

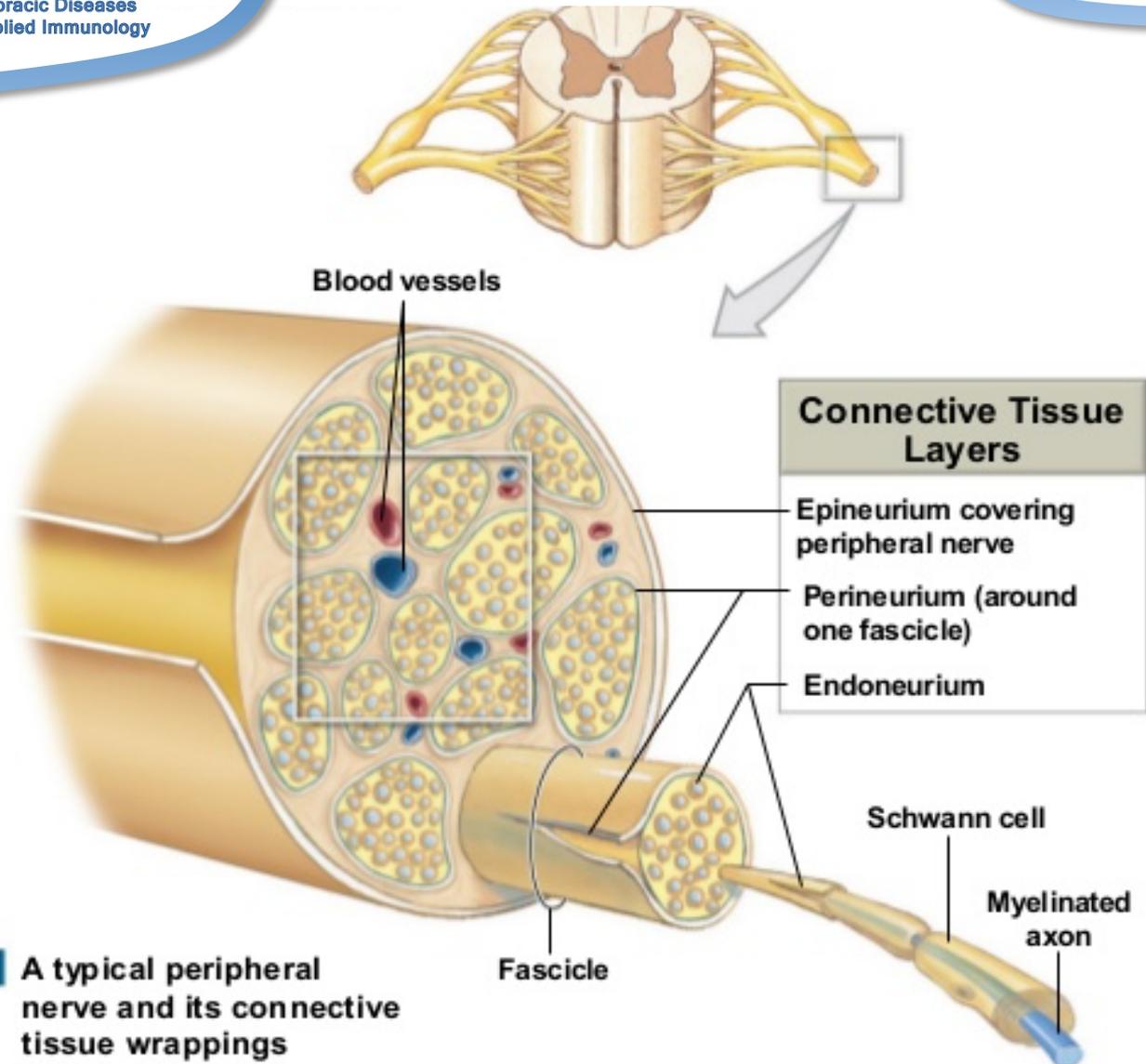
Macrophage-Induced Blood Vessels Guide Schwann Cell-Mediated Regeneration of Peripheral Nerves

Cattin AL et al.

Cell. 2015 Aug 27;162(5):1127-39

Overview

- Regeneration in peripheral nervous system
(**PNS**)
- Blood vessels
- Macrophages
- Schwann cells (SC)



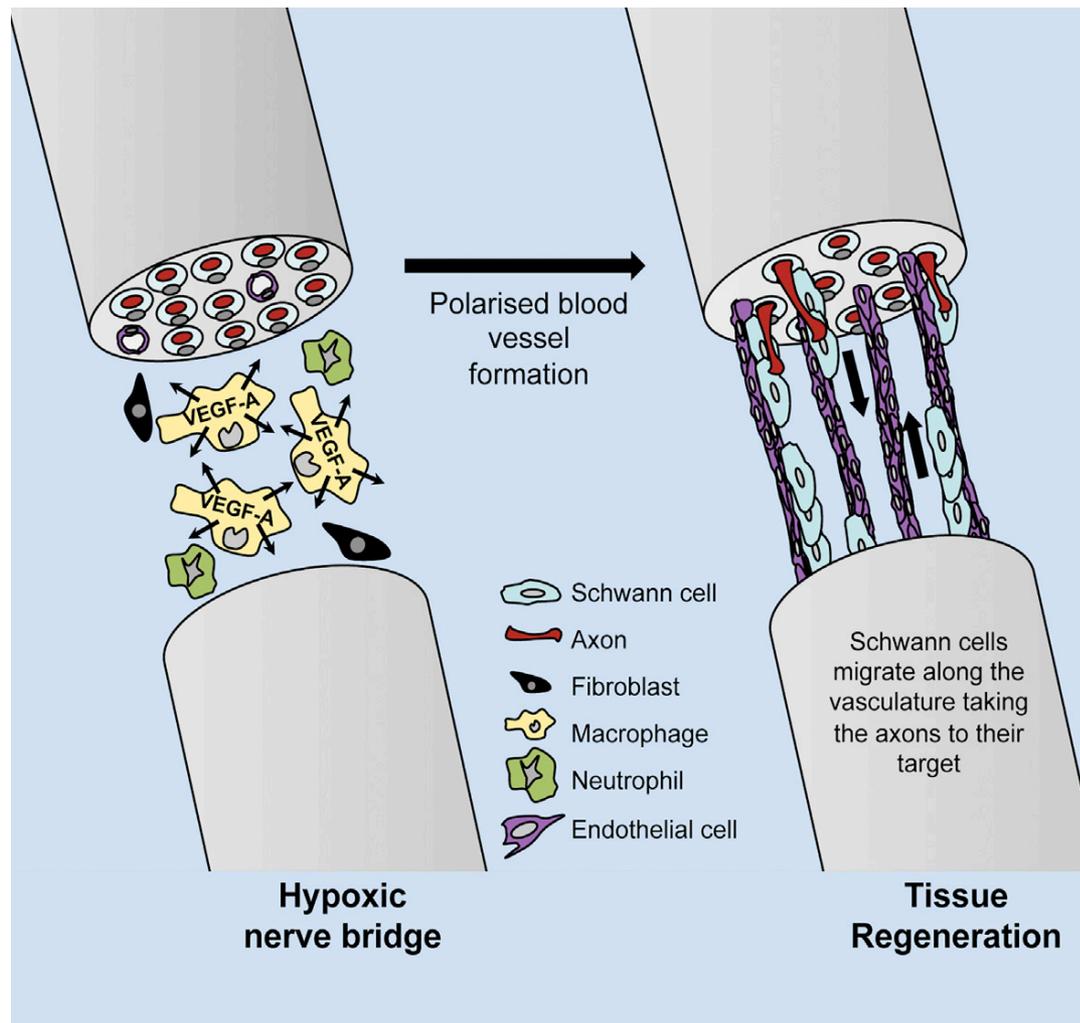
a A typical peripheral nerve and its connective tissue wrappings

„The bridge“

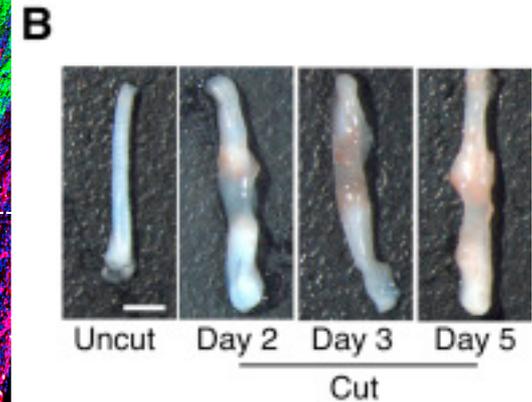
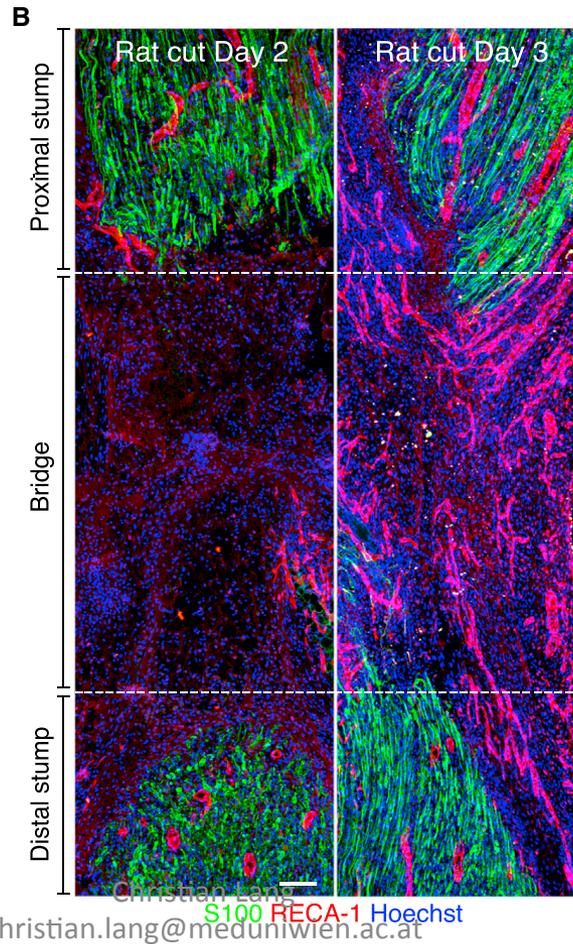
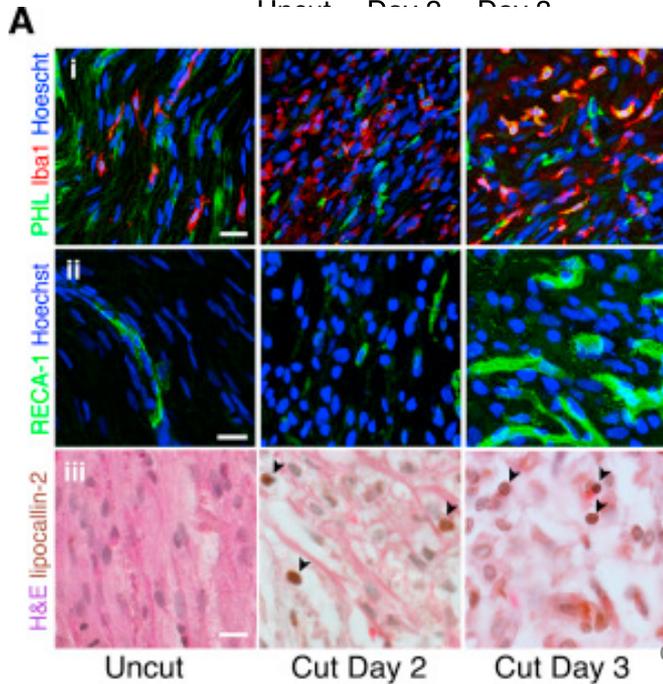
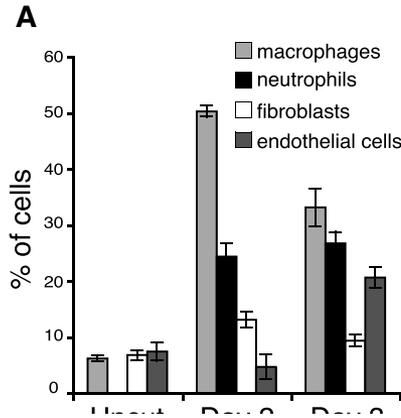
- SCs → guiding axons (proximal → distal)
 - „Organized“ migration via EphrinB/EphB2 signaling
- but: **How do SCs find their way across the bridge?**
 - **Macrophages** sensitize Hypoxia --> **VEGF-A** secretion & polarizing vasculature
 - SCs use newly formed vasculature as **tracks**



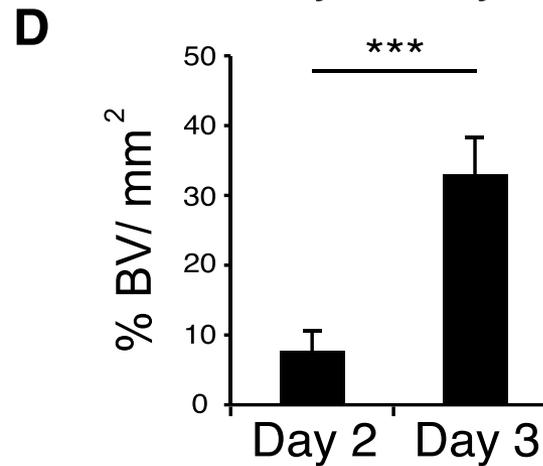
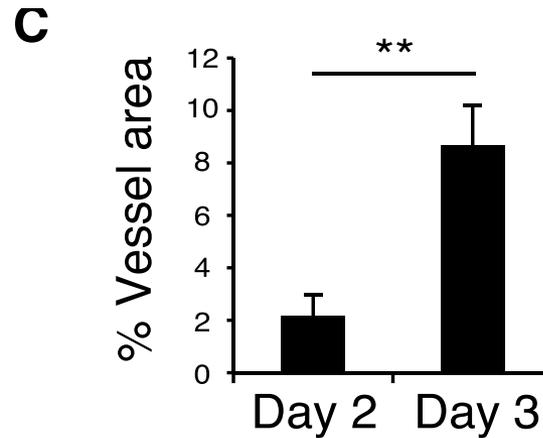
„The bridge“



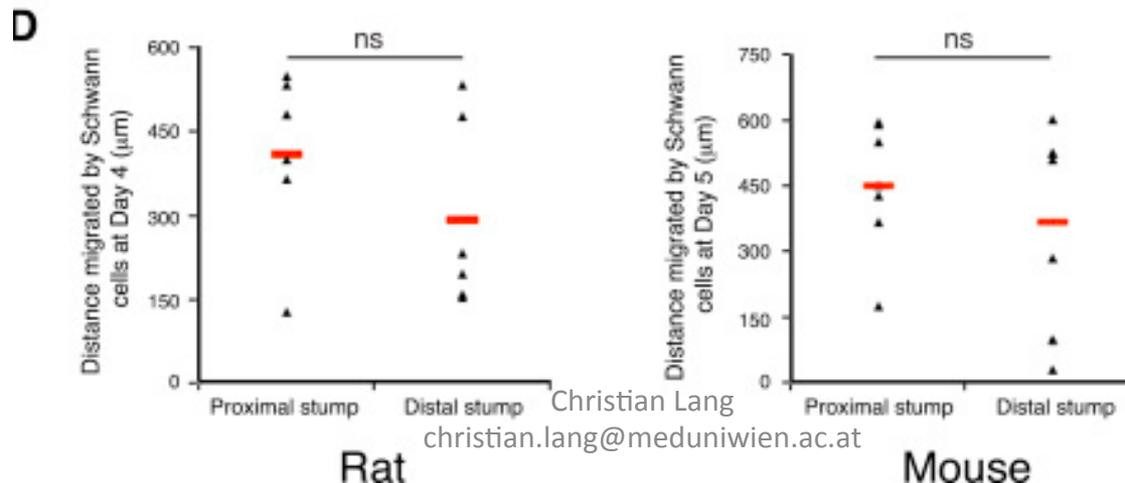
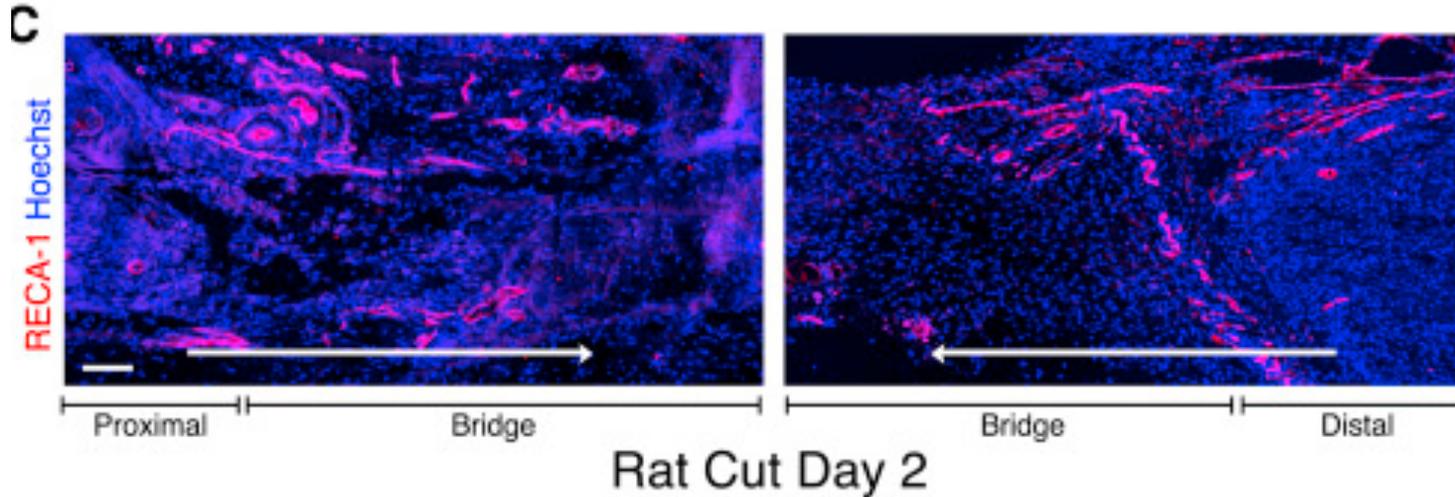
Cell composition of bridge



