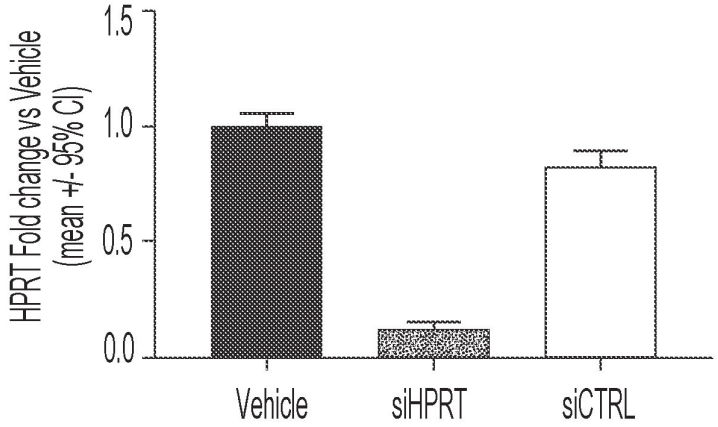

HPRT Fold change vs Vehicle
(mean +/- 95% CI)
1.5
1.0
0.5
0.0
Vehicle
siHPRT
siCTRL

FIG. 1

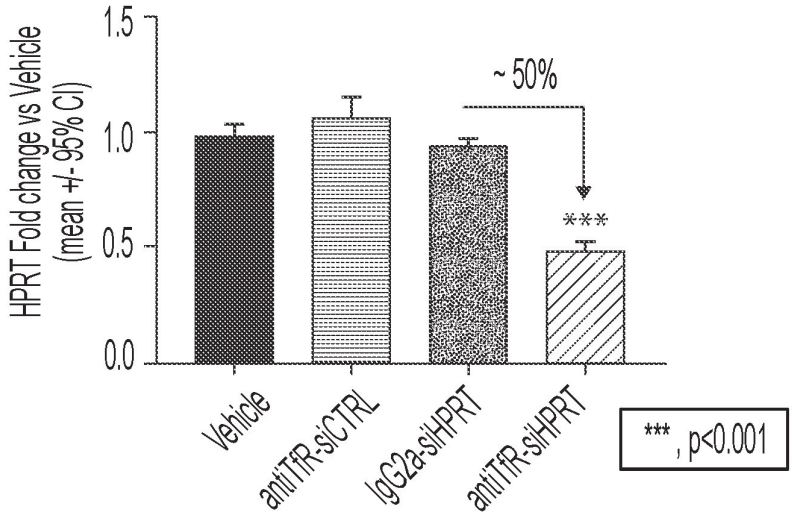

HPRT Fold change vs Vehicle
(mean +/- 95% CI)
1.5
1.0
0.5
0.0
~ 50%
***
Vehicle
antiTfR-siCTRL
IgG2a-siHPRT
antiTfR-siHPRT
***, p<0.001

FIG. 2

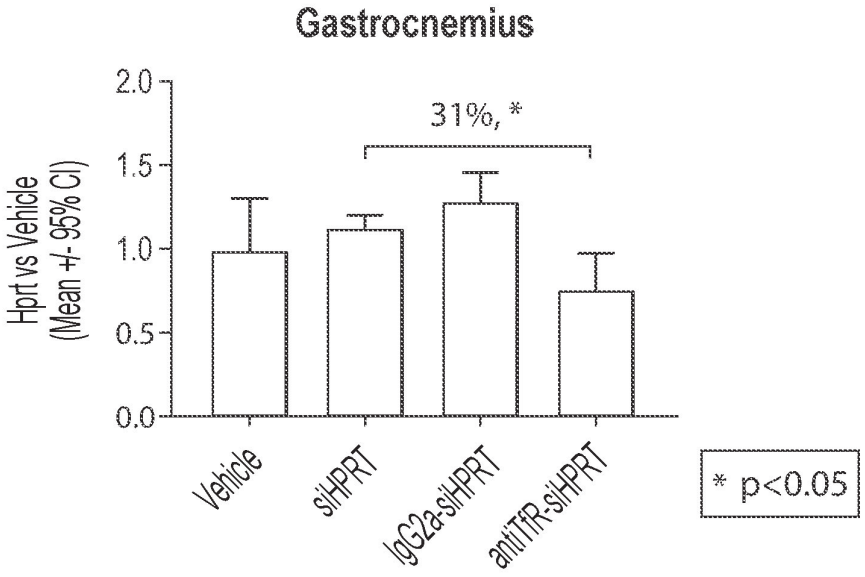

Gastrocnemius
Hprt vs Vehicle
(Mean +/- 95% CI)
2.0
1.5
1.0
0.5
0.0
31%, *
Vehicle
siHPRT
IgG2a-siHPRT
antiTfR-siHPRT
* p<0.05

FIG. 3A

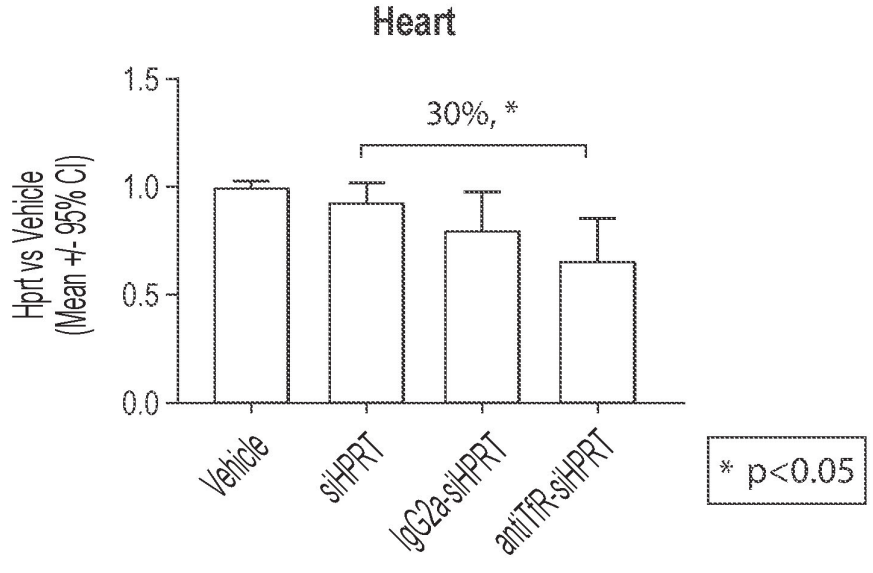

Heart
Hprt vs Vehicle
(Mean +/- 95% CI)
1.5
1.0
0.5
0.0
30%, *
Vehicle
siHPRT
IgG2a-siHPRT
antiTfR-siHPRT
* p<0.05

FIG. 3B

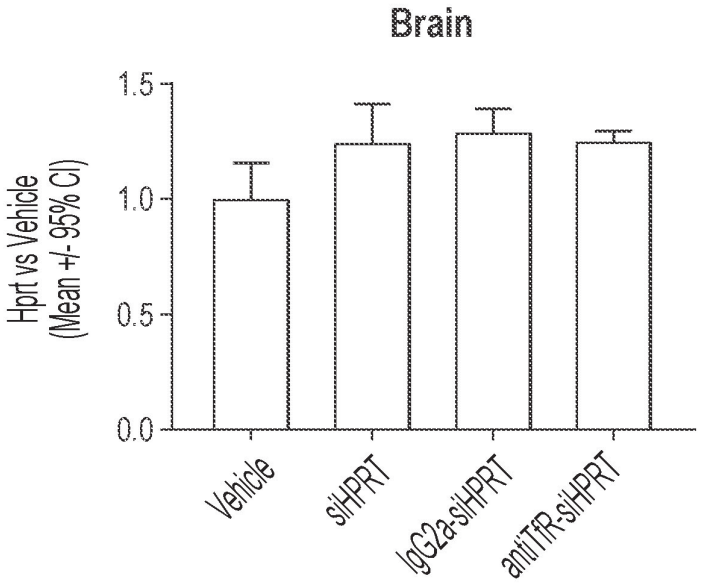

Brain
Hprt vs Vehicle
(Mean +/- 95% CI)
1.5
1.0
0.5
0.0
Vehicle
siHPRT
IgG2a-siHPRT
antiTfR-siHPRT

FIG. 4A

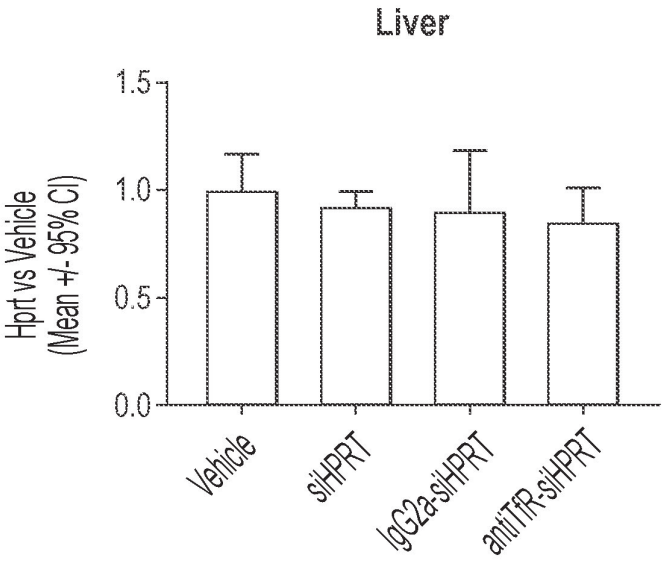

Liver
Hprt vs Vehicle
(Mean +/- 95% CI)
1.5
1.0
0.5
0.0
Vehicle
siHPRT
IgG2a-siHPRT
antiTfR-siHPRT

FIG. 4B

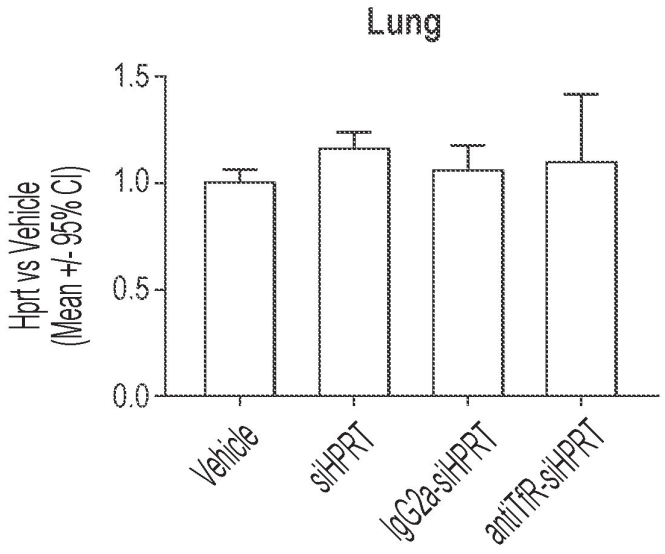

Lung
Hprt vs Vehicle
(Mean +/- 95% CI)
1.5
1.0
0.5
0.0
Vehicle
siHPRT
IgG2a-siHPRT
antiTfR-siHPRT

FIG. 4C

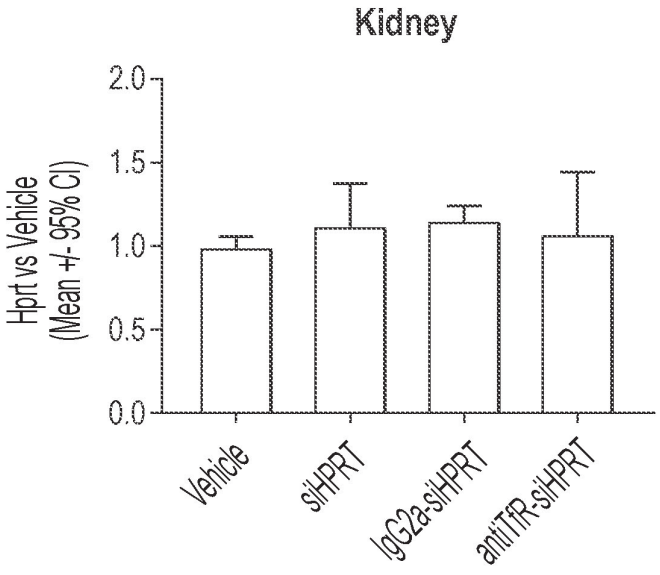

Kidney
Hprt vs Vehicle
(Mean +/- 95% CI)
2.0
1.5
1.0
0.5
0.0
Vehicle
siHPRT
IgG2a-siHPRT
antiTfR-siHPRT

FIG. 4D

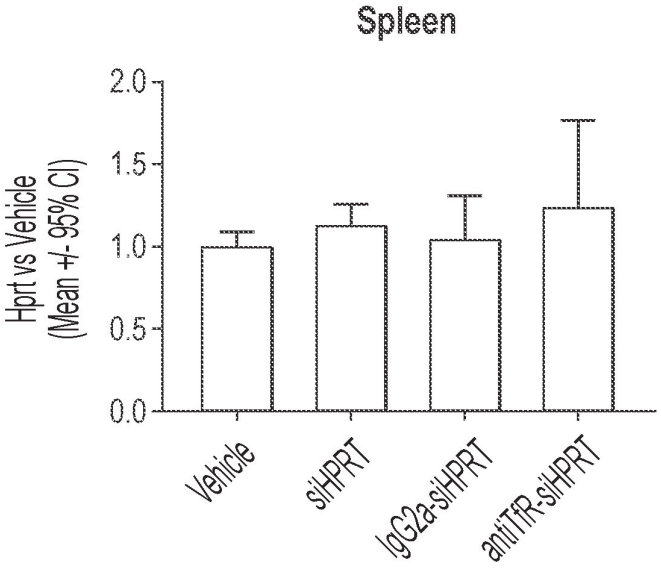

Spleen
Hprt vs Vehicle
(Mean +/- 95% CI)
2.0
1.5
1.0
0.5
0.0
Vehicle
siHPRT
IgG2a-siHPRT
antiTfR-siHPRT

