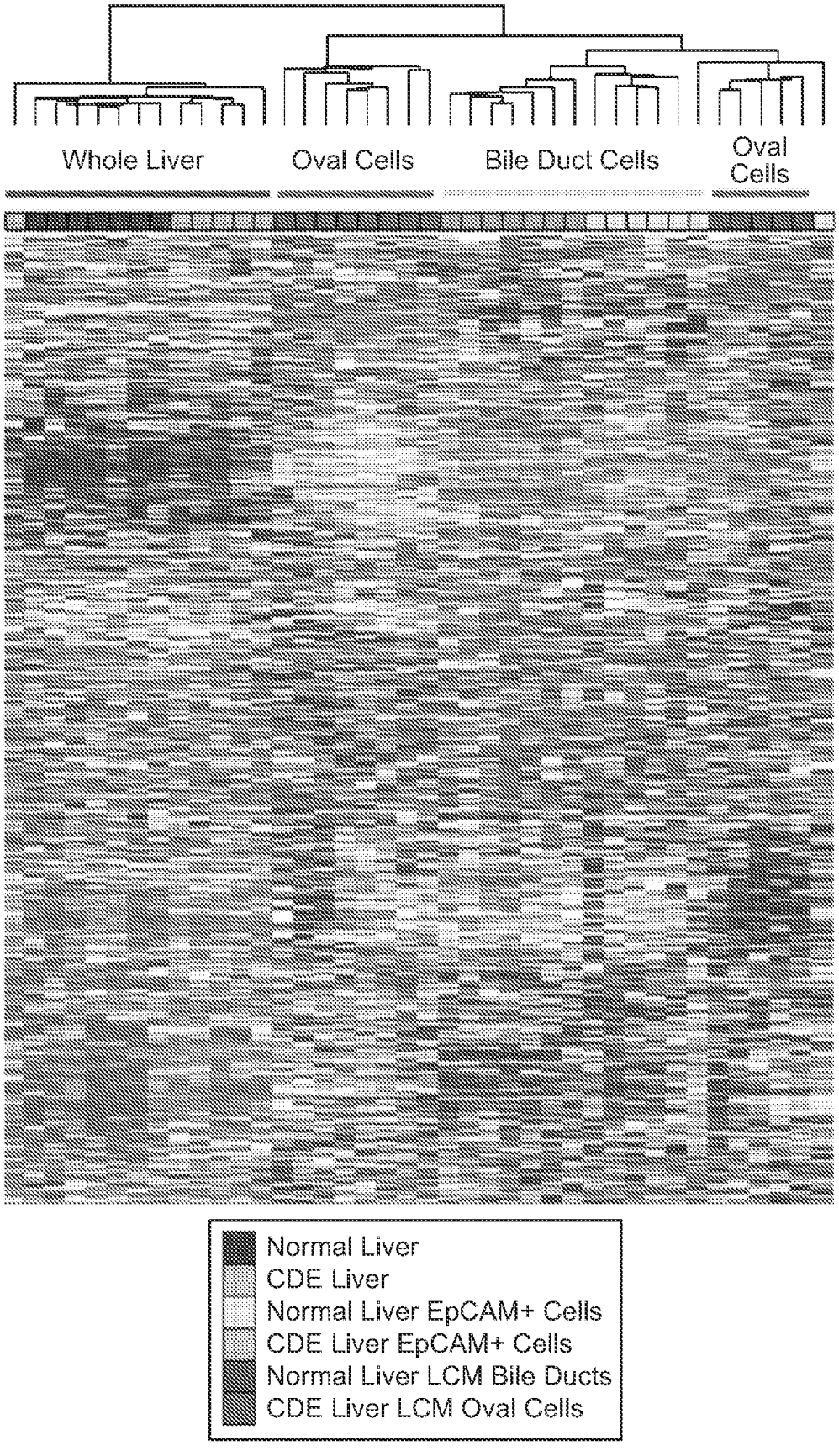

Whole Liver
Oval Cells
Bile Duct Cells
Oval Cells
Normal Liver
CDE Liver
Normal Liver EpCAM+ Cells
CDE Liver EpCAM+ Cells
Normal Liver LCM Bile Ducts
CDE Liver LCM Oval Cells

FIG. 1A

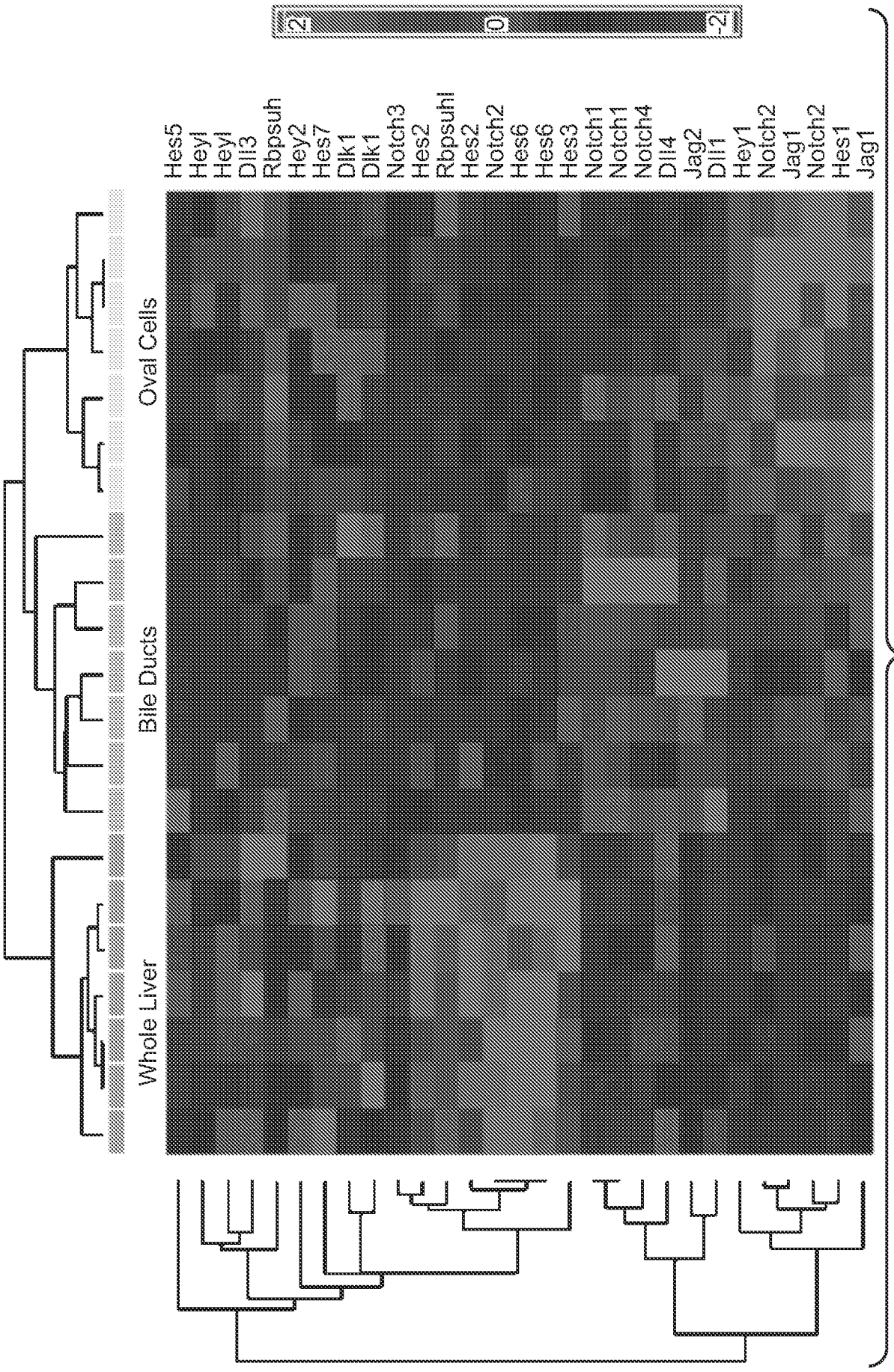

2
0
-2
Hes5
Hey1
Heyl
Dll3
Rbpsuh
Hey2
Hes7
Dlk1
Dlk1
Notch3
Hes2
Rbpsuhl
Hes2
Notch2
Hes6
Hes6
Hes3
Notch1
Notch1
Notch4
Dll4
Jag2
Dll1
Hey1
Notch2
Jag1
Notch2
Hes1
Jag1
Oval Cells
Bile Ducts
Whole Liver

