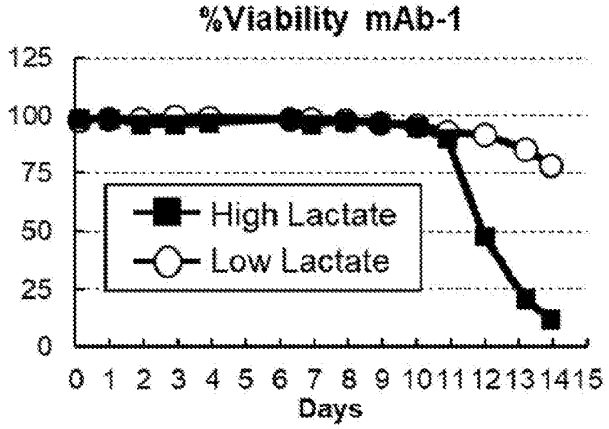


FIG. 1A

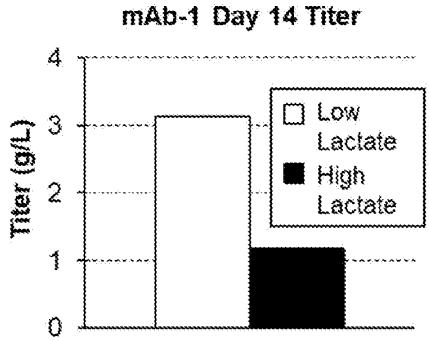


FIG. 1B

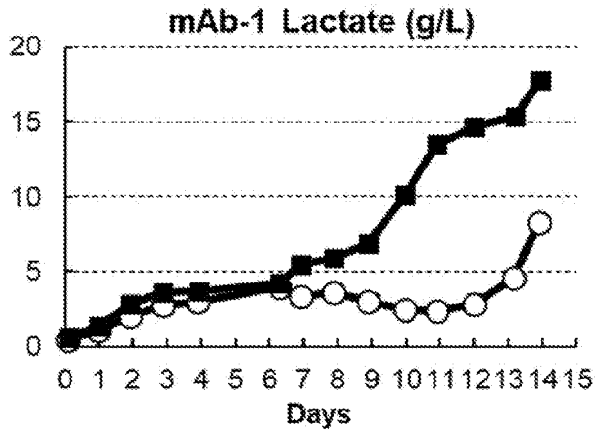


FIG. 1C

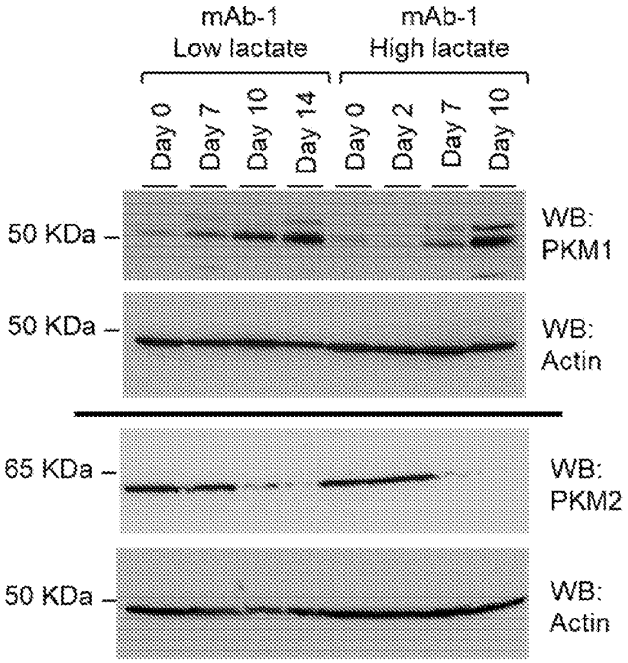

mAb-1
Low lactate
mAb-1
High lactate
Day 0
Day 7
Day 10
Day 14
Day 0
Day 2
Day 7
Day 10
50 KDa
WB:
PKM1
50 KDa
WB:
Actin
65 KDa
WB:
PKM2
50 KDa
WB:
Actin

FIG. 1D

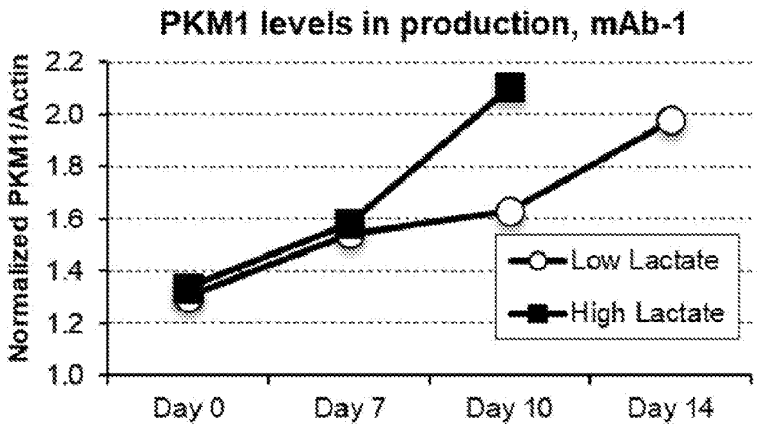

PKM1 levels in production, mAb-1
Normalized PKM1/Actin
2.2
2.0
1.8
1.6
1.4
1.2
1.0
Day 0
Day 7
Day 10
Day 14
Low Lactate
High Lactate

FIG. 1E

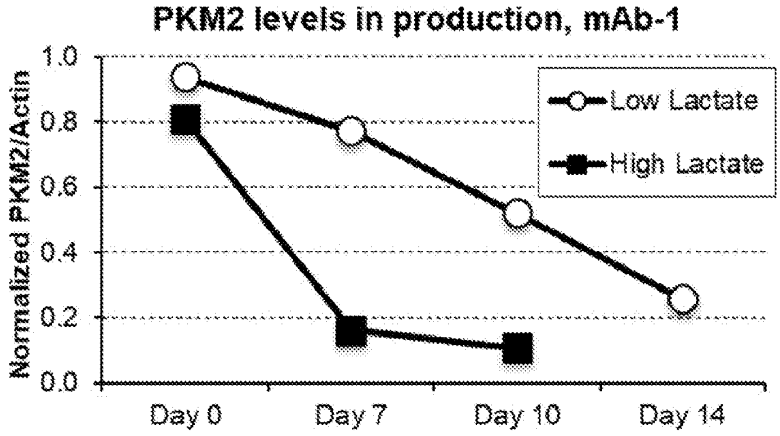

PKM2 levels in production, mAb-1
Normalized PKM2/Actin
1.0
0.8
0.6
0.4
0.2
0.0
Day 0
Day 7
Day 10
Day 14
Low Lactate
High Lactate

FIG. 1F

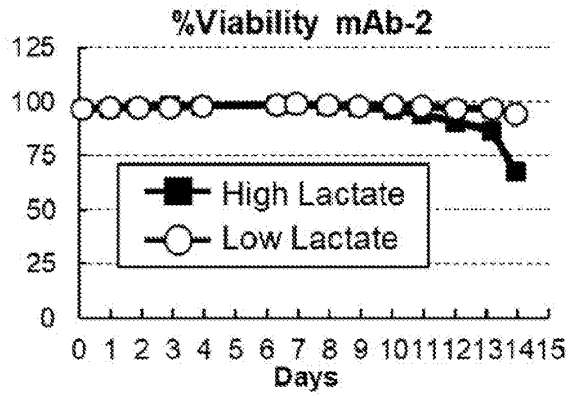


FIG. 2A

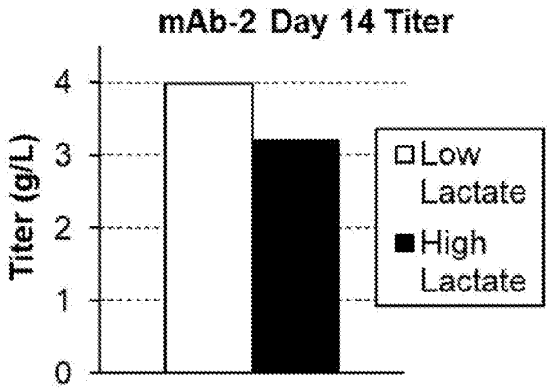


FIG. 2B

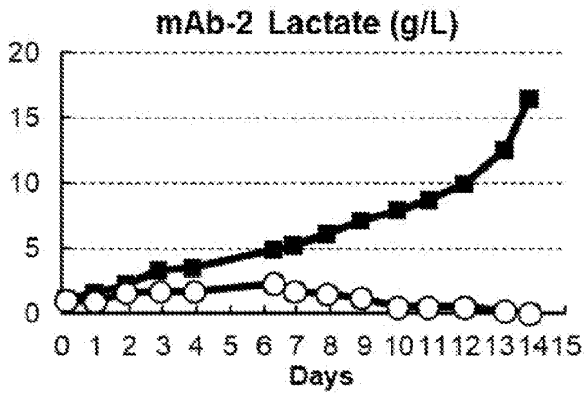


FIG. 2C

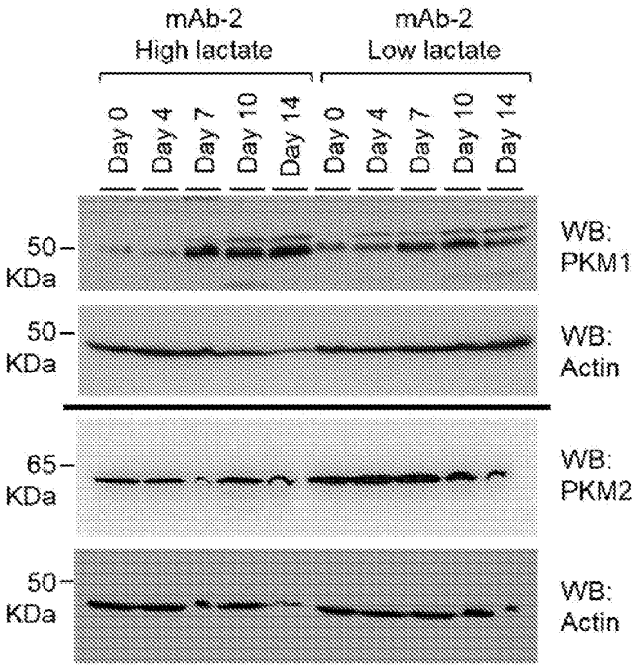

mAb-2
High lactate
mAb-2
Low lactate
Day 0
Day 4
Day 7
Day 10
Day 14
Day 0
Day 4
Day 7
Day 10
Day 14
50 KDa
WB: PKM1
50 KDa
WB: Actin
65 KDa
WB: PKM2
50 KDa
WB: Actin

