FIG. 1

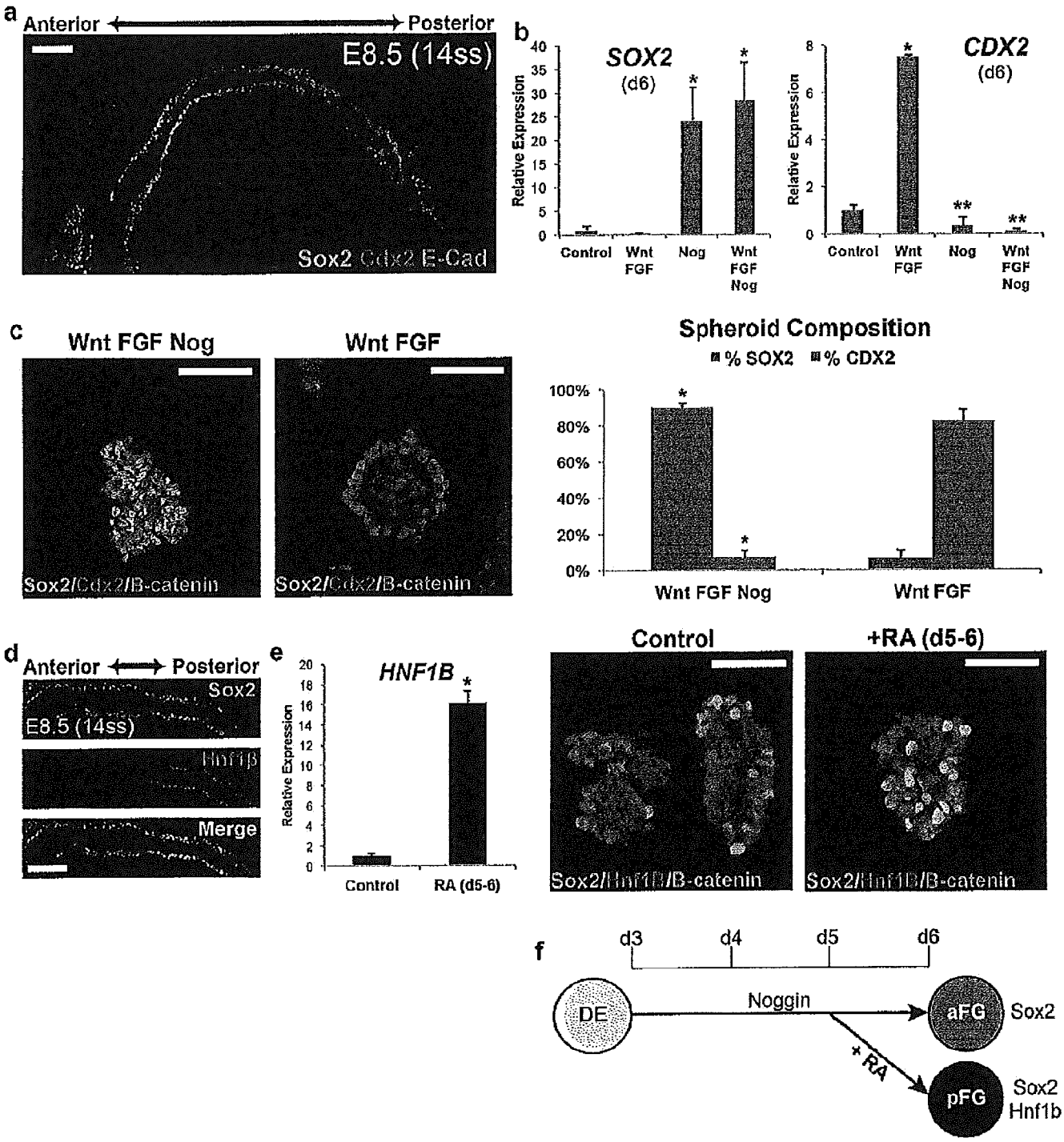

a
Anterior
Posterior
E8.5 (14ss)
Sox2 Cdx2 E-Cad
b
SOX2
(d6)
CDX2
(d6)
Relative Expression
Control
Wnt FGF
Nog
Wnt FGF Nog
c
Wnt FGF Nog
Wnt FGF
Sox2/Cdx2/B-catenin
Spheroid Composition
% SOX2
% CDX2
100%
80%
60%
40%
20%
0%
d
Anterior
Posterior
Sox2
E8.5 (14ss)
Hnf1β
Merge
e
HNF1B
Control
RA (d5-6)
Control
+RA (d5-6)
f
d3
d4
d5
d6
DE
Noggin
aFG
Sox2
+RA
pFG
Sox2
Hnf1b

FIG. 2

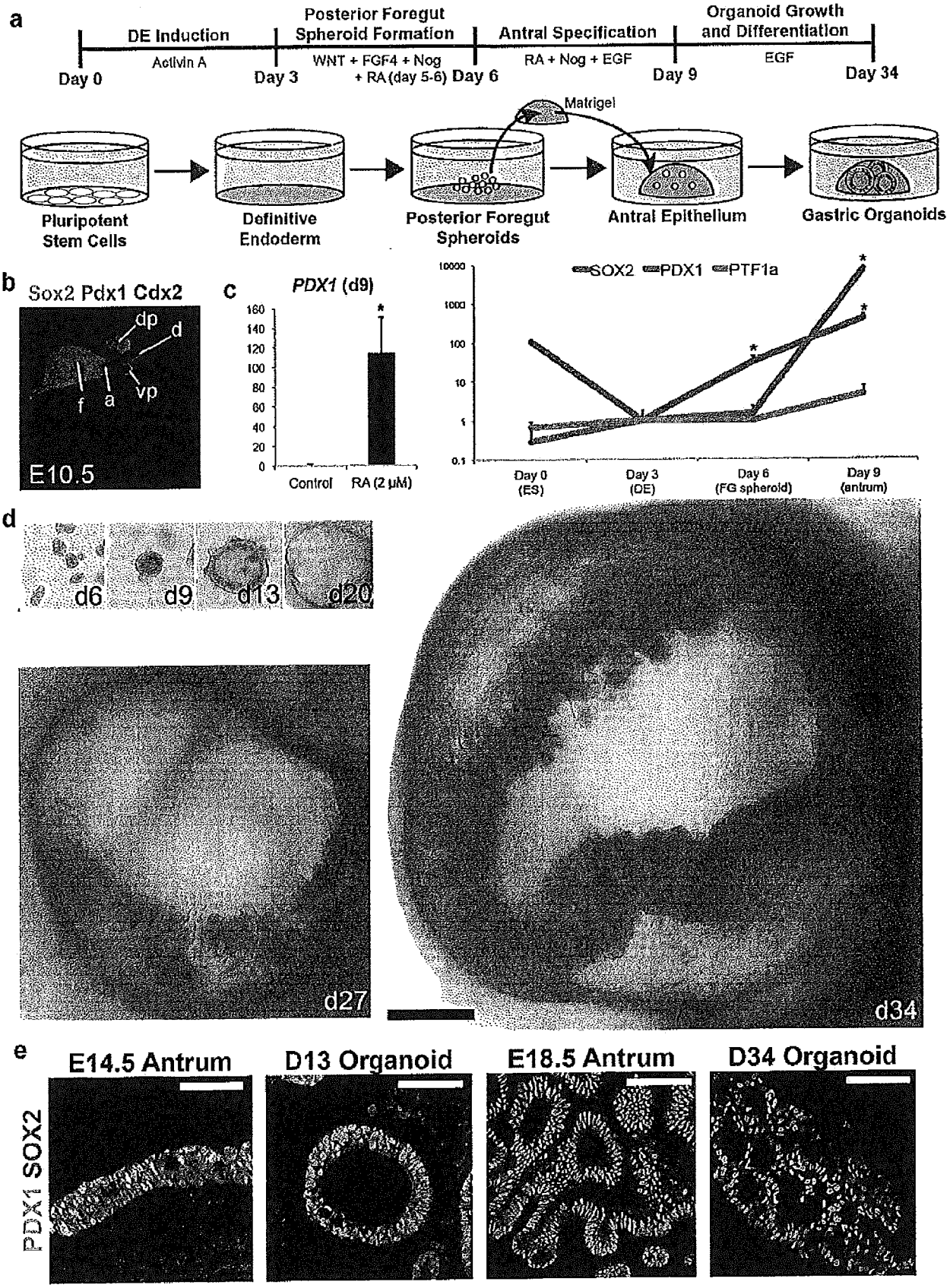

a
DE Induction
Activin A
Day 0
Day 3
Posterior Foregut Spheroid Formation
WNT + FGF4 + Nog + RA (day 5-6)
Day 6
Antral Specification
RA + Nog + EGF
Day 9
Organoid Growth and Differentiation
EGF
Day 34
Matrigel
Pluripotent Stem Cells
Definitive Endoderm
Posterior Foregut Spheroids
Antral Epithelium
Gastric Organoids
b
Sox2 Pdx1 Cdx2
dp
d
f
a
vp
E10.5
c
PDX1 (d9)
Control
RA (2 μM)
SOX2
PDX1
PTF1a
Day 0 (ES)
Day 3 (DE)
Day 6 (FG spheroid)
Day 9 (antrum)
d
d6
d9
d13
d20
d27
d34
e
E14.5 Antrum
D13 Organoid
E18.5 Antrum
D34 Organoid
PDX1 SOX2

