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DELTA LIKE 4 (DLL4), AN ENDOTHELIAL SPECIFIC NOTCH LIGAND IS CRITICAL FOR LUNG VASCULAR ARBORIZATION AND ALVEOLARIZATION



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Introduction

- Human and mouse lung development progress temporally from the embryonic, pseudo-glandular, canalicular, saccular and alveolar phase.
- Vascular development parallels lung development, resulting in an arborized network with terminal capillaries in close proximity to alveoli.
- The relative contribution of vasculogenesis vs. angiogenesis to distal lung development remains unclear. Further, the contribution of endothelial cell (EC) angiogenesis to lung development is unknown.
- Herein, we investigated the role of Delta like 4 (DLL4), a regulator of embryonic angiogenesis in vascular arborization and alveolarization.

Hypothesis and Objectives

Hypothesis: DLL4-dependent angiogenesis is essential for distal vascular arborization and normal alveolarization.

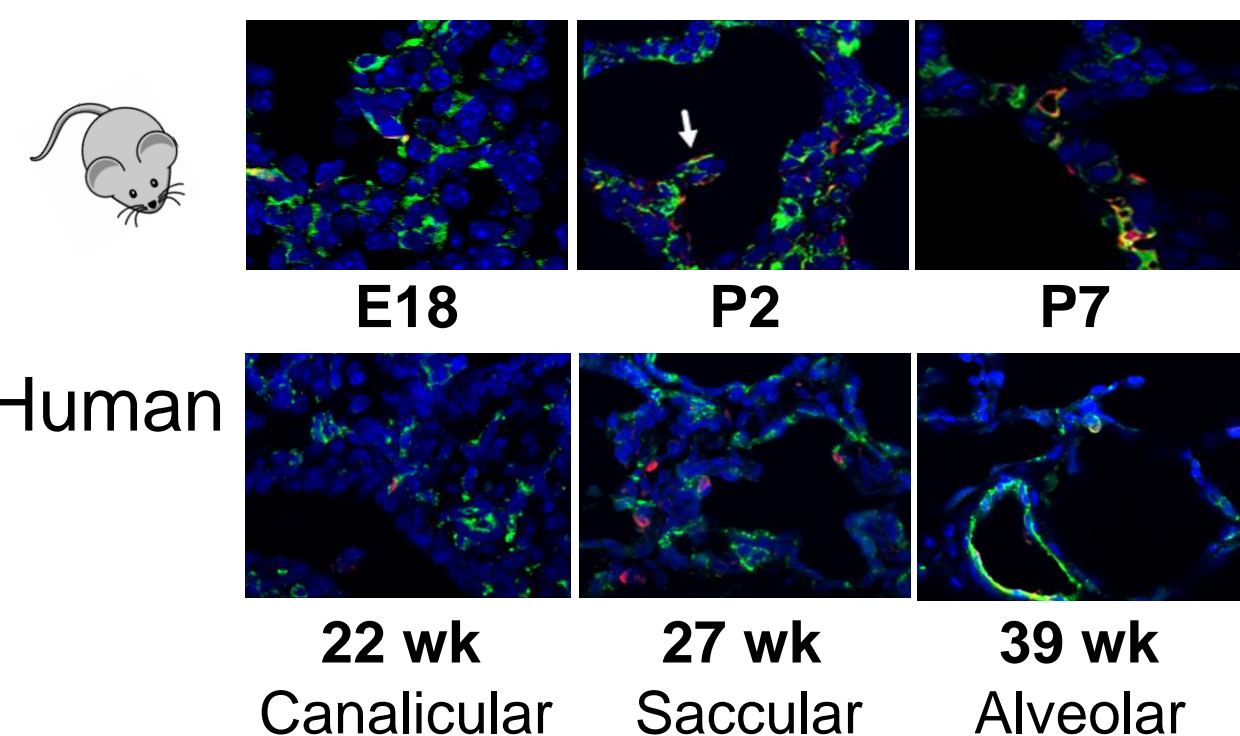
Objectives: 1) To map DLL4 during mouse and human lung development using wild-type and *Dll4-LacZ* mice. 2) To investigate the effect of DLL4 haploinsufficiency and EC-specific *Dll4* deletion on lung vascular development and alveolarization.

