Supplementary materials for Selvam et al. entitled "Histone H4 H75E mutation attenuates global genomic and Rad26independent nucleotide excision repair"

Sup	Supplementary Table S1. UV sensitivity (%) of histone H4 mutants suggested by random mutation screening.																	
		Histone H4 residues																
		S64	V65	166	R67	D68	S69	V70	T71	Y72	T73	E74	H75	A76	K77	R78	К79	T80
	Α	86	79	х	324	76	266	117	83	х	83	79	29	100	227	х	156	41
	С	х	х	173	48	100	195	х	119	56	89	43	71	141	127	х	223	84
	D	59	х	х	396	100	х	404	177	69	8	88	47	47	179	х	х	х
	Е	х	х	х	88	115	х	276	х	х	62	100	0	х	102	х	134	х
	F	317	60	х	—	34	116	61	68	117	21	29	30	х	139	х	150	4
9	G	432	х	х	267	50	254	122	114	х	59	74	х	127	98	58	156	84
ed	н	186	х	х	206	145	х	136	181	131	65	99	100	х	212	138	187	х
Itat	Т	409	х	100	125	26	х	163	510	425	133	140	х	х	169	13	112	32
л Ш	К	х	х	х	199	80	х	76	66	х	50	60	х	х	100	158	100	х
les	L	13	171	х	217	76	х	44	х	276	193	76	38	х	159	х	160	26
idu	М	х	582	340	28	68	х	558	121	х	143	17	х	12	121	27	124	66
res	Ν	232	х	х	210	109	х	81	100	20	86	93	63	х	142	49	98	45
Н4	Ρ	х	х	х	822	х	33	х	х	х	х	х	х	29	х	х	36	х
ne	Q	х	х	х	148	108	х	110	121	х	80	53	33	х	66	х	197	х
sto	R	422	х	х	100	х	х	85	56	х	100	х	52	41	126	100	129	101
Ξ	S	100	221	х	340	90	100	147	127	х	113	87	40	104	116	10	102	57
	Т	247	974	х	168	109	218	109	100	8	100	68	36	19	178	82	54	100
	v	23	100	х	317	85	121	100	85	х	326	110	45	90	100	х	141	74
	w	х	х	х	38	х	х	80	76	х	х	47	х	х	180	х	156	х
	Y	64	0	х	222	576	х	136	94	100	31	100	89	55	181	х	116	44

Mutations appeared to be 3 times less or more resistant to UV than wild types are shown in red and green, respectively. x: inviable mutations, which were present in the original plasmids but absent in the 5-FOA-selected yeast cells. —: not calculated for UV survival, as fewer than 10 sequencing reads for this H4 R67F mutation were obtained from both the original plasmid library and plasmids isolated from UV irradiated or unirradiated yeast cells.

**Supplementary Table S2.** Summary for confirmation of UV sensitivity (S) or resistance (R) of histone H4 mutants (fold change relative to wild type cells)<sup>*a*</sup>.

Mutation	Fold change	Mutation	Fold change	
S64G	3 (R)	T73D	10 (S)	
S64I	3 (R)	T73F	10 (S)	
V65T	10 (R)	T73Y	30 (S)	
V65Y <sup>b</sup>	3 (R)	E74M	10 (S)	
R67A	10 (R)	H75E	10 (S)	
R67D	10 (R)	A76P	5 (S)	
R67S	5 (R)	A76T	5 (S)	
R67V	5 (R)	R78I	50 (S)	
D68I	5 (S)	R78S	10 (S)	
D68Y <sup>c</sup>	3 (S)	T80F	10 (S)	
T71I	2 (R)	T80I	5 (S)	
Y72T	5 (S)	T80L	5 (S)	

<sup>*a*</sup> The fold changes shown are at 120 J/m<sup>2</sup> of UV and the averages of at least 3 independent experiments. <sup>*b*</sup> The screening (Supplementary Table S1) indicated that this mutation was UV sensitive. <sup>*c*</sup> The screening (Supplementary Table S1) indicated that this mutation was UV sensitive.

