Supporting Information

Lipid Nanoparticles Deliver mRNA to the Brain after an Intracerebral Injection

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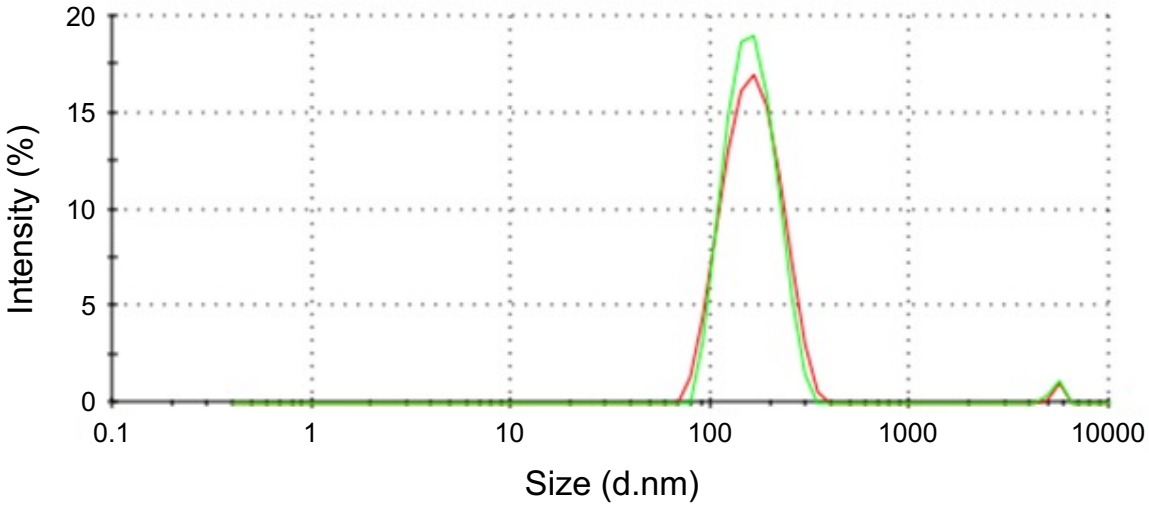

Z-Average (d.nm): 162.4
PdI: 0.219
Intercept: 0.933
Result quality: Good

	Size (d.nm)	% Intensity	St Dev.
Peak 1:	163.3	98.4	44.97
Peak 2:	5365	1.6	331.5
Peak 3:	0.000	0.0	0.000

Figure S1. Size distribution and polydispersity index (PDI) of MC3 LNP mRNA complexes. MC3 LNP mRNA complex was characterized by dynamic light scattering (DLS). For MC3 LNP preparation, a stock solution (10 mg mL^{-1}) of MC3, DOPE, Cholesterol, and DMG-PEG was made by dissolving each lipid separately in ethanol. The MC3, DOPE, Cholesterol, and DMG-PEG were mixed in 36.8:23.8:38.2:1.2 molar ratios, respectively. RNAs 1 µg $µL^{-1}$ (Cre mRNA, Cas9 mRNA/sgRNA) were dissolved in 200 mM citrate buffer (pH 4) and mixed with MC3 LNP in a 1:3 ratio (vol/vol). 20 ul MC3 LNP mRNA complex for DLS was prepared with 5 µg Cre mRNA, diluted to 100 µL total volume with PBS pH7.2, and incubated at room temperature for 1 h. The size distribution and polydispersity were determined by Malvern Zetasizer (Malvern, United Kingdom) and had an average size of 162 nm. This is from two independent readouts and number represents average of two readouts.

