

Supplementary information

LIST OF FIGURES

S1 Full western blots (A and B) shown for promoters mDlx and hSynapsin and an alternate way (C) of confirmation of AAV virus in the spinal cord sections. (A) Full blot of beta actin. (B) Full western blots of AAV2.1-mDlx and AAV9-hSynapsin viruses were shown where the replicates were represented by numbers 1, 2 and 3 (B). Note that black boxes in the images represent the cropped images shown in the main text of Figure 2. Bands at 50 KDa (B) were not taken into consideration. (C) Alternatively AAV virus expression was confirmed by sectioning the spinal cords and directly observing the sections under fluorescence microscope as shown here in example images where AAV2.1-mDlx virus expression was visualized in comparison to control spinal cord with no virus expression. Scale bars: 200 μ m and 20 μ m in the zoom-in images (C). 3

S2 mDlx expression in the mice brain. (A) Coronal section of mouse prefrontal cortex (Scale bar: 500 μ m) where the green labelling (GFP) indicates expression of AAV9 virus with mDlx promoter. (B) Zoomed-in images (of a ROI highlighted with white square in A) of viral expressed cells labelled by GFP showed co-localization with PAX2 whereas GAD67 biomarker (in red) showed lack of colocalization. Scale bars: 50 μ m in image B and 20 μ m in the zoomed-in images which represent single cell (B). Note N = 3, n = 3-4, where N represents the number of mice brains and n shows the number of sections used per mice brain. 4

S3 mDlx has some colocalization with PAX2. (A) Transverse section of the lumbar spinal cord indicating an inset region and the segregation of dorsal, central and ventral portions. (B) Combined fluorescent images of the GFP-positive neurons (green), where the AAV infected, and the PAX2 positive immunostaining (red). Colocalizing cells were counted in all three regions (bottom right). Scale bars: 200 μ m in image A and 20 μ m in the zoom-in images (B). 5

S4 mDlx enhancer expression has overlap with a putative motor neurons. (A) Immunostaining of the neurofilament protein SMI32, which is abundant in motor neurons. (B-D) The combined fluorescent images of the highlighted region in (A). Scale bars: 200 μ m in image A and 20 μ m in the zoom-in images. 6

S5 The mScarlet expression in CamKIIa neurons has some colocalization with GluA1 neurons. (A) Overview showing the infected cells in red (mScarlet). (B) Counting of cells with colocalization using GluA1 biomarker (glutamatergic neuron) in the three regions. (C) The highlighted region in (A) with GluA1 and 2. Scale bars: 200 μ m in image A and 20 μ m in the zoom-in images. 7

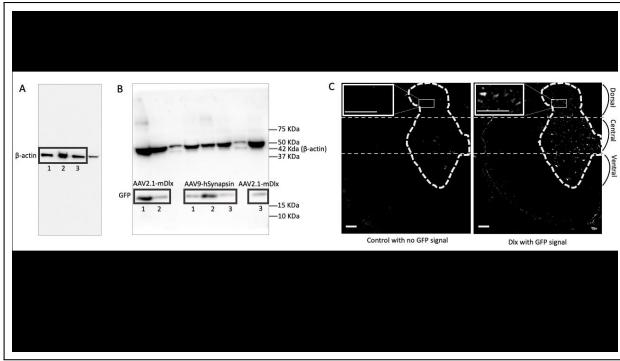


Fig. S1. Full western blots (A and B) shown for promoters mDlx and hSynapsin and an alternate way (C) of confirmation of AAV virus in the spinal cord sections. (A) Full blot of beta actin. **(B)** Full western blots of AAV2.1-mDlx and AAV9-hSynapsin viruses were shown where the replicates were represented by numbers 1, 2 and 3 (B). Note that black boxes in the images represent the cropped images shown in the main text of Figure 2. Bands at 50 KDa (B) were not taken into consideration. **(C)** Alternatively AAV virus expression was confirmed by sectioning the spinal cords and directly observing the sections under fluorescence microscope as shown here in example images where AAV2.1-mDlx virus expression was visualized in comparison to control spinal cord with no virus expression. Scale bars: 200 μ m and 20 μ m in the zoom-in images (C).

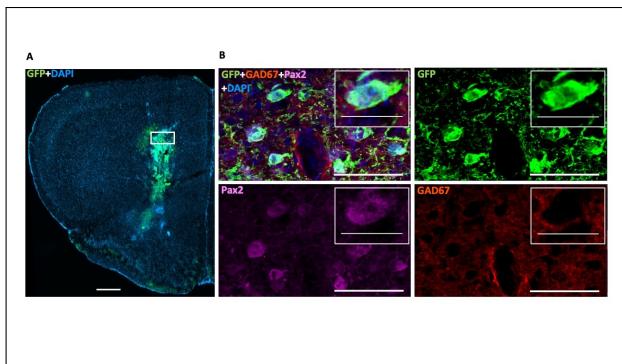


Fig. S2. mDlx expression in the mice brain. **(A)** Coronal section of mouse prefrontal cortex (Scale bar: 500 μ m) where the green labelling (GFP) indicates expression of AAV9 virus with mDlx promoter. **(B)** Zoomed-in images (of a ROI highlighted with white square in A) of viral expressed cells labelled by GFP showed co-localization with PAX2 whereas GAD67 biomaker (in red) showed lack of colocalization. Scale bars: 50 μ m in image B and 20 μ m in the zoomed-in images which represent single cell (B). Note N = 3, n = 3-4, where N represents the number of mice brains and n shows the number of sections used per mice brain.

