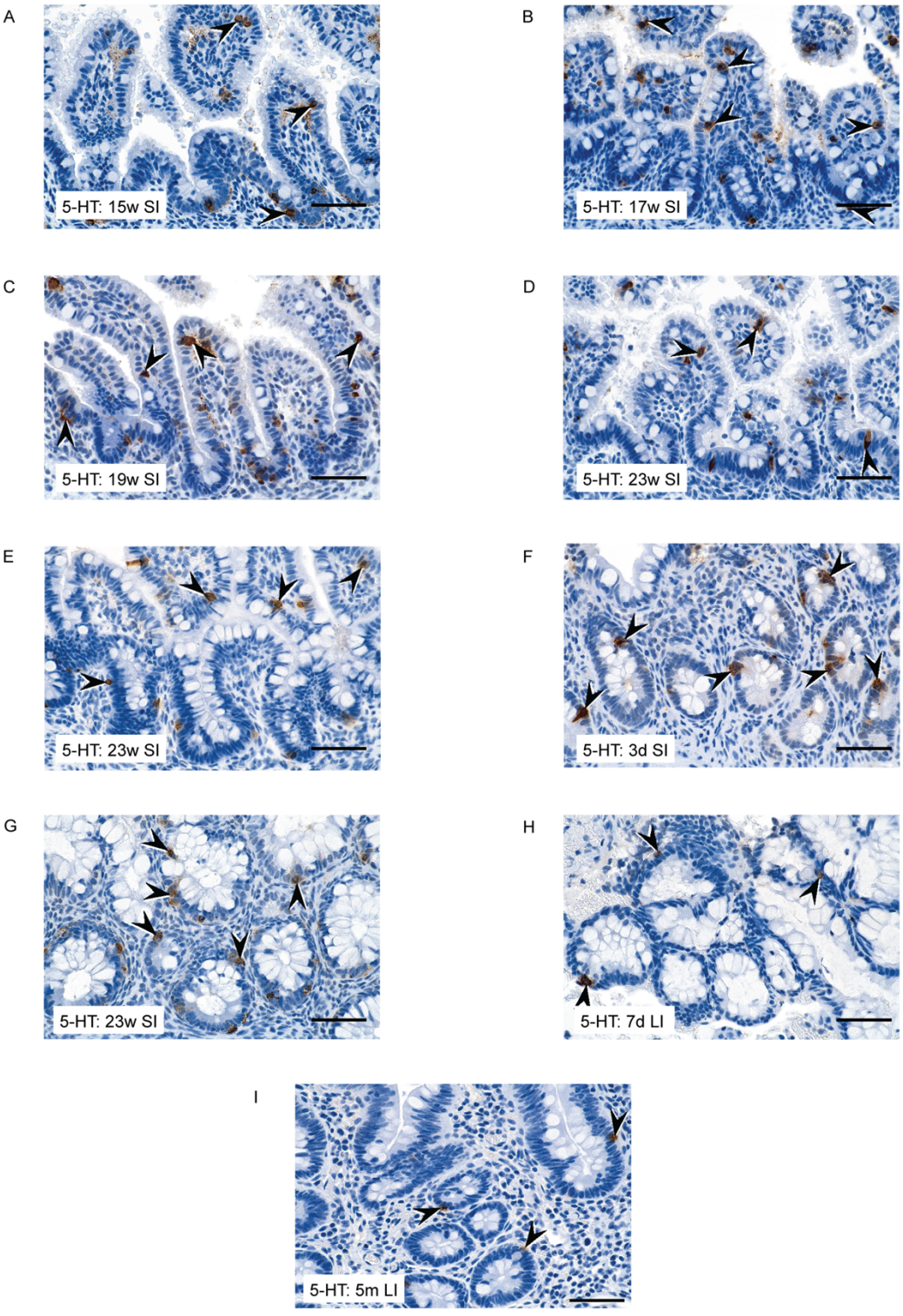

A
5-HT: 15w SI
B
5-HT: 17w SI
C
5-HT: 19w SI
D
5-HT: 23w SI
E
5-HT: 23w SI
F
5-HT: 3d SI
G
5-HT: 23w SI
H
5-HT: 7d LI
I
5-HT: 5m LI

Figure S1. Serotonin expression in early-life intestine (cont.) (A-E) Representative immunohistochemistry staining of serotonin (5HT) in fetal SI tissues, (F) neonatal SI tissue, (G-H) fetal LI tissues, (I) neonatal LI tissue, and (J) pediatrics LI tissue. Gestational ages in panels A-E and G are in weeks, neonatal ages in panels F and H are in days, and neonatal age in panel I is in months. Arrowheads identify individual enterochromaffin cells. Scale bar in panels A-I represents 50 μm.

