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## Supplemental Information

# Paraxial Mesoderm Is the Major Source of Lymphatic Endothelium

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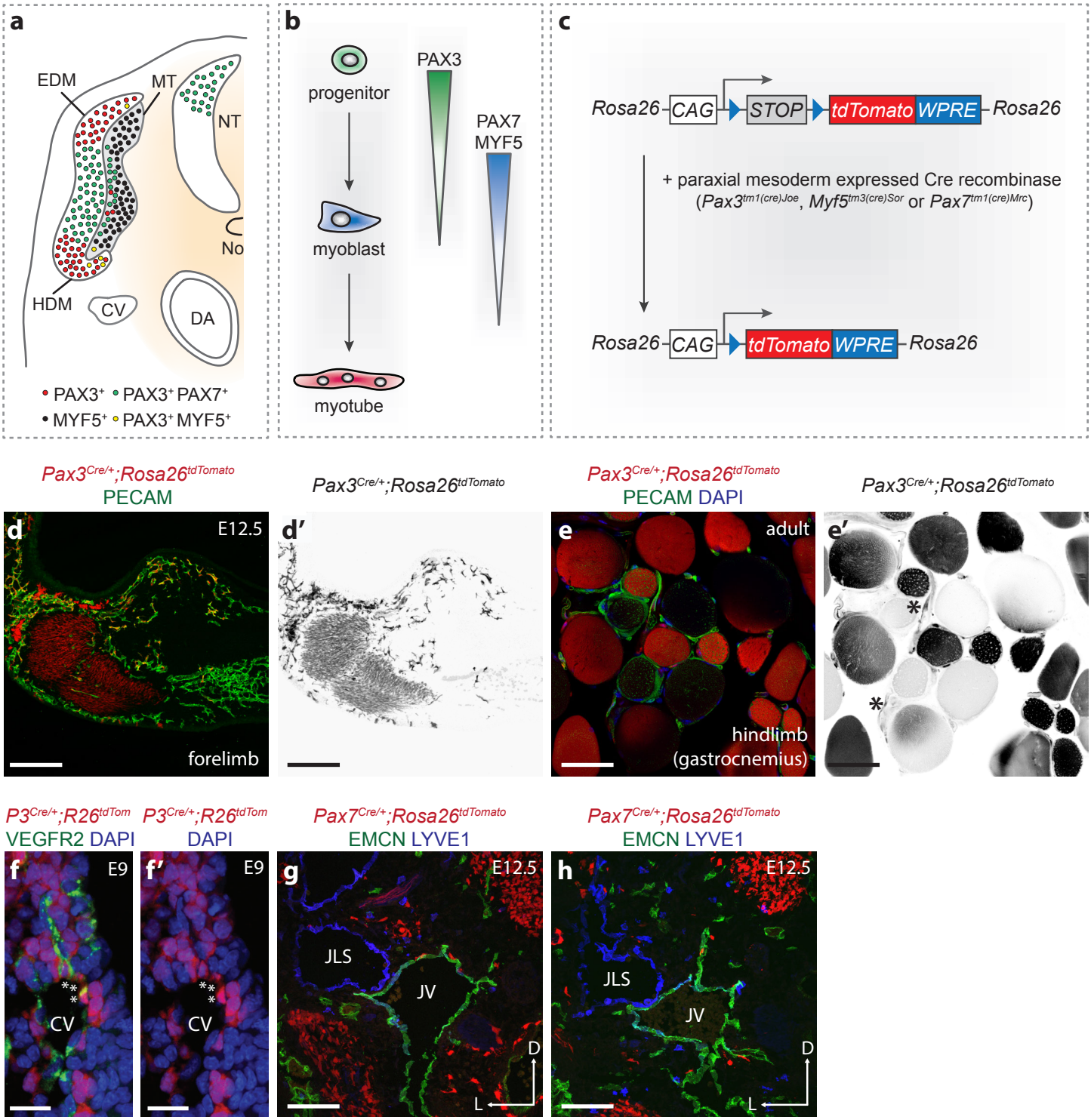
a
EDM
MT
NT
No
HDM
CV
DA
PAX3+
PAX3+ PAX7+
MYF5+
PAX3+ MYF5+
b
progenitor
myoblast
myotube
PAX3
PAX7
MYF5
c
Rosa26
CAG
STOP
tdTomato
WPRE
Rosa26
+ paraxial mesoderm expressed Cre recombinase
(Pax3tm1(cre)Joe, Myf5tm3(cre)Sor or Pax7tm1(cre)Mrc)
Rosa26
CAG
tdTomato
WPRE
Rosa26
Pax3Cre/+;Rosa26tdTomato
PECAM
d
E12.5
forelimb
Pax3Cre/+;Rosa26tdTomato
d'
Pax3Cre/+;Rosa26tdTomato
PECAM DAPI
e
adult
hindlimb
(gastrocnemius)
Pax3Cre/+;Rosa26tdTomato
e'
P3Cre/+;R26tdTom
VEGFR2 DAPI
f
E9
CV
P3Cre/+;R26tdTom
DAPI
f'
E9
CV
Pax7Cre/+;Rosa26tdTomato
EMCN LYVE1
g
E12.5
JLS
JV
D
L
Pax7Cre/+;Rosa26tdTomato
EMCN LYVE1
h
E12.5
JLS
JV
D
L

