

**Supporting Information**

**A non-canonical role for the DNA glycosylase NEIL3 in suppressing  
APE1 endonuclease-mediated ssDNA damage**

Anh Ha, Yunfeng Lin, and Shan Yan\*

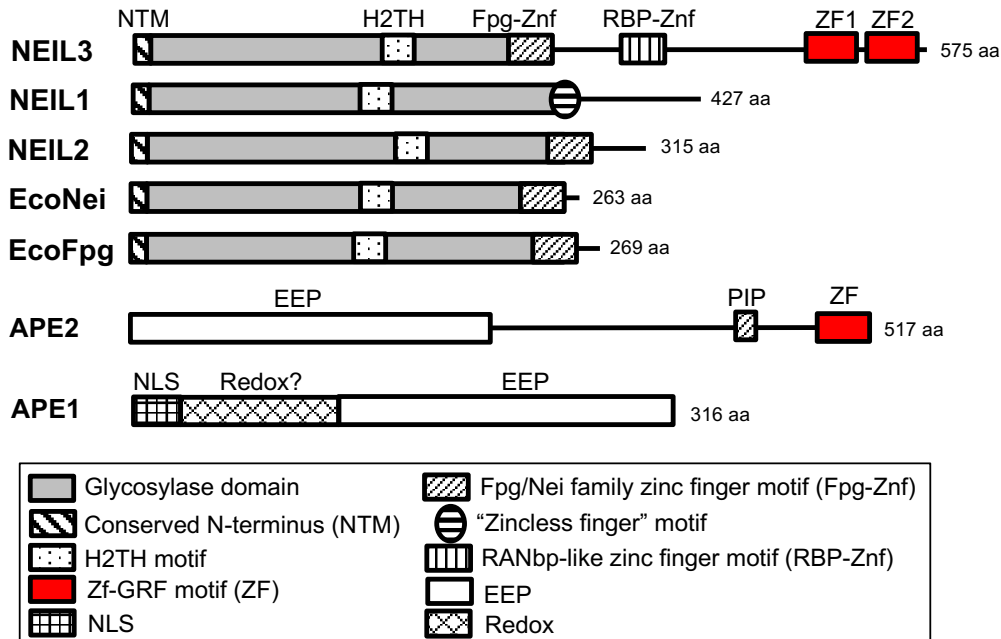
From the Department of Biological Sciences, University of North Carolina at Charlotte, Charlotte, NC  
28223, USA

\*To whom correspondence should be addressed: Shan Yan

Tel: 704-687-8528; E-mail: [shan.yan@uncc.edu](mailto:shan.yan@uncc.edu)

Supplementary figures and tables

**A**



**B**

Xl NEIL3-ZF1 (aa 484-527) PQC**S**AHNVPCALQVVRKEGENK**GRS**F**Y**TC**S**LP**R**ERR**C**Q**Y**FEWAD  
Xt NEIL3-ZF1 (aa 483-526) PNC**S**THNVPCALQVVRKEGENK**GRS**F**Y**TC**S**LP**R**ERR**C**Q**F**FEWAD  
Ms NEIL3-ZF1 (aa 506-549) PL**C**K**M**H**H**RR**C**VL**R**V**R**RD**G**ENK**GR****Q**F**Y**AC**S**L**P**RG**A**Q**C**G**F**FEWAD  
Hs NEIL3-ZF1 (aa 505-548) P**R****C**S**K**H**N**RL**C**IL**R**V**V**RD**G**ENK**GR****Q**F**Y**AC**P**L**P**RE**A**Q**C**G**F**FEWAD  
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Xl NEIL3-ZF2 (aa 531-575) P**F****C****N**-**H**G**K**R**C**I**V**R**T**V**L**K**I**G**P**N**N**G**K****N**F**Y****V****C**P**M**G**K**D**K**Q**C**N**F**F**E**W**A**K**T**E  
Xt NEIL3-ZF2 (aa 530-574) P**F****C****N**-**H**G**K**R**C**I**V**R**T**V**L**K**L**G**P**N**N**G**K****N**F**Y****V****C**P**M**G**K**D**K**Q**C**N**F**F**E**W**A**K**T**E  
Ms NEIL3-ZF2 (aa 553-597) P**F****C****R**-**H**G**K**R**S**I**M**K**T**V**L**K**I**G**P**N**N**G**K****N**F**F****V****C**P**L**E**K**K**Q**C**N**F**F**Q**W**A**E**N**G**  
Hs NEIL3-ZF2 (aa 552-596) P**F****C****N**-**H**G**K**R**S**T**M**K**T**V**L**K**I**G**P**N**N**G**K****N**F**F****V****C**P**L**G**K**E**K**Q**C**N**F**F**Q**W**A**E**N**G  
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**C**

