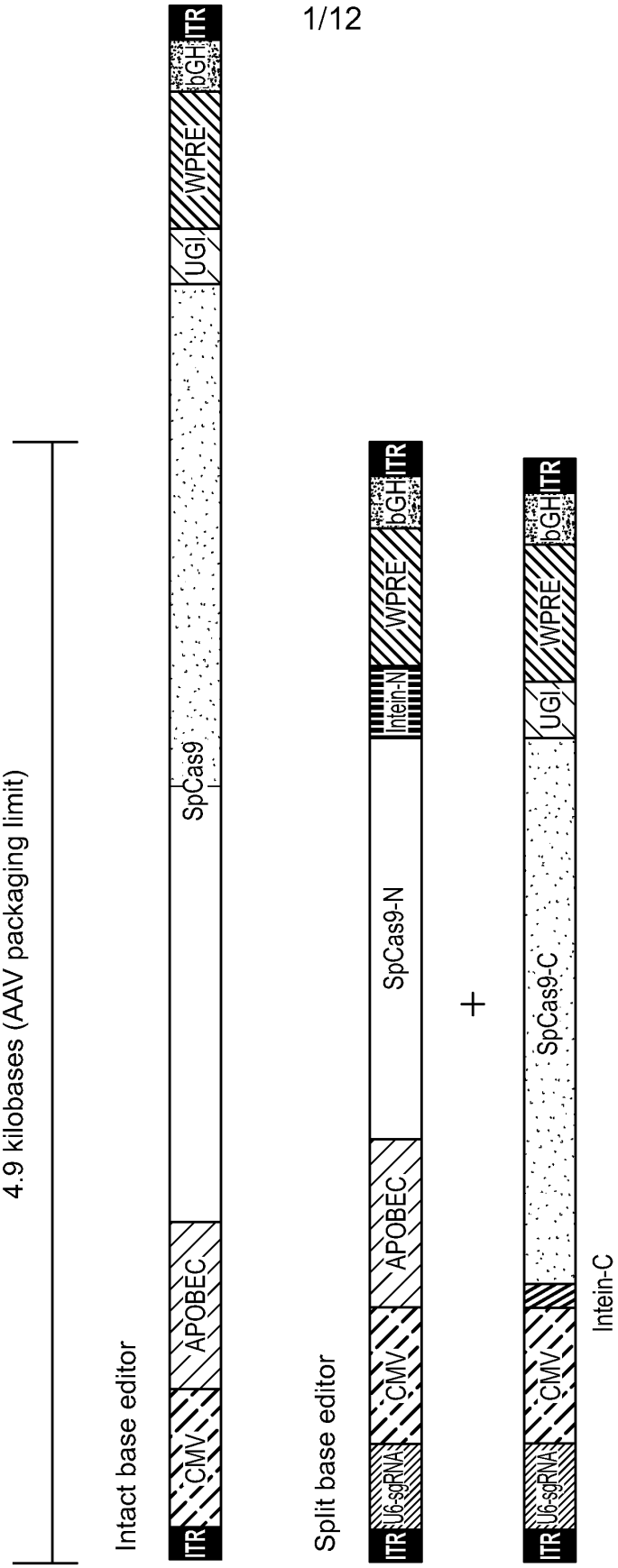

4.9 kilobases (AAV packaging limit)
Intact base editor
ITR
U6
CMV
APOBEC
SpCas9
UGI
WPRE
bGH
ITR
Split base editor
ITR
U6-sgRNA
CMV
APOBEC
SpCas9-N
Intein-N
WPRE
bGH
ITR
+
ITR
U6-sgRNA
CMV
SpCas9-C
UGI
WPRE
bGH
ITR
Intein-C

Figure 1A

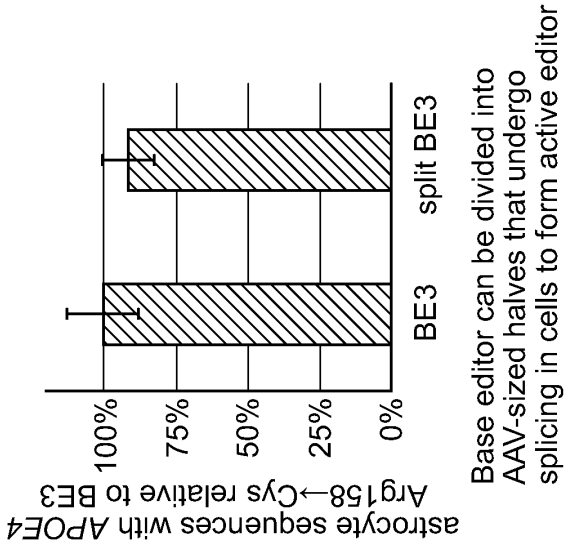

astrocyte sequences with APOE4
Arg158→Cys relative to BE3
100%
75%
50%
25%
0%
BE3
split BE3
Base editor can be divided into
AAV-sized halves that undergo
splicing in cells to form active editor

