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Supplemental Information

Scaffold-Mediated Sustained, Non-viral Delivery of miR-219/miR-338 Promotes CNS Remyelination

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Supplemental Methods

DRG neuron culture and transfection

Dorsal root ganglia were harvested from adult Sprague-Dawley rats and collected in DMEM/F12 medium in a petri dish. All DRGs were first desheathed to minimize culture contamination by other cells. Thereafter, the dissociated cells were seeded onto glass coverslips that were precoated with poly-D-lysine and laminin. Cells were maintained at 37 °C and 5% CO_2 in DMEM/F12 medium containing 1% penicillin–streptomycin, 10% horse serum, 1% of N2 and 50 ng/ml of NGF. One day after seeding, half of the medium was changed and 10 μM/well of cytosine arabinoside and 20 μM/well of 5-fluoro-2′-deoxyuridine were added to the culture. At the same time, the various transfection group and controls were implemented.

In total, 4 experimental groups containing 4 technical replicates and 3 biological replicates were established: i) Untreated cells; ii) cells treated with transfection reagent, TKO, only; iii) TKO-scrambled miR (Neg miR) complexes; and iv) TKO-miR-219/miR-338 complexes. To constitute the TKO-miR complexes, 1.5 μl of TransIT-TKO was diluted in 50 μl of DMEM before complexation with 1.5 μl of miRs (50 μM) at room temperature for 15 min. Cells were transfected for 24 h before they were fixed and immunostained.

Immunocytochemistry

DRG neuron cultures were fixed with 4% PFA in phosphate buffer containing 15% sucrose for 20 min. After washing in PBS for 3 times, cells were permeabilized with 0.3% Triton X-100 followed by blocking with 10% goat serum for 1 h at room temperature. Thereafter, the samples were incubated with mouse anti-βIII-Tubulin

(1:500 dilution) for 2 h at room temperature. Finally, all samples were incubated in goat anti-mouse Alexa Fluor 555 secondary antibody for 1.5 h at room temperature and cell nuclei were counterstained with DAPI.

In vitro DRG neurite measurements

Standardized exposure times were established for comparison between various treatment groups. Neurite measurements were performed using the ImageJ software, Simple neurite tracer plugin. At least 50 cells per condition were counted in each trial in terms of the average total neurite length and the longest neurite length.

In vivo neurofilament infiltration

A region of interest (ROI) was chosen by delimiting the area around the injury site (including scaffold) using the ImageJ software. Thereafter, all images were transformed into 8-bit gray scale to determine the number of pixels above a selected threshold. The lowest threshold was defined by selecting the pixel intensity of the scaffold without $NF200^{+}$ signal thus separating the positive signal from the background signal. The average area fraction of these pixels was measured and recorded.

Glial scar measurements

A ROI of 250 μm around the injury site was chosen for analyses as the area contained all prominent GFAP stained glial scar regions in all samples. Similar to the neurofilament infiltration measurements, all the images were transformed into 8-bit gray scale to determine the number of pixels above a selected threshold. To select the lowest threshold, GFAP intensity far away from the injury site was set as background. Thus, all signals that were above this threshold would be the reactive astrocytes

forming the glial scar. The average area fraction of these pixels was measured and recorded.