FIG. 3

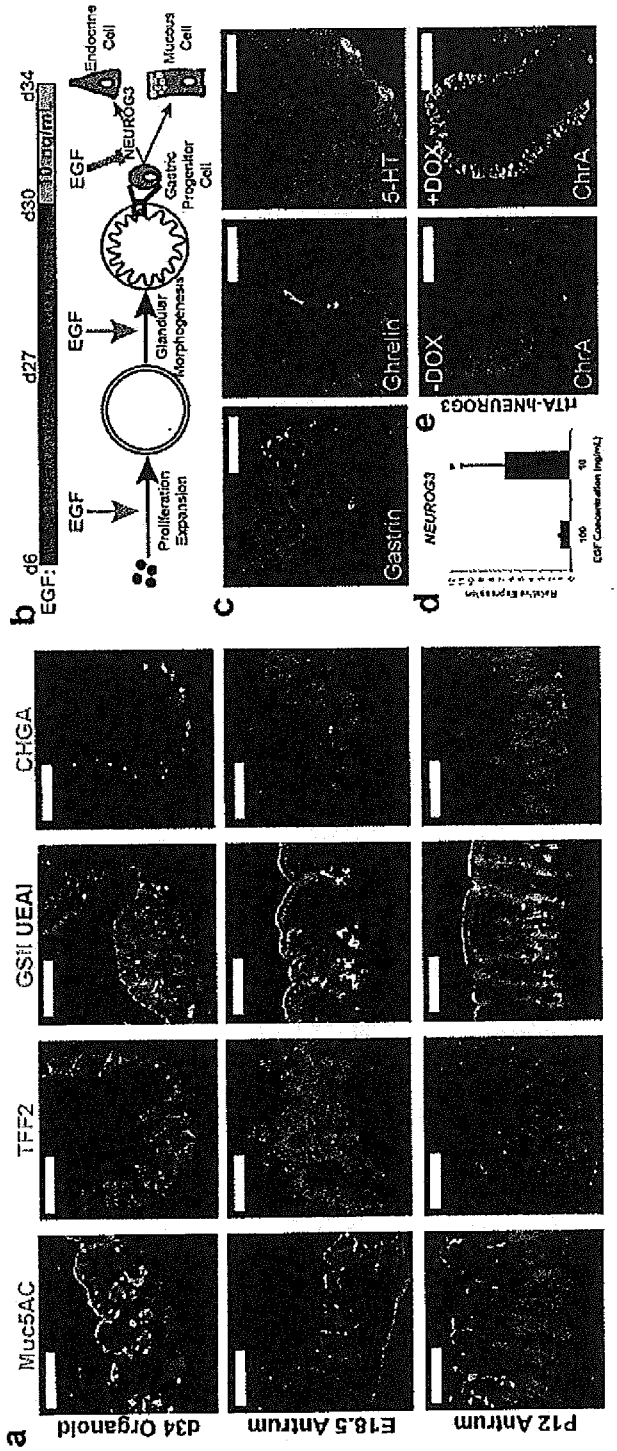

a
Muc5AC
TFF2
GSII UEAI
CHGA
d34 Organoid
E18.5 Antrum
P12 Antrum
b
EGF:
d6
d27
d30
d34
EGF
EGF
EGF
Proliferation Expansion
Glandular Morphogenesis
Gastric Progenitor Cell
NEUROG3
Endocrine Cell
Mucous Cell
c
Gastrin
Ghrelin
5-HT
d
NEUROG3
e
rtTA-hNEUROG3
-DOX
+DOX
ChrA
ChrA

FIG. 4

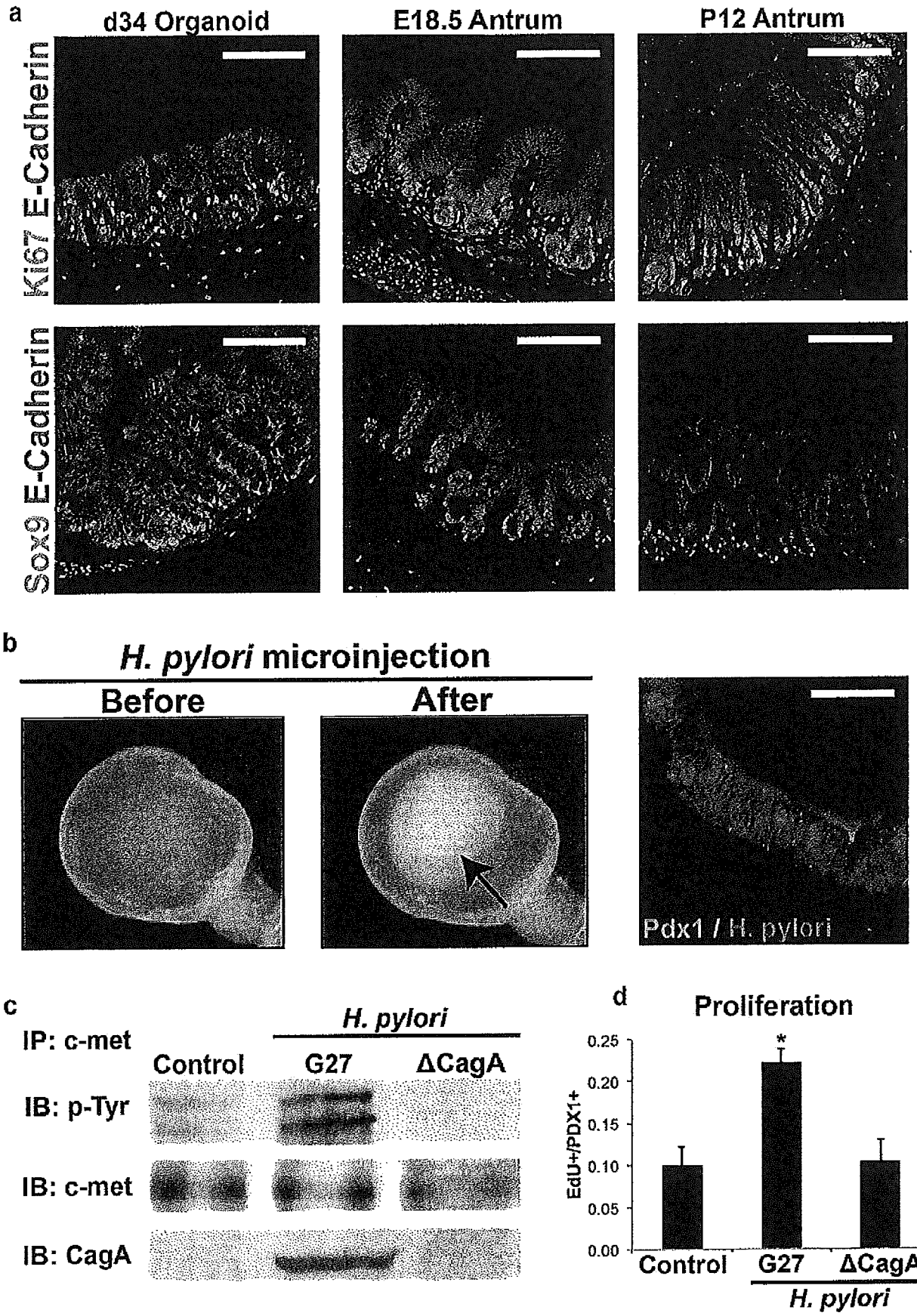

a
d34 Organoid
E18.5 Antrum
P12 Antrum
Ki67 E-Cadherin
Sox9 E-Cadherin
b
H. pylori microinjection
Before
After
Pdx1 / H. pylori
c
IP: c-met
H. pylori
Control
G27
ΔCagA
IB: p-Tyr
IB: c-met
IB: CagA
d
Proliferation
EdU+/PDX1+
0.25
0.20
0.15
0.10
0.05
0.00
*
Control
G27
ΔCagA
H. pylori

FIG. 5

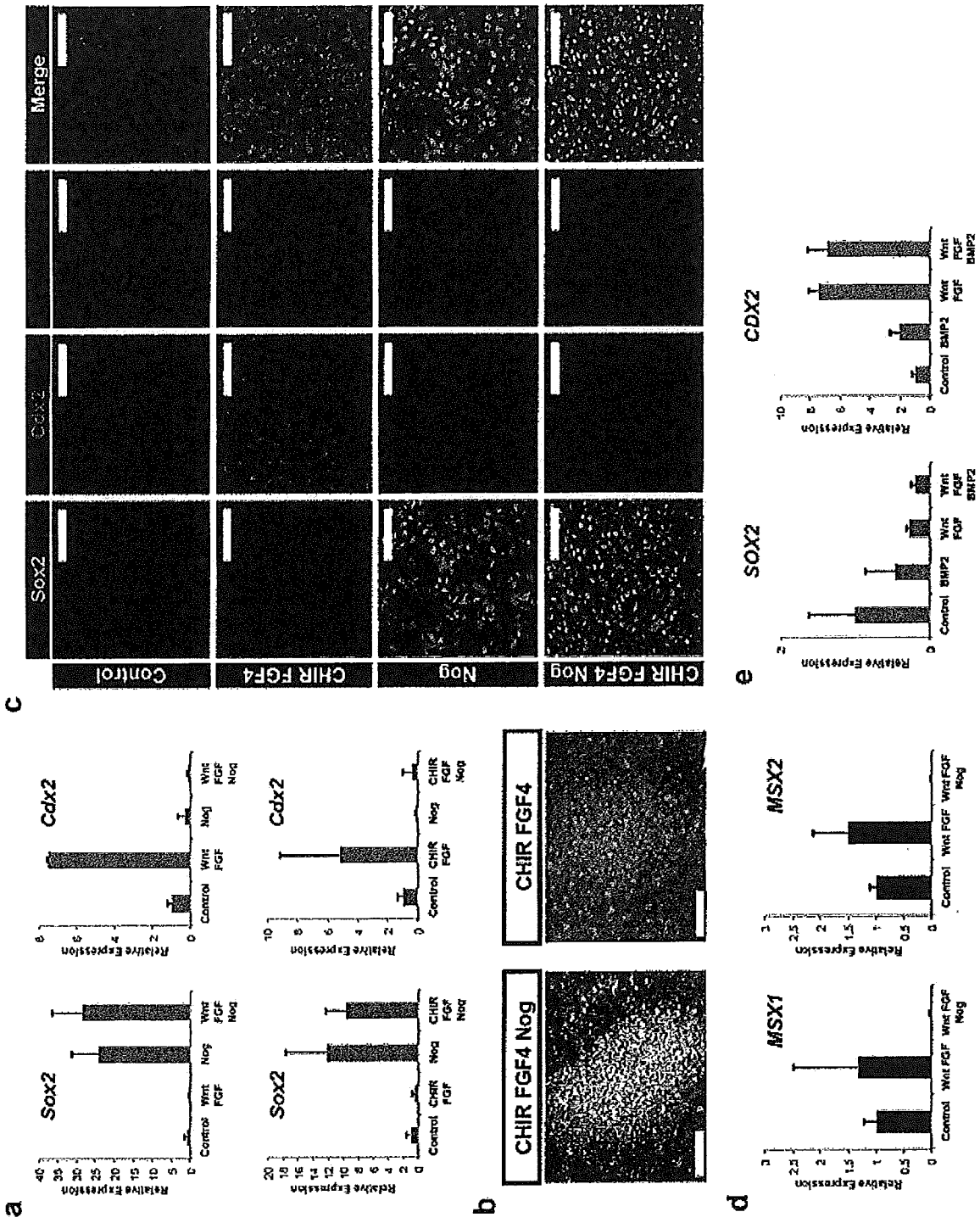

a
Sox2
Cdx2
Relative Expression
Control
Wnt FGF
Nog
CHIR FGF
Wnt FGF Nog
CHIR FGF Nog
b
CHIR FGF4 Nog
CHIR FGF4
c
Sox2
Cdx2
Merge
Control
CHIR FGF4
Nog
CHIR FGF4 Nog
d
MSX1
MSX2
e
SOX2
CDX2
BMP2
Wnt FGF BMP2

