SUPPLEMENTAL FIGURES

Supplemental Figure S1. Conditional NgR1 Gene Trageting

A, Schematic of NgR1 gene targeting. Genomic locus: exon 2 encodes all but the first 8 amino acids of NgR1. B, N, X. and H represent BamH-I, Nhe-I, Xho-I and Hind-III sites, respectively. The position of DNA probes used for Southern blotting (NgR1, black; GFP, grey) and Northern blotting (NgR1, white; GFP, grey) are shown.

B, Cre-mediated recombination from the pActin-Cre transgene abolishes expression of NgR1. Immunoblots of brain lysate from mice transgenic for pActin-Cre and heterozygous (+/Δ) or homozygous (Δ/Δ) for the (recombined) NgR1flx allele, as well as brain lysate from a constitutive heterozygote (-/+), wild-type (+/+), probed with antibodies directed against either NgR1 or GFP.

C, Analysis of genomic DNA by Southern blotting. Genomic DNA was digested with Hind-III and probed for sequences unique to NgR1 or GFP. The restriction product corresponding to NgR1 exon 2 in wild-type (+/+) but not mutant (-/-) preparations shifts to a larger size containing sequences for both exon 2 and GFP (arrowhead) (flx/- and time point 0 preceding tamoxifen injection of flx/-; cre/Esr1 mice). Following tamoxifen-induced activation of ER-Cre and recombination, both the NgR1 probe (outside the region of recombination) and GFP probe recognize a smaller fragment corresponding in size to the predicted recombination product at 14 days after tamoxifen injection (14 flx/-; cre/Esr1).

D, Analysis of mRNA expression by northern blotting. Messenger RNA recognized by the NgR1 probe in brain lysate from wild-type (+/+) but not mutants (-/-) (white arrowhead) shifts to a larger size that is also recognized by the GFP probe (grey arrowhead) (flx/- and time point 0 preceding tamoxifen injection of flx/-; cre/Esr1 mice). Note that this mRNA does not yield detectable GFP protein (see panel E below). Following tamoxifen injection, GFP probe recognizes a smaller fragment (black arrowhead) while the NgR1 probe is no longer detectable.

E, Cre-mediated recombination from the pActin-Cre/Esr1 transgene abolishes expression of NgR1 following tamoxifen injection. Immunohistology of brain from mice transgenic for pActin-Cre/Esr1 at several time points after tamoxifen injection to activate the Cre fusion protein with antibodies directed against either NgR1 or GFP. Scale bar, 2000 μm.

Supplemental Figure S2. Examples of Kinematic Analysis from Chronic Hemisection Mice after Conditional NgR1 deletion.

Representative stick tracings of limb joint motion during one gait cycle in the anterior-posterior plane. Positions are normalized to the hip (red dot) and the extent of foot swing is highlighted by the dotted lines and the red shading. The data were collected at the end of the dorsal hemisection experiment illustrated in Fig. 3A. The examples are from separate mice in either the -Cre group with NgR1 expression (left) or the +Cre group with deletion of NgR1 expression beginning 11 weeks after dorsal hemisection (right).

Supplemental Figure S3. Ankle Angle and Foot Velocity in Injured Mice.

The ankle angle and the foot velocity are plotted as a function of time for mice from the hemisection experiment of Figure 2, collected by the kinematic method of Figure 3. The

data are mean±sem from n=28-32 steps from each injured cohort. *, *P*<0.02, repeated measures ANOVA for *cre* genotype effect.

Supplemental Figure S4. Examples of Kinematic Analysis from Chronic Contusion Rats after AA-NgR(310)ecto-Fc Treatment Treatment.
Representative limb joint motion during one gait cycle in the anterior-posterior plane. Positions are normalized to the iliac crest (red dot) and the extent of foot swing is highlighted by the red bar and the gray shading. Examples are from separate rats in the IgG treatment group (A-E) and the AA-NgR-310 treatment group (F-J).