FIG. 4E

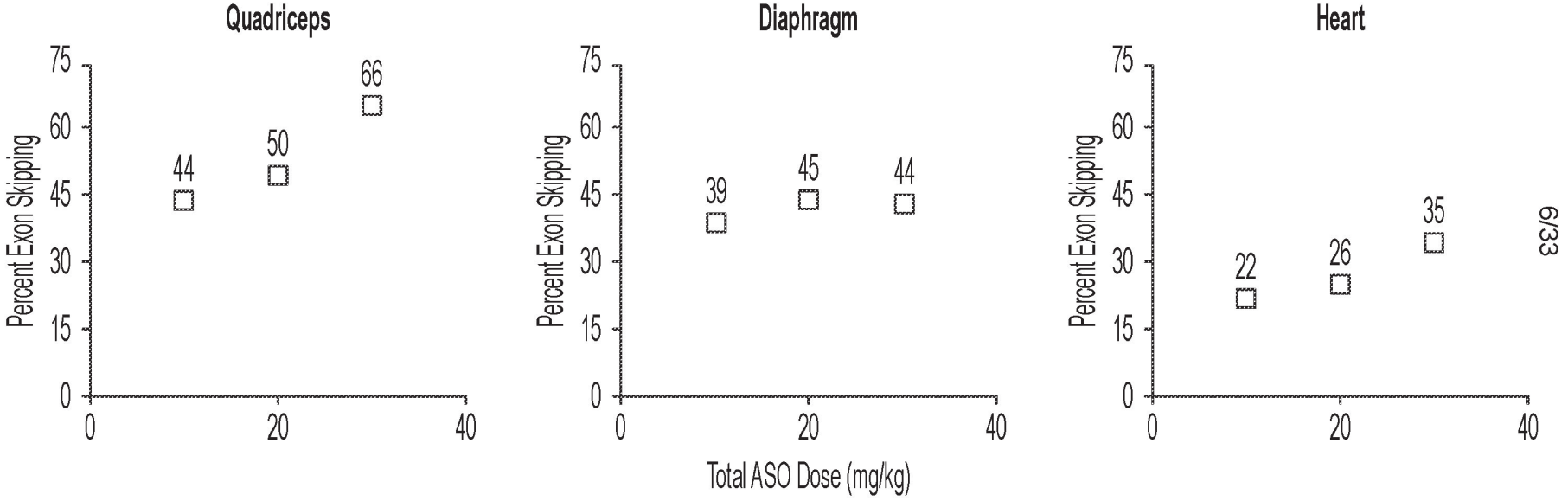

Quadriceps
Diaphragm
Heart
Percent Exon Skipping
Total ASO Dose (mg/kg)
0
15
30
45
60
75
0
20
40
44
50
66
39
45
44
22
26
35
6/33

FIG. 5

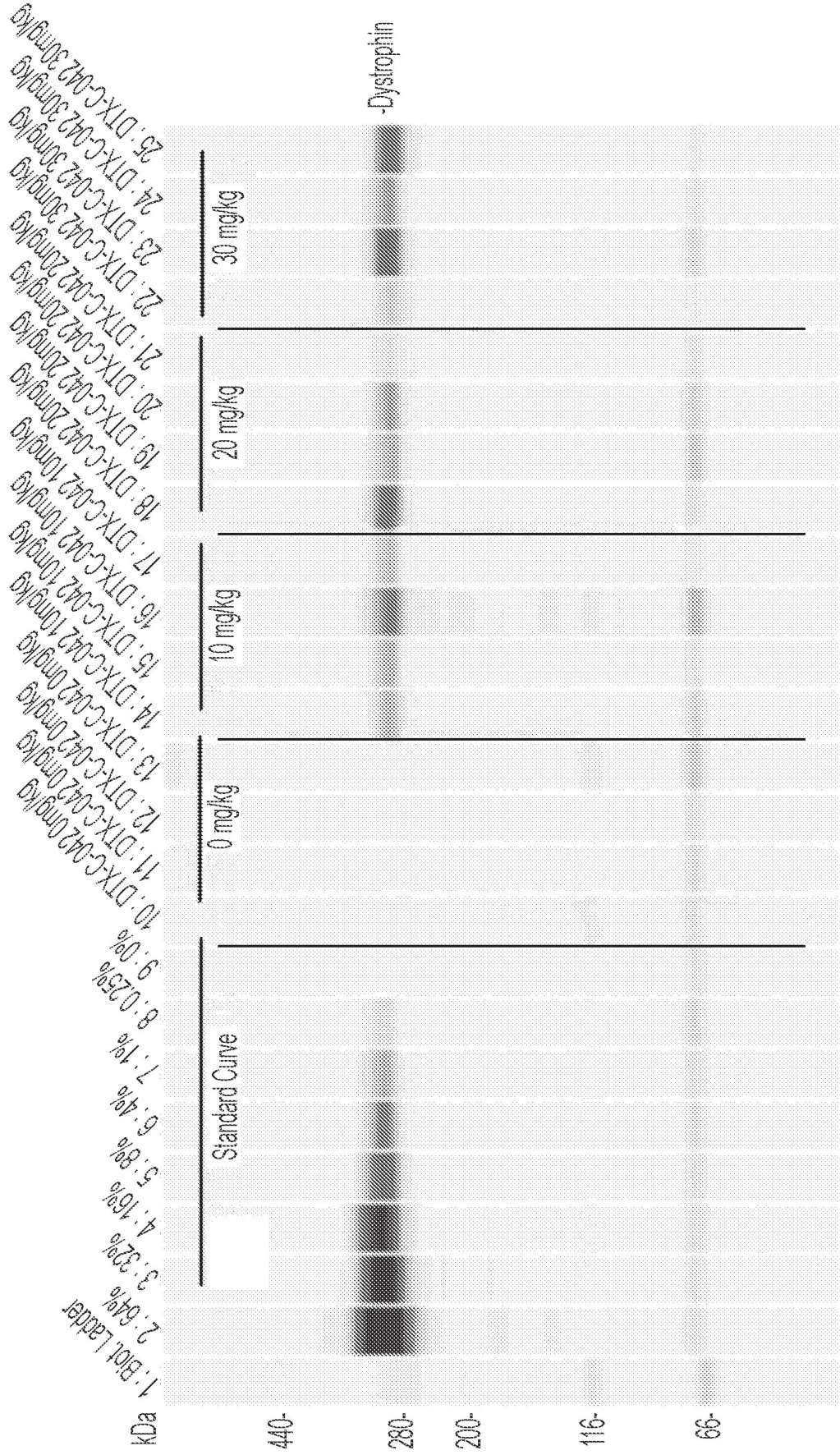

kDa
440-
280-
200-
116-
66-
1. Bio-Rad Ladder
2. 64%
3. 32%
4. 16%
5. 8%
6. 4%
7. 1%
8. 0.25%
9. 0%
10. DTX-C-042 0mg/kg
11. DTX-C-042 0mg/kg
12. DTX-C-042 0mg/kg
13. DTX-C-042 0mg/kg
14. DTX-C-042 0mg/kg
15. DTX-C-042 10mg/kg
16. DTX-C-042 10mg/kg
17. DTX-C-042 10mg/kg
18. DTX-C-042 10mg/kg
19. DTX-C-042 20mg/kg
20. DTX-C-042 20mg/kg
21. DTX-C-042 20mg/kg
22. DTX-C-042 20mg/kg
23. DTX-C-042 30mg/kg
24. DTX-C-042 30mg/kg
25. DTX-C-042 30mg/kg
Standard Curve
0 mg/kg
10 mg/kg
20 mg/kg
30 mg/kg
-Dystrophin

FIG. 6A

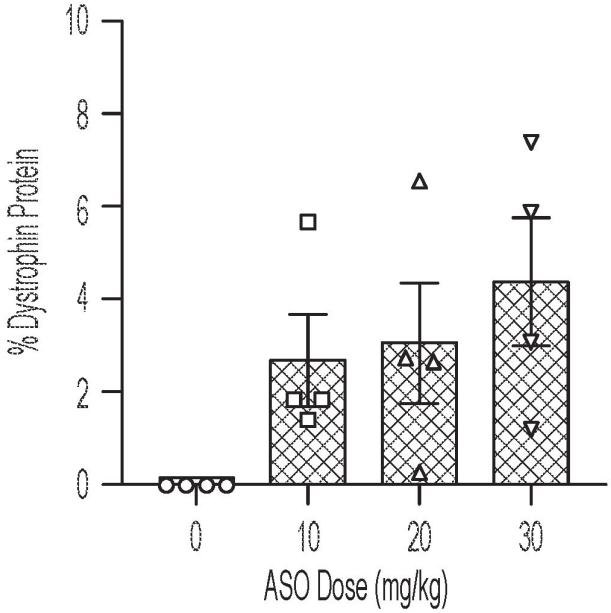


FIG. 6B

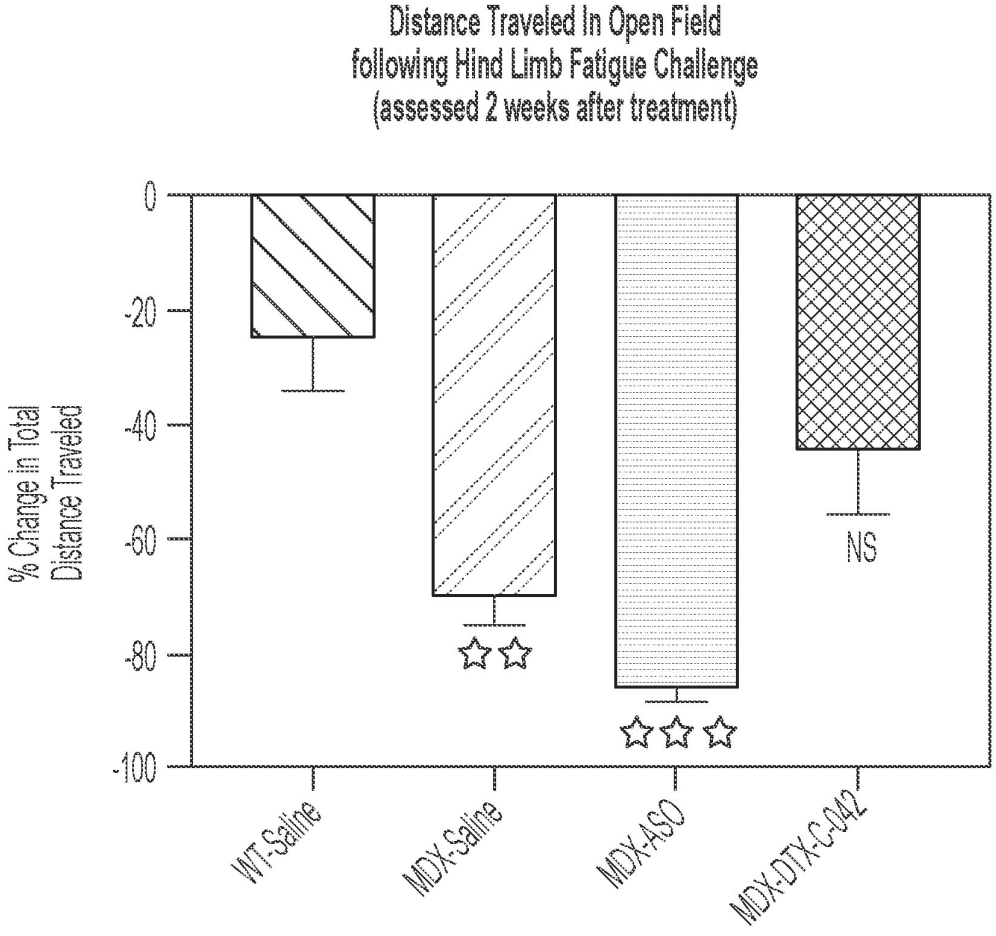

Distance Traveled In Open Field
following Hind Limb Fatigue Challenge
(assessed 2 weeks after treatment)
% Change in Total Distance Traveled
0
-20
-40
-60
-80
-100
NS
WT-Saline
MDX-Saline
MDX-ASO
MDX-DTX-C-042

FIG. 7A

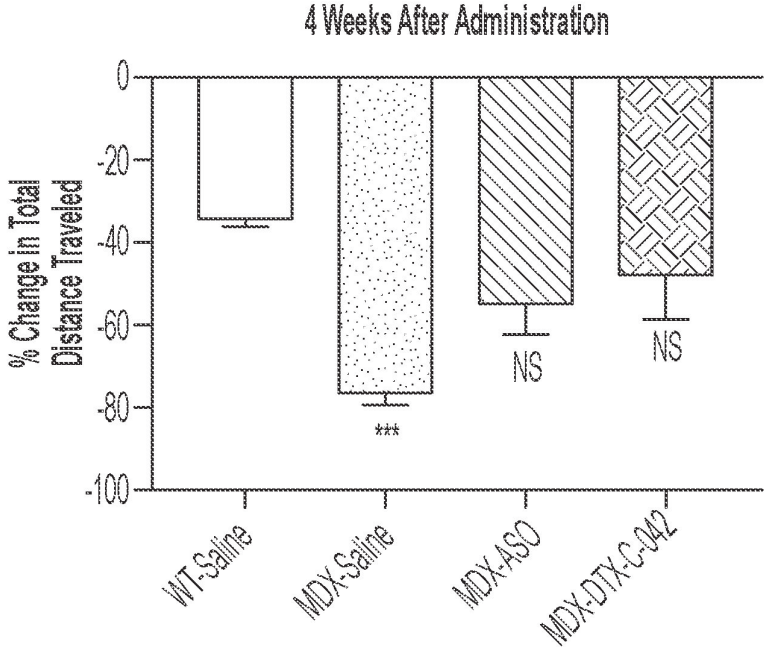

4 Weeks After Administration
% Change in Total Distance Traveled
0
-20
-40
-60
-80
-100
***
NS
NS
WT-Saline
MDX-Saline
MDX-ASO
MDX-DTX-C-042

