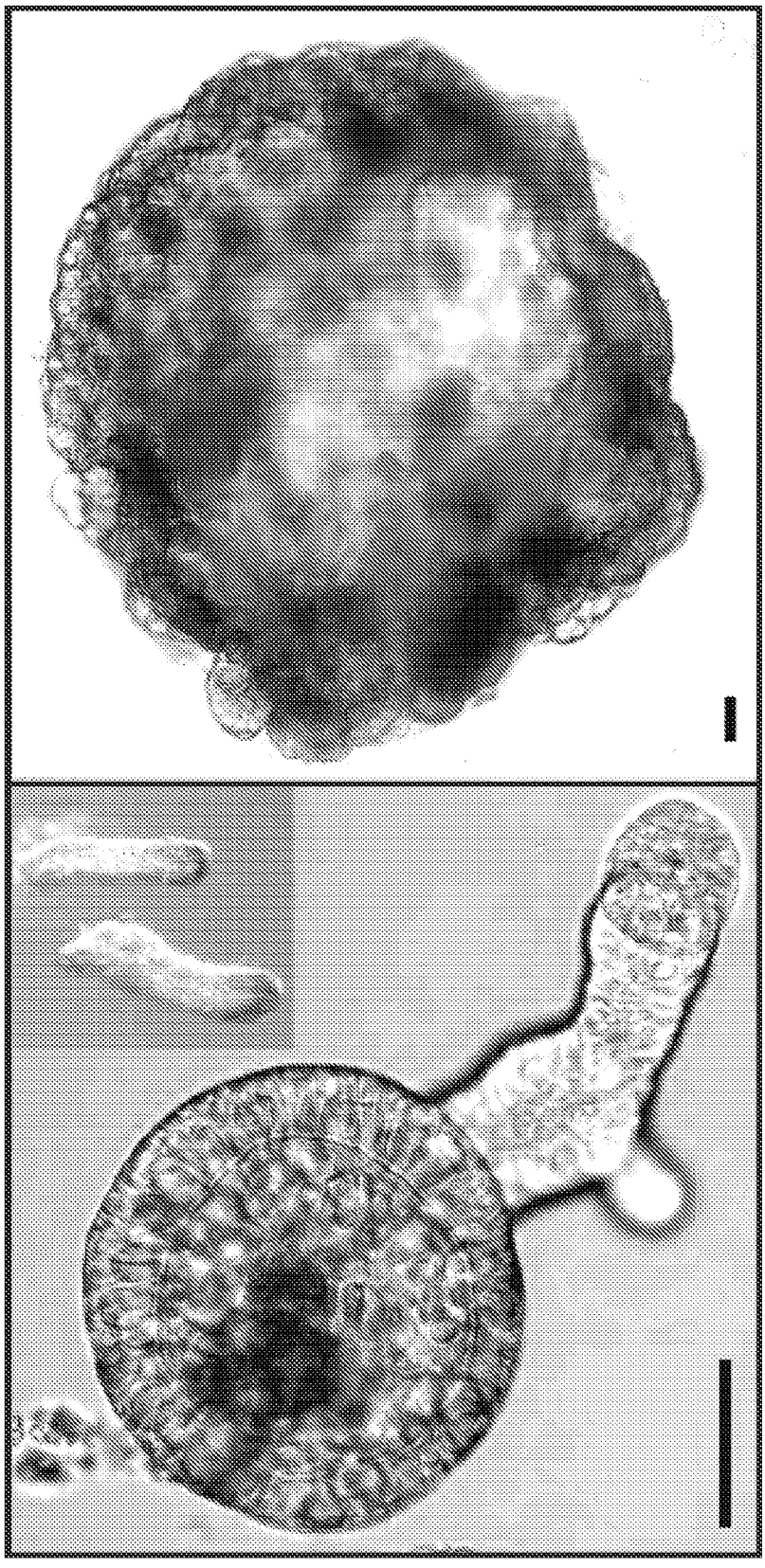
FIG. 1(a)



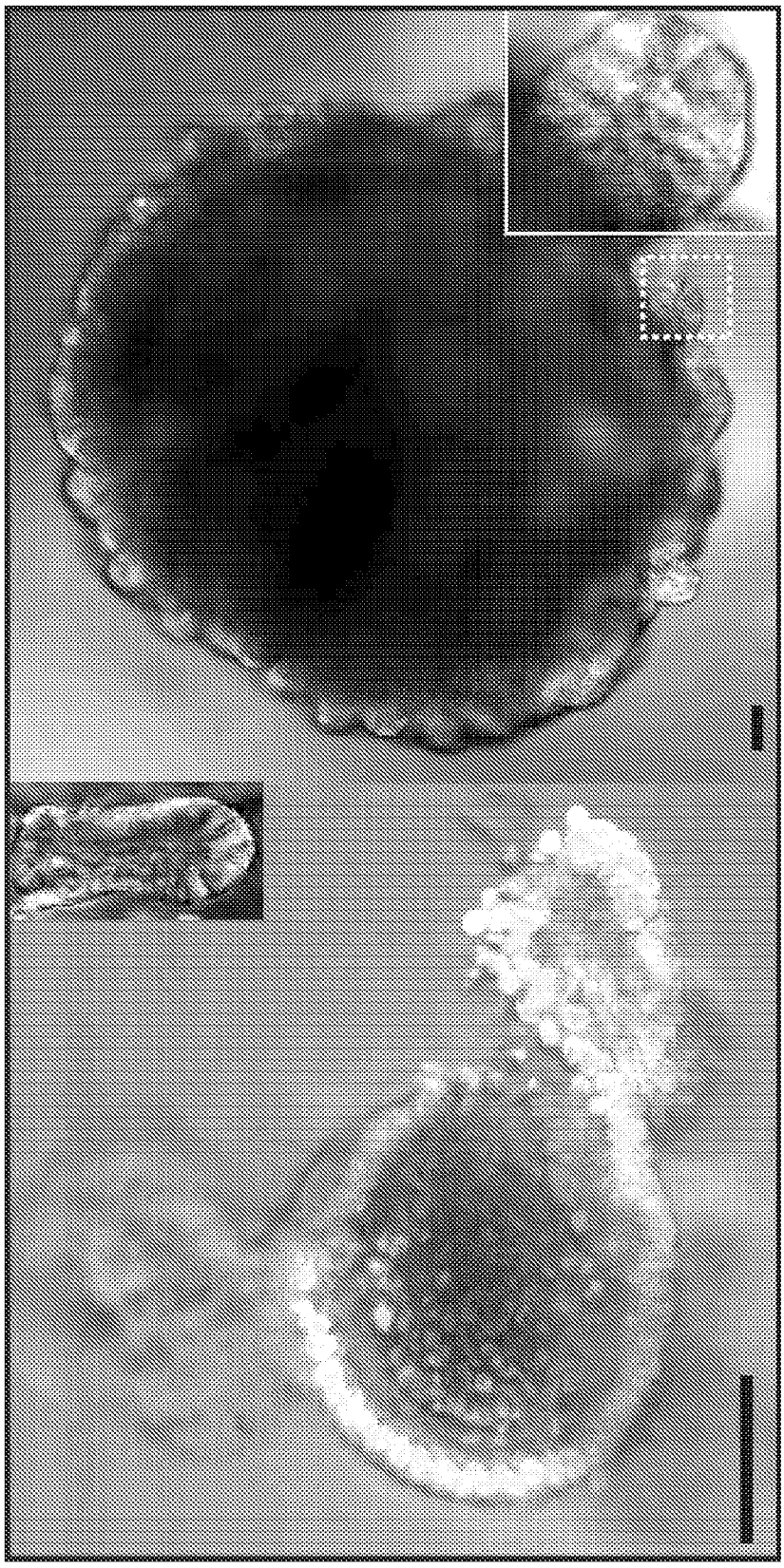


FIG. 1(b)

FIG. 1(c)

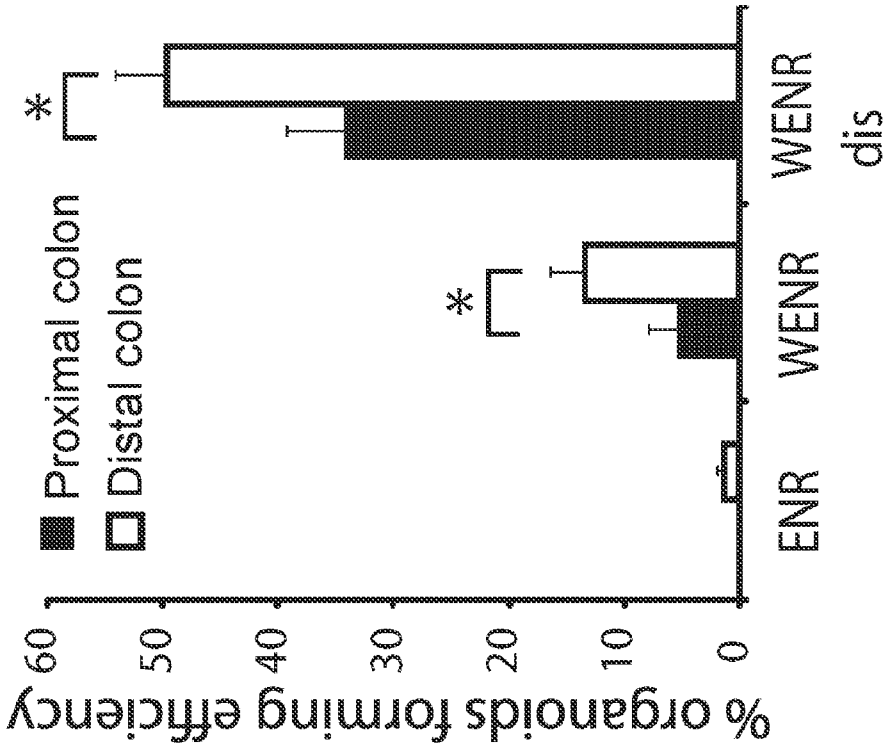
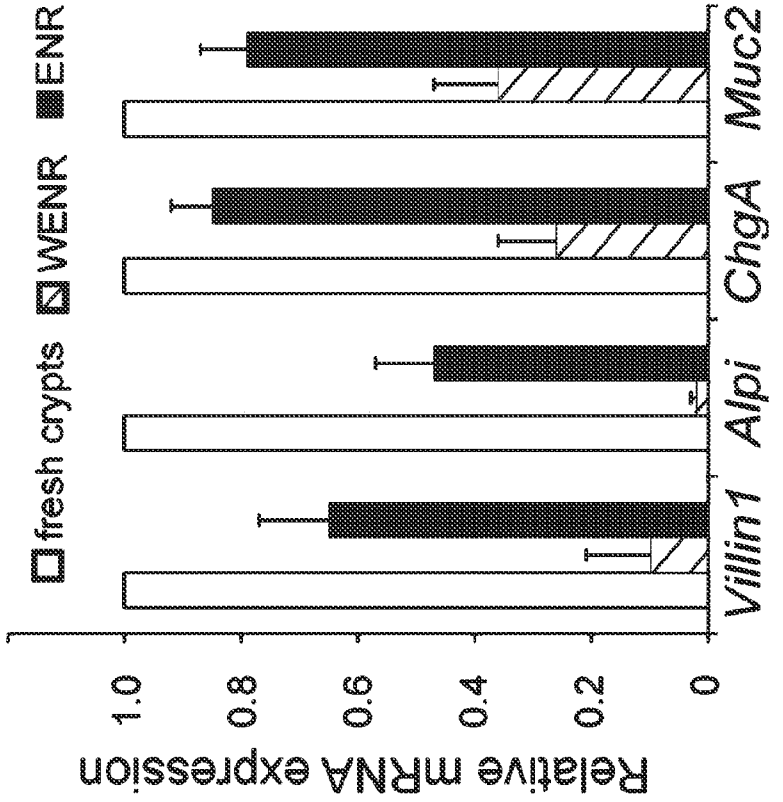

% organoids forming efficiency
60
50
40
30
20
10
0
Proximal colon
Distal colon
*
*
ENR
WENR
WENR
dis

FIG. 1(f)


Relative mRNA expression
1.0
0.8
0.6
0.4
0.2
0
fresh crypts
WENR
ENR
Villin1
Alpi
ChgA
Muc2

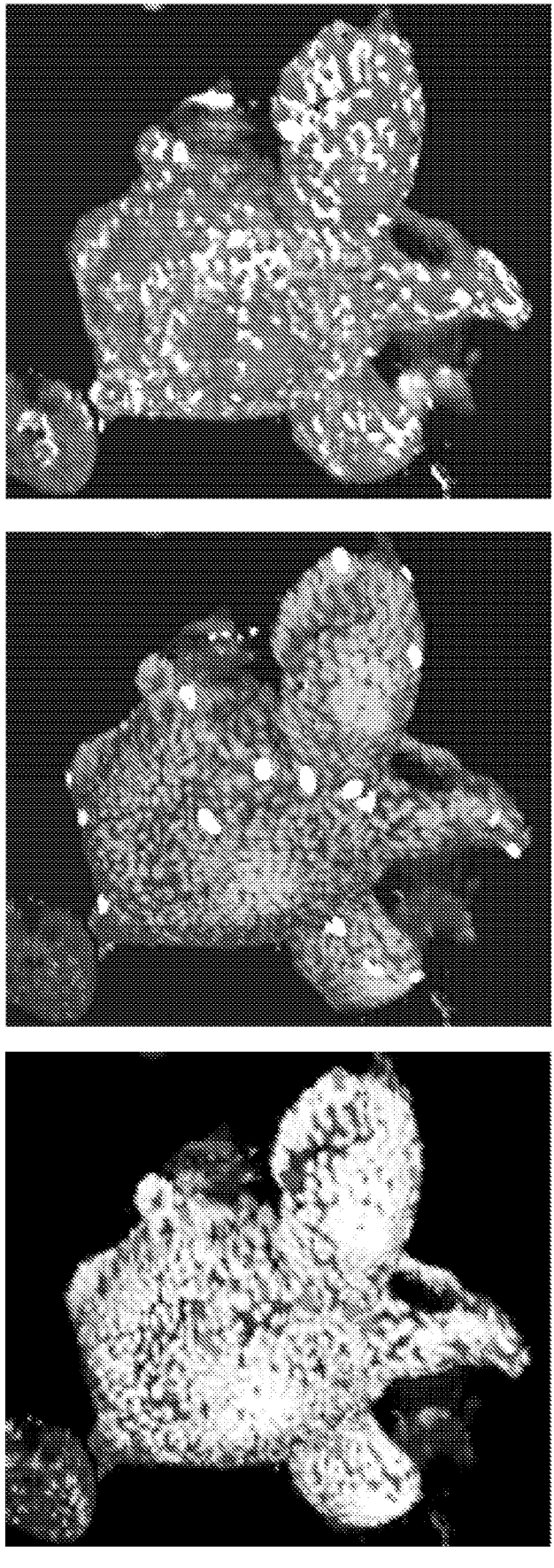
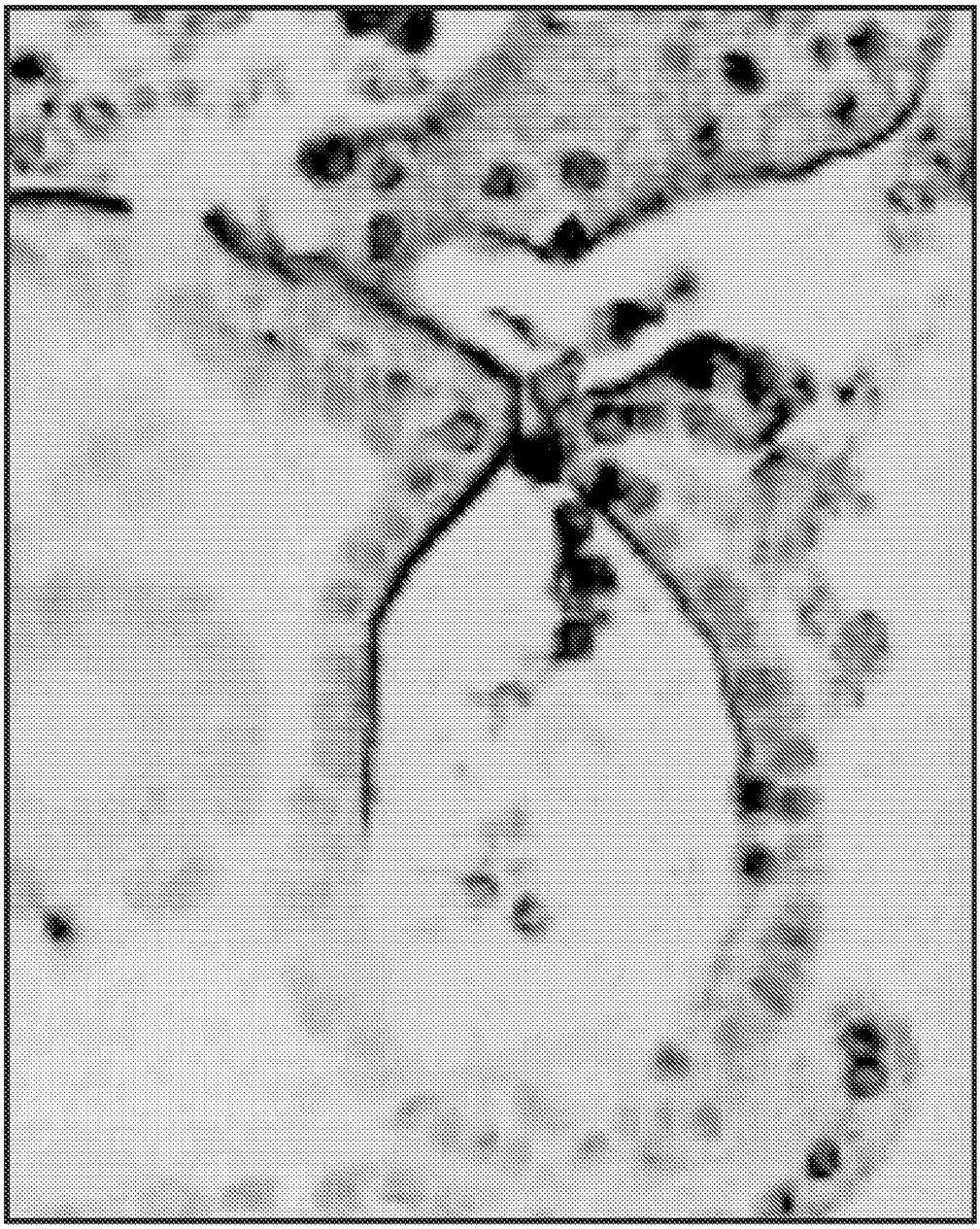


FIG. 1(d)

FIG. 1(e)



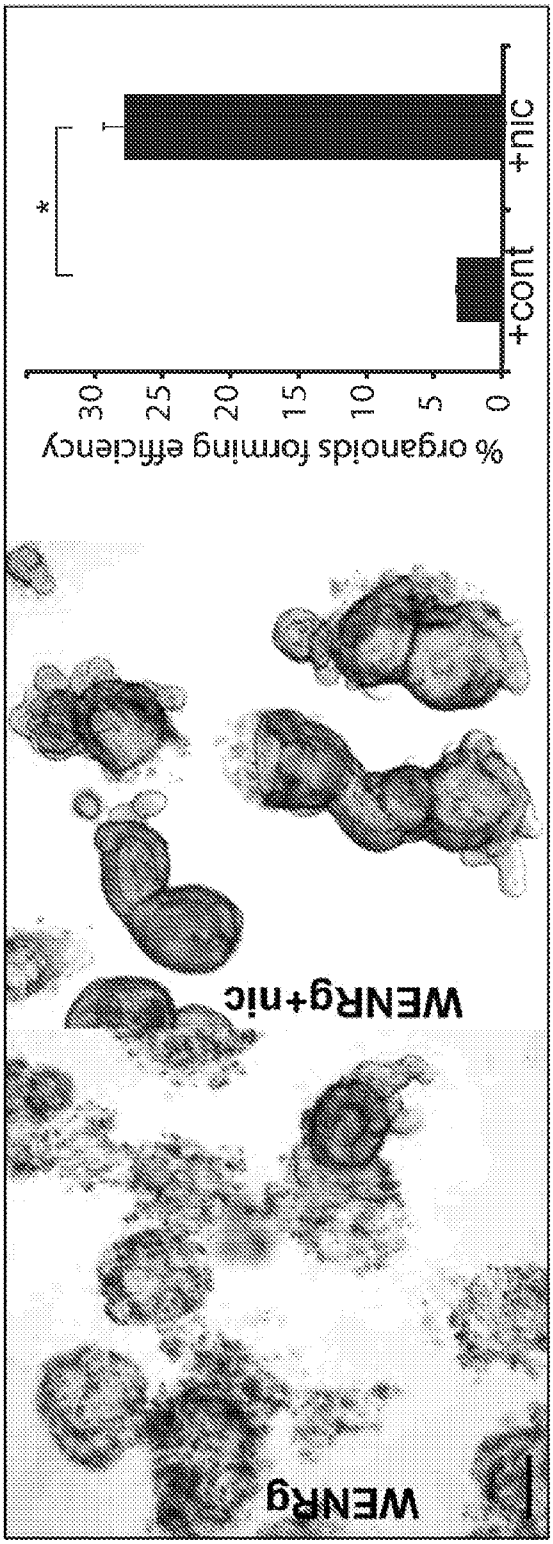

WENRg
WENRg+nic
% organoids forming efficiency
0
5
10
15
20
25
30
+cont
+nic
*

FIG. 2(a)

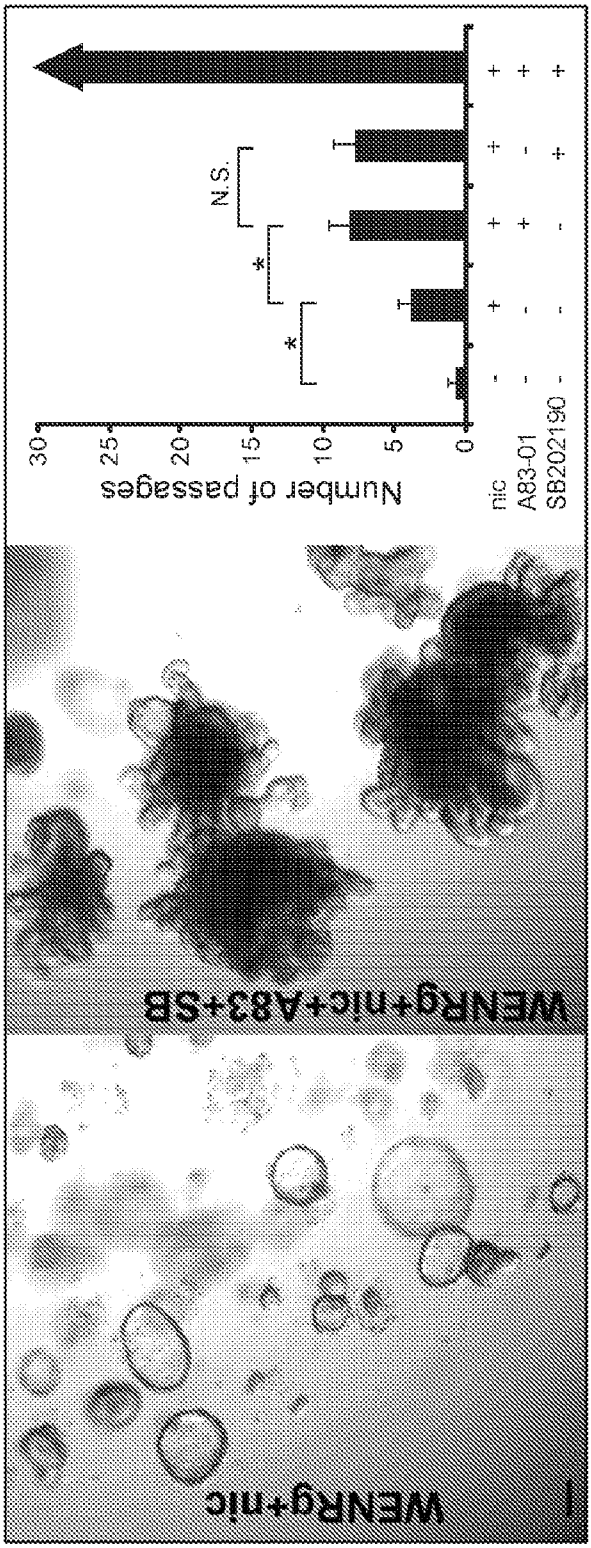

WENRg+nic
WENRg+nic+A83+SB
Number of passages
0
5
10
15
20
25
30
*
*
N.S.
nic
A83-01
SB202190

FIG. 2(b)

FIG. 2(c)

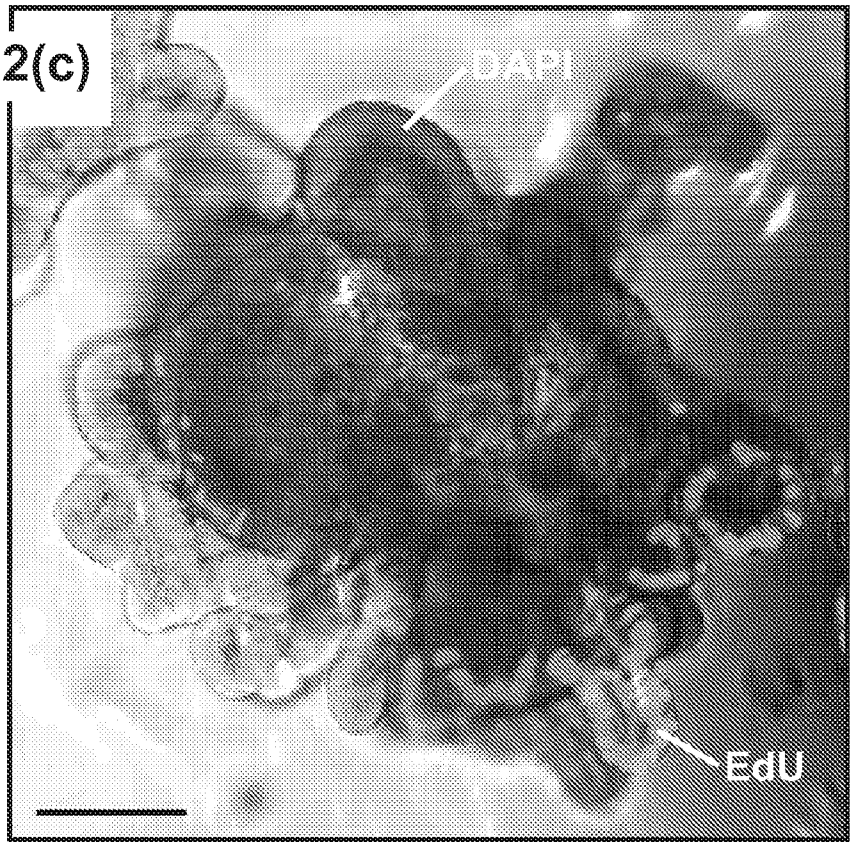
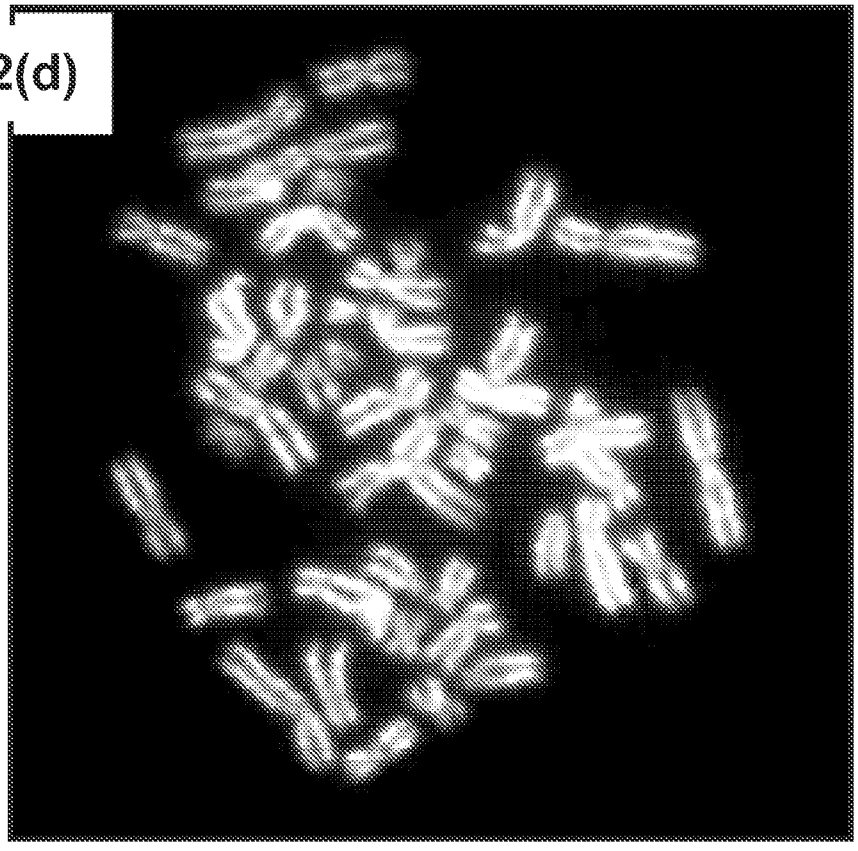


FIG. 2(d)



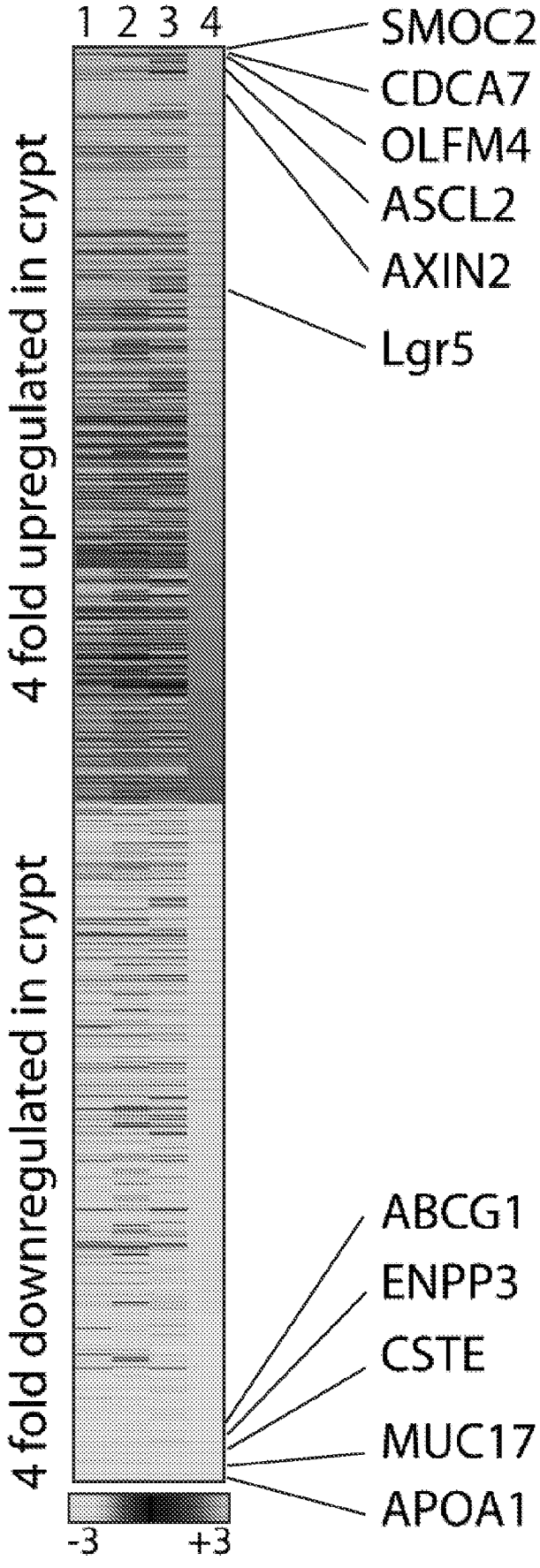

1 2 3 4
SMOC2
CDCA7
OLFM4
ASCL2
AXIN2
Lgr5
4 fold upregulated in crypt
4 fold downregulated in crypt
ABCG1
ENPP3
CSTE
MUC17
APOA1
-3
+3

FIG. 2(e)

FIG. 3(a)

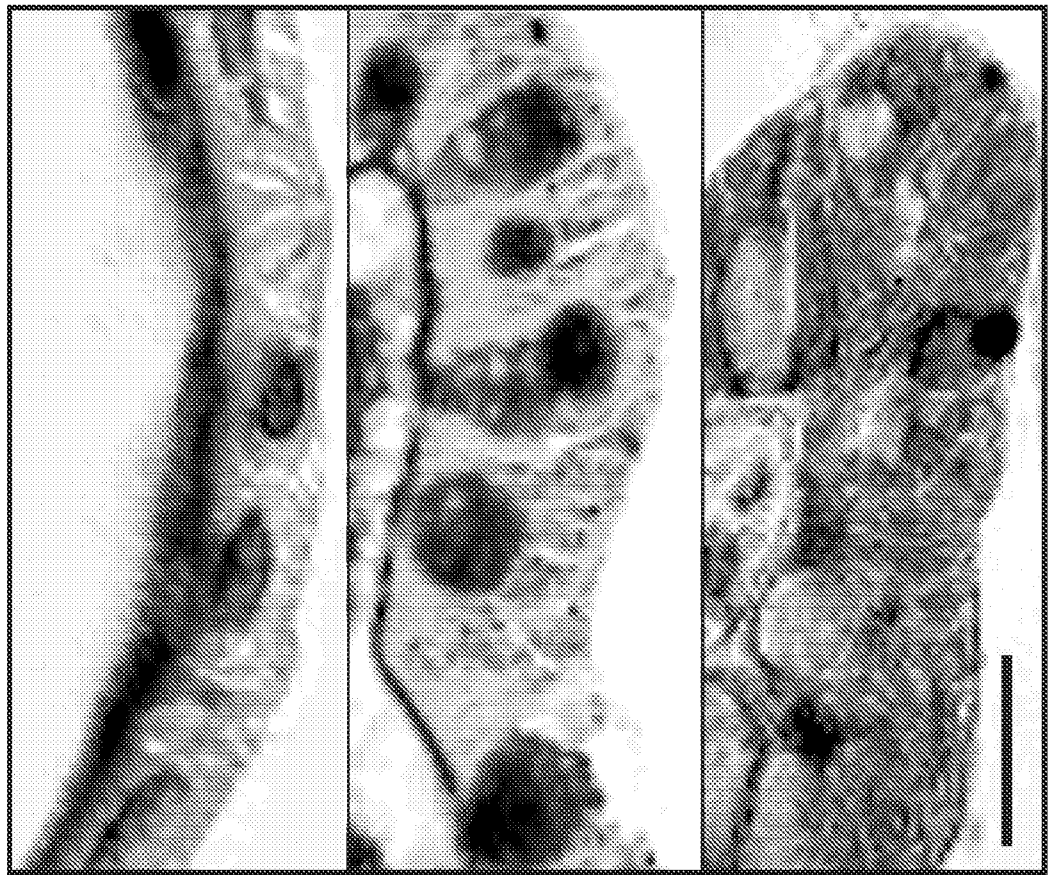


FIG. 3(b)

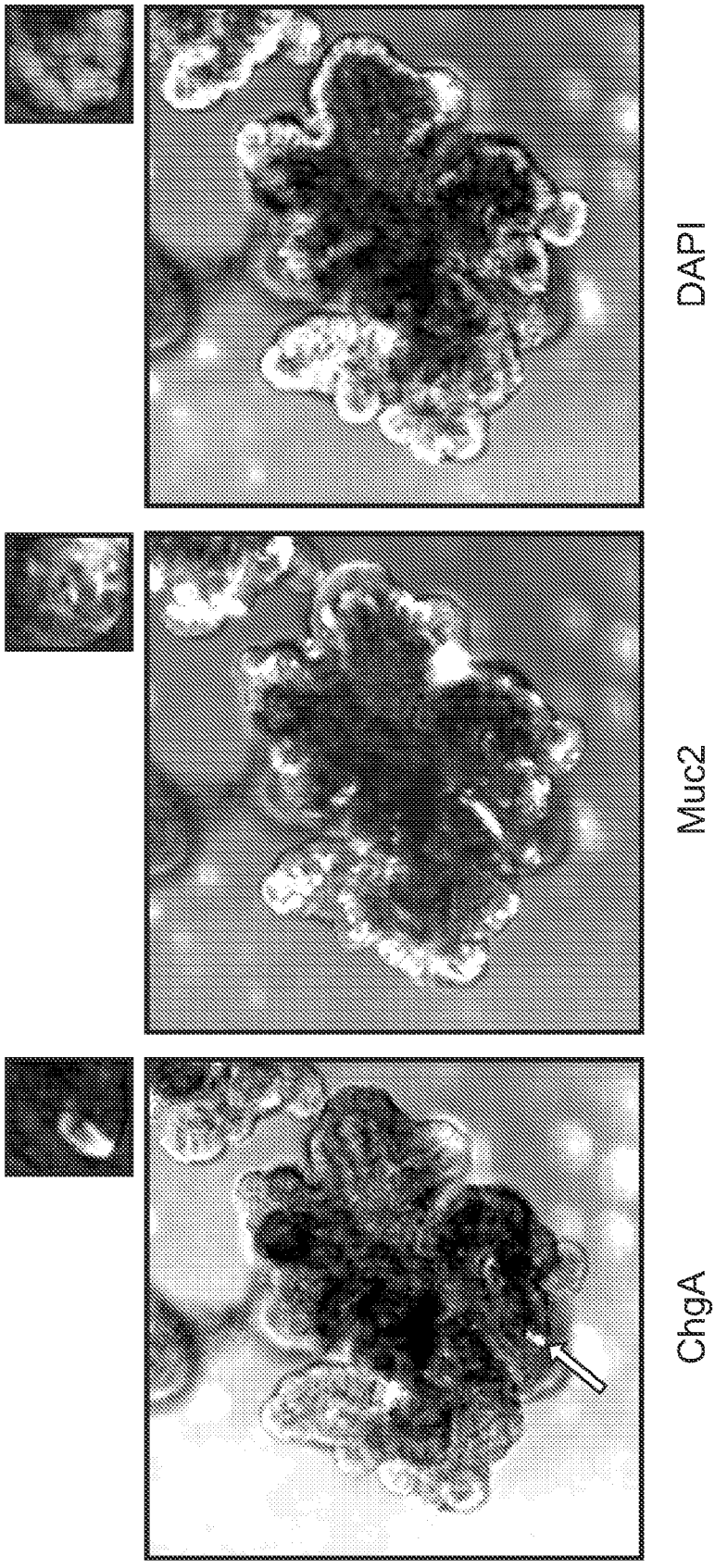
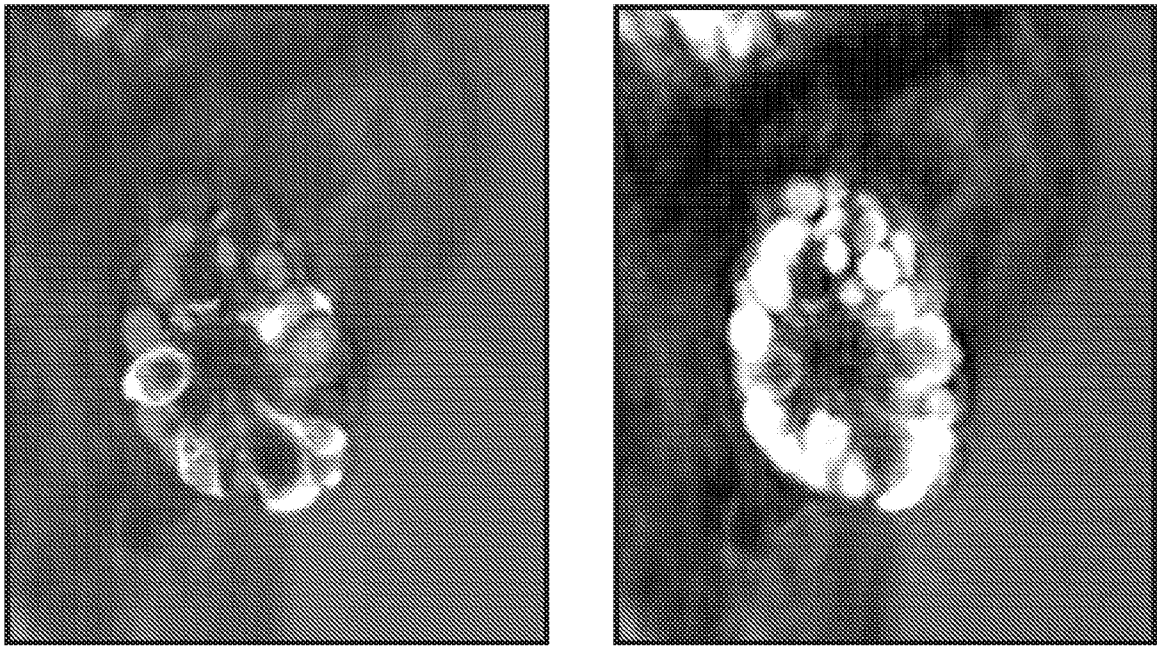


FIG. 3(c)



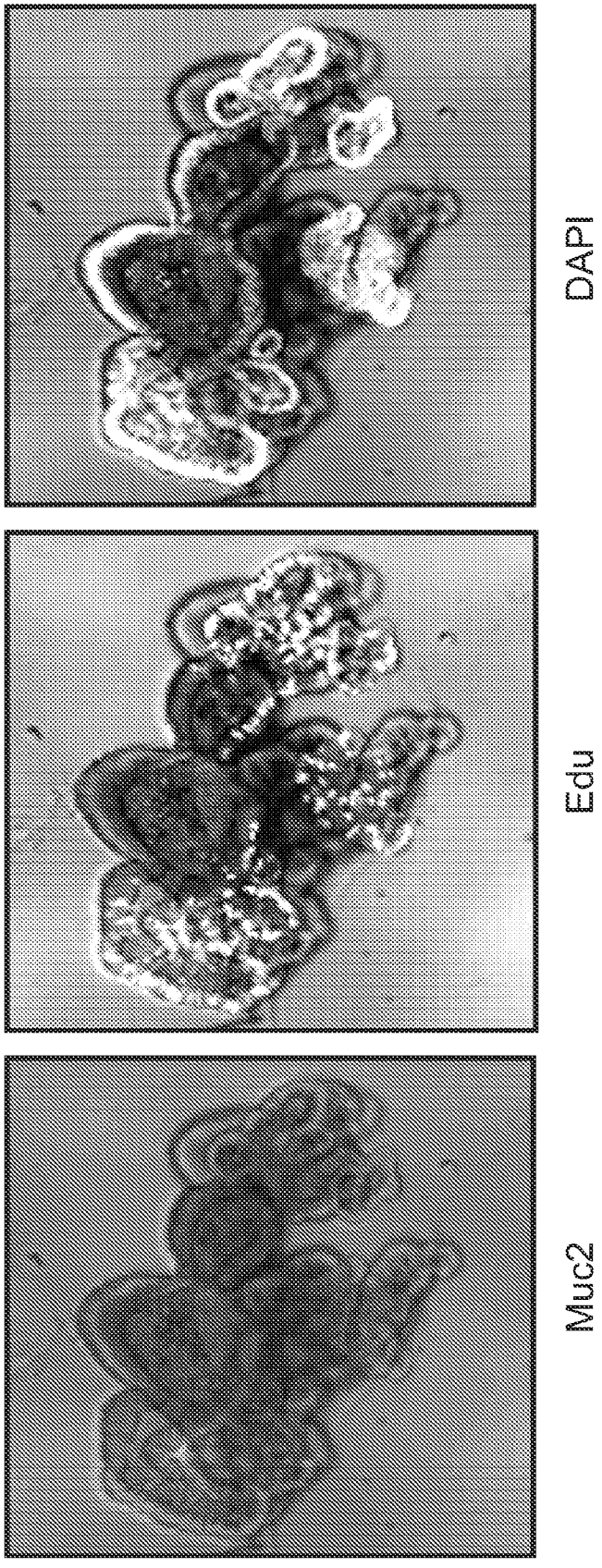
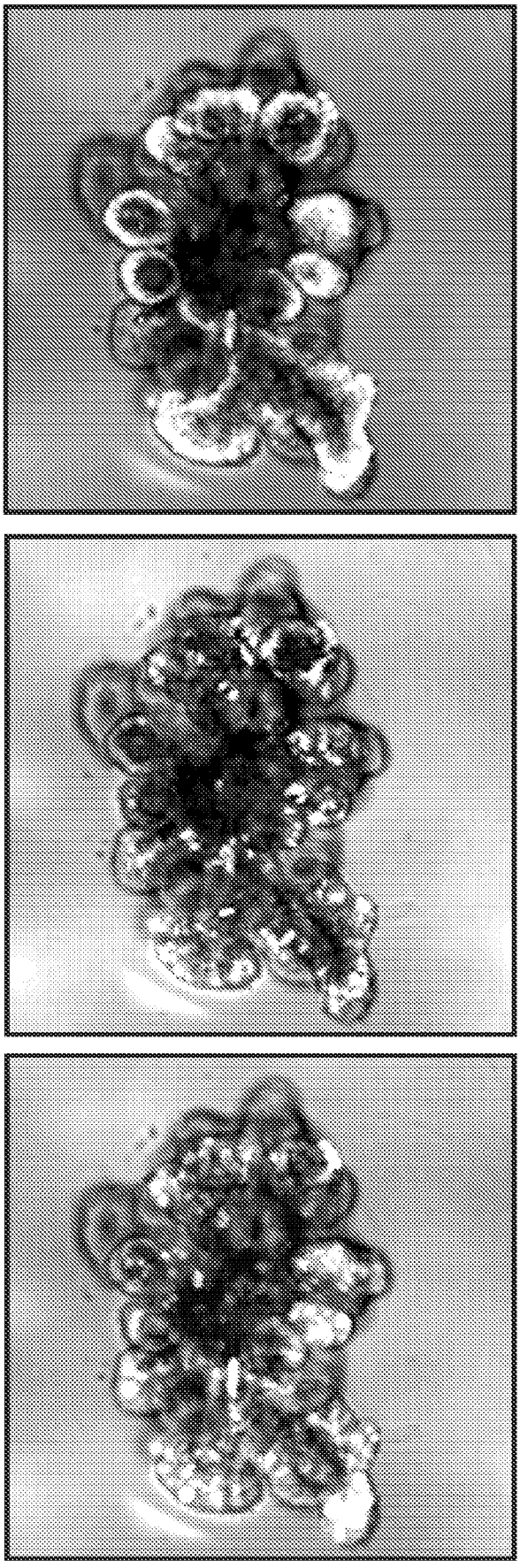


