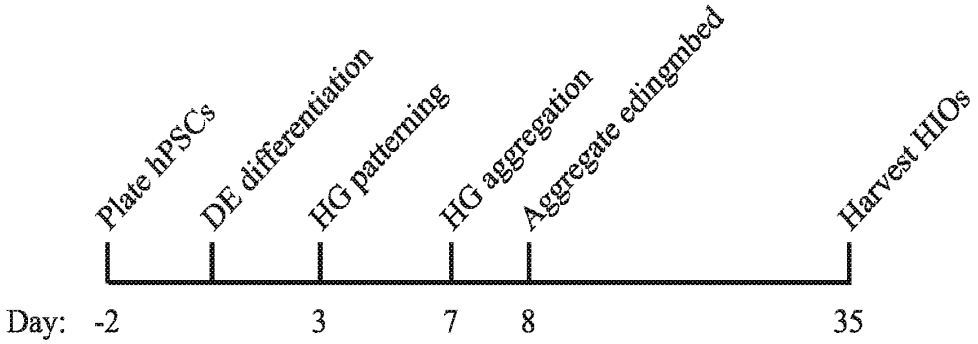


Figure 1

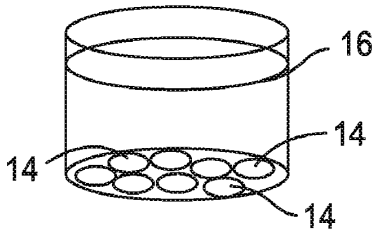


Figure 2A

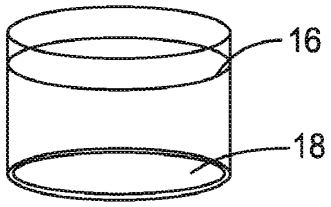


Figure 2B

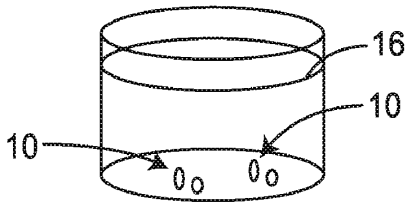


Figure 2C

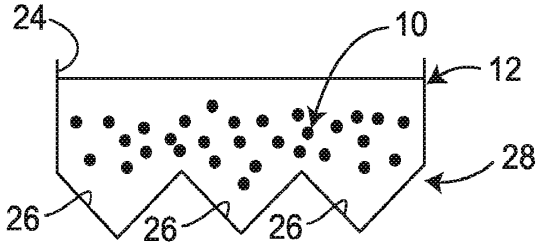


Figure 3A

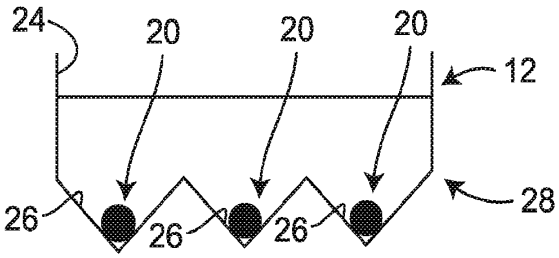


Figure 3B

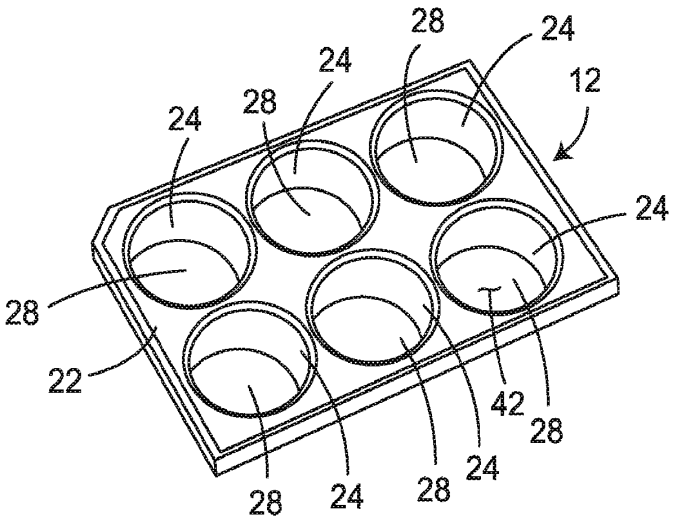


Figure 4

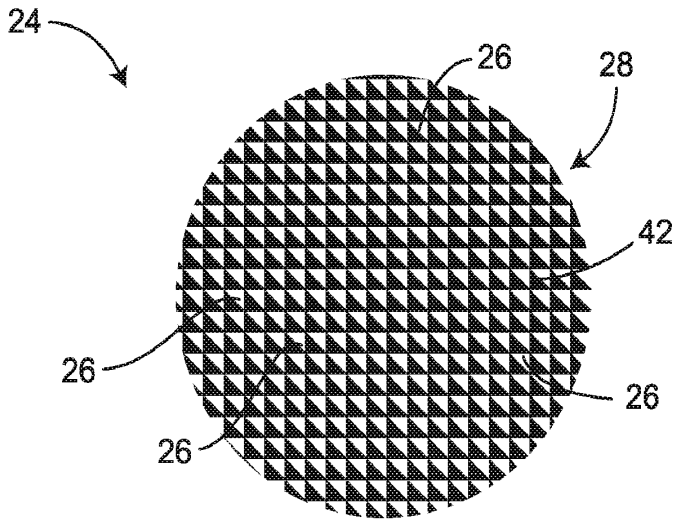


