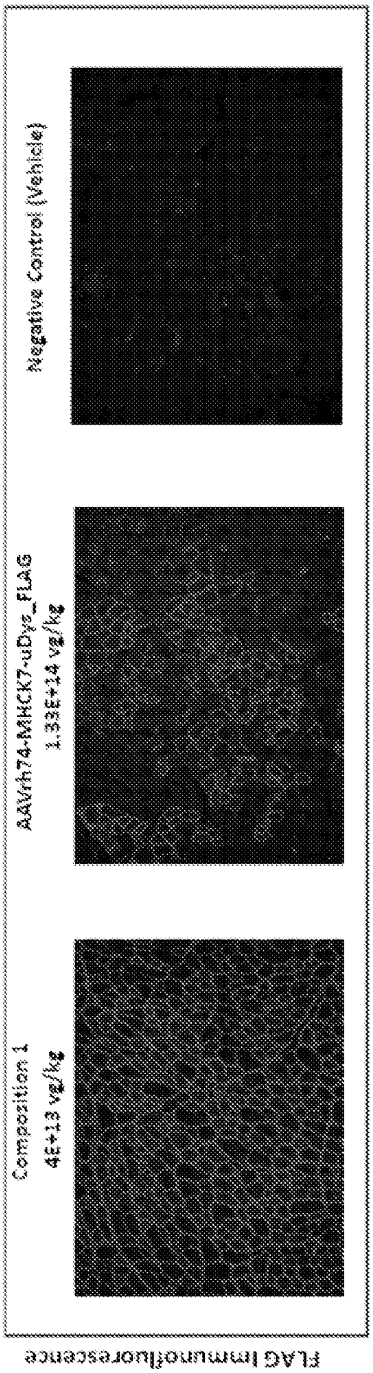

FLAG Immunofluorescence
Composition 1
4E+13 vg/kg
AAVrh74-MHCK7-uDys_FLAG
1.33E+14 vg/kg
Negative Control (Vehicle)

FIG. 1A

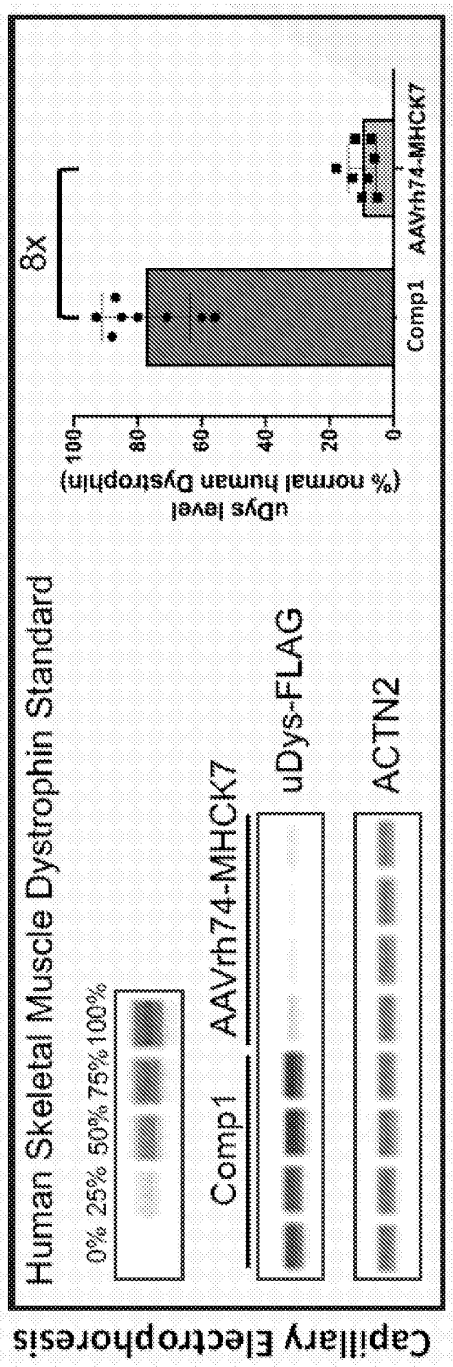

Capillary Electrophoresis
Human Skeletal Muscle Dystrophin Standard
0% 25% 50% 75% 100%
Comp1
AAVrh74-MHCK7
uDys-FLAG
ACTN2
uDys level
(% normal human Dystrophin)
100
80
60
40
20
0
8x
Comp1
AAVrh74-MHCK7

FIG. 1B

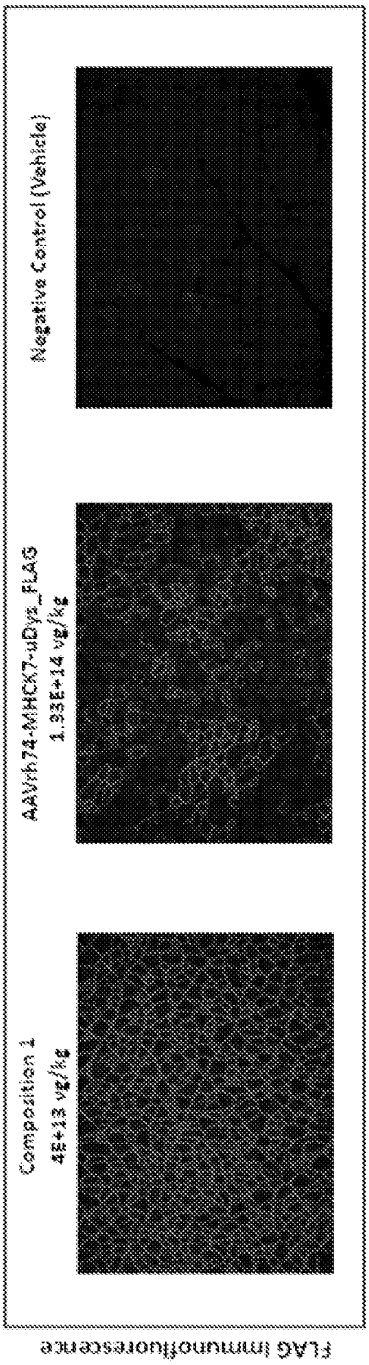

Negative Control (Vehicle)
AAVrh74-MHCK7-uDys_FLAG
1.33E+14 vg/kg
Composition 1
4E+13 vg/kg
FLAG immunofluorescence

FIG. 2A

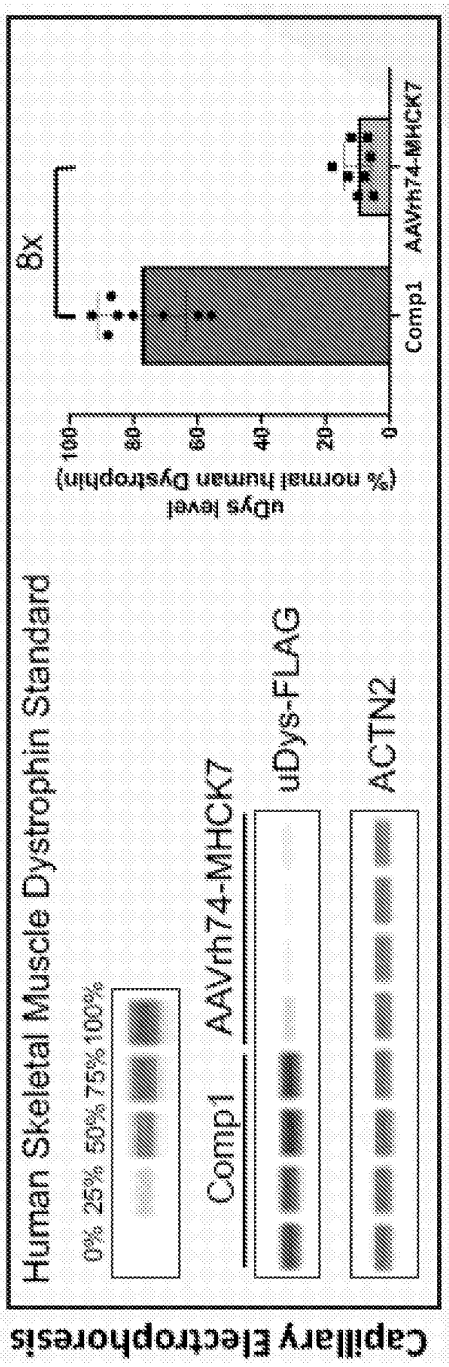

Capillary Electrophoresis
Human Skeletal Muscle Dystrophin Standard
0% 25% 50% 75% 100%
Comp1 AAVrh74-MHCK7
uDys-FLAG
ACTN2
uDys level
(% normal human Dystrophin)
100
80
60
40
20
0
8x
Comp1
AAVrh74-MHCK7

FIG. 2B

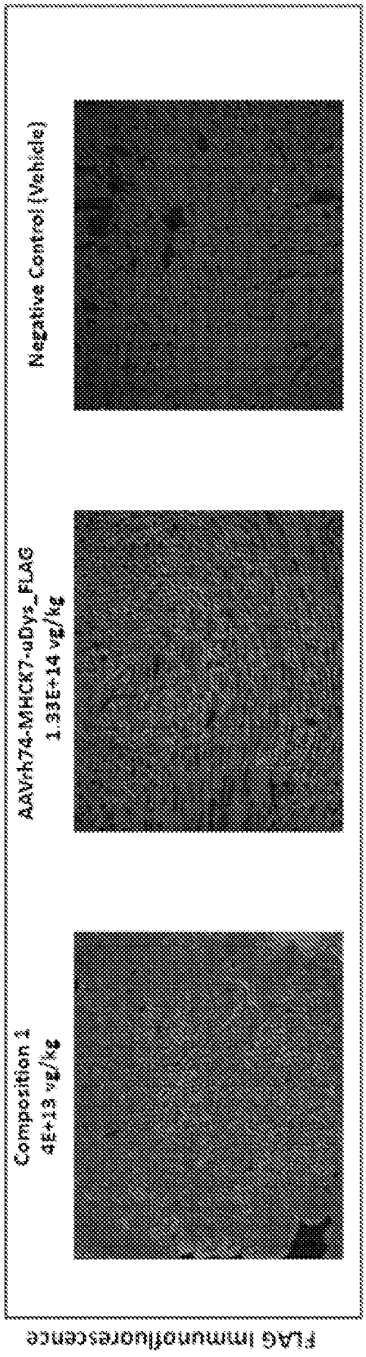

Negative Control (Vehicle)
AAVrh74-MHCK7-uDys_FLAG
1.33E+14 vg/kg
Composition 1
4E+13 vg/kg
FLAG immunofluorescence

FIG. 3A

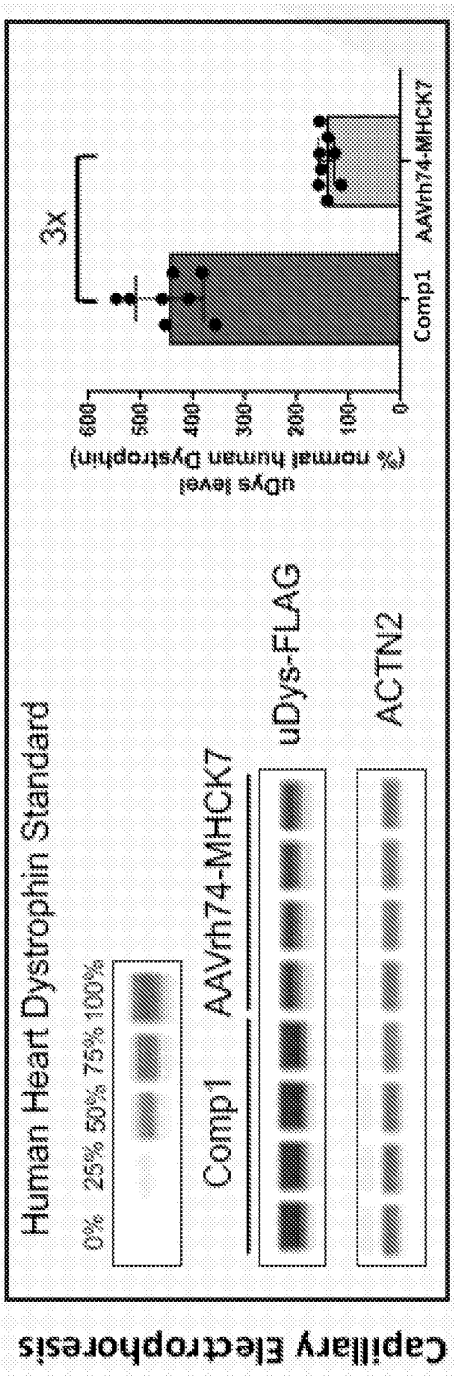

Capillary Electrophoresis
Human Heart Dystrophin Standard
0% 25% 50% 75% 100%
Comp1
AAVrh74-MHCK7
uDys-FLAG
ACTN2
uDys level
(% normal human Dystrophin)
600
500
400
300
200
100
0
3x
Comp1
AAVrh74-MHCK7

FIG. 3B

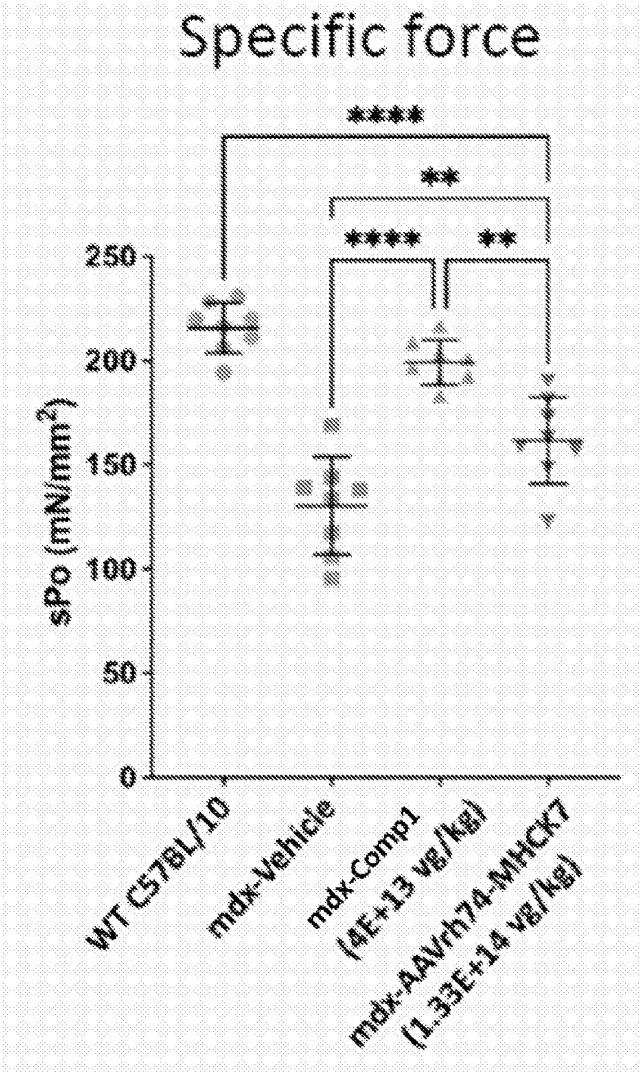

Specific force
****
**
****
**
250
200
150
100
50
0
sPo (mN/mm2)
WT C57BL/10
mdx-Vehicle
mdx-Comp1 (4E+13 vg/kg)
mdx-AAVrh74-MHCK7 (1.33E+14 vg/kg)

