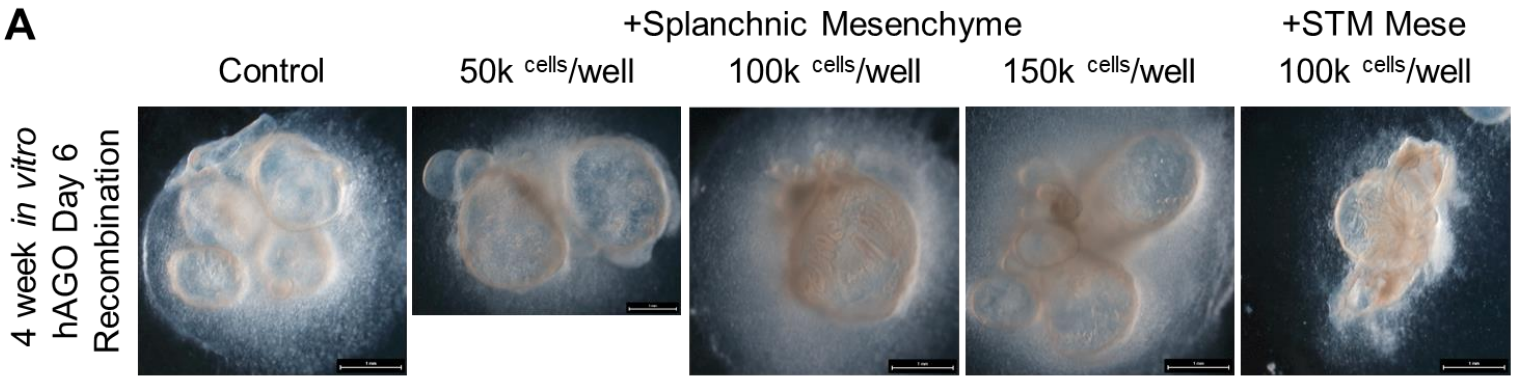
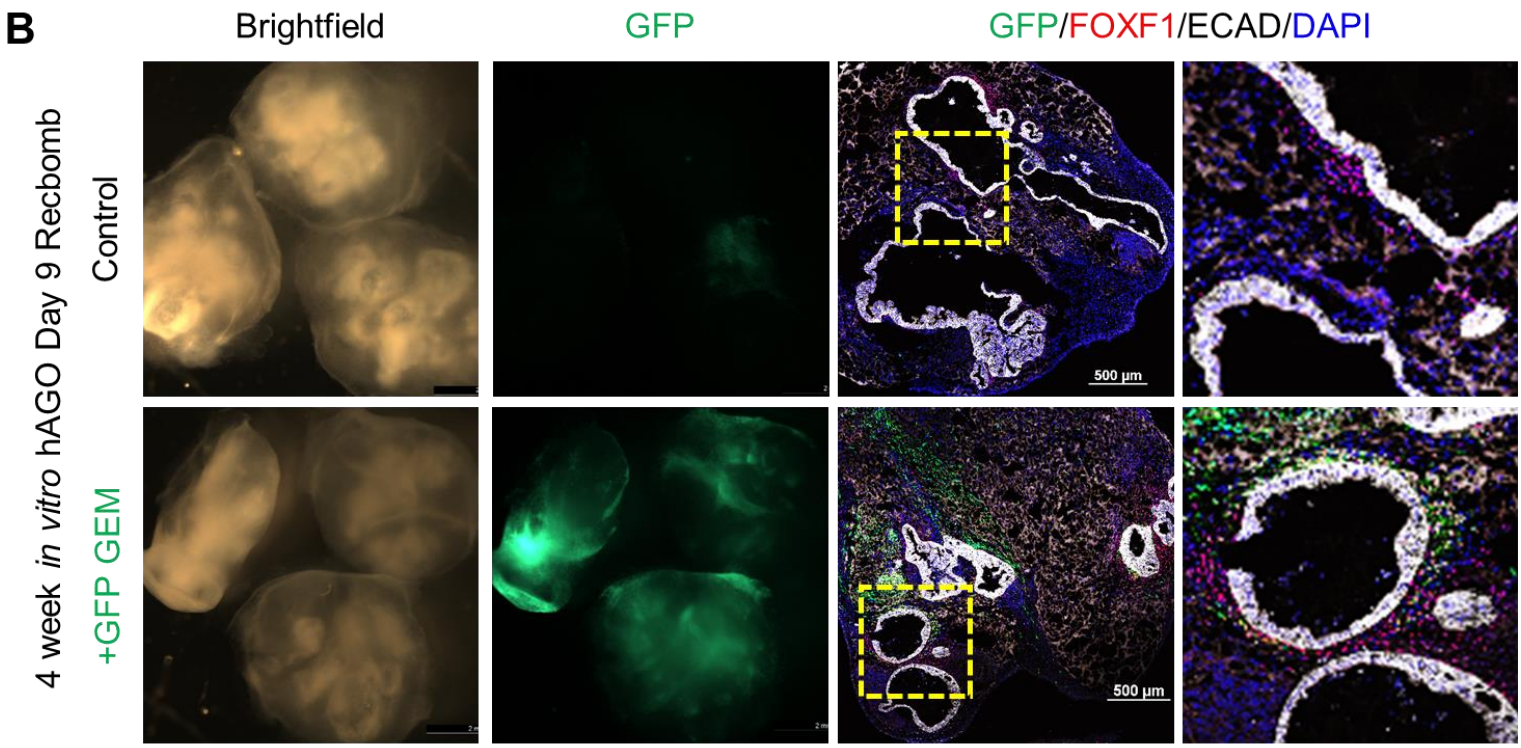
Figure S1


A
+Splanchnic Mesenchyme
+STM Mese
Control
50k cells/well
100k cells/well
150k cells/well
100k cells/well
4 week in vitro hAGO Day 6 Recombination


B
Brightfield
GFP
GFP/FOXF1/ECAD/DAPI
4 week in vitro hAGO Day 9 Recbomb
Control
+GFP GEM
500 µm
500 µm

