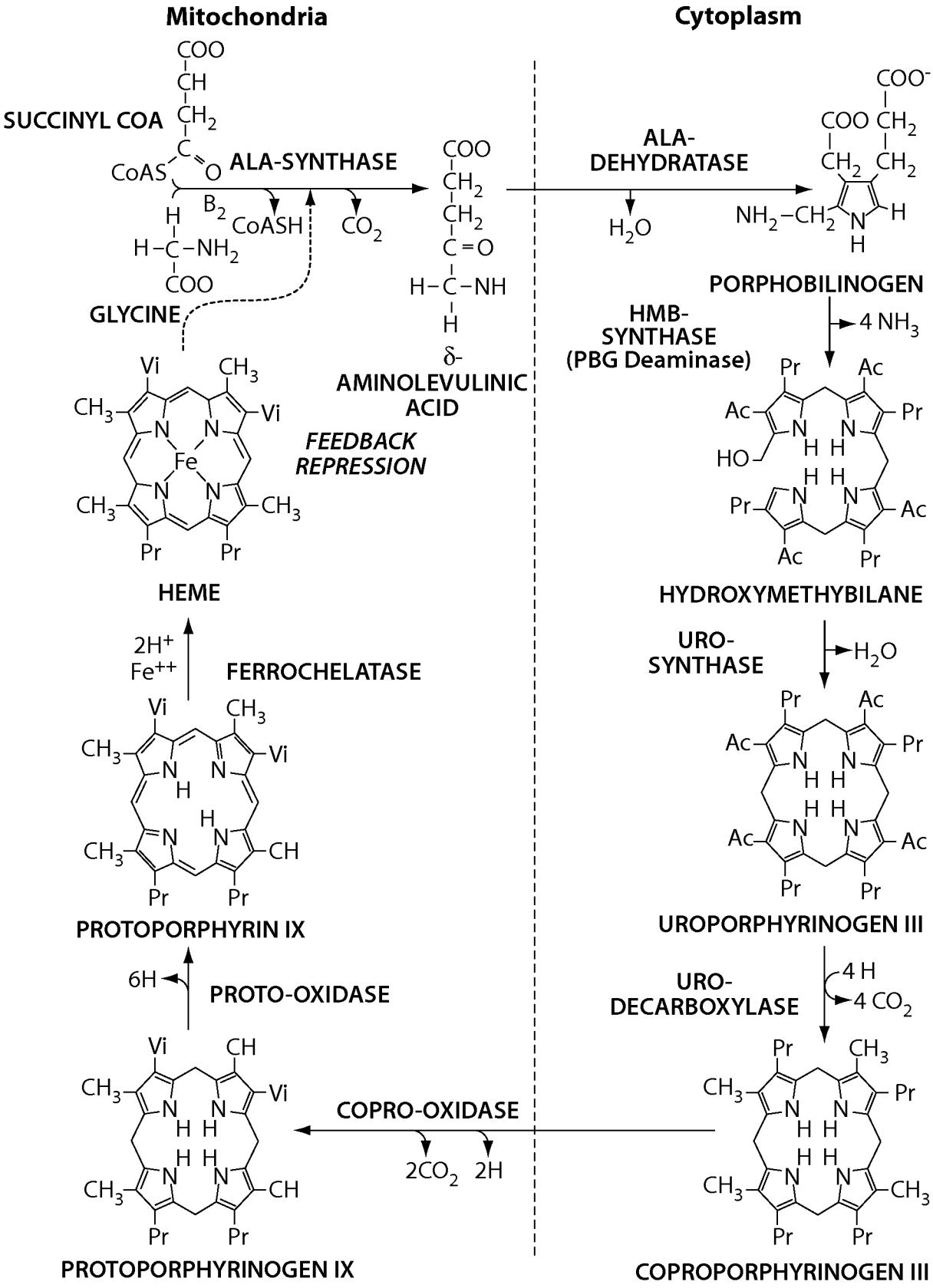

Mitochondria
Cytoplasm
SUCCINYL COA
ALA-SYNTHASE
CoASH
CO2
GLYCINE
δ-
AMINOLEVULINIC ACID
FEEDBACK REPRESSION
ALA-DEHYDRATASE
H2O
PORPHOBILINOGEN
HMB-SYNTHASE (PBG Deaminase)
4 NH3
HYDROXYMETHYBILANE
URO-SYNTHASE
H2O
UROPORPHYRINOGEN III
URO-DECARBOXYLASE
4 H
4 CO2
COPROPORPHYRINOGEN III
COPRO-OXIDASE
2CO2
2H
PROTOPORPHYRINOGEN IX
PROTO-OXIDASE
6H
PROTOPORPHYRIN IX
FERROCHELATASE
2H+
Fe++
HEME

Fig. 1

FIG. 2A

Enzyme, Chromosomal location	**Reaction Catalyzed**	**Associated Porphyria**	**Type of Porphyria**	**Typical Inheritance Pattern**	**Typical Symptoms**
δ-aminolevulinate (ALA) synthase 1 3p21	Glycine + SuccinylCoA ↓ δ-aminolevulinic acid (ALA)				
δ-aminolevulinate (ALA) synthase 2 (ALAS2) (erythroid specific) Xp11.21	Glycine + SuccinylCoA ↓ δ-aminolevulinic acid (ALA)	X-linked sideroblastic anemia (XLSA), X-linked protoporphyria (XLP)	Erythropoietic	X-linked	
δ-aminolevulinate dehydratase (ALAD) 9q34	δ-aminolevulinic acid (ALA) ↓ Porphobilinogen (PBG)	ALA dehydratase deficiency porphyria (ADP or Doss porphyria)	Hepatic	Autosomal recessive	Abdominal pain, neuropathy
PBG deaminase (PBGD) or Hydroxymethylbilane synthase (HMBS) 11q23	Porphobilinogen (PBG) ↓ Hydroxymethylbilane (HMB)	Acute intermittent porphyria (AIP)	Hepatic	Autosomal dominant	Periodic abdominal pain, peripheral neuropathy, psychiatric disorders, tachycardia

FIG. 2B

Uroporphyrinogen III Synthase (UROS) 10q26	Hydroxymethylbilane ↓ Uroporphyrinogen III (URO)	Congenital erythropoietic porphyria (CEP)	Erythropoietic	Autosomal recessive	Severe photosensitivity with erythema, swelling and blistering. Hemolytic anemia, splenomegaly
Uroporphyrinogen decarboxylase (UROD) 1q34	Uroporphyrinogen III (URO) ↓ Coprophyrinogen III	Porphyria cutanea tarda (PCT)	Hepatic	Autosomal dominant or sporadic	Photosensitivity with vesicles and bullae
Coproporphyrinogen III oxidase (CPOX)3q12	Coprophyrinogen III (COPRO) ↓ Protoporphyrinogen IX	Hereditary coproporphyria (HCP)	Hepatic	Autosomal dominant	Photosensitivity, neurologic symptoms, colic
Protoporphyrinogen oxidase (PPOX) 1q14	Protoporphyrinogen IX (PROTO) ↓ Protoporphyrin IX	Variegate prophyria (VP)	Mixed	Autosomal dominant	Photosensitivity, neurologic symptoms, developmental delay
Ferrochelatase 18q21.3	Protoporphyrin IX ↓ Heme	Erythropoietic protoporphyria (EPP)	Erythropoietic	Autosomal recessive	Photosensitivity with skin lesions. Gallstones, mild liver dysfunction

FIG. 3A

```
   1 ctgtatatta aggcgccggc gatcgcggcc tgaggctgct cccggacaag ggcaacgagc
  61 gtttcgtttg gacttctcga cttgagtgcc cgcctccttc gccgccgcct ctgcagtcct
 121 cagcgcagtt atgcccagtt cttcccgctg tggggacacg accacggagg aatccttgct
 181 tcagggactc gggaccctgc tggacccctt cctcgggttt aggggatgtg gggaccagga
 241 gaaagtcagg atccctaaga gtcttccctg cctggatgga tgagtggctt cttctccacc
 301 tagattcttt ccacaggagc cagcatactt cctgaacatg gagagtgttg ttcgccgctg
 361 cccattctta tcccgagtcc cccaggcctt tctgcagaaa gcaggcaaat ctctgttgtt
 421 ctatgcccaa aactgcccca agatgatgga agttggggcc aagccagccc ctcgggcatt
 481 gtccactgca gcagtacact accaacagat caaagaaacc cctccggcca gtgagaaaga
 541 caaaactgct aaggccaagg tccaacagac tcctgatgga tcccagcaga gtccagatgg
 601 cacacagctt ccgtctggac acccccttgcc tgccacaagc cagggcactg caagcaaatg
 661 ccctttcctg gcagcacaga tgaatcagag aggcagcagt gtcttctgca aagccagtct
 721 tgagcttcag gaggatgtgc aggaaatgaa tgccgtgagg aaagaggttg ctgaaacctc
 781 agcaggcccc agtgtggtta gtgtgaaaac cgatggaggg gatcccagtg gactgctgaa
 841 gaacttccag gacatcatgc aaaagcaaag accagaaaga gtgtctcatc ttcttcaaga
 901 taacttgcca aaatctgttt ccacttttca gtatgatcgt ttctttgaga aaaaaattga
 961 tgagaaaaag aatgaccaca cctatcgagt ttttaaaact gtgaaccggc gagcacacat
1021 cttccccatg gcagatgact attcagactc cctcatcacc aaaaagcaag tgtcagtctg
1081 gtgcagtaat gactacctag gaatgagtcg ccacccacgg gtgtgtgggg cagttatgga
1141 cactttgaaa caacatggtg ctggggcagg tggtactaga aatatttctg gaactagtaa
1201 attccatgtg gacttagagc gggagctggc agacctccat gggaaagatg ccgcactctt
1261 gttttcctcg tgctttgtgg ccaatgactc aaccctcttc accctggcta agatgatgcc
1321 aggctgtgag atttactctg attctgggaa ccatgcctcc atgatccaag ggattcgaaa
1381 cagccgagtg ccaaagtaca tcttccgcca caatgatgtc agccacctca gagaactgct
1441 gcaaagatct gacccctcag tccccaagat tgtggcattt gaaactgtcc attcaatgga
1501 tggggcggtg tgcccactgg aagagctgtg tgatgtggcc catgagtttg gagcaatcac
1561 cttcgtggat gaggtccacg cagtggggct ttatggggct cgaggcggag ggattgggga
1621 tcgggatgga gtcatgccaa aaatggacat catttctgga acacttggca aagcctttgg
1681 ttgtgttgga gggtacatcg ccagcacgag ttctctgatt gacaccgtac ggtcctatgc
1741 tgctggcttc atcttcacca cctctctgcc acccatgctg ctggctggag ccctggagtc
1801 tgtgcggatc ctgaagagcg ctgagggacg ggtgcttcgc cgccagcacc agcgcaacgt
1861 caaactcatg agacagatgc taatggatgc cggcctccct gttgtccact gccccagcca
1921 catcatccct gtgcgggttg cagatgctgc taaaaacaca gaagtctgtg atgaactaat
1981 gagcagacat aacatctacg tgcaagcaat caattaccct acggtgcccc ggggagaaga
2041 gctcctacgg attgccccca ccctcacca cacaccccag atgatgaact acttccttga
2101 gaatctgcta gtcacatgga agcaagtggg gctggaactg aagcctcatt cctcagctga
2161 gtgcaacttc tgcaggaggc cactgcattt tgaagtgatg agtgaaagag agaagtccta