Figure 5

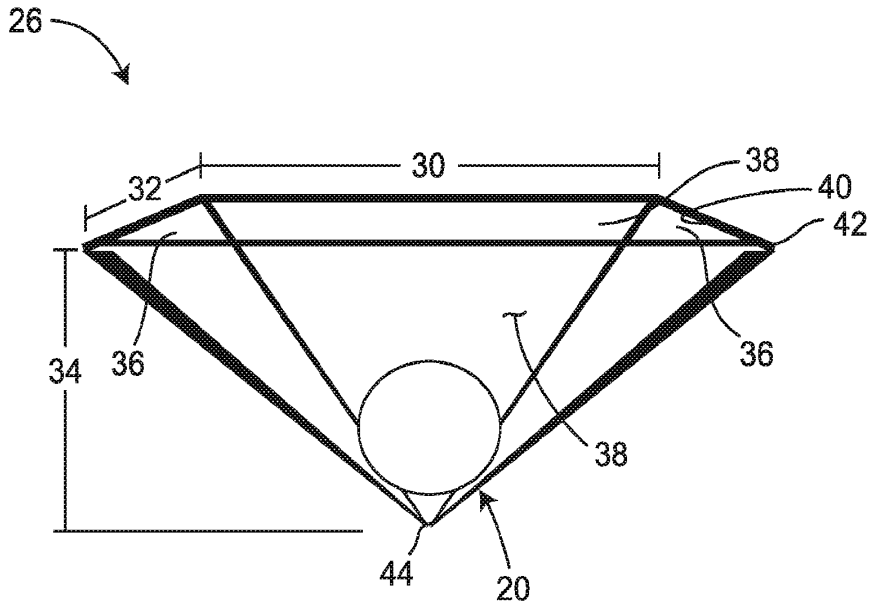


Figure 6

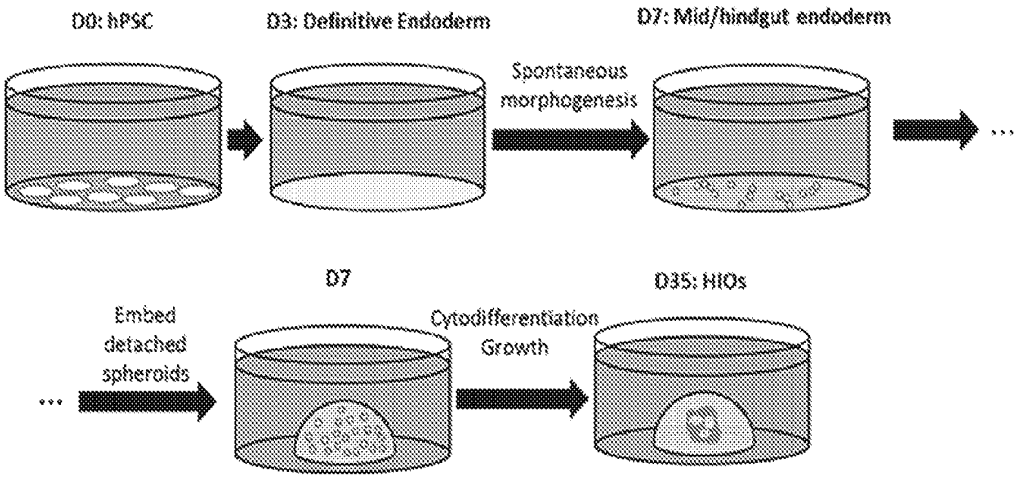


Figure 7A

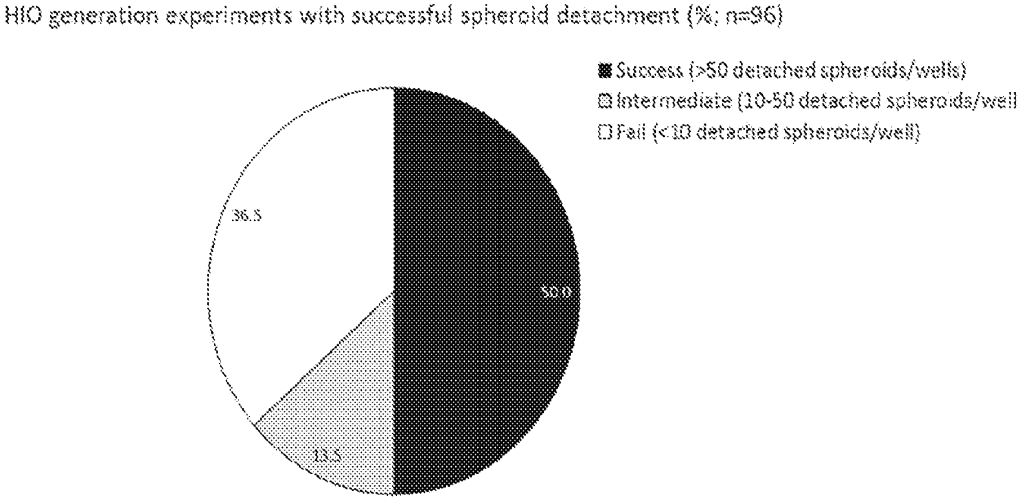


Figure 7B

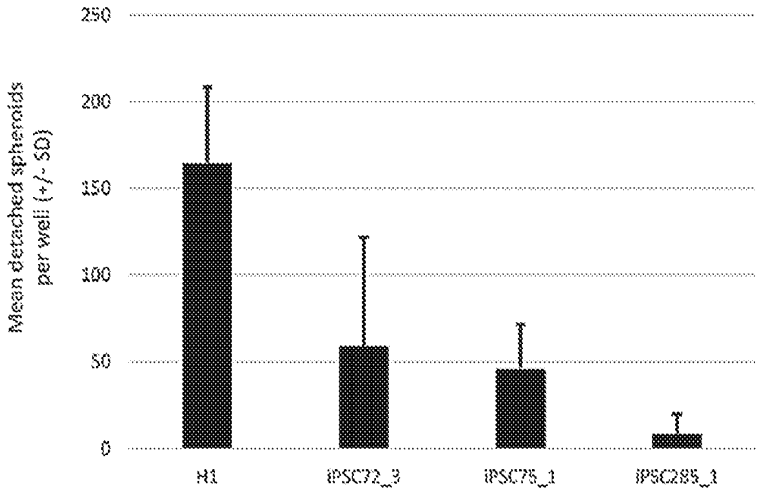


Figure 7C

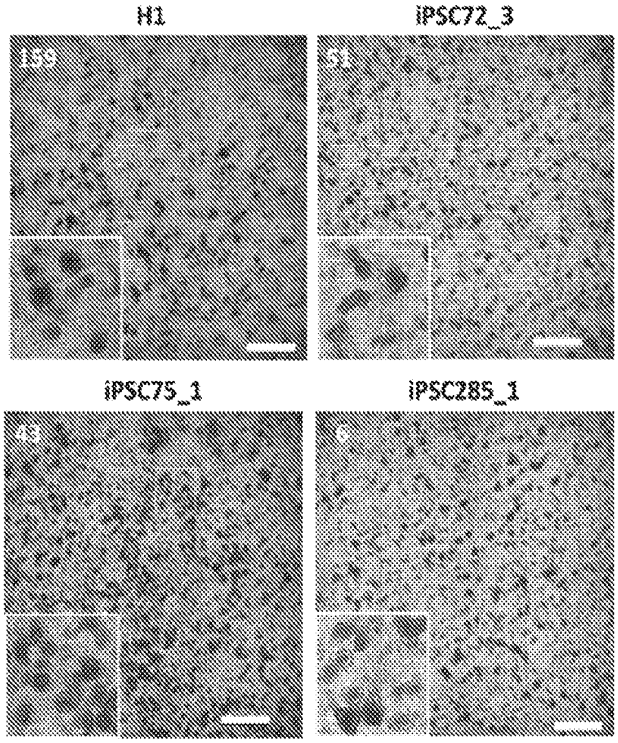


Figure 7D

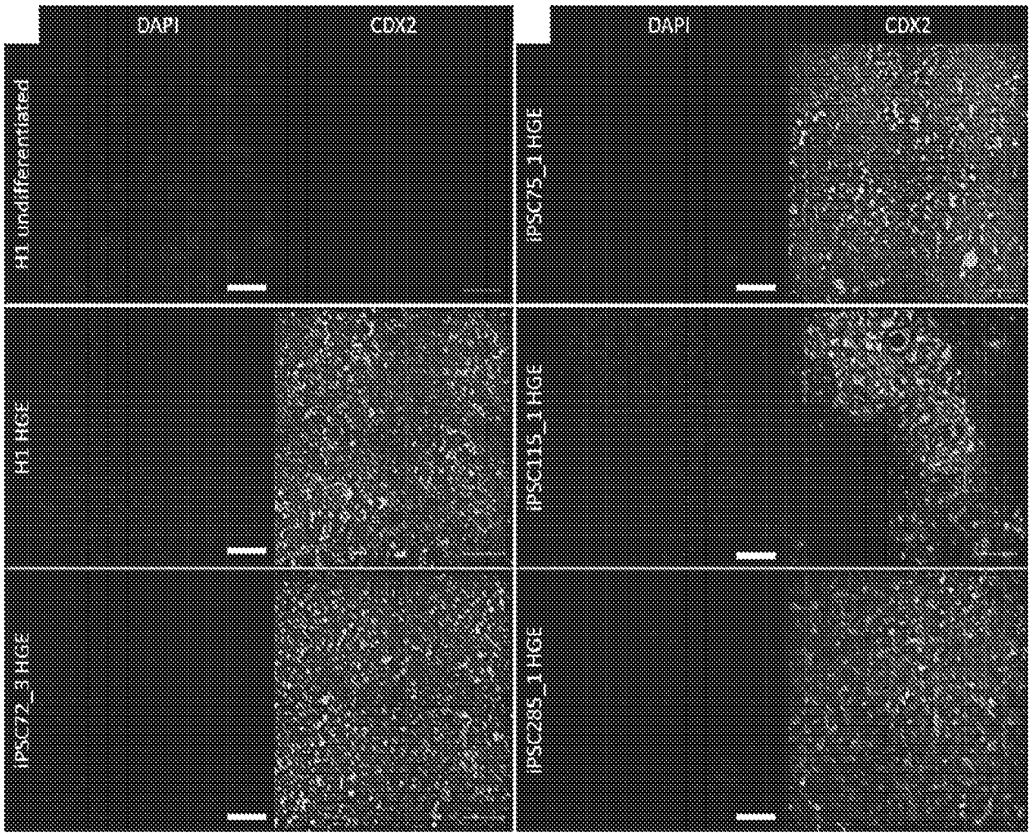

DAPI
CDX2
H1 undifferentiated
H1 HGE
iPSC72_3 HGE
DAPI
CDX2
iPSC75_1 HGE
iPSC115_1 HGE
iPSC285_1 HGE

