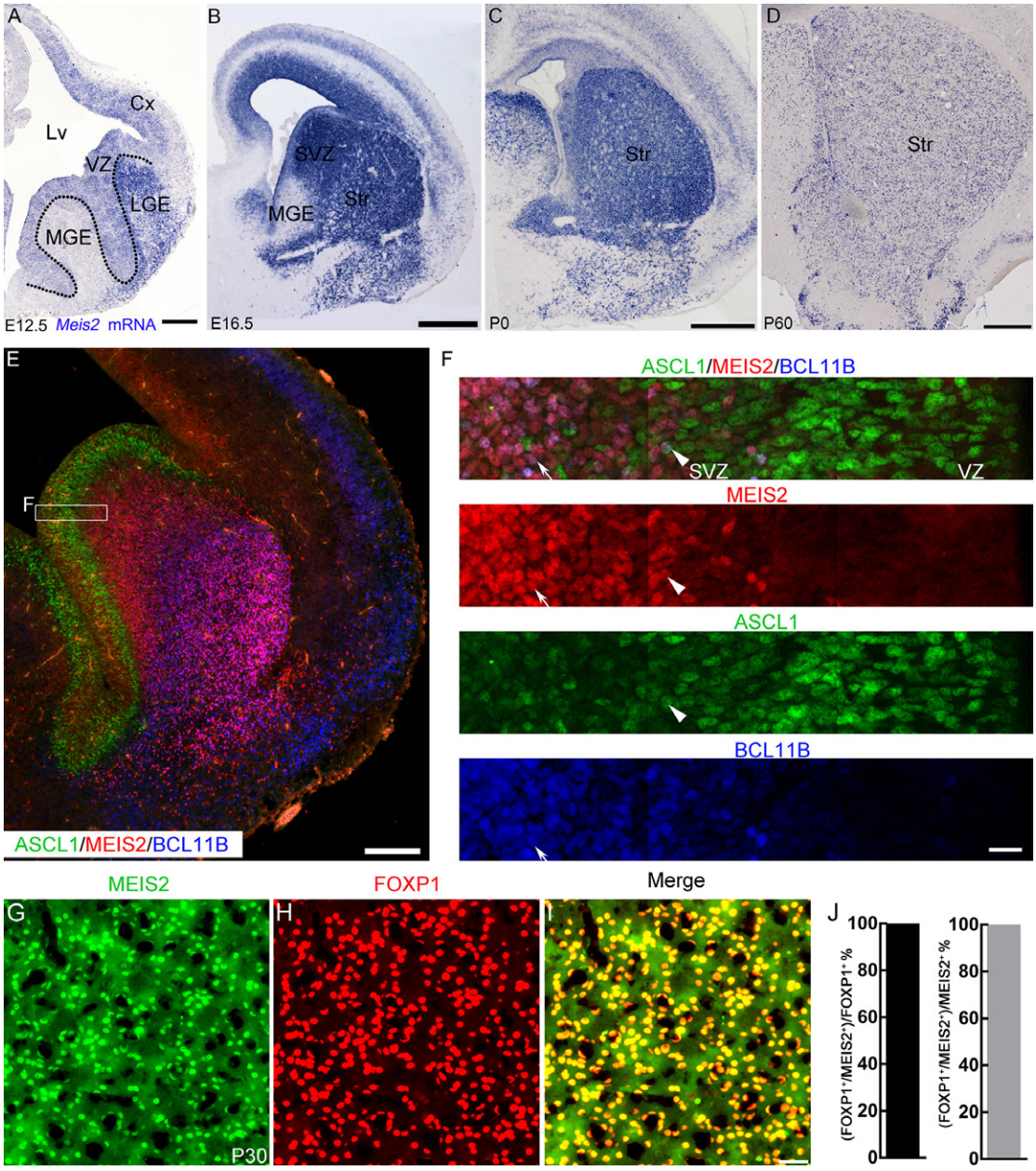


Fig. S1. *Meis2* is strongly expressed in the MSNs from early embryonic to adult stage

(A) At the early stage of E12.5, *Meis2* is strongly expressed in the LGE SVZ and relatively weakly expressed in the MGE, LGE, and cortical VZ. (B-D) ISH showed that *Meis2* was highly expressed in the whole striatum from the embryonic to adult stage. (E) ASCL1/MEIS2/BCL11B triple immunostaining coronal hemi-sections at E14.5. (F)

High magnification images of the boxed regions in E. Very few ASCL1-positive cells were co-labelled with MEIS2 (arrowhead), and the majority of BCL11B-positive cells were co-labelled with MEIS2 (arrow). (G-J) All FOXP1-positive cells were co-labelled with MEIS2 in the striatum at P30, and vice versa. Abbreviations: Cx (cortex); Lv (lateral ventricle); LGE (lateral ganglionic eminence); MGE (medial ganglionic eminence); VZ (ventricular zone); SVZ (subventricular zone); Str (striatum). Scale bars: 100 µm in A; 200 µm in B; 200 µm in C; 500 µm in D; 200 µm in E; 100 µm in F; 100 µm in I for G-I.