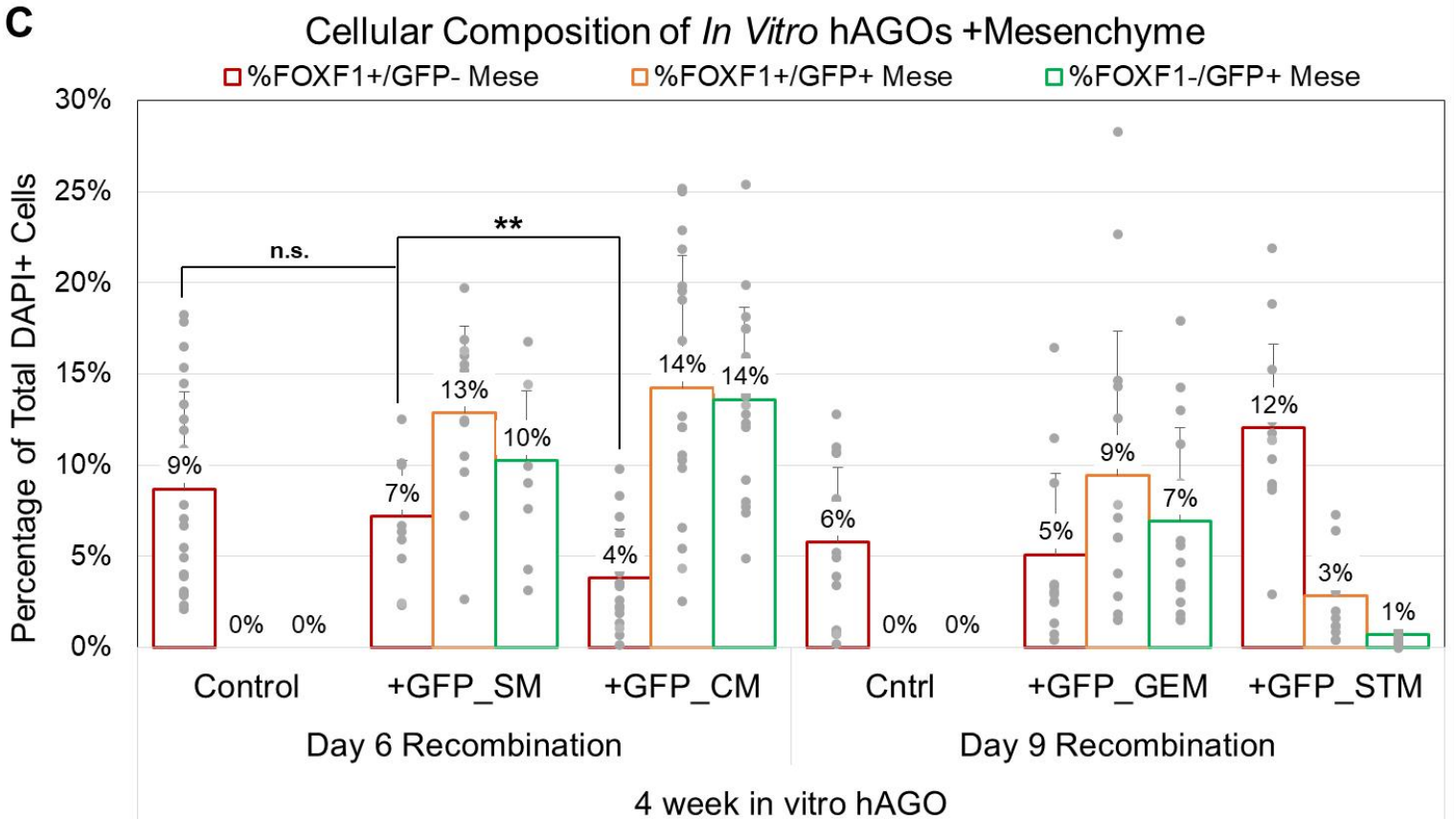

C
Cellular Composition of In Vitro hAGOs +Mesenchyme
%FOXF1+/GFP- Mese
%FOXF1+/GFP+ Mese
%FOXF1-/GFP+ Mese
Percentage of Total DAPI+ Cells
30%
25%
20%
15%
10%
5%
0%
n.s.
**
9%
0%
0%
7%
13%
10%
4%
14%
14%
6%
0%
0%
5%
9%
7%
12%
3%
1%
Control
+GFP_SM
+GFP_CM
Cntrl
+GFP_GEM
+GFP_STM
Day 6 Recombination
Day 9 Recombination
4 week in vitro hAGO

Figure S1. Splanchnic mesenchymal recombination yielded the most added exogenous mesenchyme while still retaining endogenous mesenchyme, relating to Figure 1. (**A**) Brightfield images of 4 week *in vitro* hAGOs recombined with varying concentrations of splanchnic and septum transversum (STM) mesenchyme on day 6 of hAGO protocol. (**B**) Brightfield images of hAGOs grown for 4 weeks *in vitro* with and without recombination with exogenous GFP-labeled gastric-esophageal mesenchyme (GEM) (green) costained with mesenchymal marker FOXF1 (red). Higher magnification images are shown to the right. This relates to Fig. 1D. (**C**) Quantification of various mesenchymal recombination techniques, including day 6 mesenchymal recombination (left) of either GFP+ splanchnic (SM) or cardiac (CM) mesenchyme and day 9 mesenchymal recombination (right) of either GFP+ GEM or STM mesenchyme (n=12-24 fields from at least 3 organoids per condition from one differentiation, same trend seen across at least two individually seeded differentiations, **p<0.01, Student's t-test).

Figure S2

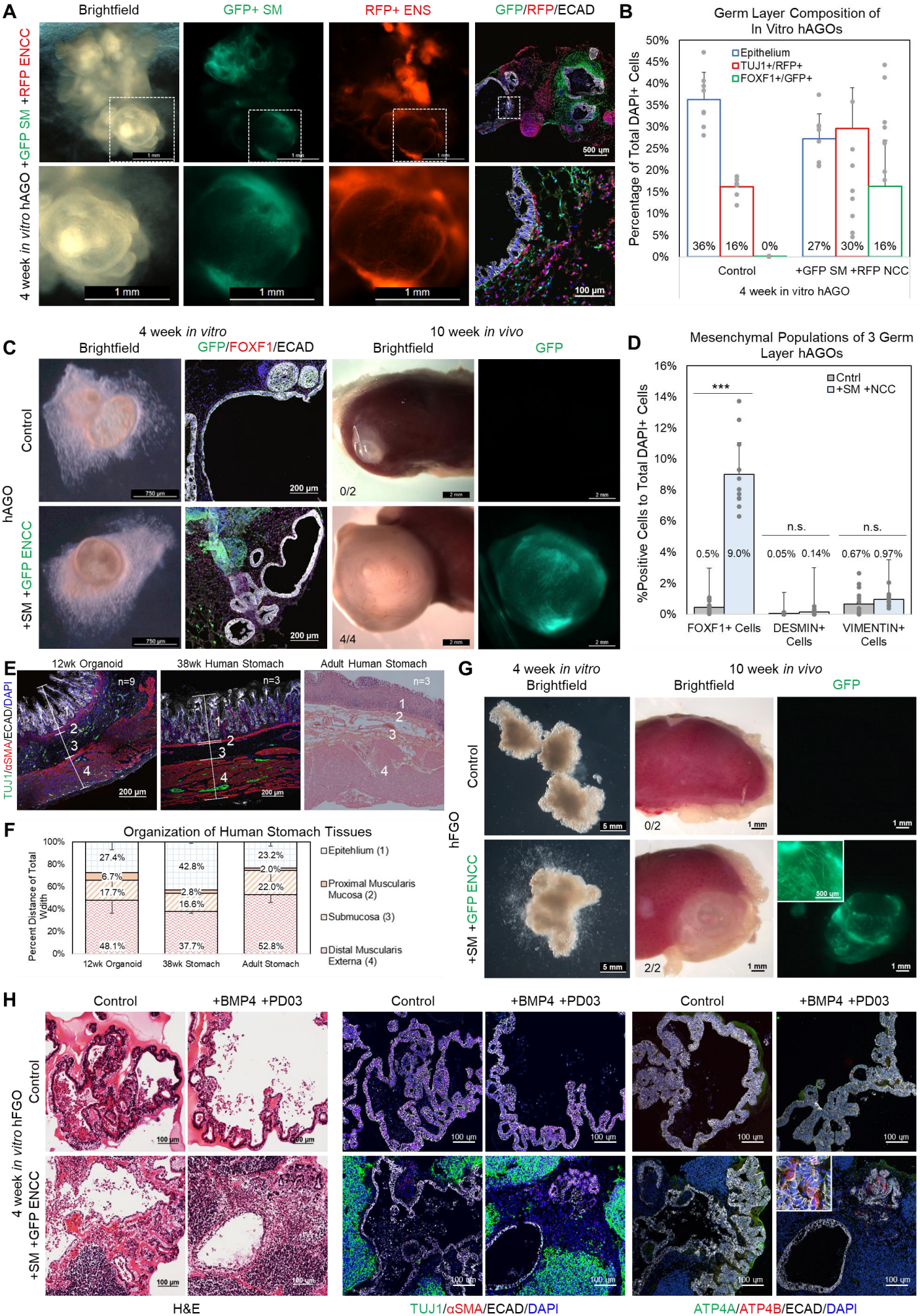
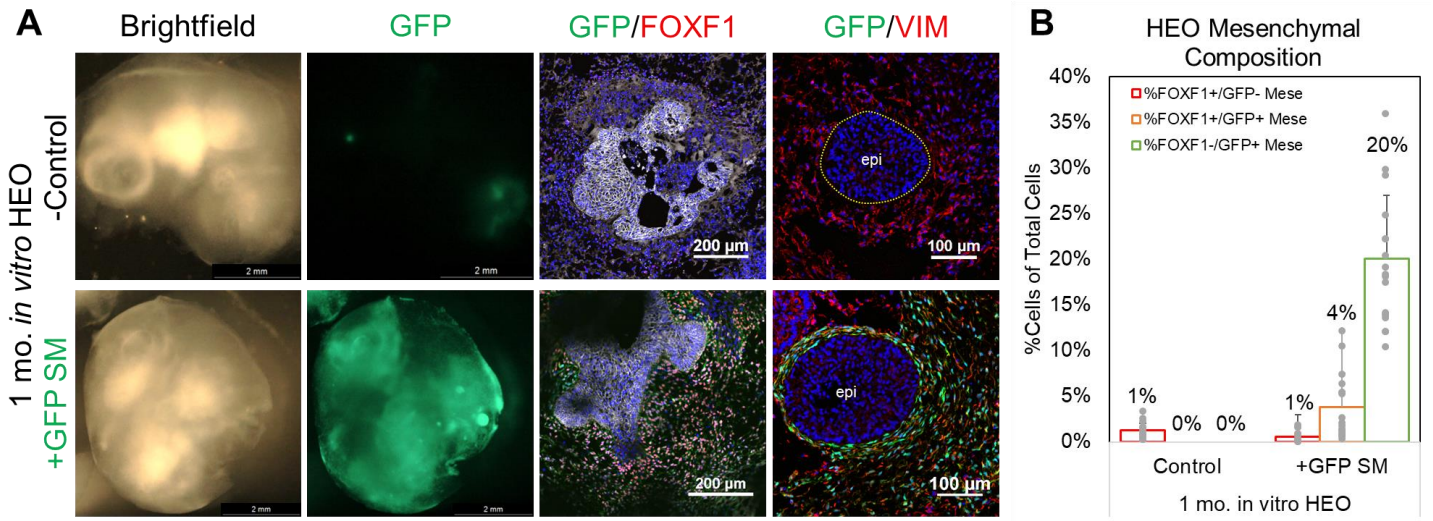
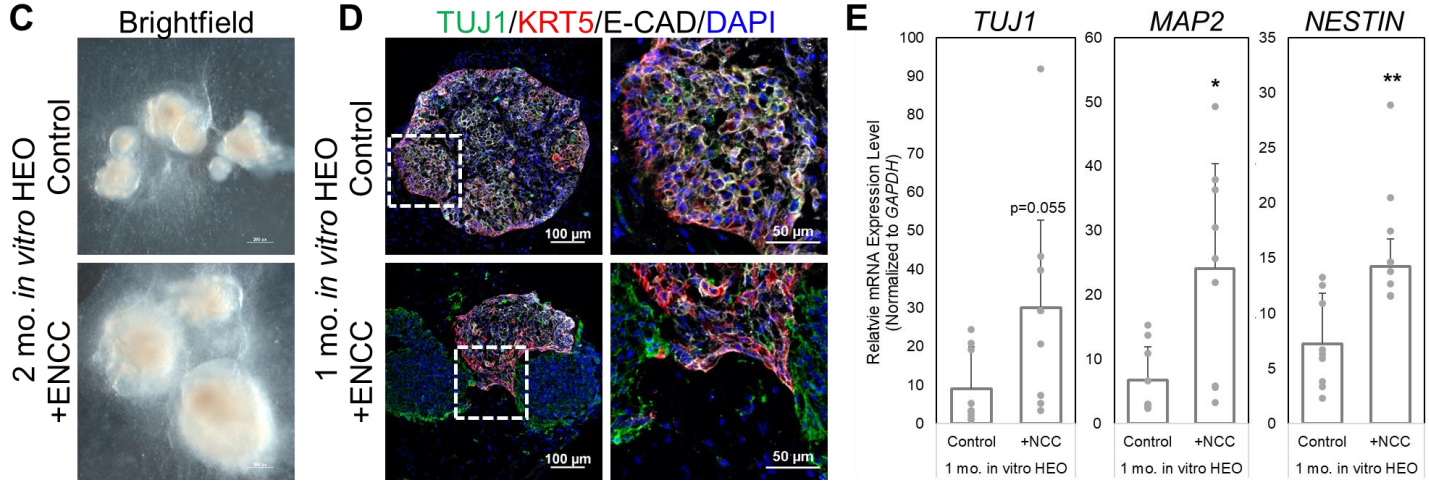
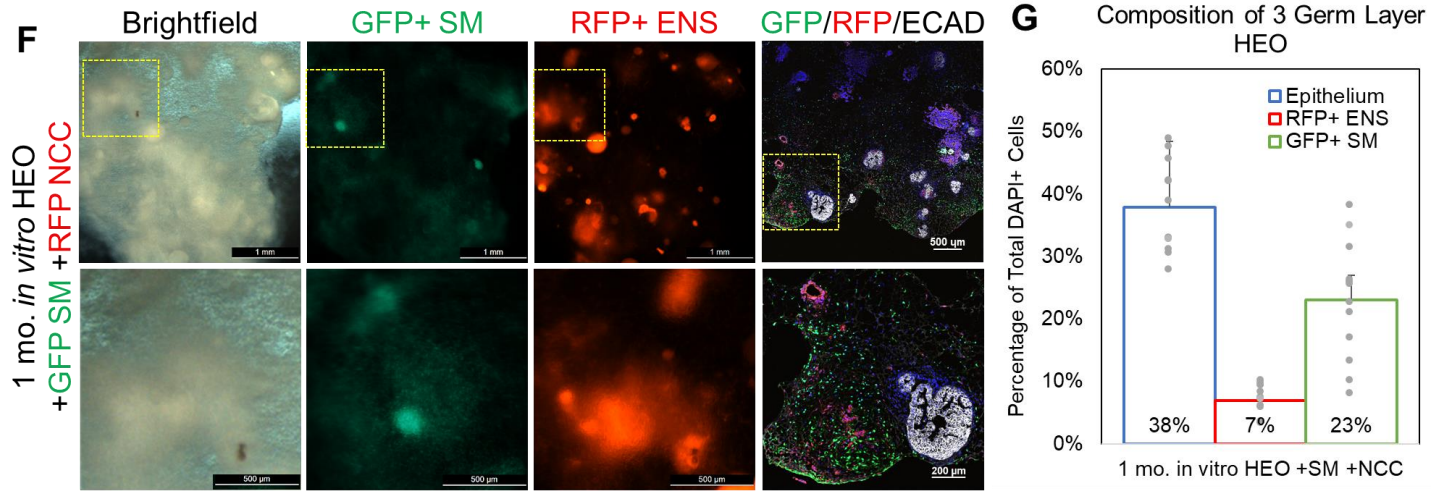