	Α	В	С	D	Е	F	G	н	I	J	κ	L
	H4S64G	H4S64I	H4V65T	H4V65Y	H4R67A	H4R67D	H4R67S	H4R67V	H4D68I	H4D68Y	H4T71I	H4Y72T
	U0124	U 0 1 2 4	U 0 1 2 4	U 0 1 2 4	U 0 1 2 4	U 0 1 2 4	U0124	U 0 1 2 4	U0124	U0124	U0124	U 0 1 2 4
-200 +1 RPB2 (NTS)												
+830	-							4482		423		

**Supplementary Fig. S1. Sequencing gels showing CPDs remaining in the nontranscribed strand of the** *RPB2* **genes in cells with the indicated histone H4 mutations.** The times (h) of repair incubation are indicated at the top. '*U*' indicates samples from unirradiated cells. Approximate nucleotide positions relative to the transcription start site (+1) of the *RPB2* gene are indicated on the left.

	Α	В	С	D	Е	F	G	н	1	J	K
	H4T73D	H4T73F	H4T73Y	H4E74M	H4A76P	H4A76T	H4R78I	H4R78S	H4T80F	H4T80I	H4T80L
	U0124	U 0 1 2 4	U0124	U 0 1 2 4	U0124	U0124	U0124	U 0 1 2 4	U0124	U0124	U 0 1 2 4
-200 +1 RPB2 (NTS)											
	NAME MONTH OFFICE AND										
+830		48-1			-					44	

**Supplementary Fig. S2. Sequencing gels showing CPDs remaining in the nontranscribed strand of the** *RPB2* genes in cells with the indicated histone H4 mutations. The times (h) of repair incubation are indicated at the top. 'U' indicates samples from unirradiated cells. Approximate nucleotide positions relative to the transcription start site (+1) of the *RPB2* gene are indicated on the left.

	Α	В	С	D	Е	F	G	Н	1	J	K
	rad7∆ rad26∆ H4S64G	rad7∆ rad26∆ H4S64I	rad7∆ rad26∆ H4V65T	rad7∆ rad26∆ H4V65Y	rad7∆ rad26∆ H4R67A	rad7∆ rad26∆ H4R67D	rad7∆ rad26∆ H4R67S	rad7∆ rad26∆ H4R67V	rad7∆ rad26∆ H4D68I	rad7∆ rad26∆ H4D68Y	rad7∆ rad26∆ H4T71I
	U 0 ½ 1 2 4	U 0 ½ 1 2 4	U 0 ½ 1 2 4	U 0 ½ 1 2 4	U 0 ½ 1 2 4	U 0 ½ 1 2 4	U 0 ½ 1 2 4	U 0 ½ 1 2 4	U 0 ½ 1 2 4	U 0 ½ 1 2 4	U 0 ½ 1 2 4
+940											
+50					2222						
<sup>+</sup> <i>RPB2</i>									1		
-40											
-60							-				

Supplementary Fig. S3. Sequencing gels showing CPDs remaining in the transcribed strand of the *RPB2* genes in *rad7* $\Delta$  *rad26* $\Delta$  cells with the indicated histone H4 mutations. The times (h) of repair incubation are indicated at the top. 'U' indicates samples from unirradiated cells. Approximate nucleotide positions relative to the transcription start site (+1) of the *RPB2* gene are indicated on the left.