FIG. 7B

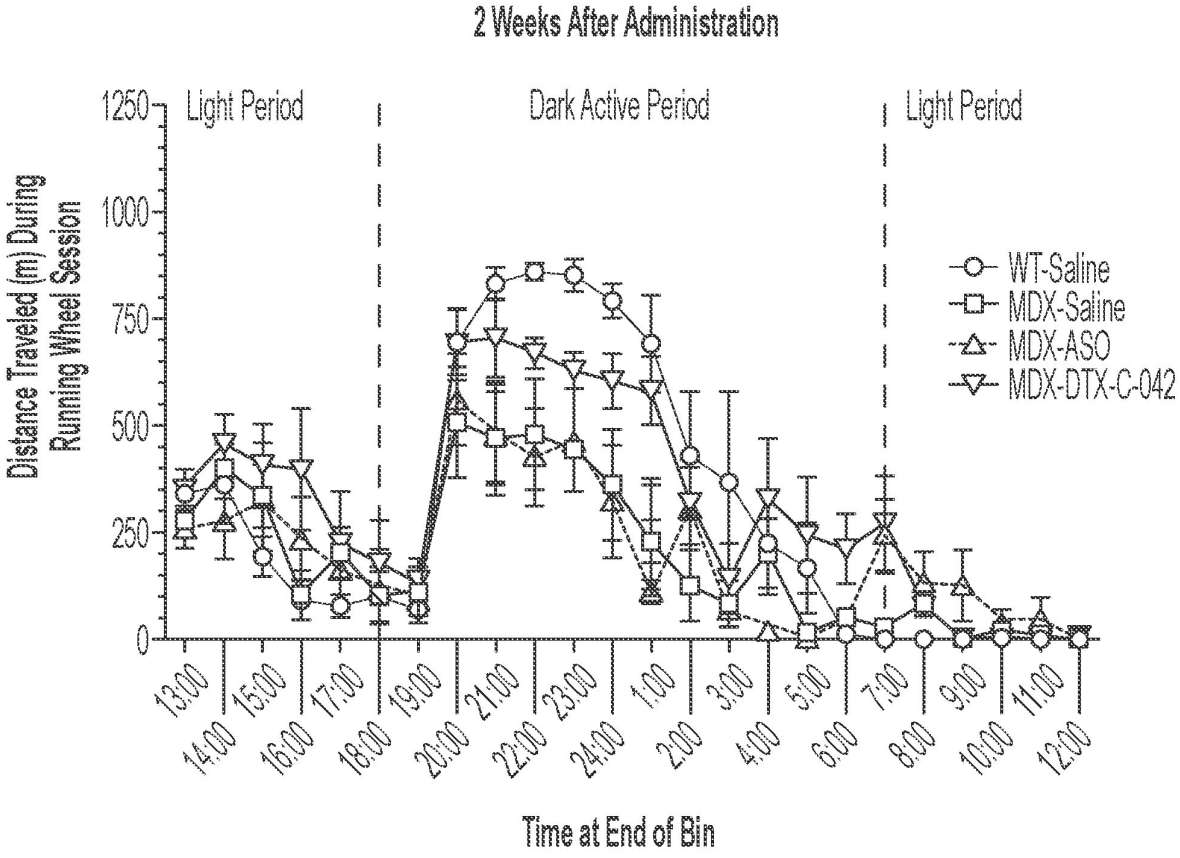

2 Weeks After Administration
Light Period
Dark Active Period
Light Period
Distance Traveled (m) During Running Wheel Session
1250
1000
750
500
250
0
WT-Saline
MDX-Saline
MDX-ASO
MDX-DTX-C-042
13:00
14:00
15:00
16:00
17:00
18:00
19:00
20:00
21:00
22:00
23:00
24:00
1:00
2:00
3:00
4:00
5:00
6:00
7:00
8:00
9:00
10:00
11:00
12:00
Time at End of Bin

FIG. 7C

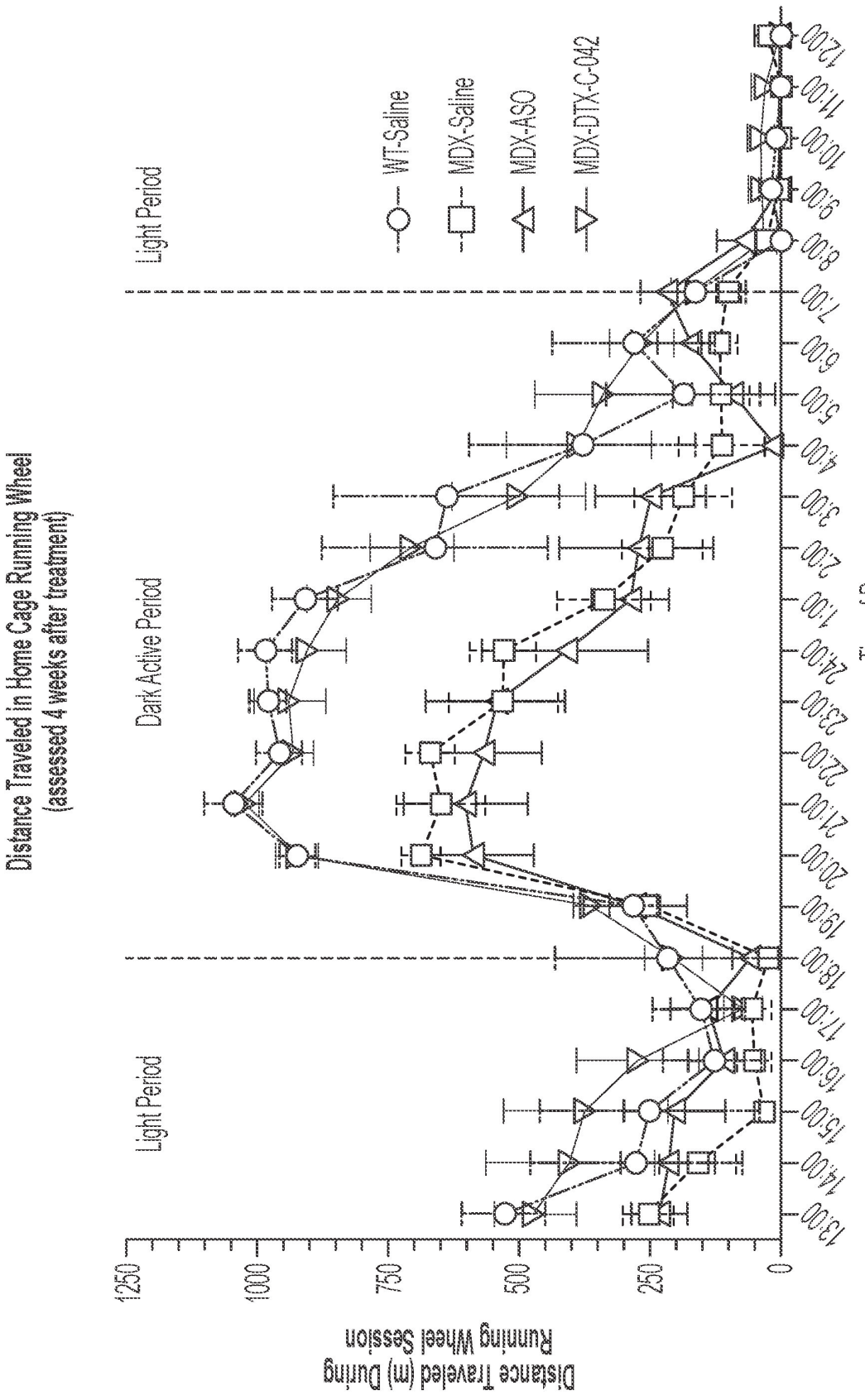

Distance Traveled in Home Cage Running Wheel
(assessed 4 weeks after treatment)
Light Period
Dark Active Period
Light Period
WT-Saline
MDX-Saline
MDX-ASO
MDX-DTX-C-042
Distance Traveled (m) During
Running Wheel Session
Time of Day
0
250
500
750
1000
1250
1300
1400
1500
1600
1700
1800
1900
2000
2100
2200
2300
2400
100
200
300
400
500
600
700
800
900
1000
1100
1200

FIG. 7D

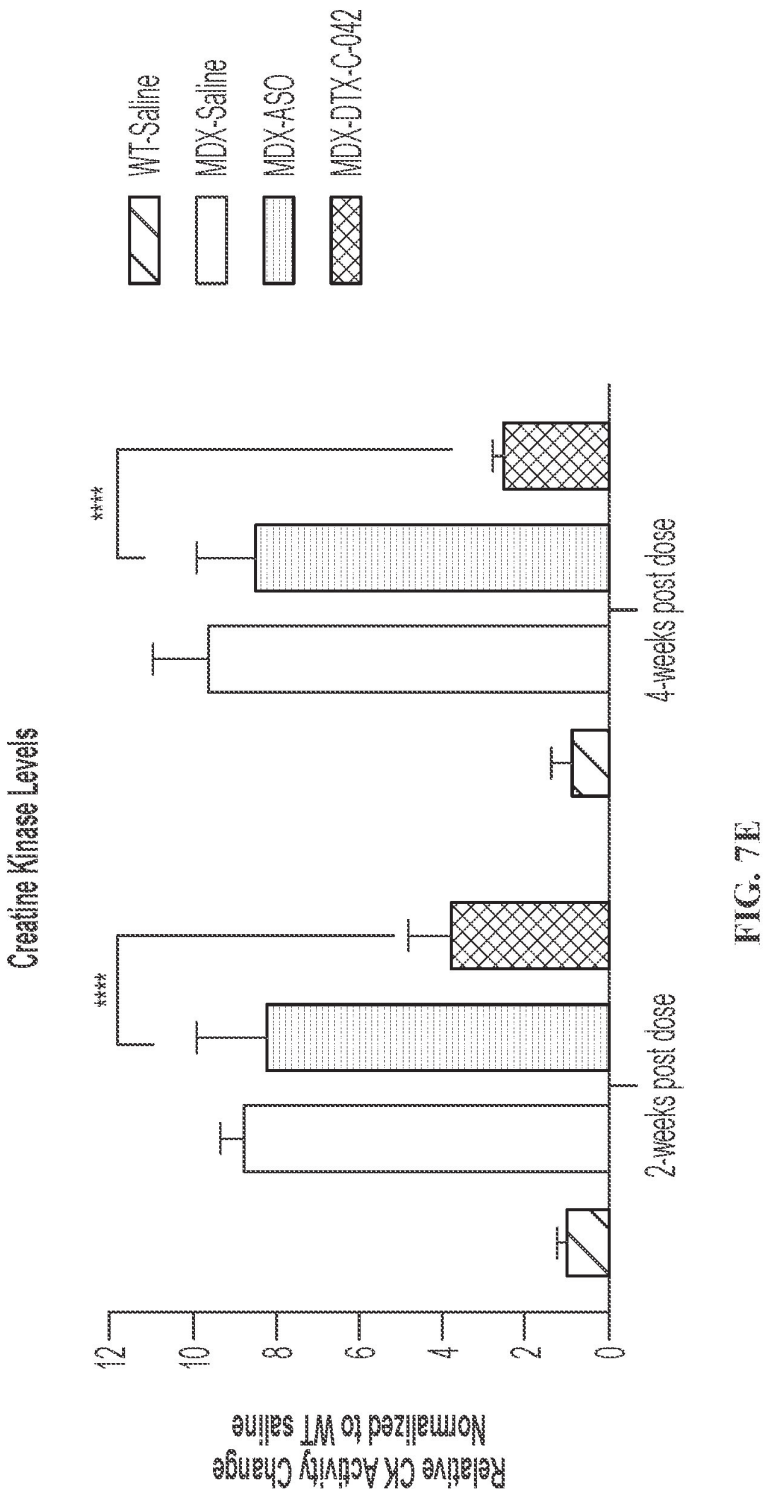

Creatine Kinase Levels
Relative CK Activity Change
Normalized to WT saline
12
10
8
6
4
2
0
2-weeks post dose
4-weeks post dose
****
****
WT-Saline
MDX-Saline
MDX-ASO
MDX-DTX-C-042

FIG. 7E

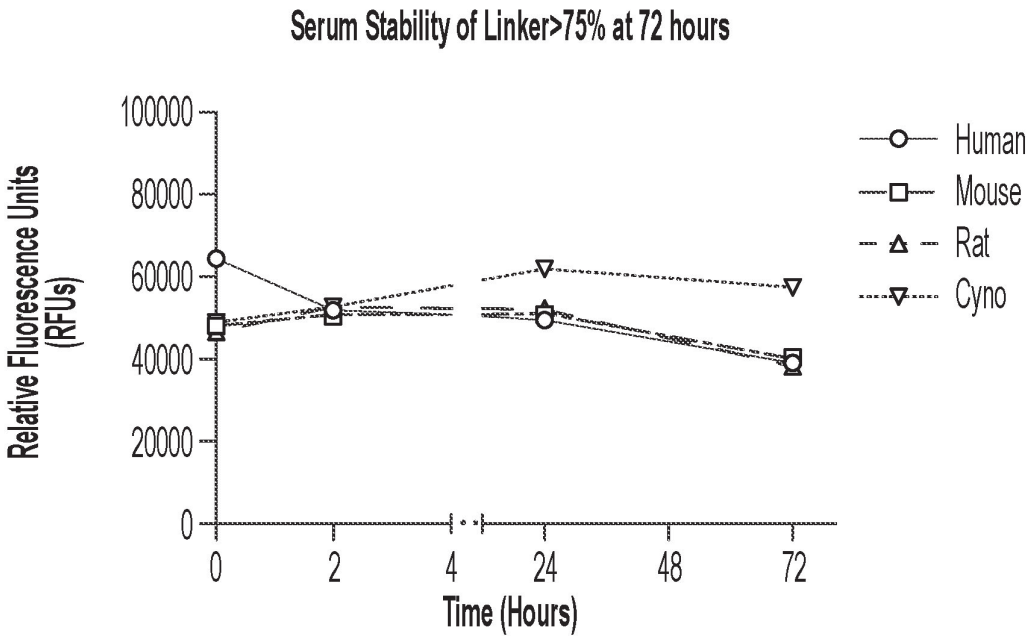

Serum Stability of Linker>75% at 72 hours
Relative Fluorescence Units (RFUs)
100000
80000
60000
40000
20000
0
0
2
4
24
48
72
Time (Hours)
Human
Mouse
Rat
Cyno