Supplemental Figure S5. Ankle Angle and Foot Velocity in Injured Rats.
A, The ankle angle and the foot velocity measurements for rats from the chronic contusion study of Figure 5 were collected from gait kinematics as in Figure 6 and are plotted as a function of time. The data are averages from 33-39 steps from each injured cohort. The vertical bars represent the time periods analyzed in B.
B, The ankle angle was averaged during the 50 msec which preceded by 200 msec the peak foot velocity time of each gait cycle, or which followed by 200 msec each foot movement. The data are averaged from 33-39 steps from each injured group, and the sem are shown. *, *P*<0.01, two-tailed *t* test.

Supplemental Figure S6. Tissue Sparing is Unaltered by AA-NgR(310)ecto-Fc Treatment.
The dorsal-ventral extent of spared tissue is plotted as a function of rostral caudal distance along the spinal cord for tissue from spinal contusion rats at 150 days post-injury as in Fig. 5B. Data are mean ± sem for n = 6-12 per group.

Supplemental Figure S7. Absence of Long Distance Corticospinal Axon Regeneration after Spinal Contusion.
A, The CST was traced by BDA injection at 150 days post spinal contusion injury. An example of a sagittal section of the spinal cord spanning the injury site stained for both GFAP (red) and CST axons (green) is from either the IgG or the AA-NgR(310)ecto-Fc treated group, as indicated. The lesion center is indicated by the vertical white line. Areas of rostral sprouting near the lesion are highlighted (blue arrows).
B, CST axon counts normalized to the labeling efficiency in the upper cervical cord are presented as a function of rostral-caudal distance above the center of the contusion site. The trend towards greater CST axon density 2 mm rostral to the contusion in the NgR treated group is not statistically significant. Data are mean ± sem for n = 6-12 per group.

