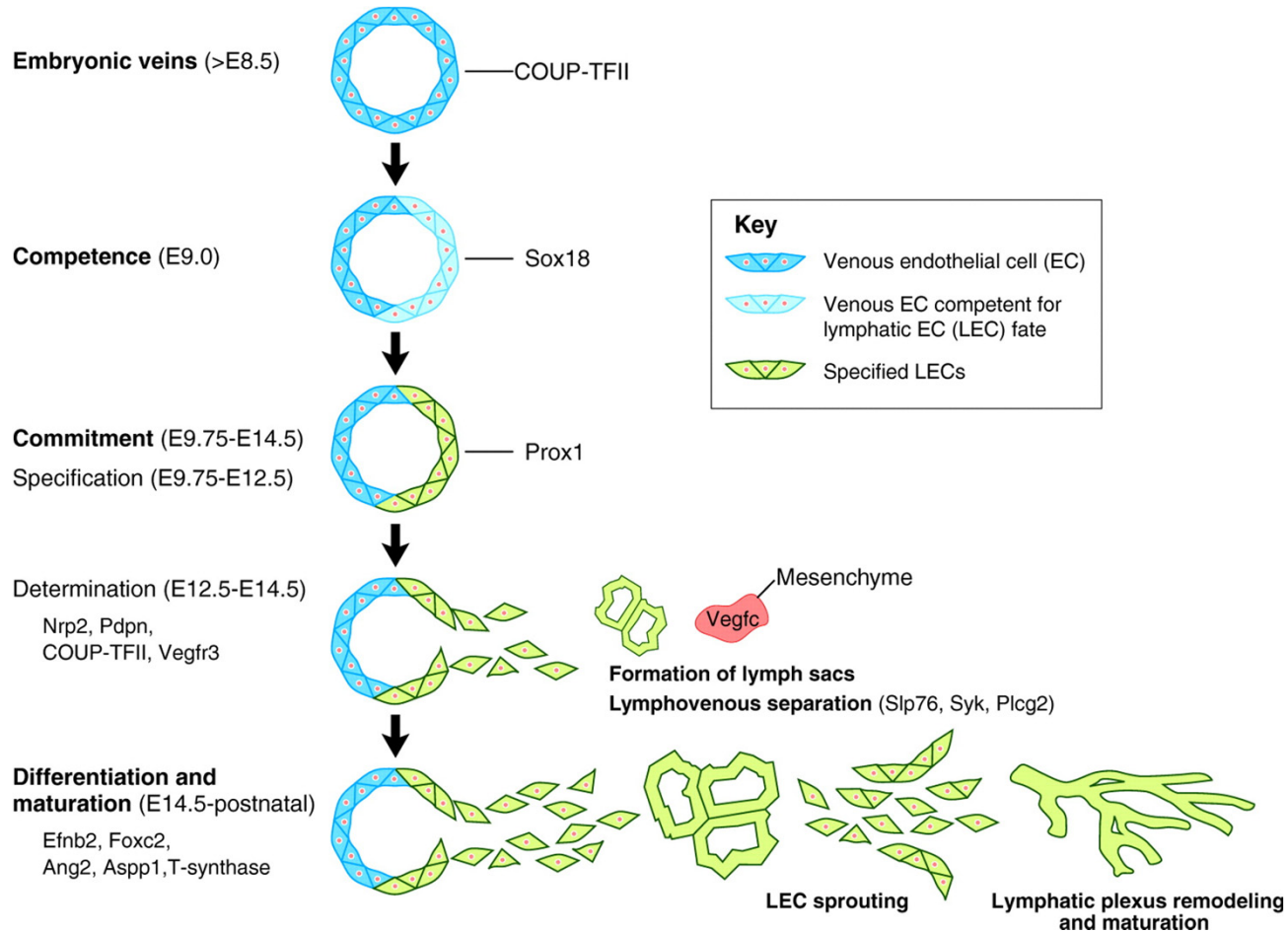


The Prox1–Vegfr3 feedback loop maintains the identity and the number of lymphatic endothelial cell progenitors

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Introduction



Introduction

Prox1 - a master switch of LEC determination

in *Prox1*-null embryos LECs are absent

conditional deletion of *Prox1* results in the loss of LEC identity

ectopic expression of *Prox1* in BECs leads to activation of LEC-specific genes

dose-dependent autoregulation of *Prox1* expression

TAMMELA T. (2010). Lymphangiogenesis: Molecular mechanisms and future promise

SRINIVASAN RS. (2014). The *Prox1*–*Vegfr3* feedback loop maintains the identity and the number of lymphatic endothelial cell progenitors

Vegfr3 - a target of *Prox1*

Vegfc being a ligand for the receptor tyrosine kinase *Vegfr3*

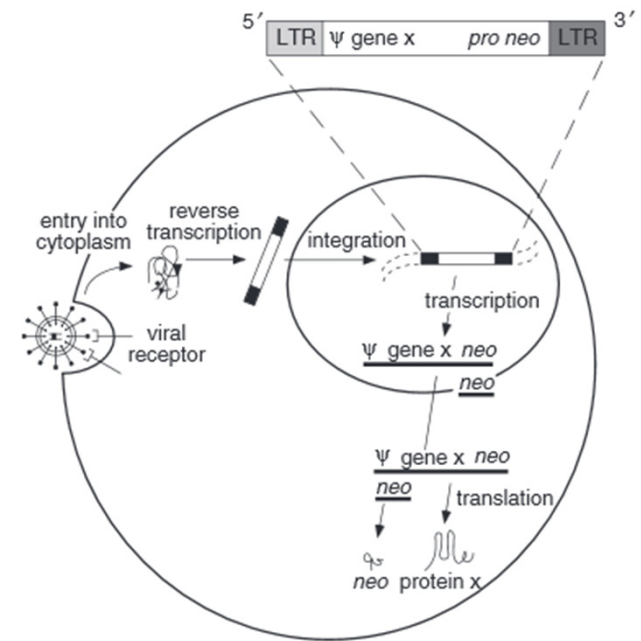
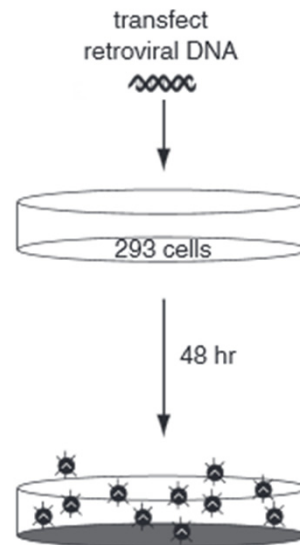
expressed in all BECs until E10.5, later on it is restricted to LECs

number of LEC progenitors and LECs is reduced in *Vegfr3*^{+/-} and *Vegfc*^{+/-} embryos

retroviral transduction

transfection of 293T cells with the retroviral vector (empty or containing avitag-Prox-1)

retroviral transduction of H5V cells by incubation with supernatant of transfected 293T cells



