Figure 1

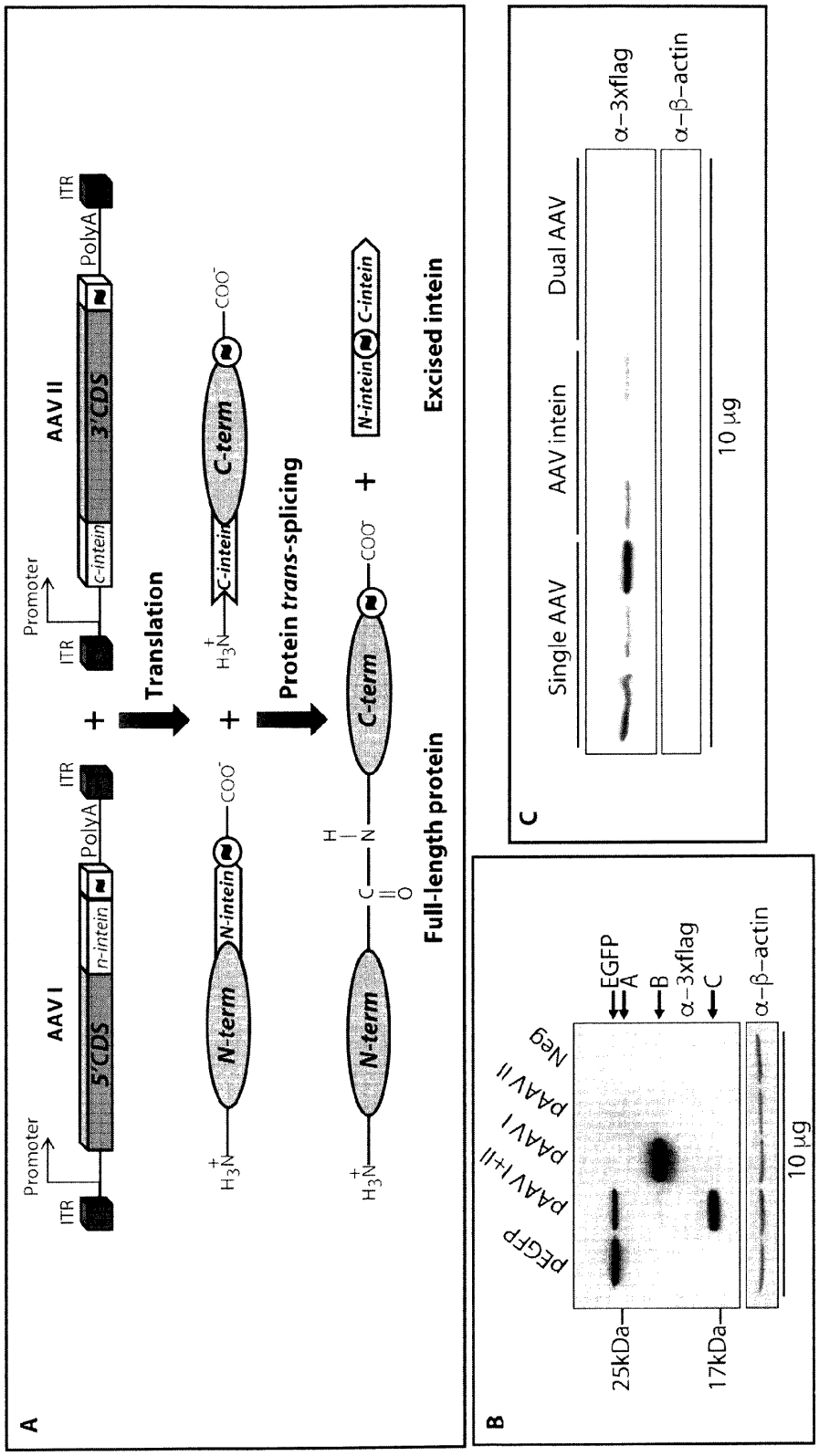

A
AAV I
AAV II
ITR
Promoter
5'CDS
n-intein
PolyA
c-intein
3'CDS
Translation
Protein trans-splicing
N-term
C-term
N-intein
C-intein
Excised intein
Full-length protein
B
pEGFP
pAAV I+II
pAAV I
pAAV II
Neg
EGFP
A
B
α-3xflag
C
α-β-actin
25kDa
17kDa
10 µg
C
Single AAV
AAV intein
Dual AAV
α-3xflag
α-β-actin
10 µg

Figure 1

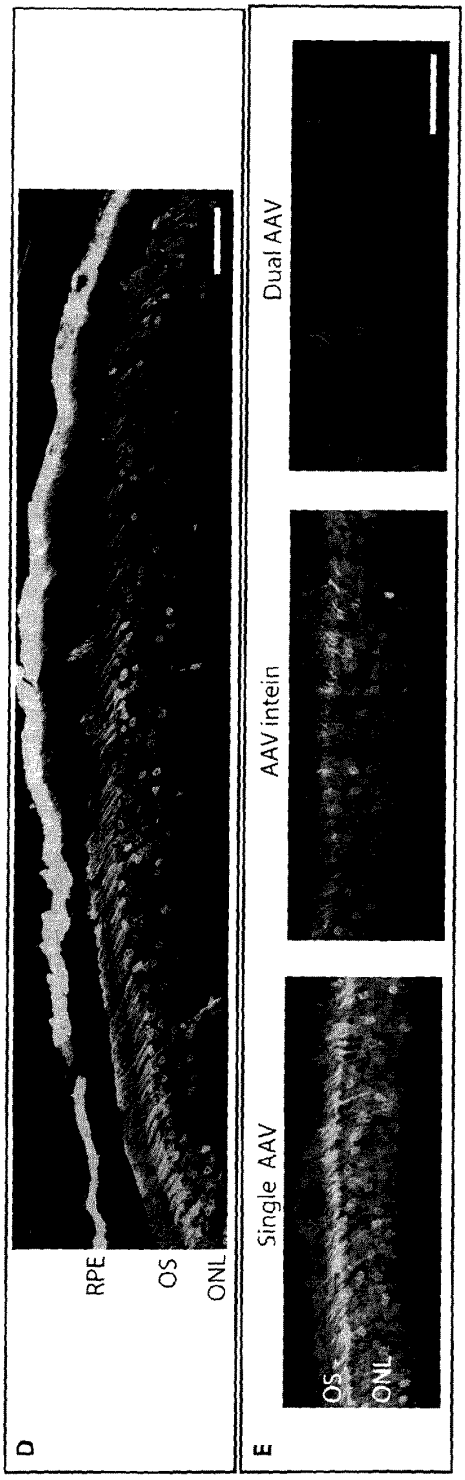


Figure 1

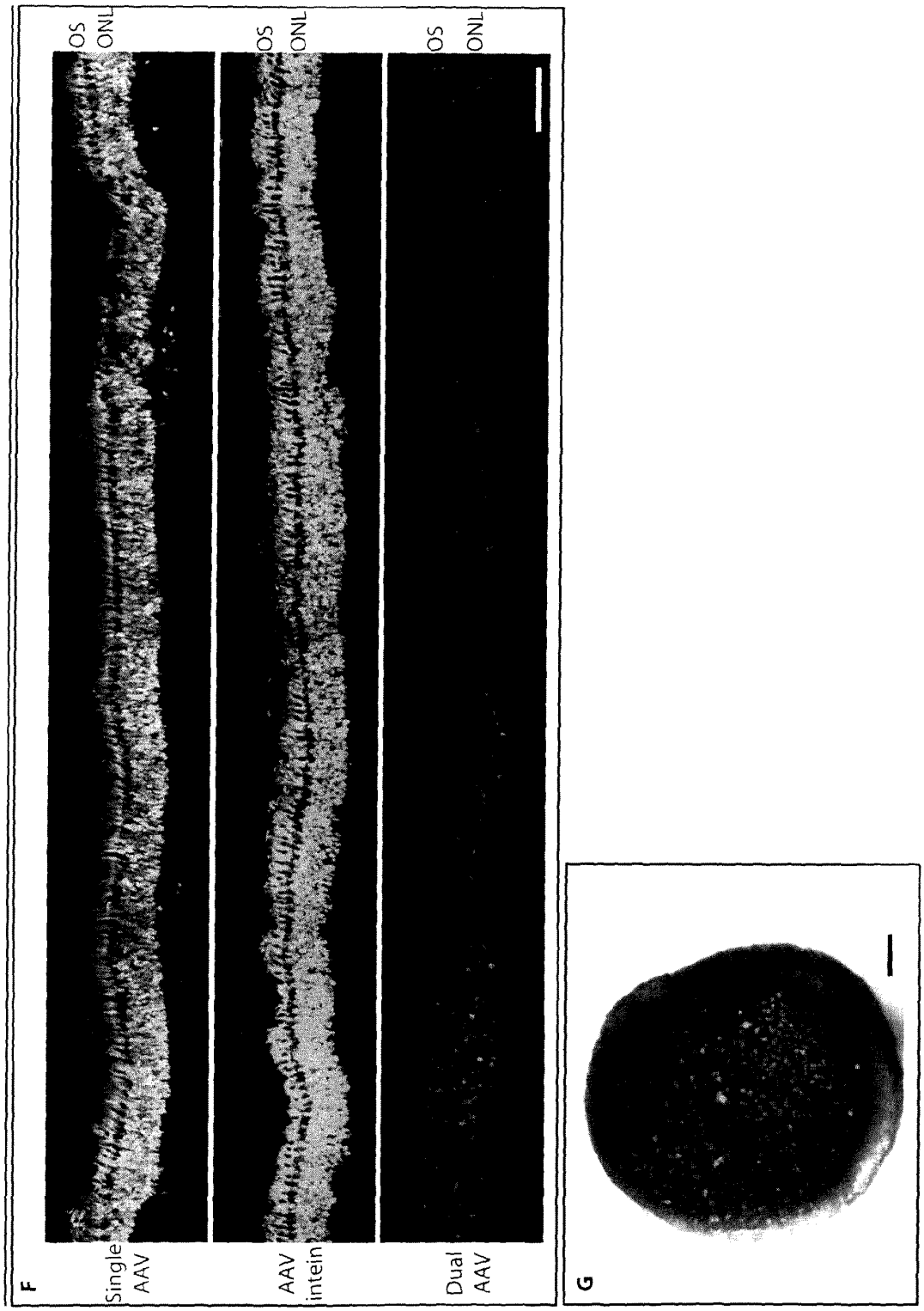


Figure 2

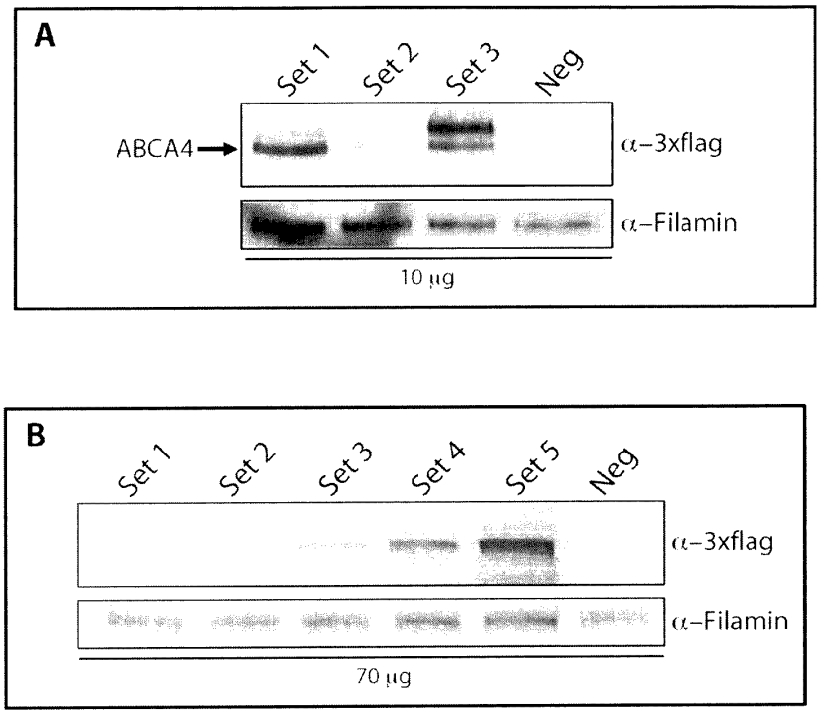

A
Set 1
Set 2
Set 3
Neg
ABCA4
α–3xflag
α–Filamin
10 µg
B
Set 1
Set 2
Set 3
Set 4
Set 5
Neg
α–3xflag
α–Filamin
70 µg