NEIL3-ZF1 (aa 471-527) P**Q**S**K**M**T**-**G**D**A**A**A**K**T**G**N**P**Q****C****S**A**H**N**V**P**C**A**L**Q**V**V**R**K**E**G**E**N**K****GR****S****F****Y****T****C****S**L**P**R**-----**ERR**C**Q**Y**FEW**A**D**-----**  
NEIL3-ZF2 (aa 531-575) -----**P****F****C**-**N****H**G**K**R**C**I**V**R**T**V**L**K**I**G**P**N**N**G**K****N**F**Y****V****C**P**M**G**K**-----**D**K**C**N**F**F**E**W**A**K**T**E**-----**  
APE2-Zf-GRF (aa 445-517) [**P**Q**T**A**F**W**K**S**L**L**K**G**P**P**P**P**P****N**C**K**G**H**S**E**P**C**V**L**R**T**V**K**K**A**G**P**N**C****GR****Q**F**Y****V****C**A**R**P**E**G**H**S**S**N**P**Q**A**R**C**N**F**F**L**W**L**T**K**K**A**G**C**E**D**  
α 1 helix Pro helix \* \* . \* : \* \* \* \* \* : \* \* \* \* \* . : : \* \* \* \* \*

**Figure S1. NEIL3 unique structure comparison among the Fpg/Nei family members.** *A*, Schematic diagram of NEIL3 (endonuclease 8-like 3, *Xenopus laevis*, 575aa, XP\_018086207.1), NEIL1 (endonuclease 8-like 1, *Xenopus laevis*, 427aa, XP\_018108460.1), NEIL2 (endonuclease 8-like 2, *Xenopus laevis*, 315 aa, XP\_018119591.1), EcoNei (nei, *Escherichia coli* O104:H4 str. C227-11, 263aa, EGT66846.1), EcoFpg (DNA-formamidopyrimidine glycosylase, *Escherichia coli* str. K-12 substr. MG1655, 269aa, NP\_418092.1), APE2 (apurinic/aprimidinic endodeoxyribonuclease 2L homeolog, *Xenopus laevis*, 517aa, AAH77433.1), and APE1 (apurinic/aprimidinic endonuclease 1, *Xenopus laevis*, 316aa, AAH72056). *B*, Sequence alignment of NEIL3-ZF1 and NEIL3-ZF2 in *Xenopus laevis* (Xl) (XP\_018086207.1), *Xenopus tropicalis* (Xt) (NP\_001017201.1), *Mus musculus* (Ms) (NP\_666320.1) and *Homo sapiens* (Hs) (NP\_060718.2) using Clustal Omega software. *C*, Amino acid sequence alignment of NEIL3-ZF1, NEIL3-ZF2, and APE2-Zf-GRF (AAH77433.1) in *Xenopus laevis* using Clustal Omega software.

