FIG. 1A- FIG. 1F

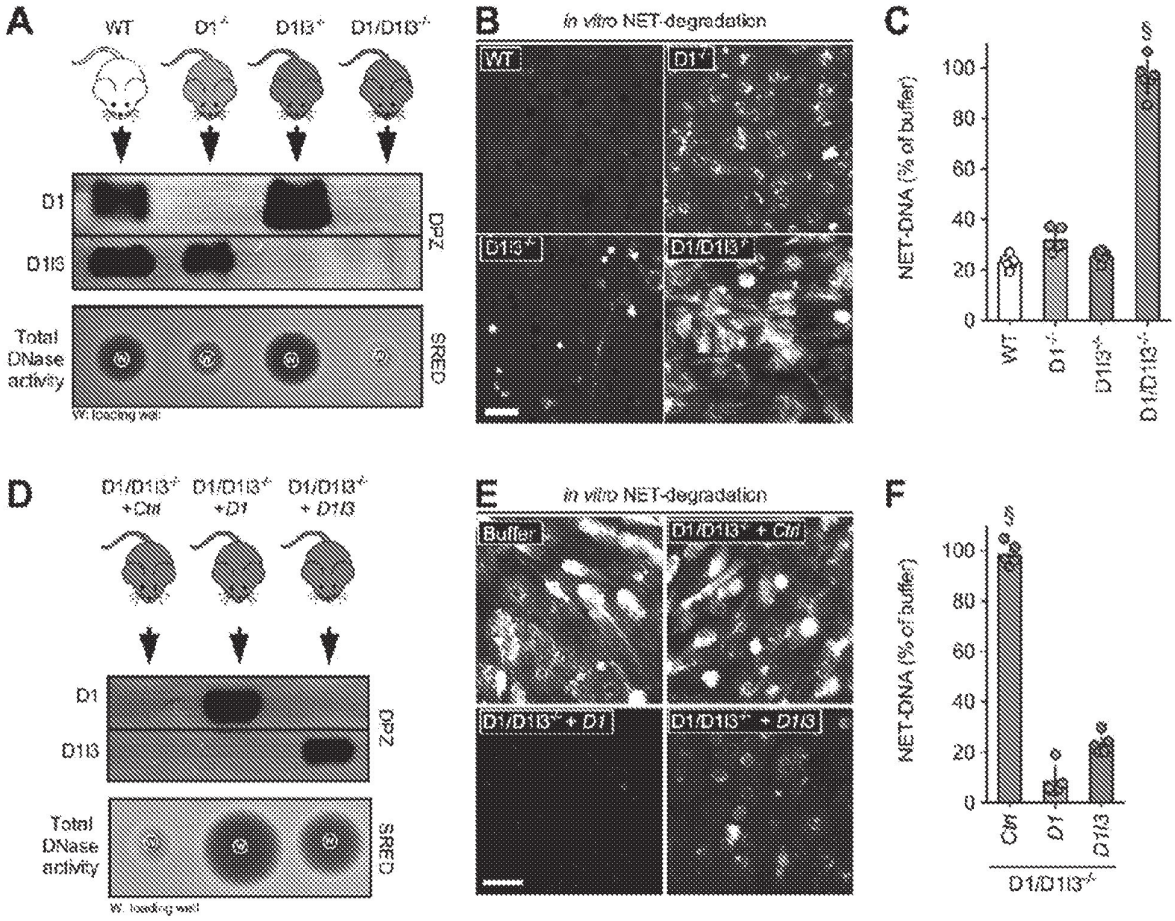

A
WT
D1-/-
D1I3-/-
D1/D1I3-/-
D1
D1I3
DpZ
Total DNase activity
SRED
B
in vitro NET-degradation
WT
D1-/-
D1I3-/-
D1/D1I3-/-
C
NET-DNA (% of buffer)
WT
D1-/-
D1I3-/-
D1/D1I3-/-
D
D1/D1I3-/- +Ctrl
D1/D1I3-/- +D1
D1/D1I3-/- +D1I3
D1
D1I3
DpZ
Total DNase activity
SRED
E
in vitro NET-degradation
Buffer
D1/D1I3-/- + Ctrl
D1/D1I3-/- + D1
D1/D1I3-/- + D1I3
F
NET-DNA (% of buffer)
Ctrl
D1
D1I3
D1/D1I3-/-

FIG. 2A- FIG.2I

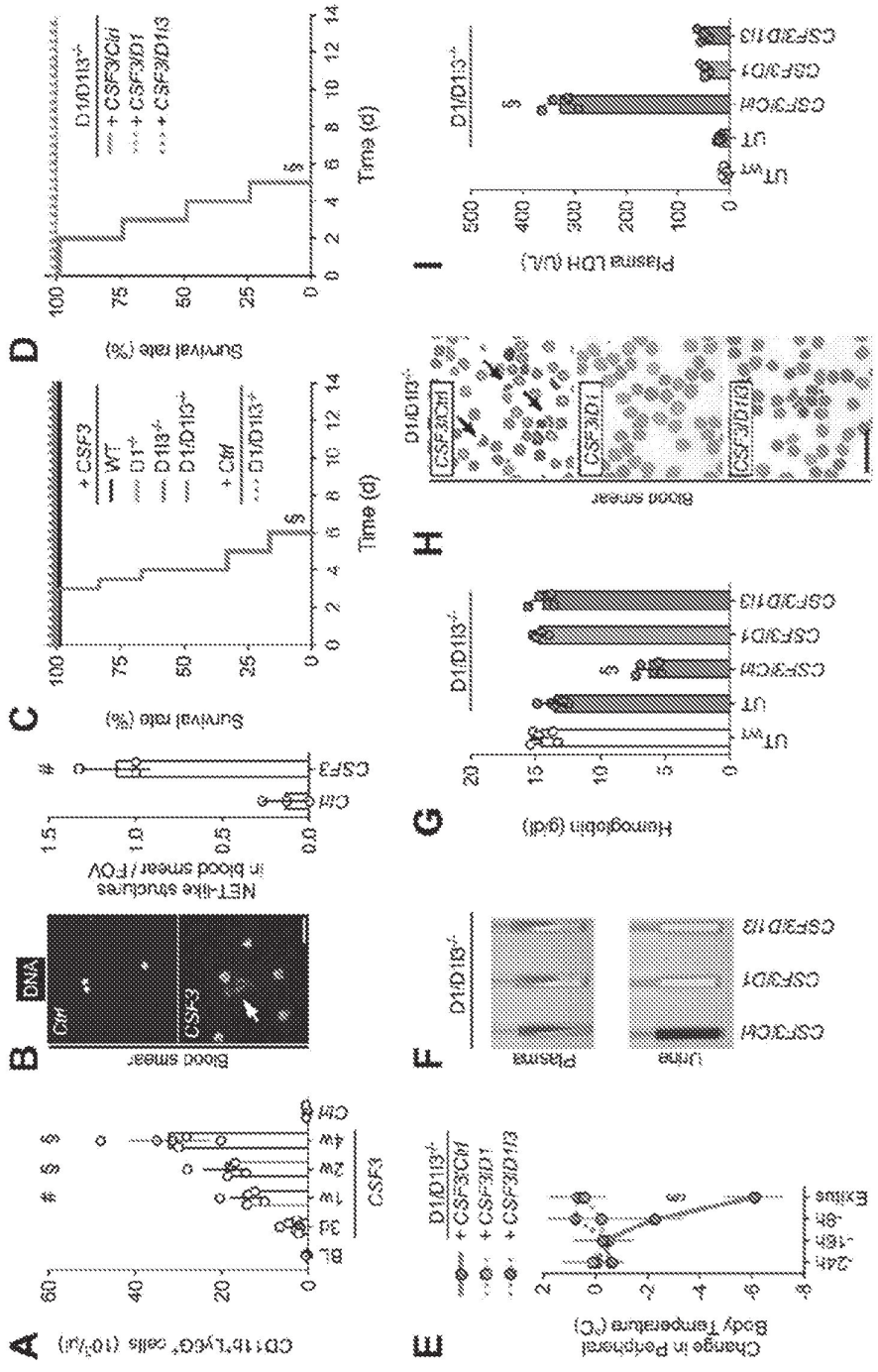
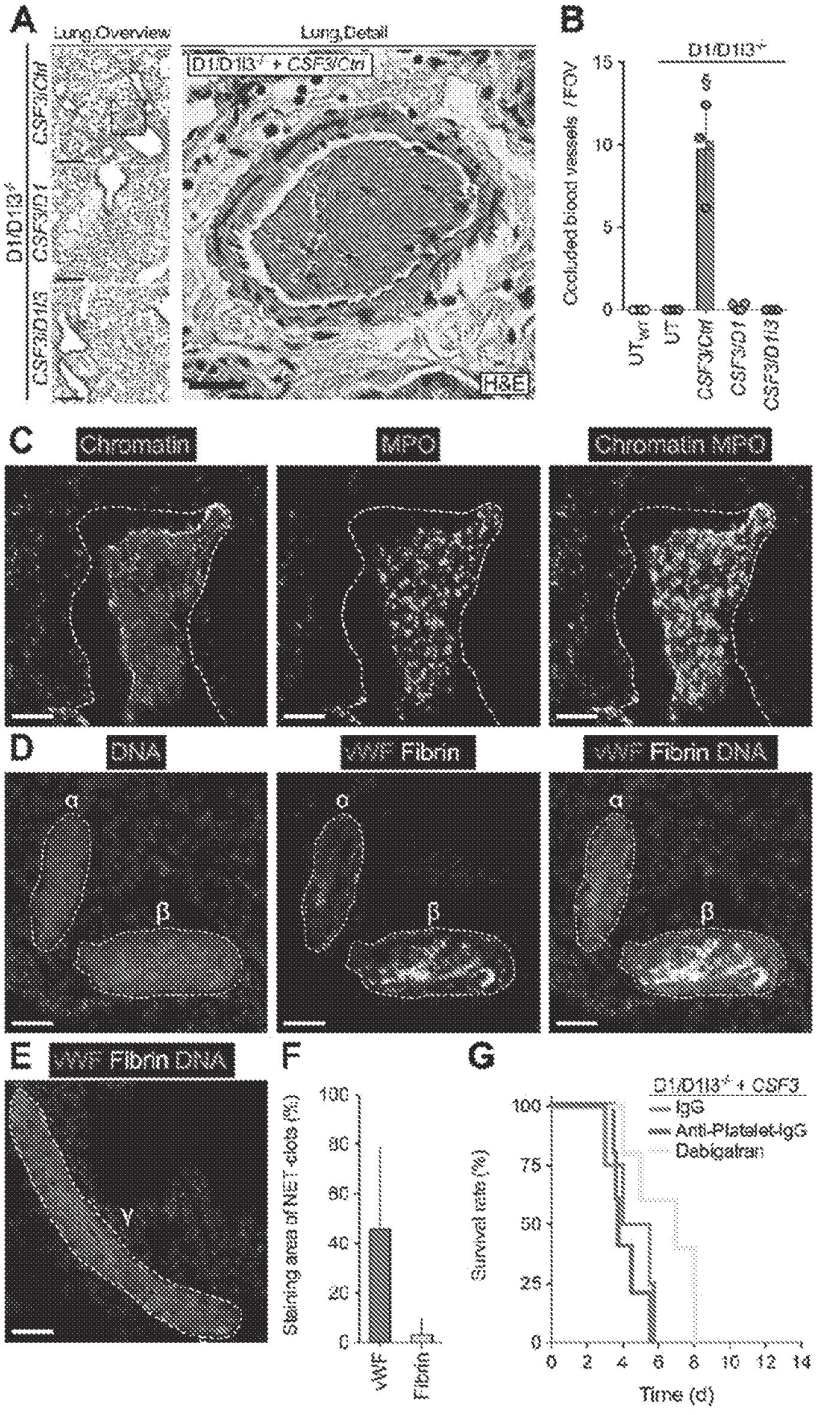

A
CD11b+Ly6G+ cells (10⁶/µl)
Ctrl
CSF3
B
DNA
Blood smear
Ctrl
CSF3
NET-like structures in blood smear / FOV
C
Survival rate (%)
+ CSF3
WT
D1-/-
D113-/-
D1/D113-/-
+ Ctrl
D1/D113-/-
Time (d)
D
Survival rate (%)
D1/D113-/-
+ CSF3Ctrl
+ CSF3D1
+ CSF3D113
Time (d)
E
Change in Peripheral Body Temperature (°C)
D1/D113-/-
+ CSF3Ctrl
+ CSF3D1
+ CSF3D113
Bleeds
F
D1/D113-/-
Plasma
Urine
CSF3Ctrl
CSF3D1
CSF3D113
G
Hemoglobin (g/dl)
D1/D113-/-
UTWT
UT
CSF3Ctrl
CSF3D1
CSF3D113
H
D1/D113-/-
CSF3Ctrl
CSF3D1
CSF3D113
Blood smear
I
Plasma LDH (U/L)
D1/D113-/-
UTWT
UT
CSF3Ctrl
CSF3D1
CSF3D113

FIG. 3A- FIG. 3G


A
Lung,Overview
Lung,Detail
D1/D113-/-
CSF3/Ctrl
CSF3/D1
CSF3/D113
D1/D113-/- + CSF3/Ctrl
H&E
B
D1/D113-/-
Occluded blood vessels / FOV
UTwt
UT
CSF3/Ctrl
CSF3/D1
CSF3/D113
C
Chromatin
MPO
Chromatin MPO
D
DNA
vWF Fibrin
vWF Fibrin DNA
α
β
E
vWF Fibrin DNA
γ
F
Staining area of NET-clots (%)
vWF
Fibrin
G
D1/D113-/- + CSF3
IgG
Anti-Platelet-IgG
Dabigatran
Survival rate (%)
Time (d)

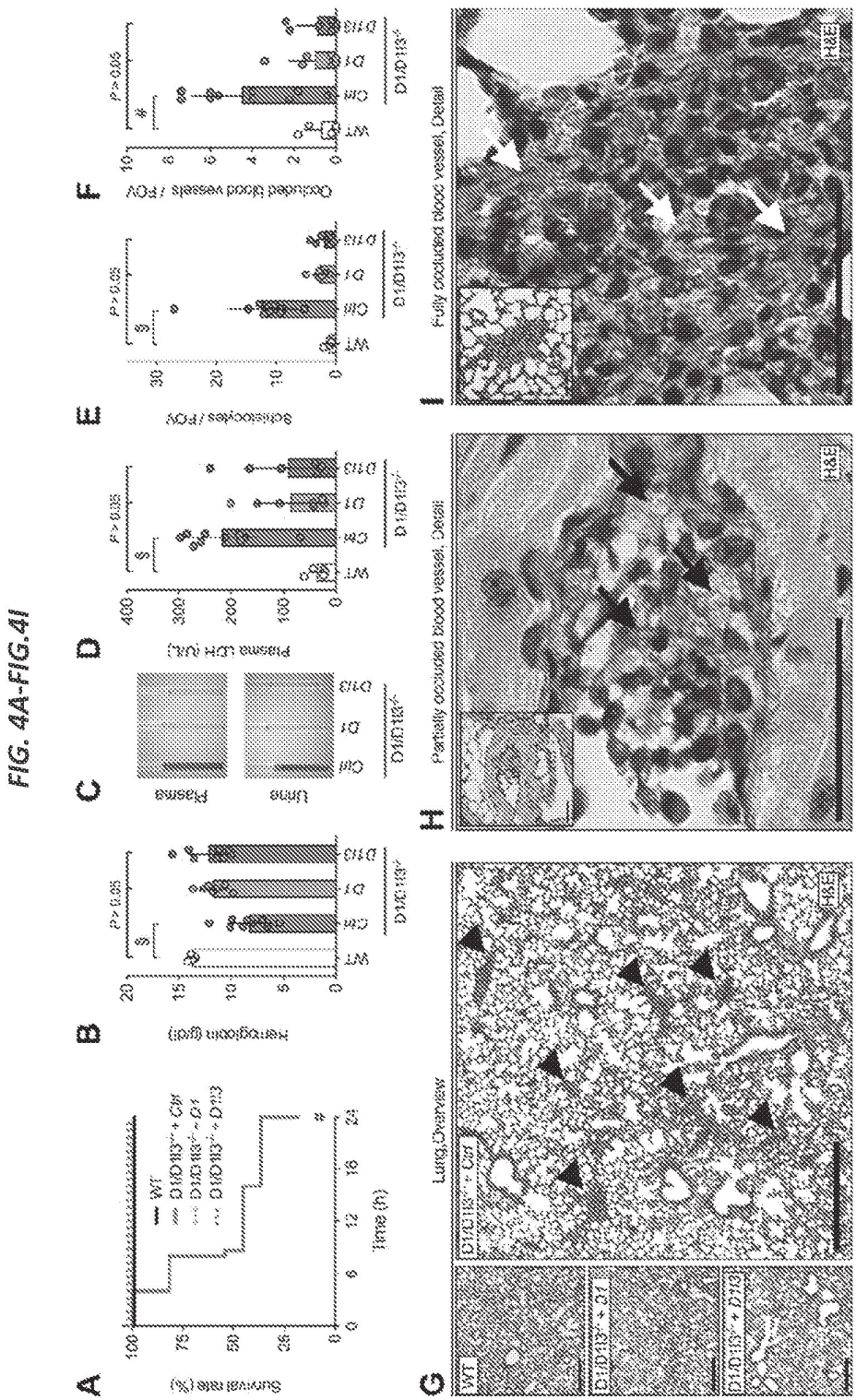
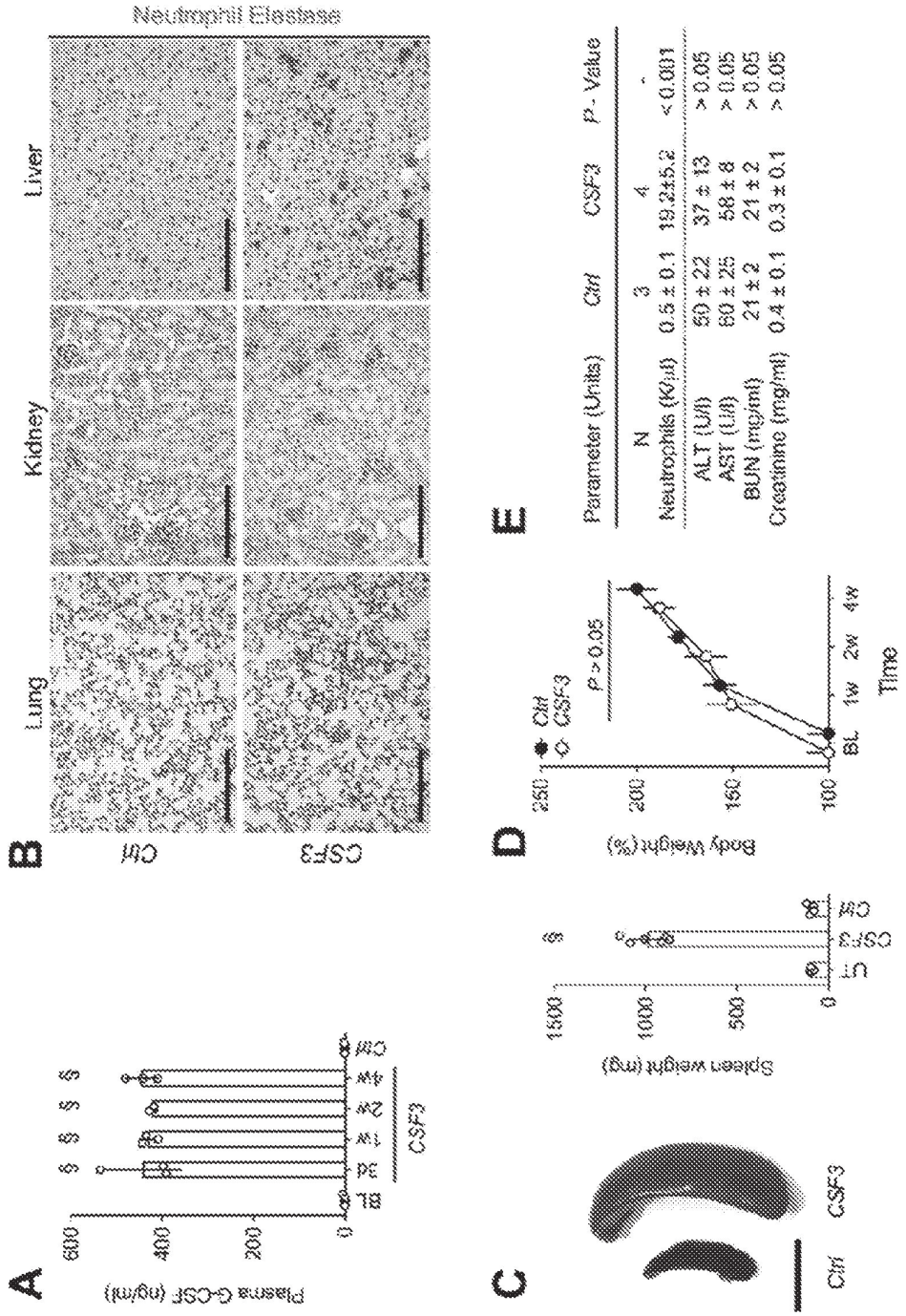