Cell composition of bridge

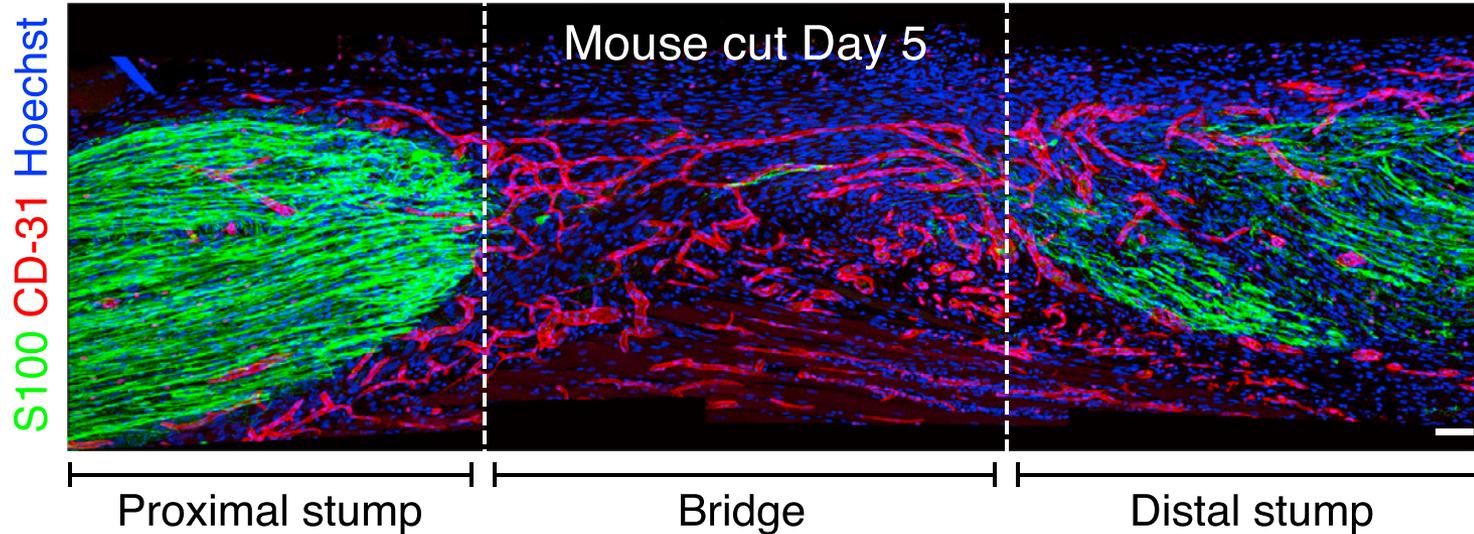


Cell composition of bridge

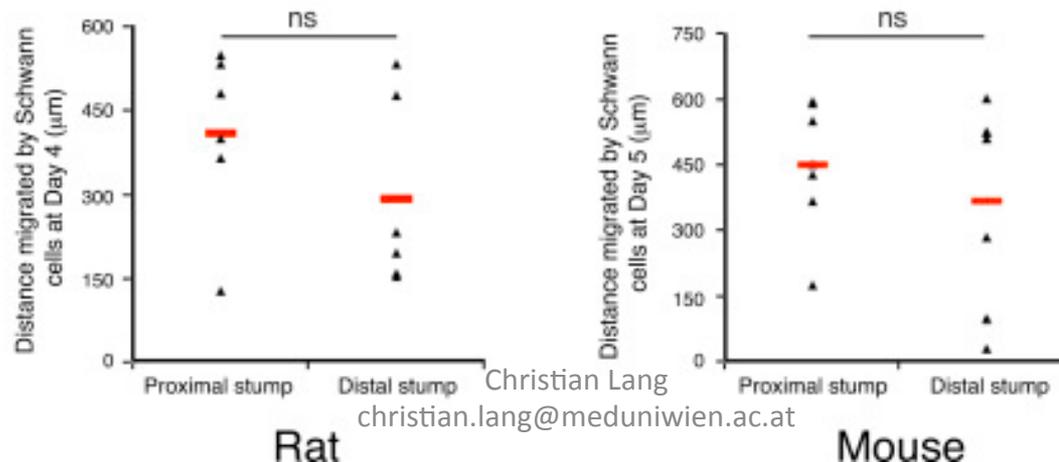


Cell composition of bridge

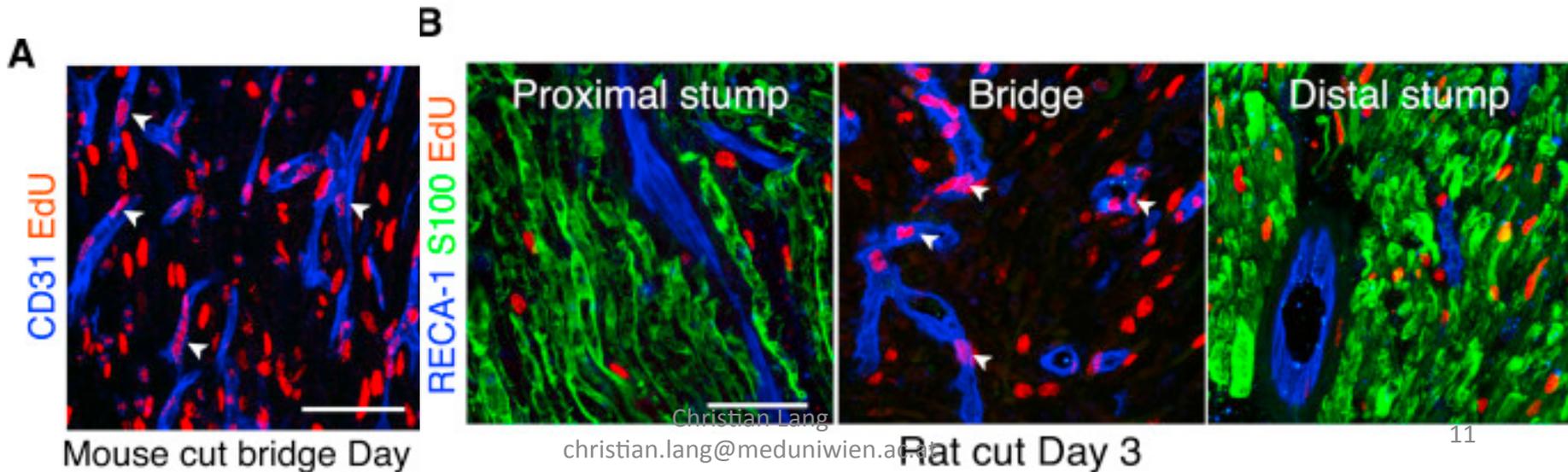
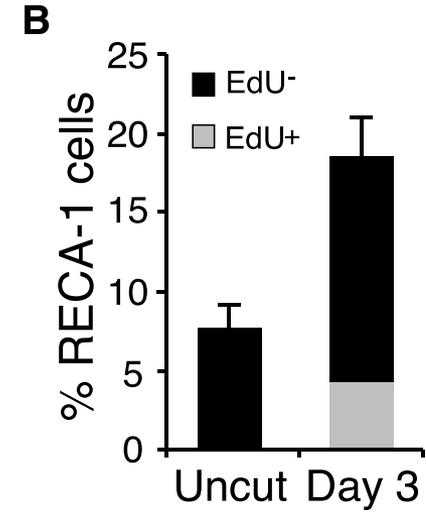
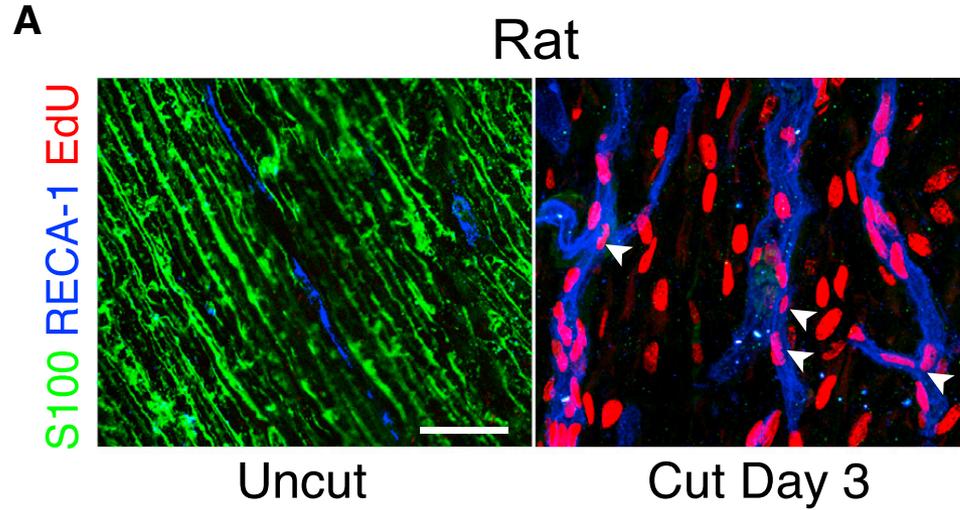
E



D



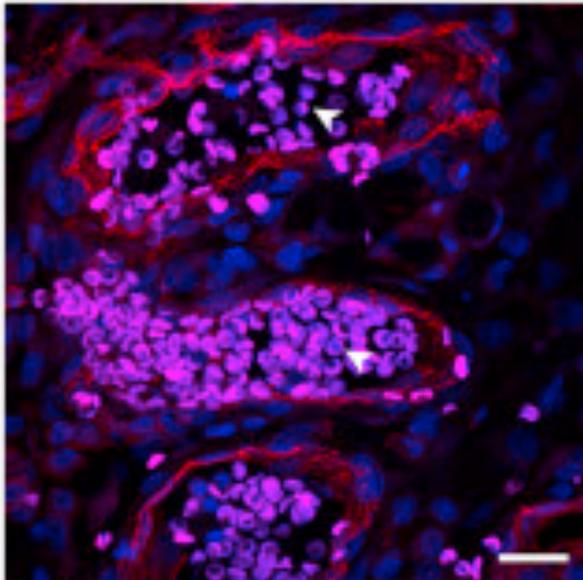
- After day 2 → significant **vascularization (ECs)**
- ECs cross bridge **prior** to SCs





C

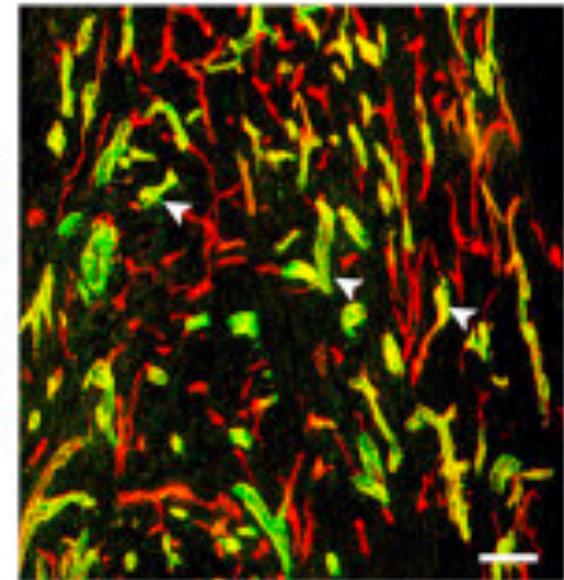
Hoechst RECA-1



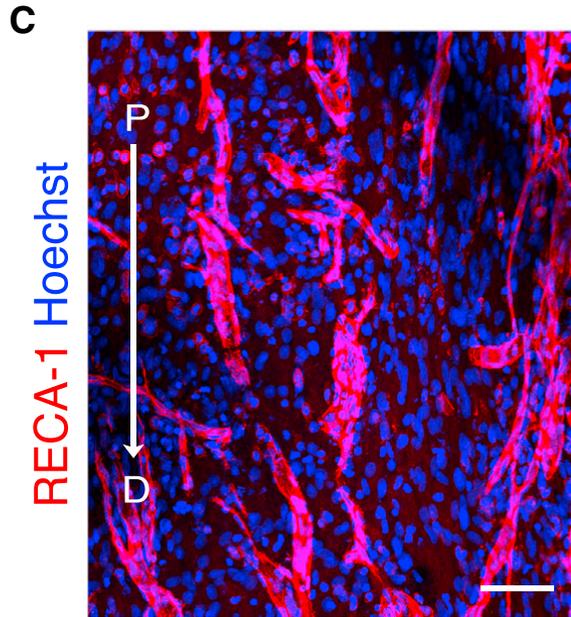
Rat cut bridge Day 3

D

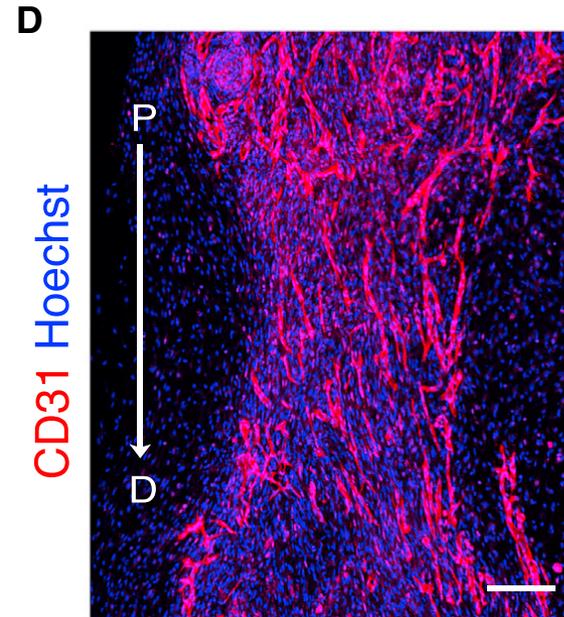
Lectin CD31



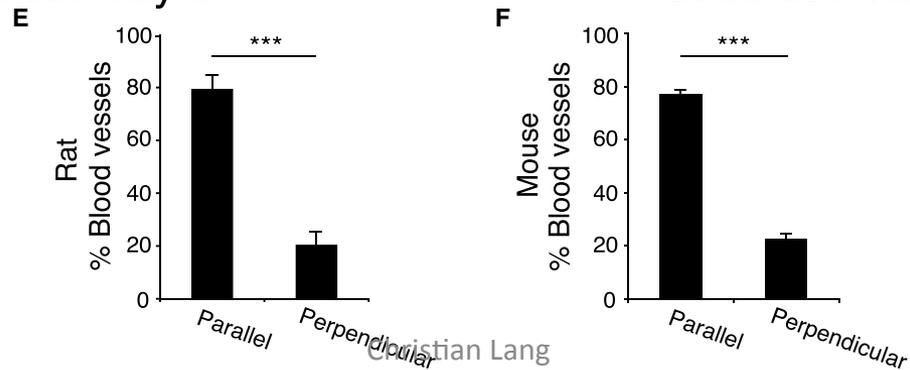
Mouse cut bridge Day 5



Rat cut Day 3

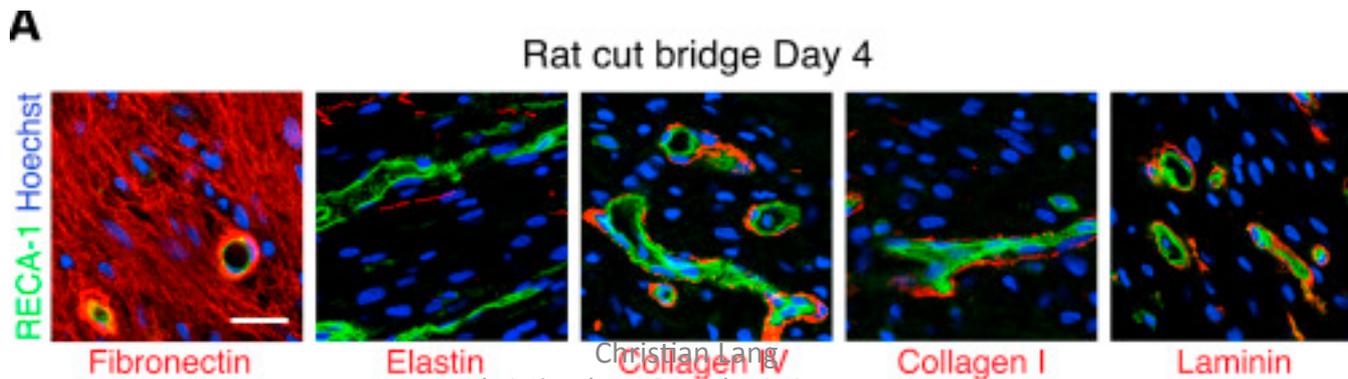
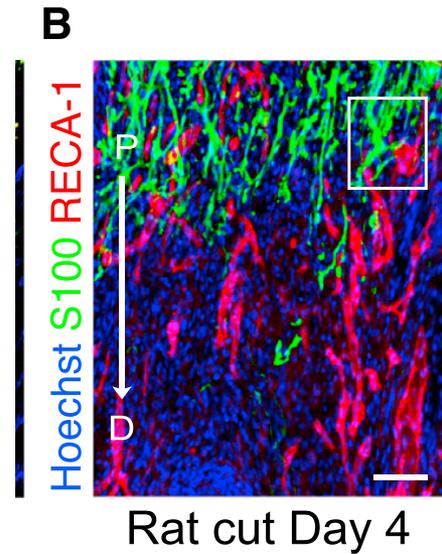
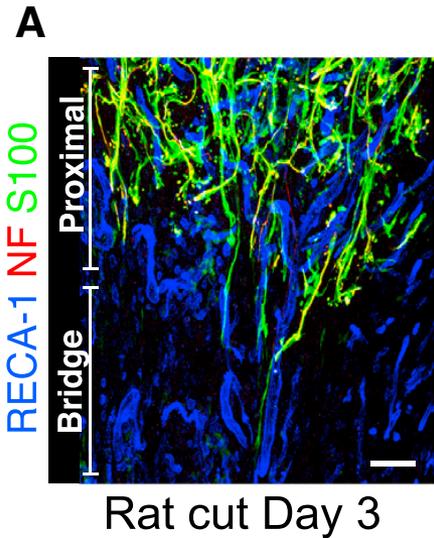