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Xl NEIL3 MVEGPGCTLNGEKIRARVQKGQCVVLELRGSVSAK-----APA 38
Xt NEIL3 MVEGPGCTLNGEKIRARVEKQSVVLELRGSVSGT-----PPS 38
Ms NEIL3 MVEGPGCTLNGEKIRARVLPQAVTGVRTALQSLGPAASPAASLADVATSAAPMNAKD 60
Hs NEIL3 MVEGPGCTLNGEKIRARVLPQAVTGVRSALRSLQGRALRLAASVTVVSPQAAALN-ND 59
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Xl NEIL3 SSHNDLSSLTGCSYAGVETLKGKELFYFGLKAMRVHFGMNGSMRINQPMKKQENGRPIP 98
Xt NEIL3 SCYNALSSLTGCSYTGVTGKELFMYFGLKAIKRVHFGMNGSIRLNQAVKKGQENSRPMP 98
Ms NEIL3 SGWKLRLRFNGYVYSGVETLKGKELFMYFGPRALRIHFGMKGSIILINPREGENRAG----A 116
Hs NEIL3 SSQNVLSLFGYVYSGVETLKGKELFMYFGPKALRIHFGMKGFIIMINPLEYKYKNG----A 115
* : * :.* * :*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:

Xl NEIL3 IAVLEVQLTKDLICFYESTVDVRNASECQEKIRFFEELDVCSKFSFPRAECEIKKQTR 158
Xt NEIL3 VAVLEVQLEKDLICFYESTVDVRNVSECEKIRFLEELDVCSKFSFSAEICEIKKQKAR 158
Ms NEIL3 SPALAVQLTRDLICFYDSSVELRNSVESQQRVVRVMEELDICSFKFSFSAEISEVKKQGR 176
Hs NEIL3 SPVLEVQLTKDLICFFDSSVELRNSMESQQRIRMMKELDVCSPEFSLRAESEVKKQKGR 175
.* ** :*:*:*:*:*:*:*. *.:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*.

Xl NEIL3 MLCDILLDQMLPGVGNIIKNEALFDSGLHPGVQAGLLTDEQVSHLVKMTDRDFTLLFYKC 218
Xt NEIL3 MLCDILLDQMLPGVGNIIKNEALFDSGLHPGVQAGLLTDMQVSHLVKMTDRDFTLLFYKC 218
Ms NEIL3 MLCDVLLDQVRLPGVGNIIKNEALFDSGLHPAVKVCQLSDKQACHLVKMTDRDFTLLFYRC 236
Hs NEIL3 MLGDVLLDQVRLPGVGNIIKNEALFDSGLHPAVKVCQLTDEQIHHLKMKMIRDFSLFYRC 235
** *.:*:*. :*:*:*:*:*:*:*:*:*:*:*. :.* * **:*:*. :*:*:*.

Xl NEIL3 RKSGSALYKHYKVKYKRPNCGQCGKITVCRLEHNRMTYFCPKCQKDKPQHVDVSKLPTR 278
Xt NEIL3 RKSGSALYKHYKVKYKRPNCGQCDKKITVCRLEGNRMTYFCPKCQNDKPQHVDVSKLPKR 278
Ms NEIL3 CKAGSAISKHCKVYKRPNCQCHSKITVCRFGENSRMTYFCPHCQKENPQCVQVQCLPTR 296
Hs NEIL3 RKAGLALSXYKVKYKRPNCGQCHCRITVCRFGDNNRMTYFCPHCQKENPQHVDICKLPTR 295
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Xl NEIL3 NSLIGWVQRTASNANEHVATSKEEHWACAVCTLINKPSDKQCDACLTLRPEVSSLAVSDE 338
Xt NEIL3 NSLIGWVQRIPPHSNEHVATSKEEHWACTVCTLINKPSDQCDACLTLRPEVSSLAESDK 338
Ms NEIL3 NTEISWTPRGEDCFTDSVARKSEEQWCVVCTLINKRPSAKACDACTLTPRPLDSVLKN-RE 355
Hs NEIL3 NTIISWTSRVDHVMDSVARKSEEHWTVCVCTLINKPSSKACDACTLRPIDSVLKS-EE 354
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Xl NEIL3 AAELNTDLVKYPCNNFAKVLPELKLNRRTAFGNTTLVLTDFGAKGLADKNSQQNILNRS 398
Xt NEIL3 AAELDADLVKYPNNFAKLLPELKLNRRTAFGNTTLVLTDFGAKEDLSDKNIQQHSLNRS 398
Ms NEIL3 NSIAFNLVKYPNNFENTHTEVKINRRTAFGNTTLVLTDLNKSALARKKRANHTIDG 415
Hs NEIL3 NSTVFSHLMKYPNCTFGKPHTEVKINRRTAFGNTTLVLTDFSNKSSTLERKTKQNQILDE 414
: . :*:*:*. : *.:*:*:*. :*:*:*. :*:*:*. :*:*:*. :*:*:*. :*:*:*.

