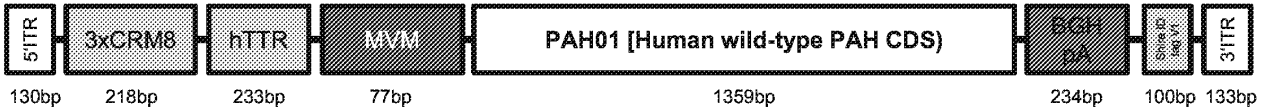
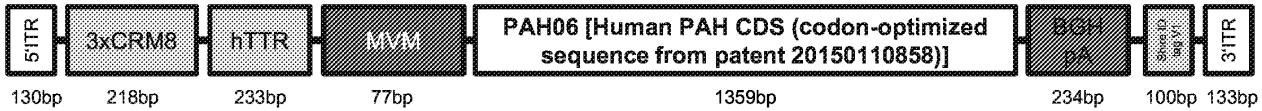
T01



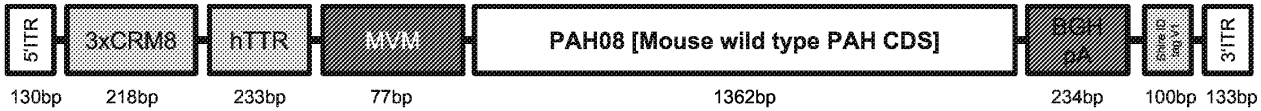
- wild type human PAH coding sequence (GenBank U49897)
- 23 CpGs
- 47.1 % GC content

T02



- Human PAH cds, codon-optimized – codon-optimzed PAH CDS described in U.S. Patent Application 20150110858
  - 110 CpGs
  - 64.6% GC content

T03



- Mouse PAH cds (wild type mouse PAH coding sequence (GenBank NM_008777.3))
  - 30 CpGs
  - 49.4% GC content

92% amino acid sequence identity between human PAH and mouse PAH

FIG. 1A

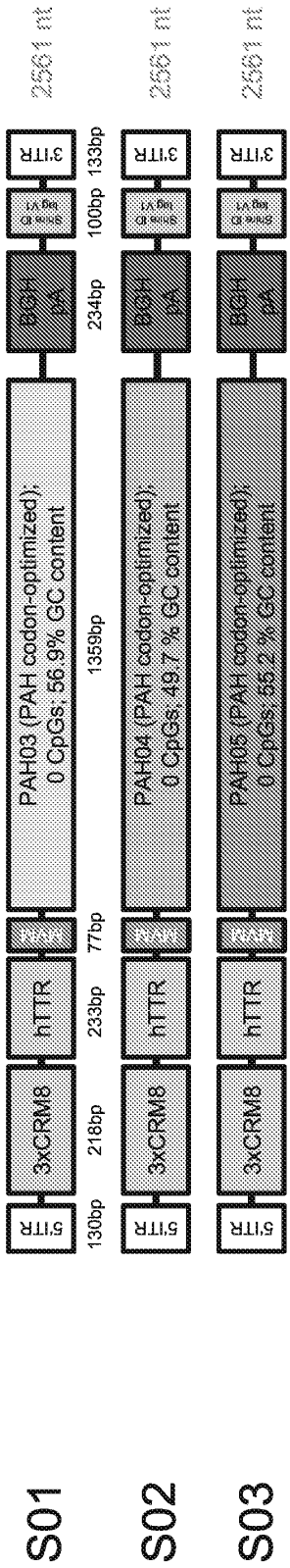

S01
S02
S03
2661 nt
2661 nt
2661 nt
5'ITR
3xCRM8
hTTR
PAH5'
PAH03 (PAH codon-optimized); 0 CpGs; 56.9% GC content
PAH04 (PAH codon-optimized); 0 CpGs; 49.7 % GC content
PAH05 (PAH codon-optimized); 0 CpGs; 55.2 % GC content
BGH pA
Stuffer ID Seq V1
3'ITR
130bp
218bp
233bp
77bp
1359bp
234bp
100bp
133bp