FIG. 4A-FIG.4I
A
Survival rate (%)
Time (h)
WT
D1/D13-/- + Ctr
D1/D13-/- + D1
D1/D13-/- + D13
B
Hemoglobin (g/dl)
P > 0.05
WT
Ctr
D1
D13
D1/D13-/-
C
Plasma
Lung
D
Plasma LDH (U/L)
E
Schistocytes / FOV
F
Occluded blood vessels / FOV
G
Lung Overview
H&E
H
Partially occluded blood vessel, Detail
I
Fully occluded blood vessel, Detail

FIG. 5A-FIG. 5E


A
Plasma G-CSF (ng/ml)
600
400
200
0
BL
3d
1w
2w
4w
Ctrl
CSF3
§
B
Neutrophil Elastase
Lung
Kidney
Liver
Ctrl
CSF3
C
Ctrl
CSF3
Spleen weight (mg)
1500
1000
500
0
UT
CSF3
Ctrl
D
Ctrl
CSF3
P > 0.05
Body Weight (%)
250
200
150
100
BL
1w
2w
4w
Time
E
Parameter (Units) Ctrl CSF3 P - Value
N 3 4 -
Neutrophils (K/µl) 0.5 ± 0.1 19.2±5.2 < 0.001
ALT (U/l) 50 ± 22 37 ± 13 > 0.05
AST (U/l) 80 ± 25 58 ± 8 > 0.05
BUN (mg/ml) 21 ± 2 21 ± 2 > 0.05
Creatinine (mg/ml) 0.4 ± 0.1 0.3 ± 0.1 > 0.05

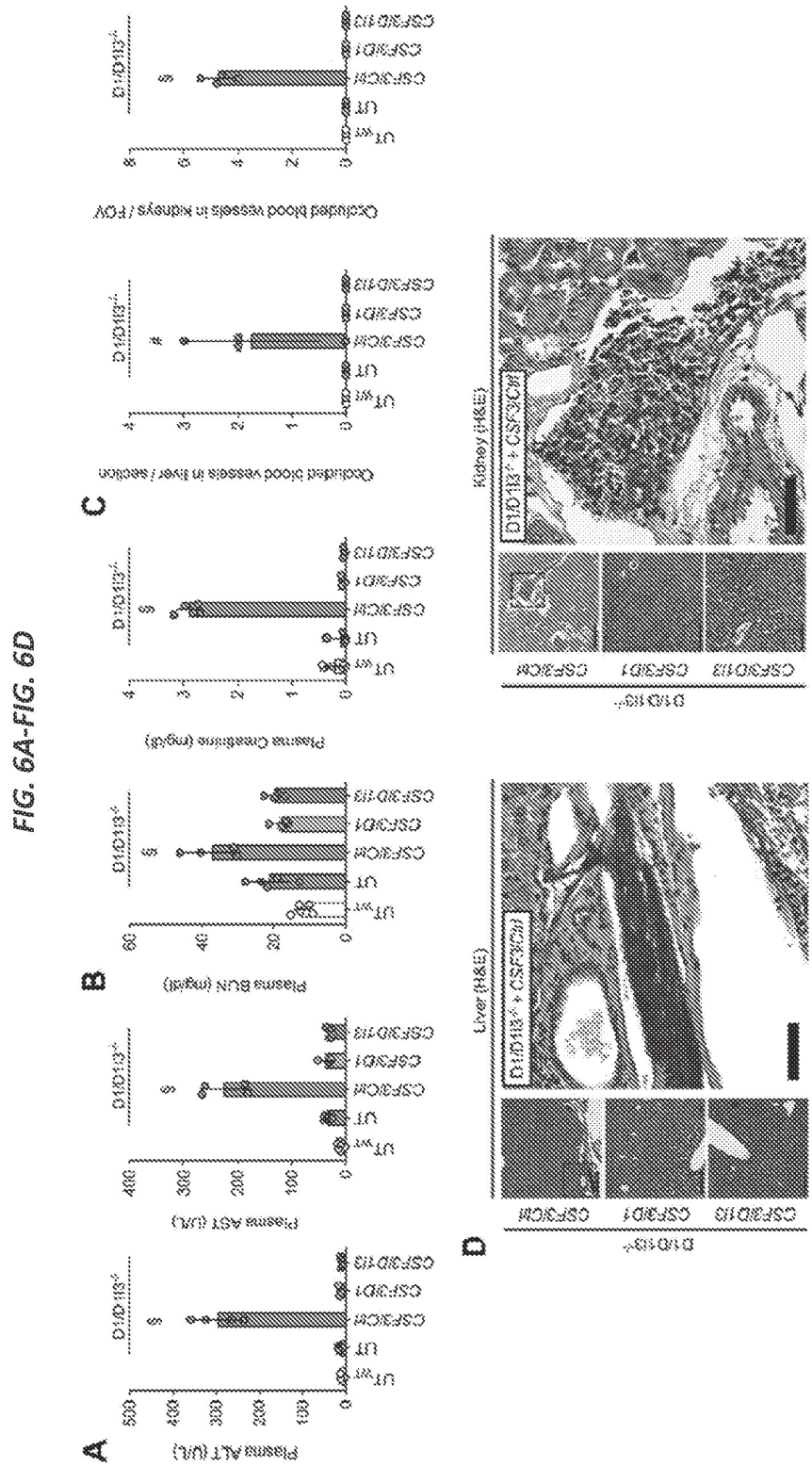

A
Plasma ALT (U/L)
B
Plasma AST (U/L)
Plasma BUN (mg/dl)
C
Plasma Creatinine (mg/dl)
Occluded blood vessels in liver / section
Occluded blood vessels in kidneys / FOV
D
Liver (H&E)
Kidney (H&E)
D1/D13-/-
UTWT
UT
CSF3/CM
CSF3/D1
CSF3/D13

FIG. 6A-FIG. 6D

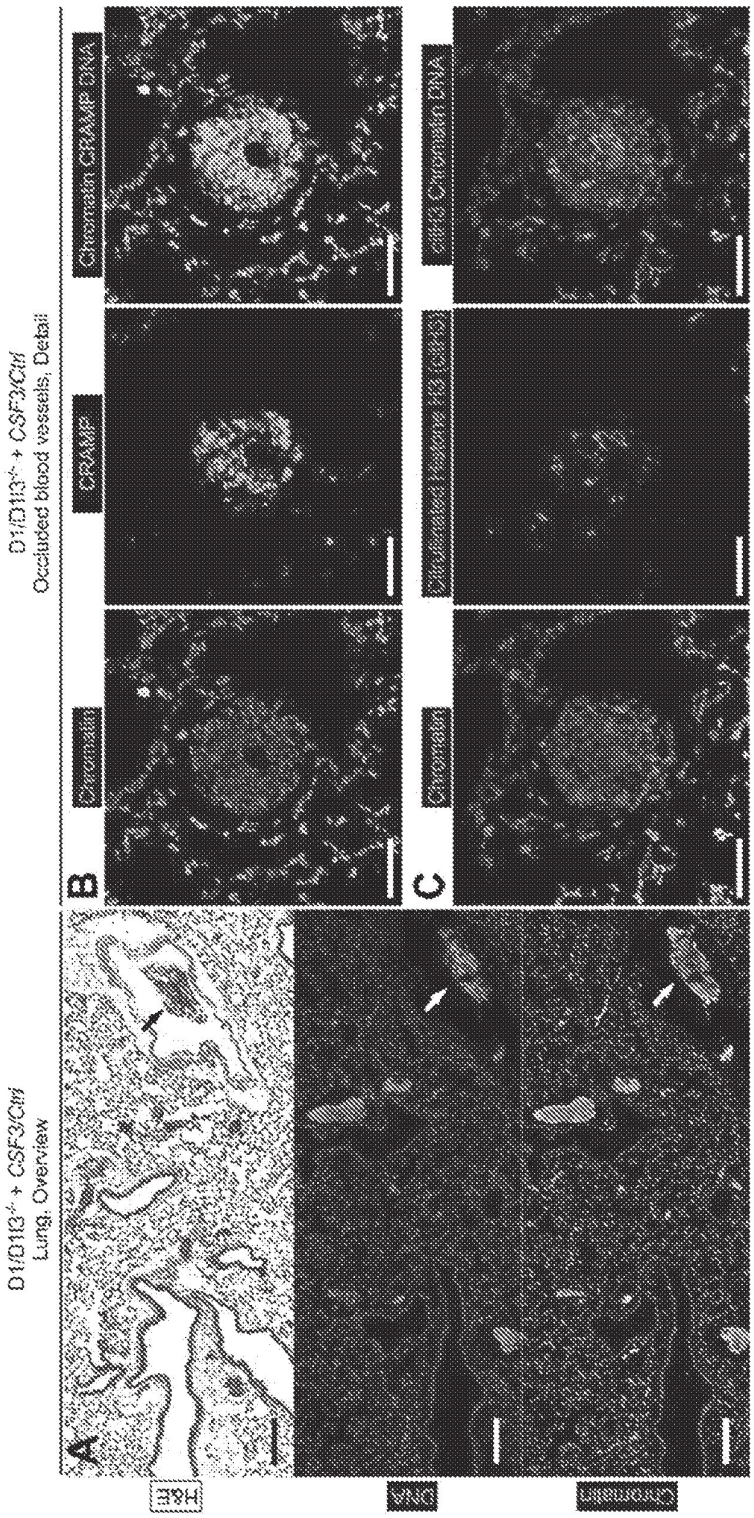
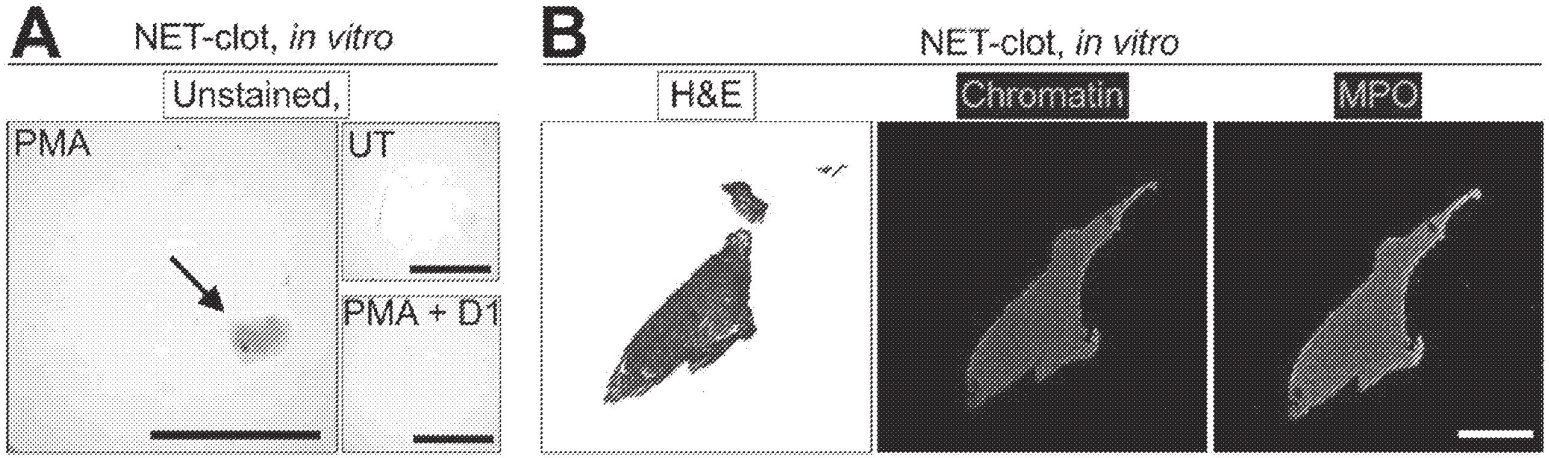

A
D1/D1β-/- + CSF3/Ctrl
Lung, Overview
H&E
B
D1/D1β-/- + CSF3/Ctrl
Occluded blood vessels, Detail
Chromatin
CRAMP
Chromatin CRAMP DNA
C
Chromatin
Citrullinated Histone H3 (Cit R2)
anti-Chromatin DNA
DNA
Chromatin

FIG. 7A-FIG. 7C

FIG. 8A and FIG. 8B



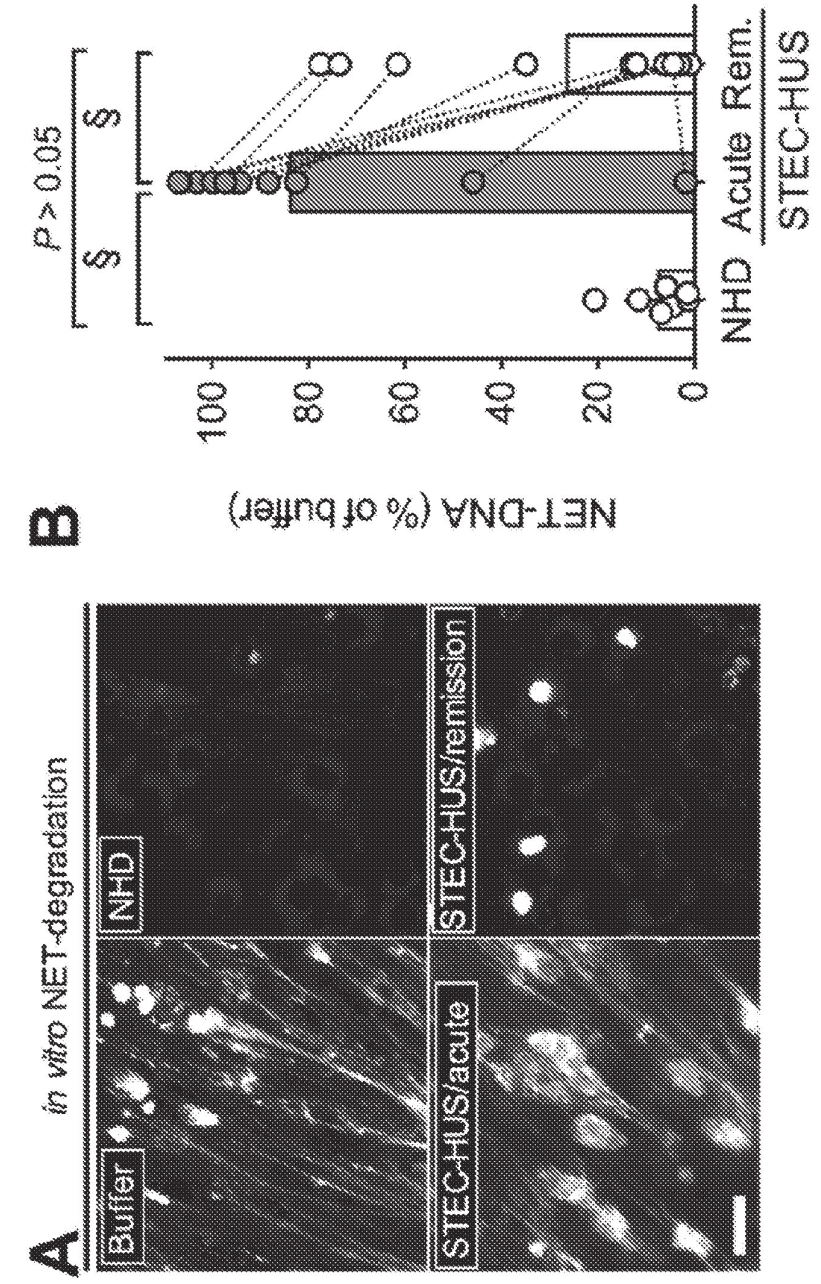
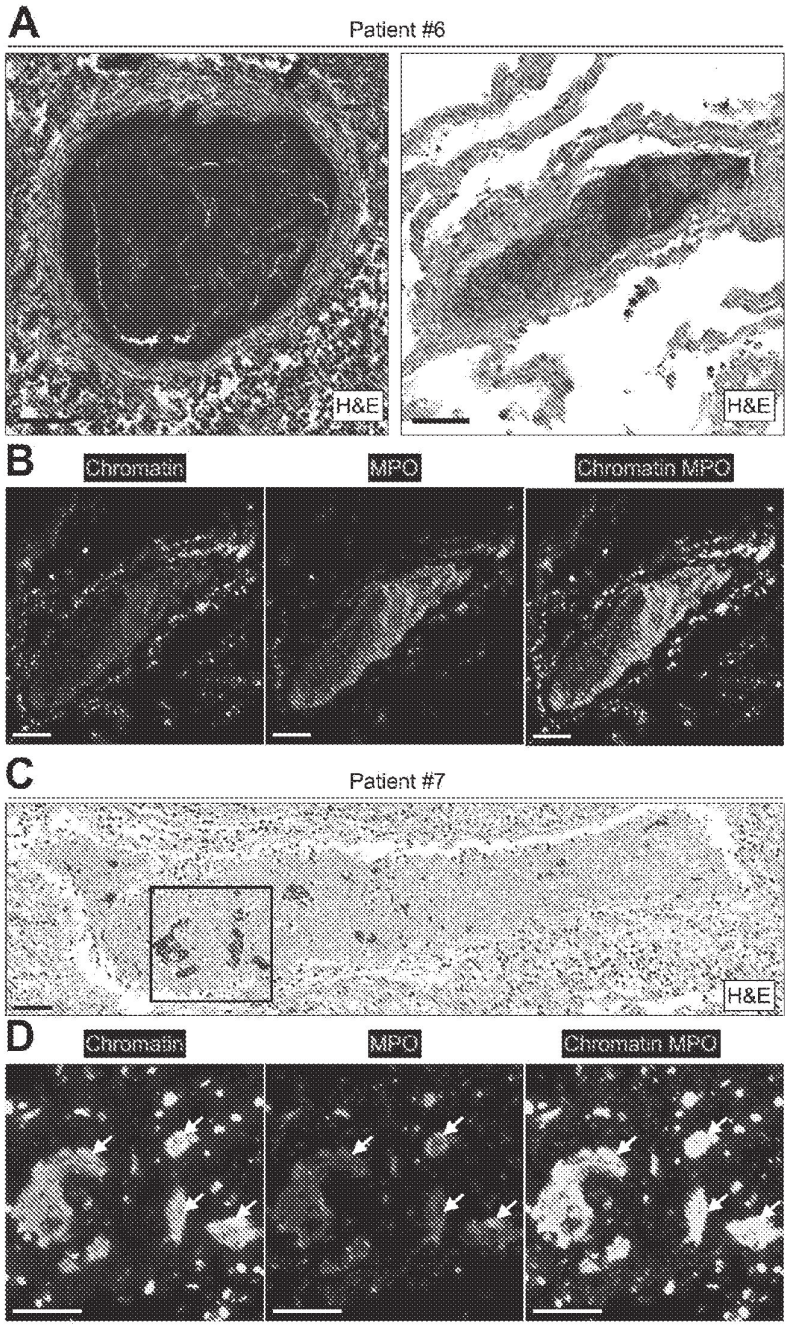