**Figure S1: Related to Figure 1. Analysis of paraxial mesodermal cell contribution to the developing vasculature.**

Schematic representations of the embryonic expression of PAX3, PAX7 and MYF5 in the developing dermomyotome, myotome and neural tube (neural crest) (**a**), lineage commitment during embryonic myogenesis (**b**) and the strategy used to lineage trace cells from the paraxial mesoderm (**c**). Immunofluorescence for tdTomato and PECAM on cryosections of embryonic *Pax3*$^{Cre/+}$*;Rosa26*$^{tdtomato}$ forelimb (**d**-**d**') and tdTomato, PECAM and DAPI on cryosections of adult *Pax3*$^{Cre/+}$*;Rosa26*$^{tdtomato}$ gastrocnemius (**e**-**e**'). (**f**-**f**') Immunofluorescence for tdTomato and VEGFR2 on transverse cryosections from a *Pax3*$^{Cre/+}$*;Rosa26*$^{tdtomato}$ embryo. (**g**, **h**) Immunofluorescence for tdTomato, EMCN and LYVE1 on transverse cryosections from *Pax7*$^{Cre/+}$*;Rosa26*$^{tdtomato}$ embryos. (CV - cardinal vein; DA - dorsal aorta; HDM - hypaxial dermomyotome; EDM - epaxial dermomyotome; MT - myotome; No - notochord; NT - neural tube; JLS - jugular lymph sac; JV - jugular vein). Scale bars: **d, d'**, **g**, **h** – 100 μm; **e**-**f**' – 50 μm.