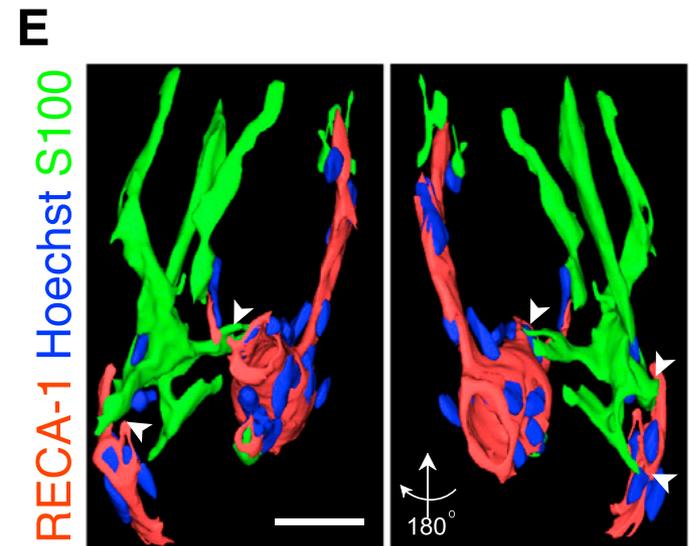
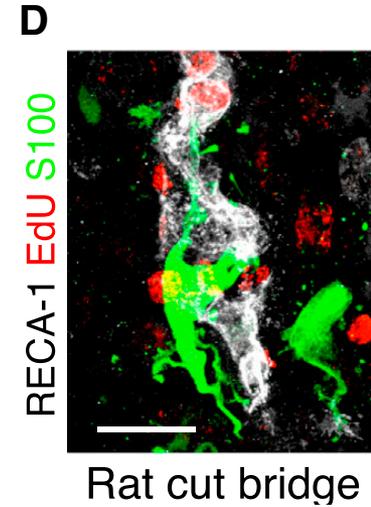
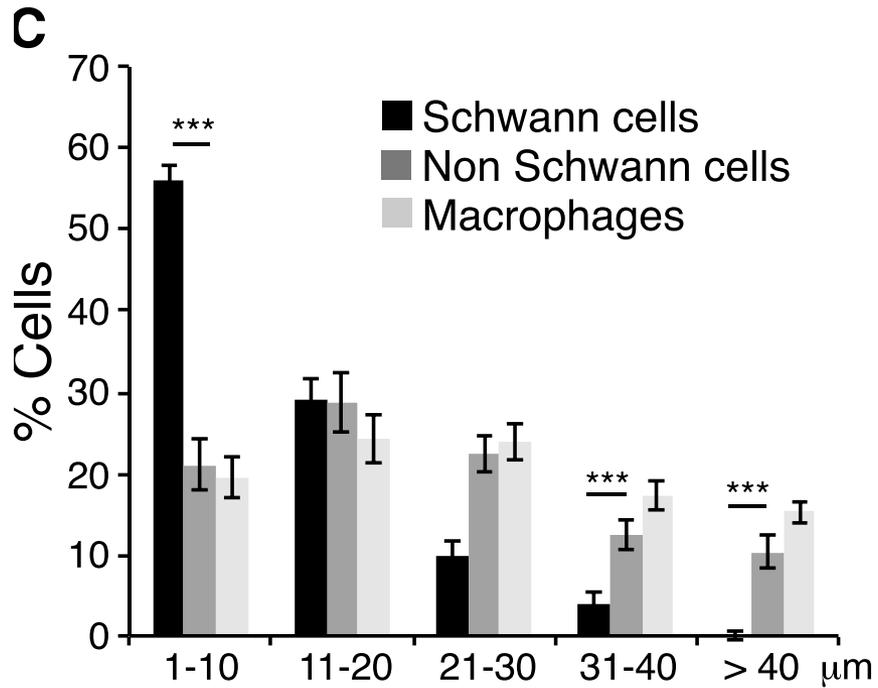
Mouse cut Day 5



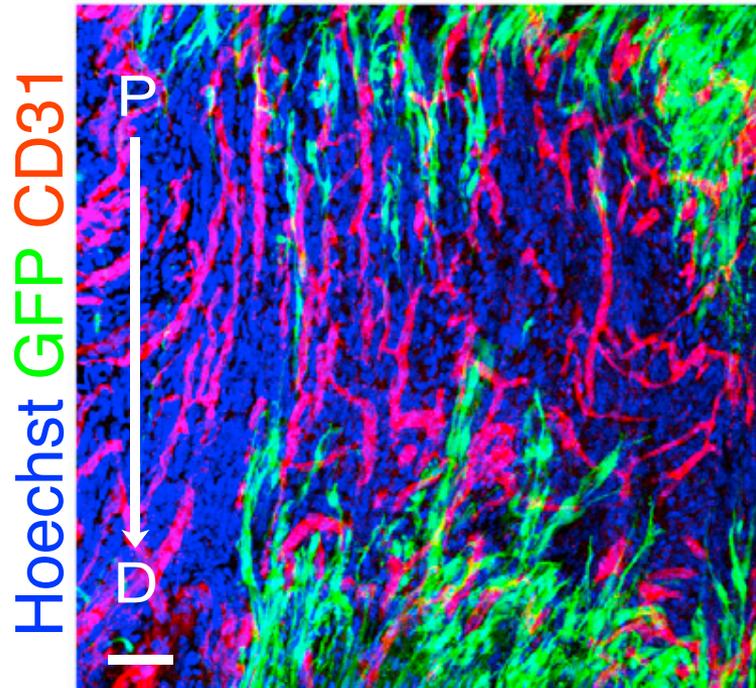
- Blood vessels **prior** to SC migration

Do blood vessels provide
directional signals
to SCs?



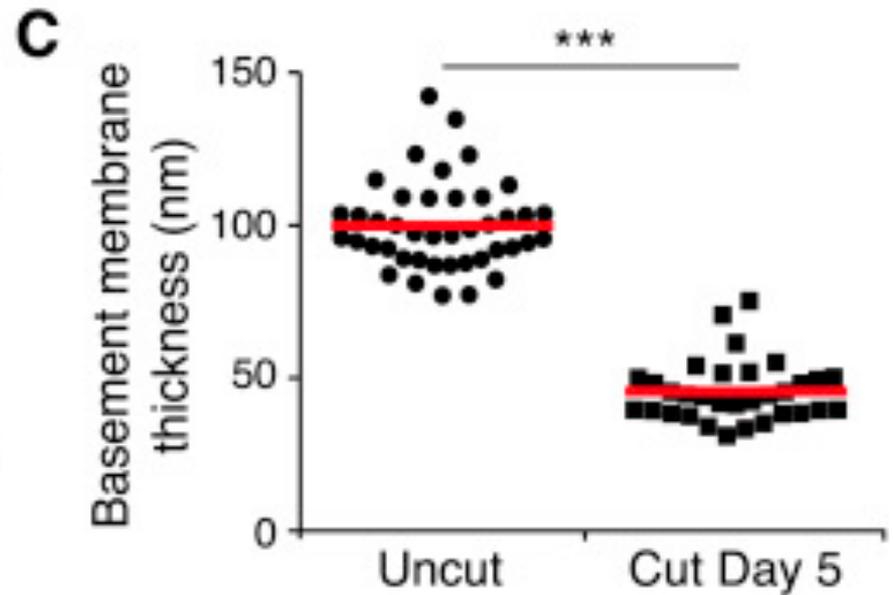
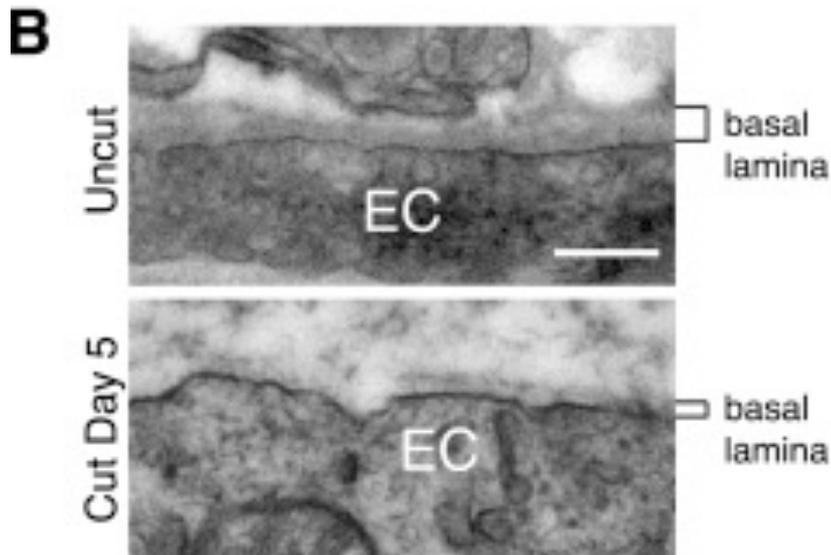


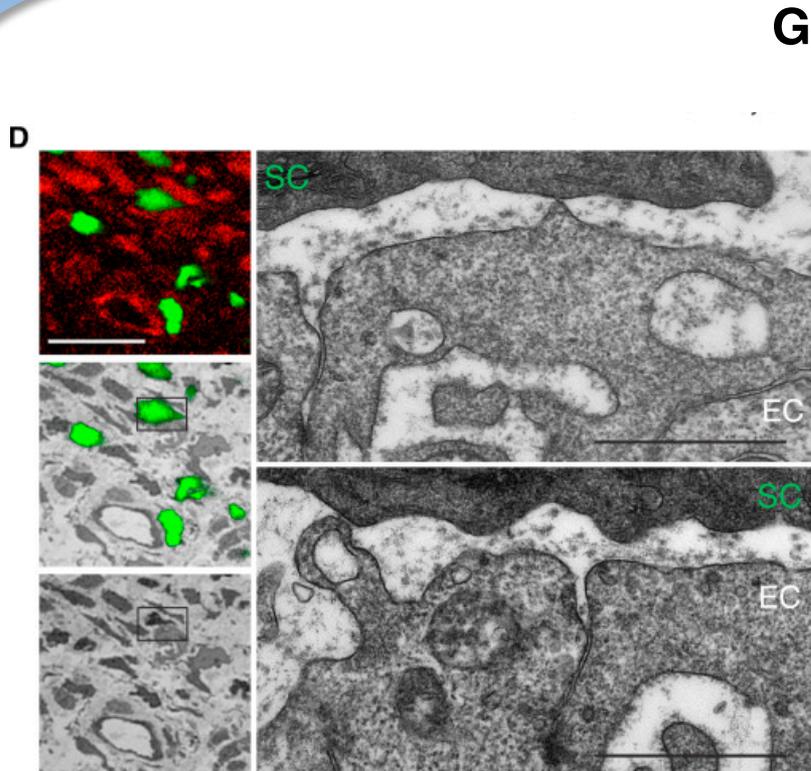
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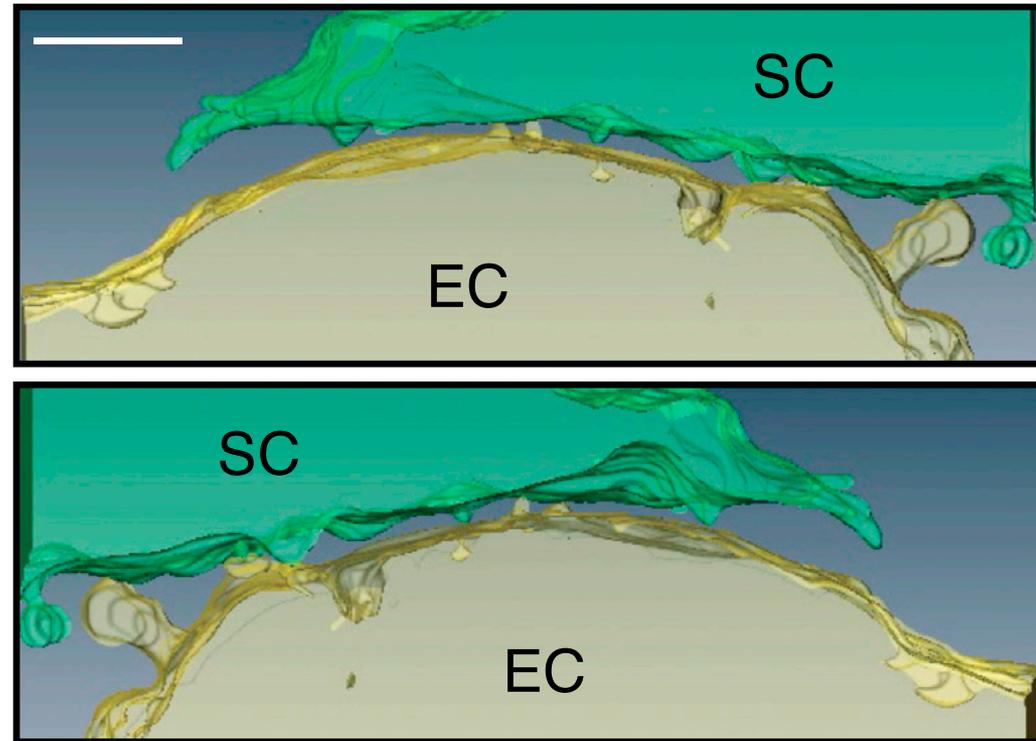
Mouse cut Day 5

- Transgenic mouse with GFP in SC





G



Mouse cut Day 5

TEM...transmission electron microscopy

CLEM...correlative light and electron microscopy
Christian Lang

christian.lang@meduniwien.ac.at

- **Interaction - SCs and blood vessels**
(rat and mouse)
 - migrating and guiding axons (throughout the bridge)

- interaction **in vitro**:
SCs and blood vessels?

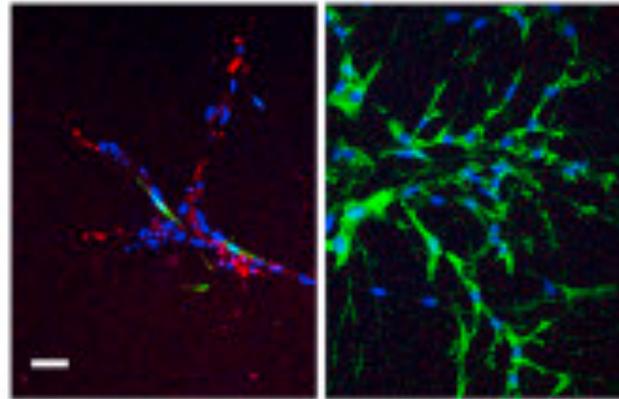


GFP+ rat SC and human umbilical vein
endothelial cells (**HUVEC**) in fibrin matrix



C

CD31 Hoechst

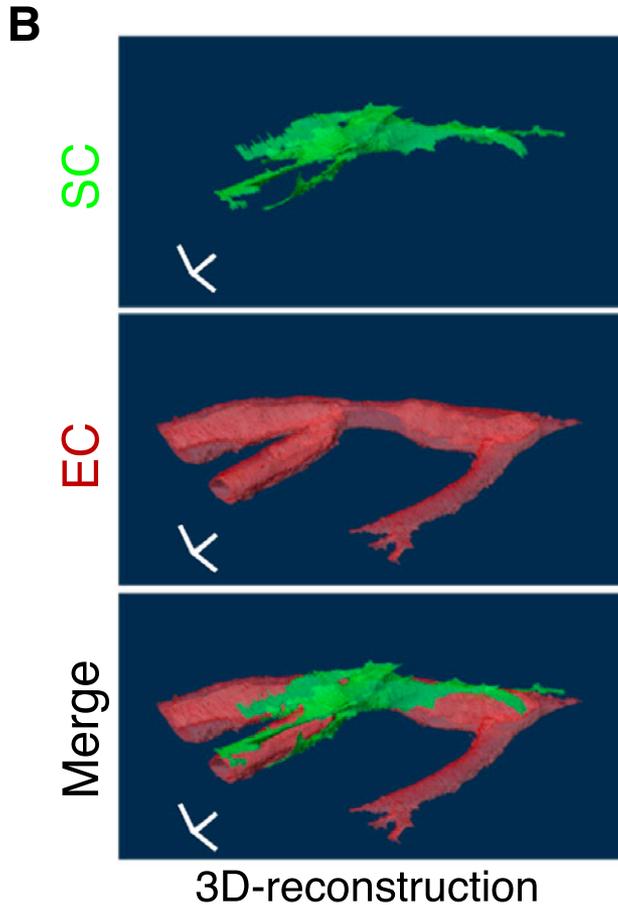


GFP SC

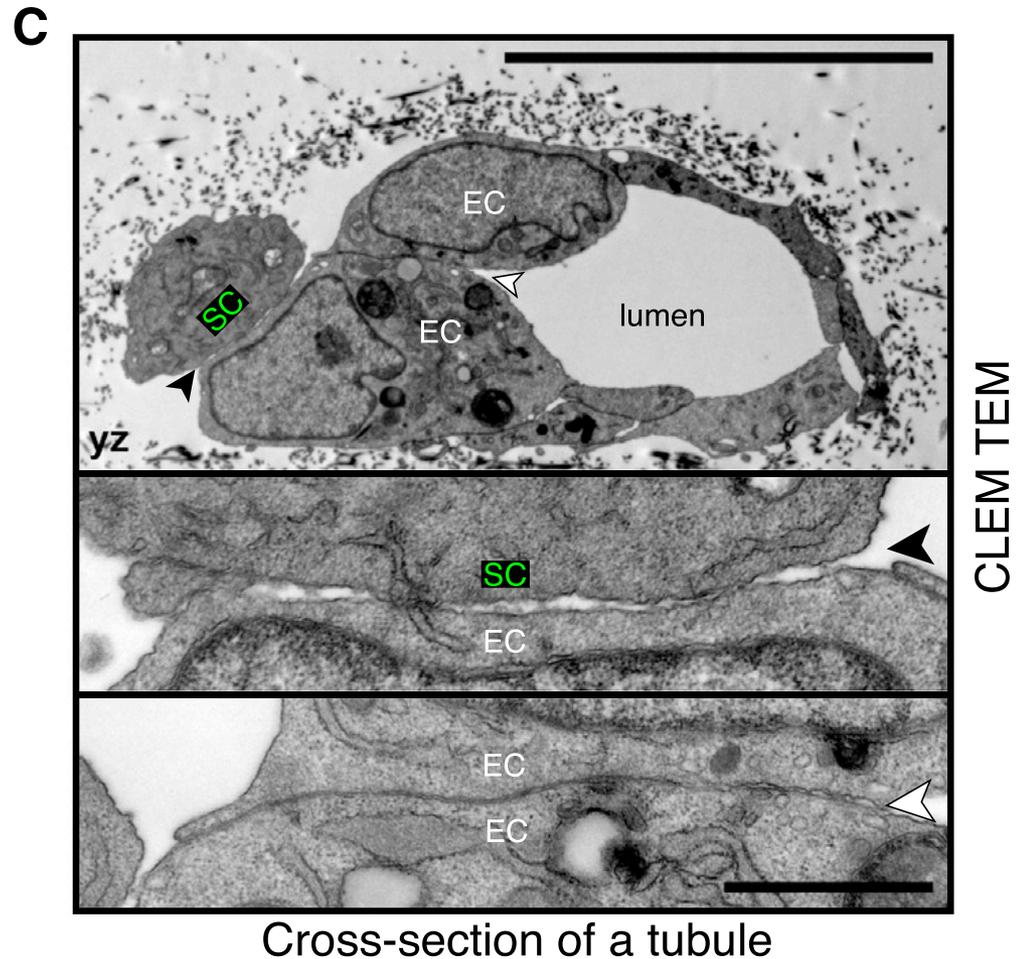
GFP Fib

- **SCs unable to migrate unless associated with EC tubules**
 - *vs. fibroblasts*