2221 tttctcaggc ttgagcaagt tggtatctgc tcaggcctga gcatgacctc aattatttca
```

FIG. 3B

```
2281 cttaacccca ggccattatc atatccagat ggtcttcaga gttgtcttta tatgtgaatt
2341 aagttatatt aaattttaat ctatagtaaa aacatagtcc tggaaataaa ttcttgctta
2401 aatggtg
```

(SEQ ID NO:1)

FIG. 4A

```
   1 cagaagaagg cagcgcccaa ggcgcatgcg cagcggtcac tcccgctgta tattaaggcg
  61 ccggcgatcg cggcctgagg ctgctcccgg acaagggcaa cgagcgtttc gtttggactt
 121 ctcgacttga gtgcccgcct ccttcgccgc cgcctctgca gtcctcagcg cagttatgcc
 181 cagttcttcc cgctgtgggg acacgaccac ggaggaatcc ttgcttcagg gactcgggac
 241 cctgctggac cccttcctcg ggtttagggg atgtggggac caggagaaag tcaggatccc
 301 taagagtctt ccctgcctgg atggatgagt ggcttcttct ccacctagat tctttccaca
 361 ggagccagca tacttcctga acatggagag tgttgttcgc cgctgcccat tcttatcccg
 421 agtcccccag gcctttctgc agaaagcagg caaatctctg ttgttctatg cccaaaactg
 481 ccccaagatg atggaagttg gggccaagcc agcccctcgg gcattgtcca ctgcagcagt
 541 acactaccaa cagatcaaag aaacccctcc ggccagtgag aaagacaaaa ctgctaaggc
 601 caaggtccaa cagactcctg atggatccca gcagagtcca gatggcacac agcttccgtc
 661 tggacacccc ttgcctgcca caagccaggg cactgcaagc aaatgccctt tcctggcagc
 721 acagatgaat cagagaggca gcagtgtctt ctgcaaagcc agtcttgagc ttcaggagga
 781 tgtgcaggaa atgaatgccg tgaggaaaga ggttgctgaa acctcagcag gccccagtgt
 841 ggttagtgtg aaaaccgatg gaggggatcc cagtggactg ctgaagaact tccaggacat
 901 catgcaaaag caaagaccag aaagagtgtc tcatcttctt caagataact tgccaaaatc
 961 tgtttccact tttcagtatg atcgtttctt tgagaaaaaa attgatgaga aaaagaatga
1021 ccacacctat cgagttttta aaactgtgaa ccggcgagca cacatcttcc ccatggcaga
1081 tgactattca gactccctca tcaccaaaaa gcaagtgtca gtctggtgca gtaatgacta
1141 cctaggaatg agtcgccacc cacgggtgtg tggggcagtt atggacactt tgaaacaaca
1201 tggtgctggg gcaggtggta ctagaaatat ttctggaact agtaaattcc atgtggactt
1261 agagcgggag ctggcagacc tccatgggaa agatgccgca ctcttgtttt cctcgtgctt
1321 tgtggccaat gactcaaccc tcttcaccct ggctaagatg atgccaggct gtgagattta
1381 ctctgattct gggaaccatg cctccatgat ccaagggatt cgaaacagcc gagtgccaaa
1441 gtacatcttc cgccacaatg atgtcagcca cctcagagaa ctgctgcaaa gatctgaccc
1501 ctcagtcccc aagattgtgg catttgaaac tgtccattca atggatgggg cggtgtgccc
1561 actggaagag ctgtgtgatg tggcccatga gtttggagca atcaccttcg tggatgaggt
1621 ccacgcagtg gggctttatg gggctcgagg cggagggatt ggggatcggg atggagtcat
1681 gccaaaaatg gacatcattt ctggaacact tggcaaagcc tttggttgtg ttggagggta
1741 catcgccagc acgagttctc tgattgacac cgtacggtcc tatgctgctg gcttcatctt
1801 caccacctct ctgccaccca tgctgctggc tggagccctg gagtctgtgc ggatcctgaa
1861 gagcgctgag ggacgggtgc ttcgccgcca gcaccagcgc aacgtcaaac tcatgagaca
1921 gatgctaatg gatgccggcc tccctgttgt ccactgcccc agccacatca tccctgtgcg
1981 ggttgcagat gctgctaaaa acacagaagt ctgtgatgaa ctaatgagca gacataacat
2041 ctacgtgcaa gcaatcaatt accctacggt gccccgggga gaagagctcc tacggattgc
2101 ccccacccct caccacacac cccagatgat gaactacttc cttgagaatc tgctagtcac
2161 atggaagcaa gtggggctgg aactgaagcc tcattcctca gctgagtgca acttctgcag
2221 gaggccactg cattttgaag tgatgagtga aagagagaag tcctatttct caggcttgag
```

FIG. 4B

```
2281 caagttggta tctgctcagg cctgagcatg acctcaatta tttcacttaa ccccaggcca
2341 ttatcatatc cagatggtct tcagagttgt ctttatatgt gaattaagtt atattaaatt
2401 ttaatctata gtaaaaacat agtcctggaa ataaattctt gcttaaatgg tgaaaaaa
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(SEQ ID NO:382)

FIG. 5

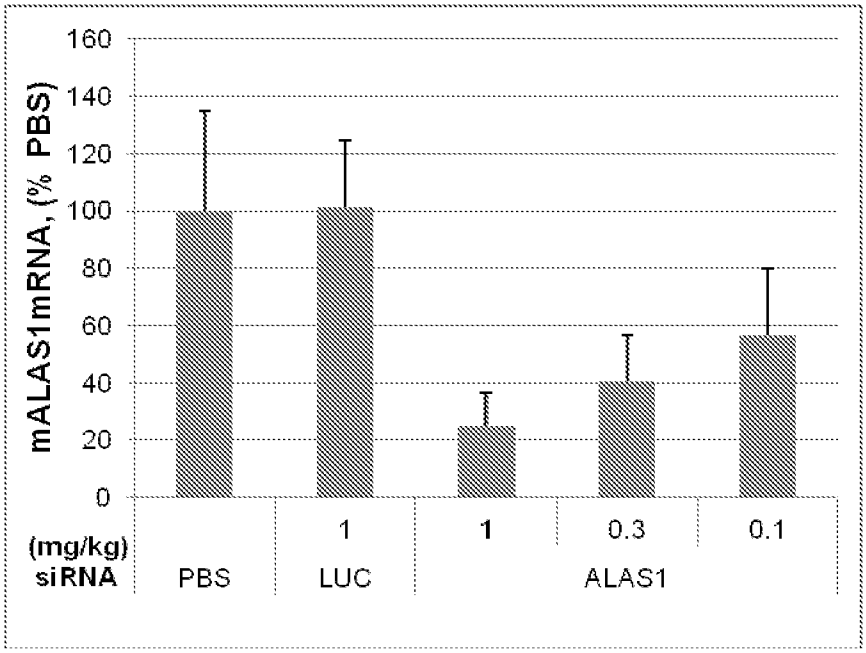

mALAS1mRNA, (% PBS)
160
140
120
100
80
60
40
20
0
(mg/kg)
siRNA
1
1
0.3
0.1
PBS
LUC
ALAS1

FIG. 6

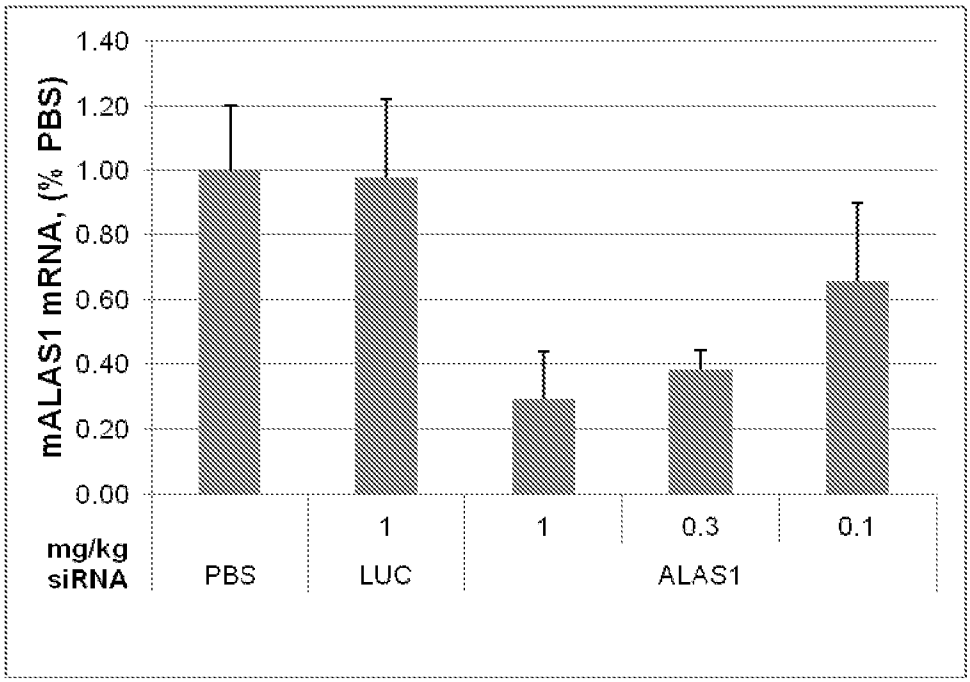

mALAS1 mRNA, (% PBS)
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
mg/kg siRNA
PBS
1
LUC
1
0.3
0.1
ALAS1

FIG. 7

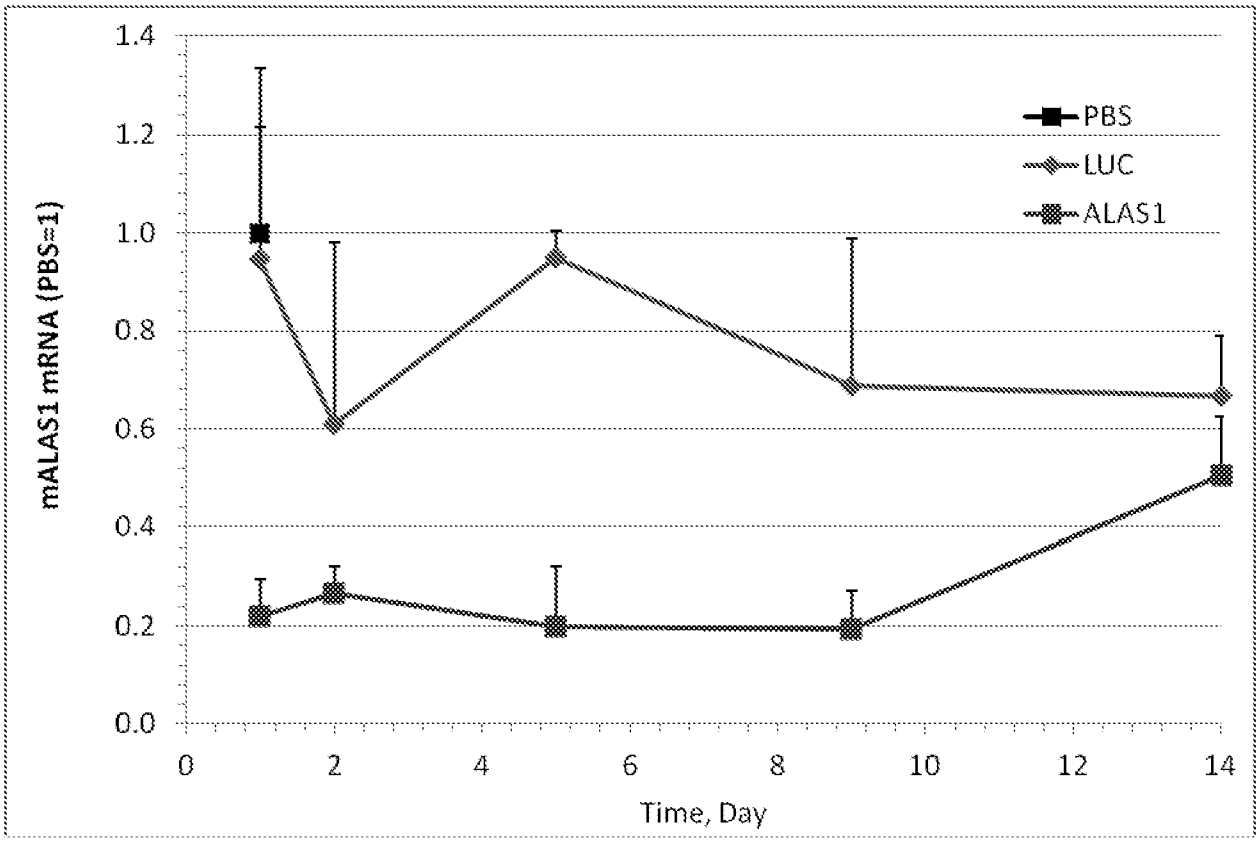

mALAS1 mRNA (PBS=1)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
14
Time, Day
PBS
LUC
ALAS1

FIG. 8

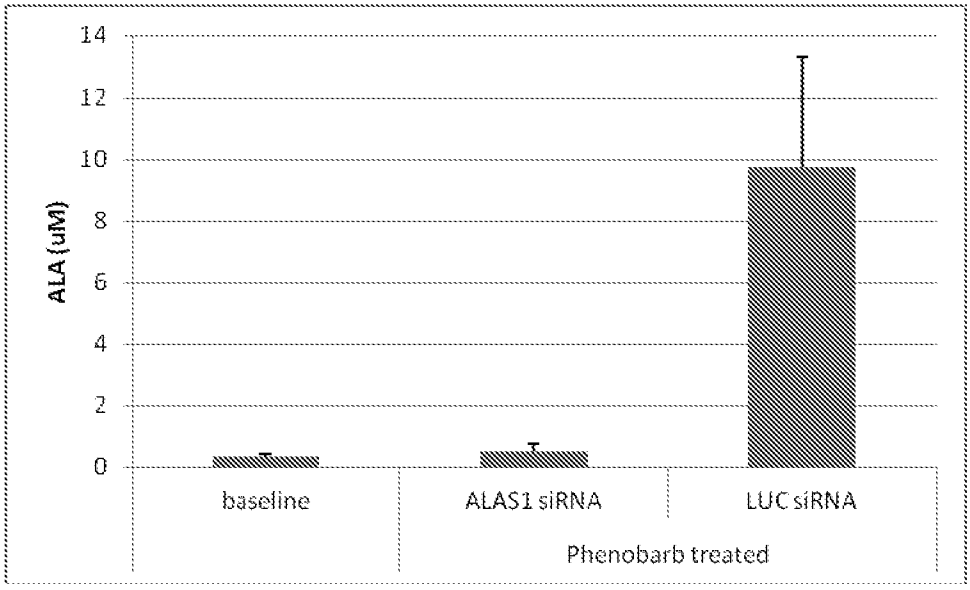

14
12
10
8
6
4
2
0
ALA (uM)
baseline
ALAS1 siRNA
LUC siRNA
Phenobarb treated

FIG. 9

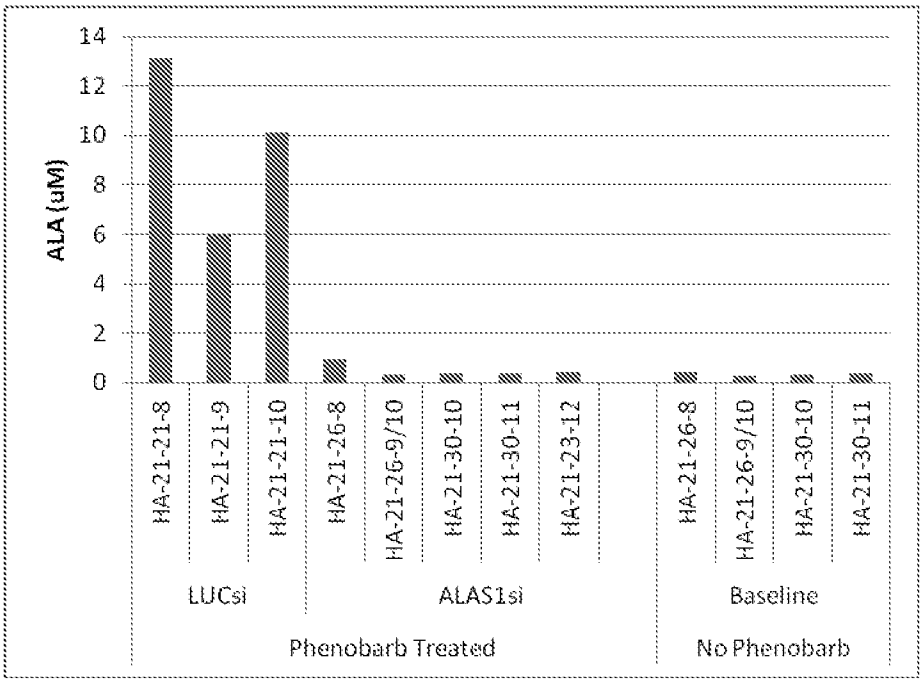

ALA (uM)
14
12
10
8
6
4
2
0
HA-21-21-8
HA-21-21-9
HA-21-21-10
HA-21-26-8
HA-21-26-9/10
HA-21-30-10
HA-21-30-11
HA-21-23-12
HA-21-26-8
HA-21-26-9/10
HA-21-30-10
HA-21-30-11
LUCsi
ALAS1si
Baseline
Phenobarb Treated
No Phenobarb

FIG. 10

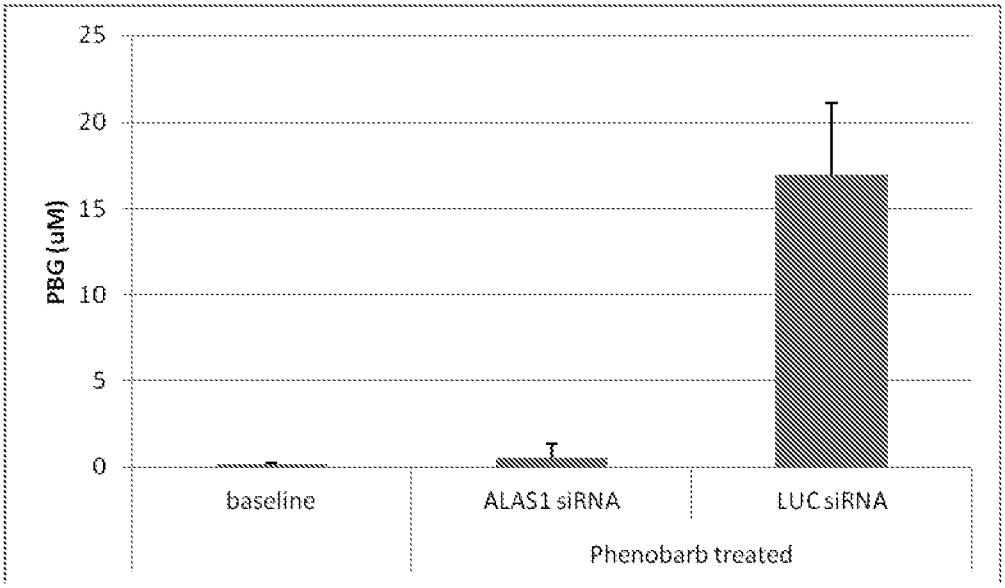

25
20
15
10
5
0
PBG (uM)
baseline
ALAS1 siRNA
LUC siRNA
Phenobarb treated

FIG. 11

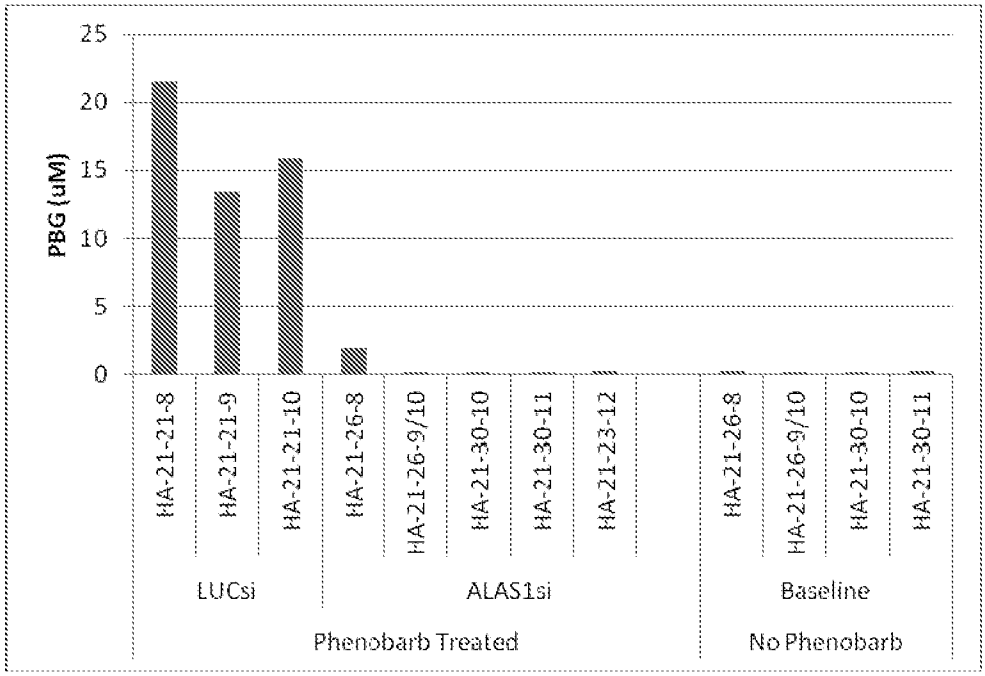

25
20
15
10
5
0
PBG (uM)
HA-21-21-8
HA-21-21-9
HA-21-21-10
HA-21-26-8
HA-21-26-9/10
HA-21-30-10
HA-21-30-11
HA-21-23-12
HA-21-26-8
HA-21-26-9/10
HA-21-30-10
HA-21-30-11
LUCsi
ALAS1si
Baseline
Phenobarb Treated
No Phenobarb

FIG. 12

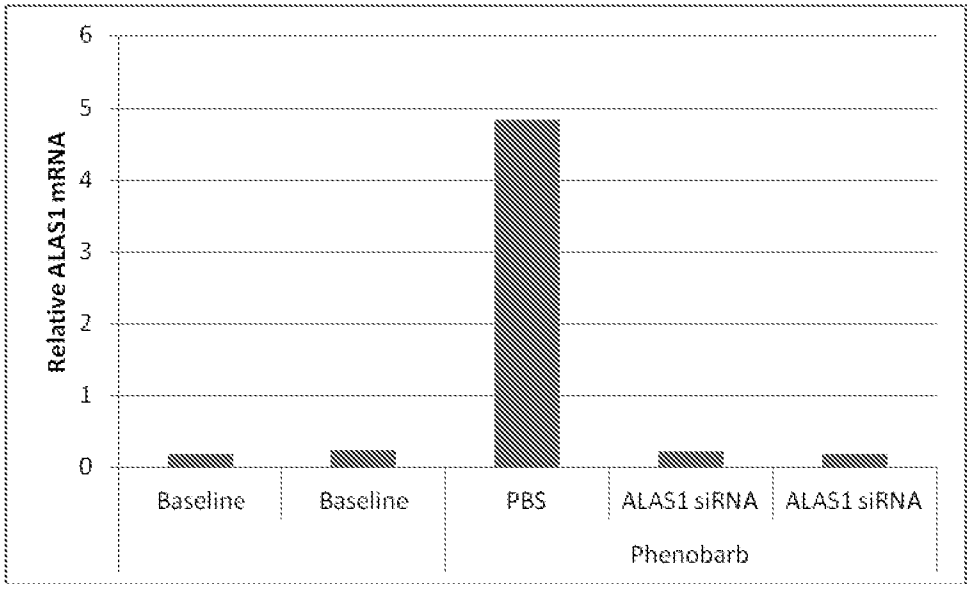

Relative ALAS1 mRNA
6
5
4
3
2
1
0
Baseline
Baseline
PBS
ALAS1 siRNA
ALAS1 siRNA
Phenobarb

FIG. 13

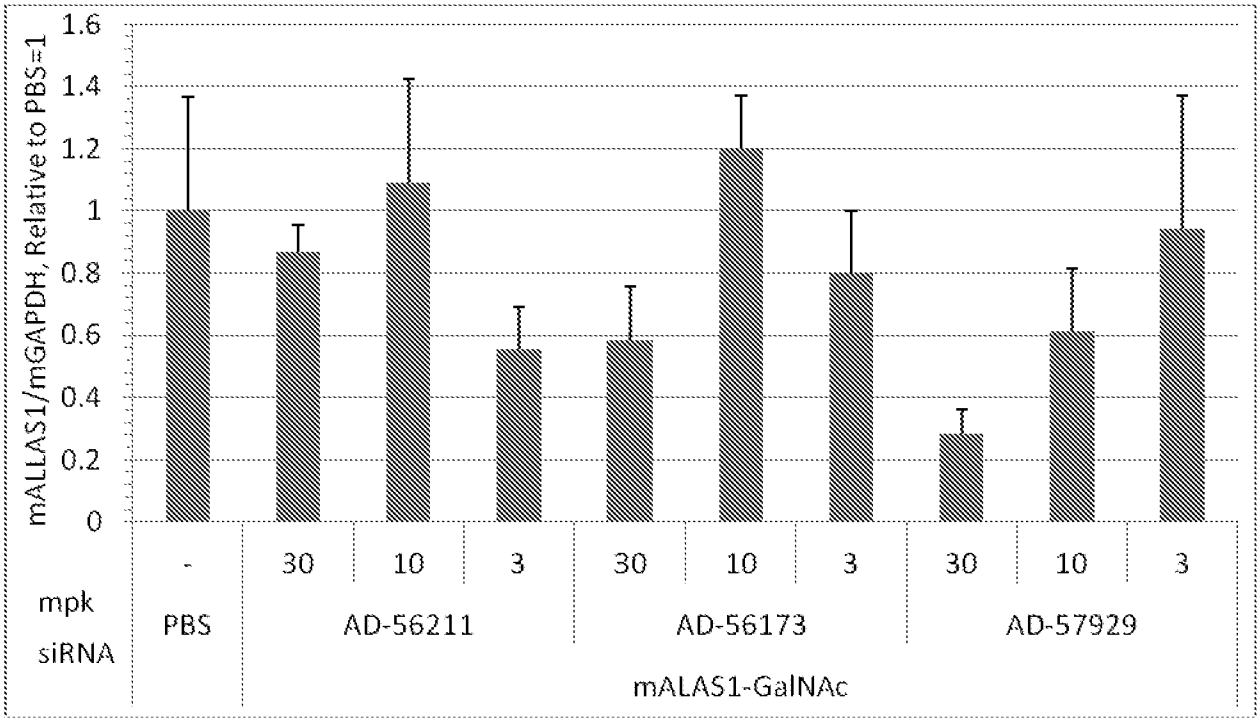
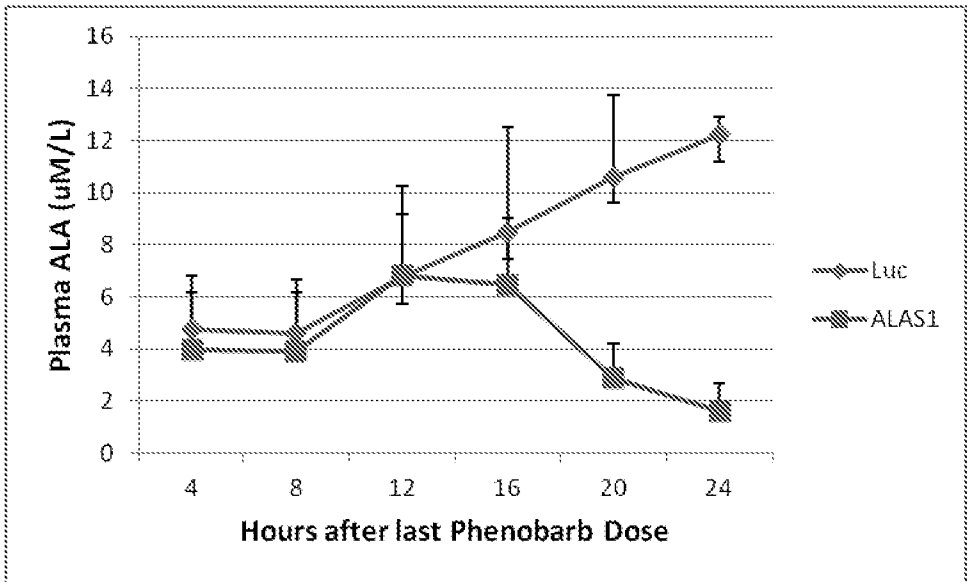

mALLAS1/mGAPDH, Relative to PBS=1
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-
30
10
3
30
10
3
30
10
3
mpk
siRNA
PBS
AD-56211
AD-56173
AD-57929
mALAS1-GalNAc

FIG. 14


16
14
12
10
8
6
4
2
0
Plasma ALA (uM/L)
4
8
12
16
20
24
Hours after last Phenobarb Dose
Luc
ALAS1

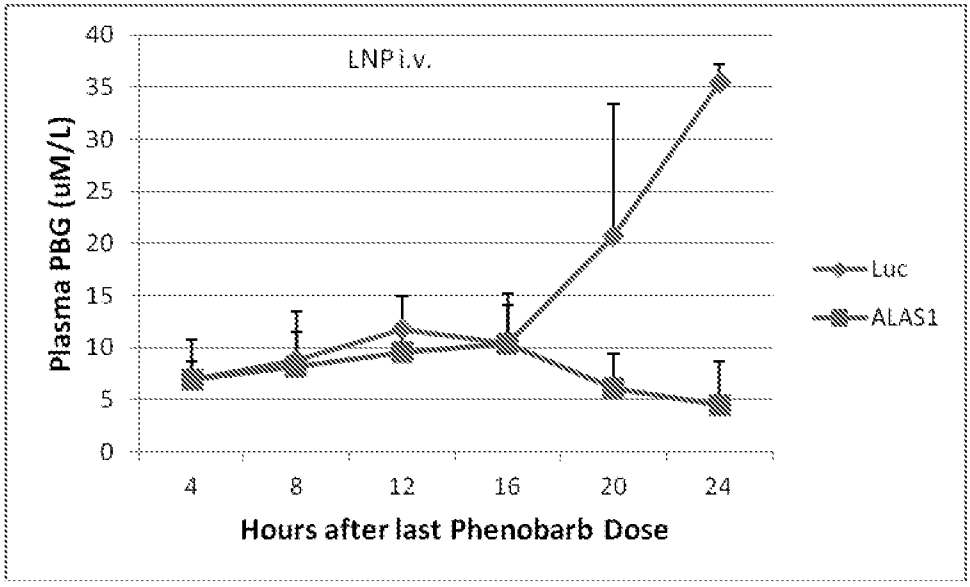

LNP i.v.