FIG. 4A

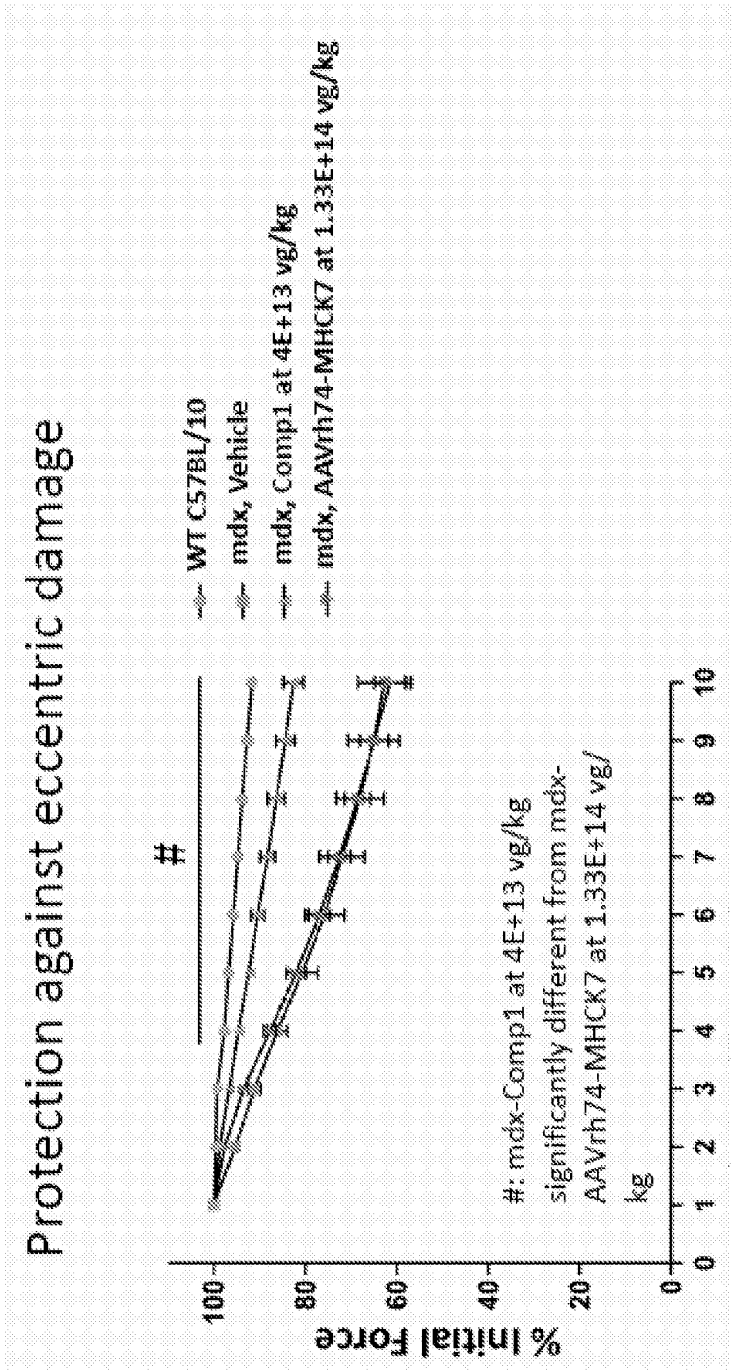

Protection against eccentric damage
% Initial Force
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10
#
WT C57BL/10
mdx, Vehicle
mdx, Comp1 at 4E+13 vg/kg
mdx, AAVrh74-MHCK7 at 1.33E+14 vg/kg
#: mdx-Comp1 at 4E+13 vg/kg significantly different from mdx-AAVrh74-MHCK7 at 1.33E+14 vg/kg

FIG. 4B

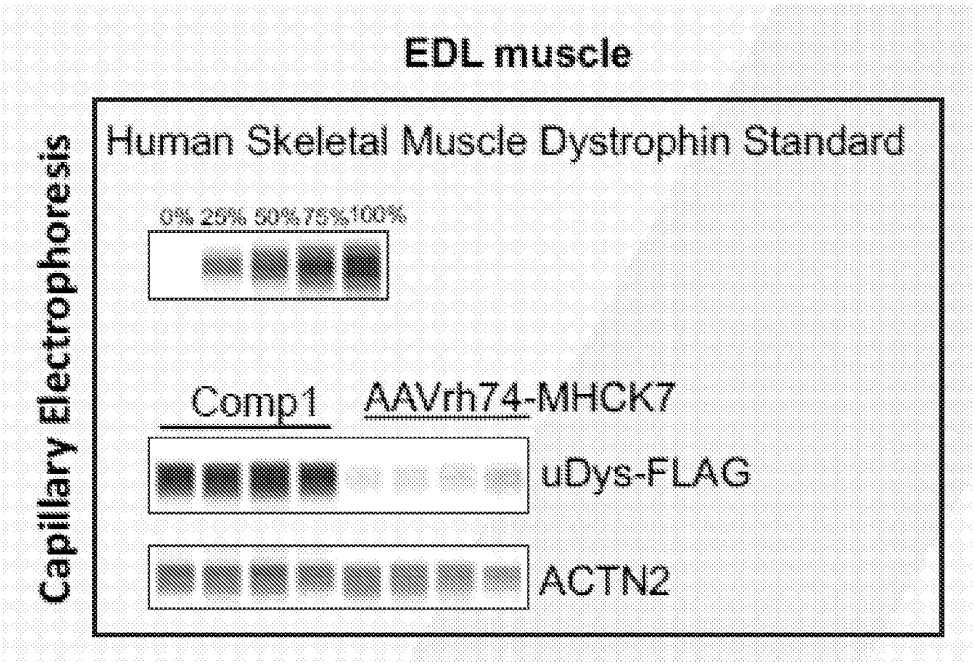

EDL muscle
Capillary Electrophoresis
Human Skeletal Muscle Dystrophin Standard
0% 25% 50% 75% 100%
Comp1
AAVrh74-MHCK7
uDys-FLAG
ACTN2

FIG. 4C

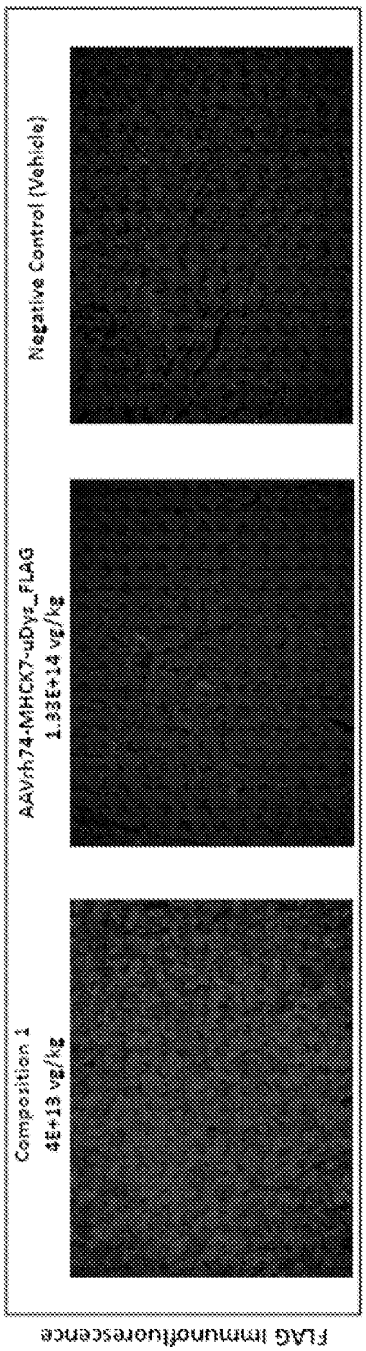

Negative Control (Vehicle)
AAVrh74-MHCK7-uDys_FLAG
1.33E+14 vg/kg
Composition 1
4E+13 vg/kg
FLAG immunofluorescence

FIG. 5A

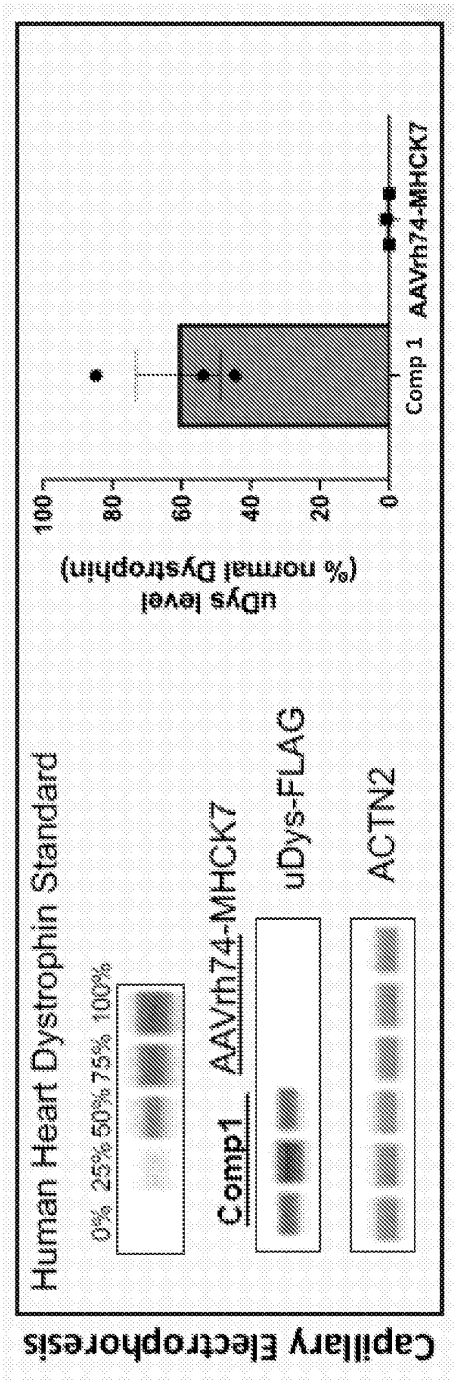

Capillary Electrophoresis
Human Heart Dystrophin Standard
0% 25% 50% 75% 100%
Comp1 AAVrh74-MHCK7 uDys-FLAG
ACTN2
uDys level
(% normal Dystrophin)
100
80
60
40
20
0
Comp 1 AAVrh74-MHCK7

FIG. 5B

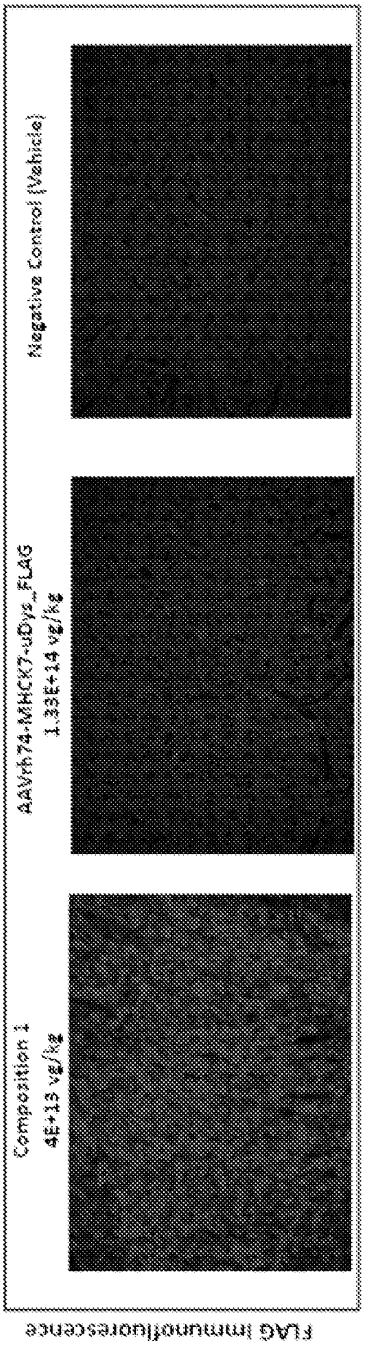

FLAG Immunofluorescence
Composition 1
4E+13 vg/kg
AAVrh74-MHCK7-uDys_FLAG
1.33E+14 vg/kg
Negative Control (Vehicle)

FIG. 6A

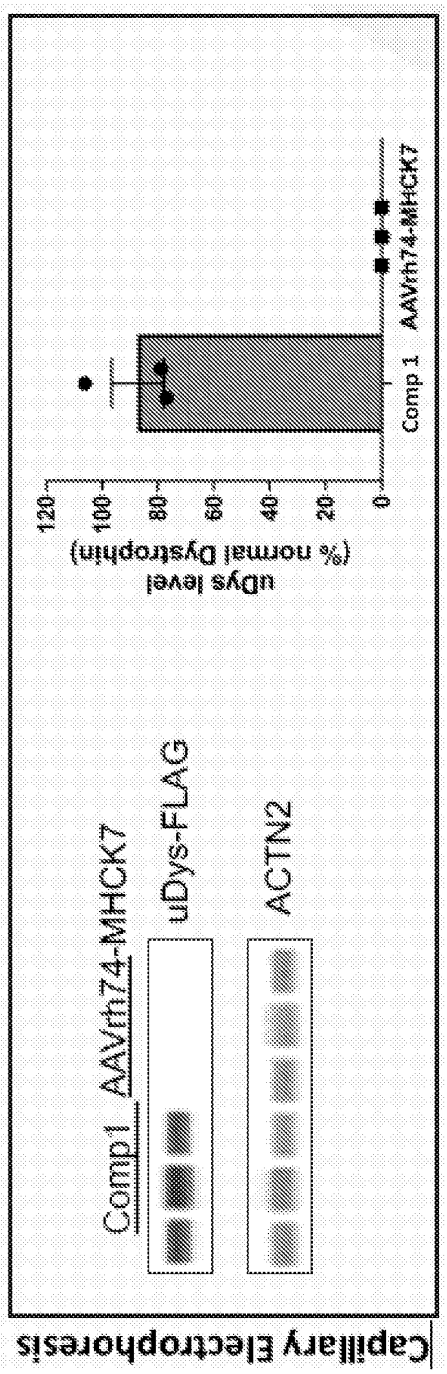

Capillary Electrophoresis
Comp1
AAVrh74-MHCK7
uDys-FLAG
ACTN2
uDys level
(% normal Dystrophin)
120
100
80
60
40
20
0
Comp 1
AAVrh74-MHCK7

FIG. 6B

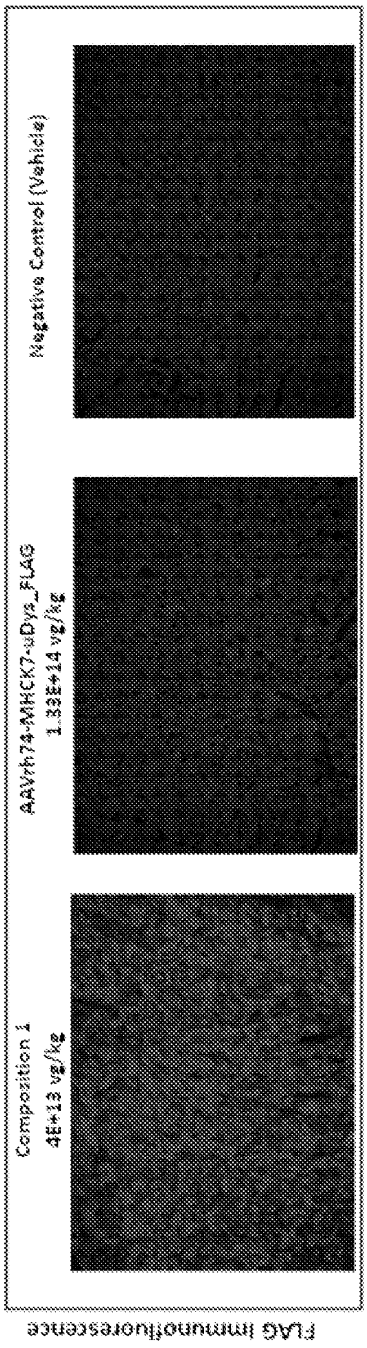

Negative Control (Vehicle)
AAVrh74-MHCK7-uDys_FLAG
1.33E+14 vg/kg
Composition 1
4E+13 vg/kg
FLAG immunofluorescence

FIG. 7A

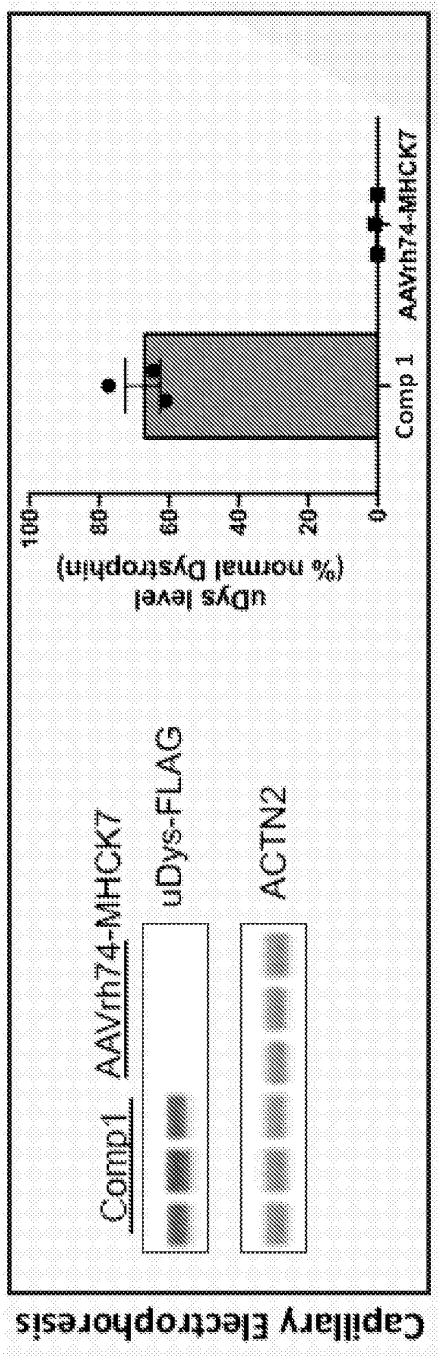

Capillary Electrophoresis
Comp1
AAVrh74-MHCK7
uDys-FLAG
ACTN2
uDys level
(% normal Dystrophin)
100
80
60
40
20
0
Comp 1
AAVrh74-MHCK7