Figure 8

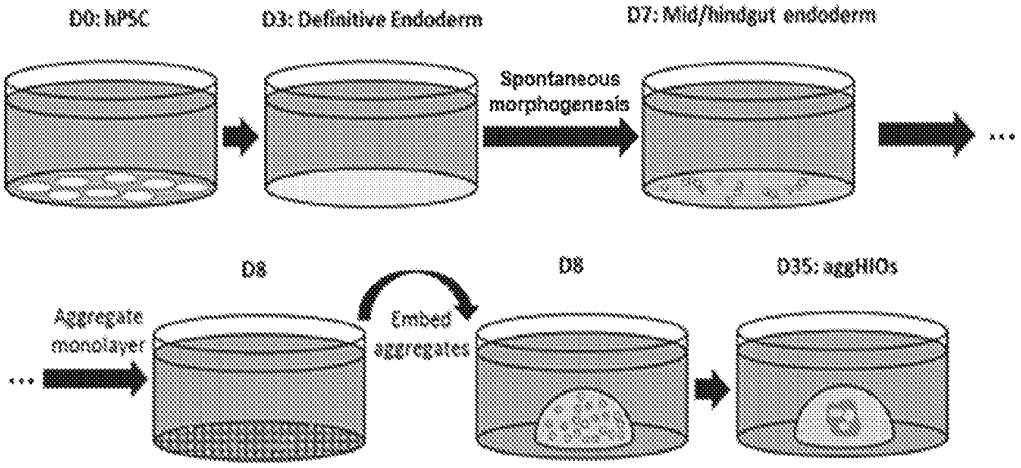


Figure 9A

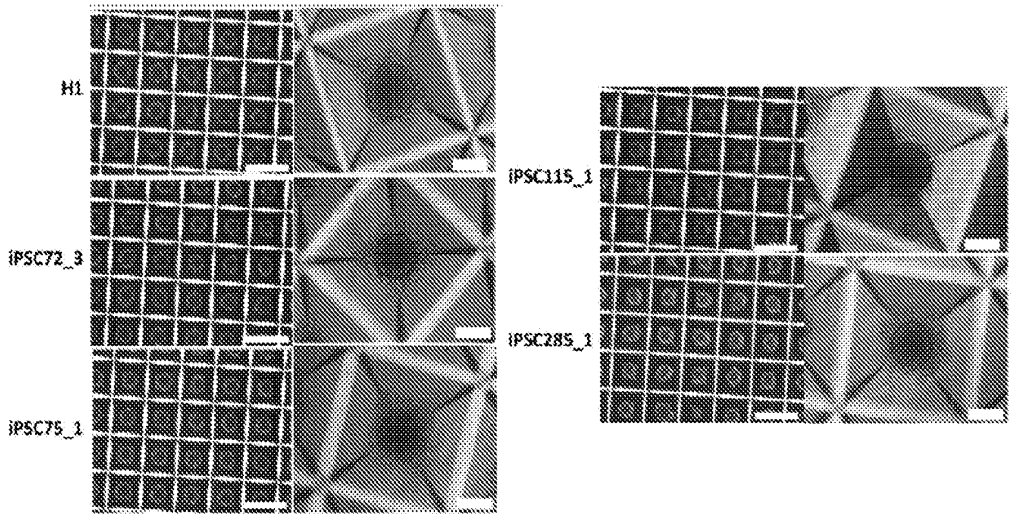


Figure 9B

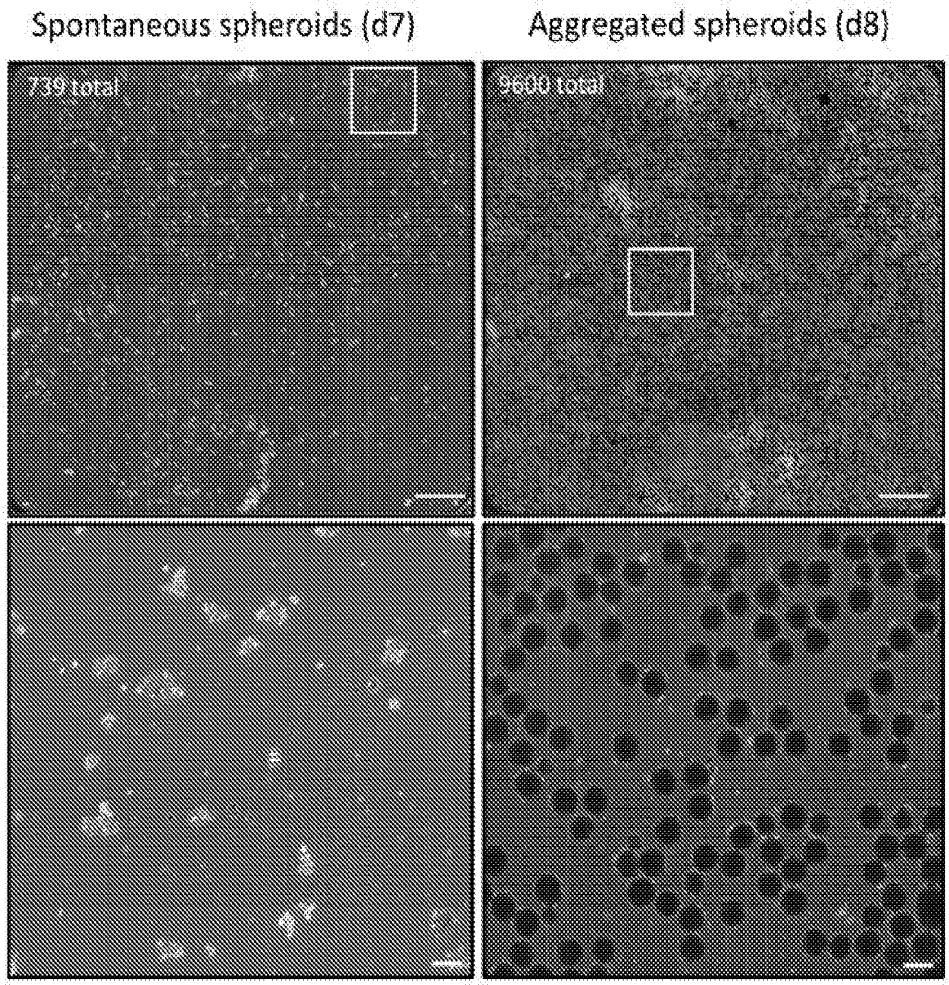

Spontaneous spheroids (d7)
Aggregated spheroids (d8)
739 total
9600 total

Figure 9C

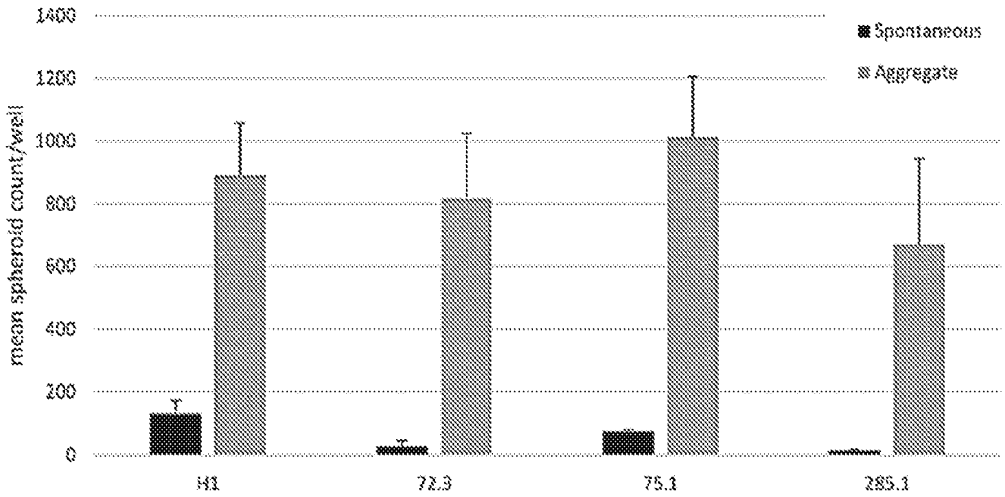