FIG. 2D

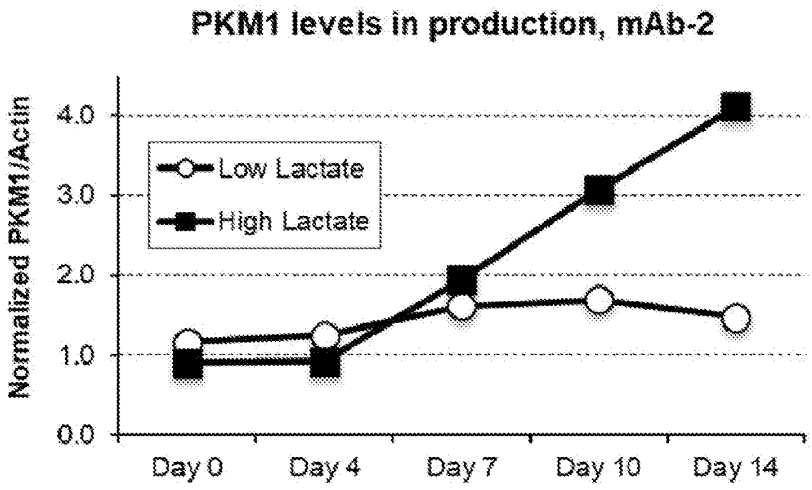

PKM1 levels in production, mAb-2
Normalized PKM1/Actin
4.0
3.0
2.0
1.0
0.0
Low Lactate
High Lactate
Day 0
Day 4
Day 7
Day 10
Day 14

FIG. 2E

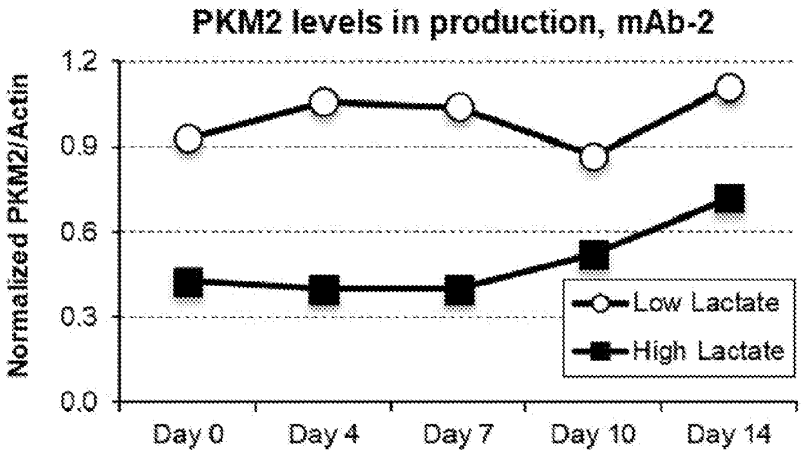

PKM2 levels in production, mAb-2
Normalized PKM2/Actin
1.2
0.9
0.6
0.3
0.0
Low Lactate
High Lactate
Day 0
Day 4
Day 7
Day 10
Day 14

FIG. 2F

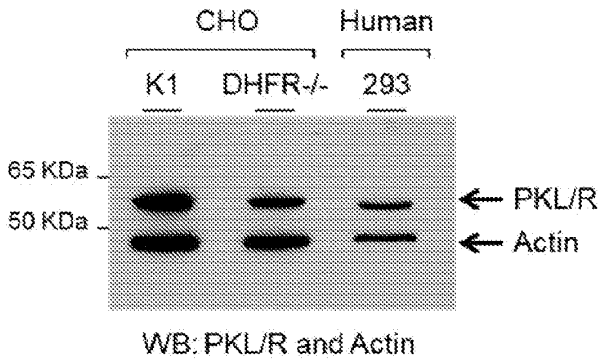


FIG. 3A

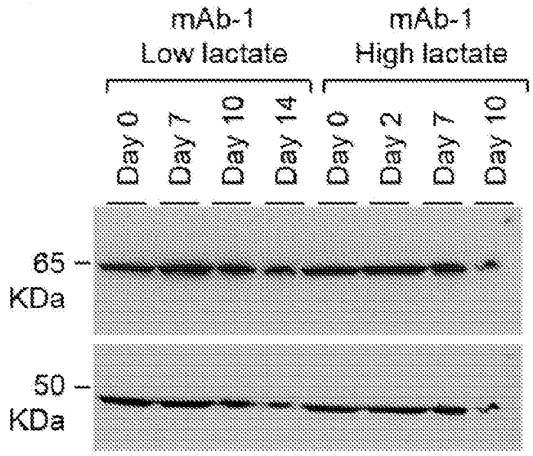


FIG. 3B

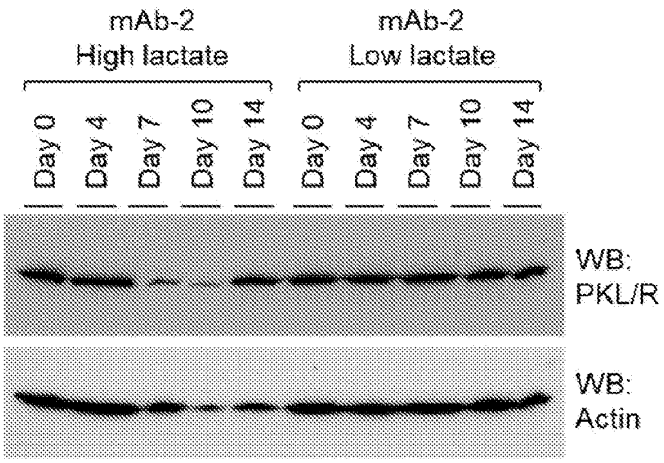


FIG. 3C

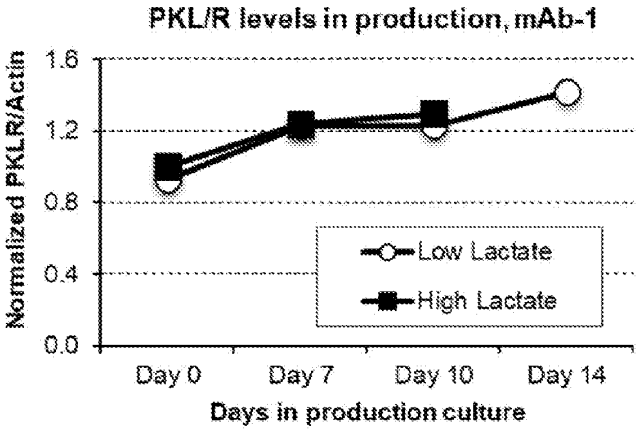

PKL/R levels in production, mAb-1
Normalized PKLR/Actin
1.6
1.2
0.8
0.4
0.0
Low Lactate
High Lactate
Day 0
Day 7
Day 10
Day 14
Days in production culture

FIG. 3D

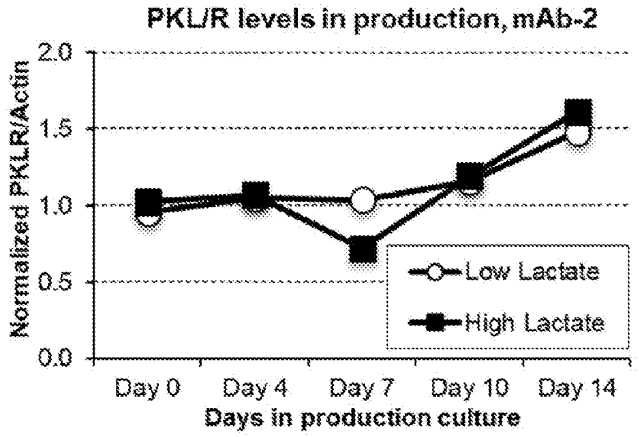

PKL/R levels in production, mAb-2
Normalized PKLR/Actin
2.0
1.5
1.0
0.5
0.0
Low Lactate
High Lactate
Day 0
Day 4
Day 7
Day 10
Day 14
Days in production culture

FIG. 3E

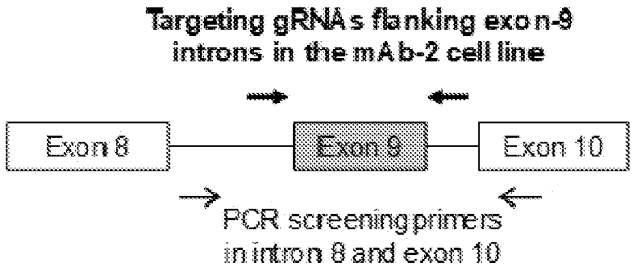

Targeting gRNAs flanking exon-9
introns in the mAb-2 cell line
Exon 8
Exon 9
Exon 10
PCR screening primers
in intron 8 and exon 10

FIG. 4A

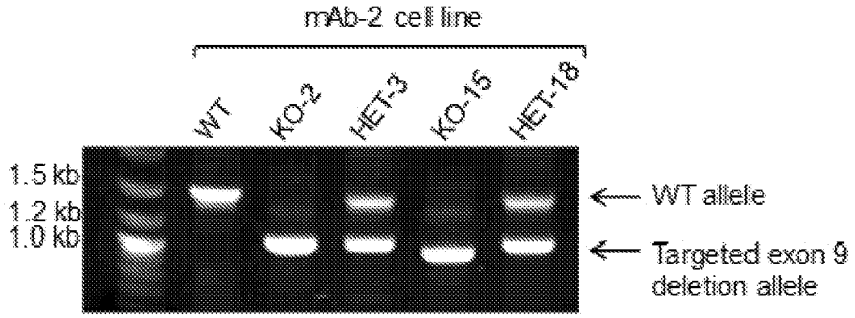

mAb-2 cell line
WT
KO-2
HET-3
KO-15
HET-18
1.5 kb
1.2 kb
1.0 kb
WT allele
Targeted exon 9
deletion allele

