

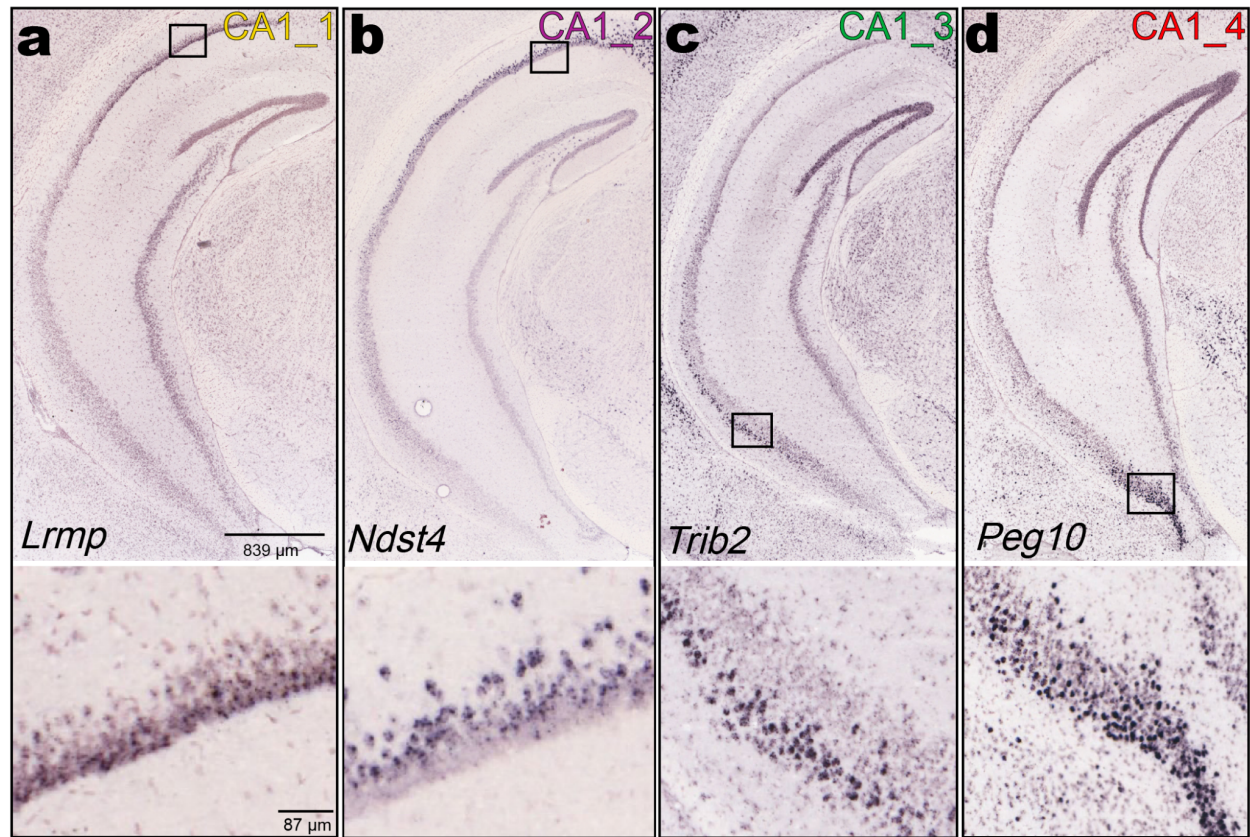
600 **Supplementary Fig. 1: Coronal CA1 Hippocampal Gene Expression Atlas**

601 Updated coronal HGEA with refined CA1 pyramidal cell layer. CA1 subregions CA1d, CA1i, CA1v, and CA1vv can be
602 defined by a distinct combination of cell type layers where CA1d consists of layers 1 and 2, CA1i contains layers 2 and 3,

603 CA1v is composed of layers 2, 3, and 4, and CA1vv is composed of layer 4. While caudal CA1 (HGEA Levels 86-9)
604 comprises layers 1, 2, and 3.