FIG. 1B

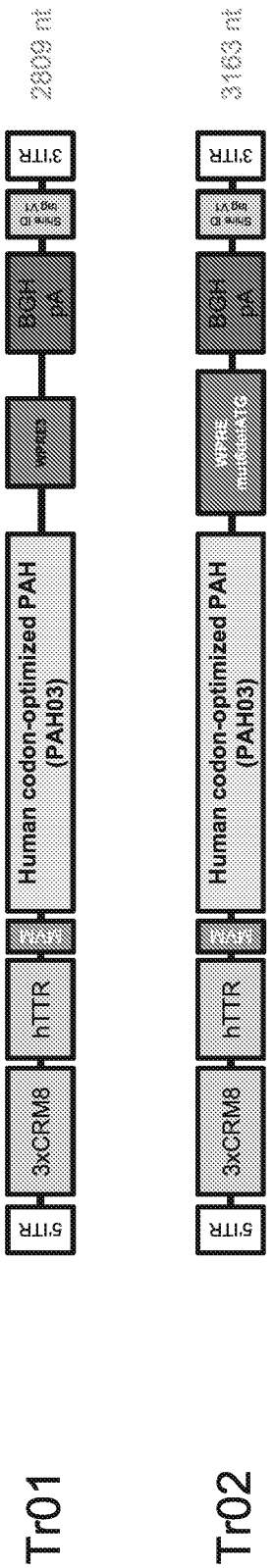

2809 nt
3'ITR
Synthetic poly(A)
BGH pA
WPRE
Human codon-optimized PAH (PAH03)
Kozak
hTTR
3xCRM8
5'ITR
Tr01
3163 nt
3'ITR
Synthetic poly(A)
BGH pA
WPRE mutated
Human codon-optimized PAH (PAH03)
Kozak
hTTR
3xCRM8
5'ITR
Tr02