FIG. 8

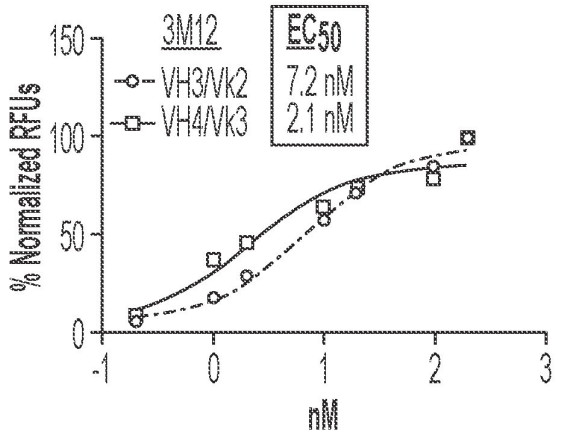


FIG. 9A

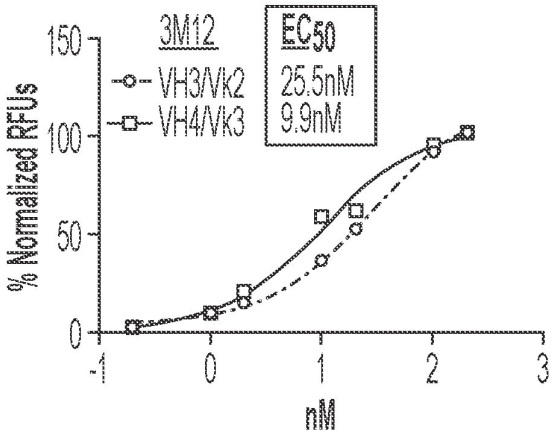


FIG. 9B

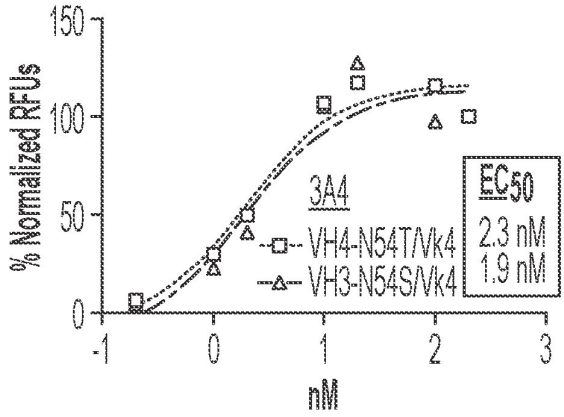


FIG. 9C

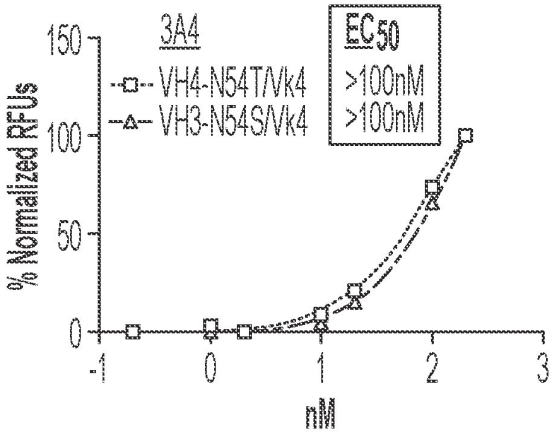


FIG. 9D

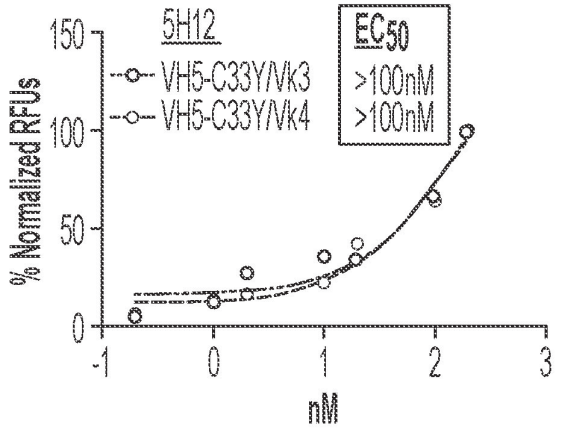


FIG. 9E

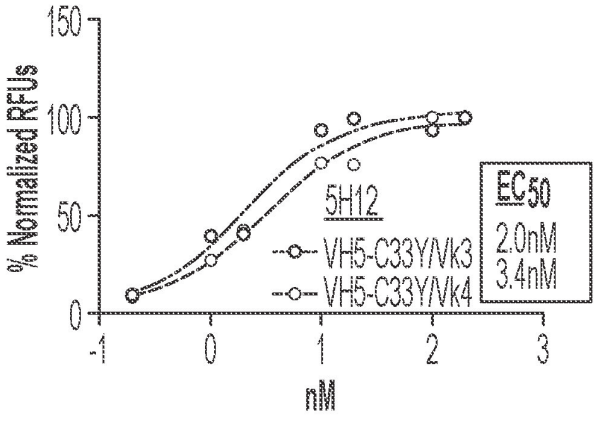


FIG. 9F

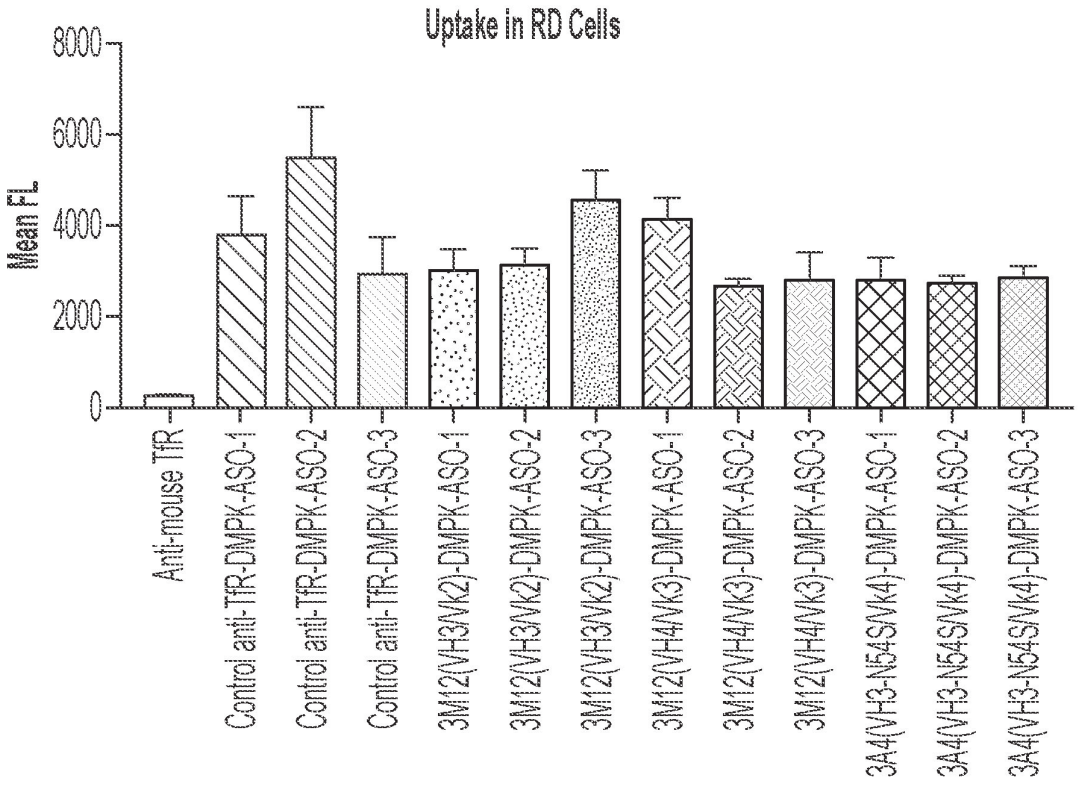

Uptake in RD Cells
Mean FL
8000
6000
4000
2000
0
Anti-mouse TfR
Control anti-TfR-DMPK-ASO-1
Control anti-TfR-DMPK-ASO-2
Control anti-TfR-DMPK-ASO-3
3M12(VH3/Vk2)-DMPK-ASO-1
3M12(VH3/Vk2)-DMPK-ASO-2
3M12(VH3/Vk2)-DMPK-ASO-3
3M12(VH4/Vk3)-DMPK-ASO-1
3M12(VH4/Vk3)-DMPK-ASO-2
3M12(VH4/Vk3)-DMPK-ASO-3
3A4(VH3-N54S/Vk4)-DMPK-ASO-1
3A4(VH3-N54S/Vk4)-DMPK-ASO-2
3A4(VH3-N54S/Vk4)-DMPK-ASO-3

