

Supplemental Figure legends

Supplemental Figure 1. (A) The breeding strategy used to obtain the EC-Smad2/3KO mice is shown. The Smad2^{fl/fl};Smad3^{+/-};Tie2-Cre mice were used as control mice in all experiments if there are no descriptions. (B) Gross morphology of Smad2^{fl/fl};Smad3^{+/-};Tie2-Cre (left panel) and EC-Smad2/3KO embryos (right panel) at E13.5.

Supplemental Figure 2. Gross morphology of EC-ALK5KO (left panel) at E12.5 and EC-Smad4KO embryos (right panel) at E9.5.

Supplemental Figure 3. Whole-mount LacZ-stained Rosa26 transgenic embryos without (left panel) or with Tie2-Cre transgene (right panel) at E11.5. The right photo in the right panel shows high magnification of the left photo in the right panel.

Supplemental Figure 4. Expression of PDGF-B (A), CNN1 (B) and S1PR family (C and D) in S2^{fl/fl} and S2/3KO MEECs. Total RNAs from each sample were used to generate 1st-strand cDNAs. Primer sets for PCR are described in Supplemental Table 3. CNN1 (calponin 1) is known to be induced by S1P (Donati et al., PLoS ONE, 6: e20389, 2011). MEECs were stimulated with either 1 mM S1P or 5 ng/ml TGF- β for 24 h. CNN1 is induced in S2/3KO MEECs upon S1P stimulation although S1P marginally

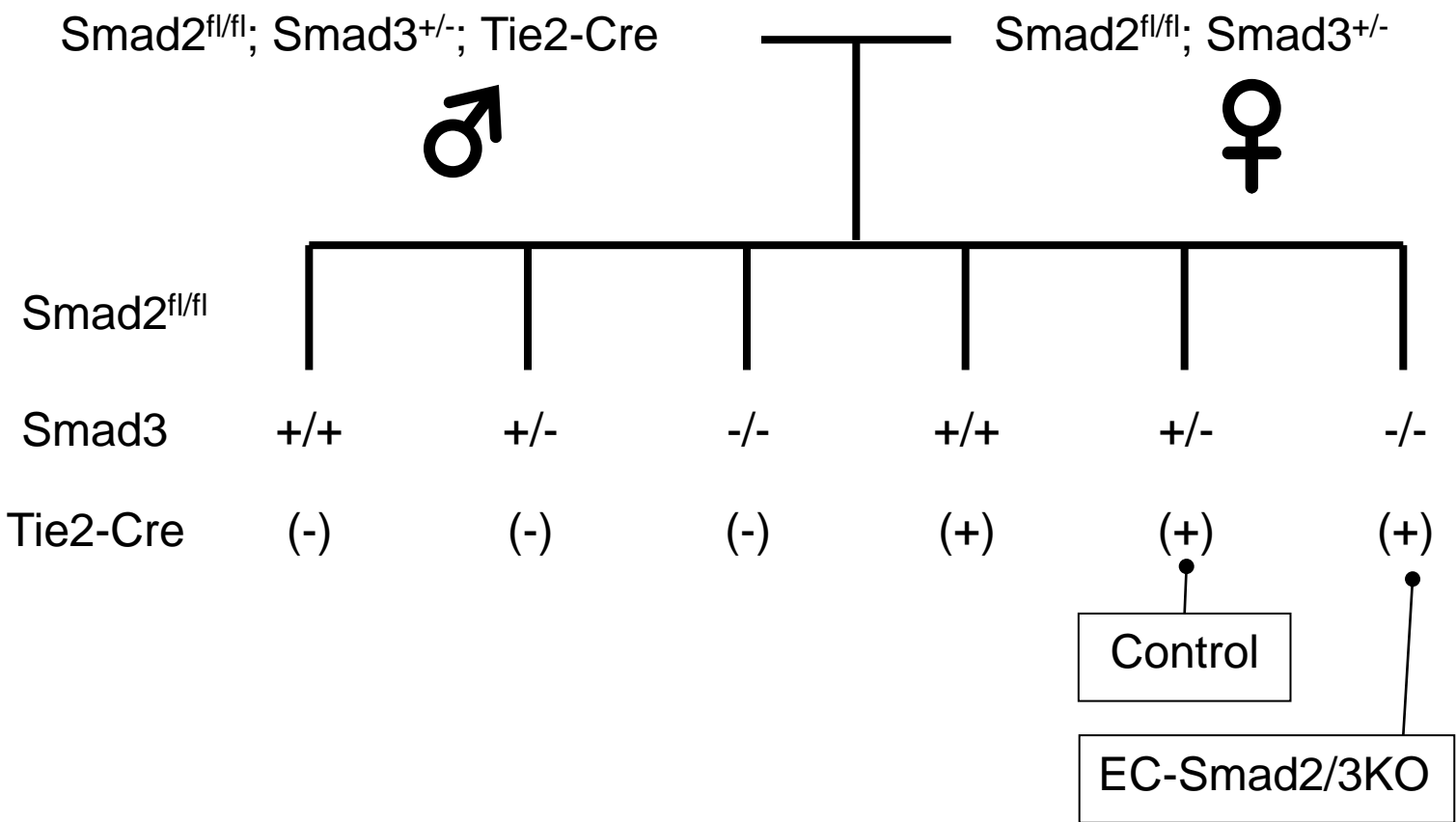
induced CNN1 mRNA in S2^{fl/fl} MEECs.

