Harnessing neurovascular interaction to guide axon growth

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SUPPLEMENTAL FIGURE LEGENDS

Fig. s1. Microvascular morphology in the cd44KD and blebbistatin conditions compared to controls. Ai) Comparison of microvessel length and Aii) microvessel diameter of cd44KD and blebbistatin conditions to normal day 5 perfused hydrogels. Data are presented as mean ± s.e.m. *P<0.05; statistical significance was calculated using Welch Two Sample t-test. Length values (n=15) and diameter values (n = 5) are from single hydrogel samples per condition.

Fig. s2. Transplantation of a scaffold containing 5 mg/mL collagen density. A) Low magnitude image showing a lack of axon infiltration in the location of the transplant following hemisection injury. Scale = 500 μm. B) A magnified image of the location denoted with dotted lines in panel A. White arrows show attenuated neurite infiltration labelled by both Tuj and RT97 staining. Scale = 100 μm.

Fig. s3. A) Regenerating axon (CGRP-positive) infiltration into the transplant three weeks after transplantation. B) Low magnitude image showing the orientation of the transplant relative to host tissue. Scale bars, 50 μm (A) and 200 μm (B).

Fig. s4. Non-cropped blots for Figure 3. Two blots were used to evaluate cd44 expression levels at days 1, 3, 5, and 7 after incubation with siRNA.

Supplementary Video 1. 3d rendering of a transplanted scaffold with aligned microvessels within the host tissue, sacrificed 3 weeks-post transplantation. Transplanted cells (green-GFP), host axons (red-anti-Tuj), and cell nuclei (blue-DAPI) are used to visualize the scaffold. The rendering is constructed from confocal stacks of serial 8 micron sections imaged with a laser scanning confocal microscope with a 10x objective.

Supplementary Video 2. 3d rendering of a transplanted scaffold with aligned microvessels at 20x magnification. The staining and method of measurement is the same as Supplementary Video 1.

