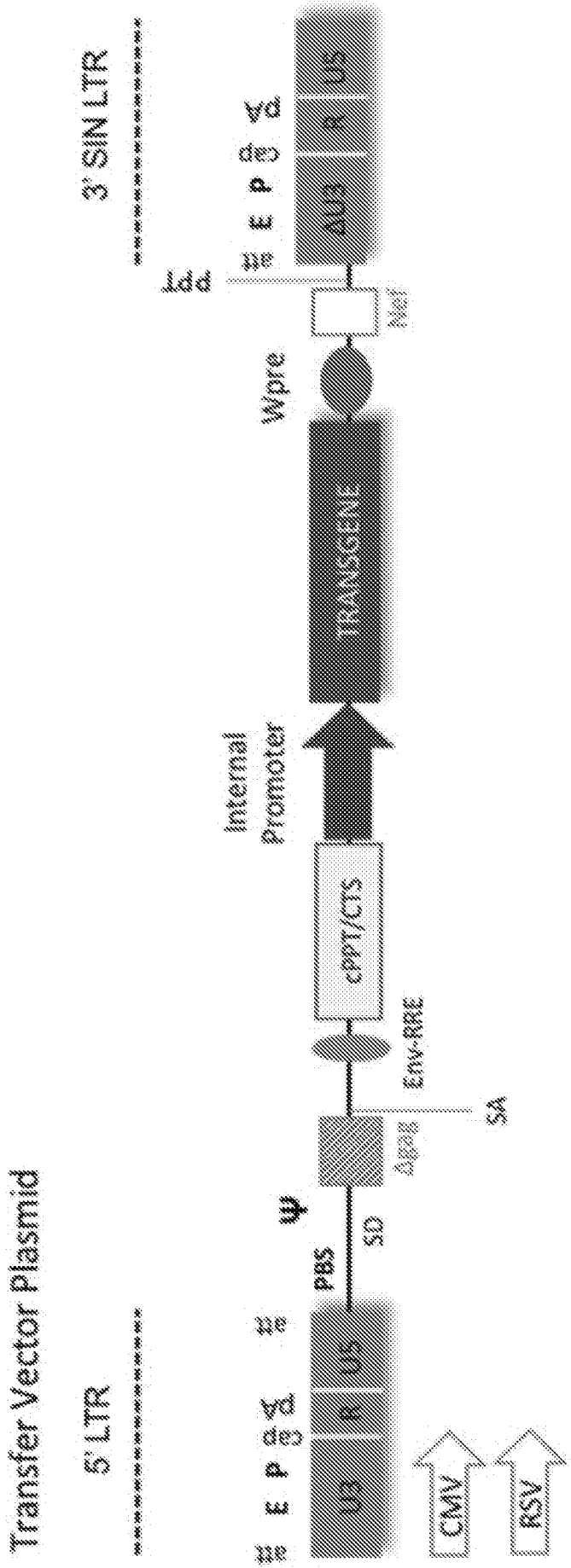

Transfer Vector Plasmid
5' LTR
3' SIN LTR
U3
R
U5
CMV
RSV
PBS
SD
SA
Env-RRE
cPPT/CTS
Internal Promoter
TRANSGENE
Wpre
Not
ΔU3
R
U5

FIG. 1

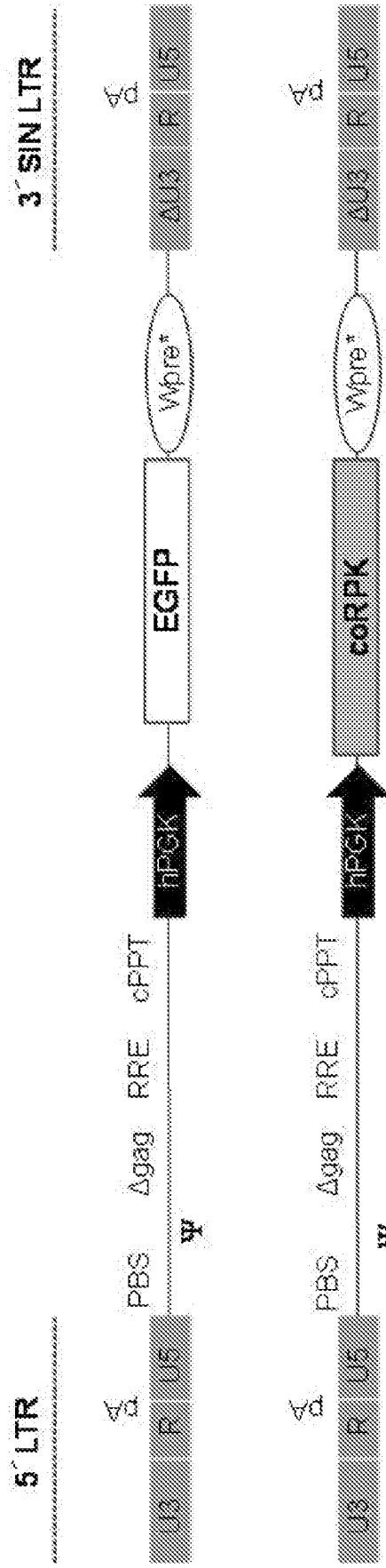

5´LTR
3´SIN LTR
∀d
U3
R
U5
PBS
Ψ
Δgag
RRE
cPPT
hPGK
EGFP
coRPK
Wpre*

FIG. 2A

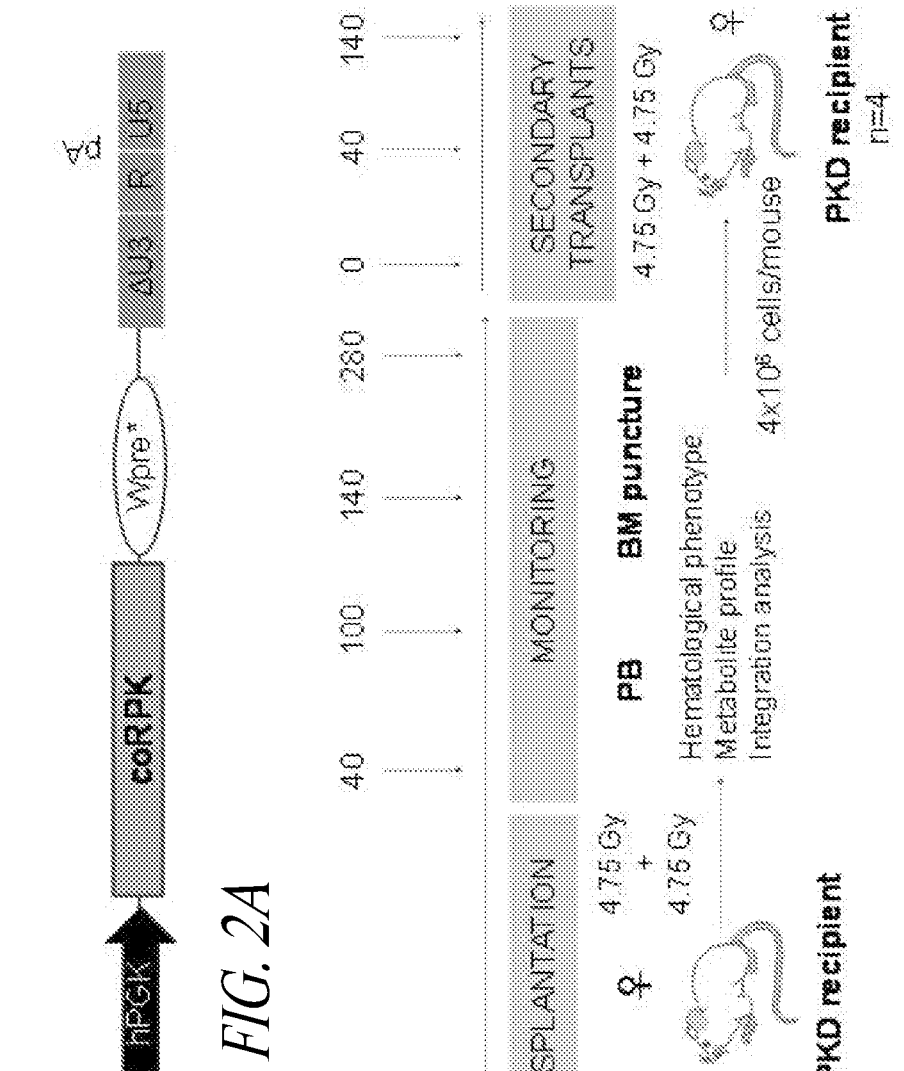

0
1
2
3
40
100
140
280
0
40
140
expansion
BM harvest
2x TRANSDUCTION
TRANSPLANTATION
MONITORING
SECONDARY TRANSPLANTS
MOI = 10
4x10⁴ – 2x10⁵ cells/mouse
4.75 Gy + 4.75 Gy
PB
BM puncture
4.75 Gy + 4.75 Gy
Hematological phenotype
Metabolite profile
Integration analysis
4x10⁶ cells/mouse
Lin-
hPGK-EGFP n=8
hPGK-coRPK n=17
PKD donor
PKD recipient
PKD recipient n=4

FIG. 2B

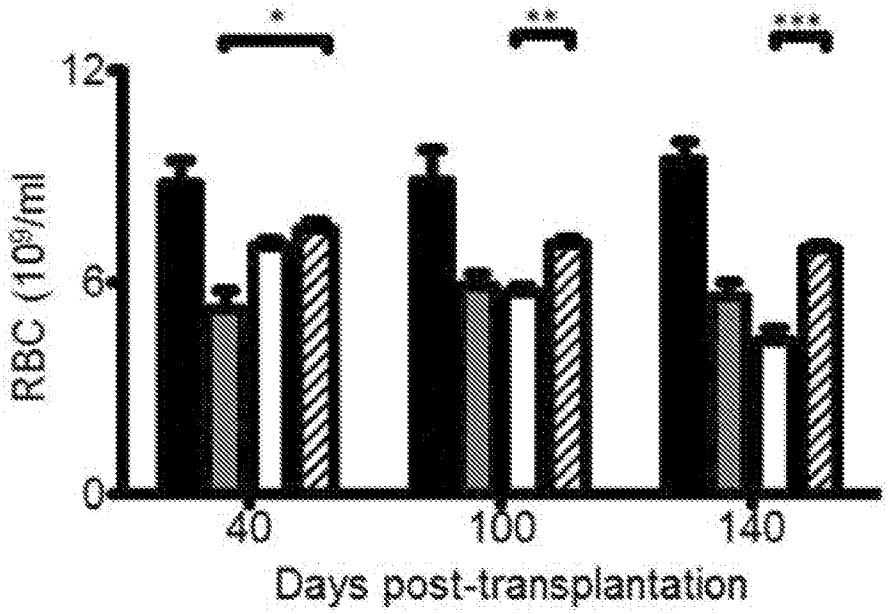

*
**
***
12
6
0
RBC (10^9/ml
40
100
140
Days post-transplantation

FIG. 3A

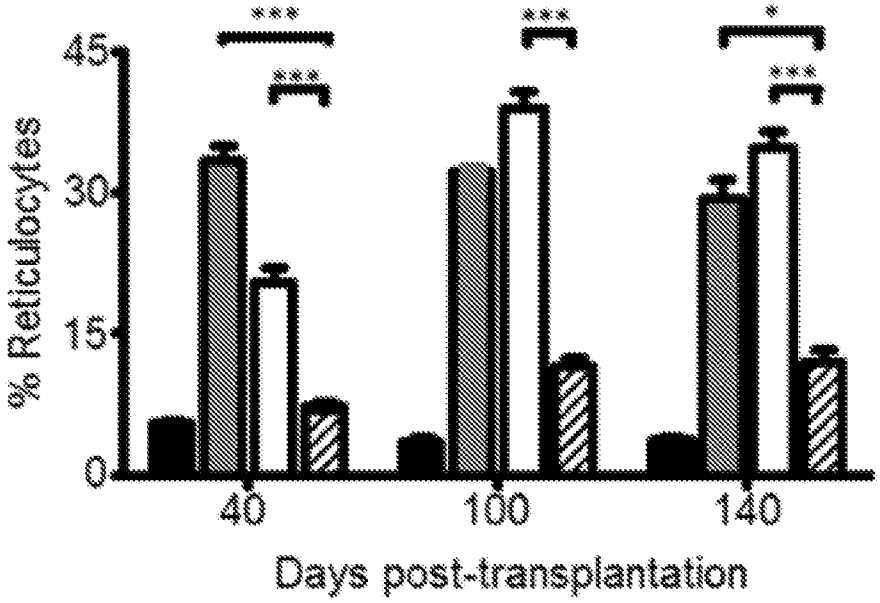

***
***
***
***
*
***
45
30
15
0
% Reticulocytes
40
100
140
Days post-transplantation

FIG. 3B

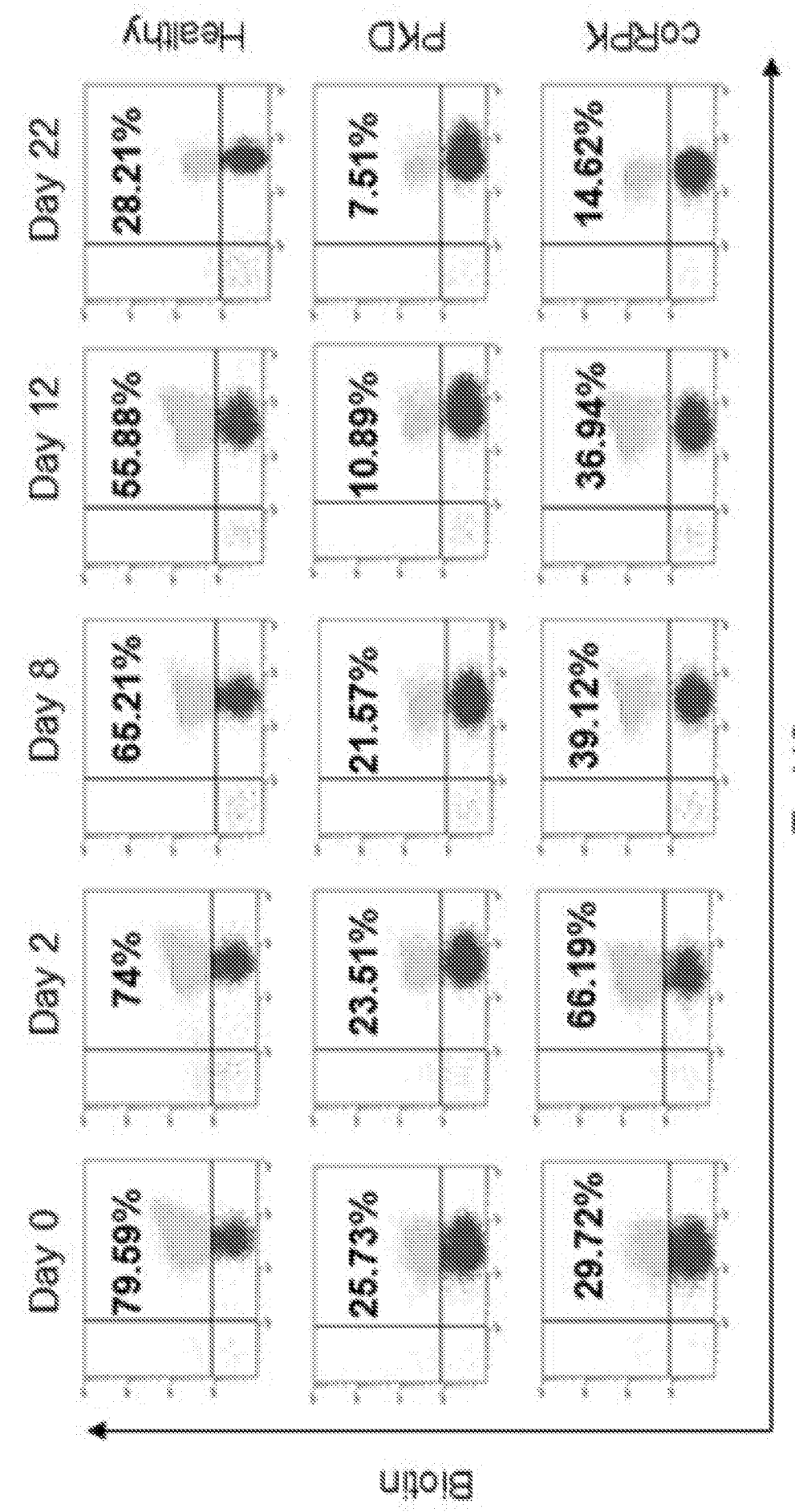

Healthy
PKD
coRPK
Day 22
Day 12
Day 8
Day 2
Day 0
28.21%
7.51%
14.62%
65.88%
10.89%
36.94%
65.21%
21.57%
39.12%
74%
23.51%
66.19%
79.59%
25.73%
29.72%
Biotin
Ter119