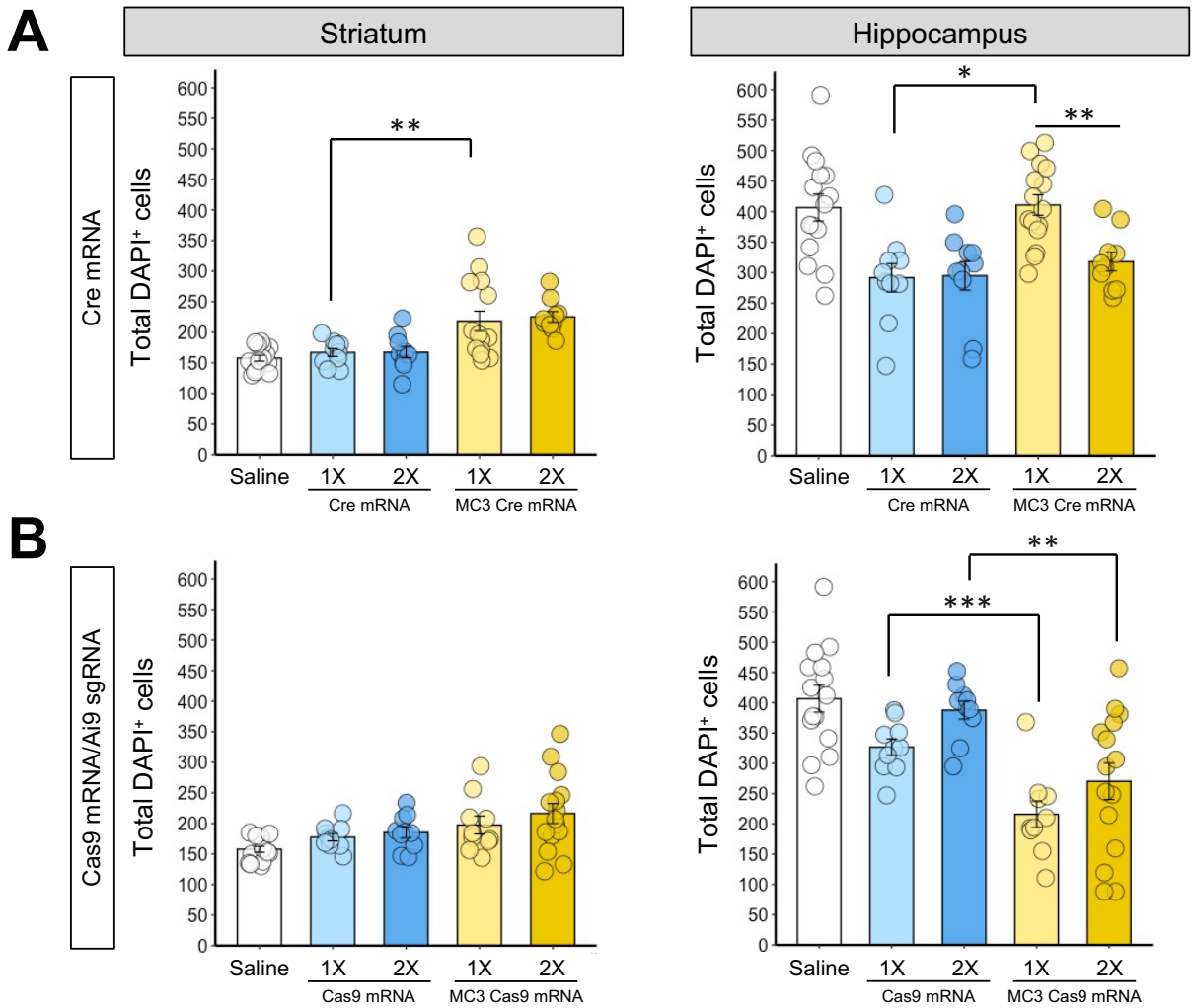

Figure S2: Quantification of total DAPI⁺ cells in the striatum and hippocampus in Ai9 mice. (A) Quantification of the total DAPI⁺ cells in the injected area of striatum (Left) or hippocampus (Right) injected with saline, Cre mRNA or MC3 LNP Cre mRNA. Cre mRNA or MC3 LNP Cre mRNA were injected with two doses: 0.125 μg μL^{-1} (1X) or 0.250 μg μL^{-1} (2X). Data were analyzed using permutation one-way ANOVA (striatum: $F_{(4,55)}$ = 8.81, p < 0.001; hippocampus: $F_{(4,55)}$ = 8.59, p < 0.001). * p < 0.05, ** p < 0.01 by post hoc permutation t-test. Post hoc p values were calculated between Cre mRNA (0.125 μg μL^{-1} or 0.250 μg μL^{-1}) and MC3 LNP Cre mRNA (0.125 μg μL^{-1} or 0.250 μg μL^{-1}) as well as between 0.125 μg μL^{-1} and 0.250 μg μL^{-1} within Cre mRNA or MC3 LNP Cre mRNA. Saline (n = 15 images from 3 mice), Cre mRNA 1X (n = 10 images from 2 mice), Cre mRNA 2X (n = 10 images from 2 mice), MC3 LNP Cre mRNA 1X (n = 15 images from 3 mice), and MC3 LNP Cre mRNA 2X (n = 10 images from 2 mice). (B) Quantification