FIG. 17

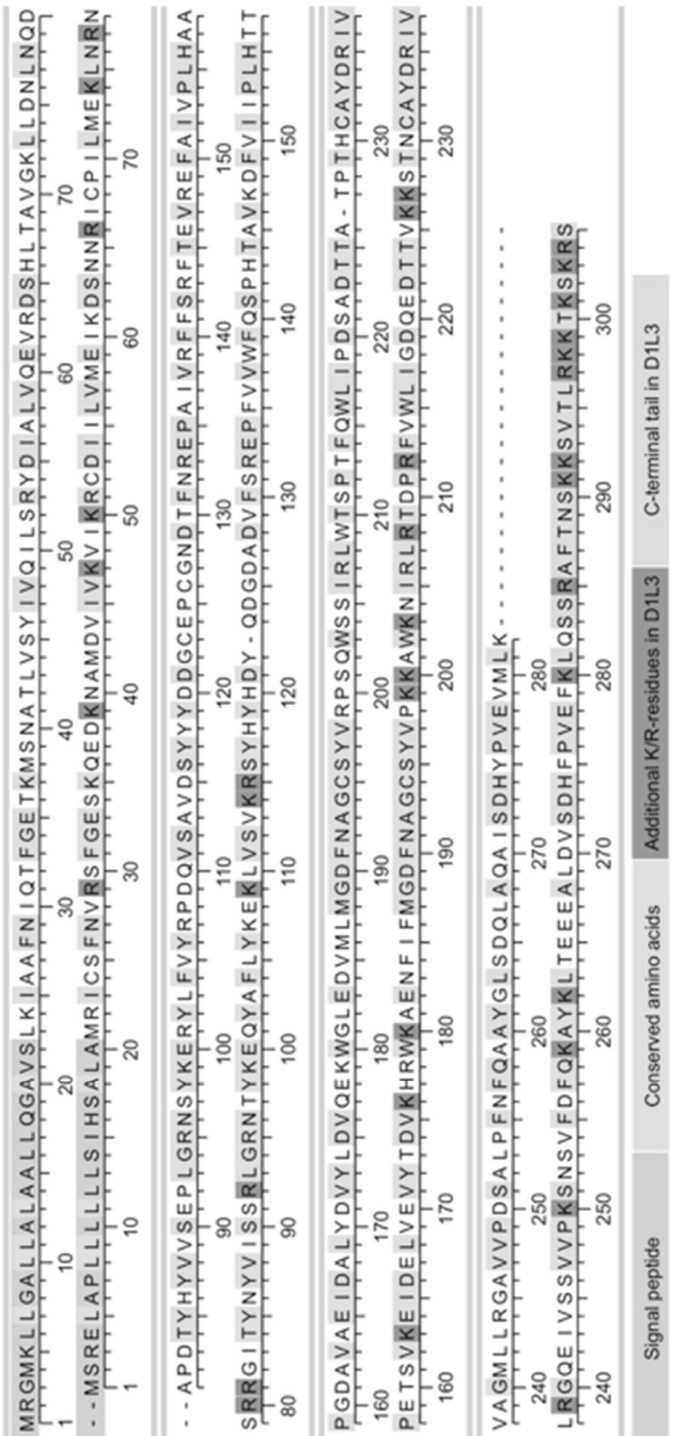

Human DNase1
Sequence Ruler
Human DNase1L3
Sequence Ruler
Signal peptide
Conserved amino acids
Additional K/R-residues in D1L3
C-terminal tail in D1L3

FIG. 19

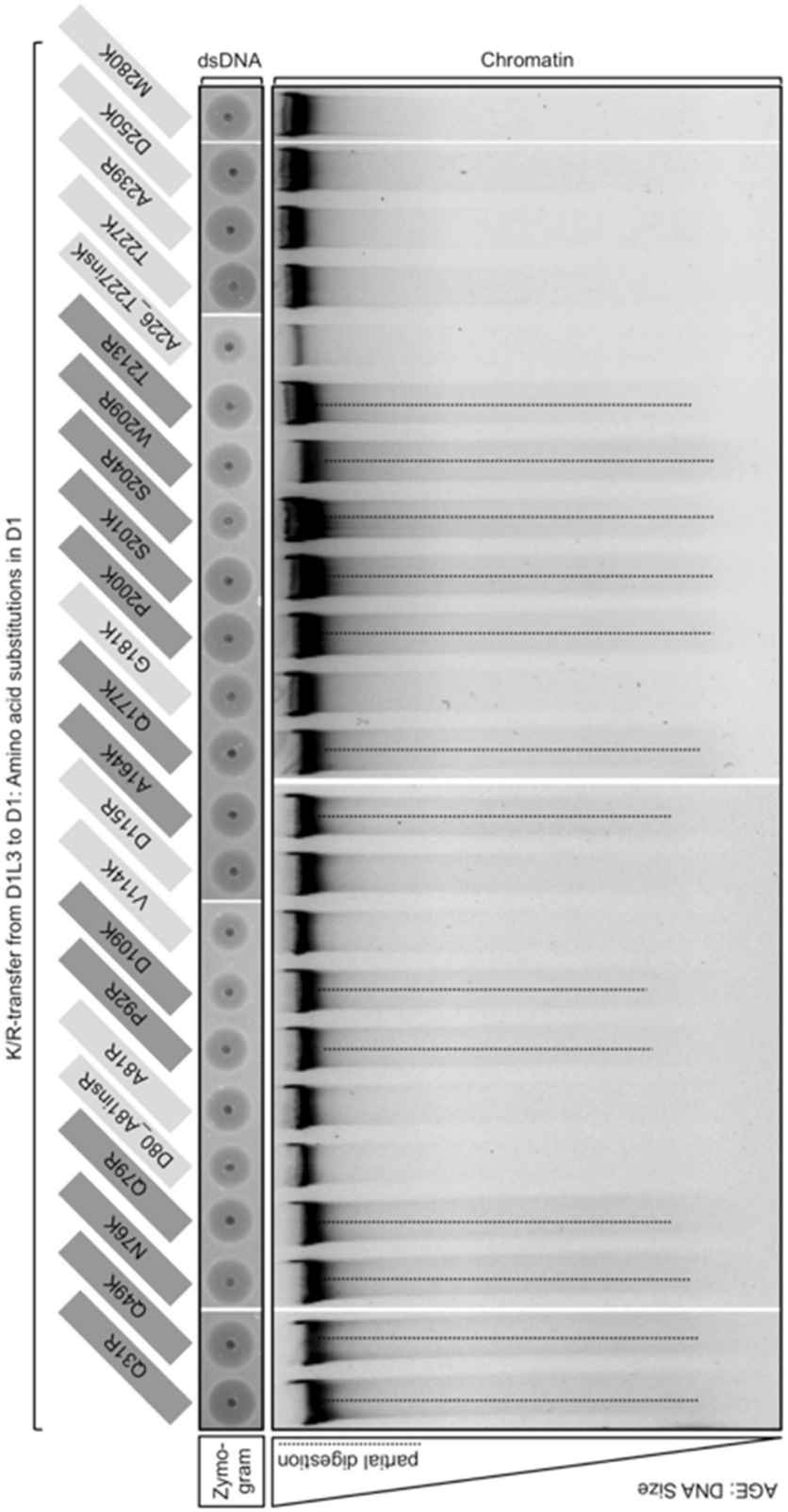

K/R-transfer from D1L3 to D1: Amino acid substitutions in D1
M280K
D250K
A239R
T227K
A226_T227insK
T213R
W209R
S204R
S201K
P200K
G181K
Q177K
A164K
D115R
V114K
D109K
P92R
A81R
D80_A81insR
C79R
N76K
Q49K
Q31R
dsDNA
Chromatin
Zymo-gram
partial digestion
AGE: DNA Size

