

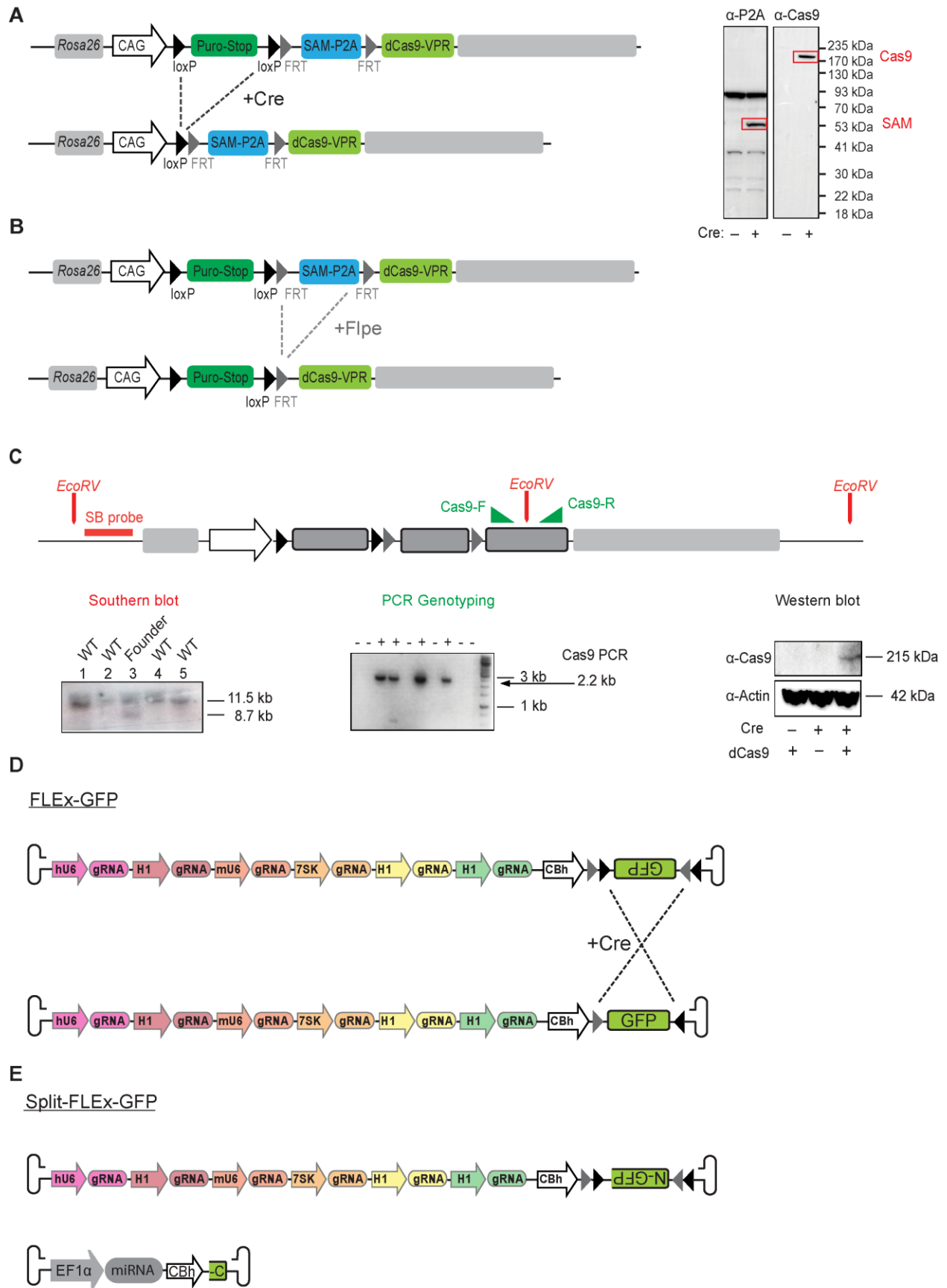
## Appendix:

### Parkinson's disease motor symptoms rescue by CRISPRa-reprogramming astrocytes into GABAergic neurons

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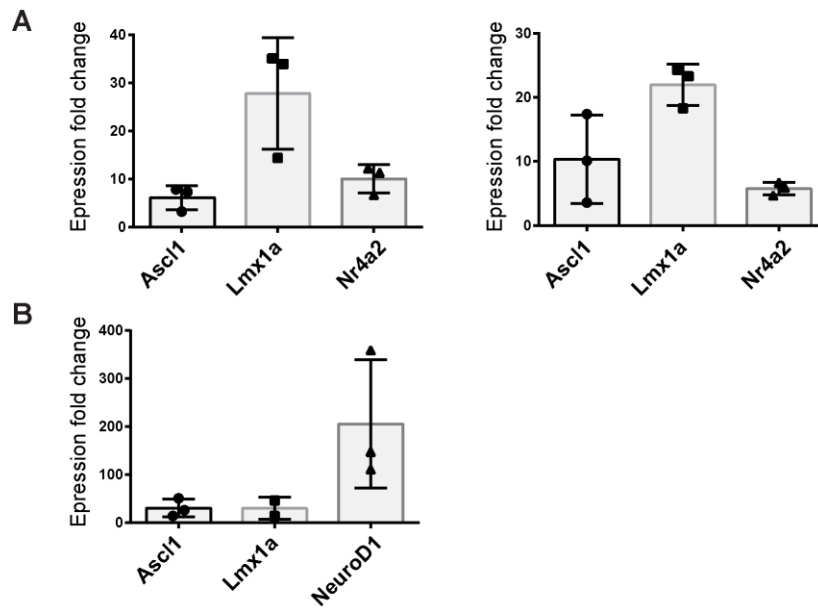
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**Appendix Figure S1: Design and evaluation of the *dCAM* line.**

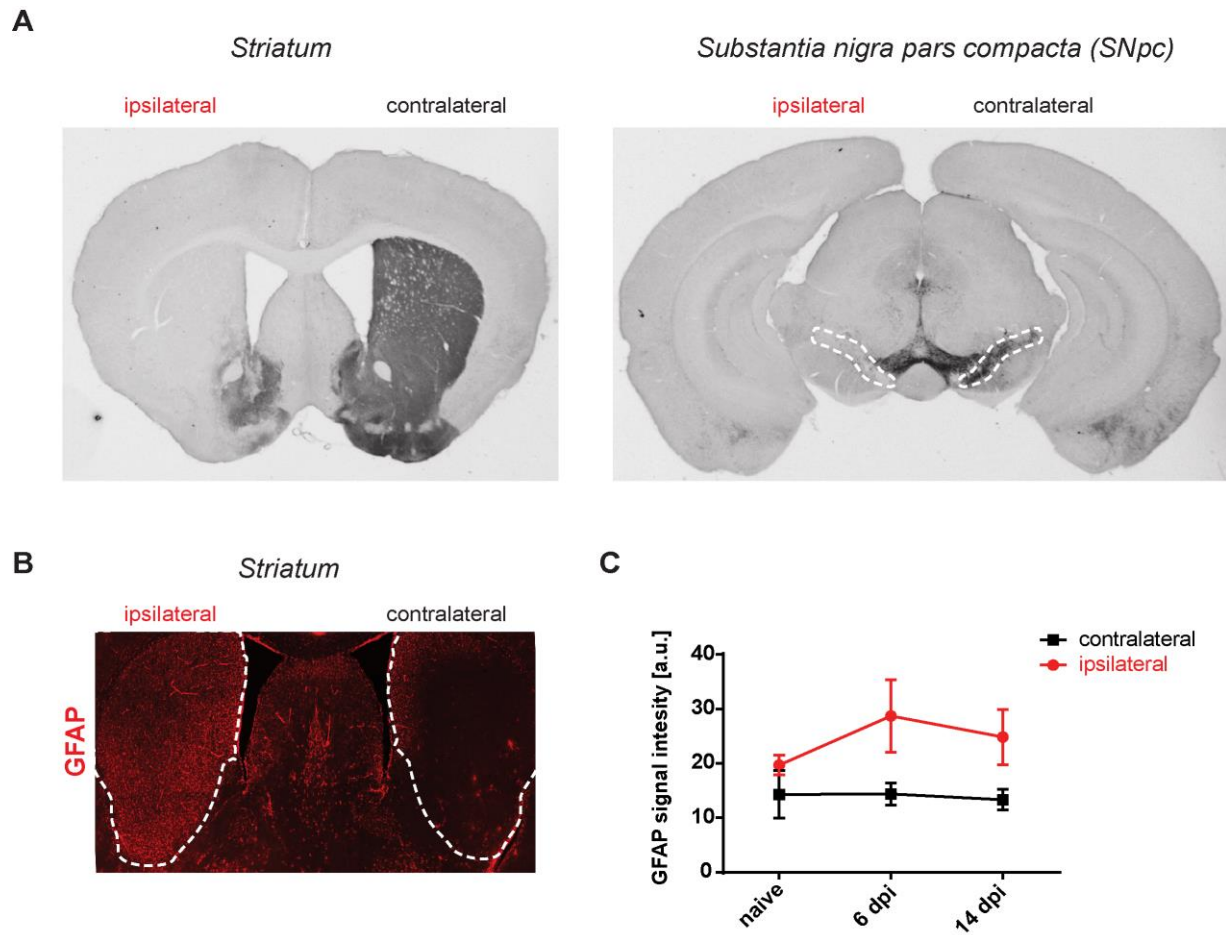
**A**, The *LoxP*-flanked puro-stop cassette ensures highly specific knock-in expression. Western blot analysis of targeting construct in Neuro2A cells. Left blot –Test of the P2A sequence for appropriate cleavage. Antibody binds 5'part of the P2A, SAM-5'-P2A and detects a 55 kDa peptide. No uncleaved fusion products are observed. Right blot – Test of the puro-stop-cassette. Without Cre no Cas9 protein is visible **B**, Variable activation levels can be achieved by removing the *FRT*-flanked SAM activator via flippase induced recombination. **C**, Rosa26 knock-in design, homology arms are used 5'arm 1 kb and 3'arm 4 kb long. Southern blot analysis of the founder animals. gDNA digest using EcoRV results in one wild type fragment of 11.5 kb and one 8.7 kb knock-in fragment indicating the heterozygous knock-in in mouse number 3, which was used for further breeding. Genotyping PCR of F1 generation using Cas9 F and Cas9 R primers, 4 (No. 3, 4, 6, 8) out of 10 animals show knock-in. Western blot from primary astrocytes of the *dCAM x GFAP-cre* line. dCas9 is only detected when Cre was expressed. **D**, For *in vivo* activation an AAV containing 6 sgRNAs and a reporter gene can be applied. **E**, If more than 6 sgRNAs shall be used for *in vivo* activation two AAVs containing 12 sgRNAs or 6 sgRNAs and a miRNA expression cassette can be applied with a split-reporter gene. AAVs contain sgRNAs, whose expression is driven by the different Pol III promoters (H1, hU6, mU6 and 7SK), and the marker gene FLEx-*GFP*, respectively split-FLEx-*GFP*, is expressed by the CBh promoter and also delivered by AAVs.