FIG. 4B

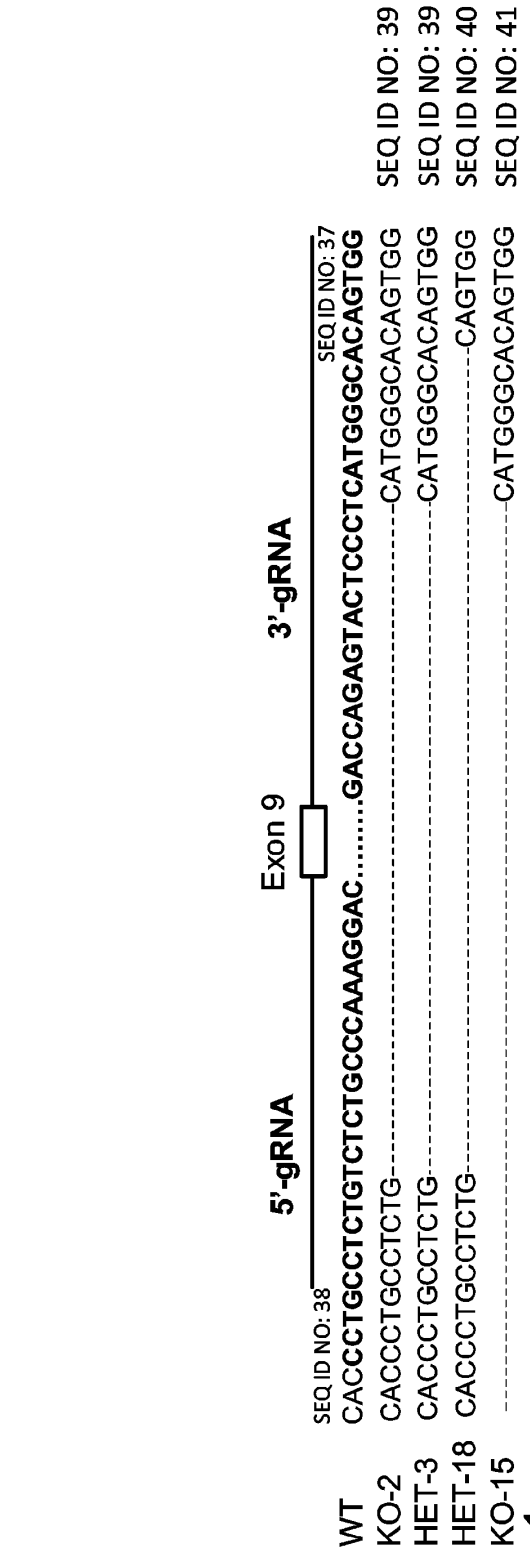

5'-gRNA
Exon 9
3'-gRNA
SEQ ID NO: 38
SEQ ID NO: 37
WT CACCCTGCCTCTGTCTCTGCCCAAAGGAC.....GACCAGAGTACTCCCTCATGGGCACAGTGG
KO-2 CACCCTGCCTCTG----------CATGGGCACAGTGG SEQ ID NO: 39
HET-3 CACCCTGCCTCTG----------CATGGGCACAGTGG SEQ ID NO: 39
HET-18 CACCCTGCCTCTG----------CAGTGG SEQ ID NO: 40
KO-15 ----------CATGGGCACAGTGG SEQ ID NO: 41
KO-15 does not match WT
for another 122 bp
upstream of the 5'-gRNA.

FIG. 4C

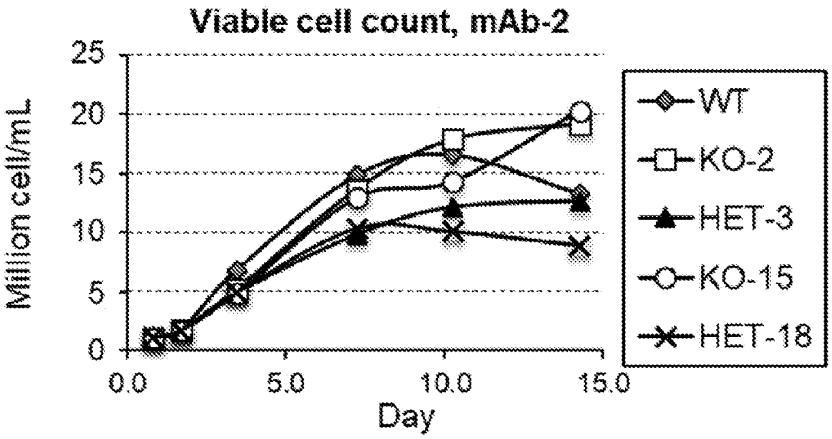

Viable cell count, mAb-2
Million cell/mL
25
20
15
10
5
0
0.0
5.0
10.0
15.0
Day
WT
KO-2
HET-3
KO-15
HET-18

FIG. 5A

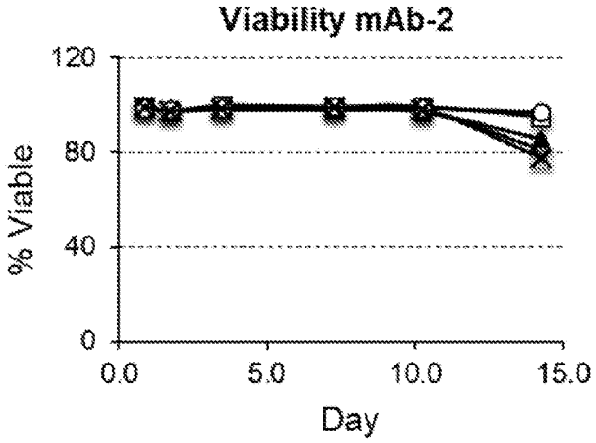

Viability mAb-2
% Viable
120
80
40
0
0.0
5.0
10.0
15.0
Day

FIG. 5B

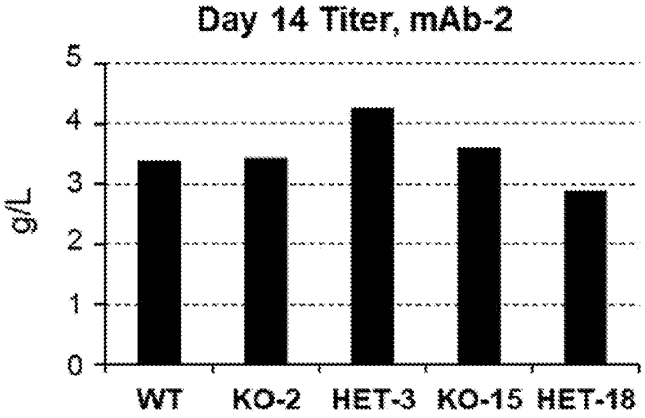

Day 14 Titer, mAb-2
g/L
5
4
3
2
1
0
WT
KO-2
HET-3
KO-15
HET-18

FIG. 5C

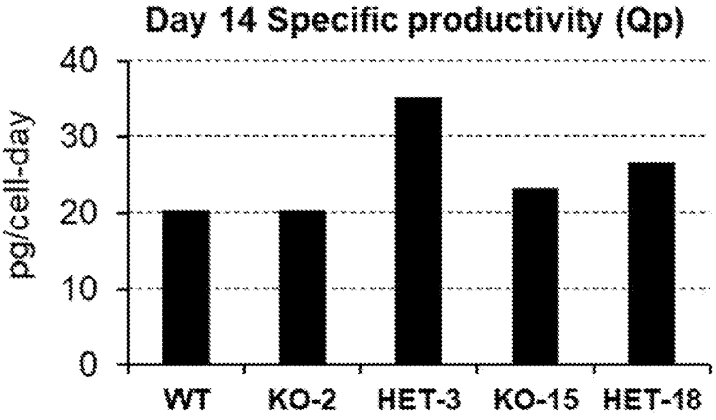

Day 14 Specific productivity (Qp)
pg/cell-day
40
30
20
10
0
WT
KO-2
HET-3
KO-15
HET-18

FIG. 5D

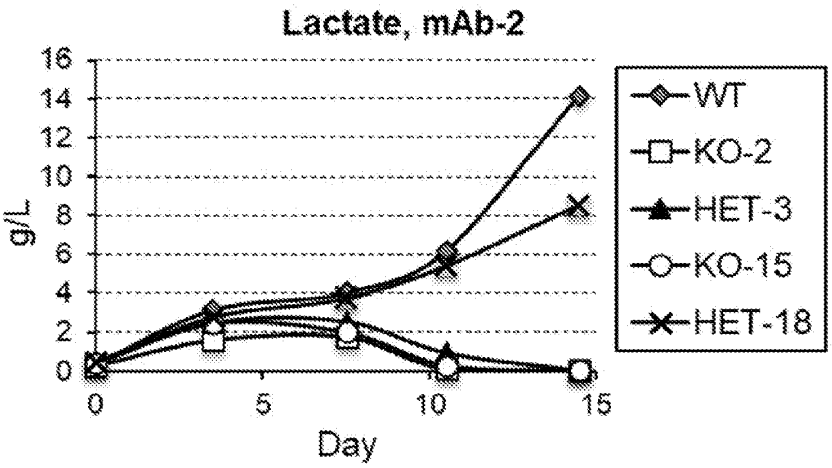

Lactate, mAb-2
16
14
12
10
8
6
4
2
0
g/L
0
5
10
15
Day
WT
KO-2
HET-3
KO-15
HET-18

