

Supplementary Information for

**A quantitative 3D Subcellular Map of Human Retinal Pigment Epithelium Discovers
Non-stochastic Cell State Transition in Establishment of Apical/Basal Polarity**

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Supplementary text

Human in the loop parameter selection

Each organelle or subcellular structure was segmented using a combination of model-based algorithms (**Table S6**). The parameters used by the algorithms were selected using a human-in-the-loop approach. To optimize the selection of parameters used by the algorithm sequence, we determined an initial set of pixel values that corresponded to the measured dimensions of each organelle and structure. These values were converted to pixels based on the pixel/micron scale. We calculated a 50 % deviation on the upper and lower side of this set of values and used this range to carry out a first round of segmentation for each organelle. Segmentation was subsequently visually inspected by two experts, who selected the parameters that produced the best quality of segmentation. The range of parameters was then reduced by 50 % starting from the newly selected values, and a new round of segmentation was performed. A total of three rounds of segmentation with a human in the loop were carried out (**Figure S10**). For example, if the initial parameter was 1, the range of parameters for the first iteration will be 0.5, 1, 1.5. If 1.5 produced the best segmentation quality, we chose this value $\pm 25\%$ (1.125, 1.5, 1.875) for the second iteration and so on. A range of cutoff values was also included in the parameter selection. These cutoff values are related to the distribution of intensities along cross sections, not to the size or shape of the objects of interest. We started from standard values for the selection process. For DSP and GJA1, a fourth round of parameter selection was carried out as the quality of the segmentation could still be improved. We were not successful in finding an algorithm that allowed us to close the segmentation of

LMNB1. In this case, we decided to stop the parameter selection at the first round and not to run the shape metric analysis for this structure.

Data type and requirements:

All stacks were in the OME-TIFF format, which is an open-source file format standard.

Segmentation algorithms for each organelle

TJP1 segmentation technique

Preprocessing

We performed auto contrast intensity normalization, which uses two scaling parameters for modifying the number of standard deviations on either side of the mean intensity. The algorithm uses 99.99% of maximum intensity as saturation intensity. Edge preserving smoothing (anisotropic diffusion filter) was performed to enhance structure edges.

Segmentation

For core segmentation, 2D Frangi vesselness was calculated for each individual frame in the stack. When compared to 3D Frangi vesselness this was found to be significantly better. Combining 2D with 3D was not significantly different from 2D vesselness, so the 2D approach was selected. Due to resolution and limitations of Frangi vesselness in intersecting filaments, some fragmentation of the segmentation is visible.

Postprocessing

No objects were removed in this phase since doing that could remove a large number of TJP1 true positive filament fragments. These fragments occurred because the filaments

are extremely thin and are not uniformly bright. As a result, parts of the filaments were not segmented.

CTNNB1 segmentation technique

Preprocessing

We performed auto contrast intensity normalization, which uses two scaling parameters for modifying the number of standard deviations on either side of the mean intensity. The algorithm uses 99.99% of maximum intensity as saturation intensity. A 3D gaussian smoothing was performed to reduce noise in the stack.

Segmentation

For core segmentation, 2D Frangi vesselness was calculated for each individual frame in the stack. When compared to 3D Frangi vesselness this was found to be significantly better. Combining 2D with 3D was not significantly different from 2D vesselness. Due to resolution and limitations of Frangi vesselness in intersecting filaments, some fragmentation of the segmentation is visible. Additionally, a 2D spot filter was used as CTNNB1 signal had some spot-like structures. To reduce the number of steps involved, both sets of parameters (vesselness and spot) were kept the same due to their similar scales. The spot filter uses a Laplacian of Gaussian (LoG), edge detection filter.

Postprocessing

Small objects were not removed to prevent the exclusion of real segmentation.

DSP segmentation technique

Preprocessing

We performed auto contrast intensity normalization, which uses two scaling parameters for modifying the number of standard deviations on either side of the mean intensity. The algorithm uses 99.99% of maximum intensity as saturation intensity. 2D slice-by-slice gaussian smoothing was performed to reduce noise in the stack.