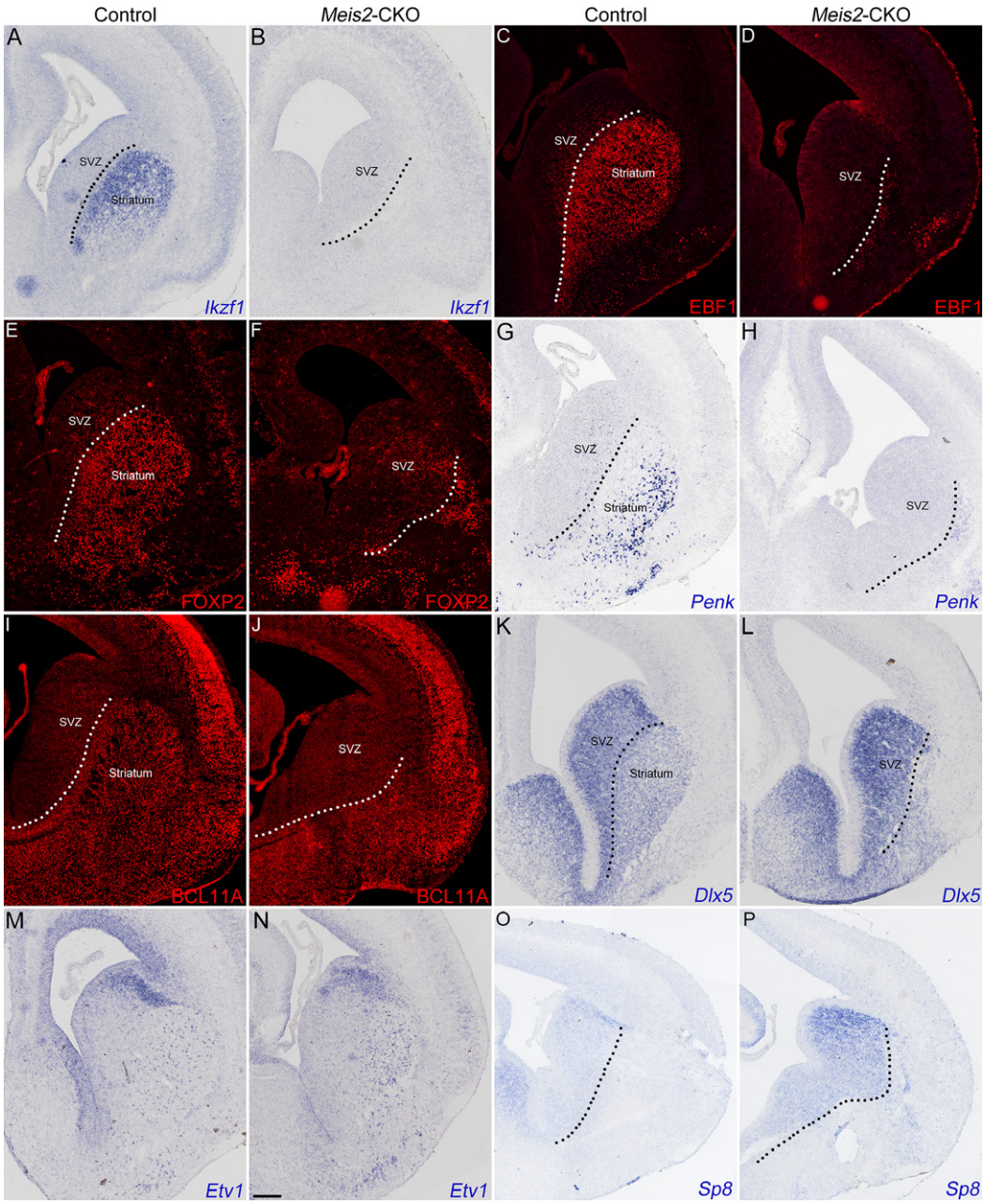


Fig. S2. *Meis2* is required for the generation of striatal MSNs

(A-J) At E16.5, the expression of *Ikzf1*, EBF1, FOXP2, *Penk* and BCL11A was greatly reduced in *Meis2*-CKO mice, compared to control mice. (K-L) Immature neurons that expressed *Dlx5* were generated in the LGE SVZ of *Meis2*-CKO mice. (M-N) There is no significant difference of the *Etv1* expression between *Meis2*-CKO mice and control mice. (O-P) The expression of the transcription factors *Sp8* are increased in the LGE. Scale bars: 200 μm in N for A-P.