FIG. 10

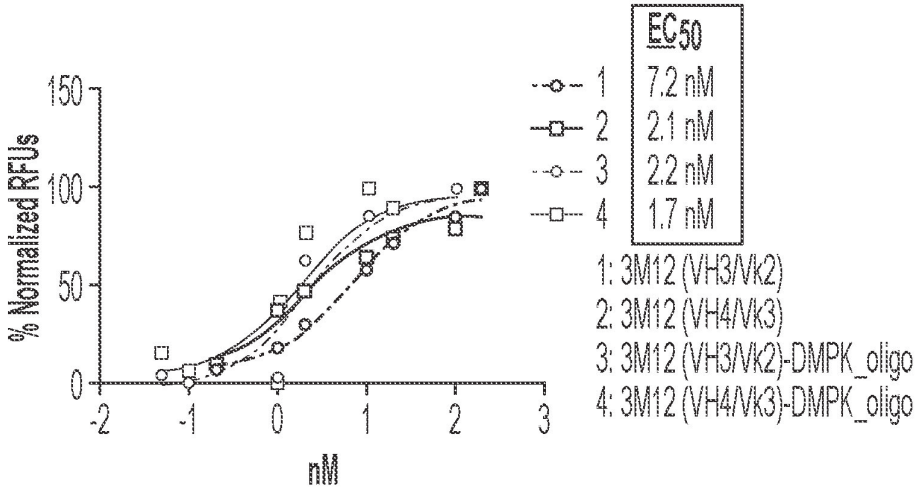


FIG. 11A

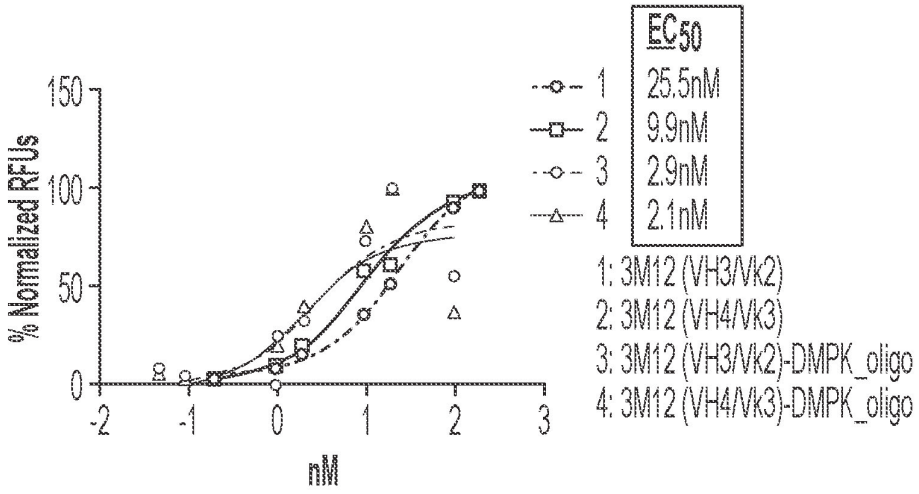


FIG. 11B

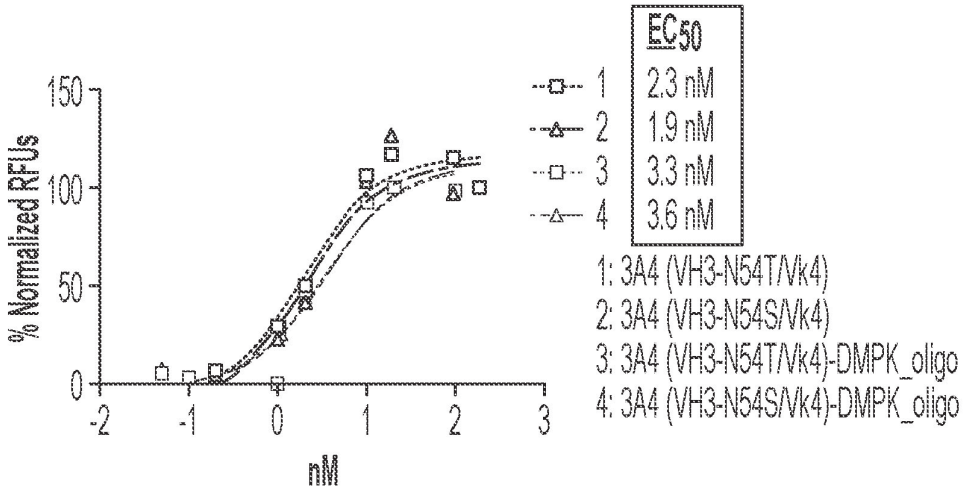


FIG. 11C

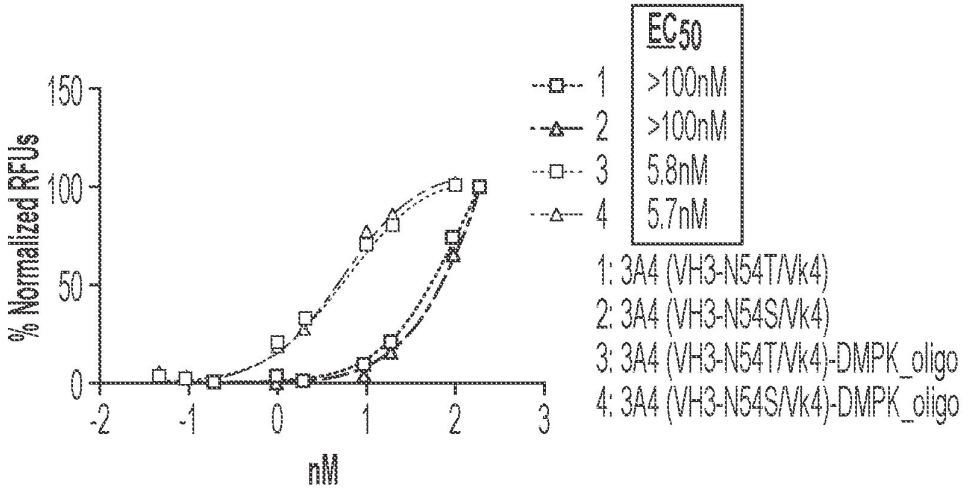


FIG. 11D

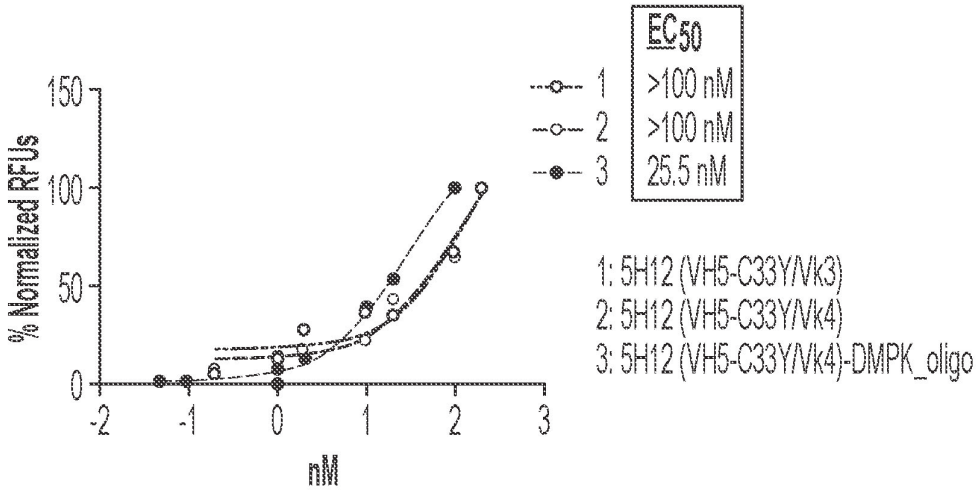


FIG. 11E

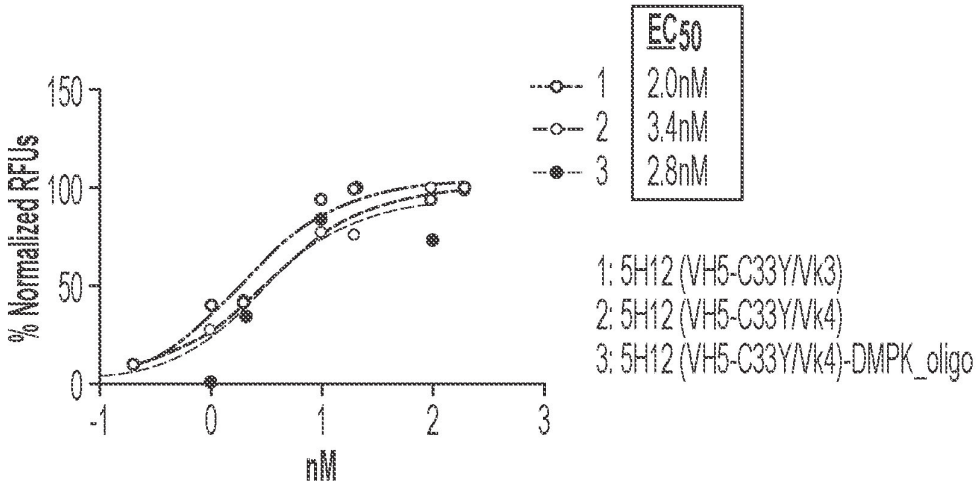


FIG. 11F

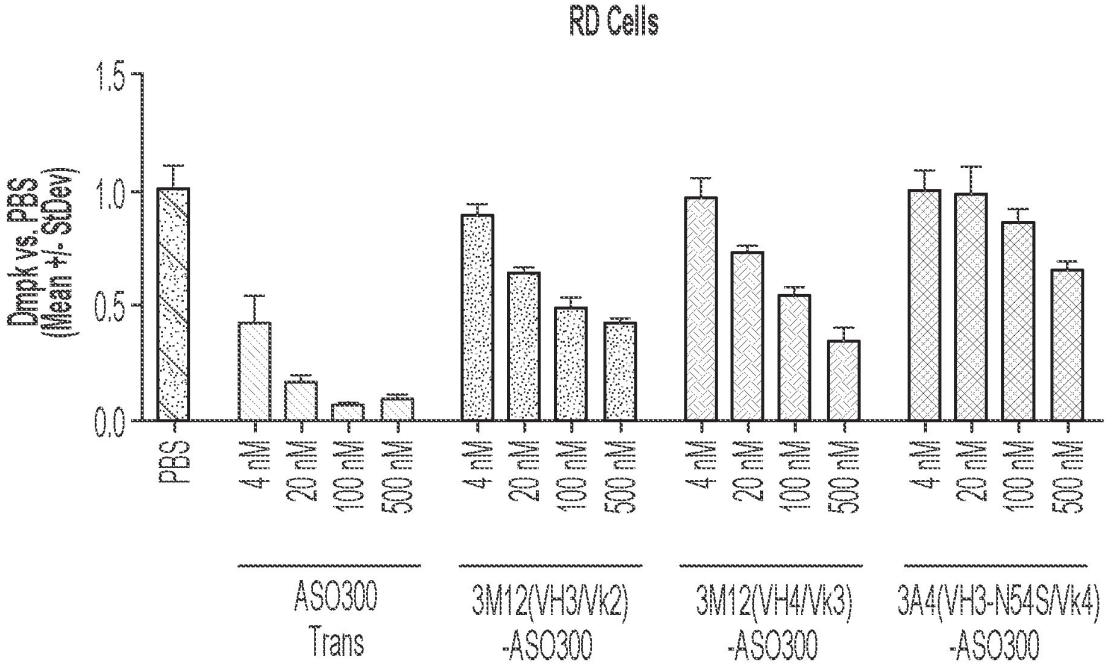

RD Cells
Dmpk vs. PBS
(Mean +/- StDev)
1.5
1.0
0.5
0.0
PBS
4 nM
20 nM
100 nM
500 nM
4 nM
20 nM
100 nM
500 nM
4 nM
20 nM
100 nM
500 nM
4 nM
20 nM
100 nM
500 nM
ASO300
Trans
3M12(VH3/Vk2)
-ASO300
3M12(VH4/Vk3)
-ASO300
3A4(VH3-N54S/Vk4)
-ASO300