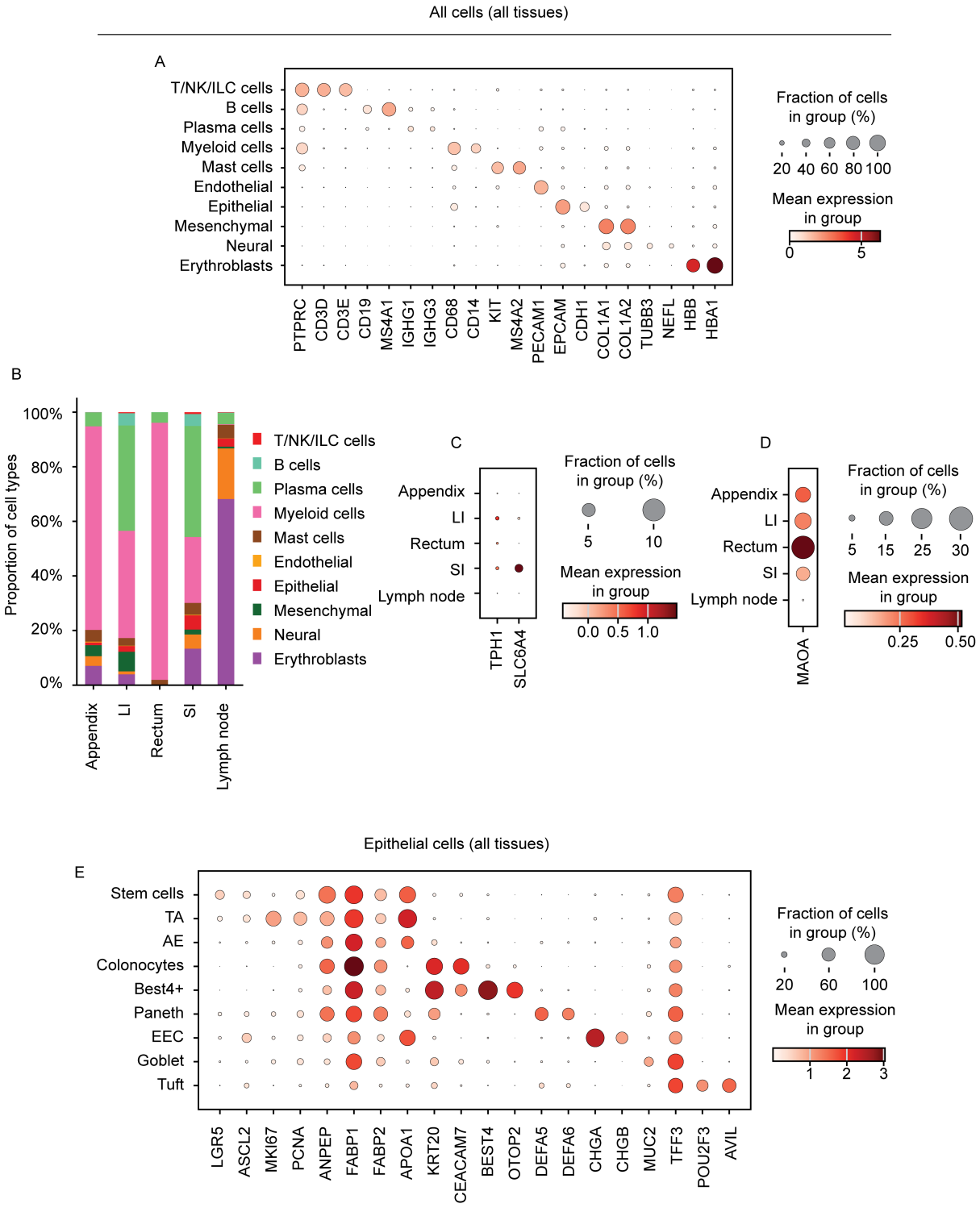

All cells (all tissues)
A
T/NK/ILC cells
B cells
Plasma cells
Myeloid cells
Mast cells
Endothelial
Epithelial
Mesenchymal
Neural
Erythroblasts
PTPRC
CD3D
CD3E
CD19
MS4A1
IGHG1
IGHG3
CD68
CD14
KIT
MS4A2
PECAM1
EPCAM
CDH1
COL1A1
COL1A2
TUBB3
NEFL
HBB
HBA1
Fraction of cells in group (%)
20 40 60 80 100
Mean expression in group
0 5
B
Proportion of cell types
100%
80%
60%
40%
20%
0%
Appendix
LI
Rectum
SI
Lymph node
T/NK/ILC cells
B cells
Plasma cells
Myeloid cells
Mast cells
Endothelial
Epithelial
Mesenchymal
Neural
Erythroblasts
C
Appendix
LI
Rectum
SI
Lymph node
TPH1
SLC6A4
Fraction of cells in group (%)
5
10
Mean expression in group
0.0 0.5 1.0
D
Appendix
LI
Rectum
SI
Lymph node
MAOA
Fraction of cells in group (%)
5 15 25 30
Mean expression in group
0.25 0.50
Epithelial cells (all tissues)
E
Stem cells
TA
AE
Colonocytes
Best4+
Paneth
EEC
Goblet
Tuft
LGR5
ASCL2
MKI67
PCNA
ANPEP
FABP1
FABP2
APOA1
KRT20
CEACAM7
BEST4
OTOP2
DEFA5
DEFA6
CHGA
CHGB
MUC2
TFF3
POU2F3
AVIL
Fraction of cells in group (%)
20 60 100
Mean expression in group
1 2 3

Figure S2. Serotonin synthesis, transportation, degradation genes in the epithelial compartment across tissues and developmental stages (cont.) (A) Dot plot of marker genes expression in cell types across intestines. Color represents normalized mean expression of markers genes in each cell type, and size indicates the proportion of cells expressing marker genes. (B) Proportion bar plot of cell types in each region. Dot plot to visualize the expression of (C) *TPH1* and *SLC6A4* and (D) *MAOA* across tissue regions. (E) Dot plot of marker genes expression in epithelial subtype types across intestines. Color represents normalized mean expression of markers genes in each cell type, and size indicates the proportion of cells expressing marker genes.