FIG. 20

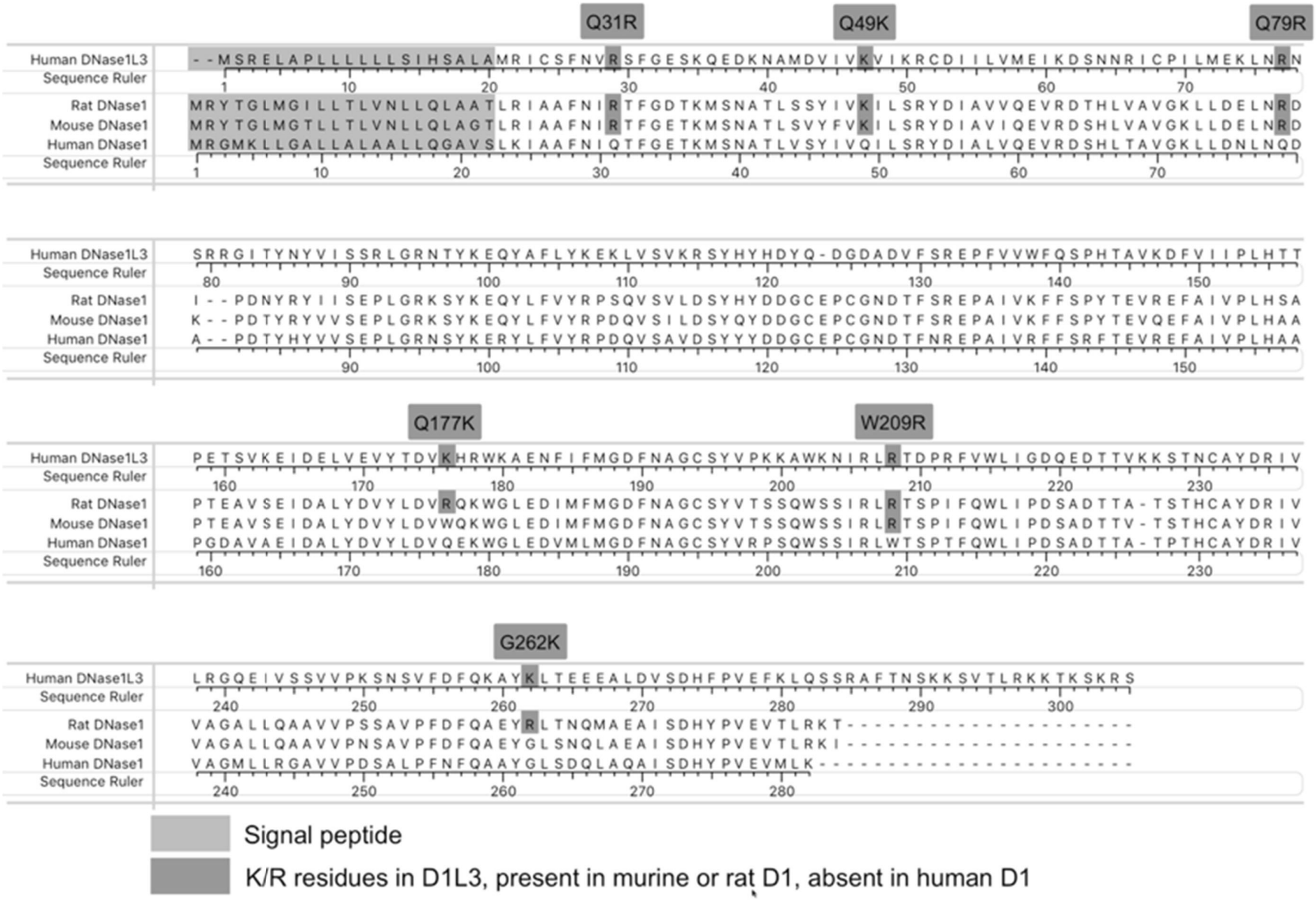

Q31R
Q49K
Q79R
Human DNase1L3
Sequence Ruler
Rat DNase1
Mouse DNase1
Human DNase1
Q177K
W209R
G262K
Signal peptide
K/R residues in D1L3, present in murine or rat D1, absent in human D1

FIG. 23


Human DNase1
Sequence Ruler
MRGMKLLGALLALAALLQGAVSLKIAAFNIQTFGETKMSNATLVSYIVQILSRYDIALVQEVRDSHLTAVGKLLDNLNQD
1 10 20 30 40 50 60 70
Human DNase1L3
Sequence Ruler
--MSRELAPLLLLLLSIHSALAMRICSFNVRSFGESKQEDKNAMDVIVKVIKRCDIILVMEIKDSNNRICPILMEKLNRN
1 10 20 30 40 50 60 70
Human DNase1
Sequence Ruler
--APDTYHYVVSEPLGRNSYKERYLFVYRPDQVSAVDSYYYDDGCEPCGNDTFNREPAIVRFFSRFTEVREFAIVPLHAA
90 100 110 120 130 140 150
Human DNase1L3
Sequence Ruler
SRRGITYNYVISSRLGRNTYKEQYAFLYKEKLVSVKRSYHYHDY-QDGDADVFSREPFVVWFQSPHTAVKDFVIIPLHTT
80 90 100 110 120 130 140 150
Human DNase1
Sequence Ruler
PGDAVAEIDALYDVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWSSIRLWTSPTFQWLIPDSADTTA-TPTHCAYDRIV
160 170 180 190 200 210 220 230
Human DNase1L3
Sequence Ruler
PETSVKEIDELVEVYTDVKHRWKAENFIFMGDFNAGCSYVPKKAWKNIRLRTDPRFVWLIGDQEDTTVKKSTNCAYDRIV
160 170 180 190 200 210 220 230
Human DNase1
Sequence Ruler
VAGMLLRGAVVPDSALPFNFQAAYGLSDQLAQAISDHYPVEVMLK----------
240 250 260 270 280
Human DNase1L3
Sequence Ruler
LRGQEIVSSVVPKSNSVFDFQKAYKLTEEEALDVSDHFPVEFKLQSSRAFTNSKKSVTLRKKTKSKRS
240 250 260 270 280 290 300
Signal peptide
Engineered AA in D1 [82 AA, 184 Mutations]
Engineered AA in D1, present in D1L3, no gain-of-function [4 Mutations]
Engineered AA in D1, present in D1L3, with gain-of-function [3 Mutations]

FIG. 24

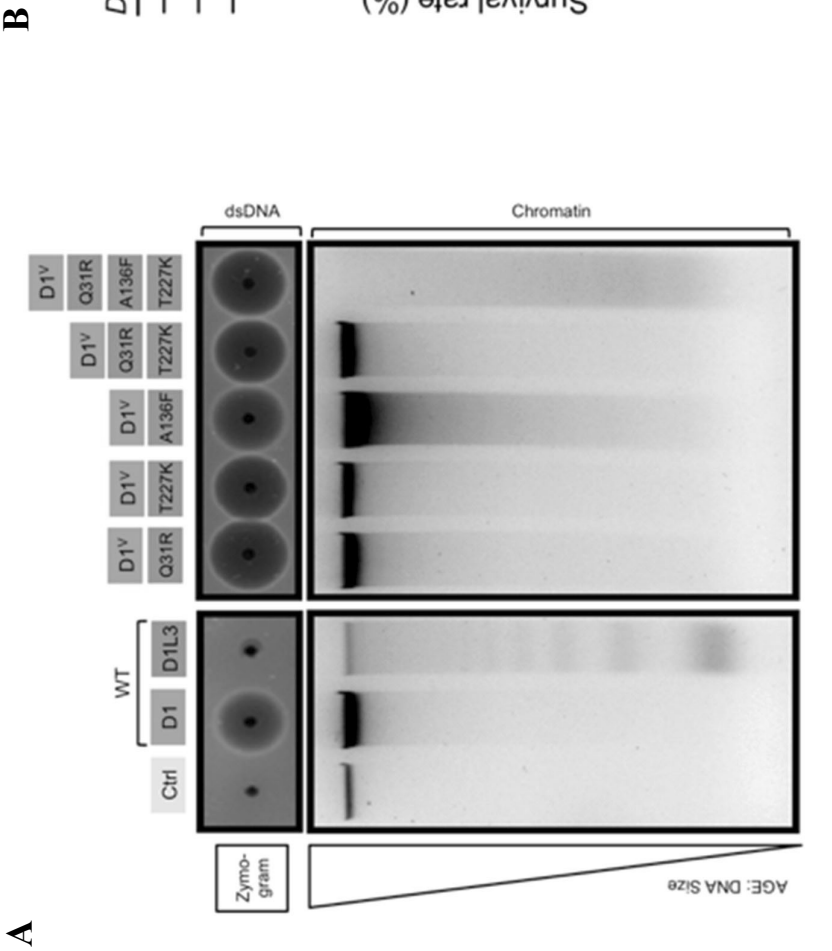

A
Zymo-gram
AGE: DNA Size
dsDNA
Chromatin
Ctrl
WT
D1
D1L3
D1V Q31R
D1V T227K
D1V A136F
D1V Q31R T227K
D1V Q31R A136F T227K
B
Dnase1-/- Dnase1l3-/-
Csf3 + Ctrl
Csf3 + [D1]
Csf3 + [D1] Q31R/A136F/T227K
P < 0.001
P < 0.05
Survival rate (%)
100
80
60
40
20
0
0
7
14
21
28
Time (d)

