

Supplementary materials for Selvam et al. entitled “Histone H4 H75E mutation attenuates global genomic and Rad26-independent nucleotide excision repair”

Supplementary Table S1. UV sensitivity (%) of histone H4 mutants suggested by random mutation screening.

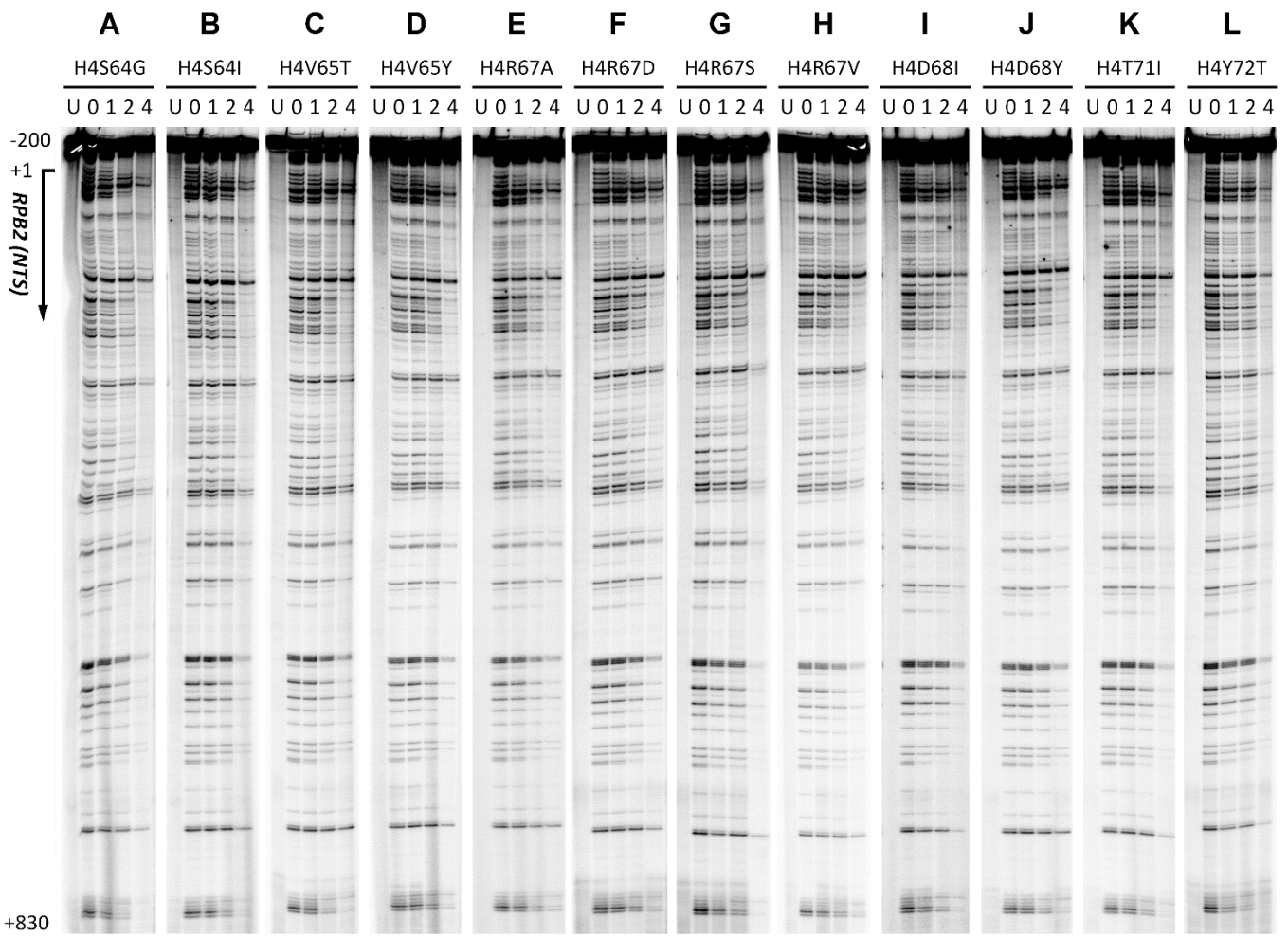
		Histone H4 residues																
		S64	V65	I66	R67	D68	S69	V70	T71	Y72	T73	E74	H75	A76	K77	R78	K79	T80
Histone H4 residues mutated to	A	86	79	x	324	76	266	117	83	x	83	79	29	100	227	x	156	41
	C	x	x	173	48	100	195	x	119	56	89	43	71	141	127	x	223	84
	D	59	x	x	396	100	x	404	177	69	8	88	47	47	179	x	x	x
	E	x	x	x	88	115	x	276	x	x	62	100	0	x	102	x	134	x
	F	317	60	x	—	34	116	61	68	117	21	29	30	x	139	x	150	4
	G	432	x	x	267	50	254	122	114	x	59	74	x	127	98	58	156	84
	H	186	x	x	206	145	x	136	181	131	65	99	100	x	212	138	187	x
	I	409	x	100	125	26	x	163	510	425	133	140	x	x	169	13	112	32
	K	x	x	x	199	80	x	76	66	x	50	60	x	x	100	158	100	x
	L	13	171	x	217	76	x	44	x	276	193	76	38	x	159	x	160	26
	M	x	582	340	28	68	x	558	121	x	143	17	x	12	121	27	124	66
	N	232	x	x	210	109	x	81	100	20	86	93	63	x	142	49	98	45
	P	x	x	x	822	x	33	x	x	x	x	x	x	29	x	x	36	x
	Q	x	x	x	148	108	x	110	121	x	80	53	33	x	66	x	197	x
	R	422	x	x	100	x	x	85	56	x	100	x	52	41	126	100	129	101
	S	100	221	x	340	90	100	147	127	x	113	87	40	104	116	10	102	57
	T	247	974	x	168	109	218	109	100	8	100	68	36	19	178	82	54	100
	V	23	100	x	317	85	121	100	85	x	326	110	45	90	100	x	141	74
	W	x	x	x	38	x	x	80	76	x	x	47	x	x	180	x	156	x
	Y	64	0	x	222	576	x	136	94	100	31	100	89	55	181	x	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.

