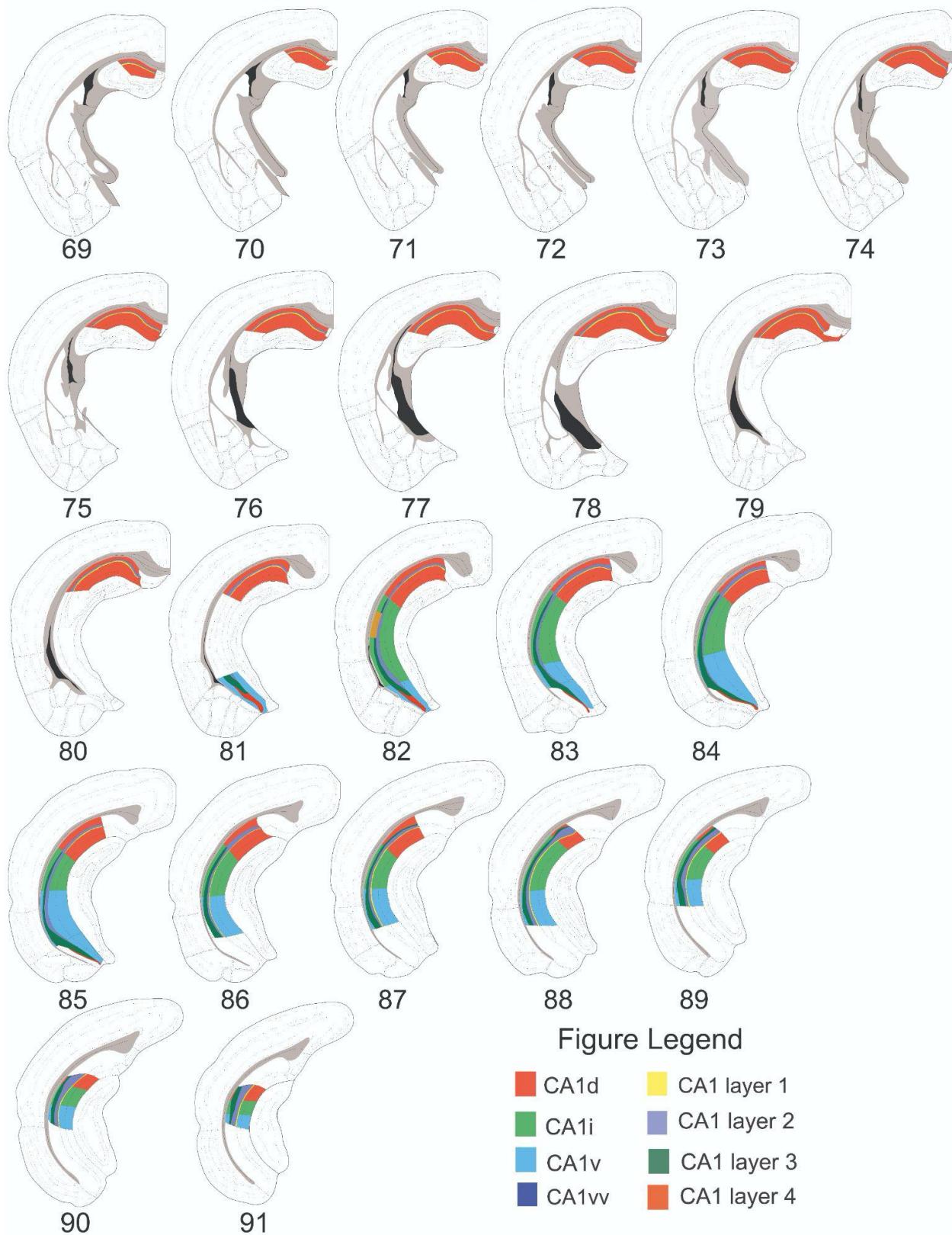
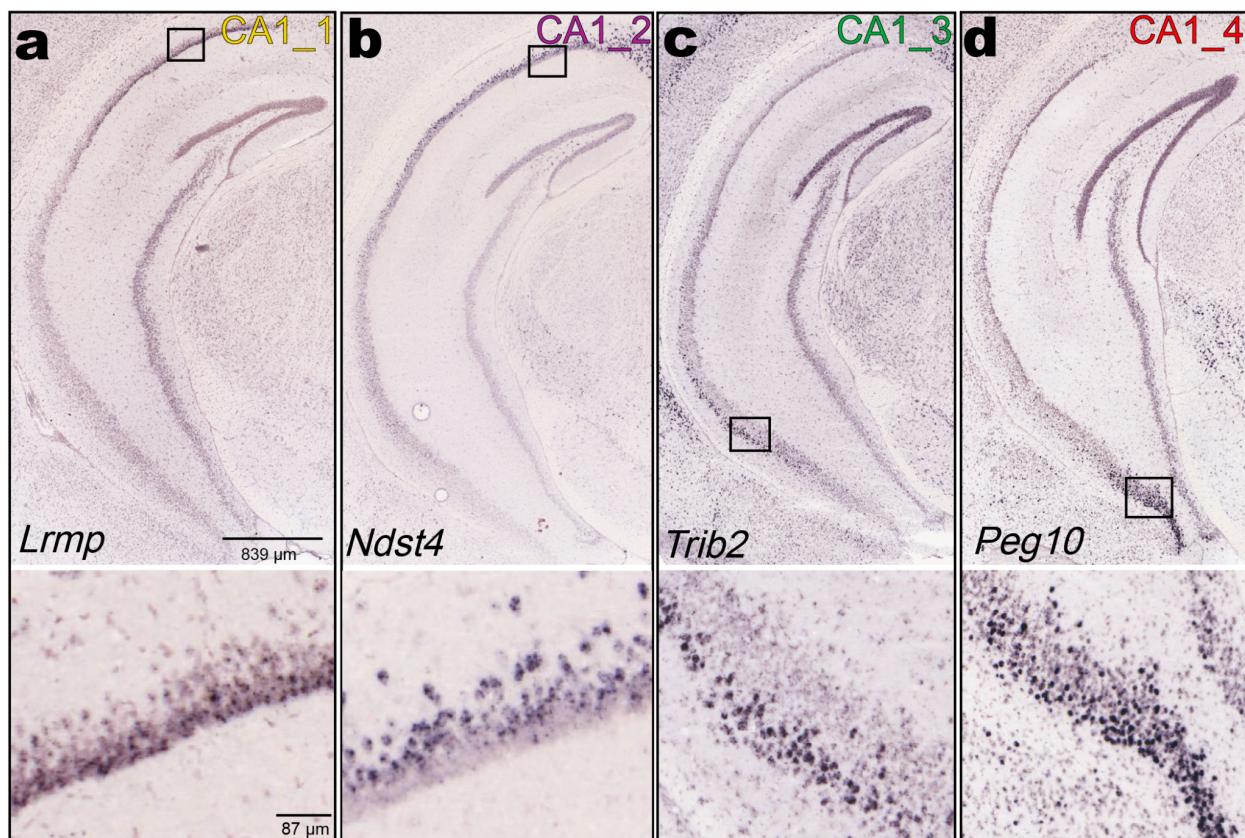