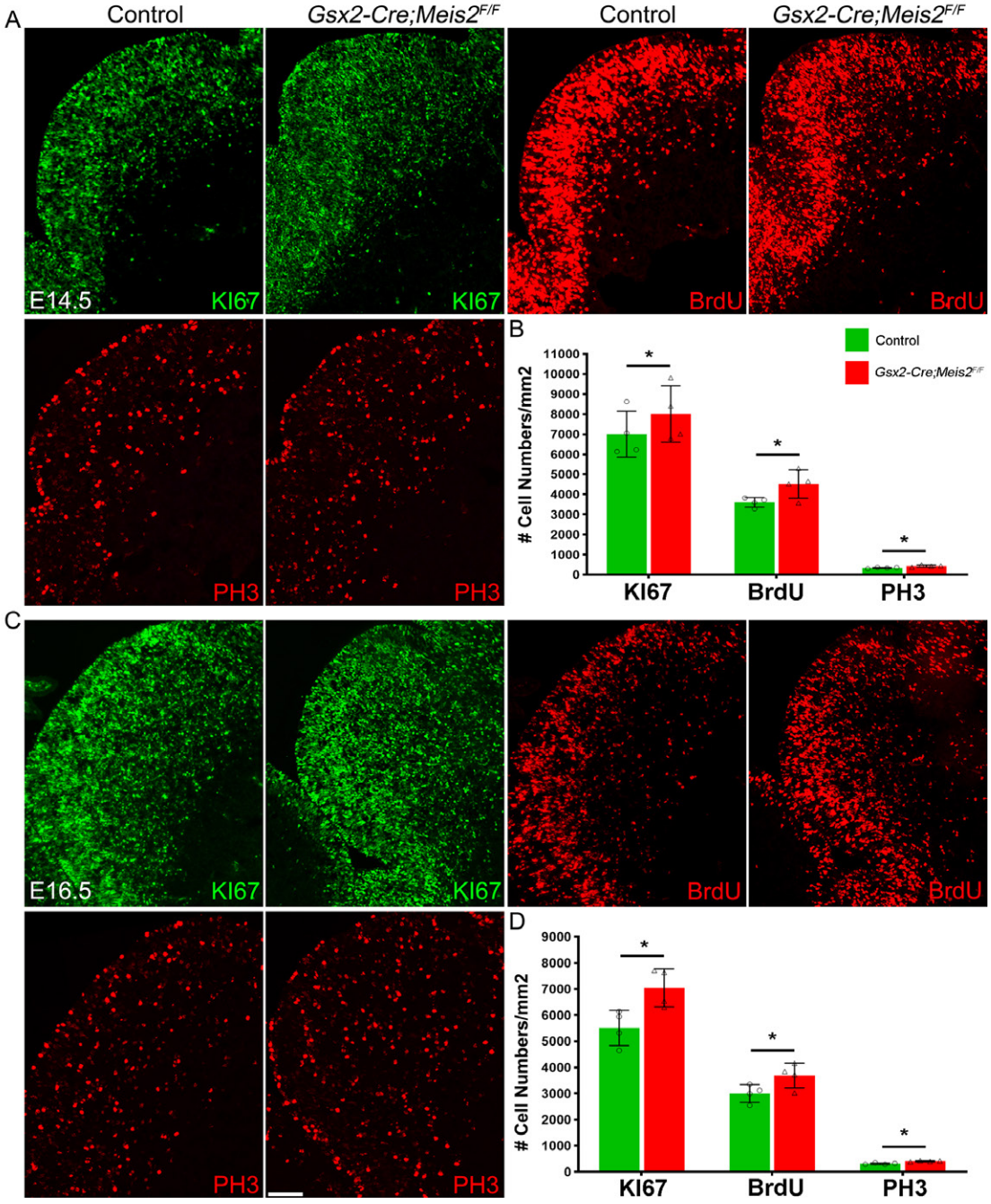


Fig. S3. Cell proliferation is also increased in *Gsx2-Cre; Meis2$^{F/F}$* mice

(A-B) At E14.5, the number of KI67^{+}, PH3^{+}, and BrdU^{+} (2 h) cells were increased in the LGE of *Gsx2-Cre; Meis2$^{F/F}$* mice versus control. (C-D) At E16.5, the numbers of KI67^{+}, PH3^{+}, and BrdU^{+} cells were also increased in the LGE of *Gsx2-Cre; Meis2$^{F/F}$* mice. Scale bars: 50 μm in C for A and C.