FIG. 3C

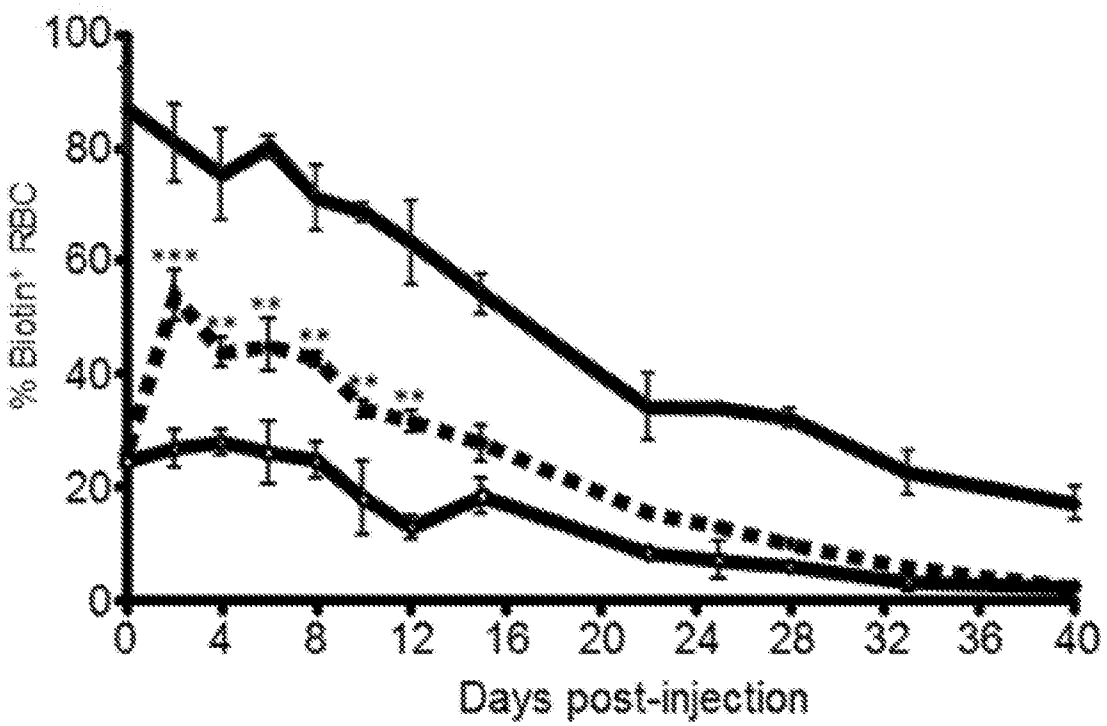

100
80
60
40
20
0
% Biotin+ RBC
***
**
**
**
*
**
0
4
8
12
16
20
24
28
32
36
40
Days post-injection

FIG. 3D

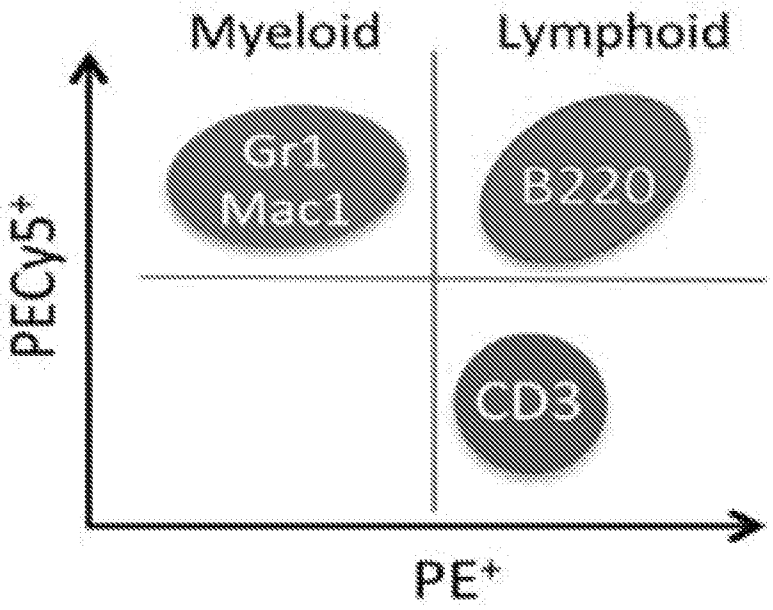

Myeloid
Lymphoid
Gr1
Mac1
B220
CD3
PECy5+
PE+

FIG. 4A

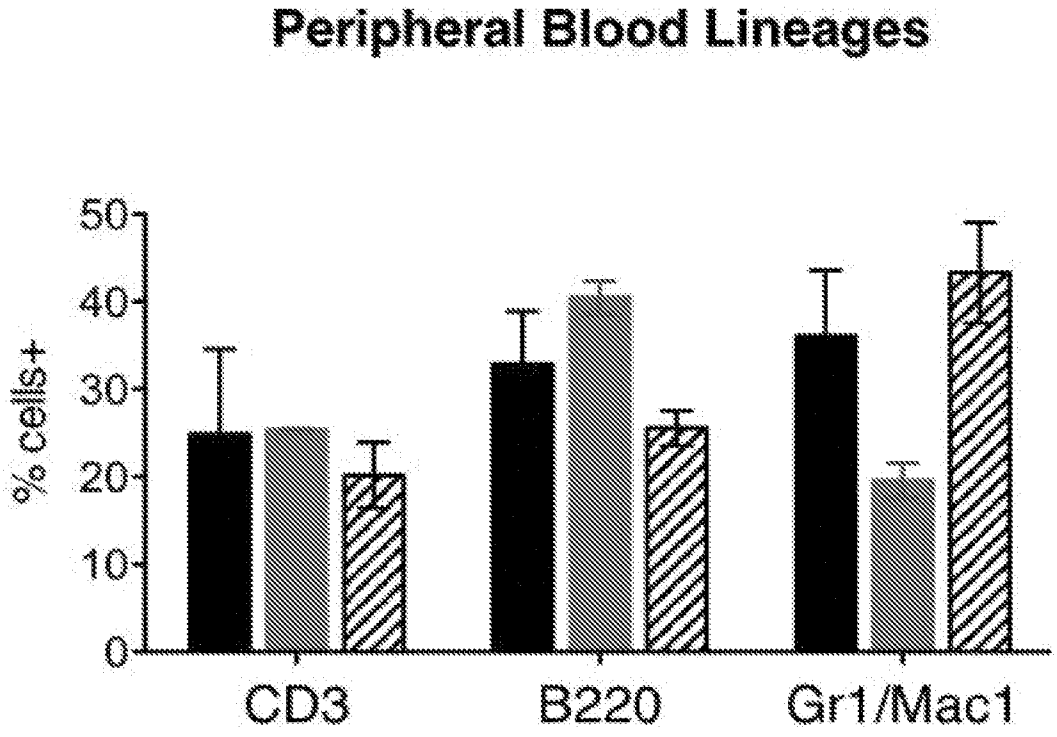

Peripheral Blood Lineages
% cells+
50
40
30
20
10
0
CD3
B220
Gr1/Mac1

FIG. 4C

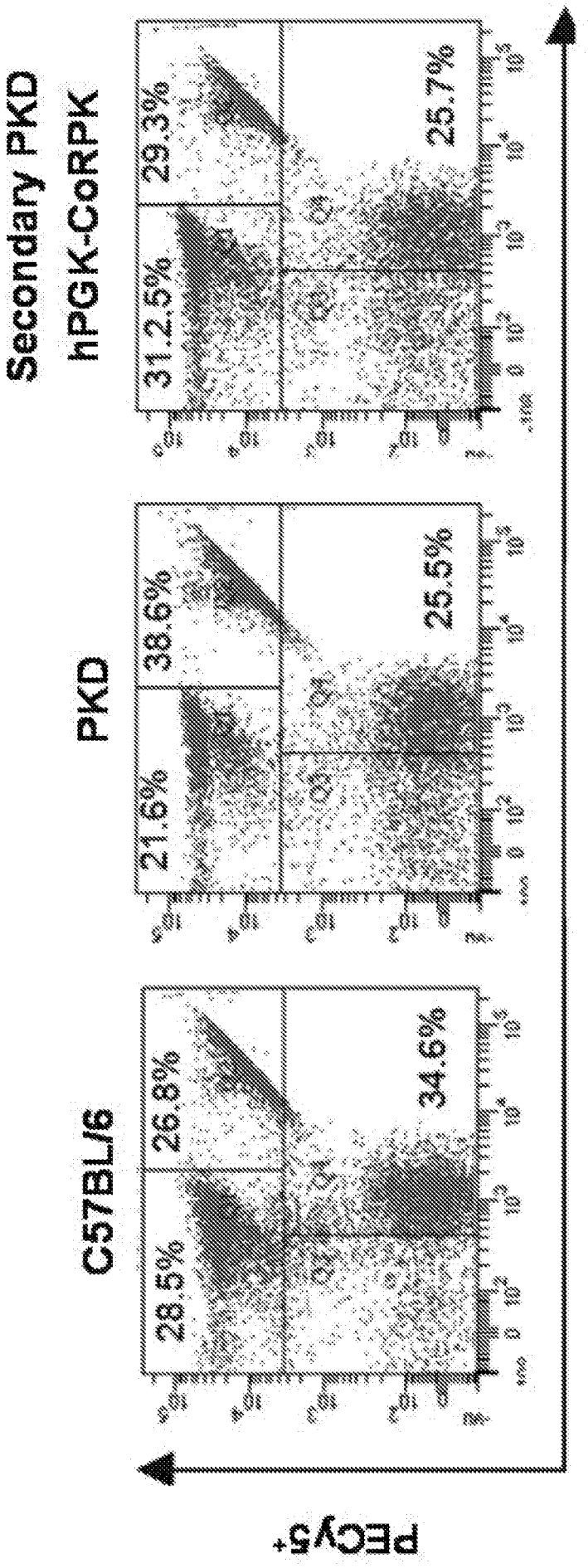

Secondary PKD
hPGK-CoRPK
PKD
C57BL/6
31.2%
29.3%
25.7%
21.6%
38.6%
25.5%
28.5%
26.8%
34.6%
PE+
PECy5+