FIG. 5E

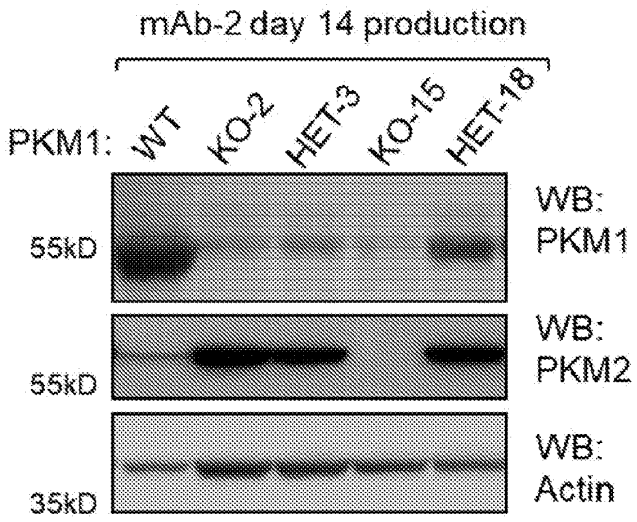

mAb-2 day 14 production
PKM1:
WT
KO-2
HET-3
KO-15
HET-18
55kD
WB:
PKM1
55kD
WB:
PKM2
35kD
WB:
Actin

FIG. 5F

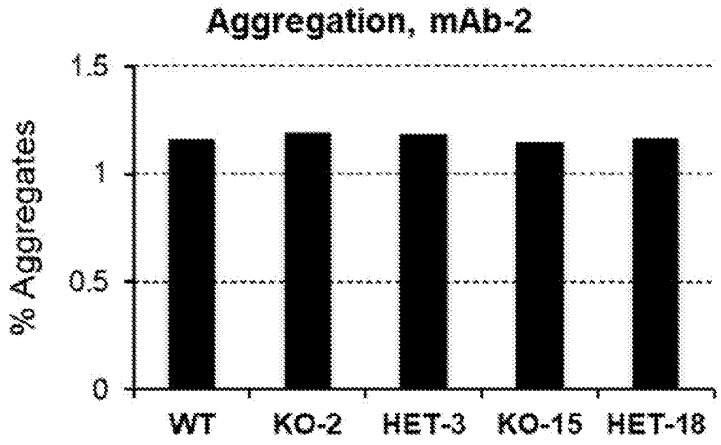

Aggregation, mAb-2
% Aggregates
1.5
1
0.5
0
WT
KO-2
HET-3
KO-15
HET-18

FIG. 5G

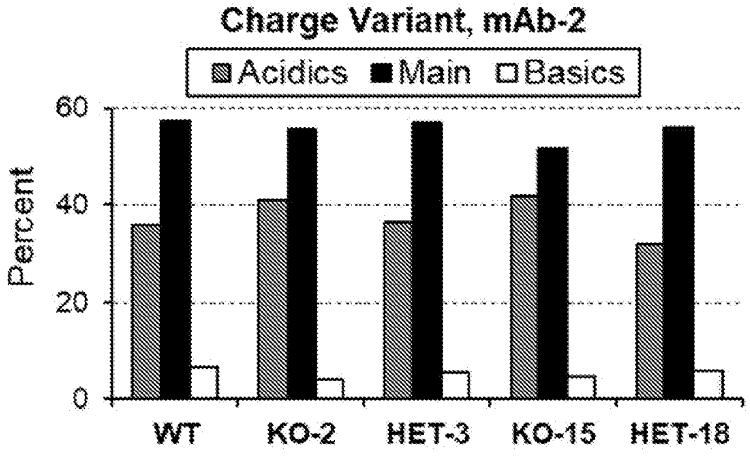

Charge Variant, mAb-2
Acidics
Main
Basics
Percent
60
40
20
0
WT
KO-2
HET-3
KO-15
HET-18

FIG. 5H

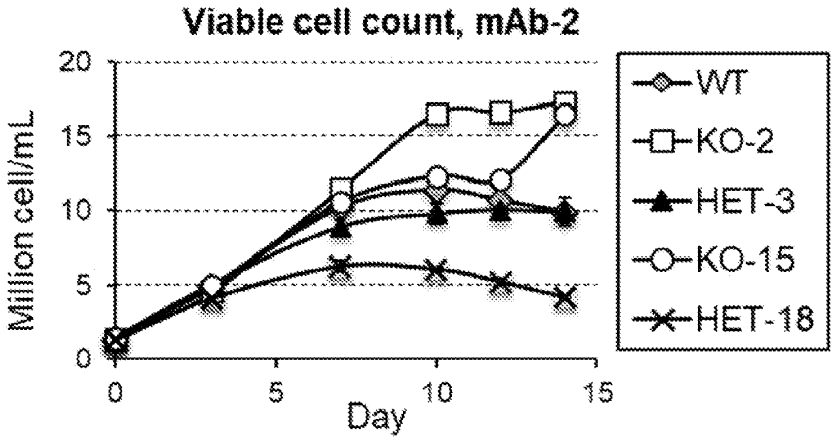

Viable cell count, mAb-2
Million cell/mL
20
15
10
5
0
0
5
10
15
Day
WT
KO-2
HET-3
KO-15
HET-18

FIG. 6A

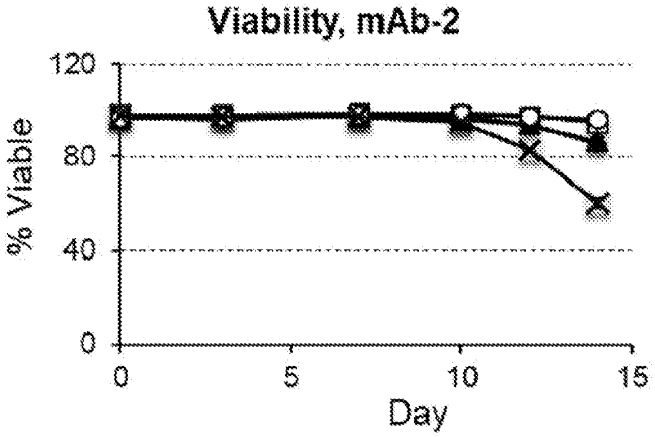

Viability, mAb-2
% Viable
120
80
40
0
0
5
10
15
Day

FIG. 6B

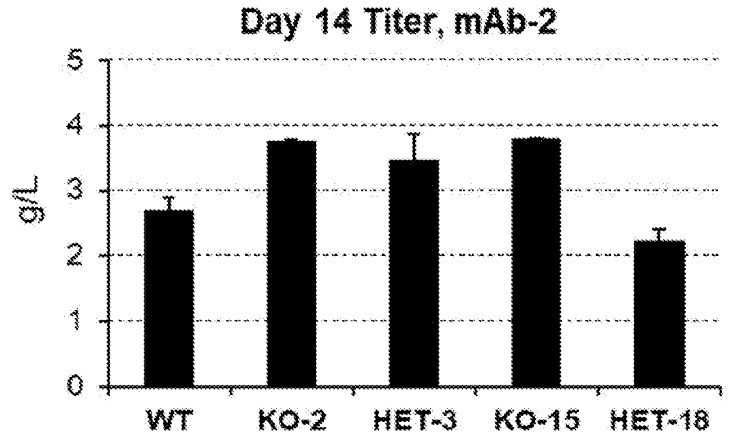

Day 14 Titer, mAb-2
g/L
5
4
3
2
1
0
WT
KO-2
HET-3
KO-15
HET-18

FIG. 6C

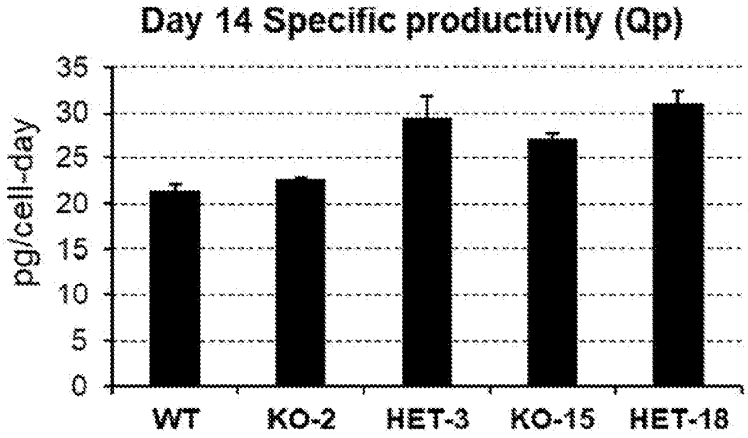

Day 14 Specific productivity (Qp)
pg/cell-day
35
30
25
20
15
10
5
0
WT
KO-2
HET-3
KO-15
HET-18