FIG. 1B

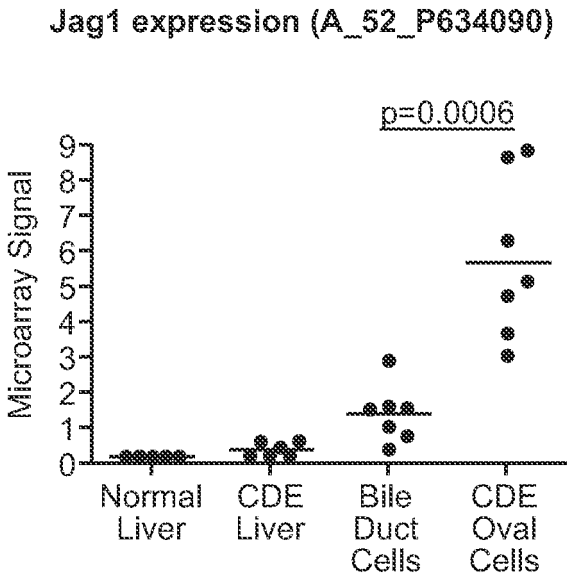


FIG. 1C

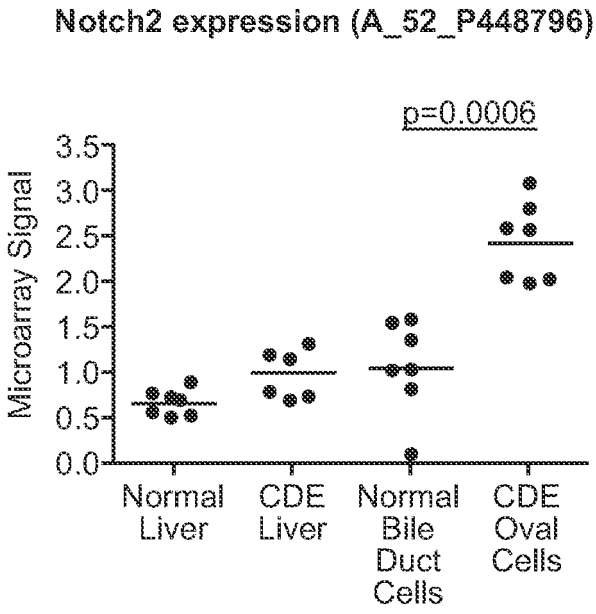


FIG. 1D

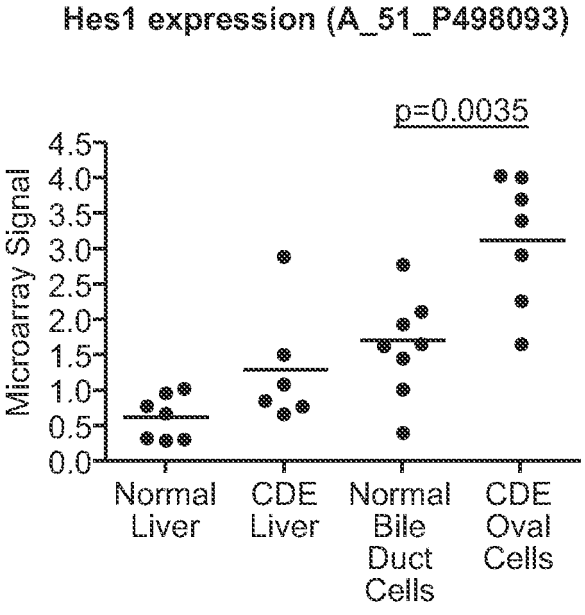


FIG. 1E

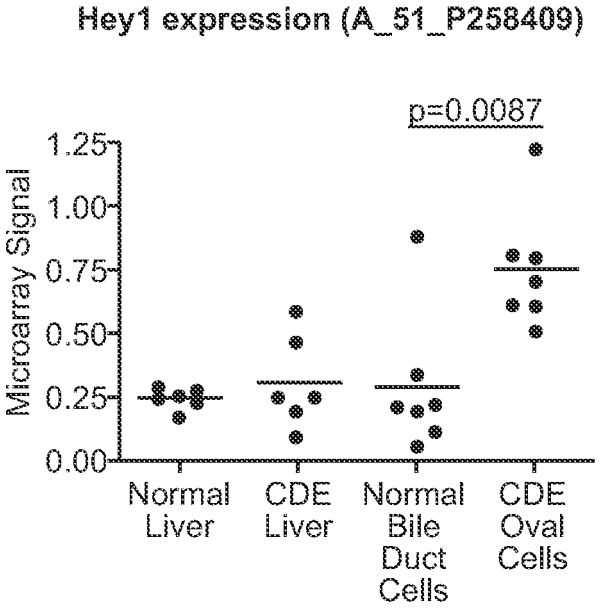


FIG. 1F

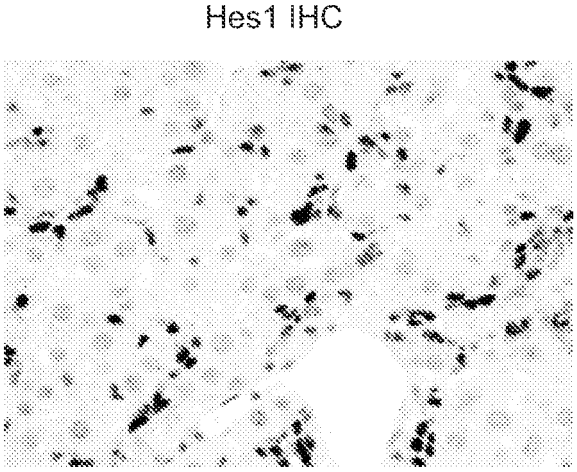


FIG. 1G

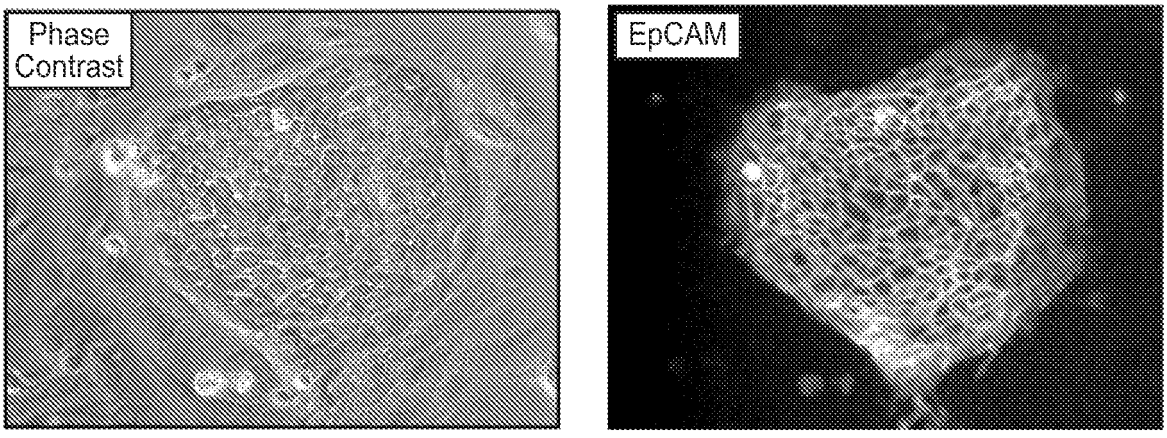


FIG. 2A

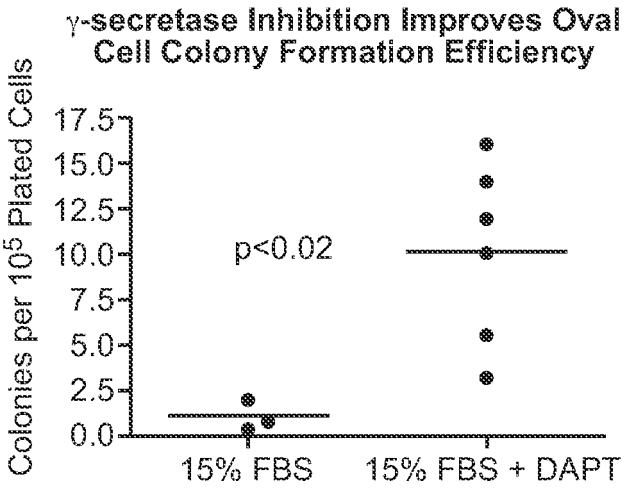


FIG. 2B

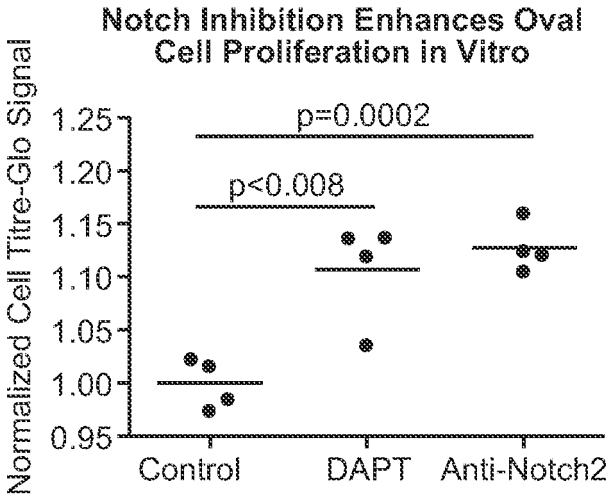


FIG. 2D

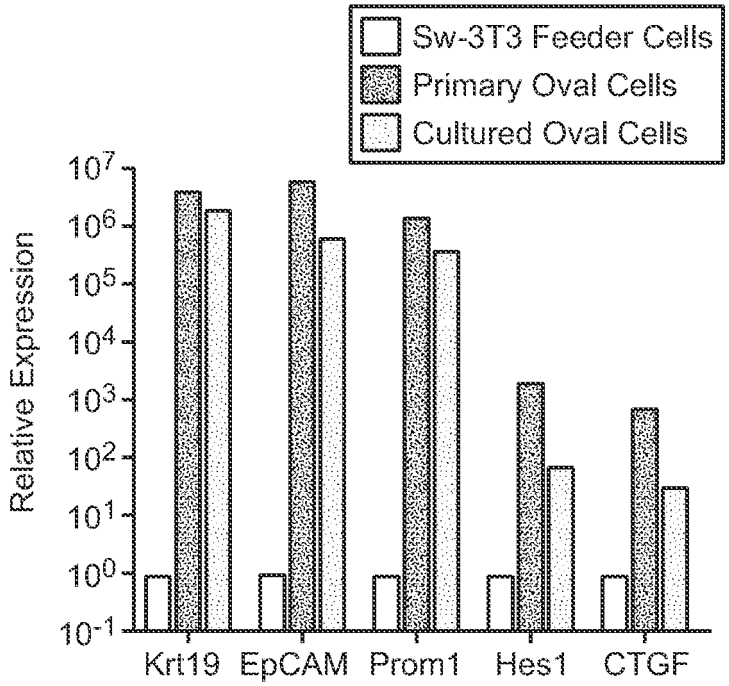


FIG. 2C

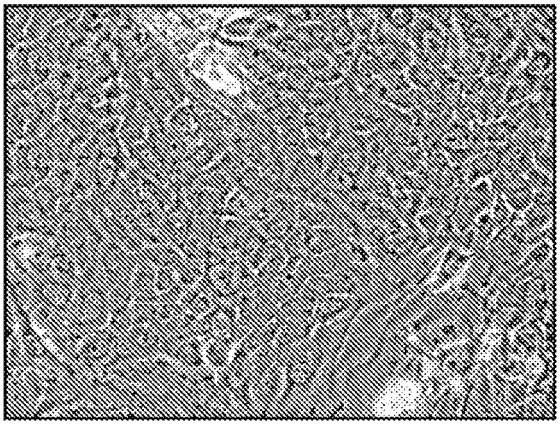


FIG. 2E

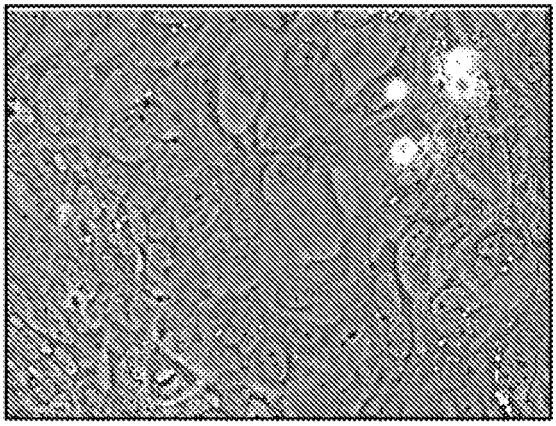


FIG. 2F

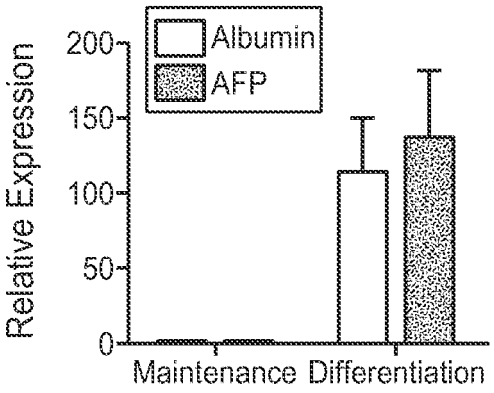


FIG. 2G

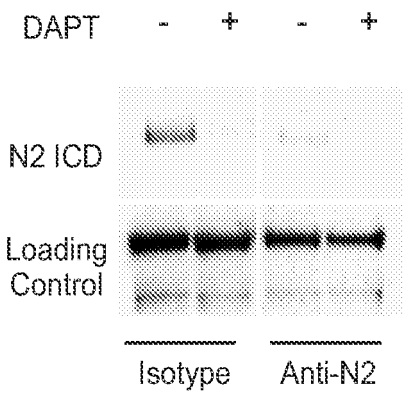


FIG. 2H

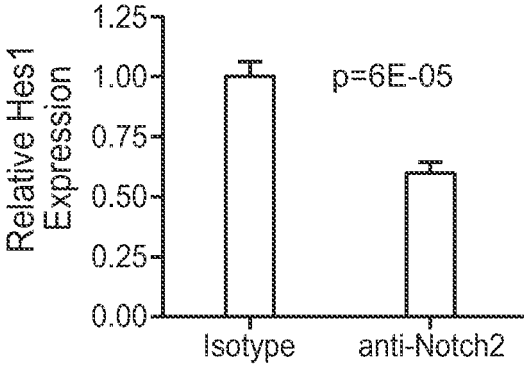


FIG. 2I

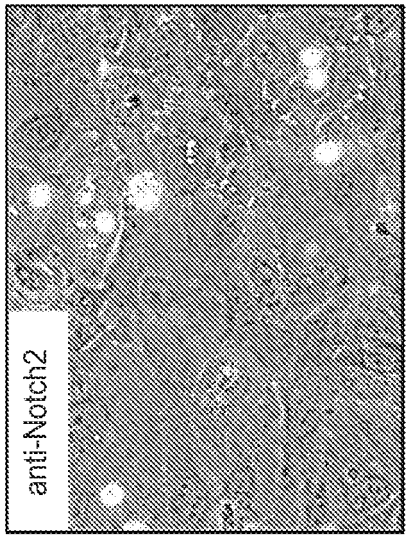


FIG. 2K

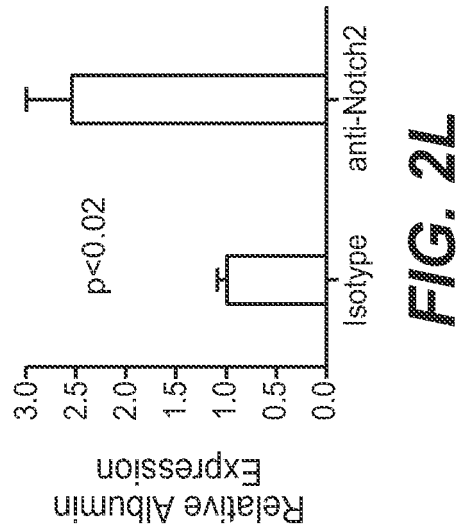


FIG. 2L

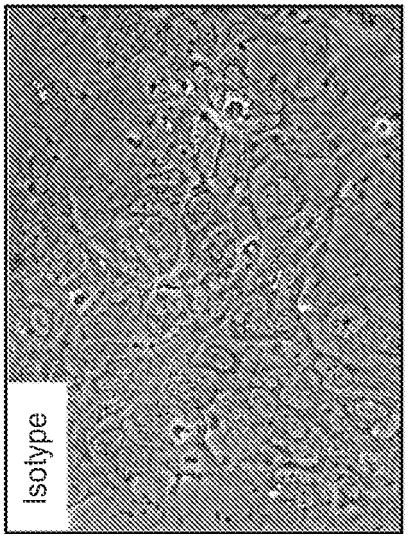


FIG. 2J

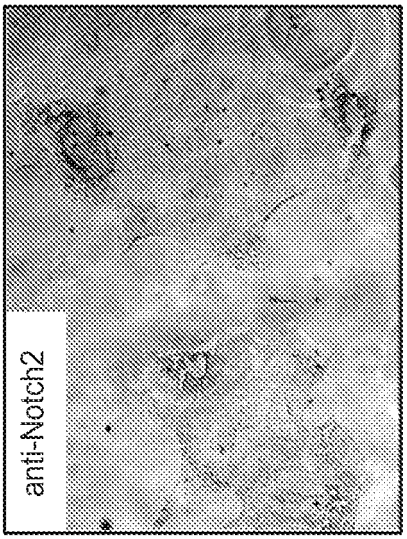


FIG. 2N

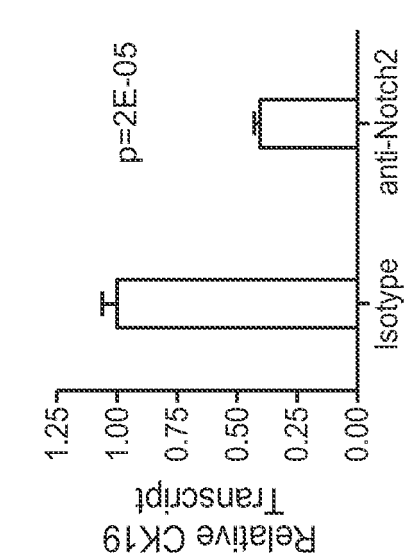


FIG. 2O

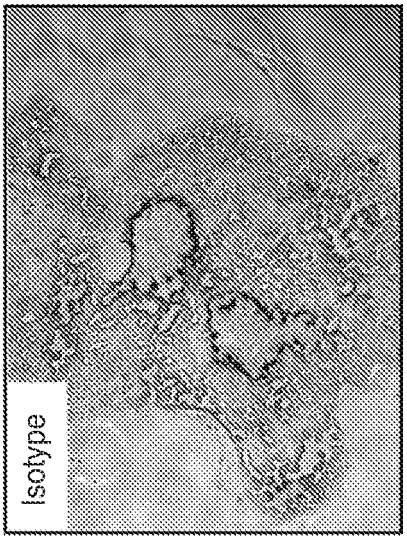
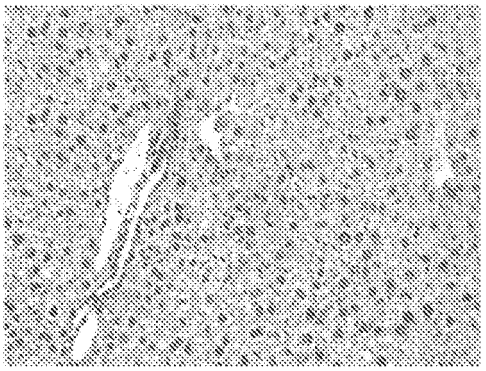


