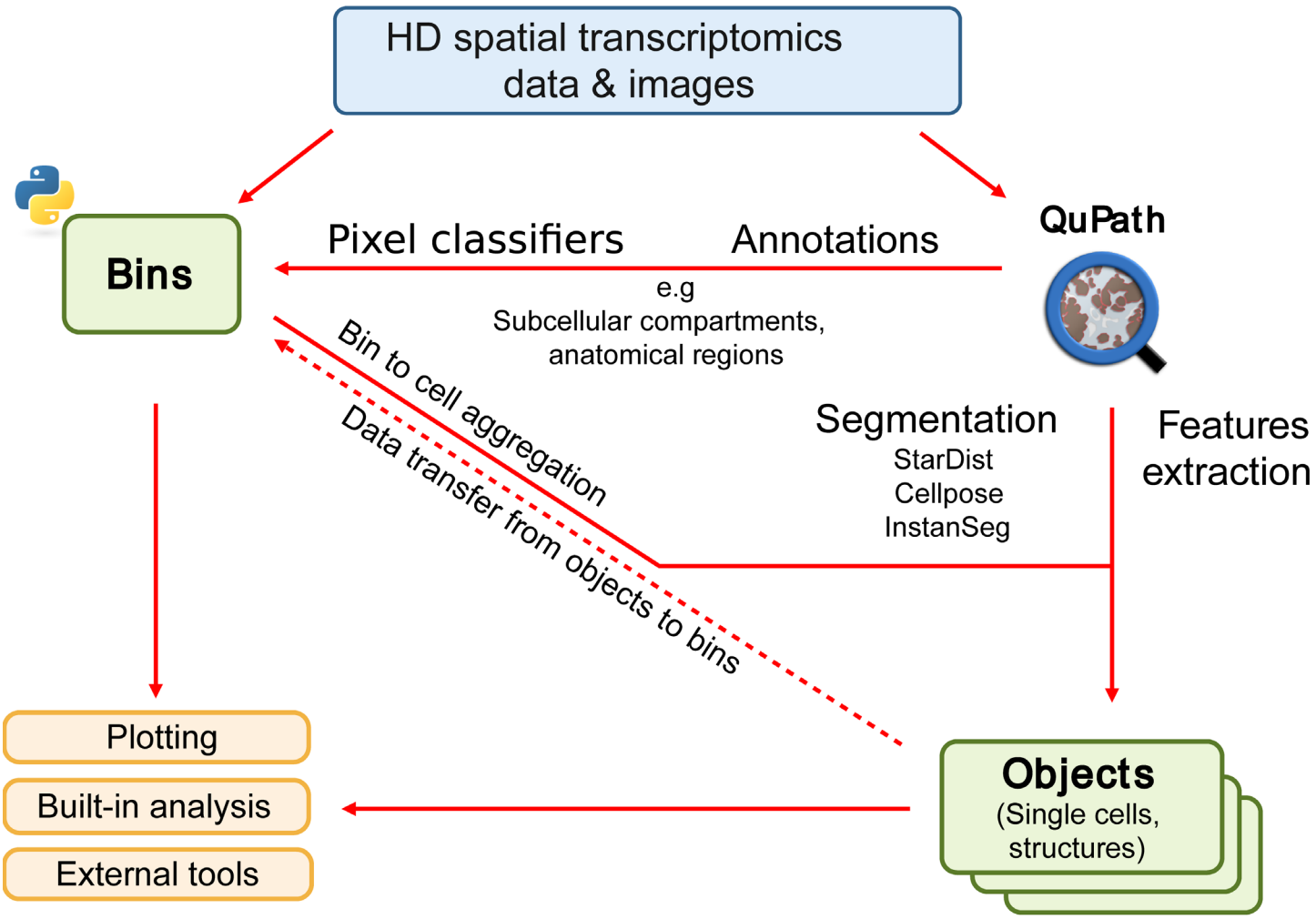
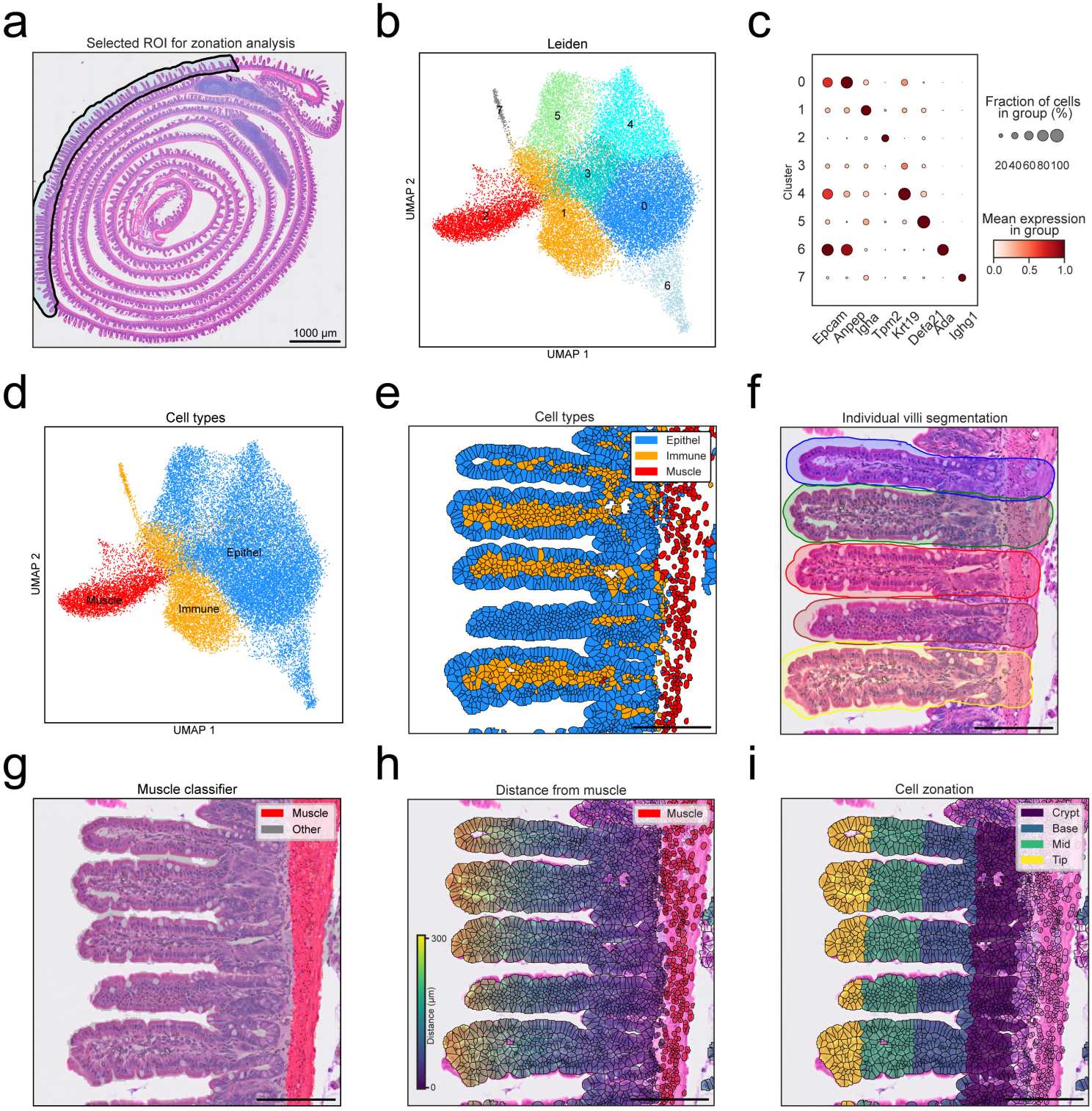
Subcellular mRNA localization patterns across tissues resolved with spatial transcriptomics