A
Brightfield
GFP+ SM
RFP+ ENS
GFP/RFP/ECAD
4 week in vitro hAGO +GFP SM +RFP ENCC
1 mm
500 µm
100 µm
B
Germ Layer Composition of In Vitro hAGOs
Epithelium
TUJ1+/RFP+
FOXF1+/GFP+
Percentage of Total DAPI+ Cells
36%
16%
0%
27%
30%
16%
Control
+GFP SM +RFP NCC
4 week in vitro hAGO
C
4 week in vitro
10 week in vivo
Brightfield
GFP/FOXF1/ECAD
Brightfield
GFP
Control
+SM +GFP ENCC
hAGO
750 µm
200 µm
0/2
4/4
2 mm
D
Mesenchymal Populations of 3 Germ Layer hAGOs
Cntrl
+SM +NCC
%Positive Cells to Total DAPI+ Cells
***
n.s.
n.s.
0.5%
9.0%
0.05%
0.14%
0.67%
0.97%
FOXF1+ Cells
DESMIN+ Cells
VIMENTIN+ Cells
E
12wk Organoid
38wk Human Stomach
Adult Human Stomach
TUJ1/αSMA/ECAD/DAPI
n=9
n=3
n=3
1
2
3
4
200 µm
F
Organization of Human Stomach Tissues
Percent Distance of Total Width
27.4%
6.7%
17.7%
48.1%
42.8%
2.8%
16.6%
37.7%
23.2%
2.0%
22.0%
52.8%
12wk Organoid
38wk Stomach
Adult Stomach
Epitehlium (1)
Proximal Muscularis Mucosa (2)
Submucosa (3)
Distal Muscularis Externa (4)
G
4 week in vitro
10 week in vivo
Brightfield
Brightfield
GFP
Control
+SM +GFP ENCC
hFGO
5 mm
0/2
2/2
1 mm
500 µm
H
Control
+BMP4 +PD03
Control
+BMP4 +PD03
Control
+BMP4 +PD03
4 week in vitro hFGO
Control
+SM +GFP ENCC
100 µm
H&E
TUJ1/αSMA/ECAD/DAPI
ATP4A/ATP4B/ECAD/DAPI

Figure S2. Three germ layer *in vitro* and *in vivo* hAGOs and hFGOs contain GFP+ splanchnic mesenchyme and RFP+ ENCC, relating to Figure 3. (**A**) Brightfield and fluorescent images of four week *in vitro* hAGO +GFP SM (green) +RFP ENCC (red) and epithelial ECAD (white). Higher magnification images are show on the bottom row. (**B**) Quantification of GFP+ mesenchyme, RFP+ neural, and ECAD+ epithelial populations within four week *in vitro* hAGOs (n=8 fields from at least 3 organoids from one differentiation, same trend seen across at least two individually seeded differentiations). (**C**) Representative images of gross *in vitro* and post transplantation hAGOs with and without incorporation of SM and GFP-labeled ENCC. GFP neurons formed networks around grafts post transplantations. (**D**) Quantification of mesenchymal populations within four week *in vitro* hAGOs (n=11-18 fields from at least 3 organoids from one differentiation, same trend seen across at least two individually seeded differentiations, ***$p<0.001$, Student's t-test). (**E**) Representative images and (**F**) quantification of the epithelial (1), proximal muscularis mucosa (2), submucosa (3), and distal muscularis externa (4) layer thickness from hAGOs 12 week post transplantation, 38 week old human fetal stomach, and adult stomach (n=3-9 fields from 3 hAGOs, 1 38 week fetal stomach, and 1 adult stomach). (**G**) Representative images of gross *in vitro* and post transplantation hFGOs with and without incorporation of SM and GFP-labeled ENCC. GFP neurons formed networks around grafts post transplantations (**H**) Representative histological (left) and immunofluorescent (middle and right) comparison of *in vitro* hFGOs with and without added SM and GFP ENCC as well as with and without added BMP4 and MEK pathway inhibitor PD03 to stimulate parietal cell differentiation. Neurons are labeled with TUJ1 (green, middle), smooth muscle with αSMA (red, middle), and parietal cells with APT4A (green, right) and ATP4B (red, right). Epithelium is labeled with ECAD (white). Inset (right) highlighting ATP4B+ parietal cell differentiation in hFGOs with added SM and ENCC. ATP4A/ATP4B staining is representative of a maxIP rendered from a z-stack of images by Nikon NIS Elements.