Abbreviations: Puro – puromycin resistance, SAM - synergistic activation mediator (MS2: MS2 bacteriophage coat protein, p65: p65 subunit of human NF- $\kappa$ B, HSF1: Heat shock factor 1), P2A – 2A self-cleaving peptide, dCas9- deadCas9 (nuclease-deficient), VPR - VP64: 4x VP16 herpes simplex virus protein vmw65, p65, Rta: Regulator of transcriptional activation, CAG - CMV early enhancer/chicken  $\beta$  actin promoter.



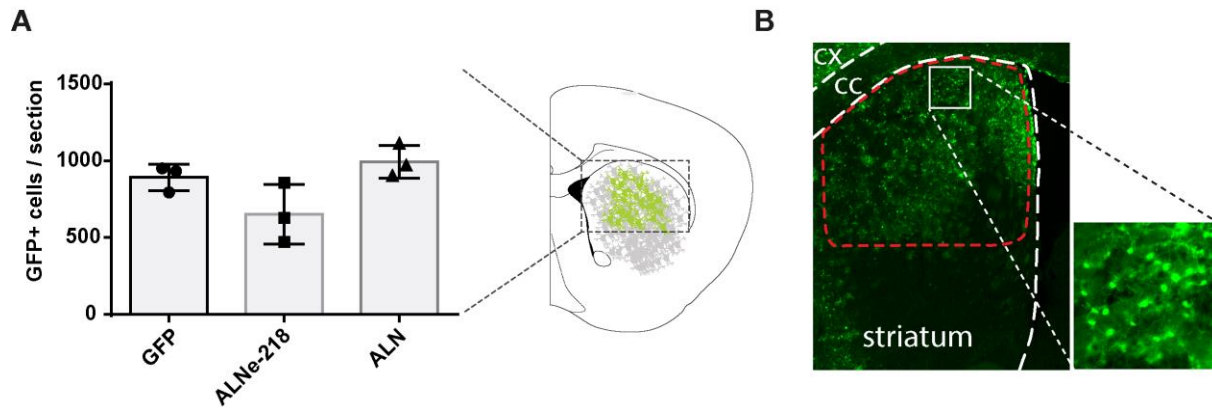
**Appendix Figure S2: Evaluation of *dCAM x Gfap-Cre* primary astrocytes for the activation capacity.**

**A**, Multiplexed activation of *Ascl1*, *Lmx1a* and *Nr4a2*; independent replicates: left: *Ascl1* 6 ± 3, *Lmx1a* 28 ± 12, *Nr4a2* 10 ± 3, right: *Ascl1* 10 ± 7, *Lmx1a* 22 ± 3, *Nr4a2* 6 ± 1) **B**, Multiplexed activation of *Ascl1*, *Lmx1a* and *NeuroD1*. (*Ascl1* 31 ± 19, *Lmx1a* 30 ± 23, *NeuroD1* 206 ± 134). Activation levels are depicted as fold change between cells transfected with and without sgRNAs. All levels were normalized to β-Actin. Error bars represent mean ± SD between technical replicates.



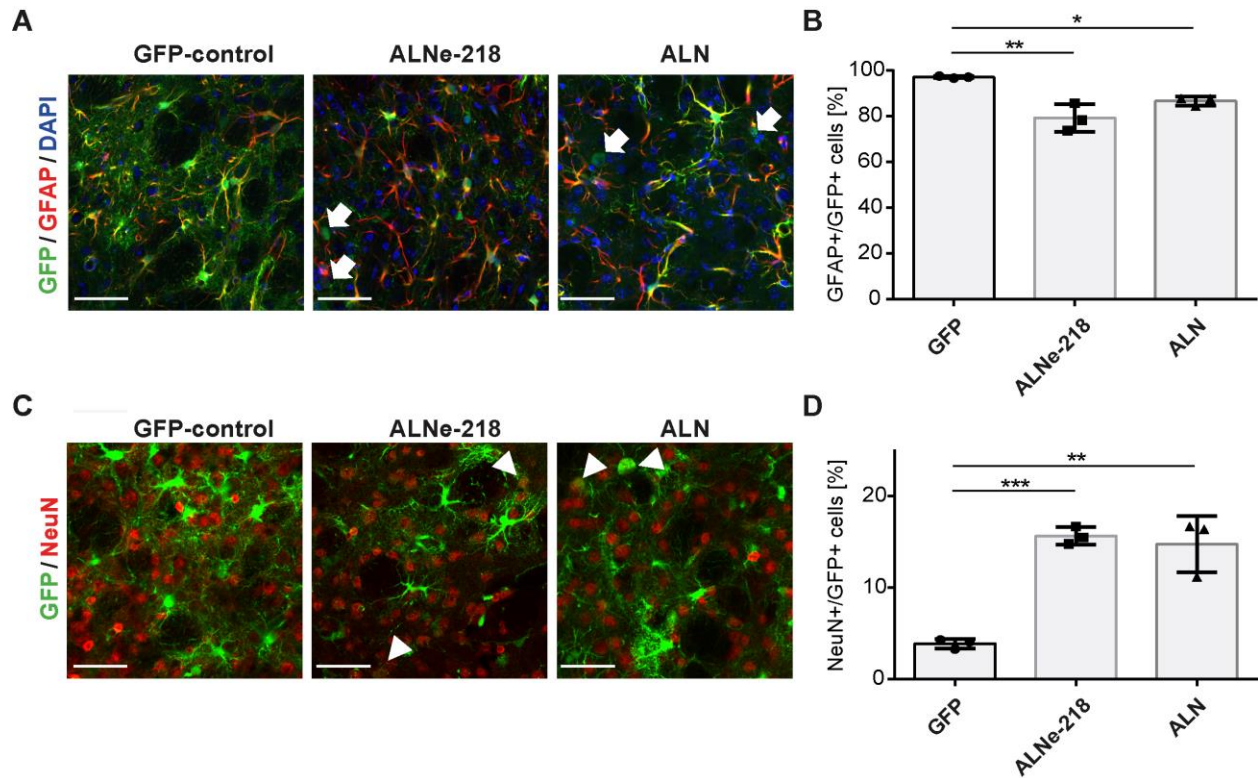
### Appendix Figure S3: Evaluation of 6-ODHA induced lesion.

**A, B**, Immunohistochemistry 14 days after the 6-OHDA injection into the medium forebrain bundle. **A**, Immunohistochemical staining of dopaminergic lesion using the marker tyrosine hydroxylase (TH). **B**, Staining with the astrocytic marker GFAP to assess the reactive gliosis. **C**, Reactive gliosis was assessed via the signal intensity of GFAP stained striata. Naïve, 6 days post lesion (dpi) and 14 dpi animals were analyzed. Per condition data was collected from two animals, from each animal ten images were analyzed, randomly taken in striatal regions. Ipsilateral: Naïve  $19.7 \pm 1.8$ , 6 dpi  $28.7 \pm 6.7$ , 14 dpi  $24.9 \pm 6.0$ , contralateral: Naïve  $14.3 \pm 4.4$ , 6 dpi  $14.4 \pm 2.0$ , 14 dpi  $13.3 \pm 1.9$ .



