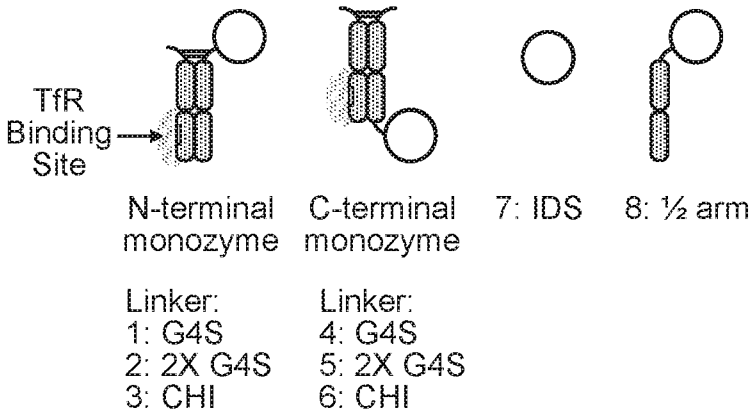

TfR
Binding
Site
N-terminal
monozyme
C-terminal
monozyme
7: IDS
8: ½ arm
Linker:
1: G4S
2: 2X G4S
3: CHI
Linker:
4: G4S
5: 2X G4S
6: CHI

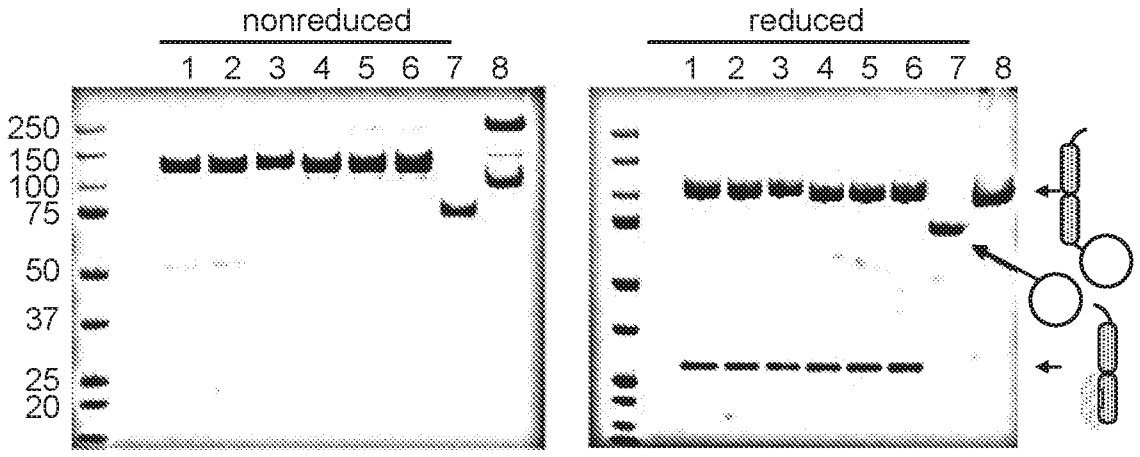
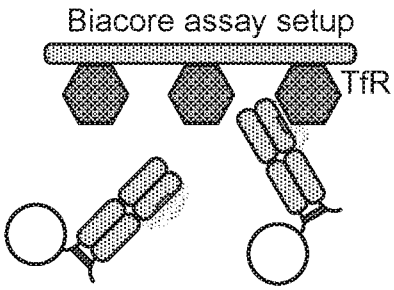
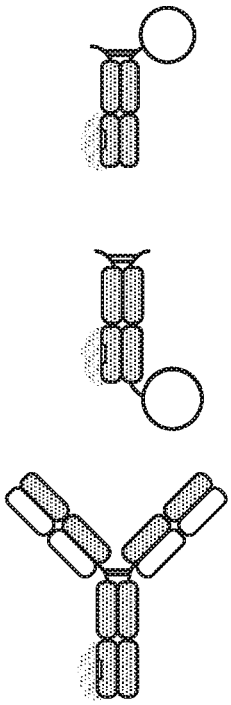

nonreduced
reduced
1 2 3 4 5 6 7 8
250
150
100
75
50
37
25
20

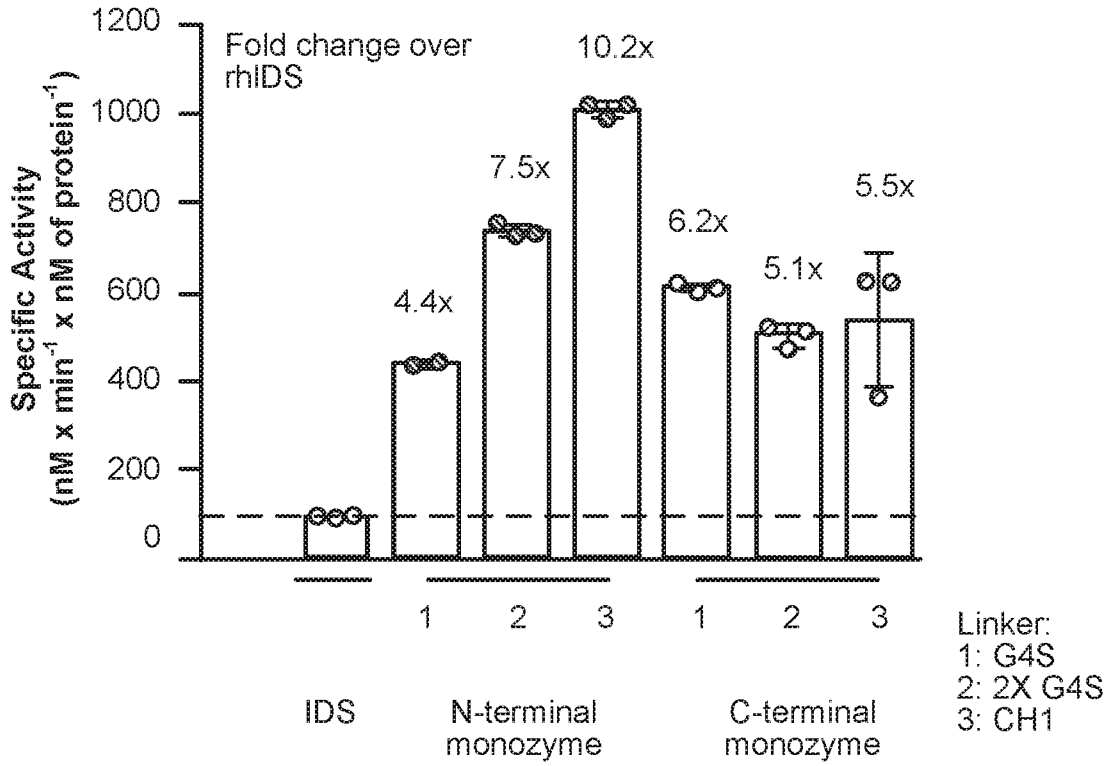
FIG. 1





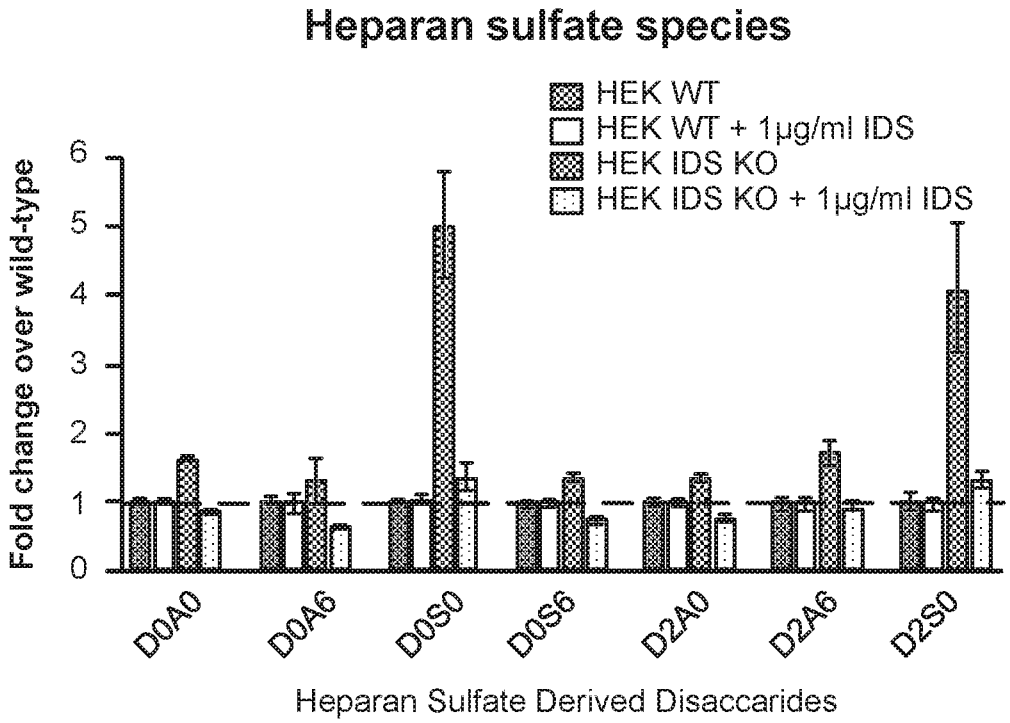
Linker	TfR affinity (nM)
G_4S	300
$(G_4S)_2$	460
CH1-VH	450
G_4S	750
$(G_4S)_2$	670
CH1-VH	700
parental ATV	270