Segmentation

A 3D LoG-based spot filter was used to perform the core segmentation. We used two sets of parameters to recognize small or large objects, and the two segmentations were combined. 3D watershed was used for separating connected objects.

Postprocessing

Further postprocessing was unnecessary as the segmentations were of acceptable quality.

GJA1 segmentation technique

Preprocessing

We performed auto contrast intensity normalization, which uses two scaling parameters for standard deviation on either side of the mean intensity. The algorithm uses 99.99% of maximum intensity as saturation intensity. 2D slice-by-slice gaussian smoothing was performed to reduce noise in the stack.

Segmentation

A 3D LoG-based spot filter was used to perform the core segmentation. We used two sets of parameters to recognize small or large objects, and the two segmentations were combined.

120 Postprocessing

121 Further postprocessing was unnecessary as the segmentations were of acceptable
122 quality.

123

124 **CETN2 segmentation technique**

125 Preprocessing

126 We performed auto contrast intensity normalization, which uses two scaling parameters
127 for modifying the number of standard deviations on either side of the mean intensity. The
128 algorithm uses 99.99% of maximum intensity as saturation intensity. A 2D slice-by-slice
129 gaussian smoothing was performed to reduce noise in the stack.

130 Segmentation

131 For core segmentation, a 3D LoG based spot filter was used to segment spot-like
132 structures. 3D watershed was used to separate fused objects.

133 Postprocessing

134 Based on pixel connectivity, objects equal to or smaller than 2 pixels were removed. This
135 value was chosen to remove a large number of false positive segmented objects.

136

137 **SEC61B segmentation technique**

138 Preprocessing

139 We performed auto contrast intensity normalization, which uses two scaling parameters
140 for modifying the number of standard deviations on either side of the mean intensity. The
141 algorithm uses 99.99% of maximum intensity as saturation intensity. Edge preserving
142 smoothing (anisotropic diffusion filter) was performed to enhance edge-like structures.

143 Segmentation

144 2D Frangi vesselness was calculated for each individual frame in the stack.

145 Postprocessing

146 Based on pixel connectivity, objects equal to or smaller than 4 pixels are removed. Note
147 that 5 pixels mean 5 connected voxels in 3D. This cutoff helped with removing large
148 number of false positive segmented objects.

149

150 **ST6GAL1 segmentation technique**

151 Preprocessing

152 We performed auto contrast intensity normalization, which uses two scaling parameters
153 for modifying the number of standard deviations on either side of the mean intensity. The
154 algorithm uses 99.99% of maximum intensity as saturation intensity. A 3D gaussian
155 smoothing was performed to reduce noise in the stack.

156 Segmentation

157 Segmentation was done using a 3D LoG filter. We experimented with combinations of
158 masked object thresholding and topologically preserving thinning prior to the LoG filter
159 but found they didn't improve the segmentation.

160 Postprocessing

161 Further postprocessing was unnecessary as the segmentations were of acceptable
162 quality.

163

164 **TOM20 segmentation technique**

165 Preprocessing

166 We performed auto contrast intensity normalization, which uses two scaling parameters
167 for modifying the number of standard deviations on either side of the mean intensity. The
168 algorithm uses 99.99% of maximum intensity as saturation intensity. A 3D gaussian
169 smoothing was performed to reduce noise in the stack.

170 Segmentation:

171 2D Frangi Vesselness using selected parameters was conducted on the normalized and
172 smoothed images for each slice of the stack.

173 Postprocessing:

174 Based on pixel connectivity, objects equal to or smaller than 2 pixels were removed. This
175 value was chosen to remove a large number of false positive segmented objects.

176

177 **LAMP1 segmentation technique**

178 Preprocessing

179 We performed auto contrast intensity normalization, which uses two scaling parameters
180 for modifying the number of standard deviations on either side of the mean intensity. The
181 algorithm uses 99.99% of maximum intensity as saturation intensity. 2D slice-by-slice
182 gaussian smoothing was performed to reduce noise in the stack.

