Figure 1A

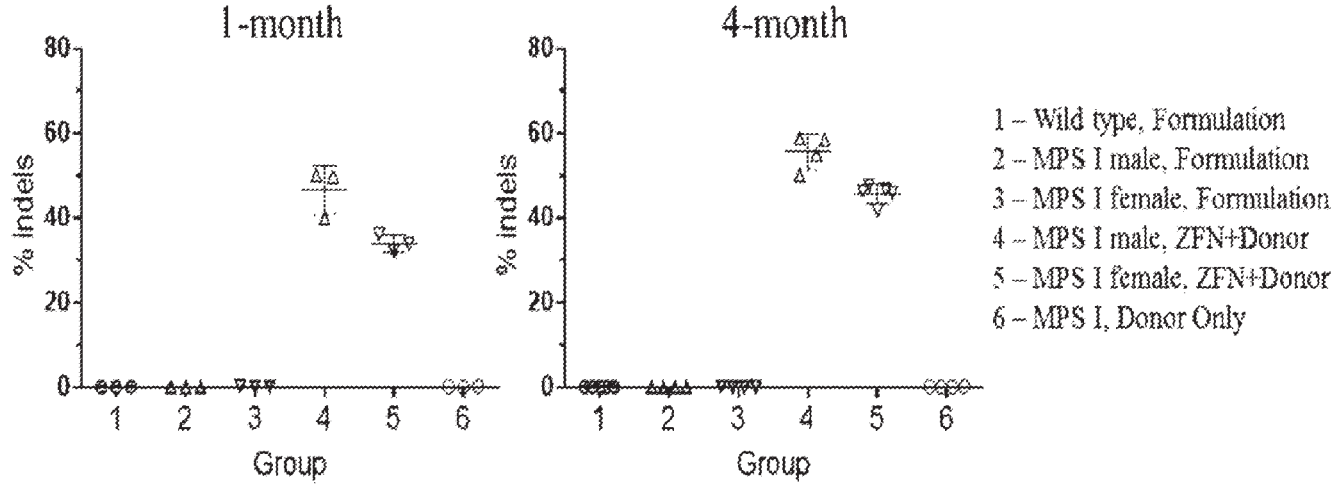
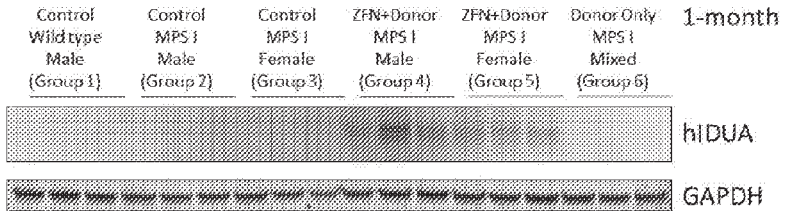


Figure 1B

i



ii

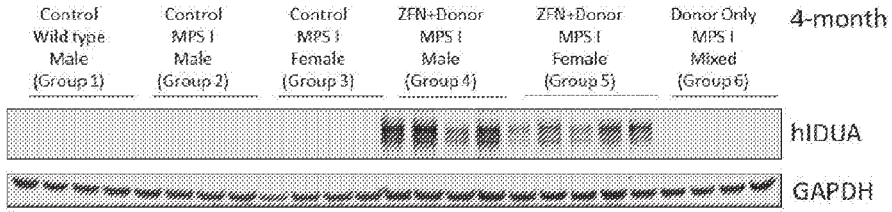


Figure 2A

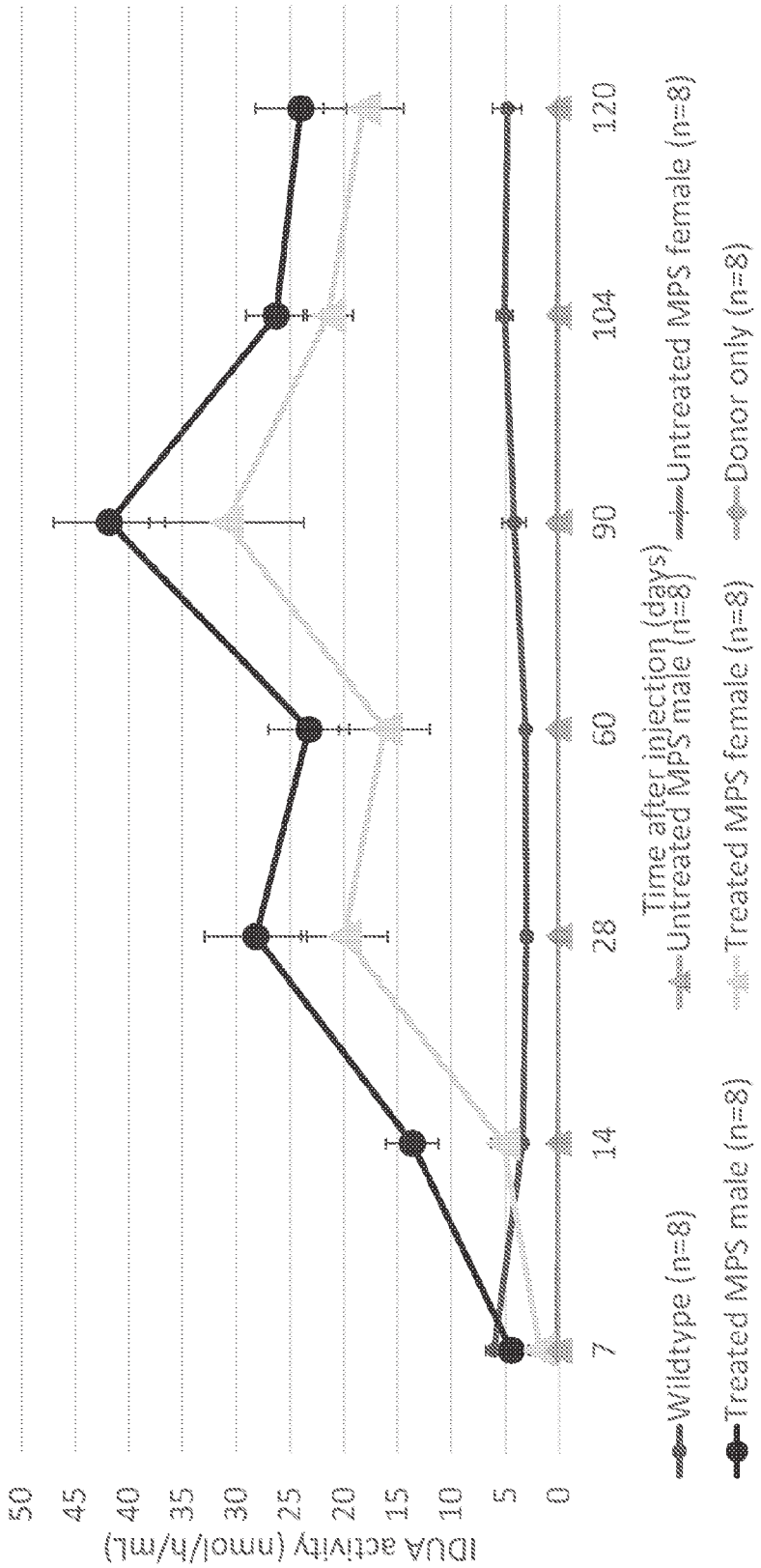

IDUA activity (nmol/h/mL)
50
45
40
35
30
25
20
15
10
5
0
7
14
28
60
90
104
120
Time after injection (days)
Wildtype (n=8)
Untreated MPS male (n=8)
Untreated MPS female (n=8)
Treated MPS male (n=8)
Treated MPS female (n=8)
Donor only (n=8)

Figure 2B

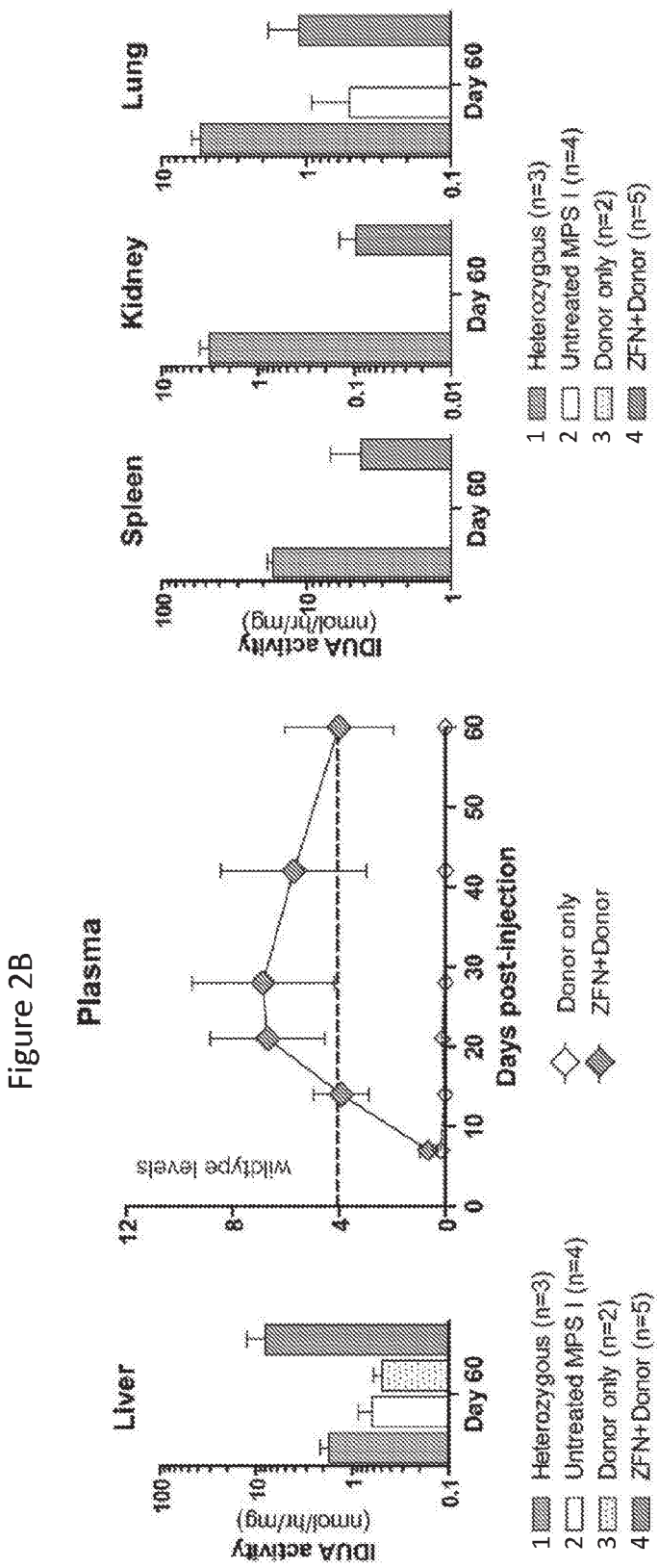
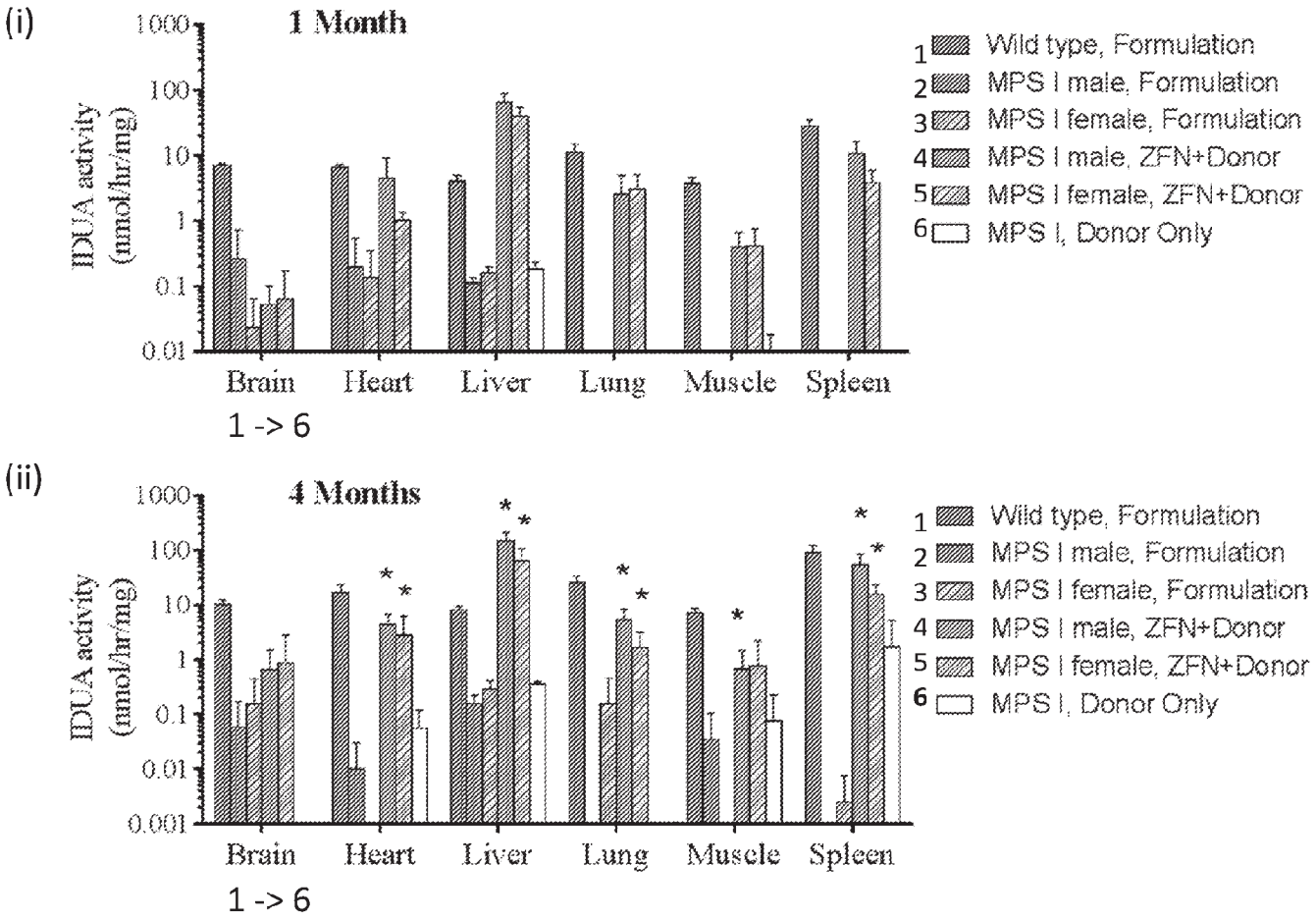