Methods

- DLL4 mapping in mouse & human:** DLL4 & PECAM IF/IHC in WT, *DLL4^{+/lacZ}* mice, human autopsy lung samples, X-gal in *DLL4^{+/lacZ}* mice.
- Mouse gene expression:** Whole lung RNA expression by qRT-PCR.
- Mouse lung morphometry:** Radial alveolar count (RAC) quantification was done to assess alveolarization in *WT* and *DLL4^{+/lacZ}* mice.
- Tamoxifen-inducible *Dll4* deletion:** *Dll4^{+/-Loxp}* (F) X *Dll4^{+/-Loxp}*; *VE-cad-CreERT2* (M). Female injected with Tamoxifen on P2, P3, P4.

Results

DLL4 expression from Canalicular to Alveolar Phase

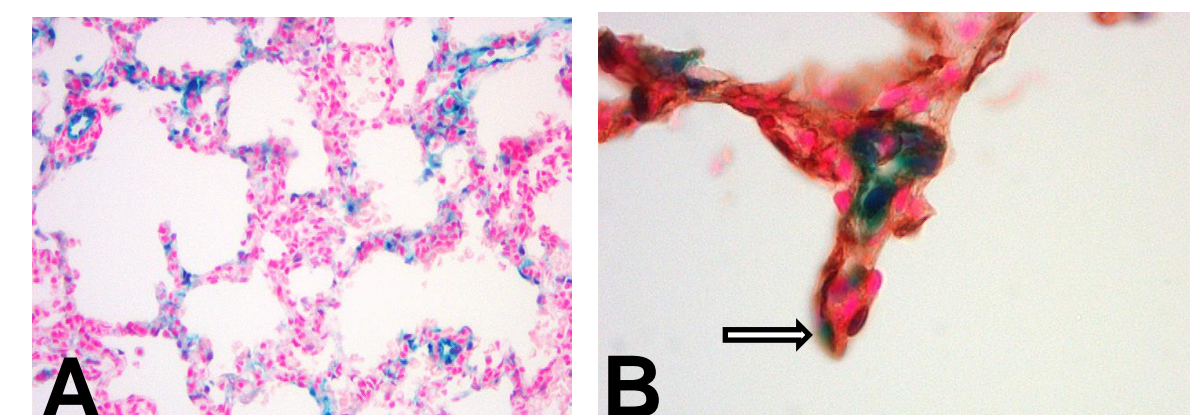


DLL4/PECAM/DAPI (nucleus)

Arrow inset represents DLL4 expression in alveolar septum in PECAM+ cells. 63x; n=3.

Results

DLL4 Lung Mapping Using *Dll4^{+/lacZ}* Reporter Mice

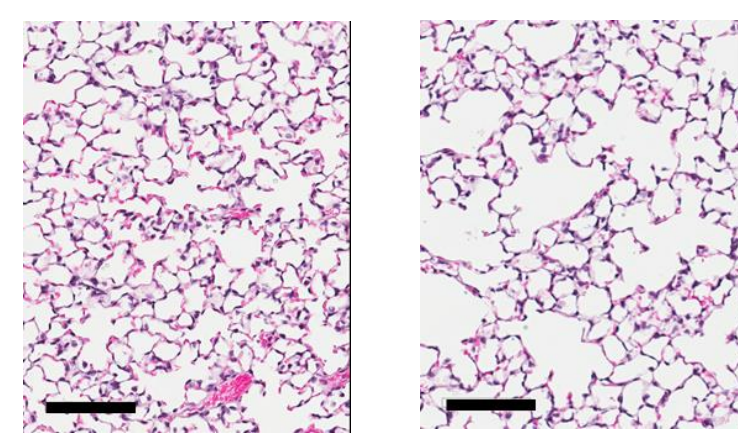


DLL4/PECAM/ Fast Red (nucleus)

