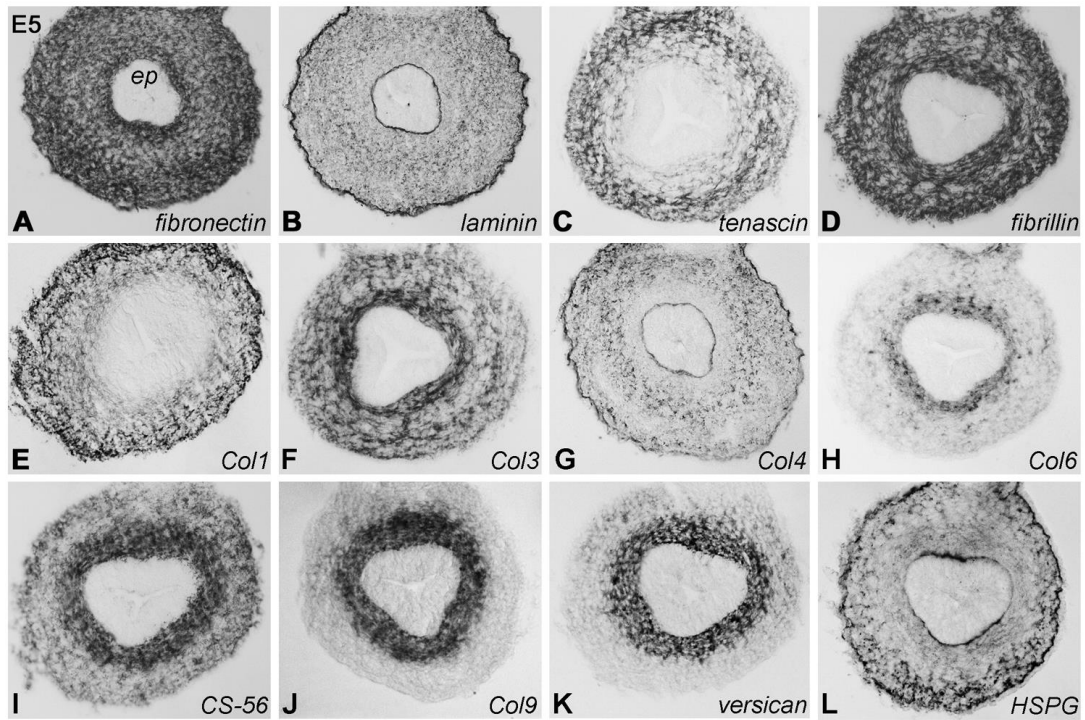
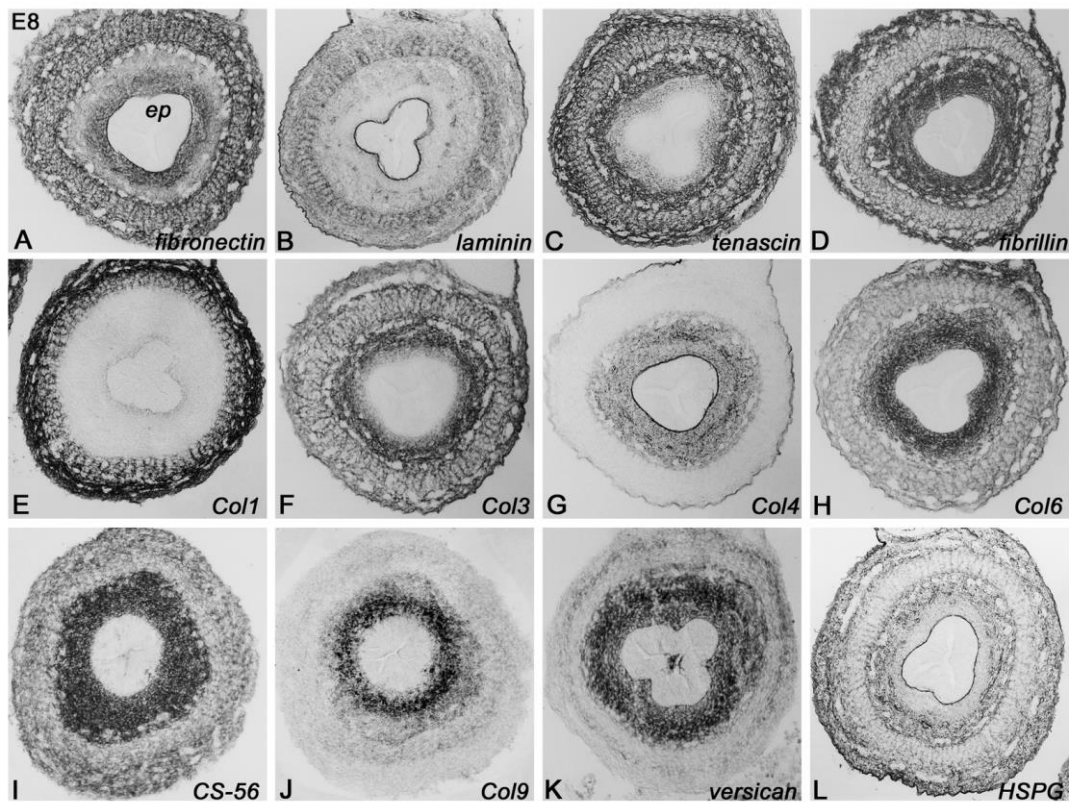


