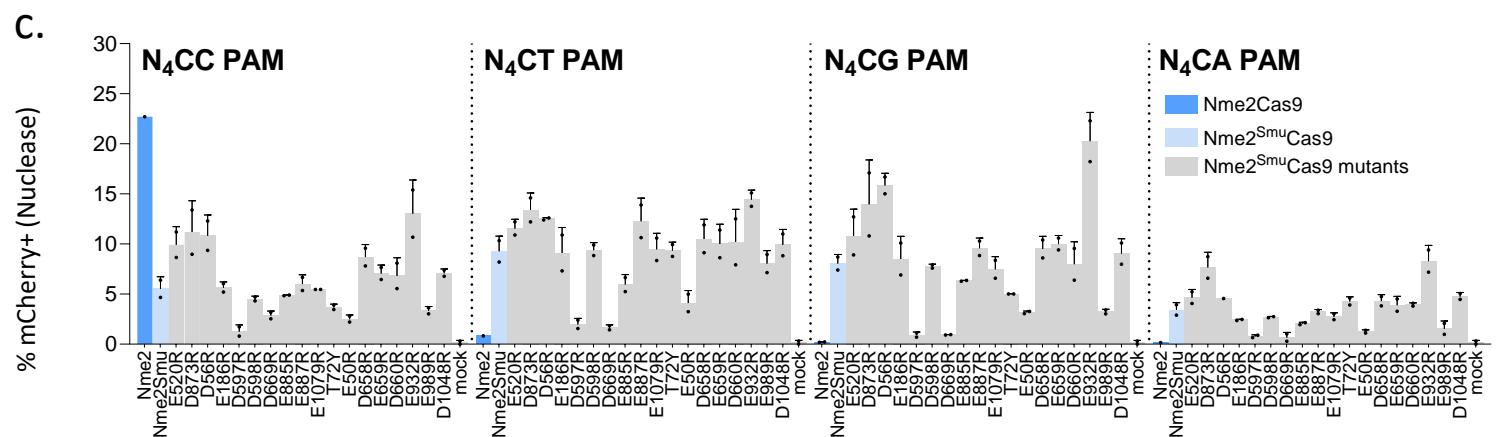
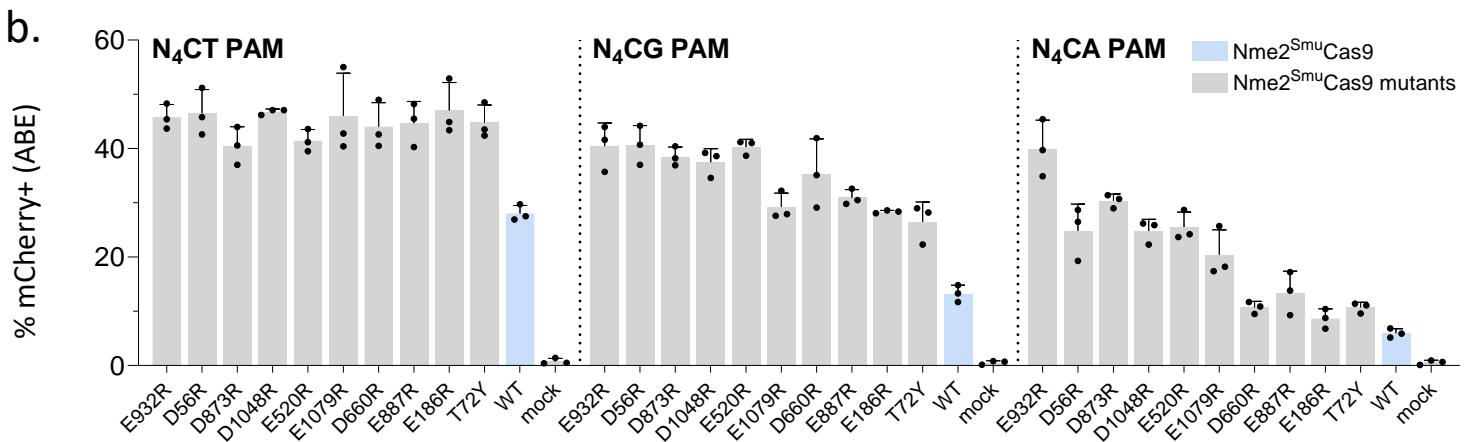
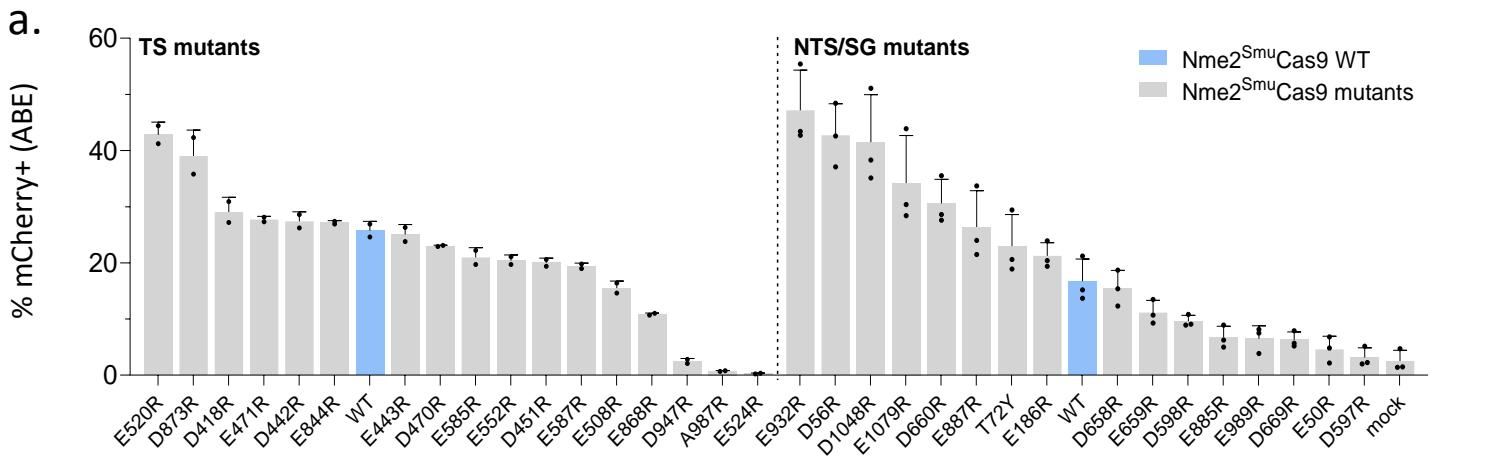
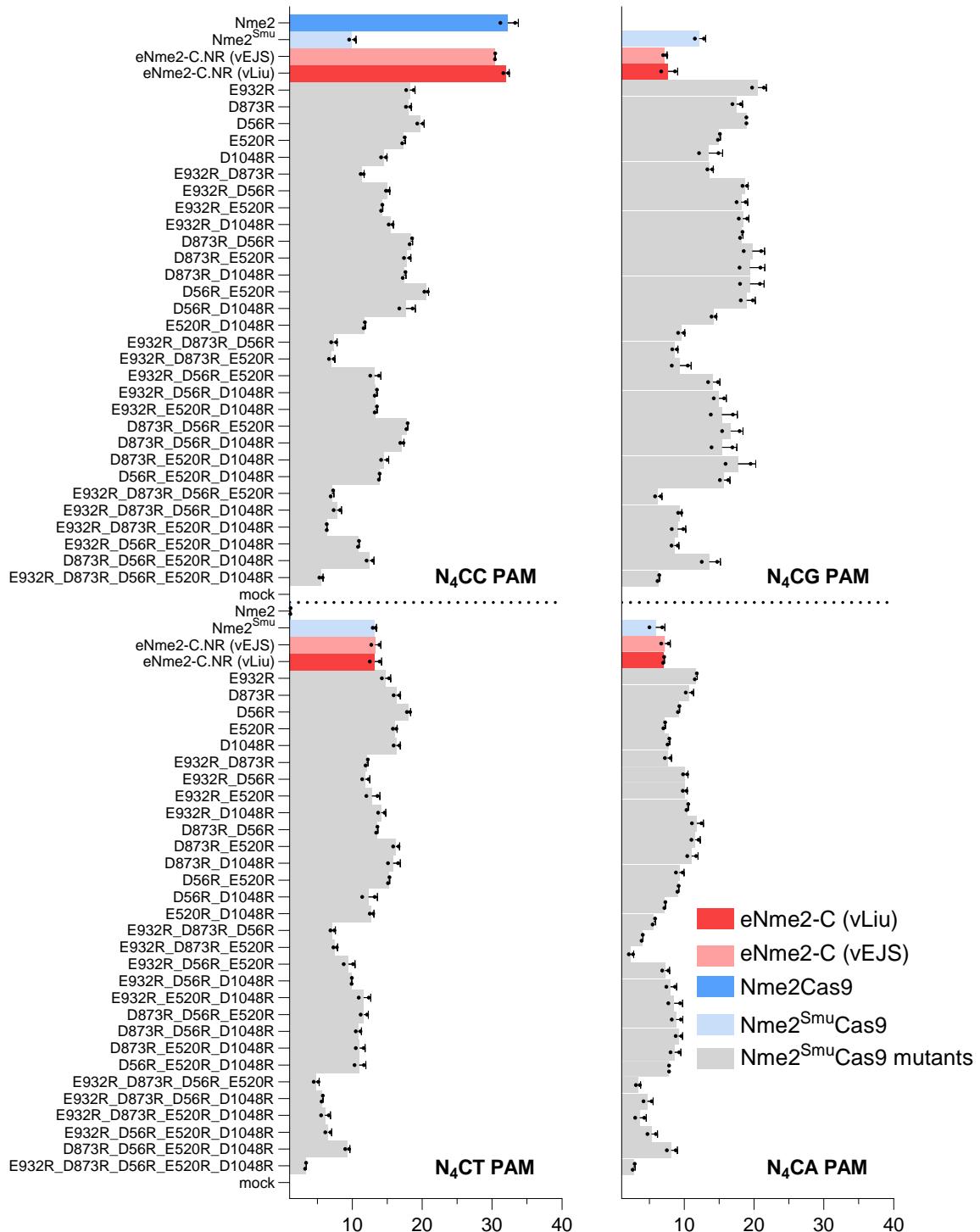


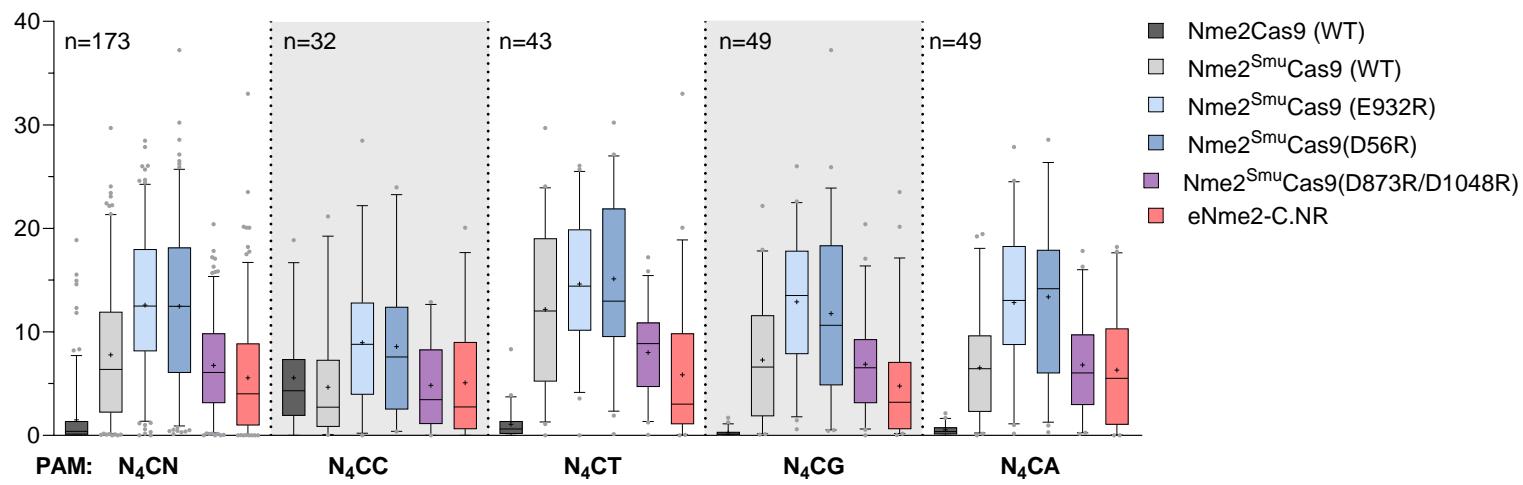
Supplementary Figure 1. PID-chimeric Nme2^{Smu}Cas9 nucleases and ABEs perform poorly at N₄CC targets compared to WT PID Nme2Cas9 effectors. (a) Nuclease editing, following transfection of Nme2- or Nme2^{Smu}Cas9 and associated sgRNA plasmids, at endogenous N₄CC PAM genomic loci in HEK293T cells. Editing efficiencies were measured by amplicon deep sequencing ($n = 3$ biological replicates; data represent mean \pm SD). (b) Editing with Nme2- or Nme2^{Smu}-ABE8e-i1 plasmids at N₄CC PAM target loci in HEK293T cells. The editing efficiency at the maximally edited adenine for each target was plotted. Editing efficiencies were measured by amplicon deep sequencing ($n = 3$ biological replicates; data represent mean \pm SD).