FIG. 6

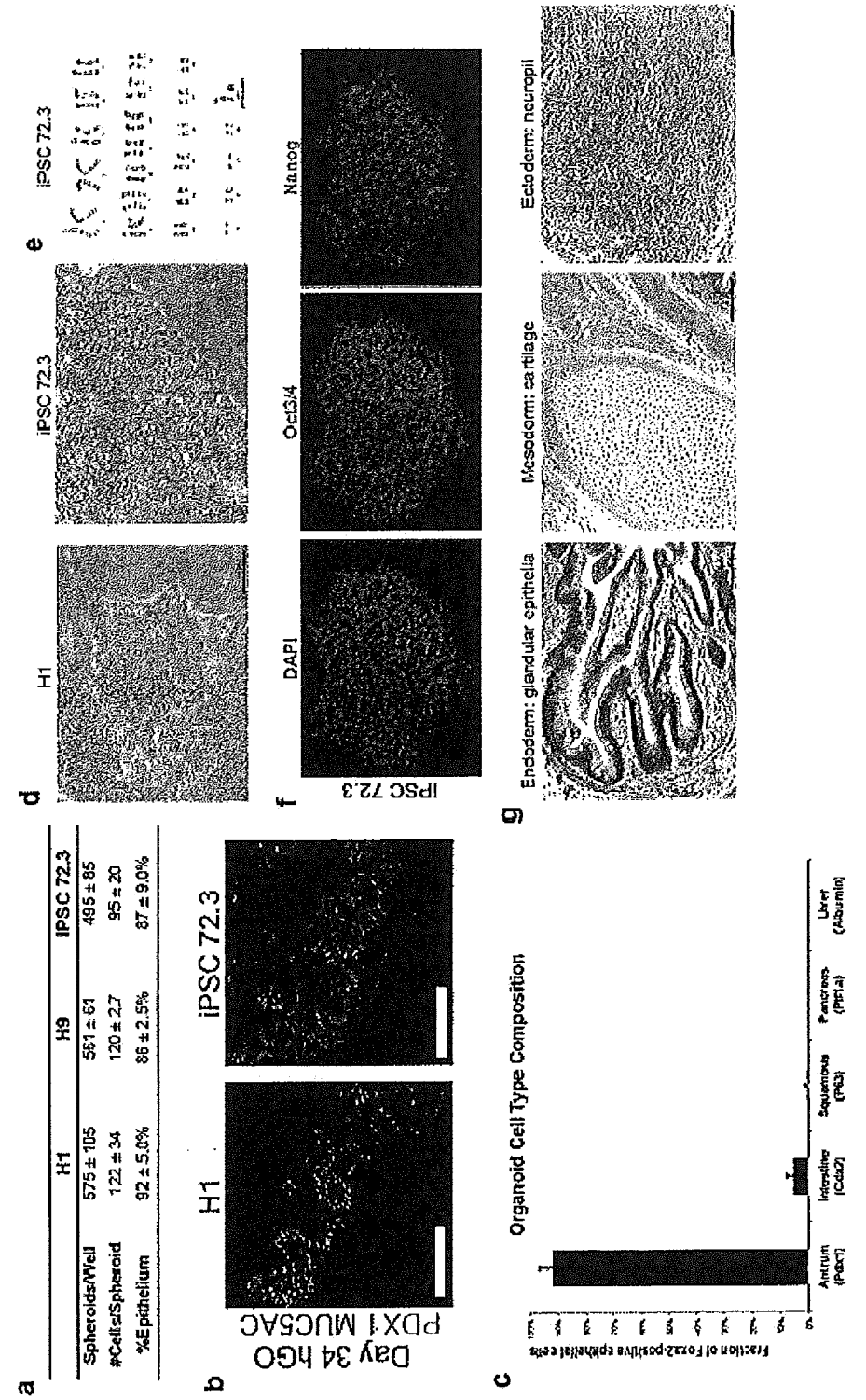
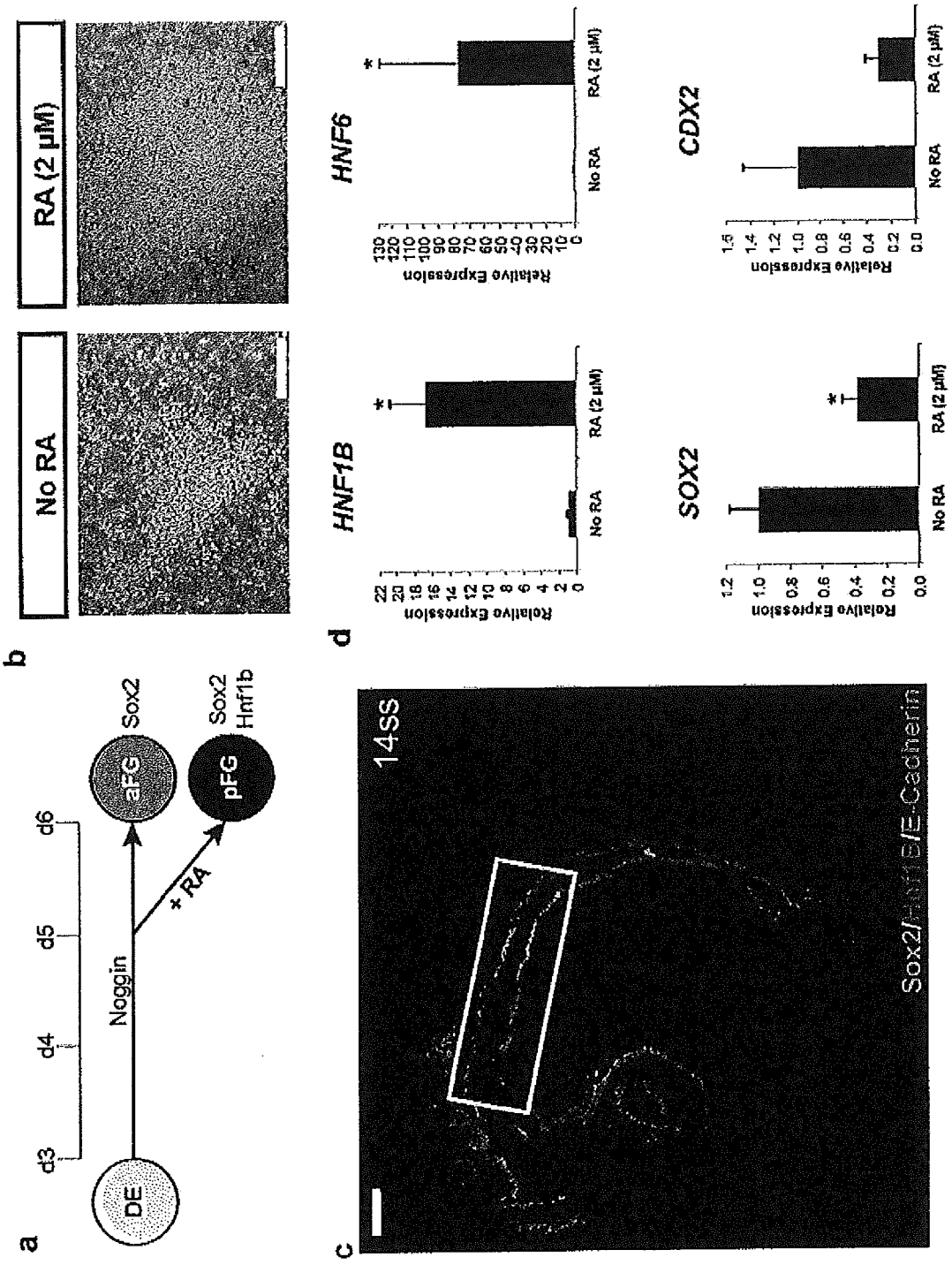

a
H1
H9
iPSC 72.3
Spheroids/Well
575 ± 105
561 ± 61
495 ± 85
#Cells/Spheroid
122 ± 34
120 ± 27
95 ± 20
%Epithelium
92 ± 5.0%
86 ± 2.5%
87 ± 9.0%
b
Day 34 hGO
PDX1 MUC5AC
H1
iPSC 72.3
c
Organoid Cell Type Composition
Fraction of Foxa2-positive epithelial cells
Antrum (PdxCre)
Intestine (Cdx2)
Squamous (P63)
Pancreas (Ptf1a)
Liver (Albumin)
d
H1
iPSC 72.3
e
iPSC 72.3
f
DAPI
Oct3/4
Nanog
iPSC 72.3
g
Endoderm: glandular epithelia
Mesoderm: cartilage
Ecto derm: neuropil

FIG. 7


a
d3
d4
d5
d6
DE
Noggin
+ RA
aFG
Sox2
pFG
Sox2
Hnf1b
b
No RA
RA (2 μM)
c
14ss
Sox2/HNF1B/E-Cadherin
d
HNF1B
HNF6
SOX2
CDX2
Relative Expression
No RA
RA (2 μM)
*

