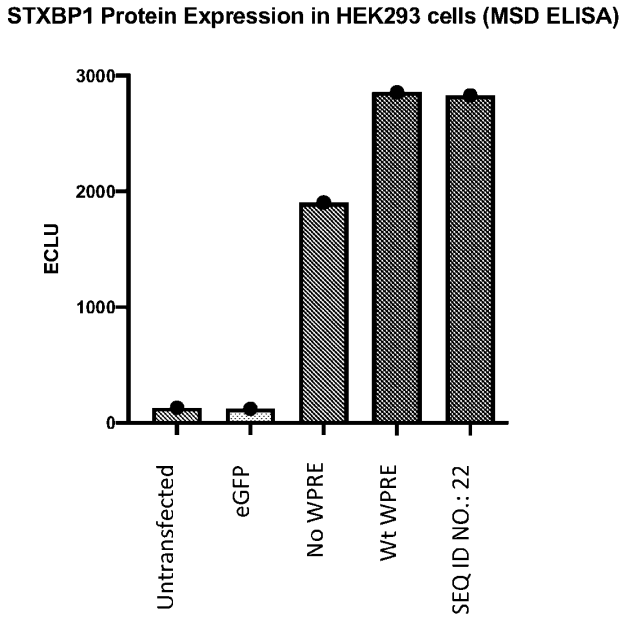

STXBP1 Protein Expression in HEK293 cells (MSD ELISA)
ECLU
3000
2000
1000
0
Untransfected
eGFP
No WPRE
Wt WPRE
SEQ ID NO.: 22

FIG. 1

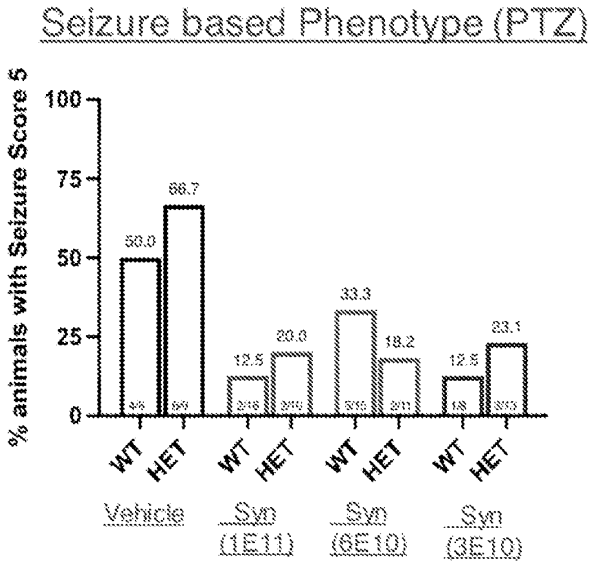

Seizure based Phenotype (PTZ)
% animals with Seizure Score 5
100
75
50
25
0
50.0
66.7
12.5
20.0
33.3
18.2
12.5
33.1
WT
HET
WT
HET
WT
HET
WT
HET
Vehicle
Syn (1E11)
Syn (6E10)
Syn (3E10)

FIG. 2

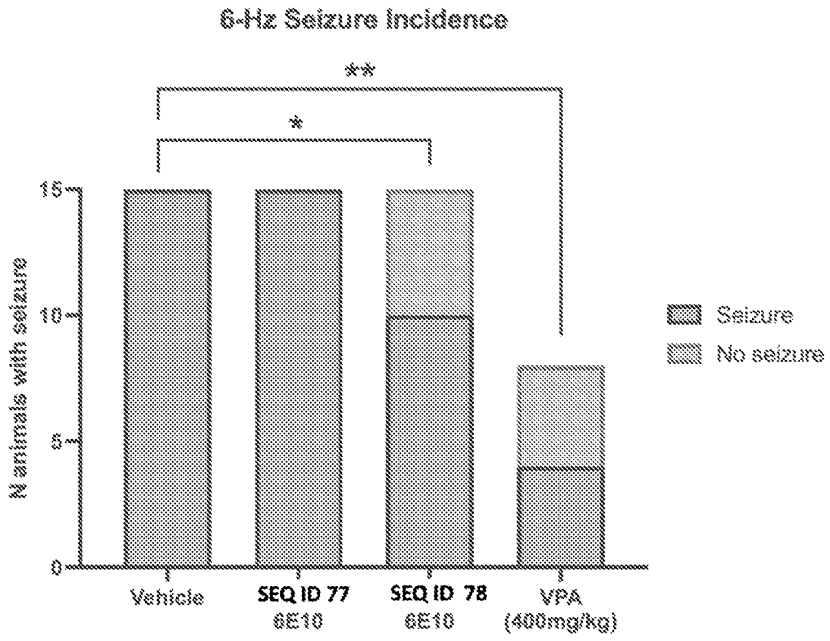

6-Hz Seizure Incidence
**
*
15
10
5
0
N animals with seizure
Seizure
No seizure
Vehicle
SEQ ID 77
6E10
SEQ ID 78
6E10
VPA
(400mg/kg)

FIG. 3

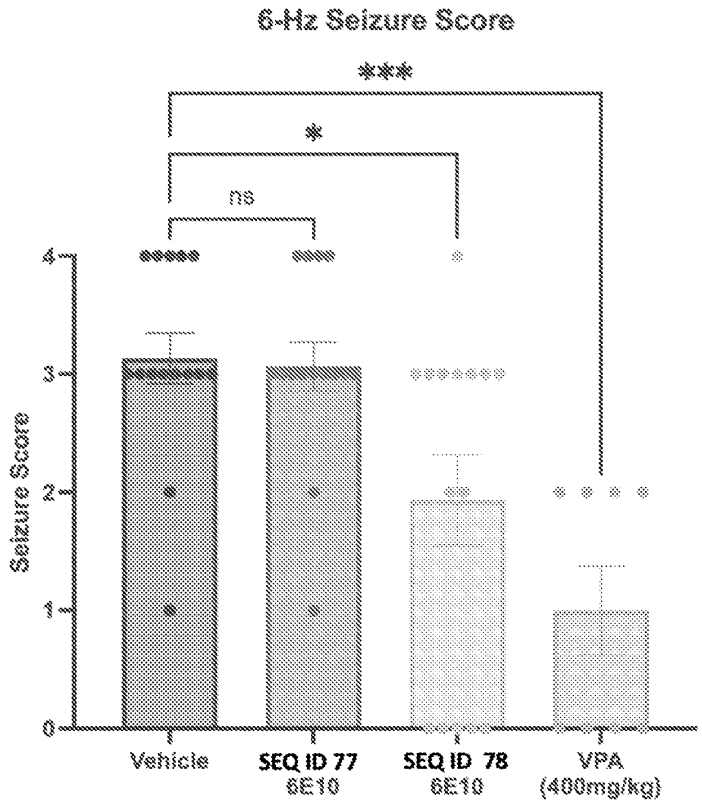

6-Hz Seizure Score
***
*
ns
Seizure Score
0
1
2
3
4
Vehicle
SEQ ID 77
6E10
SEQ ID 78
6E10
VPA
(400mg/kg)

FIG. 4

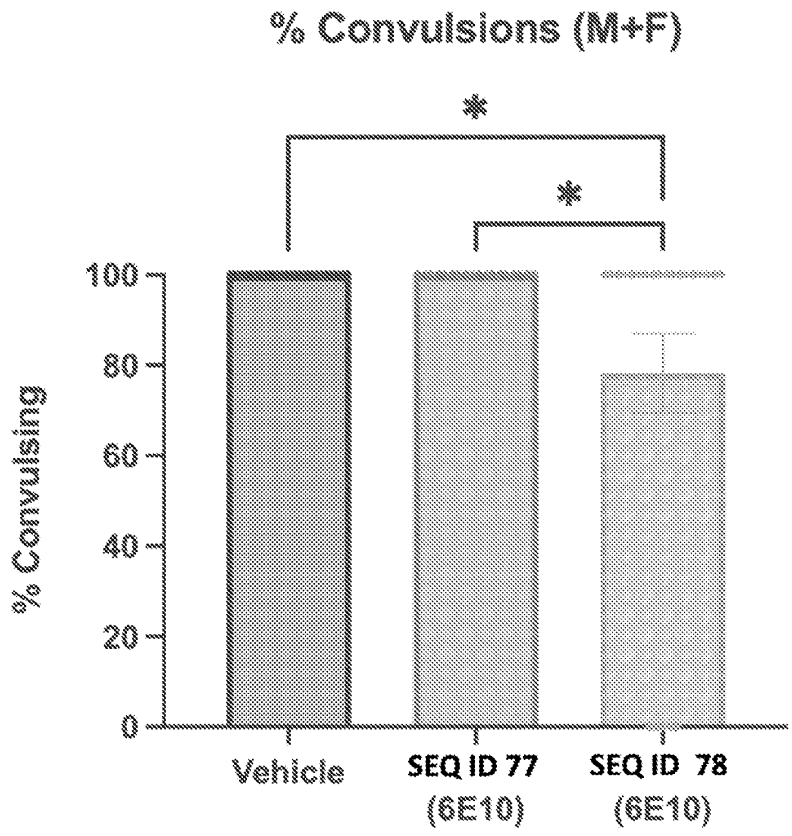

% Convulsions (M+F)
*
*
100
80
60
40
20
0
% Convulsing
Vehicle
SEQ ID 77
(6E10)
SEQ ID 78
(6E10)

FIG. 5

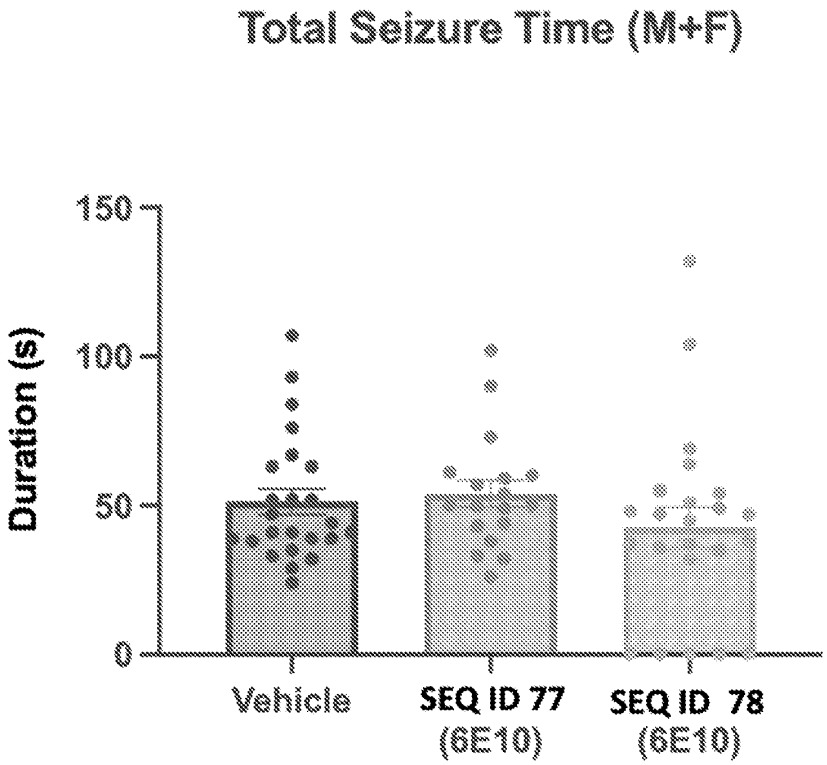

Total Seizure Time (M+F)
Duration (s)
150
100
50
0
Vehicle
SEQ ID 77
(6E10)
SEQ ID 78
(6E10)