Hippocampus Gene Expression Atlas - CA1



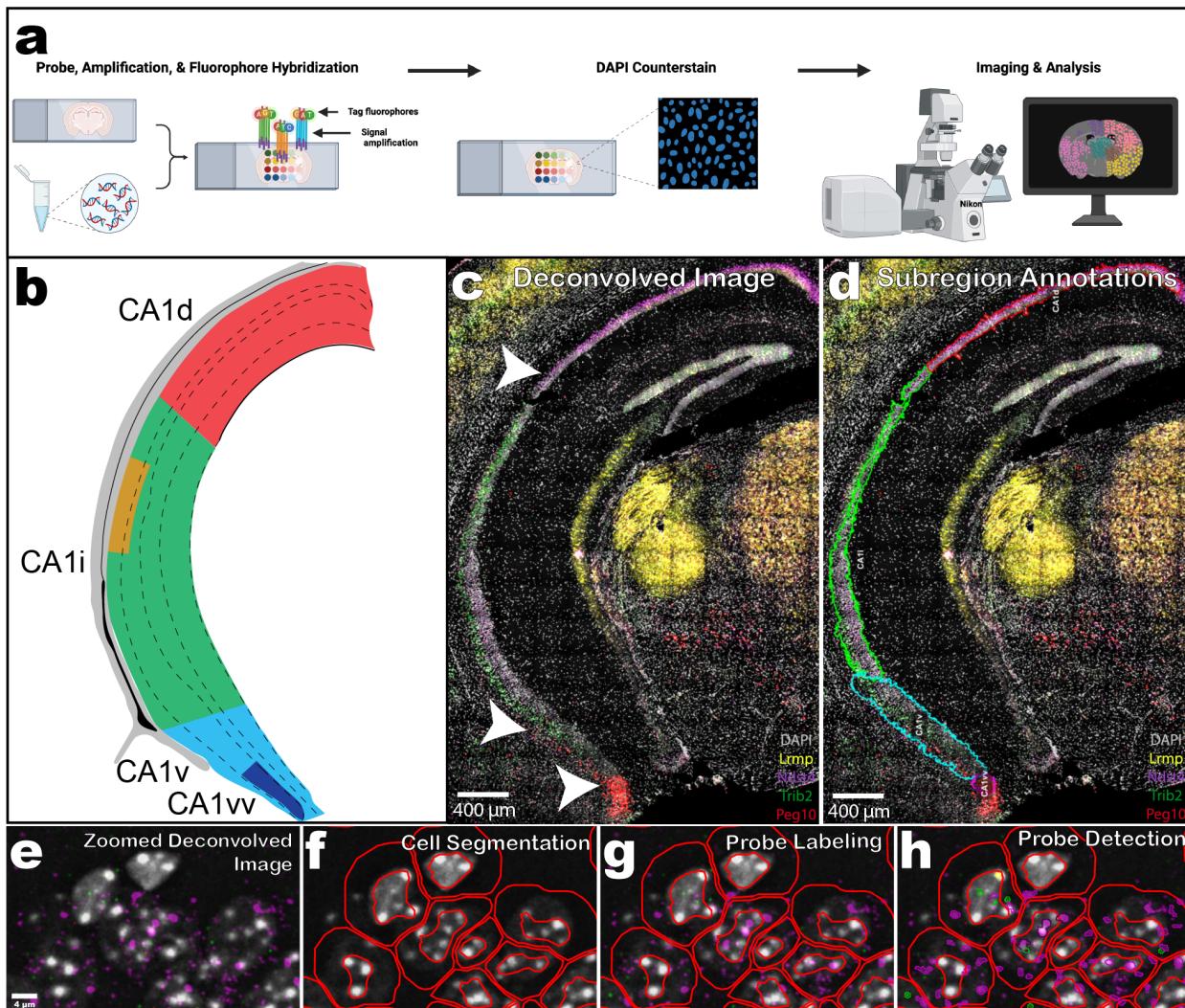
603 CA1v is composed of layers 2, 3, and 4, and CA1vv is composed of layer 4. While caudal CA1 (HGEA Levels 86-9)
604 comprises layers 1, 2, and 3.
605



606

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 μ m. (Bottom) Expanded image of black box in (top) panel showing pyramidal layer. Scale
610 bar, 131 μ m.
611
612



613
614 **Supplementary Fig. 3: RNAscope experimental workflow and QuPath single cell quantification**

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

616 <https://BioRender.com/jv9gvxo>. **b**, CA1 subregion delineation in original HGEA at Level 82. **c**, RNAscope-labeled

617 deconvolved confocal max intensity projection image of coronal tissue section corresponding to HGEA Level 82. Scale

618 bar, 400 μ m. **d**, Pyramidal layer of CA1 subregions (CA1d, CA1i, CA1v, CA1vv) annotated in QuPath to define ROIs. **e**,