FIG. 6D

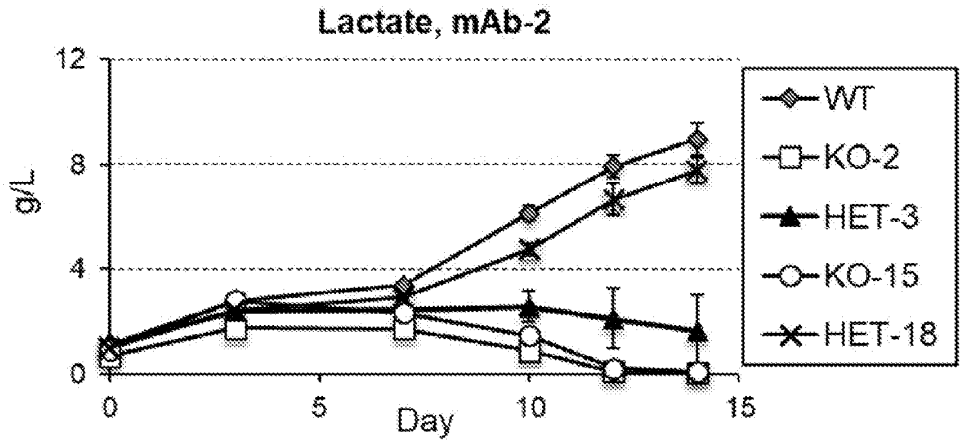


FIG. 6E

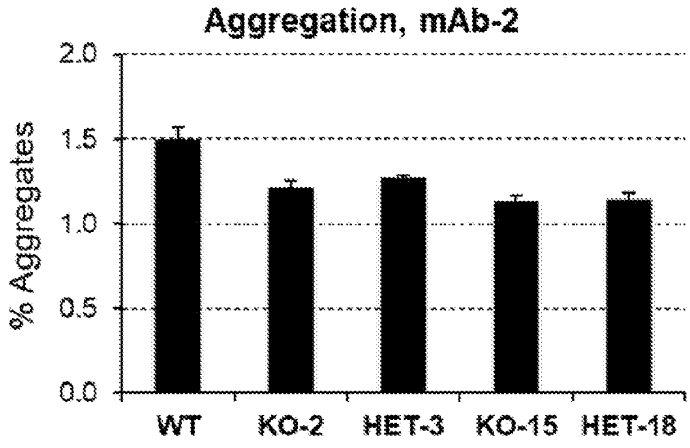


FIG. 6F

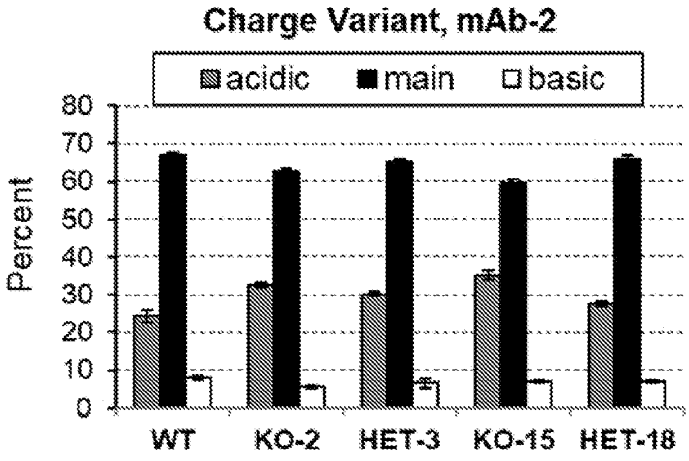


FIG. 6G

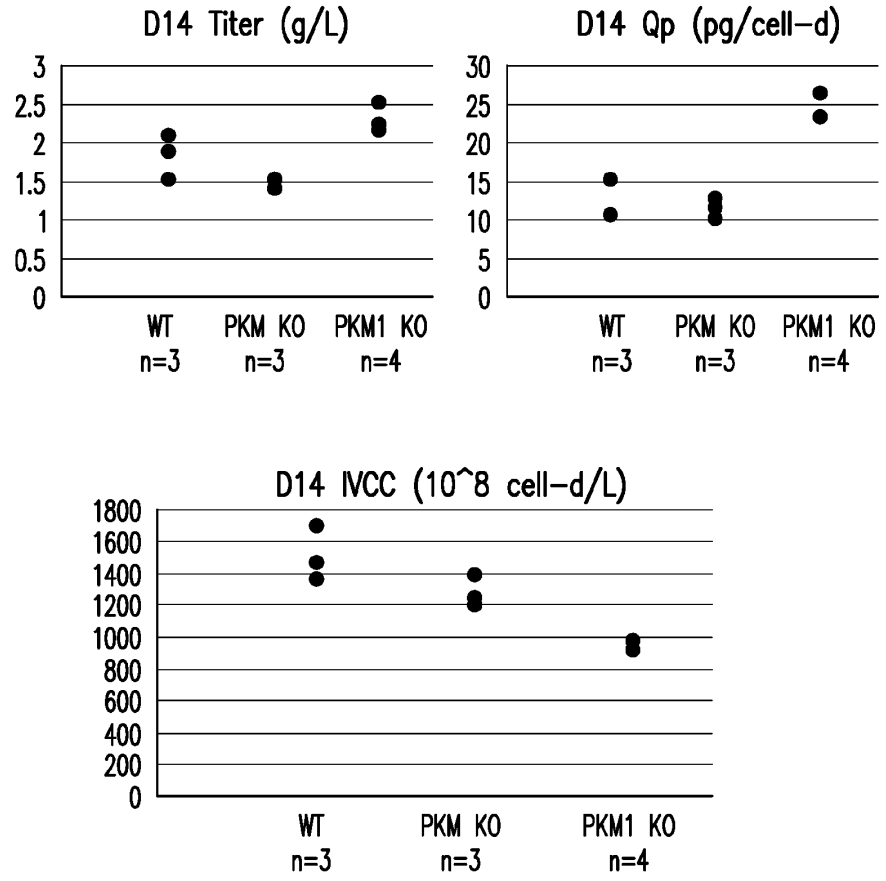

D14 Titer (g/L)
3
2.5
2
1.5
1
0.5
0
WT n=3
PKM KO n=3
PKM1 KO n=4
D14 Qp (pg/cell-d)
30
25
20
15
10
5
0
WT n=3
PKM KO n=3
PKM1 KO n=4
D14 IVCC (10^8 cell-d/L)
1800
1600
1400
1200
1000
800
600
400
200
0
WT n=3
PKM KO n=3
PKM1 KO n=4

FIG. 7

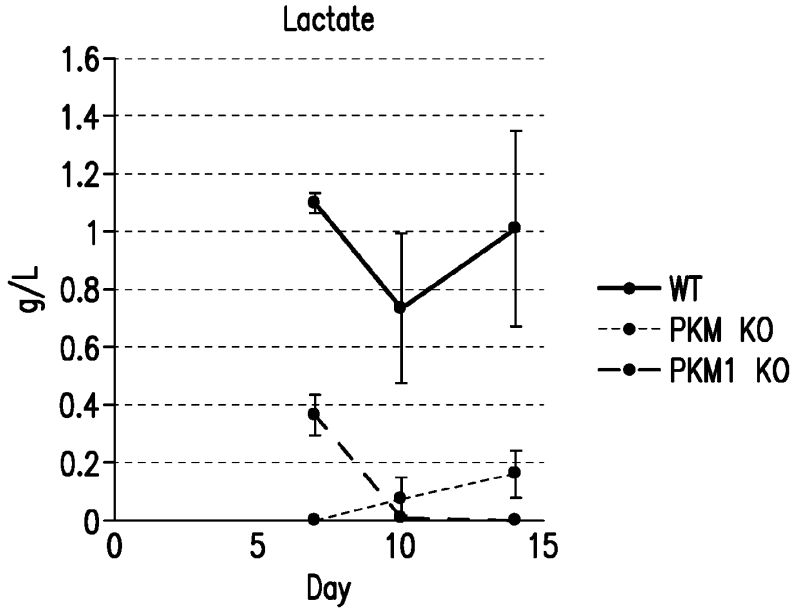

Lactate
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
g/L
0
5
10
15
Day
WT
PKM KO
PKM1 KO

FIG. 8A

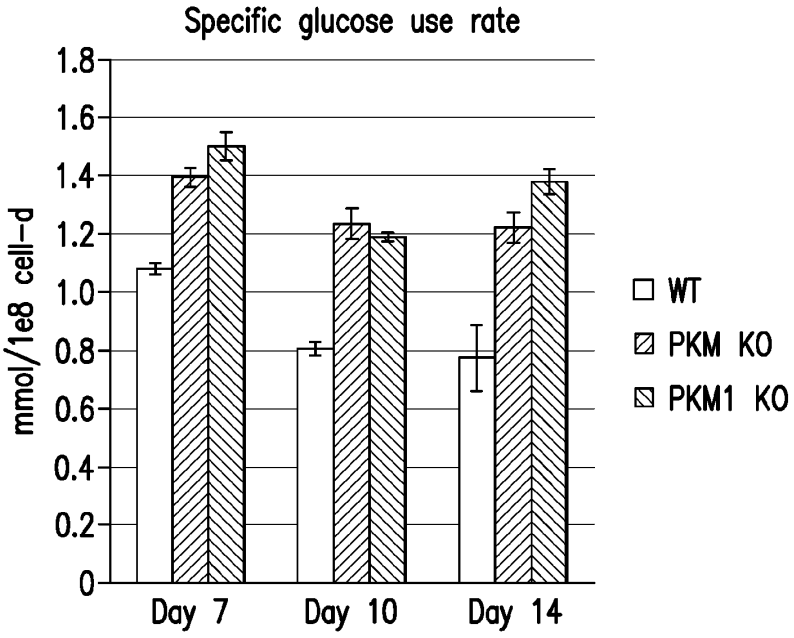

Specific glucose use rate
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
mmol/1e8 cell-d
Day 7
Day 10
Day 14
WT
PKM KO
PKM1 KO

