

Figure 1A

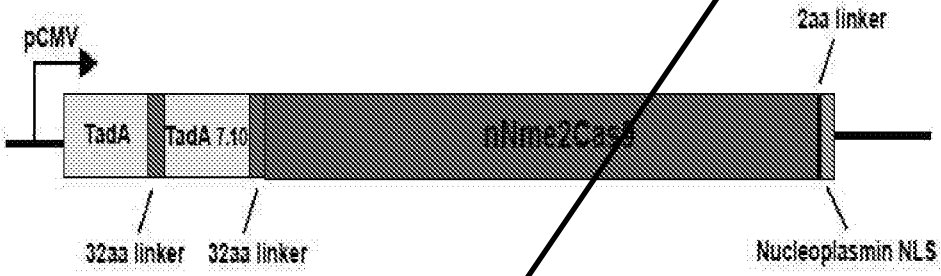


Figure 1B

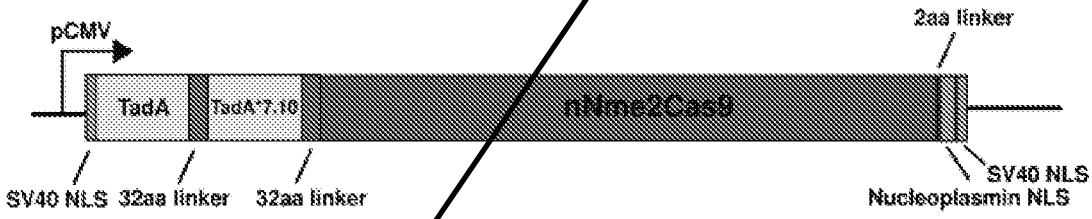


Figure 1C

Figure 1

Figure 2A

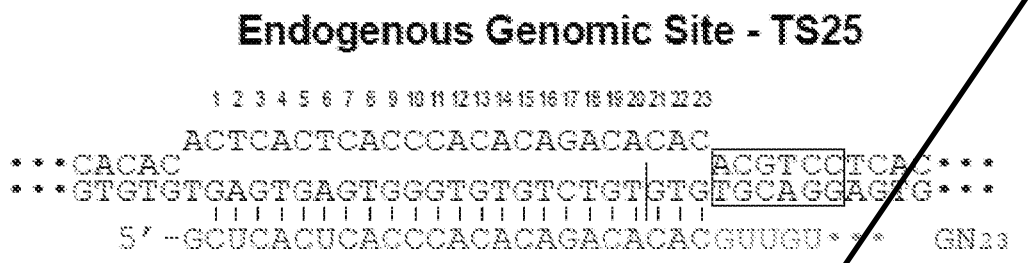


Figure 2B

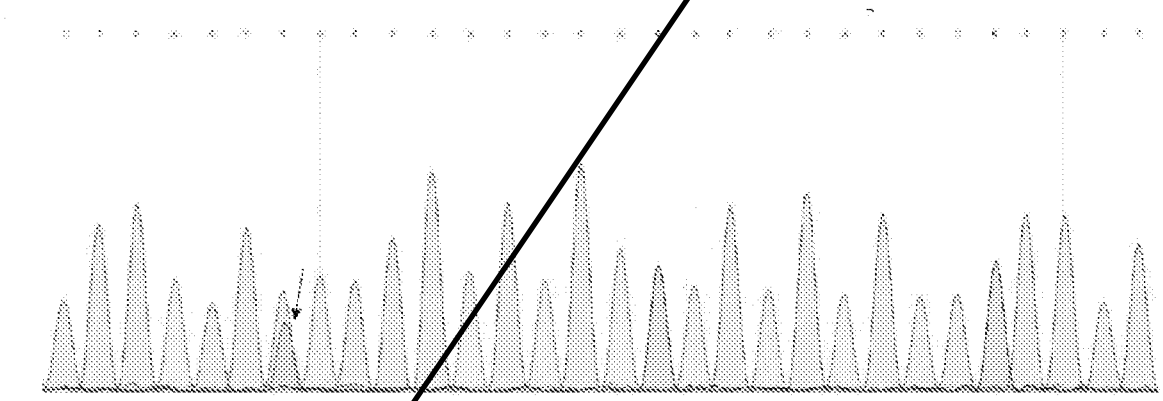


Figure 2

Figure 2C

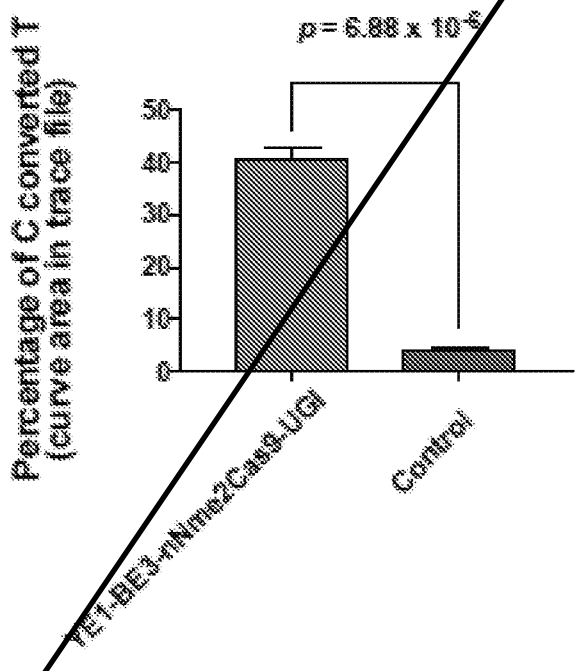
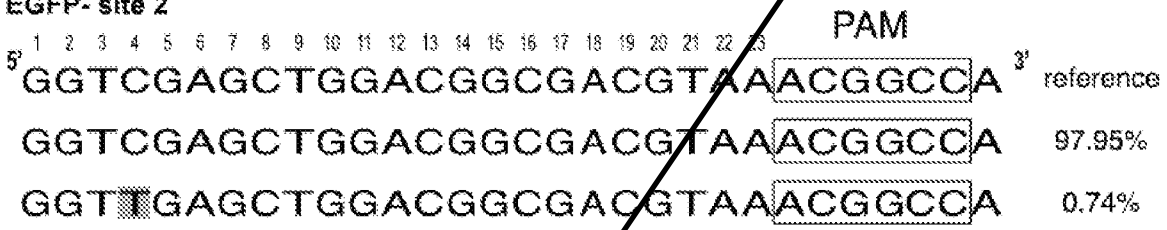


Figure 2 (cont'd)

Figure 3A



Figure 3B



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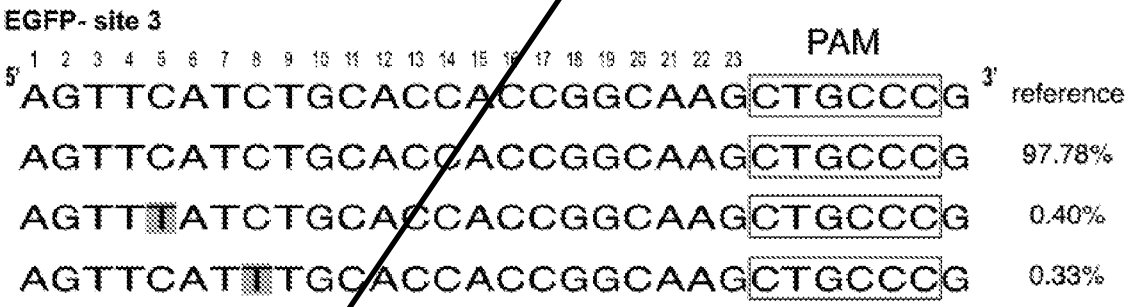
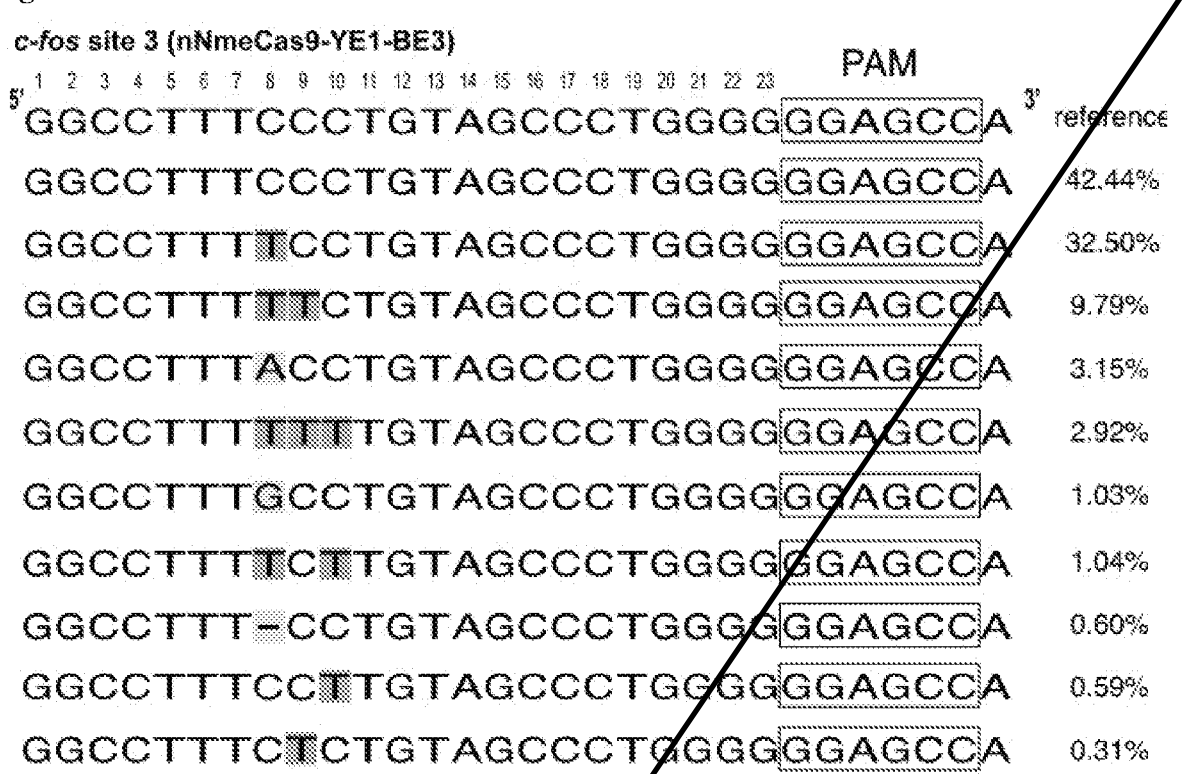


Figure 3D



Figure 3

Figure 3E



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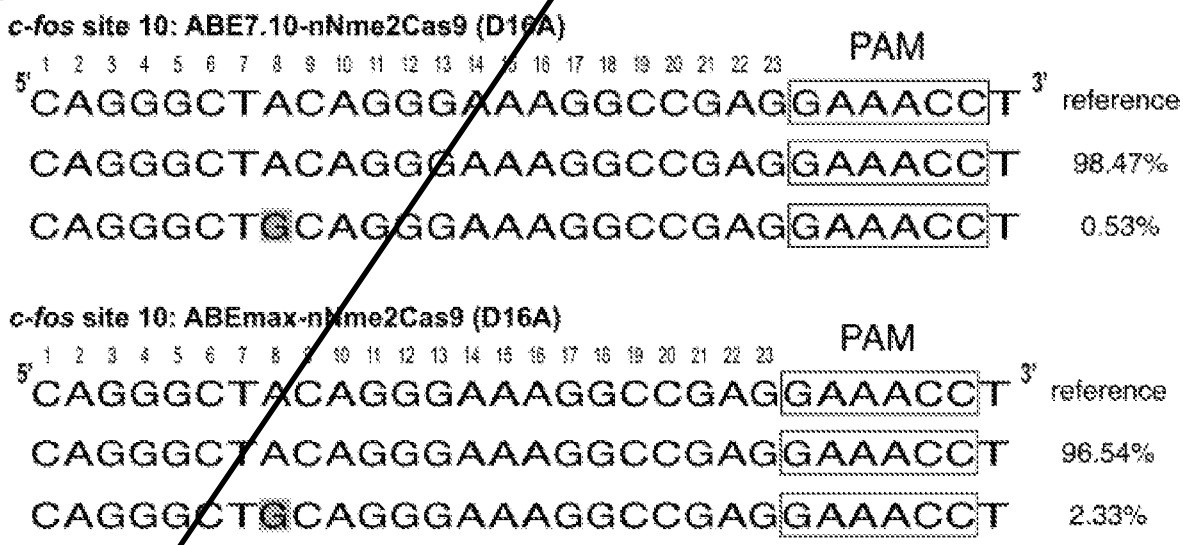

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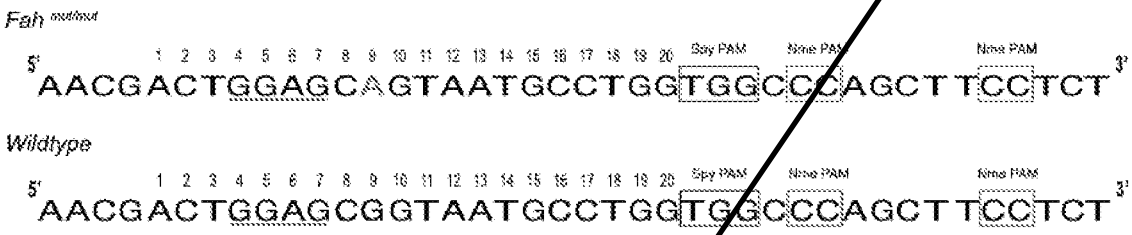

Fah mutmut
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
SpyPAM
NmePAM
NmePAM
5' AACGACTGGAGCAGTAATGCCTGGTGGCCCAGCTTCCTCT 3'
Wildtype
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
SpyPAM
NmePAM
NmePAM
5' AACGACTGGAGCGGTAATGCCTGGTGGCCCAGCTTCCTCT 3'

Figure 4

Figure 5A

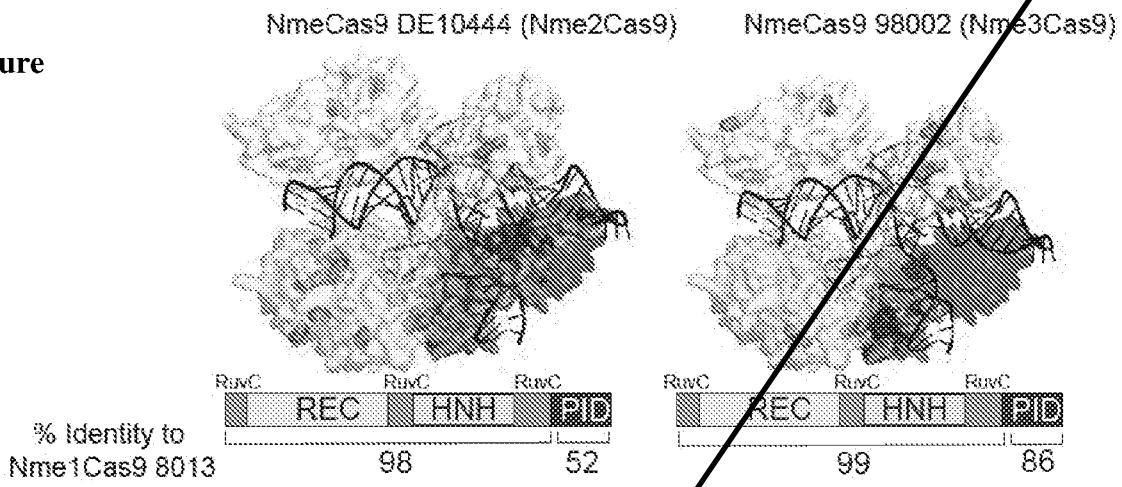


Figure 5B

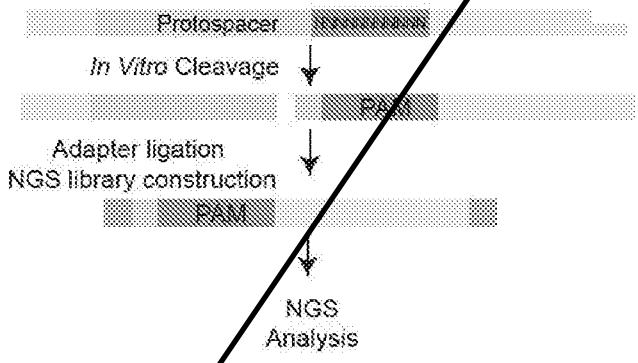


Figure 5C

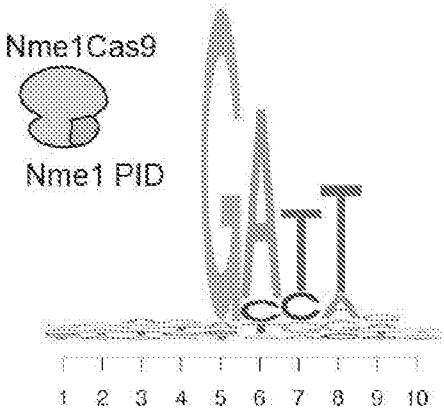
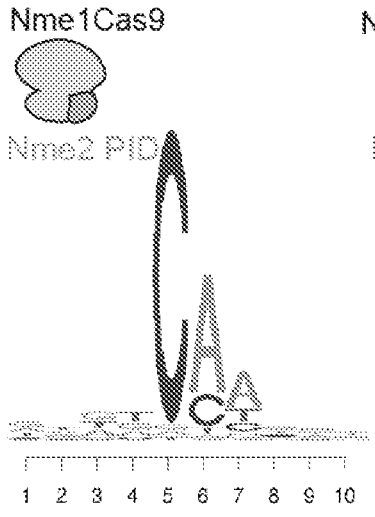


Figure 5

Figure 5D



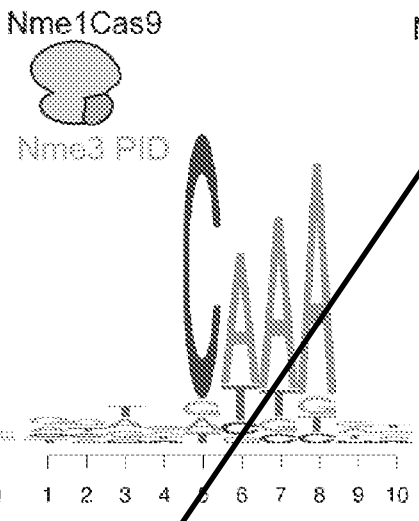


Figure 5E

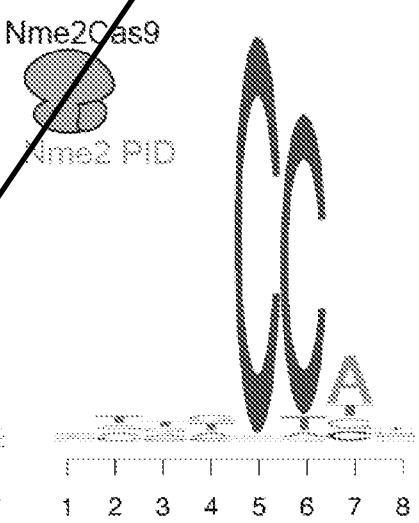


Figure 5 (cont'd)

Figure 6A

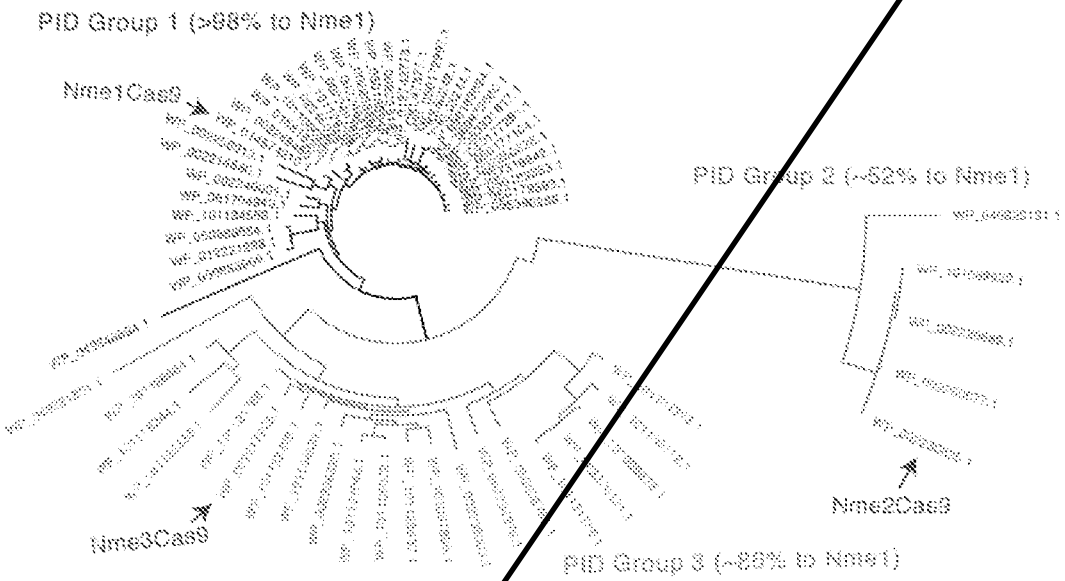


Figure 6B

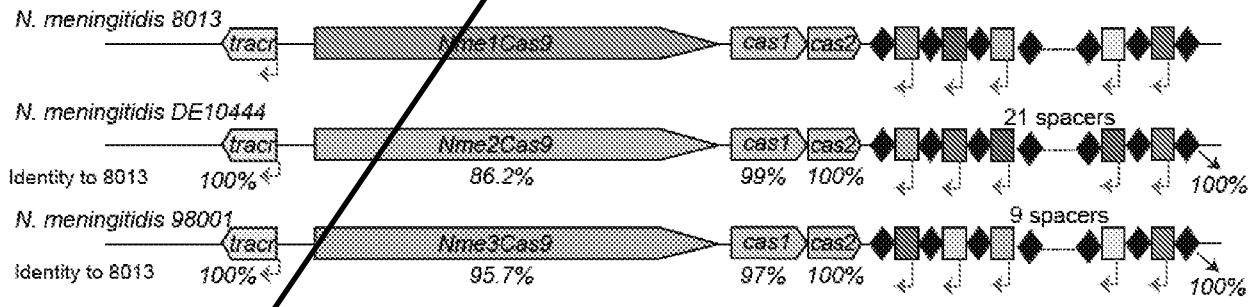


Figure 6

Figure 6C

Figure 6D

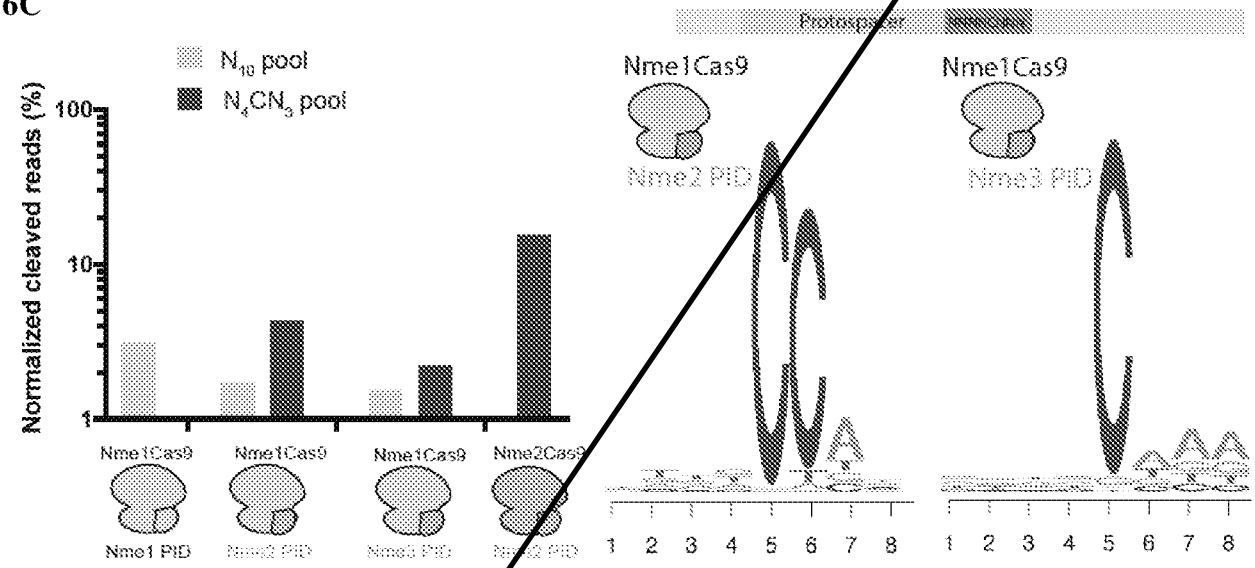


Figure 6 (cont'd)