40
35
30
25
20
15
10
5
0
Plasma PBG (uM/L)
4
8
12
16
20
24
Hours after last Phenobarb Dose
Luc
ALAS1

FIG. 15

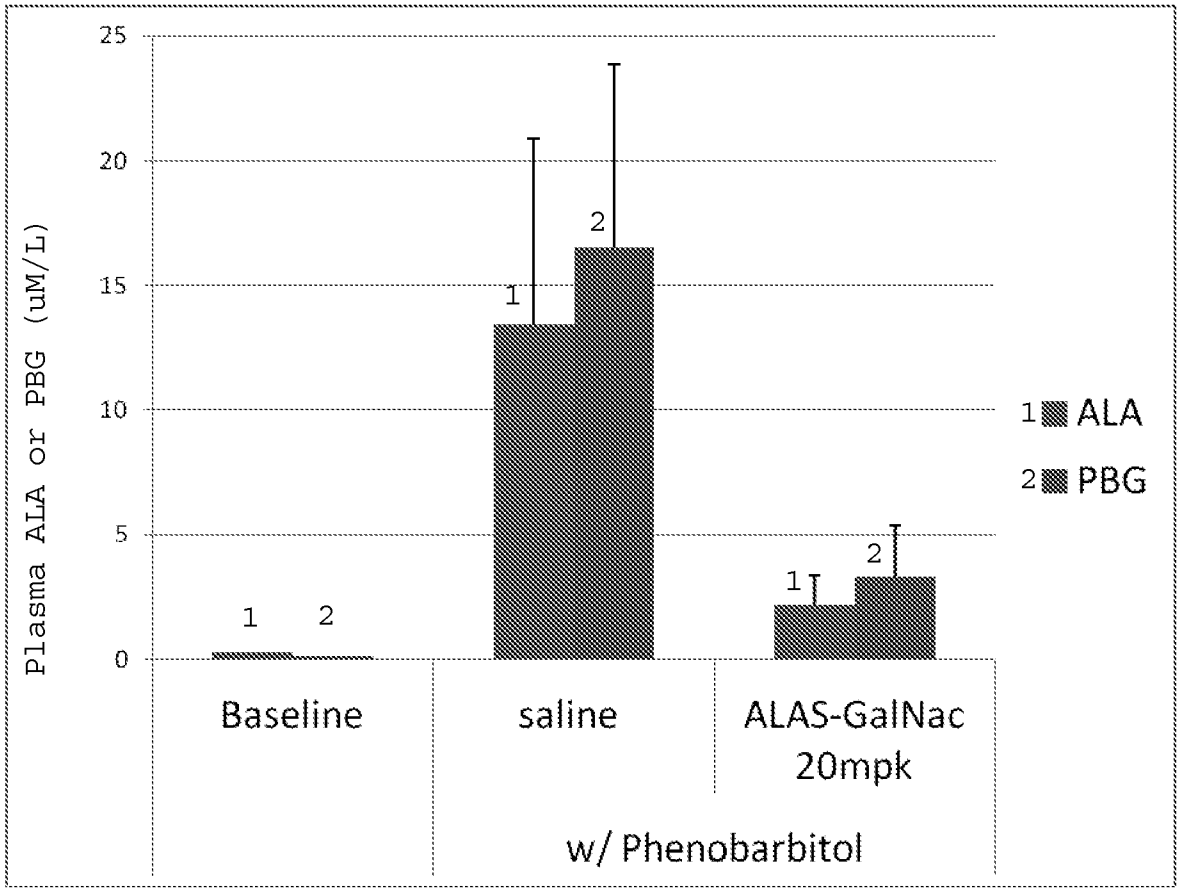

Plasma ALA or PBG (uM/L)
25
20
15
10
5
0
1
2
1
2
1
2
1 ALA
2 PBG
Baseline
saline
ALAS-GalNac 20mpk
w/ Phenobarbitol

FIG. 16

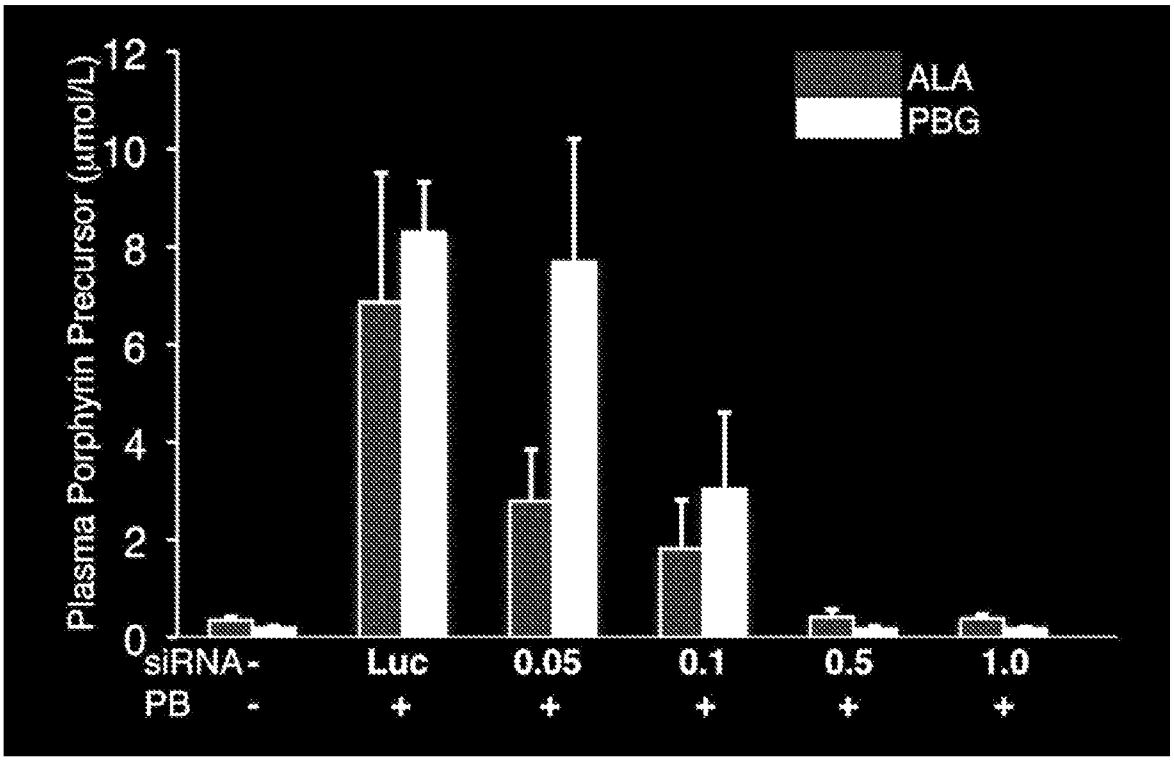
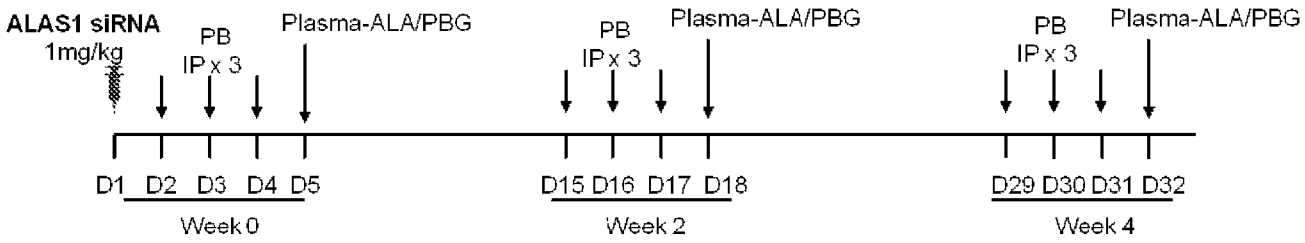

Plasma Porphyrin Precursor (μmol/L)
0
2
4
6
8
10
12
ALA
PBG
siRNA -
PB -
Luc
+
0.05
+
0.1
+
0.5
+
1.0
+

FIG. 17


ALAS1 siRNA
1mg/kg
PB
IP x 3
Plasma-ALA/PBG
PB
IP x 3
Plasma-ALA/PBG
PB
IP x 3
Plasma-ALA/PBG
D1 D2 D3 D4 D5
D15 D16 D17 D18
D29 D30 D31 D32
Week 0
Week 2
Week 4

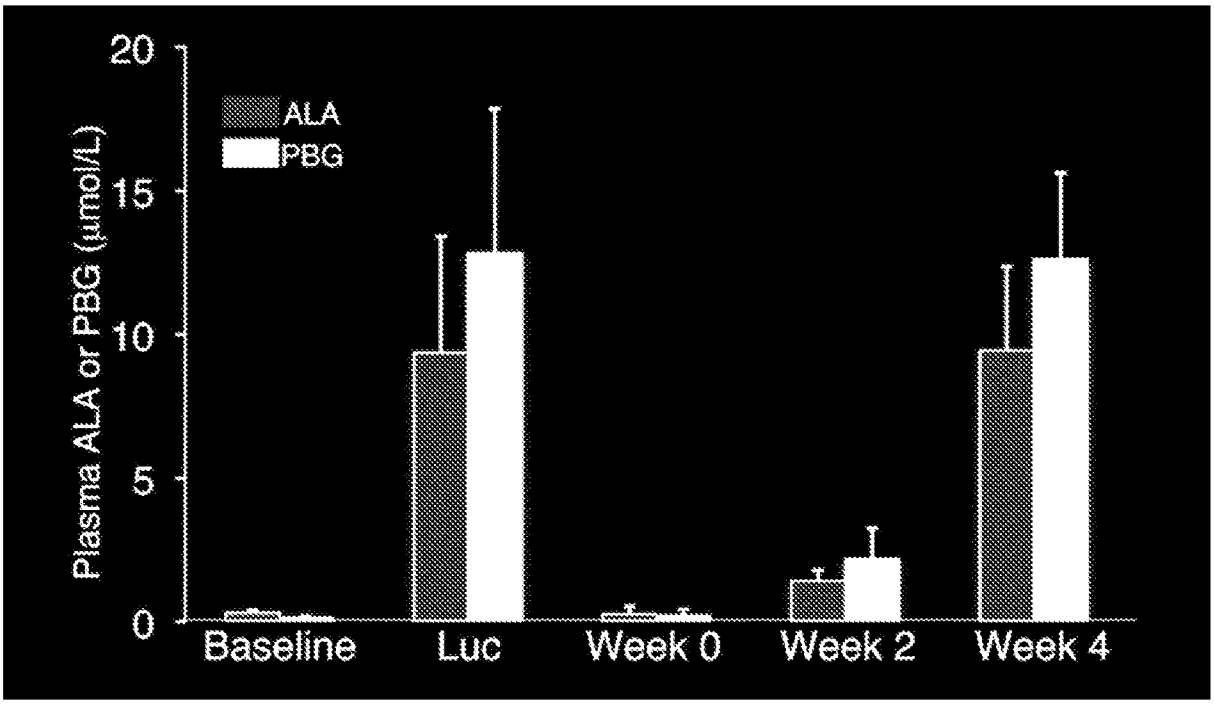
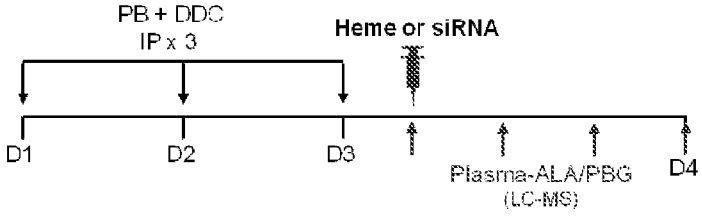
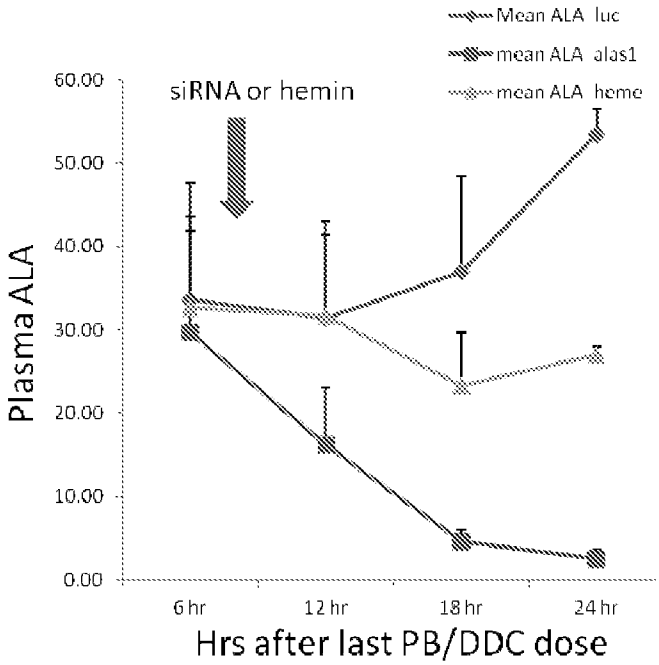

Plasma ALA or PBG (µmol/L)
20
15
10
5
0
ALA
PBG
Baseline
Luc
Week 0
Week 2
Week 4

FIG. 18


PB + DDC
IP x 3
Heme or siRNA
D1
D2
D3
Plasma-ALA/PBG
(LC-MS)
D4


Mean ALA luc
mean ALA alas1
mean ALA heme
siRNA or hemin
Plasma ALA
60.00
50.00
40.00
30.00
20.00
10.00
0.00
6 hr
12 hr
18 hr
24 hr
Hrs after last PB/DDC dose

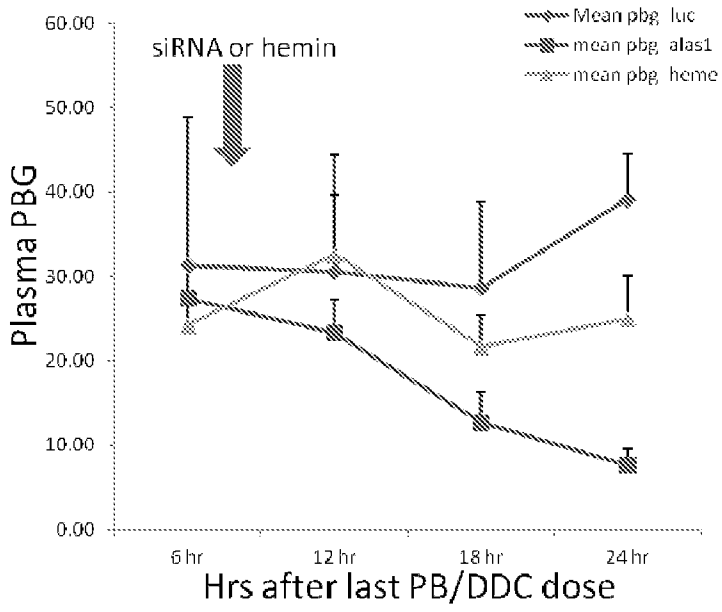

Mean pbg luc
mean pbg alas1
mean pbg heme
siRNA or hemin
Plasma PBG
60.00
50.00
40.00
30.00
20.00
10.00
0.00
6 hr
12 hr
18 hr
24 hr
Hrs after last PB/DDC dose

FIG. 19

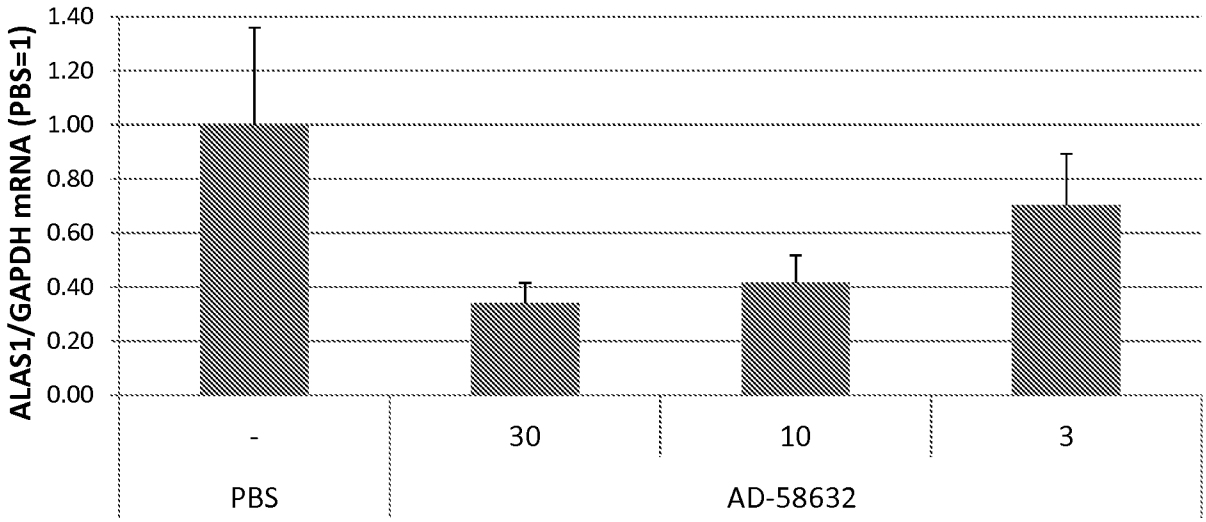

ALAS1/GAPDH mRNA (PBS=1)
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
-
30
10
3
PBS
AD-58632

FIG. 20

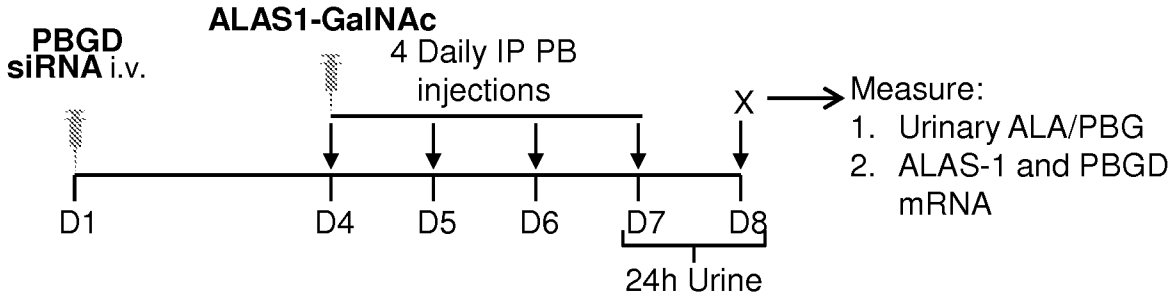
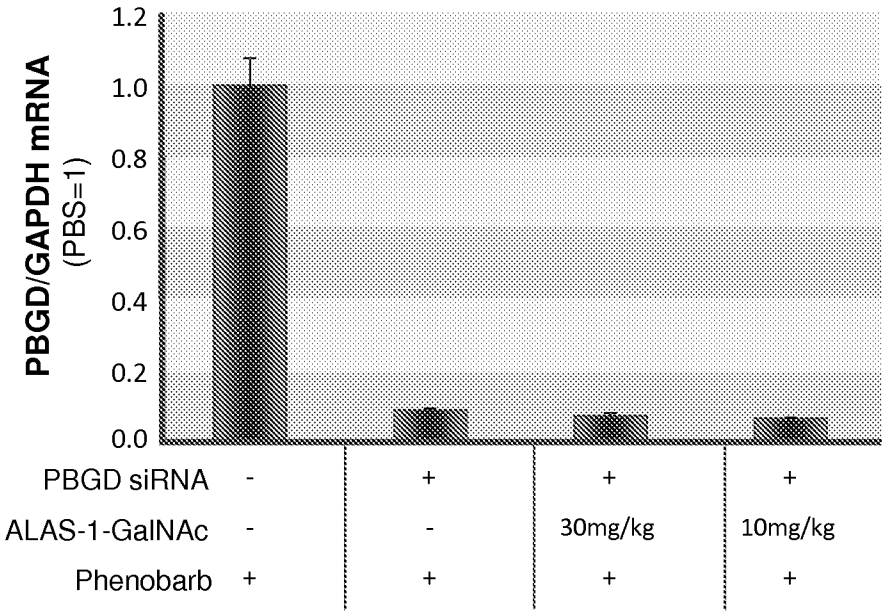

PBGD
siRNA i.v.
ALAS1-GalNAc
4 Daily IP PB
injections
X
Measure:
1. Urinary ALA/PBG
2. ALAS-1 and PBGD
mRNA
D1
D4
D5
D6
D7
D8
24h Urine

FIG. 21


PBGD/GAPDH mRNA
(PBS=1)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
PBGD siRNA - + + +
ALAS-1-GalNAc - - 30mg/kg 10mg/kg
Phenobarb + + + +

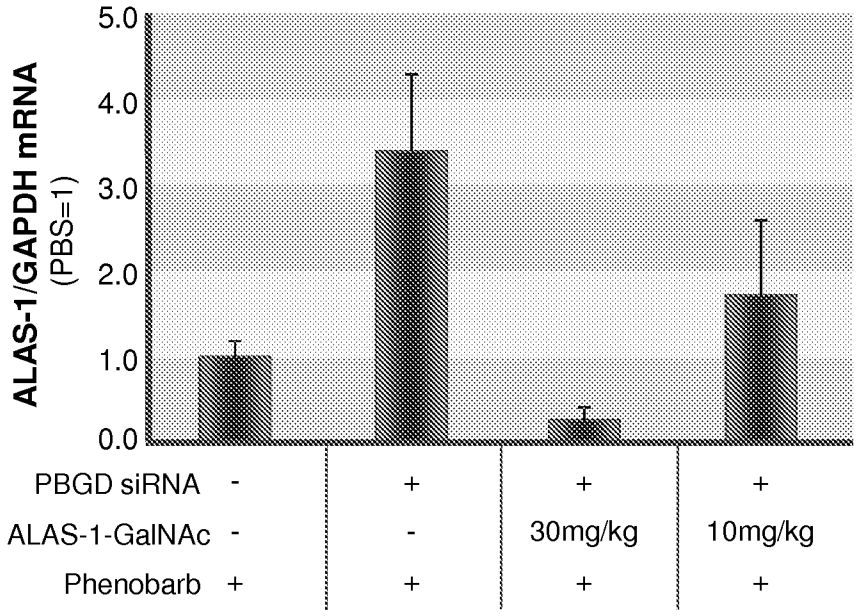
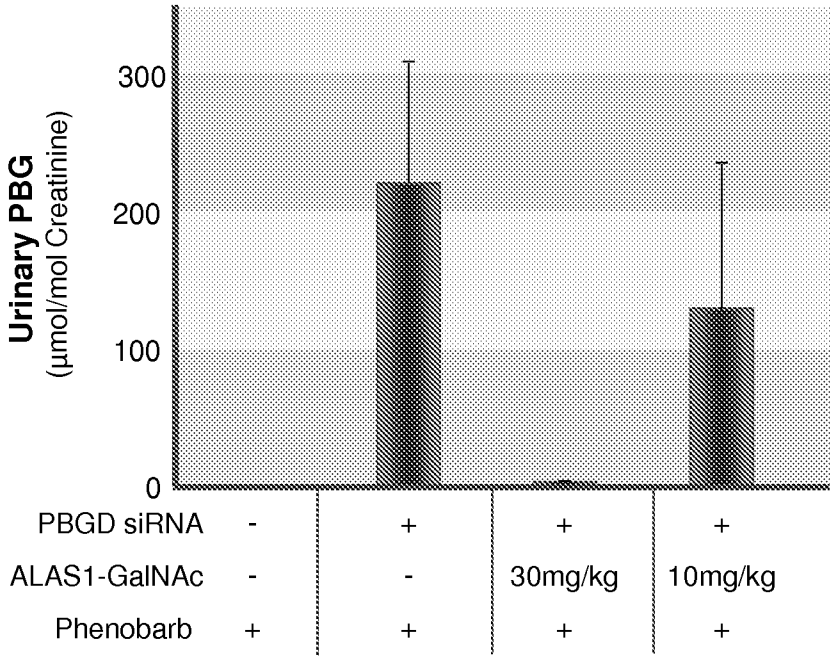

ALAS-1/GAPDH mRNA
(PBS=1)
5.0
4.0
3.0
2.0
1.0
0.0
PBGD siRNA - + + +
ALAS-1-GalNAc - - 30mg/kg 10mg/kg
Phenobarb + + + +

FIG. 22


Urinary PBG
(µmol/mol Creatinine)
300
200
100
0
PBGD siRNA - + + +
ALAS1-GalNAc - - 30mg/kg 10mg/kg
Phenobarb + + + +

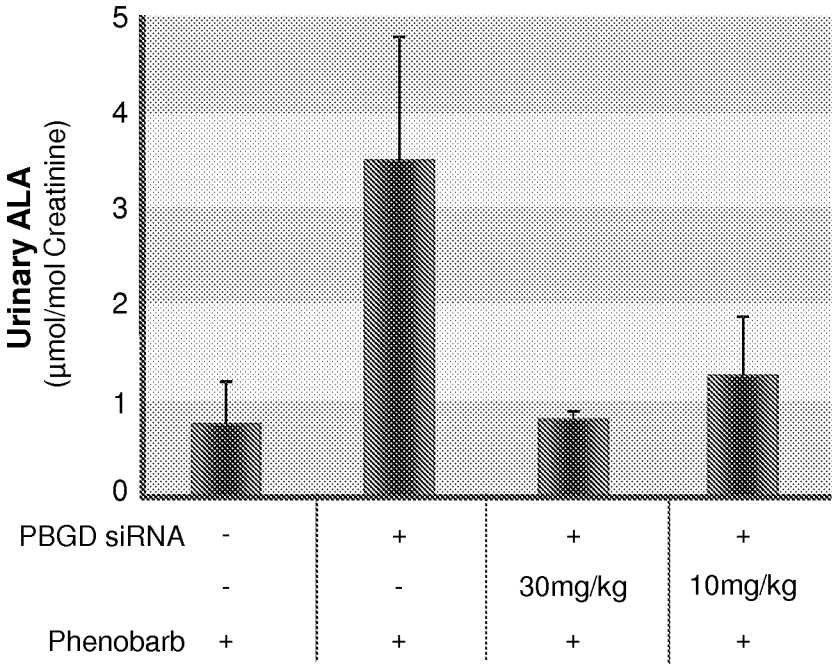

Urinary ALA
(µmol/mol Creatinine)
5
4
3
2
1
0
PBGD siRNA - + + +
- - 30mg/kg 10mg/kg
Phenobarb + + + +

FIG. 23

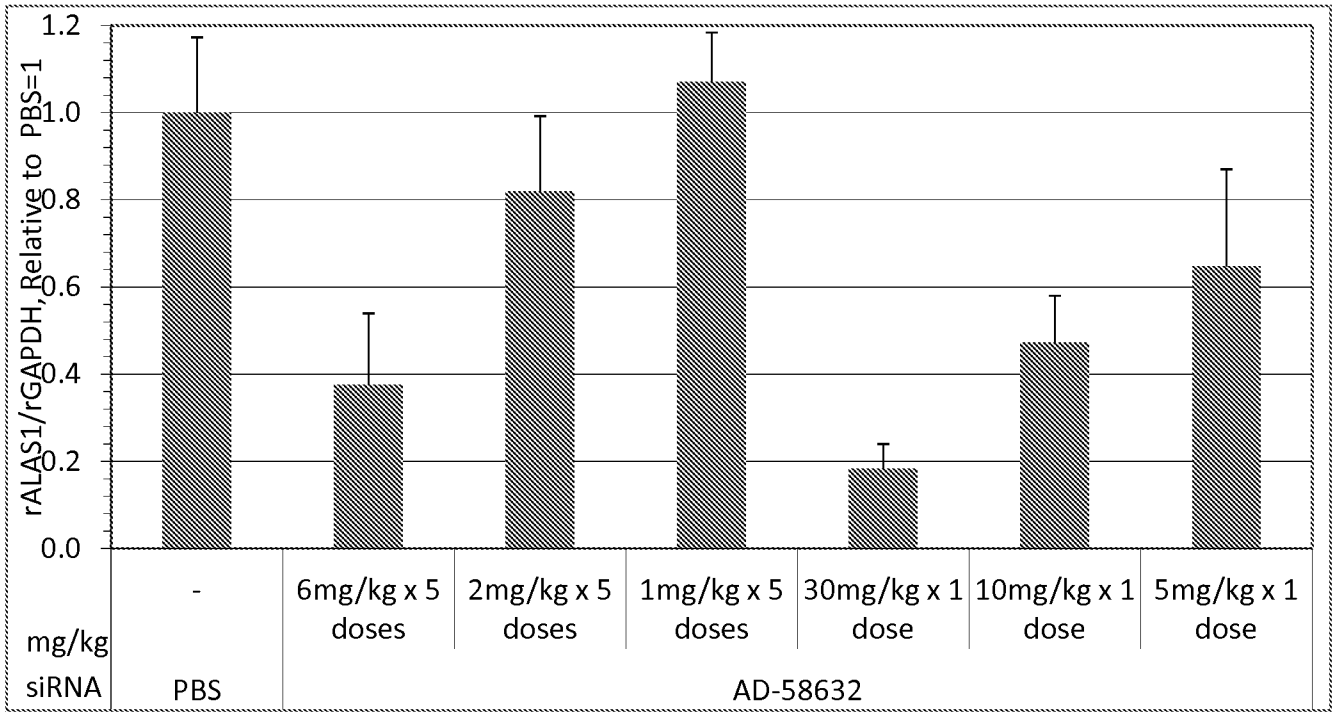