FIG. 9A and FIG. 9B

FIG. 10

#	Cause of death	Sex	Age	Tissue	Hematoxylin-rich clots	Chromatin & MPO-positive	Localization
1	ARDS	M	84	Lung	Yes	Yes	Bronchus
2	Septic cardiovascular failure + ARDS	F	63	Lung	No	Not applicable	Not applicable
3	ARDS	F	67	Lung	No	Not applicable	Not applicable
4	Septic multi-organ failure + ARDS	M	72	Lung	Yes	Yes	Bronchus
5	Septic cardiovascular failure + ARDS	F	75	Lung	No	Not applicable	Not applicable
6	Septic multi-organ failure	M	60	Lung	Yes	Yes	Blood vessel
7	Septic cardiovascular failure	F	76	Lung	Yes	Yes	Blood vessel
8	ARDS	F	68	Lung	No	Not applicable	Not applicable

FIG. 11A-FIG. 11D



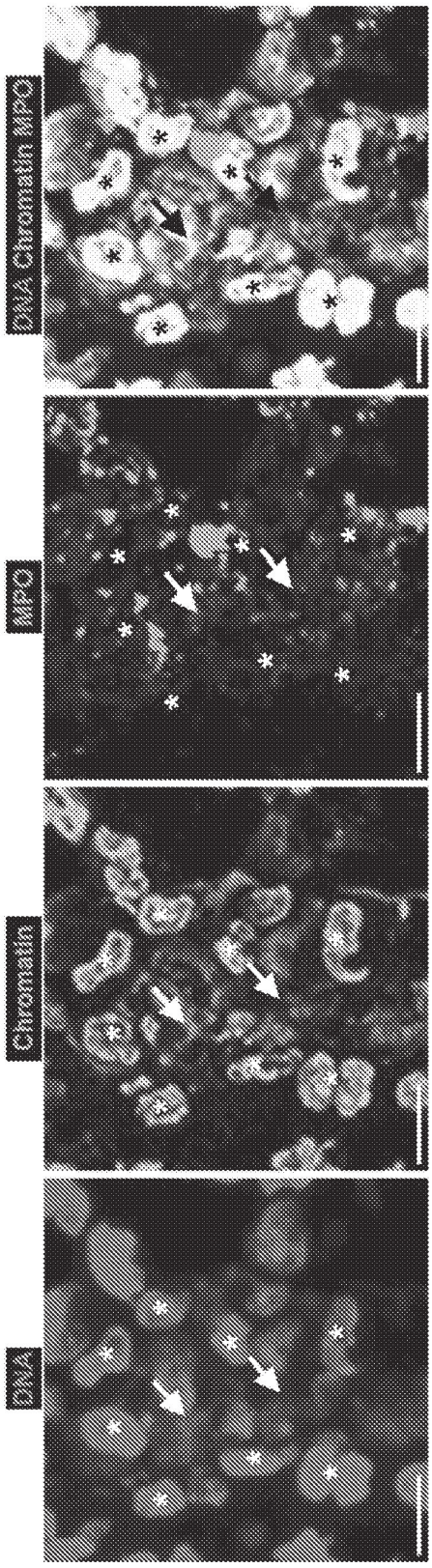


FIG. 12

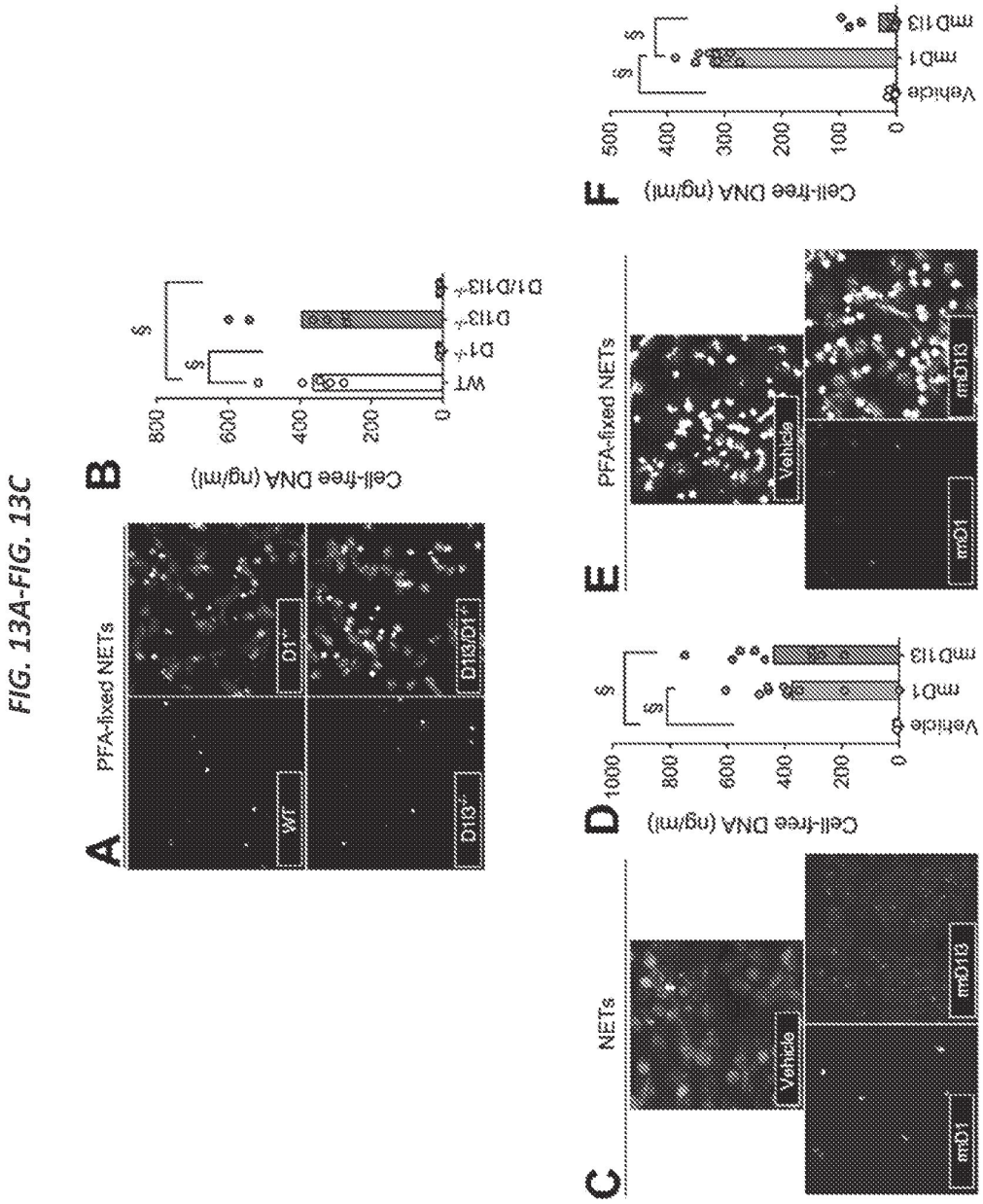


FIG. 13A-FIG. 13C

FIG. 14A-FIG. 14G

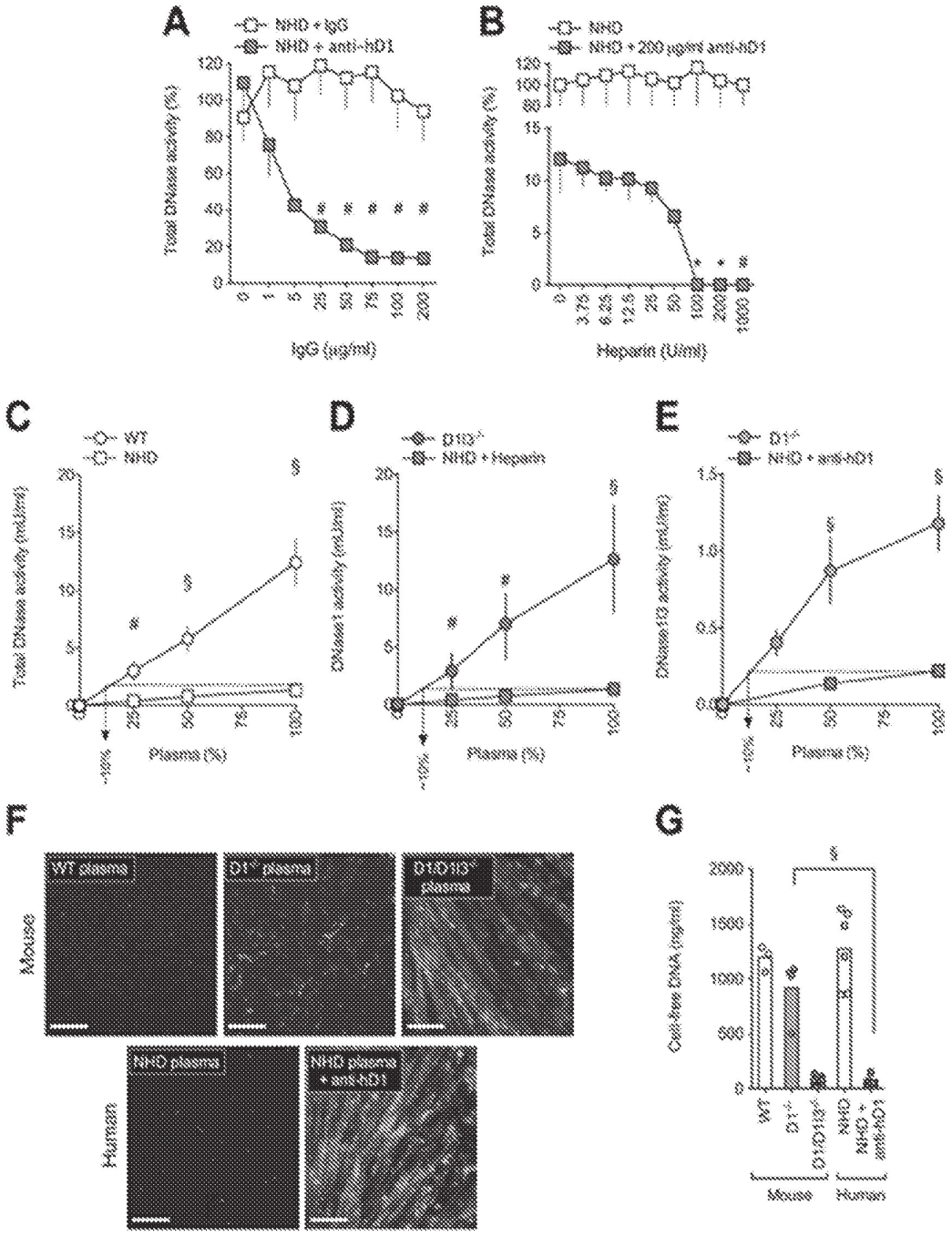


FIG. 15A-FIG. 15C

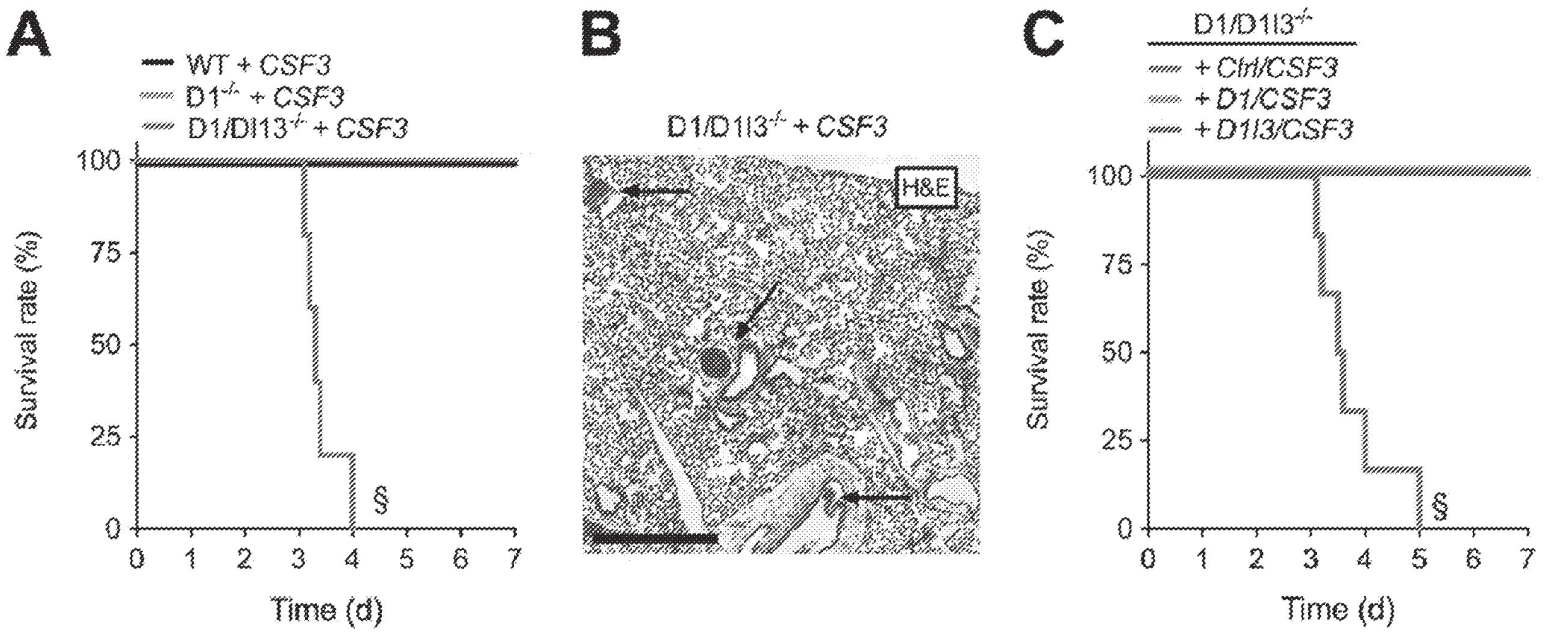


FIG. 16

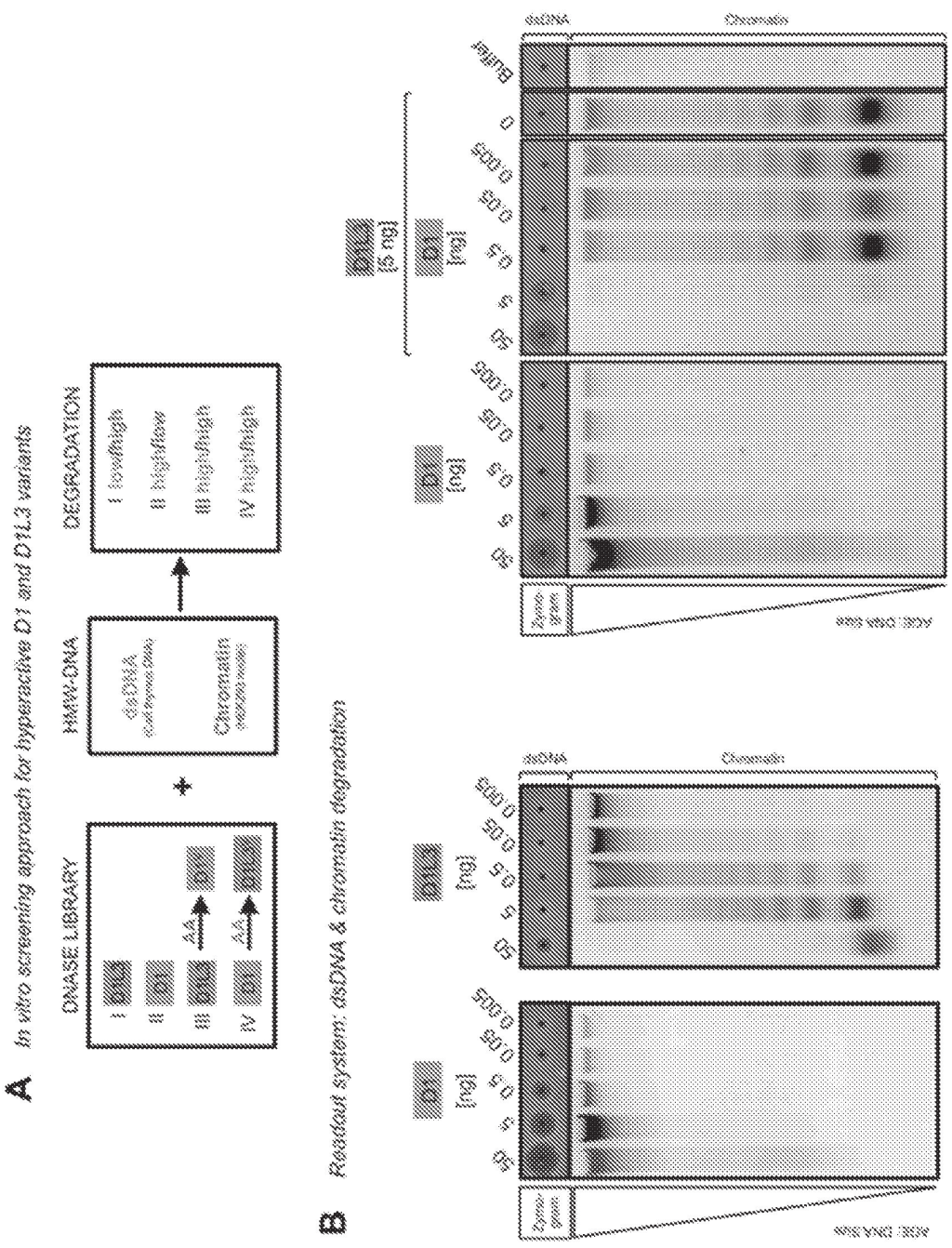

A
In vitro screening approach for hyperactive D1 and D1L3 variants
DNASE LIBRARY
I D1L3
II D1
III D1L3 Δ4 D1
IV D1 Δ4 D1L3
+
HMW-DNA
dsDNA
Chromatin
DEGRADATION
I low/high
II high/low
III high/high
IV high/high
B
Readout system: dsDNA & chromatin degradation
D1 [ng]
D1L3 [ng]
0.05
0.5
5
50
500
5000
Buffer
dsDNA
Chromatin
Agilent Bioanalyzer
Agilent Bioanalyzer