Figure 1B

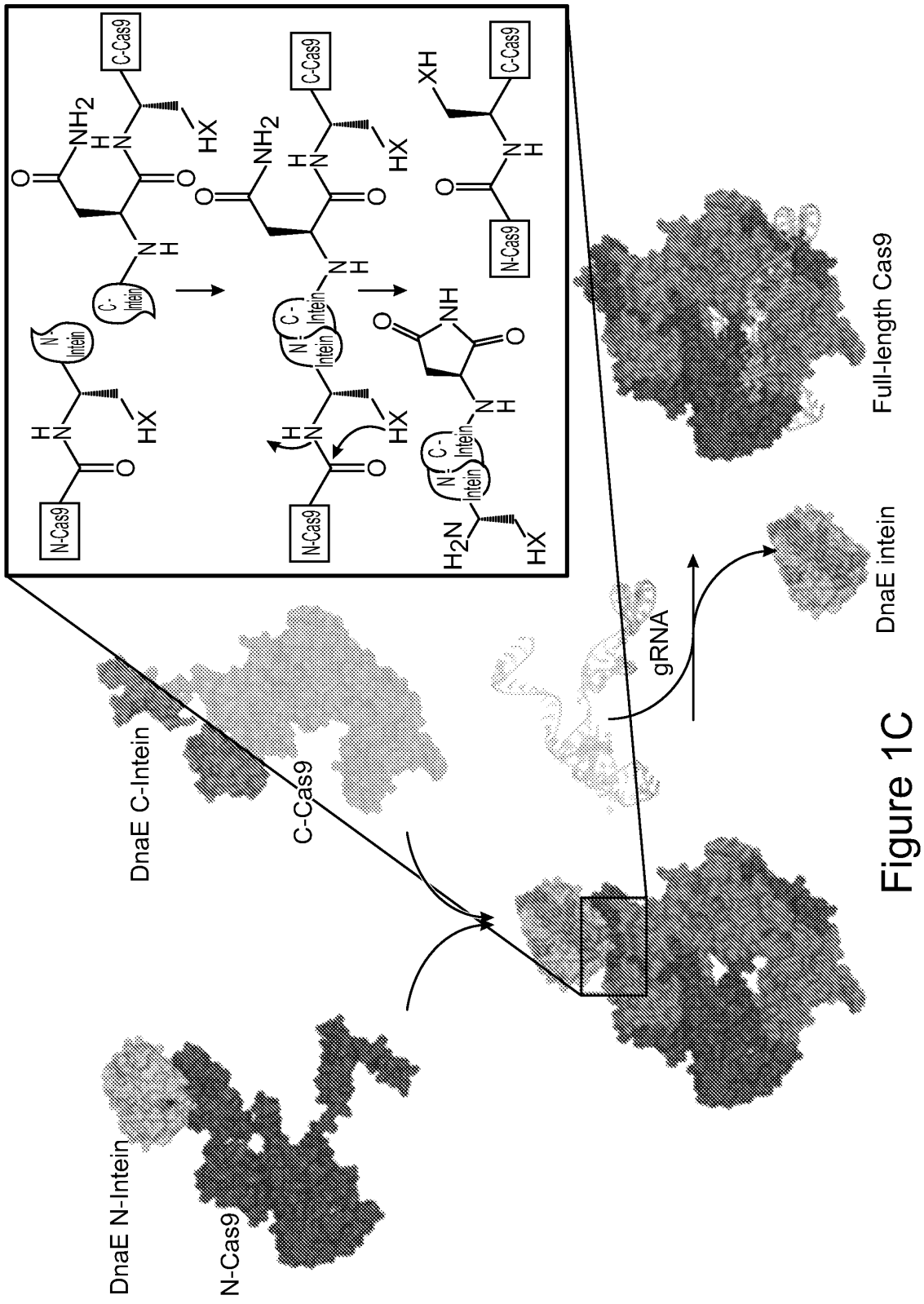

DnaE N-Intein
N-Cas9
DnaE C-Intein
C-Cas9
gRNA
DnaE intein
Full-length Cas9
N-Cas9
C-Cas9
N-Intein
C-Intein
HX
NH2
H2N