FIG. 6

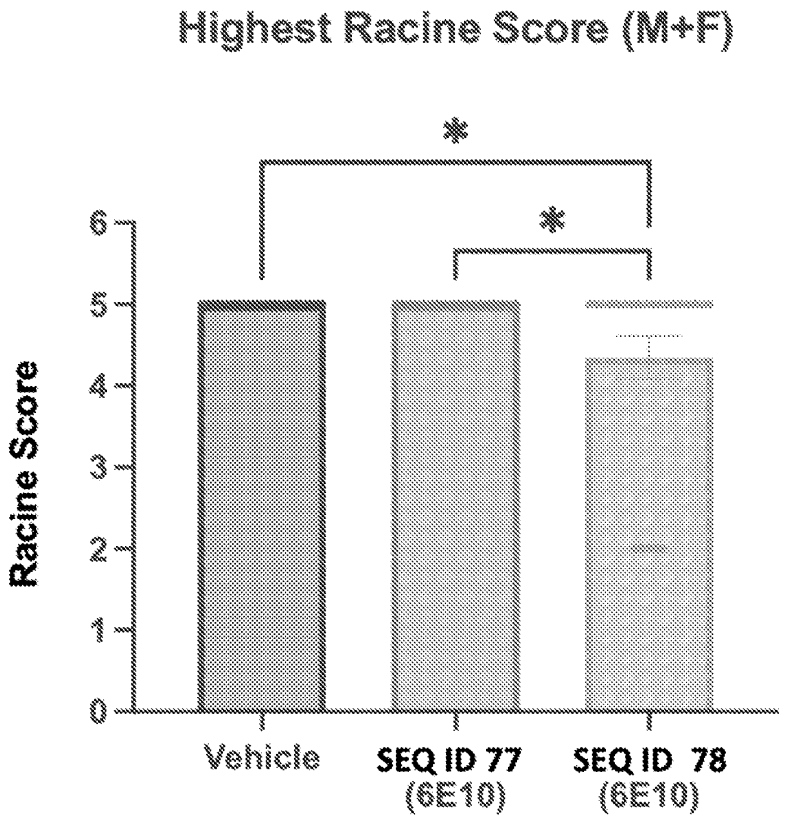

Highest Racine Score (M+F)
Racine Score
0
1
2
3
4
5
6
*
*
Vehicle
SEQ ID 77
(6E10)
SEQ ID 78
(6E10)

FIG. 7

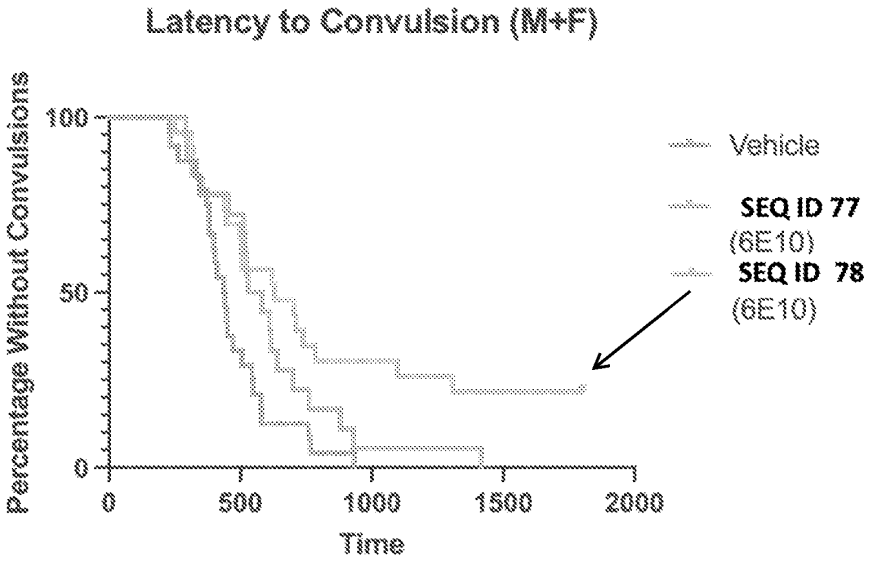

Latency to Convulsion (M+F)
Percentage Without Convulsions
100
50
0
0
500
1000
1500
2000
Time
Vehicle
SEQ ID 77
(6E10)
SEQ ID 78
(6E10)

FIG. 8

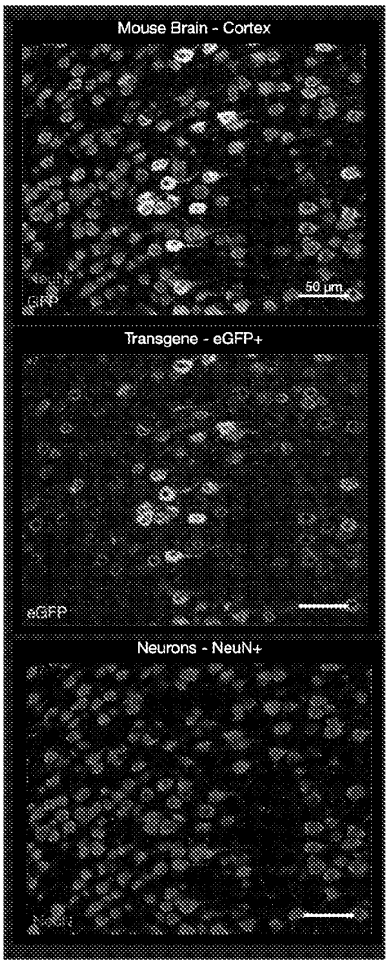


FIG. 9

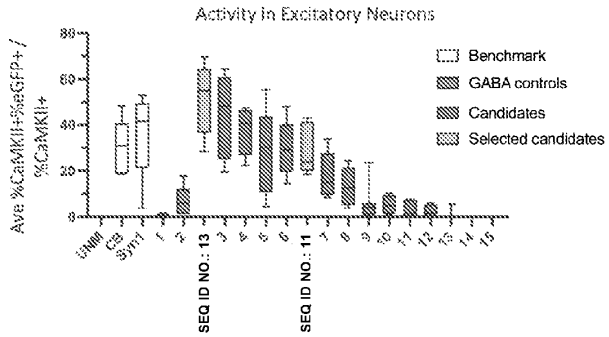

Activity in Excitatory Neurons
Ave %CaMKII+%eGFP+ / %CaMKII+
Benchmark
GABA controls
Candidates
Selected candidates
SEQ ID NO.: 13
SEQ ID NO.: 11

FIG. 10

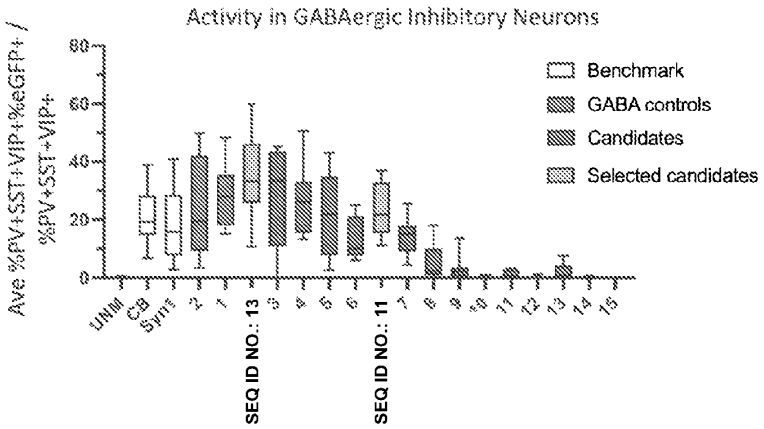

Activity in GABAergic Inhibitory Neurons
Ave %PV+SST+VIP+%eGFP+ / %PV+SST+VIP+
Benchmark
GABA controls
Candidates
Selected candidates
UNM
CB
Syn1
SEQ ID NO.: 13
SEQ ID NO.: 11

FIG. 11

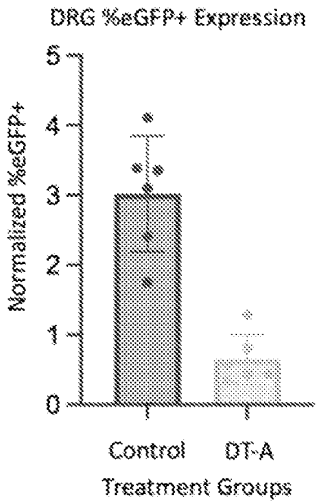


FIG. 12A

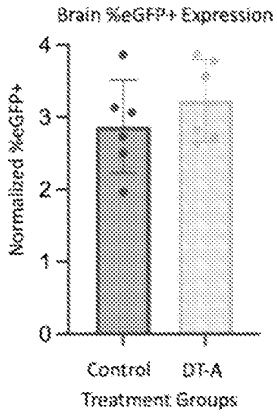


FIG. 12B

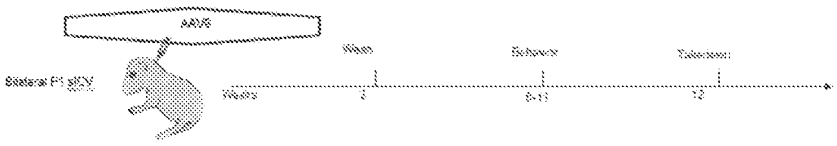


FIG. 13

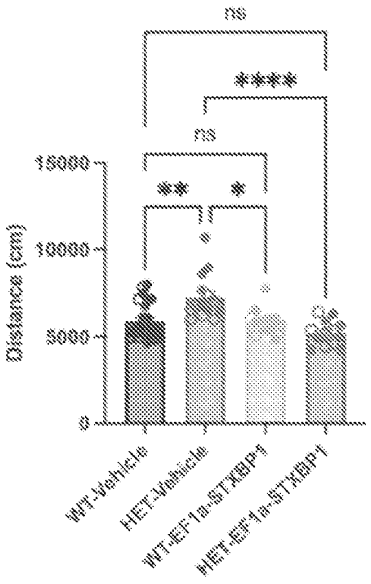

ns
****
ns
**
*
15000
10000
5000
0
Distance (cm)
WT-Vehicle
HET-Vehicle
WT-EF1a-STXBP1
HET-EF1a-STXBP1

FIG. 14

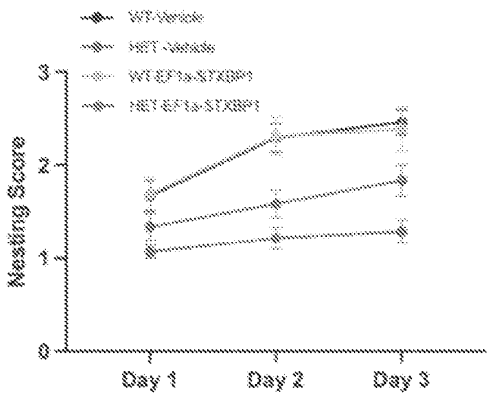


FIG. 15

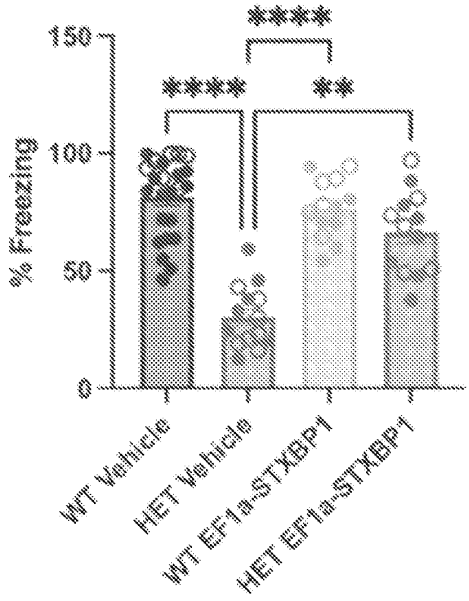

****
****
**
150
100
50
0
% Freezing
WT Vehicle
HET Vehicle
WT EF1a-STXBP1
HET EF1a-STXBP1

FIG. 16

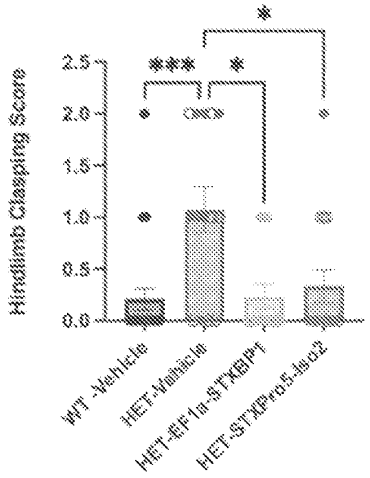

*
***
*
2.5
2.0
1.5
1.0
0.5
0.0
Hindlimb Clasping Score
WT -Vehicle
HET-Vehicle
HET-EF1a-STXBP1
HET-STXBP1-5.3e12