FIG. 3(d)

FIG. 3(e)



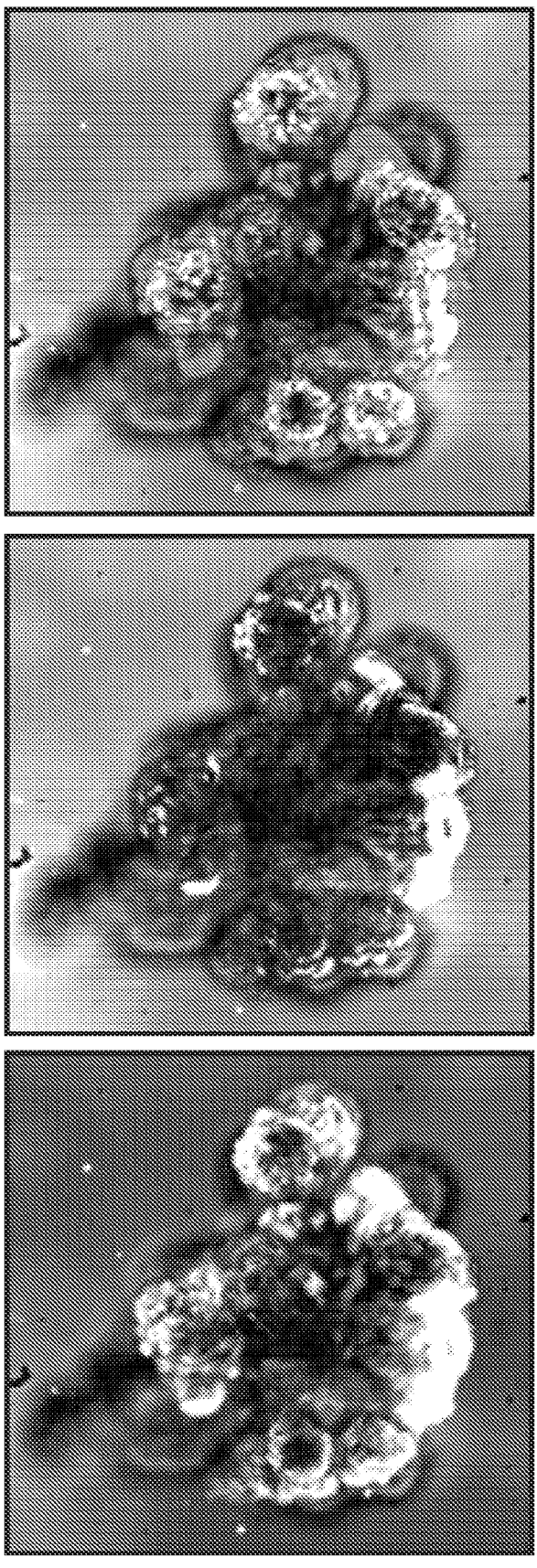


FIG. 3(f)

FIG. 4(a)

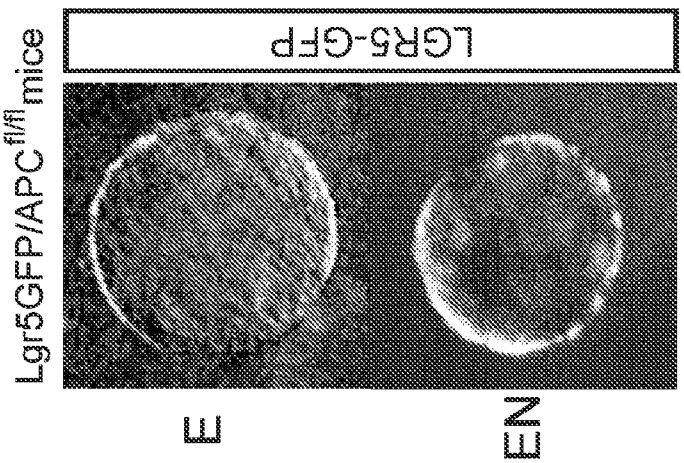


FIG. 4(b)

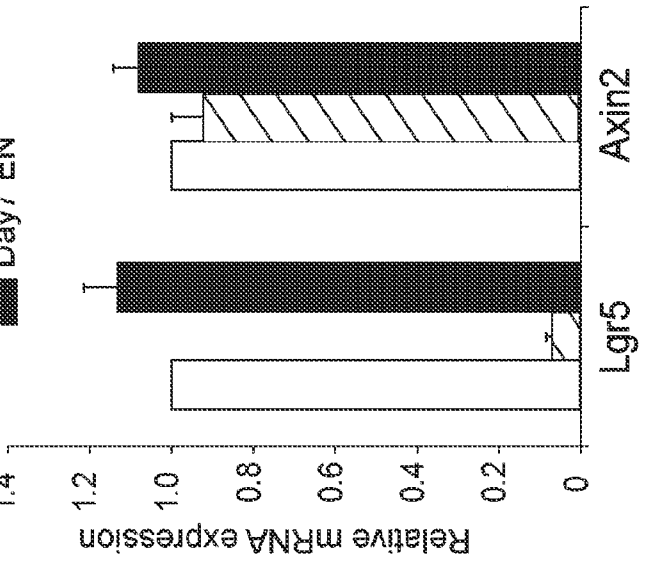
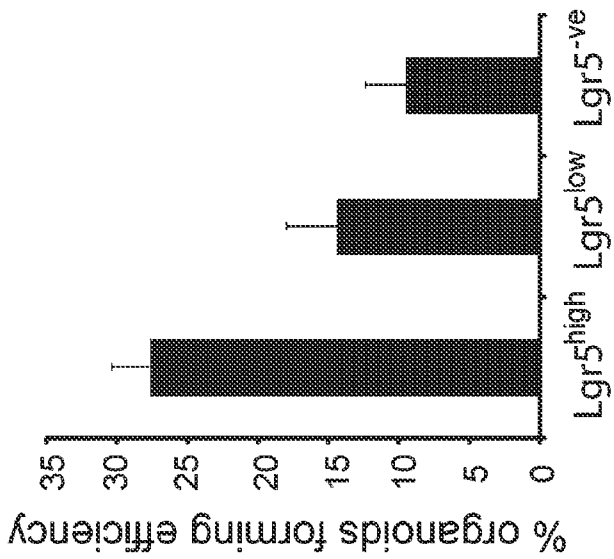


FIG. 4(c)



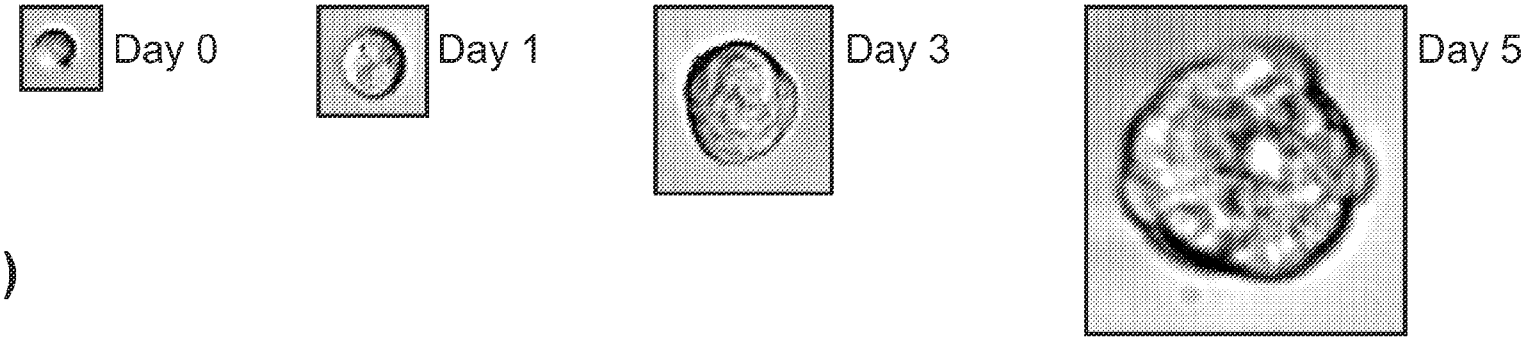


FIG. 4(d)

FIG. 5(a)

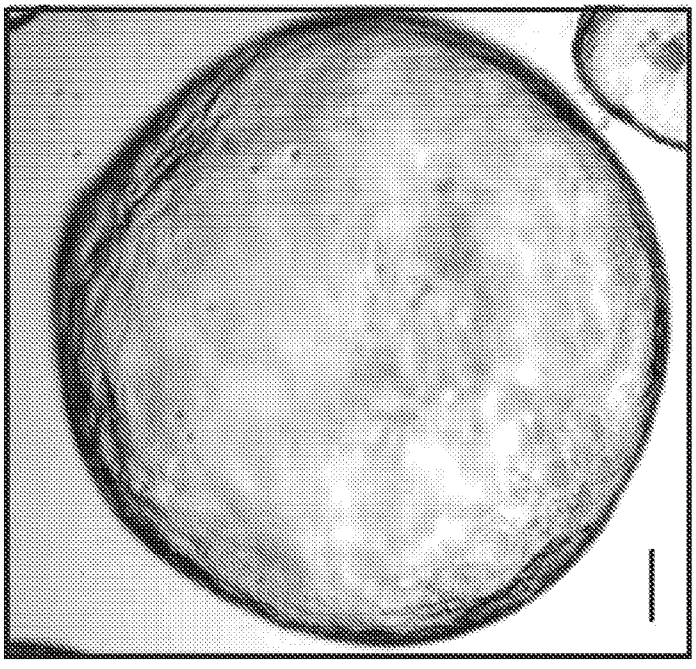
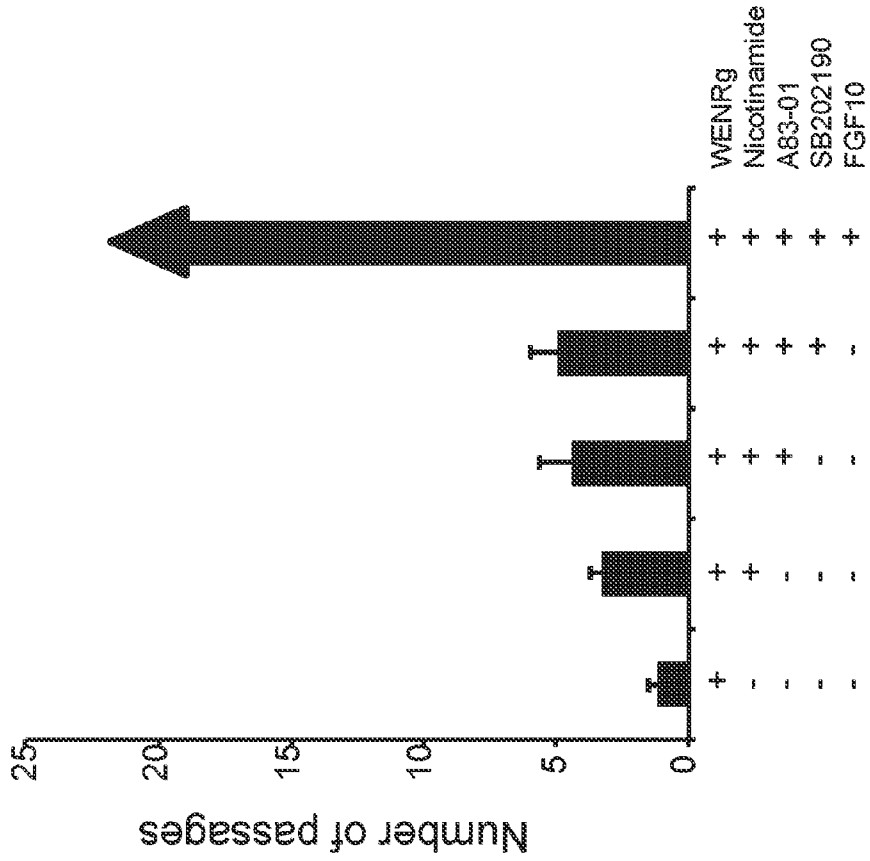
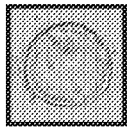


FIG. 5(b)

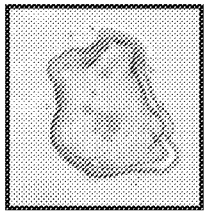

Number of passages
0
5
10
15
20
25
WENRg
Nicotinamide
A83-01
SB202190
FGF10



Day 0

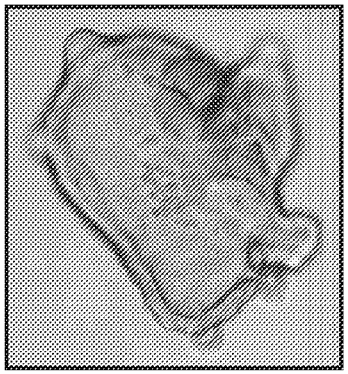


Day 2

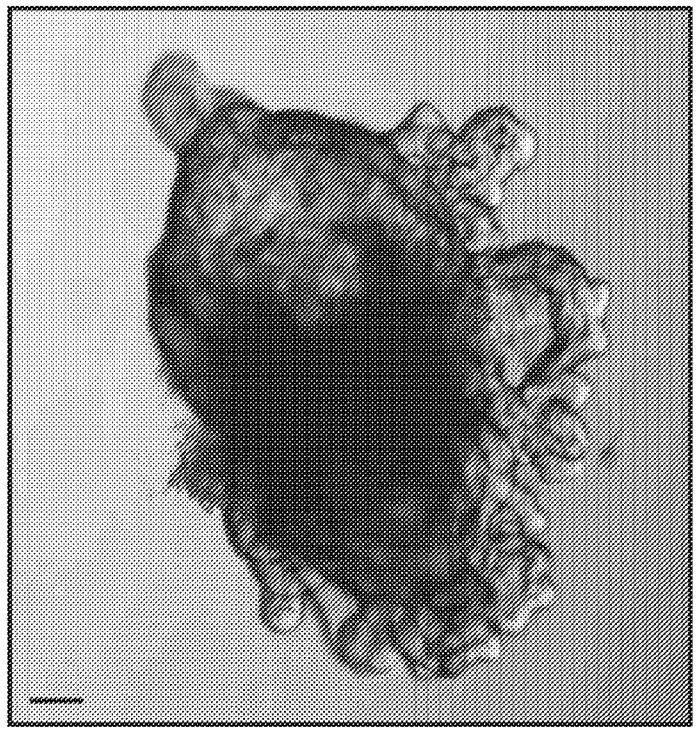


Day 4

FIG. 5(c)



Day 6



Day 10

FIG. 5(d)

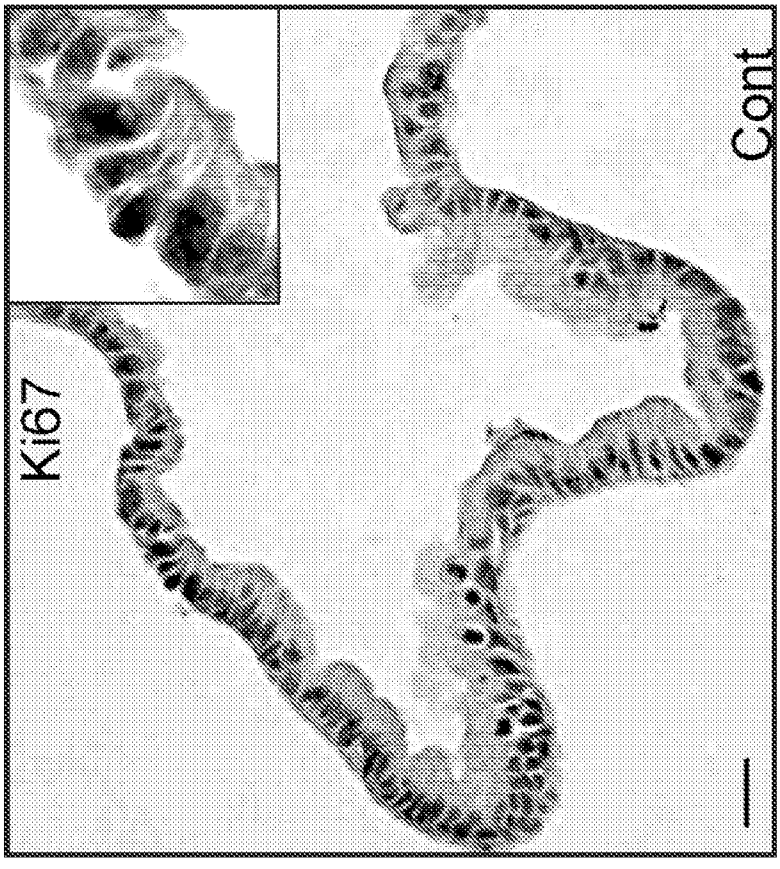


FIG. 5(e)

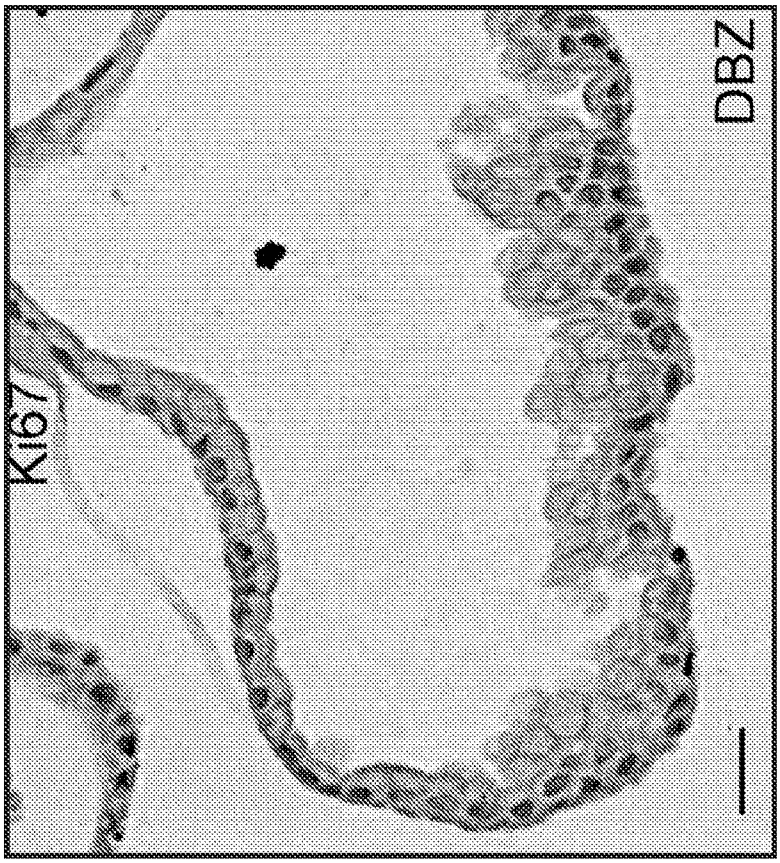


FIG. 5(f)

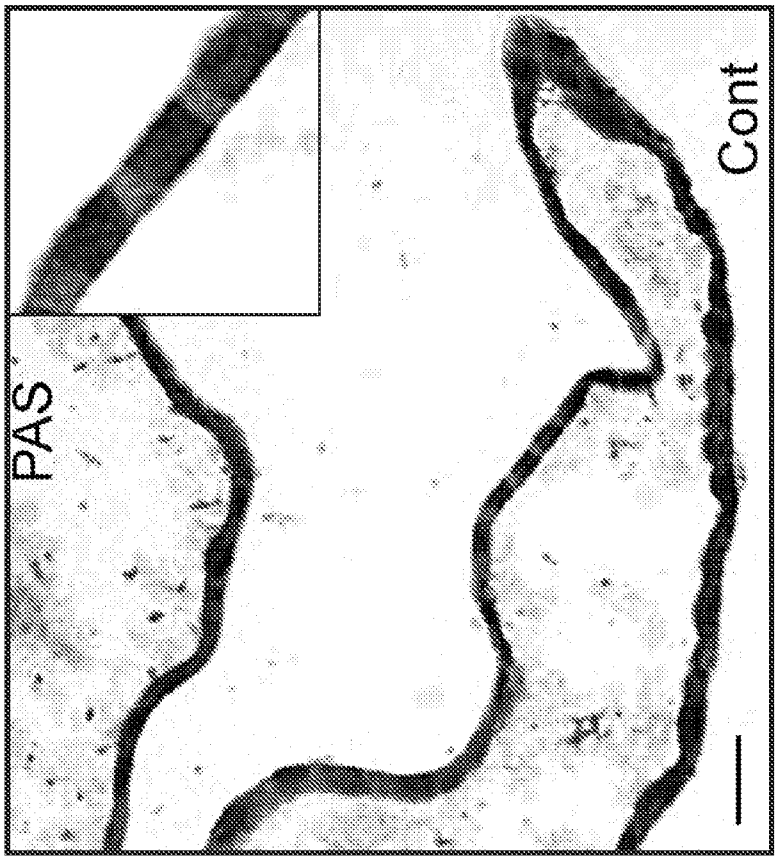


FIG. 5(g)

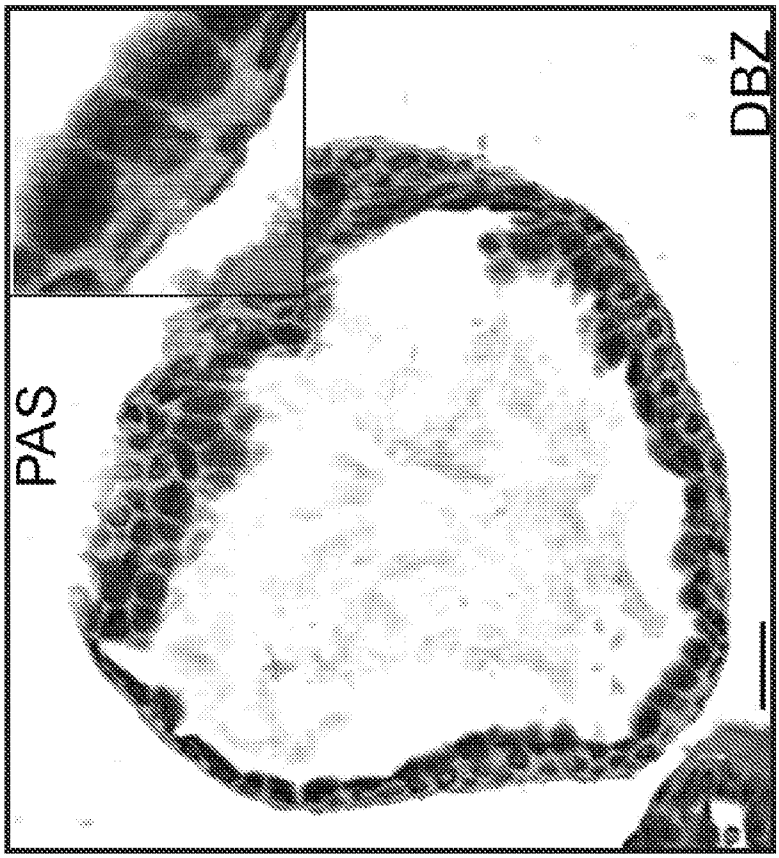


FIG. 6

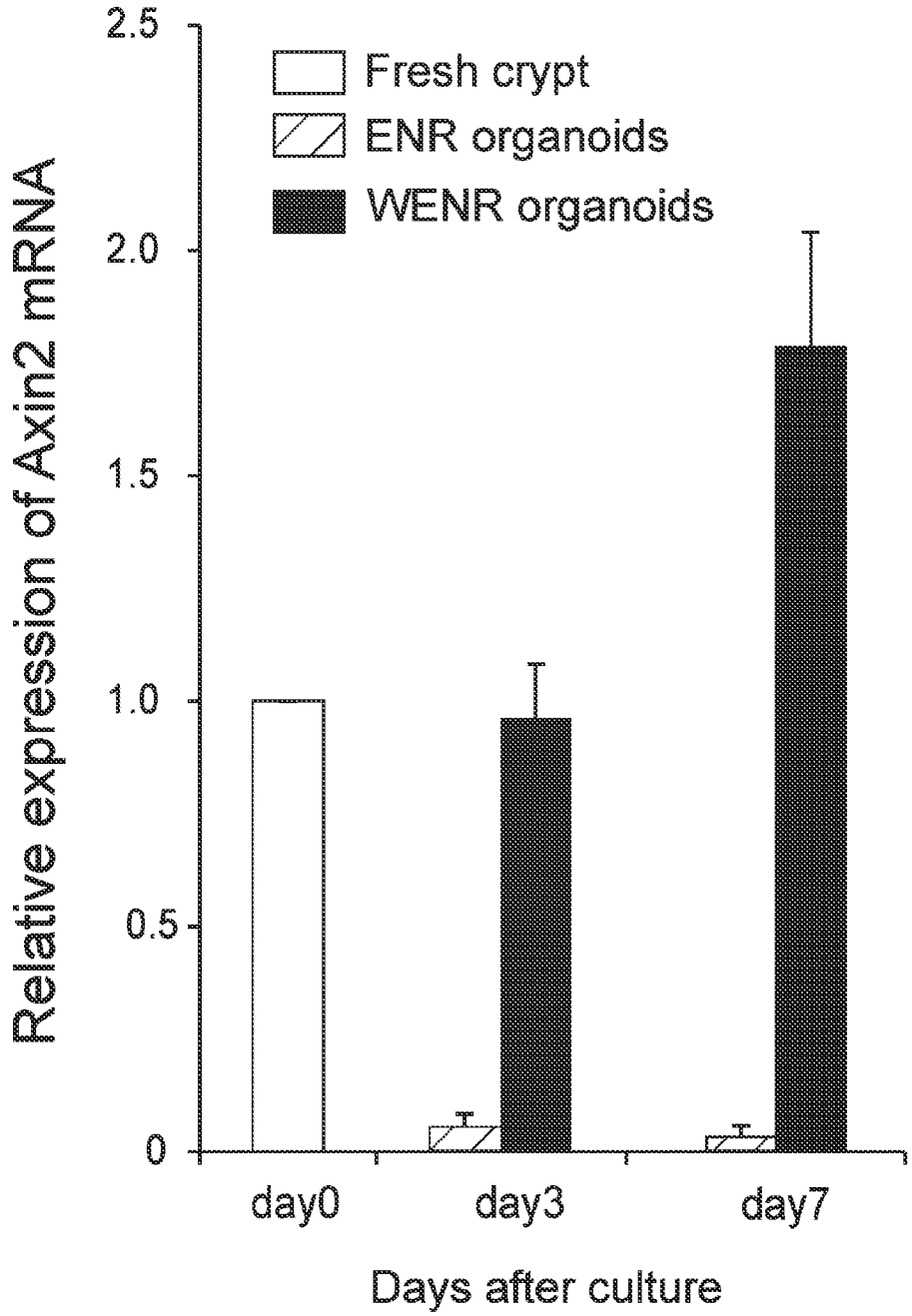

2.5
2.0
1.5
1.0
0.5
0
Relative expression of Axin2 mRNA
Fresh crypt
ENR organoids
WENR organoids
day0
day3
day7
Days after culture

FIG. 7

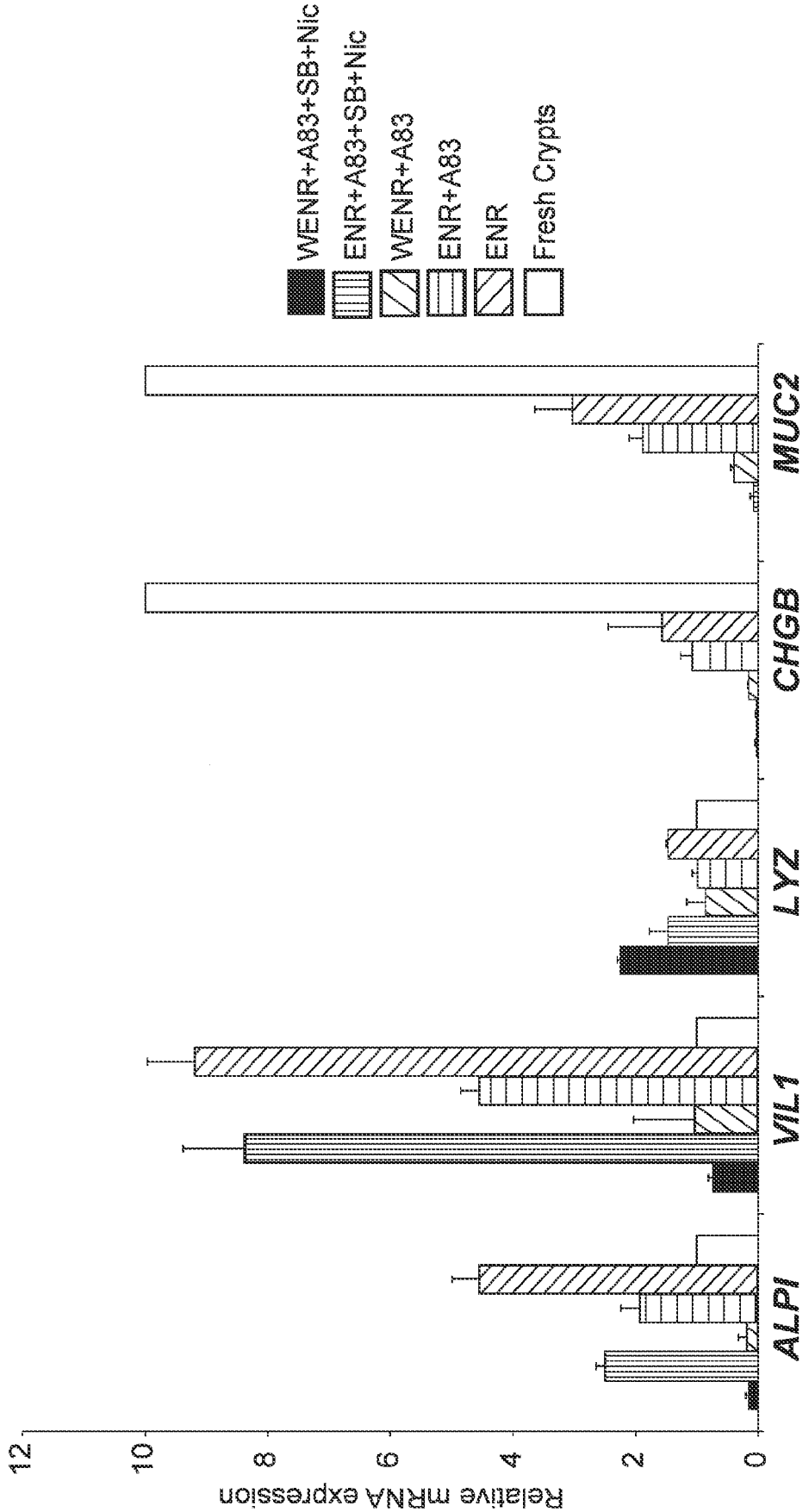
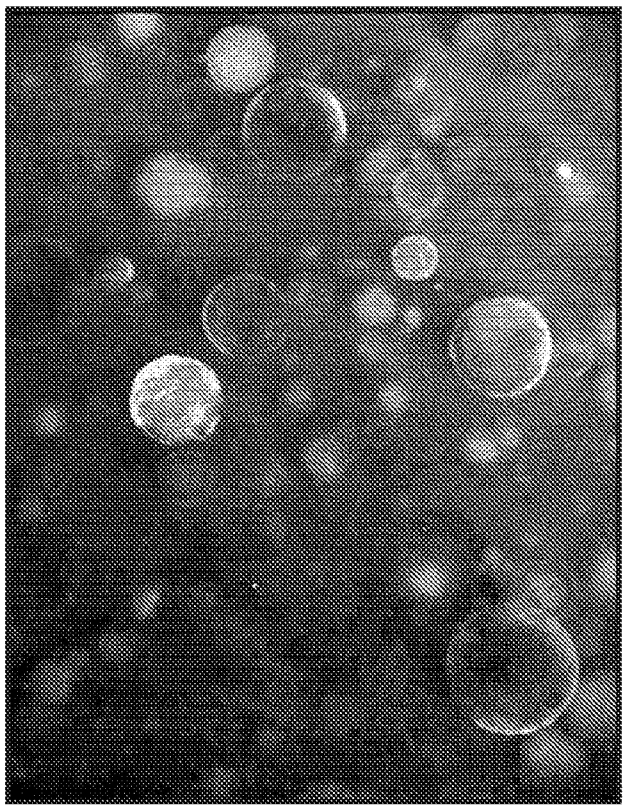

WENR+A83+SB+Nic
ENR+A83+SB+Nic
WENR+A83
ENR+A83
ENR
Fresh Crypts
Relative mRNA expression
0
2
4
6
8
10
12
ALPI
VIL1
LYZ
CHGB
MUC2

FIG. 8(b)

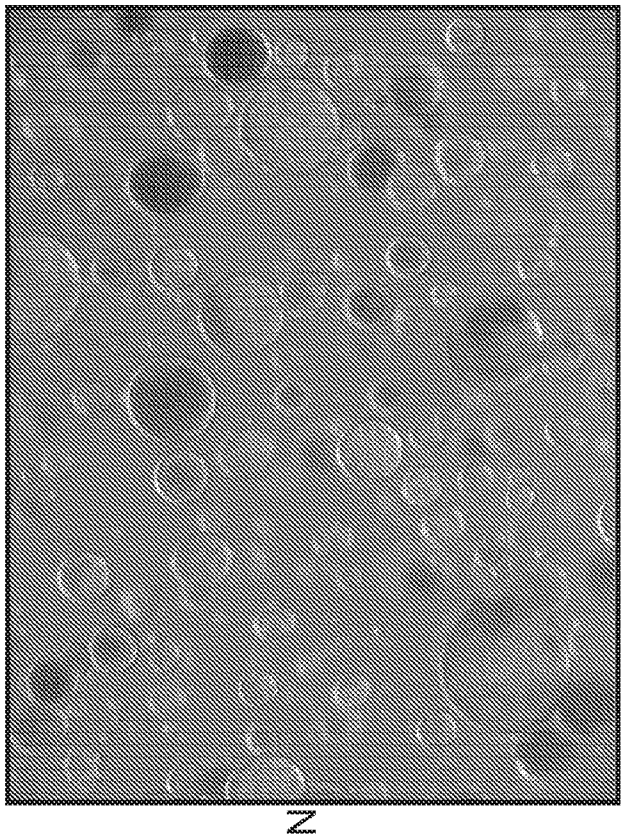
Lgr5-GFP



EN

FIG. 8(a)

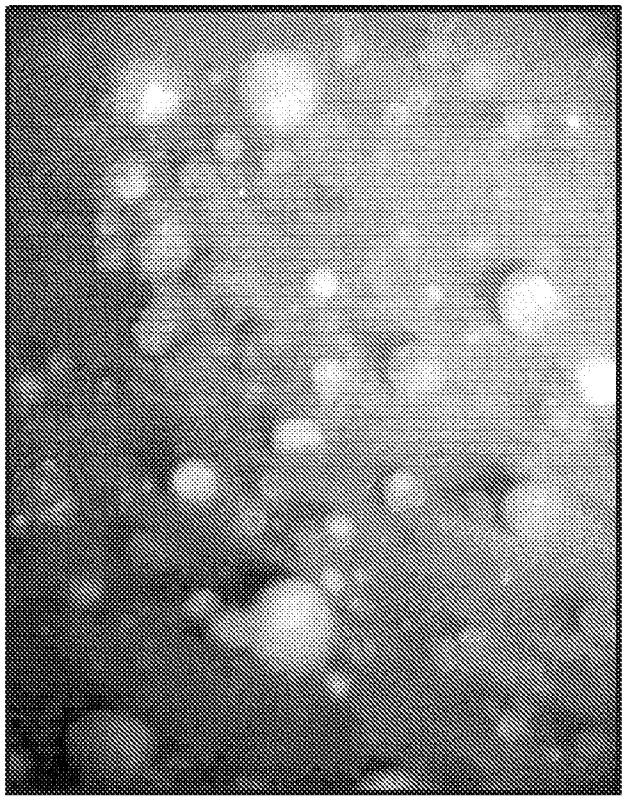
Bright field



EN

FIG. 8(d)

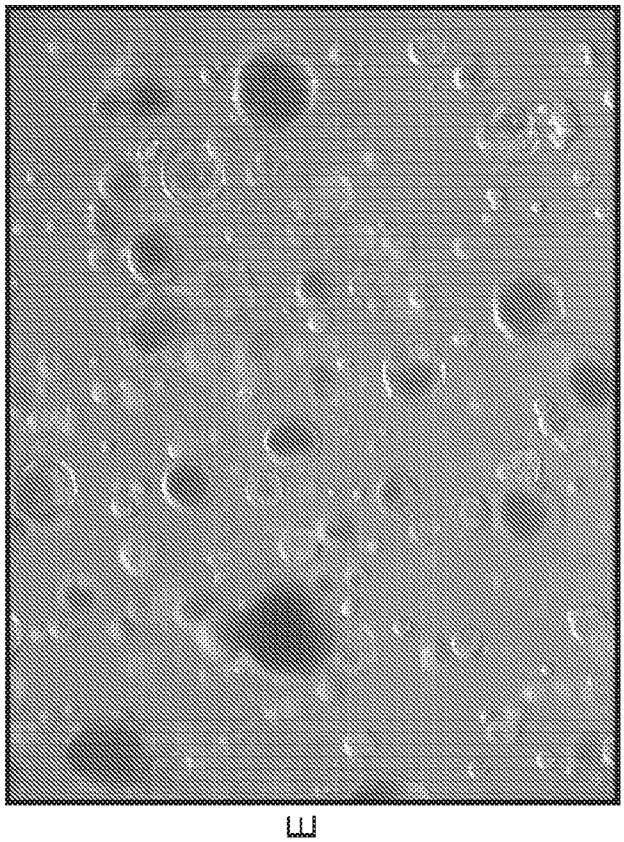
Lgr5-GFP



E

FIG. 8(c)

Bright field



E

FIG. 9

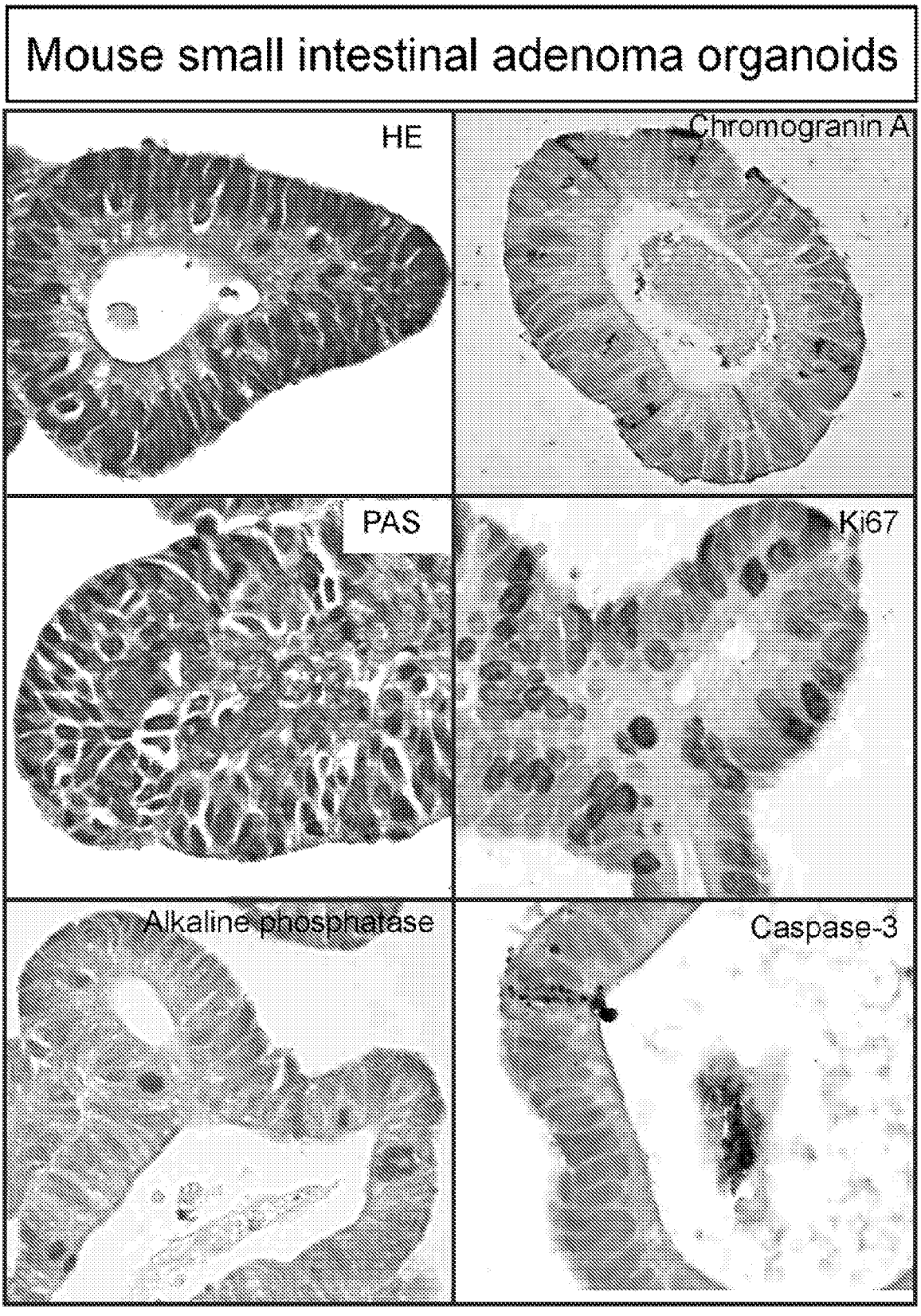

Mouse small intestinal adenoma organoids
HE
Chromogranin A
PAS
Ki67
Alkaline phosphatase
Caspase-3

FIG. 9(contd.)