FIG. 29

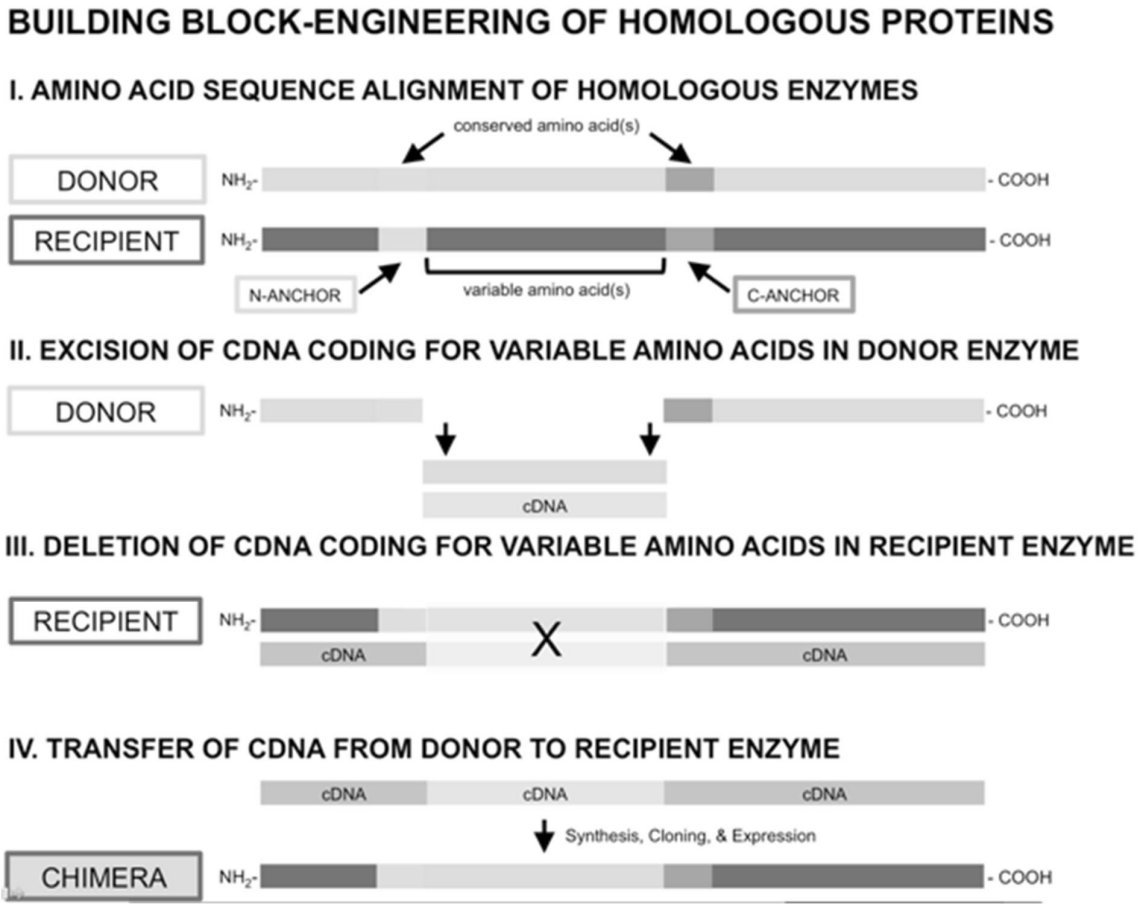

BUILDING BLOCK-ENGINEERING OF HOMOLOGOUS PROTEINS
I. AMINO ACID SEQUENCE ALIGNMENT OF HOMOLOGOUS ENZYMES
conserved amino acid(s)
DONOR
NH2-
- COOH
RECIPIENT
NH2-
- COOH
N-ANCHOR
variable amino acid(s)
C-ANCHOR
II. EXCISION OF CDNA CODING FOR VARIABLE AMINO ACIDS IN DONOR ENZYME
DONOR
NH2-
- COOH
cDNA
III. DELETION OF CDNA CODING FOR VARIABLE AMINO ACIDS IN RECIPIENT ENZYME
RECIPIENT
NH2-
- COOH
cDNA
X
cDNA
IV. TRANSFER OF CDNA FROM DONOR TO RECIPIENT ENZYME
cDNA
cDNA
cDNA
Synthesis, Cloning, & Expression
CHIMERA
NH2-
- COOH

FIG. 30

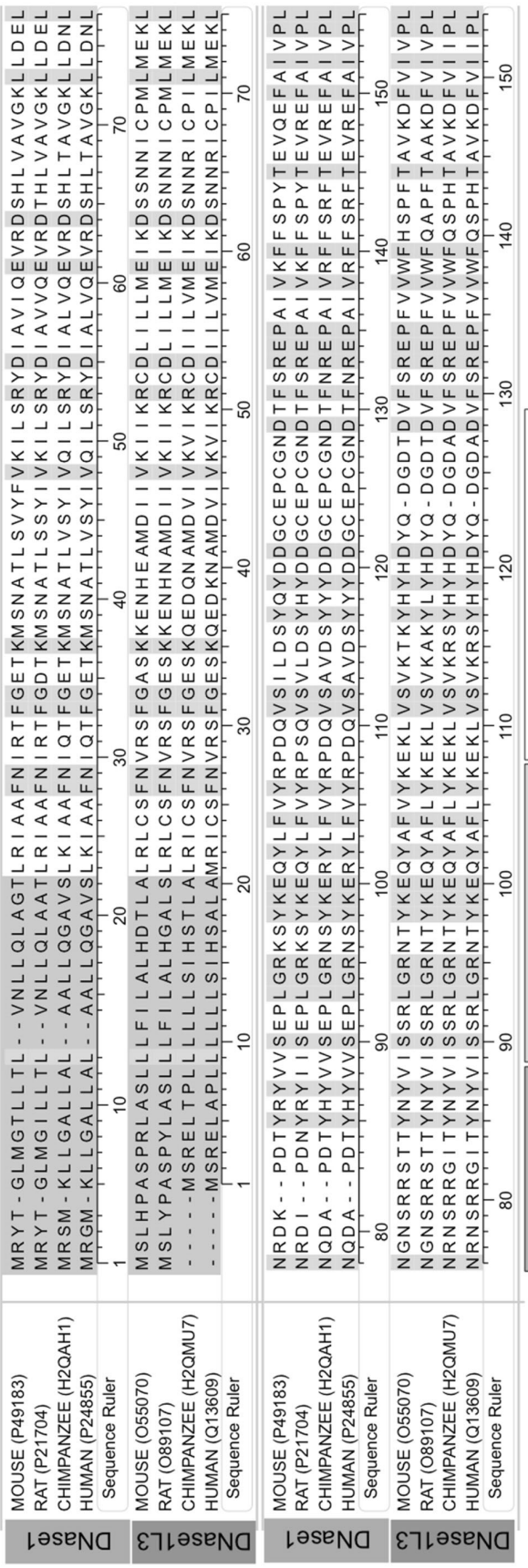

DNase1
MOUSE (P49183)
RAT (P21704)
CHIMPANZEE (H2QAH1)
HUMAN (P24855)
Sequence Ruler
DNase1L3
MOUSE (O55070)
RAT (O89107)
CHIMPANZEE (H2QMU7)
HUMAN (Q13609)
Sequence Ruler
DNase1
MOUSE (P49183)
RAT (P21704)
CHIMPANZEE (H2QAH1)
HUMAN (P24855)
Sequence Ruler
DNase1L3
MOUSE (O55070)
RAT (O89107)
CHIMPANZEE (H2QMU7)
HUMAN (Q13609)
Sequence Ruler
Signal Peptide
Conserved AA = Anchors
Variable AA = Building Blocks

FIG. 30 continued

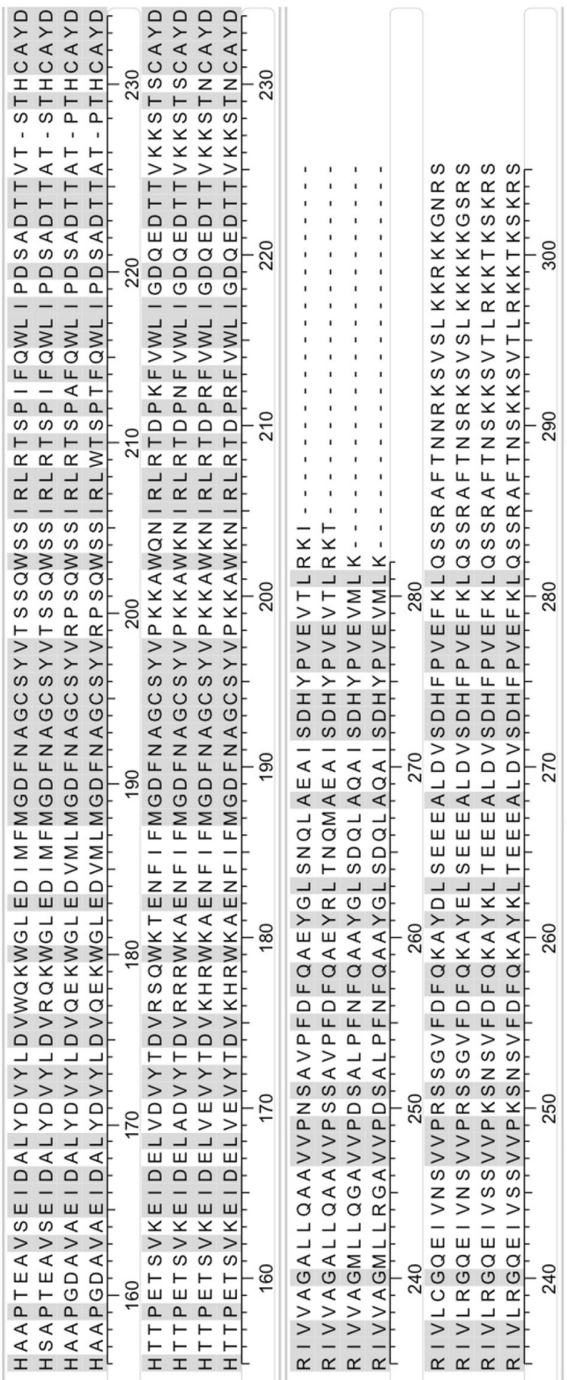


FIG. 31

A: Example of Building Block Engineering: human DNase1 (D1) variants with Building Blocks from human DNase1L3 (D1L3)