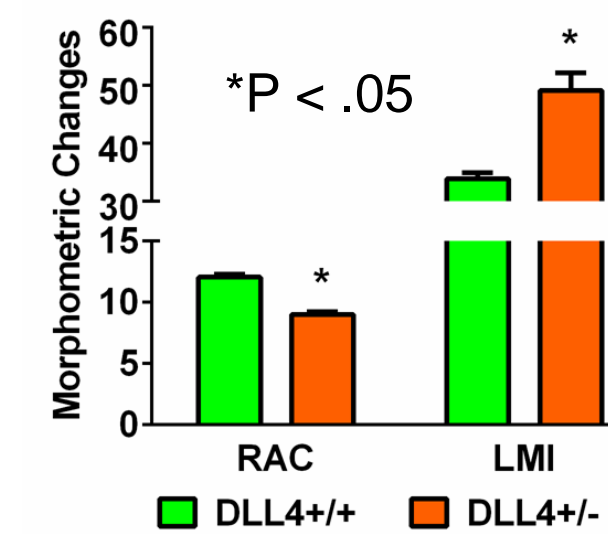
- A. X-gal (blue) indicates DLL4 in distal lung capillary EC.
 B. Arrow - DLL4 in alveolar septum in PECAM+ cells (lung EC). 40x; n=3.

