Supplementary Information

RGS4 Deficit in Prefrontal Cortex Contributes to the Behaviors related to Schizophrenia via System x_c^--mediated Glutamatergic Dysfunction in Mice

Min-Wei Huang[1,2,+], Yu-Jung Lin[3,+], Chi-Wei Chang[4], Fu-Ju Lei[3], En-Peng Ho[3], Ren-Shyan Liu[4], Woei-Cherng Shyu[3,5], Chia-Hung Hsieh[3,6,7,*]

[1]School of Medicine, China Medical University, Taichung, Taiwan
[2]Department of Psychiatry, Chiayi Branch, Taichung Veterans General Hospital, Chiayi, Taiwan
[3]Graduate Institute of Biomedical Science, China Medical University, Taichung, Taiwan
[4]National PET/Cyclotron Center and Department of Nuclear Medicine, Taipei Veterans General Hospital, Taipei, Taiwan
[5]Department of Neurology, Center for Translational Medicine, China Medical University and Hospital, Taichung, Taiwan
[6]Department of Medical Research, China Medical University Hospital, Taichung, Taiwan
[7]Department of Biomedical Informatics, Asia University, Taichung, Taiwan

[+] these authors contributed equally to this work

*Corresponding author:
Chia-Hung Hsieh, Ph.D
Mailing address: No. 91, Hsueh-Shih Road, Taichung 404, Taiwan
E-mail: chhsiehcmu@mail.cmu.edu.tw
Tel: 886-4-22052121 extn. 7712
Fax: 886-4-22333641

A. Supplementary figure legend

Figure S1. Densitometric analysis of RGS4 and xCT protein levels in organotypic brain-slice cultures with or without RGS4 knockdown or RGS4 overexpression (n = 9 samples). The density of proteins in each control (untreated) group was used as a standard (1 arbitrary unit) to compare relative densities in the other groups. *P < 0.001 compared to control groups, Two-way ANOVA followed by Bonferroni test.

Figure S2. Ex vivo biodistribution of ^{18}F-labelled alkylthiophenyl guanidine (^{18}F-FSAG, a specific radioligand for PCP sites of the NMDAR) in the microinjection sites at 21 days after virus injection. Before biodistribution studies, mice were pre-treated with NAC (500 mg/kg/day) or vehicle for 1 week. N=8 per group. *P < 0.01 compared to control mice; $^{\#}$ P < 0.05 compared to vehicle-treated mice, one-way ANOVA followed by Tukey test.

Figure S3. There is no significance between RGS4 knockdown mice and control mice in prefrontal cortical glutathione (GSH) content. GSH assay was carried out at 21 days after virus injection. For the NAC treatment, mice were pre-treated with NAC (500 mg/kg/day) or vehicle for 1 week before GSH assay. N=9 per group.

Figure S4. No significant difference between RGS4 knockdown mice and control mice was observed in total distance traveled or resting time for open field test and in open-arm to total entries or distance traveled for elevated plus-maze testing. All of behaviors were carried out at 21 days after virus injection. For the NAC treatment, mice were pre-treated with NAC (500 mg/kg/day) or vehicle for 1 week before GSH assay. N=12 per group.

Figure S5. A whole image of xCT gels indicates the specificity of xCT antibody in wild-type (WT) and xCT knockout (xCT$^{-/-}$) brain tissue lysate.

