miR-21 improves the neurological outcome after traumatic brain injury in rats

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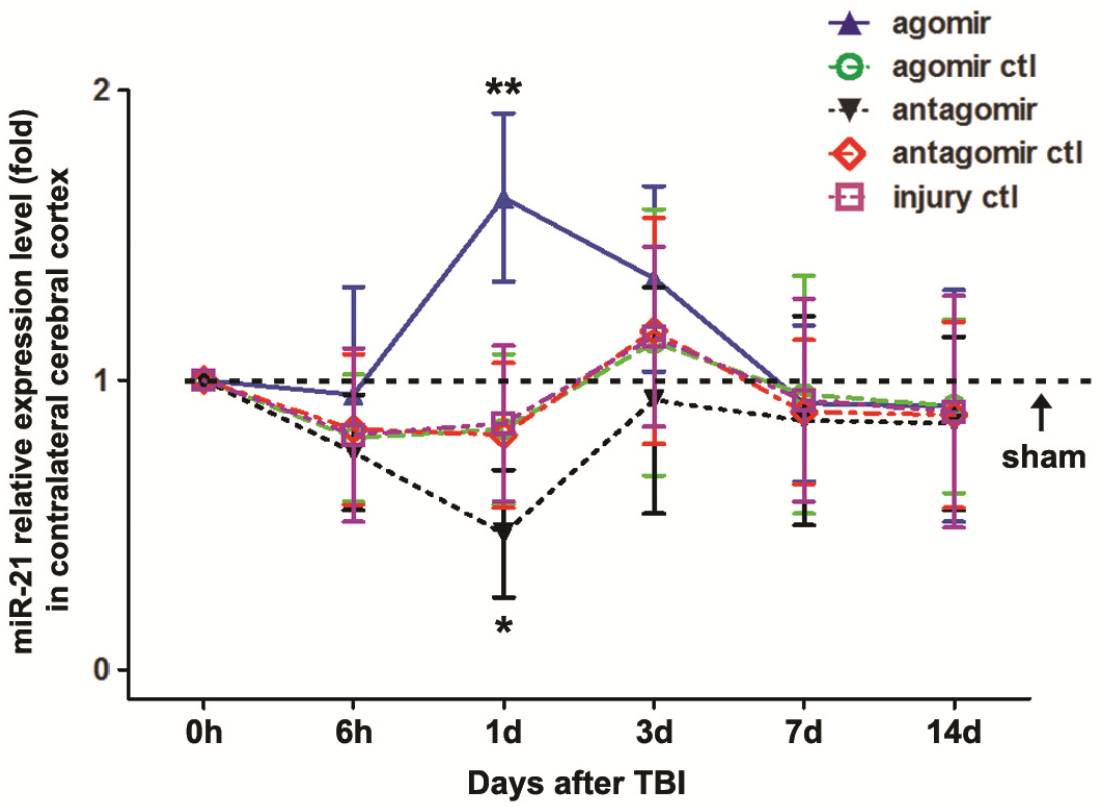
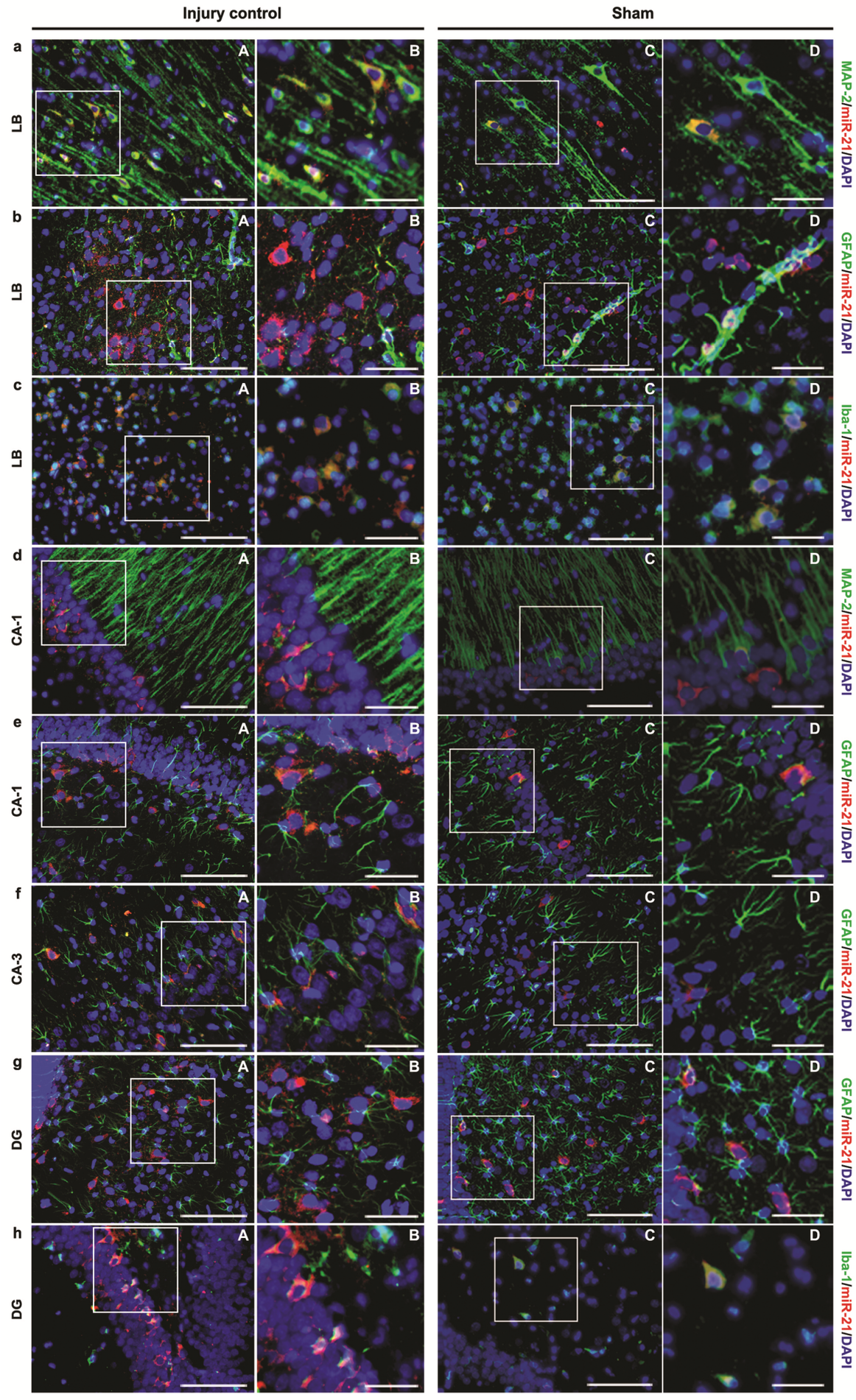
Supplementary Figure 1. Altered miR-21 level in the contralateral cerebral cortex after TBI and miR-21 oligomers intervention.