DLL4 Haploinsufficiency Causes Defective Alveolarization in Mice

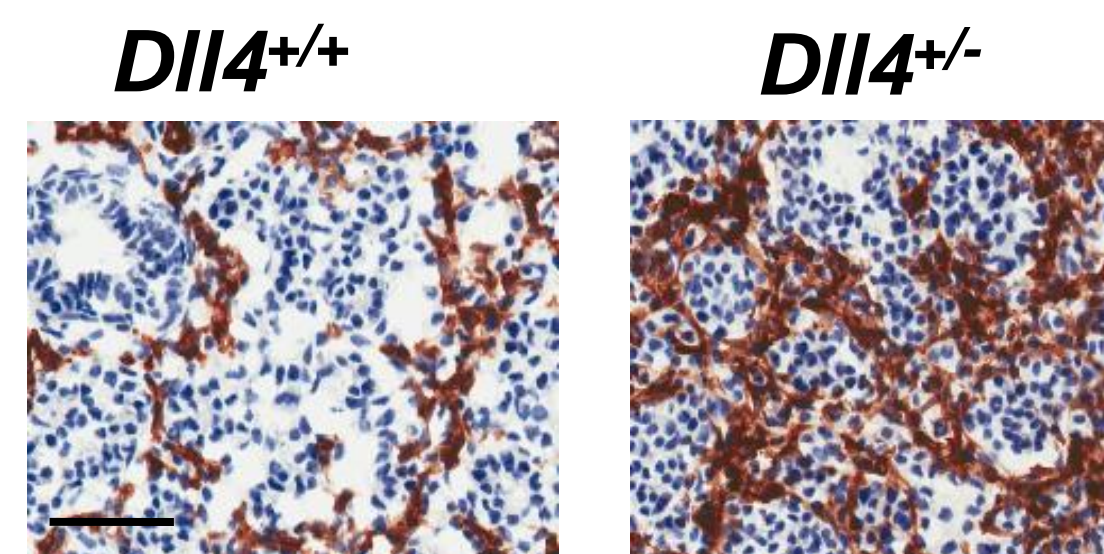
DLL4^{+/+} *DLL4^{+/-}*



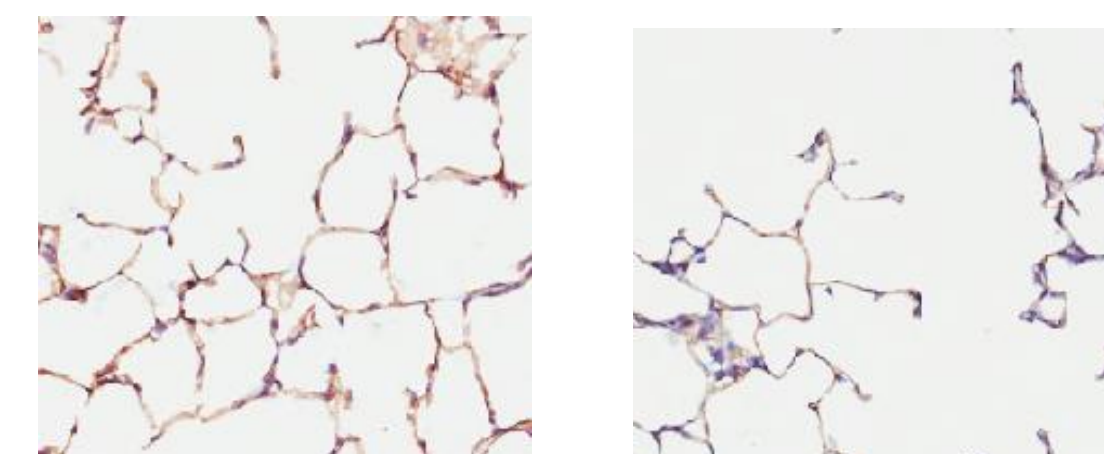
Inflation-fixed P28 mouse lungs
Scale bar = 100 μm