rALAS1/rGAPDH, Relative to PBS=1
1.2
1.0
0.8
0.6
0.4
0.2
0.0
mg/kg
siRNA
-
PBS
6mg/kg x 5 doses
2mg/kg x 5 doses
1mg/kg x 5 doses
30mg/kg x 1 dose
10mg/kg x 1 dose
5mg/kg x 1 dose
AD-58632

FIG. 24

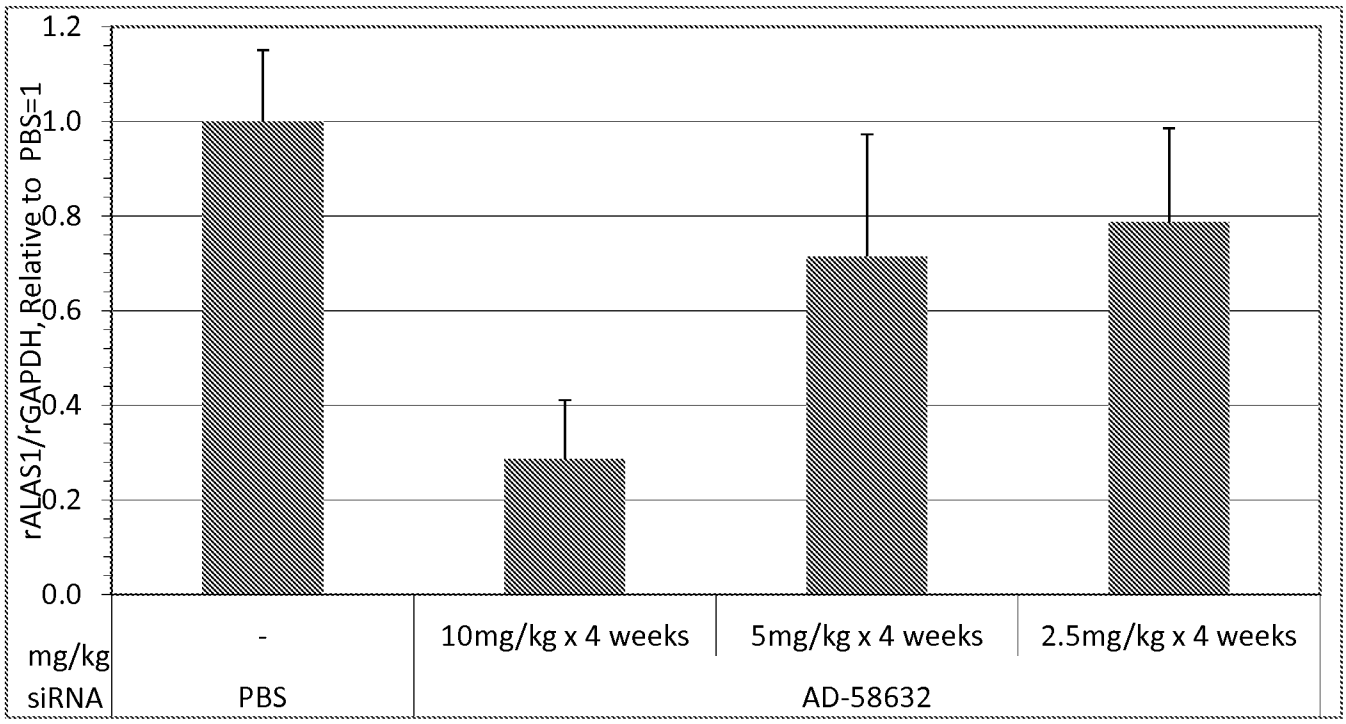

rALAS1/rGAPDH, Relative to PBS=1
1.2
1.0
0.8
0.6
0.4
0.2
0.0
mg/kg
siRNA
-
PBS
10mg/kg x 4 weeks
5mg/kg x 4 weeks
2.5mg/kg x 4 weeks
AD-58632

FIG. 25

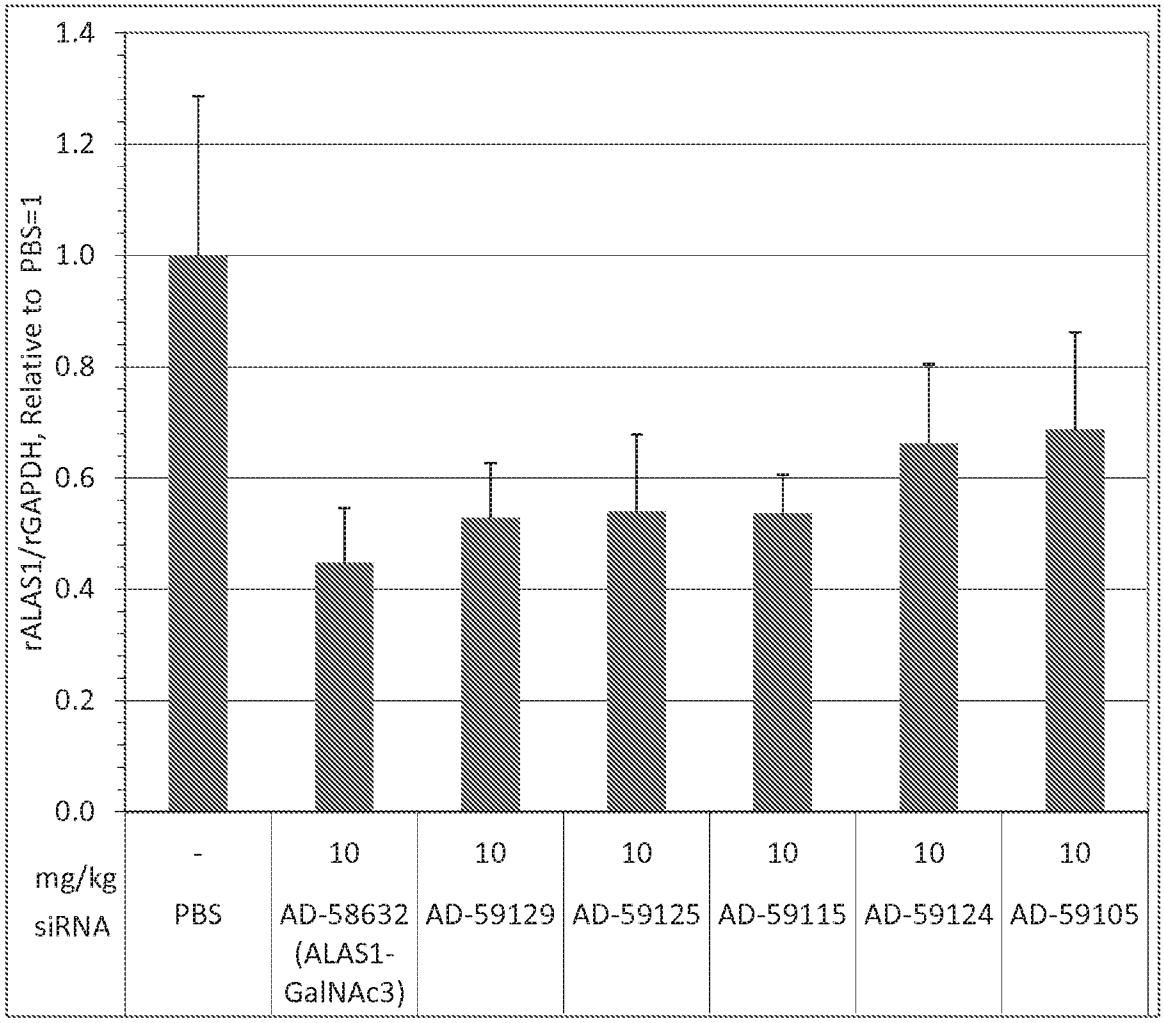
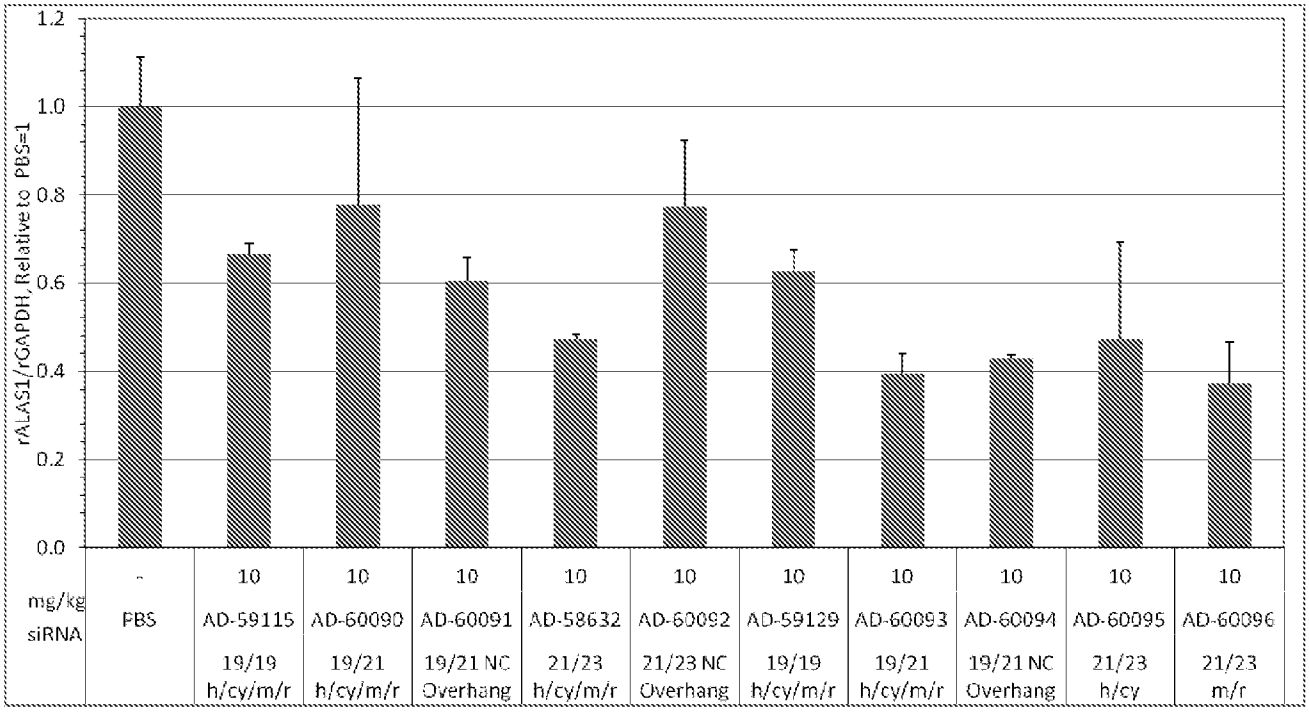

rALAS1/rGAPDH, Relative to PBS=1
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
mg/kg
siRNA
-
PBS
10
AD-58632 (ALAS1-GalNAc3)
10
AD-59129
10
AD-59125
10
AD-59115
10
AD-59124
10
AD-59105

FIG. 26


rALAS1/rGAPDH, Relative to PBS=1
1.2
1.0
0.8
0.6
0.4
0.2
0.0
mg/kg
siRNA
-
PBS
10
AD-59115
19/19
h/cy/m/r
10
AD-60090
19/21
h/cy/m/r
10
AD-60091
19/21 NC
Overhang
10
AD-58632
21/23
h/cy/m/r
10
AD-60092
21/23 NC
Overhang
10
AD-59129
19/19
h/cy/m/r
10
AD-60093
19/21
h/cy/m/r
10
AD-60094
19/21 NC
Overhang
10
AD-60095
21/23
h/cy
10
AD-60096
21/23
m/r

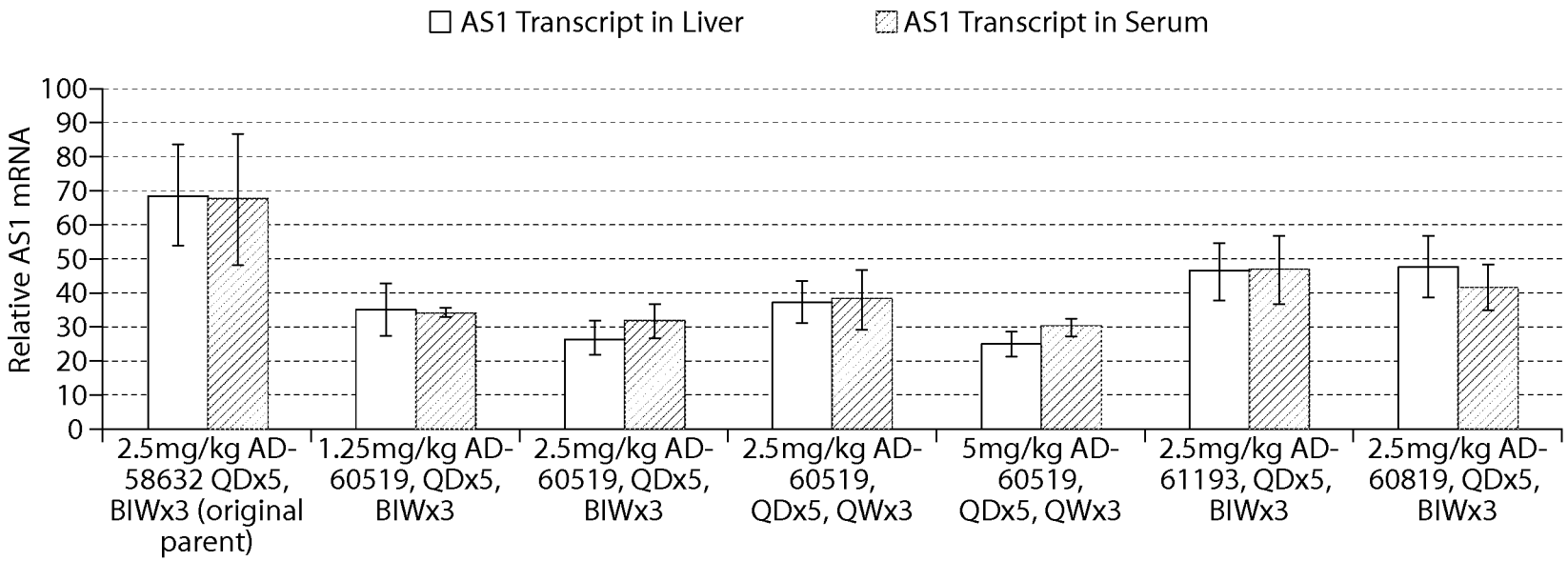

AS1 Transcript in Liver
AS1 Transcript in Serum
Relative AS1 mRNA
100
90
80
70
60
50
40
30
20
10
0
2.5mg/kg AD-58632 QDx5, BIWx3 (original parent)
1.25mg/kg AD-60519, QDx5, BIWx3
2.5mg/kg AD-60519, QDx5, BIWx3
2.5mg/kg AD-60519, QDx5, QWx3
5mg/kg AD-60519, QDx5, QWx3
2.5mg/kg AD-61193, QDx5, BIWx3
2.5mg/kg AD-60819, QDx5, BIWx3

Fig. 27

FIG. 28

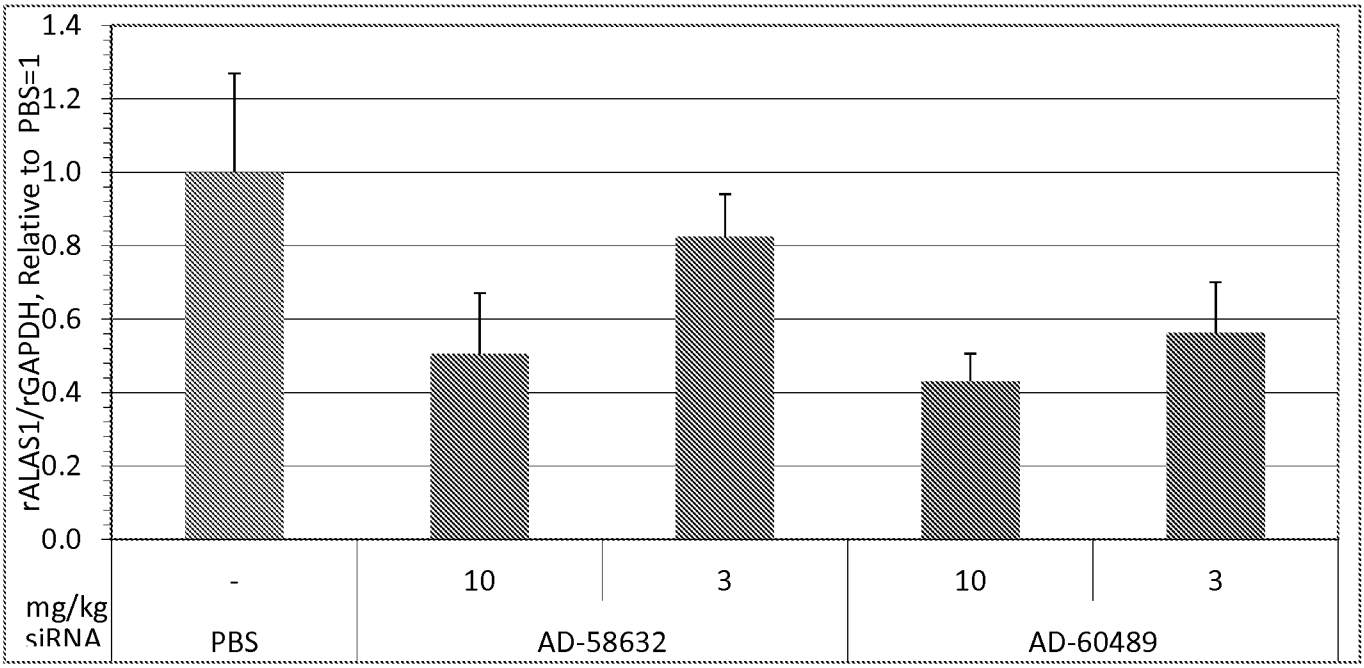
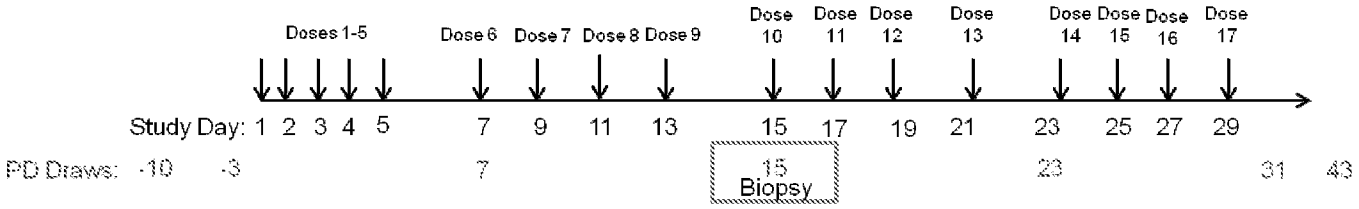

rALAS1/rGAPDH, Relative to PBS=1
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
mg/kg
siRNA
-
10
3
10
3
PBS
AD-58632
AD-60489

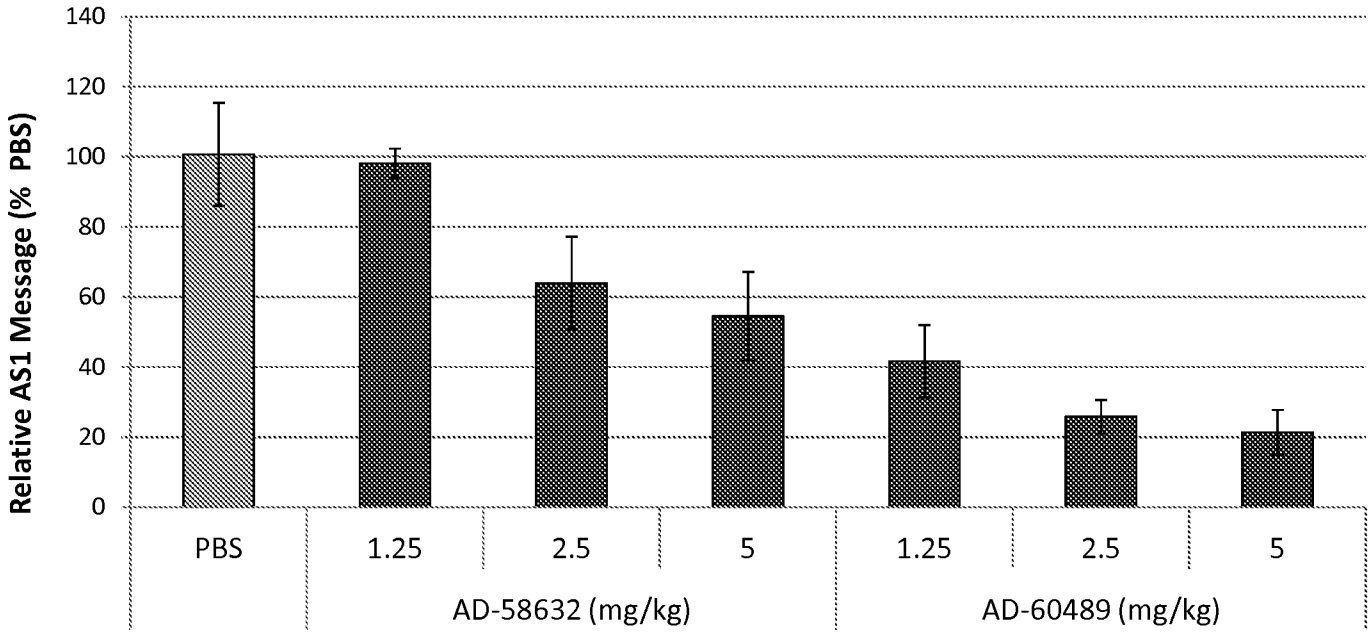
FIG. 29



Group Number	Number of Mates	Test Article	Dose Level (mg/kg)	Dose Conc (mg/mL)	Dose Regimen	Dose Volume (mL/kg)	Dose Route
1	3	PBS	0	0	QDx5 followed by q2d for 3 weeks	2	SC
2	3	AD-58632	5	2.5			
3	3	AD-58632	2.5	1.25			
4	3	AD-58632	1.25	0.625			
5	3	AD-60489	5	2.5			
6	3	AD-60489	2.5	1.25			
7	3	AD-60489	1.25	0.625			

Serum for Exploratory mRNA detection : Days -10, -3, 7*, 15*, 23*, 31, and 43 (*All blood samples collected prior to re-dose)
Liver biopsy for mRNA: Day 15** (Serum collected prior to biopsy)
Serum for Clin Chem Panel (ALT, AST, ALP): Days -3, 6, 30, 43
Necropsy: No
Injection site observation: Yes

FIG. 30