Figure 2

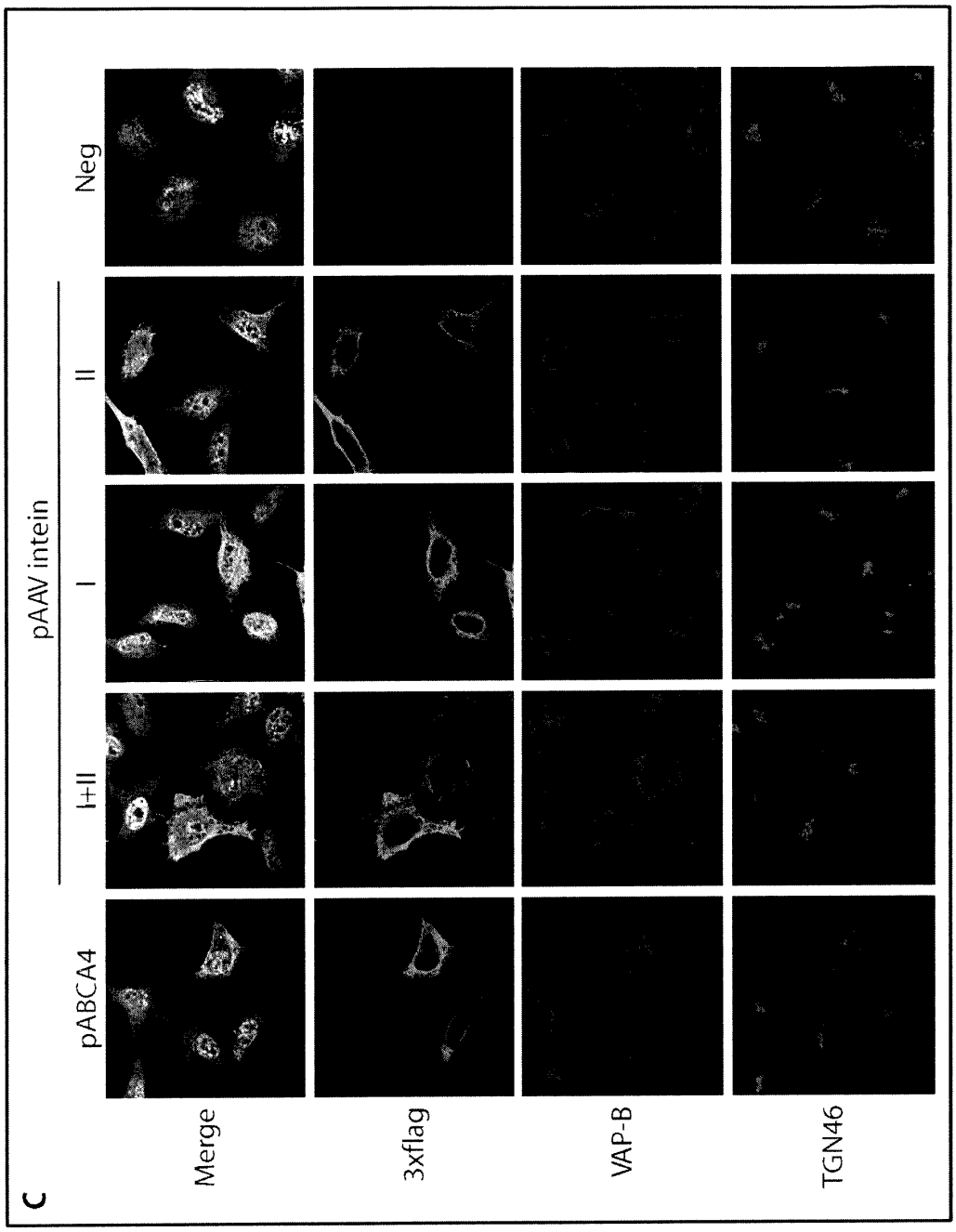


Figure 2

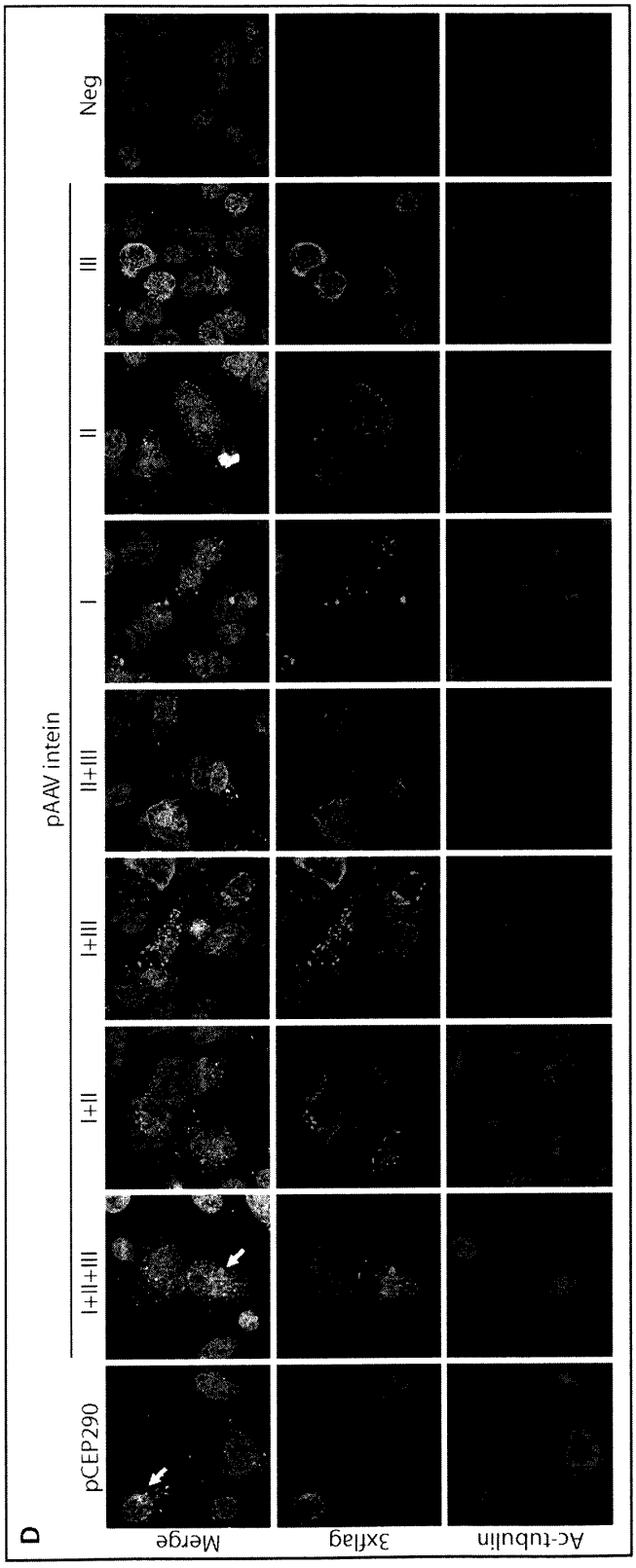
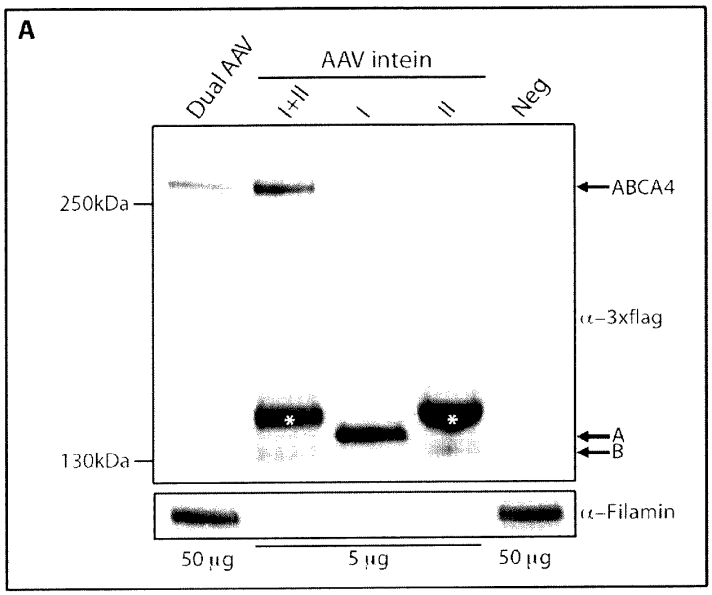

D
Merge
3xflag
Ac-tubulin
pCEP290
I+II+III
I+II
I+III
II+III
I
II
III
Neg
pAAV intein

Figure 3


A
Dual AAV
AAV intein
I+II
I
II
Neg
250kDa
130kDa
ABCA4
α-3xflag
A
B
α-Filamin
50 µg
5 µg
50 µg

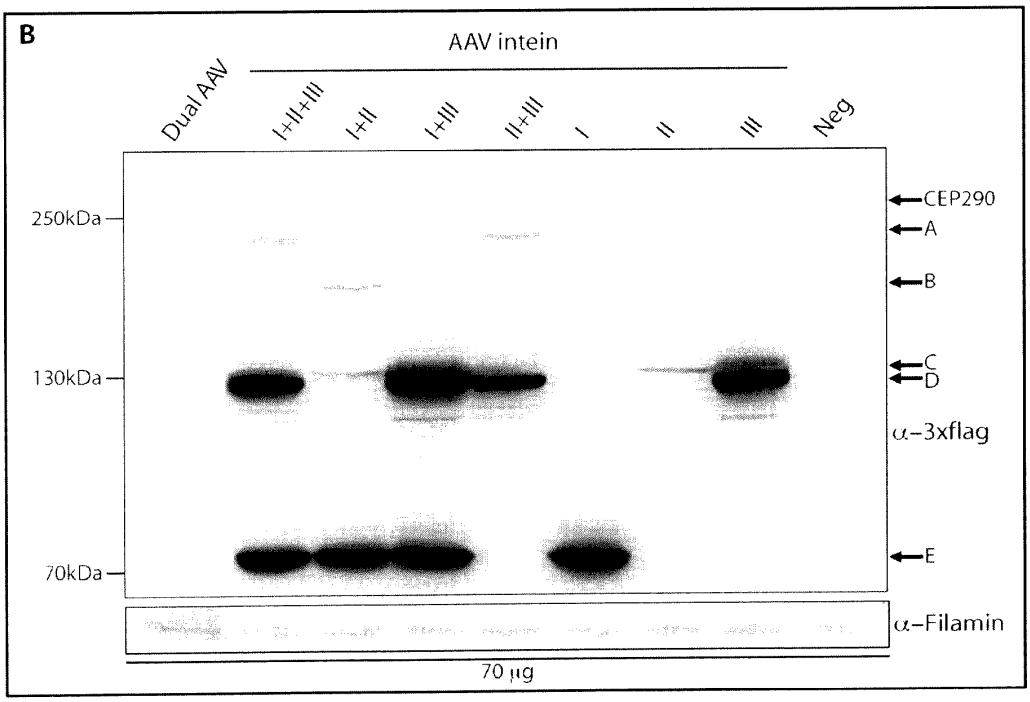

B
AAV intein
Dual AAV
I+II+III
I+II
I+III
II+III
I
II
III
Neg
250kDa
130kDa
70kDa
CEP290
A
B
C
D
α-3xflag
E
α-Filamin
70 µg

