Supplementary Material

Mutational analysis of the damage-recognition and catalytic mechanism of human SMUG1 DNA glycosylase

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Table S1. Oligonucleotide primers used to generate hSMUG1 mutants[a]

Primer	Mutation	Sequence (5' →3')[b]
T1-N85A	Asn85Ala	CCAAAAGGTCCAGGG***GC***CATGCCCAGGAAGAG
T1-G87A	Gly87Ala	CCATGCCAAAAGGT***G***CAGGGTTCATGCCC
T1-G87S	Gly87Ser	GGCCATGCCAAAAGG***GC******T***AGGGTTCATGCCCAGG
T1-G87F	Gly87Phe	GGGCCATGCCAAAAGG***AAA***AGGGTTCATGCCCAGGAAG
T1-F89G	Phe89Gly	CTGGGCCATGCCA***CC***AGGTCCAGGGTTC
T1-F89A	Phe89Ala	CTGGGCCATGCCA***GC***AGGTCCAGGGTTC
T1-F89S	Phe89Ser	CTGGGCCATGCCA***G***AAGGTCCAGGG
T1-G90A	Gly90Ala	CAGTCTGGGCCATG***G***CAAAAGGTGCAGGG
T1-M91A	Met91Ala	CCCCAGTCTGGGCC***GC***GCCAAAAGGTCCAG
T1-F98H	Phe98His	TGCTTACTTCCCC***ATG***GGG**T**ACCCCAGTCTGGGCC
T1-F98L	Phe98Leu	TGCTTACTTCCCC**C**A**G**GGG**T**ACCCCAGTCTGGGCC
T1-N163D	Asn163Asp	GCAGAGGGCGGCATAGAT***C***GTGGACAAAACAGTGATG
T1-H239N	His239Asn	CGGGGAGAGGGAT***T***CAGGAGCCCTTCC
T1-H239L	His239Leu	GGTTACGGGGAGAGGGC***AG***CAGGAGCCCTTCCAC

[a] Only the sequence of the sense-strand primer is shown. For site-directed mutagenesis, a set of sense-strand and antisense-strand primers were used.

[b] Italics indicate the substituted bases, and underlining indicates the mutagenized codon. In Phe98Leu and Phe98His mutants, an additional synonymous codon change (G**C**A→G**T**A, indicated in bold) was introduced to facilitate the screening of mutants.

Table S2. Kinetic parameters of selected hSMUG1 mutants for excision of hmU:G[a]

Mutant	k_{cat} (min^{-1})	K_m (nM)	k_{cat}/K_m ($min^{-1}.nM^{-1}$)	Relative k_{cat}/K_m (%)
Wild-type	10	120	0.083	100
N85A	0.85	300	0.0028	3.3
H239L	0.00077	760	0.0000010	0.012
N163D	0.24	320	0.00075	0.90

[a] Wild-type and mutant hSMUG1s were incubated with varying concentrations of 25HMU/G at 37 °C. After incubation, the sample was treated with 0.1 M NaOH, and nicked products were quantified by PAGE analysis. The concentration of the substrate, the amount of enzyme, and incubation time were adjusted to appropriate ranges depending on the enzyme activity such that the yield of products was linearly related to the amount of protein and incubation time. The maximum substrate concentration and incubation time, and the amount of enzyme were following: wild-type (500 nM, 5 min, 0.2 ng), N85A (1 µM, 10 min, 5 ng), H239L (2.5 µM, 60 min, 100 ng), and N163D (1 µM, 15 min, 1 ng).

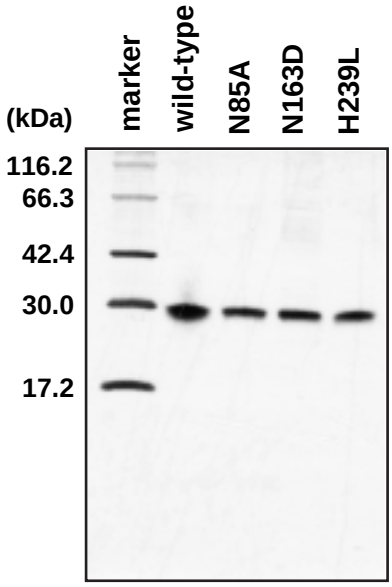

Figure S1. SDS-PAGE analysis of the wild-type and selected mutant hSMUG1 proteins.