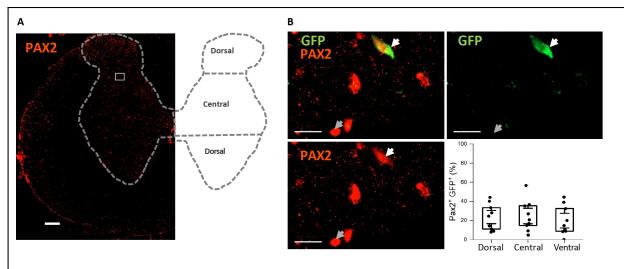


Fig. S3. mDlx has some colocalization with PAX2. (A) Transverse section of the lumbar spinal cord indicating an inset region and the segregation of dorsal, central and ventral portions. (B) Combined fluorescent images of the GFP-positive neurons (green), where the AAV infected, and the PAX2 positive immunostaining (red). Colocalizing cells were counted in all three regions (bottom right). Scale bars: 200 μ m in image A and 20 μ m in the zoom-in images (B).

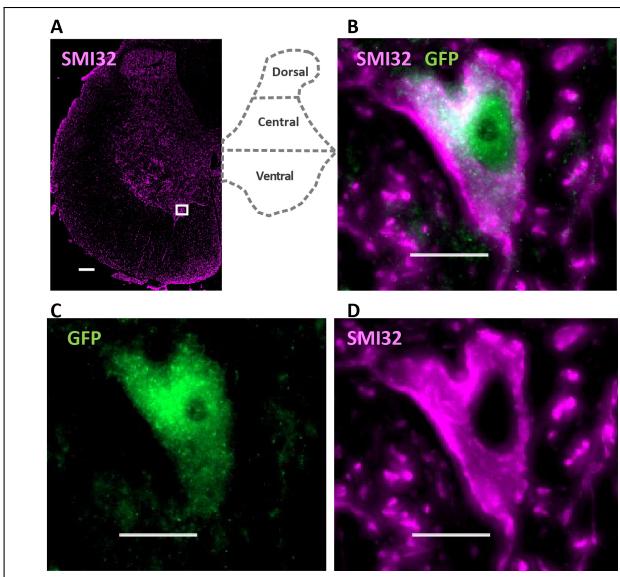


Fig. S4. mDlx enhancer expression has overlap with a putative motor neurons. (A) Immunostaining of the neurofilament protein SMI32, which is abundant in motor neurons. (B-D) The combined fluorescent images of the highlighted region in (A). Scale bars: 200 μ m in image A and 20 μ m in the zoom-in images.

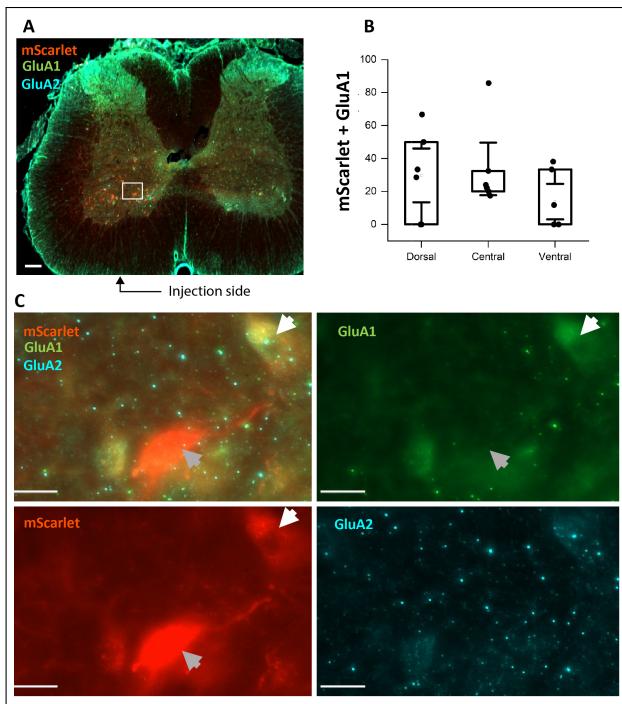


Fig. S5. The mScarlet expression in CamKIIa neurons has some colocalization with GluA1 neurons. (A) Overview showing the infected cells in red (mScarlet). (B) Counting of cells with colocalization using GluA1 biomarker (glutamatergic neuron) in the three regions. (C) The highlighted region in (A) with GluA1 and 2. Scale bars: 200 μ m in image A and 20 μ m in the zoom-in images.