Figure 1

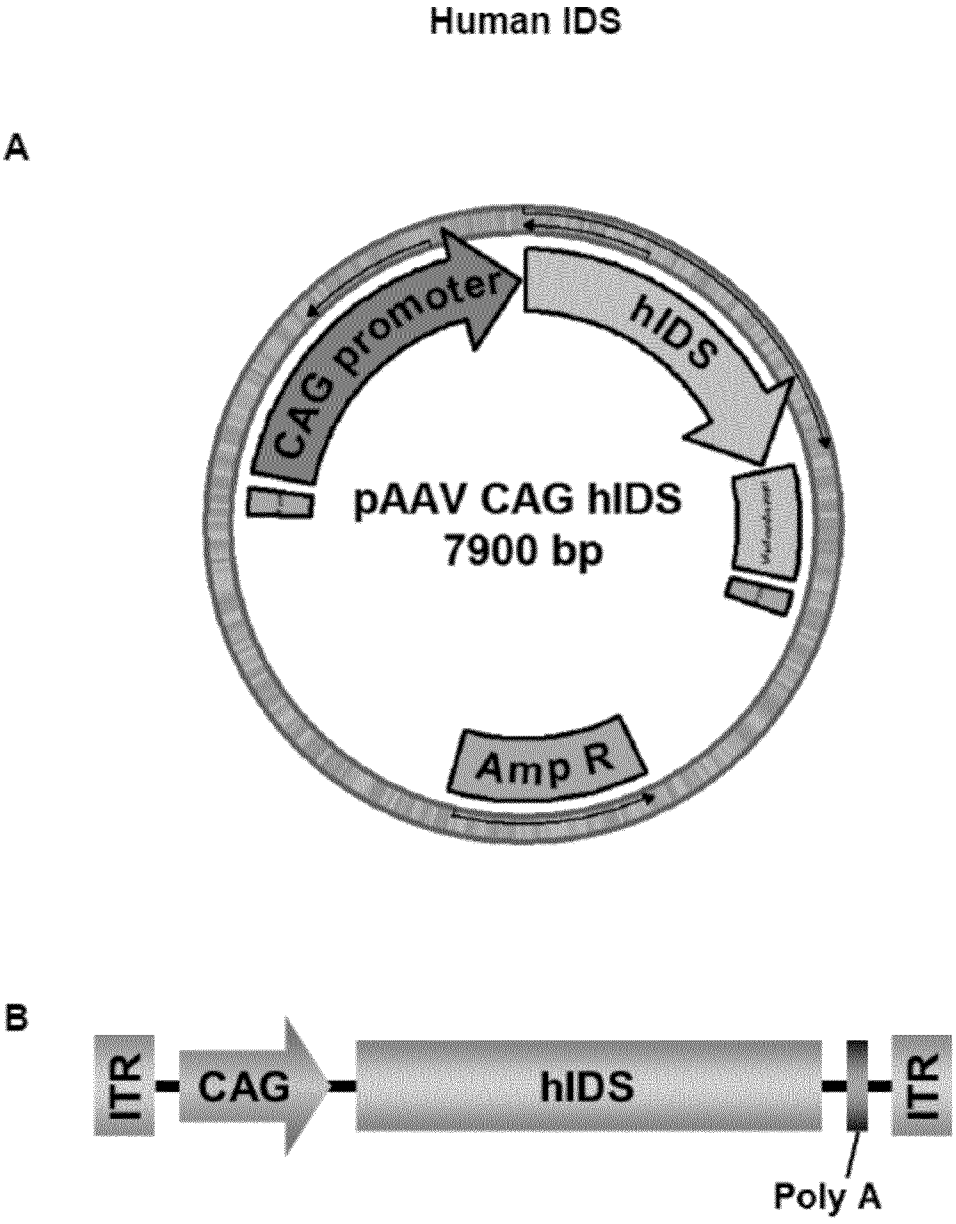

Human IDS
A
CAG promoter
hIDS
pAAV CAG hIDS
7900 bp
Amp R
B
ITR
CAG
hIDS
ITR
Poly A

Figure 2

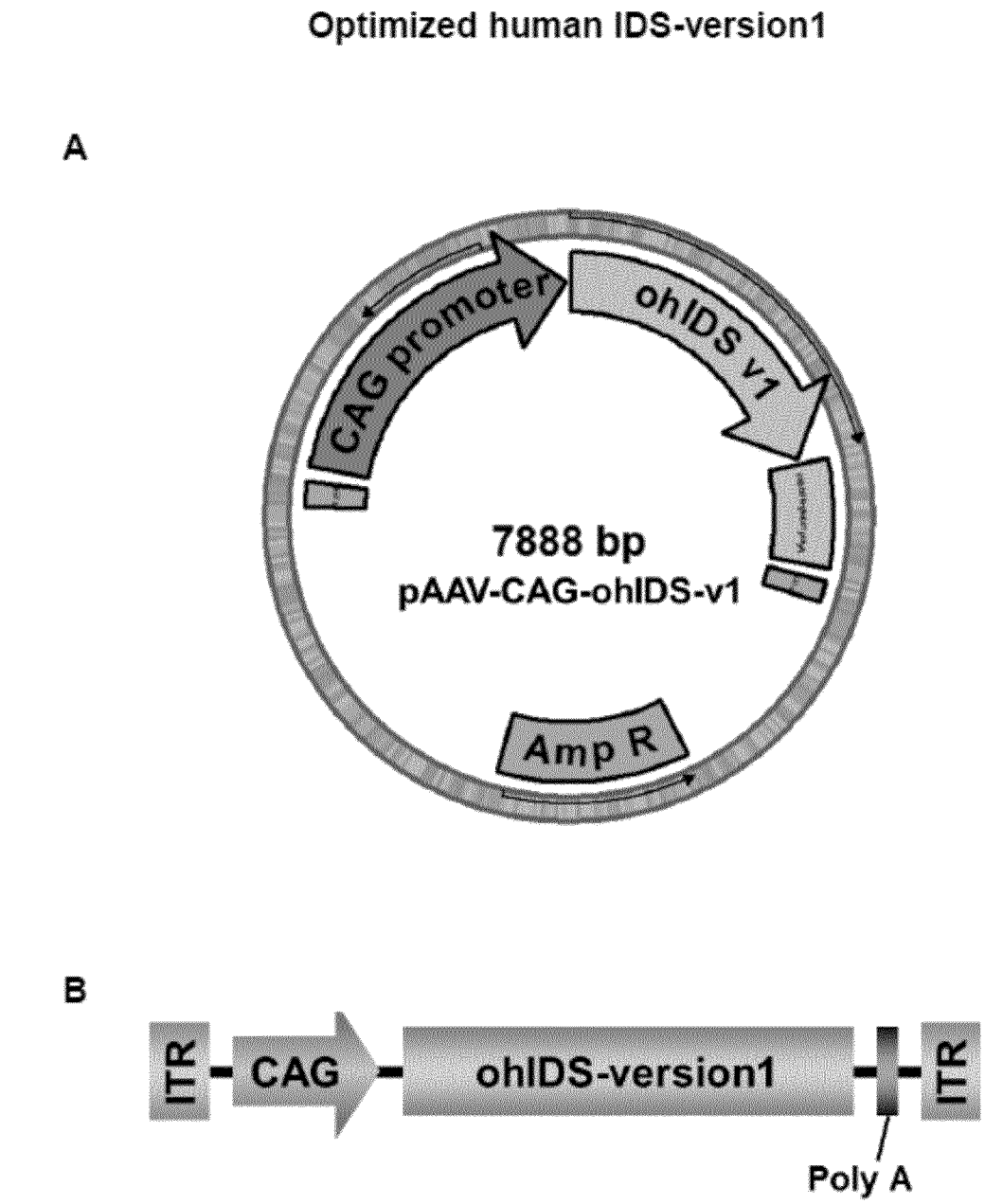

Optimized human IDS-version1
A
CAG promoter
ohIDS v1
7888 bp
pAAV-CAG-ohIDS-v1
Amp R
B
ITR
CAG
ohIDS-version1
ITR
Poly A