FIG. 2



Technical replicates n = 3, data shown as mean ± SD

FIG. 3



Data shown as mean and s.e.m. from three IDS KO cell lines

FIG. 4

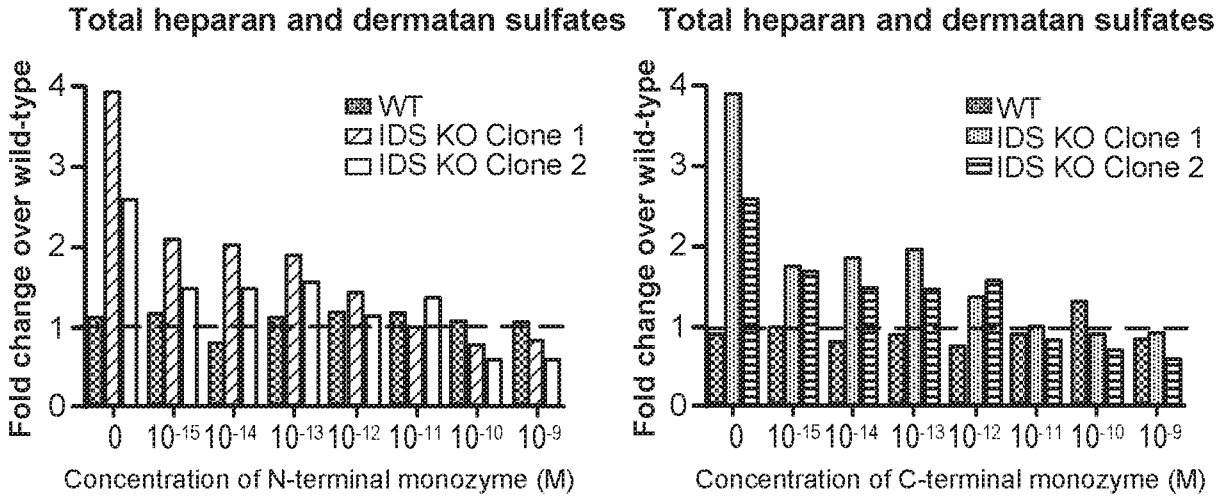

Total heparan and dermatan sulfates
Fold change over wild-type
WT
IDS KO Clone 1
IDS KO Clone 2
Concentration of N-terminal monozyme (M)
Total heparan and dermatan sulfates
Fold change over wild-type
WT
IDS KO Clone 1
IDS KO Clone 2
Concentration of C-terminal monozyme (M)

FIG. 5A

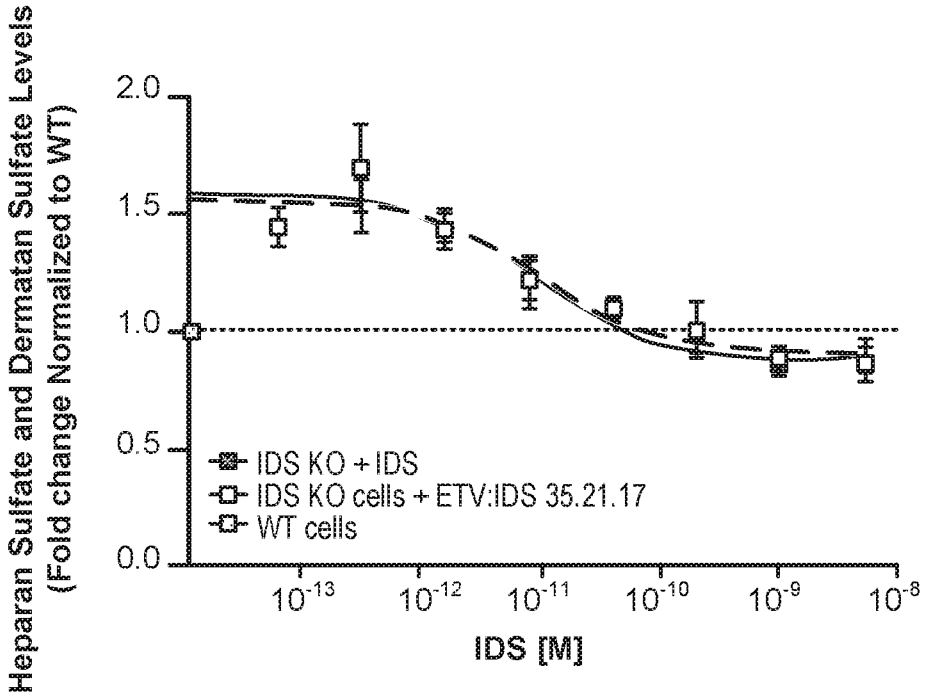

Heparan Sulfate and Dermatan Sulfate Levels
(Fold change Normalized to WT)
IDS KO + IDS
IDS KO cells + ETV:IDS 35.21.17
WT cells
IDS [M]

FIG. 5B

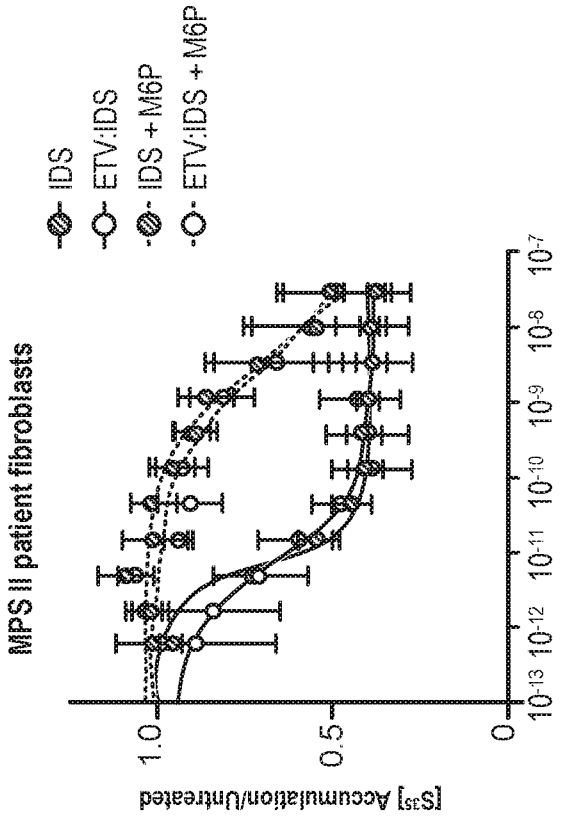

MPS II patient fibroblasts
IDS
ETV:IDS
IDS + M6P
ETV:IDS + M6P
[S35] Accumulation/Untreated
1.0
0.5
0
10-13 10-12 10-11 10-10 10-9 10-8 10-7
IDS (M)

FIG. 5D

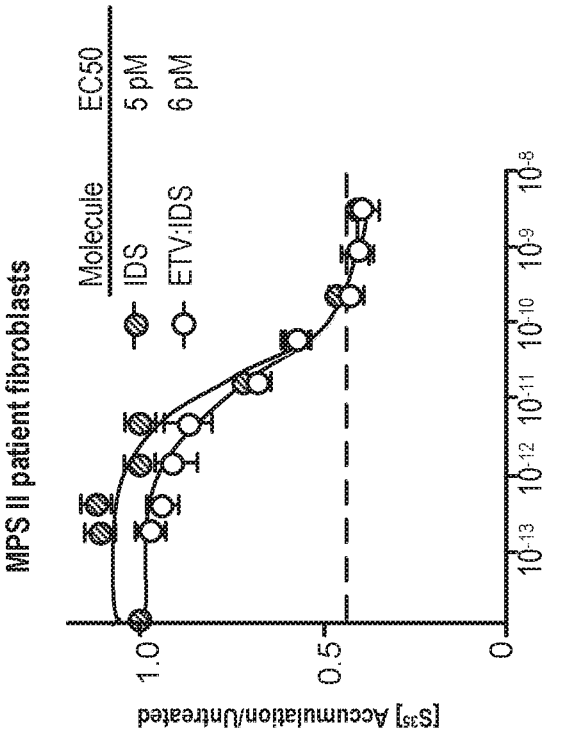

MPS II patient fibroblasts
Molecule EC50
IDS 5 pM
ETV:IDS 6 pM
[S35] Accumulation/Untreated
1.0
0.5
0
10-13 10-12 10-11 10-10 10-9 10-8
IDS (M)

FIG. 5C

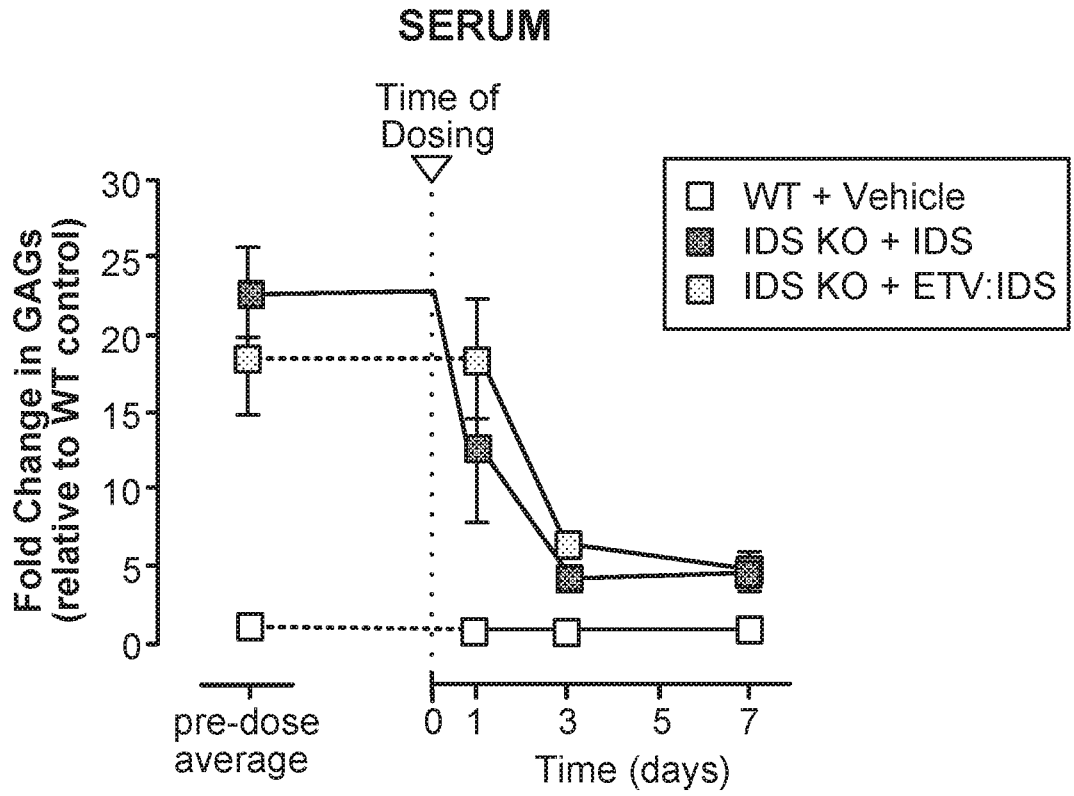

SERUM
Time of Dosing
Fold Change in GAGs (relative to WT control)
30
25
20
15
10
5
0
WT + Vehicle
IDS KO + IDS
IDS KO + ETV:IDS
pre-dose average
0 1 3 5 7
Time (days)

