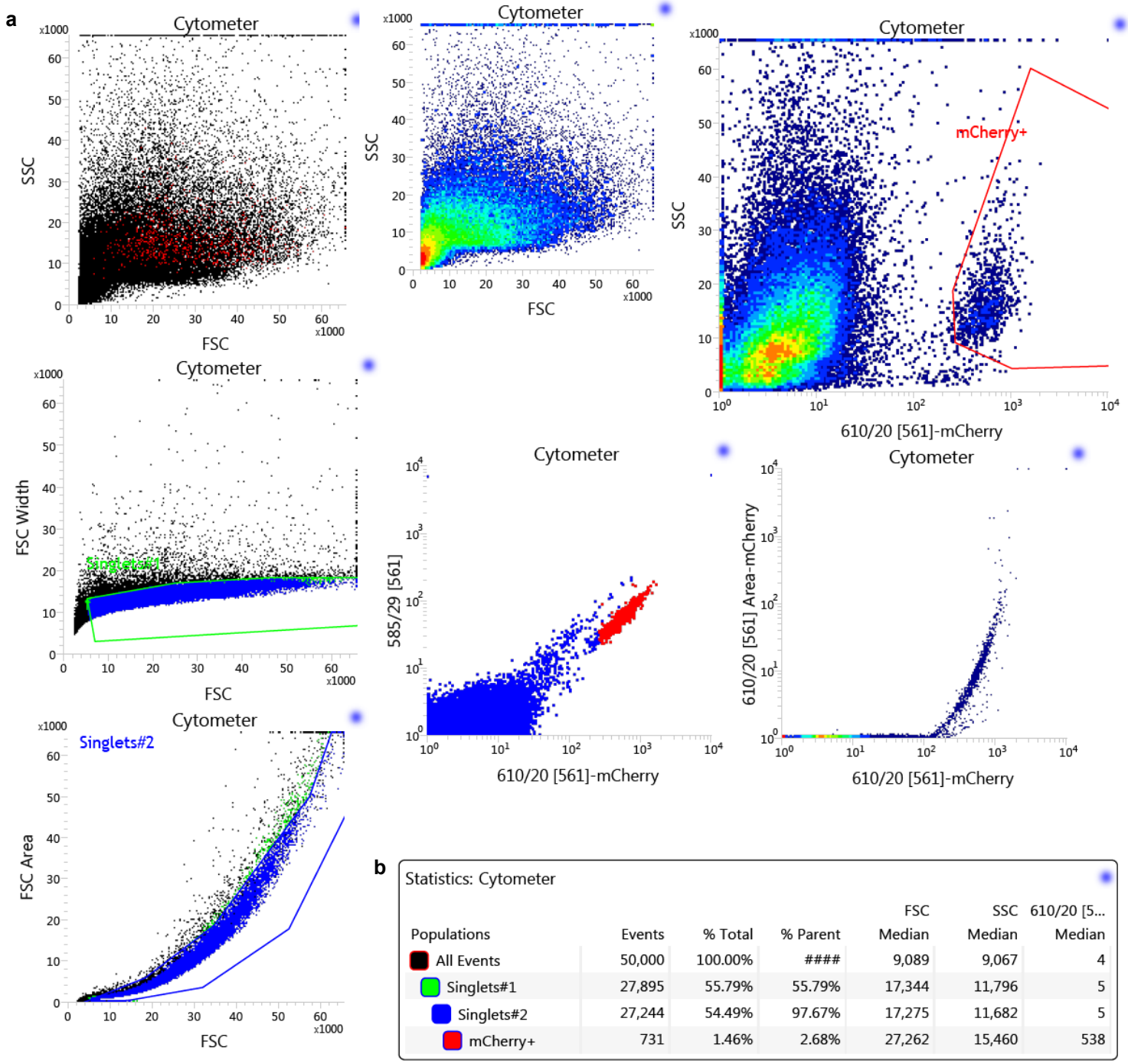
Supplementary Information

Spatio-molecular domains identified in the mouse subthalamic nucleus and neighboring glutamatergic and GABAergic brain structures