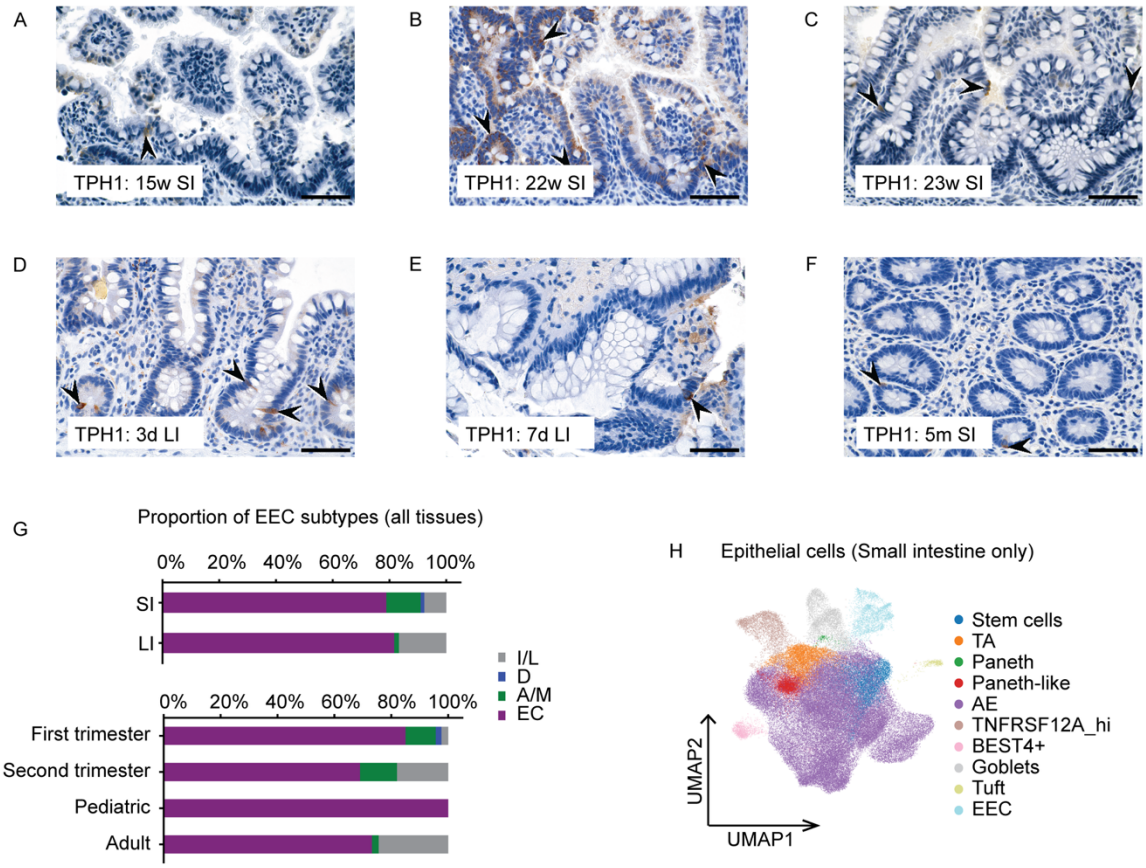

A
TPH1: 15w SI
B
TPH1: 22w SI
C
TPH1: 23w SI
D
TPH1: 3d LI
E
TPH1: 7d LI
F
TPH1: 5m SI
G
Proportion of EEC subtypes (all tissues)
0% 20% 40% 60% 80% 100%
SI
LI
I/L
D
A/M
EC
0% 20% 40% 60% 80% 100%
First trimester
Second trimester
Pediatric
Adult
H
Epithelial cells (Small intestine only)
UMAP2
UMAP1
Stem cells
TA
Paneth
Paneth-like
AE
TNFRSF12A_hi
BEST4+
Goblets
Tuft
EEC
Epithelial cells (Small intestine only)
I
Stem cells
TA
Paneth
Paneth-like
AE
TNFRSF12A_hi
BEST4+
Goblets
Tuft
EEC
LGR5 OLFM4 MKI67 PCNA DEFA5 DEFA6 LYZ ALPI SI SLC5A1 TNFRSF12A BEST4 OTOP2 MUC2 TFF3 SPINK4 AVIL POU2F3 CHGA CHGB
Fraction of cells in group (%)
20 60 100
Mean expression in group
0.0 2.5
J
SLC6A4
UMAP2
UMAP1
2.0
1.5
1.0
0.5
0.0
K
Absorptive enterocytes (Small intestine only)
early AE
immature AE
mature AE
LGR5 CCL25 MKI67 FABP1 FABP2 SI ANPEP APOA4 SLC5A1 APOC3 ACE GUCA2A FABP6
Fraction of cells in group (%)
20 40 60 80
Mean expression in group
1 2
L
Pseudotime
0.0 0.2 0.4 0.6 0.8 1.0
early AE
immature AE
mature AE

Figure S3. *TPH1* and *SLC6A4* expression across cell types in the epithelial compartment (cont.) (A-C) Representative immunohistochemistry staining of TPH1 in fetal SI tissues, (D) neonatal SI tissue, (E) neonatal LI tissue, and (F) pediatrics LI tissue. Gestational ages in panels A-C are in weeks, neonatal ages in panels D and E are in days, and neonatal age in panel F is in months. Arrowheads identify individual enterochromaffin cells. Scale bar in panels A-F represents 50 μm. (G) Proportion bar plot of enteroendocrine subtypes in each region and age group. (H) UMAP visualization of epithelial compartment of the small intestine only, colored by subtypes. (I) Dot plot of marker genes expression in epithelial subtype types across small intestine only. (J) UMAP visualization of epithelial compartment of the small intestine only, colored by *SLC6A4* expression. (K) Dot plot of marker genes expression in absorptive enterocytes subtypes across small intestine only. (L) Box plot distribution of diffusion pseudotime values for each absorptive enterocyte subtype. The central line indicates the median pseudotime, the box spans the interquartile range (IQR), and whisker extends to 1.5xIQR. Outliers are shown as individual points.