FIG. 1C

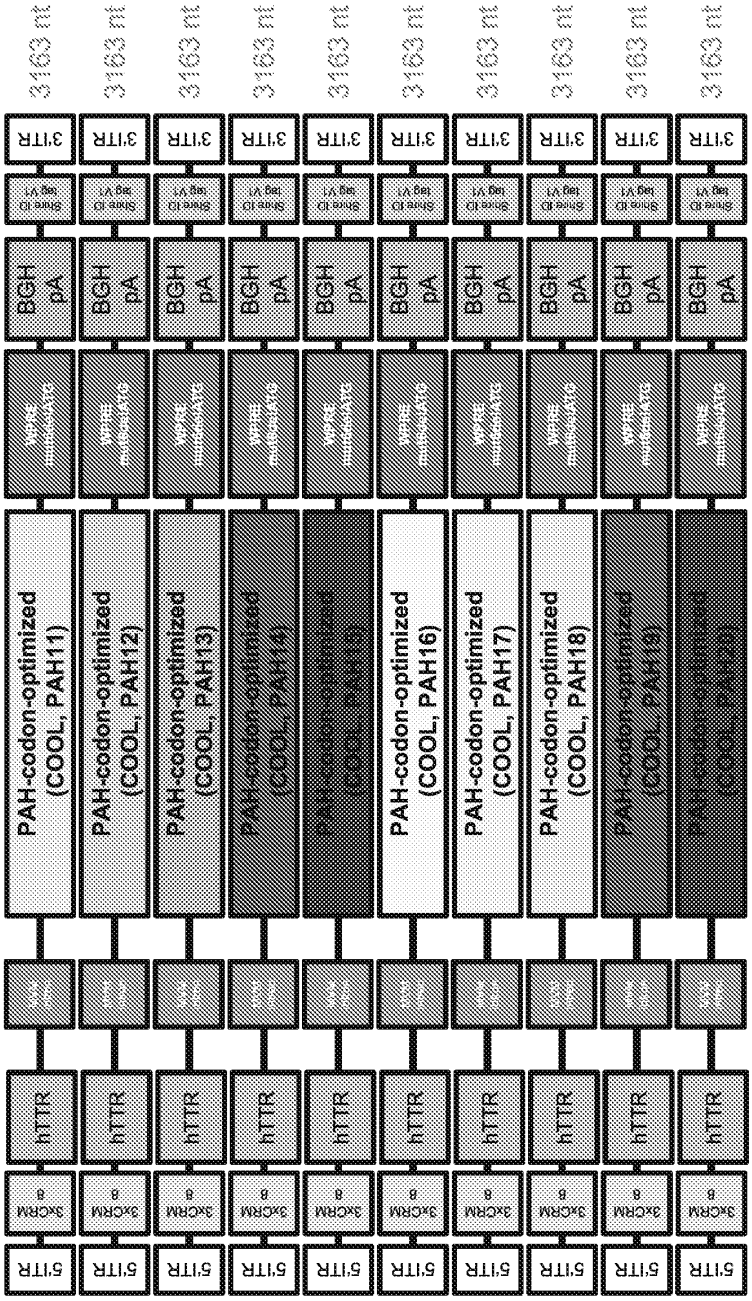

3163 nt
3'ITR
Stuffer ID tag V1
BGH pA
PAH-codon-optimized (COOL, PAH11)
PAH-codon-optimized (COOL, PAH12)
PAH-codon-optimized (COOL, PAH13)
PAH-codon-optimized (COOL, PAH14)
PAH-codon-optimized (COOL, PAH15)
PAH-codon-optimized (COOL, PAH16)
PAH-codon-optimized (COOL, PAH17)
PAH-codon-optimized (COOL, PAH18)
PAH-codon-optimized (COOL, PAH19)
PAH-codon-optimized (COOL, PAH20)
hTTR
3xCRM 8
5'ITR
P01
P02
P03
P04
P05
P06
P07
P08
P09
P10

FIG. 1D

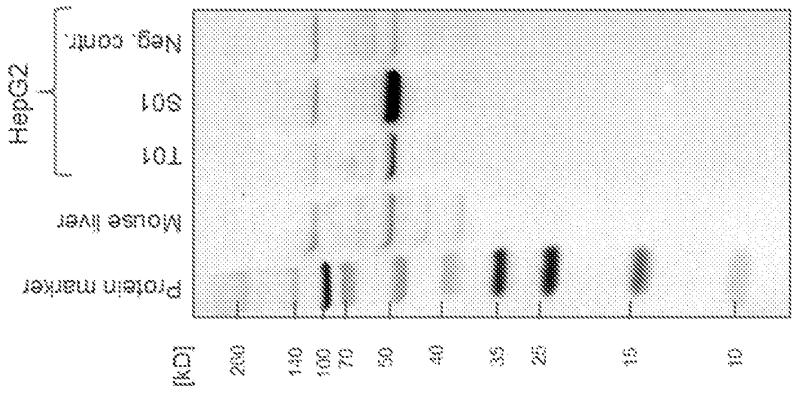

Protein marker
Mouse liver
T01
S01
Neg. contr.
HepG2
[kD]
260
140
100
70
50
40
35
25
15
10