of the total DAPI⁺ cells in the injected area of striatum (Left) or hippocampus (Right) injected with saline, Cas9 mRNA/Ai9 sgRNA or MC3 LNP Cas9 mRNA/Ai9 sgRNA. Cas9 mRNA/Ai9 sgRNA or MC3 LNP Cas9 mRNA/Ai9 sgRNA were injected with two doses: 0.125 μg μL^{-1} (1X) or 0.250 μg μL^{-1} (2X). Data were analyzed using permutation one-way ANOVA (striatum: $F_{(4,55)}$ = 4.24, p = 0.003; hippocampus: $F_{(4,55)}$ = 11.21, p < 0.001). ** p < 0.01, *** p < 0.001 by post hoc permutation t-test. Post hoc p values were calculated between Cas9 mRNA/Ai9 sgRNA (0.125 μg μL^{-1} or 0.250 μg μL^{-1}) and MC3 LNP Cas9 mRNA/Ai9 sgRNA (0.125 μg μL^{-1} or 0.250 μg μL^{-1}) as well as between 0.125 μg μL^{-1} and 0.250 μg μL^{-1} within Cas9 mRNA/Ai9 sgRNA or MC3 LNP Cas9 mRNA/Ai9 sgRNA. Saline (n = 15 images from 3 mice), Cas9 mRNA/Ai9 sgRNA 1X (n = 10 images from 2 mice), Cas9 mRNA/Ai9 sgRNA 2X (n = 10 images from 2 mice), MC3 LNP Cas9 mRNA/Ai9 sgRNA 1X (n = 10 images from 2 mice), and MC3 LNP Cas9 mRNA/Ai9 sgRNA 2X (n = 15 images from 3 mice). All data are presented as mean ± SEM.