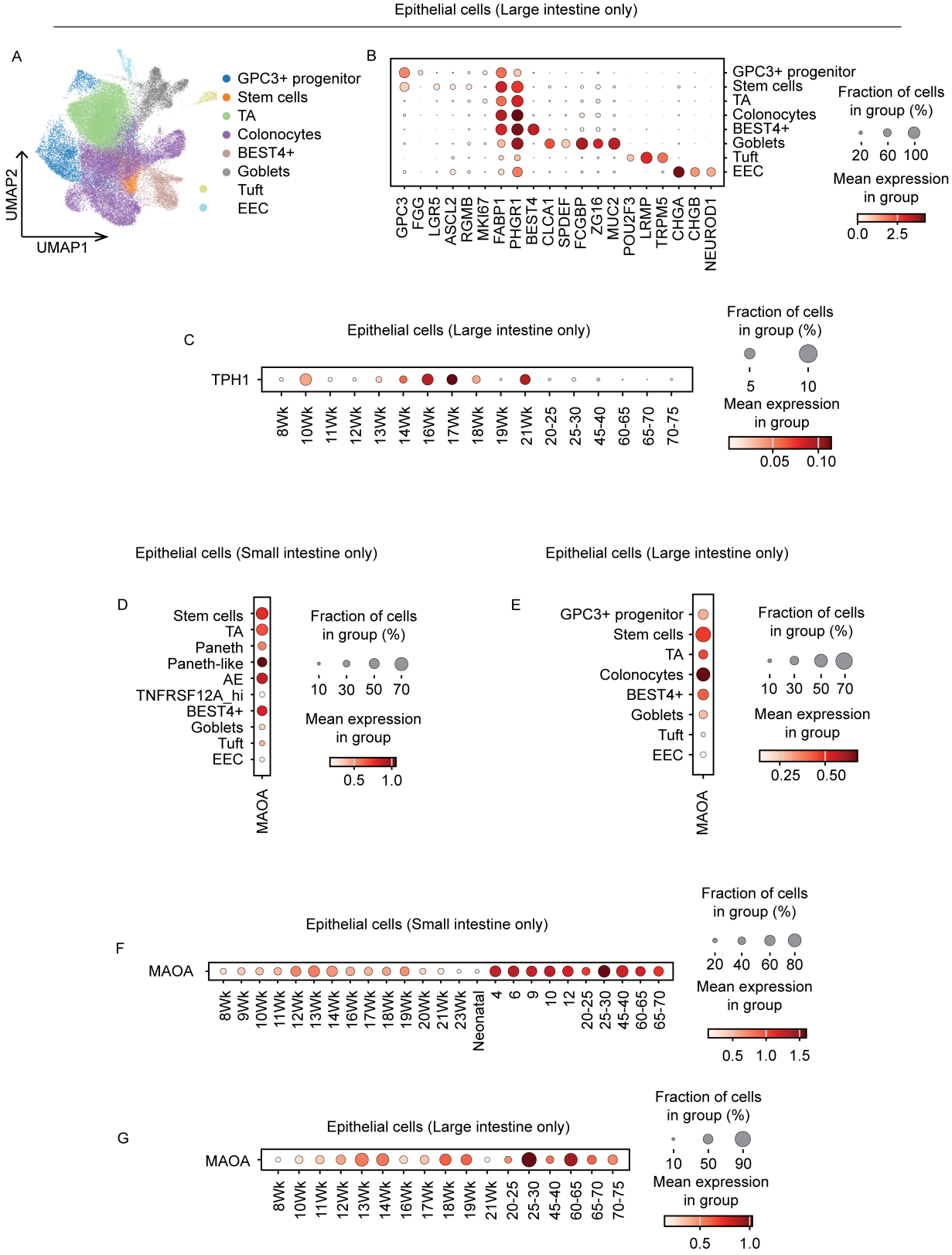

Epithelial cells (Large intestine only)
A
GPC3+ progenitor
Stem cells
TA
Colonocytes
BEST4+
Goblets
Tuft
EEC
UMAP2
UMAP1
B
GPC3+ progenitor
Stem cells
TA
Colonocytes
BEST4+
Goblets
Tuft
EEC
GPC3
FGG
LGR5
ASCL2
RGMB
MKI67
FABP1
PHGR1
BEST4
CLCA1
SPDEF
FCGBP
ZG16
MUC2
POU2F3
LRMP
TRPM5
CHGA
CHGB
NEUROD1
Fraction of cells in group (%)
20 60 100
Mean expression in group
0.0 2.5
C
Epithelial cells (Large intestine only)
TPH1
8Wk 10Wk 11Wk 12Wk 13Wk 14Wk 16Wk 17Wk 18Wk 19Wk 21Wk 20-25 25-30 45-40 60-65 65-70 70-75
Fraction of cells in group (%)
5 10
Mean expression in group
0.05 0.10
Epithelial cells (Small intestine only)
D
Stem cells
TA
Paneth
Paneth-like
AE
TNFRSF12A_hi
BEST4+
Goblets
Tuft
EEC
MAOA
Fraction of cells in group (%)
10 30 50 70
Mean expression in group
0.5 1.0
Epithelial cells (Large intestine only)
E
GPC3+ progenitor
Stem cells
TA
Colonocytes
BEST4+
Goblets
Tuft
EEC
MAOA
Fraction of cells in group (%)
10 30 50 70
Mean expression in group
0.25 0.50
Epithelial cells (Small intestine only)
F
MAOA
8Wk 9Wk 10Wk 11Wk 12Wk 13Wk 14Wk 16Wk 17Wk 18Wk 19Wk 20Wk 21Wk 23Wk Neonatal 4 6 9 10 12 20-25 25-30 45-40 60-65 65-70
Fraction of cells in group (%)
20 40 60 80
Mean expression in group
0.5 1.0 1.5
Epithelial cells (Large intestine only)
G
MAOA
8Wk 10Wk 11Wk 12Wk 13Wk 14Wk 16Wk 17Wk 18Wk 19Wk 21Wk 20-25 25-30 45-40 60-65 65-70 70-75
Fraction of cells in group (%)
10 50 90
Mean expression in group
0.5 1.0

Figure S4. *TPH1, SLC6A4,* and *MAOA* expression across developmental ages in the epithelial compartment (cont.) (A) UMAP visualization of epithelial compartment of the large intestine only, colored by subtypes. (B) Dot plot of marker genes expression in epithelial subtype types across large intestine only. (C) Dot plot visualization of *TPH1* expression in the epithelial compartment of large intestines across ages. Dot plot visualization of *MAOA* expression in the epithelial compartment across epithelial subtypes of (D) small intestine and (E) large intestine. (F) Dot plot visualization of *MAOA* expression in the epithelial compartment of small intestines across ages. (G) Dot plot visualization of *MAOA* expression in the epithelial compartment of large intestines across ages.