Figure 1C

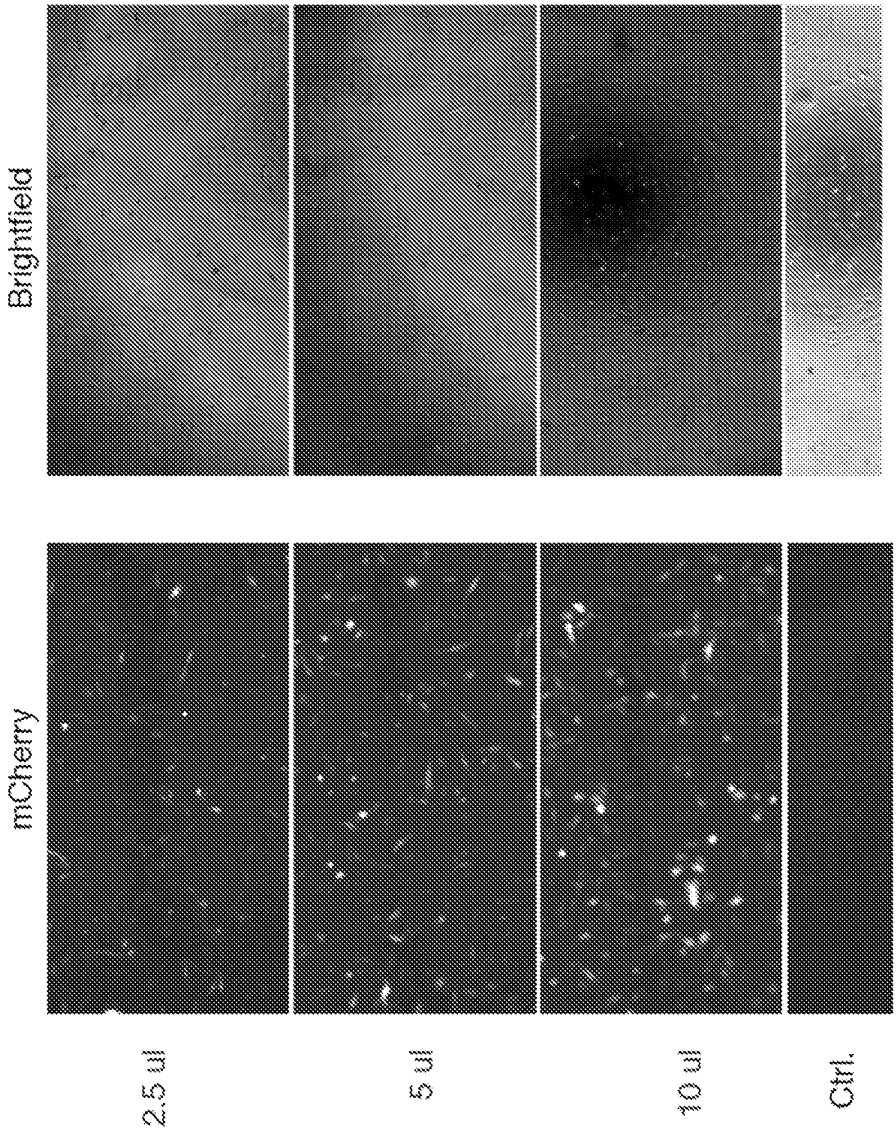


Figure 2

Virus harvested at 24 hours

72 hours:

C1

N1

	C7	C8	C7	C8	C7	C8	C7	C8	C7	C8	C7	C8
U118	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	99.8	99.8	99.9	99.9	99.9	100.0	99.8	99.9	100.0	100.0	100.0	100.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.2	0.2	0.1	0.1	0.1	0.0	0.2	0.1	0.0	0.0	0.0	0.0
HEK	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	97.0	97.1	99.8	99.8	99.8	99.8	99.6	99.7	99.5	99.5	99.8	99.8
	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2.9	2.9	0.2	0.2	0.2	0.2	0.3	0.3	0.5	0.5	0.2	0.2

Control – lipid transfection with BE3 and PRNP guide(U118 top row; HEK bottom row)

Gly			Ser -> Ser		Arg -> stop			Tyr				Pro			Gly				Glu				
G	G	C4	A	G	C7	C8	G	A	T	A	C	C	C	G	G	G	G	C	A	G	G	G
0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	100.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
0.0	0.0	99.8	0.0	0.0	98.0	98.1	0.0	0.0	0.0	0.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0
100.0	100.0	0.0	0.0	100.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	0.0	0.0	100.0	100.0	100.0
0.0	0.0	0.2	0.0	0.0	1.9	1.9	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

G	G	C4	A	G	C7	C8	G	A	T	A	C	C	C	G	G	G	G	C	A	G	G	G
0.0	0.0	0.1	100.0	0.1	1.3	0.6	0.1	99.9	0.0	100.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
0.0	0.0	96.0	0.0	0.0	68.8	70.8	0.0	0.0	0.0	0.0	99.8	99.7	99.9	0.0	0.1	0.1	0.0	100.0	0.0	0.0	0.0	0.0
100.0	100.0	0.0	0.0	99.9	2.1	2.0	99.9	0.0	0.0	0.0	0.0	0.0	0.0	99.9	99.9	99.9	99.9	0.0	0.0	100.0	100.0	100.0
0.0	0.0	3.8	0.0	0.0	27.9	26.6	0.0	0.0	100.0	0.0	0.2	0.2	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0

Figure 3A

SUBSTITUTE SHEET (RULE 26)