BB #	D1 N-Anchor: Conserved AA in D1	D1 C-Anchors: Conserved AA in D1	D1 Building Blocks: Variable AA in D1	D1L3 Building Blocks: Variable AA in D1L3	Building Block Mutations of D1 variants	BB Cluster
0	N/A	N/A	1-22 (MRGMKLL GALLALAALLQGAVS)	1-20 (MSRELAPLLLLLLSIHSALA)	M1_S22delinsMSRELAPLLL LLLSIHSALA	BB_0
1	Signal peptide	F28/N29	23-27 (LKIAA)	21-25 (MRICS)	L23_A27delinsMRICS	BB_{1-3}
2	F28/N29	F33/G34	30-32 (IQT)	28-30 (VRS)	I30_T32delinsVRS	
3	F33/G34	K37	35-36 (ET)	33-34 (ES)	E35_T36delinsES	
4	K37	V48	38-47 (MSNATLVSYI)	36-45 (QEDKNAMDVI)	M38_I47delinsQEDKNAMDVI	BB_4
5	V48	R53	49-52 (QILS)	47-50 (KVIK)	Q49_S52delinsKVIK	BB_{5-7}
6	R53	D55	54 (Y)	52 (C)	Y54C	
7	D55	E61	56-60 (IALVQ)	54-58 (IILVM)	I56_Q60delinsIILVM	
8	E61	D64	62-63 (VR)	60-61 (IK)	V62_R63delinsIK	BB_{8-10}
9	D64	L73	65-72 (SHLTAVGK)	63-70 (SNNRICPI)	S65_K72delinsSNNRICPI	
10	L73	L77/N78	74-76 (LDN)	72-74 (MEK)	L74_N76delinsMEK	
11	L77/N78	Y85	79-84 (QDAPDT)	77-84 (RNSRRGIT)	Q79_T84delinsRNSRRGIT	BB_{11}
12	Y85	Y87	86 (H)	86 (N)	H86N	BB_{12-14}
13	Y87	S90	88-89 (VV)	88-89 (VI)	V88_V89delinsVI	
14	S90	L93/G94/R95	91-92 (EP)	91-92 (SR)	E91_P92delinsSR	
15	L93/G94/R95	Y98/K99/E100	96-97 (NS)	96-97 (NT)	N96_S97delinsNT	BB_{15-18}
16	Y98/K99/E100	Y102	101 (R)	101 (Q)	R101Q	
17	Y102	F104	103 (L)	103 (A)	L103A	
18	F104	Y106	105 (V)	105 (L)	V105L	
19	Y106	V111/S112	107-110 (RPDQ)	107-110 (KEKL)	R107_Q110delinsKEKL	BB_{19-22}
20	Y111/S112	Y117	113-116 (AVDS)	113-116 (VKRS)	A113_S116delinsVKRS	
21	Y117	Y119	118 (Y)	118 (H)	Y118H	
22	Y119	D121	120 (D)	120 (H)	D120H	
23	D121	D129	122-128 (GCEPCGN)	122-127 (YQDGDA)	G122_N128delinsYQDGDA	BB_{23-25}
24	D129	F131	130 (T)	129 (V)	T130S	
25	F131	R133/E134/P135	132 (N)	131 (S)	N132S	
26	R133/E134/P135	V138	136-137 (AI)	135-136 (FV)	A136_I137delinsFV	BB_{26}
27	V138	F140	139 (R)	138 (W)	R139W	B_{27-34}
28	F140	T145	141-144 (FSRF)	140-143 (QSPH)	F141_F144delinsQSPH	
29	T145	F150	146-149 (EVRE)	145-148 (AVKD)	E146_E149delinsAVKD	
30	F150	I152	151 (A)	150 (V)	A151V	
31	I152	P154/L155/H156	153 (V)	152 (I)	V153I	
32	P154/L155/H156	P159	157-158 (AA)	156-157 (TT)	A157_A158delinsTT	
33	P159	V163	160-162 (GDA)	159-161 (ETS)	G160_A162delinsETS	
34	V163	E165/I166/D167	164 (A)	163 (K)	A164K	

FIG. 31 continued

A: continued

BB #	D1 N-Anchor: Conserved AA in D1	D1 C-Anchors: Conserved AA in D1	D1 Building Blocks: Variable AA in D1	D1L3 Building Blocks: Variable AA in D1L3	Building Block Mutations of D1 variants	BB Cluster
35	E165/I166/D167	L169	168 (A)	167 (E)	A168E	
36	L169	V172/Y173	170-171 (YD)	169-171 (VE)	Y170_D171delinsVE	BB_{35-37}
37	V172/Y173	D175/V176	174 (L)	173 (T)	L174T	
38	D175/V176	W180	177-179 (QEK)	176-178 (KHR)	Q177_K179delinsKHR	
39	W180	E183	181-182 (GL)	180-181 (KA)	G181_L182delinsKA	BB_{38-40}
40	E183	M187-GDFNAGCSY-V198	184-187 (DVML)	183-187 (NFIF)	D184_L187delinsNFIF	
41	M187-GDFNAGCSY-V198	W203	199-202 (RPSQ)	198-201 (PKKA)	R199_Q202delinsPKKA	
42	W203	I206/R207/L208	204-205 (SS)	203-204 (KN)	S204_S205delinsKN	
43	I206/R207/L208	T210	209 (W)	208 (R)	W209R	
44	T210	P212	211 (S)	210 (D)	S211D	BB_{41-48}
45	P212	F214	213 (T)	212 (R)	T213R	
46	F214	W216/L217/I218	215 (Q)	214 (V)	Q215V	
47	W216/L217/I218	D220	219 (P)	218 (G)	P219G	
48	D220	D223/T224/T225	221-222 (SA)	220-221 (QE)	S221_A222delinsQE	
49	D223/T224/T225	T229	226-228 (ATP)	225-228 (VKKS)	A226_P228delinsVKKS	BB_{49}
50	T229	C231-AYDRI-V237	230 (H)	230 (N)	H230N	
51	C231-AYDRI-V237	G240	238-239 (VA)	238-239 (LR)	V238_A239delinsLR	
52	G240	V247/V248/P249	241-246 (MLLRGA)	241-246 (QEIVSS)	M241_A246delinsQEIVSS	BB_{50-54}
53	V247/V248/P249	S251	250 (D)	250 (K)	D250K	
54	S251	F255	252-254 (ALP)	252-254 (NSV)	A252_P254delinsNSV	
55	F255	F257/Q258	256 (N)	256 (D)	N256D	
56	F257/Q258	Y261	259-260 (AA)	259-260 (KA)	A259_A260delinsKA	
57	Y261	L263	262 (G)	262 (K)	G262K	BB_{55-59}
58	L263	A268	264-267 (SDQL)	264-267 (TEEE)	S264_L267delinsTEEE	
59	A268	S272/D273/H274	269-271 (QAI)	269-271 (LDV)	Q269_I271delinsLDV	
60	S272/D273/H274	P276/V277/E278	275 (Y)	275 (F)	Y275F	
61	P276/V277/E278	L281	279-280 (VM)	279-280 (FK)	V279_M280delinsFK	
62	L281	-	282 (K)	282-305 (QSSRAFTNSKK SVTLRKKTKSKRS)	K282delinsQSSRAFTNSKKSVTLRKKTKSKRS	BB_{60-62}

FIG. 31 continued

B: **Example of Building Block Engineering:** human DNase1L3 (D1L3) variants with Building Blocks from human DNase1 (D1)