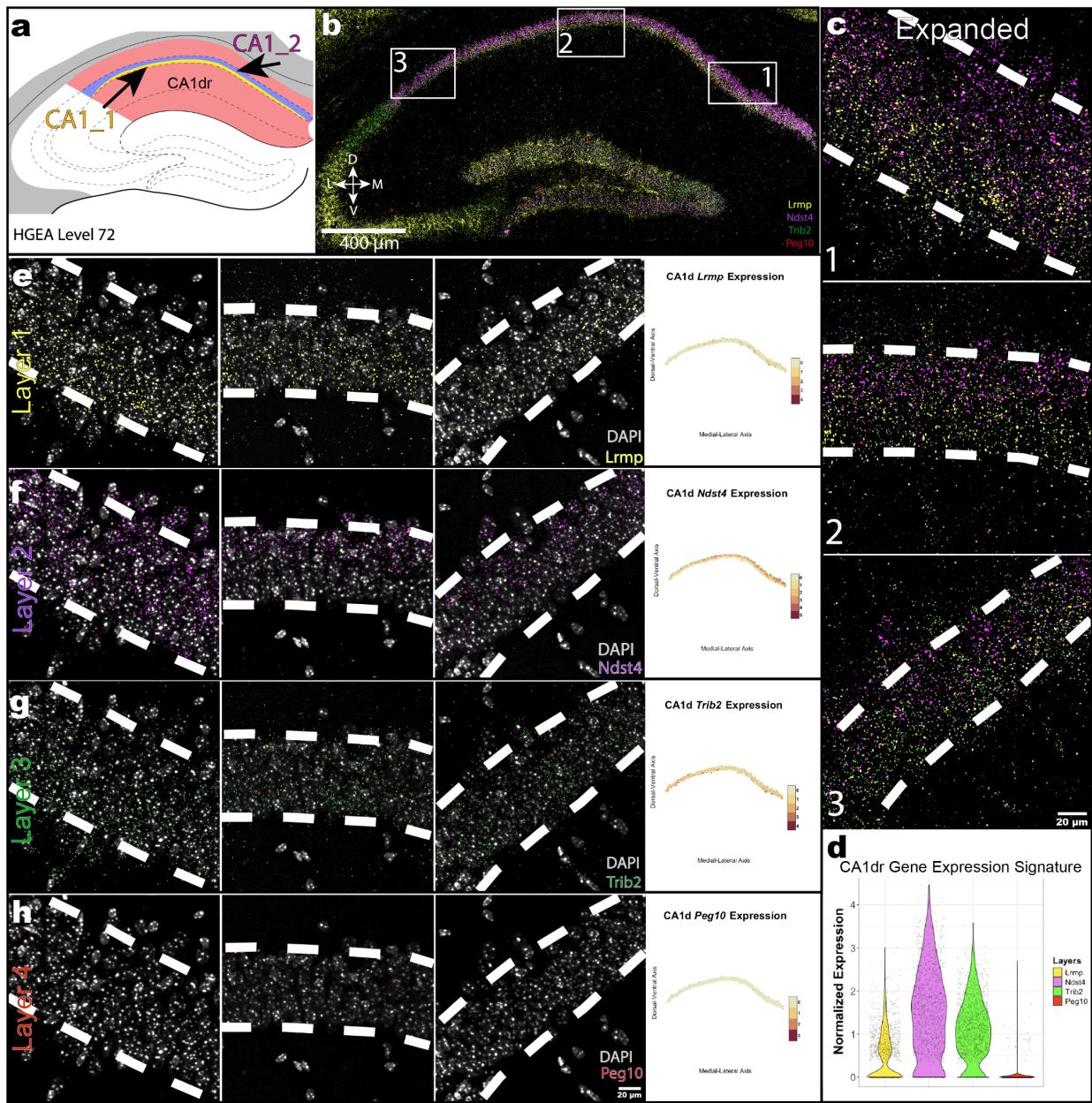
619 Zoom-in of a deconvolved image showing individual fluorescent spots identifying single-RNA transcript molecules ('dots')