FIG. 12

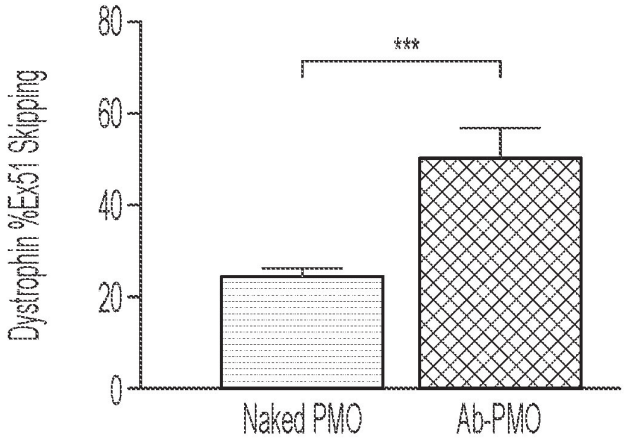


FIG. 13

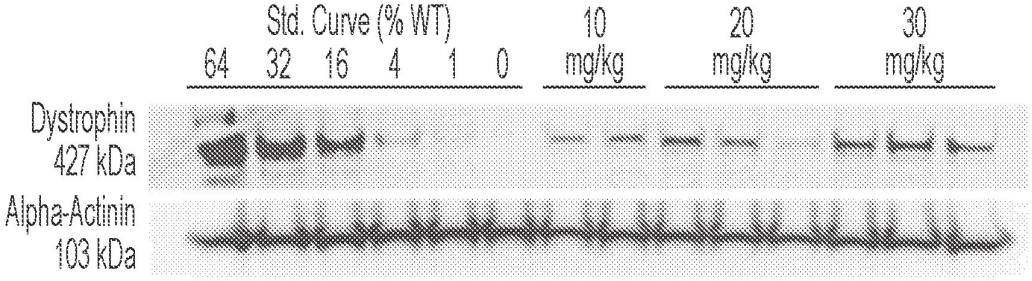


FIG. 14

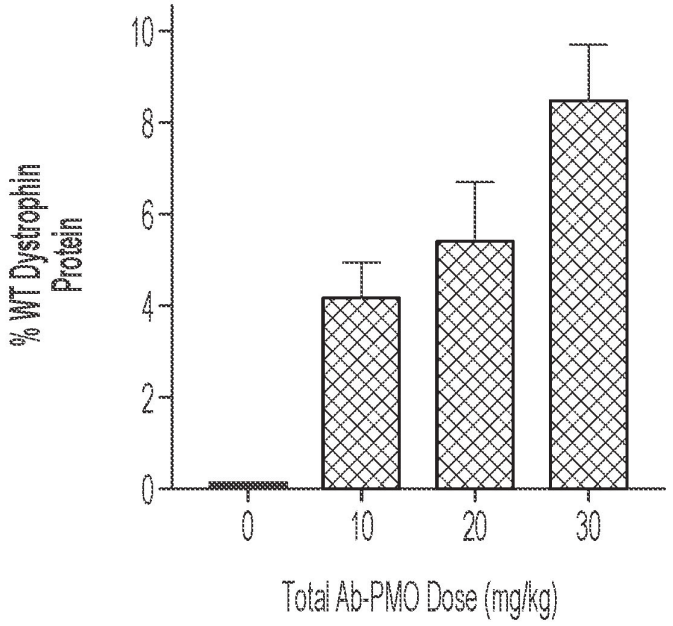


FIG. 15

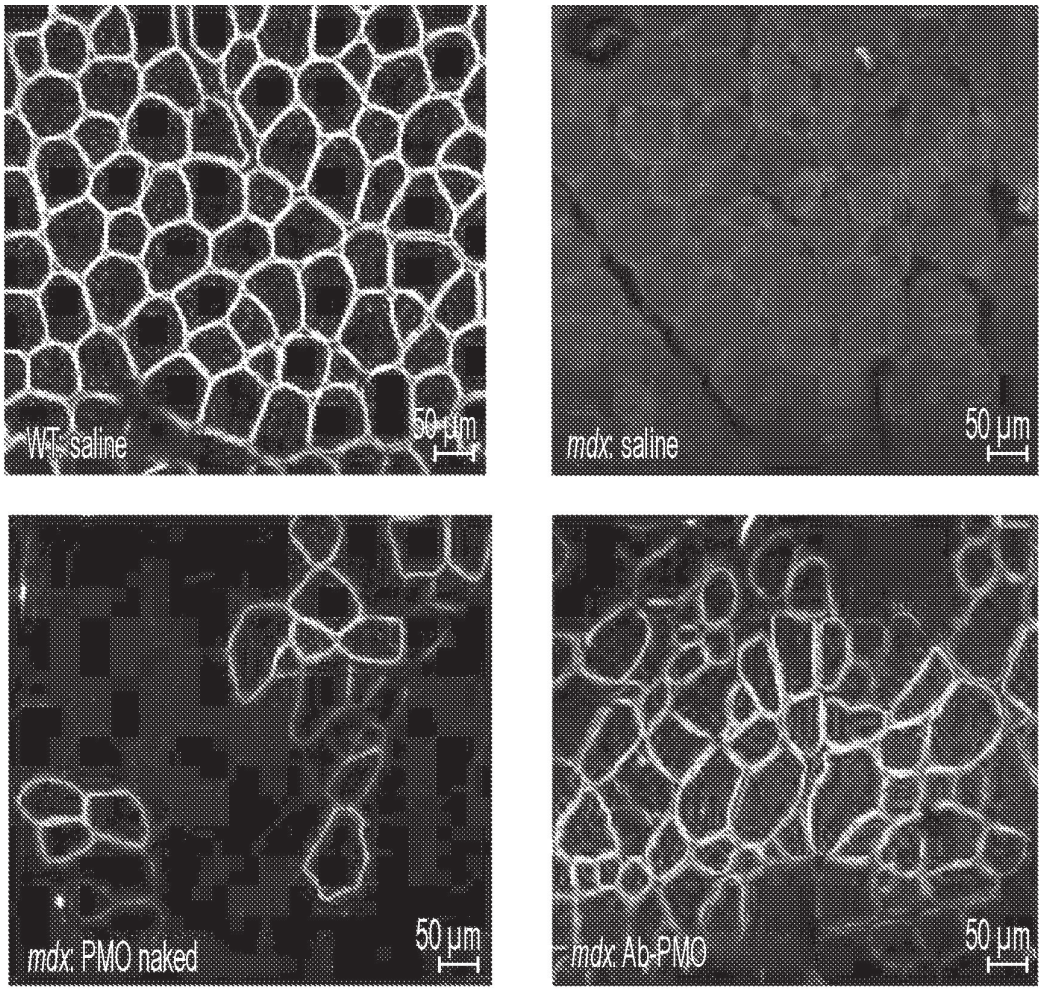

WT: saline
50 μm
mdx: saline
50 μm
mdx: PMO naked
50 μm
mdx: Ab-PMO
50 μm

FIG. 16

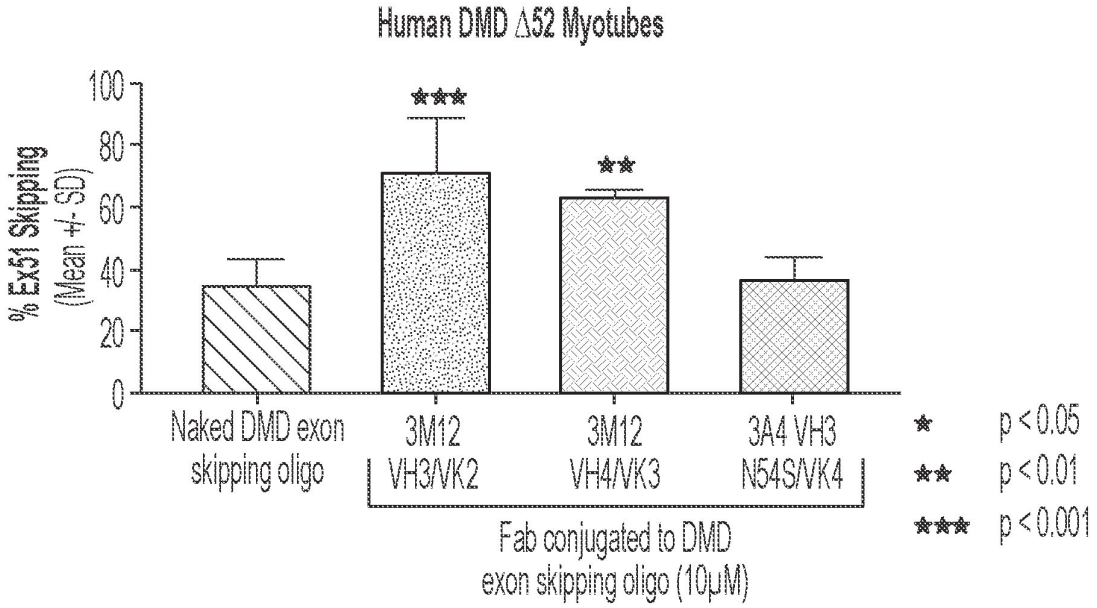

Human DMD Δ52 Myotubes
% Ex51 Skipping (Mean +/- SD)
100
80
60
40
20
0
***
**
Naked DMD exon skipping oligo
3M12 VH3/VK2
3M12 VH4/VK3
3A4 VH3 N54S/VK4
Fab conjugated to DMD exon skipping oligo (10μM)
* p < 0.05
** p < 0.01
*** p < 0.001

FIG. 17

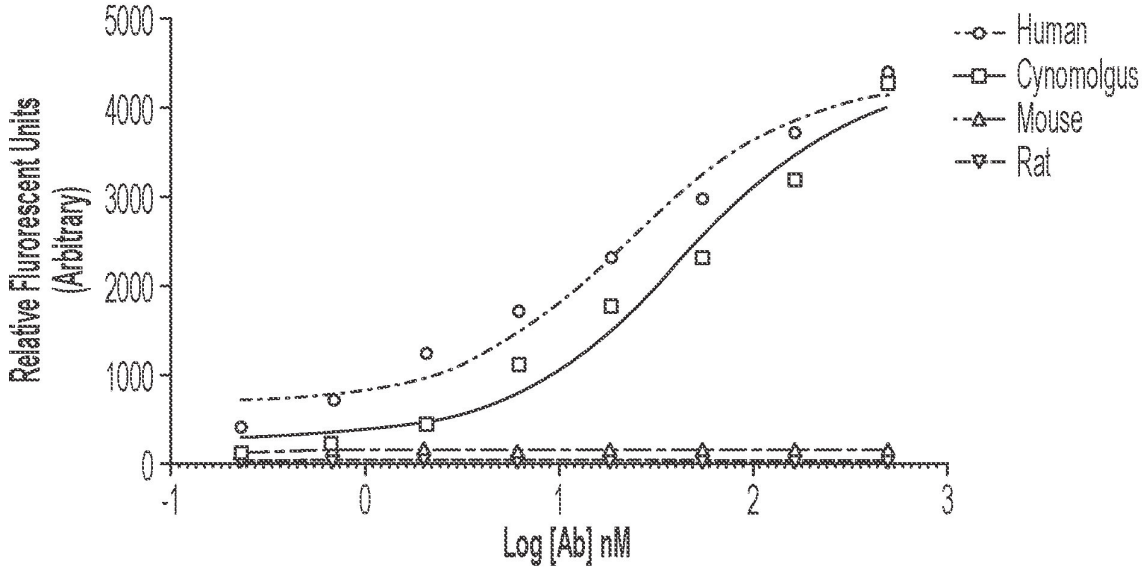

Relative Fluorescent Units (Arbitrary)
5000
4000
3000
2000
1000
0
-1
0
1
2
3
Log [Ab] nM
Human
Cynomolgus
Mouse
Rat

FIG. 18

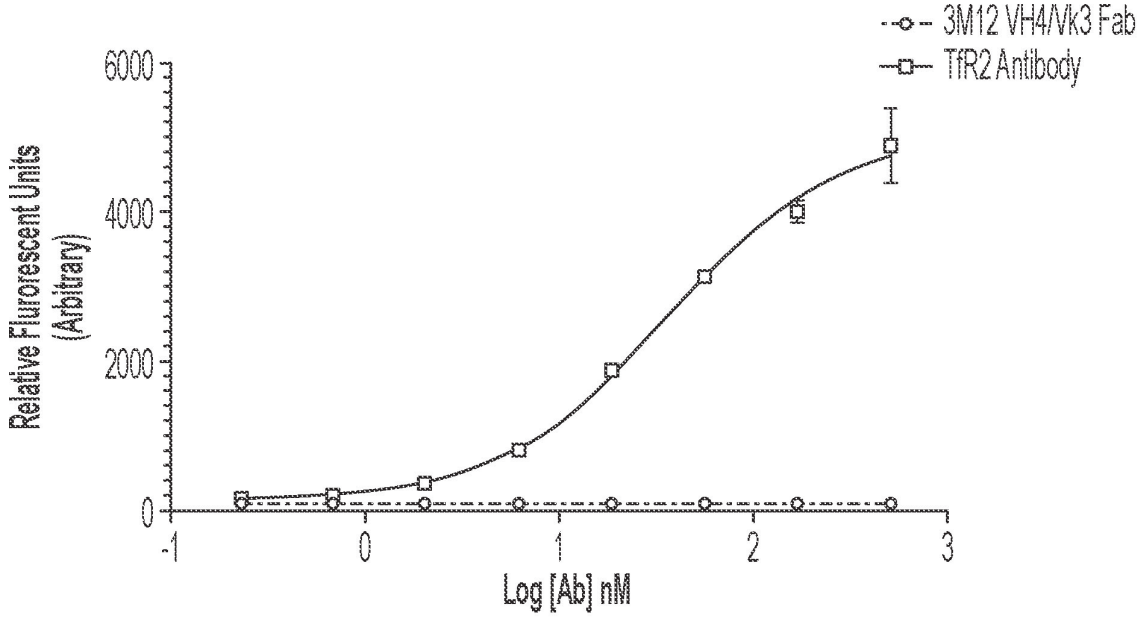

Relative Fluorescent Units (Arbitrary)
6000
4000
2000
0
-1
0
1
2
3
Log [Ab] nM
3M12 VH4/Vk3 Fab
TfR2 Antibody

FIG. 19

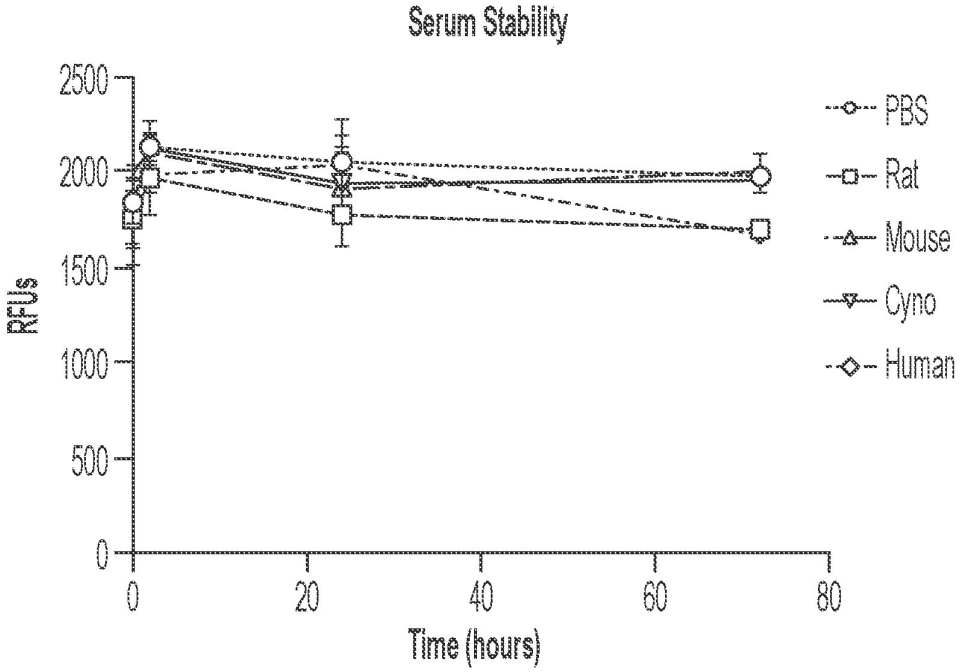

Serum Stability
2500
2000
1500
1000
500
0
RFUs
0
20
40
60
80
Time (hours)
PBS
Rat
Mouse
Cyno
Human

