

Supplementary Information

In vivo transplantation of mammalian vascular organoids onto the chick chorioallantoic membrane reveals the formation of a hierarchical vascular network

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vessels enveloped with α SMA⁺ cells are present in both models. E-H) Single-channel endothelial staining further shows hierarchical changes between the organoid (E and G) and limb skin (F and H). I-L) α SMA⁺ mural cells were absent or minimal in the immature vasculature (I and J), but were recruited in later stages (K and L). M) The illustration depicts the transition from a vessel plexus formed by vasculogenesis to a mature branched geometry formed through angiogenic remodeling. Created with Biorender.com.

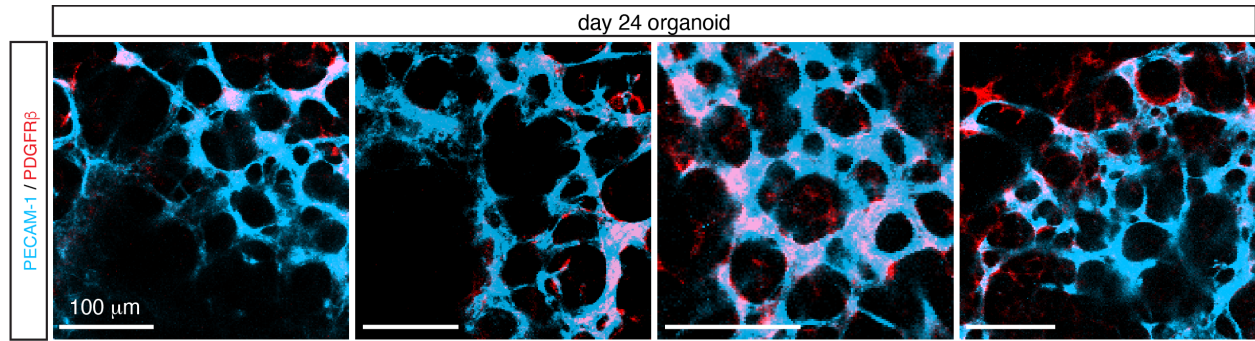


Figure S2. Mural cell progenitors associated with endothelial networks expressed PDGFR β in addition to NG2 (see Figure 2).

Table S1. Primary and secondary antibodies for immunostaining

antibody	host	vendor/product	dilution
α SMA-FITC	Mouse, clone 1A4	Sigma, F3777	1:500
NG2	Guinea Pig	Wm. Stallcup, Sanford Burnham Prebys	1:300
NG2	Rabbit	Millipore, AB5320	1:300
PECAM-1	Armenian Hamster, clone 2H8	Millipore, MAB1398Z	1:300
PDGFR β	Rabbit	Wm. Stallcup, Sanford Burnham Prebys	1:300
SM22 α	Rabbit	Abcam, ab14106	1:500

secondary antibody	fluorophore	vendor/product	dilution
Goat anti-Armenian Hamster	Cy3	Jackson Immuno, 127-165-160	1:250
Goat anti-Guinea Pig	AF488	Jackson Immuno, 106-546-003	1:250
Goat anti-Rabbit	AF647	Jackson Immuno, 111-605-144	1:250
		Invitrogen, A21244	1:250

Table S2. Gene-specific oligonucleotide primers for qRT-PCR ^{1,2}

gene	sense (5' to 3')	antisense (3' to 5')
<i>Nanog</i>	TCTTCCTGGTCCCCACAGTTT	GCAAGAATAGTTCTCGGGATGAA
<i>Oct3/4</i>	CACCATCTGTCGCTTCGAGG	AGGGTCTCCGATTGTCATATCT
<i>Pecam1</i>	AACAGAAACCCGTGGAGATG	GTCTCTGTGGCTCTCGTTCC
<i>Cdh5</i>	TCAACGCATCTGTGCCAGAGAT	CACGATTTGGTACAAGACAGTG
<i>Kdr</i>	TTTGGCAAATACAACCCTTCAGA	GCAGAAGATACTGTCACCACC
<i>Efnb2</i>	AGGAATCACGGTCCAACAAG	GTCTCCTGCGGTACTTGAGC
<i>Dll4</i>	TTCCAGGCAACCTTCTCCGA	ACTGCCGCTATTCTTGTC
<i>Hey1</i>	GCGCGGACGAGAATGGAAA	TCAGGTGATCCACAGTCATCTG
<i>EphB4</i>	GGTCAGCGCTCTGGACAAGATG	AGCCGAATCCAGCCGCTGCAA
<i>Nr2f2</i>	TGGAGAAGCTCAAGGCACTG	ACGAAGCAAGAGCTTTCCGA
<i>Aplnr</i>	TGGTGTTCCGTTCCACAGAC	GGTCACTACAAGCACCACGA
<i>Pdgfrb</i>	TTCCAGGAGTGATACCAGCTT	AGGGGGCGTGATGACTAGG
<i>Acta2</i>	GTCCCAGACATCAGGGAGTAA	TCGGATACTTCAGCGTCAGGA

References

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- 2 Poh, Y. C. *et al.* Generation of organized germ layers from a single mouse embryonic stem cell. *Nat Commun* **5**, 4000 (2014).
<https://doi.org:10.1038/ncomms5000>