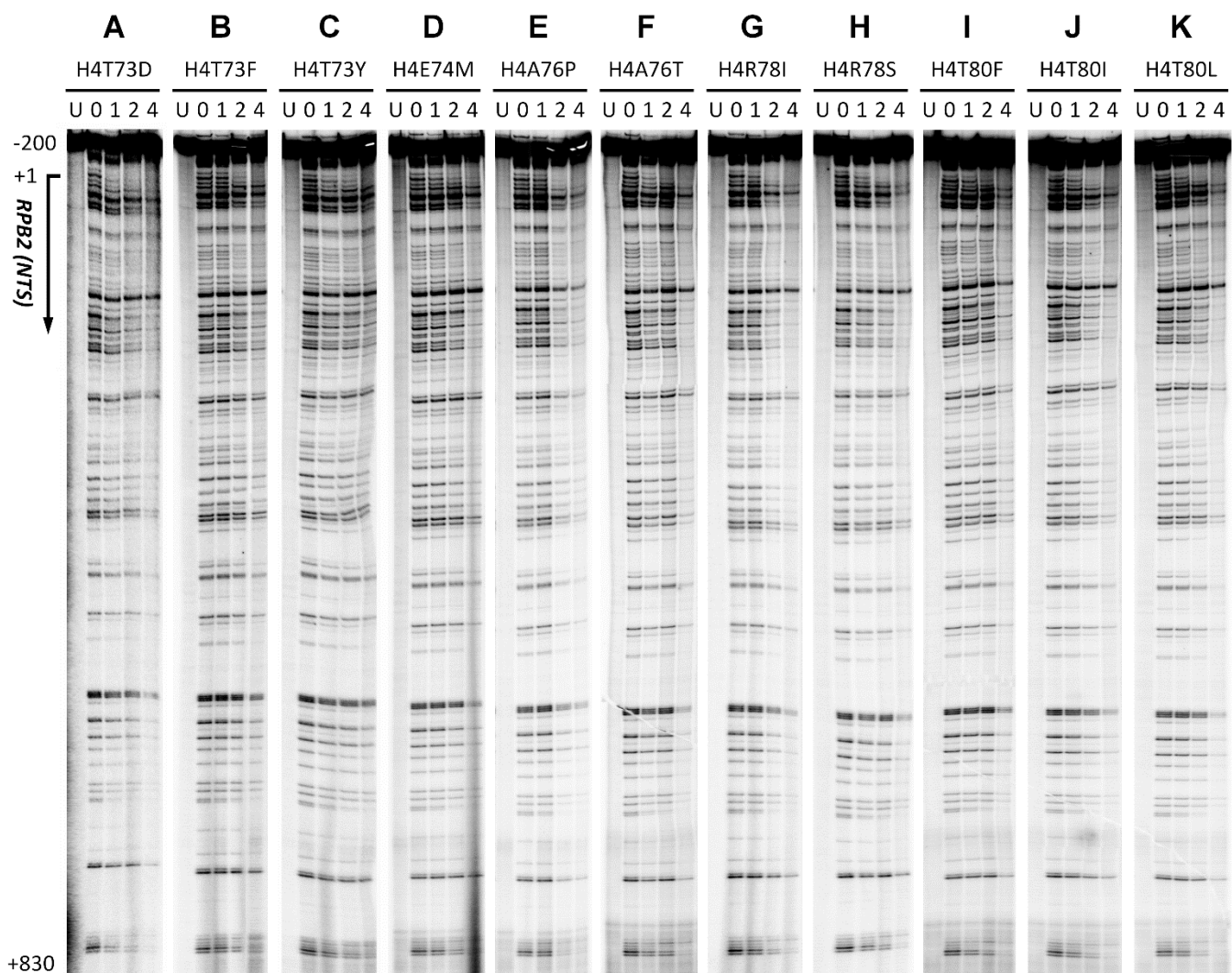
Supplementary Table S2. Summary for confirmation of UV sensitivity (S) or resistance (R) of histone H4 mutants (fold change relative to wild type cells)^a.