Figure 9D

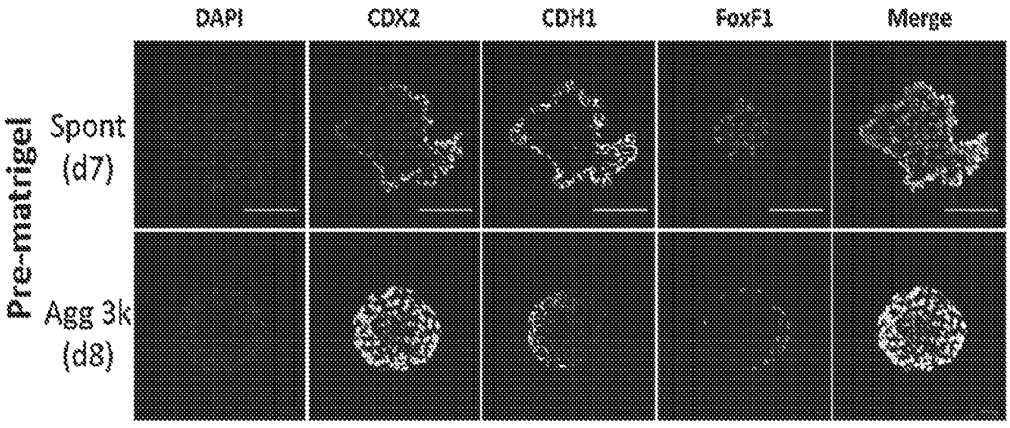


Figure 9E

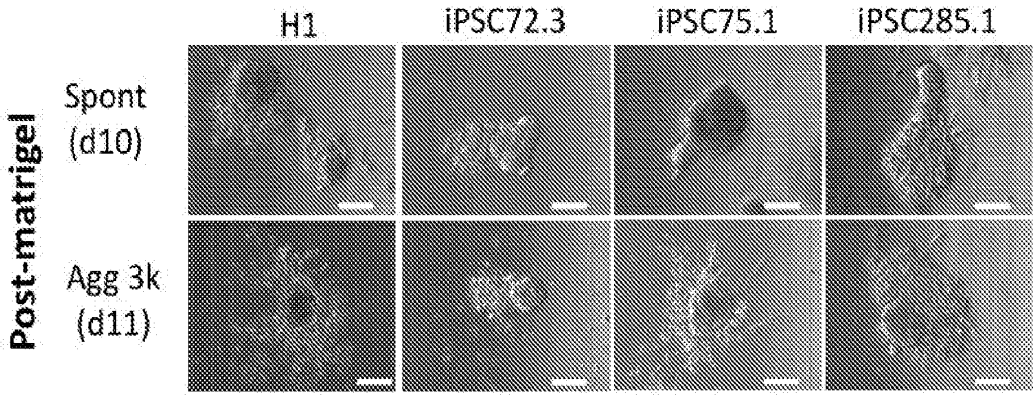


Figure 10A

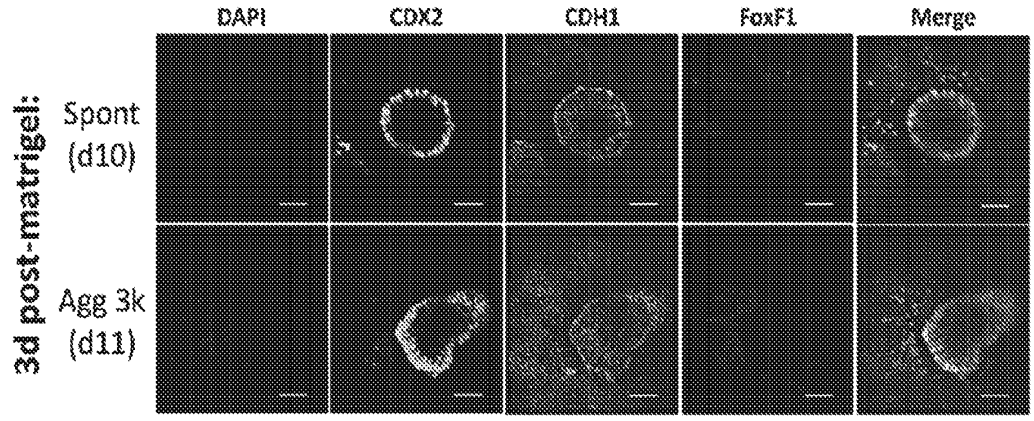


Figure 10B

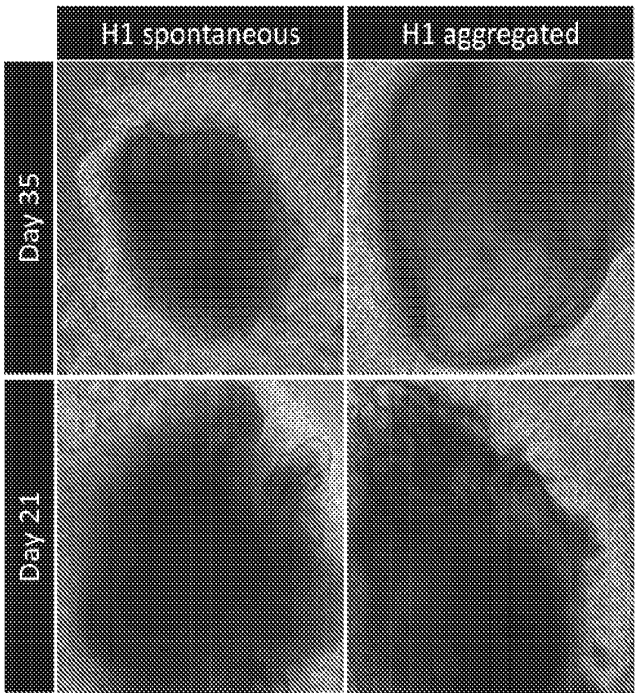


Figure 11A

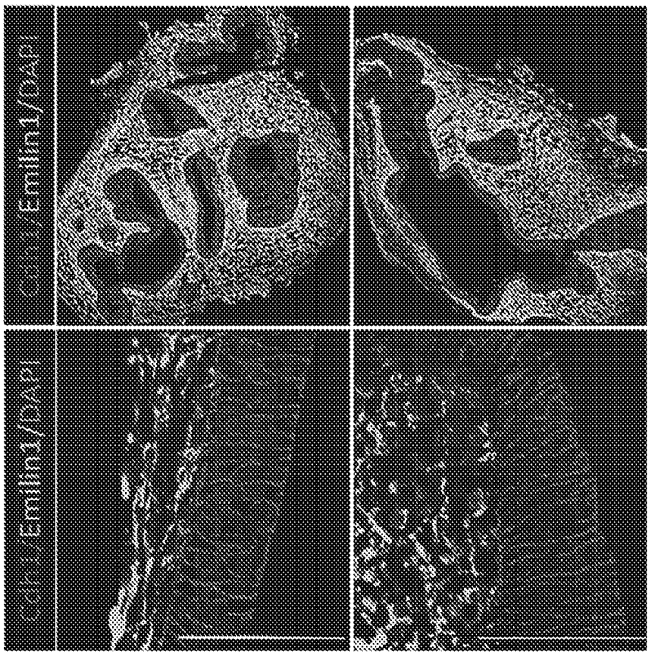