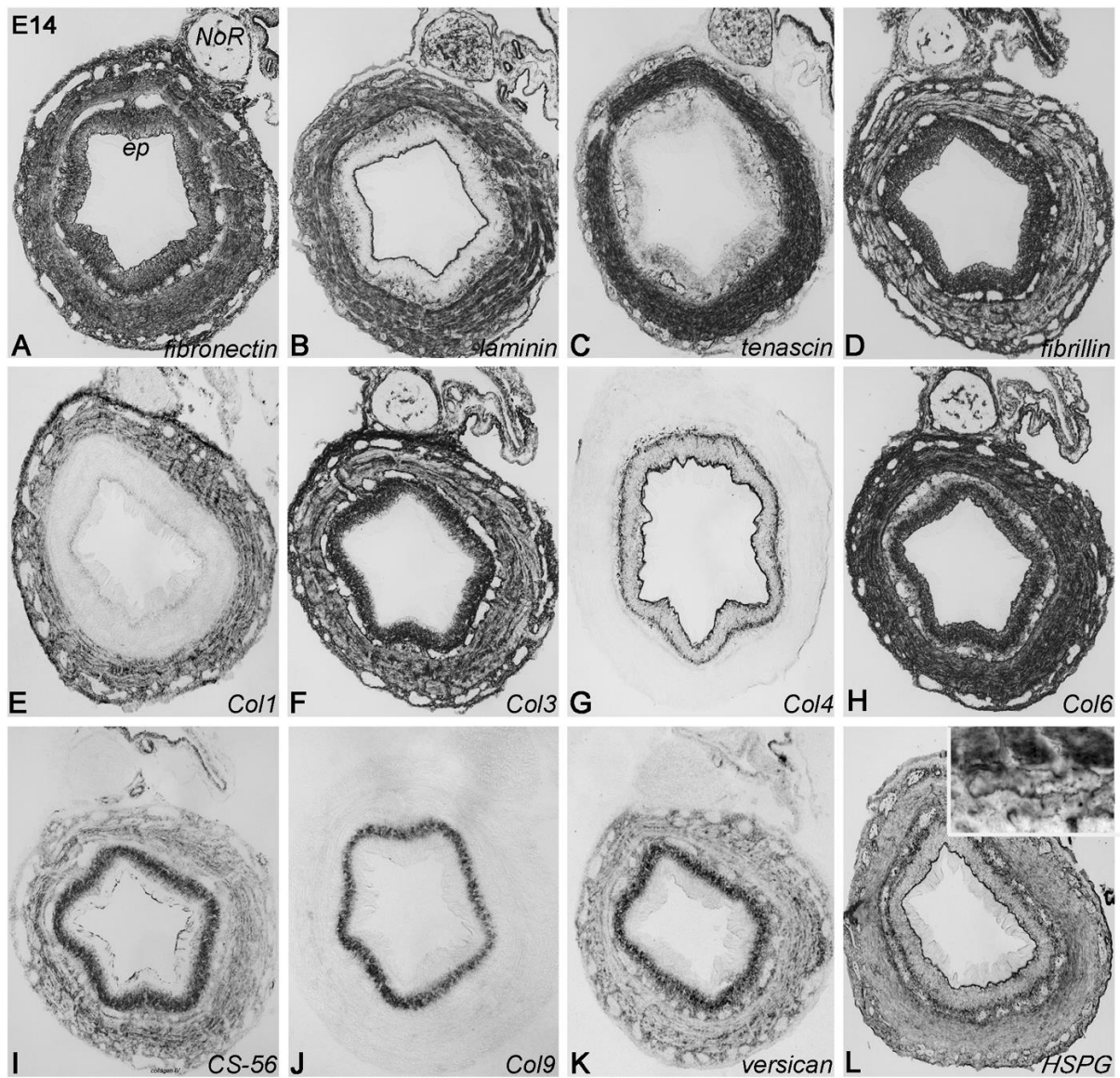
### Supplementary materials



**Figure S1. ECM expression in E5 chick hindgut.** The expression of 12 different ECM proteins was examined in the mid-hindgut of E5 chick embryo.

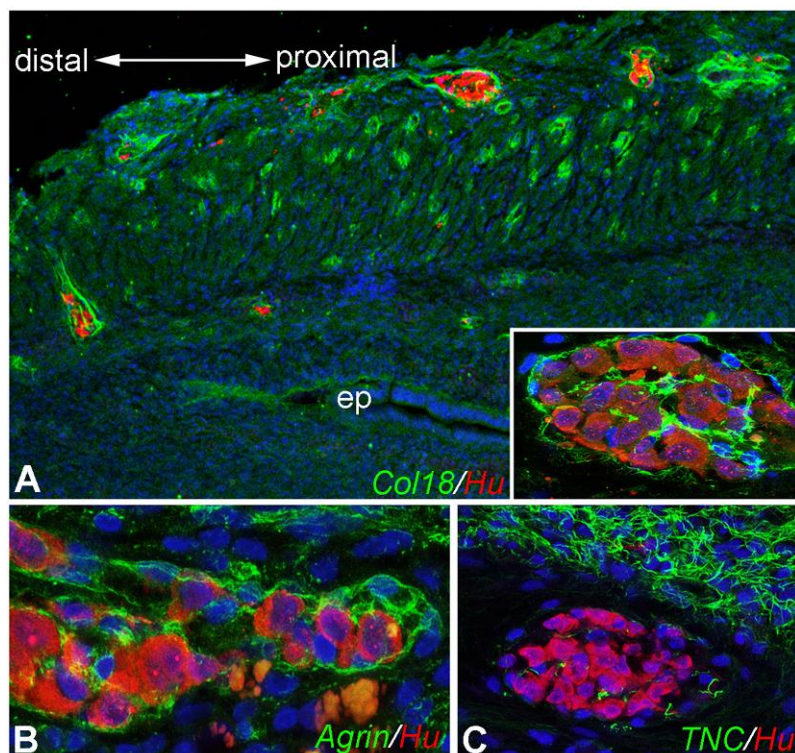


**Figure S2. ECM expression in E8 chick hindgut.** The expression of 12 different ECM proteins was examined in the mid-hindgut of E8 chick embryo.



**Figure S3. ECM expression in E14 chick hindgut.** The expression of 12 different ECM proteins was examined in the mid-hindgut of E14 chick embryo.





**Figure S4. Sacral neural crest-derived ENCCs express Col18 and agrin.** E5 chick hindgut was explanted, with cloaca and without ceca, onto the chorioallantoic membrane of a host chick to generate hindguts containing only sacral crest-derived ENCCs. The resulting ENS contains scattered Hu<sup>+</sup> enteric ganglia that express Col18 (A) and agrin (B), but not tenascin-C (C).