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 ^b	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 ^c	3 (S)	T80F	10 (S)
T71I	2 (R)	T80I	5 (S)
Y72T	5 (S)	T80L	5 (S)

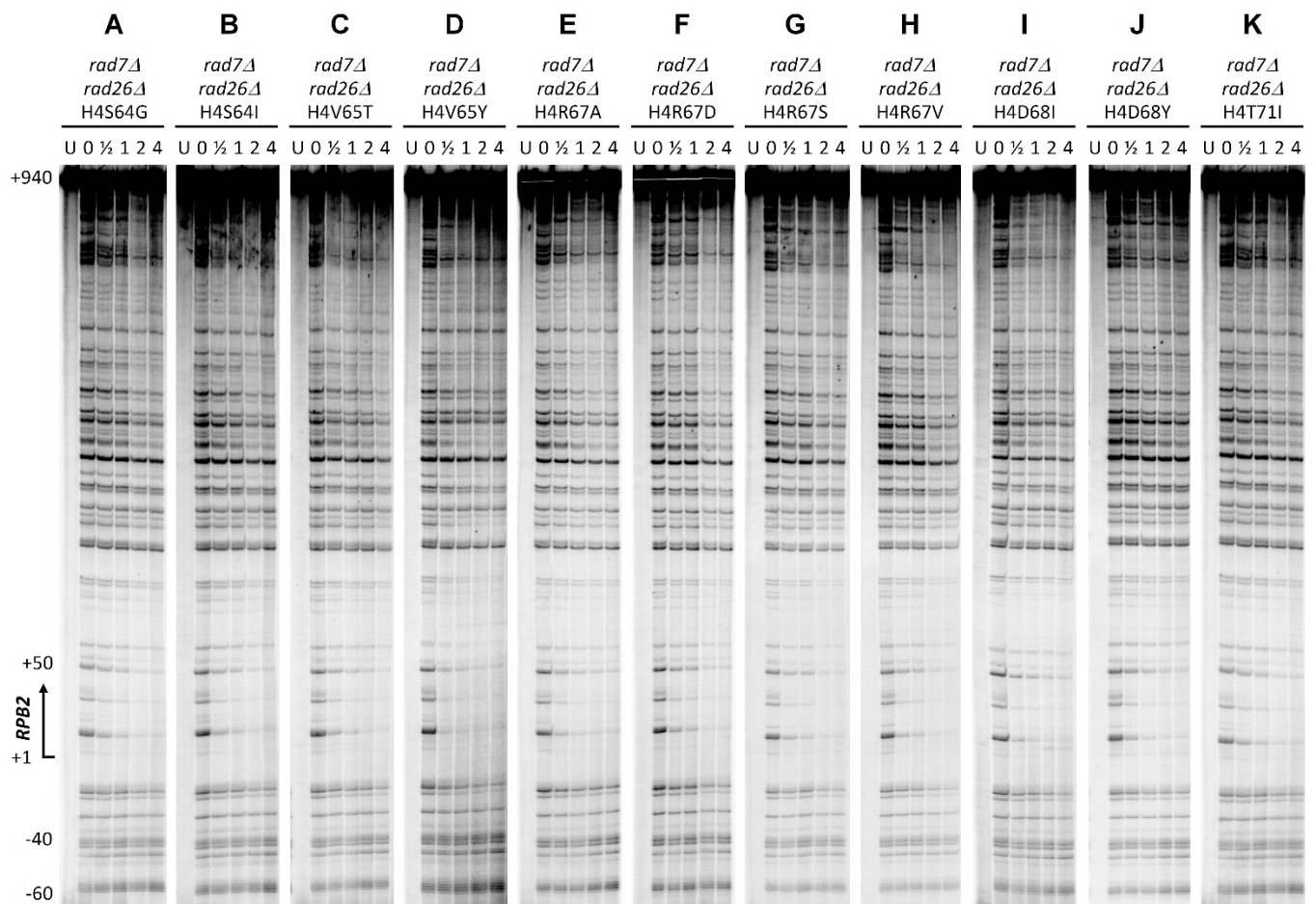
^a The fold changes shown are at 120 J/m² of UV and the averages of at least 3 independent experiments. ^b The screening (Supplementary Table S1) indicated that this mutation was UV sensitive. ^c The screening (Supplementary Table S1) indicated that this mutation was UV resistant.