FIG. 4B

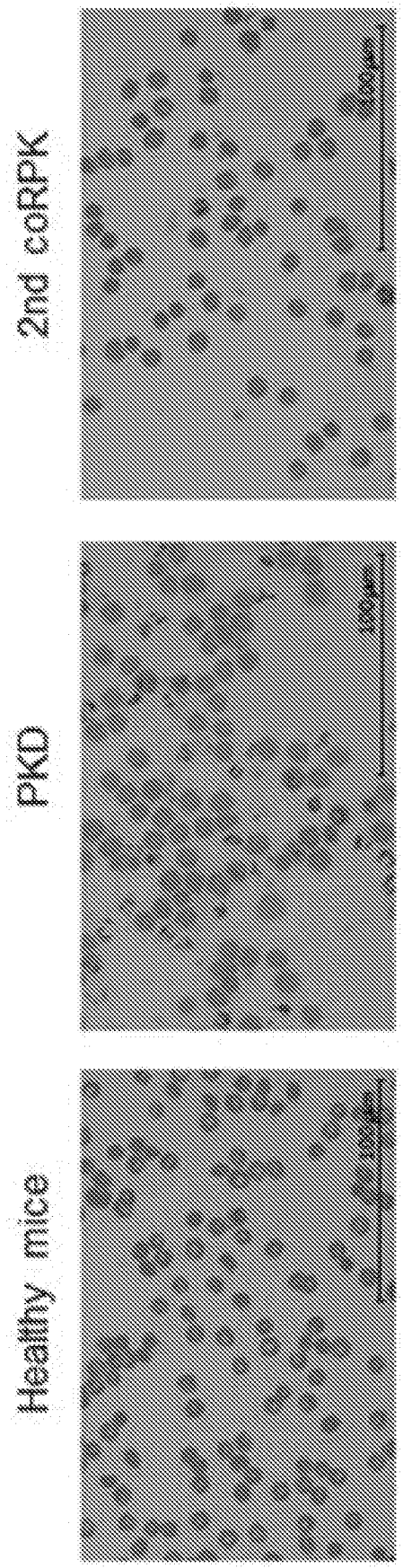

Healthy mice
PKD
2nd coRPK

FIG. 5A

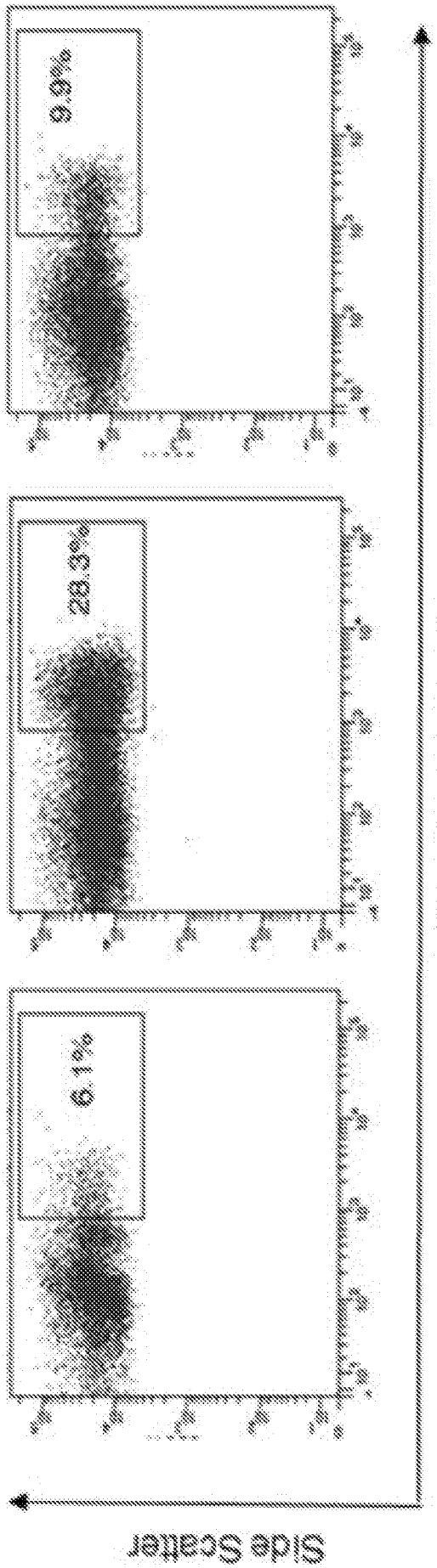

6.1%
28.3%
9.9%
Side Scatter
Erythroid RNA+ fraction

FIG. 5B

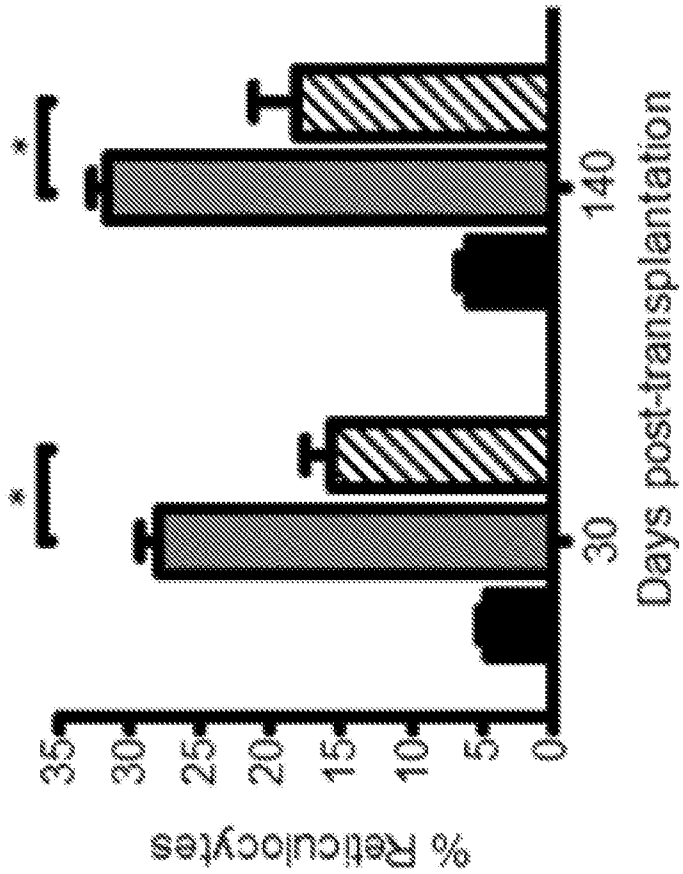

% Reticulocytes
0
5
10
15
20
25
30
35
30
140
Days post-transplantation
*
*

FIG. 5D

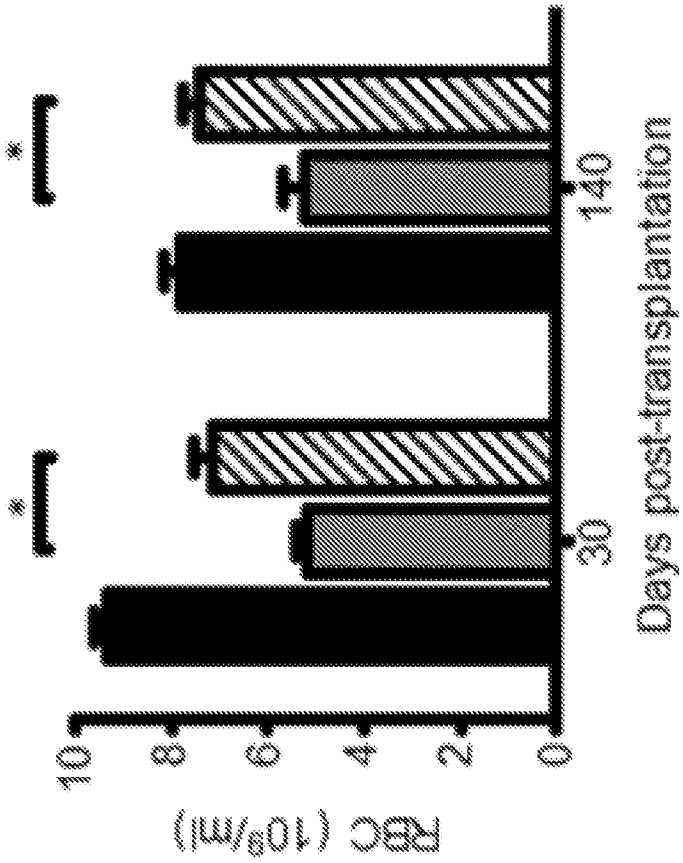

RBC (10^9/ml)
0
2
4
6
8
10
30
140
Days post-transplantation
*
*

FIG. 5C

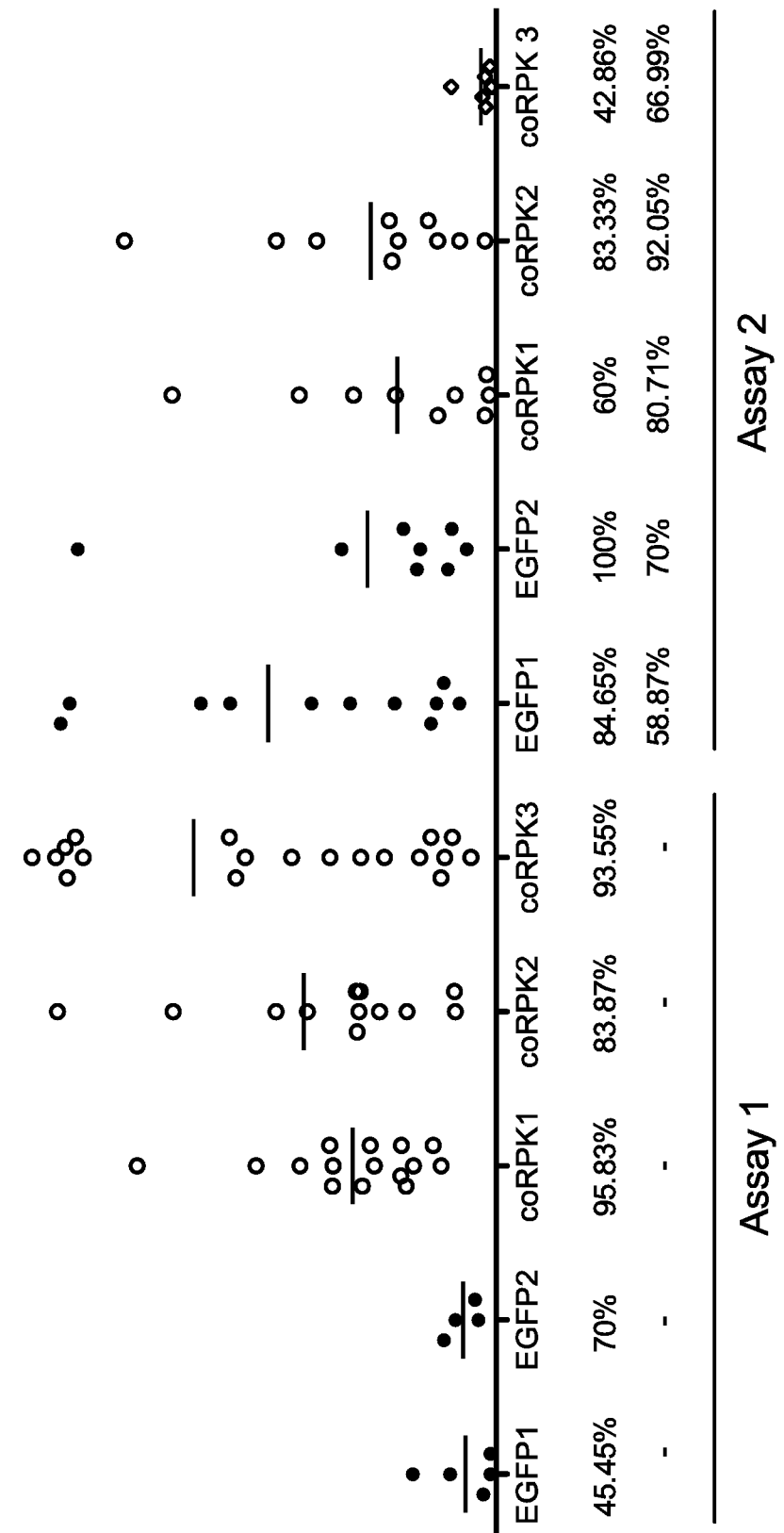

VCN / CFU-C
24
16
8
6
4
2
0
EGFP1
EGFP2
coRPK1
coRPK2
coRPK3
EGFP1
EGFP2
coRPK1
coRPK2
coRPK 3
Transduction % 45.45% 70% 95.83% 83.87% 93.55% 84.65% 100% 60% 83.33% 42.86%
Chimerism % - - - - - 58.87% 70% 80.71% 92.05% 66.99%
Assay 1
Assay 2

FIG. 6A

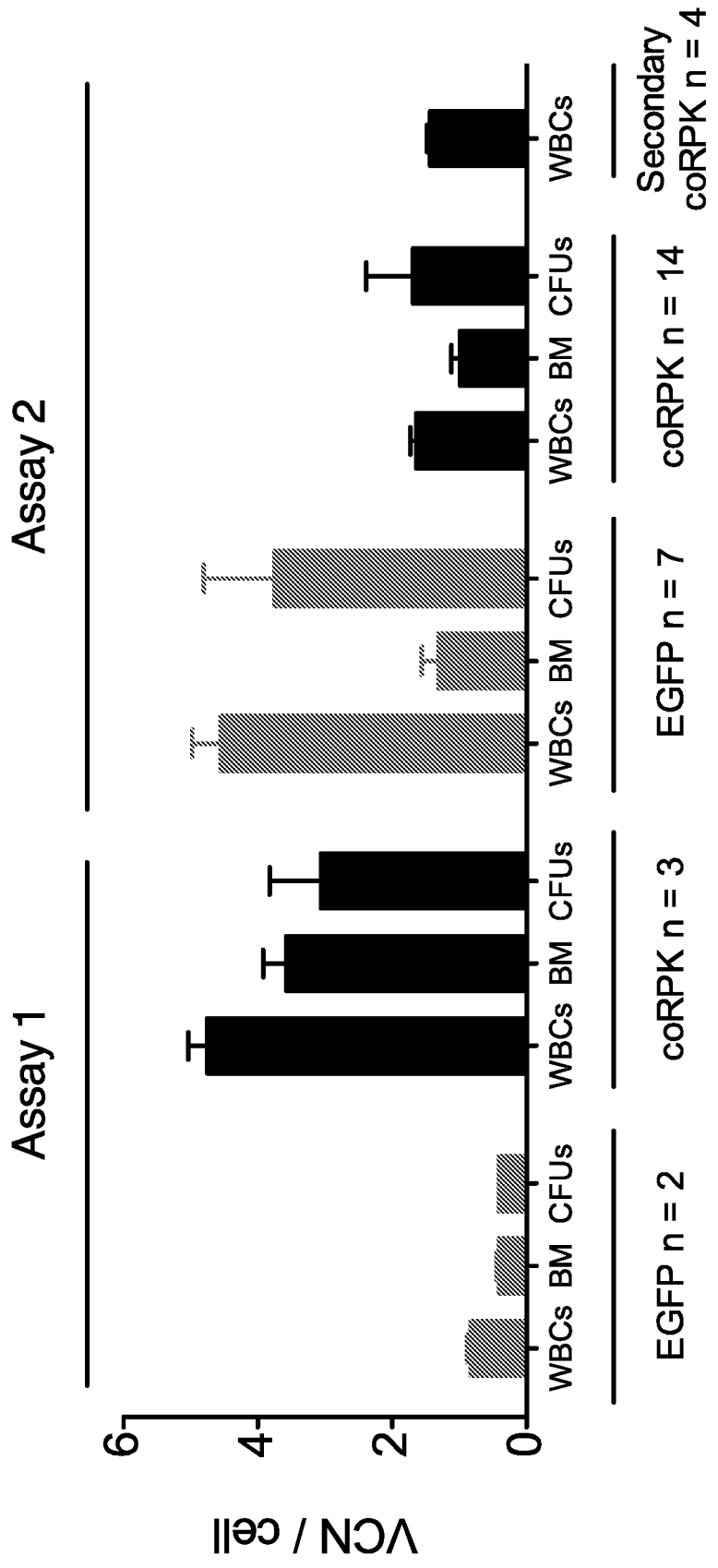

VCN / cell
0
2
4
6
Assay 1
Assay 2
WBCs
BM
CFUs
WBCs
BM
CFUs
WBCs
BM
CFUs
WBCs
EGFP n = 2
coRPK n = 3
EGFP n = 7
coRPK n = 14
Secondary coRPK n = 4

FIG. 6B

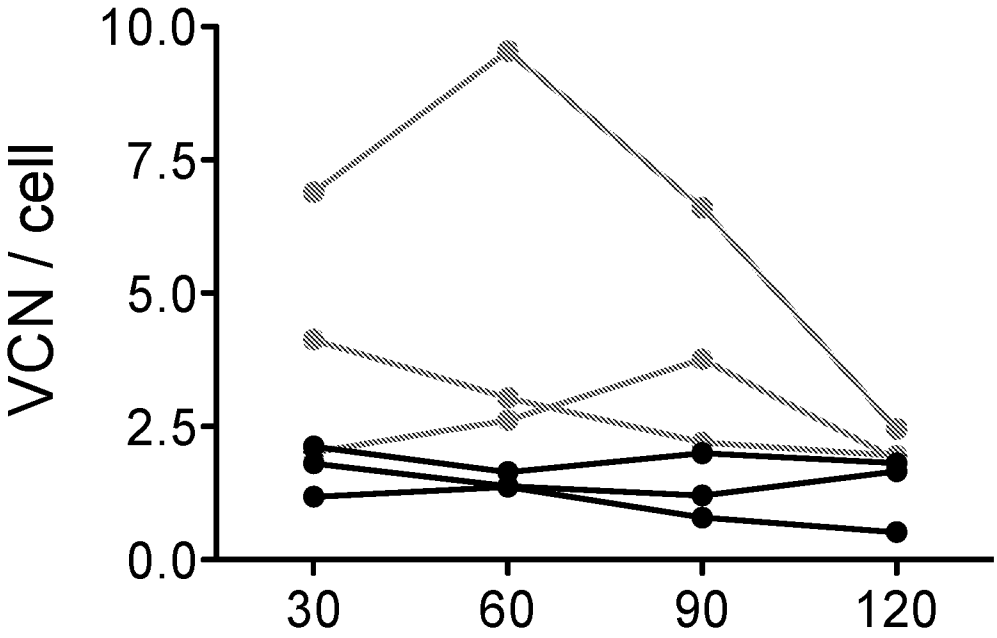

10.0
7.5
5.0
2.5
0.0
VCN / cell
30
60
90
120
Lin- EGFP VCN = 14.39
Lin- coRPK VCN = 12.74

FIG. 6C

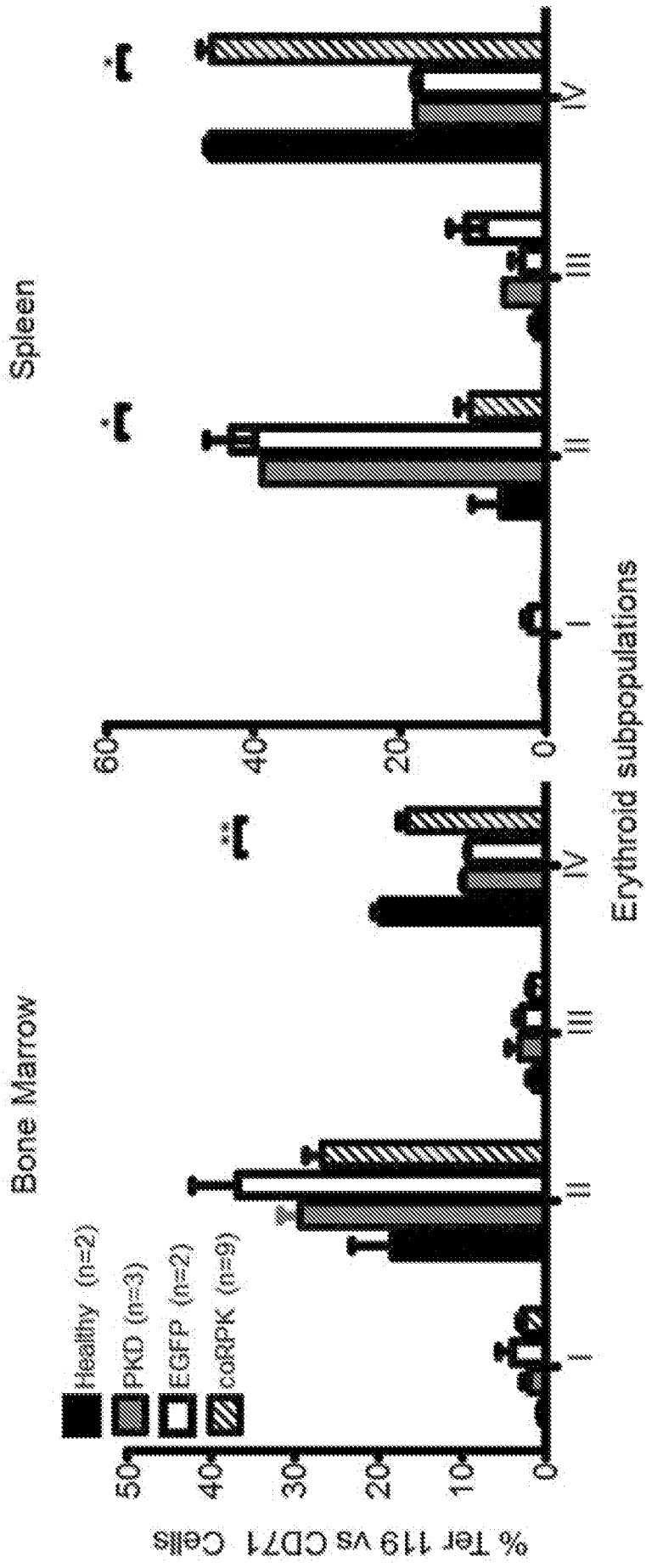

% Ter 119 vs CD71 Cells
0
10
20
30
40
50
Healthy (n=2)
PKD (n=3)
EGFP (n=2)
cpk8PK (n=9)
Bone Marrow
Spleen
I
II
III
IV
Erythroid subpopulations
*
**
*