Figure 3

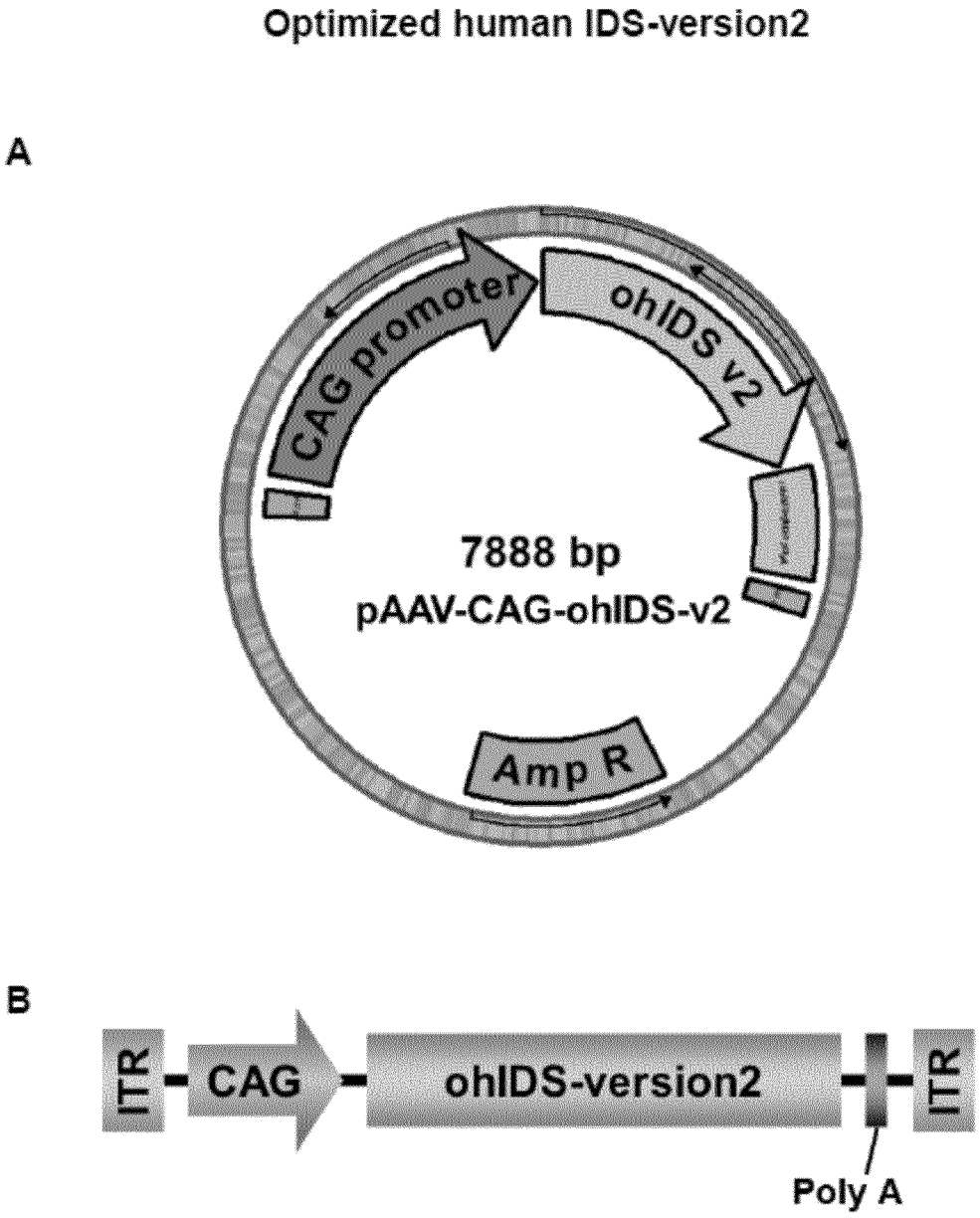

Optimized human IDS-version2
A
CAG promoter
ohIDS v2
7888 bp
pAAV-CAG-ohIDS-v2
Amp R
B
ITR
CAG
ohIDS-version2
ITR
Poly A

Figure 4

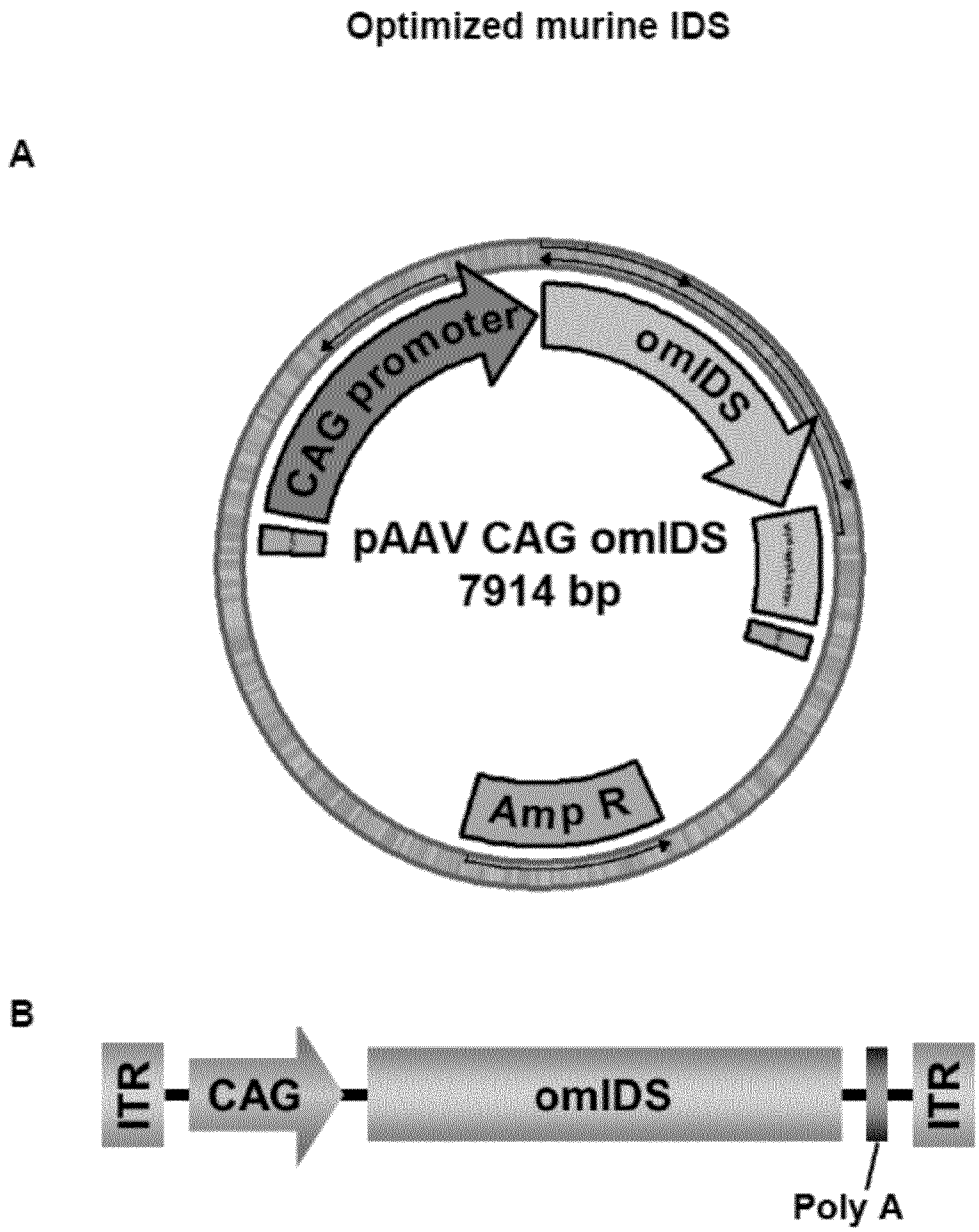

Optimized murine IDS
A
CAG promoter
omIDS
pAAV CAG omIDS
7914 bp
Amp R
B
ITR
CAG
omIDS
ITR
Poly A

Figure 5

Hydrodynamic injection of pAAV-CAG-hIDS, pAAV-CAG-ohIDS-version1 and pAAV-CAG-ohIDS-version2 plasmids in healthy mice

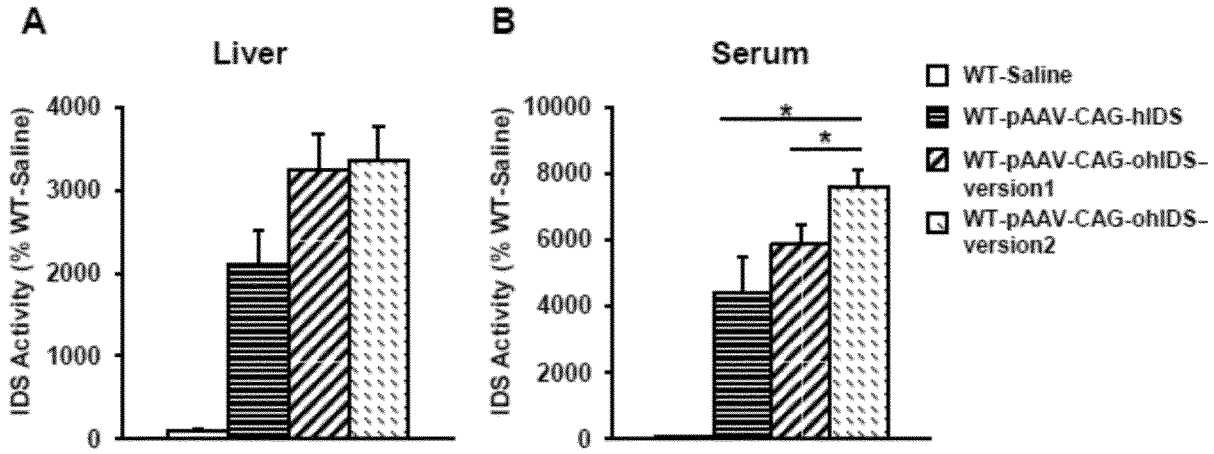


Figure 6

Hydrodynamic injection of pAAV-CAG-hIDS, pAAV-CAG-ohIDS-version1 and pAAV-CAG-ohIDS-version2 plasmids in MPSII mice