Supplementary figures: 6

Supplementary tables: 3

Supplementary Figure 1 related to Figure 1



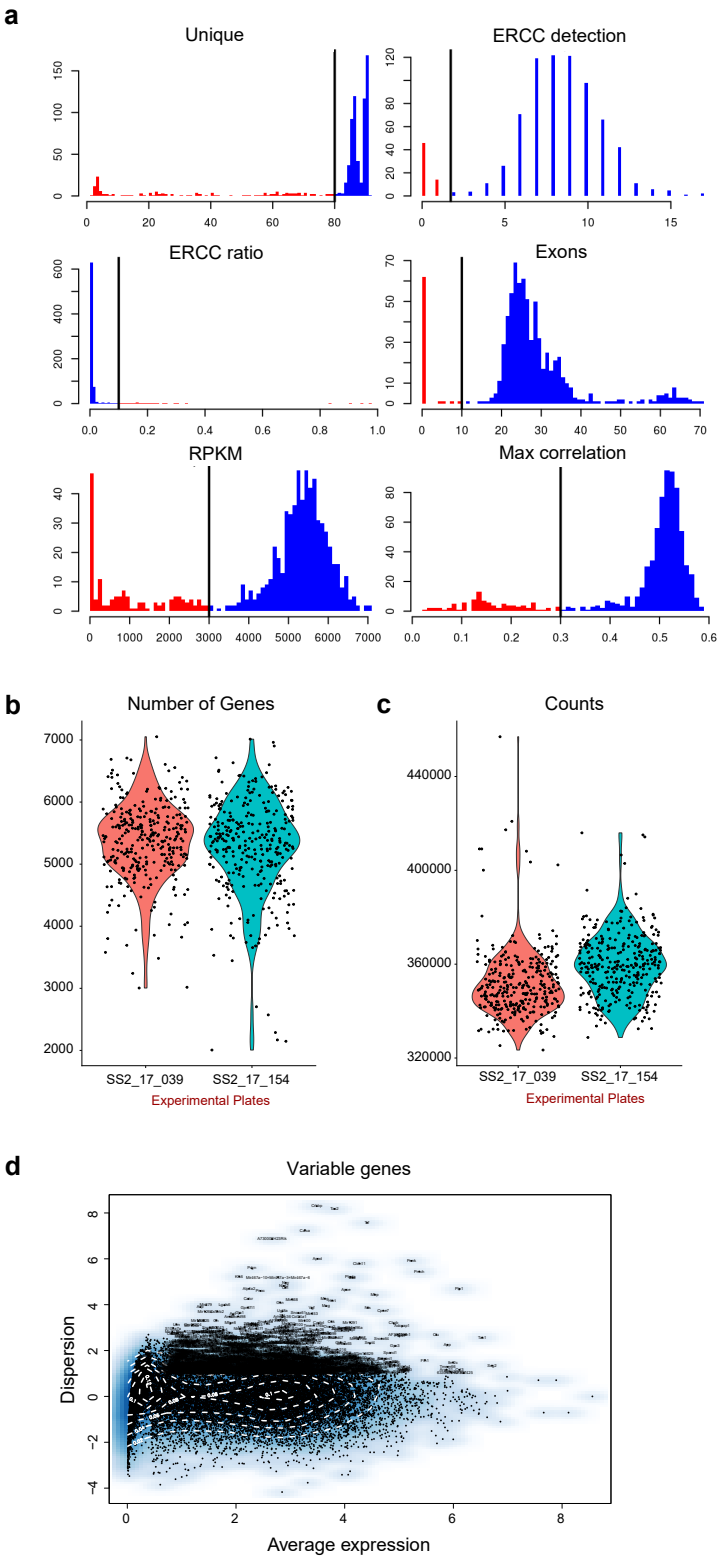
b

Statistics: Cytometer

Populations	Events	% Total	% Parent	FSC Median	SSC Median	610/20 [5... Median
All Events	50,000	100.00%	####	9,089	9,067	4
Singlets#1	27,895	55.79%	55.79%	17,344	11,796	5
Singlets#2	27,244	54.49%	97.67%	17,275	11,682	5
mCherry+	731	1.46%	2.68%	27,262	15,460	538