Figure 11B

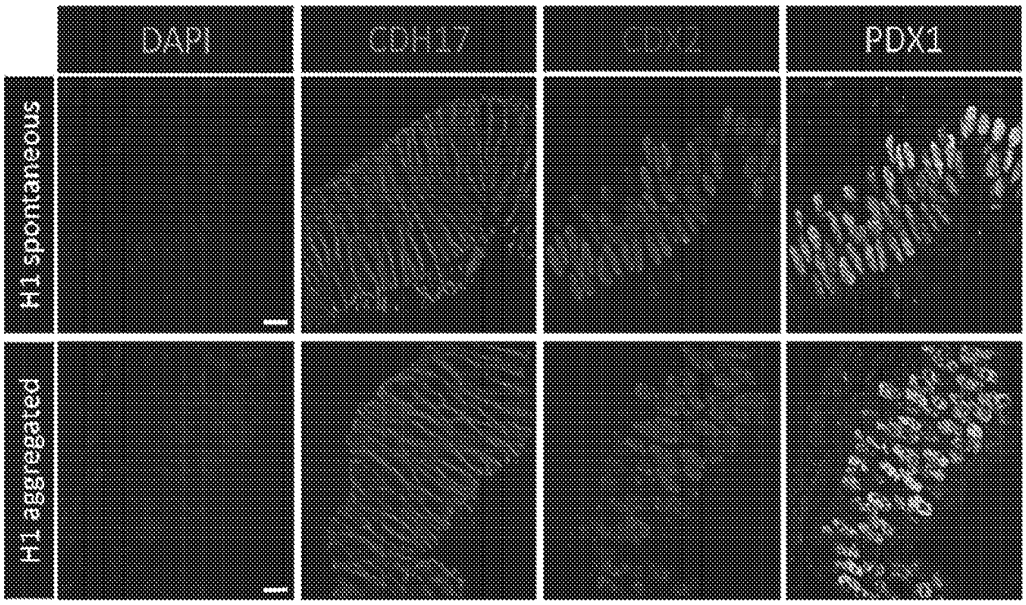


Figure 11C

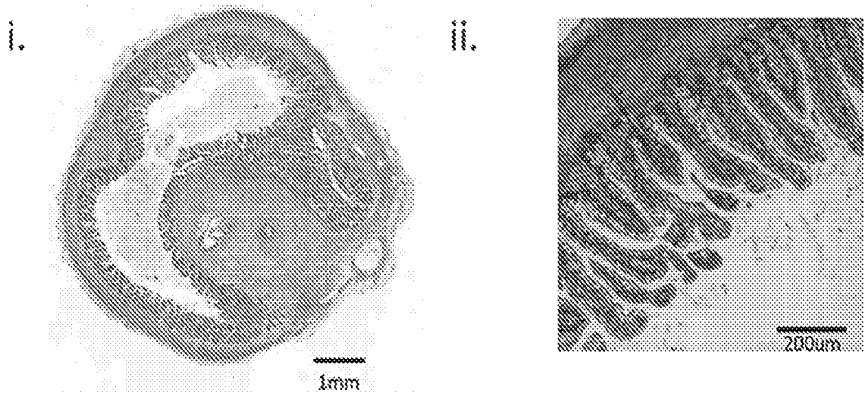


Figure 12A

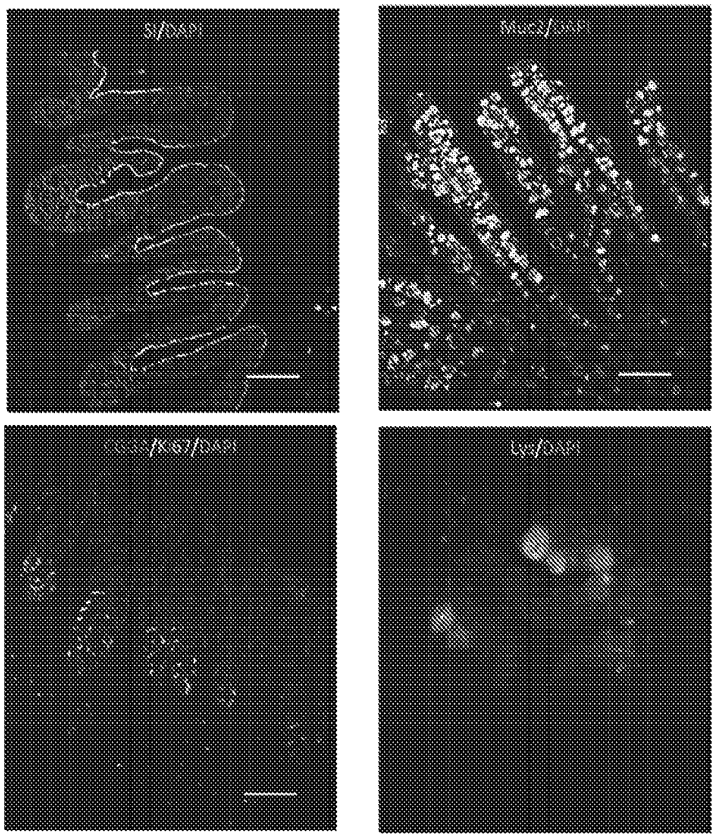


Figure 12B

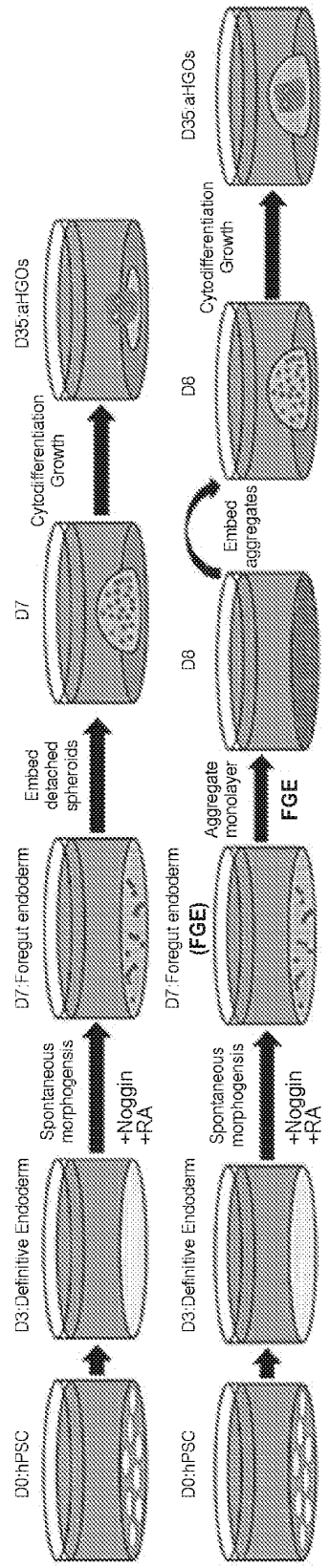


Figure 13A

