

Supplemental material

Supplemental Table 1 **Clinical characteristics of study participants.** NIL = neurological injury level. C = cervical; T = thoracic; L = lumbar; AIS = ASIA Impairment Score. A = motor and sensory complete SCI; B = motor complete and sensory incomplete SCI; C = Motor incomplete SCI (more than half of key muscle functions below the NIL have a muscle grade less than 3); D = Motor incomplete SCI (at least half of key muscle functions below the NIL have a muscle grade of 3 or greater); Δ = change; UEMS = upper extremity motor score; LEMS = lower extremity motor score; TMS = total motor score; LT = light touch sensation; PP = pin prick sensation. * Included patients had >3 month follow up (range 3-6 months)

Patient ID	Sex	Age	NIL	Baseline AIS	Follow-up AIS*	Follow-up NIL*	Δ UEMS	Δ LEMS	Δ TMS	Δ LT	Δ PP
1	M	20	L1	C	A	L2	0	-2	-2	4	4
2	M	76	C5	B	C	C7	23	22	55	-1	-2
3	M	46	C7	A	D	C7	20	26	46	44	44
7	M	79	C4	C	D	C4	39	11	50	0	0
8	F	45	C4	A	A	C4	10	10	20	0	0
9	M	25	T11	A	A	T11	0	0	0	0	0
10	M	60	C5	A	B	C4	-5	-1	-6	-1	-1
11	M	22	C5	A	A	C5	0	0	0	0	0
12	F	69	C5	C	D	C5	0	16	16	0	0
13	M	22	T6	A	A	T6	0	0	0	0	0
14	M	68	C5	B	C	C4	6	11	17	0	0
15	M	35	C6	B	D	C6	3	32	35	0	0
17	M	59	C3	A	A	C3	2	0	2	2	2
19	M	47	C4	A	B	C4	6	0	6	2	3
20	M	34	T4	A	A	T4	0	0	0	0	0
21	F	32	C3	A	A	C2	0	0	0	-4	-4
22	M	52	C4	B	C	C4	18	18	36	20	60
24	M	49	C7	B	C	C4	-17	20	3	33	32
27	M	68	C4	C	D	C8	48	4	52	50	50
28	M	59	C4	C	A	C4	0	5	5	22	22
29	M	78	C4	C	D	C4	28	12	40	0	0
31	M	57	C4	B	D	C4	37	28	65	48	48
33	M	28	C5	C	D	C5	2	30	32	9	0
34	M	30	C4	B	C	C4	3	25	28	8	8
35	M	71	C4	A	B	C4	0	0	0	14	14

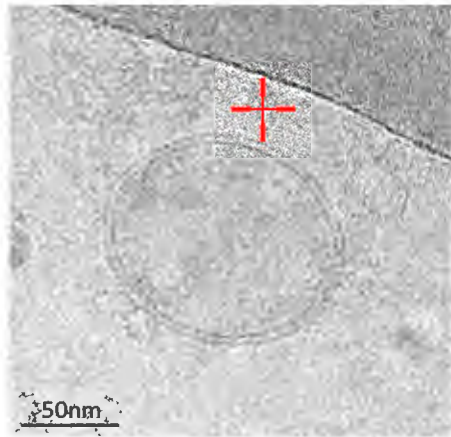
Supplemental Table 2 **The hub genes identified by WGCNA.** The hub genes were filtered by $|MM| > 0.8$ and $|GS| > 0.2$.