Xl NEIL3 TFD--VPLNNKYHTKTPSNKRSNENEHWTNTLNAVNGHSAASNNVFNHPQKCLKTGHT 456
Xt NEIL3 TFD--VPLSKHF-NGKNSNKRSNENEHWTNTLNAVNGSGTSNNVFNHPQKCLKTVHT 455
Ms NEIL3 ESQMFLPTDIGFSDSQHPSKEGINIYITQPSNKVNI-SPTVCAQSKLFSAHKKFKPAHTS 474
Hs NEIL3 EFQNSPPASVCLNDIQHPSKKTNDITQPSKVNII-SPTISSESKLFSAPHKKPKTAQYS 473
: * . : :*:*. : * : :*:*. : :*:*. : :*:*. : :*:*. :*:*. :*:*.

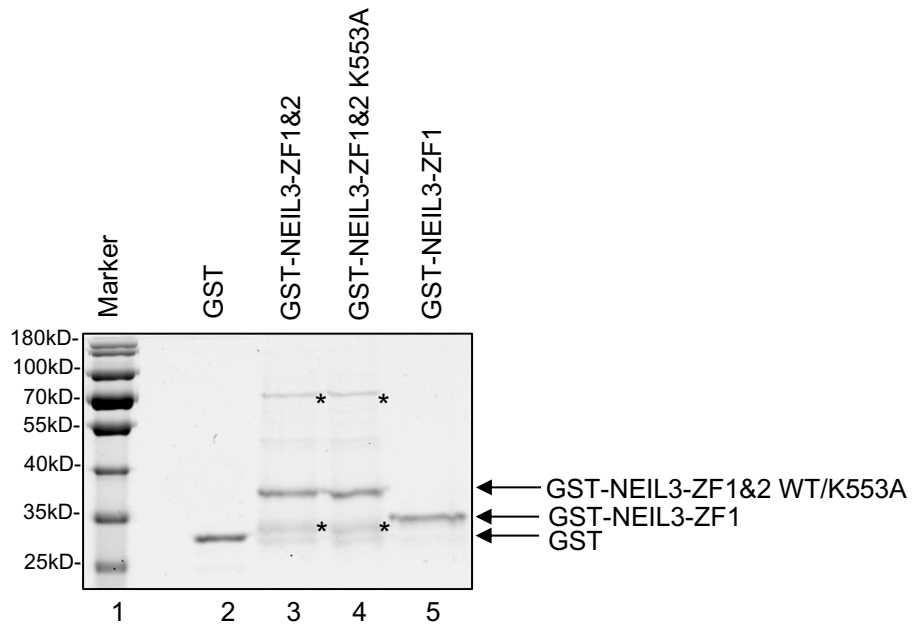
Xl NEIL3 SNTIHL-SSTIS--SPQS-KMTGDAAAKTGNPQCSAHNVPCALQVVRKEGENKGRSFYTC 512
Xt NEIL3 PNKTNI-RSTIS--SSQS-TMADAANTGGPNCSTHNVPALQVVRKEGENKGRSFYTC 511
Ms NEIL3 ATELKSYNSGLSNSELQTNRTRGHHSKSDGSPKCMHHRRCVLRVVRKDGENKGRQFYAC 534
Hs NEIL3 SPELKS CNPGYSNSELQINMTDGPRTLNDSPKCSKHNRCLILRVVRKDGENKGRQFYAC 533
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Xl NEIL3 SLPRERRCQYFEWADLHFPFCNHGKRCIVRTVLKIGPNNGNFYVCPMGKDKQCNEFFEWA 572
Xt NEIL3 SLPRERRCQYFEWADLHFPFCNHGKRCIVRTVLKIGPNNGNFYVCPMGKDKQCNEFFEWA 571
Ms NEIL3 SLPRGAQCQYFEWADLHFPFCNHGKRSIMKTVLKIGPNNGNFYVCPLEKKKQCNEFFQWA 594
Hs NEIL3 PLPREAQCGYFEWADLHFPFCNHGKRSTMKTVLKIGPNNGNFYVCPPLGKERQCNEFFQWA 593
** * : * :*:*:*. :*:*:*. :*:*:*. :*:*:*. :*:*:*. :*:*:*. :*:*:*. :*:*:*.