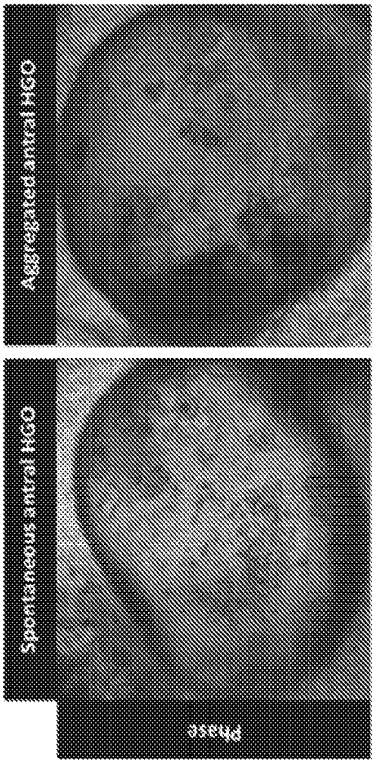


Figure 13B

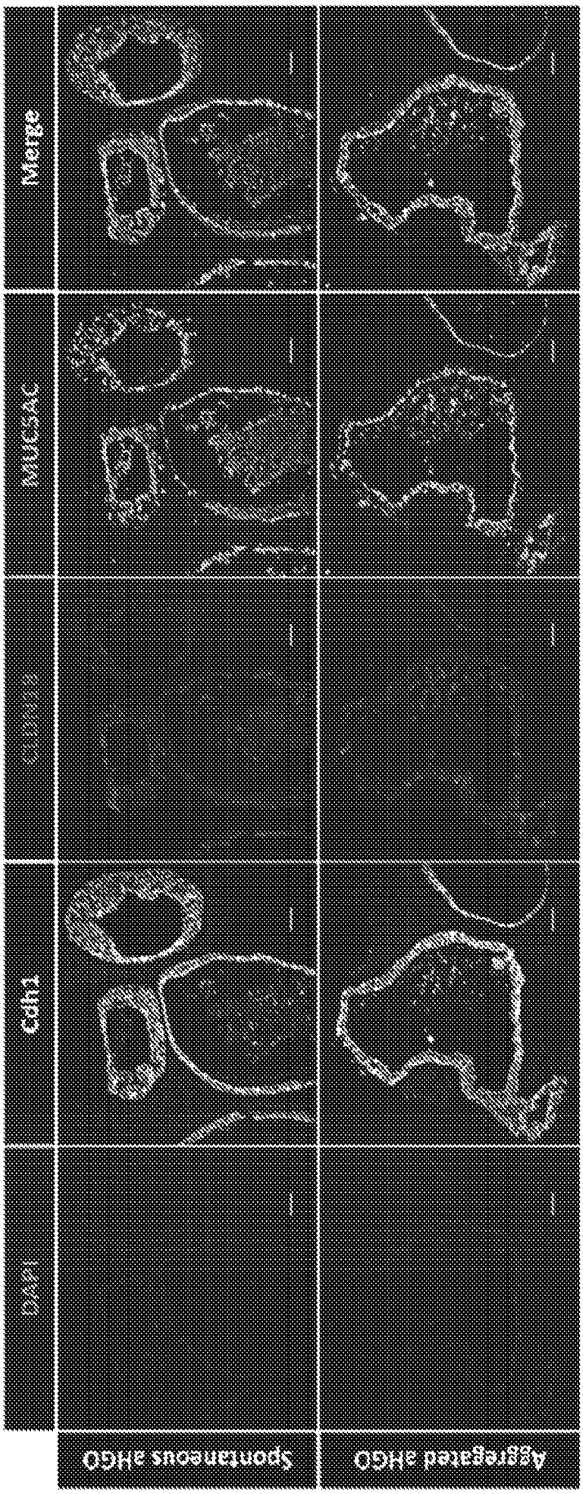

Merge
MUC5AC
CLDN18
Cdh1
DAPI
Spontaneous aHGO
Aggregated aHGO

Figure 13C

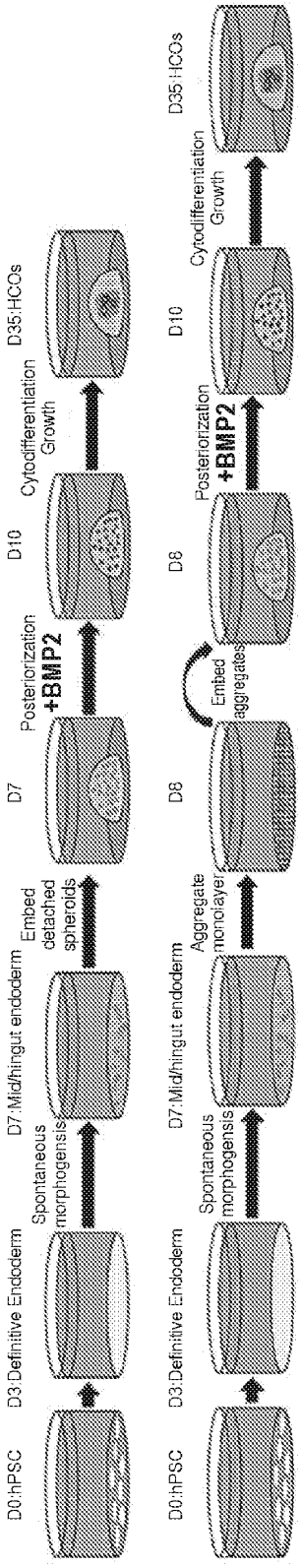


Figure 14A

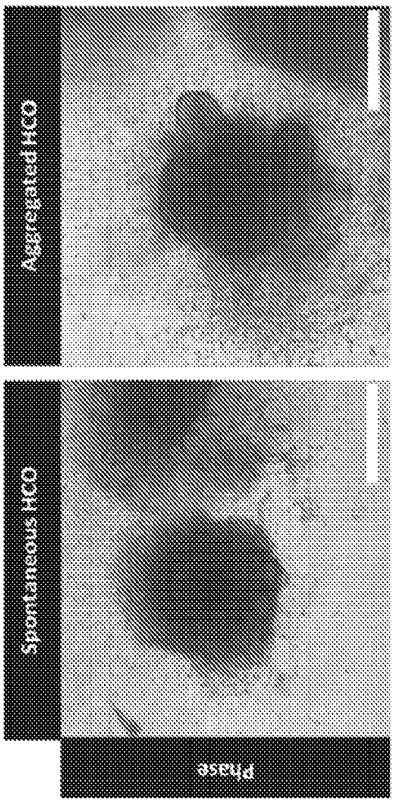


Figure 14B