Figure S3


A
Brightfield
GFP
GFP/FOXF1
GFP/VIM
1 mo. in vitro HEO
-Control
+GFP SM
epi
200 µm
100 µm
B
HEO Mesenchymal Composition
%FOXF1+/GFP- Mese
%FOXF1+/GFP+ Mese
%FOXF1-/GFP+ Mese
%Cells of Total Cells
1%
0%
0%
1%
4%
20%
Control
+GFP SM
1 mo. in vitro HEO


C
Brightfield
2 mo. in vitro HEO
Control
+ENCC
D
TUJ1/KRT5/E-CAD/DAPI
1 mo. in vitro HEO
Control
+ENCC
100 µm
50 µm
E
TUJ1
MAP2
NESTIN
Relative mRNA Expression Level (Normalized to GAPDH)
p=0.055
*
**
Control
+NCC
1 mo. in vitro HEO


F
Brightfield
GFP+ SM
RFP+ ENS
GFP/RFP/ECAD
1 mo. in vitro HEO
+GFP SM +RFP NCC
500 µm
200 µm
G
Composition of 3 Germ Layer HEO
Epithelium
RFP+ ENS
GFP+ SM
Percentage of Total DAPI+ Cells
38%
7%
23%
1 mo. in vitro HEO +SM +NCC

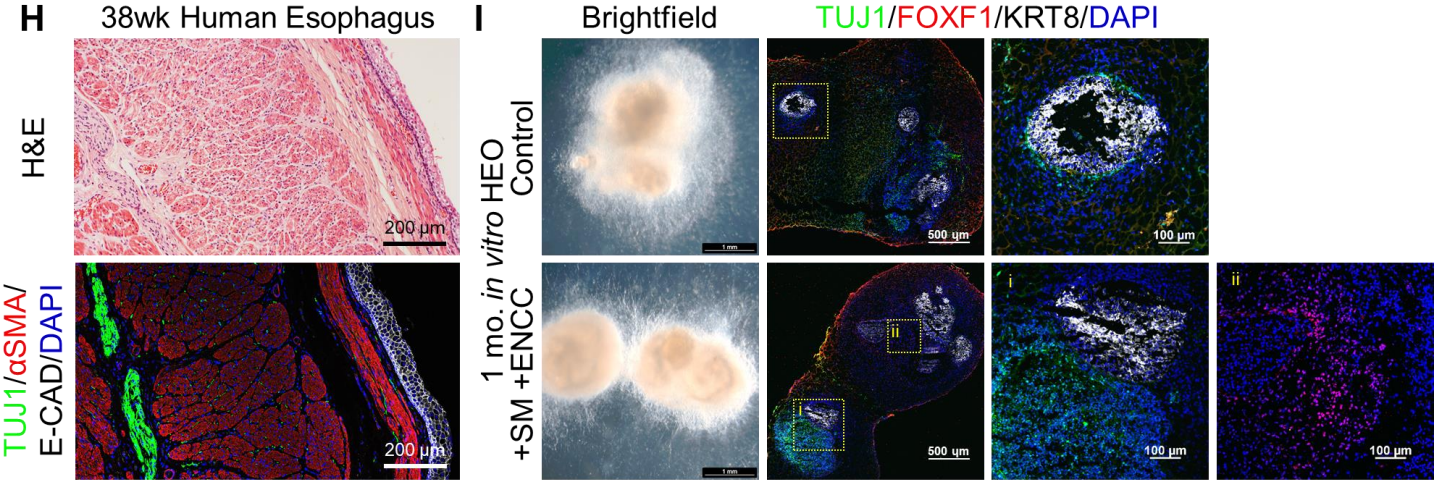

H
38wk Human Esophagus
H&E
TUJ1/αSMA/
E-CAD/DAPI
200 µm
I
Brightfield
TUJ1/FOXF1/KRT8/DAPI
1 mo. in vitro HEO
Control
+SM +ENCC
500 µm
100 µm
i
ii

Figure S3. Constructing three germ layer organoids *in vitro* is applicable to human esophageal organoids, relating to Figure 3. (**A**) Brightfield and GFP-fluorescent images of 1 mo. *in vitro* HEOs. GFP cells label exogenous hPSC-derived SM. Immunofluorescent images of representative HEOs depicting GFP+ (green), FOXF1+ and Vimentin+ (red) mesenchymal cells. (**B**) Quantification of different mesenchymal populations within 1 mo. *in vitro* HEOs. FOXF1+/GFP- cells mark endogenous mesenchyme, while both GFP+ groups represent exogenous SM (n=16-18 fields from at least 3 organoids per condition from one differentiation, same trend seen across at least two individually seeded differentiations, **p<0.01, ***p<0.001, Student's t-test). (**C**) Brightfield images of 2 mo. *in vitro* HEOs +/- ENCC. (**D**) Representative images of 1 mo. *in vitro* HEOs depicting TUJ1+ (green) enteric neurons surrounding the KRT5+ (red) and ECAD+ (white) epithelium of HEOs +ENCC. Higher magnification images are shown to the right. (**E**) Relative expression of neuronal-specific genes including tubulin genes, *TUJ1* and *MAP2*, and filament genes, *Nestin* within 1 mo. HEOs +ENCC (n=3, representative of 3 individual experiments, **p<0.01, ***p<0.001, Student's t-test). (**F**) Brightfield and fluorescent images of 1 mo. *in vitro* HEOs +GFP SM +RFP ENCC and epithelial marker ECAD (white). Higher magnification images are show on the bottom row. (**G**) Quantification of GFP+ mesenchymal, RFP+ neural, and ECAD+ epithelial populations within 1 mo. *in vitro* HEOs (n=12 fields from at least 3 organoids from one differentiation, same trend seen across at least two individually seeded differentiations). (**F**) Representative histological (top) and immunofluorescence (bottom) images of human 38 week esophagus stained with TUJ1 (green), αSMA (red), and ECAD (white). (I) Representative brightfield and immunofluorescent images of 1 mo. *in vitro* HEOs +/- SM +/- ENCC marked with TUJ1+ (green) enteric neurons, FOXF1+ (red) mesenchyme, and KRT8+ (white) epithelium. Higher magnification images are shown to the right.