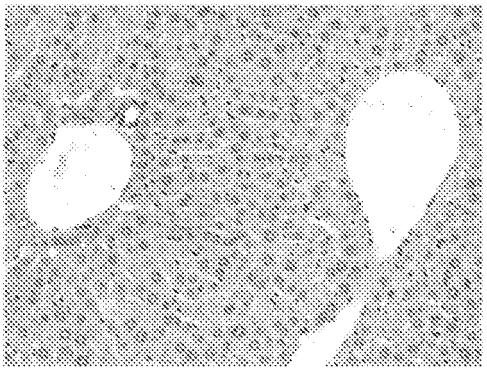
FIG. 2M

Isotype

anti-Notch2

H&E



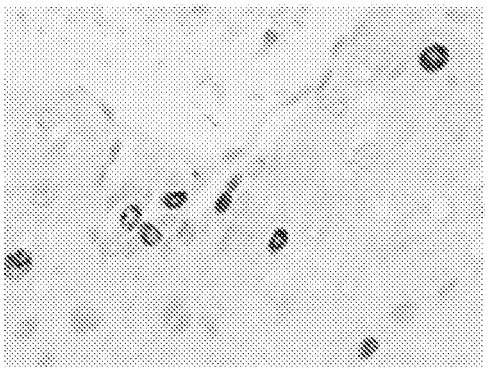


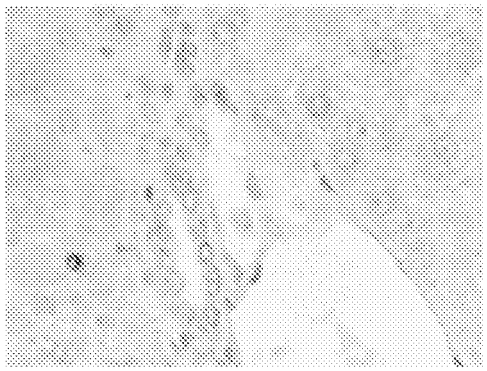
2 Weeks Post phx

FIG. 3A-1

FIG. 3A-2

BrdU



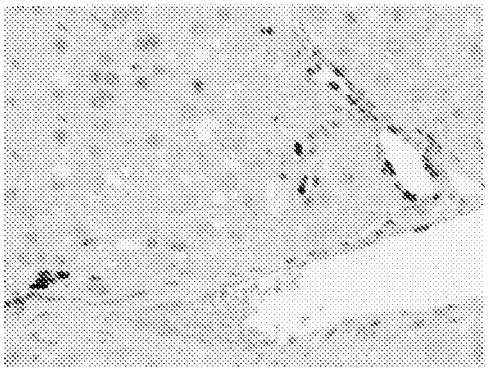


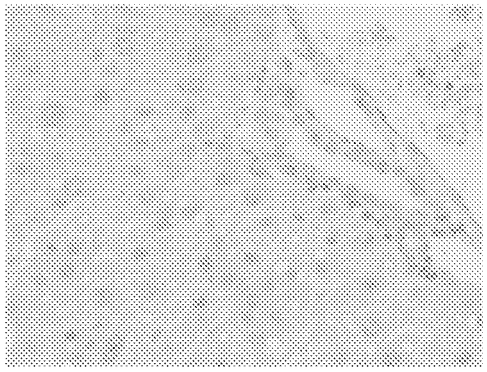
40 Hrs after 2/3 phx

FIG. 3B-1

FIG. 3B-2

Hes1



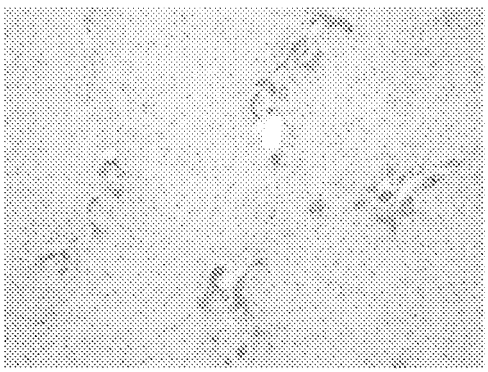


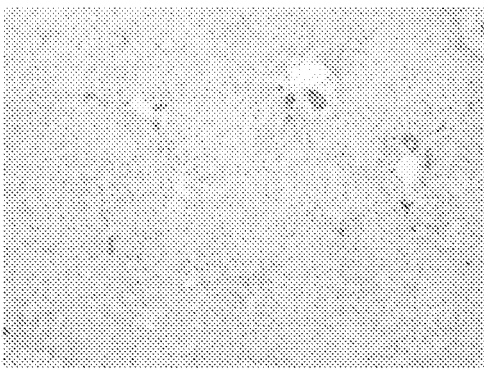
40 Hrs after 2/3 phx

FIG. 3C-1

FIG. 3C-2

pan-CK





DDC Diet 4 Weeks

FIG. 3D-1

FIG. 3D-2

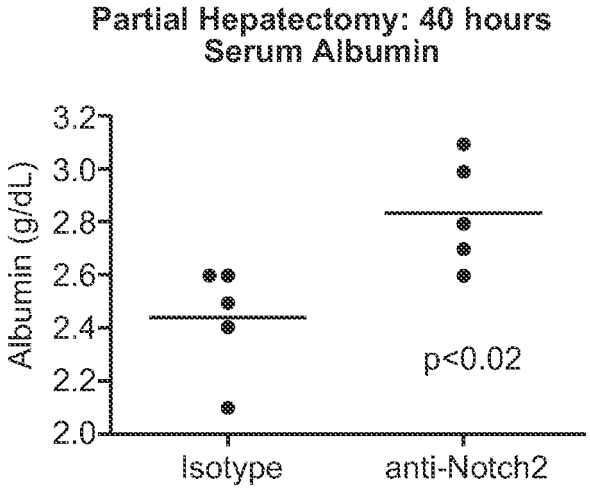


FIG. 3E

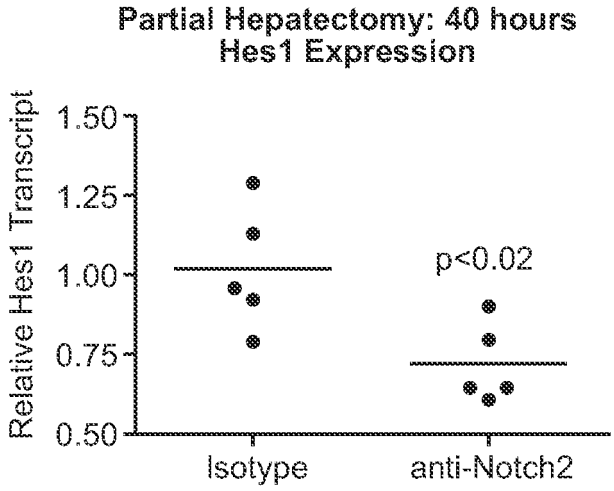


FIG. 3F

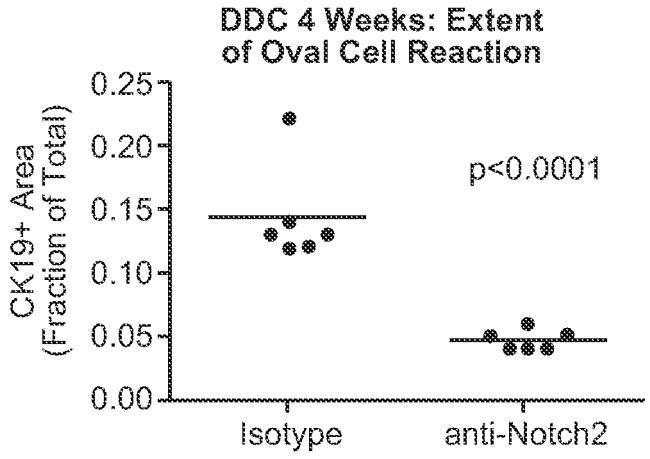


FIG. 3G

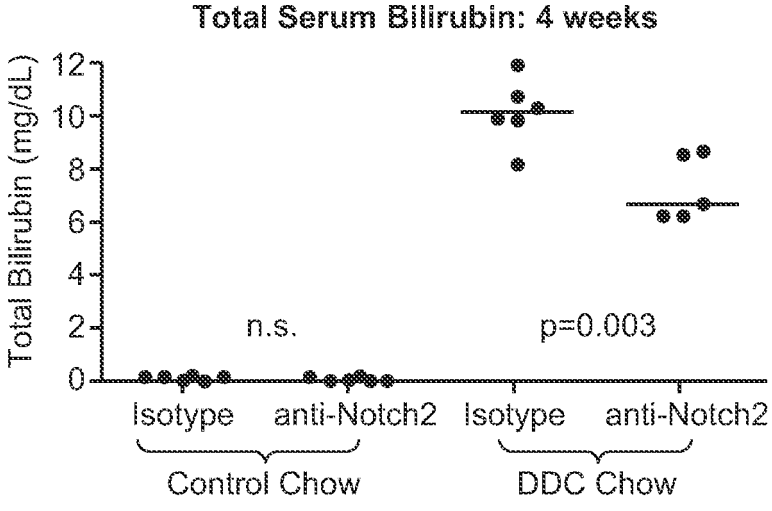


FIG. 3H

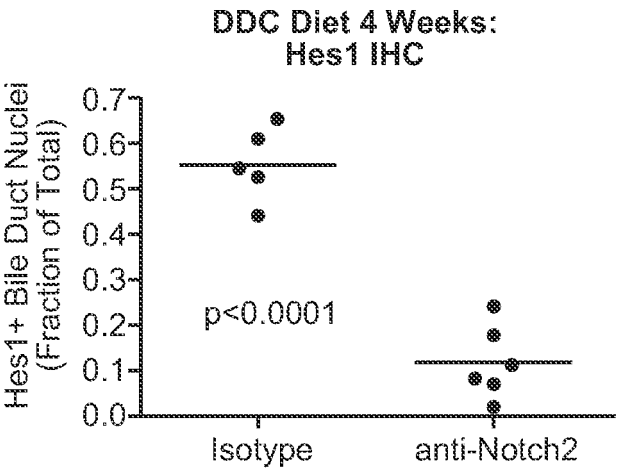


FIG. 3I

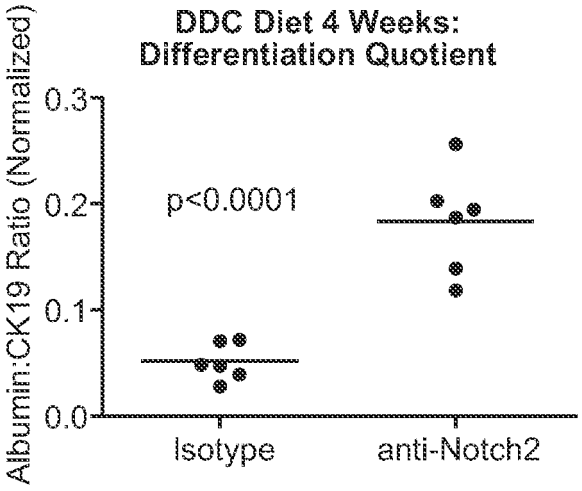


FIG. 3J

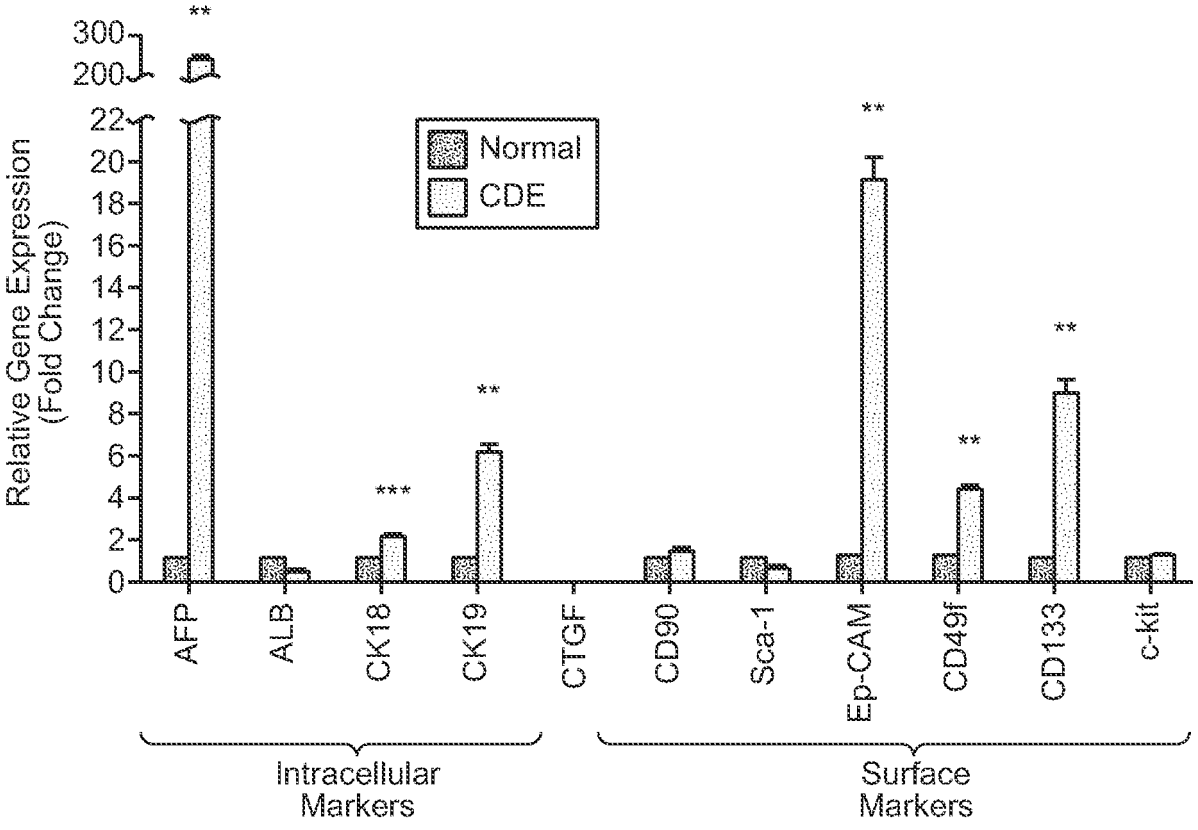


FIG. 4A

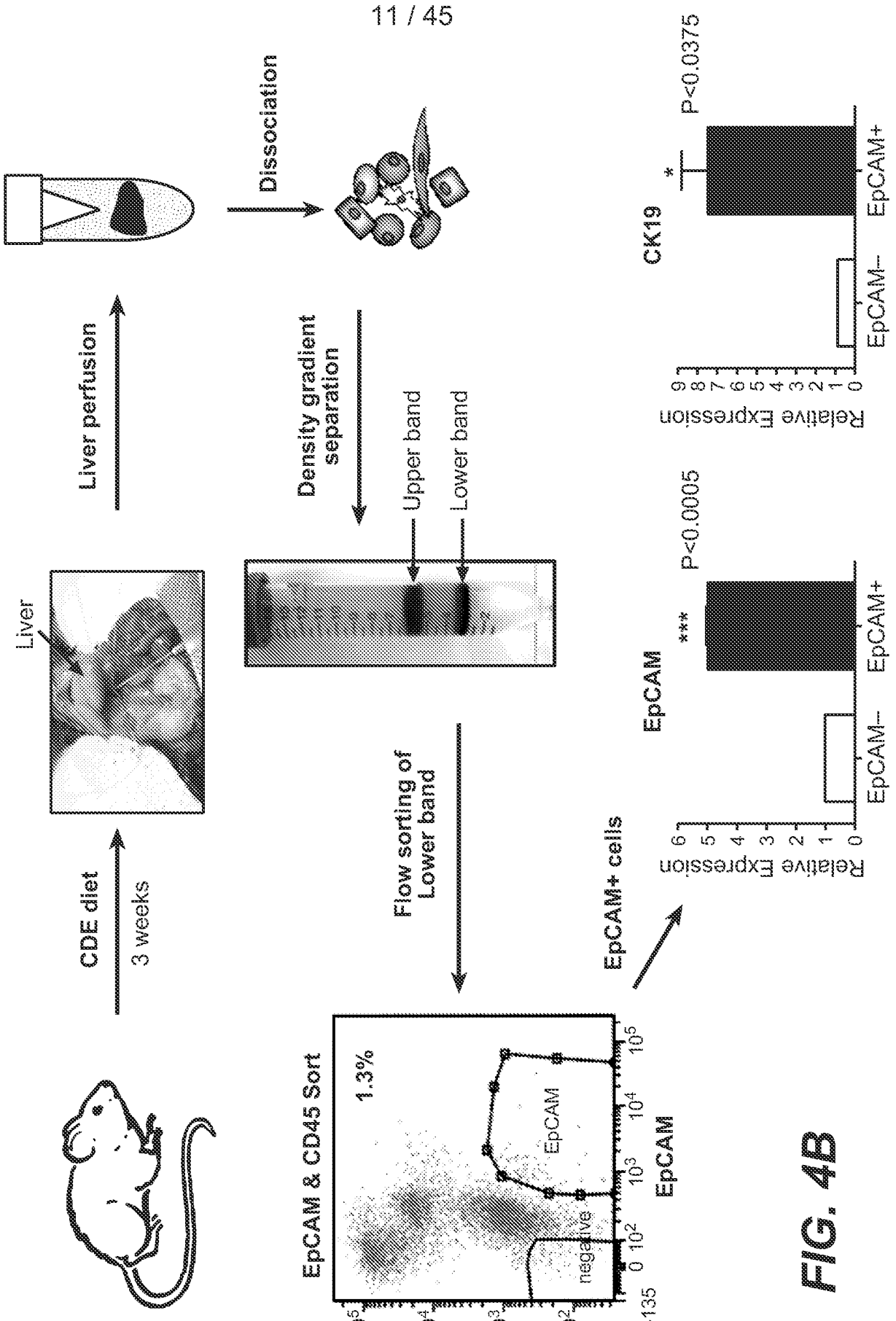
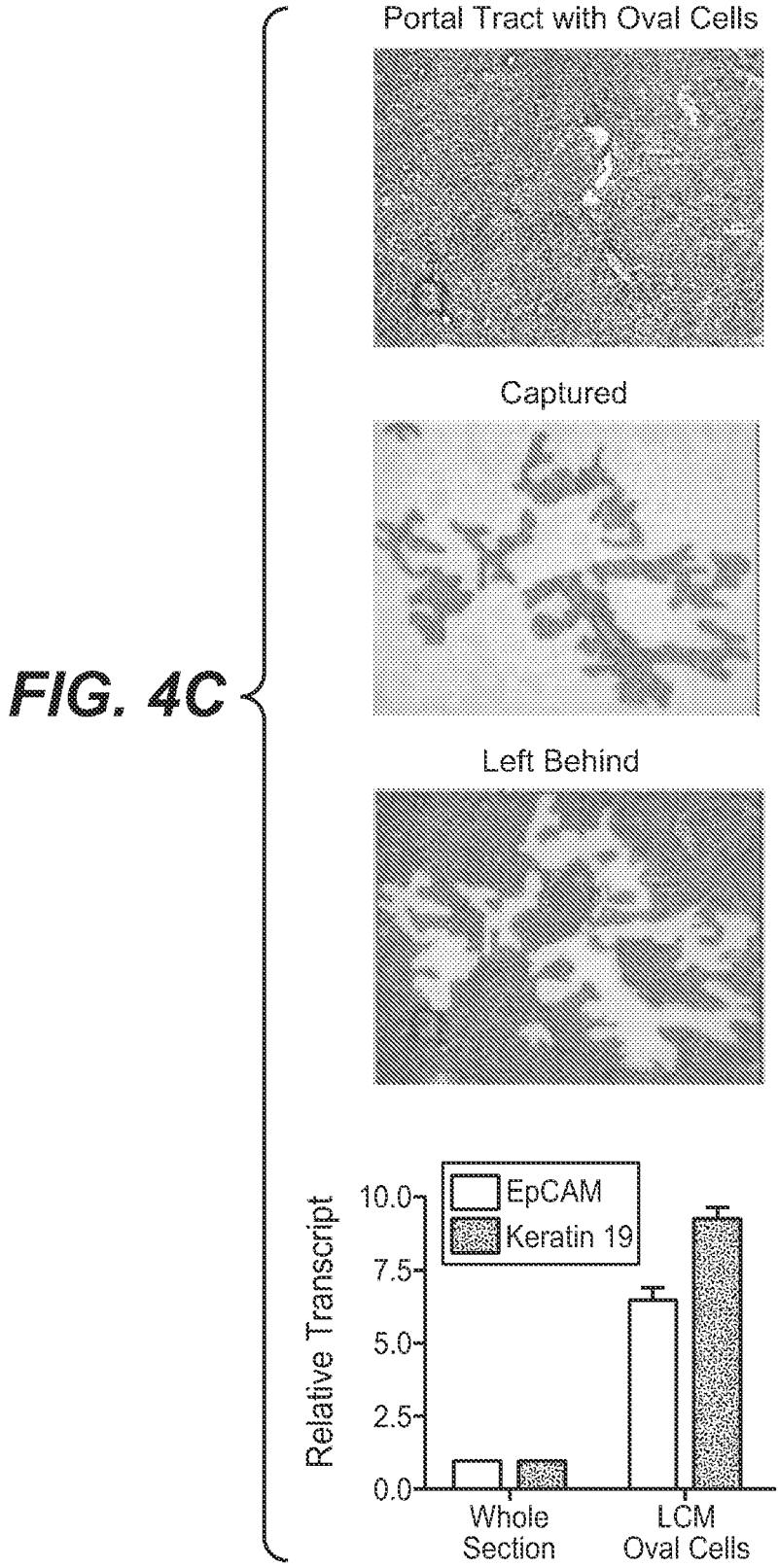

CDE diet
3 weeks
Liver
Liver perfusion
Dissociation
Density gradient separation
Upper band
Lower band
Flow sorting of Lower band
EpCAM+ cells
EpCAM & CD45 Sort
1.3%
EpCAM
negative
CD45
EpCAM
EpCAM
***
P<0.0005
Relative Expression
EpCAM–
EpCAM+
CK19
*
P<0.0375
Relative Expression
EpCAM–
EpCAM+

FIG. 4B

FIG. 4C


Portal Tract with Oval Cells
Captured
Left Behind
Relative Transcript
10.0
7.5
5.0
2.5
0.0
EpCAM
Keratin 19
Whole Section
LCM Oval Cells

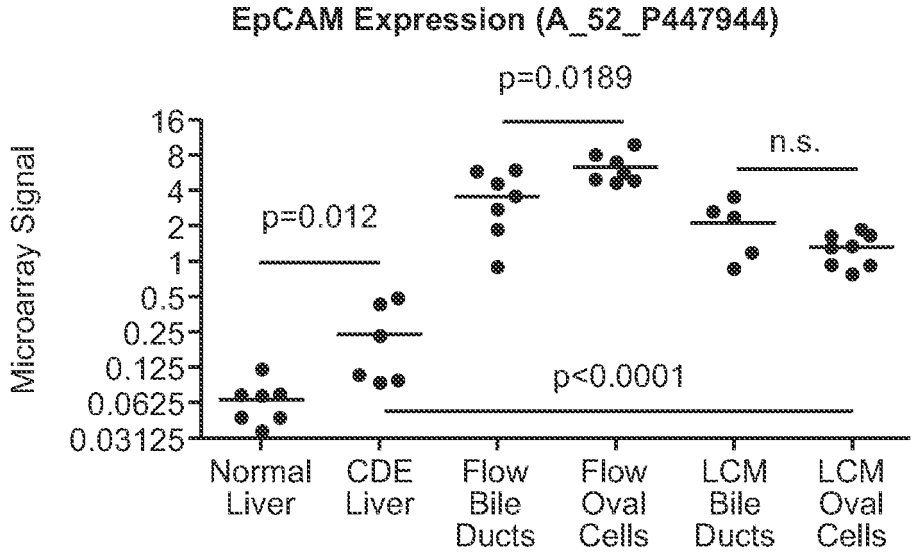

EpCAM Expression (A_52_P447944)
p=0.0189
n.s.
p=0.012
p<0.0001
Microarray Signal
16
8
4
2
1
0.5
0.25
0.125
0.0625
0.03125
Normal Liver
CDE Liver
Flow Bile Ducts
Flow Oval Cells
LCM Bile Ducts
LCM Oval Cells

FIG. 4D

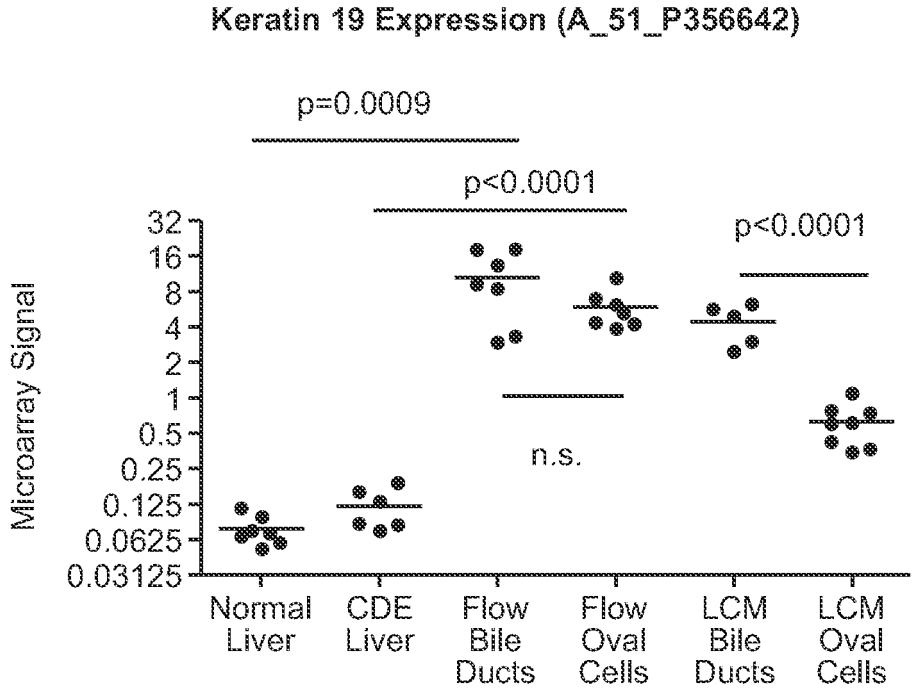

Keratin 19 Expression (A_51_P356642)
p=0.0009
p<0.0001
p<0.0001
n.s.
Microarray Signal
32
16
8
4
2
1
0.5
0.25
0.125
0.0625
0.03125
Normal Liver
CDE Liver
Flow Bile Ducts
Flow Oval Cells
LCM Bile Ducts
LCM Oval Cells

FIG. 4E

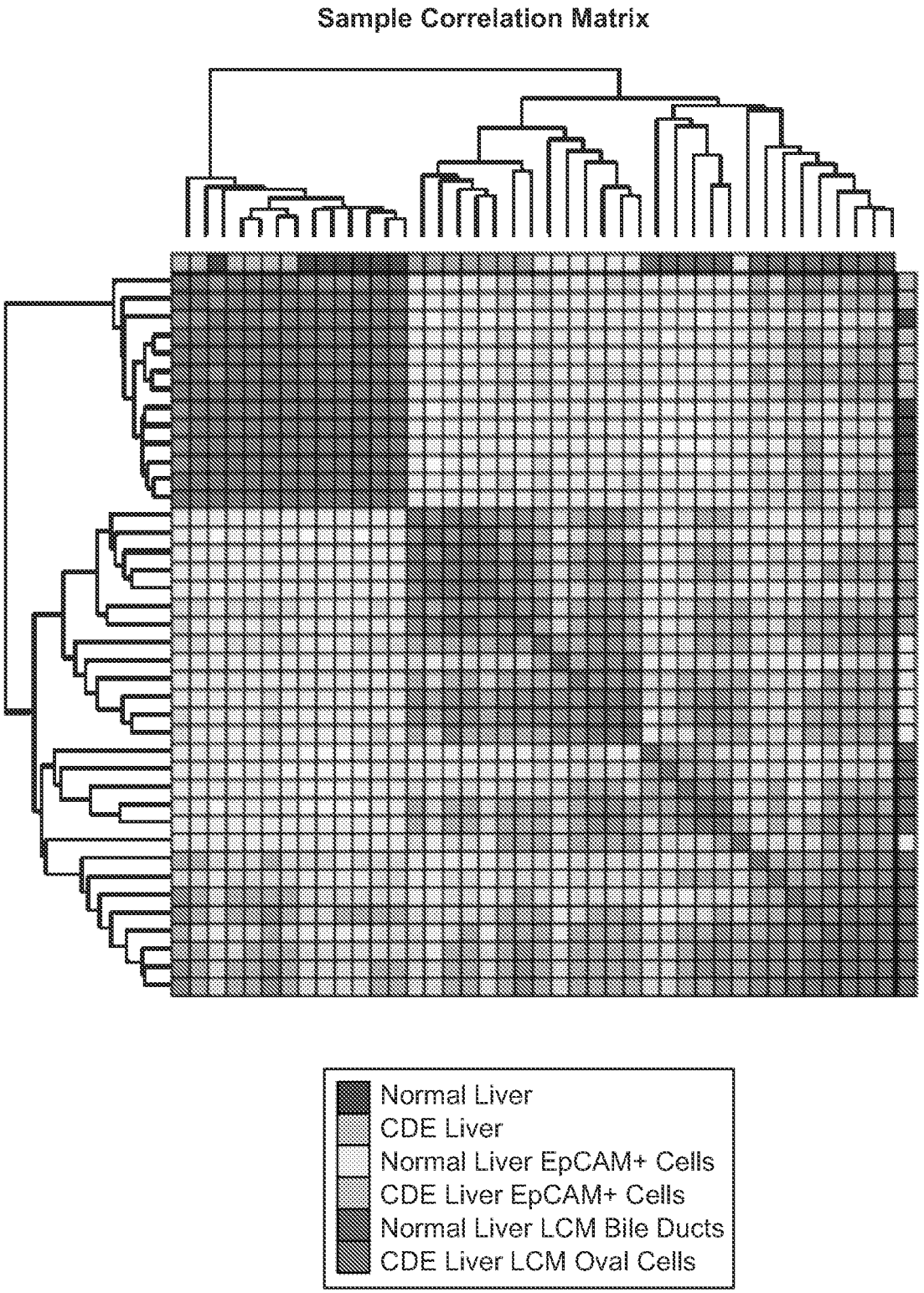

Sample Correlation Matrix
Normal Liver
CDE Liver
Normal Liver EpCAM+ Cells
CDE Liver EpCAM+ Cells
Normal Liver LCM Bile Ducts
CDE Liver LCM Oval Cells

FIG. 5A

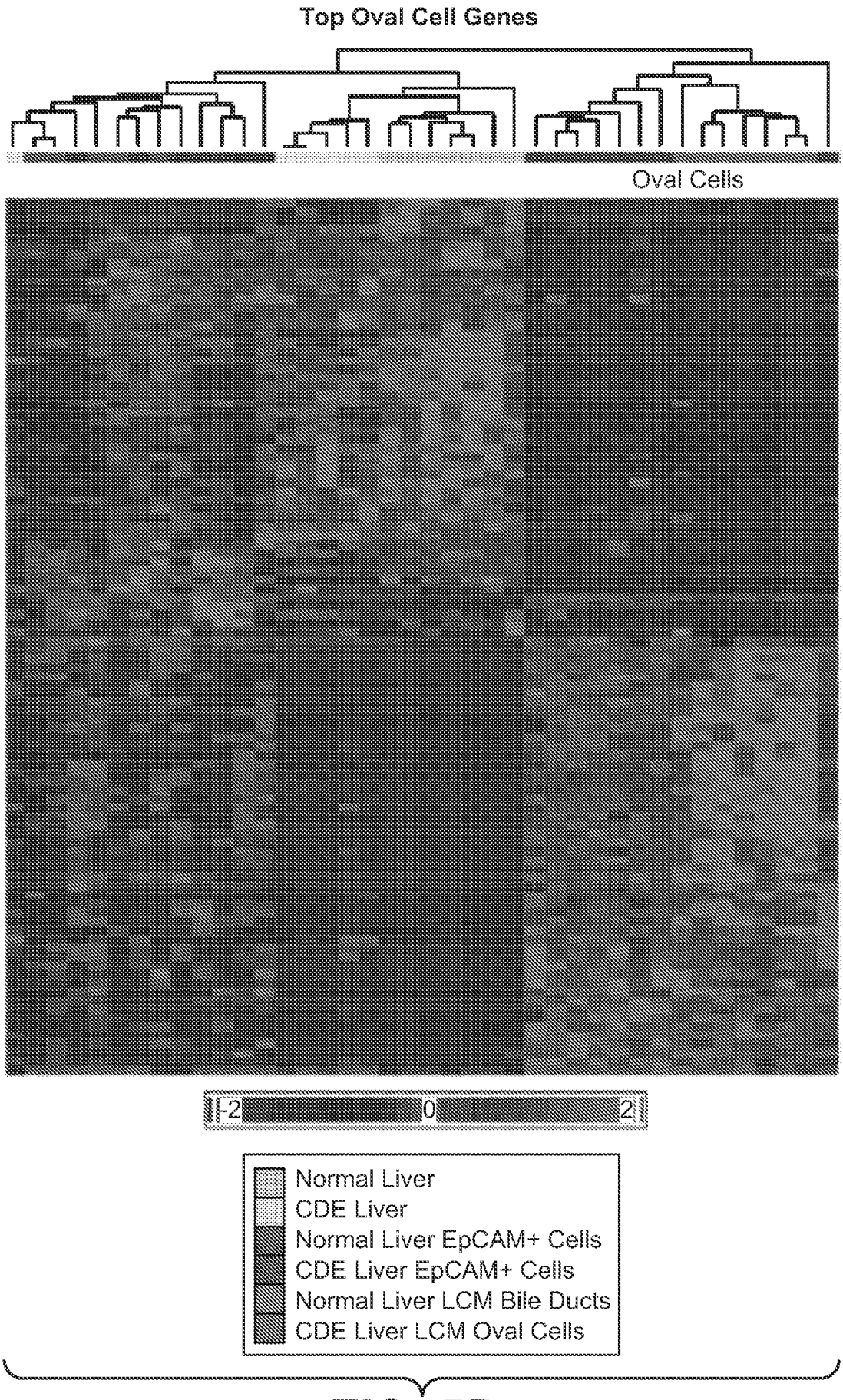

Top Oval Cell Genes
Oval Cells
-2
0
2
Normal Liver
CDE Liver
Normal Liver EpCAM+ Cells
CDE Liver EpCAM+ Cells
Normal Liver LCM Bile Ducts
CDE Liver LCM Oval Cells

FIG. 5B

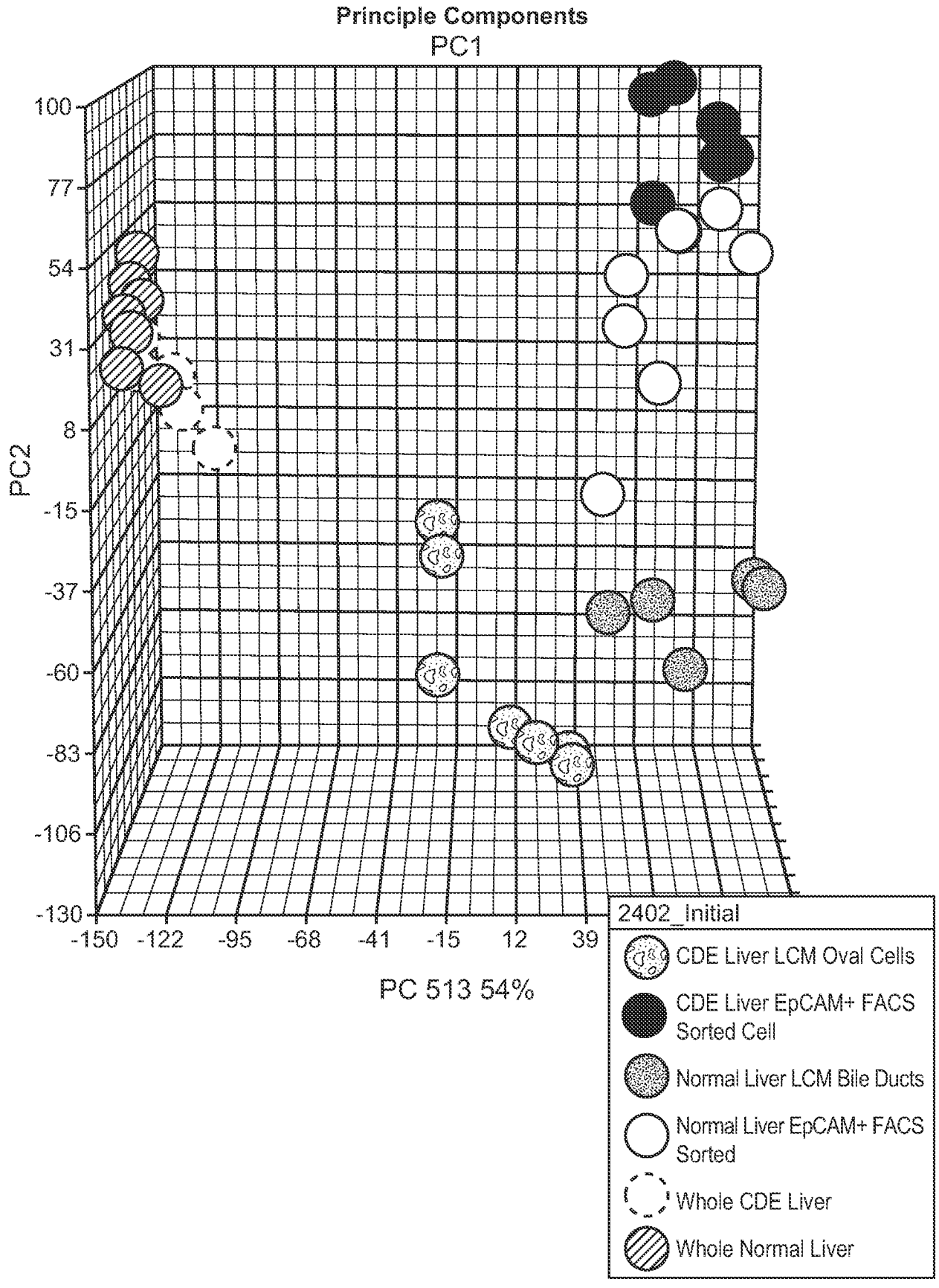

Principle Components
PC1
PC2
100
77
54
31
8
-15
-37
-60
-83
-106
-130
-150 -122 -95 -68 -41 -15 12 39
PC 513 54%
2402_Initial
CDE Liver LCM Oval Cells
CDE Liver EpCAM+ FACS Sorted Cell
Normal Liver LCM Bile Ducts
Normal Liver EpCAM+ FACS Sorted
Whole CDE Liver
Whole Normal Liver