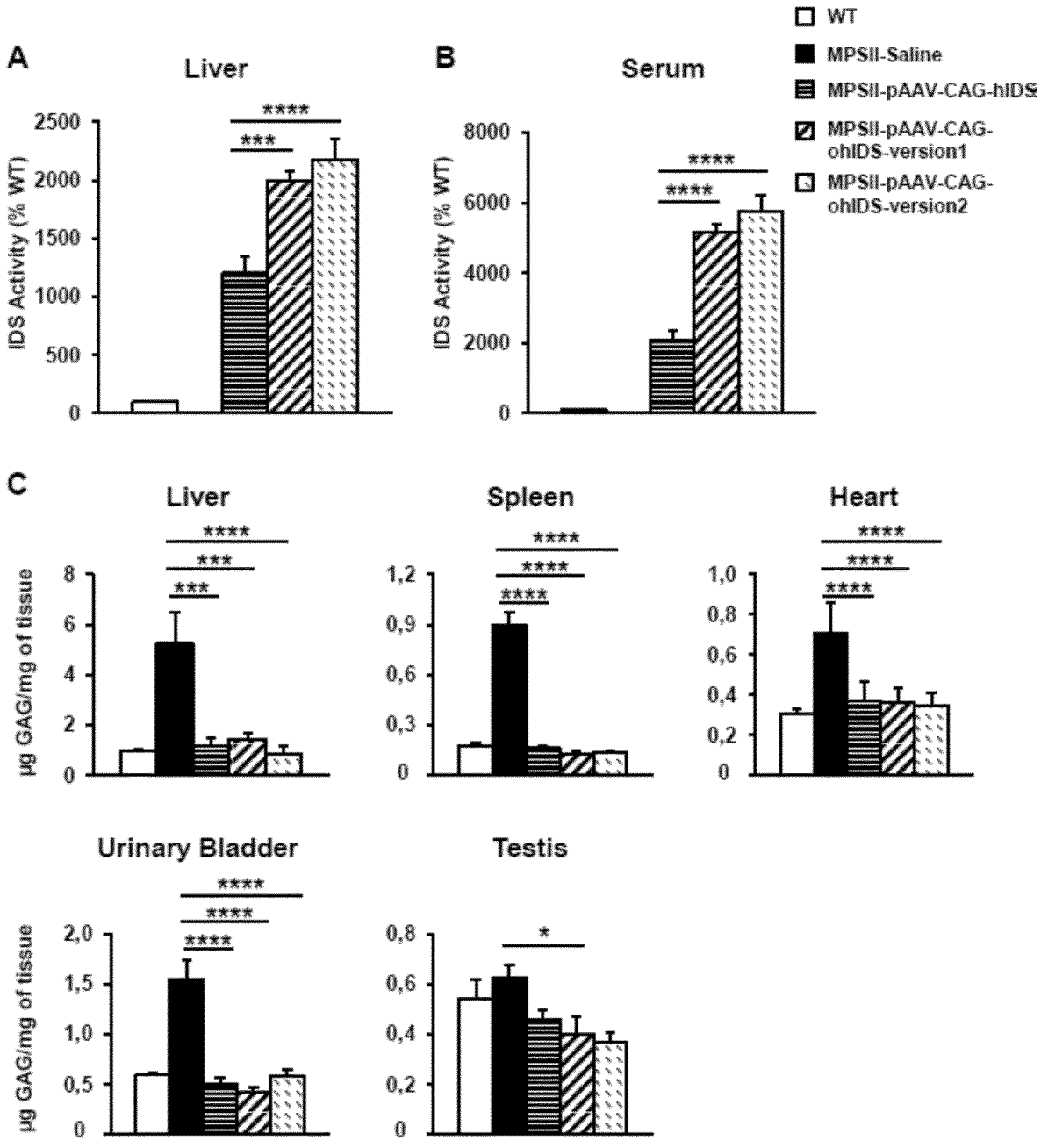


Figure 7

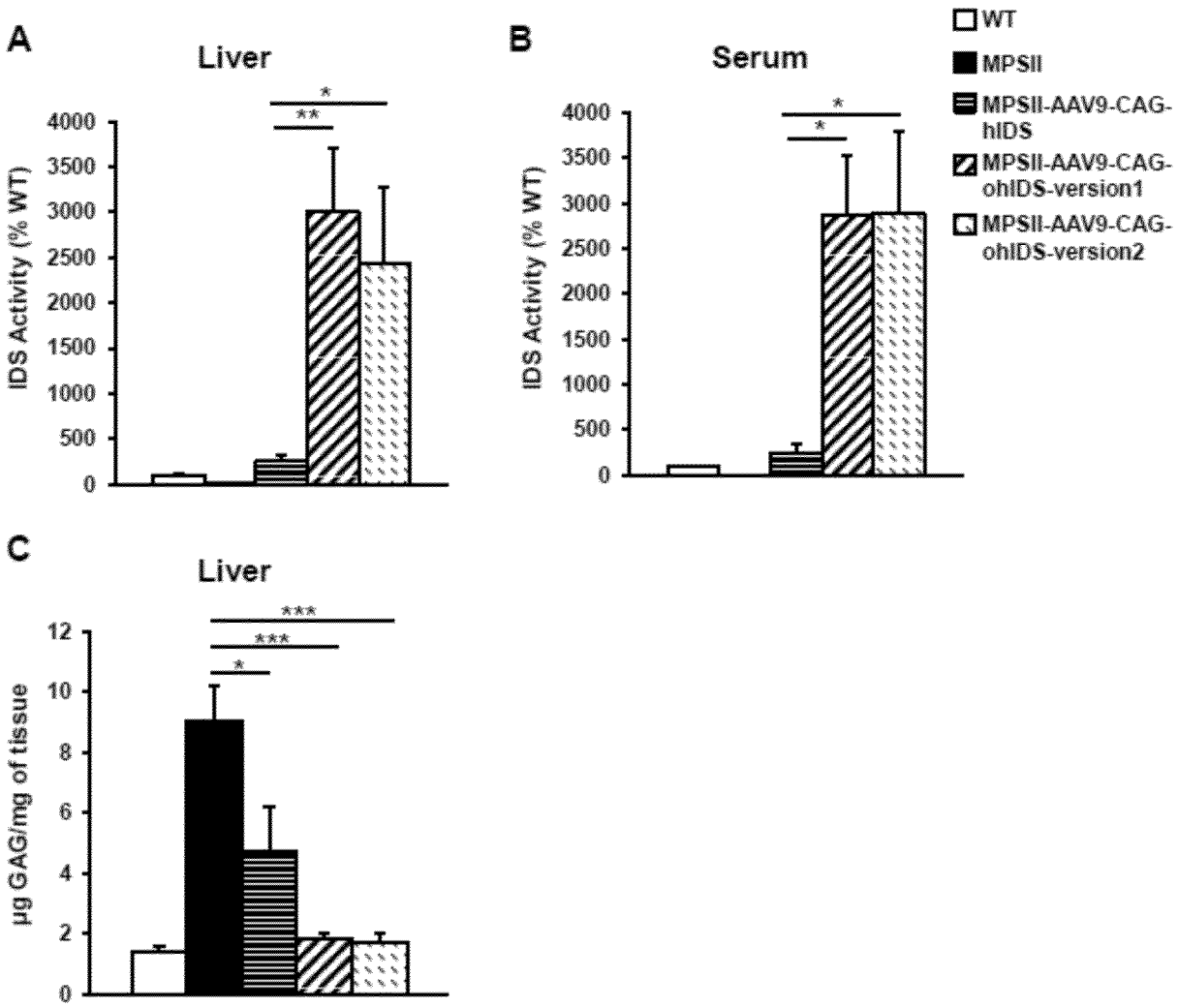

Intravenous injection of AAV9-CAG-hIDS, AAV9-CAG-ohIDS-version1 and AAV9-CAG-ohIDS-version2 vectors in MPSII mice
A
Liver
IDS Activity (% WT)
4000
3500
3000
2500
2000
1500
1000
500
0
*
**
B
Serum
IDS Activity (% WT)
4000
3500
3000
2500
2000
1500
1000
500
0
*
*
WT
MPSII
MPSII-AAV9-CAG-hIDS
MPSII-AAV9-CAG-ohIDS-version1
MPSII-AAV9-CAG-ohIDS-version2
C
Liver
µg GAG/mg of tissue
12
10
8
6
4
2
0
***
***
*

Figure 8

Intra-CSF injection of AAV9-CAG-hIDS, AAV9-CAG-ohIDS-version1 and AAV9-CAG-ohIDS-version2 vectors in MPSII mice