Figure S4

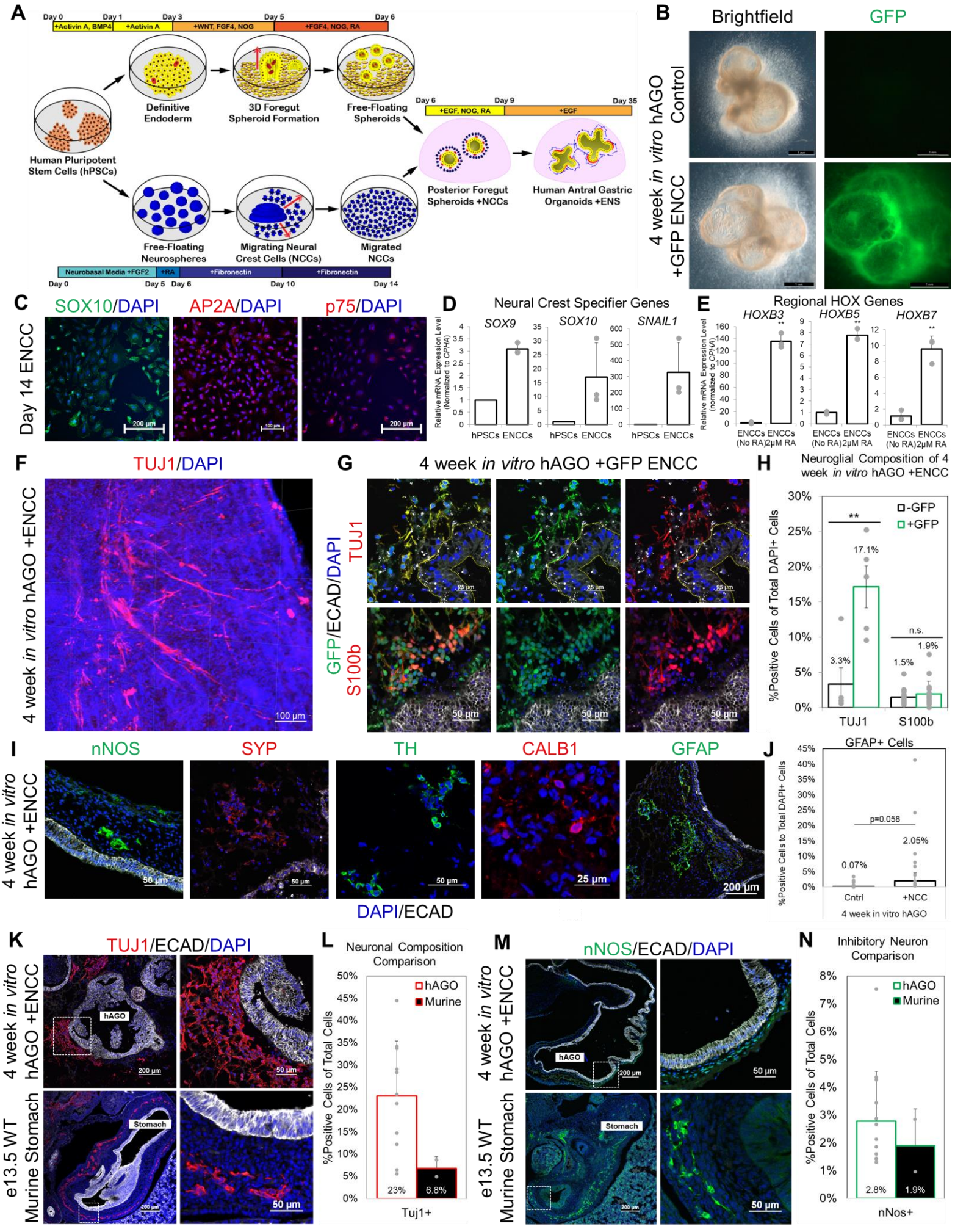
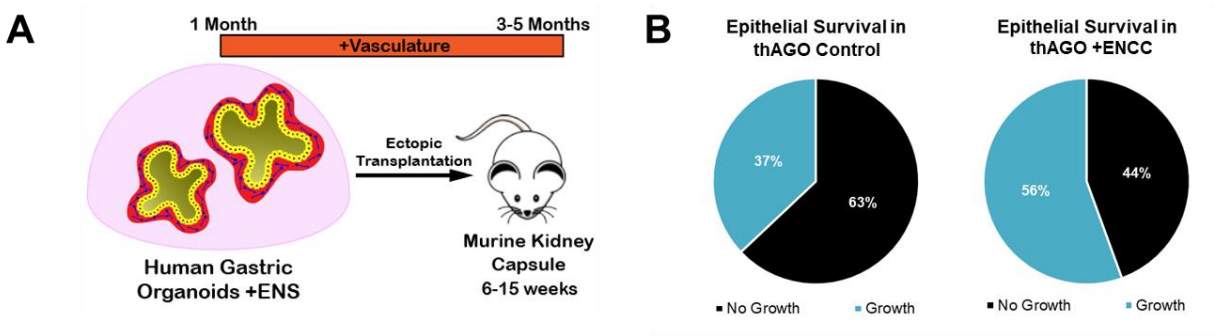
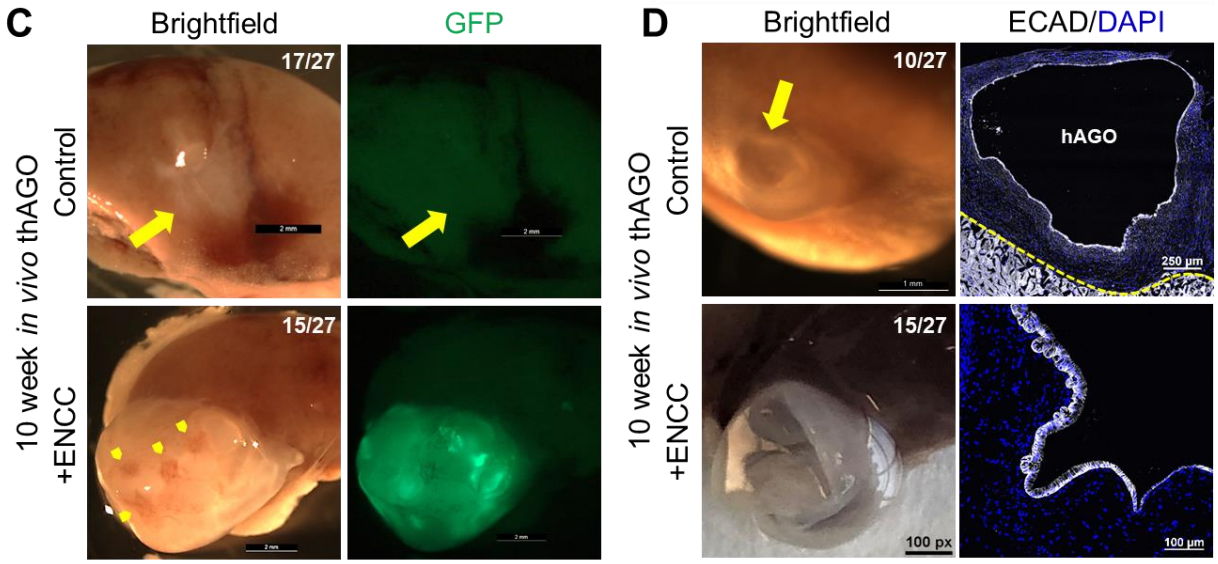
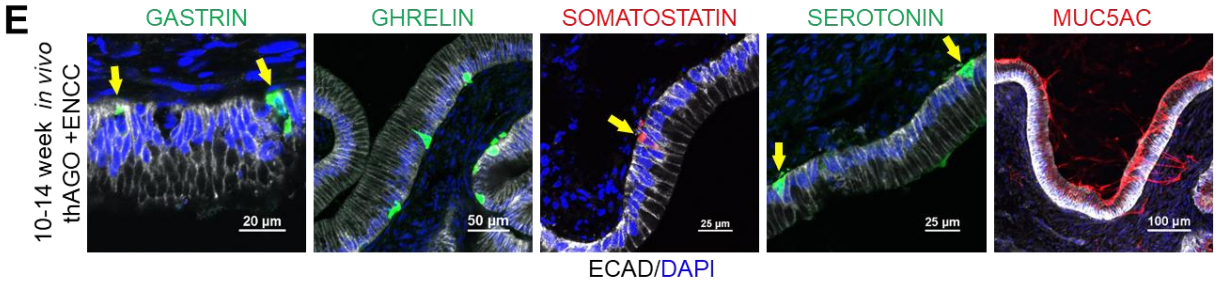

A
Day 0
Day 1
Day 3
Day 5
Day 6
+Activin A, BMP4
+Activin A
+WNT, FGF4, NOG
+FGF4, NOG, RA
Definitive Endoderm
3D Foregut Spheroid Formation
Free-Floating Spheroids
Human Pluripotent Stem Cells (hPSCs)
Day 6
Day 9
Day 35
+EGF, NOG, RA
+EGF
Posterior Foregut Spheroids +NCCs
Human Antral Gastric Organoids +ENS
Free-Floating Neurospheres
Migrating Neural Crest Cells (NCCs)
Migrated NCCs
Neurobasal Media +FGF2
+RA
+Fibronectin
+Fibronectin
Day 0
Day 5
Day 6
Day 10
Day 14
B
Brightfield
GFP
4 week in vitro hAGO Control
4 week in vitro hAGO +GFP ENCC
C
SOX10/DAPI
AP2A/DAPI
p75/DAPI
Day 14 ENCC
200 µm
100 µm
200 µm
D
Neural Crest Specifier Genes
SOX9
SOX10
SNAIL1
Relative mRNA Expression Level (Normalized to CPHA)
hPSCs ENCCs
hPSCs ENCCs
hPSCs ENCCs
E
Regional HOX Genes
HOXB3
HOXB5
HOXB7
Relative mRNA Expression Level (normalized to CPHA)
ENCCs (No RA) ENCCs 2µM RA
ENCCs (No RA) ENCCs 2µM RA
ENCCs (No RA) ENCCs 2µM RA
F
TUJ1/DAPI
4 week in vitro hAGO +ENCC
100 µm
G
4 week in vitro hAGO +GFP ENCC
TUJ1
GFP/ECAD/DAPI
S100b
50 µm
50 µm
50 µm
H
Neuroglial Composition of 4 week in vitro hAGO +ENCC
-GFP
+GFP
%Positive Cells of Total DAPI+ Cells
**
17.1%
3.3%
n.s.
1.9%
1.5%
TUJ1
S100b
I
nNOS
SYP
TH
CALB1
GFAP
4 week in vitro hAGO +ENCC
50 µm
50 µm
50 µm
25 µm
200 µm
DAPI/ECAD
J
GFAP+ Cells
%Positive Cells to Total DAPI+ Cells
p=0.058
0.07%
2.05%
Cntrl
+NCC
4 week in vitro hAGO
K
TUJ1/ECAD/DAPI
4 week in vitro hAGO +ENCC
e13.5 WT Murine Stomach
hAGO
Stomach
200 µm
50 µm
200 µm
50 µm
L
Neuronal Composition Comparison
hAGO
Murine
%Positive Cells of Total Cells
23%
6.8%
Tuj1+
M
nNOS/ECAD/DAPI
4 week in vitro hAGO +ENCC
e13.5 WT Murine Stomach
hAGO
Stomach
200 µm
50 µm
200 µm
50 µm
N
Inhibitory Neuron Comparison
hAGO
Murine
%Positive Cells of Total Cells
2.8%
1.9%
nNos+

Figure S4. hPSC-derived ENCCs differentiated into neuroglial subtypes when engineered into hAGOs without exogenous mesenchyme, relating to Figure 5. (**A**) Schematic depicting detailed method of deriving and innervating hAGOs. (**B**) Representative brightfield (left) and GFP fluorescent (right) images of four week *in vitro* hAGOs with and without GFP+ ENCC. (**C**) Representative images of end time point, day 14, monolayer ENCCs stained for ENCC markers SOX10 (green, left), AP2A (red, middle), and p75 (red, right). (**D**) Relative expression of neural crest specifier genes (*SOX9*, *SOX10*, and *SNAIL2*), and (**E**) regional hox patterning genes (*HOXB3*, *HOXB5*, *HOXB7*) (n=3 wells from one differentiation, same trend seen across at least four individually seeded differentiations, **p<0.01, Student's t-test). (**F**) Wholemount immunofluorescence of four week *in vitro* hAGO +ENCC labeled with TUJ1+ (red) neurons. (**G**) Representative images (**G**) and quantification (**H**) of TUJ1+ neurons (top) and S100b+ glial cells (bottom) co-expressed with GFP labeled ENCCs (n=6 fields from one differentiation, same trend seen across at least two individually seeded differentiations, ***p<0.001, Student's t-test). (**I**) Immunofluorescent images of specific neuronal subtypes, including inhibitory neurons (nNOS) and synaptophysin (SYNAP), dopaminergic neurons (TH), sensory neurons (CALB1), and glial fibrillary acidic protein (GFAP) in hAGOs +ENCC. (**J**) Quantification of GFAP+ cells in four week *in vitro* hAGO +ENCC (n=11-16 fields from at least 3 organoids from one differentiation, p-value determined using Student's t-test). Representative images (**K,M**) and quantification (**L,N**) of (**K,L**) TUJ1+ neurons (red) and (**M,N**) nNOS+ inhibitory neurons (green) within four week *in vitro* hAGOs +ENCC (top) and e13.5 WT murine stomach (bottom) (n>2 fields from one differentiation and one mouse; there is no significant difference). Epithelium is labelled with ECAD (white). Right panels are higher magnification insets of left panels.