FIG. 8B

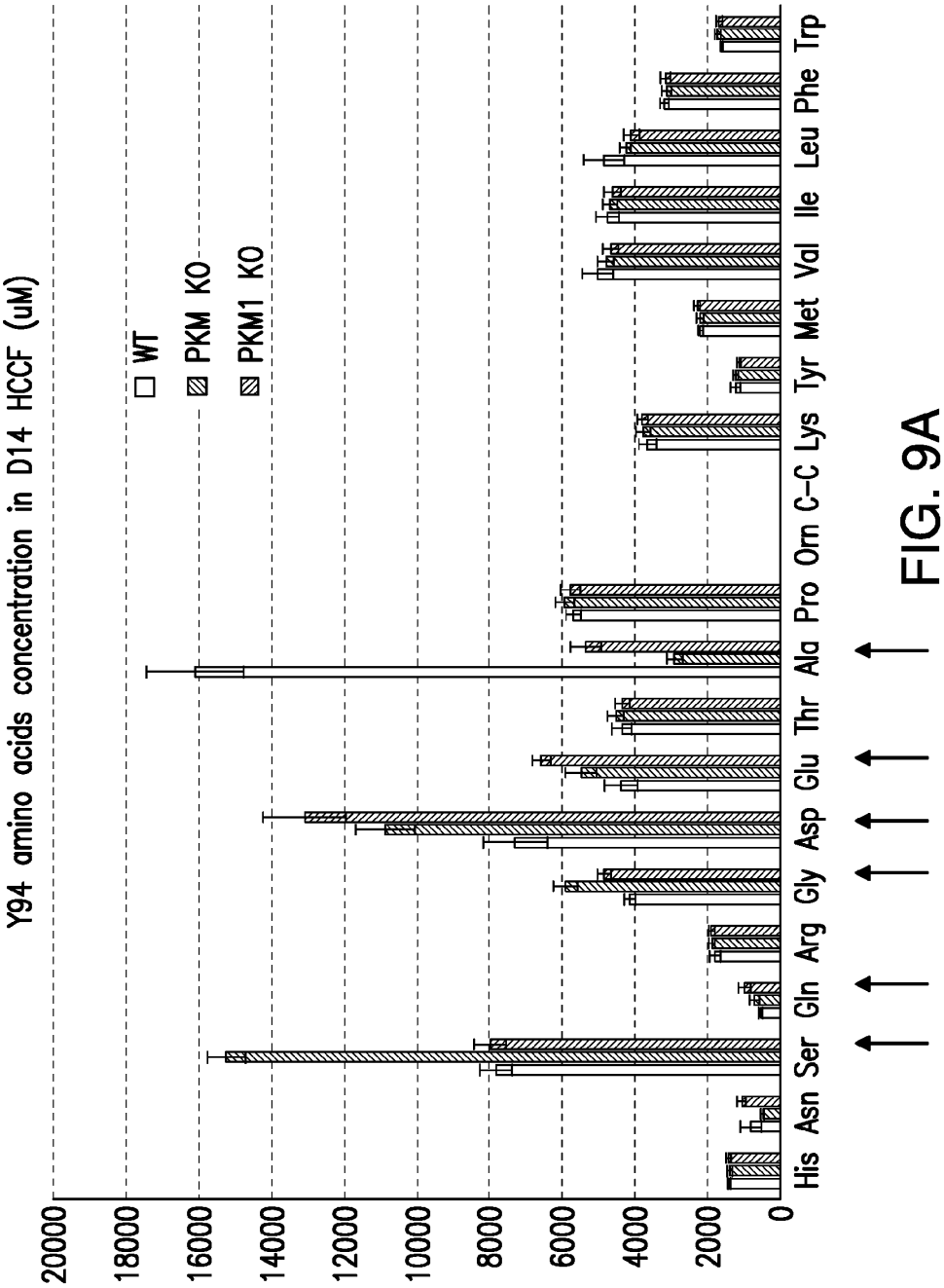

Y94 amino acids concentration in D14 HCCF (uM)
WT
PKM KO
PKM1 KO
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
His Asn Ser Gln Arg Gly Asp Glu Thr Ala Pro Orn C-C Lys Tyr Met Val Ile Leu Phe Trp

FIG. 9A

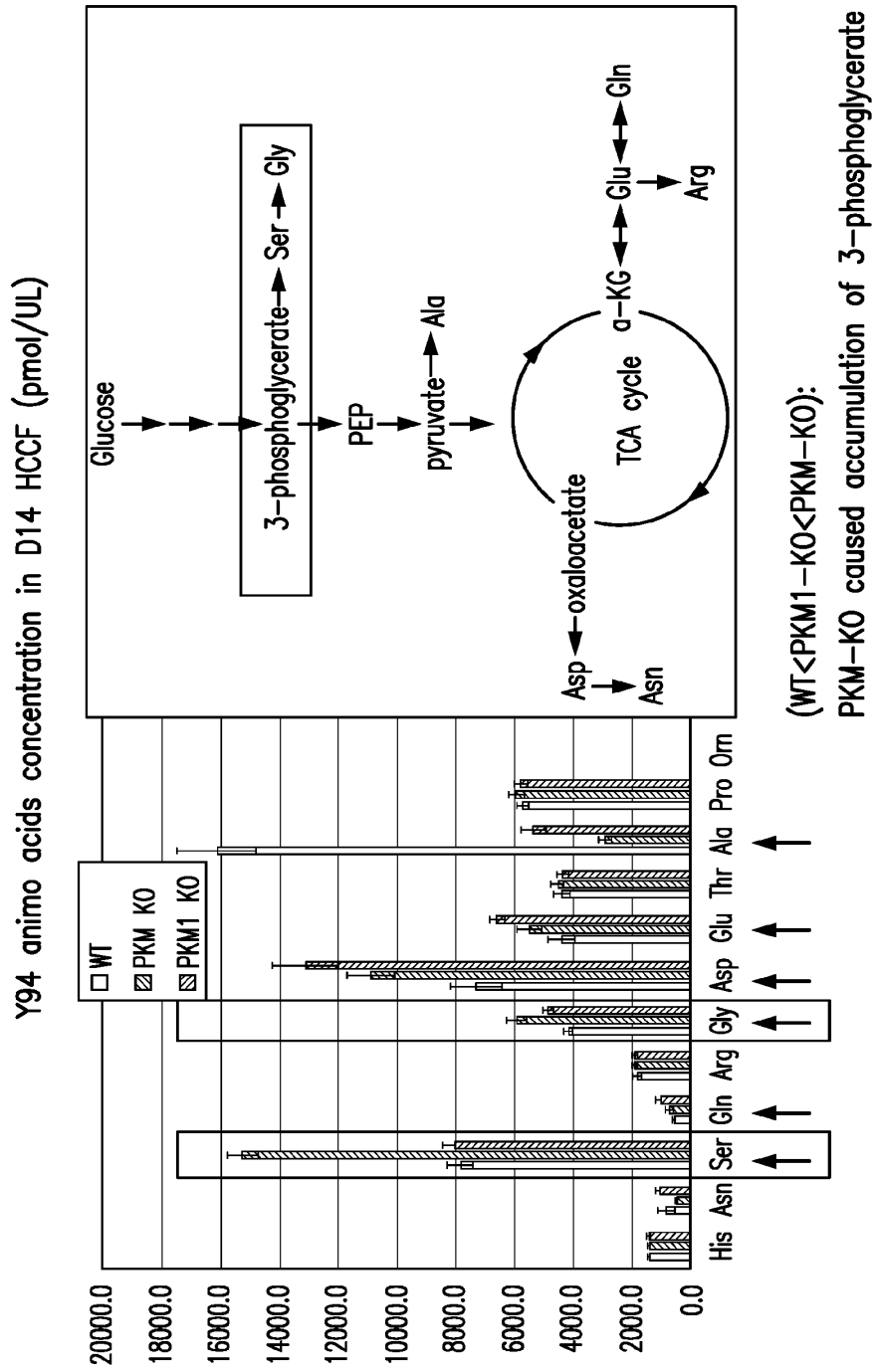

Y94 animo acids concentration in D14 HCCF (pmol/UL)
WT
PKM KO
PKM1 KO
20000.0
18000.0
16000.0
14000.0
12000.0
10000.0
8000.0
6000.0
4000.0
2000.0
0.0
His Asn Ser Gln Arg Gly Asp Glu Thr Ala Pro Orn
Glucose
3-phosphoglycerate
Ser
Gly
PEP
pyruvate
Ala
TCA cycle
a-KG
Glu
Gln
Arg
Asp
oxaloacetate
Asn
(WT<PKM1-KO<PKM-KO):
PKM-KO caused accumulation of 3-phosphoglycerate

