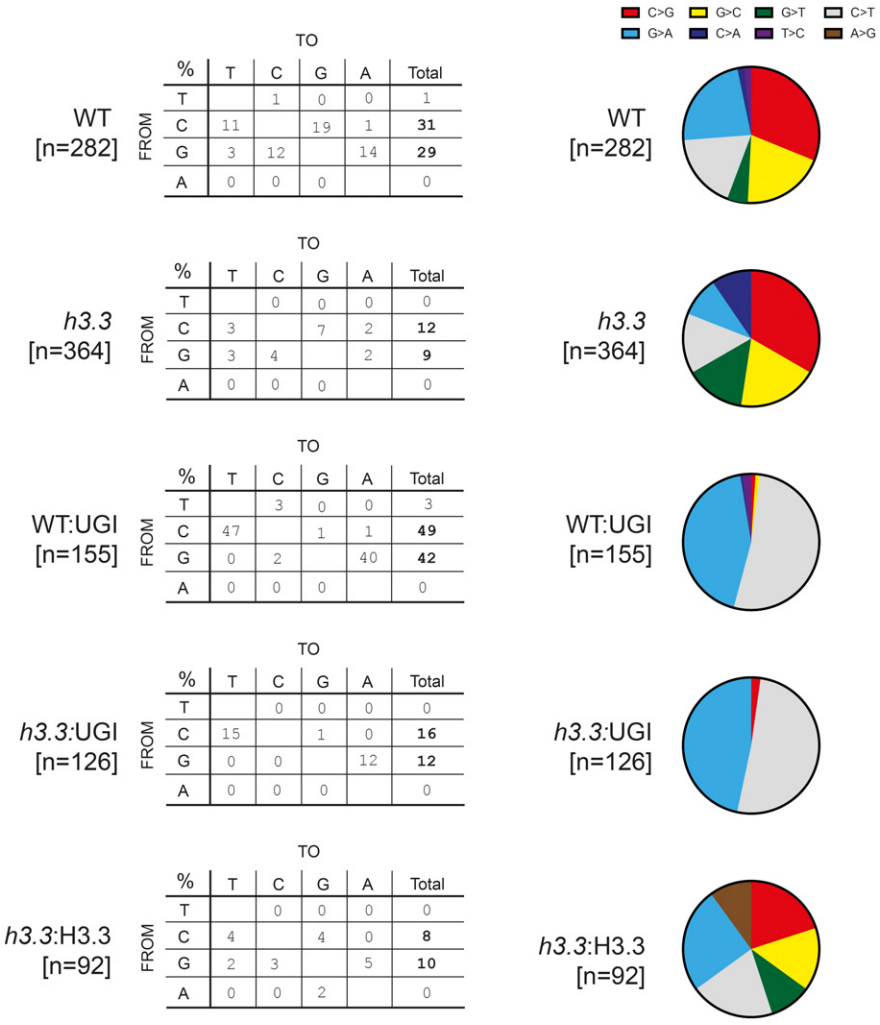
Expanded View Figures



WT [n=282]	%	T	C	G	A	Total
FROM	T		1	0	0	1
	C	11		19	1	**31**
	G	3	12		14	**29**
	A	0	0	0		0

h3.3 [n=364]	%	T	C	G	A	Total
FROM	T		0	0	0	0
	C	3		7	2	**12**
	G	3	4		2	**9**
	A	0	0	0		0

WT:UGI [n=155]	%	T	C	G	A	Total
FROM	T		3	0	0	3
	C	47		1	1	**49**
	G	0	2		40	**42**
	A	0	0	0		0

h3.3:UGI [n=126]	%	T	C	G	A	Total
FROM	T		0	0	0	0
	C	15		1	0	**16**
	G	0	0		12	**12**
	A	0	0	0		0

h3.3:H3.3 [n=92]	%	T	C	G	A	Total
FROM	T		0	0	0	0
	C	4		4	0	**8**
	G	2	3		5	**10**
	A	0	0	2		0

Figure EV1. The pattern of Ig point mutation is not affected by loss of H3.3.
Tables showing the pattern of non-templated point mutations in each cell line presented as absolute numbers in the table and as a proportion in the adjacent colour-coded pie chart. *n* gives the number of sequencing reads analysed.