FIG. 2

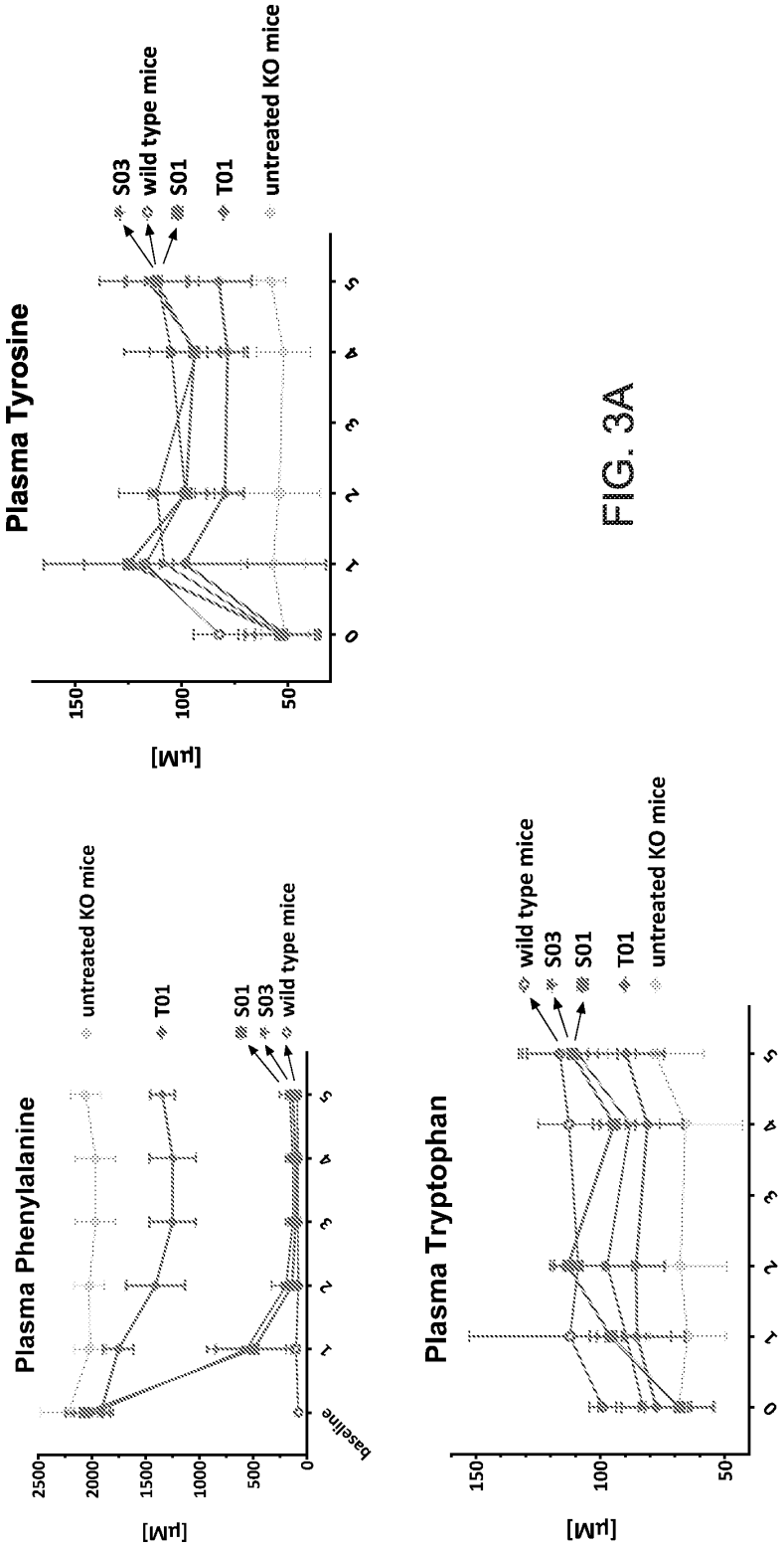

Plasma Tyrosine
S03
wild type mice
S01
T01
untreated KO mice
[µM]
150
100
50
0
1
2
3
4
5
Plasma Phenylalanine
untreated KO mice
T01
S01
S03
wild type mice
[µM]
2500
2000
1500
1000
500
0