Supplemental Figure 5. Statistical analysis for the length of tight junction between ECs. The length of tight junction was measured using Image-J (NIH). All values are expressed as mean \pm SD. Comparisons were performed by 1-way ANOVA followed by Bonferroni's post hoc test. Statistics were performed using Statview-J 5.0 (SAS Institute) and differences were considered statistically significant as * $p < 0.05$.

Supplemental Figure 6. Statistical analysis for vascular sprouting from OMDs. The length of vascular sprouting was measured using Image-J (NIH). All values are expressed as mean \pm SD. Comparisons were performed by 1-way ANOVA followed by Bonferroni's post hoc test. Statistics were performed using Statview-J 5.0 (SAS Institute) and differences were considered statistically significant as * $p < 0.05$.

Supplemental Figure 7. Decreased expression of VE-cadherin in aorta from EC-Smad2/3KO embryos. Transverse sections of aorta from E11.5 embryos were stained with anti-VE-cadherin (green) and anti-PECAM-1 (red) antibodies. (A) Smad2^{fl/fl};Smad3^{+/-};Tie2-Cre (B) EC-Smad2/3KO embryos.

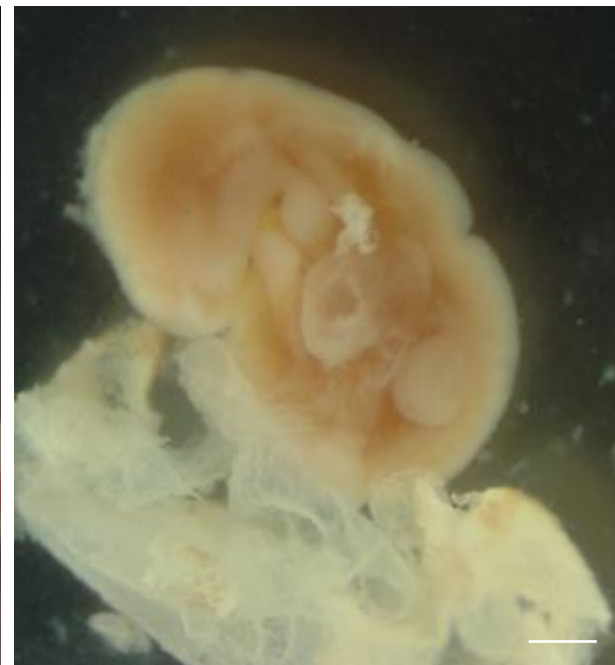
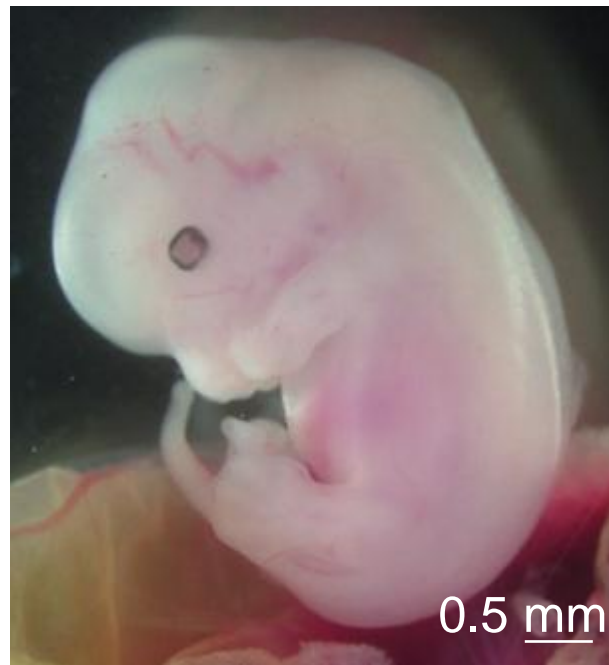
(A)



