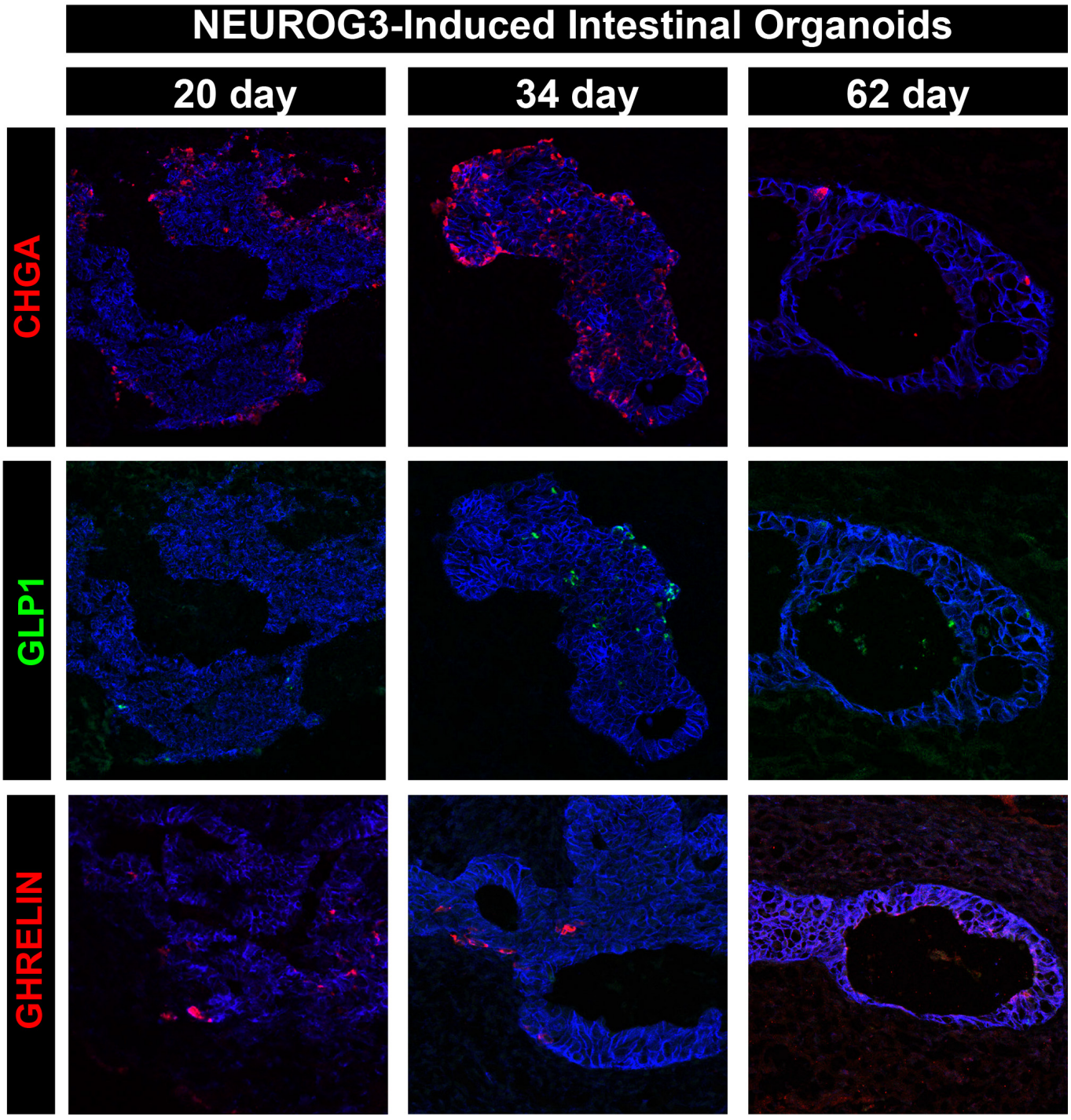

Fig. S1. Related to Figure 1. 34 day-old organoids are optimal for NEUROG3 Induction
20, 34, and 62-day old HIOs were given doxycycline to induce NEUROG3 expression. While 20 day and 34 day HIOs were capable of inducing many CHGA positive cells, 62 day HIOs did not respond as readily to forced NEUROG3 expression. In addition, staining with hormone-specific markers, we found that 34-day old HIOs give rise to more differentiated subtypes, which are not found in 20-day HIOs. From these data, we conclude that 34-day HIOs are optimally competent for NEUROG3 induction and EEC specification.