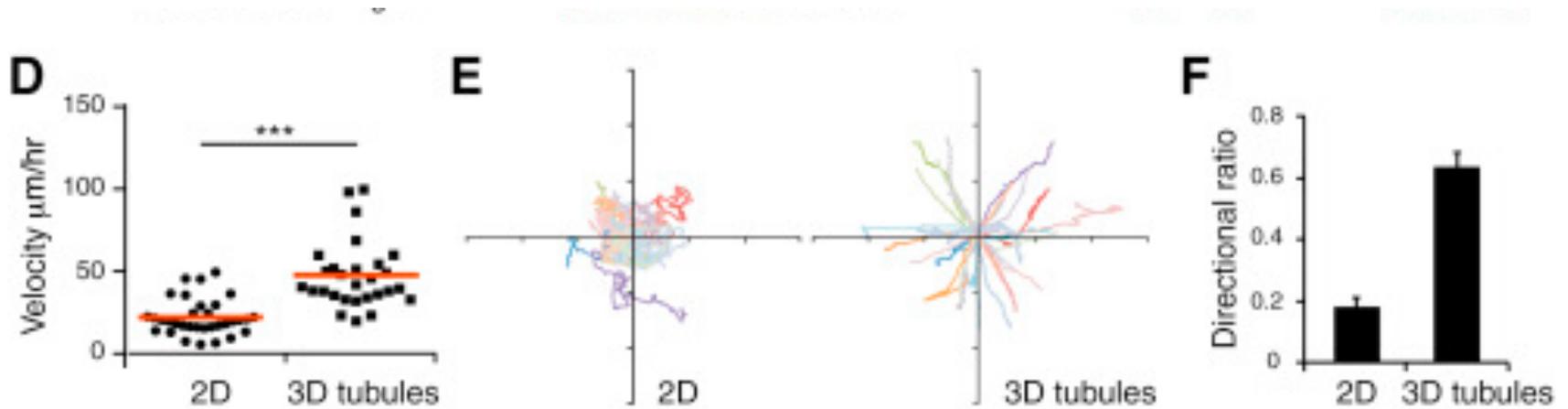
Nature of interaction

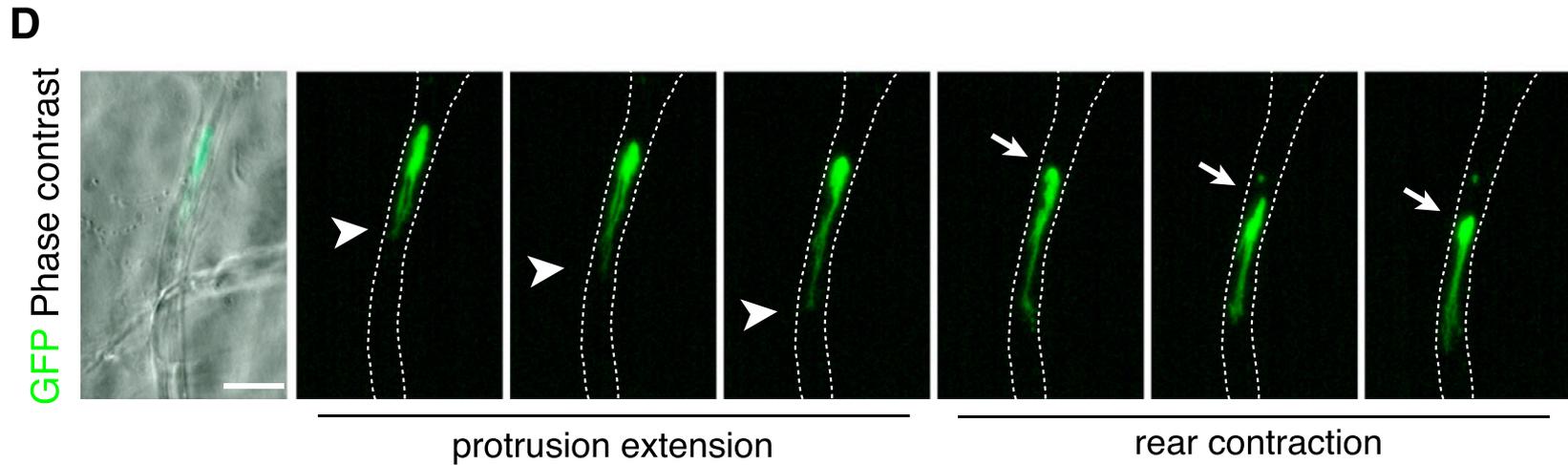


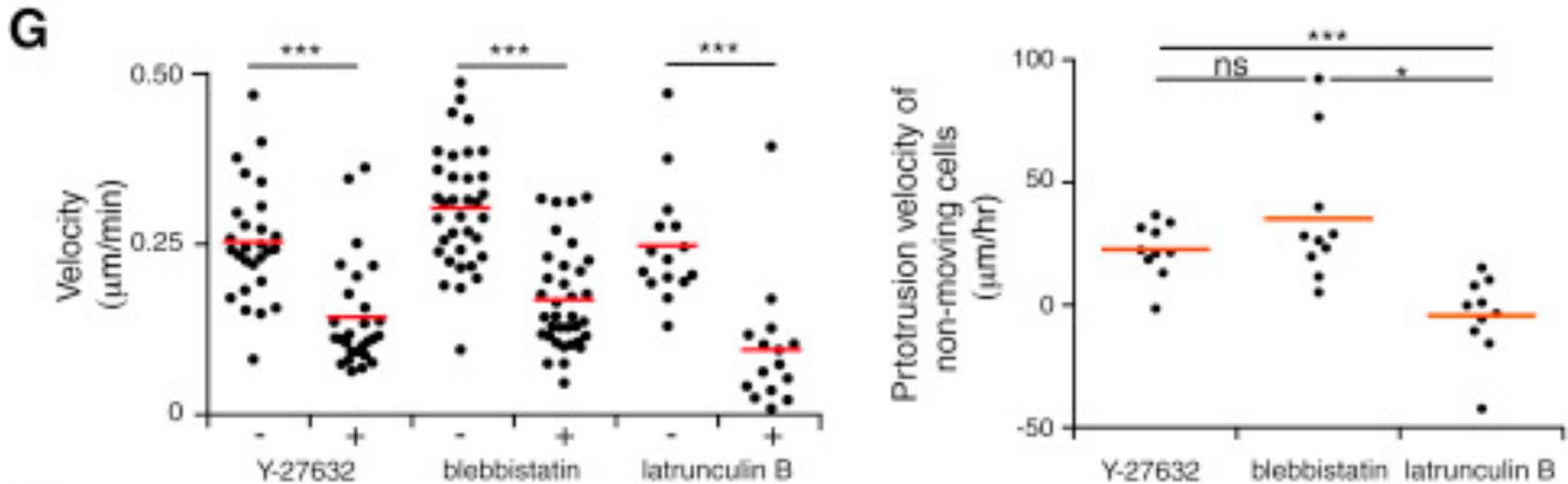
CLEM SBF SEM



Mode of migrating





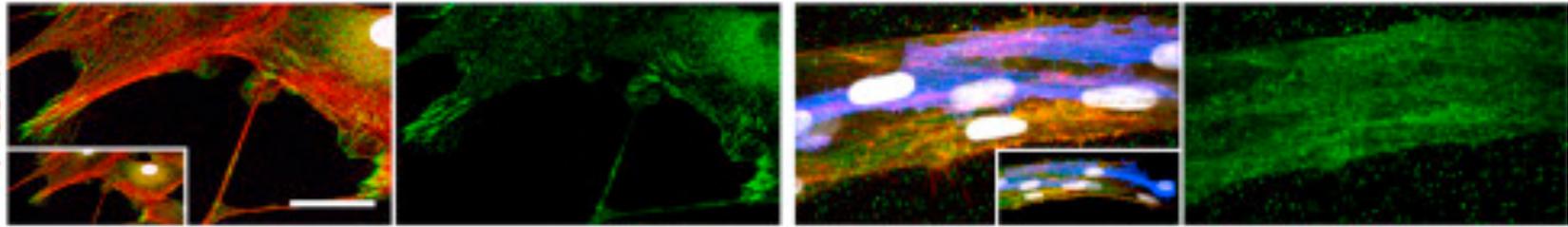


actomyosin contractility is required for migration



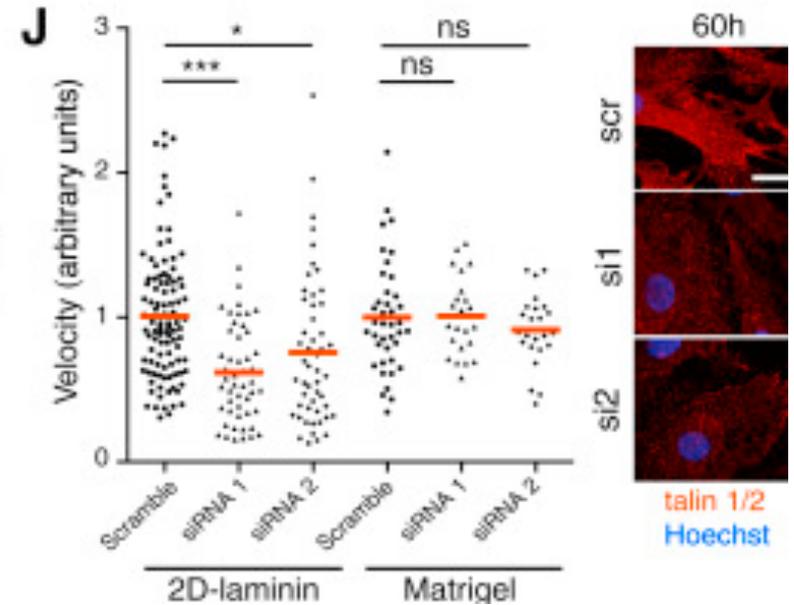
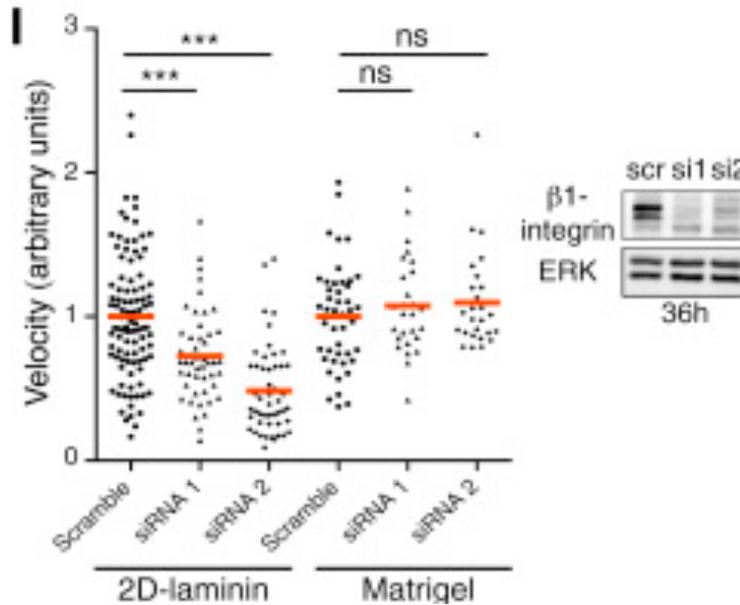
H

GFP Paxillin
F-actin



2D- laminin

3D- fibrin gel



- Blood vessels provide ideal surface for **actomyosin-driven, amoeboid-like SCs migration (3D)**

Summary of the first part

1. blood vessels within the bridge **prior** to SC migration (in vivo)
2. **Interaction** SC – blood vessels (in vivo) → SC **migration** along endothelial cells (in vitro)

Macrophages as sensors for hypoxia

Hypoxia 



HIF 1 alpha 

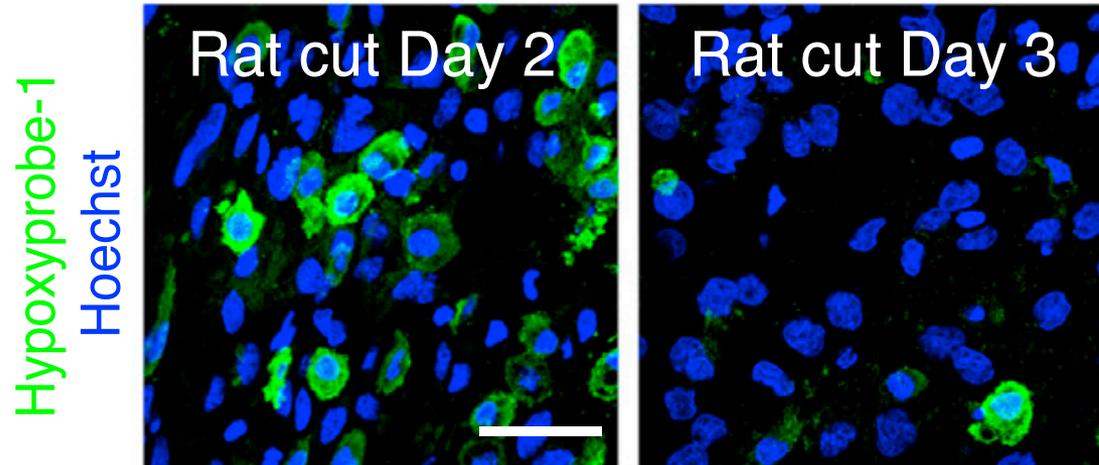


VEGF 

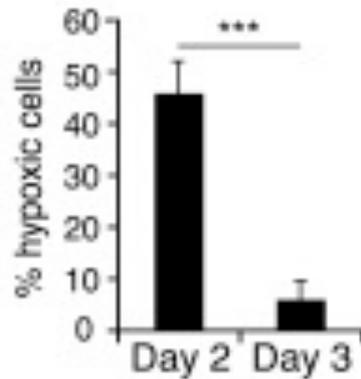


Newly formed blood vessels

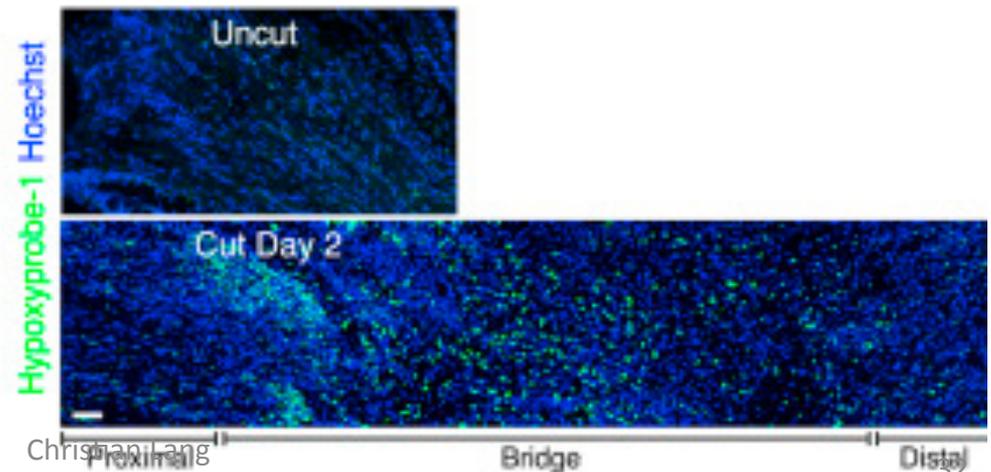
A



A

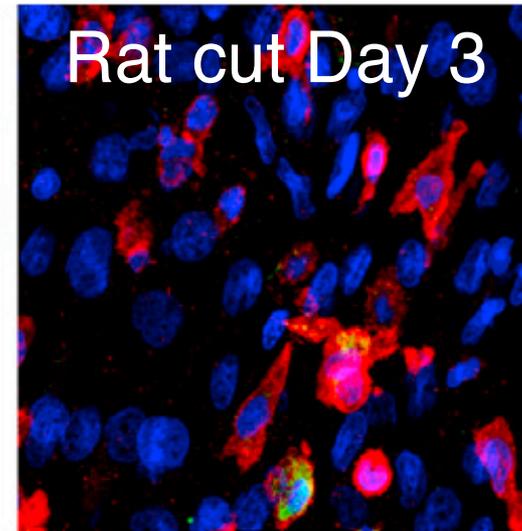
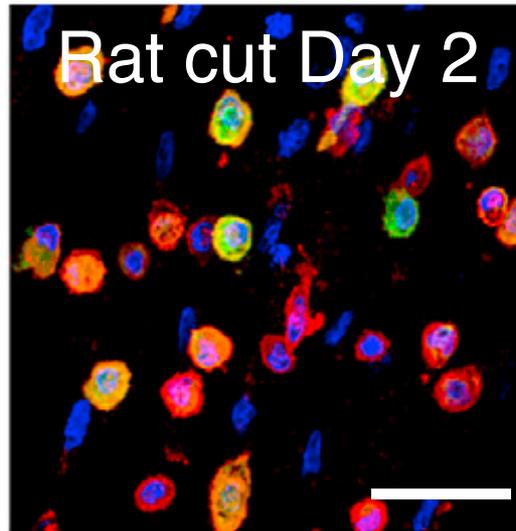