Liver
Spleen
Kidney
Lung
IDUA activity
(nmol/hr/mg)
Day 60
Plasma
wildtype levels
Days post-injection
Donor only
ZFN+Donor
1 Heterozygous (n=3)
2 Untreated MPS I (n=4)
3 Donor only (n=2)
4 ZFN+Donor (n=5)

Figure 2C



* Indicates significance ($p<0.05$) as compared to gender-matched MPS I, Formulation group

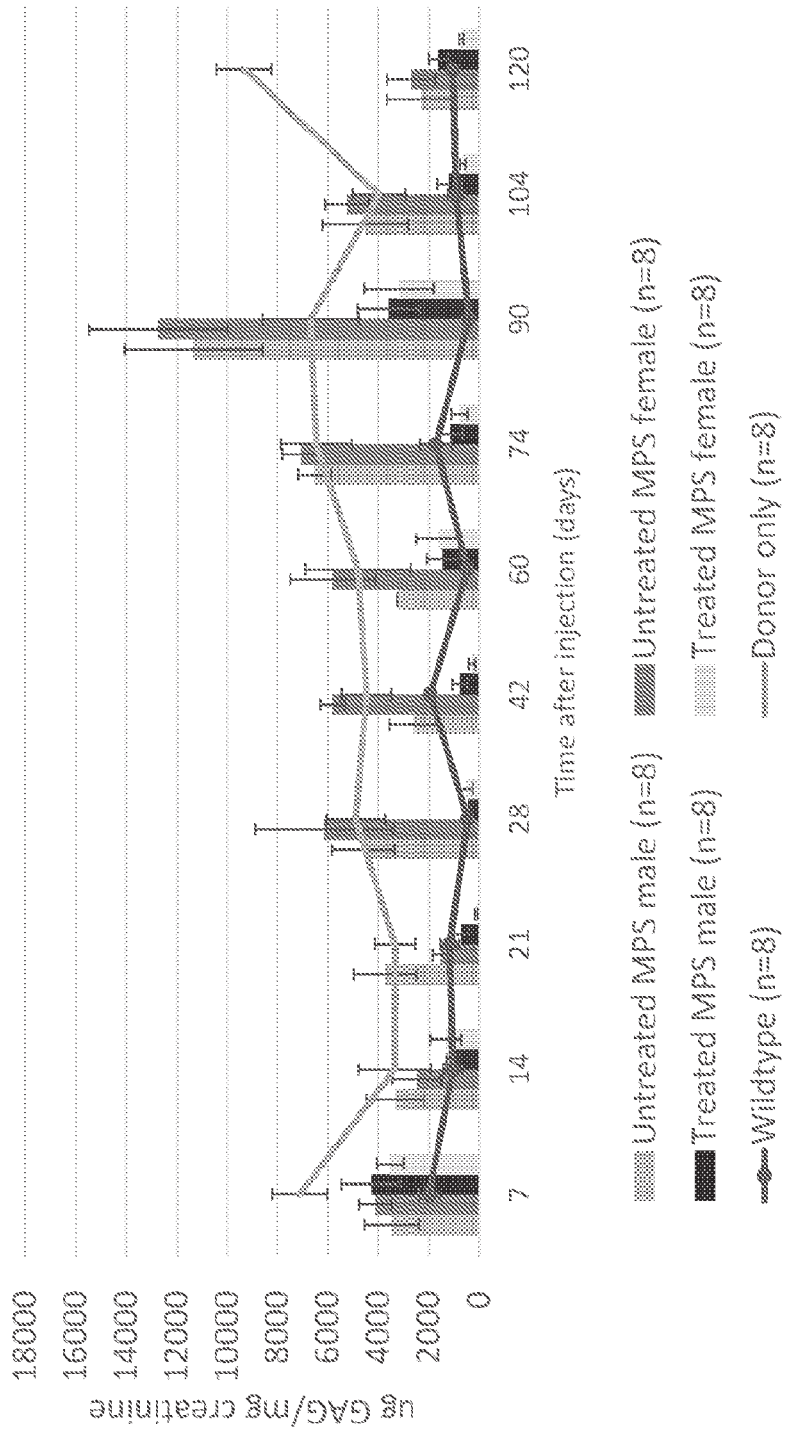
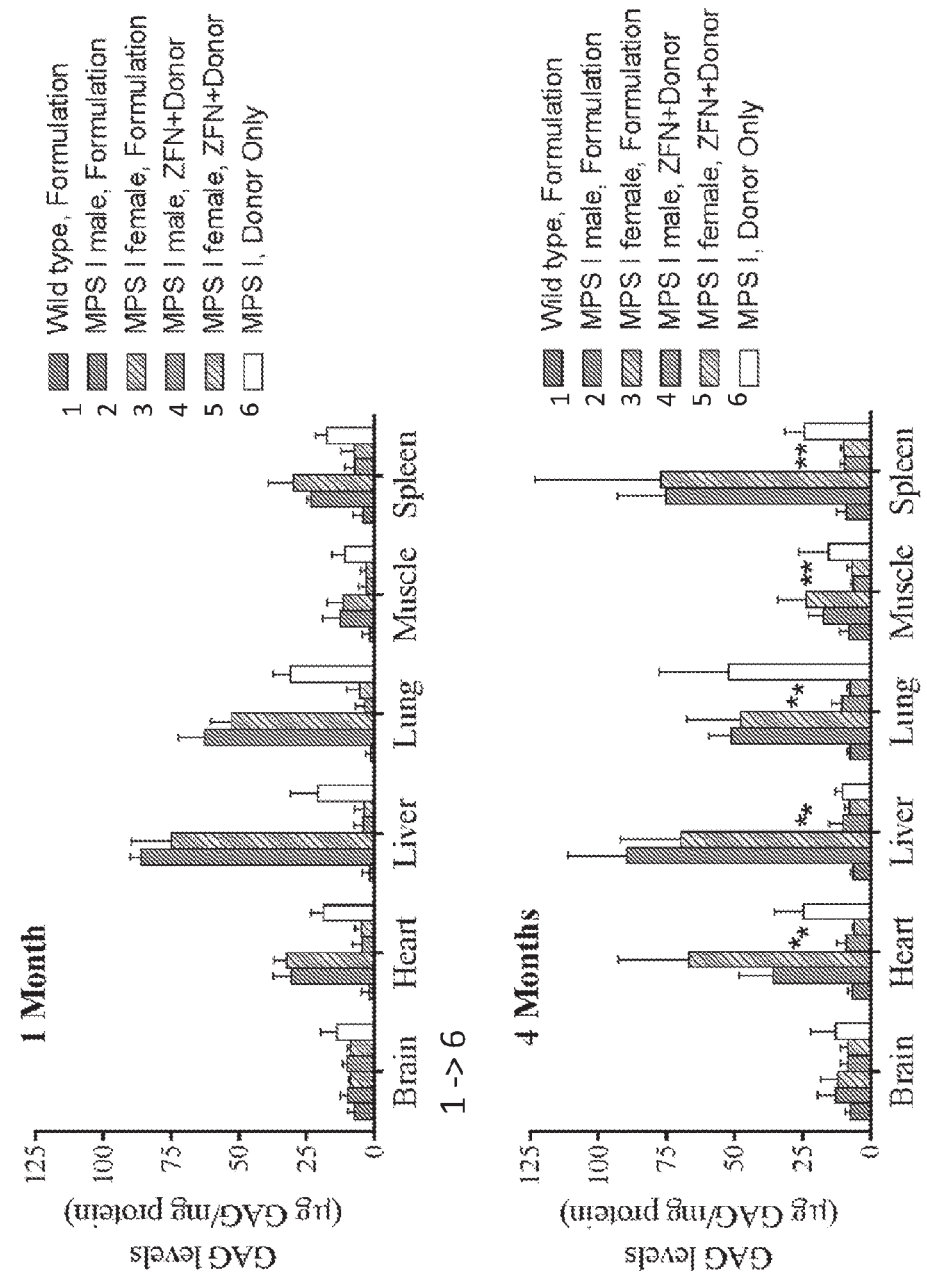

18000
16000
14000
12000
10000
8000
6000
4000
2000
0
uG GAG/mg creatinine
7
14
21
28
42
60
74
90
104
120
Time after injection (days)
Untreated MPS male (n=8)
Untreated MPS female (n=8)
Treated MPS male (n=8)
Treated MPS female (n=8)
Wildtype (n=8)
Donor only (n=8)

Figure 3A

Figure 3B


(i)
1 Month
GAG levels
(µg GAG/mg protein)
125
100
75
50
25
0
Brain
Heart
Liver
Lung
Muscle
Spleen
1 -> 6
1 Wild type, Formulation
2 MPS I male, Formulation
3 MPS I female, Formulation
4 MPS I male, ZFN+Donor
5 MPS I female, ZFN+Donor
6 MPS I, Donor Only
(ii)
4 Months
GAG levels
(µg GAG/mg protein)
125
100
75
50
25
0
Brain
Heart
Liver
Lung
Muscle
Spleen
1 -> 6
1 Wild type, Formulation
2 MPS I male, Formulation
3 MPS I female, Formulation
4 MPS I male, ZFN+Donor
5 MPS I female, ZFN+Donor
6 MPS I, Donor Only

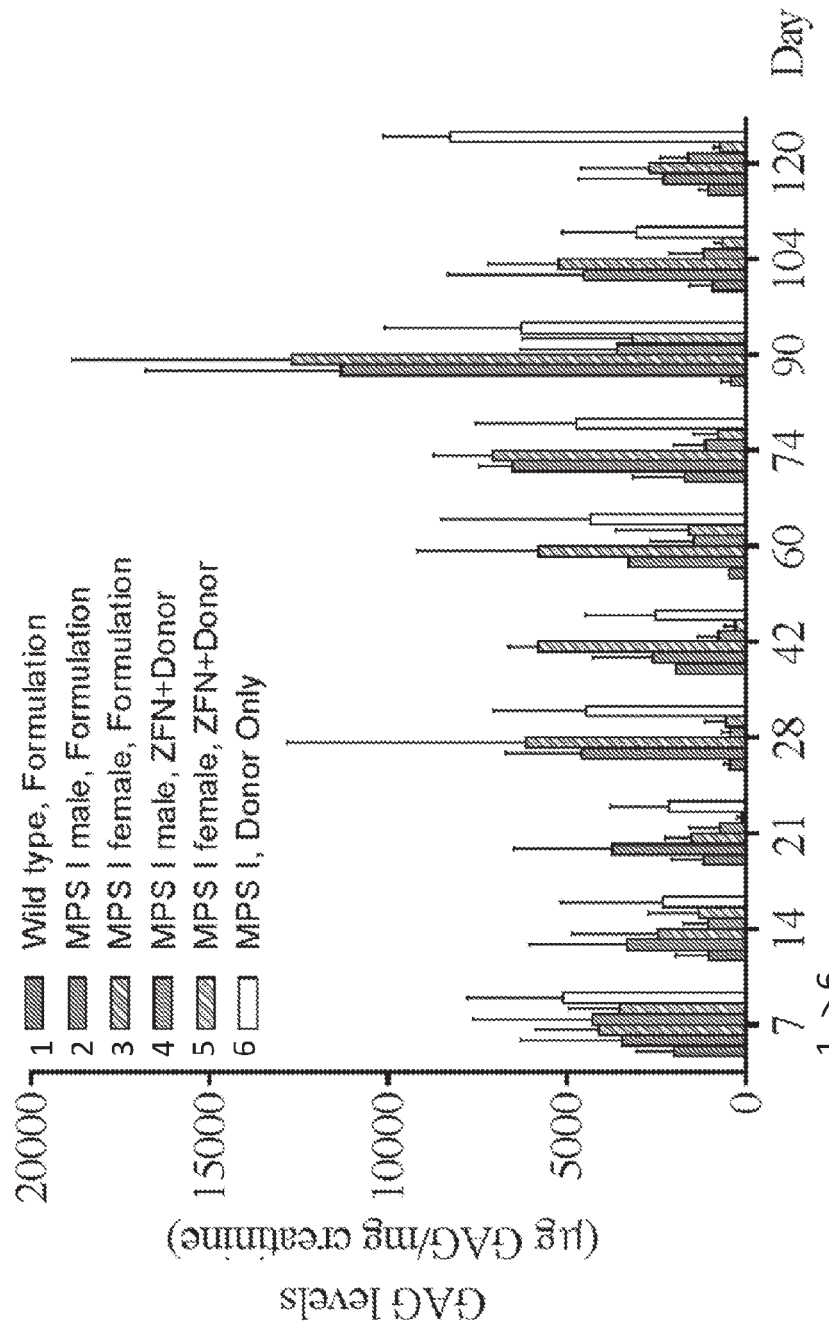

GAG levels
(µg GAG/mg creatinine)
20000
15000
10000
5000
0
1 Wild type, Formulation
2 MPS I male, Formulation
3 MPS I female, Formulation
4 MPS I male, ZFN+Donor
5 MPS I female, ZFN+Donor
6 MPS I, Donor Only
1 -> 6
7
14
21
28
42
60
74
90
104
120
Day