Relative AS1 Message (% PBS)
140
120
100
80
60
40
20
0
PBS
1.25
2.5
5
1.25
2.5
5
AD-58632 (mg/kg)
AD-60489 (mg/kg)

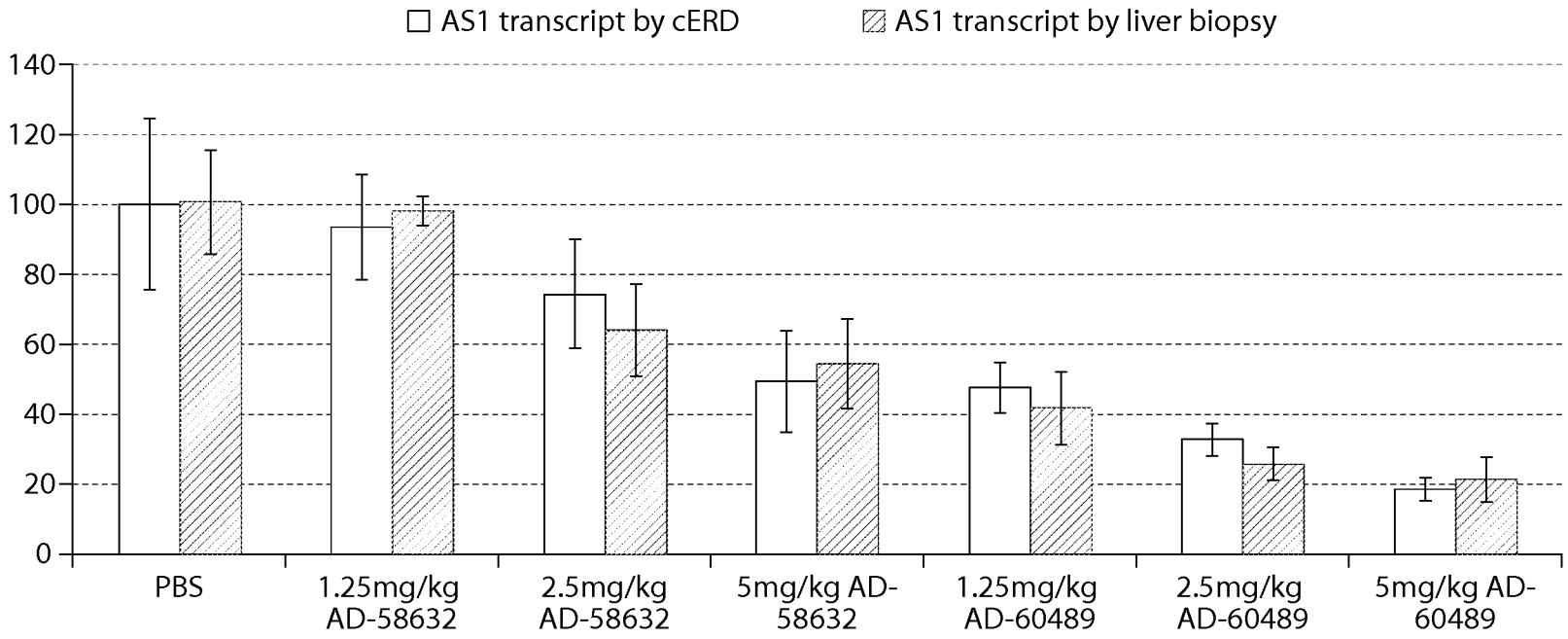
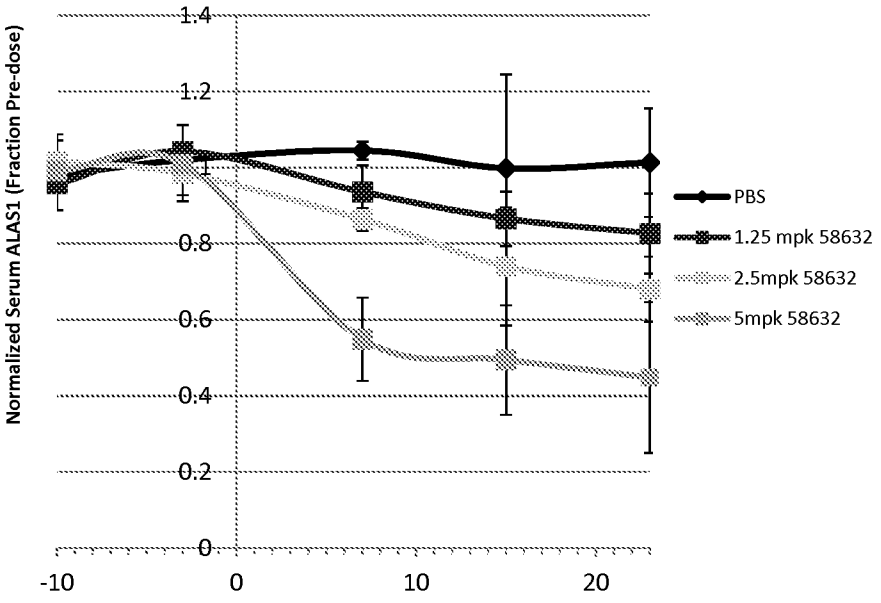

AS1 transcript by cERD
AS1 transcript by liver biopsy
140
120
100
80
60
40
20
0
PBS
1.25mg/kg AD-58632
2.5mg/kg AD-58632
5mg/kg AD-58632
1.25mg/kg AD-60489
2.5mg/kg AD-60489
5mg/kg AD-60489

Fig. 31

FIG. 32


Normalized Serum ALAS1 (Fraction Pre-dose)
1.4
1.2
0.8
0.6
0.4
0.2
0
-10
0
10
20
PBS
1.25 mpk 58632
2.5mpk 58632
5mpk 58632

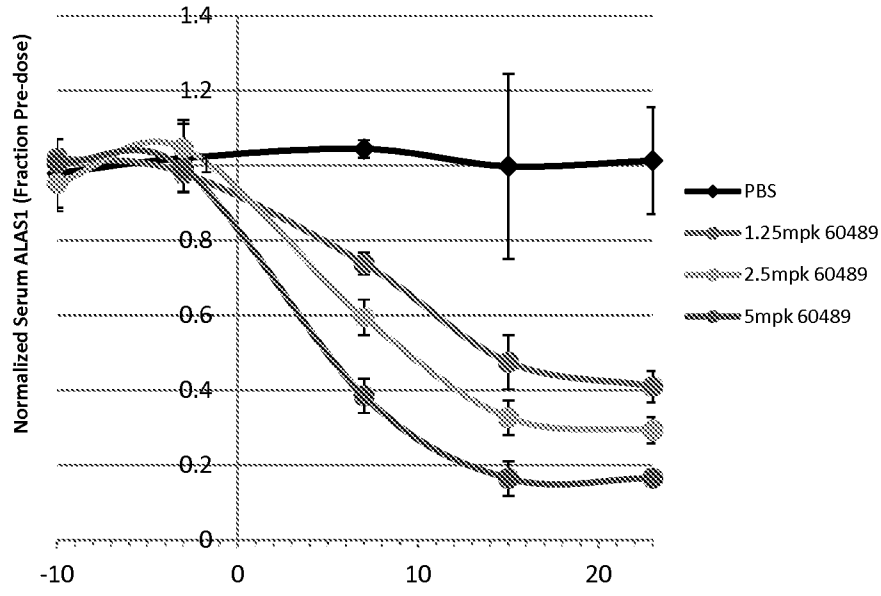

Normalized Serum ALAS1 (Fraction Pre-dose)
1.4
1.2
0.8
0.6
0.4
0.2
0
-10
0
10
20
PBS
1.25mpk 60489
2.5mpk 60489
5mpk 60489

FIG. 33

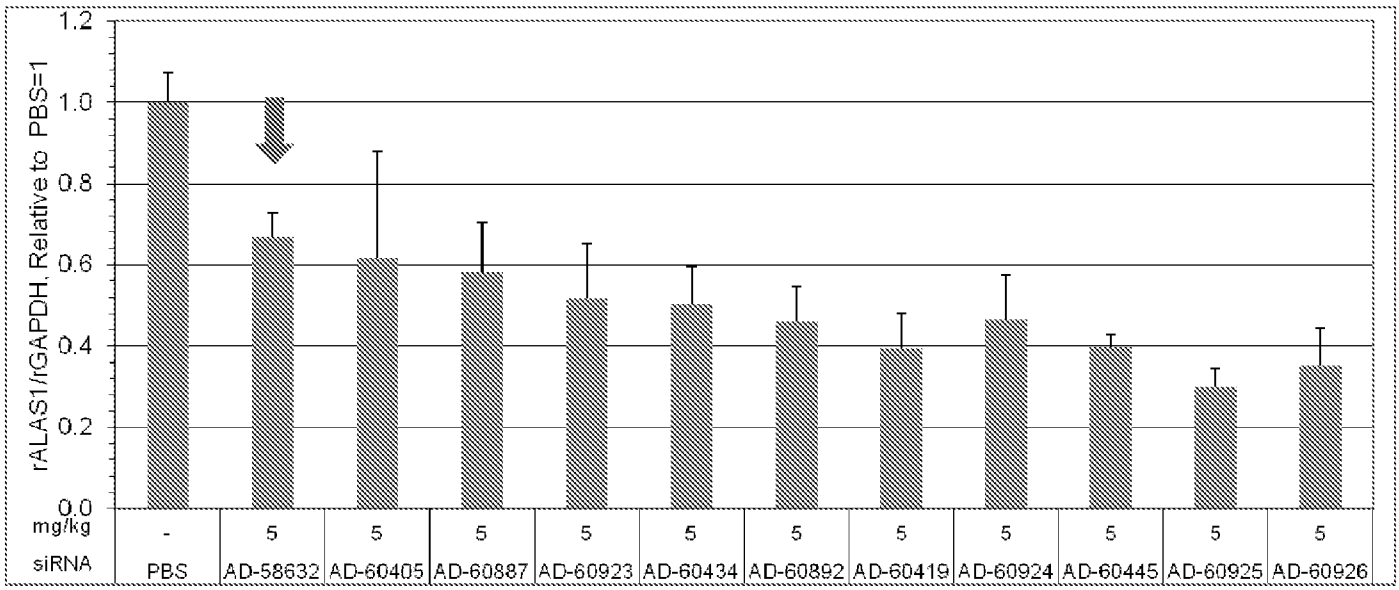

rALAS1/rGAPDH, Relative to PBS=1
1.2
1.0
0.8
0.6
0.4
0.2
0.0
mg/kg
siRNA
- 5 5 5 5 5 5 5 5 5 5 5
PBS AD-58632 AD-60405 AD-60887 AD-60923 AD-60434 AD-60892 AD-60419 AD-60924 AD-60445 AD-60925 AD-60926

FIG. 34

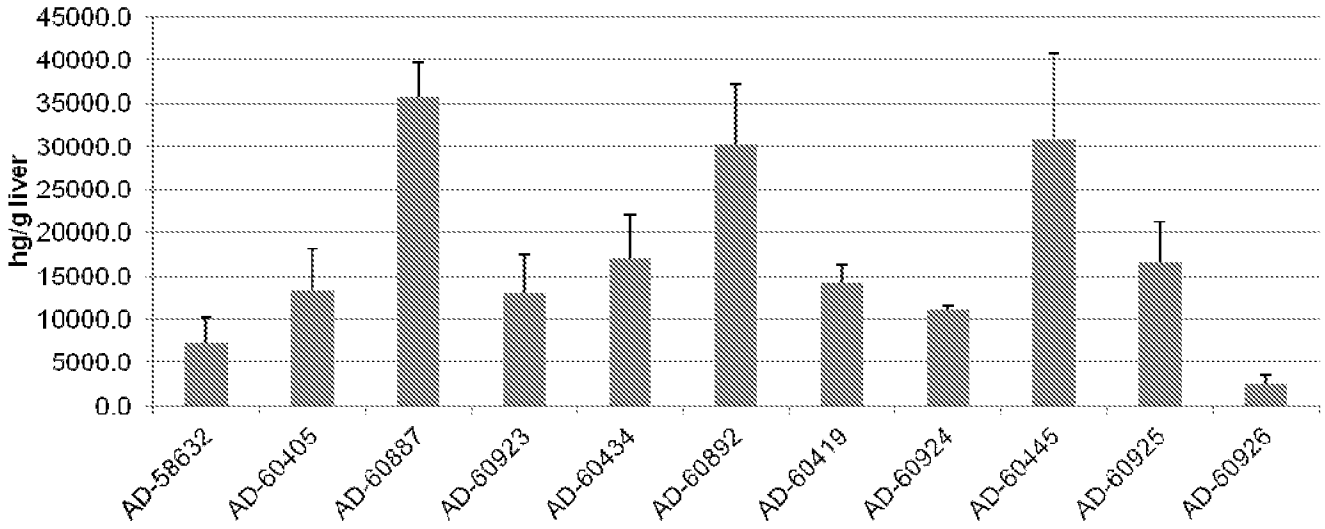
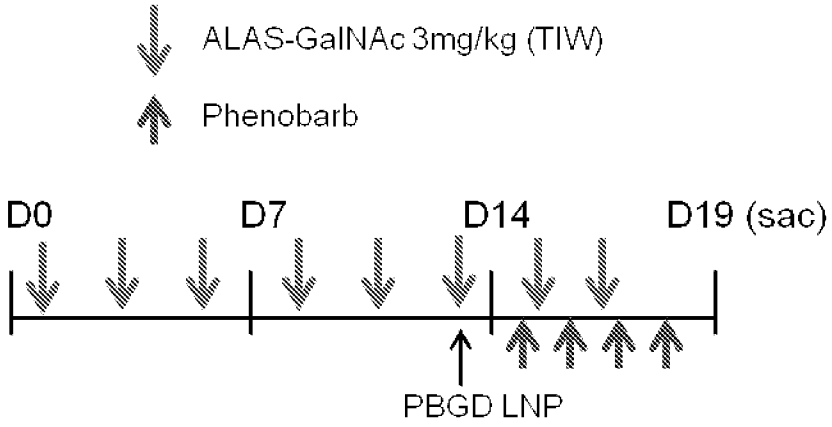

45000.0
40000.0
35000.0
30000.0
25000.0
20000.0
15000.0
10000.0
5000.0
0.0
hg/g liver
AD-58632
AD-60405
AD-60887
AD-60923
AD-60434
AD-60892
AD-60419
AD-60924
AD-60445
AD-60925
AD-60926

FIG. 35


ALAS-GalNAc 3mg/kg (TIW)
Phenobarb
D0
D7
D14
D19 (sac)
PBGD LNP

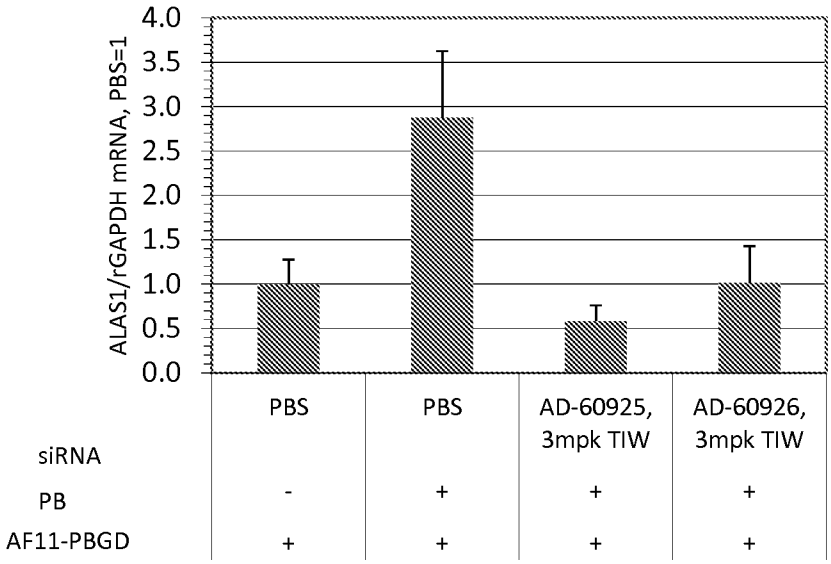
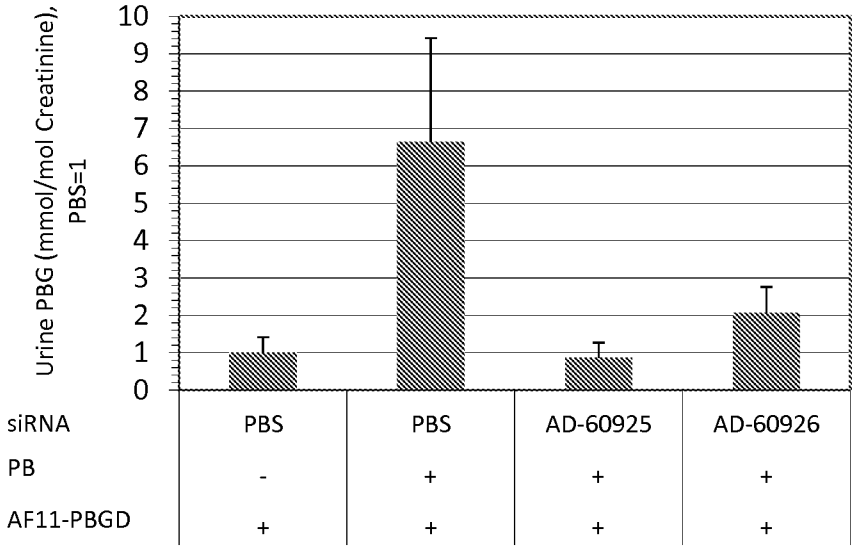

ALAS1/rGAPDH mRNA, PBS=1
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
PBS
PBS
AD-60925, 3mpk TIW
AD-60926, 3mpk TIW
siRNA
PB
AF11-PBGD
-
+
+
+
+
+
+
+

FIG. 36


Urine PBG (mmol/mol Creatinine), PBS=1
10
9
8
7
6
5
4
3
2
1
0
siRNA
PB
AF11-PBGD
PBS
PBS
AD-60925
AD-60926
-
+
+
+
+
+
+
+

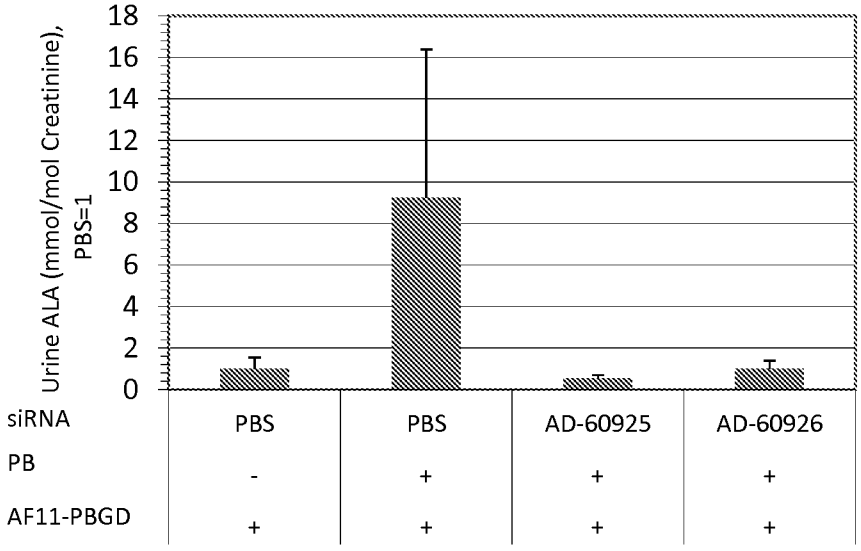
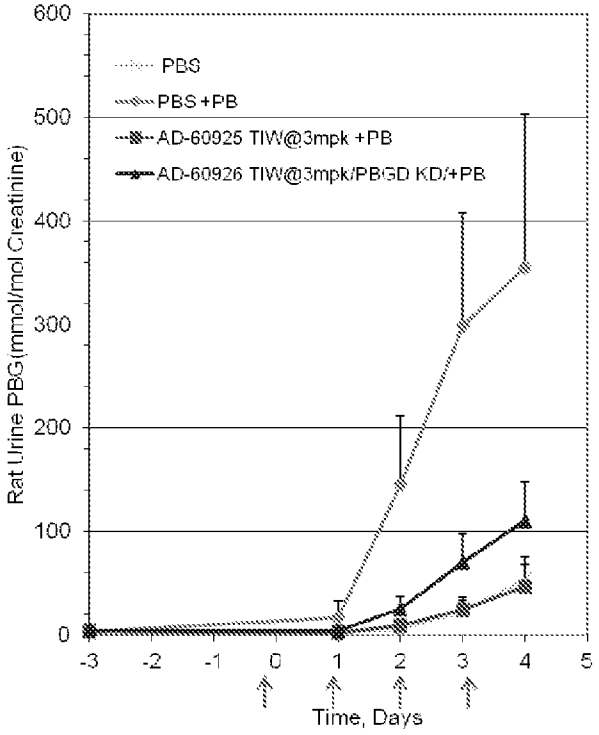

Urine ALA (mmol/mol Creatinine), PBS=1
18
16
14
12
10
8
6
4
2
0
siRNA
PB
AF11-PBGD
PBS
PBS
AD-60925
AD-60926
-
+
+
+
+
+
+
+

FIG. 37


600
500
400
300
200
100
0
PBS
PBS+PB
AD-60925 TIW@3mpk +PB
AD-60926 TIW@3mpk/PBGD KD/+PB
Rat Urine PBG(mmol/mol Creatinine)
-3
-2
-1
0
1
2
3
4
5
Time, Days

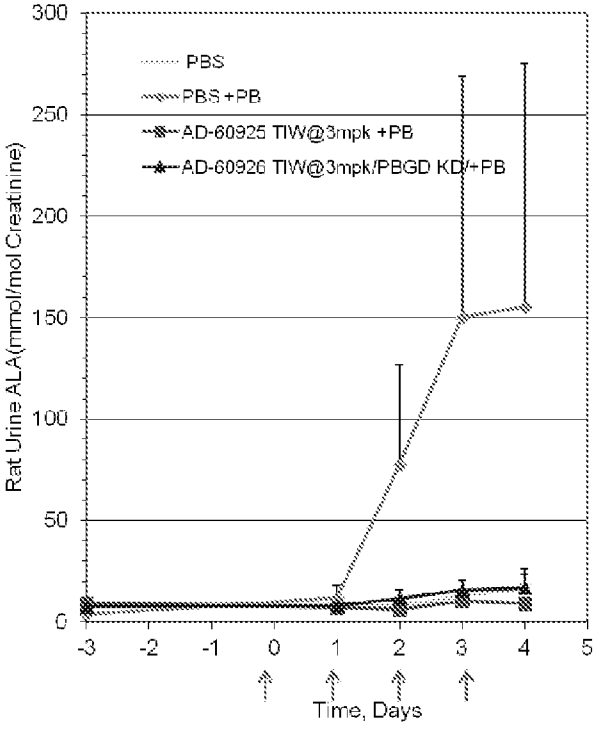

300
250
200
150
100
50
0
PBS
PBS+PB
AD-60925 TIW@3mpk +PB
AD-60926 TIW@3mpk/PBGD KD/+PB
Rat Urine ALA(mmol/mol Creatinine)
-3
-2
-1
0
1
2
3
4
5
Time, Days

FIG. 38

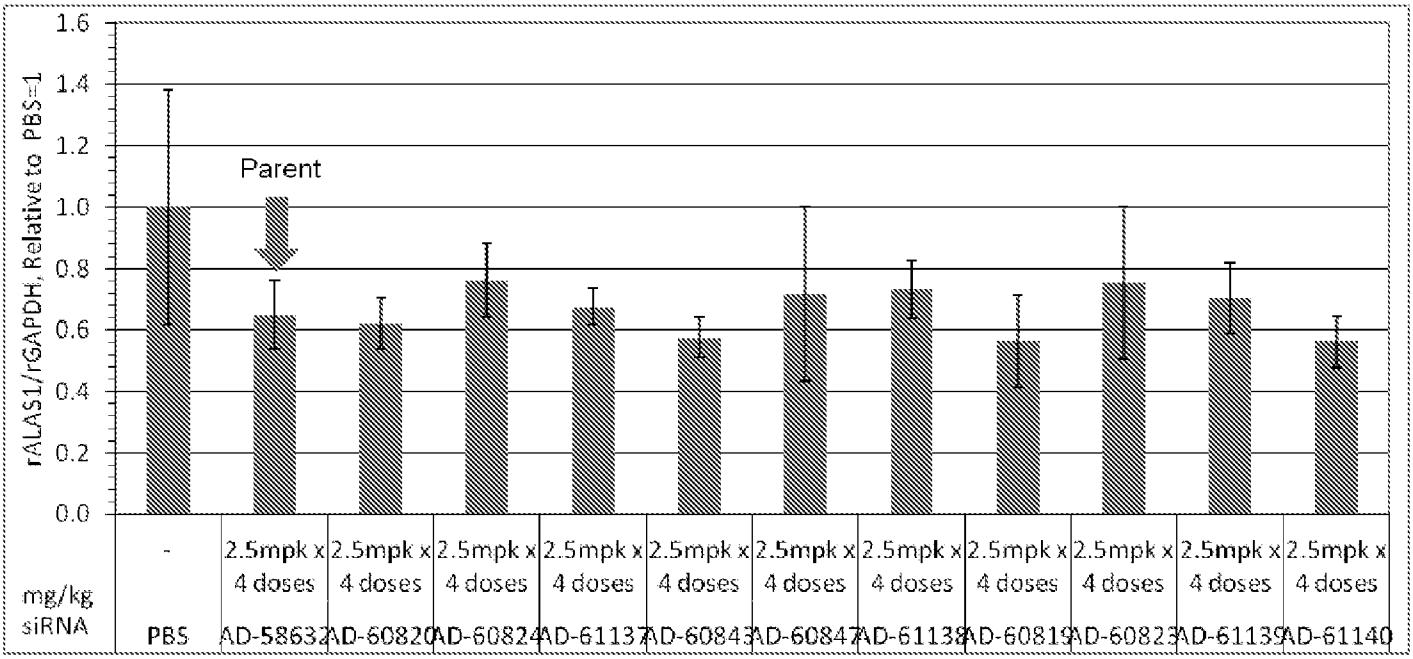

rALAS1/rGAPDH, Relative to PBS=1