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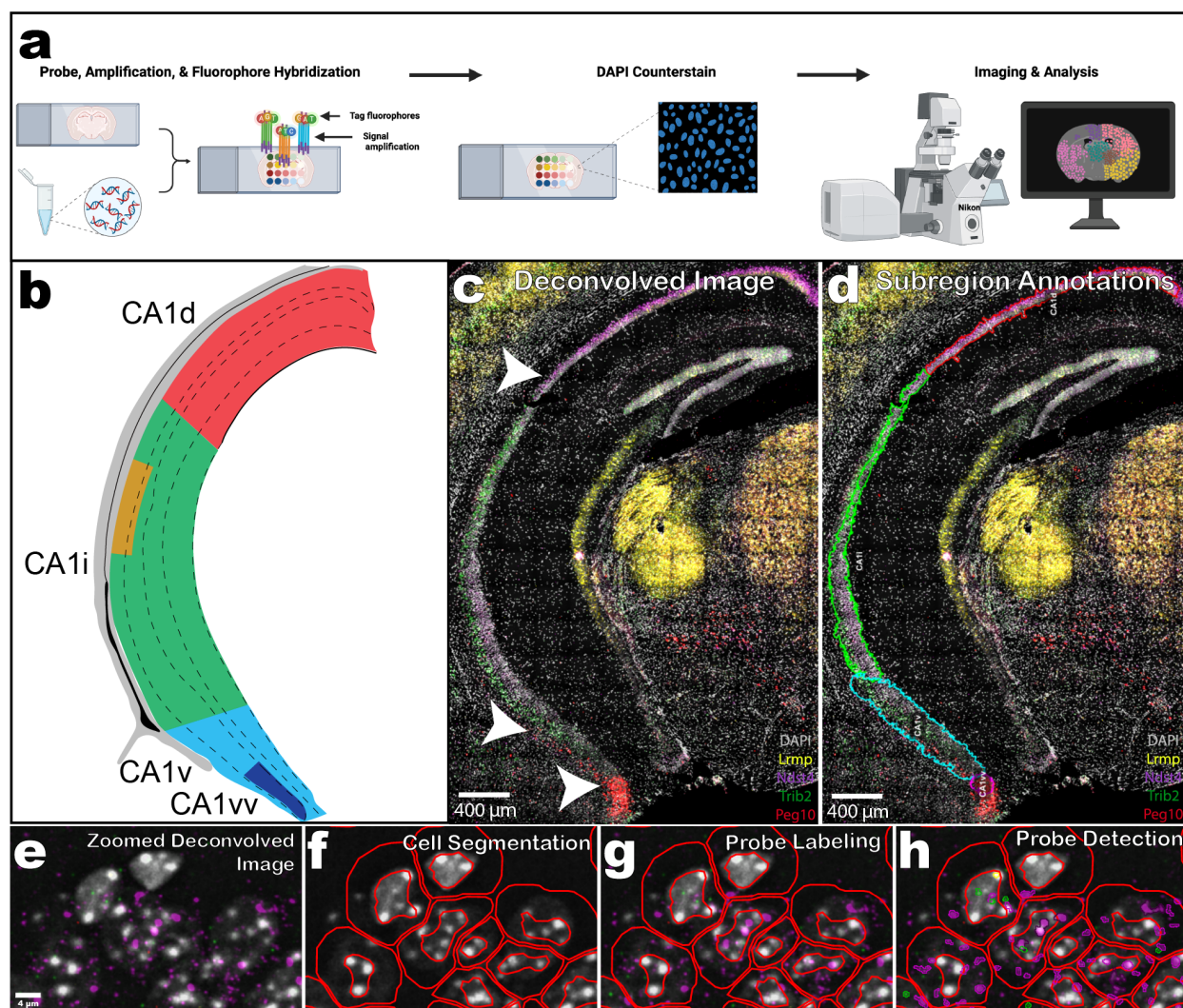


607 **Supplementary Fig. 2: Four exemplar CA1 cell type gene markers**

608 **a-d**, (Top) Allen Institute ISH images of CA1 layer cell type markers *Lrmp* (layer 1), *Ndst4* (layer 2), *Trib2* (layer 3), and
609 *Peg10* (layer 4). Scale bar, 839 μm. (Bottom) Expanded image of black box in (top) panel showing pyramidal layer. Scale
610 bar, 131 μm.