FIG. 17

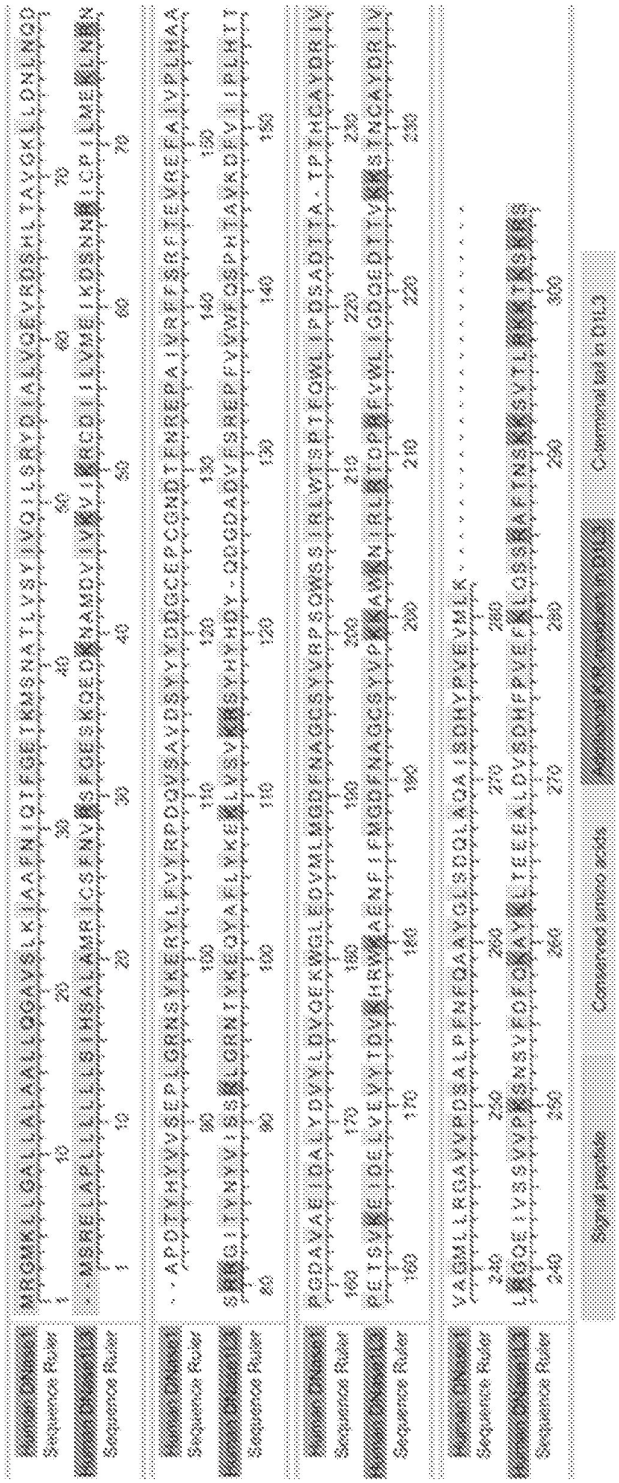


FIG. 18

#	DNase1L3	DNase1	Mutation in DNase1	Analyzed in this application
1	R29 (R9)	Q31 (Q9)	Q31R (Q9R)	+
2	K39 (R19)	A41 (A19)	A41K (A19K)	-
3	K47 (K27)	Q49 (Q27)	Q49K (Q27K)	+
4	K50 (K30)	S52 (S30)	S52K (S30K)	-
5	R66 (R46)	T68 (T46)	T68R (T46R)	-
6	K74 (K54)	N76 (N54)	N76K (N54K)	+
7	R77 (R57)	Q79 (Q57)	Q79R (Q57R)	+
8	R80 (R60)	-	D80_A81insR (D60_A61insR)	+
9	R81 (R61)	A81 (A59)	A81R (A61R)	+
10	R92 (R72)	P92 (P70)	P92R (P70R	+
11	K109 (K89)	D109 (D87)	D109K (D87K)	+
12	K114 (K94)	V114 (V92)	V114K (V92)	+
13	R115 (K95)	D115 (D93)	D115R (D92R)	+
14	K163 (K143)	A164 (A142)	A164K (A142K)	+
15	K176 (K156)	Q177 (Q155)	Q177K (Q115K)	+
16	K180 (K160)	G181 (G159)	G181K (G159K)	+
17	K199 (K179)	P200 (P178)	P200K (P178K)	-
18	K200 (K180)	S201 (S179)	S201K (S179K)	+
19	K203 (K183)	S204 (S182)	S204K (S182K)	+
20	R208 (R188)	W209 (W187)	W209R (W187R)	+
21	R212 (R192)	T213 (T191)	T213R (T191R)	+
22	K226 (K206)	-	A226_T227insK (A204_T205insK)	+
23	K227 (K207)	T227 (T205)	T227K (T205K)	+
24	R239 (R219)	A239 (A217)	A239R (A217R)	+
25	K250 (K230)	D250 (D228)	D250K (D228K)	+
26	K259 (K239)	A259 (A237)	A259K (A237K)	-
27	K262 (K262)	G262 (G240)	G262K (G240K)	-
28	K280 (K260)	M280 (M258)	M280K (M258K)	+
29	R285 (K265)	-	-	-
30	K291 (K271)	-	-	-
31	K292 (K272)	-	-	-
32	R297 (K277)	-	-	-
33	K298 (K278)	-	-	-
34	K299 (K279)	-	-	-
35	K301 (K281)	-	-	-
36	K303 (K283)	-	-	-
37	R304 (K284)	-	-	-

FIG. 19

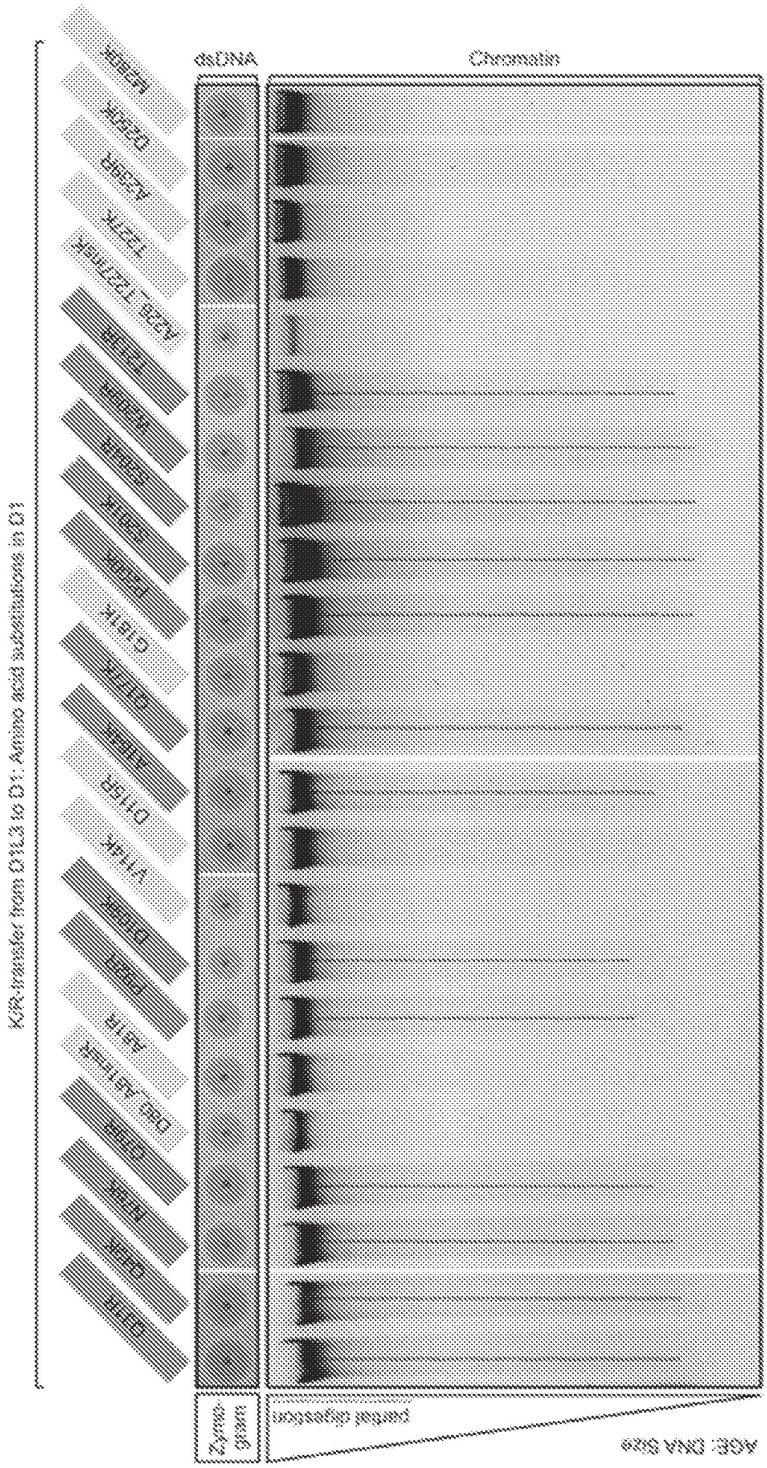

KIR-transfer from G1L3 to G1; Amino acid substitutions in G1
dsDNA
Chromatin
Partial digestion
AGE: DNA Size

FIG. 20

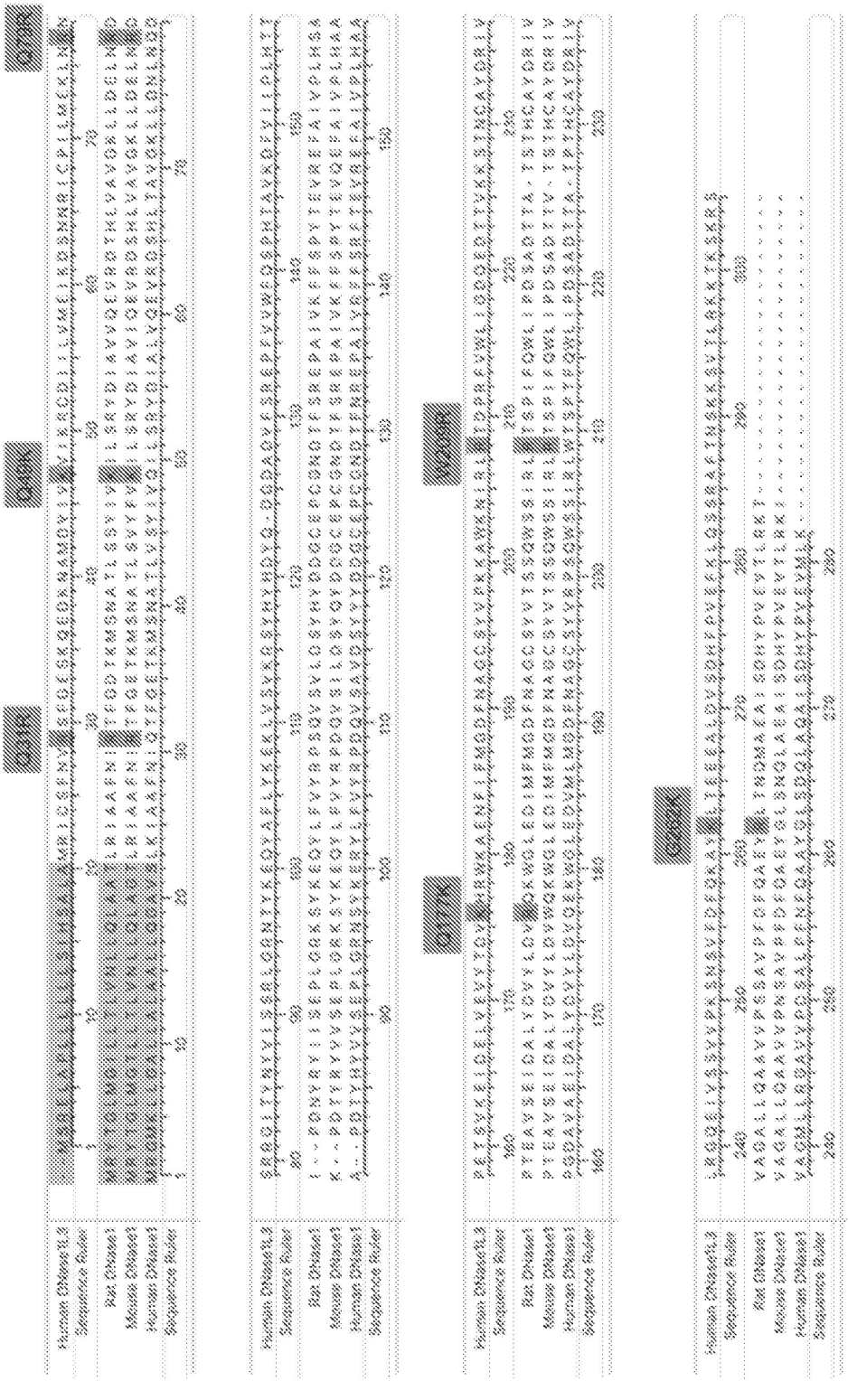

Q1R
Q4R
Q7R
K17R
K20R
K23R
Human DNase1L3
Sequence Rulet
Rat DNase1
Mouse DNase1
Human DNase1
Sequence Rulet
Signal peptide
K/R residues in D1L3, present in murine or rat D1, absent in human D1

FIG. 21

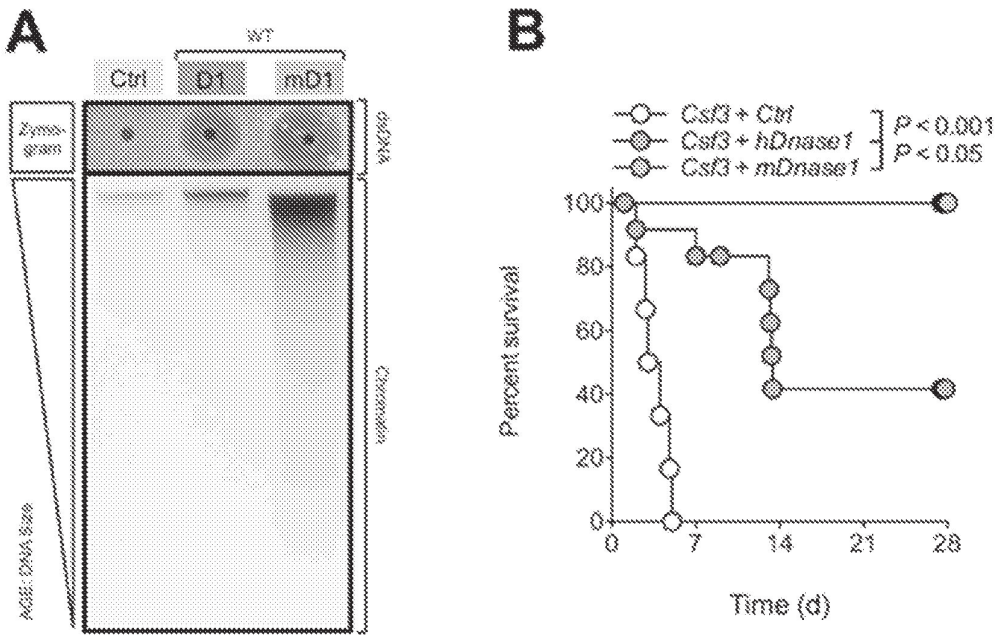
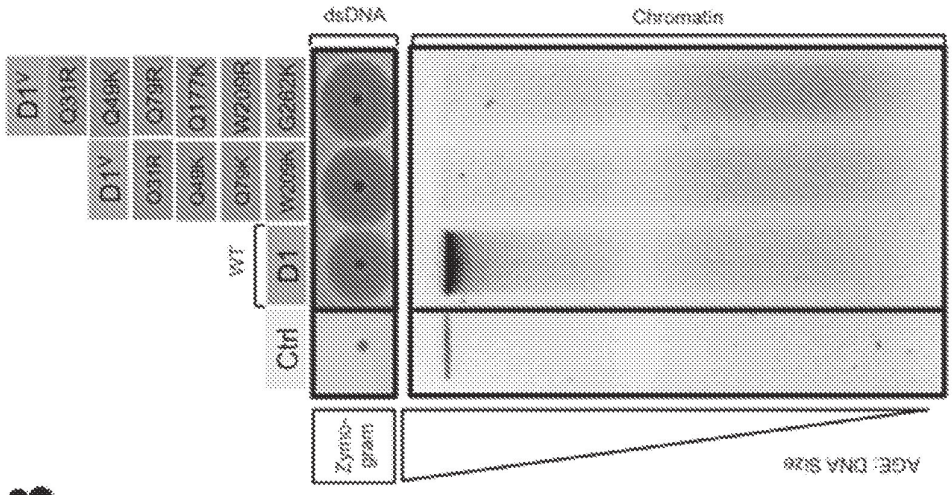

A
WT
Ctrl
D1
mD1
Agarose gel
wells
origin
Chromatin
AGE: DNA Size
B
Csf3 + Ctrl
Csf3 + hDnase1
Csf3 + mDnase1
P < 0.001
P < 0.05
Percent survival
100
80
60
40
20
0
0
7
14
21
28
Time (d)

FIG. 22


B
Ctrl
WT
D1
D1V Q31R
D1V Q49K
Q49K Q79R
Q79R W209R
Q49K Q177K
W209R G262K
dsDNA
Chromatin
Zymo-gram
AGE: DNA size

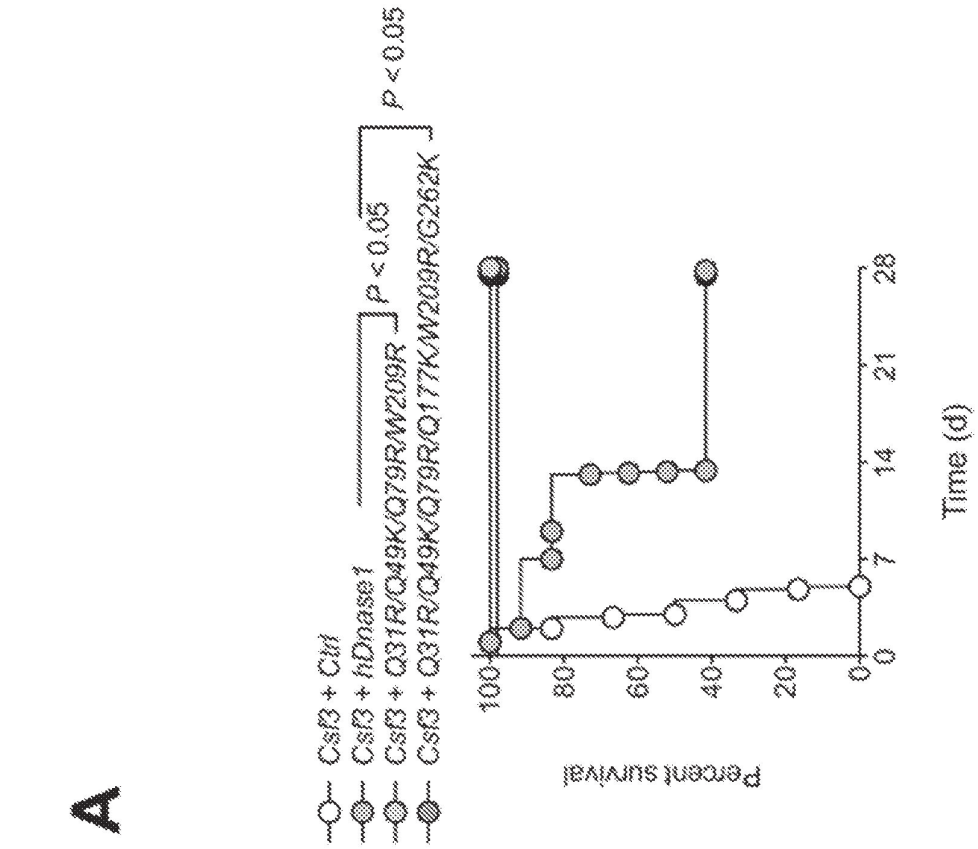

A
Csf3 + Ctrl
Csf3 + hDnase1
Csf3 + Q31R/Q49K/Q79R/W209R
Csf3 + Q31R/Q49K/Q79R/Q177K/W209R/G262K
P < 0.05
P < 0.05
Percent survival
100
80
60
40
20
0
0
7
14
21
28
Time (d)