FIG. 5C

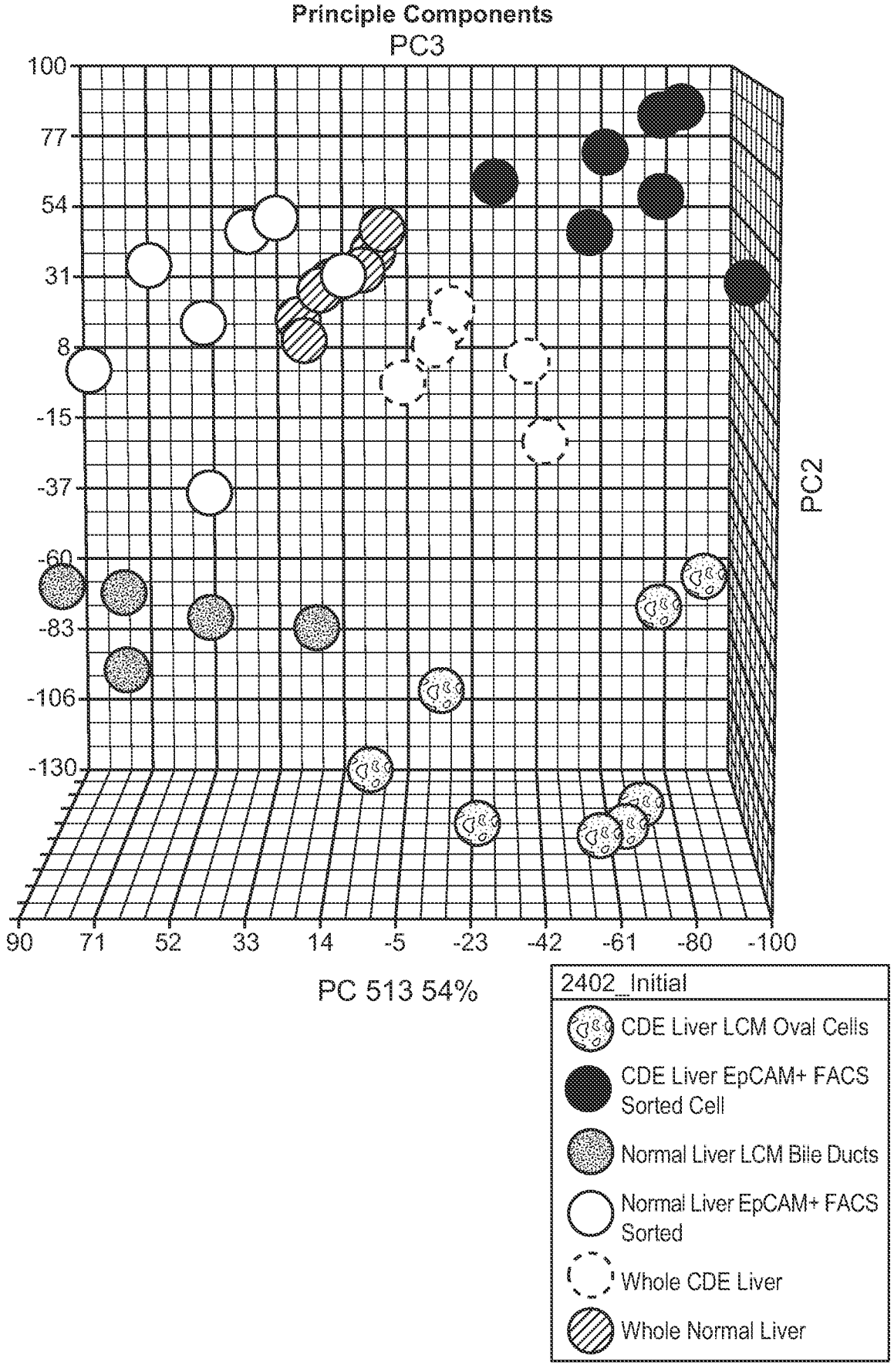

Principle Components
PC3
100
77
54
31
8
-15
-37
-60
-83
-106
-130
90
71
52
33
14
-5
-23
-42
-61
-80
-100
PC2
PC 513 54%
2402_Initial
CDE Liver LCM Oval Cells
CDE Liver EpCAM+ FACS Sorted Cell
Normal Liver LCM Bile Ducts
Normal Liver EpCAM+ FACS Sorted
Whole CDE Liver
Whole Normal Liver

FIG. 5D

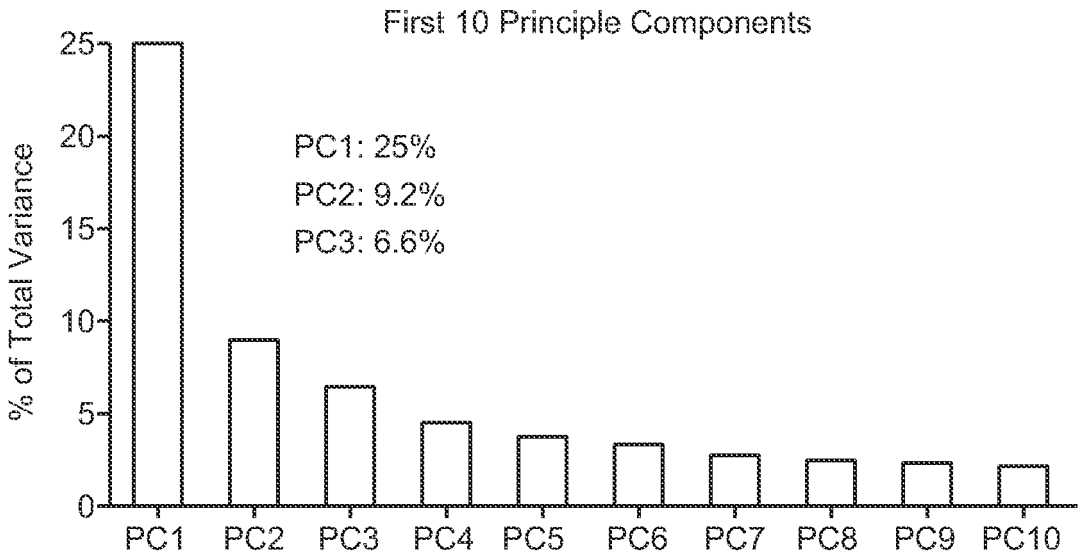

First 10 Principle Components
% of Total Variance
25
20
15
10
5
0
PC1: 25%
PC2: 9.2%
PC3: 6.6%
PC1
PC2
PC3
PC4
PC5
PC6
PC7
PC8
PC9
PC10

FIG. 5E

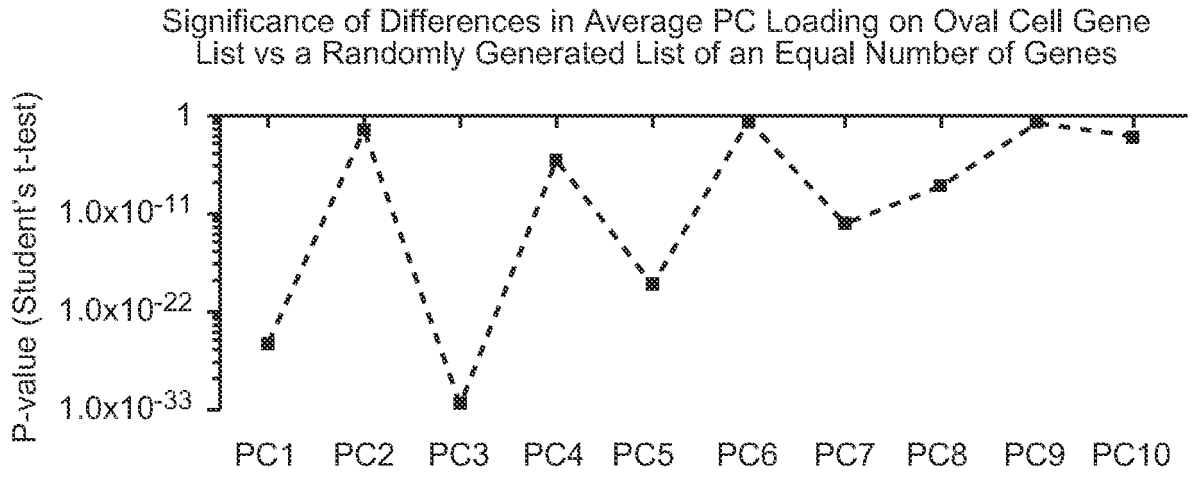

Significance of Differences in Average PC Loading on Oval Cell Gene List vs a Randomly Generated List of an Equal Number of Genes
P-value (Student's t-test)
1
1.0x10-11
1.0x10-22
1.0x10-33
PC1
PC2
PC3
PC4
PC5
PC6
PC7
PC8
PC9
PC10

FIG. 5F

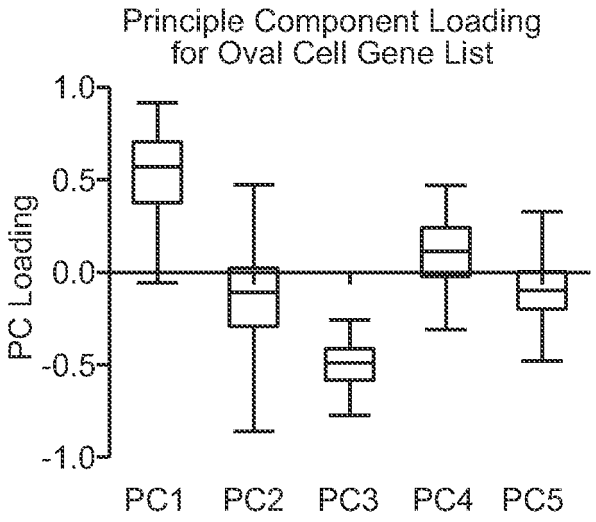

Principle Component Loading
for Oval Cell Gene List
PC Loading
1.0
0.5
0.0
-0.5
-1.0
PC1
PC2
PC3
PC4
PC5

FIG. 5G

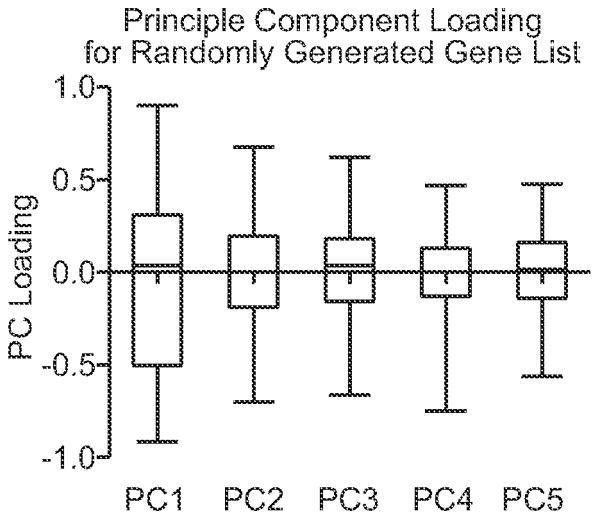

Principle Component Loading
for Randomly Generated Gene List
PC Loading
1.0
0.5
0.0
-0.5
-1.0
PC1
PC2
PC3
PC4
PC5

FIG. 5H

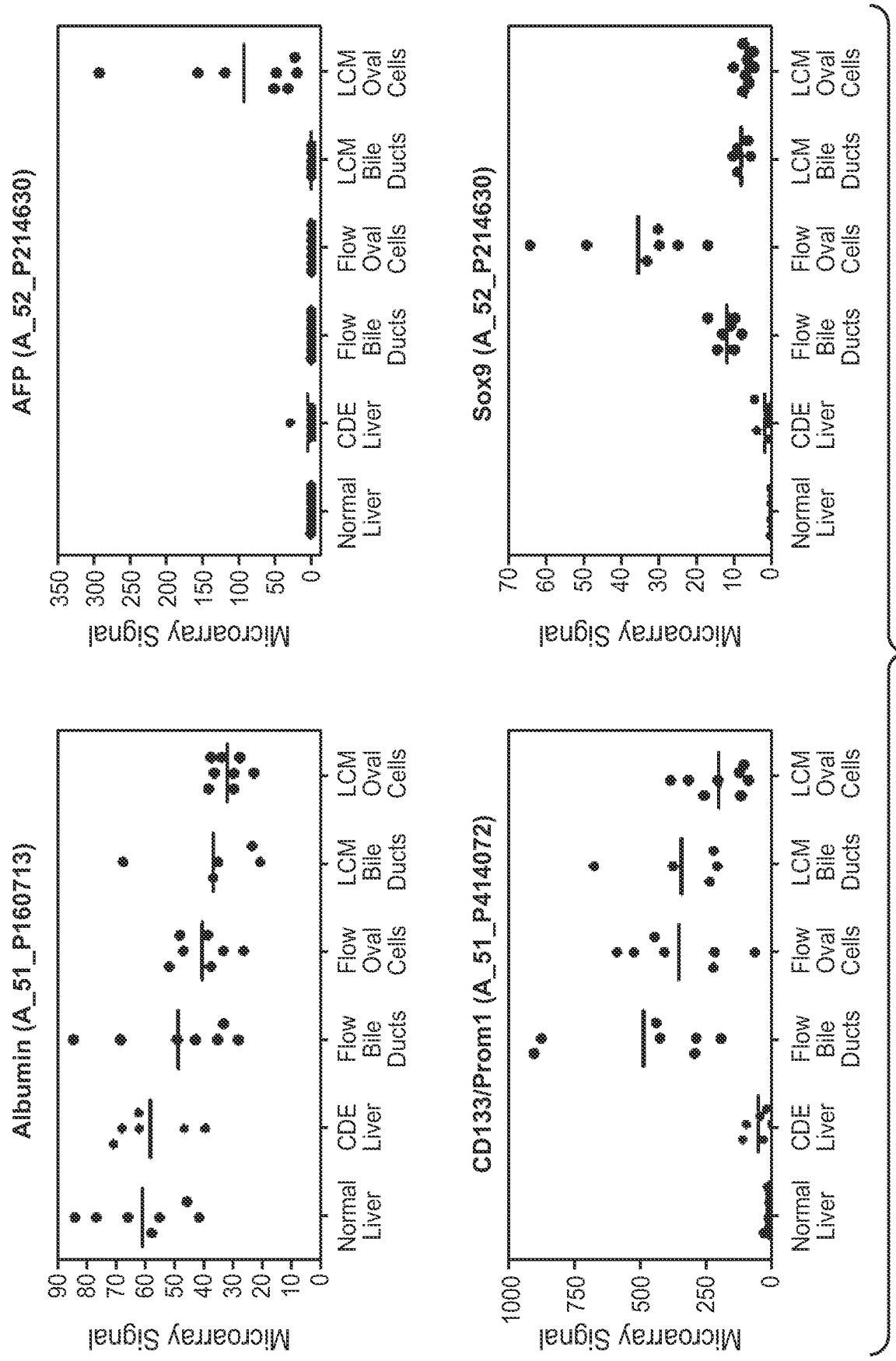

AFP (A_52_P214630)
Microarray Signal
0
50
100
150
200
250
300
350
Normal Liver
CDE Liver
Flow Bile Ducts
Flow Oval Cells
LCM Bile Ducts
LCM Oval Cells
Sox9 (A_52_P214630)
Microarray Signal
0
10
20
30
40
50
60
70
Normal Liver
CDE Liver
Flow Bile Ducts
Flow Oval Cells
LCM Bile Ducts
LCM Oval Cells
Albumin (A_51_P160713)
Microarray Signal
0
10
20
30
40
50
60
70
80
90
Normal Liver
CDE Liver
Flow Bile Ducts
Flow Oval Cells
LCM Bile Ducts
LCM Oval Cells
CD133/Prom1 (A_51_P414072)
Microarray Signal
0
250
500
750
1000
Normal Liver
CDE Liver
Flow Bile Ducts
Flow Oval Cells
LCM Bile Ducts
LCM Oval Cells

FIG. 6A-1

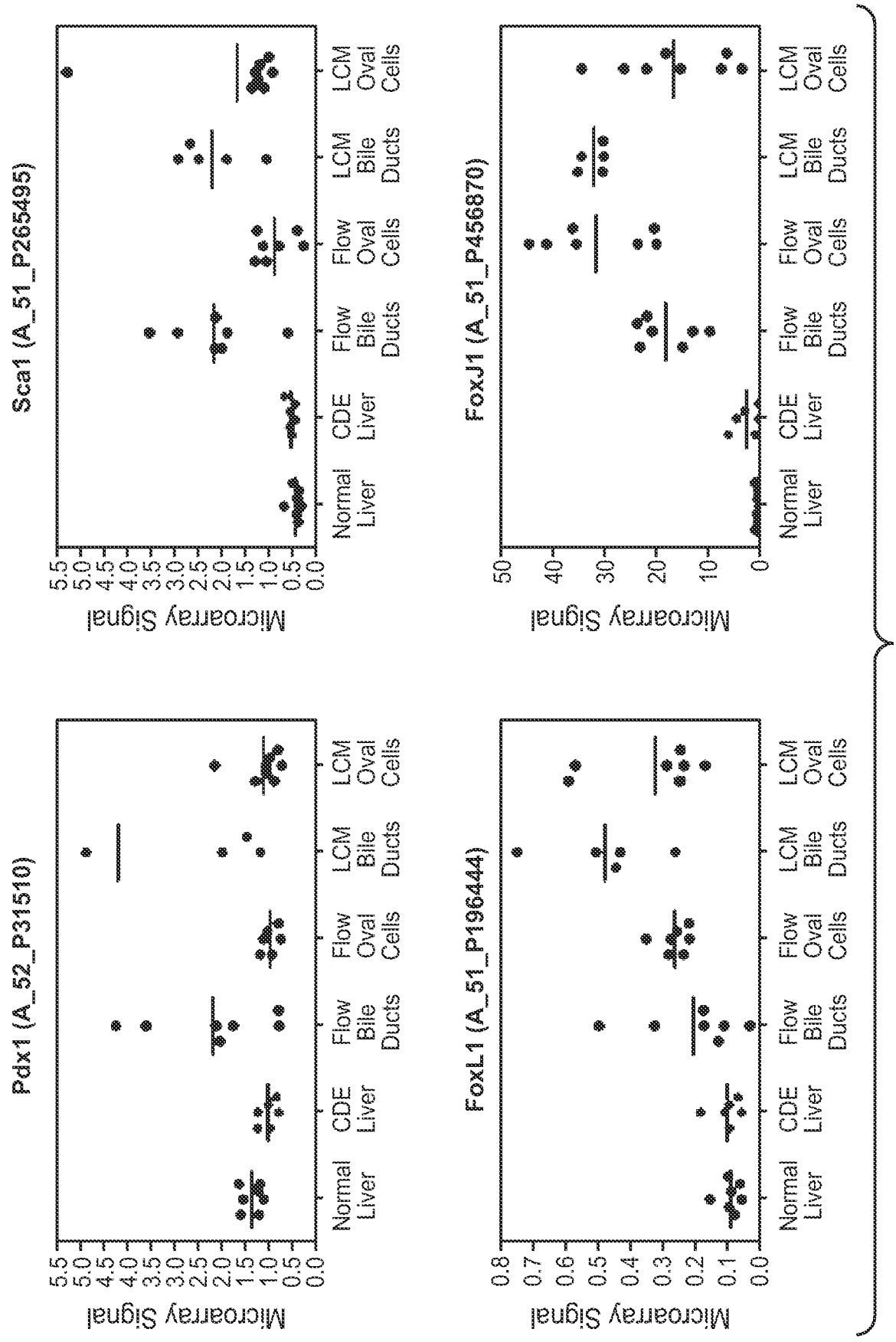

Pdx1 (A_52_P31510)
Sca1 (A_51_P265495)
FoxL1 (A_51_P196444)
FoxJ1 (A_51_P456870)
Microarray Signal
Normal Liver
CDE Liver
Flow Bile Ducts
Flow Oval Cells
LCM Bile Ducts
LCM Oval Cells

FIG. 6A-2

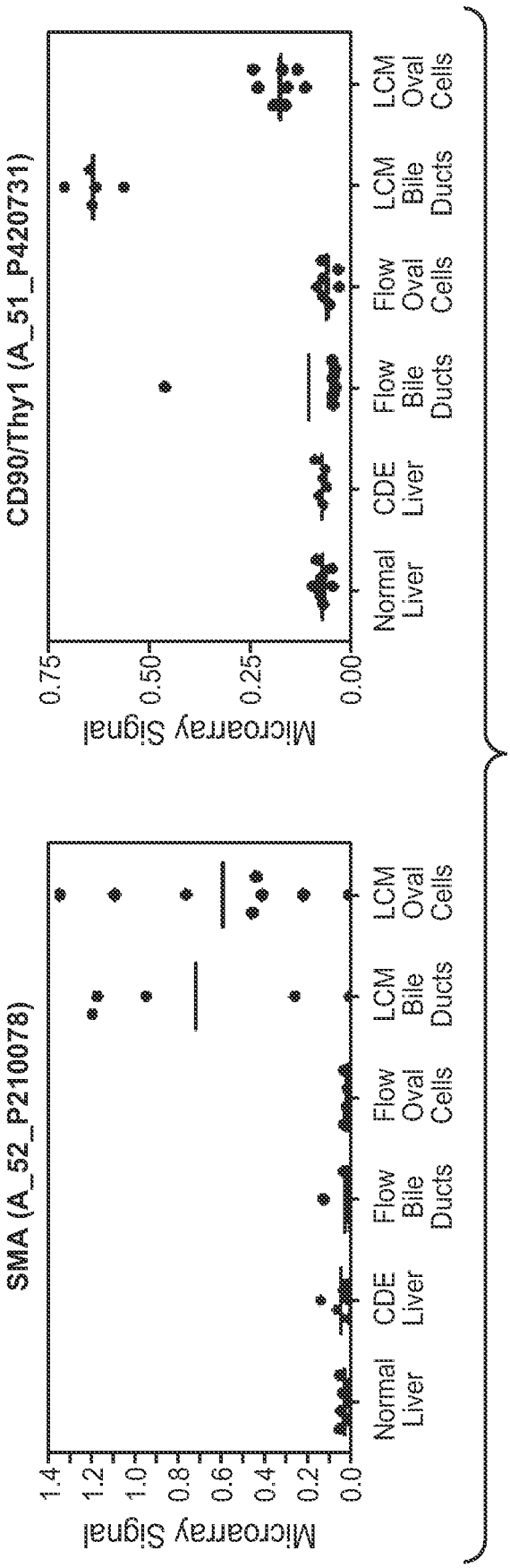

SMA (A_52_P210078)
Microarray Signal
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Normal Liver
CDE Liver
Flow Bile Ducts
Flow Oval Cells
LCM Bile Ducts
LCM Oval Cells
CD90/Thy1 (A_51_P420731)
Microarray Signal
0.75
0.50
0.25
0.00
Normal Liver
CDE Liver
Flow Bile Ducts
Flow Oval Cells
LCM Bile Ducts
LCM Oval Cells