FIG. 7B

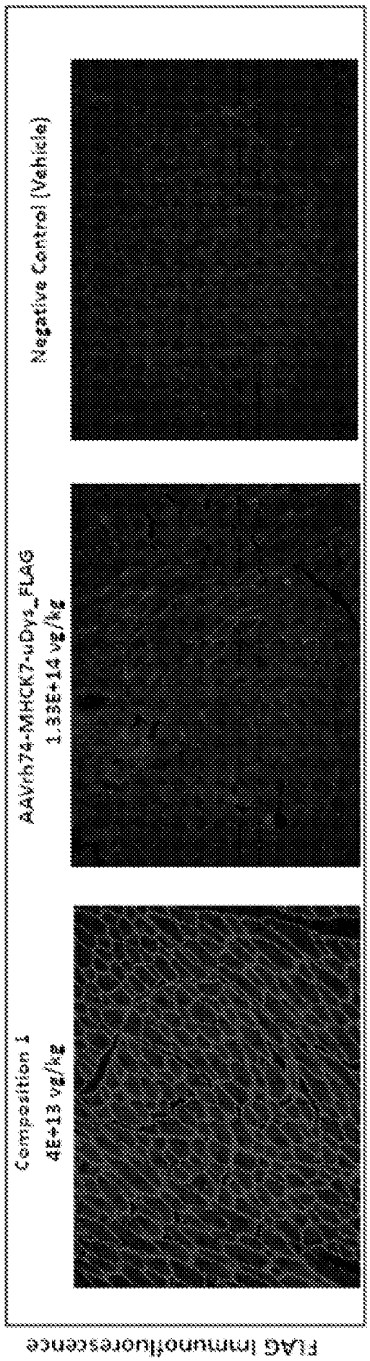

FLAG immunofluorescence
Composition 1
4E+13 vg/kg
AAVrh74-MHCK7-uDys_FLAG
1.33E+14 vg/kg
Negative Control (Vehicle)

FIG. 8A

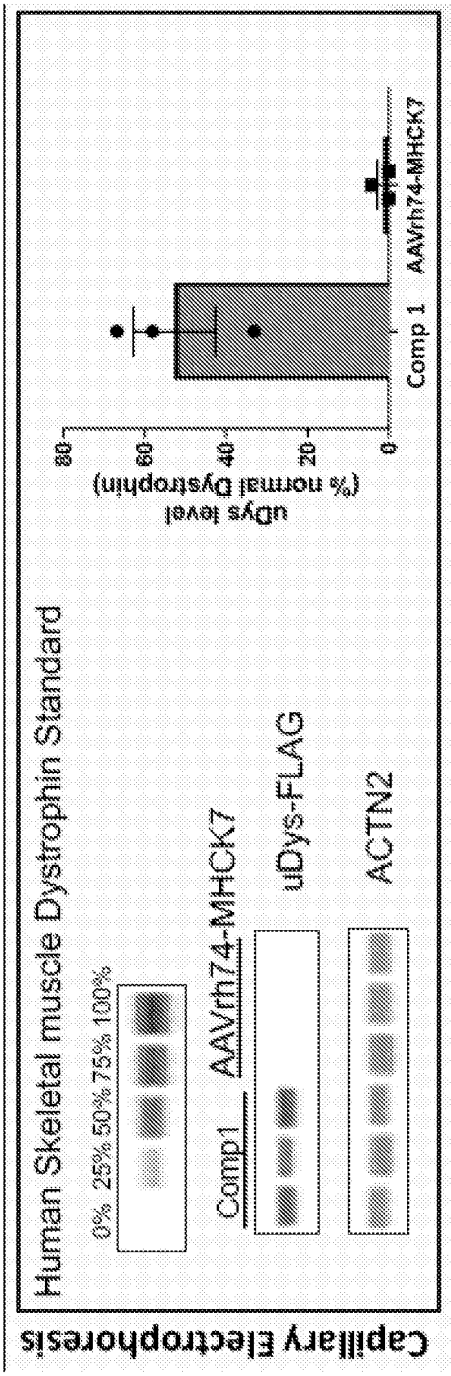

Capillary Electrophoresis
Human Skeletal muscle Dystrophin Standard
0% 25% 50% 75% 100%
Comp1
AAVrh74-MHCK7
uDys-FLAG
ACTN2
uDys level
(% normal Dystrophin)
80
60
40
20
0
Comp 1
AAVrh74-MHCK7

FIG. 8B

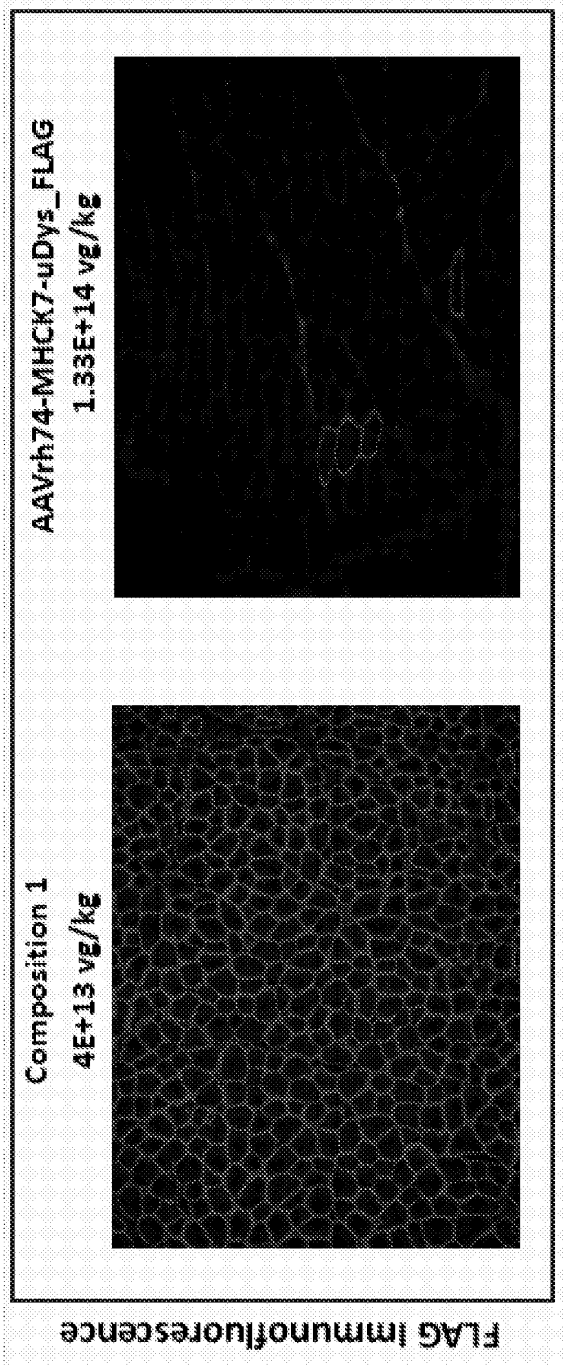

FLAG immunofluorescence
Composition 1
4E+13 vg/kg
AAVrh74-MHCK7-uDys_FLAG
1.33E+14 vg/kg

FIG. 9A

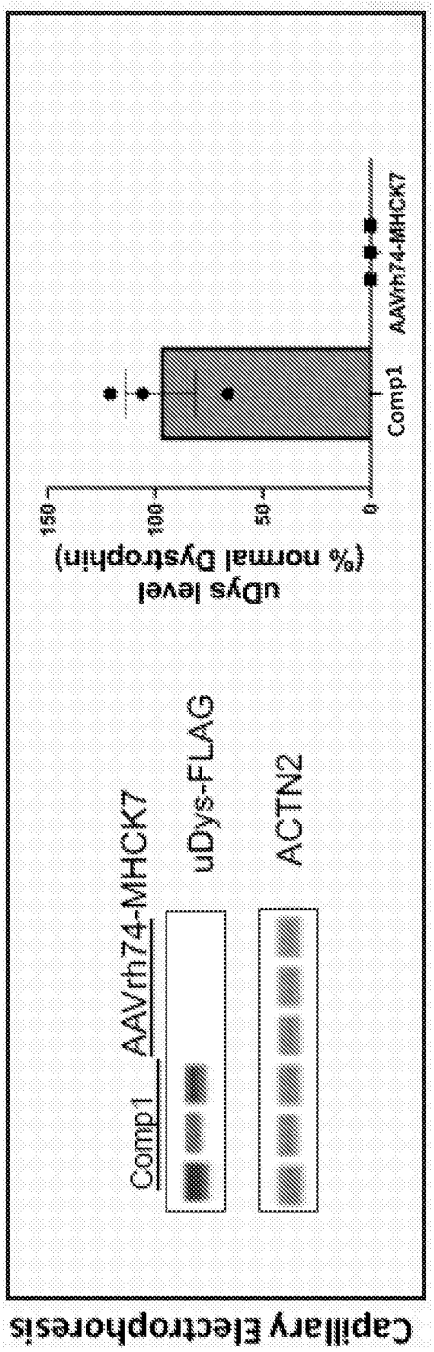

Capillary Electrophoresis
Comp1
AAVrh74-MHCK7
uDys-FLAG
ACTN2
uDys level
(% normal Dystrophin)
150
100
50
0
Comp1
AAVrh74-MHCK7

FIG. 9B

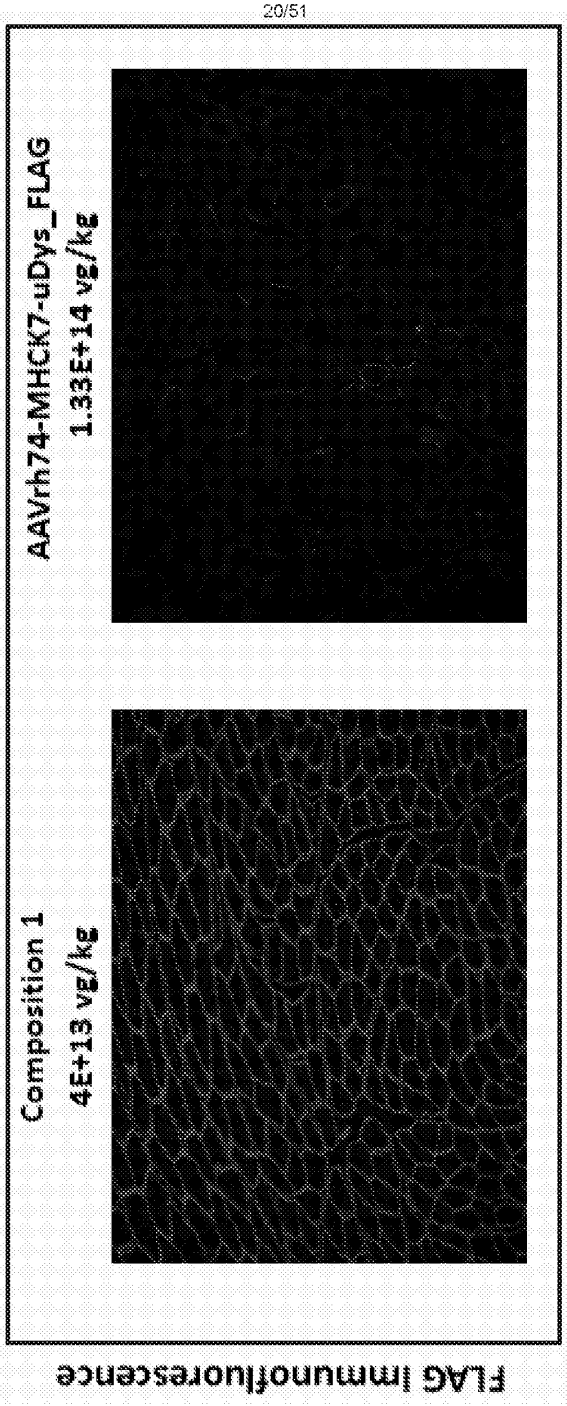

FLAG immunofluorescence
Composition 1
4E+13 vg/kg
AAVrh74-MHCK7-uDys_FLAG
1.33E+14 vg/kg

FIG. 10A

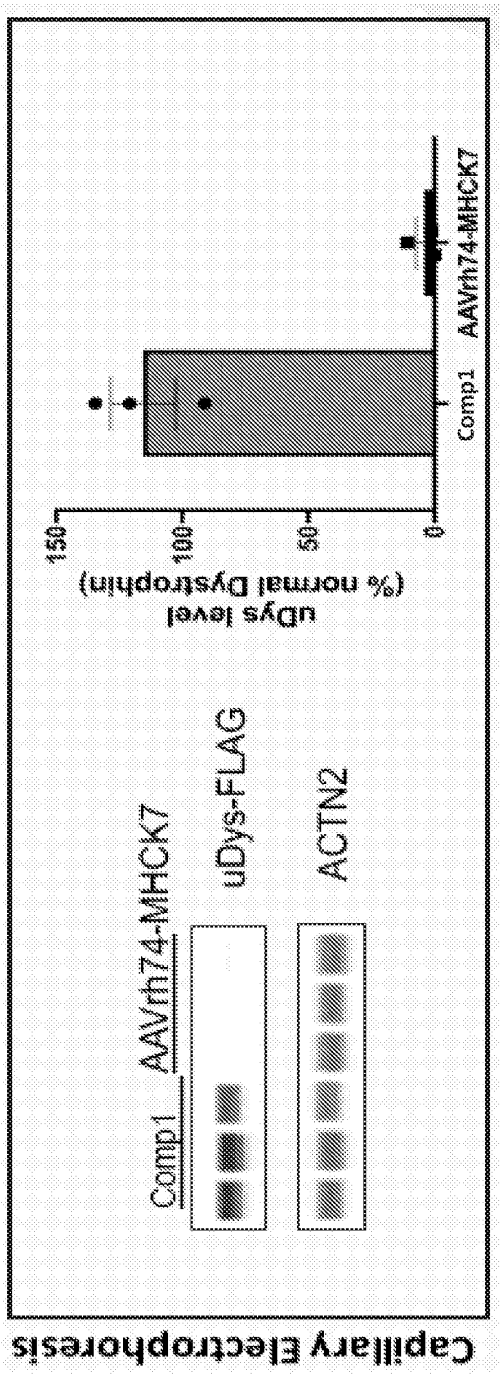

Capillary Electrophoresis
Comp1 AAVrh74-MHCK7
uDys-FLAG
ACTN2
uDys level
(% normal Dystrophin)
150
100
50
0
Comp1
AAVrh74-MHCK7

FIG. 10B

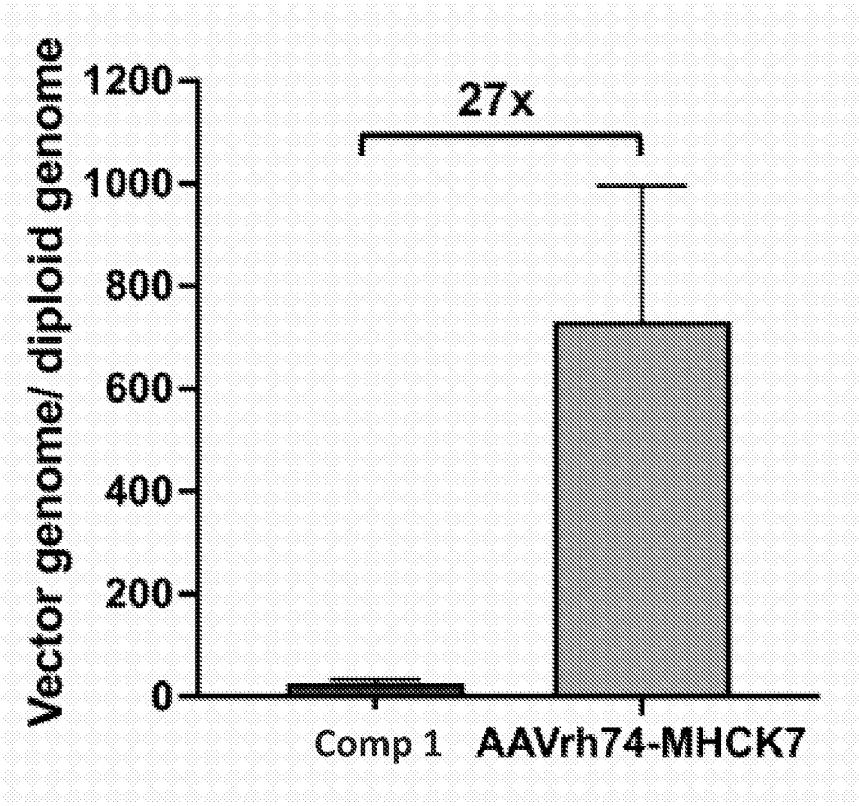

Vector genome/ diploid genome
1200
1000
800
600
400
200
0
27x
Comp 1
AAVrh74-MHCK7