FIG. 9B

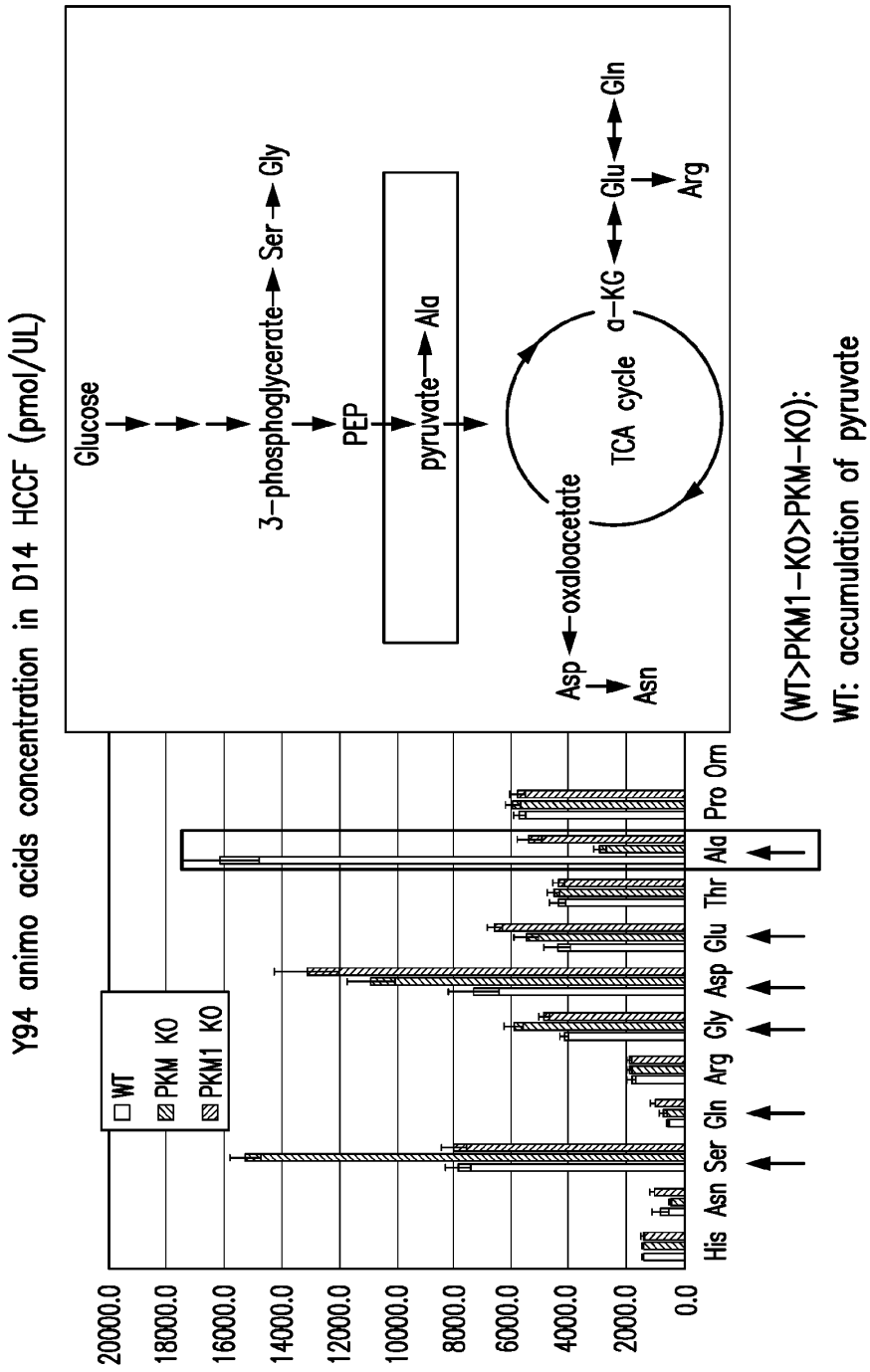

Y94 animo acids concentration in D14 HCCF (pmol/UL)
WT
PKM KO
PKM1 KO
20000.0
18000.0
16000.0
14000.0
12000.0
10000.0
8000.0
6000.0
4000.0
2000.0
0.0
His Asn Ser Gln Arg Gly Asp Glu Thr Ala Pro Orn
Glucose
3-phosphoglycerate
Ser
Gly
PEP
pyruvate
Ala
Asp
oxaloacetate
Asn
TCA cycle
α-KG
Glu
Gln
Arg
(WT>PKM1-KO>PKM-KO):
WT: accumulation of pyruvate

FIG. 9C

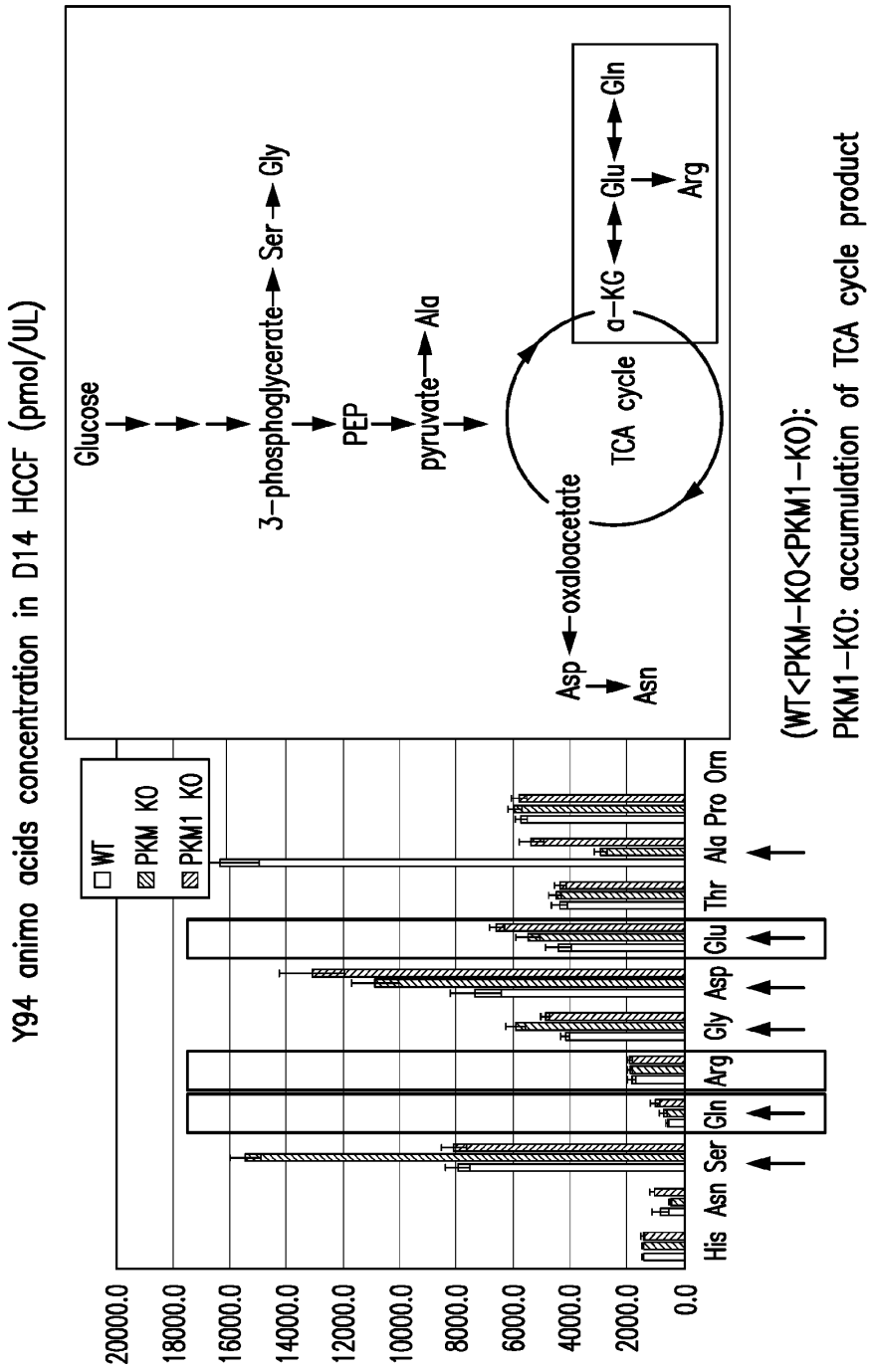

Y94 animo acids concentration in D14 HCCF (pmol/UL)
WT
PKM KO
PKM1 KO
20000.0
18000.0
16000.0
14000.0
12000.0
10000.0
8000.0
6000.0
4000.0
2000.0
0.0
His Asn Ser Gln Arg Gly Asp Glu Thr Ala Pro Orn
Glucose
3-phosphoglycerate
Ser
Gly
PEP
pyruvate
Ala
Asp
oxaloacetate
Asn
TCA cycle
a-KG
Glu
Gln
Arg
(WT<PKM-KO<PKM1-KO):
PKM1-KO: accumulation of TCA cycle product

FIG. 9D

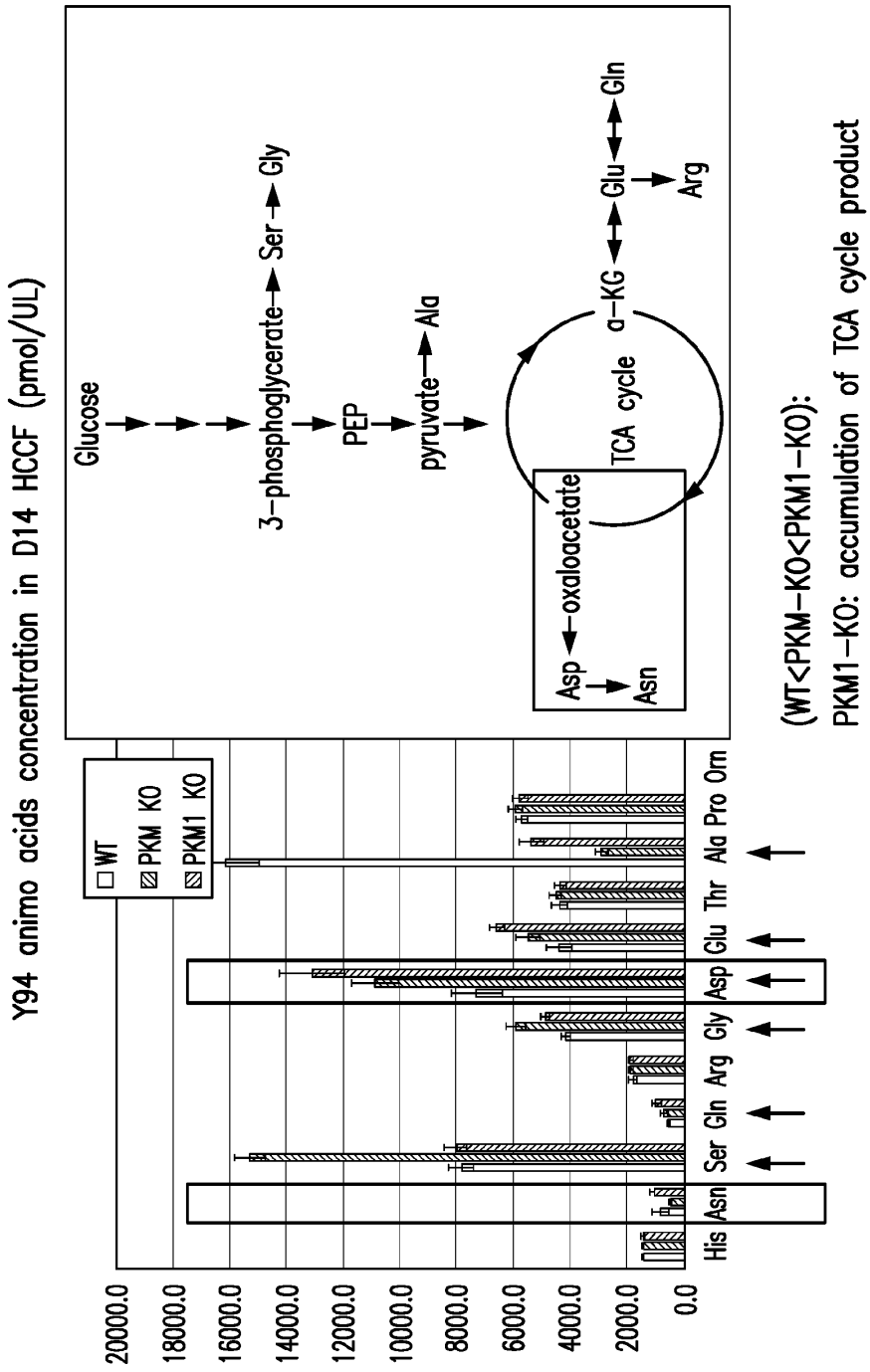

Y94 animo acids concentration in D14 HCCF (pmol/UL)
WT
PKM KO
PKM1 KO
20000.0
18000.0
16000.0
14000.0
12000.0
10000.0
8000.0
6000.0
4000.0
2000.0
0.0
His Asn Ser Gln Arg Gly Asp Glu Thr Ala Pro Orn
Glucose
3-phosphoglycerate
Ser
Gly
PEP
pyruvate
Ala
TCA cycle
a-KG
Glu
Gln
Arg
Asp
oxaloacetate
Asn
(WT<PKM-KO<PKM1-KO):
PKM1-KO: accumulation of TCA cycle product

