Figure 2

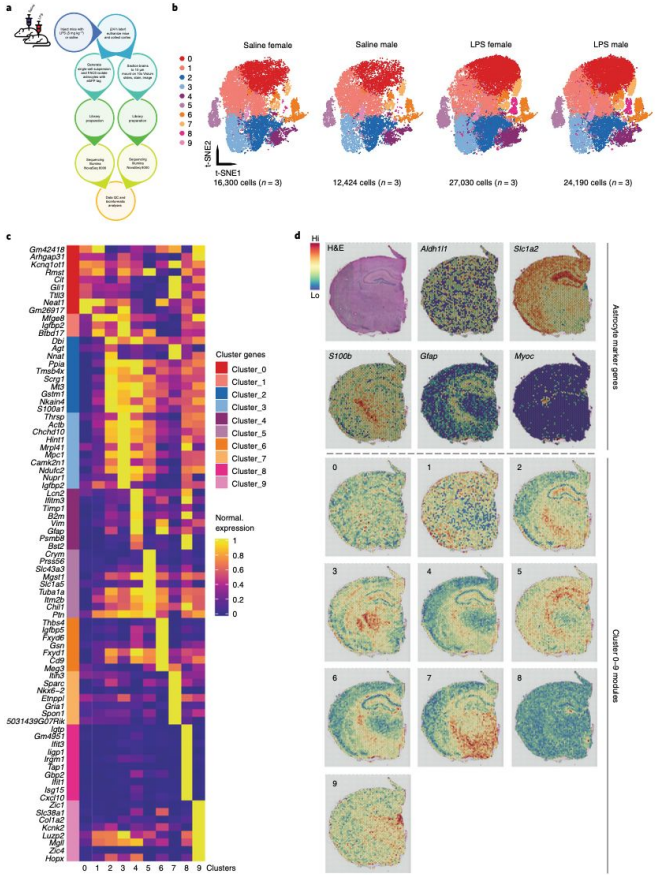


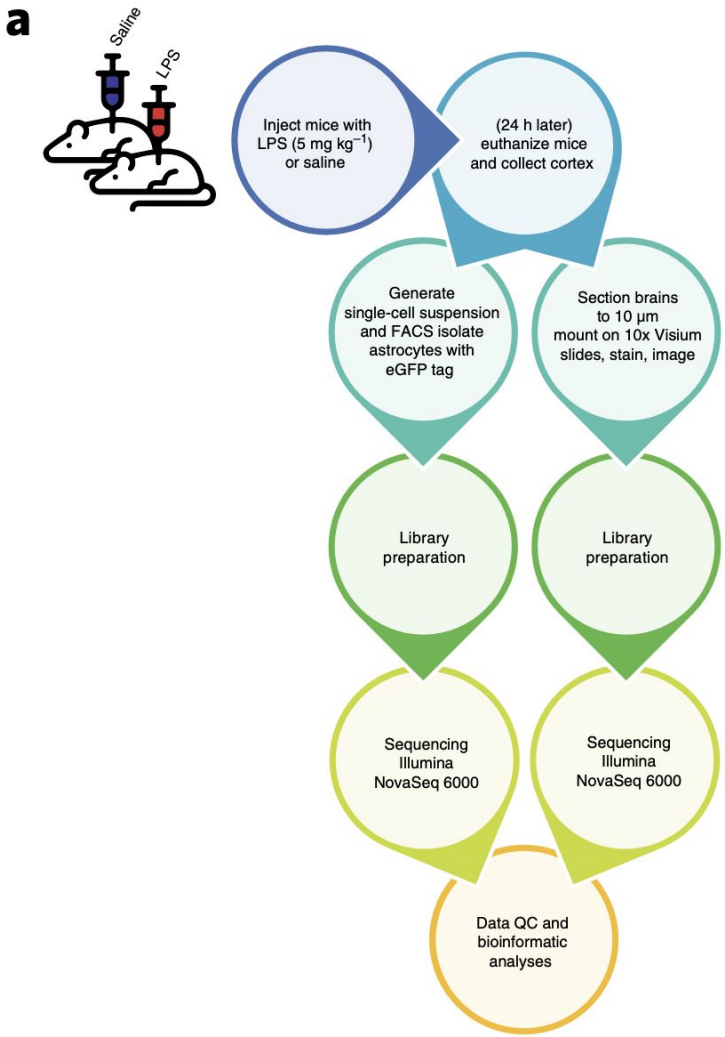
Fig. 2 | Astrocytic subtypes as identified by single-cell RNA-seq and localized using spatial transcriptomics in the brain of saline-treated mice. **a**, Schematic of experimental approach for astrocyte single-cell RNA-seq and spatial transcriptomics of saline- and LPS-injected *Aldh1l1eGFP* mice. **b**, After removal of non-astrocytic cells, 79,944 astrocytes were re-clustered based on variable features calculated for each sample and used to anchor the 12 samples. t-SNE plots are split across all four conditions: Female saline, male saline, female LPS and male LPS (left to right, *n* = 3 animals each). Colors highlight the ten identified astrocyte clusters. **c**, Genes statistically enriched in each cluster using *seurat's* FindAllClusters function. Shown is the normalized average expression of the top enriched genes for each cluster. **d**, Visium spatial transcriptomics highlights classical astrocyte-specific markers in one of the six coronal brain sections (here, saline-treated) as well as showing the expression of astrocyte clusters identified using single-cell RNA-seq using a module-based approach.