FIG. 6

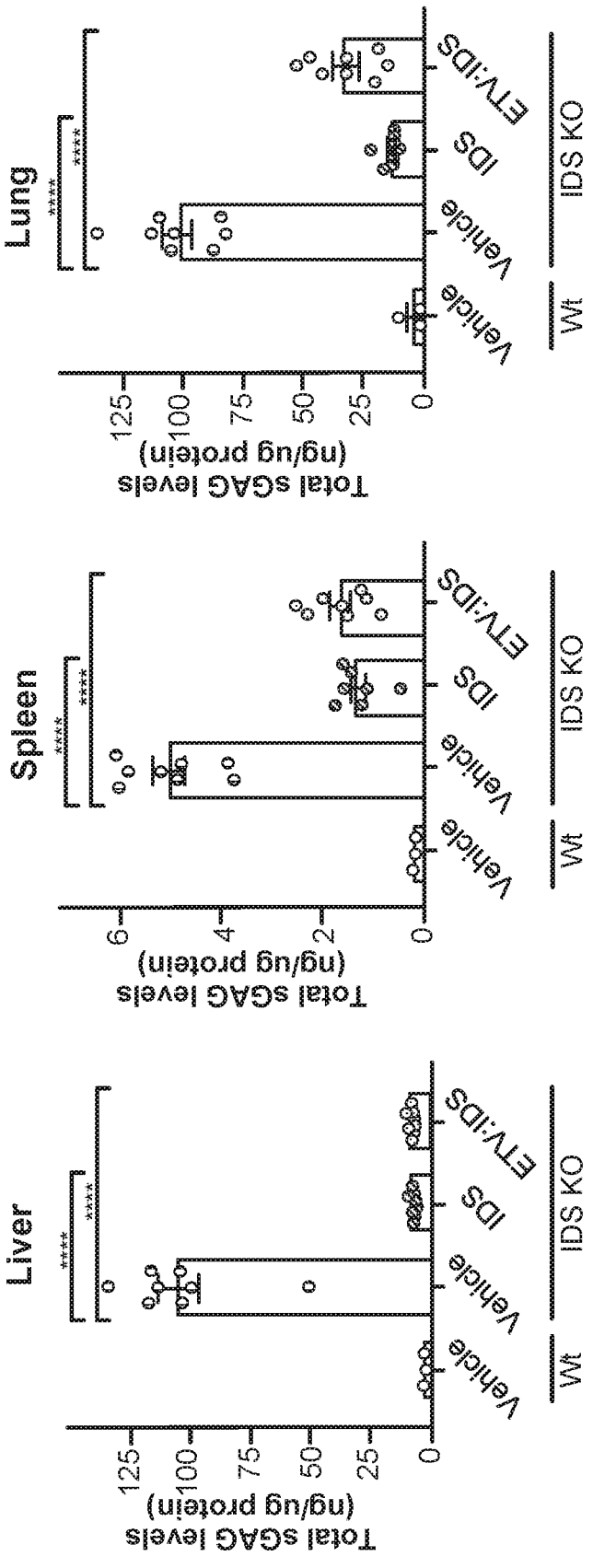

Liver
Spleen
Lung
Total sGAG levels
(ng/ug protein)
0
25
50
75
100
125
2
4
6
****
Vehicle
Vehicle
IDS
ETV:IDS
Wt
IDS KO

FIG. 7

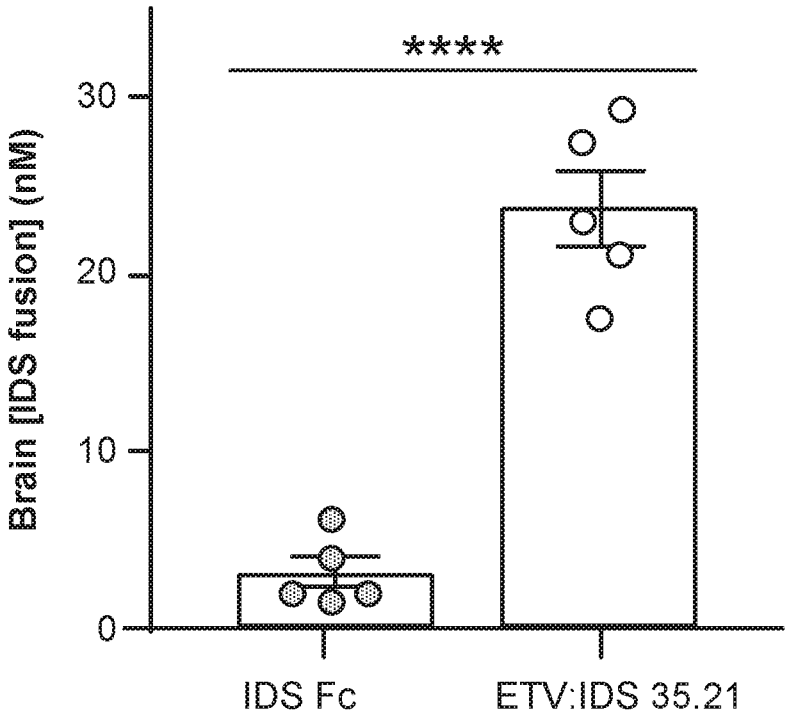


FIG. 8

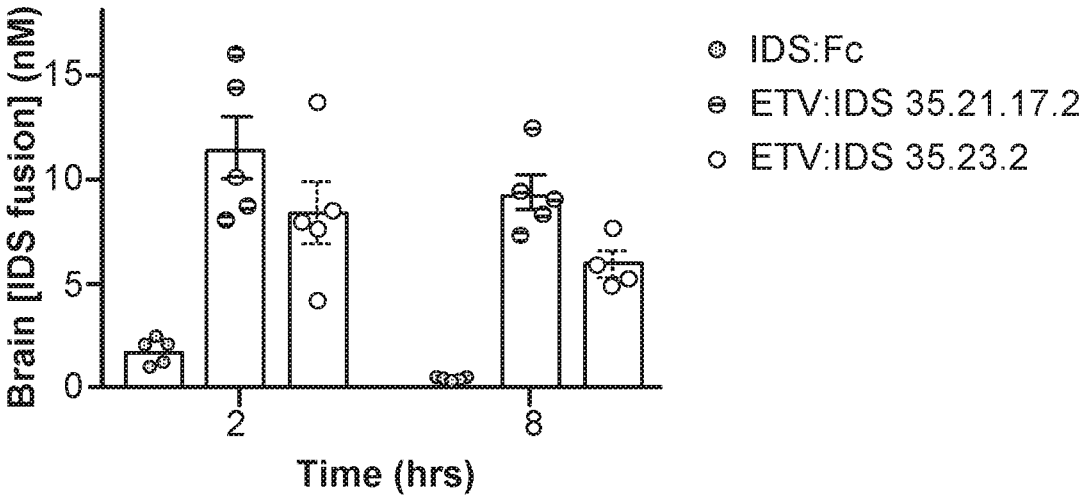

Brain [IDS fusion] (nM)
15
10
5
0
2
8
Time (hrs)
IDS:Fc
ETV:IDS 35.21.17.2
ETV:IDS 35.23.2

FIG. 9A

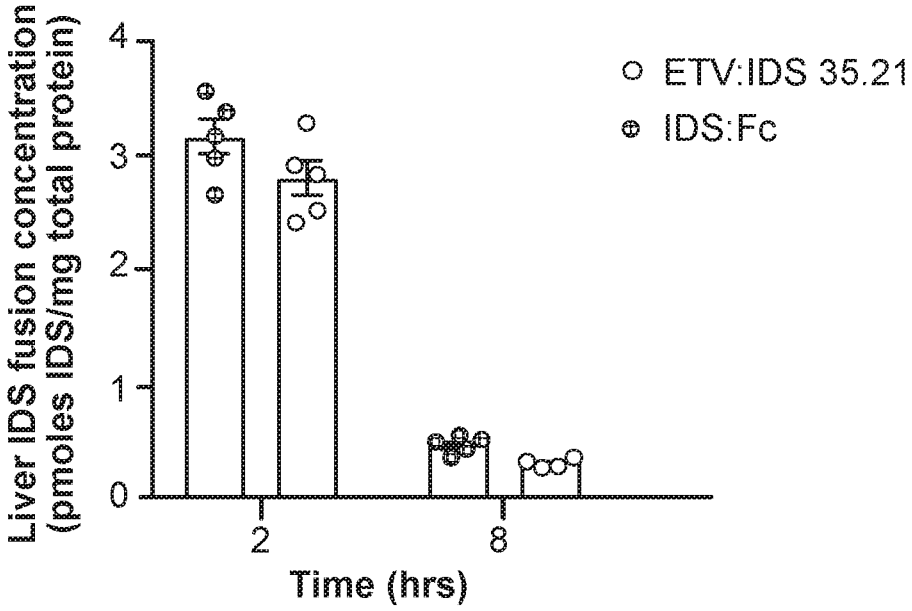

Liver IDS fusion concentration
(pmoles IDS/mg total protein)
4
3
2
1
0
2
8
Time (hrs)
ETV:IDS 35.21
IDS:Fc