Supplementary Figure 2. Arginine mutagenesis improves Nme2^{Smu}Cas9 effector activity. (a) Activities of Nme2^{Smu}-ABE8e-i1 (denoted as WT, blue bar) and nucleic acid-interacting arginine mutants [target DNA strand (TS), single guide RNA (SG), and non-target DNA strand (NTS)] denoted by amino acid substitution (grey bars) in the mCherry ABE reporter cell line (activated upon A-to-G editing). After plasmid transfection with an N₄CC PAM-targeting sgRNA plasmid and an effector-expressing plasmid, editing activities were measured by flow cytometry ($n = 2$ or 3 biological replicates; data represent mean \pm SD). (b) Activities of Nme2^{Smu}-ABE8e-i1 and the top 10 performing arginine mutants in the mCherry ABE reporter cell line (activated upon A-to-G editing) at N₄CD (D = not C) PAM targets. After plasmid transfection with the associated sgRNA plasmid and the effector-expressing plasmid, activities were measured by flow cytometry ($n = 3$ biological replicates; data represent mean \pm SD). (c) Activities of Nme2Cas9 nuclease variants within the HEK293T TLR-MCV1 reporter at N₄CC, N₄CT, N₄CG and N₄CA PAM targets, comparing Nme2Cas9 (dark blue), Nme2^{Smu}Cas9 (light blue), and Nme2^{Smu}Cas9 arginine mutants (grey). After parallel plasmid transfection with the sgRNA and nuclease effector plasmids, activities were measured by flow cytometry ($n = 2$ biological replicates; data represent mean \pm SD).

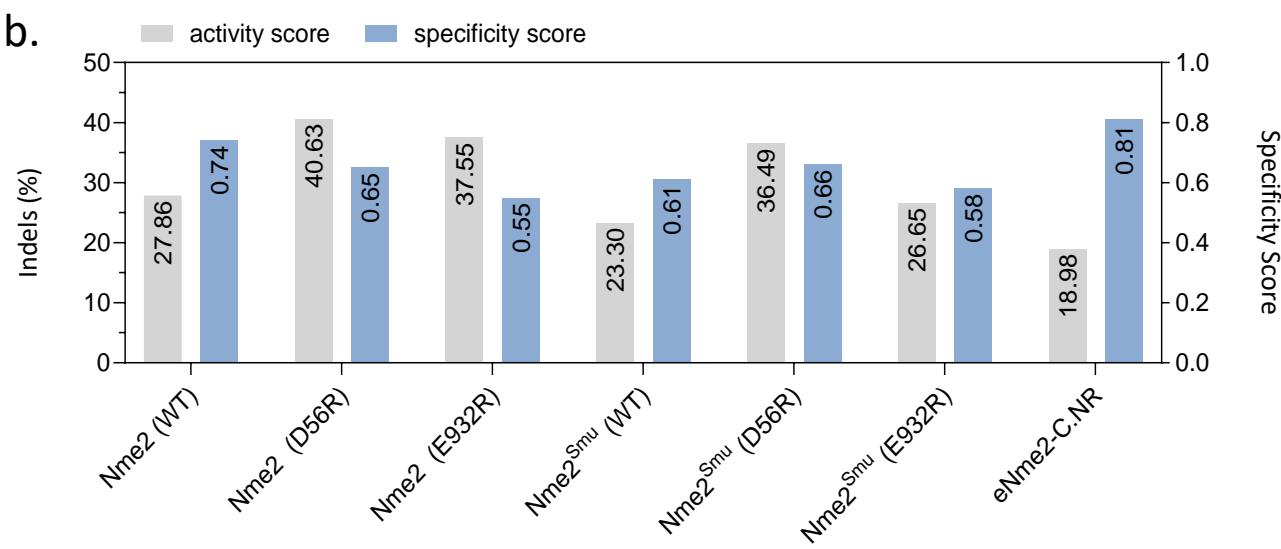
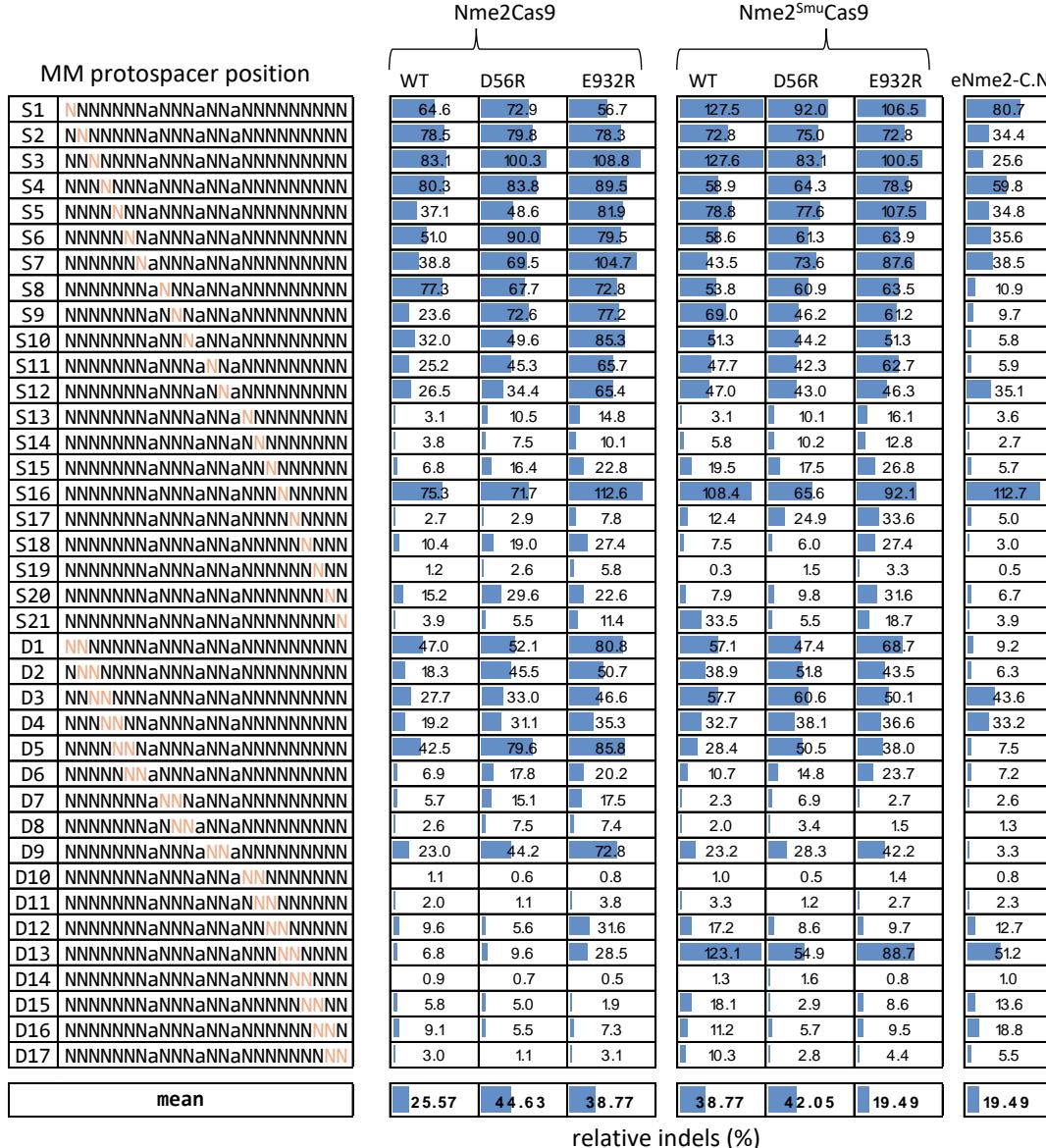


Supplementary Figure 3. Arginine mutagenesis improves Nme2^{Smu}Cas9 nuclease activity. Activities of Nme2Cas9 nuclease variants with the HEK293T TLR-MCV1 reporter at N₄CC, N₄CT, N₄CG and N₄CA PAM targets, comparing Nme2Cas9, eNme2-C.NR (vLiu), eNme2-C.NR (vEJS), Nme2^{Smu}Cas9, and Nme2^{Smu}Cas9 single and multiple arginine mutants. For eNme2-C.NR, vLiu is the original plasmid obtained from Addgene (#185672), whereas vEJS is the same effector re-cloned into the expression plasmid backbone used for all other effectors in this experiment. Activities were measured after parallel plasmid transfection with sgRNA and nuclease editor plasmids, followed by flow cytometry (n = 2 biological replicates; data represent mean ± SD).



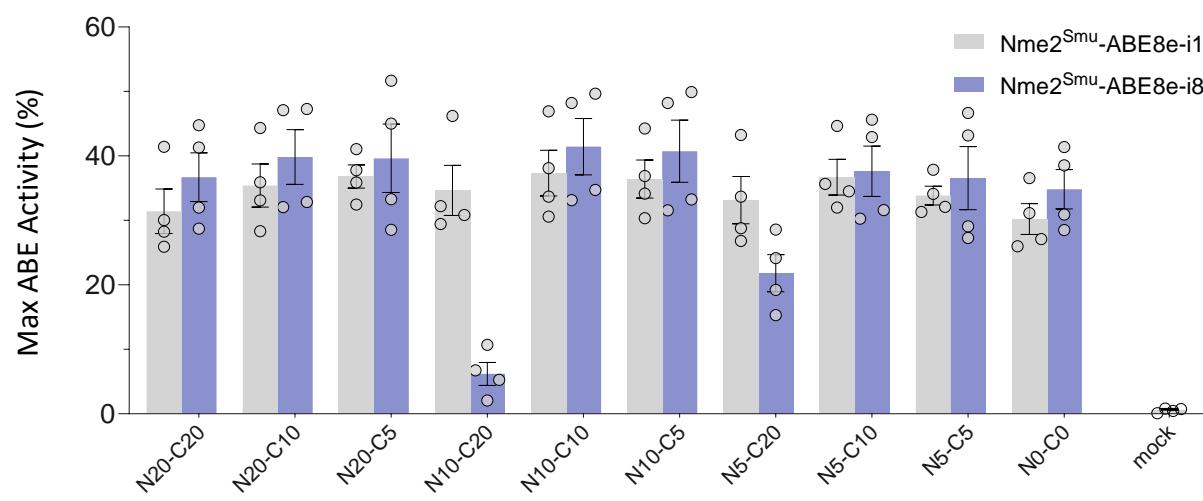
Supplementary Figure 4. Activities of Nme2^{Smu}Cas9 nuclease variants. Nuclease-induced indels in experimental panel 1 of the guide-target activity library following plasmid transfection of Nme2Cas9 (WT), Nme2^{Smu}Cas9 (WT and E932R, D56R, and E520R/D873R variants) or eNme2-C.NR into HEK293T cells. The editing efficiencies for 173 target sites were plotted. Editing activities were measured by amplicon sequencing ($n = 3$ biological replicates; boxplots represent median and interquartile range; whiskers indicate 5th and 95th percentiles and the cross represents the mean).

a.



Supplementary Figure 5. Specificities of Nme2- and Nme2^{Smu}Cas9 nucleases at N₄CC PAM targets. (a) Indel editing frequencies of Nme2Cas9, Nme2^{Smu}Cas9 variants, and eNme2-C.NR across single- (S) or di-nucleotide (D) mismatched target sites within the guide-target mismatch library. Activities for each mismatched target were normalized to the mean efficiency of their respective perfectly-matched target site. Orange nucleotides represent protospacer positions of the transversion mutation(s) present within the mismatched target site. (b) indel activity vs. specificity score for nuclease variants from (a) across the mismatched guide-target library. Nuclease activity was compiled from editing data for three perfectly matched N₄CC target sites (0 MM) (**Supplementary data file**). The specificity score was calculated as one minus the tiled mismatched editing mean from (a), normalized to a scale of one to 100. Editing activities were measured by amplicon sequencing (n = 3 biological replicates).

a.