Figure 7A

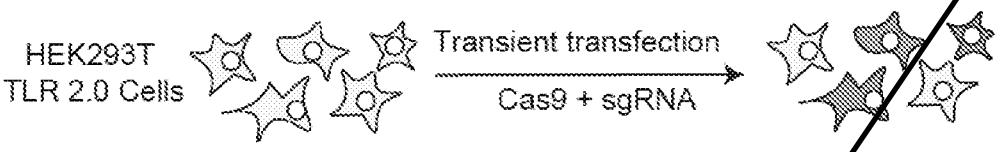


Figure 7B

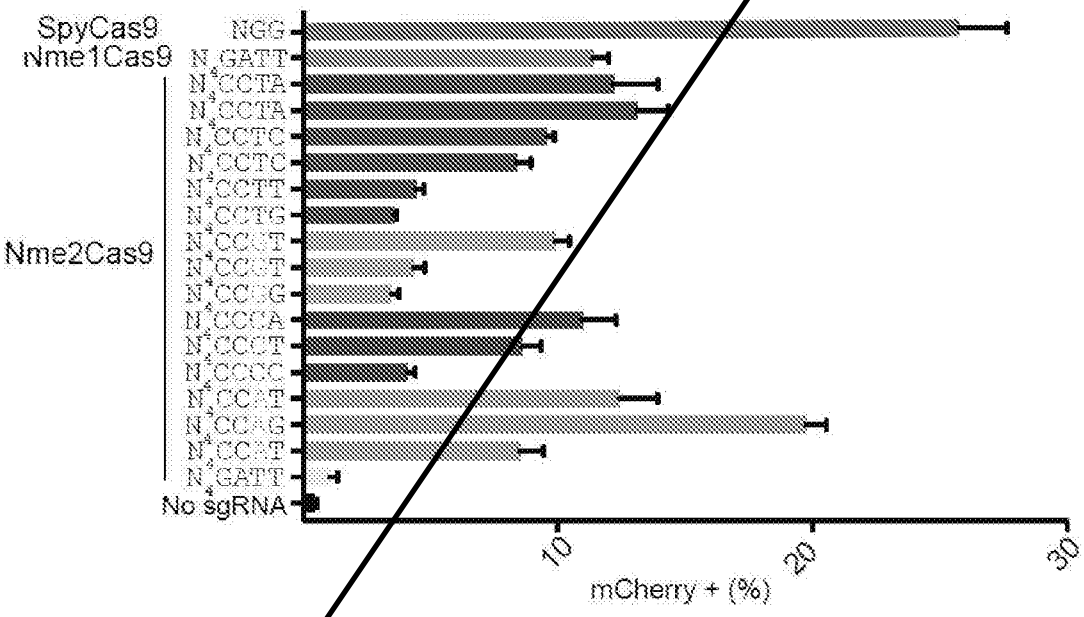


Figure 7

Figure 7C

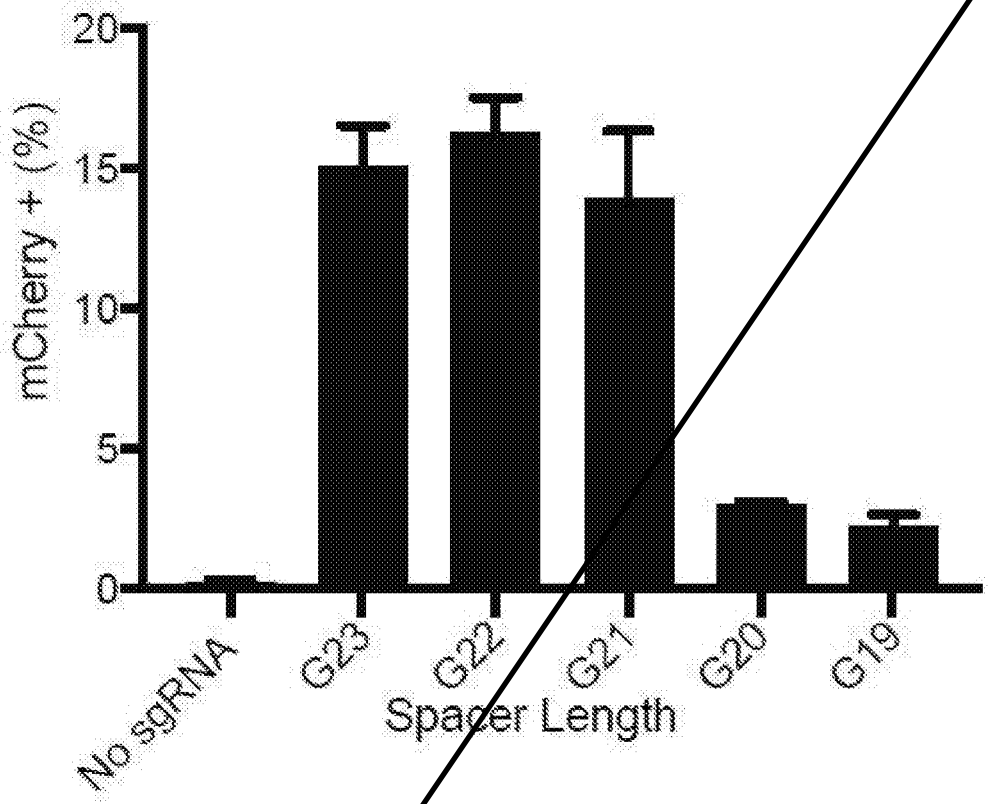


Figure 7D

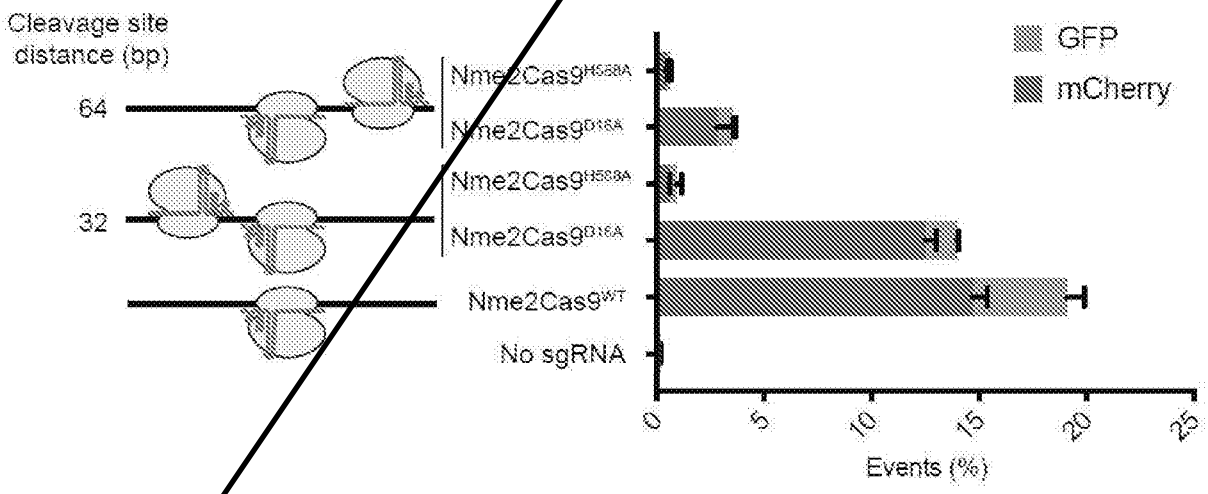


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Figure 8A

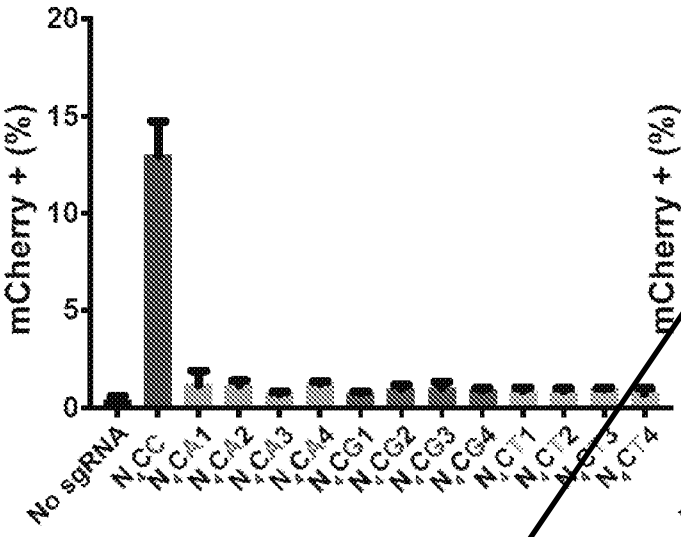


Figure 8B

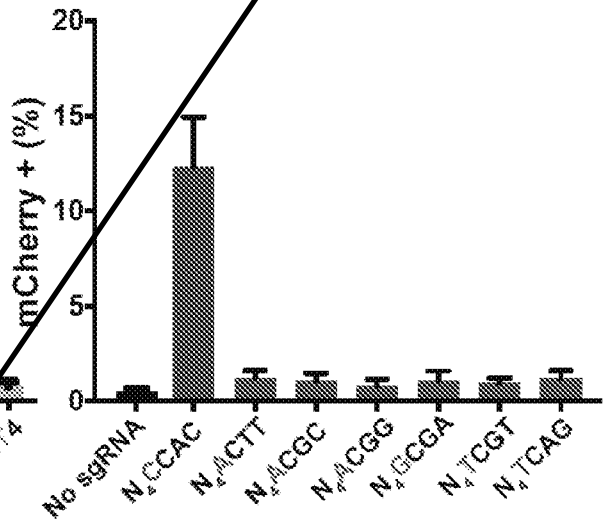
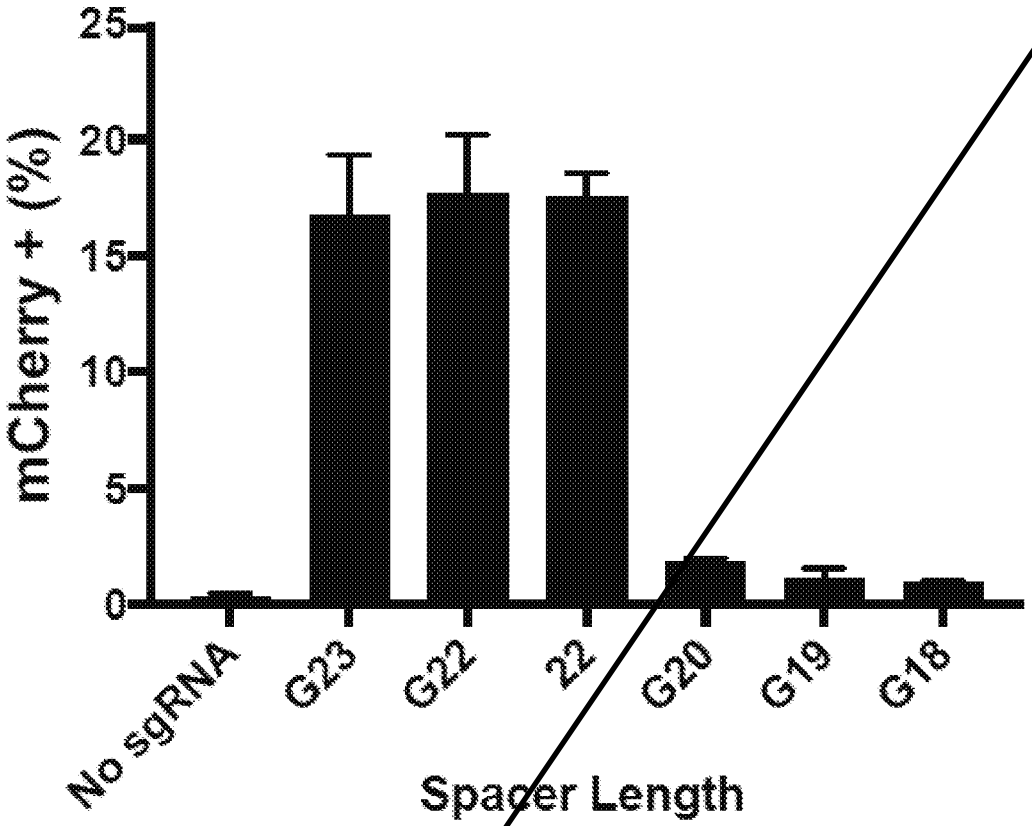


Figure 8

Figure 8C



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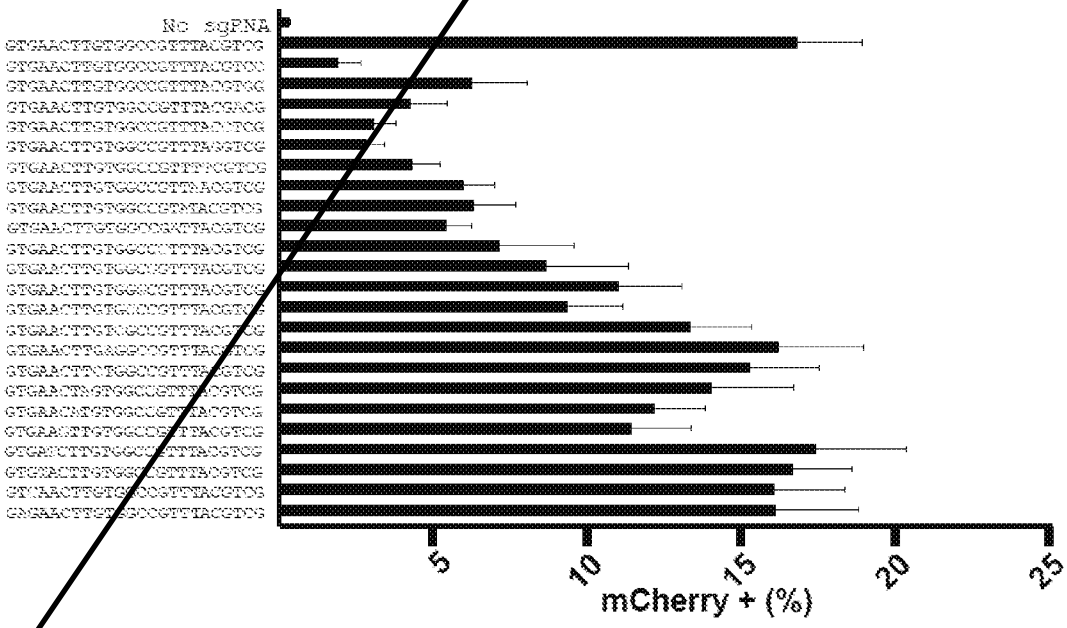


Figure 8 (cont'd)

Figure 9A

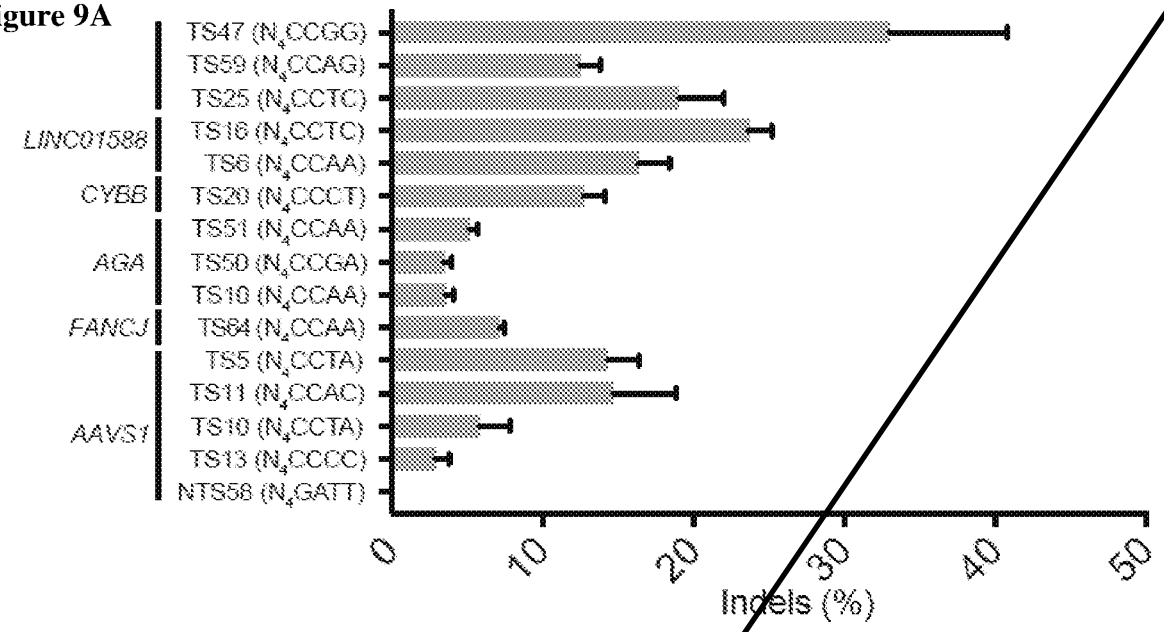


Figure 9B

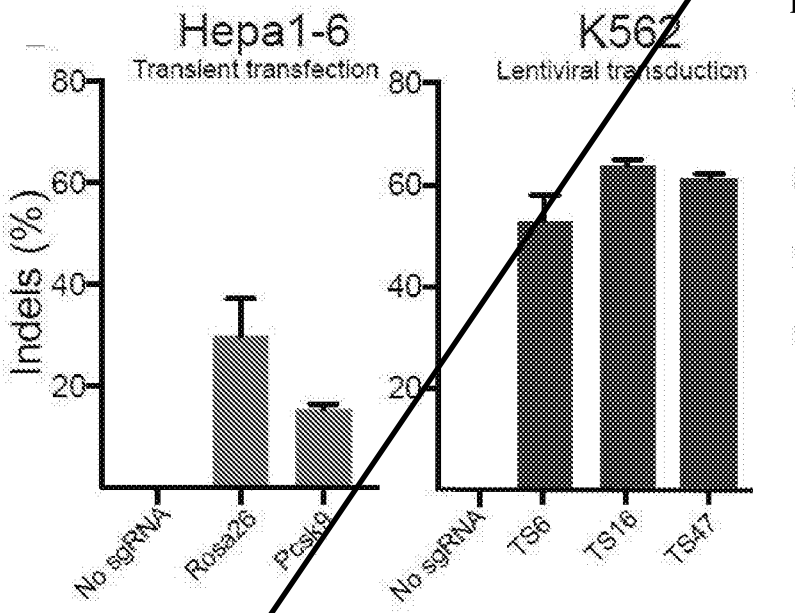


Figure 9C

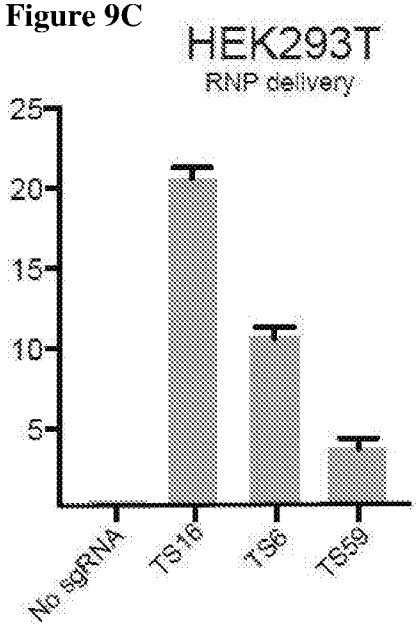


Figure 9

Figure 10A

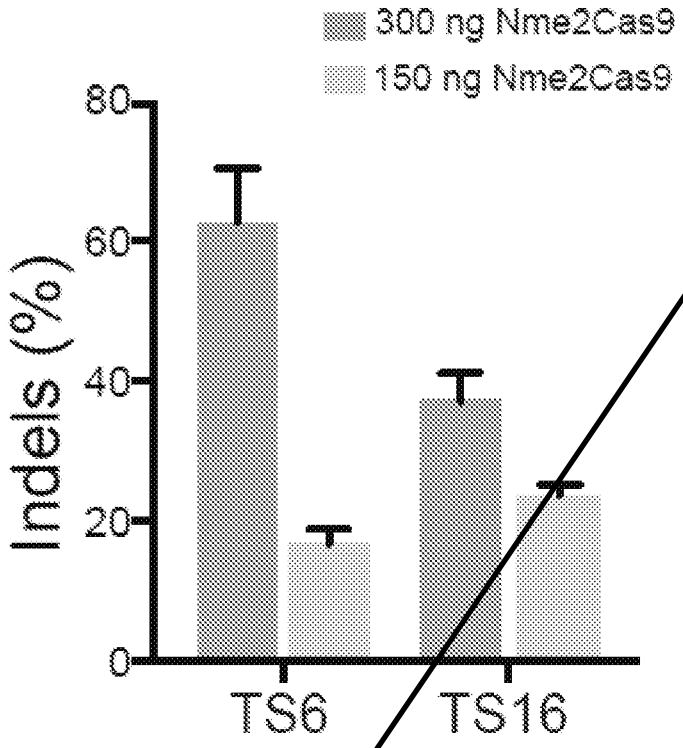


Figure 10B

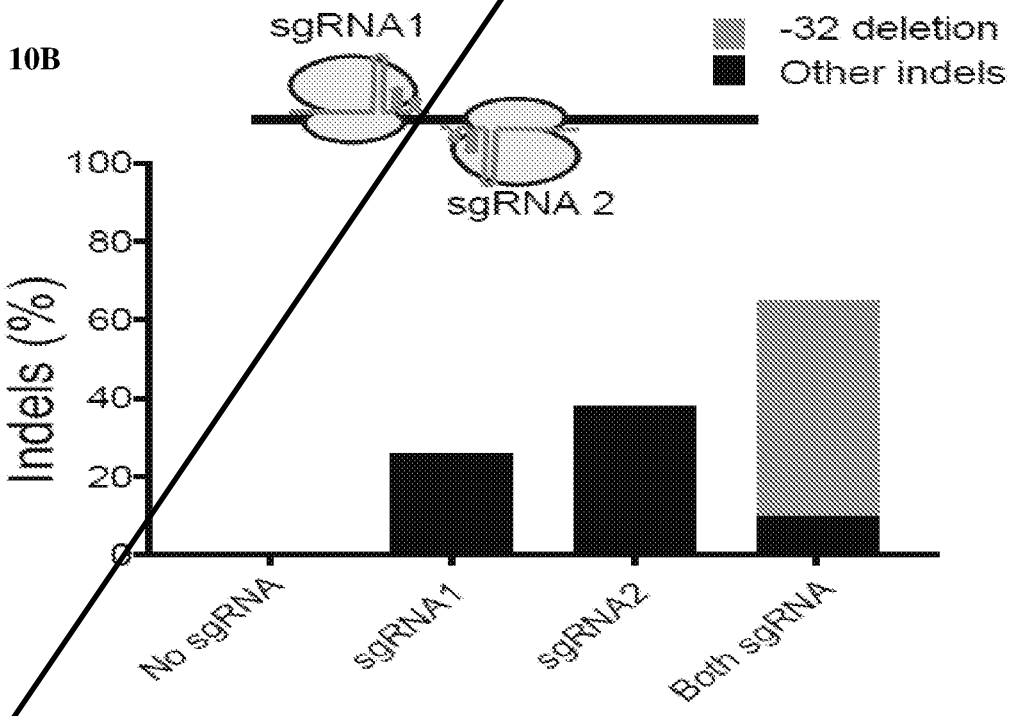


Figure 10

Figure 11A

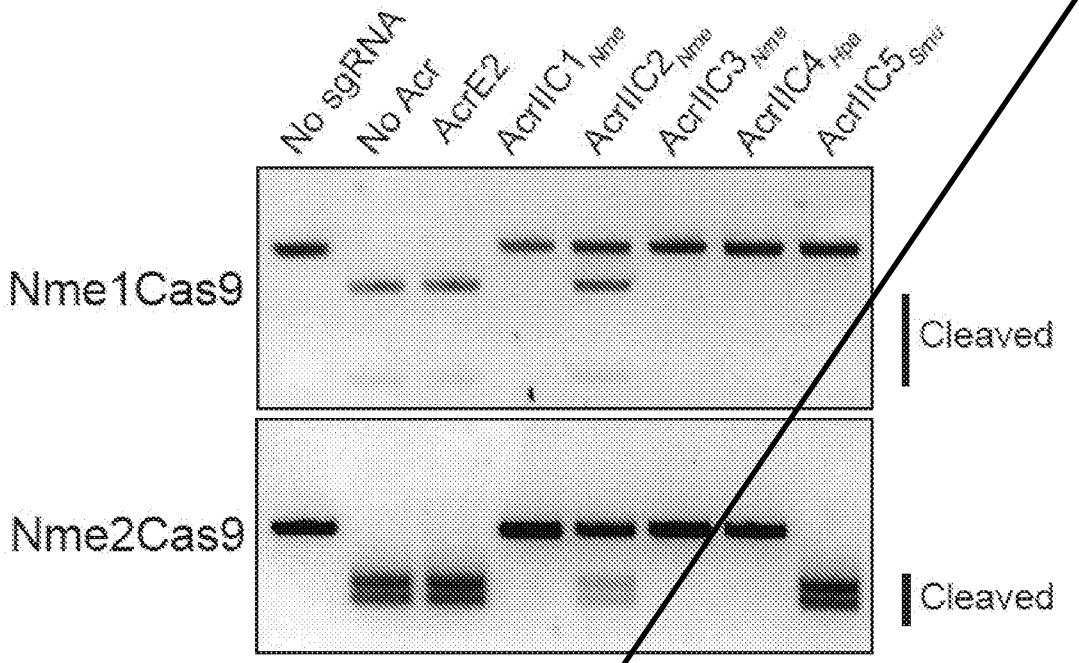


Figure 11B

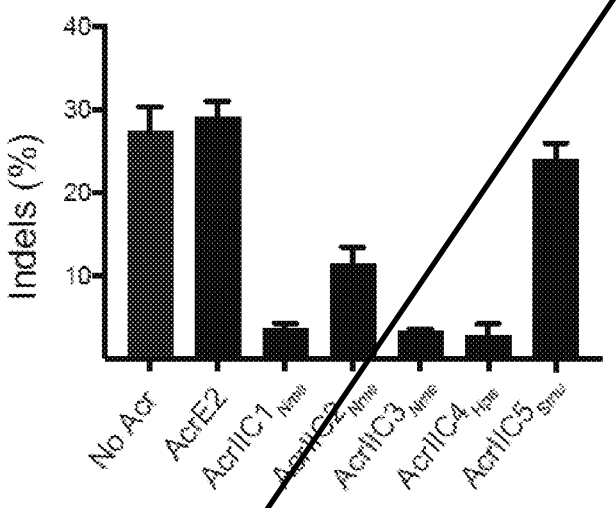


Figure 11C

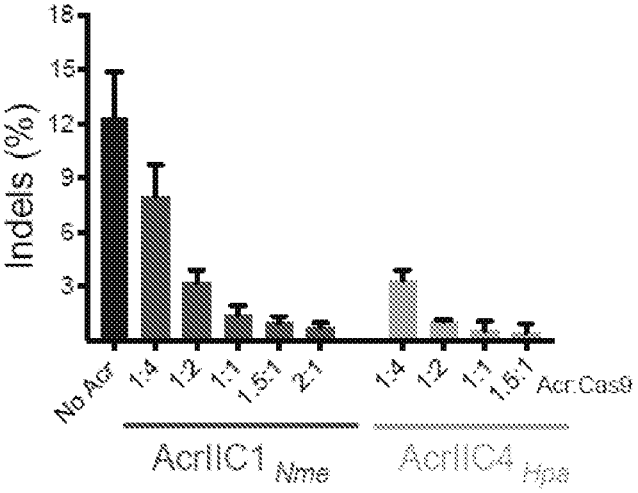
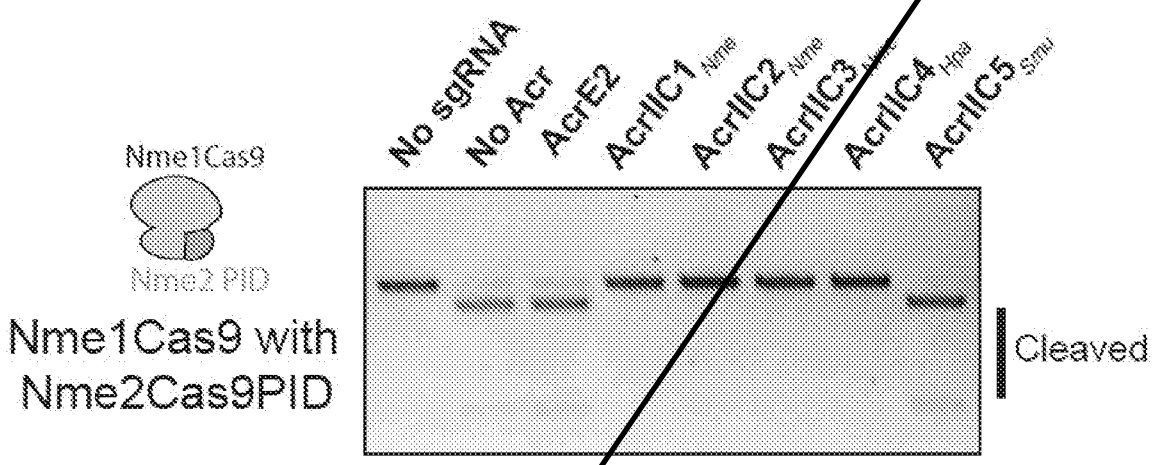


Figure 11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No Acr
AcrE2
AcrIIC1 Nme
AcrIIC2 Nme
AcrIIC3 Nme
AcrIIC4 Hpa
AcrIIC5 Smu
Nme1Cas9
Nme2 PID
Nme1Cas9 with
Nme2Cas9PID
Cleaved