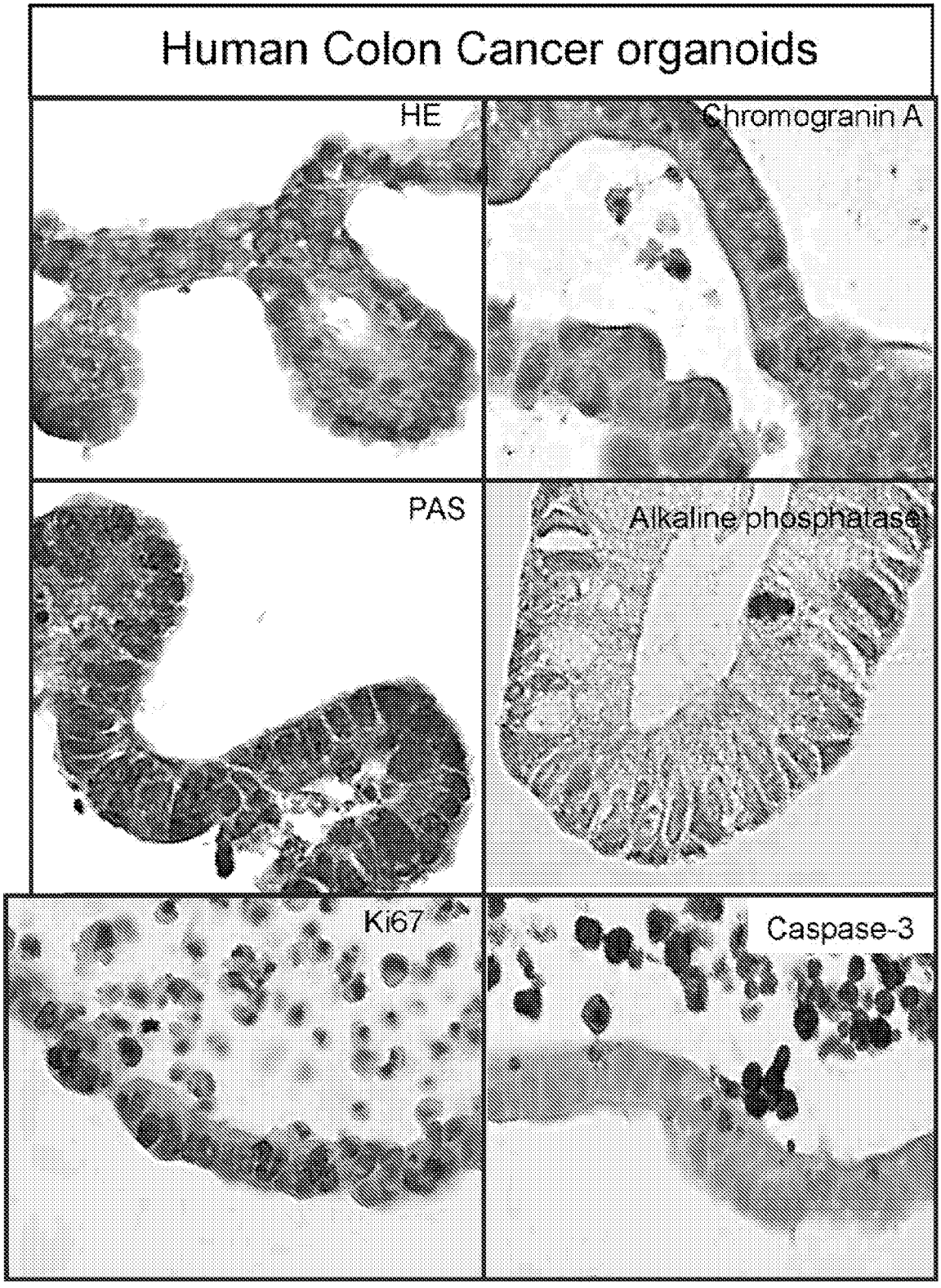

Human Colon Cancer organoids
HE
Chromogranin A
PAS
Alkaline phosphatase
Ki67
Caspase-3

FIG. 10



FIG. 11

Reagent name	Supplier	Cat No.	Solvent	Stock solution	final conc
Matrigel, GFR, phenol free	BD bioscience	356231			
GlutaMAX-I	Invitrogen	35050-079		200 mM	2 mM
Penicillin/Streptomycin	Invitrogen	15140-122		10000/10000 U/ml	100/100 U/ml
N2 supplement	Invitrogen	17502-048		100x	1x
B27 supplement	Invitrogen	17504-044		50x	1x
N-Acetylcysteine	Sigma-Aldrich	A9165-5G	DW	500 mM=81.5 mg/ml	1 mM
EDTA	Sigma-Aldrich	431788-25g	DW	500 mM=14.6g/100ml	2 mM
mouse recombinant noggin	Peprotech	250-38 100 µg	PBS/BSA	100 µg/ml	100 ng/ml
mouse recombinant EGF	Invitrogen	PMG8043	PBS/BSA	500 µg/ml	50 ng/ml
human recombinant R-spondin	Nuvelo		PBS/BSA	1 mg/ml	1 µg/ml
human recombinant FGF10	Peprotech	100-26	PBS/BSA	100 µg/ml	100 ng/ml
mouse recombinant Wnt-3A	Millipore	GF-160	PBS	10 µg/ml	100 ng/ml
Y-27632	Sigma-Aldrich	Y0503	PBS	10 mM=1g/338 µl	10 µM
A-83-01	Tocris	2939	DMSO	500 µM	500 nM
SB202190	Sigma-Aldrich	S7067	DMSO	30 mM	10 µM
Nicotinamide	Sigma-Aldrich	N0636	DW	1M	10 mM
[Leu15]-Gastrin I	Sigma-Aldrich	G9145	PBS/BSA	100 µM	10 nM
DNase	Sigma-Aldrich	DN25-1g	PBS	200000 U/ml	2000 U/ml

All stock solutions are stored in -20 °C. Matrigel is aliquoted and stored in -20 °C

Reagent name	Supplier	Cat No.
HEPES 1M	Invitrogen	15630-056
Advanced DMEM/F12	Invitrogen	12634-028
TrypLE express	Invitrogen	12605-036
Collagenase type XI	Sigma-Aldrich	C9407
Dispase	Invitrogen	17105-041
70um Cell strainer	BD falcon	352350

FIG. 11(contd.)

List of primers and probes for Real-time PCR

	Gene name	Forward primer	Reverse primer	Taqman probe (UPL library number)
Human	Villin1	GCAGCATTACCTGCTCTACGTT	GCTTGATAAGCTGATGCTGTAATTT	#71 gcagccag
	Alkaline phosphatase, intestinal	CATGGACCGCTTCCCATA	GGCACCTGTCTGTCCACAT	#21 tggctctg
	Chromogranin B	CAGCCAACGCTGCTTCTC	TGGCATGGAATTGACAGC	#79 cctcctgg
	Lysozyme	CCGCTACTGGTGTAATGATGG	CATCAGCGATGTTATCTTGCAG	#68 aggagcag
	Mucin2	GCCAGCTCATCAAGGACAG	GCAGGCATCGTAGTAGTGCTG	#61 ttgcccag
	Hprt	TGACCTTGATTTATTTTGCATACC	CGAGCAAGACGTTCAGTCCT	#73
Mouse	Villin1	gcttgccacaacttcctaagat	tcagtttagtcatggtggacga	#77 ggtggtgg
	Muc2	tgtggaacccgggaagatg	gaccacaggtatggttctgga	#22 tggtggag
	Chromogranin A	cgatccagaaagatgatggtc	cggaagcctctgtctttcc	#58 ggatggag
	Hprt	tcctcctcagaccgctttt	cctggttcatcatcgctaatc	#95 actcccag

FIG. 12

	Reagent name	function	Company	Final concentration	Activity scale
Hormones, vitamines etc	Hydrocortison		Sigma	500 nM	0
	[Leu15]-Gastrin I		Sigma	1 mM	1+
	Exendin4	GLP1 analog	Sigma	100 nM	0
	Nicotinamide	Vitamin B delivative	Sigma	10 mM	3+
	L-Ascorbic acid	Vitamin C	Sigma	10 mM	0
	anti-oxidant mixture		Sigma	1x	0
	Lipid mixture		Sigma	1x	0
	PGE2		Sigma	10 mM	1+(Cystic)
	Cholera Toxin		Sigma	100 nM	1+ (Cystic)
Growth factors	IGF		Peprotech	100 ng/ml	0
	HGF		Peprotech	100-500 ng/ml	0
	BDNF		Peprotech	100 ng/ml	0
	GDNF		Peprotech	100 ng/ml	0
	FGF2		Peprotech	100 ng/ml	0
	FGF10		Peprotech	100 ng/ml	0
	Follistatin		Peprotech	100 ng/ml	0
	Cyr61		Peprotech	1 mg/ml	0
	LIF		Millipore	1000 U/ml	0

Reagents tested for optimization of human intestinal organoid culture. Each reagent was added to WENR culture condition

Activity scale:plating efficiency was compared with control after 4 days culture:

0=no change, 1+=<50% increase, 2+=50- 100% increase, 3+= >100% increase, 1-=0-50%, 2-=50-100% decrease, 3-=>100% decrease

FIG. 13

	Reagent name	function	Company	Final concentration	Activity scale
Small molecule inhibitors	PD98059	ERK inhibitor	Sigma	10 mM	1-
	SB203580	p38 inhibitor	Sigma	1-10 mM	2+
	SB202190	p38 inhibitor	Sigma	1-10 mM	2+
	SP600125	JNK inhibitor	Sigma	10 mM	0
	PS48	PDK1 activator	Sigma	5 mM	0
	Y27632	ROCK inihibitor	Sigma	10 mM	1+(cystic)
	Cyclopamine	Hedgehog inhibitor	Sigma	100 nM	2-
	Trichostatin A	HDAC inhibitor	Stemolecule	100 nM	1-
	5 Azacytidin	DNA methylase inhibitor	Stemolecule	20 mM	1-
	Dorsomorphin	BMP inhibitor	Stemolecule	10 mM	0
	A83-01	ALK4,5,7 inhibitor	Tocris	50 n-1 mM	3+
	VO-OHpic trihydrate	PTEN inhibitor	Sigma	500 nM	3-
	cyclic Pifithrin-α	p53 inhibitor	Sigma	50 nM	0
	BIX01294	G9a HMTase inhibitor	Stemolecule	10 mM	1-
	DBZ	Notch inhibitor	Ref.1	10 mM	0

Small molecule inhibitors for optimization of human intestinal organoid culture.

Each inhibitor was added to WENR+ gastrin +Nicotinamide culture condition.

Activity scale:plating efficiency was compared with control after 4 days culture:

0=no change, 1+=<50% increase, 2+=50- 100% increase, 3+= >100% increase, 1-=0-50%, 2-=50-100% decrease, 3-=>100% decrease

Reference 1: van Es JH et al, Nature 2005, 435, 959-63

FIG. 14

SI top 25 upregulated genes

	Small intestinal organoids		Colon organoids	
	Gene symbol	Fold difference vs small intestinal villi	Gene symbol	Fold difference vs small intestinal villi
1	CCL26	9.60	ADORA2B	10.19
2	ADORA2B	9.54	NOX1	9.02
3	LPL	9.11	LEFTY1	8.99
4	LCN2	8.26	CCL26	8.90
5	DSG3	8.04	CD24	8.72
6	SMOC2	7.75	EYA2	8.05
7	CLDN1	7.64	RHBDL2	7.93
8	BX415272	7.50	SMOC2	7.72
9	CXCL3	7.48	SPINK5	7.65
10	AK022042	7.48	TFCP2L1	7.58
11	LGR6	7.17	LCN2	7.49
12	CTTNBP2	7.00	C1orf125	7.48
13	CD24	6.89	BX415272	7.34
14	MMP1	6.85	CXCL3	7.29
15	SERPINB5	6.76	KIAA1199	7.00
16	KIAA1199	6.61	CTTNBP2	6.92
17	CD44	6.37	IL20RA	6.91
18	CEP55	6.36	CEP55	6.83
19	C18orf56	5.90	E2F8	6.63
20	LRRN1	5.89	CD44	6.49
21	AK095715	5.89	MYB	6.26
22	JUB	5.85	AK021425	6.18
23	GPX2	5.84	IRX2	6.11
24	HPDL	5.82	GPX2	6.06
25	RHBDL2	5.82	CCNA2	6.05

FIG. 14(contd.)

SI bottom 25 downregulated genes

	Small intestinal organoids		Colon organoids	
	Gene symbol	Fold difference vs small intestinal villi	Gene symbol	Fold difference vs small intestinal villi
1	APOA1	-9.41	APOB	-10.68
2	CD36	-9.28	CD36	-9.69
3	KLRB1	-7.88	SLC13A1	-9.39
4	HBB	-7.71	APOA1	-9.14
5	CYP2C19	-7.48	APOC3	-9.06
6	APOC3	-7.45	NTS	-8.87
7	SEPT3	-7.17	LRAT	-8.51
8	FAM101B	-6.98	KCNJ16	-8.29
9	SLC15A1	-6.87	SLC8A1	-8.07
10	GZMK	-6.81	SLC15A1	-7.63
11	AV691872	-6.70	BE766438	-7.51
12	APOB	-6.62	CYP2C19	-7.48
13	GNLY	-6.58	SEMA3G	-7.44
14	CLDN8	-6.21	SEPP1	-7.38
15	CD48	-6.17	HBB	-7.28
16	CD69	-6.05	TRAF3IP3	-7.28
17	CD96	-5.88	SEPT3	-7.17
18	GPR171	-5.85	GIMAR6	-7.10
19	CD247	-5.82	FAM101B	-7.05
20	RGS1	-5.76	FGF19	-6.90
21	BMP8B	-5.73	GZMK	-6.81
22	SLCO2A1	-5.68	AV691872	-6.70
23	IL18RAP	-5.65	ADAMDEC1	-6.50
24	ARG2	-5.64	DFNA5	-6.42
25	C1QA	-5.57	CD48	-6.39

FIG. 15

		Organoids in stem cell culture condition			Organoids in ifferentiation condition		
Species	Organ	Proliferation	Differeniation	Apoptosis	Proliferation	Differeniation	Apoptosis
Mouse	Small intestine	20-30%	AGEP	<1%			
	Colon	20-30%	GE	<1%	10-20%	AGE	<1%
	Adenoma	40-60%	GEP	<1%			
Human	Small intestine	20-30%	P	<1%	10-20%	AGEP	<1%
	Colon	20-30%	(-)	<1%	10-20%	AGE	<1%
	Colon Cancer	50-90%	GE	<1%			
	Barrett's epithelium	20-30%	(-)	<1%	<1%	GP	<1%

Proliferation was determined by Ki67 staining.

Apoptosis was determined by Caspase-3 staining

Differentiation markers; A: absorptive enterocyte, G: goblet cells, E: enteroendocrine cells, P: Paneth cells

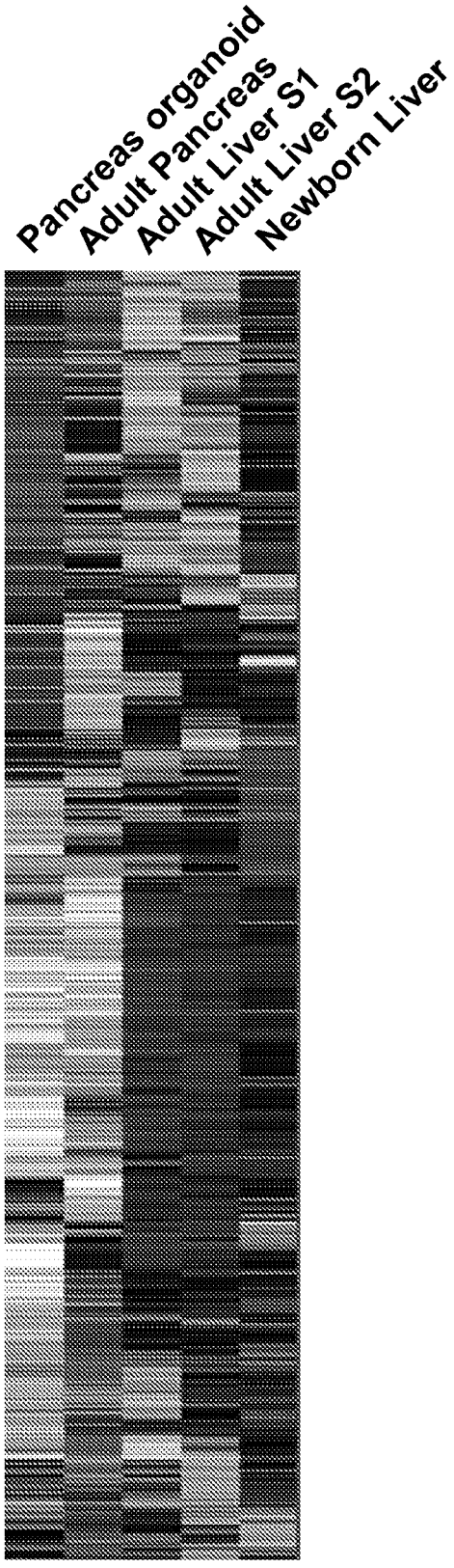
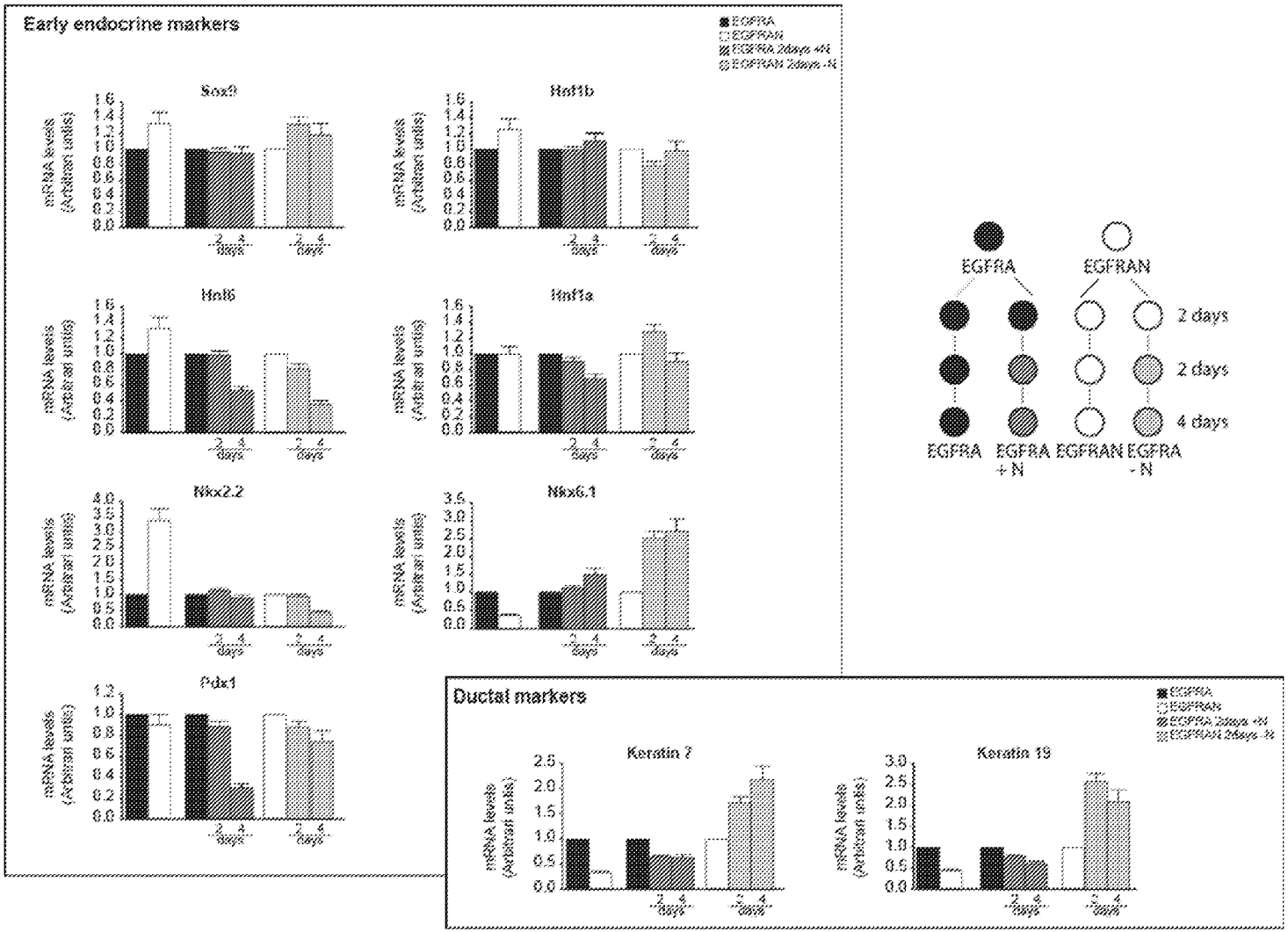

Pancreas organoid
Adult Pancreas
Adult Liver S1
Adult Liver S2
Newborn Liver

Figure 16A

Figure 16B

Ductal markers		Adult liver	Adult pancreas	Pancreas organoid	Liver organoid
ref|Mus musculus leucine aminopeptidase 3 (Lap3), mRNA [NM_024434]	**Lap3**	11826	922	**3286**	2655
ref|Mus musculus keratin 19 (Krt19), mRNA [NM_008471]	**Krt19**	302	566	**76191**	29904
ref|Mus musculus keratin 7 (Krt7), mRNA [NM_033073]	**Krt7**	237	369	**147227**	108352
ref|Mus musculus SRY-box containing gene 9 (Sox9), mRNA [NM_011448]	**Sox9**	235	185	**2365**	2492
ref|Mus musculus HNF1 homeobox B (Hnf1b), mRNA [NM_009330]	**Hnf1b**	234	115	**608**	764
ref|Mus musculus one cut domain, family member 1 (Onecut1), mRNA [NM_008262]	**Hnf6**	1239	120	**1297**	1454
Transcription Factors necessary for Ngn3 expression					
ref|Mus musculus SRY-box containing gene 9 (Sox9), mRNA [NM_011448]	**Sox9**	235	185	**2365**	2492
ref|Mus musculus forkhead box A2 (Foxa2), mRNA [NM_010446]	**Foxa2**	8695	1415	**12948**	9579
ref|Mus musculus HNF1 homeobox B (Hnf1b), mRNA [NM_009330]	**Hnf1b**	234	115	**608**	764
ref|Mus musculus one cut domain, family member 1 (Onecut1), mRNA [NM_008262]	**Hnf6**	1239	120	**1297**	1454
Endocrine markers					
ref|Mus musculus neurogenin 3 (Neurog3), mRNA [NM_009719]	**Neurog3**	226.5	108	**110**	118
gb|Mouse hepatocyte nuclear factor 1 (HNF-1) mRNA, complete cds. [M37966]	**Hnf1a**	5709.5	693	**4929**	6185
ref|Mus musculus hepatic nuclear factor 4, alpha (Hnf4a), mRNA [NM_008261]	**Hnf4a**	2947	90.5	**385**	404
ref|Mus musculus pancreatic and duodenal homeobox 1 (Pdx1), mRNA [NM_008814]	**Pdx1**	137	102	**201.5**	198.5
ref|Mus musculus neurogenic differentiation 1 (Neurod1), mRNA [NM_010894]	**Neurod1**	87	76	**88**	78.5
ref|Mus musculus ISL1 transcription factor, LIM/homeodomain (Isl1), mRNA [NM_021459]	**Isl1**	92	88.5	**93**	239
ref|Mus musculus NK2 transcription factor related, locus 2 (Drosophila) (Nkx2-2), mRNA [NM_010919]	**Nkx2-2**	100	105	**158**	76
ref|Mus musculus NK2 transcription factor related, locus 6 (Drosophila) (Nkx2-6), mRNA [NM_010920]	**Nkx2-6**	159	93	**94**	99
ref|Mus musculus paired box gene 6 (Pax6), mRNA [NM_013627]	**Pax6**	157	88.5	**100.5**	114
ref|Mus musculus insulin II (Ins2), mRNA [NM_008387]	**Ins2**	195.5	113183	**107.5**	85.5
ref|Mus musculus solute carrier family 2 (facilitated glucose transporter), member 2 (Slc2a2), mRNA [NM_031197]	**Slc2a2**	1212.5	100	**188**	143
ref|Mus musculus pyruvate kinase liver and red blood cell (Pklr), transcript variant 1, mRNA [NM_013631]	**Pklr**	237	124.5	**288**	186
ref|Mus musculus glucokinase (Gck), mRNA [NM_010292]	**Gck**	1771	184	**261**	162

Figure 17A


Early endocrine markers
EGFRA
EGFRAN
EGFRA 2days +N
EGFRAN 2days -N
Sox9
Hnf1b
Hnf6
Hnf1a
Nkx2.2
Nkx6.1
Pdx1
mRNA levels (Arbitrari units)
2 4 days
Ductal markers
Keratin 7
Keratin 19
EGFRA
EGFRAN
2 days
2 days
4 days
EGFRA
EGFRA + N
EGFRAN
EGFRA - N

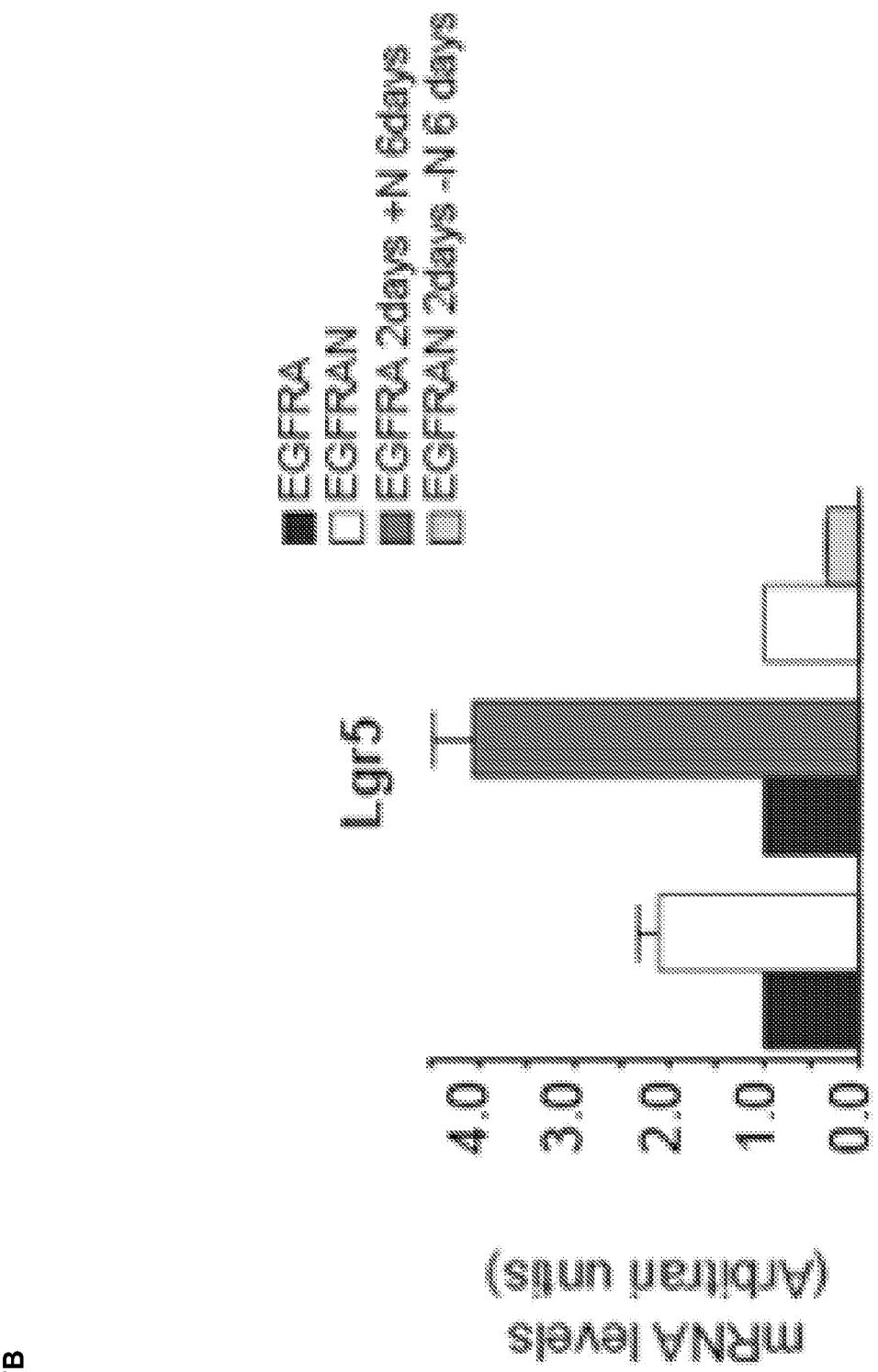

EGFRA
EGFRAN
EGFRA 2days +N 6days
EGFRAN 2days -N 6 days
Lgr5
mRNA levels
(Arbitran units)
4.0
3.0
2.0
1.0
0.0

Figure 17B

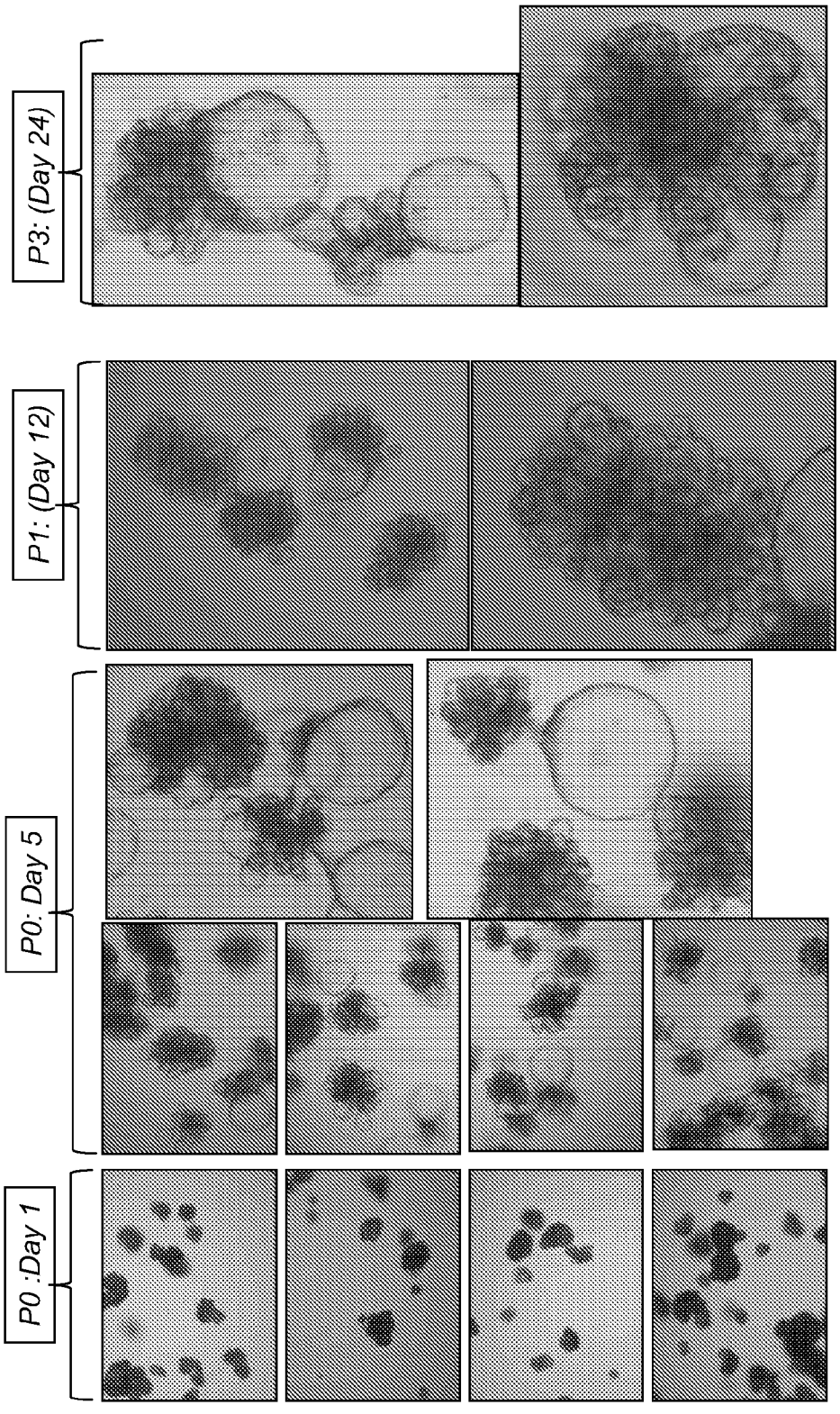
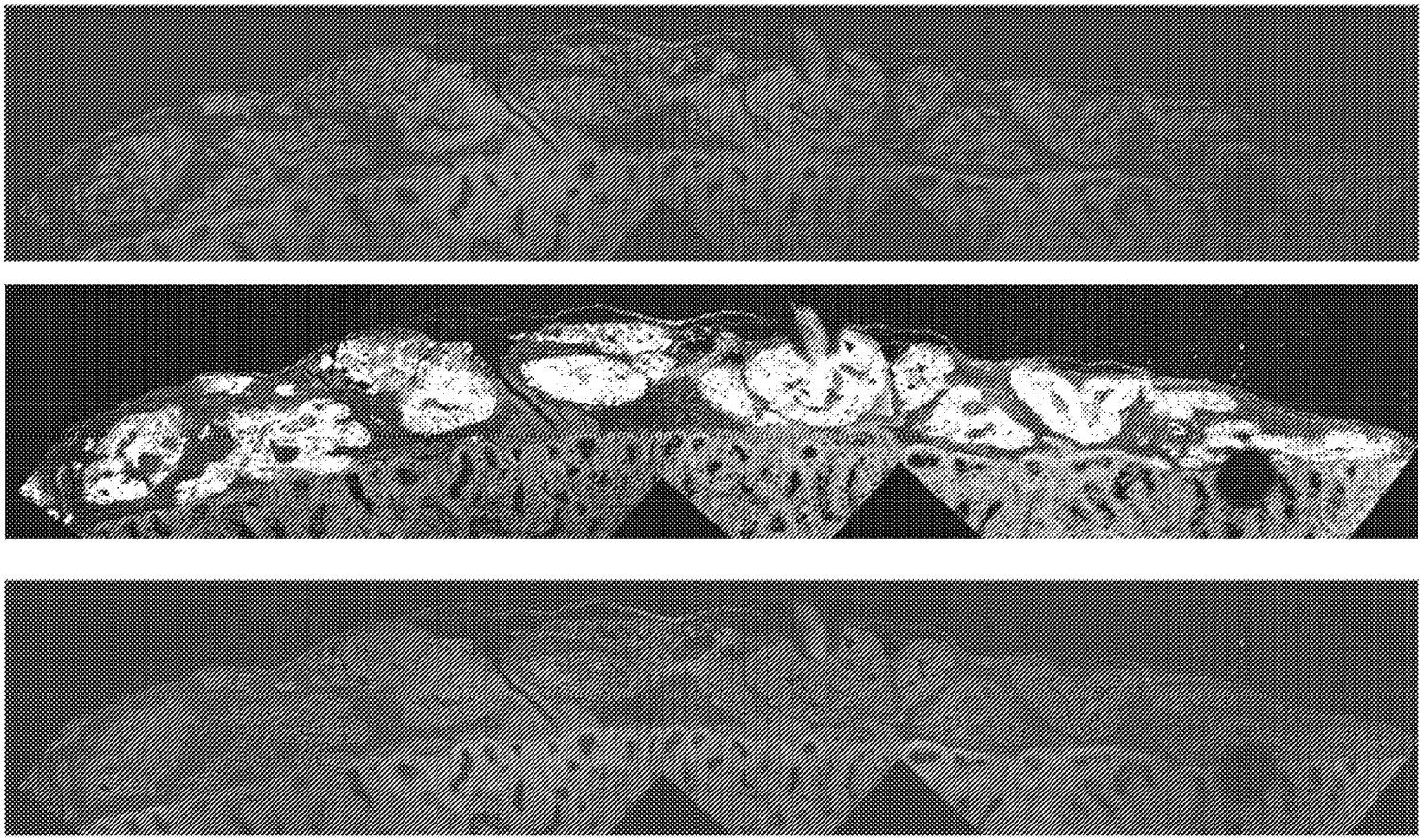


Figure 18A

Figure 18B

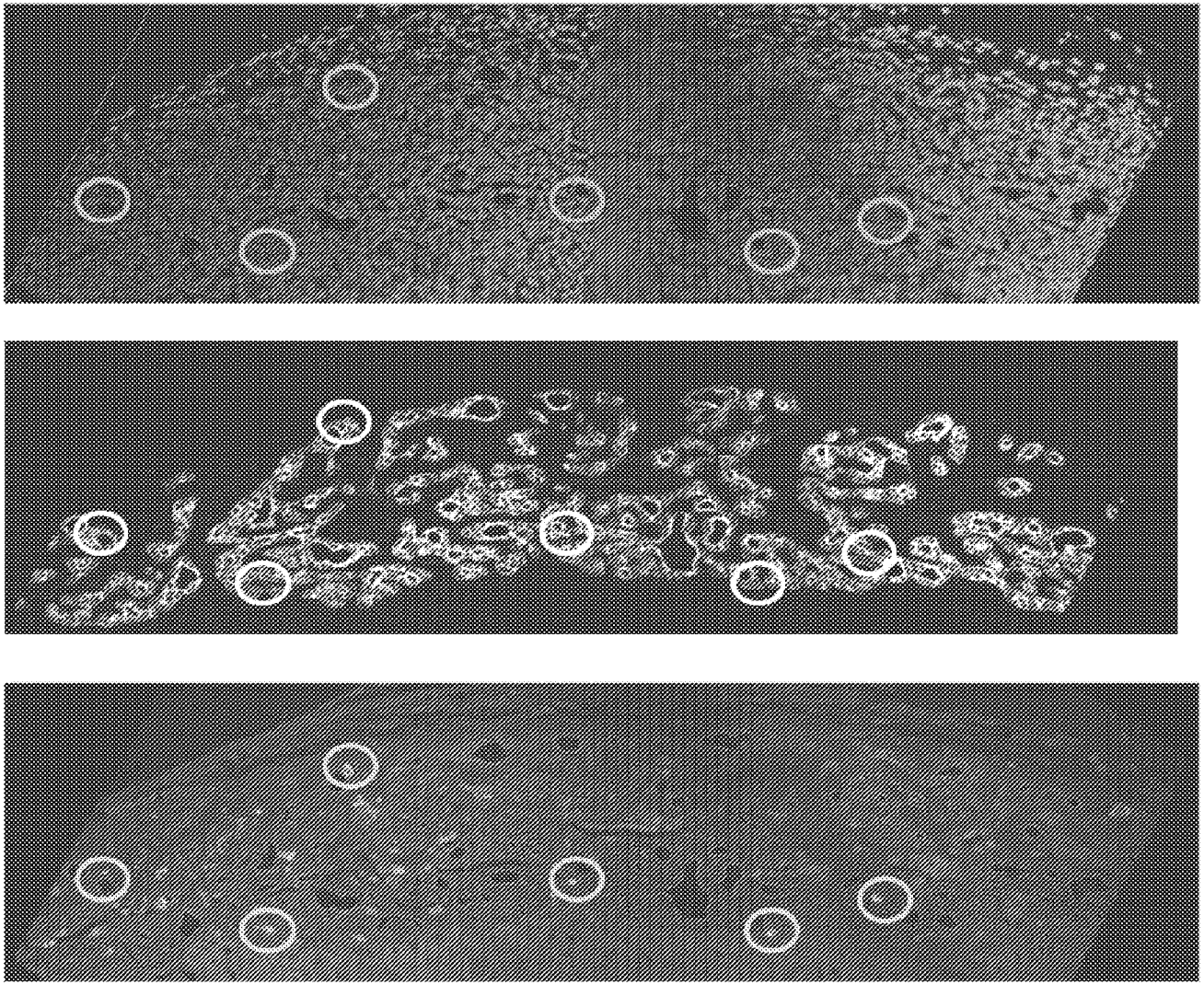


DAPI (nuclear marker) staining; indicates all cells.

CK19 (ductal marker); indicates all transplanted cells.

Insulin staining.

Figure 18C



DAPI (nuclear marker) staining; indicates all cells.