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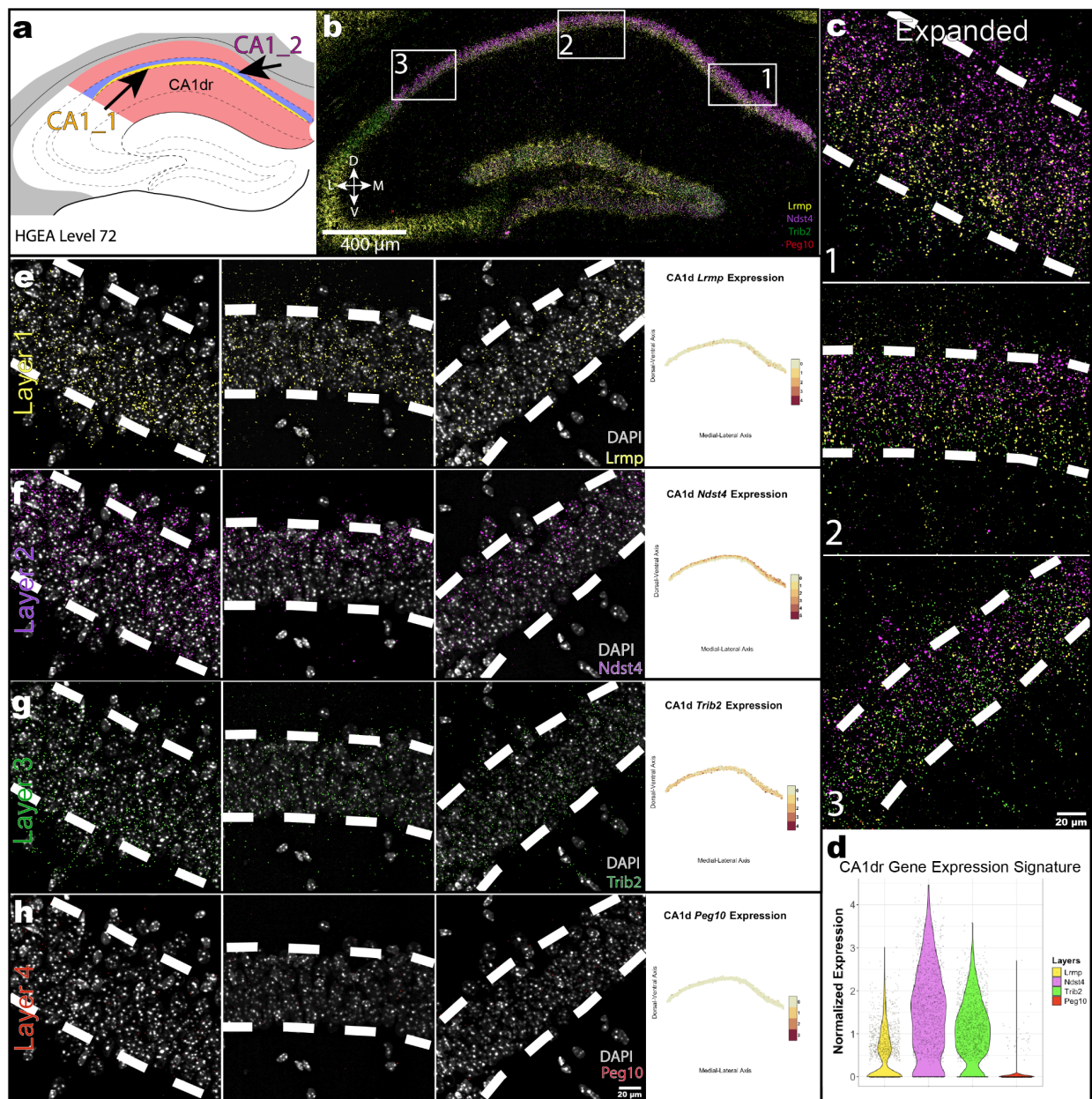
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Supplementary Fig. 3: RNAscope experimental workflow and QuPath single cell quantification

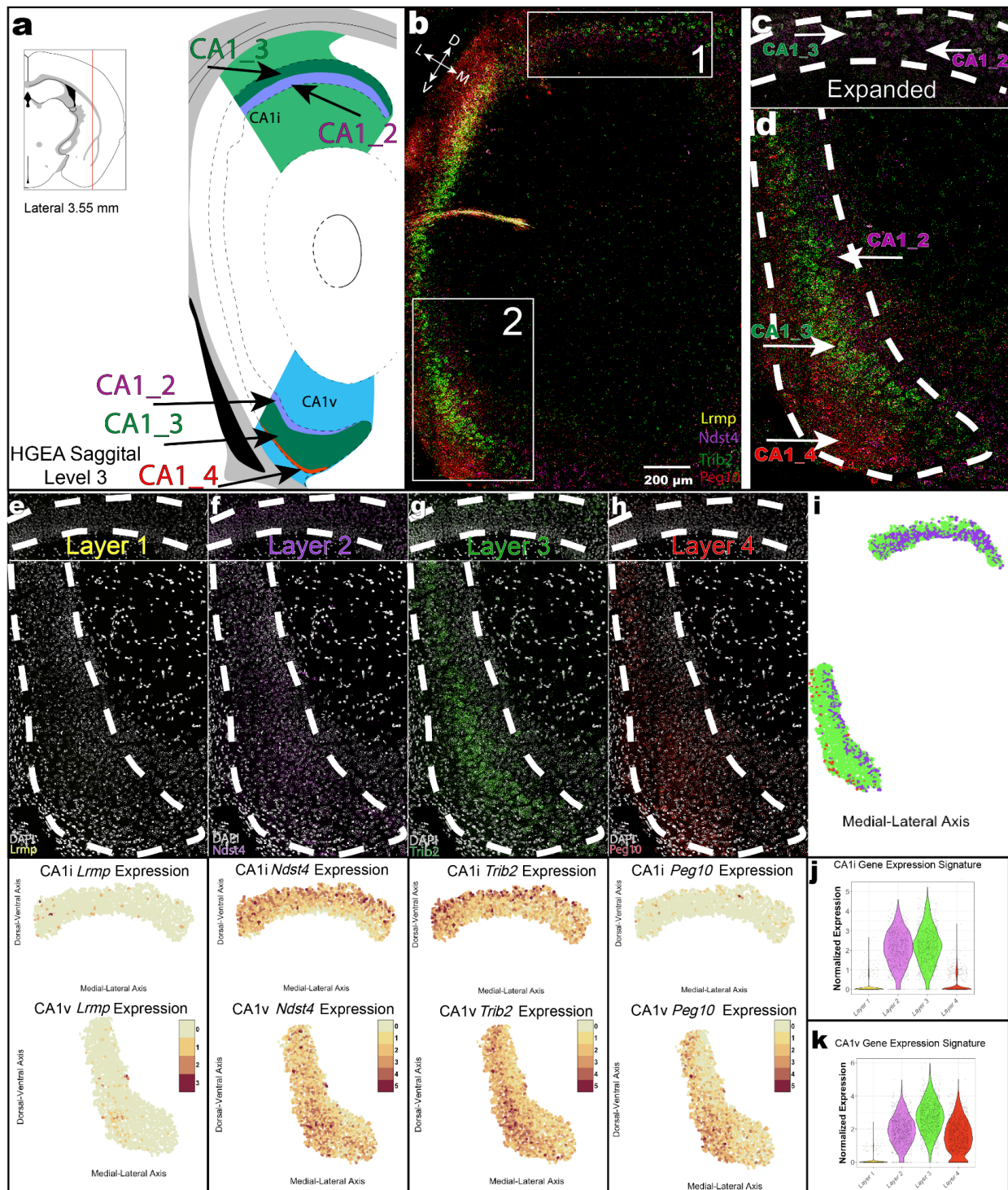
a, General RNAscope ISH workflow; see Methods. Created in BioRender. Pachicano, M. (2025)