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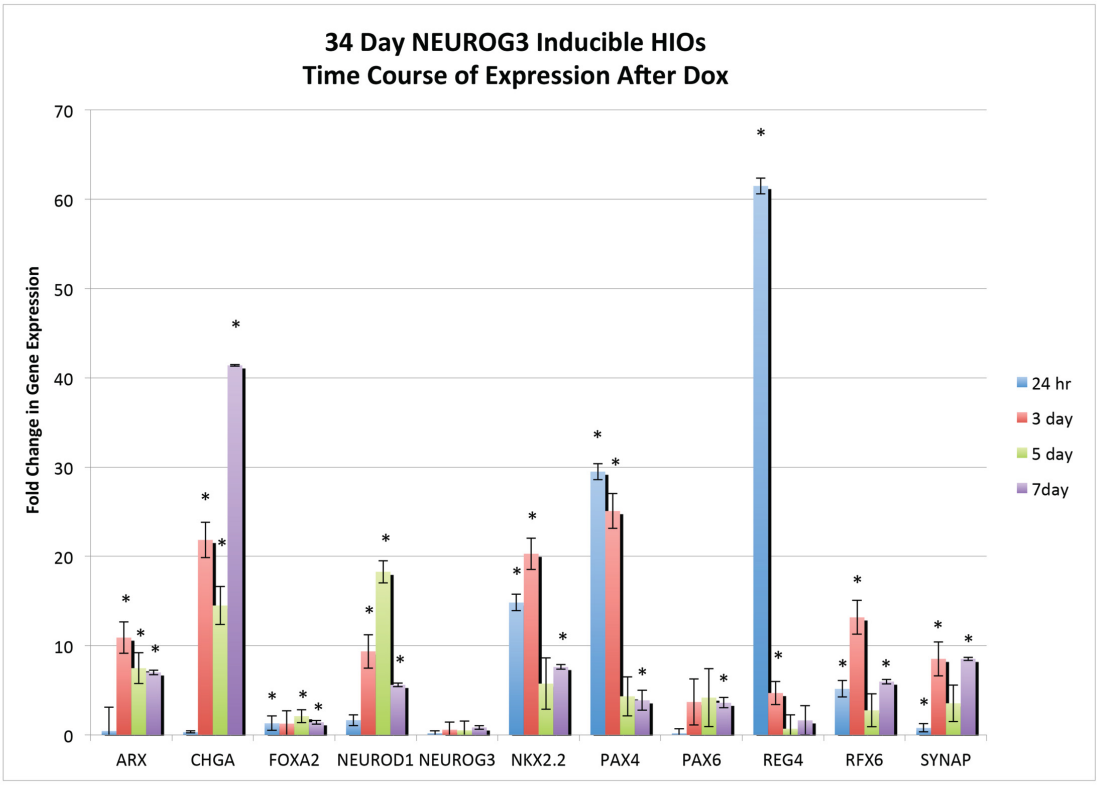
A