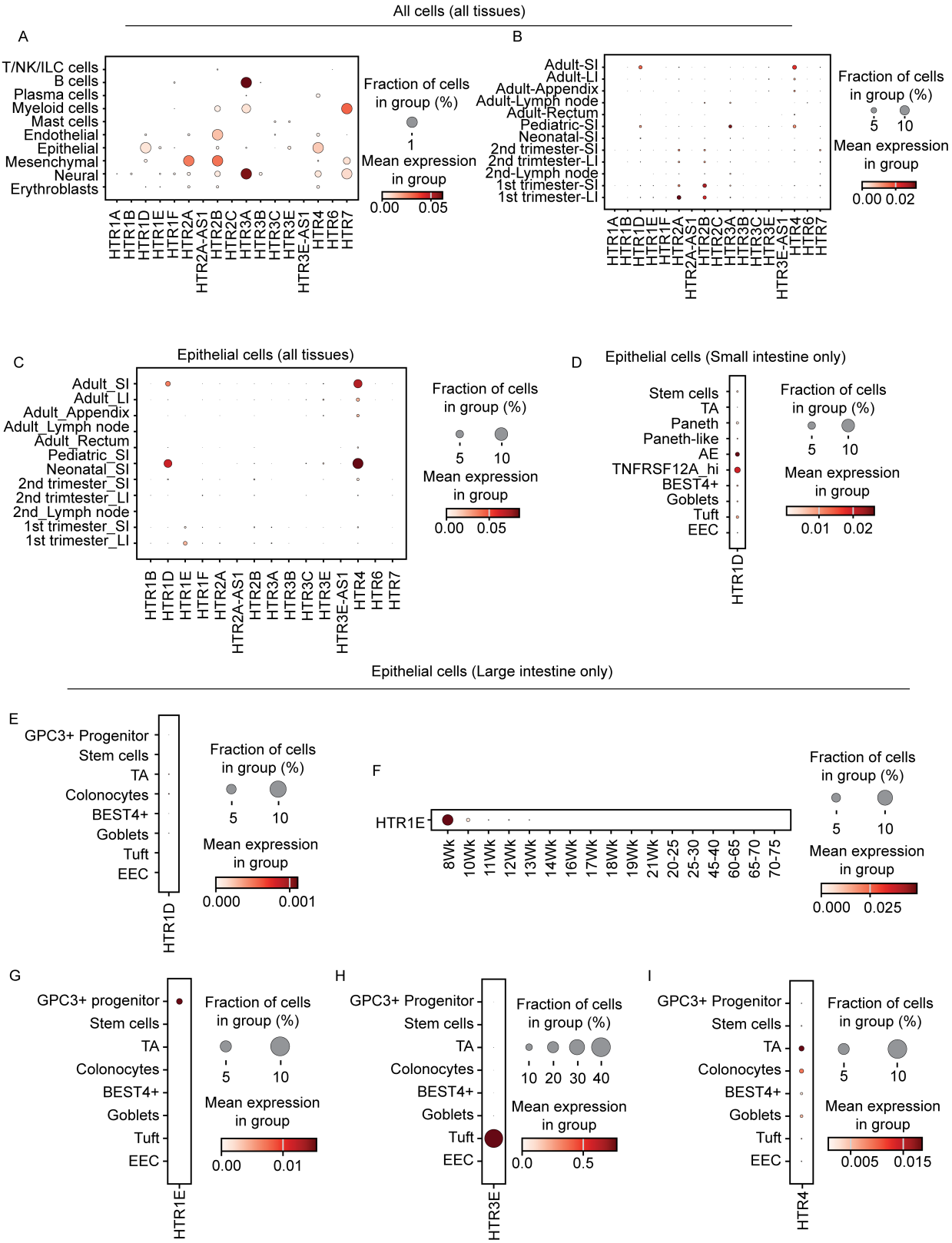

All cells (all tissues)
A
T/NK/ILC cells
B cells
Plasma cells
Myeloid cells
Mast cells
Endothelial
Epithelial
Mesenchymal
Neural
Erythroblasts
HTR1A HTR1B HTR1D HTR1E HTR1F HTR2A HTR2A-AS1 HTR2B HTR2C HTR3A HTR3B HTR3C HTR3E HTR3E-AS1 HTR4 HTR6 HTR7
Fraction of cells in group (%)
1
Mean expression in group
0.00 0.05
B
Adult-SI
Adult-LI
Adult-Appendix
Adult-Lymph node
Adult-Rectum
Pediatric-SI
Neonatal-SI
2nd trimester-SI
2nd trimtester-LI
2nd-Lymph node
1st trimester-SI
1st trimester-LI
HTR1A HTR1B HTR1D HTR1E HTR1F HTR2A HTR2A-AS1 HTR2B HTR2C HTR3A HTR3B HTR3C HTR3E HTR3E-AS1 HTR4 HTR6 HTR7
Fraction of cells in group (%)
5 10
Mean expression in group
0.00 0.02
C
Epithelial cells (all tissues)
Adult_SI
Adult_LI
Adult_Appendix
Adult_Lymph node
Adult_Rectum
Pediatric_SI
Neonatal_SI
2nd trimester_SI
2nd trimtester_LI
2nd_Lymph node
1st trimester_SI
1st trimester_LI
HTR1B HTR1D HTR1E HTR1F HTR2A HTR2A-AS1 HTR2B HTR3A HTR3B HTR3C HTR3E HTR3E-AS1 HTR4 HTR6 HTR7
Fraction of cells in group (%)
5 10
Mean expression in group
0.00 0.05
D
Epithelial cells (Small intestine only)
Stem cells
TA
Paneth
Paneth-like
AE
TNFRSF12A_hi
BEST4+
Goblets
Tuft
EEC
HTR1D
Fraction of cells in group (%)
5 10
Mean expression in group
0.01 0.02
Epithelial cells (Large intestine only)
E
GPC3+ Progenitor
Stem cells
TA
Colonocytes
BEST4+
Goblets
Tuft
EEC
HTR1D
Fraction of cells in group (%)
5 10
Mean expression in group
0.000 0.001
F
HTR1E
8Wk 10Wk 11Wk 12Wk 13Wk 14Wk 16Wk 17Wk 18Wk 19Wk 21Wk 20-25 25-30 45-40 60-65 65-70 70-75
Fraction of cells in group (%)
5 10
Mean expression in group
0.000 0.025
G
GPC3+ progenitor
Stem cells
TA
Colonocytes
BEST4+
Goblets
Tuft
EEC
HTR1E
Fraction of cells in group (%)
5 10
Mean expression in group
0.00 0.01
H
GPC3+ Progenitor
Stem cells
TA
Colonocytes
BEST4+
Goblets
Tuft
EEC
HTR3E
Fraction of cells in group (%)
10 20 30 40
Mean expression in group
0.0 0.5
I
GPC3+ Progenitor
Stem cells
TA
Colonocytes
BEST4+
Goblets
Tuft
EEC
HTR4
Fraction of cells in group (%)
5 10
Mean expression in group
0.005 0.015

Figure S5. Serotonin receptors *HTR1E*, *HTR3E* and *HTR4* expression across cell types in the epithelial compartment (cont.) (A) Dot plot visualization of serotonin receptor gene expression across cell types. (B) Dot plot visualization of serotonin receptor gene expression across all cell types by tissue regions and developmental stages. (C) Dot plot visualization of serotonin receptor gene expression in the epithelial compartment by tissue regions and developmental stages. (D) Dot plot visualization of *HTR1D* expression in the epithelial compartment of small intestines across epithelial subtypes. (E) Dot plot visualization of *HTR1D* expression in the epithelial compartment of large intestines across epithelial subtypes. (F) Dot plot visualization of *HTR1E* expression in the epithelial compartment of large intestines across ages. Dot plot visualization of (G) *HTR1E* expression, (H) *HTR3E* expression, and (I) *HTR4* expression in the epithelial compartment of large intestines across epithelial subtypes.