FIG. 7A

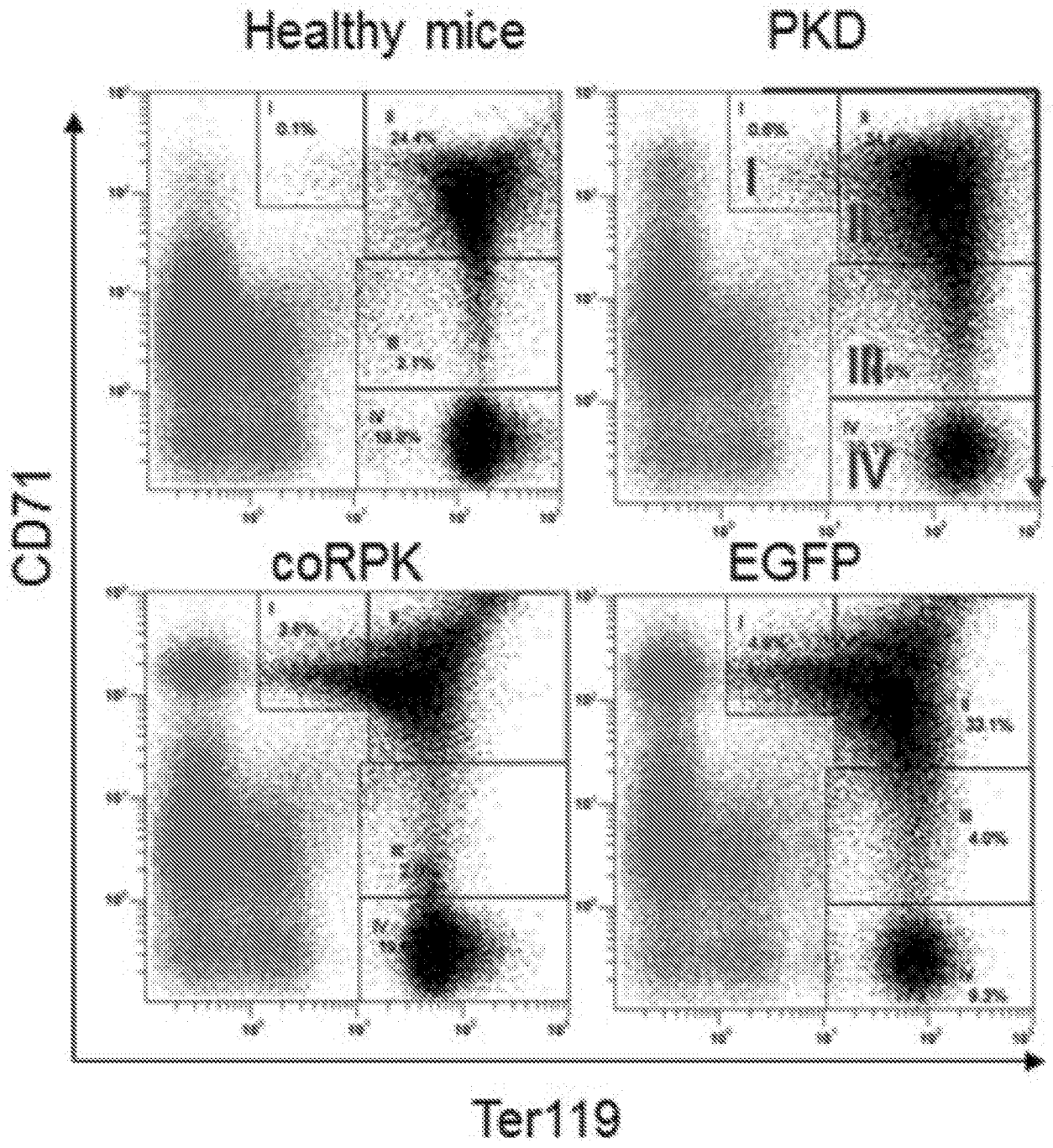


FIG. 7B

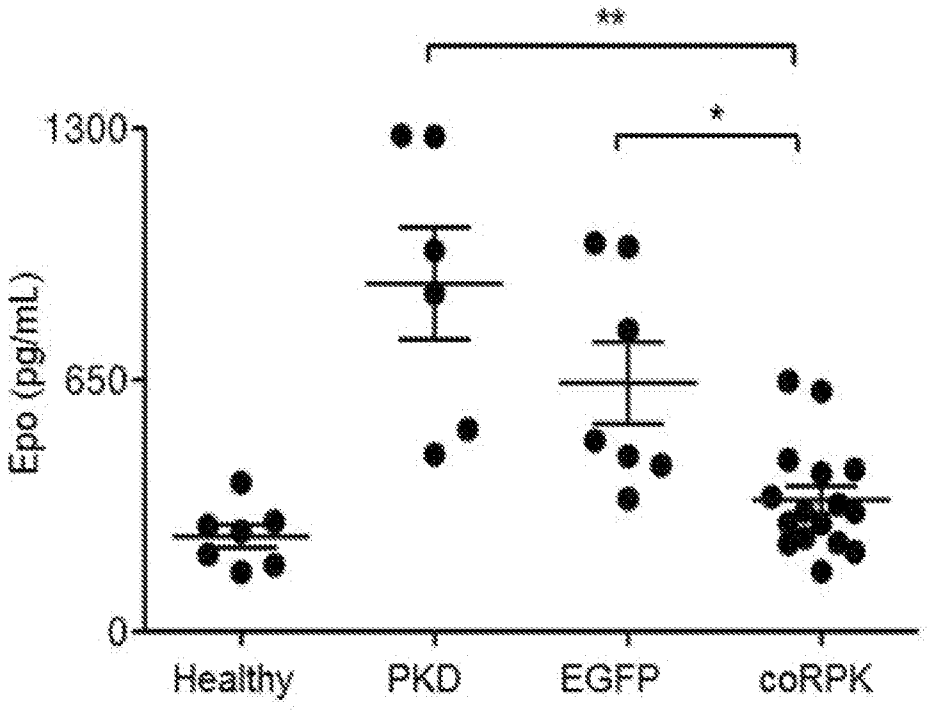


FIG. 7C

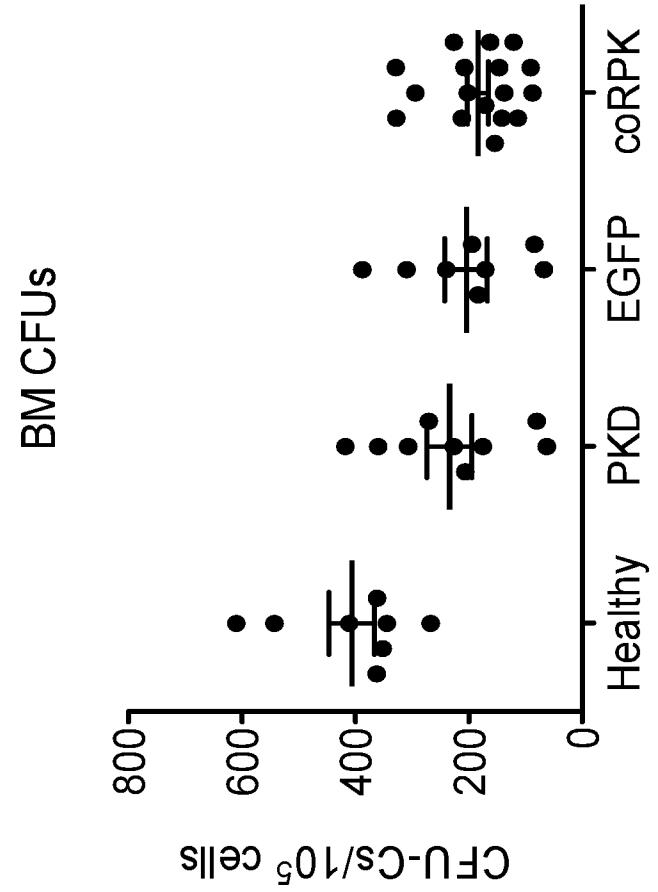

BM CFUs
CFU-Cs/10^5 cells
800
600
400
200
0
Healthy
PKD
EGFP
coRPK

FIG. 8B

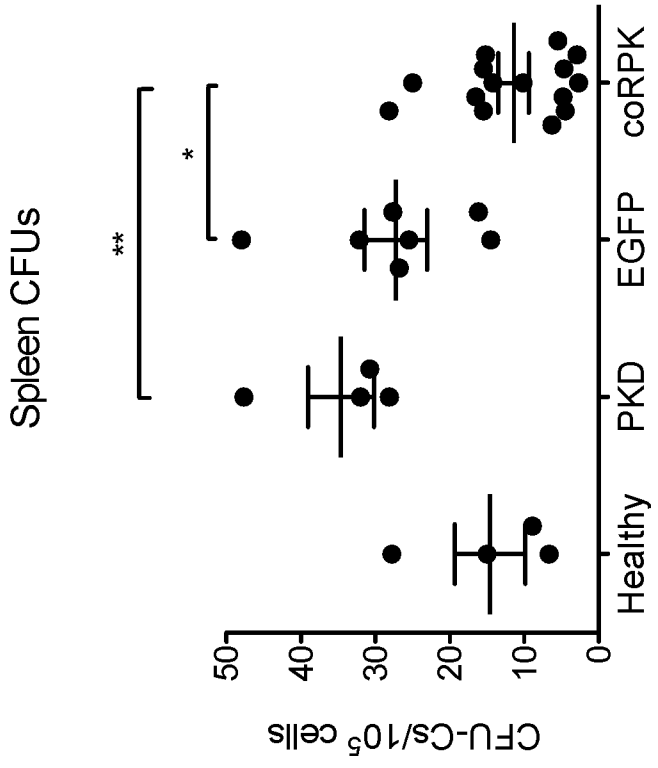

Spleen CFUs
CFU-Cs/10^5 cells
50
40
30
20
10
0
**
*
Healthy
PKD
EGFP
coRPK

FIG. 8A

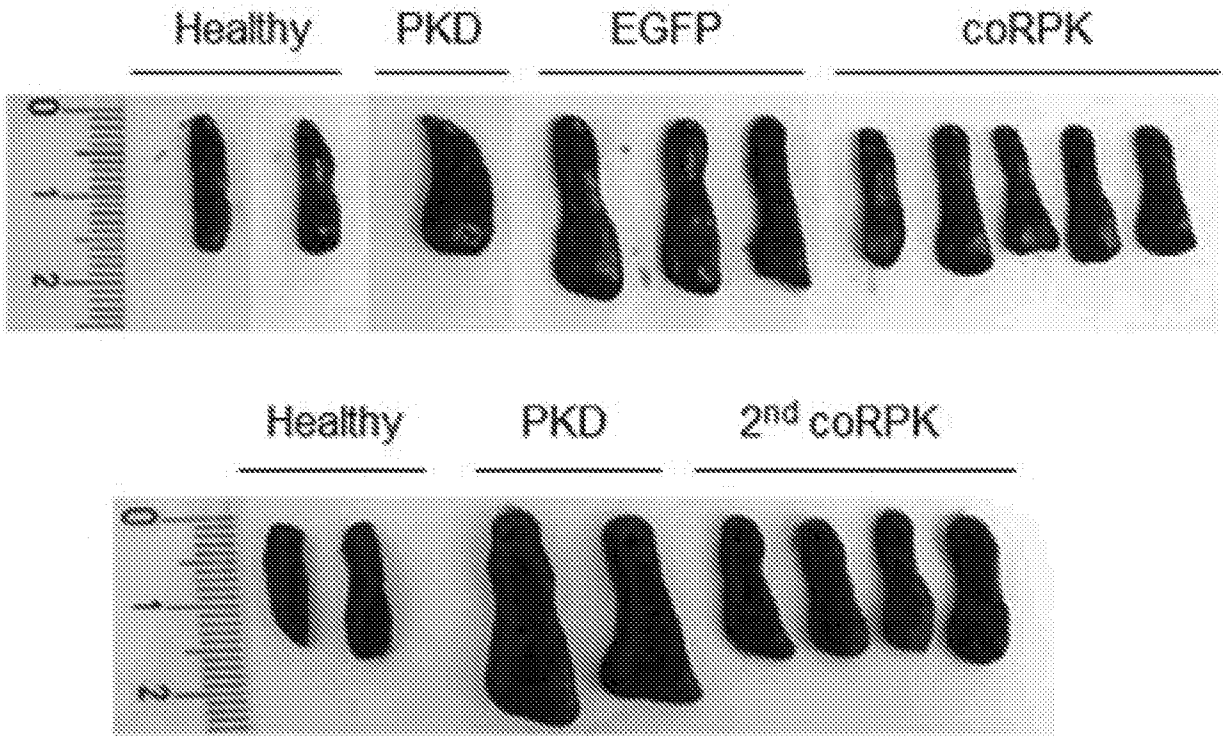

Healthy
PKD
EGFP
coRPK
Healthy
PKD
2nd coRPK

FIG. 9A

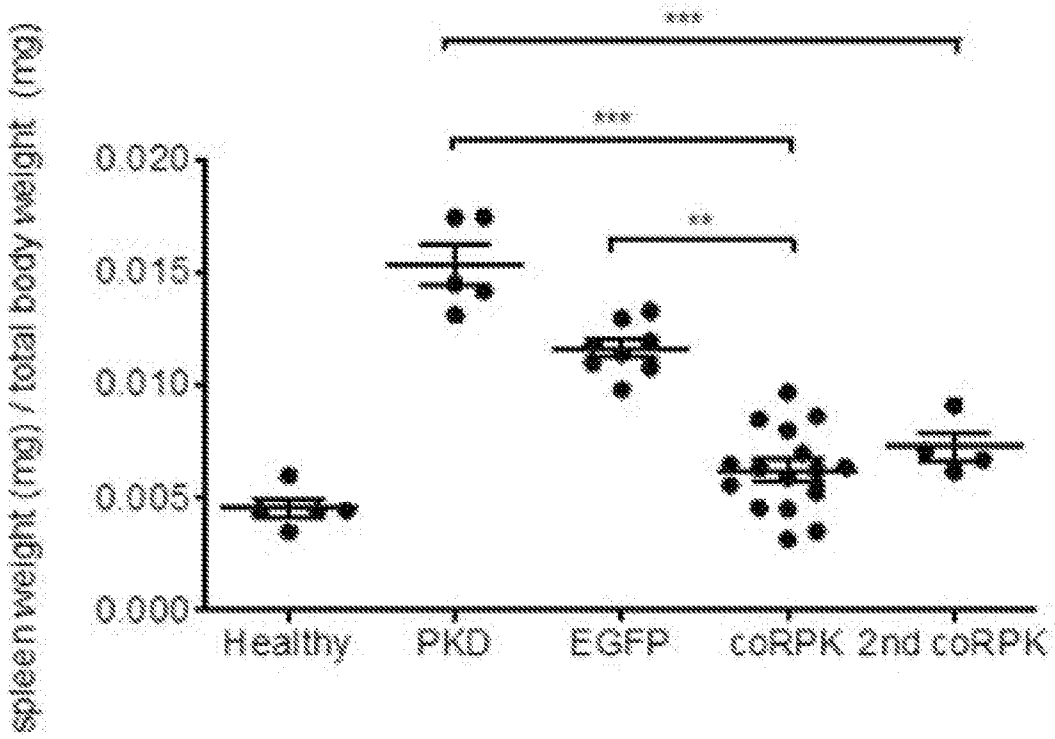

spleen weight (mg) / total body weight (mg)
0.020
0.015
0.010
0.005
0.000
***
***
**
Healthy
PKD
EGFP
coRPK
2nd coRPK