FIG. 17

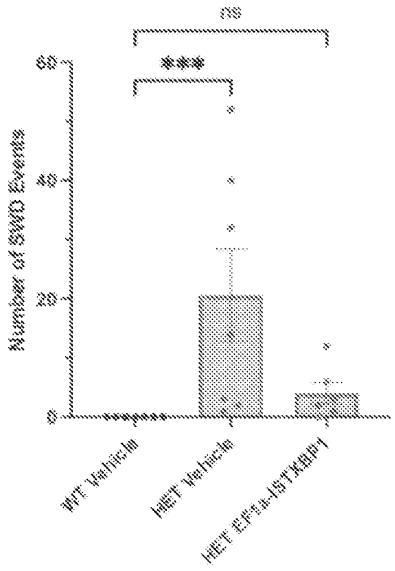

ns
***
60
40
20
0
Number of SWD Events
WT Vehicle
HET Vehicle
HET EPE-1ST1KBP1

FIG. 18

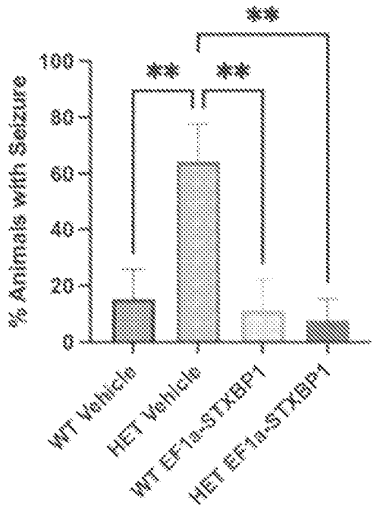

**
**
**
100
80
60
40
20
0
% Animals with Seizure
WT Vehicle
HET Vehicle
WT EF1a-STXBP1
HET EF1a-STXBP1

FIG. 19

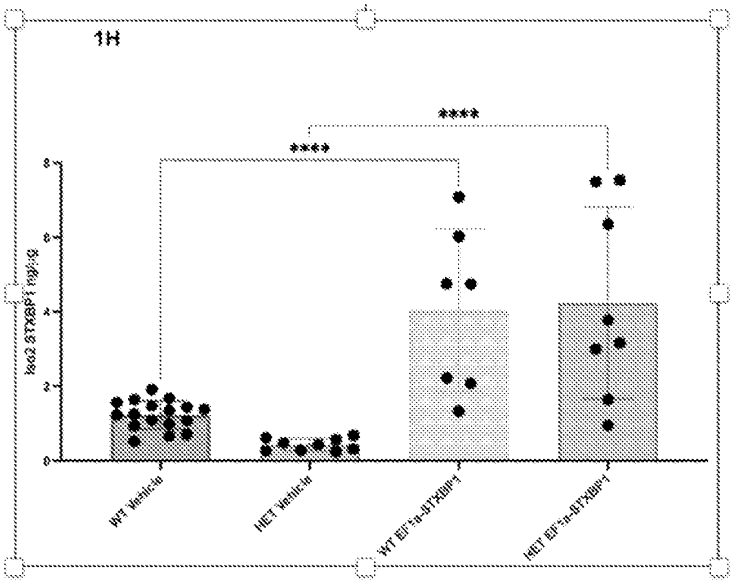

1H
****
****
WT Vehicle
HET Vehicle
WT EIF2a-STX8IP1
HET EIF2a-STX8IP1

FIG. 20

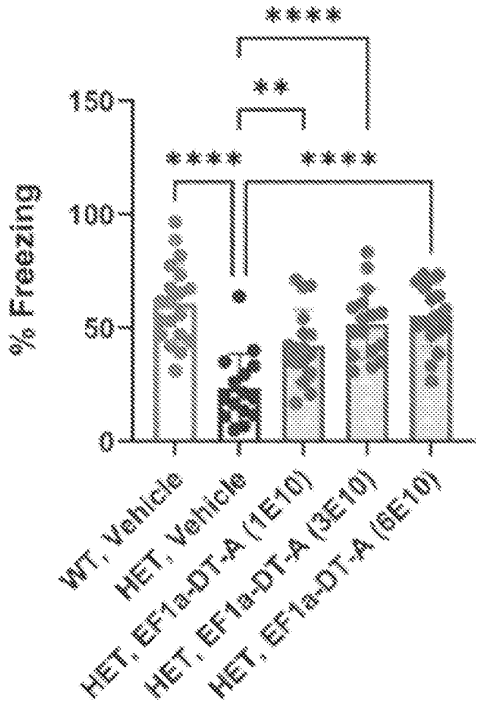

****
**
****
****
150
100
50
0
% Freezing
WT, Vehicle
HET, Vehicle
HET, EF1a-DT-A (1E10)
HET, EF1a-DT-A (3E10)
HET, EF1a-DT-A (6E10)

FIG. 21

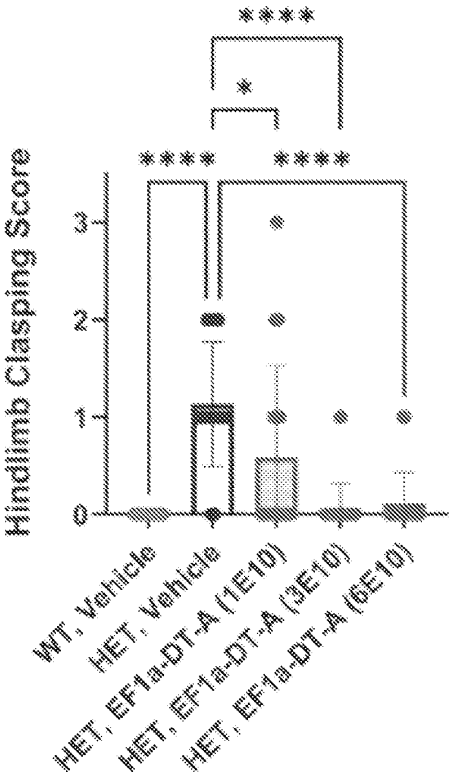

****
*
****
****
Hindlimb Clasping Score
0
1
2
3
WT, Vehicle
HET, Vehicle
HET, EF1a-DT-A (1E10)
HET, EF1a-DT-A (3E10)
HET, EF1a-DT-A (6E10)

FIG. 22

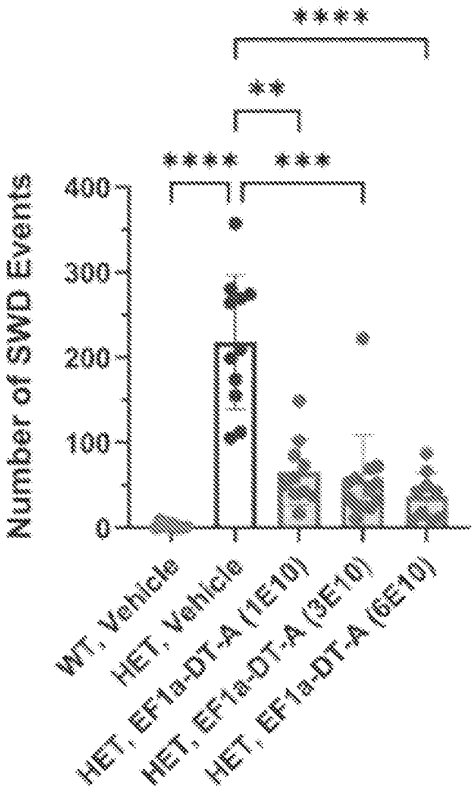

****
**
****
***
Number of SWD Events
400
300
200
100
0
WT, Vehicle
HET, Vehicle
HET, EF1a-DT-A (1E10)
HET, EF1a-DT-A (3E10)
HET, EF1a-DT-A (6E10)

FIG. 23

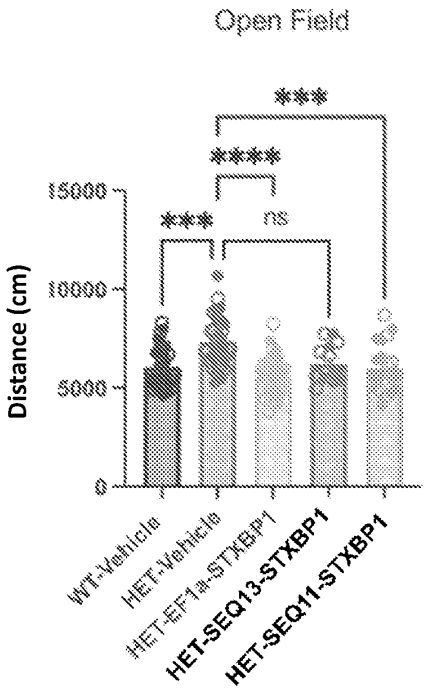


FIG. 24A

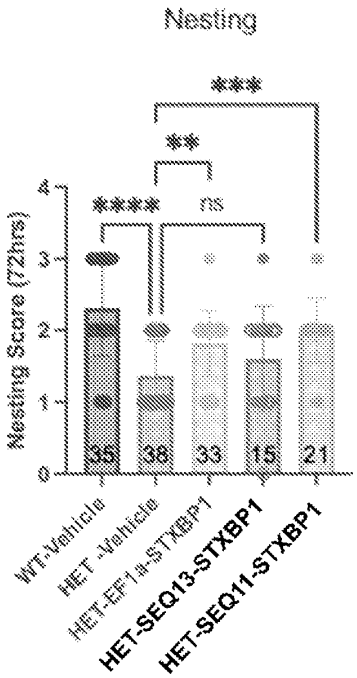


FIG. 24B

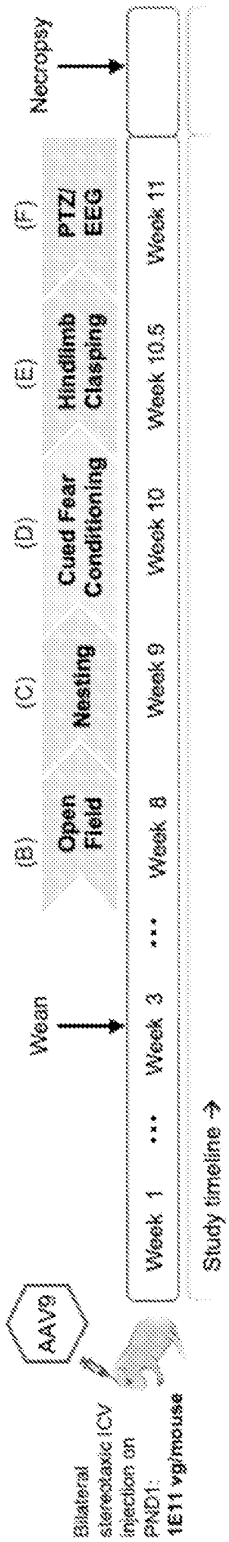

Bilateral stereotaxic ICV injection on PND1: 1E11 vg/mouse
AAV9
Wean
Study timeline
Week 1
Week 3
Week 8
Week 9
Week 10
Week 10.5
Week 11
(B) Open Field
(C) Nesting
(D) Cued Fear Conditioning
(E) Hindlimb Clasping
(F) PTZ/ EEG
Necropsy

FIG. 24C

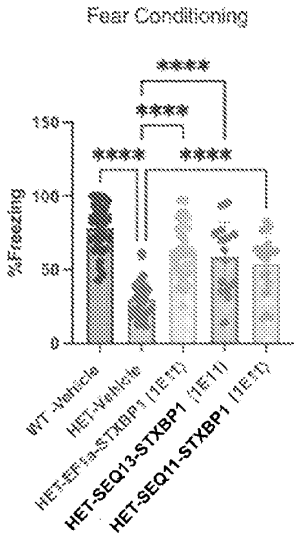

Fear Conditioning
%Freezing
150
100
50
0
****
****
****
****
WT -Vehicle
HET-Vehicle
HET-EF1a-STXBP1 (1E11)
HET-SEQ13-STXBP1 (1E11)
HET-SEQ11-STXBP1 (1E11)

