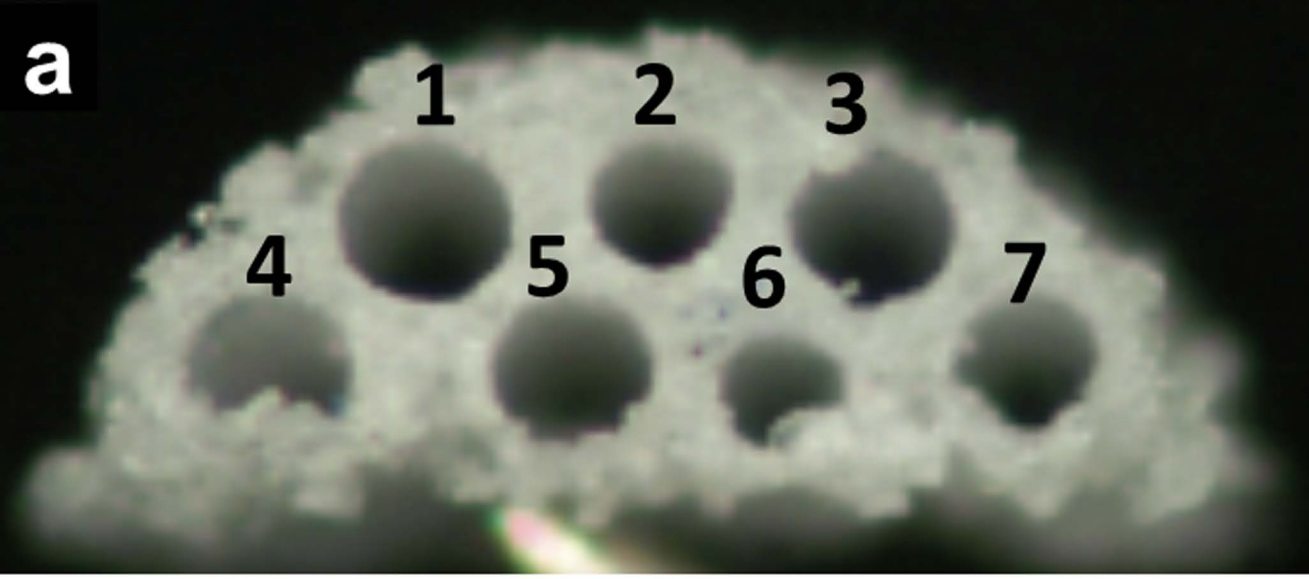
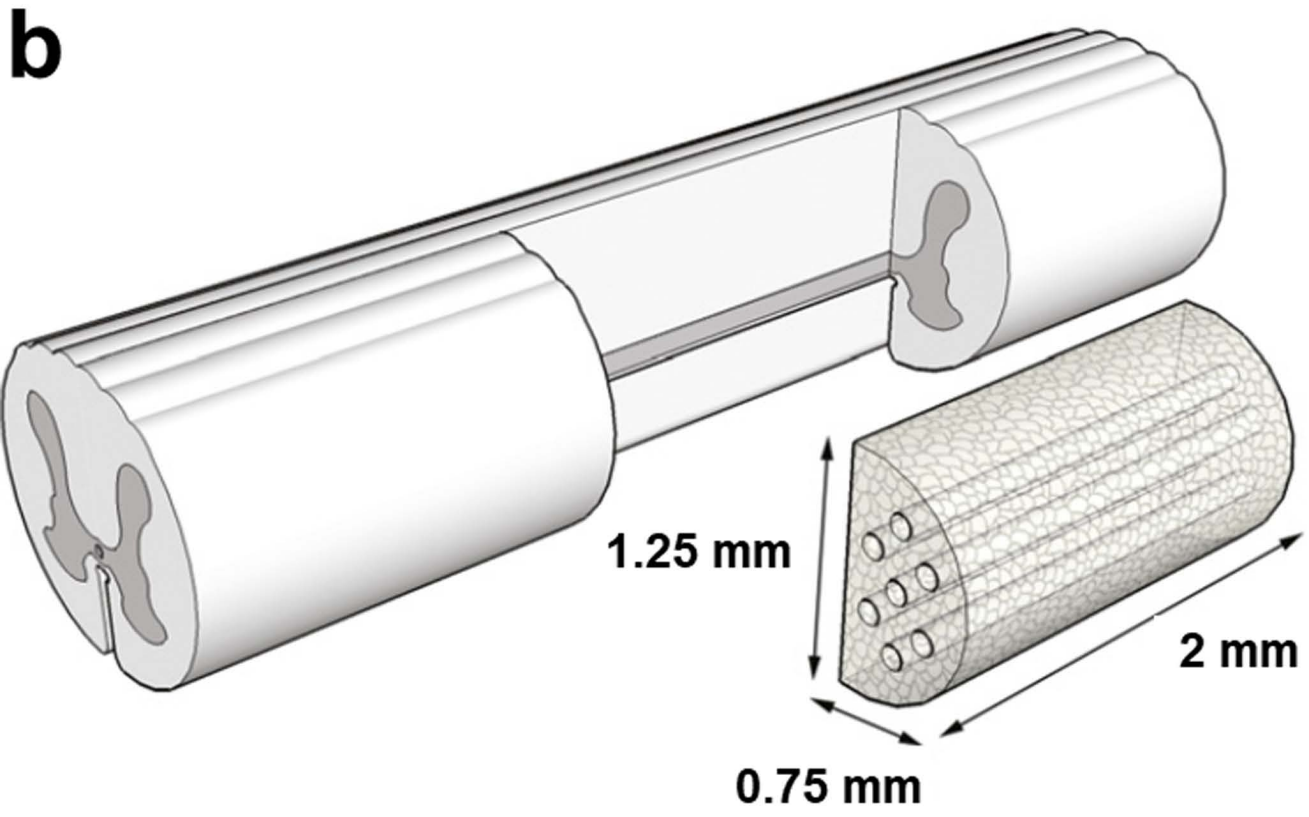
YMTHE, Volume *26*