FIG. 9E

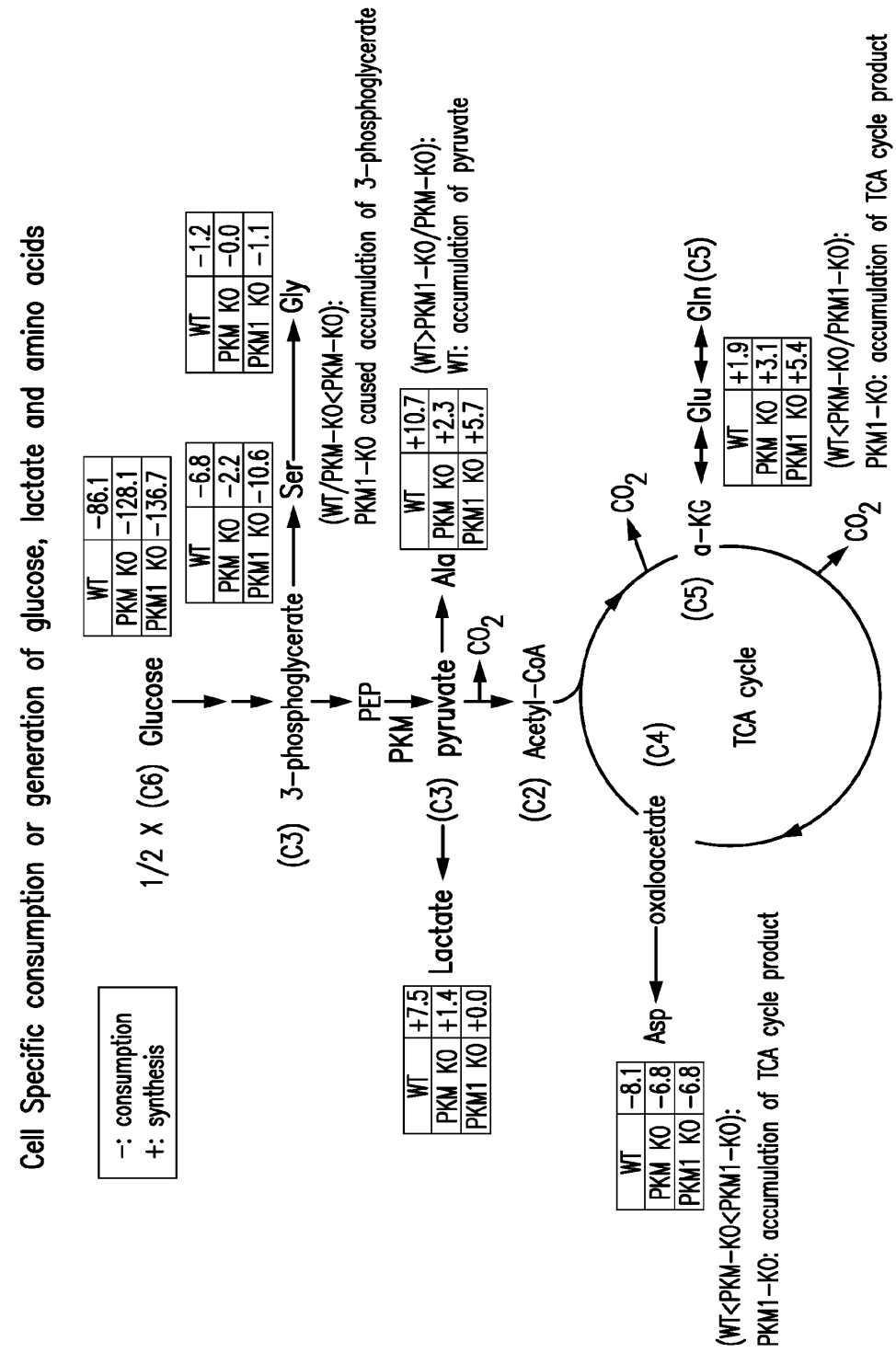

Cell Specific consumption or generation of glucose, lactate and amino acids
-: consumption
+: synthesis
1/2 X (C6) Glucose
WT -86.1
PKM KO -128.1
PKM1 KO -136.7
(C3) 3-phosphoglycerate
WT -6.8
PKM KO -2.2
PKM1 KO -10.6
Ser
WT -1.2
PKM KO -0.0
PKM1 KO -1.1
Gly
(WT/PKM-KO<PKM-KO):
PKM1-KO caused accumulation of 3-phosphoglycerate
PEP
PKM
(C3) pyruvate
WT +10.7
PKM KO +2.3
PKM1 KO +5.7
Ala
(WT>PKM1-KO/PKM-KO):
WT: accumulation of pyruvate
Lactate
WT +7.5
PKM KO +1.4
PKM1 KO +0.0
(C3)
CO2
(C2) Acetyl-CoA
CO2
(C5) α-KG
Glu
Gln (C5)
WT +1.9
PKM KO +3.1
PKM1 KO +5.4
(C4)
TCA cycle
CO2
Asp
oxaloacetate
WT -8.1
PKM KO -6.8
PKM1 KO -6.8
(WT<PKM-KO<PKM1-KO):
PKM1-KO: accumulation of TCA cycle product
(WT<PKM-KO/PKM1-KO):
PKM1-KO: accumulation of TCA cycle product

FIG. 10

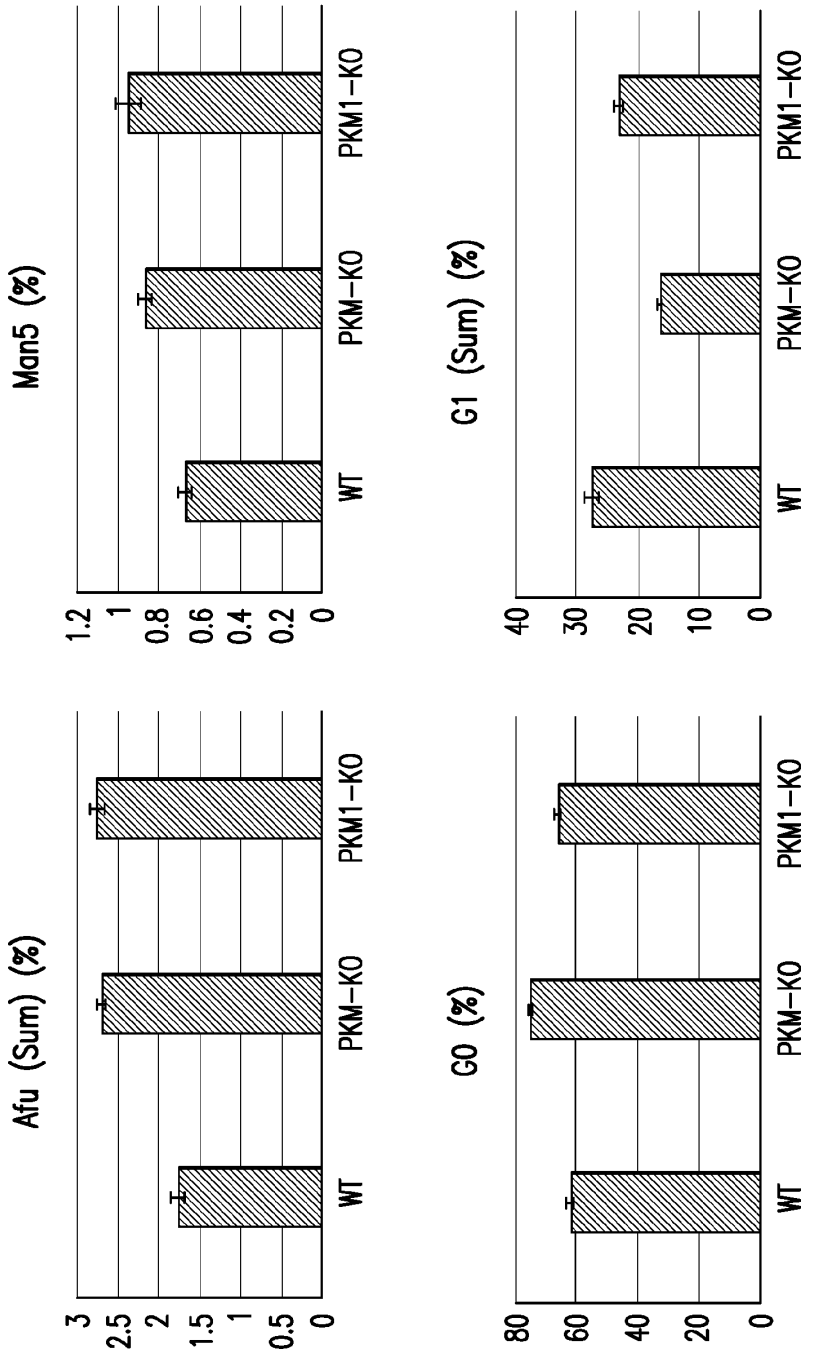

Man5 (%)
1.2
1
0.8
0.6
0.4
0.2
0
WT
PKM-KO
PKM1-KO
G1 (Sum) (%)
40
30
20
10
0
WT
PKM-KO
PKM1-KO
Afu (Sum) (%)
3
2.5
2
1.5
1
0.5
0
WT
PKM-KO
PKM1-KO
G0 (%)
80
60
40
20
0
WT
PKM-KO
PKM1-KO

FIG. 11