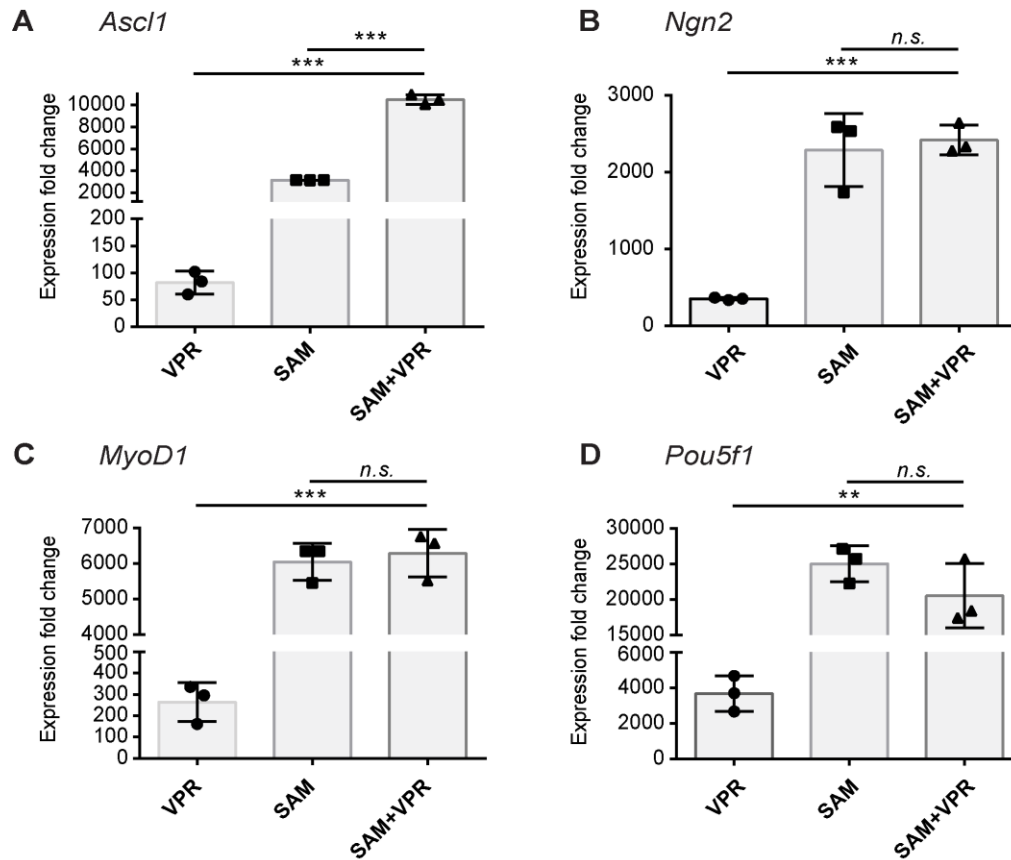
**Appendix Figure S4: Total amount and regional distribution of GFP<sup>+</sup> cells *in vivo* in *dCAM x Gfap-Cre* mice injected with FLEX-GFP reporter.**

**A**, GFP<sup>+</sup> cells in the ipsilateral dorsal striatum of one slide after five weeks of injection. No significant difference could be observed between the different reprogramming conditions and the GFP control. *GFP* 892.0 ± 85.4, *ALNe-218* 652.7 ± 193.6, *ALN* 993.3 ± 106.6. Error bars represent mean ± SD. **B**, Immunohistochemical staining of GFP positive cells 13 wpi with ALN illustrates the regional distribution of the infected and reprogrammed cells. Quantifications are performed in the dorsal striatum (red dashed line) excluding the subventricular zone. Abbreviations: CX – cortex, CC - corpus callosum.



**Appendix Figure S5: *In vivo* reprogramming 5 weeks after AAV injection in dCAM x Gfap-Cre mice.**

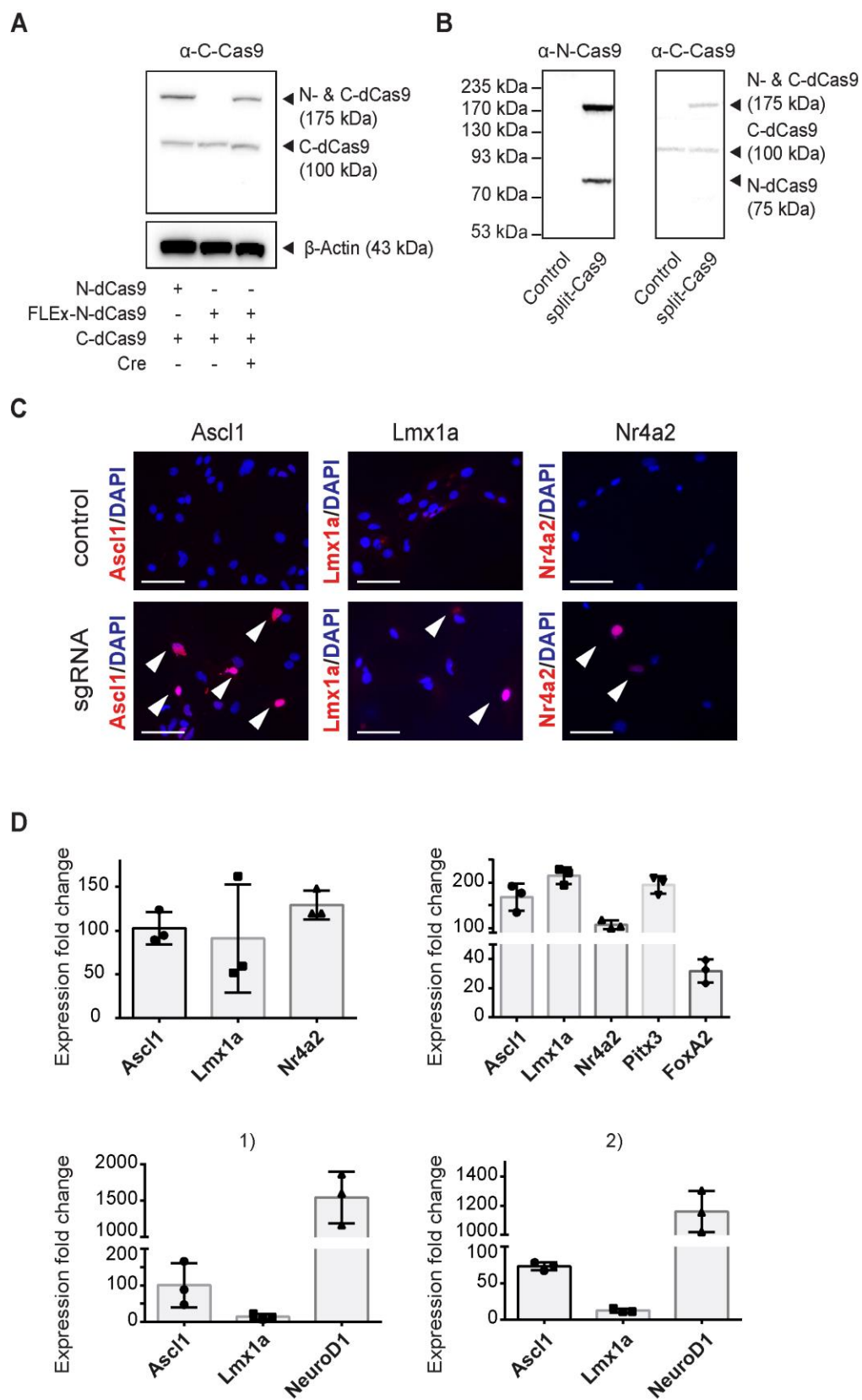
**A**, Photomicrographs showing GFP<sup>+</sup>/Gfap<sup>+</sup> double positive cells 5 wpi. Arrows indicating GFP<sup>+</sup>/Gfap<sup>+</sup> cells. **B**, Quantification GFAP<sup>+</sup>/GFP<sup>+</sup> cells. *GFP* 97.13 ± 0.45%, *ALNe-218* 79.33 ± 6.05 %, and *ALN* 86.70 ± 1.90%. *GFP* vs. *ALNe-218* *P*=0.0025, *GFP* vs. *ALN* *P*=0.03. Multiple comparison ANOVA *F*(2,6)=17.78. **C**, Photomicrographs showing GFP<sup>+</sup>/NeuN<sup>+</sup> neurons 5 wpi. Arrow heads indicating GFP<sup>+</sup>/NeuN<sup>+</sup> cells. **D**, Quantification NeuN<sup>+</sup>/GFP<sup>+</sup> cells. *GFP* 3.9 ± 0.53%, *ALNe-218* 15.67 ± 0.96% and *ALN* 14.77 ± 3.09%. *GFP* vs. *ALNe-218* *P*=0.0007, *GFP* vs. *ALN* *P*=0.001. Multiple comparison ANOVA *F*(2,6)=35-85. Scale bar indicates 50 µm. Error bars represent mean ± SD. Tukey's multiple comparisons test \* *P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001, \*\*\*\**P*<0.0001.