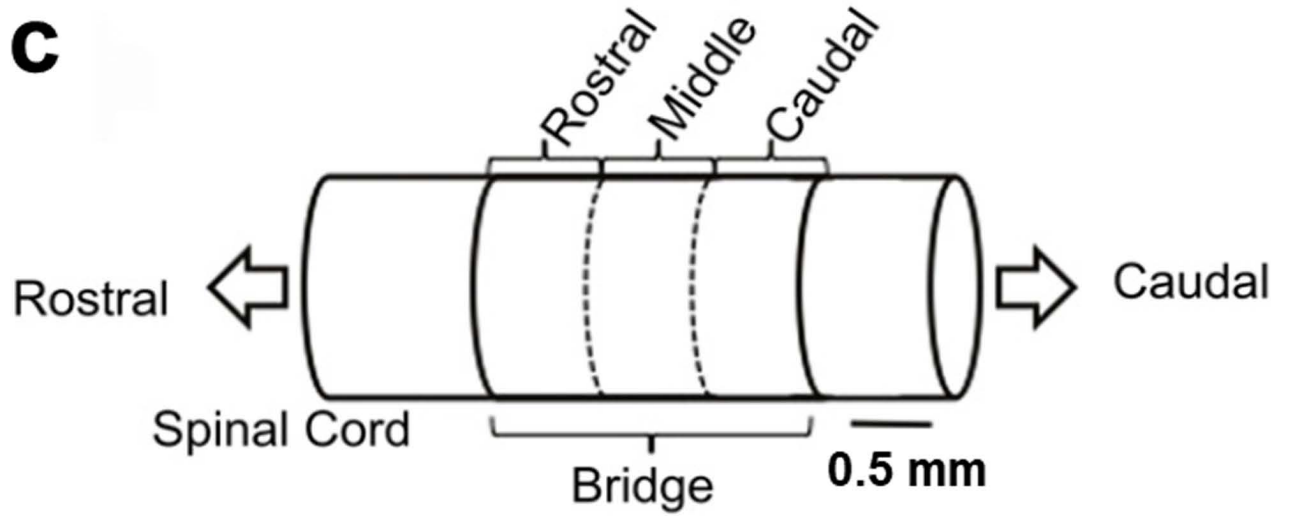
Supplemental Information

Local Immunomodulation with Anti-inflammatory Cytokine-Encoding Lentivirus Enhances Functional Recovery after Spinal Cord Injury