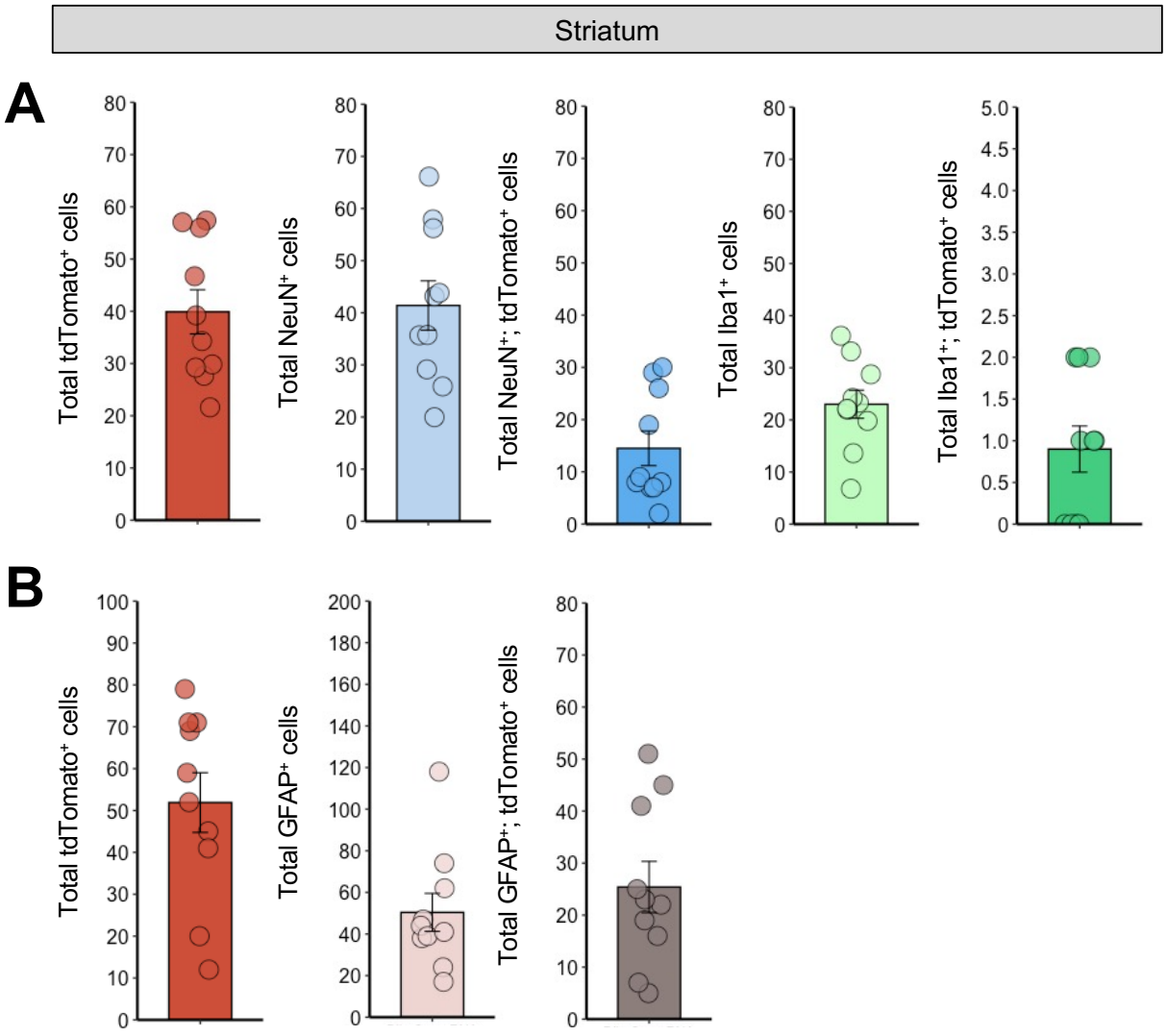

Figure S3: Total number of all Cre-recombined (tdTomato$^+$) cells, total number of NeuN$^+$, Iba1$^+$ or GFAP$^+$ as well as total number of Cre-recombined NeuN$^+$, Iba1$^+$ or GFAP$^+$ cells in the striatum after the injection with MC3 LNP Cre mRNA (0.250 µg µL^{-1}). (A) Quantification of total number of Cre-recombined cells (tdTomato$^+$), neurons (NeuN$^+$) and Cre-recombined neurons (NeuN$^+$; tdTomato$^+$) as well as total number of microglia (Iba1$^+$) and Cre-recombined microglia (Iba1$^+$; tdTomato$^+$). (B) Quantification of total number of Cre-recombined cells (tdTomato$^+$), astrocytes (GFAP$^+$) and Cre-recombined astrocytes (GFAP$^+$; tdTomato$^+$) in MC3 LNP Cre mRNA (0.250 µg µL^{-1})-injected area in the striatum. The quantification was performed in samples stained from the same animal with either (A) tdTomato, NeuN and Iba1 or (B) tdTomato and GFAP. Mean ± SEM, *n* = 10 images from 2 mice.