Supplemental Figures

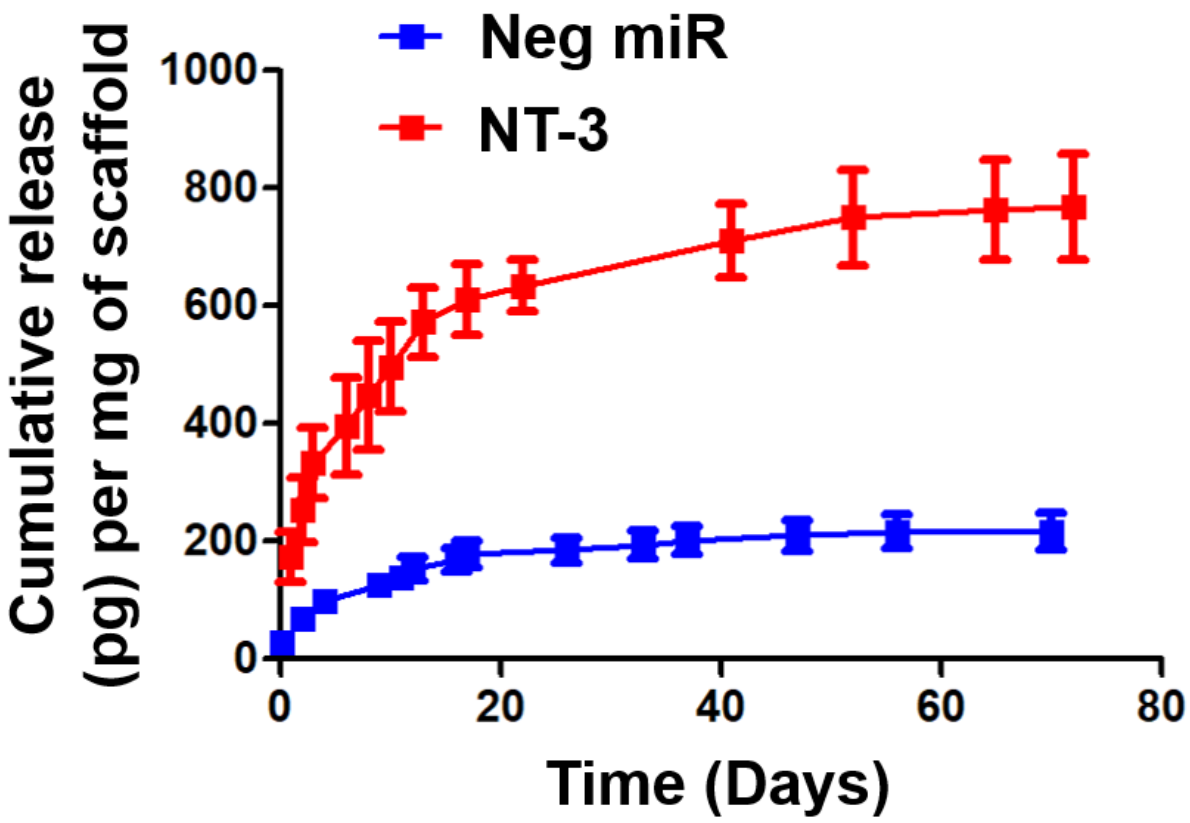

Figure S1. Cumulative release profile of Neg miR and NT-3. Data represented as mean ± SD.