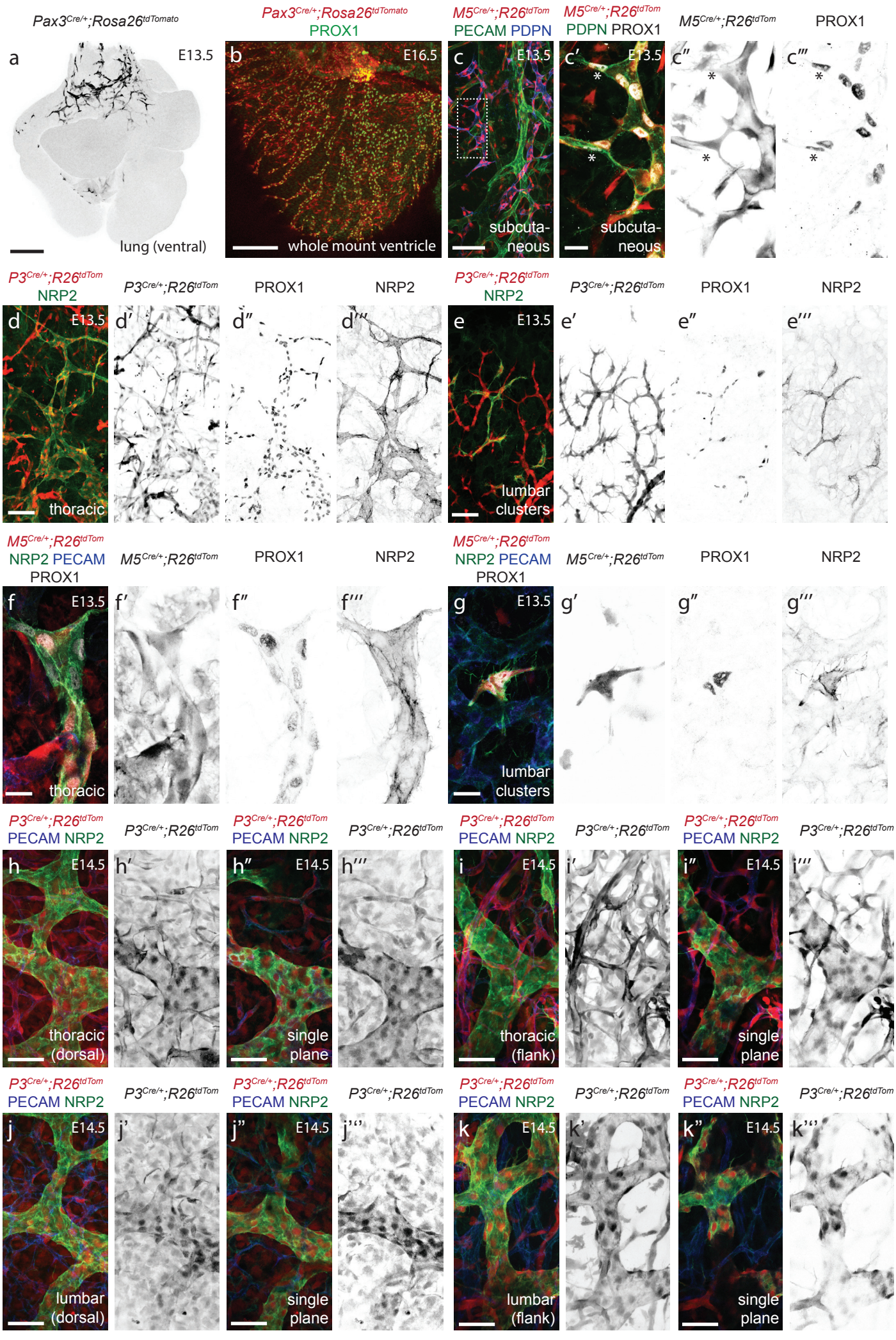
Pax3Cre/+;Rosa26tdTomato
a
E13.5
lung (ventral)
Pax3Cre/+;Rosa26tdTomato
PROX1
b
E16.5
whole mount ventricle
M5Cre/+;R26tdTom
PECAM PDPN
c
E13.5
subcuta-
neous
M5Cre/+;R26tdTom
PDPN PROX1
c′
E13.5
subcuta-
neous
M5Cre/+;R26tdTom
c″
PROX1
c‴
P3Cre/+;R26tdTom
NRP2
d
E13.5
thoracic
P3Cre/+;R26tdTom
d′
PROX1
d″
NRP2
d‴
P3Cre/+;R26tdTom
NRP2
e
E13.5
lumbar
clusters
P3Cre/+;R26tdTom
e′
PROX1
e″
NRP2
e‴
M5Cre/+;R26tdTom
NRP2 PECAM
PROX1
f
E13.5
thoracic
M5Cre/+;R26tdTom
f′
PROX1
f″
NRP2
f‴
M5Cre/+;R26tdTom
NRP2 PECAM
PROX1
g
E13.5
lumbar
clusters
M5Cre/+;R26tdTom
g′
PROX1
g″
NRP2
g‴
P3Cre/+;R26tdTom
PECAM NRP2
h
E14.5
thoracic
(dorsal)
P3Cre/+;R26tdTom
h′
P3Cre/+;R26tdTom
PECAM NRP2
h″
E14.5
single
plane
P3Cre/+;R26tdTom
h‴
P3Cre/+;R26tdTom
PECAM NRP2
i
E14.5
thoracic
(flank)
P3Cre/+;R26tdTom
i′
P3Cre/+;R26tdTom
PECAM NRP2
i″
E14.5
single
plane
P3Cre/+;R26tdTom
i‴
P3Cre/+;R26tdTom
PECAM NRP2
j
E14.5
lumbar
(dorsal)
P3Cre/+;R26tdTom
j′
P3Cre/+;R26tdTom
PECAM NRP2
j″
E14.5
single
plane
P3Cre/+;R26tdTom
j‴
P3Cre/+;R26tdTom
PECAM NRP2
k
E14.5
lumbar
(flank)
P3Cre/+;R26tdTom
k′
P3Cre/+;R26tdTom
PECAM NRP2
k″
E14.5
single
plane
P3Cre/+;R26tdTom
k‴