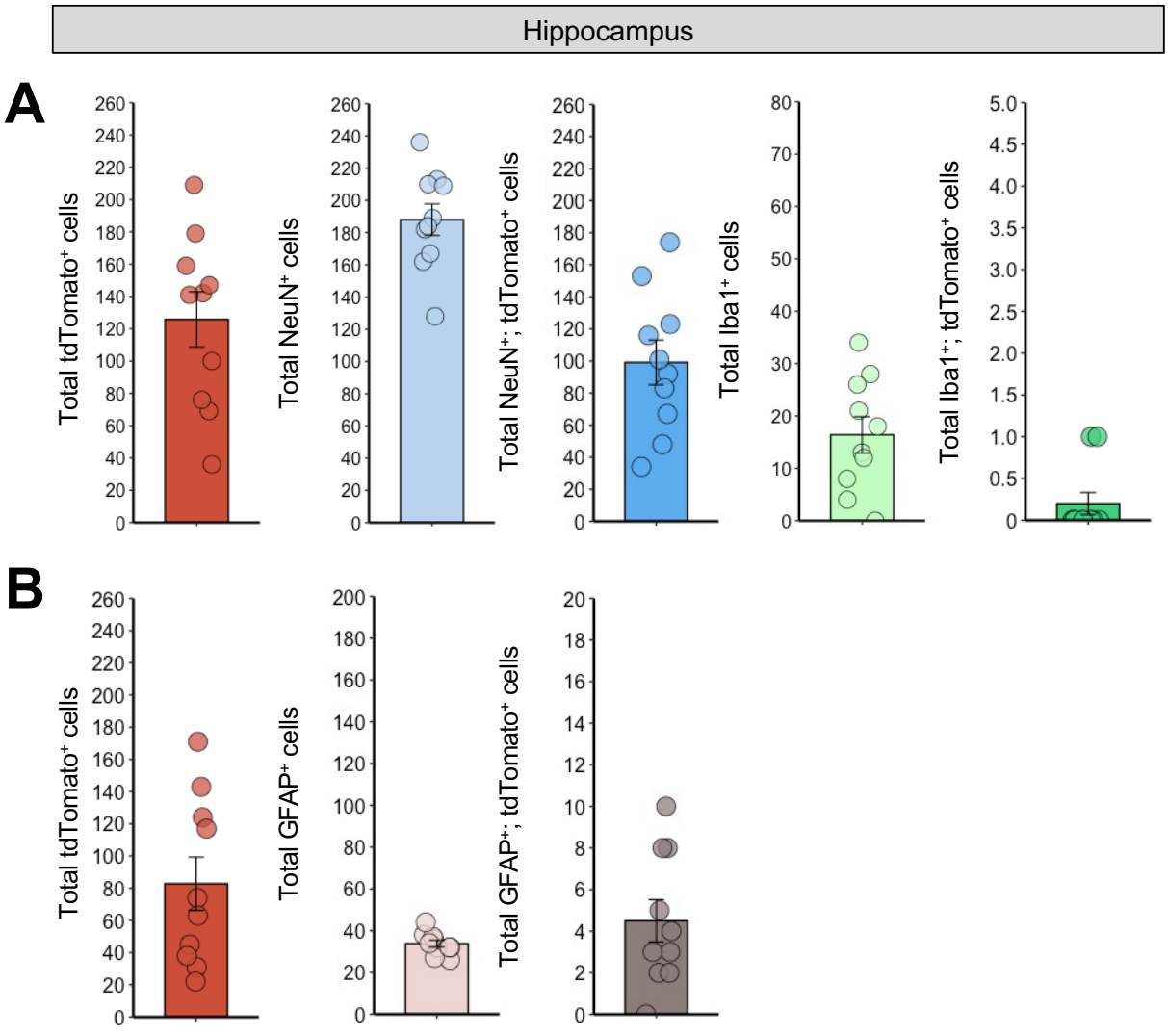

Figure S4: Total number of all Cre-recombined (tdTomato$^+$) cells, total number of NeuN$^+$, Iba1$^+$ or GFAP$^+$ as well as total number of Cre-recombined NeuN$^+$, Iba$^+$ or GFAP$^+$ cells in the hippocampus after the injection with MC3 LNP Cre mRNA (0.250 µg µL^{-1}). (A) Quantification of total number of Cre-recombined cells (tdTomato$^+$), neurons (NeuN$^+$) and Cre-recombined neurons (NeuN$^+$; tdTomato$^+$) as well as total number of microglia (Iba1$^+$) and Cre-recombined microglia (Iba1$^+$; tdTomato$^+$). (B) Quantification of total number of Cre-recombined cells (tdTomato$^+$), astrocytes (GFAP$^+$) and Cre-recombined astrocytes (GFAP$^+$; tdTomato$^+$) in MC3 LNP Cre mRNA (0.250 µg µL^{-1})-injected area in the hippocampus. The quantification was performed in samples from the same animal stained with either (A) tdTomato, NeuN and Iba1 or (B) tdTomato and GFAP. Mean ± SEM, *n* = 10 images from 2 mice.