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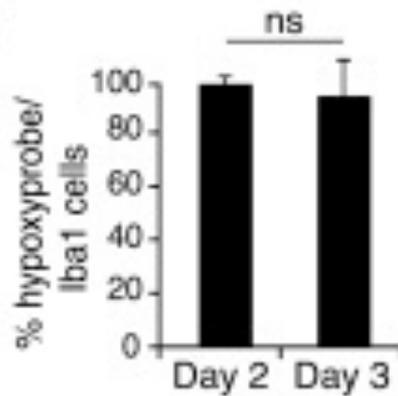


B

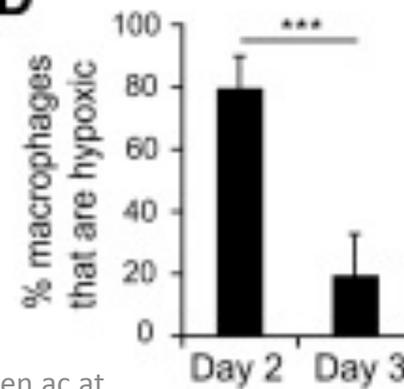
Hypoxyprobe-1
Iba1 Hoechst



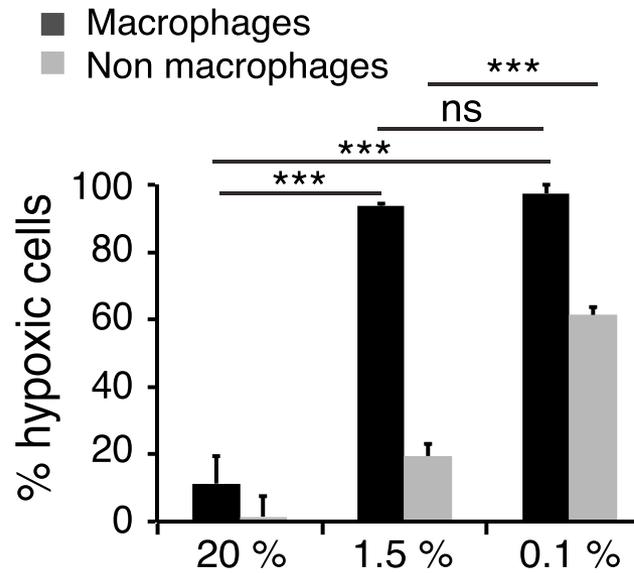
C



D

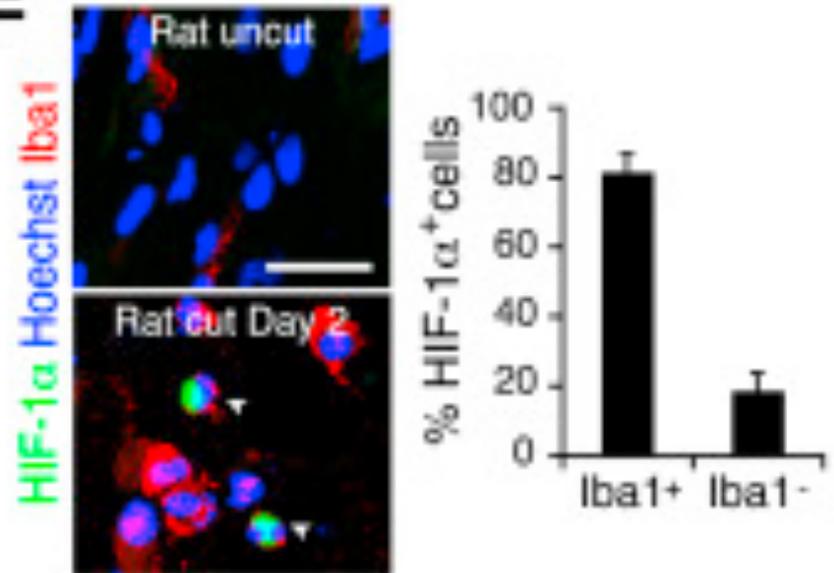


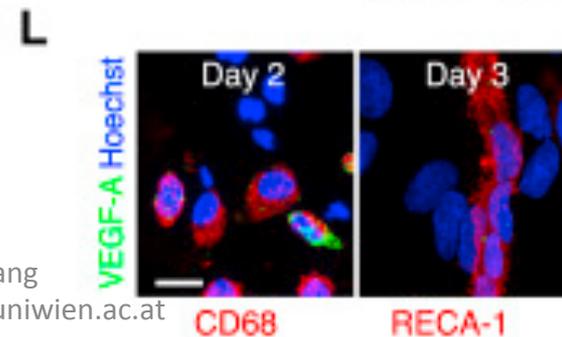
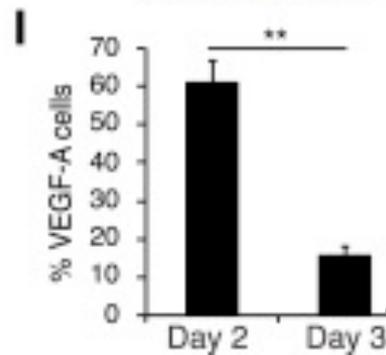
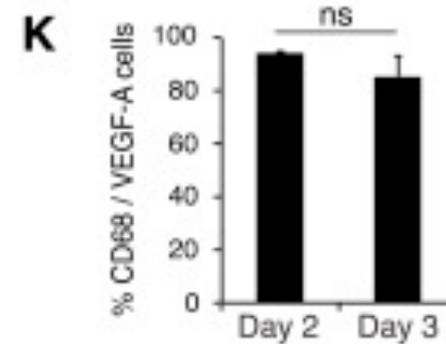
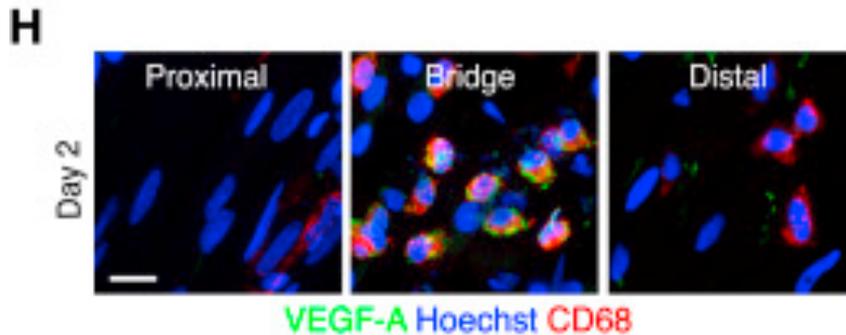
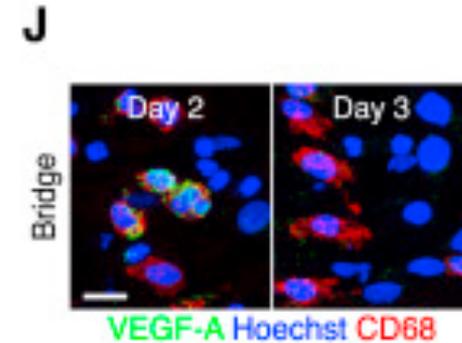
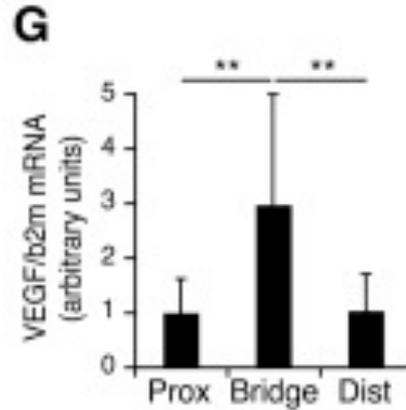
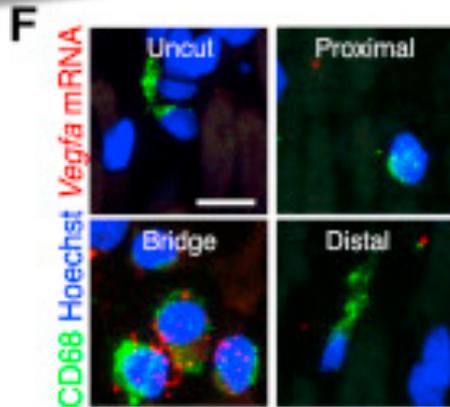
C



In vitro test

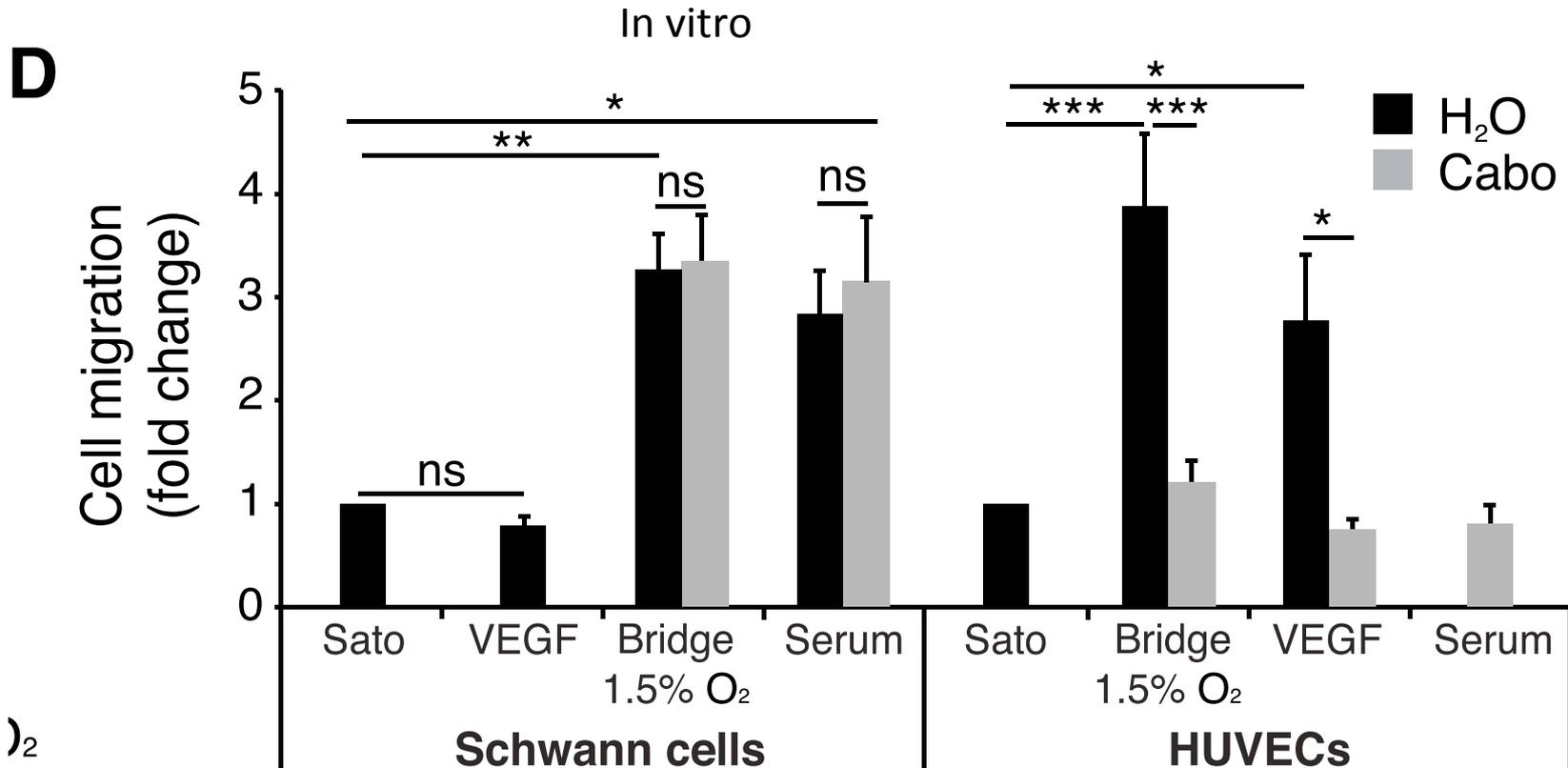
E





- Hypoxia sensitized by macrophages → VEGF-A secretion
- VEGF-A → does is directly attract SCs?

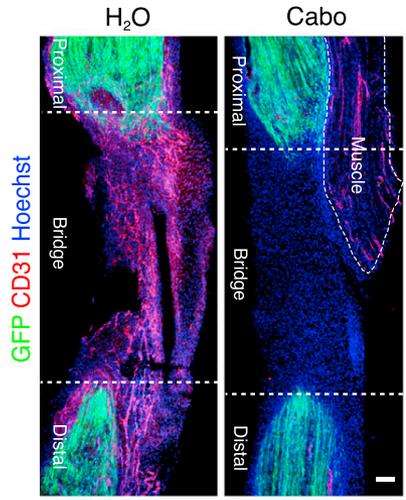
D



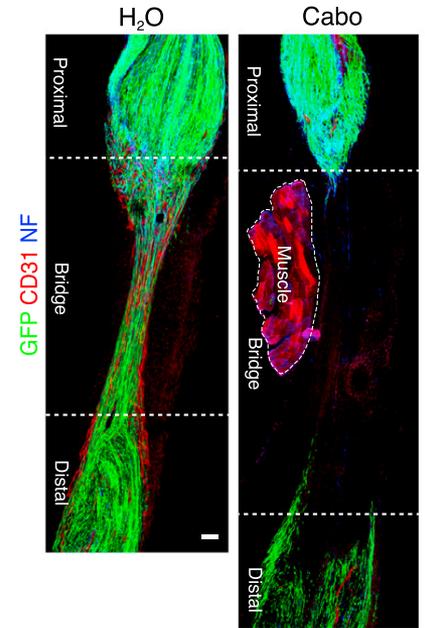
Transwell assay...specific test to study migratory response to angiogenetic inducers

In vivo

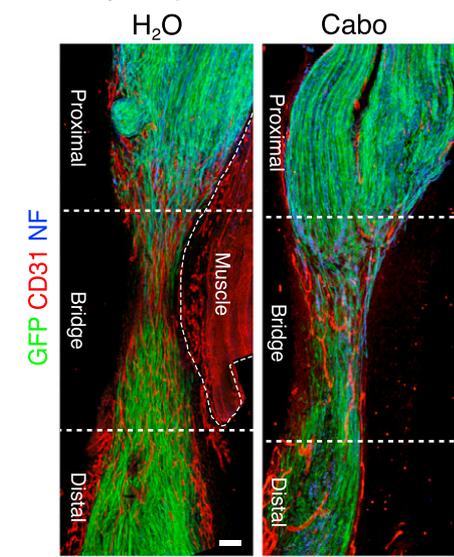
E Day 5 - pre-vascularisation



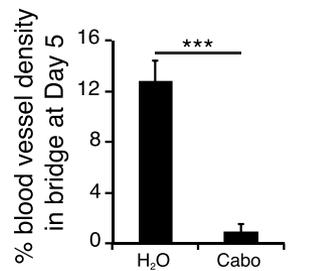
F Day 7 - pre-vascularisation



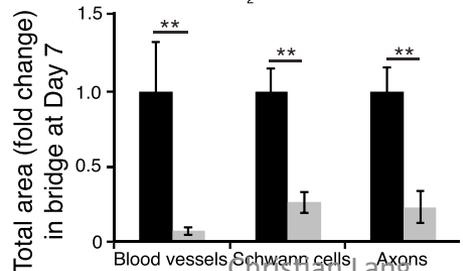
I Day 6 - post-vascularisation



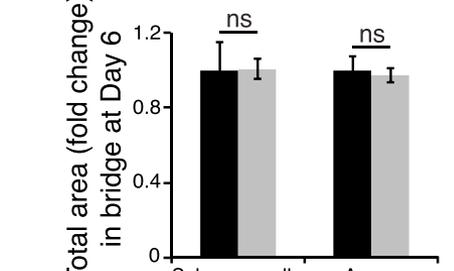
G Day 5 - pre-vascularisation



H Day 7 - pre-vascularisation



J Day 6 - post-vascularisation



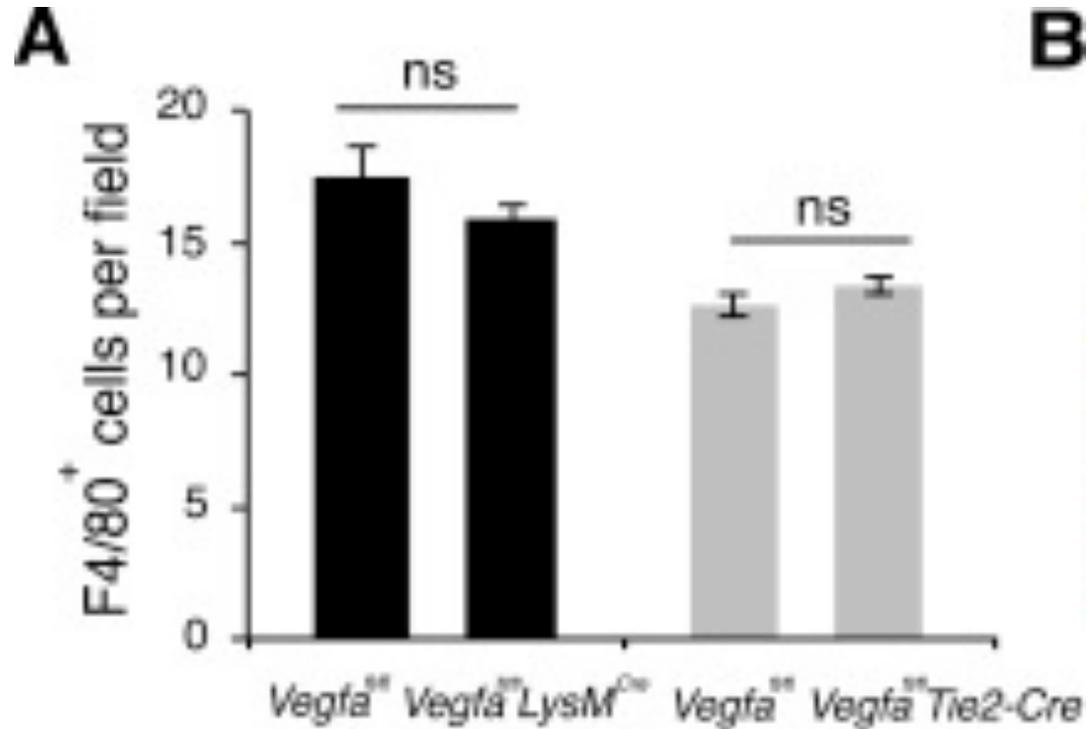
- **Macrophages** sensitize hypoxia → VEGF-A secretion
- VEGF-A is required for ECs crossing
- If blood vessels already formed, VEGF-A is not required for SCs and axons