siRNA analysis

human dermal LECs

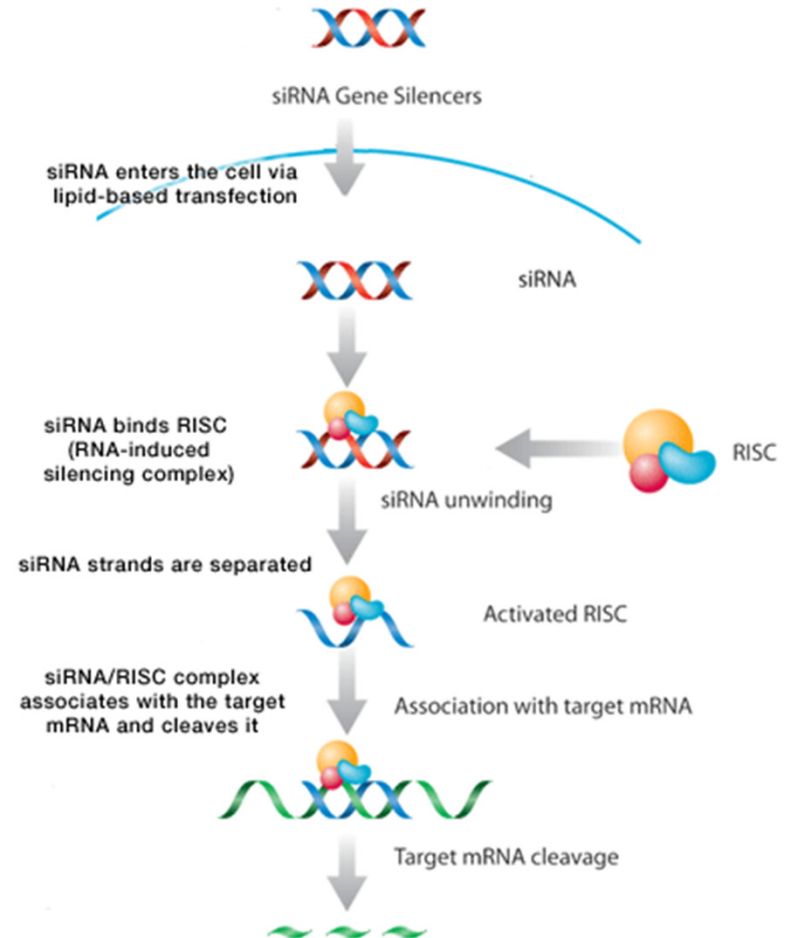
target RNA: *Prox 1*
Vegfr3

knockdown was examined by

western blot

immunofluorescence

qPCR

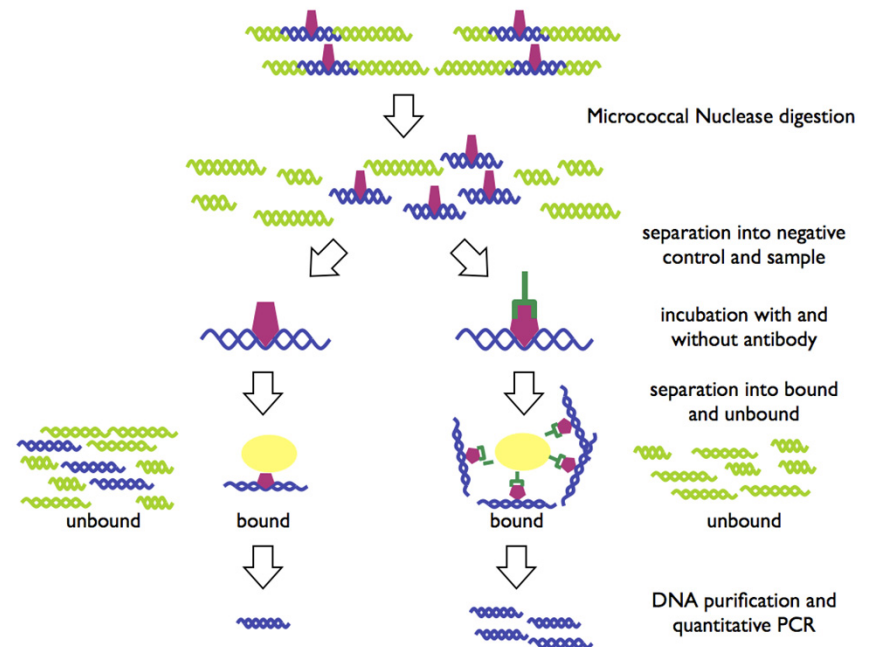


Chromatin immunoprecipitation

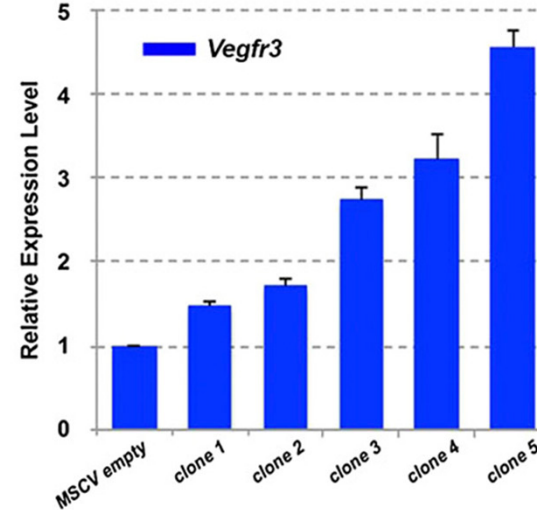
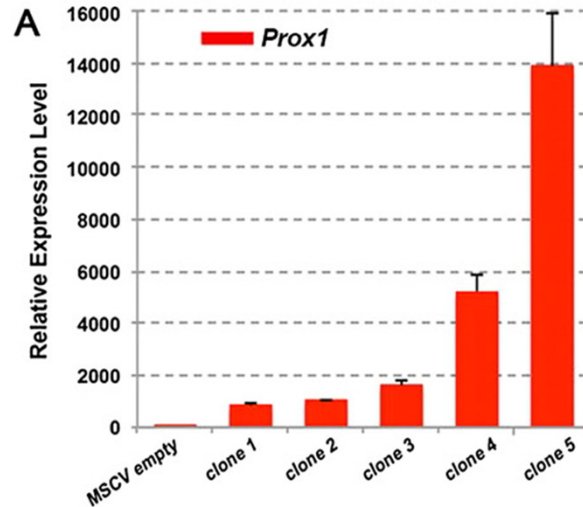
mouse primary LECs from E 14.5 were isolated by flow cytometry