CK19 (ductal marker); indicates all transplanted cells

Insulin-producing cells

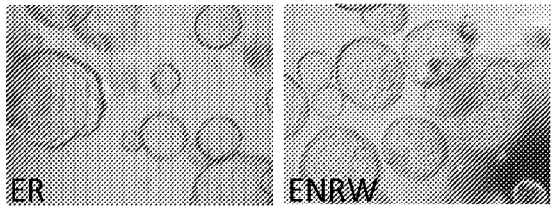
Figure 19

background cutoff >??fold; dyeswap to Cy3/Cy5 (Cy3-samples; Cy5-reference RNA)				Adult pancreas	Pancreas organoid	Liver organoid ER
FeatureNum	**ProbeName**	**Description**	**GeneName**	**gMedianSignal**	**gMedianSignal**	**gMedianSignal**
5886	A_52_P242199	gb|Mus musculus 12 days embryo spinal ganglion cDNA, RIKEN full-length enriched library, clone:D130061E05 product:unclassifiable, full insert sequence. [AK051635]	**Aaas**	84	98473	92
15415	A_51_P406105	ref|Mus musculus ribosomal protein S4, Y-linked 2 (Rps4y2), non-coding RNA [NR_003634]	**Rps4y2**	408	1980	127
14485	A_51_P189121	ref|Mus musculus ATPase, Ca++ transporting, type 2C, member 2 (Atp2c2), mRNA [NM_026922]	**Atp2c2**	1606	2198	144
35066	A_52_P306305	ref|Mus musculus A kinase (PRKA) anchor protein 2 (Akap2), transcript variant 1, mRNA [NM_001035533]	**Akap2**	333	1718	115
3713	A_51_P334689	ref|Mus musculus urotensin 2 (Uts2), mRNA [NM_011910]	**Uts2**	72	1563	112
39077	A_52_P279368	ref|Mus musculus SRY-box containing gene 17 (Sox17), mRNA [NM_011441]	**Sox17**	75	658	88
38112	A_51_P209122	ref|Mus musculus anterior gradient 2 (Xenopus laevis) (Agr2), mRNA [NM_011783]	**Agr2**	160	467	79

Figure 20

Mouse liver culture- 24months old

A



B

n chromosomes	counts
40	10/15
80	2/15
42	1/15
36	1/15
85	1/15

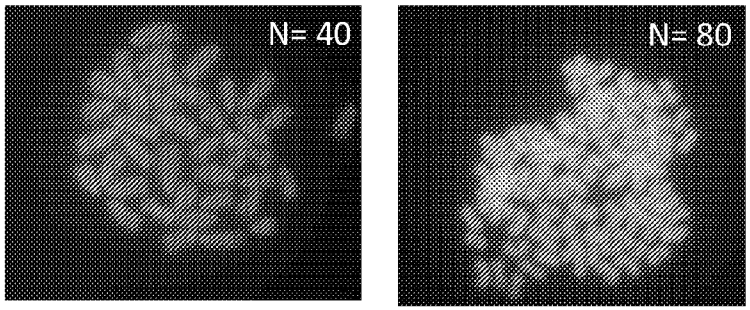
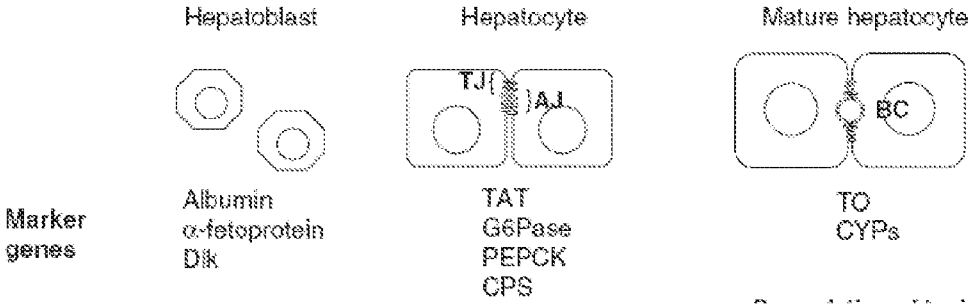


Figure 21

A


Hepatoblast
Hepatocyte
Mature hepatocyte
TJ
AJ
BC
Marker genes
Albumin
α-fetoprotein
Dlk
TAT
G6Pase
PEPCK
CPS
TO
CYPs

B

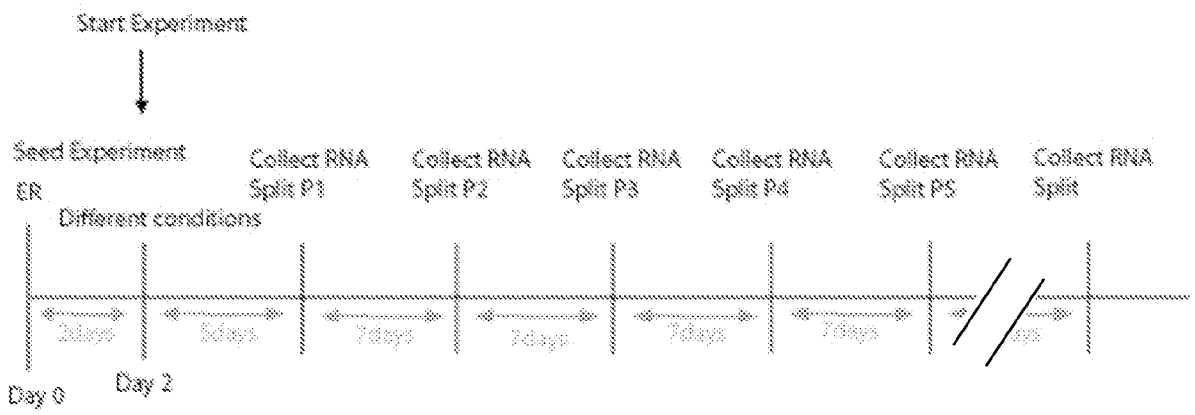

Start Experiment
Seed Experiment
ER
Different conditions
Collect RNA Split P1
Collect RNA Split P2
Collect RNA Split P3
Collect RNA Split P4
Collect RNA Split P5
Collect RNA Split
2days
5days
7days
7days
7days
7days
Day 0
Day 2

Figure 21

C After first split

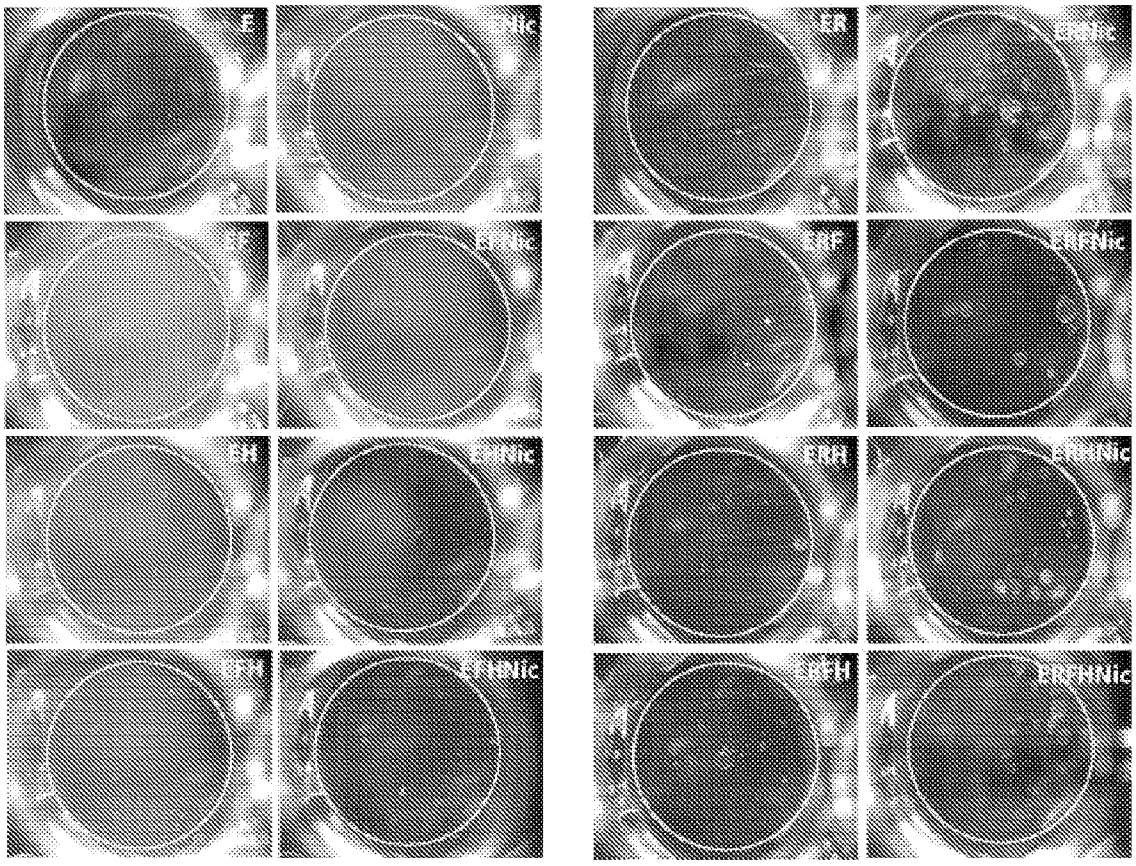
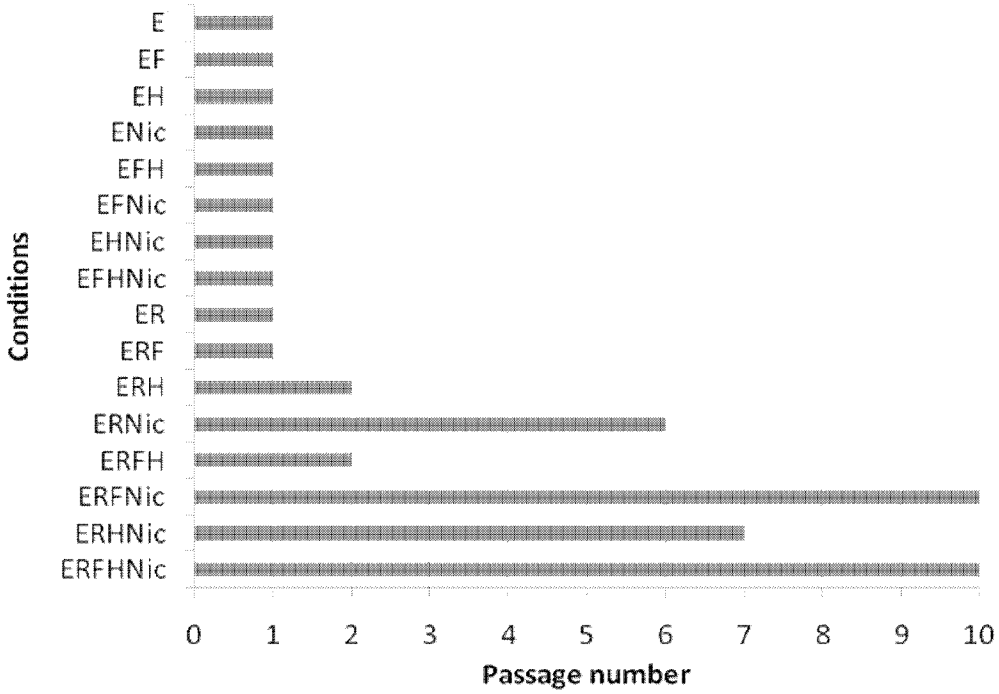
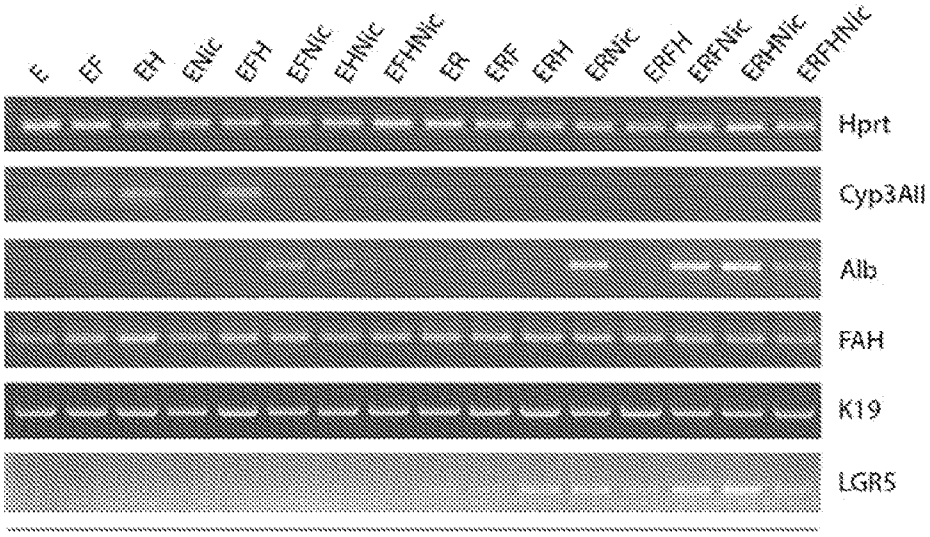


Figure 21

D



E



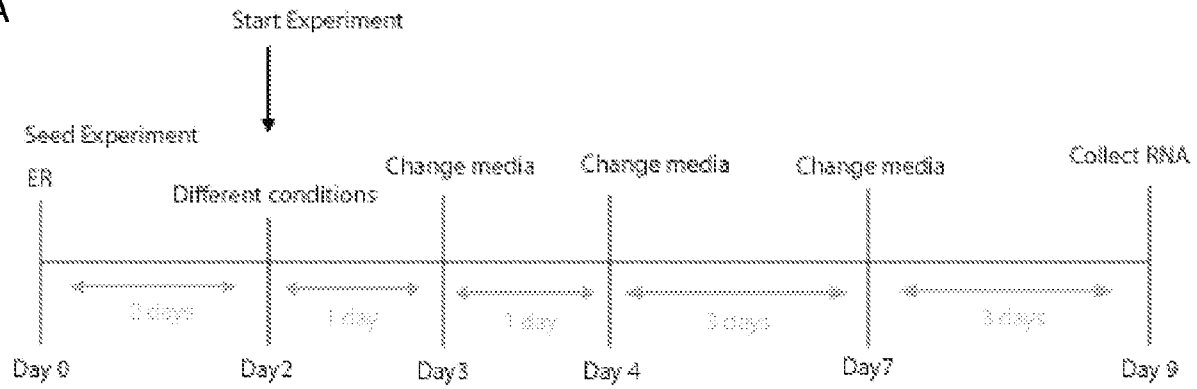
5 days after differentiation

Figure 22

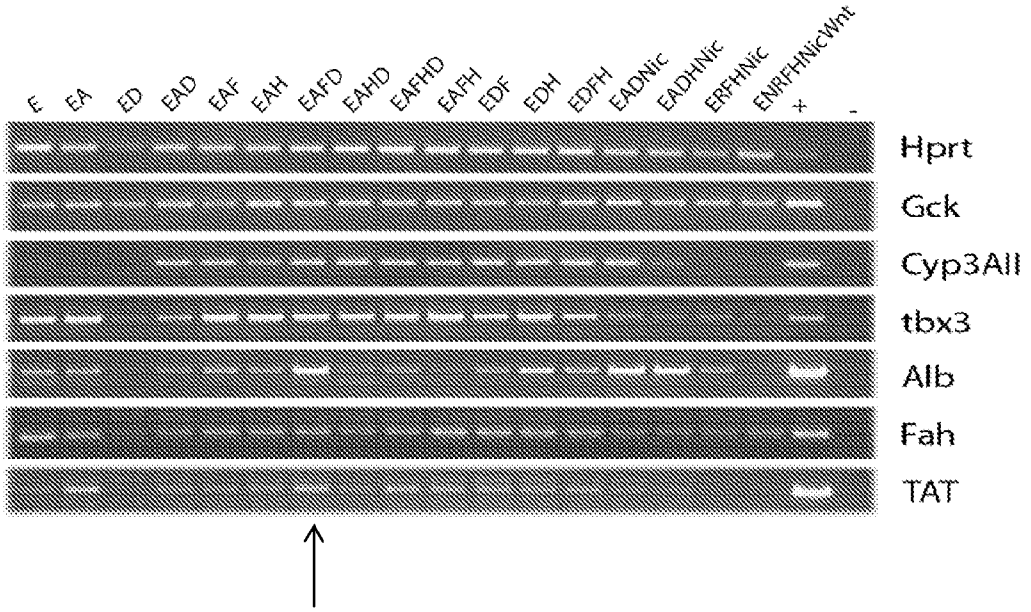
Bile duct specific TF	E	ER	ER	ENRW	Mouse adult liver
Hnf1b	3118,5	2357	2682	2770,5	361
Onecut2	34223,5	13172	21655	14926,5	6826
Onecut1	6435	2473,5	4083	2721	1038,5
Foxa2	16123	10962,5	10970	11872	5788
Hes1	1752	965	1453,5	1018	193
Sox9	4861,5	7490	6738,5	7547	426
HC specific TF					
Gata6	4981,5	5381	5124	5184	740
Cebpa	1431	1188	856	946	10640
Hnf1a	11640	10980	9865	6176	8334
Hnf4a	2573	1336	1283	1086	4033
Prox1	495	219,5	379,5	319	2139
Tbx3	196,5	214	208	180	1840,5
Nr5a2	132	139	131,5	141	1234
Notch signaling					
Jag1	**6832,5**	**3638,5**	**4111**	**3394**	**343**
Dll1	4035	3758	2791,5	2714,5	415,5
Notch3	557	718	476	788	234
TGFb signaling					
Tgfbr1	3439	2971	4281	3664	733
Tgfbr1	245	234	243	210	116
Tgfbr2	8442	9942	13874	11392	1364

Figure 23

A



B



E=EGF 50ng/ml

A=A8301 50nM

D=DAPT 10nM

F=FGF10 100ng/ml

H=HGF 50ng/ml

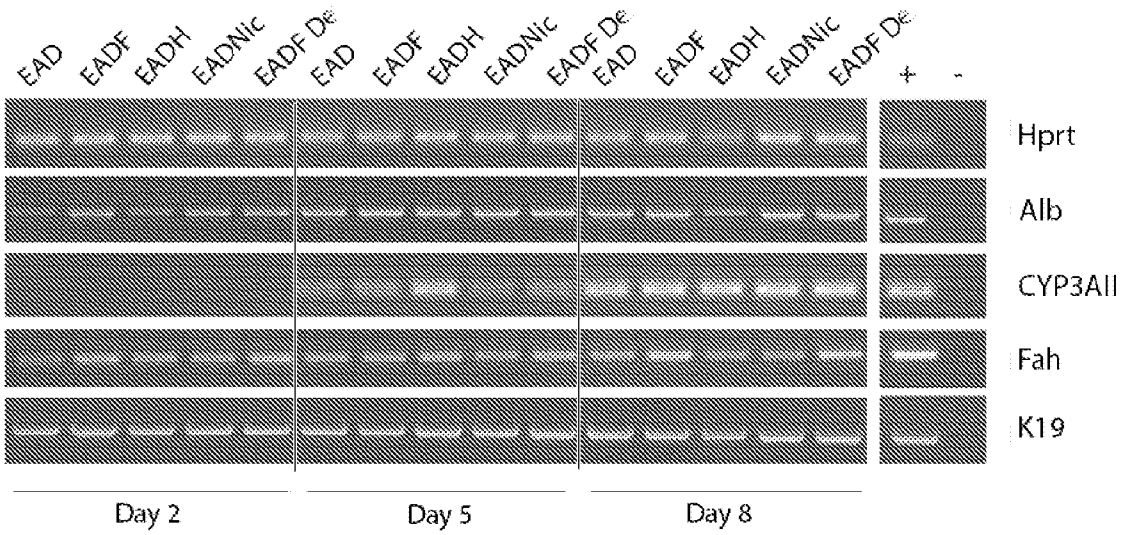
Nic=Nicotinamide 10nM

R=Rspondin1 500ng/ml

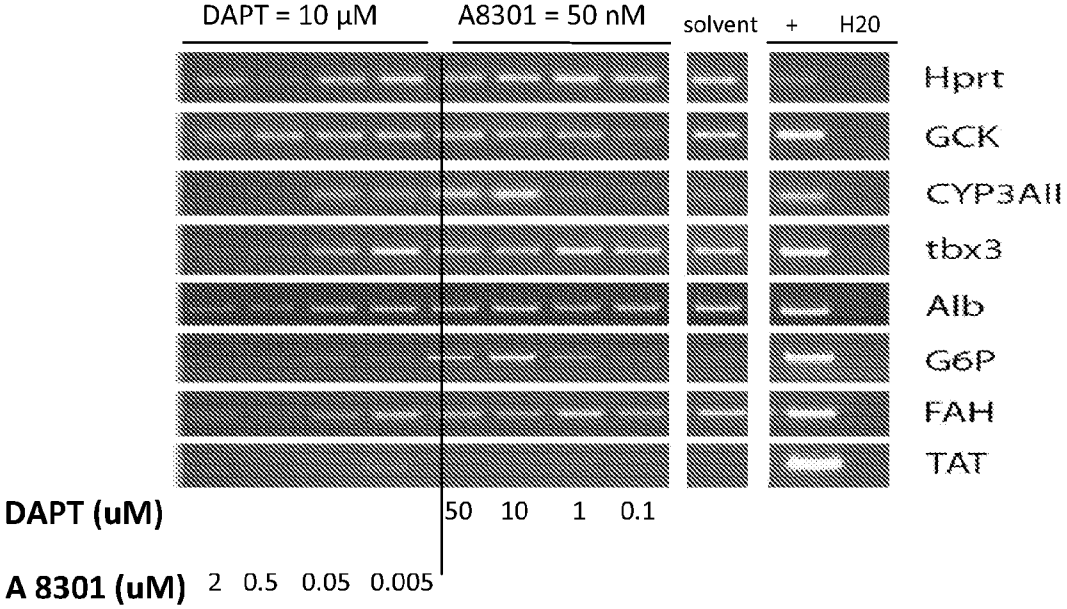
Wnt= Wnt conditioned media 50%

Figure 23

C



D



E=EGF 50ng/ml

A=A8301 50nM

D=DAPT 10nM

F=FGF10 100ng/ml

H=HGF 50ng/ml

Nic=Nicotinamide 10nM

R=Rspondin1 500ng/ml

Wnt= Wnt conditioned media 50%

De= dexamethasone 10mM

Figure 23

E Staining for liver markers after 8 days of differentiation condition EADF

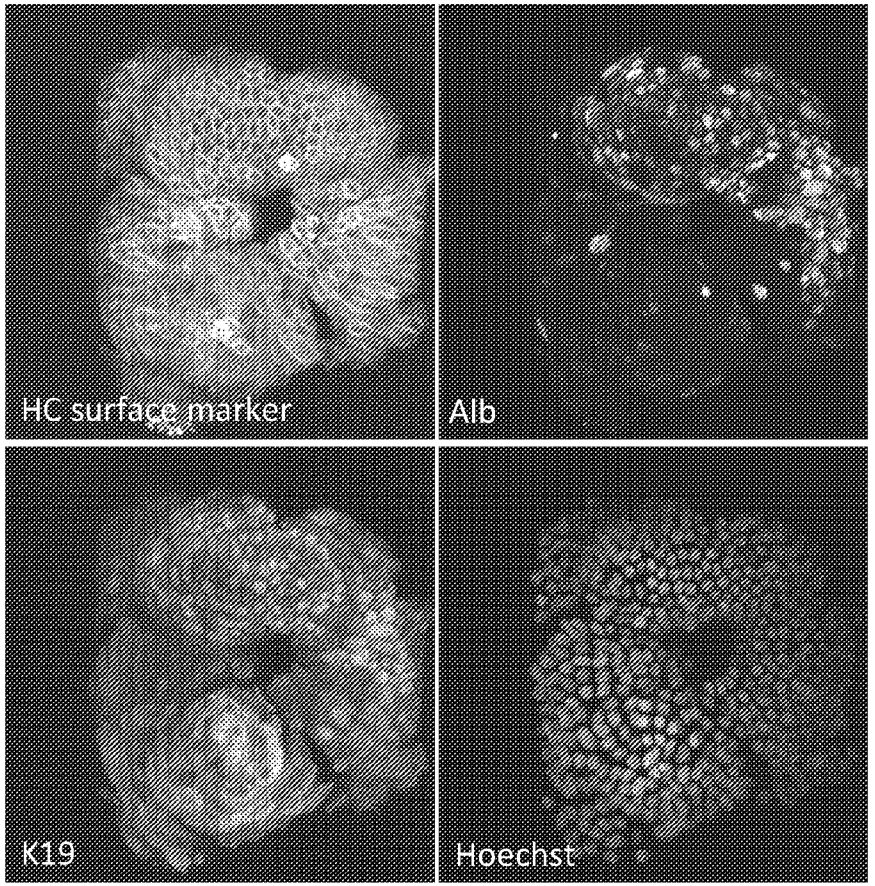
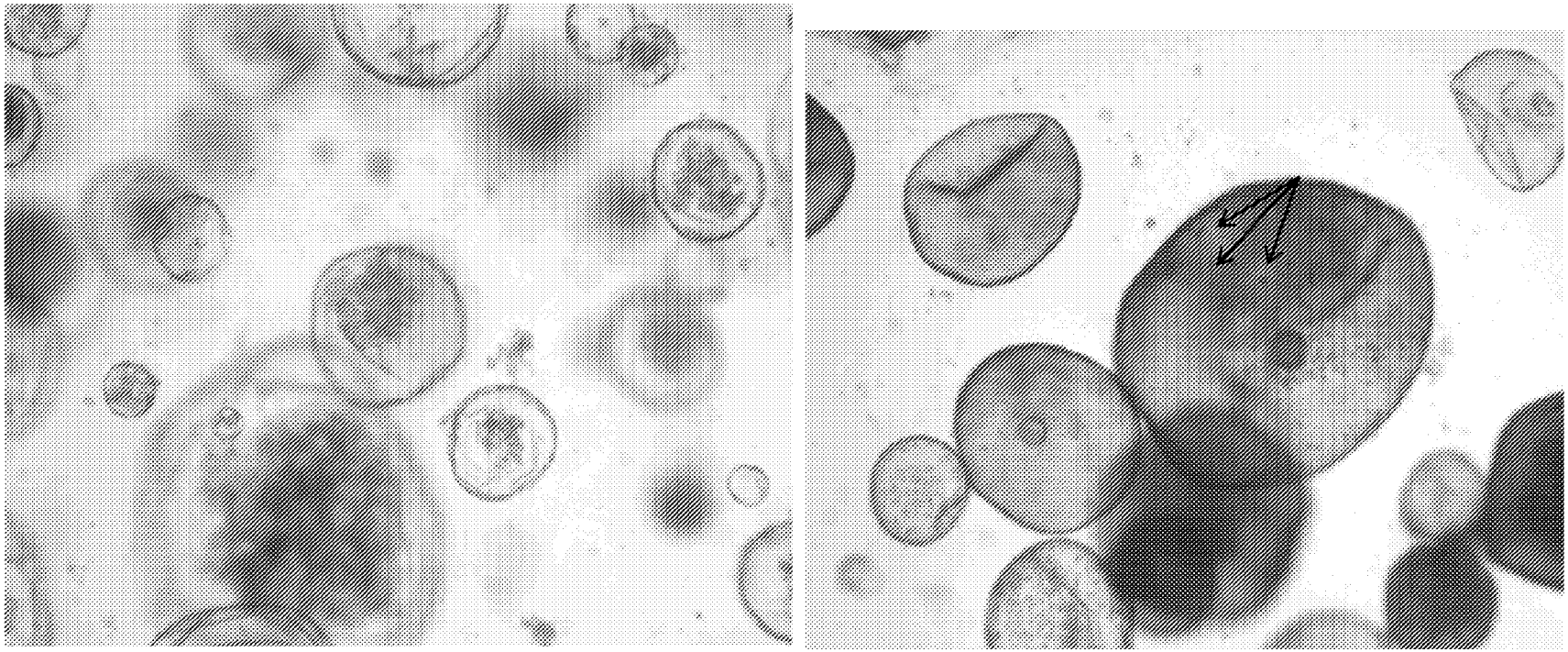


Figure 23

F Albcreert2LacZ derived organoids, (tamoxifen inducible system)



Control not induced with tamoxifen

Tamoxifen induced culture

Figure 24

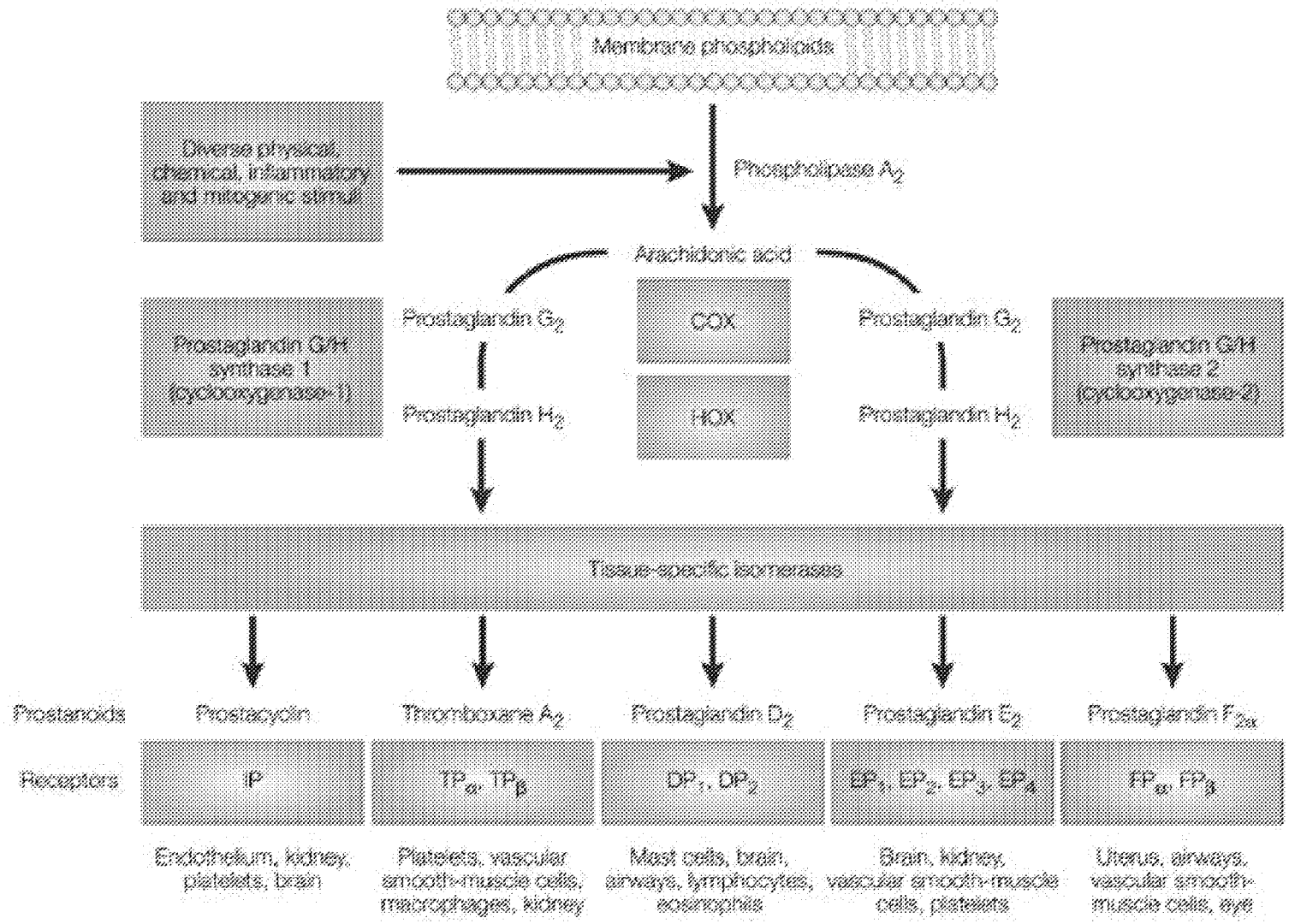
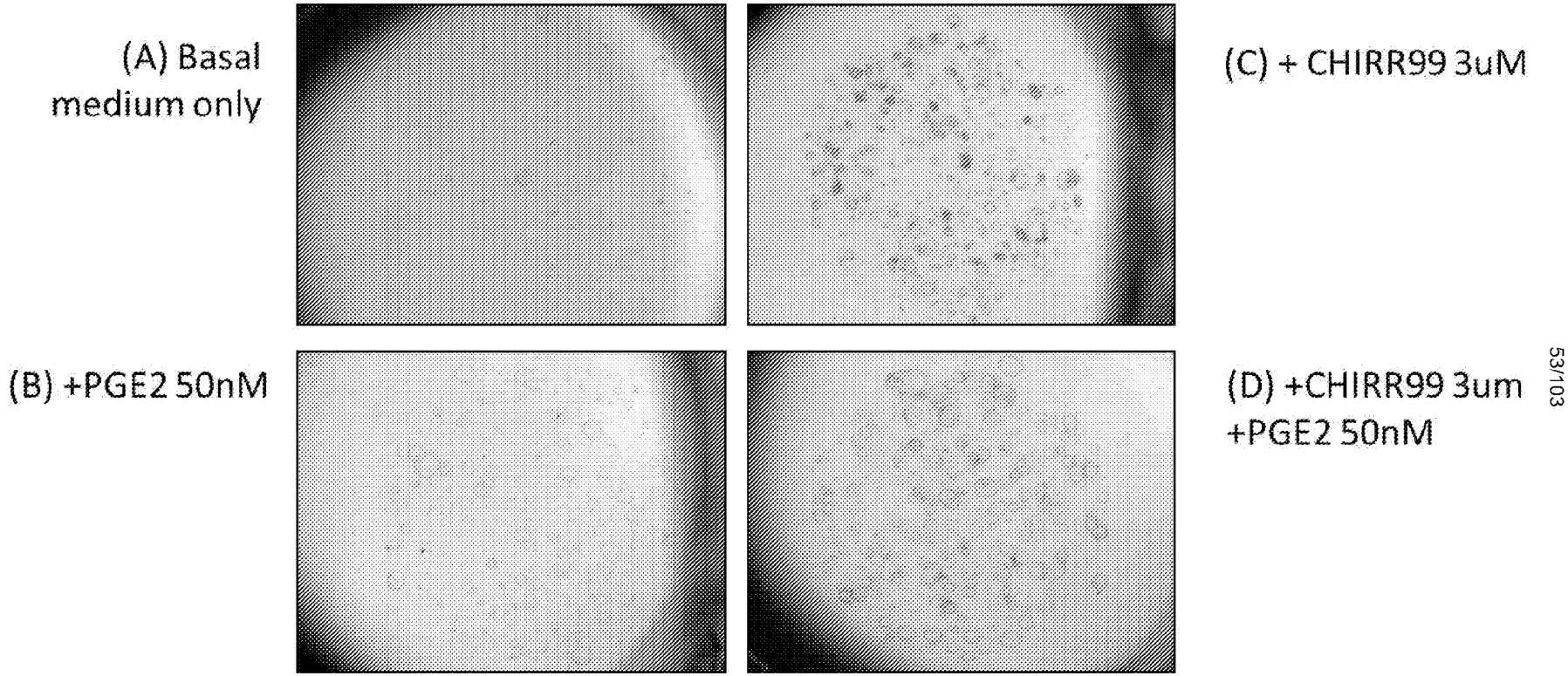

Membrane phospholipids
Diverse physical, chemical, inflammatory and mitogenic stimuli
Phospholipase A_2
Arachidonic acid
Prostaglandin G/H synthase 1 (cyclooxygenase-1)
Prostaglandin G_2
Prostaglandin H_2
COX
HOX
Prostaglandin G_2
Prostaglandin H_2
Prostaglandin G/H synthase 2 (cyclooxygenase-2)
Tissue-specific isomerases
Prostanoids
Prostacyclin
Thromboxane A_2
Prostaglandin D_2
Prostaglandin E_2
Prostaglandin $F_{2\alpha}$
Receptors
IP
TP_α, TP_β
DP_1, DP_2
EP_1, EP_2, EP_3, EP_4
FP_α, FP_β
Endothelium, kidney, platelets, brain
Platelets, vascular smooth-muscle cells, macrophages, kidney
Mast cells, brain, airways, lymphocytes, eosinophils
Brain, kidney, vascular smooth-muscle cells, platelets
Uterus, airways, vascular smooth-muscle cells, eye

Figure 25


(A) Basal medium only
(C) + CHIRR99 3uM
(B) +PGE2 50nM
(D) +CHIRR99 3um +PGE2 50nM

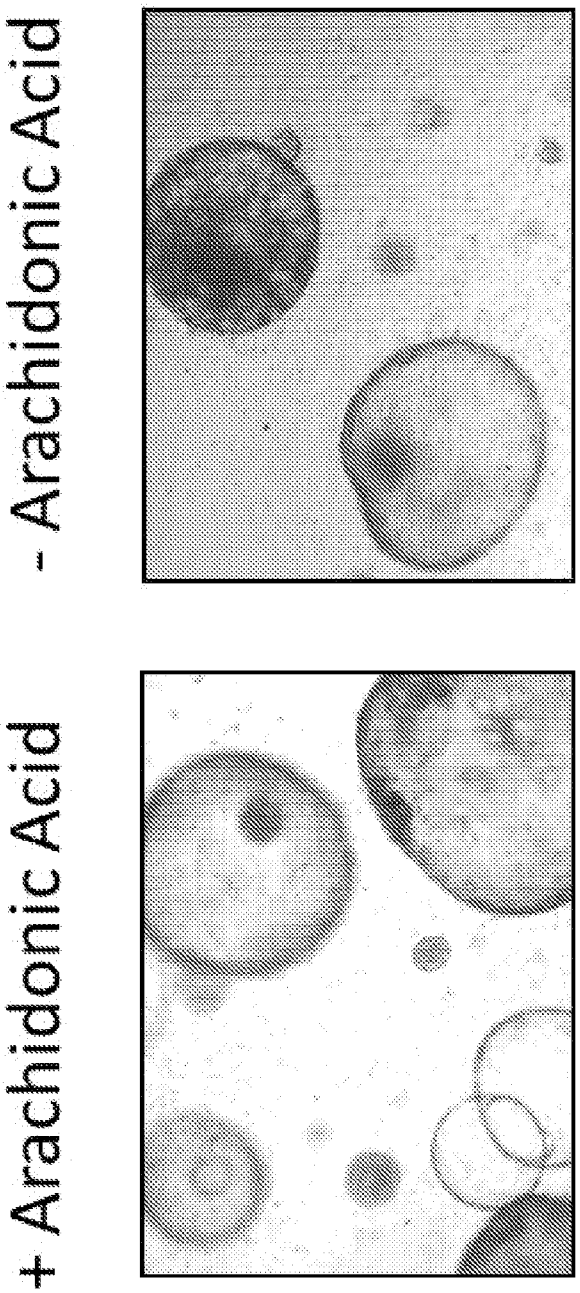


Figure 26

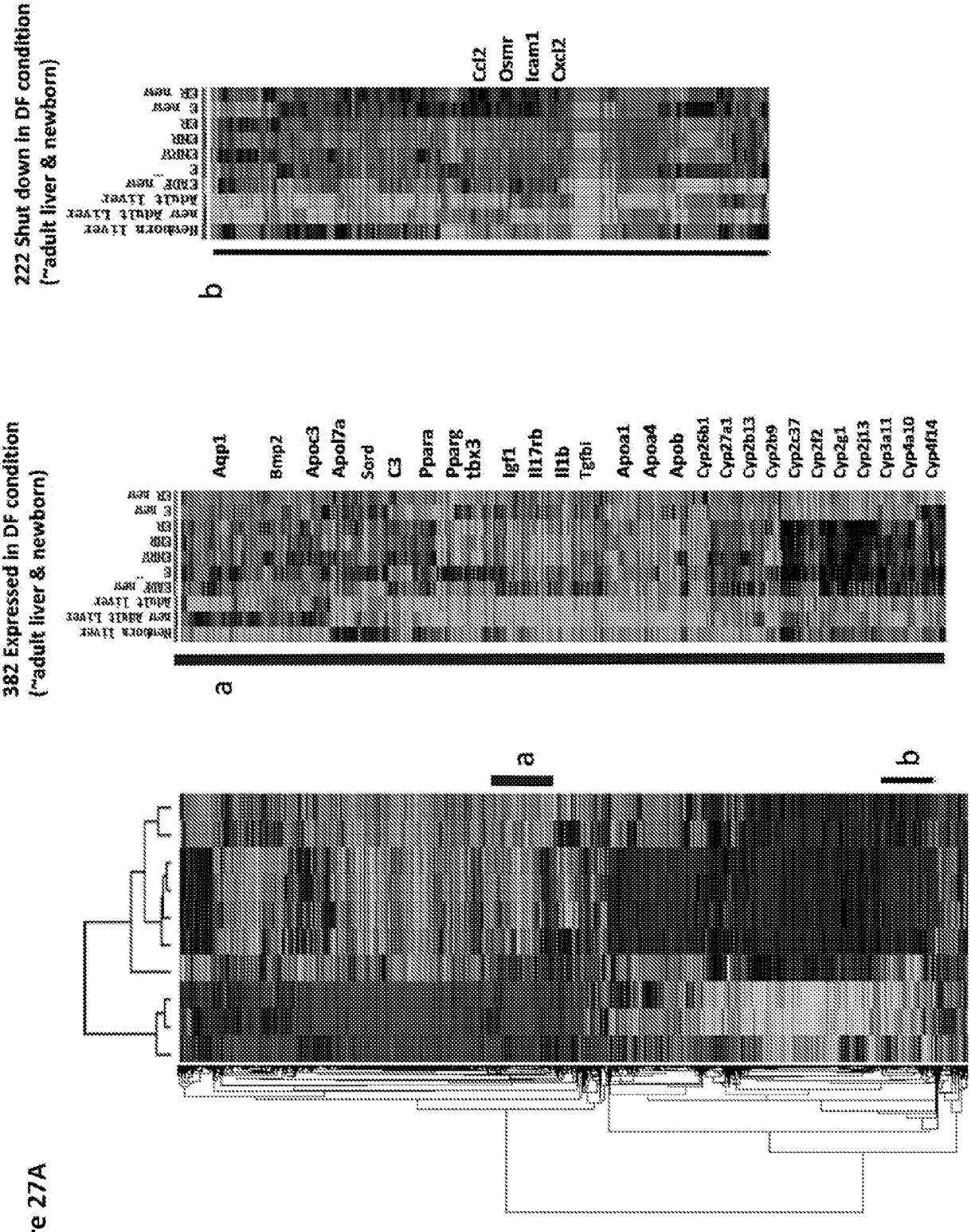

382 Expressed in DF condition
("adult liver & newborn)
222 Shut down in DF condition
("adult liver & newborn)
a
b
Aqp1
Bmp2
Apoc3
Apol7a
Sord
C3
Ppara
Pparg
tbx3
Igf1
Il17rb
Il1b
Tgfbi
Apoa1
Apoa4
Apob
Cyp26b1
Cyp27a1
Cyp2b13
Cyp2b9
Cyp2c37
Cyp2f2
Cyp2g1
Cyp2j13
Cyp3a11
Cyp4a10
Cyp4f14
Ccl2
Osmr
Icam1
Cxcl2
Newborn Liver
new Adult Liver
Adult Liver
E18F new
E18F
E18M
E18
E new
E18 new

Figure 27A

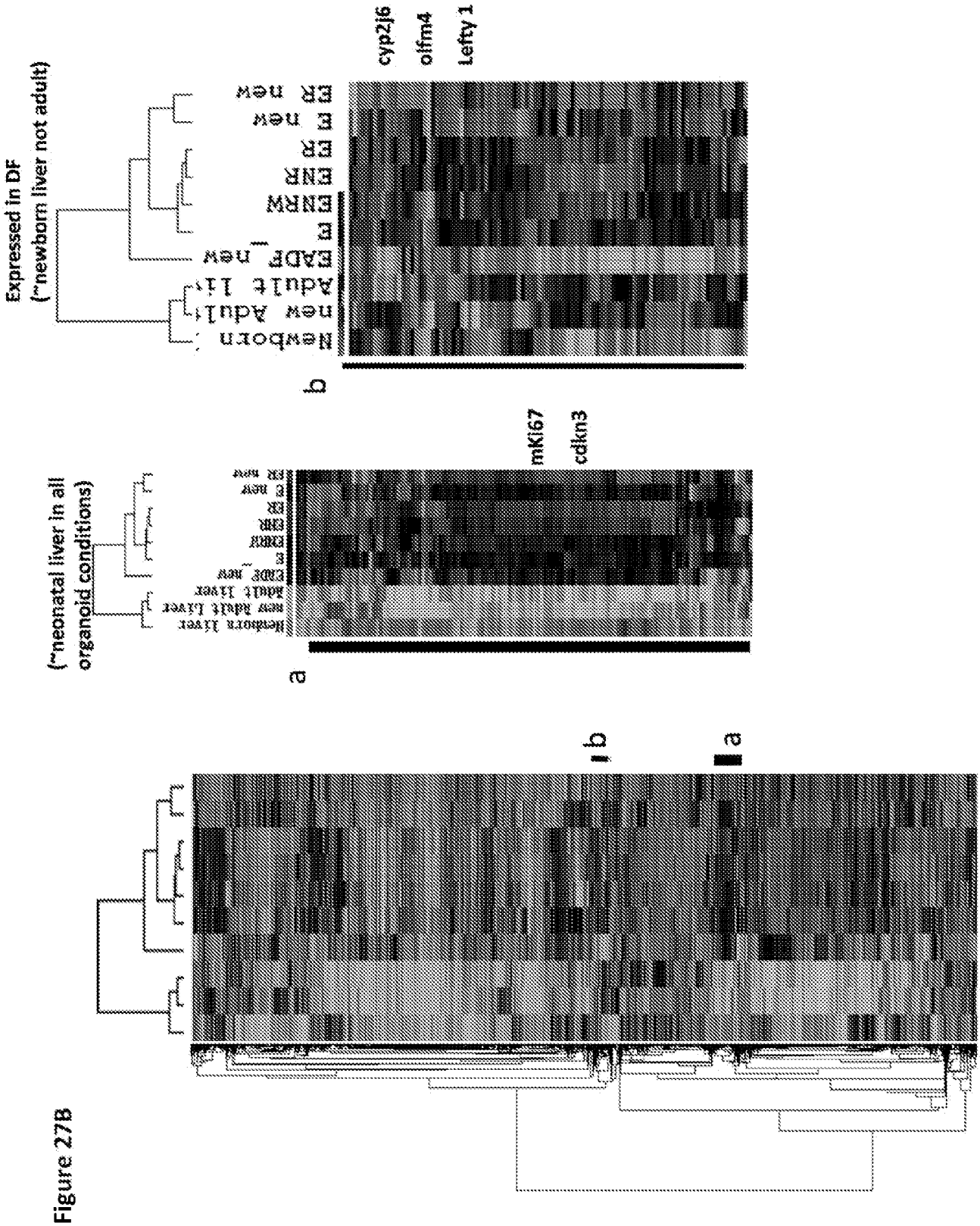

Expressed in DF
(~newborn liver not adult)
cyp2j6
olfm4
Lefty 1
Newborn
new Adult
Adult li
EADF_new
E
ENRW
ENR
ER
E new
ER new
b
(~neonatal liver in all
organoid conditions)
mKi67
cdkn3
a
b
a