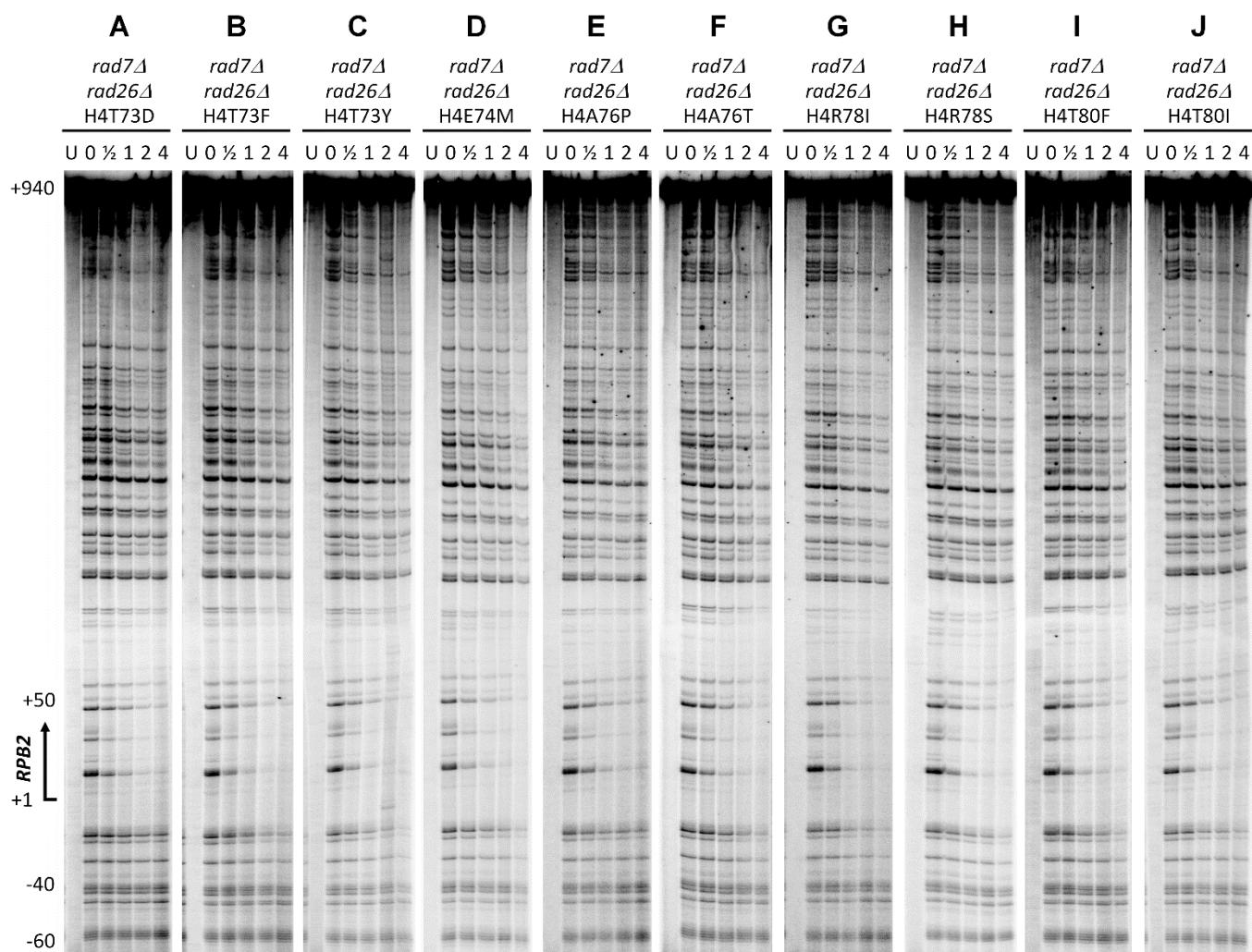
Supplementary Fig. S1. Sequencing gels showing CPDs remaining in the nontranscribed strand of the *RPB2* genes in cells with the indicated histone H4 mutations. The times (h) of repair incubation are indicated at the top. 'U' indicates samples from unirradiated cells. Approximate nucleotide positions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