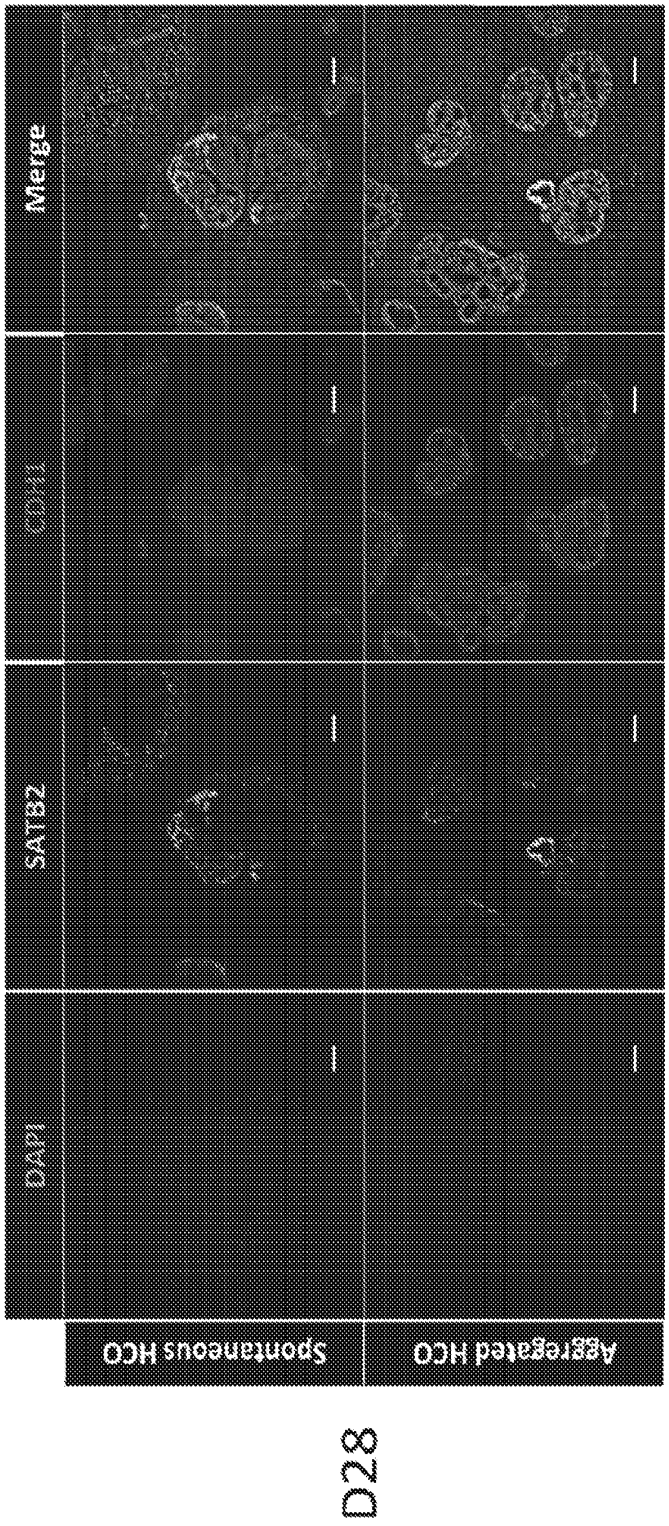


Figure 14C

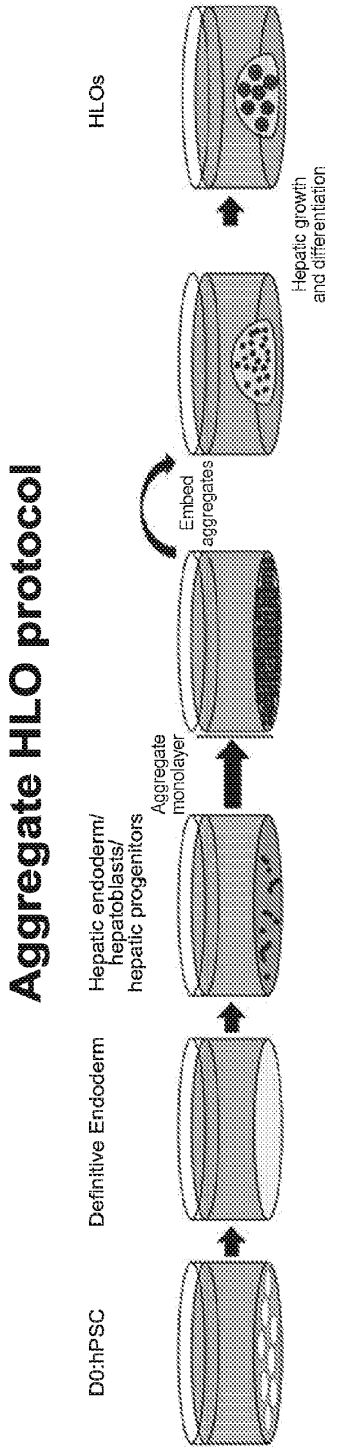

Aggregate HLO protocol
D0:hPSC
Definitive Endoderm
Hepatic endoderm/
hepatoblasts/
hepatic progenitors
Aggregate
monolayer
Embed
aggregates
Hepatic growth
and differentiation
HLOs