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Parent
mg/kg
siRNA
-
2.5mpk x 4 doses
PBS
AD-58632
AD-60820
AD-60824
AD-61137
AD-60843
AD-60847
AD-61138
AD-60819
AD-60823
AD-61139
AD-61140

FIG. 39

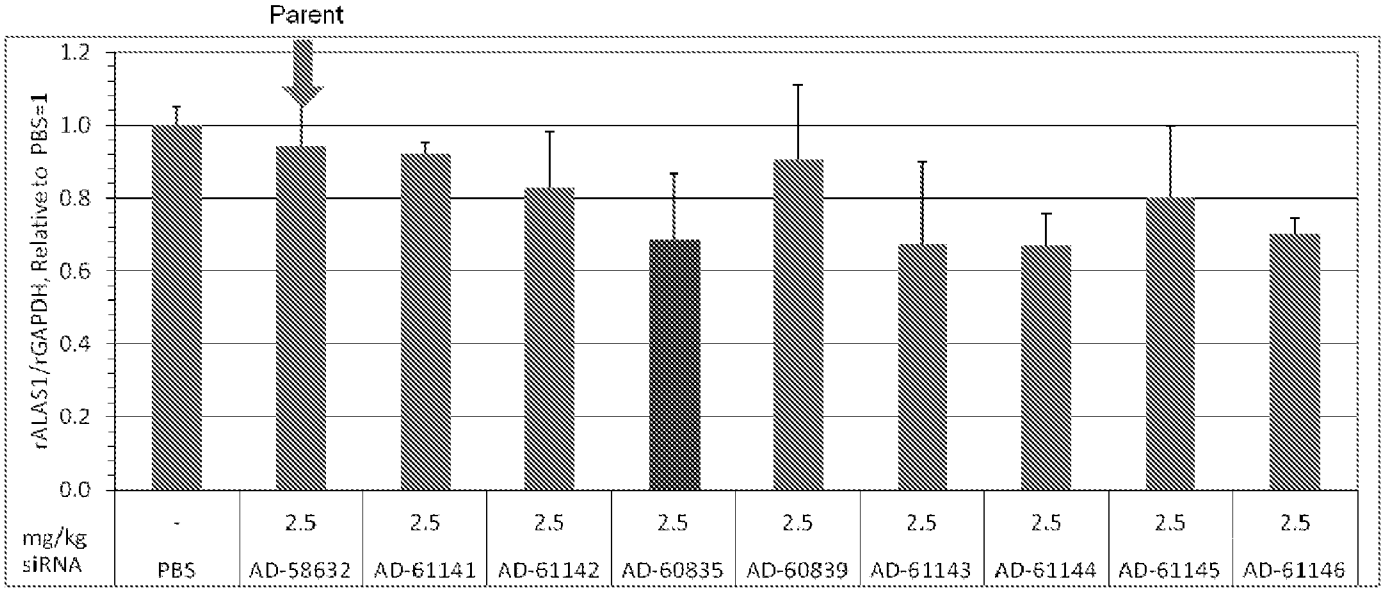
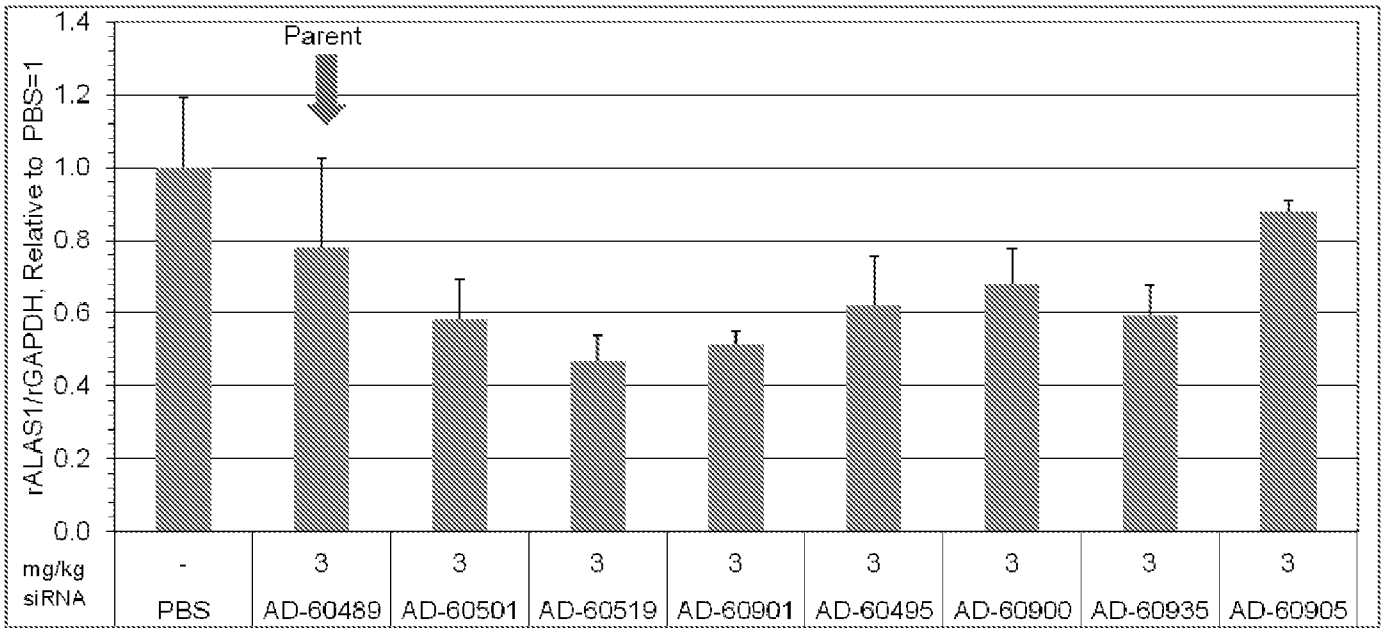

Parent
rALAS1/rGAPDH, Relative to PBS=1
1.2
1.0
0.8
0.6
0.4
0.2
0.0
mg/kg
siRNA
-
2.5
PBS
AD-58632
AD-61141
AD-61142
AD-60835
AD-60839
AD-61143
AD-61144
AD-61145
AD-61146

FIG. 40


rALAS1/rGAPDH, Relative to PBS=1
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Parent
mg/kg
siRNA
-
PBS
3
AD-60489
3
AD-60501
3
AD-60519
3
AD-60901
3
AD-60495
3
AD-60900
3
AD-60935
3
AD-60905

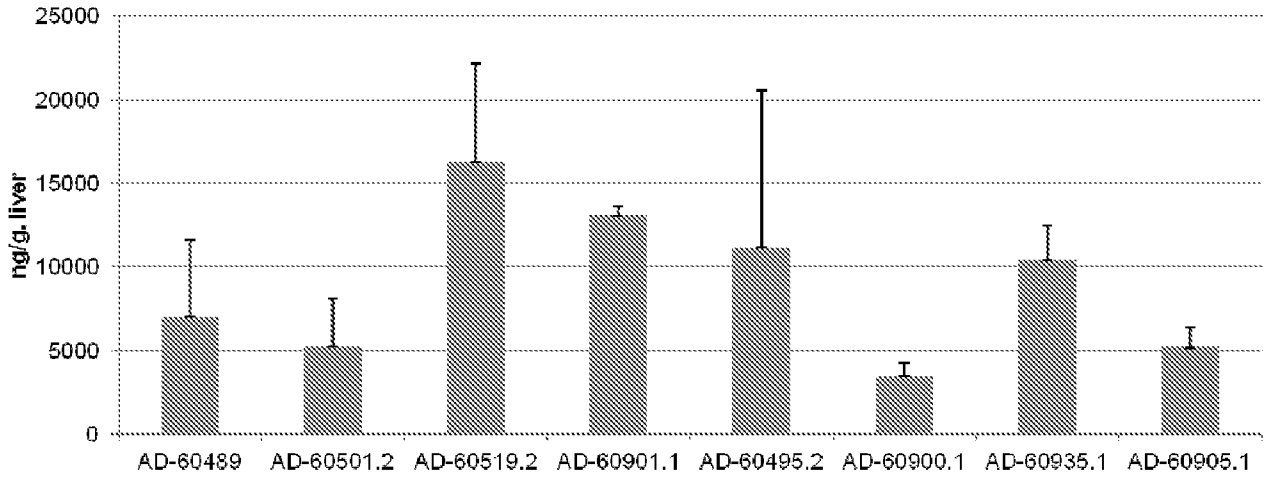
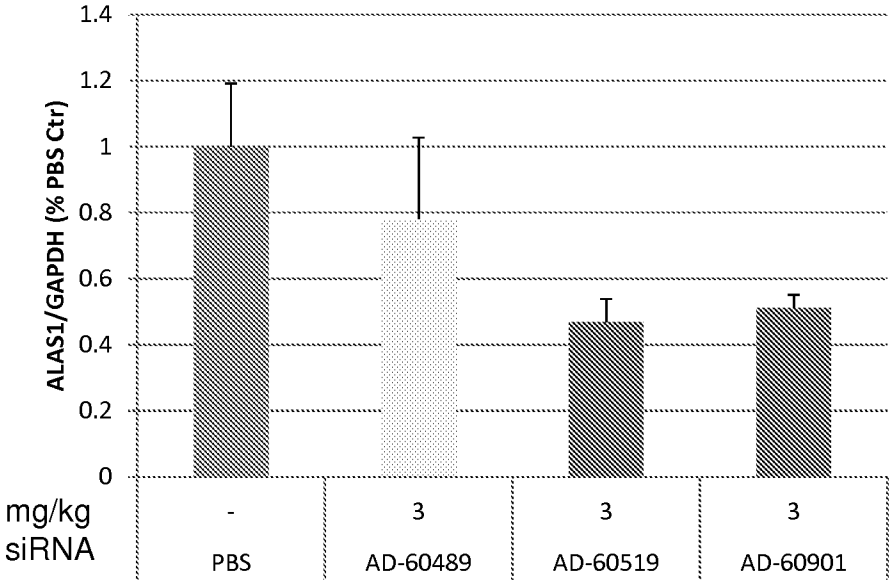

ng/g. liver
25000
20000
15000
10000
5000
0
AD-60489
AD-60501.2
AD-60519.2
AD-60901.1
AD-60495.2
AD-60900.1
AD-60935.1
AD-60905.1

FIG. 41


ALAS1/GAPDH (% PBS Ctr)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
mg/kg
siRNA
-
3
3
3
PBS
AD-60489
AD-60519
AD-60901

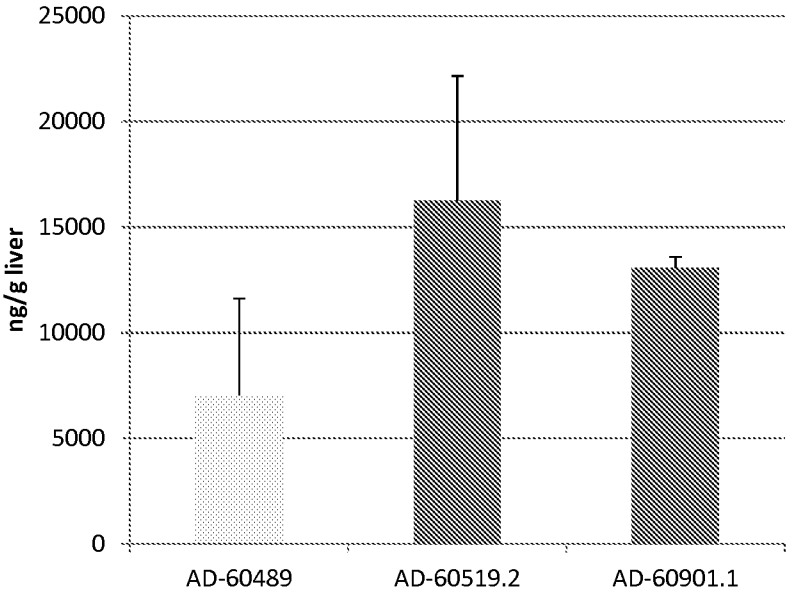

ng/g liver
25000
20000
15000
10000
5000
0
AD-60489
AD-60519.2
AD-60901.1

FIG. 42

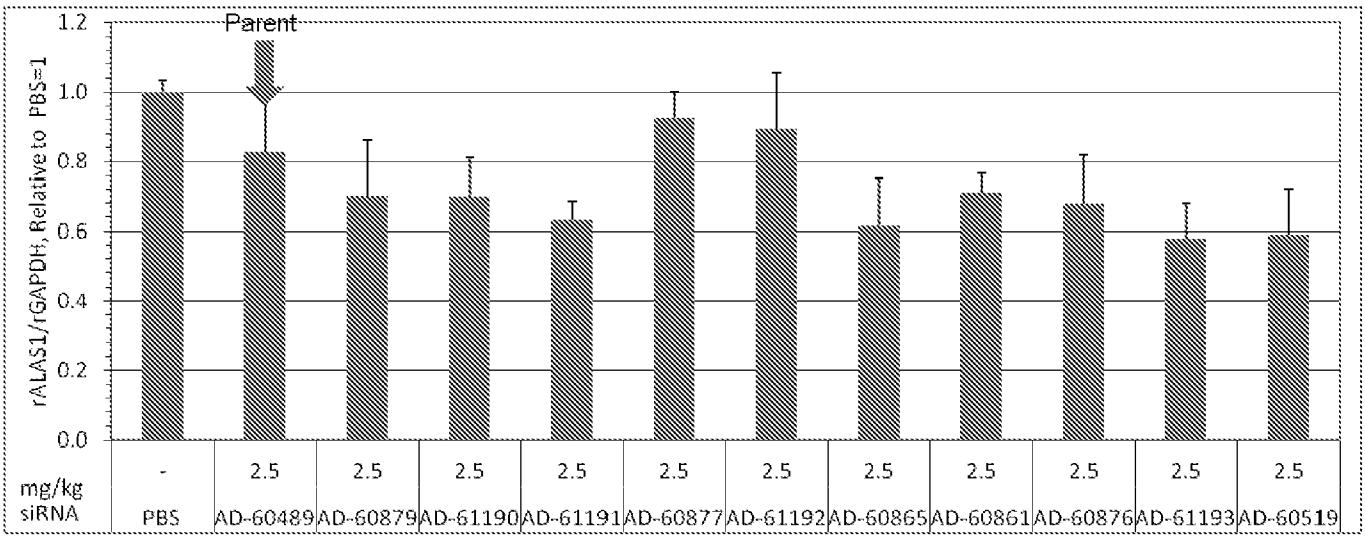

Parent
rALAS1/rGAPDH, Relative to PBS=1
1.2
1.0
0.8
0.6
0.4
0.2
0.0
mg/kg
siRNA
-
PBS
2.5
AD-60489
2.5
AD-60879
2.5
AD-61190
2.5
AD-61191
2.5
AD-60877
2.5
AD-61192
2.5
AD-60865
2.5
AD-60861
2.5
AD-60876
2.5
AD-61193
2.5
AD-60519

FIG. 43

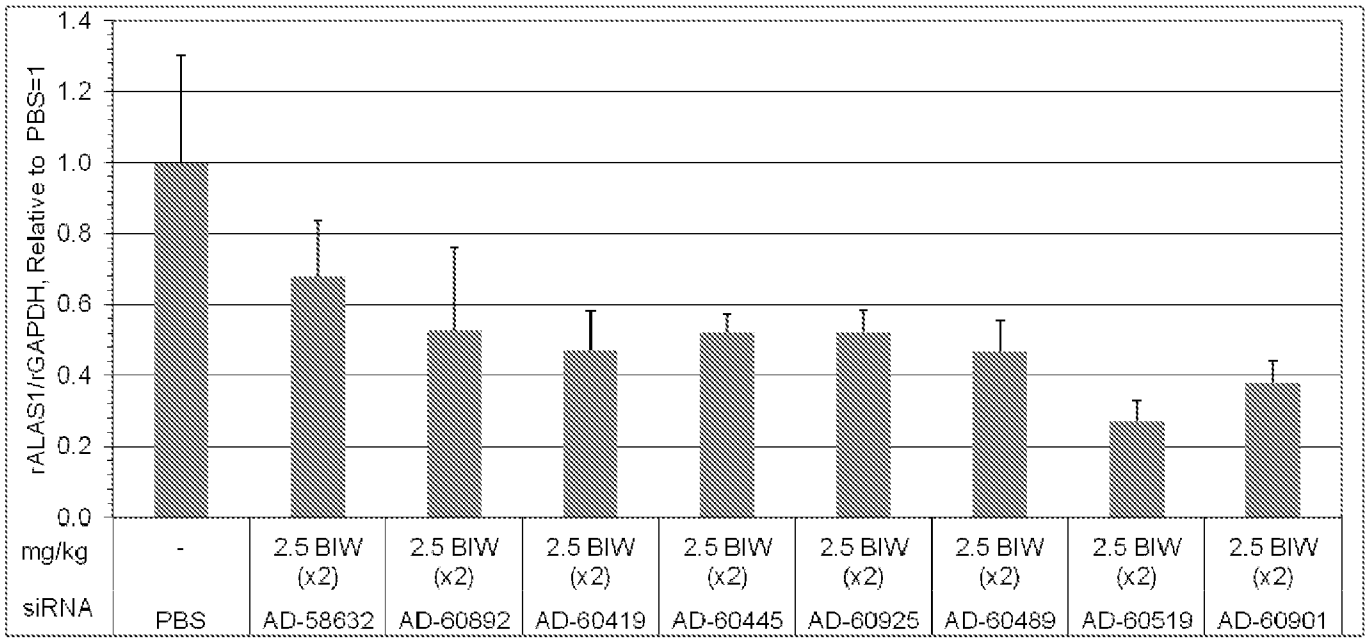

rALAS1/rGAPDH, Relative to PBS=1
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
mg/kg
siRNA
-
PBS
2.5 BIW (x2)
AD-58632
2.5 BIW (x2)
AD-60892
2.5 BIW (x2)
AD-60419
2.5 BIW (x2)
AD-60445
2.5 BIW (x2)
AD-60925
2.5 BIW (x2)
AD-60489
2.5 BIW (x2)
AD-60519
2.5 BIW (x2)
AD-60901

FIG. 44

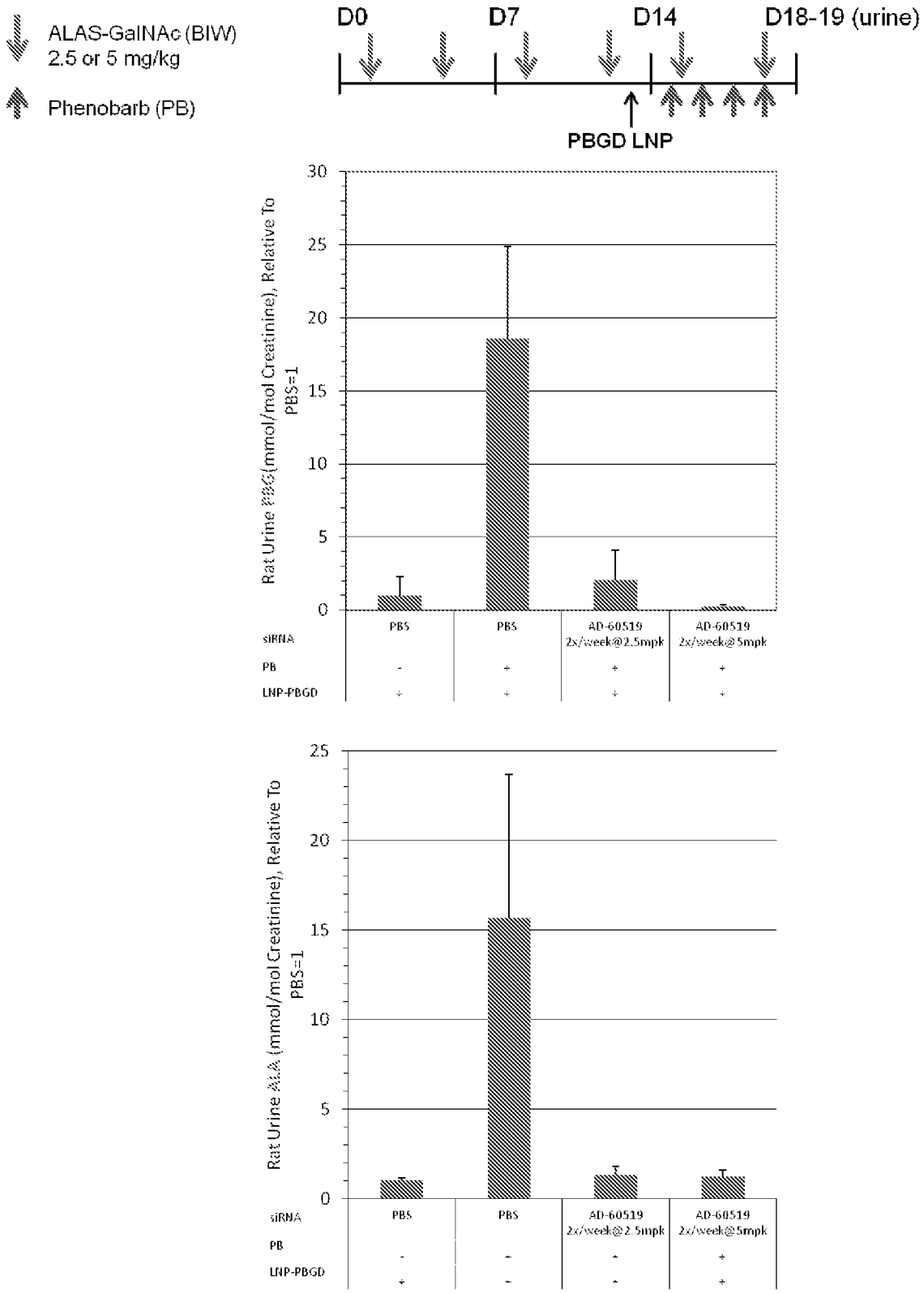
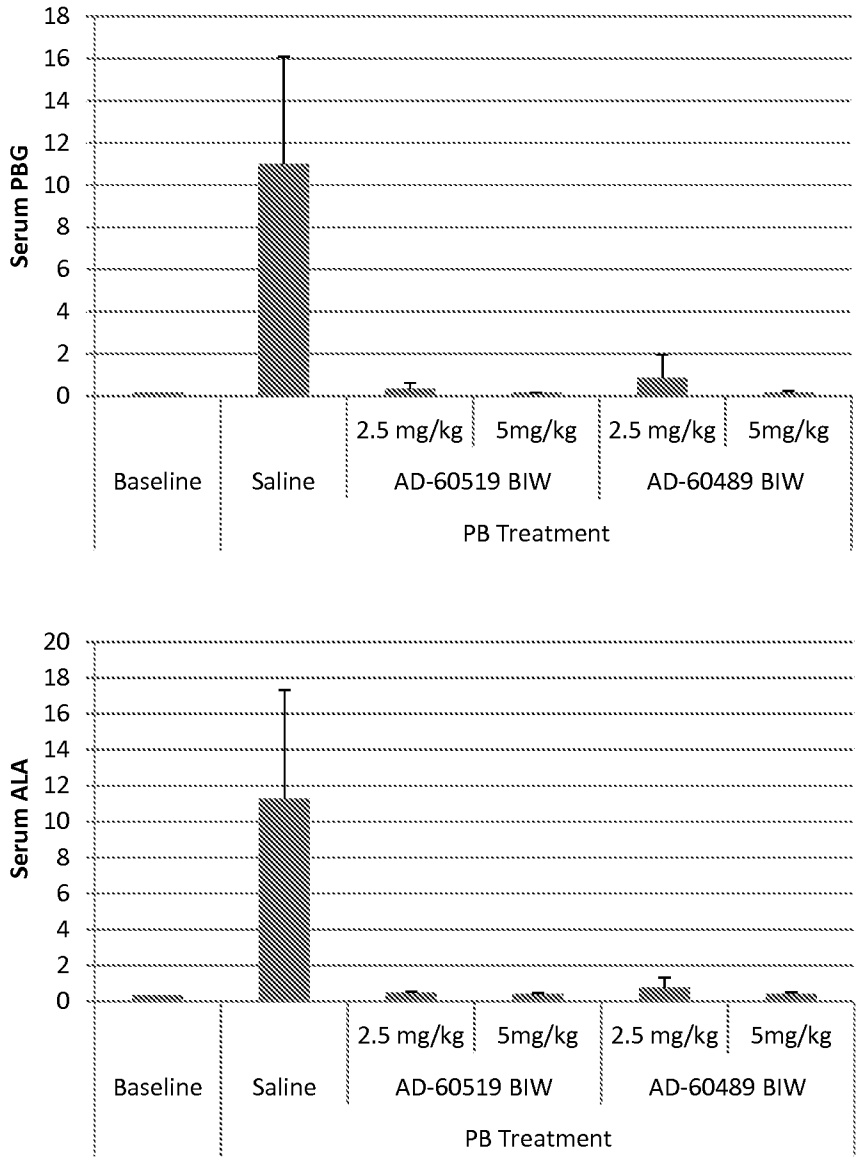

ALAS-GalNAc (BIW)
2.5 or 5 mg/kg
Phenobarb (PB)
D0
D7
D14
D18-19 (urine)
PBGD LNP
Rat Urine PBG (mmol/mol Creatinine), Relative To PBS=1
30
25
20
15
10
5
0
siRNA
PB
LNP-PBGD
PBS
PBS
AD-60519 2x/week@2.5mpk
AD-60519 2x/week@5mpk
Rat Urine ALA (mmol/mol Creatinine), Relative To PBS=1
25
20
15
10
5
0
siRNA
PB
LNP-PBGD
PBS
PBS
AD-60519 2x/week@2.5mpk
AD-60519 2x/week@5mpk

FIG. 45


Serum PBG
18
16
14
12
10
8
6
4
2
0
Baseline
Saline
2.5 mg/kg
5mg/kg
AD-60519 BIW
2.5 mg/kg
5mg/kg
AD-60489 BIW
PB Treatment

Serum ALA
20
18
16