Figure 12

Figure 13A

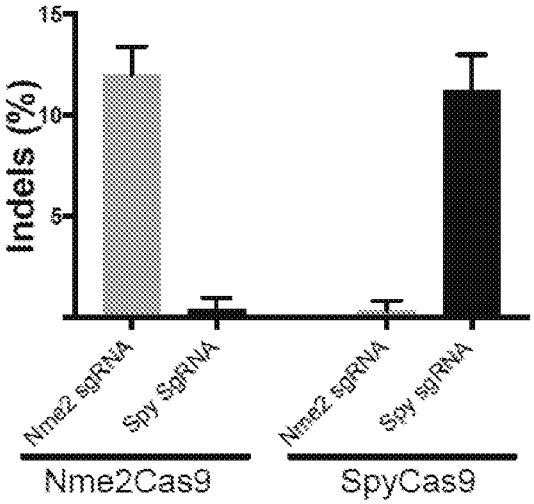

Figure 13B

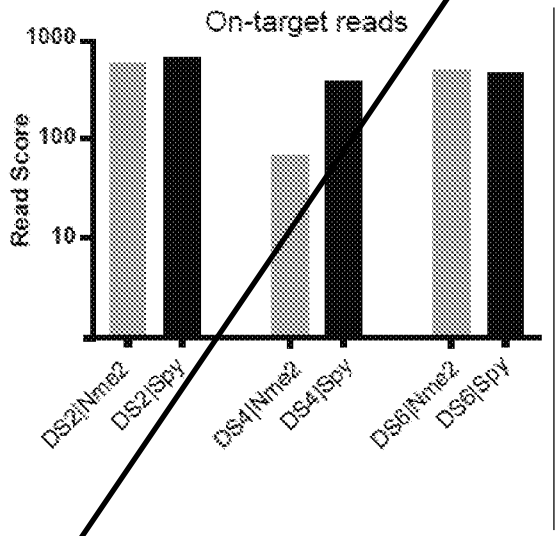

Figure 13C

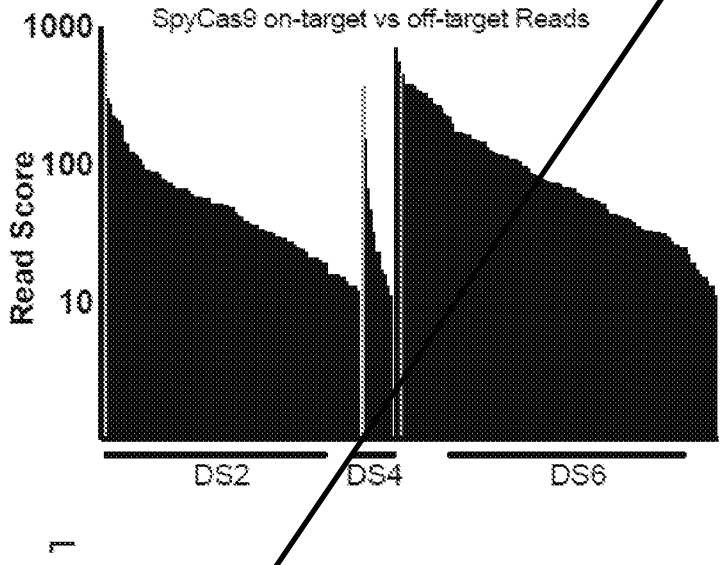

Figure 13D

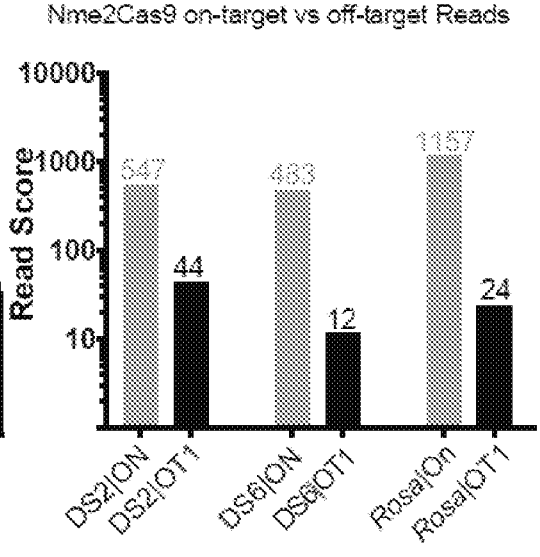

Figure 13

Figure 13E

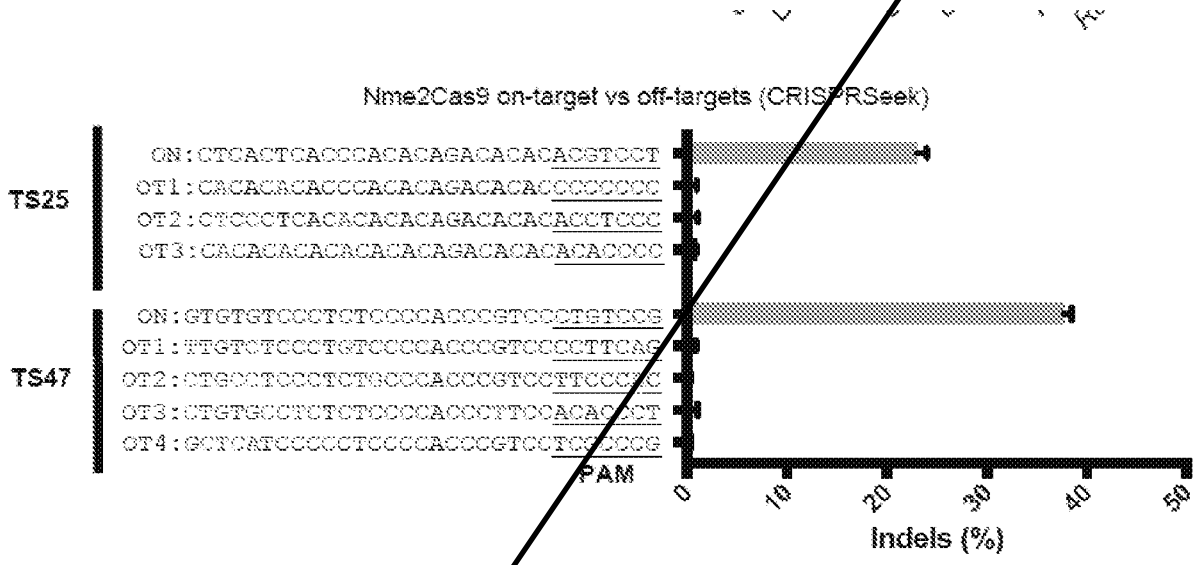

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Figure 14A

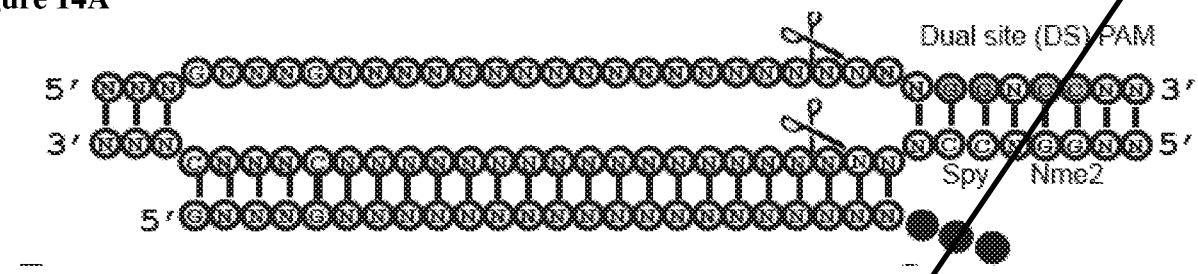


Figure 14B

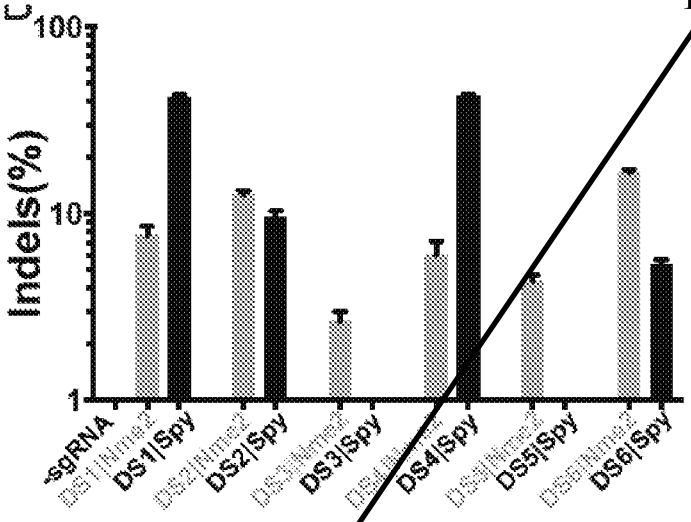


Figure 14C

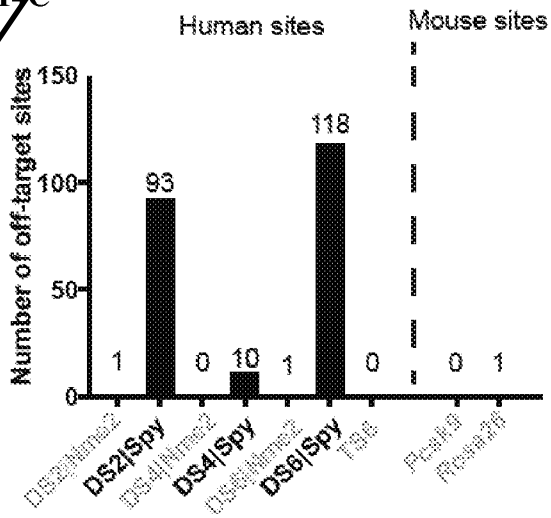


Figure 14

Figure 14D

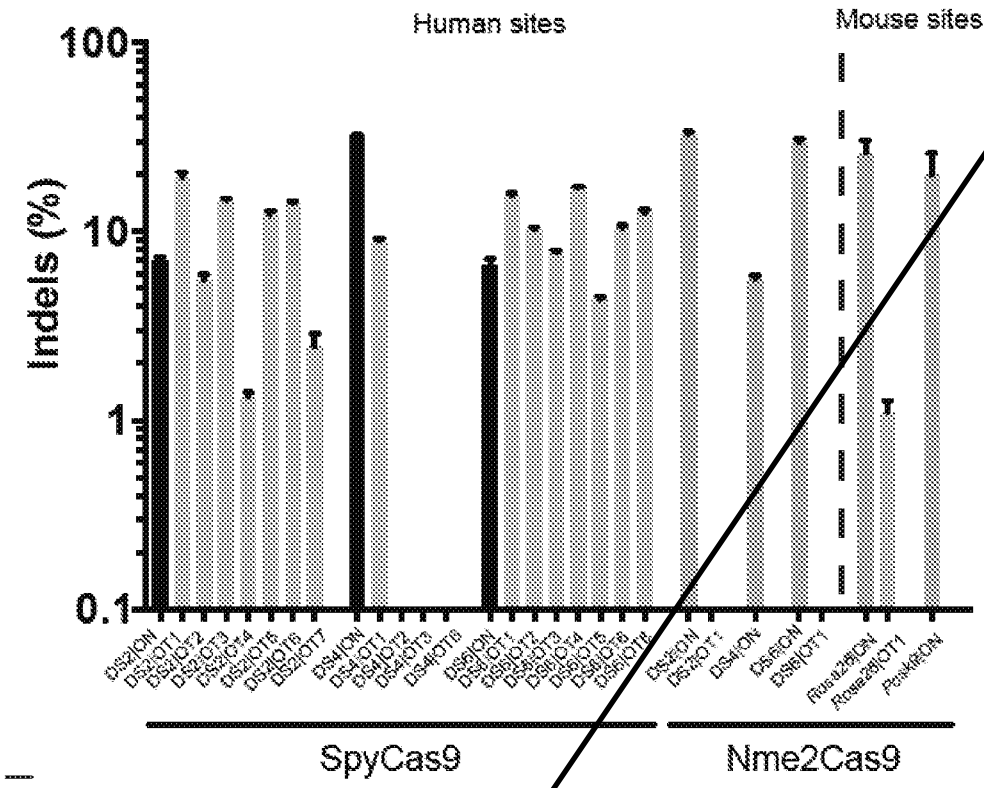


Figure 14E

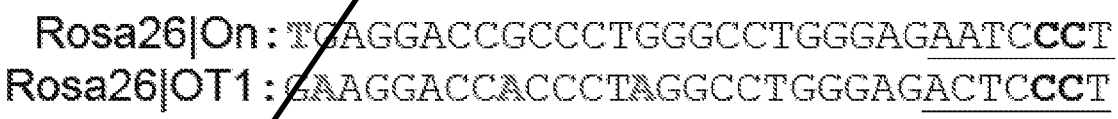


Figure 14 (cont'd)

Figure 15A

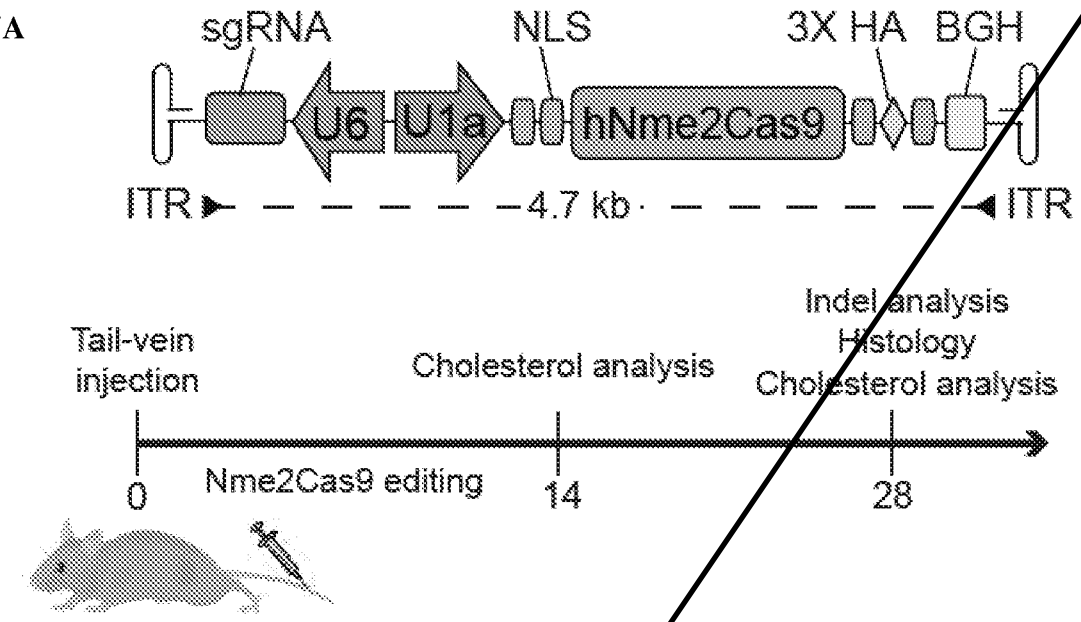


Figure 15B

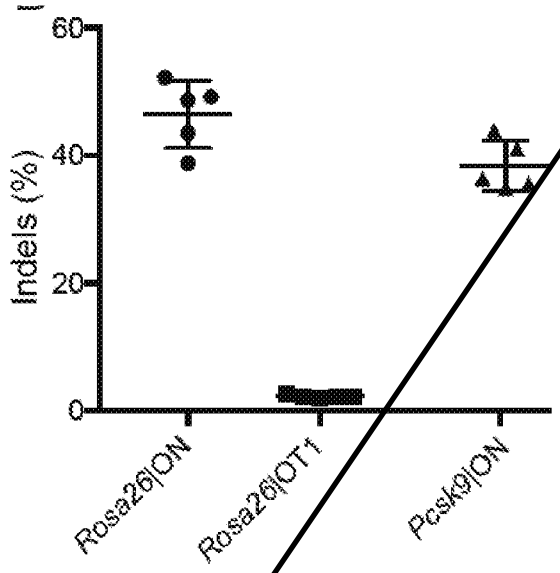


Figure 15C

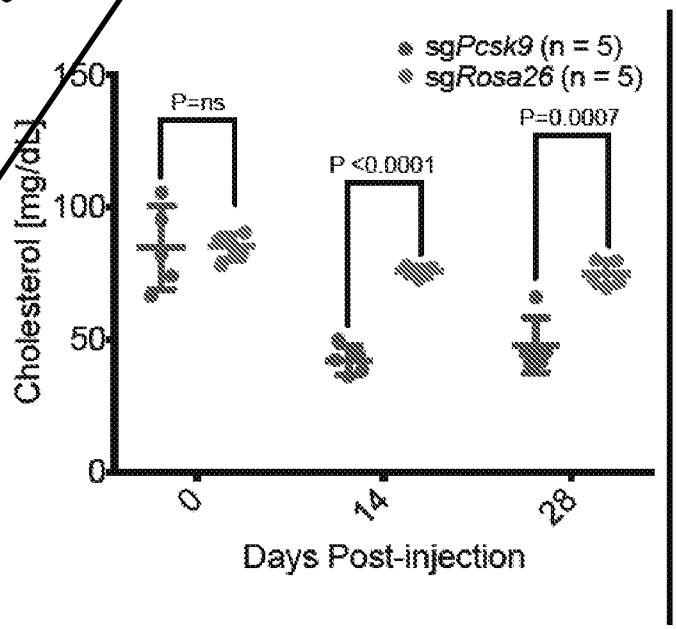


Figure 15

Figure 16A

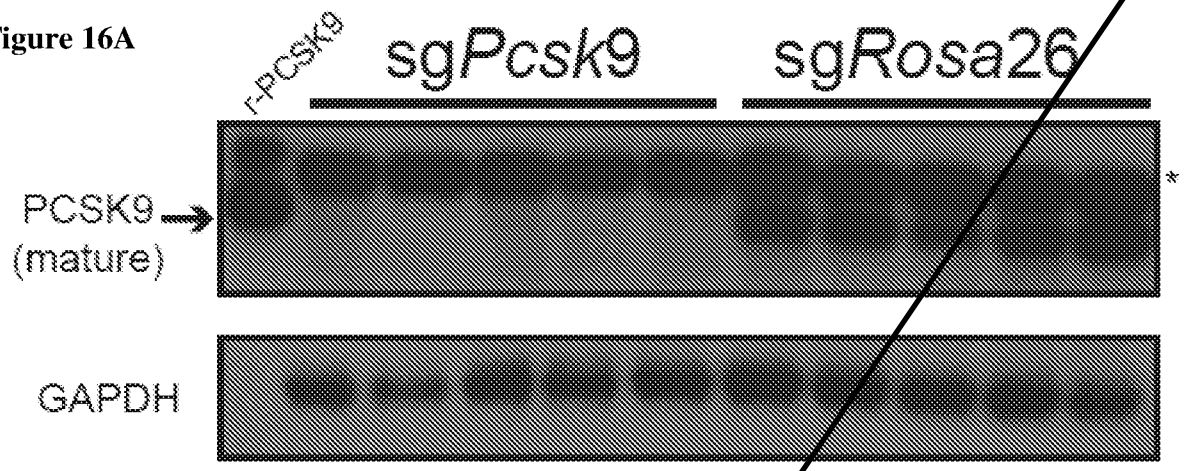


Figure 16B

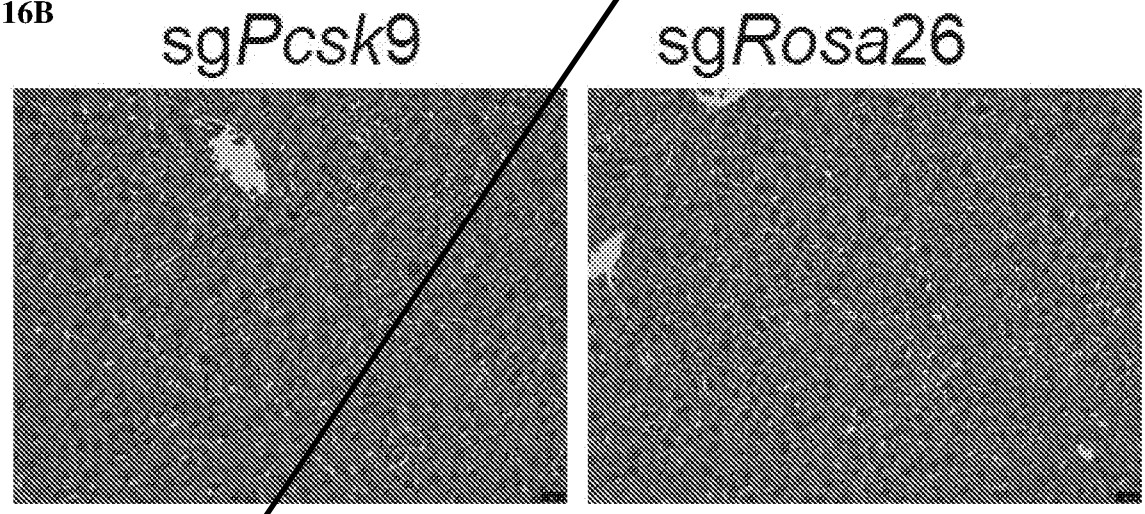


Figure 16

Figure 17A

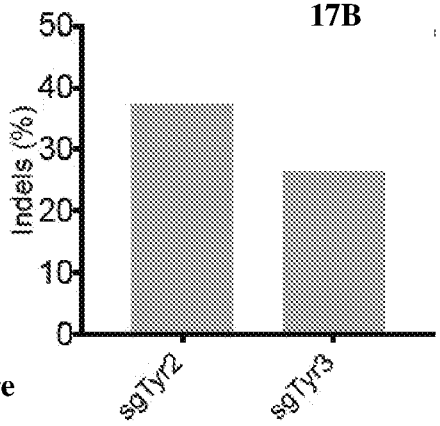


Figure 17B

Mouse ID	AAV dosage	Indels (%)	Coat color
9-1	3 x 10^9 GCs	100	Albino
9-2	3 x 10^9 GCs	100	Albino
9-3	3 x 10^9 GCs	84	mosaic and hypomorphic
9-4	3 x 10^9 GCs	98	mosaic and hypomorphic
9-5	3 x 10^9 GCs	100	mosaic and hypomorphic
8-1	3 x 10^8 GCs	100	mosaic and hypomorphic
8-2	3 x 10^8 GCs	96	mosaic and hypomorphic

Figure 17C

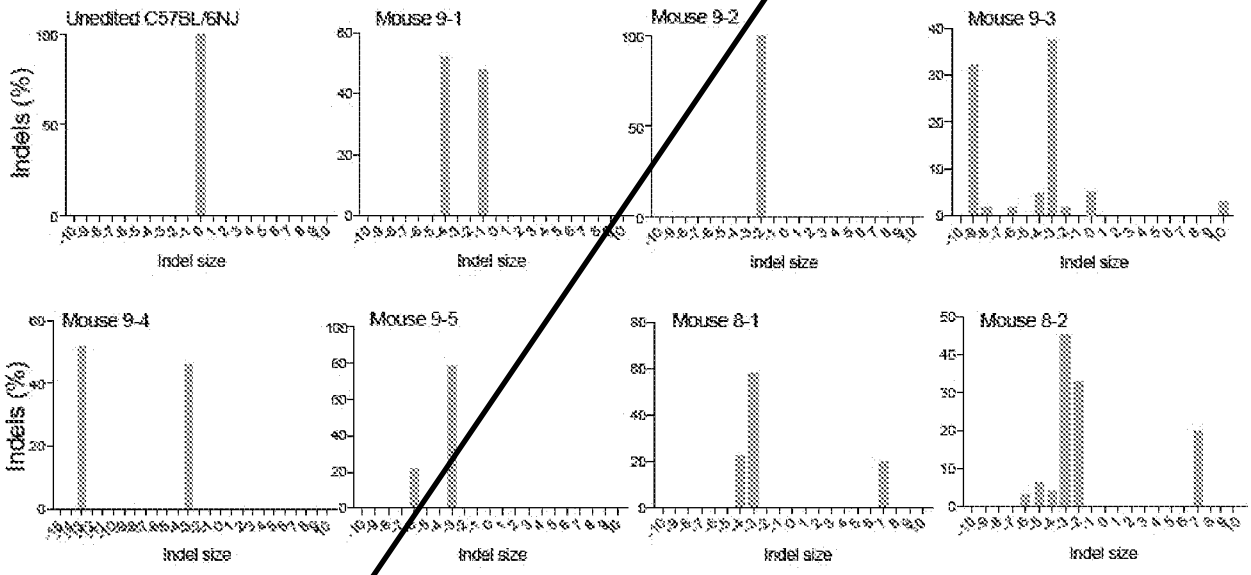


Figure 17

Figure 18A

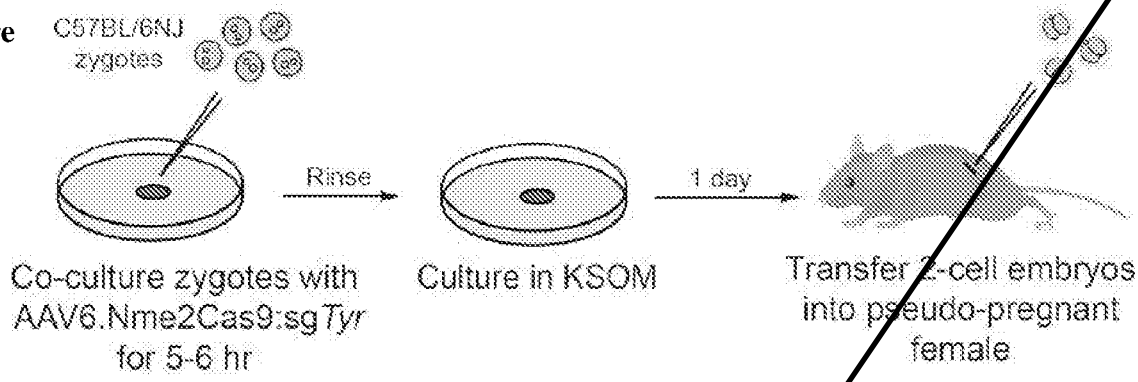


Figure 18B

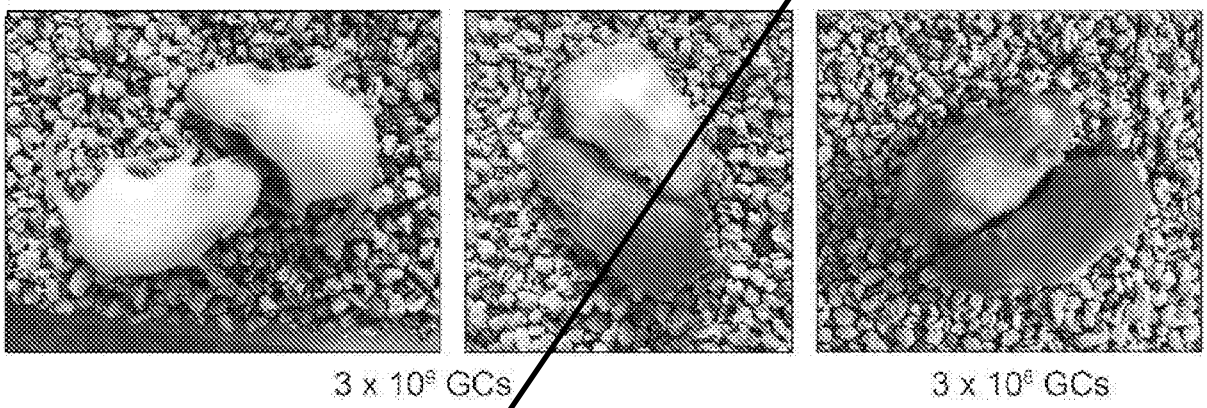


Figure 18C

AAV Dosage	Number of embryos transferred	Number of pups recovered	Number of *Tyr*-edited pups (albino or chinchilla)	*Tyr* editing frequency (%)
3 x 10^9 GCs	50	5	5 (3)	100
3 x 10^8 GCs	28	2*	2 (1)	100