FIG. 23

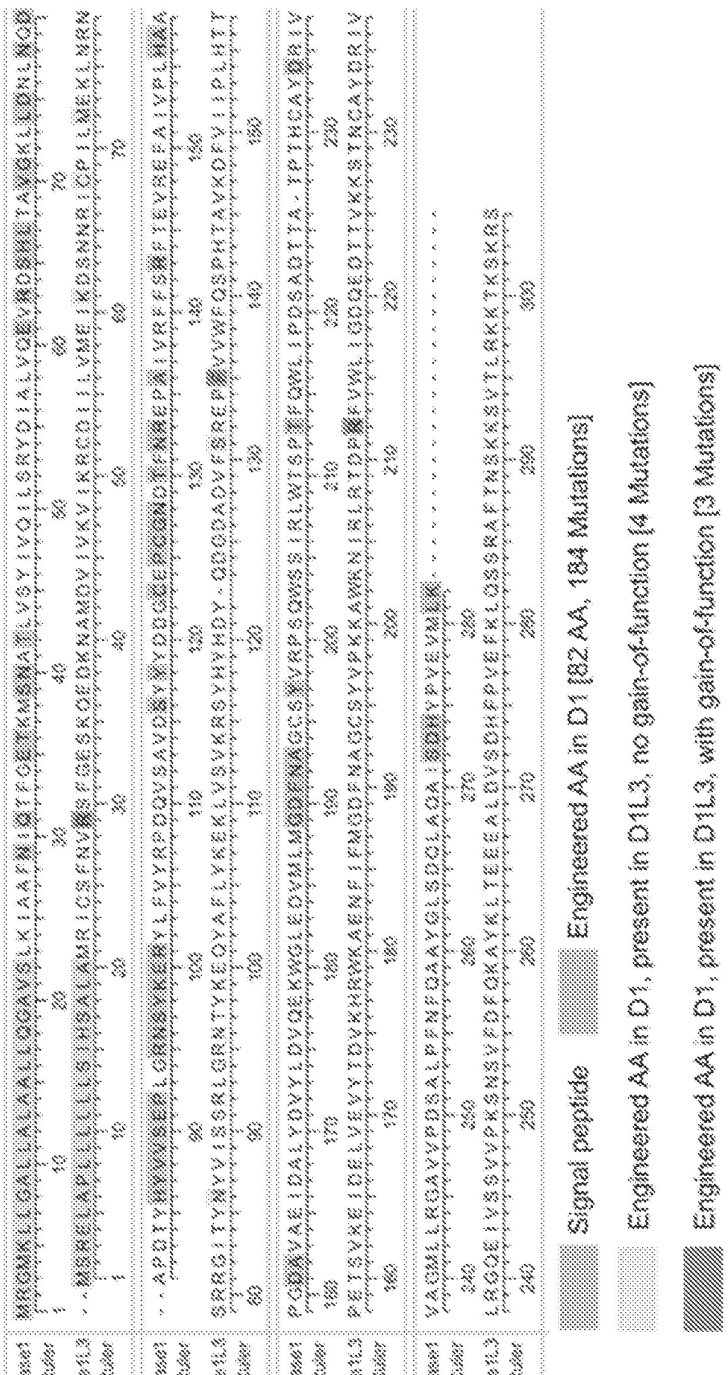

Human DNase1 Sequence Ruler
Human DNase1L3 Sequence Ruler
Signal peptide
Engineered AA in D1 [82 AA, 184 Mutations]
Engineered AA in D1, present in D1L3, no gain-of-function [4 Mutations]
Engineered AA in D1, present in D1L3, with gain-of-function [3 Mutations]

FIG. 24

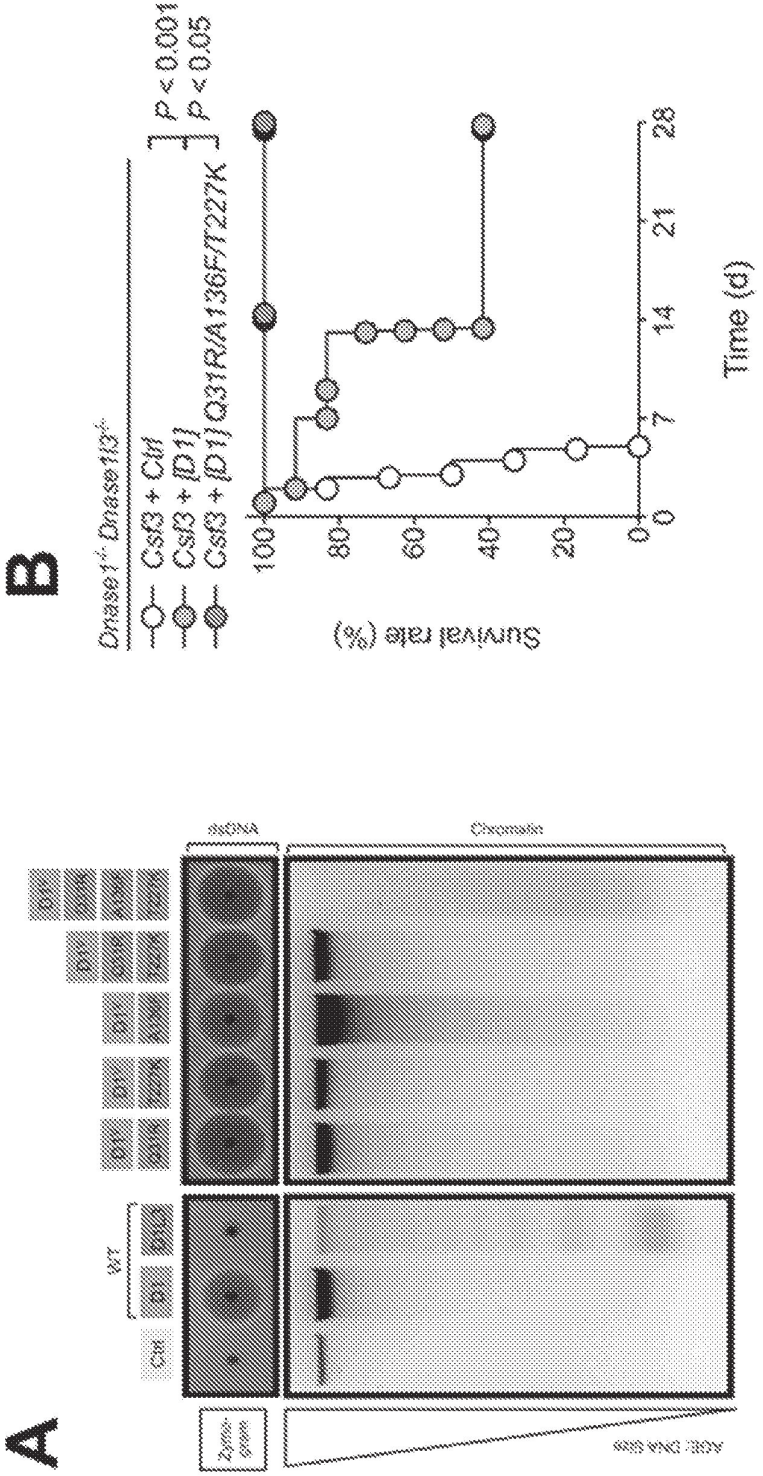

A
Cre
WT
B1
B1/B2
B1/B2
B1/B2
B1/B2
B1/B2
B1/B2
Zymolyase
rsDNA
Chromatin
ADE: DNA size
B
Dnase1l3-/- Dnase1l3-/-
Csf3 + Ctrl
Csf3 + [D1]
Csf3 + [D1] Q31R/A136F/T227K
P < 0.001
P < 0.05
Survival rate (%)
100
80
60
40
20
0
0
7
14
21
28
Time (d)

FIG. 25

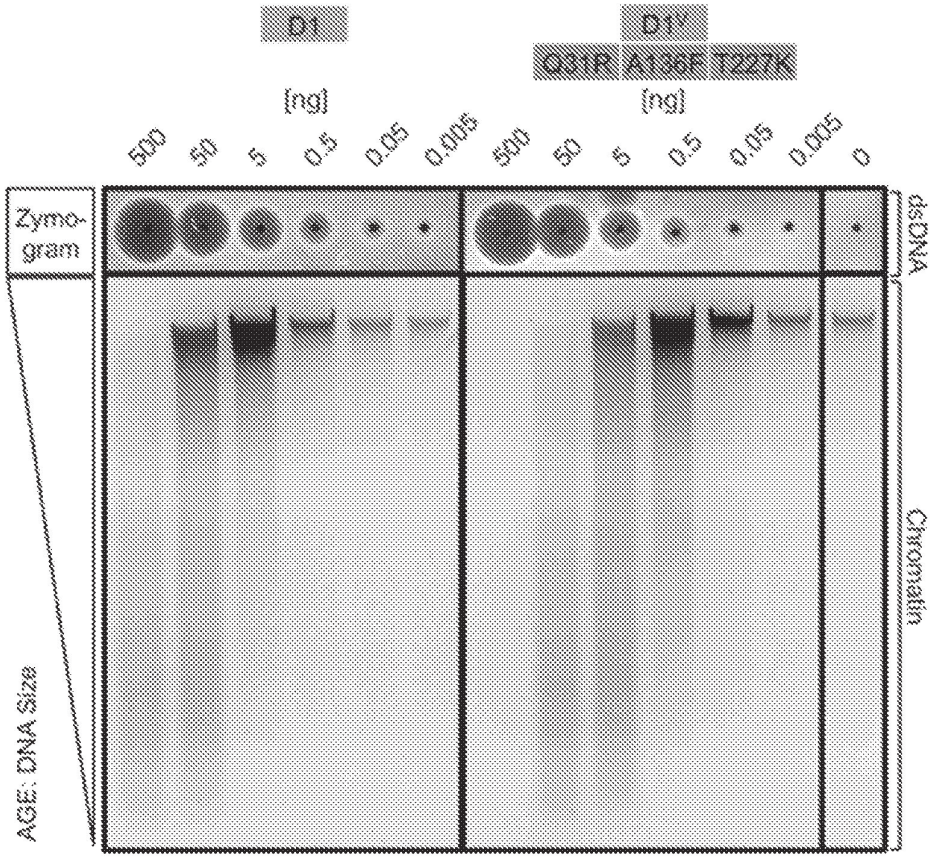

D1
D1Y
Q31R A136F T227K
[ng]
[ng]
500 50 5 0.5 0.05 0.005
500 50 5 0.5 0.05 0.005 0
Zymo-gram
dsDNA
Chromatin
AGE: DNA Size

FIG. 26

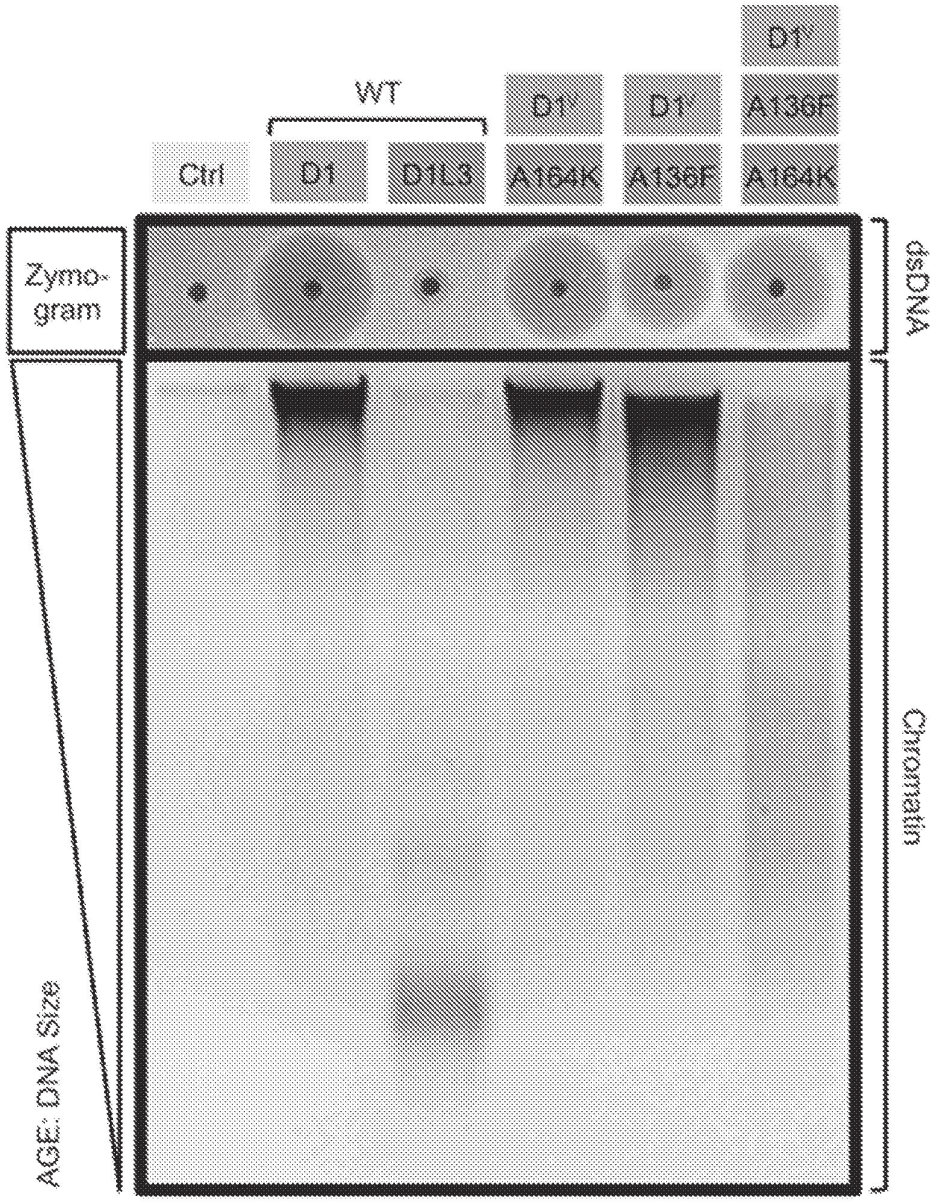

Ctrl
WT
D1
D1L3
D1'
A164K
D1'
A136F
D1'
A136F
A164K
Zymo-
gram
dsDNA
Chromatin
AGE: DNA Size

FIG. 27

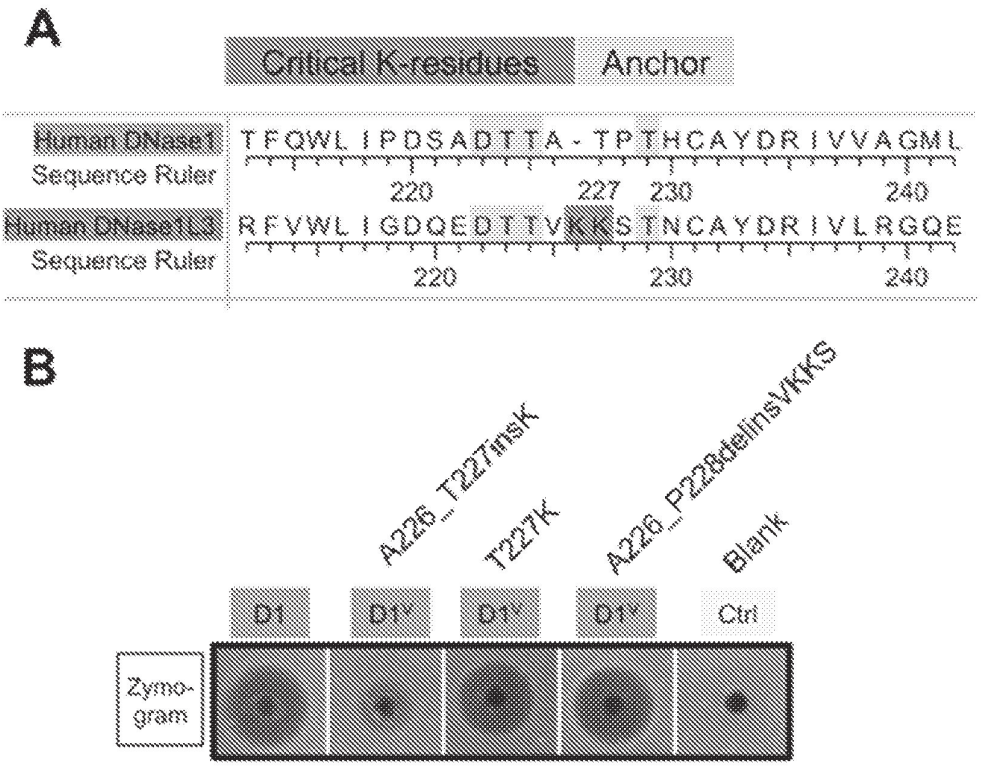

A
Critical K-residues
Anchor
Human DNase1
Sequence Ruler
T F Q W L I P D S A D T T A - T P T H C A Y D R I V V A G M L
220
227
230
240
Human DNase1L3
Sequence Ruler
R F V W L I G D Q E D T T V K K S T N C A Y D R I V L R G Q E
220
230
240
B
A226_T227insK
T227K
A226_P228delinsVKKS
Blank
D1
D1*
D1*
D1*
Ctrl
Zymo-gram

FIG. 28

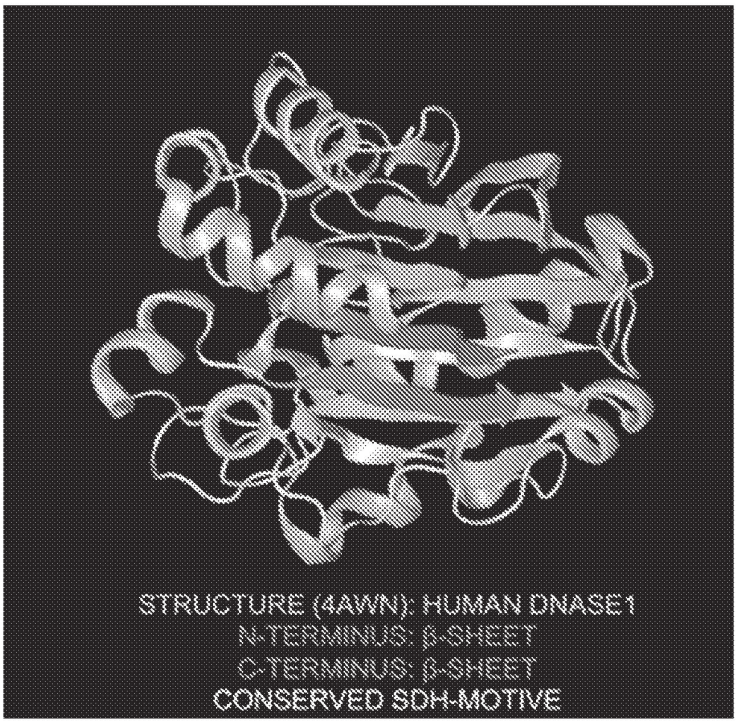

STRUCTURE (4AWN): HUMAN DNASE1
N-TERMINUS: β-SHEET
C-TERMINUS: β-SHEET
CONSERVED SDH-MOTIVE

FIG. 29

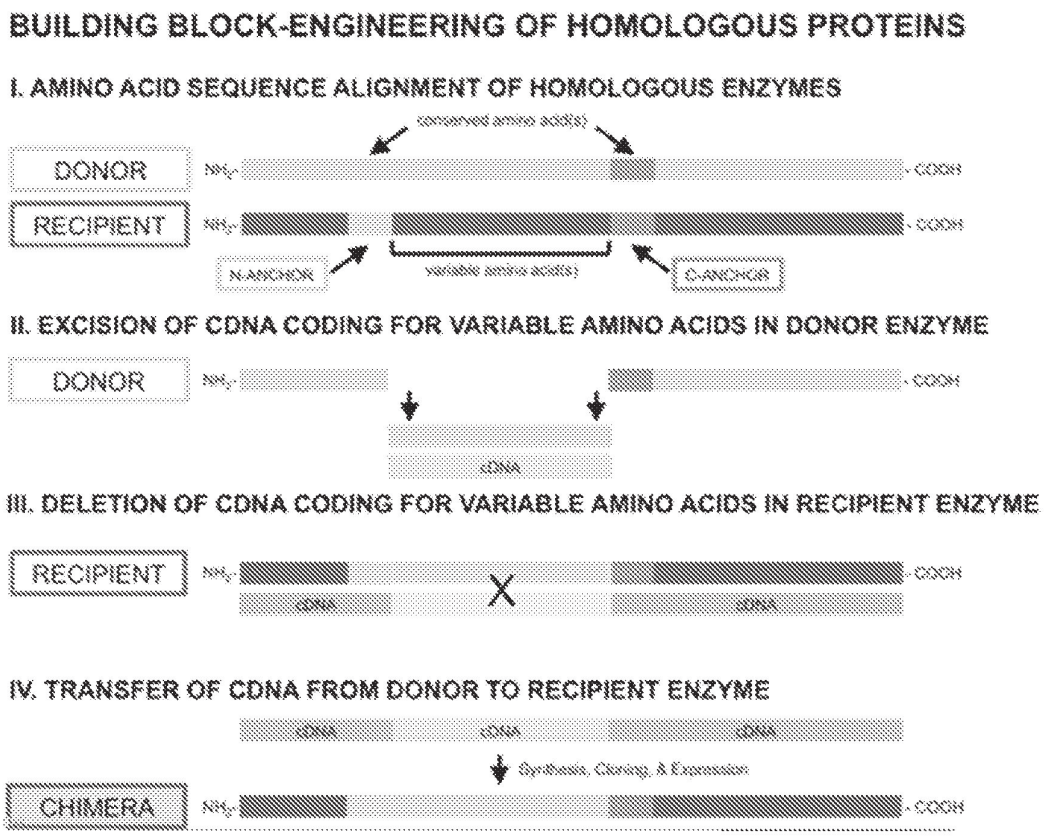

BUILDING BLOCK-ENGINEERING OF HOMOLOGOUS PROTEINS
I. AMINO ACID SEQUENCE ALIGNMENT OF HOMOLOGOUS ENZYMES
DONOR
RECIPIENT
N-ANCHOR
C-ANCHOR
II. EXCISION OF CDNA CODING FOR VARIABLE AMINO ACIDS IN DONOR ENZYME
DONOR
III. DELETION OF CDNA CODING FOR VARIABLE AMINO ACIDS IN RECIPIENT ENZYME
RECIPIENT
IV. TRANSFER OF CDNA FROM DONOR TO RECIPIENT ENZYME
CHIMERA

