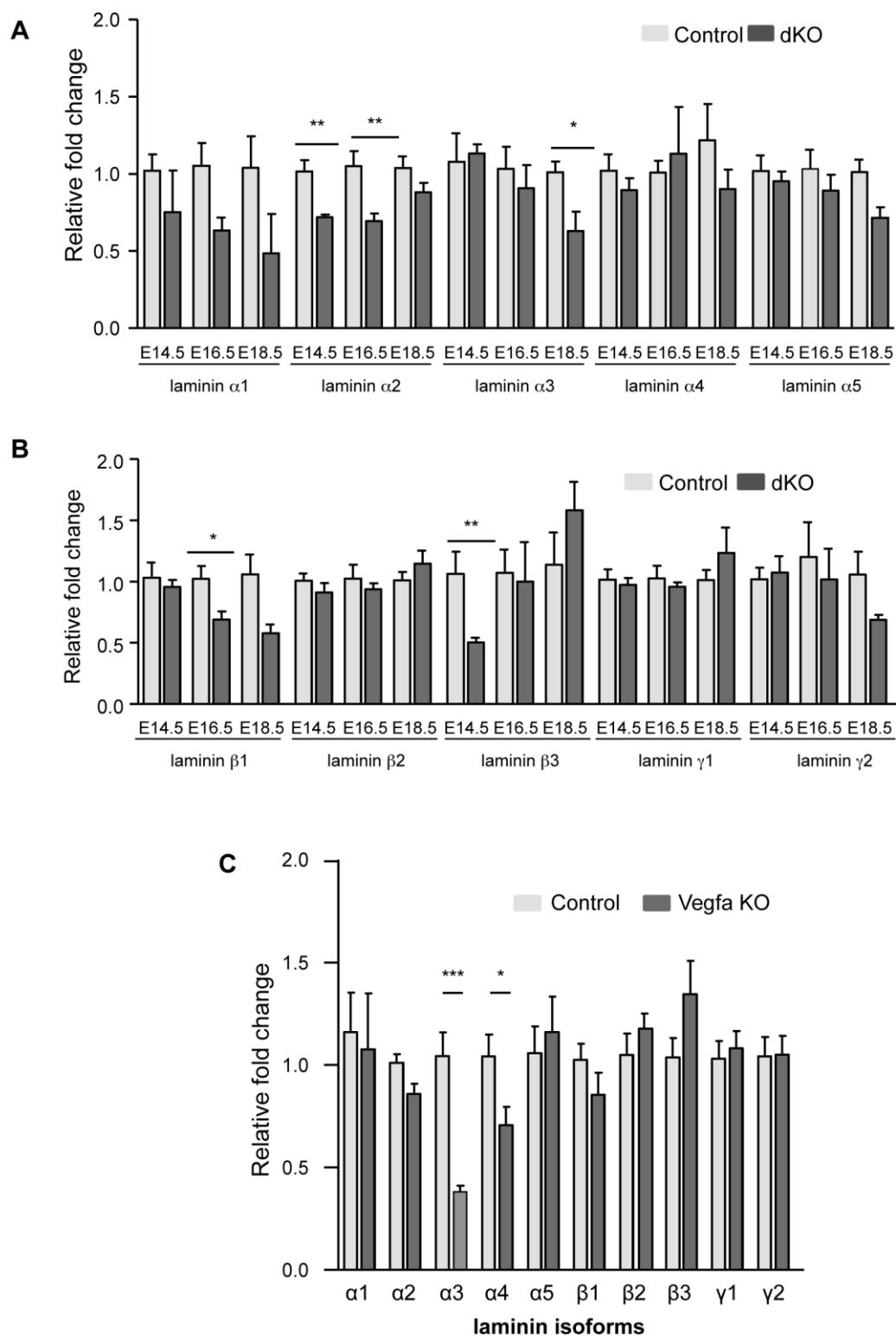


Figure S1. Apical polarity is not affected in Smad1/5^{dKO} at E18.5. (A) Apical polarity marker, Par3 and cytoplasmic stratification marker, F-actin, show no difference in signal intensity and localization between Smad1/5^{dKO} and control. Numbers illustrate counting of cells facing lumina (see Fig. 3D) (B) Transmission electron microscopy of control thyroid reveals cuboidal epithelial cells surrounding a large lumen filled with homogeneous colloid (*), in which tight junctions and apical microvilli are projecting. In Smad1/5^{dKO}, columnar thyrocytes also display tight junctions and apical microvilli projecting in non-expanded and smaller lumen (*). Inset illustrates microvilli inclusion body at a distance from apical lumen. Bar, (A) 10 µm and (B) 2 µm.