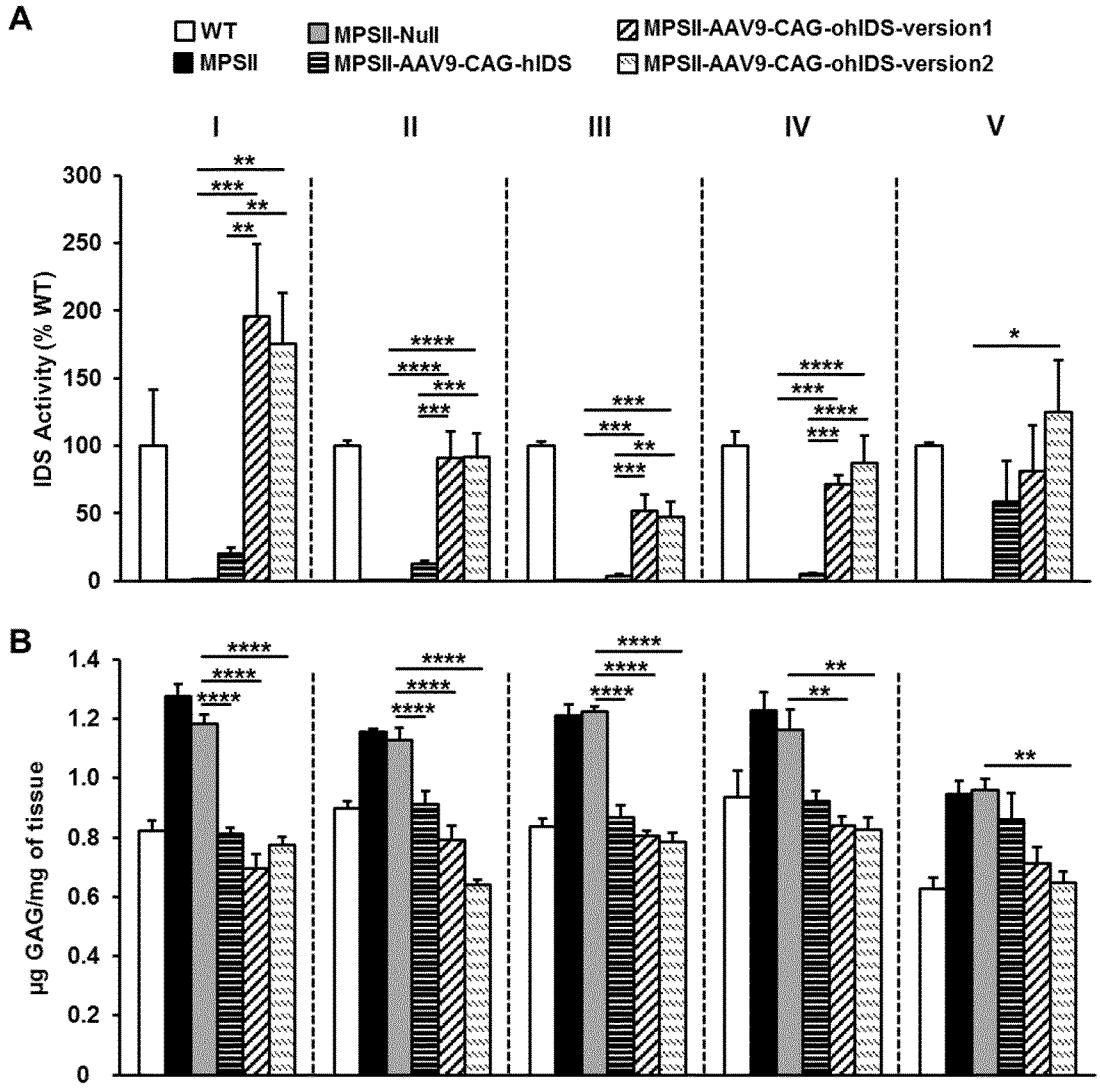


Figure 9

Intra-CSF injection of AAV9-CAG-hIDS, AAV9-CAG-ohIDS-version1 and AAV9-CAG-ohIDS-version2 vectors in MPSII mice

