SUPPLEMENTAL MATERIAL

Activation of Neuronal Ras-Related C3 Botulinum Toxin Substrate 1 (Rac1) Improves Post-Stroke Recovery and Axonal Plasticity in Mice

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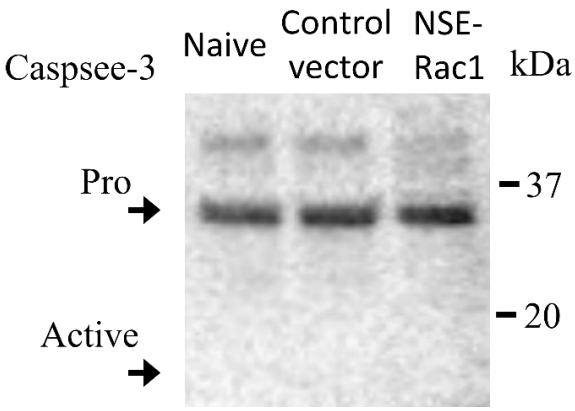

Figure S1. Delayed cerebral injection of lentiviral vector encoding Rac1 and GFP with NSE promotor did not cause cell death in mice. Ipsilateral hemisphere was dissected and homogenized 7 days after injection to detect cleaved caspase-3 by western blotting. Sham mice were treated by control vectors or NSE-Rac1 vectors without stroke surgery. Mice without vectors injection and stroke surgery were used as naïve control. The result shows that similar as the naïve group, there is no active caspase-3 (cleaved) was detected in the sham-operated groups, suggesting that lentiviral vector did not lead to cell death in our preparation.