Supplementary Figures

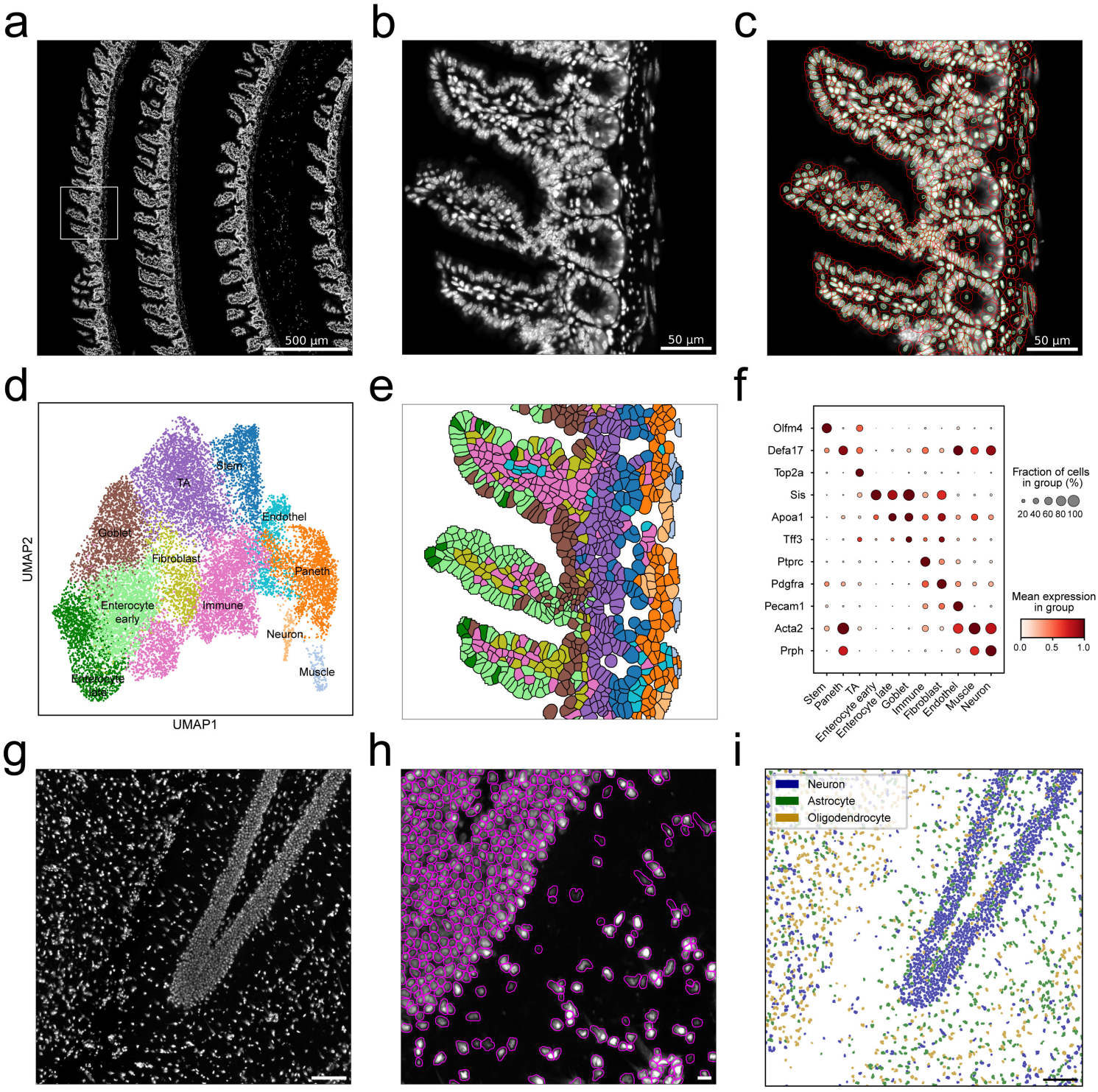


Supplementary Figure 1 – Diagram of the HiVis tool
HiVis is a user-friendly platform for high-definition spatial transcriptomics analysis. Image analysis is performed with QuPath, whereas all other steps are implemented in Python, including aggregation of bins into cells or other objects. HiVis operates at both the bin and aggregation