Figure 4

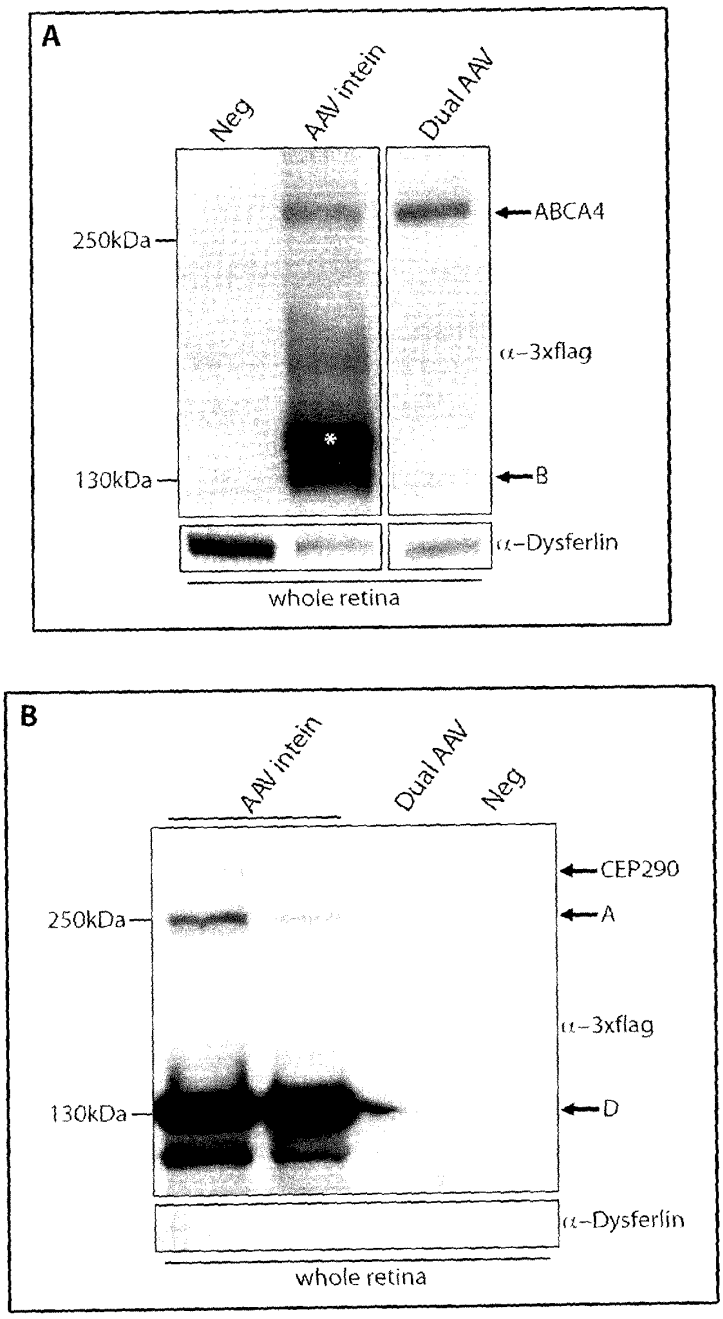

A
Neg
AAV intein
Dual AAV
250kDa
130kDa
ABCA4
α-3xflag
B
α-Dysferlin
whole retina
B
AAV intein
Dual AAV
Neg
250kDa
130kDa
CEP290
A
α-3xflag
D
α-Dysferlin
whole retina

Figure 4

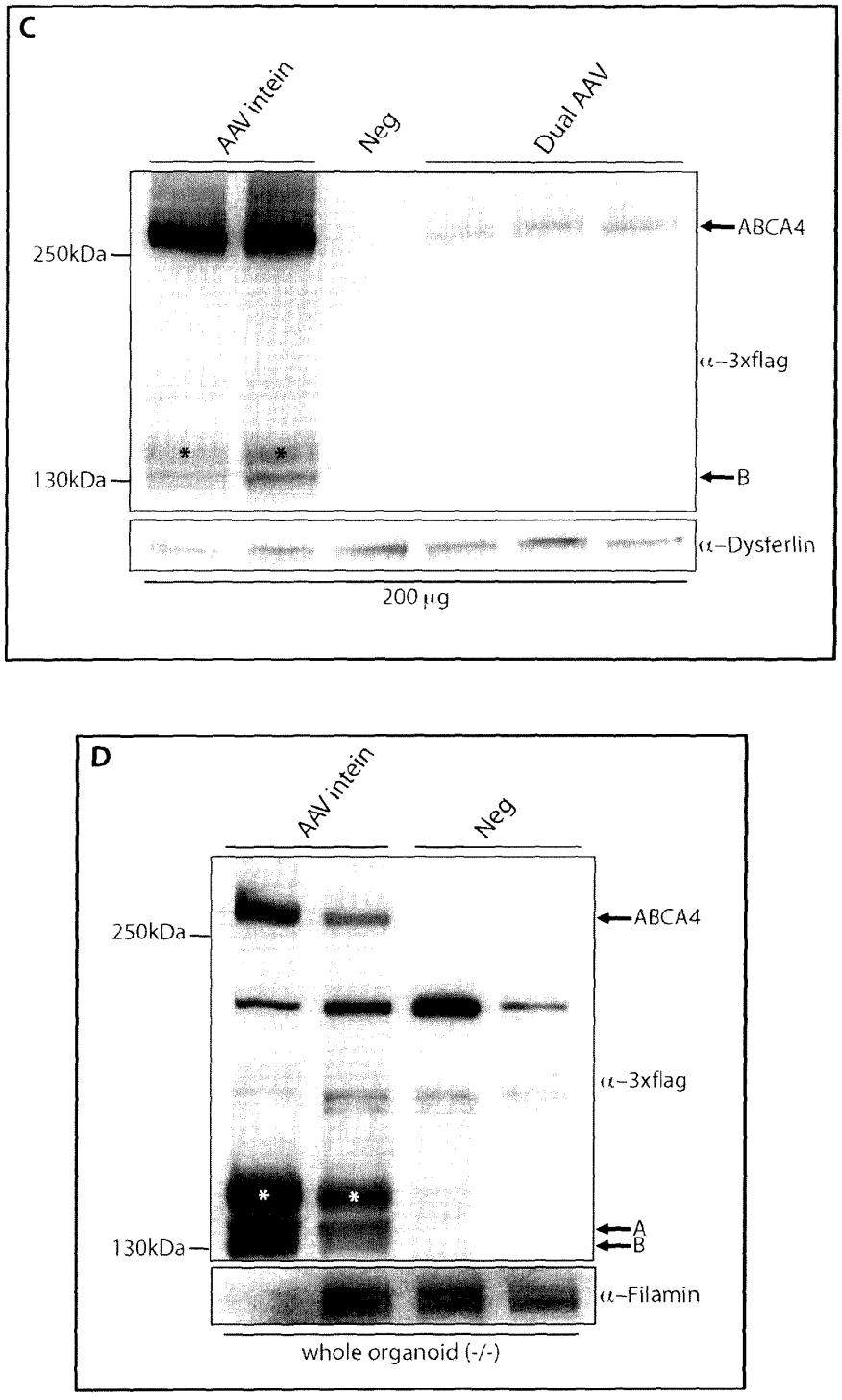

C
AAV intein
Neg
Dual AAV
250kDa
130kDa
ABCA4
α-3xflag
B
α-Dysferlin
200 µg
D
AAV intein
Neg
250kDa
130kDa
ABCA4
α-3xflag
A
B
α-Filamin
whole organoid (-/-)

Figure 5

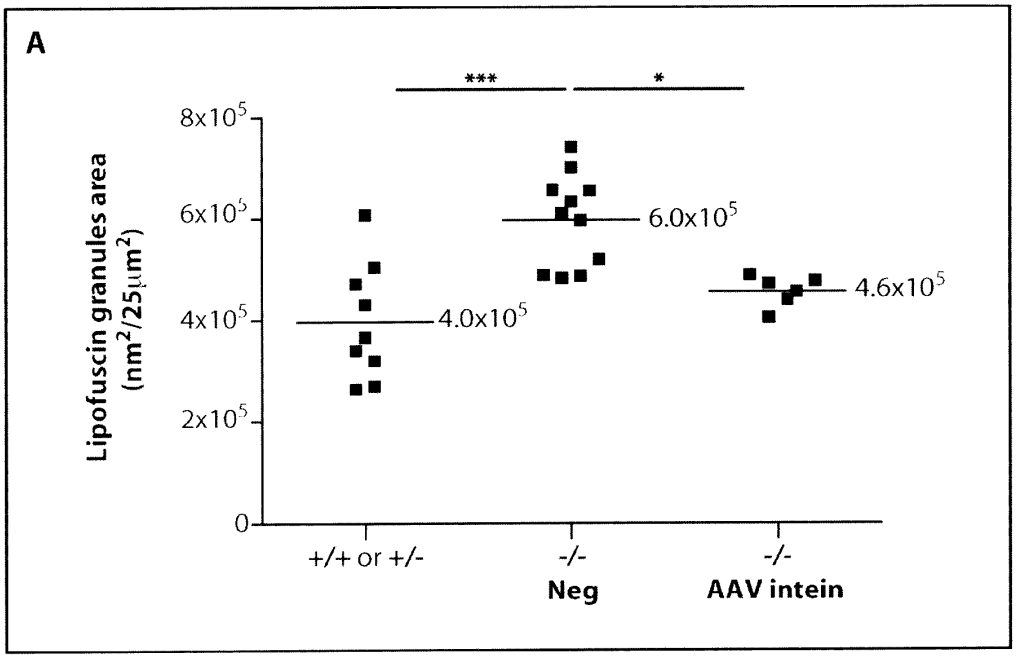

A
***
*
8x10^5
6x10^5
4x10^5
2x10^5
0
Lipofuscin granules area
(nm^2/25μm^2)
4.0x10^5
6.0x10^5
4.6x10^5
+/+ or +/-
-/-
Neg
-/-
AAV intein