Figure 3C

Groups:
1: Wildtype (N=8)
2: Untreated MPS male (N=8)
3: Untreated MPS female (N=8)
4: Treated MPS male (N=8)
5: Treated MPS female (N=8)
6: Donor only (N=8)

Figure 4A

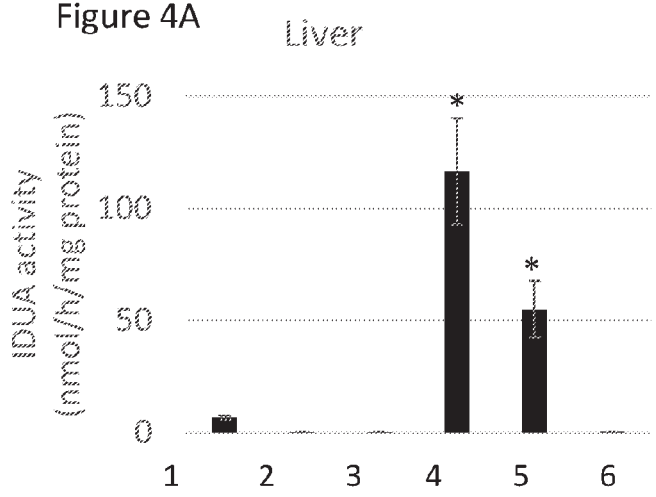


Figure 4B

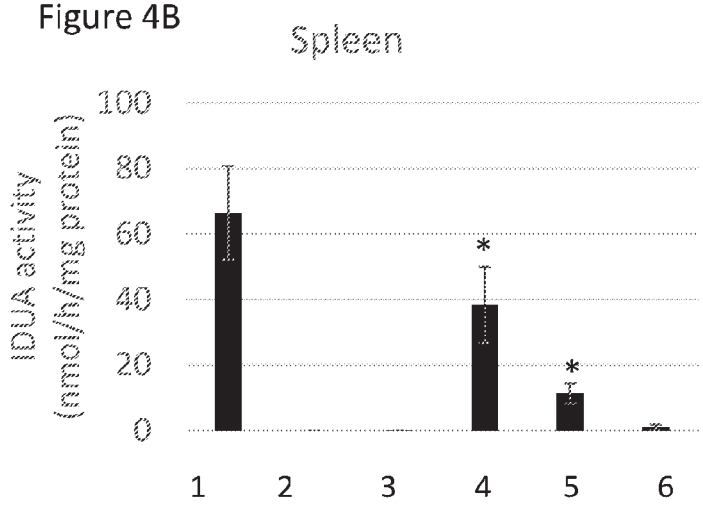


Figure 4C

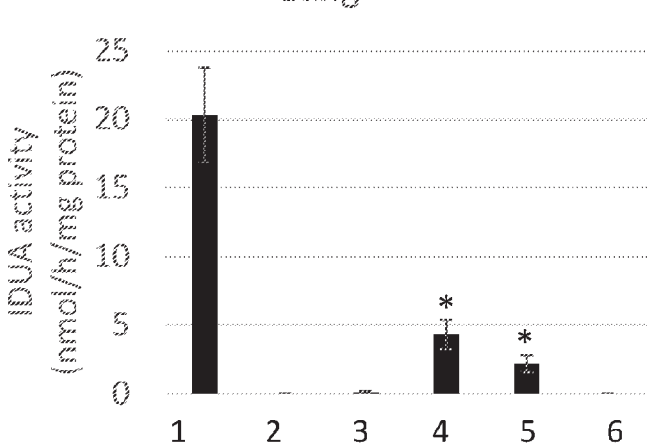


Figure 4D

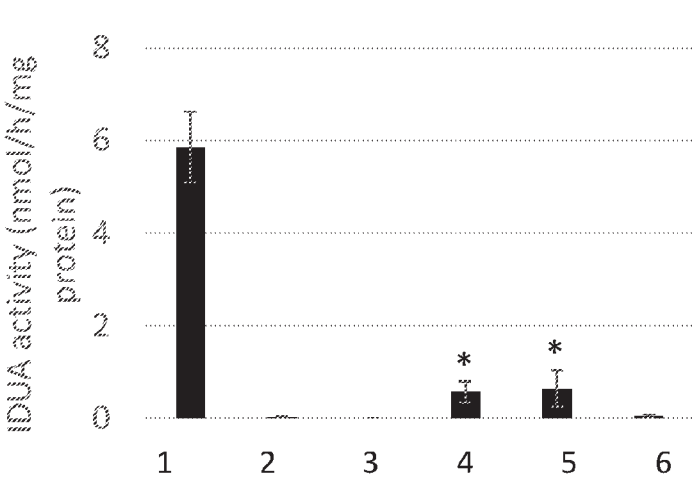


Figure 4F

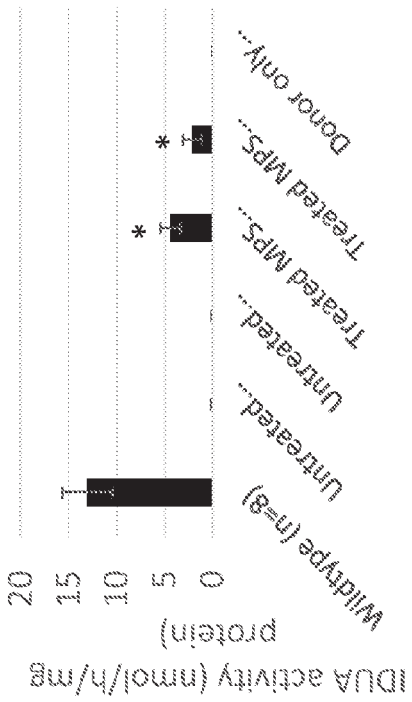
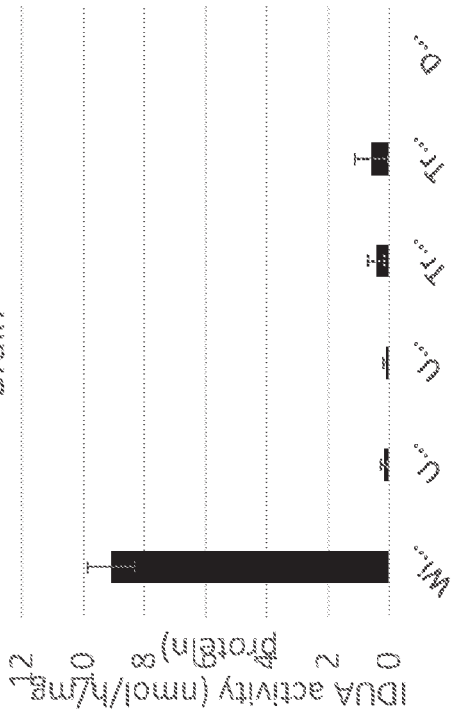


Figure 4E



Groups:
1: Wildtype (N=8)
2: Untreated MPS male (N=8)
3: Untreated MPS female (N=8)
4: Treated MPS male (N=8)
5: Treated MPS female (N=8)
6: Donor only (N=8)

Figure 5A

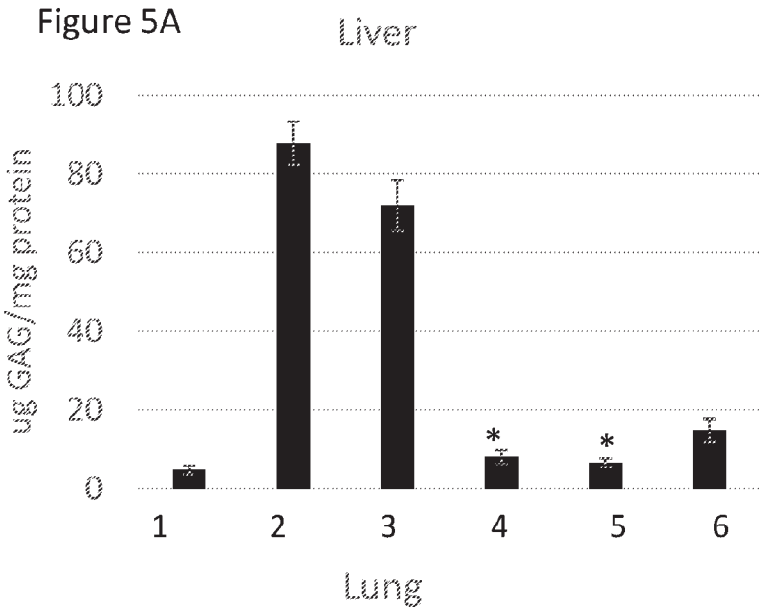
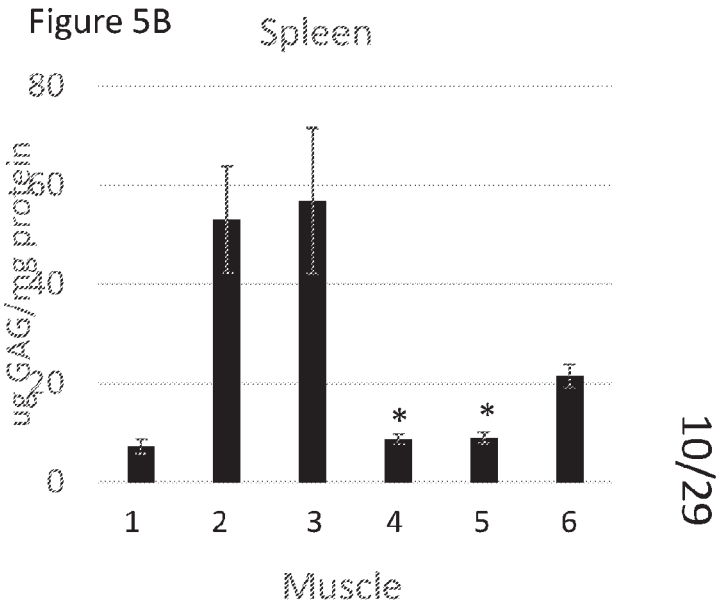