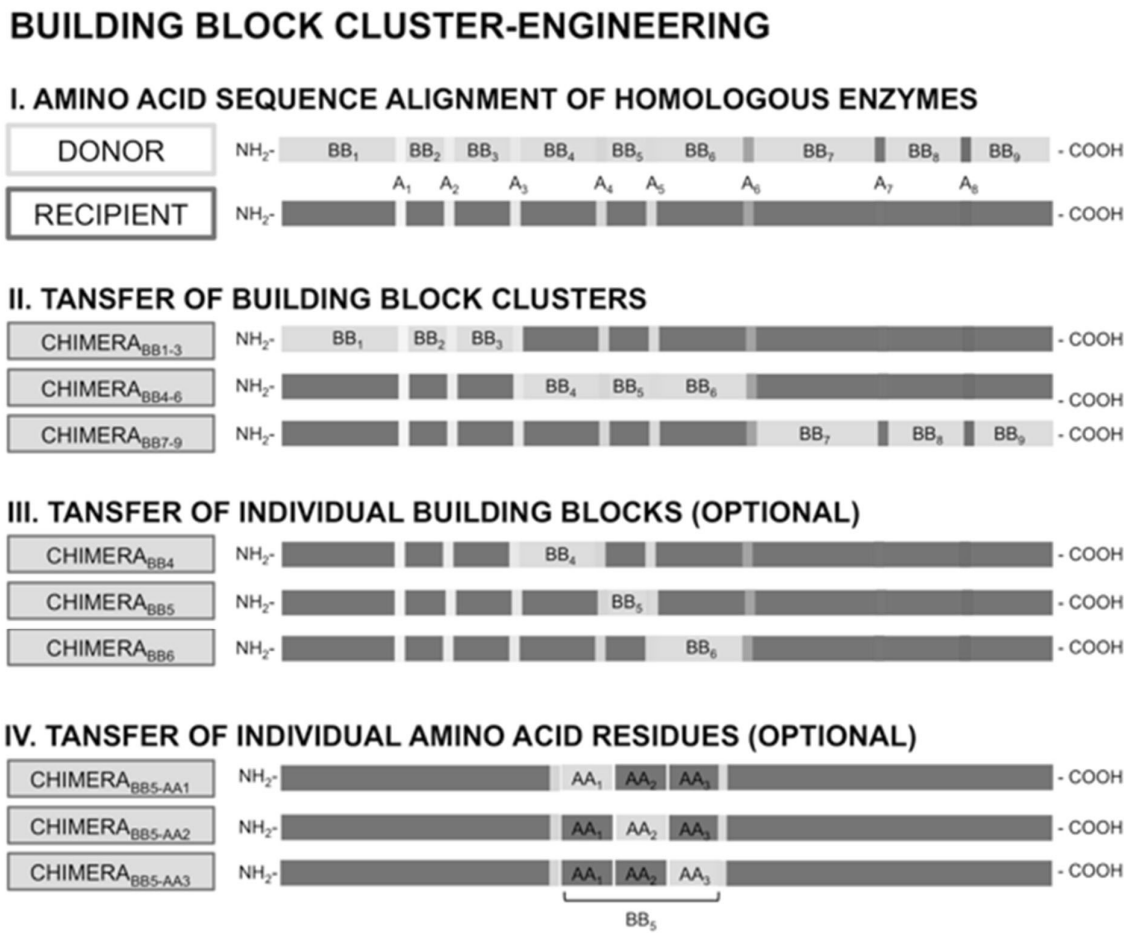
BB #	D1L3 N-Anchor: Conserved AA in D1	D1L3 C-Anchors: Conserved AA in D1	D1L3 Building Blocks: Variable AA in D1L3	D1 Building Blocks: Variable AA in D1	Building Block Mutations of D1L3 variants	BB Cluster
0	N/A	N/A	1-20 (MSRELAPLLLLLLSIHSALA)	1-22 (MRGMKLL GALLALAALLQGAVS)	M1_A20delinsMRGMKLLGALLALA ALLQGAVS	BB_0
1	Signal peptide	F26/N27	21-25 (MRICS)	23-27 (LKIAA)	M21_S25delinsLKIAA	BB_{1-3}
2	F26/N27	F31/G32	28-30 (VRS)	30-32 (IQT)	V28_S30delinsIQT	
3	F33/G34	K35	33-34 (ES)	35-36 (ET)	E33_S34delinsET	
4	K35	V46	36-45 (QEDKNAMDVI)	38-47 (MSNATLVSYI)	Q36_I45delinsMSNATLVSYI	BB_4
5	V46	R51	47-50 (KVIK)	49-52 (QILS)	K47_K50delinsQILS	BB_{5-7}
6	R51	D53	52 (C)	54 (Y)	C52Y	
7	D53	E59	54-58 (IILVM)	56-60 (IALVQ)	I54_M58delinsIALVQ	
8	E59	D62	60-61 (IK)	62-63 (VR)	I60_K61delinsVR	BB_{8-10}
9	D62	L71	63-70 (SNNRICPI)	65-72 (SHLTAVGK)	S63_I70delinsSHLTAVGK	
10	L71	L75/N76	72-74 (MEK)	74-76 (LDN)	M72_K74delinsLDN	
11	L75/N76	Y85	77-84 (RNSRRGIT)	79-84 (QDAPDT)	R77_T84delinsQDAPDT	BB_{11}
12	Y85	Y87	86 (N)	86 (H)	N86H	BB_{12-14}
13	Y87	S90	88-89 (VI)	88-89 (VV)	V88_I89delinsVV	
14	S90	L93/G94/R95	91-92 (SR)	91-92 (EP)	S91_R92delinsEP	
15	L93/G94/R95	Y98/K99/E100	96-97 (NT)	96-97 (NS)	N96_T97delinsNS	BB_{15-18}
16	Y98/K99/E100	Y102	101 (Q)	101 (R)	Q101R	
17	Y102	F104	103 (A)	103 (L)	A103L	
18	F104	Y106	105 (L)	105 (V)	L105V	
19	Y106	V111/S112	107-110 (KEKL)	107-110 (RPDQ)	K107_L110delinsRPDQ	BB_{19-22}
20	Y111/S112	Y117	113-116 (VKRS)	113-116 (AVDS)	V113_S116delinsAVDS	
21	Y117	Y119	118 (H)	118 (Y)	H118Y	
22	Y119	D121	120 (H)	120 (D)	H120D	
23	D121	D128	122-127 (YQDGDA)	122-128 (GCEPCGN)	Y122_A127delinsGCEPCGN	BB_{23-25}
24	D128	F130	129 (V)	130 (T)	V129T	
25	F130	R132/E133/P134	131 (S)	132 (N)	S131N	
26	R132/E133/P134	V137	135-136 (FV)	136-137 (AI)	135F_136VdelinsAI	BB_{26}
27	V137	F139	138 (W)	139 (R)	W138R	B_{27-34}
28	F139	T144	140-143 (QSPH)	141-144 (FSRF)	Q140_H143delinsFSRF	
29	T144	F149	145-148 (AVKD)	146-149 (EVRE)	A145_D148delinsAVKD	
30	F149	I151	150 (V)	151 (A)	V150A	
31	I151	P153/L154/H155	152 (I)	153 (V)	I152A	
32	P153/L154/H155	P157	156-157 (TT)	157-158 (AA)	T156_T157delinsAA	
33	P157	V162	159-161 (ETS)	160-162 (GDA)	E159_S161delinsGDA	
34	V162	E164/I165/D166	163 (K)	164 (A)	K163A	