Figure 5

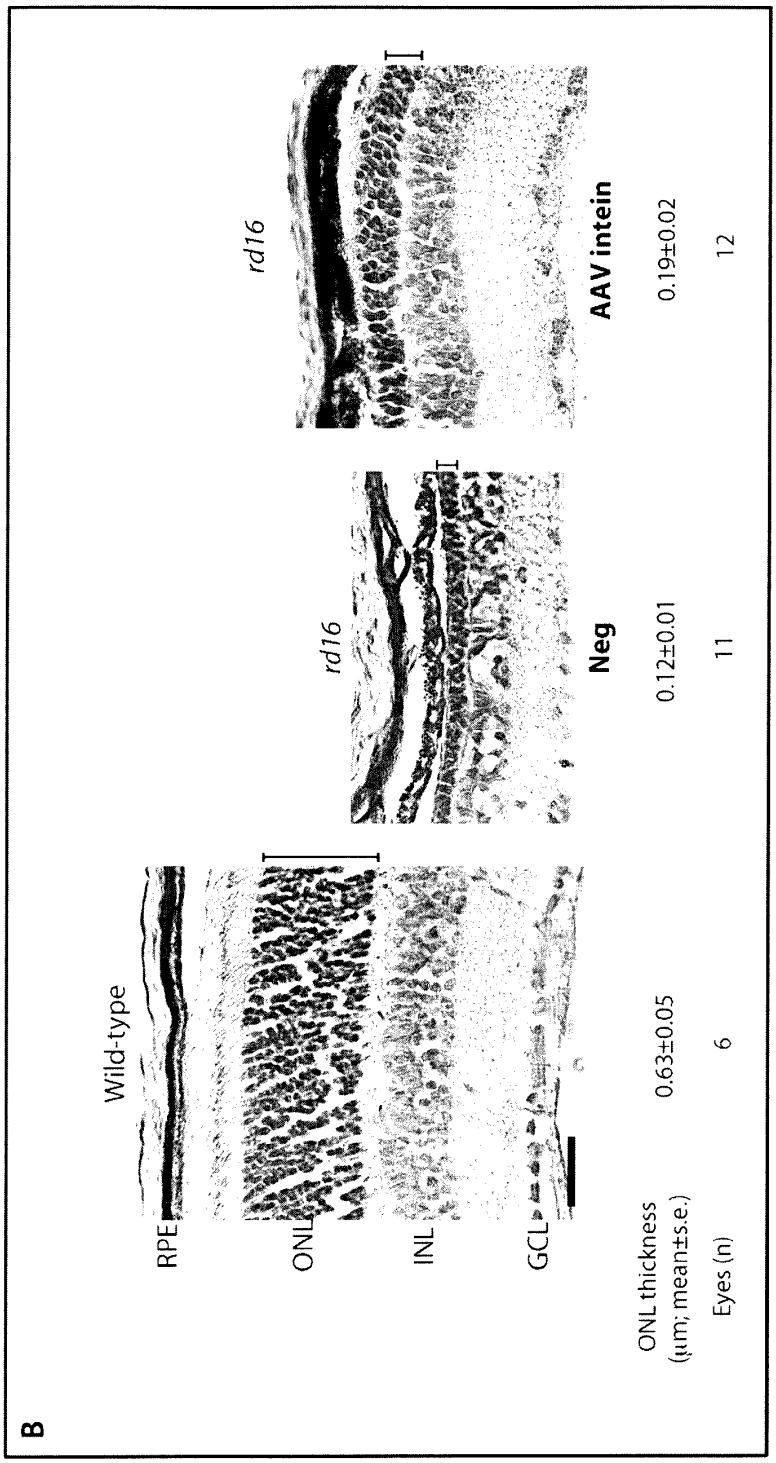

B
Wild-type
RPE
ONL
INL
GCL
rd16
Neg
rd16
AAV intein
ONL thickness (µm; mean±s.e.)
0.63±0.05
0.12±0.01
0.19±0.02
Eyes (n)
6
11
12

Figure 5

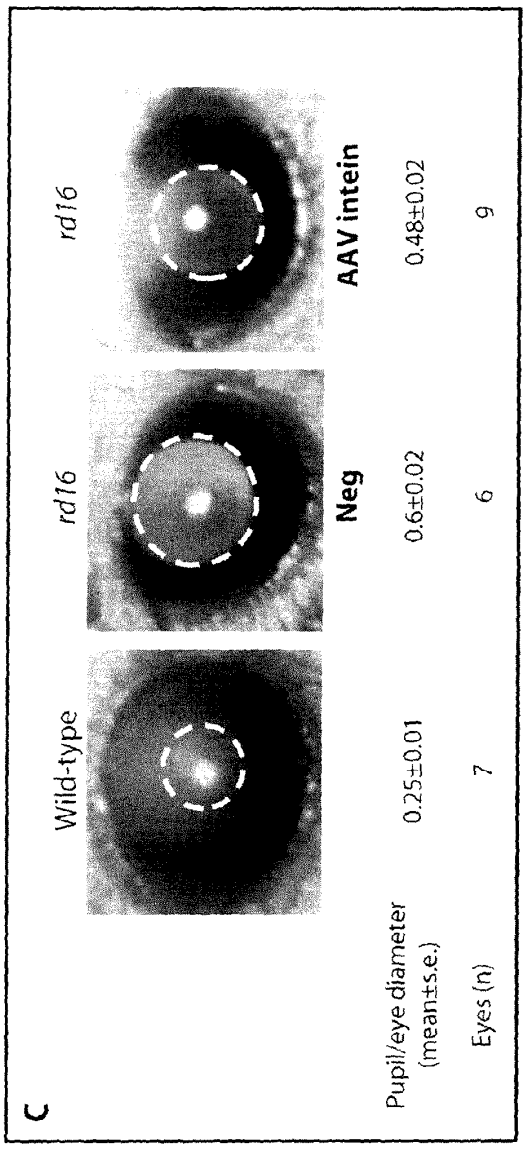
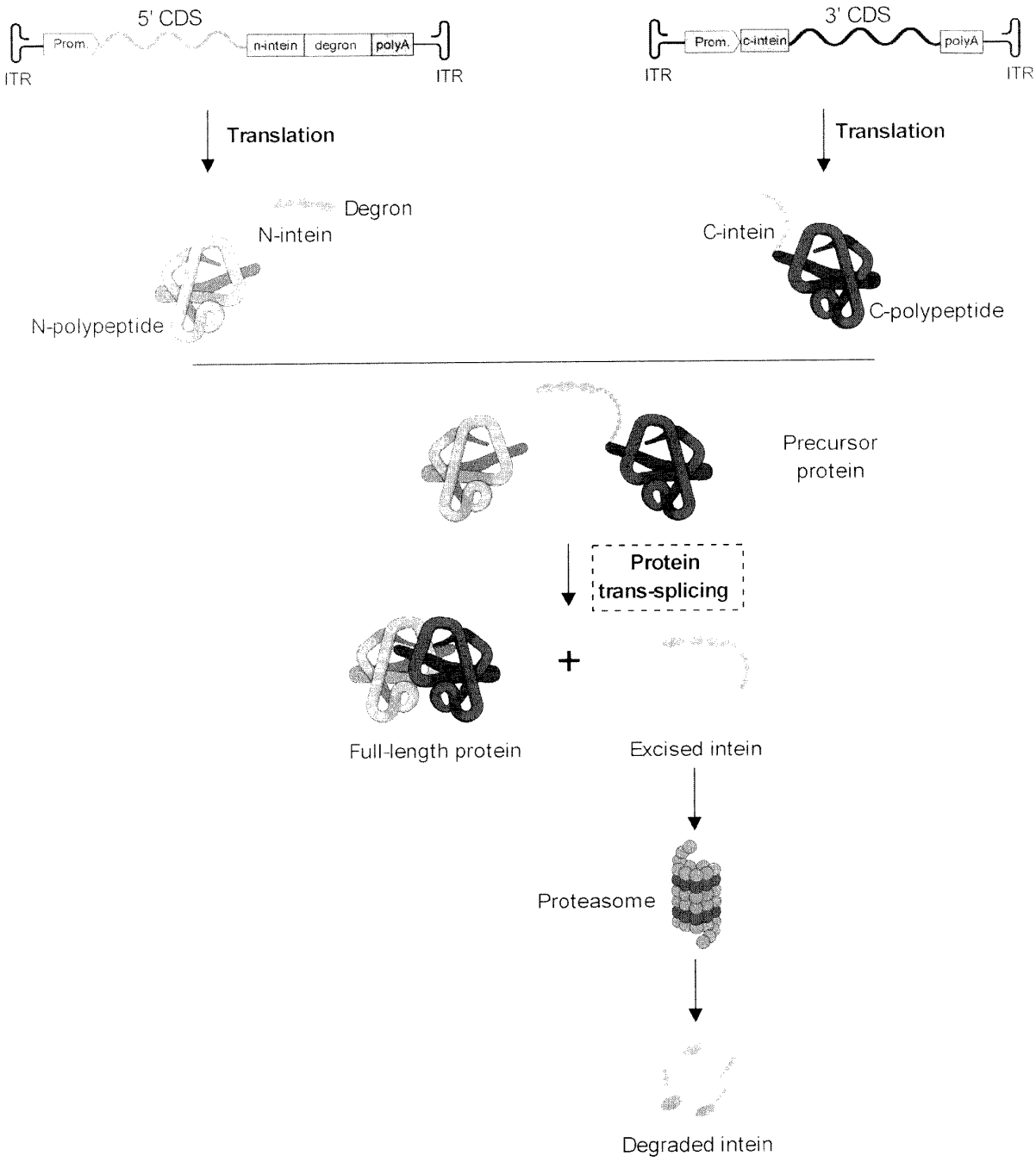

C
Wild-type
rd16
rd16
Neg
AAV intein
Pupil/eye diameter (mean±s.e.)
0.25±0.01
0.6±0.02
0.48±0.02
Eyes (n)
7
6
9

Figure 6


5' CDS
Prom.
n-intein
degron
polyA
ITR
ITR
3' CDS
Prom.
c-intein
polyA
ITR
ITR
Translation
Translation
Degron
N-intein
N-polypeptide
C-intein
C-polypeptide
Precursor protein
Protein trans-splicing
+
Full-length protein
Excised intein
Proteasome
Degraded intein

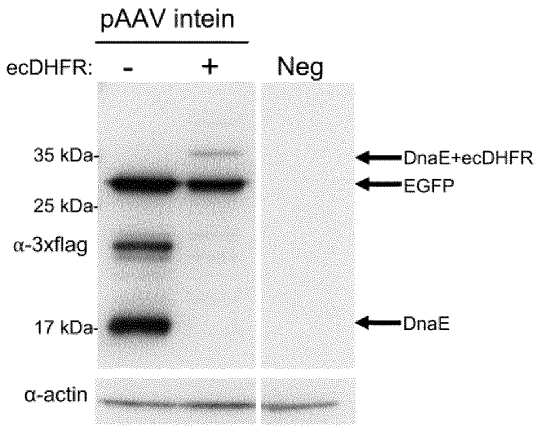

pAAV intein
ecDHFR: - + Neg
35 kDa-
25 kDa-
α-3xflag
17 kDa-
α-actin
DnaE+ecDHFR
EGFP
DnaE

Fig. 7

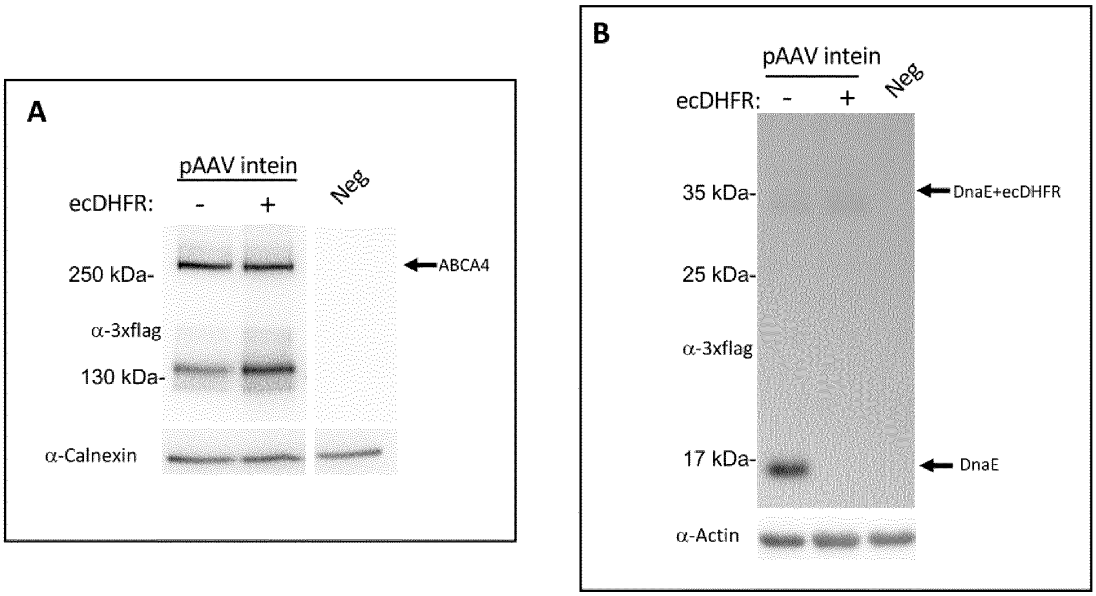

A
pAAV intein
ecDHFR: - +
Neg
250 kDa-
ABCA4
α-3xflag
130 kDa-
α-Calnexin
B
pAAV intein
ecDHFR: - +
Neg
35 kDa-
DnaE+ecDHFR
25 kDa-
α-3xflag
17 kDa-
DnaE
α-Actin