183 Segmentation

184 A 2D LoG Filter was used on each frame to obtain segmentation.

185 Postprocessing

186 Based on pixel connectivity, objects equal to or smaller than 3 pixels are removed. Note
187 that 3 pixels means 3 connected voxels in 3D. This cutoff helped with removing a large

188 number of false positive segmented objects. Inversely, connected toroidal areas with
189 holes in the segmentation were filled.

190

191 **LC3B segmentation technique**

192 Preprocessing

193 We performed auto contrast intensity normalization, which uses two scaling parameters
194 for modifying the number of standard deviations on either side of the mean intensity. The
195 algorithm uses 99.99% of maximum intensity as saturation intensity. 2D slice-by-slice
196 gaussian smoothing was performed to reduce noise in the stack.

197 Segmentation

198 A 3D LoG Filter was used to obtain particle segmentation. Subsequently, 3D Watershed
199 was performed to separate connected objects into individual particles.

200 Postprocessing

201 Based on pixel connectivity, objects equal to or smaller than 4 pixels are removed. Note
202 that 4 pixels mean 4 connected voxels in 3D. This cutoff helped with removing a large
203 number of false positive segmented objects.

204

205 **RAB5 segmentation technique**

206 Preprocessing

207 We performed auto contrast intensity normalization, which uses two scaling parameters
208 for modifying the number of standard deviations on either side of the mean intensity. The
209 algorithm uses 99.99% of maximum intensity as saturation intensity. 2D slice-by-slice
210 gaussian smoothing was performed to reduce noise in the stack.

211 Segmentation

212 A 2D LoG filter was used to obtain segmentation of RAB5 particles.

213 Postprocessing

214 Based on pixel connectivity, objects equal to or smaller than 4 pixels are removed. Note
215 that 4 pixels mean 4 connected voxels in 3D. This cutoff helped with removing a large
216 number of false positive segmented objects. Additionally, any enclosed empty areas were
217 filled slice-by-slice.

218

219 **SLC25A17 segmentation technique**

220 Preprocessing

221 We performed auto contrast intensity normalization, which uses two scaling parameters
222 for modifying the number of standard deviations on either side of the mean intensity. The
223 algorithm uses 99.99% of maximum intensity as saturation intensity. A 2D slice-by-slice
224 gaussian smoothing was performed to reduce noise in the stack.

225 Segmentation

226 A 3D LoG Filter was used to obtain object segmentation. Subsequently, connected
227 objects were separated using 3D watershed.

228 Postprocessing

229 Further postprocessing was unnecessary as the segmentations were of acceptable
230 quality.

231

232 **ACTB segmentation technique**

233 Preprocessing

234 We performed auto contrast intensity normalization, which uses two scaling parameters
235 for modifying the number of standard deviations on either side of the mean intensity. The
236 algorithm uses 99.99% of maximum intensity as saturation intensity. Edge preserving
237 smoothing (anisotropic diffusion filter) was performed to enhance edge-like structures.

238 Segmentation

239 2D Frangi vesselness was calculated for each individual frame in the stack.

240 Postprocessing

241 No objects were removed in this phase since that may remove a large number of actin
242 filaments. These fragments occurred because the filaments are extremely thin and are
243 not uniformly bright. As a result, parts of the filaments were not segmented.

244

245 **MYH10 segmentation technique**

246 Preprocessing

247 We performed auto contrast intensity normalization, which uses two scaling parameters
248 for modifying the number of standard deviations on either side of the mean intensity. The
249 algorithm uses 99.99% of maximum intensity as saturation intensity. Edge preserving
250 smoothing (anisotropic diffusion filter) was performed to enhance edge-like structures.

251 Segmentation

252 2D Frangi vesselness was calculated for each individual frame in the stack.