Figure 18

Figure 19A

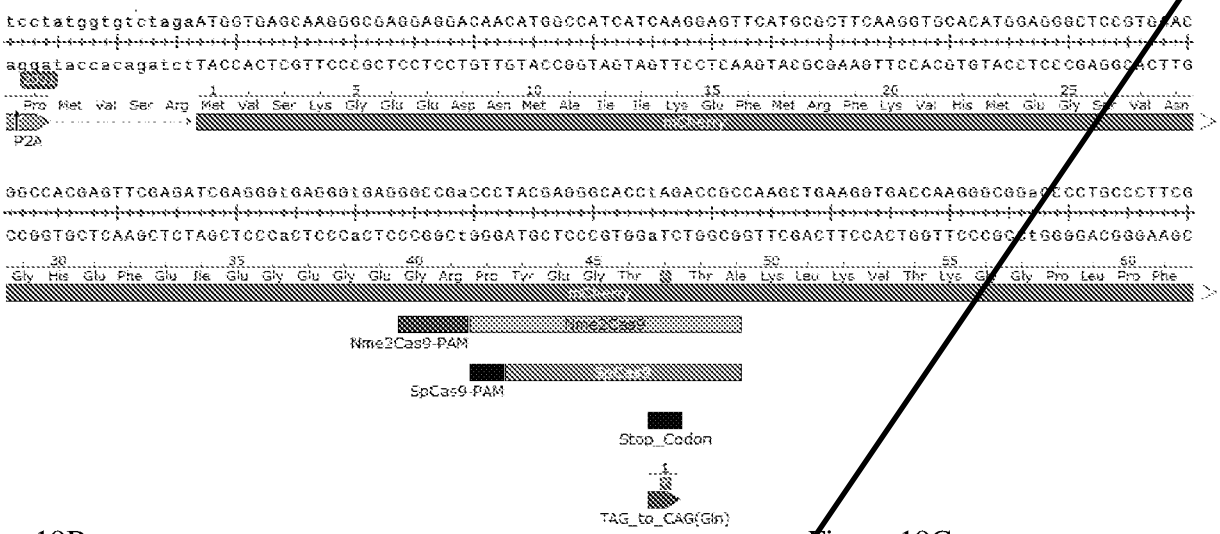


Figure 19B

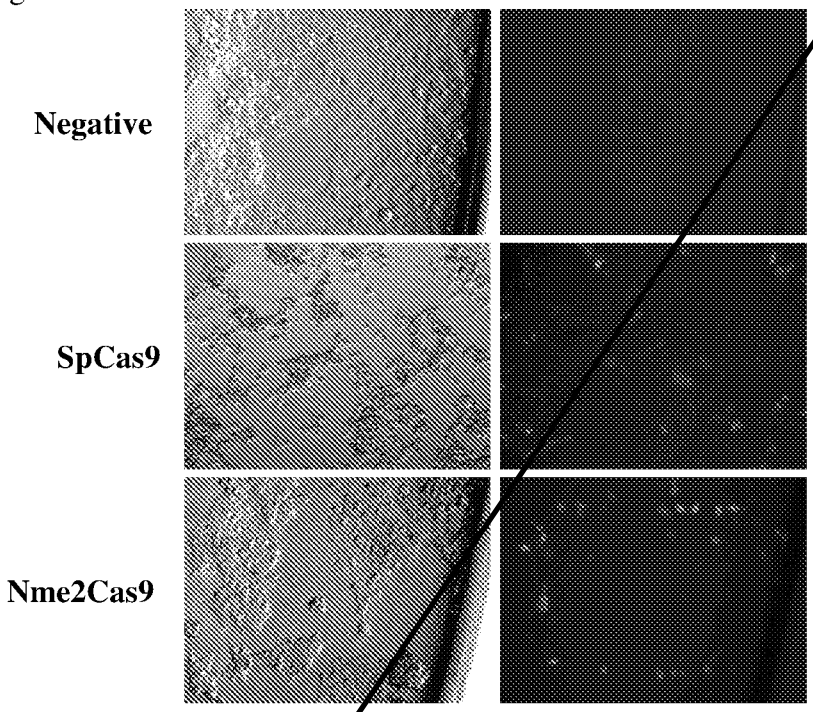


Figure 19C

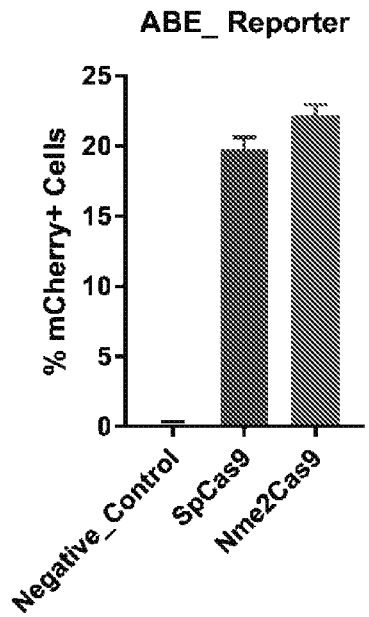


Figure 19

Figure 20A

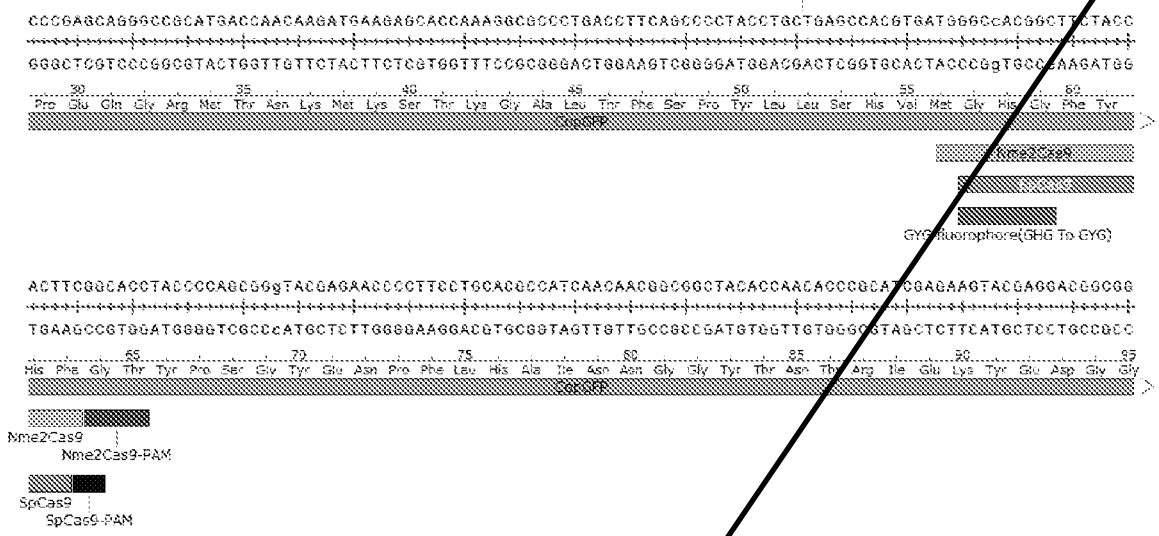


Figure 20B

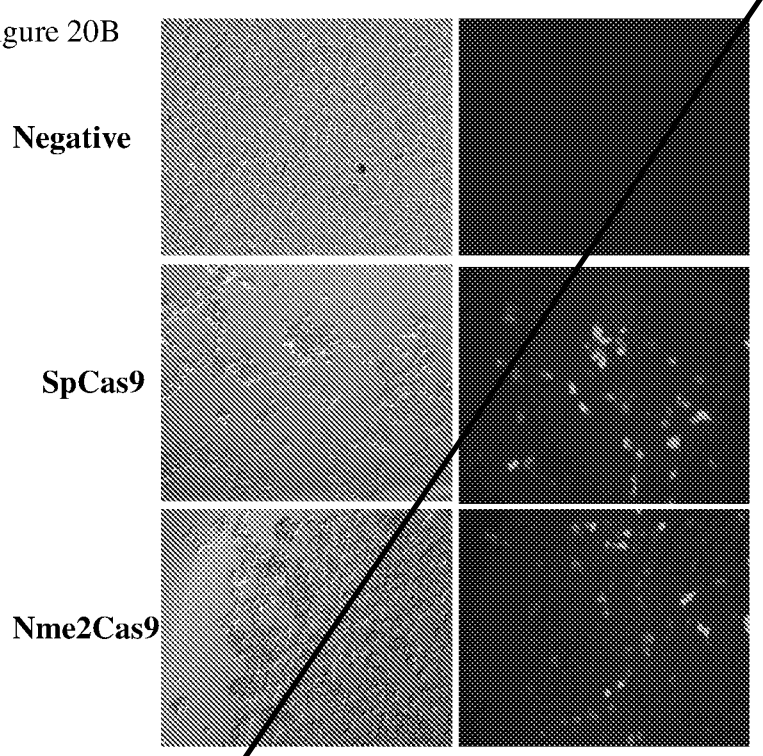


Figure 20C

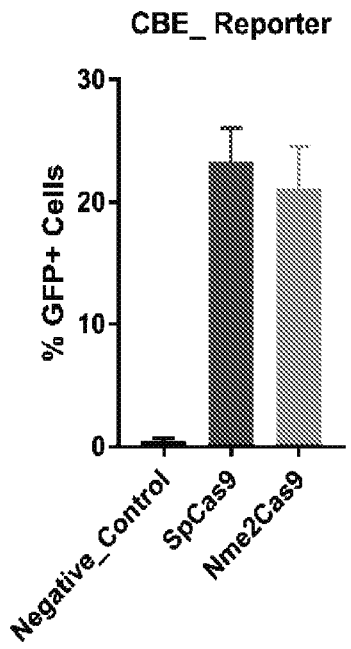


Figure 20

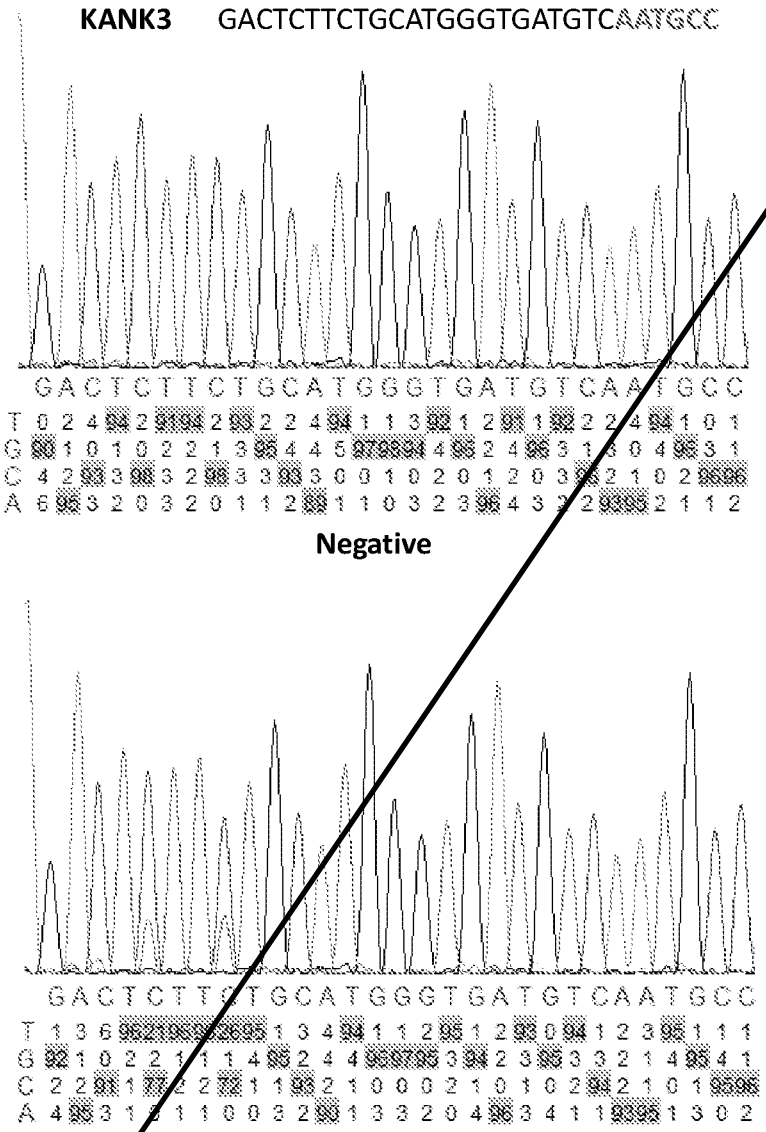

KANK3 GACTCTTCTGCATGGGTGATGTCAATGCC
Negative
Nme2Cas9-CBE

Figure 21

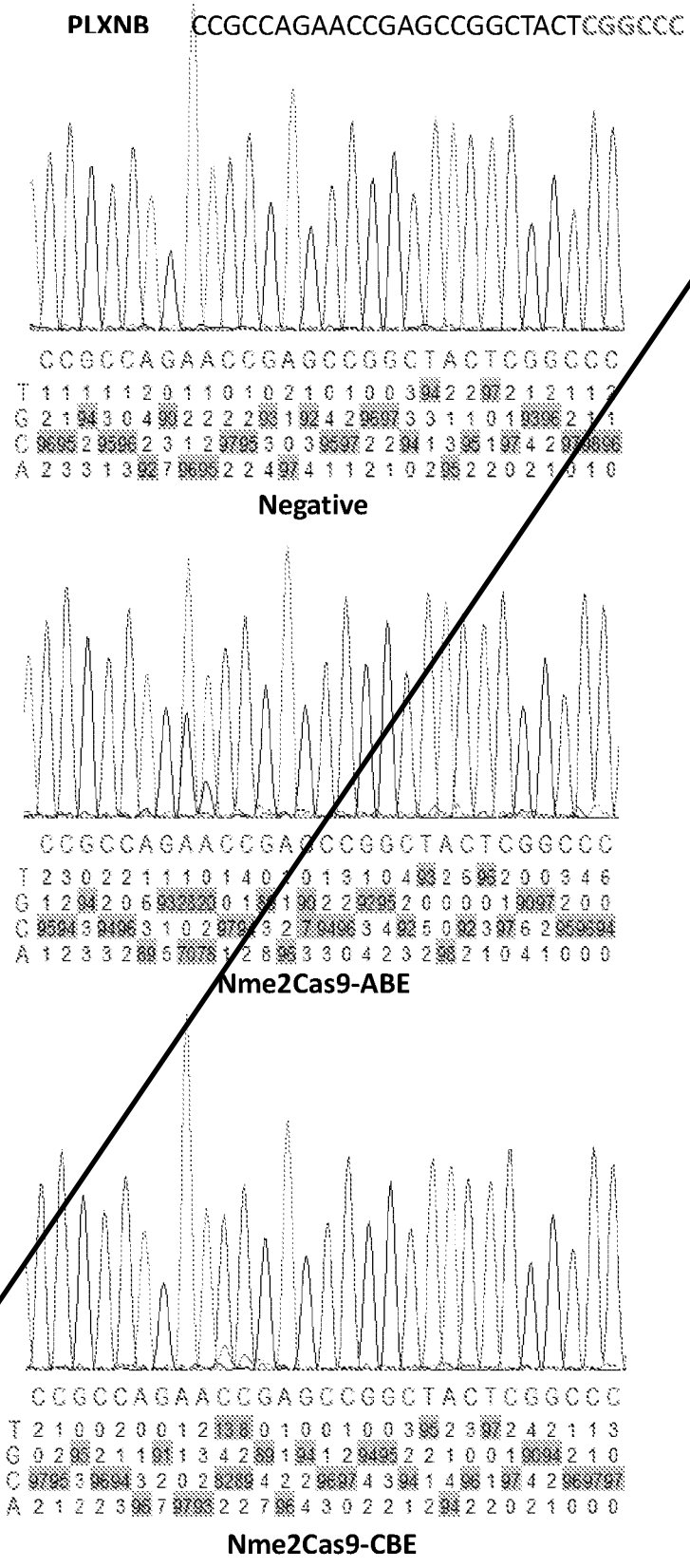

PLXNB CCGCCAGAACCGAGCCGGCTACTCGGCCC
Negative
Nme2Cas9-ABE
Nme2Cas9-CBE

Figure 22



Figure 1A



Figure 1B



Figure 1C



Figure 1D



Figure 1E

Figure 1

Figure 2A

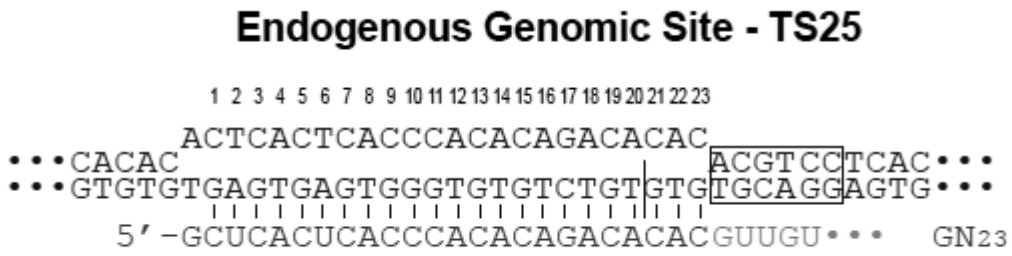


Figure 2B

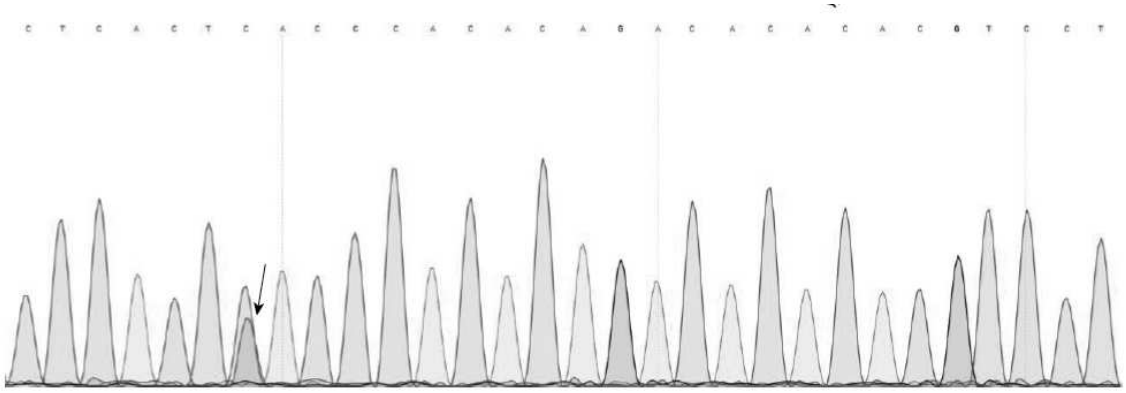


Figure 2

Figure 2C

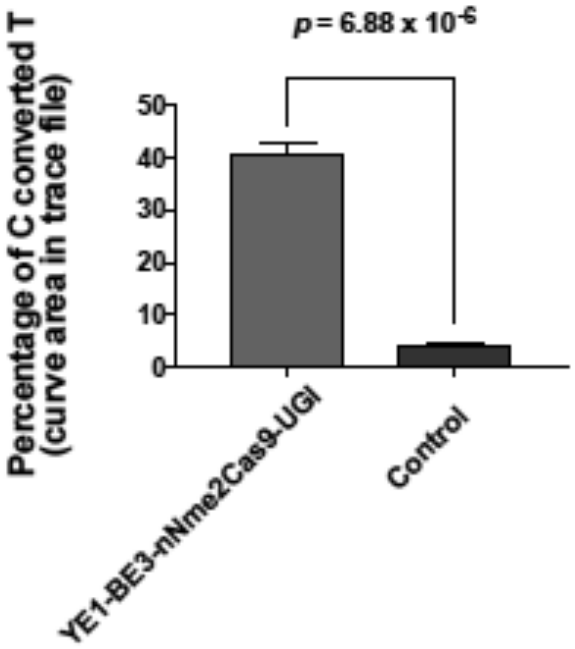


Figure 2 (cont'd)

Figure 3A

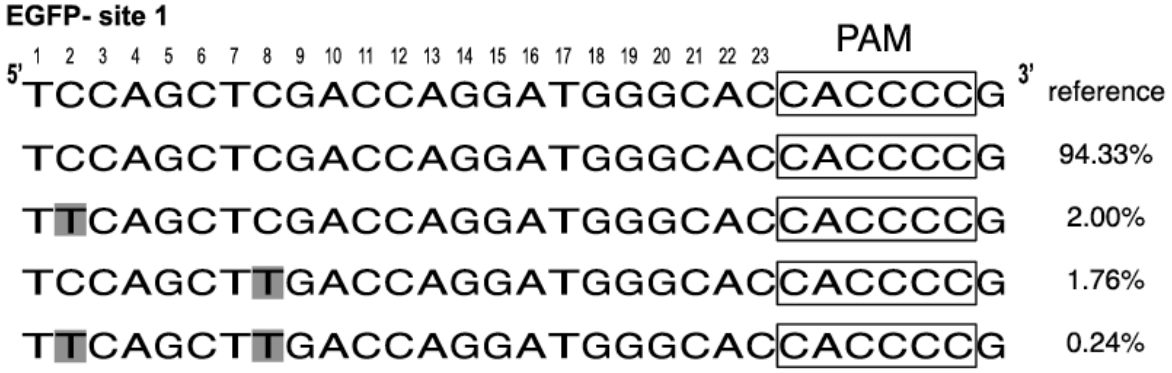


Figure 3B

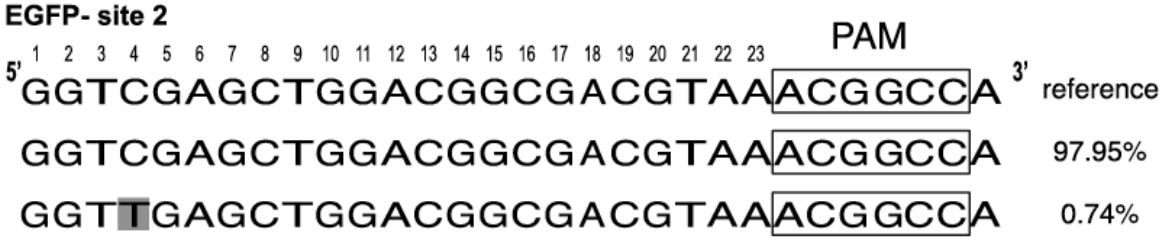


Figure 3C

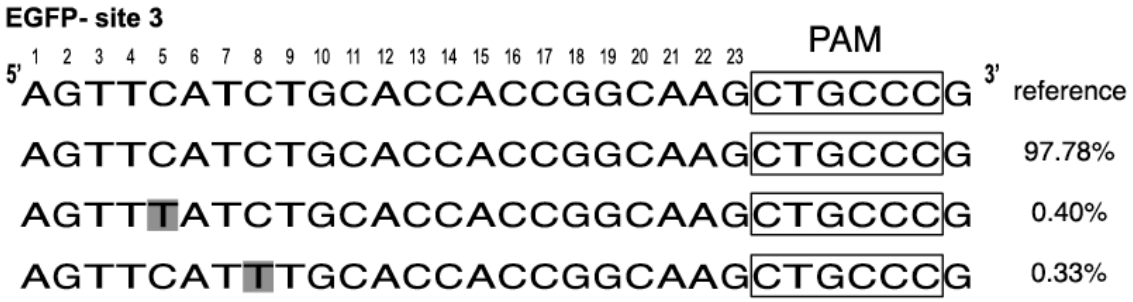


Figure 3D

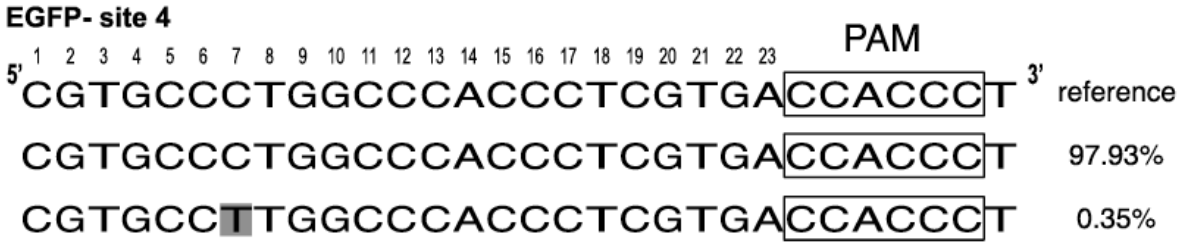


Figure 3

Figure 3E

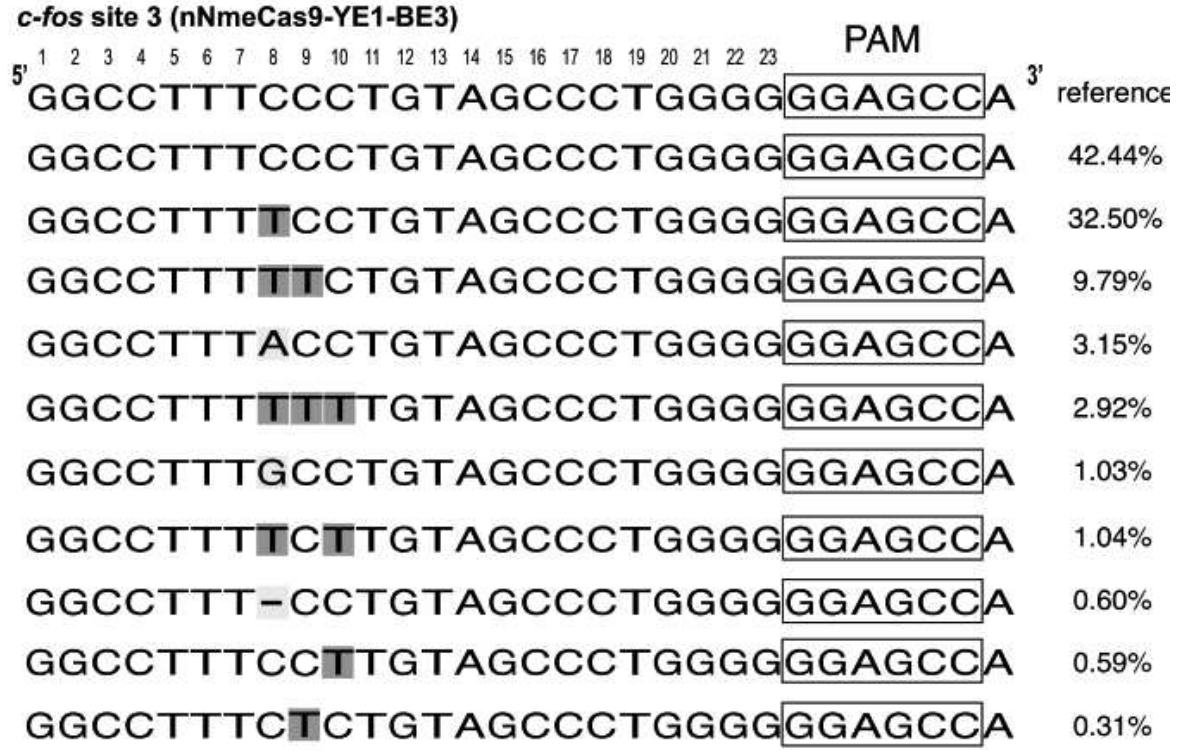


Figure 3F

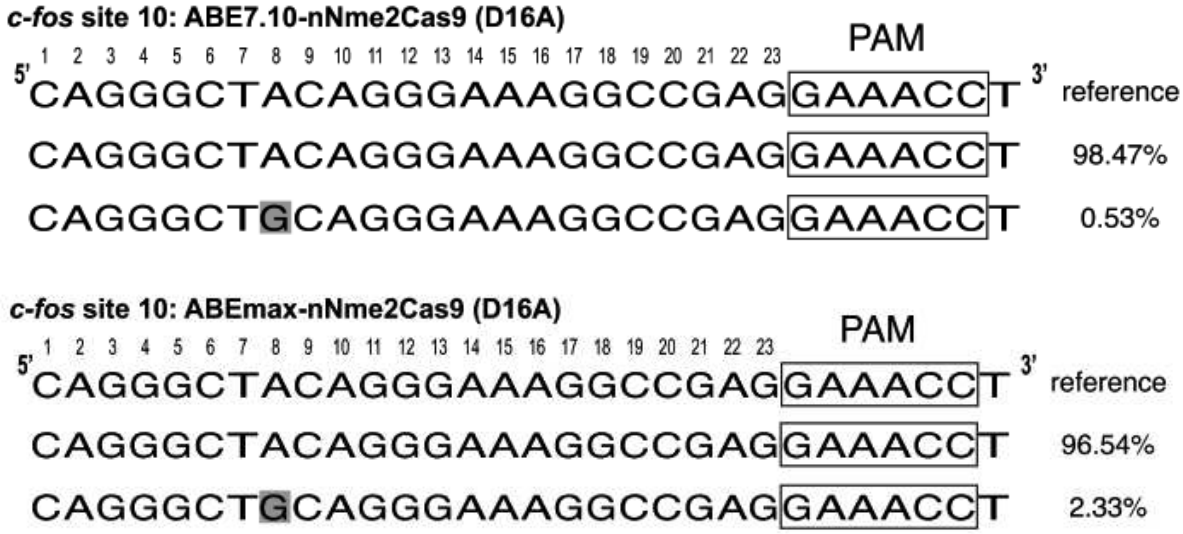


Figure 3 (cont'd)