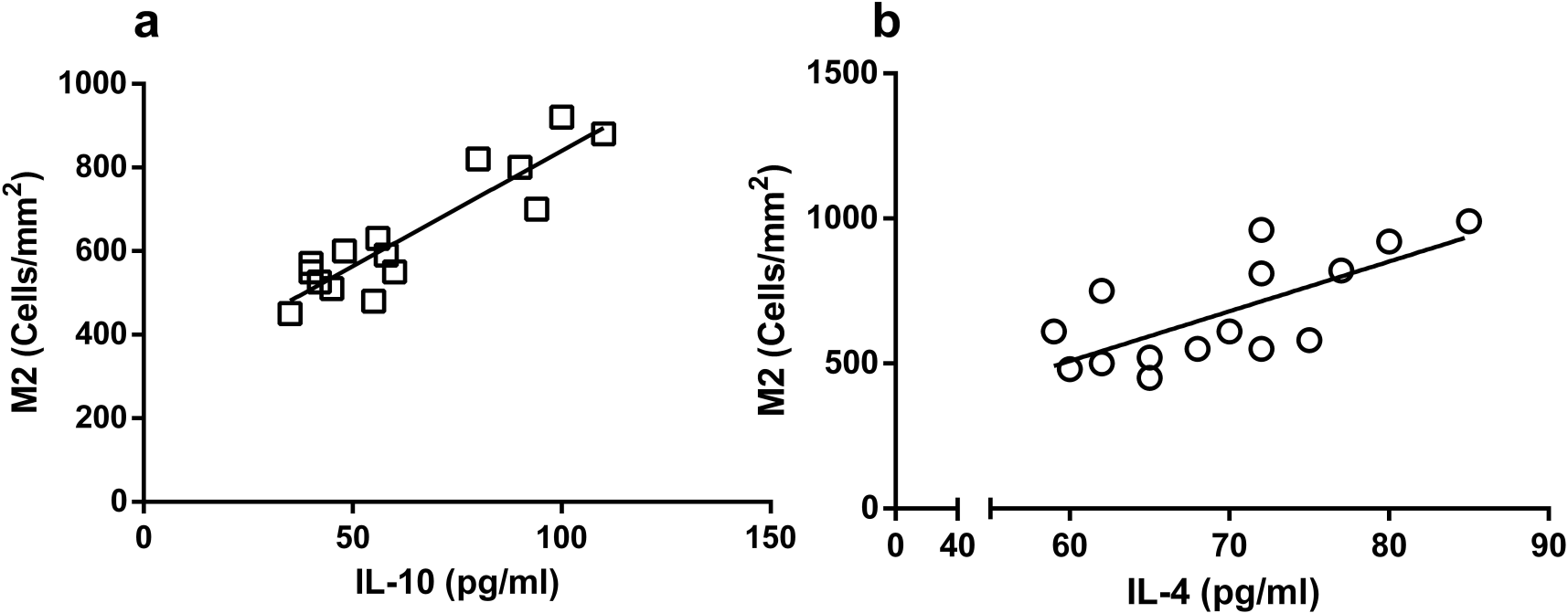
Jonghyuck Park, Joseph T. Decker, Daniel J. Margul, Dominique R. Smith, Brian J. Cummings, Aileen J. Anderson, and Lonnie D. Shea