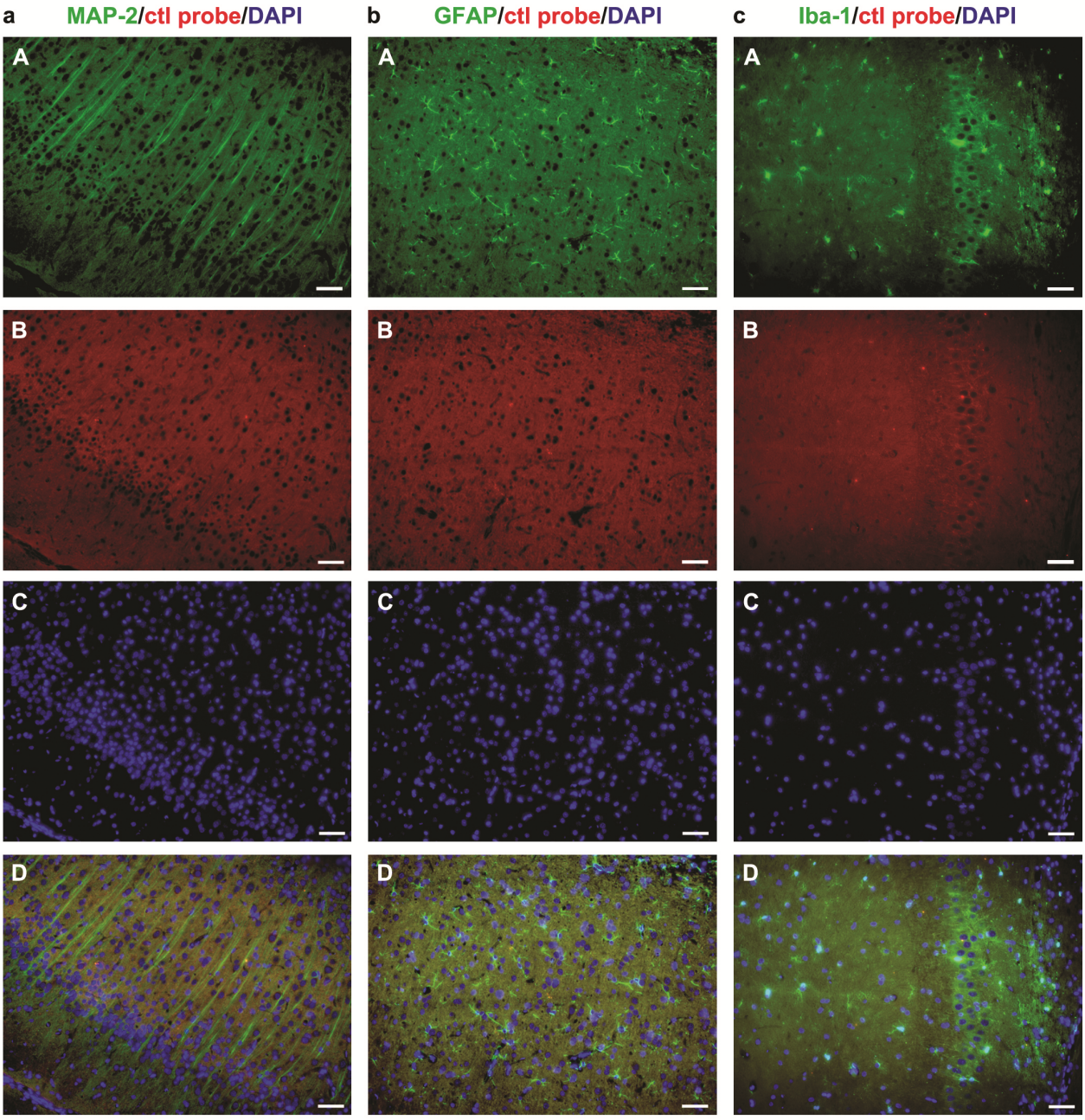
The temporal profile (from 0 h to 14 d post-injury) of miR-21 level in the contralateral cerebral cortex determined by qRT-PCR. The quantitative data were analyzed using the $2^{-\Delta\Delta Ct}$ method, in which the miR-21 levels of the *sham* group (presented as the dotted line) were used as controls. Note that the miR-21 level in the contralateral cerebral cortex were not altered after TBI (*injury ctl* group). The miR-21 level at 1 d post-injury can be upregulated (or downregulated) after infusing miR-21 agomir (or antagomir), but its variation extent is much smaller (less than 2-fold alteration) than that in the traumatic foci (more than 7-fold alteration). No difference was observed at other time-points between the *agomir* (or *antagomir*) group and *injury ctl* group. The data are expressed as mean ± SD. (n = 6) (*P<0.05, **P<0.01 versus the *injury ctl* group)