levels, synchronizing data across them. It provides flexible, fully customizable plotting, offers built-in analysis methods, and is compatible with external tools such as Scanpy[1] and Squidpy[2]. The diagram was created with BioRender.com and available at: https://biorender.com/ewh3f8t.



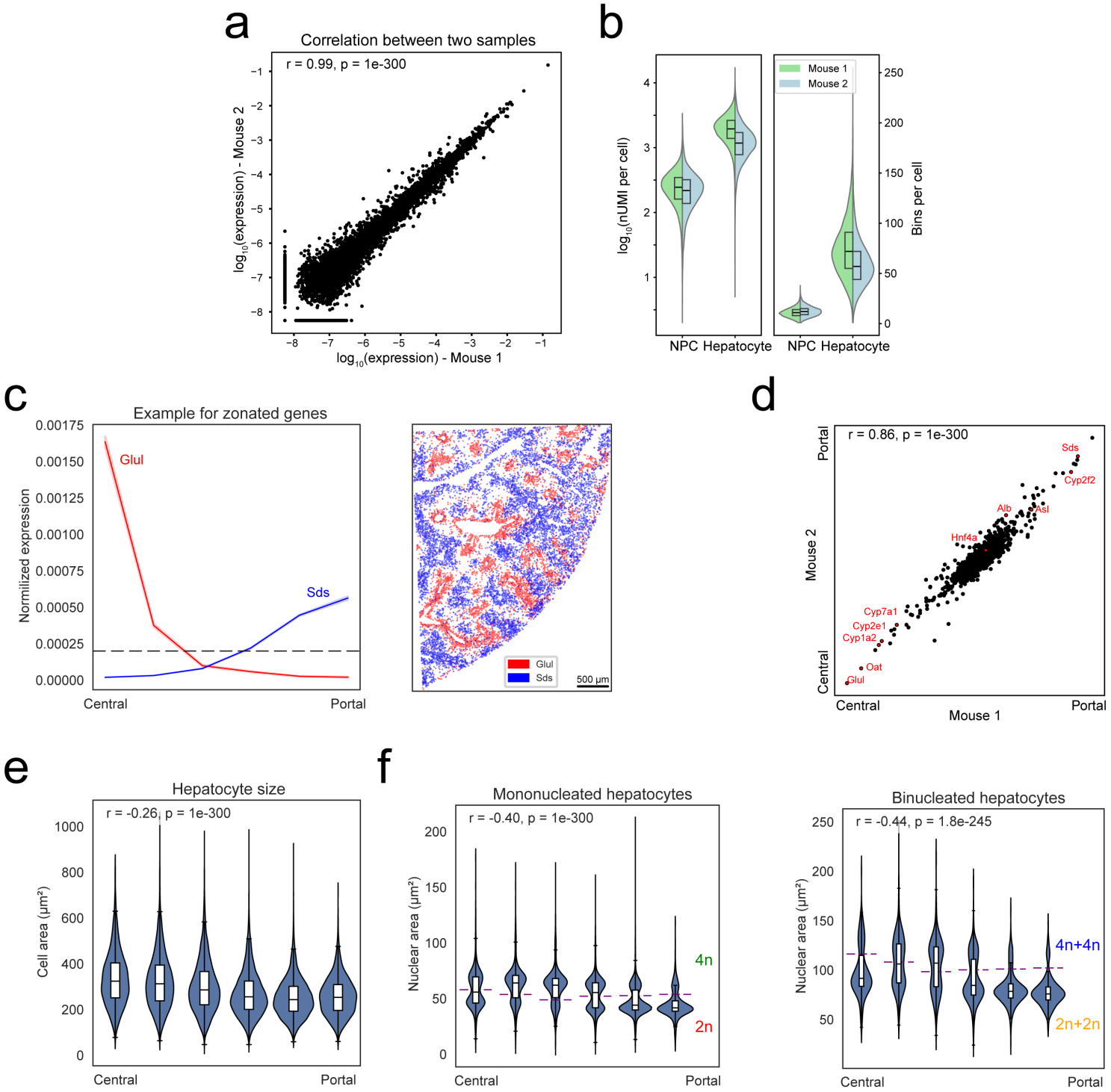
Supplementary Figure 2 – Reconstructing zonation along villi in mouse small intestine

