

p35 and Rac1 underlie the neuroprotection and cognitive improvement induced by CDK5 silencing

Rafael Andres Posada-Duque¹, Alejandro López-Tobón¹, Diego Piedrahita¹, Christian González-Billault², Gloria Patricia Cardona-Gomez^{1*}

¹Cellular and Molecular Neurobiology Area, Group of Neuroscience of Antioquia, Faculty of Medicine, SIU, University of Antioquia UdeA, Calle 70 No. 52-21, Medellín, Colombia.

²Laboratory of Cell and Neuronal Dynamics, Department of Biology and Institute for Cell Dynamics and Biotechnology, Faculty of Sciences, Universidad de Chile, Las Palmeras 3425, Ñuñoa, Santiago, Chile.

Running title: p35 contributes to neuroprotection and cognitive improvement following CDK5 RNAi

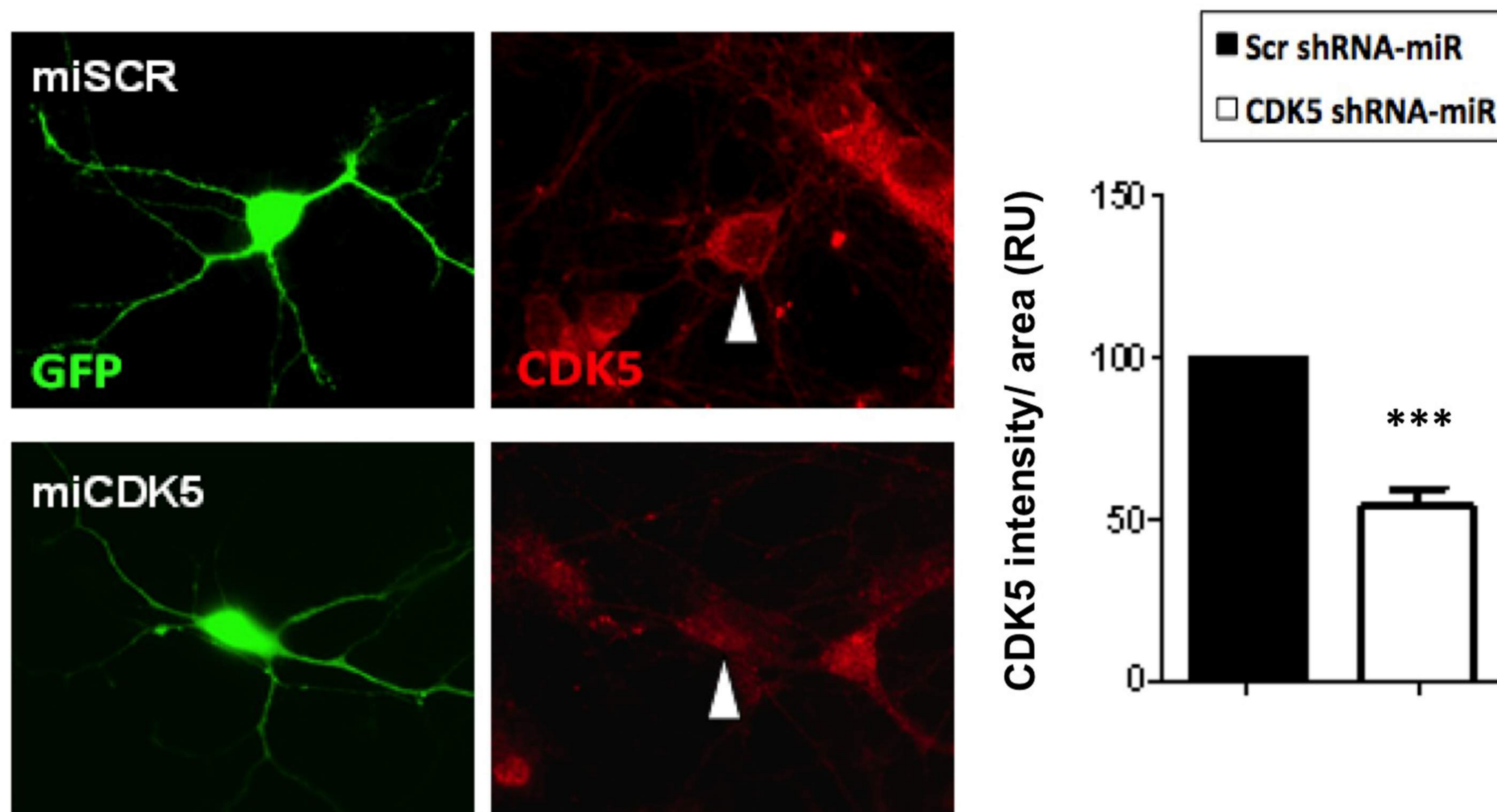
*Corresponding author: Gloria Patricia Cardona-Gómez, Universidad de Antioquia, Sede de Investigación Universitaria (SIU), Calle 62 # 52 – 59; Torre 1, Piso 4, Laboratorio 412; Medellín, Colombia. Phone: 57-4-2196458; Fax: 57-4-2196444; E-mail: patricia.cardonag@udea.edu.co