FIG. 25

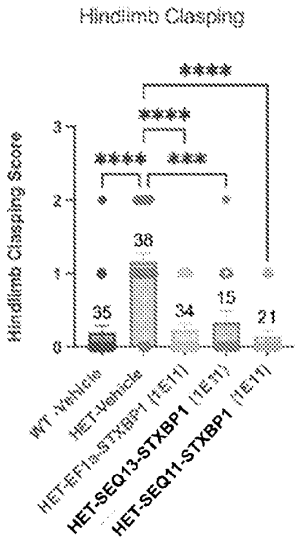

Hindlimb Clasping
Hindlimb Clasping Score
3
2
1
0
****
****
****
***
35
38
34
15
21
WT -Vehicle
HET-Vehicle
HET-EF1a-STXBP1 (1E11)
HET-SEQ13-STXBP1 (1E11)
HET-SEQ11-STXBP1 (1E11)

FIG. 26

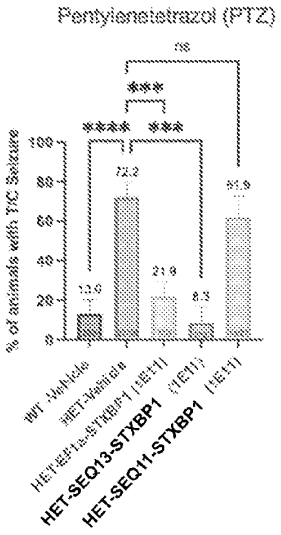

Pentylenetetrazol (PTZ)
ns
***
****
***
% of animals with TIC Seizure
100
80
60
40
20
0
12.9
72.2
21.9
8.3
95.8
WT -Vehicle
HET-Vehicle
HET-SEQ13-STXBP1 (+/E11)
HET-SEQ13-STXBP1 (-/E11)
HET-SEQ11-STXBP1 (+/E11)

FIG. 27

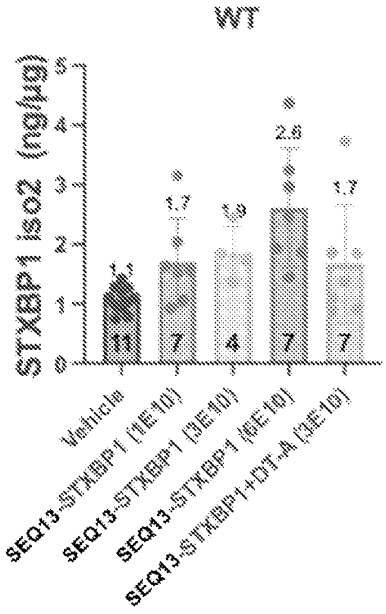

WT
STXBP1 iso2 (mg/ug)
0
1
2
3
4
5
1.7
1.7
1.9
2.6
1.7
11
7
4
7
7
Vehicle
SEQ13-STXBP1 (1E10)
SEQ13-STXBP1 (3E10)
SEQ13-STXBP1 (6E10)
SEQ13-STXBP1+DTrA (3E10)

FIG. 28A

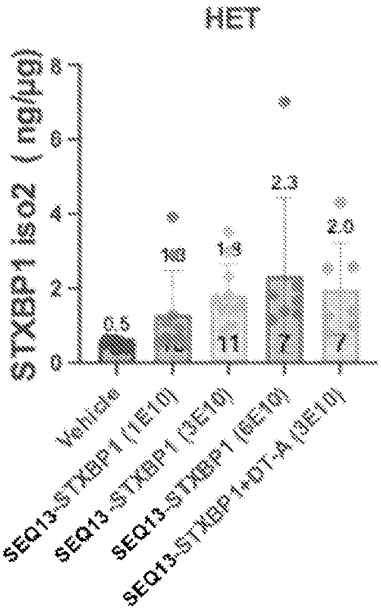


FIG. 28B

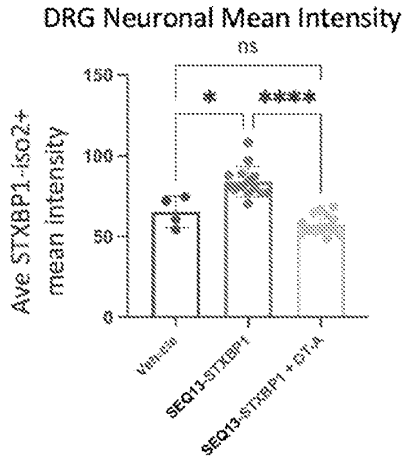


FIG. 28C

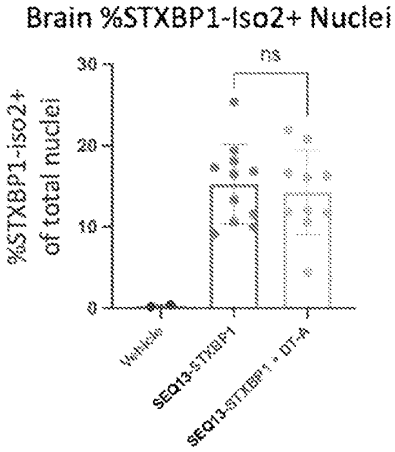

Brain %STXBP1-Iso2+ Nuclei
%STXBP1-iso2+ of total nuclei
ns
30
20
10
0
Vehicle
SEQ13-STXBP1
SEQ13-STXBP1 + DT-A

FIG. 28D

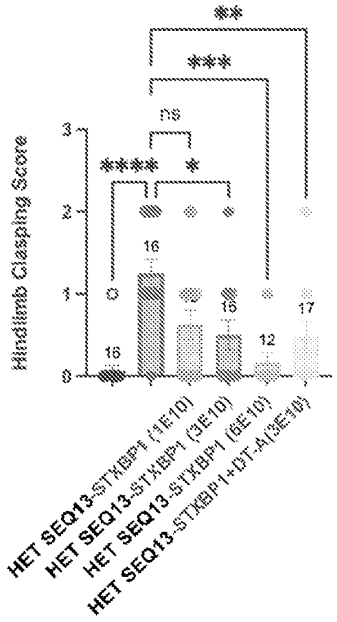

**
***
ns
****
*
Hindlimb Clasping Score
3
2
1
0
16
16
15
12
17
HET SEQ13-STXBP1 (1E10)
HET SEQ13-STXBP1 (3E10)
HET SEQ13-STXBP1 (6E10)
HET SEQ13-STXBP1+GT-A(3E10)

FIG. 29

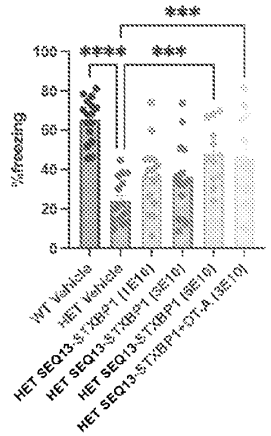

***
****
***
%freezing
100
80
60
40
20
0
WT Vehicle
HET Vehicle
HET SEQ13-STXBP1 (1E10)
HET SEQ13-STXBP1 (3E10)
HET SEQ13-STXBP1 (6E10)
HET SEQ13-STXBP1+GT-A (3E10)

FIG. 30

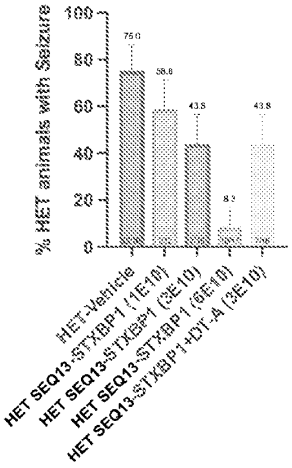

% MET animals with Seizure
100
80
60
40
20
0
75.0
58.6
43.8
8.3
43.8
HET-Vehicle
HET SEQ13-STXBP1 (1E10)
HET SEQ13-STXBP1 (3E10)
HET SEQ13-STXBP1 (6E10)
HET SEQ13-STXBP1+DT-A (3E10)