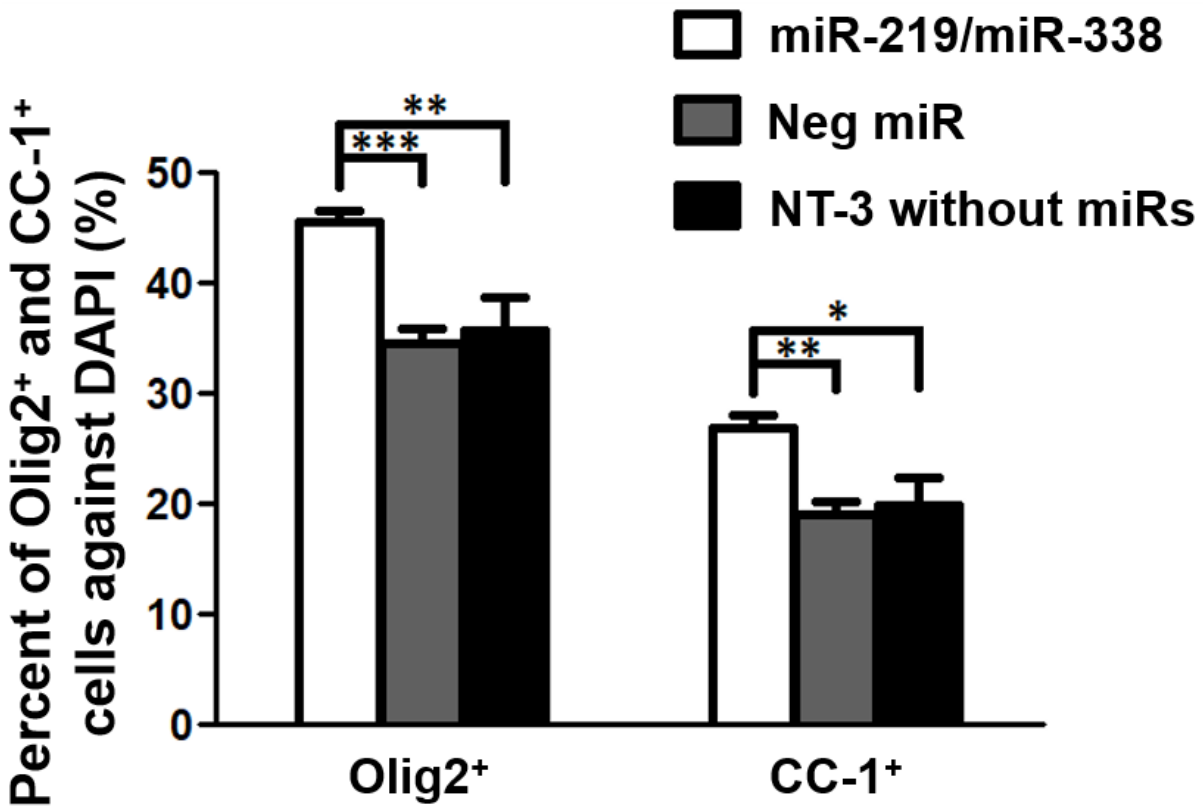

Figure S2. NT-3 alone did not prevent loss of oligodendroglial lineage Olig2$^+$ cells nor induced OPC maturation after SCI. *** p<0.001, ** p<0.01, * p<0.05 (ANOVA). Data represented as mean ± SEM.

