Angiogenesis and tissue formation driven by an arteriovenous loop in the mouse

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Supplementary Information



Figure S1: Appearance of the contents of chamber with AVL at (A) day 4, (B) day 21 and (C) day 28; and no AVL controls at (D) day 0, (E) day 4, (F) day 21 and (G) day 28. Scale bar = 1mm



Figure S2: Immuno-staining of control chambers with no AVL at 28 days show that there is little blood vessel or tissue formation with ingress occurring via the pores (arrows). Scale bar = 1mm. (a) CD31 (b) laminin (c) α -SMA (d) VEGF (e) BrdU (f) Hsp47.



Figure S3: Immuno-staining and histology of no-flow AVL control chambers at 28 days show that there is little blood vessel or tissue formation around the ligated vessel. The vessel provides a potential source of blood and may have resulted in a haematoma appearance within the fibrin matrix. The staining and structure is different to that of the AVL samples (Figure S5). Scale bar = 1mm except (b) which is 200 μ m. (a) CD31 (b) CD31 with higher magnification (c) laminin (d) α -SMA (e) VEGF (f) H&E stain.



Figure S4: The graph shows the average number of blood vessels counted in 4 central sections of chambers (N=3) stained by CD31. Counts were differentiated between the venous side (blue) and the arterial side (red) of the AVL. White = no AVL control. Vessels counted show that there was a significant increase in vessels from day 14 onwards in AVL chambers. A greater number of vessels were also found from the vein side of the AVL than the arterial side on day 28. Student T-test comparisons *** P<0.0001, ** P<0.001, * P<0.05.



Figure S5(a) Cross-section of AVL at 14 days with CD31 staining. Vessels can be observed around the artery. Scale bar = $300 \,\mu m$



Figure S5(b) Cross-section of AVL at 14 days with laminin staining. A number of vessels can be observed around the artery. Scale bar = $300 \,\mu m$



Figure S5(c) Cross-section of AVL at 14 days with α -SMA staining. The vein is visible on the left with the vessel looping from the artery (off-screen). Scale bar = 200 μ m



Figure S5(d) Cross-section of AVL at 28 days with CD31 staining. A greater number of vessels can be observed around the vein (left) than the artery (right). Scale bar = $300 \,\mu m$



Figure S5(e) Cross-section of AVL at 28 days with laminin staining. A greater number of vessels can be observed around the vein (left) than the artery (right). Scale bar = $300 \,\mu\text{m}$



Figure S5(f) Cross-section of AVL at 28 days with α -SMA staining. A greater number of vessels can be observed around the vein (left) than the artery (right). Scale bar = 300 μ m