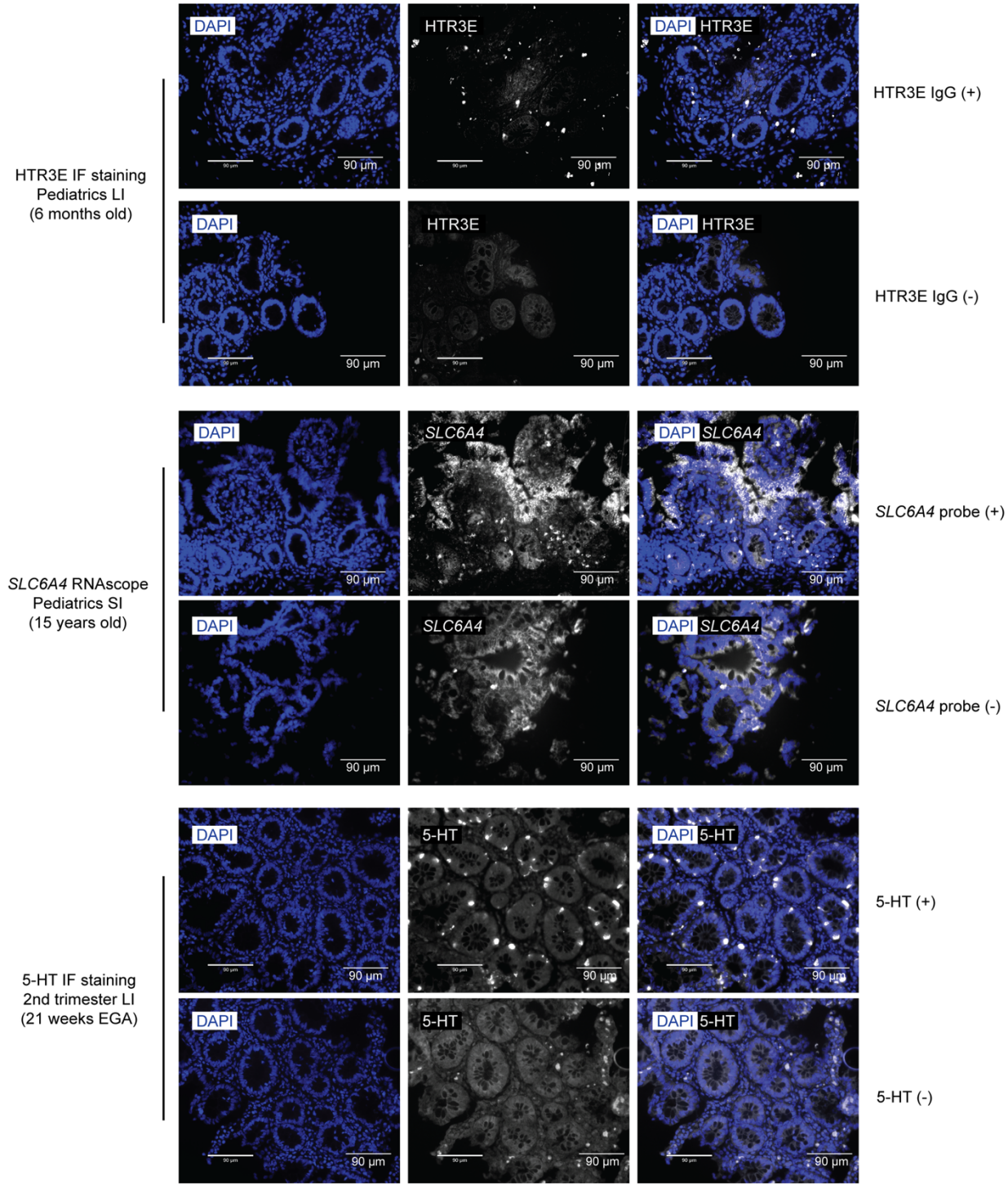

HTR3E IF staining
Pediatrics LI
(6 months old)
DAPI
HTR3E
DAPI HTR3E
HTR3E IgG (+)
HTR3E IgG (-)
90 µm
SLC6A4 RNAscope
Pediatrics SI
(15 years old)
SLC6A4
DAPI SLC6A4
SLC6A4 probe (+)
SLC6A4 probe (-)
5-HT IF staining
2nd trimester LI
(21 weeks EGA)
5-HT
DAPI 5-HT
5-HT (+)
5-HT (-)

Figure S6. Positive and negative controls validating HTR3E and 5-HT immunofluorescence staining and *SLC6A4* RNAscope detection.