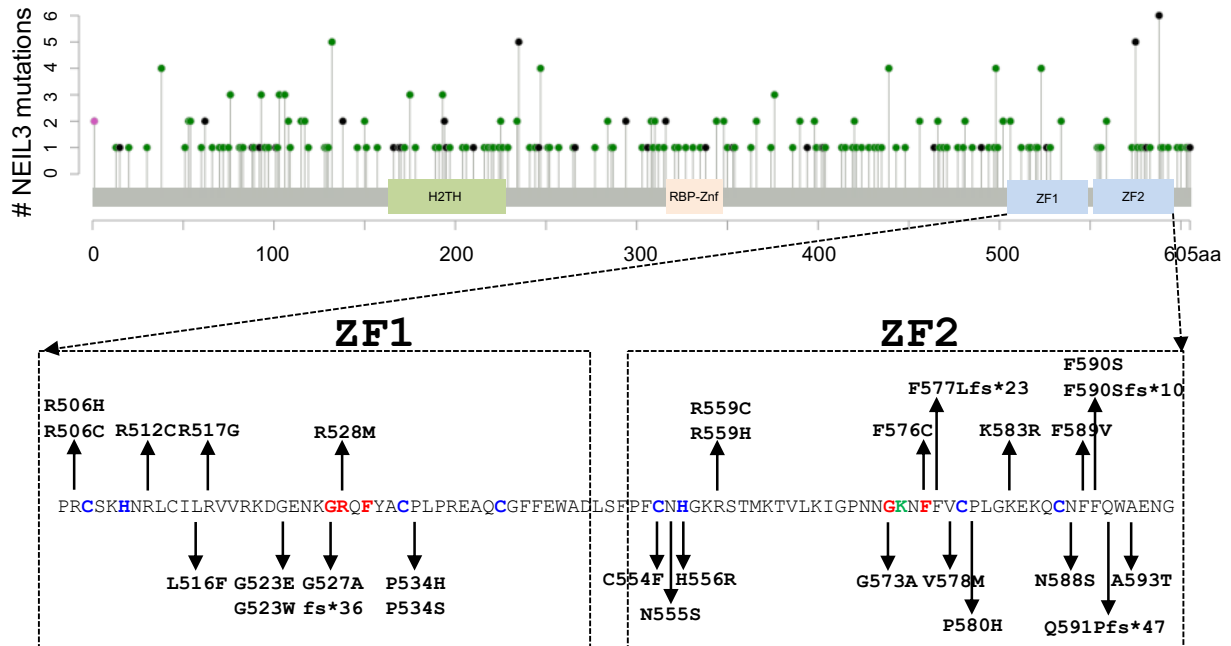
Xl NEIL3 KTE----- 575
Xt NEIL3 KTE----- 574
Ms NEIL3 ENCPGMEIVPGC 606
Hs NEIL3 ENCPGIKIIPGC 605
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**Figure S2. Amino acid alignment of NEIL3 proteins using the Clustal Omega software.** Xl NEIL3 (*Xenopus laevis*, XP\_018086207.1); Xt NEIL3 (*Xenopus tropicalis*, NP\_001017201.1); Ms NEIL3 (*Mus musculus*, NP\_666320.1); Hs NEIL3 (*Homo sapiens*, NP\_060718.2). -, gaps in the alignment; \* indicates identical residues; : indicates highly conserved residues; . indicates moderately conserved residues. ZF1 and ZF2 are highlighted with red/green box.