Importance of **macrophage**-derived VEGF-A in vivo



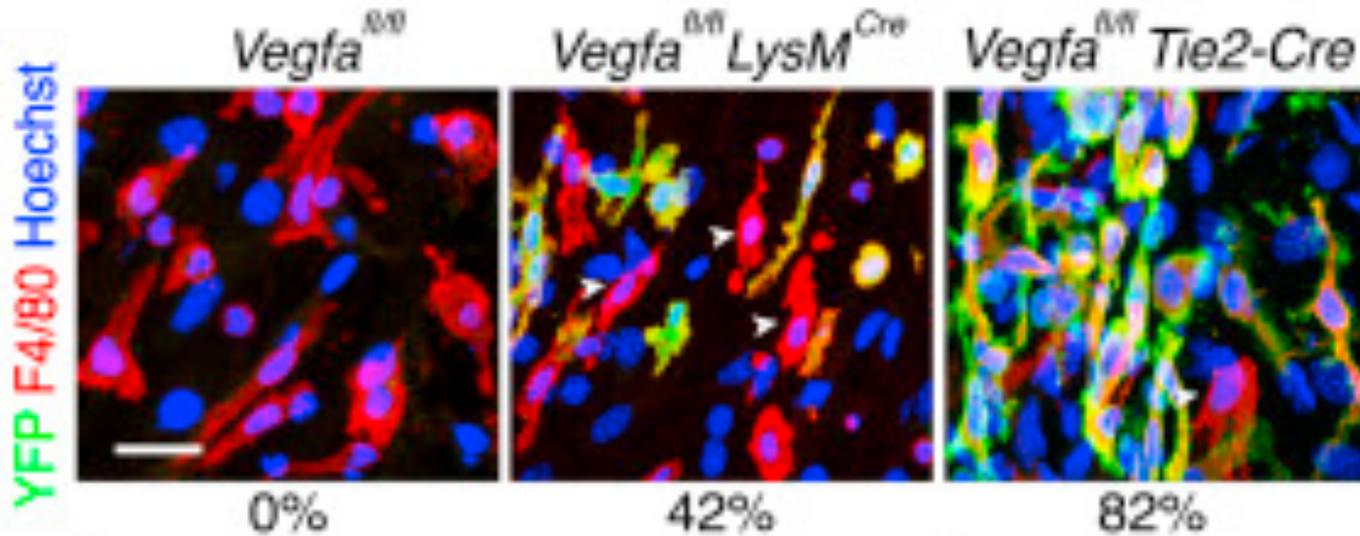
Inactivation of VEGF in macrophages in two mouse models

1. $Vegfa^{fl/fl} Lysm^{Cre}$ → **macrophages** and granulocytes
2. $Vegfa^{fl/fl} Tie2-Cre$ → hematopoietic and **endothelial cells**



Number of macrophages within the bridge , at day 5

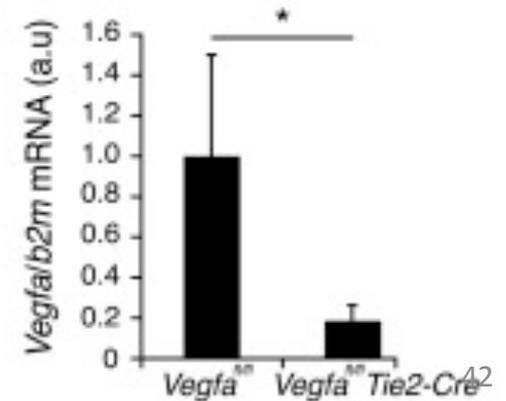
B



Cre

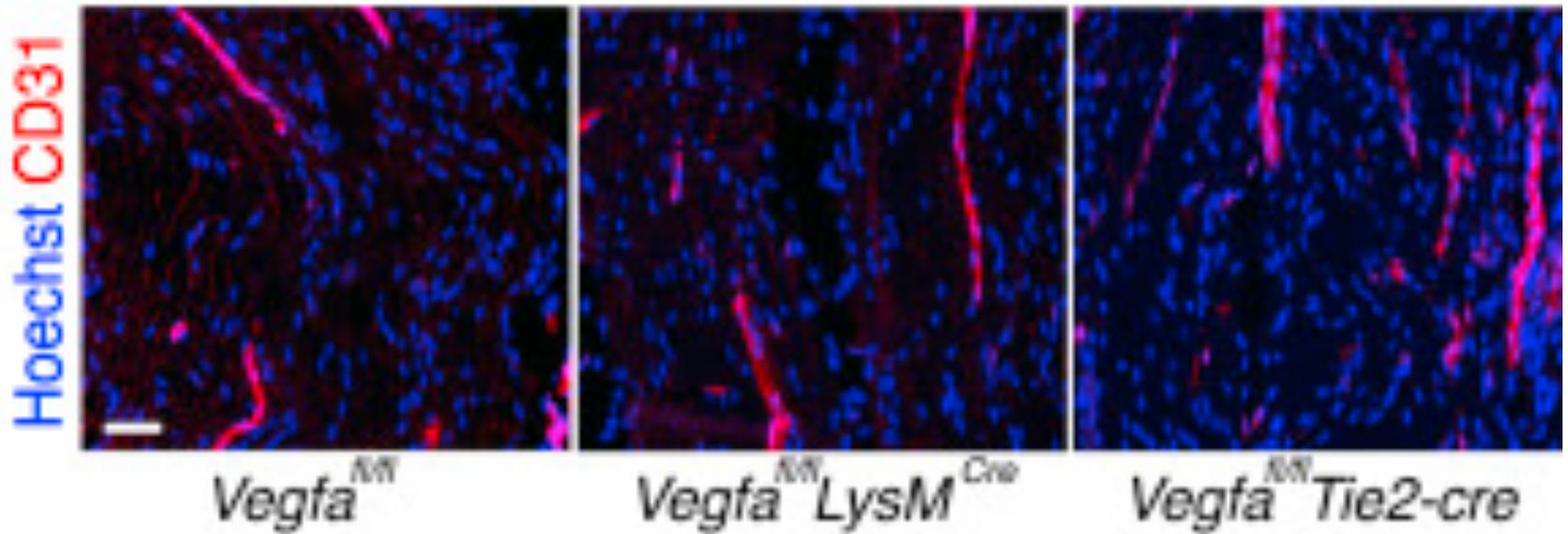
(YFP F4/80⁺) / (F4/80⁺)