Supplementary Figure 2. Representative images of the in-situ immunostained miR-21 in neurons, astrocytes and microglias in different areas of injured brain at 72 h post-injury compared to that of sham group.



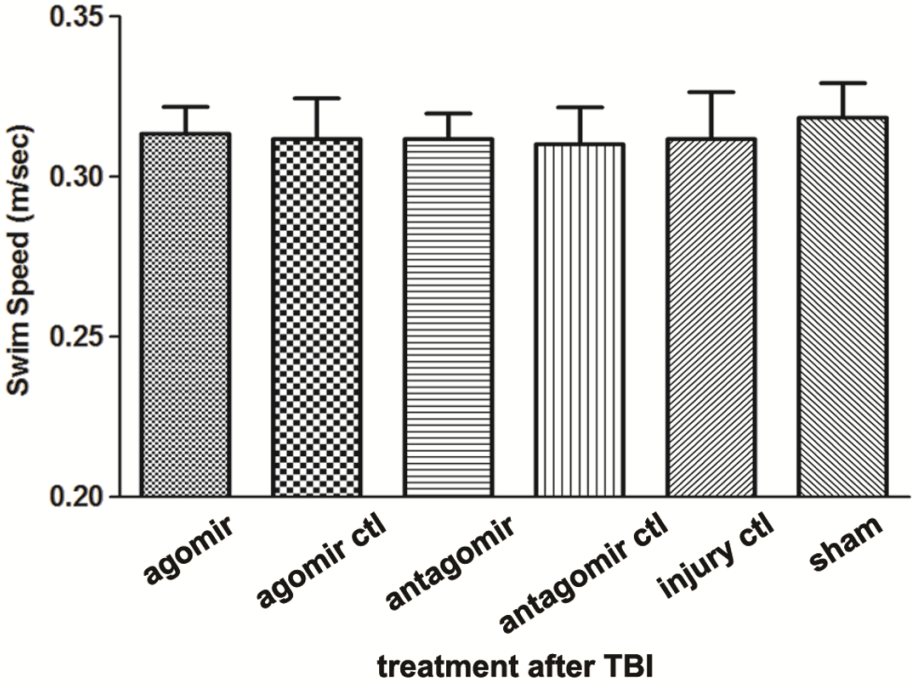
The combined staining of miR-21/MAP-2 (a), miR-21/GFAP (b), miR-21/Iba-1 (c) in the lesioned boundary of cerebral cortex (LB, scope see Figure 3e). The combined staining of miR-21/MAP-2 (d) and miR-21/GFAP (e) in the CA1 (a subdivision of Ammon's horn). The combined staining of miR-21/GFAP (f) in the CA3 (a subdivision of Ammon's horn). The combined staining of miR-21/GFAP (g) and miR-21/Iba-1 (h) in the dentate gyrus (DG). In each set of the figures, Figure A