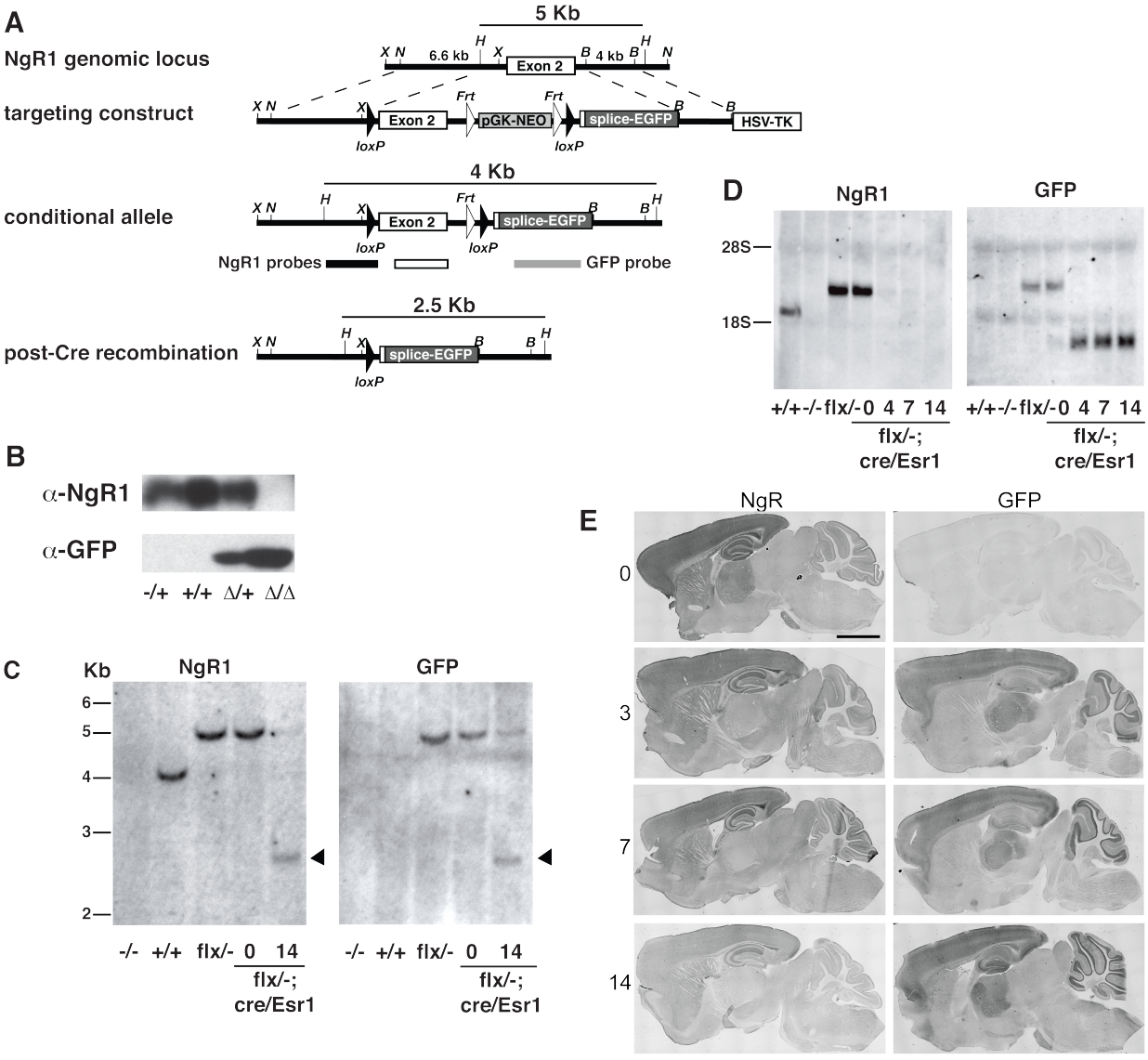

A
NgR1 genomic locus
5 Kb
X N 6.6 kb H X Exon 2 B 4 kb B H N
targeting construct
X N X Exon 2 Frt pGK-NEO Frt splice-EGFP B B HSV-TK
loxP loxP
conditional allele
4 Kb
X N H X Exon 2 Frt splice-EGFP B B H
loxP loxP
NgR1 probes
GFP probe
post-Cre recombination
2.5 Kb
X N H X splice-EGFP B B H
loxP
B
α-NgR1
α-GFP
-/+ +/+ Δ/+ Δ/Δ
C
Kb
NgR1
GFP
6
5
4
3
2
-/- +/+ flx/- 0 14
flx/-; cre/Esr1
-/- +/+ flx/- 0 14
flx/-; cre/Esr1
D
NgR1
GFP
28S
18S
+/+ -/- flx/- 0 4 7 14
flx/-; cre/Esr1
+/+ -/- flx/- 0 4 7 14
flx/-; cre/Esr1
E
NgR
GFP
0
3
7
14