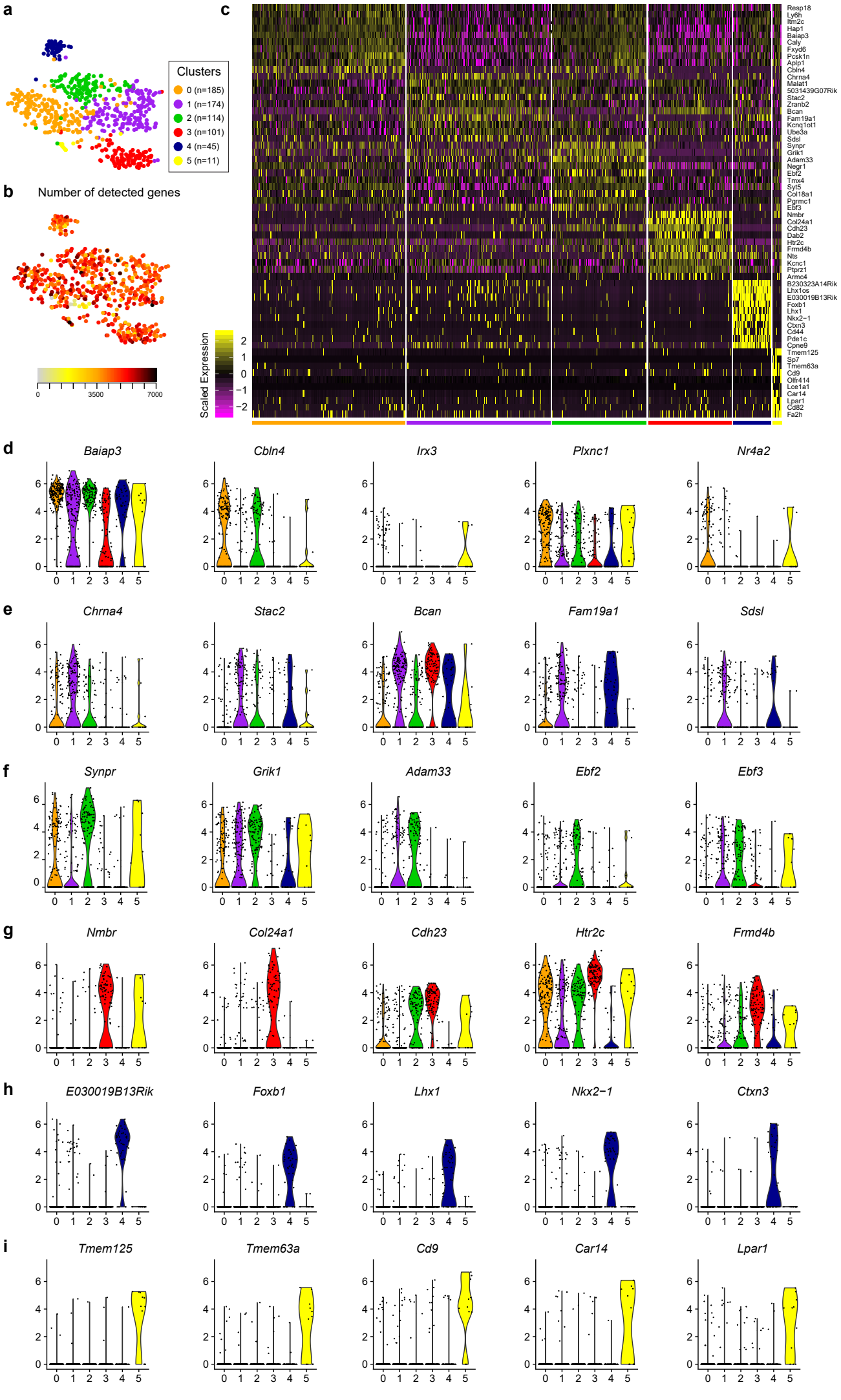
Supplementary Figure 1 related to Figure 1: Information related to the sorting layout and sorting parameters. **(a)** FACS methodology and gating parameters. **(b)** Statistics cytometer showing percentage of sorted population.