Supplementary figure 1. Silencing of CDK5 reduces neurite branching in early neuronal primary cultures. (A) Neurons labelled CDK5 (Red) exhibit differences in the fluorescence intensity in the somas (arrows) 40X. CDK5 shRNA-miR n= 78 and SCR shRNA-miR n=74 fields. (B) The neurite number was determined with GFP using ImagePro software. Individual counts CDK5 shRNA-miR n= 51 and SCR shRNA-miR n=54 fields. \pm S.E.M. ** = $p < 0,01$. *** = $p < 0,005$. (C) Schematic experimental design: Primary hippocampal neurons were transduced with the AAV viral vector eGFP-tagged SCR or CDK5 shRNA-miR (DIV7) and DIV12 transduced with control or p35 shRNA for 7 days.

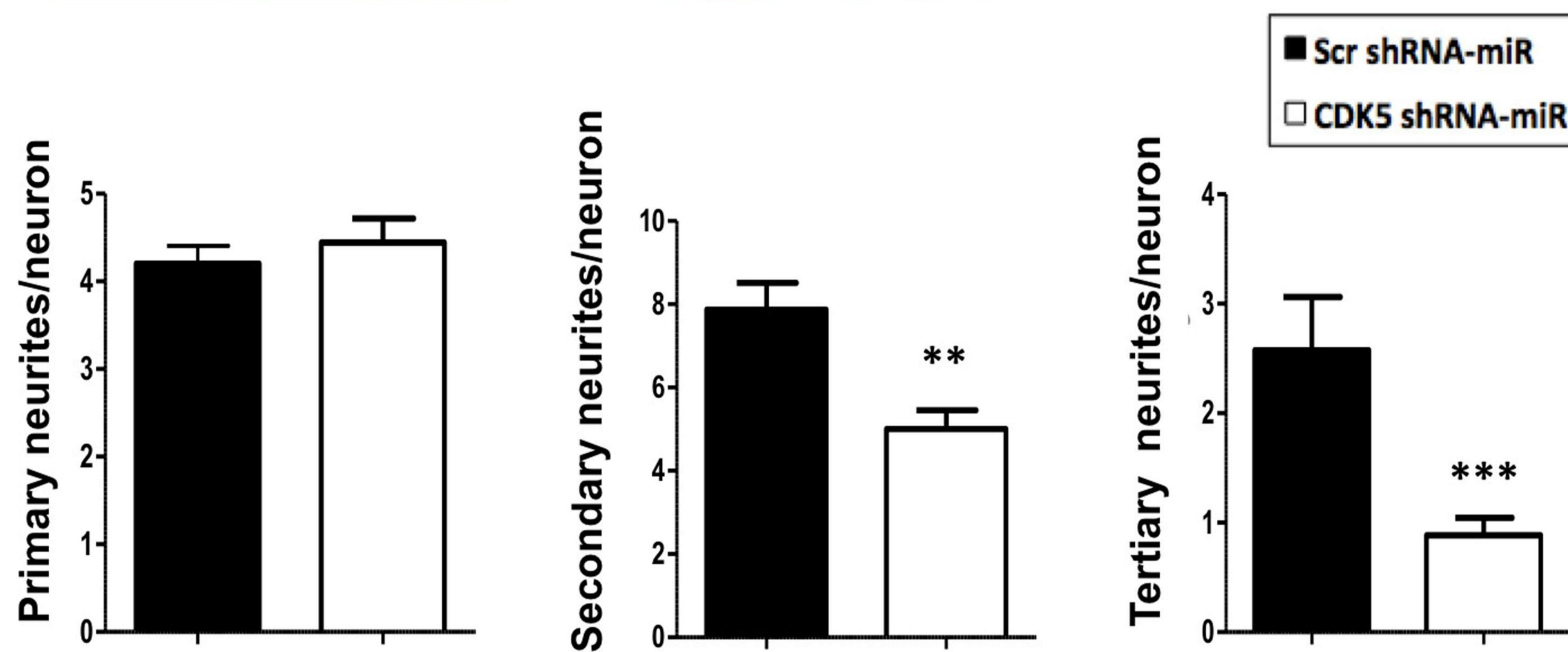
Supplementary figure 2. CDK5 inhibition protects against glutamate excitotoxicity: p35-dependent. Neurons (DIV8) 48 h post transfection and after 24 h of Ros treatment. Morphological characteristics are shown for neurons transfected with pBI-p35 Tet-OFF. Nuclei were stained with Hoechst (blue), and PSD95 was labelled with Alexa 594 dye (red). The arrowhead shows the condensed nucleus compared with the normal nucleus (arrow). Binary images were used to determine the number and area of the PSD95 clusters. Magnification, 60X with 50% zoom. Scale bar, 20 μ m. n=3 per duplicate.