	Α	В	С	D	Е	F	G	н	I	J
	rad7∆ rad26∆ H4T73D	rad7∆ rad26∆ H4T73F	rad7∆ rad26∆ H4T73Y	rad7∆ rad26∆ H4E74M	rad7∆ rad26∆ H4A76P	rad7∆ rad26∆ H4A76T	rad7∆ rad26∆ H4R78I	rad7∆ rad26∆ H4R78S	rad7∆ rad26∆ H4T80F	rad7∆ rad26∆ H4T80I
	U 0 ½ 1 2 4	U 0 ½ 1 2 4	U 0 ½ 1 2 4	U 0 ½ 1 2 4	U 0 ½ 1 2 4	U 0 ½ 1 2 4	U 0 ½ 1 2 4	U 0 ½ 1 2 4	U 0 ½ 1 2 4	U 0 ½ 1 2 4
+940										
+50				And And sold and		The second second second second second				
<b>↑</b>										
+1 RPB2			-							
40			· · · · · · · · · · · · · · · · · · ·							
-40										
-60					10 10 10 <b>10 10</b>				-	

Supplementary Fig. S4. Sequencing gels showing CPDs remaining in the transcribed strand of the *RPB2* genes in *rad7* $\Delta$  *rad26* $\Delta$  cells with the indicated histone H4 mutations. The times (h) of repair incubation are indicated at the top. 'U' indicates samples from unirradiated cells. Approximate nucleotide positions relative to the transcription start site (+1) of the *RPB2* gene are indicated on the left.

	A Rad1	B Rad2	C Rad3	D Rad7
Min after UV	WT H4 H75E WT WT WT H4 H75E H4 H75E M4 H75E	WT H4 H75E WT WT WT WT H4 H75E 02 0	WT H4 H75E WT WT H4 H75E MT H4 H75E H4 H75E	0 0 0 0 0 0 0 0 0 0 0 0 0 0
Anti-FLAG				
Anti-H3				
	<b>E</b> Rad10	<b>F</b> Rad14	G Rad16	H Rad23
Min after UV	E Rad10 A H4H H4H H4H H4H H4H H4H H4H H4H	F Rad14 0 30 31 32 44 44 44 44 45 45 45 45 45 45 45 45 45	G Rad16 0 33 35 24 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	H Rad23
Min after UV Anti-FLAG	E Rad10 0 30 120 352H 74 M H H M H H M H H M H H M H H H M H H H H H H H H H H H H H H H H H H H H	$F_{Rad14}$ $0 30 420 H H H H H H H H H H H H H H H H H H H$	G Rad16 0 30 35 2 4 4 4 4 7 35 2 4 4 4 4 4 5 4 4 4 7 35 35 35 35 4 4 5 4 4 5 4 5 4 5 4 5 4 5	H Rad23 0 30 31 32 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4

**Supplementary Fig. S5. H4H75E mutation does not significantly affect cellular levels of NER proteins.** (A-H) Western blots showing cellular levels of 3×FLAG tagged NER proteins. Total histone H3 serves as loading control.

	A Rad1		<b>B</b> Ra	ad2	C Ra	ad3	D Rad7		
	WT	H4H75E	WT	H4H75E	WT	H4H75E	WT	H4H75E	
Min after UV	0 30 00	0 30 60	0 30 90	0 30 60	0 90 00	0 30	0 90 90	0 00	
Anti-FLAG				-					
Anti-H3	000			-	-	-			
	E Rad10		F		G		H Rad23		
	Ra	d10	Ra	d14	Rad	16	Ra	d23	
	Ra WT	d10 H4H75E	Ra WT	d14 H4H75E	Rad WT	16 H4H75E	Rai WT	d23 H4H75E	
Min after UV		d10 H4H75E 0 8 9		d14 H4H75E 0 8 9	Rad WT	16 H4H75E o G		d23 H4H75E o ෆි ගි	
Min after UV Anti-FLAG		d10 H4H75E o ෆි ගි	Ran WT ୦ ନି ଓ	d14 H4H75E o & @	Rad WT o G	16 H4H75E o 饧		d23 H4H75E o ෆි ගි	

**Supplementary Fig. S6. H4H75E mutation does not dramatically affect the association of NER proteins (other than Rad4) with chromatin.** (A-H) Western blots showing the levels of chromatin-associated 3×FLAG tagged NER proteins. Histone H3 serves as loading control.