<https://BioRender.com/jv9gvxo>. **b**, CA1 subregion delineation in original HGEA at Level 82. **c**, RNAscope-labeled deconvolved confocal max intensity projection image of coronal tissue section corresponding to HGEA Level 82. Scale bar, 400 µm. **d**, Pyramidal layer of CA1 subregions (CA1d, CA1i, CA1v, CA1vv) annotated in QuPath to define ROIs. **e**, Zoom-in of a deconvolved image showing individual fluorescent spots identifying single-RNA transcript molecules ('dots' in color) surrounding DAPI-labeled cell nuclei (gray). Scale bar, 4 µm. **f**, QuPath cell segmentation of DAPI nuclei and estimated 5 µm surrounding cell body area (red outline). **g**, Probe-labeled RNA transcript 'dots' inside detected cell bodies. **h**, Subcellular probe detection (outline in color) within cellular ROI areas.



Supplementary Fig. 4: Rostral CA1 Gene Expression Signature: Layer 1 and Layer 2

a, New HGEA Level 72 showing CA1 layer delineation. **b**, Deconvolved confocal image of CA1 coronal section corresponding to HGEA Level 72. Dorsoventral and mediolateral orientation shown by crosshairs. Scale bar, 400 μm. **c**, Expanded images of white boxes in panel (b) showing the distribution of *Lrmp*, *Ndst4*, *Trib2*, and *Peg10* transcripts within layers 1 and 2. White dashed lines delineate the boundary of the pyramidal cell layer. **d**, Violin plot of log-normalized gene expression for *Lrmp*, *Ndst4*, *Trib2*, and *Peg10* within the CA1d region at HGEA Level 72. **e-h**, (Left) Individual labeling of each gene marker on CA1 DAPI nuclei (gray) from panel (c) and (right) full distribution spatial heatmap of log-normalized gene expression within individual cellular ROIs plotted to the dorsoventral (Y axis) and mediolateral (X axis) anatomical position within the CA1d region. Similar to the gene expression signature shown in Fig.4, the rostral CA1d pyramidal cell layer comprises layers 1 and 2.