and B were captured from the *injury ctl* group, and Figure C and D were captured from the *sham* group. The immunostained areas in the white box were magnified from Figure A to B and from Figure C to D. Note that the expression of miR-21 in neurons, astrocytes and microglias were increased at 72 h post-injury. Scale bars: A, C 50μm; B, D 20μm. (n = 6)

Supplementary Figure 3. The in-situ staining of control probe in neurons, astrocytes and microglias in brain.



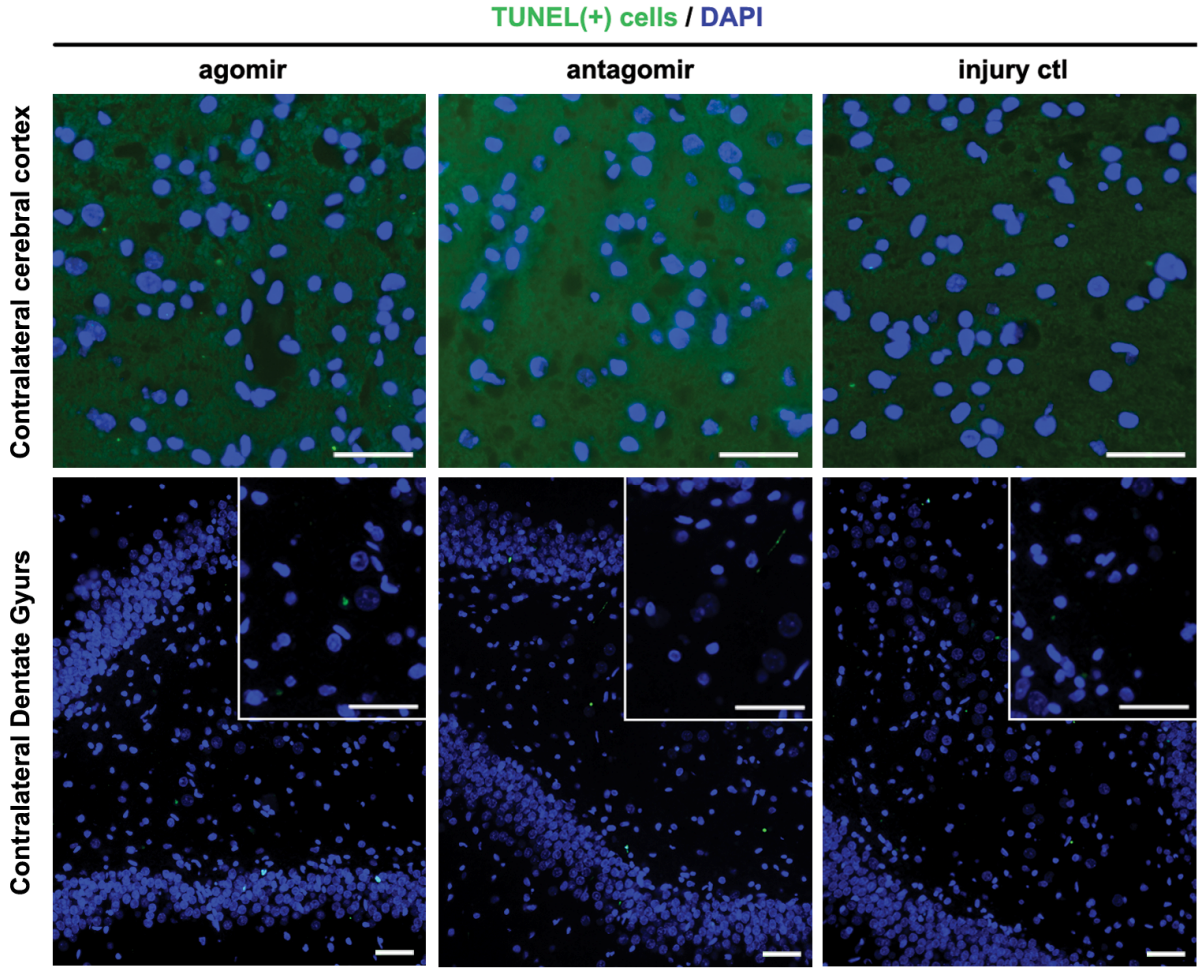
The combined staining of control probe with MAP-2 (a), GFAP (b), and Iba-1 (c) in brain. No co-labeling were observed in the sections. All figures were captured from the sham group. Scale bars: 50μm. (n = 6)