**Figure S2: Related to Figure 2. Paraxial mesodermal cells give rise to the subcutaneous and dermal lymphatics.**

(**a**) Representative confocal projection of *Pax3*$^{Cre/+}$*;Rosa26*$^{tdtomato}$ whole mount embryonic lungs. (**b**) Representative confocal projection of a *Pax3*$^{Cre/+}$*;Rosa26*$^{tdtomato}$ whole mount embryonic ventricle co-immunostained for PROX1. (**c-c**’’’) Immunofluorescence staining for tdTomato, PECAM, PDPN and PROX1 on a sagtital vibratome section at the level of the subcutaneous lymphatic network from a *Myf5*$^{Cre/+}$*;Rosa26*$^{tdtomato}$ embryo. (**d-e**’’’) Immunofluorescence for tdTomato, NRP2 and PROX1 on whole mount embryonic skin from *Pax3*$^{Cre/+}$*;Rosa26*$^{tdtomato}$ embryos at the indicated stage and locations. (**f-g**’’’) Immunofluorescence for tdTomato, NRP2 and PROX1 on whole mount embryonic skin from *Myf5*$^{Cre/+}$*;Rosa26*$^{tdtomato}$ embryos at the indicated stage and locations. High resolution and single confocal plane images of immunofluorescence for tdTomato, PECAM and NRP2 on whole mount embryonic skin from *Pax3*$^{Cre/+}$*;Rosa26*$^{tdtomato}$ embryos at the indicated stage and locations (**h-k’’’**). Scale bars: a, b – 200 μm; c, d, e – 100 μm; c’-c’’’, f, g – 20 μm; h-k – 50 μm.