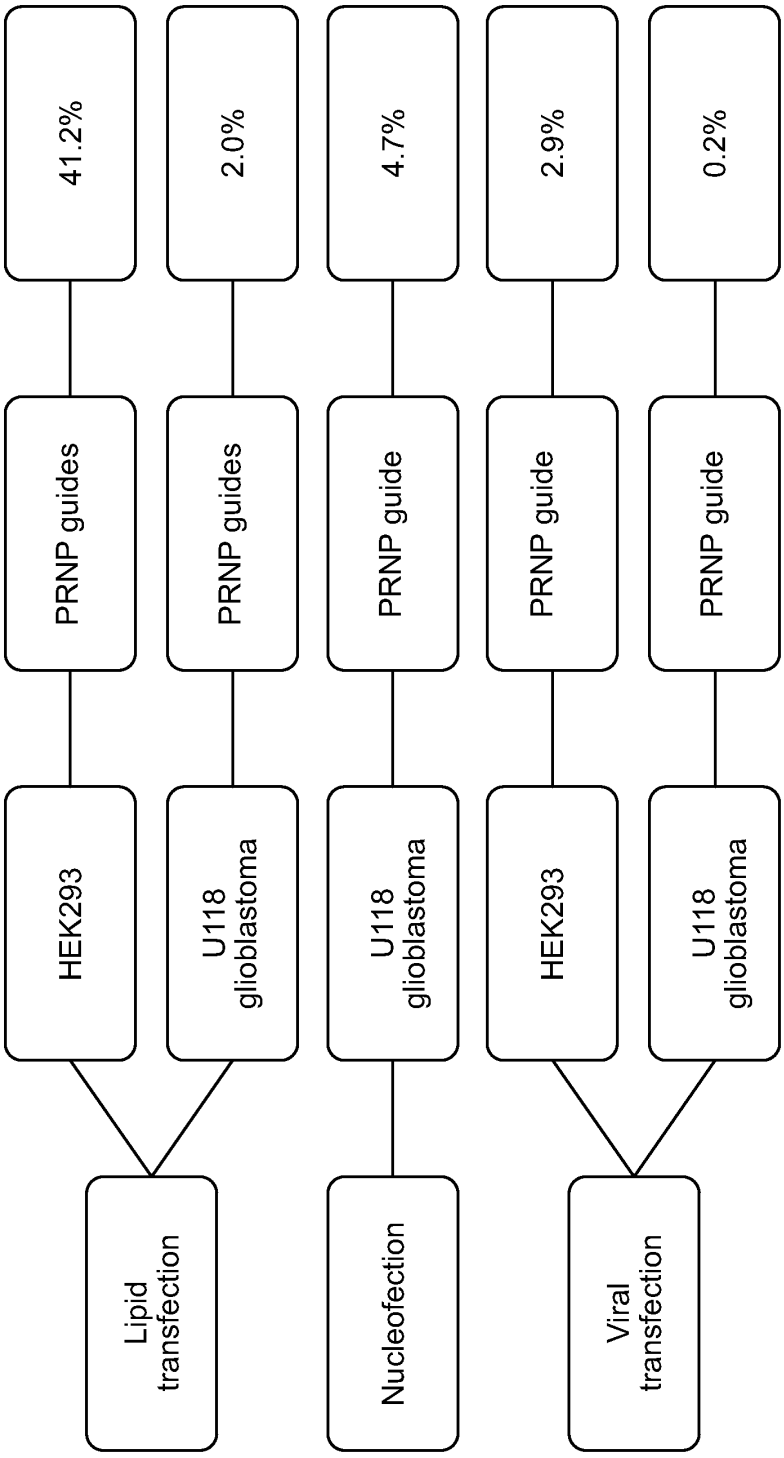

Lipid transfection
Nucleofection
Viral transfection
HEK293
U118 glioblastoma
U118 glioblastoma
HEK293
U118 glioblastoma
PRNP guides
PRNP guides
PRNP guide
PRNP guide
PRNP guide
41.2%
2.0%
4.7%
2.9%
0.2%