Model	Number	hub genes
purple	6	hsa-miR-6746-3p, hsa-miR-451a, hsa-miR-197-3p, hsa-miR-424-3p, hsa-miR-877-3p, hsa-miR-7111-3p
midnightblue	7	hsa-miR-9-3p, hsa-miR-136-3p, hsa-miR-1185-1-3p, hsa-miR-374b-5p, hsa-miR-4290, hsa-miR-374c-3p, hsa-miR-6809-3p
cyan	7	hsa-miR-125b-1-3p, hsa-miR-485-5p, hsa-miR-1306-5p, hsa-miR-3154, hsa-miR-5009-5p, hsa-miR-5193, hsa-miR-6741-3p
turquoise	29	hsa-miR-99a-5p, hsa-miR-29a-3p, hsa-miR-103a-3p, hsa-miR-103b, hsa-miR-29b-3p, hsa-miR-449b-5p, hsa-miR-210-3p, hsa-miR-125a-5p, hsa-let-7d-5p, hsa-miR-141-3p, hsa-miR-200c-3p, hsa-miR-15b-5p, hsa-miR-449a, hsa-miR-10b-5p, hsa-miR-21-5p, hsa-miR-429, hsa-miR-449c-5p, hsa-miR-31-5p, hsa-miR-24-3p, hsa-miR-34c-5p, hsa-miR-7-5p, hsa-miR-3529-3p, hsa-miR-20a-5p, hsa-miR-361-5p, hsa-miR-191-5p, hsa-miR-200b-3p, hsa-miR-130a-3p, hsa-miR-99b-5p, hsa-miR-3182
Brown	23	hsa-miR-4787-5p, hsa-miR-7853-5p, hsa-miR-32-5p, hsa-miR-200a-5p, hsa-miR-6869-5p, hsa-miR-6126, hsa-miR-302a-3p, hsa-miR-302b-3p, hsa-miR-28-5p, hsa-miR-342-3p, hsa-miR-365a-3p, hsa-miR-365b-3p, hsa-miR-4785, hsa-miR-324-5p, hsa-miR-1303, hsa-miR-8485, hsa-miR-27a-5p, hsa-miR-32-3p, hsa-miR-5196-5p, hsa-miR-4429, hsa-miR-941, hsa-miR-518b, hsa-miR-148b-3p
blue	14	hsa-miR-133a-3p, hsa-miR-133b, hsa-let-7i-5p, hsa-miR-1-3p, hsa-miR-378a-3p, hsa-miR-22-5p, hsa-miR-6529-5p, hsa-miR-199a-5p, hsa-miR-193b-3p, hsa-miR-30e-3p, hsa-miR-378c, hsa-miR-133a-5p, hsa-miR-206, hsa-miR-27b-3p

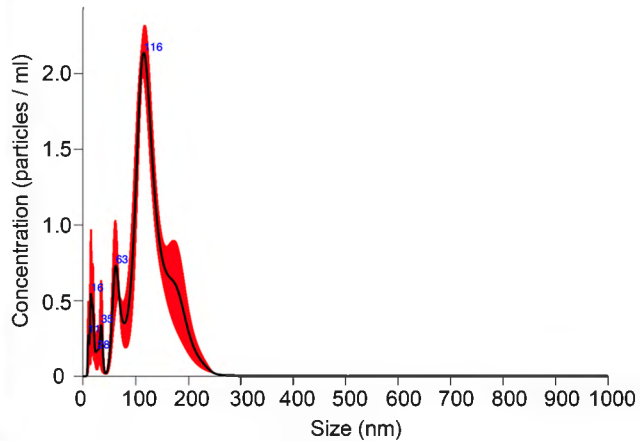
Supplemental Table 3 miRNA mimic/inhibitor sequences

Cat. Number	Description microRNA mimics	Estimated bus sequence
C-300491-03	Human hsa- miR-20a-5p	UAAAGUGCUUAUAGUGCAGGUAG
C-300723-03	Human hsa- miR-449a	UGGCAGUGUAUUGUUAGCUGGU
C-301180-01	Human hsa- miR-16-2-3p	CCAAUAUUACUGUGCUGCUUUA
C-300682-05	Human hsa- miR-375	UUUGUUCGUUCGGCUCGCGUGA
C-300542-05	Human hsa- miR-30c-5p	UGUAAACAUCCUACACUCUCAGC
C-300664-05	Human hsa- miR-362-5p	AAUCCUUGGAACCUAGGUGUGAGU
C-303064-00	Human hsa- miR-6873-3p	UUCUCUCUGUCUUUCUCUCUCAG
C-301238-01	Human hsa- miR-708-5p	AAGGAGCUUACAAUCUAGCUGGG
IH-303098-01	Human has- miR-7111-3p inhibitor	AUCCUCUCUUCCCUCCUCCAG
IH-300643-05	Human hsa- miR-195-5p inhibitor	UAGCAGCACAGAAAUUUGGC
CN-001000-01	miRIDIAN microRNA Mimic Negative Control #1	