253 Postprocessing

254 No objects were removed in this phase since that may remove a large number of myosin
255 filaments. These fragments occurred because the filaments are extremely thin and are
256 not uniformly bright. As a result, parts of the filaments were not segmented.

257

258 **FBL segmentation technique**

259 Preprocessing

260 We performed auto contrast intensity normalization, which uses two scaling parameters
261 for modifying the number of standard deviations on either side of the mean intensity. The
262 algorithm uses 99.99% of maximum intensity as saturation intensity. A 2D slice-by-slice
263 gaussian smoothing was performed to reduce noise in the stack.

264 Segmentation

265 Following the preprocessing, a 2D LoG filter was used to obtain particle segmentation.

266 Postprocessing

267 Based on pixel connectivity, objects equal to or smaller than 5 pixels are removed. Note
268 that 5 pixels mean 5 connected voxels in 3D. This cutoff helped with removing a large
269 number of false positive segmented objects.

270

271 **LMNB1 segmentation technique**

272 Preprocessing

273 We performed auto contrast intensity normalization, which uses two scaling parameters
274 for modifying the number of standard deviations on either side of the mean intensity. The
275 algorithm uses 99.99% of maximum intensity as saturation intensity. A 3D Gaussian
276 smoothing was performed to reduce noise in the stack.

277 Segmentation

278 The middle frame of each stack was obtained from the intensity distribution using an Otsu
279 threshold. A 2D Frangi Vesselness filter was then used to obtain nuclei outlines in the

280 middle frame. The middle frame is subsequently filled using hole-filling. Logical XOR was
281 used on the frame obtained from Frangi vesselness and the one from filling holes. The
282 resultant array is used to generate a 3D seed (as described on the Allen Cell and
283 Structure Segmenter, <https://github.com/AllenCell/aics-segmentation>). Watershed was
284 done to obtain each region inside the object. Logical XOR was then conducted on this
285 segmentation and the same segmentation after a pixel dilation to obtain the object
286 boundary.

287 Postprocessing

288 No postprocessing was performed.

289 **Note:** Because many objects were not segmented and because we were not able to close
290 the segmentation of the nuclear envelope, we did not use this signal for calculating shape
291 metrics.