FIG. 31

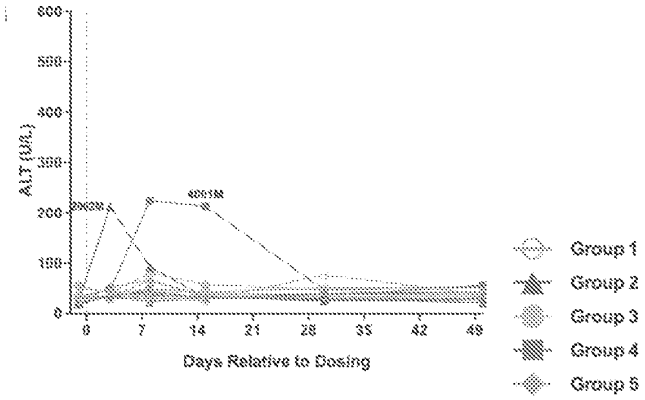


FIG. 32A

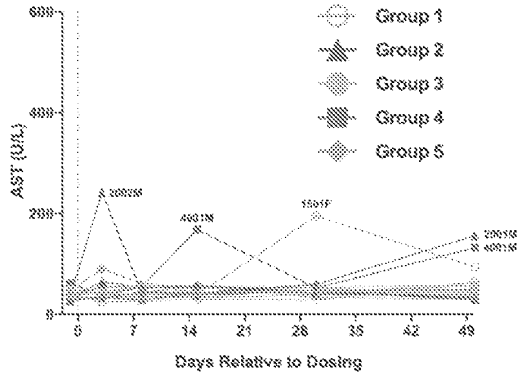


FIG. 32B

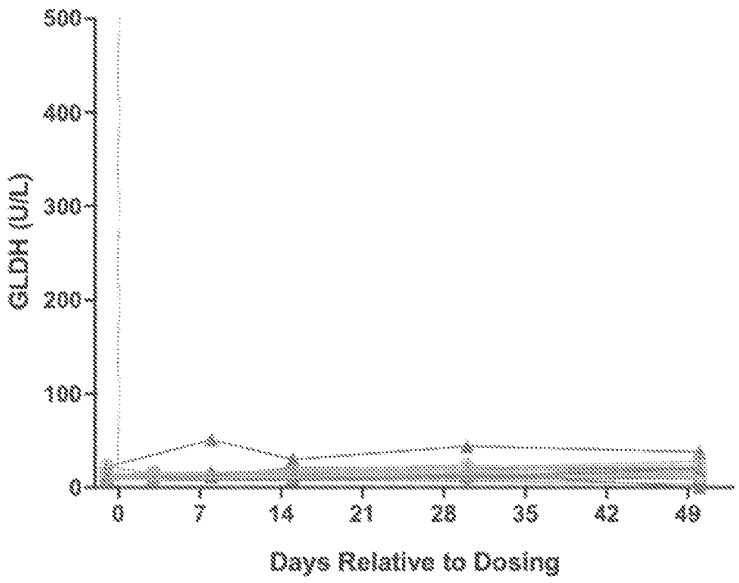


FIG. 32C

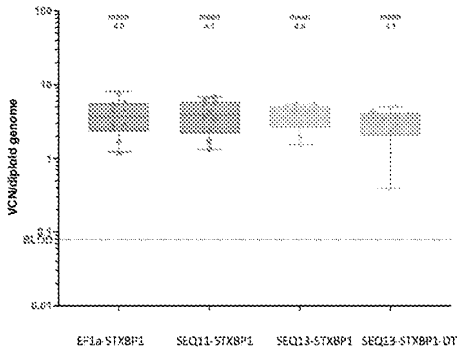


FIG. 33

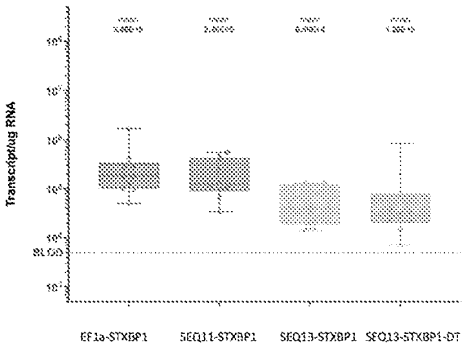

Transcript/ug RNA
EF1a-STXBP1
SEQ11-STXBP1
SEQ13-STXBP1
SEQ13-STXBP1-DT

FIG. 34

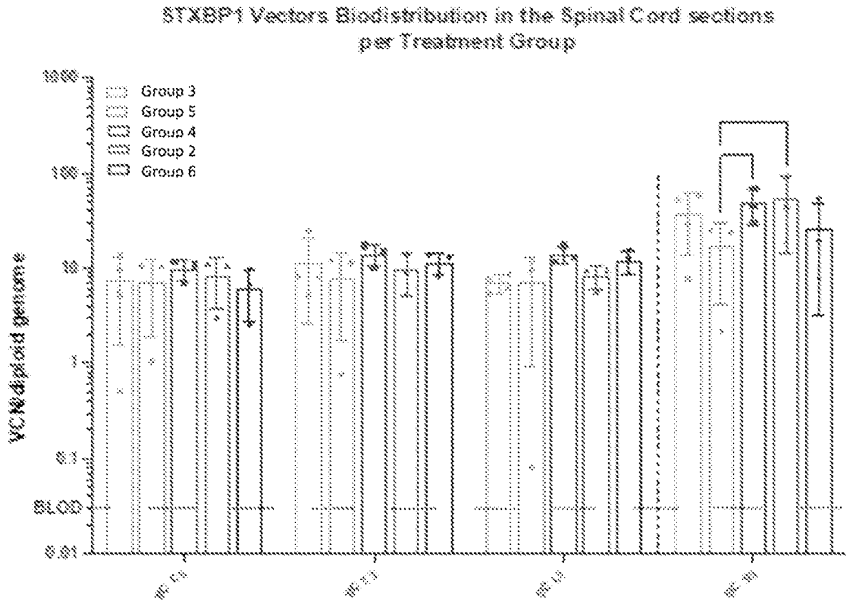

STXBP1 Vectors Biodistribution in the Spinal Cord sections per Treatment Group
Group 3
Group 5
Group 4
Group 2
Group 6
VCN/diploid genome
BLOD

FIG. 35

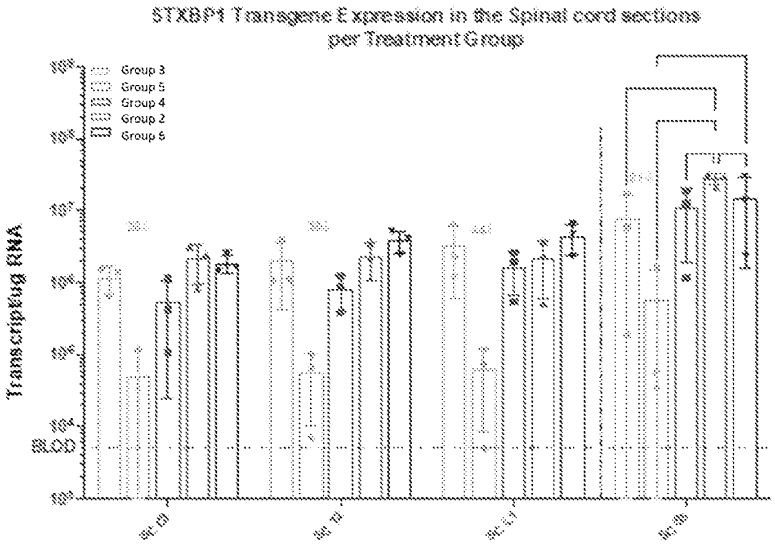

STXBP1 Transgene Expression in the Spinal cord sections
per Treatment Group
Group 3
Group 5
Group 4
Group 2
Group 6
Transcript/ug RNA
BLOD

FIG. 36

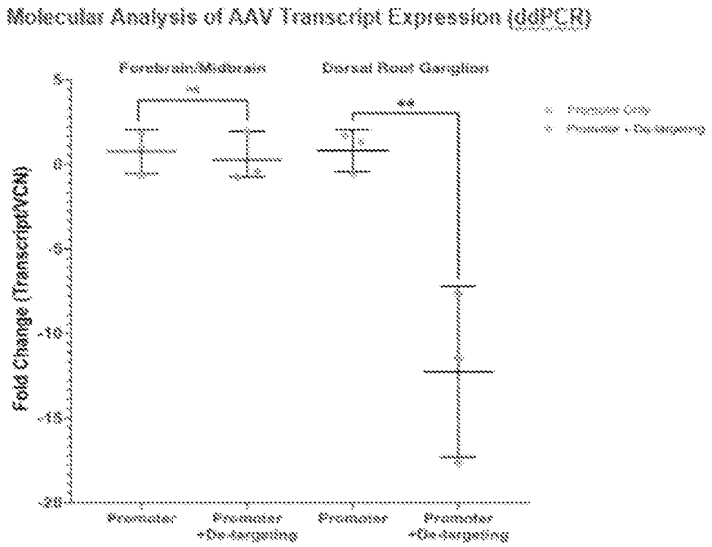

Molecular Analysis of AAV Transcript Expression (ddPCR)
Forebrain/Midbrain
Dorsal Root Ganglion
Fold Change (Transcript/VCN)
Promoter
Promoter +De-targeting
Promoter
Promoter +De-targeting

FIG. 37

Region		Histopathology Test Article Related-Findings	(EPAG-hvL) 1.0E14 vg/animal			Group 4			Group 3				Group 5		
			2001-M	2002-M	2001-F	4001-M	4002-M	4001-F	3001-M	3002-M	3001-F	3002-F	5001-M	5001-F	5002-F
Spinal Cord	Cervical	Degeneration, nerve fiber, dorsal funiculus, bilateral	1	2	2	2	1	1	1	2		1	1	1	
	Thoracic	Degeneration, nerve fiber, dorsal funiculus, bilateral	1	2	2	2	1	1	1	2	1	1	1	1	
	Lumbar	Degeneration, nerve fiber, dorsal funiculus, bilateral	1	2	2	2	1	1	1	2	1	1	1	1	
Dorsal Root Ganglion	Cervical	Necrosis, neuron													
		Cellularity, decreased, neuron								1					
		Autophagy, neuron													
		Cellularity, increased, satellite glial cell								1					
		Cellularity, increased, Schwann cell, dorsal nerve root													
		Degeneration, nerve fiber, dorsal nerve root													
		Infiltrate, mononuclear cell								1					
	Thoracic	Autophagy, neuron													
		Infiltrate, mononuclear cell													
	Lumbar	Necrosis, neuron								1					
		Cellularity, decreased, neuron				1				1					
		Autophagy, neuron												1	
		Cellularity, increased, satellite glial cell								1					
		Cellularity, increased, Schwann cell, dorsal nerve root	1	2	1	2				1			1	1	
		Degeneration, nerve fiber, dorsal nerve root	1	2	1	2				1			1		
		Infiltrate, mononuclear cell	1	1	1				1				1		
	Sacral	Necrosis, neuron				1									
		Cellularity, decreased, neuron		1		1				1					
		Chromatolysis, neuron				1		1							
		Autophagy, neuron							1					1	1
		Cellularity, increased, satellite glial cell		1		1	1	1		1		1		1	
		Cellularity, increased, Schwann cell, dorsal nerve root	1	2	2	1	1			1	1				
		Degeneration, nerve fiber, dorsal nerve root	1	2	2	1	1				1		1		
		Infiltrate, mononuclear cell		1		1							1		
Nerve Conduction Test Article-Related Findings						No P,So,M		So↑, M	0			So↑,P,M			
Serum NfL (Neurofilament Light Chain) Transient Peak Levels (pg/ml)			762	1710	661	2409	195	227	288	1604	654	344	670	78.1	28.6

FIG. 38

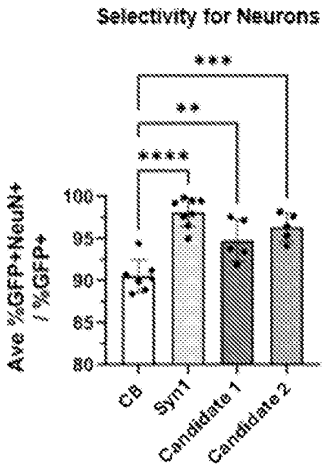


FIG. 39

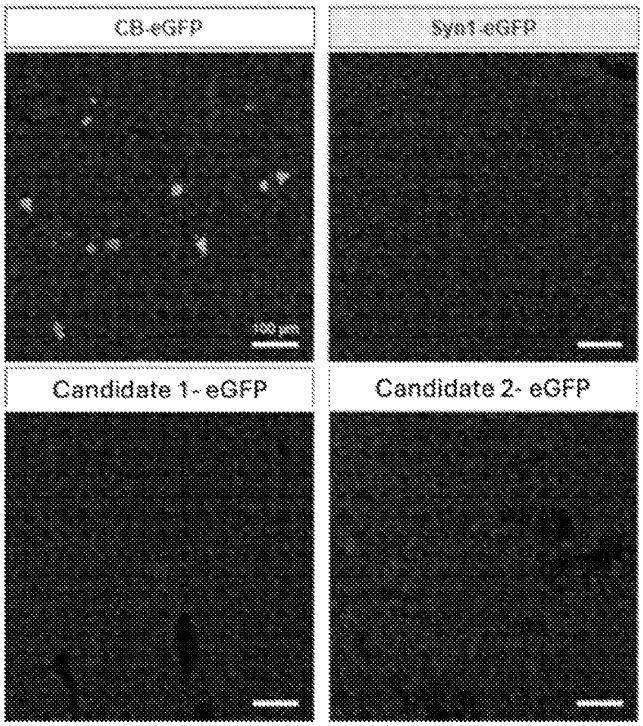


FIG. 40

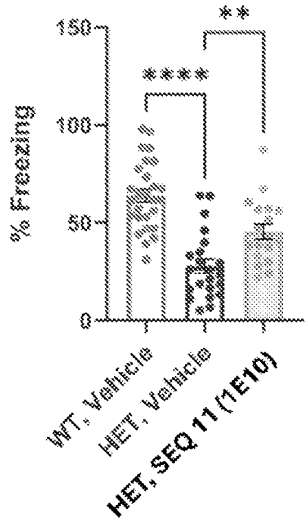

% Freezing
150
100
50
0
****
**
WT, Vehicle
HET, Vehicle
HET, SEQ 11 (1E10)

FIG. 41

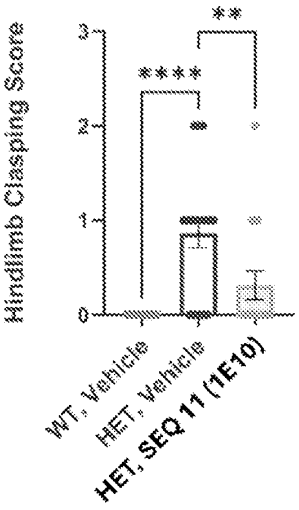

Hindlimb Clasping Score
2
1
0
****
**
WT, Vehicle
HET, Vehicle
HET, SEQ 11 (1E10)