Figure S1

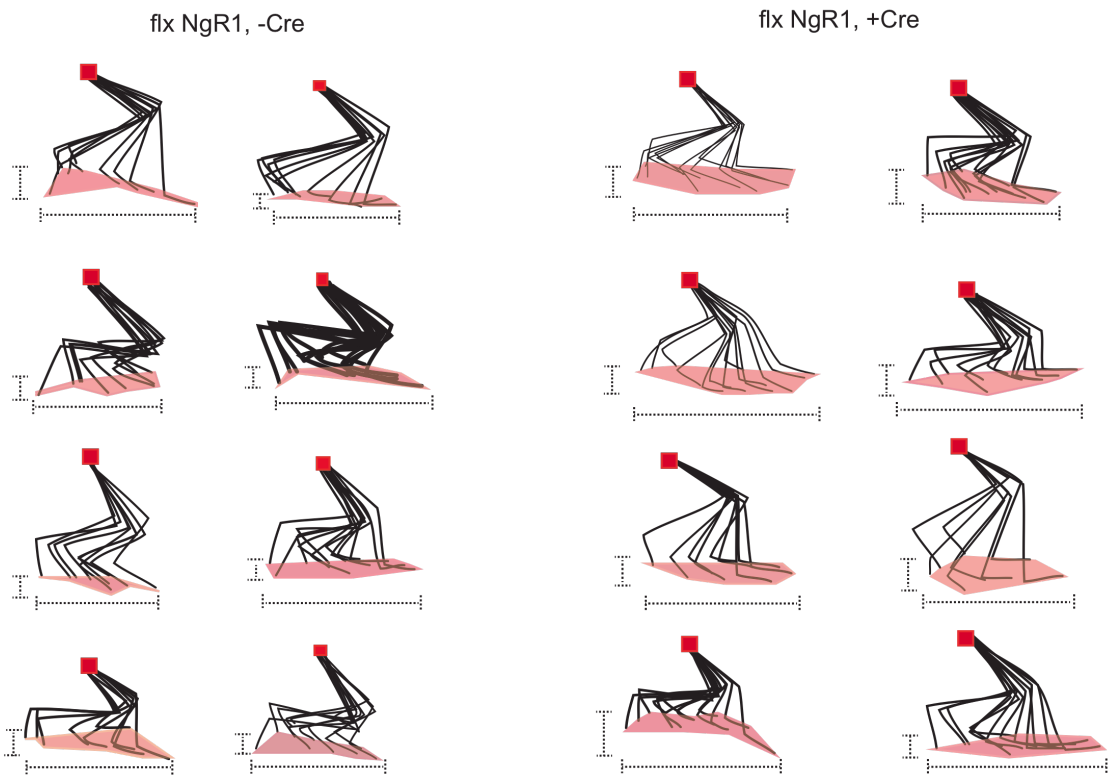


Figure S2

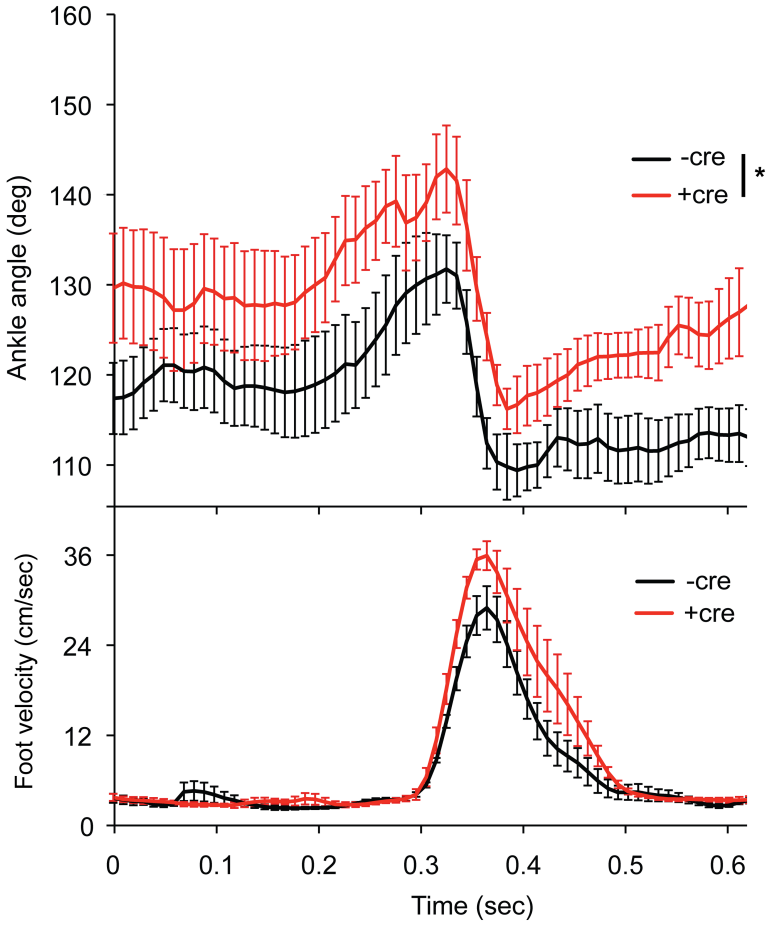

160
150
140
130
120
110
Ankle angle (deg)
-cre
+cre
*
36
24
12
0
Foot velocity (cm/sec)
-cre
+cre
0
0.1
0.2
0.3
0.4
0.5
0.6
Time (sec)