FIG. 20

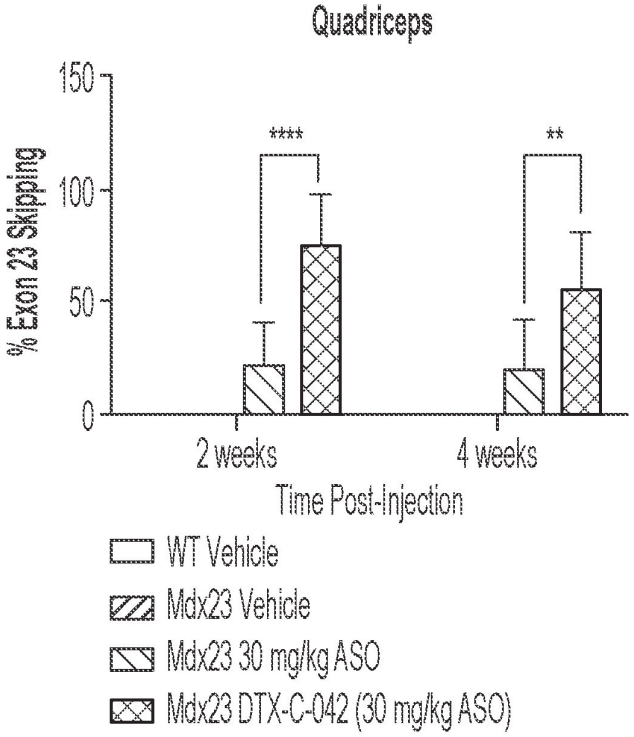


FIG. 21A

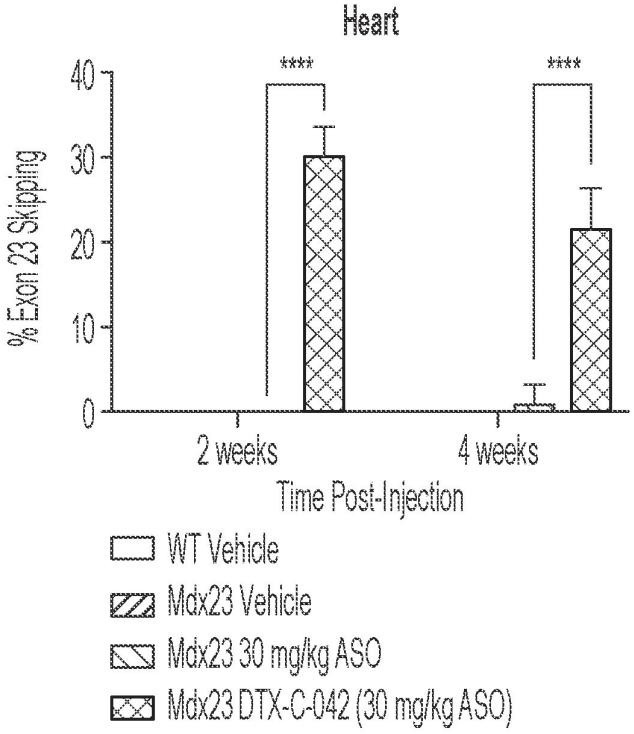


FIG. 21B

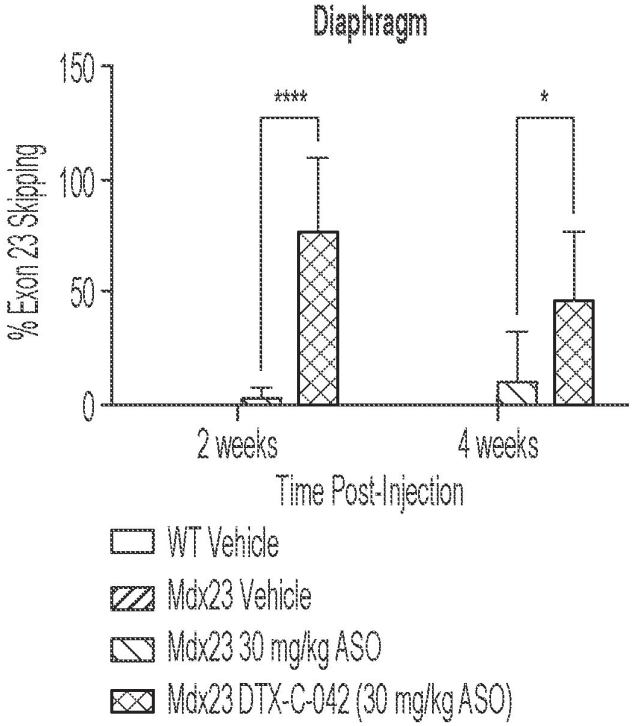


FIG. 21C

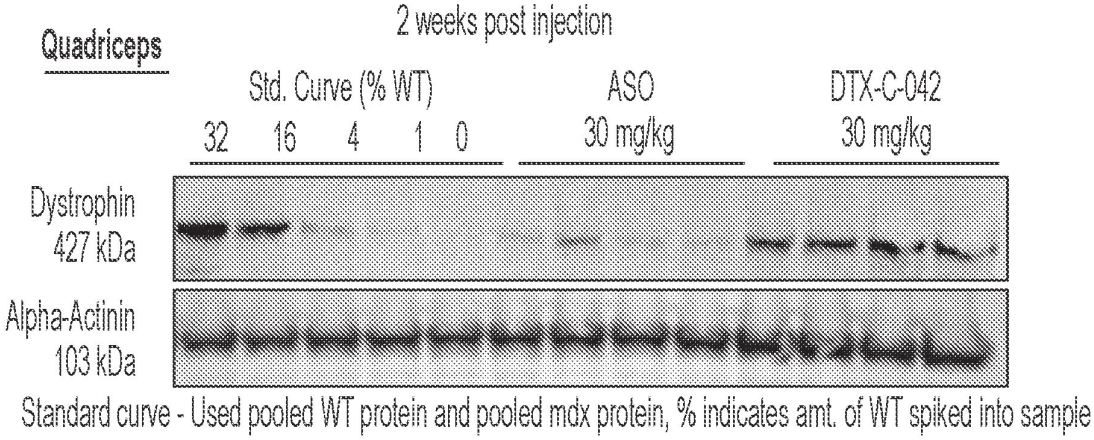


FIG. 22A

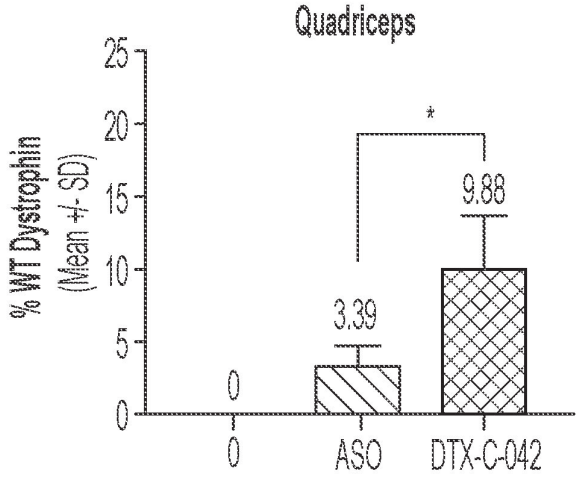


FIG. 22B

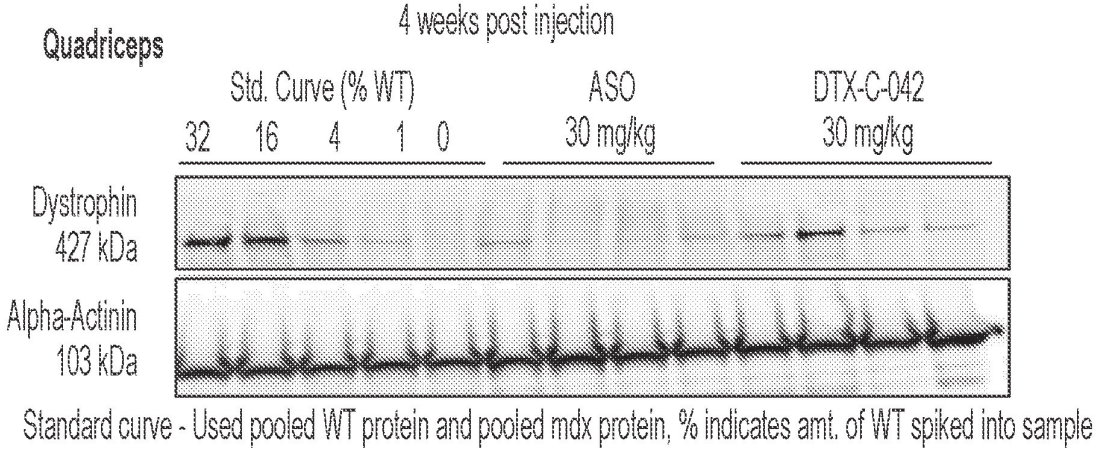


FIG. 22C

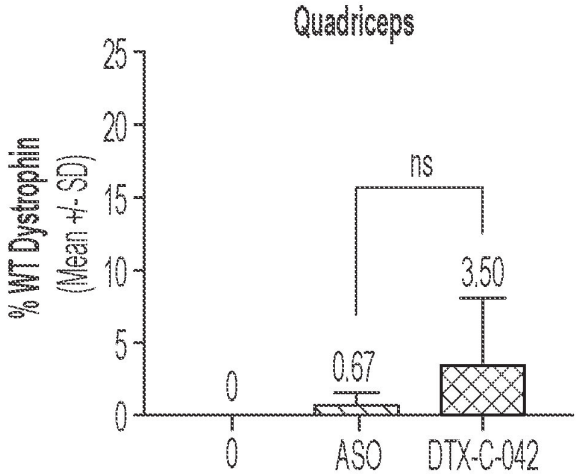
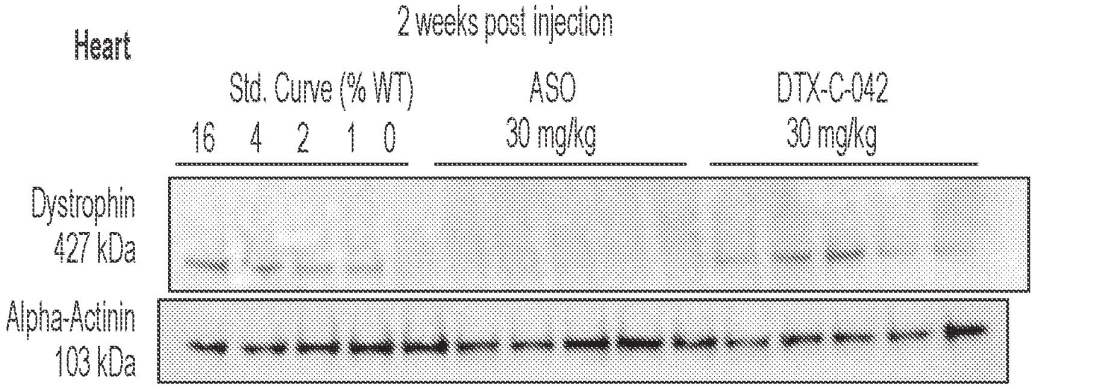


FIG. 22D



Standard curve - Used pooled WT protein and pooled mdx protein, % indicates amt. of WT spiked into sample

FIG. 23A

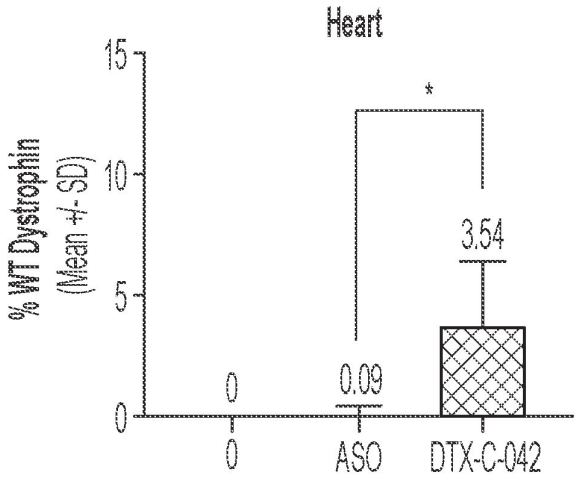
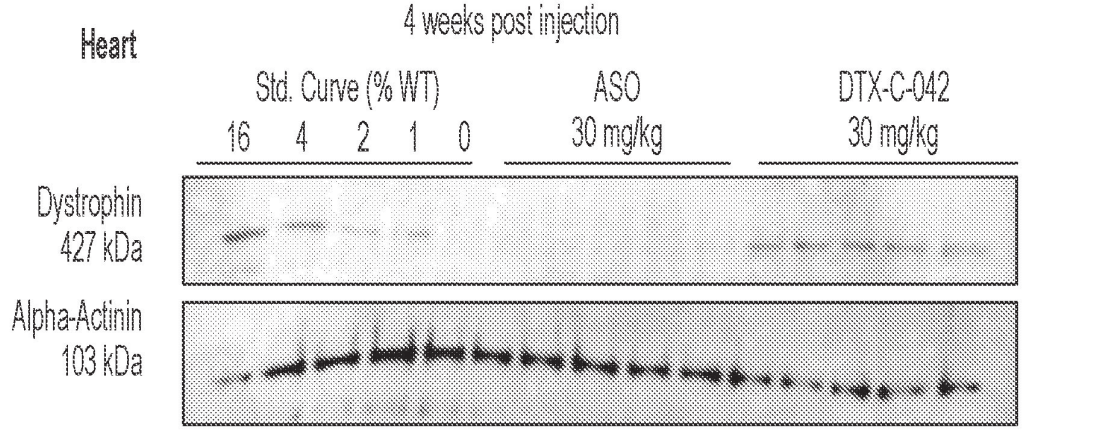


FIG. 23B



Standard curve - Used pooled WT protein and pooled mdx protein, % indicates amt. of WT spiked into sample

FIG. 23C

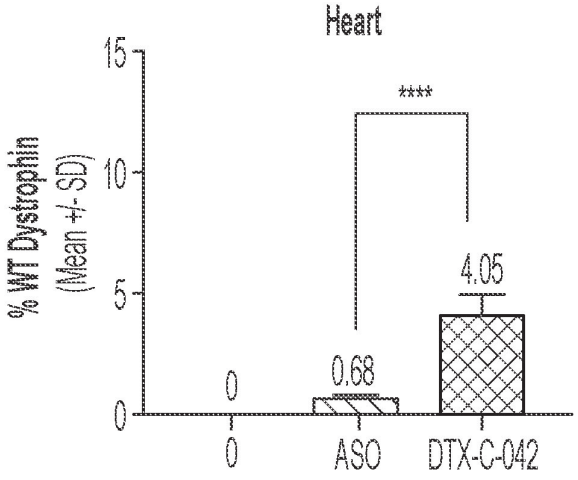
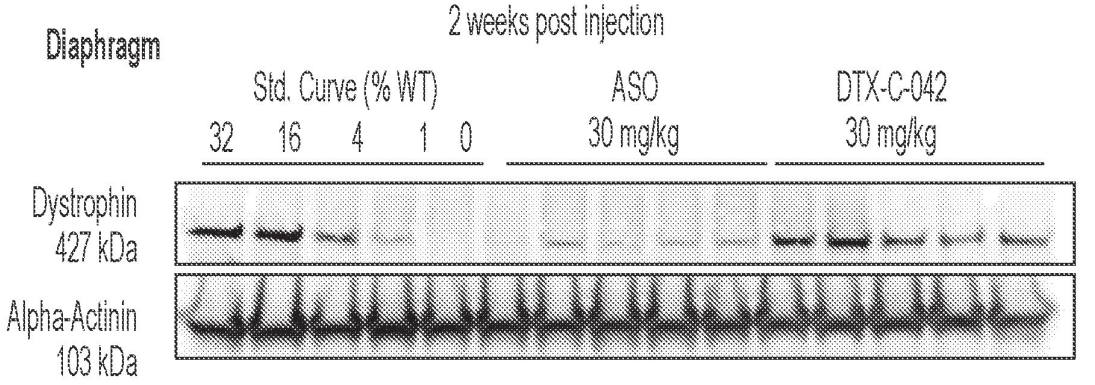


FIG. 23D



Standard curve - Used pooled WT protein and pooled mdx protein, % indicates amt. of WT spiked into sample