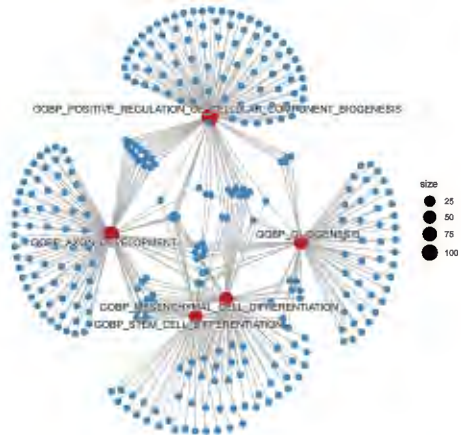
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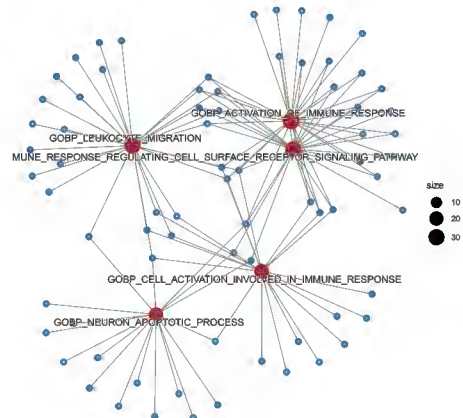
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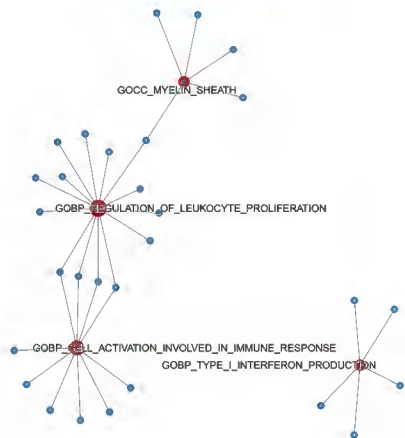
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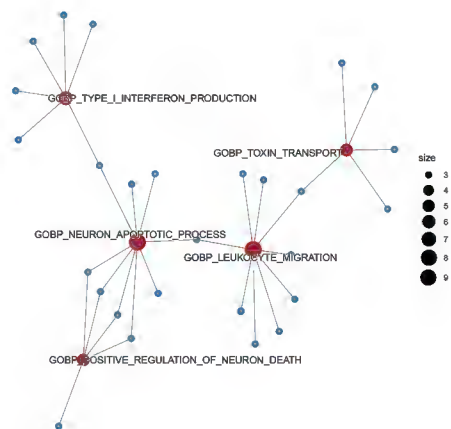
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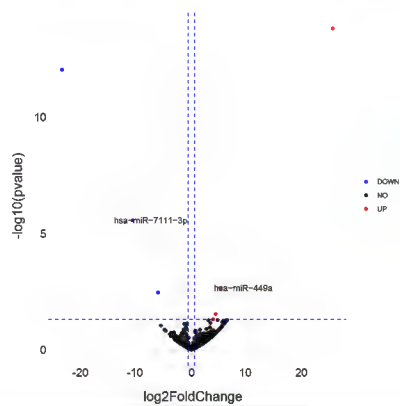