Figure S3

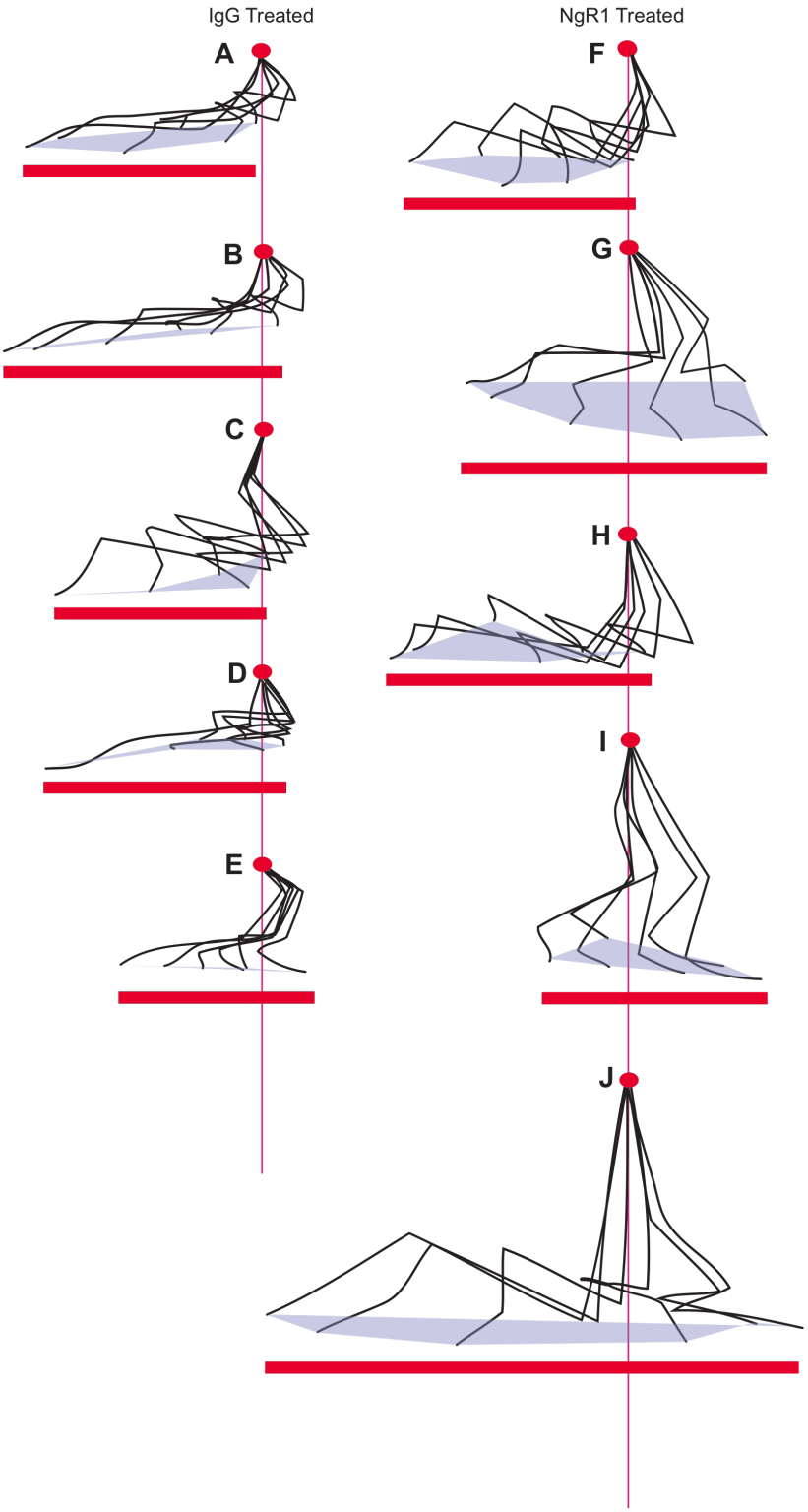


Figure S4

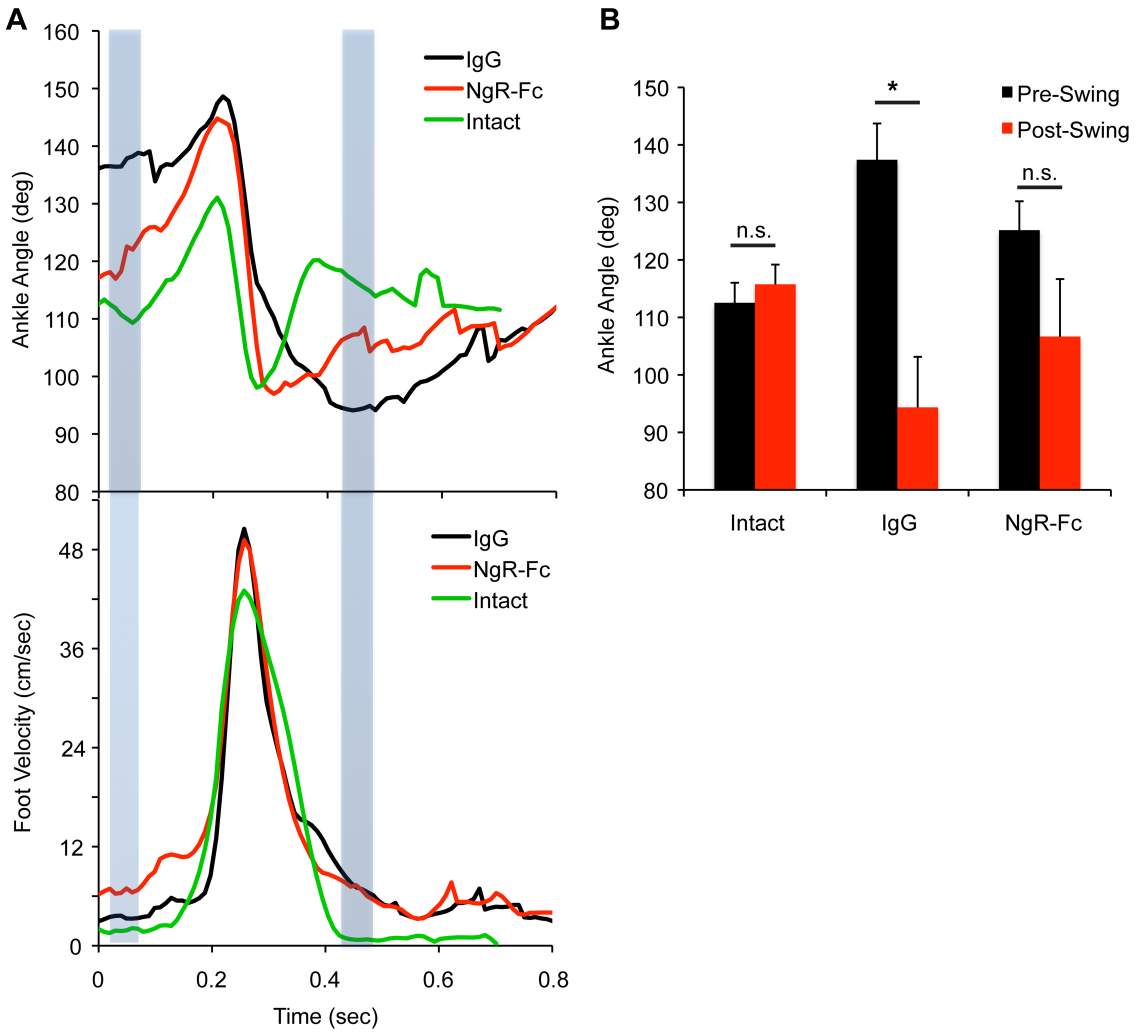

A
B
IgG
NgR-Fc
Intact
Ankle Angle (deg)
Foot Velocity (cm/sec)
Time (sec)
Pre-Swing
Post-Swing
n.s.
*
n.s.
Intact
IgG
NgR-Fc