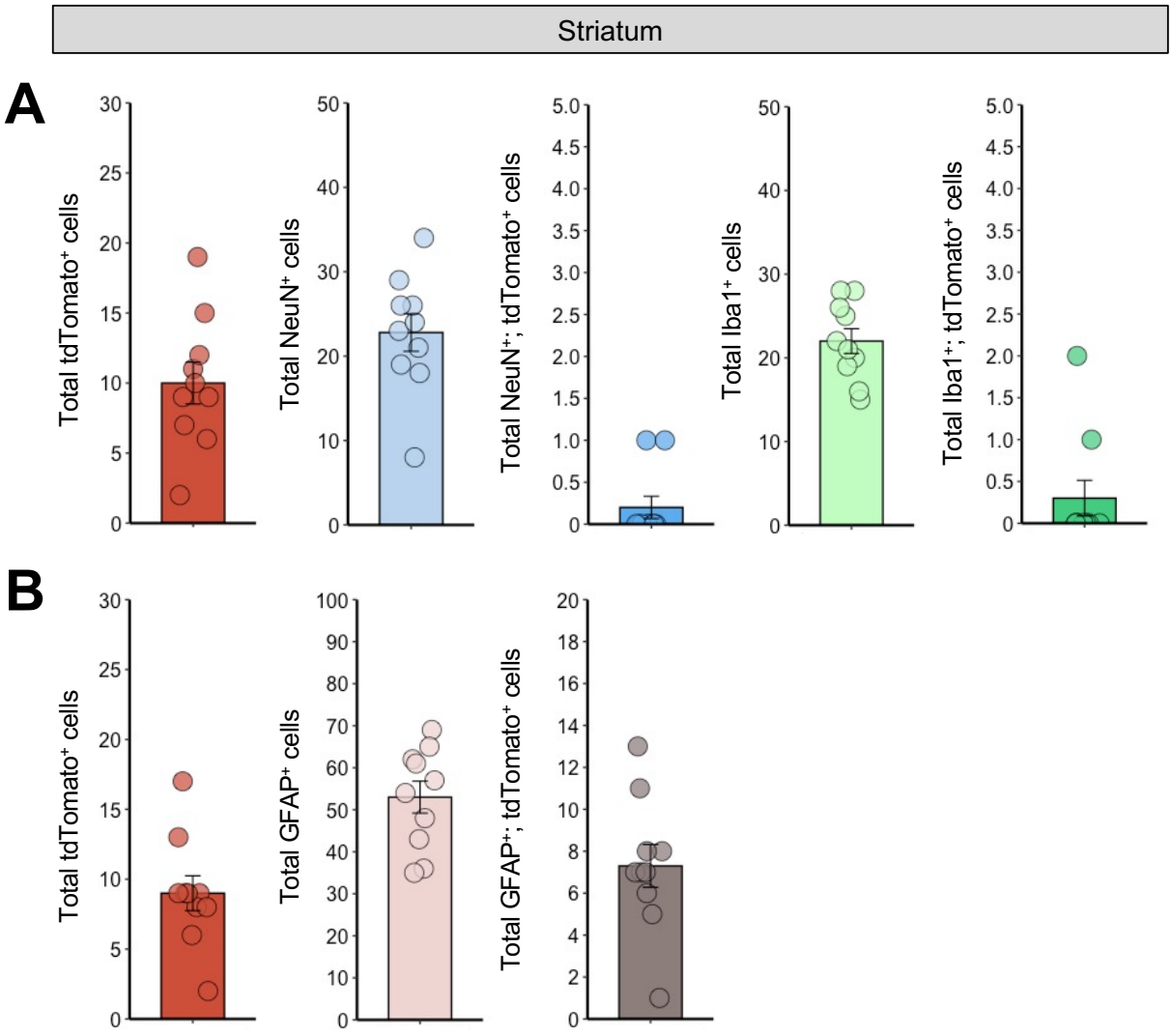

Figure S5: Total number of gene-edited (tdTomato$^+$) cells, total number of NeuN$^+$, Iba1$^+$ and GFAP$^+$ as well as total number of gene-edited NeuN$^+$, Iba1$^+$ and GFAP$^+$ cells in the striatum after the injection with MC3 LNP Cas9 mRNA/Ai9 sgRNA (0.250 µg µL^{-1}). (A) Quantification of total number of gene-edited cells (tdTomato$^+$), neurons (NeuN$^+$) and gene-edited neurons (NeuN$^+$; tdTomato$^+$) as well as total number of microglia (Iba1$^+$) and gene-edited microglia (Iba1$^+$; tdTomato$^+$). (B) Quantification of total number of gene-edited cells (tdTomato$^+$), astrocytes (GFAP$^+$) and gene-edited astrocytes (GFAP$^+$; tdTomato$^+$) in MC3 LNP Cas9 mRNA/Ai9 sgRNA (0.250 µg µL^{-1})-injected area in the striatum. The quantification was performed in samples from the same animal stained with either (A) tdTomato, NeuN and Iba1 or (B) tdTomato and GFAP. Mean ± SEM, *n* = 10 images from 2 mice.