Figure S5


A
1 Month
3-5 Months
+Vasculature
Ectopic Transplantation
Human Gastric Organoids +ENS
Murine Kidney Capsule 6-15 weeks
B
Epithelial Survival in thAGO Control
37%
63%
No Growth
Growth
Epithelial Survival in thAGO +ENCC
56%
44%
No Growth
Growth


C
Brightfield
GFP
10 week in vivo thAGO Control
17/27
+ENCC
15/27
D
Brightfield
ECAD/DAPI
10 week in vivo thAGO Control
10/27
hAGO
250 µm
+ENCC
15/27
100 px
100 µm


E
GASTRIN
GHRELIN
SOMATOSTATIN
SEROTONIN
MUC5AC
10-14 week in vivo thAGO +ENCC
20 µm
50 µm
25 µm
25 µm
100 µm
ECAD/DAPI

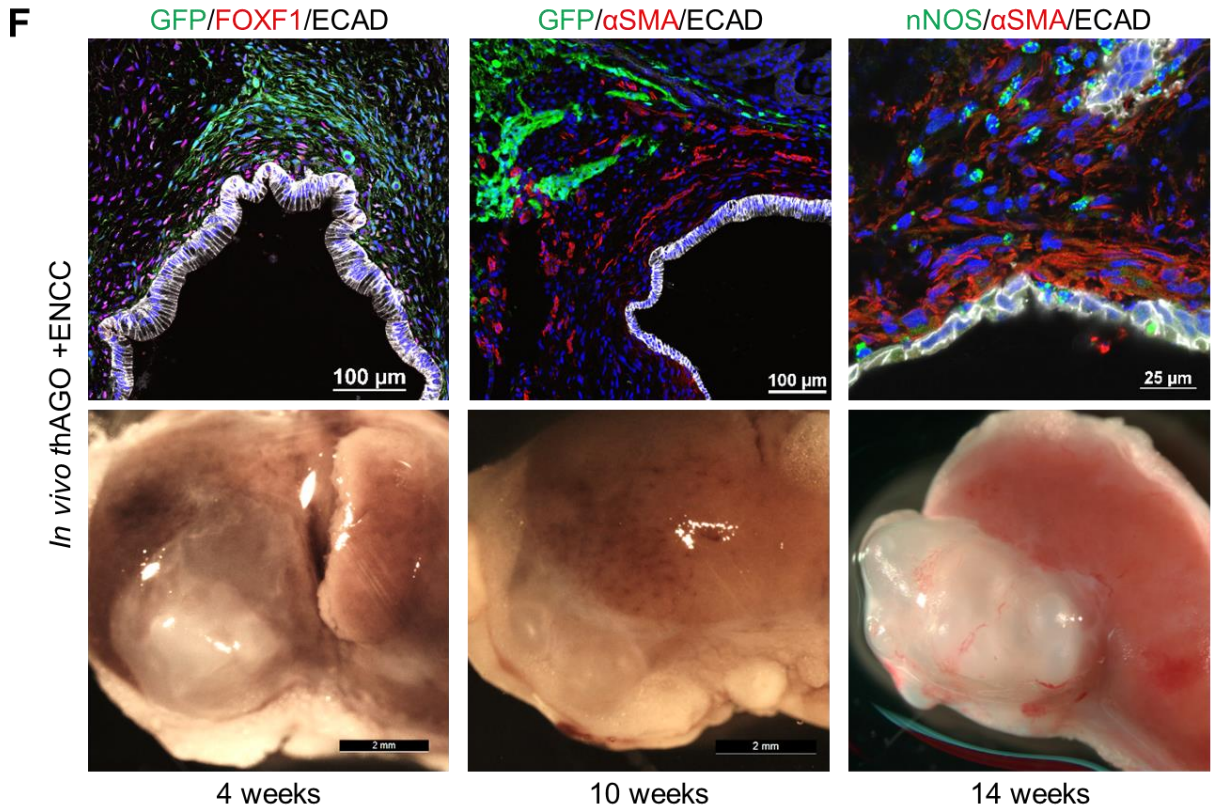

F
GFP/FOXF1/ECAD
GFP/αSMA/ECAD
nNOS/αSMA/ECAD
In vivo thAGO +ENCC
100 µm
100 µm
25 µm
2 mm
2 mm
4 weeks
10 weeks
14 weeks

Figure S5. ENS cells support *in vivo* growth and survival of hAGOs, relating to Figure 6. (**A**) Schematic illustration the method of transplanting hAGOs +ENCC. (**B**) Quantification of epithelial growth from thAGOs with and without ENCC (n=46-48 transplants per condition from 6 individual differentiations). (**C**) Representative brightfield (left) and GFP fluorescent (right) images of thAGOs with and without GFP+ ENCC following *in vivo* transplantation (n=29). (**D**) Brightfield (left) and immunofluorescent (right) images of ECAD+ epithelium (white) from *in vivo* thAGOs with or without ENCC cystic grafts. Representative images of (**E**) differentiated antral epithelial and (**F**) mesenchymal and neuronal cell types in hAGOs +ENCC following *in vivo* growth. (**E**) Endocrine cells (yellow arrow) are marked with gastrin, ghrelin, somatostatin, and serotonin, as well as surface mucous cells marked by MUC5AC. (**F**) Mesenchymal cells are marked with FOXF1+ with smooth muscle marked with αSMA. Lineage-traced hPSC-derived ENCCs are marked by GFP and differentiated inhibitory neurons are marked with nNOS. Sections were counterstained with epithelial marker ECAD (white) and nuclear DAPI (blue).