FIG. 6A-3

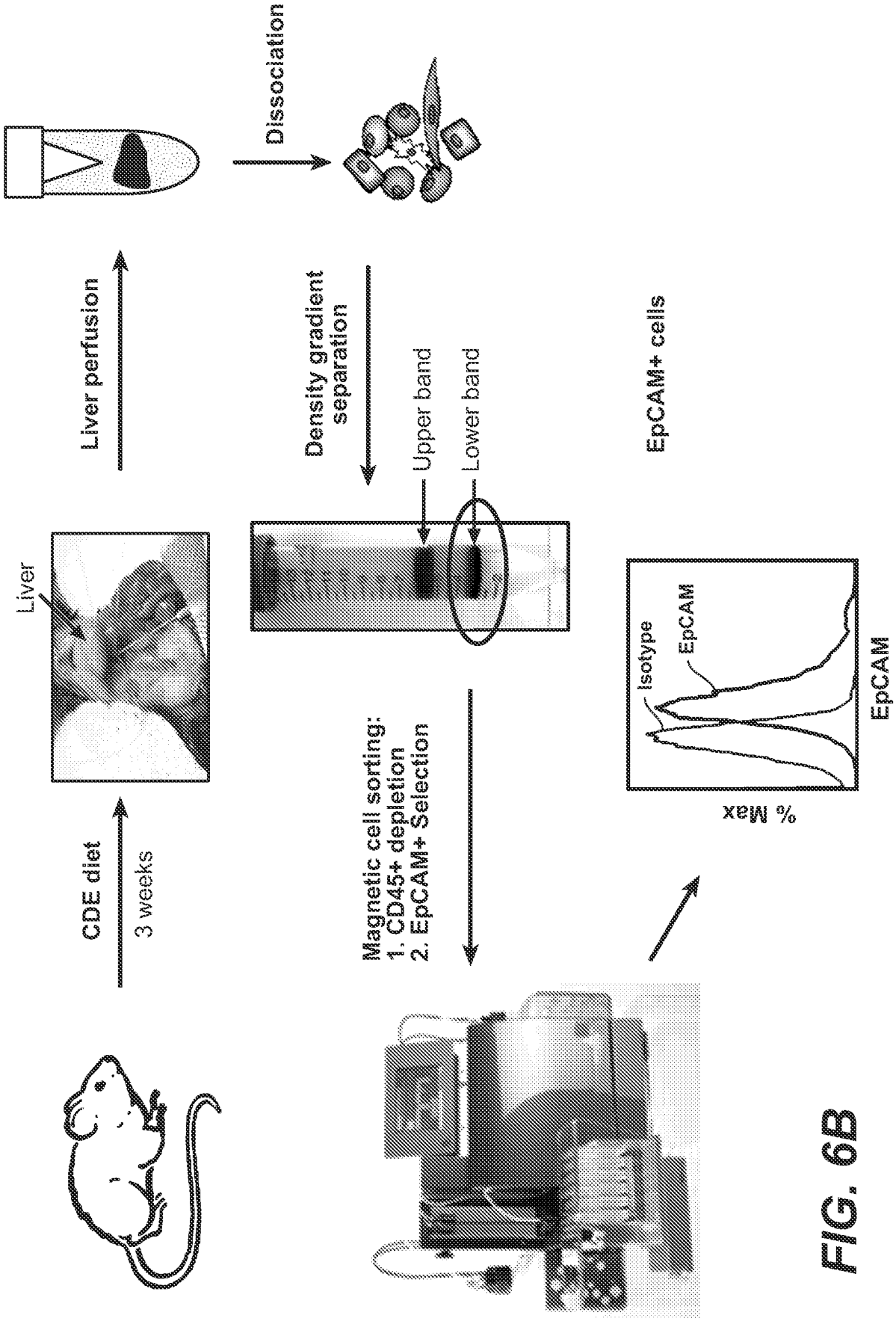

CDE diet
3 weeks
Liver
Liver perfusion
Dissociation
Density gradient separation
Upper band
Lower band
Magnetic cell sorting:
1. CD45+ depletion
2. EpCAM+ Selection
EpCAM+ cells
Isotype
EpCAM
% Max
EpCAM

FIG. 6B

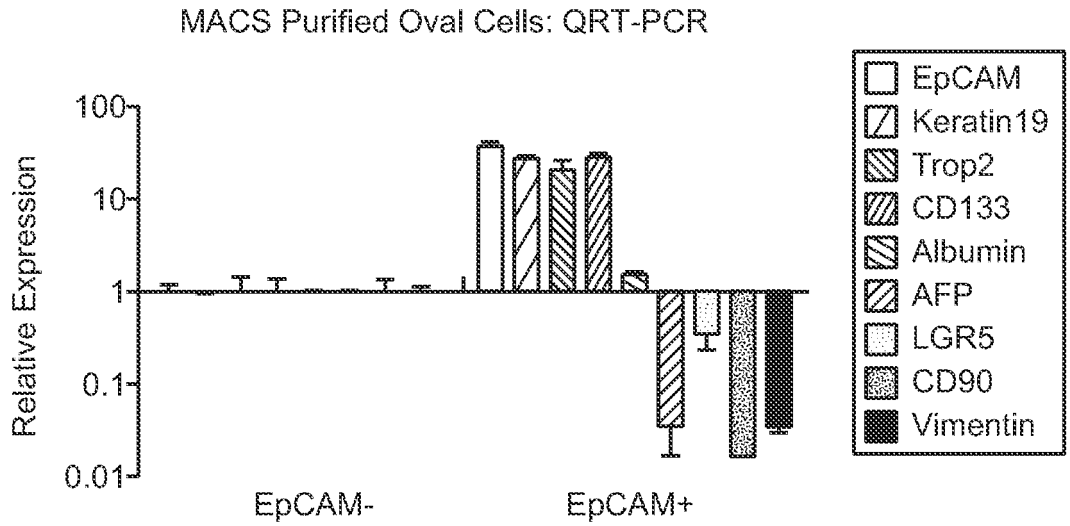

MACS Purified Oval Cells: QRT-PCR
Relative Expression
100
10
1
0.1
0.01
EpCAM-
EpCAM+
EpCAM
Keratin19
Trop2
CD133
Albumin
AFP
LGR5
CD90
Vimentin

FIG. 6C

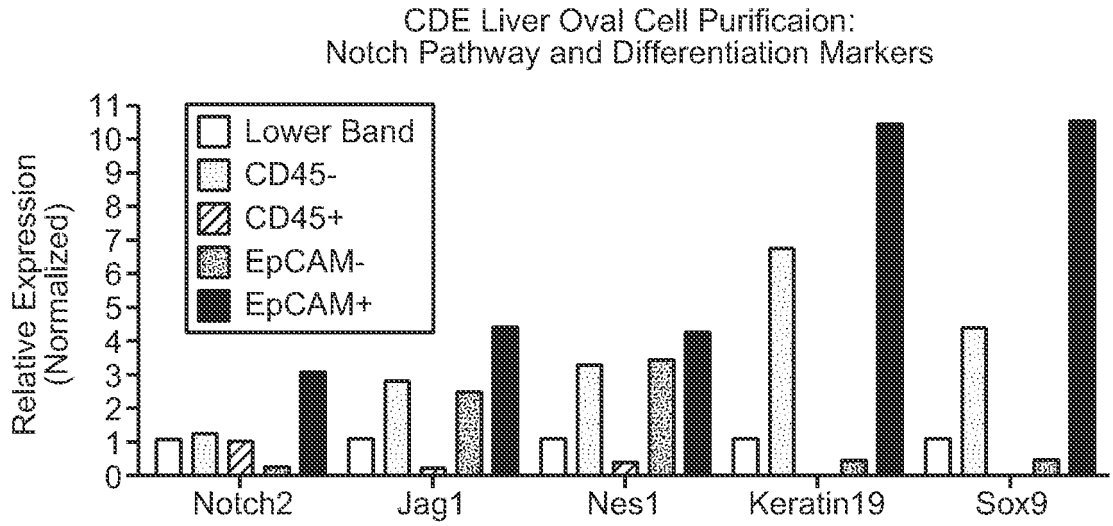

CDE Liver Oval Cell Purificaion:
Notch Pathway and Differentiation Markers
Relative Expression
(Normalized)
11
10
9
8
7
6
5
4
3
2
1
0
Lower Band
CD45-
CD45+
EpCAM-
EpCAM+
Notch2
Jag1
Nes1
Keratin19
Sox9

FIG. 6E

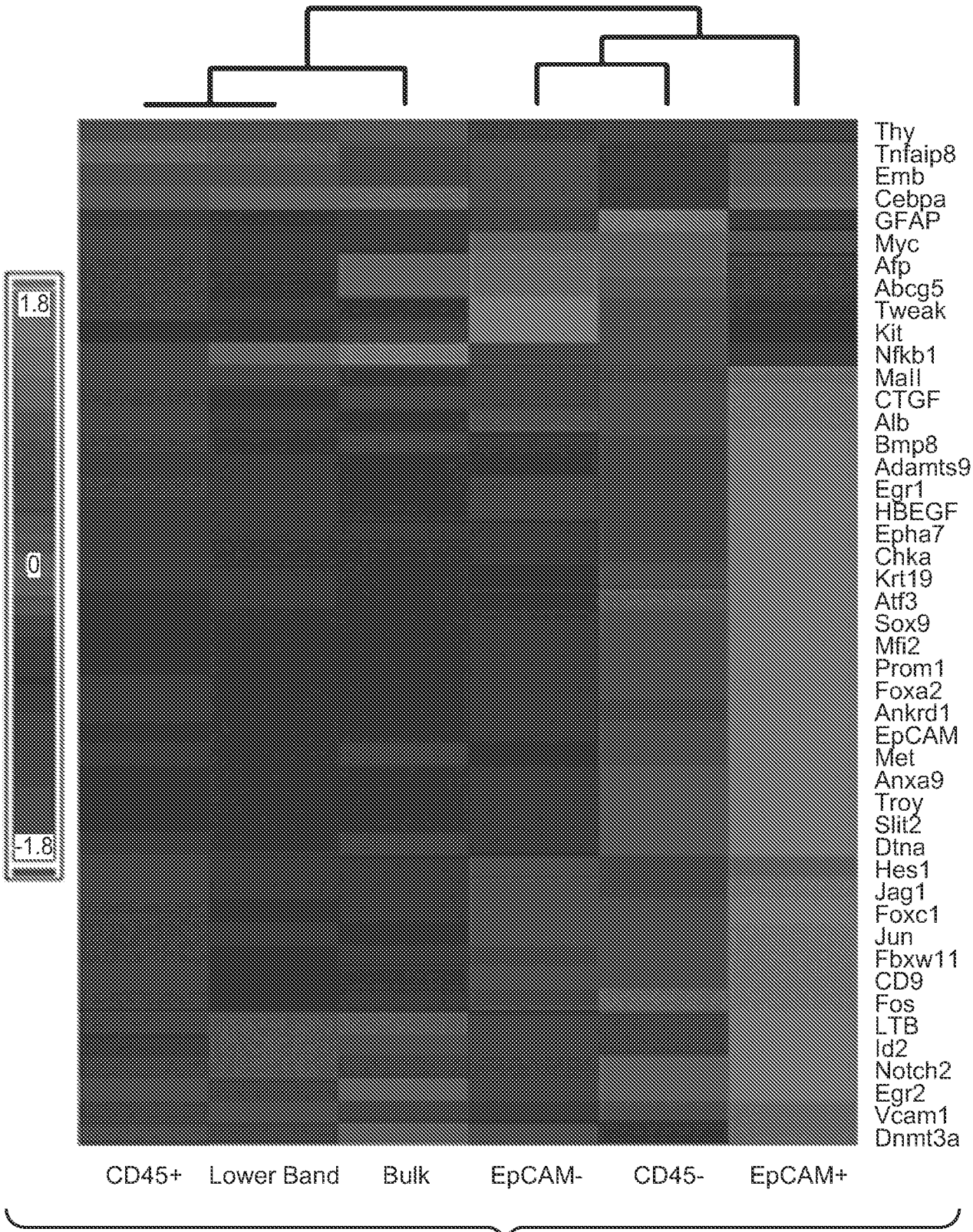

1.8
0
-1.8
Thy
Tnfaip8
Emb
Cebpa
GFAP
Myc
Afp
Abcg5
Tweak
Kit
Nfkb1
Mall
CTGF
Alb
Bmp8
Adamts9
Egr1
HBEGF
Epha7
Chka
Krt19
Atf3
Sox9
Mfi2
Prom1
Foxa2
Ankrd1
EpCAM
Met
Anxa9
Troy
Slit2
Dtna
Hes1
Jag1
Foxc1
Jun
Fbxw11
CD9
Fos
LTB
Id2
Notch2
Egr2
Vcam1
Dnmt3a
CD45+
Lower Band
Bulk
EpCAM-
CD45-
EpCAM+

FIG. 6D

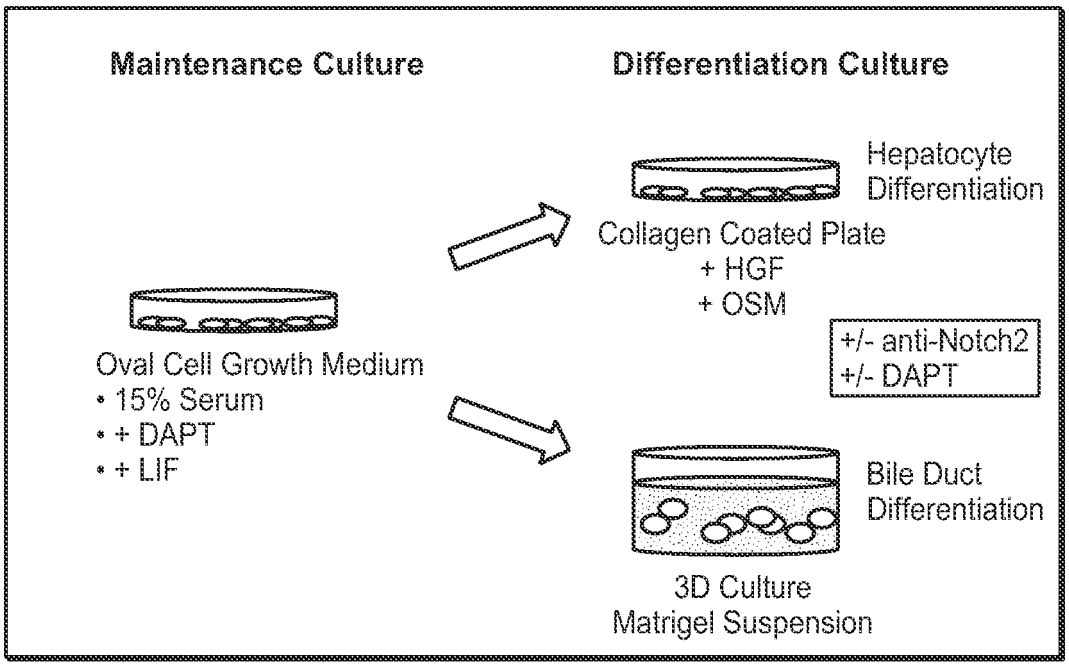

Maintenance Culture
Differentiation Culture
Hepatocyte Differentiation
Collagen Coated Plate
+ HGF
+ OSM
+/- anti-Notch2
+/- DAPT
Oval Cell Growth Medium
• 15% Serum
• + DAPT
• + LIF
Bile Duct Differentiation
3D Culture
Matrigel Suspension

FIG. 7A

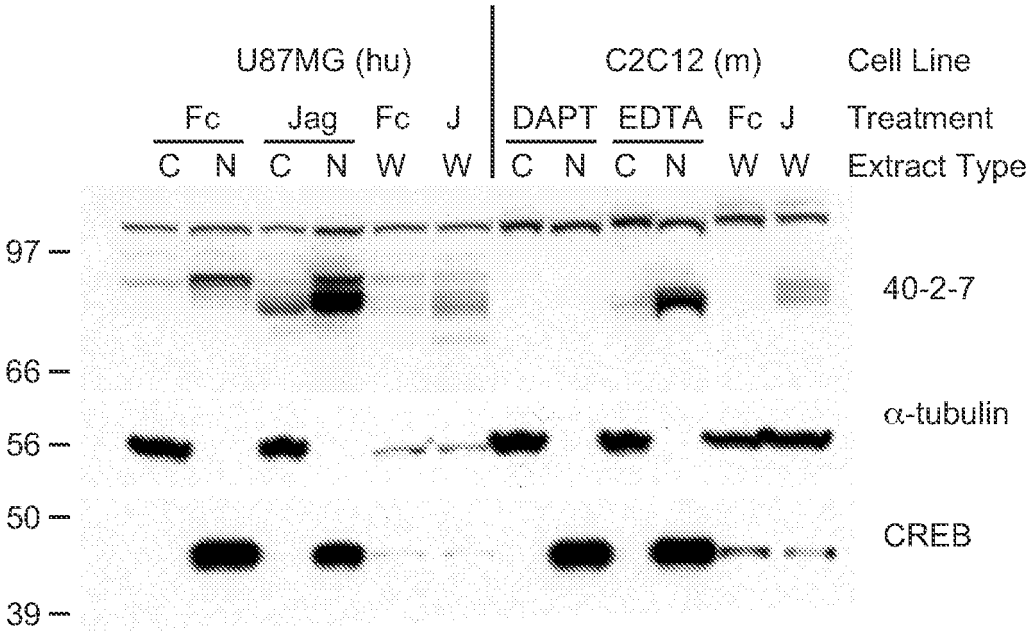

U87MG (hu)
C2C12 (m)
Cell Line
Fc
Jag
Fc
J
DAPT
EDTA
Fc
J
Treatment
C N C N W W C N C N W W
Extract Type
97
66
56
50
39
40-2-7
α-tubulin
CREB

FIG. 7B

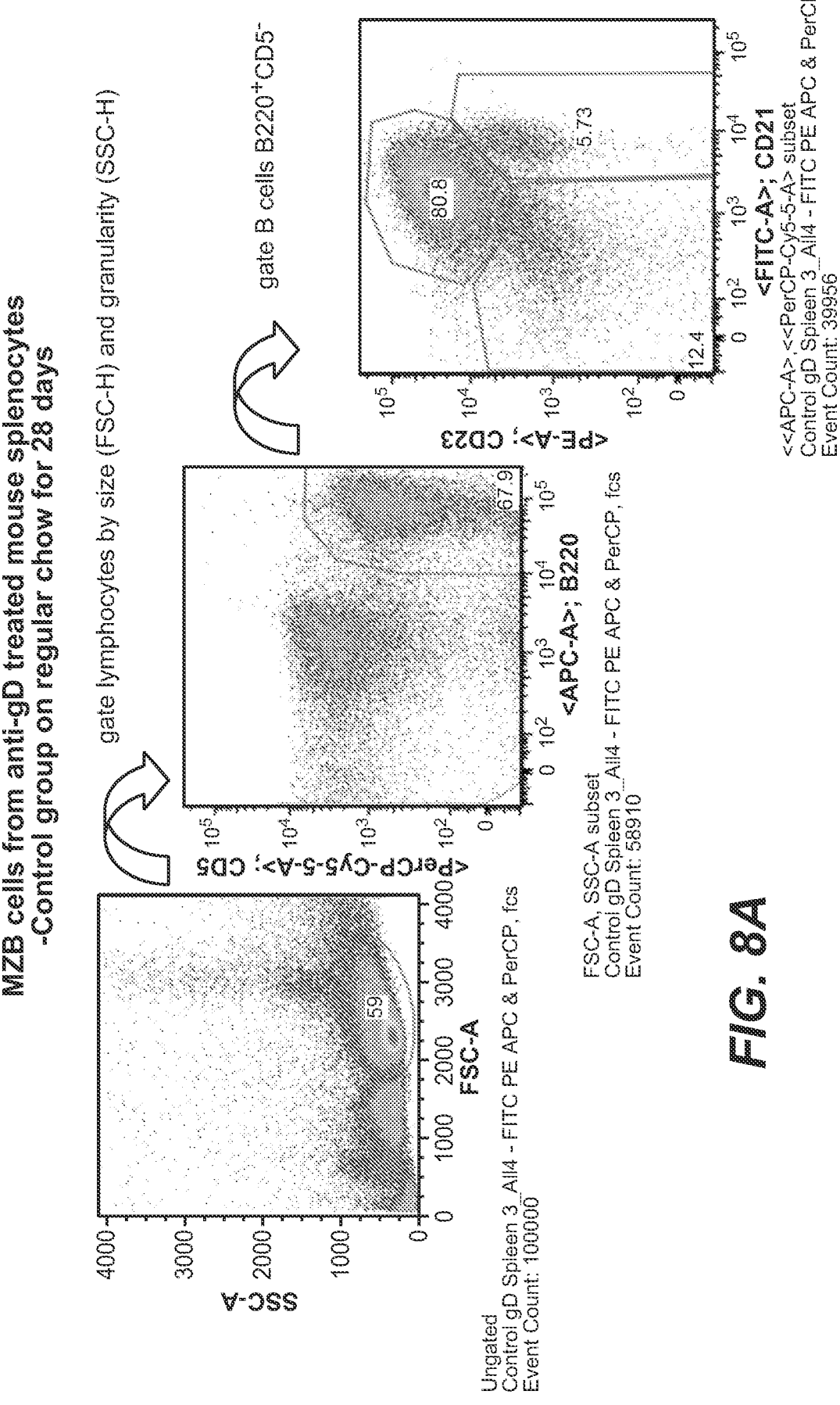

MZB cells from anti-gD treated mouse splenocytes
-Control group on regular chow for 28 days
gate lymphocytes by size (FSC-H) and granularity (SSC-H)
gate B cells B220+CD5-
SSC-A
FSC-A
Ungated
Control gD Spleen 3_All4 - FITC PE APC & PerCP, fcs
Event Count: 100000
59
PerCP-Cy5-5-A>; CD5
<APC-A>; B220
FSC-A, SSC-A subset
Control gD Spleen 3_All4 - FITC PE APC & PerCP, fcs
Event Count: 58910
67.9
<PE-A>; CD23
<FITC-A>; CD21
80.8
5.73
12.4
<<APC-A>,<<PerCP-Cy5-5-A> subset
Control gD Spleen 3_All4 - FITC PE APC & PerCP, fcs
Event Count: 39956