**Table S1: Primary antibodies.**

Antibody (clone)	Host	Structure/cells identified	Dilution	Source of antibody, catalogue number
p75	Rabbit polyclonal	Chick ENCDCs	1:7000	Kind gift of Dr. Louis Reichardt, (Weskamp and Reichardt, 1991)
p75	Rabbit polyclonal	Mouse ENCDCs	1:300	Promega (G3231)
HNK1	Mouse IgM	Chick ENCDCs	1:50	NeoMarkers
Tuj1 (B1195)	Mouse IgG2a	Neurons	1:200	Covance (MMS-435P)
HuC/HuD (clone: 16A11)	Mouse IgG2a	Neurons	1:100	Invitrogen (A-21271)

Antibody (clone)	Host	Structure/cells identified	Dilution	Source of antibody, catalogue number
CN	Mouse IgG	chicken-specific neurite marker	1:10	Kind gift of Dr. Hideaki Tanaka, (Tanaka et al., 1990)
Fibronectin (B3/D6)	Mouse IgG2a	Chick extracellular matrix	1:5	DSHB*
Laminin (31 or 31-2)	Mouse IgG1	Chick extracellular matrix	1:40	DSHB
Tenascin-C (M1B4)	Mouse IgG1	Chick extracellular matrix	1:10	DSHB
Fibrillin 2-like (JB3)	Mouse IgG1	Chick extracellular matrix	1:10	DSHB
Collagen type I (DD4/F12)	Mouse IgG2a	Chick extracellular matrix	1:2	Kind gift of Dr. Thomas F. Linsenmayer, (Birk et al., 1988)
Collagen type III (3B2)	Mouse IgG1	Chick extracellular matrix	1:10	DSHB
Collagen type IV (IF8)	Mouse IgG2a	Chick extracellular matrix	1:2	Kind gift of Dr. Thomas F. Linsenmayer, (Fitch et al., 1990)
Collagen type VI (39)	Mouse IgG1	Chick extracellular matrix	1:5	DSHB
Chondroitin sulphate proteoglycan (CS-56)	Mouse IgM	glycosaminoglycan portion of native CSPG	1:2000	Sigma (C8350)
Collagen type IX (2B9)	Mouse IgM	Chick extracellular matrix	1:2	DSHB
Versican	Rabbit polyclonal	Extracellular matrix	1:500	Kind gift of Dr. Maria T. Dours-Zimmermann, (Dutt et al., 2011)
Heparan sulfate proteoglycan proteoglycan (33 or 33-2)	Mouse IgG	Chick extracellular matrix	1:5	DSHB
Collagen type XVIII (6C4)	Mouse IgG1	Chick extracellular matrix	1:2	DSHB
Agrin (6D2)	Mouse IgG1	Chick extracellular matrix	1:5	DSHB
Perlecan (5C9)	Mouse IgG1	Chick extracellular matrix	1:5	DSHB
Collagen type XVIII	Rabbit	Mouse extracellular	1:1000	Kind gift of Dr.

Antibody (clone)	Host	Structure/cells identified	Dilution	Source of antibody, catalogue number
(NC11)	polyclonal	matrix		Takako Sasaki, (Sasaki et al., 1998)
Collagen type XVIII (NC1)	Rabbit polyclonal	Mouse extracellular matrix	1:300	Kind gift of Dr. Takako Sasaki, (Sasaki et al., 1998)
Endostatin (1092)	Rabbit polyclonal	Mouse extracellular matrix	1:100	Kind gift of Dr. Takako Sasaki, (Sasaki et al., 1998)
Agrin (Mab5204)	Mouse IgG1	Mouse extracellular matrix	1:10	Millipore
Agrin (AF550)	Goat polyclonal	Mouse extracellular matrix	1:100	R&D Systems
MEP21 ( $\alpha$ -chicken podocalyxin)	Mouse IgG1	Chick endothelial cells	1:200	Kind gift of Dr. Kelly McNagny, (McNagny et al., 1997)
Beta-dystroglycan (43DAG1/8)	Mouse IgGa	Receptor for agrin	1:50	Leica Biosystems
Laminin (ab11575)	Rabbit polyclonal	Mouse extracellular matrix	1:200	Abcam
PHOX2B	Guinea pig	Enteric neural crest cells and glial precursors	1:500	Kind gift of Dr. Hideki Enomoto. (Nagashimada et al., 2012)
SOX10 (20B7)	Mouse IgG1	Enteric neural crest cells and neural precursors	1:50	ThermoFisher

\*DSHB (Developmental Studies Hybridoma Bank. Iowa, USA)

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