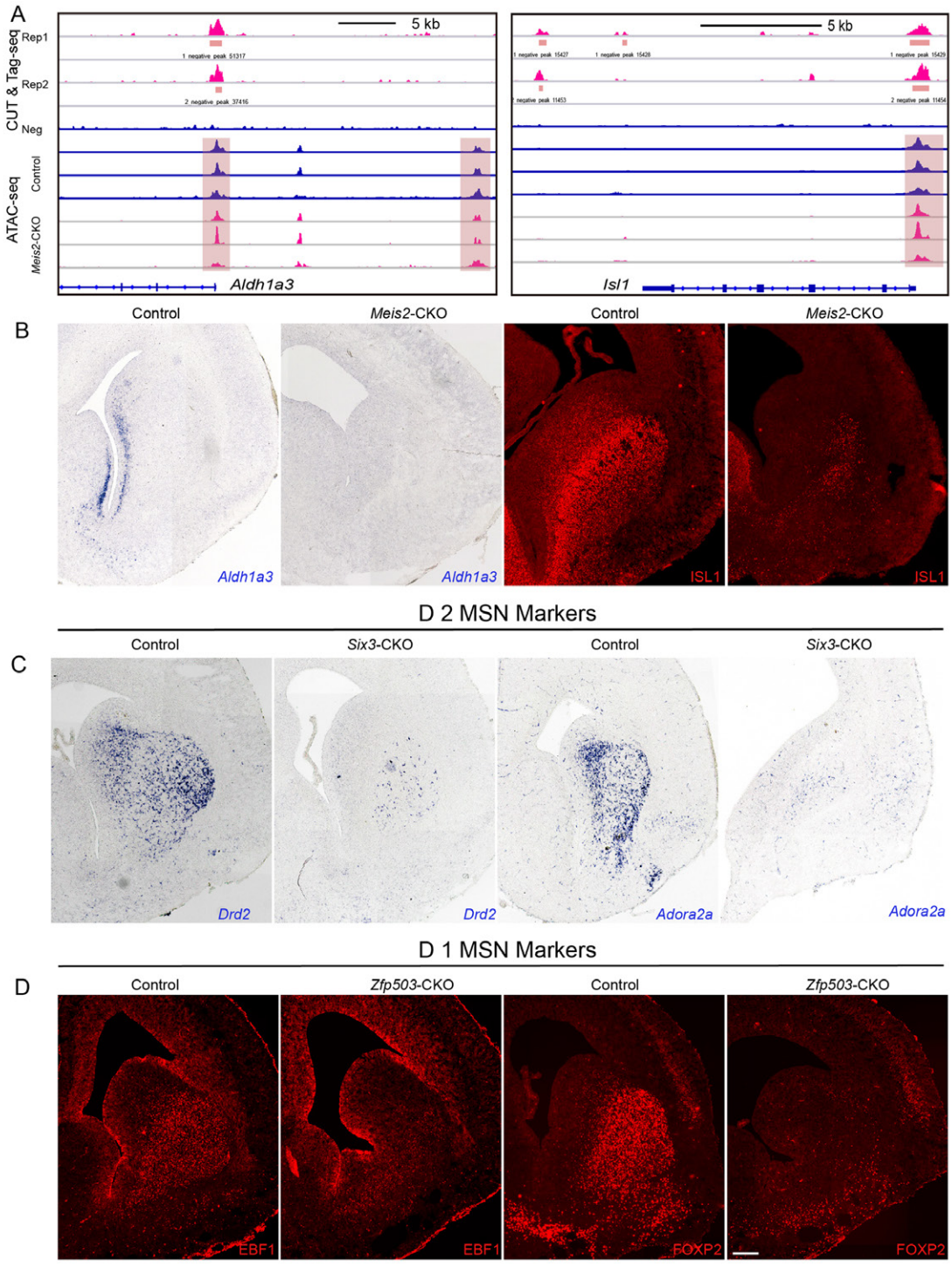


Fig. S4: *Meis2* is required for the normal differentiation process of the MSNs

(A) CUT&Tag-seq and ATAC-seq data showed that the expression of *Aldh1a3* and *Isl1* is directly regulated by *Meis2*. (B) At E16.5, the expression of *Aldh1a3* and *Isl1* was greatly reduced in *Meis2*-CKO mice compared to control mice. (C) ISH showed that the expression of *Drd2* and *Adora2a* was significantly reduced in the *Six3*-CKO mice. (D) Immunostaining showed that the expression of D1 MSN markers (EBF1 and FOXP2) was significantly reduced in *Zfp503*-CKO mice. N = 3 mice per group; Scale bar: 200 μm in D for B-D.