FIG. 24A

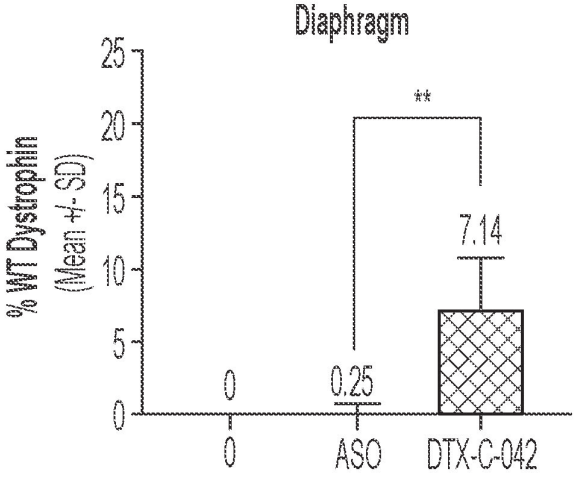


FIG. 24B

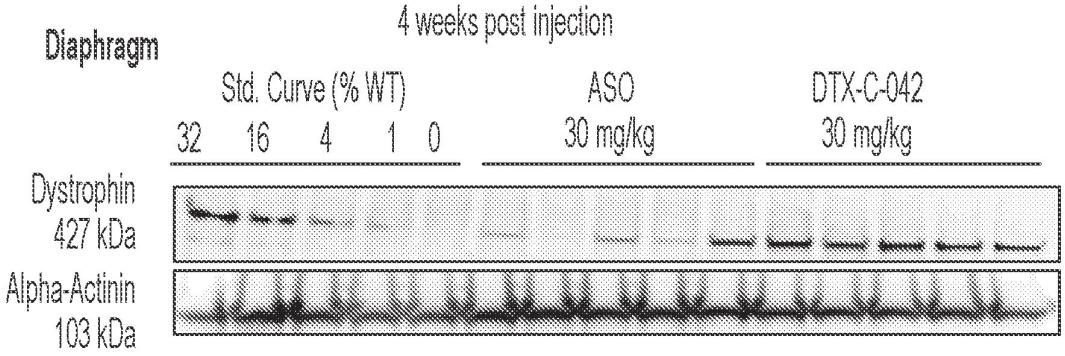


FIG. 24C

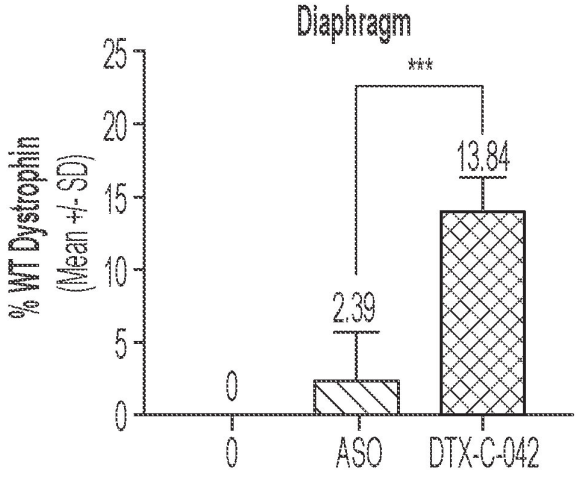


FIG. 24D

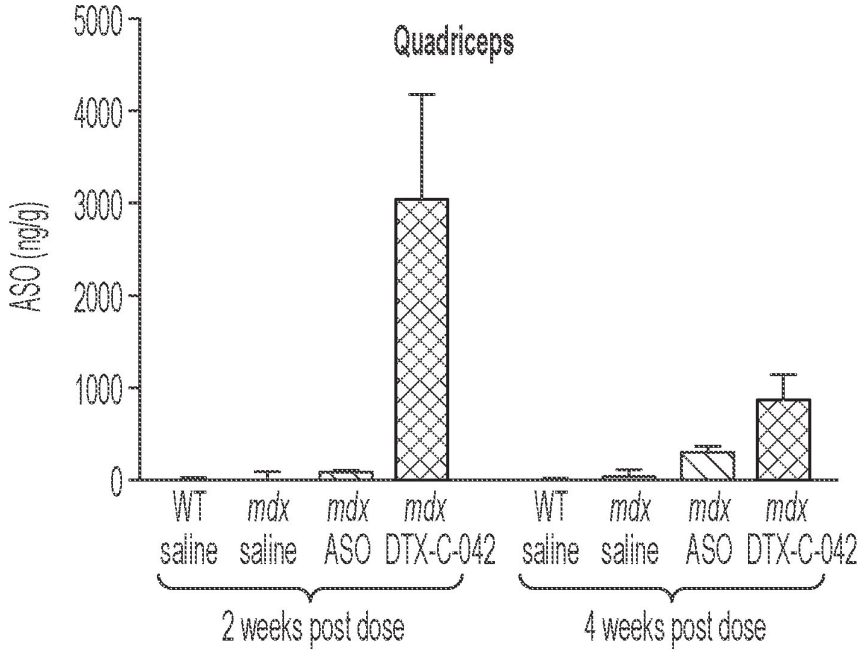


FIG. 25A

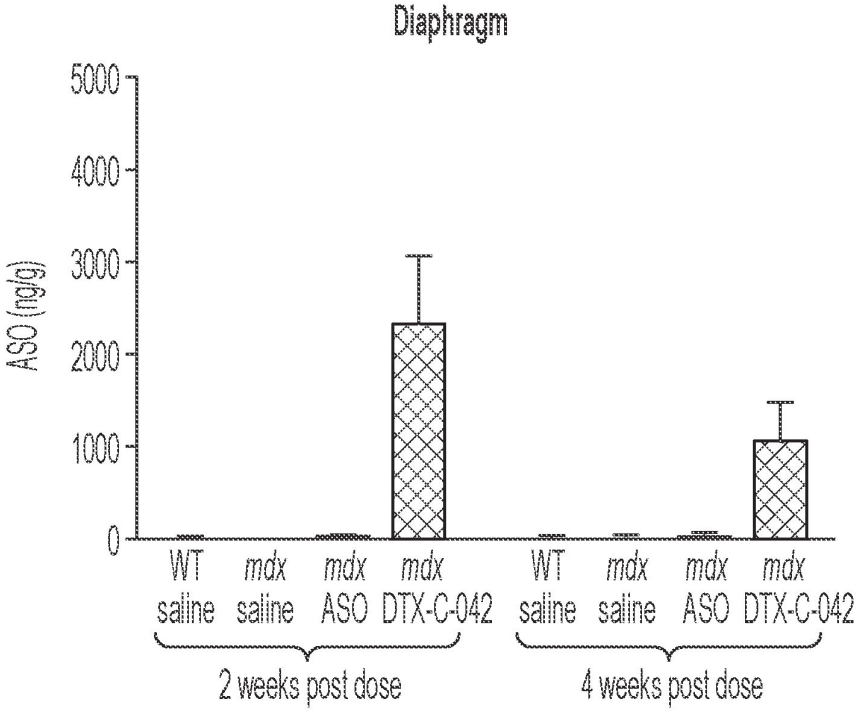


FIG. 25B

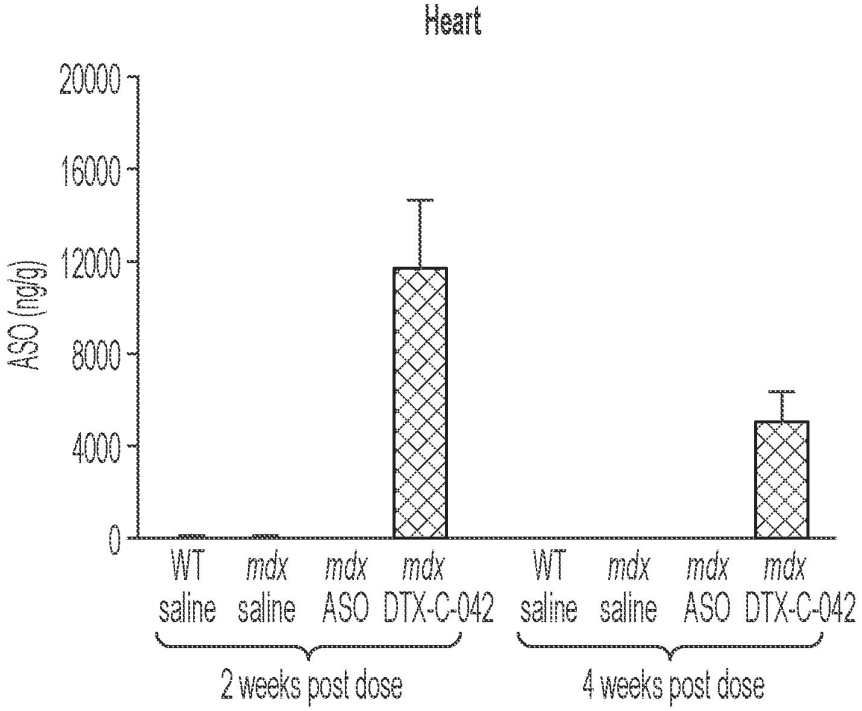


FIG. 25C

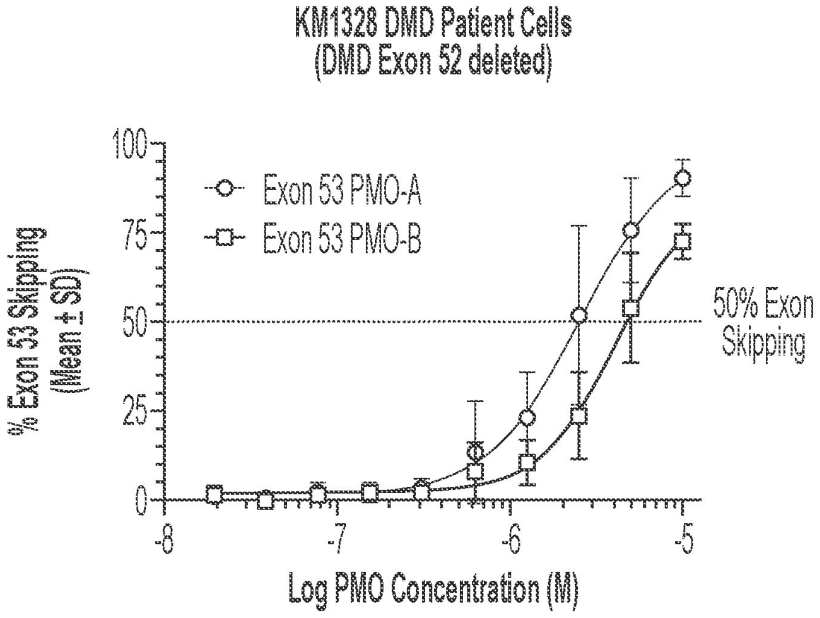

KM1328 DMD Patient Cells
(DMD Exon 52 deleted)
% Exon 53 Skipping
(Mean ± SD)
100
75
50
25
0
Exon 53 PMO-A
Exon 53 PMO-B
50% Exon
Skipping
-8
-7
-6
-5
Log PMO Concentration (M)

FIG. 26

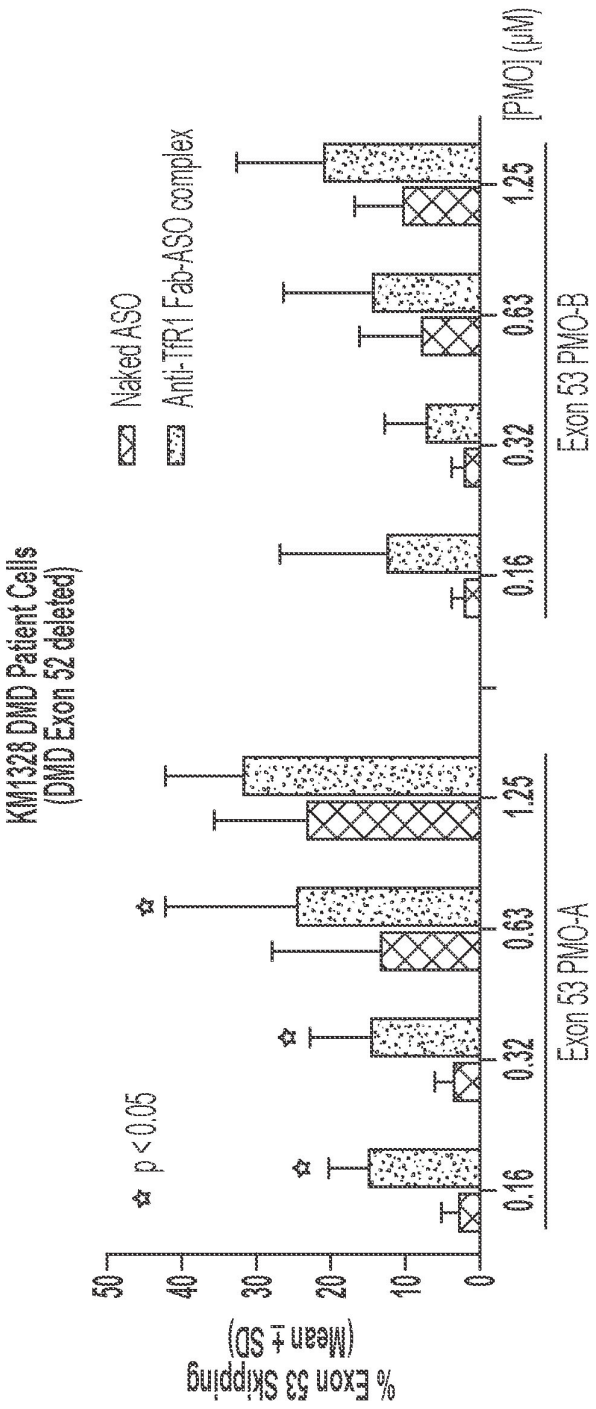

KM1328 DMD Patient Cells
(DMD Exon 52 deleted)
Naked ASO
Anti-TfR1 Fab-ASO complex
p < 0.05
% Exon 53 Skipping
(Mean ± SD)
50
40
30
20
10
0
0.16
0.32
0.63
1.25
Exon 53 PMO-A
0.16
0.32
0.63
1.25
Exon 53 PMO-B
[PMO] (μM)

FIG. 27