B

Smad2^{fl/fl};Smad3^{+/-};Tie2-Cre

EC-Smad2/3KO





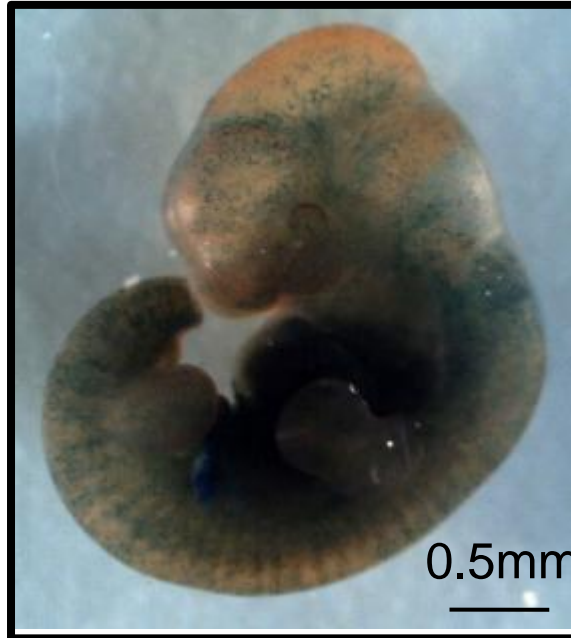
EC-ALK5KO



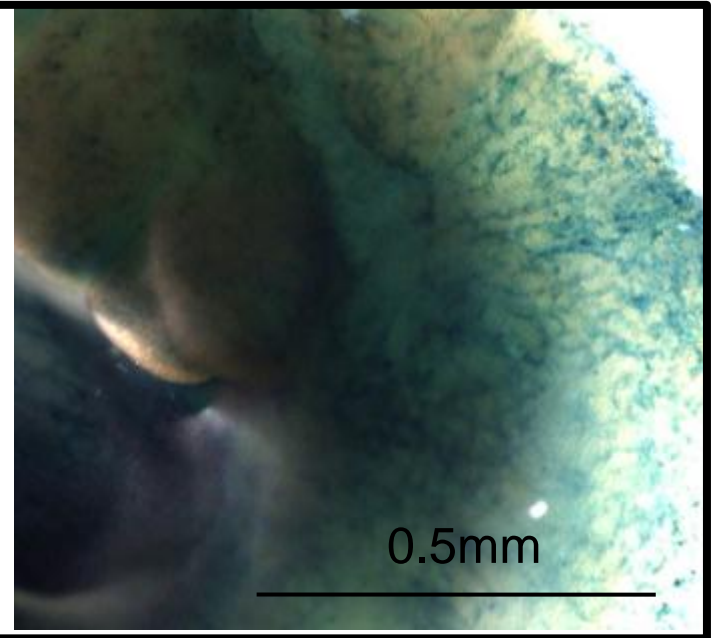
EC-Smad4KO