Lyve-1⁺, CD31⁺ and CD45⁻ population

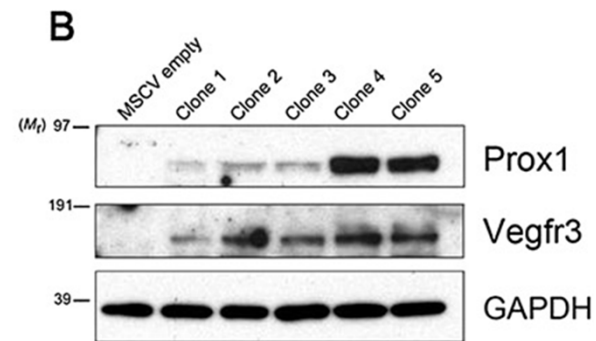
real-time PCR

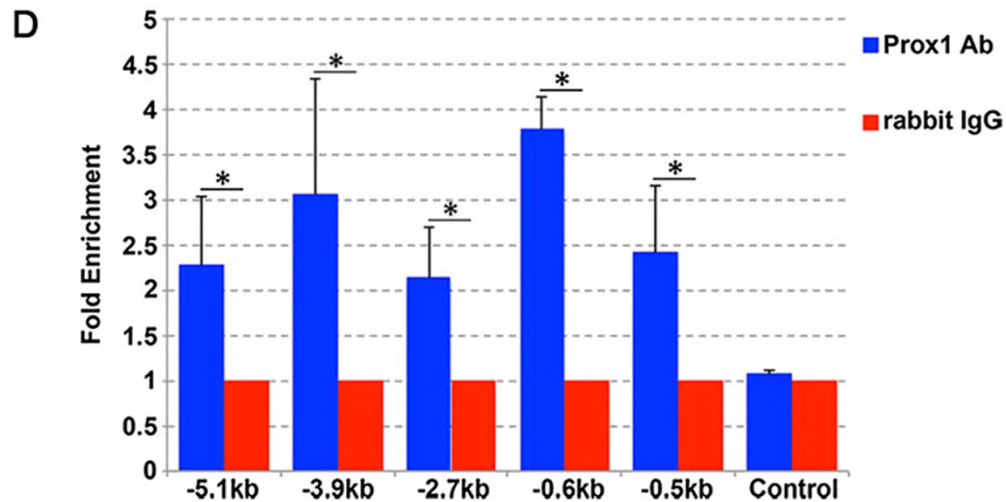
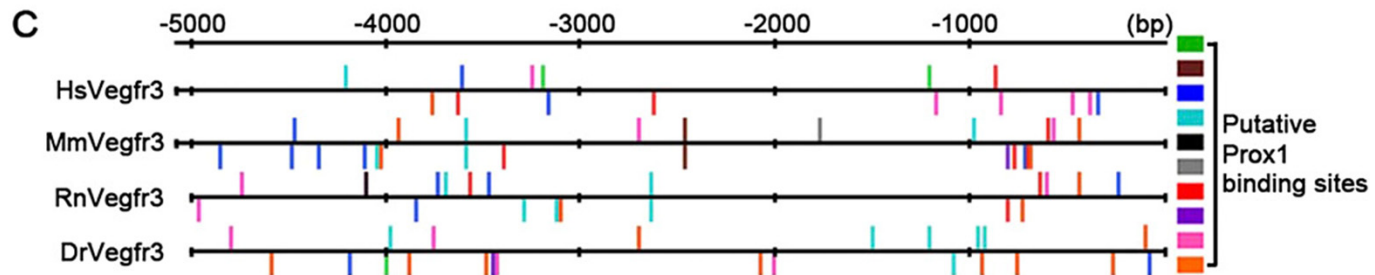


Vegfr3 is a dosage-dependent target of *Prox1*



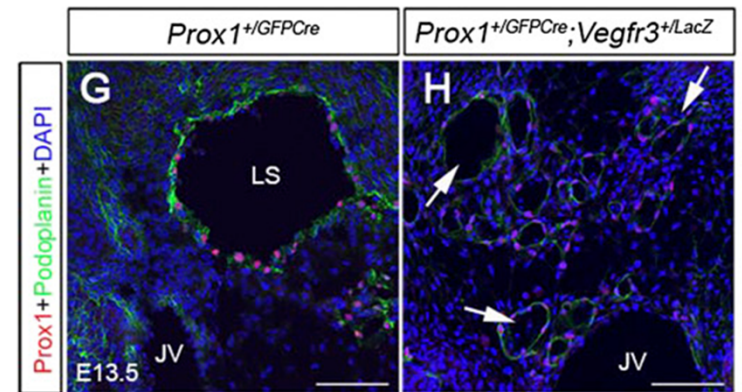
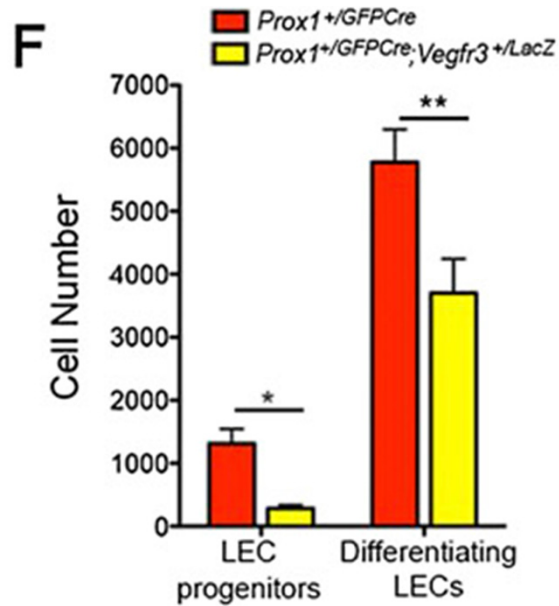
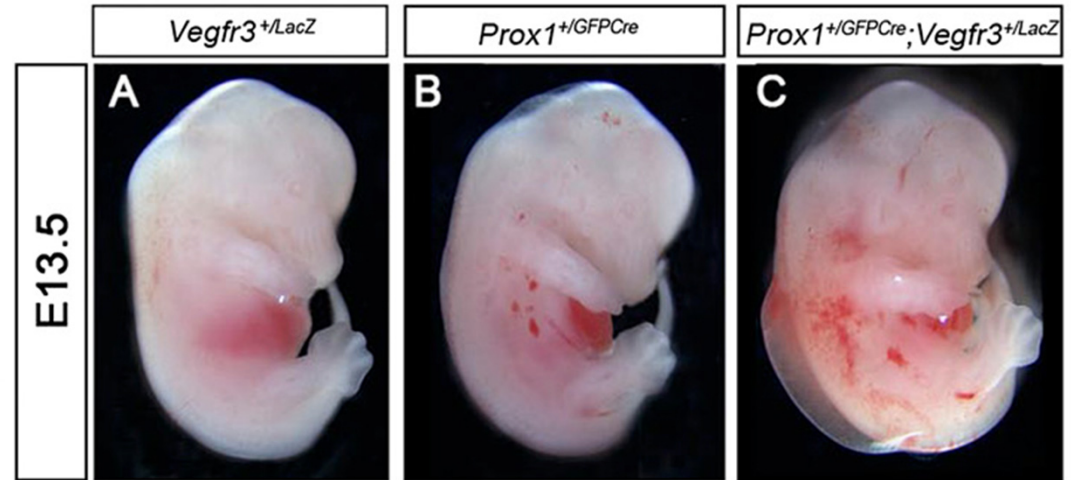
other BEC- and LEC-specific genes such as *Foxc2*, *Integrin α 9*, *Coupled-TFII*, *Reelin*, *Tie2*, *Nrp1*, *Nrp2*, *PECAM1*, *VE-Cadherin* and *Lyve1* showed no correlation with *Prox1* expression