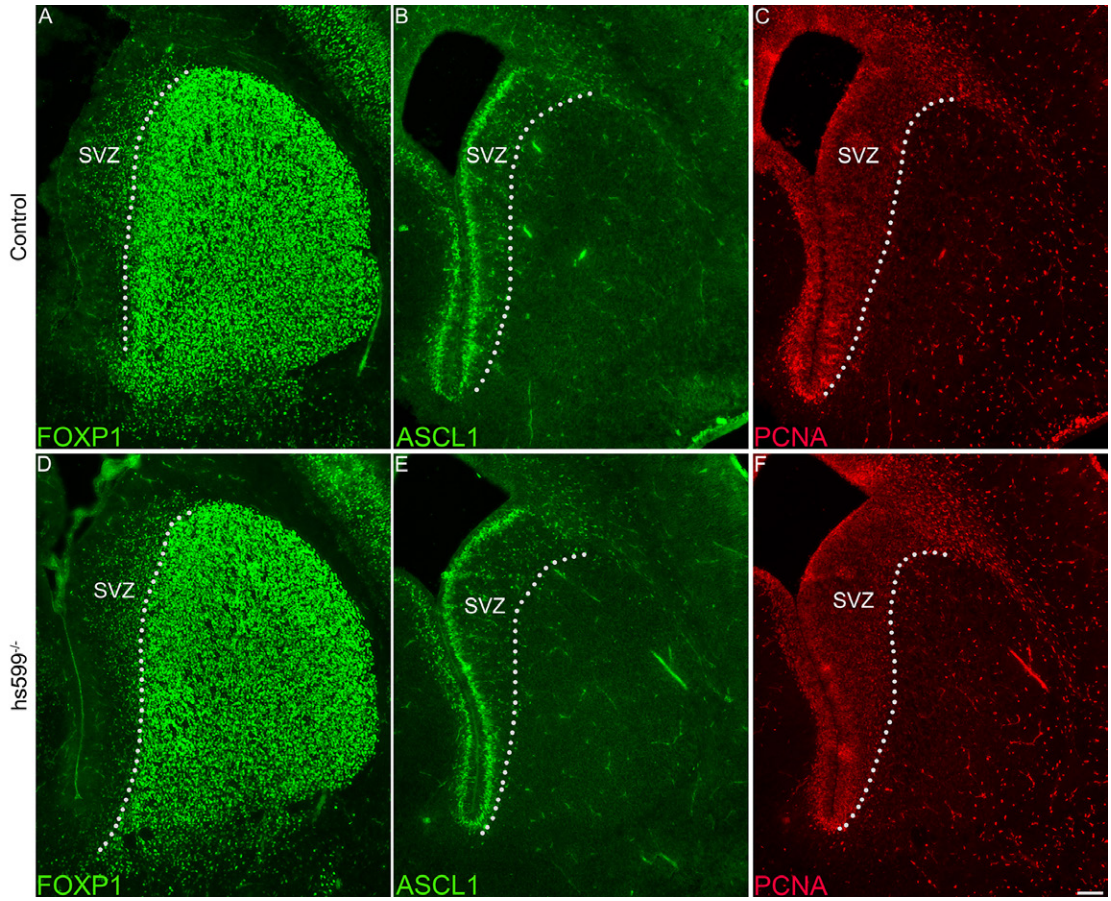
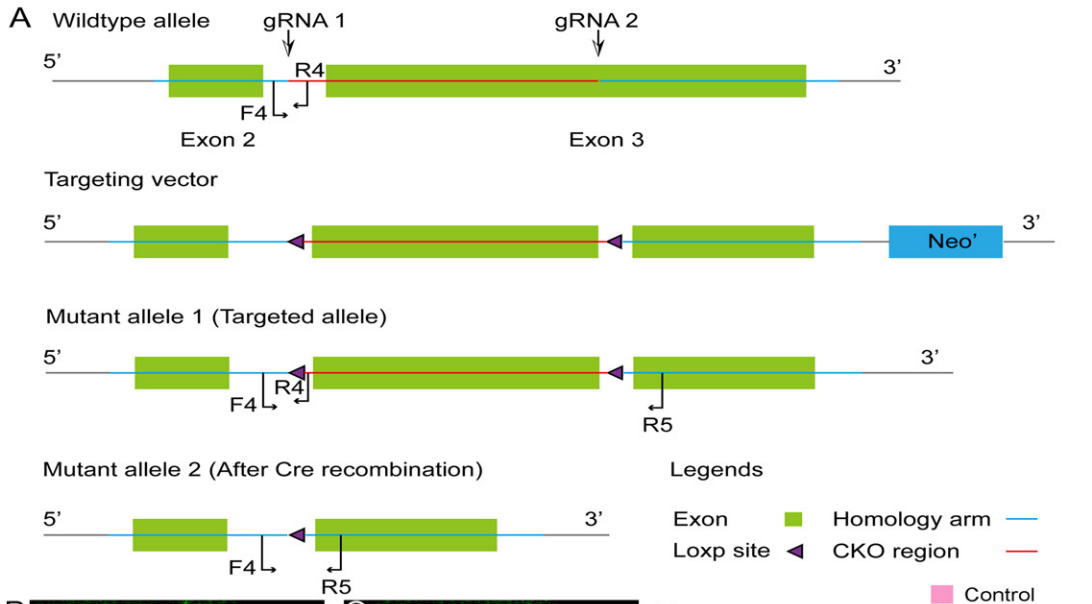
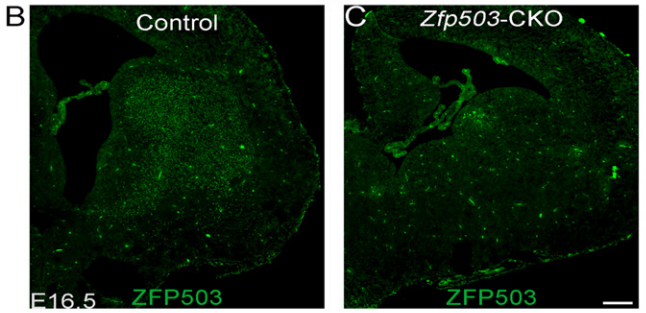
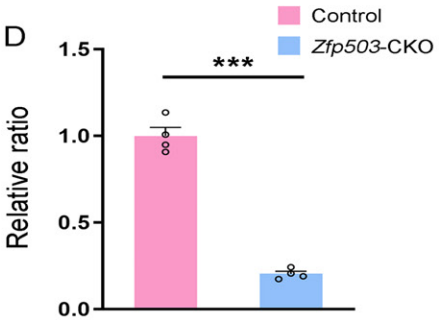


Fig. S5. The development of the striatal MSNs is not significantly changed in *hs599*$^{-/-}$ mice

(A, D) The expression of FOXP1 is not affected in the striatum of hs599 mutant mice at E16.5. (B-C, E-F) The expression of the ASCL1 and PCNA is slightly increased in the *hs599* mutants compared to the control mice. Dotted lines mark the border of the LGE SVZ and striatum. Subventricular zone: SVZ. N = 3 mice per group; Scale bar: 100 μm in F for A-F.