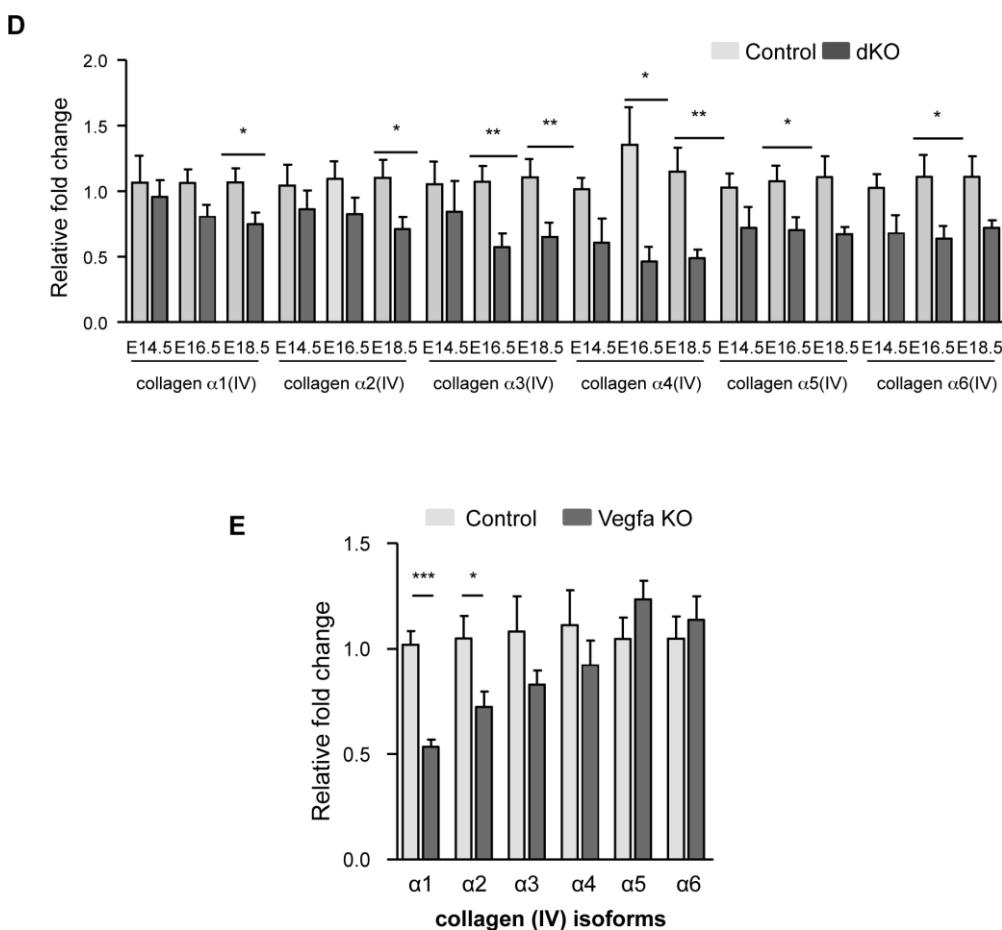


Figure S2. Expression of laminin α , β and γ chains in Smad1/5^{dKO} and Vegfa^{KO} mice. (A) Quantification of laminin α in control and Smad1/5^{dKO} from E14.5 to E18.5. Expression of laminin $\alpha 1$ shows a trend to a decrease from E14.5 to E18.5. Variability may come from remaining parathyroid cells, highly expressing this laminin isoform. Laminin $\alpha 2$ expression was significantly reduced at E14.5 and E16.5 but was normal at E18.5. Laminin $\alpha 3$ was significantly reduced at E18.5. Expression levels of laminin $\alpha 4$ and $\alpha 5$ were statistically not different from E14.5 to E18.5. (B) Quantification of laminin β and γ in control and Smad1/5^{dKO} from E14.5 to E18.5. Expression of laminin $\beta 1$ was reduced from E16.5. Expression of laminin $\beta 3$ showed 50% reduction at E14.5 and no change at E18.5. Laminin $\beta 1$ and $\gamma 2$ expression were comparable to control. (C) Quantification of laminins in Vegfa^{KO} at P0. Laminin $\alpha 3$ and $\alpha 4$ were significantly reduced as compared to control. All the other laminins were normally expressed. (D) Quantification of collagen IV genes in Smad1/5^{dKO} from E14.5 to E18.5. Expression of collagen IV $\alpha 1$ and IV $\alpha 2$, showed no change at E14.5 and E16.5 but their expression levels were significantly reduced at E18.5. Collagen IV $\alpha 3$ and IV $\alpha 4$ showed no difference at E14.5 but significantly reduced at E16.5 and E18.5. Collagen IV $\alpha 5$ and IV $\alpha 6$ were reduced from E14.5 onwards, but only statistically at E16.5. (E) Quantification of collagen IV genes in Vegfa^{KO} at P0. collagen IV $\alpha 1$, $\alpha 2$ were significantly reduced as compared to control. All the other collagen IV genes were normally expressed. All mRNAs were normalized to either RPL27 or β -actin *p<0.05; ** p<0.001; *** p<0.0001 using Mann Whitney U test (n≥3). Data are presented as means ± S.E.M.