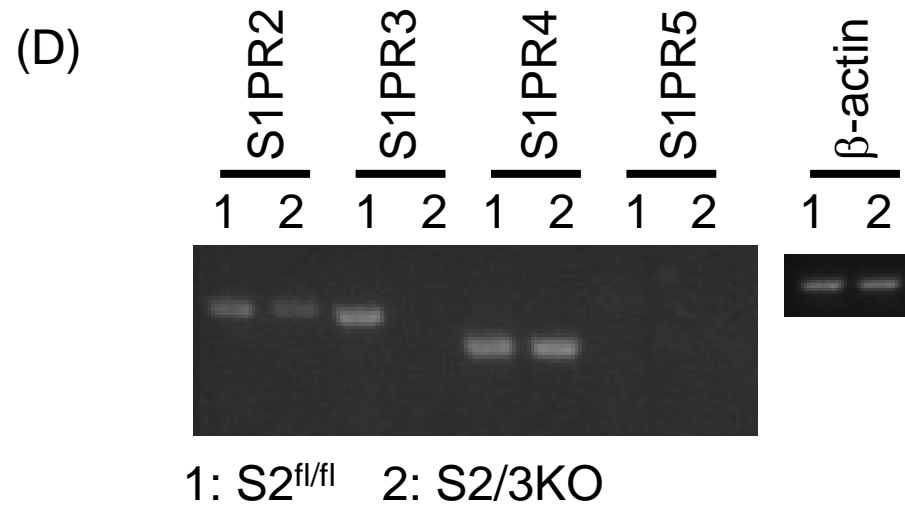
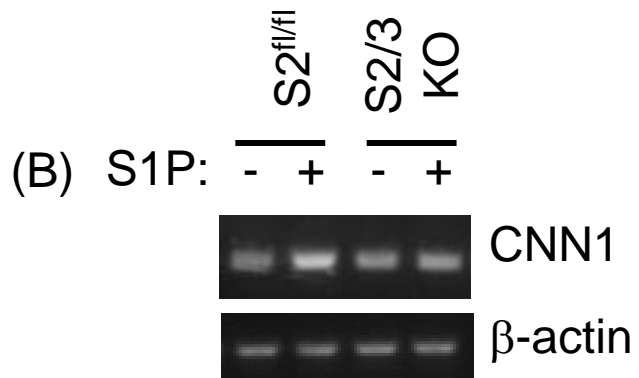
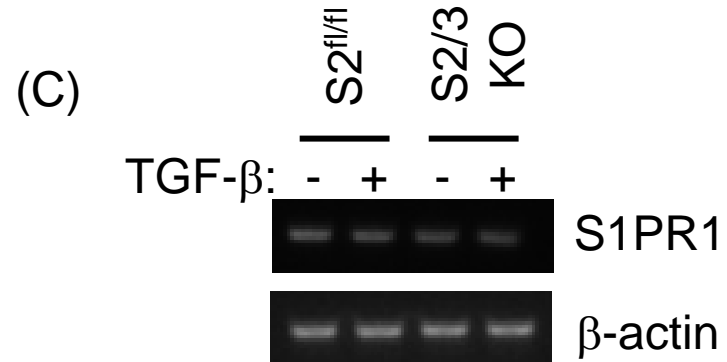
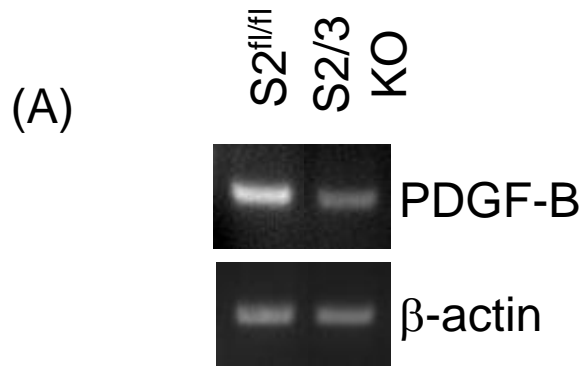
Tie2-Cre