FIG. 31 continued

B: continued

BB #	D1L3 N-Anchor: Conserved AA in D1L3	D1L3 C-Anchors: Conserved AA in D1L3	D1L3 Building Blocks: Variable AA in D1L3	D1 Building Blocks: Variable AA in D1	Building Block Mutations of D1L3 variants	BB Cluster
35	E164/I165/D166	L168	167 (E)	168 (A)	E167A	BB_{35-37}
36	L168	V171/Y172	169-170 (VE)	170-171 (YD)	V169_E170delinsYD	
37	V171/Y172	D174/V175	173 (T)	174 (L)	T173L	
38	D174/V175	W179	176-178 (KHR)	177-179 (QEK)	K176_R178delinsQEK	BB_{38-40}
39	W179	E182	180-181 (KA)	181-182 (GL)	K180_A181delinsGL	
40	E182	M187-GDFNAGCSY-V197	183-186 (NFIF)	184-187 (DVML)	N183_F186delinsDVML	
41	M187-GDFNAGCSY-V197	W202	198-201 (PKKA)	199-202 (RPSQ)	198P_201AdelinsRPSQ	BB_{41-48}
42	W202	I205/R206/L207	203-204 (KN)	204-205 (SS)	K203_N204delinsSS	
43	I205/R206/L207	T209	208 (R)	209 (W)	R208W	
44	T209	P211	210 (D)	211 (S)	D210S	
45	P211	F213	212 (R)	213 (T)	R212T	
46	F213	W215/L216/I217	214 (V)	215 (Q)	V214Q	
47	W215/L216/I217	D219	218 (G)	219 (P)	G218P	
48	D219	D222/T223/T224	220-221 (QE)	221-222 (SA)	Q220_E221delinsSA	
49	D222/T223/T224	T229	225-228 (VKKS)	226-228 (ATP)	V225_S228delinsATP	BB_{49}
50	T229	C231-AYDRI-V237	230 (N)	230 (H)	N230H	BB_{50-54}
51	C231-AYDRI-V237	G240	238-239 (LR)	238-239 (VA)	L238_R239delinsVA	
52	G240	V247/V248/P249	241-246 (QEIVSS)	241-246 (MLLRGA)	Q241_S246delinsMLLRGA	
53	V247/V248/P249	S251	250 (K)	250 (D)	K250D	
54	S251	F255	252-254 (NSV)	252-254 (ALP)	N252_V254delinsALP	
55	F255	F257/Q258	256 (D)	256 (N)	D256N	BB_{55-59}
56	F257/Q258	Y261	259-260 (KA)	259-260 (AA)	K259_A260delinsAA	
57	Y261	L263	262 (K)	262 (G)	K262G	
58	L263	A268	264-267 (TEEE)	264-267 (SDQL)	T264_E267delinsSDQL	
59	A268	S272/D273/H274	269-271 (LDV)	269-271 (QAI)	L269_V271delinsQAI	
60	S272/D273/H274	P276/V277/E278	275 (F)	275 (Y)	F275Y	BB_{60-62}
61	P276/V277/E278	L281	279-280 (FK)	279-280 (VM)	F279_K280delinsVM	
62	L281	-	282-305 (QSSRAFTNSKK SVTLRKKTKSKRS)	282 (K)	Q282_S305delinsK	

FIG. 32


BUILDING BLOCK CLUSTER-ENGINEERING
I. AMINO ACID SEQUENCE ALIGNMENT OF HOMOLOGOUS ENZYMES
DONOR
RECIPIENT
NH2-
- COOH
BB1 BB2 BB3 BB4 BB5 BB6 BB7 BB8 BB9
A1 A2 A3 A4 A5 A6 A7 A8
II. TANSFER OF BUILDING BLOCK CLUSTERS
CHIMERA BB1-3
CHIMERA BB4-6
CHIMERA BB7-9
III. TANSFER OF INDIVIDUAL BUILDING BLOCKS (OPTIONAL)
CHIMERA BB4
CHIMERA BB5
CHIMERA BB6
IV. TANSFER OF INDIVIDUAL AMINO ACID RESIDUES (OPTIONAL)
CHIMERA BB5-AA1
CHIMERA BB5-AA2
CHIMERA BB5-AA3
AA1 AA2 AA3
BB5

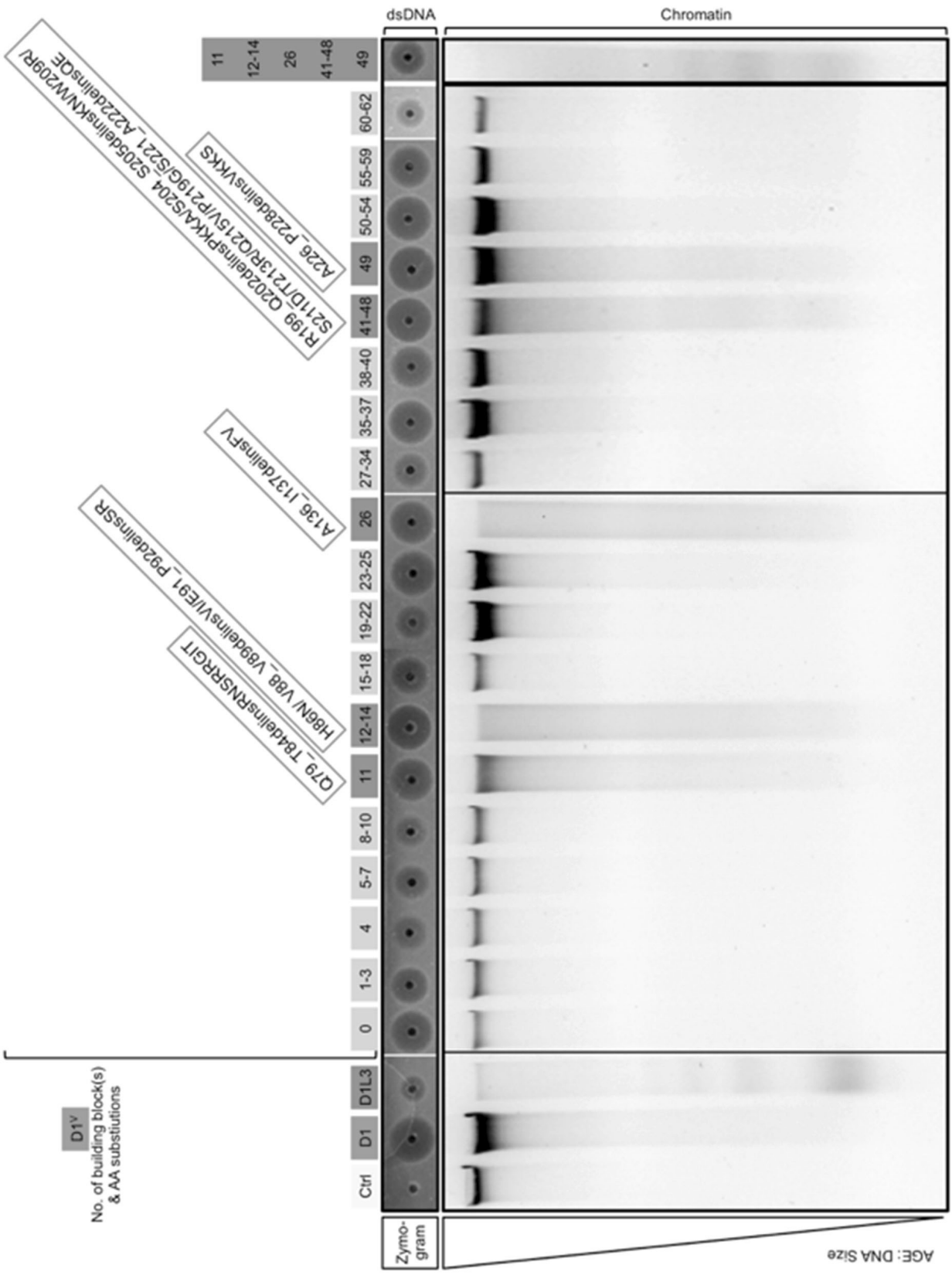
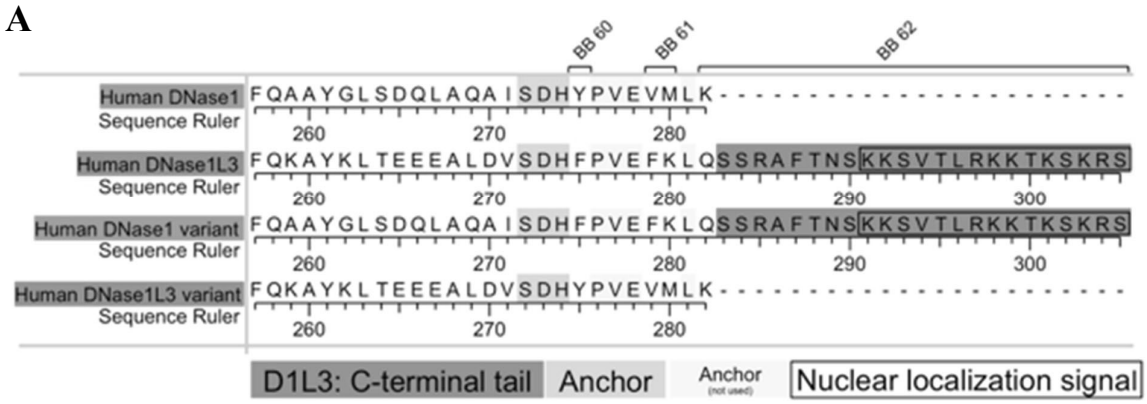

Zymo-gram
dsDNA
Chromatin
AGE: DNA Size
Ctrl
D1
D1L3
0
1-3
4
5-7
8-10
11
12-14
15-18
19-22
23-25
26
27-34
35-37
38-40
41-48
49
50-54
55-59
60-62
11
12-14
26
41-48
49
D1V
No. of building block(s) & AA substiutions
Q79_T84delinsRNSRRGIT
H86N/ V88_V89delinsVI/E91_P92delinsSR
A136_I137delinsFV
R199_Q202delinsPKKA/S204_S205delinsKN/W209RV
S211D/T213R/Q215V/P219G/S221_A222delinsQE
A226_P228delinsVKKS