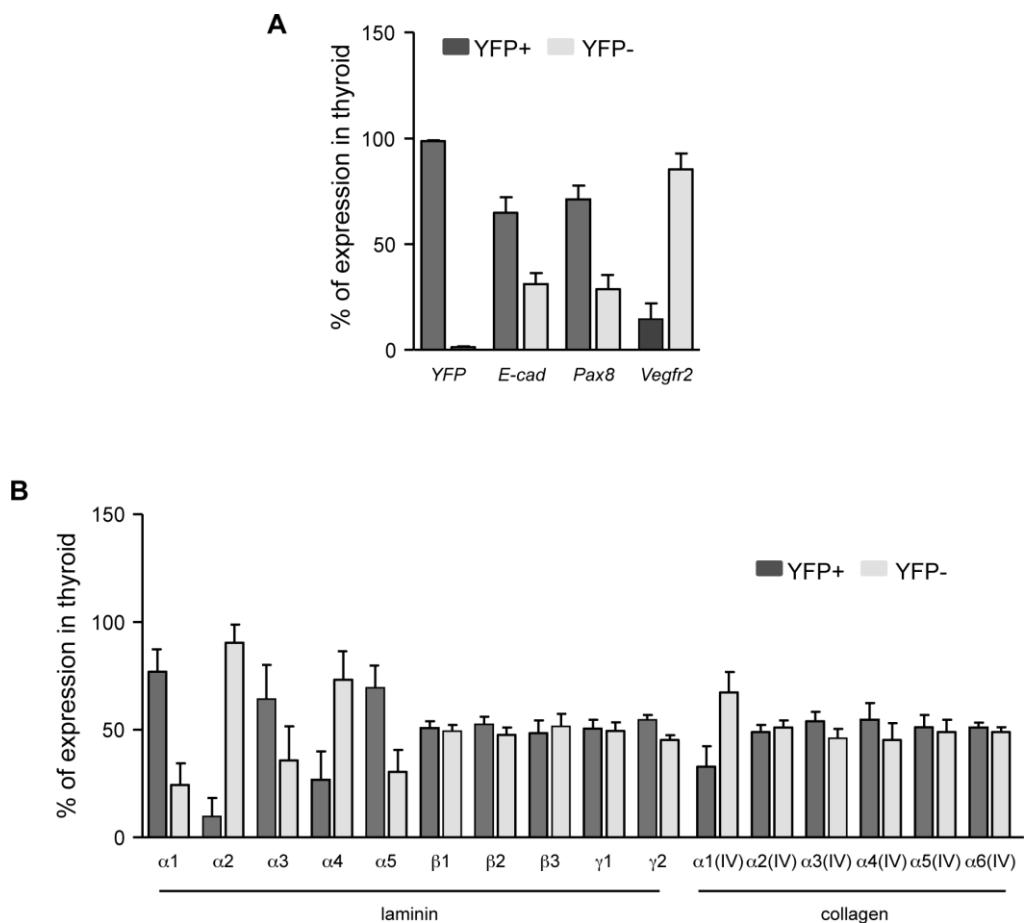


Figure S3. Expression of laminin and collagen in FACS-sorted Pax8/CRE⁺ cells. (A) Percent of expression of selected genes in YFP⁺ and YFP⁻ populations reveals enrichment of *YFP*, *E-cadherin* and *Pax8* in YFP⁺ (i.e. thyrocytes progenitors) population and absence of *Vegfr2* in this population. (B) Percent of expression of laminin α , β , and γ and collagen type IV in YFP⁺ and YFP⁻ populations reveals enrichment of laminin $\alpha 1$ and $\alpha 5$ in YFP⁺ population and enrichment