292

293

Supplementary figures

Figure S1

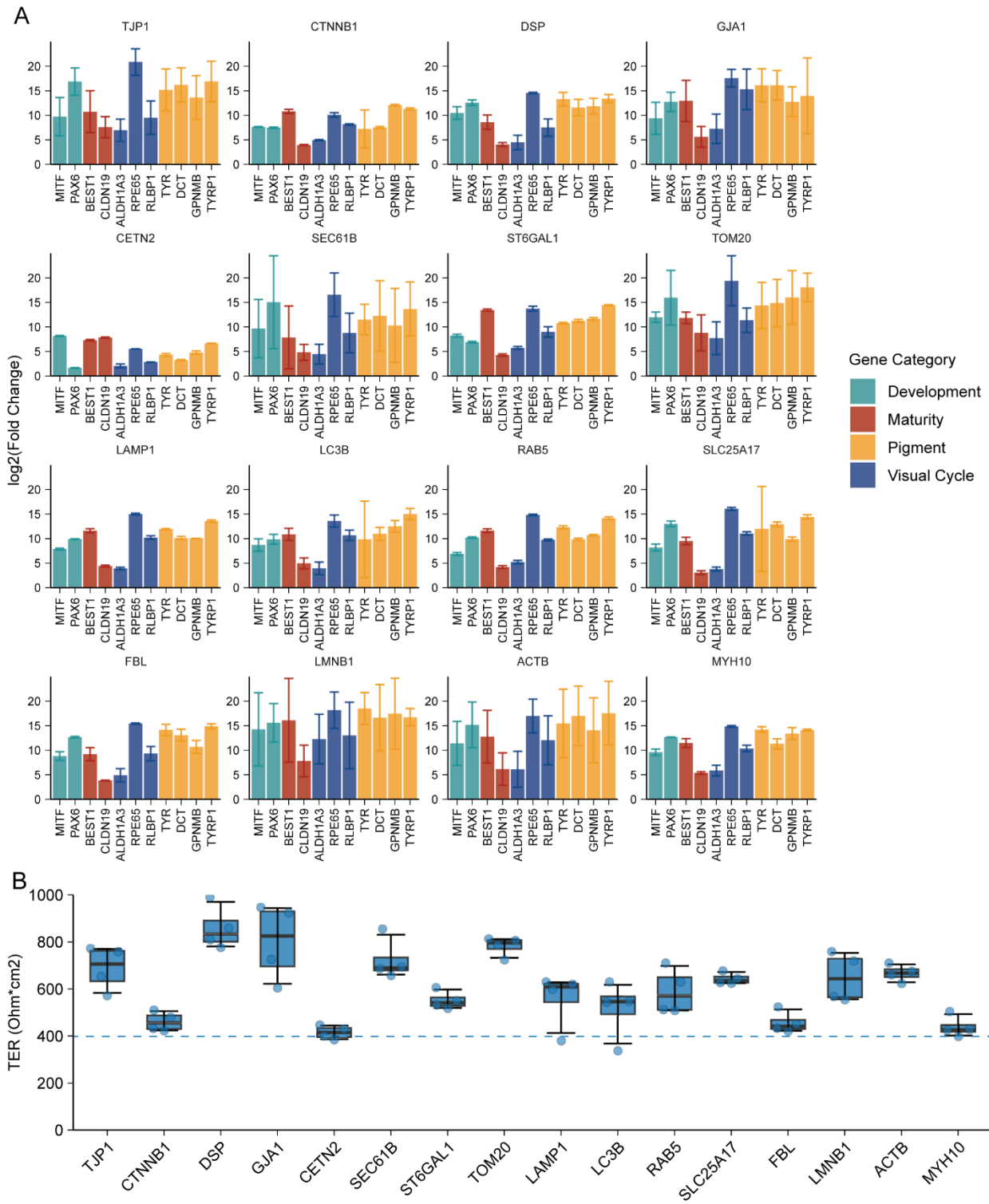


Figure S1: Validation of fully mature iPSC-RPE lines. (A) Differential expression of RPE signature genes in fully mature iPSC-RPE cells as compared to iPSC for each of the 16 tagged lines. (B) Boxplot showing the trans-epithelial electrical resistance of fully mature iPSC-RPE for each line. All lines have a TER of several hundred Ohms*cm², showing the good quality of the differentiations. The dashed line at 400 Ohms*cm² represents the quality control cutoff that was used for this study.

Figure S2

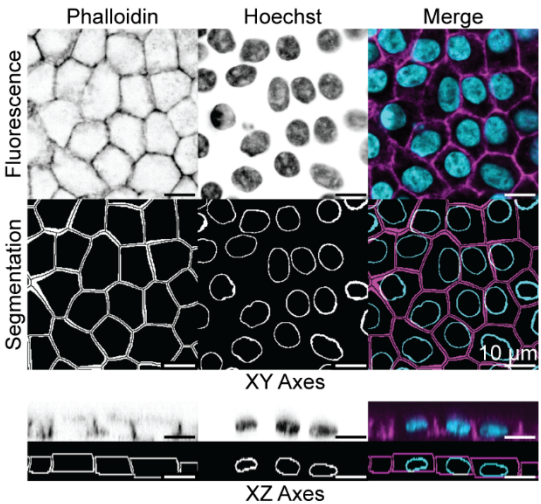


Figure S2: Representative images of phalloidin and Hoechst staining with their segmentation. Two panels showing W4 PGE2-treated iPSC-RPE cells stained with phalloidin and Hoechst and their respective AI-generated segmentations. Images in the top panel were obtained from a representative X/Y plane of an image stack, while images in the bottom panel show an XZ plane (magenta = phalloidin, cyan = Hoechst; scale bar = 10 μ m).