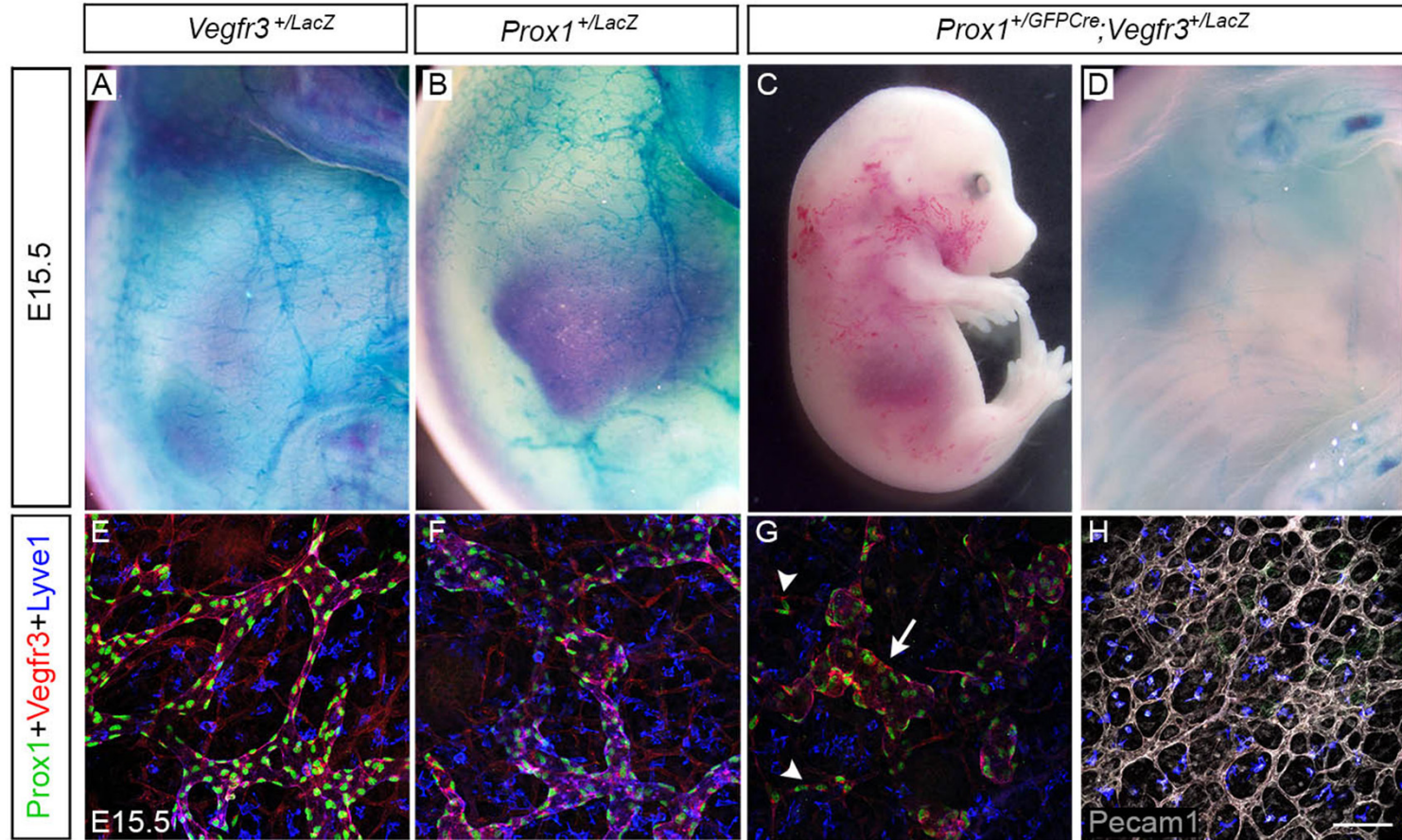




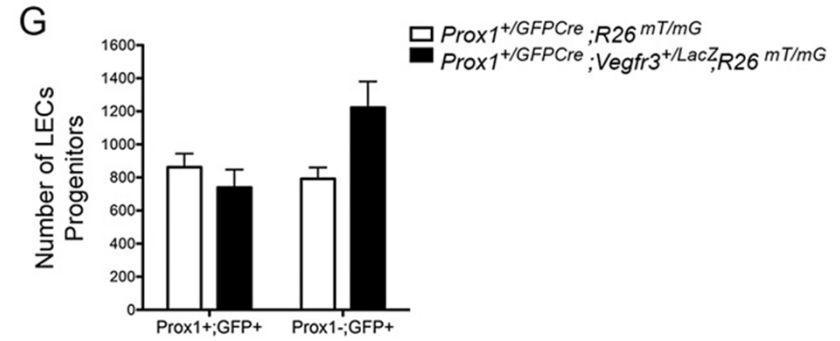
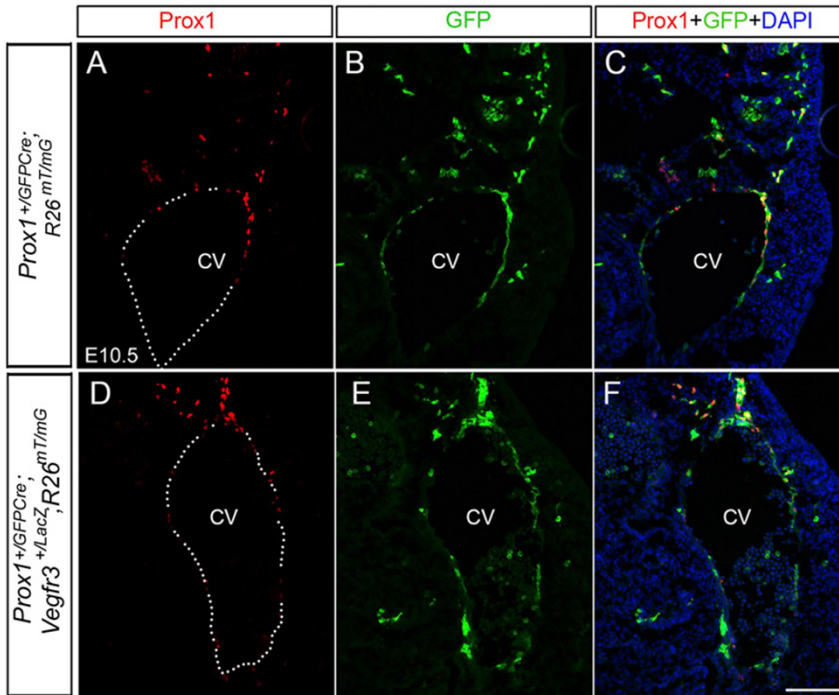
Lymphatic vasculature is defective in
Prox1^{+/GFPCre};Vegfr3^{+/LacZ} embryos