Figure 27B

Figure 27C

Ductal markers		Adult liver	Adult pancreas	Pancreas organoid	Liver organoid
ref|Mus musculus leucine aminopeptidase 3 (Lap3), mRNA [NM_024434]	**Lap3**	11826	922	**3286**	2635
ref|Mus musculus keratin 19 (Krt19), mRNA [NM_008471]	**Krt19**	302	566	**76191**	29904
ref|Mus musculus keratin 7 (Krt7), mRNA [NM_033073]	**Krt7**	237	369	**147227**	108352
ref|Mus musculus SRY-box containing gene 9 (Sox9), mRNA [NM_011448]	**Sox9**	235	185	**2365**	2492
ref|Mus musculus HNF1 homeobox B (Hnf1b), mRNA [NM_009330]	**Hnf1b**	234	115	**608**	764
ref|Mus musculus one cut domain, family member 1 (Onecut1), mRNA [NM_008262]	**Hnf6**	1239	120	**1297**	1454
Transcription Factors necessary for Ngn3 expression					
ref|Mus musculus SRY-box containing gene 9 (Sox9), mRNA [NM_011448]	**Sox9**	235	185	**2365**	2492
ref|Mus musculus forkhead box A2 (Foxa2), mRNA [NM_010446]	**Foxa2**	8695	1415	**12948**	9579
ref|Mus musculus HNF1 homeobox B (Hnf1b), mRNA [NM_009330]	**Hnf1b**	234	115	**608**	764
ref|Mus musculus one cut domain, family member 1 (Onecut1), mRNA [NM_008262]	**Hnf6**	1239	120	**1297**	1454
Endocrine markers					
ref|Mus musculus neurogenin 3 (Neurog3), mRNA [NM_009719]	**Neurog3**	226.5	108	**110**	118
gb|Mouse hepatocyte nuclear factor 1 (HNF-1) mRNA, complete cds. [M57966]	**Hnf1a**	5709.5	693	**4929**	6185
ref|Mus musculus hepatic nuclear factor 4, alpha (Hnf4a), mRNA [NM_008261]	**Hnf4a**	2947	90.5	**385**	404
ref|Mus musculus pancreatic and duodenal homeobox 1 (Pdx1), mRNA [NM_008814]	**Pdx1**	137	102	**201.5**	158.5
ref|Mus musculus neurogenic differentiation 1 (Neurod1), mRNA [NM_010894]	**Neurod1**	87	76	**88**	78.5
ref|Mus musculus ISL1 transcription factor, LIM/homeodomain (Isl1), mRNA [NM_021459]	**Isl1**	82	88.5	**93**	239
ref|Mus musculus NK2 transcription factor related, locus 2 (Drosophila) (Nkx2-2), mRNA [NM_010919]	**Nkx2-2**	100	105	**158**	76
ref|Mus musculus NK2 transcription factor related, locus 6 (Drosophila) (Nkx2-6), mRNA [NM_010920]	**Nkx2-6**	159	93	**94**	99
ref|Mus musculus paired box gene 6 (Pax6), mRNA [NM_013627]	**Pax6**	157	88.5	**100.5**	114
ref|Mus musculus insulin II (Ins2), mRNA [NM_008387]	**Ins2**	195.5	113183	**107.5**	85.5
ref|Mus musculus solute carrier family 2 (facilitated glucose transporter), member 2 (Slc2a2), mRNA [NM_031197]	**Slc2a2**	1212.5	100	**188**	143
ref|Mus musculus pyruvate kinase liver and red blood cell (Pklr), transcript variant 1, mRNA [NM_013631]	**Pklr**	237	124.5	**288**	186
ref|Mus musculus glucokinase (Gck), mRNA [NM_010292]	**Gck**	1771	184	**261**	162

Figure 28

stem cell signature mouse liver		**liver/HC CG specific signature**		**reprograming genes**	
positive for	**negative for**	**positive for in expansion**	**negative for**	**positive**	**negative**
lgr5	lgr6	Hnf1a	afp	Klf4	Pou5f1
lgr4		Hnf1b	Ins1	Myc	Sox2
epcam		Hnf4a	Ins2		
Cd44		Hhex	Gcg		
Tnfrsf19		Onecut1	Ptf1a		
Sox9		Onecut2	Cela1		
Sp5		Prox1	Cela2a		
Cd24a		Cdh1	Cela3b		
Prom1		Foxa2	Neurod1		
Cdca7		Gata6	Neurod2		
Elf3		Foxm1	Neurog1		
		Cebpa	Neurog2		
		Cebpb	Neurog3		
		Cebpd	Amy2a4		
		Cebpg	Igf1r		
		Glul	Igf2		
		Krt7	Cd34		
		Krt19			
		Met			

Figure 29

*background cutoff >3.5fold (gMedian); dyeswap to Cy3/Cy5 (Cy3-samples; Cy5-reference RNA)				251485067708_1	251485067708_2	251485067708_3	251485067708_4	ENRW_pat10511	ER_pat10511	EADF_pat10511	liver tissue_pat10511
FeatureNum	**SystematicName**	**Description**	**GeneName**	ENRW_pat10511	ER_pat10511	EADF_pat10511	liver tissue_pat10511	**gMedianSignal**	**gMedianSignal**	**gMedianSignal**	**gMedianSignal**
Stem cell signature mouse liver											
40197	NM_003667	ref|Homo sapiens leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5), mRNA [NM_003667]	**LGR5**					85	82.5	83	80
40602	NM_018490	ref|Homo sapiens leucine-rich repeat-containing G protein-coupled receptor 4 (LGR4), mRNA [NM_018490]	**LGR4**	1.3710	1.5826	1.8852	1.1145	1602	1684	2689	190.5
4087	NM_002354	ref|Homo sapiens tumor-associated calcium signal transducer 1 (TACSTD1), mRNA [NM_002354]	**TACSTD1/Epcam**	3.0978	3.5264	4.2412	-3.2251	16341	23939	42144	118
18697	NM_000610	ref|Homo sapiens CD44 molecule (Indian blood group) (CD44), transcript variant 1, mRNA [NM_000610]	**CD44**	0.8742	0.8495	-1.5025	-3.8418	10117	11624.5	2586.5	148.5
31576	NM_018647	ref|Homo sapiens tumor necrosis factor receptor superfamily, member 19 (TNFRSF19), transcript variant 1, mRNA [NM_018647]	**TNFRSF19**					83	85.5	81	81
2504	NM_000346	ref|Homo sapiens SRY (sex determining	**SOX9**	2.2553	2.3538	1.5394	-2.0445	484	448.5	355	88

		region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal) (SOX9), mRNA [NM_000346]									
6370	NM_001003845	ref|Homo sapiens Sp5 transcription factor (SP5), mRNA [NM_001003845]	**SP5**	-0.0509	0.4409	0.0402	3.3653	162	217.5	199	239.5
40879	A_23_P85250	gb|Homo sapiens CD24 signal transducer mRNA, complete cds and 3' region. [L33930]	**CD24**	3.2316	3.5288	1.9579	-2.4236	15141.5	17882	7049	125
1705	NM_006017	ref|Homo sapiens prominin 1 (PROM1), mRNA [NM_006017]	**PROM1**	3.2512	2.6437	2.4209	-1.5556	445	340	346	84
43572	NM_031942	ref|Homo sapiens cell division cycle associated 7 (CDCA7), transcript variant 1, mRNA [NM_031942]	**CDCA7**	-2.4618	-3.6148	-7.7368	-5.0979	476	218	87	83
1226	NM_004433	ref|Homo sapiens E74-like factor 3 (ets domain transcription factor, epithelial-specific) (ELF3), transcript variant 1, mRNA [NM_004433]	**ELF3**	3.3588	3.9163	4.2213	0.4499	6378.5	8700.5	14527	161
Reprogramming genes											
19017	NM_004235	ref|Homo sapiens Kruppel-like factor 4 (gut) (KLF4), mRNA [NM_004235]	**KLF4**	0.3754	2.3397	1.9646	-0.9306	291	1176.5	919	105
6084	NM_002467	ref|Homo sapiens v-myc myelocytomatosis viral oncogene homolog (avian) (MYC), mRNA [NM_002467]	**MYC**	-1.1416	-0.8254	-3.3457	-2.1032	3006	3896	871	323
11740	NM_002701	ref|Homo sapiens POU class 5 homeobox 1 (POU5F1), transcript variant 1, mRNA [NM_002701]	**POU5F1**	-3.9024	-3.5281	-3.8577	-1.8681	1643	2251.5	2261	1173
34630	NM_003106	ref|Homo sapiens SRY (sex determining region Y)-box 2 (SOX2), mRNA [NM_003106]	**SOX2**	-4.0411	-4.0509	-4.3534	-2.6317	131	131.5	129	98

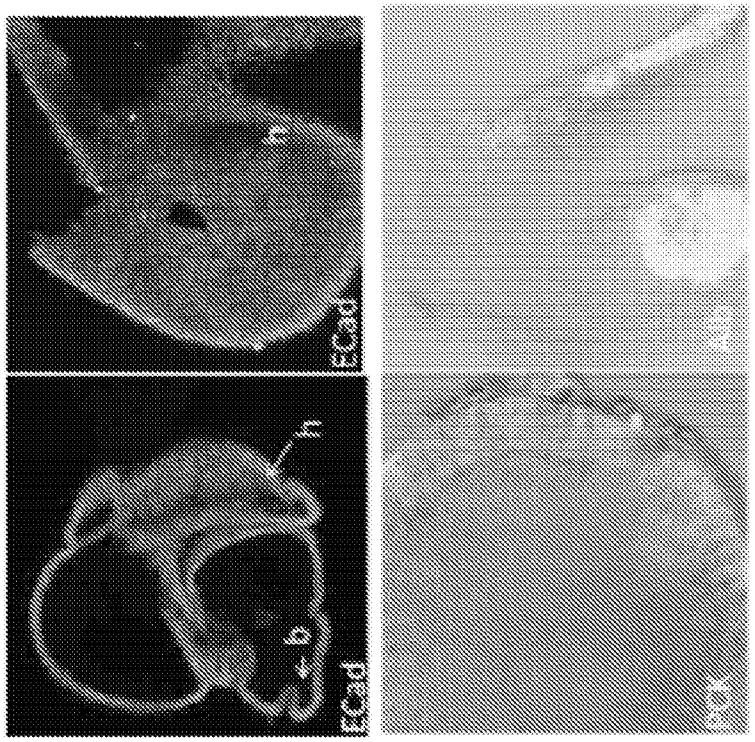
Liver/HC CG specific signature: positive for expansion											
22258	NM_000545	ref|Homo sapiens HNF1 homeobox A (HNF1A), mRNA [NM_000545]	**HNF1A**	0.1593	0.4237	0.3115	2.1337	287.5	366.5	367.5	240
19971	NM_000458	ref|Homo sapiens HNF1 homeobox B (HNF1B), mRNA [NM_000458]	**HNF1B**	3.3524	3.3315	3.1792	0.0000	200.5	200	141	86
7418	NM_000457	ref|Homo sapiens hepatocyte nuclear factor 4, alpha (HNF4A), transcript variant 2, mRNA [NM_000457]	**HNF4A**	-0.9981	1.7168	1.0493	2.0374	84.5	119.5	108	103
25738	NM_002729	ref|Homo sapiens hematopoietically expressed homeobox (HHEX), mRNA [NM_002729]	**HHEX**	0.8347	0.9290	1.4544	0.2789	1025	1263	1931	178
28260	NM_004498	ref|Homo sapiens one cut homeobox 1 (ONECUT1), mRNA [NM_004498]	**ONECUT1**	0.5189	2.1647	2.9438	5.8053	120	211	327	387
30379	NM_004852	ref|Homo sapiens one cut homeobox 2 (ONECUT2), mRNA [NM_004852]	**ONECUT2**	3.4671	3.7373	3.5642	2.8300	4693	6965	6460	498
20882	NM_002763	ref|Homo sapiens prospero homeobox 1 (PROX1), mRNA [NM_002763]	**PROX1**	2.4930	1.0227	1.1415	2.4817	98	98	97.5	101
12860	NM_004360	ref|Homo sapiens cadherin 1, type 1, E-cadherin (epithelial) (CDH1), mRNA [NM_004360]	**CDH1**	3.3253	3.6614	4.1391	2.4935	10803	16648	25328	1076.5
33903	NM_021784	ref|Homo sapiens forkhead box A2 (FOXA2), transcript variant 1, mRNA [NM_021784]	**FOXA2**	2.8637	3.2434	3.0108	2.6774	289.5	306	398	129.5
39806	NM_005257	ref|Homo sapiens GATA binding protein 6 (GATA6), mRNA [NM_005257]	**GATA6**	3.0052	3.5536	3.8552	1.2789	1620	2038	3585	136
16668	NM_202002	ref|Homo sapiens forkhead box M1 (FOXM1), transcript variant 1, mRNA [NM_202002]	**FOXM1**	-1.9203	-3.6288	-5.0418	-4.5112	329	156.5	117.5	94

9214	NM_004364	ref|Homo sapiens CCAAT/enhancer binding protein (C/EBP), alpha (CEBPA), mRNA [NM_004364]	**CEBPA**	-0.9322	1.2903	-0.0086	4.2303	304	1257	647	1586.5
14125	NM_005194	ref|Homo sapiens CCAAT/enhancer binding protein (C/EBP), beta (CEBPB), mRNA [NM_005194]	**CEBPB**	-0.1111	-0.2252	0.4127	2.5221	5257	5653.5	10124	5163.5
37458	NM_005195	ref|Homo sapiens CCAAT/enhancer binding protein (C/EBP), delta (CEBPD), mRNA [NM_005195]	**CEBPD**	-0.1615	0.9115	0.4583	4.1610	1525	3232	2733	2661
145	NM_001806	ref|Homo sapiens CCAAT/enhancer binding protein (C/EBP), gamma (CEBPG), mRNA [NM_001806]	**CEBPG**	0.9187	2.0261	1.8780	0.7494	2185	4317.5	4980	364
41435	NM_002065	ref|Homo sapiens glutamate-ammonia ligase (glutamine synthetase) (GLUL), transcript variant 1, mRNA [NM_002065]	**GLUL**	-0.1834	0.0758	-0.8704	0.4736	1365.5	1338	876	311
30469	NM_005556	ref|Homo sapiens keratin 7 (KRT7), mRNA [NM_005556]	**KRT7**	5.1396	4.8373	4.3723	-0.0401	7538.5	4712	2627	96
13920	NM_002276	ref|Homo sapiens keratin 19 (KRT19), mRNA [NM_002276]	**KRT19**	4.1830	3.7196	3.6914	-2.4731	365708	424094	308350	1571
5427	NM_000245	ref|Homo sapiens met proto-oncogene (hepatocyte growth factor receptor) (MET), mRNA [NM_000245]	**MET**	1.3614	1.0945	1.2510	-0.3692	7623.5	6639	8627	406
Liver/HC CG specific signature: negative											
16839	NM_001134	ref|Homo sapiens alpha-fetoprotein (AFP), mRNA [NM_001134]	**AFP**	-8.2963	-6.4426	-8.2926	-5.5894	95	141	107	103
33640	NM_002054	ref|Homo sapiens glucagon (GCG), mRNA [NM_002054]	**GCG**	0.0000	0.0000	0.6162	0.0000	85	90	88	78
34885	NP233946	gb|Homo sapiens bHLH transcription	**PTF1A**	0.1838	0.4898	0.4317	0.7839	105	111	112	93

		factor p48 mRNA, partial cds. [AF181999]									
40134	NM_024019	ref|Homo sapiens neurogenin 2 (NEUROG2), mRNA [NM_024019]	**NEUROG2**					73	79.5	81.5	75
5505	NM_000875	ref|Homo sapiens insulin-like growth factor 1 receptor (IGF1R), mRNA [NM_000875]	**IGF1R**					78.5	81	82.5	78
5632	NM_001773	ref|Homo sapiens CD34 molecule (CD34), transcript variant 2, mRNA [NM_001773]	**CD34**					74	70	75	79
Hepatocyte specific gene signature											
991	NM_000371	ref|Homo sapiens transthyretin (prealbumin, amyloidosis type I) (TTR), mRNA [NM_000371]	**TTR**	-0.5692	0.0924	0.1884	9.0077	1243.5	1660	2331	107880
23323	NM_000477	ref|Homo sapiens albumin (ALB), mRNA [NM_000477]	**ALB**	-3.0821	-2.9215	-3.2544	6.3642	5970	10841	7266	306897
10807	NM_000137	ref|Homo sapiens fumarylacetoacetate hydrolase (fumarylacetoacetase) (FAH), mRNA [NM_000137]	**FAH**	-0.1297	-0.5453	-0.2095	2.2811	716	520	783	618
29558	NM_000353	ref|Homo sapiens tyrosine aminotransferase (TAT), nuclear gene encoding mitochondrial protein, mRNA [NM_000353]	**TAT**	0.1829	0.3011	-0.8291	6.6911	97	101	95	381.5
2423	NM_000765	ref|Homo sapiens cytochrome P450, family 3, subfamily A, polypeptide 7 (CYP3A7), mRNA [NM_000765]	**CYP3A7**	5.5511	7.1260	6.6750	8.9567	3649	10927	9853	5989
1710	NM_000039	ref|Homo sapiens apolipoprotein A-I (APOA1), mRNA [NM_000039]	**APOA1**	-3.5478	-2.8913	-2.1400	5.2314	370	536	1014.5	24580

8511	NM_002130	ref|Homo sapiens 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble) (HMGCS1), transcript variant 2, mRNA [NM_002130]	**HMGCS1**	1.9834	1.8579	-1.2536	1.9013	1518	1248	264	271
2331	NM_138711	ref|Homo sapiens peroxisome proliferator-activated receptor gamma (PPARG), transcript variant 3, mRNA [NM_138711]	**PPARG**	1.9376	3.5421	1.0259	-0.9723	794	2235	489	102
42261	NM_000767	ref|Homo sapiens cytochrome P450, family 2, subfamily B, polypeptide 6 (CYP2B6), mRNA [NM_000767]	**CYP2B6**	3.1696	3.8429	3.7438	7.0424	691	835	1376	23535
40086	NM_000772	ref|Homo sapiens cytochrome P450, family 2, subfamily C, polypeptide 18 (CYP2C18), mRNA [NM_000772]	**CYP2C18**	7.6099	8.9559	7.5195	8.1267	441	938	458.5	231
18649	NM_000771	ref|Homo sapiens cytochrome P450, family 2, subfamily C, polypeptide 9 (CYP2C9), mRNA [NM_000771]	**CYP2C9**	7.4072	9.3744	7.5571	10.9138	371.5	1144	765	3195
1249	NM_000775	ref|Homo sapiens cytochrome P450, family 2, subfamily J, polypeptide 2 (CYP2J2), mRNA [NM_000775]	**CYP2J2**	2.6839	2.5692	2.5672	5.4100	839	595.5	802	682.5
37457	NM_017460	ref|Homo sapiens cytochrome P450, family 3, subfamily A, polypeptide 4 (CYP3A4), mRNA [NM_017460]	**CYP3A4**	6.0960	6.9956	7.3093	9.3567	400.5	1260	1142	496.5
33810	ENST00000439761	gb|Homo sapiens CYP3A5 mRNA, allele CYP3A5*3, exon 4B and partial cds, alternatively spliced. [AF355801]	**CYP3A5**	-0.3739	0.1067	0.0937	1.4438	736	962.5	1172	403.5
2423	NM_000765	ref|Homo sapiens cytochrome P450, family 3, subfamily A, polypeptide 7 (CYP3A7), mRNA [NM_000765]	**CYP3A7**	5.5511	7.1260	6.6750	8.9567	3649	10927	9853	5989

14586	NM_007253	ref|Homo sapiens cytochrome P450, family 4, subfamily F, polypeptide 8 (CYP4F8), mRNA [NM_007253]	**CYP4F8**	2.7132	4.1535	2.5950	5.9628	686	2541.5	825.5	1176
14415	NM_207352	ref|Homo sapiens cytochrome P450, family 4, subfamily V, polypeptide 2 (CYP4V2), mRNA [NM_207352]	**CYP4V2**	2.9194	2.9333	2.7663	5.2822	853	995	923.5	822
29103	NM_005505	ref|Homo sapiens scavenger receptor class B, member 1 (SCARB1), transcript variant 1, mRNA [NM_005505]	**SCARB1**	-0.6468	-0.4215	-1.0325	2.0324	14042	23763	13137	22012



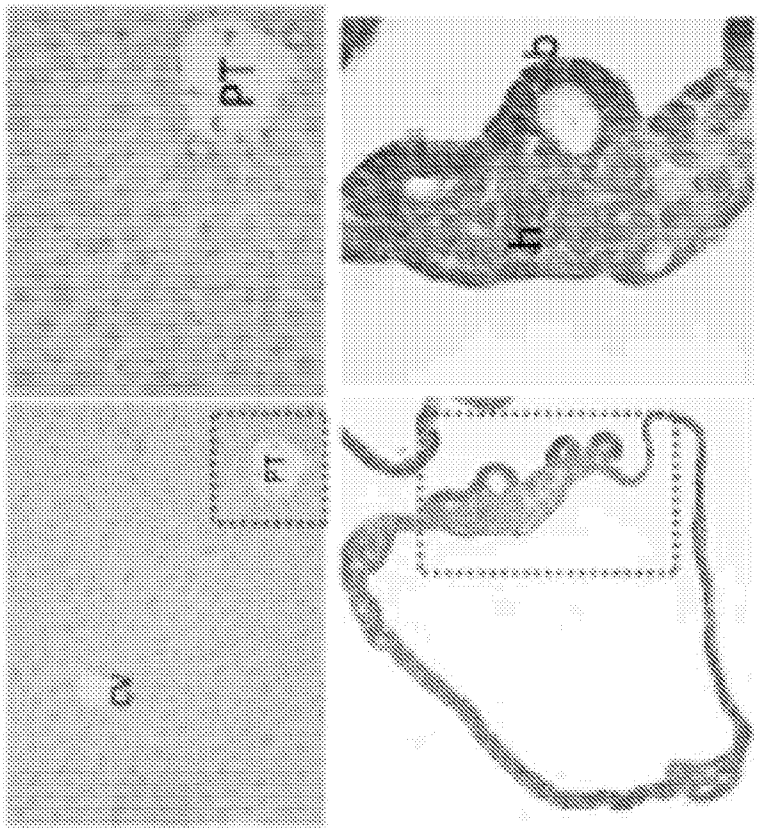
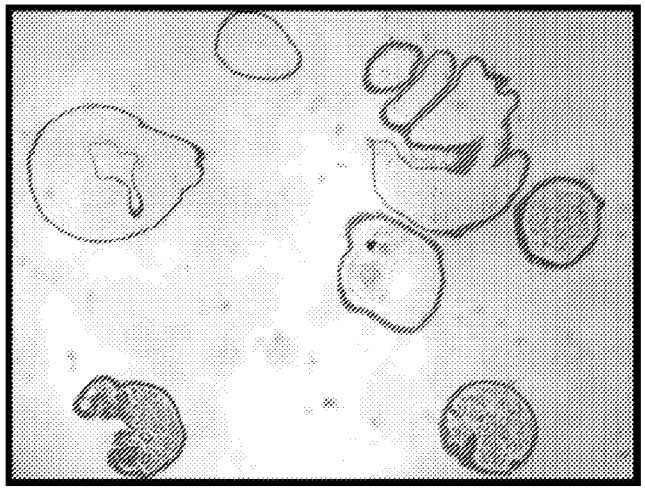


Figure 30

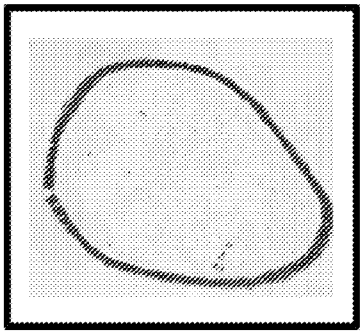
FIG. 31

H&E staining in paraffin sections of pancreas organoids culture in $EGFNRA_{83\text{-}01}$ during 3 months (passage 12)

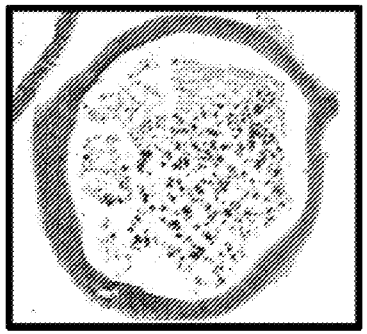
4x magnification



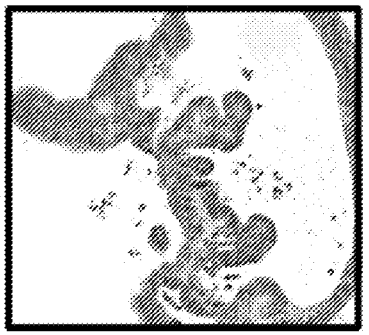
20x magnification



Single layer



(pseudo) Stratified



(pseudo)Stratified and/or single layers folded

FIG. 32(a)

Quantification of forskolin-induced murine organoid swellin. Dekkers *et al.*

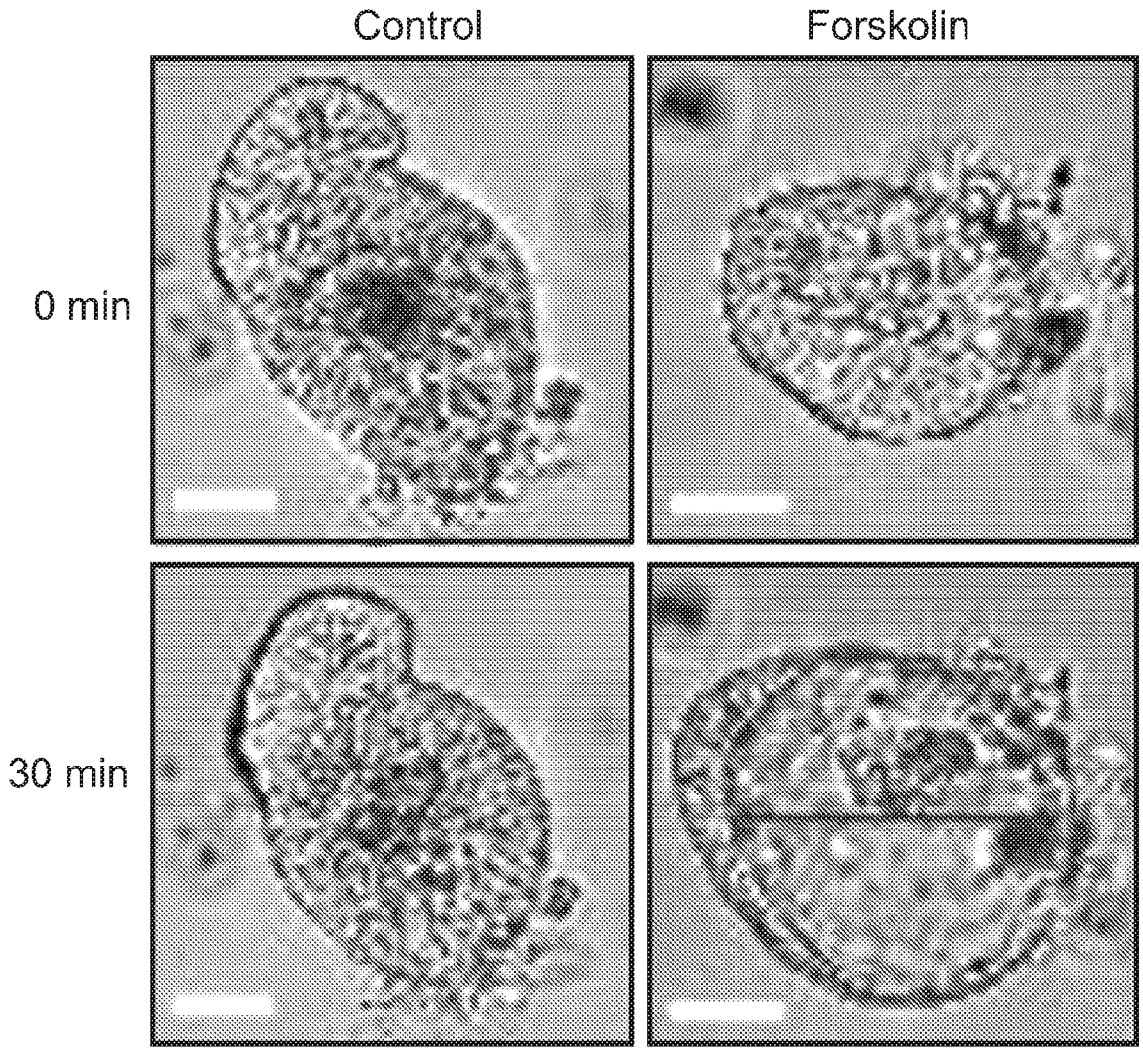


FIG. 32(b)

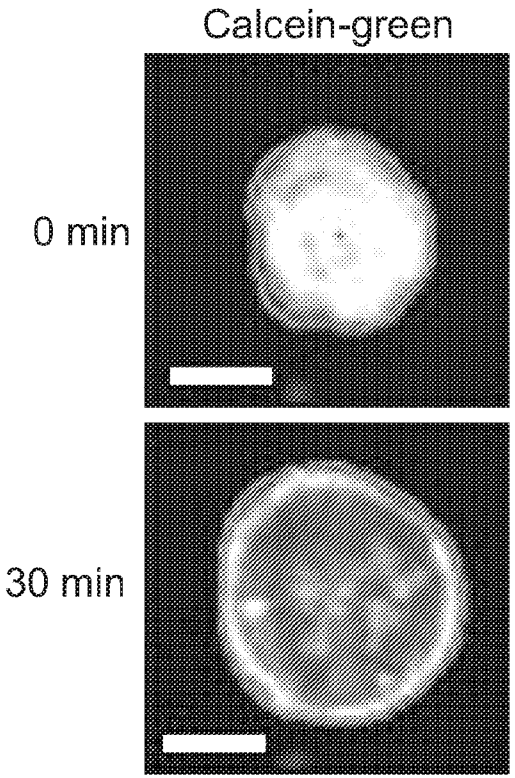


FIG. 32(c)

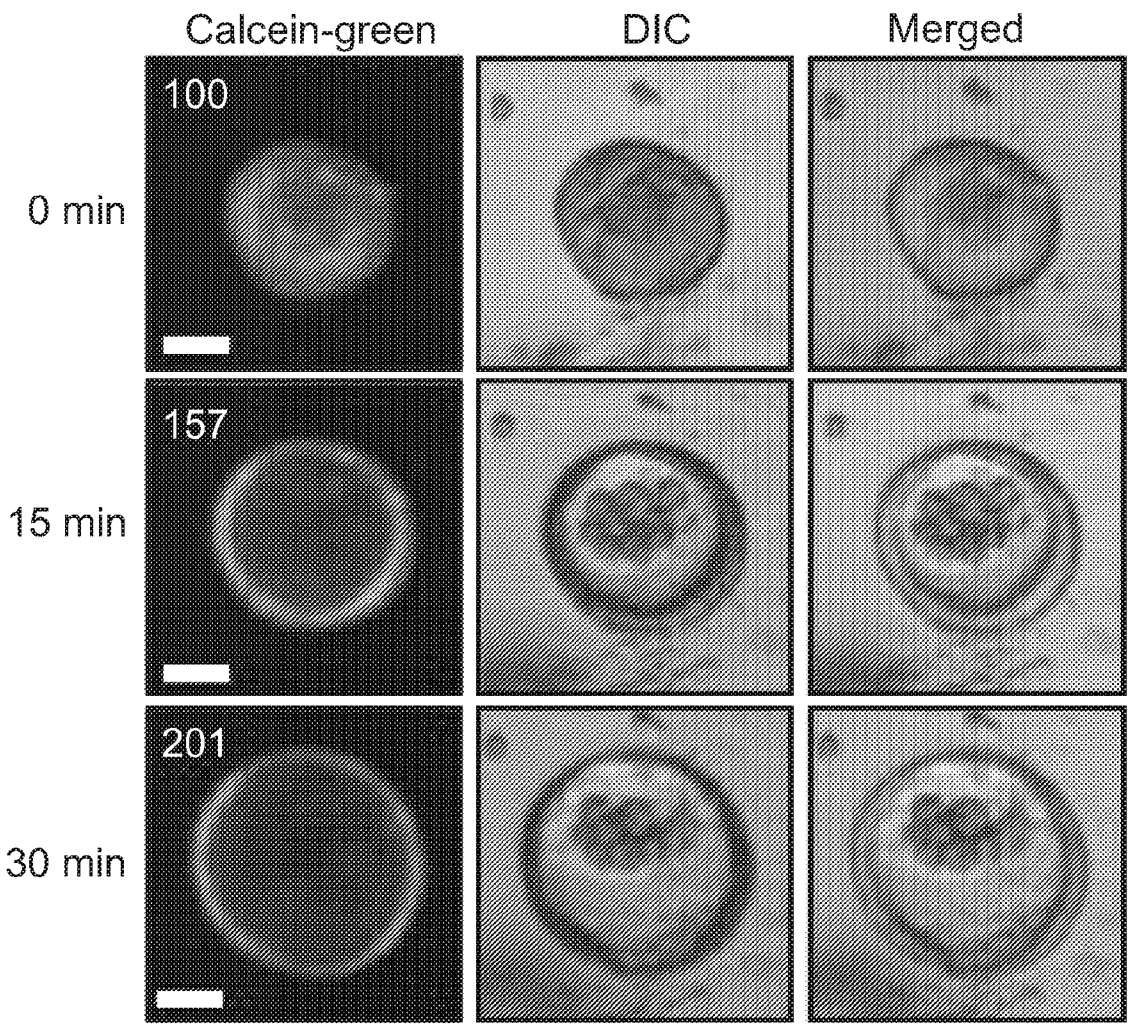


FIG. 32(d)

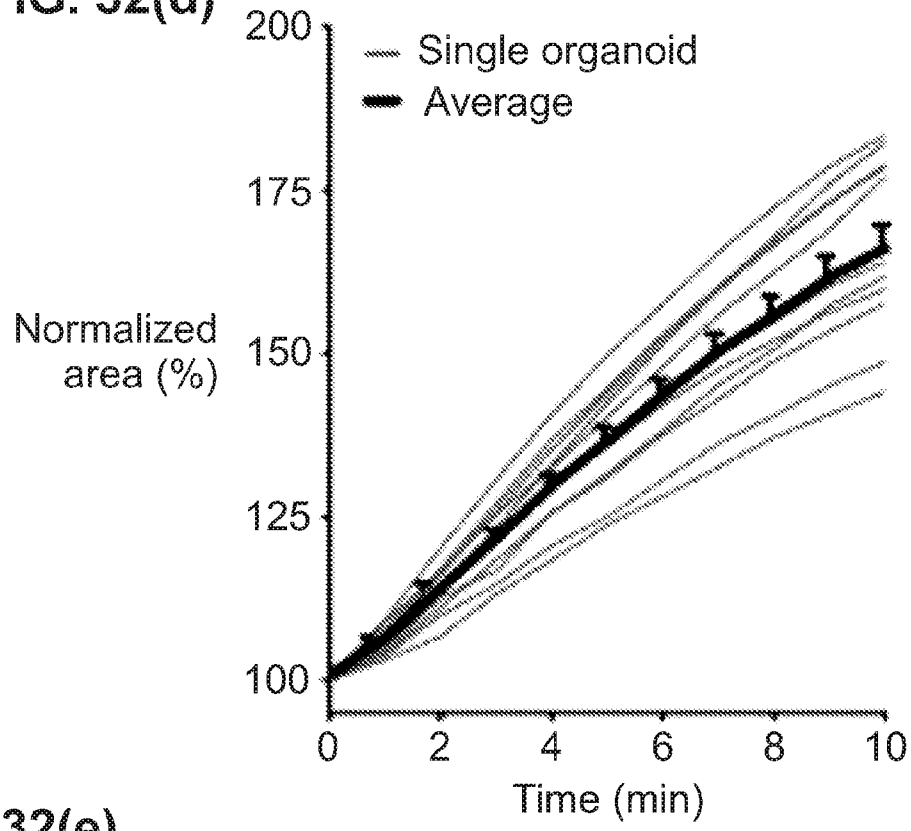


FIG. 32(e)

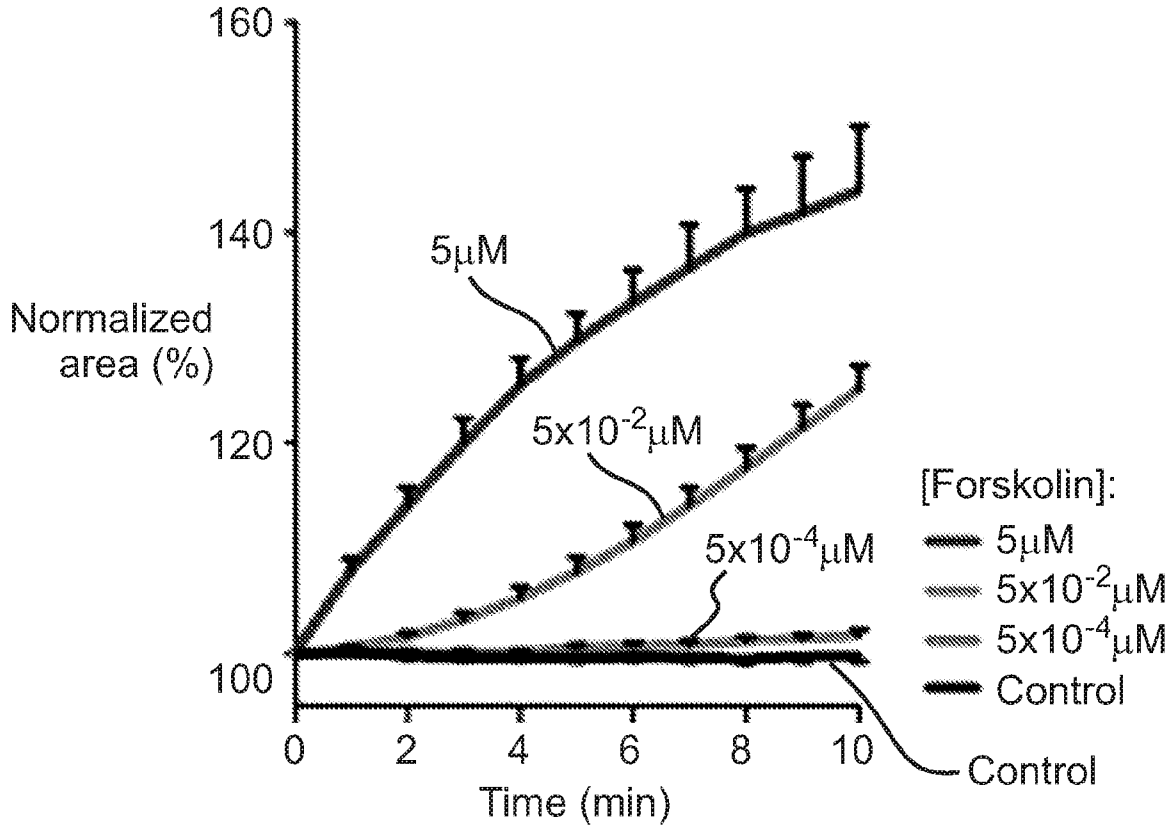


FIG. 33(a)

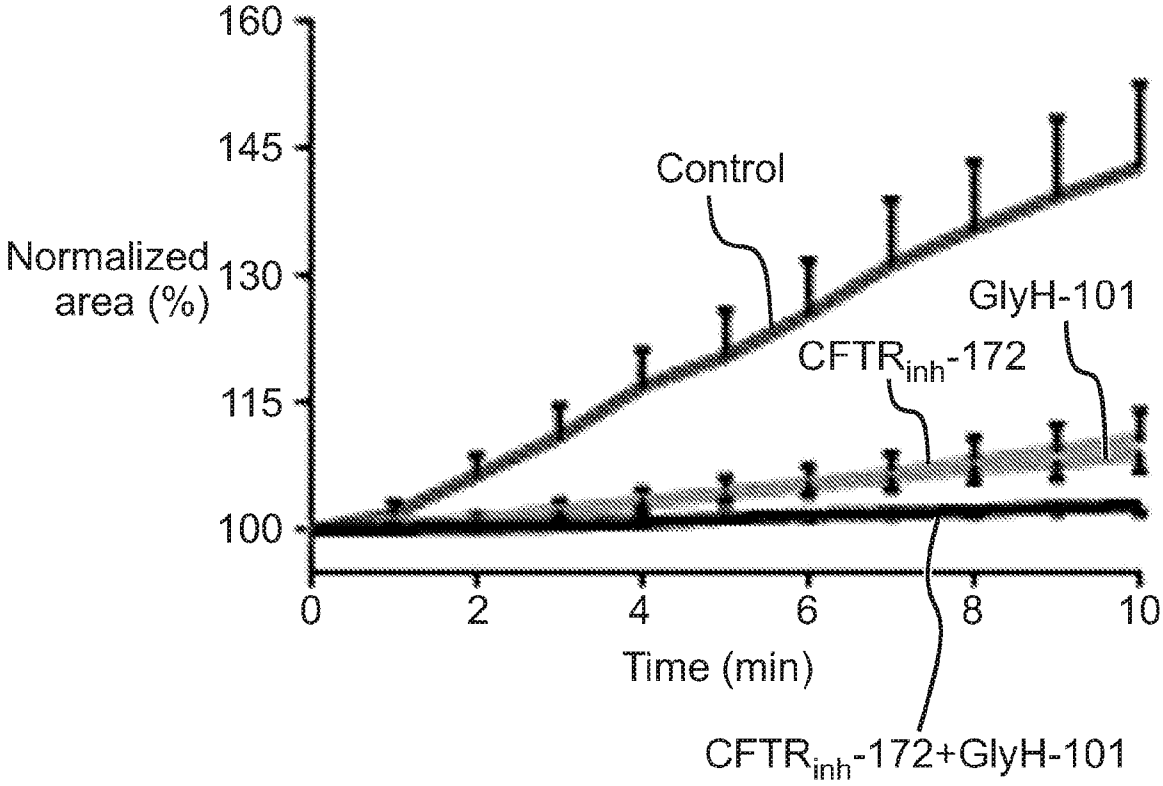


FIG. 33(b)

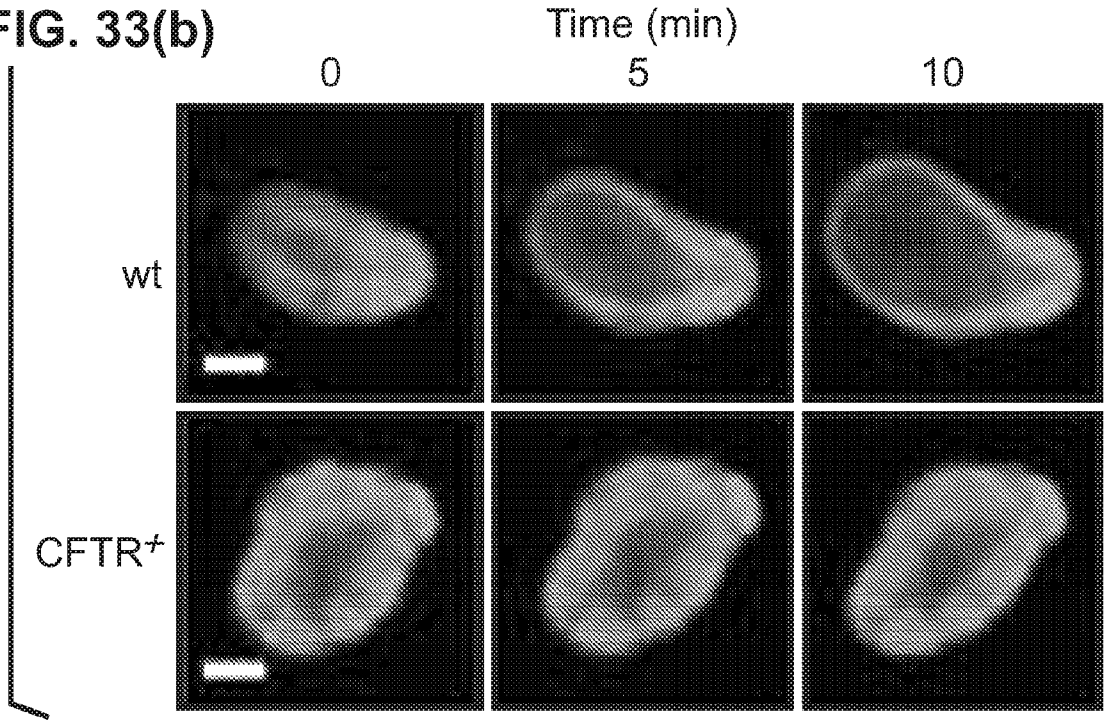


FIG. 33(c)