baseline
1
2
3
4
5
Plasma Tryptophan
wild type mice
S03
S01
T01
untreated KO mice
[µM]
150
100
50
0
1
2
3
4
5

FIG. 3A

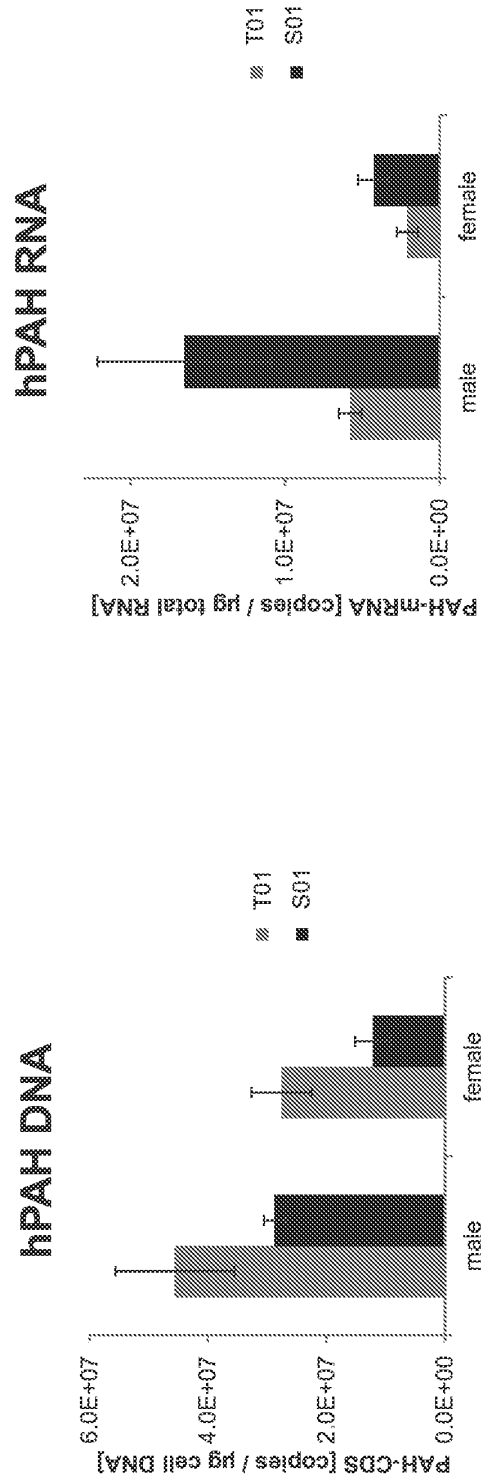
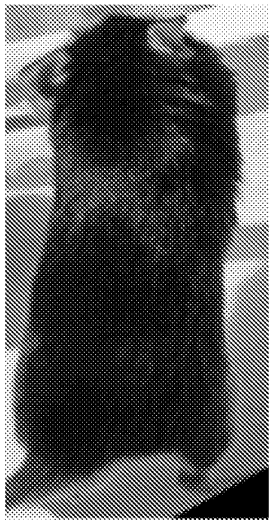
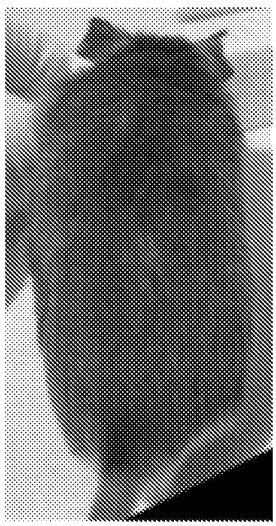