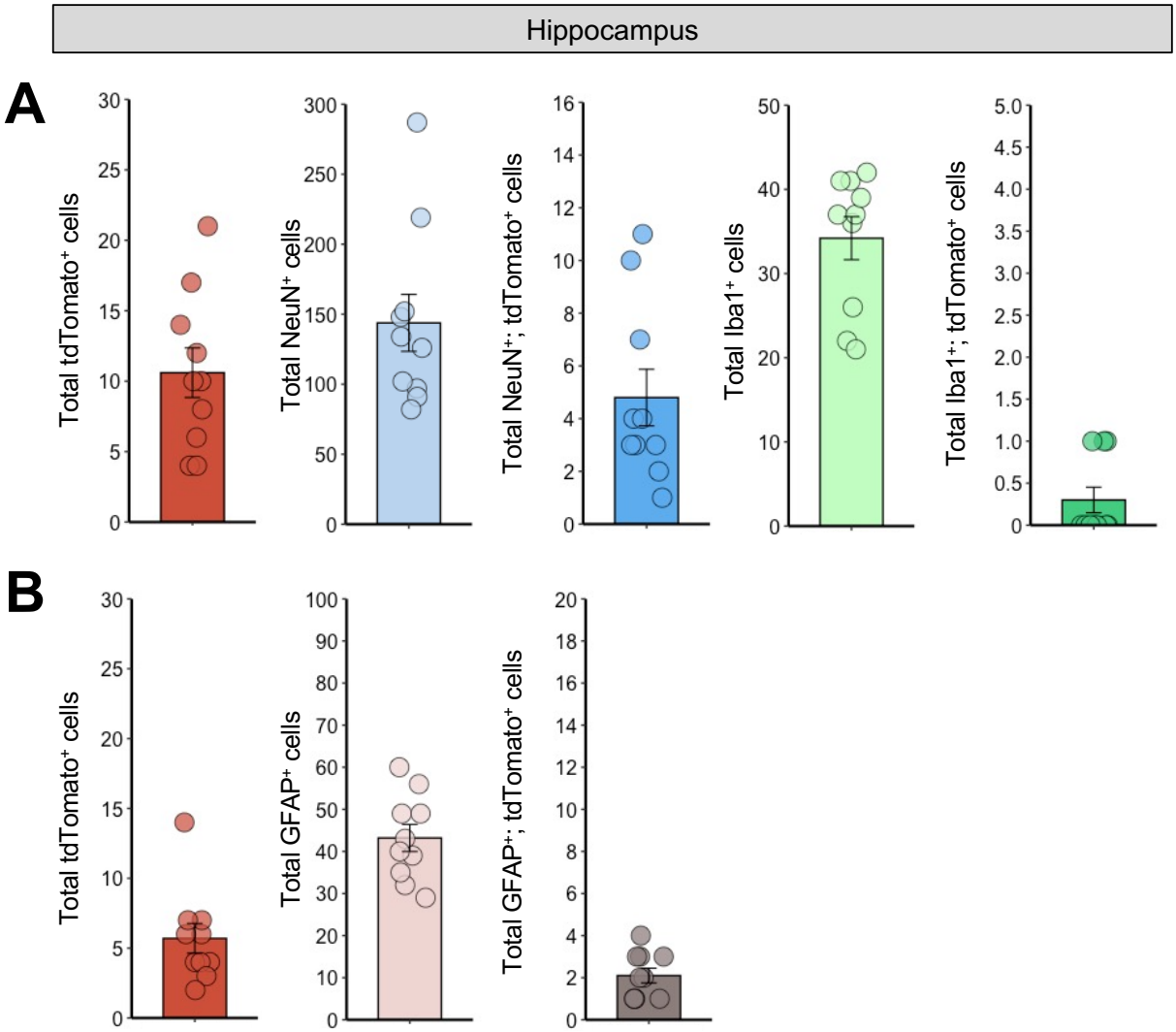

Figure S6: Total number of gene-edited (tdTomato$^+$) cells, total number of NeuN$^+$, Iba1$^+$ and GFAP$^+$ as well as total number of gene-edited NeuN$^+$, Iba1$^+$ and GFAP$^+$ cells in the hippocampus after the injection with MC3 LNP Cas9 mRNA/Ai9 sgRNA (0.250 µg µL^{-1}). (A) Quantification of total number of gene-edited cells (tdTomato$^+$), neurons (NeuN$^+$) and gene-edited neurons (NeuN$^+$; tdTomato$^+$) as well as total number of microglia (Iba1$^+$) and gene-edited microglia (Iba1$^+$; tdTomato$^+$). (B) Quantification of total number of gene-edited cells (tdTomato$^+$), astrocytes (GFAP$^+$) and gene-edited astrocytes (GFAP$^+$; tdTomato$^+$) in MC3 LNP Cas9 mRNA/Ai9 sgRNA (0.250 µg µL^{-1})-injected area in the hippocampus. The quantification was performed in samples from the same animal stained with either (A) tdTomato, NeuN and Iba1 or (B) tdTomato and GFAP. Mean ± SEM, *n* = 10 images from 2 mice.