For each figure plot/result, focus on:

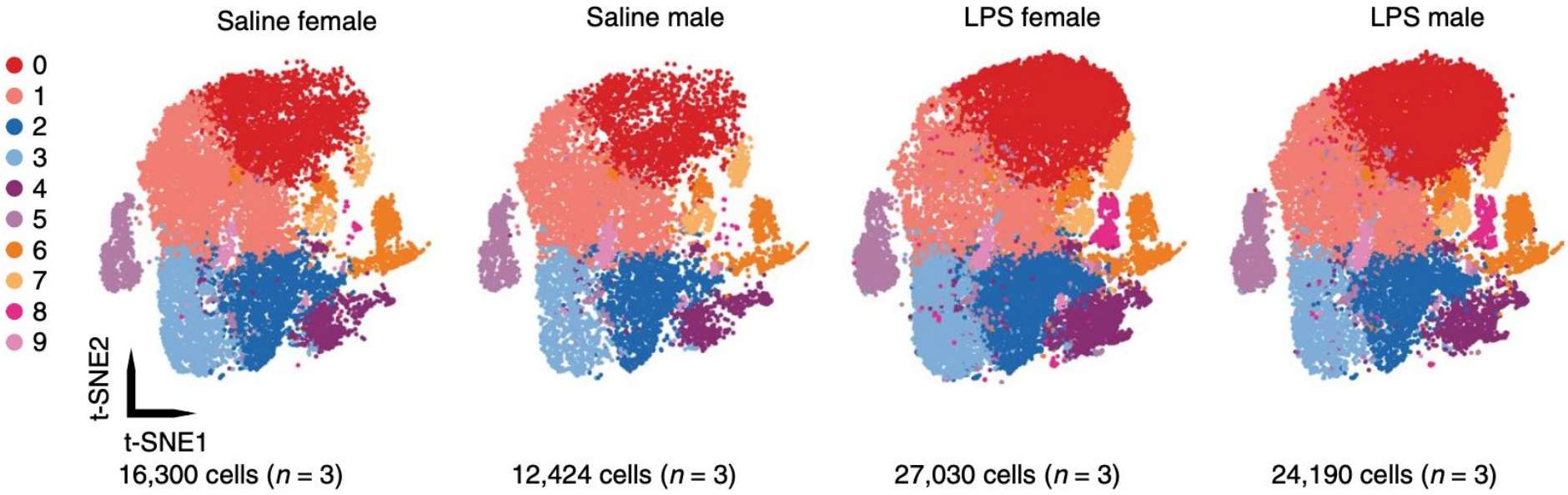
- What is a dot, column, and/or row represents
- X-axis unit
- Y-axis unit
- Overarching conclusion

