

SUPPLEMENTARY INFORMATION

ColonyQuant: Automated quantification and morphometric analysis of pluripotent stem cell colonies

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Running title: Automated ESC Colony Analysis.

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Supplemental Figures

Figure S1. Overview of the ColonyQuant workflow and module integration

Schematic overview of the end-to-end ColonyQuant pipeline, illustrating how raw bright-field images are transformed into quantitative colony phenotypes. The workflow proceeds through five integrated stages: (i) image preprocessing and colony detection, including background correction, adaptive thresholding, and contour extraction; (ii) AP intensity quantification to compute per-colony mean and total staining metrics; (iii) morphometric feature extraction, generating area, perimeter, solidity, circularity, eccentricity, aspect ratio, extent, and equivalent diameter; (iv) visualization and distributional profiling across images and treatment groups; and (v) statistical classification and multivariate analysis to identify discriminative features and stratify conditions. Solid arrows indicate data flow between modules, and boxed elements denote intermediate and final outputs.

Figure S2. Comparative PR benchmarking of ColonyQuant and Cellpose.

ColonyQuant precision-recall (PR) curve overlaid with all Cellpose PR curves generated from a parameter sweep. Cellpose was run across a grid of permissive settings, e.g., flow_threshold 0-2.0, cellprob_threshold, 1.0 to 0.1, tile_norm_blocksize 0/100/200/300, diameter 0/60/90/120 (0 = auto), min_size 0/16/32. For each resulting variant, a PR curve was obtained by sweeping the probability threshold on its probability map; curves are lightly alpha-blended to show the spread. CQ remains along or above most Cellpose traces at high precision.

Figure S3. Additional morphometric feature distributions of ESC colonies

Supplementary morphometric descriptors quantified by ColonyQuant that capture complementary aspects of ESC colony geometry and size. **(A)** Extent (mask area / bounding-box area) indicating how efficiently colonies fill their enclosing bounding boxes. **(B)** Eccentricity (focal-distance / major-axis length) distinguishing circular from elongated colony shapes. **(C)** Aspect ratio (major-axis length / minor-axis length) further characterizing elongation variability. **(D)** Equivalent diameter (diameter of a circle with the same area as the colony mask) providing a scale-independent size metric. **(E)** Perimeter length distribution capturing variation in colony edge length and boundary complexity.

Figure S4. Supervised UMAP visualization of colony phenotypic space across treatments.

(A) Supervised UMAP embedding (initialized from PCA, with supervision weight $w = 0$) projects the leading principal components into two dimensions under categorical guidance, yielding compact clusters that reflect treatment-specific organization in colony phenotypic space.

Figure S5. Segmentation of X-gal–stained ESC colonies using ColonyQuant

Example output from ColonyQuant applied to X-gal (β -galactosidase)-stained ESC colonies. **(A)** X-gal-stained images showing dark chromogenic precipitates associated with β -galactosidase activity. **(B)** Segmentation boundary overlays generated by ColonyQuant. **(C)** Binary segmentation masks for detected colonies.

ColonyQuant pipeline overview

Detection, quantification, profiling, and classification from input images to final reports