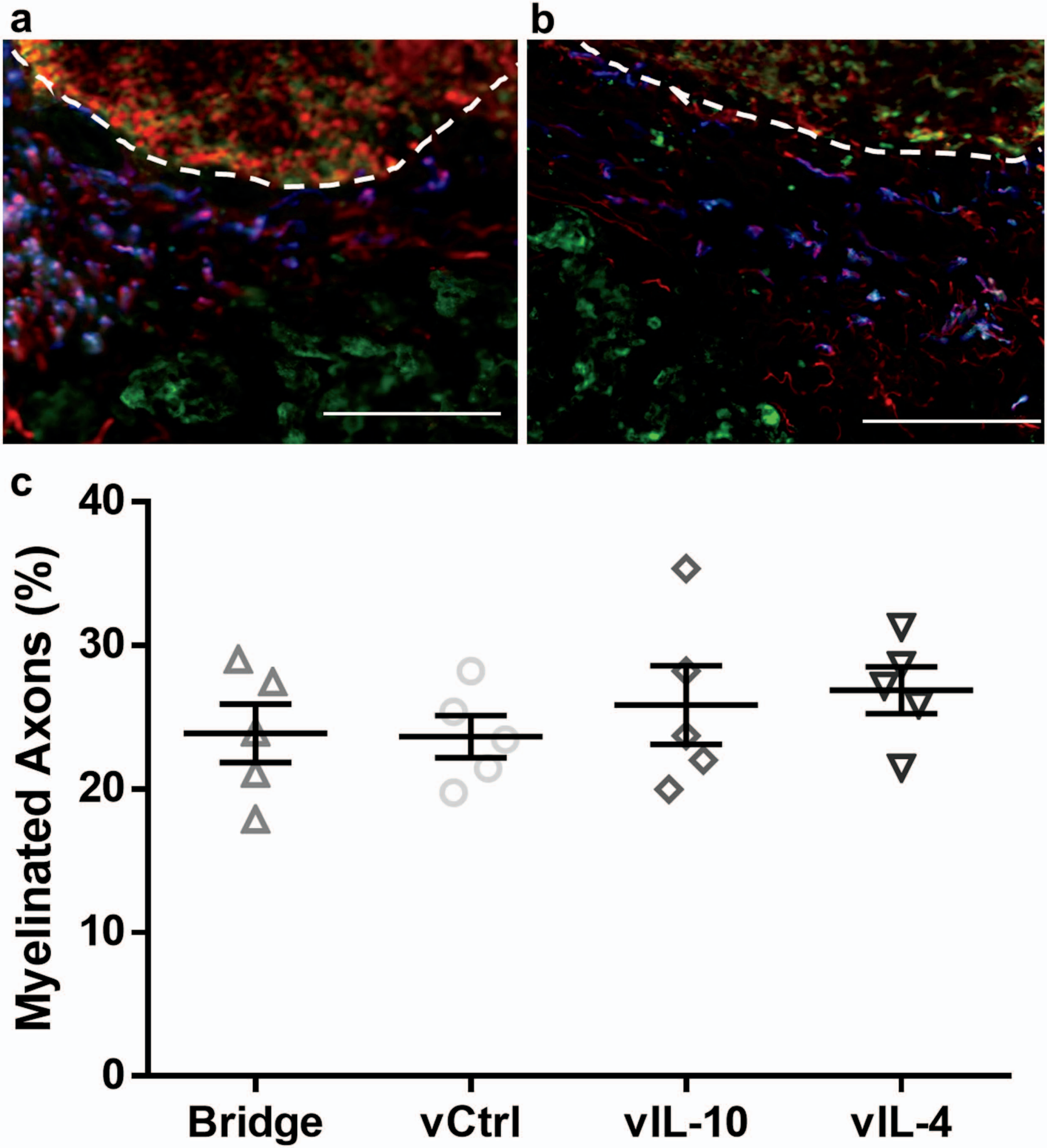

a
1
2
3
4
5
6
7

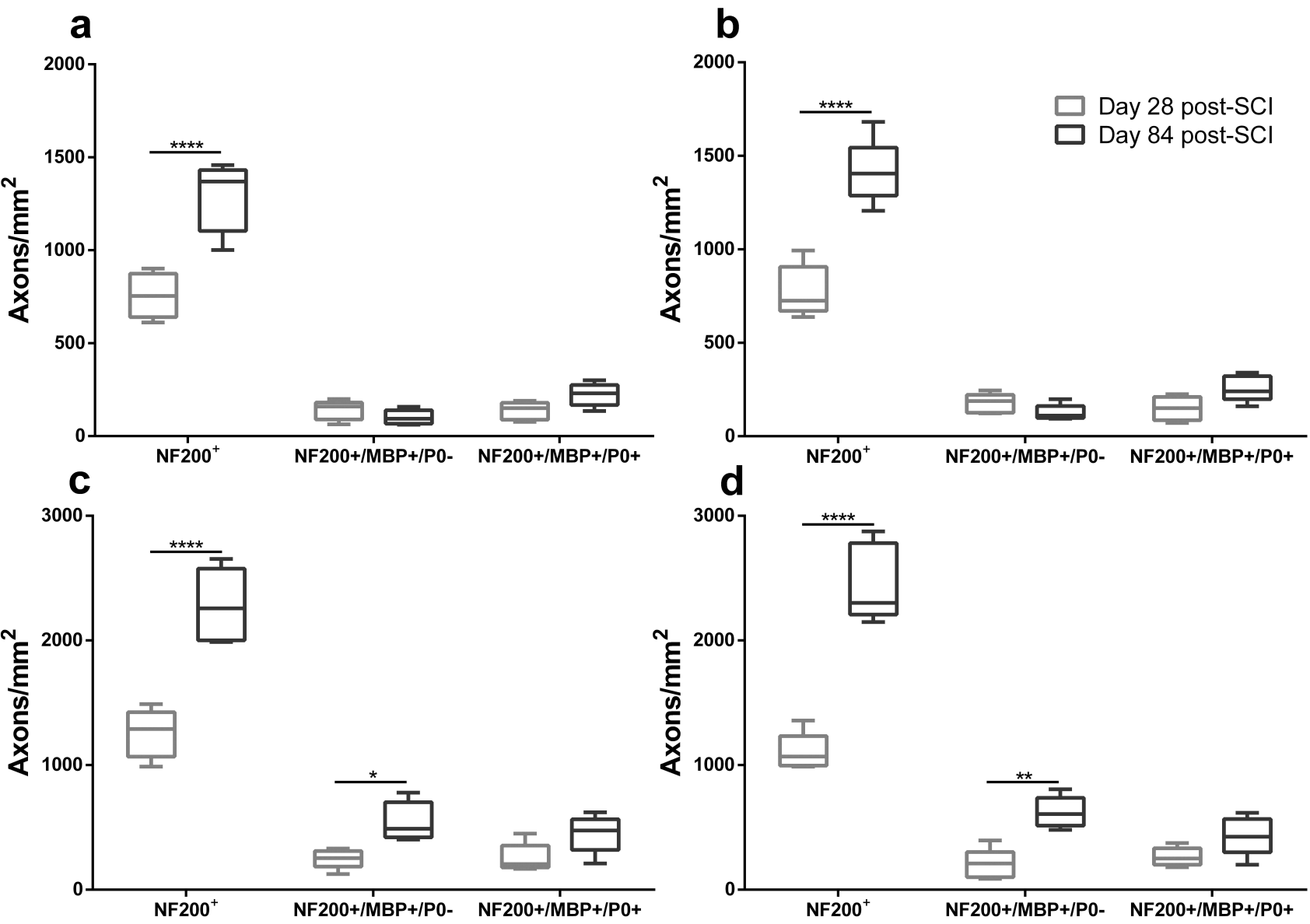

b
1.25 mm
2 mm
0.75 mm

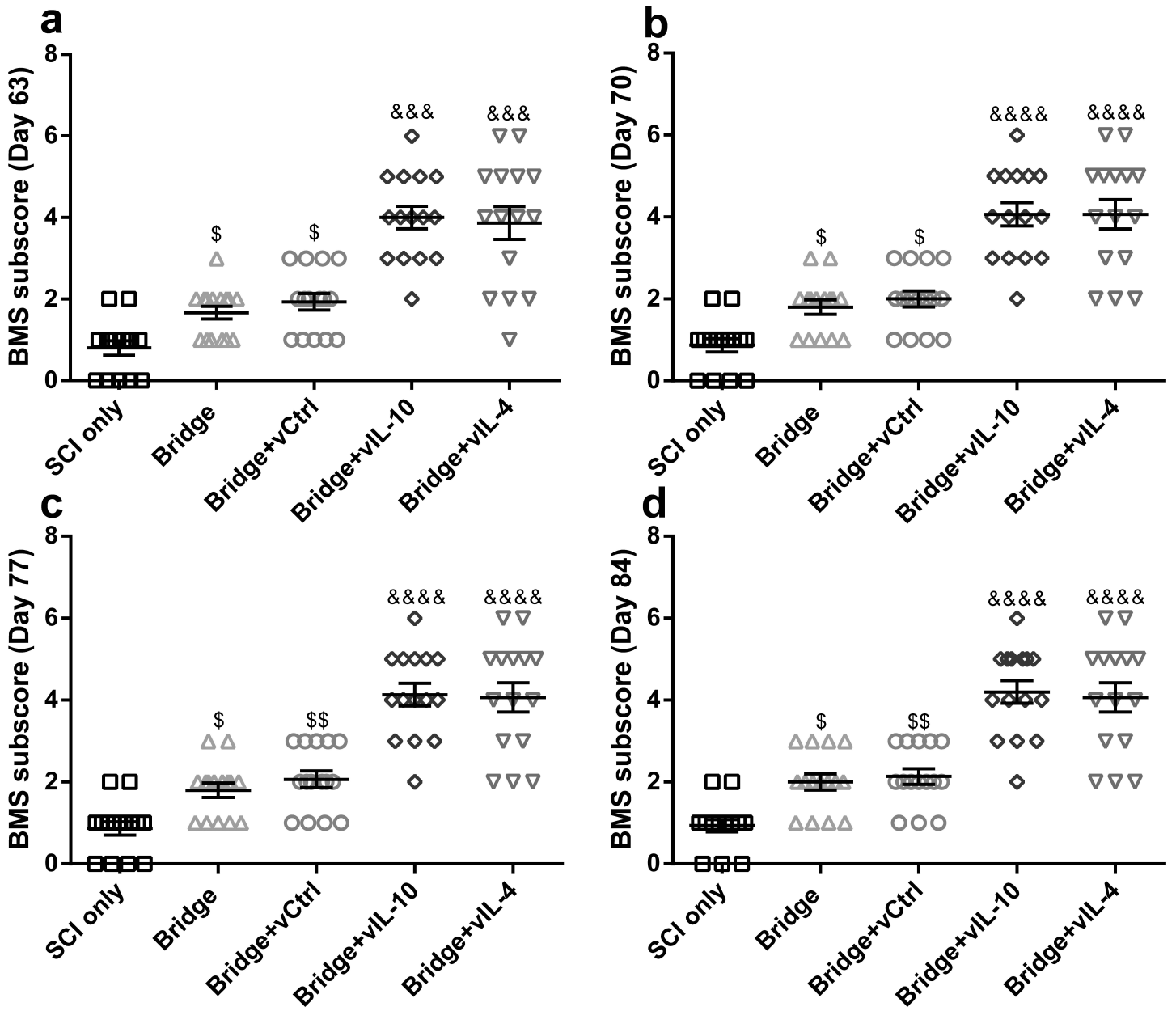

c
Rostral
Middle
Caudal
Rostral
Caudal
Spinal Cord
Bridge
0.5 mm

a
Cd28
Slc11a1
Fcgr4
Cd48
Cxcr4
Itgb2
Stat6
Havcr2
Milr1
Cd300a
Dock2
Zfp36l1
Syk
Cd180
Lcp1
Lgals1
Il7r
Myo1f
Vav1
Nfatc1
Dock8
Anxa1
Blnk
Lbp
Cdk6
Cxcl5
Anxa3
Tec
Lat2
Pilrb1
Cxcl12
Bridge + vCtrl
Bridge + vIL-10
Bridge + vI-L4