hPAH DNA
T01
S01
PAH-CDS [copies / µg cell DNA]
6.0E+07
4.0E+07
2.0E+07
0.0E+00
male
female
hPAH RNA
T01
S01
PAH-mRNA [copies / µg total RNA]
2.0E+07
1.0E+07
0.0E+00
male
female

FIG. 3B



PKU (3 weeks post treatment)



PKU (before treatment)

FIG. 4

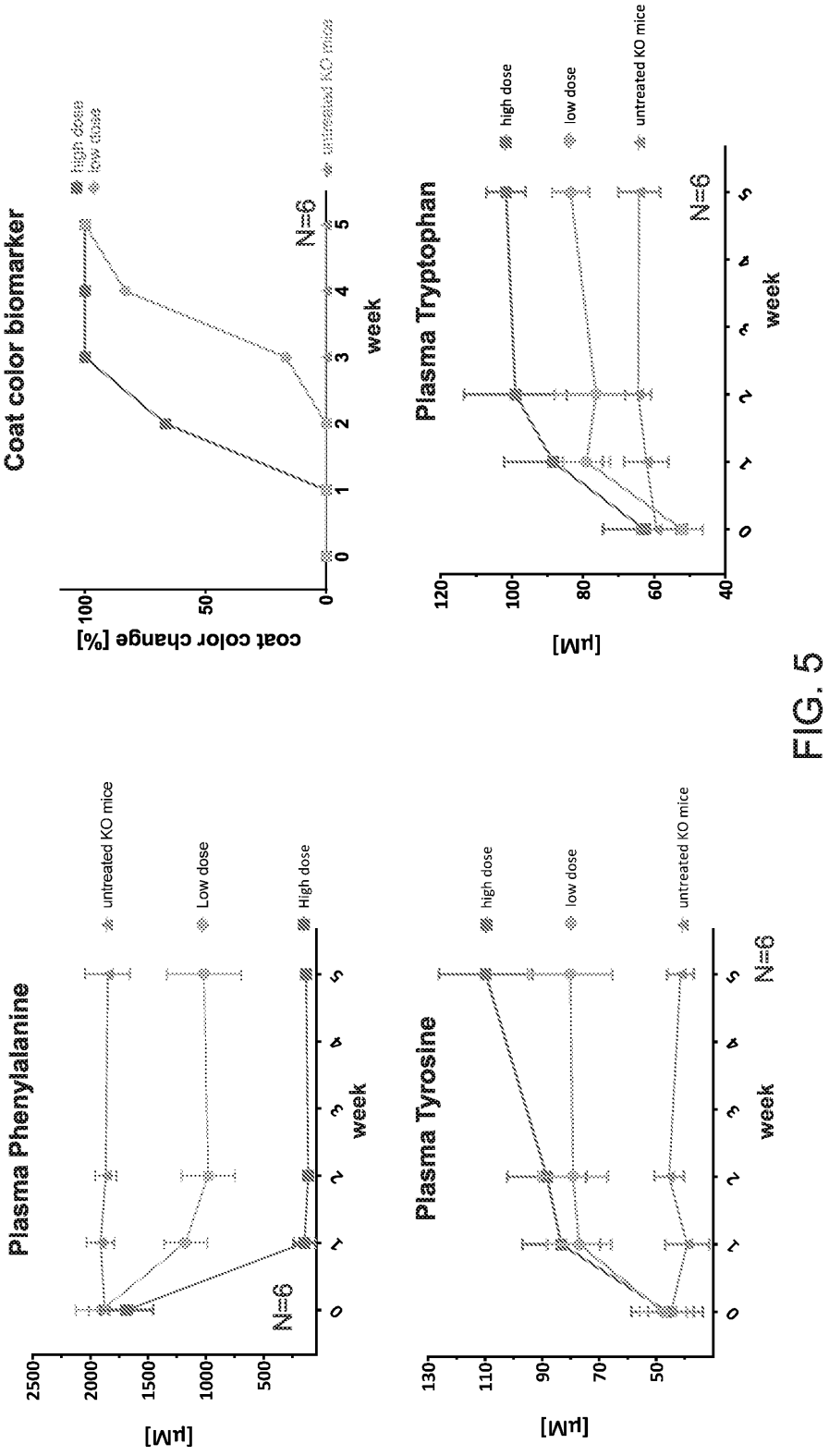

Coat color biomarker
high dose
low dose
untreated KO mice
N=6
coat color change [%]
week
Plasma Tryptophan
high dose
low dose
untreated KO mice
N=6
[µM]
week
Plasma Phenylalanine
untreated KO mice
Low dose
High dose
N=6
[µM]
week
Plasma Tyrosine
high dose
low dose
untreated KO mice
N=6
[µM]
week