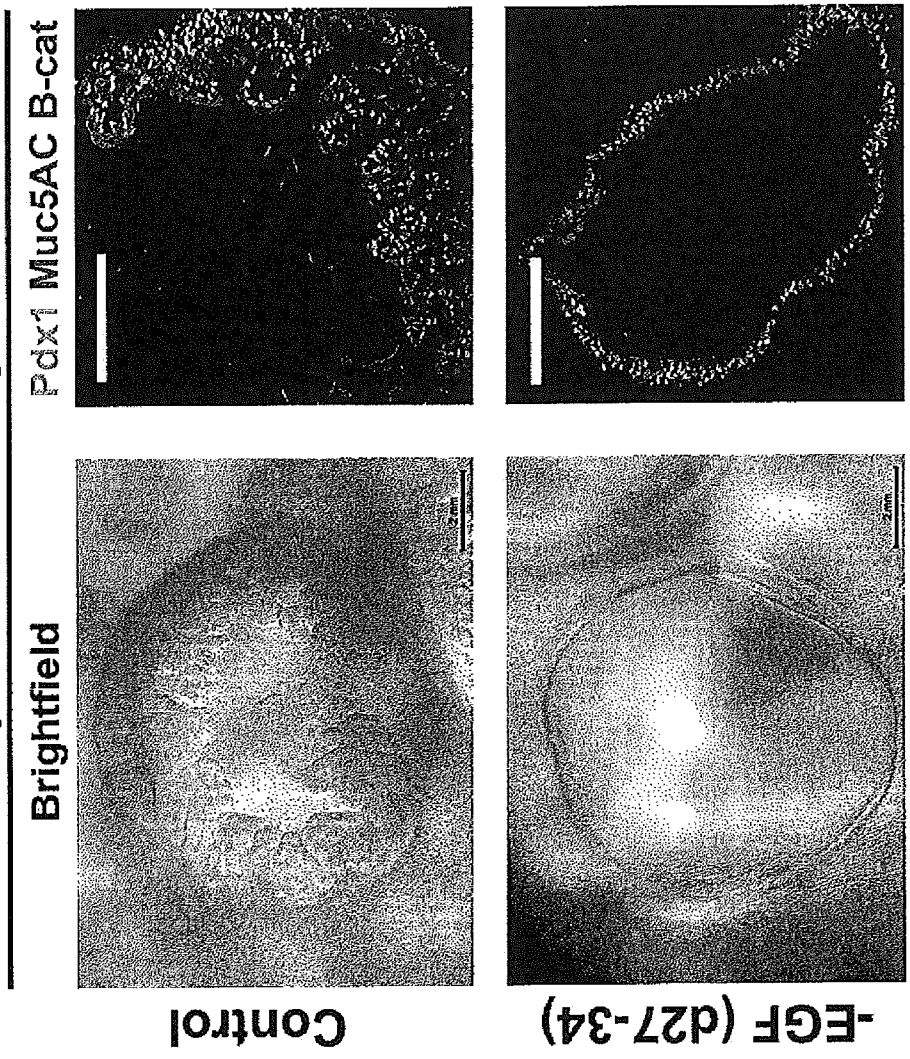
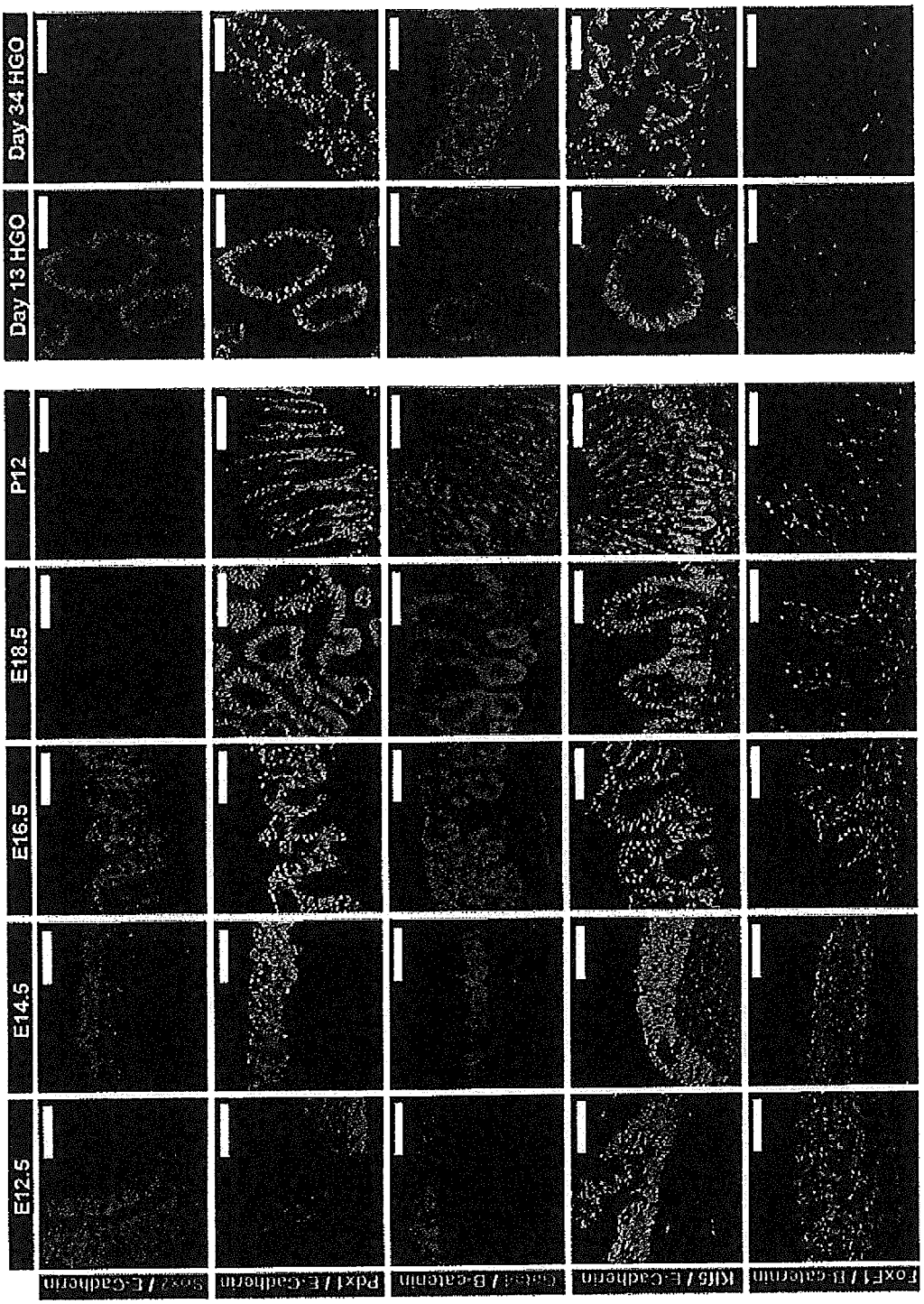

Day 34 Gastric Organoids
Brightfield
Pdx1 Muc5AC B-cat
Control
-EGF (d27-34)

FIG. 8

FIG. 9


E12.5
E14.5
E16.5
E18.5
P12
Day 13 HGO
Day 34 HGO
Sox2 / E-Cadherin
Pdx1 / E-Cadherin
Cdx2 / B-catenin
Klf5 / E-Cadherin
FoxF1 / B-catenin