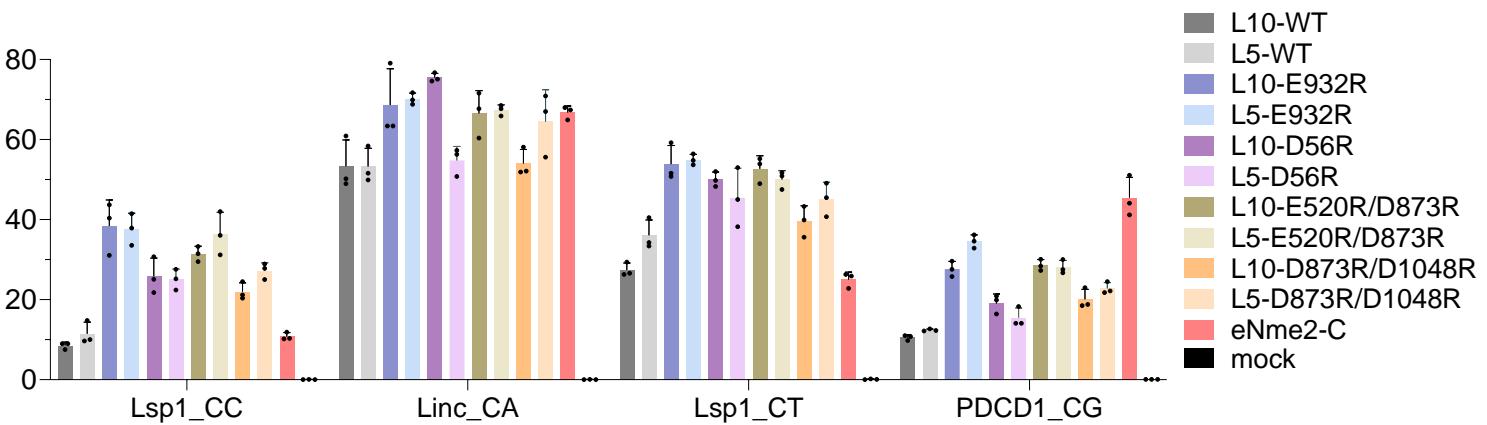


b.

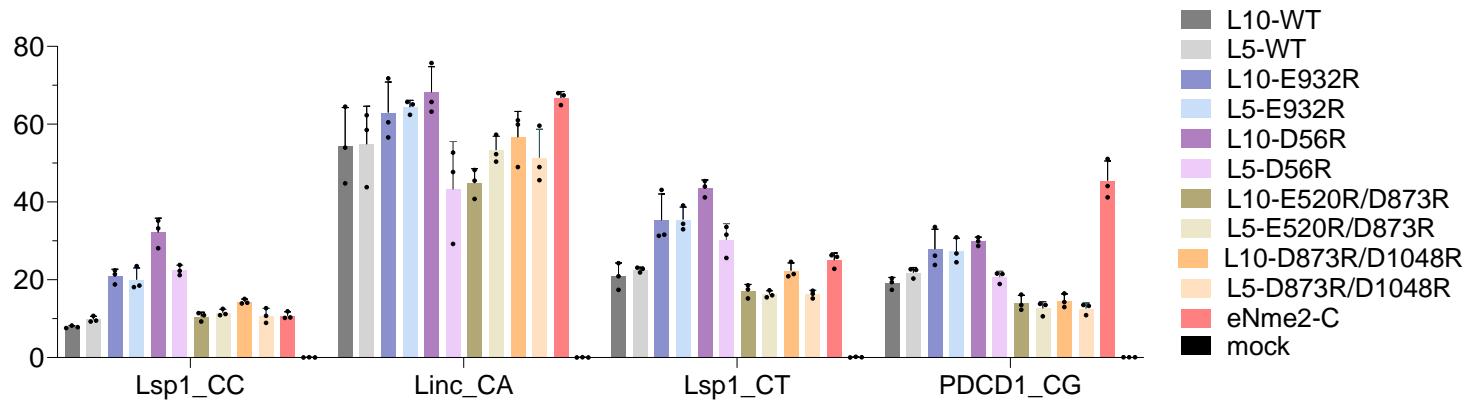
Effector	N-Linker Length	C-Linker Length
Nme2 ^{Smu} -ABE8e-i1	20	20
Nme2 ^{Smu} -ABE8e-i1	20	10
Nme2 ^{Smu} -ABE8e-i1	20	5
Nme2 ^{Smu} -ABE8e-i1	10	20
Nme2 ^{Smu} -ABE8e-i1	10	10
Nme2 ^{Smu} -ABE8e-i1	10	5
Nme2 ^{Smu} -ABE8e-i1	5	20
Nme2 ^{Smu} -ABE8e-i1	5	10
Nme2 ^{Smu} -ABE8e-i1	5	5
Nme2 ^{Smu} -ABE8e-i1	0	0
Nme2 ^{Smu} -ABE8e-i8	20	20
Nme2 ^{Smu} -ABE8e-i8	20	10
Nme2 ^{Smu} -ABE8e-i8	20	5
Nme2 ^{Smu} -ABE8e-i8	10	20
Nme2 ^{Smu} -ABE8e-i8	10	10
Nme2 ^{Smu} -ABE8e-i8	10	5
Nme2 ^{Smu} -ABE8e-i8	5	20
Nme2 ^{Smu} -ABE8e-i8	5	10
Nme2 ^{Smu} -ABE8e-i8	5	5
Nme2 ^{Smu} -ABE8e-i8	0	0

Supplementary Figure 6. Domain-inlaid Nme2^{Smu}-ABE8e linker length optimization. (a) A-to-G editing at four endogenous HEK293T genomic loci with Nme2^{Smu}-ABE8e-i1 (grey bars) or Nme2^{Smu}-ABE8e-i8 (blue bars) carrying N- or C-terminal (Nx-Cx) linker variants following plasmid transfection. The editing efficiencies at the maximally edited adenine for each target was plotted and aggregated. Data for individual target sites are in the **Supplementary data file**. Editing efficiencies were measured by amplicon deep sequencing ($n = 3$ biological replicates; data represent mean \pm SD).

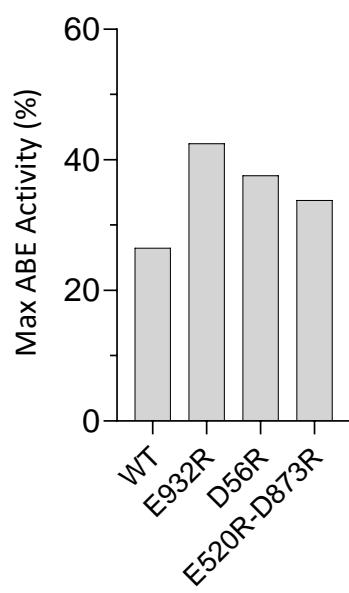
a.



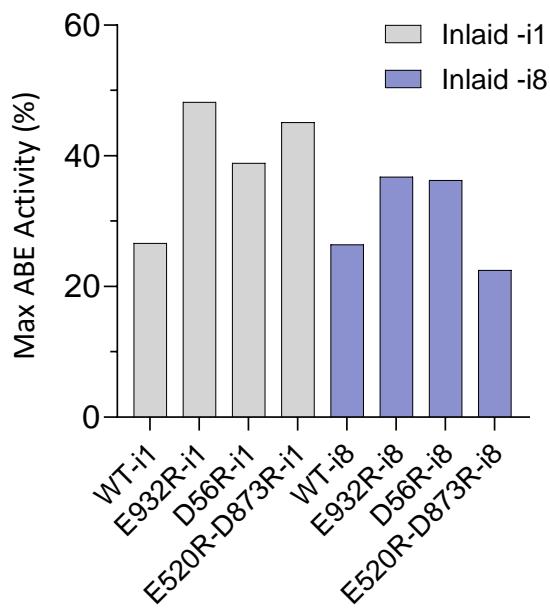
b.



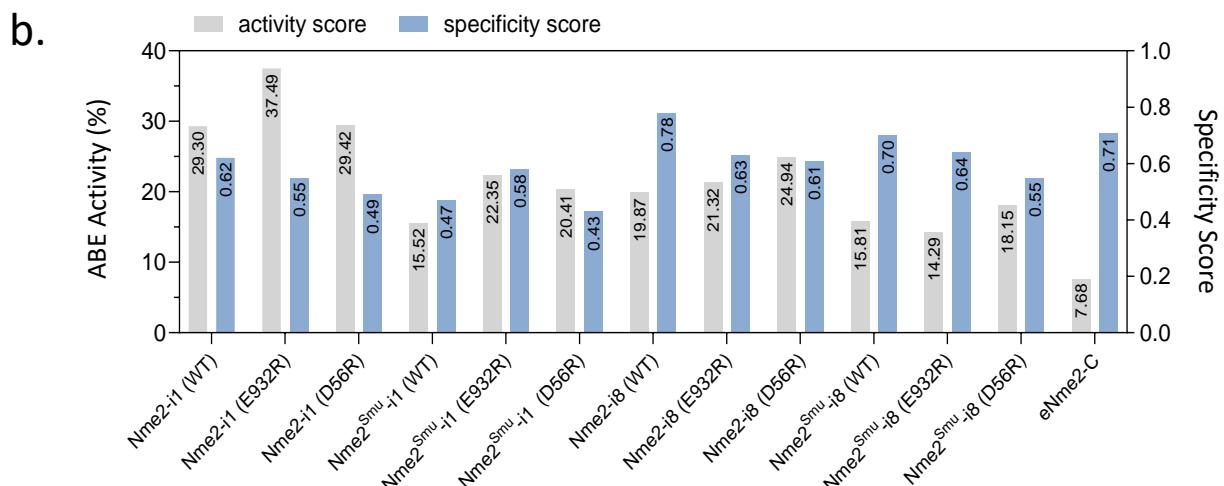
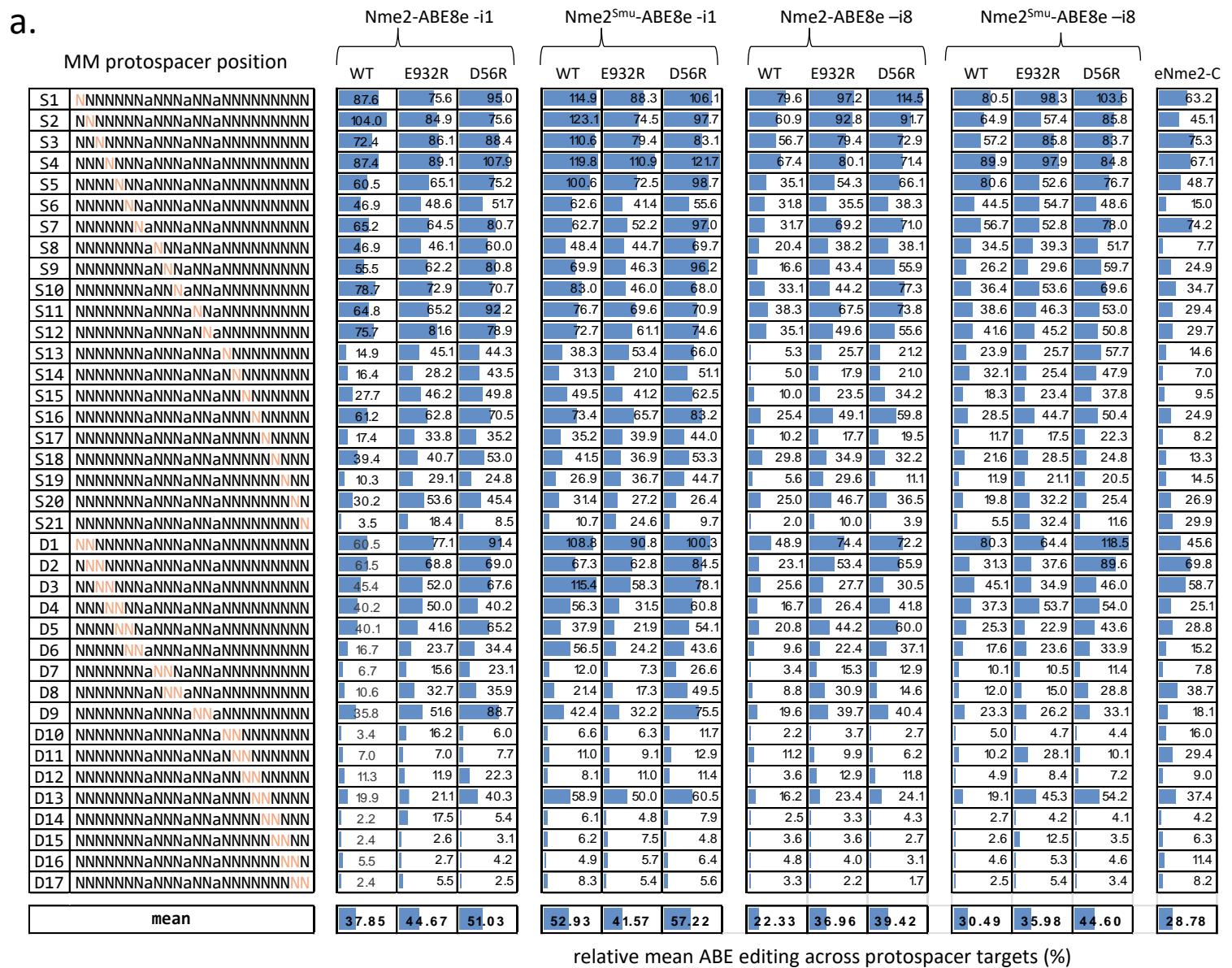
c.