Supplementary Figure 4. Swim speeds of each group in the Morris Water Mass test.



In the Morris Water Mass test, no difference was observed in swim speed among the groups (one-way ANOVA, $F(5, 30) = 0.070$, $P = 0.996$). The data are expressed as mean ± SD. ($n = 6$)

Supplementary Figure 5. The impact of miR-21 on cellular apoptosis in contralateral brain after TBI.



The immnuostaining of apoptotic cells in the cerebral cortex and the dentate gyrus of contralateral brain at 72 h post-injury. No difference was observed in apoptosis among the groups, suggesting that the impact of miR-21 on cellular apoptosis in brain after TBI is specific to the injury area. (n = 6)

Supplementary Table 1. Information about the primary antibodies used in the study.

Antibody	Manufacturer	Catalogue	Cellular Localization	Application
CD31	Santa Cruz	sc-53526	Membrane	MVD
MAP-2	Abcam	ab32454	Cytoplasm	combined FISH and IF staining
GFAP	ZSJB-BIO	ZA-0117	Cytoplasm	combined FISH and IF staining
Iba-1	Abcam	ab5076	Cytoplasm	combined FISH and IF staining
PTEN	CST	9559	Cytoplasm	WB
p-AKT(Ser473)	CST	9271	Cytoplasm	WB
AKT	CST	9272	Cytoplasm	WB
Bcl-2	CST	2876	Mitochondrion membrane	WB
Bax	Abcam	ab7977	Mitochondrion membrane	WB
Caspase-3	CST	9665	Cytoplasm	WB
VEGF	Abcam	ab68334	Secreted	IF & WB
Ang-1	Abcam	ab133425	Secreted	IF & WB
Tie-2	Millipore	Ab33 05-584	Membrane	IF & WB
GAPDH	ZSJB-BIO	TA-08	Cytoplasm	WB
α-tubulin	Boster	BM1452	Cytoplasm	WB

Supplementary Table 2. The sequences of miRNA oligomers.

miRNA oligomers	Sequences
miR-21 agomir	5′-UAGCUUAUCAGACUGAUGUUGA-3′
agomir negative control	5′-UUCUCCGAACGUGUCACGUTT-3′
miR-21 antagomir	5′-UCAACAUCAGUCUGAUAAGCUA-3′
antagomir negative control	5′-CAGUACUUUUGUGUAGUACAA-3′