FIG. 9B

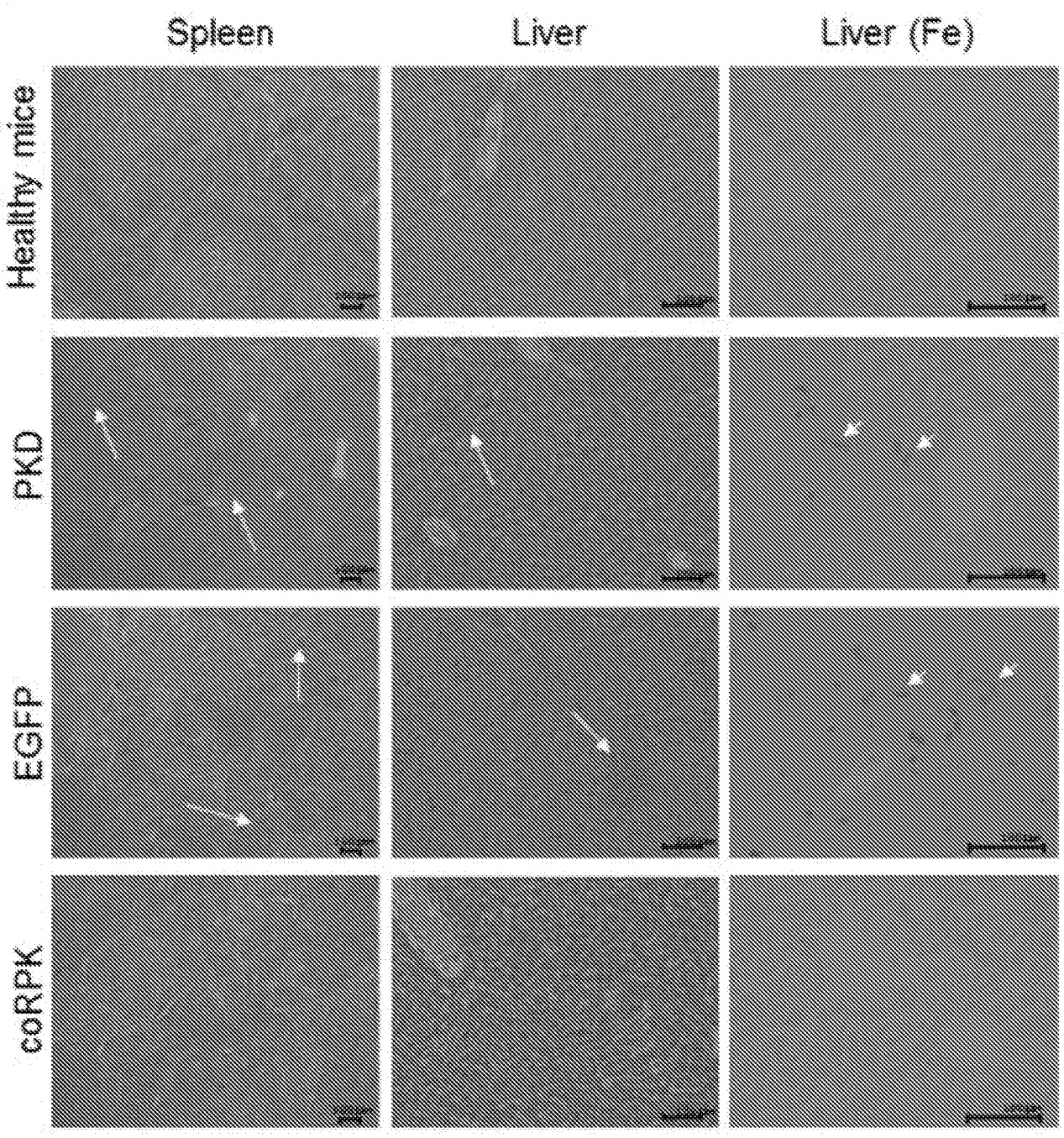


FIG. 9C

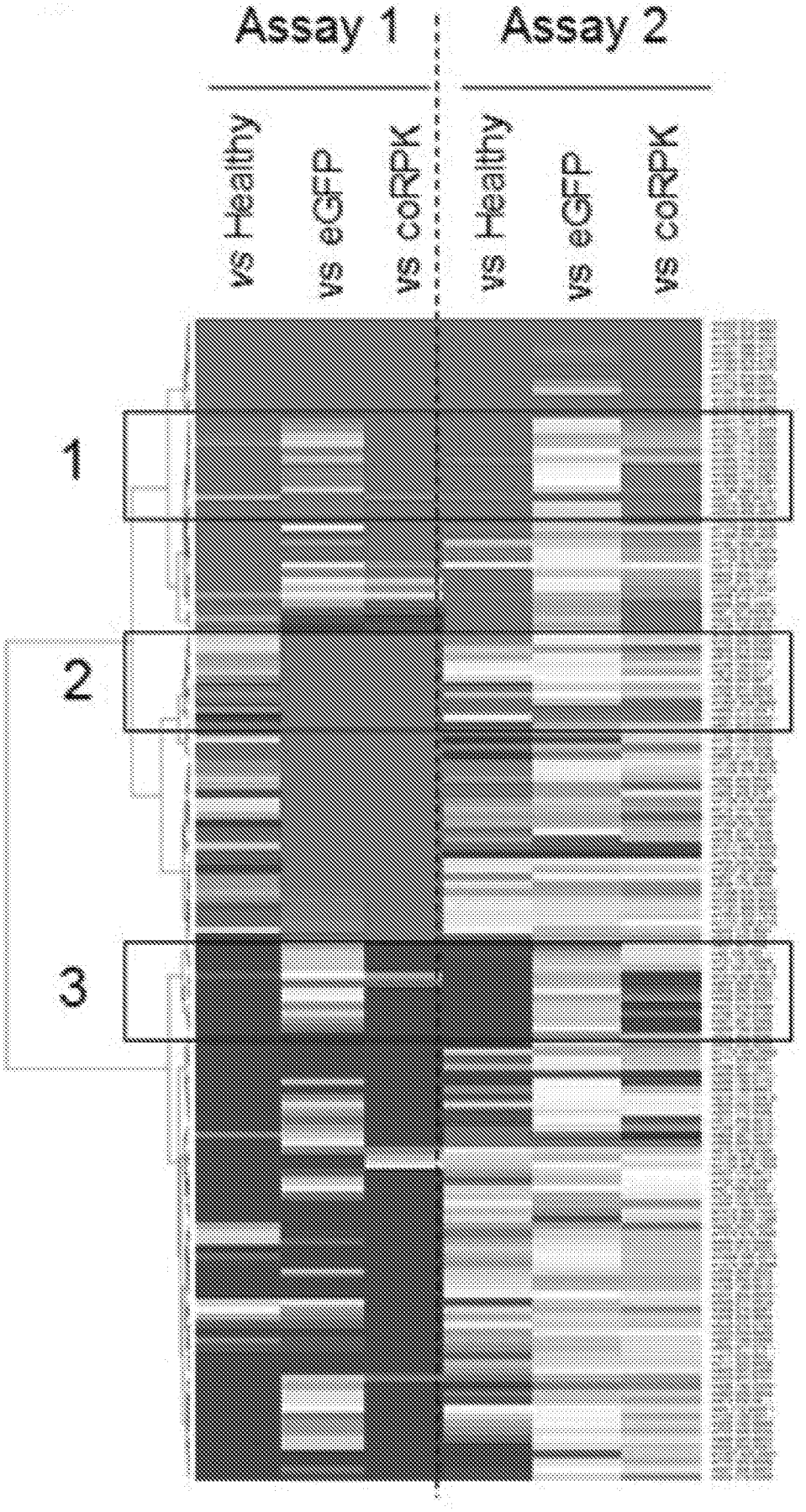

Assay 1
Assay 2
vs Healthy
vs eGFP
vs coRPK
vs Healthy
vs eGFP
vs coRPK
1
2
3

FIG. 10A

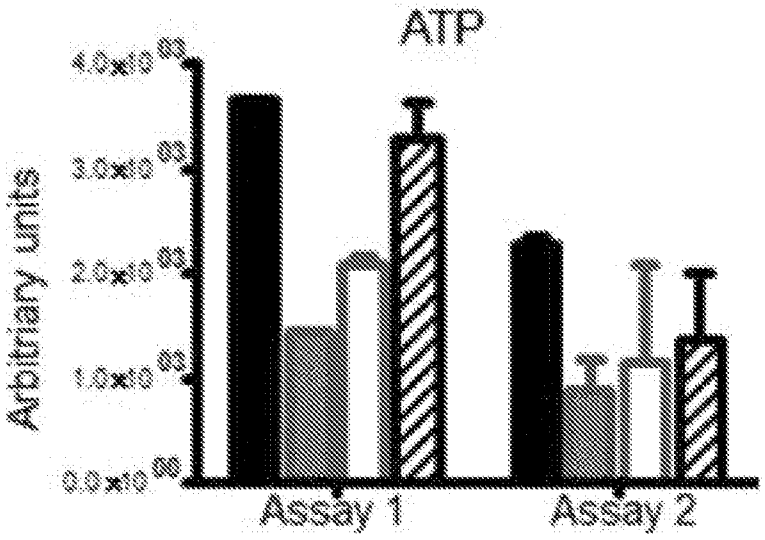


FIG. 10B

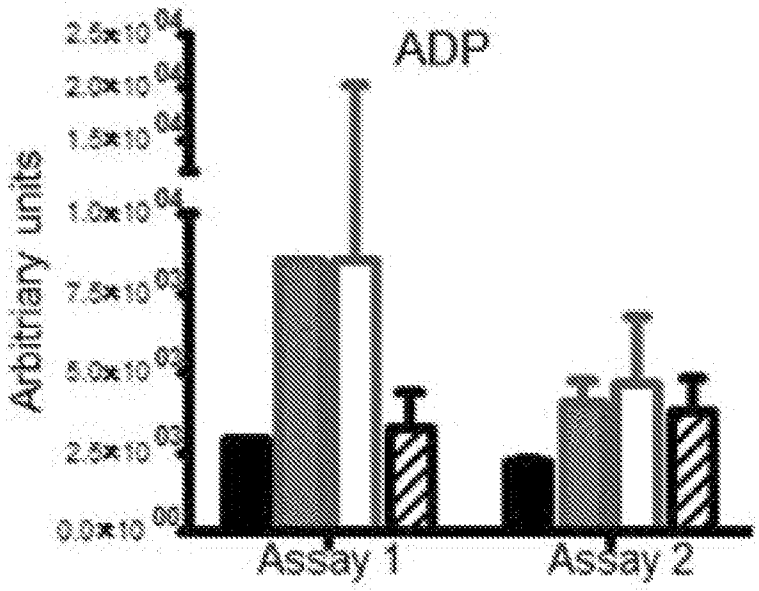


FIG. 10C

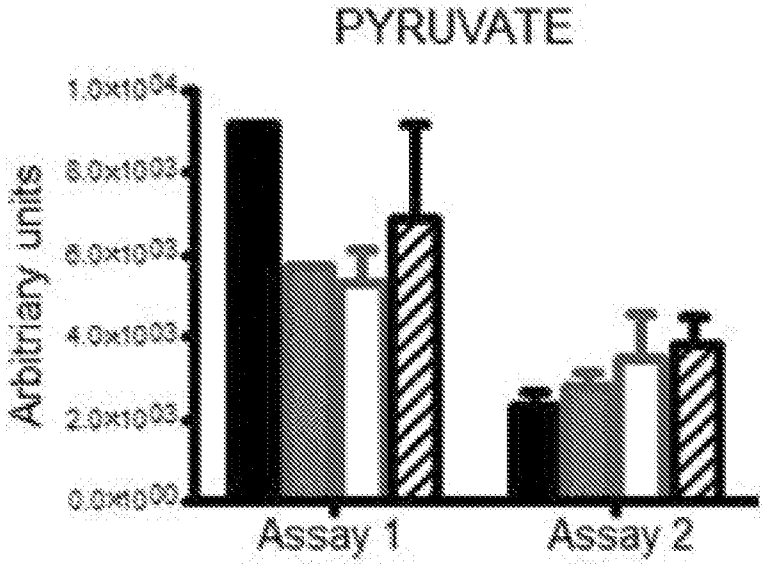


FIG. 10D

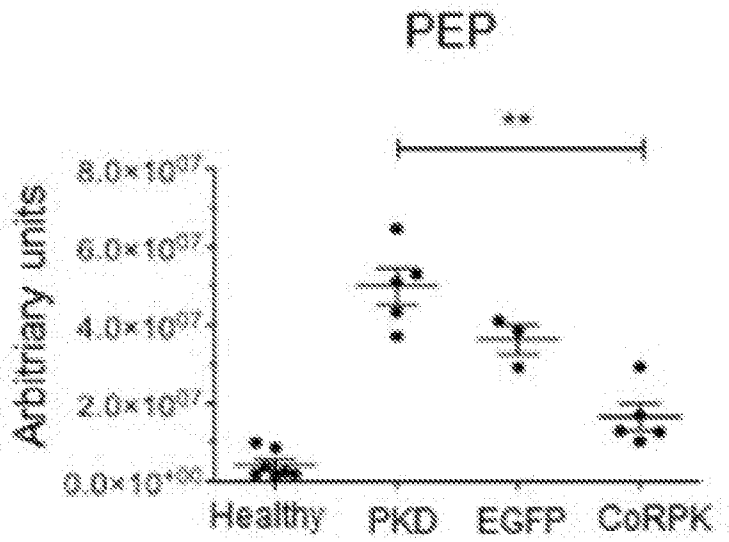


FIG. 10E

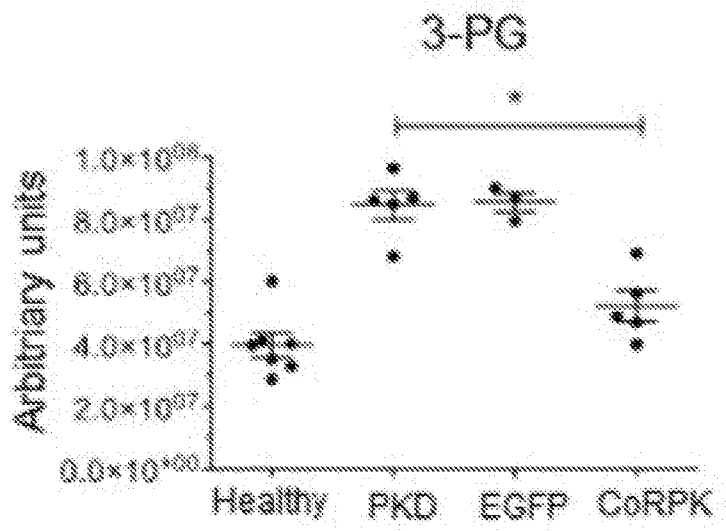


FIG. 10F

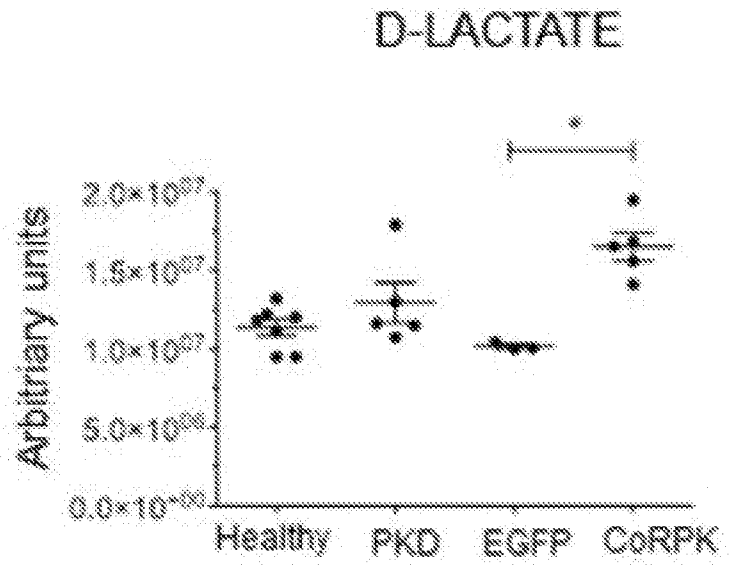


FIG. 10G

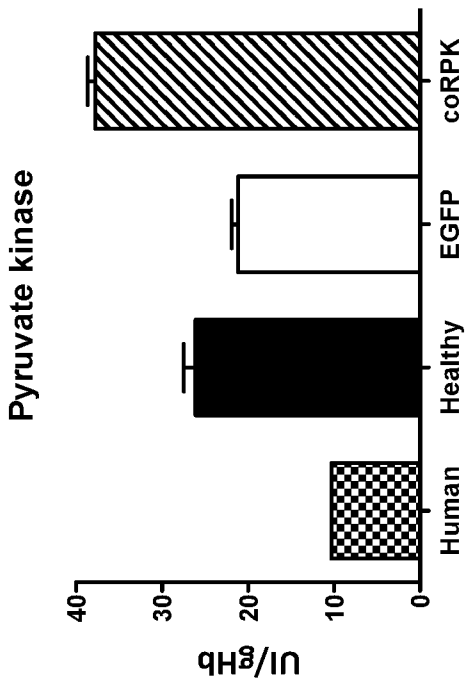


FIG. 11A

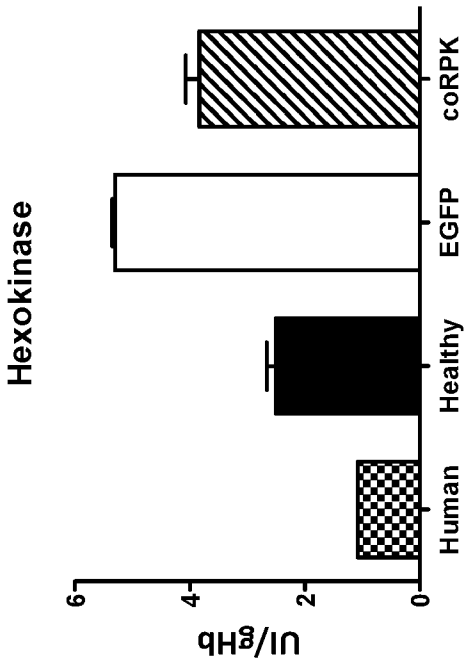


FIG. 11B

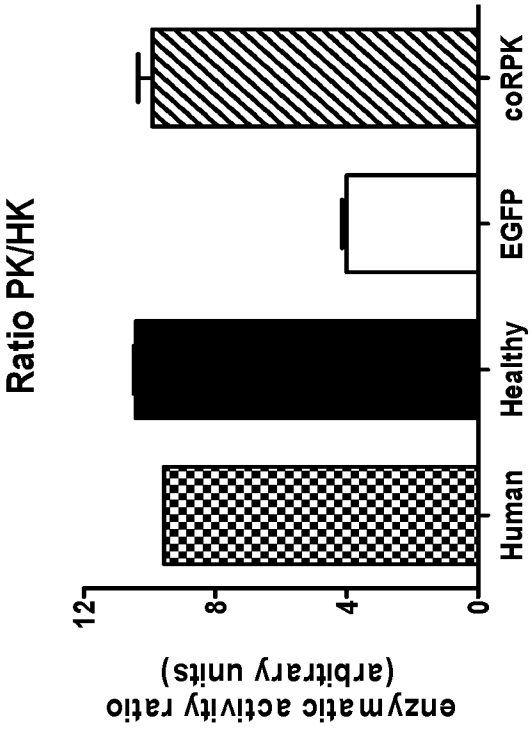


FIG. 11C

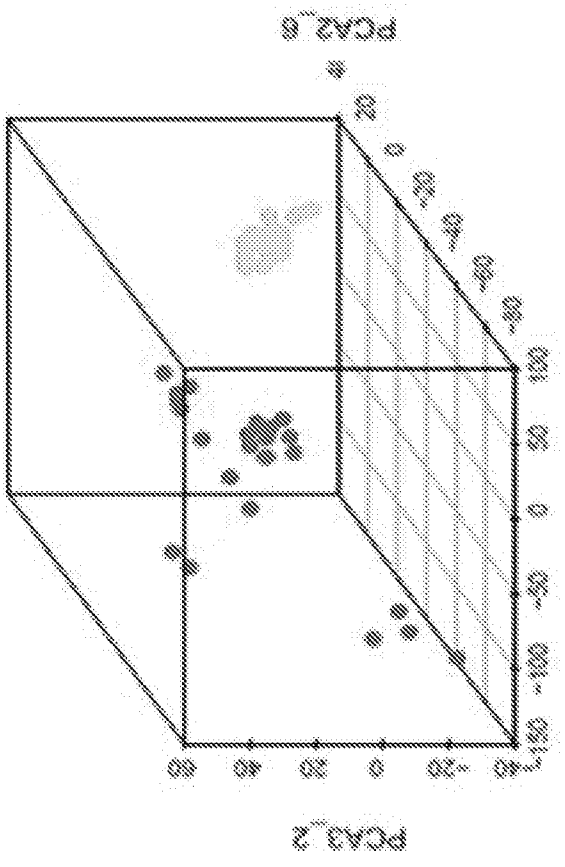


FIG. 12A