Figure 5B



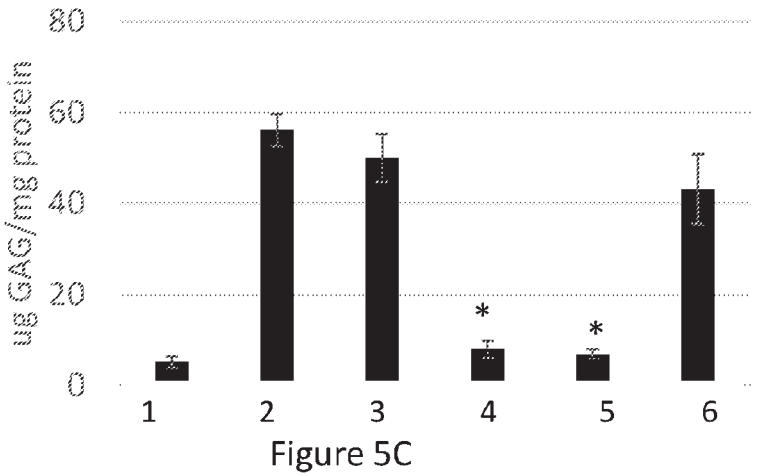


Figure 5C

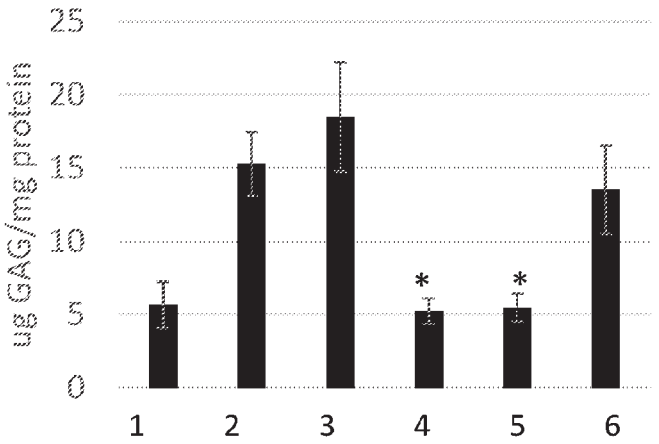


Figure 5D

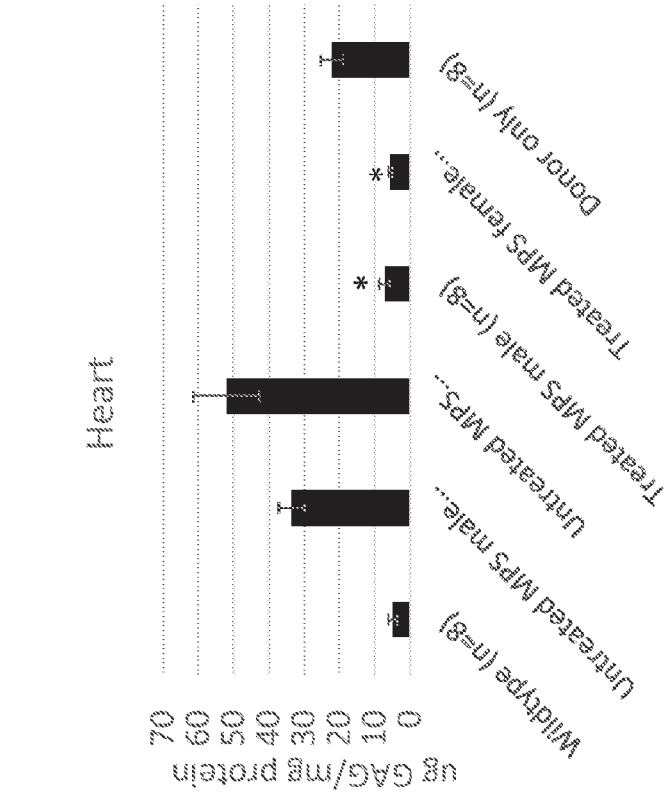


Figure 5F

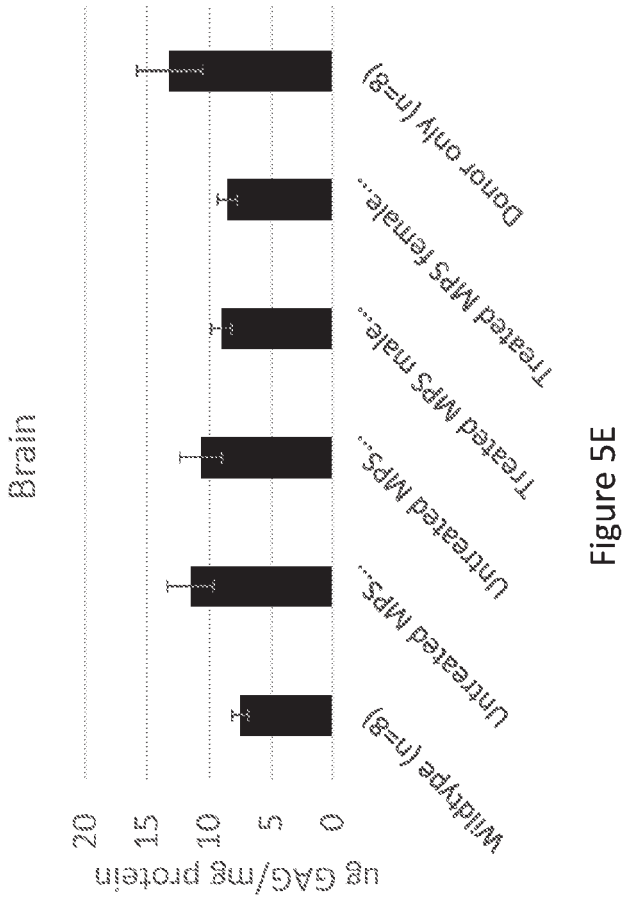


Figure 5E

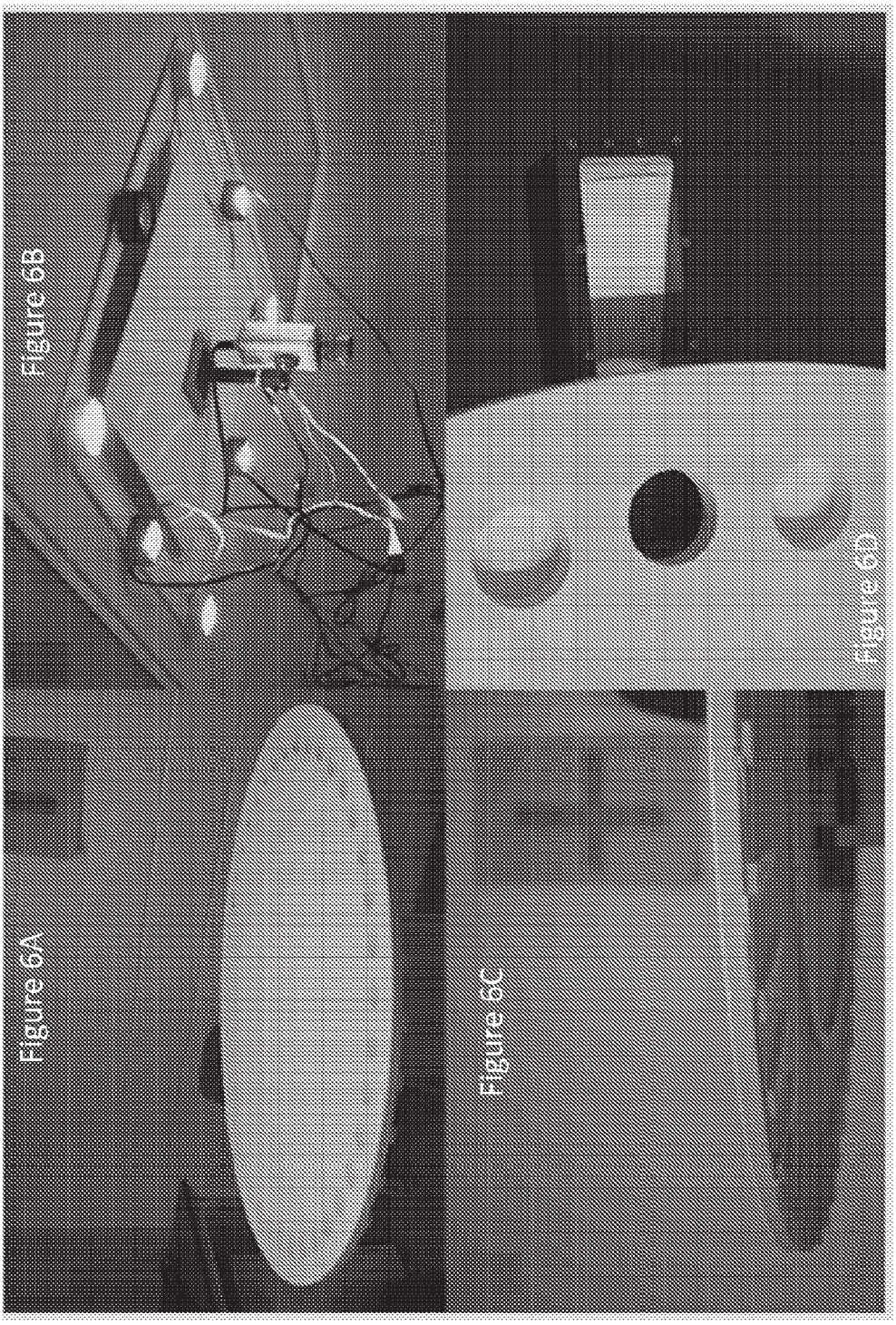


Figure 6A
Figure 6B
Figure 6C
Figure 6D

Figure 7A

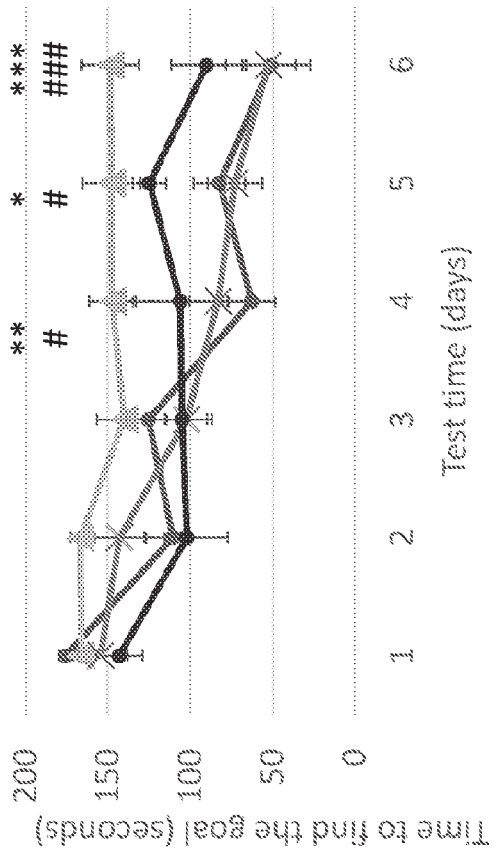


Figure 7B

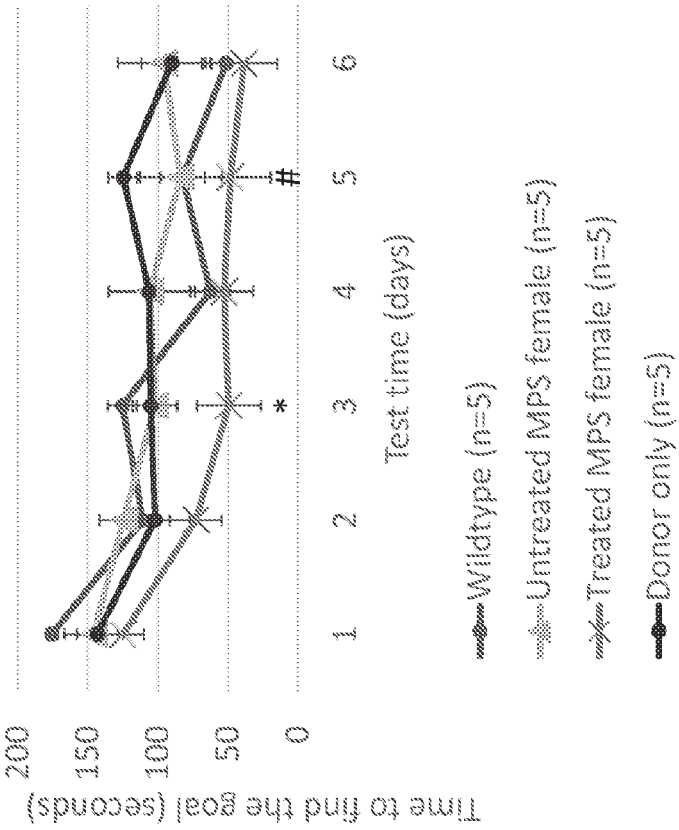
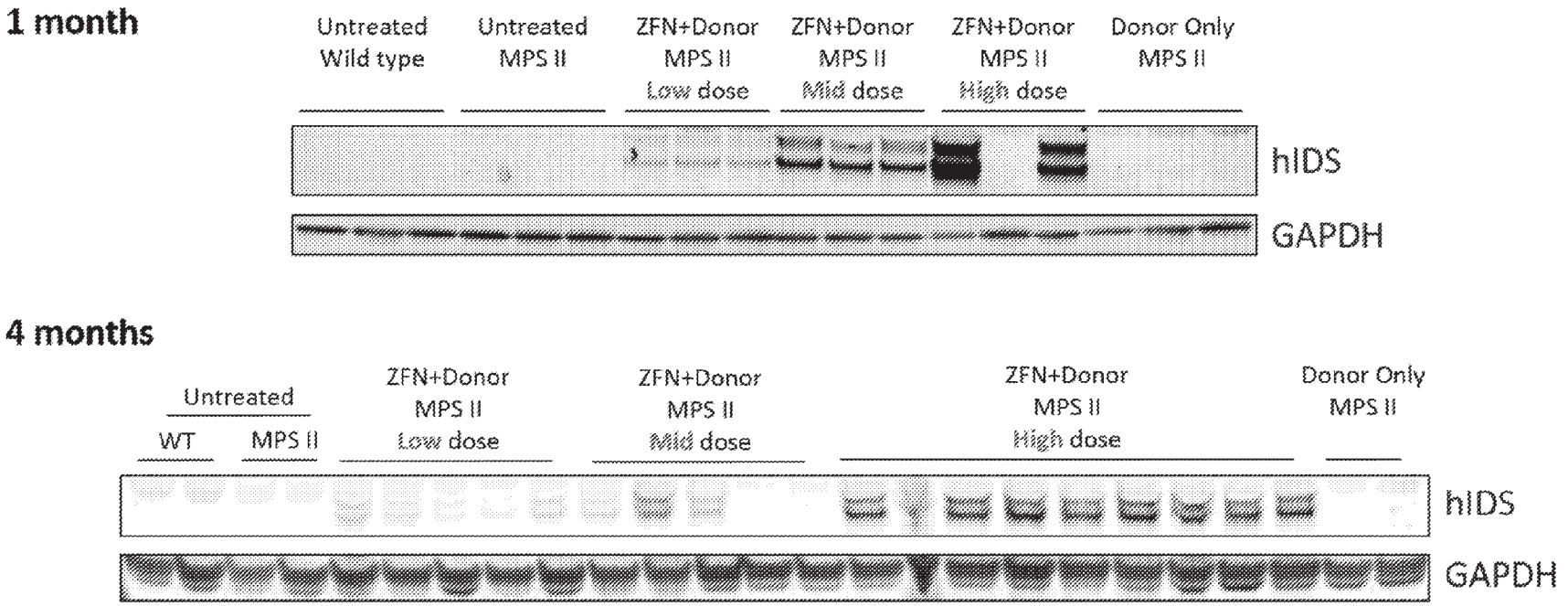