FIG. 9B

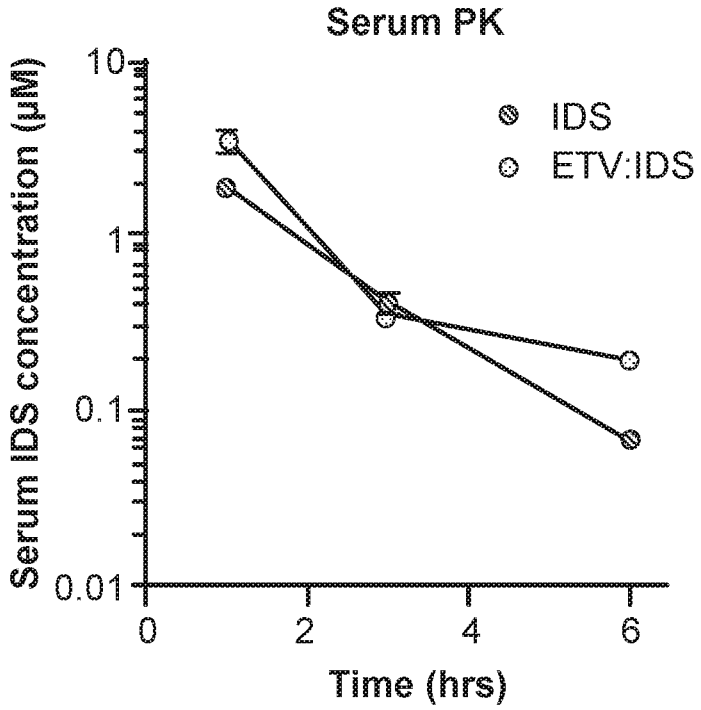


FIG. 10A

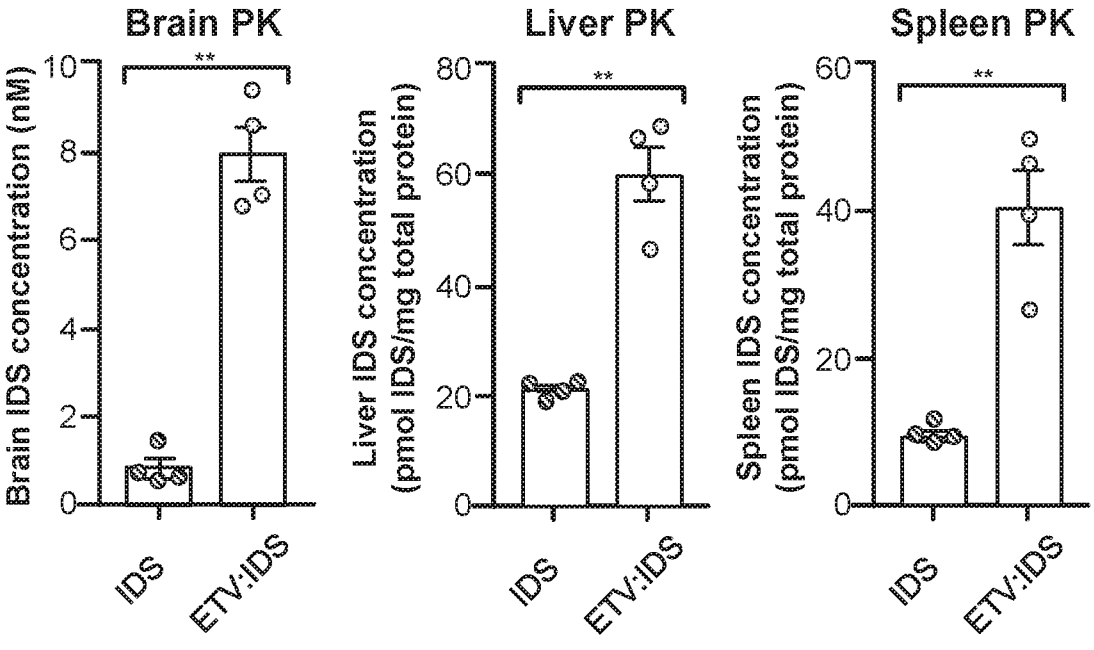


FIG. 10B

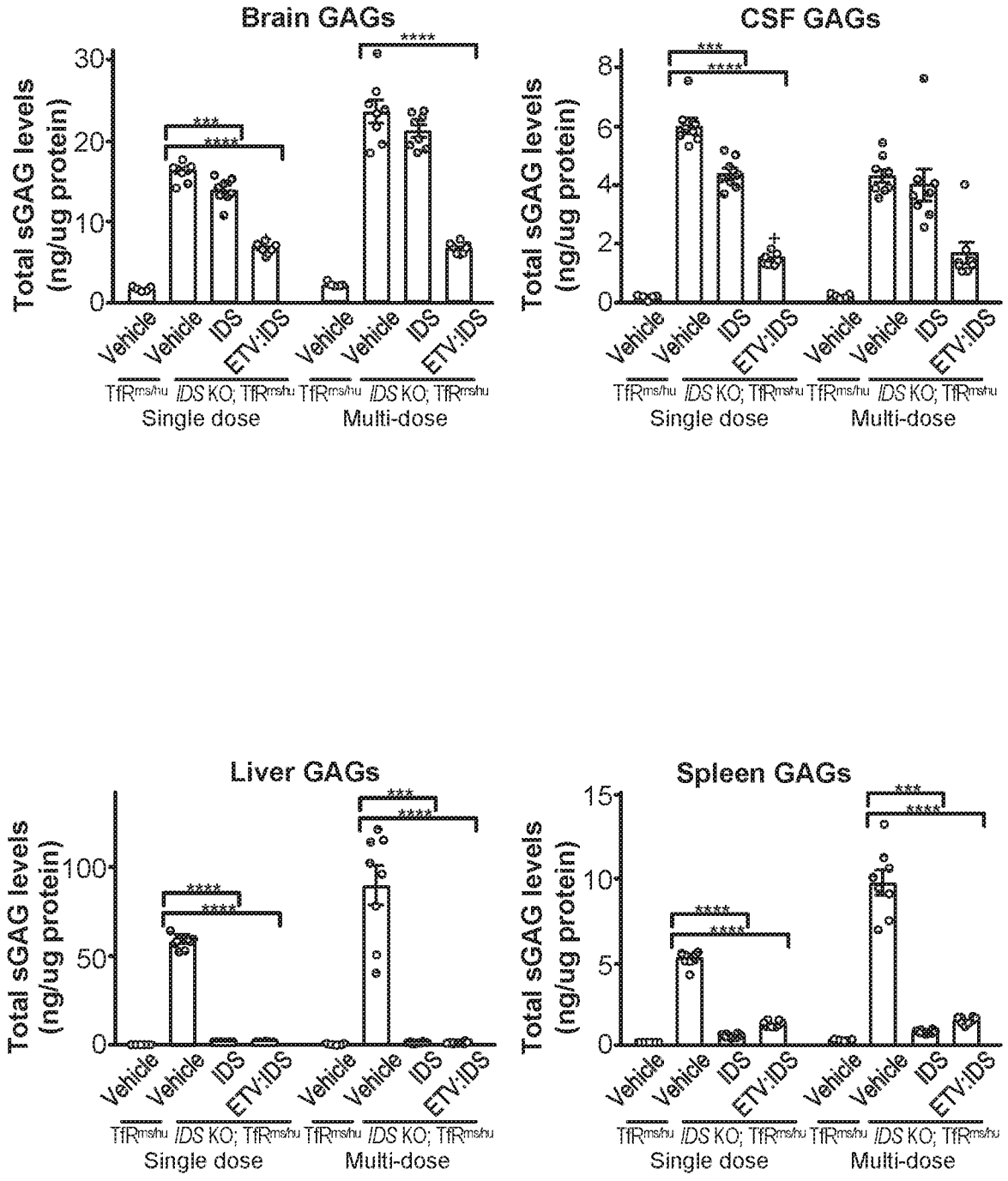

Brain GAGs
CSF GAGs
Liver GAGs
Spleen GAGs
Total sGAG levels (ng/ug protein)
Vehicle
Vehicle
IDS
ETV:IDS
TfRms/hu
IDS KO; TfRms/hu
Single dose
Multi-dose