Figure 15

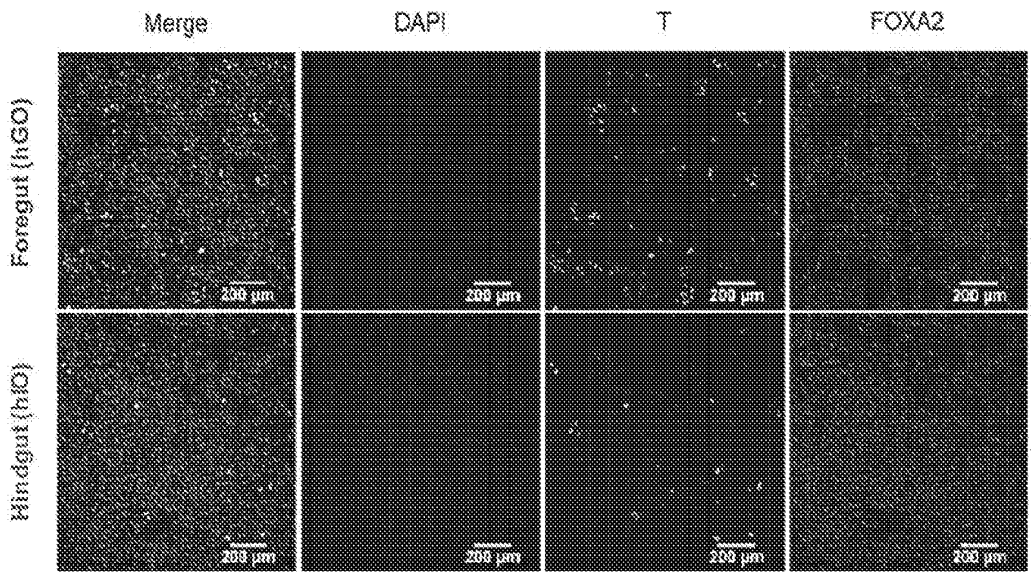


Figure 16A

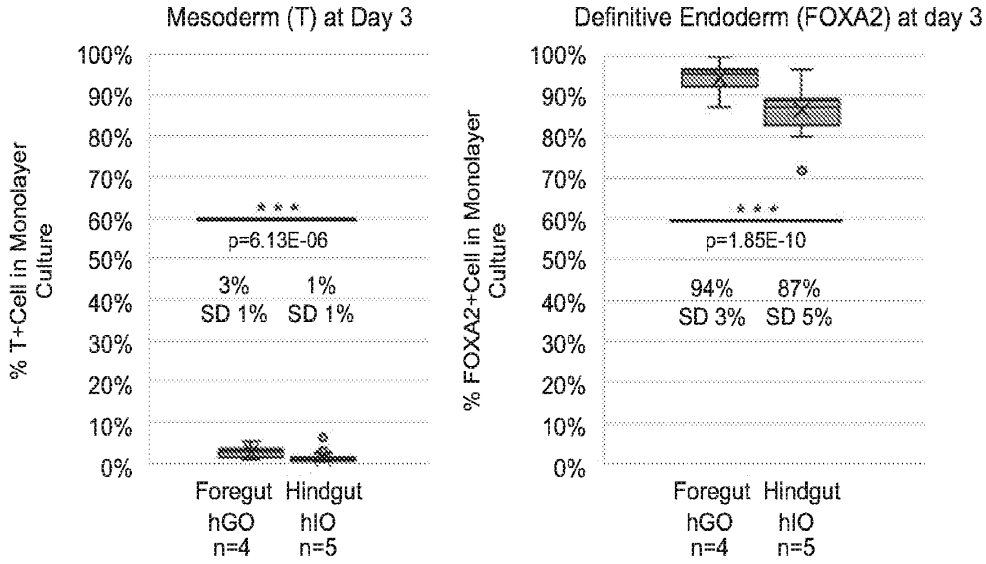


Figure 16B

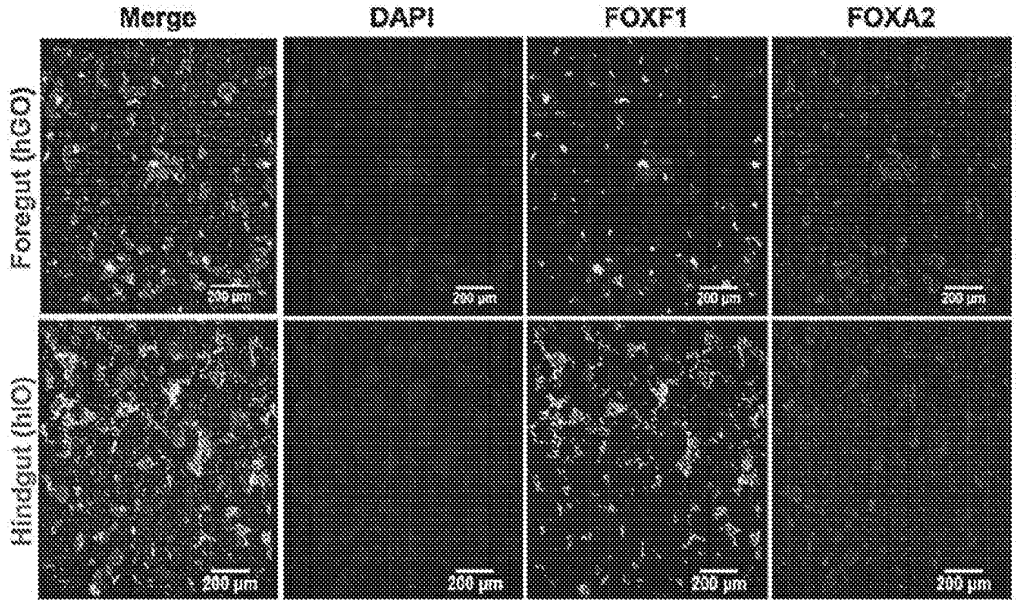


Figure 16C

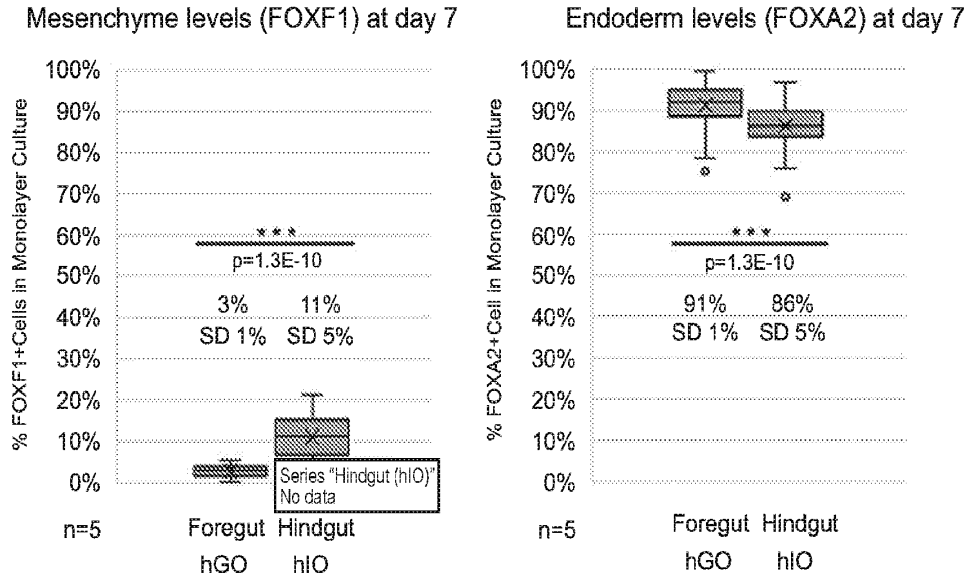


Figure 16D

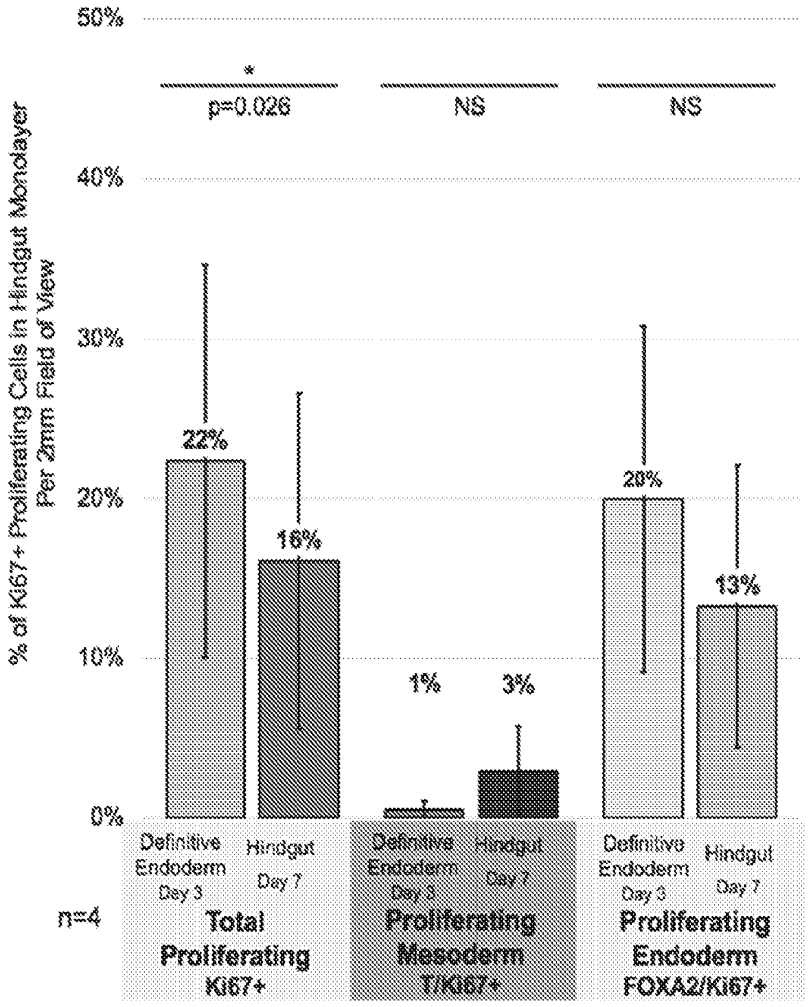

50%
40%
30%
20%
10%
0%
% of Ki67+ Proliferating Cells in Hindgut Monolayer Per 2mm Field of View
*
p=0.026
NS
NS
22%
16%
1%
3%
20%
13%
n=4
Definitive Endoderm Day 3
Hindgut Day 7
Total Proliferating Ki67+
Definitive Endoderm Day 3
Hindgut Day 7
Proliferating Mesoderm T/Ki67+
Definitive Endoderm Day 3
Hindgut Day 7
Proliferating Endoderm FOXA2/Ki67+

Figure 16E