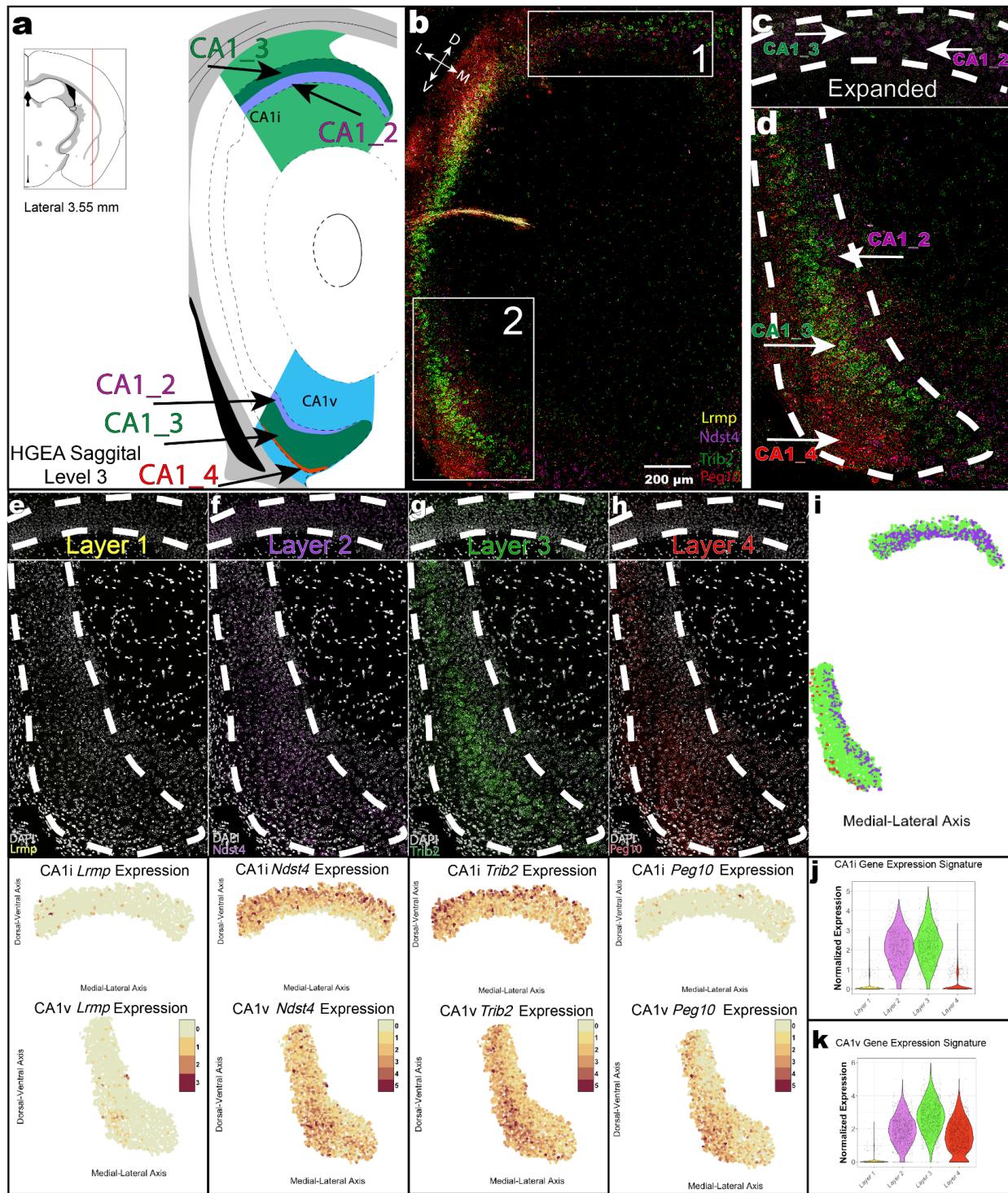
620 in color) surrounding DAPI-labeled cell nuclei (gray). Scale bar, 4 μ m. **f**, QuPath cell segmentation of DAPI nuclei and