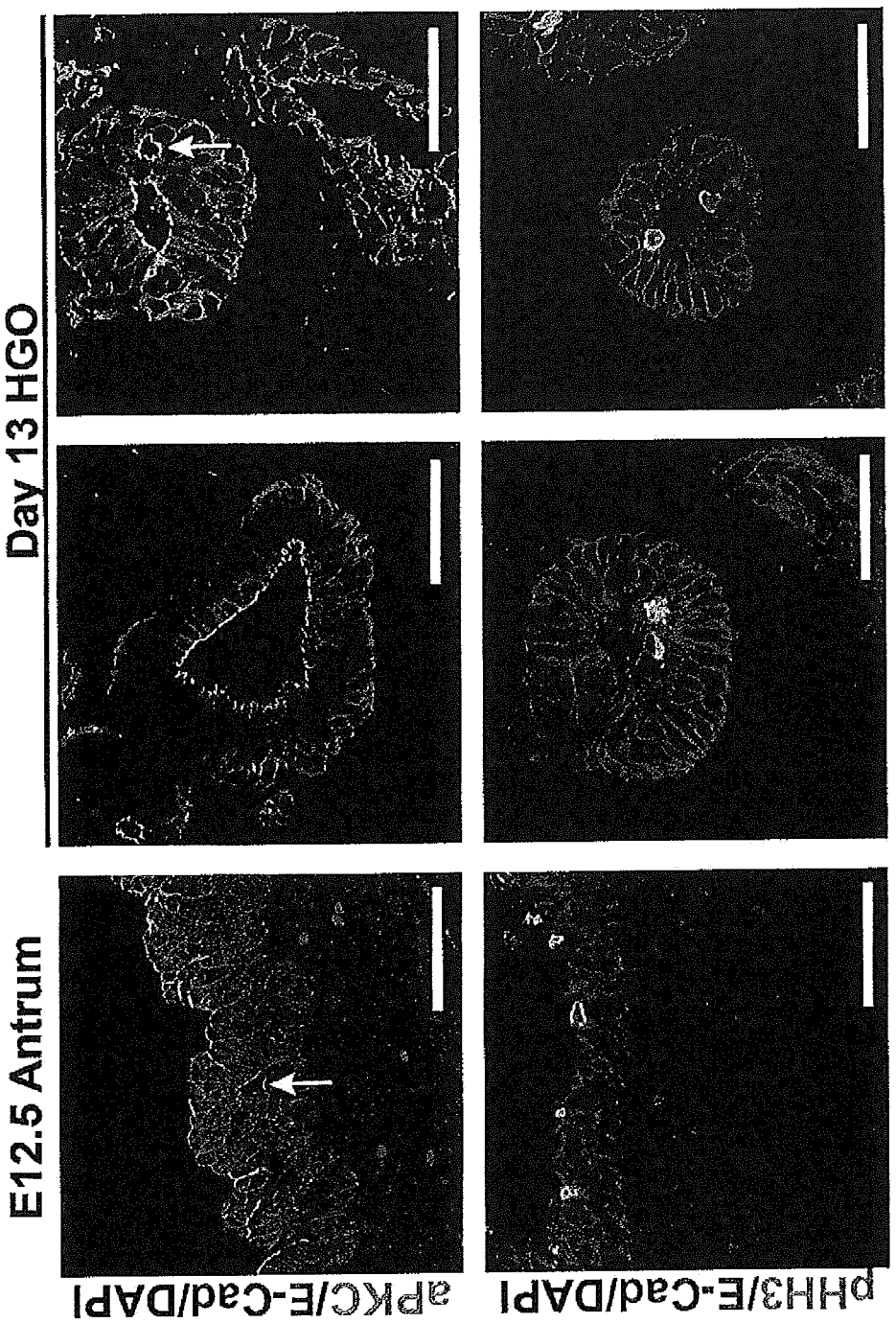
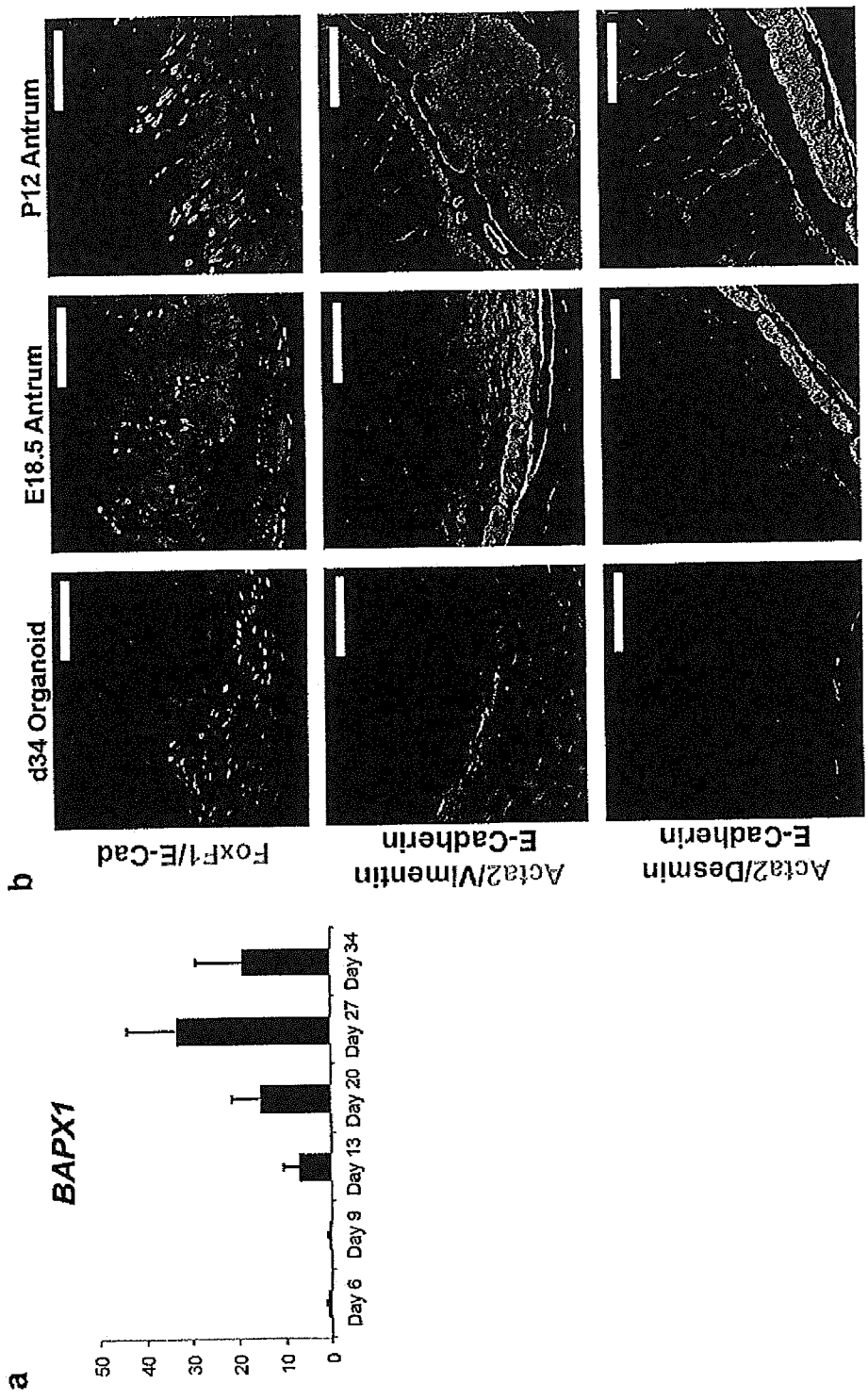


FIG. 10

FIG. 11


a
BAPX1
50
40
30
20
10
0
Day 6
Day 9
Day 13
Day 20
Day 27
Day 34
b
d34 Organoid
E18.5 Antrum
P12 Antrum
FoxF1/E-Cad
Acta2/Vimentin
E-Cadherin
Acta2/Desmin
E-Cadherin