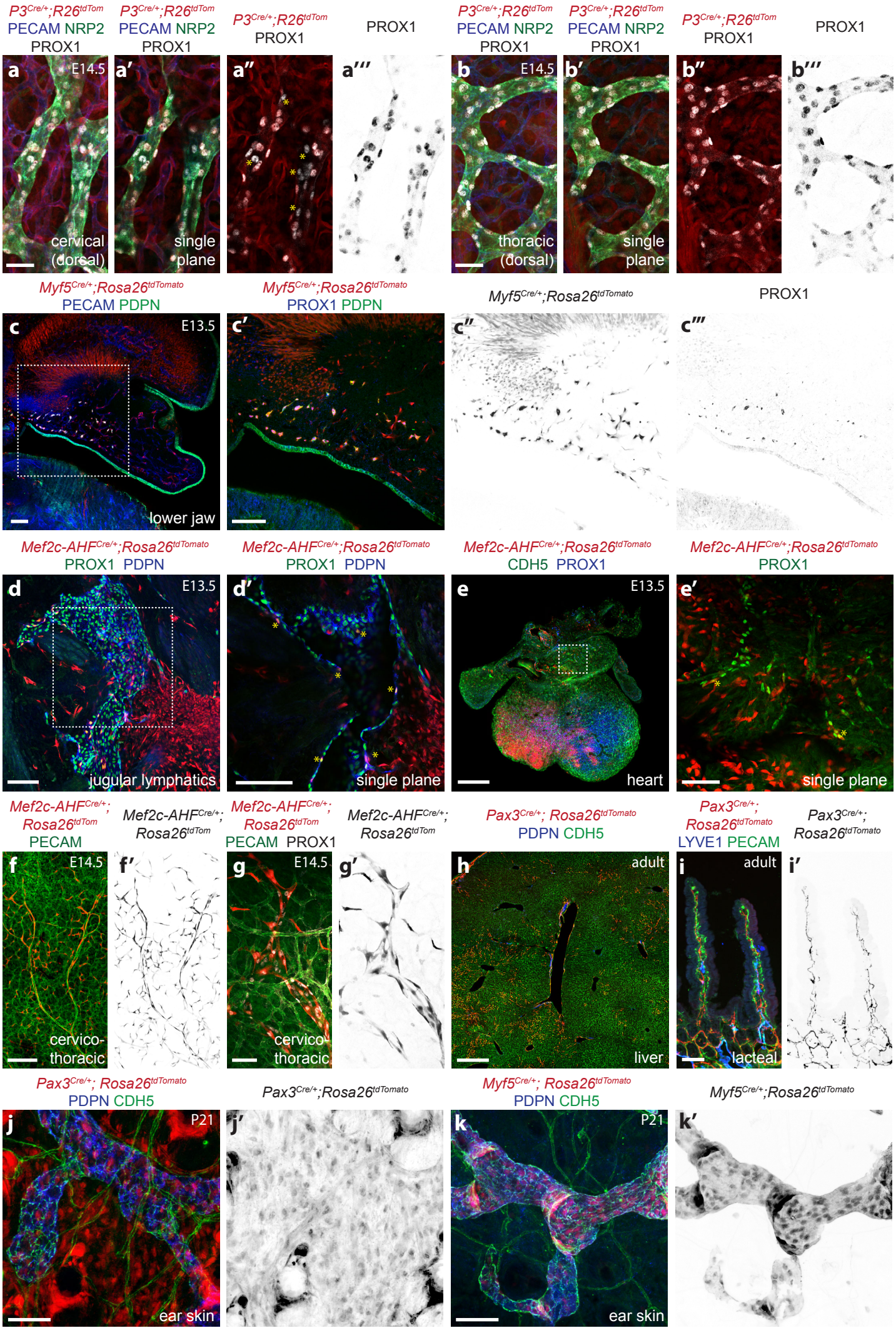
P3Cre/+;R26tdTom
PECAM NRP2
PROX1
a
E14.5
cervical (dorsal)
a′
single plane
a″
a‴
b
thoracic (dorsal)
b′
b″
b‴
Myf5Cre/+;Rosa26tdTomato
PECAM PDPN
c
E13.5
lower jaw
PROX1 PDPN
c′
c″
c‴
Mef2c-AHFCre/+;Rosa26tdTomato
PROX1 PDPN
d
jugular lymphatics
d′
single plane
CDH5 PROX1
e
heart
e′
Mef2c-AHFCre/+;
Rosa26tdTom
PECAM
f
cervico-thoracic
f′
PECAM PROX1
g
g′
Pax3Cre/+; Rosa26tdTomato
PDPN CDH5
h
adult
liver
Pax3Cre/+;
Rosa26tdTomato
LYVE1 PECAM
i
lacteal
i′
j
P21
ear skin
j′
k
k′

**Figure S3: Related to Figures 2 and 3. Contribution of multiple muscle progenitor populations to organ and tissue specific lymphatic vascular beds.**