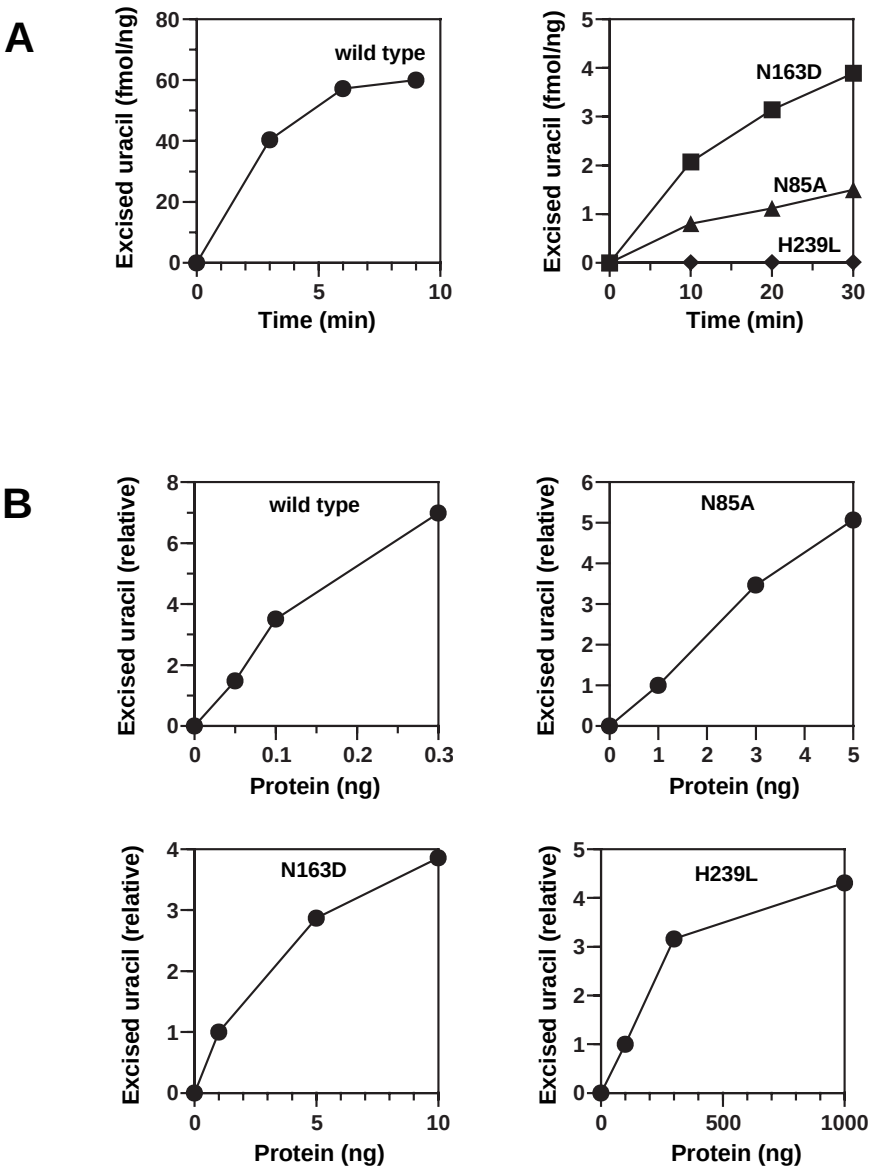

Figure S2. Typical data for the variation of the yield of excised uracil with incubation time and the amount of hSMUG1. 19U/G were incubated with wild-type or indicated mutant hSMUG1. (**A**) Relationship between incubation time and the yield of excised uracil. The amount of protein used were 0.1 ng (wild-type), 1 ng (N163D), 2 ng (N85A), and 100 ng (H239L) (**B**) Relationship between the amount of hSMUG1 and the yield of excised uracil.

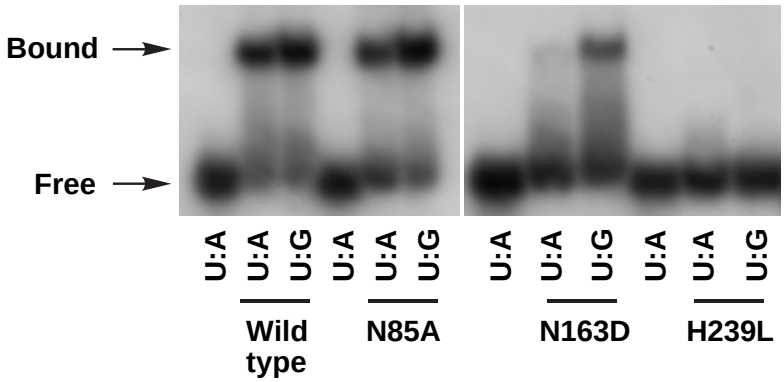

Figure S3. Gel electrophoretic mobility shift assays of the binding of the wild-type and mutant hSMUG1 proteins to 19U/A (U:A) and 19U/G (U:G). The assays were performed as described in MATERIALS AND METHODS.