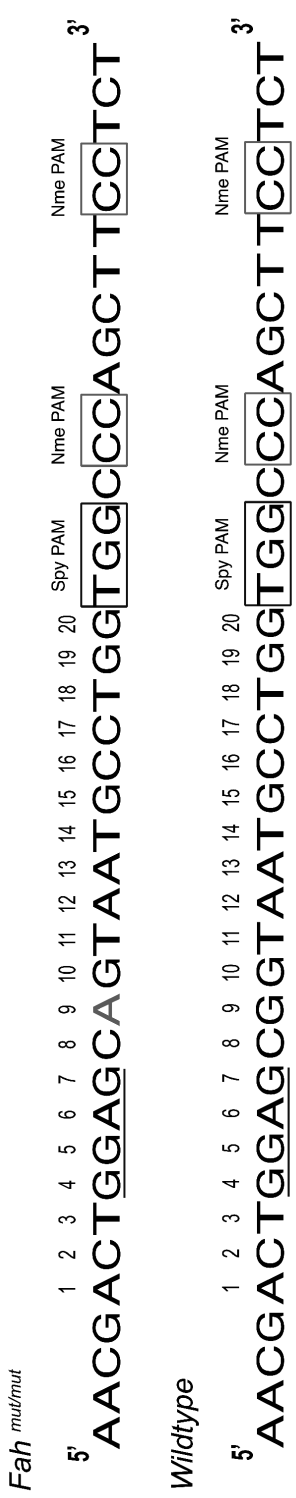

Fah mut/mut
5'
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
AACGACTGGAGCAGTAATGCCTGGTGGCCAGCTTCCTCT
Spy PAM
Nme PAM
3'
Wildtype
5'
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
AACGACTGGAGCGGTAATGCCTGGTGGCCAGCTTCCTCT
Spy PAM
Nme PAM
3'

Figure 4

Figure 5A

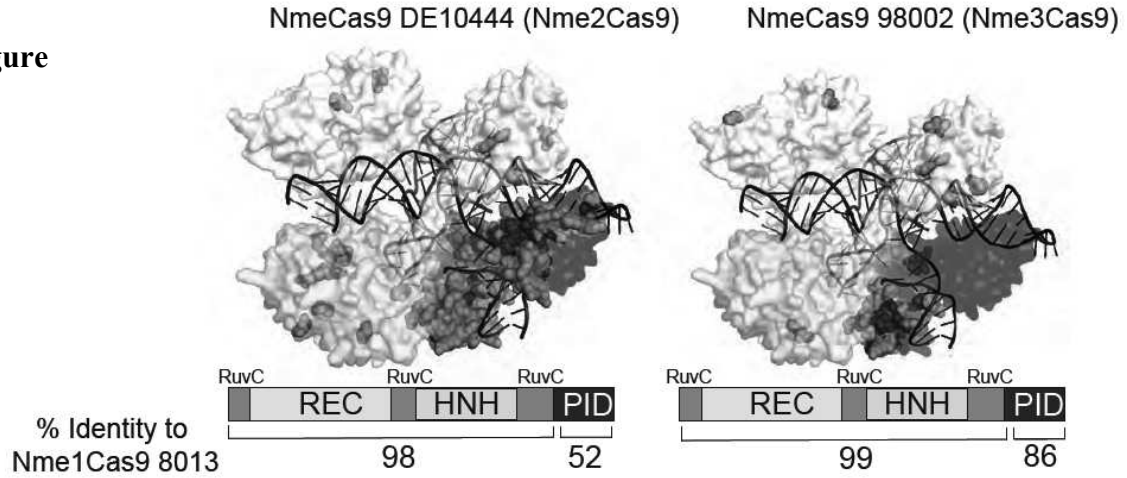


Figure 5B

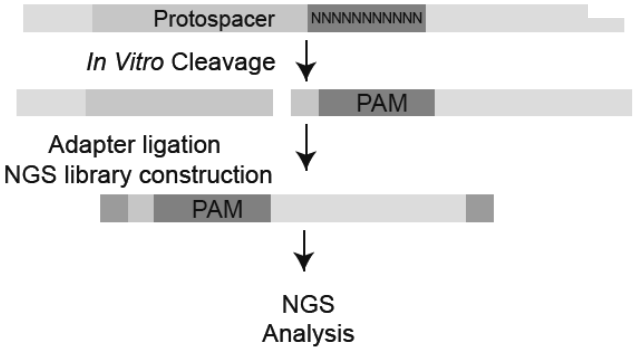


Figure 5C

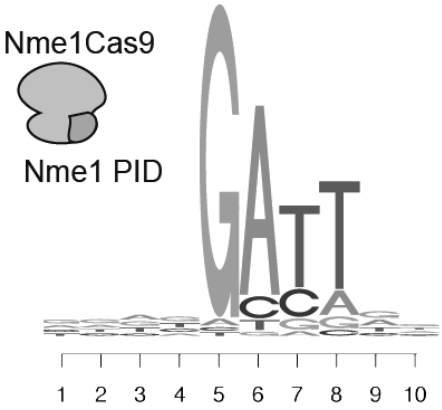


Figure 5

Figure 5D

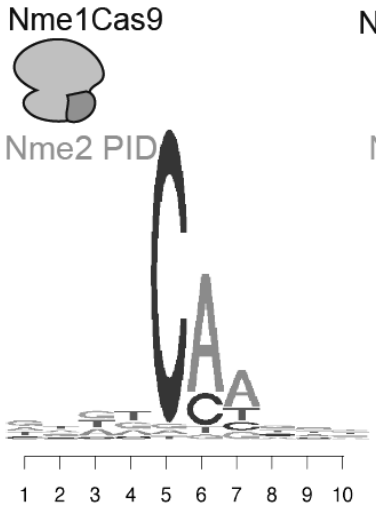


Figure 5E

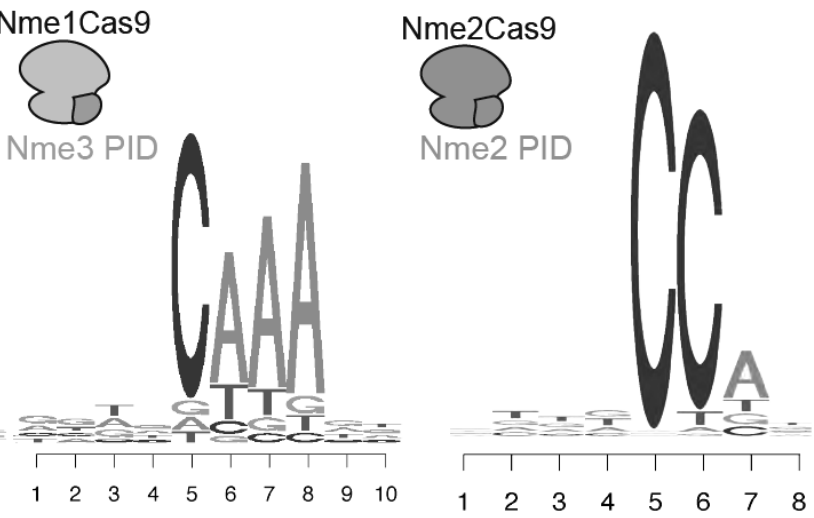


Figure 5 (cont'd)

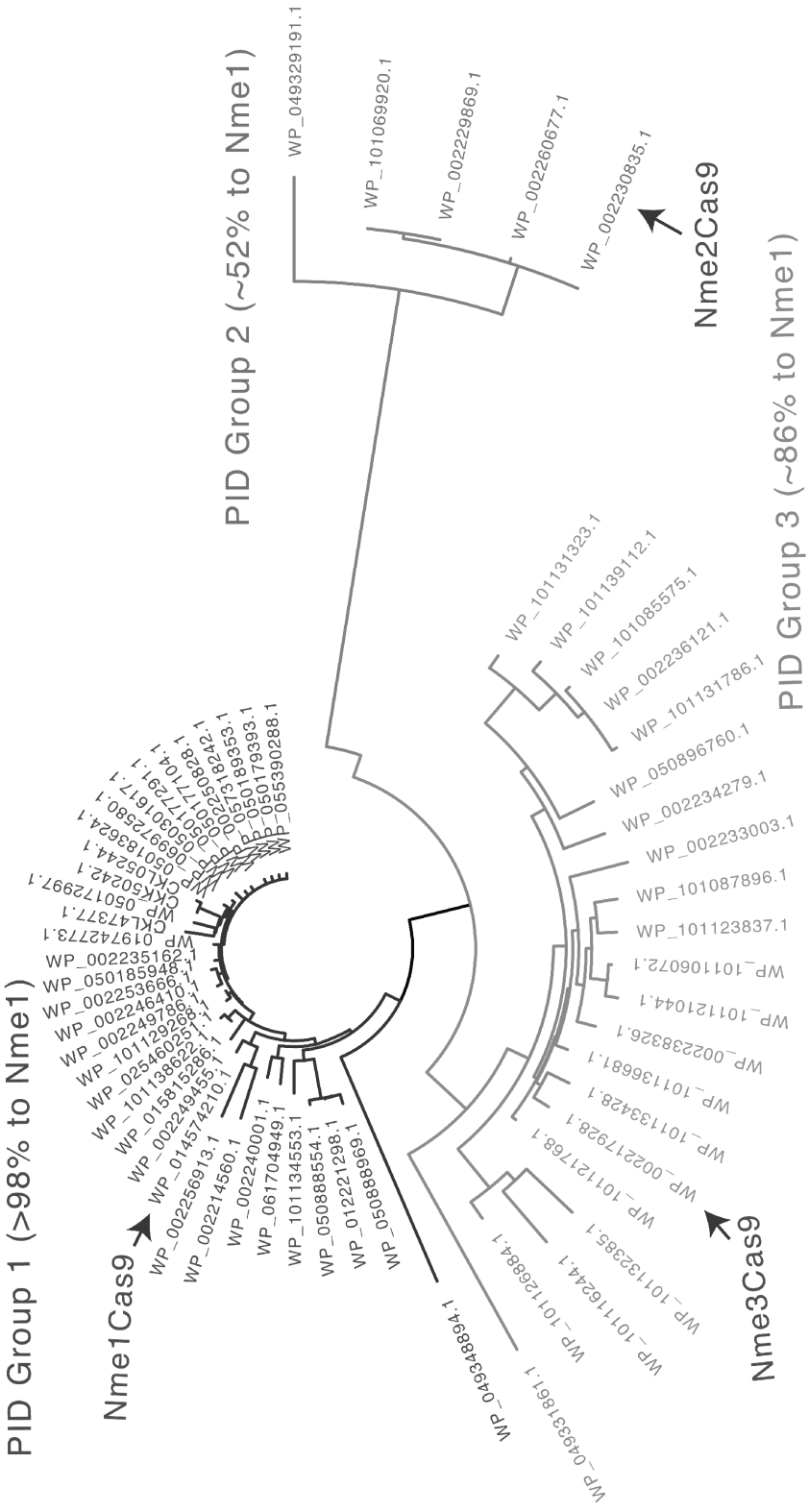
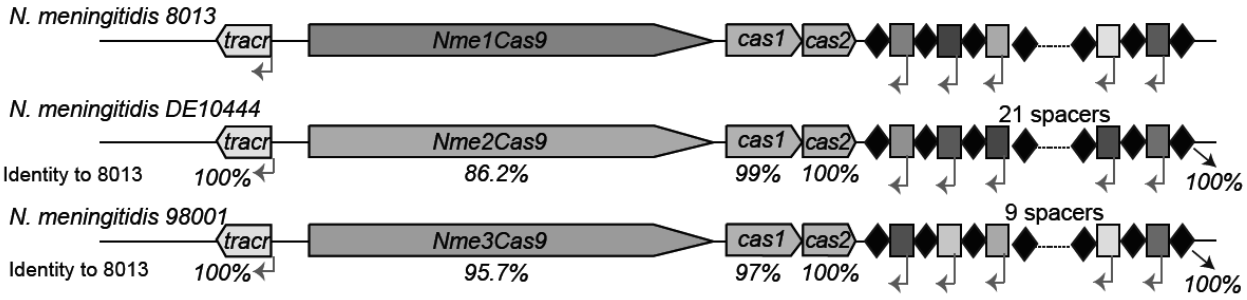

PID Group 1 (>98% to Nme1)
PID Group 2 (~52% to Nme1)
PID Group 3 (~86% to Nme1)
Nme1Cas9
Nme2Cas9
Nme3Cas9
WP_049329191.1
WP_101069920.1
WP_002229869.1
WP_002260677.1
WP_002230835.1
WP_101131323.1
WP_101139112.1
WP_101085575.1
WP_002236121.1
WP_101131786.1
WP_050896760.1
WP_002234279.1
WP_002233003.1
WP_101087896.1
WP_101123837.1
WP_101106072.1
WP_101121044.1
WP_002238326.1
WP_101136681.1
WP_101133428.1
WP_002217928.1
WP_101121768.1
WP_101132385.1
WP_101116244.1
WP_101126884.1
WP_049331861.1
WP_049348894.1
WP_050888969.1
WP_012221298.1
WP_050888554.1
WP_101134553.1
WP_061704949.1
WP_002240001.1
WP_002214560.1
WP_002256913.1
WP_014574210.1
WP_002249455.1
WP_015815286.1
WP_101138622.1
WP_025460251.1
WP_101129268.1
WP_002249786.1
WP_002246410.1
WP_002253666.1
WP_050185948.1
WP_002235162.1
WP_019742773.1
CKL47377.1
WP_050172997.1
CKK50242.1
WP_050183624.1
WP_050177291.1
WP_050177104.1
WP_050250828.1
WP_050189353.1
WP_050179393.1
WP_055390288.1

Figure 6A

Figure 6B



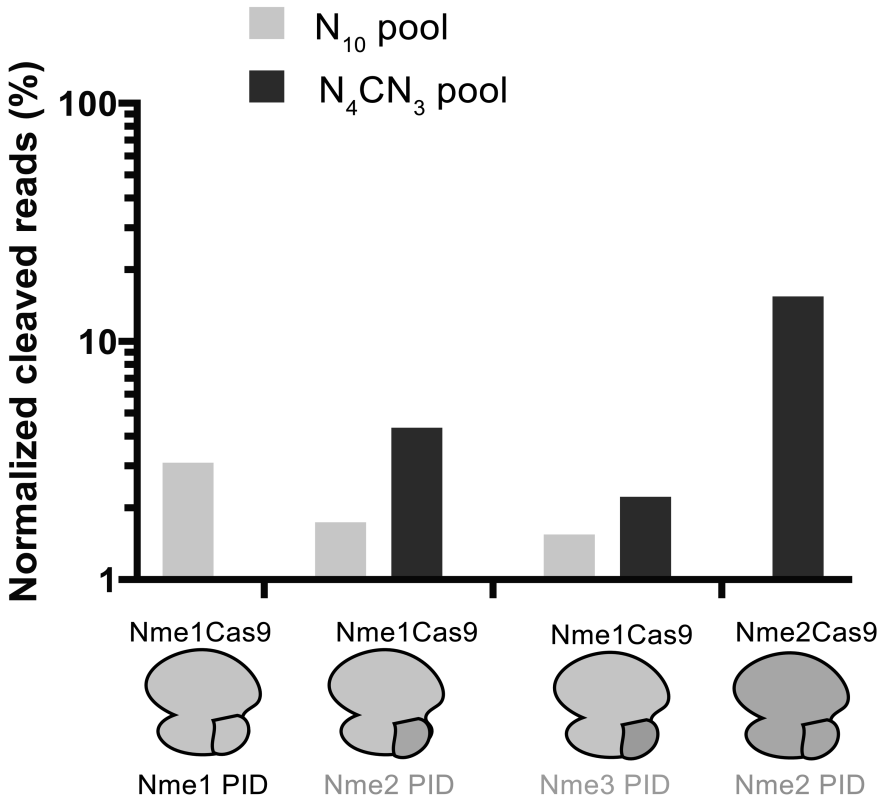


Figure 6C

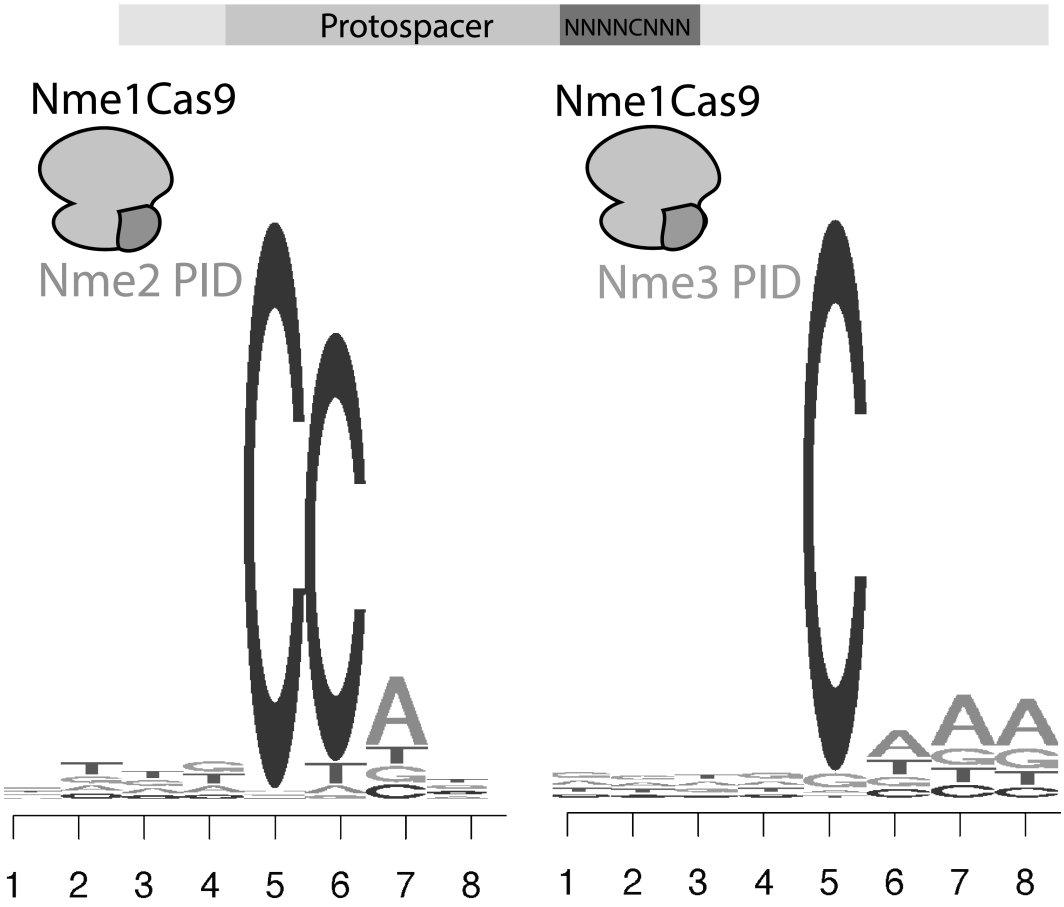


Figure 6D

Figure 6 (cont'd)

Figure 7A

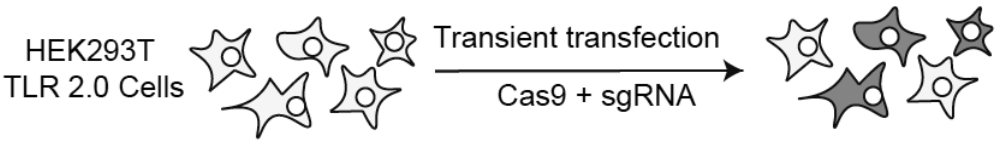


Figure 7

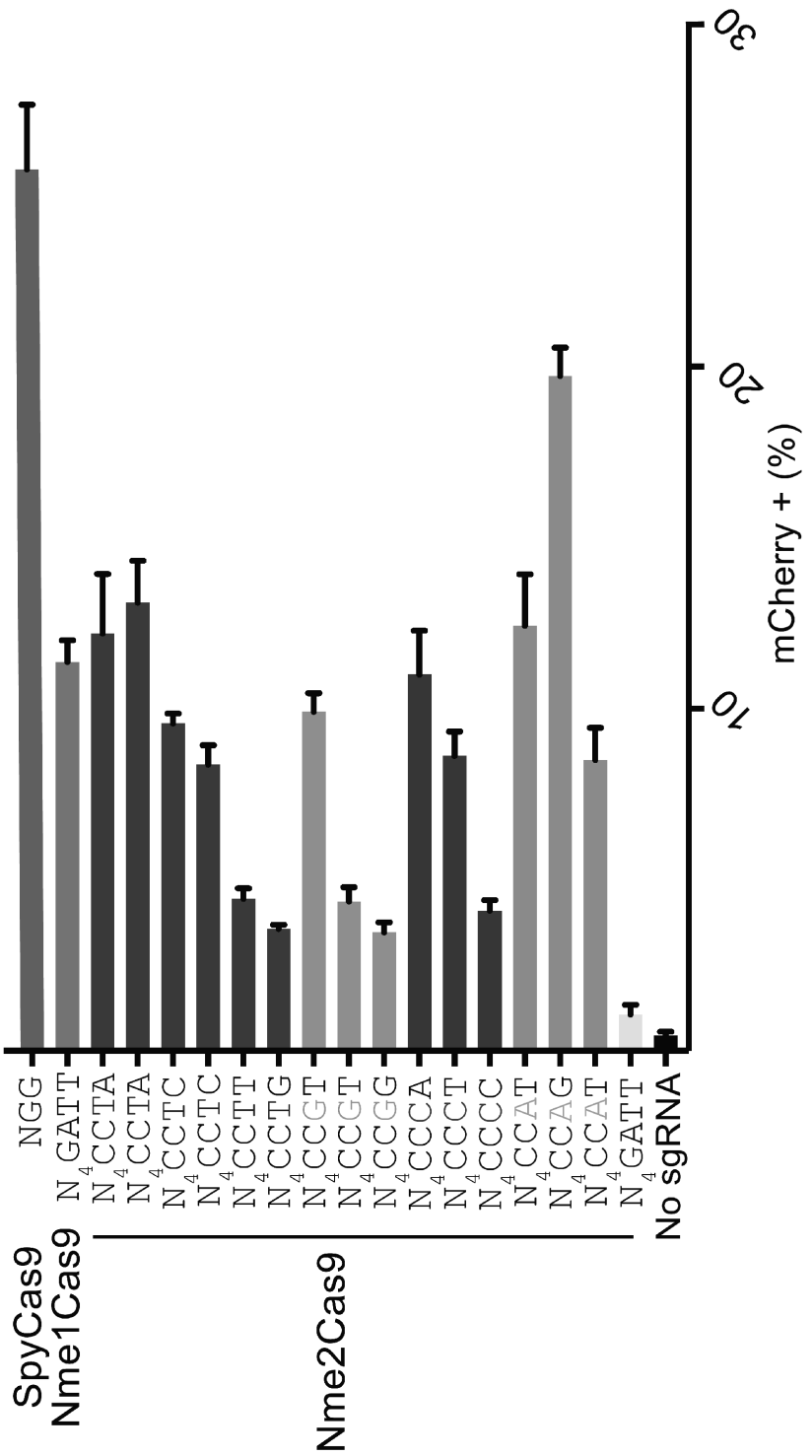


Figure 7B

Figure 7 (cont'd)

Figure 7C

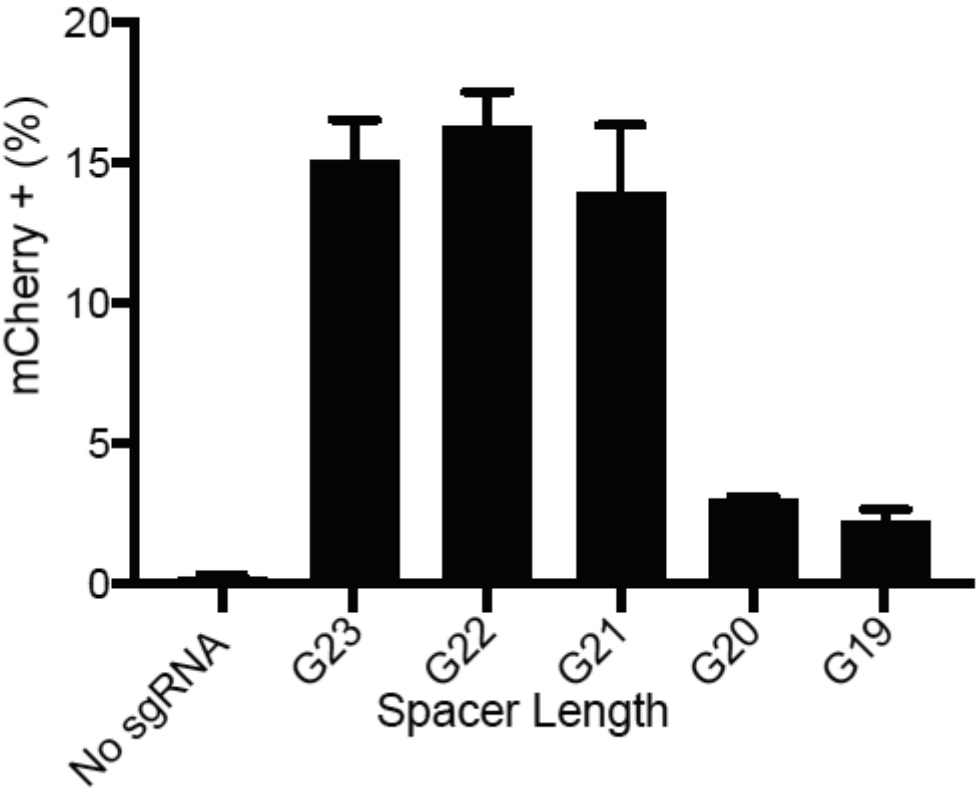


Figure 7 (cont'd)