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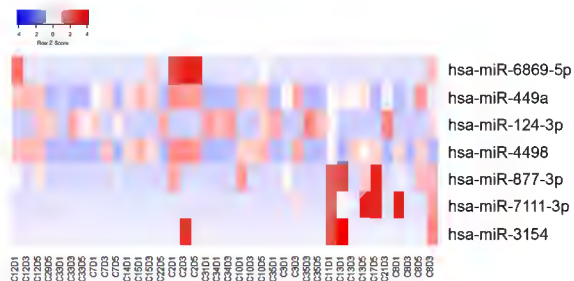
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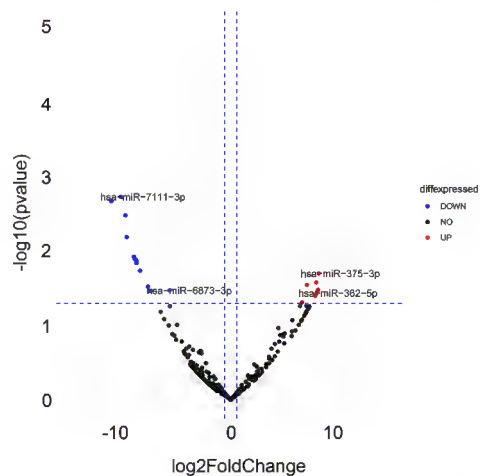
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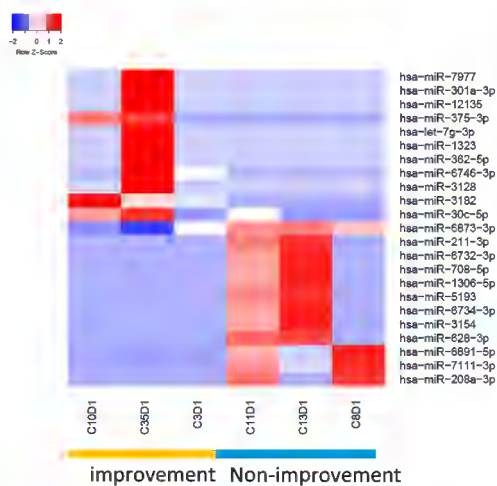
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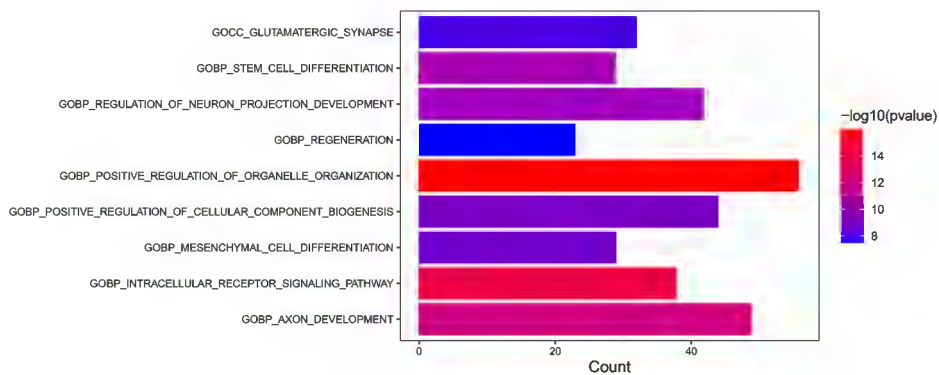
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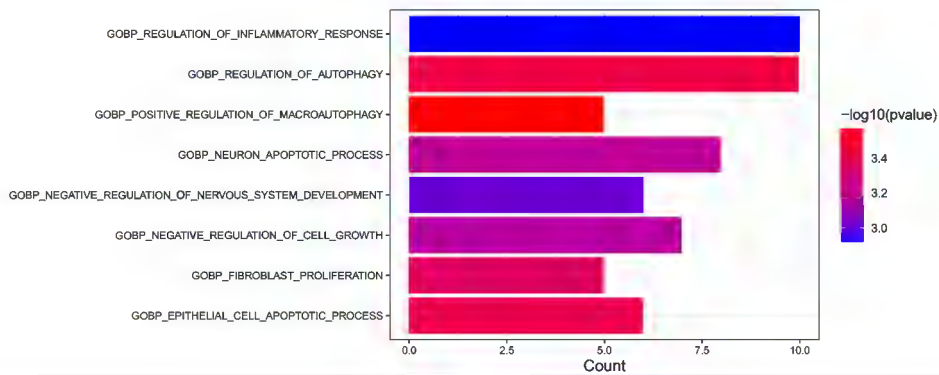