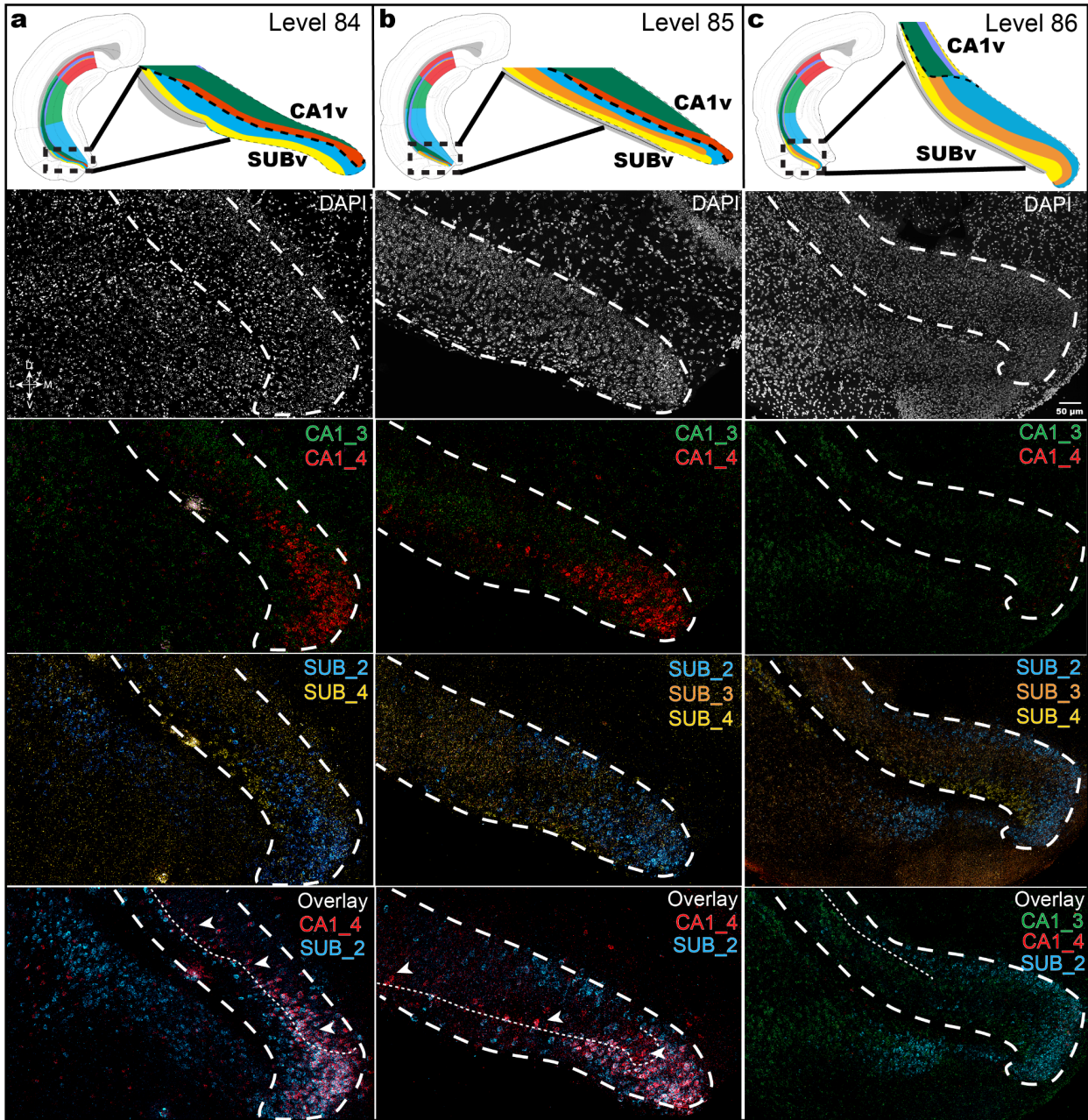


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637 **Supplementary Fig. 5: Gene expression signatures conserved in sagittal CA1**

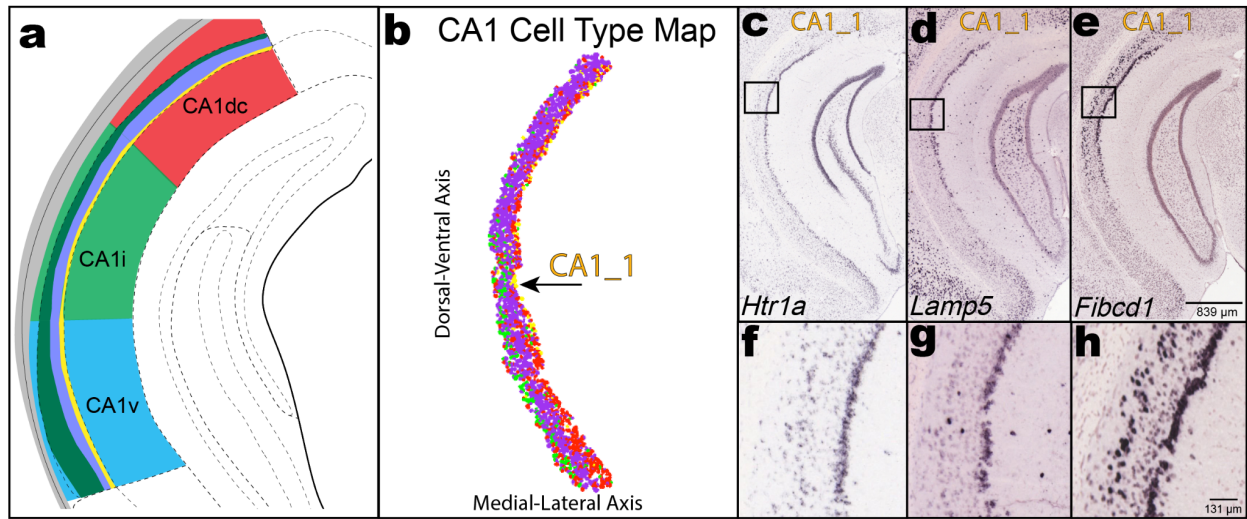
638 **a**, New sagittal HGEA at Level 3 showing CA1 layer delineation. **b**, Deconvolved confocal image of CA1 coronal section
 639 corresponding to sagittal HGEA Level 3. Dorsoventral and mediolateral orientation shown by crosshairs. Scale bar,
 640 200 μ m. **c-d**, Zoomed images of white boxes in panel (**b**) showing the distribution of *Lrmp*, *Ndst4*, *Trib2*, and *Peg10*
 641 transcripts within layers 1, 2, 3 and 4. White dashed lines delineate the boundary of the pyramidal cell layer. **e-h**, (Top)
 642 Individual labeling of each gene marker on CA1 DAPI nuclei (gray) from panel (**c**) and (bottom) full distribution spatial
 643 heatmap of log-normalized gene expression within individual cellular ROIs plotted to the dorsoventral (Y axis) and
 644 mediolateral (X axis) anatomical position within CA1i and CA1v regions. (**i**) Cell type topography map of individual cellular
 645 ROIs where each cell is assigned a color (layer 1 = yellow, layer 2 = purple, layer 3 = green, layer 4 = red) based on
 646 highest log-normalized gene expression value plotted to the dorsoventral (Y axis) and mediolateral (X axis) anatomical
 647 position within CA1i and CA1v regions. Note the layer-specific cell type profiles observed in coronal CA1 are maintained