Figure 3B

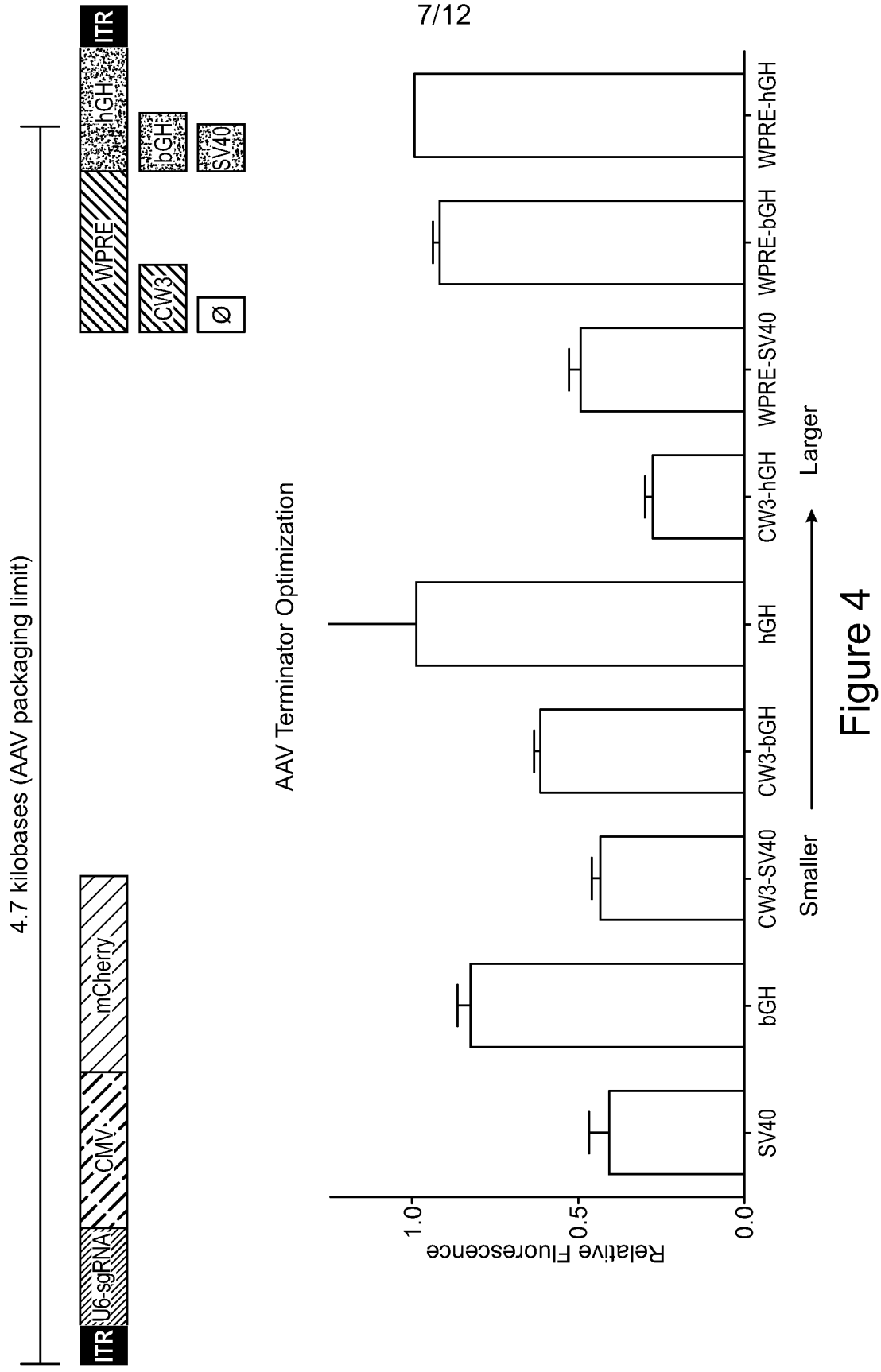
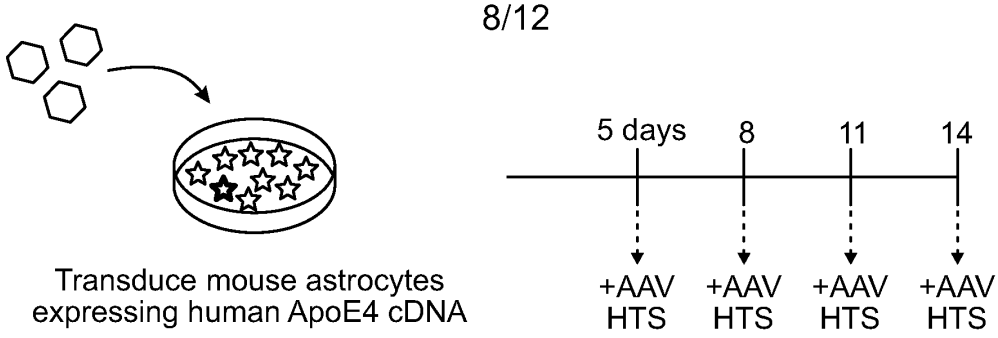

4.7 kilobases (AAV packaging limit)
ITR
U6-sgRNA
CMV
mCherry
WPRE
hGH
ITR
CW3
bGH
Ø
SV40
AAV Terminator Optimization
Relative Fluorescence
0.0
0.5
1.0
SV40
bGH
CW3-SV40
CW3-bGH
hGH
CW3-hGH
WPRE-SV40
WPRE-bGH
WPRE-hGH
Smaller
Larger