FIG. 5

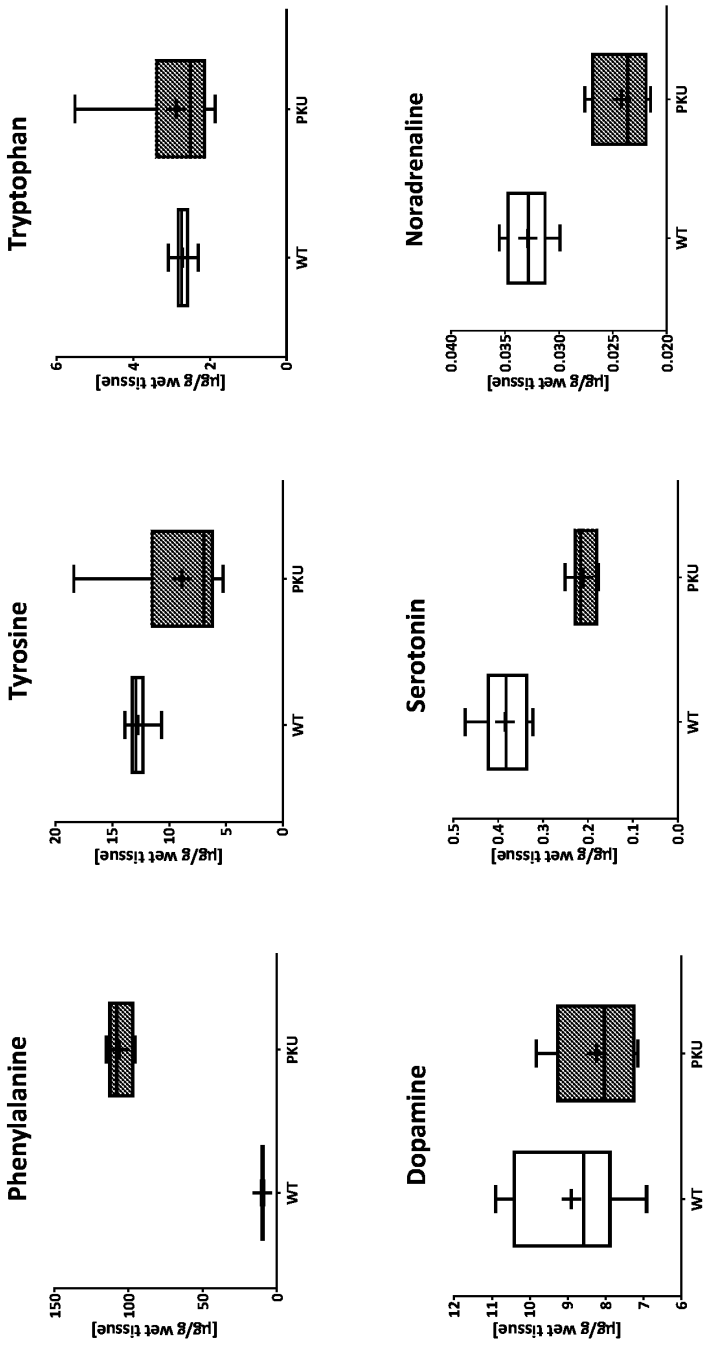

Tryptophan
[µg/g wet tissue]
0
2
4
6
WT
PKU
Noradrenaline
[µg/g wet tissue]
0.020
0.025
0.030
0.035
0.040
WT
PKU
Tyrosine
[µg/g wet tissue]
0
5
10
15
20
WT
PKU
Serotonin
[µg/g wet tissue]
0.0
0.1
0.2
0.3
0.4
0.5
WT
PKU
Phenylalanine
[µg/g wet tissue]
0
50
100
150
WT
PKU
Dopamine
[µg/g wet tissue]
6
7
8
9
10
11
12
WT
PKU

FIG. 6

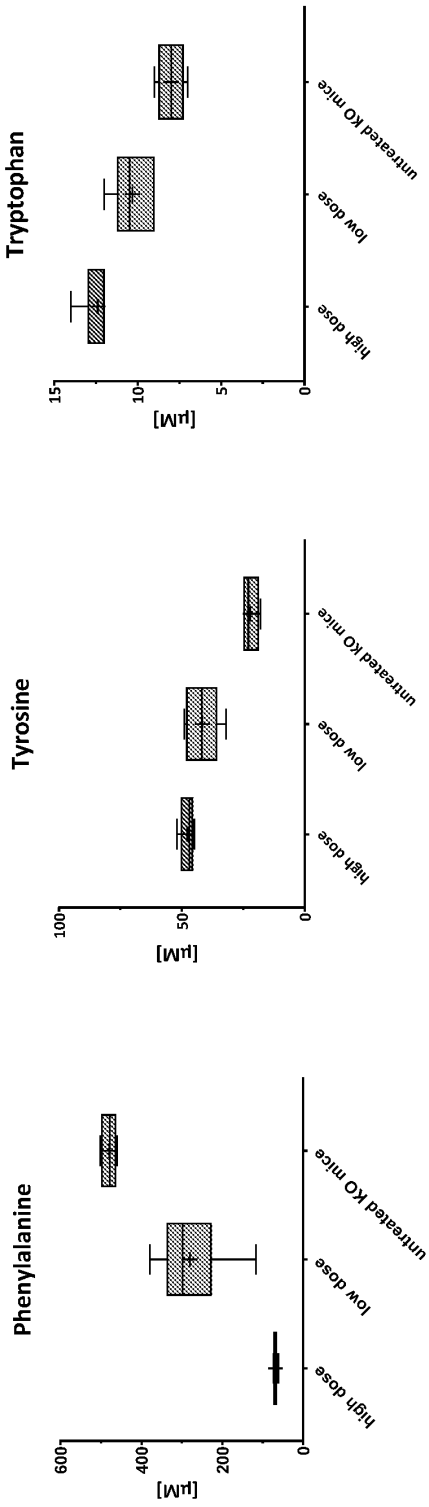

Phenylalanine
[µM]
0
200
400
600
high dose
low dose
untreated KO mice
Tyrosine
[µM]
0
50
100
high dose
low dose
untreated KO mice
Tryptophan
[µM]
0
5
10
15
high dose
low dose
untreated KO mice