Supplementary Figure 2 related to Figure 1

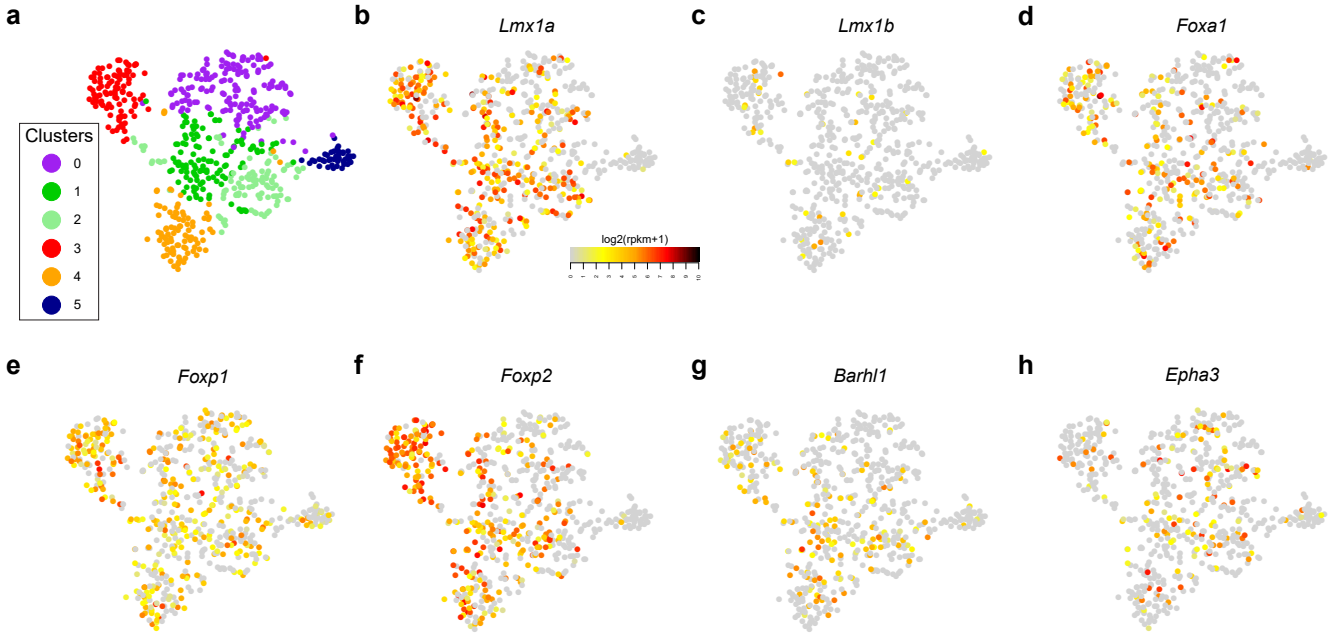


Supplementary Figure 2 related to Figure 1: Quality control of snRNASeq experiment. (a) Quality control of the two experimental plates with histograms of percent uniquely mapping (Unique), Spike-in detection (ERCC), Spike-in ratio (ERCC-ratio), percent mapped to exons (Exons), detected genes with RPKM>1 (RPKM) and highest correlation to another cell (Max correlation). Nuclei which failed cutoffs are colored in red. **(b)** Violin plots showing the number of detected genes of each nuclei (5384) across the two experiment plates according to Seurat unsupervised clustering. **(c)** Violin plots showing the average number of transcripts (354578) across the two experiment plates. **(d)** Identification of highly variable genes (out of 18697 genes 1776 selected as variable). The dispersion of the genes (variance/log-average expression) is plotted against the average expression (normalized log-expression, on x-axis). The minimum threshold to be considered highly variable was set at a dispersion of 1 on the y-axis.