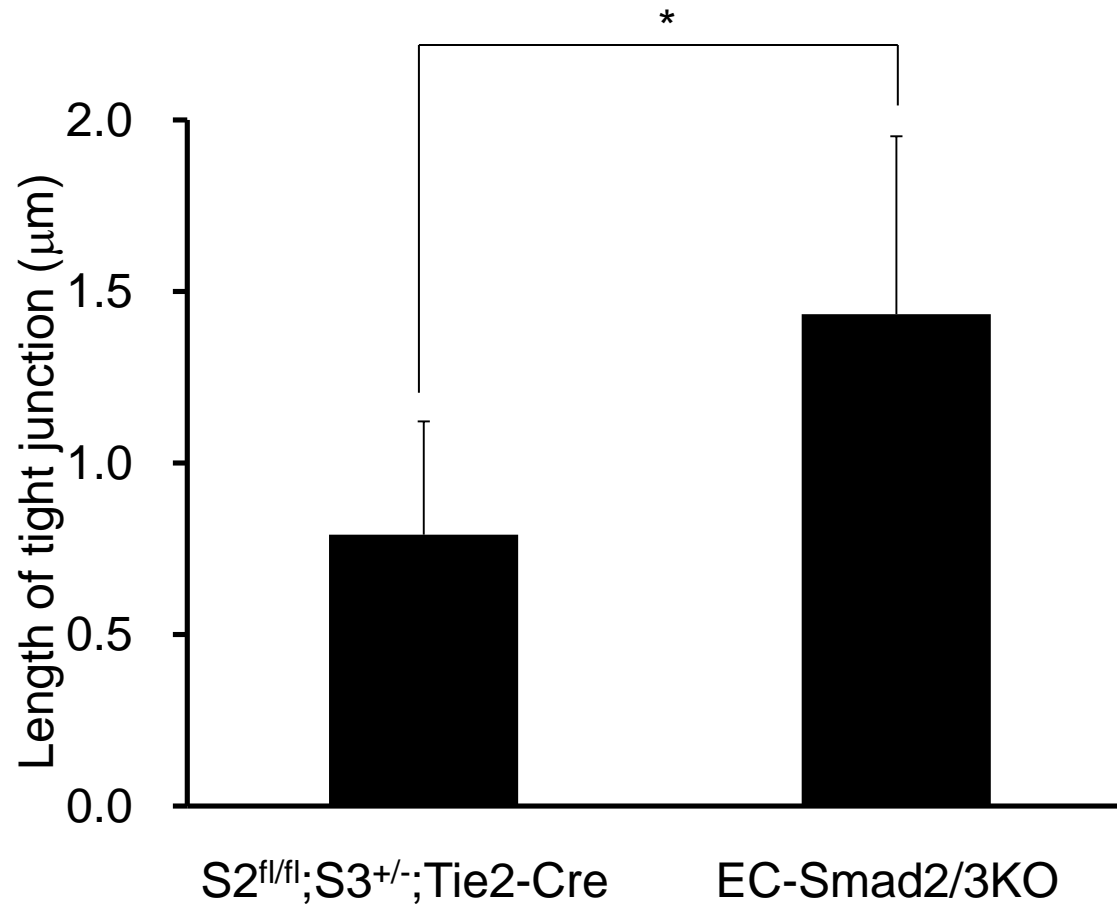
Tie2-Cre;Rosa26



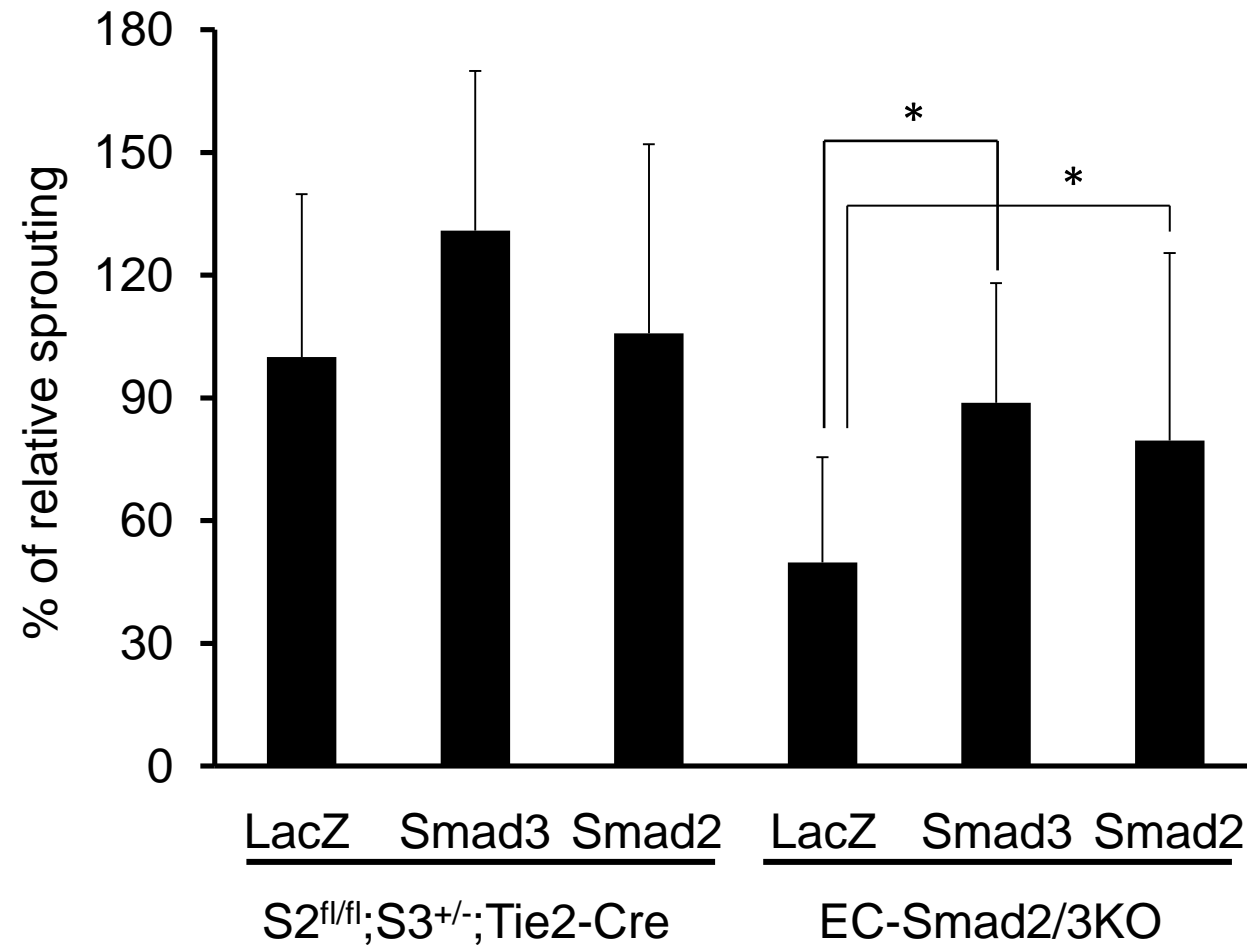
Supplemental Figure 4 (Itoh F et al)

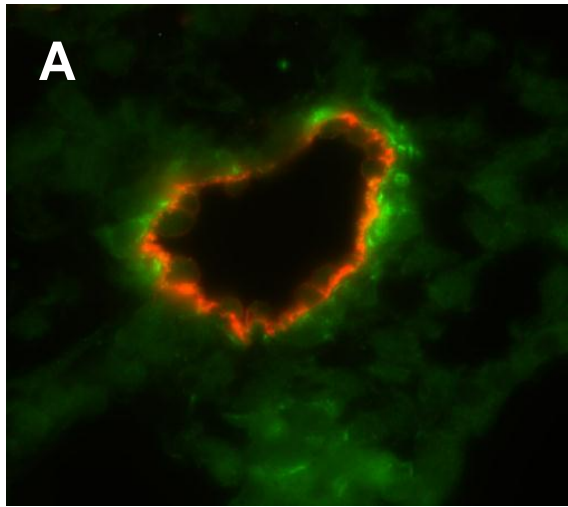


Supplemental Figure 5 (Itoh F et al)