C

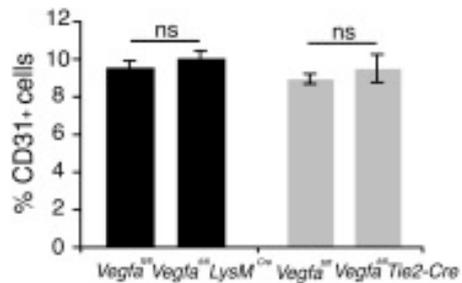


D

Mouse Uncut

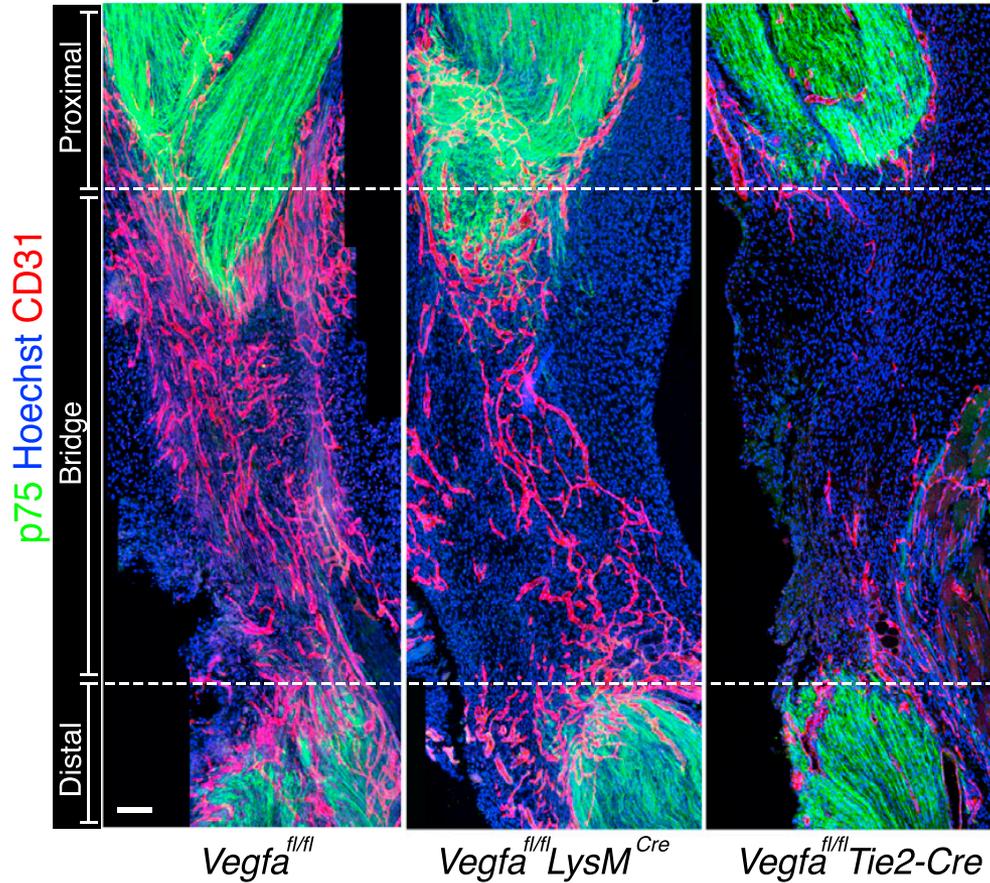


E

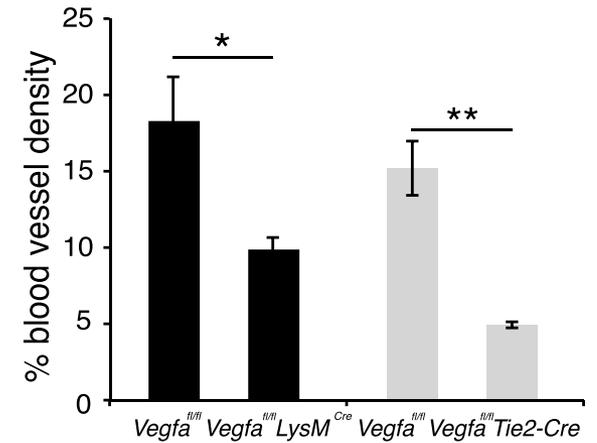


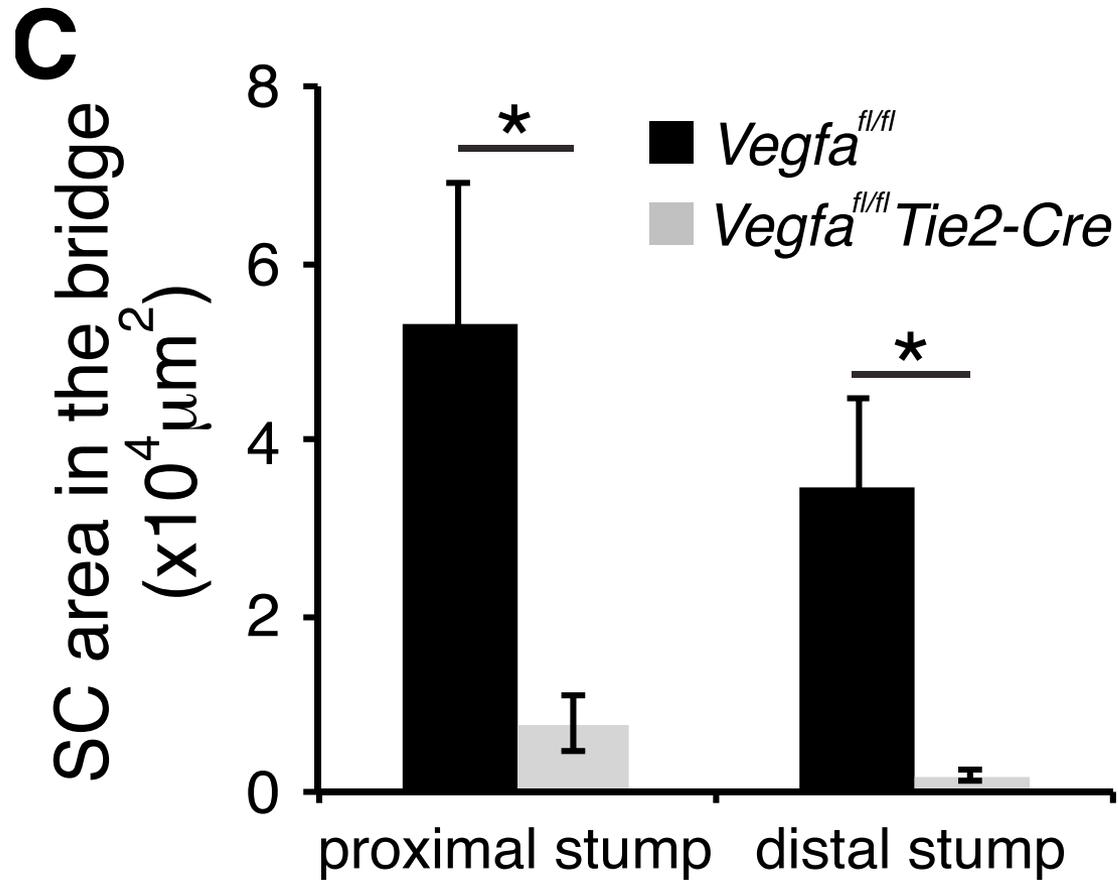
A

Mouse cut Day 5

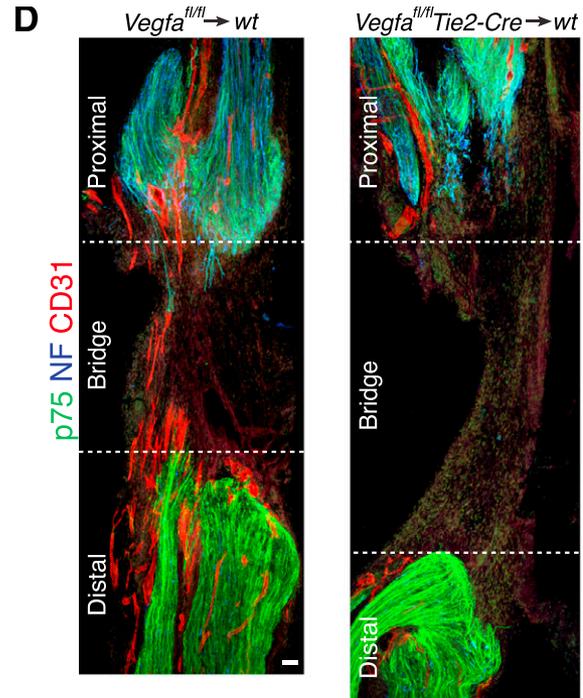


B

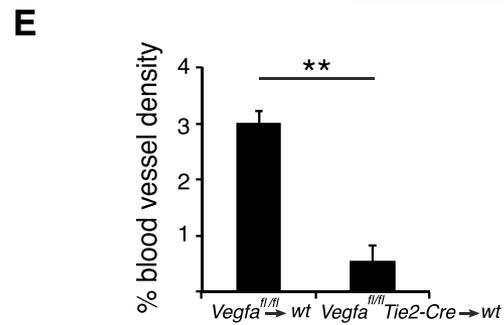




Due to loss of VEGF-A expression in ECs?
→ Bone mark transplantation

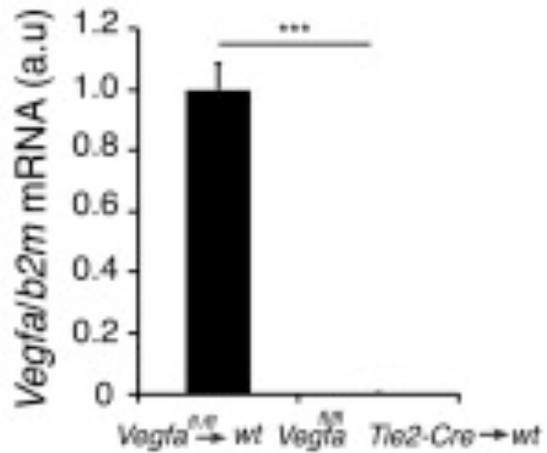


Day 5 after transection

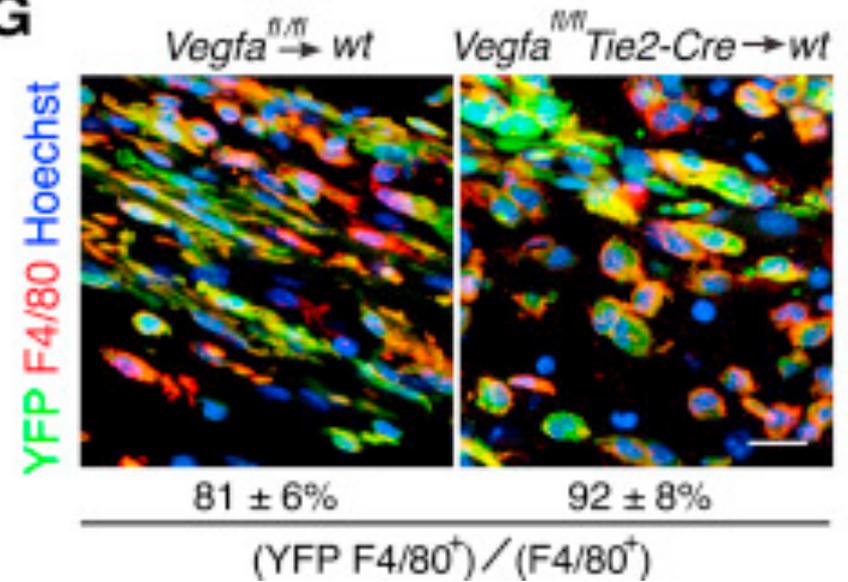




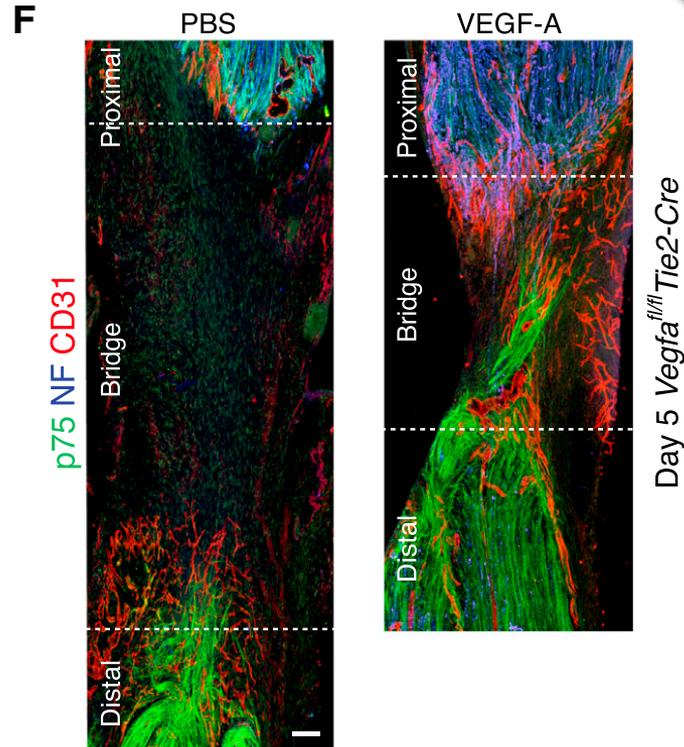
F



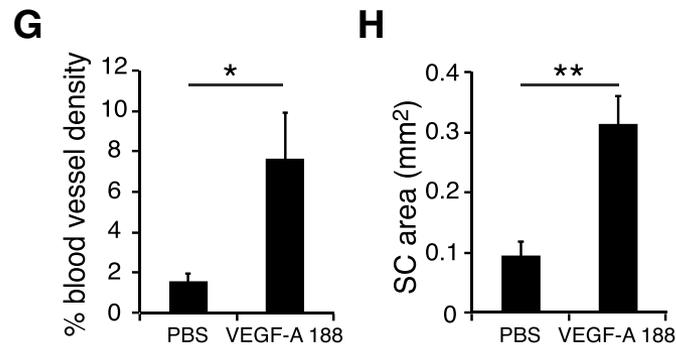
G



„rescue experiments“



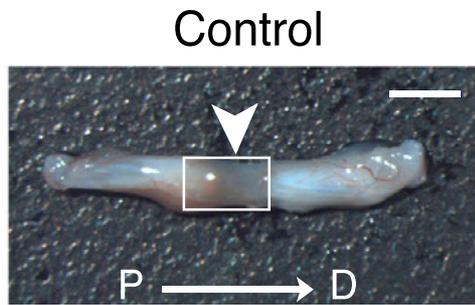
Injection at day 4



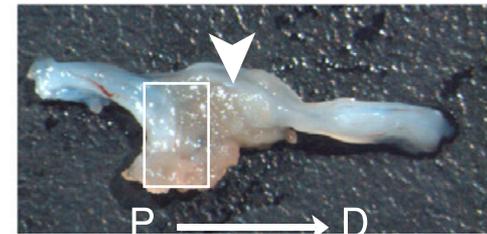
- ECs **deleted** for VEGF-A → **regeneration possible**
- **Macrophages** secrete VEGF-A → EC → SC → regeneration

SCs use vasculature for guiding axons

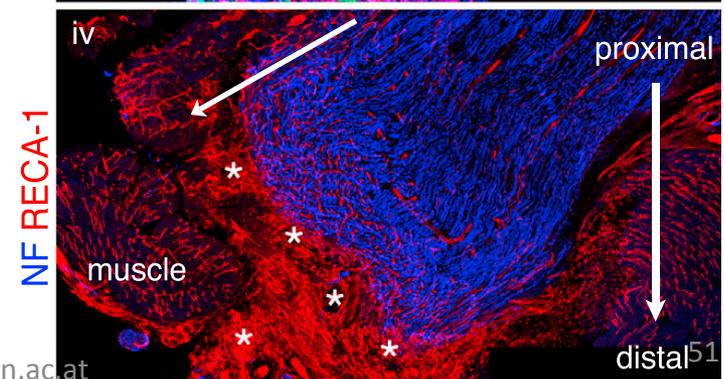
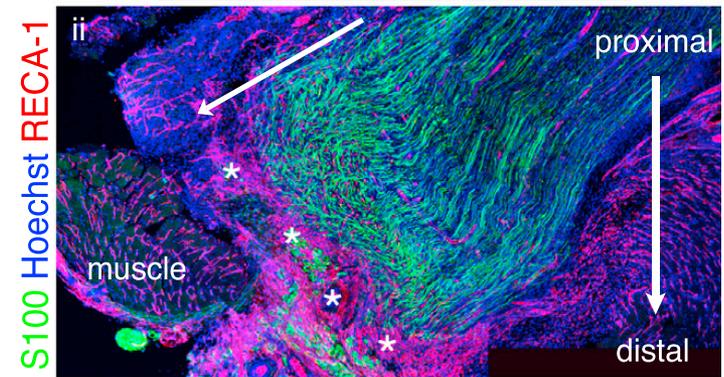
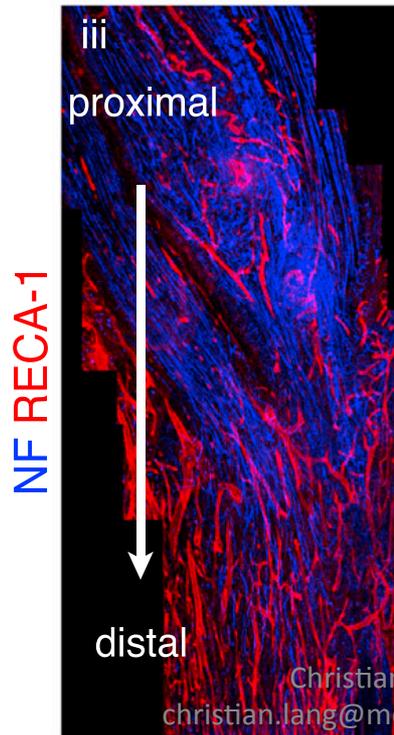
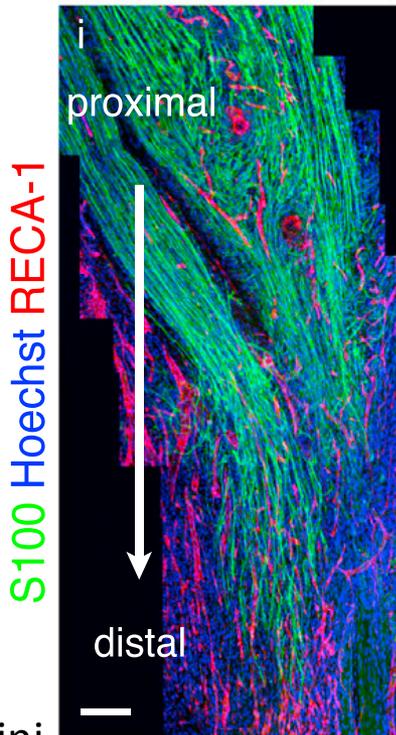
A



VEGF-A misdirected

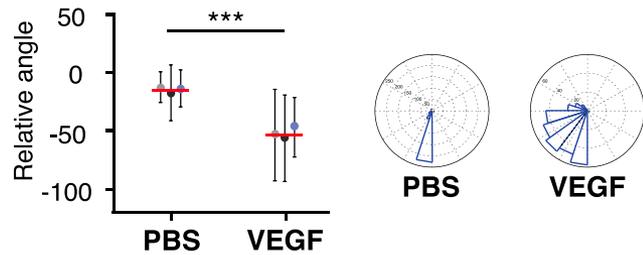


B

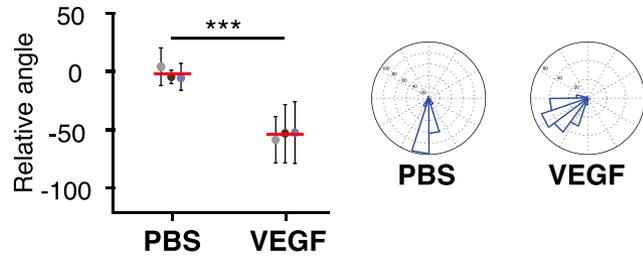


Day 6 after inj.

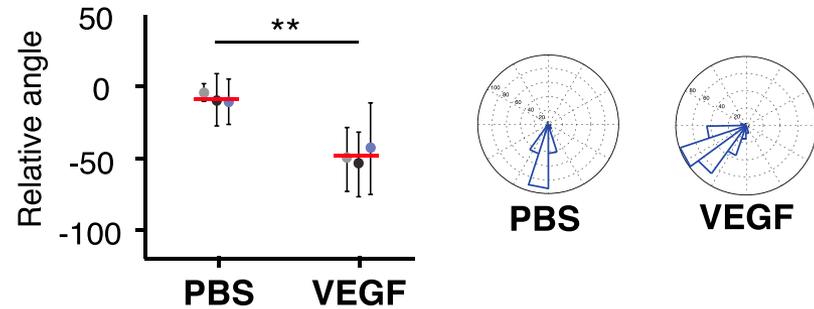
C Blood vessels vs prox/dist axis



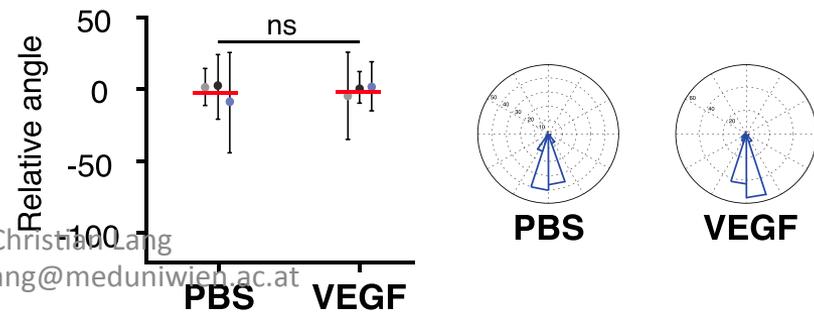
D Schwann cells vs prox/dist axis

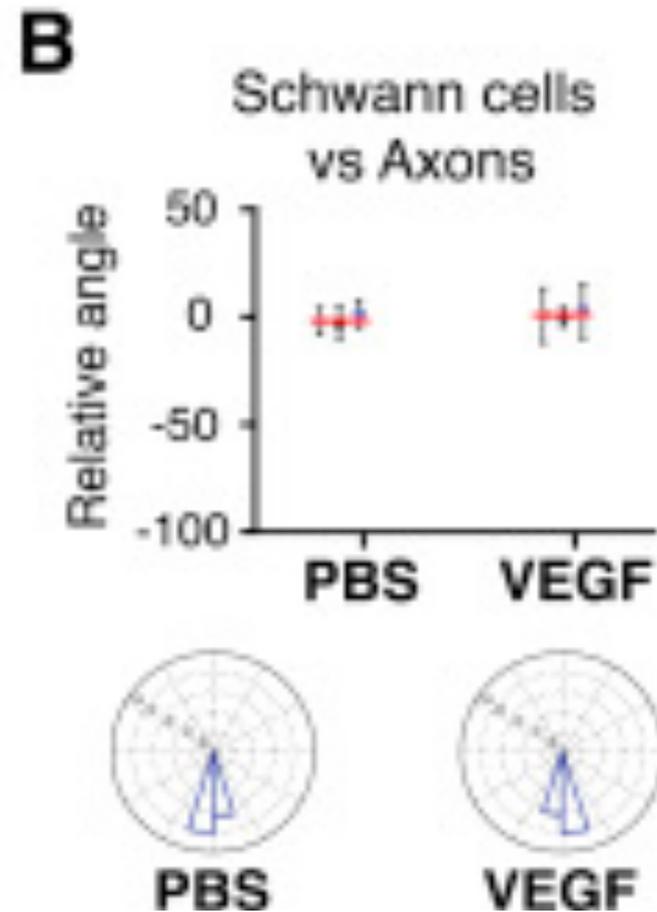
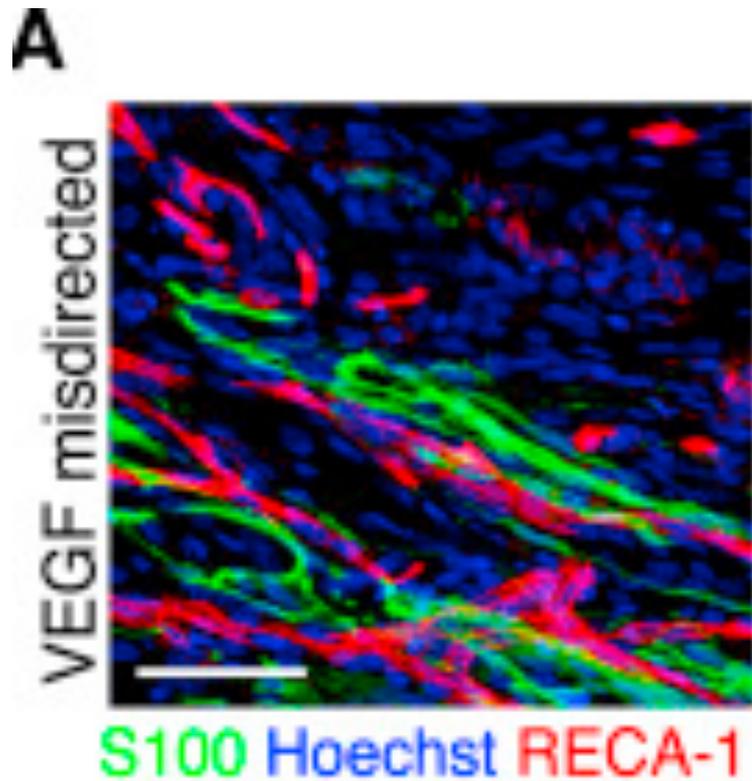