in the sagittal view. **j-k**, Violin plot of log-normalized gene expression for *Lrmp*, *Ndst4*, *Trib2*, and *Peg10* within the CA1i and CA1v regions at sagittal HGEA Level 3.



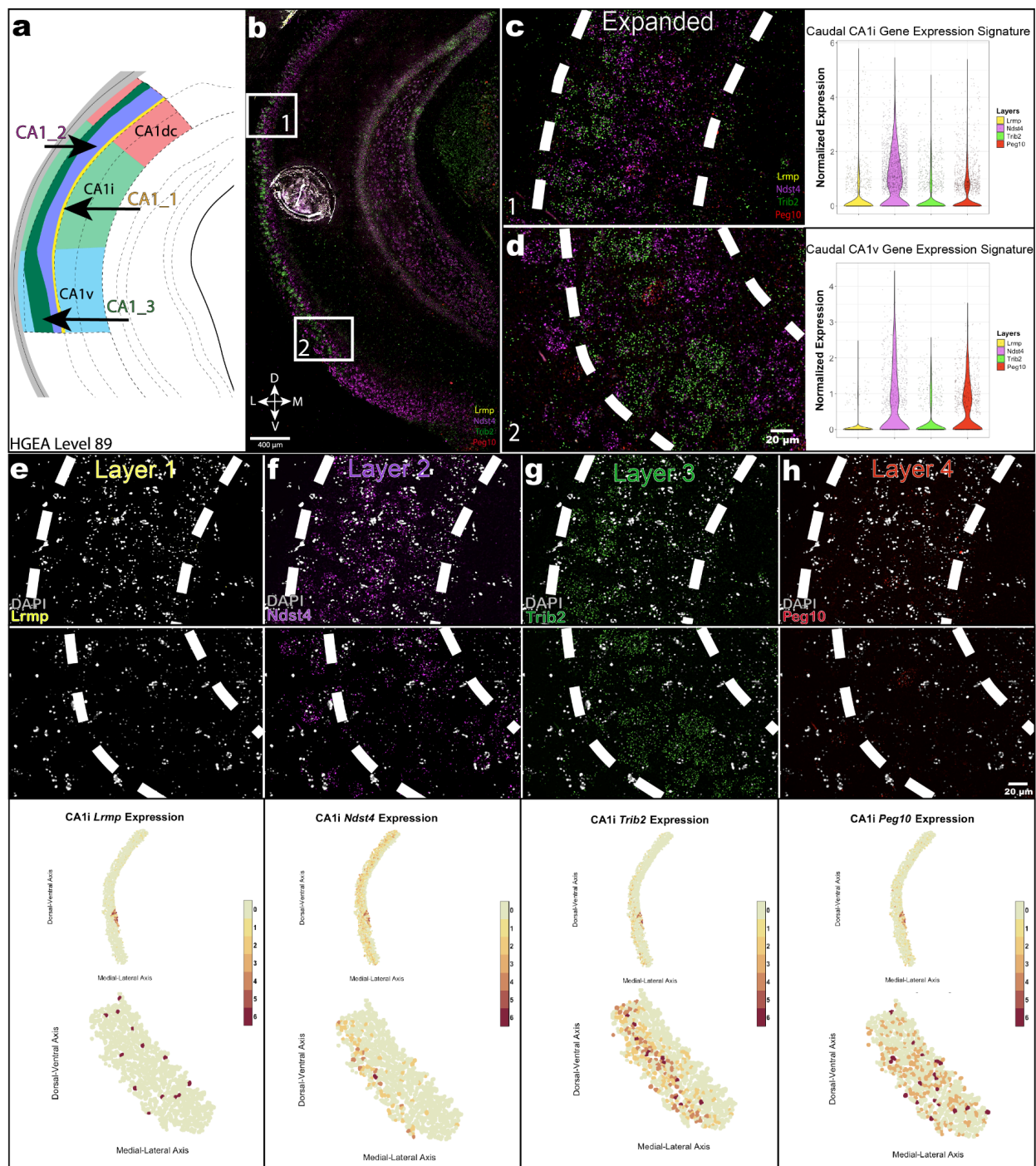
Supplementary Fig. 6: CA1v and SUB cell type layers define a distinct border

