

Figure S1: Identification of cell clusters in hypothalamic reference datasets. **(A)** UMAP of hypothalamic single cell RNA sequencing dataset. Raw data by Yoo et al. **(B)** Featureplots of cell-type specific marker genes identifying cell clusters in Supp Figure 1A. **(C)** Violin plots of cell-type specific marker genes identifying the cell clusters in Supp. Figure 1A. **(D)** UMAP of hypothalamic single cell RNA sequencing dataset. Raw data by Campbell et al. **(E)** Featureplots of cell-type specific marker genes identifying the cell clusters in Supp. Figure 1D. **(F)** Violin plots of cell-type specific marker genes identifying the cell clusters in Supp. Figure 1D. **(G)** UMAP of hypothalamic single nucleus RNA sequencing dataset. Raw data by Deng et al. **(H)** Featureplots of cell-type specific marker genes identifying the cell clusters in Supp. Figure 1G. **(I)** Violin plots of cell-type specific marker genes identifying the cell clusters in Supp. Figure 1G. **(J)** UMAP of hypothalamic single cell RNA sequencing dataset. Raw data by Chen et al. **(K)** Featureplots of cell-type specific marker genes identifying the cell clusters in Supp. Figure 1J. **(L)** Violin plots of cell-type specific marker genes identifying the cell clusters in Supp. Figure 1J.

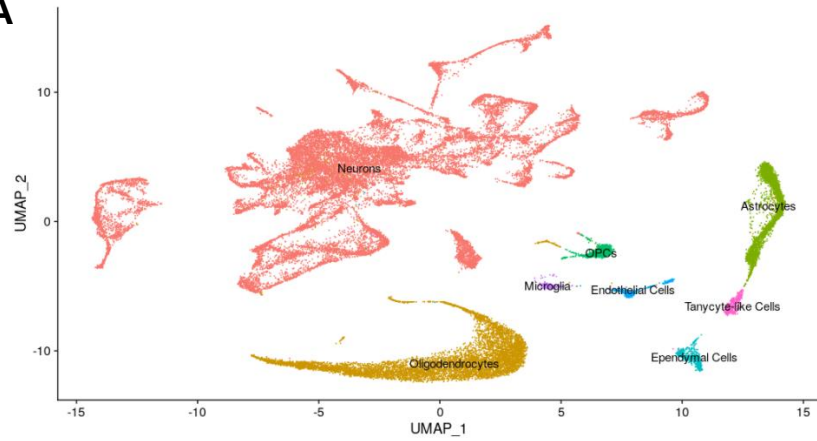