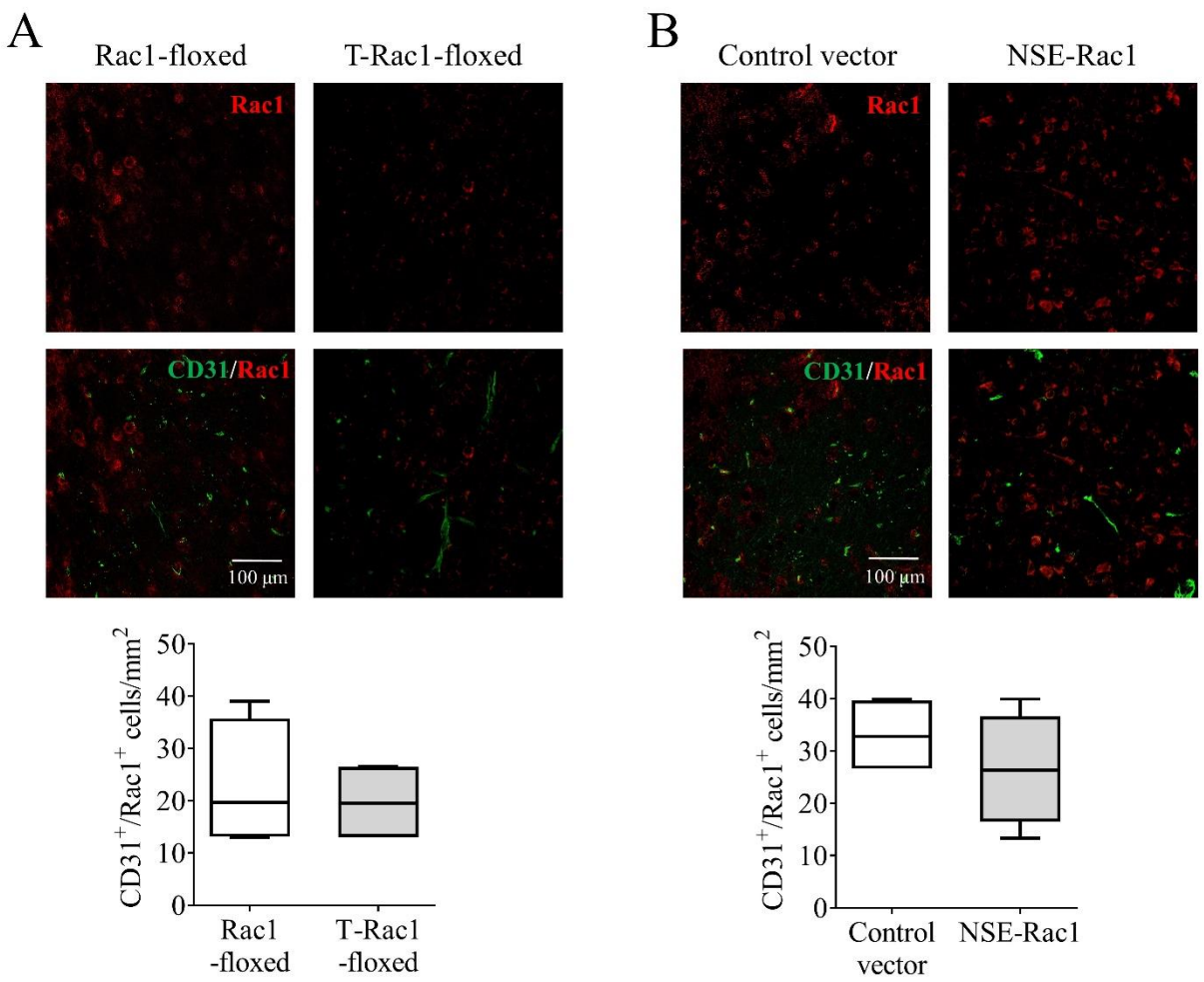

Figure S2. Neither delayed deletion nor overexpression of neuronal Rac1 altered the expression of Rac1 in endothelial cells after stroke in mice. Cells were double-labeled for CD31 (green, anti-goat Alexa Fluor 594) and Rac1 (red, anti-goat Alexa Fluor 647). The number of double-label cells was assayed in the peri-infarct zone 28 days after MCAO injury. (A) Delayed deletion of neuronal Rac1 was performed by intraperitoneal injection of tamoxifen on T-Rac1-floxed mice 7 days after MCAO. Rac1-floxed mice received tamoxifen and served as controls. (B) Delayed overexpression of neuronal Rac1was performed by cerebral injection of lentiviral vectors carrying GFP-Rac1 with neuronal promotor NSE into WT mice at 6 days after MCAO. Vectors in the absence of Rac1 was used as control. N (number of animals) = 4 for each group. Data represent as mean ± SEM from independent experiments, and compared by Mann-Whitney test between two individual groups.