of laminin $\alpha 2$, $\alpha 4$ and collagen $\alpha 1(IV)$ in YFP⁻ population. All mRNAs were normalized to β -actin. Data are presented as means \pm S.E.M. (n=4).

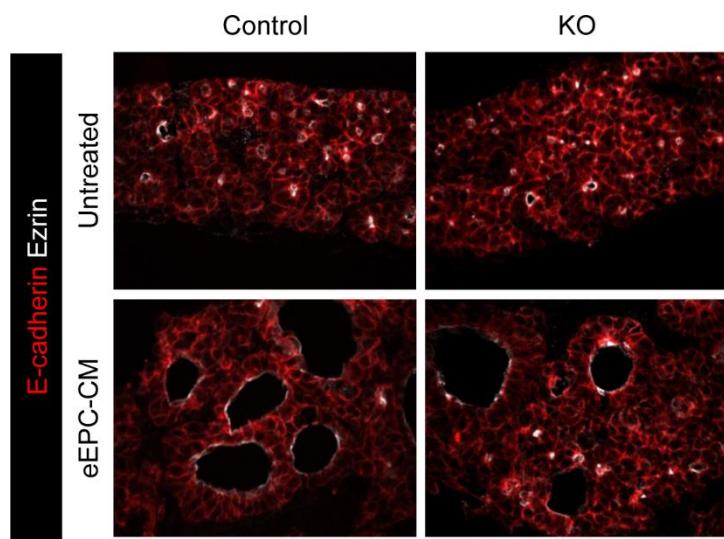


Figure S4. eEPC-CM rescues follicle formation defect in Vegfa^{KO} thyroid glands.

Treatment of E14.5 thyroid glands of control and Vegfa^{KO} mice with eEPC-CM during 3 days, stimulates follicle formation and lumen size enlargement, as visualized by ezrin (white) and E-cadherin (red) labeling. Bar, 20 μm .