FIG. 8A

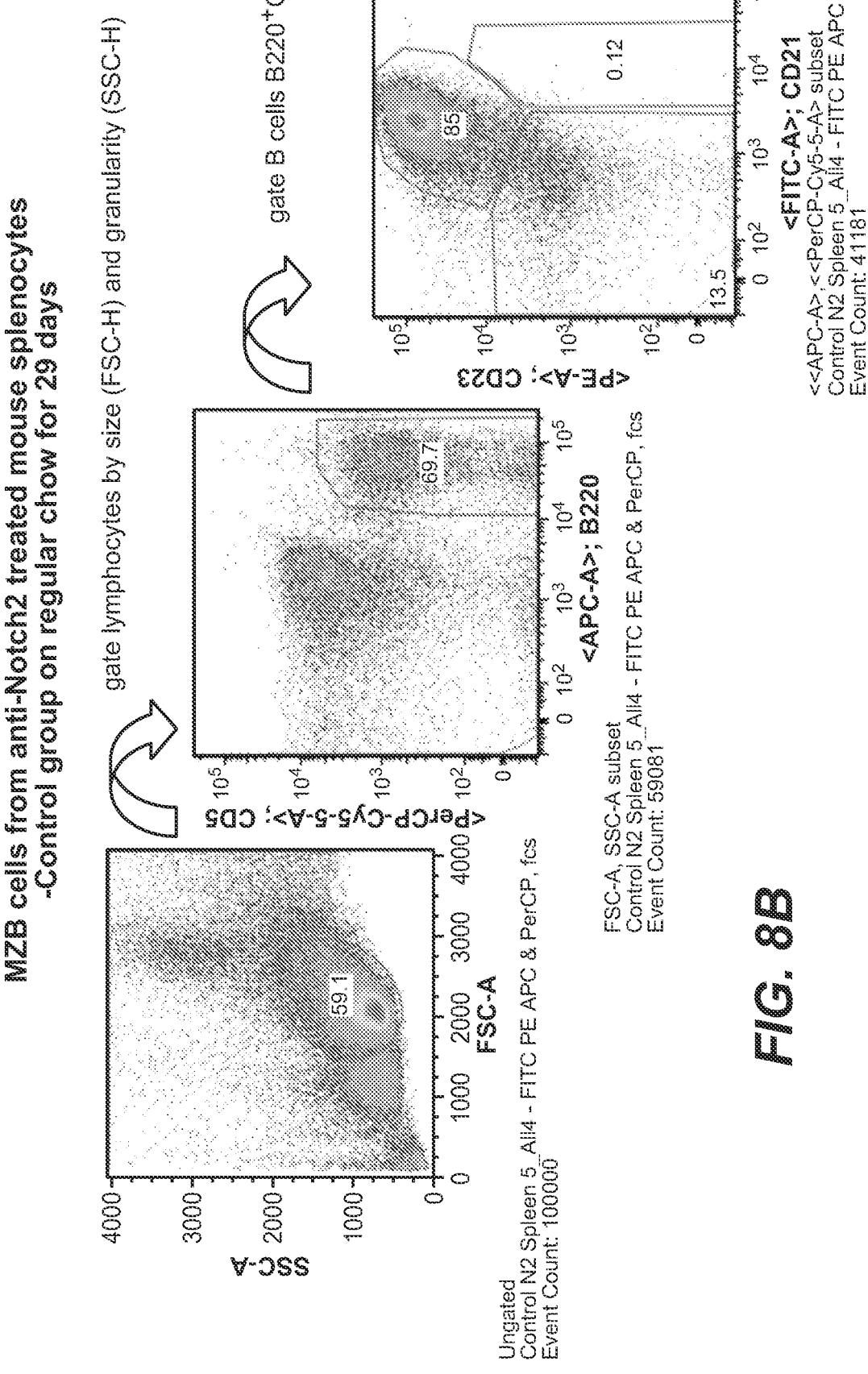

MZB cells from anti-Notch2 treated mouse splenocytes
-Control group on regular chow for 29 days
gate lymphocytes by size (FSC-H) and granularity (SSC-H)
gate B cells B220+CD5-
SSC-A
FSC-A
59.1
Ungated
Control N2 Spleen 5_All4 - FITC PE APC & PerCP, fcs
Event Count: 100000
<PerCP-Cy5-5-A>; CD5
<APC-A>; B220
69.7
FSC-A, SSC-A subset
Control N2 Spleen 5_All4 - FITC PE APC & PerCP, fcs
Event Count: 59081
<PE-A>; CD23
<FITC-A>; CD21
85
0.12
13.5
<APC-A>, <PerCP-Cy5-5-A> subset
Control N2 Spleen 5_All4 - FITC PE APC & PerCP, fcs
Event Count: 41181

FIG. 8B

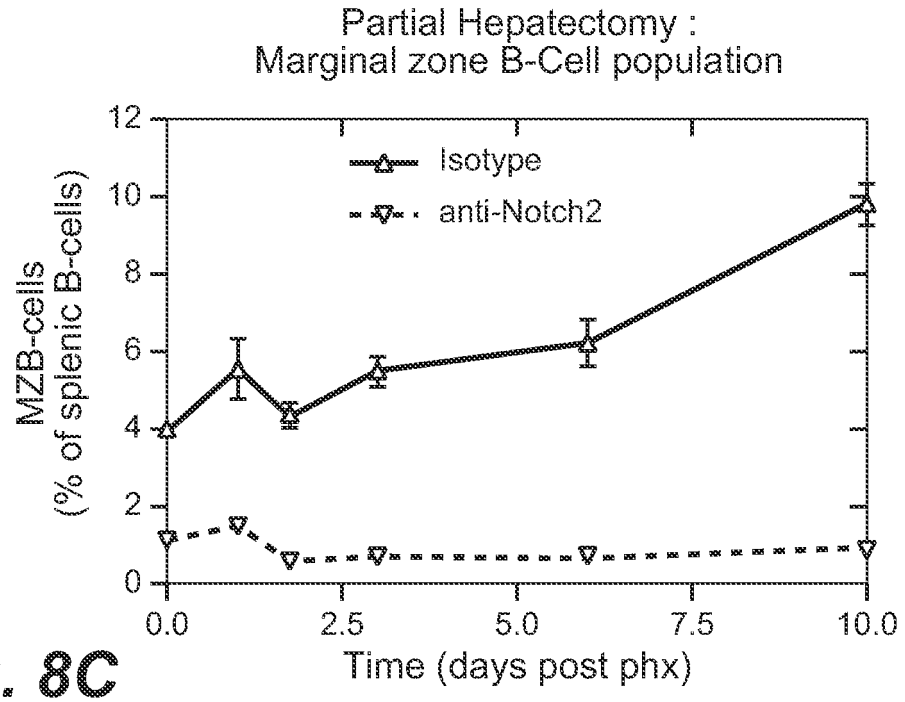

Partial Hepatectomy :
Marginal zone B-Cell population
MZB-cells
(% of splenic B-cells)
Isotype
anti-Notch2
0
2
4
6
8
10
12
0.0
2.5
5.0
7.5
10.0
Time (days post phx)

FIG. 8C

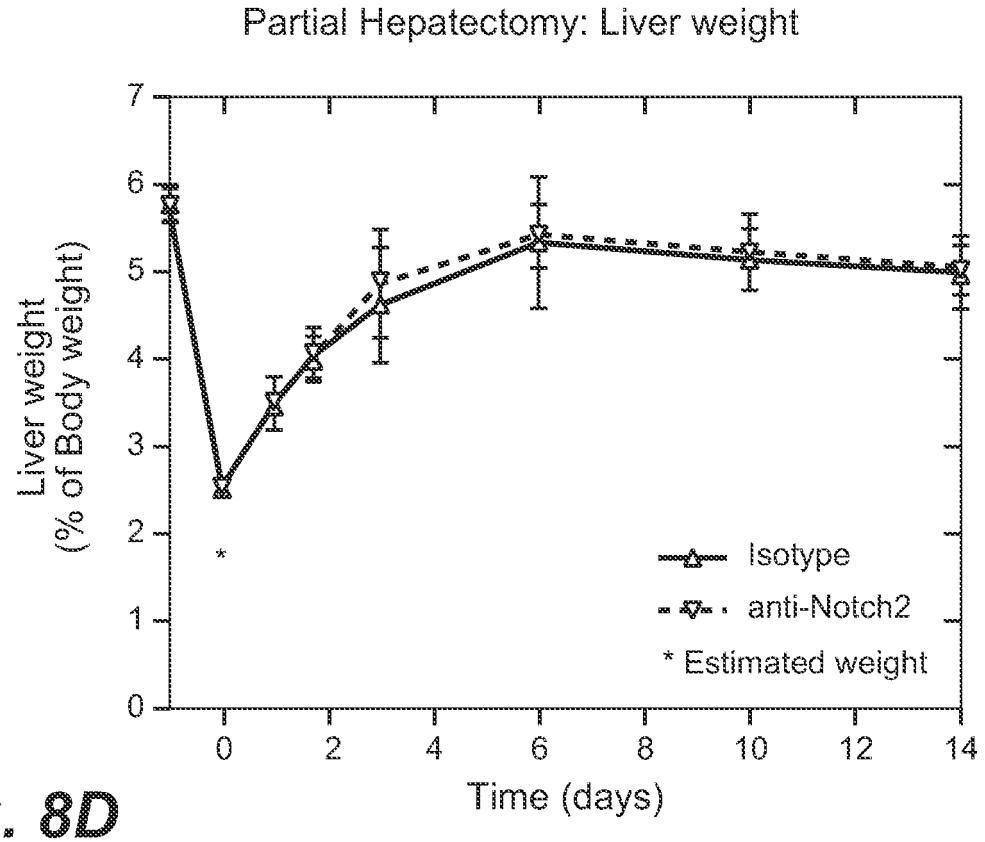

Partial Hepatectomy: Liver weight
Liver weight
(% of Body weight)
*
Isotype
anti-Notch2
* Estimated weight
0
1
2
3
4
5
6
7
0
2
4
6
8
10
12
14
Time (days)

FIG. 8D

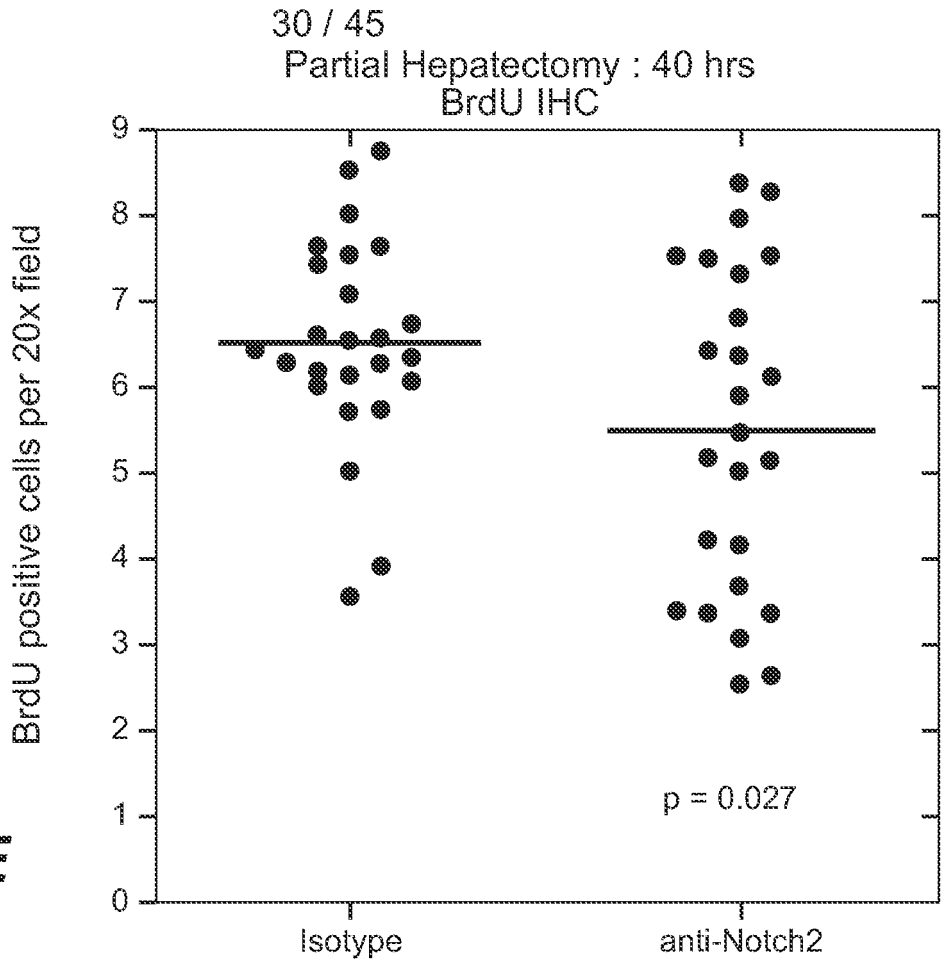

Partial Hepatectomy : 40 hrs
BrdU IHC
BrdU positive cells per 20x field
0
1
2
3
4
5
6
7
8
9
p = 0.027
Isotype
anti-Notch2

FIG. 8E

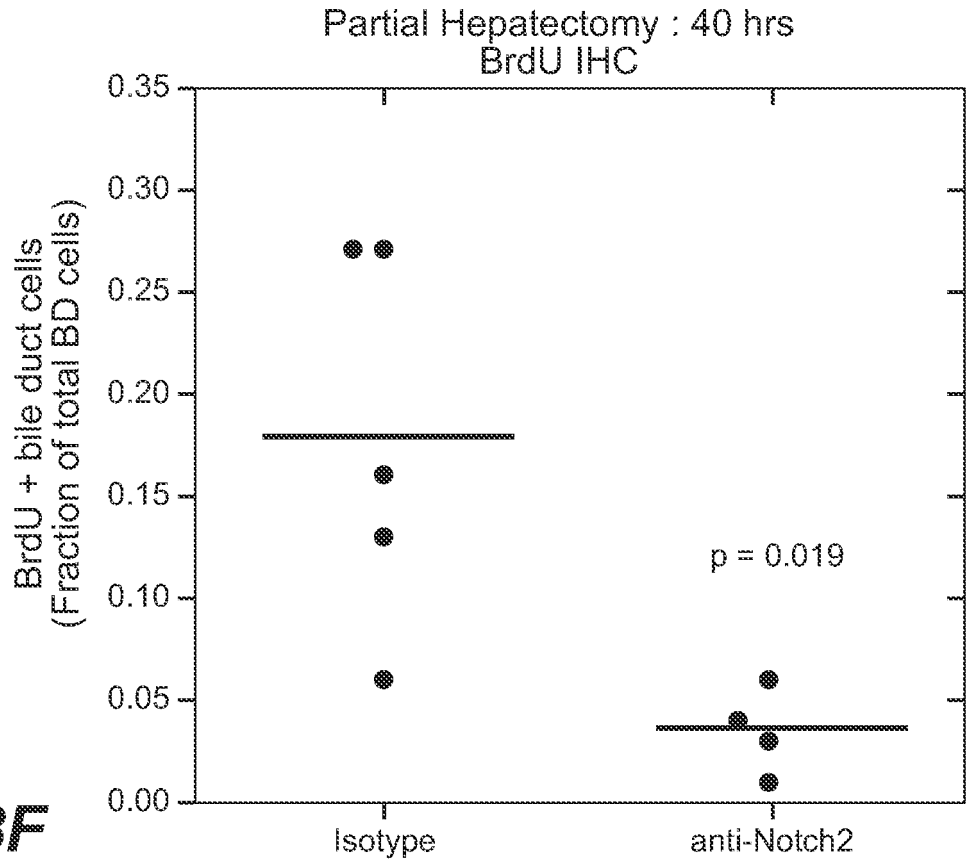

Partial Hepatectomy : 40 hrs
BrdU IHC
BrdU + bile duct cells
(Fraction of total BD cells)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
p = 0.019
Isotype
anti-Notch2