FIG. 11

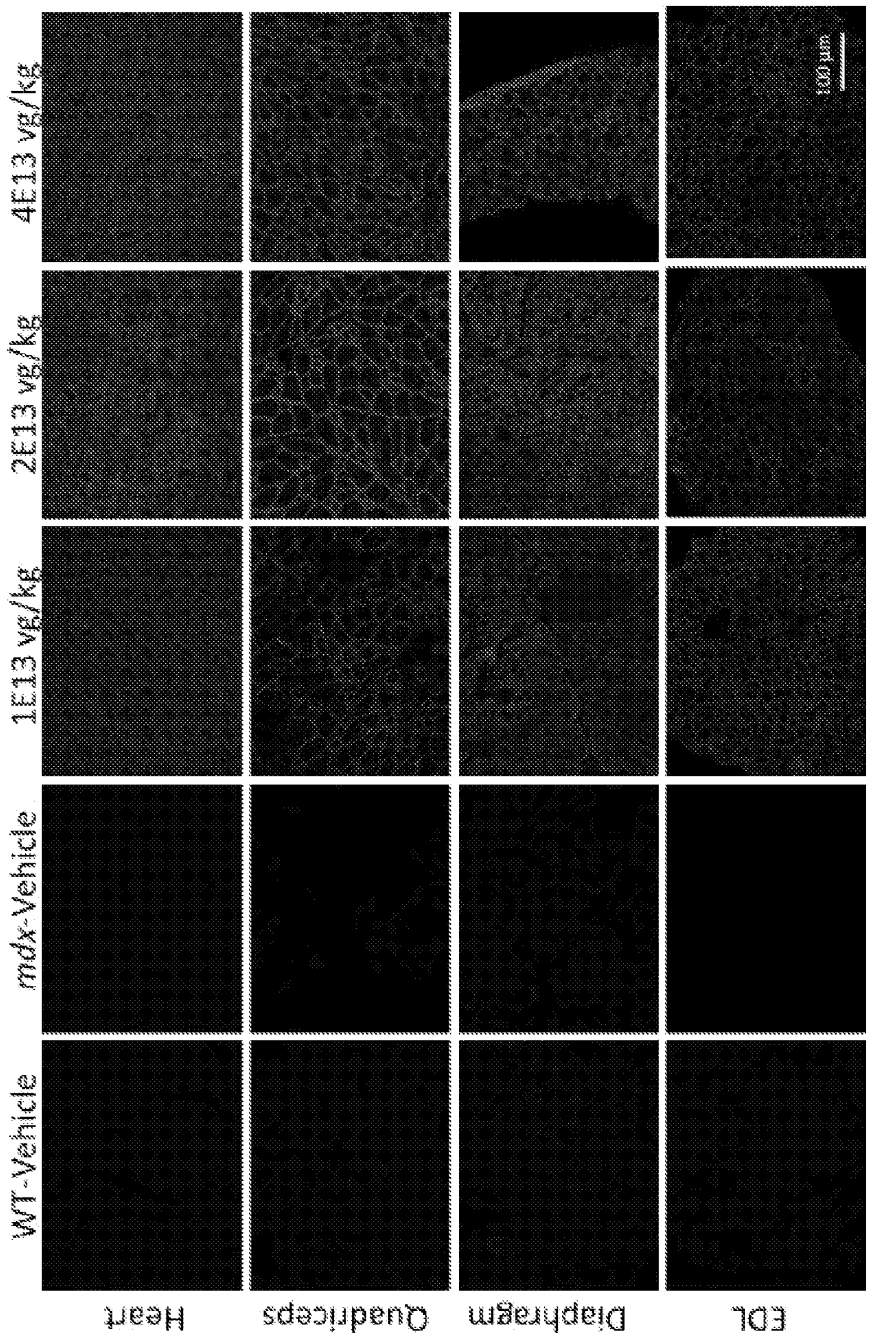

WT-Vehicle
mdx-Vehicle
1E13 vg/kg
2E13 vg/kg
4E13 vg/kg
Heart
Quadriceps
Diaphragm
EDL
100 µm

FIG. 12

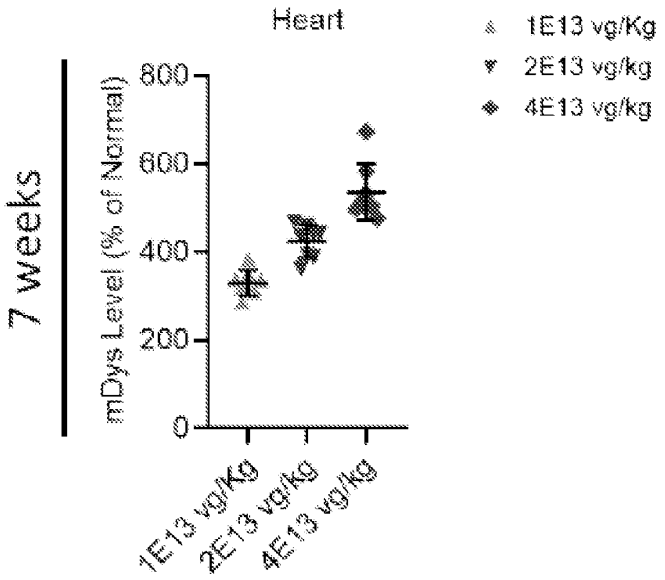


FIG. 13A

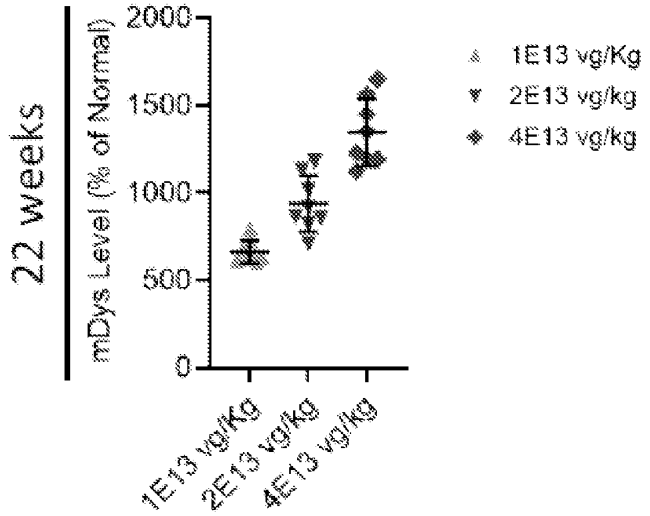


FIG. 13B

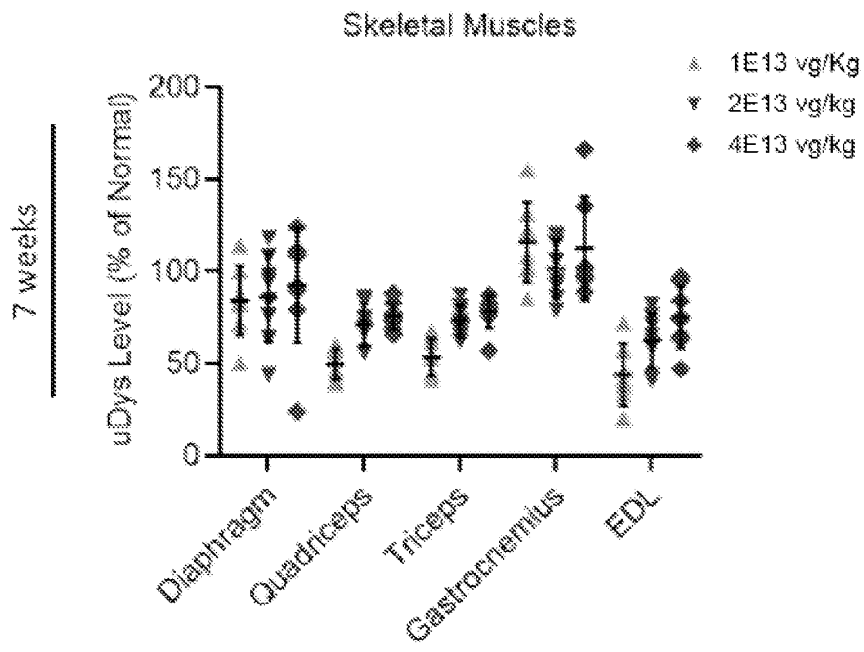


FIG. 14A

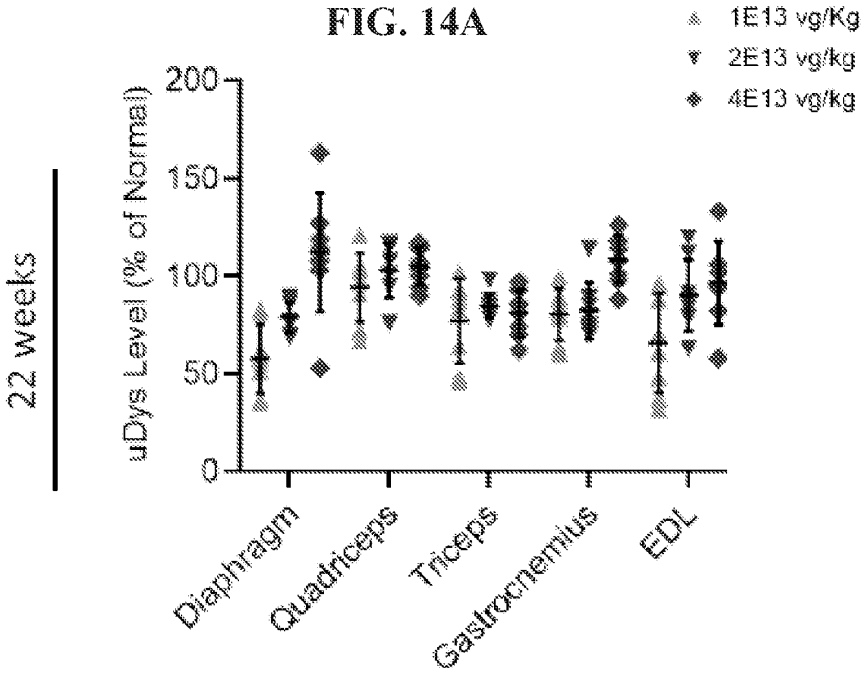


FIG. 14B

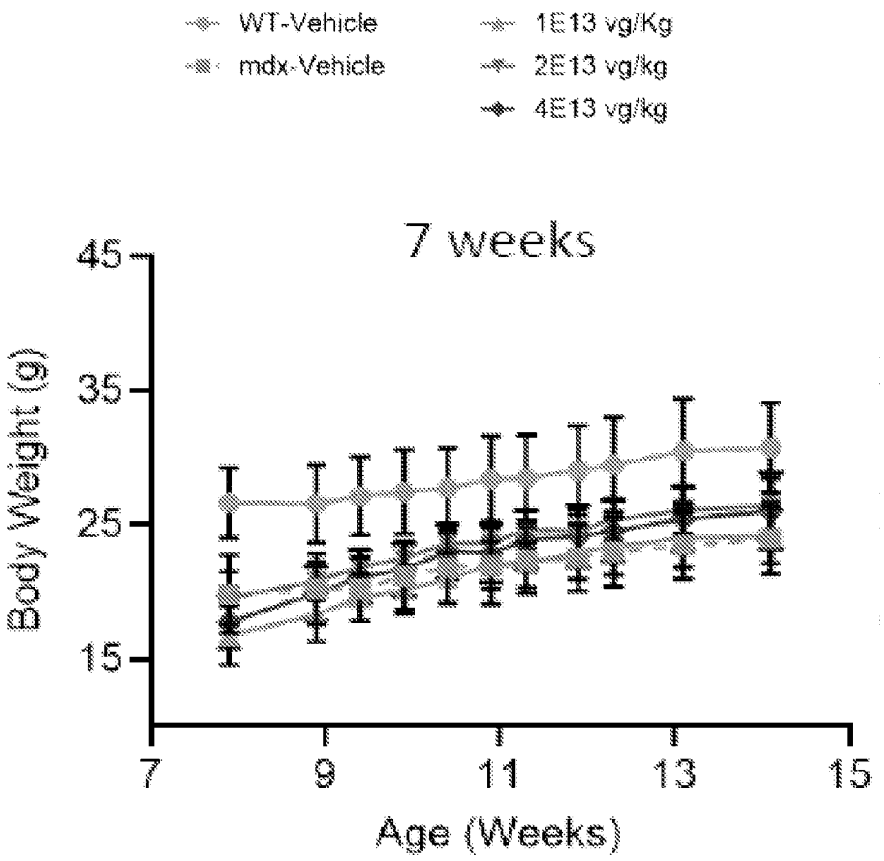

WT-Vehicle
mdx-Vehicle
1E13 vg/Kg
2E13 vg/kg
4E13 vg/kg
7 weeks
Body Weight (g)
45
35
25
15
7
9
11
13
15
Age (Weeks)

FIG. 15A

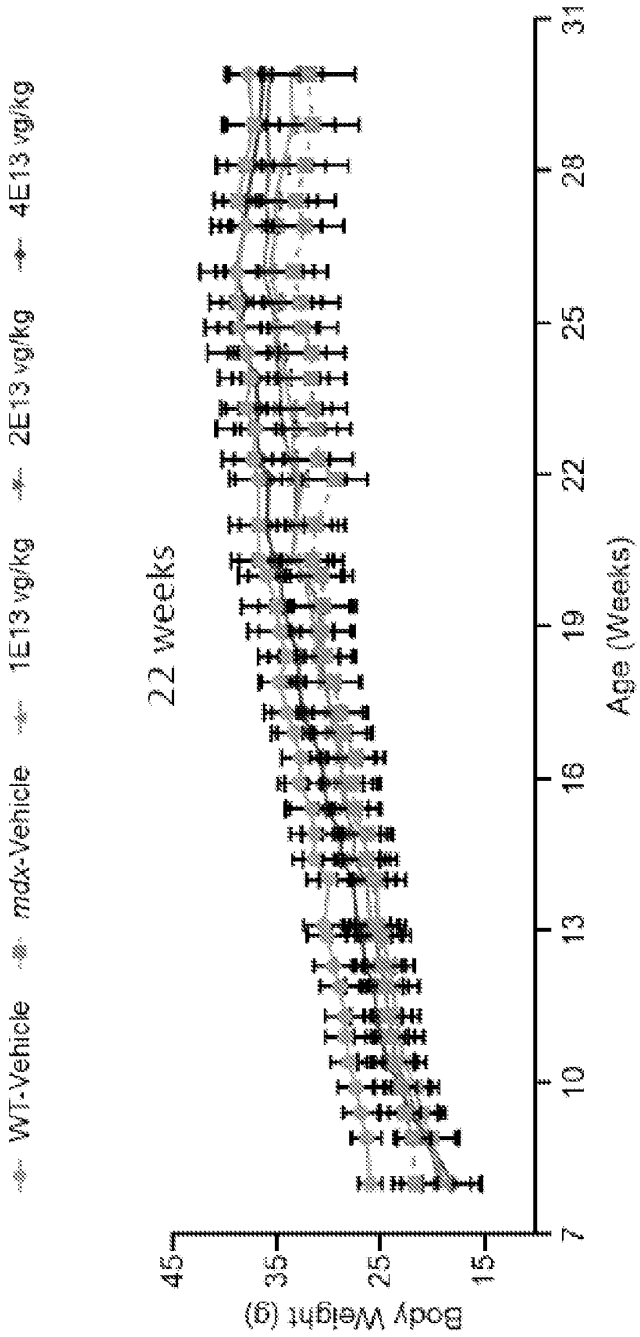

WT-Vehicle
mdx-Vehicle
1E13 vg/kg
2E13 vg/kg
4E13 vg/kg
22 weeks
Body Weight (g)
45
35
25
15
7
10
13
16
19
22
25
28
31
Age (Weeks)

