0%	1	1.1%
5′- <u>TT</u>	\rightarrow		5'-GT	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5′- <u>TT</u>	\rightarrow		5'-AA	0	0.0%	0	0.0%	1	1.1%	0	0.0%
5′- <u>∏</u>	\rightarrow		5'-AC	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5′- <u>TT</u>	\rightarrow		5'-CA	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5′- <u>TT</u>	\rightarrow		5'-CC	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5′- <u>∏</u>	\rightarrow		5'-GC	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5′-C <u>TT</u>	\rightarrow		5'-TTT	43	48.9%	29	30.9%	21	23.9%	10	11.2%
5′-C <u>TT</u>	\rightarrow		5'-ATT	1	1.1%	1	1.1%	1	1.1%	1	1.1%
5'-CTT	\rightarrow		5'-GTT	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5'-CTT	\rightarrow		5'-TAT	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5'-CTT	\rightarrow		5'-TTA	2	2.3%	3	3.2%	3	3.4%	2	2.2%
5'-CTT	\rightarrow		5'-TTC	0	0.0%	2	2.1%	0	0.0%	0	0.0%
5'-CTT	\rightarrow		5'-TTG	0	0.0%	0	0.0%	0	0.0%	1	1.1%
5′-CTT	\rightarrow		5'-TAA	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5'-CTT	\rightarrow		5'-AAT	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5'-CTT	\rightarrow		5'-AAA	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5′-C <u>TT</u>	\rightarrow		5'-ATA	0	0.0%	0	0.0%	0	0.0%	1	1.1%
5′-C <u>TT</u>	\rightarrow		5'-ATC	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5′-TC <u>TT</u>	\rightarrow		5'-ACTT	0	0.0%	0	0.0%	1	1.1%	0	0.0%
5′-TC <u>TT</u>	\rightarrow		5'-ACTA	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5′-TC <u>TT</u>	\rightarrow		5'-ATTT	0	0.0%	0	0.0%	1	1.1%	1	1.1%
5′-TC <u>TT</u>	\rightarrow		5'-ATAT	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5′-TC <u>TT</u>	\rightarrow		5'-AAAC	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5′-TC <u>TT</u>	\rightarrow		5'-ATTA	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5′-TC <u>TT</u>	\rightarrow		5'-CTTA	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5'-ATC <u>TT</u>	\rightarrow		5'-TTCTT	0	0.0%	0	0.0%	0	0.0%	0	0.0%
deletion				1	1.1%	2	2.1%	2	2.3%	8	9.0%
5′- <u>TT</u>	\rightarrow	5′-A		0	0.0%	0	0.0%	0	0.0%	0	0.0%
5′- <u>TT</u>	\rightarrow	5′-C		0	0.0%	0	0.0%	0	0.0%	0	0.0%
5′-C <u>∏</u>	\rightarrow	5′-TT		1	1.1%	1	1.1%	1	1.1%	1	1.1%
5′-C <u>TT</u>	\rightarrow		5'-TA	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5′- <u>TT</u> C	\rightarrow		5'-TT	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5′-TC <u>TT</u>	\rightarrow		5'-CTT	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5'-ATC <u>TT</u>	\rightarrow		5'-TCTT	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5′-C <u>TT</u> C	\rightarrow		5'-CC	0	0.0%	1	1.1%	0	0.0%	4	4.5%
5′-C <u>TT</u> C	\rightarrow		5'-C	0	0.0%	0	0.0%	1	1.1%	1	1.1%
5′-C <u>TT</u> CA	\rightarrow		5'-CC	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5′-C <u>TT</u> CA	\rightarrow		5′-C	0	0.0%	0	0.0%	0	0.0%	0	0.0%
5′-TC <u>TT</u> C	\rightarrow		5′-T	0	0.0%	0	0.0%	0	0.0%	1	1.1%
5′-TC <u>TT</u> C	\rightarrow		5'-C	0	0.0%	0	0.0%	0	0.0%	1	1.1%
insertion				0	0.0%	0	0.0%	0	0.0%	0	0.0%
large deletion /inse	rtion		1	1	1.1%	1	1.1%	0	0.0%	0	0.0%
sum				88	100%	94	100%	88	100%	89	100%