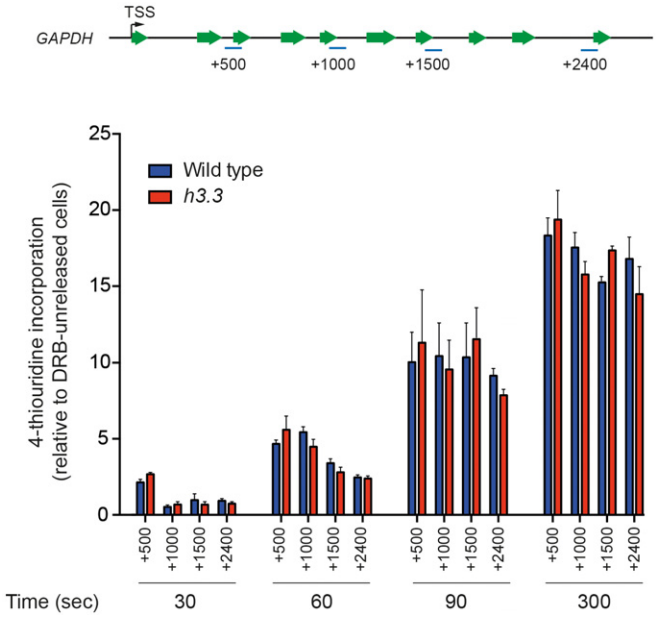


Figure EV2. Kinetics of transcription along the *GAPDH* locus measured by 4sUDRB.
4sUDRB mapping of transcription kinetics through the *GAPDH* locus. Representative locations of qPCR amplicons (blue) are shown relative to the *GAPDH* gene, and their distance from the TSS shown in parenthesis. qPCR signals are normalised to the enrichment at time zero (before release). Error bars represent ± SD of qPCR triplicates.

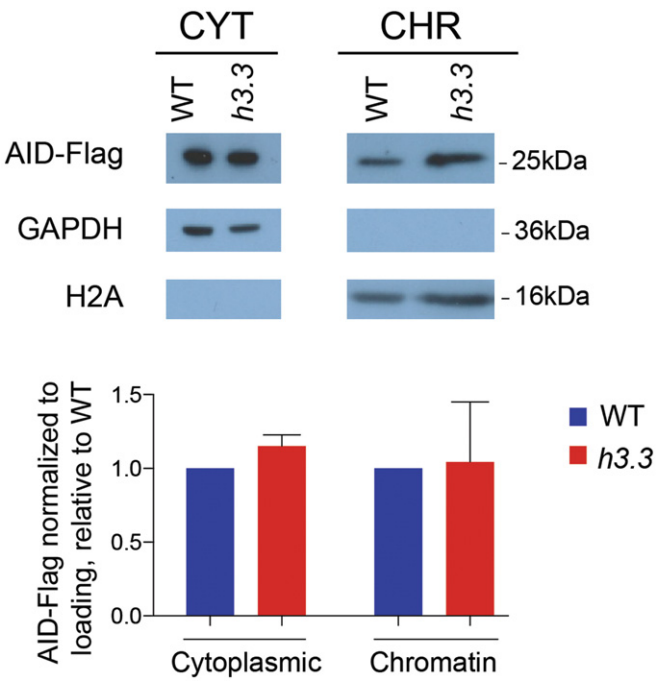


Figure EV3. The subcellular distribution of AID is not influenced by H3.3.
The subcellular localisation of AID is not grossly altered in the absence of H3.3. Detection of $hAID^{up}$ in cytoplasmic and chromatin fractions of WT and *h3.3* cells that overexpress $hAID^{up}$ to around 30 times the WT AID level. GAPDH is used as a cytoplasm-specific control and H2A as a chromatin-specific control. The graphs show the quantification of two biological repeats with ± SD.

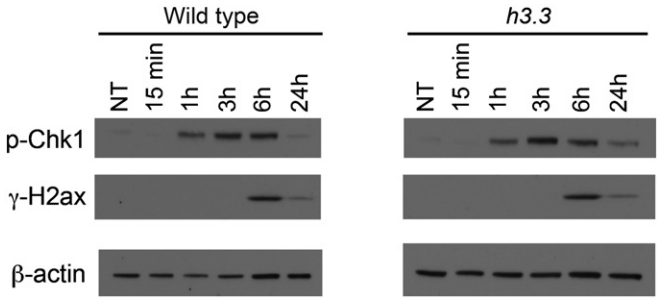


Figure EV4. The kinetics of CHK1 and H2Ax phosphorylation following exposure to UV light is not altered by the absence of H3.3.
Phospho-Chk1(S345) and γH2Ax, detected by Western blot in total protein extract of WT and *h3.3* cells, at the indicated time points post-UVC irradiation (265 nm at 8 J/m^2). β-actin is shown as a loading control.