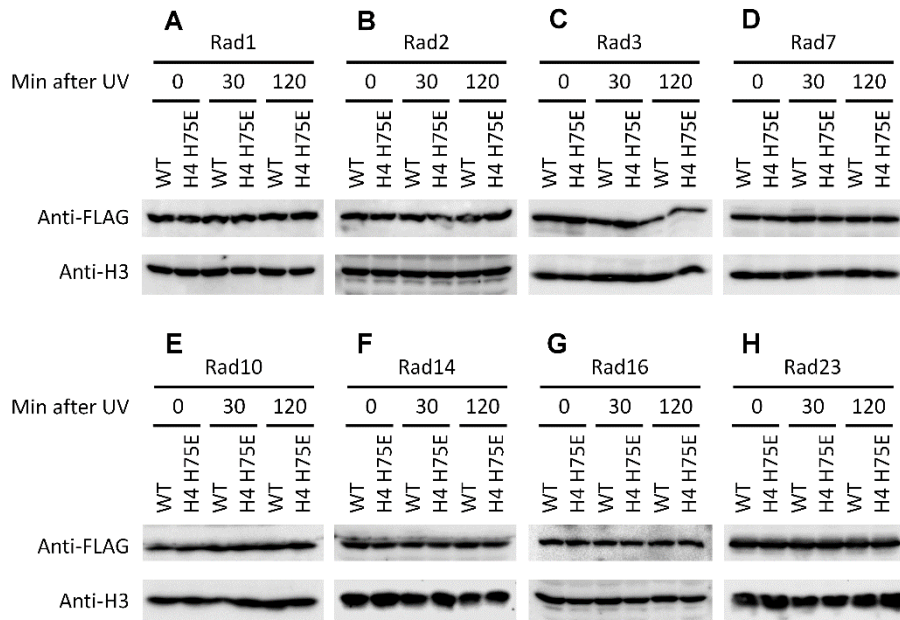
Supplementary Fig. S2. Sequencing gels showing CPDs remaining in the nontranscribed strand of the *RPB2* genes in cells with the indicated histone H4 mutations. The times (h) of repair incubation are indicated at the top. 'U' indicates samples from unirradiated cells. Approximate nucleotide positions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



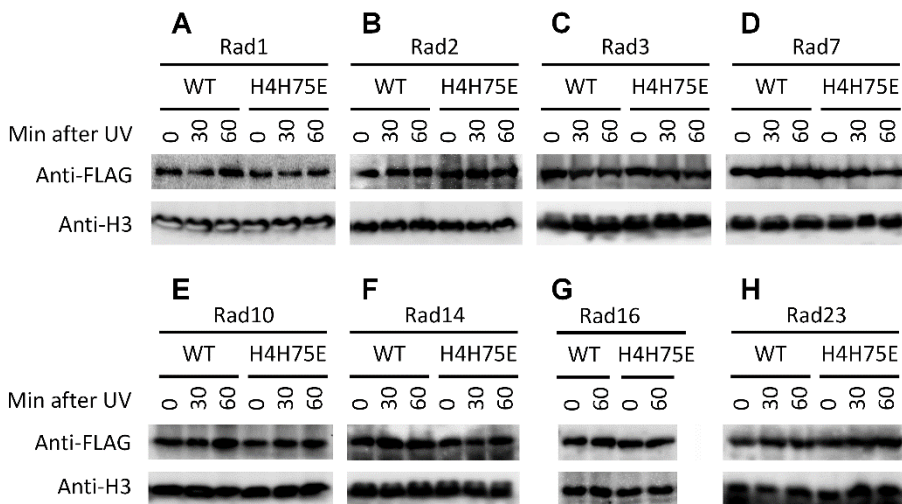
Supplementary Fig. S3. Sequencing gels showing CPDs remaining in the transcribed strand of the *RPB2* genes in *rad7Δ rad26Δ* cells with the indicated histone H4 mutations. The times (h) of repair incubation are indicated at the top. 'U' indicates samples from unirradiated cells. Approximate nucleotide positions relative to the transcription start site (+1) of the *RPB2* gene are indicated on the left.



Supplementary Fig. S4. Sequencing gels showing CPDs remaining in the transcribed strand of the *RPB2* genes in *rad7Δ rad26Δ* 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.



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



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 3xFLAG tagged NER proteins. Histone H3 serves as loading control.