Table S1. Candidate folliculogenic factors found by mass spectrometry analysis of eEPC-CM

Accession	Name	Score	MW (kDa)
Q5NCU4	SPARC	71.04	34.3
P07724	Serum albumin	67.97	68.6
F8VQJ3	Laminin subunit gamma 1	60.83	177.1
P06151	L-lactate dehydrogenase A chain	53.01	36.5
P09103	Protein disulfide-isomerase	50.57	57.0
P02469	Laminin subunit beta 1	48.94	197.0
P08113	Endoplasmin	40.42	92.4
G3x9D5	Histone H2B	35.83	13.4
P14211	Calreticulin	35.32	48.0
F8VQ40	Laminin subunit alpha-1	34.57	337.9
P60710	Actin, cytoplasmic1	31.37	41.7
P68033	Actin, alpha cardiac muscle	25.63	42.0
Q01768	Nucleoside diphosphate kinase B	23.24	17.4
P20029	78 kDa glucose-regulated protein	21.38	72.4
Q5SQB0	Nucleophosmin	19.06	29.5

Table S2. Primary antibodies

Primary antibody	species	dilution	Source	Catalog number
E-cadherin	mouse	1/200	BD Biosciences	610182
Pan-Laminin	rabbit	1/100	Sigma	L9393
PECAM	rat	1/100	BD Biosciences	550274
PECAM	rat	1/20	Dianova	DIA 310
Ezrin	mouse	1/300	Thermo Scientific	MS-661
Calcitonin	rabbit	1/1000	Dako	A0576
GFP	rabbit	1/200	Cell Signaling	2956S
Par3	rabbit	1/200	Merck/Millipore	07-330
Laminin α 1	rabbit	1/1000	Gift from Dr. T. Sasaki	
Laminin α 5	rabbit	1/1000	Gift from Dr. T. Sasaki	
Collagen IV	rabbit	1/500	Merck/Millipore	AB756P
Thyroglobulin	mouse	1/500	Dako	M0781
pSmad158	rabbit	1/100	Cell Signaling	9511S
pSmad15	rabbit	1/100	Cell Signaling	9516S
iodo-thyroglobulin	mouse	1/100	Gift from Dr. Ris-Stalpers	

Table S3. Primers

Gene	Primer sequences (5'-3')	Fragment size (bp)
E-cadherin	AGGGAGCTGTCTACCAAAGTG CCAGTCCTCGTTCTGTCTTC	146
VE-cadherin	GGATGTGGTGCCAGTAAACC ACCCCCTTGTCTGAGATGAG	173
Vegfr2	GCATGGAAGAGGATTCTGGA CGGCTTTCGCTTACTGTT	142
Vegfa mouse	GTACCTCCACCATGCCAAGT CTGCATGGTGTGTTGCTCT	265
Vegfa rat	GAGTATATCTTCAAGCCGTCCTGT TTTCTGGCTTGTCTATCTTC	193
β-actin	TCCTGAGCGCAAGTACTCTGT CTGATCACATCTGCTGGAAG	77
Nkx2.1	ATCTGAGCTGGGTGCTGGG GCCCTGTCTGTACGCTGCGA	244
Pax8	TGCCTTCCCCATGCTGCCTCCGTGTA GGTGGGTGGTGCCTGGCCTGATGTAG	298
Foxe1	GGCGGCATCTACAAGTTCAT GGATCTTGAGGAAGCAGTCG	115
Hhex	TCAGAACATGCCGAGCTAAAT ACTGCGAACGATCCAAAGAG	152
Tpo	TGCCAACAGAACGATGGCAAC GCACAAAGTTCCCATTGTCCAC	424
Tg	TGGGACGTGAAAGGGGAATGGTGC GTGAGCTTTGGAATGGCAGGCAG	394
Nis	AGCAGGCTTAGCTGTATCCC AGCCCCGTAGTAGAGATAGGAG	235
Tshr	CTGCGGGCAAAGAGTGTGC AGGGGAGCTGTCAAGGCA	325
Calcitonin	TGGTTGTCAAGCATCTTGCTC CTTAGATCTGGGCTGTCCA	221
Smad 1	GCCTCTGGAATGCTGTGAGT GAACTGAGCCAGAACGGCTGT	137
Smad 5	GCAGAGCCATCACGAGCTAA CCAGAAGGCTGTGTTGTGGA	169
Smad 8	CACCGACCCTTCCAATAAC CTGGACAAAGATGCTGCTG	153
RPL 27	GCCCTGGTGGCTGGAATTGACC AAACTTGACCTTGGCCTCCCGC	233
ID2	CATCCTGTCTTGCAAGGCAT CCATTCAACGTGTTCTCCTGG	199
PECAM	ATAGGCATCAGCTGCCAGTC TCCGCTCTGCACTGGTATTG	157
Pcdh12	TGCCCTCACCAACCAATTAC GTGCTGCCCAACAAACATTG	171
Tie 1	CAGGGACCTTGACCTTGACC ATCATGGCCGGATCACTTG	135