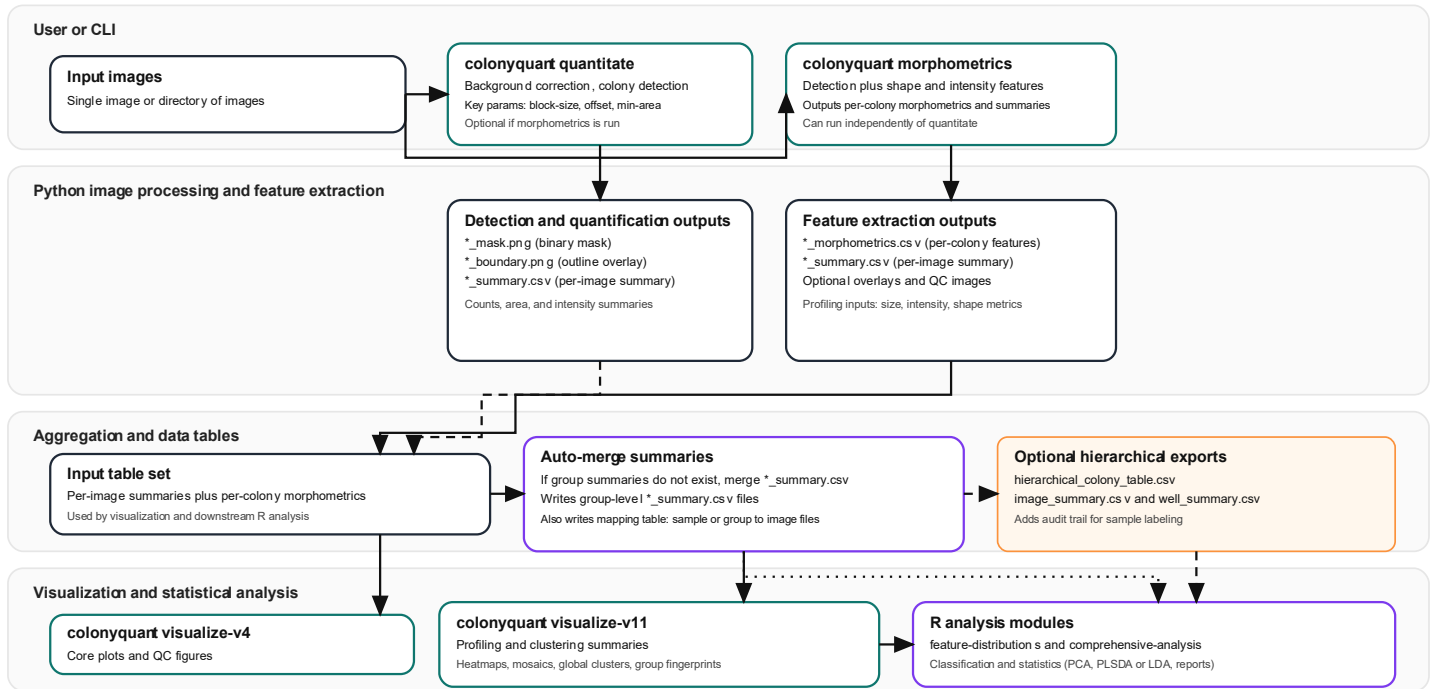


Figure S1

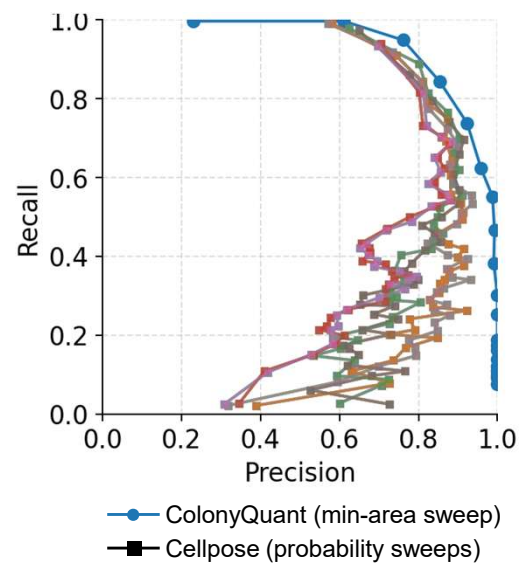


Figure S2

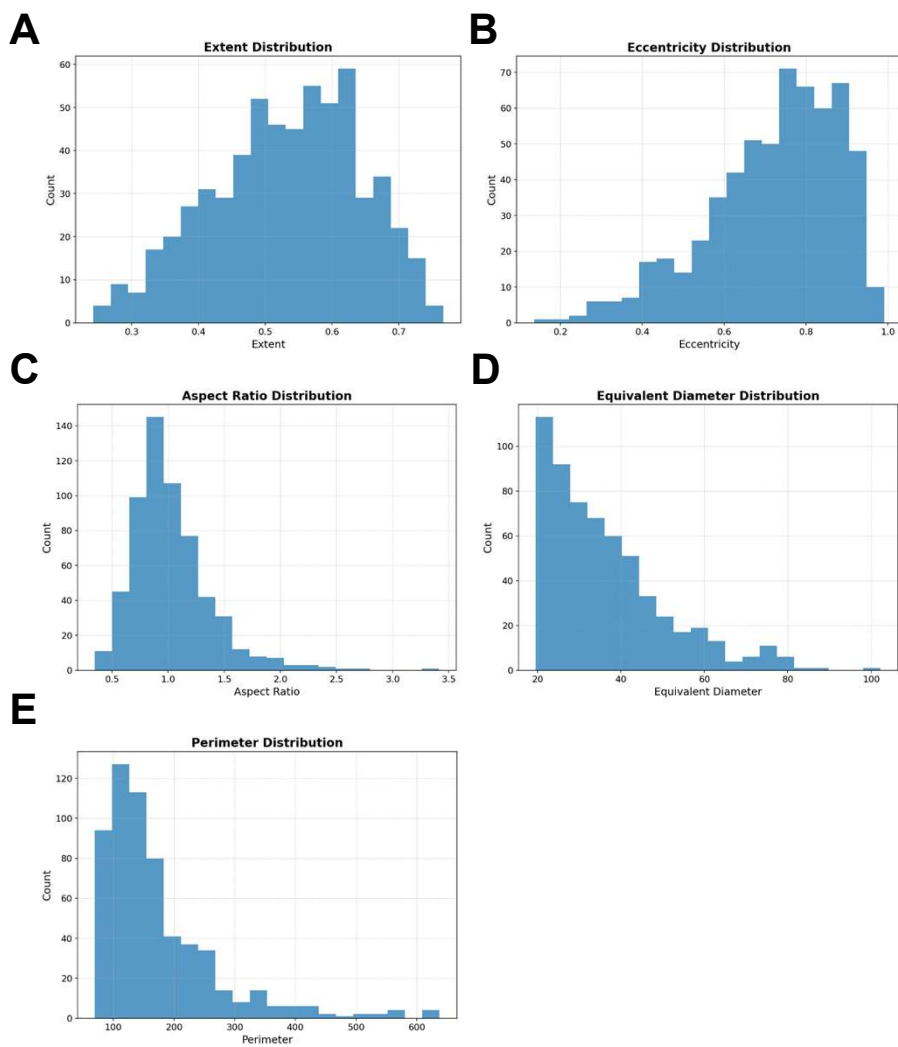