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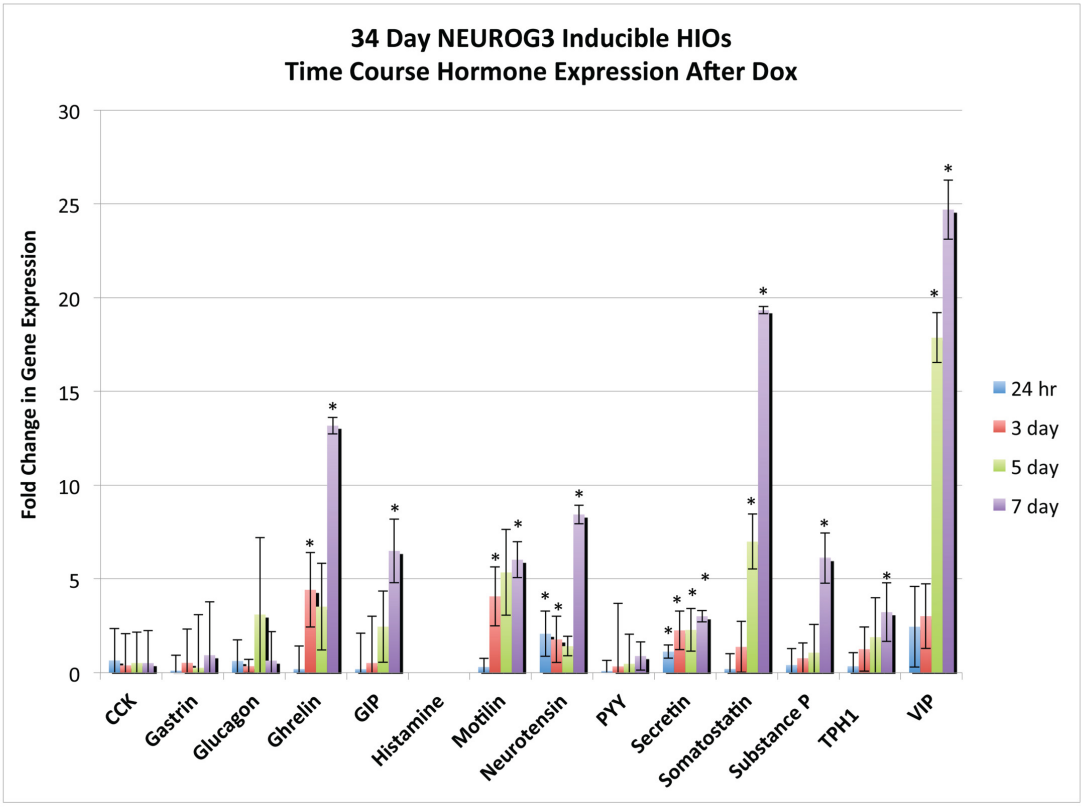

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Fig. S2. Related to Figure 3. Time-course of hormone and transcription factor expression in NEUROG3-induced HIOs

(A) Gene expression in HIOs 24-hr, 3 day, 5 day, and 7 days after the 8-hr pulse of doxycycline. Certain TF's that are known direct targets of NEUROG3 (*NKX2.2*) are up-regulated at 24-hr, whereas later genes that differentiate hormone subtypes and indicate vesicle maturity (*ARX*, *CHGA*, respectively) are up-regulated and maintained at 3-, 5-, and 7 days post doxycycline. **(B)** Gene expression of hormone subtypes 24-hr, 3 days, 5 days, and 7 days after doxycycline. Interesting, proximal subtypes CCK and Gastrin were not readily up-regulated at any time-point. Serotonin is produced from the *TPH1* gene. Values in graphs represent mean ± S.E.M. *P<0.05, (n=3).