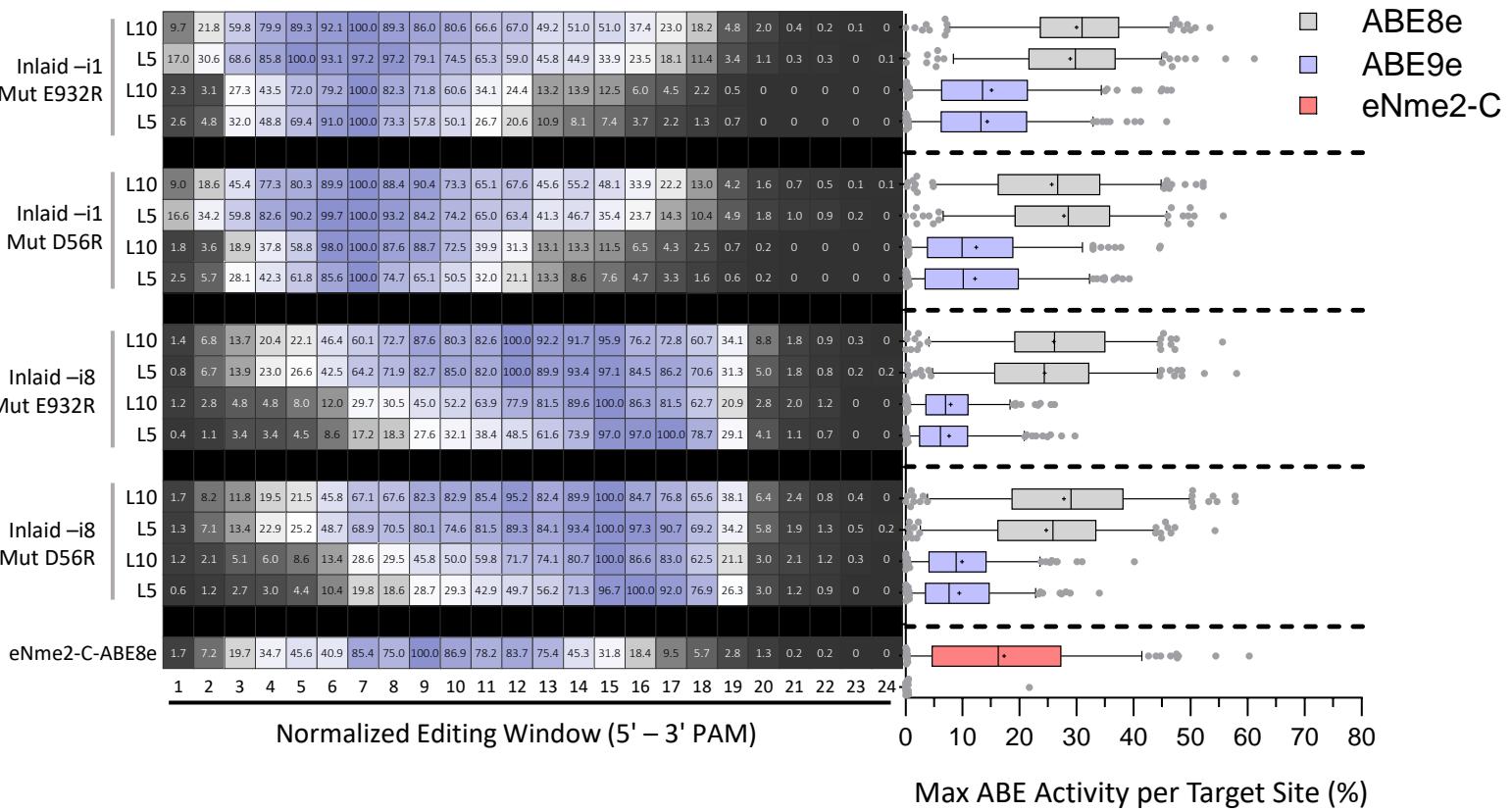
d.



Supplementary Figure 7. Domain-inlaid Nme2^{Smu}-ABE8e linker length and activity optimization (continued). (a-b) A-to-G editing at four endogenous HEK293T genomic loci with Nme2^{Smu}-ABE8e-i1 (a) or Nme2^{Smu}-ABE8e-i8 (b) arginine mutants (WT and E932R, D56R, E520R/D873R, D873R/D1048R) and linker (L10, L5) variants by plasmid transfection. The editing efficiency at the maximally edited adenine for each target was plotted. Editing efficiencies were measured by amplicon deep sequencing ($n = 3$ biological replicates; data represent mean \pm SD). Data for individual target sites are in the **Supplementary data file**. (c-d) Summary data from endogenous maximum activity aggregated from (a) and (b). (c) Nme2^{Smu}-ABE8e and arginine mutant activity independent of domain insertion site and linker length, or (d) separated by position of domain insertion (-i1 vs. -i8).

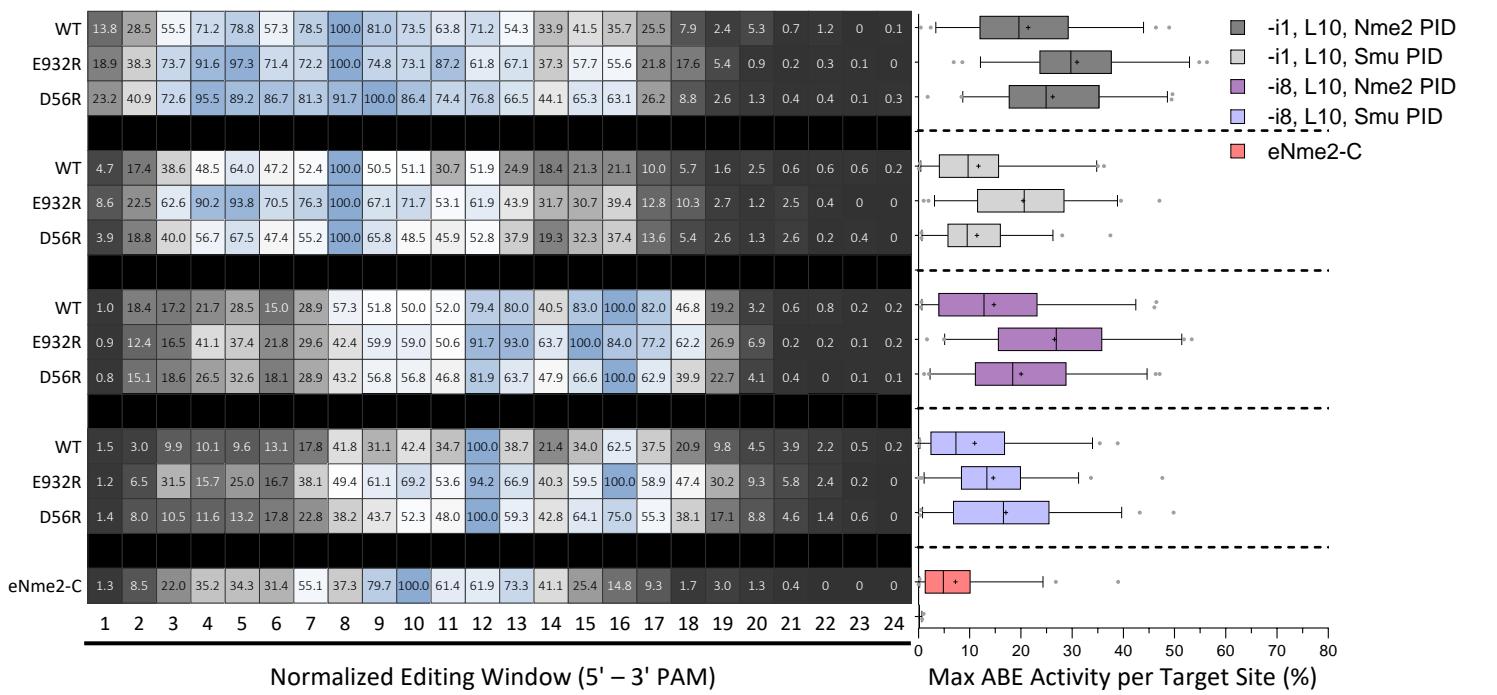


Supplementary Figure 8. Specificities of domain-inlaid Nme2- and Nme2^{Smu}-ABE8e variants at N₄CC PAM targets. (a) Mean A-to-G editing frequencies of domain-inlaid Nme2^{Smu}-ABE8e variants or eNme2-C across single- (S) or di-nucleotide (D) mismatched target sites within the guide-target mismatch library. Activities for each mismatched target were normalized to the mean efficiency of their respective perfectly matched target site. Orange nucleotides represent protospacer position of the transversion mutation(s) present within the mismatched target site. **(b)** ABE activity vs. specificity score for base editing variants in (a) across the mismatched guide-target library. ABE activity was compiled from editing data for three perfectly matched N₄CC target sites (0 MM) (**Supplementary data file**). The specificity scores were calculated as one minus the tiled mismatched editing mean in (a) normalized to a scale of one to 100. Editing activities were measured by amplicon sequencing (n = 3 biological replicates).

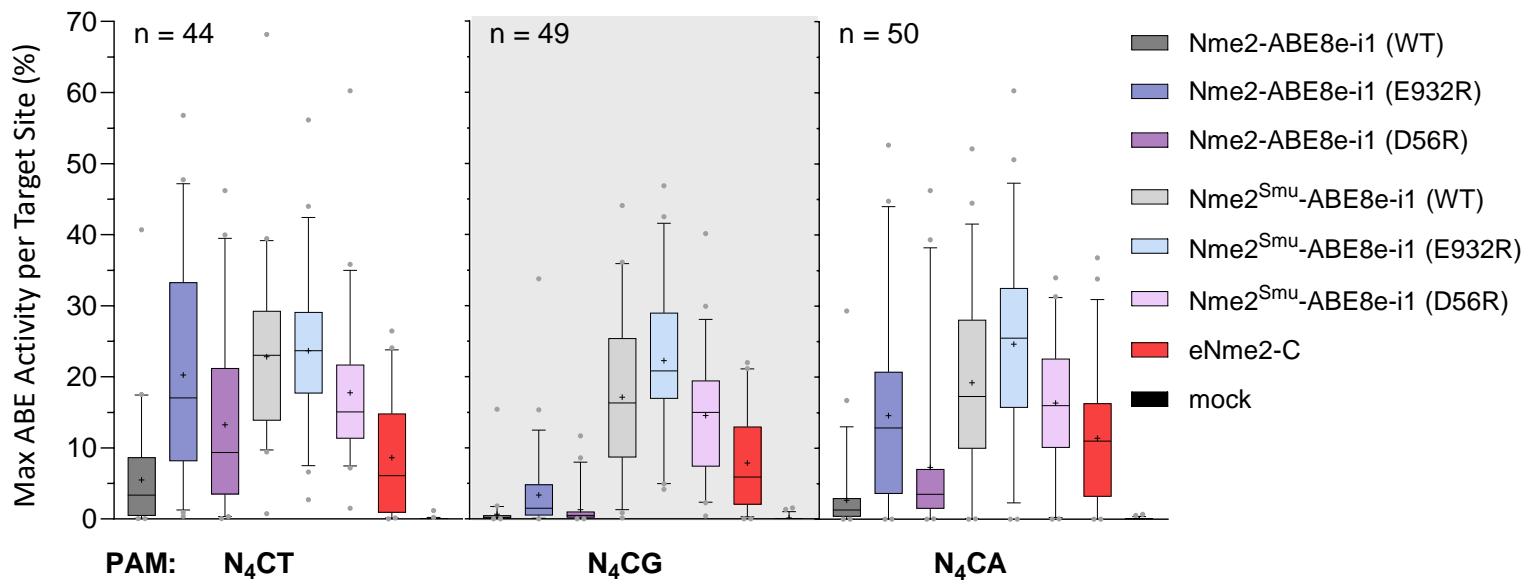


Supplementary Figure 9. Editing windows of domain-inlaid Nme2^{Smu}-ABEs with narrow-window adenine deaminases. Assessment of editing windows and activities from experimental panel 3 of the guide-target activity library (193 sites) for broad- or narrow-window deaminases (ABE8e or ABE9e, respectively). Test subjects include Nme2^{Smu}-ABE -i1 or -i8 with E932R or D56R arginine mutants, in combination with deaminase linker lengths (L10 and L5). The eNme2-C variant was also included (bottom). Editing was assessed following plasmid transfection of the ABE variants into HEK293T cells with the integrated guide-target library. Left: average editing windows across the target sites, normalized on a scale of 0 – 100 (%) against adenine positions with the highest observed edited efficiencies within the window. Right: activity at the maximally edited adenine for each target was plotted. Editing activities were measured by amplicon sequencing ($n = 3$ biological replicates; boxplots represent median and interquartile ranges; whiskers indicate 5th and 95th percentiles; the cross represents the mean).

a.