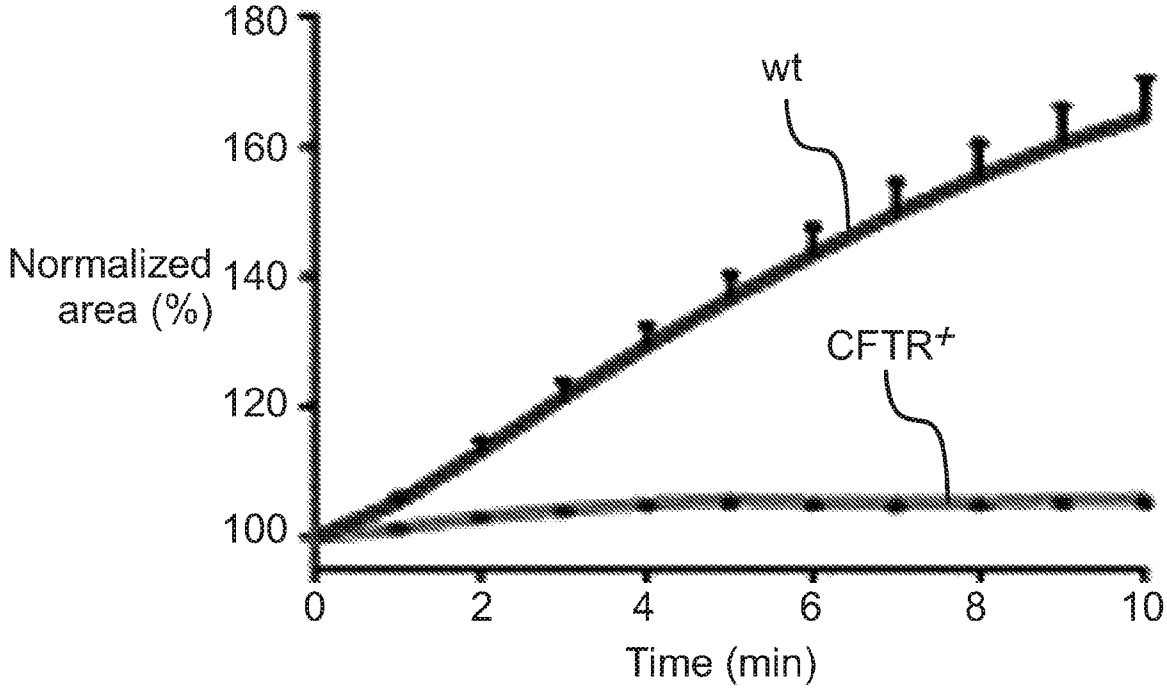


FIG. 33(d)

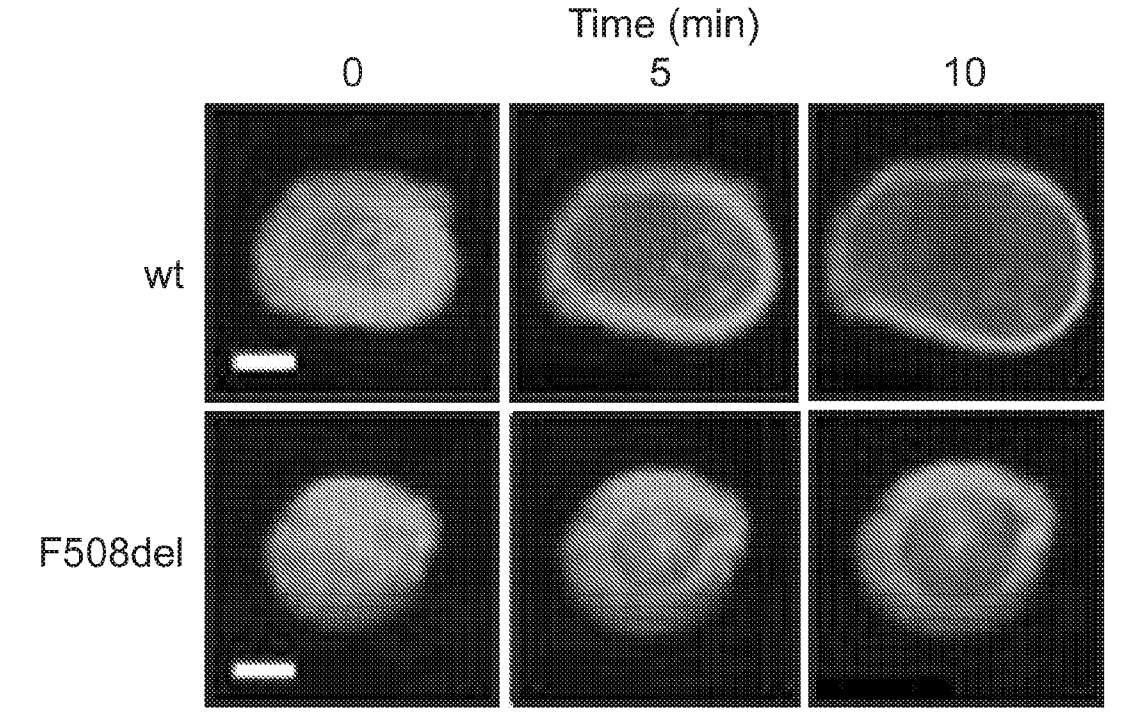


FIG. 33(e)

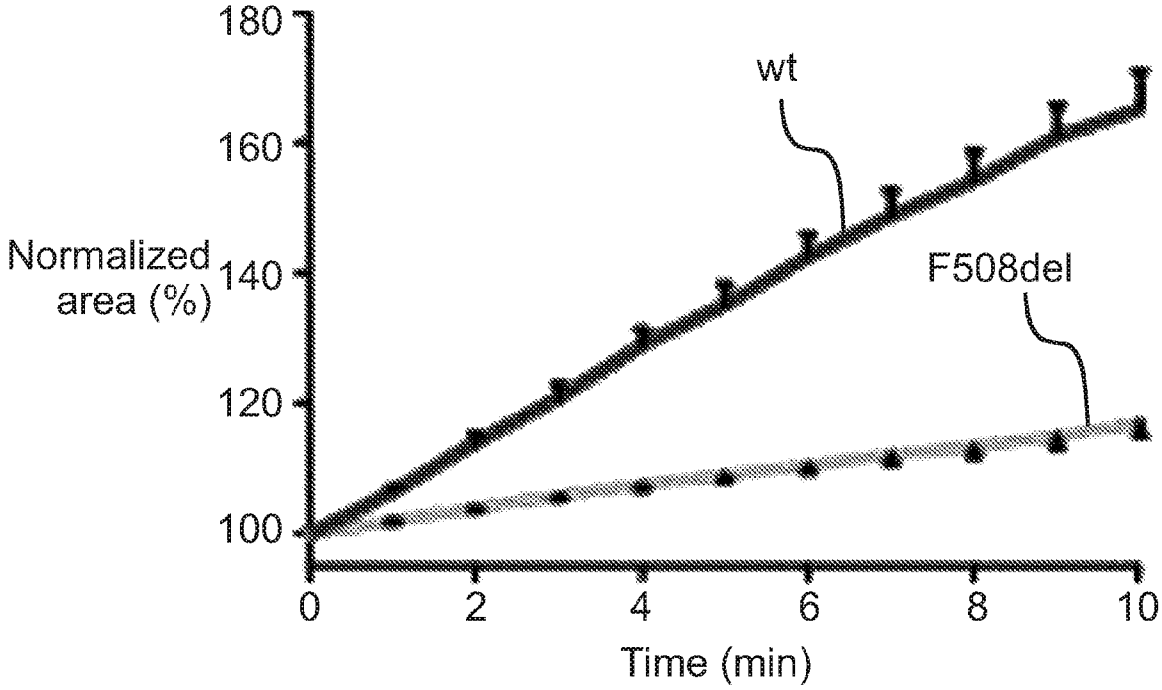


FIG. 33(f)

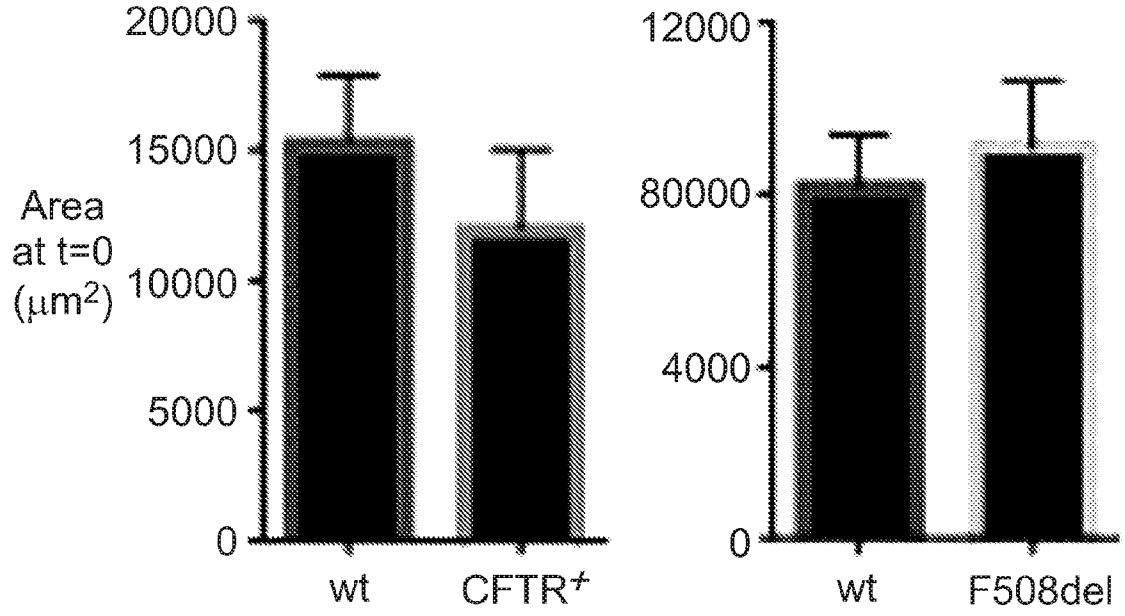


FIG. 33(g)

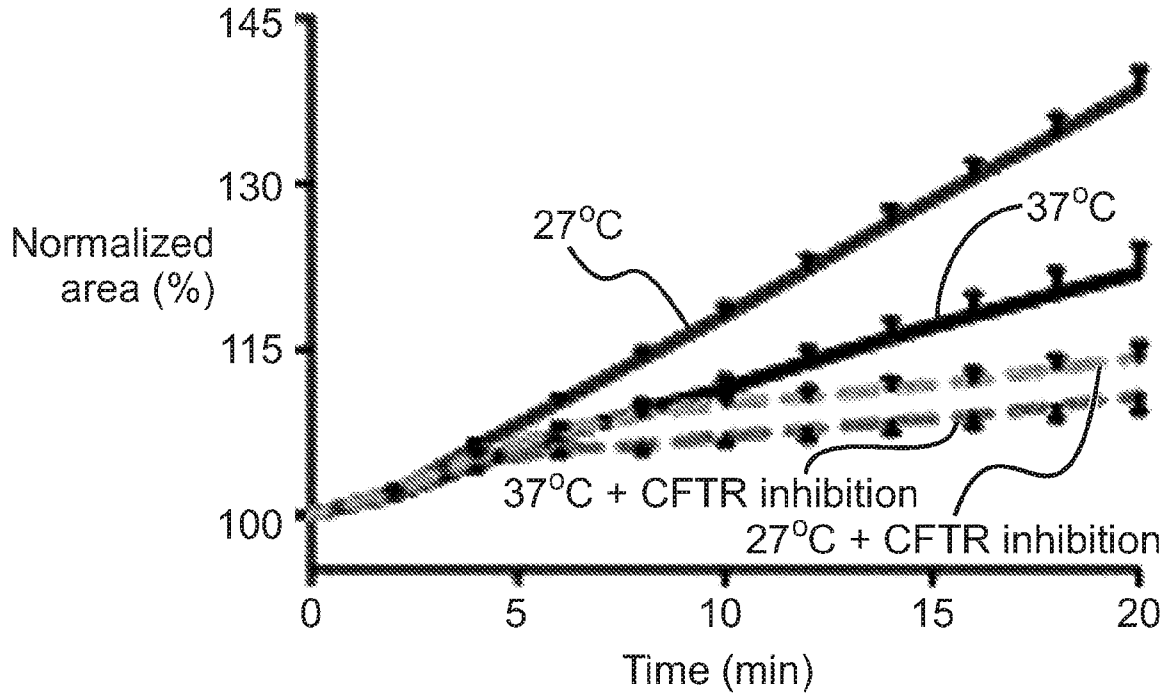


FIG. 33(h)

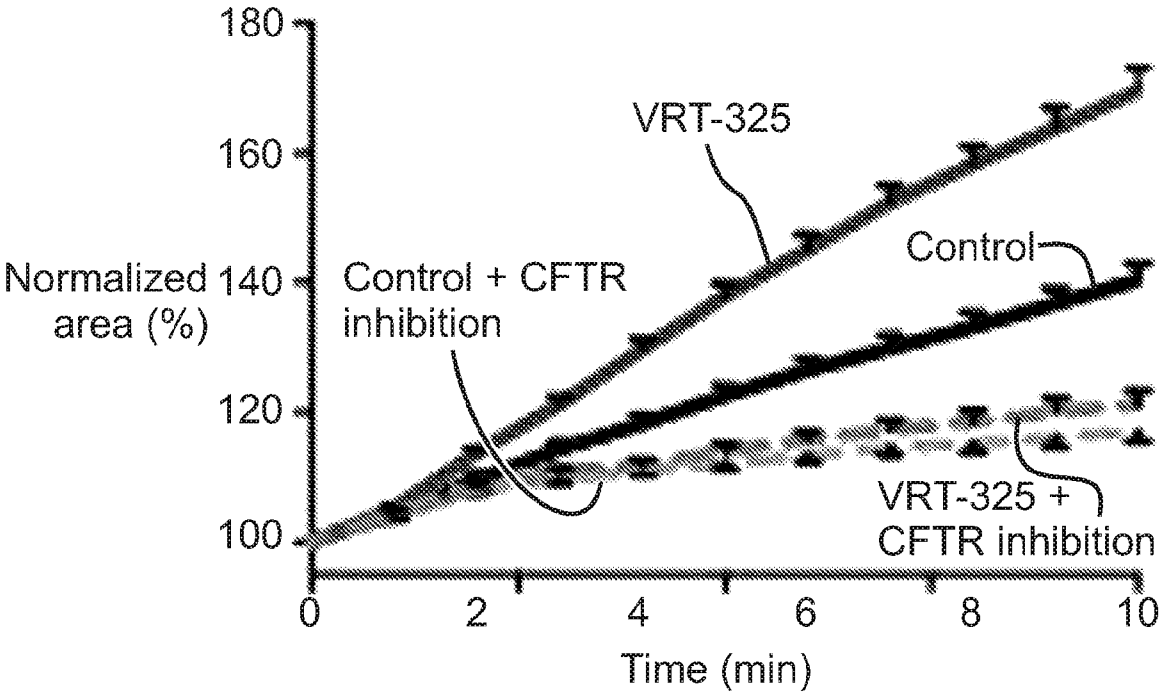


FIG. 33(i)

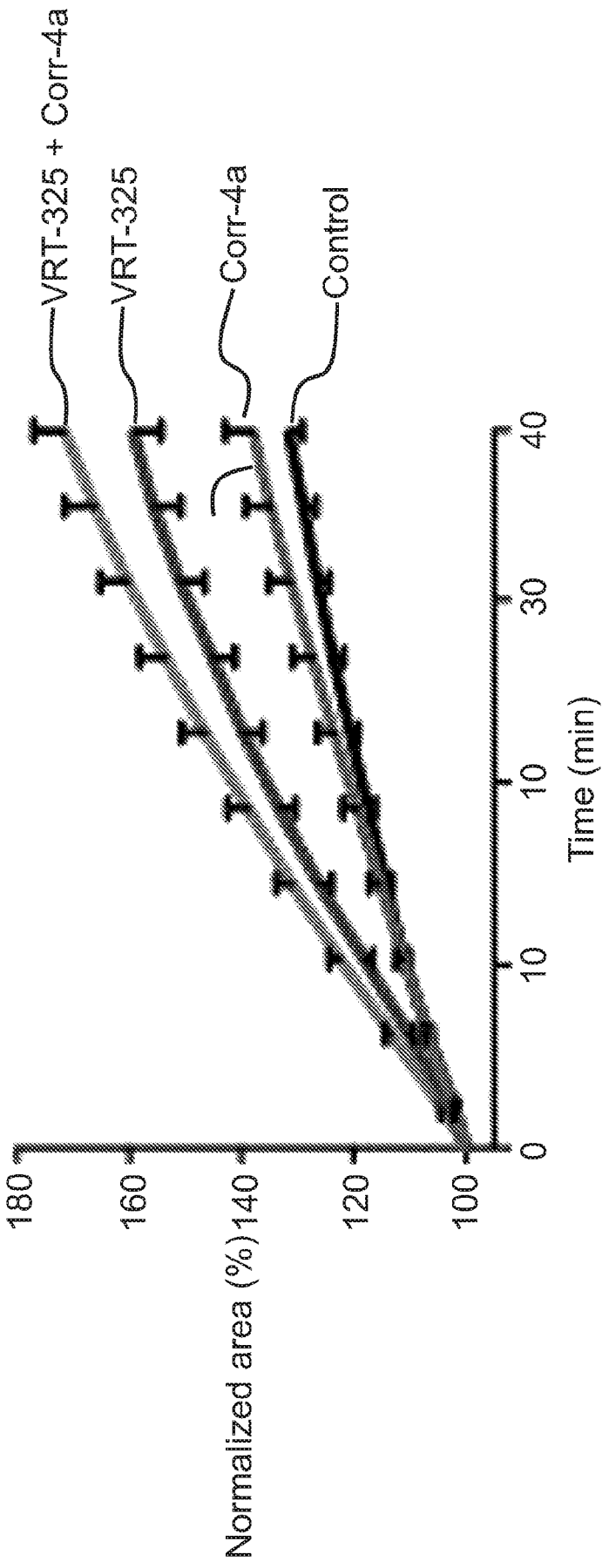

VRT-325 + Corr-4a
VRT-325
Corr-4a
Control
Normalized area (%)
180
160
140
120
100
0
10
10
30
40
Time (min)

FIG. 34(a)

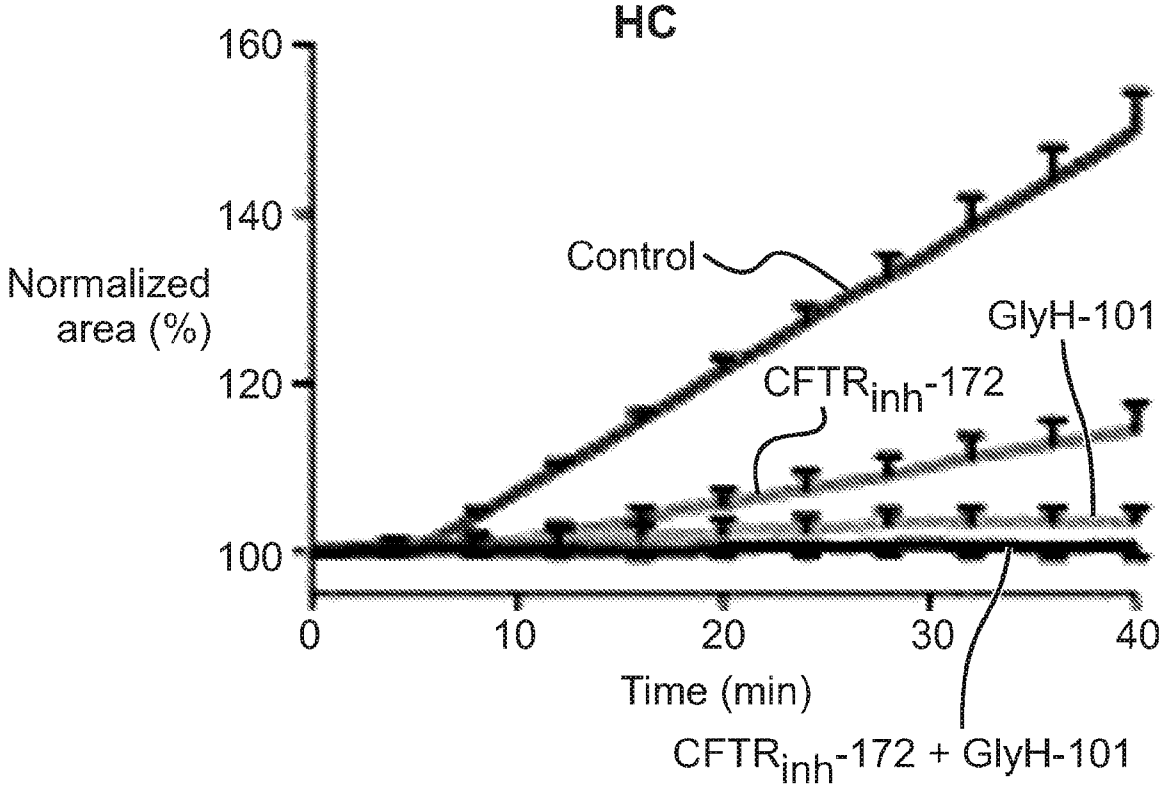


FIG. 34(b)

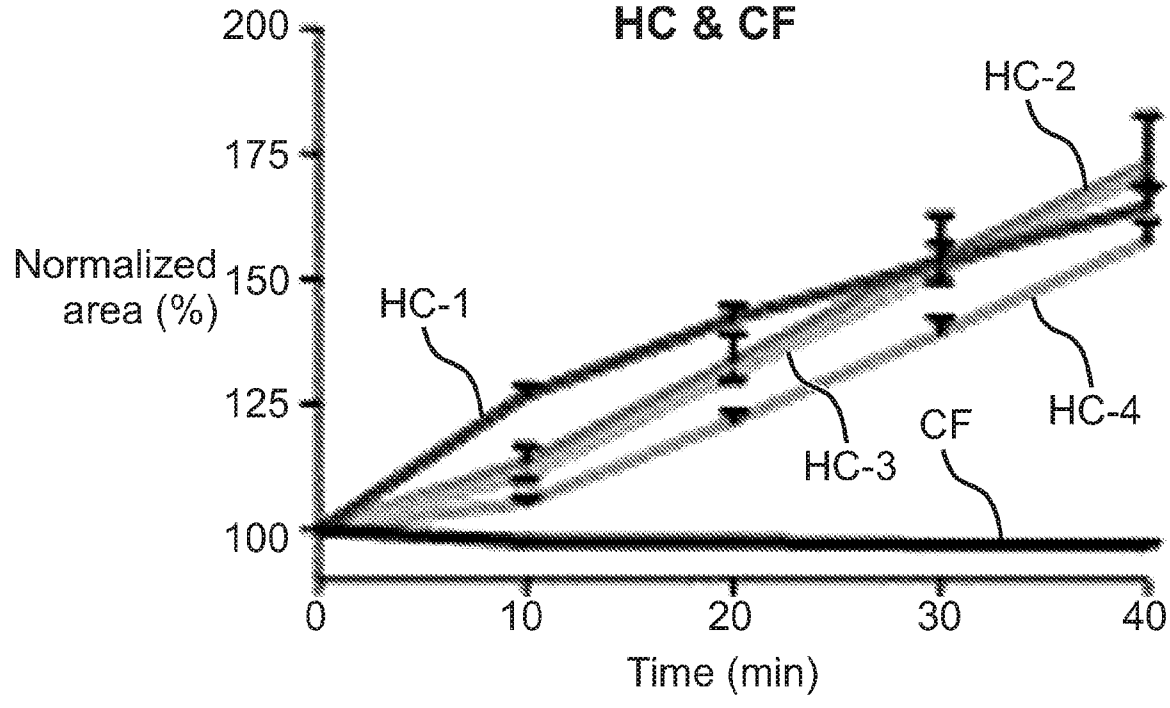


FIG. 34(c)

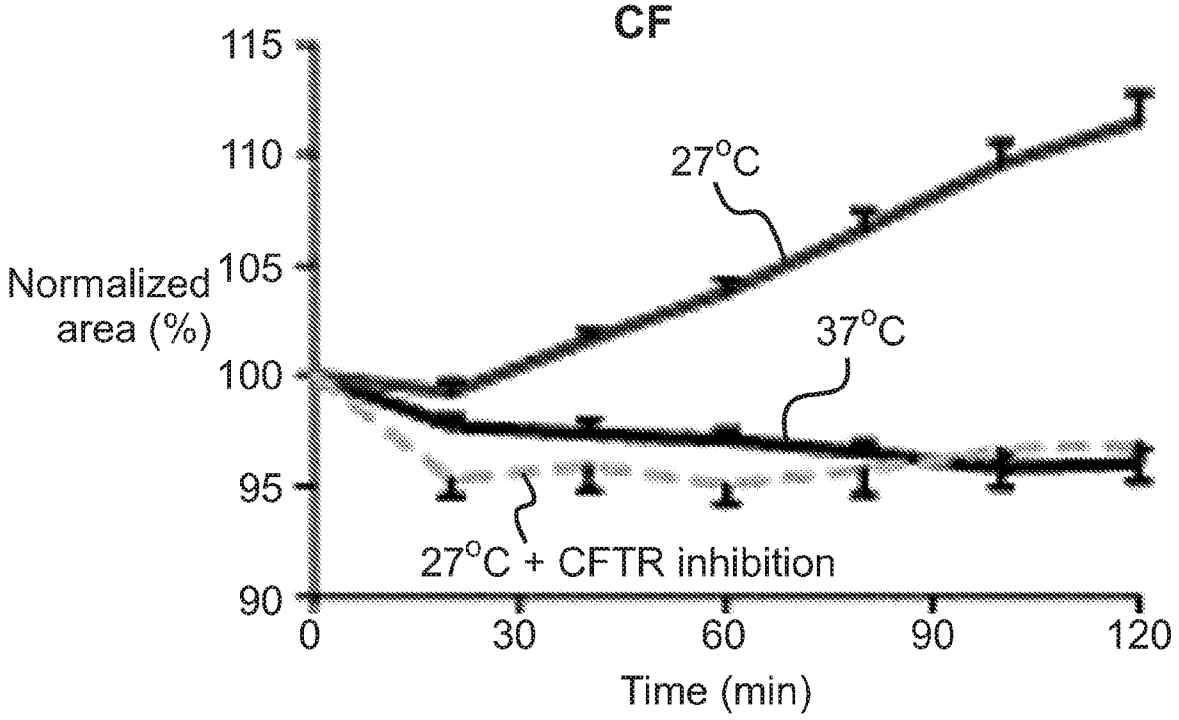


FIG. 34(e)

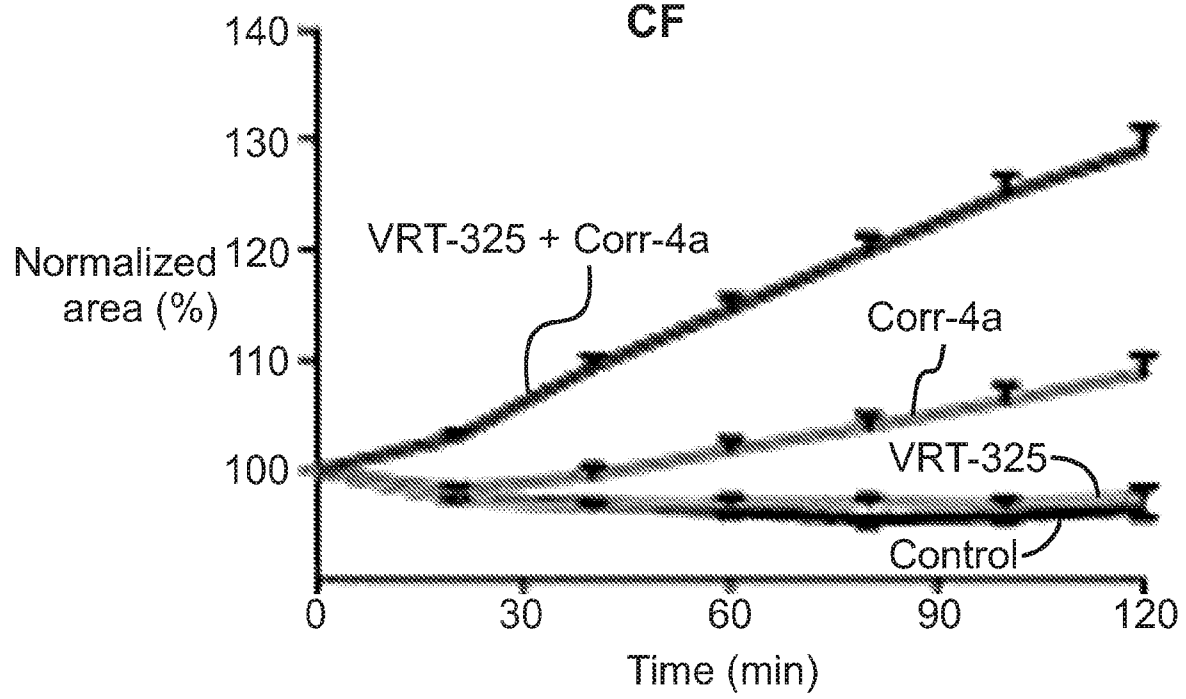


FIG. 34(d)

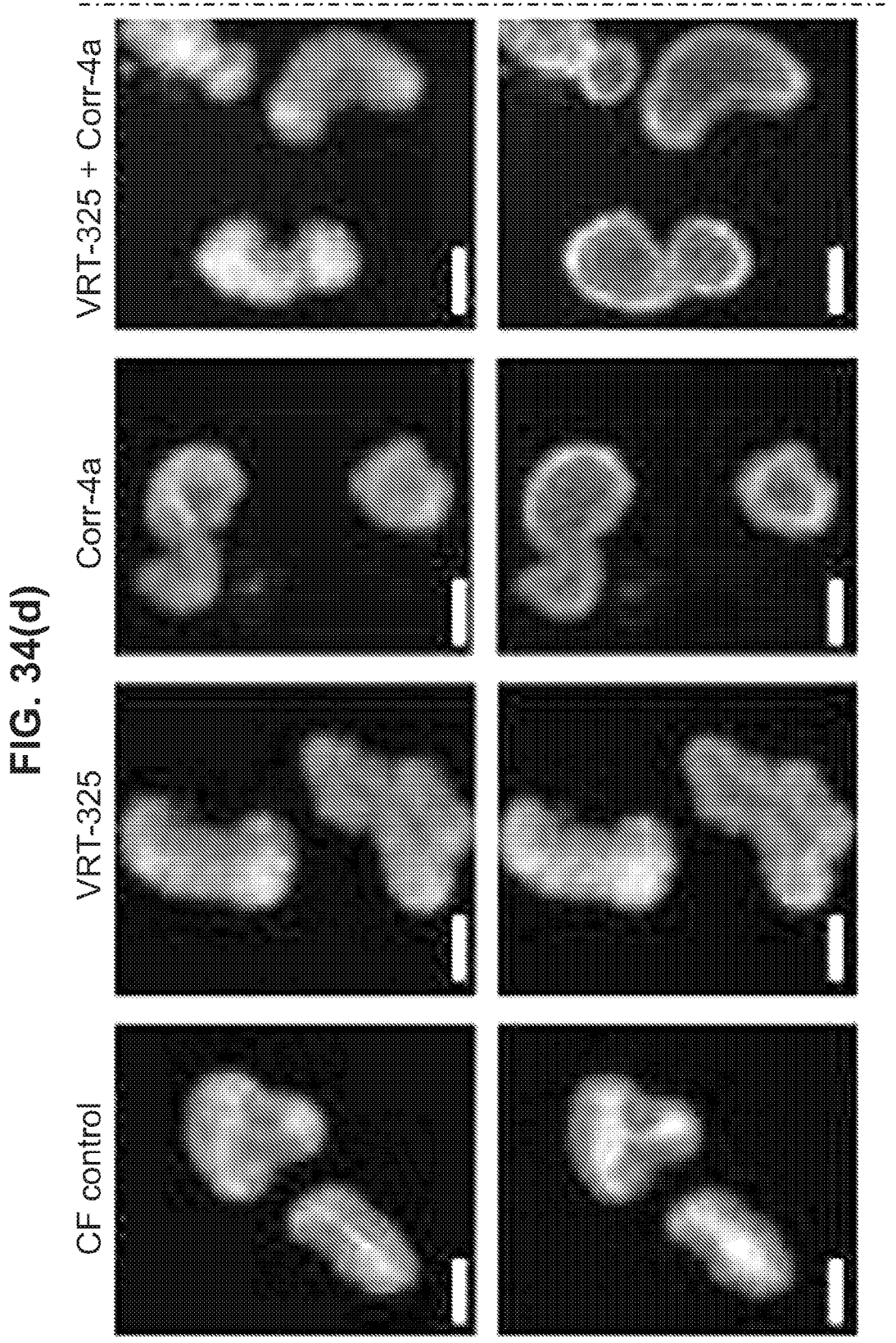


FIG. 34(d)contd.

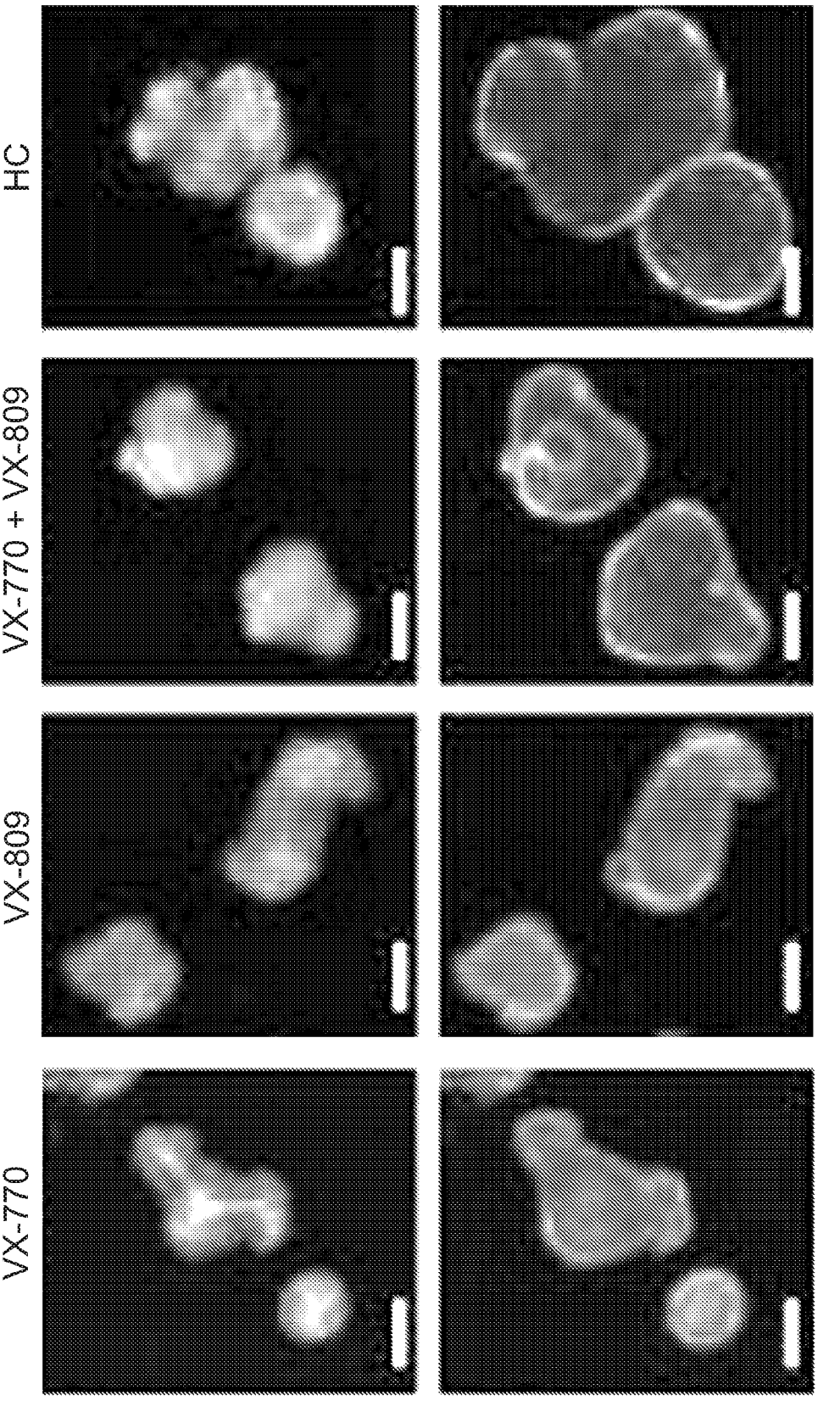


FIG. 34(f)

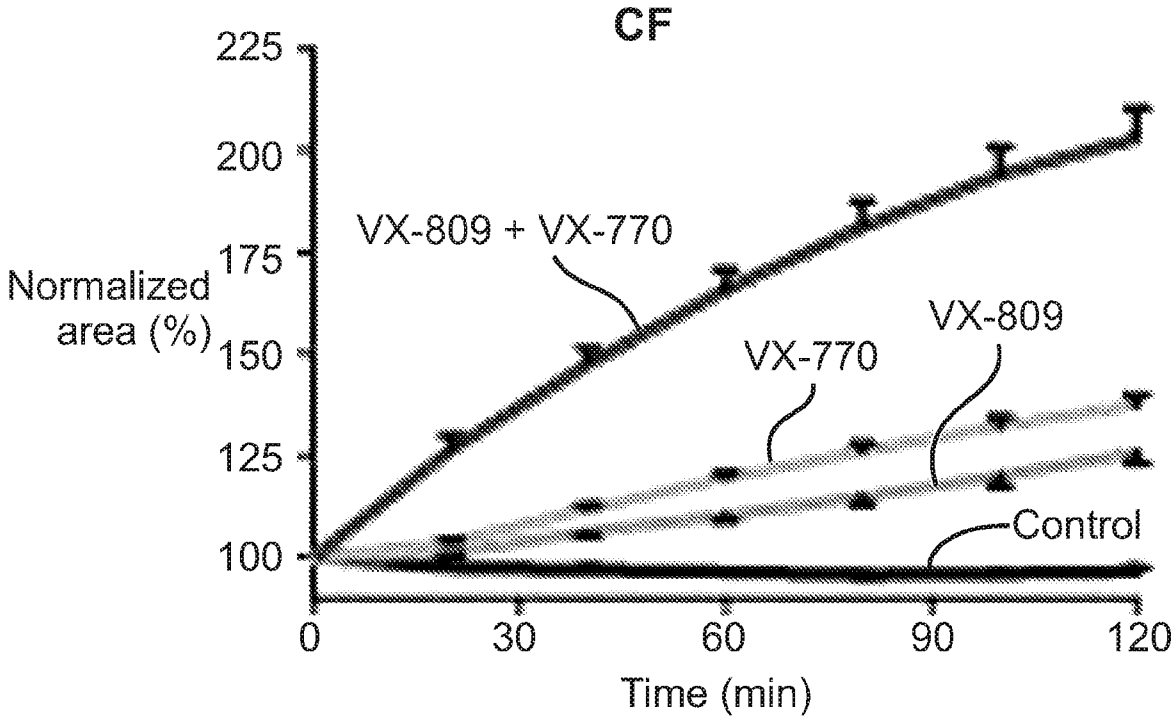


FIG. 34(g)