14
12
10
8
6
4
2
0
Baseline
Saline
2.5 mg/kg
5mg/kg
AD-60519 BIW
2.5 mg/kg
5mg/kg
AD-60489 BIW
PB Treatment

FIG. 46

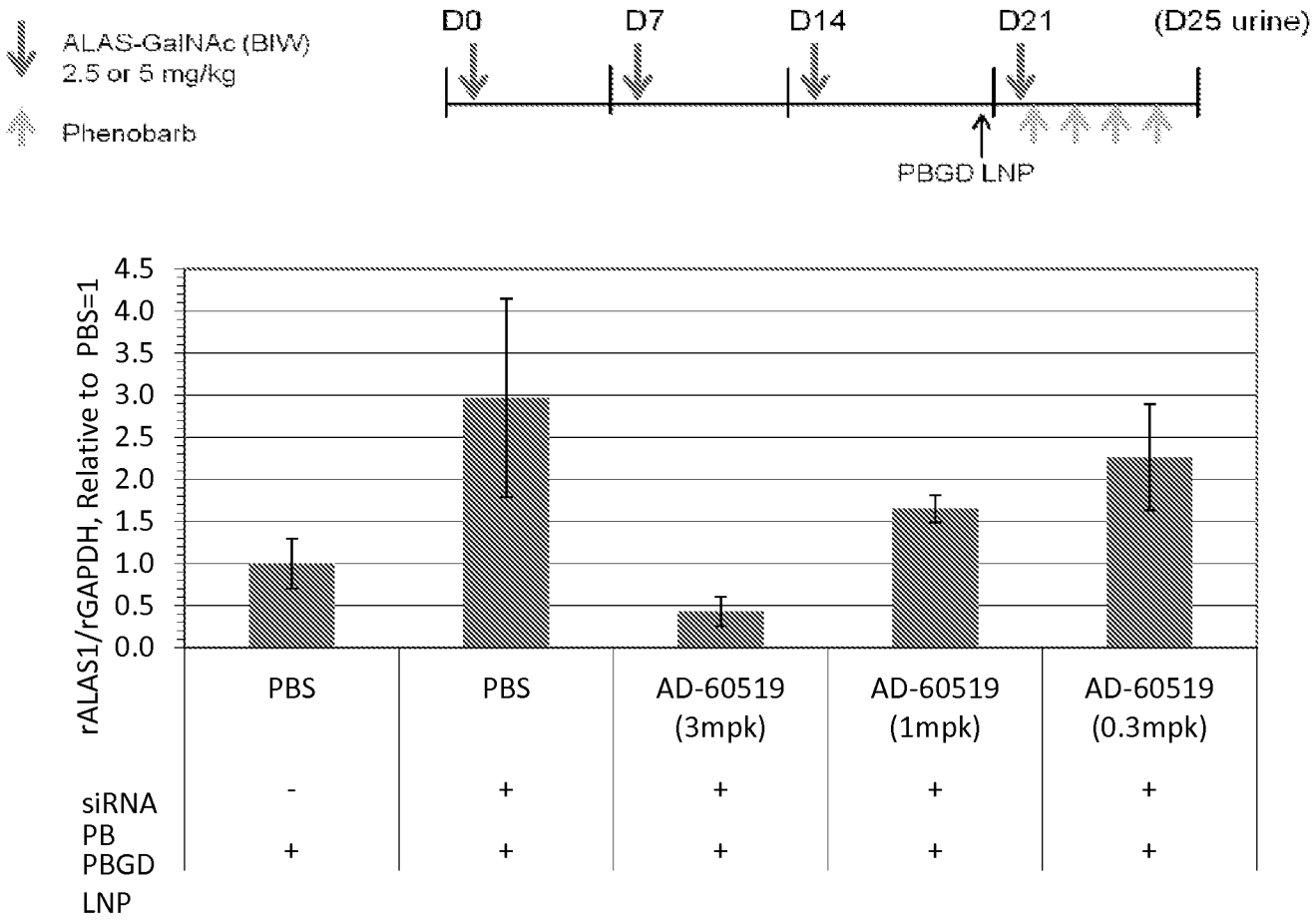
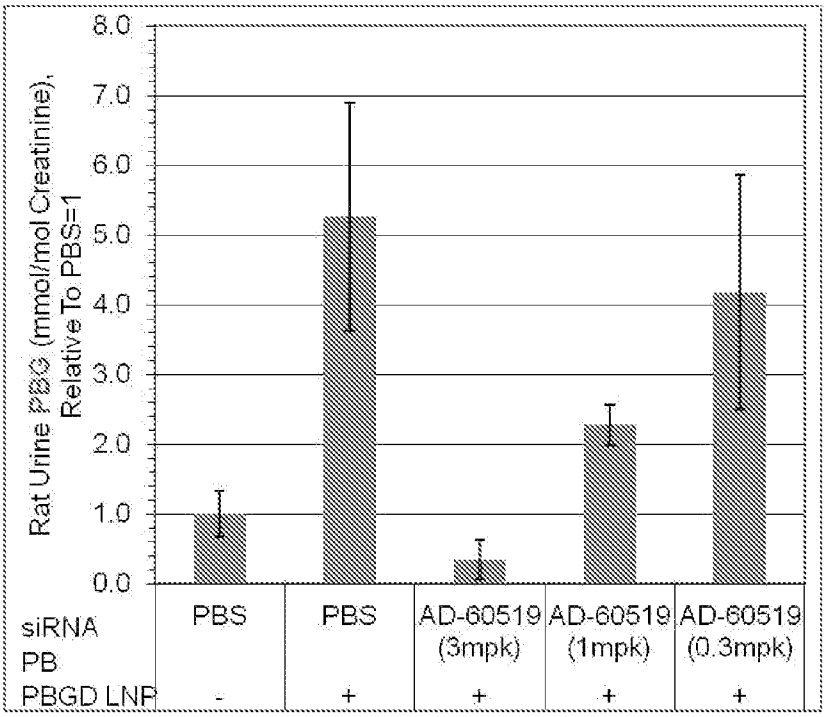

ALAS-GalNAc (BIW)
2.5 or 5 mg/kg
Phenobarb
D0
D7
D14
D21
(D25 urine)
PBGD LNP
rALAS1/rGAPDH, Relative to PBS=1
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
PBS
PBS
AD-60519 (3mpk)
AD-60519 (1mpk)
AD-60519 (0.3mpk)
siRNA
PB
PBGD
LNP
-
+
+
+
+
+
+
+
+
+

FIG. 47


Rat Urine PBG (mmol/mol Creatinine), Relative To PBS=1
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
siRNA
PB
PBGD LNP
PBS
PBS
AD-60519 (3mpk)
AD-60519 (1mpk)
AD-60519 (0.3mpk)
-
+
+
+
+

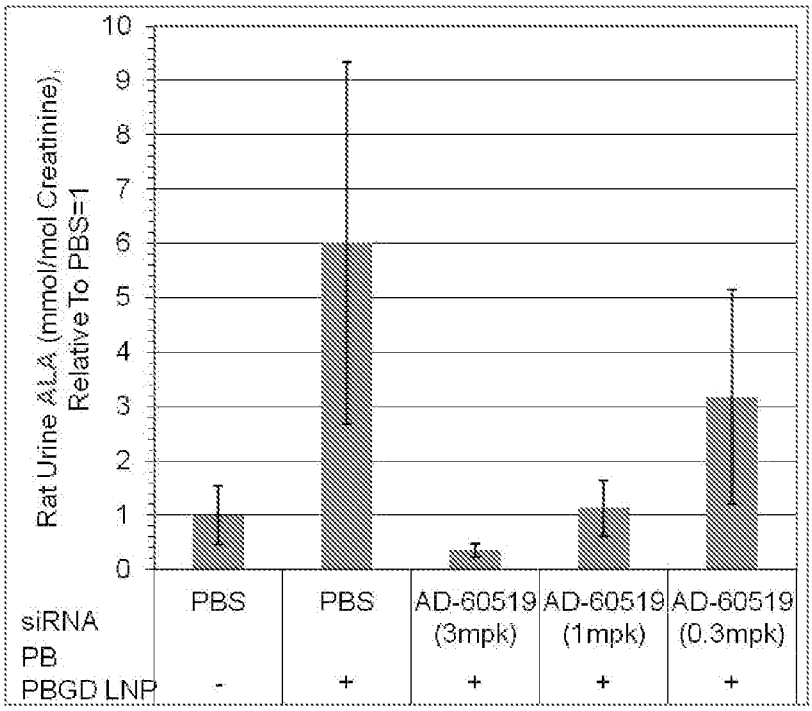

Rat Urine ALA (mmol/mol Creatinine), Relative To PBS=1
10
9
8
7
6
5
4
3
2
1
0
siRNA
PB
PBGD LNP
PBS
PBS
AD-60519 (3mpk)
AD-60519 (1mpk)
AD-60519 (0.3mpk)
-
+
+
+
+

FIG. 48

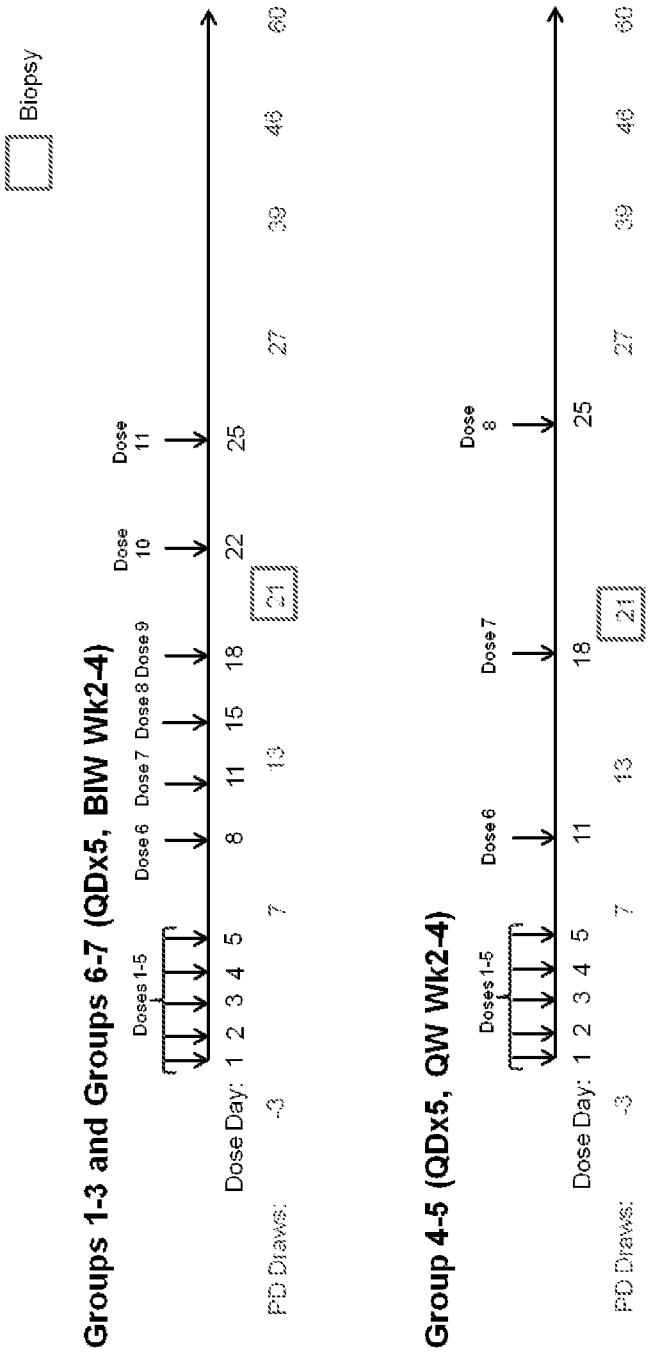
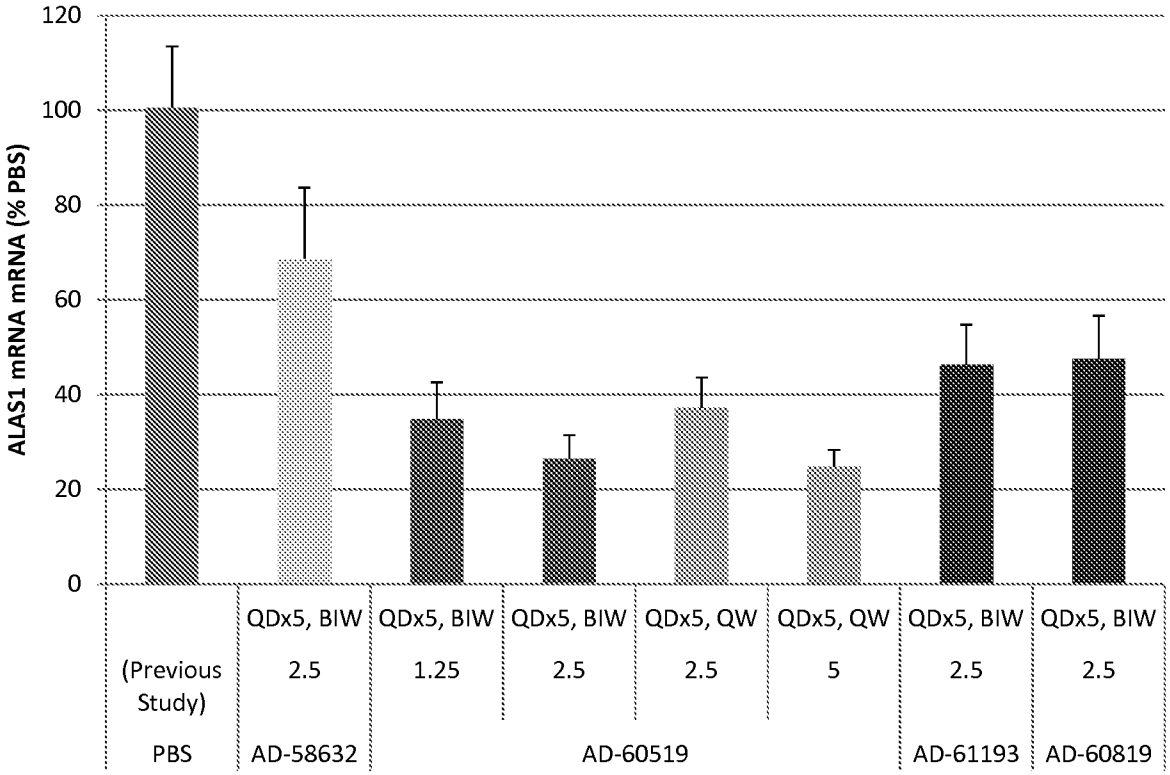

Biopsy
Groups 1-3 and Groups 6-7 (QDx5, BIW Wk2-4)
Doses 1-5
Dose 6
Dose 7
Dose 8
Dose 9
Dose 10
Dose 11
Dose Day: 1 2 3 4 5 8 11 15 18 22 25
PD Draws: -3 7 13 21 27 39 46 60
Group 4-5 (QDx5, QW Wk2-4)
Doses 1-5
Dose 6
Dose 7
Dose 8
Dose Day: 1 2 3 4 5 11 18 25
PD Draws: -3 7 13 21 27 39 46 60

FIG. 49


ALAS1 mRNA mRNA (% PBS)
120
100
80
60
40
20
0
(Previous Study)
PBS
QDx5, BIW
2.5
AD-58632
QDx5, BIW
1.25
QDx5, BIW
2.5
QDx5, QW
2.5
QDx5, QW
5
AD-60519
QDx5, BIW
2.5
AD-61193
QDx5, BIW
2.5
AD-60819

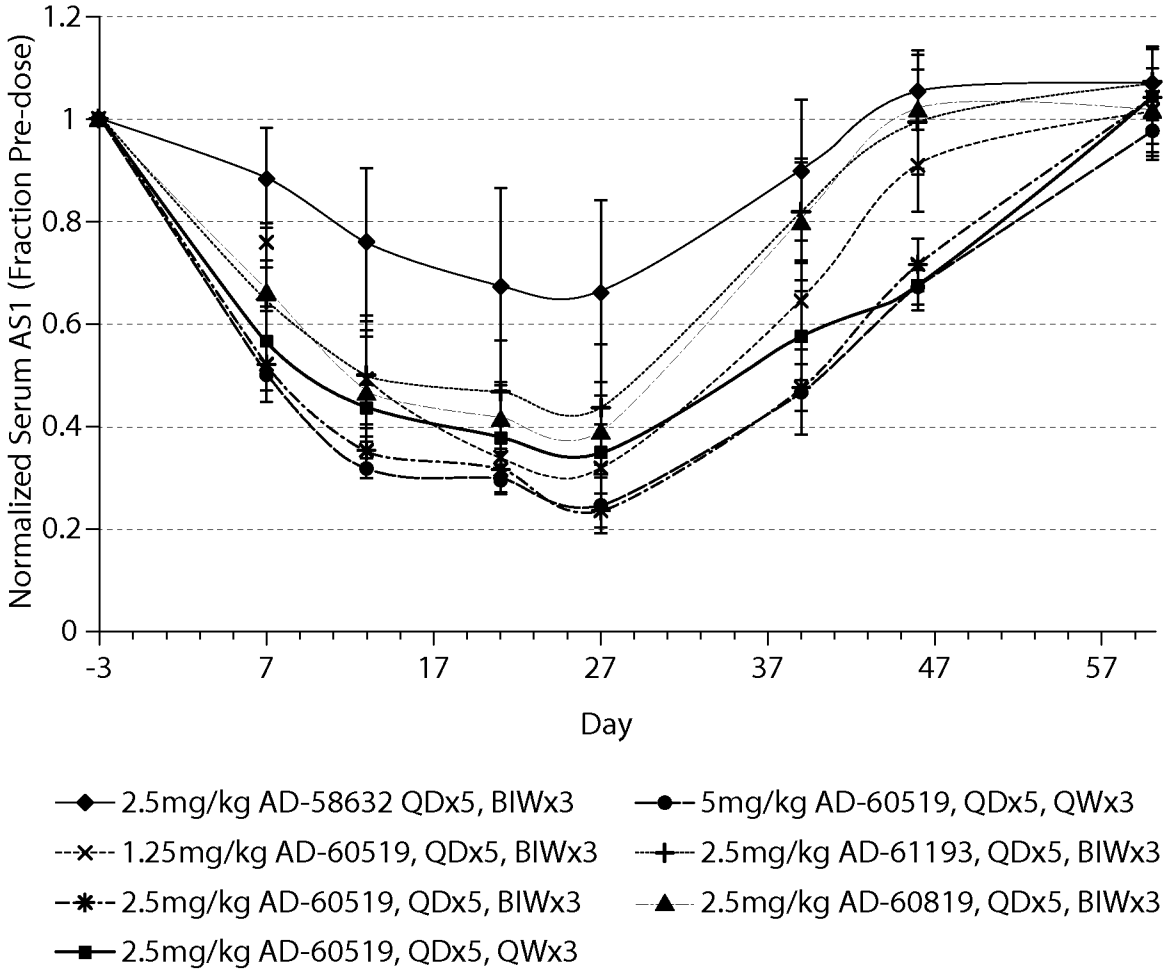

Normalized Serum AS1 (Fraction Pre-dose)
1.2
1
0.8
0.6
0.4
0.2
0
-3
7
17
27
37
47
57
Day
2.5mg/kg AD-58632 QDx5, BIWx3
5mg/kg AD-60519, QDx5, QWx3
1.25mg/kg AD-60519, QDx5, BIWx3
2.5mg/kg AD-61193, QDx5, BIWx3
2.5mg/kg AD-60519, QDx5, BIWx3
2.5mg/kg AD-60819, QDx5, BIWx3
2.5mg/kg AD-60519, QDx5, QWx3

Fig. 50

FIG. 51

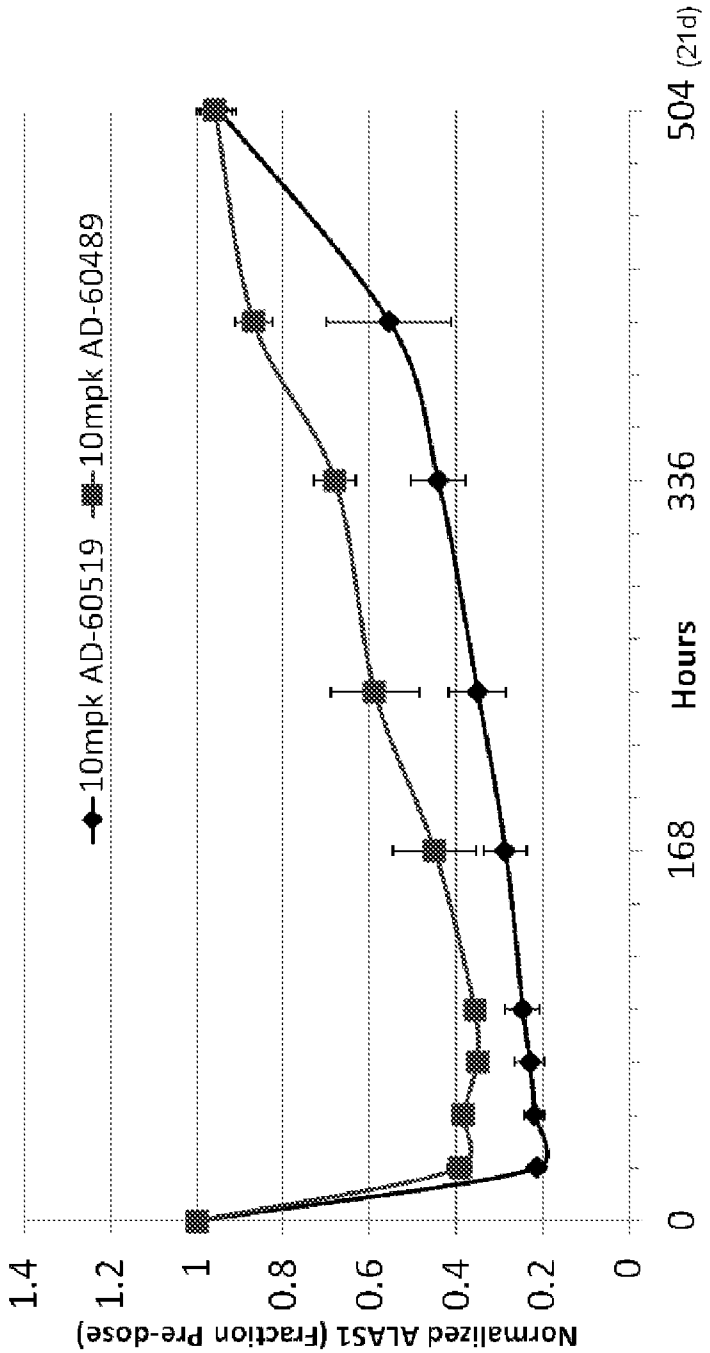

Normalized ALAS1 (Fraction Pre-dose)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
168
336
504 (21d)
Hours
10mpk AD-60519
10mpk AD-60489

FIG. 52

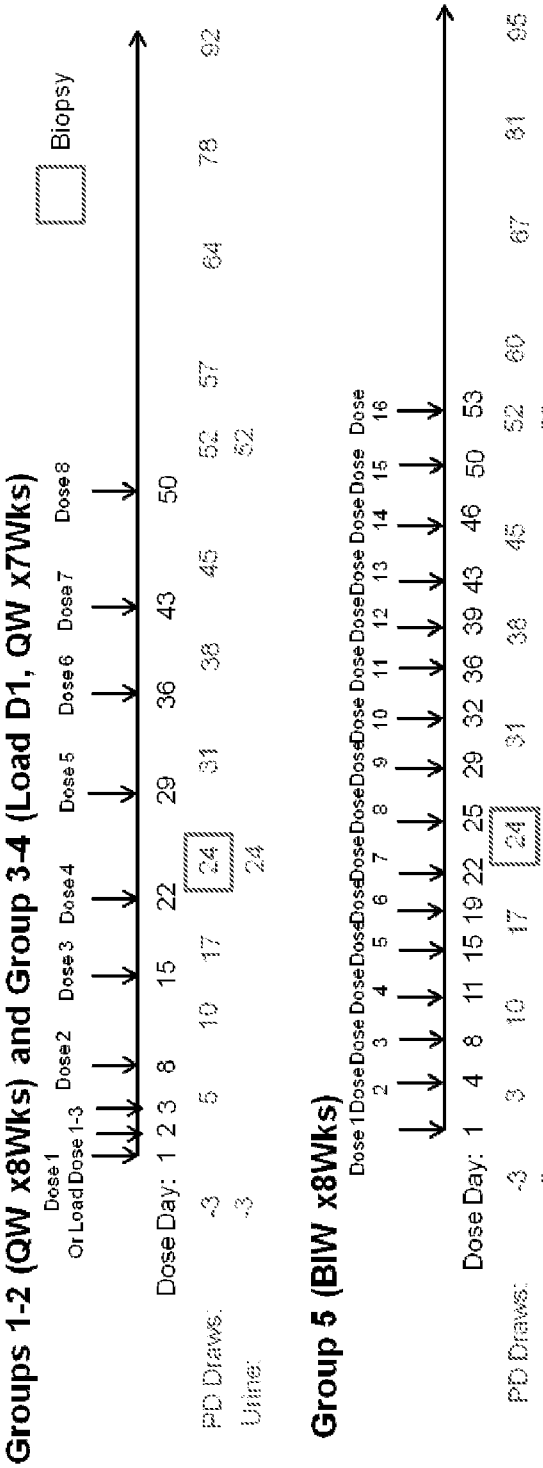
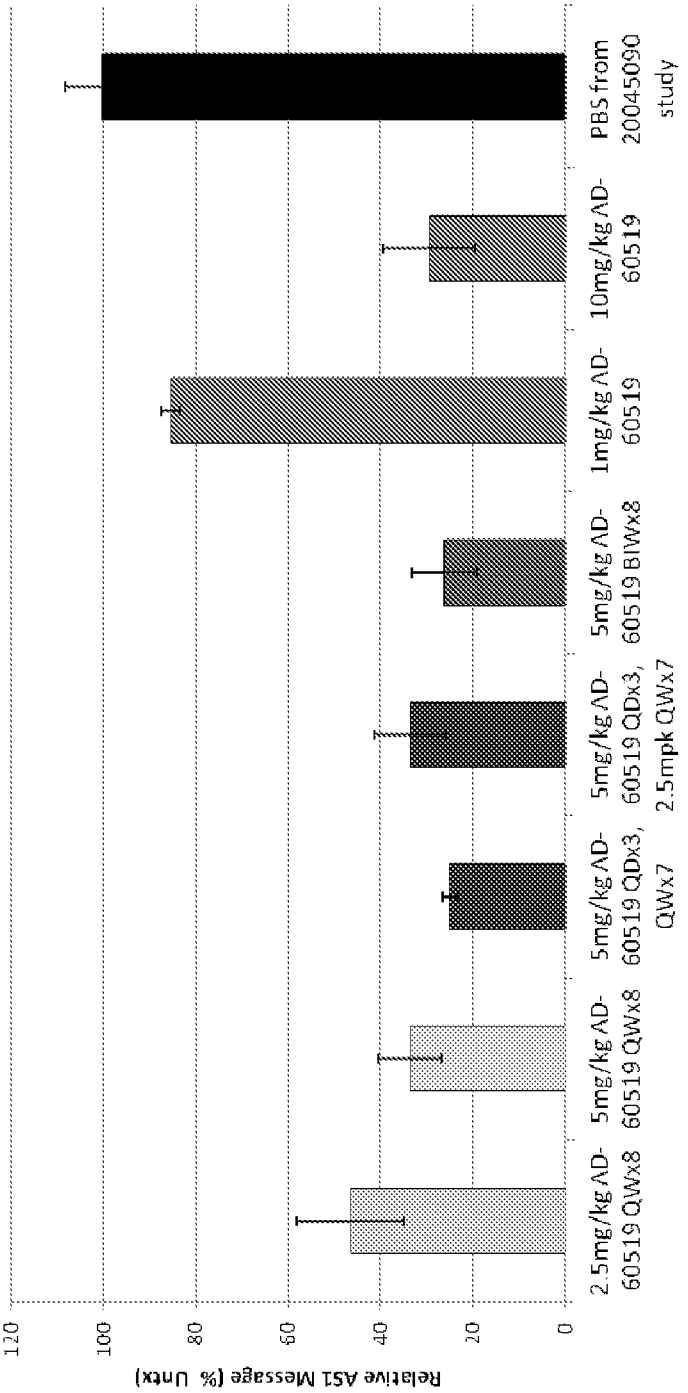

Groups 1-2 (QW x8Wks) and Group 3-4 (Load D1, QW x7Wks)
Biopsy
Dose 1 Or Load
Dose 1-3
Dose 2
Dose 3
Dose 4
Dose 5
Dose 6
Dose 7
Dose 8
Dose Day: 1 2 3 8 15 22 29 36 43 50
PD Draws: -3 24 31 38 45 52 57 64 78 92
Urine: -3 24 52