621 estimated 5 μ m surrounding cell body area (red outline). **g**, Probe-labeled RNA transcript 'dots' inside detected cell

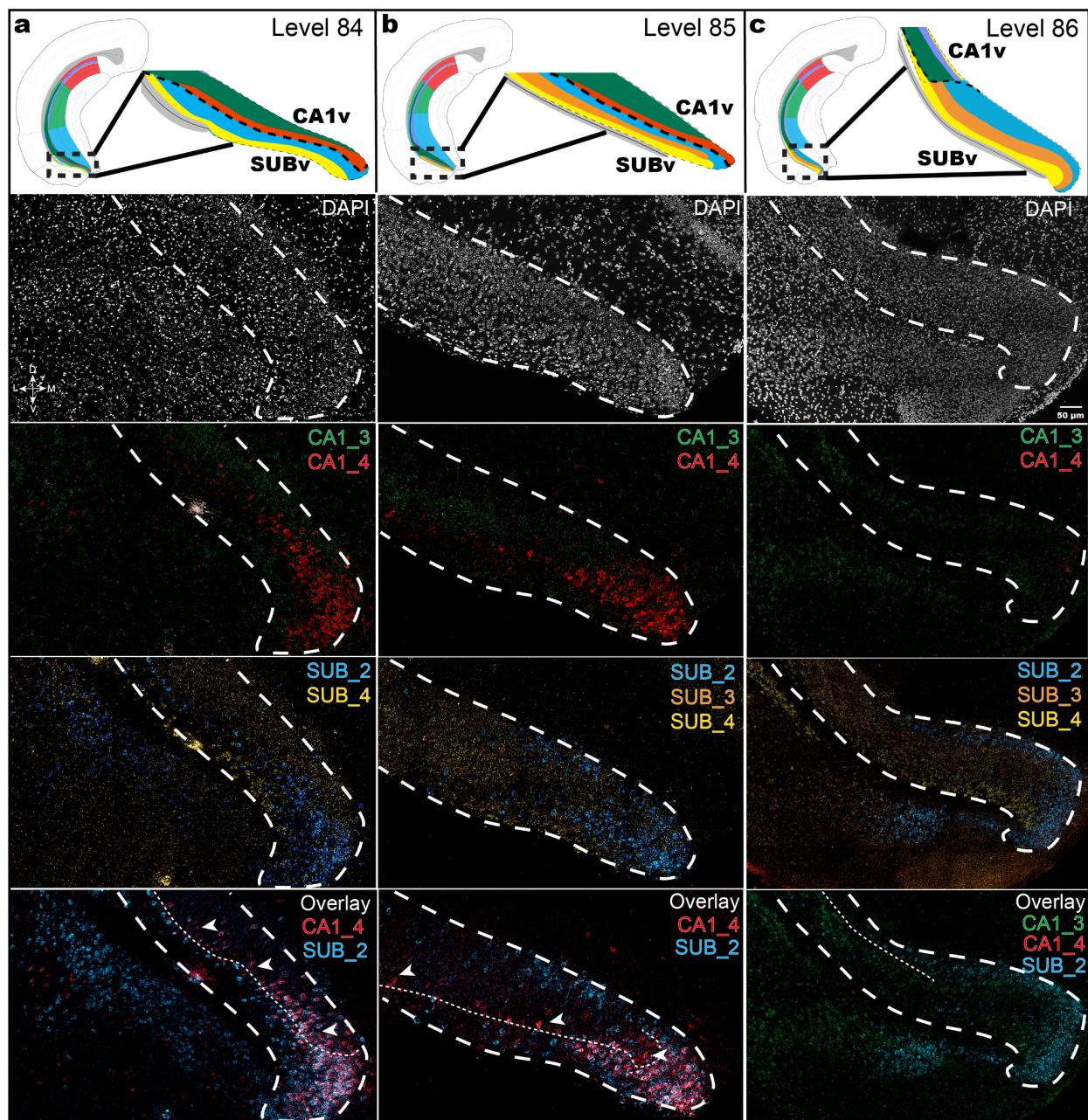
622 bodies. **h**, Subcellular probe detection (outline in color) within cellular ROI areas.