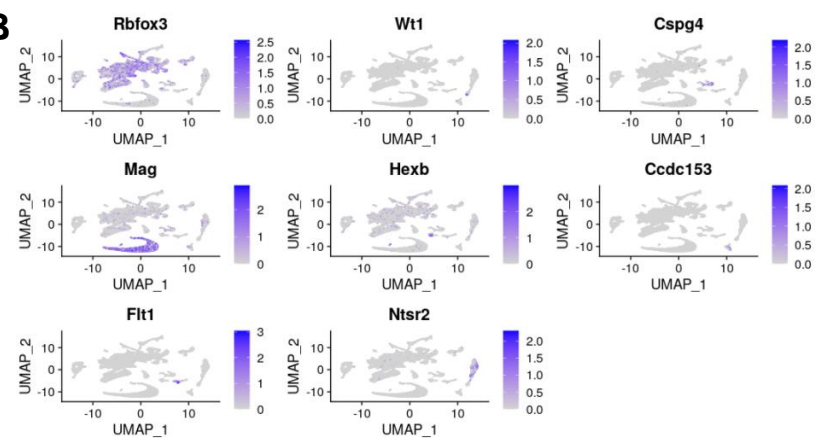
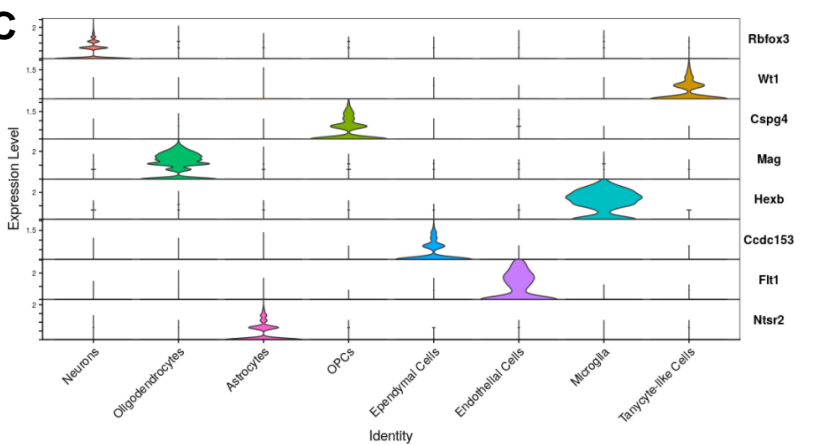
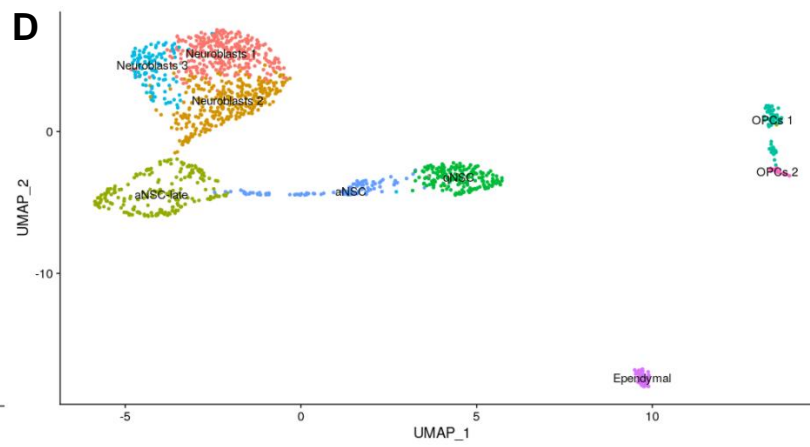
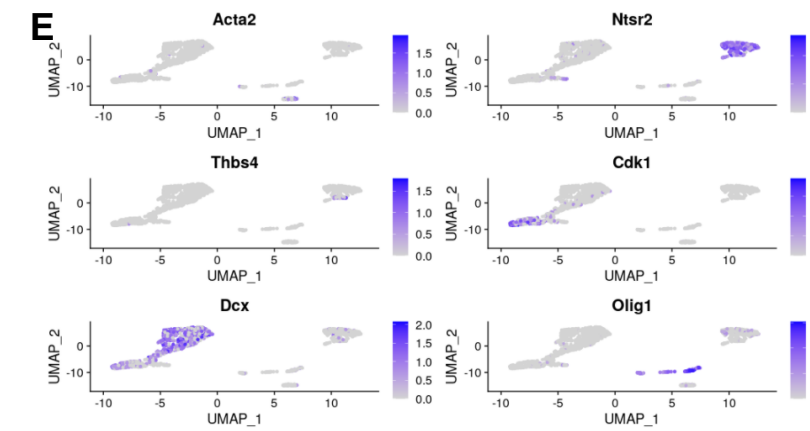
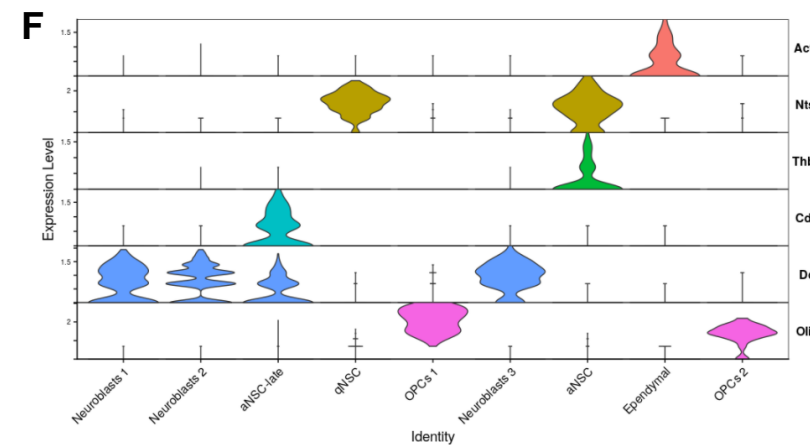
A**B****C****D****E****F**

Figure S2: Identification of cell clusters in hindbrain and NSC reference datasets. **(A)** UMAP of combined hindbrain single cell RNA sequencing dataset. Raw data by Dowsett et al. and Ludwig et al. **(B)** Feature plots of cell-type specific marker genes identifying the cell clusters in Supp. Figure 2A. **(C)** Violin plots of cell-type specific marker genes identifying the cell clusters in Supp. Figure 2A. **(D)** UMAP of neural stem cell single cell RNA sequencing dataset. Raw data by Shah et al. **(E)** Featureplots of cell-type specific marker genes identifying the cell clusters in Supp Figure 2D. **(F)** Violin plots of cell-type specific marker genes identifying the cell clusters in Supp Figure 2D.

Table S1: Summary of datasets utilized in study. The following is a table containing the original publication title, author, gene expression omnibus (GEO) access code, sample type utilized from each dataset, the exact number of biological samples used from each condition, and the next generation sequencing platform used in each sample collection.