SUPPLEMENTAL FIGURES

Fig. s1

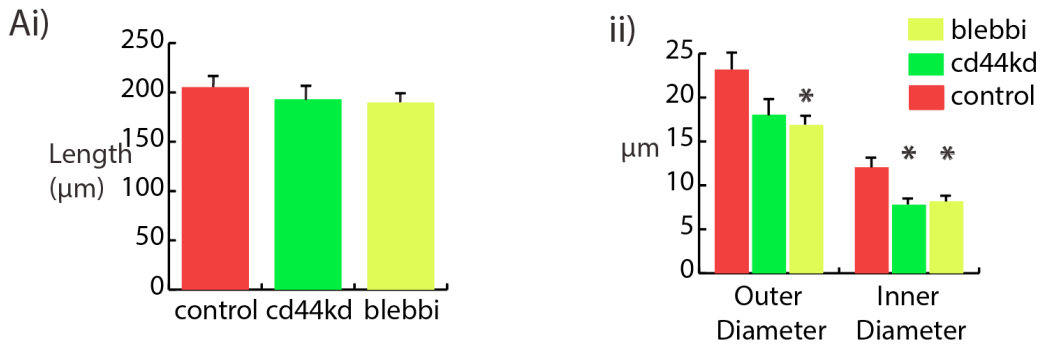


Fig. s2

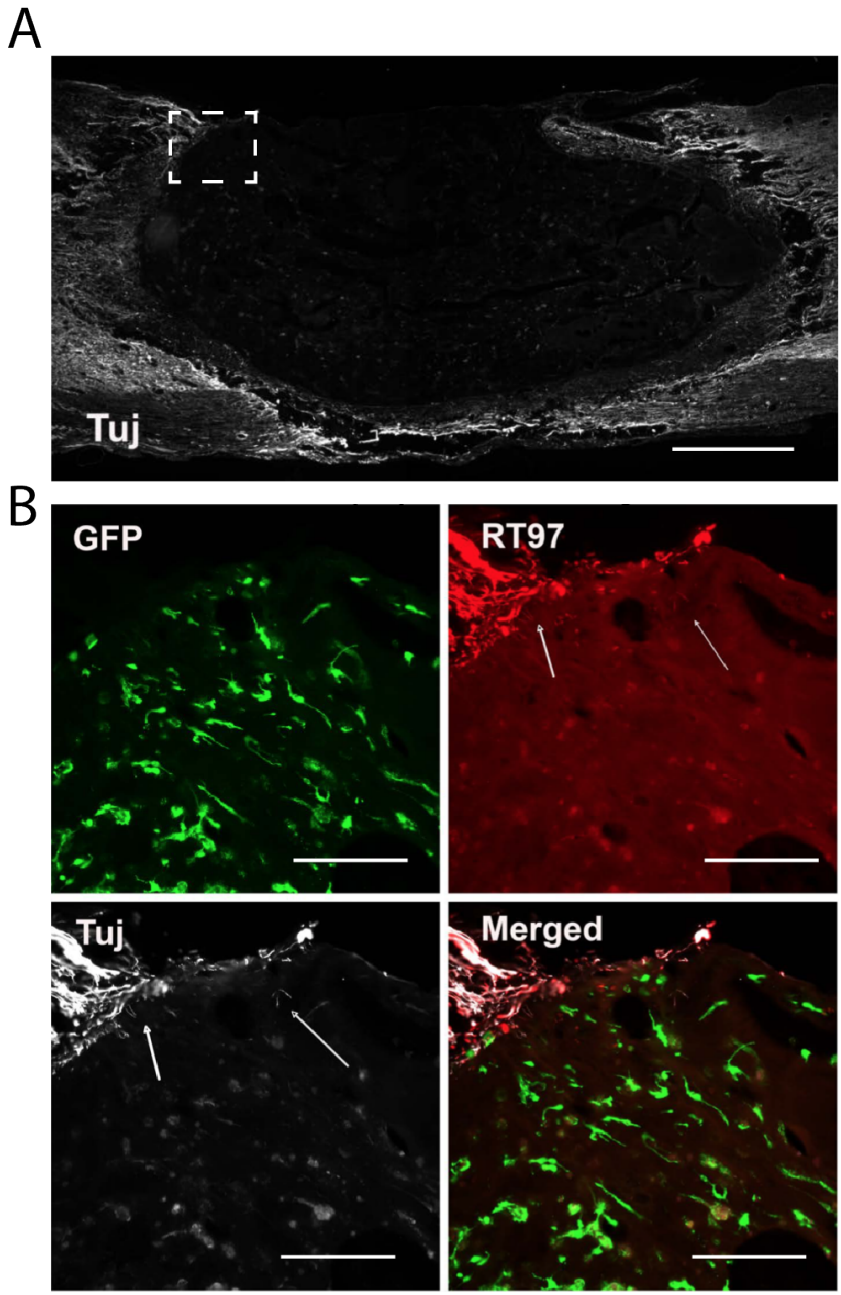


Fig. s3

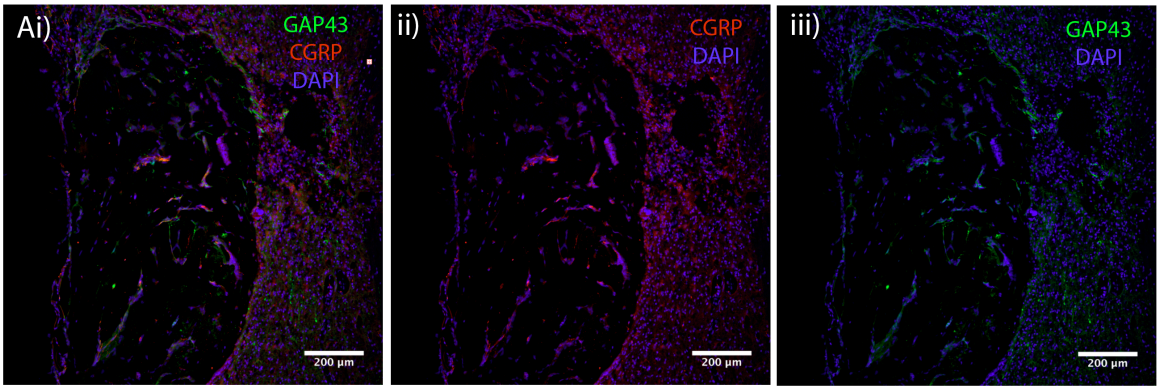