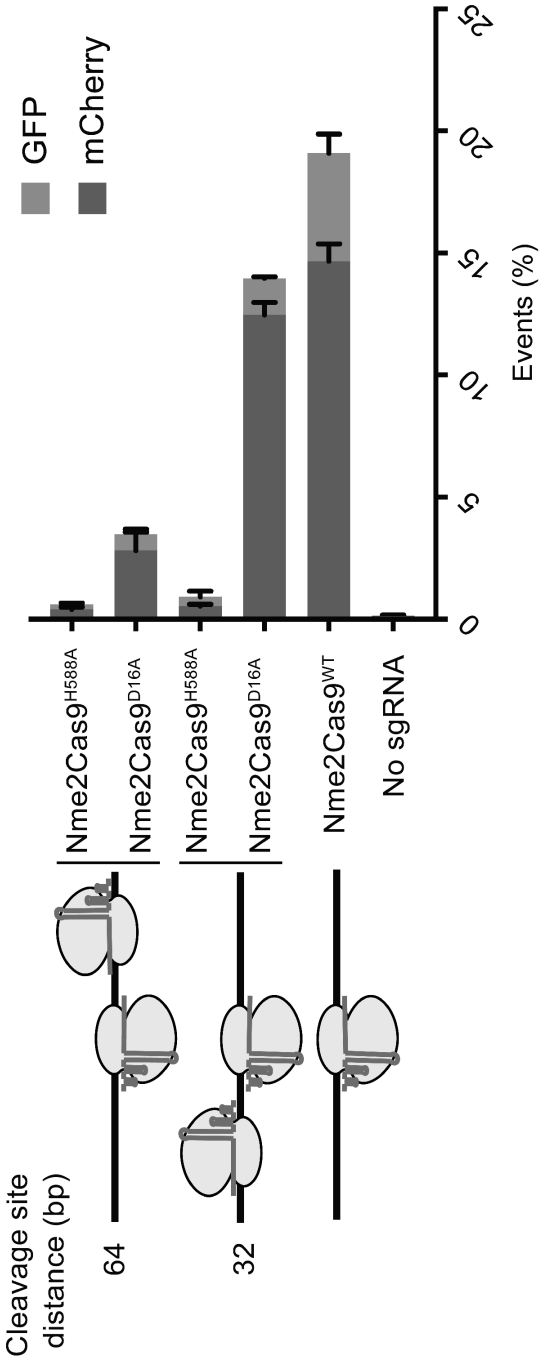


Figure 7D

Figure 7 (cont'd)

Figure 8A

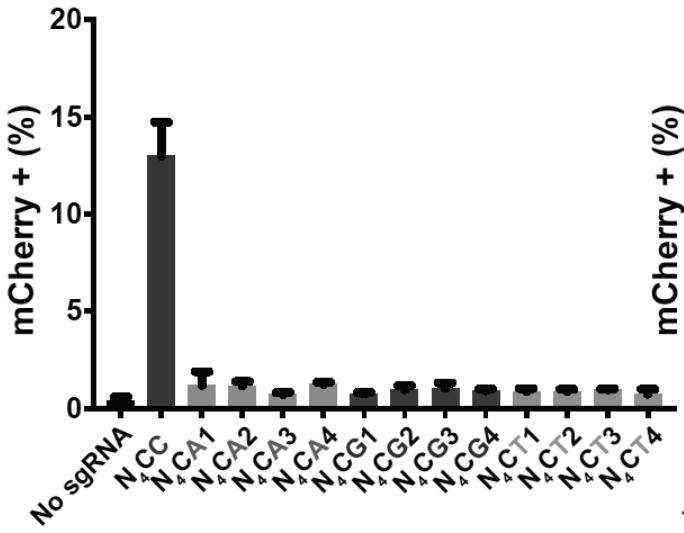


Figure 8B

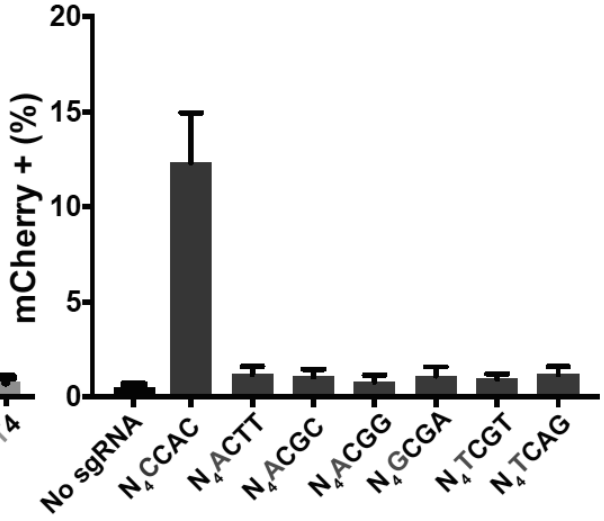
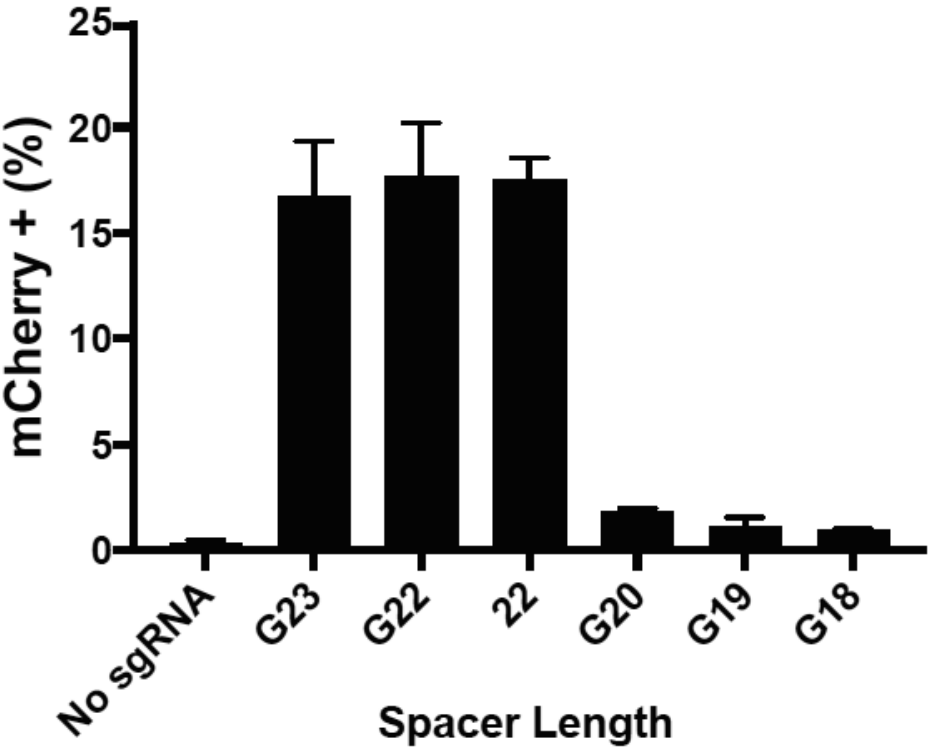


Figure 8

Figure 8C


25
20
15
10
5
0
mCherry + (%)
No sgRNA
G23
G22
22
G20
G19
G18
Spacer Length

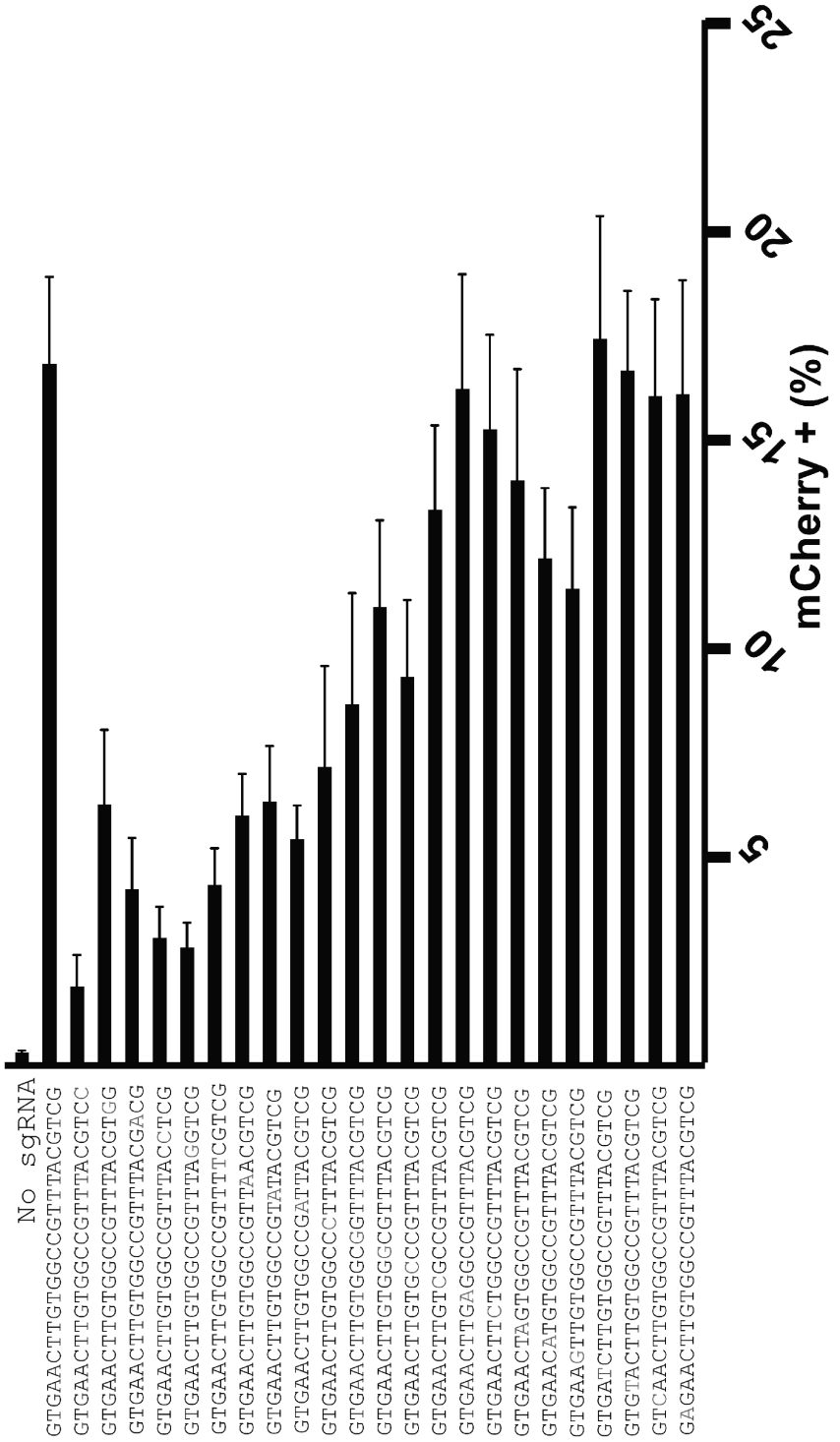


Figure 8D

Figure 8 (cont'd)

Figure 9A

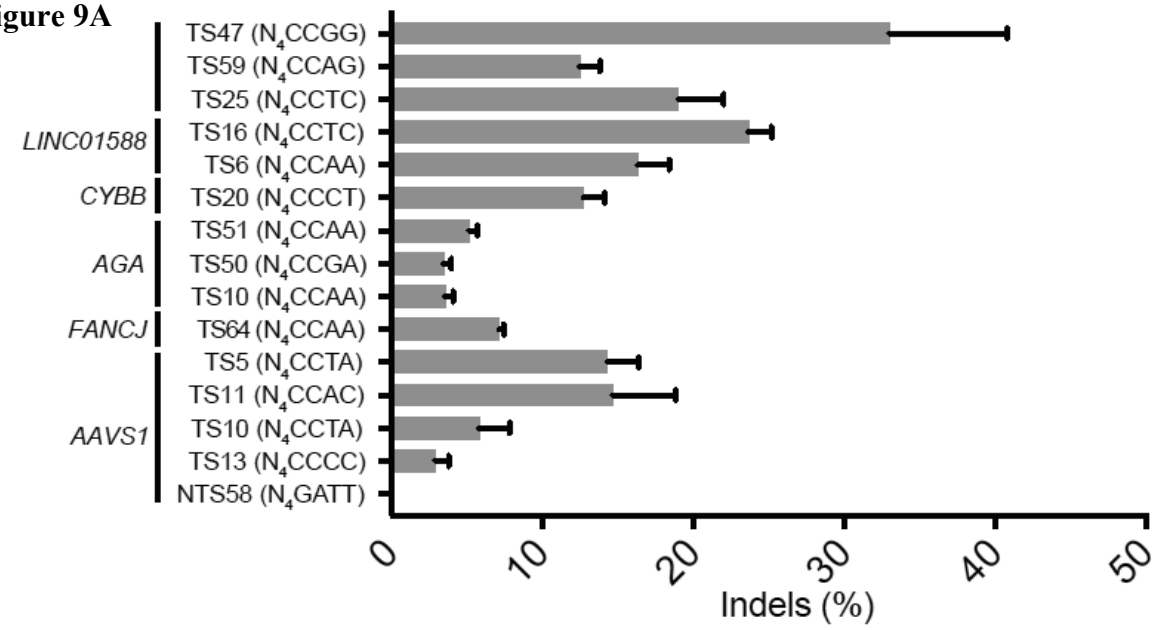
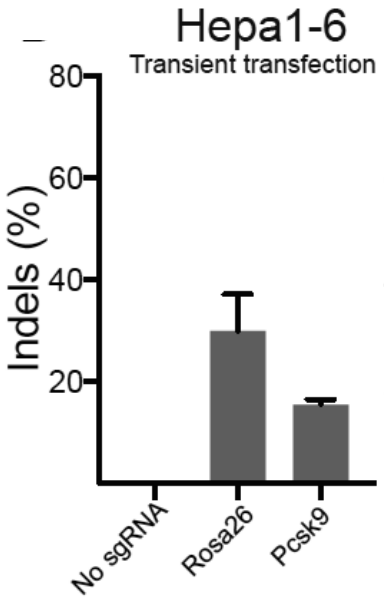


Figure 9B



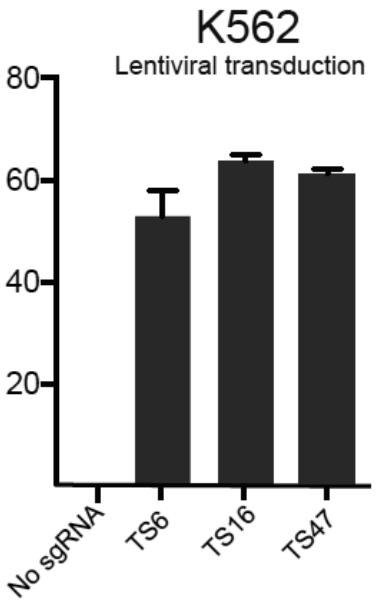


Figure 9C

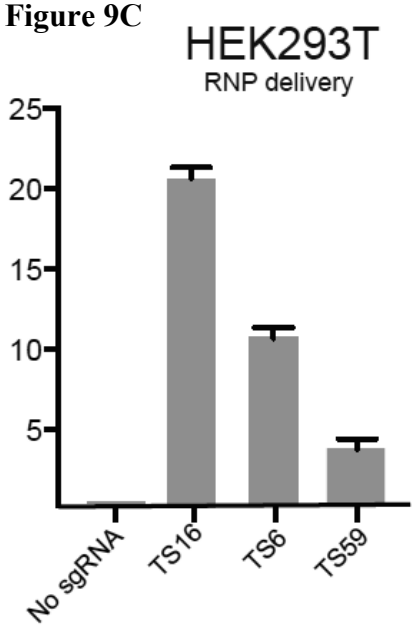


Figure 9

Figure 10A

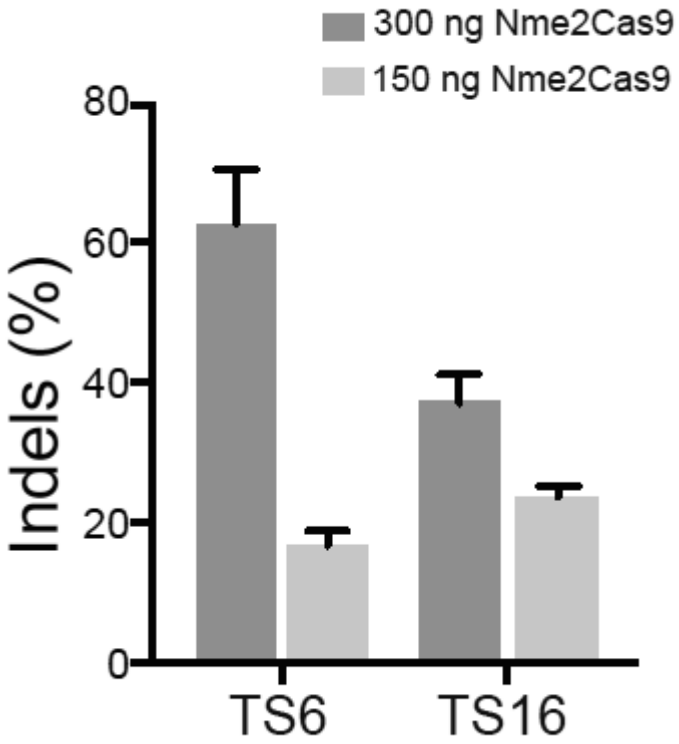


Figure 10B

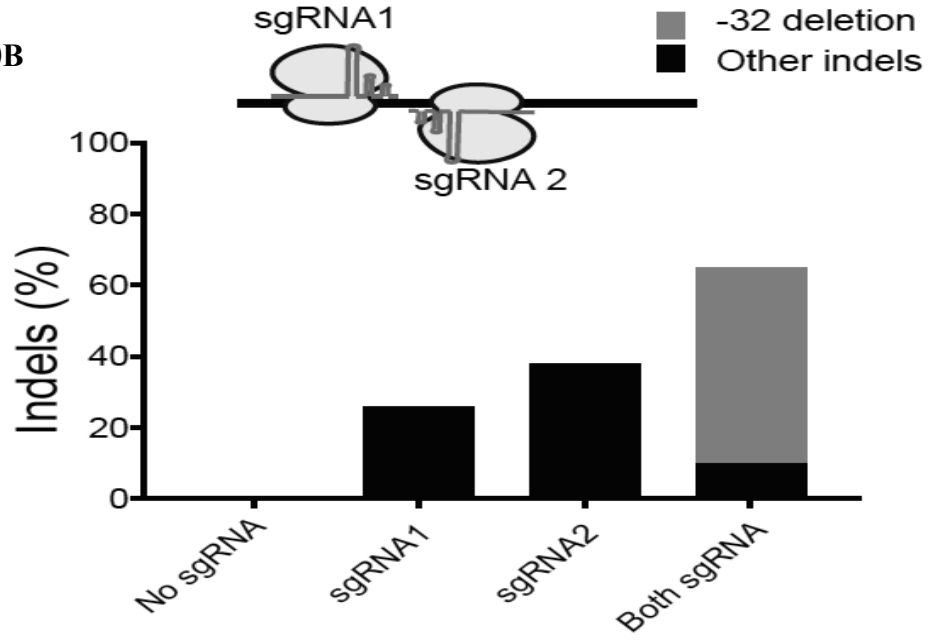
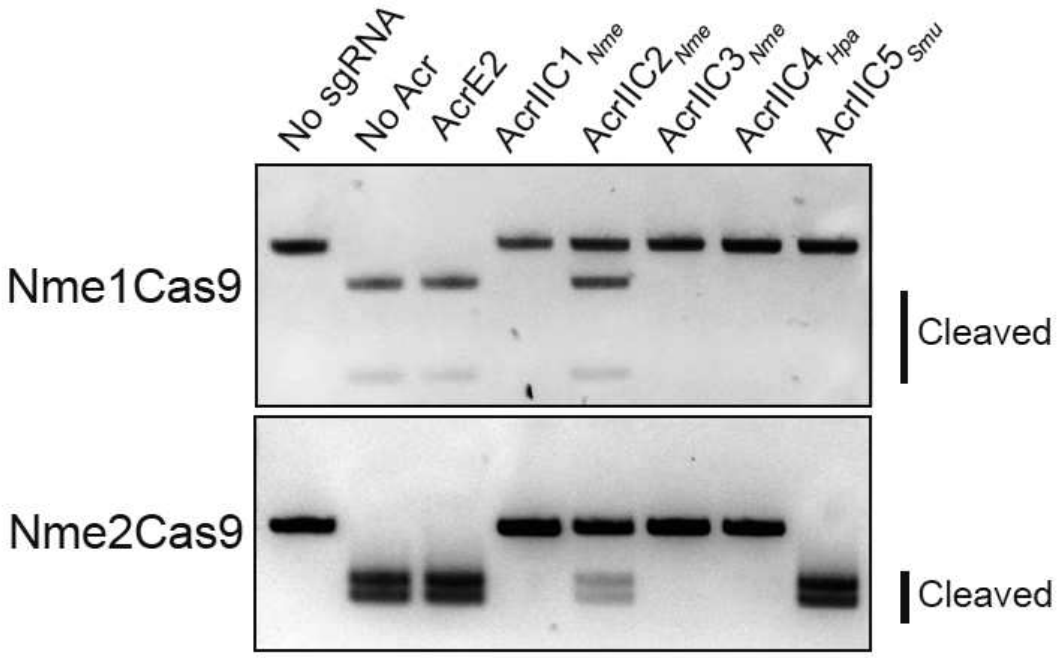


Figure 10

Figure 11A



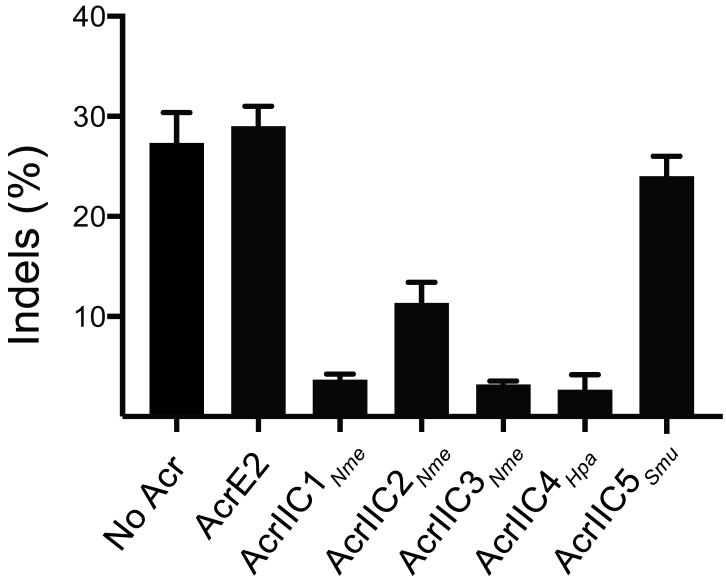


Figure 11B

Figure 11

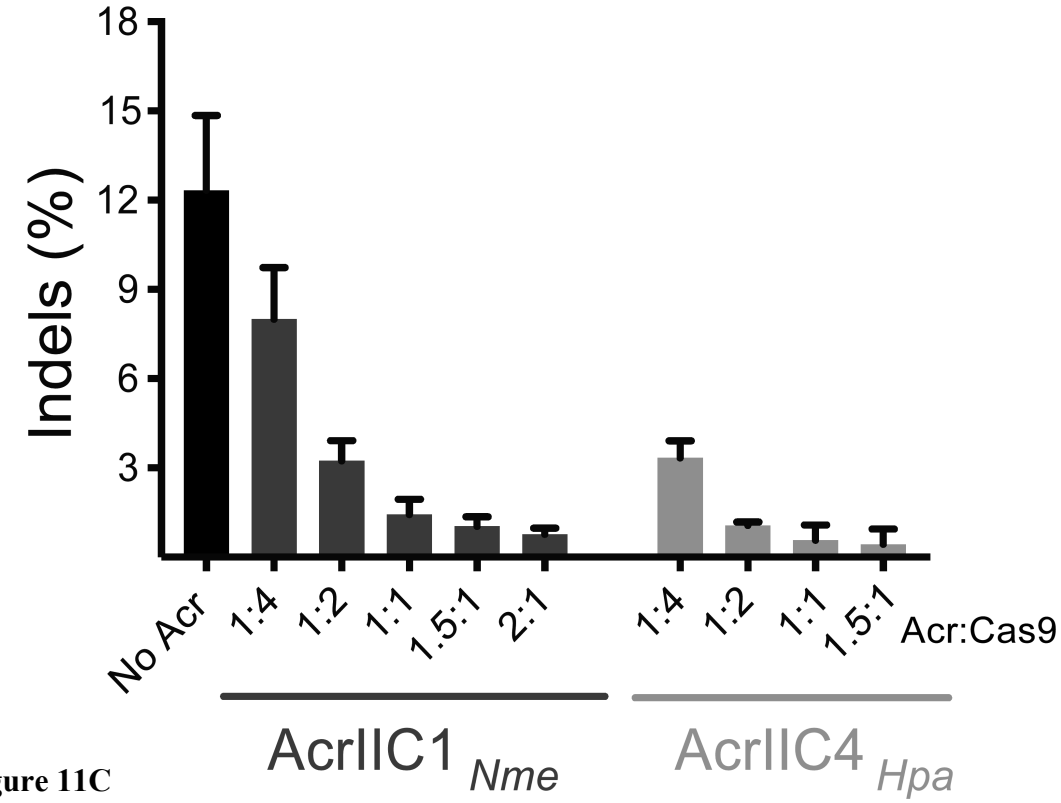


Figure 11C

Figure 11 (cont'd)

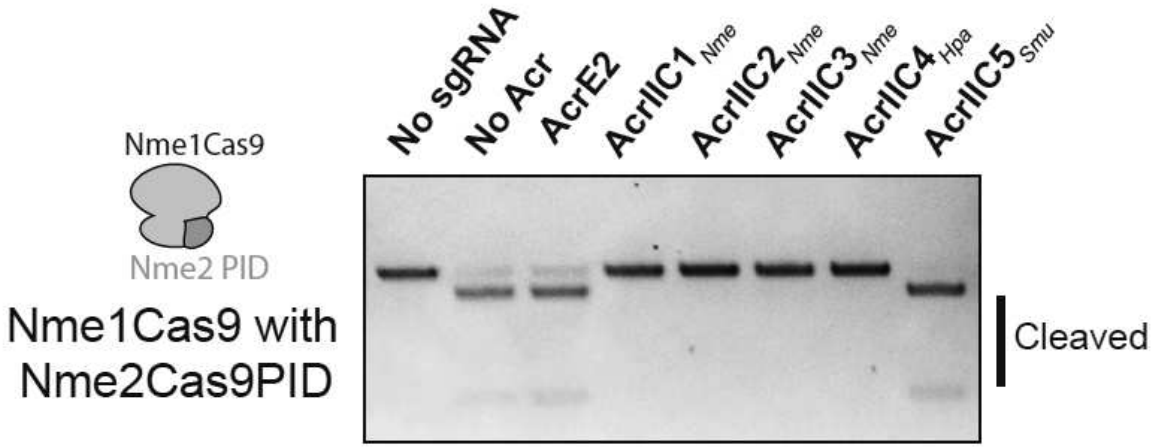

No sgRNA
No Acr
AcrE2
AcrIIC1 Nme
AcrIIC2 Nme
AcrIIC3 Nme
AcrIIC4 Hpa
AcrIIC5 Smu
Nme1Cas9
Nme2 PID
Nme1Cas9 with
Nme2Cas9PID
Cleaved