FIG. 15B

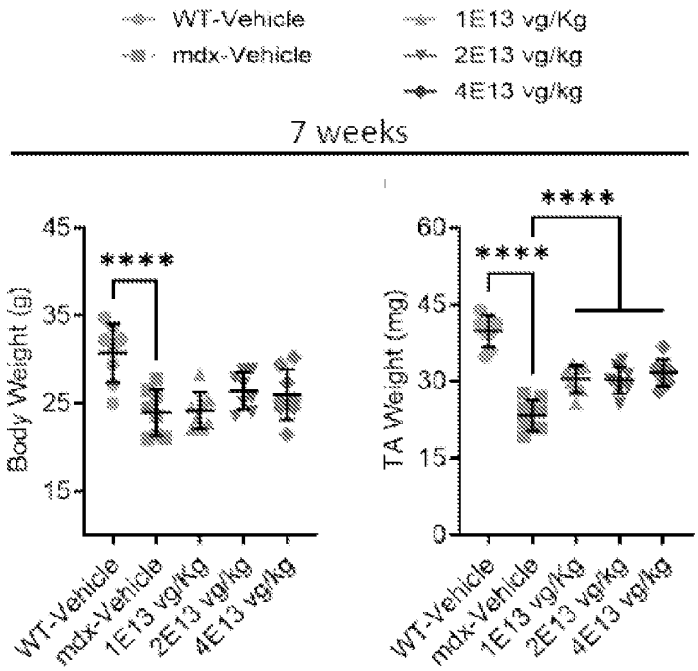


FIG. 16A

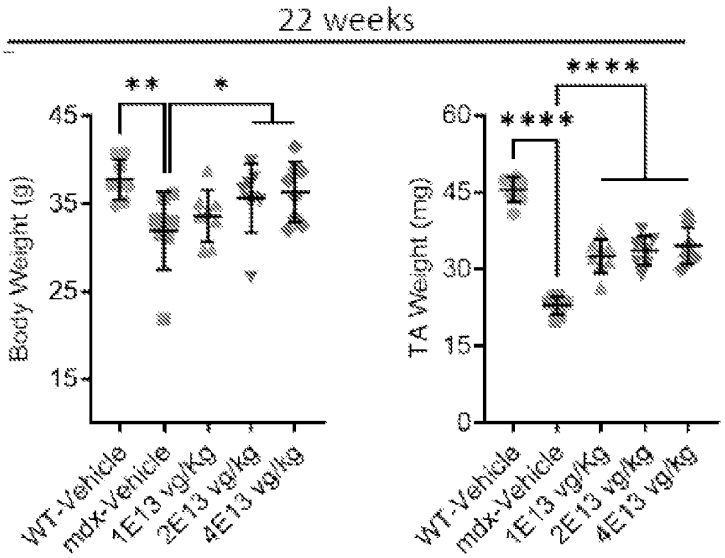


FIG. 16B

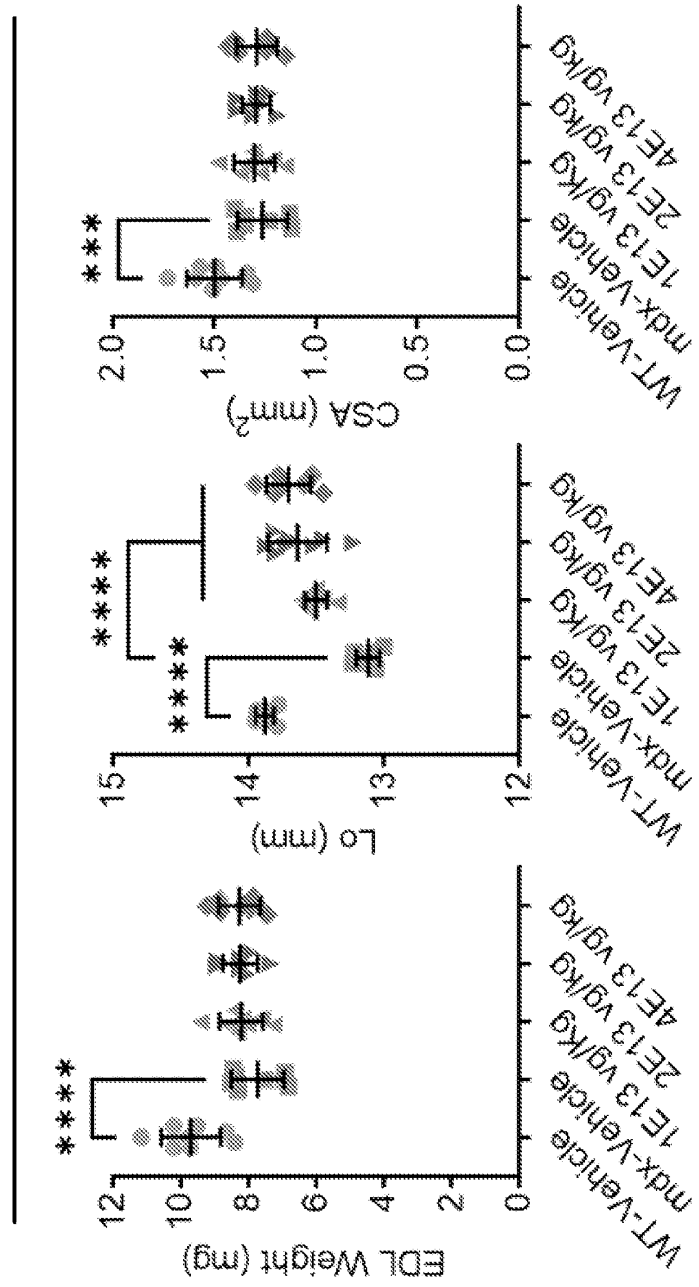

WT-Vehicle
mdx-Vehicle
1E13 vg/kg
2E13 vg/kg
4E13 vg/kg
7 weeks
EDL Weight (mg)
Lo (mm)
CSA (mm²)
WT-Vehicle
mdx-Vehicle
1E13 vg/kg
2E13 vg/kg
4E13 vg/kg

FIG. 17A

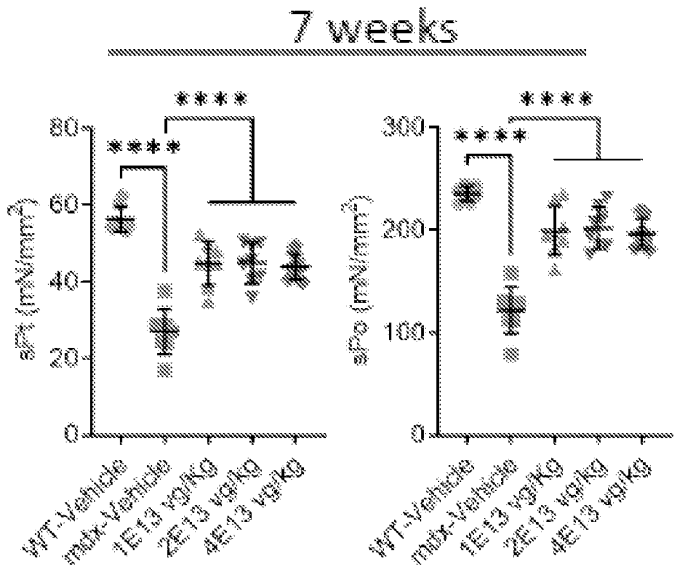


FIG. 17B

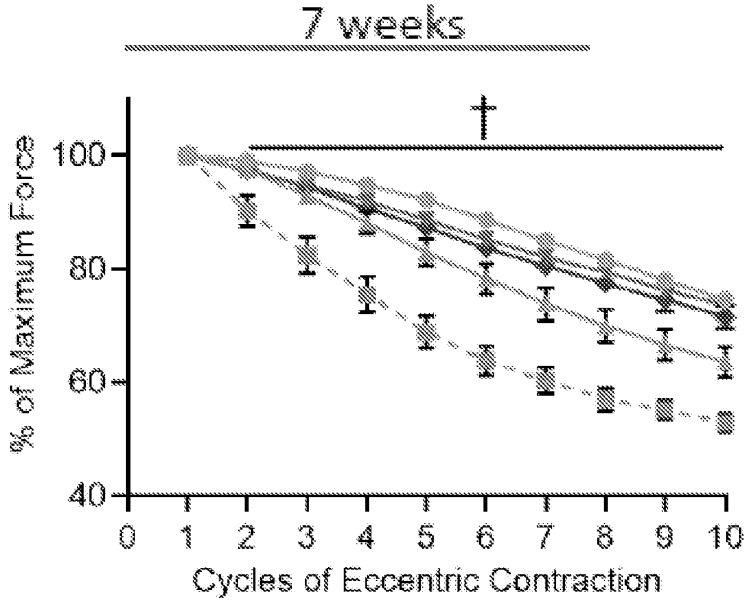


FIG. 17C

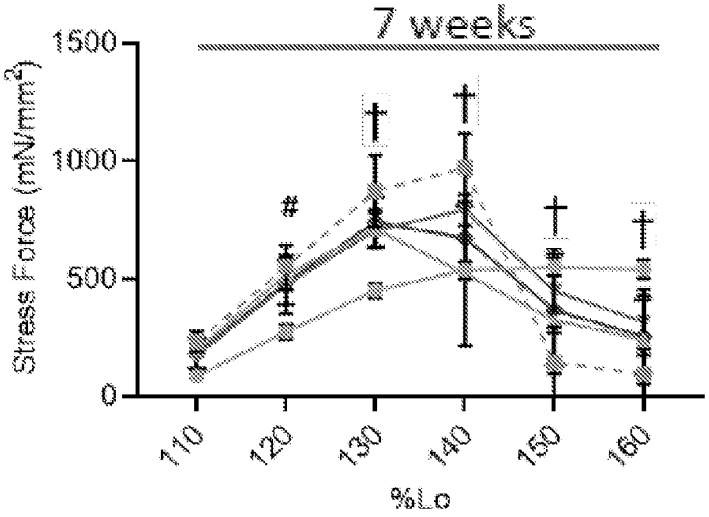


FIG. 17D

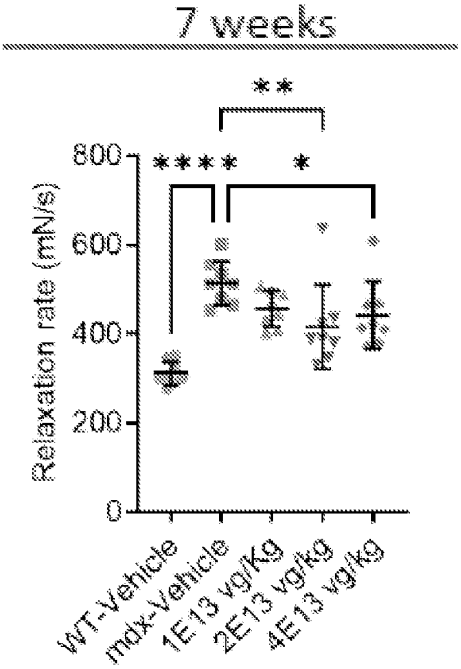


FIG. 17E

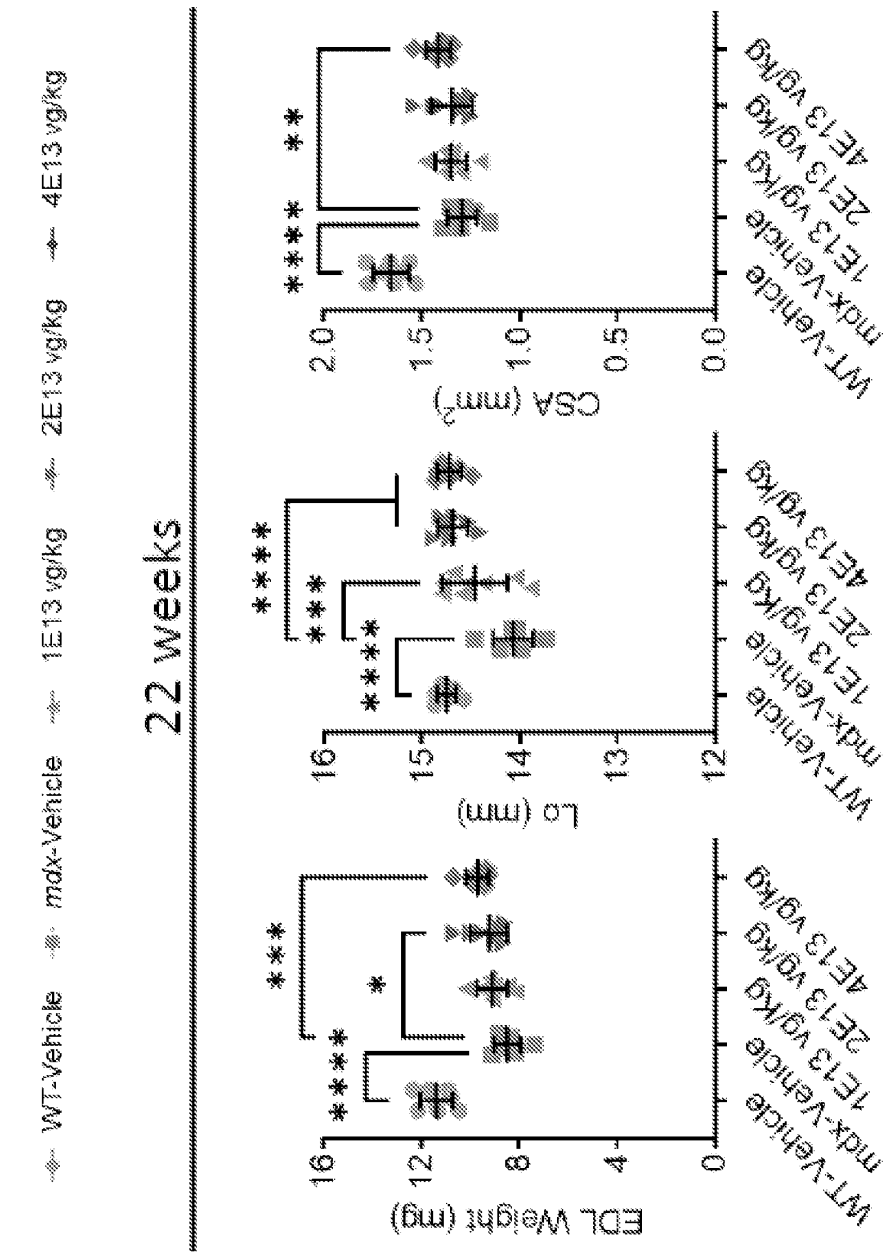

WT-Vehicle
mdx-Vehicle
1E13 vg/kg
2E13 vg/kg
4E13 vg/kg
22 weeks
EDL Weight (mg)
Lo (mm)
CSA (mm²)
WT-Vehicle
mdx-Vehicle
1E13 vg/kg
2E13 vg/kg
4E13 vg/kg