Publication	Author	Access	Sample Type	Number of Each Biological Samples Used	NGS platform
Single-Cell RNA-Seq Reveals Hypothalamic Cell Diversity	Renchao Chen	GSE87544	All	7 fasted, 10 chow	Illumina HiSeq 2500
Control of neurogenic competence in mammalian hypothalamic tanycytes	Sooyeon Yoo	GSE160378	scRNA-seq controls	3	Illumina NextSeq
A Molecular Census of Arcuate Hypothalamus and Median Eminence Cell Types	John N. Campbell	Lowell lab website	All but HFD	24 chow, 14 fast, 7 fast and re-fed, 3 low-fat diet	Illumina NextSeq 500
Single-Nucleus RNA Sequencing of the Hypothalamic Arcuate Nucleus of C57BL/6J Mice After Prolonged Diet-Induced Obesity	Guorui Deng	Direct access	Chow	7	Illumina HiSeq 4000
Single-Cell Transcriptomics and Fate Mapping of Ependymal Cells Reveals an Absence of Neural Stem Cell Function	Prajay T. Shah	GSE100320	V-SVZ	5	Illumina HiSeq 2500
A survey of the mouse hindbrain in the fed and fasted state using single-nucleus RNA sequencing.	Georgina K C Dowsett	GSE168737	All	6 chow, 6 fast	Illumina NovaSeq 6000
A genetic map of the murine dorsal vagal complex and its role in obesity	Mette Q Ludwig	GSE166649	snRNA-seq vehicles	6	NextSeq 500 platform

Table S2: Software Versions. The following is a list of the version of each R software package utilized in this study.