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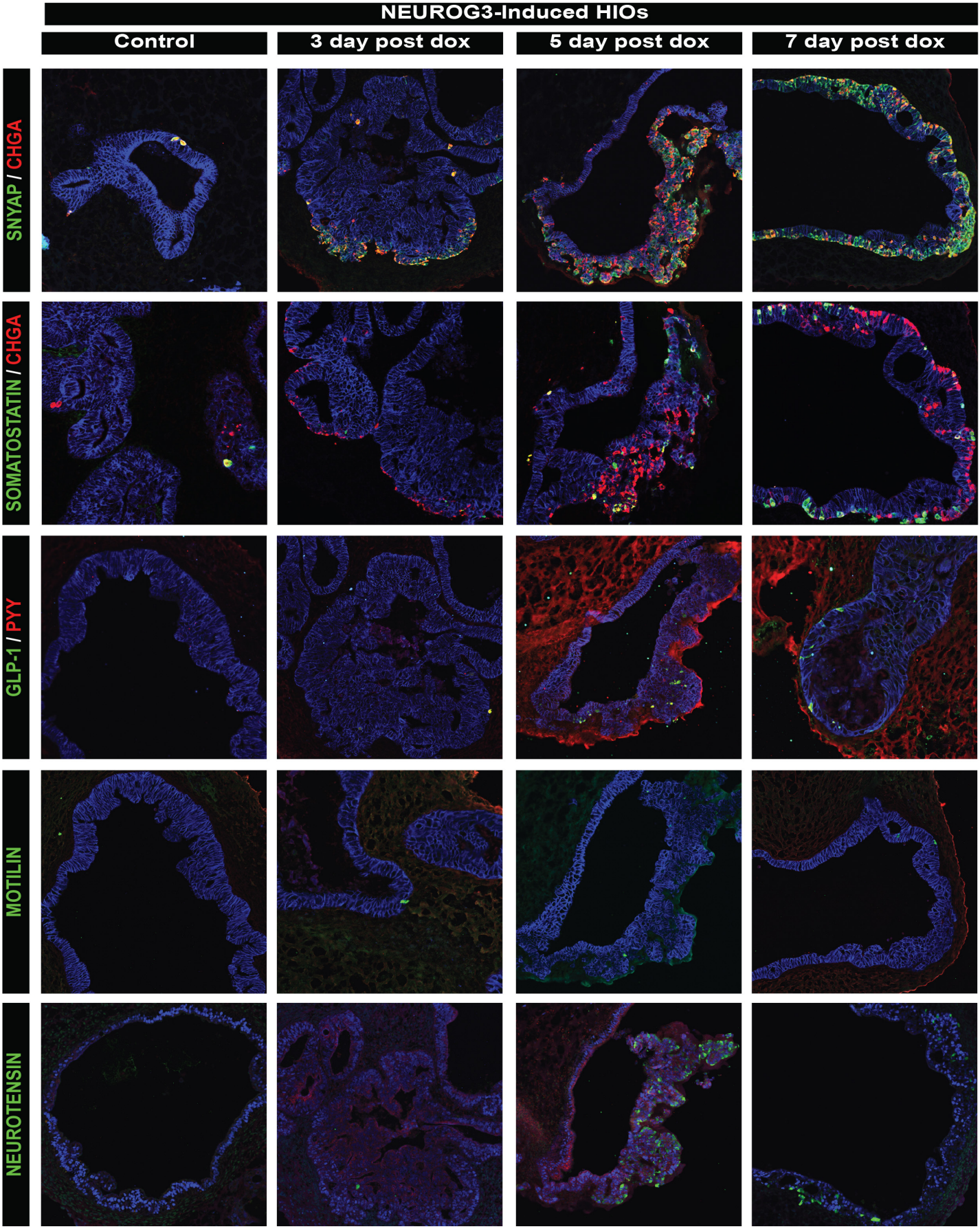
NEUROG3-Induced HIOs
Control
3 day post dox
5 day post dox
7 day post dox
SNYAP / CHGA
SOMATOSTATIN / CHGA
GLP-1 / PYY
MOTILIN
NEUROTENSIN

Fig. S3. Related to Figure 4. Time-course of protein expression of NEUROG3-induced hormones
A subset of hormone-positive subtypes are induced over the course of a week after NEUROG3 induction. Hormone staining and subtype differentiation at 3 days, 5 days, and 7 days post doxycycline induction. Few EECs are observed in the control, as marked by CHGA and SYNAP. However, after induction, significantly more EECs are specified and go onto differentiate into Somatostatin, GLP-1, Motilin, and Neurotensin-positive cells. Images are meant to highlight the presence and variety of cell types induced in the HIO, but are not representative of the entire HIO epithelium.