Figure S5

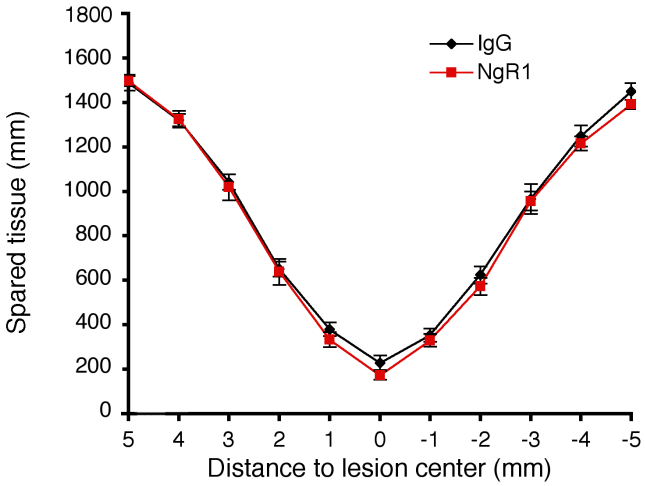
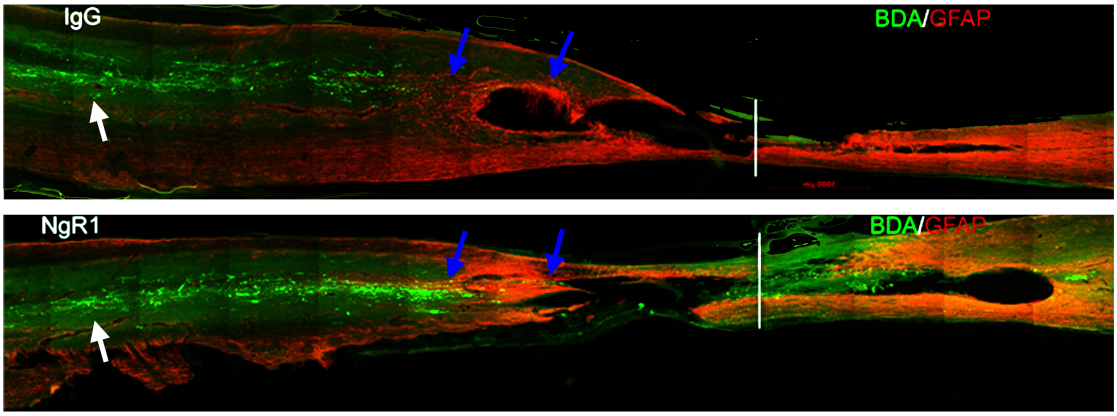

1800
1600
1400
1200
1000
800
600
400
200
0
Spared tissue (mm)
IgG
NgR1
5
4
3
2
1
0
-1
-2
-3
-4
-5
Distance to lesion center (mm)

Figure S6

A


IgG
BDA/GFAP
NgR1
BDA/GFAP

B

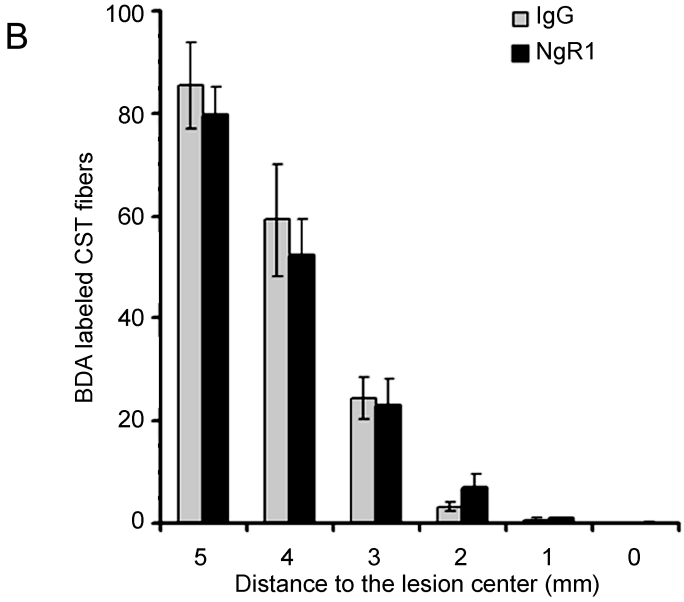

IgG
NgR1
BDA labeled CST fibers
100
80
60
40
20
0
5
4
3
2
1
0
Distance to the lesion center (mm)

Figure S7