The number of LEC progenitors and differentiating LECs
is severely reduced in *Prox1^{+/GFPCre};Vegfr3^{+/LacZ}* embryos

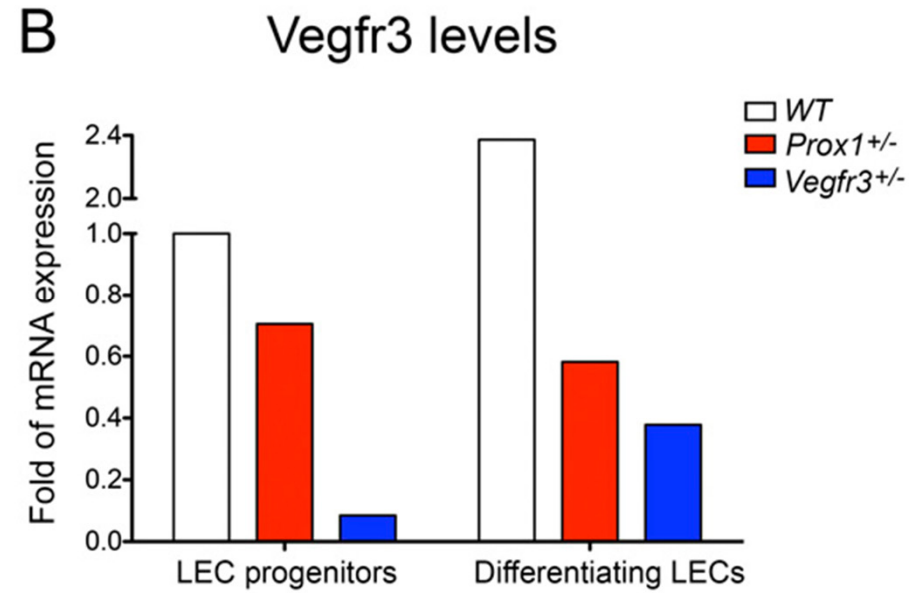
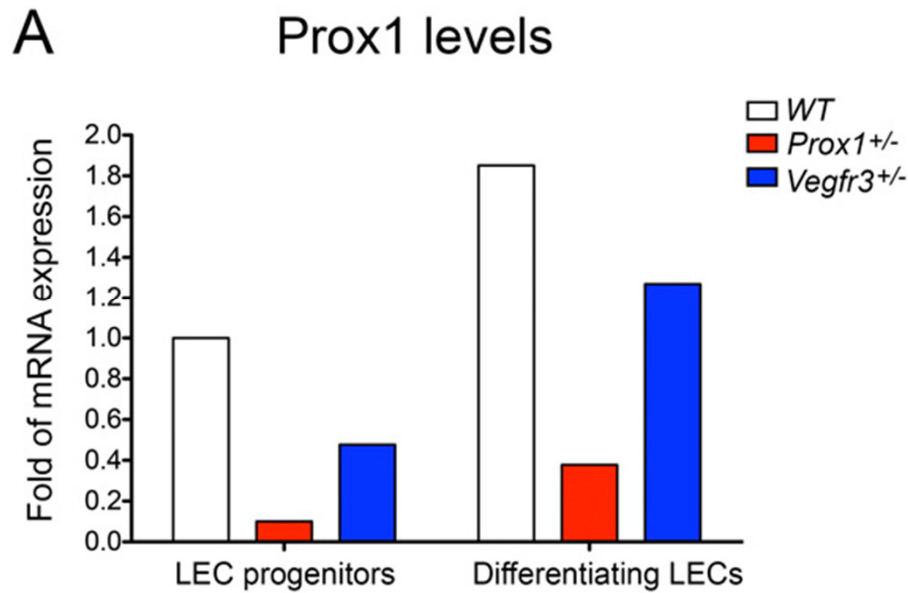