E Axons vs prox/dist axis



F Schwann cells vs Blood vessels



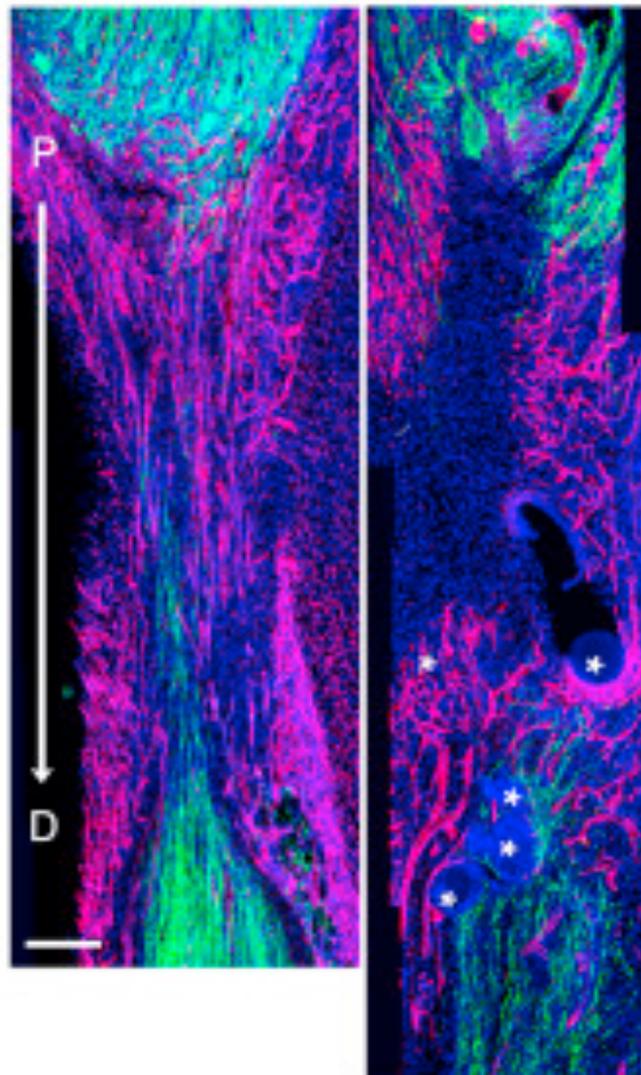


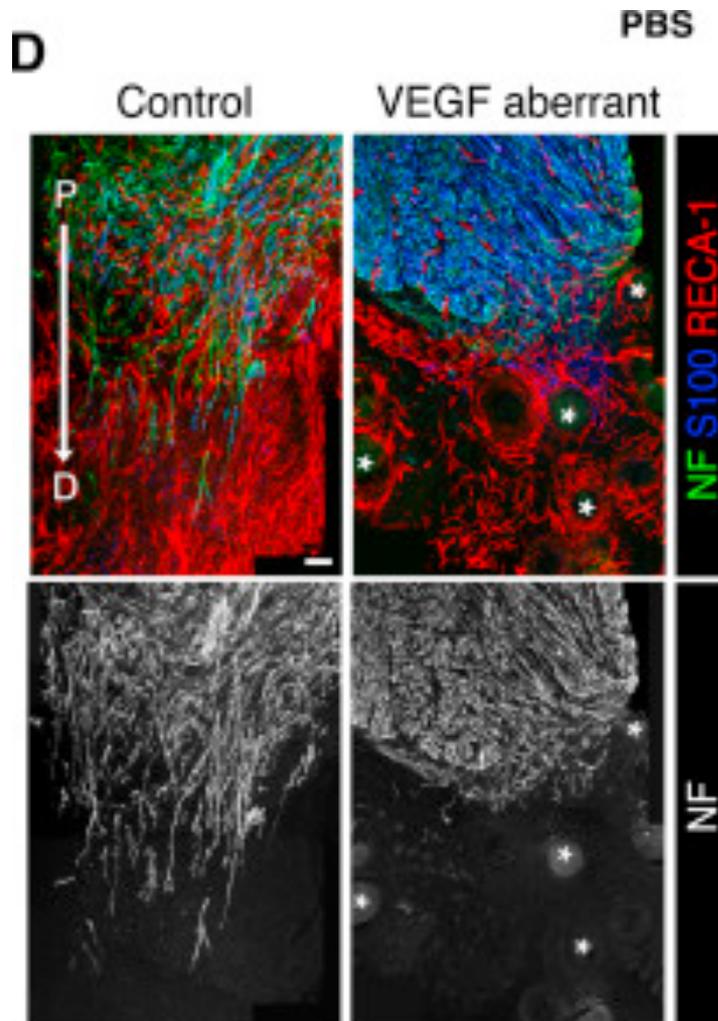


C

Control

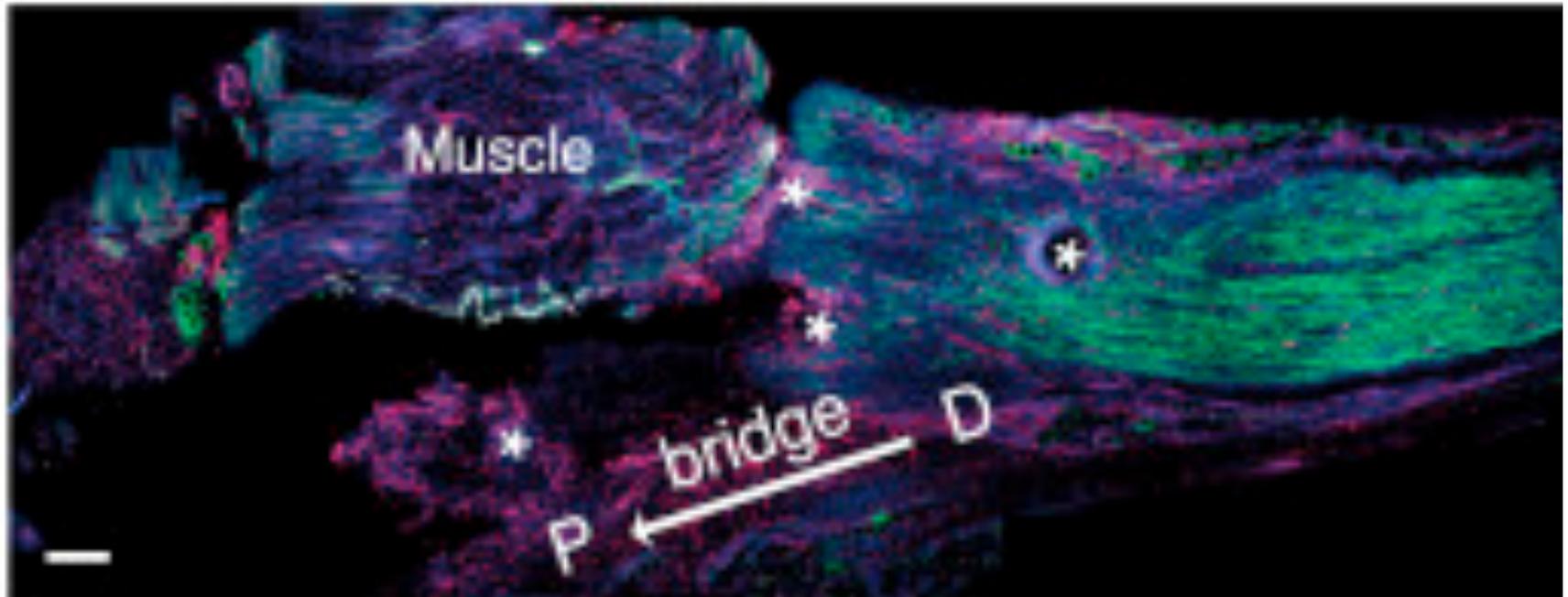
VEGF aberrant





E

VEGF distal misdirected

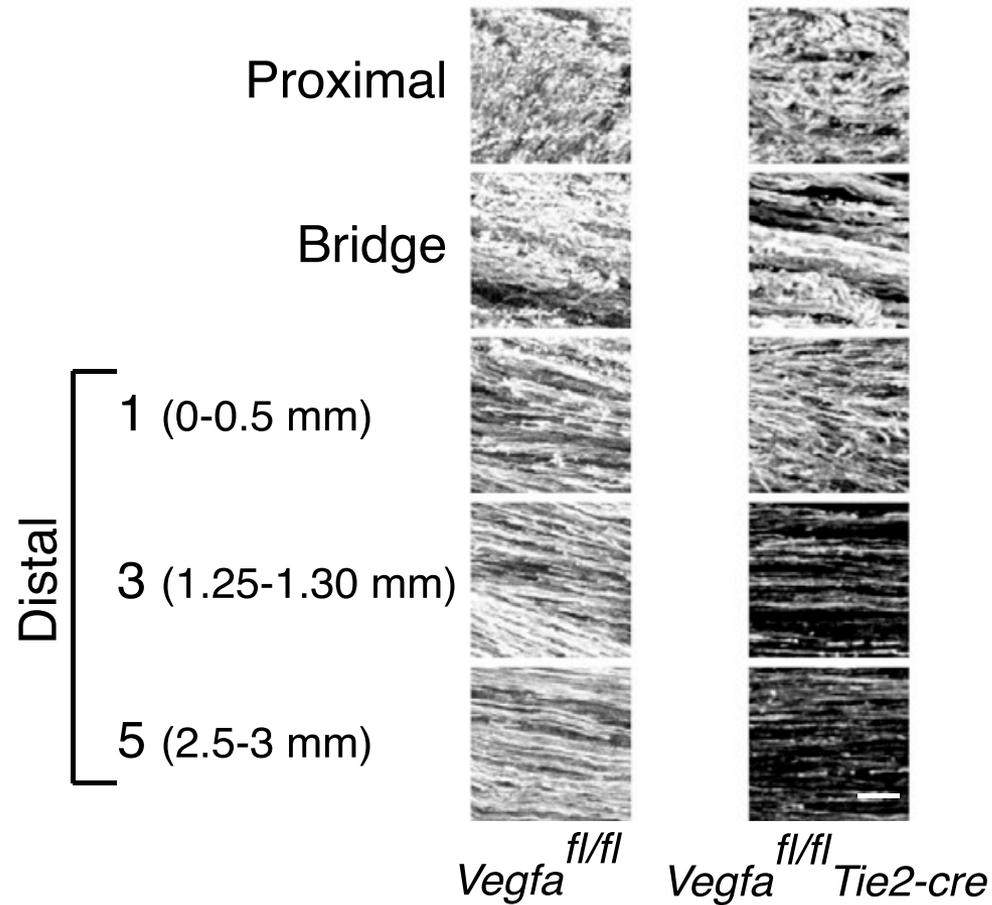


S100 **Hoechst** **RECA-1** **NF**

- **VEGF-induced blood vessels** → guide SCs & enable nerve **regeneration**

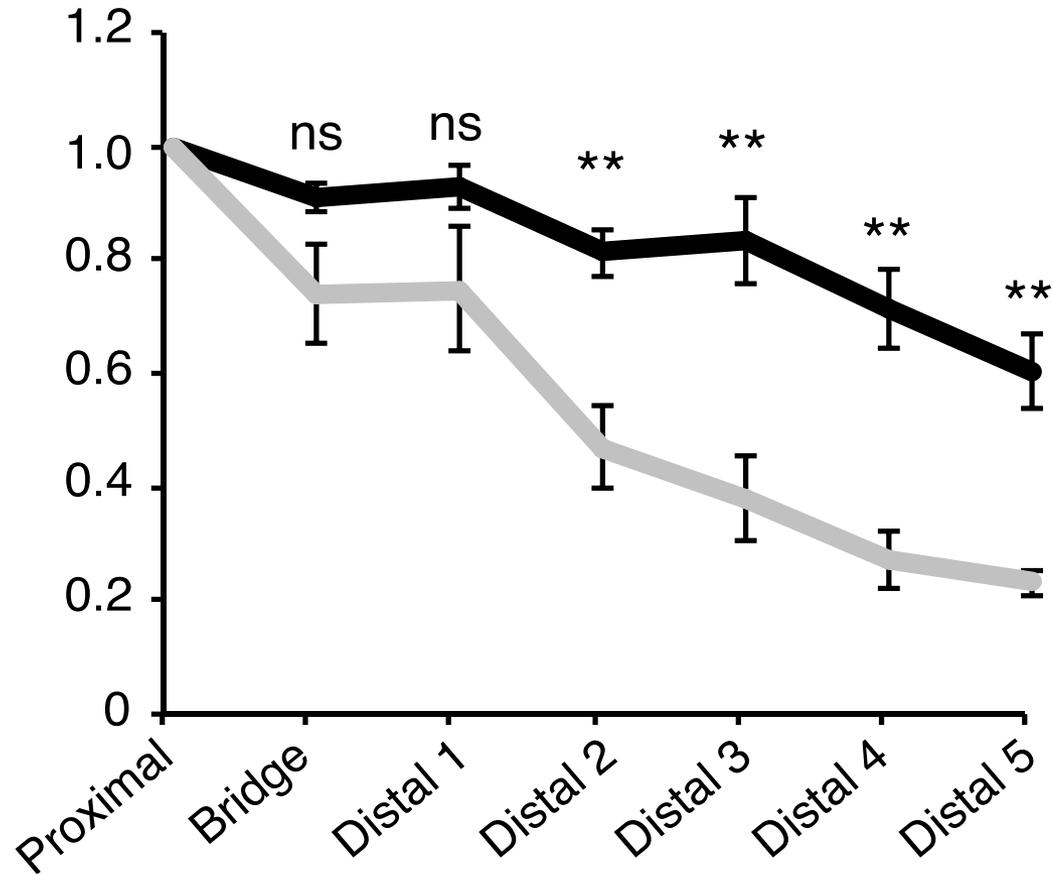
Long term observation

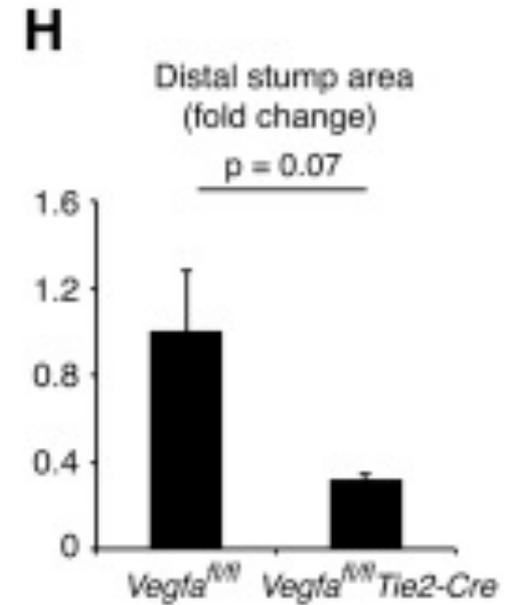
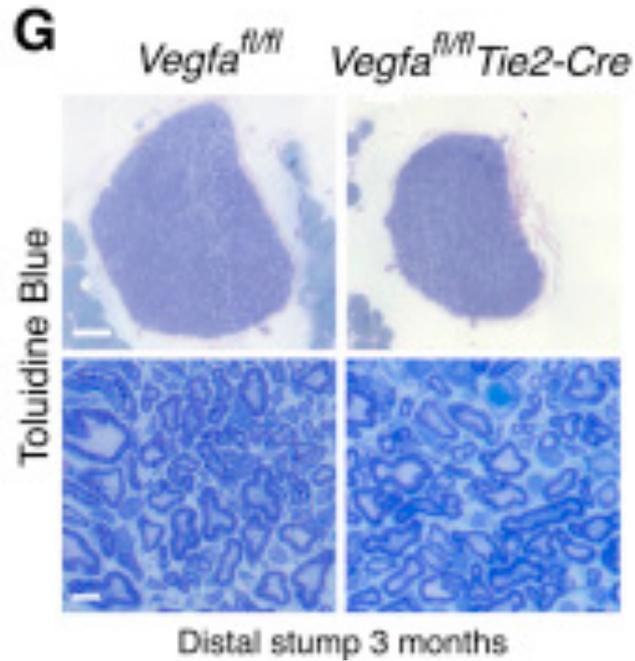
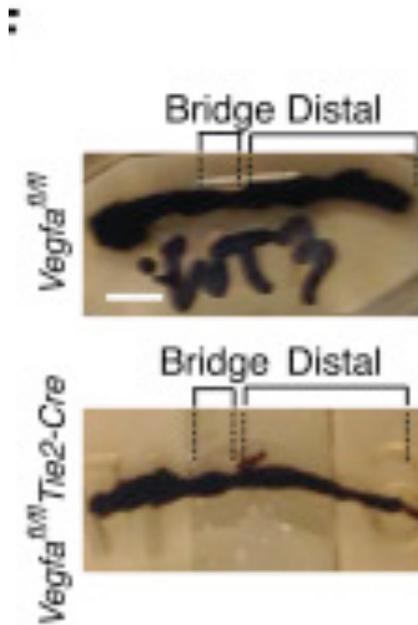
G



H

Neurofilament intensity relative
to the proximal (arbitrary units)





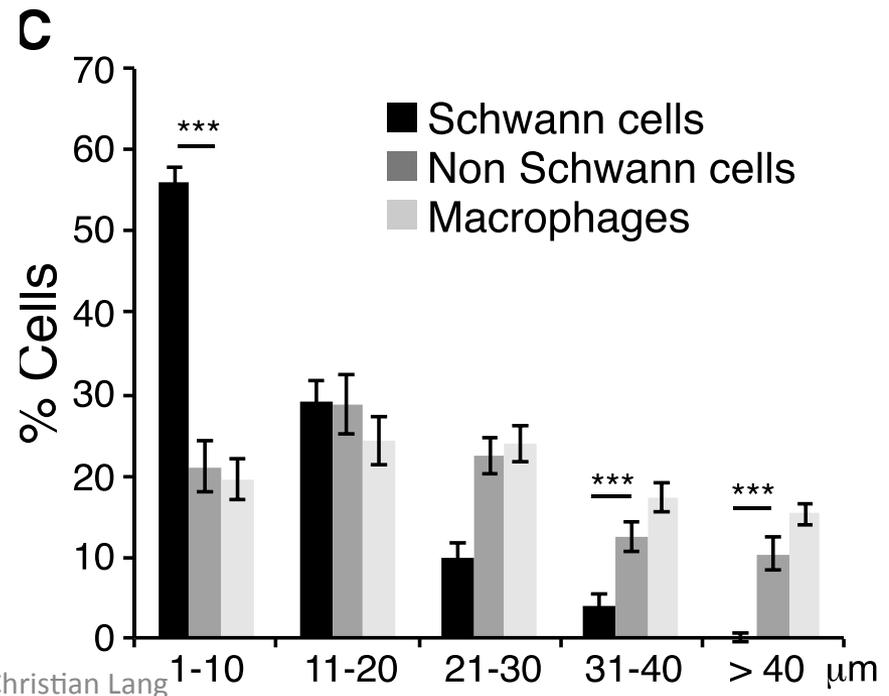
- „Long term“ observation → defects in mutant model

Final Summary

- **Macrophage-derived VEGF-A → vascularization of the bridge**
- **Vascularization necessary for amoeboid-like SC-migration and therefore axonal regrowth / PNS-regeneration**
- **Applicability**
 - The use of pre-existing structures after **stroke** / in **cancer**
 - Mimicking polarization in nerve **grafts** → improved regeneration?

Personal feedback

- Logical & transparent structure
- Nice graphics / video footages
- Critics
 - Fig. 3C
 - „Long term“ observation
- Applicability
 - Cancer
 - + Grafts



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