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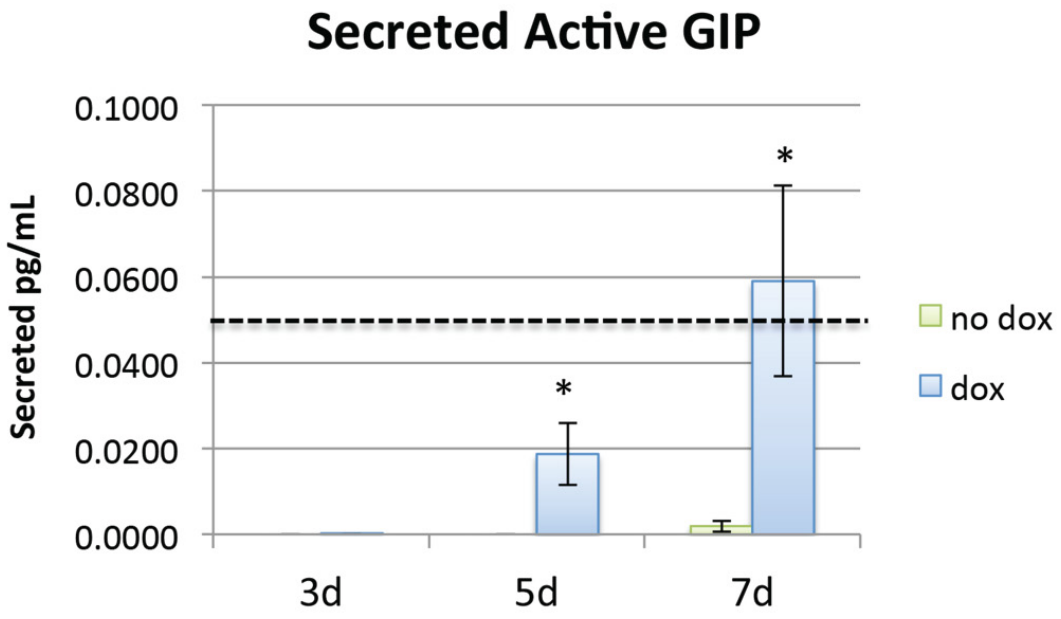
A



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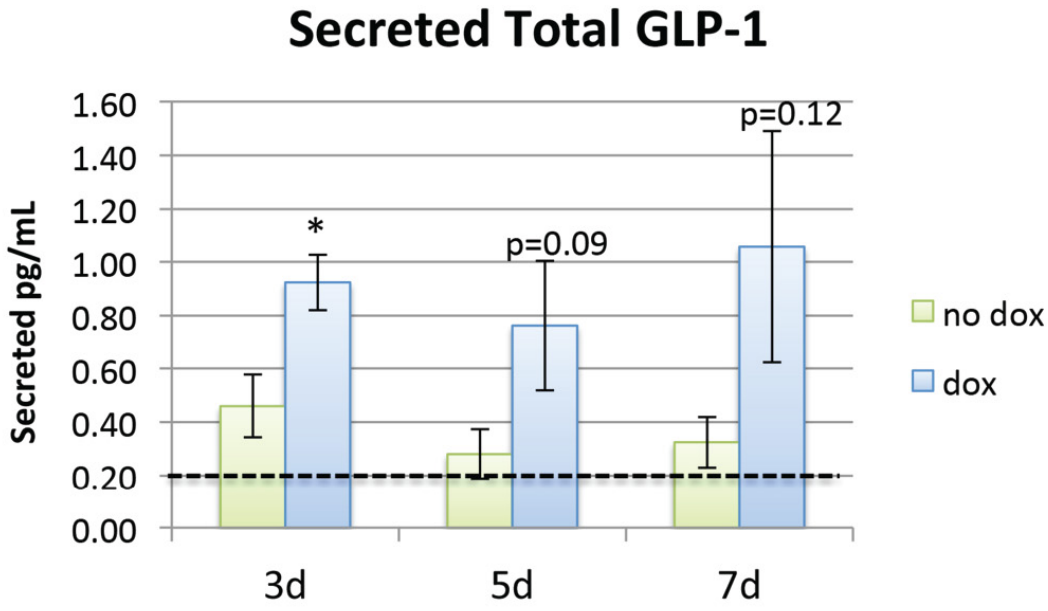