RStudio 4.0.2	futile.logger 14.3	limma 3.46.0	Rhdf5lib 1.12.1	spatstat.geom 2.2-0	cli 3.1.0	gargle 1.2.0	lifecycle 1.0.1	quantmod 0.4.18	SQUAREM 2021.1
abind 14-5	futile.options 10.1	listenv 0.8.0	RhpcBLASctl 0.20-137	spatstat.linnet 2.2-1	clipr 0.7.1	generics 0.1.1	listenv 0.8.0	r2d3 0.2.5	stringi 1.7.5
batchelor 16.3	future 1.21.0	loomR 0.2.19000	Rhtslib 1.22.0	spatstat.sparse 2.0-0	colorspace 2.0-2	gerit 14.1	lmtest 0.9-38	R6 2.5.1	stringr 14.0
beachmat 2.6.4	future.apply 1.7.0	lsa 0.73.2	ROCR 10-11	spatstat.utils 2.2-0	commonmark 1.7	ggplot2 3.3.5	lubridate 18.0	randomForest 4.6-14	sys 3.4
Biobase 2.50.0	gdata 2.18.0	Matrix.utils 0.9.8	rsample 0.10	spData 0.3.10	config 0.3.1	gh 13.0	magrittr 2.0.1	rappdirs 0.3.3	testthat 3.1.0
BiocGenerics 0.36.1	GenomeInfoDb 1.26.7	MatrixGenerics 1.2.1	Rsamtools 2.6.0	spdep 1.1-8	cpp11 0.4.0	gitcreds 0.1.1	memoise 2.0.0	rmrcheck 14.0	tibble 3.1.5
BiocManager 1.30.16	GenomeInfoDbData 1.2.4	matrixStats 0.59.0	RSpectra 0.16-0	speedglm 0.3-3	crayon 14.1	globals 0.14.0	mime 0.12	RColorBrewer 1.1-2	tidyr 1.1.4
BiocNeighbors 18.2	GenomicRanges 142.0	miniUI 0.1.1.1	rsvd 10.5	stringr 14.0	credentials 1.3.1	glue 14.2	ModelMetrics 1.2.2.2	Rcpp 10.7	tidyselect 1.1.1
BiocParallel 1.24.1	ggforce 0.3.3	monocle3 10.0	Rtsne 0.15	SummarizedExperiment 120.0	curl 4.3.2	googledrive 2.0.0	modelr 0.18	RcppArmadillo 0.10.7.0.0	tidyverse 1.3.1
BiocSingular 16.0	ggfortify 0.4.12	parallelly 1.26.1	s2 10.6	tensor 1.5	data.table 114.2	googlesheets4 10.0	munsell 0.5.0	readr 2.0.2	timeDate 3043.102
BiocVersion 3.12.0	ggrepel 0.9.1	patchwork 1.1.1	S4Vectors 0.28.1	tweenr 10.2	DBI 1.1.1	gower 0.2.2	numDeriv 2016.8-1.1	readxl 1.3.1	tinytex 0.34
Biostrings 2.58.0	ggribbes 0.5.3	pbapply 14-3	scattermore 0.7	units 0.7-2	dbplyr 2.1.1	gtable 0.3.0	nyctlights13 10.2	recipes 0.117	tseries 0.10-48
bitops 10-7	ggseqlogo 0.1	pbmcapply 15.0	scrantransform 0.3.2	uwot 0.110	desc 14.0	haven 24.3	openssl 14.5	rematch 10.1	TTR 0.24.2