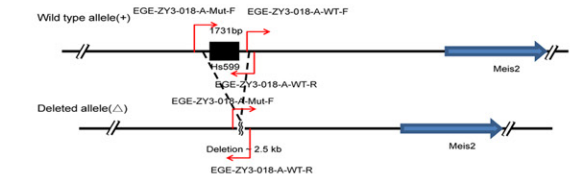


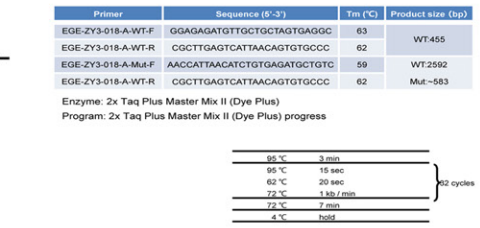


RNA design

E

Guide	Sequence (5'-3')
5'Guide #1	CACCGAGTAAGGTGACCAGC AGG
3'Guide #2	AAAAGAAGCGAAGGTCGGGA GGG





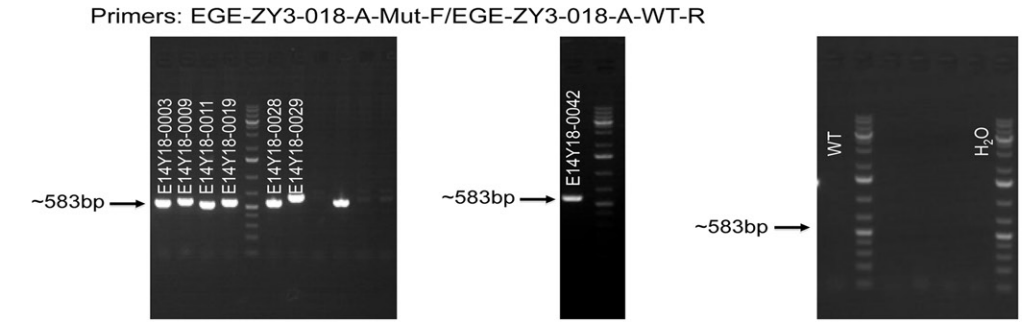


Fig. S6. Generation of *Zfp503*-CKO and *hs599*$^{+/-}$ mice

(A) Using the CRISPR/Cas9 strategy to generate *Zfp503*$^{F/+}$ mice. The coding region exon 3 was flanked by Loxp sites. After Cre recombination, exon 3 was deleted. (B-D) At E16.5, deletion of the *Zfp503* gene was confirmed at the protein level by immunostaining in the *Zfp503-CKO* striatum. ***$P < 0.001$ (two-tailed, unpaired Student's *t*-test), n = 4 per group; Scale bar, 200 μm. (E) The primer sequence of the gRNA. (F-H) Genotyping to confirm enhancer deletions and gel images showing genotyping results from wild-type (WT) and enhancer deletion mice. Genotyping from wild-type (WT) and enhancer deletion mice to confirm enhancer deletions and gel image.