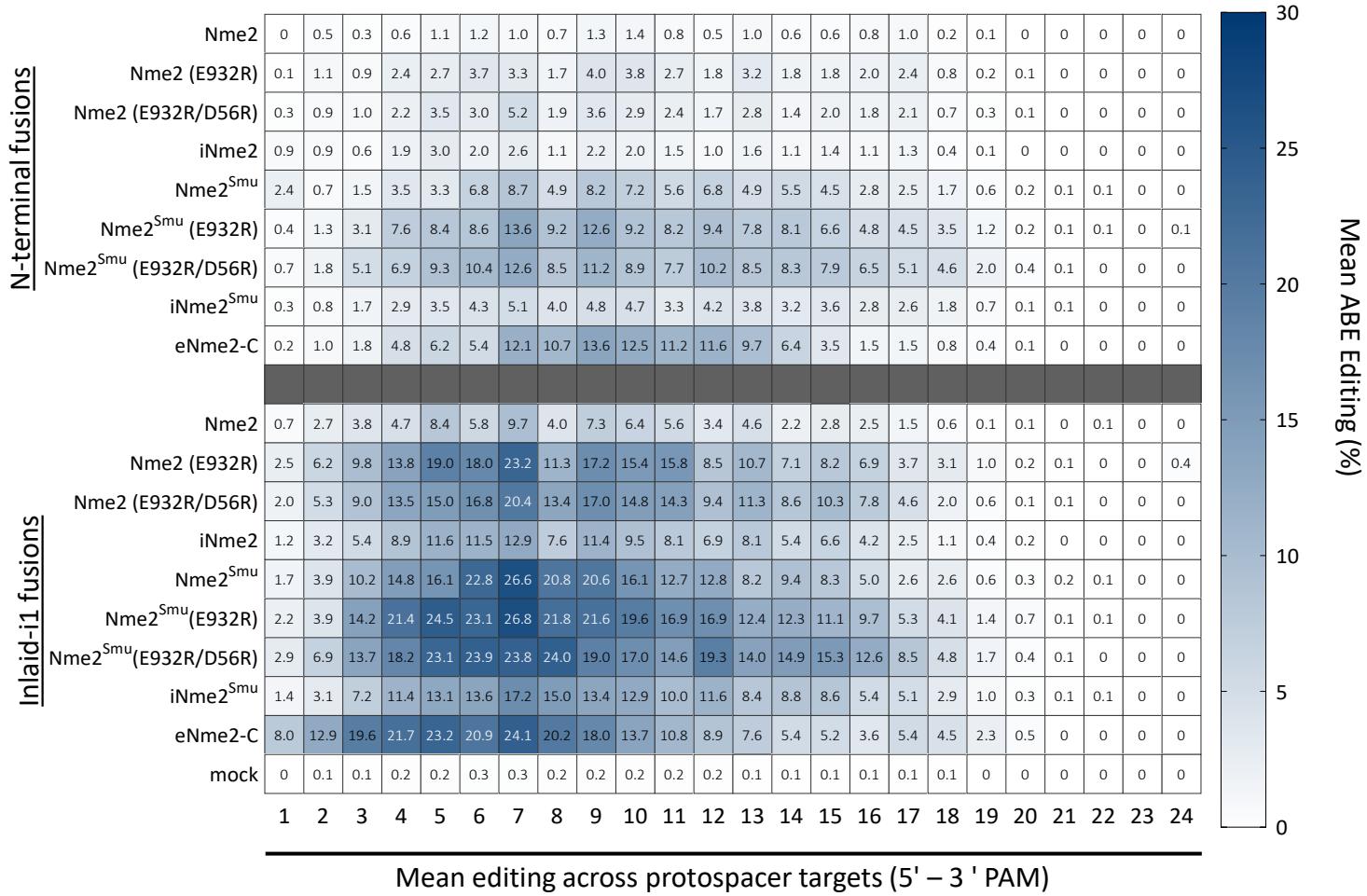
b.



Supplementary Figure 10. Activities and editing windows of domain-inlaid Nme2- and Nme2^{Smu}-ABE8e variants at N₄CC or N₄CN PAM targets. Assessment of editing windows and activities from experimental panel 2 of the guide-target activity library (192 sites) for Nme2- and Nme2^{Smu}-ABE8e -i1 or -i8, with and without the E932R or D56R arginine mutations, in combination with the L10 deaminase linker. The eNme2-C variant was also included (bottom). **(a)** Editing data for N₄CC PAM targets only (49 sites). Following plasmid transfection of the ABE variants into HEK293T cells with the integrated guide-target library, editing activities were measured by amplicon sequencing. Left: average editing windows across target sites, normalized on a scale of 0 – 100 (%) against adenine positions with the highest observed edited efficiencies within the window. Right: editing efficiency at the maximally edited adenine for each target. **(b)** Nme2- or Nme2^{Smu}-ABE8e-i1 editing data at N₄CD PAM target sites. The maximally edited adenine for each target was plotted. n , the number of target sites with each PAM ($n = 3$ biological replicates; boxplots represent median and interquartile ranges; whiskers indicate 5th and 95th percentiles; the cross represents the mean).

181 N₄CN PAM Target Sites

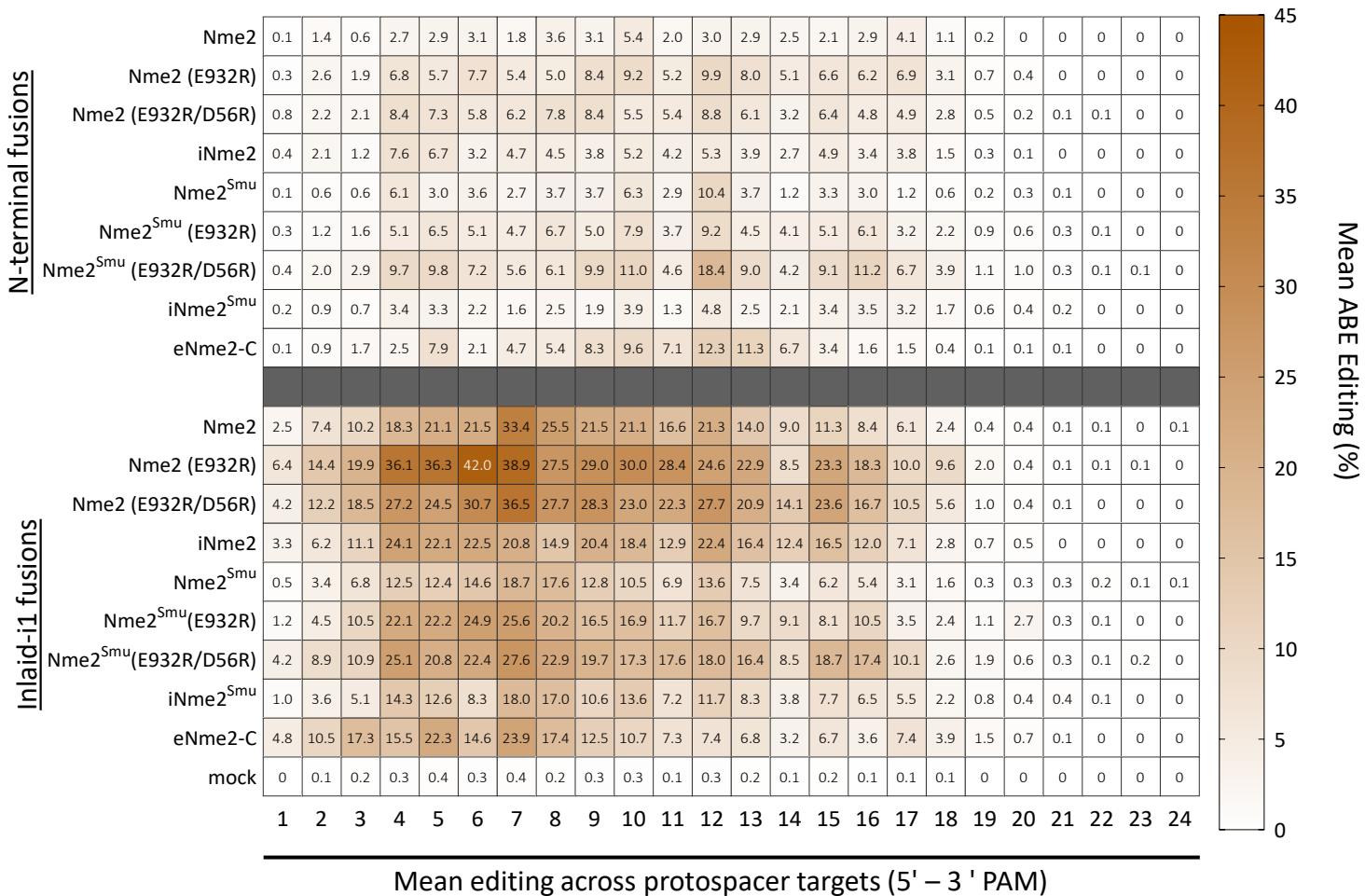
a.



Supplementary Figure 11. Activities and editing windows of engineered Nme2Cas9 ABE8e variants at N₄CN PAM targets. Assessment of editing windows and activities from experimental panel 4 of the guide-target activity library (181 N₄CN PAM sites) for Nme2-, Nme2^{Smu}-, iNme2-, iNme2^{Smu}- and eNme2-C variants in either the N-terminal or inlaid-i1 (linker 10) formats. Mean A-to-G editing activities and editing windows across protospacer positions in the activity guide-target library are shown for the engineered Nme2Cas9 ABE8e variants. Editing activities were measured by amplicon sequencing (n = 3 biological replicates).

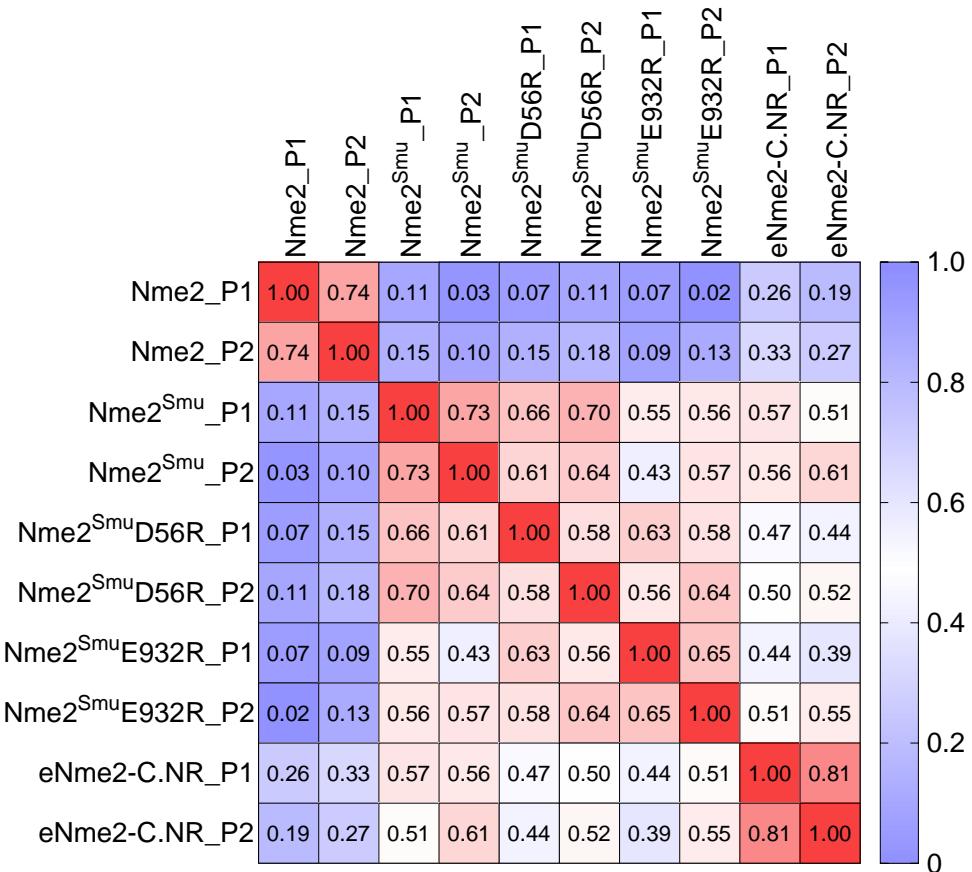
38 N₄CC PAM Target Sites

a.



Supplementary Figure 12. Activities and editing windows of engineered Nme2Cas9 ABE8e variants at N₄CC PAM targets. Assessment of editing windows and activities from experimental panel 4 of the guide-target activity library (38 N₄CC PAM sites) for Nme2-, Nme2^{Smu}-, iNme2-, iNme2^{Smu}- and eNme2-C variants in either the N-terminal or inlaid-i1 (linker 10) formats. Mean A-to-G editing activities and editing windows across protospacer positions in the activity guide-target library are shown for the engineered Nme2Cas9 ABE8e variants. Editing activities were measured by amplicon sequencing (n = 3 biological replicates).

a.