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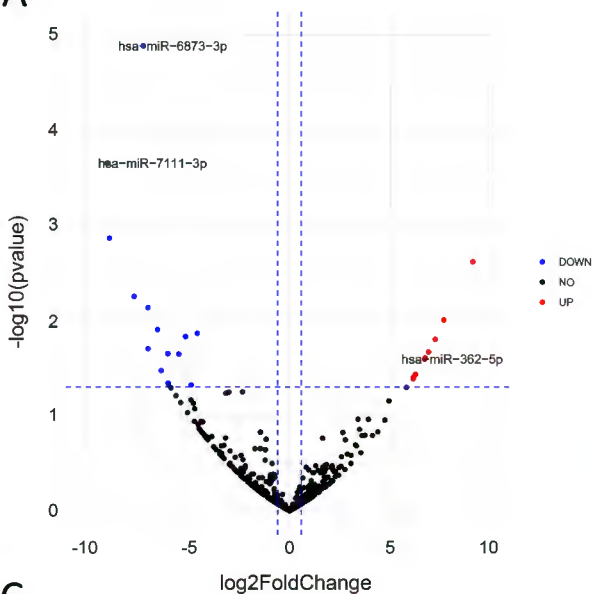
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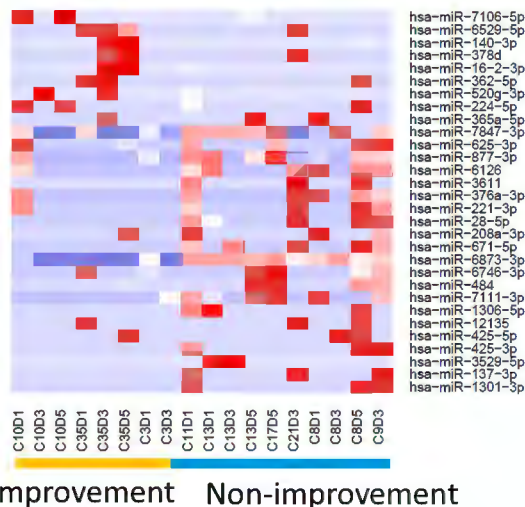
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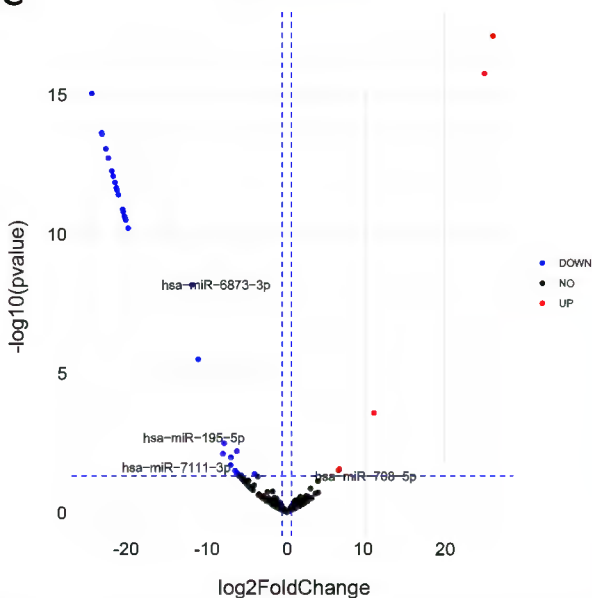
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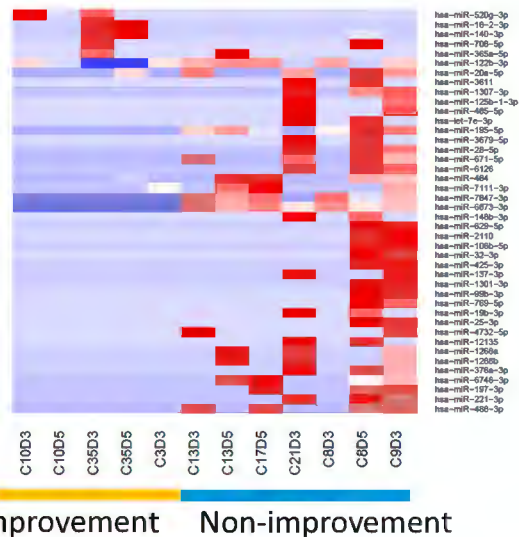
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C