623



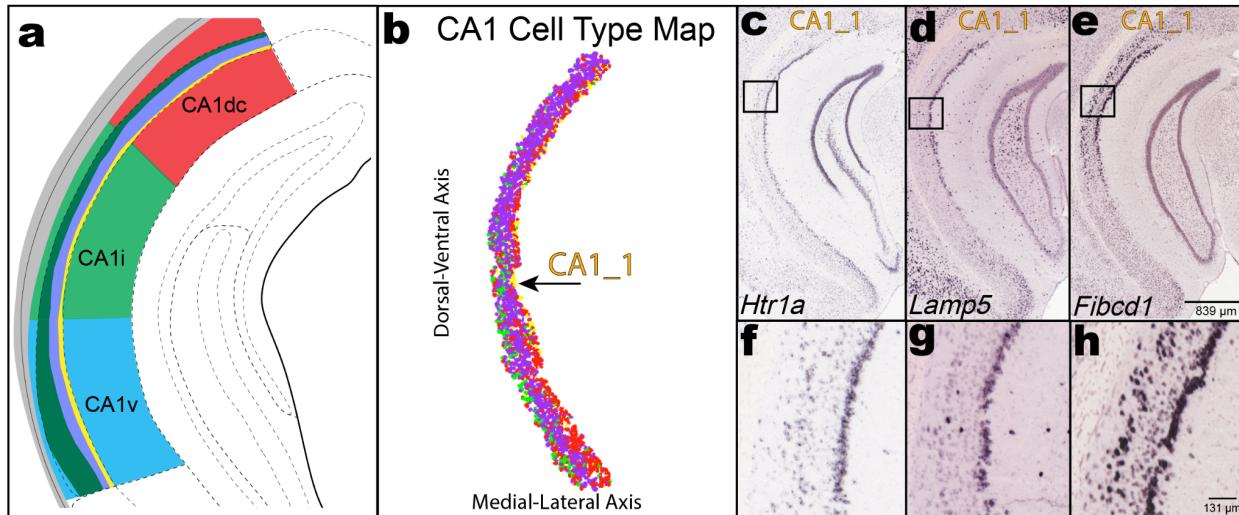


648 in the sagittal view. **j-k**, Violin plot of log-normalized gene expression for *Lrmp*, *Ndst4*, *Trib2*, and *Peg10* within the CA1i
649 and CA1v regions at sagittal HGEA Level 3.
650



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

653 **a-c**, New HGEA Levels 84, 85, and 86, with zoomed ventral HGEA with the bold dashed line representing the newly found
654 CA1v and SUB border. Below top panels are zoomed ventral hippocampus DAPI nuclei (gray) for sections corresponding
655 to HGEA Levels 84, 85, and 86, respectively; CA1 distribution of layer 3 and layer 4 gene markers *Trib2* and *Peg10*
656 across HGEA Levels 84-86; SUB distribution of layers 2, 3, and 4 SUB gene markers *Dlk1*, *Tle4*, *Teddm3* across HGEA
657 levels 84-86; and bottom panel shows CA1_4 and SUB_2 gene markers overlaid onto the same tissue sections
658 corresponding to HGEA Levels 84-86. Right panel also includes CA1_3 marker expression to show the upward shift of
659 ventral CA1 at HGEA Level 86. White dashed lines delineate the boundary of the pyramidal cell layer, smaller white
660 dashed lines (with arrows) represent the refined CA1v and SUB border. Dorsoventral and mediolateral orientation shown
661 by crosshairs. Scale bar for all sections, 50 μ m.
662