FIG. 18A

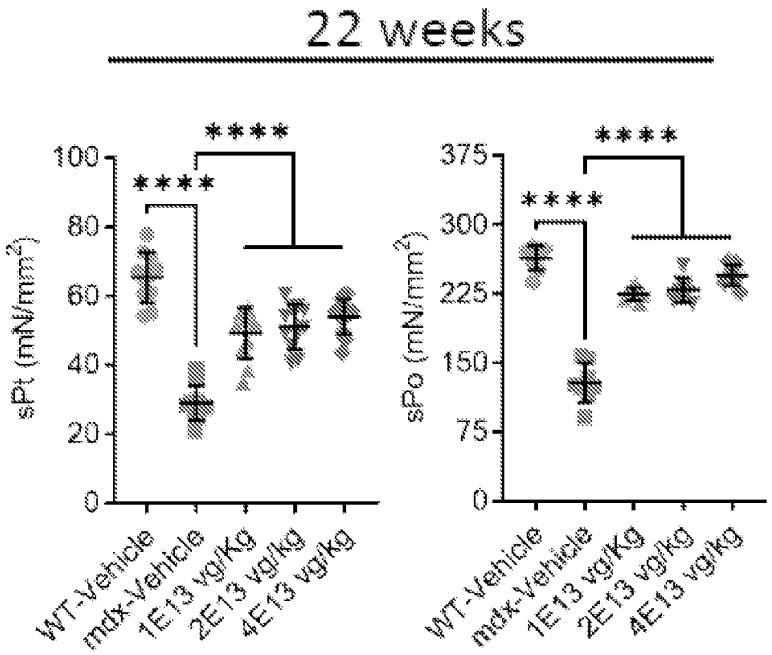


FIG. 18B

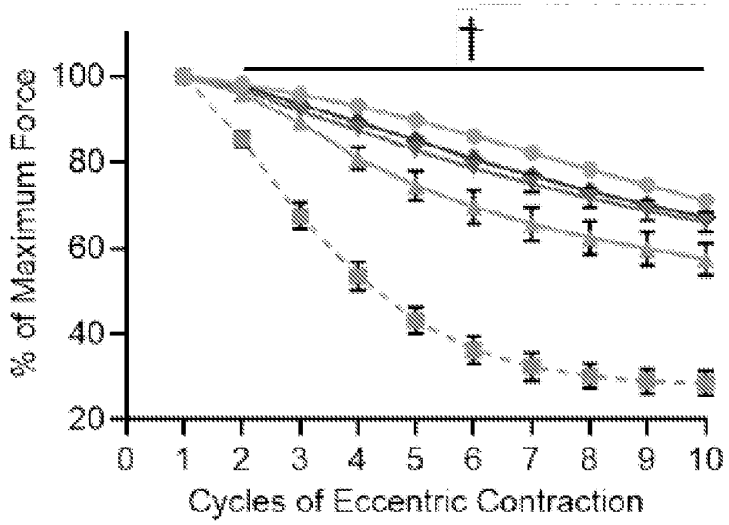


FIG. 18C

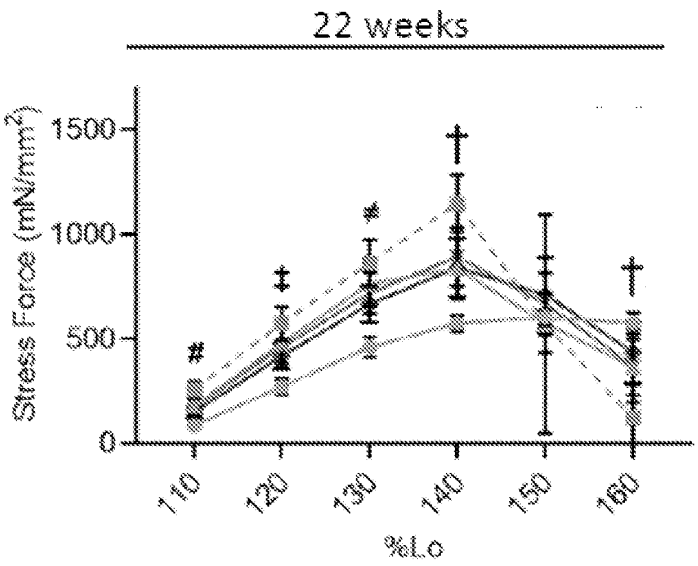


FIG. 18D

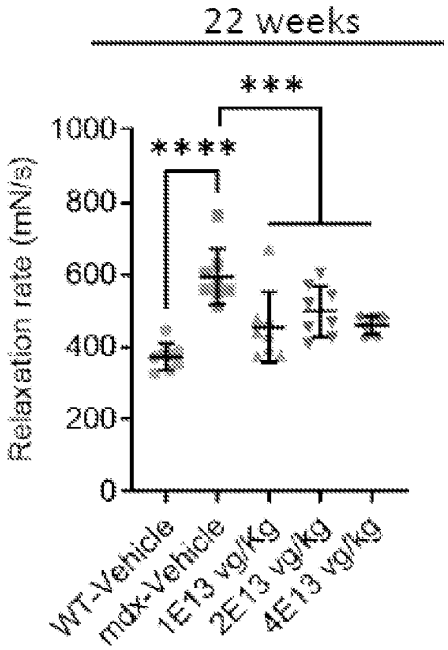


FIG. 18E

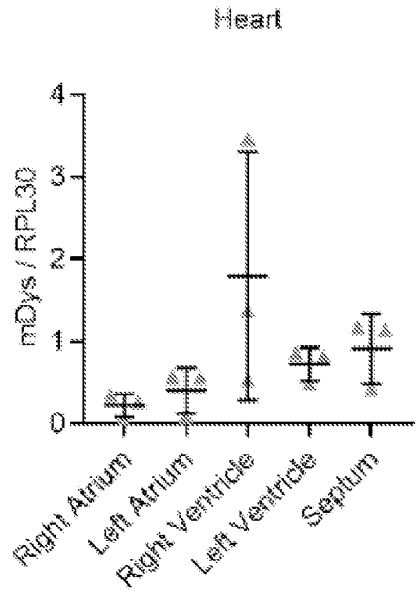


FIG. 19A

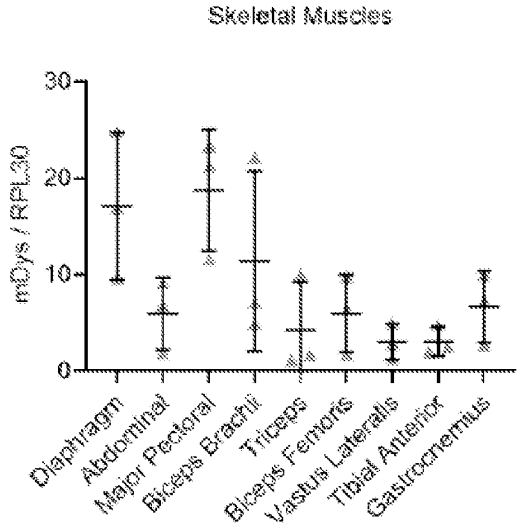


FIG. 19B

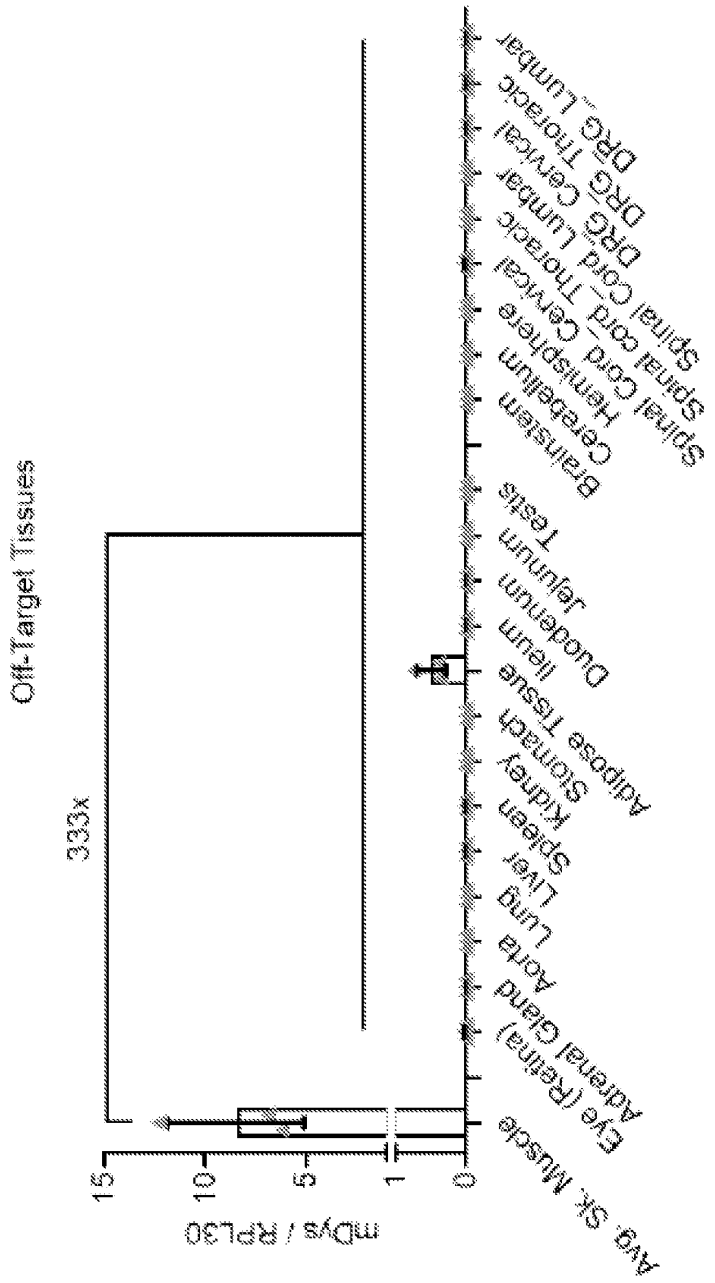

Off-Target Tissues
333x
mDys / RPL30
15
10
5
1
0
Avg. Sk. Muscle
Eye (Retina)
Adrenal Gland
Aorta
Lung
Liver
Spleen
Kidney
Stomach
Adipose Tissue
Ileum
Duodenum
Jejunum
Testis
Brainstem
Cerebellum
Hemisphere
Spinal Cord_Cervical
Spinal Cord_Thoracic
Spinal Cord_Lumbar
DRG_Cervical
DRG_Thoracic
DRG_Lumbar

FIG. 19C

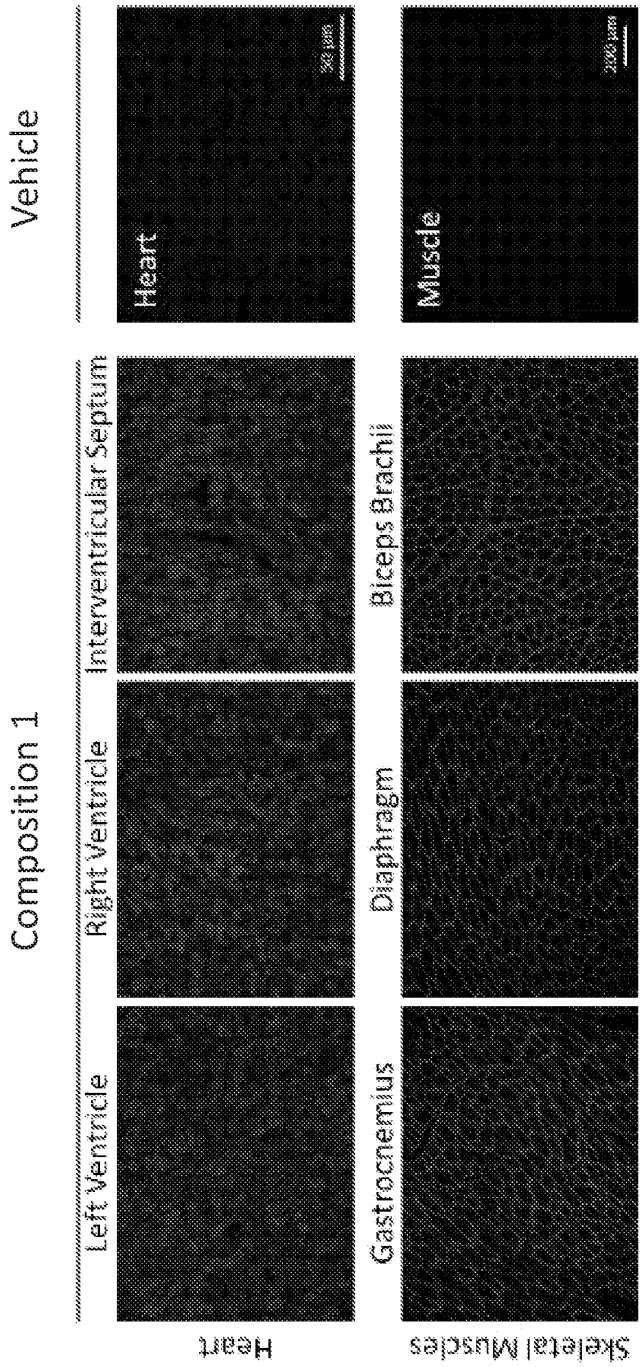

Vehicle
Heart
Muscle
Composition 1
Left Ventricle
Right Ventricle
Interventricular Septum
Gastrocnemius
Diaphragm
Biceps Brachii
Heart
Skeletal Muscles

FIG. 20

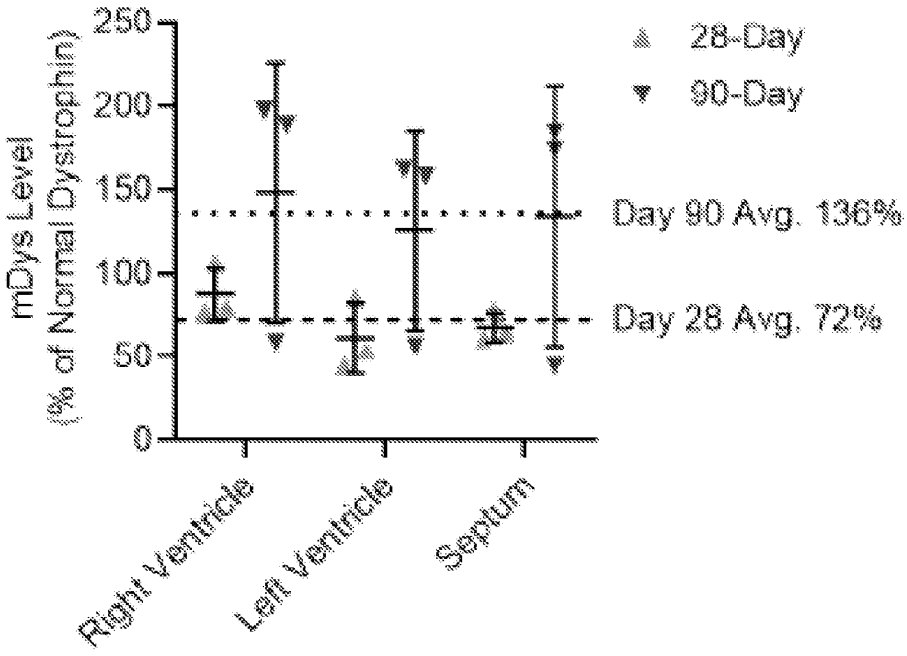

mDys Level
(% of Normal Dystrophin)
250
200
150
100
50
0
28-Day
90-Day
Day 90 Avg. 136%
Day 28 Avg. 72%
Right Ventricle
Left Ventricle
Septum

FIG. 21A

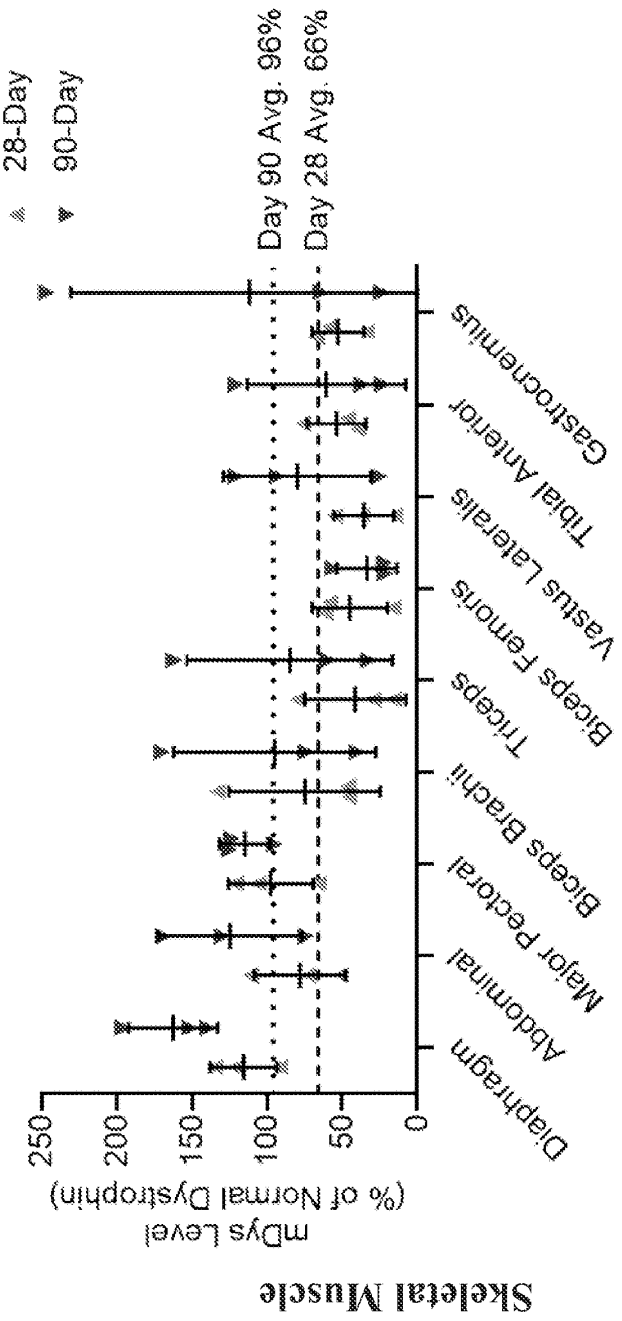

Skeletal Muscle
mDys Level
(% of Normal Dystrophin)
250
200
150
100
50
0
Diaphragm
Abdominal
Major Pectoral
Biceps Brachii
Triceps
Biceps Femoris
Vastus Lateralis
Tibial Anterior
Gastrocnemius
Day 90 Avg. 96%
Day 28 Avg. 66%
28-Day
90-Day