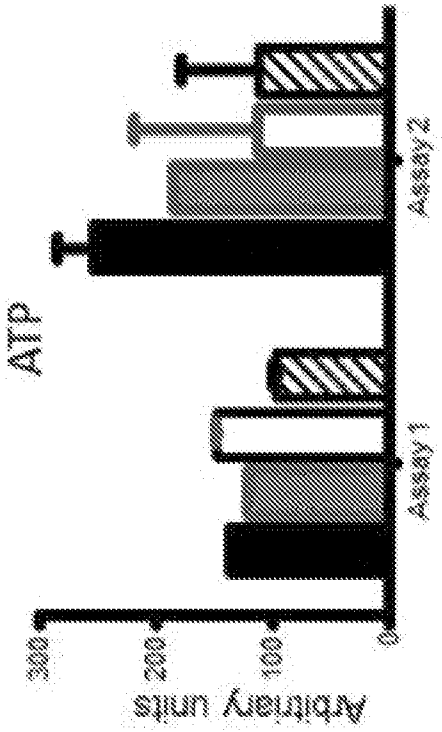


FIG. 12B

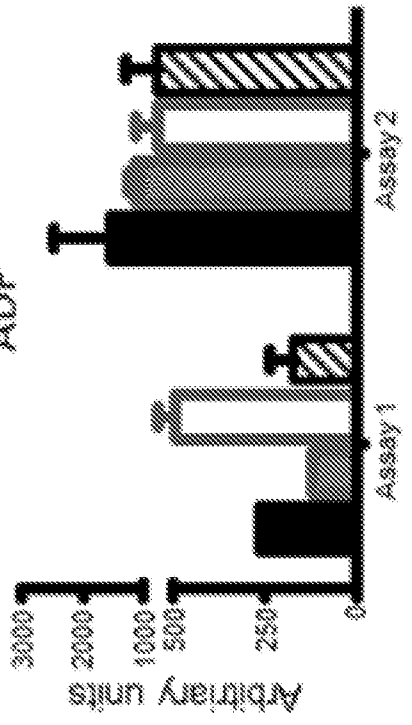


FIG. 12C

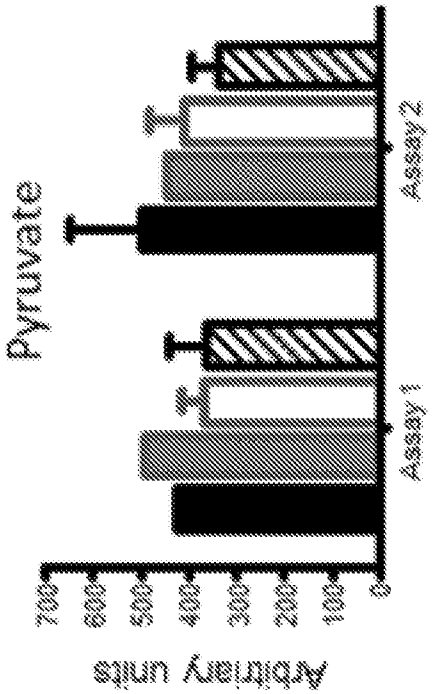


FIG. 12D

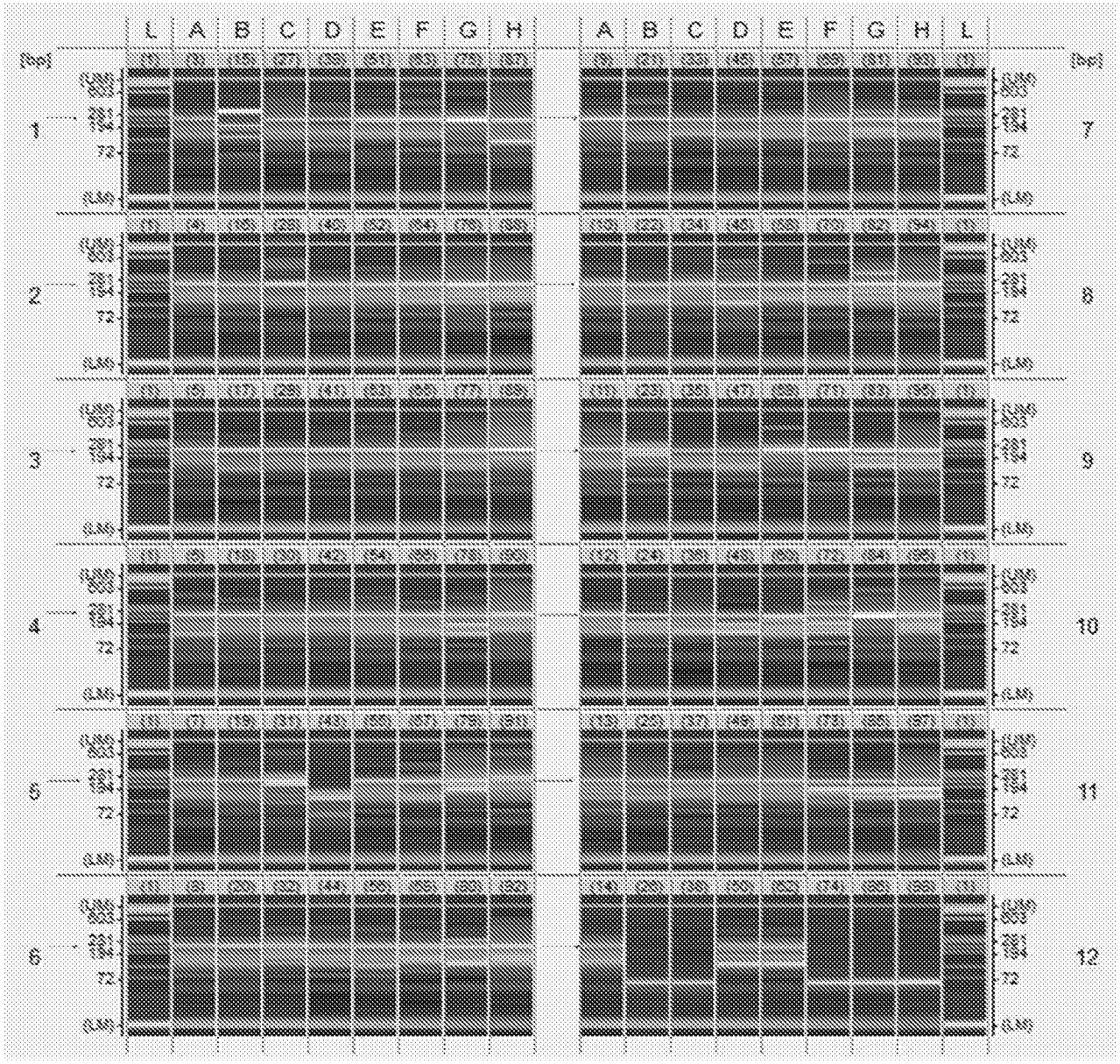


FIG. 13

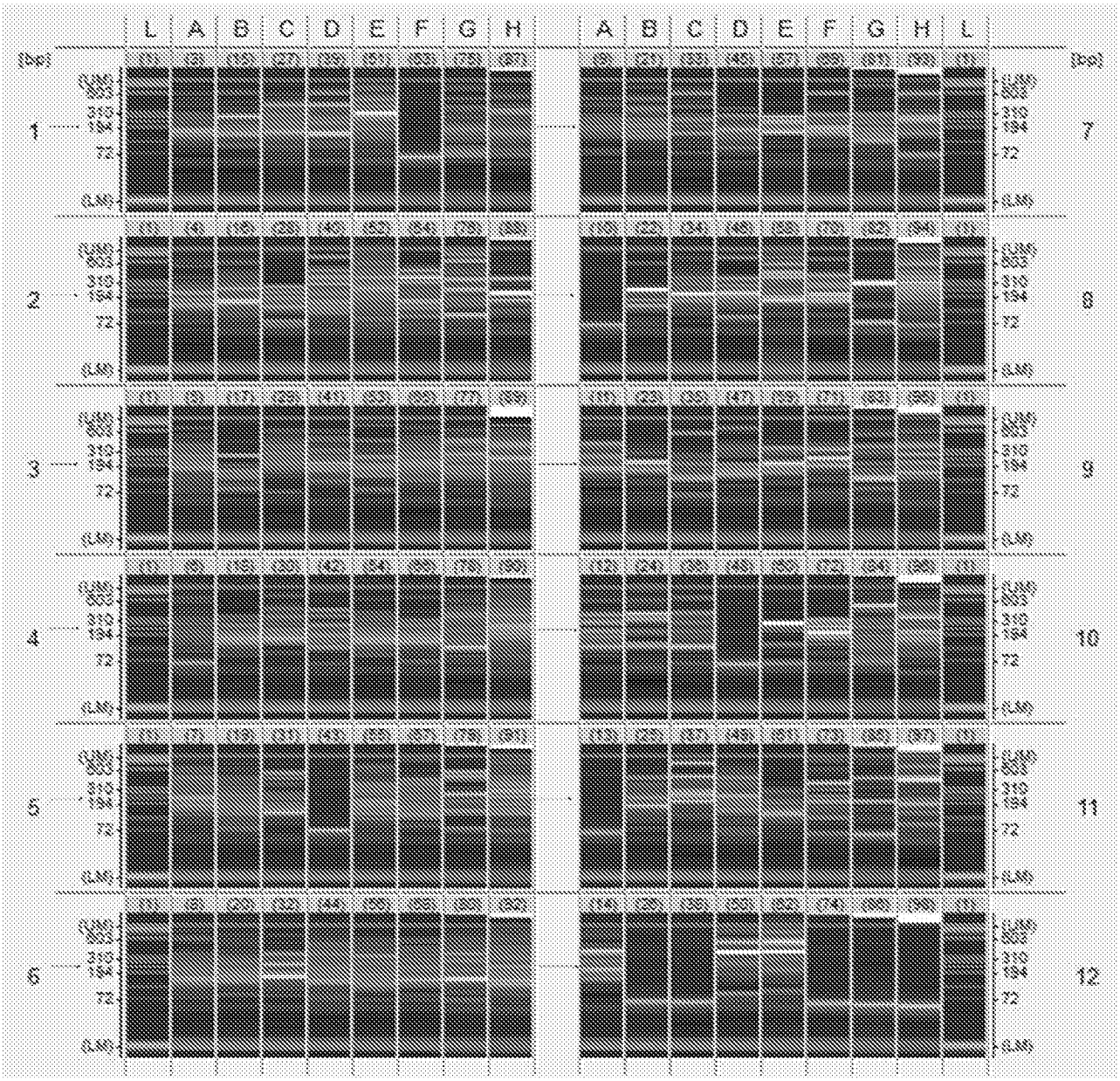


FIG. 14

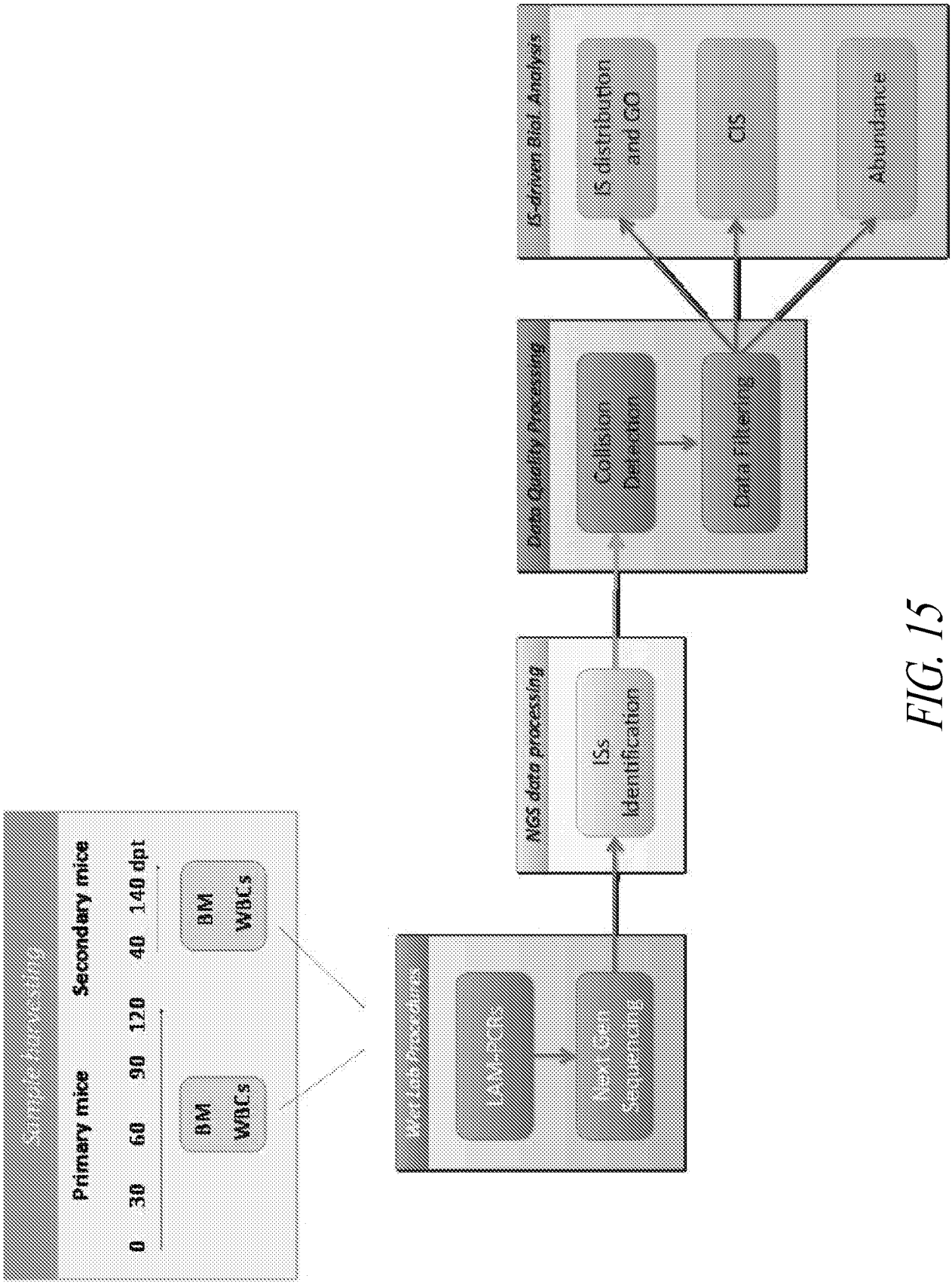

Primary mice
Secondary mice
0
30
60
90
120
40
140 dpt
BM
WBCs
BM
WBCs
NGS data processing
ISs
Identification
Data Quality Processing
Collision
Detection
Data Filtering
IS-driven Biol. Analyses
IS distribution
and CO
CIS
Abundance

FIG. 15

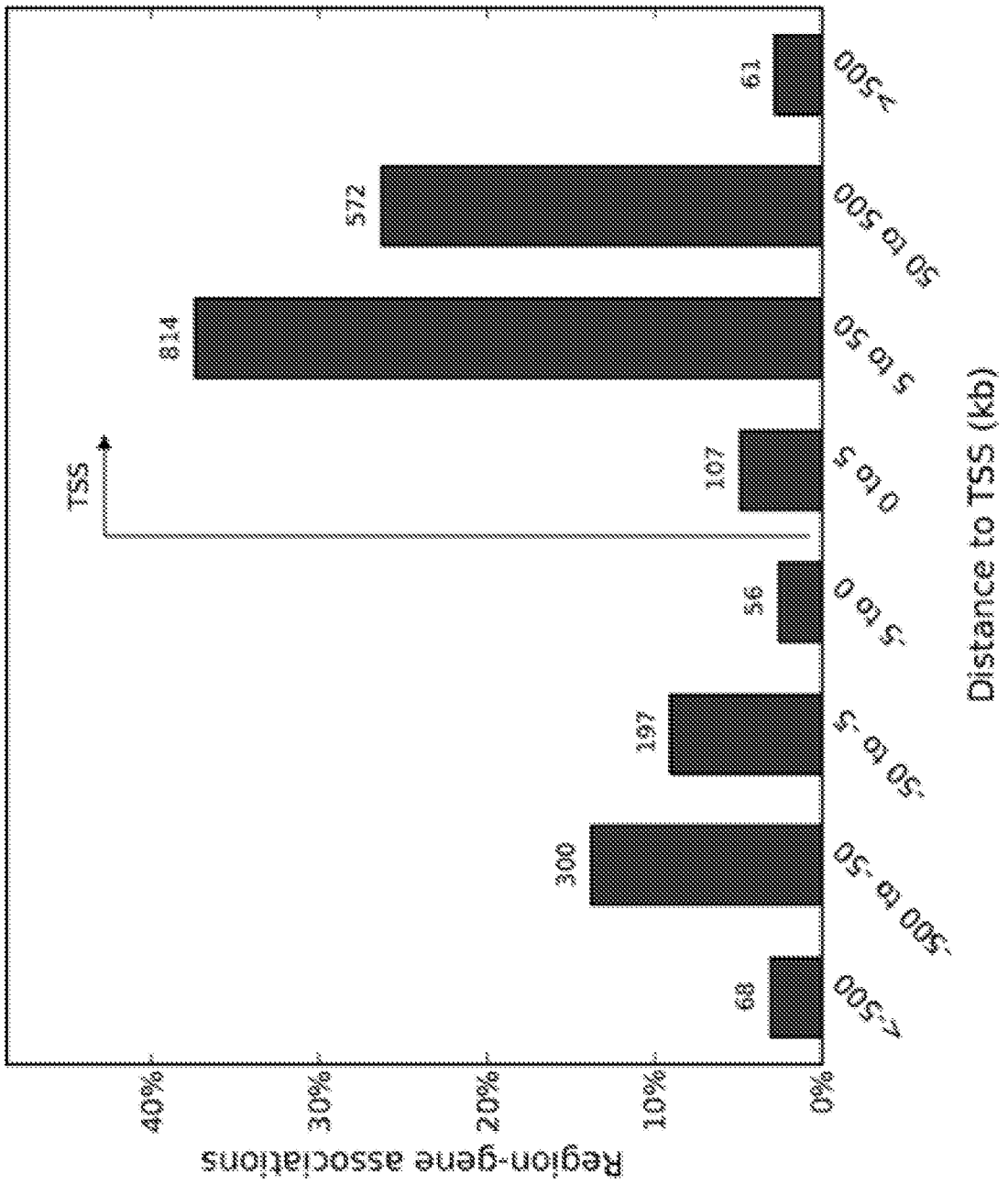

Region-gene associations
40%
30%
20%
10%
0%
TSS
69
300
197
56
107
814
572
91
-500 to -500
-500 to -50
-50 to -5
-5 to 0
0 to 5
5 to 50
50 to 500
>500
Distance to TSS (kb)