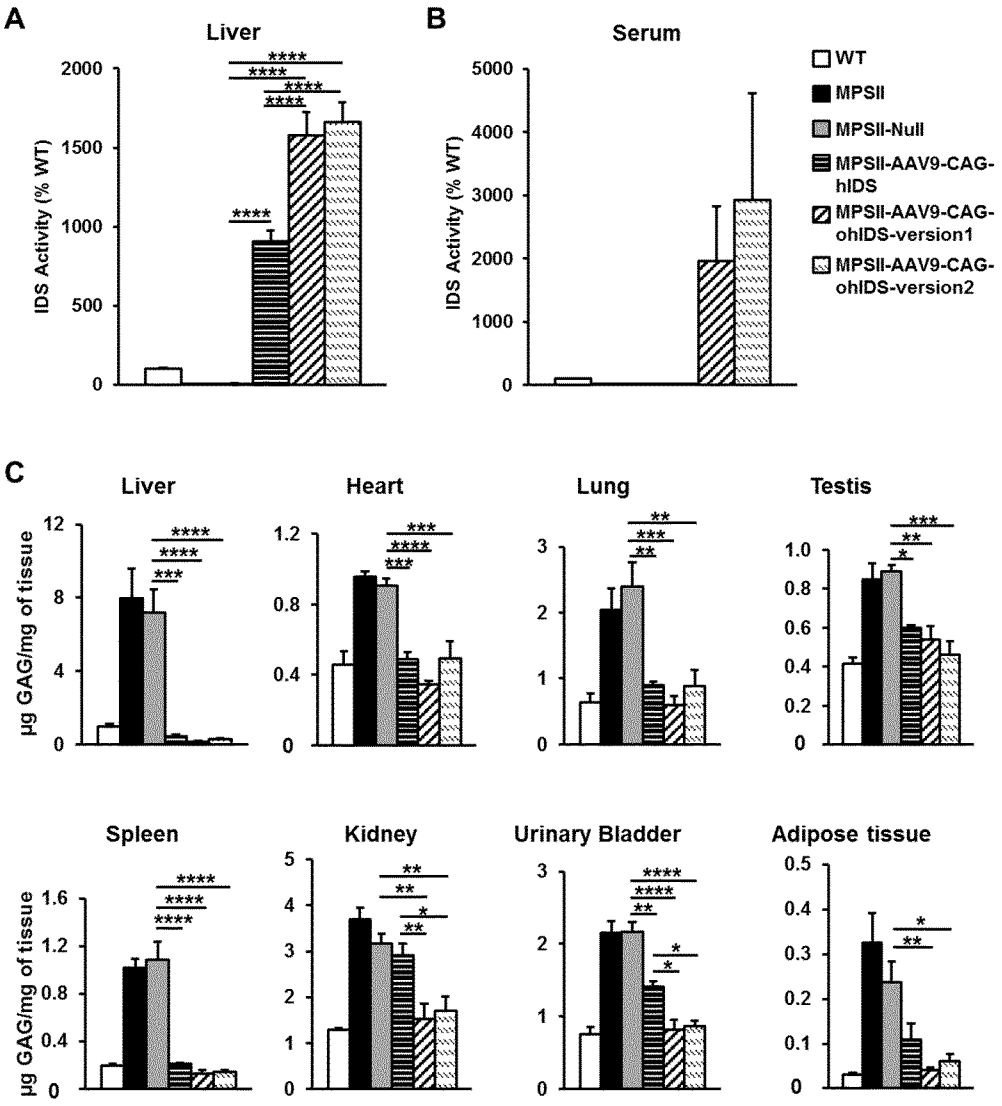


Figure 10

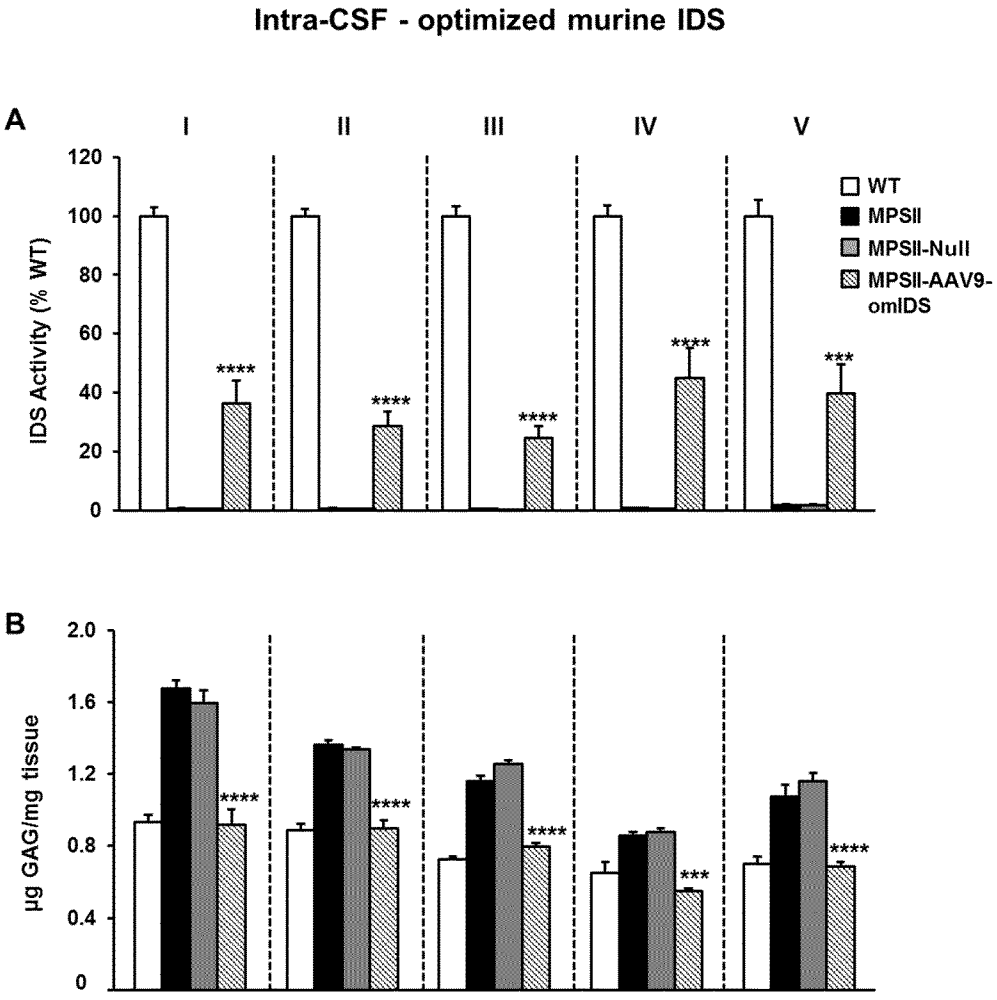

Intra-CSF - optimized murine IDS
A
I
II
III
IV
V
IDS Activity (% WT)
120
100
80
60
40
20
0
WT
MPSII
MPSII-Null
MPSII-AAV9-omIDS
****
****
****
****
***
B
µg GAG/mg tissue
2.0
1.6
1.2
0.8
0.4
0
****
****
****
***
****

Figure 11

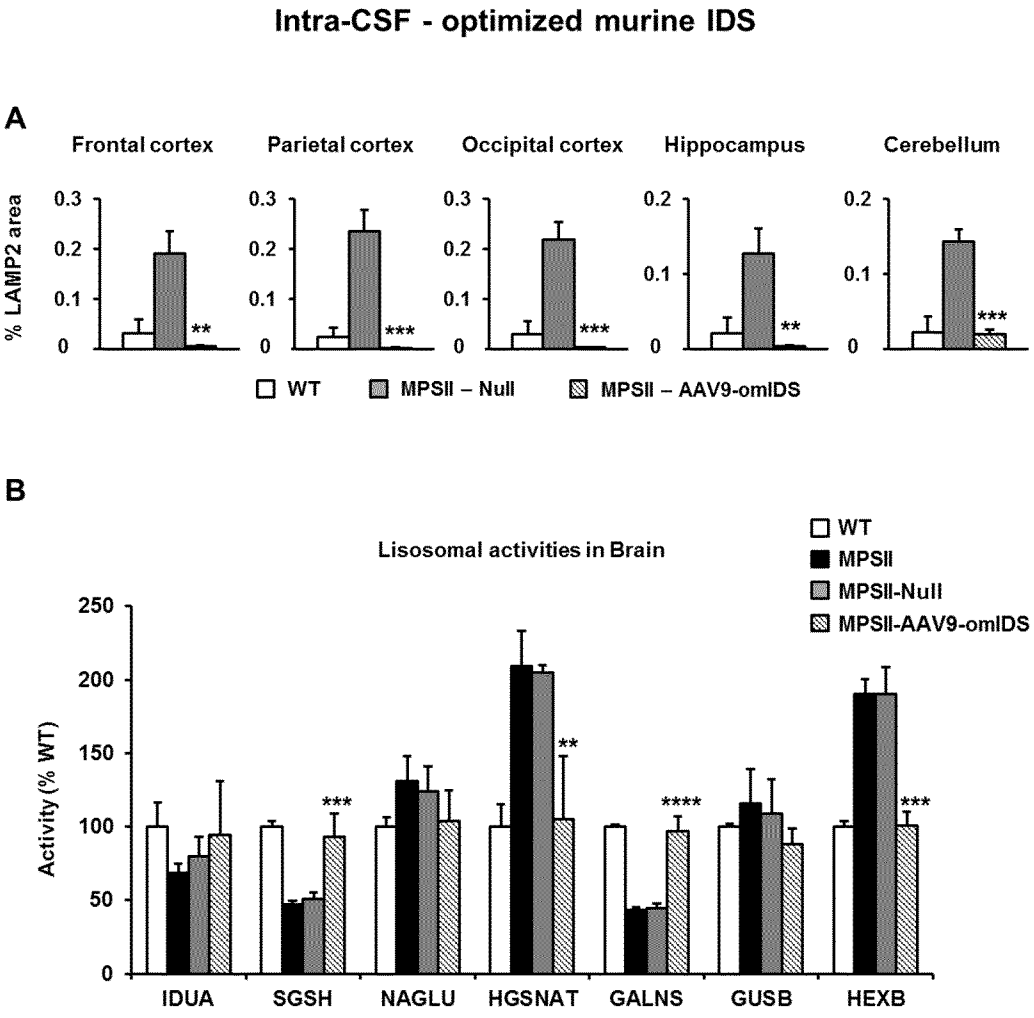

Intra-CSF - optimized murine IDS
A
Frontal cortex
Parietal cortex
Occipital cortex
Hippocampus
Cerebellum
% LAMP2 area
WT
MPSII – Null
MPSII – AAV9-omIDS
B
Lisosomal activities in Brain
WT
MPSII
MPSII-Null
MPSII-AAV9-omIDS
Activity (% WT)
IDUA
SGSH
NAGLU
HGSNAT
GALNS
GUSB
HEXB

Figure 12

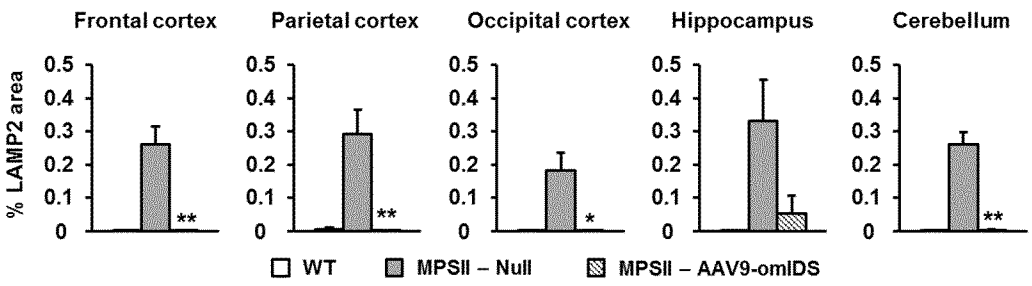

Intra-CSF - optimized murine IDS (long-term)
Frontal cortex
Parietal cortex
Occipital cortex
Hippocampus
Cerebellum
% LAMP2 area
0.5
0.4
0.3
0.2
0.1
0
**
**
*
**
WT
MPSII – Null
MPSII – AAV9-omIDS

Figure 13

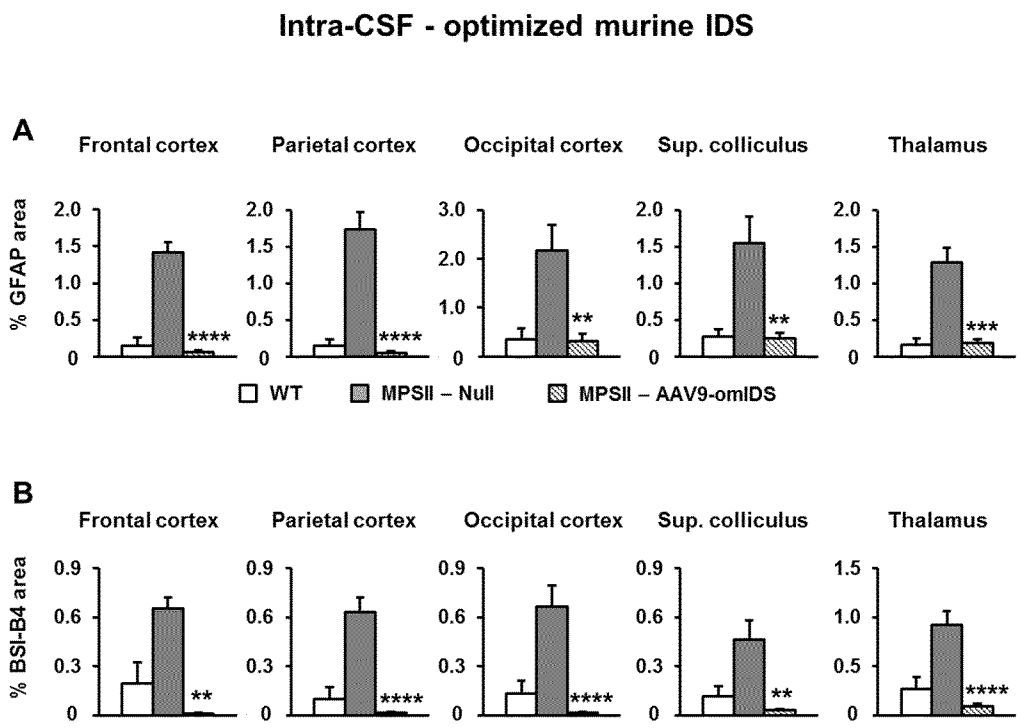

Intra-CSF - optimized murine IDS
A
Frontal cortex
Parietal cortex
Occipital cortex
Sup. colliculus
Thalamus
% GFAP area
WT
MPSII – Null
MPSII – AAV9-omIDS
B
% BSI-B4 area