**Figure S3. Verification of GST-NEIL3-ZF1&2, GST-NEIL3-ZF1&2 K553A, and GST-NEIL3-ZF1.** Isolated GST-NEIL3-ZF1&2, GST-NEIL3-ZF1&2 K553A, and GST-NEIL3-ZF1 with GST as control were examined via PAGE using Coomassie staining (~3.5 $\mu$ g per lane for lane 2-5). \* indicates a couple of unspecific bands or degradation products.



**Figure S4. Cbioportal analysis of human NEIL3 mutations in cancer patients.** A total of 336 mutations events (265 missense, 67 truncating, 4 non-start, and 2 fusion) were found from Cbioportal analysis in a query of 83,622 samples in 80,293 cancer patients from 269 studies on April 16, 2020. The missense mutation and truncating events in the NEIL3 ZF1 and ZF2 region were highlighted.

**Table S1. The sequences of oligos used in this study.**

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**FP#1:** 5'-CCCCCGGATCCCCACAGTGCAGTGCACACAATGTTC-3'  
**FP#2:** 5'-GAAGATTGGCCCAAACAACGGAGCGAATTTTTACGTCTGCCCGATGG-3'  
**FP#3:** 5'-CCCCCGAATTCACCACAGTGCAGTGCACACAATGTTC-3'  
**FP#4:** 5'-CCCCCGAATTCACCATTCTGCAACCATGGGAAAC-3'  
**FP#5:** 5'-GACCCACACATCATGTGTCTCCAGCAAATAAAATGTGCAGAGAAATTG-3'  
**FP#6:** 5'-GAAGCCATTAATACTGTGTGGTAATCTGAATGTGGCGCACCAGG -3'  
**FP#7:** FAM-5'-TGCTCGTCAAGAGTTCGTAA(THF)ATGCCTACACTGGAGATC -3'  
**FP#8:** FAM-5'-TGCTCGTCAAGAGTTCGTAAACATGCCTACACTGGAGATC -3'  
**FP#9:** FAM-5'-TCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATG  
CAAGCTTGGCGTAATCATGGTCATAGCTGT -3'  
**FP#10:** Cy5-5'-TCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATG  
CAAGCTTGGCGTAATCATGGTCATAGCTGT -3'

**RP#1:** 5'-CCCCCCTCGAGCTACTCTGTTTTTGCCCATTC-3'  
**RP#2:** 5'-CCCCCCTCGAGCTAGTCTGCCATTCAAATATTGACAAC-3'  
**RP#3:** 5'-CCATCGGGCAGACGTAAAATTCGCTCCGTTGTTTGGGCCAATCTTC-3'  
**RP#5:** 5'-CAATTTCTCTGCACATTTTATTTGCTGGAGACACATGATGTGTGGGTC -3'  
**RP#6:** 5'-CCTGGTGCGCCACATTCAGATTACCACACAGTATTAATGGCTTC -3'  
**RP#7:** 5'-GATCTCCAGTGTAGGCATCTTACGAACTCTTGACGAGCA -3'

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**Table S2. The sequences of ssDNA with different lengths (10, 20, 40, or 60 nt) with 5'-Biotin label.**

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**Biotin- 10nt:** [Biotin]-5'-GGTCGACTCT-3'  
**Biotin- 20nt:** [Biotin]-5'-GGTCGACTCTAGAGGATCCC-3'  
**Biotin- 40nt:** [Biotin]-5'-GGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTC-3'  
**Biotin- 60nt:** [Biotin]-5'-GGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTC  
ACTGGCCGTCGTTTTACAAC-3'

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