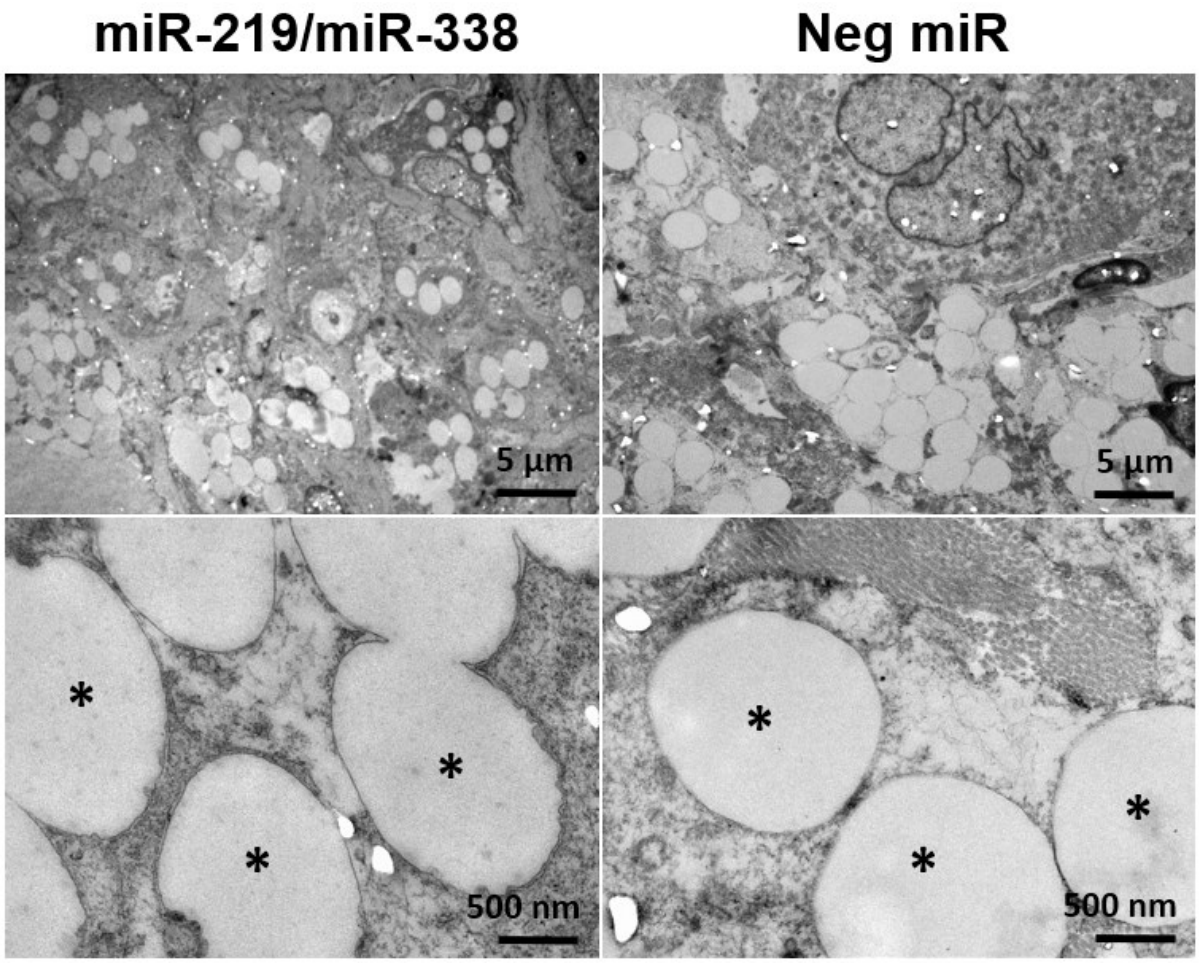

Figure S3. Representative transmission electron microscopy images within the scaffold of both miR-219/miR-338 and Neg miR group. Black asterisk denotes a cross sectional view of an individual electrospun PCLEEP fiber. No compact myelin sheaths were observed around the electrospun fibers screened in both groups.