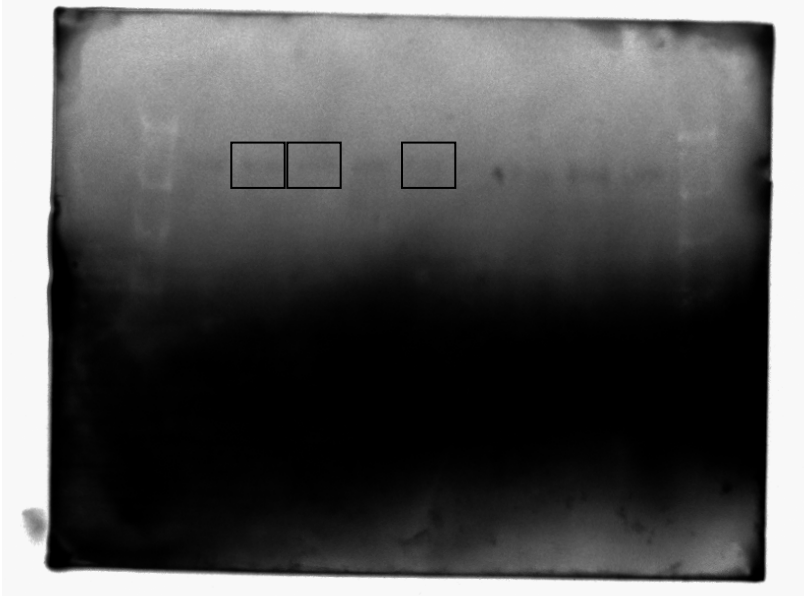
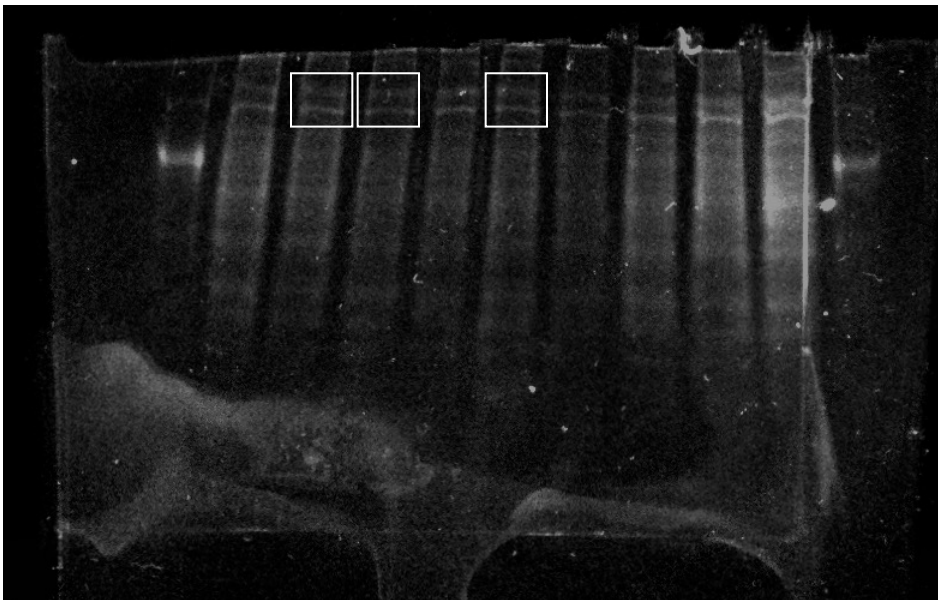


Fig. s4
Blot 1 – anti-cd44
(representative lanes highlighted in black)