**Appendix Figure S6: Activation of endogenous genes using the dCas9-VPR and the SAM activator system in Neuro2A cells.**

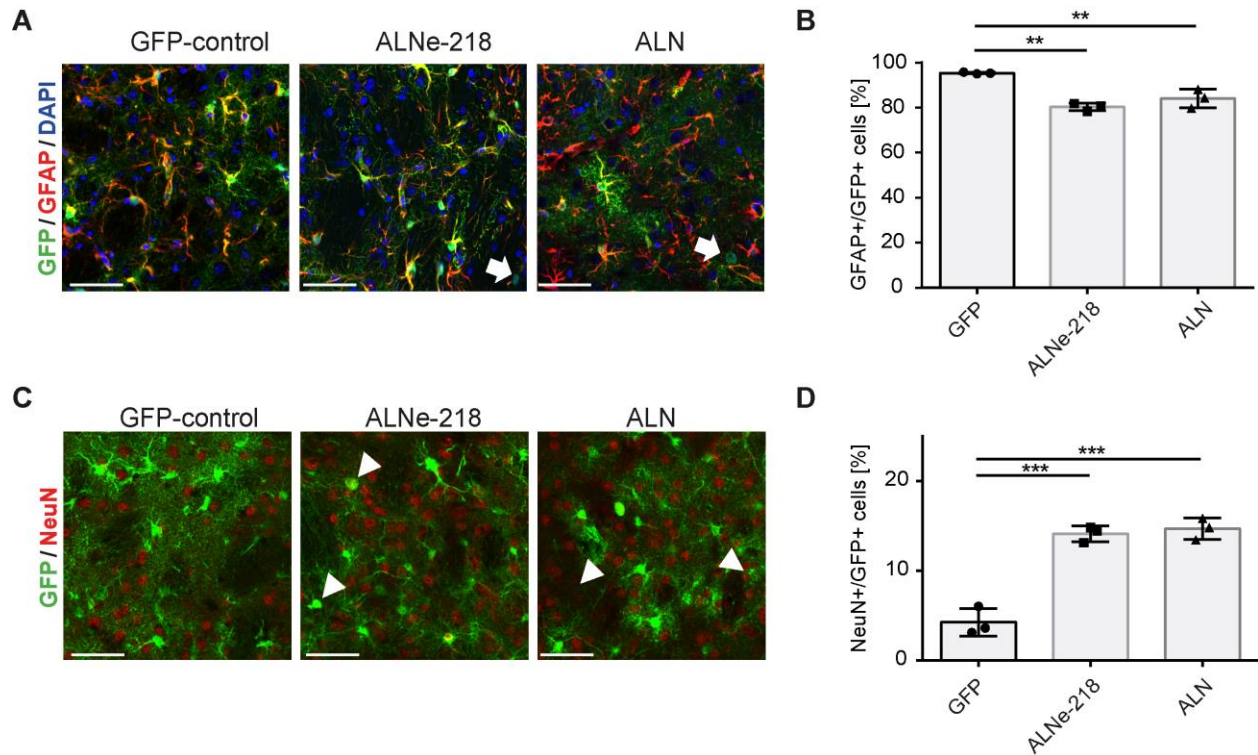
**A**, Activation of *Ascl1*. (VPR  $82 \pm 21$ , SAM  $3158 \pm 10$ , SAM&VPR  $10493 \pm 432$ ) **B**, Activation of *Ngn2*. (VPR  $355 \pm 17$ , SAM  $2290 \pm 476$ , SAM&VPR  $2422 \pm 195$ ) **C**, Activation of *MyoD1*. (VPR  $264 \pm 91$ , SAM  $6047 \pm 517$ , SAM&VPR  $6285 \pm 669$ ) **D**, Activation of *Pou5f1*. (VPR  $3682 \pm 1003$ , SAM  $25041 \pm 2507$ , SAM&VPR  $20534 \pm 4521$ ). Each gene was activated by two sgRNAs targeting a region -200bp to 1bp upstream of the transcriptional start side. Activation levels are depicted as fold change between cells transfected with and without sgRNAs. All levels were normalized to  $\beta$ -Actin.  $n=1$ , with three technical replicates. Error bars represent mean  $\pm$  SD between technical replicates.





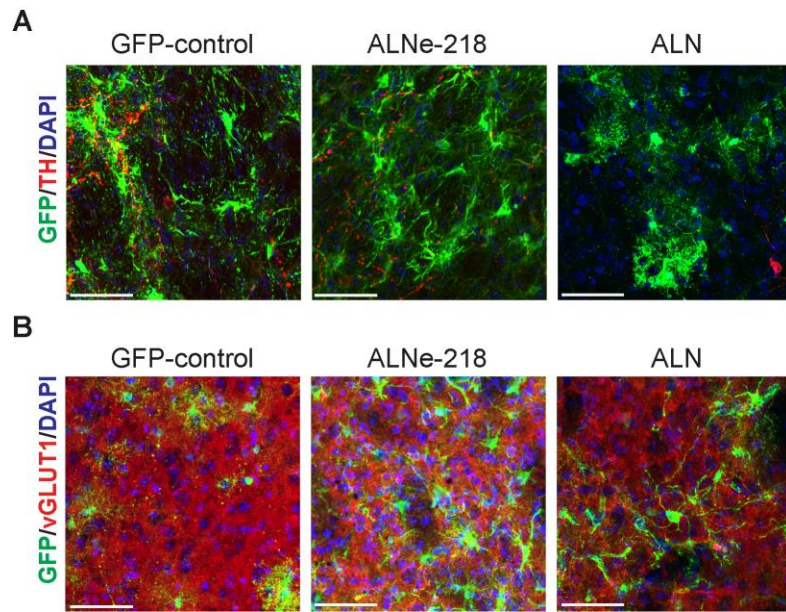
**Appendix Figure S7: Evaluation of AAV-dCAS system *in vitro*.**

**A**, Western blot analysis evaluating the FLEEx-N-dCas9 system in Neuro2A cells, using a C-Cas9 antibody. **B**, Western blot analysis evaluating the split-dCas9 system in Neuro2A cells, left blot – N-Cas9 antibody, right blot – C-Cas9 antibody. Correct fusion of the split-dCas9 parts at 175 kDa. **C**, Immunocytochemistry analysis on primary astrocytic cultures. Activation of *Ascl1*, *Lmx1a* and *Nr4a2*. Upper lane – Transfection of dCas9-activators without sgRNAs. Lower lane - Transfection of dCas9-activators with sgRNAs. Red channel staining for the respective protein. Scale bars indicate 10  $\mu$ m. **D**, RT-qPCR levels. Multiplexed activation of *Ascl1*, *Lmx1a*, *Nr4a2* (*Ascl1*  $103 \pm 19$ , *Lmx1a*  $91 \pm 62$ , *Nr4a2*  $129 \pm 16$ ) and *Ascl1*, *Lmx1a*, and *NeuroD1* (left (1): *Ascl1*  $100 \pm 61$ , *Lmx1a*  $14 \pm 7.5$ , *NeuroD1*  $1542 \pm 352$ , right (2): *Ascl1*  $73 \pm 6$ , *Lmx1a*  $12 \pm 3$ , *NeuroD1*  $1160 \pm 142$ ) and *Ascl1*, *Lmx1a*, *Nr4a2*, *Pitx3*, *FoxA2* (*Ascl1*  $167 \pm 30$ , *Lmx1a*  $215 \pm 18$ , *Nr4a2*  $107 \pm 10$ , *Pitx3*  $195 \pm 19$ , *FoxA2*  $32 \pm 8$ ) in primary astrocytic cultures. Activation levels are depicted as fold change between cells transfected with and without sgRNAs. All levels were normalized to  $\beta$ -Actin. Error bars represent mean  $\pm$  SD between technical replicates.



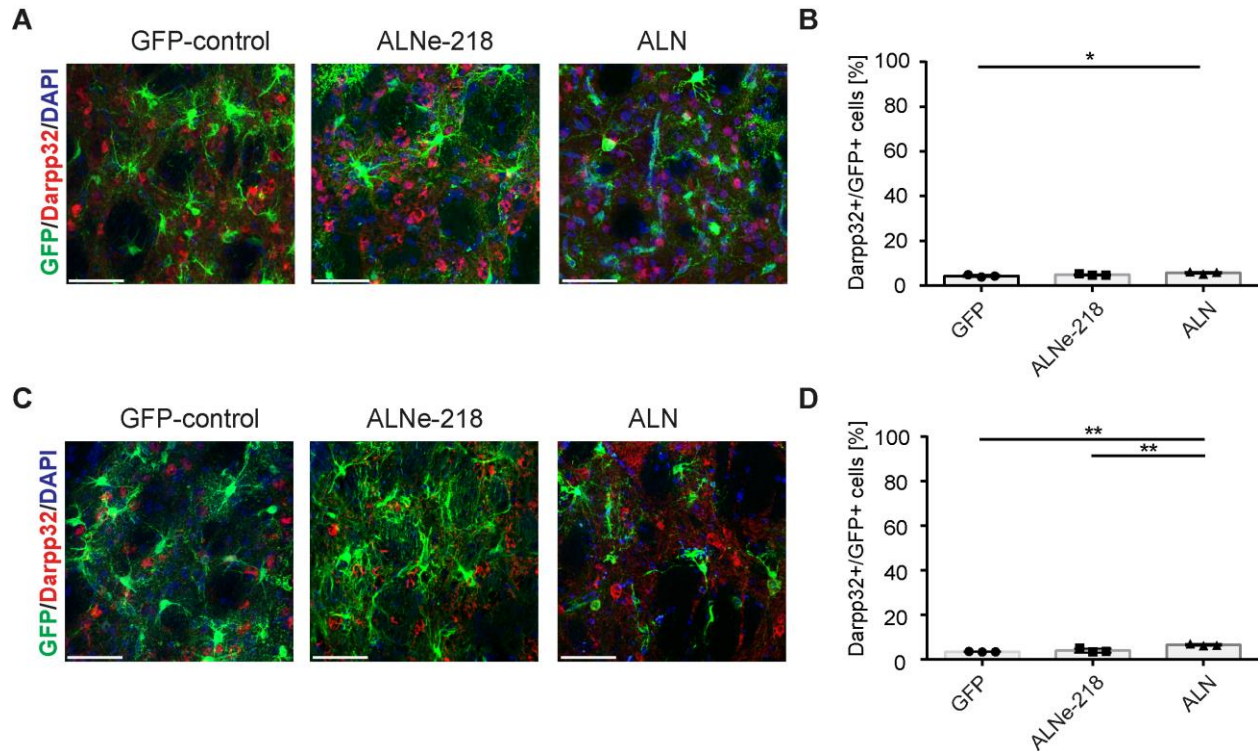
**Appendix Figure S8: *In vivo* reprogramming 5 wpi – AAV-dCAS reprogramming in Gfap-Cre mice.**