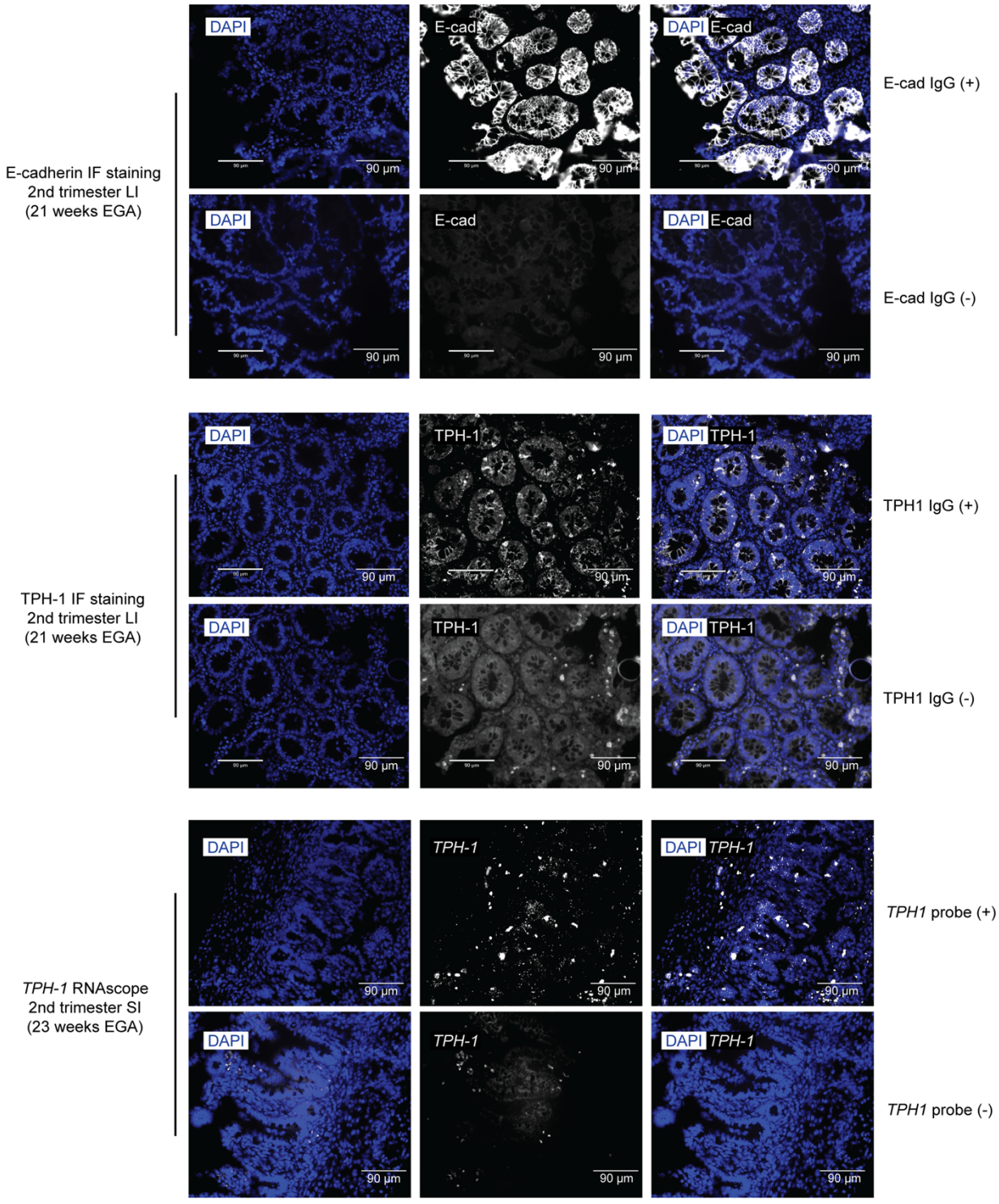

E-cadherin IF staining
2nd trimester LI
(21 weeks EGA)
DAPI
E-cad
DAPI E-cad
E-cad IgG (+)
E-cad IgG (-)
TPH-1 IF staining
2nd trimester LI
(21 weeks EGA)
TPH-1
DAPI TPH-1
TPH1 IgG (+)
TPH1 IgG (-)
TPH-1 RNAscope
2nd trimester SI
(23 weeks EGA)
TPH-1
DAPI TPH-1
TPH1 probe (+)
TPH1 probe (-)
90 µm

Figure S7. Positive and negative controls validating E-cadherin, and TPH-1 immunofluorescence staining and *TPH-1* RNAscope detection.