Figure S6

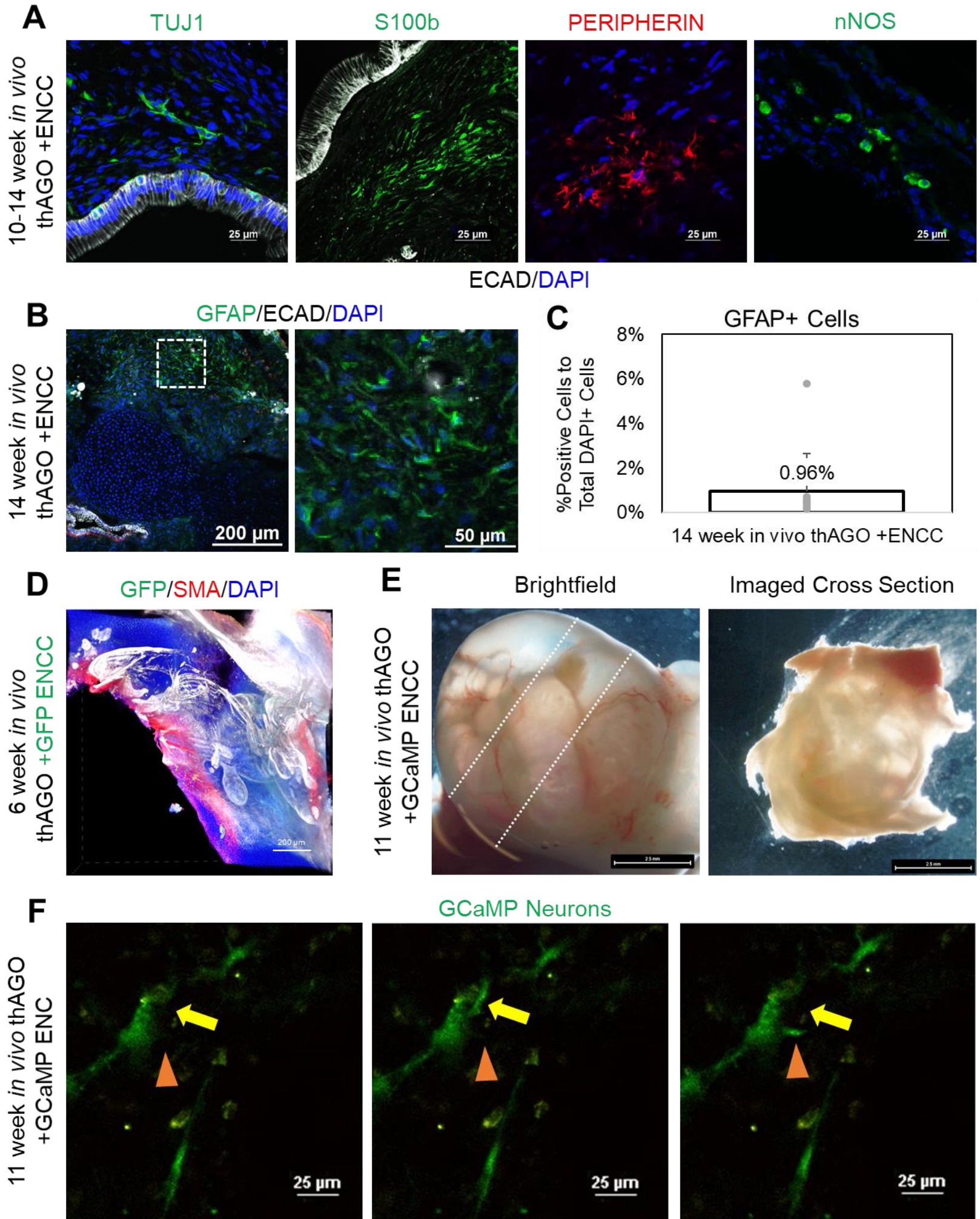

A
TUJ1
S100b
PERIPHERIN
nNOS
10-14 week in vivo thAGO +ENCC
25 µm
ECAD/DAPI
B
GFAP/ECAD/DAPI
14 week in vivo thAGO +ENCC
200 µm
50 µm
C
GFAP+ Cells
%Positive Cells to Total DAPI+ Cells
8%
6%
4%
2%
0%
0.96%
14 week in vivo thAGO +ENCC
D
GFP/SMA/DAPI
6 week in vivo thAGO +GFP ENCC
200 µm
E
Brightfield
Imaged Cross Section
11 week in vivo thAGO +GCaMP ENCC
F
GCaMP Neurons
11 week in vivo thAGO +GCaMP ENC
25 µm
25 µm
25 µm

Figure S6. Transplanted hAGO grafts +ENCC contain neuroglial cell types that are able to efflux calcium, relating to Figure 6. (**A-B**) Immunofluorescent images of *in vivo* hAGOs +ENCC show presence of (**A**) TUJ1+ (green, left) neural and S100b+ (green, middle) glial cells as well as differentiated neuronal subtypes marked by peripherin (red, middle) and nNOS (green, right) and (**B**) GFAP+ glial cells (green). ECAD (white) marks the epithelium. (**C**) Quantification of GFAP+ cells in 14 week *in vivo* hAGO +ENCC (n=10 fields from at least 3 organoids from one differentiation). (**D**) Wholemount immunohistochemistry of *in vivo* hAGO +GFP ENCC show a 3D network formation of GFP+ (green) neurons within αSMA+ (red) smooth muscle layers; ECAD (white) marks epithelium. (**E**) Gross morphology of *in vivo* hAGO +ENCC grafts used to obtain live images of GCaMP neuronal firing. (**F**) Static images of two individual GFP+ GCaMP neurons taken from live-imaged movie with neuronal firing indicated by a yellow arrow and orange triangle.

Figure S7

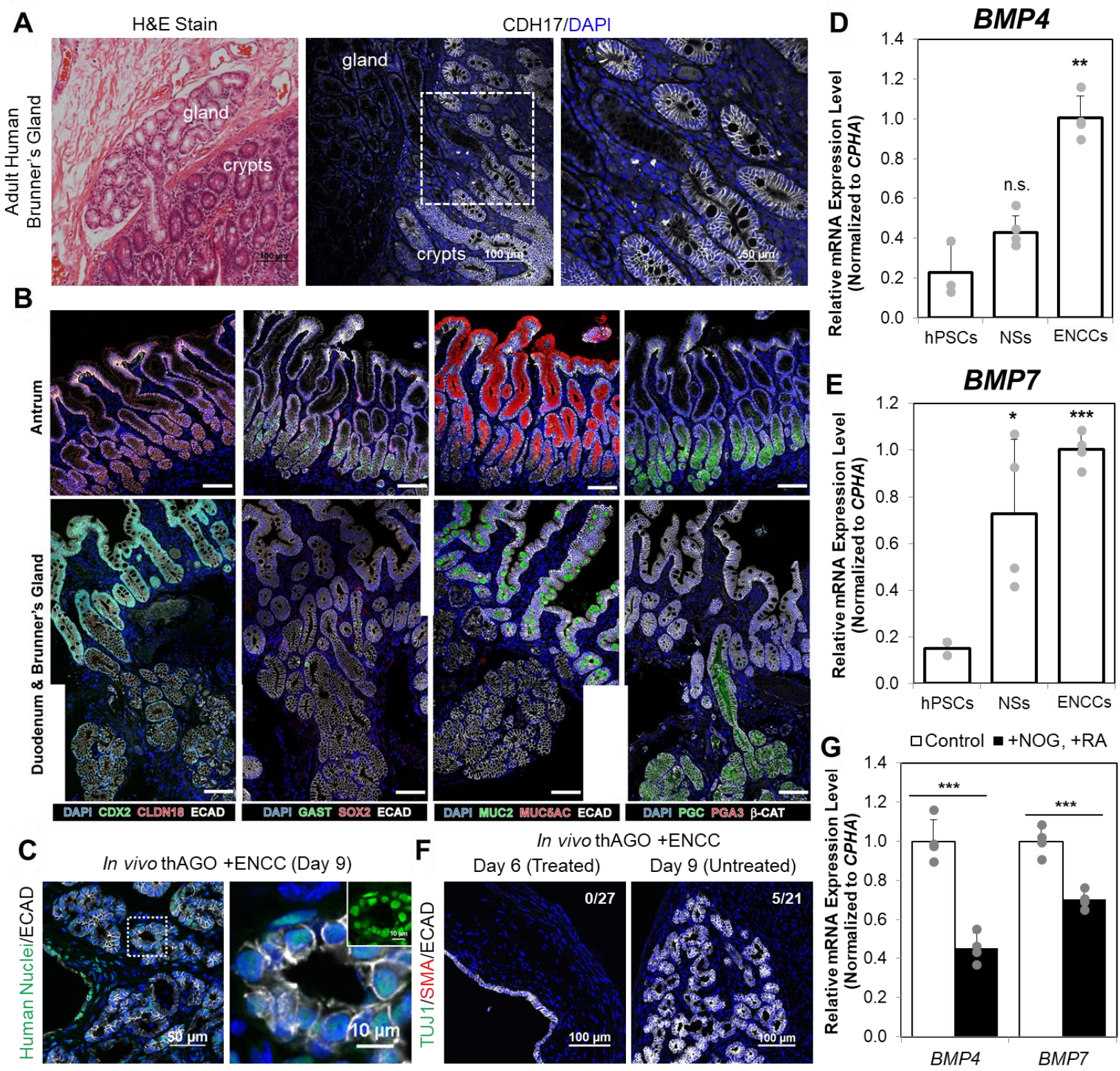
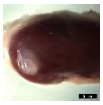
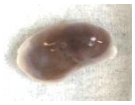
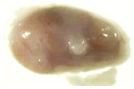
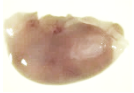
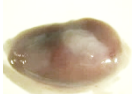
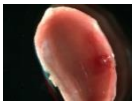
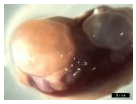
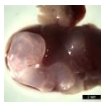
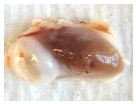
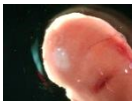
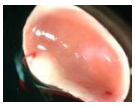
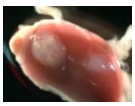
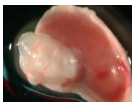
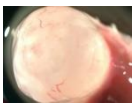

A
H&E Stain
CDH17/DAPI
Adult Human Brunner's Gland
gland
crypts
100 µm
50 µm
B
Antrum
Duodenum & Brunner's Gland
DAPI CDX2 CLDN18 ECAD
DAPI GAST SOX2 ECAD
DAPI MUC2 MUC5AC ECAD
DAPI PGC PGA3 β-CAT
C
In vivo thAGO +ENCC (Day 9)
Human Nuclei/ECAD
50 µm
10 µm
F
In vivo thAGO +ENCC
Day 6 (Treated)
Day 9 (Untreated)
TUJ1/SMA/ECAD
0/27
5/21
100 µm
D
BMP4
Relative mRNA Expression Level (Normalized to CPHA)
n.s.
**
hPSCs
NSs
ENCCs
E
BMP7
*
***
G
Control
+NOG, +RA
BMP4
BMP7