a-c, New HGEA Levels 84,85, and 86, with zoomed ventral HGEA with the bold dashed line representing the newly found CA1v and SUB border. Below top panels are zoomed ventral hippocampus DAPI nuclei (gray) for sections corresponding to HGEA Levels 84, 85, and 86, respectively; CA1 distribution of layer 3 and layer 4 gene markers *Trib2* and *Peg10* across HGEA Levels 84-86; SUB distribution of layers 2, 3, and 4 SUB gene markers *Dlk1*, *Tle4*, *Teddm3* across HGEA levels 84-86; and bottom panel shows CA1_4 and SUB_2 gene markers overlaid onto the same tissue sections corresponding to HGEA Levels 84-86. Right panel also includes CA1_3 marker expression to show the upward shift of ventral CA1 at HGEA Level 86. White dashed lines delineate the boundary of the pyramidal cell layer, smaller white dashed lines (with arrows) represent the refined CA1v and SUB border. Dorsoventral and mediolateral orientation shown by crosshairs. Scale bar for all sections, 50 μm.



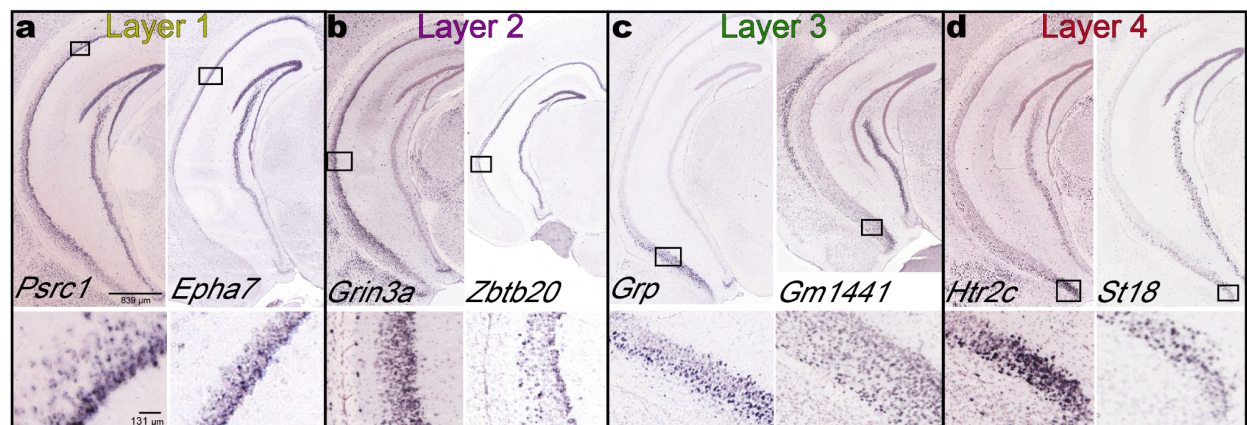
Supplementary Fig. 7: Caudal CA1 Gene Expression Signature defined by Layers 1, 2, and 3.

a, New HGEA Level 89 showing CA1 layer delineation. **b**, Caudal CA1 cell type topography map (same as in Fig.8F) corresponding to HGEA Level 89. **c**, ABA image of CA1 layer 1 gene marker *Htr1a*. Scale bar, 839 μm. **d**, ABA image of CA1 layer 1 gene marker *Lamp5*. Scale bar, 839 μm. **e**, ABA image of CA1 layer 1 gene marker *Fibcd1*. Scale bar, 839 μm. **f**, Zoomed image of black box in panel (c) showing localized *Htr1a* gene expression within the superficial CA1 pyramidal cell layer in caudal CA1. Scale bar, 131 μm. **g**, Zoomed image of black box in panel (d) showing localized *Lamp5* gene expression within the superficial CA1 pyramidal cell layer in caudal CA1. Scale bar, 131 μm. **h**, Zoomed image of black box in panel (e) showing localized *Fibcd1* gene expression within the superficial CA1 pyramidal cell layer in caudal CA1. Scale bar, 131 μm. ISH images in panels (c-h) are from the Allen Institute website.



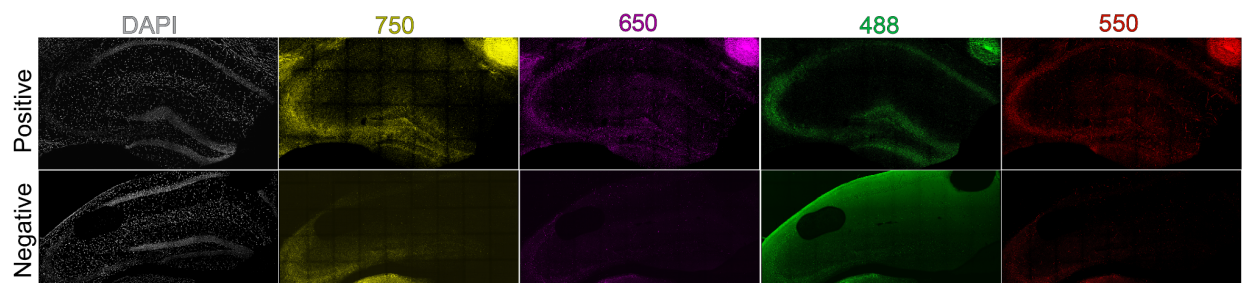
Supplementary Fig. 8: Caudal CA1 Gene Expression Signature: Layers 1, 2 and 3.