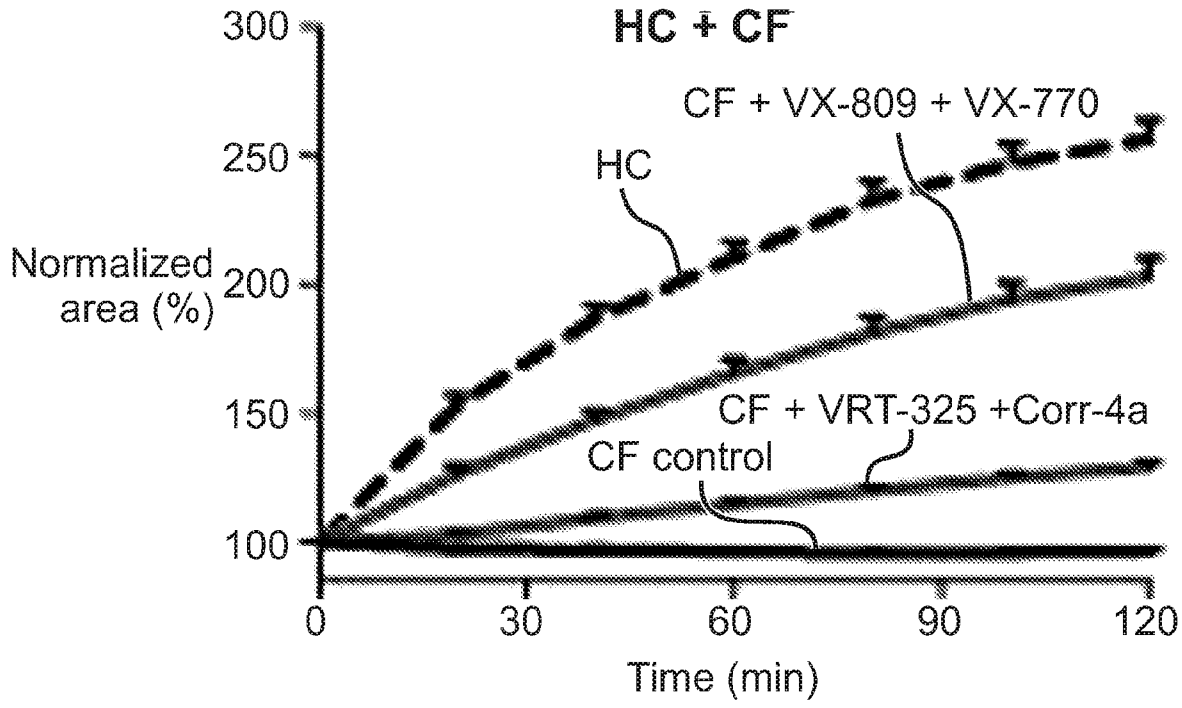


FIG. 35

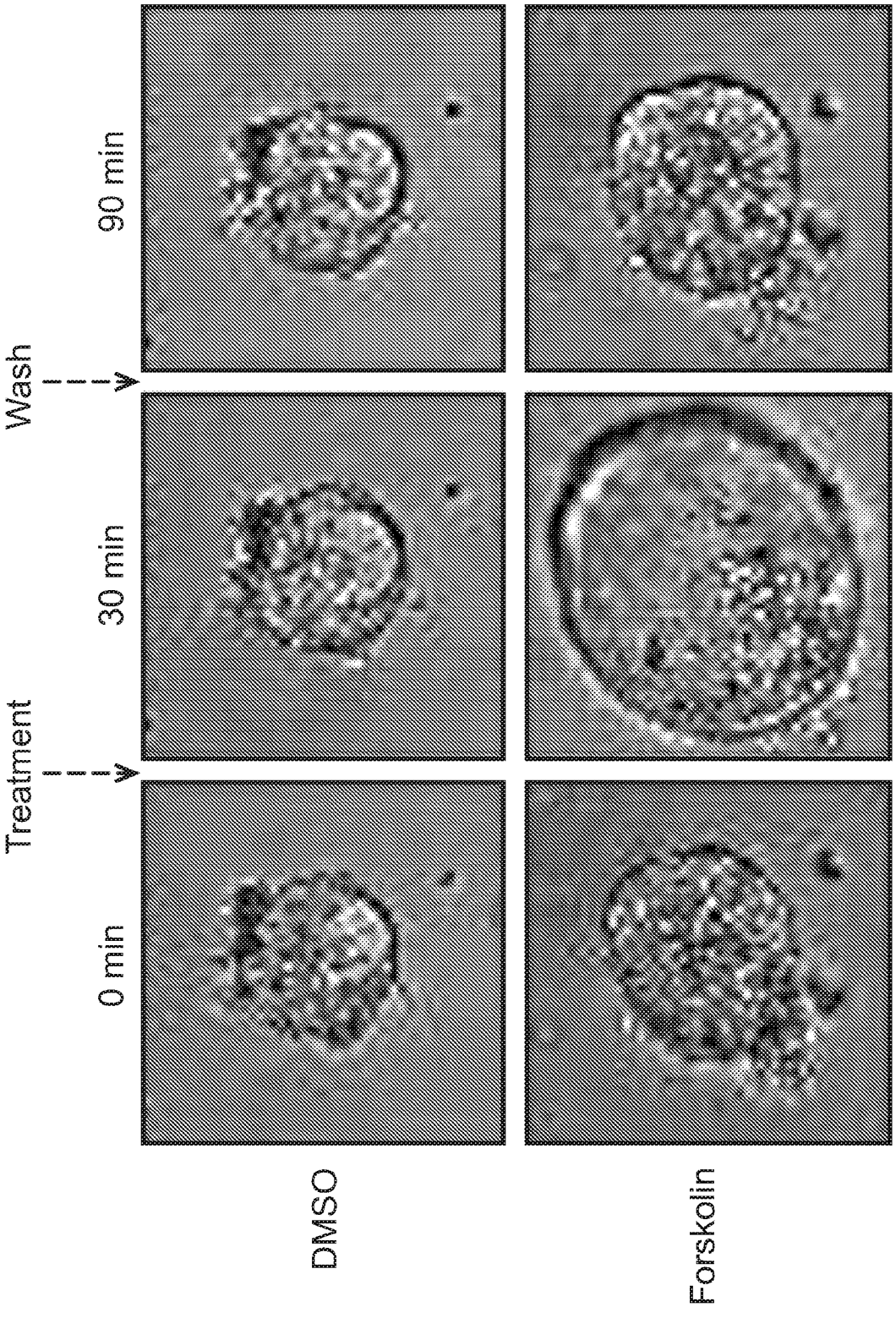


FIG. 36

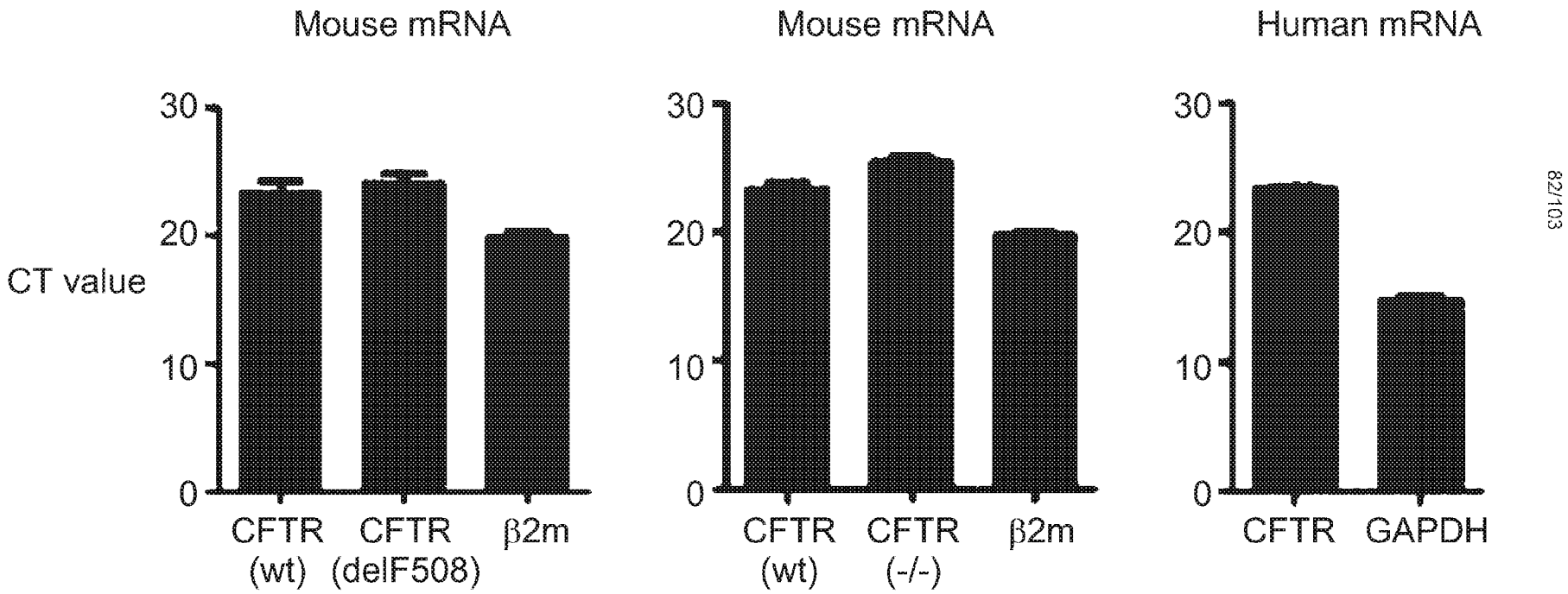

Mouse mRNA
Mouse mRNA
Human mRNA
CT value
30
20
10
0
CFTR (wt)
CFTR (delF508)
β2m
CFTR (wt)
CFTR (-/-)
β2m
CFTR
GAPDH

FIG. 37(a)

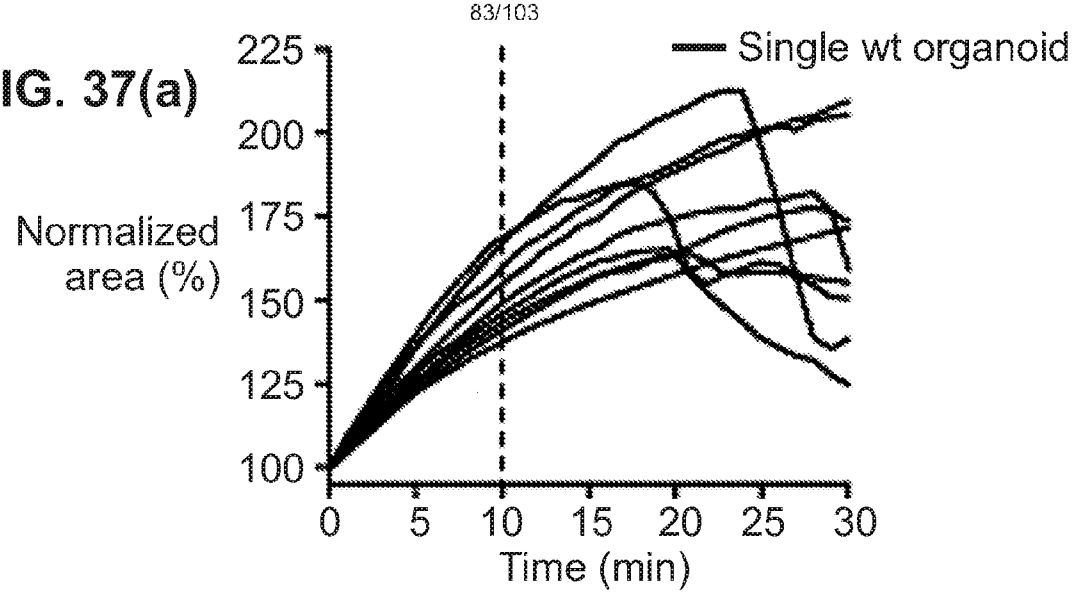


FIG. 37(b)

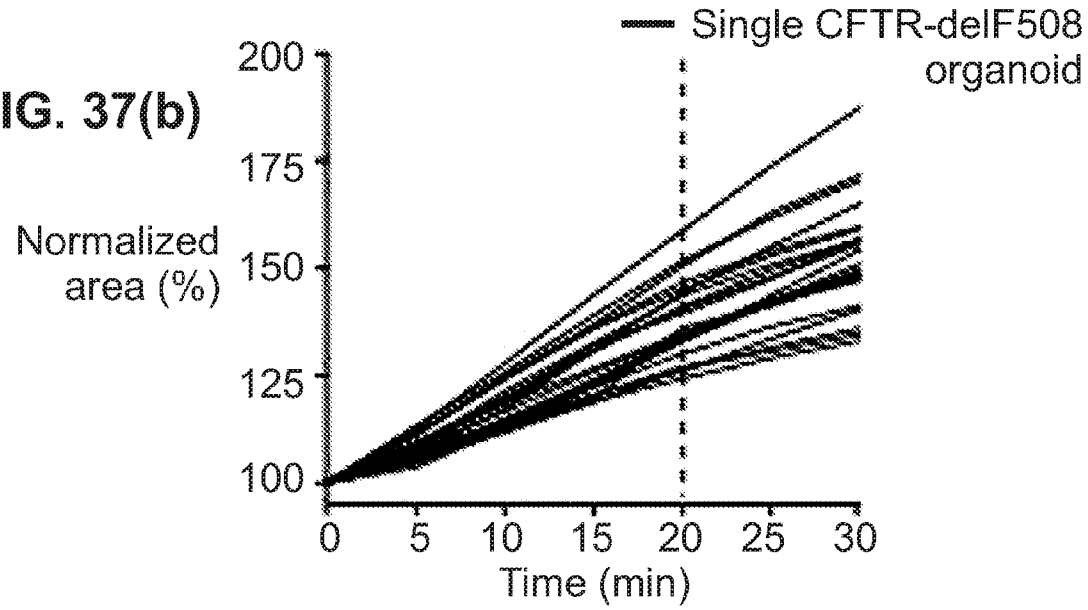


FIG. 37(c)

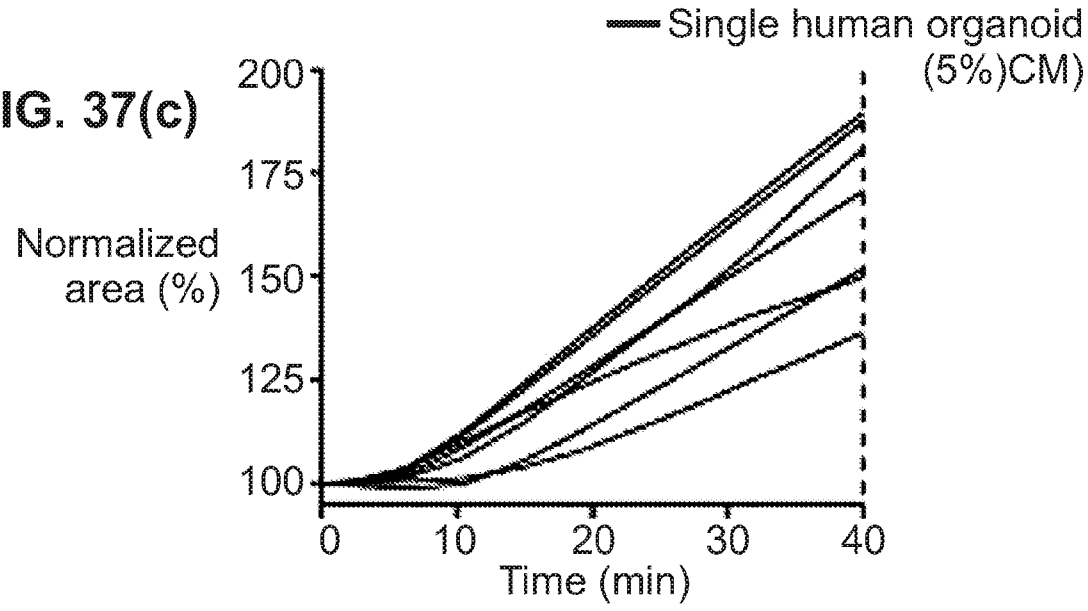


FIG. 38
Human organoids

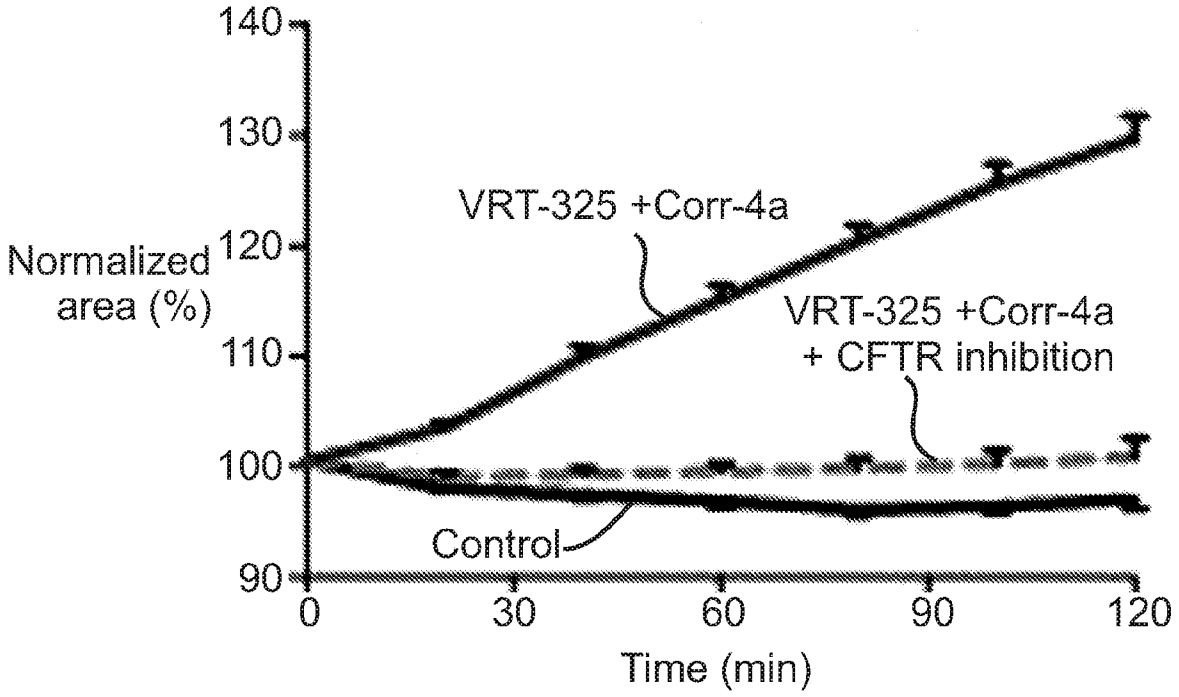
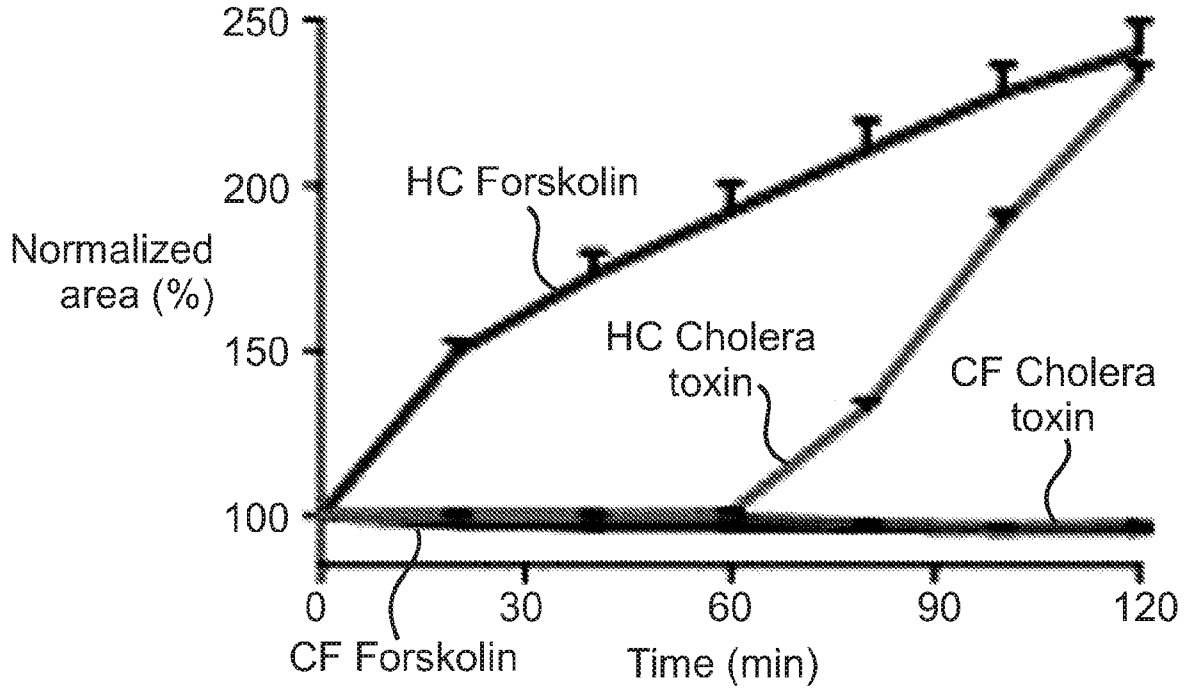


FIG. 39
Human organoids



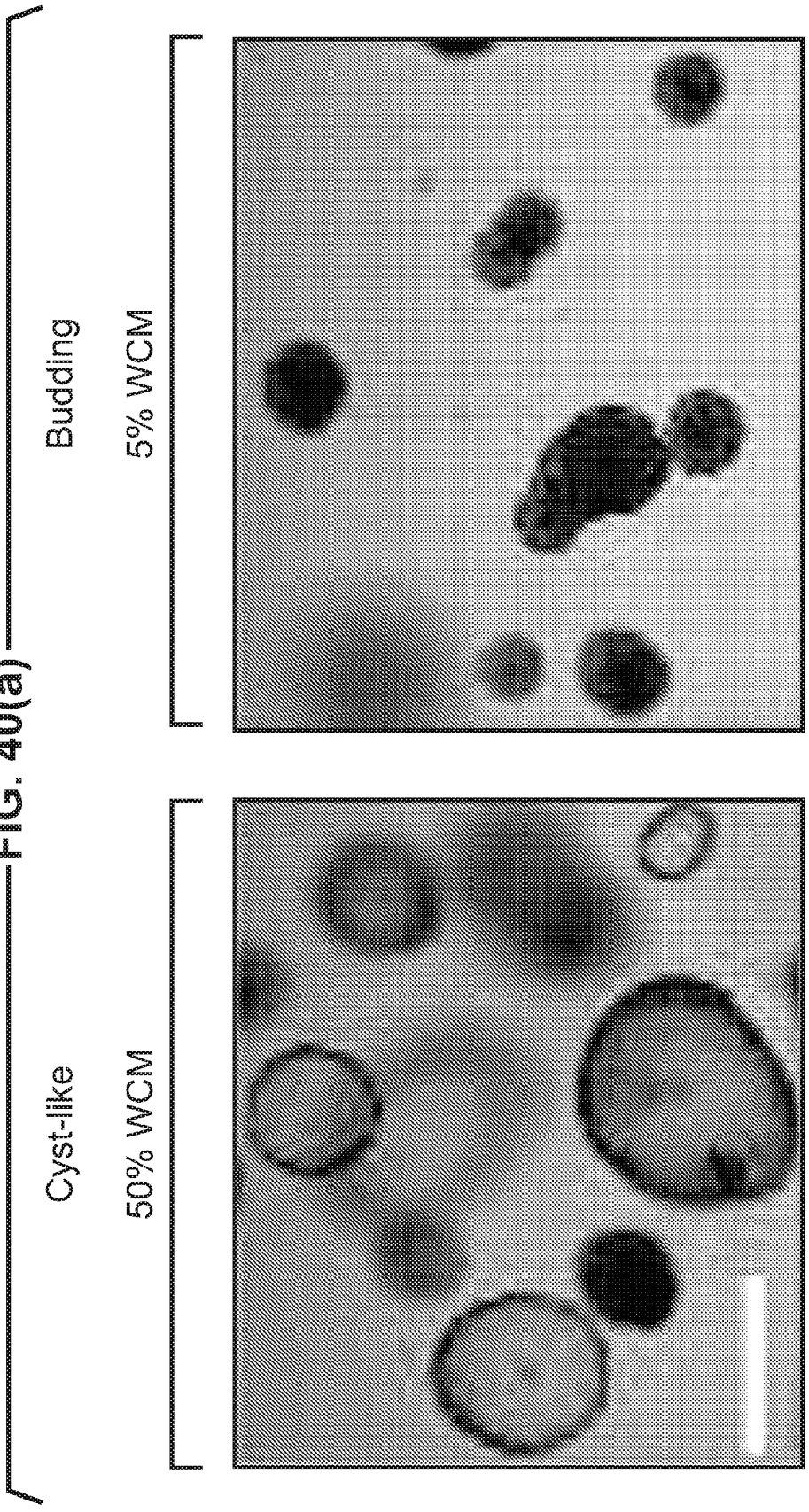


FIG. 40(a)

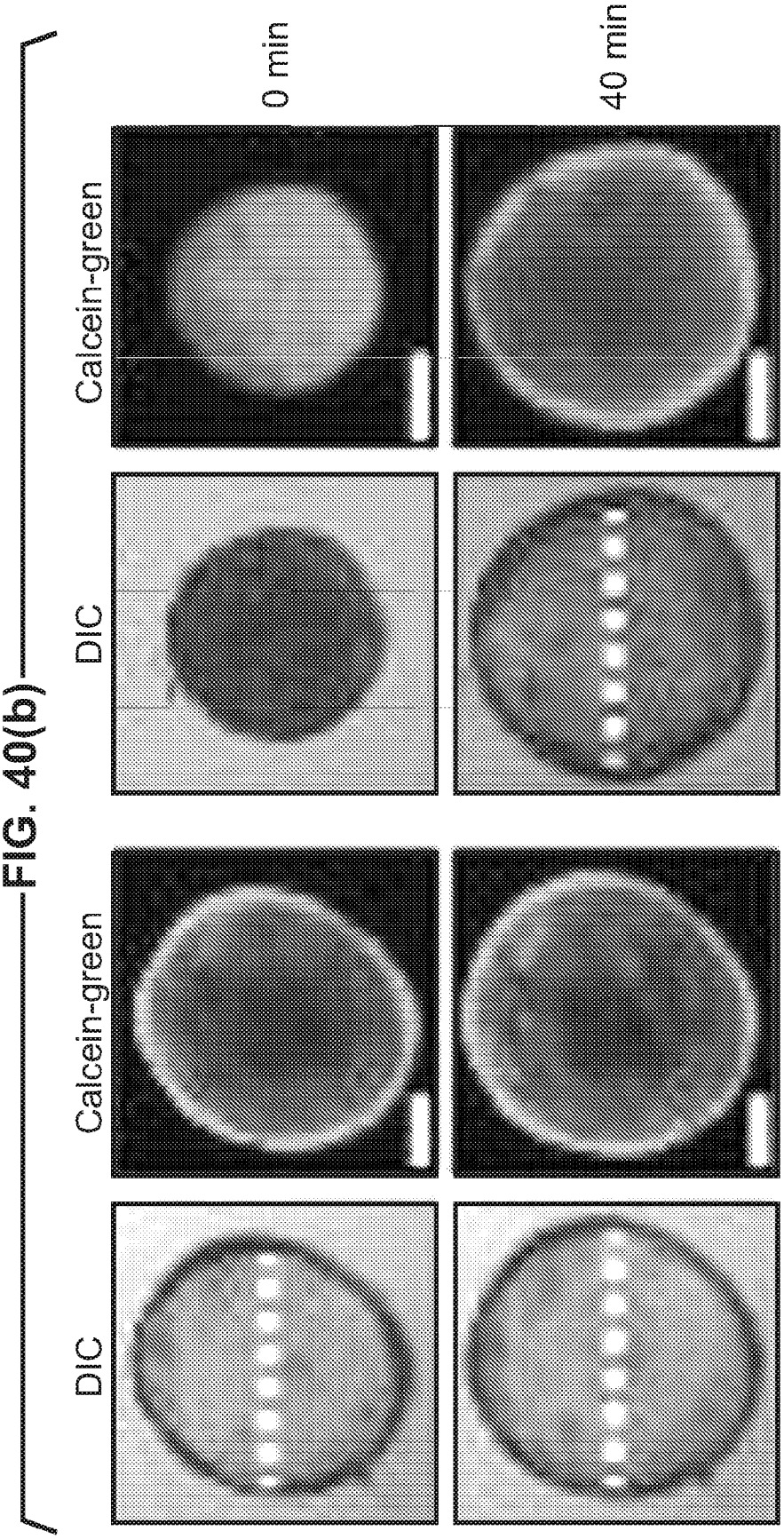


FIG. 40(b)

FIG. 40(c)

505 wcm

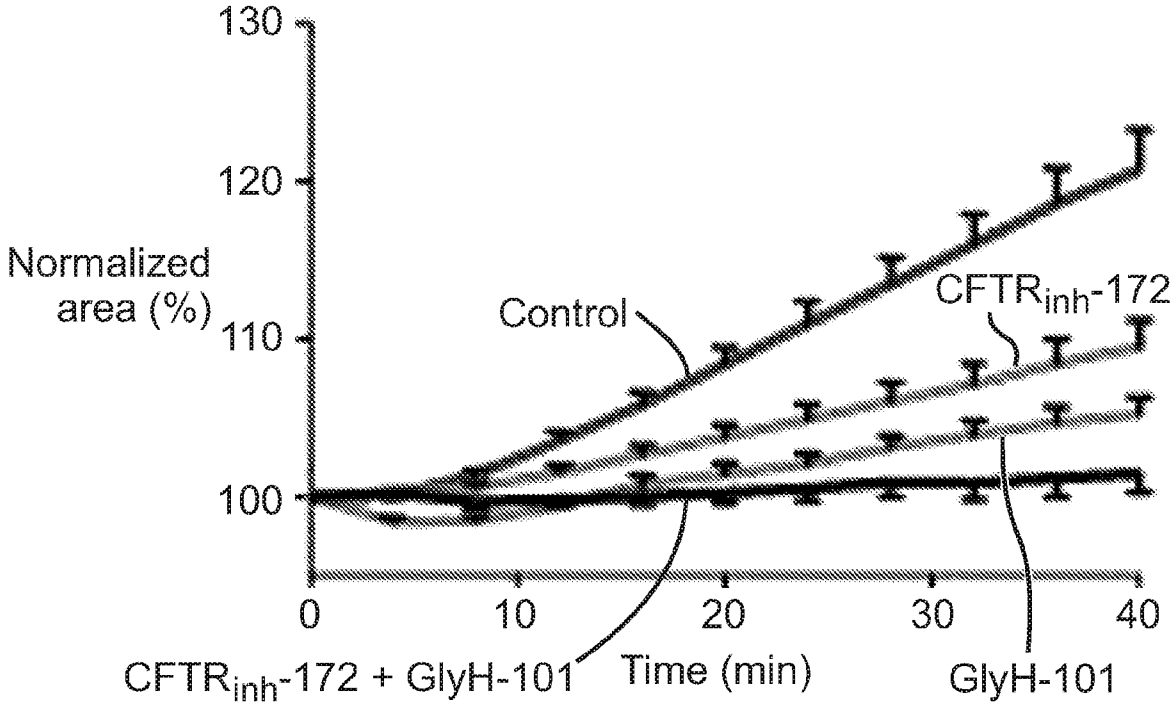


FIG. (40d)

Budding phenotype

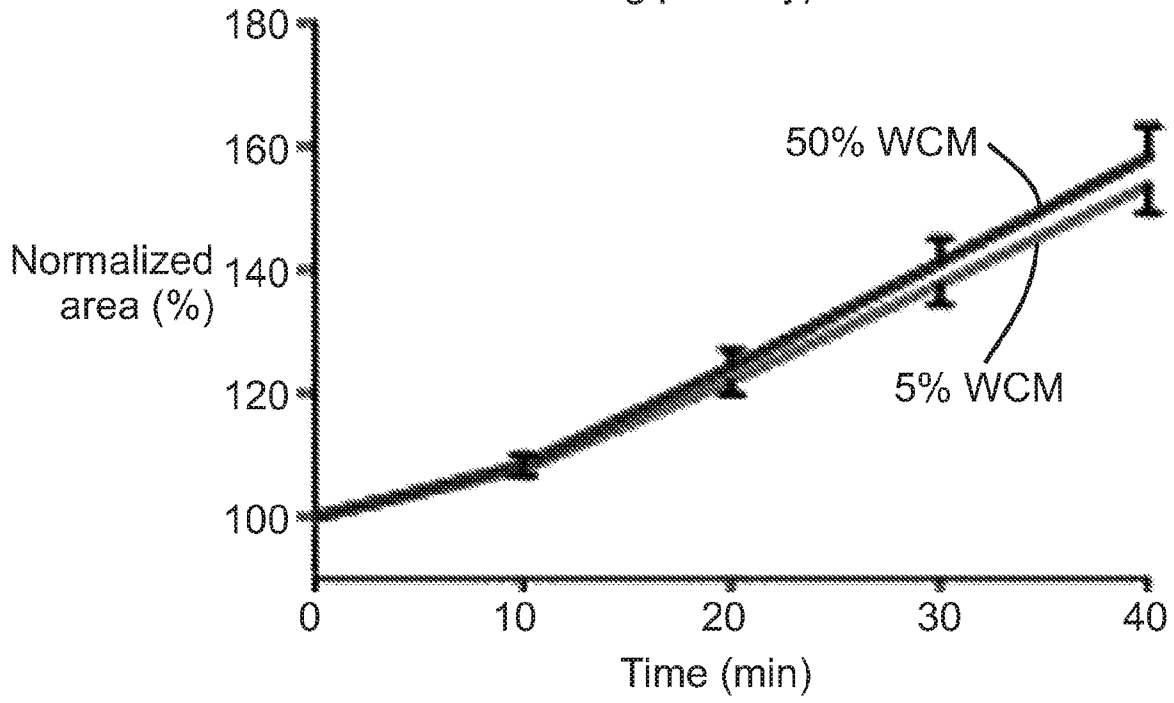


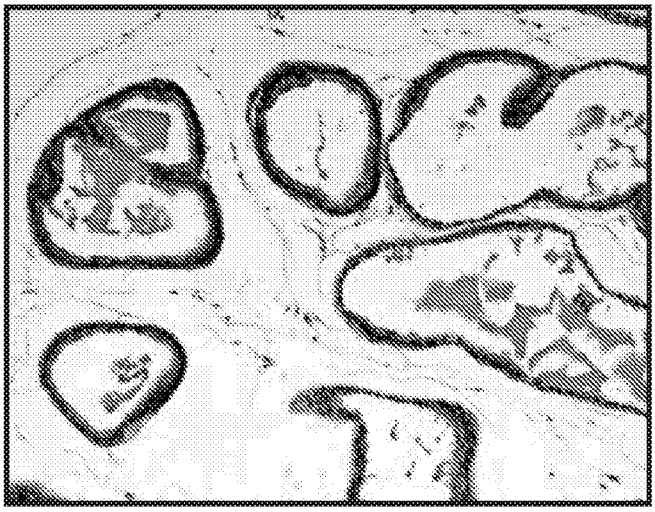
FIG. 41

H&E after 3month culture 1 passage a week 5x split

Prostate
(whole organ)



Prostate
(whole organ)



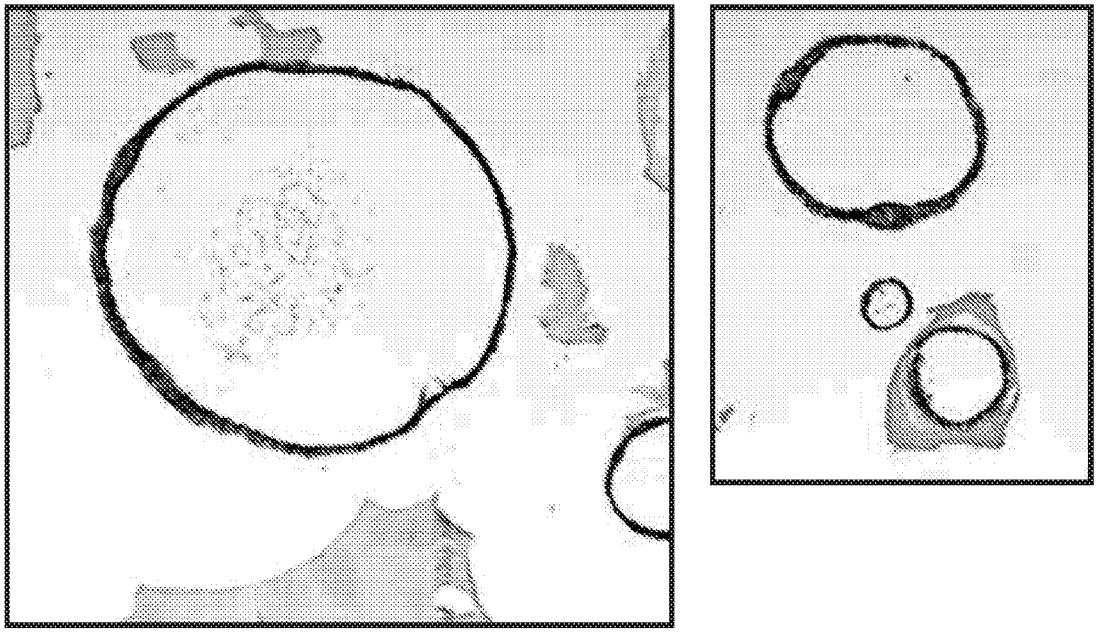
Prostate
(whole organ)



FIG. 41(contd.)

H&E after 3month culture 1 passage a week 5x split

Culture after 25 weeks EGF/Noggin/ RSp



Culture after 25 weeks EGF/Noggin/ RSp + Testosterone

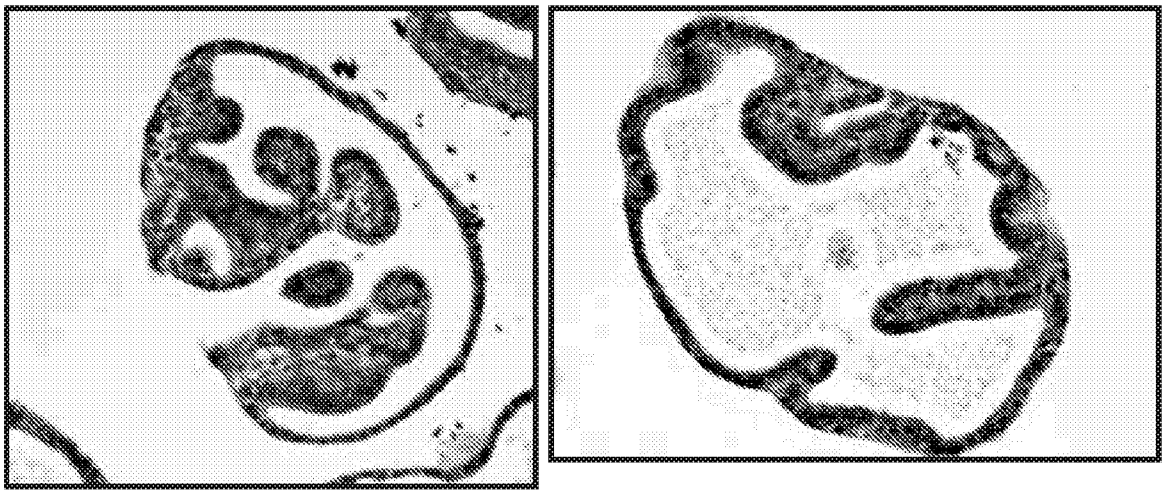
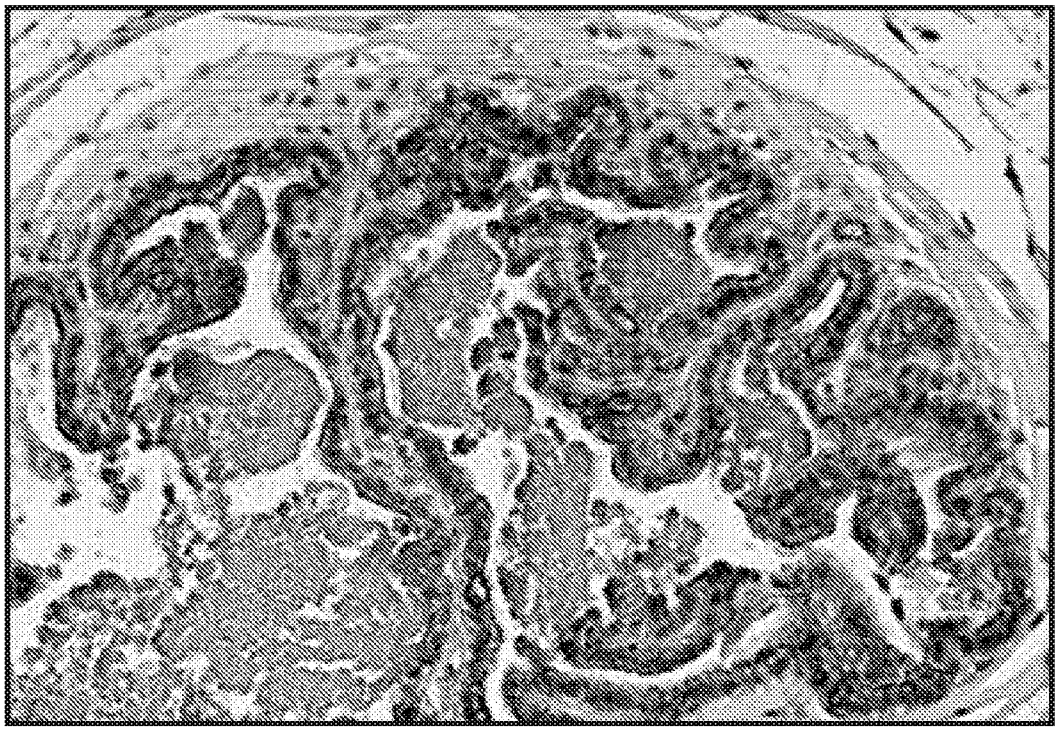


FIG. 42 CK8 staining (a differentiation marker)

Prostate (whole organ)



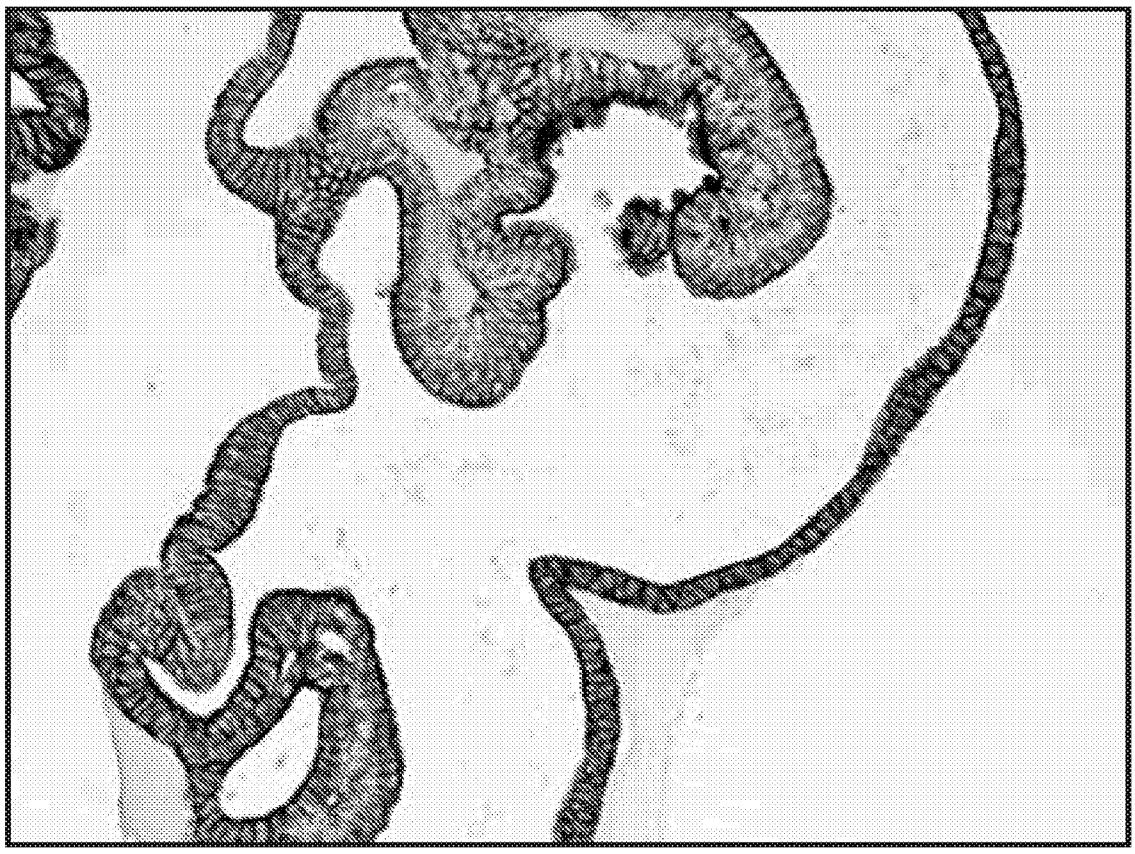
Culture after 25 weeks EGF/Noggin/ RSp



FIG. 42(contd.)