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Fig. S4. Related to Figure 4. Secretion of Hormones in NEUROG3-induced HIOs
Time-course of EEC hormone secretion post doxycycline exposure. **(A)** Active GIP secretion is only observed at 7 days after forced NEUROG3 expression. **(B)** While some detectable GLP-1 was secreted at 3-, 5-, and 7 days after NEUROG3 induction, we did not observe a significant increase at 5- and 7-days as compared to un-induced controls. This was possibly owing to the fact that more proximal HIOs induce differentiation and secretion of proximal hormones. Dotted lines represent detection limits for ELISA assays. iPSC lines were used for the secretion studies. (n=6 for each time-point)

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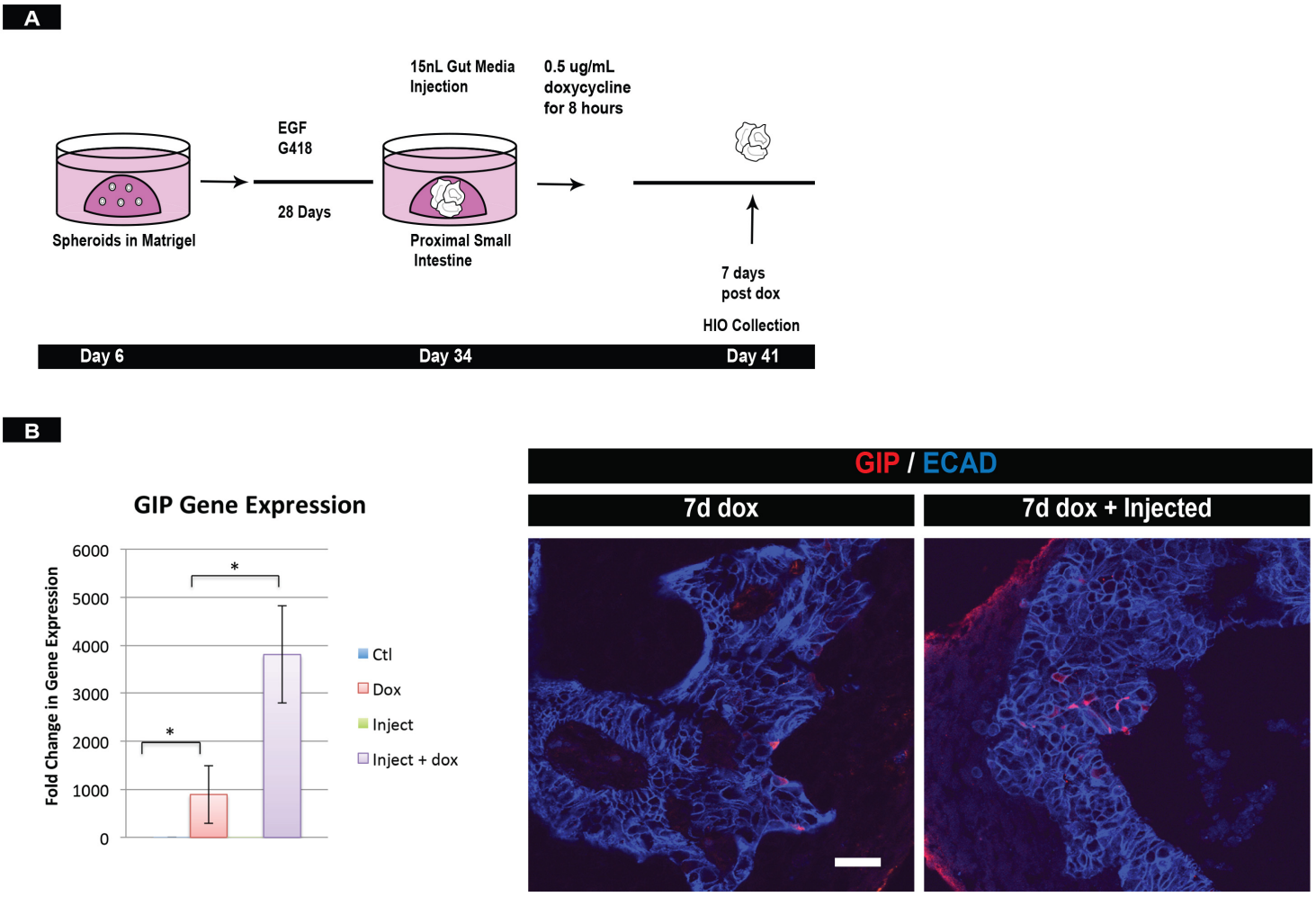