FIG. 21B

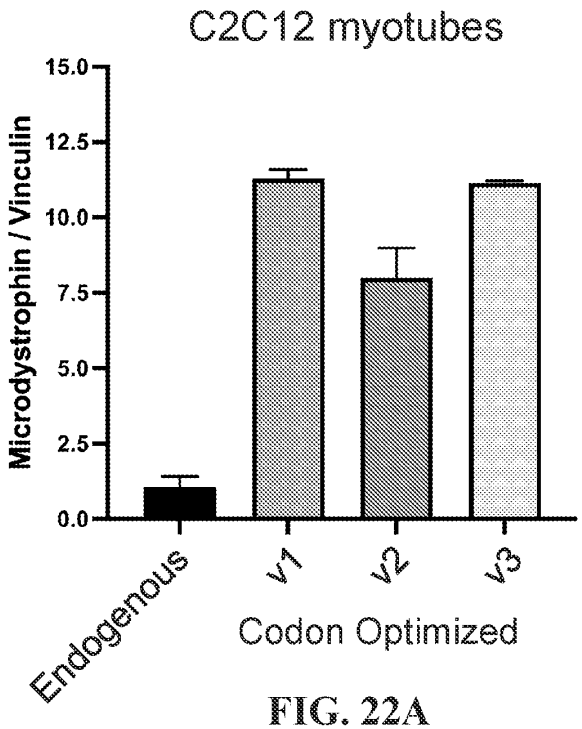


FIG. 22A

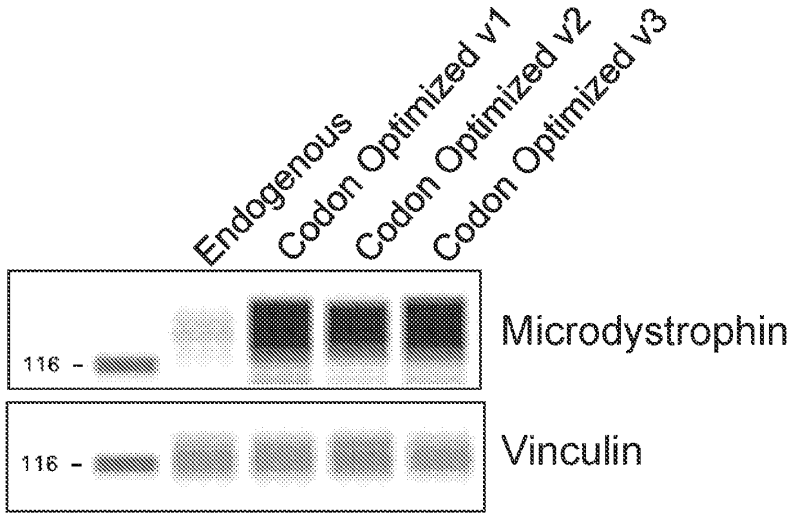


FIG. 22B

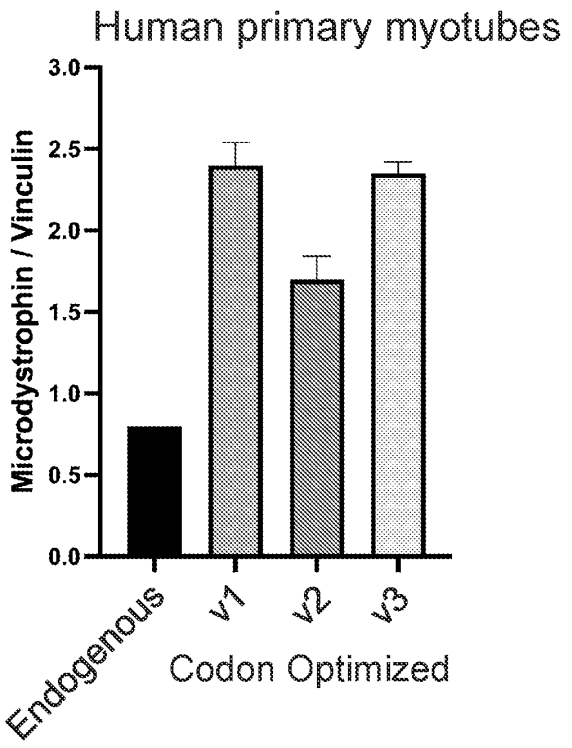


FIG. 23A

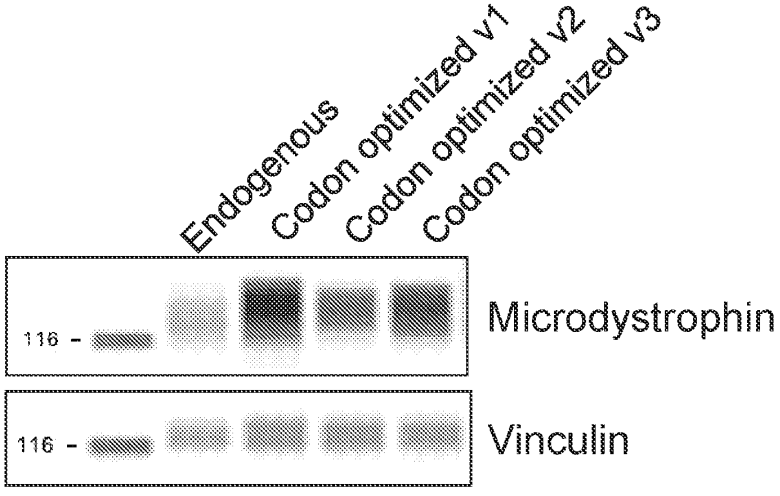


FIG. 23B

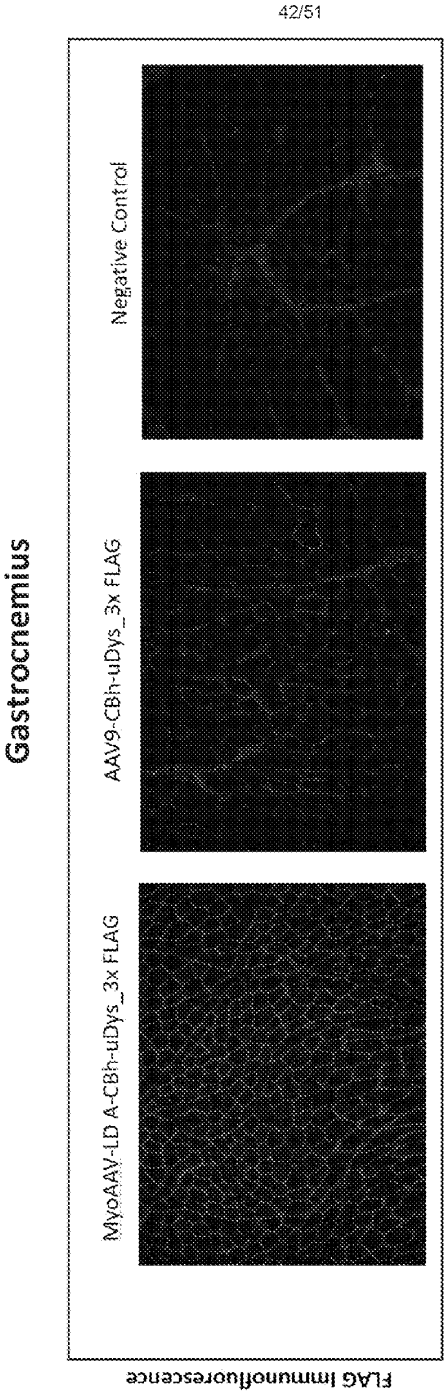

Gastrocnemius
MyoAAV-1D A-CBh-uDys_3x FLAG
AAV9-CBh-uDys_3x FLAG
Negative Control
FLAG Immunofluorescence

FIG. 24A

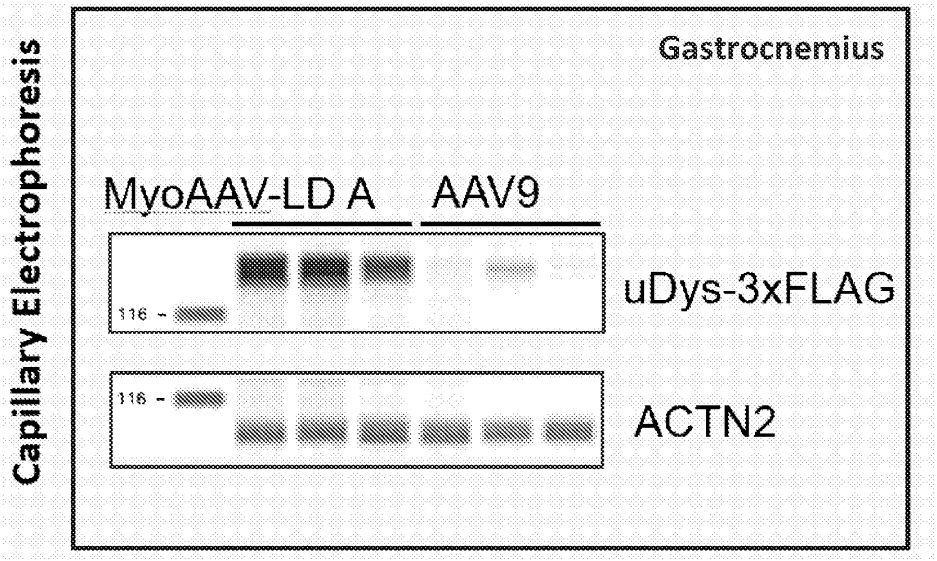


FIG. 24B

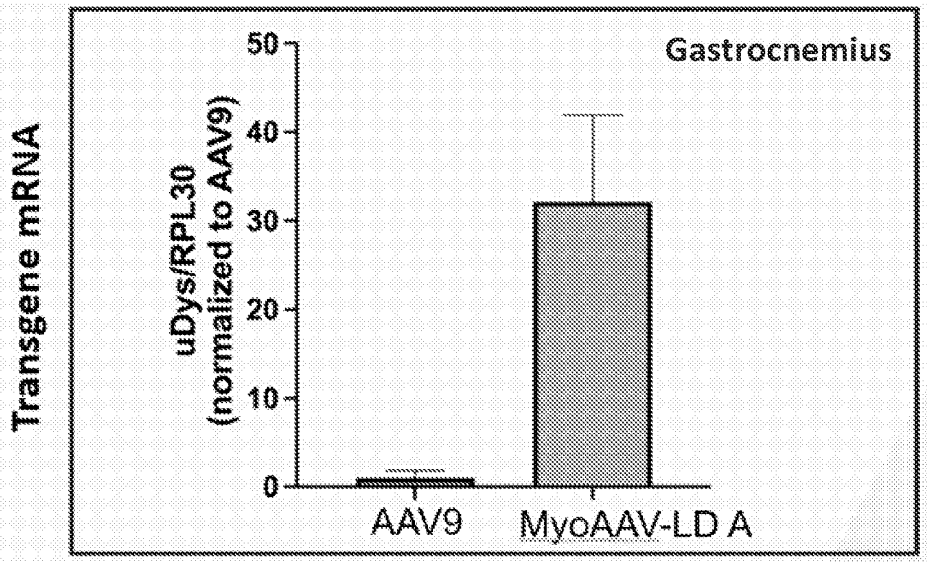


FIG. 24C

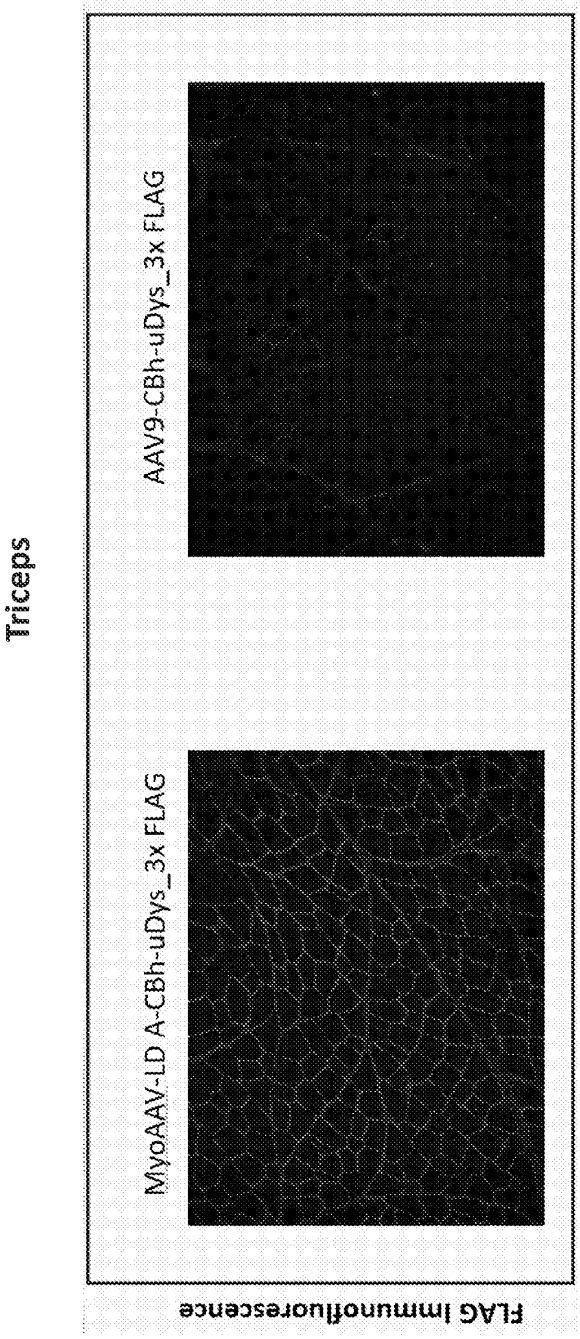

Triceps
AAV9-CBh-uDys_3x FLAG
MyoAAV-LD A-CBh-uDys_3x FLAG
FLAG Immunofluorescence

FIG. 25A

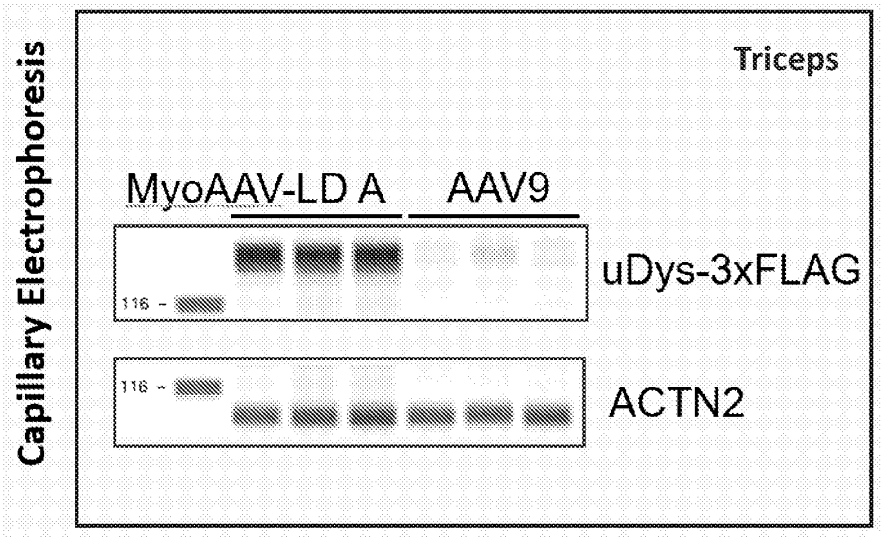


FIG. 25B

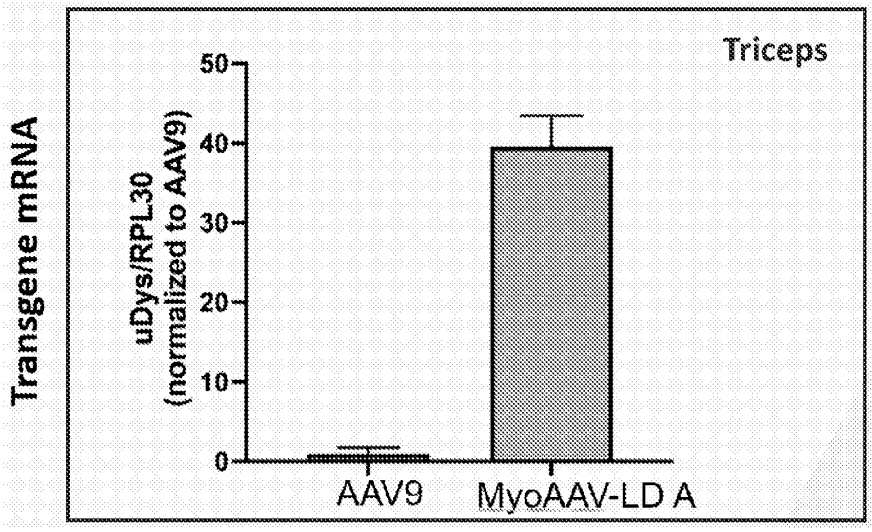


FIG. 25C

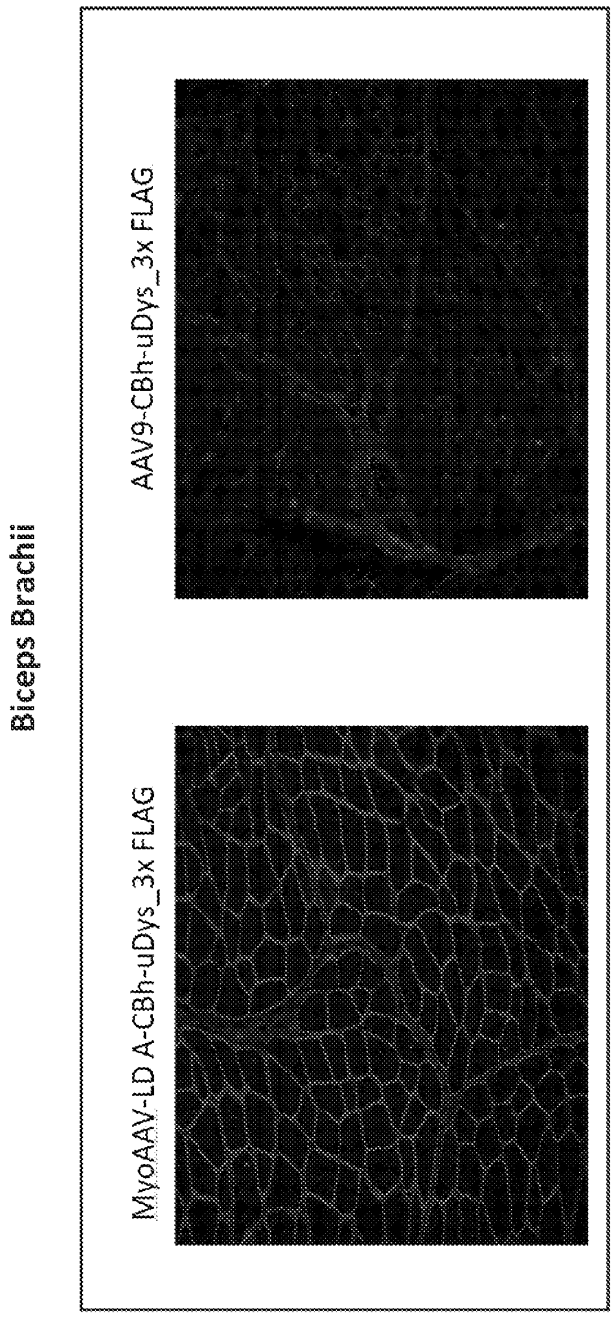

Biceps Brachii
MyoAAV-LD A-CBh-uDys_3x FLAG
AAV9-CBh-uDys_3x FLAG
FLAG Immunofluorescence