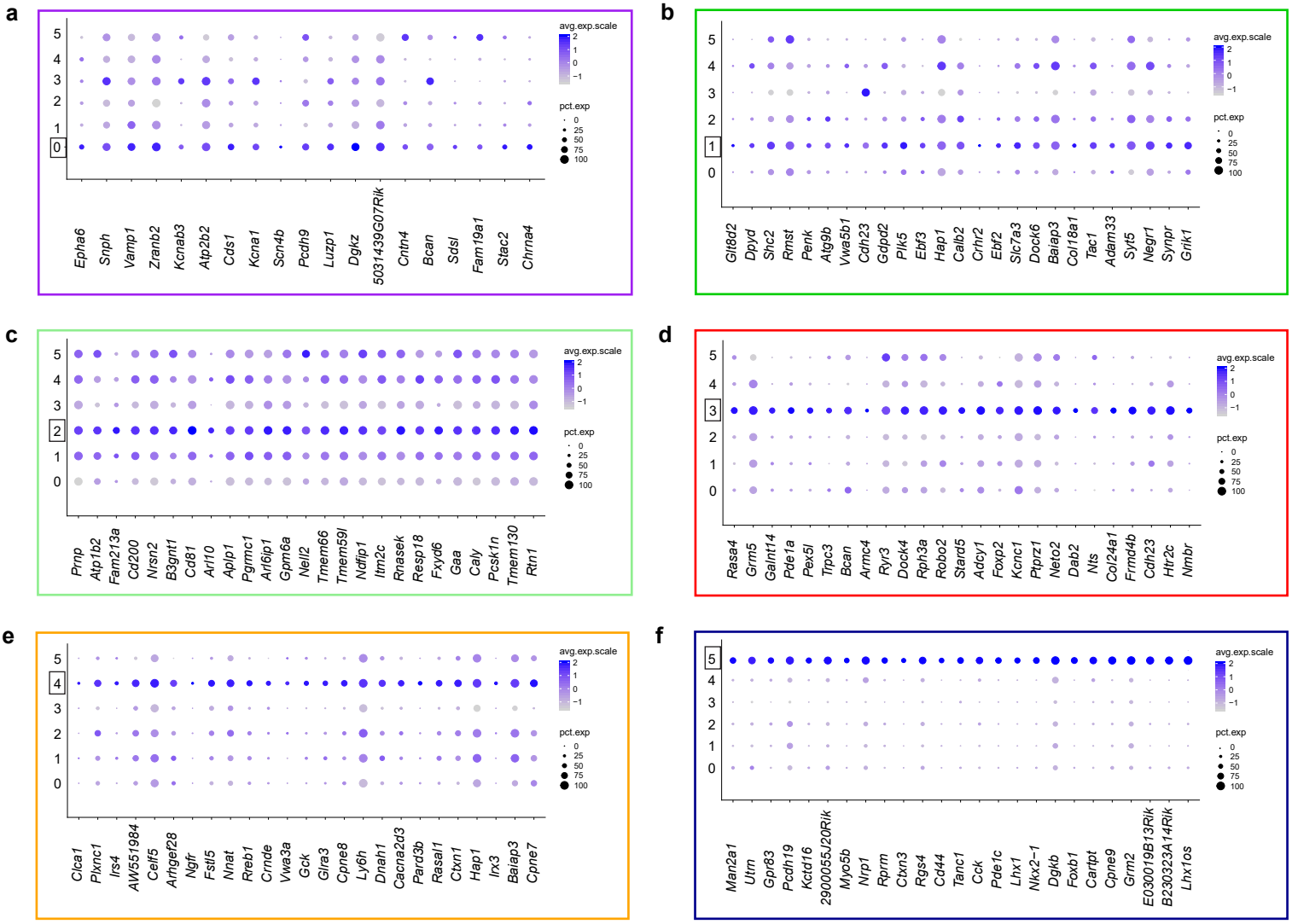
Supplementary Figure 3 related to Figure 2