(a) VisiumHD H&E image of a mouse small intestine sample, overlayed with proximal intestine annotation for further analysis. **(b)** UMAP of single cells colored by leiden clusters. **(c)** Dot plot of normalized gene expression for selected markers across leiden clusters. **(d)** Annotated UMAP of single cells colored by cell type. **(e)** Cells colored by cell type. **(f)** VisiumHD H&E image of mouse proximal intestine, overlayed with individual villi manual annotations. **(g)** Mouse proximal intestine VisiumHD H&E image overlayed with 2×2µm bins colored by muscle identity, based on a pixel classifier. **(h)** Mouse proximal intestine VisiumHD H&E image overlayed with cells colored by the distance from the muscle layer. **(i)** Mouse proximal intestine VisiumHD H&E image overlayed with cells colored by the villus zone. Scale bar in (e-i) – 100µm.



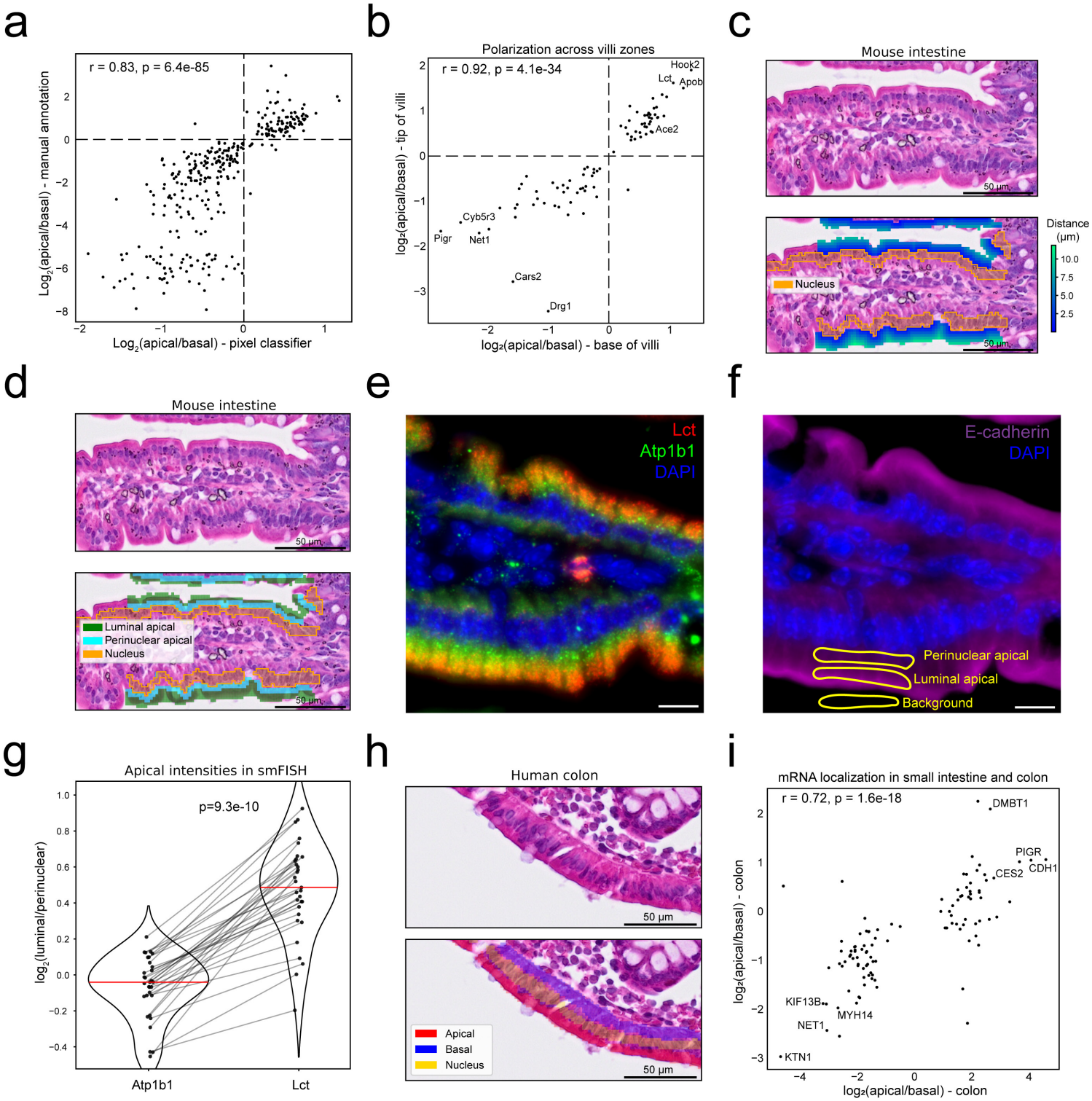
Supplementary Figure 3 – Cross-platform application of HiVis