FIG. 30

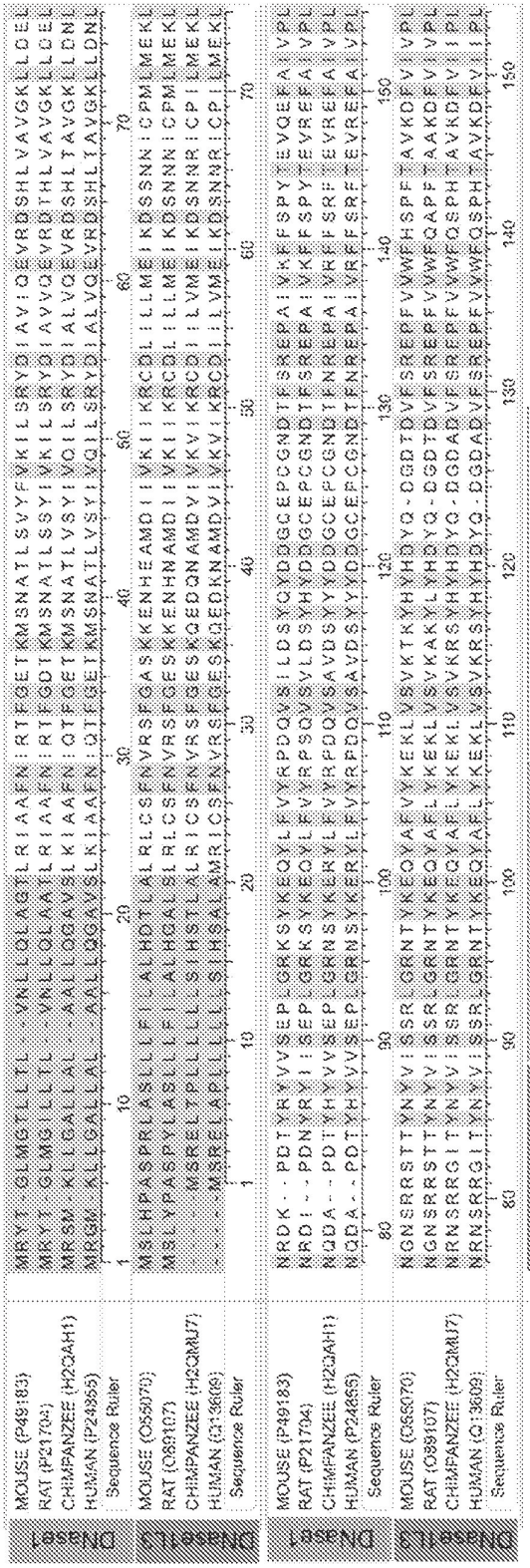

DNase1
DNase1L1
DNase1L2
DNase1L3
MOUSE (P49183)
RAT (P21704)
CHIMPANZEE (H2QAH1)
HUMAN (P24855)
Sequence Ruler
MOUSE (O55070)
RAT (O89107)
CHIMPANZEE (H2QMJ7)
HUMAN (Q13609)
Signal Peptide
Conserved AA = Anchors
Variable AA = Building Blocks

FIG. 30 continued

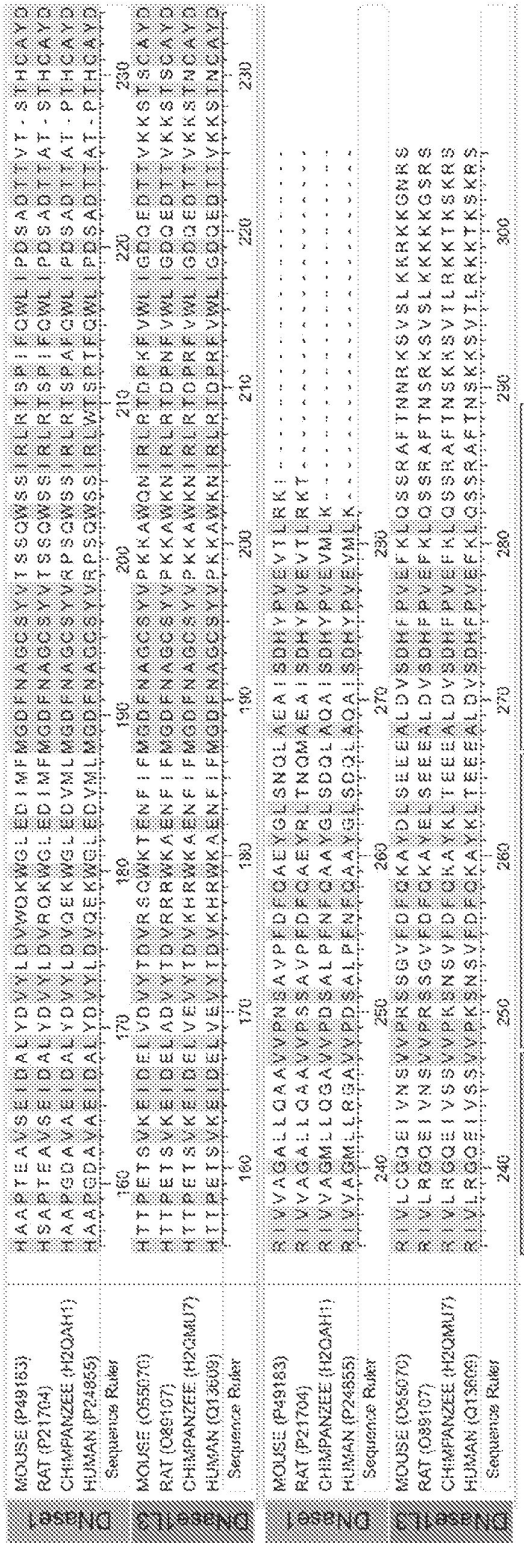
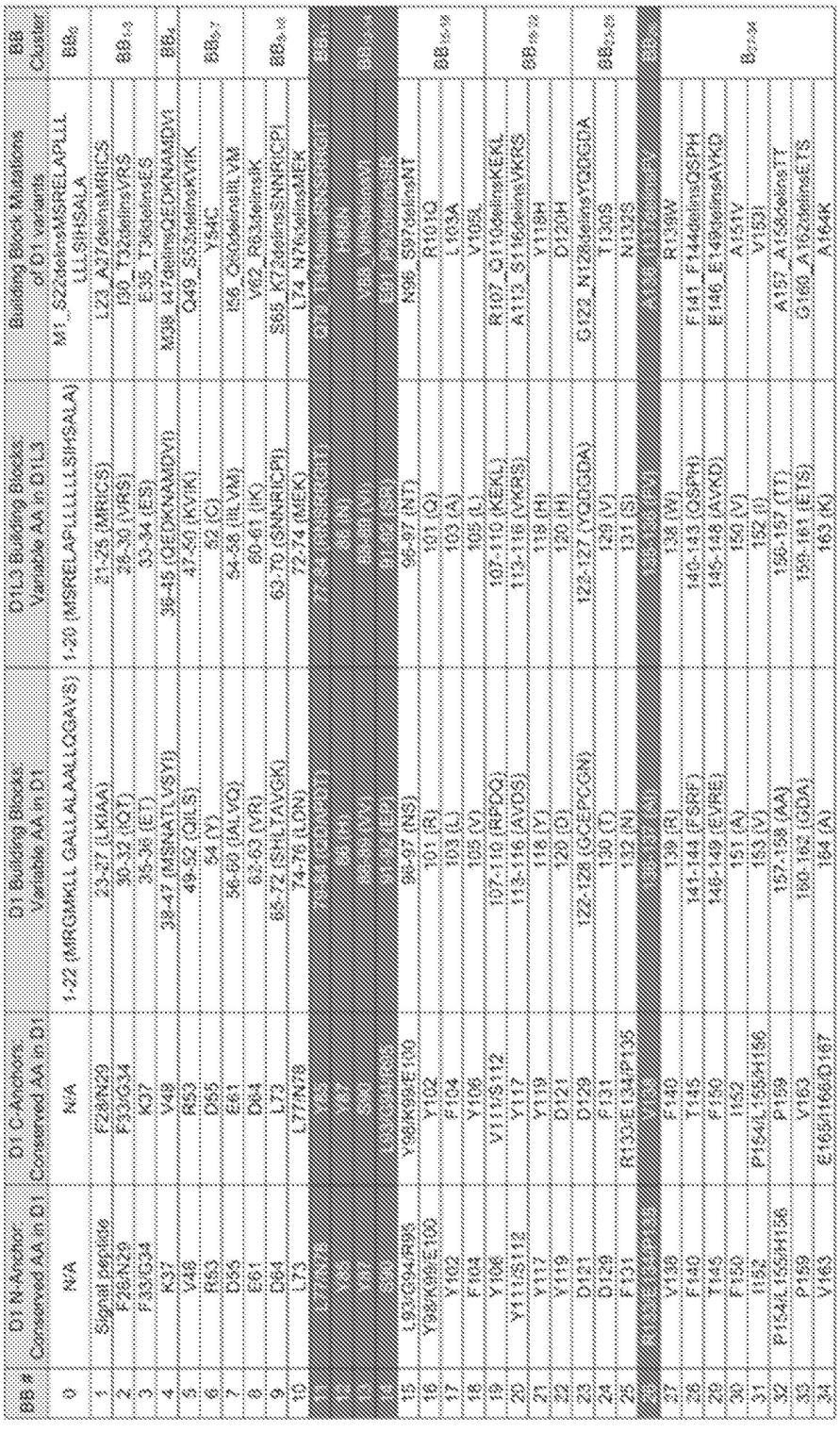


FIG. 31

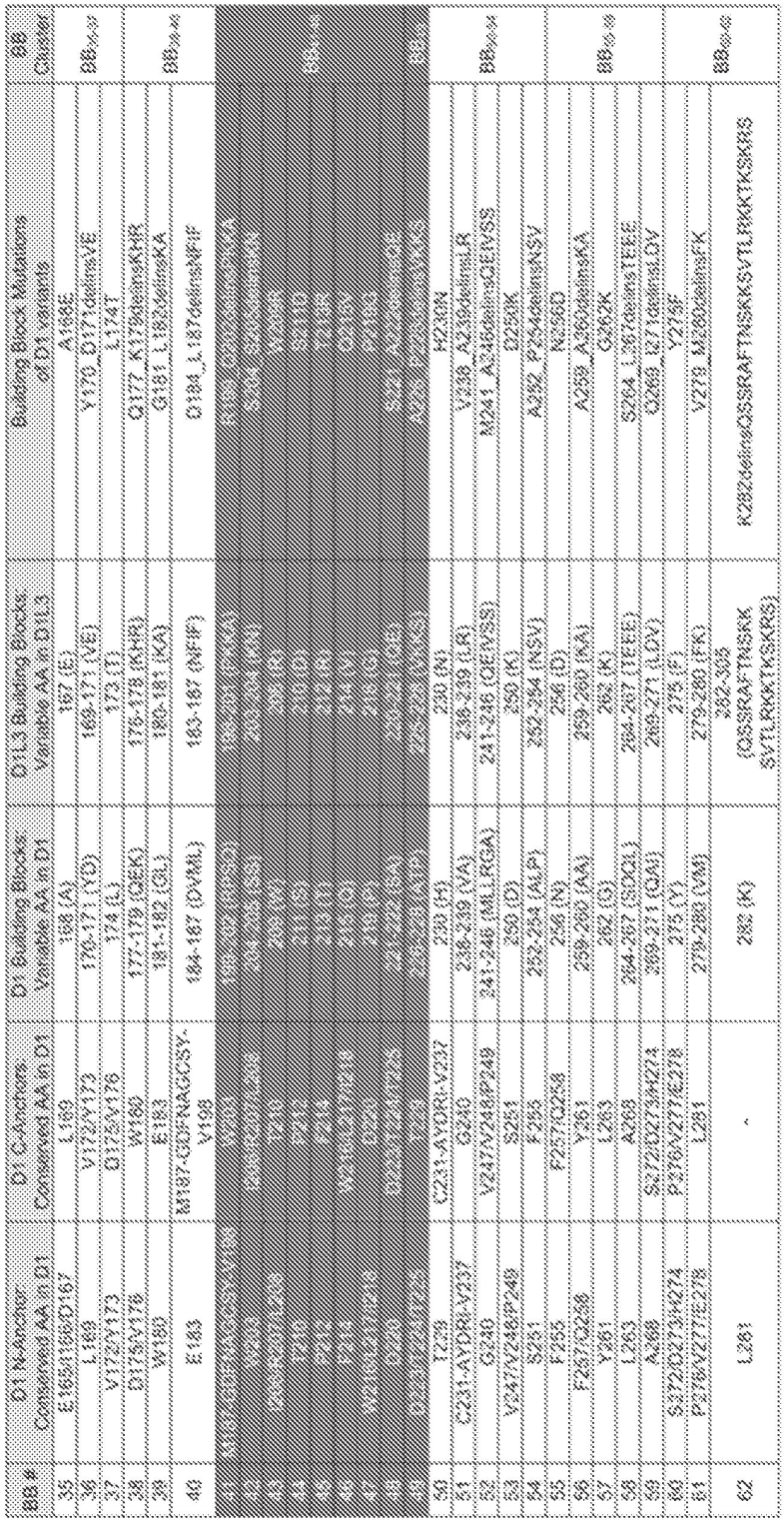
A. Example of Building Block Engineering: human DNase1 (D1) variants with Building Blocks from human DNase1L3 (D1L3)



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 (MRGWKLLGALALAALQGAVS)	1-20 (MSRELAPLLLLLLSHSALA)	M1_S22delinsMSRELAPLLLLLSHSALA	BB_0
1	Signal peptide	F23/N29	23-27 (LKIAA)	21-25 (MRICS)	L23_A27delinsMRICS	$BB_{1.0}$
2	F23/N29	F33/G34	30-32 (IQT)	26-30 (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_0
5	V48	R53	49-52 (QILS)	47-50 (KYIK)	Q49_S53delinsKYIK	
6	R53	D55	54 (Y)	52 (C)	Y54C	$BB_{0.1}$
7	D55	E61	56-60 (ALVQI)	54-58 (RLVM)	I56_C60delinsRLVM	
8	E61	D64	62-63 (VR)	60-61 (IK)	V62_R63delinsIK	
9	D64	L73	65-72 (SHLTAVGK)	63-70 (SNNRICPI)	S65_K72delinsSNNRICPI	$BB_{0.10}$
10	L73	L77/N78	74-76 (LDN)	72-74 (MEK)	L74_N76delinsMEK	
11	L77/N78	Y96	79-95 (GECPCGN)	77-94 (GEDPCGNAHGT)	G77_T95delinsGEDPCGNAHGT	$BB_{0.11}$
12	Y96	S97	96 (N)	95 (N)	N96_S97delinsNT	$BB_{0.12}$
13	S97	S99/R99/E100	97-100 (TVIQ)	96-99 (NTVQ)	S97delinsNTVQ	
14	S99/R99/E100	L93/G94/R95	98-97 (NS)	96-97 (NT)	N96_S97delinsMT	$BB_{0.13}$
15	L93/G94/R95	Y96/R99/E100	96-97 (NS)	96-97 (NT)	N96_S97delinsMT	
16	Y96/R99/E100	Y102	101 (R)	101 (Q)	R101Q	$BB_{0.14}$
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	$BB_{0.15}$
20	V111/S112	Y117	113-116 (AVDS)	112-116 (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)	123-127 (YQDGDA)	G123_N128delinsYQDGDA	$BB_{0.16}$
24	D129	F131	130 (T)	129 (V)	T130S	
25	F131	R133/E134/P135	132 (N)	131 (S)	N132S	
26	R133/E134/P135	Y117	133-117 (T)	132-117 (T)	T132_Y117T	$BB_{0.17}$
27	Y106	F140	139 (R)	138 (W)	R139W	$B_{0.1-0.4}$
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	157-159 (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 NAnchor Conserved AA in D1	D1 CAnchors Conserved AA in D1	D1 Building Block Variable AA in D1	D1L3 Building Block Variable AA in D1L3	Building Block Mutations of D1 variants	BB Cluster
35	E165/I166/D167	L169	158 (A)	167 (E)	A166E	BB_{35-37}
36	L169	V172/Y173	170-171 (YD)	169-171 (VE)	Y170_D171delinsVE	
37	V172/Y173	D175/V176	174 (L)	173 (I)	L174T	
38	D175/V176	W180	177-179 (QEK)	176-178 (KHR)	Q177_K179delinsKHR	BB_{38-40}
39	W180	E183	181-182 (GL)	180-181 (KA)	G181_L182delinsKA	
40	E183	M187-GDFNAGCSY-V195	184-187 (DVML)	183-187 (NFIF)	D184_L187delinsNFIF	
41	V195-S211/T222-K236	P206	196-221 (TEE)	188-206 (SSSRAFTNSKKSVTLRKKTKS)	S196_S205delinsTEE	BB_{41}
42	W211	Q210/T222	222-230 (QSSRAFTNSKK)	207-210 (QSSR)	S207_S209delinsQSSR	
43	P206-T222	T222	231-239 (SVTLRKKTK)	211-222 (AFTNSKKSVTLR)	A208_R221delinsFTNSKKSVTLR	
44	T222	T229	240-246 (SKRKRS)	223-229 (KKTKSKR)	K224_R230delinsKTKSKRS	
45	T229	C231-AYDRI-V237	247-253 (VSSRAFT)	230 (N)	H230N	
46	C231-AYDRI-V237	G240	238-239 (VA)	236-239 (LR)	V238_A239delinsLR	BB_{46-48}
47	G240	V247/V248/P249	241-246 (MLLRGA)	241-246 (QEIVSS)	M241_A246delinsQEIVSS	
48	V247/V248/P249	S261	250 (D)	250 (K)	D250K	
49	S261	F265	252-254 (ALP)	252-254 (NSV)	A252_P254delinsNSV	
50	F255	F257/Q258	256 (N)	256 (D)	N256D	
51	F257/Q258	Y261	259-260 (AA)	259-260 (KA)	A259_A260delinsKA	BB_{49-50}
52	Y261	L263	262 (Q)	262 (K)	G262K	
53	L263	A268	264-267 (SDQL)	264-267 (TEEE)	S264_L267delinsTEEE	
54	A268	S272/Q273/H274	269-271 (QAI)	269-271 (LDV)	Q269_I271delinsLDV	
55	S272/Q273/H274	P276/V277/E278	275 (Y)	275 (F)	Y275F	
56	P276/V277/E278	L281	279-280 (VM)	279-280 (FK)	V279_M280delinsFK	BB_{55-56}
57	L281	-	282 (K)	282-305 (QSSRAFTNSKKSVTLRKKTKSKRS)	K282delinsQSSRAFTNSKKSVTLRKKTKSKRS	