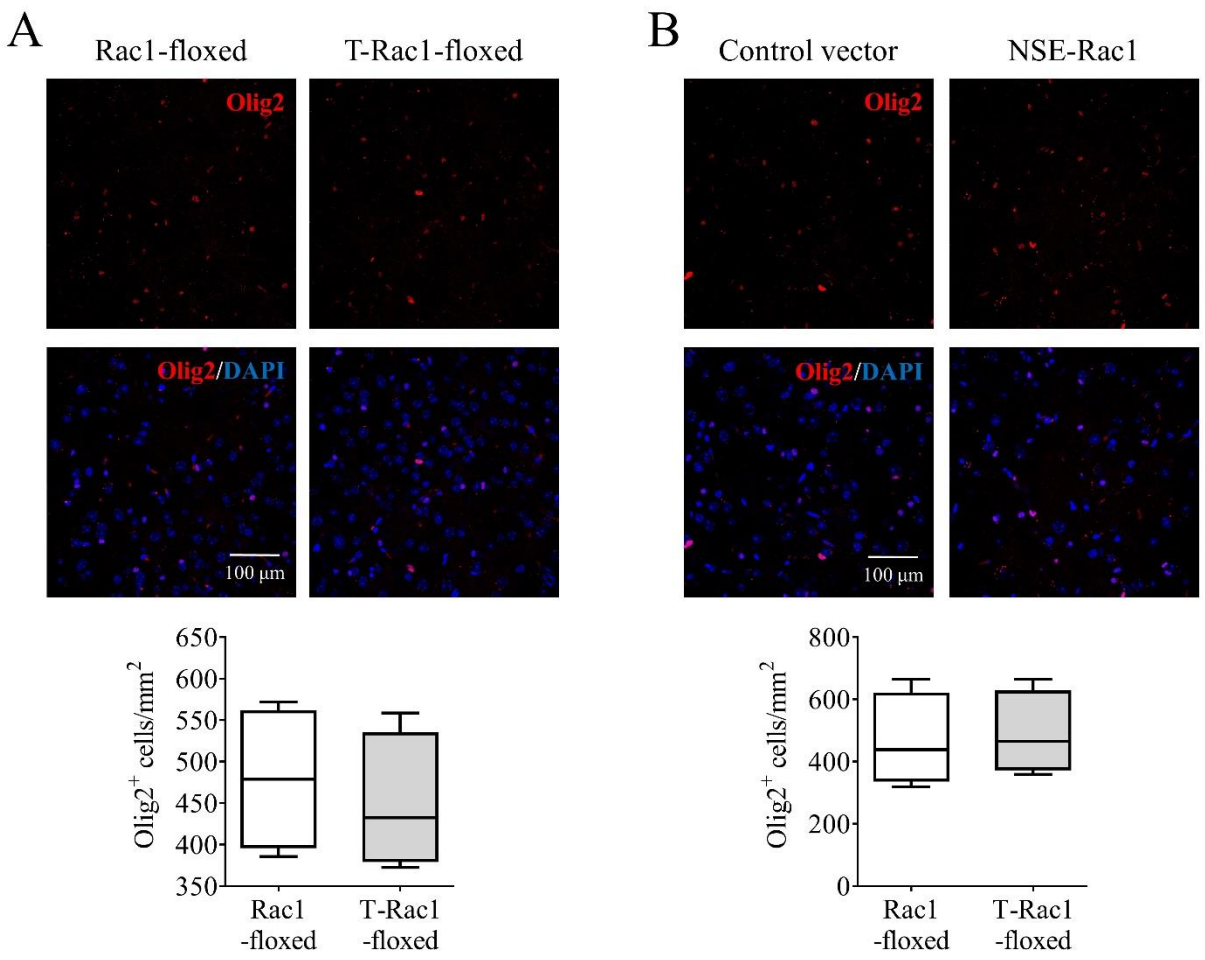

Figure S3. Neither delayed deletion nor overexpression of neuronal Rac1 altered the number of oligodendrocytes after stroke in mice. Cells were double-labeled for olig2 (red, anti-goat Alexa Fluor 594) and DAPI (blue). The number of olig2 was assayed in the peri-infarct zone 28 days after MCAO injury. (A) Delayed deletion of neuronal Rac1 was performed by intraperitoneal injection of tamoxifen on T-Rac1-floxed mice 7 days after MCAO. Rac1-floxed mice received tamoxifen and served as controls. (B) Delayed overexpression of neuronal Rac1was performed by cerebral injection of lentiviral vectors carrying GFP-Rac1 with neuronal promotor NSE into WT mice 6 days after MCAO. Vectors in the absence of Rac1 was used as control. N (number of animals) = 4 for each group. Data represent as mean ± SEM from independent experiments, and compared by Mann-Whitney test between two individual groups.