(a) DAPI image of a mouse intestine Xenium sample from Ameku et al.[3]. Box denotes selected FOV in (b,c,e). Scalebar 500µm. **(b)** DAPI image of a mouse intestine Xenium sample, selected FOV. Scalebar 50µm. **(c)** Same ROI as (b), with single cells segmented in red. Nuclei in green. Scalebar 50µm. **(d)** Annotated UMAP of single cells colored by cell type. **(e)** Same FOV as (b), with cells colored by cell type. Colors correspond to the cell-type colors in (d). **(f)** Dot plot of normalized gene expression for selected markers across cell types. **(g)** ssDNA image of mouse brain Stereo-seq dataset from Qiu et al.[4]. Scalebar 100µm. **(h)** Single cells segmented in mouse brain Stereo-seq dataset. Scalebar 10µm. **(i)** Same FOV as (g) with cells colored by cell-type. Scalebar 100µm.



Supplementary Figure 4 – Reconstructing hepatocyte zonation across liver lobules

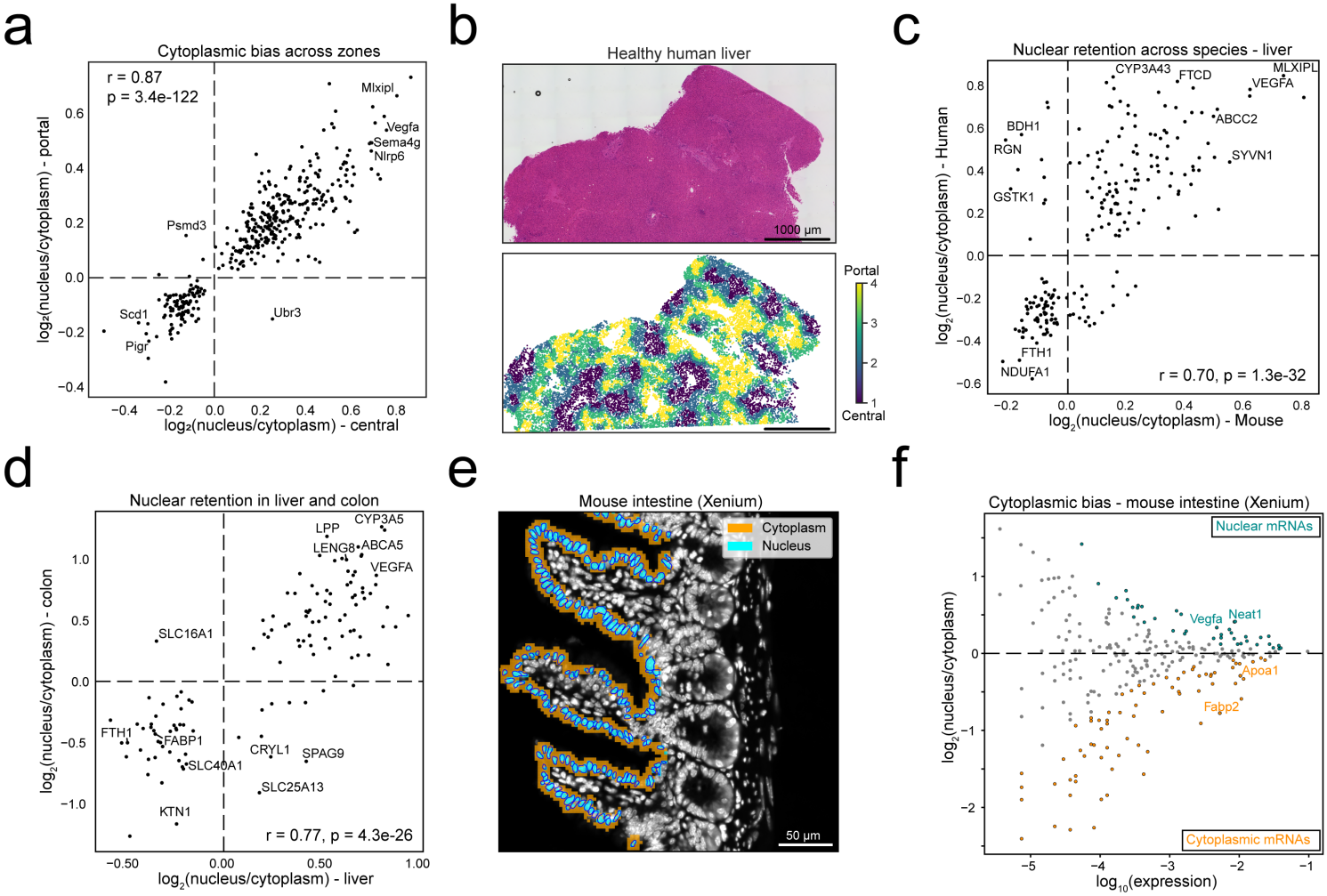
(a) Spearman correlation (two-sided) of normalized gene expression levels between both mice in the experiment. **(b)** Distribution of nUMI (left) and number of 2×2 µm bins (right) in cells from two VisiumHD mouse liver samples. NPC – non parenchymal cells. **(c)** Examples of two zonated genes in single-cell data from mouse liver VisiumHD. Left – normalized expression across liver lobules, data is mean expression, and patch is standard error of the mean. Right – spatial plot of single cells colored by expression of each of the genes. Cells are colored if above the normalized expression > 0.0002. **(d)** Spearman correlation (two-sided) plots of center of mass across liver zones from the two mice in the experiment. **(e)** Violin plots with boxplots showing cell areas of hepatocytes across liver zones. Data is from two samples. Correlation is spearman. **(f)** Violin plots with boxplots showing nuclear area of hepatocytes across liver zones for mononucleated (left) and binucleated (right) hepatocytes. The purple dashed lines mark thresholds separating ploidy classes, indicated by colored labels. Data is from two samples. Correlation is spearman. In boxplots (b, e-f) – horizontal bars are medians, boxes delineate the 25–75 percentiles.