FIG. 8F

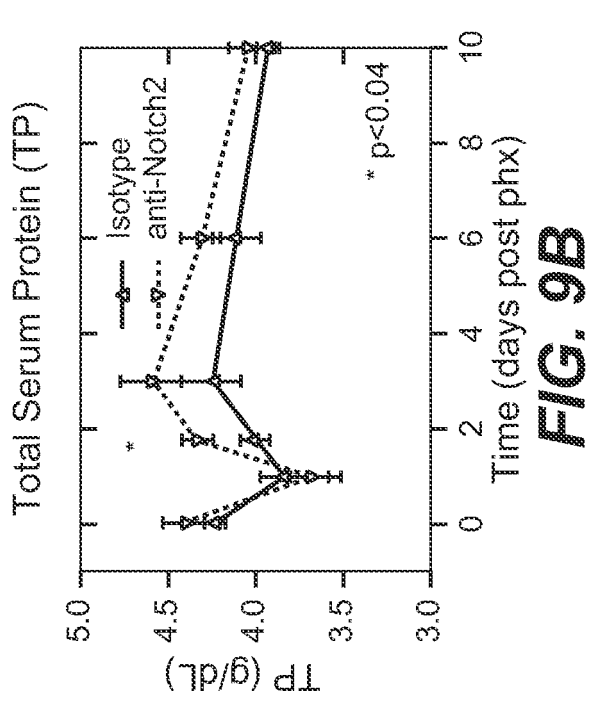


FIG. 9B

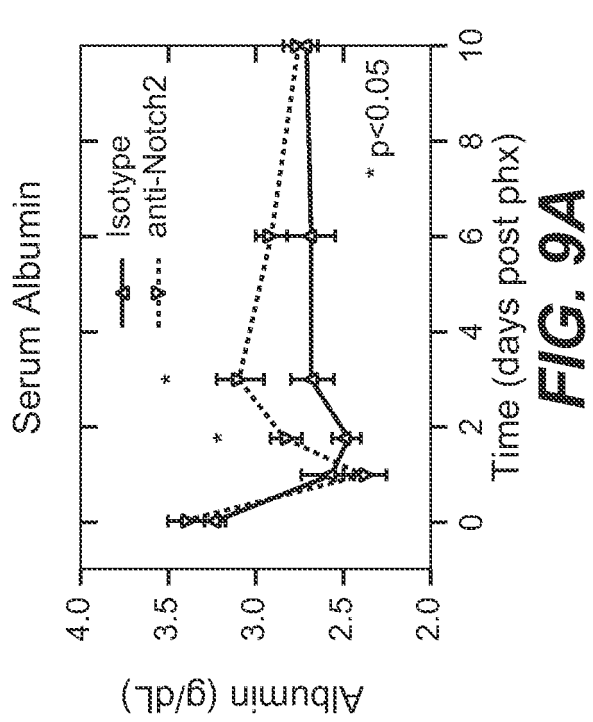


FIG. 9A

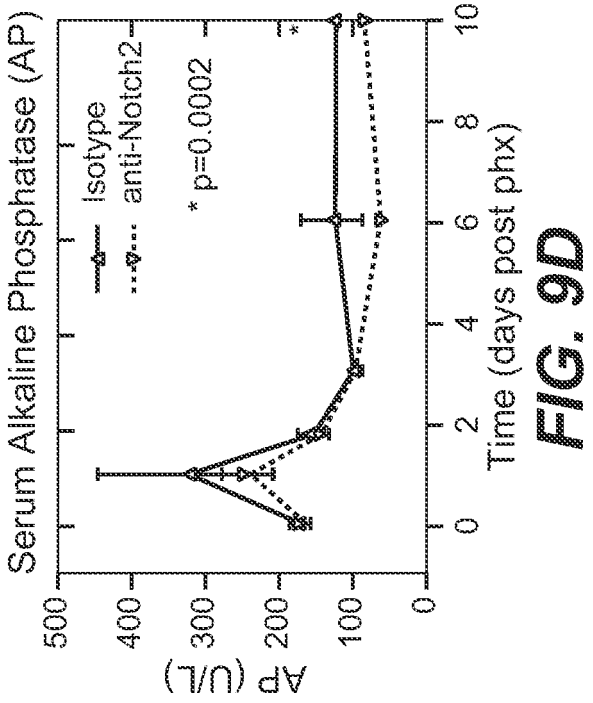


FIG. 9D

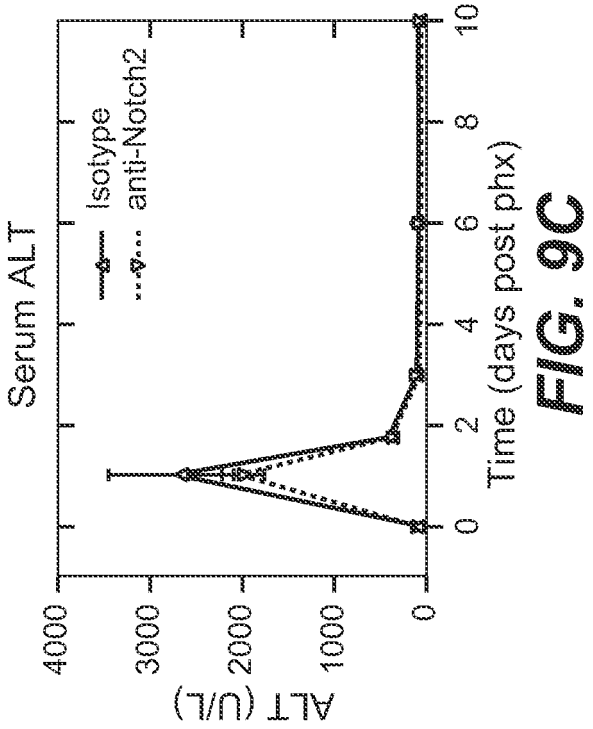


FIG. 9C

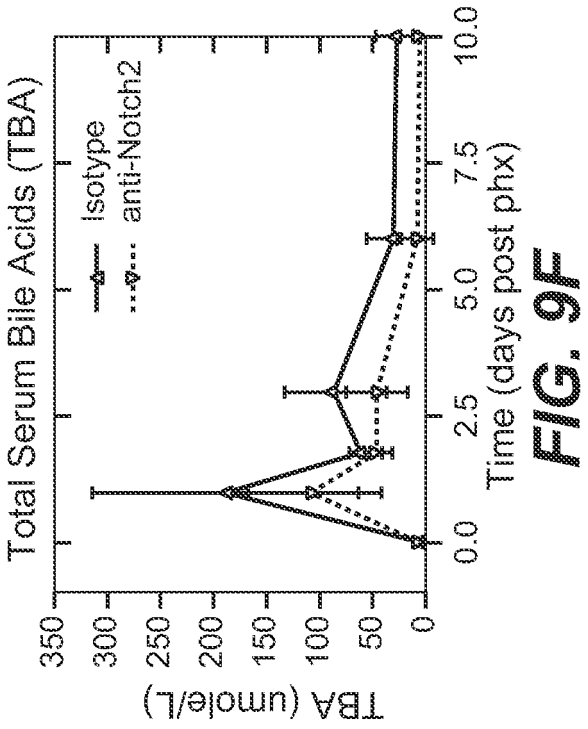


FIG. 9F

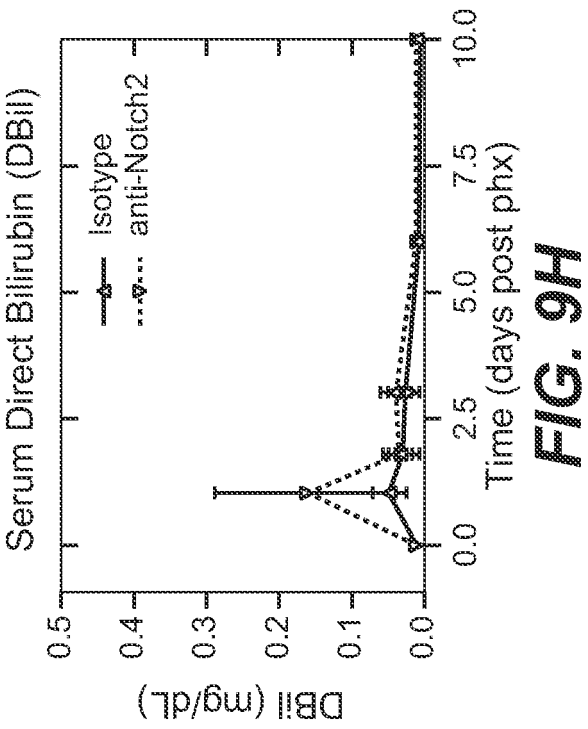


FIG. 9H

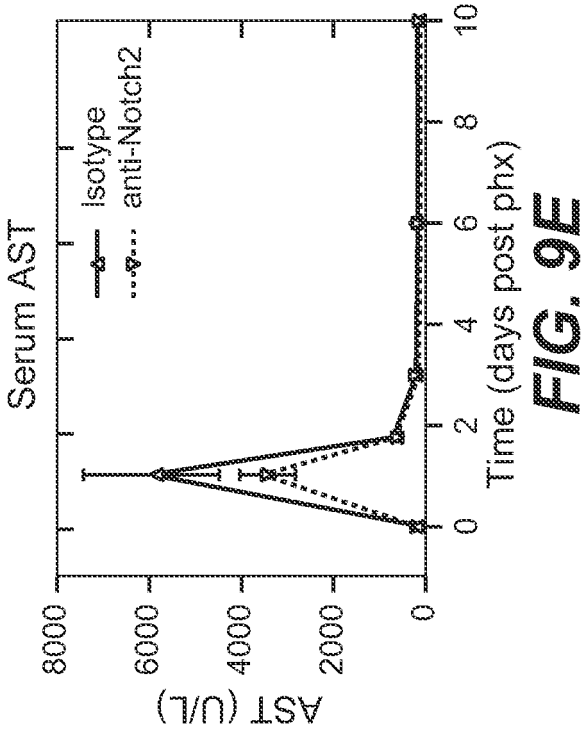


FIG. 9E

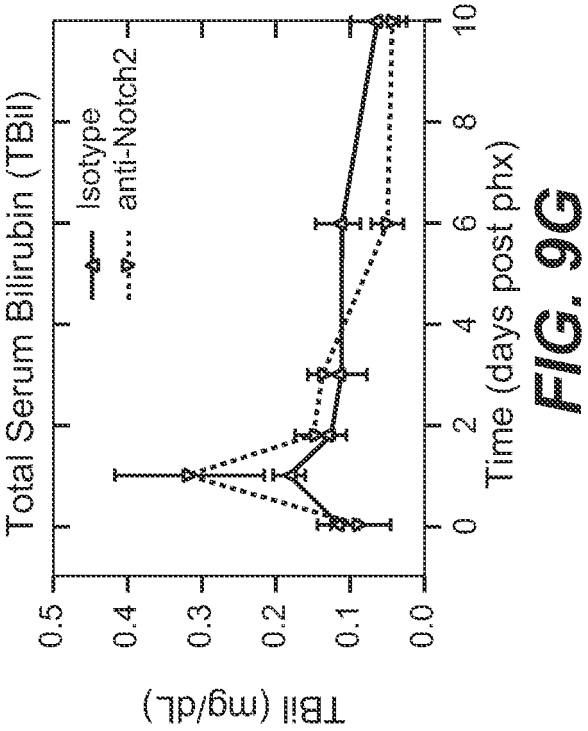


FIG. 9G

FIG. 10A

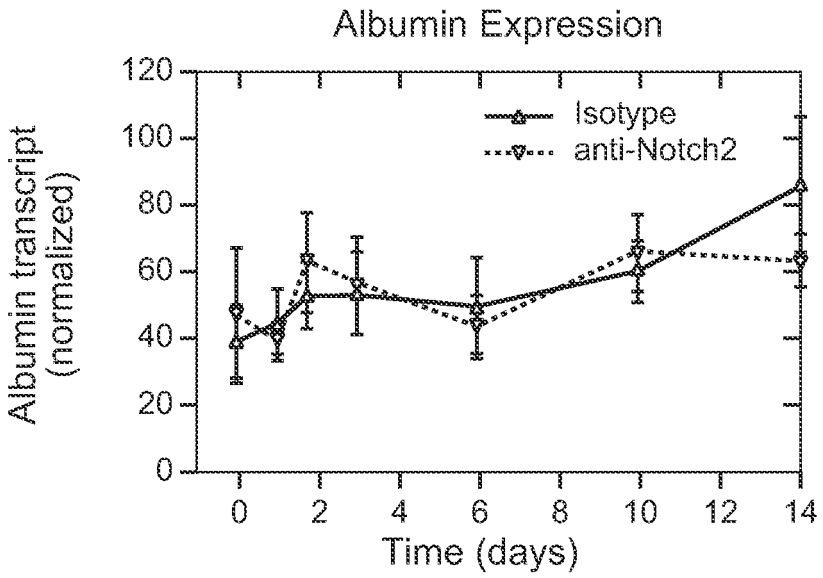


FIG. 10C

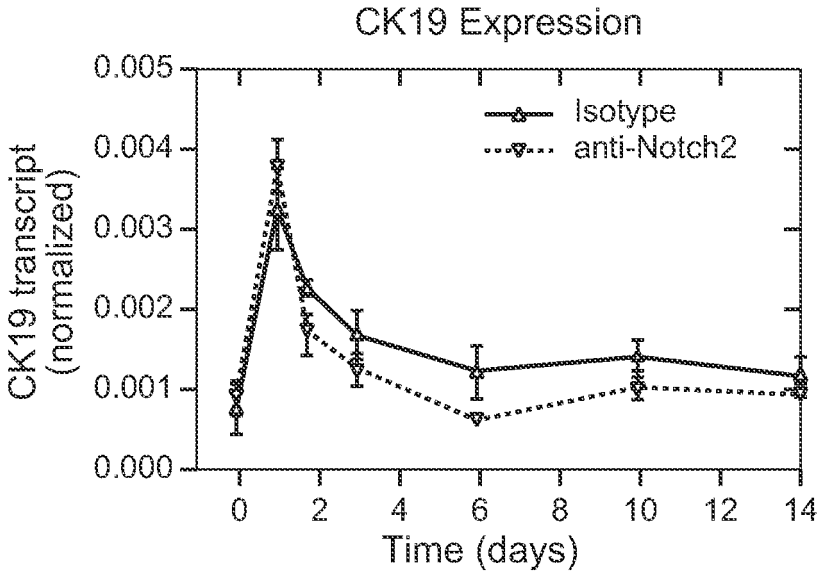


FIG. 10E

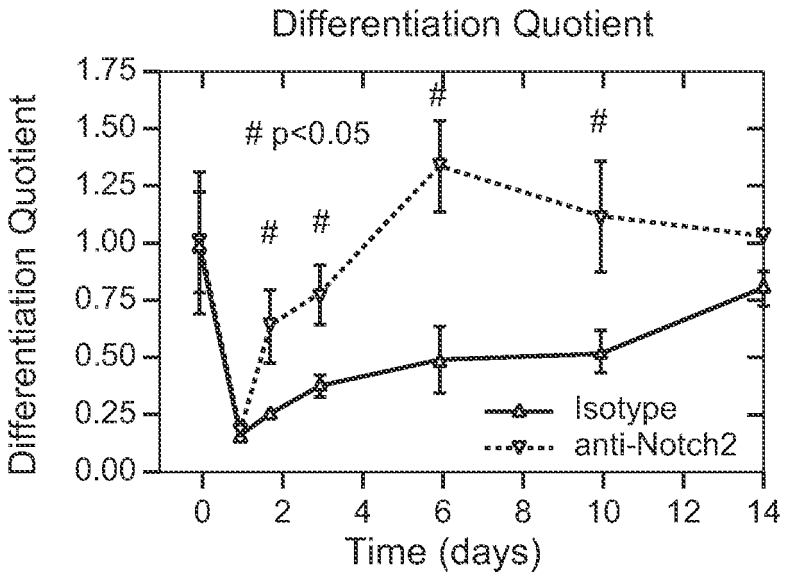


FIG. 10B

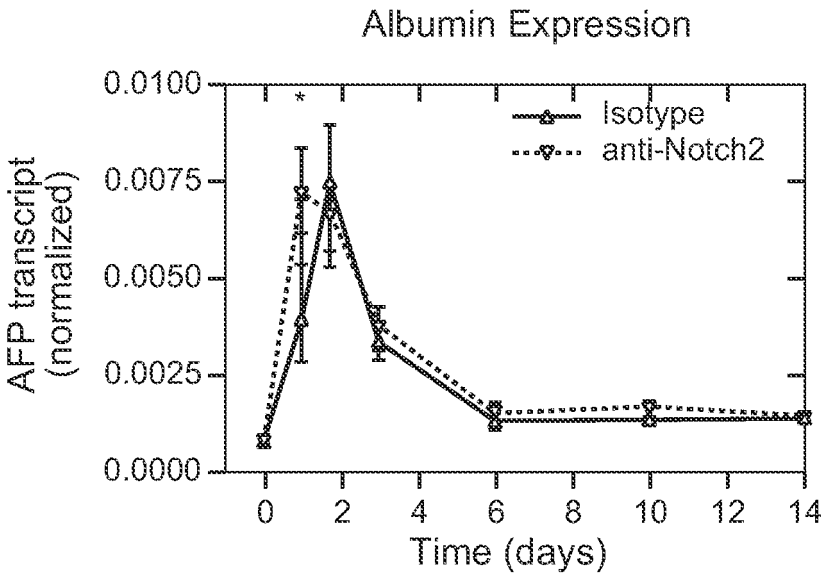


FIG. 10D

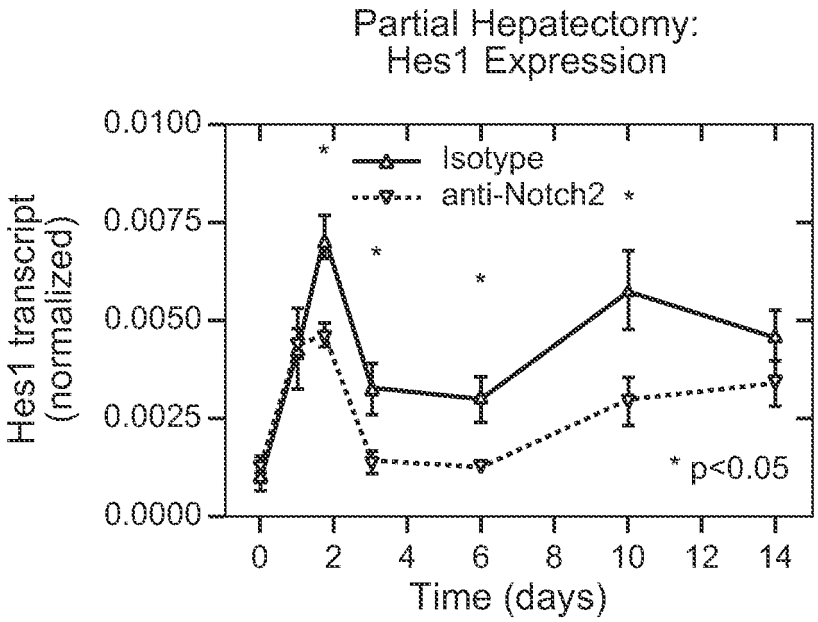


FIG. 10F

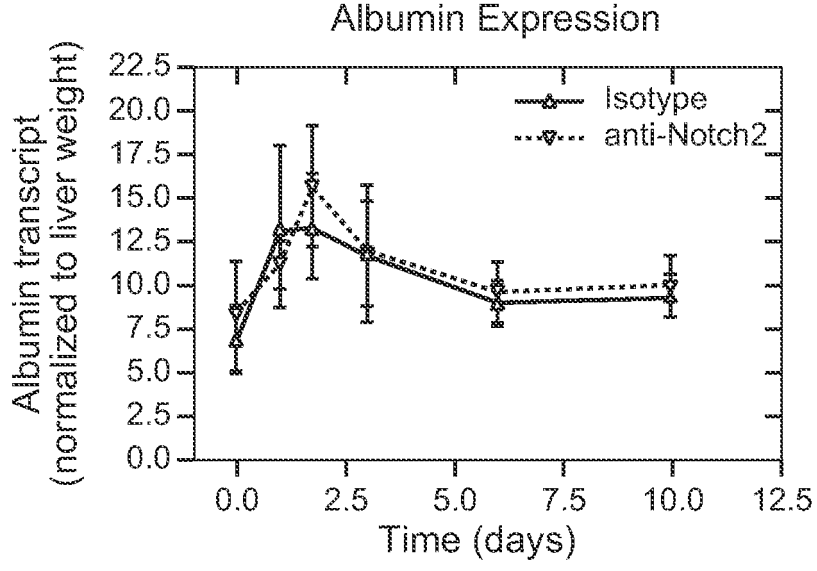


FIG. 11A

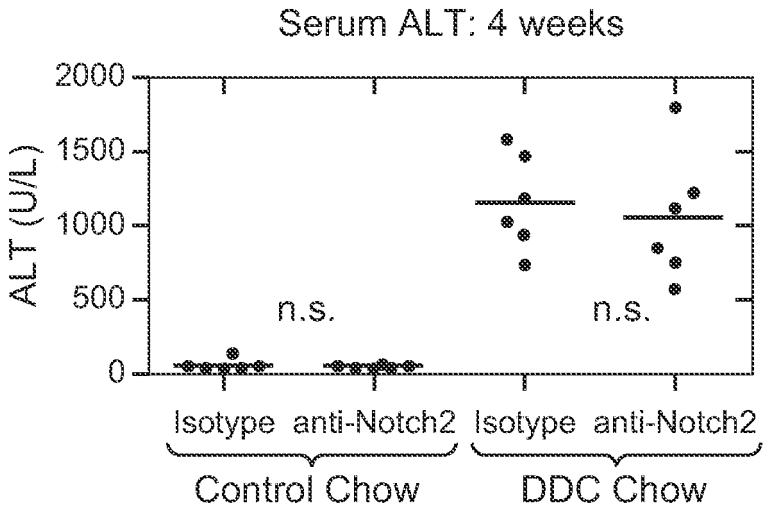


FIG. 11B

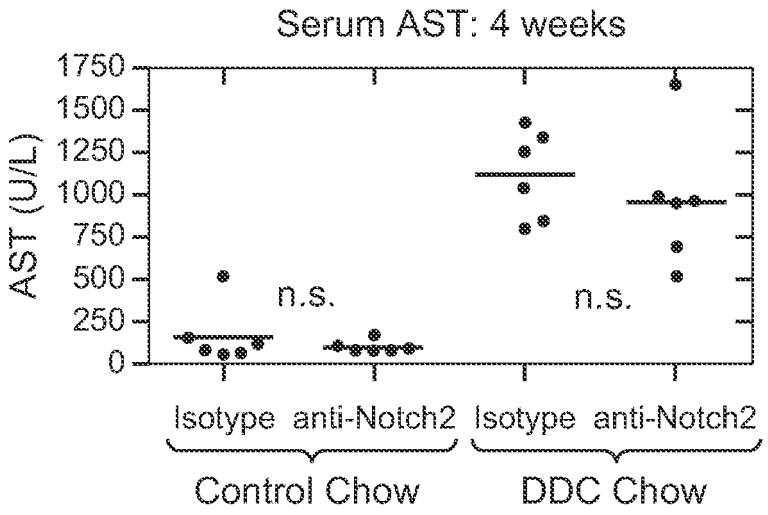


FIG. 11C

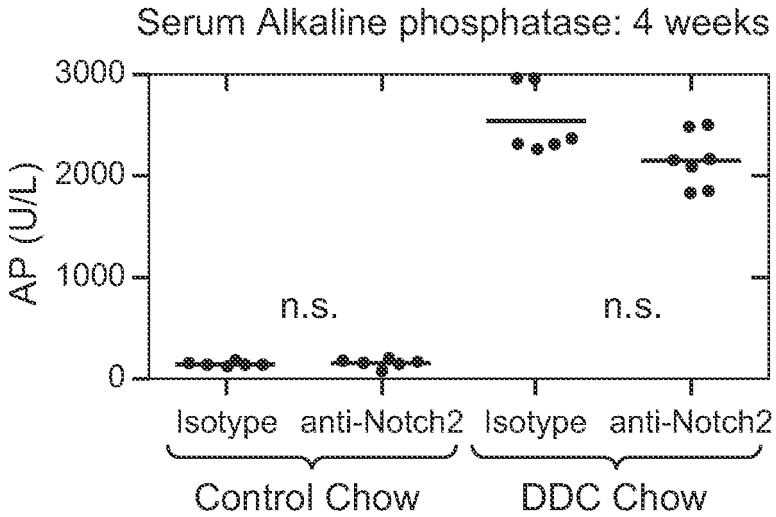


FIG. 11D

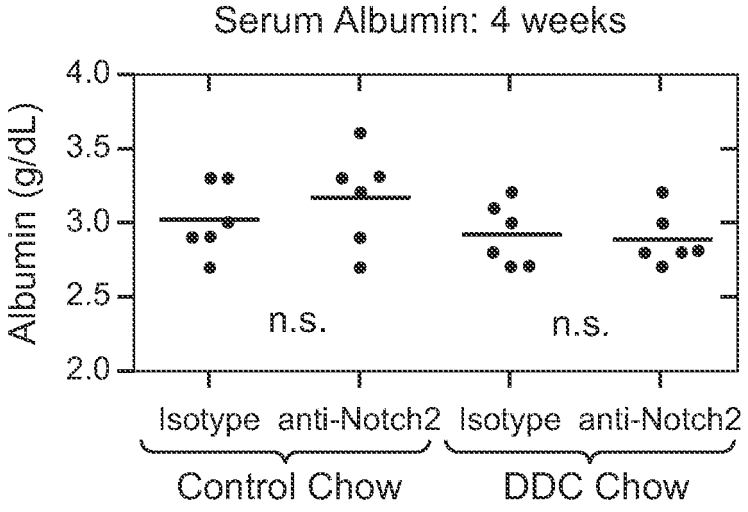


FIG. 11E

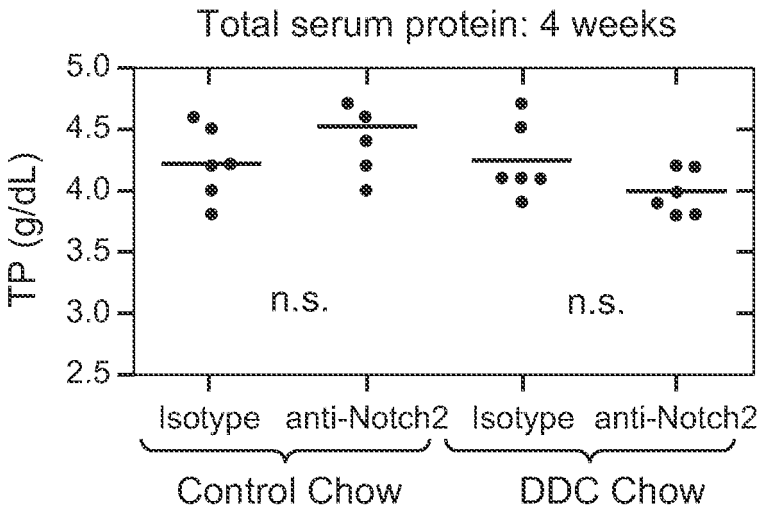


FIG. 11F

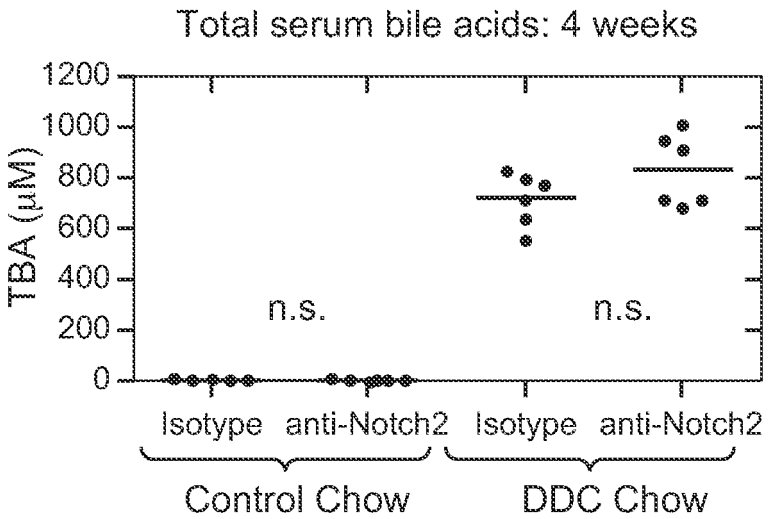


FIG. 11G

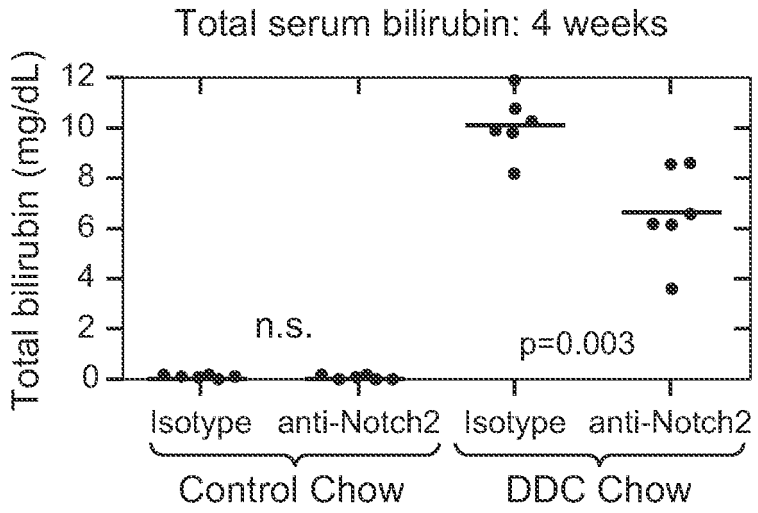


FIG. 11H

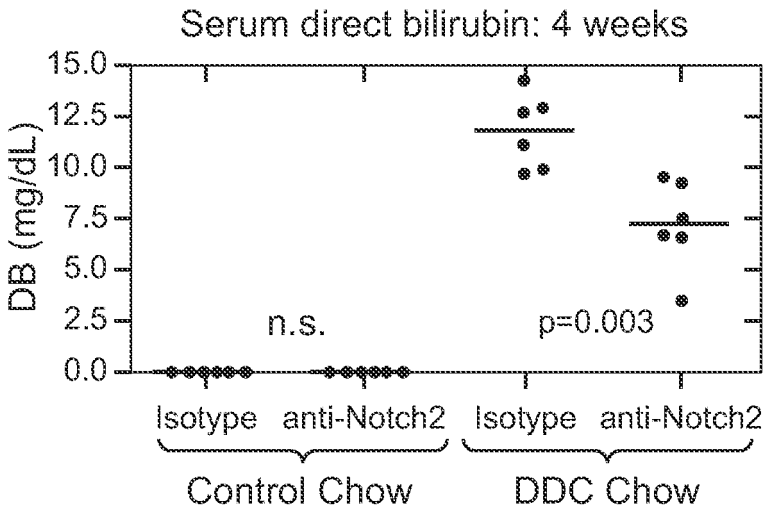
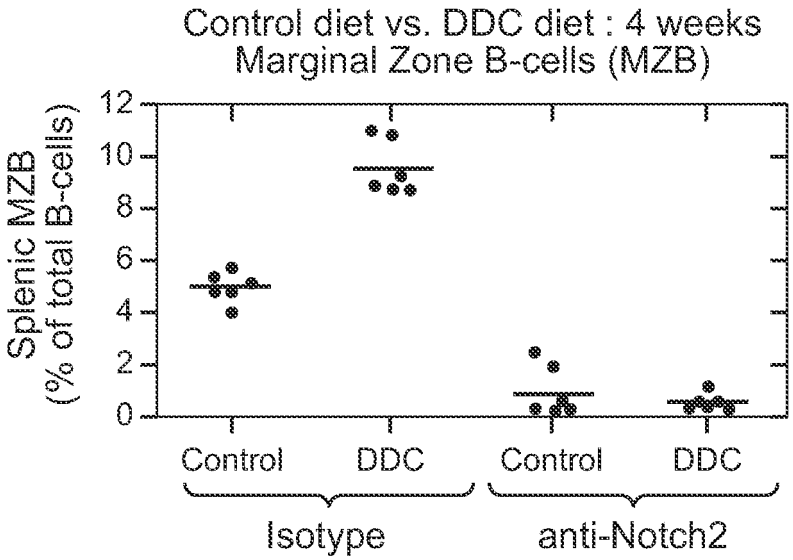