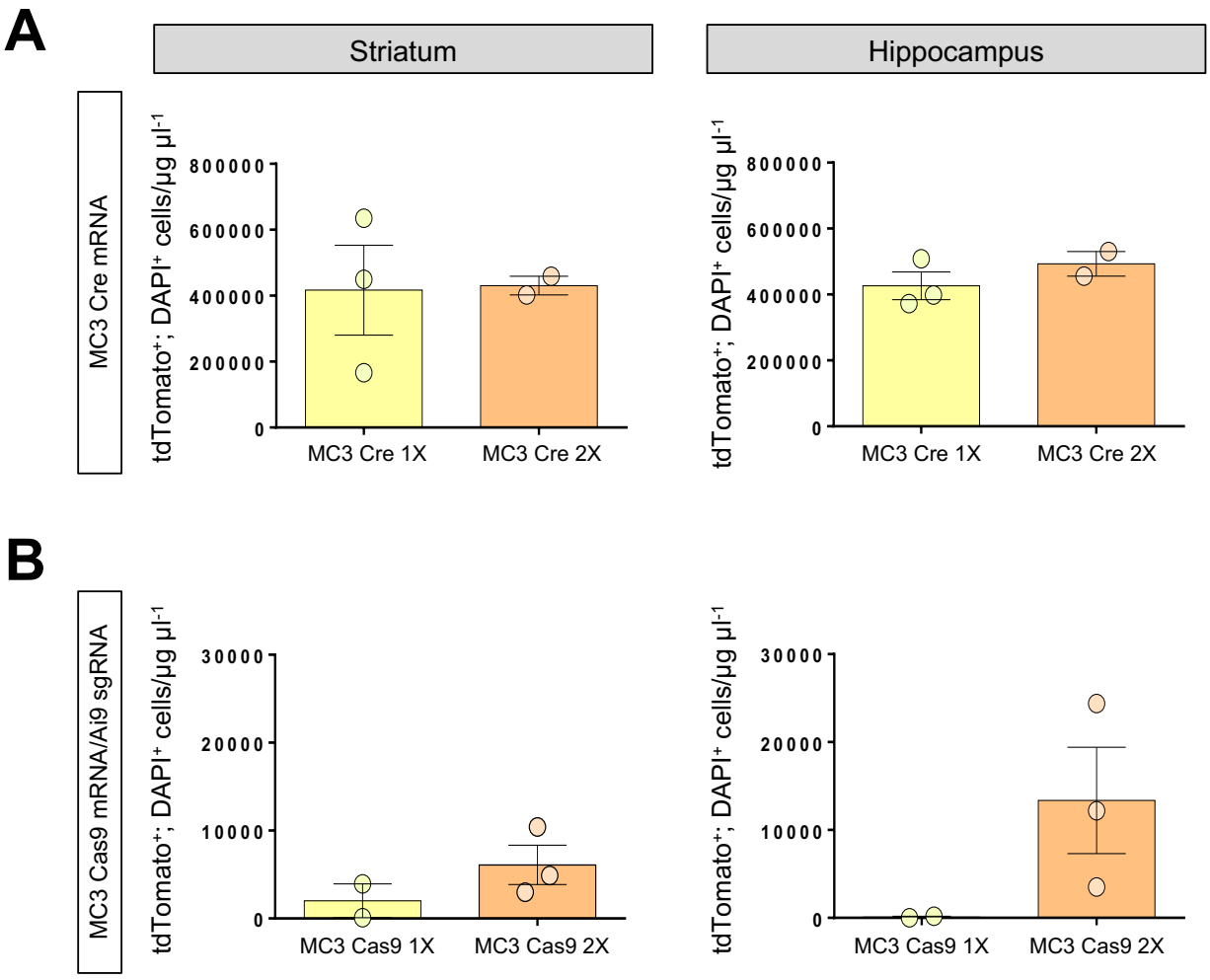

Figure S7: Quantification of tdTomato$^+$; DAPI$^+$ cells normalized by injection concentration of MC3 LNP mRNA. (A) Quantification of tdTomato$^+$; DAPI$^+$ cells per µg µl^{-1} MC3 LNP Cre mRNA in either striatum (Left) or hippocampus (Right) injected with low dose of MC3 LNP Cre mRNA (0.125 µg µL^{-1}; MC3 Cre 1X; *n* = 3 mice) and high dose of MC3 LNP Cre mRNA (0.250 µg µL^{-1}; MC3 Cre 2X; *n* = 2 mice). (B) Quantification of tdTomato$^+$; DAPI$^+$ cells per µg µl^{-1} MC3 LNP Cas9 mRNA/Ai9 sgRNA in either striatum (Left) or hippocampus (Right) injected with low dose of MC3 LNP Cas9 mRNA/Ai9 sgRNA (0.125 µg µL^{-1}; MC3 Cas9 1X; *n* = 2 mice) and high dose of MC3 LNP Cas9 mRNA/Ai9 sgRNA (0.250 µg µL^{-1}; MC3 Cas9 2X; *n* = 3 mice).