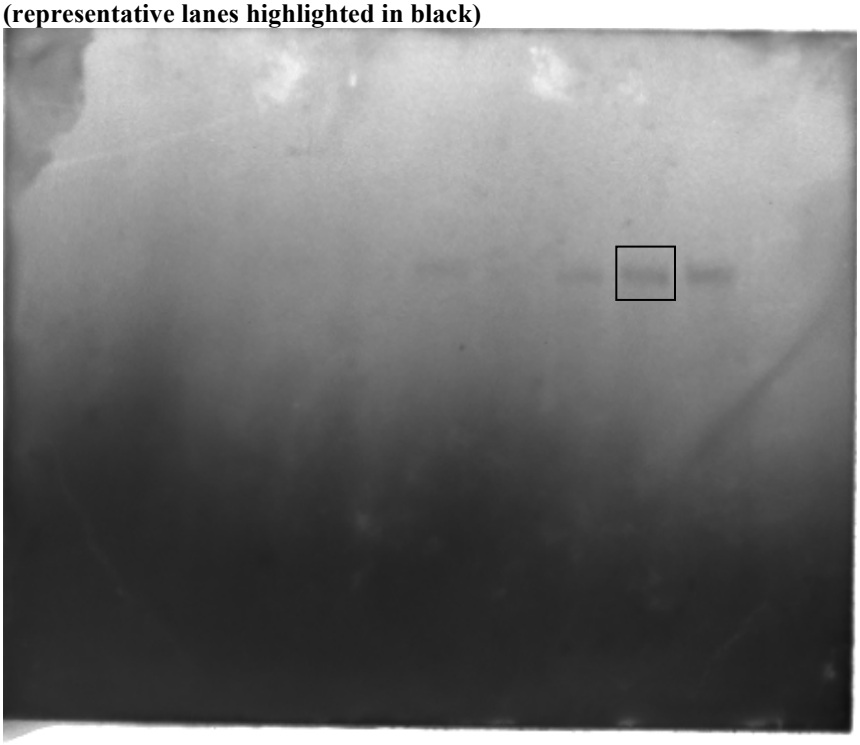
Lanes (1-9): Day1b, Day1a*, Day5b*, Day5a, Day3b*, Day3a, Day3c, Day5c, Day 7a

Coomassie Blue stain of Gel 1
(representative lanes highlighted in white)



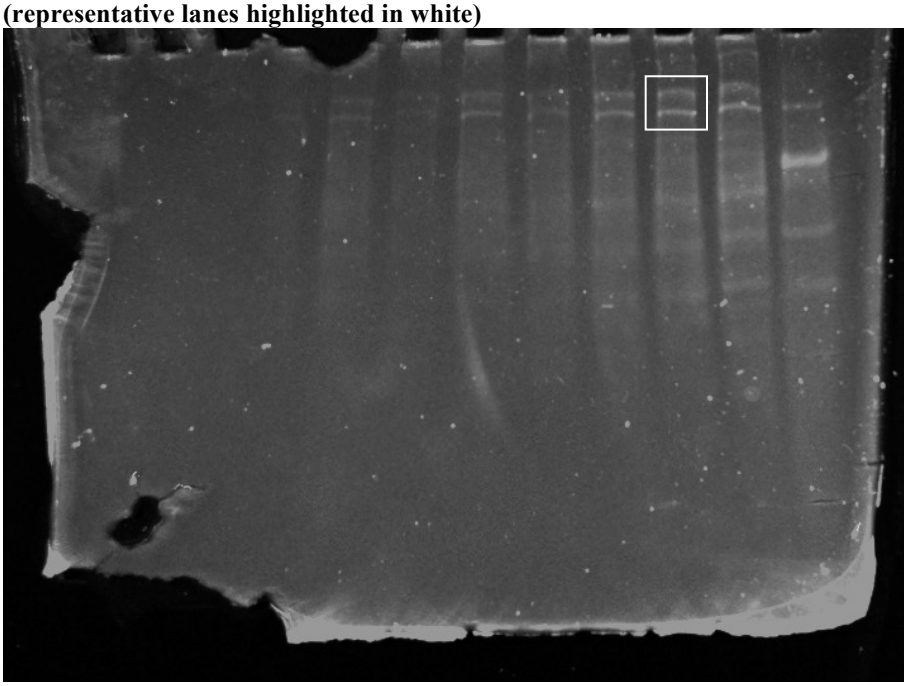
Lanes (1-9): Day1b, Day1a*, Day5b*, Day5a, Day3b*, Day3a, Day3c, Day5c, Day 7a

Blot 2 – anti-cd44
(representative lanes highlighted in black)



Lanes (1-7): Day3e, Day5d, Day7c, Day3d, Day1c, Day7b*, Day7a

Coomassie Blue stain of Gel 2
(representative lanes highlighted in white)



Lanes (1-7): Day3e, Day5d, Day7c, Day3d, Day1c, Day7b*, Day7a