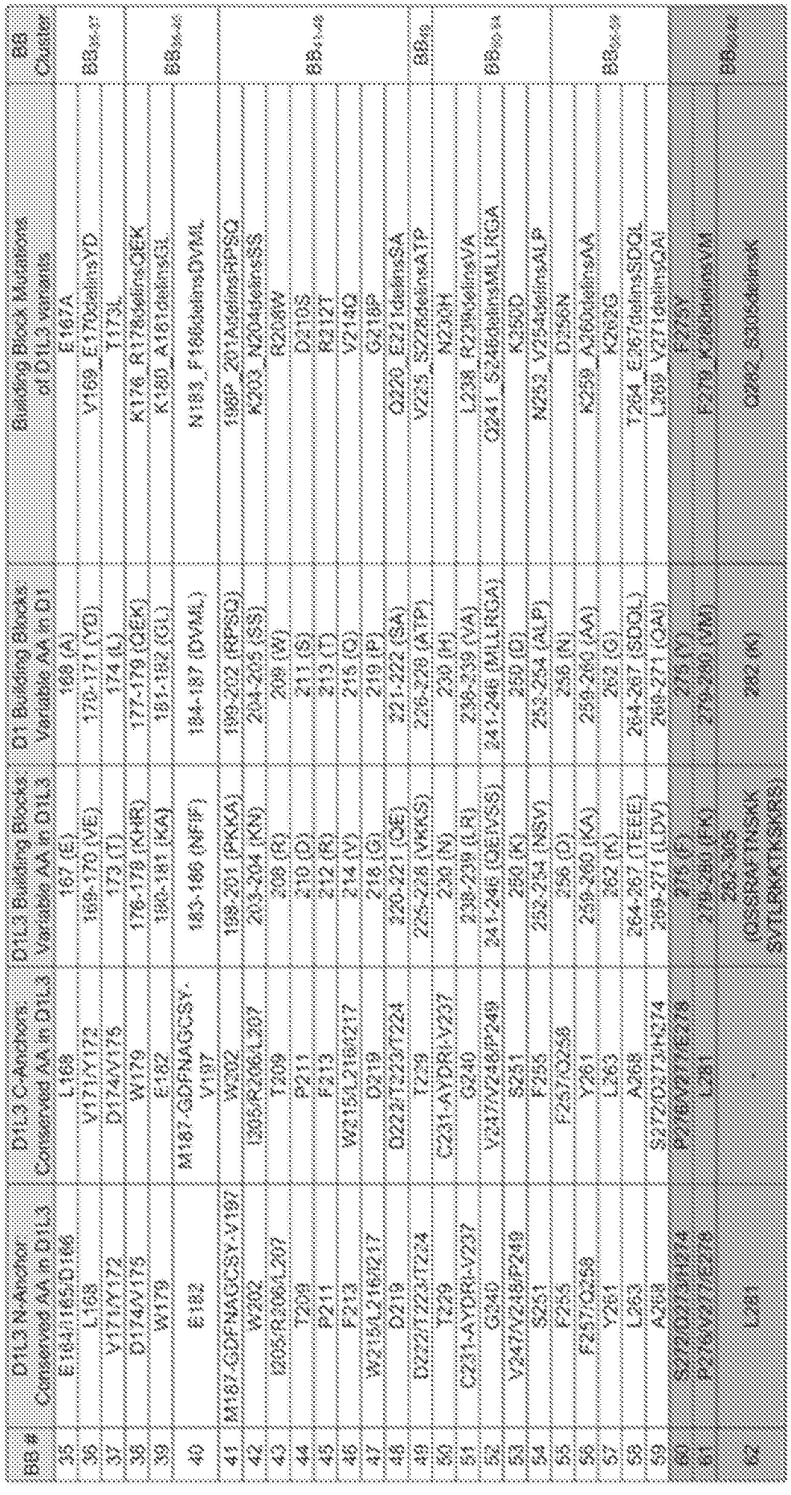
FIG. 31 continued

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-Anchor, 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 (MSKELAPLLLLLLSHSALA)	1-22 (MPGMKLLGALLALAALLQGAVS)	M1_A2delinsMPGMKLLGALLALAALLQGAVS	BB_0
1	Signal peptide	F26/N27	21-28 (MRICS)	23-27 (LKIAA)	M21_S25delinsLKIAA	$BB_{1\text{-}3}$
2	F26/N27	F31/G32	28-30 (VRS)	30-32 (IQT)	V28_S30delinsIDT	
3	F31/G32	K35	33-34 (ES)	35-39 (ETT)	E33_S34delinsETT	
4	K35	Y46	36-45 (EPSAAPKCLL)	40-47 (MSKALVSV)	S36_E45delinsMSKALVSV	BB_4
5	V46	R51	47-50 (KVIK)	49-53 (QILS)	K47_K50delinsQILS	$BB_{5\text{-}7}$
6	R51	D53	52 (C)	54 (Y)	C52Y	
7	D53	E58	54-58 (ILYVM)	56-60 (ALVQI)	I54_M58delinsIALVQ	
8	E58	D62	60-61 (IK)	62-63 (VR)	I60_A61delinsVR	$BB_{8\text{-}10}$
9	D62	L71	63-70 (SNVRICPI)	65-72 (SHLTAVGK)	S63_I70delinsSHLTAVGK	
10	L71	L75/N76	72-74 (MEK)	74-76 (LDN)	M72_K74delinsLDN	
11	L75/N76	Y85	77-84 (PNSRRGIT)	79-94 (GDAPDITI)	R77_T84delinsGDAPDIT	BB_{11}
12	Y85	Y87	86 (N)	96 (H)	N86H	
13	Y87	S90	88-89 (VI)	98-99 (VV)	V88_I89delinsVV	$BB_{13\text{-}14}$
14	S90	L93/S94/R95	91-92 (SR)	91-92 (EP)	S91_R92delinsEP	
15	L93/S94/R95	Y98/K99/E100	96-97 (NT)	96-97 (NS)	N95_T97delinsNS	
16	Y98/K99/E100	Y102	101 (Q)	101 (R)	Q101R	$BB_{16\text{-}18}$
17	Y102	F104	103 (A)	103 (L)	A103L	
18	F104	Y109	106 (L)	105 (V)	L105V	
19	Y109	Y111/S112	107-110 (KENL)	107-110 (RPDQ)	K107_L110delinsRPDQ	$BB_{19\text{-}20}$
20	Y111/S112	Y117	113-116 (VKRS)	113-116 (AVDS)	V113_S116delinsAVDS	
21	Y117	Y119	118 (H)	118 (Y)	H116Y	$BB_{21\text{-}22}$
22	Y119	D121	120 (H)	120 (D)	H120D	
23	D121	D126	122-125 (GCEP)	122-129 (GCEPCGRA)	Y122_A125delinsGCEPCGR	$BB_{23\text{-}24}$
24	D126	F130	127 (N)	131 (T)	Y126T	
25	F130	R132E133/P134	130 (S)	132 (N)	S130N	$BB_{25\text{-}26}$
26	R132E133/P134	Y137	135-136 (FY)	136-137 (AI)	I35F_L36YdelinsAI	BB_{26}
27	Y137	F139	138 (W)	139 (R)	W138R	BB_{27}
28	F139	T144	140-143 (QSPH)	141-144 (FSRF)	Q140_H143delinsFSRF	
29	T144	E149	145-148 (AVKD)	145-149 (EVRE)	A145_D148delinsAVKD	
30	E149	I151	150 (V)	150 (A)	V150A	
31	I151	P163/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/H165/D166	163 (K)	164 (A)	K163A	

FIG. 31 continued

B; continued



BB #	D1L3 N-Anchor	D1L3 C-Anchors	D1L3 Building Blocks	D1 Building Blocks	Building Block Mutations	BB
	Conserved AA in D1L3	*Conserved AA in D1L3*	*Variable AA in D1L3*	*Variable AA in D1*	*of D1L3 versions*	*Cluster*
35	E184/I185/D186	L168	167 (E)	166 (A)	E167A	
36	L168	Y171/Y172	169-170 (VE)	170-171 (YD)	V169_E170delinsYD	BB_{35-37}
37	Y171/Y172	D174/V175	173 (T)	174 (L)	T173L	
38	D174/V175	W179	176-178 (KAR)	177-179 (QEK)	K176_R178delinsQEK	
39	W179	E182	180-181 (KA)	181-182 (GL)	K180_A181delinsGL	BB_{38-40}
40	E182	M187-GDPNAGCSY-V197	183-186 (NFIF)	184-187 (DYML)	N183_F186delinsDYML	
41	M187-GDPNAGCSY-V197	W302	198-201 (PKKA)	199-202 (RPSQ)	198P_201Adelins4PSQ	
42	W302	I305/R306/L307	203-204 (KN)	204-205 (SS)	K203_N204delinsSS	
43	I305/R306/L307	T309	208 (R)	208 (W)	R208W	
44	T309	P311	210 (Q)	211 (S)	Q210S	BB_{41-46}
45	P311	F313	212 (R)	213 (T)	R212T	
46	F313	W315/L216/I217	214 (V)	215 (Q)	V214Q	
47	W315/L216/I217	Q219	218 (G)	219 (P)	G218P	
48	Q219	D222/T223/T224	220-221 (QE)	221-222 (SA)	Q220_E221delinsSA	
49	D222/T223/T224	T229	225-228 (VKKS)	226-229 (ATP)	V225_S228delinsATP	BB_{48}
50	T229	C231-AYDRI-V237	230 (N)	230 (H)	N230H	
51	C231-AYDRI-V237	G240	238-239 (LR)	236-239 (VA)	L238_R239delinsVA	
52	G240	V247/Y248/P249	241-246 (QEIVSS)	241-246 (MLLRGA)	Q241_S246delinsMLLRGA	BB_{49-54}
53	V247/Y248/P249	S251	250 (K)	250 (D)	K250D	
54	S251	F255	252-254 (NSV)	253-254 (ALP)	N252_Y254delinsALP	
55	F255	F257/Q258	256 (Q)	256 (N)	D256N	
56	F257/Q258	Y261	259-260 (KA)	259-260 (AA)	K259_A260delinsAA	
57	Y261	L263	262 (K)	262 (G)	K262G	BB_{55-59}
58	L263	A268	264-267 (TEEE)	264-267 (SDQL)	T264_E267delinsSDQL	
59	A268	S272/Q273/H274	269-271 (LDV)	269-271 (QAI)	L269_V271delinsQAI	
60	S272/Q273/H274	P276/P277/E278	276 (F)	276 (T)	F275T	
61	P276/P277/E278	L281	279-280 (NK)	279-280 (VM)	F279_K280delinsVM	BB_{60-61}
62	L281		282-285 (GSSQ&FTNSKK SVTLPHKTKSYRS)	282 (K)	Q282_S285delinsK	

FIG. 32

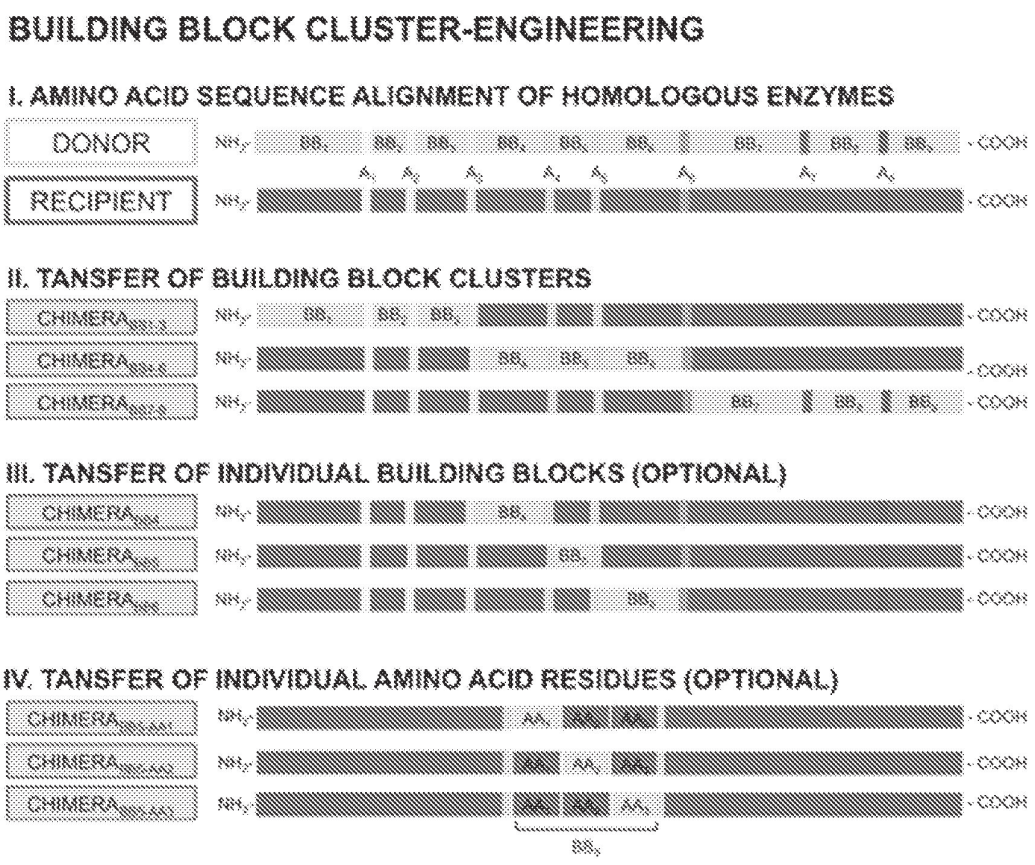

BUILDING BLOCK CLUSTER-ENGINEERING
I. AMINO ACID SEQUENCE ALIGNMENT OF HOMOLOGOUS ENZYMES
DONOR
RECIPIENT
II. TANSFER OF BUILDING BLOCK CLUSTERS
III. TANSFER OF INDIVIDUAL BUILDING BLOCKS (OPTIONAL)
IV. TANSFER OF INDIVIDUAL AMINO ACID RESIDUES (OPTIONAL)
COOH

FIG. 33

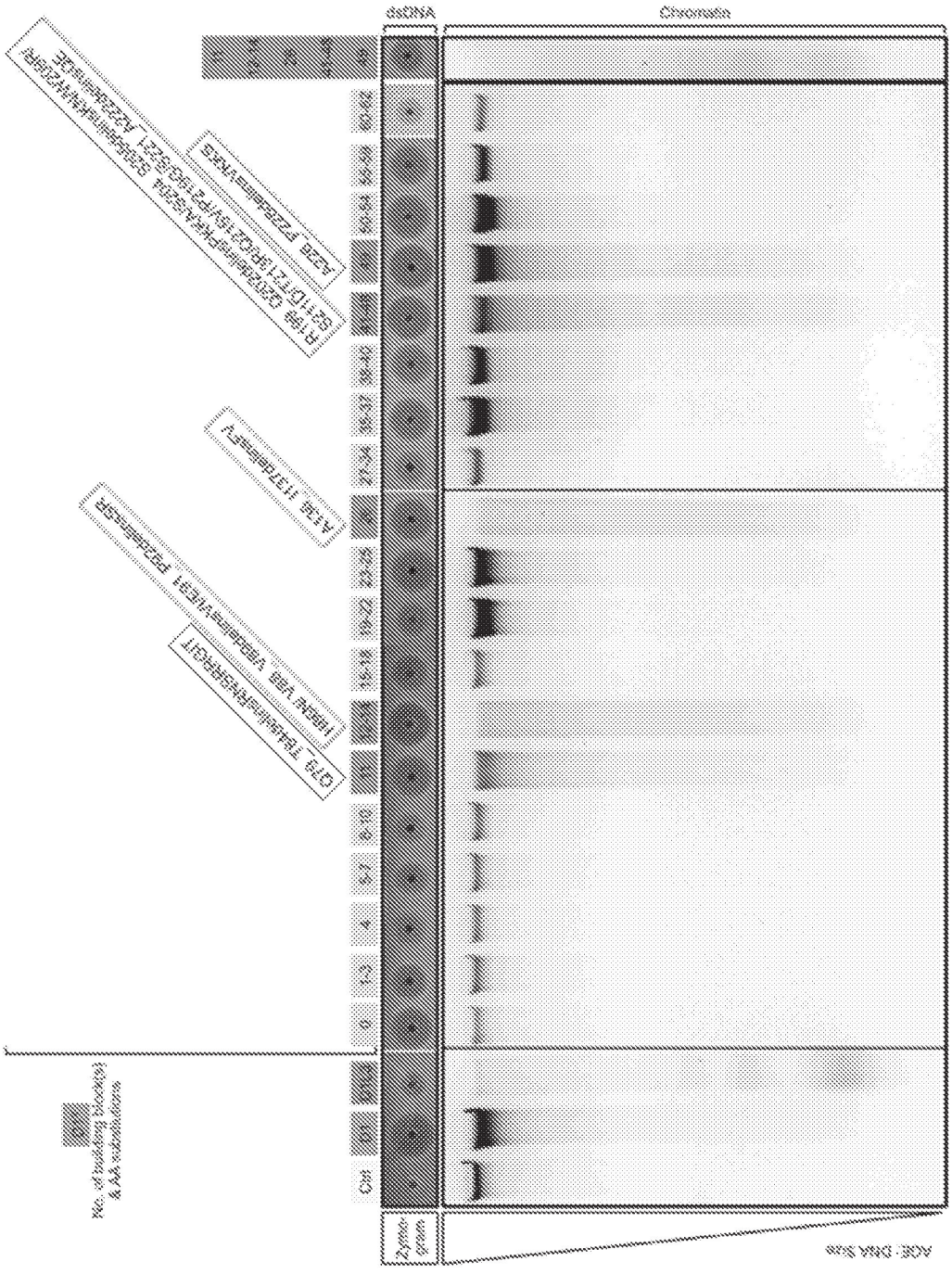


FIG. 34

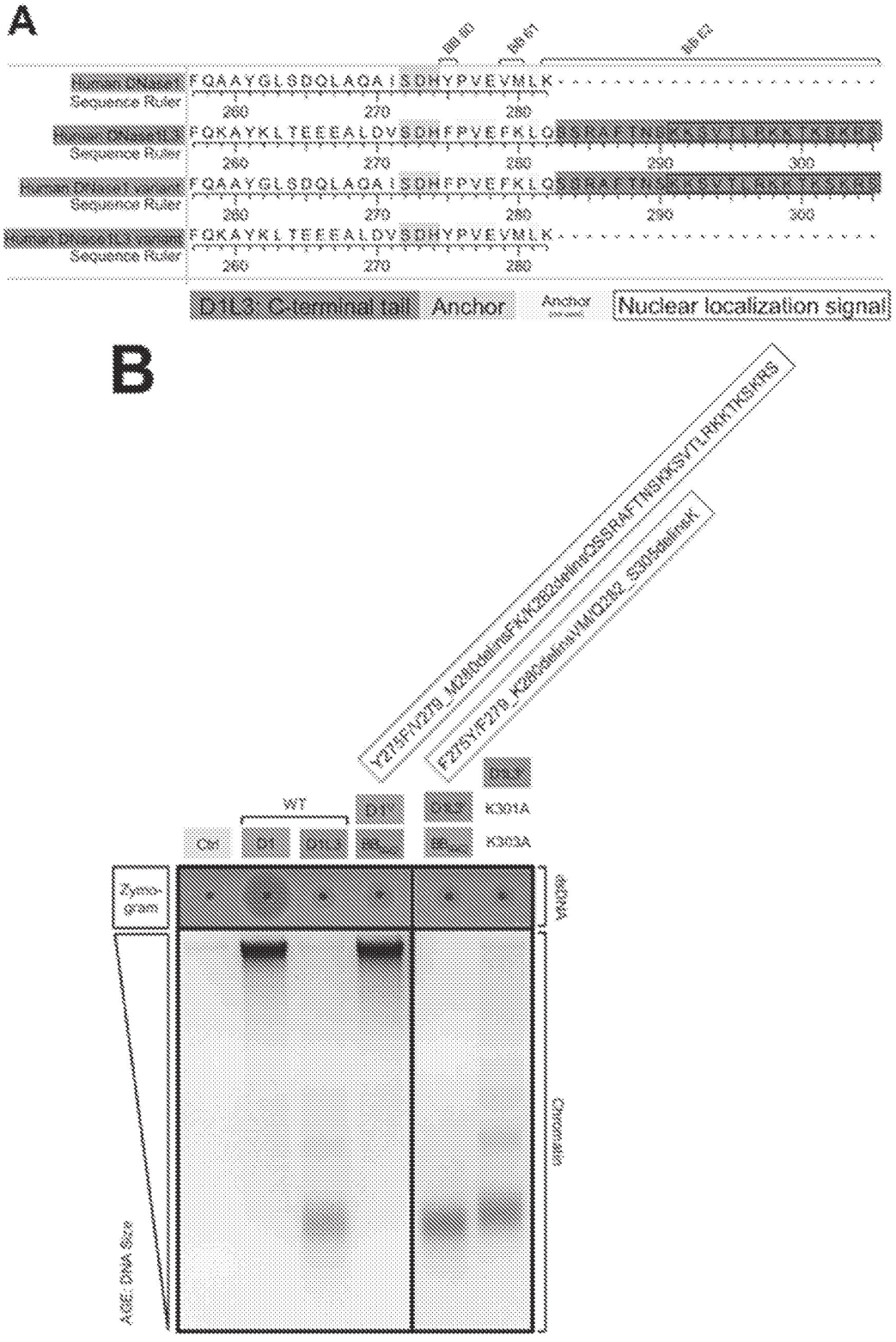

A
Sequence Ruler
Anchor
Nuclear localization signal
B
WT
Ctrl
K301A
K303A
Zymogram
AGE: DNA Size
WB:HA
Chromatin