B. Supplementary figures

Figure S1

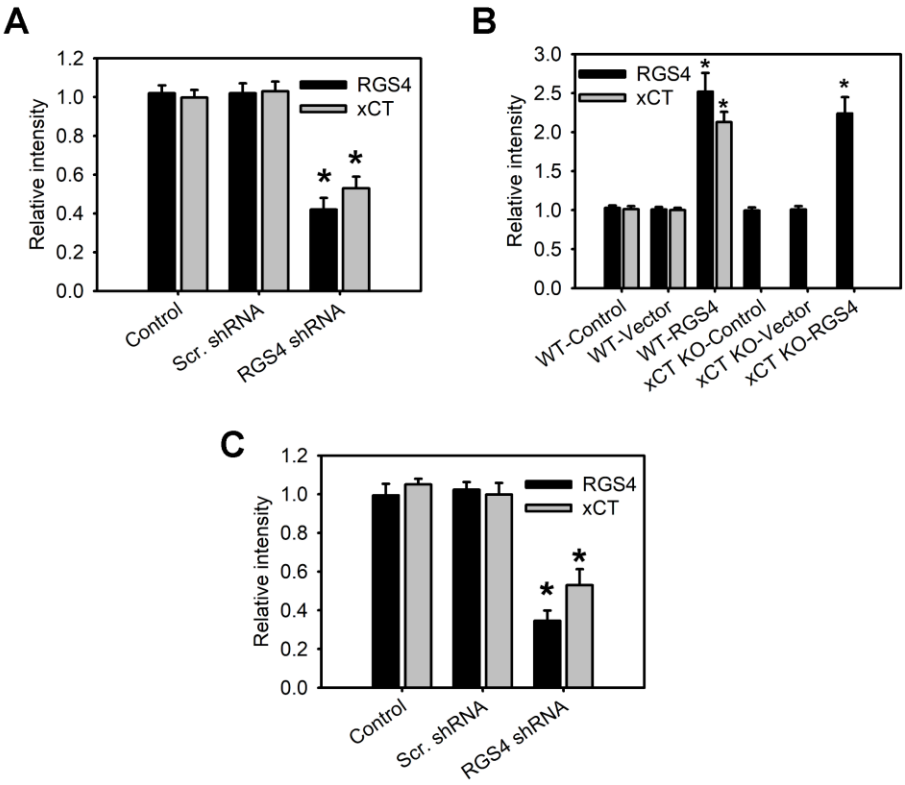


Figure S2

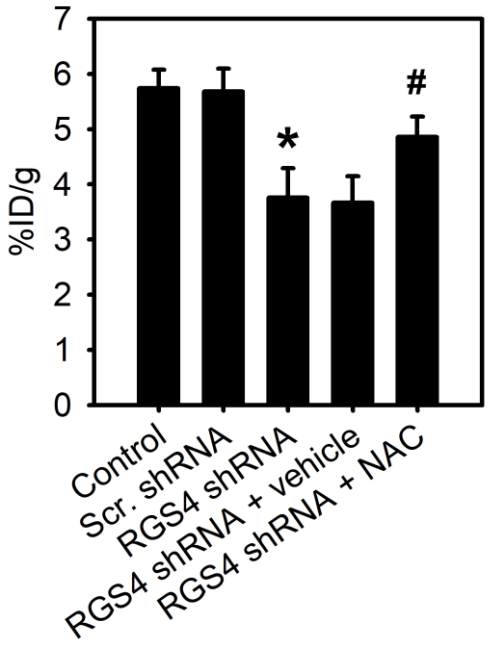