FIG. 10C

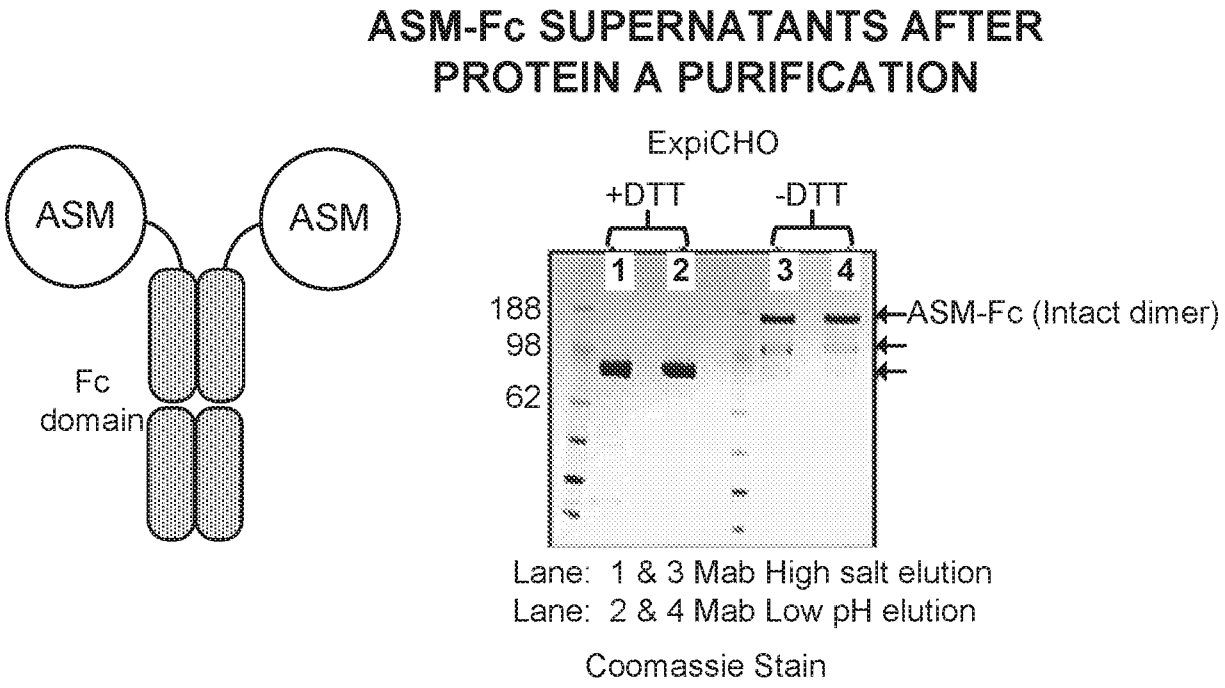

ASM-Fc SUPERNATANTS AFTER PROTEIN A PURIFICATION
ASM
ASM
Fc domain
ExpiCHO
+DTT
-DTT
1
2
3
4
188
98
62
ASM-Fc (Intact dimer)
Lane: 1 & 3 Mab High salt elution
Lane: 2 & 4 Mab Low pH elution
Coomassie Stain

FIG. 11

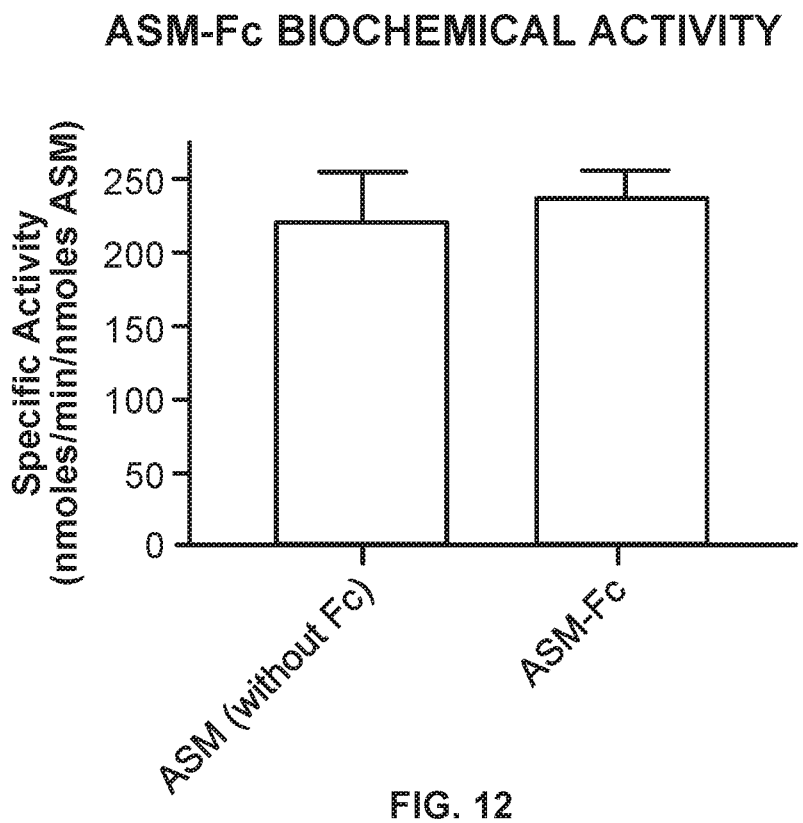

ASM-Fc BIOCHEMICAL ACTIVITY
Specific Activity
(nmoles/min/nmoles ASM)
250
200
150
100
50
0
ASM (without Fc)
ASM-Fc

FIG. 12

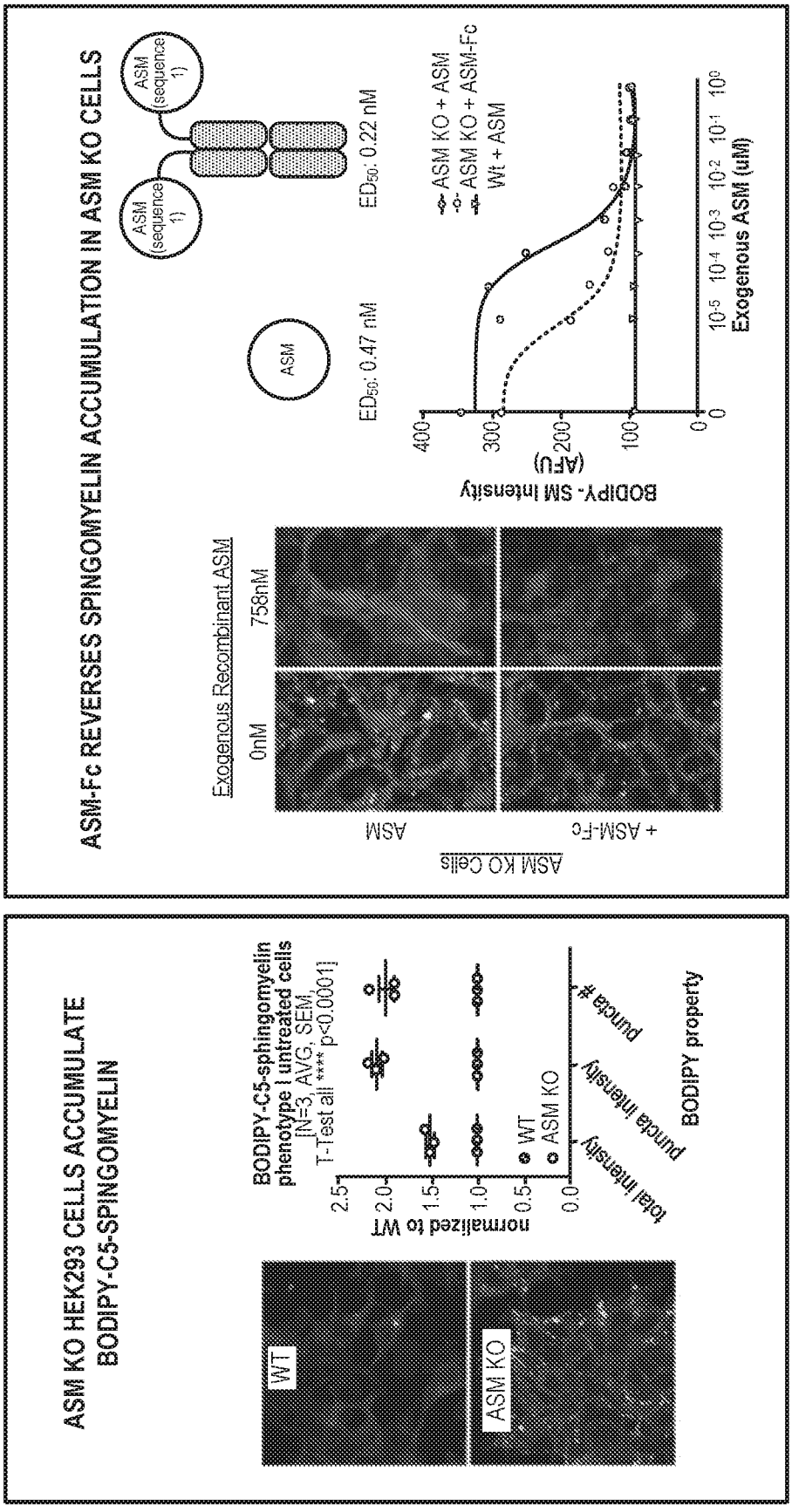

ASM KO HEK293 CELLS ACCUMULATE BODIPY-C5-SPINGOMYELIN
WT
ASM KO
BODIPY-C5-sphingomyelin phenotype I untreated cells
[N=3, AVG, SEM, T-Test all **** p<0.0001]
normalized to WT
2.5
2.0
1.5
1.0
0.5
0.0
WT
ASM KO
total intensity
puncta intensity
puncta #
BODIPY property
ASM-Fc REVERSES SPINGOMYELIN ACCUMULATION IN ASM KO CELLS
ASM (sequence 1)
ASM (sequence 1)
ASM
ED50: 0.47 nM
ED50: 0.22 nM
ASM KO + ASM
ASM KO + ASM-Fc
Wt + ASM
BODIPY- SM intensity (AFU)
400
300
200
100
0
0
10-5 10-4 10-3 10-2 10-1 100
Exogenous ASM (uM)
Exogenous Recombinant ASM
0nM
758nM
ASM
+ ASM-Fc
ASM KO Cells