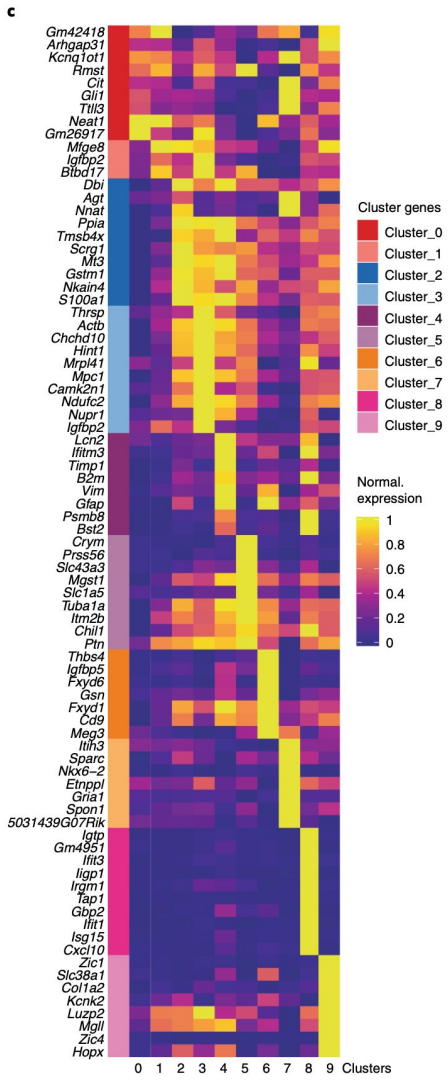
Try not to:

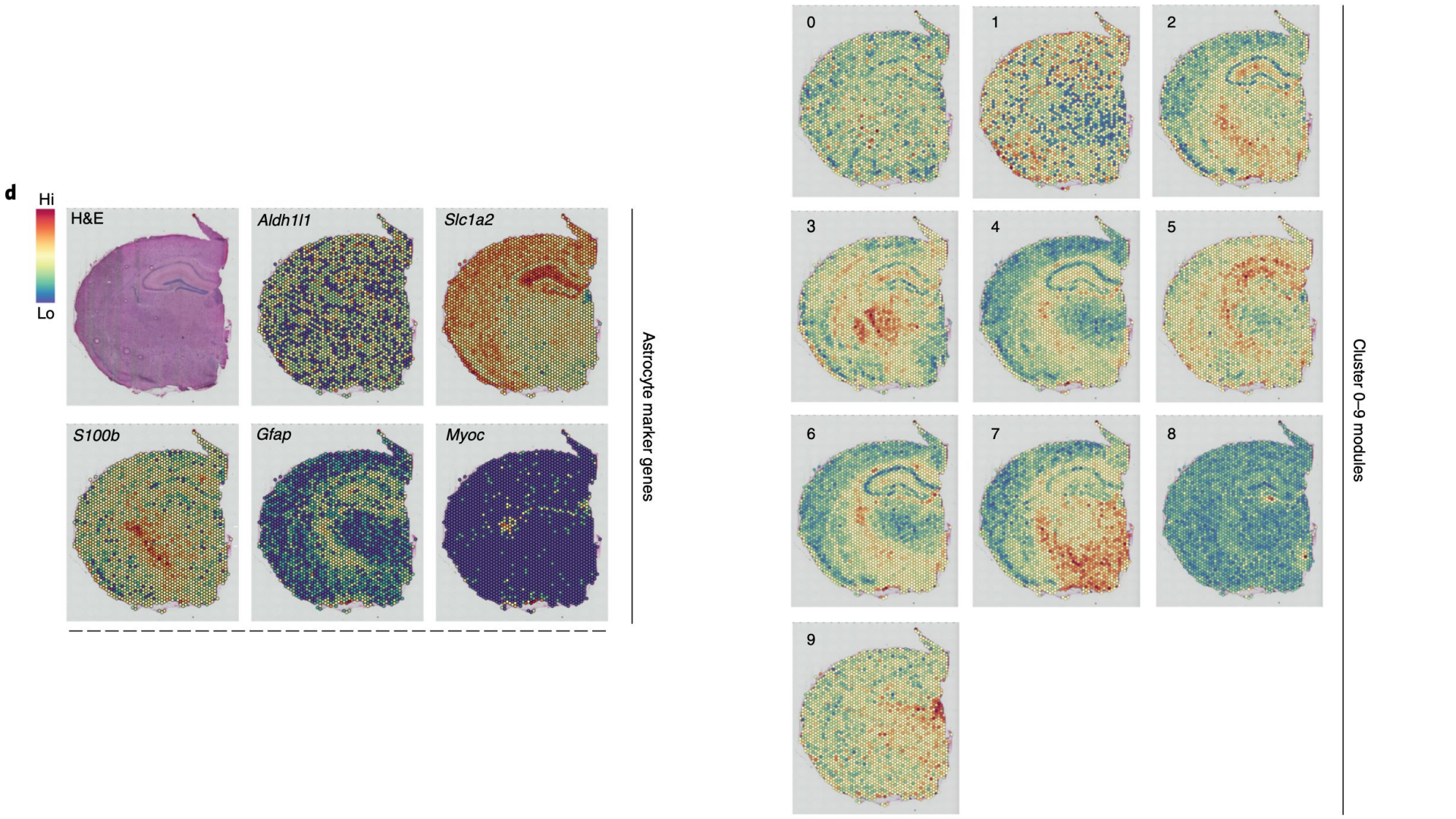
- Get hung-up on the technical minutiae


a
Saline
LPS
Inject mice with LPS (5 mg kg^{-1}) or saline
(24 h later) euthanize mice and collect cortex
Generate single-cell suspension and FACS isolate astrocytes with eGFP tag
Section brains to 10 µm mount on 10x Visium slides, stain, image
Library preparation
Library preparation
Sequencing Illumina NovaSeq 6000
Sequencing Illumina NovaSeq 6000
Data QC and bioinformatic analyses

b


Saline female
Saline male
LPS female
LPS male
0
1
2
3
4
5
6
7
8
9
t-SNE2
t-SNE1
16,300 cells (n = 3)
12,424 cells (n = 3)
27,030 cells (n = 3)
24,190 cells (n = 3)


c
Gm42418
Arhgap31
Kcnq1ot1
Rmst
Cit
Gli1
Ttll3
Neat1
Gm26917
Mfge8
Igfbp2
Btbd17
Dbi
Agt
Nnat
Ppia
Tmsb4x
Scrg1
Mt3
Gstm1
Nkain4
S100a1
Thrsp
Actb
Chchd10
Hint1
Mrpl41
Mpc1
Camk2n1
Ndufc2
Nupr1
Igfbp2
Lcn2
Ifitm3
Timp1
B2m
Vim
Gfap
Psmb8
Bst2
Crym
Prss56
Slc43a3
Mgst1
Slc1a5
Tuba1a
Itm2b
Chil1
Ptn
Thbs4
Igfbp5
Fxyd6
Gsn
Fxyd1
Cd9
Meg3
Itih3
Sparc
Nkx6–2
Etnppl
Gria1
Spon1
5031439G07Rik
Igtp
Gm4951
Ifit3
Iigp1
Irgm1
Tap1
Gbp2
Ifit1
Isg15
Cxcl10
Zic1
Slc38a1
Col1a2
Kcnk2
Luzp2
Mgll
Zic4
Hopx
0 1 2 3 4 5 6 7 8 9 Clusters
Cluster genes
Cluster_0
Cluster_1
Cluster_2
Cluster_3
Cluster_4
Cluster_5
Cluster_6
Cluster_7
Cluster_8
Cluster_9
Normal. expression
1
0.8
0.6
0.4
0.2
0


d
Hi
Lo
H&E
Aldh1l1
Slc1a2
S100b
Gfap
Myoc
Astrocyte marker genes
0
1
2
3
4
5
6
7
8
9
Cluster 0–9 modules