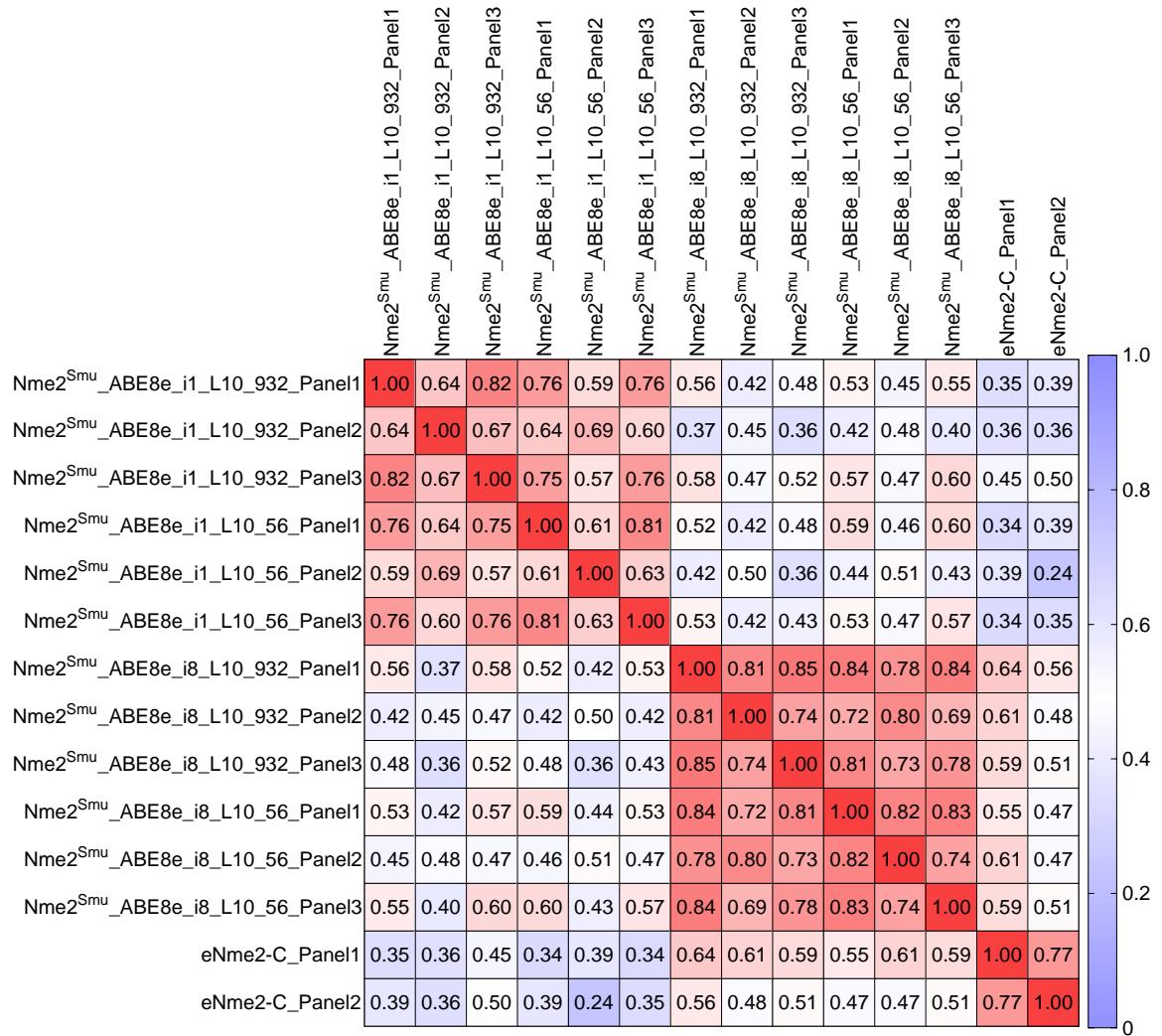


b.

Sample	Pearson r	p value	CI	n
Nme2Cas9	0.737	1.656E-33	0.6642 to 0.7964	188
Nme2 ^{Smu} Cas9	0.735	3.332E-33	0.6614 to 0.7945	188
Nme2 ^{Smu} Cas9 D56R	0.582	2.005E-18	0.4786 to 0.6693	188
Nme2 ^{Smu} Cas9 E932R	0.654	2.486E-24	0.5638 to 0.7290	188
eNme2-C.NR	0.811	3.826E-45	0.7554 to 0.8548	188

Supplementary Figure 13. Guide-target library quality control for nuclease editing panels. (a) Pearson correlation between experimental panels of nuclease guide-target library experiments, comparing the mean editing efficiencies of library member target sites. Each experimental panel consisted of 3 biological replicates (Rep1, 2 or 3), compiled from **Supplementary Figure 4** [panel 1, (P1)] and **Figure 2a** [panel 2, (P2)]. (b) Summary statistics from (a); CI indicates confidence interval, and n indicates number of comparisons used.

a.



b.

ABE panels 1 vs. 2 vs. 3		Pearson r						p value					
Sample		Panel1 vs 2	Panel1 vs 3	Panel2 vs 3	Panel 1 vs 2	Panel 1 vs 3	Panel 2 vs 3	Panel 1 vs 2	Panel 1 vs 3	Panel 2 vs 3			
Nme2 ^{Smu} _ABE8e_i1_L10 (E932R)		0.645		0.824		0.670		7.217E-23		1.696E-46		3.378E-25	
Nme2 ^{Smu} _ABE8e_i1_L10 (D56R)		0.607		0.808		0.627		8.391E-20		1.745E-43		2.215E-21	
Nme2_ABE8e_i8_L10 (E932R)		0.809		0.851		0.744		1.276E-43		1.840E-52		1.399E-33	
Nme2_ABE8e_i8_L10 (D56R)		0.822		0.833		0.742		4.906E-46		2.162E-48		3.106E-33	
eNme2.C_ABE8e		0.766		n/a		n/a		1.518E-36		n/a		n/a	
		CI						n					
Sample		Panel 1 vs 2	Panel 1 vs 3	Panel 2 vs 3	Panel 1 vs 2	Panel 1 vs 3	Panel 2 vs 3	Panel 1 vs 2	Panel 1 vs 3	Panel 2 vs 3			
Nme2 ^{Smu} _ABE8e_i1_L10 (E932R)		0.5510 to 0.7221	0.7709 to 0.8655	0.5814 to 0.7428	183		183	183		183			
Nme2 ^{Smu} _ABE8e_i1_L10 (D56R)		0.5065 to 0.6912	0.7512 to 0.8532	0.5301 to 0.7077	183		183	183		183			
Nme2_ABE8e_i8_L10 (E932R)		0.7521 to 0.8538	0.8051 to 0.8865	0.6720 to 0.8028	183		183	183		183			
Nme2_ABE8e_i8_L10 (D56R)		0.7680 to 0.8637	0.7824 to 0.8726	0.6687 to 0.8007	183		183	183		183			
eNme2.C_ABE8e		0.6983 to 0.8198	n/a	n/a	183		183	n/a		n/a			

Supplementary Figure 14. Guide-target quality control for ABE editing panels. (a) Pearson correlation between experimental panels of Nme2^{Smu}-ABE8e and eNme2-C effectors for the guide-target library experiments, comparing the mean editing rates across the adenines of a library member target site. Each experimental panel consisted of 3 biological replicates (Rep1, 2 or 3) compiled from **Figure 3c** (panel 1), **Supplementary Figure 10** (panel 2), and **Supplementary Figure 9** (panel 3). (b) Summary statistics for data in (a); CI indicates confidence interval, and n indicates number of comparisons.

AA Mutation Location and Identity in nuclease	Codon Usage	Nucleic Acid Proximity in Homology Model	Domain	Note
D56R	CGT	sgRNA	Nme2 - Bridge Helix	
E186R	CGT	sgRNA	Nme2 - REC1	
D597R	CGT	sgRNA	Nme2 - HNH	
D598R	CGT	sgRNA	Nme2 - HNH	
D669R	CGT	sgRNA	Nme2 - RuvC III	
E885R	CGT	sgRNA	Nme2 - WEDGE	
E887R	CGT	sgRNA	Nme2 - WEDGE	
E1079R	CGT	sgRNA	Nme2 ^{Smu} - PID	Specific to Nme2 ^{Smu} Effectors
T72Y	TAT	sgRNA	Nme2 - Bridge Helix	
E50R	CGT	non-target DNA	Nme2 - RuvC I	
D658R	CGT	non-target DNA	Nme2 - Linker II	
E659R	CGT	non-target DNA	Nme2 - Linker II	
D660R	CGT	non-target DNA	Nme2 - Linker II	
E932R	CGT	non-target DNA	Nme2 - WEDGE	
E989R	CGT	target & non-target DNA	Nme2 ^{Smu} - WEDGE	
D1048R	CGT	target & non-target DNA	Nme2 ^{Smu} - PID	Specific to Nme2 ^{Smu} Effectors
D418R	CGT	Target DNA	Nme2 - REC1	
D442R	CGT	Target DNA	Nme2 - REC1	
E443R	CGT	Target DNA	Nme2 - REC1	
D451R	CGT	Target DNA	Nme2 - REC1	
D470R	CGT	Target DNA	Nme2 - RuvC II	
E471R	CGT	Target DNA	Nme2 - RuvC II	
E508R	CGT	Target DNA	Nme2 - RuvC II	
E520R	CGT	Target DNA	Nme2 - Linker I	
E524R	CGT	Target DNA	Nme2 - Linker I	
E552R	CGT	Target DNA	Nme2 - HNH	
E585R	CGT	Target DNA	Nme2 - HNH	
E587R	CGT	Target DNA	Nme2 - HNH	
E844R	CGT	Target DNA	Nme2 - WEDGE	
E868R	CGT	Target DNA	Nme2 - WEDGE	
E873R	CGT	Target DNA	Nme2 - WEDGE	
D947R	CGT	Target DNA	Nme2 ^{Smu} - PID	Specific to Nme2 ^{Smu} Effectors
A987R	CGT	Target DNA	Nme2 ^{Smu} - PID	Equivalent to D987 in Nme2

Supplementary Table 1. Amino acid substitutions of Nme2Cas9 and Nme2^{Smu}Cas9 arginine mutants

eNme2-C	eNme2-C_NR	iNme2
P6S		D844G
E33G		E868K
K104T	K104T	K870R
D152A	D152A	D873A
F260L	F260L	D911G
A263T	A263T	K929R
A303S	A303S	E932K
D451V	D451V	
E520A		
R646S		
F696V		
G711R		
I758V		
H767Y		
E932K	E932K	
N1031S	N1031S	
R1033G	R1033G	
K1044R	K1044R	
Q1047R	Q1047R	
V1056A	V1056A	

Supplementary Table 2. Amino acid substitutions present within previously published PAM-relaxed Nme2Cas9 variants. Black-highlighted amino acid mutations are unique to specific Nme2Cas9 variants, while red-highlighted mutations indicate overlapping positions between previously characterized mutations and/or the Nme2Cas9 arginine variants described in this study.

Supplementary Note 1. Nucleotide sequence of key nuclease and base editing constructs described in this manuscript

Nme2Cas9 (WT): [BPSV40-NLS](#), [Nme2Cas9_Linkers](#)

MKRTADGSEFESPKKKRKVEDMAAFKPNPINYLGLDIGIASVGWAMVEIDEENPIRLIDLGVRVFERAEVPKTGDSLAMARRLARSVRR
LTRRRRAHRLRARRLLKREGVLQAADFDENLIKSLPNTPWQLRAAALDRKLTPLEWSAVLLHLIKHRGYLSQRKNEGETADKELGALLKG
VANNAHALQTGDFRTPAELALNKFEKESGHIRNQRGDYSHTFSRKDLQAEILLFEKQKEFGNPHVSGGLKEGIETLLMTQRPAISGDAV
QKMLGHCTFEPKAAKNTYTAERFIWLTKLNNLRILEQGSERPLTDTERATLMDEPYRKS KLTYAQARKLLGLEDTAFFKGLRYGKDNE
EASTLMEMKAYHAISRALEKEGLDKKSPLNLSSELQD EIGTAFSLFKTDEDITGRLKDRVQPEILEALLKHISFDKFVQISLKA LRRIVPLMEQ
GKRYDEACEIYGDHYGKKNTEEKYLPPIPADEIRNPVVLRALSQARKVINGVVRYYGSPARIHIETAREVGKSFKDRKEIEKRQEENRKDR
EKAAAKFREYFPNFVGEPKSKDILKRLYEQQHGKCLYSGKEINLVRNLNEKG YVEIDHALPFSRTWDDSFNNKVLV LGSENQNKG NQTPYE
YFNGKDNSREWQE FKARVETSRPRSKKQRILLQKFEDGFKECNLDTRYVNRFLCQFVADHILLTGKGKRRVFASNGQITNLLRGFWG
LRKVRAENDRHHALDAVVVACSTVAMQQKITRFVRYKEMNAFDGKTIDKETGKLHQKTHFPQPWEFFAQEV MIRVFGKPDGKPEFEE
ADTPEKLRTLLAEKLSSRPEAVHEYVTPLFVSRAPNRKMSGAHKDLRSAKRFVKHNEKISVKRVWLTEIKLADLENMVNYKNGREIELYEA
LKARLEAYGGNAKQAFDPKDNPFYKKGGQLVKAVRVEKTQESGVLLNNKNA YTADNGDMVRVDVFCKVDKGKKNQYFIVPIYAWQVA
ENILPDIDCKGYRIDD SYTFCFSLHKYDLIAFQKDEKS KVEFAYYINCDSN GRFYLA WHDKGSKEQQFRISTQNLVLIQKYQVNELGKEIRPC
RLKKRPPVREDKRTADGSEFEPKKKRKV

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Nme2Cas9 (E932R): BPSV40-NLS, Nme2Cas9_Linkers

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Nme2Cas9 (D56R): [BPSV40-NLS](#), Nme2Cas9_Linkers

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Nme2^{Smu}Cas9 (WT): [BPSV40-NLS](#), Nme2Cas9 – delta PID, [SmuCas9 PID](#), Linkers

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Nme2^{Smu}Cas9 (E932R): BPSV40-NLS, Nme2Cas9 – delta PID, SmuCas9 PID, Linkers

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Nme2^{Smu}Cas9 (D56R): [BPSV40-NLS](#), Nme2Cas9 – delta PID, [SmuCas9 PID](#), Linkers