FIG. 13

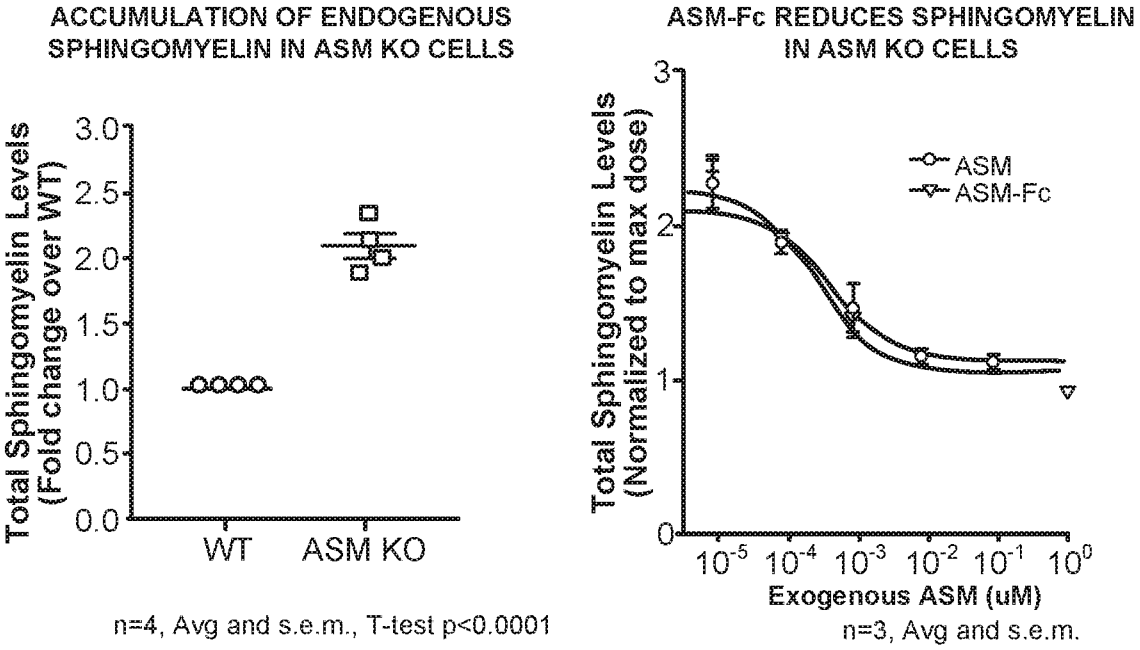

ACCUMULATION OF ENDOGENOUS SPHINGOMYELIN IN ASM KO CELLS
Total Sphingomyelin Levels (Fold change over WT)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
WT
ASM KO
n=4, Avg and s.e.m., T-test p<0.0001
ASM-Fc REDUCES SPHINGOMYELIN IN ASM KO CELLS
Total Sphingomyelin Levels (Normalized to max dose)
3
2
1
0
ASM
ASM-Fc
10-5 10-4 10-3 10-2 10-1 100
Exogenous ASM (uM)
n=3, Avg and s.e.m.

FIG. 14

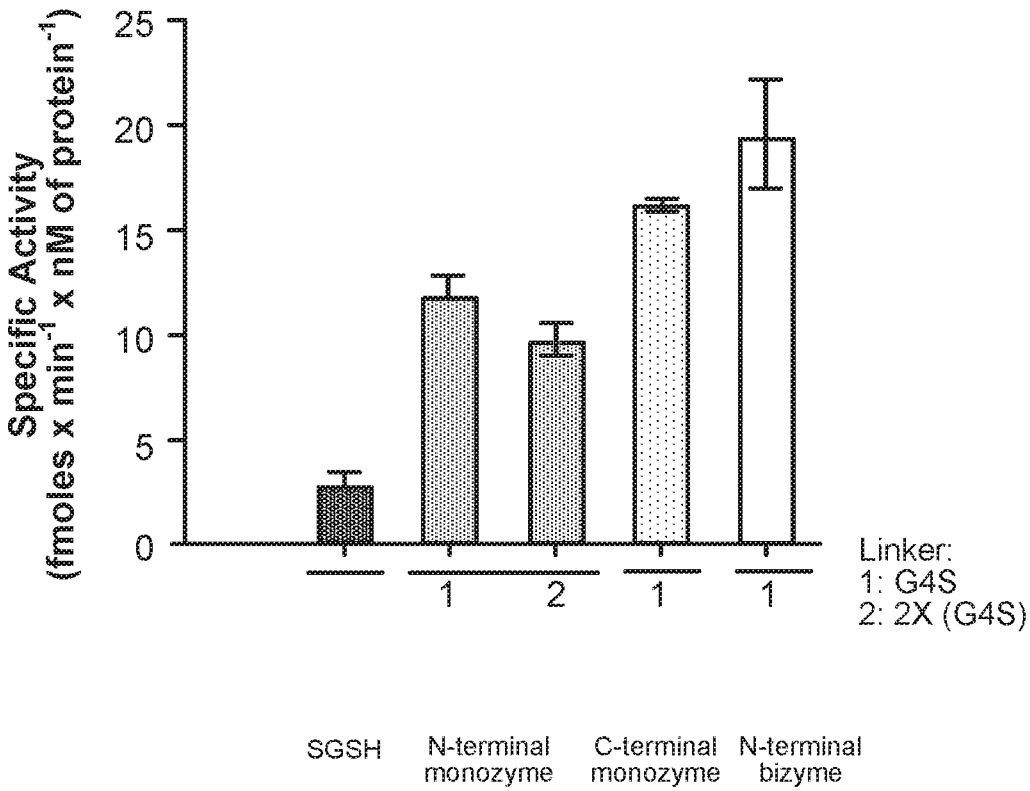

Specific Activity
(fmoles x min-1 x nM of protein-1)
25
20
15
10
5
0
1
2
1
1
Linker:
1: G4S
2: 2X (G4S)
SGSH
N-terminal monozyme
C-terminal monozyme
N-terminal bizyme

FIG. 15

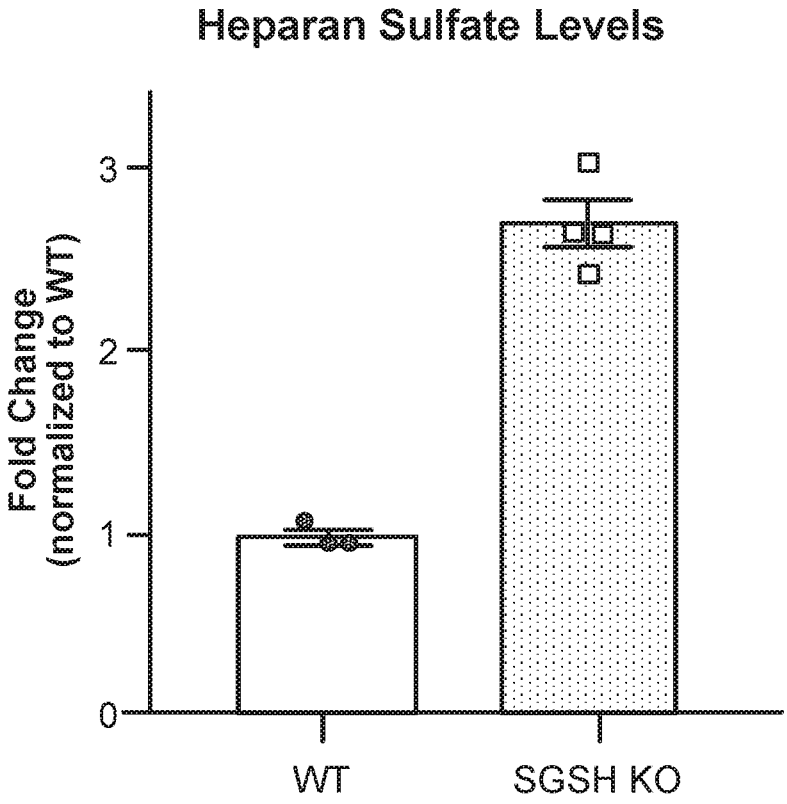

Heparan Sulfate Levels
Fold Change
(normalized to WT)
3
2
1
0
WT
SGSH KO

FIG. 16

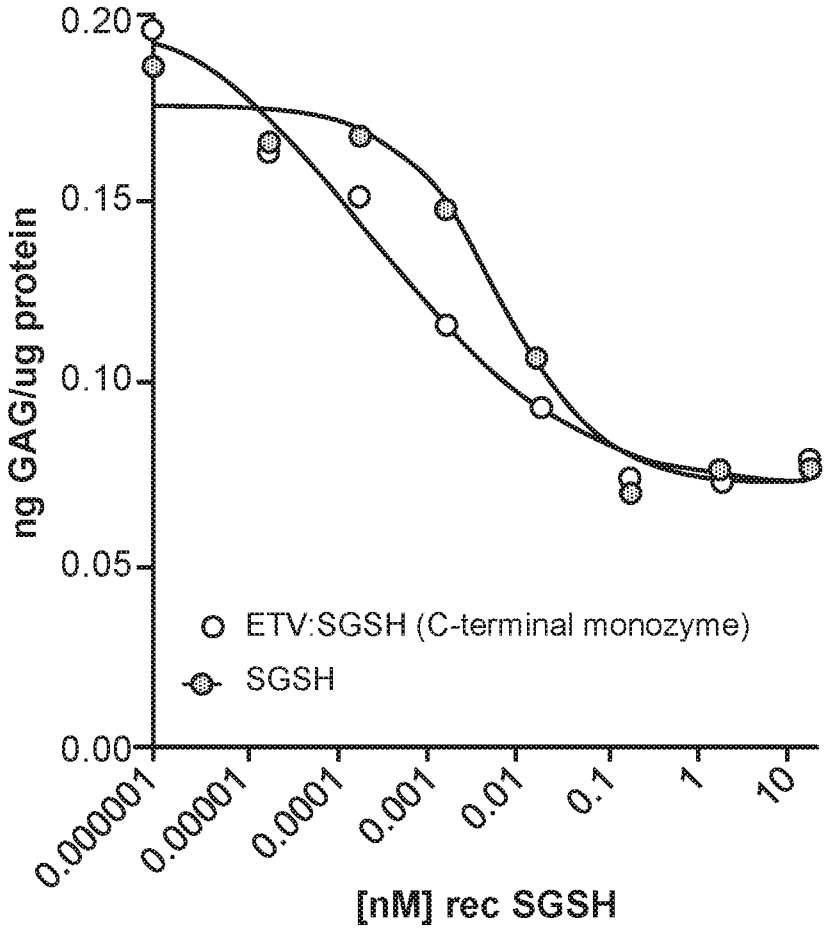

0.20
0.15
0.10
0.05
0.00
ng GAG/ug protein
ETV:SGSH (C-terminal monozyme)
SGSH
0.000001
0.00001
0.0001
0.001
0.01
0.1
1
10
[nM] rec SGSH