Figure S3

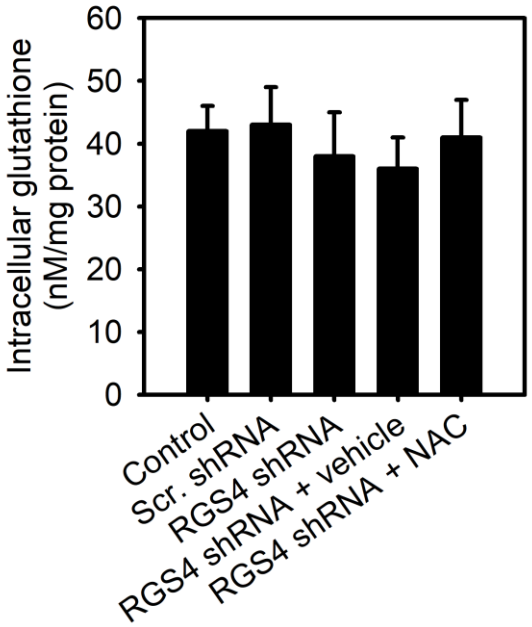
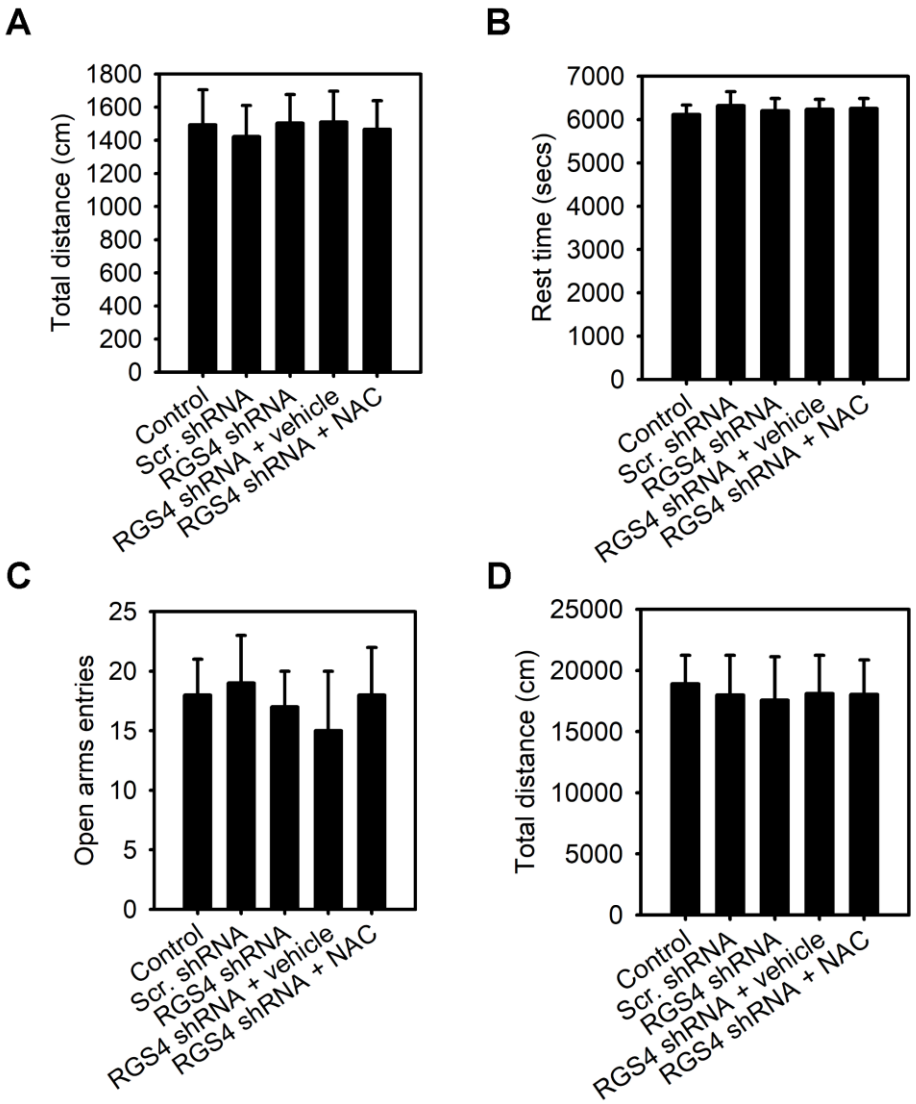