FIG. 26A

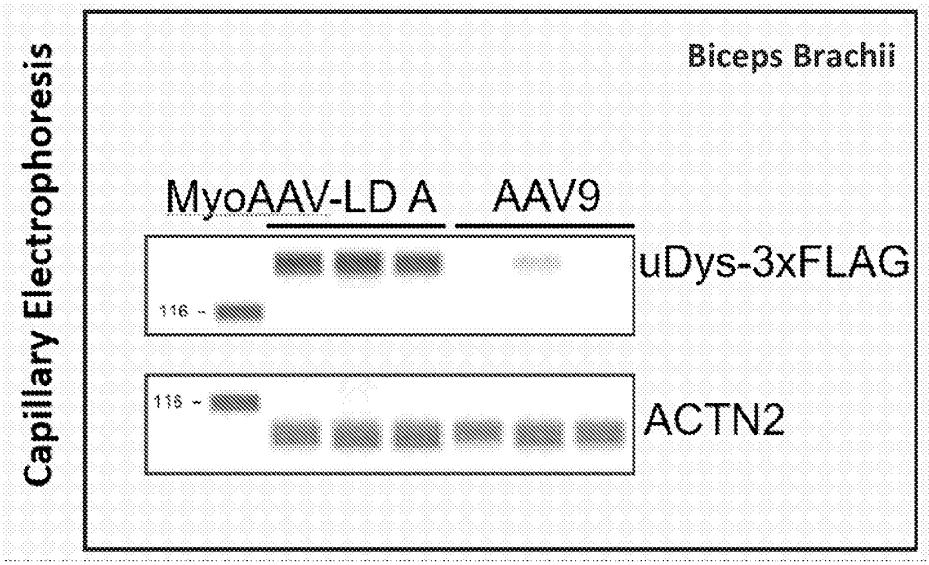


FIG. 26B

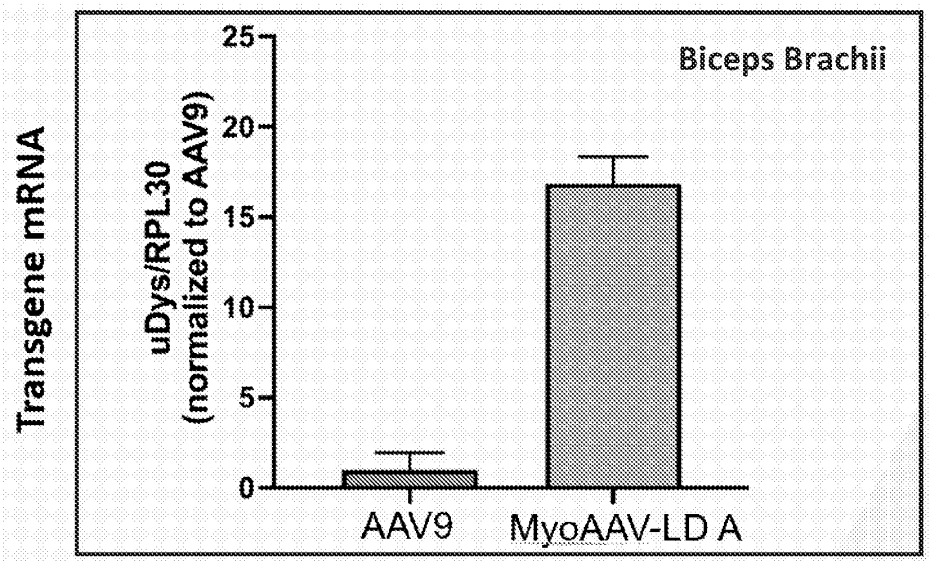


FIG. 26C

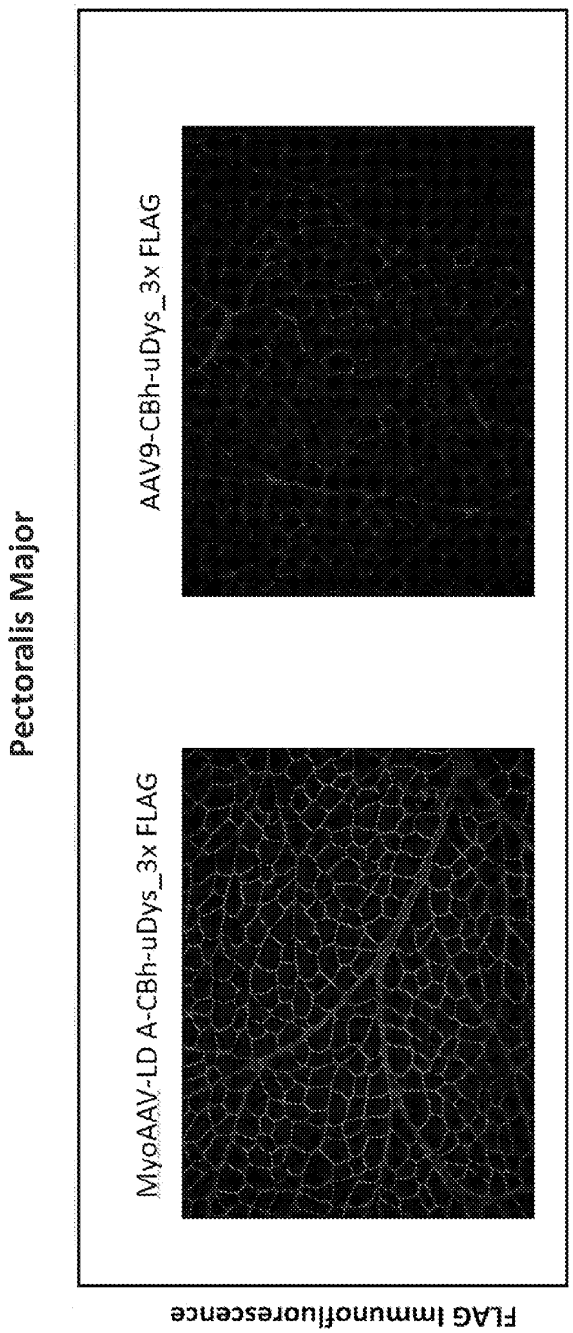

Pectoralis Major
AAV9-CBh-uDys_3x FLAG
MyoAAV-1D A-CBh-uDys_3x FLAG
FLAG Immunofluorescence

FIG. 27A

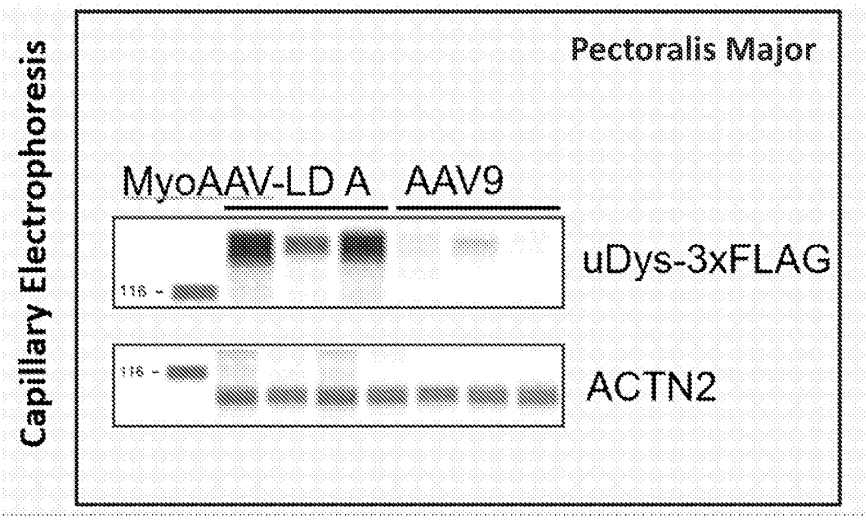


FIG. 27B

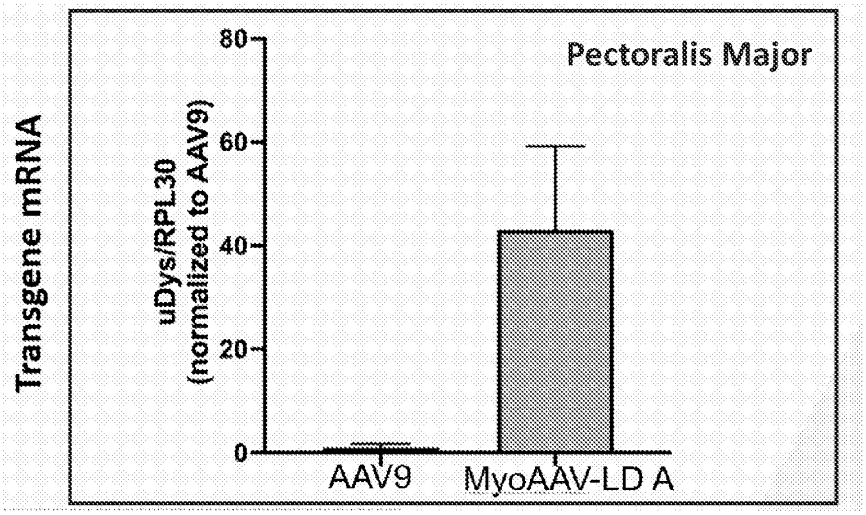


FIG. 27C

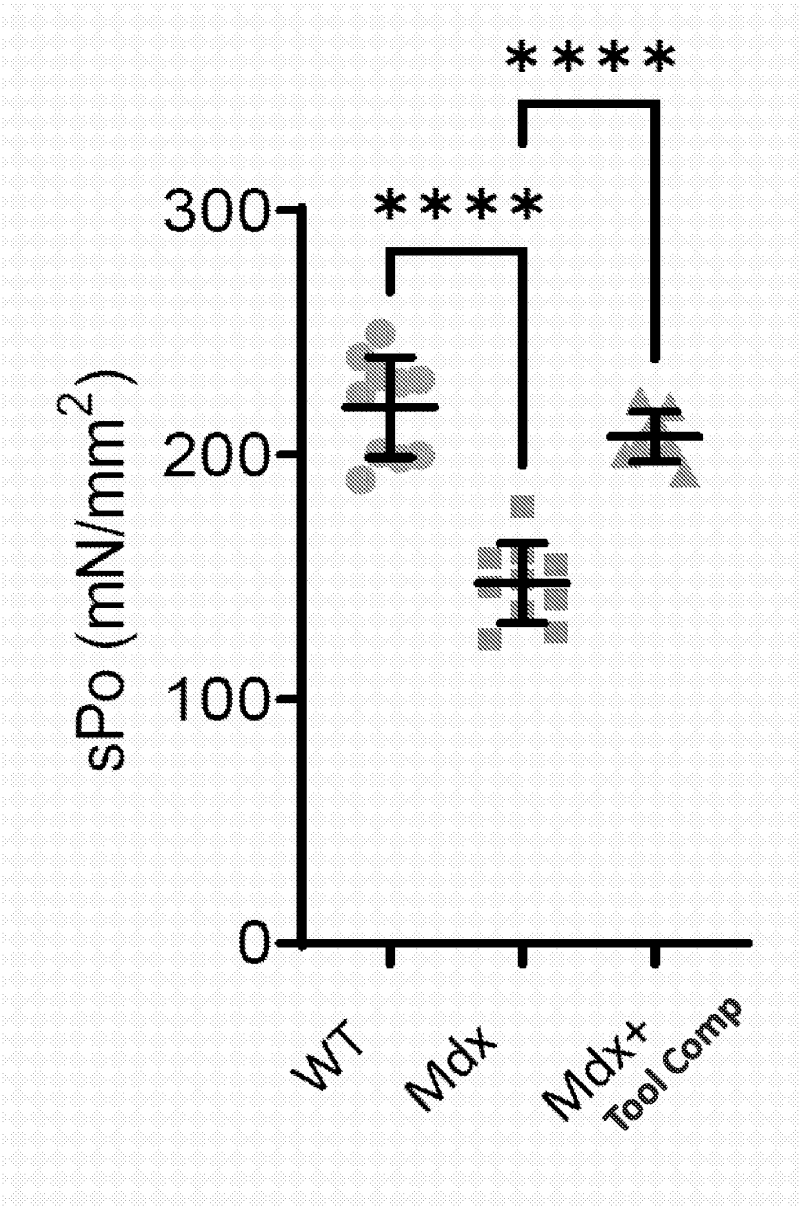


FIG. 28A

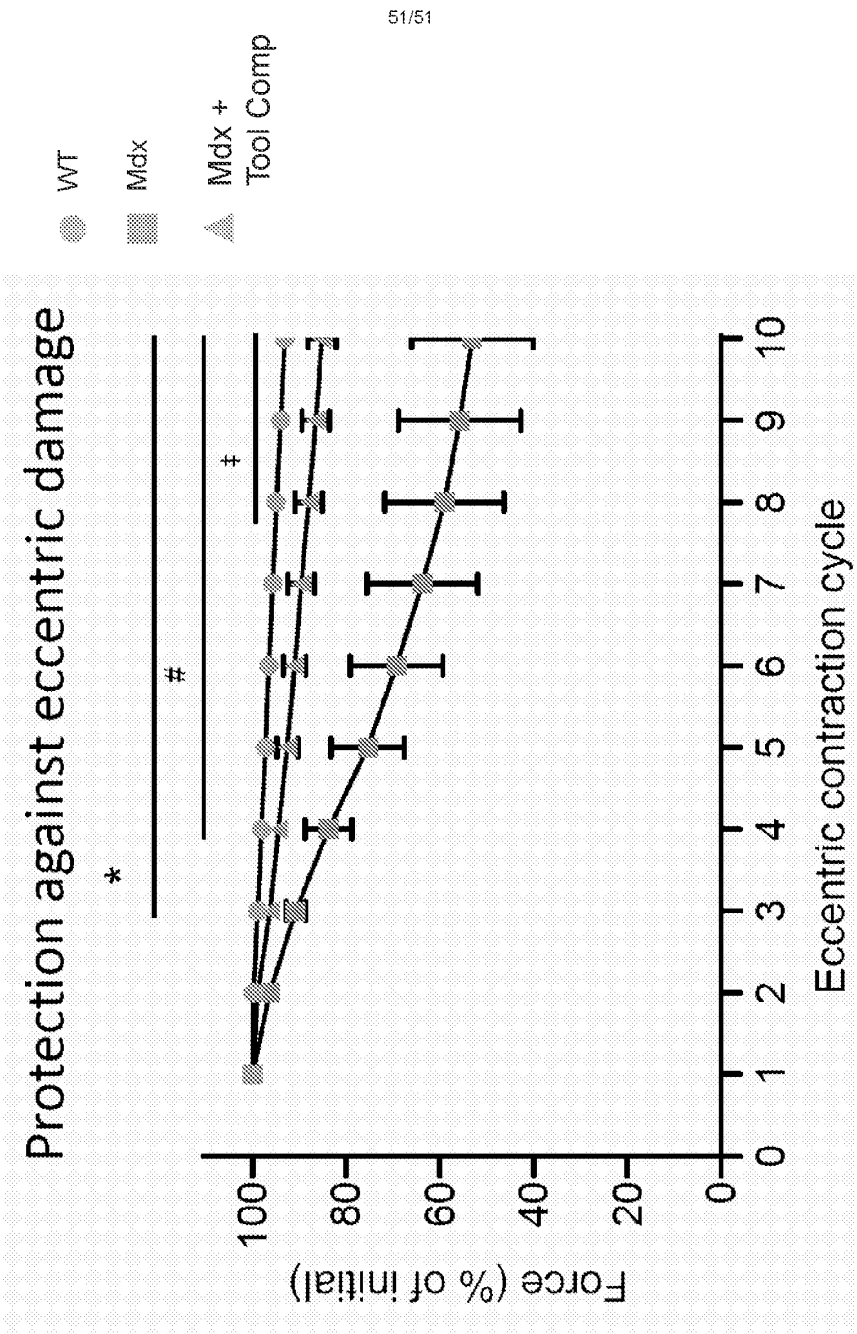

Protection against eccentric damage
WT
Mdx
Mdx +
Tool Comp
*
#
‡
Force (% of initial)
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
9
10
Eccentric contraction cycle

FIG. 28B