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RESPONSE TO LETTER TO THE EDITOR FROM DR. CHUN AND COLLEAGUES

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We thank Dr. Chun and colleagues for their interest in our recent work, in which we show that homozygous deletion of the *Erg* or *Aplnr* gene results in pulmonary veno-occlusive disease in mice.¹ In our paper, we cited three studies demonstrating that *Aplnr* signaling had direct effects on venous circulation and stand by this statement. In the first study entitled, “Venous dilator effect of apelin, an endogenous peptide ligand for the orphan APJ receptor, in conscious rats”, Cheng and colleagues showed that in rats, pretreated with mecamylamine to cause ganglionic blockade followed by noradrenaline to restore venomotor tone, apelin caused a dose-dependent decrease in mean circulatory filling pressure despite greater reduction in mean arterial pressure, with the authors concluding that “apelin has a prominent venodilator action” and “is a more efficacious venodilator than verapamil, flunarizine, and nifedipine”.² In the second citation, Cox and colleagues reported that apelin stimulated outgrowth of endothelial cells from the posterior cardinal vein toward an apelin-impregnated bead in xenopus embryos.³ We agree this does not exclude non-venous cells sprouting in the same vicinity, although none were reported. In the third paper, Eyries and colleagues studied the role of apelin in a zebrafish fin amputation model.⁴ Although the model is not specific to venous regeneration alone, endothelial cells in regenerated tissue formed a plexus characterized by immature thin-walled vessels that remodeled into new veins and arteries with arteriovenous anastomosis. The results of these papers demonstrate that modulation of apelin or *Aplnr* signaling had direct effects on venous circulation.

We realize that distinction between small pulmonary arteries and veins in the mouse may be difficult for vessels <200µm in diameter, given the lack of biomarkers completely specific for each of these structures. Nonetheless, β-gal-stained vessels in our global and endothelial-specific *Aplnr-lacZ* knock-in mice strains had the histologic characteristics of veins (thin-

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walled, absence of medial layer) as well as stained positive for neuropilin-2 and Ephrin-B4 (vein-specific endothelial markers), and had no staining using antibodies to neuropilin-1 and Ephrin-B2 (arterial-specific endothelial markers). We cannot comment on the finding of pulmonary arteriolar expression of *Aplnr* in *Aplnr*CreER:RosamTmG mice, as you mention, as this work has not been published. When available, it will be interesting to compare *Aplnr* expression within all three gene knockout animal models (*Aplnr*^{-/-}:*nlacZ*, *Aplnr*^{fl/fl}:*nlacZ*-Flk-1Cre, and *Aplnr*CreER:RosamTmG).

It is well-known that Erg selectively regulates different genes depending on gestational age, cell type, organ-specificity, and type of organism. Our findings that Erg selectively upregulates *Aplnr* in pulmonary venous endothelium, while not inducing genes specific to arterial endothelium or other cell types in veins, is itself a novel finding. Finally, within the adult mouse lung, the pattern of Erg and *Aplnr* expression is not “disparate”. Rather, Erg and *Aplnr* expression are tightly-linked temporally and spatially to pulmonary venous endothelial cells during embryonic development and in the adult mouse. The fact that Erg is also seen in arterial endothelium within the adult lung simply suggests that it may control different genes and have other functions in cell types other than venous endothelium.

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