Supplementary Figure 5 – Mapping apical-basal polarization in mouse small intestine and human colon

(a) Spearman correlation (two-sided) of apical-basal bias in mouse proximal intestine using manual annotation and pixel classifier. *Genes with normalized expression > 10^{-5} and q<0.05 are used.* **(b)** Spearman correlation (two-sided) of apical-basal bias in base and tip of villi in mouse proximal intestine. Only genes with q<0.05 and normalized expression > 10^{-5} in both zones are used. Selected gene names are shown. **(c)** Mouse proximal intestine VisiumHD H&E image (top) and overlayed with 2×2µm bins colored by the distance to the nuclei (bottom). **(d)** Mouse proximal intestine VisiumHD H&E image (top) and overlayed with 2×2µm bins colored by apical sub-compartments (bottom). **(e)** smFISH staining of Lct and Atp1b1 in mouse proximal intestine. Image is representative of 2 mice. Scale bar, 10 µm. **(f)** Example annotation of apical sub-compartments and background, used for quantification of smFISH intensity. Annotations are based on Immunofluorescence staining of E-cadherin and nuclei. Same FOV as (e). **(g)** Quantification of apical smFISH signal as log2(luminal/perinuclear) intensity for Atp1b1 and Lct. Lines connect

paired measurements across annotated apical sub-compartments. Red lines indicate medians. p-value calculated by Wilcoxon signed-rank test. Annotations are from two mice. **(h)** Human colon VisiumHD H&E image (top) and overlayed with 2×2µm bins colored by apical/basal assignment (bottom). Representative image from 5 samples. **(i)** Spearman correlation (two-sided) of apical-basal bias in colon and Jejunum. Jejunum data is based on previously published LCM RNA-seq[14]. Only genes with q<0.25 in both datasets are used. Selected gene names are shown.



Supplementary Figure 6 – Mapping nuclear retention in livers and Xenium

(a) Spearman correlation (two-sided) of nuclear/cytoplasmic bias in pericentral and periportal mouse liver zones. Only genes with q<0.05 and normalized expression > 10^{-4} in both zones are used. Selected gene names are shown. **(b)** Top – VisiumHD H&E image of a human liver. Bottom – single-cell VisiumHD data of human liver, colored by zones along the central-portal axis. **(c)** Spearman correlation (two-sided) of nuclear/cytoplasmic bias in human and mouse livers. Only genes with q<0.05 and normalized expression > 10^{-4} in both species are used. Selected gene names are shown. **(d)** Spearman correlation (two-sided) of nuclear/cytoplasmic bias in human colon and liver. Only genes with q<0.05 and normalized expression > 10^{-4} in both organs are used. Selected gene names are shown. **(e)** Mouse intestine Xenium[3] DAPI image overlayed with 3×3µm bins colored by nuclear/cytoplasmic assignment, and with nuclear segmentation. **(f)** MA-plot of differential gene expression results in mouse intestine (Xenium). Significant genes are colored cyan and orange (q<0.1). Selected gene names are shown. q-values are computed by applying the Benjamini–Hochberg correction to p-values from one-sided Fisher's exact tests.

References

1. Wolf, F. A., Angerer, P. & Theis, F. J. SCANPY: large-scale single-cell gene expression data analysis. *Genome Biol* **19**, 15 (2018).
2. Palla, G. *et al.* Squidpy: a scalable framework for spatial omics analysis. *Nat Methods* **19**, 171–178 (2022).
3. Ameku, T. *et al.* Growth of the maternal intestine during reproduction. *Cell* **188**, 2738-2756.e22 (2025).
4. Qiu, X. *et al.* Spatiotemporal modeling of molecular holograms. *Cell* **187**, 7351-7373.e61 (2024).