Figure 4



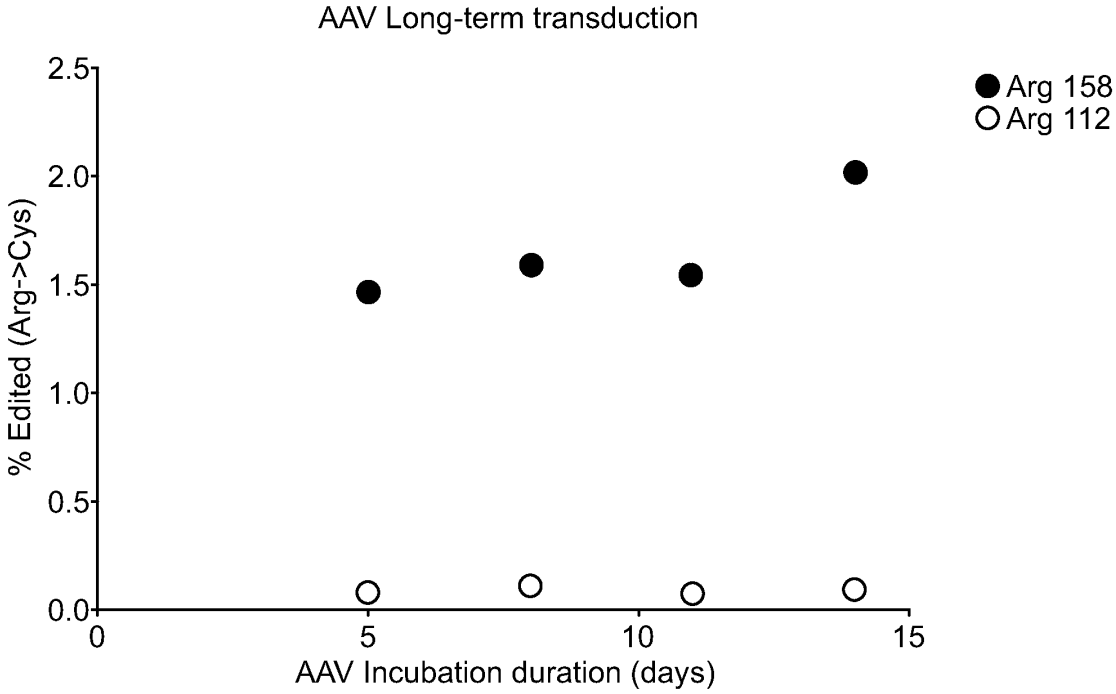


Figure 5A

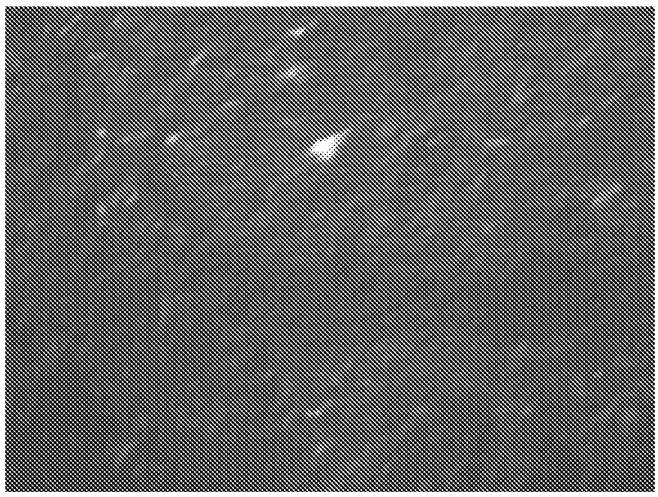


Figure 5B

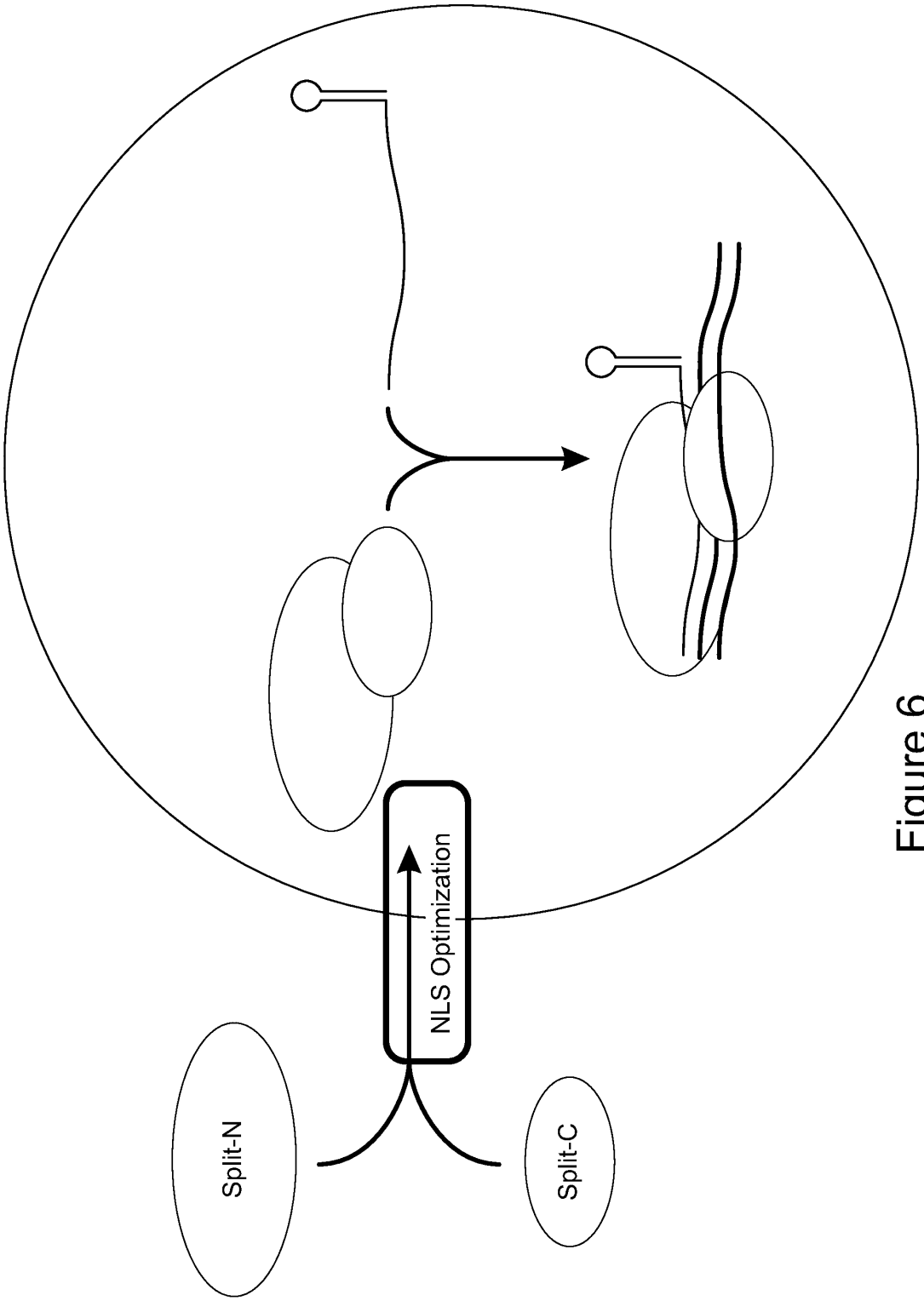


Figure 6

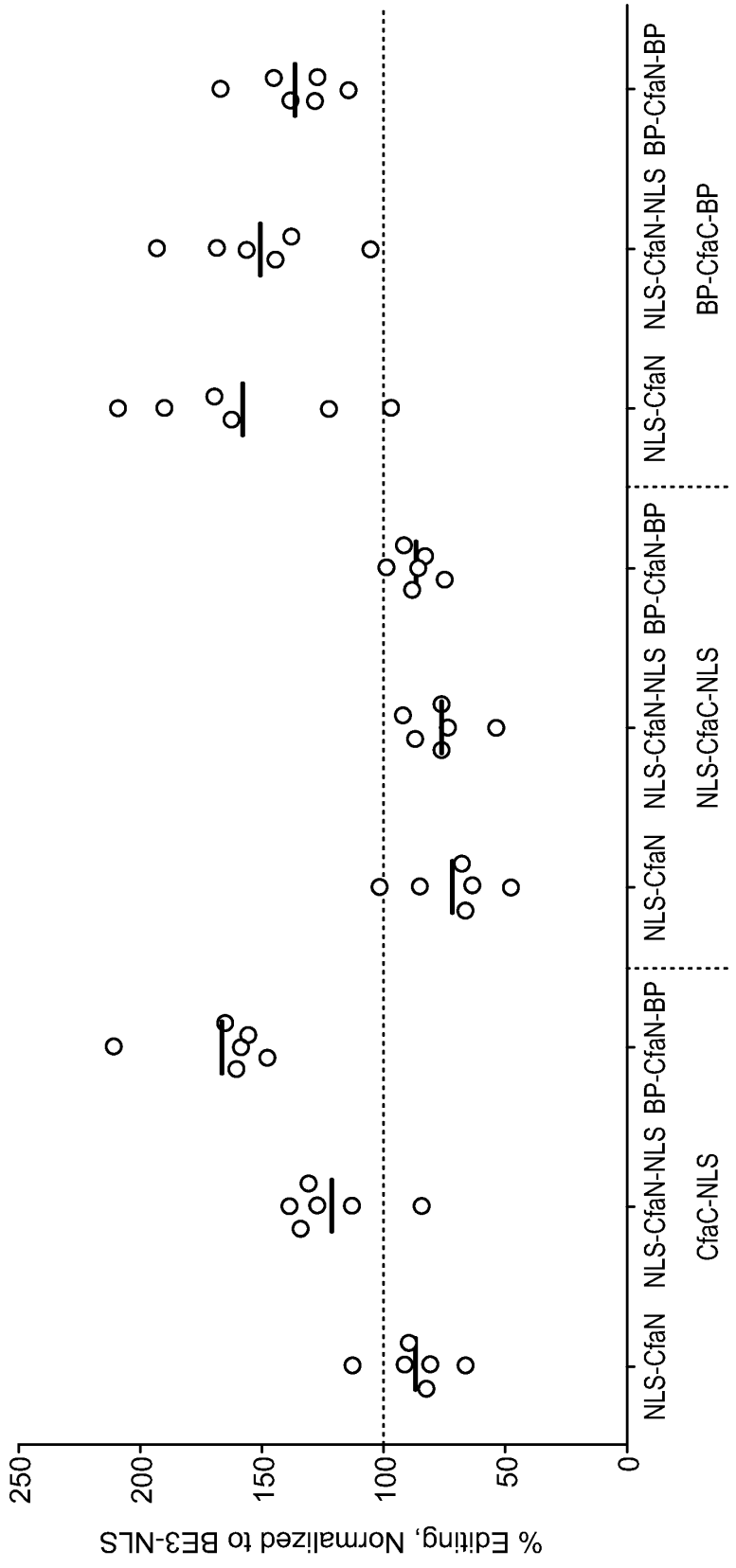

% Editing, Normalized to BE3-NLS
250
200
150
100
50
0
NLS-CfaN
NLS-CfaN-NLS
BP-CfaN-BP
CfaC-NLS
NLS-CfaN
NLS-CfaN-NLS
BP-CfaN-BP
NLS-CfaC-NLS
NLS-CfaN
NLS-CfaN-NLS
BP-CfaN-BP
BP-CfaC-BP