Group 5 (BIW x8Wks)
Dose 1 Dose Dose Dose Dose Dose Dose Dose Dose Dose Dose Dose Dose Dose Dose Dose Dose
Dose Day: 1 2 3 4 8 11 15 19 22 25 29 32 36 39 43 46 50 53
PD Draws: -3 24 31 38 45 52 60 67 81 95
Urine: -3 24 52
Groups 6-7 (Single Dose)
Dose 1
Dose Day: 1
PD Draws: -3 2 4 6 9 15 22 29 36
Urine: -3 4 36
PK draws for Gp 7 -3 2 3 4 5 6 9 15 22 29 36

FIG. 53


Relative AS1 Message (% Untx)
120
100
80
60
40
20
0
2.5mg/kg AD-60519 QWx8
5mg/kg AD-60519 QWx8
5mg/kg AD-60519 QDx3, QWx7
5mg/kg AD-60519 QDx3, 2.5mpk QWx7
5mg/kg AD-60519 BIWx8
1mg/kg AD-60519
10mg/kg AD-60519
PBS from 20045090 study

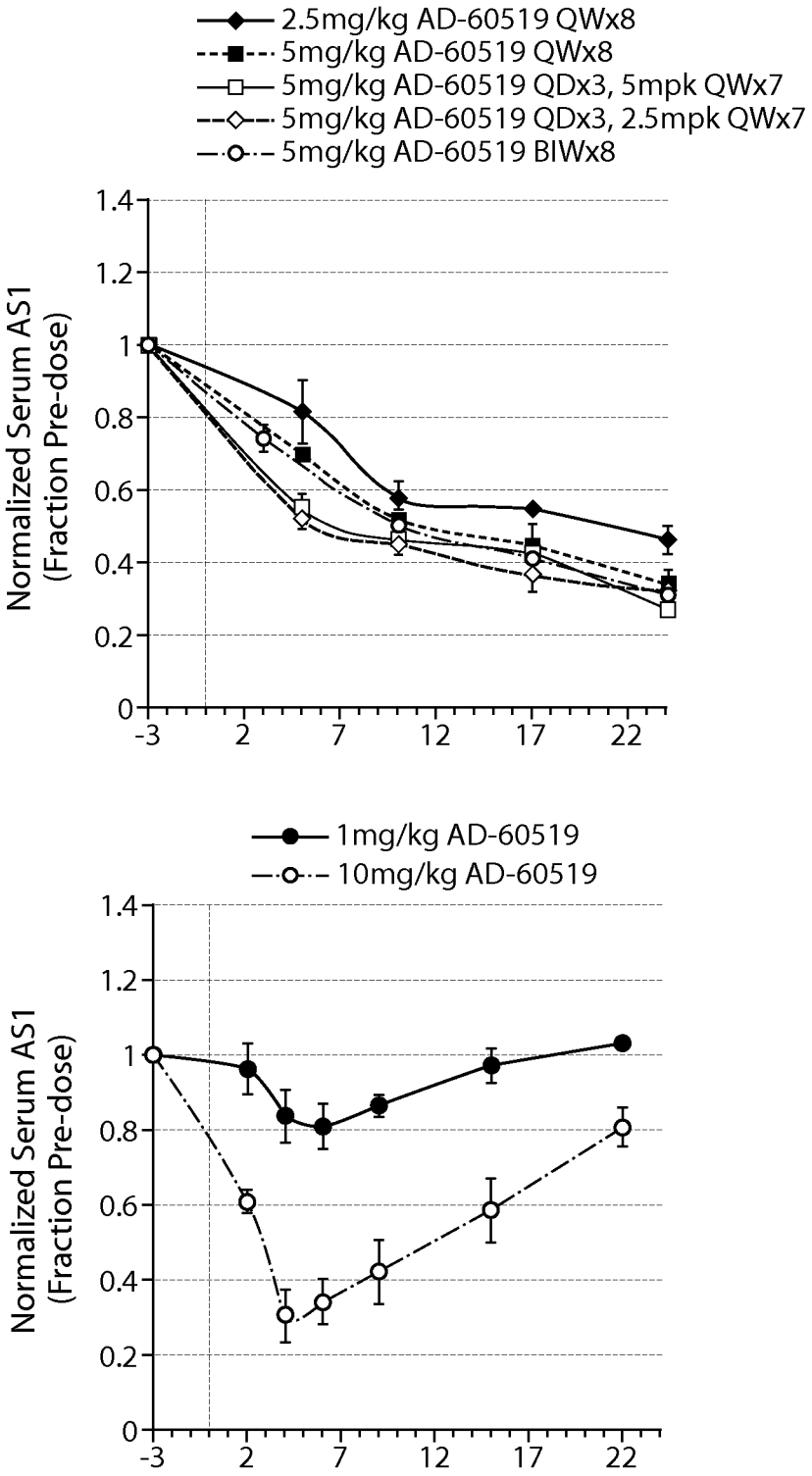
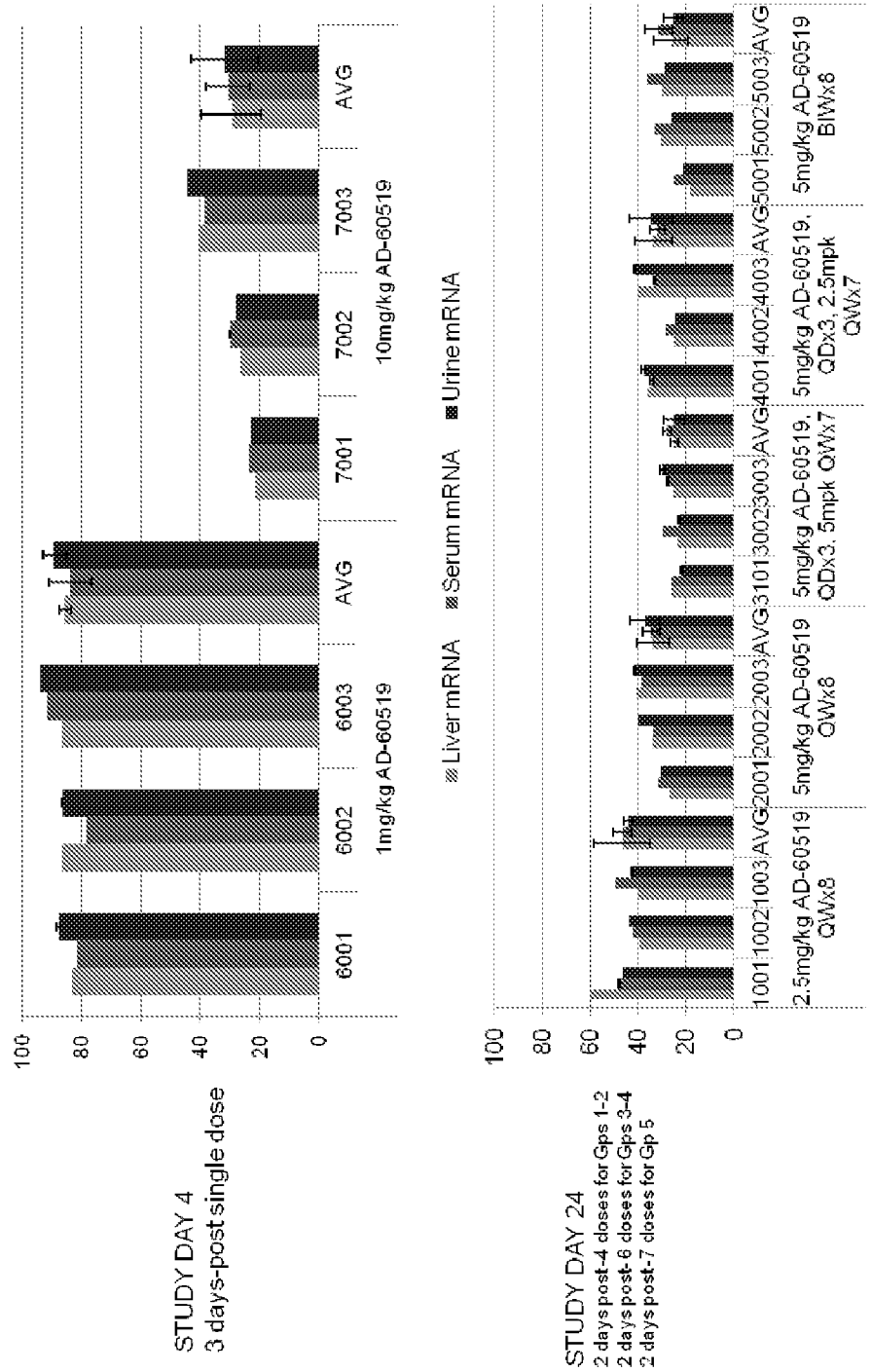

2.5mg/kg AD-60519 QWx8
5mg/kg AD-60519 QWx8
5mg/kg AD-60519 QDx3, 5mpk QWx7
5mg/kg AD-60519 QDx3, 2.5mpk QWx7
5mg/kg AD-60519 BIWx8
Normalized Serum AS1 (Fraction Pre-dose)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-3
2
7
12
17
22
1mg/kg AD-60519
10mg/kg AD-60519
Normalized Serum AS1 (Fraction Pre-dose)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-3
2
7
12
17
22

FIG. 54

FIG. 55


Liver mRNA
Serum mRNA
Urine mRNA
100
80
60
40
20
0
6001
6002
6003
AVG
7001
7002
7003
AVG
1mg/kg AD-60519
10mg/kg AD-60519
STUDY DAY 4
3 days-post single dose
Liver mRNA
Serum mRNA
Urine mRNA
100
80
60
40
20
0
10011002 1003 AVG 2001 2002 2003 AVG 3101 3002 3003 AVG 4001 4002 4003 AVG 5001 5002 5003 AVG
2.5mg/kg AD-60519 QWx8
5mg/kg AD-60519 QWx8
5mg/kg AD-60519, QDx3, 5mpk QWx7
5mg/kg AD-60519, QDx3, 2.5mpk QWx7
5mg/kg AD-60519 BIWx8
STUDY DAY 24
2 days post-4 doses for Gps 1-2
2 days post-6 doses for Gps 3-4
2 days post-7 doses for Gp 5

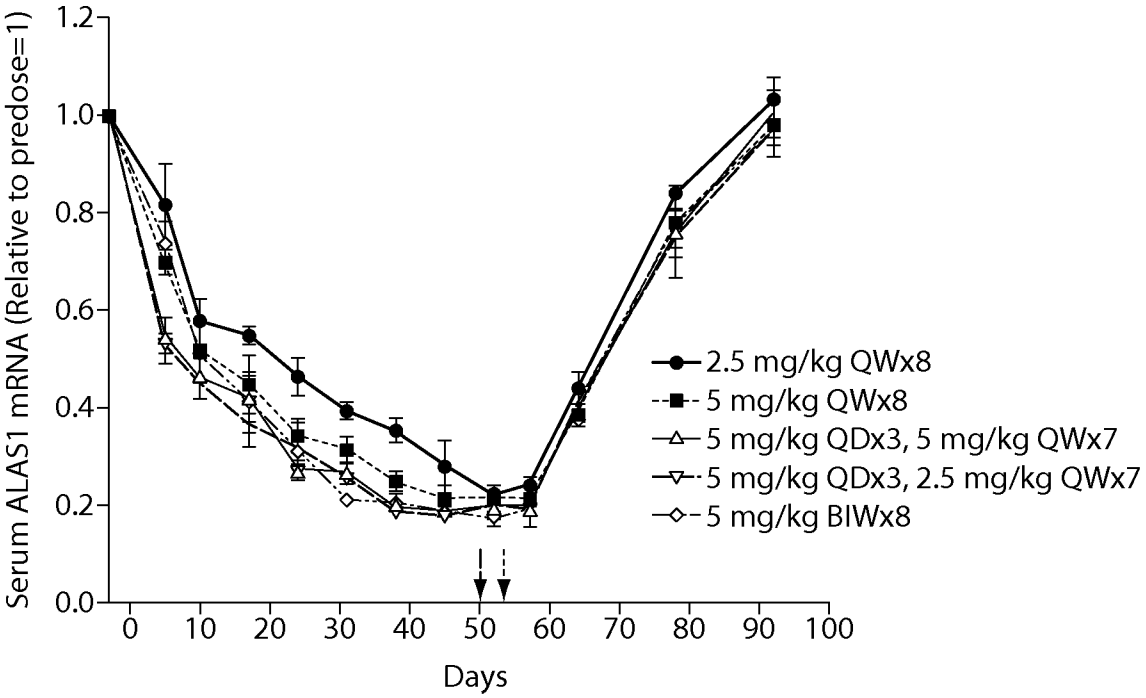
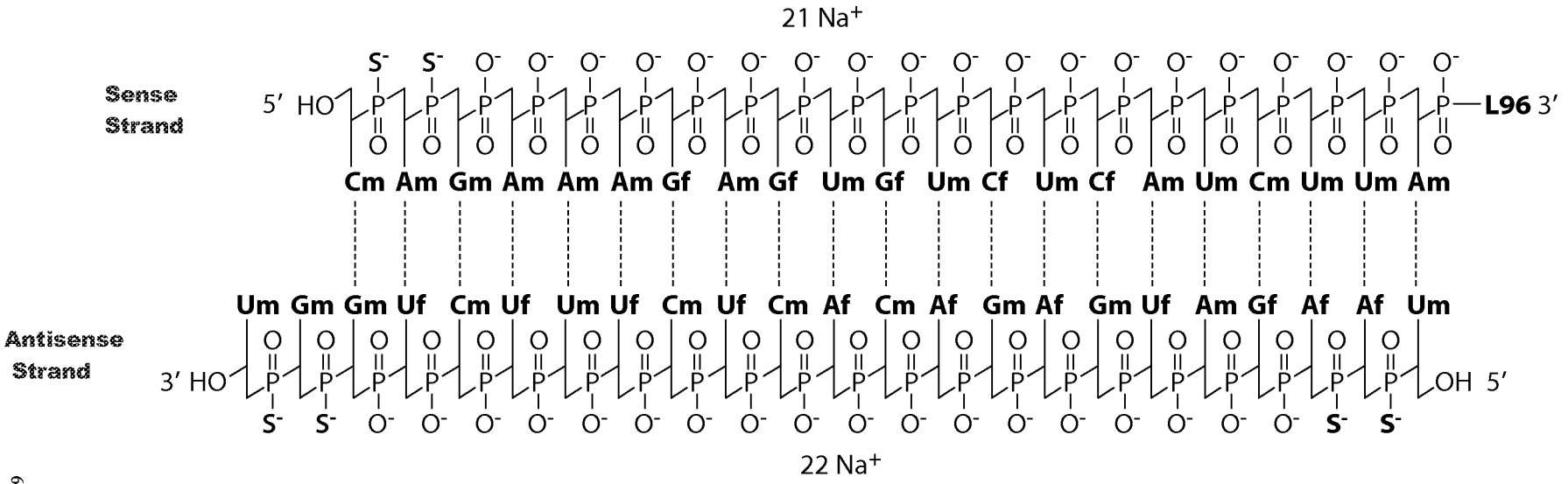

Serum ALAS1 mRNA (Relative to predose=1)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
70
80
90
100
Days
2.5 mg/kg QWx8
5 mg/kg QWx8
5 mg/kg QDx3, 5 mg/kg QWx7
5 mg/kg QDx3, 2.5 mg/kg QWx7
5 mg/kg BIWx8

Fig. 56



19/09

ALN-60519

Af, Cf, Gf, Uf = 2′-F ribonucleosides

Am, Cm, Gm, Um = 2′-OMe ribonucleosides

S = phosphorothioate

L96 =

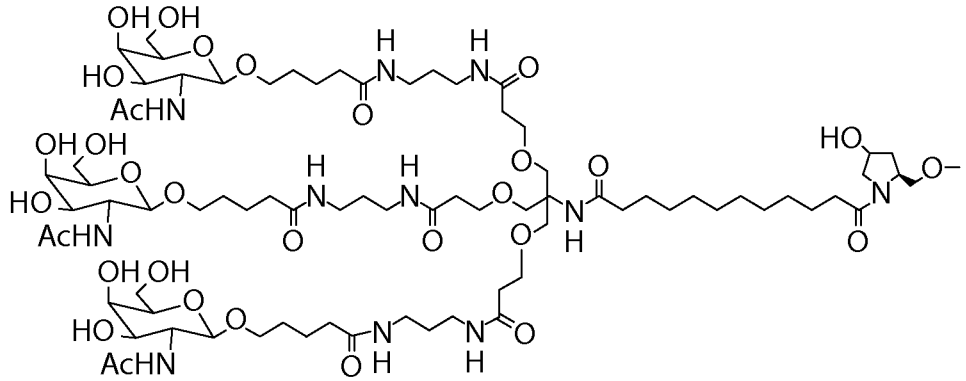
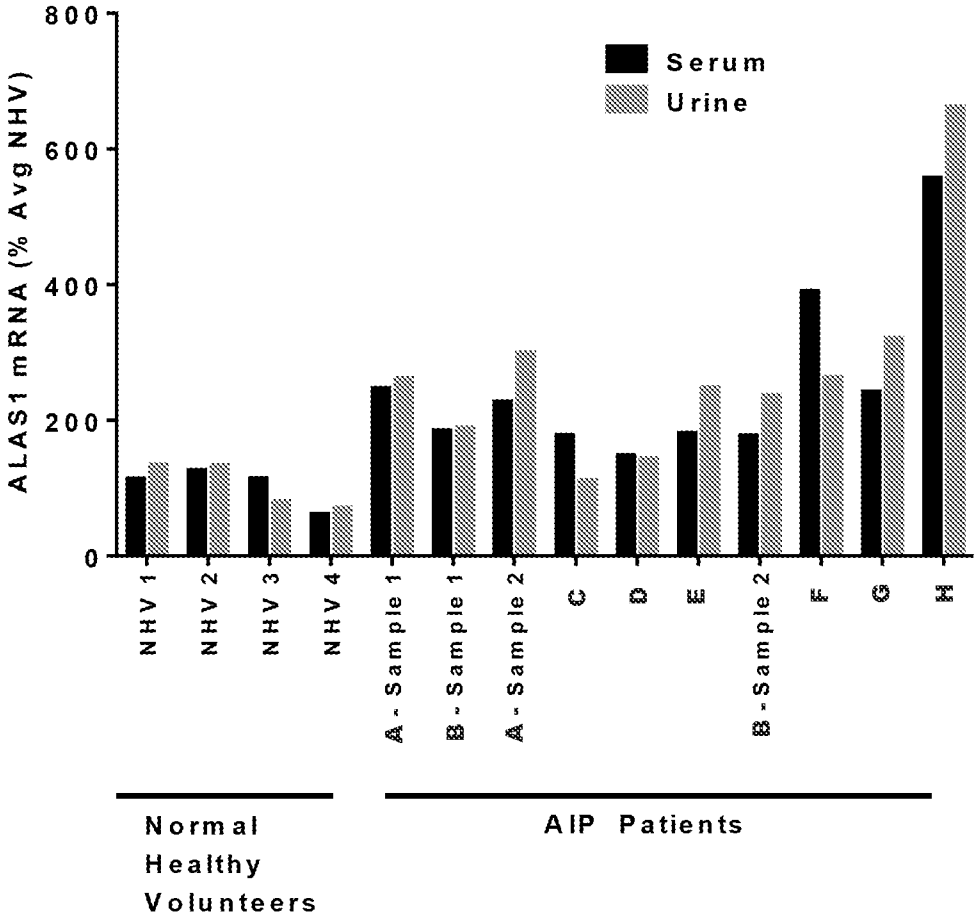


Fig. 57

FIG. 58


800
600
400
200
0
ALAS1 mRNA (% Avg NHV)
Serum
Urine
NHV 1
NHV 2
NHV 3
NHV 4
A - Sample 1
B - Sample 1
A - Sample 2
C
D
E
B - Sample 2
F
G
H
Normal Healthy Volunteers
AIP Patients