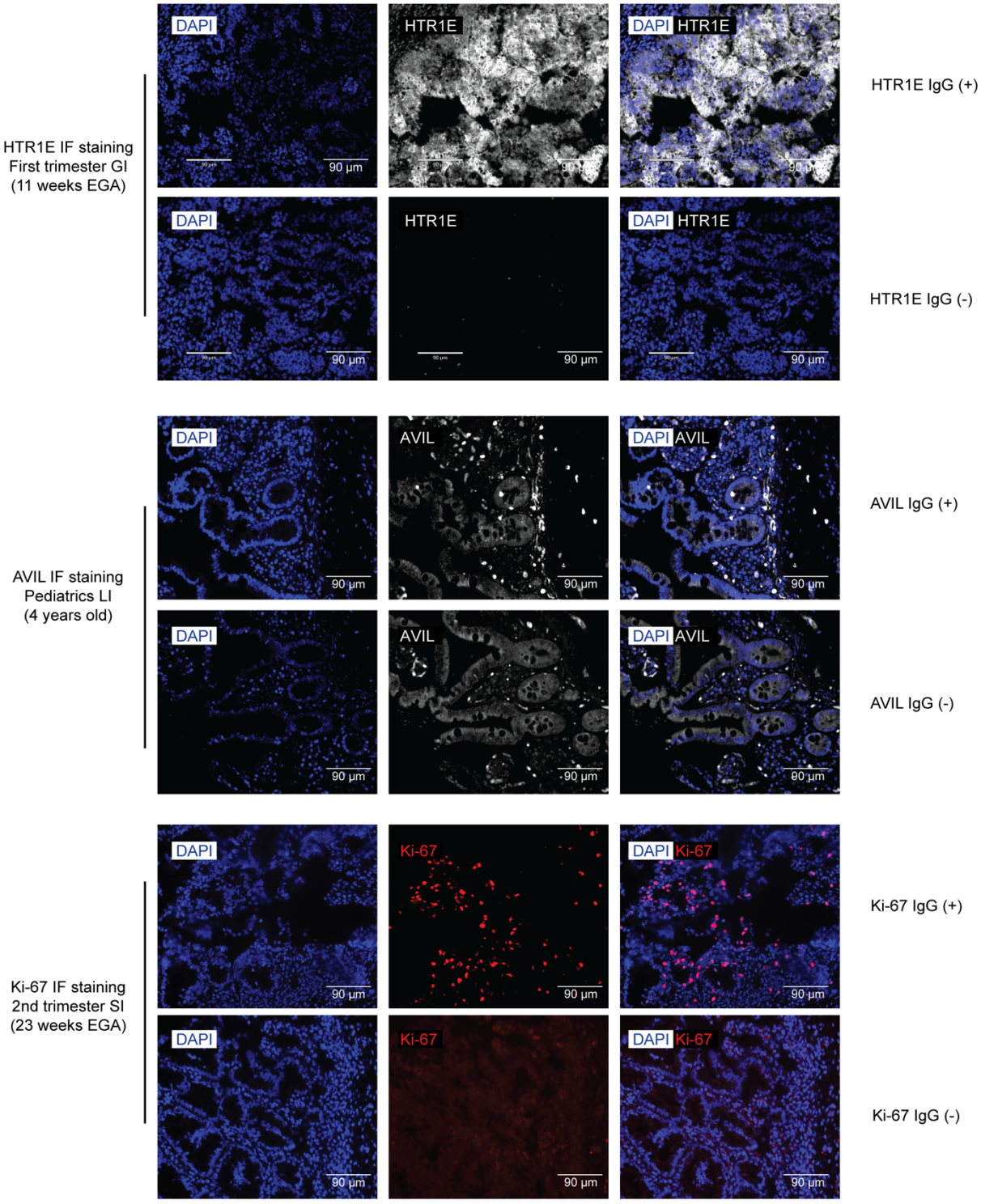

HTR1E IF staining
First trimester GI
(11 weeks EGA)
DAPI
HTR1E
DAPI HTR1E
HTR1E IgG (+)
DAPI
HTR1E
DAPI HTR1E
HTR1E IgG (-)
AVIL IF staining
Pediatrics LI
(4 years old)
DAPI
AVIL
DAPI AVIL
AVIL IgG (+)
DAPI
AVIL
DAPI AVIL
AVIL IgG (-)
Ki-67 IF staining
2nd trimester SI
(23 weeks EGA)
DAPI
Ki-67
DAPI Ki-67
Ki-67 IgG (+)
DAPI
Ki-67
DAPI Ki-67
Ki-67 IgG (-)
90 µm

Figure S8. Positive and negative controls validating HTR1E, AVIL, and Ki-67 immunofluorescence staining.

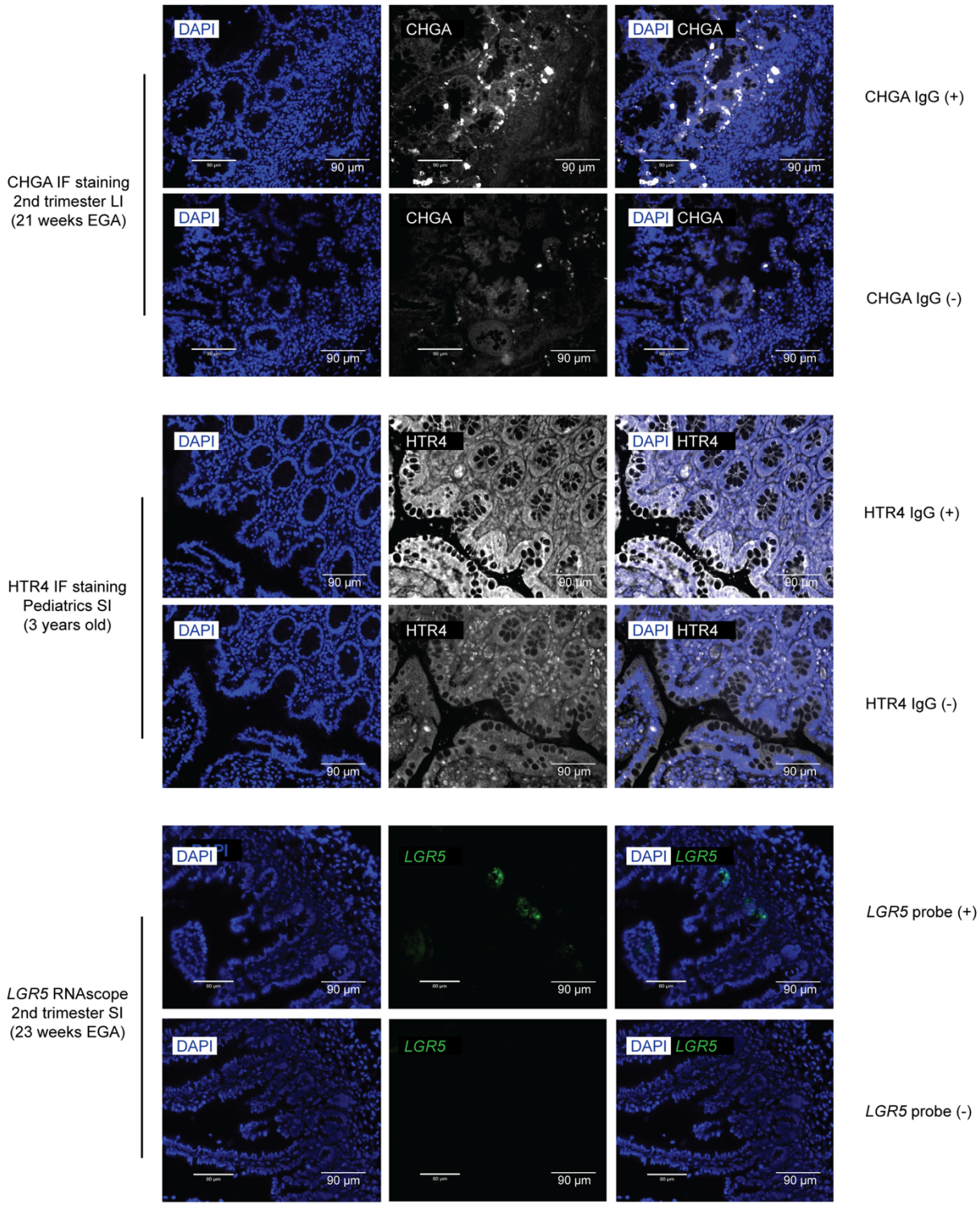

CHGA IF staining
2nd trimester LI
(21 weeks EGA)
DAPI
CHGA
DAPI CHGA
90 µm
CHGA IgG (+)
CHGA IgG (-)
HTR4 IF staining
Pediatrics SI
(3 years old)
HTR4
DAPI HTR4
HTR4 IgG (+)
HTR4 IgG (-)
LGR5 RNAscope
2nd trimester SI
(23 weeks EGA)
LGR5
DAPI LGR5
LGR5 probe (+)
LGR5 probe (-)

Figure S9. Positive and negative controls validating CHGA, and HTR4 immunofluorescence staining and *LGR5* RNAscope detection.