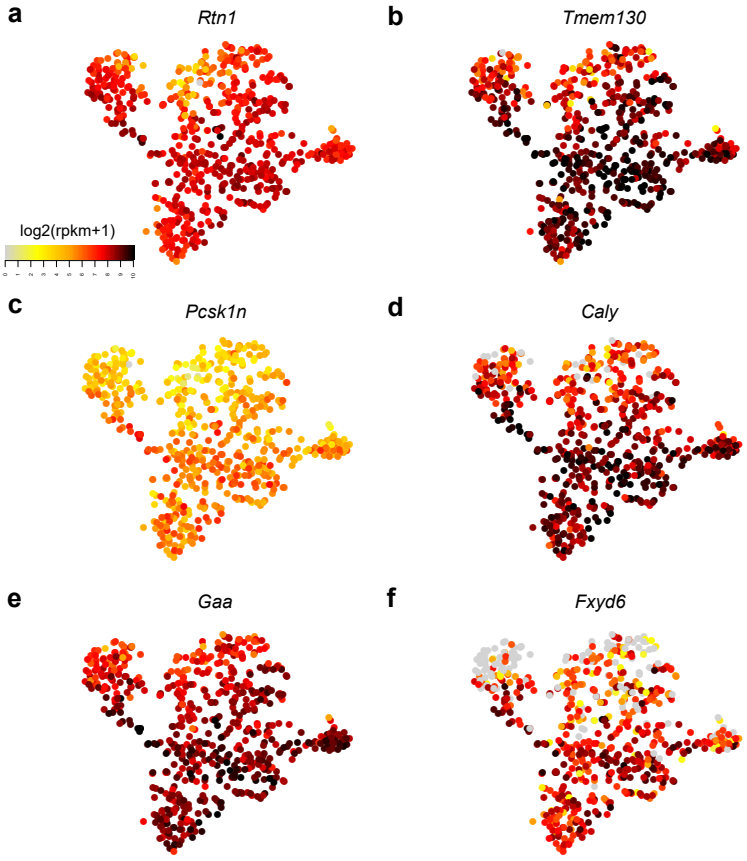
Supplementary Figure 3 related to Figure 2: Identification of 6 different glutamatergic *Pitx2*-positive subpopulations. (a) t-SNE plot with 6 clusters (orange, purple, green, red, blue and yellow). **(b)** Number of detected genes per nuclei (expression levels ranging from light grey (low) to black (high). **(c)** Heatmap showing the top ten genes considered unique for and characterizing each cluster **(d-i)** Violin plots of 5 of the top 10 identified discriminatory markers of each cluster.



Supplementary Figure 4 related to Figure 2: t-SNE of previously established markers for the developing STN. (a) t-SNE plot with 6 clusters (purple cluster0; green cluster1; light-green cluster2; red cluster3; orange cluster4 and blue cluster5). **(b-h)** Expression levels of *Lmx1a*, *Lmx1b*, *Foxa1*, *Foxp1*, *Foxp2*, *Barhl1* and *Epha3*, genes previosuly associated with the STN (References 1-3), visualized in the t-SNE plot.



Supplementary Figure 5 related to Figure 2: Dotplot of upregulated genes across each cluster (a-f) Dotplots of up to 25 significantly upregulated genes of each cluster *vs* the rest using MAST R package.



Supplementary Figure 6 related to Figure 2: t-SNE-plots of selected genes showing gradient of expression. **(a)** *Rtn1* **(b)** *Tmem130* **(c)** *Pcsk1n* **(d)** *Caly* **(e)** *Gaa* **(f)** *Fxyd6* showing gradient of expression along the t-SNE-2 axis.