FIG. 33

FIG. 34


A
BB 60
BB 61
BB 62
Human DNase1
Sequence Ruler
FQAAYGLSDQLAQAISDHYPVEVMLK
260
270
280
Human DNase1L3
Sequence Ruler
FQKAYKLTEEEALDVSDHFPVEFKLQSSRAFTNSKKSVTLRKKTKSKRS
260
270
280
290
300
Human DNase1 variant
Sequence Ruler
FQAAYGLSDQLAQAISDHFPVEFKLQSSRAFTNSKKSVTLRKKTKSKRS
260
270
280
290
300
Human DNase1L3 variant
Sequence Ruler
FQKAYKLTEEEALDVSDHYPVEVMLK
260
270
280
D1L3: C-terminal tail
Anchor
Anchor
(not used)
Nuclear localization signal

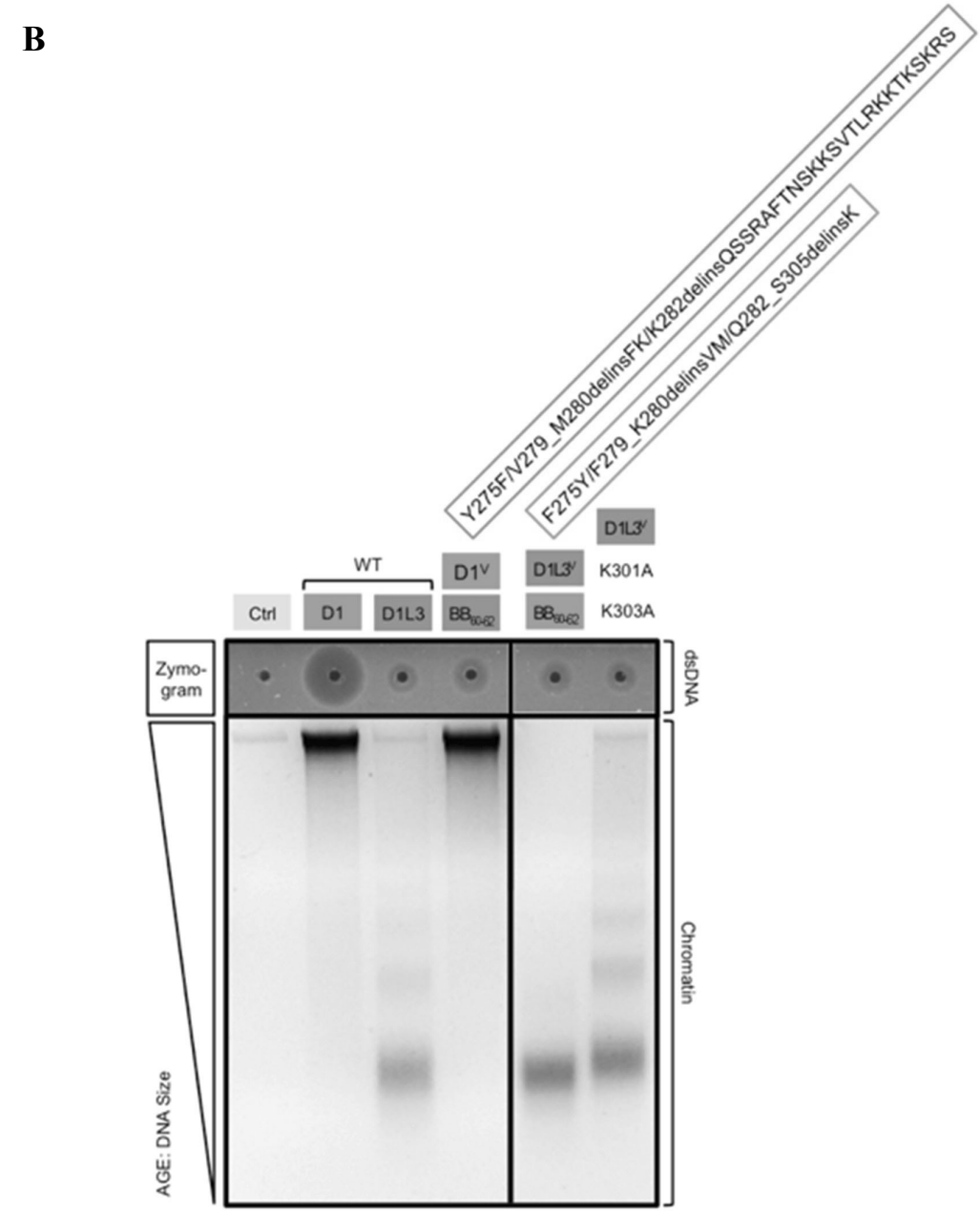

B
Y275F/N279_M280delinsFK/K282delinsQSSRAFTNSKKSVTLRKKTKSKRS
F275Y/F279_K280delinsVM/Q282_S305delinsK
D1L3^{Y}
WT
D1^{V}
D1L3^{Y}
K301A
Ctrl
D1
D1L3
BB$_{60-62}$
BB$_{60-62}$
K303A
Zymo-gram
dsDNA
Chromatin
AGE: DNA Size

FIG. 35

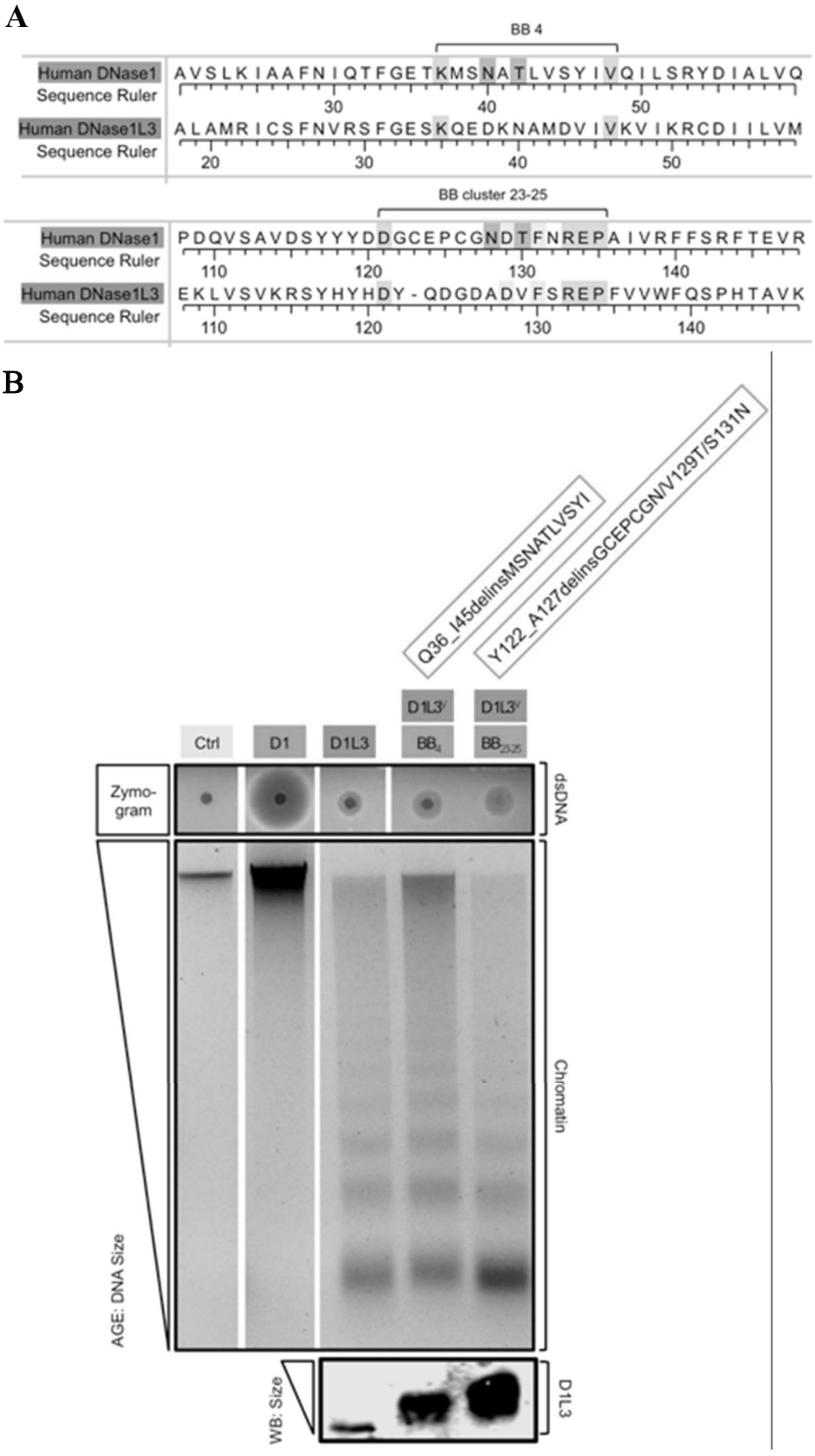

A
BB 4
Human DNase1
Sequence Ruler
A V S L K I A A F N I Q T F G E T K M S N A T L V S Y I V Q I L S R Y D I A L V Q
30
40
50
Human DNase1L3
Sequence Ruler
A L A M R I C S F N V R S F G E S K Q E D K N A M D V I V K V I K R C D I I L V M
20
30
40
50
BB cluster 23-25
Human DNase1
Sequence Ruler
P D Q V S A V D S Y Y Y D D G C E P C G N D T F N R E P A I V R F F S R F T E V R
110
120
130
140
Human DNase1L3
Sequence Ruler
E K L V S V K R S Y H Y H D Y - Q D G D A D V F S R E P F V V W F Q S P H T A V K
110
120
130
140
B
Q36_I45delinsMSNATLVSYI
Y122_A127delinsGCEPCGN/V129T/S131N
D1L3
D1L3
Ctrl
D1
D1L3
BB4
BB23-25
Zymo-gram
dsDNA
Chromatin
AGE: DNA Size
WB: Size
D1L3