b
Ly96
Slc11a1
Fn1
Mapkapk2
Itgb2
Havcr2
Aoc3
Cd300a
Ccl9
Ccl6
Ccl3
Syk
Cd180
Stab1
Cd14
Lipa
Il1rn
Thbs1
Lbp
Il1b
Cd5l
Ecm1
Ptafr
Tnfrsf1b
Cxcl5
C3ar1
Tgfb1
Il4ra
C5ar1
Mrgpra3
Nupr1
Adam8
Cyba
Casp4
Ccr1
Tlr13
Cybb
Tlr8
Tlr7
Bridge + vCtrl
Bridge + vIL-10
Bridge + vI-L4
c
Thbs1
Lbp
Il1b
Ecm1
Ptafr
Cxcl5
Cxcl12
C3ar1
Tgfb1
C5ar1
Adam8
Ccr1
d
Ly96
Cd28
Slc11a1
Cd84
Ikbke
Slamf7
Stat6
Havcr2
Milr1
Cd300a
Dock2
Bcl6b
Ccl9
Ccl6
Ccl3
Syk
Cd180
Bridge + vCtrl
Bridge + vIL-10
Bridge + vIL-4
e
Gabrb2
Cntnap1
Nptx1
Spock1
Kcnma1
Chat
Slitrk1
Hoxc10
Faim2
Htr7
Hoxd10
Slc12a5
Grin1
Stxbp1
Chn1
Lingo4
Olfm3
Efna3
Cnr1
Sh3gl2
Lhx5
Cntnap2
Cntn6
Cntn4
Atp2b2
Bridge + vCtrl
Bridge + vIL-10
Bridge + vIL-4
f
Syt2
Rims1
Syn3
Gabrb2
Gabrg2
Glra1
Nrxn3
Cadps
Kcnma1
Chat
Fgf14
Syt10
Slc5a7
Pcdhb3
Htr7
Syt13
Slc12a5
Oprl1
Grin1
Dnm1
Stxbp1
Napb
Chrna4
Glrb
Gabrg1
Bridge + vCtrl
Bridge + vIL-10
Bridge + vIL-4
2
0
-2