FIG. 35

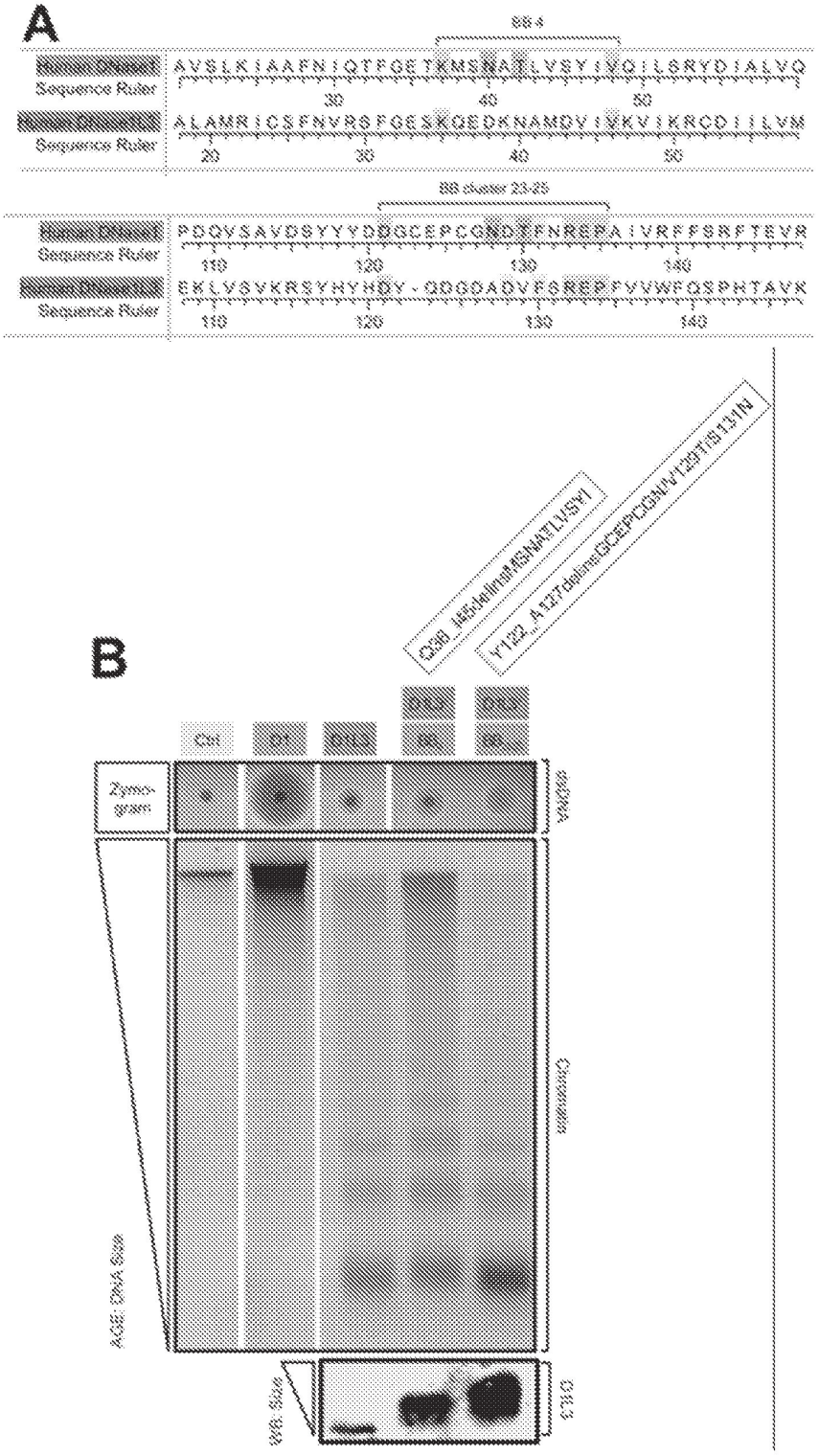

A
Sequence Ruler
Sequence Ruler
Sequence Ruler
Sequence Ruler
B
Ctrl
DTT
WT
Zymo gel
AGE: DNA Size
WB: Size
Coomassie
anti-DNA
anti-His

FIG. 36

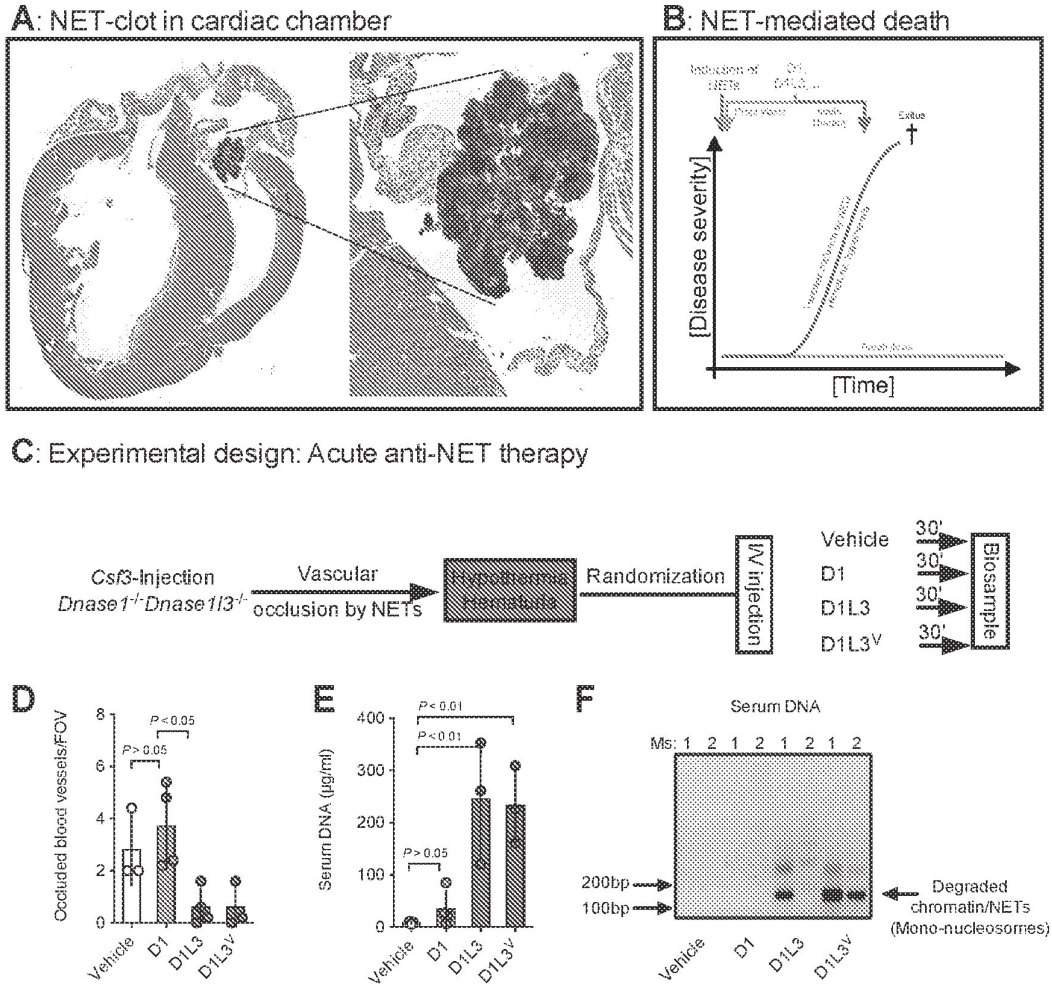

A: NET-clot in cardiac chamber
B: NET-mediated death
[Disease severity]
[Time]
C: Experimental design: Acute anti-NET therapy
Csf3-Injection
Dnase1-/-Dnase1l3-/-
Vascular occlusion by NETs
Hypothermia Normothermia
Randomization
IV injection
Vehicle
D1
D1L3
D1L3V
30'
Biosample
D
Occluded blood vessels/FOV
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. 37

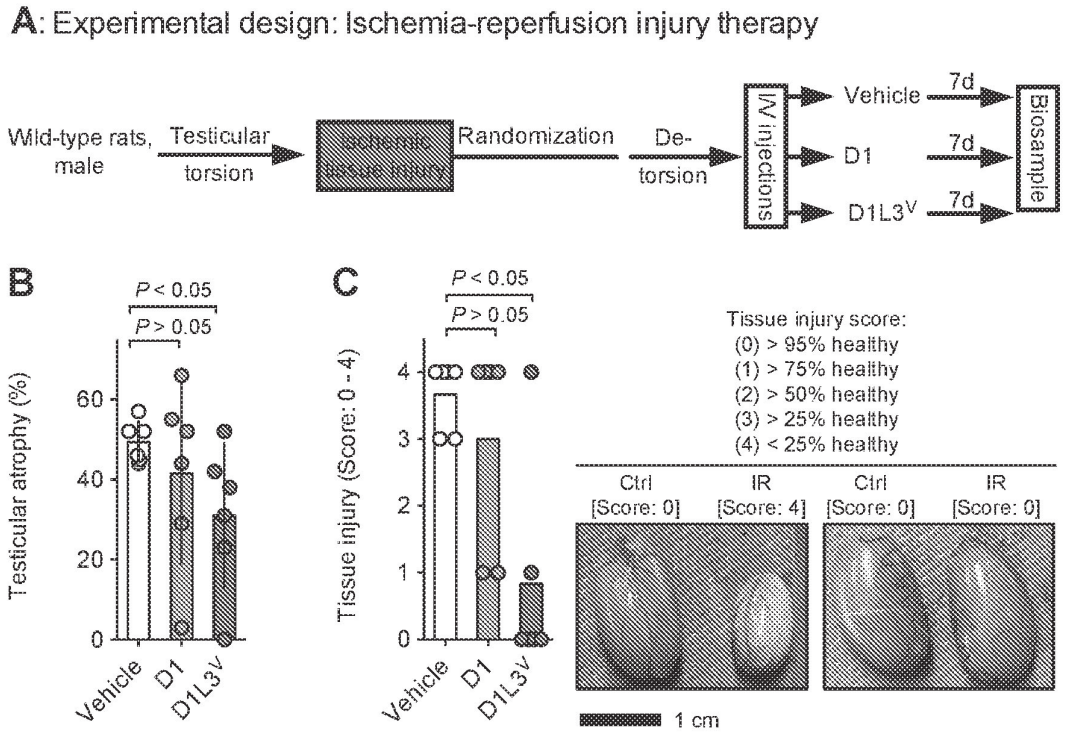

A: Experimental design: Ischemia-reperfusion injury therapy
Wild-type rats, male
Testicular torsion
Ischemic tissue injury
Randomization
De-torsion
I/V injections
Vehicle
D1
D1L3V
7d
7d
7d
Biosample
B
P < 0.05
P > 0.05
Testicular atrophy (%)
60
40
20
0
Vehicle
D1
D1L3V
C
P < 0.05
P > 0.05
Tissue injury (Score: 0 - 4)
4
3
2
1
0
Vehicle
D1
D1L3V
Tissue injury score:
(0) > 95% healthy
(1) > 75% healthy
(2) > 50% healthy
(3) > 25% healthy
(4) < 25% healthy
Ctrl
[Score: 0]
IR
[Score: 4]
Ctrl
[Score: 0]
IR
[Score: 0]
1 cm

FIG. 38

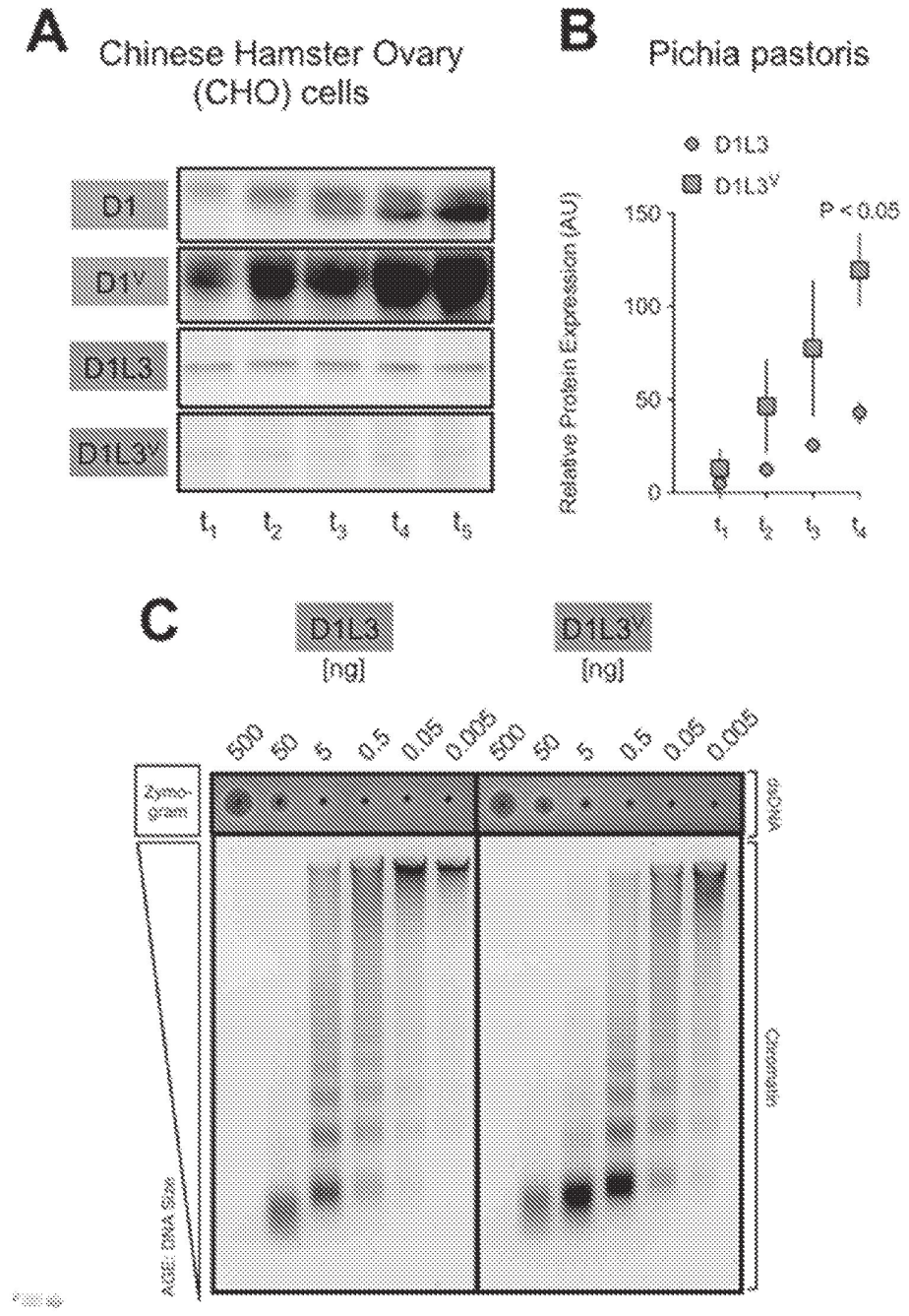

A
Chinese Hamster Ovary (CHO) cells
D1
D1V
D1L3
D1L3V
t1 t2 t3 t4 t5
B
Pichia pastoris
D1L3
D1L3V
P < 0.05
Relative Protein Expression (AU)
150
100
50
0
t1 t2 t3 t4
C
D1L3 [ng]
D1L3V [ng]
500 50 5 0.5 0.05 0.005 500 50 5 0.5 0.05 0.005
Wells
Chromatin
AGE: DNA Size

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/W269K** (Q31R/Q33K/Q57K/W167K)	-	1
6	DNase1 variant	**Q31R/Q49K/Q79K/Q117K/W269K/Q262K** (Q31R/Q27K/Q33K/Q155K/W167K/Q240K)	-	1
7	DNase1 variant	**Q31R/A136F/T227K** (Q31R/A114F/T205K)	-	1
8	DNase1 variant	**A136F/A164K** (A114F/A142K)	-	1
10	DNase1 variant	**Q79_T84delinsRNSRRGIT** (Q57_T62delinsRNSRRGIT)	+ ($BB_{1:1}$)	1
11	DNase1 variant	**H86N/V88_V89delinsVIE91_P92delinsSR** (H64N/V66_V67delinsVIE69_P70delinsSR)	+ ($BB_{1:1}$)	1
12	DNase1 variant	**A136_I137delinsFV** (A114_I115delinsFV)	+ ($BB_{1:1}$)	1
13	DNase1 variant	**R199_Q202delinsPKKA/S204_S205delinsKNW/280R/S211D/T213R/Q215V/ P219G/S221_A222delinsQE** (R177_Q180delinsPKKA/S182_S185delinsKNW/187R/S189D/T191R/Q193V/ P197G/S199_A200delinsQE)	+ ($BB_{1:1}$)	1
14	DNase1 variant	**A226_P228delinsVKKS** (A204_P206delinsVKKS)	+ ($BB_{1:1}$)	1
15	DNase1 variant	**Q79_T84delinsRNSRRGITH86N/V88_V89delinsVIE91_P92delinsSR/ A136_I137delinsFV/ R199_Q202delinsPKKA/S204_S205delinsKNW/280R/S211D/ T213R/Q215V/P219G/S221_A222delinsQE/A226_P228delinsVKKS** (Q57_T62delinsRNSRRGITH64N/V66_V67delinsVIE69_P70delinsSR/ A114_I115delinsFV/R177_Q180delinsPKKA/S182_S185delinsKNW/187R/S189D/ T191R/Q193V/P197G/S199_A200delinsQE/ A204_P206delinsVKKS)	+ ($BB_{1:1}$, $BB_{1:1:1:1}$, $BB_{2:1}$, $BB_{2:1:1:1}$, $BB_{1:1}$)	1
17	DNase1L3 variant	**F275Y/F279_K280delinsVM/Q282_S285delinsK** (F285Y/F289_K290delinsVM/Q292_S295delinsK)	+ ($BB_{1:1:1}$)	2
19	DNase1L3 variant	**Q36_I45delinsMSNATLVSY1** (Q16_I25delinsMSNATLVSY1)	+ ($BB_{1:1}$)	2
20	DNase1L3 variant	**Y122_A127delinsGCEPCGN/V129T/S131N** (Y108_A107delinsGCEPCGN/V109T/S111N)	+ ($BB_{1:1:1}$)	2