Fig. S5. Related to Figure 5. NEUROG3-induced HIOs respond to luminal nutrients.
(A) Schematic of injection experiment. 34-day HIOs were given 15nL of gut media (glucose, essential amino acids, serum) and then pulsed with doxycycline to induce NEUROG3. 7 days after doxycycline, HIOs were collected. **(B)** qPCR of induced-injected HIOs reveals an increase in *GIP* mRNA, indicating HIOs are transcriptionally responsive to luminal nutrients. Immunofluorescence for GIP protein indicates that induced-injected HIOs also have more GIP+ cells, indicating luminal nutrients may potentiate GIP EEC differentiation. *P<0.05, (n=3).

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Table S1. Summary Location of Gut Enteroendocrine Hormones

Cell	Hormone	Stomach	Duodenum	Jejunum	Ileum	Colon
G	Gastrin					
X/A	Ghrelin/Motilin	Ghrelin Only				
S	Secretin					
I	CCK					
K	GIP					
L	GLP-1					
(L)	PYY					
(L)	INSL5					
N	Neurotensin					
D	Somatostatin					
EC	Substance-P/5'HT					

***The intensity of blue shading indicates the localization of the hormone within that region. As the shading gets lighter, the enteroendocrine cell and hormone presence is less prevalent in that region of the intestine. As seen, most hormones have a gradient of expression pattern within the intestinal epithelium, with the exception of D and EC cells, which are found throughout the gastrointestinal tract.**

Table S2. Primary Antibody List

Primary Antibody	Source	Catalog Number	Dilution
Goat anti-Somatostatin	Santa Cruz	SC-7819	1:200
Goat anti-Glut2	Santa Cruz	SC-7580	1:500
Mouse anti-Muc6	Abcam	Ab49462	1:500
Mouse anti-Cdx2	BioGenex	Cdx2-88	1:500
Goat anti-Pdx1	Abcam	Ab94931	1:5000
Mouse anti-Ecad	BD Transduction	610182	1:500
Rat anti-Ecad	R&D	MAB7481	1:1000
Goat anti-Ecad	R&D	AF648	1:500

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Rabbit anti-PYY	Abcam	22663	1:1000
Rabbit anti-ChromograninA	Immunostar	20086	1:500
Goat anti-ChromograninA	Santa Cruz	Sc-1488	1:200
Goat anti-Ghrelin	Santa Cruz	SC-10368	1:300
Mouse anti-GLP-1	Biovision	3104-100	1:200
Goat anti-CCK	Santa Cruz	SC-21617	1:200
Goat anti-GIP	Santa Cruz	SC-23554	1:500
Mouse anti-Motilin	Santa Cruz	SC-376605	1:100
Rabbit anti-5'HT	Immunostar	20080	1:1000
Guinea Pig anti-Synaptophysin	Synaptic Systems	101004	1:1000
Rabbit anti-Gastrin	DAKO	A0568	1:500

Table S3. Primer List
Primers sets for qPCR detection of human hormone transcript. Those not listed were obtained from a predesigned 96-well TaqMan array plate fom Life Technologies.

Hormone Transcript	**Primers**
GHRELIN	Forward: GCT GGT ACT GAA CCC CTG AC Reverse: GAT GGA GGT CAA GCA GAA GG
GIP	Forward: GTT GAG GGC TGC TCA CCT TA Reverse: GGC AGT GGG ACT AGG AGA GA
MOTILIN	Forward: TCC AAT TTC CAG AGG AGC AG Reverse: CCC TGA GTG TAT GGC AGA GG
GCG	Forward: CAG CAA GTA TCT GGA CTC CAG G Reverse: CCA GTT TAT AAA GTC CCT GGC
CHGA	Forward: TGT GTC GGA GAT GAC CTC AA Reverse: GTC CTG GCT CTT CTG CTC TG

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