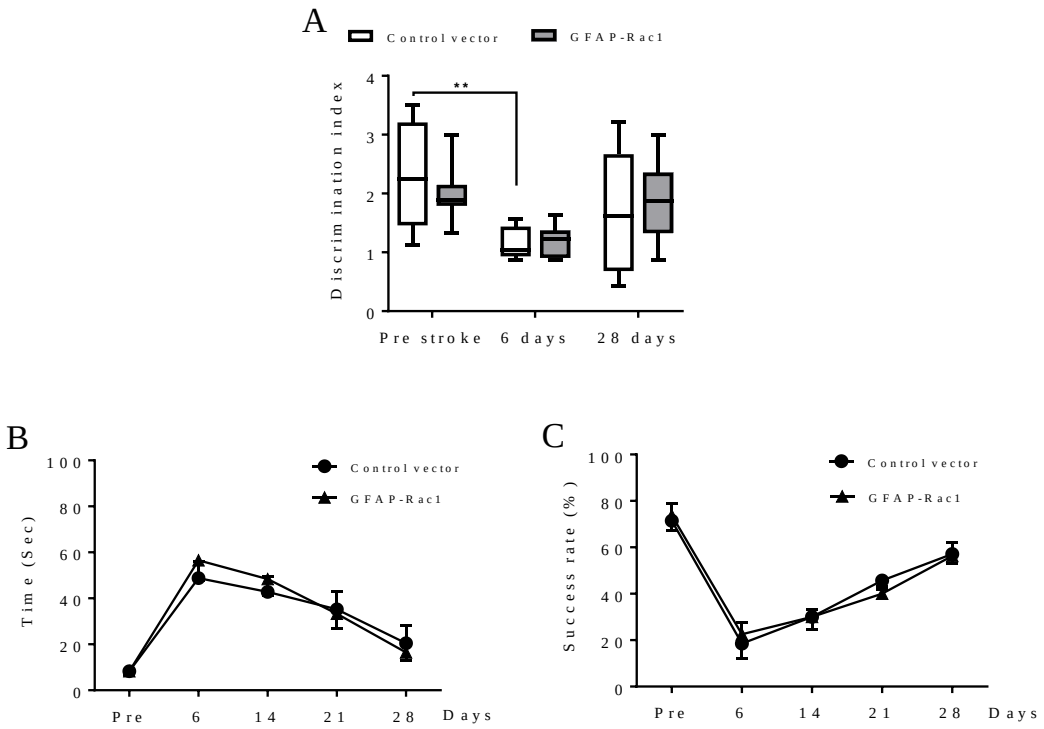

Figure S4. Delayed overexpression of astrocytic Rac1 has no effect to the functional recovery after ischemic stroke in mice. Wide type mice were subjected to 60-minutes middle cerebral artery occlusion (MCAO). Highly concentrated lentiviral vectors (1×10^9 transducing units/mL) encoding Rac1 with astrocytes promotor GFAP (Custom Mouse Rac1 lentiviral vector (pLenti-GIII-GFAP-GFP-2A-Puro), #LV002-c(41219), Abm) were injected into both the cortex and striatum of mice 7 days after stroke following established approach (methods in supplementary document). Same process was performed using lentivirus without Rac1 sequence as controls. Cognitive and sensorimotor deficits were assessed before (Pre) and up to 28 days after MCAO by a battery of behavioral tests. (A) Novel object recognition test. (B) Adhesive removal test. (C) Pellet reaching test. Ischemic stroke produced a cognitive impairment in the control group (2.35 ± 0.35 pre stroke vs 1.14 ± 0.98 at day 6, $^{**}p < 0.01$, panel A). Delayed administration of GFAP-Rac1 to mice brain had no effect to recognitive or sensorimotor functional recovery compared with the control vector group (N = 7 in the control vector group, N = 9 in the GFAP-Rac1 group). N = number of animals. Data represent the mean and SEM. Two-way ANOVA with subsequent Bonferonni test was used for comparisons across groups.

Table S1. The case information of postmortem brain tissues.

Cohort	Sample code	Age	Sex	Cohort	Sample code	Age	Sex	Stroke age
Old control	CW01-097	76	M	Ischemic stroke	CW05-003	83	M	Old
	CW02-017	75	M		CW05-022	84	M	Late subacute to old
	CW94-027	77	M		CW13-001	78	M	Subacute
	CW96-024	61	M		CW15-008	88	M	Subacute
	CW98-019	75	M		CW98-014	90	M	Old
	CW94-043	68	M		CW13-013	82	M	Old
	CW01-099	95	M		CW03-070	82	M	Subacute
	CW98-025	82	M		CW09-010	69	M	Old
	CW00-117	62	M		CW12-011	88	M	Old
	CW00-026	87	M		CW09-011	74	M	Old
Maximal value		95		Maximal value		90		
Minimum value		61		Minimum value		69		
Average		75.8 [ns]		Average		81.8		
MSE		3.34		MSE		2.08		

[ns] There was no significant difference in the mean age between control and stroke groups. N = number of humans. P = 0.15. Unpairedt-test.