Figure 14

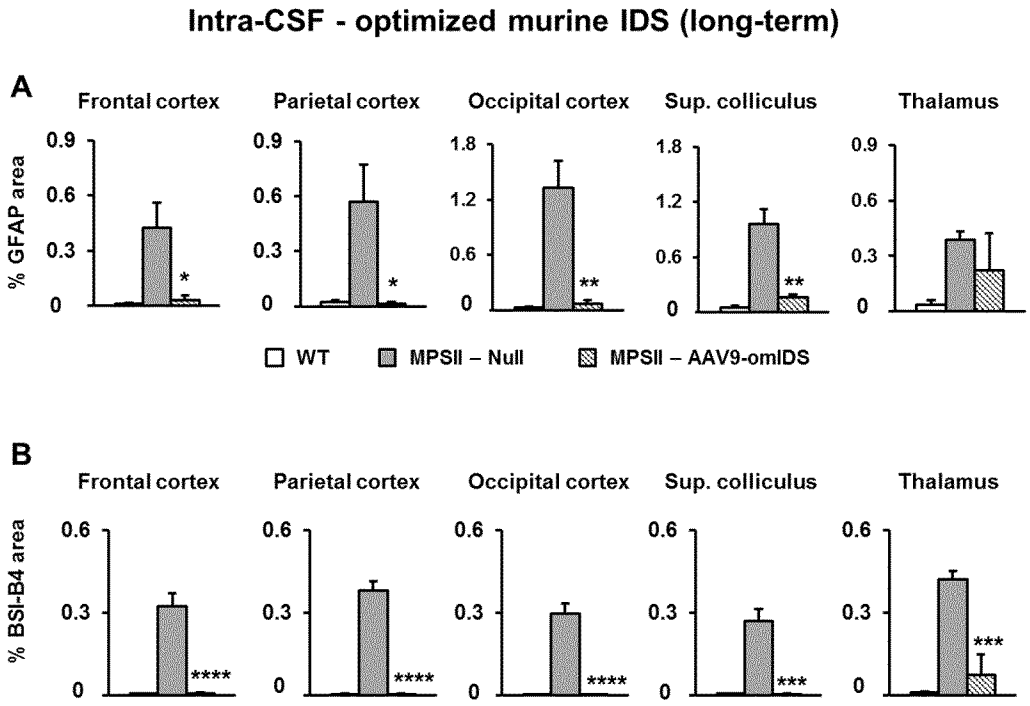

Intra-CSF - optimized murine IDS (long-term)
A
Frontal cortex
Parietal cortex
Occipital cortex
Sup. colliculus
Thalamus
% GFAP area
0.9
0.6
0.3
0
1.8
1.2
0.6
*
**
WT
MPSII – Null
MPSII – AAV9-omIDS
B
% BSI-B4 area
****
***

Figure 15

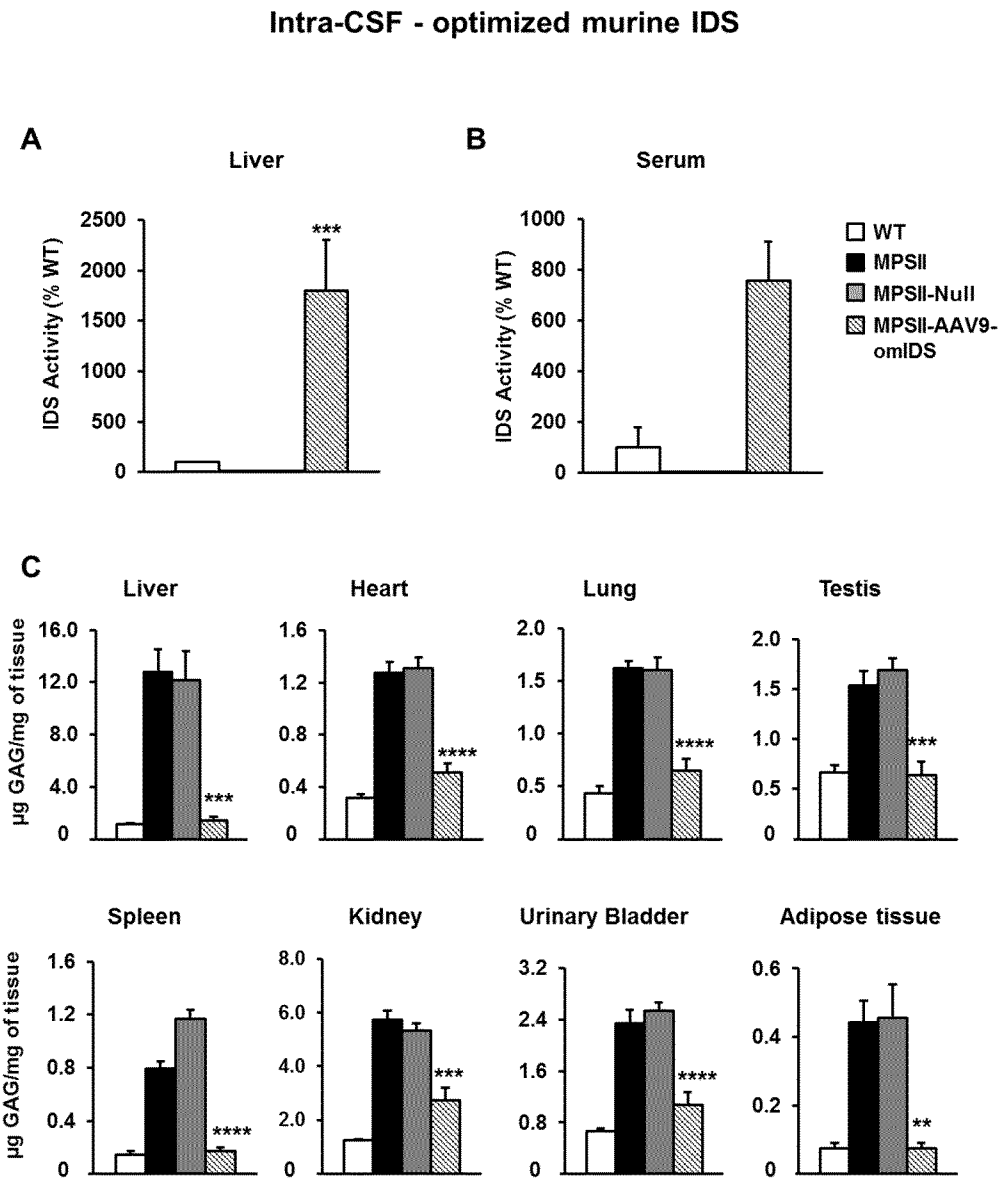
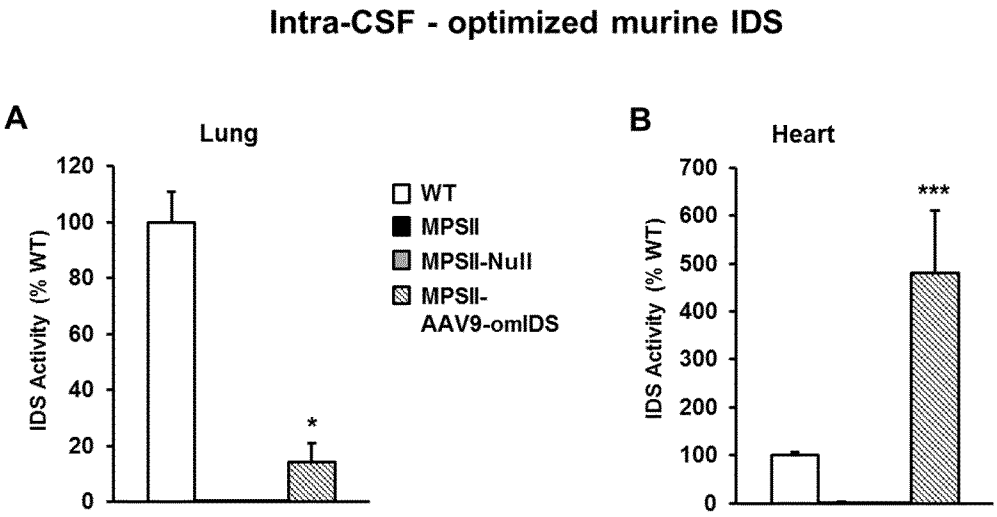

Intra-CSF - optimized murine IDS
A
Liver
IDS Activity (% WT)
2500
2000
1500
1000
500
0
***
B
Serum
IDS Activity (% WT)
1000
800
600
400
200
0
WT
MPSII
MPSII-Null
MPSII-AAV9-omIDS
C
Liver
Heart
Lung
Testis
µg GAG/mg of tissue
16.0
12.0
8.0
4.0
0
1.6
1.2
0.8
0.4
2.0
1.5
1.0
0.5
***
****
Spleen
Kidney
Urinary Bladder
Adipose tissue
3.2
2.4
6.0
4.0
2.0
0.6
0.2
**