Figure 7

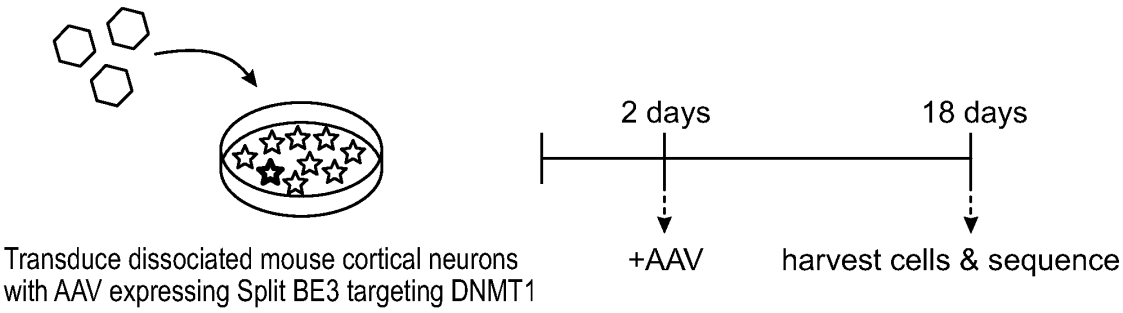


Figure 8A

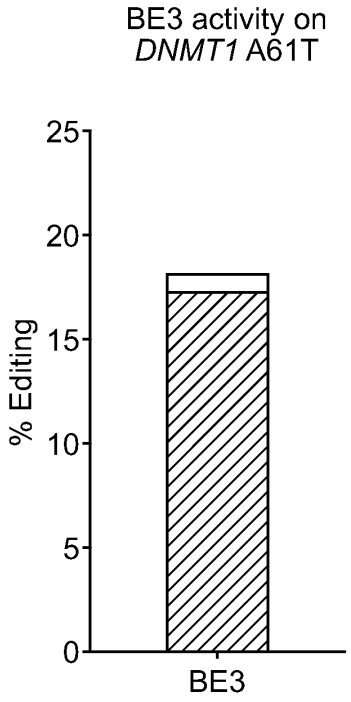


Figure 8B

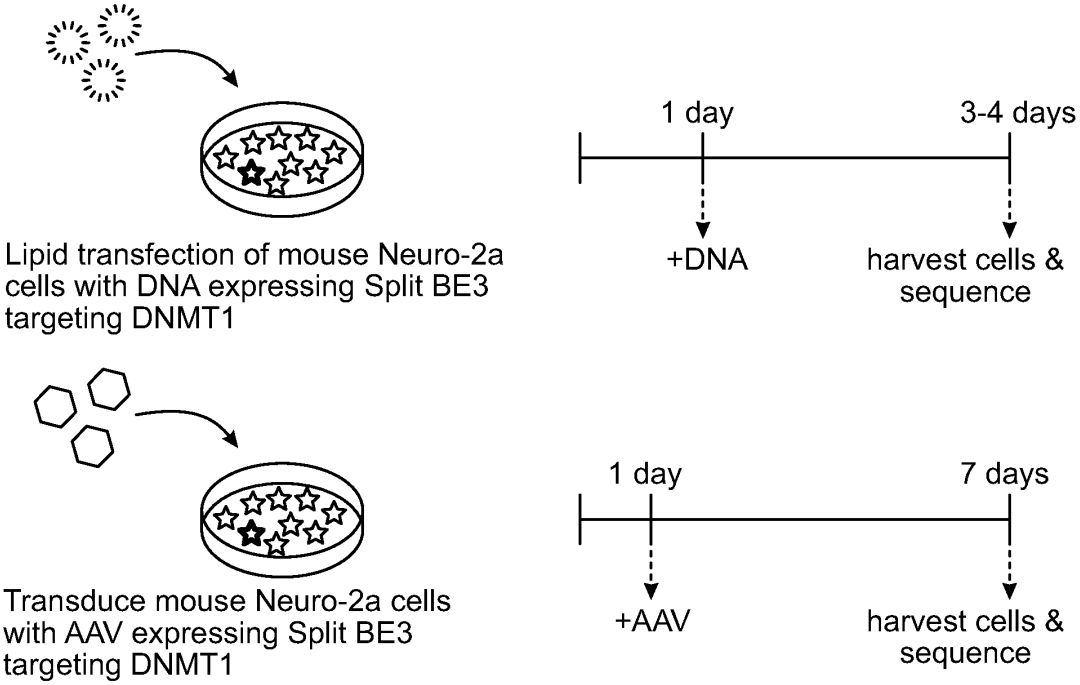


Figure 9A

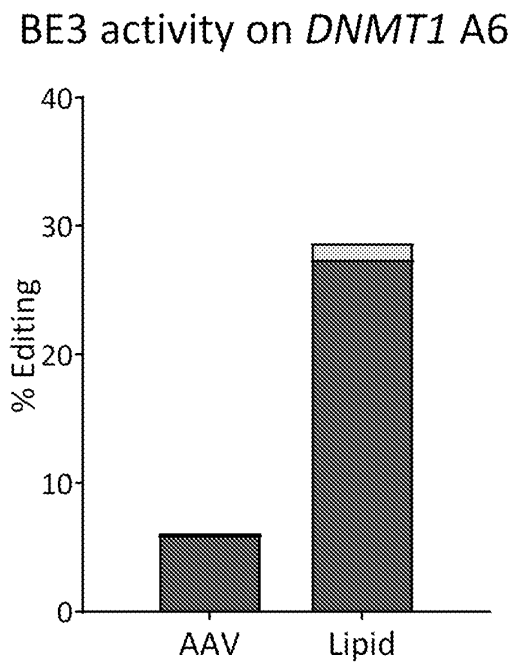


Figure 9B