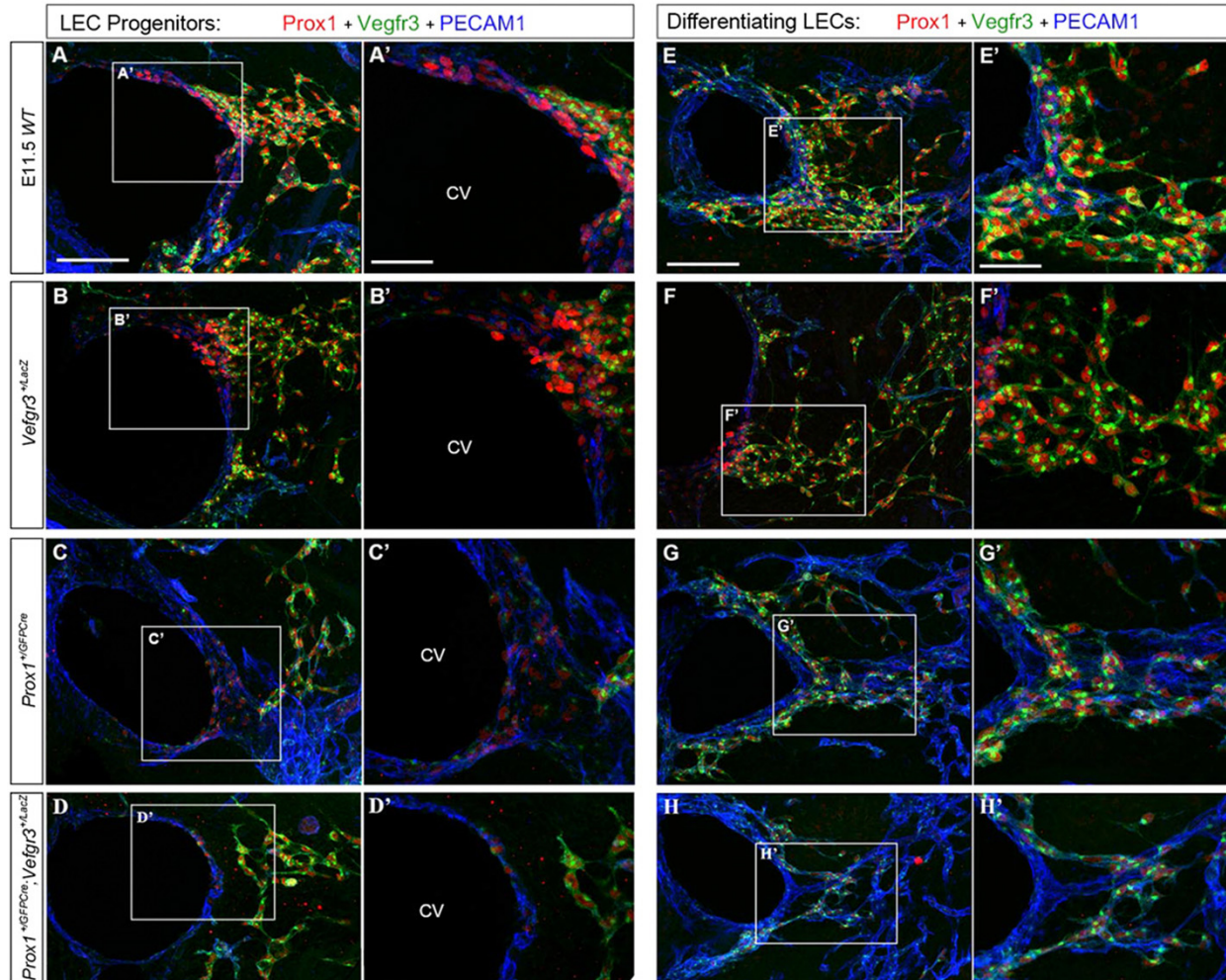


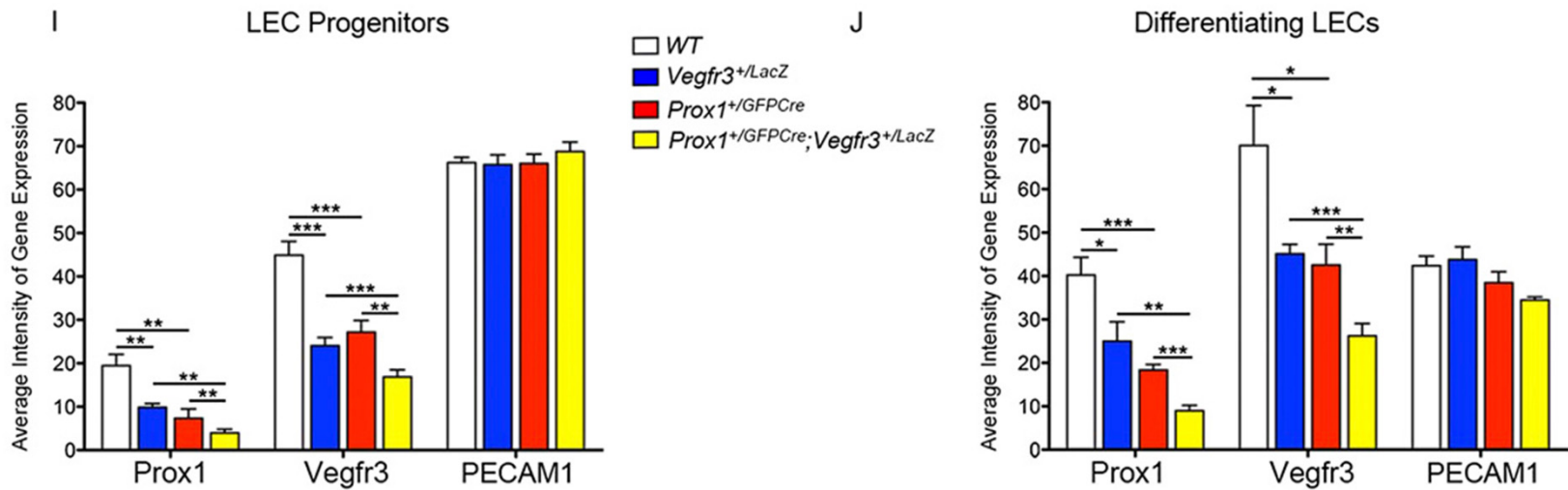
LEC progenitor identity is lost in
Prox1⁺/GFPCre;Vegfr3⁺/LacZ embryos

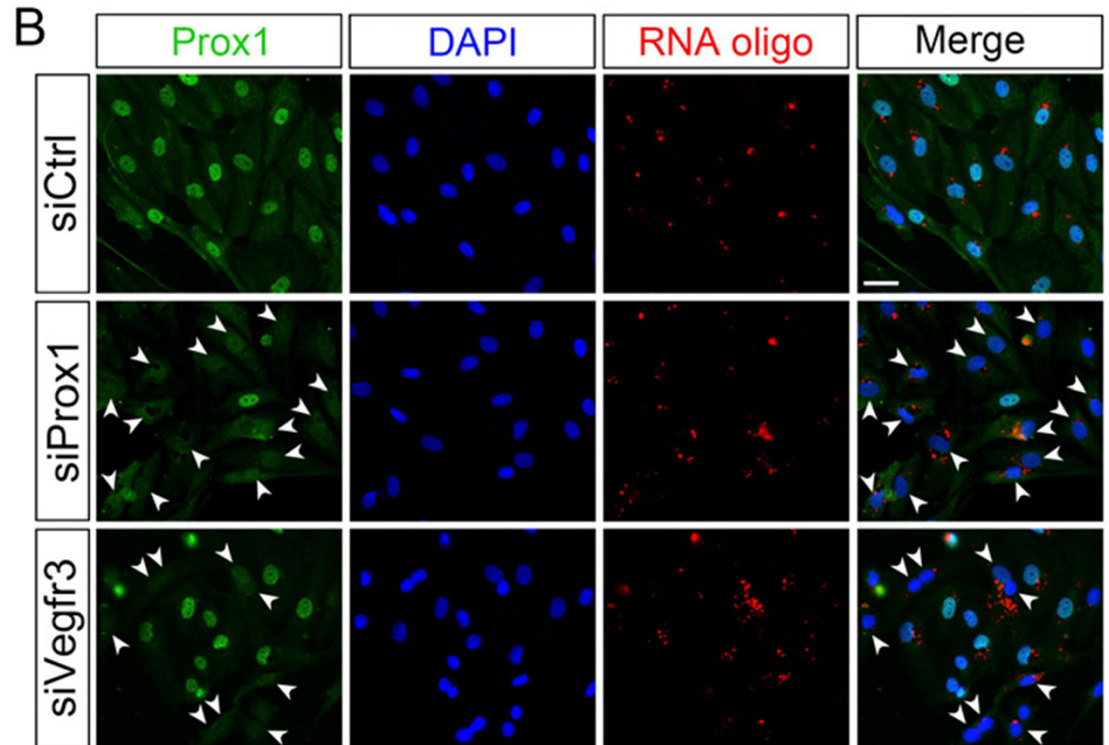
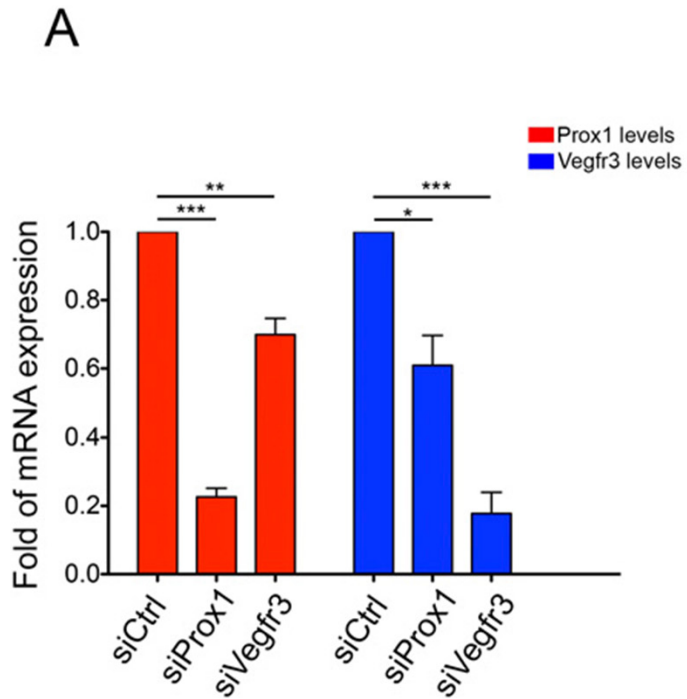