Figure 16



C

	Vector genome copy number/ diploid genome (vg/dg)						
	Untreated MPSII	AAV9-Ids-treated MPSII					
	1	1	2	3	4	5	Mean ± SEM
Liver	0.002	0.401	0.073	0.159	0.195	0.384	0.243 ± 0.064
Lung	0.002	0.004	0.003	0.004	0.003	0.005	0.004 ± 0.000
Heart	0.002	0.007	0.007	0.006	0.007	0.005	0.006 ± 0.000

Figure 17

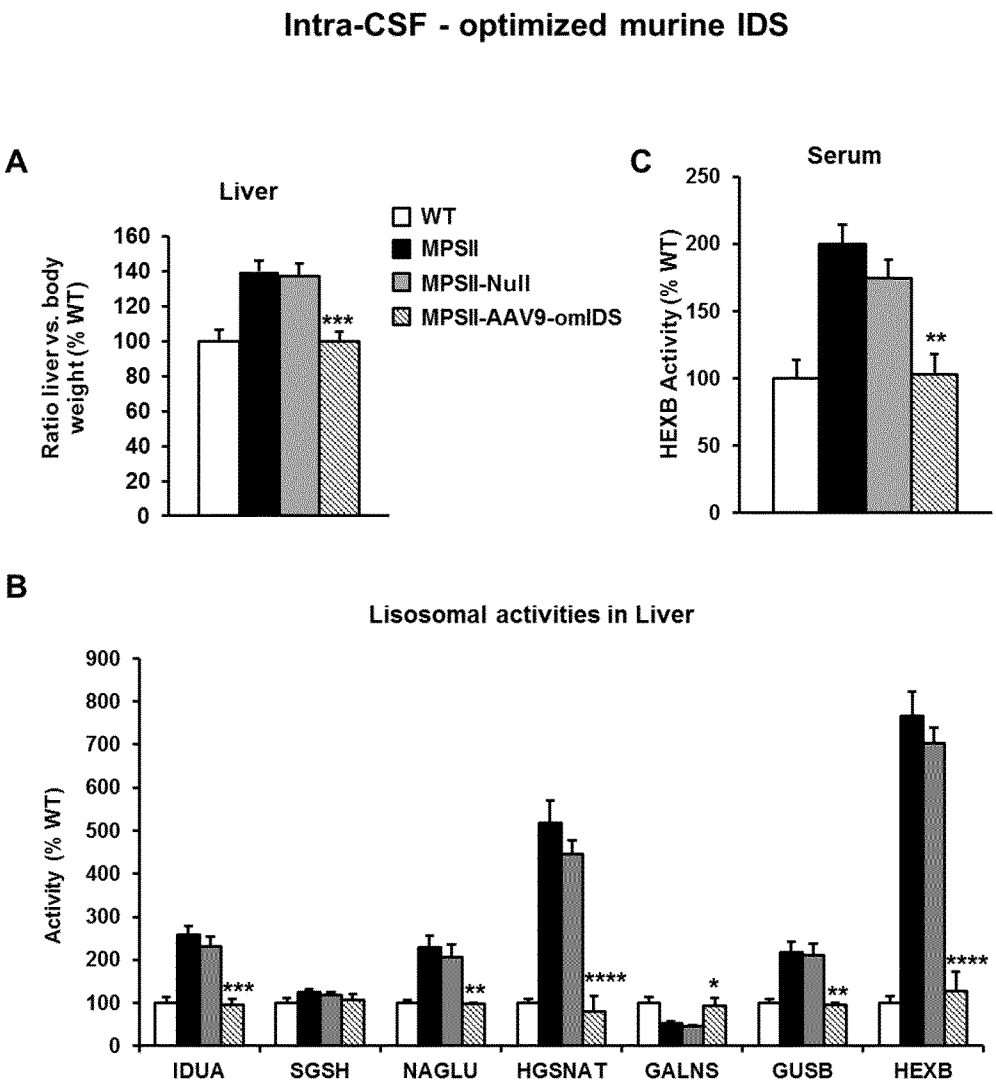

Intra-CSF - optimized murine IDS
A
Liver
Ratio liver vs. body weight (% WT)
160
140
120
100
80
60
40
20
0
***
WT
MPSII
MPSII-Null
MPSII-AAV9-omIDS
C
Serum
HEXB Activity (% WT)
250
200
150
100
50
0
**
B
Lisosomal activities in Liver
Activity (% WT)
900
800
700
600
500
400
300
200
100
0
***
**
****
*
**
****
IDUA
SGSH
NAGLU
HGSNAT
GALNS
GUSB
HEXB

Figure 18

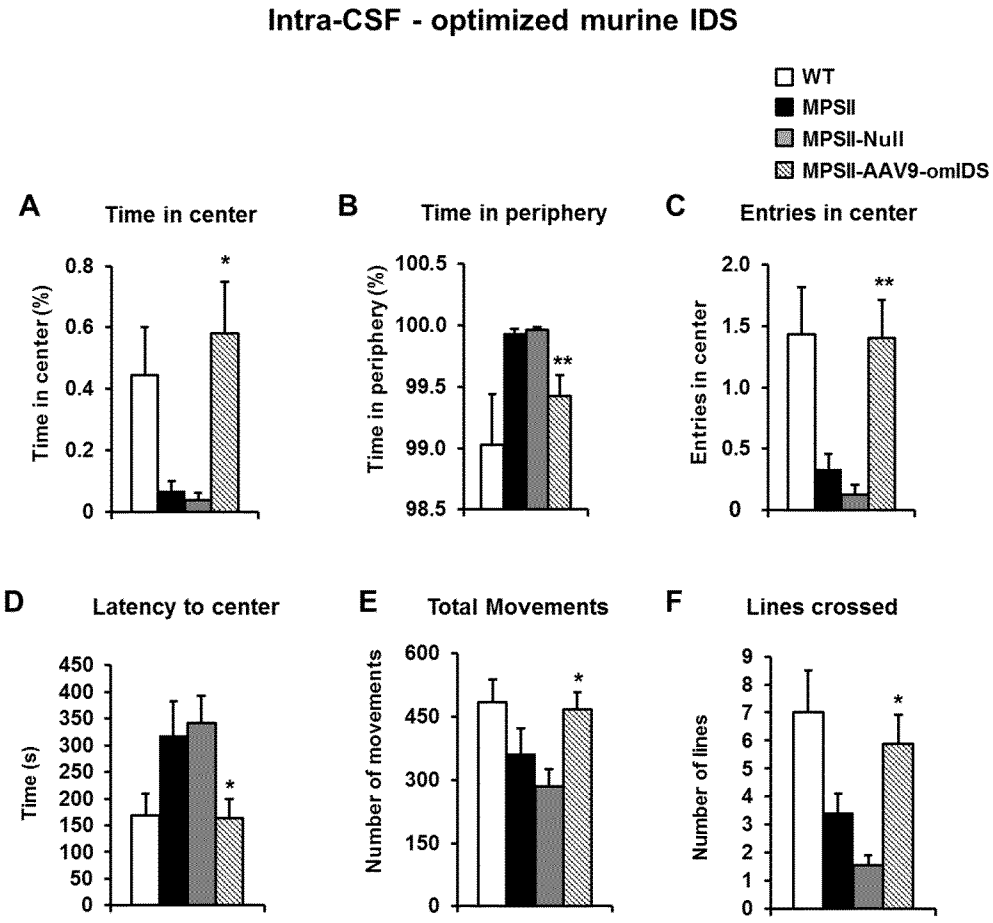

Intra-CSF - optimized murine IDS
WT
MPSII
MPSII-Null
MPSII-AAV9-omIDS
A
Time in center
Time in center (%)
0.8
0.6
0.4
0.2
0
*
B
Time in periphery
Time in periphery (%)
100.5
100.0
99.5
99.0
98.5
**
C
Entries in center
Entries in center
2.0
1.5
1.0
0.5
0
**
D
Latency to center
Time (s)
450
400
350
300
250
200
150
100
50
0
*
E
Total Movements
Number of movements
600
450
300
150
0
*
F
Lines crossed
Number of lines
9
8
7
6
5
4
3
2
1
0
*

Figure 19

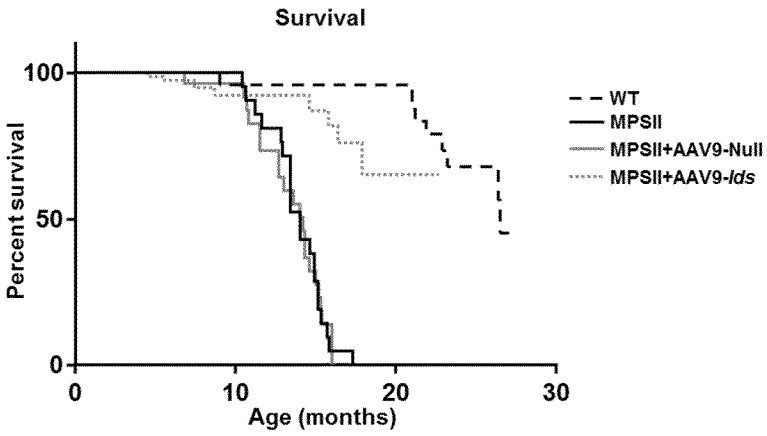

Intra-CSF - optimized murine IDS
Survival
100
50
0
Percent survival
0
10
20
30
Age (months)
WT
MPSII
MPSII+AAV9-Null
MPSII+AAV9-Ids