FIG. 16A

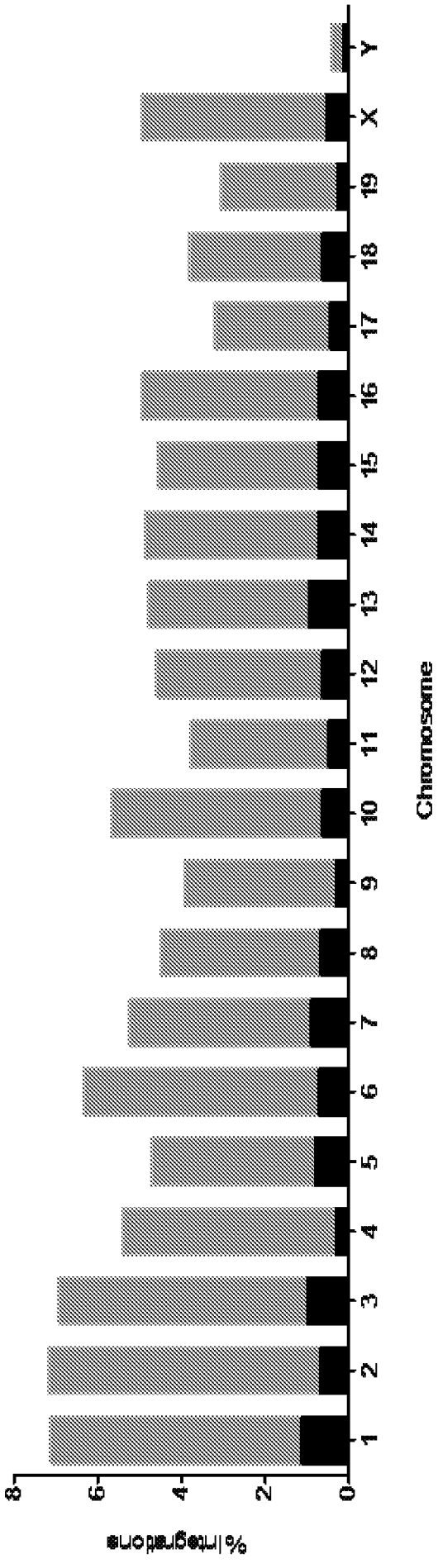

% Integrations
0
2
4
6
8
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
X
Y
Chromosome

FIG. 16B

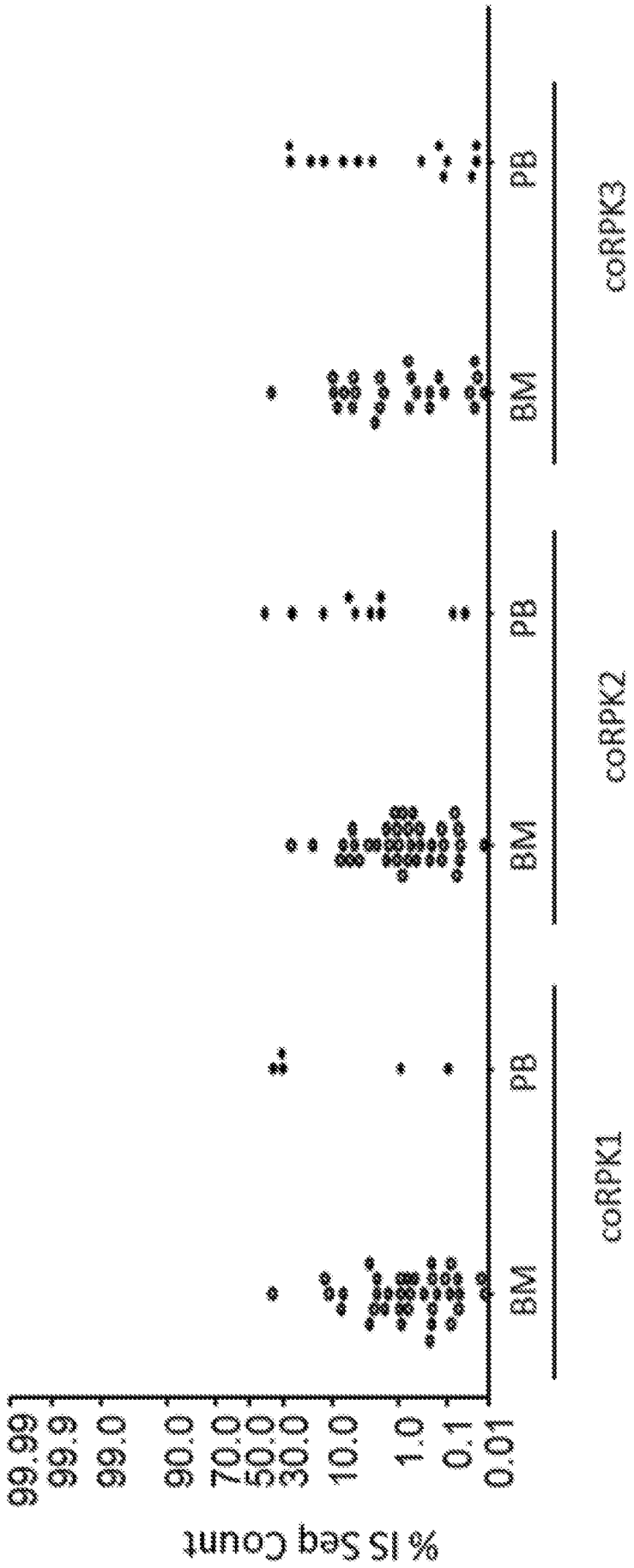

% IS Seq Count
99.99
99.9
99.0
90.0
70.00
50.0
30.0
10.0
1.0
0.1
0.01
BM
PB
BM
PB
BM
PB
coRPK1
coRPK2
coRPK3

FIG. 17A

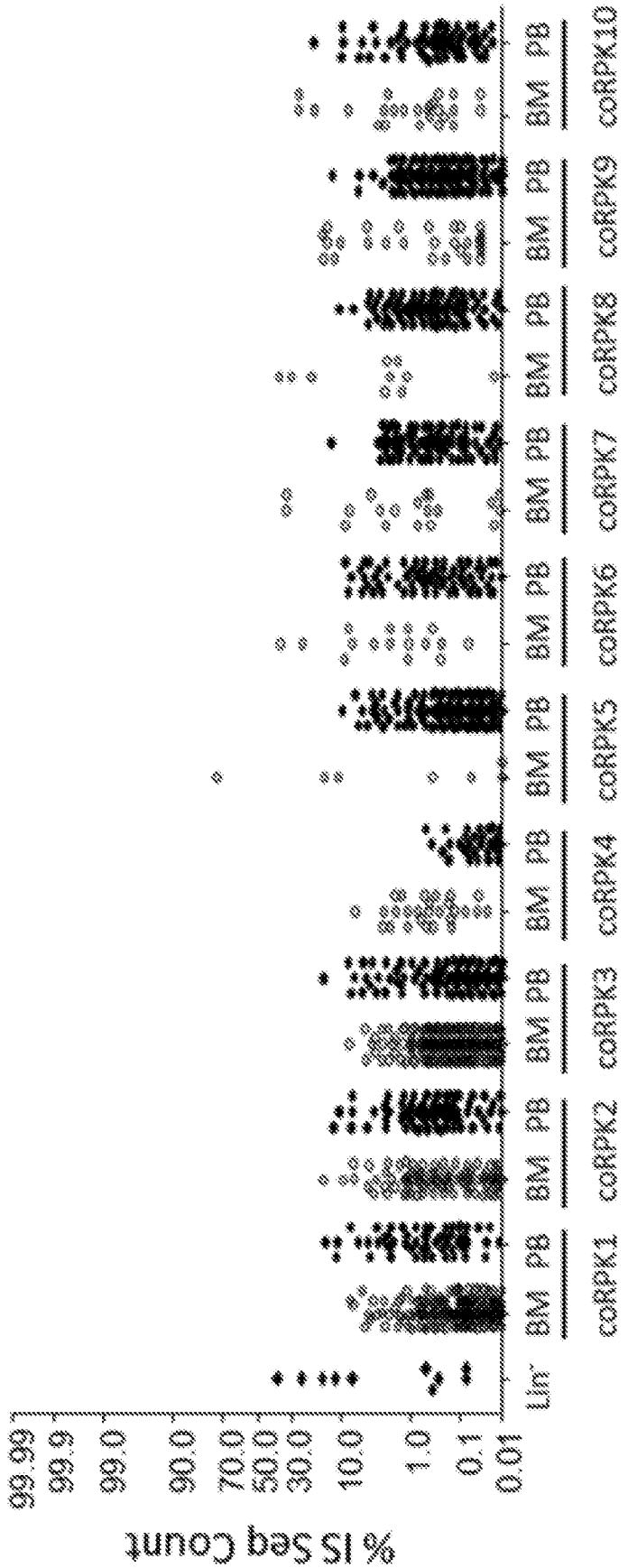

% IS seq Count
99.99
99.9
99.0
90.0
70.0
50.0
30.0
10.0
1.0
0.1
0.01
Lin⁻
BM PB
coRPK1
BM PB
coRPK2
BM PB
coRPK3
BM PB
coRPK4
BM PB
coRPK5
BM PB
coRPK6
BM PB
coRPK7
BM PB
coRPK8
BM PB
coRPK9
BM PB
coRPK10

FIG. 17B

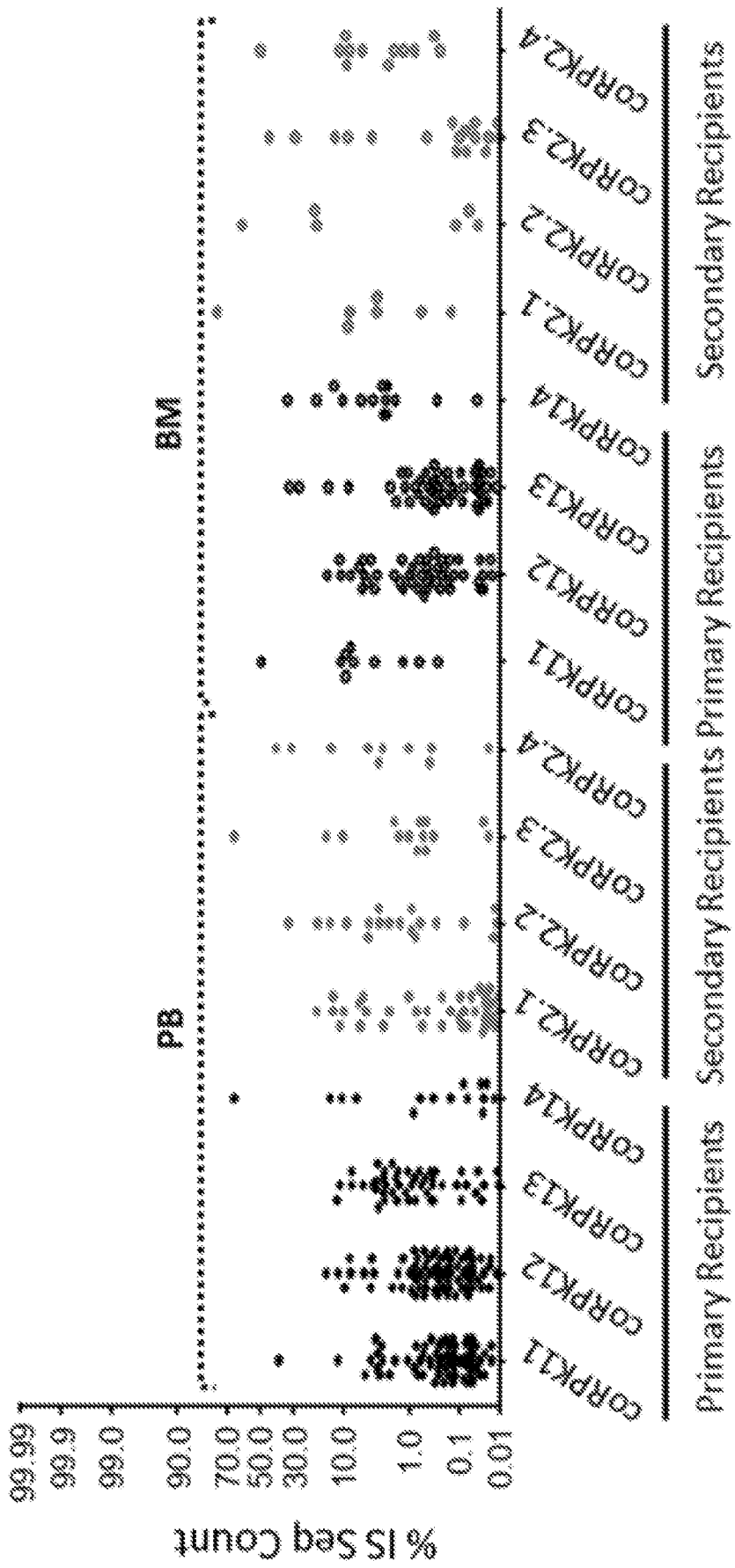

% IS Seq Count
99.99
99.9
99.0
90.0
70.0
50.0
30.0
10.0
1.0
0.1
0.01
PB
BM
CORPK1.1
CORPK1.2
CORPK1.3
CORPK1.4
CORPK2.1
CORPK2.2
CORPK2.3
CORPK2.4
CORPK1.1
CORPK1.2
CORPK1.3
CORPK1.4
CORPK2.1
CORPK2.2
CORPK2.3
CORPK2.4
Primary Recipients
Secondary Recipients
Primary Recipients
Secondary Recipients

FIG. 17C

			Primary recicipients				Secondary Recipients			
	Non Redundant IS		99	168	87	19	113	35	32	27
	Total Sequence Reads		14102	340586	314455	69343	206932	152162	200987	180905
Chr	Integration Locus	RefGene	coRPK11	coRPK12	coRPK13	coRPK14	coRPK2.1	coRPK2.2	coRPK2.3	coRPK2.4
16	52114775	Cblb			8,87	48,57	5,29	12,35	0,02	
18	35737739	Matr3			4,58		4,79	15,84	14,36	17,41
9	59671229	Myo9a			2,39		2,69	3,05	0,52	0,74
8	23308857	Vps36			6,41		9,34	21,52		4,97
1	41860552	4930448I06Rik			1,77		1,68	13,02	8,18	
10	37953519	Rfpl4b			1,39	6,17		1,03	0,39	
1	125478355	Dpp10			0,91		1,10	3,18		1,63
2	148695277	Cst3			0,44		3,02		0,35	2,64
6	80828813	Lrrtm4			0,16		0,29	0,44		0,22
7	4446040	Ppp1r12c			0,01		0,01	0,01	0,02	
10	7744574	Tab2			3,27		9,12			4,07
5	18735403	Magi2	33,36	0,81			0,01			
18	57525115	Prrc1	17,40				6,26	2,22		
X	105969090	Brwd3	1,95	0,71			0,11			
3	148528413	Lphn2	1,76	0,45						0,24
5	13643926	Sema3a	1,58				0,05	0,53		
4	24449640	Mms22l	0,96	0,35			0,11			
1	107487037	Pign				12,04			0,35	
19	27467465	D19Bwg1357e				9,75		0,63		
13	73458709	Ndufs6				9,58			0,00	
14	86078480	4930529K09Rik				0,00				0,00
12	72161794	Arid4a			9,44				0,00	
1	109398282	Serpinb2			5,61					0,00
17	18006611	Fpr1			1,35				0,00	
2	38132209	Dennd1a			1,28			0,00		
16	81433433	Ncam2			0,04					0,00
6	56736516	Kbtbd2		6,40					0,03	
7	79224238	Mctp2		3,31						22,26
16	58006735	Col8a1		3,16					0,00	
4	85596276	Adamtsl1		2,37			0,02			
7	111583985	Trim30a		2,29			0,01			
16	33948595	Itgb5		1,74			0,01			
13	7150736	Pfkp		1,11			0,01			
6	9899432	Nxph1		0,82			0,00			
4	66828139	Tlr4		0,78			0,01			
19	34647666	Ifit2		0,70			0,08			
2	174443138	Zfp831		0,54						3,79
19	23412889	Mamdc2		0,41			0,00			
14	96290784	4921530L21Rik		0,33			0,00			
X	82997642	Gyk		0,30			0,00			
6	43431106	9430018G01Rik		0,16			0,00			
11	98079745	Cdk12		0,10			0,00			
6	13808636	2610001J05Rik		0,03			0,00			
X	83019292	Gyk	3,15					0,00		
4	144607235	Vps13d	1,06							0,11
10	53745139	Man1a	0,07							0,00
2	104684227	Prrg4	0,04					0,00		