MKRTADGSEFESPKKKRKV EDMAAFKPNNPINYLGLDIGIASVGWAMVEIDEEENPIRLIDLGVRFERAEPKTGRSLAMARRLARSVRRLTRRRAHRLRARRLLKREGVLQAADFDENGLIKSLPNTWPQLRAAALDRKLTPLEWSAVLLHLIKRGYLSQRKNEGETADKELGALLKG
VANNAHALQTGDFRTPAELALNKFEKESGHIRNQRGDYSHTFSRKDLQAEILLFEKQKEFGNPHVSGGLKEGIETLLMTQRPALSGDAV
QKMLGHCTFEPKAAKNTYTAERFIWLTKLNNLRILEQGSERPLTDERATLMDEPYRKSKLTYAQARKLLGLEDTAKFKGLRYGKDNEASTLMEMKAYHAISRALEKEGLDKKSPLNLSSQEDEIGTAFSLFKTDEDITGRLKDRVQPEILEALLKHISFDKFVQISLKALRRIVPLMEQ
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EKAAGKFREYFPNFVGEPKSKDILKRLYEQQHKGCLSGKEINLVRNEKYVEIDHALPFSRTWDDSFNNKVLVLSSENQNKGNNQTPYE
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ADTPEKLRTLLAEKLSSRPEAVHEYVTPLFVSRAPNRKMSGAHKDTLRSAKR芙KHNEKISVKRVLTEIKLADLENMVNYKNGREIELYEA
LKRLEAYGGNAKQAFDPKDNPFYKKGGQLVKAVRVEKTQESGVLLNNKAYTIA
DNA
TMRVDVYTAKGKNYLV
PVY
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SKK
ED
KRTADGSEFEPKKKRKV

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aggataaaagaacccgcacggcagcggcgtaccatgtggccatctggcggccatccatgtggcggccatccatgtggcggccatccatgtggcggccatccatgtgg

eNme2-C.NR (vLiu): [BPSV40-NLS](#), eNme2-C.NR , Linkers

David Liu Lab evolved Nme2Cas9 nuclease for N4CN PAM targeting without alterations

MKRTADGSEFESPKKKRKVAAFKPNPINYLGLDIGIASVGWAMVEIDEENPIRLIDLGVRFERAEPKTGDSLAMARRLARSVRRLTRRAHRLRARRLLKREGVLQAADFDEGLITSPLNTPQLRAAALDRKLTLPEWSAVLHLIKHRGYLSQRKNEGETAAKELGALLKGVANNAHALQTGDFRTPAELALNKFEKESGHIRNQRGDYSHTFSRKDLQAEILLFEKQKEFGNPHVSGGLKEGIETLLMTQRPALSGDAVQKMLGHCTLEPTEPKAAKNTYTAERFIWLTKLNNLRILEQGSERPLTDERSTLMDEPYRKSCLTYAQARKLLGEDTAFFKGLRYGKDNAEASTLMEMKAYHAISRALEKEGLDKKSPLNLSSELQDEIGTAFSLFKTDEDITGRLKDRVQPEILEALLKHISFDKFVQISLKAIRRIVPLMEQGKRYDEACAEIYGVHYGKKNTEEKIYLPPIPADEIRNPVVLRALSQARKVINGVVRRYGSARIHETAREVGKSFKDRKEIEKRQEENRKDREKAAAKFREYFPNFVGEPKSKDILKRLYEQQHGKCLYSGKEINLVRNEKGYVEIDHALPFSRTWDDSFNNKVVLGSENQNKGNTQPYEFNGKDNSREWQEFKARVETSFRPRSKKQQRILLQKFDEDGFKECNLDTRYVNRFLCQFVAZHILLTGKGKRRVFASNGQITNLLRGFWGLRKVRAENDRHHALDAVVVACSTVAMQQKITRFVRYKEMNAFDGKTIDKETGKVLHQKTHFPQPWEFFAQEVIMRVFGKPDGKPEEEADTPEKLRTLAAKLSSRPEAVHEYVTPLFVSAPNRKMSGAHKDTLSAKRFVKHNEKISVKRVWLTEIKLADLENMVNYKNGREIELYEALKARLEAYGGNAKQAFDPKDNPFYKGGQLVKAVRVEKTQKSGVLLNKNAYTIADNGDMVRDVFCVDKKGKNQYFIVPIYAWQVAENILPDIDCKGYRIDDSTFCFLSHKYDLIAFKQDEKSKVEFAYYINCDSGGFYLAWHDKGSREQFRISTQNLALIQKYQVNELGKEIRPCRLKKRPPVRSIGGSKRTADGSEFEPKKKRKV

eNme2-C.NR (vEJS): [BPSV40-NLS](#), eNme2-C.NR , Linkers

David Liu Lab evolved Nme2Cas9 nuclease for N4CN PAM targeting with linker and nuclear localization signals in the same framework as Nme2- and Nme2^{Smu}Cas9 nucleases described in this work.

MKRTADGSEFESPKKKRKVEDA AFKP NPI YILGL DIGIA SVGWAM VEIDEEN PIRL IDLG VRFERA E VPKT GDSL AMARR LARS VRRLT
RRRAH RLL RARR LLKREG VLQA ADFD ENGL ITS PNT PWQL RAA ALDR KLT PLEWS AV LH LIK HRG YLS QRKNE GETAA KEL GALL KGVA
NNAHAL QTGDFRTPAELALNKFEKESGHIRNQRGDYSHTFSRKDLQAE LILLFEKQKEFGNPHVSGGLKEGIETLLMTQRPA LSGDAVQK
MLGHCT LEPTEPKA AKNTYTAERFIWLTKLNNLRILEQGSERPLTDTERSTLMDEPYRKS KLTYAQARKLLGLED TAFFKG LRYGKD NAEAS
TLMEMKAYH AISRALEKEGLDKKSPLNLSELQD EIGTAFSLFKT DEDITGRLKDRVQPEILEALLKHISFDKFVQISLKA LRIVPLM EQGKR
YDEACAEIYGVHYGKKNTEEKIYLPPIPADEIRNPVVLRALSQARKVINGVVR RYGS PARIHETAREVGKSF KDRKEIEKRQEE NRKDREKA
AAKFREYFPNFVGEPKSKDILKLRLYEQQHGKCL YSGKEINLVRN EKG YVEIDHALPF SRTWDDSFNNKVLV LGSENQNKG NQTPYEFN
GK DNSREWQEFKARVETSRP RSKKQ RILLQKFEDGFKECNLNDTRYVNRFLCQFVADHILLTGKGKRRVFA NGQITNLLRGFWGLRK
VRAENDRH HALDAVV VACSTVAMQQK ITRFVRYKEMNAFDGKTIDKETGKV LHQK THFPQPWEFFA QEV MIRVFGKPDGKPE FEEAD
TPEKLRTLLAEKLSSRPEAVHEYVTPLFVSAPNRKMSGAHKDTLRSAKRFVKHNEKISV KRVWL TEIKLADLENMVNYKNGREIELYEA
KARLEAYGGNAKQAFDPKDNPFYKKGGQLVKAVRVEKTQKSGVLLNKKNAYTIADNGDMVRDVFCVKDKKGK NQYFIVPI AWQVAE
NILPDIDCKGYRIDD SYTFCFSLHKYDLIAFQKDEKS KVEFAYYINCDS SGGFYLA WHDKG SREQFRISTQNLALIQKYQVNELGKEIPCR
LKKRPPVREDKRTADGSEFEPKKKRKV

Nme2-ABE8e-i1_linker10 (WT): BPSV40-NLS, nNme2Cas9, TadA8e, Linkers

MKRTADGSEFESPKKRKVEDMAAFKPNPINYLGLAIGIASVGWAMVEIDEEENPIRLIDLGVRFERAEVPKTGDLSMARRLARSVRLTRR
RAHRLLRARRLLKREGVLQAADFDENLIKSLPNTPWQLRAAALDRKLTPLEWSAVLLHLIKHRYGLSQRKNEGETADKELGALLKGVANNAHAL
QTGDFRTPAELALNKEFEKGIRNQRGDYSHTFSRKDLQAEILLFEKQKEFGNPHVSGGLKEGIETLLMTQRPALSGDAVQKMLGHCTFPEAE
PKAAKNTYTAERFIWLTKLNNLRILEQSGGSGGSGGSSEVEFSHEYWMRHALTLAKRARDEREVPVGAFLVLRNVRIGEGWNRAIGLHDPTAH
AEIMALRQGGLVMQNYRLIDATLYTFEPVCVMCAGAMIHSRIGRVVFGVRNSKRGAAAGSLMNVNYPGMNHRVEITEGILADECACALLCDFYR
MPRQVFNAQKKAQSSINETPGTSESATGSERPLTDTERATLMDEPYRKSCLTYAQARKLLGLEDTAFFKGLRYGKDNEAESTLMEMKAYHAISR
ALEKEGLKDKKSPNLSELQDEIGTAFSLFKTDEDITGRLKDRVQPEILEALLKHISFDKFVQISLKLRRIVPLMEQGKRYDEACAEIYGDHYGKKN
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RFVRYKEMNAFDGKTIDKETGKVLHQKTHFPQPWEFFAQEVIMRFGKPDGKPEFEEADTPEKLRTLAEKLSSRPEAVHEYVTPLFVSRAPNRK
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QESGVLLNKKNAYTIADNGDMVRDVFKVDKGKNQYFIVPIYAWQVAENILPDIDCKGYRIDDSTFCFLSHKYDLIAFKQDEKSKVEFAYYIN
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aaagtc

Nme2-ABE8e-i1_linker10 (E932R): BPSV40-NLS, nNme2Cas9, TadA8e, Linkers

MKRTADGSEFESPKKKRKVEDMAAFKPNIYILGLAIGIASVGWAMVEIDEEENPIRLIDLGVRFERAEVPKTGDLSMARRLARSVRLTRR
RAHRLRARRLLKREGVLQAADFDENLIKSLPNTPWQLRAAALDRKLTPLEWSAVLLHLIKHRYGLSQRKNEGETADKEGLALLKGVANNAHAL
QTGDFRTPAELALNKFKESEGHIRNQRGDYSHTFSRKDLQAEILLFEKQKEFGNPHVSGGLKEGIETLLMTQRPALSGDAVKMLGHCTFPEAE
PKAAKNTYTAERFIWLTKLNNLRILEQSGGSGGSGGSSEVEFSHEYWMRHALTAKARDEREVPVGAVLVLNNRVIGEGWNRAIGLHDPTAH
AEIMALRQGGLVMQNYRLIDATLYTFEPVCVMCAGAMIHSRIGRFFGVRNSKRGAAAGSLMNVLYPGMNRVIEITEGILADECACALLCDFYR
MPRQVFNAQKKAQSSINETPGTSESATGSERPLTDTERATLMDEPYRKSCLTYAQARKLLGLEDTAFFKGLRYGKDNEAESTLMEMKAYHAISR
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Nme2^{Smu}-ABE8e-i1_linker10 (WT): BPSV40-NLS, Nme2Cas9 – delta PID, TadA8e, SmuCas9 PID, Linkers

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Nme2^{Smu}-ABE8e-i1_linker10 (E932R): BPSV40-NLS, Nme2Cas9 – delta PID, TadA8e, SmuCas9 PID, Linkers

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eNme2-C-ABE8e-i1_linker10: [BPSV40-NLS](#), nNme2Cas9, [TadA8e](#), Linkers

David Liu Lab, eNme2-C ABE8e variant in domain-inlaid-i1 format

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Nme2-ABE8e-i8_linker10 (D56R): BPSV40-NLS, Nme2Cas9 – delta PID, TadA8e, SmuCas9 PID, Linkers

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Nme2^{Smu}-ABE8e-i8_linker10 (WT): BPSV40-NLS, Nme2Cas9 – delta PID, TadA8e, SmuCas9 PID, Linkers

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iNme2^{Smu}-ABE8e-nt: BPSV40-NLS, Nme2Cas9 – delta PID, TadA8e, SmuCas9 PID, Linkers

Jenifer Dounda Lab, iNme2Cas9 (D16A) variant with SmuCas9 PID swap in n-terminally fused ABE8e format

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ALRQGGGLVMQNRYLIDATLYVTFEPVCVMCAGAMIHSRIGRVFGVRNSKRGAAAGSLMVNLNPGMNHRVEITEGILADECAALLCDFYRMPR
QVFNAQKKAQSSINSGGSSGGSSGSETPGTSESATPESSGGSSGSMAAFKPNPINYILGLAIGIASVGWAMVEIDEEENPIRLIDLGVRFERAE
VPKTGDSLAMARRLARSVRLTRRRAHRLRARRLLKREGVLQAADFENDENGLIKSLPNTPWQLRAAALDRKLITLEWSAVLHLIKRGYLSRK
NEGETADKELGALLKGVANNAHALQTGDFRTPAELALNKFEKESGHIRNQRGDYSHTFSRKDLQAEILLFEKQKEFGNPHVSGGLKEGIETLLM
TQRPALSGDAVKMLGHCTFEPKAAKNTYTAERFIWLTKLNNLRILEQGSERPLTDTERATLMDEPYRKSCLKTYAQARKLLGEDTAFFKGL
RYGKDNEAESTLMECKAYHAISRALEKEGLDKKSPLNLSSELQDIEGTAFLSKTDEDITGRLKDRVQPEILEALLKHISFDKFVQISLKALRRIVPL
MEQGKRYDEACAEIYGDHYGKKNTEEKIYLPPADEIRNPVVRLALSQARKVINGVVRYYGSPARIHETAREVGKSFKDRKEIEKRQEENRKDR
KAAAKFREYFPNFVGEPKSKDILKLRLYEQQHGKCLYSGKEINLVRNEGYVEIDHALPSRTWDDSFNNKVLVLSHENQNKGNQTPYEYFNGK
DNSREWQEFKARVETSFRPRSKKQRILLQKFDEDGFKECNLNDTRYVNRFCLQFVADHILLTGKGKRRVFASNGQITNLLRGFWGLRKVRAEND
RHHALDAVVVACSTVAMQQKITRPFVRYKEMNAFDGKTIDKETGKVLHQKTHFPQPWEFFFAQEVIMIRVFGKPDGKPEFEEADTPEKLRTLLAEK
LSSRPEAVHEYVTPLFVSRAPNRKMSGAHGKTLRSAKRFVKhNEKISVKRWLTKIRLALENMVNYKNGREIELYEALKARLEAYGGNAKQAFD
PKGPNFYKKGGQLVKAVRVERTQKSGVLLNKKNAYTIADNATMVRDVYTAKGKNYLPVYVWQVAQGILPRAVTSGKSEADWDLIDESFE
FKFSLSRGDLVEMISNKGRIFGYYNGLDRANGSIGIREHDLEKSKGKDGVHRGBVKTATAFNKYHVDPGLGKEIHCSSEPRPTLKIKSKKEDKRTA
DGSEFEPKKKRKV

Nucleotide and amino acid sequences of deaminase linkers used in this study and their orientation with *n*Cas9 domains.