FIG. 42

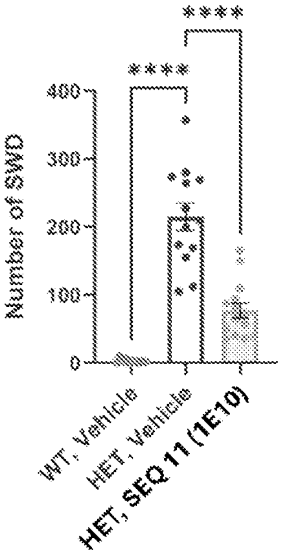

****
****
400
300
200
100
0
Number of SWD
WT, Vehicle
HET, Vehicle
HET, SEQ 11 (1E10)

FIG 43

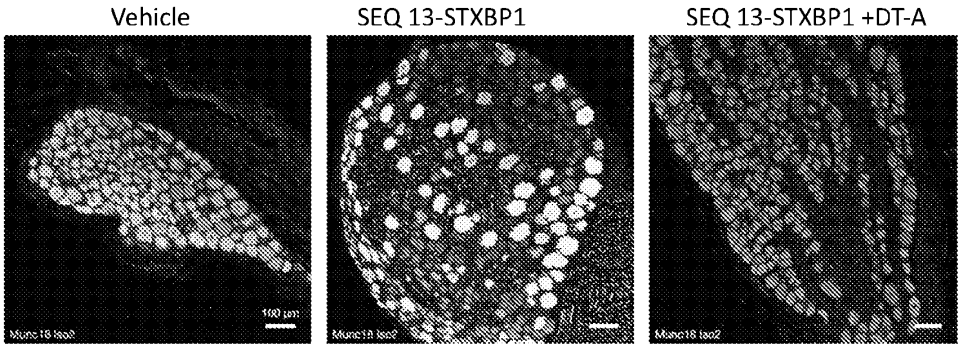

Vehicle
SEQ 13-STXBP1
SEQ 13-STXBP1 +DT-A

FIG 44

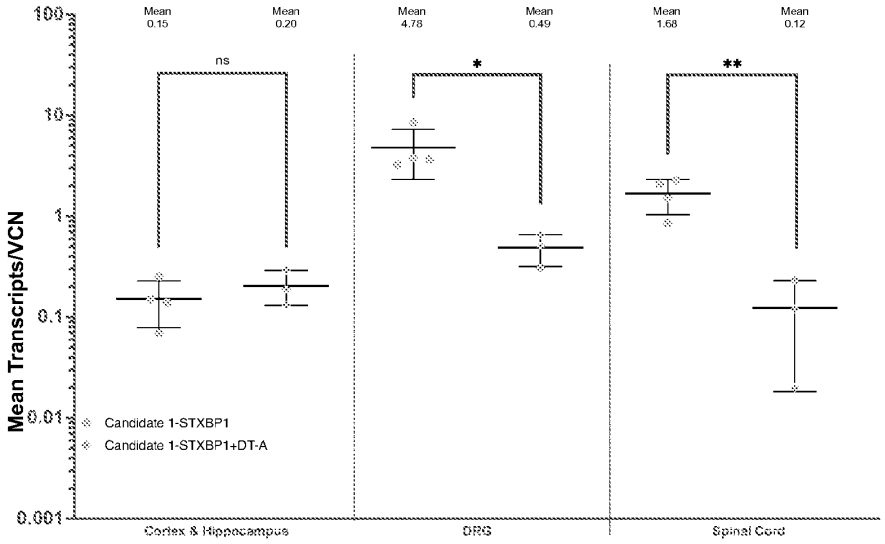

Mean
0.15
Mean
0.20
Mean
4.78
Mean
0.49
Mean
1.68
Mean
0.12
ns
*
**
100
10
1
0.1
0.01
0.001
Mean Transcripts/VCN
Candidate 1-STXBP1
Candidate 1-STXBP1+DT-A
Cortex & Hippocampus
DRG
Spinal Cord

FIG 45

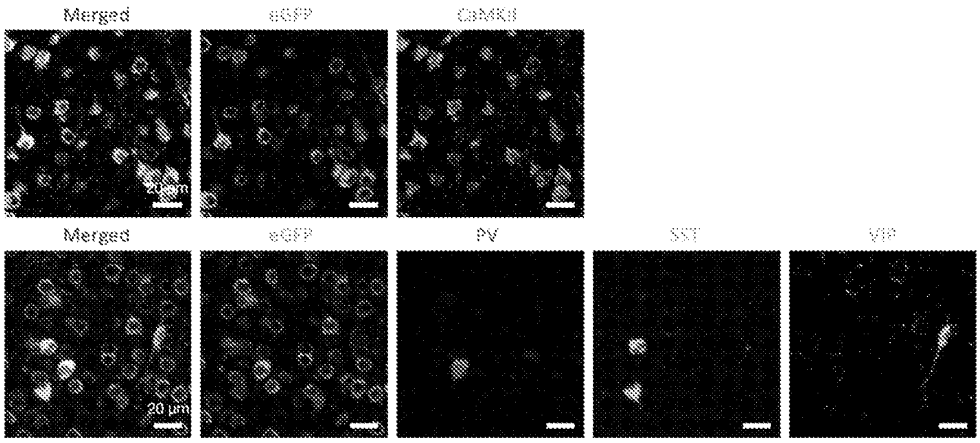


FIG. 46A

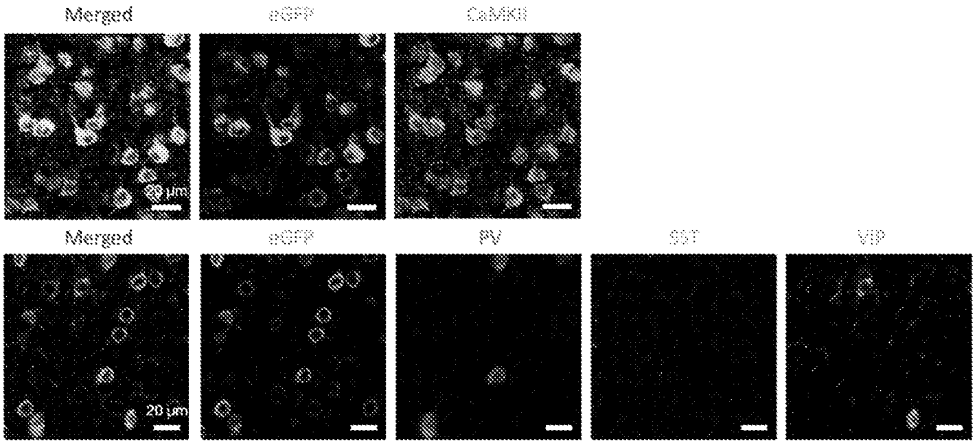


FIG. 46B

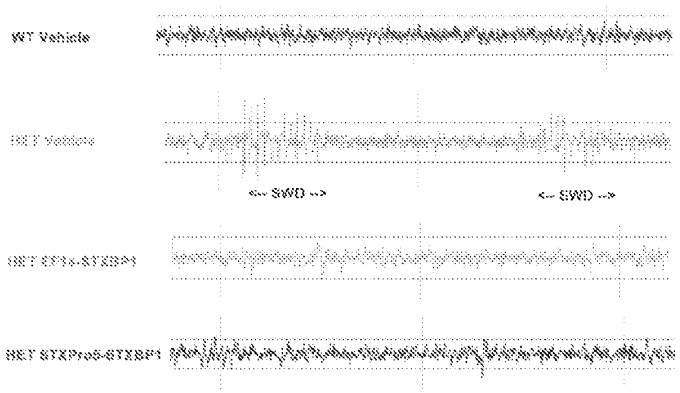


FIG. 47A

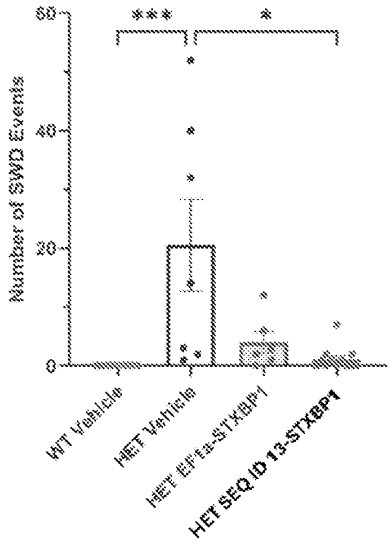


FIG. 47B

Hippocampus

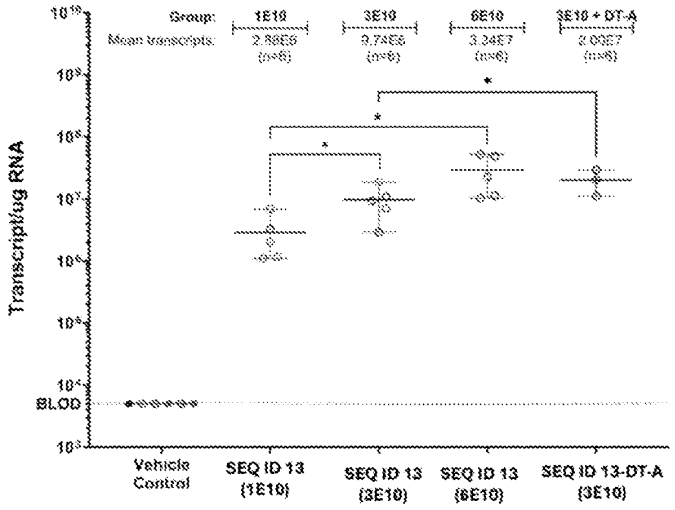


FIG. 48A

DRG

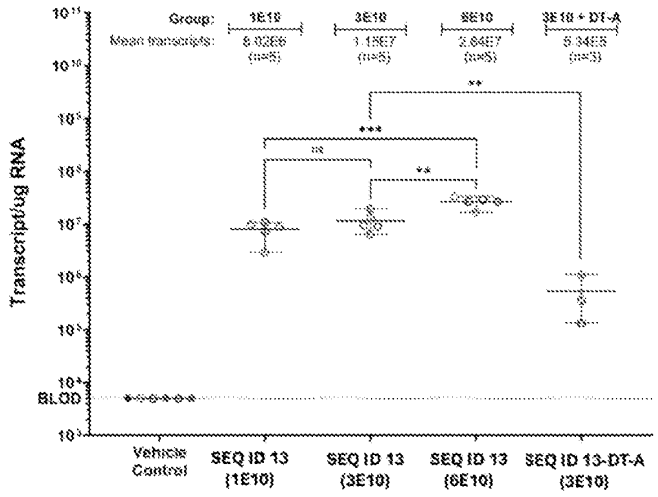


FIG. 48B

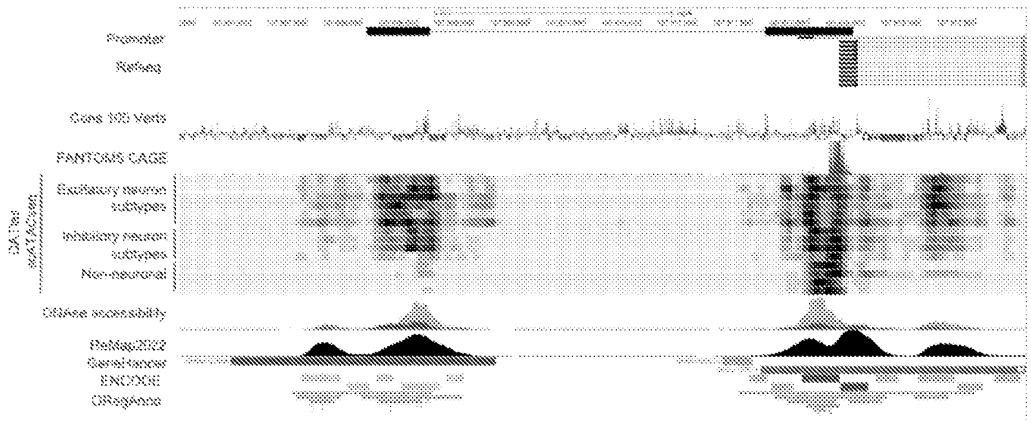


FIG. 49

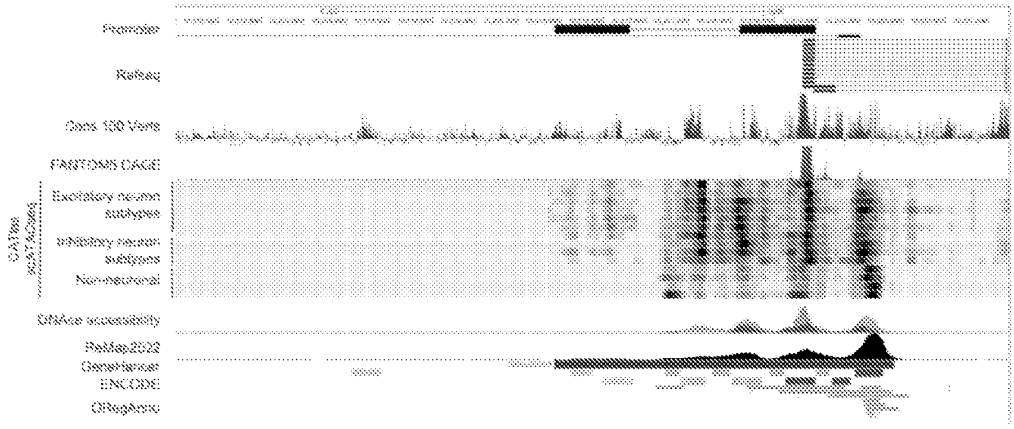

Promoter
RefSeq
Cons 100 Vert
FANTOM5 CAGE
Excitatory neuron subtypes
Inhibitory neuron subtypes
Non-neuronal
DNAse accessibility
ReMap 2022
GeneHancer
ENCODE
ORegAnno