Fig. 8

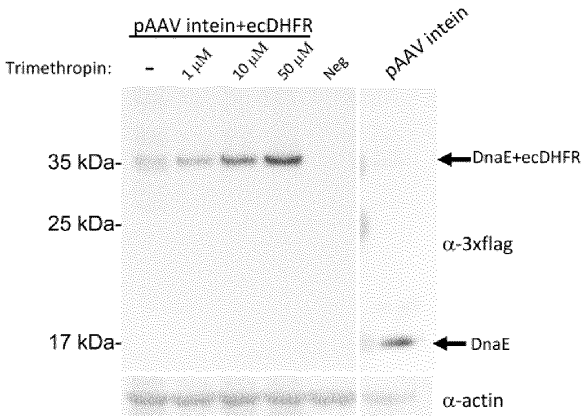

pAAV intein+ecDHFR
Trimethropin:
-
1 µM
10 µM
50 µM
Neg
pAAV intein
35 kDa-
25 kDa-
17 kDa-
DnaE+ecDHFR
α-3xflag
DnaE
α-actin

Fig. 9

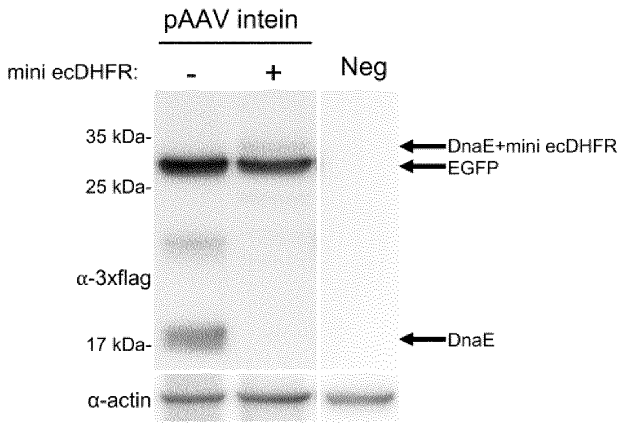

pAAV intein
mini ecDHFR:
-
+
Neg
35 kDa-
25 kDa-
α-3xflag
17 kDa-
α-actin
DnaE+mini ecDHFR
EGFP
DnaE

Fig. 10

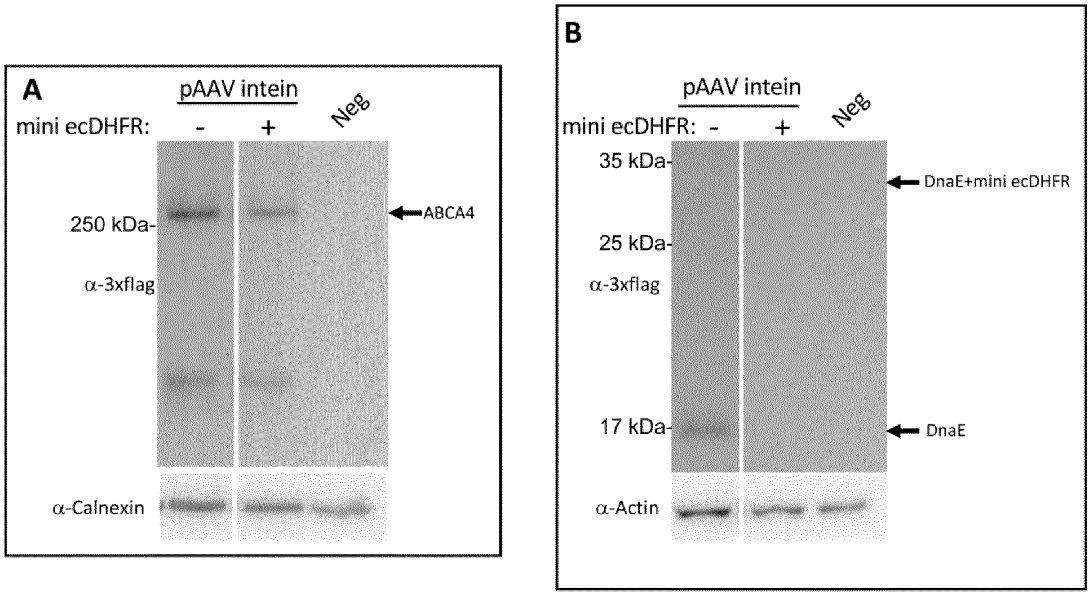

A
pAAV intein
mini ecDHFR: - +
Neg
250 kDa-
α-3xflag
ABCA4
α-Calnexin
B
pAAV intein
mini ecDHFR: - +
Neg
35 kDa-
25 kDa-
α-3xflag
DnaE+mini ecDHFR
17 kDa-
DnaE
α-Actin

Fig. 11

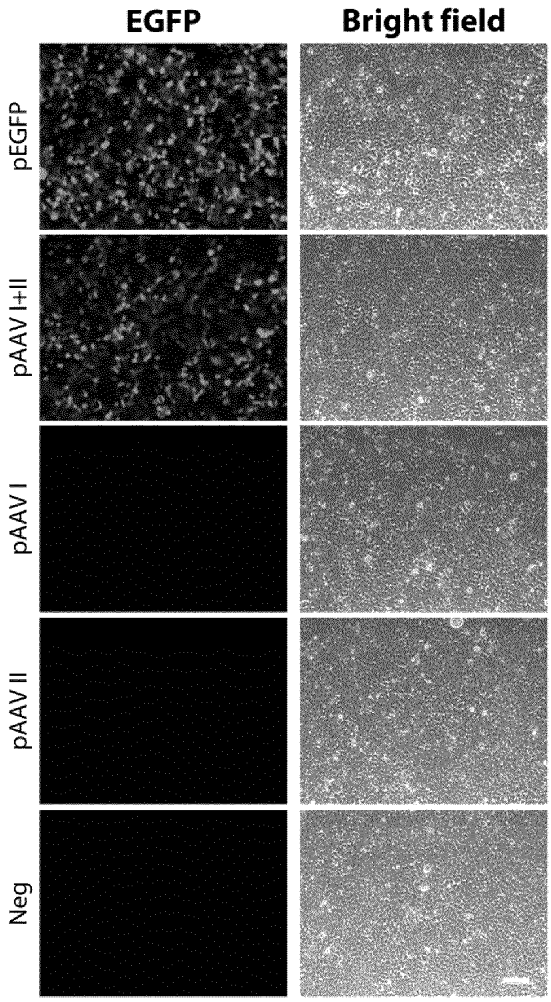


Fig. 12

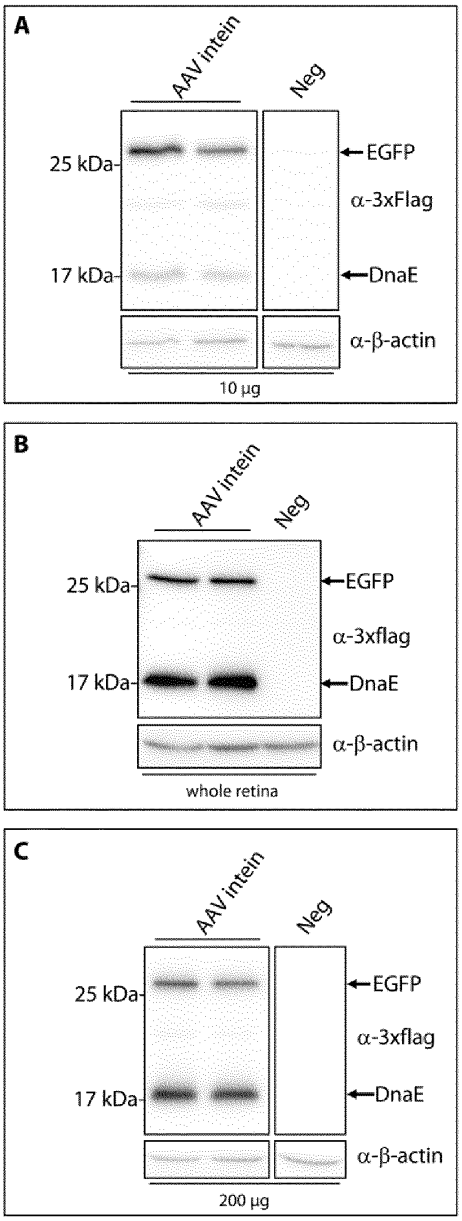

A
AAV intein
Neg
25 kDa
17 kDa
EGFP
α-3xFlag
DnaE
α-β-actin
10 µg
B
AAV intein
Neg
25 kDa
17 kDa
EGFP
α-3xflag
DnaE
α-β-actin
whole retina
C
AAV intein
Neg
25 kDa
17 kDa
EGFP
α-3xflag
DnaE
α-β-actin
200 µg

Fig. 13

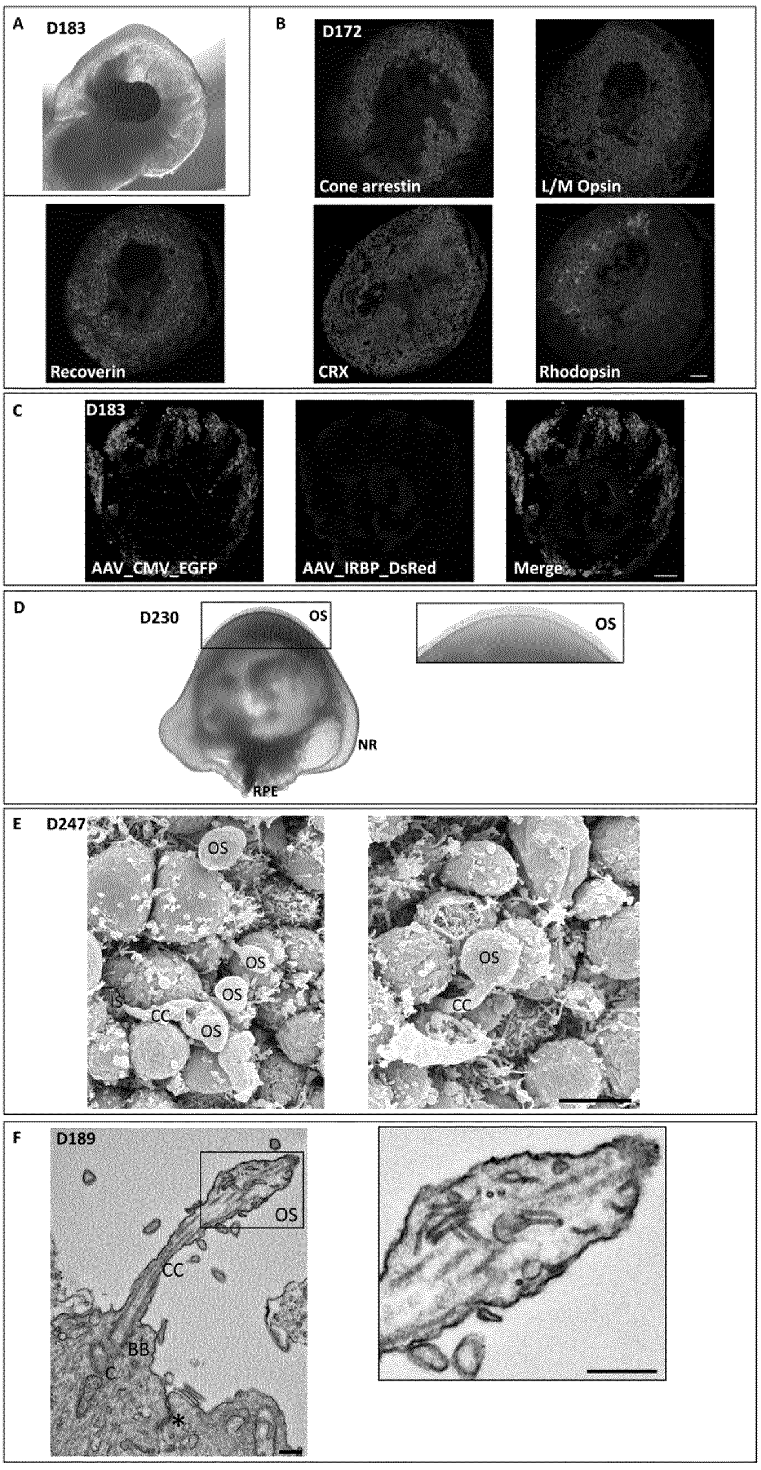

A
D183
B
D172
Cone arrestin
L/M Opsin
Recoverin
CRX
Rhodopsin
C
D183
AAV_CMV_EGFP
AAV_IRBP_DsRed
Merge
D
D230
OS
NR
RPE
OS
E
D247
OS
CC
F
D189
OS
CC
BB
C
*