Fig. 36

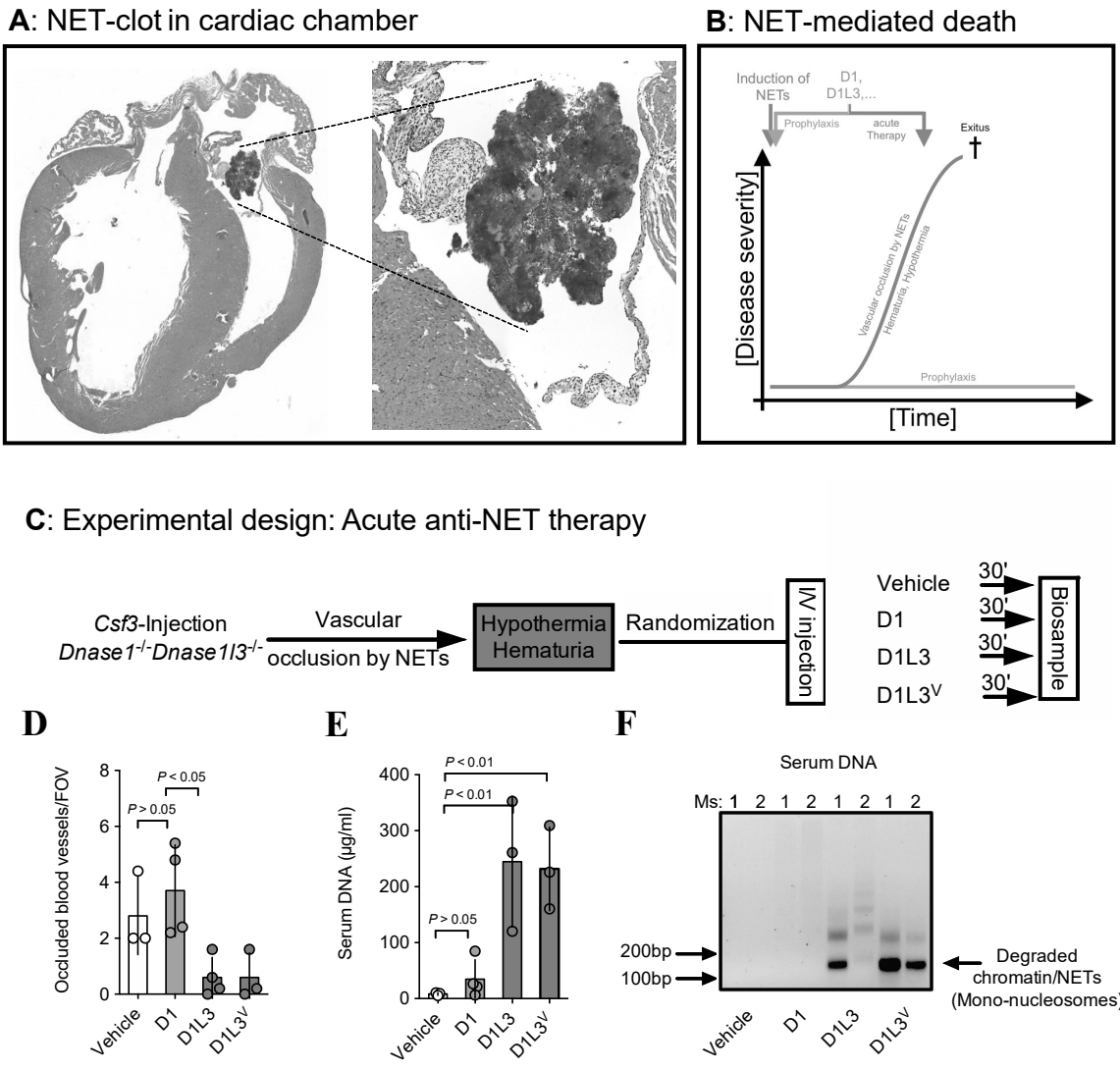

A: NET-clot in cardiac chamber
B: NET-mediated death
Induction of NETs
D1, D1L3,...
Prophylaxis
acute Therapy
Exitus
[Disease severity]
Vascular occlusion by NETs
Hematuria Hypothermia
Prophylaxis
[Time]
C: Experimental design: Acute anti-NET therapy
Csf3-Injection
Dnase1-/-Dnase1l3-/-
Vascular occlusion by NETs
Hypothermia Hematuria
Randomization
I/V injection
Vehicle
D1
D1L3
D1L3V
30'
Biosample
D
Occluded blood vessels/FOV
P > 0.05
P < 0.05
Vehicle
D1
D1L3
D1L3V
E
Serum DNA (µg/ml)
P < 0.01
P < 0.01
P > 0.05
F
Serum DNA
Ms: 1 2 1 2 1 2 1 2
200bp
100bp
Degraded chromatin/NETs (Mono-nucleosomes)

FIG. 39

SEQ ID NO	Description	**Mutation** (Mature protein without signal peptide)	Building Block Engineering	Parental SEQ ID NO
5	DNase1 variant	**Q31R/Q49K/Q79K/W209K** (Q9R/Q27K/Q57K/W187K)	-	1
6	DNase1 variant	**Q31R/Q49K/Q79K/Q177K/W209K/G262K** (Q9R/Q27K/Q57K/Q155K/W187K/G240K)	-	1
7	DNase1 variant	**Q31R/A136F/T227K** (Q9R/A114F/T205K)	-	1
9	DNase1 variant	**A136F/A164K** (A114F/A142K)	-	1
10	DNase1 variant	**Q79_T84delinsRNSRRGIT** (Q57_T62delinsRNSRRGIT)	+ (BB_{11})	1
11	DNase1 variant	**H86N/V88_V89delinsVI/E91_P92delinsSR** (H64N/V66_V67delinsVI/E69_P70delinsSR)	+ (BB_{12-14})	1
12	DNase1 variant	**A136_I137delinsFV** (A114_I115delinsFV)	+ (BB_{26})	1
13	DNase1 variant	**R199_Q202delinsPKKA/S204_S205delinsKN/W209R/S211D/T213R/Q215V/ P219G/S221_A222delinsQE** (R177_Q180delinsPKKA/S182_S183delinsKN/W187R/S189D/T191R/Q193V/ P197G/S199_A200delinsQE)	+ (BB_{41-48})	1
14	DNase1 variant	**A226_P228delinsVKKS** (A204_P206delinsVKKS)	+ (BB_{49})	1
15	DNase1 variant	**Q79_T84delinsRNSRRGIT/H86N/V88_V89delinsVI/E91_P92delinsSR/ A136_I137delinsFV/ R199_Q202delinsPKKA/S204_S205delinsKN/W209R/S211D/ T213R/Q215V/P219G/S221_A222delinsQE/A226_P228delinsVKKS** (Q57_T62delinsRNSRRGIT/H64N/V66_V67delinsVI/E69_P70delinsSR/ A114_I115delinsFV/R177_Q180delinsPKKA/S182_S183delinsKN/W187R/S189D/ T191R/Q193V/P197G/S199_A200delinsQE/ A204_P206delinsVKKS)	+ (BB_{11}, BB_{12-14}, BB_{26}, BB_{41-48}, BB_{49})	1
17	DNase1L3 variant	**F275Y/F279_K280delinsVM/Q282_S305delinsK** (F255Y/F259_K260delinsVM/Q262_S285delinsK)	+ (BB_{60-62})	2
19	DNase1L3 variant	**Q36_I45delinsMSNATLVSYI** (Q16_I25delinsMSNATLVSYI)	+ (BB_{4})	2
20	DNase1L3 variant	**Y122_A127delinsGCEPCGN/V129T/S131N** (Y102_A107delinsGCEPCGN/V109T/S111N)	+ (BB_{23-25})	2