**A**, Photomicrographs showing GFP<sup>+</sup>/GFAP<sup>+</sup> cells 5 wpi. Arrows indicating GFP<sup>+</sup>/GFAP<sup>-</sup> cells. **B**, Quantification GFP<sup>+</sup>/GFAP<sup>+</sup> cells. *GFP* 95.37 ± 0.40%, *ALNe-218* 80.33 ± 1.75% and *ALN* 84.10 ± 4.16%. *GFP* vs. *ALNe-218* *P*=0.001, *GFP* vs. *ALN* *P*=0.0045. Multiple comparison ANOVA *F*(2,6)=26.85. **C**, Photomicrographs showing GFP<sup>+</sup>/NeuN<sup>+</sup> neurons 5 wpi. Arrow heads indicating GFP<sup>+</sup>/NeuN<sup>+</sup> cells. **D**, Quantification NeuN<sup>+</sup>/GFP<sup>+</sup> cells. *GFP* 4.23 ± 1.55%, *ALNe-218* 14.10 ± 0.89%, *ALN* 14.67 ± 1.21%. *GFP* vs. *ALNe-218* *P*=0.0002, *GFP* vs. *ALN* *P*=0.001. Multiple comparison ANOVA *F*(2,6)=66.67. Scale bar indicates 50 μm. Error bars represent mean ± SD. Tukey's multiple comparisons test \* *P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001, \*\*\*\**P*<0.0001.



**Appendix Figure S9: Neurotransmitter identities of reprogrammed neurons using AAV-dCAS.**

**A, B,** Confocal images showing co-localization of GFP with specific markers for neurotransmitter subtypes. **A,** Tyrosine hydroxylase – dopaminergic neurons **B,** Vesicular glutamate transporter 1 – glutamatergic neurons. Scale bars indicate 50  $\mu$ m.



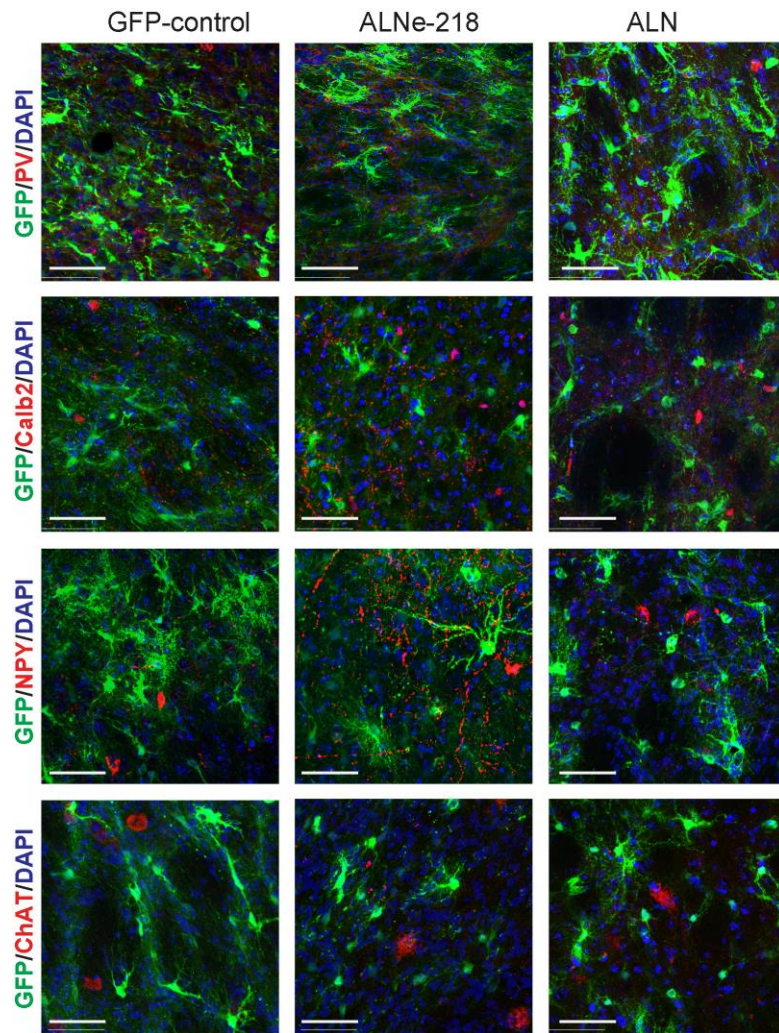
### Appendix Figure S10: Darpp32 staining and quantification 13 wpi.

**A, B**, Evaluation Darpp32 staining in *dCAM* model. **A**, Confocal images showing co-localization of GFP and Darpp32. **B**, Quantification Darpp32<sup>+</sup>/GFP<sup>+</sup> cells. *GFP* 4.3  $\pm$  0.5%, *ALNe-218* 4.76  $\pm$  0.38% and *ALN* 5.67  $\pm$  0.49%. *GFP* vs. *ALN*  $P=0.023$ . Multiple comparison ANOVA  $F(2,6)=7.078$ .

**C, D**, Evaluation Darpp32 staining in *AAV-dCAS* model. **C**, Confocal images showing co-localization of GFP and Darpp32. **D**, Quantification Darpp32<sup>+</sup>/GFP<sup>+</sup> cells. *GFP* 3.4  $\pm$  0.1%, *ALNe-218* 3.97  $\pm$  0.99% and *ALN* 6.43  $\pm$  0.42%. *GFP* vs. *ALN*  $P=0.0024$ , *ALN* vs. *ALNe-218*  $P=0.0067$ . Multiple comparison ANOVA  $F(2,6)=20.24$ . Scale bars indicate 50  $\mu$ m. Error bars represent mean  $\pm$  SD.

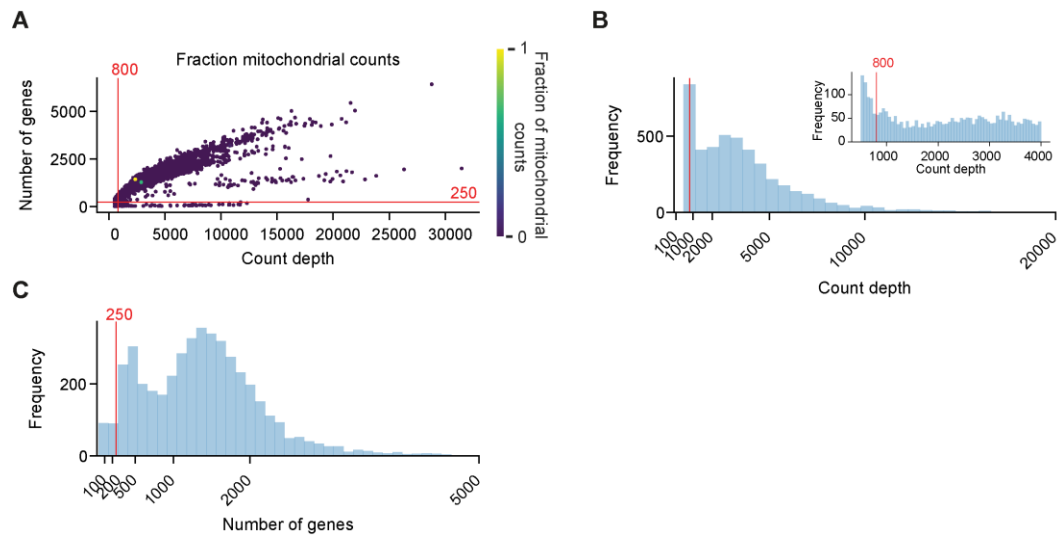
Tukey's multiple comparisons test \*  $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ .





**Appendix Figure S11: Phenotypical characterization of AAV-dCAS reprogrammed neurons.**

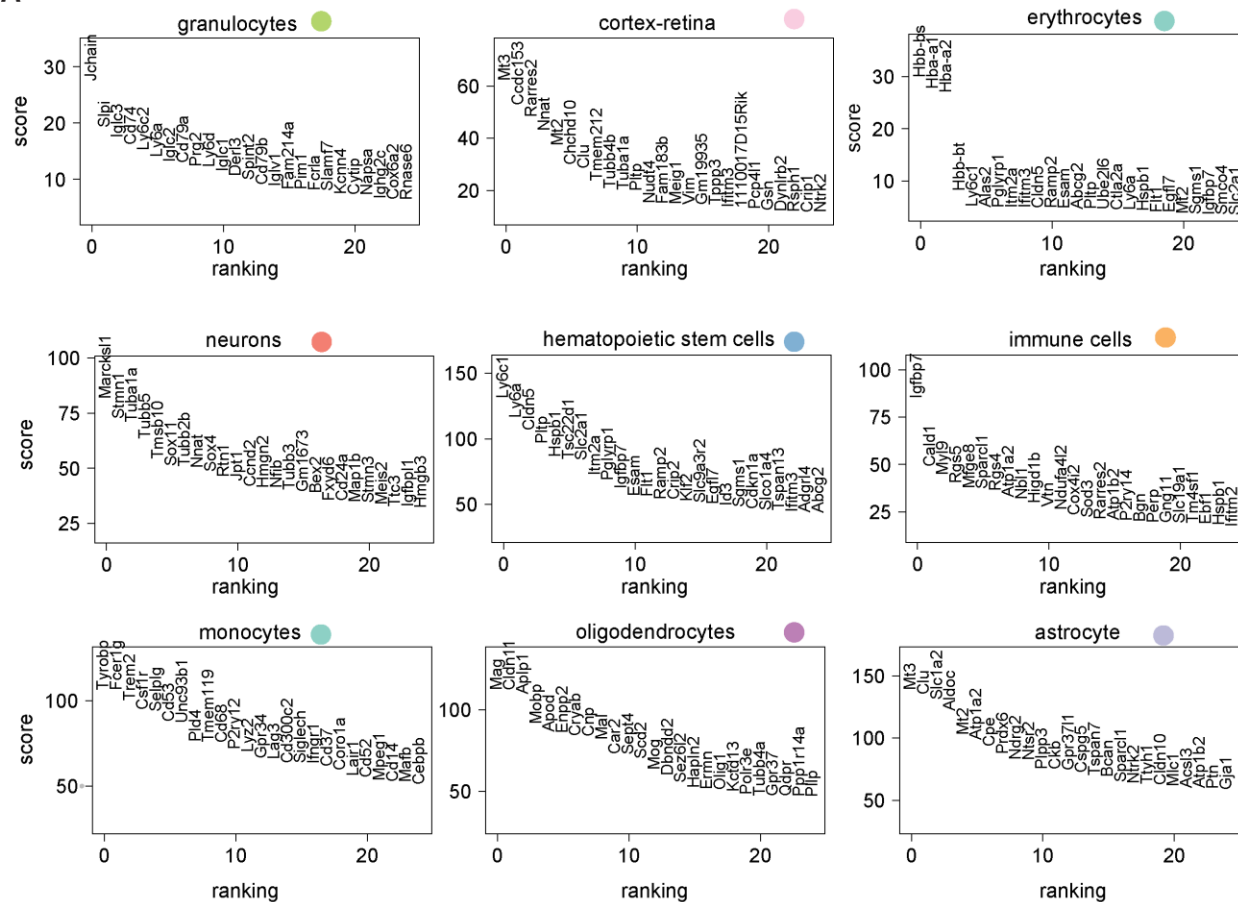
Confocal images demonstrating the absence of several interneuron subtype markers in GFP+ cells: Parvalbumin (PV), Calretinin (Calb2), Neuropeptide Y (NPY) and Choline acetyl transferase (ChAT). Scale bars indicate 50  $\mu$ m.



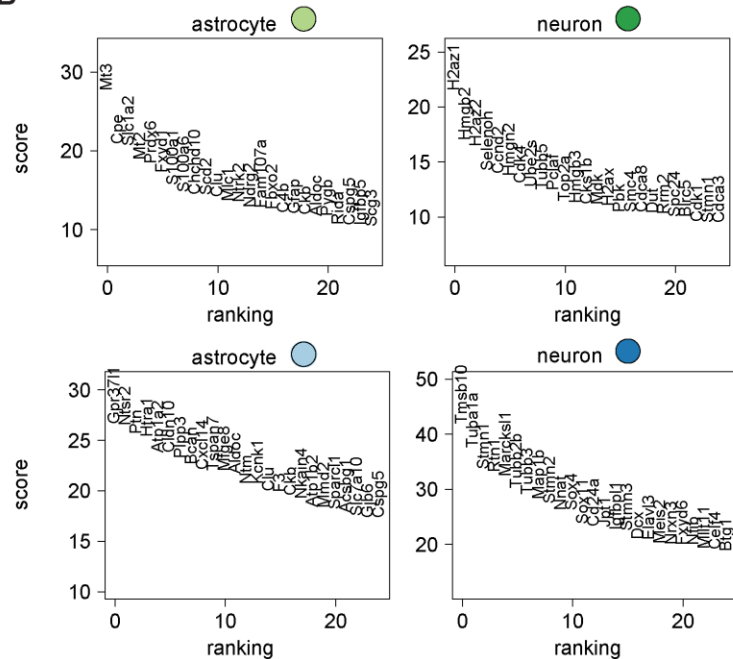
**Appendix Figure S12: Quality control of single cell RNA-seq at 13 wpi of AAVs in dCAM x GFAP-Cre mice striatal tissue.**

**A**, Number of genes (y-axis) versus count depth (x-axis) per cell. Color highlights fraction of mitochondrial reads. Quality control thresholds of 800 and 250 for number of genes and minimum cell depth are defined, respectively, obtaining 3,899 cells. **B**, Distributions of count depth for all cells. Inset shows count depth distribution from for all cells with fewer than 4000 counts. The count depth threshold of 800 is shown as a red, vertical line. **C**, Distribution of number of genes detected per cell. Red line indicates thresholds as in A.

**A**



**B**

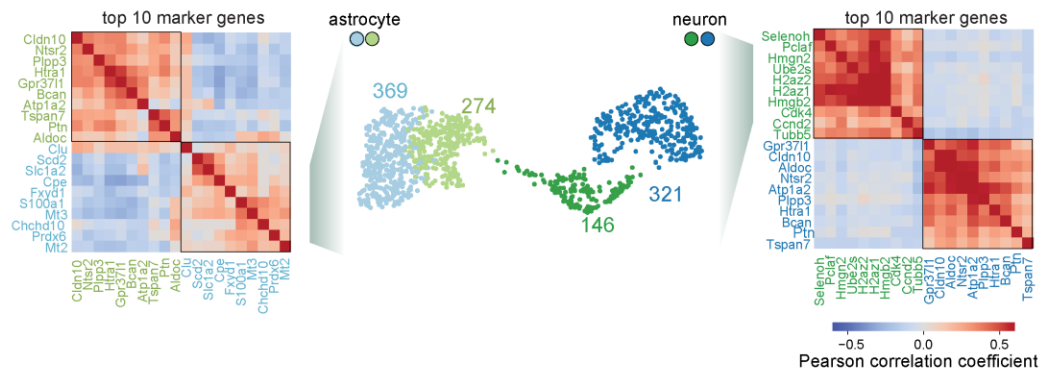




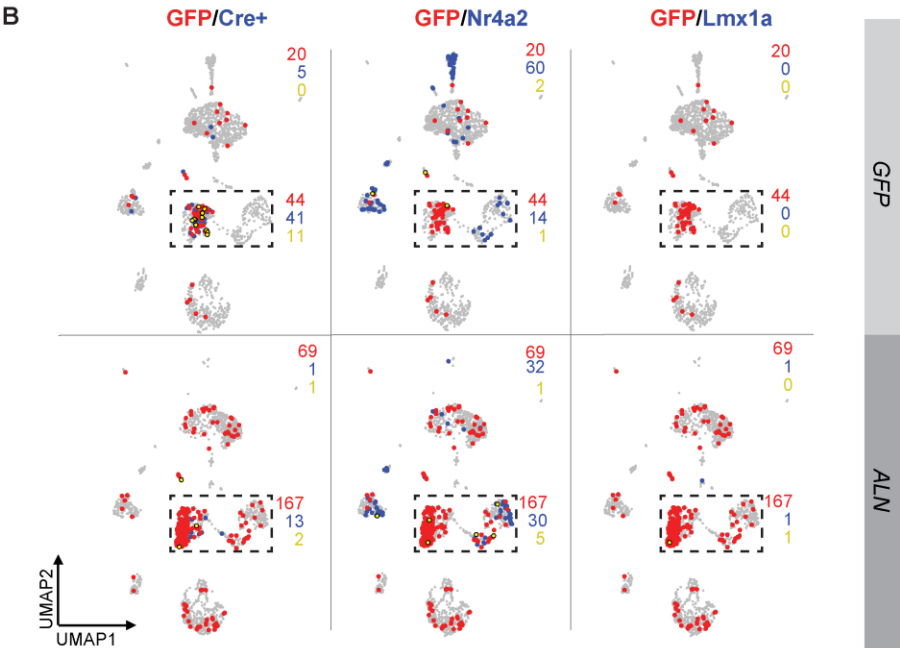
**Appendix Figure S13: Rank\_genes\_groups plots from Scanpy showing the top 25 marker genes using a t-test between log-normalized expression values.**

**A**, Top 25 marker genes ranked by their using  $t$ -test statistic when comparing normalized cell counts between annotated group against all other groups. **B**, Same as in A but using four astrocytic-neuronal subclusters (n=1,110 cells). Colors as in Figure 4 a, b.