FIG. 7A

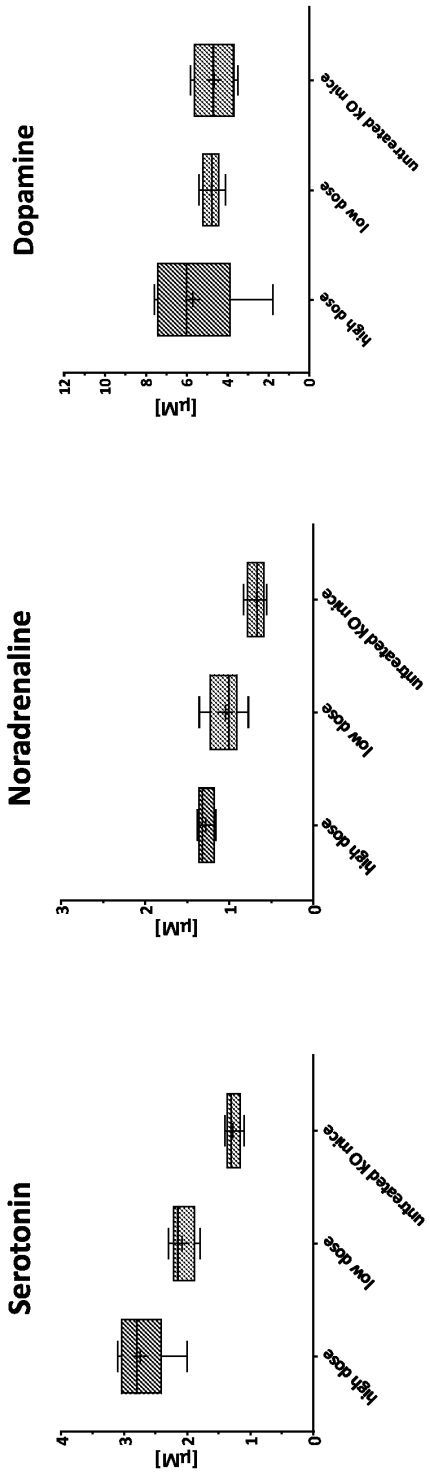

Dopamine
[µM]
0
2
4
6
8
10
12
high dose
low dose
untreated KO mice
Noradrenaline
[µM]
0
1
2
3
high dose
low dose
untreated KO mice
Serotonin
[µM]
0
1
2
3
4
high dose
low dose
untreated KO mice

FIG. 7B

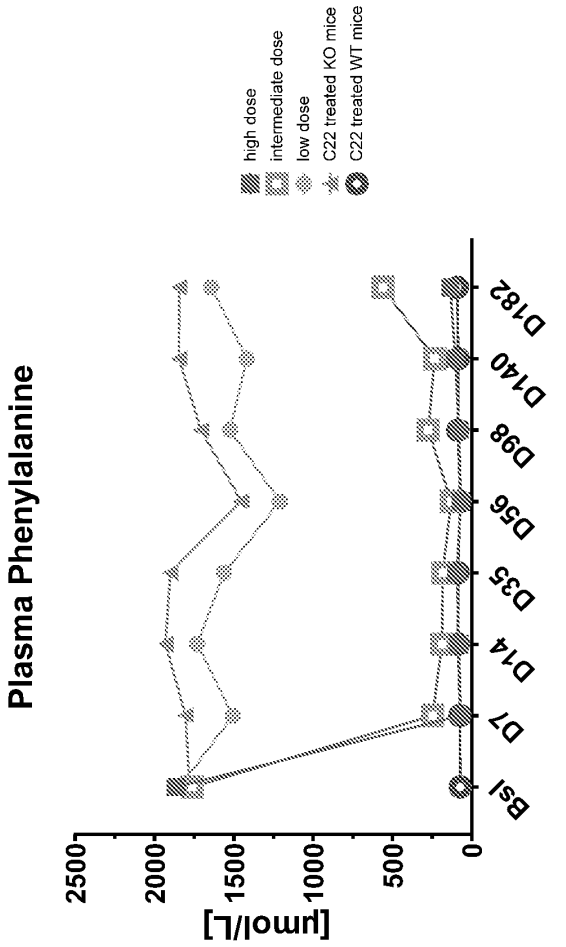

Plasma Phenylalanine
[µmol/L]
2500
2000
1500
1000
500
0
Bsl
D7
D14
D35
D56
D98
D140
D182
high dose
intermediate dose
low dose
C22 treated KO mice
C22 treated WT mice

FIG. 8