(**a-b'''**) Immunofluorescence for tdTomato, PECAM, NRP2 and PROX1 on whole mount embryonic skin from *Pax3*$^{Cre/+}$*;Rosa26*$^{tdtomato}$ embryos at the indicated stage and locations . Yellow asterisks highlight PROX1$^{+}$ cells that are not labeled by *Pax3*$^{Cre/+}$*;Rosa26*$^{tdtomato}$ in the cervical skin. (**c-c'''**) Immunofluorescence for tdTomato, PECAM, PDPN and PROX1 on sagittal vibratome sections at the level of the lower jaw from *Myf5*$^{Cre/+}$*;Rosa26*$^{tdTomato}$ embryos. (**d-d'**) Immunofluorescence for tdTomato, PROX1 and PDPN on sagittal vibratome sections at the level of the jugular lymph sac from *Mef2c-AHF*$^{Cre/+}$*;Rosa26*$^{tdTomato}$ embryos - ellow asterisks indicate LECs derived from the *Mef2c-AHF* lineage. (**e-e'**) Representative confocal images of a *Mef2c-AHF*$^{Cre/+}$*;Rosa26*$^{tdtomato}$ whole mount embryonic heart, co-immunostained for CDH5 and PROX1 - yellow asterisks indicate LECs derived from the *Mef2c-AHF* lineage. Immunofluorescence for tdTomato and PECAM (**f-f'**), and tdTomato, PECAM and PROX1 (**g-g'**) on whole mount embryonic cervico-thoracic skin from *Mef2c-AHF*$^{Cre/+}$*;Rosa26*$^{tdtomato}$ embryos. (**h**) Immunofluorescence for tdTomato, PDPN and CDH5 on cryosectioned liver tissue from an adult *Pax3*$^{Cre/+}$*;Rosa26*$^{tdtomato}$ mouse. (**i-i'**) Immunofluorescence for tdTomato, LYVE1 and PECAM on cryosectioned jejunum from an adult *Pax3*$^{Cre/+}$*;Rosa26*$^{tdtomato}$ mouse. Immunofluorescence for tdTomato, PDPN and CDH5 on whole mount ear skin from *Pax3*$^{Cre/+}$*;Rosa26*$^{tdtomato}$ (**j**, **j'**) or *Myf5*$^{Cre/+}$*;Rosa26*$^{tdtomato}$ (**k**, **k'**) mice at post-natal day 21. Scale bars: a-c''', e', g-g' – 50 μm; d-d', g-g', i-i', j-k' – 100 μm; e – 1 mm; f-f' – 200 μm; h – 500 μm.