Figure 8A


1 month
Untreated Wild type
Untreated MPS II
ZFN+Donor MPS II Low dose
ZFN+Donor MPS II Mid dose
ZFN+Donor MPS II High dose
Donor Only MPS II
hIDS
GAPDH
4 months
Untreated
WT
MPS II
ZFN+Donor MPS II Low dose
ZFN+Donor MPS II Mid dose
ZFN+Donor MPS II High dose
Donor Only MPS II
hIDS
GAPDH

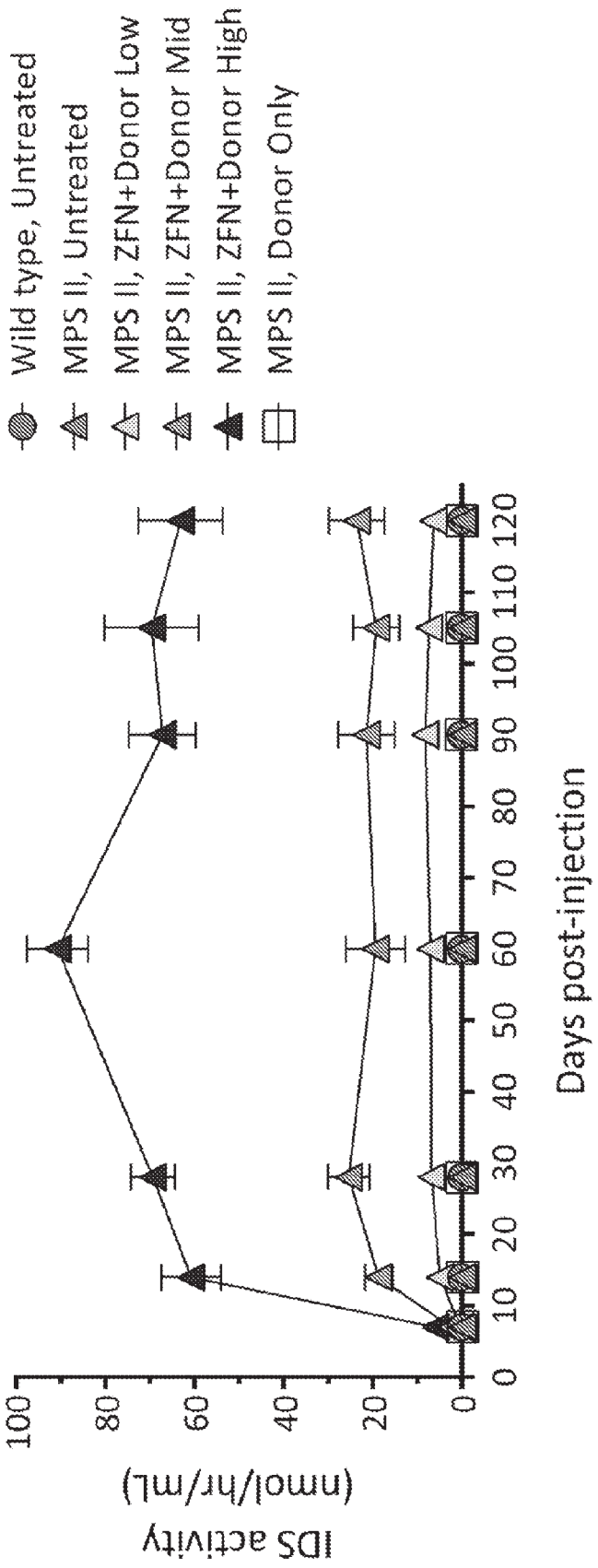

Wild type, Untreated
MPS II, Untreated
MPS II, ZFN+Donor Low
MPS II, ZFN+Donor Mid
MPS II, ZFN+Donor High
MPS II, Donor Only
IDS activity
(nmol/hr/mL)
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
90
100
110
120
Days post-injection

Figure 8B

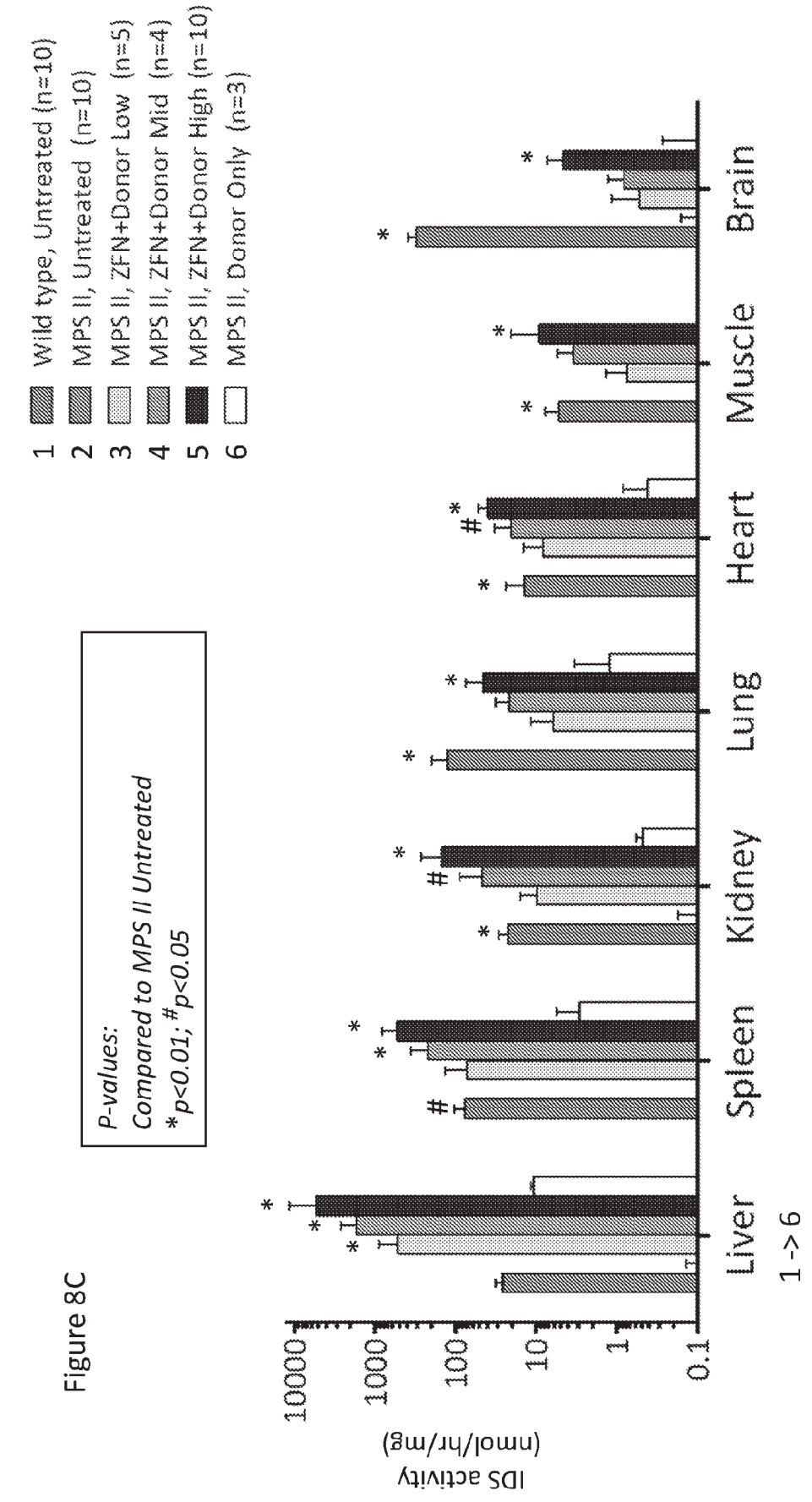

1 Wild type, Untreated (n=10)
2 MPS II, Untreated (n=10)
3 MPS II, ZFN+Donor Low (n=5)
4 MPS II, ZFN+Donor Mid (n=4)
5 MPS II, ZFN+Donor High (n=10)
6 MPS II, Donor Only (n=3)
P-values:
Compared to MPS II Untreated
*p<0.01; #p<0.05
IDS activity
(nmol/hr/mg)
10000
1000
100
10
1
0.1
Liver
Spleen
Kidney
Lung
Heart
Muscle
Brain
1 -> 6

Figure 8C

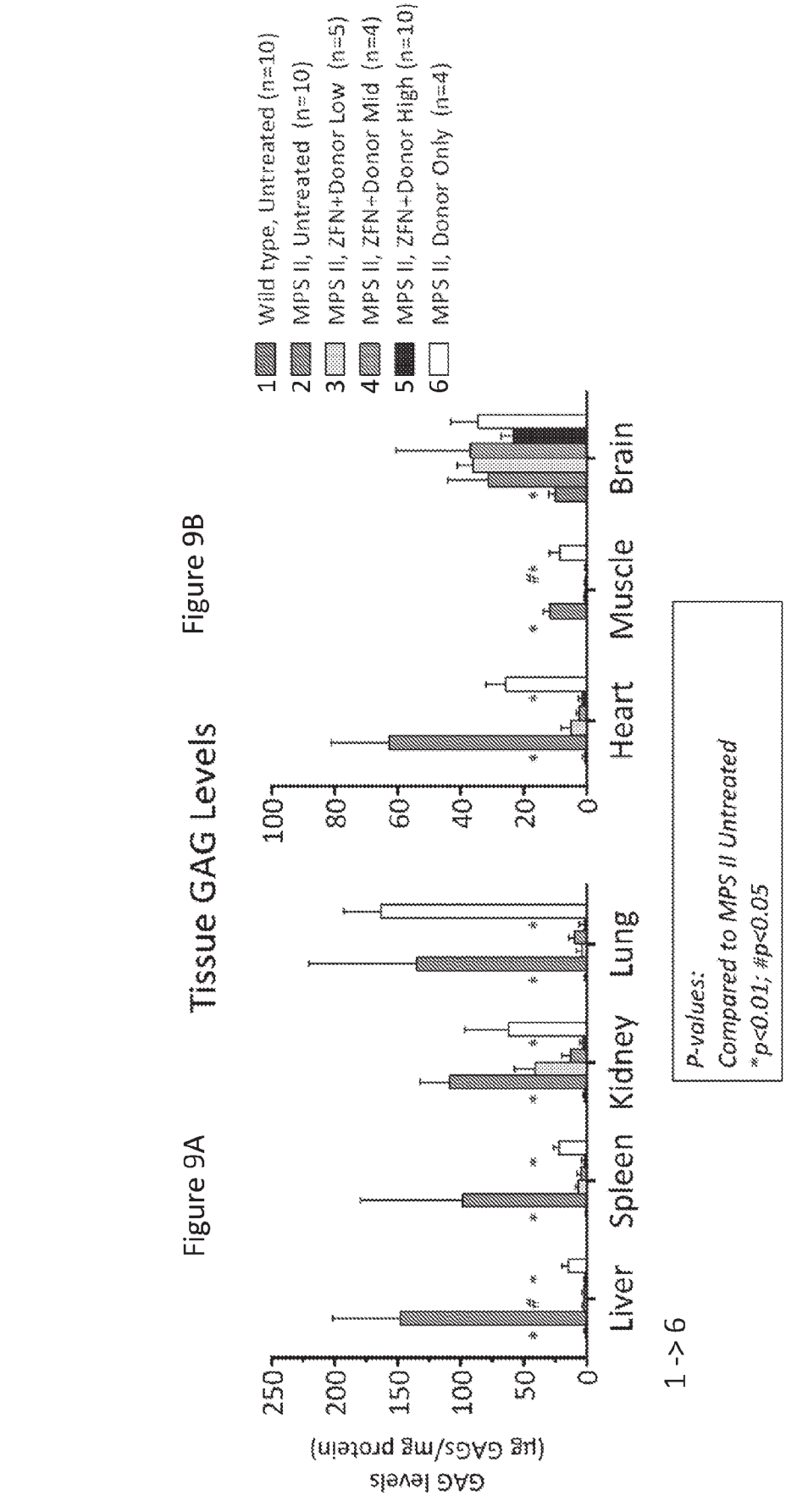


Figure 9A
Tissue GAG Levels
Figure 9B
GAG levels
(µg GAGs/mg protein)
250
200
150
100
50
0
Liver
Spleen
Kidney
Lung
Heart
Muscle
Brain
1 -> 6
1 Wild type, Untreated (n=10)
2 MPS II, Untreated (n=10)
3 MPS II, ZFN+Donor Low (n=5)
4 MPS II, ZFN+Donor Mid (n=4)
5 MPS II, ZFN+Donor High (n=10)
6 MPS II, Donor Only (n=4)
P-values:
Compared to MPS II Untreated
*p<0.01; #p<0.05