Fig. 14

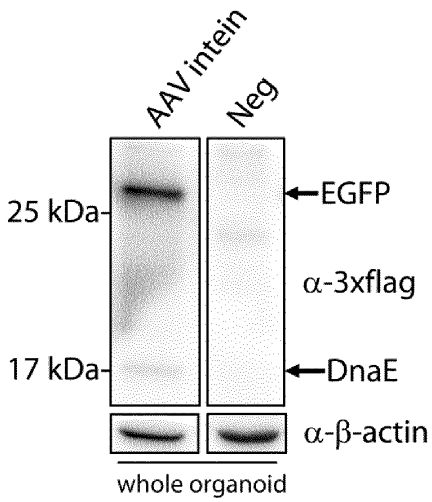

AAV intein
Neg
25 kDa
17 kDa
EGFP
α-3xflag
DnaE
α-β-actin
whole organoid

Fig. 15

Figure 16

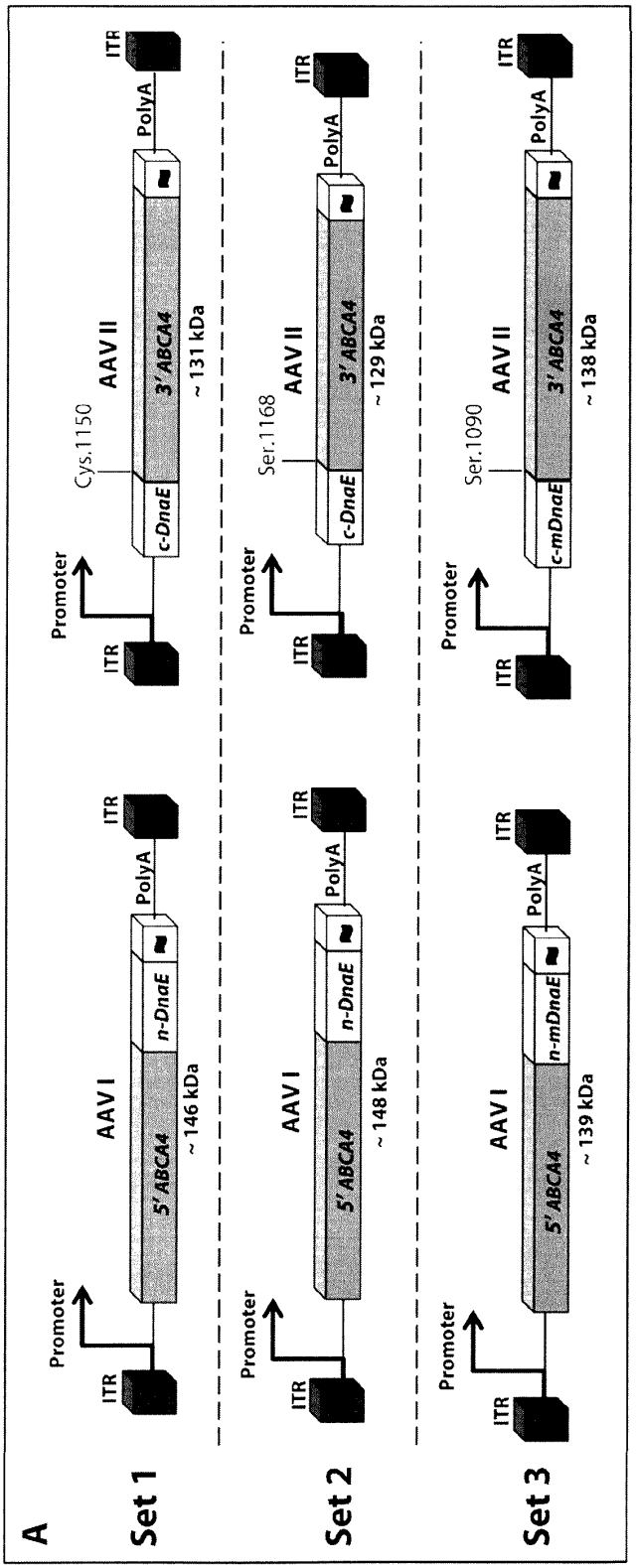
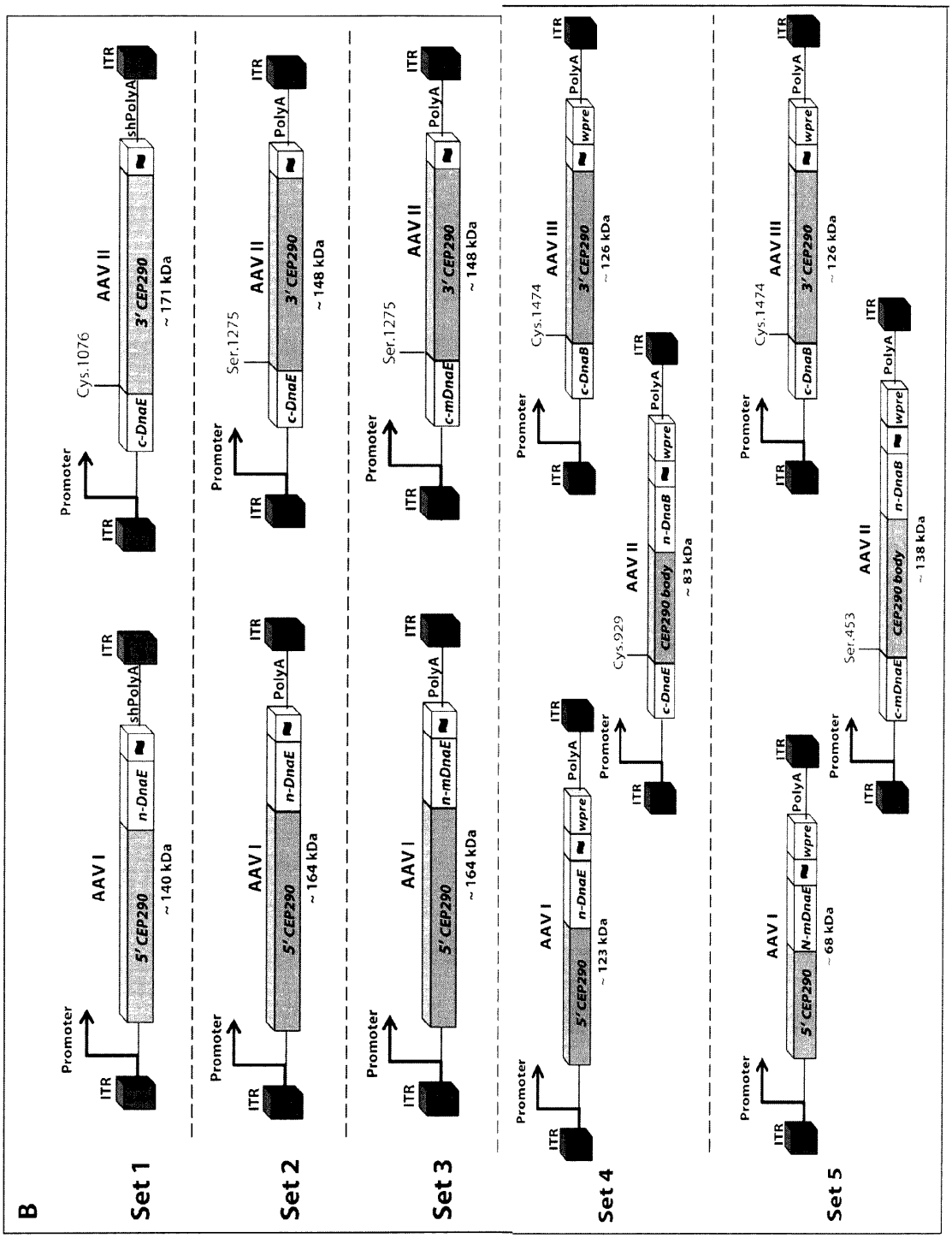

A
Set 1
AAV I
ITR
Promoter
5' ABCA4
n-DnaE
PolyA
ITR
~ 146 kDa
AAV II
ITR
Promoter
Cys.1150
c-DnaE
3' ABCA4
PolyA
ITR
~ 131 kDa
Set 2
AAV I
ITR
Promoter
5' ABCA4
n-DnaE
PolyA
ITR
~ 148 kDa
AAV II
ITR
Promoter
Ser.1168
c-DnaE
3' ABCA4
PolyA
ITR
~ 129 kDa
Set 3
AAV I
ITR
Promoter
5' ABCA4
n-mDnaE
PolyA
ITR
~ 139 kDa
AAV II
ITR
Promoter
Ser.1090
c-mDnaE
3' ABCA4
PolyA
ITR
~ 138 kDa

Figure 16


B
Set 1
AAV I
Promoter
ITR
5' CEP290
n-DnaE
shPolyA
~ 140 kDa
AAV II
Cys.1076
c-DnaE
3' CEP290
~ 171 kDa
Set 2
Ser.1275
~ 164 kDa
~ 148 kDa
Set 3
Set 4
Cys.1474
AAV III
c-DnaB
wpre
~ 123 kDa
Cys.929
CEP290 body
n-DnaB
~ 83 kDa
~ 126 kDa
Set 5
N-mDnaE
~ 68 kDa
Ser.453
c-mDnaE
~ 138 kDa

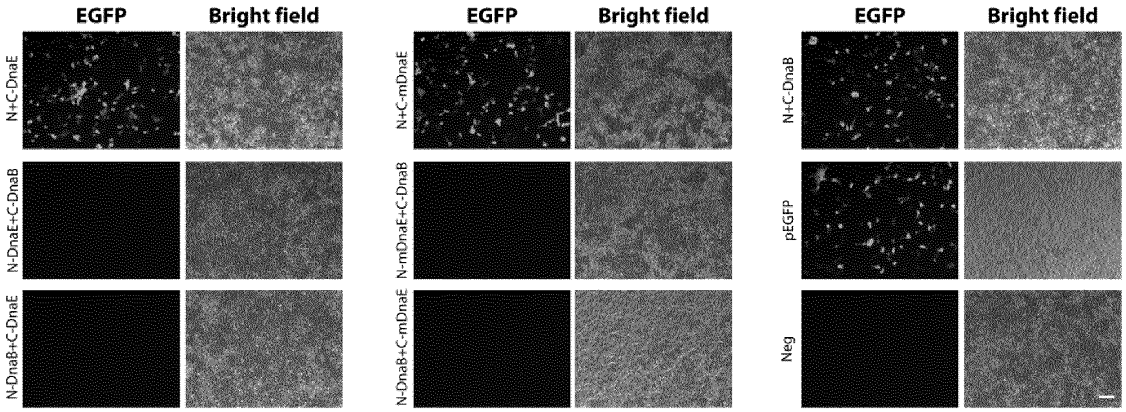

EGFP
Bright field
N+C-DnaE
N-DnaE+C-DnaB
N-DnaB+C-DnaE
N+C-mDnaE
N-mDnaE+C-DnaB
N-DnaB+C-mDnaE
N+C-DnaB
pEGFP
Neg