FIG. 50

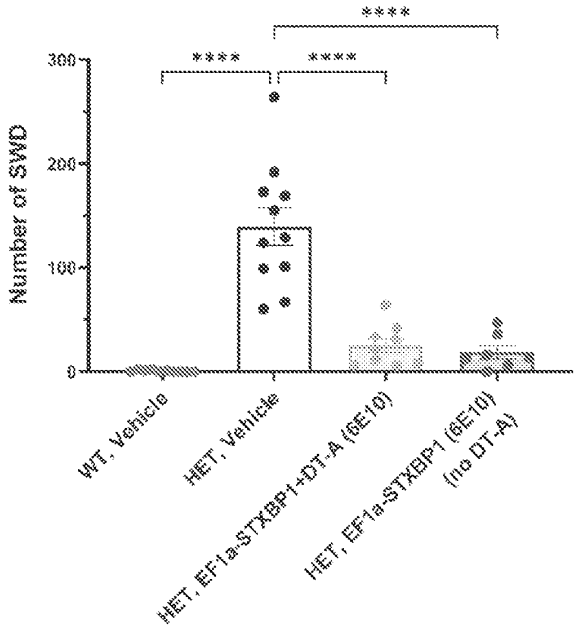

****
****
****
300
200
100
0
Number of SWD
WT, Vehicle
HET, Vehicle
HET, EF1a-STXBP1+DT-A (6E10)
HET, EF1a-STXBP1 (6E10)
(no DT-A)

FIG. 51

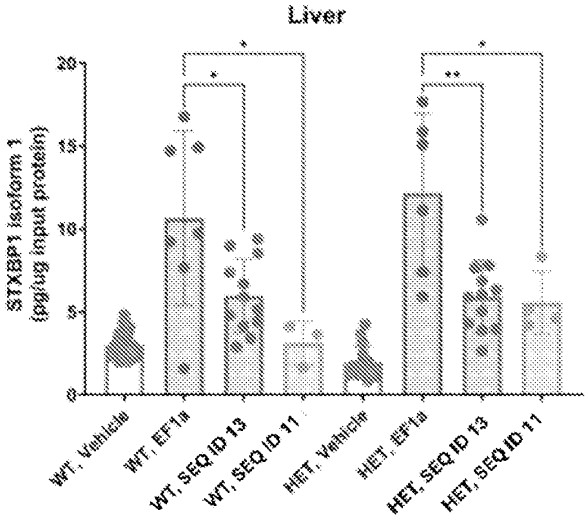


FIG. 52A

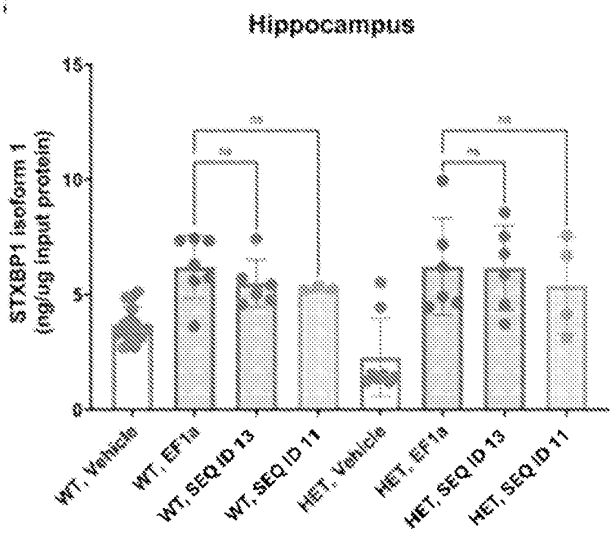


FIG. 52B

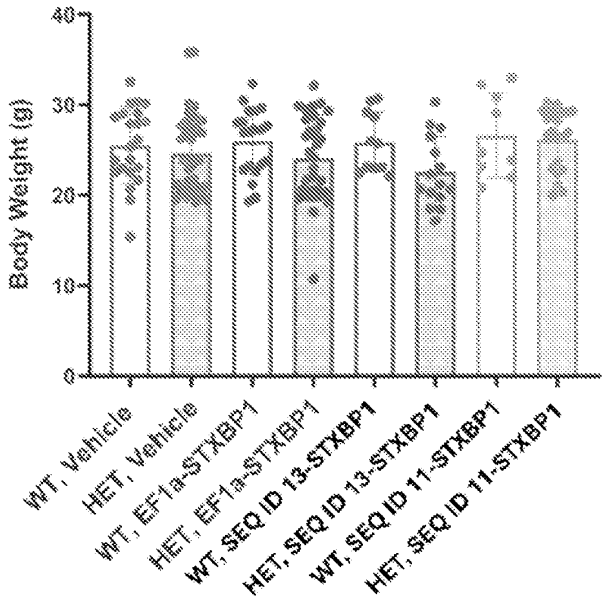

Body Weight (g)
40
30
20
10
0
WT, Vehicle
HET, Vehicle
WT, EF1a-STXBP1
HET, EF1a-STXBP1
WT, SEQ ID 13-STXBP1
HET, SEQ ID 13-STXBP1
WT, SEQ ID 11-STXBP1
HET, SEQ ID 11-STXBP1

FIG. 53

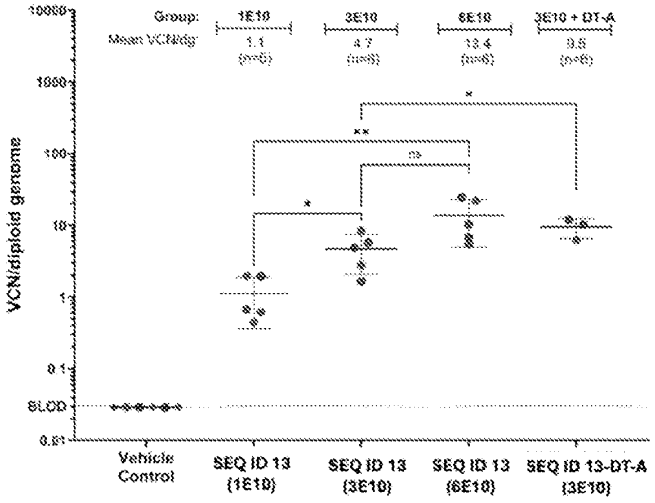


FIG. 54A

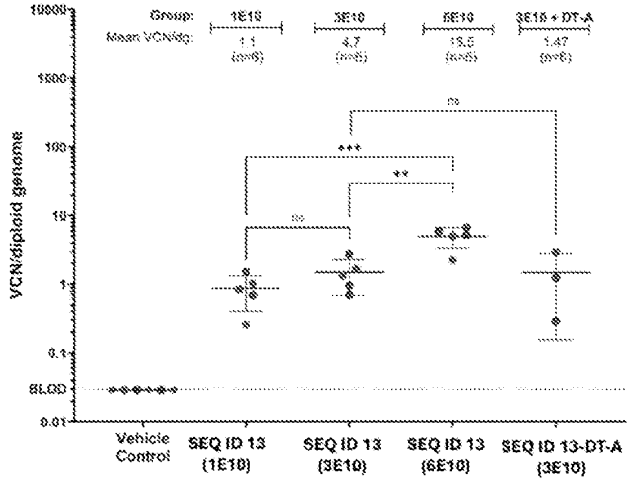


FIG. 54B

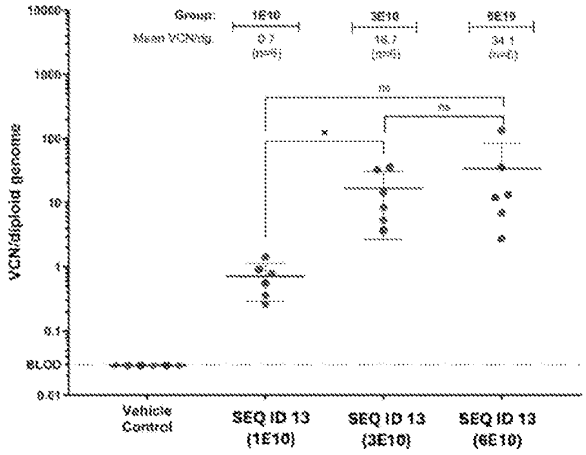


FIG. 54C

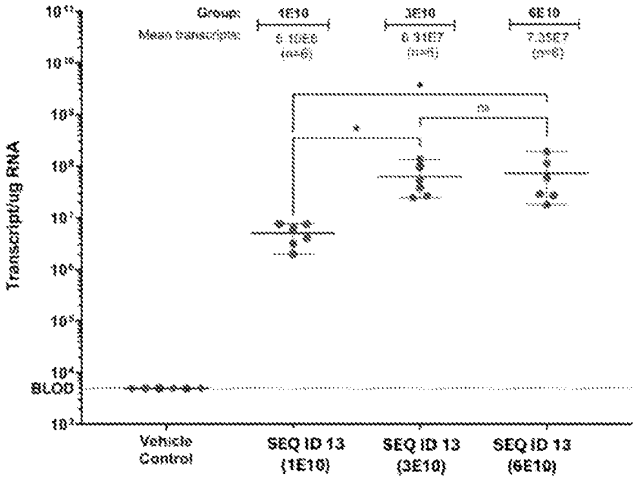


FIG. 55

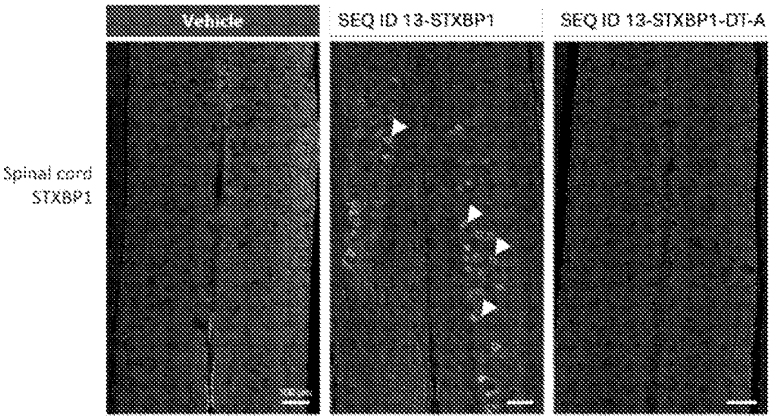

Vehicle
SEQ ID 13-STXBP1
SEQ ID 13-STXBP1-DT-A
Spinal cord
STXBP1

FIG. 56

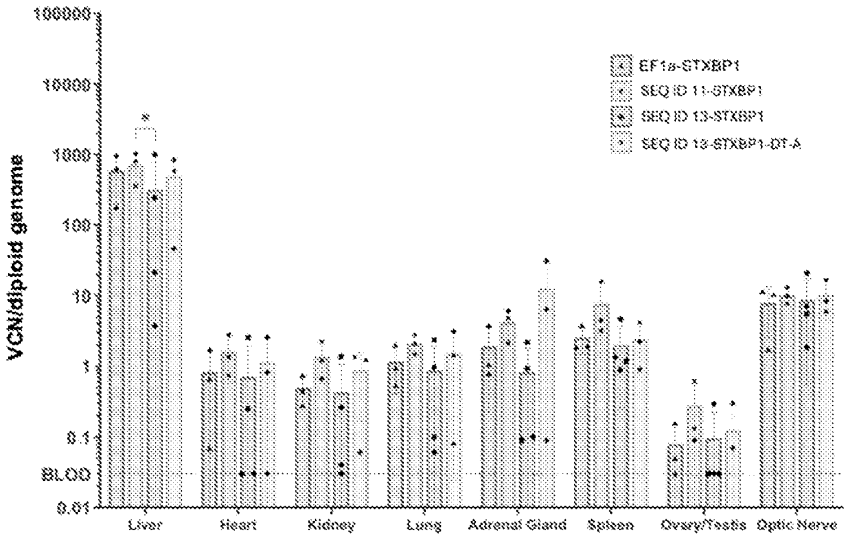

VCN/diploid genome
100000
10000
1000
100
10
1
0.1
BLOD
0.01
EF1a-STXBP1
SEQ ID 11-STXBP1
SEQ ID 13-STXBP1
SEQ ID 13-STXBP1-DT-A
Liver
Heart
Kidney
Lung
Adrenal Gland
Spleen
Ovary/Testis
Optic Nerve