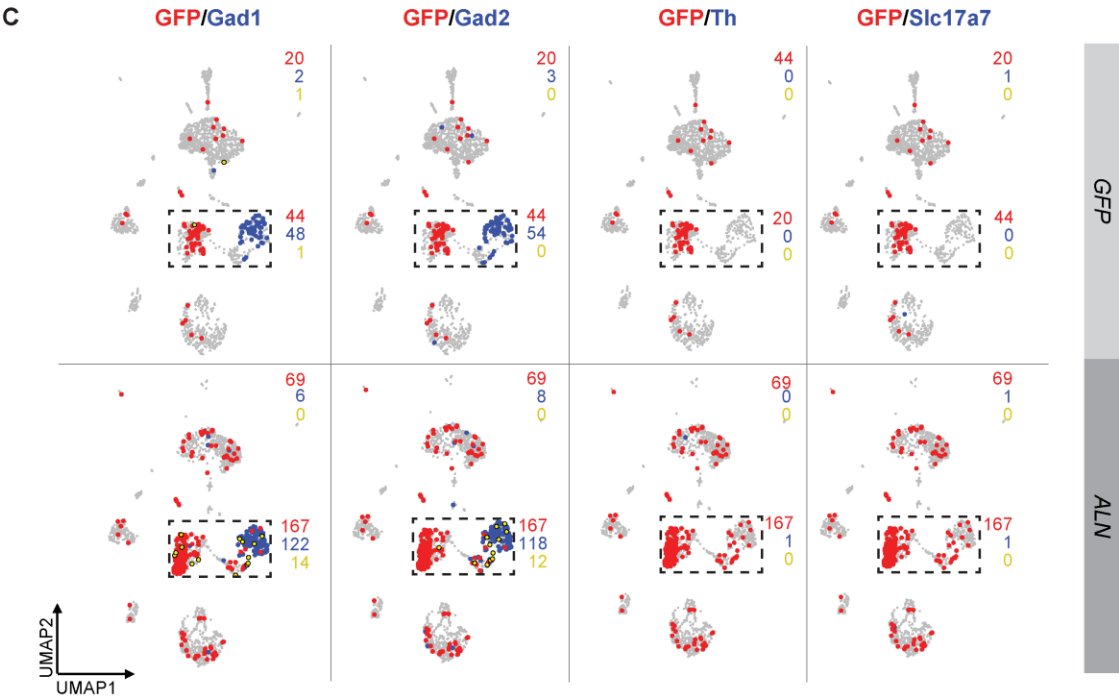
A



B

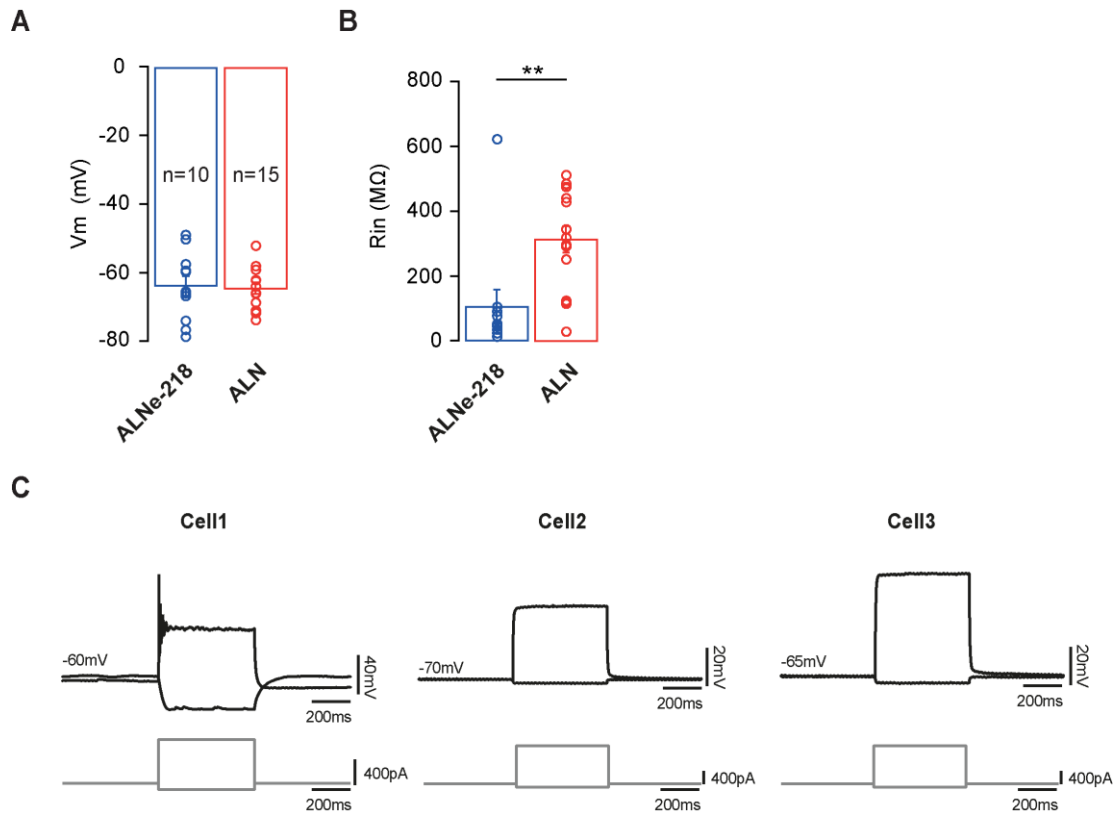


C

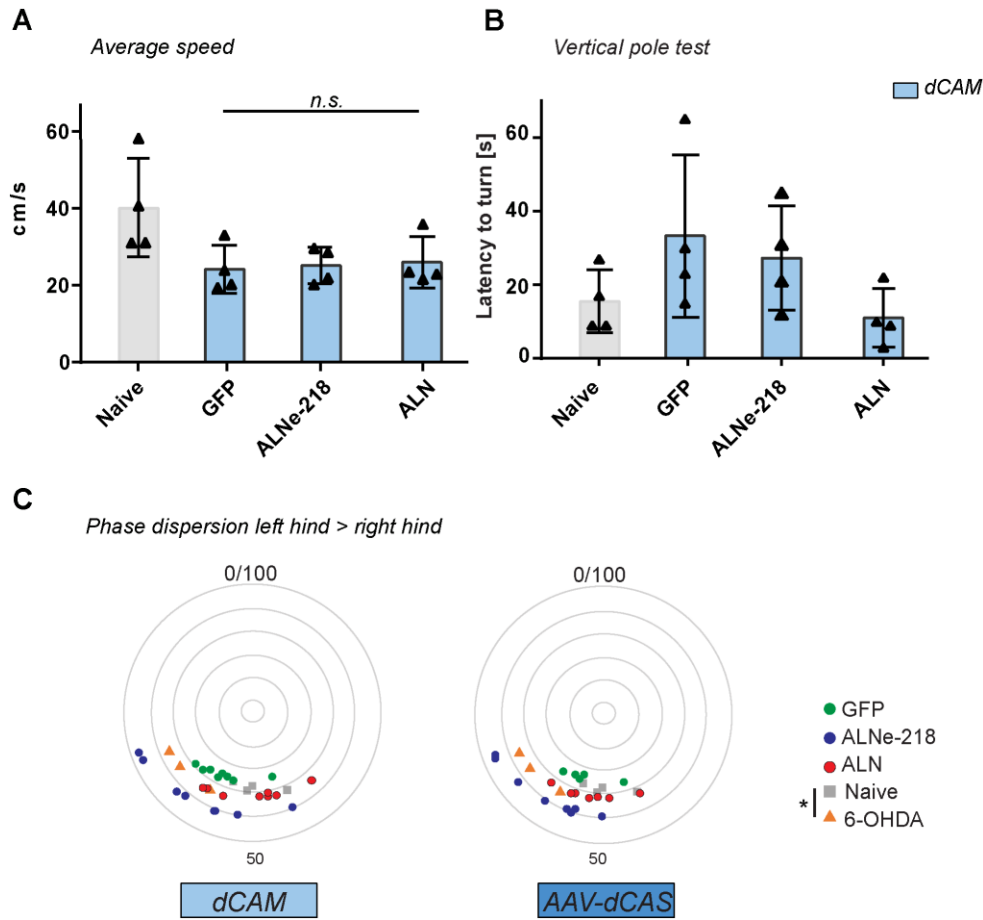


**Appendix Figure S14: scRNA-seq analysis 13 wpi in dCAM x GFAP-Cre mice.**

**A**, Pearson's correlation coefficient of top-10 marker gene expression levels of cells in astrocytic subgroups (n=643) and in neuronal cell subgroups (n=467). **B**, Counts for GFP<sup>+</sup> cells (red), markers *Cre*, *Nr4a2*, and *Lmx1a* (blue) and co-detection of cell with both markers (yellow) in *GFP control* and *ALN* reprogramming. **B**, Same as in B, but showing *Gad1*, *Gad2*, *Th* and *Slc17a7* as markers genes.

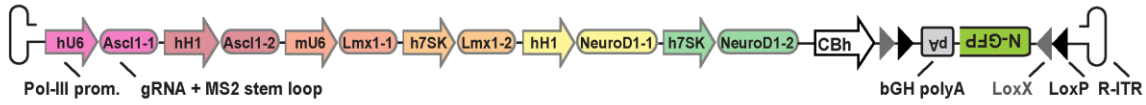
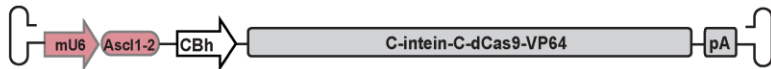
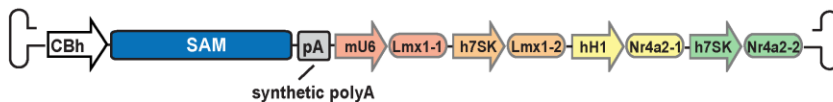
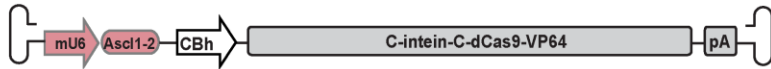
**Appendix Figure S15: Additional electrophysiological measurements 13 and 5 weeks after injection in the AAV-dCAS setting.**

**A**, The resting membrane potential ( $V_m$  in mV) is similar between different reprogramming conditions. **B**, Input resistance ( $R_{in}$  in  $m\Omega$ ) is significantly different between the different conditions (p=0.002, Kruskal-Wallis test). The input resistance of cells measured in the *ALNe-218* condition are similar to immature neurons/glia-like cells, whereas *ALN* reprogrammed cells exhibit an input resistance within the range of endogenous neurons. Kruskal-Wallis test \*\*P<0.01. Error bars represent mean  $\pm$  SEM. **C**, Firing pattern of induced neurons 5 weeks after *ALN* injection. Neurons exhibit electrophysiological properties of immature neurons (cell 1 exhibited one action potential) respectively of glial cells (cell 2 and 3).