Figure 20

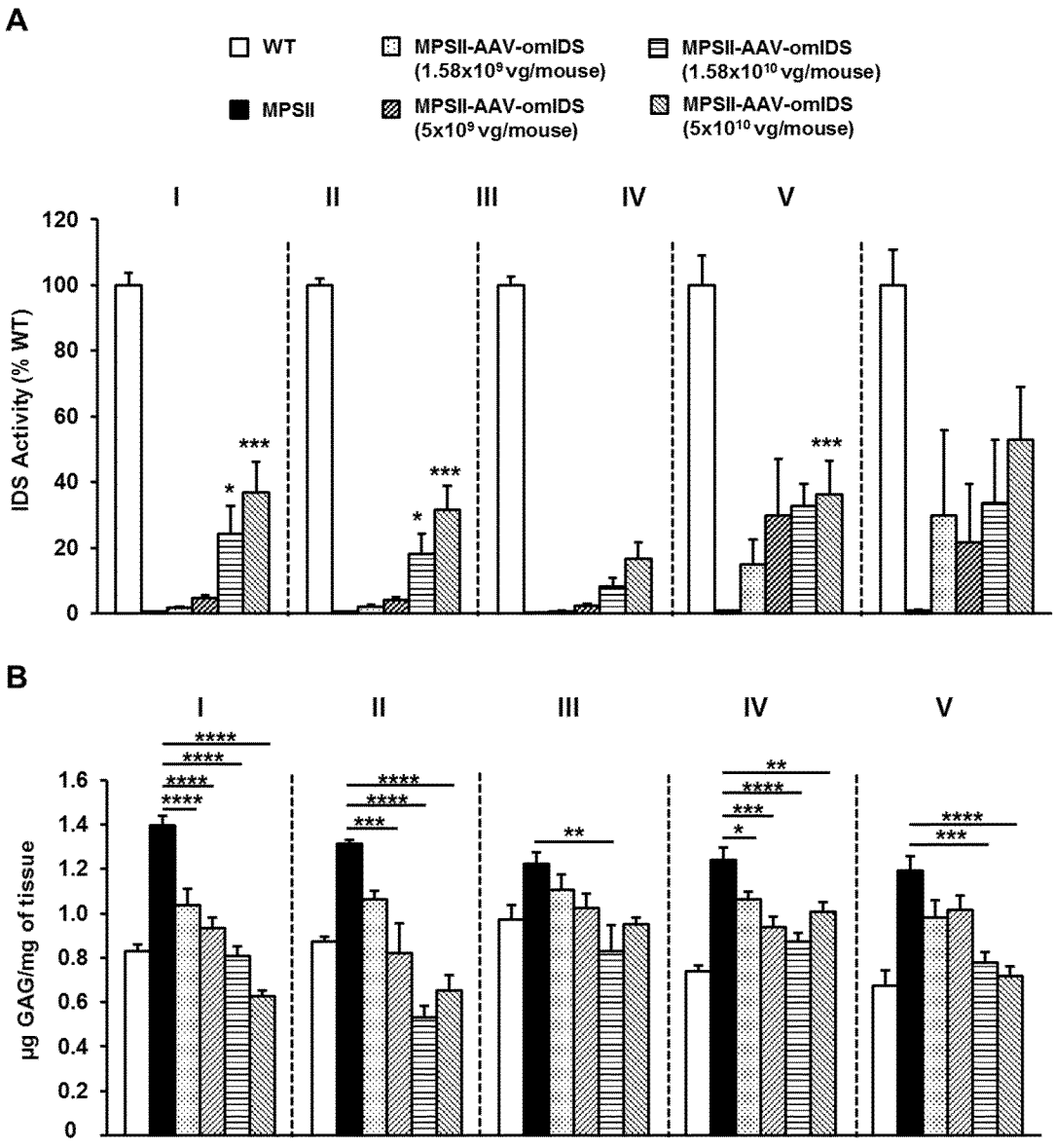
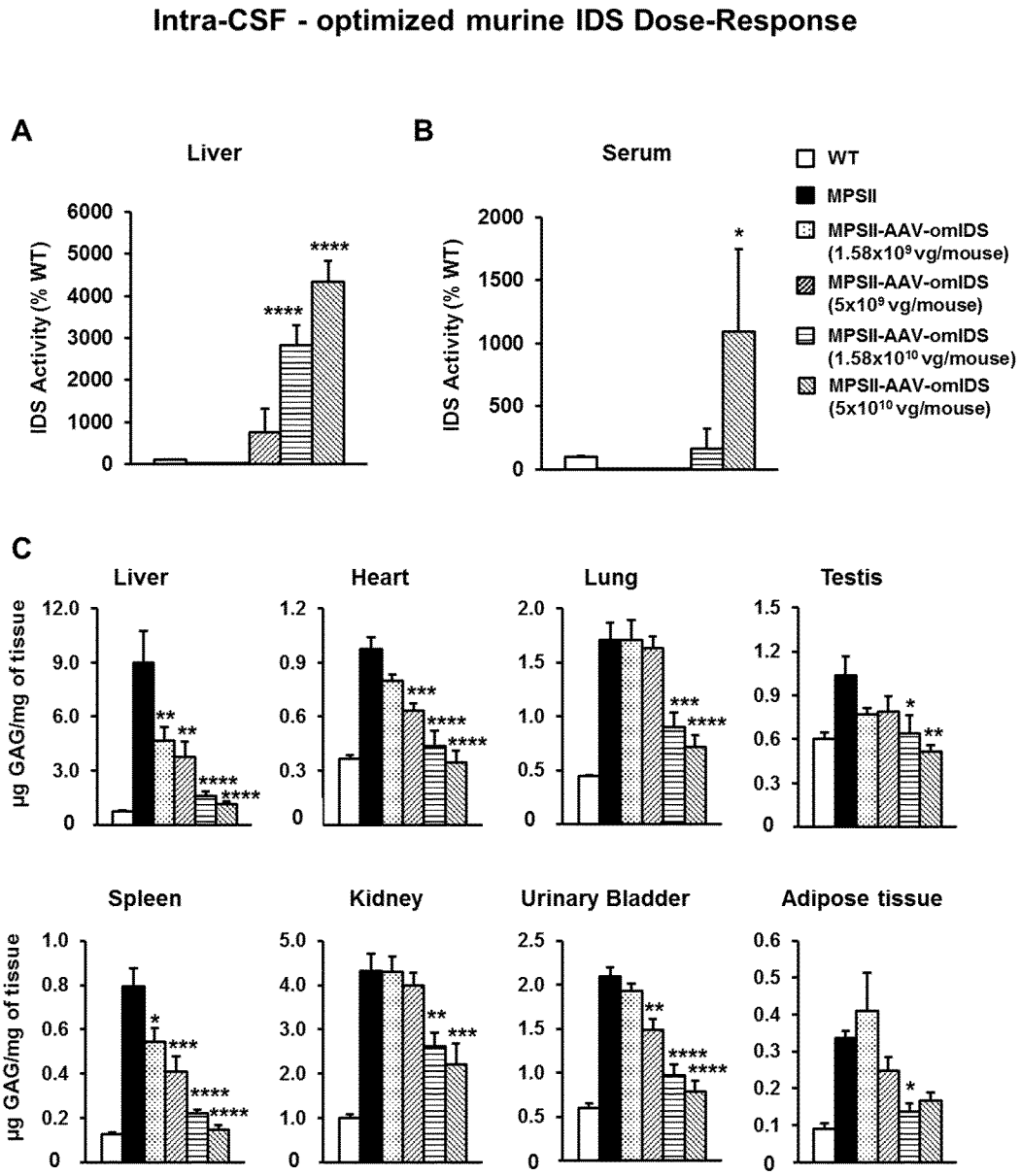

Intra-CSF - optimized murine IDS Dose-Response
A
WT
MPSII
MPSII-AAV-omIDS (1.58x10⁹ vg/mouse)
MPSII-AAV-omIDS (5x10⁹ vg/mouse)
MPSII-AAV-omIDS (1.58x10¹⁰ vg/mouse)
MPSII-AAV-omIDS (5x10¹⁰ vg/mouse)
I
II
III
IV
V
IDS Activity (% WT)
120
100
80
60
40
20
0
B
µg GAG/mg of tissue
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0

Figure 21


Intra-CSF - optimized murine IDS Dose-Response
A
Liver
IDS Activity (% WT)
B
Serum
IDS Activity (% WT)
WT
MPSII
MPSII-AAV-omIDS (1.58x10^9 vg/mouse)
MPSII-AAV-omIDS (5x10^9 vg/mouse)
MPSII-AAV-omIDS (1.58x10^10 vg/mouse)
MPSII-AAV-omIDS (5x10^10 vg/mouse)
C
Liver
Heart
Lung
Testis
Spleen
Kidney
Urinary Bladder
Adipose tissue
µg GAG/mg of tissue