a
1000
800
600
400
200
0
M2 (Cells/mm2)
0
50
100
150
IL-10 (pg/ml)
b
1500
1000
500
0
M2 (Cells/mm2)
0
40
60
70
80
90
IL-4 (pg/ml)


a
b
c
Myelinated Axons (%)
40
30
20
10
0
Bridge
vCtrl
vIL-10
vIL-4


a
b
c
d
Axons/mm2
2000
1500
1000
500
0
3000
****
*
**
Day 28 post-SCI
Day 84 post-SCI
NF200+
NF200+/MBP+/P0-
NF200+/MBP+/P0+


a
BMS subscore (Day 63)
8
6
4
2
0
&&&
&&&
$
$
SCI only
Bridge
Bridge+vCtrl
Bridge+vIL-10
Bridge+vIL-4
b
BMS subscore (Day 70)
&&&&
&&&&
$
$
c
BMS subscore (Day 77)
&&&&
&&&&
$
$$
d
BMS subscore (Day 84)
&&&&
&&&&
$
$$

Supplemental figure legends

Figure. S1. PLG multichannel bridge and hemi-sectional SCI model. (a) Photomicrograph of a bridge with 200 μm multichannel. (b) Schematic representation of hemi-section SCI at T9-T10 and multichannel bridge implantation in the injury site. Illustrating approximate bridge dimensions. (c) Schematic multichannel bridge regions where the bridge was divided for analysis. From the rostral edge of the bridge/tissue interface, rostral region analysis was done at 300 μm, middle at 900 μm, and caudal at 1600 μm.

Figure. S2. Local delivery of anti-inflammatory cytokine lentiviral vectors alters (a) Leukocyte activation (GO:0045321)-, (b) Inflammatory response (GO:0006954)-, (c) Leukocyte migration (GO:0002685)-, (d) Immune response (GO:0006955)- (e) Neuron differentiation (GO:0030182)- and (f) Synapse organization (GO:0050808)-related gene ontologies at day 7 post-SCI.

Figure. S3. Overexpression of anti-inflammatory cytokines influences the number of M2 phenotypes within the bridges. (a) The transgene expression of IL-10 was associated with the increased number of M2 phenotypes (Pearson coefficient=0.9093; $P<0.0001$; $r^2=0.8268$). (b) Overexpression of IL-4 also correlated to increased number of M2 macrophages at the injury site (Pearson coefficient=0.7067; $P=0.0032$; $r^2=0.4994$).

Figure. S4. In the sub-acute phase, extensive co-localization of MBP^+ and $P0^+$ myelin was observed around and outer surface of multichannel bridges in the rostral position for (a) vIL-10 and (b) vIL-4 (scale bar 50 μm). The dashed lines indicate the interface between bridge implants and spinal cord tissue. (c) Percentage of axons that were myelinated within the bridge area at day 28 post-SCI. No difference was observed between groups. One-way ANOVA with Tukey's post hoc test (mean±SEM, n=5/group).