Vegfr3 regulates *Prox1* expression in LEC progenitors and LECs

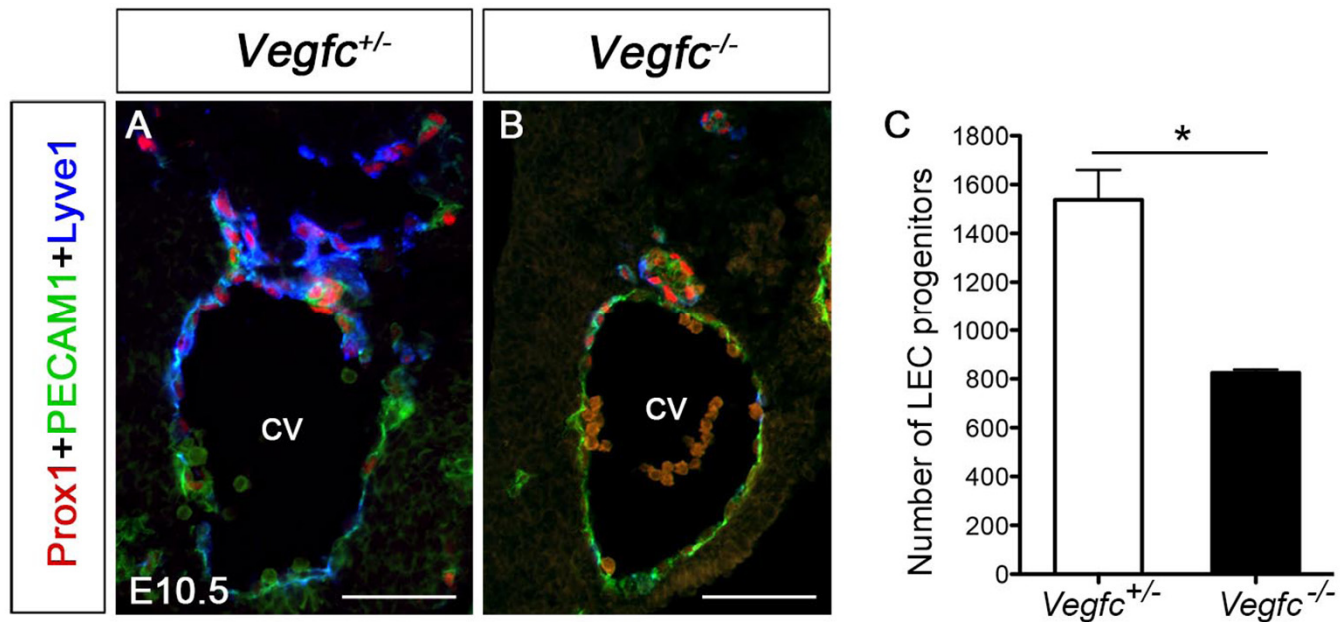


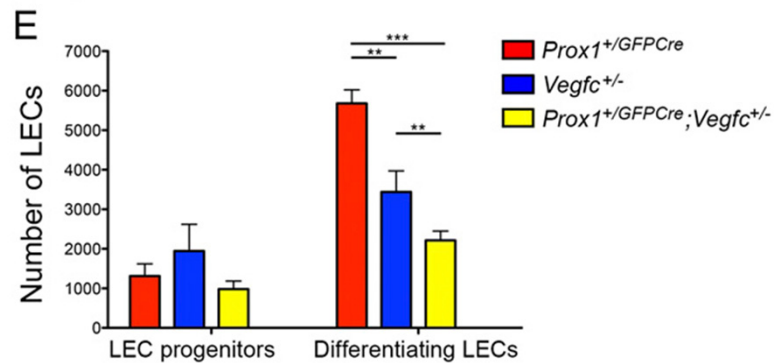
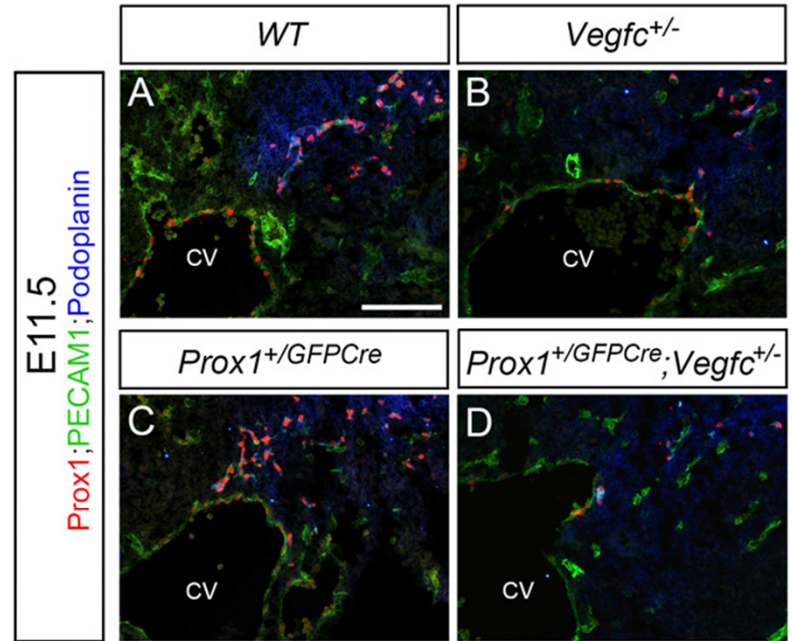