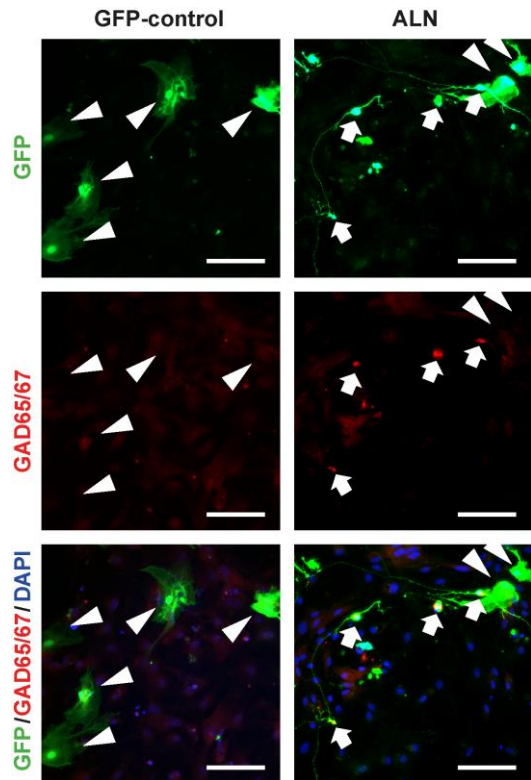
### Appendix Figure S16: Motor behavior analysis.

**A**, Gait analysis using the CatWalk XT system. Average speed of tread. *dCAM* animals 5 wpi,  $n=4$ . Data in cm/s: Naïve  $40.30 \pm 12.78$ , *GFP*  $24.19 \pm 6.25$ , *ALNe-218*  $25.14 \pm 4.74$ , *ALN*  $26.03 \pm 6.69$ . **B**, Vertical pole test for *dCAM* animals. Latency time to turn,  $n=4$ . Data in s: Naïve  $15.50 \pm 8.54$ , *GFP*  $33.25 \pm 22.04$ , *ALNe-218*  $27.25 \pm 14.15$ , *ALN*  $11.00 \pm 7.96$ . *GFP* vs *ALN*  $P=0.17$ . **C**, Phase dispersion left hind paw to right hind paw (LH>RH). Controls: Grey square – naïve, orange triangle – 6-OHDA treated animals. Data in %: Naïve  $52.42 \pm 3.58$ , 6-OHDA  $62.53 \pm 4.08$ . Naïve vs. 6-OHDA  $P=0.0174$ . Treatments: Green – *GFP*, blue – *ALNe-218*, red – *ALN*. *dCAM*: *GFP*  $54.47 \pm 3.66$ , *ALNe-218*  $56.84 \pm 7.28$ , *ALN*  $49.82 \pm 5.14$ . *ALN* vs. *ALNe-218*  $P=0.0549$ , multiple comparison ANOVA  $F(2,20)=3.250$ . *dCAS*: *GFP*  $53.77 \pm 3.56$ , *ALNe-218*  $58.04 \pm 5.42$ , *ALN*  $52.38 \pm 3.84$ . *ALN* vs. *ALNe-218*  $P=0.0653$ , multiple comparison ANOVA  $F(2,17)=3.193$ . Error bars represent mean  $\pm$  SD.

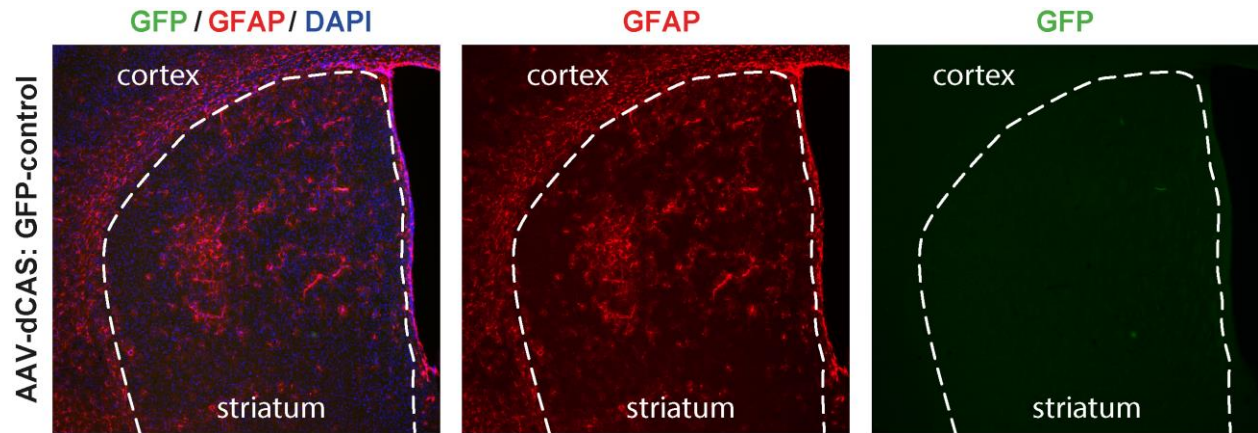
**A***dCAM: GFP control**dCAM: ALNe-218**dCAM: ALN***B***dCAS: GFP control**dCAS: ALNe-218**dCAS: ALN*

**Appendix Figure S17: AAV combinations.**

A representation of the AAV combinations, which were used for the different approaches and experimental groups with detailed information to promoter and gRNA position and regulatory elements. **A**, Combinations used for the dCas9 activator mouse experiments. **B**, Combinations used for the adeno-associated virus (AAV)-based intein-split-dCas9 activator system (AAV-dCAS).

**Appendix Figure S18: dCAS reprogramming of primary astrocytes.**

Reprogramming primary astrocytes by dCAS-mediated induction of *Ascl1*, *Lmx1a* and *Nr4a2* results in *Gad65/67*-positive neurons: Immunocytochemical analysis showing GFP+/GAD65/67+ double positive cells 3 weeks after transduction of primary astrocytes. Arrows indicate double positive GFP+/GAD65/67+ cells, arrowheads indicate GFP+/GAD65/67- cells. Primary astrocytes, (after 8 days in culture) were transfected using LTX+ reagent with AAV plasmids in an equimolar ratio (total 500ng). GFP control contains the non-flexed version of the CBh driven GFP control plasmid. ALN reprogrammed cells were transfected with the non-flexed versions of the AAV-dCas9 ALN combination as indicated in Appendix Figure S17. Cells were fixed and 21 days after transfection. Scale bars indicate 100  $\mu$ m.



**Appendix Figure S19: AAV-dCAS injection into Gfap-Cre negative mice.**

Immunohistochemical staining for GFAP and GFP in Gfap-Cre negative mice 13 weeks after injection of the AAV-dCAS GFP-control virus. Absence of any GFP expression indicates the functionality of the FLE<sub>x</sub> (Cre-ON) reporter system.

gRNA	Gene	Sequence 5' - 3'
Ascl1-1	Ascl1	GGGAGCCGCTCGCTGCAGCAGCG
Ascl1-2	Ascl1	GGGGCTGAATGGAGAGTTTGCA
Lmx1a-1	Lmx1a	GGGAGCAAAGGAGTCGCCTTG
Lmx1a-2	Lmx1a	GAATGCATCCAAGAGTGAACC
Nr4a2-1	Nr4a2	GGCGGTGGGTCATTGTTTCCG
Nr4a2-2	Nr4a2	GTGCCAGTGACGCCGGCCTGG
NeuroD1-1	NeuroD1	GGTTCTGGGAGGGGTGAATGA
NeuroD1-2	NeuroD1	GGCCATATGGCGCATGCCGGGG
Neurog2-1	Ngn2	ATAAGCTGGGGAGGGAGAGC
Neurog2-2	Ngn2	AAACAATCAGATCTGCCCCG
Pitx3-1	Pitx3	ATTCACCTTTATGGCAACCCA
Pitx3-2	Pitx3	GCTAGCCTGGGAGAGCCCAG
FoxA2-1	FoxA2	GAAAGTAACCTTGAAACACCG
FoxA2-2	FoxA2	GGGTAGCCAGAAAGAGGACTG
MyoD1-1	MyoD1	CCAATAGGAGTGTAGTAGGG
MyoD1-2	MyoD1	GAGAGACTGGCAGCCATACG
Pou5f1-1	Pou5f1	ATCTGCCTGTGTCTTCCAGA
Pou5f1-2	Pou5f1	TGTCCGGTGACCCAAGGCAG

**Appendix Table S1: Sequences of gRNAs used in this study**



Gene	TaqMan probe (Assay ID)
Ascl1/Mash1	Mm03058063_m1
Lmx1a	Mm00473947_m1
Nr4a2/NR4A2	Mm00443060_m1
NeuroD1	Mm01280117_m1
Ngn2	Mm00437603_g1
Pitx3	Mm01194166_g1
FoxA2	Mm00839704_mH
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B-Actin	Mm00607939_s1

### Appendix Table S2: TaqMan probes used in this study

**Sequence data:**

>dCas9-SAM-P2A-VPR

[illegible]

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