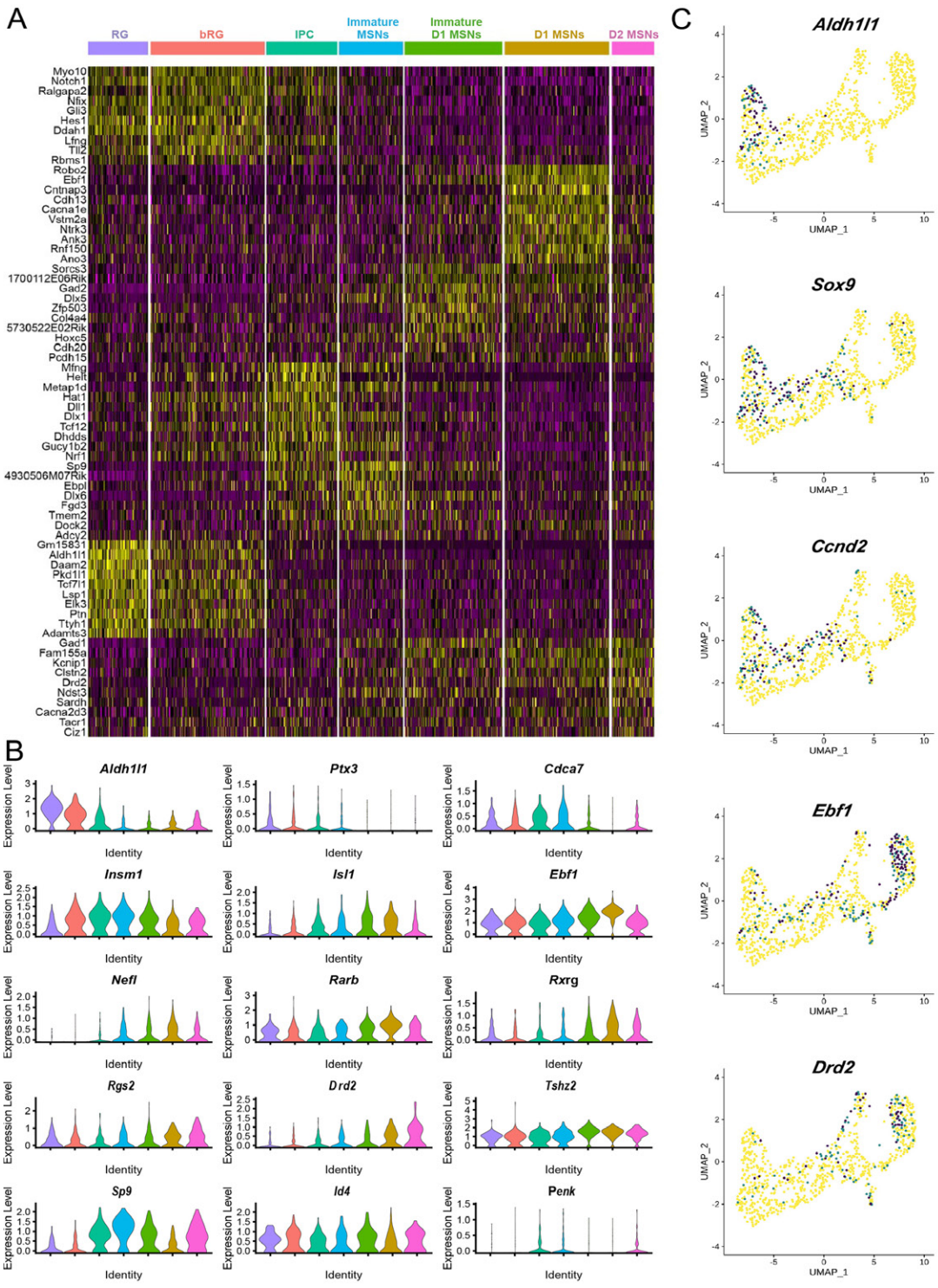


Fig. S7. Analysis of sc-ATAC-seq at E14.5

(A) Heat map showing the top 10 genes in the 7 clusters. (B) Violin plots showing the expression of the subtype cell markers. (C) UMAP plots showing distinct chromatin accessibility profiles of marker genes. progenitor cells.

Table S1. RNA-seq data of the LGE at E14.5

Click here to download Table S1

Table S2. Key resources table

Reagent or Resource	Source	Identifier
Antibodies		
Rabbit Anti-ASCL1	Cosmo Bio	SK-T01-003, Dilution: 1:2000
Rat Anti-BCL11B	Abcam	ab18465, Dilution: 1:200
Rat Anti-BrdU	Accurate Chemical and Scientific Corporation	OBT0030S, Dilution: 1:200
Rabbit Anti-CRE	Millipore	69050, Dilution: 1:1000
Rabbit Anti-DCX	Abcam	ab18723, Dilution: 1:1000
Guinea Pig Anti-DLX2	Gift from Kazuaki Yoshikawa	Dilution: 1:2000
Rabbit Anti-EBF1	Santa Cruz Biotechnology	sc-15888, Dilution: 1:500
Rabbit Anti-FOXP1	Abcam	ab16645, Dilution: 1:2000
Goat Anti-FOXP2	Abcam	ab58599, Dilution: 1:500
Chicken Anti-GFP	Aves labs	GFP-1020, Dilution: 1:2000
Rabbit Anti-GSX2	Millipore	ABN162, Dilution: 1:2000
Rabbit Anti-ISL1	Abcam	ab20670, Dilution: 1:800
Mouse Anti-MEIS2	Santa Cruz Biotechnology	sc-515470, Dilution: 1:500
Mouse Anti-PCNA	Abcam	ab29, Dilution: 1:1000
Rabbit Anti-PH3	Millipore	06-570, Dilution: 1:1500
Rabbit Anti-SP9	Our Laboratory	Dilution: 1:500

Rabbit Anti-KI67	Vector Labs	VP-K451, Dilution: 1:500
Mouse Anti- SIX3	Santa Cruz Biotechnology	sc-398797, Dilution: 1:800
Mouse Anti-TUBB3	Covance	MMS-435P, Dilution: 1:500

Experimental models

Mouse: C57BL/6	Department of laboratory Animal science at Fudan University	http://10.107.12.196/
Mouse: CD1	Department of laboratory Animal science at Fudan University	http://10.107.12.196/
Mouse: *Meis2*$^{F/+}$	This manuscript	N/A; Available from the authors
Mouse: *Six3*$^{F/+}$	Gift from Guillermo Oliver at Northwestern University	N/A; Available from the authors
Mouse: *Dlx5/6-CIE*	Gift from Kenneth Campbell at University of Cincinnati College of Medicine	N/A; Available from the authors
Mouse: *Gsx2-Cre*	The Jackson Laboratory	025806
Mouse: *Dlx1/2*$^{+/-}$	Gift from John L. Rubenstein at University of California	N/A; Available from the authors
Mouse: *Zfp503*$^{F/+}$	This manuscript	N/A; Available from the authors

Software and algorithms

Photoshop CC	Adobe Systems Inc	RRID: SCR_014199
Olympus VS120 digital slice scanning system	Olympus	N/A
Prism v.7	GraphPad	N/A
CellRanger v2.1.1	10X Genomics	https://www.10xgenomics.com/

		solutions/single-cell/
FASTQC v0.11.5	Babraham Bioinformatics	https://www.bioinformatics.babraham.ac.uk/projects/fastqc/
UMI Tools v0.5.4	（(Li et al., 2021)）	https://github.com/CGATOxford/UMI-tools

Recombinant DNA

pCAGIG	Addgene	Cat#11159
pGL4.10	Promega	Cat#E6651
pGL4.23	Promega	Cat#E8411

All secondary antibodies were from Jackson ImmunoResearch Labs.