FIG. 57

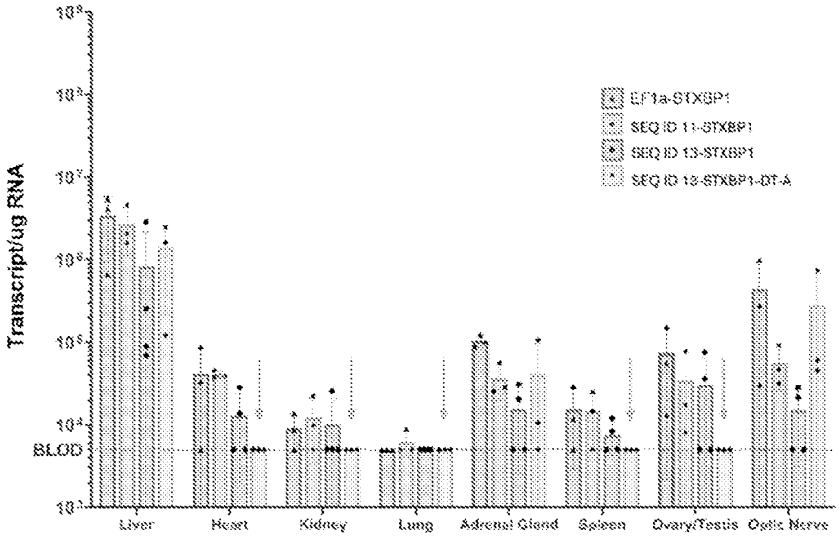

Transcript/ug RNA
BLOD
EF1a-STXBP1
SEQ ID 11-STXBP1
SEQ ID 13-STXBP1
SEQ ID 13-STXBP1-DT-A
Liver
Heart
Kidney
Lung
Adrenal Gland
Spleen
Ovary/Testis
Optic Nerve

FIG. 58

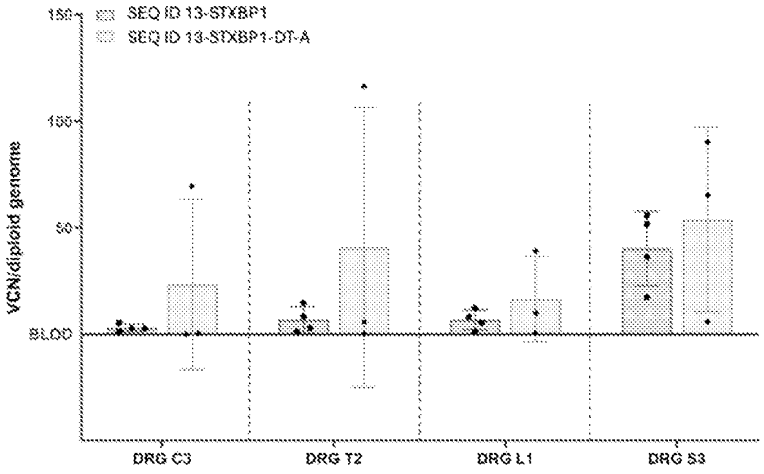

SEQ ID 13-STXBP1
SEQ ID 13-STXBP1-DT-A
vCN/diploid genome
150
100
50
BLOD
DRG C3
DRG T2
DRG L1
DRG S3

FIG. 59

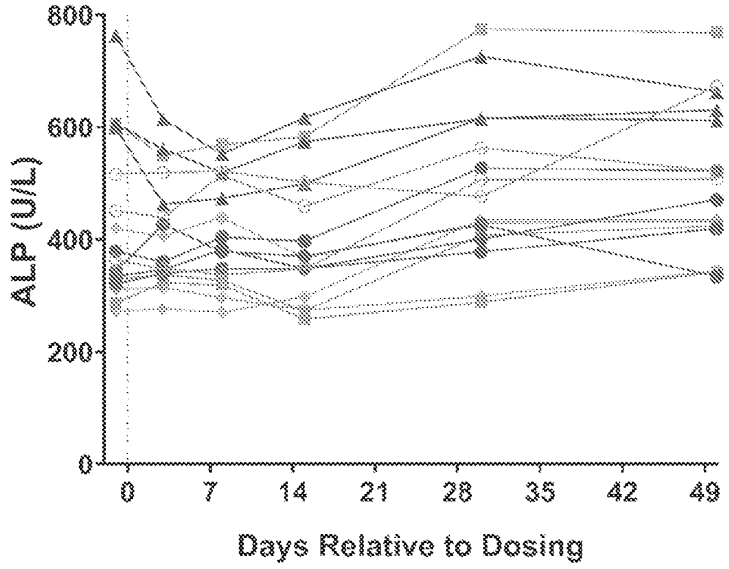

800
600
400
200
0
ALP (U/L)
0
7
14
21
28
35
42
49
Days Relative to Dosing

FIG. 60

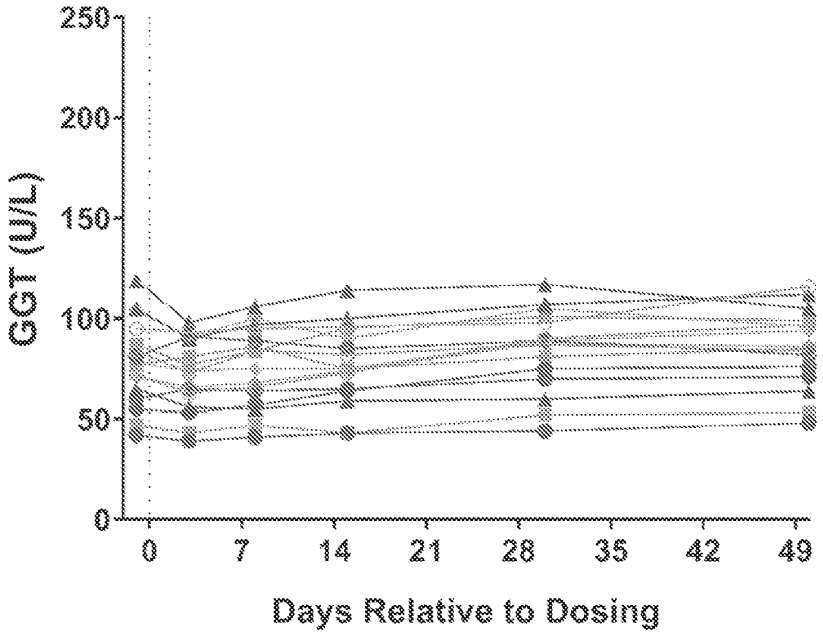

250
200
150
100
50
0
GGT (U/L)
0
7
14
21
28
35
42
49
Days Relative to Dosing

FIG. 61

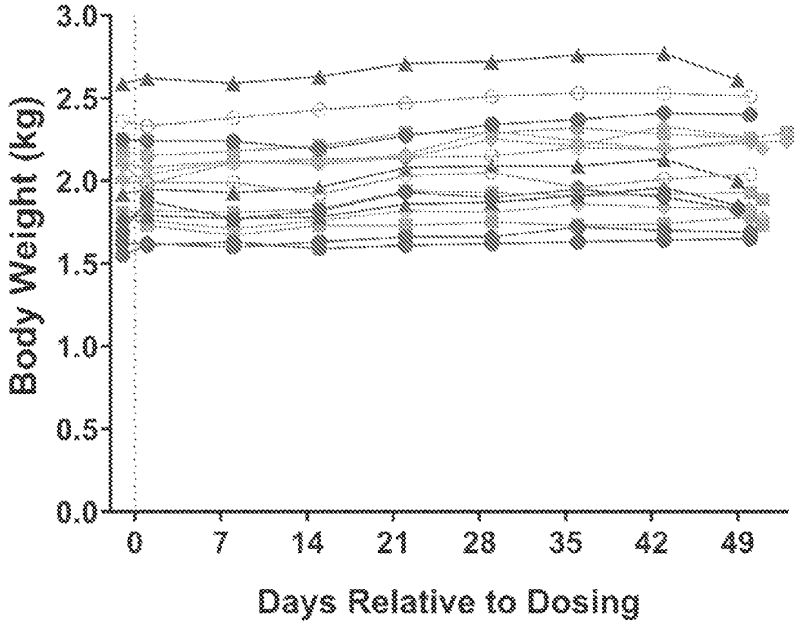

Body Weight (kg)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
7
14
21
28
35
42
49
Days Relative to Dosing

FIG. 62

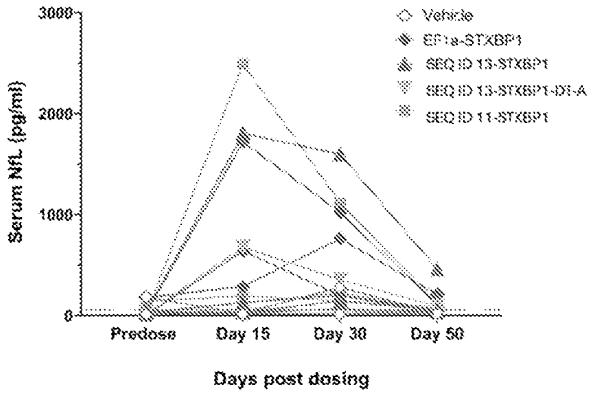

Serum NfL (pg/ml)
3000
2000
1000
0
Predose
Day 15
Day 30
Day 50
Days post dosing
Vehicle
EF1a-STXBP1
SEQ ID 13-STXBP1
SEQ ID 13-STXBP1-D1-A
SEQ ID 11-STXBP1

FIG. 63

Group	Sex	Sensory Nerves					Motor Nerve
		Sural	Peroneal	Saphenous	Radial	Median	Tibial
Vehicle	F	-	-	-	-	-	-
	M	-	-	-	-	-	-
	M	-	-	-	-	-	-
AAV9-EF1α-STXBP1	M	-	-	-	-	-	-
	M	-	-	-	-	-	-
	F	-	-	-	-	-	-
AAV9- SEQ ID 13-STXBP1	M	-	-	-	-	-	-
	F	-	-	-	-	-	-
	M	-	-	-	↓[3]	-	-
	F	*	↓	-	-	↓	-
AAV9-SEQ ID 11-STXBP1	M	-	-	-	-	-	-
	M	↓↓	↓	↓[1]	-	↓↓	-
	F	*	-	-	-	↓↓[1]	-
AAV9-SEQ ID 13-STXBP1-DT-A	F	-	-	-	-	-	-
	F	-	-	-	-	-	-
	M	-	-	-	-	-	-

FIG. 64