Supplementary figure 1

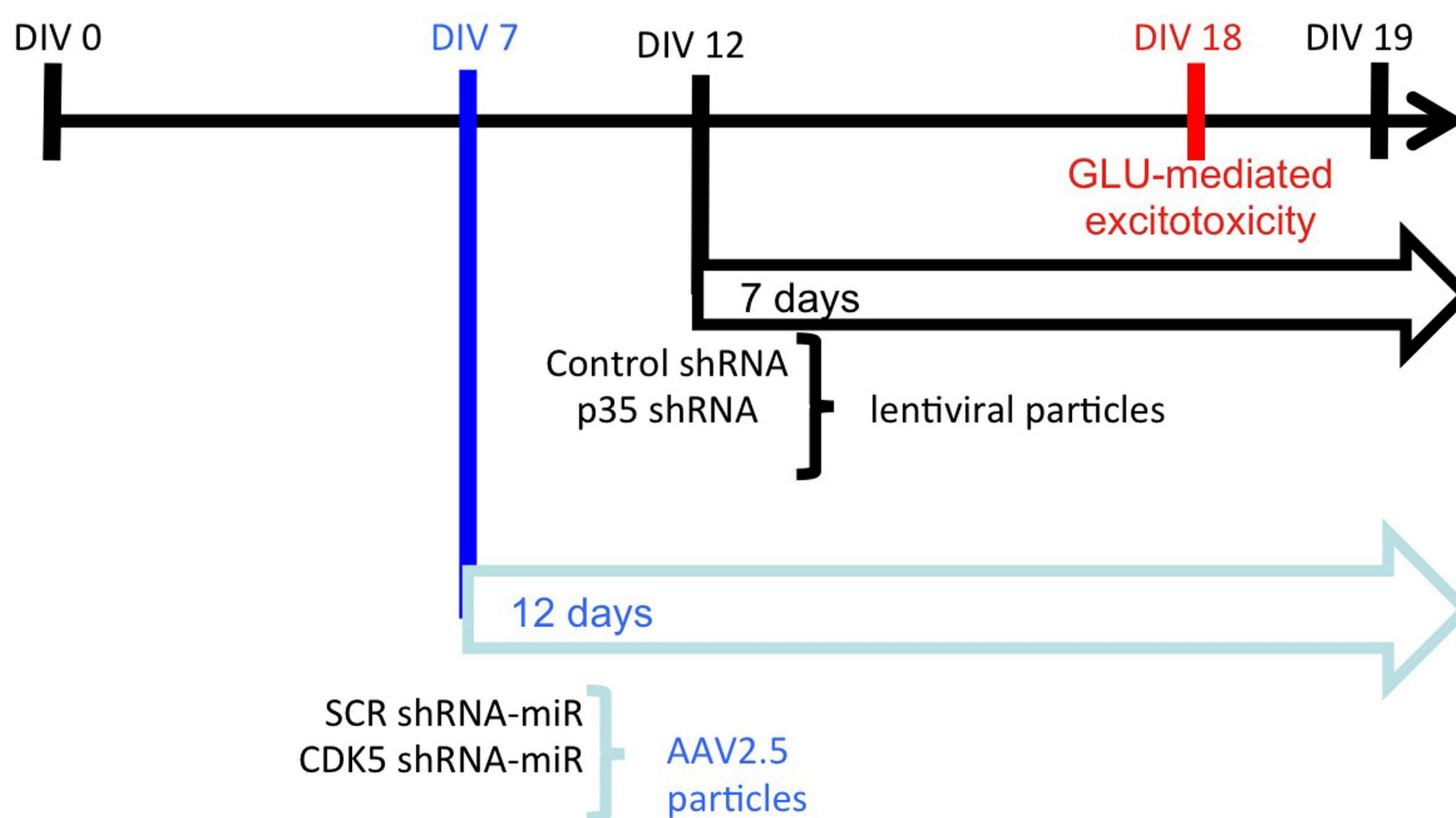
A



B



C



Supplementary figure 2

pBI-p35 Tet-OFF