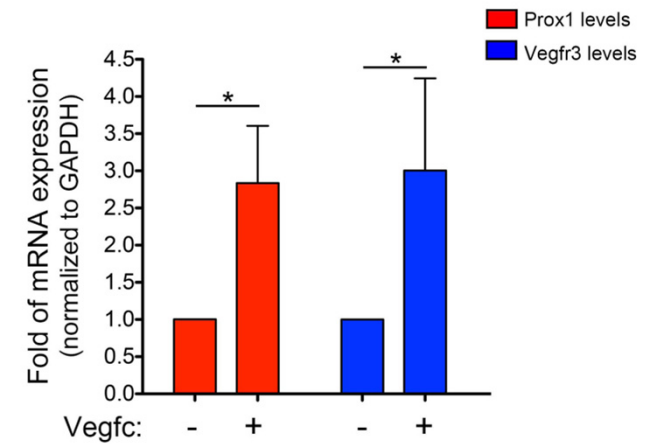
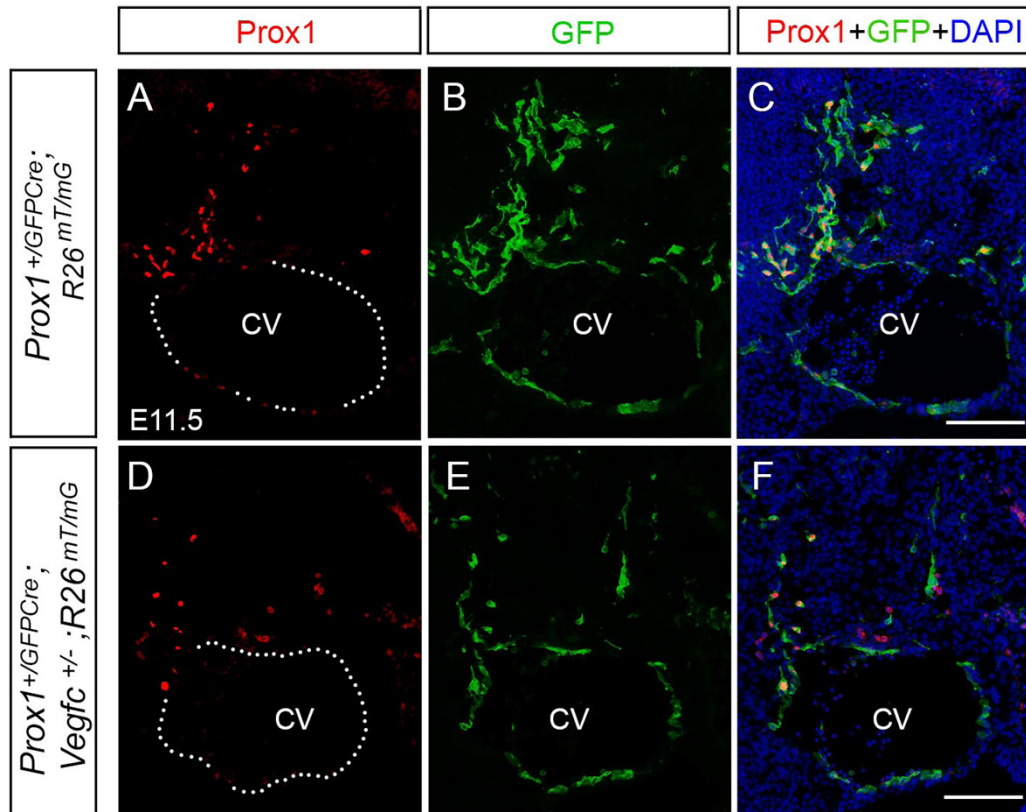




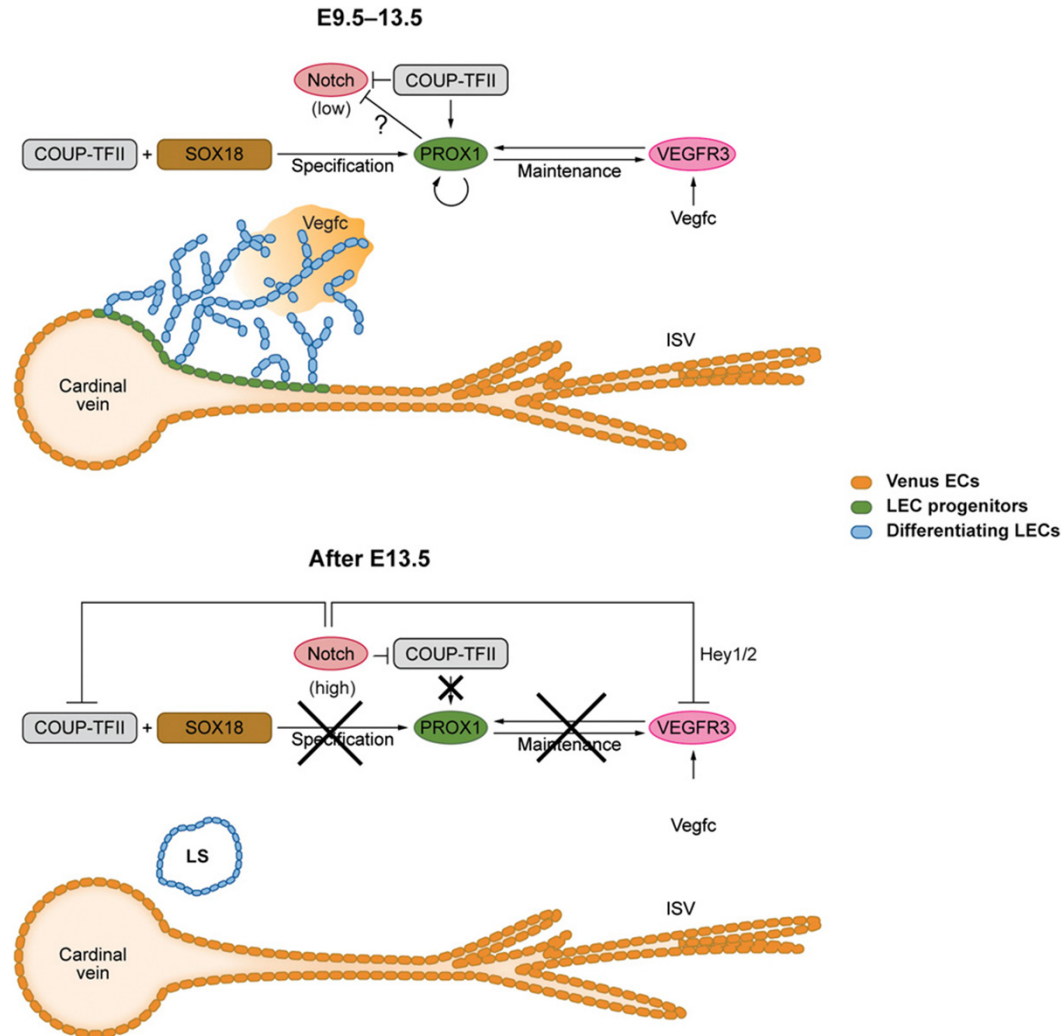
*Vegfc signaling helps maintain LEC
progenitor identity*







Conclusion



Conclusion

Prox1⁺ LEC progenitors require *Vegfr3* to maintain their identity and bud from the CV

this regulation is mediated through the activation of *Vegfr3* by *Vegfc*

alterations in *Vegfc/Vegfr3* signalling leads to the loss of *Prox1* expression in LEC progenitors and their reversal to venous EC fate