Figure S3

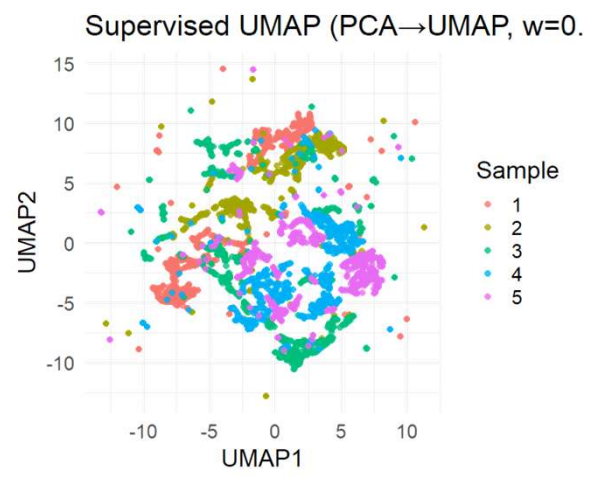


Figure S4

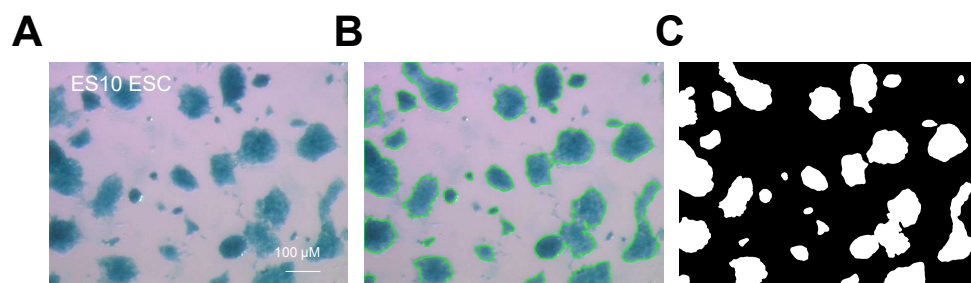


Figure S5