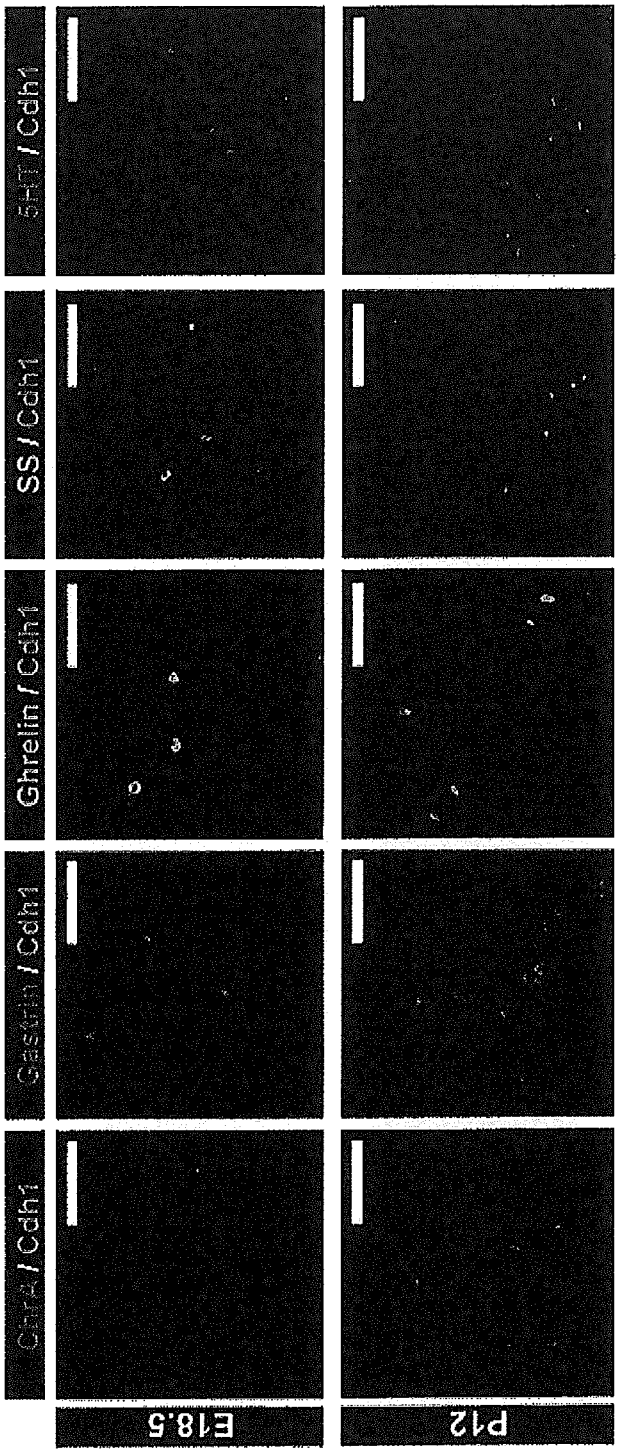

Ghrl / Cdh1
Gastrin / Cdh1
Ghrelin / Cdh1
SS / Cdh1
5-HT / Cdh1
E18.5
P12

FIG. 12

FIG. 13

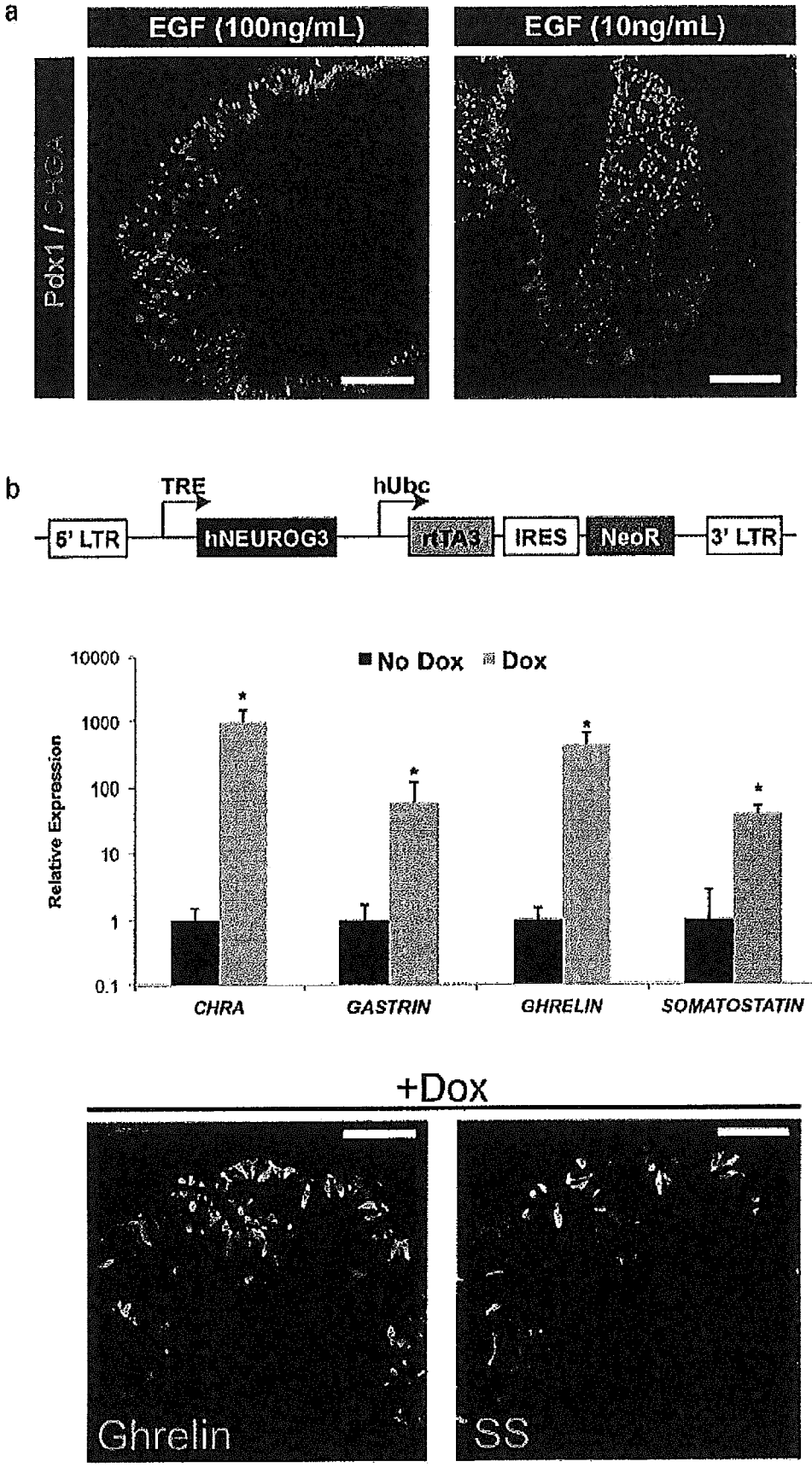

a
EGF (100ng/mL)
EGF (10ng/mL)
Pdx1 / CHGA
b
TRE
hUbc
5' LTR
hNEUROG3
rtTA3
IRES
NeoR
3' LTR
No Dox
Dox
Relative Expression
10000
1000
100
10
1
0.1
CHRA
GASTRIN
GHRELIN
SOMATOSTATIN
+Dox
Ghrelin
SS

FIG. 14

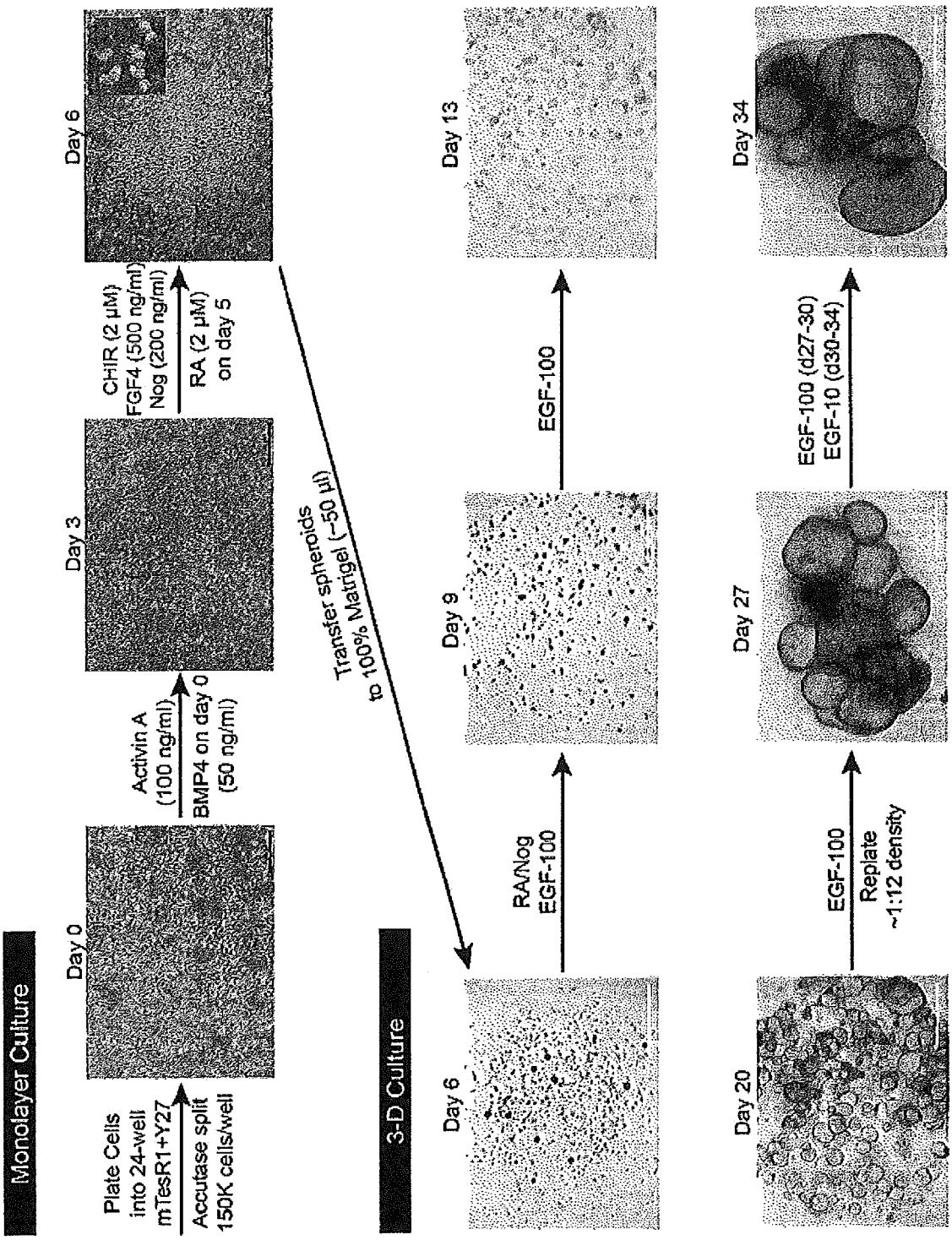
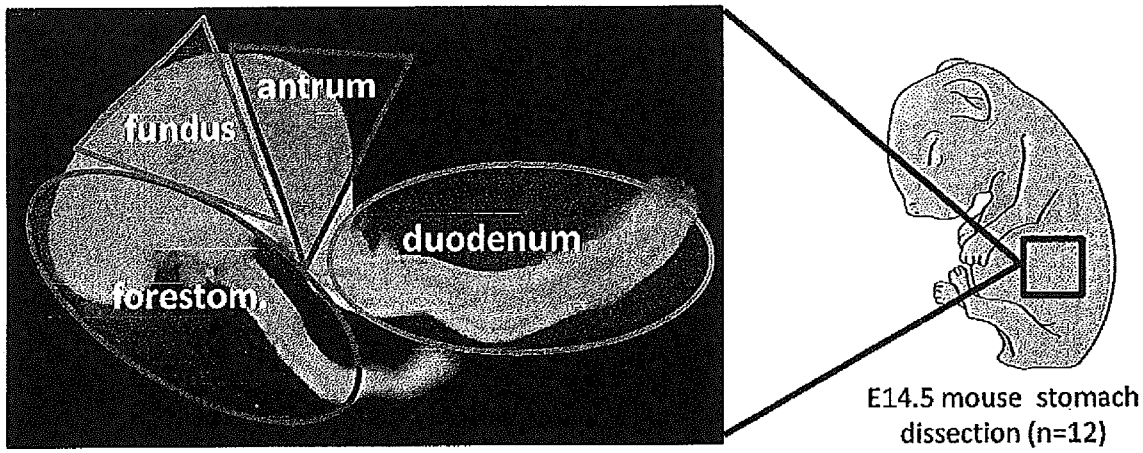

Monolayer Culture
Day 0
Plate Cells into 24-well mTesR1+Y27
Accutase split 150K cells/well
Activin A (100 ng/ml) BMP4 on day 0 (50 ng/ml)
Day 3
CHIR (2 µM) FGF4 (500 ng/ml) Nog (200 ng/ml)
RA (2 µM) on day 5
Day 6
Transfer spheroids to 100% Matrigel (~50 µl)
3-D Culture
Day 6
RA/Nog EGF-100
Day 9
EGF-100
Day 13
EGF-100
Replate ~1:12 density
Day 20
EGF-100 (d27-30) EGF-10 (d30-34)
Day 27
Day 34