Esm 1	ACCTTCGGGATGGAATGCAA AGAGGTCTGCTGGGAGATT	125
YFP	CCTCGTGACCACCTTCGG CTCAGGTAGTGGTTGTCGG	400
BMP 2	CAGCGCAATCTCCATGTTG GGGAAATATTAAAGTGTCACTGG	197
BMP 4	GGATCTTACCGGCTCCAG CCAGATGTTCTCGTGATGG	130
BMP 5	TTCCACATGGAGAAGCAGTG AAGCCCAAATTGTTCTGTGG	246
BMP 7	TCCGGTTGATCTTCCAAG TGGCTGTGATGTCAAACACC	224
Laminin α 1	AGCTGTGTGCTCTGGCTAC TCACTGTCACCTTCCACGAC Or CCGACAAACCTCCTCTTCTACC TCTCCACTGCGAGAAAGTCA	123 or 59
Laminin α 2	CTGGAGTTGGTCTCTCAGC TGAACATCAACCTCACGGGC Or TTGCCCTCTGCCAACTGAAT TGAACATCAACCTCACGGGC	240 Or 135
Laminin α 3	ATGAACAGTGAGGCAGGTGG GGACGCCTCCAATGTGTAGT	198
Laminin α 4	ACGGGAAATACCTGAACGTG TCTGTGCCATCTGCCATCAC	124
Laminin α 5	ACCCAAGGACCCACCTGTAG TCATGTGTGCGTAGCCTCTC	168
Laminin β 1	TGGACAAGAGCAACGAGGAC TTCTGTAACTGCTGTGGCGT	147
Laminin β 2	GTGTGGCTTGCATAGCCCT TCCGATGACTATTGGTTGTCT	121
Laminin β 3	GGGAGACCATGGAAATGATG GATCTGCTCCACACGCTTCT	121
Laminin γ 1	TGCCGGAGTTGTTAATGCC CTGGTTGTTGAGTCGGTCAG	184
Laminin γ 2	GGCAGTCAGCATCAGAACAG CCCCACGTAGTGCTCAGAAG	125
Integrin α 3	CCCTTCAGACACCTCAAAC ACCACAGCTCAATCTCAGCC	230
Integrin α 6	TGGACATTCTCCTGAGGGCT TGAGGGAAACACCGTCACTC	100
Integrin α 7	AAGGTGGAGCCTAGCACATC TCAAAGCTGTAGAGTGGGCAG	121
Integrin β 1	ATGGCCGGGTATTGTGAA GAAGTGGGAGCACTCCTGTG	181
Integrin β 4	CAGGGAGGCTGGCTTCAAT TTCTTGGGGTTGTCACAGAG	171
Collagen IV α 1	AACAACGTCTGCAACTTCGC CTTCACAAACCGCACACCTG	135
Collagen IV α 2	GGATGCCAGGGCTTAAAGGT CTGTCTCCAGGCAAACCTCC	159

Collagen IV a3	GGCCCTGAGTGGAAAGGAAAG GAATCCTTGGGCTCCCTTG	138
Collagen IV a4	GCCAGAAAGGACCAATGGGA TACTGGCCCTTTCTGCCTG	106
Collagen IV a5	CCCCAGGACCAGATGGATTG TACTGAAGCGACGAAGGCAG	224
Collagen IV a6	GGCCTGAAAGGAGACCAAGG CTCAAATGTGCGACCAGGTG	128