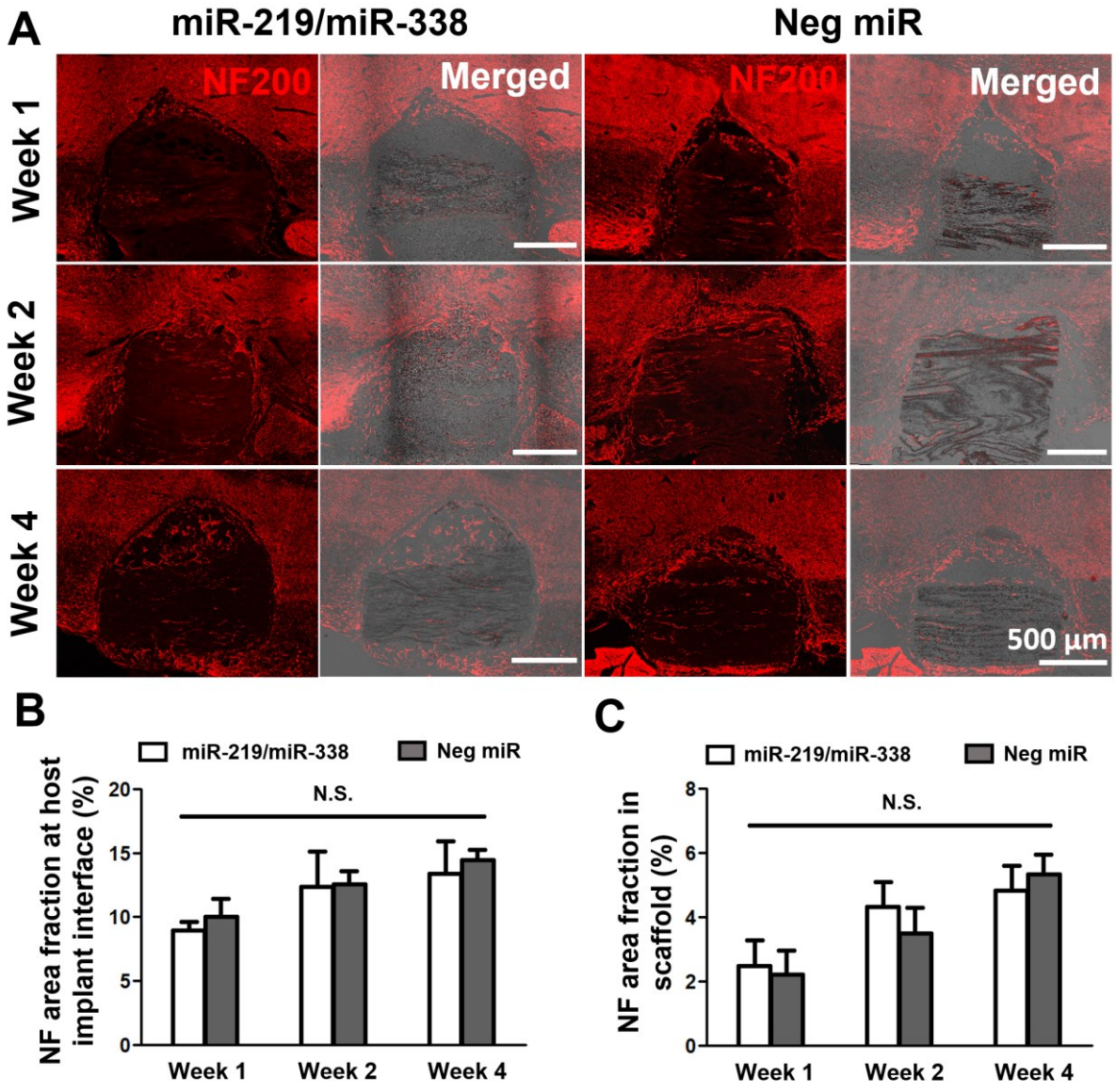

Figure S4. Scaffold-mediated non-viral delivery of miR-219/miR-338 did not affect neurite regeneration *in vivo*. (A) NF200 expression (red) at injury sites and light micrograph of fiber-hydrogel scaffolds in miR-219/miR-338 (left) and Neg miR group (right). **(B)** Percentage of host-implant interface area occupied by neurofilament ingrowth. **(C)** Percentage of scaffold area occupied by neurofilament ingrowth. N.S. - not significant (ANOVA). Data represented as mean ± SEM. Scale bars represent 500 µm.