FIG. 15

1. Fundus marker - Investigation in mouse E14.5 mouse stomach regions

Experimental procedure



Known regional markers

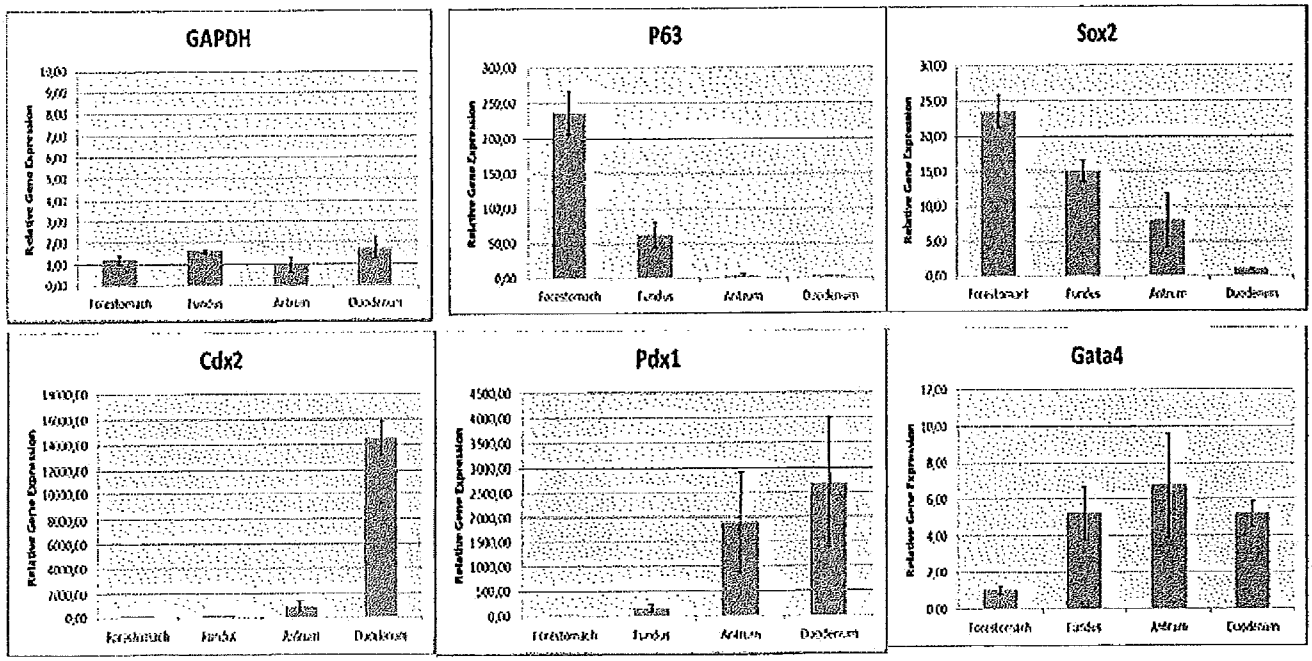


FIG. 16

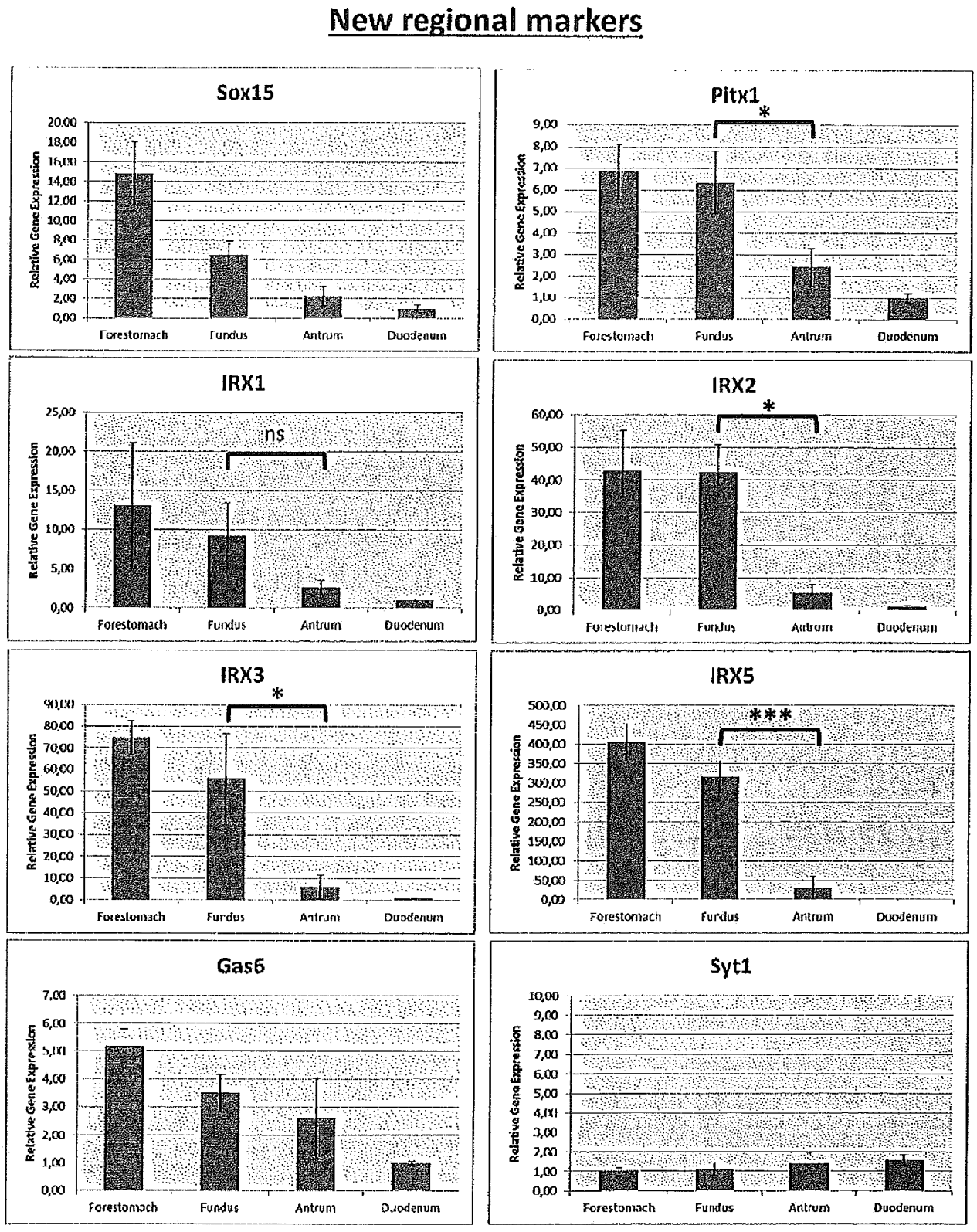
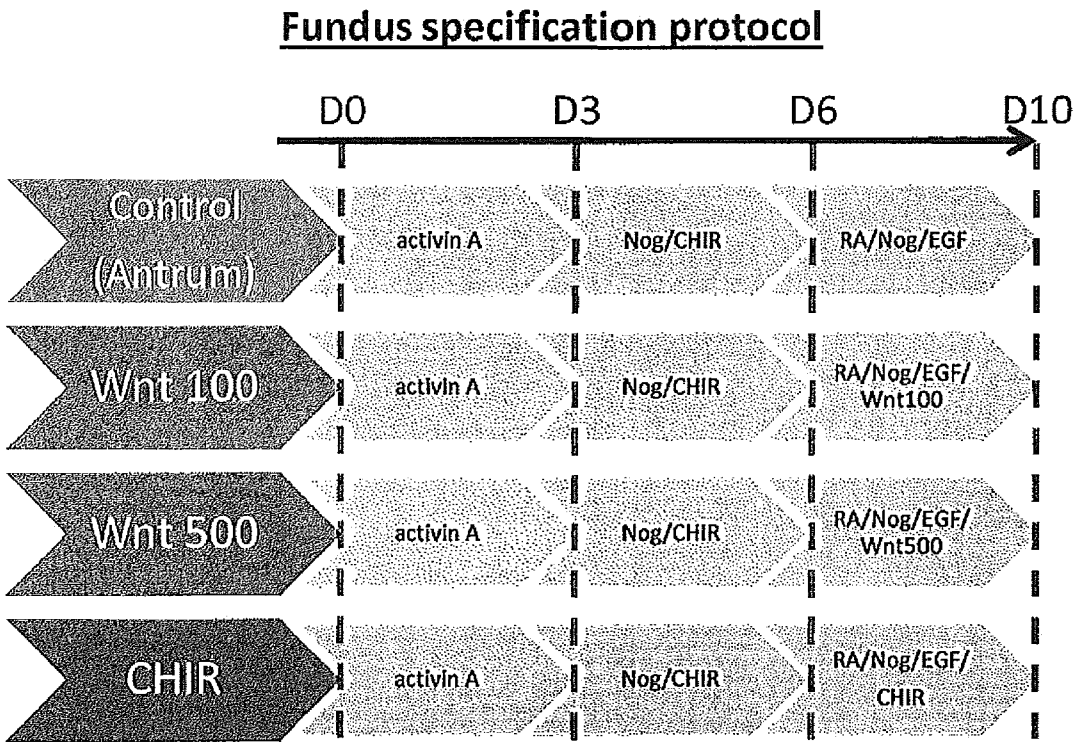

New regional markers
Sox15
Relative Gene Expression
20,00
18,00
16,00
14,00
12,00
10,00
8,00
6,00
4,00
2,00
0,00
Forestomach
Fundus
Antrum
Duodenum
Pitx1
*
9,00
8,00
7,00
6,00
5,00
4,00
3,00
2,00
1,00
0,00
IRX1
ns
25,00
20,00
15,00
10,00
5,00
0,00
IRX2
*
60,00
50,00
40,00
30,00
20,00
10,00
0,00
IRX3
*
90,00
80,00
70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00
IRX5
***
500,00
450,00
400,00
350,00
300,00
250,00
200,00
150,00
100,00
50,00
0,00
Gas6
7,00
6,00
5,00
4,00
3,00
2,00
1,00
0,00
Syt1
10,00
9,00
8,00
7,00
6,00
5,00
4,00
3,00
2,00
1,00
0,00
T test : p<0.05 * , p<0.01 ** , p<0.001 ***

FIG. 17


Fundus specification protocol
D0
D3
D6
D10
Control (Antrum)
activin A
Nog/CHIR
RA/Nog/EGF
Wnt 100
activin A
Nog/CHIR
RA/Nog/EGF/ Wnt100
Wnt 500
activin A
Nog/CHIR
RA/Nog/EGF/ Wnt500
CHIR
activin A
Nog/CHIR
RA/Nog/EGF/ CHIR