caTools 118.2	glmGamPoi 1.2.0	pheatmap 10.12	scuttle 10.4	viridis 0.6.1	devtools 2.4.2	hexbin 128.2	parallelly 128.1	rematch2 2.1.2	tzdb 0.2.0
classInt 04-3	gmodels 2.18.1	plotly 4.9.4.1	Seurat 4.0.3	warp 0.2.0	diffobj 0.3.5	highr 0.9	pbdZMQ 0.3-5	remotes 2.4.1	urca 13-0
coda 0.19-4	goftest 12-2	png 0.1-7	SeuratDisk 0.0.0.9019	wk 0.4.1	digest 0.6.28	hms 1.1.1	pillar 16.4	repr 1.13	usethis 2.1.3
cowplot 1.1.1	ggplots 3.1.1	polyclip 110-0	SeuratObject 4.0.2	XVector 0.30.0	dplyr 10.7	htmltools 0.5.2	pkgbuild 1.2.0	reprx 2.0.1	utf8 12.2
crossstalk 1.1.1	gridExtra 2.3	proxy 04-26	SeuratWrappers 0.3.0	zlibbioc 136.0	dtplyr 1.1.0	htmlwidgets 15.4	pkgconfig 2.0.3	reshape2 14.4	uuid 10-2
DelayedArray 0.16.3	grr 0.9.5	pscl 15.5	sf 10-1	askpass 1.1	e1071 1.7-9	httpuv 16.3	pkgload 1.2.3	rlang 04.12	votrs 0.3.8
DelayedMatrixStats 1.12.3	gtools 3.9.2	qlcMatrix 0.9.7	Signac 1.2.1	assertthat 0.2.1	ellipsis 0.3.2	httr 14.2	plogr 0.2.0	markdown 2.11	viridisLite 04.0
deldir 0.2-10	harmony 0.10	RANN 2.6.1	SingleCellExperiment 112.0	backports 1.3.0	evaluate 0.14	ids 10.1	plyr 18.6	roxygen2 7.1.2	vroom 15.5
devtools 2.4.2	HDF5Array 118.1	raster 34-13	sitmo 2.0.1	base64enc 0.1-3	fansi 0.5.0	ini 0.3.1	praise 10.0	rprojroot 2.0.2	waldo 0.3.1
docopt 0.7.1	hdf5r 13.3	RcppAnnoy 0.0.18	slam 0.1-48	bit 4.0.4	farver 2.10	ipred 0.9-12	prettyunits 1.1.1	RSQLite 2.2.8	whisker 0.4
dqrng 0.3.0	ica 10-2	RcppEigen 0.3.3.9.1	slider 0.2.2	bit64 4.0.5	fastmap 11.0	IRdisplay 10	pROC 118.0	rstudioapi 0.13	withr 24.2
DropSeq.util 2.0	igraph 12.6	RcppHNSW 0.3.0	snakecase 0.11.0	blob 12.2	fontawesome 0.2.2	IRkernel 12	processx 3.5.2	rversions 2.1.1	xfun 0.27
e1071 1.7-7	IRanges 2.24.1	RcppProgress 0.4.2	snow 04-3	brew 10-6	forcats 0.5.1	isoband 0.2.5	prodlim 2019.11.13	rvest 10.2	xml2 13.2
expm 0.999-6	irlba 2.3.3	RcppRoll 0.3.0	SnowballC 0.7.0	brio 11.2	foreach 15.1	iterators 10.13	progress 12.2	sass 04.0	xopen 10.0