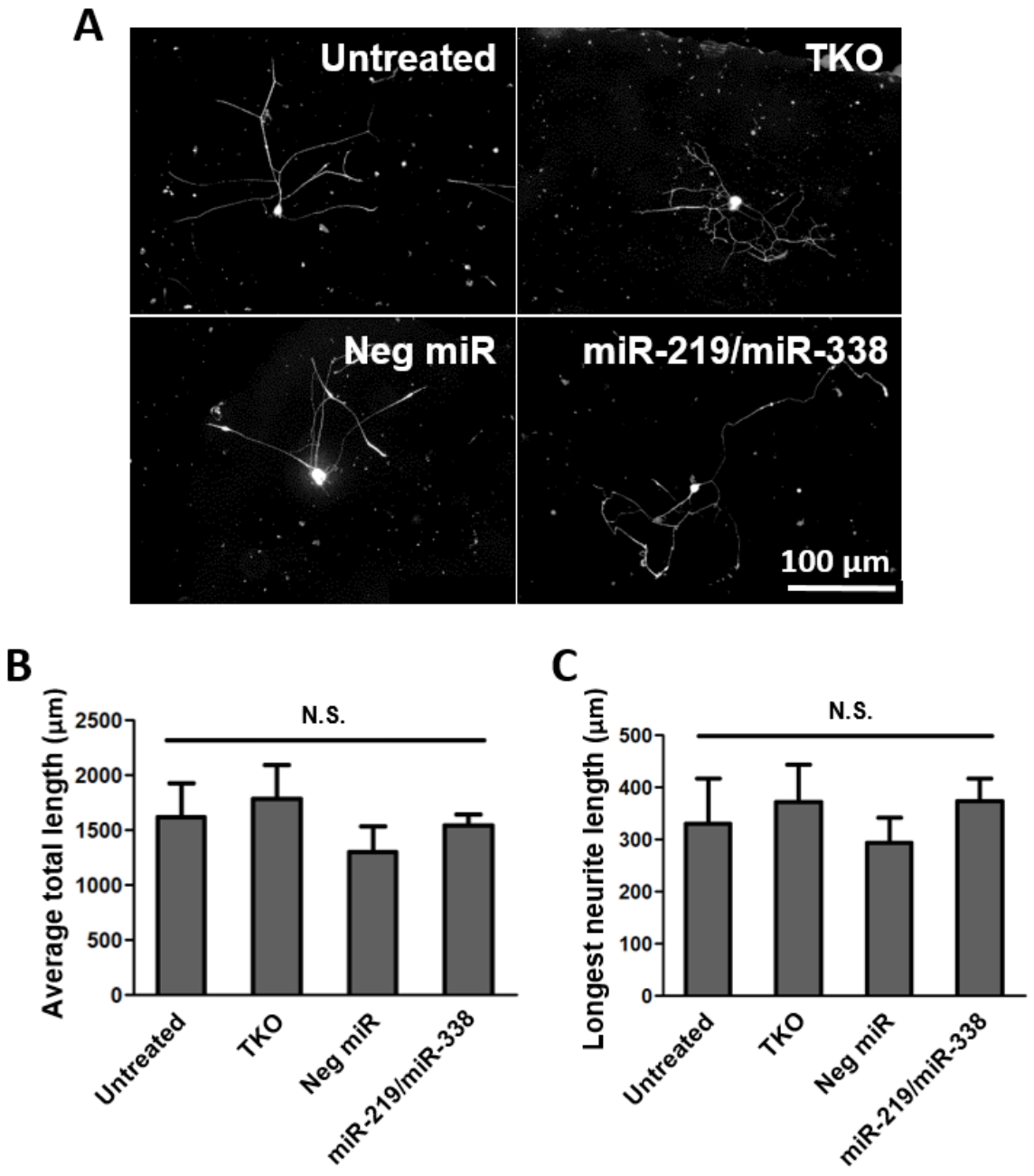

Figure S5. miR-219/miR-338 has no effect on neurite growth *in vitro*. (A). Representative images of Untreated, TKO, Neg miR and miR-219/miR-338. **(B)** Average total length of neurites. **(C)** Average longest length of neurite. N.S. - not significant (ANOVA). Data represented as mean ± SEM.