Supplementary Table 1: PCR primer sequences used for genotyping of transgenic mice employed in the study.

Transgene	Direction	PCR primer sequence
Pitx2-Cre	Fw	5'-ACGAGTGATGAGGTTCGCAAGA-3'
Pitx2-Cre	Rev	5'-ACCGACGATGAAGCATGTTTAG-3'
R26-Rpl10a	Fw (mutant)	5'- TAC ACC ATC GTG GAA CAG TAC -3'
R26-Rpl10a	Rev (mutant)	5'- GTA GTT CTT CAG GCT GAT CTG -3'
R26-wt	Fw (WT)	5'- GCG GAT CAC AAG CAA TAA TA -3'
R26-wt	Rev (WT)	5'- TTT CTG GGA GTT CTC TGC TG -3'

Supplementary Table 2: Table of NM accession number and primer sequences for riboprobe synthesis of mRNAs selected for analysis in mouse brain tissue by fluorescent *in situ* hybridization (FISH)

PCR Primer Name	PCR Primer Sequence	NM	bases
Pitx2-S-T3	AATTAACCCTCACTAAAGGGACCCCGCCCAACTCCATCTCA	NM_001042504.2	792-1579
Pitx2-AS-T7	TAATACGACTCACTATAGGGTCCTTTGCTCGCAAGCGAAAAATC		
Nmbr-S-T3	AATTAACCCTCACTAAAGGGACCCCAGGTCTCTCTCCAACC	NM_008703.3	348-1280
Nmbr-AS-T7	TAATACGACTCACTATAGGGCTCAGAACCCGGGCCACTA		
Col24a1-S-T3	AATTAACCCTCACTAAAGGGAAGGGACCTCCTGGCACTGAG	NM_027770.3	3722-4593
Col24a1-AS-T7	TAATACGACTCACTATAGGGGCCGTCGACTCCAGGGTATC		
Kcnab3-S-T3	AATTAACCCTCACTAAAGGGAGCAGCTGCCTGAGCTCTACC	NM_010599.4	1228-2184
Kcnab3-AS-T7	TAATACGACTCACTATAGGGCAGGCAGGAGGCAGGAGTC		
Stxbp2-S-T3	AATTAACCCTCACTAAAGGGAATGCCAACGTGCAGTCGTACA	NM_001357168.1	1342-1907
Stxbp2-AS-T7	TAATACGACTCACTATAGGGGGGAAGACAGAAGGGAAGCGTTT		
Baiap3-S-T3	AATTAACCCTCACTAAAGGGAGACCACGACTGGCTGTCCAC	NM_001163270.1	3415-4384
Baiap3-AS-T7	TAATACGACTCACTATAGGGGAGCCCTTCAGCCTGGTCTC		
Cacna2d3-S-T3	AATTAACCCTCACTAAAGGGATGGTGAGGTGGAAGGAGCTG	NM_009785.1	2731-2750
Cacna2d3-AS-T7	TAATACGACTCACTATAGGGGGCCTGAAGACTCGATGCAC		
Synpr-S-T3	AATTAACCCTCACTAAAGGGACTAGATGACTATTTGATGAATCACATCATGCATGC	NM_028052.4	1453-2458
Synpr-AS-T7	TAATACGACTCACTATAGGGATAACTTTATTGAGGTCAAATAGATATCACAGTACAGG		
Nxph4-S-T3	AATTAACCCTCACTAAAGGGACTCCCGGAATGGCTCCTCT	NM_183297.2	137-991
Nxph4-AS-T7	TAATACGACTCACTATAGGGCTGCACCAGTTTGTAGTCAAAACTGAGG		
Fgf11-S-T3	AATTAACCCTCACTAAAGGGACTAATCAGTACTGAACGGATCAAGAACCACT	NM_010198.3	2205-3005
Fgf11-AS-T7	TAATACGACTCACTATAGGGAGCCCCTCCCTTAGGACCTC		
Glra3-S-T3	AATTAACCCTCACTAAAGGGAGGAGATTCTGGCTCTTGGACCTAGT	NM_080438.3	43-692
Glra3-AS-T7	TAATACGACTCACTATAGGGGAGGACCTTTGAAGTTGGGTCTGATTCT		
Adcyap1-S-T3	AATTAACCCTCACTAAAGGGAGCTTCCCTGGGATCAGACC	NM_009625.3	547-1510
Adcyap1-AS-T7	TAATACGACTCACTATAGGGAGCCTGCCCCAGACTCAGA		
Nxph1-S-T3	AATTAACCCTCACTAAAGGGAACTACAACTATCACAGTGACACACCTTACTTTC	NM_008751.5	1008-1783
Nxph1-AS-T7	TAATACGACTCACTATAGGGAAGATAAGACAGACACTTATAACTTACATGGTGTCTGC		