FIG. 18

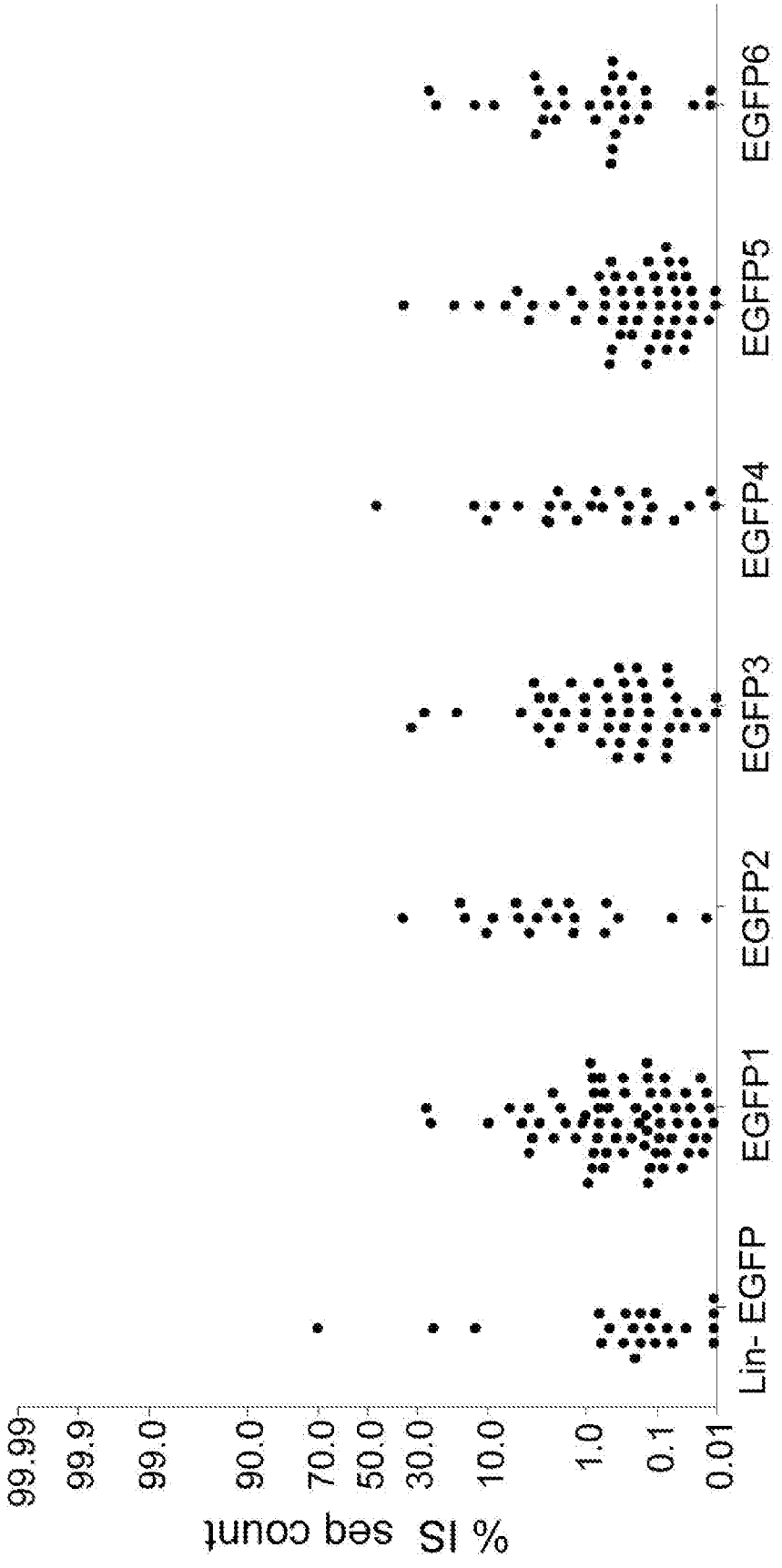

% IS seq count
99.99
99.9
99.0
90.0
70.0
50.0
30.0
10.0
1.0
0.1
0.01
Lin- EGFP
EGFP1
EGFP2
EGFP3
EGFP4
EGFP5
EGFP6

FIG. 19

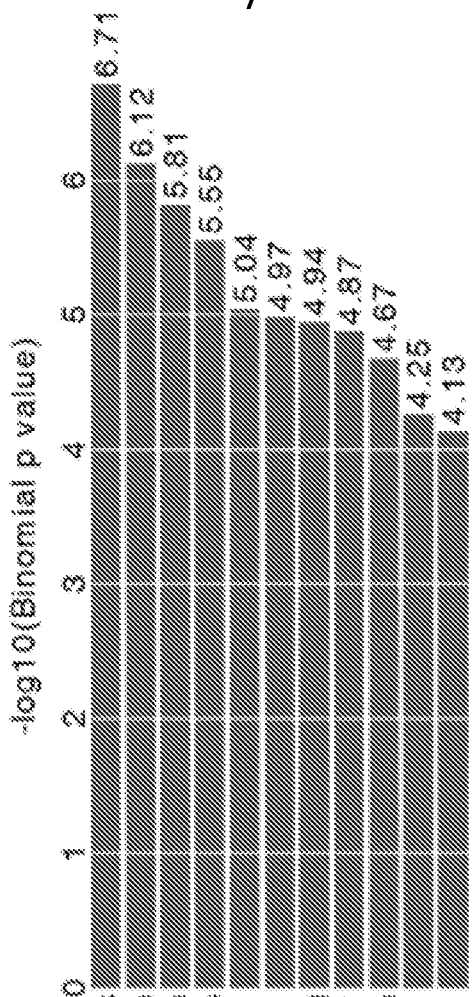

-log10(Binomial p value)
0
1
2
3
4
5
6
protein catabolic process
regulation of B cell proliferation
regulation of B cell activation
modification-dependent macromolecule catabolic process
cell projection assembly
determination of left/right symmetry
neuronal ion channel clustering
specification of symmetry
regulation of DNA replication
response to activity
early endosome to late endosome transport
6.71
6.12
5.81
5.55
5.04
4.97
4.94
4.87
4.67
4.25
4.13

FIG. 20

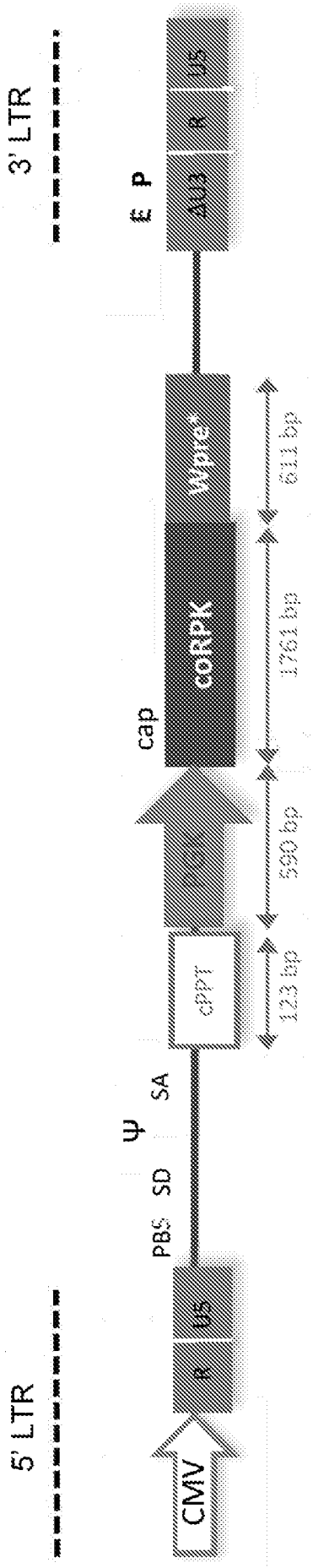

5' LTR
CMV
R
U5
PBS
SD
Ψ
SA
cPPT
123 bp
cap
coRPK
590 bp
Wpre*
1761 bp
611 bp
Δ U3
R
U5
3' LTR

FIG. 21

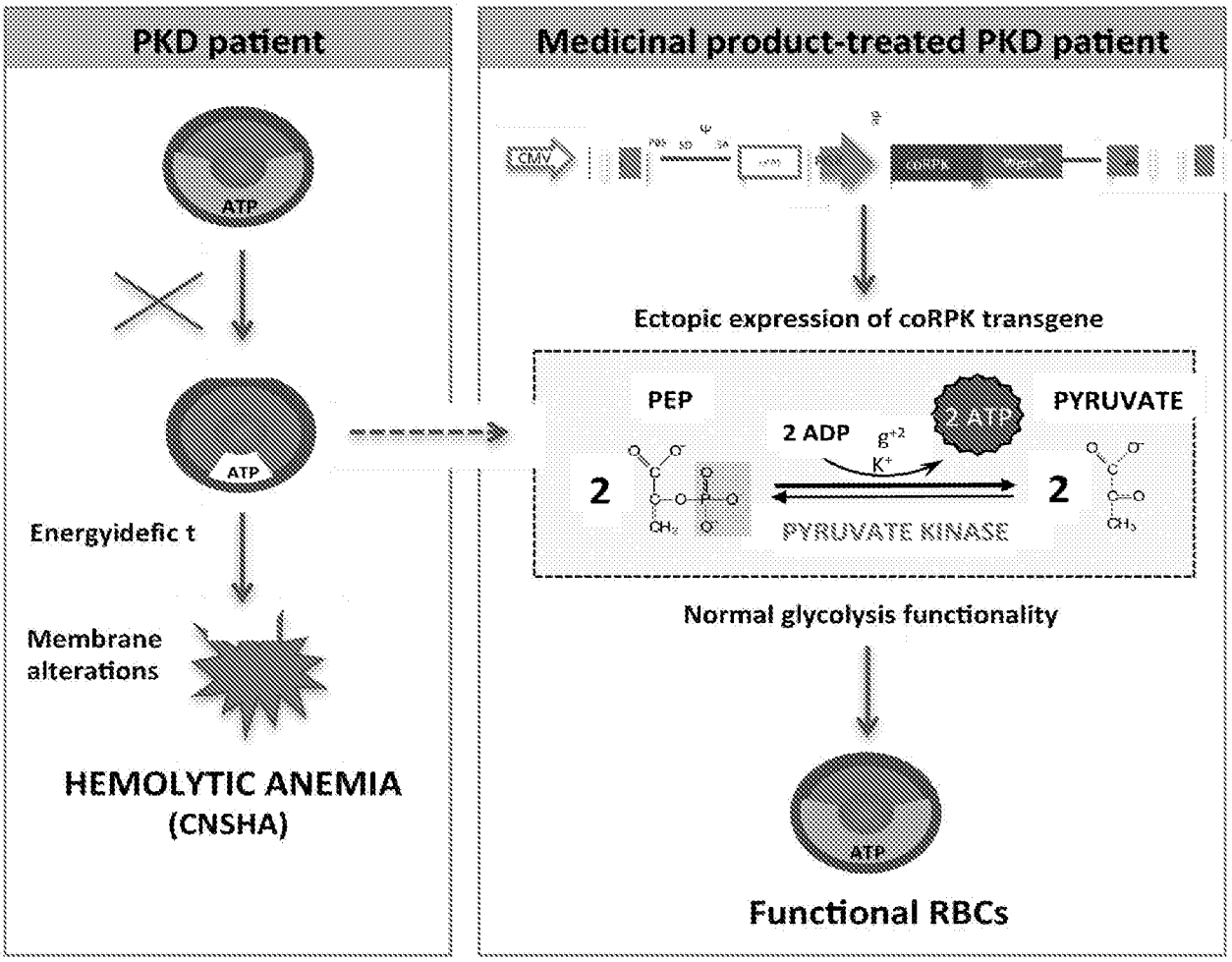

PKD patient
ATP
ATP
Energyidefic t
Membrane alterations
HEMOLYTIC ANEMIA (CNSHA)
Medicinal product-treated PKD patient
CMV
Ectopic expression of coRPK transgene
PEP
2 ADP
2 ATP
PYRUVATE
2
2
PYRUVATE KINASE
Normal glycolysis functionality
ATP
Functional RBCs

FIG. 22A

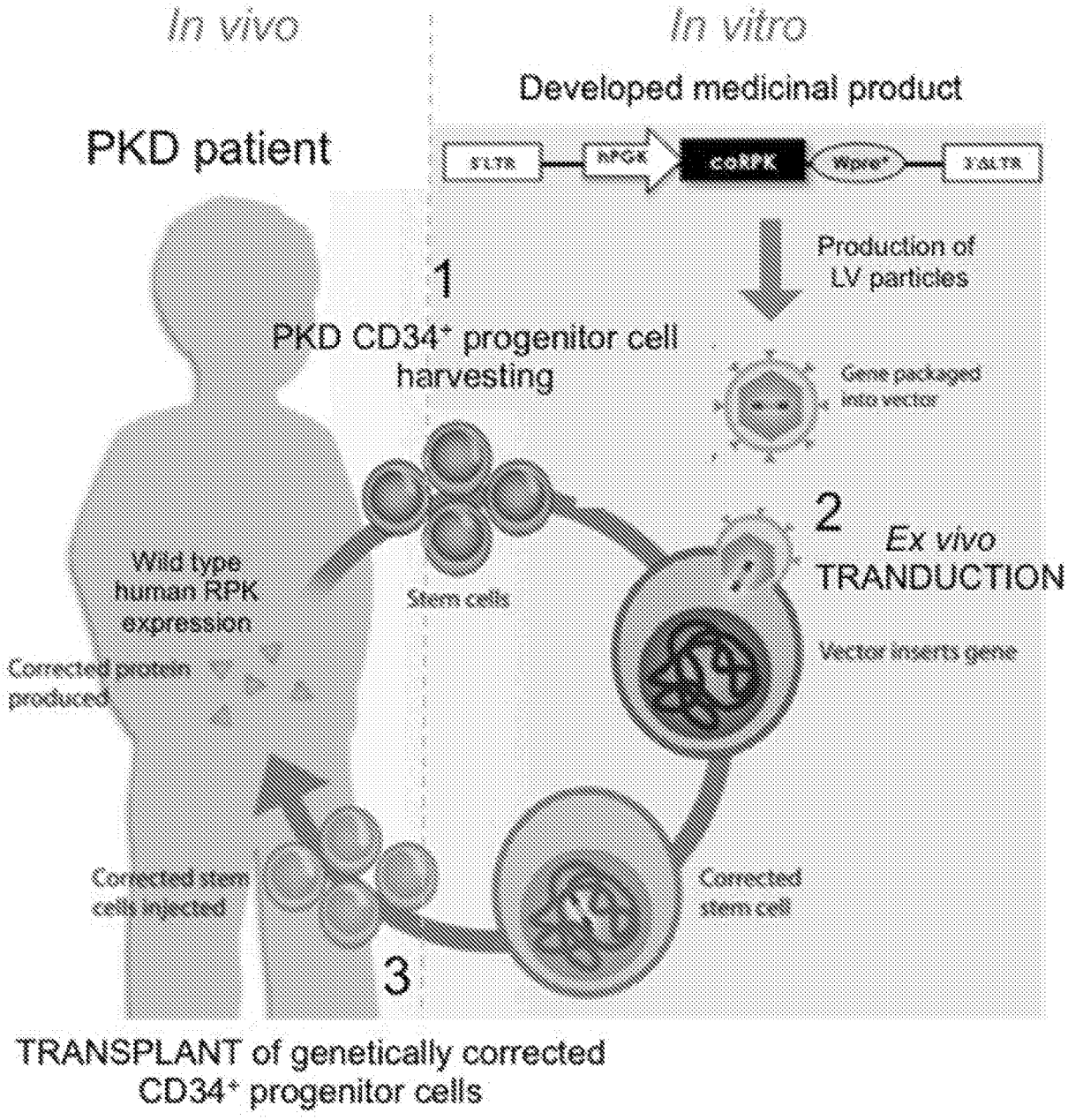

In vivo
In vitro
Developed medicinal product
PKD patient
5'LTR
RPGR
coRPK
WPRE*
3'ΔLTR
Production of LV particles
1
PKD CD34+ progenitor cell harvesting
Gene packaged into vector
2
Ex vivo TRANDUCTION
Wild type human RPK expression
Stem cells
Vector inserts gene
Corrected protein produced
Corrected stem cells injected
Corrected stem cell
3
TRANSPLANT of genetically corrected CD34+ progenitor cells

FIG. 22B