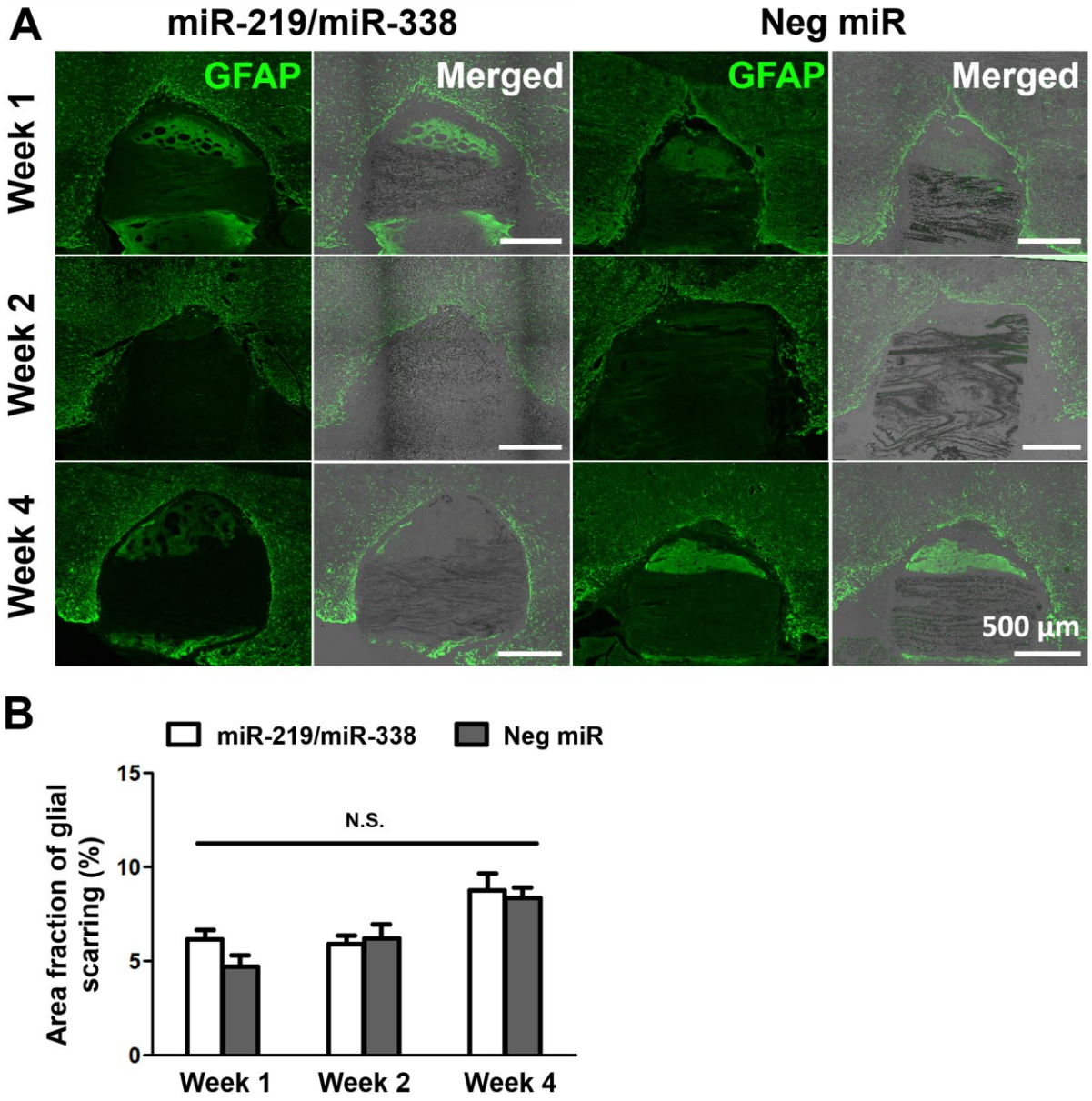

Figure S6. Scaffold-mediated non-viral delivery of miR-219/miR-338 did not affect glial scar formation. **(A)** GFAP expression (green) at injury sites and light micrograph of fiber-hydrogel scaffolds in miR-219/miR-338 (left) and Neg miR group (right). **(B)** Area fraction of glial scar formed at perilesion region. N.S. - not significant (ANOVA). Data represented as mean ± SEM. Scale bars represent 500 µm.

A

Olig2$^+$ cell density analysis		
Treatment	vs	p-value
Week 1 Neg miR	Week 4 miR-219/miR-338	0.002
	Week 4 Neg miR	0.021
Week 2 miR-219/miR-338	Week 2 Neg miR	0.001
	Week 4 Neg miR	0.015
Week 2 Neg miR	Week 4 miR-219/miR-338	0.004
Week 4 miR-219/miR-338	Week 4 Neg miR	0.045

B

PDGFRα$^+$ cell density analysis		
Treatment	vs	p-value
Week 1 miR-219/miR-338	Week 2 miR-219/miR-338	0.000
	Week 2 Neg miR	0.000
	Week 4 miR-219/miR-338	0.000
	Week 4 Neg miR	0.000
Week 1 Neg miR	Week 2 miR-219/miR-338	0.000
	Week 2 Neg miR	0.000
	Week 4 miR-219/miR-338	0.000
	Week 4 Neg miR	0.000
Week 2 miR-219/miR-338	Week 4 miR-219/miR-338	0.001
	Week 4 Neg miR	0.0206
Week 2 Neg miR	Week 4 miR-219/miR-338	0.001
	Week 4 Neg miR	0.011

C

CC-1$^+$ cell density analysis		
Treatment	vs	p-value
Week 1 miR-219/miR-338	Week 2 miR-219/miR-338	0.014
	Week 4 miR-219/miR-338	0.000
	Week 4 Neg miR	0.014
Week 1 Neg miR	Week 2 miR-219/miR-338	0.016
	Week 4 miR-219/miR-338	0.000
	Week 4 Neg miR	0.0016
Week 2 miR-219/miR-338	Week 2 Neg miR	0.01
	Week 4 miR-219/miR-338	0.001
Week 2 Neg miR	Week 4 miR-219/miR-338	0.000
	Week 4 Neg miR	0.0011
Week 4 miR-219/miR-338	Week 4 Neg miR	0.005

Table S1. Statistical significance of differences between **(A)** Olig2$^+$; **(B)** PDGFRa$^+$; and **(C)** CC-1$^+$ cell density in different experimental groups (ANOVA).