Nme2^{Smu}-ABE8e-i1 (WT): [BPSV40-NLS](#), Nme2Cas9 – delta PID, [TadA8e](#), [SmuCas9 PID](#), Deaminase Flanking Linkers

[MKRTADGSEFESPKKKRKV](#)[EDMAAFKPNPINYLGLAIGIASVGWAMVEIDEENPIRLIDLGVRFERAEVPKTGDLSMARRLARSVRRLTRRAHRLRARRLLKREGVLQAA](#)[FDENGLIKSLPNTPWQLRAALDRKLTPLEWSAVLHLIKHRYLSQRKNEGETADKELGALLKGVNNAHALQTGDFRTPAELALNKFEKESGHIRNQRGDYSHTFSRKDQLQAEIILLFEK](#)[QKEFGNPHVSGGLKEGIETLLMTQRPAQMLGHCTFEPKAQKNTYTAERFIWLTKLNNRL](#)[\(N_Link\)](#)[SEVEFSHEYWMRHALTLAKRARDEREVPGAVLVN](#)[NRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPVCVMCAGAMIHSRIGRVVFGVRNSKRGAGSLMNVLNYPGMNHRVEITEGILADECAALLCDFYRMP](#)[RQVFNAQKKAQSSIN](#)[\(C_Link\)](#)[GSERPLTDTERATLMDEPYRKS](#)[KLTYAQARKLLGLEDTAFFKGLRYKD](#)[DNAEASTL](#)[MEMKAYHAISRALEKEGLKD](#)[KSPLNLS](#)[SELQDEIGTAFSLF](#)[KTDEDITGR](#)[LKDRVQPEILEALLKHISFDKFVQISLKA](#)[RRI](#)[VPLMEQGKRYDEACAEIYGDHYGK](#)[KNTEEKYL](#)[LPPIPADEIRNPVVL](#)[RALSQARKVINGV](#)[VRRYGS](#)[PARIHETAREV](#)[GKSE](#)[KDR](#)[KEIEKR](#)[QEEN](#)[KDREKAAK](#)[FREY](#)[FPNV](#)[GEPKS](#)[KDILKLRLYEQQHGK](#)[CLYS](#)[GEINL](#)[VRLNE](#)[KGYVEID](#)[HALP](#)[FSRTWDDSFNN](#)[VVL](#)[GSENQNKG](#)[NQTPYE](#)[FNGK](#)[DN](#)[AFD](#)[GK](#)[TID](#)[KET](#)[GKVLHQ](#)[KTHFP](#)[QPWEFF](#)[AQEV](#)[MIR](#)[VFGK](#)[PDGK](#)[PEFE](#)[ADT](#)[PEKL](#)[RTLLAEKLSSR](#)[PEAV](#)[HEYV](#)[TPLF](#)[VSAP](#)[RNKMS](#)[GAHKDT](#)[LRS](#)[AKRFV](#)[KHNE](#)[KISV](#)[KRV](#)[WLT](#)[EIKLADLEN](#)[MVNY](#)[KNGREIEL](#)[YEALKAR](#)[LEAYGGNA](#)[QAFDP](#)[KDNPFY](#)[KKGGQL](#)[VKAVR](#)[VEKT](#)[QESGV](#)[VLLNKK](#)[NAYTIA](#)[DNATMVRDVY](#)[TAKG](#)[KNYLP](#)[PVYVWQ](#)[VAQGILPNRA](#)[TSGKSEADWDLIDES](#)[FEFKF](#)[SLRGDL](#)[VEMIS](#)[NKGRIFGYY](#)[NGLRANGSIGIREH](#)[DLEKSKG](#)[KDGV](#)[HRVG](#)[VKT](#)[TAFN](#)[KYHV](#)[DPLG](#)[KEI](#)[HRC](#)[SSEPR](#)[PTL](#)[KIKS](#)[KK](#)[ED](#)[KRTADGSEFEP](#)[KKR](#)

Nme2^{Smu}-ABE8e-i8 (WT): [BPSV40-NLS](#), Nme2Cas9 – delta PID, [TadA8e](#), [SmuCas9 PID](#), Deaminase Flanking Linkers

[MKRTADGSEFESPKKKRKV](#)[EDMAAFKPNPINYLGLAIGIASVGWAMVEIDEENPIRLIDLGVRFERAEVPKTGDLSMARRLARSVRRLTRRAHRLRARRLLKREGVLQAA](#)[FDENGLIKSLPNTPWQLRAALDRKLTPLEWSAVLHLIKHRYLSQRKNEGETADKELGALLKGVNNAHALQTGDFRTPAELALNKFEKESGHIRNQRGDYSHTFSRKDQLQAEIILLFEK](#)[QKEFGNPHVSGGLKEGIETLLMTQRPAQMLGHCTFEPKAQKNTYTAERFIWLTKLNNRL](#)[\(N_Link\)](#)[SEVEFSHEYWMRHALTLAKRARDEREVPGAVLVN](#)[NRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPVCVMCAGAMIHSRIGRVVFGVRNSKRGAGSLMNVLNYPGMNHRVEITEGILADECAALLCDFYRMP](#)[RQVFNAQKKAQSSIN](#)[\(C_Link\)](#)[GSERPLTDTERATLMDEPYRKS](#)[KLTYAQARKLLGLEDTAFFKGLRYKD](#)[DNAEASTL](#)[MEMKAYHAISRALEKEGLKD](#)[KSPLNLS](#)[SELQDEIGTAFSLF](#)[KTDEDITGR](#)[LKDRVQPEILEALLKHISFDKFVQISLKA](#)[RRI](#)[VPLMEQGKRYDEACAEIYGDHYGK](#)[KNTEEKYL](#)[LPPIPADEIRNPVVL](#)[RALSQARKVINGV](#)[VRRYGS](#)[PARIHETAREV](#)[GKSE](#)[KDR](#)[KEIEKR](#)[QEEN](#)[KDREKAAK](#)[FREY](#)[FPNV](#)[GEPKS](#)[KDILKLRLYEQQHGK](#)[CLYS](#)[GEINL](#)[VRLNE](#)[KGYVEID](#)[HALP](#)[FSRTWDDSFNN](#)[VVL](#)[GSENQNKG](#)[NQTPYE](#)[FNGK](#)[DN](#)[AFD](#)[GK](#)[TID](#)[KET](#)[GKVLHQ](#)[KTHFP](#)[QPWEFF](#)[AQEV](#)[MIR](#)[VFGK](#)[PDGK](#)[\(N_Link\)](#)[SEVEFSHEYWMRHALTLAKRARDEREVPGAVLVN](#)[NRVIGEGWNRAIGLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPVCVMCAGAMIHSRIGRVVFGVRNSKRGAGSLMNVLNYPGMNHRVEITEGILADECAALLCDFYRMP](#)[RQVFNAQKKAQSSIN](#)[\(C_Link\)](#)[EFEEADT](#)[PEKL](#)[RTLLAEKLSSR](#)[PEAV](#)[HEYV](#)[TPLF](#)[VSAP](#)[RNKMS](#)[GAHKDT](#)[LRS](#)[AKRFV](#)[KHNE](#)[KISV](#)[KRV](#)[WLT](#)[EIKLADLEN](#)[MVNY](#)[KNGREIEL](#)[YEALKAR](#)[LEAYGGNA](#)[QAFDP](#)[KDNPFY](#)[KKGGQL](#)[VKAVR](#)[VEKT](#)[QESGV](#)[VLLNKK](#)[NAYTIA](#)[DNATMVRDVY](#)[TAKG](#)[KNYLP](#)[PVYVWQ](#)[VAQGILPNRA](#)[TSGKSEADWDLIDES](#)[FEFKF](#)[SLRGDL](#)[VEMIS](#)[NKGRIFGYY](#)[NGLRANGSIGIREH](#)[DLEKSKG](#)[KDGV](#)[HRVG](#)[VKT](#)[TAFN](#)[KYHV](#)[DPLG](#)[KEI](#)[HRC](#)[SSEPR](#)[PTL](#)[KIKS](#)[KK](#)[ED](#)[KRTADGSEFEP](#)[KKR](#)

N-Linker Length	N-Linker AA. Seq	N-Linker nuc. Seq
20	GGSGGGGGGGGGGGGGGG	ggcggtatcaggaggctctggcggttcagggtggatcaggcggtacggcggtacggagggttcagggtgt
10	GGGGGGGGG	tctggcggttcagggtggatcaggcggtacgc
5	GGGGG	ggcggttcagggtggaa
5	GGGGG	ggcggttcagggtggaa
0	n/a	n/a

C-linker Length	C-Linker AA. Seq	C-Linker nuc. Seq
20	GSSGSETPGTSESATPESSG	ggctccctggctctgagacacctggcacaaggcagagcgcaacacctgaaagcagccgc
10	ETPGTSESAT	gagacacctggcacaaggcagagcgcaaca
5	GTSES	ggcacaaggcagagcg
0	n/a	n/a

Narrow window TadA deaminase variants used in this study

TAD9e: Tu et al. *Mol Ther.* 2022. DOI: 10.1016/j.ymthe.2022.07.010

TCTGAGGTGGAGTTTCCCACGAGTACTGGATGAGACATGCCCTGACCCCTGGCCAAGAGGGCACCGCATGAGAGGGAGGTGCCTGTGGAGCCGTGCTGGTGTGAACAATAGAGTGTGACATTGAGCCTTGCCTGATGTGCCCTGAGACAGGGCGGCCATGCAGAACTACAGACTGATTGACGCCACCTGTACGTGACATTGAGCCTTGCCTGATGTGCCCTGAGACAGGGCGGCCATGCAGAACTACAGACTGCGCAGGCTCCCTGATGAACGTGCTGAACCTACCCGGCATGAATAAGCACCGCGTCGAAATTACCGAGGGAATCCTGGCAGATGAATGTGCCGCCCTGCTGTGCGACTTCTACCGGATGCCTAGAAGAACAGGTGTTCAATGCTCAGAAGAAGGCCAGAGCTCCATCAAC

TAD9: Chen et al. *Nat Chem Biol.* 2023. DOI: 10.1038/s41589-022-01163-8.

TCTGAGGTGGAGTTTCCCACGAGTACTGGATGAGACATGCCCTGACCCCTGGCCAAGAGGGCACGGATGAGAGGGAGGTGCCTGTGGAGCCGTGCTGGTGCTGAACAATAGAGTGTGACGCCACCTGTACGTGACATTGAGCCTTGCCTGATGTGCCCTGAGACAGGGCGGCCCTGGTCATGCAGAACTACAGACTGATTGACGCCACCTGTACGTGACATTGAGCCTTGCCTGATGTGCCCTGAGACAGGGCGGCCATGCAGAACTACAGACTGCGCAGGCTCCCTGATGAACGTGCTGAACCTACCCGGCATGAATCACCGCGTCGAAATTACCGAGGGAATCCTGGCAGATGAATGTGCCGCCCTGCTGTGCGACTTCTACCGGTTGGCGTGAGGCAGTCAAAAGAGGCGCCGCAGGCTCCCTGATGAACGTGCTGAACCTACCCGGCATGAATCACCGCGTCGAAATTACCGAGGGAATCCTGGCAGATGAATGTGCCGCCCTGACCTGCGATTCTATCGGATGCCCTAGACAGGTGTTCAATGCTCAGAAGAAGGCCAGAGCTCCATCAAC