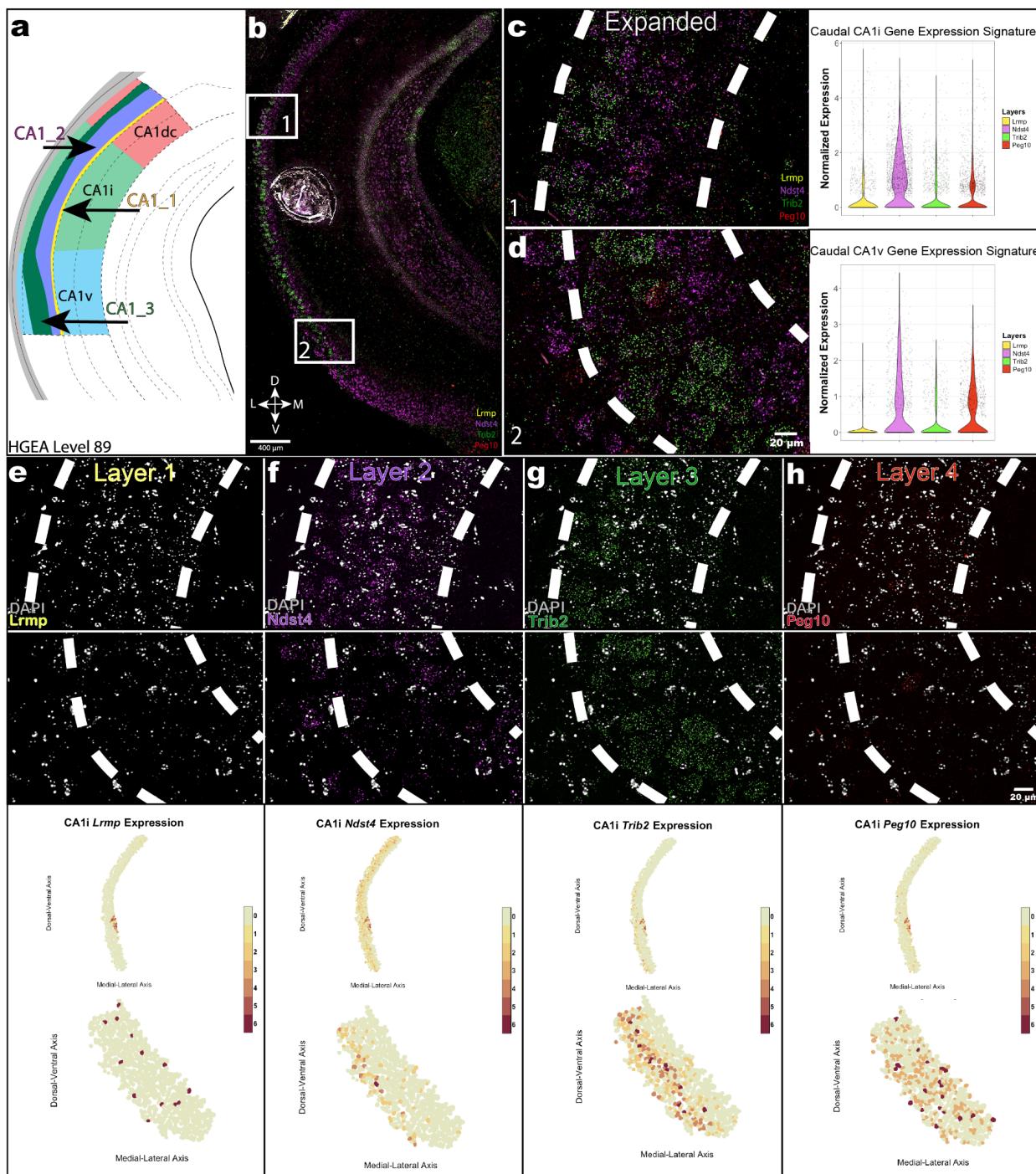


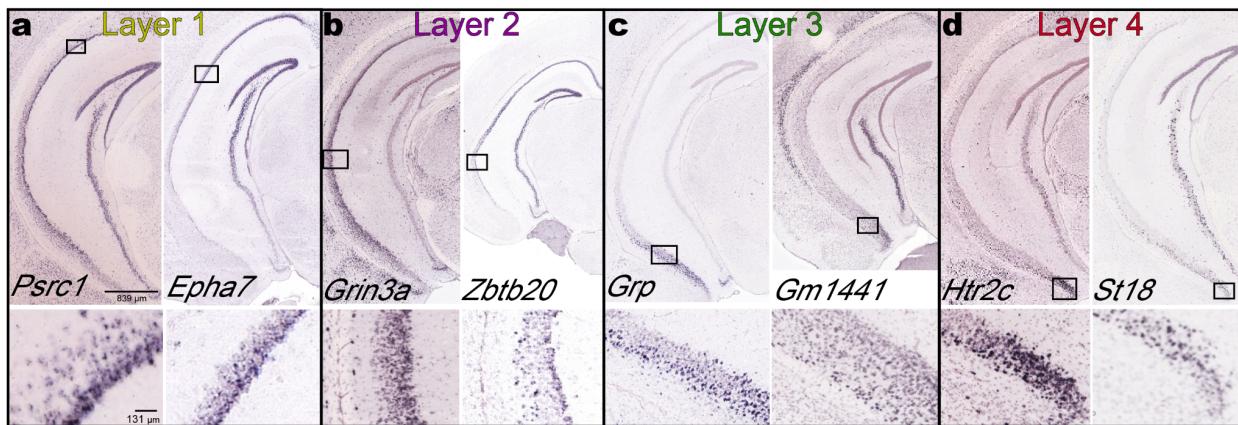
664 **Supplementary Fig. 7: Caudal CA1 Gene Expression Signature defined by Layers 1, 2, and 3.**

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

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674



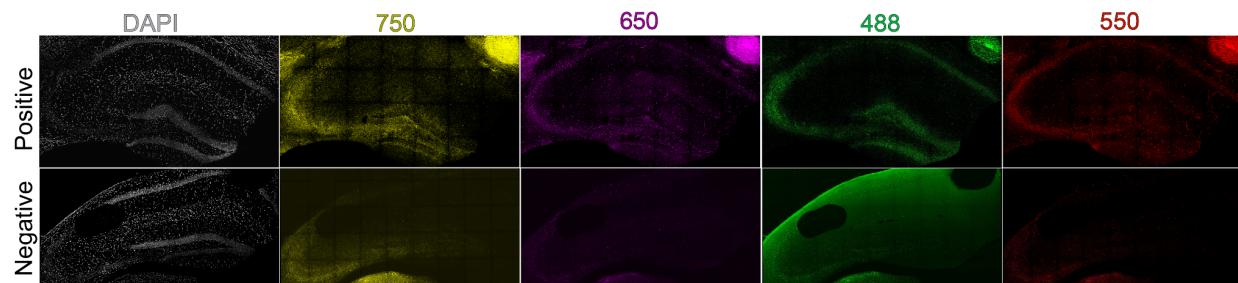


688

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

690 a, (Top) ABA image of layer 1 markers *Psnc1* and *EphA7*. Scale bar, 839 μ m. (Bottom) Expanded image of black box in
 691 (top) panel showing *Psnc1* and *EphA7* gene expression localization in CA1 layer 1. Scale bar, 131 μ m. b, (Top) ABA
 692 image of layer 2 markers *Grin3a* and *Zbtb20*. Scale bar, 839 μ m. (Bottom) Expanded image of black box in (top) panel
 693 showing *Grin3a* and *Zbtb20* gene expression localization in CA1 layer 2. Scale bar, 131 μ m. c, (Top) ABA image of layer
 694 3 markers *Grp* and *Gm1441*. Scale bar, 839 μ m. (Bottom) Expanded image of black box in (top) panel showing *Grp* and
 695 *Gm1441* gene expression localization in CA1 layer 3. Scale bar, 131 μ m. d, (Top) ABA image of layer 4 markers *Htr2c*
 696 and *St18*. Scale bar, 839 μ m. (Bottom) Expanded image of black box in (top) panel showing *Htr2c* and *St18* gene
 697 expression localization in CA1 layer 4. Scale bar, 131 μ m. ISH images are from Allen Institute website.

698



699

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

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

704 **Supplementary information**705 Supplementary Table 1.

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

710 Supplementary Table 2.

711 Raw cell-by-gene matrix data for CA1i region (Fig. 5). Subcellular probe detection data exported from
 712 QuPath for CA1i ROI. Each row represents individual cells within the ROI and columns contain cellular
 713 area and subcellular detection counts in the form of individual probe 'spots' for CA1 gene markers
 714 *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.

730