Fig. 17

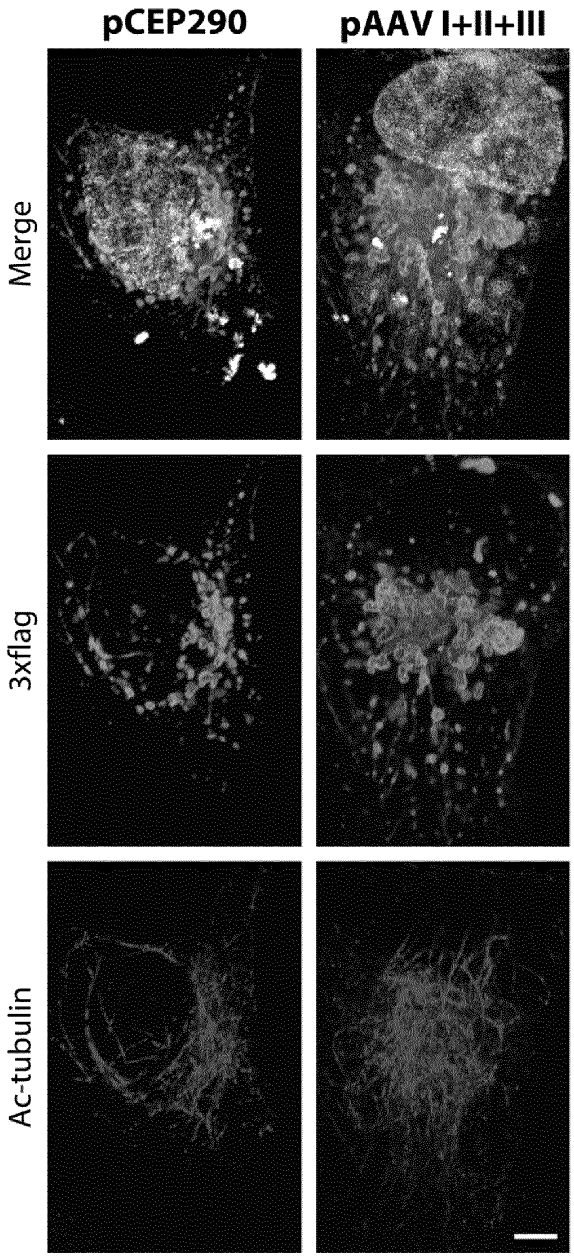


Fig. 18

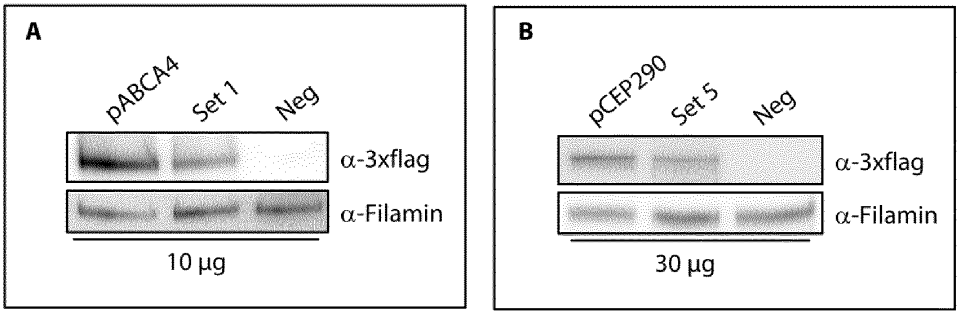

A
pABCA4
Set 1
Neg
α-3xflag
α-Filamin
10 µg
B
pCEP290
Set 5
Neg
α-3xflag
α-Filamin
30 µg

Fig. 19

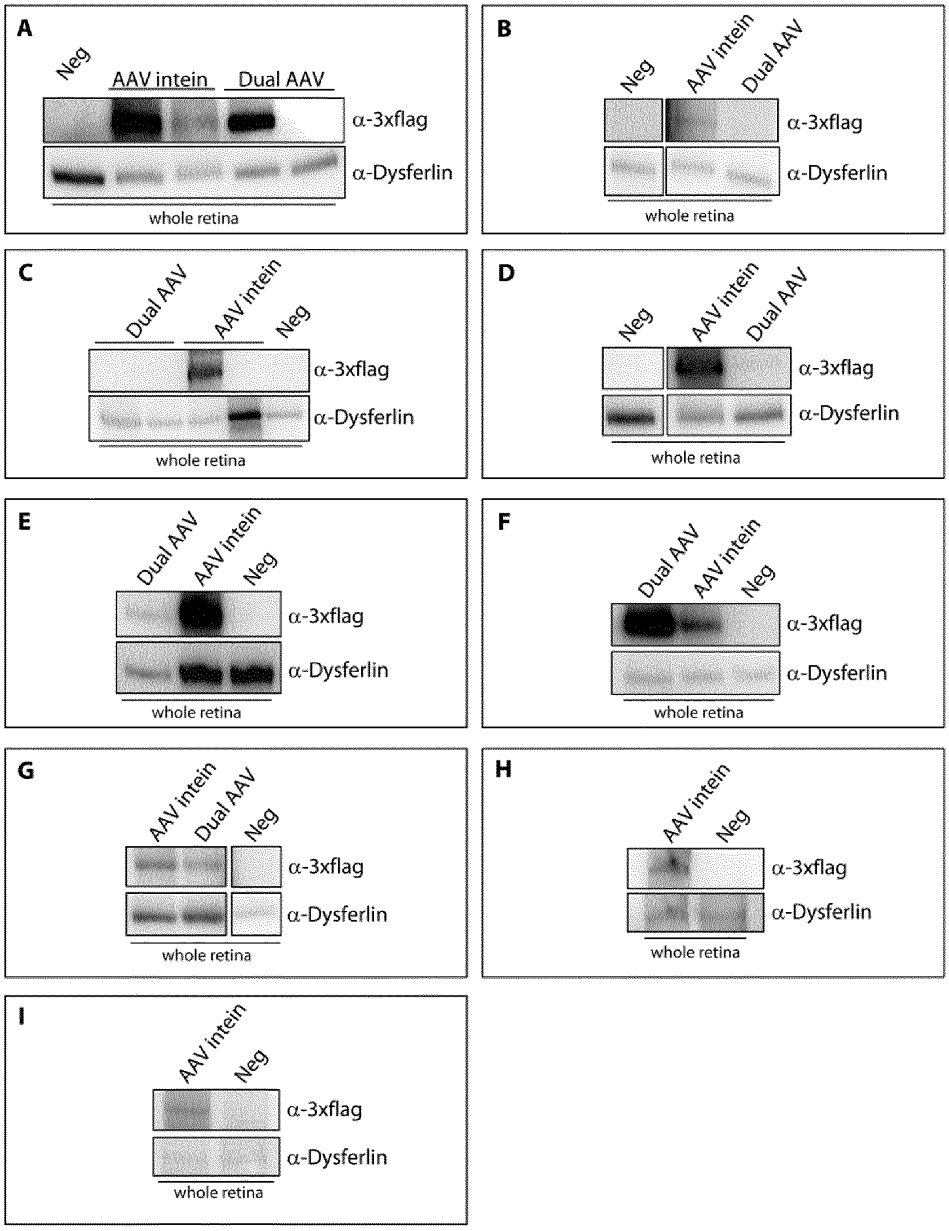

A
Neg
AAV intein
Dual AAV
α-3xflag
α-Dysferlin
whole retina
B
Neg
AAV intein
Dual AAV
α-3xflag
α-Dysferlin
whole retina
C
Dual AAV
AAV intein
Neg
α-3xflag
α-Dysferlin
whole retina
D
Neg
AAV intein
Dual AAV
α-3xflag
α-Dysferlin
whole retina
E
Dual AAV
AAV intein
Neg
α-3xflag
α-Dysferlin
whole retina
F
Dual AAV
AAV intein
Neg
α-3xflag
α-Dysferlin
whole retina
G
AAV intein
Dual AAV
Neg
α-3xflag
α-Dysferlin
whole retina
H
AAV intein
Neg
α-3xflag
α-Dysferlin
whole retina
I
AAV intein
Neg
α-3xflag
α-Dysferlin
whole retina

Fig. 20

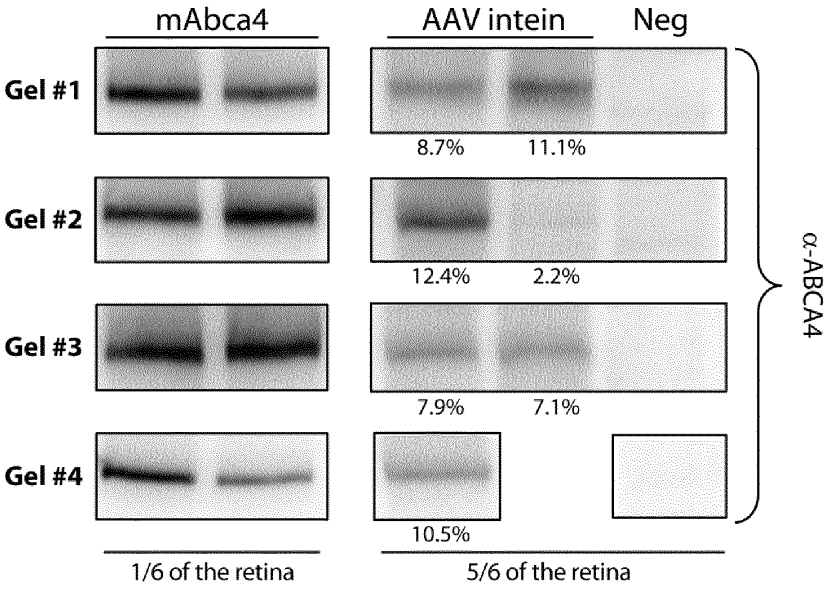

mAbca4
AAV intein
Neg
Gel #1
Gel #2
Gel #3
Gel #4
8.7%
11.1%
12.4%
2.2%
7.9%
7.1%
10.5%
α-ABCA4
1/6 of the retina
5/6 of the retina

Fig. 21

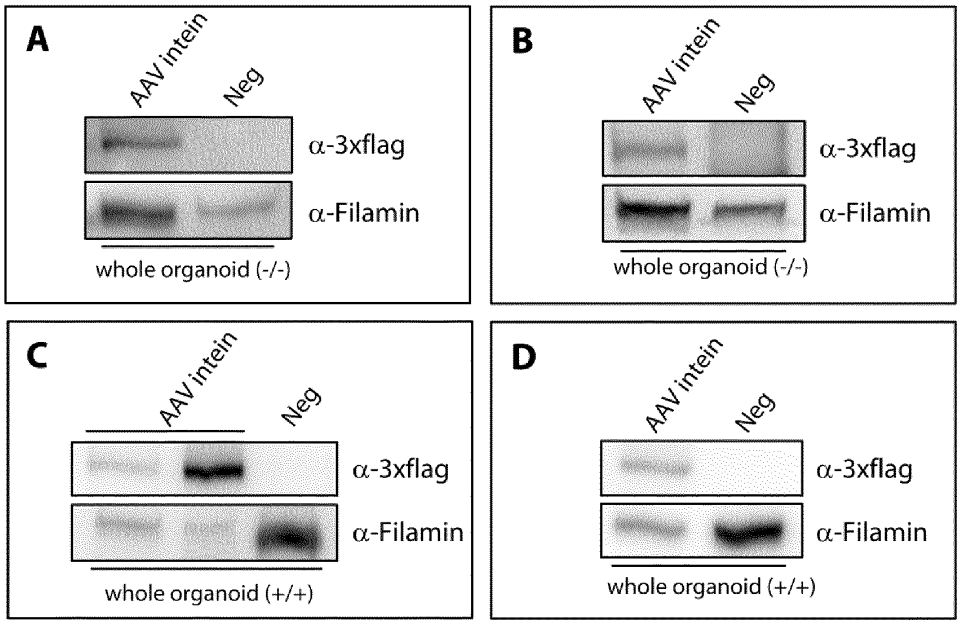

A
AAV intein
Neg
α-3xflag
α-Filamin
whole organoid (-/-)
B
AAV intein
Neg
α-3xflag
α-Filamin
whole organoid (-/-)
C
AAV intein
Neg
α-3xflag
α-Filamin
whole organoid (+/+)
D
AAV intein
Neg
α-3xflag
α-Filamin
whole organoid (+/+)