Figure 10

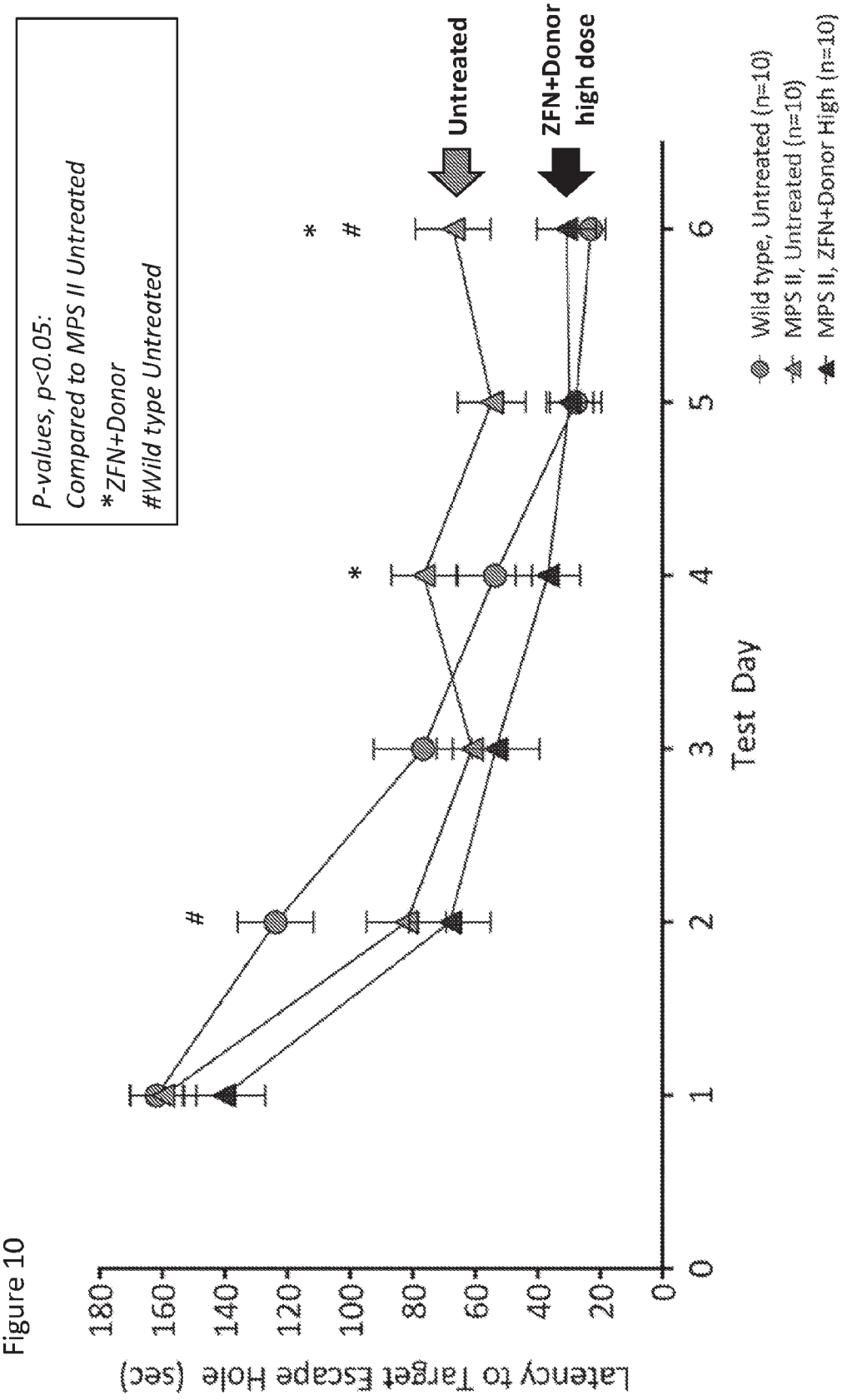

P-values, p<0.05:
Compared to MPS II Untreated
*ZFN+Donor
#Wild type Untreated
Untreated
ZFN+Donor high dose
Latency to Target Escape Hole (sec)
Test Day
Wild type, Untreated (n=10)
MPS II, Untreated (n=10)
MPS II, ZFN+Donor High (n=10)

Figure 11A

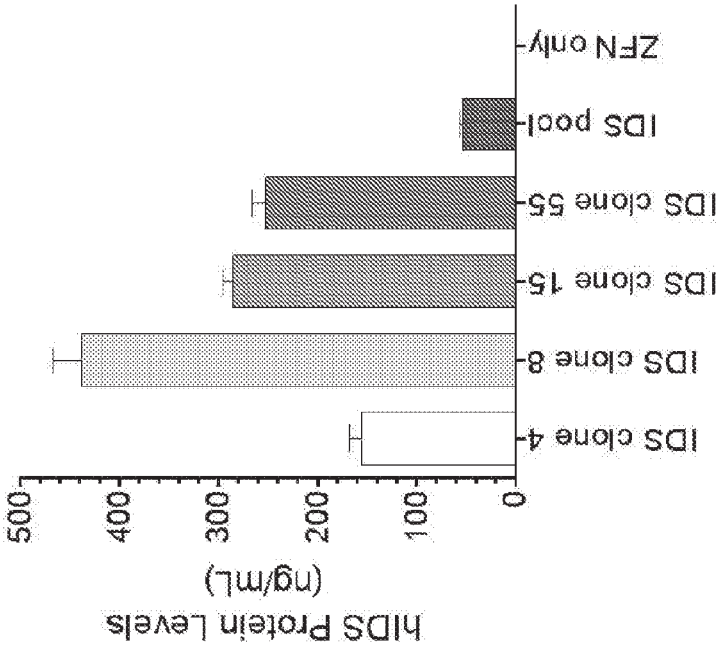


Figure 11B

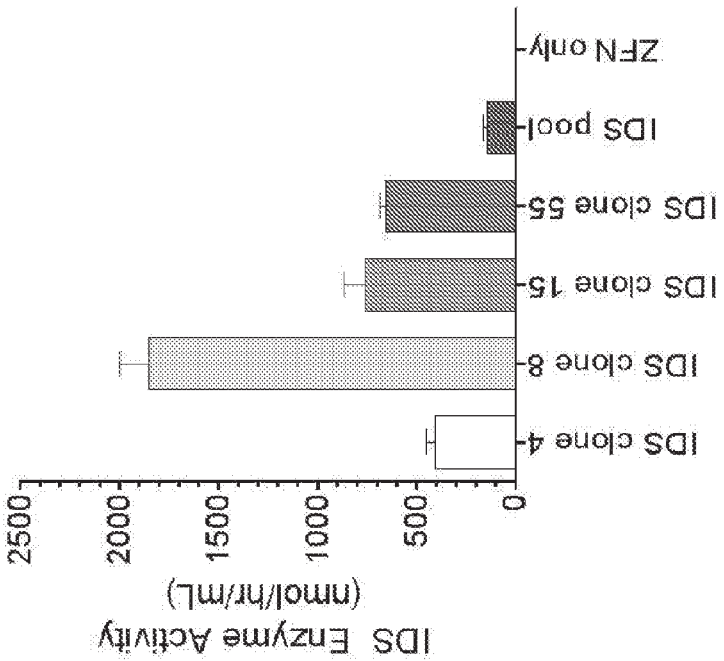


Figure 11C

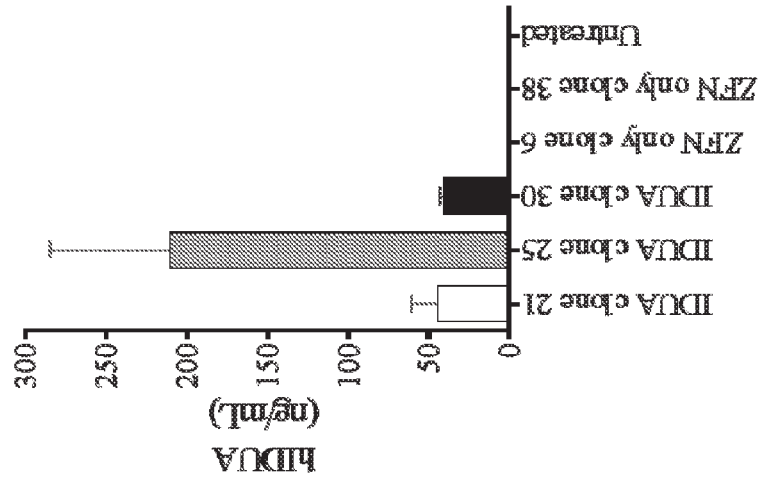


Figure 11D

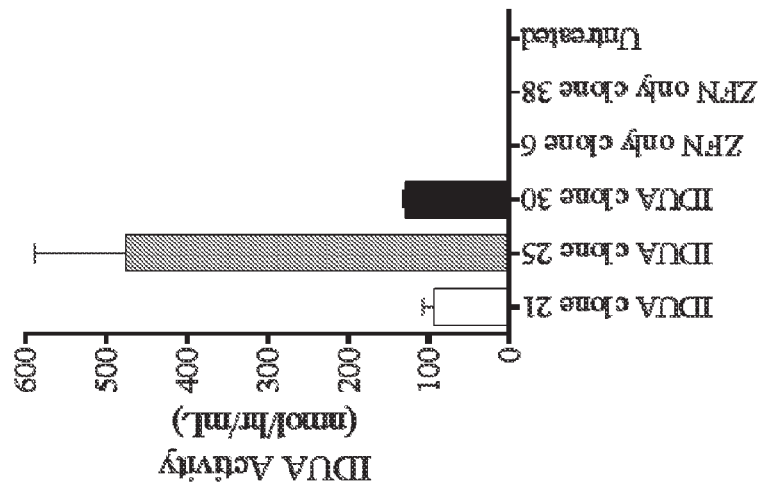


Figure 12A

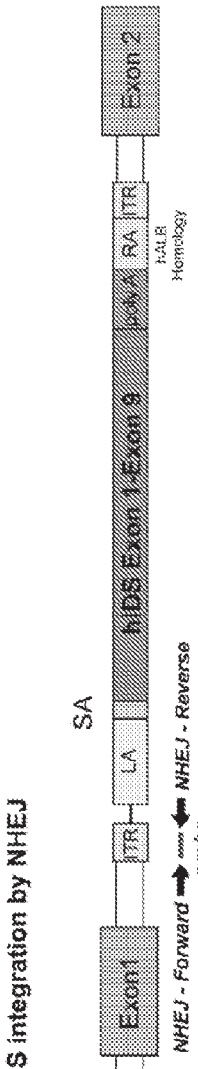


Figure 12B

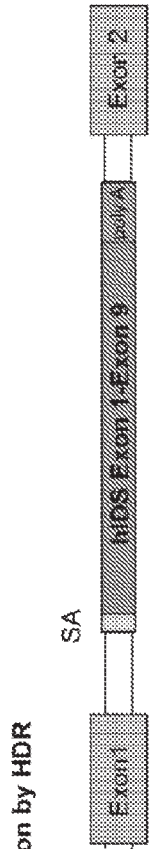


Figure 12C

HepG2 Clone #	NHEJ	HDR
IDS #4	positive (3/3)	negative (2/3)
IDS #8	positive (3/3)	negative (2/3)
IDS #15	negative (3/3)	positive (3/3)
IDS #55	negative (3/3)	positive (3/3)
NHEJ positive control	positive	negative
HDR positive control	negative	positive

Figure 12D

Sanger Sequencing
NHEJ
NHEJ
HDR
HDR
N.A.
N.A.