FIG. 17

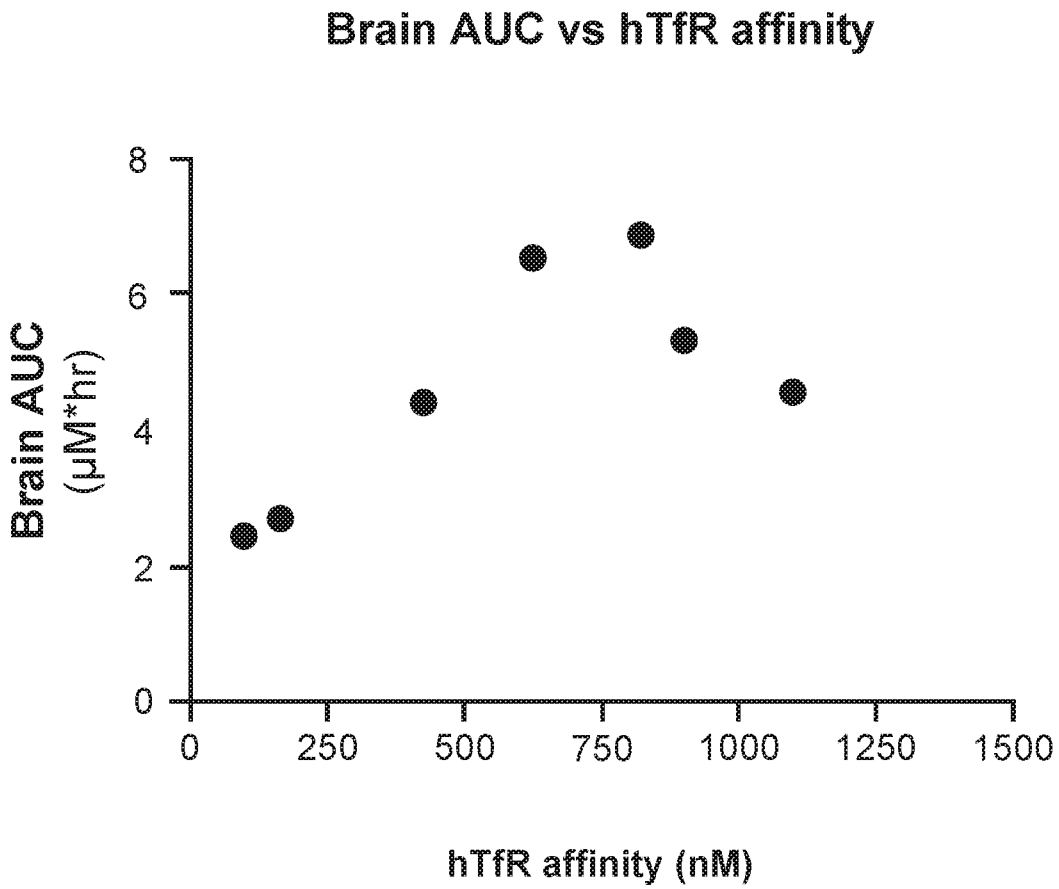
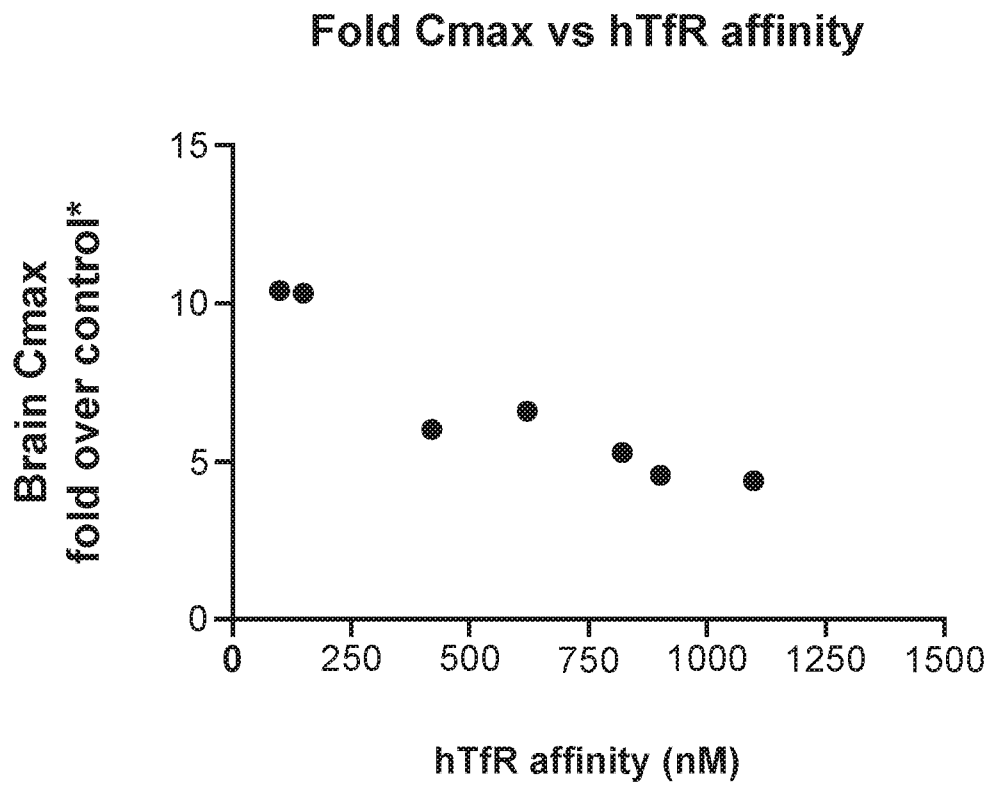

Brain AUC vs hTfR affinity
Brain AUC (μM*hr)
8
6
4
2
0
0
250
500
750
1000
1250
1500
hTfR affinity (nM)

FIG. 18



**Fold over anti-BACE1 Cmax within same study*

FIG. 19

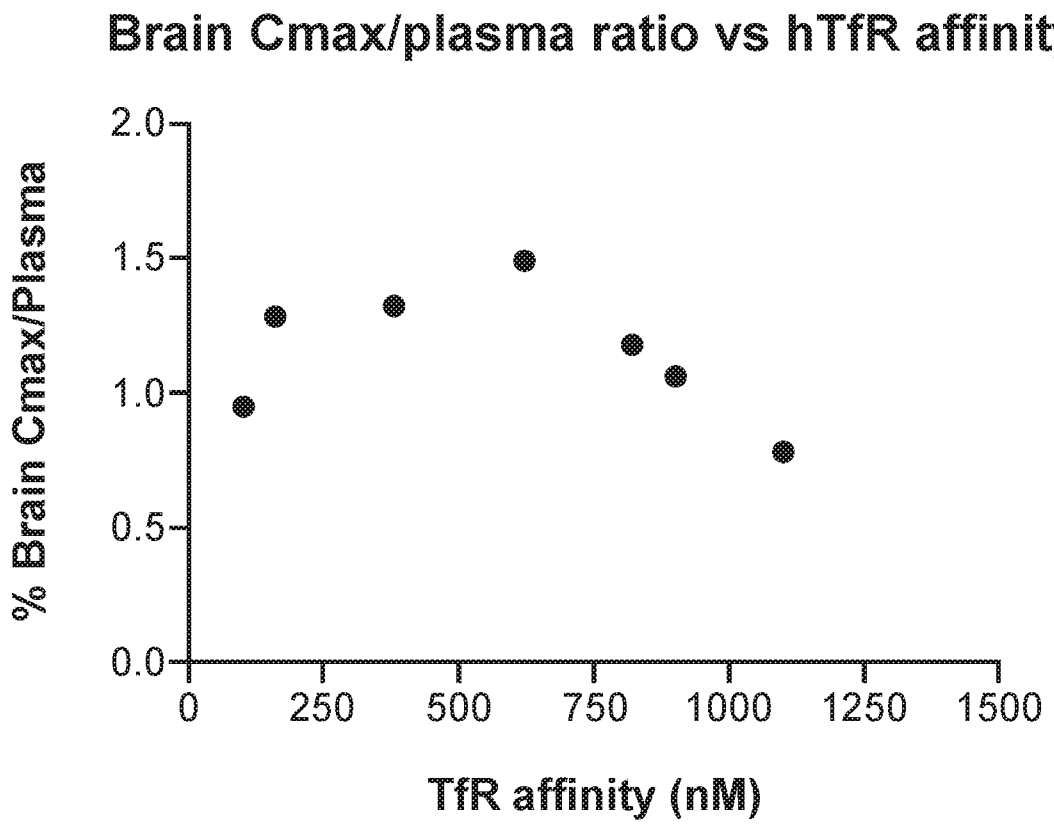

Brain Cmax/plasma ratio vs hTfR affinity
% Brain Cmax/Plasma
2.0
1.5
1.0
0.5
0.0
0
250
500
750
1000
1250
1500
TfR affinity (nM)