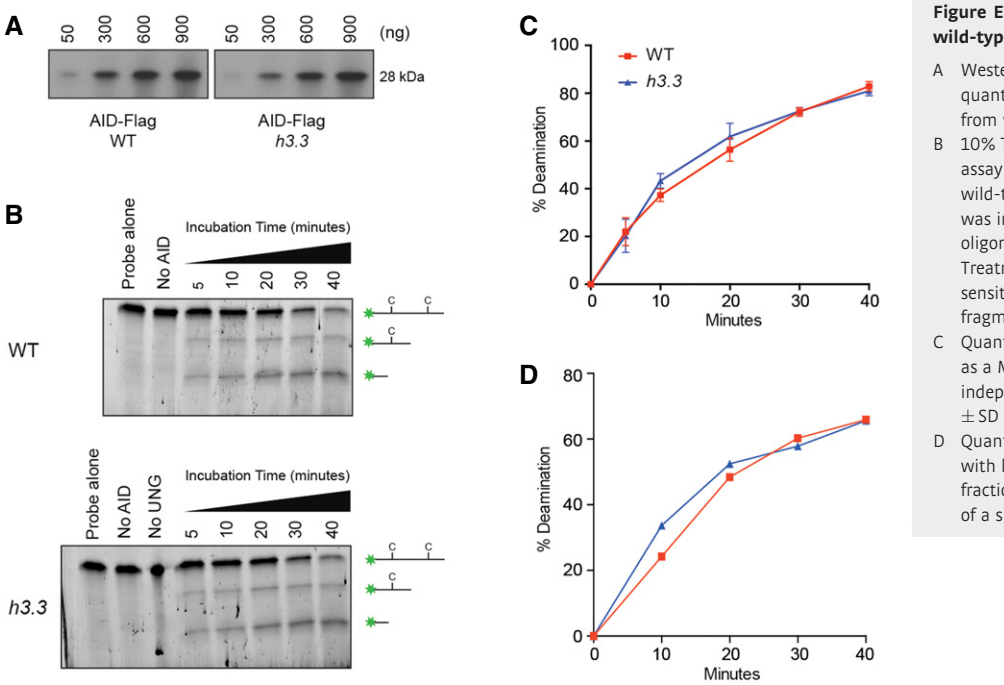


Figure EV5. hAID^{up} protein pulled down from wild-type and *h3.3* cells is equally active.

A Western blot confirmation of colorimetric quantification of hAID^{up} protein pulled down from wild-type and *h3.3* cells.

B 10% TBE-urea gel image of *in vitro* deamination assay with hAID^{up} protein pulled down from wild-type and *h3.3* cells. About 600 ng of hAID^{up} was incubated with fluorescein-labelled oligonucleotides containing two dC bases. Treatment with UNG renders deaminated sites sensitive to cleavage in alkali and the resulting fragments are visualised on the gel.

C Quantification of the deamination assay shown as a Michaelis–Menten graph of two independent experiments. Error bars represent ± SD of two independent experiments.

D Quantification of the deamination assay done with hAID^{up} pulled down from the total nuclear fraction, shown as a Michaelis–Menten analysis of a single experiment.

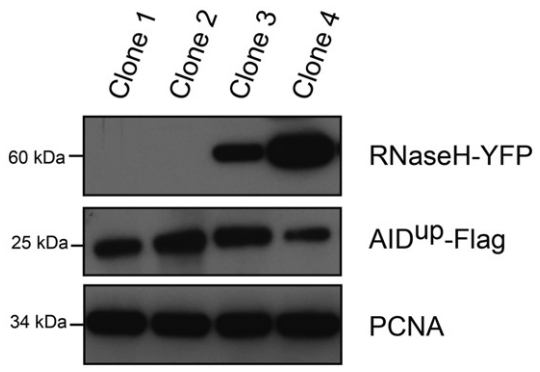


Figure EV6. Expression of RNase HI in DT40 cells.
Western blot for expression of hAID^{up}-FLAG (anti-FLAG) and chicken RNase HI-YFP (anti-YFP) in the four DT40 clones studied in Fig 5C. PCNA provides a loading control.