Fig. 22

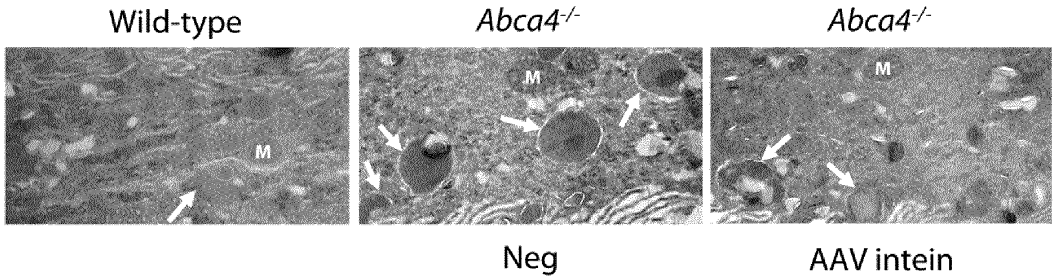


Fig. 23

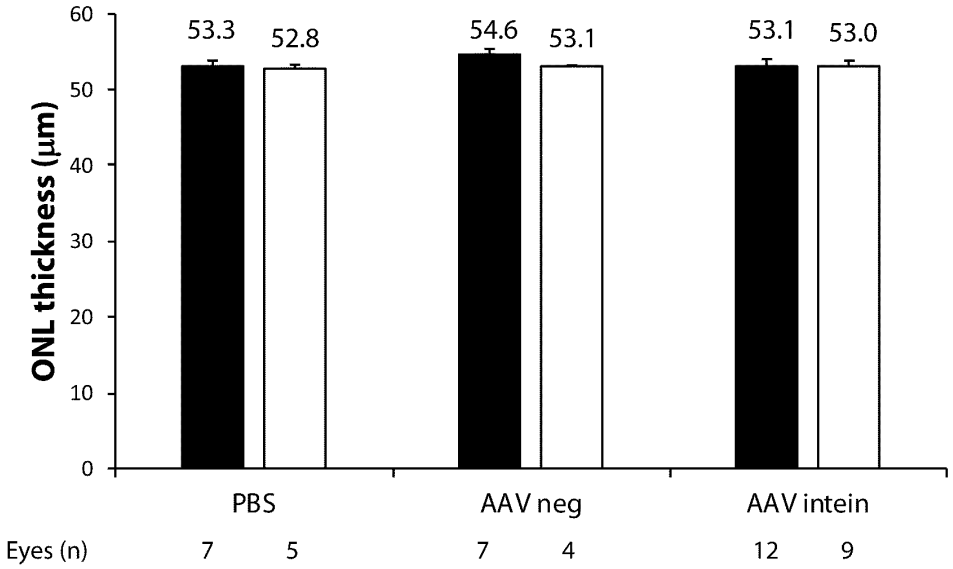

ONL thickness (µm)
60
50
40
30
20
10
0
53.3
52.8
54.6
53.1
53.1
53.0
PBS
AAV neg
AAV intein
Eyes (n)
7
5
7
4
12
9

Fig. 24

Figure 25

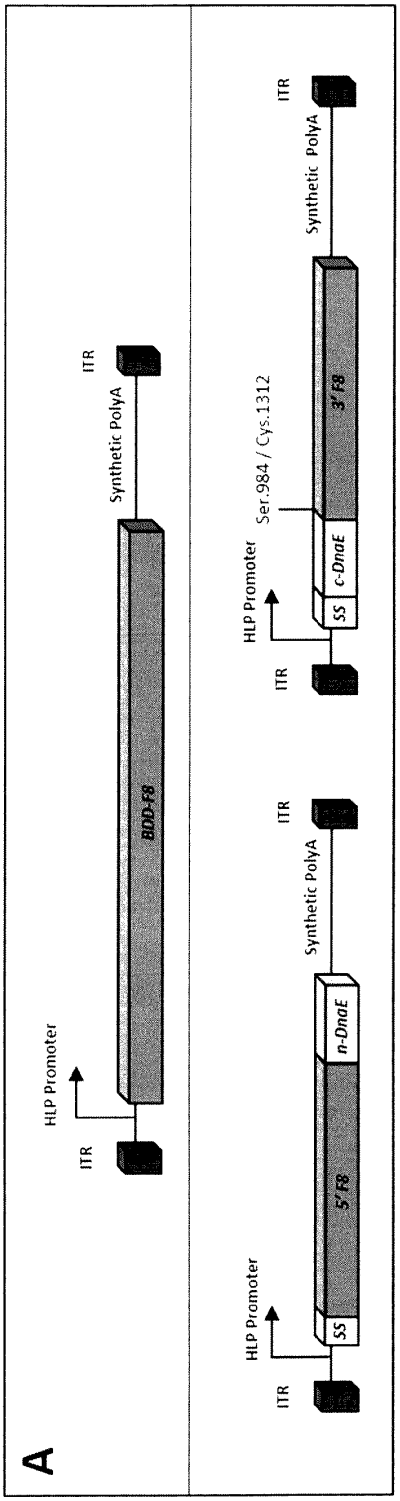

A
HLP Promoter
ITR
BDD-F8
Synthetic PolyA
ITR
HLP Promoter
ITR
SS
5' F8
n-DnaE
Synthetic PolyA
ITR
HLP Promoter
ITR
SS
c-DnaE
Ser.984 / Cys.1312
3' F8
Synthetic PolyA
ITR

Figure 25

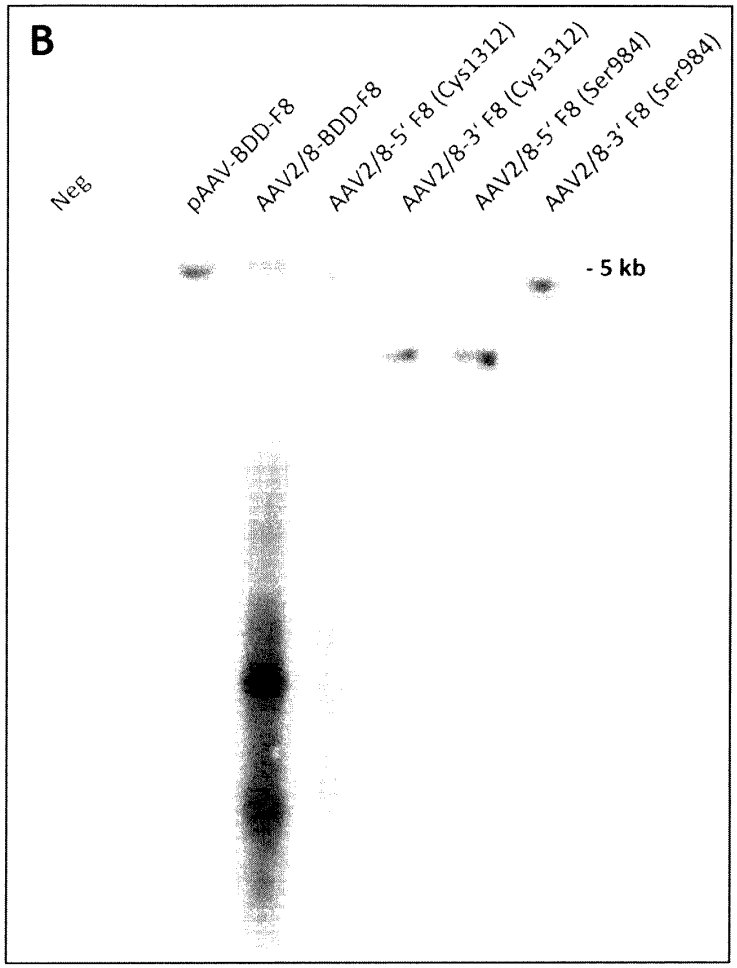
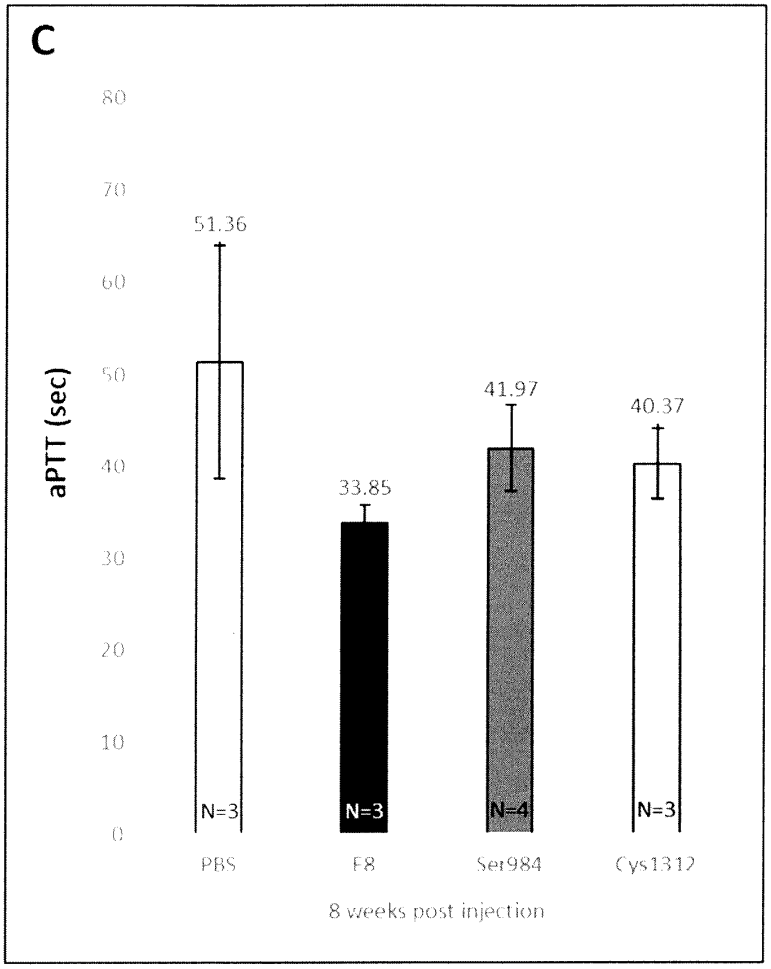

B
Neg
pAAV-BDD-F8
AAV2/8-BDD-F8
AAV2/8-5' F8 (Cys1312)
AAV2/8-3' F8 (Cys1312)
AAV2/8-5' F8 (Ser984)
AAV2/8-3' F8 (Ser984)
- 5 kb

Figure 25


C
aPTT (sec)
80
70
60
50
40
30
20
10
0
51.36
33.85
41.97
40.37
N=3
N=3
N=4
N=3
PBS
F8
Ser984
Cys1312
8 weeks post injection