Figure 12

Figure 13A

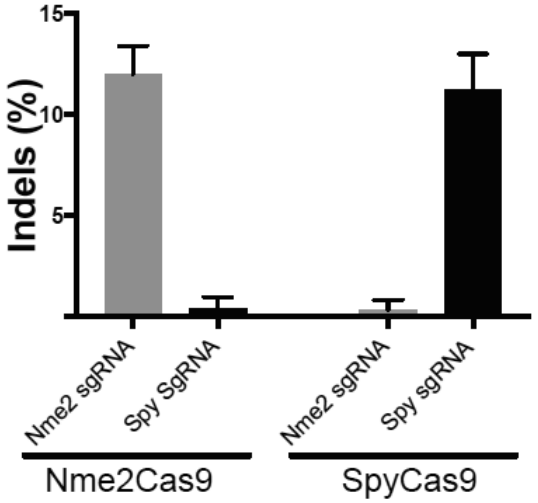


Figure 13B

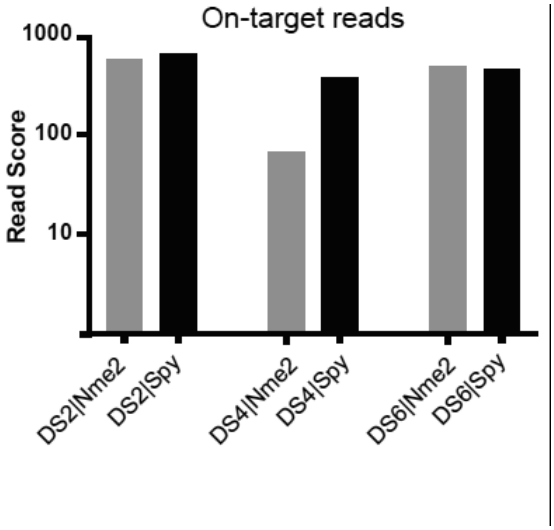


Figure 13C

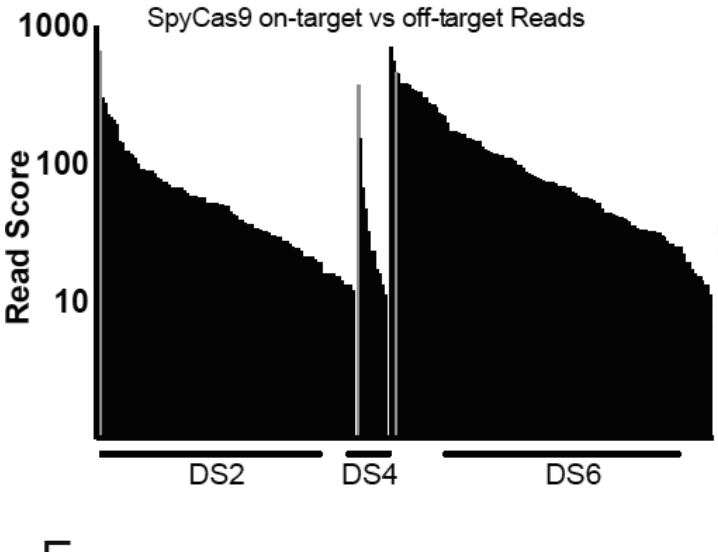


Figure 13D

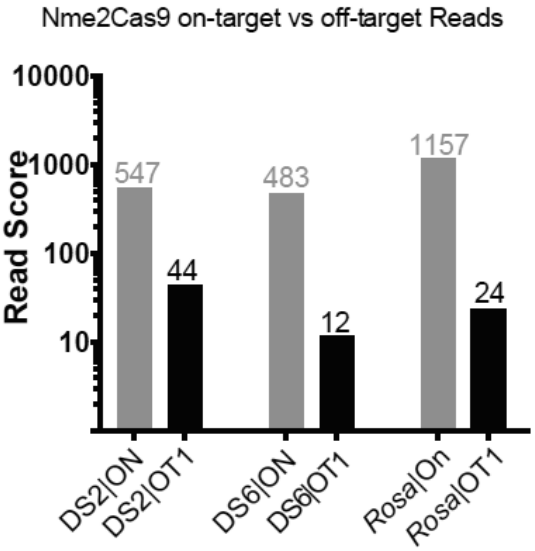


Figure 13

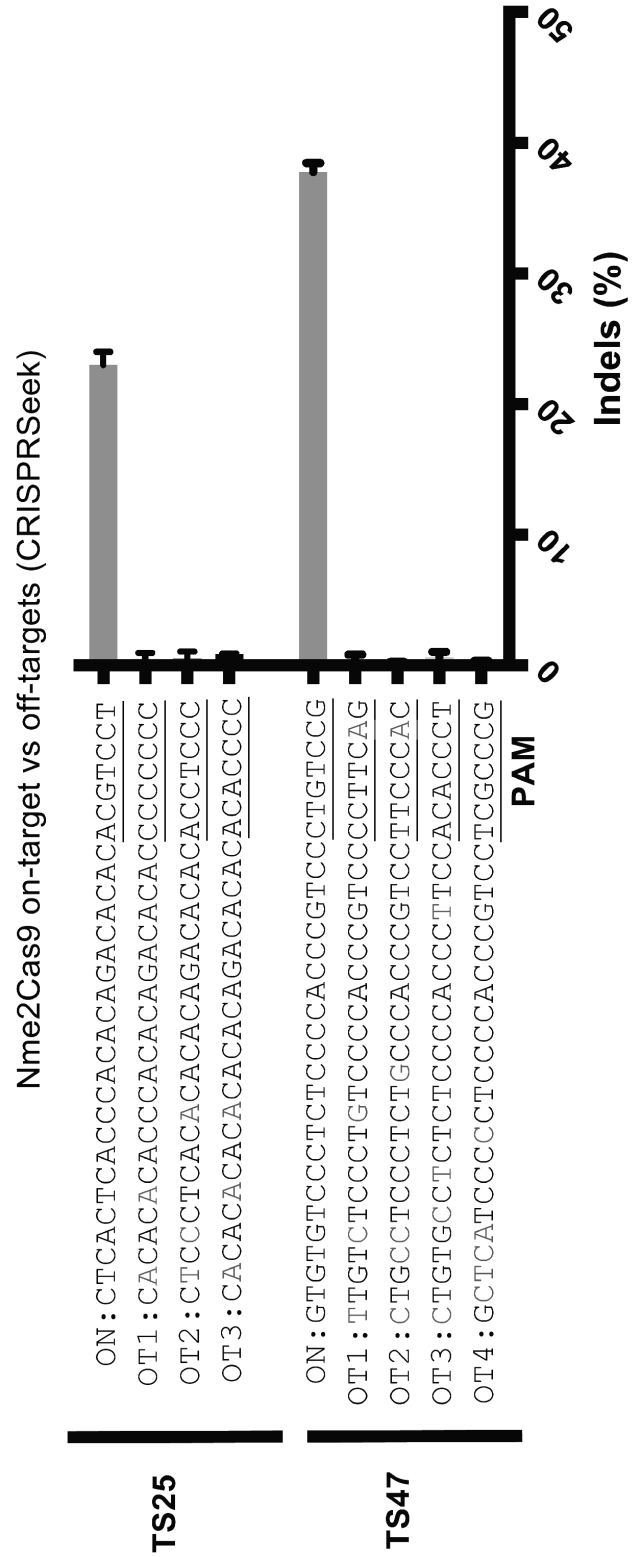


Figure 13E

Figure 13 (cont'd)

Figure 14A



Figure 14B

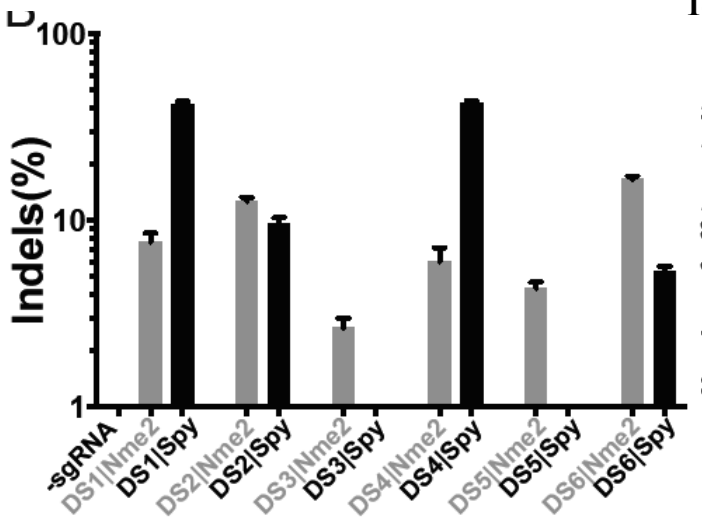


Figure 14C

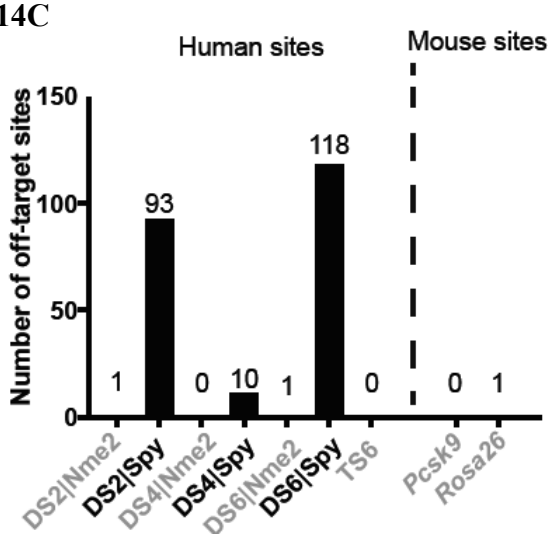


Figure 14

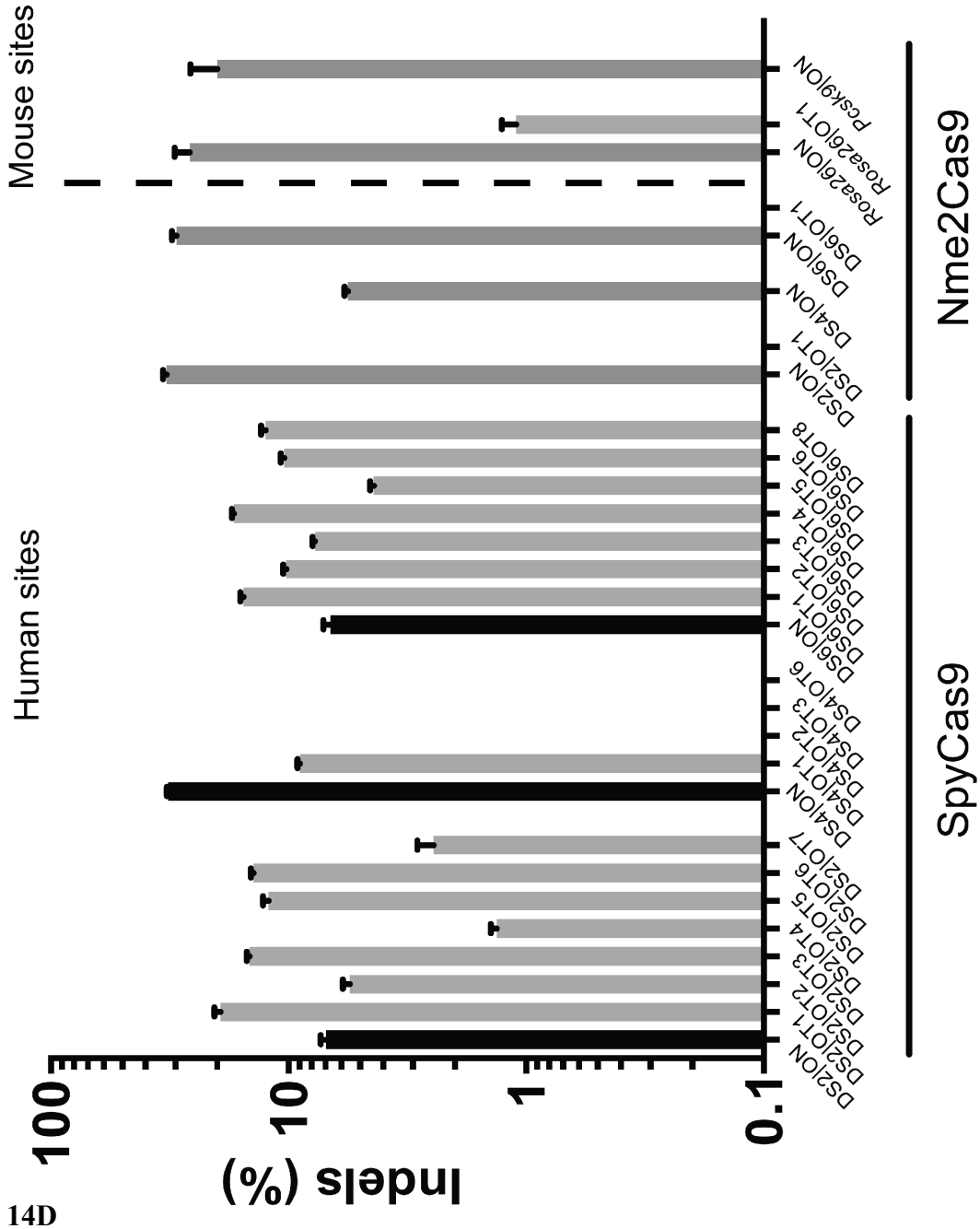

Indels (%)
100
10
1
0.1
Human sites
Mouse sites
SpyCas9
Nme2Cas9
DS2ON
DS2IOT1
DS2IOT2
DS2IOT3
DS2IOT4
DS2IOT5
DS2IOT6
DS2IOT7
DS4ON
DS4IOT1
DS4IOT2
DS4IOT3
DS4IOT6
DS6ON
DS6IOT1
DS6IOT2
DS6IOT3
DS6IOT4
DS6IOT5
DS6IOT6
DS6IOT8
DS2ON
DS2IOT1
DS4ON
DS6ON
DS6IOT1
Rosa26ON
Rosa26IOT1
Pcsk9ON

Figure 14D

Figure 14E

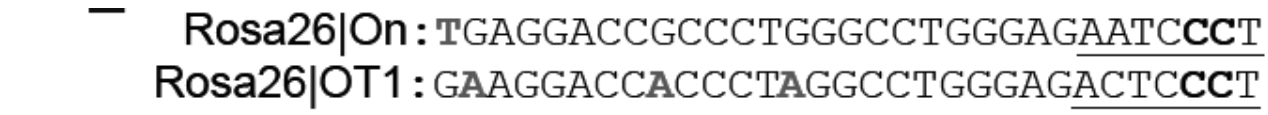


Figure 14 (cont'd)

Figure 15A

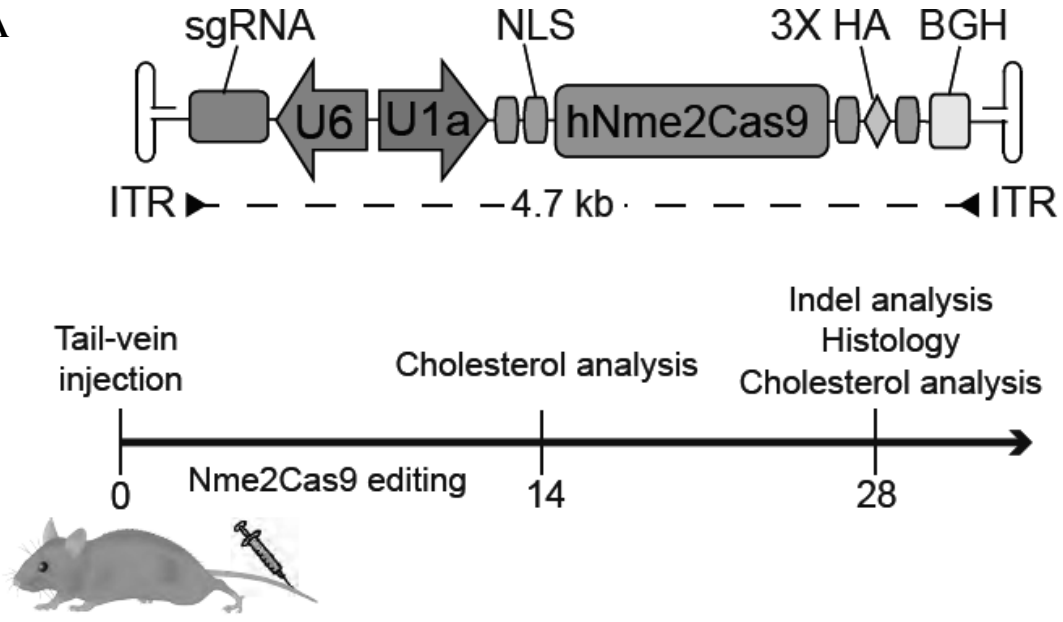


Figure 15B

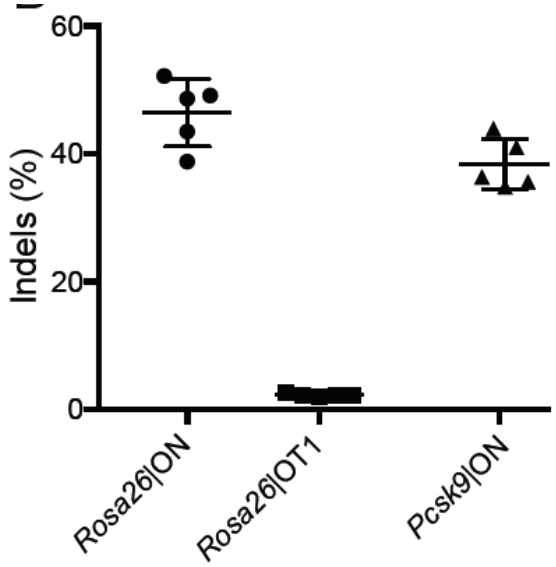


Figure 15C

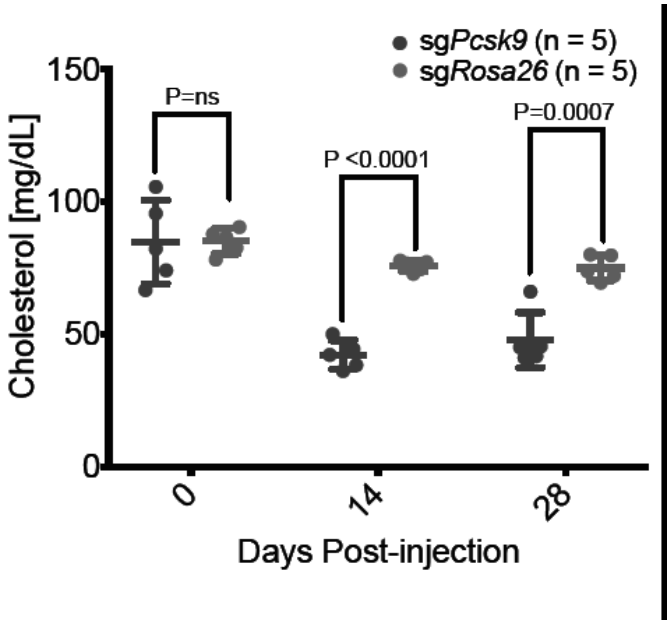


Figure 15

Figure 16A

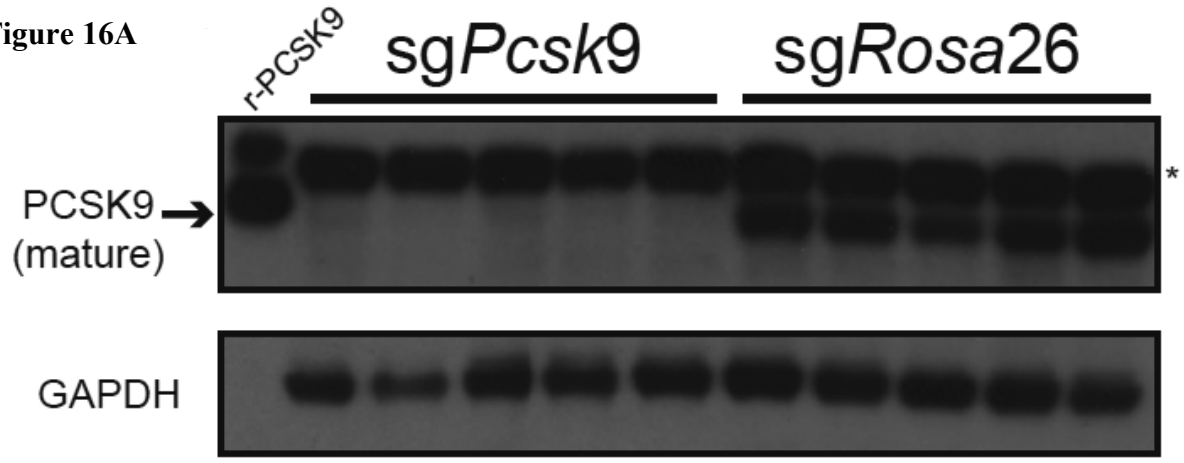


Figure 16B

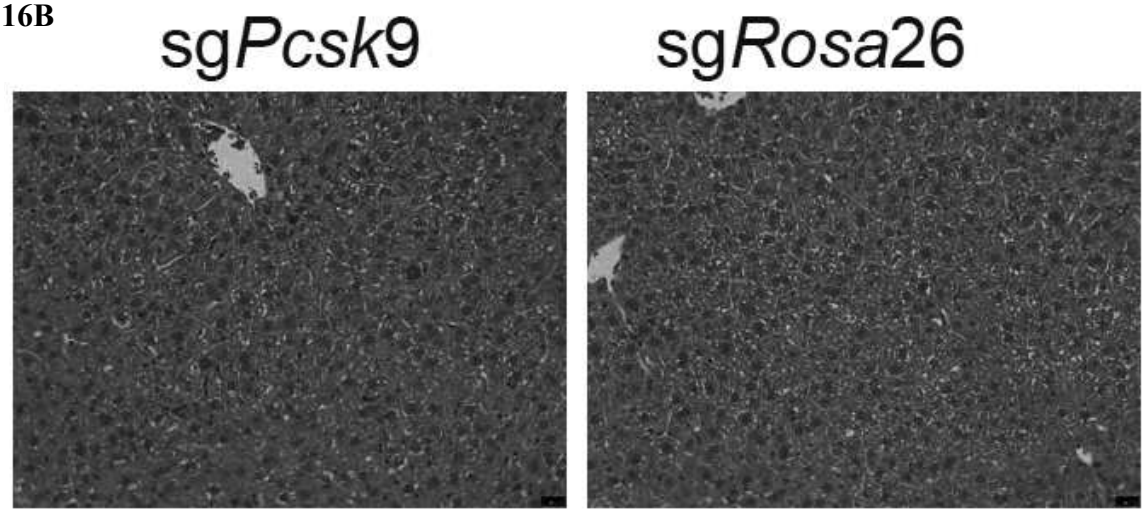


Figure 16

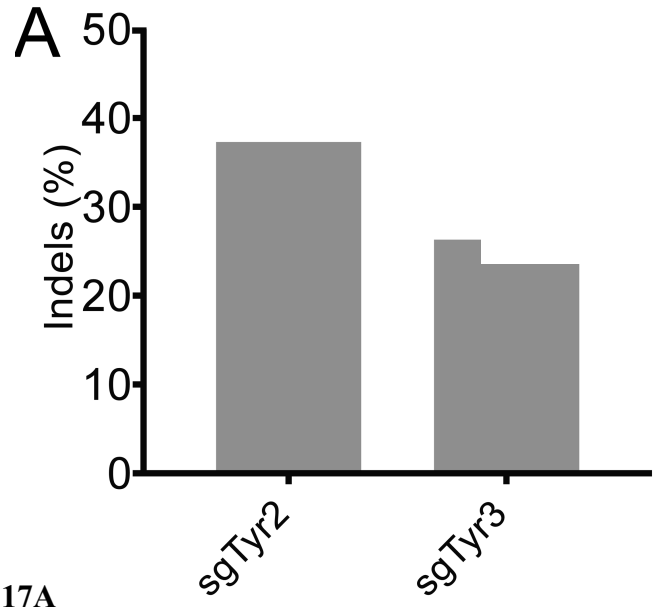


Figure 17A

Mouse ID	AAV dosage	Indels (%)	Coat color
9-1	3 x 10^9 GCs	100	Albino
9-2	3 x 10^9 GCs	100	Albino
9-3	3 x 10^9 GCs	84	mosaic and hypomorphic
9-4	3 x 10^9 GCs	98	mosaic and hypomorphic
9-5	3 x 10^9 GCs	100	mosaic and hypomorphic
8-1	3 x 10^8 GCs	100	mosaic and hypomorphic
8-2	3 x 10^8 GCs	96	mosaic and hypomorphic

Figure 17B

Figure 17

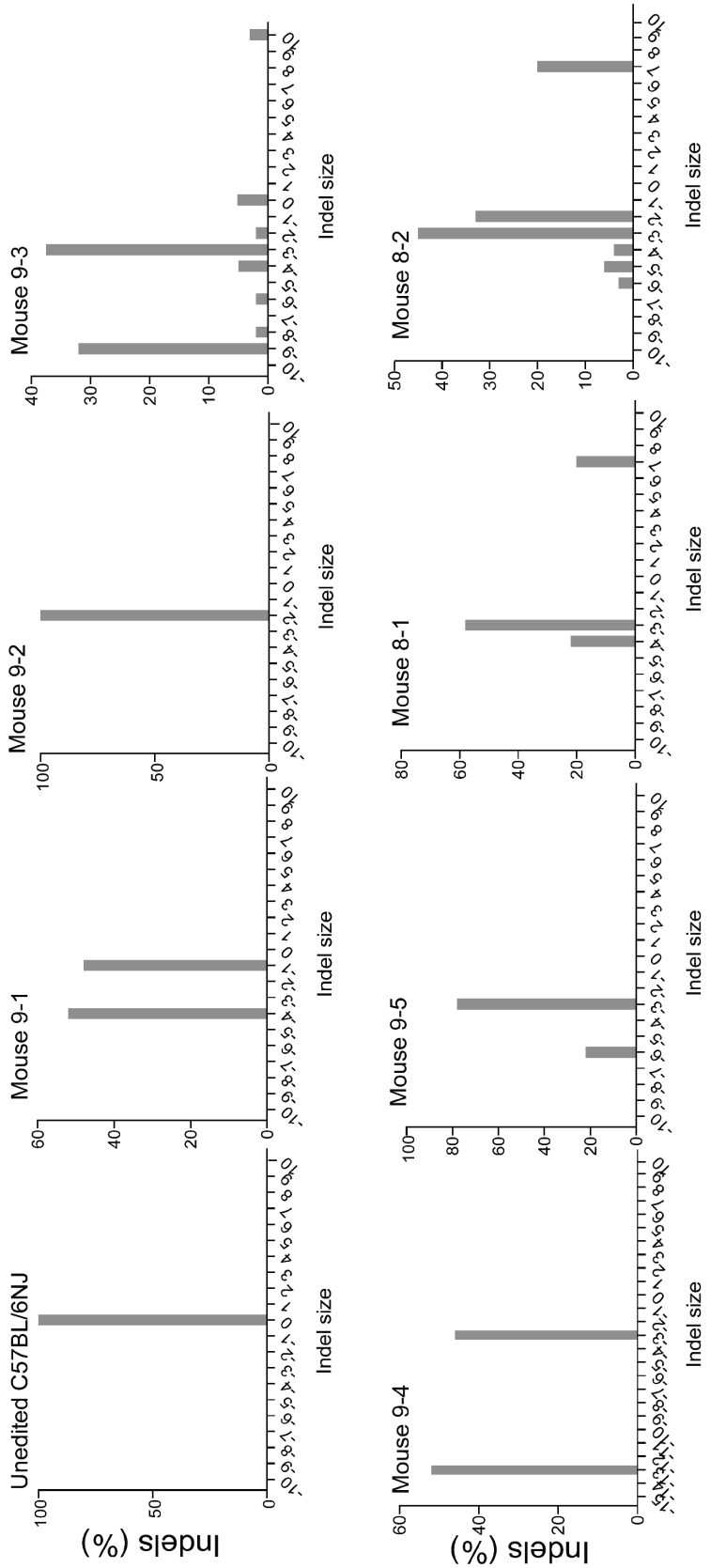


Figure 17C

Figure 17 (cont'd)