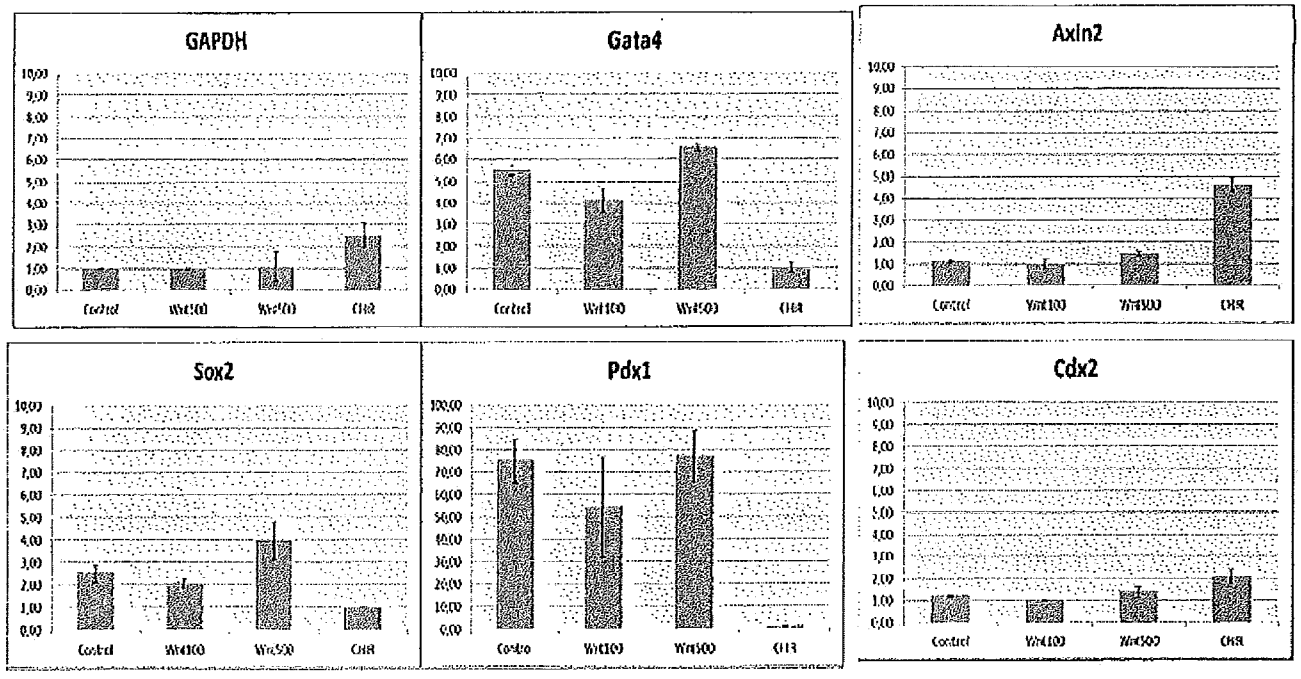
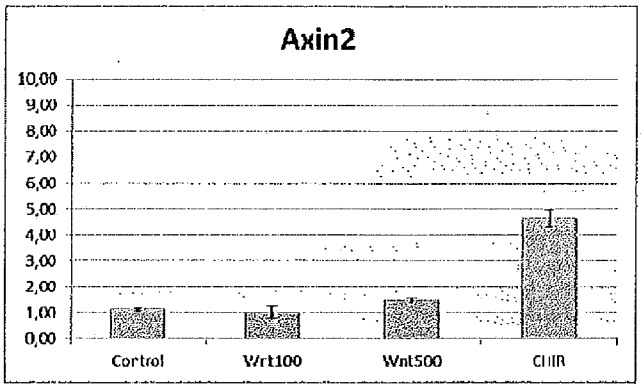
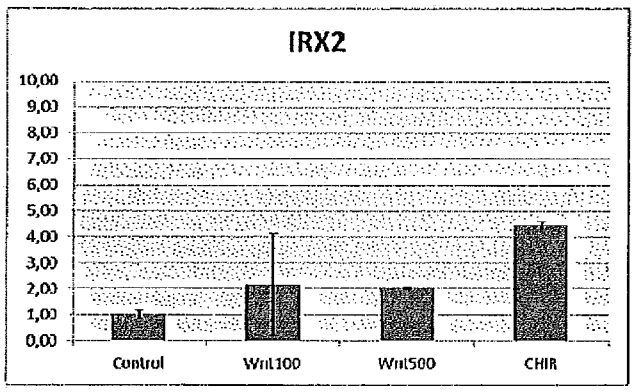

GAPDH
Gata4
Axin2
Sox2
Pdx1
Cdx2

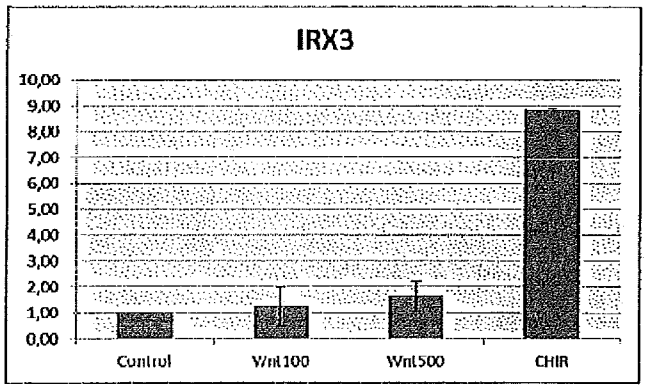
FIG. 18

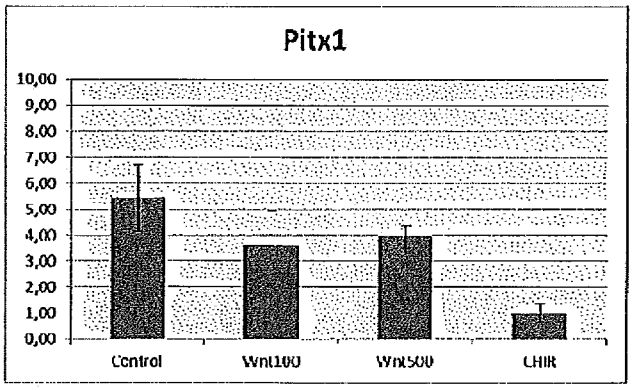
2. *Fundus specification* – Wtn pathway influence

Results – Protocol 2









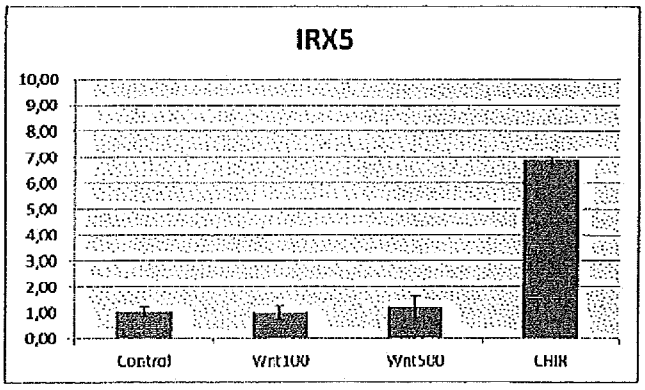
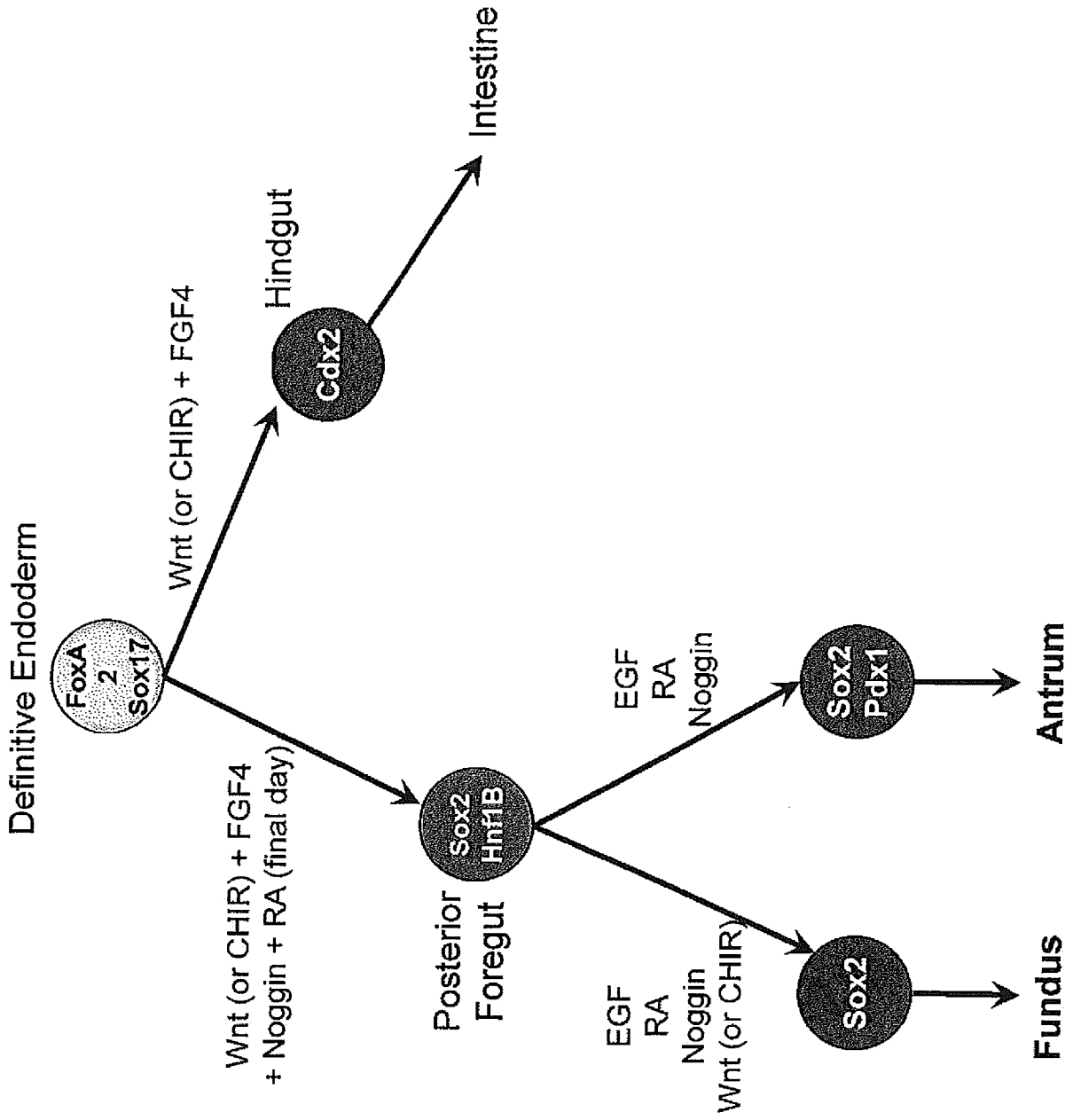


FIG. 19


Definitive Endoderm
FoxA 2 Sox17
Wnt (or CHIR) + FGF4
Hindgut
Cdx2
Intestine
Wnt (or CHIR) + FGF4 + Noggin + RA (final day)
Sox2 Hnf1B
Posterior Foregut
EGF RA Noggin
Sox2 Pdx1
Antrum
EGF RA Noggin Wnt (or CHIR)
Sox2
Fundus