fansi 0.5.0	janitor 2.1.0	RCurl 138-1.3	sp 14-5	broom 0.7.9	forecast 8.15	jquerrylib 0.14	progressr 0.9.0	scales 1.1.1	xtable 18-4
fastmatch 1.1-0	lambda.r 12.4	remotes 24.0	sparseMatrixStats 12.1	bslib 0.3.1	forge 0.2.0	jsonlite 1.7.2	promises 12.0.1	selectr 04-2	sts 0.12.1
fidistrplus 11-5	lazyeval 0.2.2	ResidualMatrix 10.0	sparseSvd 0.2	oacchem 10.6	fractdiff 1.5-1	knitr 136	proxy 04-26	sessioninfo 1.1.1	yaml 2.2.1
FNN 1.1.3	LearnBayes 215.1	reticulate 1.20	spatstat 2.2-0	calr 3.7.0	fs 15.0	labeling 04.2	ps 16.0	shiny 17.1	zip 2.2.0
formatR 1.11	leiden 0.3.8	rhdf5 2.34.0	spatstat.core 2.2-0	caret 6.0-90	future 1.22.1	later 13.0	purrr 0.34	sourceTools 0.17	zoo 18-9
furrr 0.2.3	leidenbase 0.1.3	rhdf5filters 1.2.1	spatstat.data 2.1-0	cellranger 1.10	future.apply 18.1	lava 16.10	quadprog 15-8	sparklyr 1.7.2	

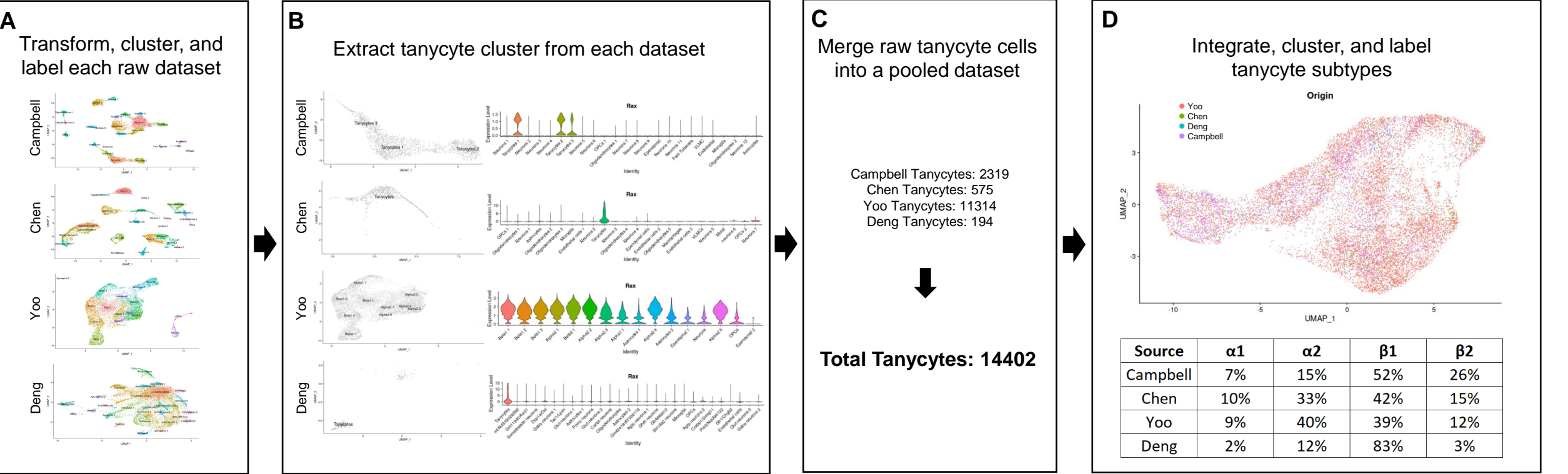


Figure S3: Workflow of tanyocyte isolation and integration. **(A)** First, each raw dataset was transformed, clustered, and labeled to allow for extraction of tanyocytes (specific functions utilized to achieve this can be found in the methods). **(B)** Tanyocyte populations from each dataset were isolated. Clusters “Tanyocytes 1,” “Tanyocytes 2,” and “Tanyocytes 3” were isolated from the Campbell dataset. Cluster “Tanyocytes” was isolated from the Chen dataset. Clusters “Alpha1 1,” “Alpha2 1,” “Alpha2 2,” “Alpha2 3,” “Alpha2 4,” “Alpha2 5,” “Beta1 1,” “Beta1 2,” “Beta1 3,” “Beta2 1” were isolated from the Yoo dataset. Cluster “Tanyocytes” was isolated from the Deng dataset. **(C)** The raw cells from each dataset’s isolated tanyocyte clusters were then merged into one dataset. **(D)** This merged dataset was then integrated by origin, clustered, and finally labeled by tanyocyte subtype. The table contains the percent of each dataset that clustered as each tanyocyte subtype.