FIG. 11I



HVR-H1 Sequences - Antibody D and Affinity Matured Antibodies Derived from Antibody D

		Kabat Number									
Antibody #	SEQ ID NO:	26	27	28	29	30	31	32	33	34	35
D	1	G	Y	S	F	T	S	Y	G	M	S
D-1, D-2, D-3	2	G	Y	T	F	S	S	Y	G	M	S
Consensus	3	G	Y	S/T	F	S/T	S	Y	G	M	S

HVR-H2 Sequence - Antibody D and Affinity Matured Antibodies Derived from Antibody D

		Kabat Number																
Antibody #	SEQ ID NO:	49	50	51	52	52a	53	54	55	56	57	58	59	60	61	62	63	64	65
D, D-1, D-2, D-3	4	S	Y	I	Y	P	Y	S	G	A	T	Y	Y	A	D	S	V	K	G

HVR-H3 Sequence - Antibody D and Affinity Matured Antibodies Derived from Antibody D

		Kabat Number												
Antibody #	SEQ ID NO:	95	96	97	98	99	100	100A	100B	100C	100D	100K	101	102
D, D-1, D-2, D-3	5	H	S	G	Y	Y	R	I	S	S	A	M	D	V

FIG. 12

HVR-L1 Sequences - Antibody D and Affinity Matured Antibodies Derived from Antibody D

		Kabat Number											
Antibody #	SEQ ID NO:	24	25	26	27	28	29	30	31	32	33	34
D	6	R	A	S	Q	S	I	S	S	Y	L	A
D-1	7	R	A	S	Q	S	N	R	R	F	L	A
D-2	8	R	A	S	Q	S	V	R	S	F	L	A
D-3	9	R	A	S	Q	N	I	K	R	F	L	A
Consensus	10	R	A	S	Q	S/N	I/N/V	S/R/K	S/R	Y/F	L	A

HVR-L2 Sequences - Antibody D and Affinity Matured Antibodies Derived from Antibody D

		Kabat Number						
Antibody #	SEQ ID NO:	50	51	52	53	54	55	56
D, D-1	11	G	A	S	S	R	A	S
D-2	12	R	A	S	I	R	A	S
D-3	13	G	A	S	T	R	E	S
Consensus	14	G/R	A	S	S/I/T	R	A/E	S

HVR-L3 Sequences - Antibody D and Affinity Matured Antibodies Derived from Antibody D

		Kabat Number								
Clone #	SEQ ID NO:	89	90	91	92	93	94	95	96	97
D	15	Q	Q	Y	Y	S	S	P	L	T
D-1	16	Q	Q	Y	Y	I	S	P	L	T
D-2	17	Q	Q	Y	Y	I	S	P	W	T
D-3	18	Q	Q	Y	Y	R	S	P	H	T
Consensus	19	Q	Q	Y	Y	S/I/R	S	P	L/W/H	T

FIG. 13

Kabat#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	35A	35B	36	37	38	39	40	41	42	43
Antibody D	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A	A	S	G	Y	S	F	T	S	Y	G	M	S			W	V	R	Q	A	P	G	K
Antibody D-1	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A	A	S	G	Y	T	F	S	S	Y	G	M	S			W	V	R	Q	A	P	G	K
Antibody D-2	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A	A	S	G	Y	T	F	S	S	Y	G	M	S			W	V	R	Q	A	P	G	K
Antibody D-3	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L	S	C	A	A	S	G	Y	T	F	S	S	Y	G	M	S			W	V	R	Q	A	P	G	K

Kabat#	44	45	46	47	48	49	50	51	52	A	B	C	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	A
Antibody D	G	L	E	W	V	S	Y	I	Y	P			Y	S	G	A	T	Y	Y	A	D	S	V	K	G	R	F	T	I	S	A	D	T	S	K	N	T	A	Y	L	Q	M	N
Antibody D-1	G	L	E	W	V	S	Y	I	Y	P			Y	S	G	A	T	Y	Y	A	D	S	V	K	G	R	F	T	I	S	A	D	T	S	K	N	T	A	Y	L	Q	M	N
Antibody D-2	G	L	E	W	V	S	Y	I	Y	P			Y	S	G	A	T	Y	Y	A	D	S	V	K	G	R	F	T	I	S	A	D	T	S	K	N	T	A	Y	L	Q	M	N
Antibody D-3	G	L	E	W	V	S	Y	I	Y	P			Y	S	G	A	T	Y	Y	A	D	S	V	K	G	R	F	T	I	S	A	D	T	S	K	N	T	A	Y	L	Q	M	N

Kabat#	B	C	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	A	B	C	D	E	F	G	H	I	J	K	101	102	103	104	105	106	107	108	109	110	111	112	113	
Antibody D	S	L	R	A	E	D	T	A	V	Y	Y	Y	C	A	R	E	S	G	Y	Y	R	I	S	S	A						M	D	V	W	G	Q	G	T	L	V	T	V	S	A	SEQ ID NO: 20
Antibody D-1	S	L	R	A	E	D	T	A	V	Y	Y	Y	C	A	R	E	S	G	Y	Y	R	I	S	S	A						M	D	V	W	G	Q	G	T	L	V	T	V	S	A	SEQ ID NO: 21
Antibody D-2	S	L	R	A	E	D	T	A	V	Y	Y	Y	C	A	R	E	S	G	Y	Y	R	I	S	S	A						M	D	V	W	G	Q	G	T	L	V	T	V	S	A	SEQ ID NO: 21
Antibody D-3	S	L	R	A	E	D	T	A	V	Y	Y	Y	C	A	R	E	S	G	Y	Y	R	I	S	S	A						M	D	V	W	G	Q	G	T	L	V	T	V	S	A	SEQ ID NO: 21

FIG. 14

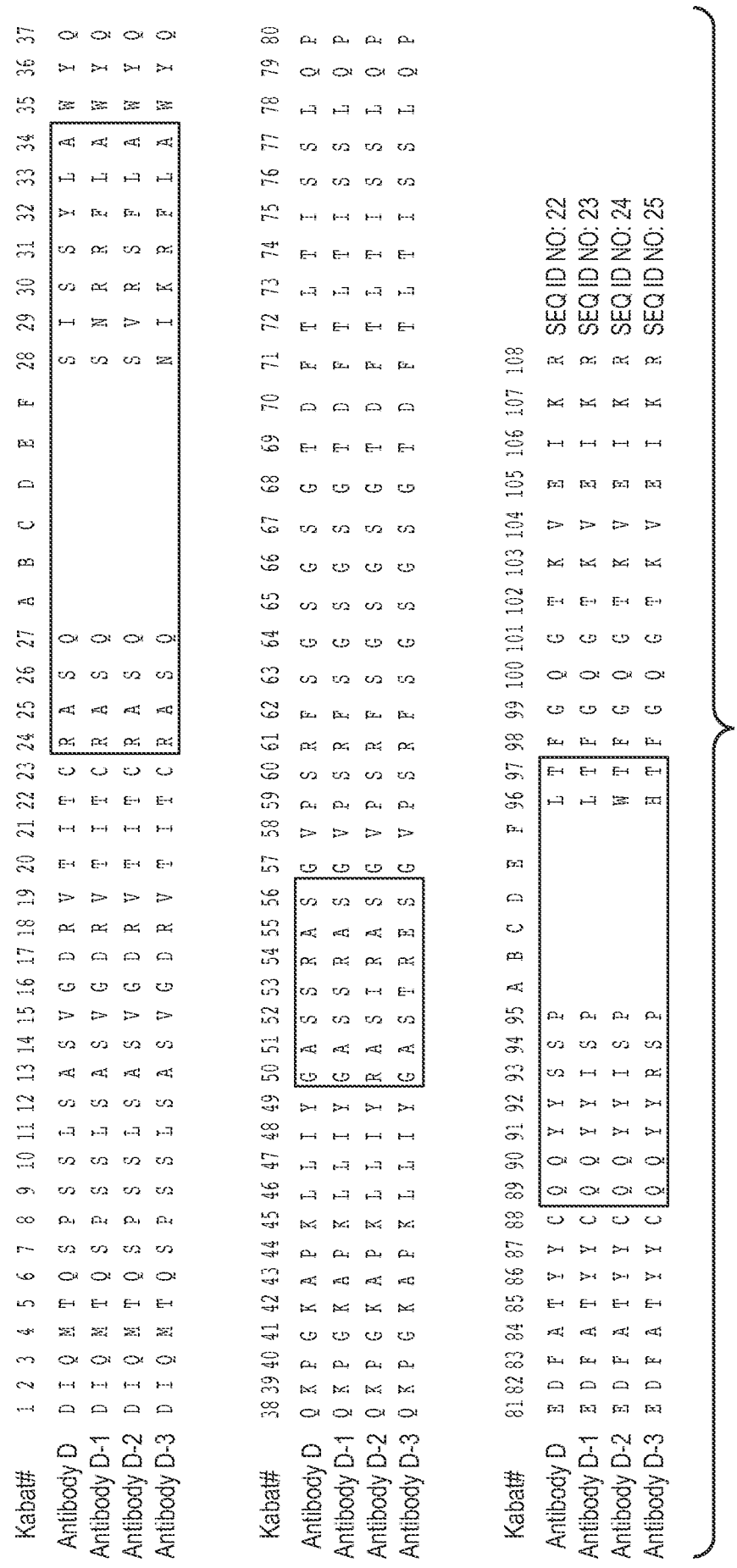

Kabat# 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 A B C D E F 28 29 30 31 32 33 34 35 36 37
Antibody D D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q S I S S Y L A W Y Q
Antibody D-1 D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q S N R R F L A W Y Q
Antibody D-2 D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q S V R S F L A W Y Q
Antibody D-3 D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q N I K R F L A W Y Q
Kabat# 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80
Antibody D Q K P G K A P K L L I Y G A S S R A S G V P S R F S G S G T D F T L T I S S L Q P
Antibody D-1 Q K P G K A P K L L I Y G A S S R A S G V P S R F S G S G T D F T L T I S S L Q P
Antibody D-2 Q K P G K A P K L L I Y R A S I R A S G V P S R F S G S G T D F T L T I S S L Q P
Antibody D-3 Q K P G K A P K L L I Y G A S T R E S G V P S R F S G S G T D F T L T I S S L Q P
Kabat# 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 A B C D E F 96 97 98 99 100 101 102 103 104 105 106 107 108
Antibody D E D F A T Y Y Y C Q Q Y Y S S P L T F G G G T K V E I K R SEQ ID NO: 22
Antibody D-1 E D F A T Y Y Y C Q Q Y Y I S P L T F G Q G T K V E I K R SEQ ID NO: 23
Antibody D-2 E D F A T Y Y Y C Q Q Y Y I S P W T F G Q G T K V E I K R SEQ ID NO: 24
Antibody D-3 E D F A T Y Y Y C Q Q Y Y R S P H T F G Q G T K V E I K R SEQ ID NO: 25

FIG. 15

FR1
FR2
-H1-
-H2-
I
A QVQLVQSGAEVKKPGASVKVSCKASGYTFT WVRQAPGQGLEWMG RVTIT
B QVQLVQSGAEVKKPGASVKVSCKAS -H1- WVRQAPGQGLEWM -H2- RVTIT
C QVQLVQSGAEVKKPGASVKVSCKAS -H1- WVRQAPGQGLEWM -H2- RVTIT
D QVQLVQSGAEVKKPGASVKVSCKAS -H1- WVRQAPGQGLEWM -H2- RVTIT
II
A QVQLQESGPGLVKPSQTLSLTCTVSGGSVS WIRQPPGKGLEWIG RVTIS
B QVQLQESGPGLVKPSQTLSLTCTVS -H1- WIRQPPGKGLEWI -H2- RVTIS
C QVQLQESGPGLVKPSQTLSLTCTVS -H1- WIRQPPGKGLEWI -H2- RVTIS
D QVQLQESGPGLVKPSQTLSLTCTVS -H1- WIRQPPGKGLEWI -H2- RVTIS
III
A EVQLVESGGGLVQPGGSLRLSCAASGFTFS WVRQAPGKGLEWVS RFTIS
B EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS
C EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS
D EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS
Acceptor - 1
A EVQLVESGGGLVQPGGSLRLSCAASGFNIK WVRQAPGKGLEWVS RFTIS
B EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS
C EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS
Acceptor - 2
A EVQLVESGGGLVQPGGSLRLSCAASGFNIK WVRQAPGKGLEWVS RFTIS
B EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS
C EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS
D EVQLVESGGGLVQPGGSLRLSCAAS -H1- WVRQAPGKGLEWV -H2- RFTIS

FIG. 16A

	FR3		FR4	SEQ ID NOs of FR1, FR2, FR3, FR4
I				
A	ADTSTSTAYMELSSLRSEDTAVYYCAR	-H3-	WGQGTLVTVSS	SEQ ID NO.: 32, 33, 34, 35
B	ADTSTSTAYMELSSLRSEDTAVYYCAR	-H3-	WGQGTLVTVSS	SEQ ID NO.: 36, 37, 34, 35
C	ADTSTSTAYMELSSLRSEDTAVYYCA	-H3-	WGQGTLVTVSS	SEQ ID NO.: 36, 37, 38, 35
D	ADTSTSTAYMELSSLRSEDTAVYYC	-H3-	WGQGTLVTVSS	SEQ ID NO.: 36, 37, 39, 35
II				
A	VDTSKNQFSLKLSSVTAADTAVYYCAR	-H3-	WGQGTLVTVSS	SEQ ID NO.: 40, 41, 42, 35
B	VDTSKNQFSLKLSSVTAADTAVYYCAR	-H3-	WGQGTLVTVSS	SEQ ID NO.: 43, 44, 42, 35
C	VDTSKNQFSLKLSSVTAADTAVYYCA	-H3-	WGQGTLVTVSS	SEQ ID NO.: 43, 44, 45, 35
D	VDTSKNQFSLKLSSVTAADTAVYYC	-H3-	WGQGTLVTVSS	SEQ ID NO.: 43, 44, 46, 35
III				
A	RDNSKNTLYLQMNSLRAEDTAVYYCAR	-H3-	WGQGTLVTVSS	SEQ ID NO.: 47, 48, 49, 35
B	RDNSKNTLYLQMNSLRAEDTAVYYCAR	-H3-	WGQGTLVTVSS	SEQ ID NO.: 50, 51, 49, 35
C	RDNSKNTLYLQMNSLRAEDTAVYYCA	-H3-	WGQGTLVTVSS	SEQ ID NO.: 50, 51, 52, 35
D	RDNSKNTLYLQMNSLRAEDTAVYYC	-H3-	WGQGTLVTVSS	SEQ ID NO.: 50, 51, 53, 35
Acceptor - 1				
A	[A/D]TSKNTAYLQMNSLRAEDTAVYYC[S/R]	-H3-	WGQGTLVTVSS	SEQ ID NO.: 54, 48, 55, 35
B	[A/D]TSKNTAYLQMNSLRAEDTAVYYC[S/R]	-H3-	WGQGTLVTVSS	SEQ ID NO.: 50, 51, 55, 35
C	[A/D]TSKNTAYLQMNSLRAEDTAVYYC[S]	-H3-	WGQGTLVTVSS	SEQ ID NO.: 50, 51, 56, 35
Acceptor - 2				
A	[A/D]TSKNTAYLQMNSLRAEDTAVYYCAR	-H3-	WGQGTLVTVSS	SEQ ID NO.: 54, 48, 57, 35
B	[A/D]TSKNTAYLQMNSLRAEDTAVYYCAR	-H3-	WGQGTLVTVSS	SEQ ID NO.: 50, 51, 57, 35
C	[A/D]TSKNTAYLQMNSLRAEDTAVYYCA	-H3-	WGQGTLVTVSS	SEQ ID NO.: 50, 51, 58, 35
D	[A/D]TSKNTAYLQMNSLRAEDTAVYYC	-H3-	WGQGTLVTVSS	SEQ ID NO.: 50, 51, 59, 35

FIG. 16B

FR1
FR2
FR3
kv1 DIQMTQSPSSLSASVGDRVTITC -L1- WYQQKPGKAPKLLIY -L2- GVPSRFSGSGSGTDFTLTISSLQP
kv2 DIVMTQSPLSLPVTPGEPASISC -L1- WYLQKPGQSPQLLIY -L2- GVPDRFSGSGSGTDFTLKISRVEA
kv3 EIVLTQSPGTLSLSPGERATLSC -L1- WYQQKPGQAPRLLIY -L2- GIPDRFSGSGSGTDFTLTISRLEP
kv4 DIVMTQSPDSLAVSLGERATINC -L1- WYQQKPGQPPKLLIY -L2- GVPDRFSGSGSGTDFTLTISSLQA
FR4
SEQ ID NOs of
FR1, FR2, FR3, FR4
EDFATYYC -L3- FGQGTKVEIK SEQ ID NO.: 60, 61, 62, 63
EDVGVYYC -L3- FGQGTKVEIK SEQ ID NO.: 64, 65, 66, 63
EDFAVYYC -L3- FGQGTKVEIK SEQ ID NO.: 67, 68, 69, 63
EDVAVYYC -L3- FGQGTKVEIK SEQ ID NO.: 70, 71, 72, 63

FIG. 17

Framework Sequences of huMAb4D5-8 Light Chain Variable Domain

LC-FR1 ^{1}Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys^{23} (SEQ ID NO: 60)

LC-FR2 ^{35}Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr^{49} (SEQ ID NO: 61)

LC-FR3 ^{57}Gly Val Pro Ser Arg Phe Ser Gly Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys^{88} (SEQ ID NO: 30)

LC-FR4 ^{98}Phe Gly Gln Gly Thr Lys Val Glu Ile Lys^{107} (SEQ ID NO: 63)

Framework Sequences of huMAb4D5-8 Heavy Chain Variable Domain

HC-FR1 ^{1}Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser^{25} (SEQ ID NO: 50)

HC-FR2 ^{36}Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val^{48} (SEQ ID NO: 51)

HC-FR3 ^{66}Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu Gln Met Asn^{83} Ser^{83a} Leu^{83b} Arg^{83c} Ala Glu Asp Thr Ala Val Tyr Tyr Cys^{92} (SEQ ID NO: 59)

HC-FR4 ^{103}Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser^{113} (SEQ ID NO: 35)

FIG. 18

Framework Sequences of huMAb4D5-8 Light Chain Variable Domain Modified at Positions 66 and 99 (Underlined)

LC-FR1 ^{1}Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys^{23} (SEQ ID NO: 60)

LC-FR2 ^{35}Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr^{49} (SEQ ID NO: 61)

LC-FR3 ^{57}Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys^{88} (SEQ ID NO: 62)

LC-FR4 ^{98}Phe Arg Gln Gly Thr Lys Val Glu Ile Lys^{107} (SEQ ID NO: 31)

Framework Sequences of huMAb4D5-8 Heavy Chain Variable Domain Modified at Postions 71, 73 and 78 (Underlined)

HC-FR1 ^{1}Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser^{25} (SEQ ID NO: 50)

HC-FR2 ^{36}Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val^{48} (SEQ ID NO: 51)

HC-FR3 ^{66}Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn83 Ser83a Leu83b Arg83c Ala Glu Asp Thr Ala Val Tyr Tyr Cys^{92} (SEQ ID NO: 53)

HC-FR4 ^{103}Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser^{113} (SEQ ID NO: 35)

FIG. 19