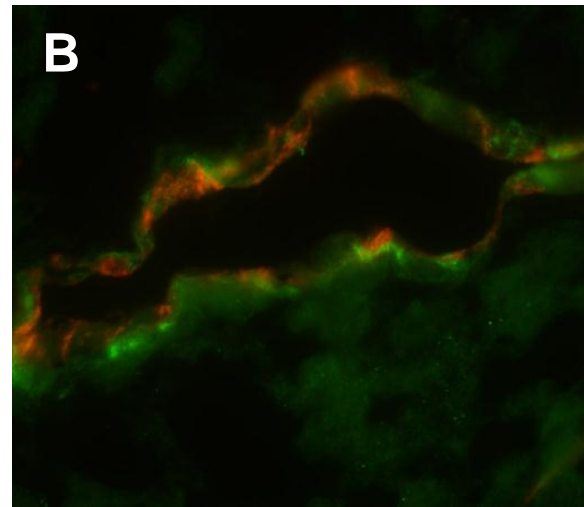


Supplemental Figure 6 (Itoh F et al)





Smad2^{fl/fl};Smad3^{+/-};Tie2-Cre



EC-Smad2/3KO

Supplemental Table 1

Number of embryos obtained from crosses between $Smad2^{fl/fl};Smad3^{+/-};Tie2-Cre$ and $Smad2^{fl/fl};Smad3^{+/-}$ mice

	Smad2 ^{flox/flox}						Absorbed	Total
	Smad3: +/+	Smad3: +/-	Smad3: -/-	control +/+	control +/-	CDKO -/-		
Tie2-Cre:	-	-	-	+	+	+		
E 10.5	47	95	46	59	98	48	0	393
E 11.5	36	48	25	34	57	26	0	226
E 12.5	13	23	6	15	20	3	0	80
E 13.5	12	21	9	14	20	0	4	80
Post Natal	55	104	36	36	29	0	-	260

The number of live embryos recovered from each developmental stage are shown.

Supplemental Table 2

Number of embryos obtained from crosses between ALK5^{fl/+}:Tie2-Cre and ALK5^{fl/fl} mice

ALK5: Tie2-Cre:	fl/+ -	fl/fl -	fl/+ +	fl/fl +	Total
E 10.5	6	8	6	4	24
E 11.5	18	19	14	23(1)	74
E 12.5	8	12	7	15(2)	42
E 13.5	6	3	3	4(4)	16
Post Natal	20	17	19	0	56

The numbers of live embryos recovered from each developmental stage are shown. The numbers of embryos that show no heart beating are in parentheses.

Primer sets for PCR

S1PR1	forward	5' - TCGTCTTGCAAAAGTGGTGCGG - 3'
	reverse	5' - TACAGCAAAGCCAGGTCAGCGA - 3'
S1PR2	forward	5' - TAGACCGAGCACAGCCAACAGT - 3'
	reverse	5' - ACGATGGCGCAGCACAAAGAT - 3'
S1PR3	forward	5' - ACAAGCCTAGCGGGAGAGAAA - 3'
	reverse	5' - TGGTTGCCATGGCTTCCTAG - 3'
S1PR4	forward	5' - ATACAGTTGGAACAGTTGGG - 3'
	reverse	5' - AACTGTGGGTGTGACTCTGG - 3'
S1PR5	forward	5' - TCCAATAGCCGCTCTCCT - 3'
	reverse	5' - CACCAAGAGCACAGCCAAGT - 3'
CNN1	forward	5' - ATGAGCTCCAGCACCTTCAG - 3'
	reverse	5' - ACAGGGTCTGCCTTCTGACT - 3'
PDGF-B	forward	5' - TTCCTTCCTCTCTGCTGCTA - 3'
	reverse	5' - TCGATGAGGTTCCGAGAGAT - 3'
β-actin	forward	5' - GCTCATAGCTCTTCTCCAGGG - 3'
	reverse	5' - TGAACCCTAAGGCCAACCGTG - 3'