Figure 18A

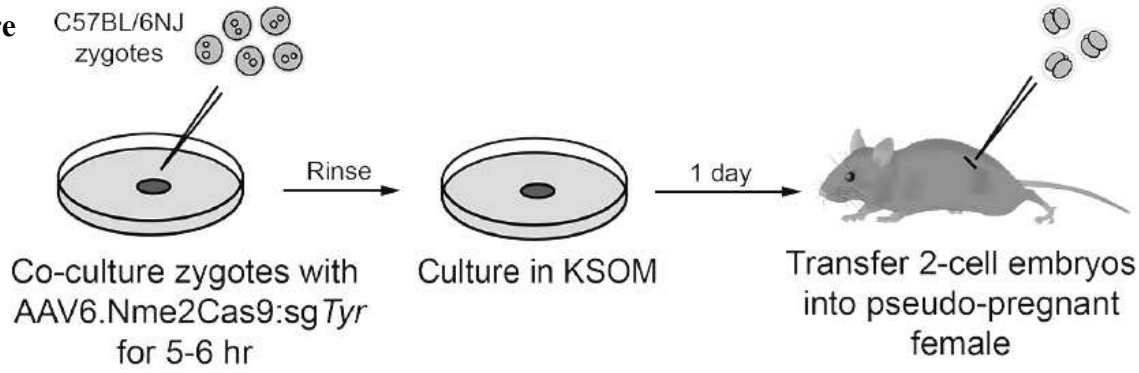


Figure 18B



Figure 18C

AAV Dosage	Number of embryos transferred	Number of pups recovered	Number of *Tyr*-edited pups (albino or chinchilla)	*Tyr* editing frequency (%)
3 x 10^9 GCs	50	5	5 (3)	100
3 x 10^8 GCs	28	2*	2 (1)	100

Figure 18

Figure 19A

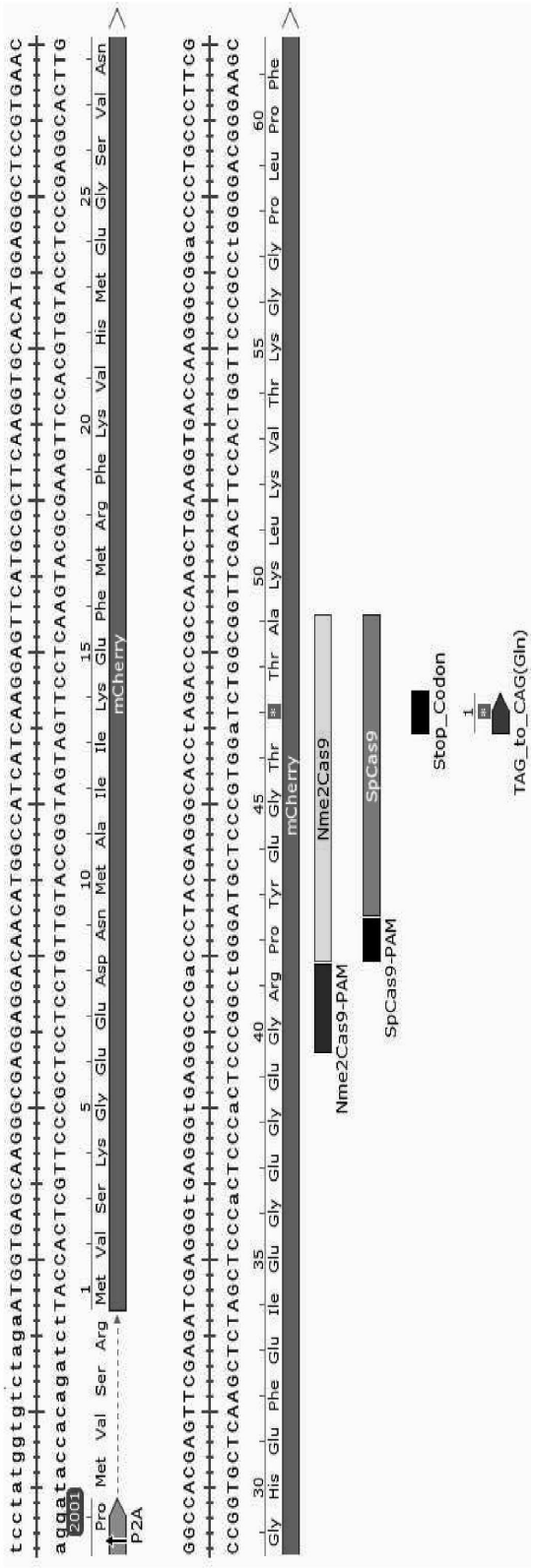


Figure 19

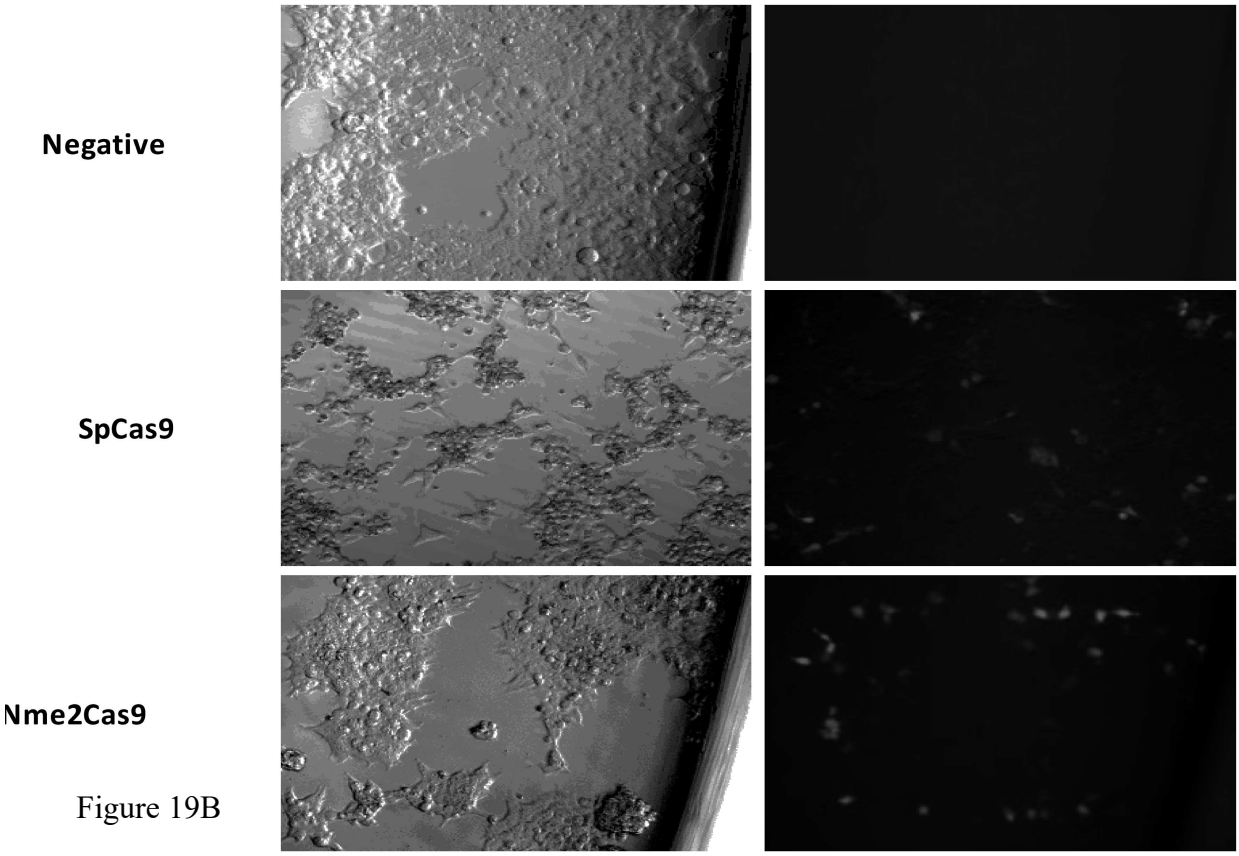


Figure 19B

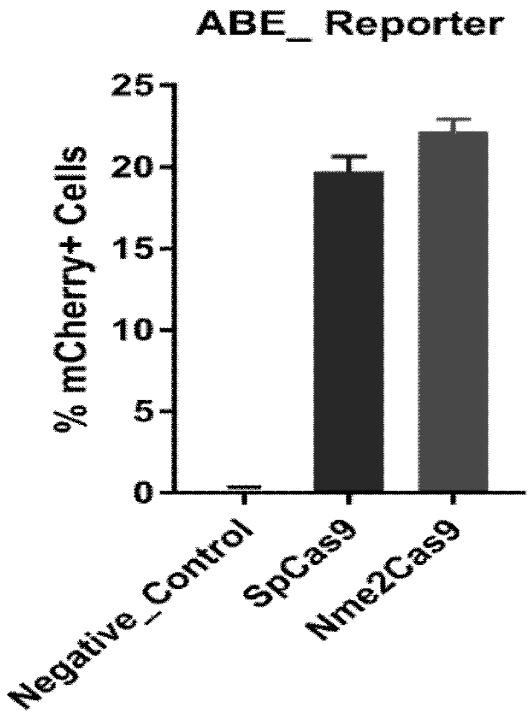


Figure 19C

Figure 19 (cont'd)

Figure 20A

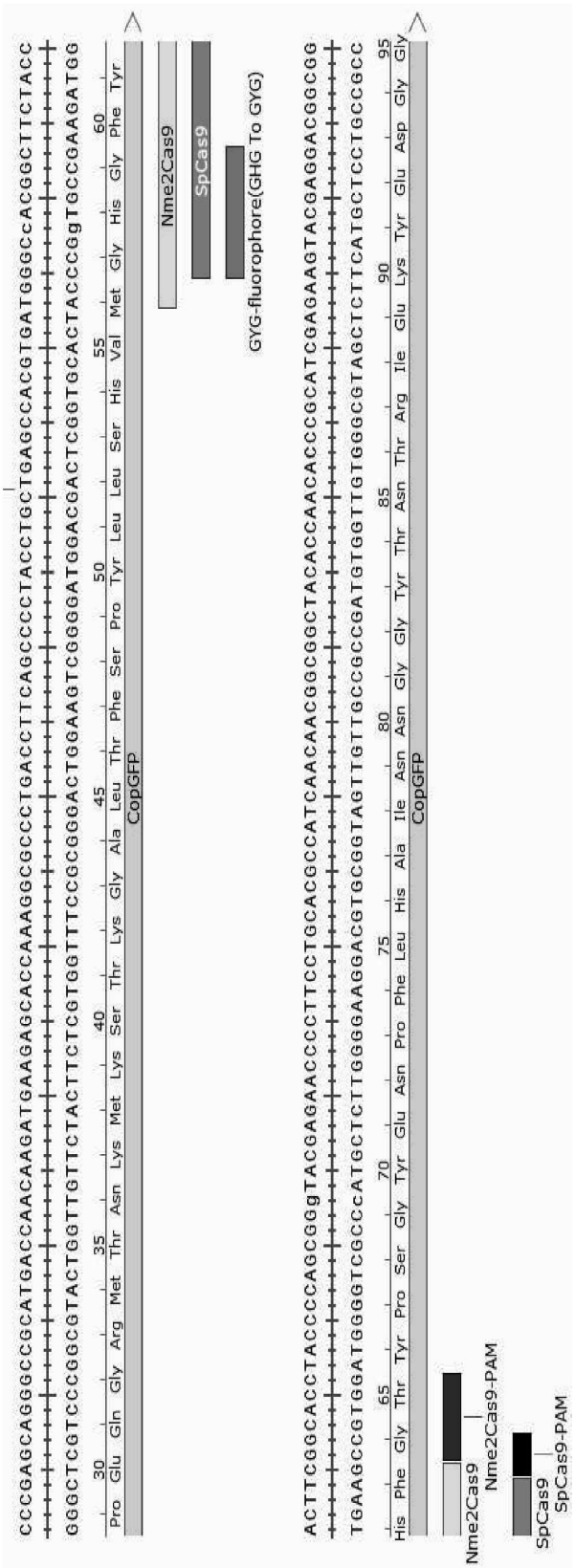


Figure 20

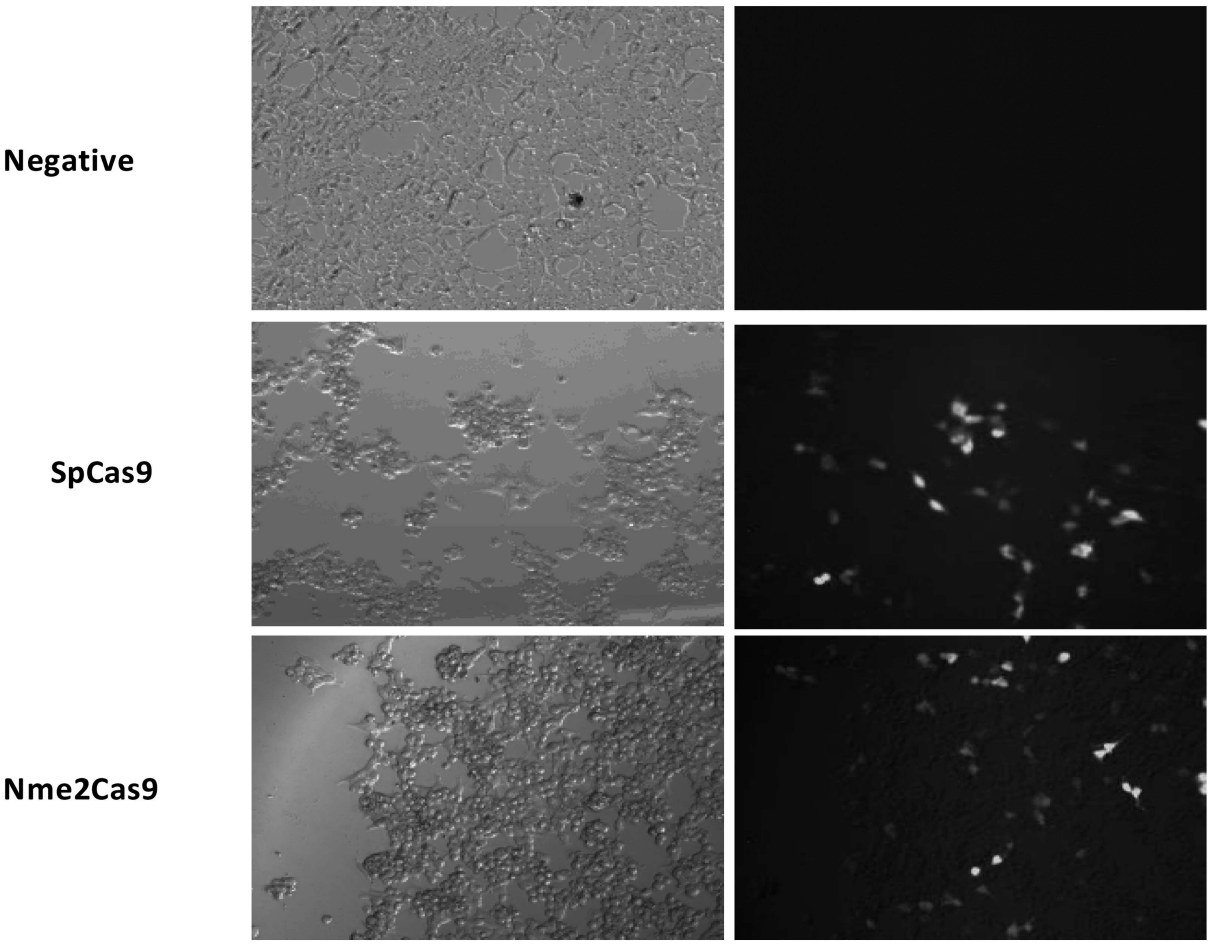


Figure 20B

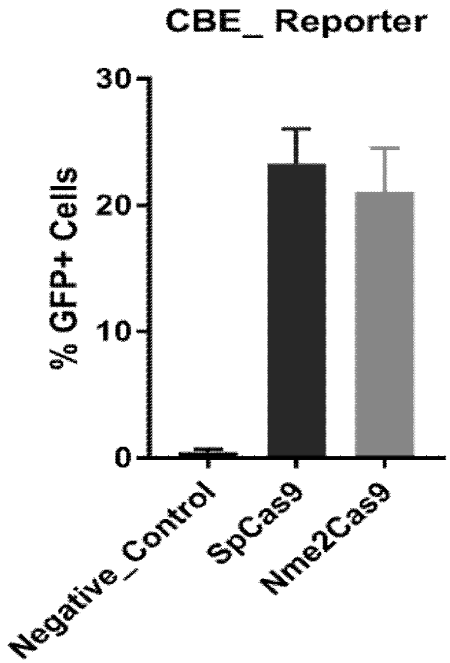
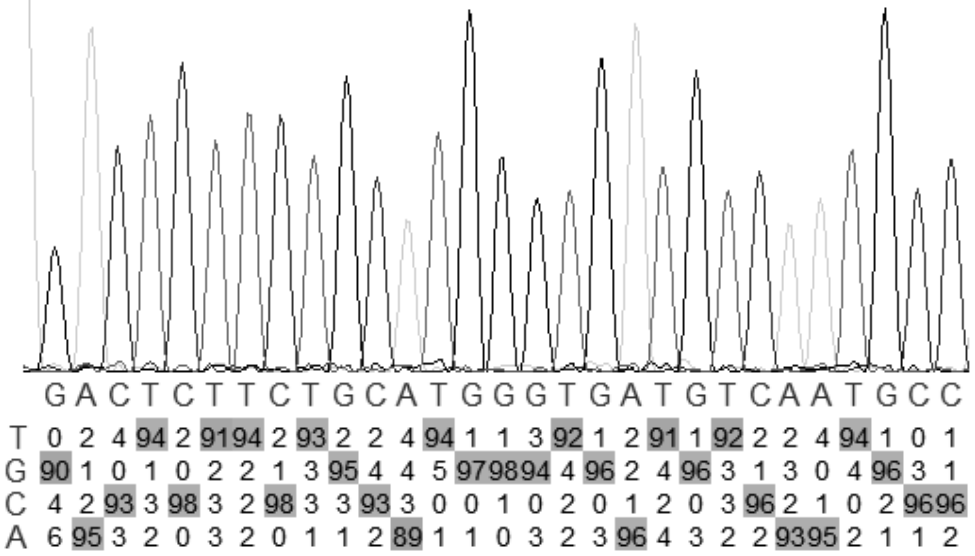


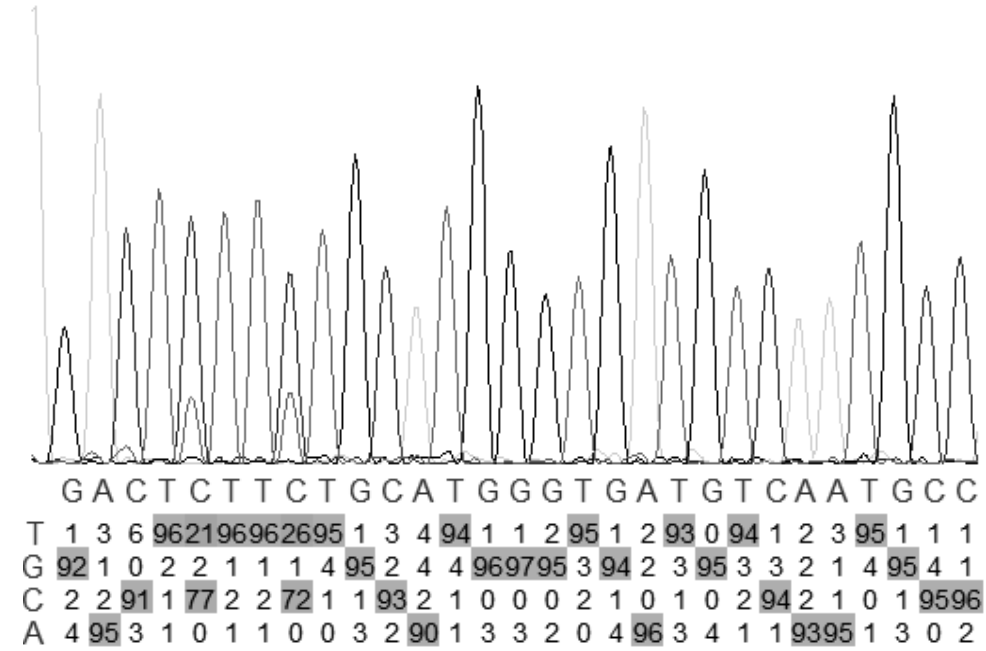
Figure 20C

Figure 20 (cont'd)

KANK3 GACTCTTCTGCATGGGTGATGTC**AATGCC**



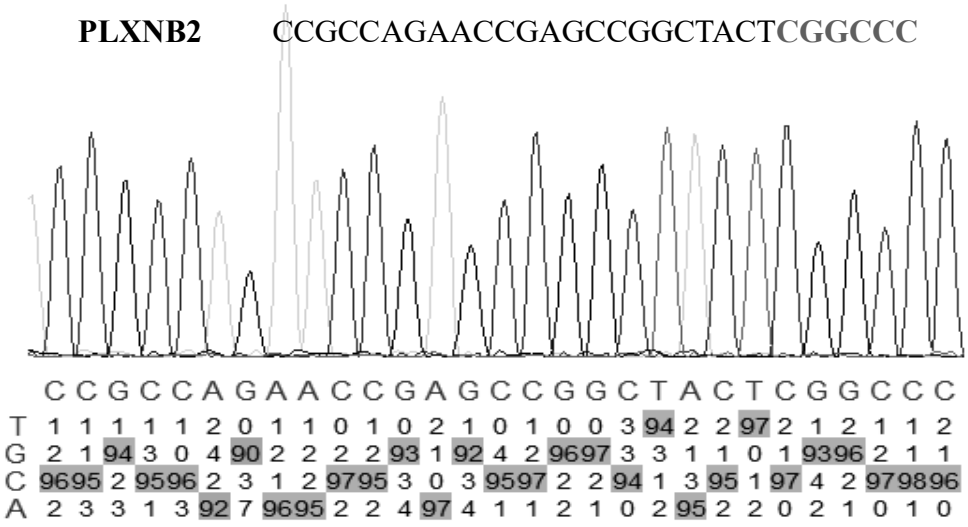
Negative



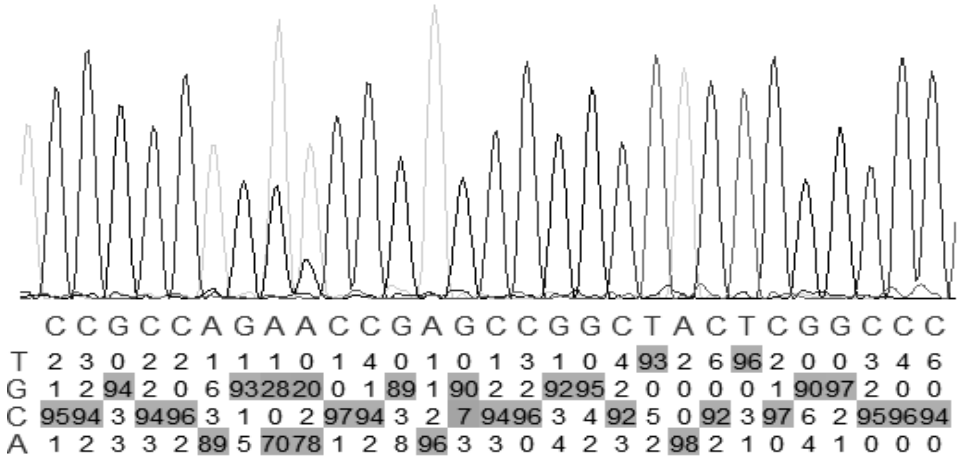
Nme2Cas9-CBE

Figure 21

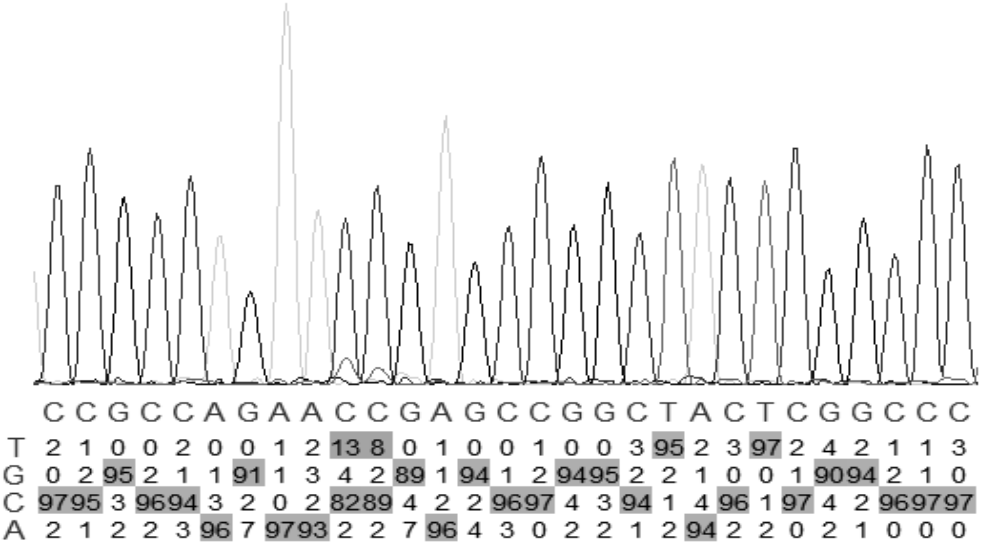
PLXNB2 CCGCCAGAACCGAGCCGGCTACTCGGCCC



Negative



Nme2Cas9-ABE



Nme2Cas9-CBE

Figure 22