Aberrant Pulmonary Vascular Development in *Dll4^{+/-}* Mice

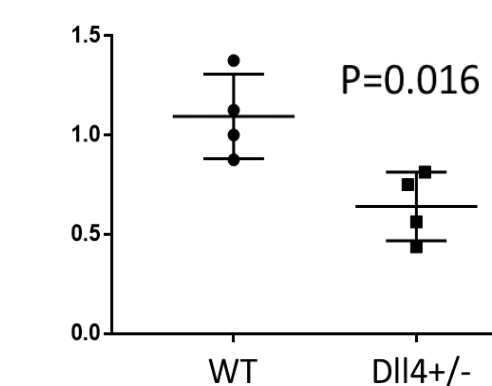


E17.5 Canalicular Stage

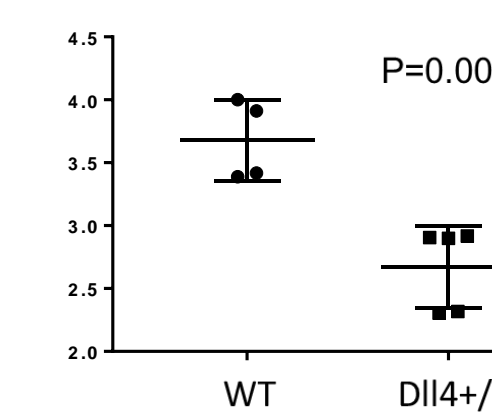


P14 Alveolar Stage

P14



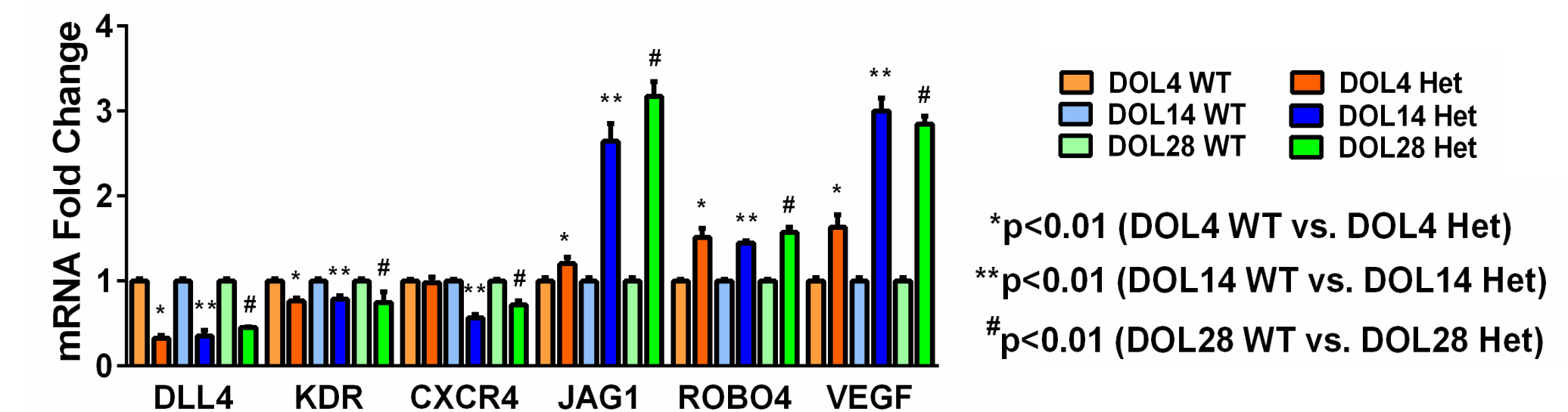
Blood vessels >10μM/40X field



Capillaries <10μM/40X field

Results

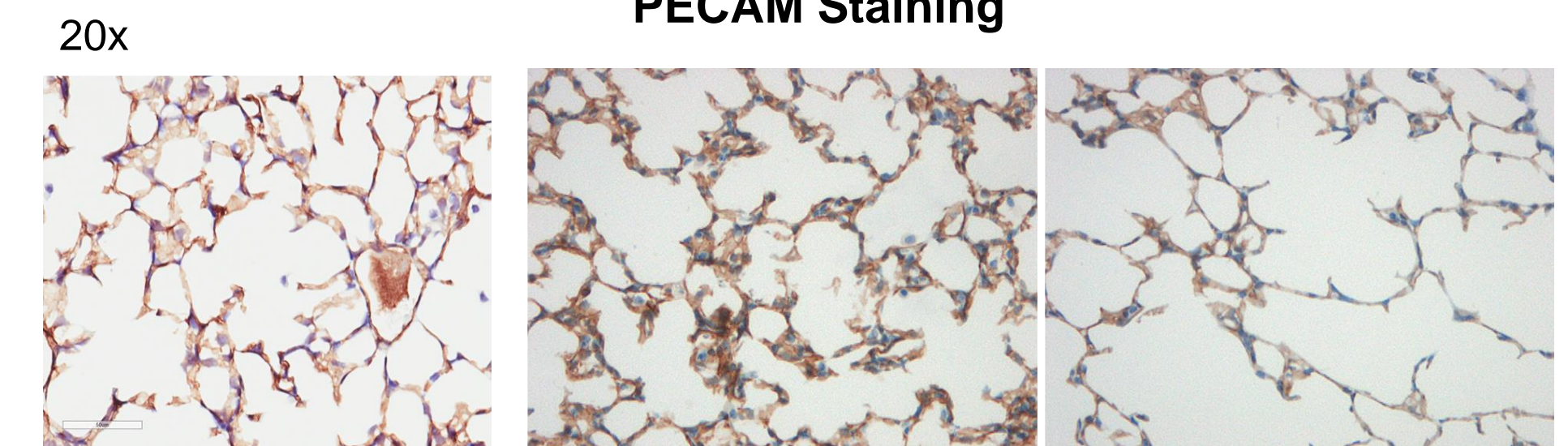
DLL4 Haploinsufficiency Causes Defective Alveolarization in Mice



Dll4 Deletion in EC Impairs Lung alveolarization

Dll4^{+/-Loxp} (F) crossed with *Dll4^{+/-Loxp}*; *VE-cad-CreERT2* (M)
 Dam treated with tamoxifen - P4 and P5

P14 Mid-Alveolar Phase
PECAM Staining



WT

Dll4loxp+/-;cre

Dll4loxp/loxp;cre

Conclusion

- DLL4 is expressed from the canalicular to the alveolar phase of lung development in mice & humans, indicating that DLL4-dependent angiogenesis contributes to vascular development.
- DLL4^{+/-}* mice have decreased expression of tip cell EC fate markers, with increased expression of stalk cell EC markers and VEGF. *DLL4^{+/-}* mice have dysmorphic microvasculature, impaired angiogenesis and disrupted alveolar development.
- Pilot data suggest that EC-*Dll4* deletion impairs alveolarization.
- Future:** Investigate how EC-DLL4 directs alveolarization, and *Dll4*'s role in defective Bronchopulmonary Dysplasia vasculature.