Figure 12E

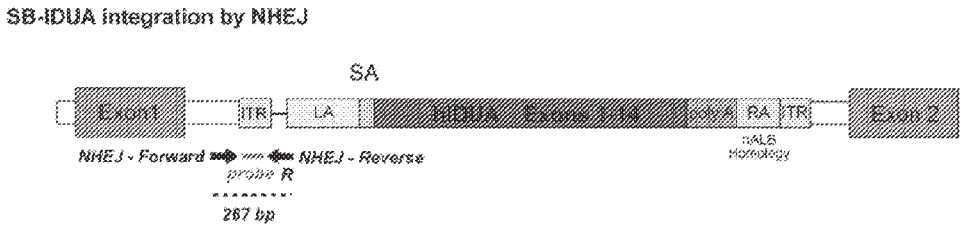


Figure 12F

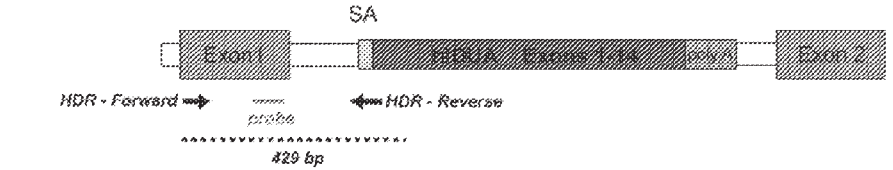


Figure 12G

HepG2 Clone #	NHEJ	HDR
IDUA #21	negative	positive
IDUA #25	positive	positive
IDUA #30	positive	positive
NHEJ positive control	positive	negative
HDR positive control	negative	positive

Figure 12H

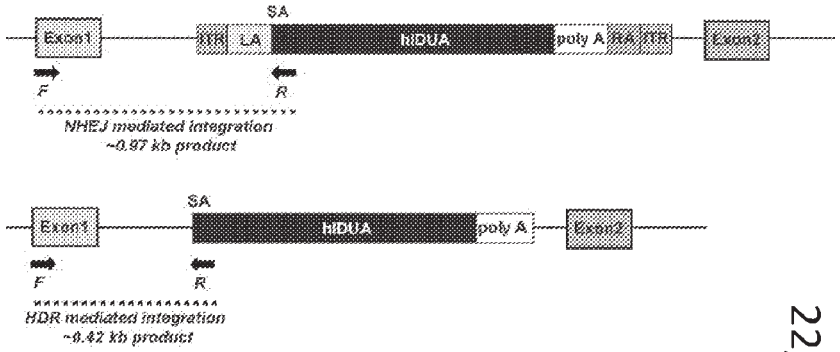
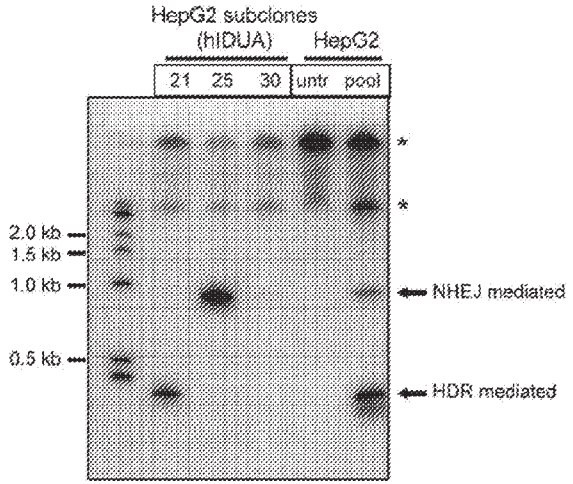


Figure 12I



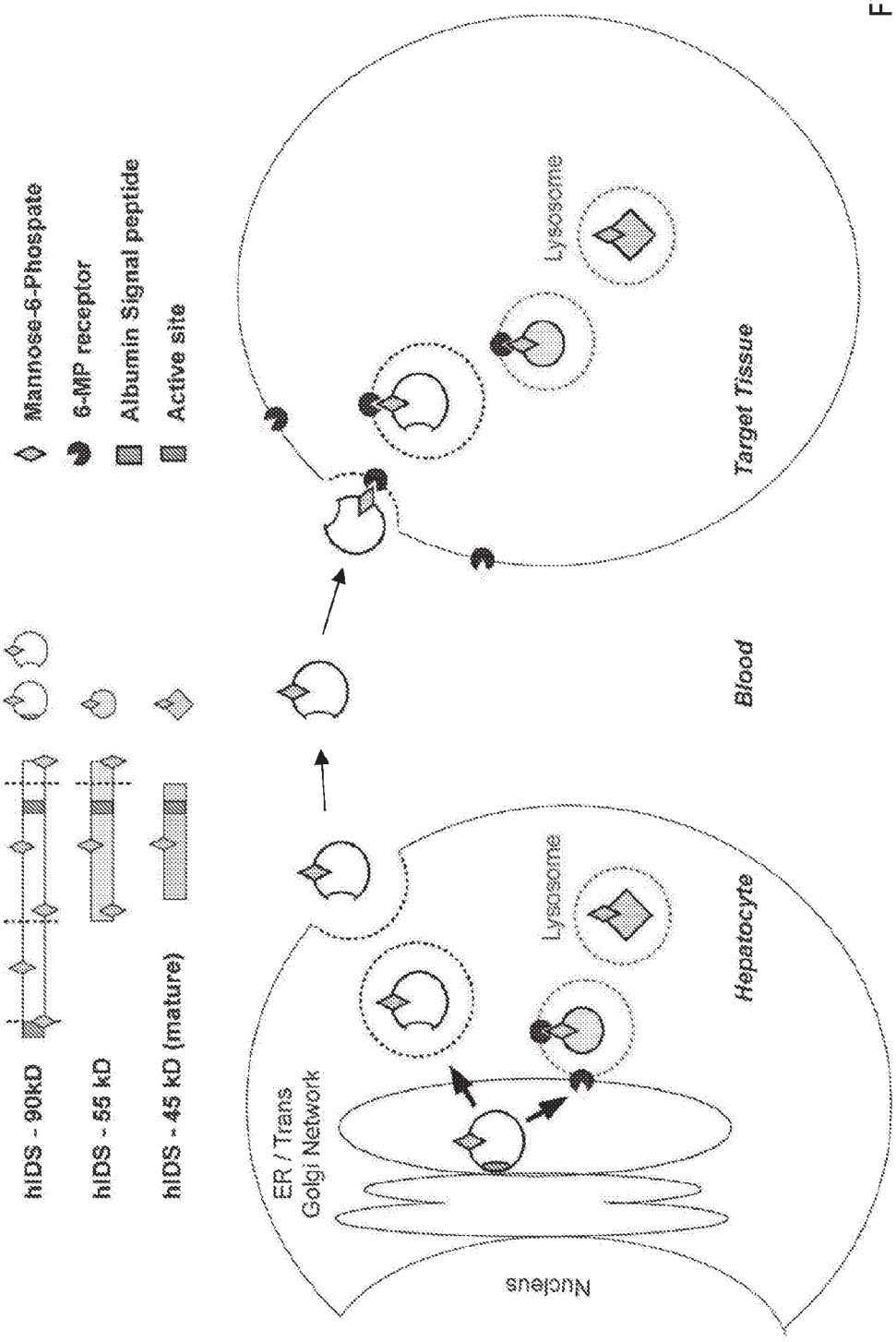
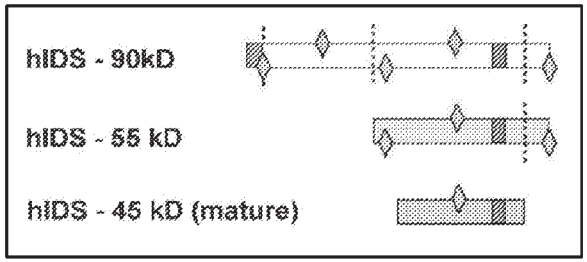

hIDS - 90kD
hIDS - 55 kD
hIDS - 45 kD (mature)
Mannose-6-Phospate
6-MP receptor
Albumin Signal peptide
Active site
ER / Trans Golgi Network
Nucleus
Lysosome
Hepatocyte
Blood
Lysosome
Target Tissue

Figure 13

Figure 14A



HepG2 subclone supernatant

Concentration Step

Add to primary hepatocyte culture

Figure 14B

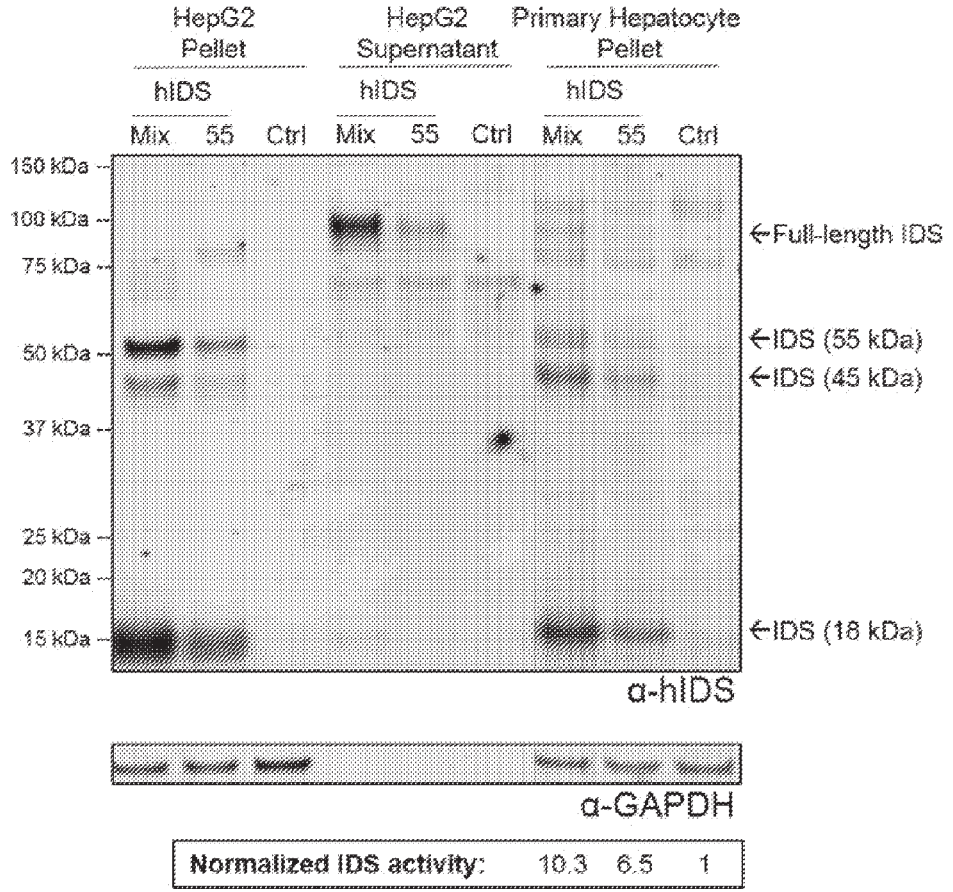


Figure 15A

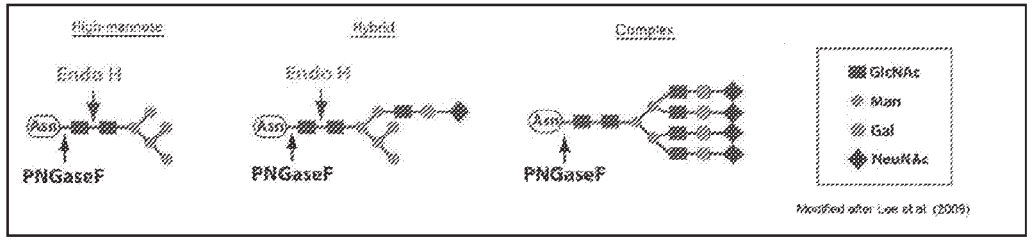
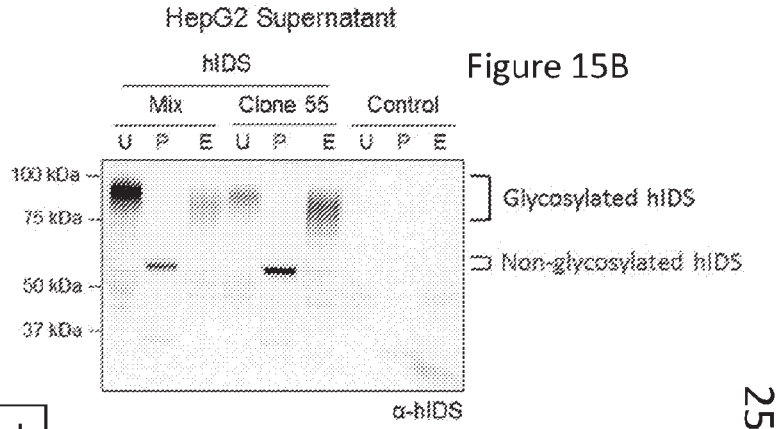