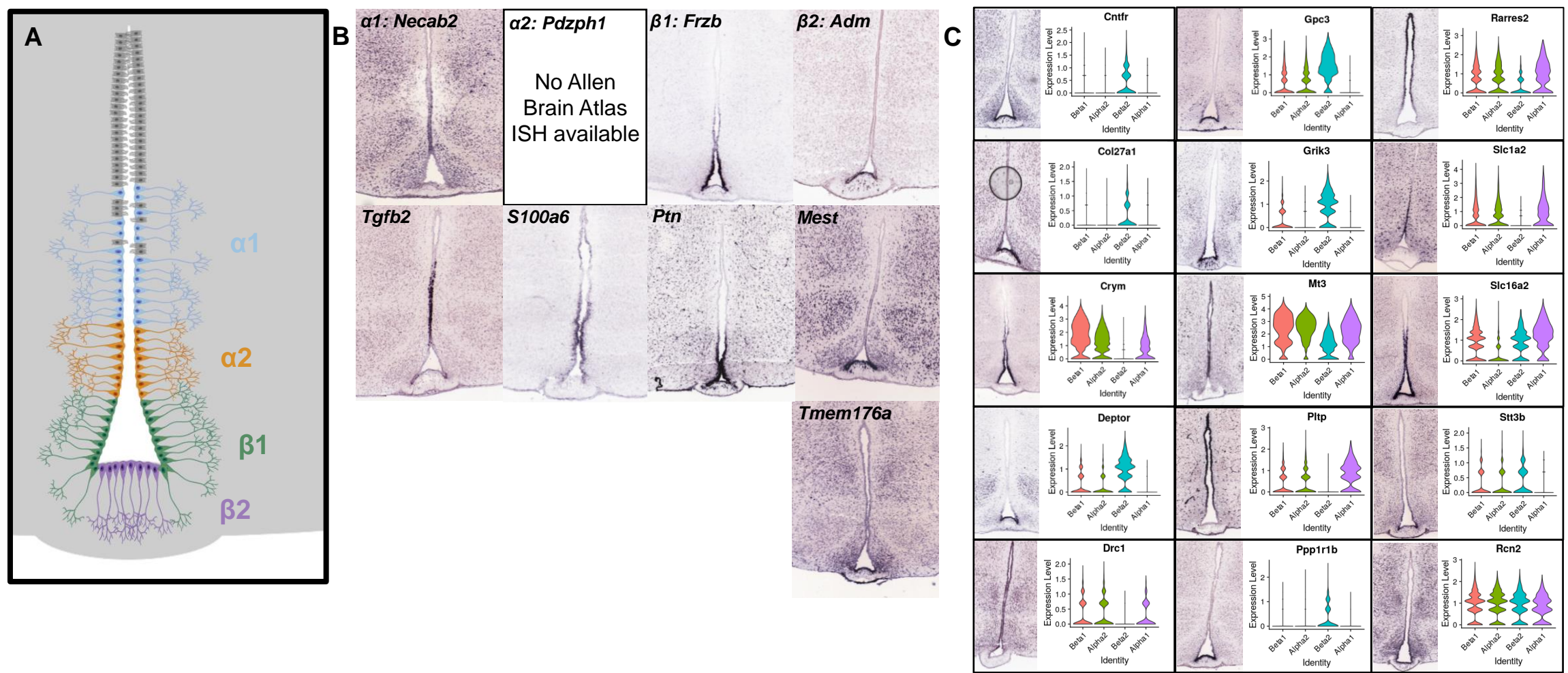


Figure S4: Allen brain atlas ISH secondary verification of tanycyte subtype classification. **(A)** Schematic representation of the tanycyte subtypes. **(B)** In situ hybridization (ISH) of subtype marker transcripts (top row) and of sample top ten transcripts upregulated in each subtype cluster (other rows). Only Allen brain atlas ISH deposits that were coronal, passed quality control parameters, and showed expression in the third ventricle were included. **(C)** ISH of transcripts from the Allen brain atlas third ventricle fine structure atlas. 41 of the genes in this atlas showed uniform expression across all tanycyte subtypes. The 15 genes that did not show uniform ISH expression in all tanycytes are shown above with accompanying violin plots showing their expression in the combined tanycyte dataset.