Figure. S5. vIL-10 and vIL-4 delivery increases the number of axons and oligodendrocyte-derived myelinated axons as a function of time at the injury. The total number of axons, oligodendrocyte-derived and Schwann cell-derived myelinated axons were quantified in delivery of (a) bridge only, (b) vCtrl, (c) vIL-10, and (d) vIL-4 in the sub-acute (day 28) and chronic phase (day 84). The statistical tests were completed using a two-way ANOVA with Šidák correction for the multiple comparisons. (*$P<0.05$, **$P<0.01$ and ****$P<0.0001$, n=5/group)

Figure. S6. Multichannel bridge with anti-inflammatory cytokines improves specific components of locomotion recovery from SCI. (a-d) The BMS subscore in vIL-4 and vIL-10 groups was significantly increased relative to bridge only and vCtrl throughout day 84. Furthermore, bridge itself increased the frequency of plantar stepping and fore limb-hind limb coordination compared to SCI only as a function of time. Statistical test was completed using a one-way ANOVA with Tukey's post hoc test. $^{\$}P<0.05$, and $^{\$\$}P<0.01$ compared to SCI only, and $^{\&\&\&}P<0.001$ and $^{\&\&\&\&}P<0.0001$ compared to vCtrl; mean±SEM and n=15/group

Table S1. Gene ontology accession number

Name	Accession Number
Inflammatory response	GO:0006954
Leukocyte activation	GO:0045321
Nervous system development	GO:0007399
Locomotor behavior	GO:0007626
Synapse organization	GO:0050808
Immune system process	GO:0002682
Leukocyte migration	GO:0002685
Immune response	GO:0006955
Neuron differentiation	GO:0030182
Chemical synaptic transmission	GO:0007268

Table S2. Primer sequences for qRT-PCR

Gene	Accession number (GenBank)	Forward (5'-3')	Reverse (5'-3')
Il7r	NM_008372.4	GCTGTACACAGTGCAAACCG	GTGGAGATGGGCTGTCTCTG
Cd28	NM_007642.4	TGGCTTGCTAGTGACAGTGG	CATTGGTGGCCCAGTAGAGG
Cdk6	NM_009873.3	GCATCGTGATCTGAAACCGC	CCACGTCTGAACTTCCACGA
Arginase1	U51805.1	GAACACGGCAGTGGCTTTAAC	TGCTTAGCTCTGTCTGCTTTGC
CD206	NM_008625.2	TCTTTGCCTTTCCCAGTCTCC	TGACACCCAGCGGAATTTC
Retnla	NM_020509.3	GGTCCCAGTGCATATGGATGAGACCATAGA	CACCTCTTCACTCGAGGGACAGTTGGCAGC
CD86	NM_019388.3	TTGTGTGTGTTCTGGAAACGGAG	AACTTAGAGGCTGTGTTGCTGGG
iNOS	U58677.1	CCCTTCAATGGTTGGTACATGG	ACATTGATCTCCGTGACAGCC
MHC-II	NM_207105.3	GACGCTCAACTTGTCCCAAAAC	GCAGCCGTGAACTTGTTGAAC
18s-rRNA	NR_003278.3	GCAATTATTCCCCATGAACG	GGCCTCACTAAACCATCCAA-3
Hoxc10	NM_010462.5	TGTACAGTGCAGAGAAGCGG	GTGTCTGGACTGGAGTCTGC
Chat	NM_009891.2	GGCTTTTGTGCAAGCCATGA	CACAGGGCCATAACAGCAGA
Efna3	NM_010108.1	CTACATCTCCACGCCCACTC	CCTGGGGATTCTCTCCCTCA
Hapln4	NM_177900.4	GGTCACAAGATGATCGTGCC	TGACCTTAAGAAGCCGAGCA
Trpc5	NM_009428.3	AACTCCCTCTACCTGGCAAC	TTCTGCAATCAGAGTCGGGT
Lhx5	NM_008499.5	CAGGATCCGTTACAGGACGA	AACCACACCTGAATGACCCT
Scn1a	NM_001313997.1	GTGTGCTCAAGCTCATCTCG	GCACCTTTGATCAGGCGTAG
Hoxd10	NM_013554.5	AACCAGCAATTGGCTCACTG	TTACTGATCTCTAGGCGGCG
Negr1	NM_177274.4	CGGTGCTCAGGTGTTACTTG	GAAACTCGAGGGTCCACTGA