Htr2c-S-T3	AATTAACCCTCACTAAAGGGAGCAGGTGACCAGAATGAGGCACA	NM_008312.4	2456-3446
Htr2c-AS-T7	TAATACGACTCACTATAGGGGAGAGCAGCAGGCCCACGAA		
Calb2-S-T3	AATTAACCCTCACTAAAGGGACGCAGCAGCAGCCCCCTTAC	NM_007586.1	80-793
Calb2-AS-T7	TAATACGACTCACTATAGGGTGGTGAGCTGTTGGATGTTCATCTCC		
Vglut2-S-T3	AATTAACCCTCACTAAAGGGACCTTGGGCAGACCCTGAGGAA	NM_080853.3	2315-3244
Vglut2-AS-T7	TAATACGACTCACTATAGGGGGGGGAGCATGGAGCATACCC		
Gad1-S-T3	AATTAACCCTCACTAAAGGGACACGCCTTCGCCTGCAACCT	NM_008077.5	284-819
Gad1-AS-T7	TAATACGACTCACTATAGGGGGTGACCTGTGCGAACCCCG		

Supplementary Table 3: Full names of mRNAs analyzed in mouse brain sections by fluorescent *in situ* hybridization (FISH)

Abbreviation, mRNA	**Full name**
Adcyap1	Adenylate Cyclase Activating Polypeptide 1
Baiap3	BAI1 Associated Protein 3
Cacna2d3	Calcium Voltage-Gated Channel Auxiliary Subunit Alpha2delta 3
Calb2	Calbindin 2
Col24a1	Collagen Type XXIV Alpha 1 Chain
Fgf11	Fibroblast Growth Factor 11
Gad1	Glutamate decarboxylase 1
Glra3	Glycine Receptor Alpha 3
Htr2c	5-Hydroxytryptamine Receptor 2C
Kcnab3	Potassium Voltage-Gated Channel Subfamily A Regulatory Beta Subunit 3
Nmbr	Neuromedin B Receptor
Nxph1	Neurexophilin 1
Nxph4	Neurexophilin 4
Pitx2	Paired Like Homeodomain 2
Pvalb2	Parvalbumin
Stxbp2	Syntaxin Binding Protein 2
Synpr	Synaptoporin
Tac1	Tachykinin Precursor 1
Vglut2	Vesicular glutamate transporter 2

Supplementary References

1. Skidmore, J. M., Cramer, J. D., Martin, J. F. & Martin, D. M. Cre fate mapping reveals lineage specific defects in neuronal migration with loss of Pitx2 function in the developing mouse hypothalamus and subthalamic nucleus. Mol. Cell. Neurosci. 37, 696–707 (2008).

2. Gasser, E., Johannssen, H. C., Rülicke, T., Zeilhofer, H. U. & Stoffel, M. Foxa1 is essential for development and functional integrity of the subthalamic nucleus. Sci Rep 6, 38611 (2016).

3. Kee, N. et al. Single-Cell Analysis Reveals a Close Relationship between Differentiating Dopamine and Subthalamic Nucleus Neuronal Lineages. Cell Stem Cell 20, 29–40 (2017).