FIG. 20

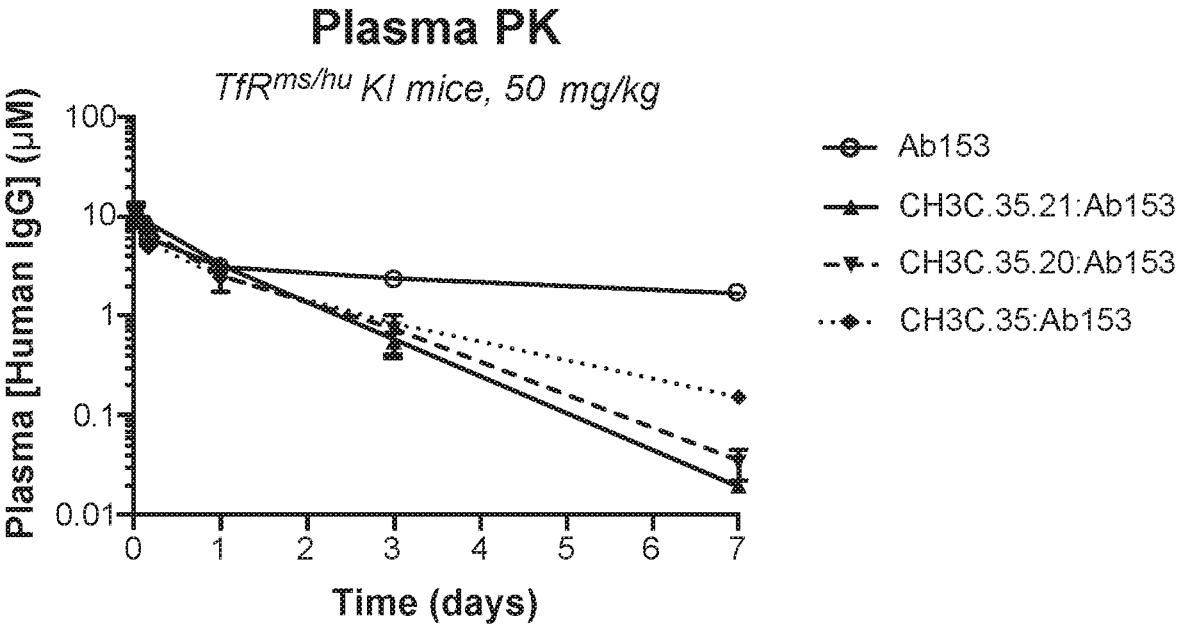

Plasma PK
TfR ms/hu KI mice, 50 mg/kg
Plasma [Human IgG] (μM)
100
10
1
0.1
0.01
0
1
2
3
4
5
6
7
Time (days)
Ab153
CH3C.35.21:Ab153
CH3C.35.20:Ab153
CH3C.35:Ab153

FIG. 21A

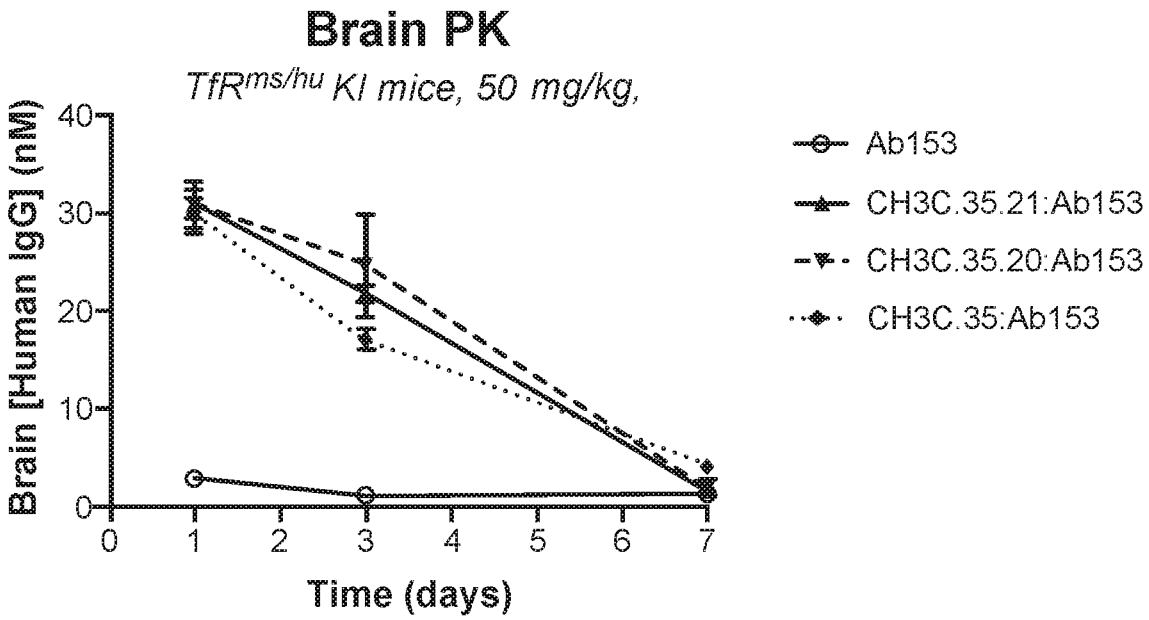

Brain PK
TfR^ms/hu KI mice, 50 mg/kg,
Brain [Human IgG] (nM)
40
30
20
10
0
0
1
2
3
4
5
6
7
Time (days)
Ab153
CH3C.35.21:Ab153
CH3C.35.20:Ab153
CH3C.35:Ab153

FIG. 21B

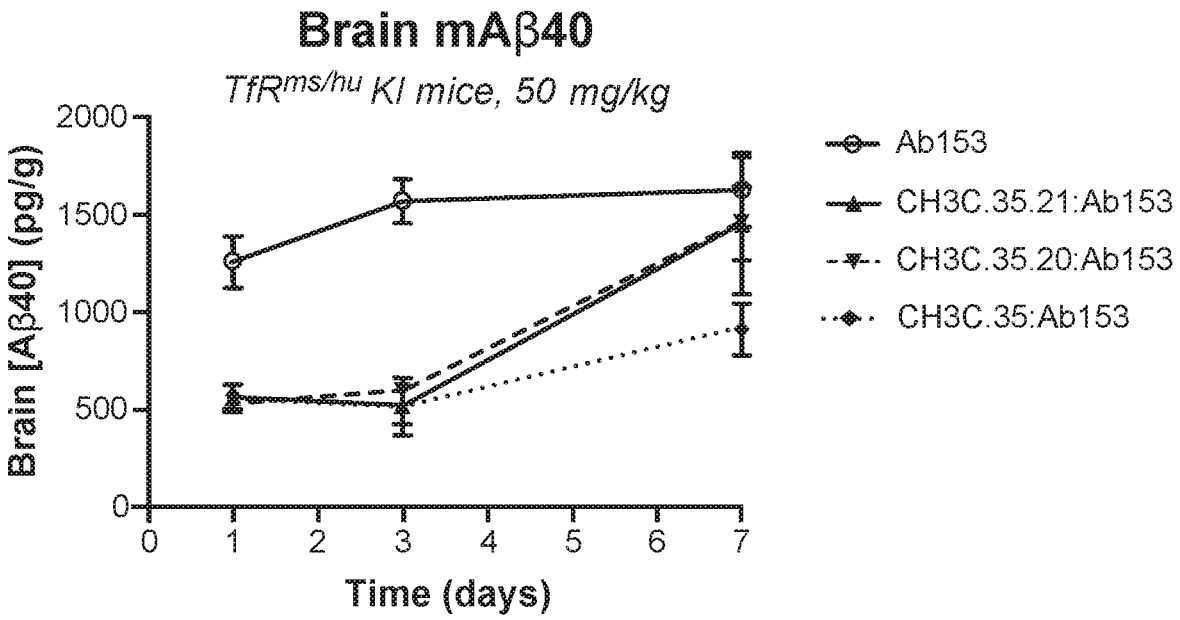

Brain mAβ40
TfR^ms/hu KI mice, 50 mg/kg
Brain [Aβ40] (pg/g)
2000
1500
1000
500
0
0 1 2 3 4 5 6 7
Time (days)
Ab153
CH3C.35.21:Ab153
CH3C.35.20:Ab153
CH3C.35:Ab153

FIG. 21C

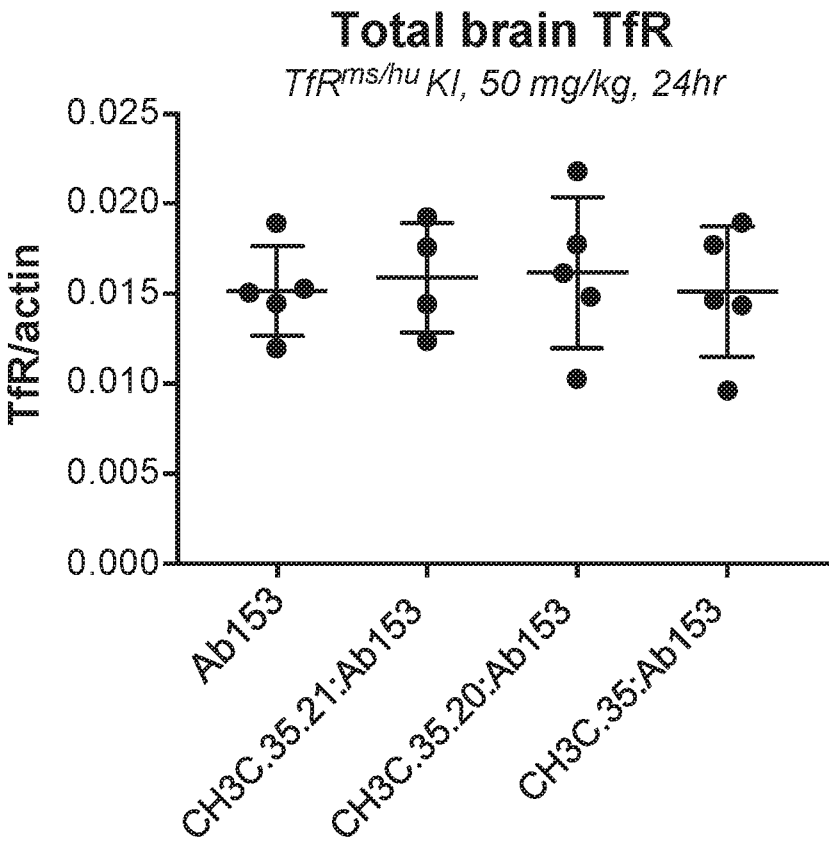

Total brain TfR
TfR^ms/hu KI, 50 mg/kg, 24hr
TfR/actin
0.025
0.020
0.015
0.010
0.005
0.000
Ab153
CH3C.35.21:Ab153
CH3C.35.20:Ab153
CH3C.35:Ab153

FIG. 21D