a, New HGEA Level 89 showing CA1 layer delineation. **b**, Deconvolved confocal image of CA1 coronal section corresponding to HGEA Level 89. Dorsoventral and mediolateral orientation shown by crosshairs. Scale bar, 400µm. **c-d**, (Left) Expanded images of white boxes in panel (**b**) showing the distribution of *Lrmp*, *Ndst4*, *Trib2*, and *Peg10* transcripts within layers 1, 2 and 3. White dashed lines delineate the boundary of the pyramidal cell layer. (Right) Violin plot of log-normalized gene expression for *Lrmp*, *Ndst4*, *Trib2*, and *Peg10* within caudal CA1 at HGEA Level 89. **e-h**, (Top) Individual labeling of each gene marker on CA1 DAPI nuclei (gray) from panel (**c**) and (bottom) full distribution spatial heatmap of log-normalized gene expression within individual cellular ROIs plotted to the dorsoventral (Y axis) and mediolateral (X axis) anatomical position within the caudal CA1 pyramidal cell layer.



Supplementary Fig. 9: Additional CA1 layer markers show similar spatial distribution.

a, (Top) ABA image of layer 1 markers *Psrc1* and *Epha7*. Scale bar, 839 μ m. (Bottom) Expanded image of black box in (top) panel showing *Psrc1* and *Epha7* gene expression localization in CA1 layer 1. Scale bar, 131 μ m. **b**, (Top) ABA image of layer 2 markers *Grin3a* and *Zbtb20*. Scale bar, 839 μ m. (Bottom) Expanded image of black box in (top) panel showing *Grin3a* and *Zbtb20* gene expression localization in CA1 layer 2. Scale bar, 131 μ m. **c**, (Top) ABA image of layer 3 markers *Grp* and *Gm1441*. Scale bar, 839 μ m. (Bottom) Expanded image of black box in (top) panel showing *Grp* and *Gm1441* gene expression localization in CA1 layer 3. Scale bar, 131 μ m. **d**, (Top) ABA image of layer 4 markers *Htr2c* and *St18*. Scale bar, 839 μ m. (Bottom) Expanded image of black box in (top) panel showing *Htr2c* and *St18* gene expression localization in CA1 layer 4. Scale bar, 131 μ m. ISH images are from Allen Institute website.



Supplementary Fig. 10: Positive and negative control sections show target probe and fluorophore reliability.

Positive and negative control containing ACDBio RTU target probes to show positively-labeled gene expression for all fluorophores (488 nm, 550 nm, 650nm, 750 nm) (top) and negatively-labeled (or lack of) gene expression for all fluorophores (bottom).

Supplementary information

Supplementary Table 1.

Raw cell-by-gene matrix data for CA1d region (Fig. 4). Subcellular probe detection data exported from QuPath for CA1d ROI. Each row represents individual cells within the ROI and columns contain cellular area and subcellular detection counts in the form of individual probe 'spots' for CA1 gene markers *Lrmp*, *Ndst4*, *Trib2*, and *Peg10*.

Supplementary Table 2.

Raw cell-by-gene matrix data for CA1i region (Fig. 5). Subcellular probe detection data exported from QuPath for CA1i ROI. Each row represents individual cells within the ROI and columns contain cellular area and subcellular detection counts in the form of individual probe 'spots' for CA1 gene markers *Lrmp*, *Ndst4*, *Trib2*, and *Peg10*.

715 Supplementary Table 3.

716 Raw cell-by-gene matrix data for CA1v region (Fig. 6). Subcellular probe detection data exported from QuPath
717 for CA1v ROI. Each row represents individual cells within the ROI and columns contain cellular area and
718 subcellular detection counts in the form of individual probe 'spots' for CA1 gene markers *Lrmp*, *Ndst4*, *Trib2*,
719 and *Peg10*.

720 Supplementary Table 4.

721 Raw cell-by-gene matrix data for CA1vv region (Fig. 7). Subcellular probe detection data exported from
722 QuPath for CA1vv ROI. Each row represents individual cells within the ROI and columns contain cellular area
723 and subcellular detection counts in the form of individual probe 'spots' for CA1 gene markers *Lrmp*, *Ndst4*,
724 *Trib2*, and *Peg10*.

725 Supplementary Video 1.

726 Three dimensional HGEA CA1 model. 3D rendering of original HGEA CA1 subregions along with the updated
727 HGEA CA1 layers within the pyramidal CA1 layer. Movie shows CA1d, CA1i, CA1v, CA1vv, with CA1 layers 1,
728 2, 3, and 4 as the camera rotates around CA1. All subregion and layer colors correspond to the updated CA1
729 HGEA atlas in Supplementary Fig. 1.
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