D



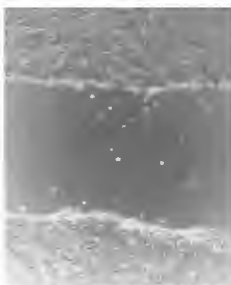
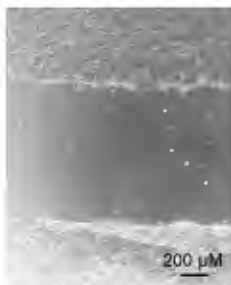
A

Control

hsa-miR-16-2-3p

hsa-miR-20a-5p

0hrs



36hrs

**B**

Control

hsa-miR-362-5p

0hrs

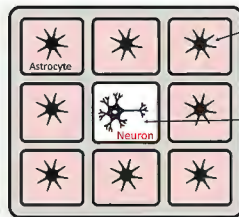


36hrs



Coculture system

ibidi coculture chamber slide



Human Astrocytes (SVG-p12)
in the peripheral chambers
and treated with miRNAs

Neurons (SH-SY5Y) cells

DMEM + 2.5% hiFBS
1% GlutaMAX
1% B27
1% Pen/Strep
10 μ M RA

Medium A
Day 0-4

Neurobasal + DMEM
1% hiFBS
1% GlutaMAX
1% B27
1% Pen/Strep
20 mM KCl
10 μ M RA

Medium B
Day 4-7

Neurobasal
1% hiFBS
1% GlutaMAX
1% N2 supplement
1% Pen/Strep

Neurite Growth Medium
Day 7-11

Astrocytes seeded
(Day 5)

Astrocyte scratch
(Day 7)

miRNA treatment
(Day 8)

Excess medium added
(Day 9)

0

1

2

3

4

5

6

7

8

9

10

11

Day of Culture

Supplemental Figure 1 (A) Cryo-EM images of EVs isolated from human CSF of spinal cord injury patients. Scale bar: 50 nm. (B) Nano-tracking analysis (NTA) of CSF.

Supplemental Figure 2 (A-D) The network in each module visualization highlights enriched functional categories and their associated genes. Functional enrichment analysis (GO) for module Blue (A), Cyan (B), Midnight blue (C) and Purple (D) was performed based on the target genes of hub miRNAs. Target genes of these hub miRNAs were determined using multiMiR, referencing TarBase (v9.0) and miRTarBase (v9.0). Hub miRNAs were identified in the module using the criteria $|MM| > 0.8$ and $|GS| > 0.2$. Blue dots indicate DEGs with fold-changes ≤ -1.5 and p-value < 0.05 , while red dots represent DEGs with fold-changes ≥ 1.5 and p-value < 0.05 (E) Volcano plot and (F) heatmap of differentially expressed genes (DEGs) between patients with improvement and non-improvement injuries among all CSF samples.

Supplemental Figure 3 (A) Volcano plot and (B) heatmap of DEGs between patients with improvement and non-improvement injuries on day 1. Blue dots indicate DEGs with fold-changes ≤ -1.5 and p-value < 0.05 , while red dots represent DEGs with fold-changes ≥ 1.5 and p-value < 0.05 . (C, D) Gene ontology enrichment analysis of significant miRNA target genes, highlighting enrichment in the improvement group (C) and the non-improvement group (D). Bar plot color represents the $-\log_{10}(\text{p-value})$, while "Count" indicates the number of genes enriched in each GO term.

Supplemental Figure 4 (A-D). The Volcano plot and heatmap of DEGs between patients with improvement and non-improvement injuries on day 1/3/5 with samples from day1, day 3 and day 5 combined (A, B) and day 3/5, with samples from day 3 and day 5 combined (C, D)

Supplemental Figure 5 Results of scratch assay at 36 hours without significant changes after miRNA mimic or inhibitor treatments. A and B: images of scratch area from groups at 0- and 36-hour time points

Supplemental Figure 6 Diagram illustrating the workflow of astrocyte-neuron co-culture