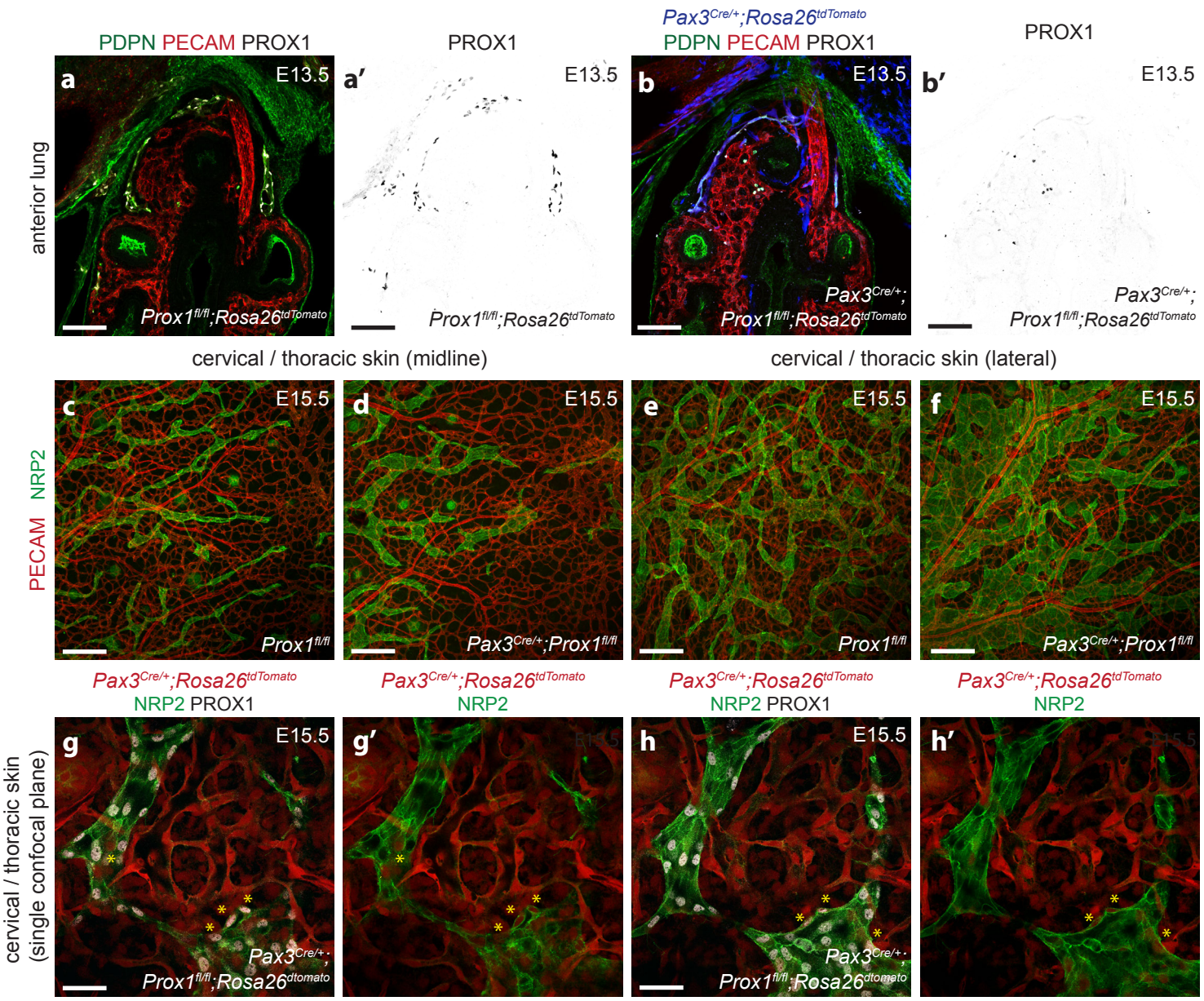
PDPN PECAM PROX1
PROX1
Pax3Cre/+;Rosa26tdTomato
PDPN PECAM PROX1
PROX1
anterior lung
a
E13.5
Prox1fl/fl;Rosa26tdTomato
a′
E13.5
Prox1fl/fl;Rosa26tdTomato
b
E13.5
Pax3Cre/+;
Prox1fl/fl;Rosa26tdTomato
b′
E13.5
Pax3Cre/+;
Prox1fl/fl;Rosa26tdTomato
cervical / thoracic skin (midline)
cervical / thoracic skin (lateral)
PECAM NRP2
c
E15.5
Prox1fl/fl
d
E15.5
Pax3Cre/+;Prox1fl/fl
e
E15.5
Prox1fl/fl
f
E15.5
Pax3Cre/+;Prox1fl/fl
Pax3Cre/+;Rosa26tdTomato
NRP2 PROX1
Pax3Cre/+;Rosa26tdTomato
NRP2
Pax3Cre/+;Rosa26tdTomato
NRP2 PROX1
Pax3Cre/+;Rosa26tdTomato
NRP2
cervical / thoracic skin
(single confocal plane)
g
E15.5
Pax3Cre/+;
Prox1fl/fl;Rosa26dtomato
g′
h
E15.5
Pax3Cre/+;
Prox1fl/fl;Rosa26dtomato
h′

**Figure S4: Related to Figure 4. Analysis of *Prox1* deletion in the paraxial mesoderm derived cells.**

Immunofluorescence for tdTomato, PDPN, PECAM and PROX1 on sagittal vibratome sections at the level of the cardiac outflow tract from $Prox1^{fl/fl};Rosa26^{tdTomato}$ (**a-a'**) and $Pax3^{Cre/+};Prox1^{fl/fl};Rosa26^{tdTomato}$ embryos (**b-b'**). Immunofluorescence for PECAM and NRP2 on whole mount cervical/thoracic skin from $Prox1^{fl/fl}$ and $Pax3^{Cre/+};Prox1^{fl/fl}$ embryos, at midline (**c-d**) and lateral positions (**e-f**). Immunofluorescence for tdTomato, NRP2 and PROX1 on whole mount cervical/thoracic skin from $Pax3^{Cre/+};Prox1^{fl/fl}$ embryos (**g-h'**) - yellow asterisks highlight $PROX1^{+}$ cells that are labeled by $Pax3^{Cre/+};Rosa26^{tdtomato}$ in the cervical/thoracic skin. Scale bars: a, b, d, e, l, m– 50 μm; c, l-m – 100 μm; h-k – 200 μm.