Figure 15B



U= Untreated
P= PNGaseF
E= Endo H

Figure 15C

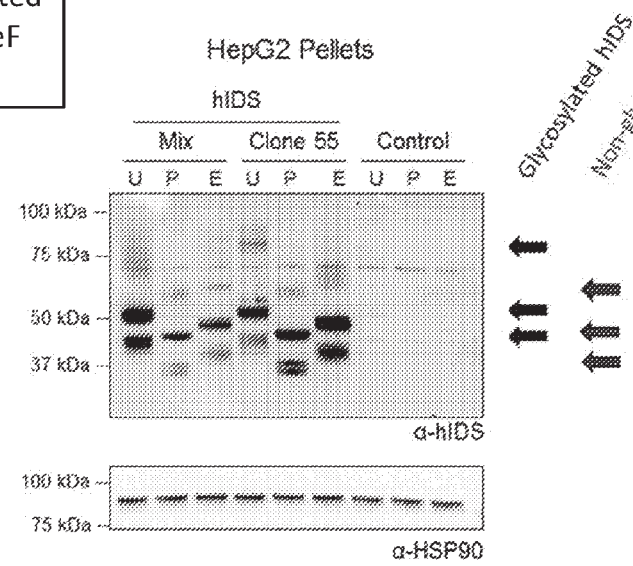


Figure 15D

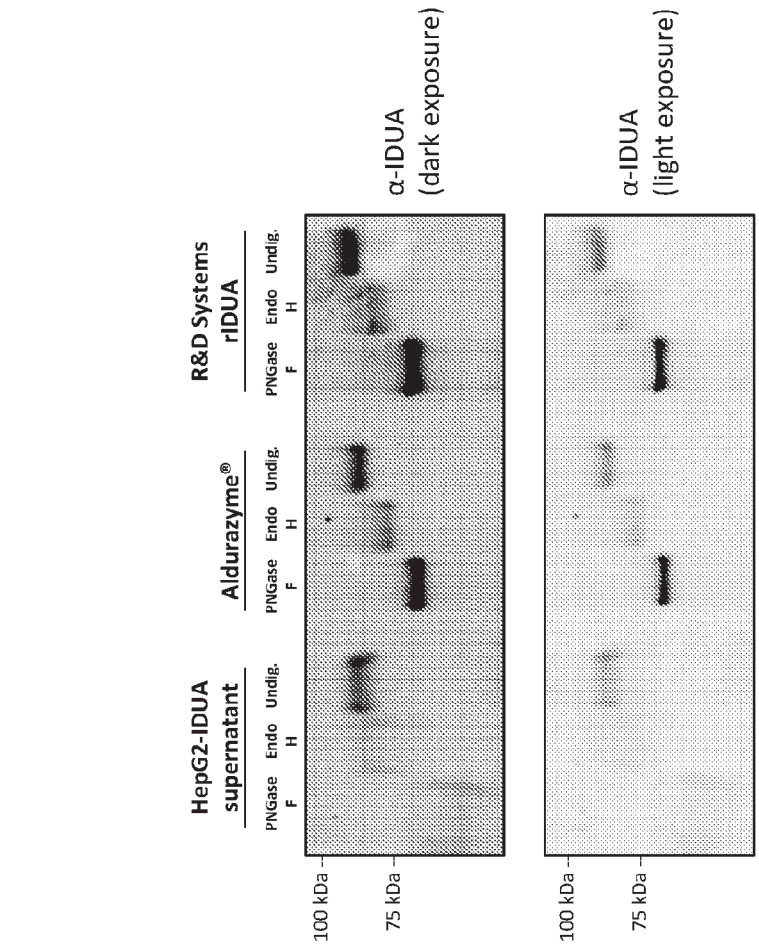

HepG2-IDUA supernatant
Aldurazyme®
R&D Systems rIDUA
PNGase F
Endo H
Undig.
100 kDa
75 kDa
α-IDUA (dark exposure)
α-IDUA (light exposure)

Figure 16A

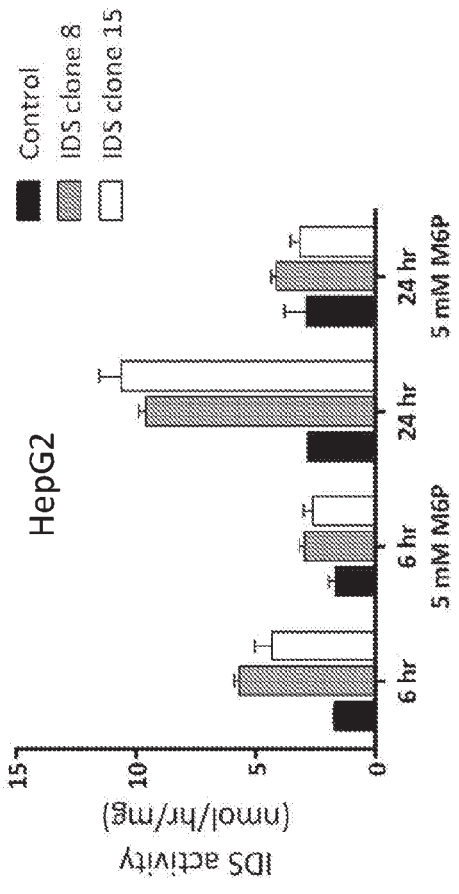


Figure 16B

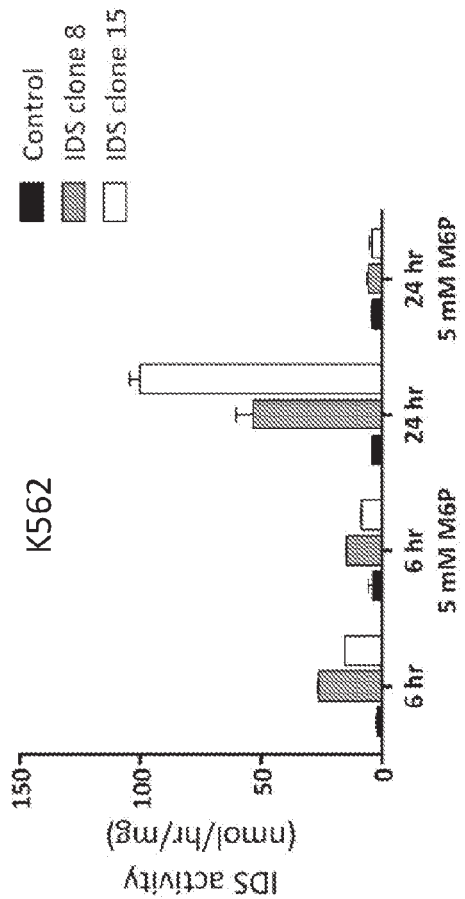
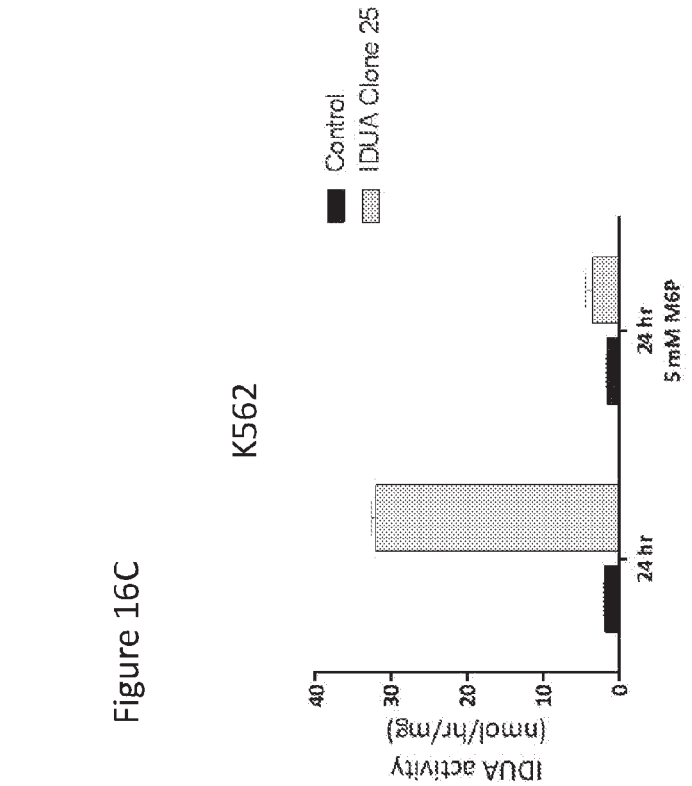


Figure 16C



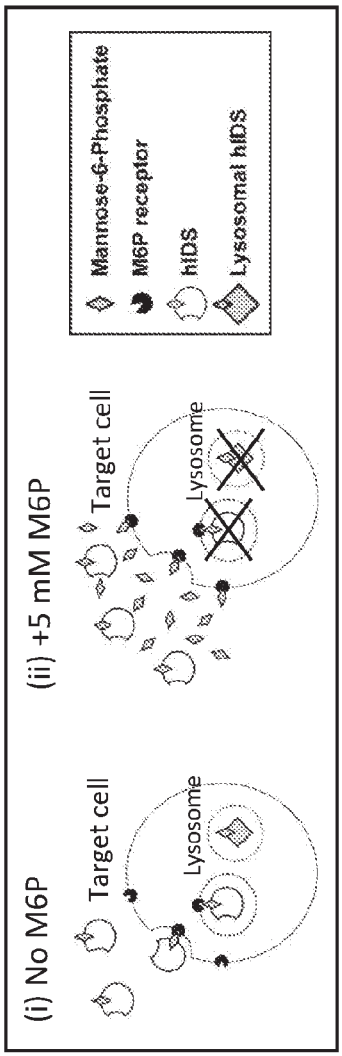
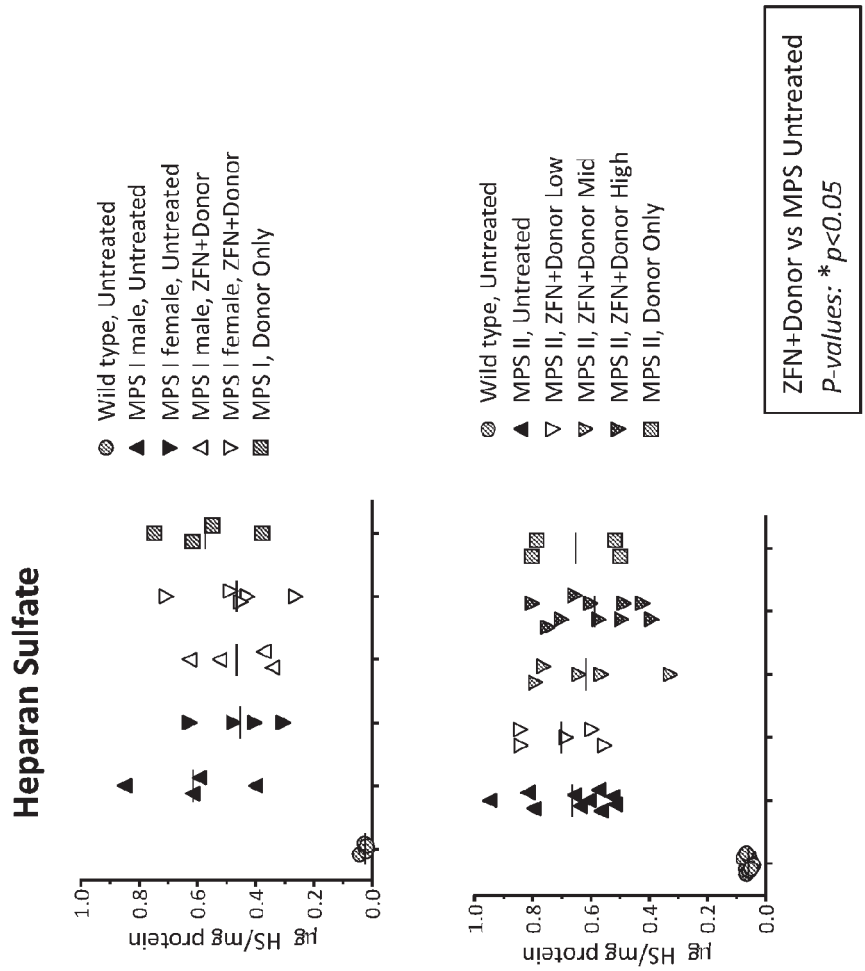


Figure 16D



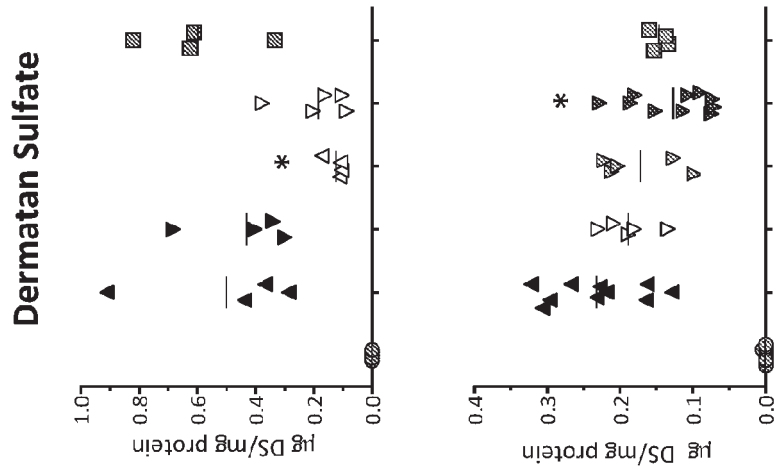


Figure 17A
MPS I

Figure 17B
MPS II