Secondary Antibodies	Cat Number	Dilution Ratio
Alexa®488-conjugated Affinipure Donkey Anti-Rabbit IgG (H^+L)	711-545-152	1:500
Cy™3-conjugated Affinipure Donkey Anti-Rabbit IgG (H^+L)	711-165-152	1:500
Alexa®488-conjugated Affinipure Donkey Anti-Mouse IgG (H^+L)	715-545-151	1:500
Cy™3-conjugated Affinipure Donkey Anti-Mouse IgG (H^+L)	715-165-151	1:500
Alexa®488-conjugated Affinipure Donkey Anti-Chicken IgY^{++} (IgG) (H^+L)	703-545-155	1:500
Cy™3-AffiniPure Donkey Anti-Goat IgG (H^+L)	705-505-147	1:500
Cy™3-AffiniPure Donkey Anti-Rat IgG (H^+L)	712-165-150	1:500
Cy™3-AffiniPure Donkey Anti-Guinea Pig IgG (H^+L)	706-165-148	1:500

Table S3. Primer sequences

Probe Name	Primer Sequence
Rarb	Primer F: AAGGGCTTTTTCCGCAGAAGTAT
	Primer R: AGCAGTGGTGACTGACTGACTCCA
Rxrg	Primer F: GGACAGATCCTCAGGGAAGCACTA
	Primer R: GCCACCTTTAACAGCCGTACAAAA
Six3	Primer F: AGAGTTGTCCATGTTCCAGTTG
	Primer R: CTGATTTCGGTTTGTTCTAGGG
Zfp503	Primer F: TCCCAGGGACAGACAAACTGCT
	Primer R: TACAAGGGATCGGAGGGTTTGTT
Meis2	Primer F: AGAGCATGCCAGGGGACTACGT
	Primer R: CTCCGCAGCATGGTTCTTTTCTC
Ikzf1	Primer F: TTGTTCACTGGTAGCTGAGGTTTCC
	Primer R: GGGAACCTGTACTGGTCACACTGTG
Gad1	Primer F: ATGGCATCTTCCACTCCTTCG
	Primer R: TTACAGATCCTGACCCAACCTCTC
Penk	Primer F: TCGGAAGGACAGGATGTCATCA
	Primer R: CGTCAGGAGAGATGAGGTAACAAAC
Dlx5	Primer F: CAGCTTTCAGCTGGCCGCTT
	Primer R: CAAGGCACCATTGATAGTGTCCACA
Aldh1a3	Primer F: GTGCTCACCAGGGAGTGTTCTTCA
	Primer R: TCTGCCTTAGATGGGCCTATGTCG
Adora2a	Primer F: ATGGGCTCCTCGGTGTACATCATG
	Primer R: TCAGGAAGGGGCAAACTCTGAAGAC
Drd2	Primer F: CGGGAGCTGGAAGCCTCGA
	Primer R: TGCAGGGTCAAGAGAAGGCCG

Supplementary Materials and Methods

Signac package code:

```
library(Signac)
library(Seurat)
library(GenomeInfoDb)
library(EnsDb.Mmusculus.v79)
library(ggplot2)
library(patchwork)
library(dplyr)
set.seed(1234)
counts <- Read10X_h5(filename = ".../filtered_peak_bc_matrix.h5")
metadata <- read.csv(file = ".../singlecell.csv",header = TRUE, row.names = 1)
chrom_assay <- CreateChromatinAssay(counts = counts,sep = c(":", "-"),genome =
'mm10', fragments = '.../fragments.tsv.gz', min.cells = 1)
pbmc <- CreateSeuratObject(counts = chrom_assay, assay = "peaks", meta.data =
metadata)
annotations <- GetGRangesFromEnsDb(ensdb = EnsDb.Mmusculus.v79)
seqlevelsStyle(annotations) <- 'UCSC' genome(annotations) <- "mm10"
Annotation(pbmc) <- annotations
pbmc <- NucleosomeSignal(object = pbmc)
pbmc <- TSSEnrichment(object = pbmc, fast = FALSE)
pbmc$pct_reads_in_peaks <- pbmc$peak_region_fragments / pbmc$passed_filters *
100
pbmc$blacklist_ratio <- pbmc$blacklist_region_fragments /
pbmc$peak_region_fragments
pbmc$high.tss <- ifelse(pbmc$TSS.enrichment > 2, 'High', 'Low')
TSSPlot(pbmc, group.by = 'high.tss') + NoLegend()
pbmc$nucleosome_group <- ifelse(pbmc$nucleosome_signal > 4, 'NS > 4', 'NS < 4')
FragmentHistogram(object = pbmc, group.by = 'nucleosome_group')
```

```
pbmc <- subset(x = pbmc, subset = peak_region_fragments > 3000
&peak_region_fragments < 80000 &pct_reads_in_peaks > 40 &blacklist_ratio < 0.01
& nucleosome_signal < 4 & TSS.enrichment > 2.5)
pbmc <- RunTFIDF(pbmc)
pbmc <- FindTopFeatures(pbmc, min.cutoff = 'q0')
pbmc <- RunSVD(pbmc)
pbmc <- RunUMAP(object = pbmc, reduction = 'lsi', dims = c(2:50))
pbmc <- FindNeighbors(object = pbmc, reduction = 'lsi', dims = c(2:50))
pbmc <- FindClusters(object = pbmc, verbose = FALSE, algorithm = 3,resolution = 1)
gene.activities <- GeneActivity(pbmc)
pbmc[['ATAC']] <- CreateAssayObject(counts = gene.activities)
pbmc <- NormalizeData(object = pbmc,assay = 'ATAC',normalization.method =
'LogNormalize', scale.factor = 10000)
```