Figure S4


A
Total distance (cm)
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1600
1400
1200
1000
800
600
400
200
0
Control
Scr. shRNA
RGS4 shRNA
RGS4 shRNA + vehicle
RGS4 shRNA + NAC
B
Rest time (secs)
7000
6000
5000
4000
3000
2000
1000
0
Control
Scr. shRNA
RGS4 shRNA
RGS4 shRNA + vehicle
RGS4 shRNA + NAC
C
Open arms entries
25
20
15
10
5
0
Control
Scr. shRNA
RGS4 shRNA
RGS4 shRNA + vehicle
RGS4 shRNA + NAC
D
Total distance (cm)
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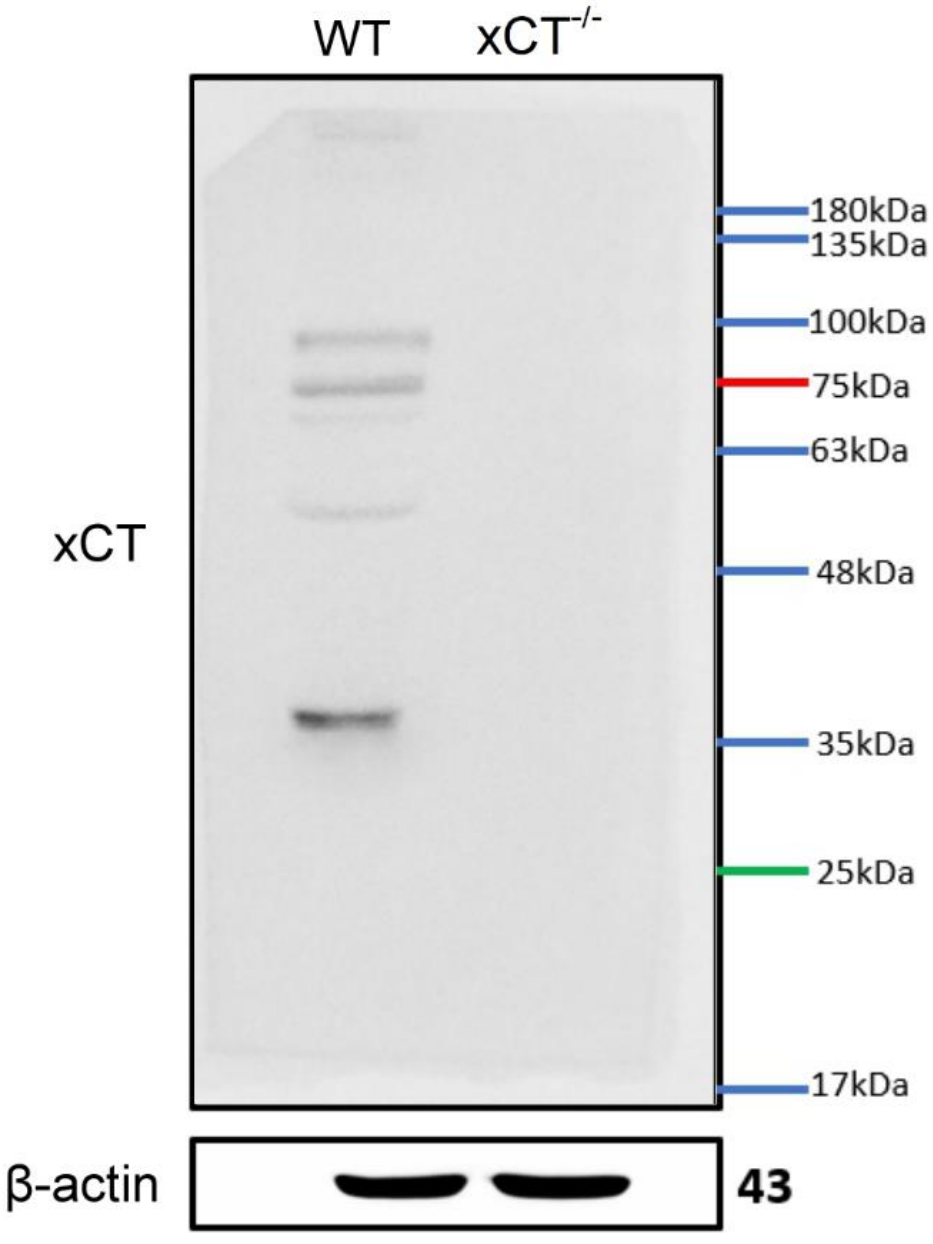
20000
15000
10000
5000
0
Control
Scr. shRNA
RGS4 shRNA
RGS4 shRNA + vehicle
RGS4 shRNA + NAC

Figure S5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xCT-/-
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135kDa
100kDa
75kDa
63kDa
xCT
48kDa
35kDa
25kDa
17kDa
β-actin
43