Figure S7. Markers that define human Brunner's glands, their use to study PSC-derived Brunner's gland-like epithelium, and a possible role for ENCC-derived BMP ligands in Brunner's gland development, relating to Figure 7. (**A**) H&E (left) and immunofluorescent (right) images of adult human Brunner's glands labeled with intestinal epithelial marker CDH17 (white). (**B**) Immunofluorescent comparison of adult human antrum (top) and duodenum and Brunner's glands (bottom). The gastric epithelial cell types are labeled with CLDN18, SOX2, MUC5AC, PGA3 (red), and PGC (green, right). Intestinal cell types are labeled with markers CDX2, and MUC2 (green). Endocrine hormone GAST (green, middle left) was observed in all regions. Only PGC and GAST (green) were consistently observed in Brunner's glands. Epithelium was labeled with ECAD or β-catenin (white). Duodenum and Brunner's glands images (bottom) are a composite of 2 individual 20x images manually overlayed. (**C**) Representative images of organoids with ECAD+ epithelium (white) and human nuclei expression (green) from thAGOs +ENCC at day 9 of hAGO protocol; higher magnification is shown to the right. (**D-E**) Relative expression of BMP ligands, (**D**) *BMP4* and (**E**) *BMP7*, at different points of ENCC differentiation. hPSCs and day 6 neurospheres (NSs) were used to compare to ENCCs (n=3-4 wells of cells per condition, *p<0.05, **p<0.01, ***p<0.001, Student's t-test). (**F**) Representative images of organoids with ECAD+ epithelium (white) from thAGOs +ENCC following recombination at either day 6 or day 9 of hAGO protocol. (**G**) Relative expression of BMP ligands with and without NOG and RA treatment (n=3-4 wells of cells per condition, ***p<0.001, Student's t-test).

Table S1 Additional bright field images of *in vivo* hAGO grafts recombined with either SM, ENCCs, neither, or both, relating to Figure 2.

SPONTANEOUS			
hAGO (Control)			
Recomb	Age	Bright field	Growth
Recombined/ Embedded on day 6 of hAGO protocol	6 wks		none
			none
			very little
	10 wks		very little
			very little
	11 wks		none
			small growth
			large growth
	12 wks		none
			none
			none
			small growth
	14 wks		none
Recombined/ Embedded on day 9 of hAGO protocol	6 wks		very little
			small growth
	10 wks		large growth
			cystic growth
	11 wks		small growth
			large growth
hAGO +ENCC			
Recomb	Age	Bright field	Growth
Recombined/ Embedded on day 6 of hAGO protocol	6 wks		none
			very little
			small growth; fluid filled cyst
	7 wks		none
	10 wks		large growth
	11 wks		large growth with cartilage
			large growth
			large cystic growth

	14wks		none
			very little
			very little
			large growth
			large growth
			cystic growth
			cystic growth
Recombined/ Embedded on day 9 of hAGO protocol	6 wks		none
			small growth
	7 wks		none
			very little
			none
	11 wks		large growth w/ some cartilage
			large growth

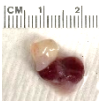
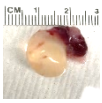
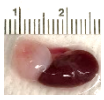
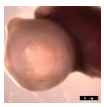
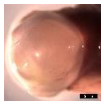
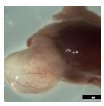
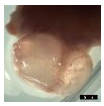
hAGO +SM			
Recomb	Age	Bright field	Growth
Recombined/ Embedded on day 6 of hAGO protocol	10 wks		large growth
			large growth
hAGO +SM +ENCC			
Recomb	Age	Bright field	Growth
Recombined/ Embedded on day 6 of hAGO protocol	10 wks		large growth
			large growth
			large growth
			large growth
Recombined/ Embedded on day 9 of hAGO protocol	10 wks		cystic growth
			cystic growth

Table S2 List of all neural markers assessed within *in vitro* and *in vivo* organoid cultures, relating to Figure 5.

Protein Name Used in Figures	Protein Name/Type	Neuroglia Cell Type	Functionality	Organoid Expression	Quant-ification for hAGO
TUJ1	neuron-specific class III β-tubulin protein	differentiated human neural progenitor cells	microtubule stability in neuronal cell bodies and axons; axonal transport	4 wk *in vitro* hAGOs +ENCC (Fig. 5B, Supp. Figs. S4G,K); 4 wk *in vitro* HEOs +ENCC (Fig. S3D); 10-12 wk *in vivo* hAGOs and hFGOs +SM +ENCC (Figs. 3C-D, S6A)	~20-25% *in vitro* (Fig. S4H)
S100β	calcium-binding protein B	pan-glial; astrocytes	regulates proliferation and differentiation; microtubule assembly and neurite extension; calcium efflux	4 wk *in vitro* hAGOs +ENCC (Fig. S4G); 10-14 wk *in vivo* hAGOs +ENCC (Fig. S6A)	~5% *in vitro* (Fig. S4H)
nNOS	nitric oxide synthase enzyme	nitrergic inhibitory motoneurons	relaxation of smooth muscle	4 wk *in vitro* hAGOs +ENCC (Figs. S4I,M); 10-14 wk *in vivo* hAGOs +ENCC (Figs. S5F, S6A)	~3% *in vitro* (Fig. S4N)
GFAP	glial fibrillary acidic protein, type III intermediate filament	non-myelinating Schwann cells; enteric glial cells	maintaining cytoskeletal structure/mechanical strength	4 wk *in vitro* hAGOs +ENCC (Figs. S4I); 14 wk *in vivo* hAGOs +ENCC (Fig. S6B)	~1% *in vivo* (Fig. S6C)
SYP	synaptophysin, major synaptic vesicle protein	interneurons	neuro-transmitter	4 wk *in vitro* hAGOs +ENCC (Fig. S4I)	N/A
CHAT	choline acetyltransferase	cholinergic neurons' motoneurons	neuro-transmitter	10 wk *in vivo* hAGO +SM +ENCC (Fig. 2D)	N/A
TH	tyrosine hydroxylase enzyme	dopaminergic peripheral sympathetic neurons	neuro-transmitter	4 wk *in vitro* hAGOs +ENCC (Fig. S4I); 10 wk *in vivo* hAGO +SM +ENCC (Fig. 2D)	N/A
CALB1	calbindin 1, calcium-binding protein	excitatory motoneurons; intrinsic sensory neurons	mediates calcium absorption	4 wk *in vitro* hAGOs +ENCC (Fig. S4I)	N/A
PRPH	peripherin, type III intermediate filament	peripheral neurons	neurite elongation and axonal guidance during development	10-14 wk *in vivo* hAGO +ENCC (Fig. S6A)	N/A

Table S3 List of oligonucleotides, relating to Key Resource Table.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
BAPX1 F: CAACACCGTCGTCCTCG	Integrated DNA Technologies (IDT)	N/A (synthesized)
BAPX1 R: CCGCTTCCAAAGACCTAGAG	IDT	N/A (synthesized)
BARX1 F: CCAGTGGGAACTTGAACACC	IDT	N/A (synthesized)
BARX1 R: CTGAAGTTCGGCGTGCAG	IDT	N/A (synthesized)
BMP4 F: CAAACTTGCTGGAAAGGCTC	IDT	N/A (synthesized)
BMP4 R: CCGCTACTGCAGGGACCTAT	IDT	N/A (synthesized)
BMP7 F: CGATTTCAGCCTGGACAACG	IDT	N/A (synthesized)
BMP7 R: CCTGGGTACTGACACGG	IDT	N/A (synthesized)
CPHA F: CCCACCGTGTTCTTCGACATT	IDT	N/A (synthesized)
CPHA R: GGACCCGTATGCTTTAGGATGA	IDT	N/A (synthesized)
FGF10 F: TGCTGTTAATGGCTTTGACG	IDT	N/A (synthesized)
FGF10 R: AGAAGAACGGGAAGGTCAGC	IDT	N/A (synthesized)
FOXF1 F: CGTCCTCTTCCATGCACTCG	IDT	N/A (synthesized)
FOXF1 R: TTTTGAGGGAGTGAGGAAGG	IDT	N/A (synthesized)
GAPDH F: CCCATCACCATCTTCCAGGAG	IDT	N/A (synthesized)
GAPDH R: CTTCTCCATGGTGGTGAAGACG	IDT	N/A (synthesized)
ISL1 F: TCACGAAGTCGTTCTTGCTG	IDT	N/A (synthesized)
ISL1 R: CATGCTTTGTTAGGGATGGG	IDT	N/A (synthesized)
MAP2 F: TTCGTTGTGTCGTGTTCTCA	IDT	N/A (synthesized)
MAP2 R: AACCGAGGAAGCATTGATTG	IDT	N/A (synthesized)
NESTIN F: GAGGGAAGTCTTGGAGCCAC	IDT	N/A (synthesized)
NESTIN R: AAGATGTCCCTCAGCCTGG	IDT	N/A (synthesized)
SIX2 F: CGGGTTGTGGCTGTTAGAAT	IDT	N/A (synthesized)
SIX2 R: CACCACACAGGTCAGCAACT	IDT	N/A (synthesized)
TUJ1 F: CGATGCCATGCTCATCAC	IDT	N/A (synthesized)
TUJ1 R: CCCAGTATGAGGGAGATCGT	IDT	N/A (synthesized)