CK8 staining (a differentiation marker)

Culture after 25 weeks EGF/Noggin/ RSp + Testosterone



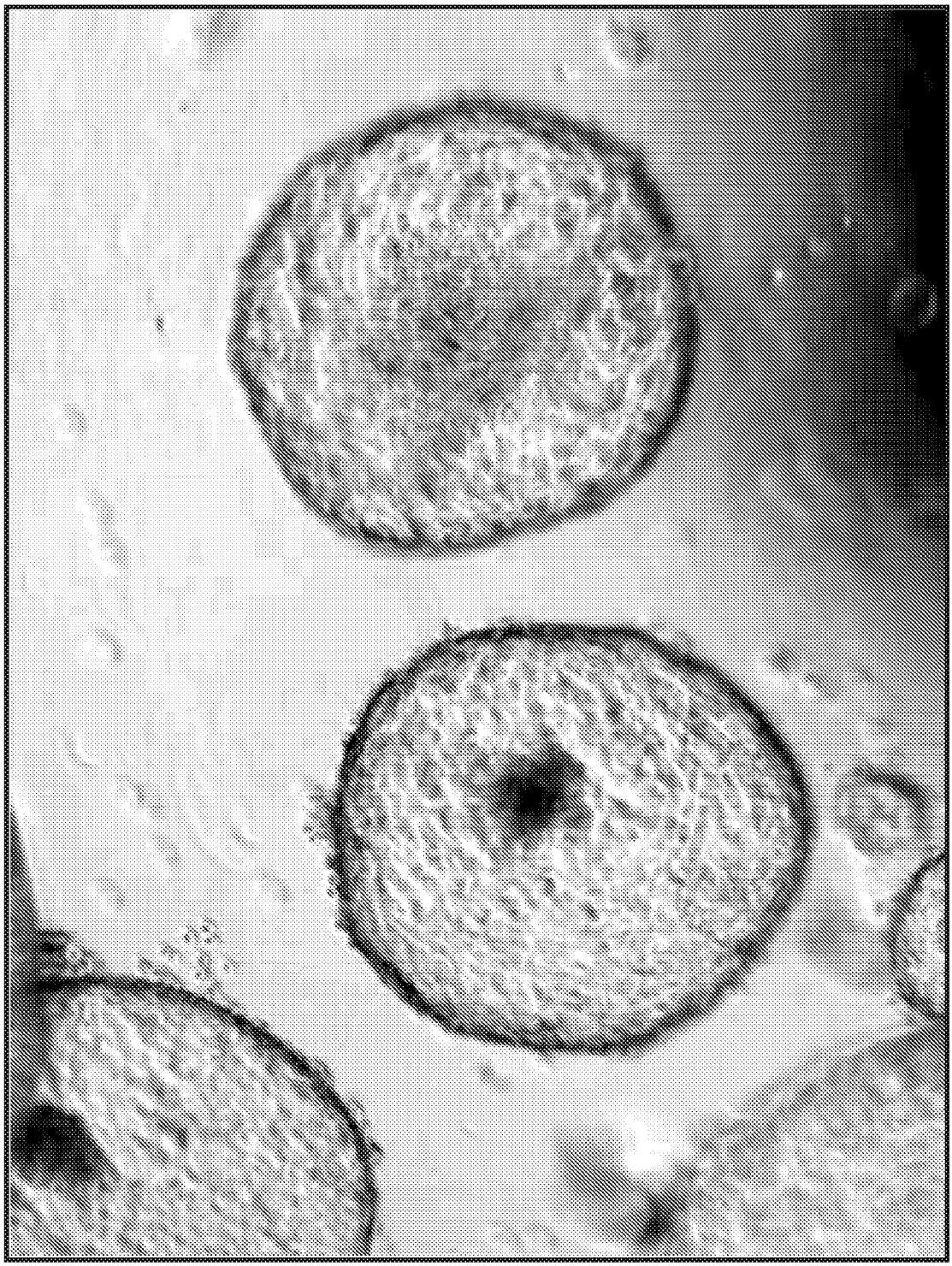


FIG. 43

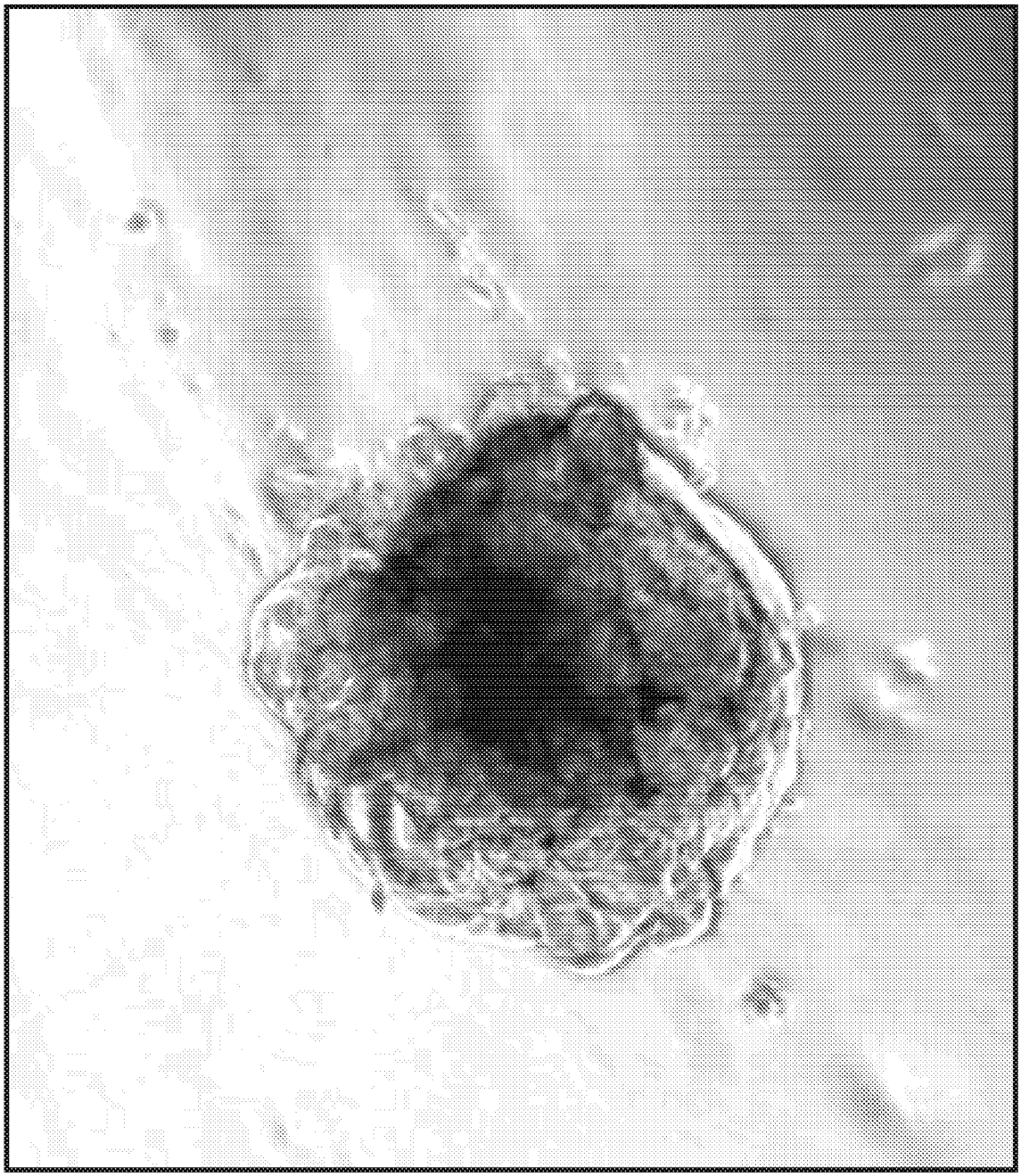


FIG. 43(contd.)

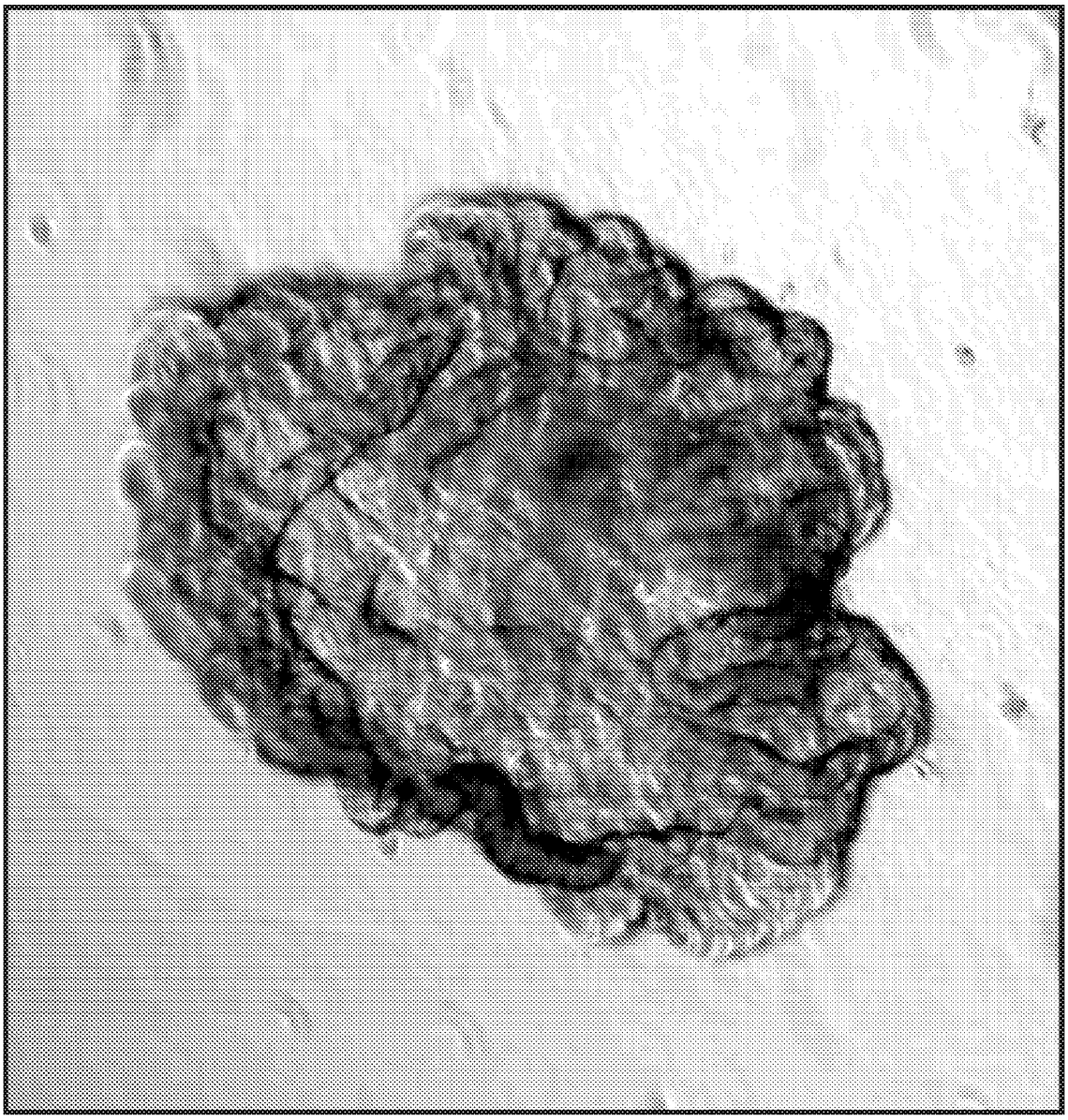


FIG. 43(contd.)

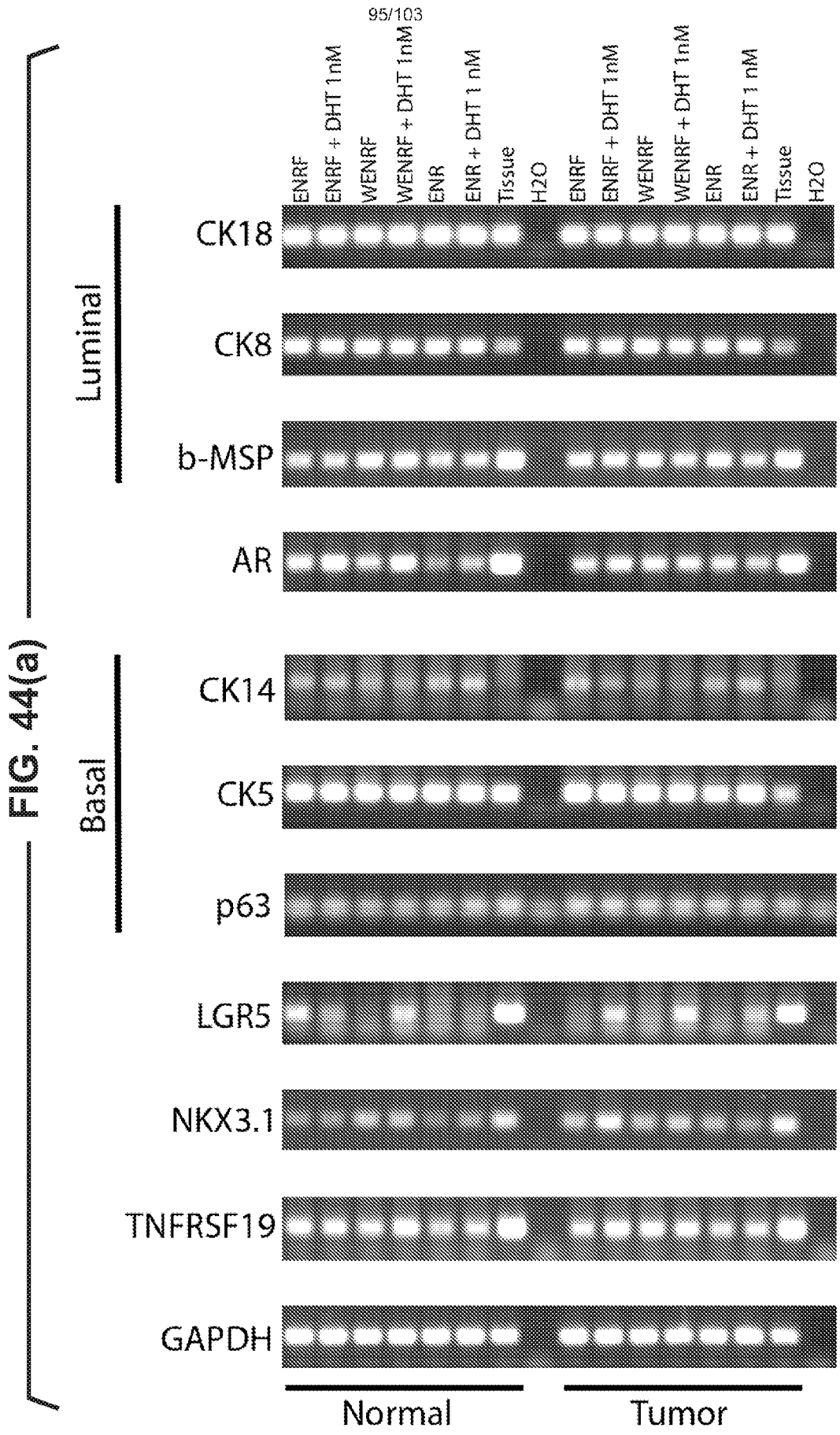

ENRF
ENRF + DHT 1nM
WENRF
WENRF + DHT 1nM
ENR
ENR + DHT 1 nM
Tissue
H2O
ENRF
ENRF + DHT 1nM
WENRF
WENRF + DHT 1nM
ENR
ENR + DHT 1 nM
Tissue
H2O
Luminal
CK18
CK8
b-MSP
AR
Basal
CK14
CK5
p63
LGR5
NKX3.1
TNFRSF19
GAPDH
Normal
Tumor

FIG. 44(a)

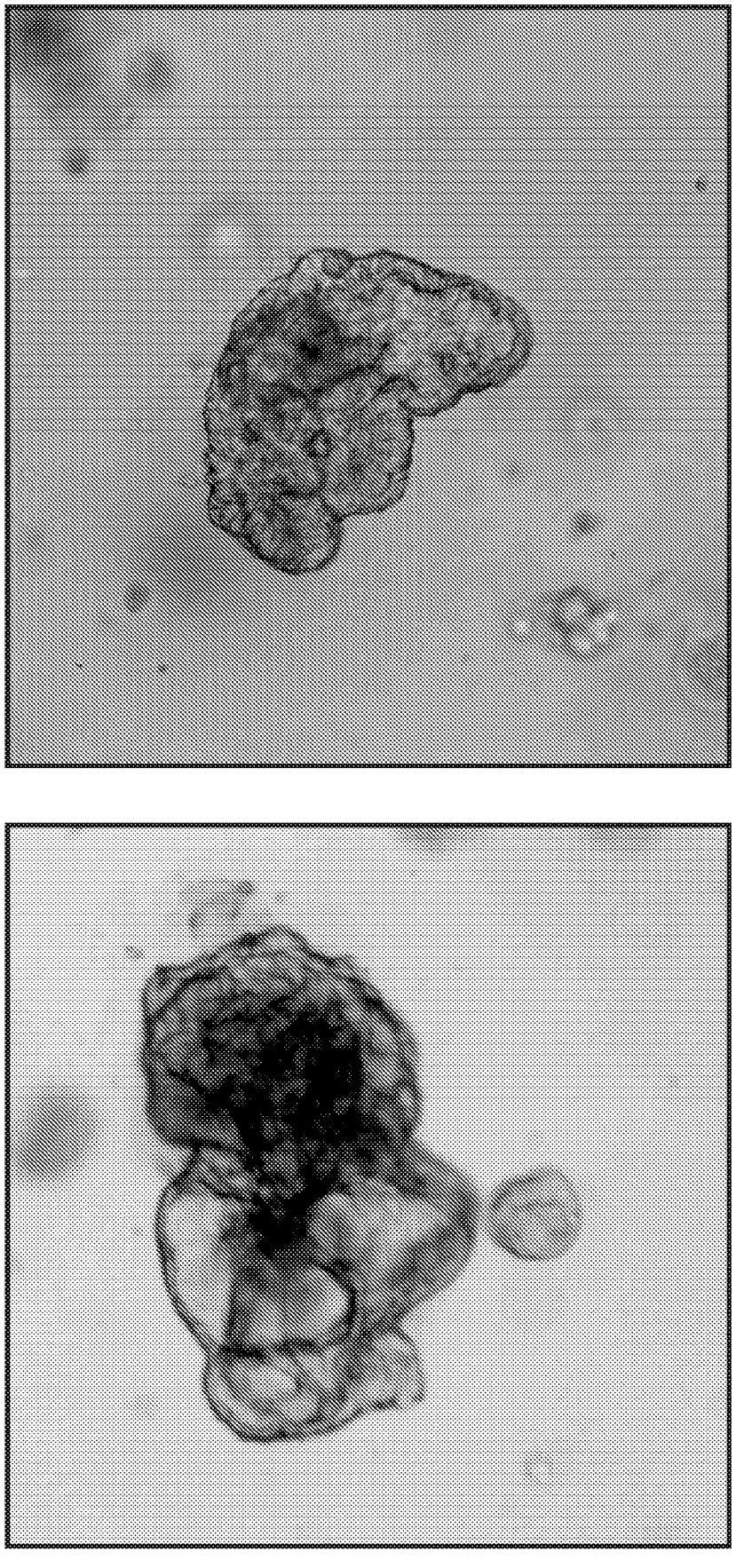


FIG. 44(b)

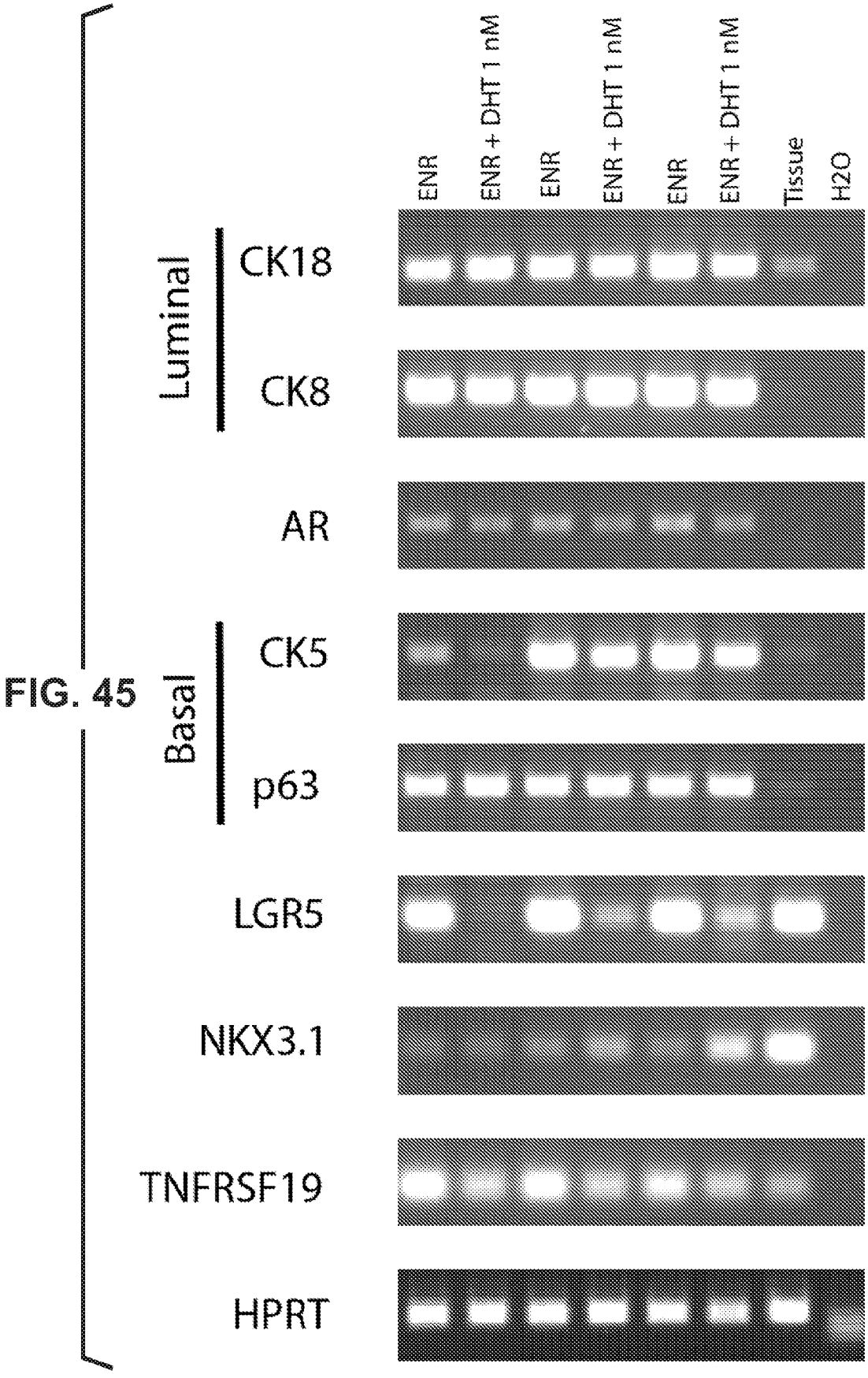

ENR
ENR + DHT 1 nM
ENR
ENR + DHT 1 nM
ENR
ENR + DHT 1 nM
Tissue
H2O
Luminal
CK18
CK8
AR
Basal
CK5
p63
LGR5
NKX3.1
TNFRSF19
HPRT

FIG. 45

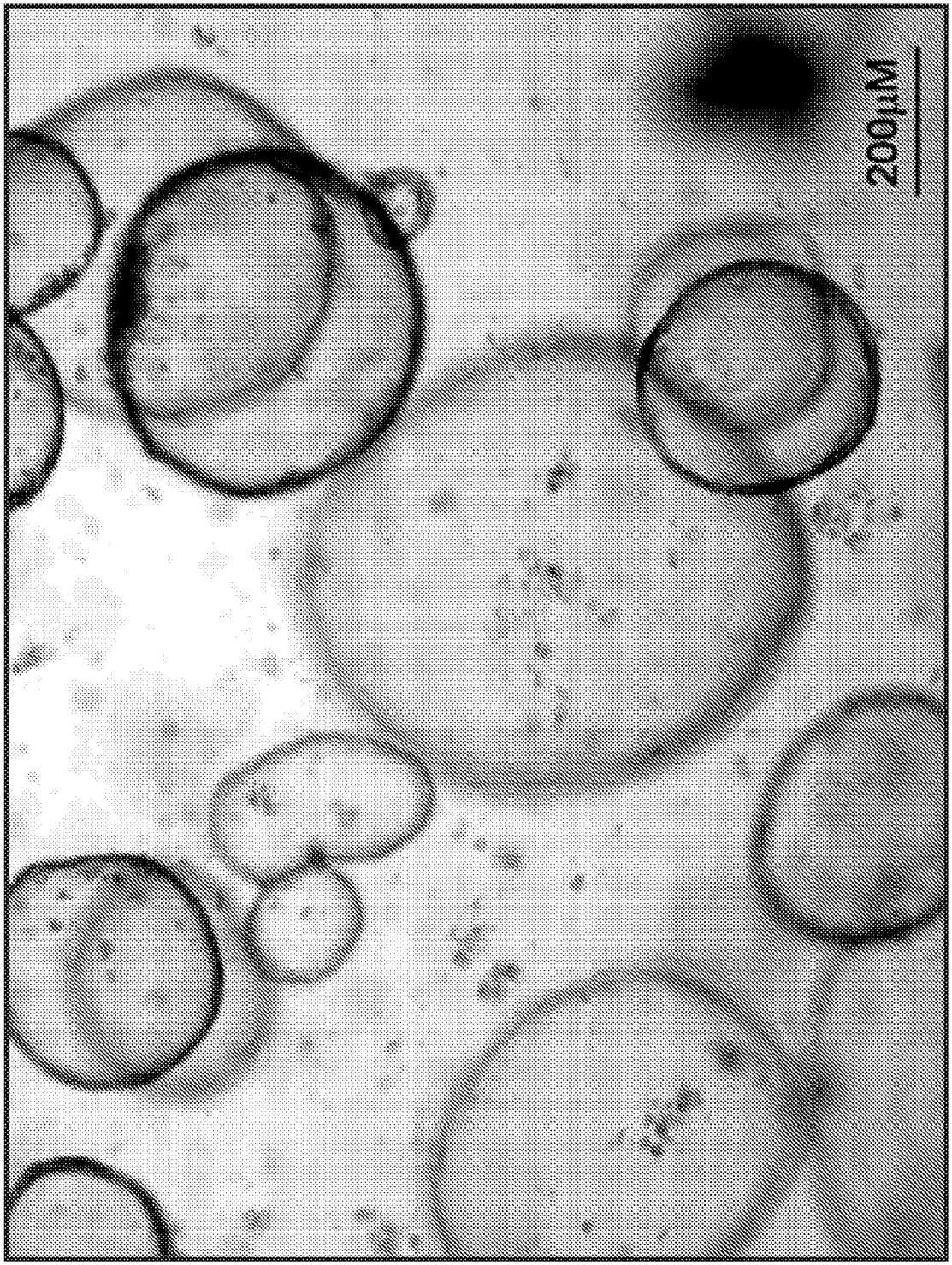
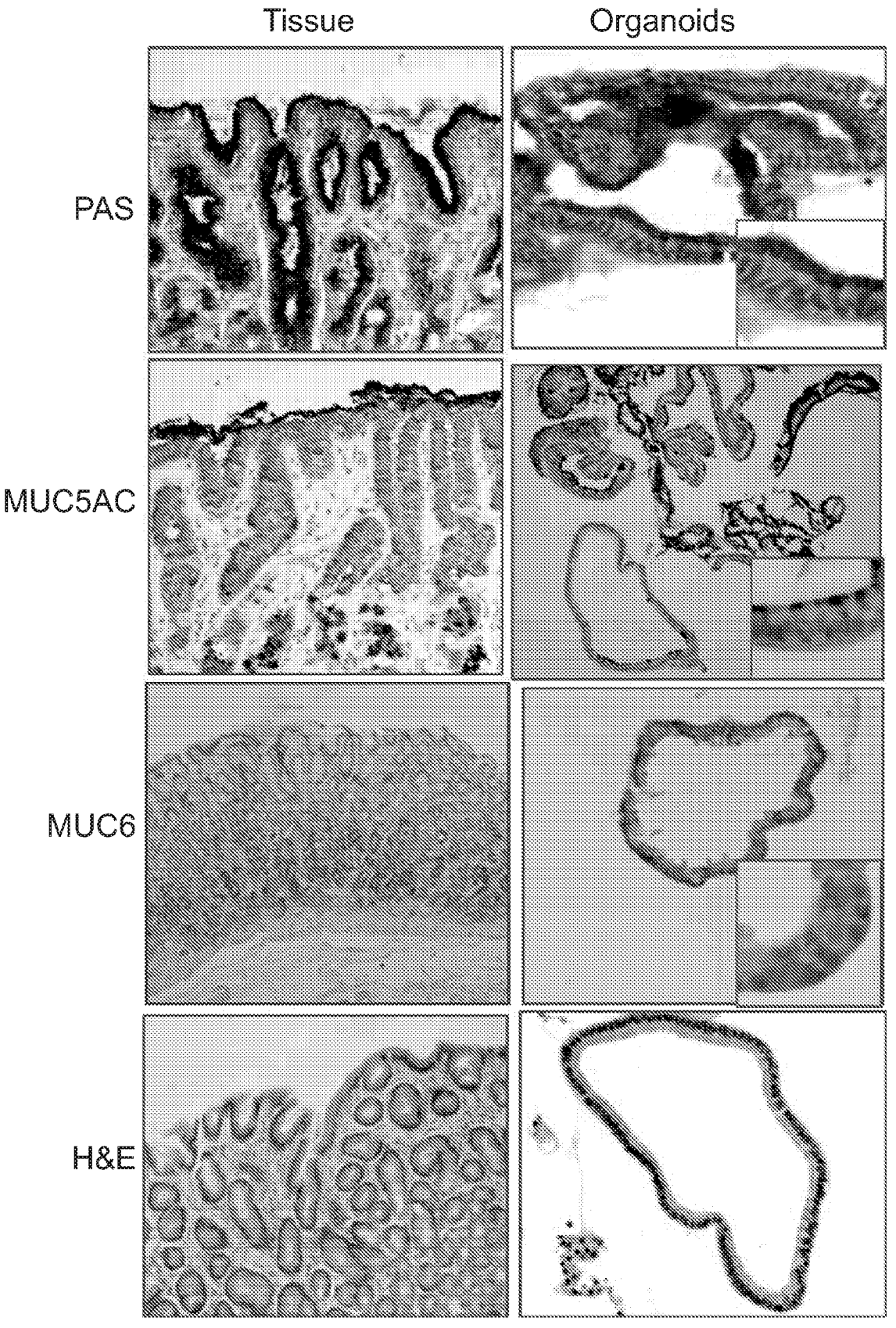


FIG. 46(a)

FIG. 46(b)



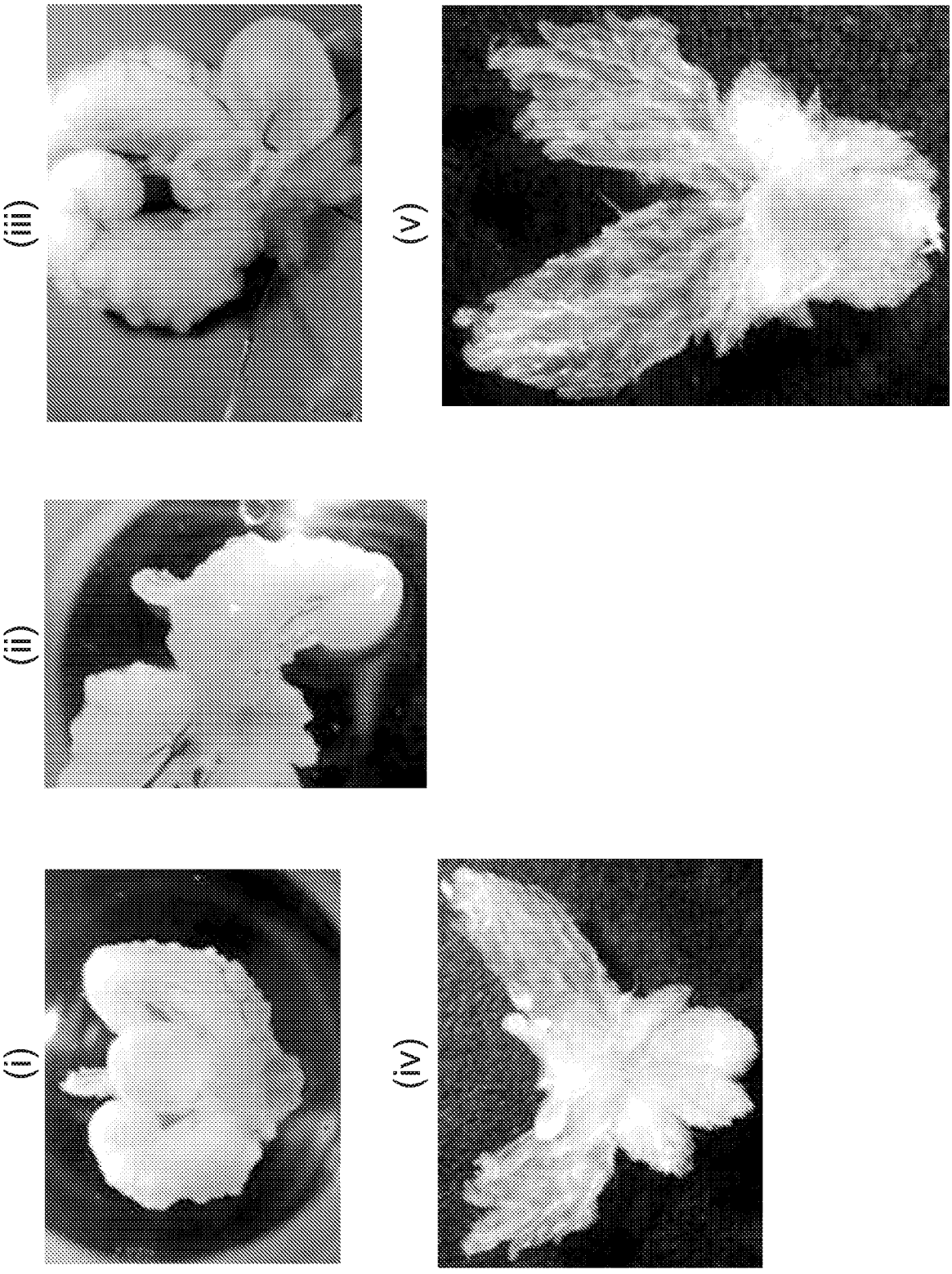
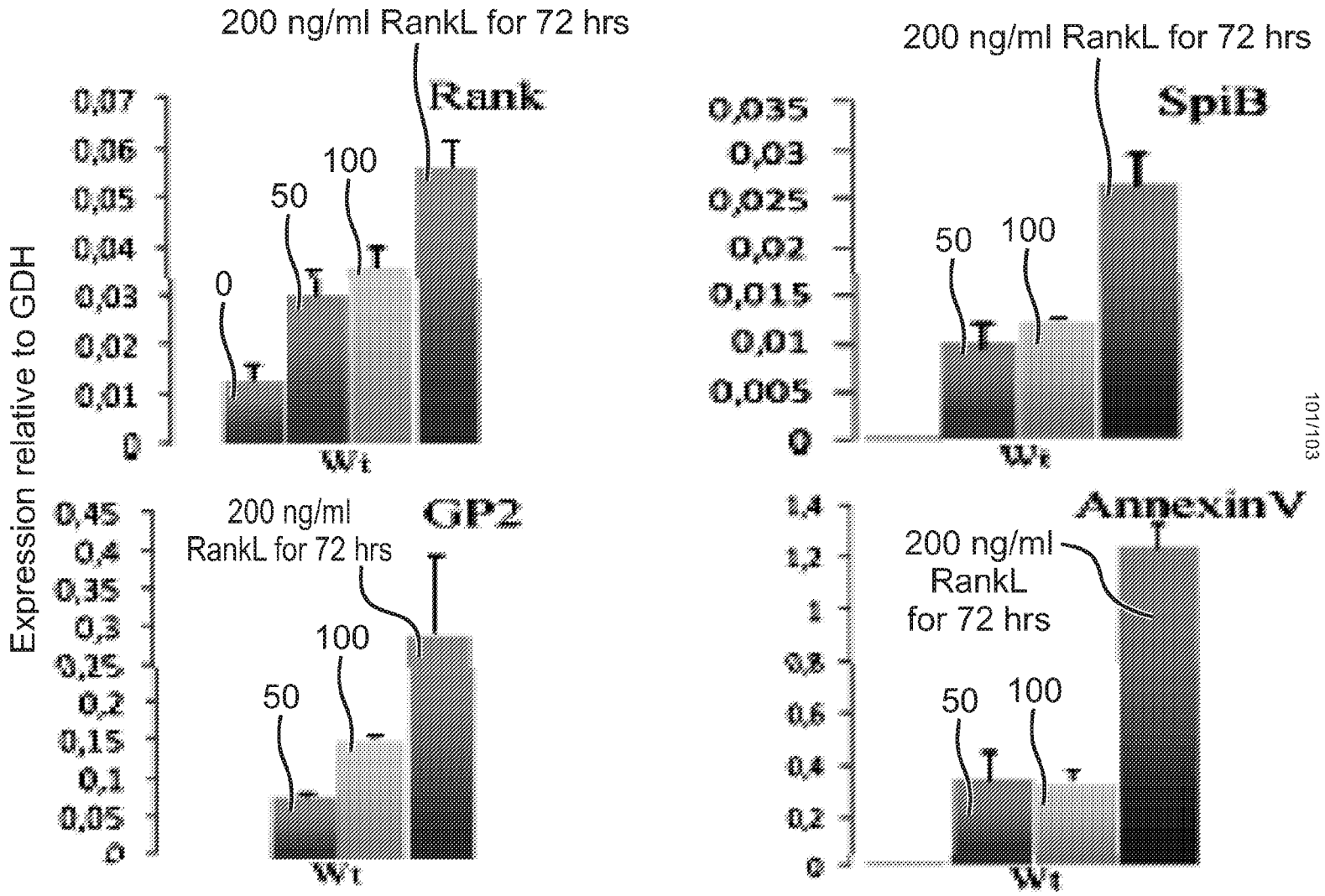


FIG. 47

FIG. 48(a)



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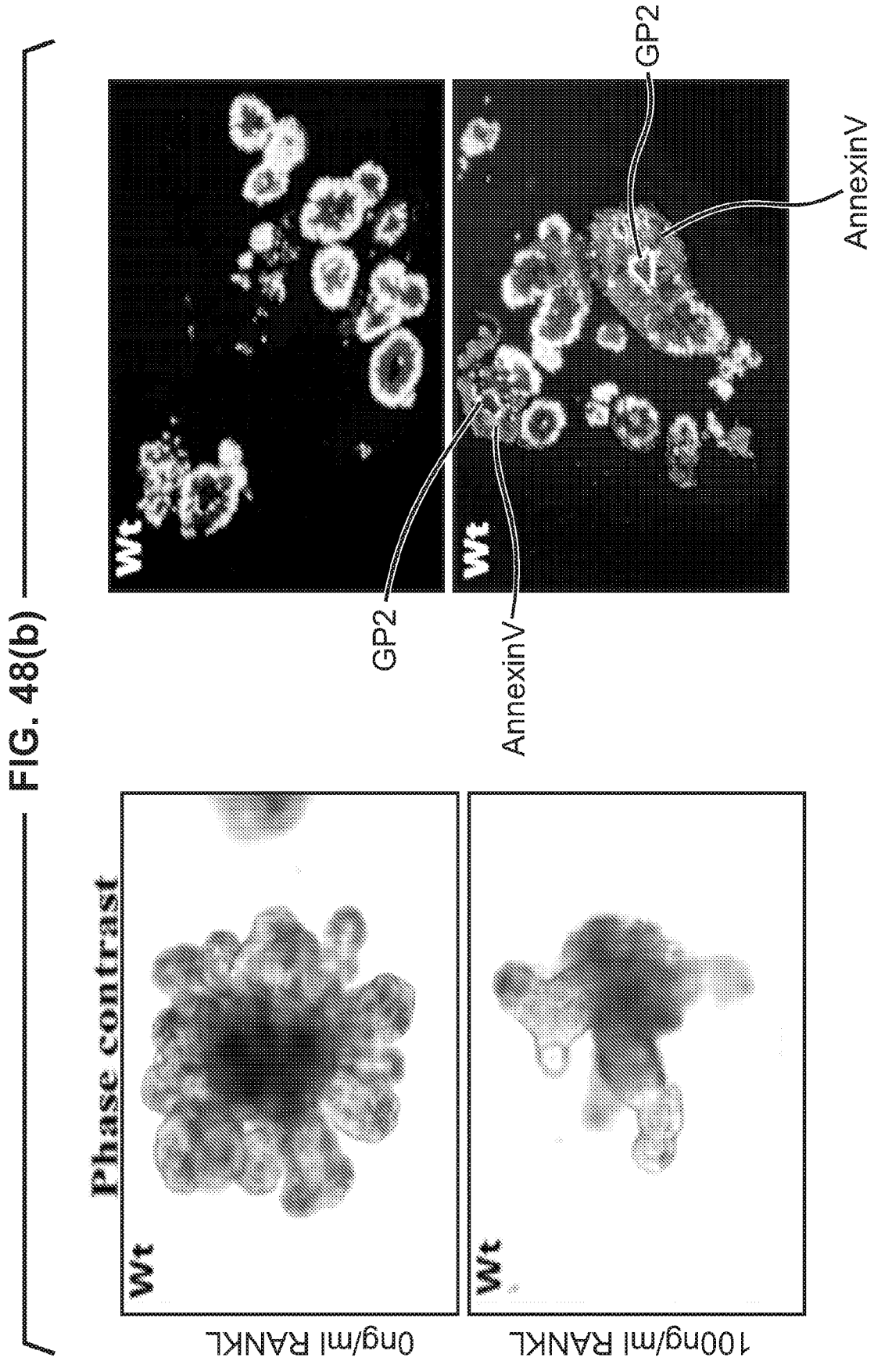
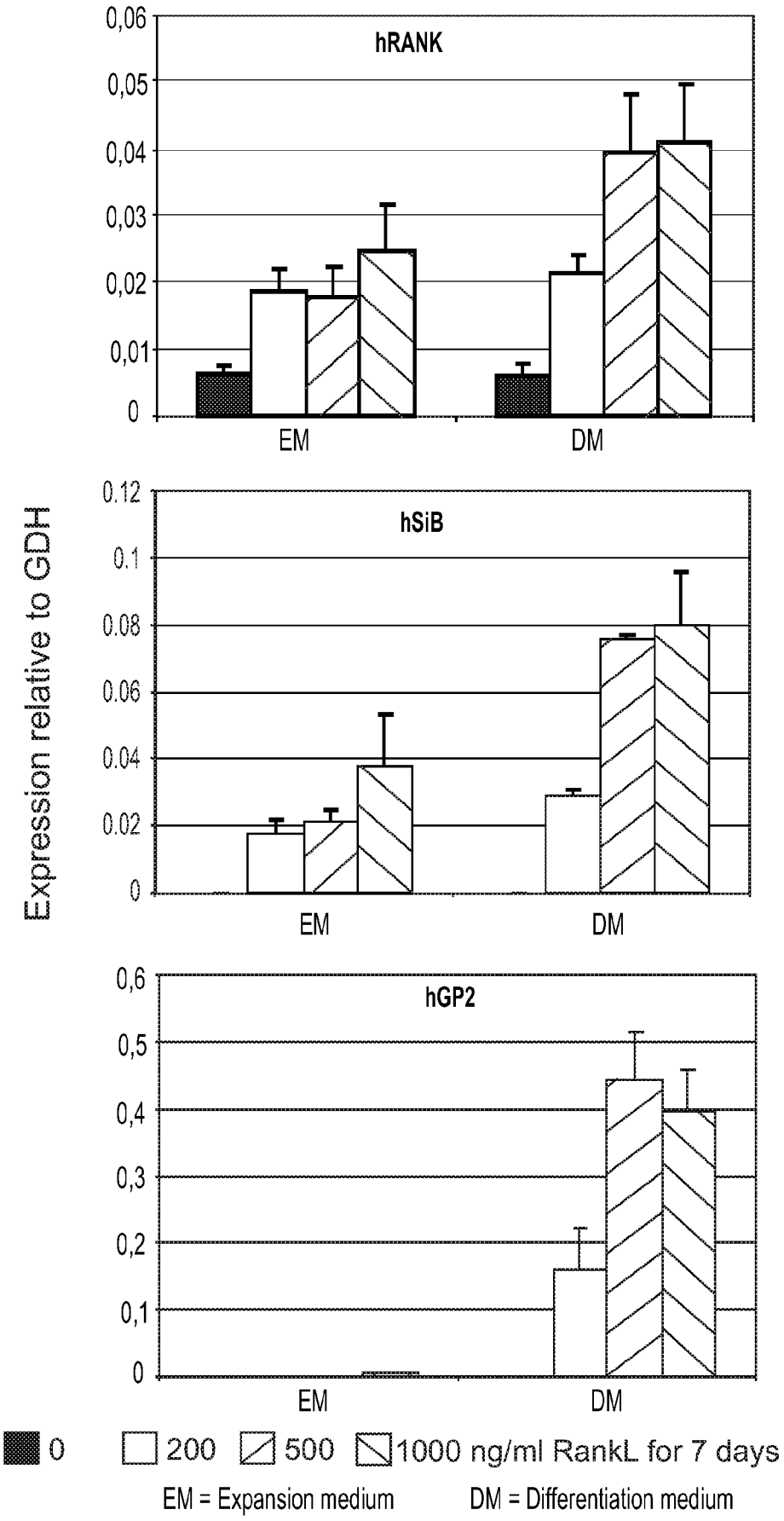

Phase contrast
Wt
0ng/ml RANKL
Wt
100ng/ml RANKL
Wt
GP2
AnnexinV
Wt
GP2
AnnexinV

FIG. 48(b)

FIG. 49


hRANK
0,06
0,05
0,04
0,03
0,02
0,01
0
EM
DM
hSiB
0.12
0.1
0.08
0.06
0.04
0.02
0
EM
DM
hGP2
0,6
0,5
0,4
0,3
0,2
0,1
0
EM
DM
Expression relative to GDH
0
200
500
1000 ng/ml RankL for 7 days
EM = Expansion medium
DM = Differentiation medium