Figure S3

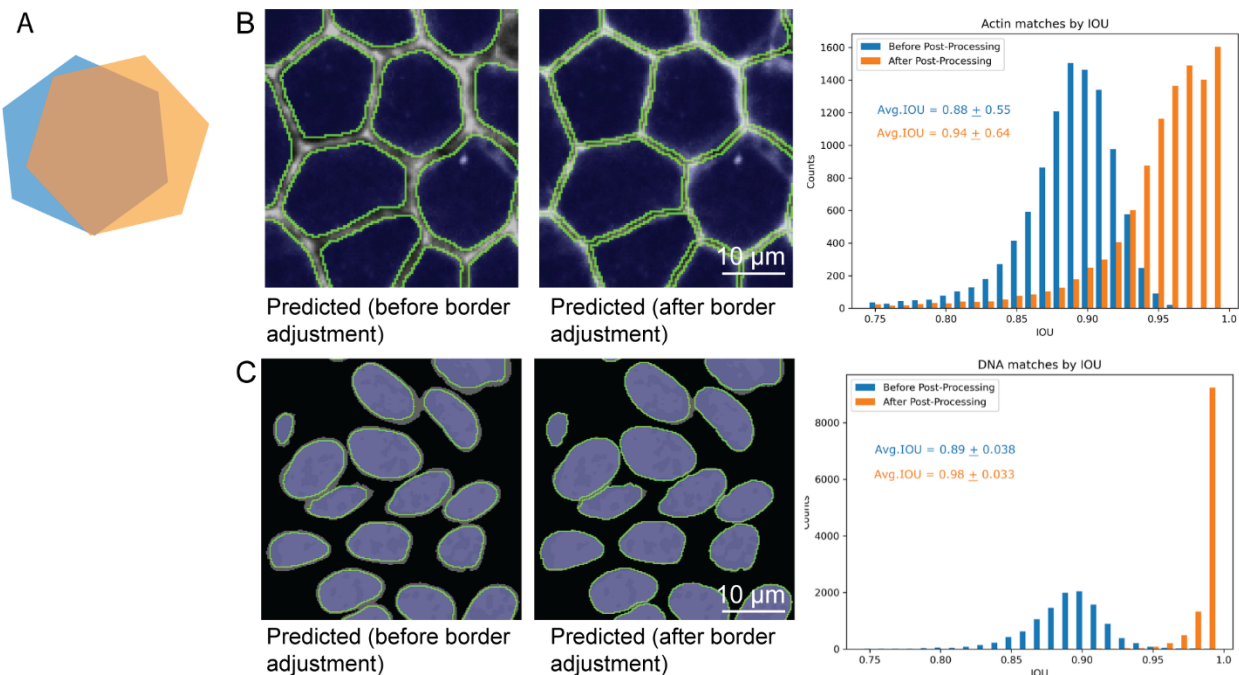
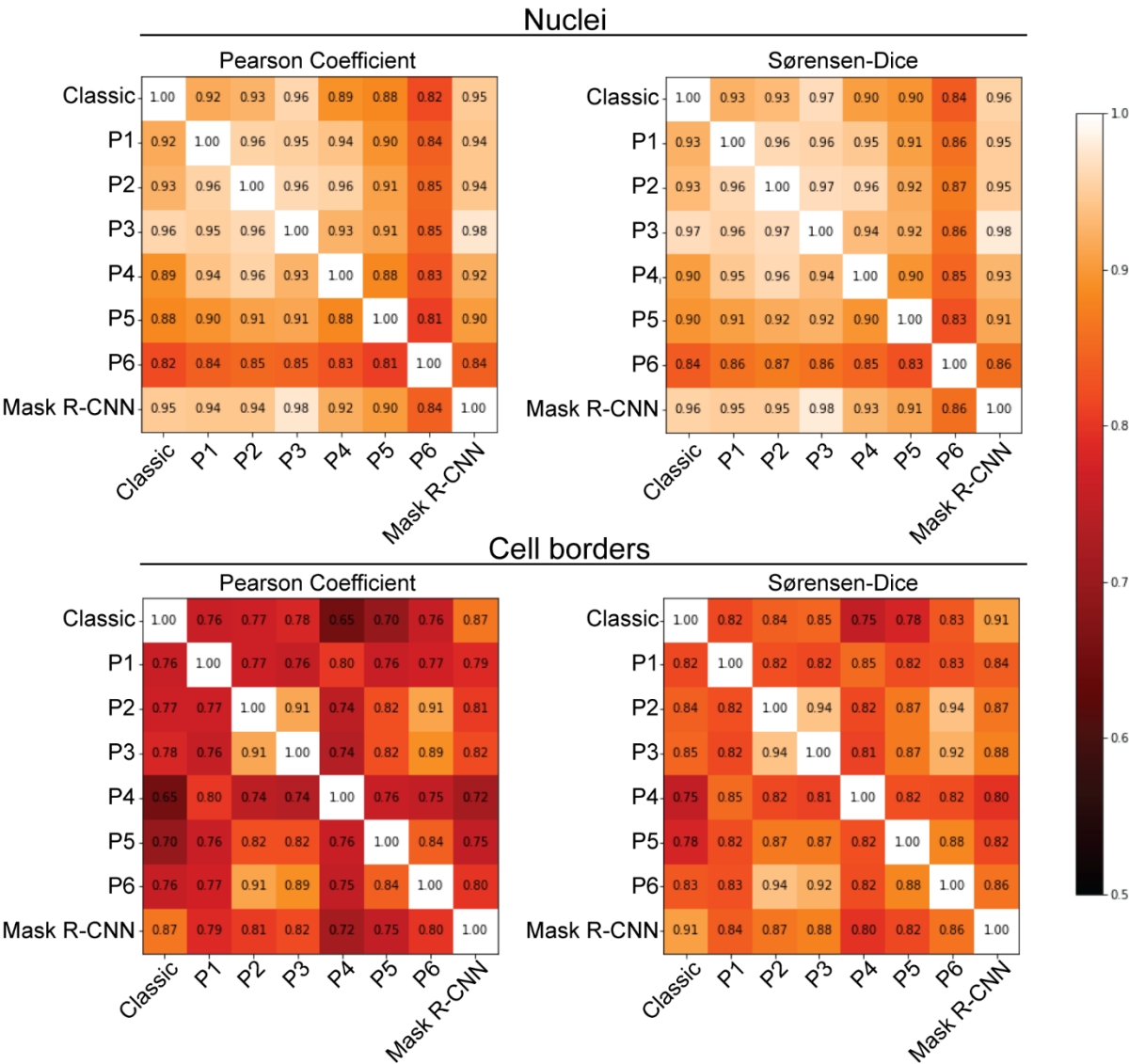


Figure S3: 3D assembly. (A) An IoU score was calculated to find the best match between 3D objects across different Z planes. If the score was above a threshold of 0.6, the objects were combined. (B) Representative images of cell border segmentation (blue masks with green outlines) from actin staining before and after dilation. Postprocessing dilation improved the IoU score, as shown in the bar graph. (C) Representative images of nuclei border segmentation (blue masks with green outlines) from Hoechst staining before and after dilation. Postprocessing dilation improved the IoU score, as shown in the bar graph. (Data from one stack at week 1 and one at week 2 that were not used for training).

325 **Figure S4**



326

327 **Figure S4: Pearson correlation and Sorensen-Dice coefficient.** The four heatmaps

328 show the degree of variation resulting from the generation of ground truth data by six

329 different operators for nuclei (top) and cell borders (bottom). The segmentation produced

330 by classic algorithms and Mask R-CNN was also included as reference. (1.0 = high

331 degree of correlation, 0.0 = low degree of correlation).

332

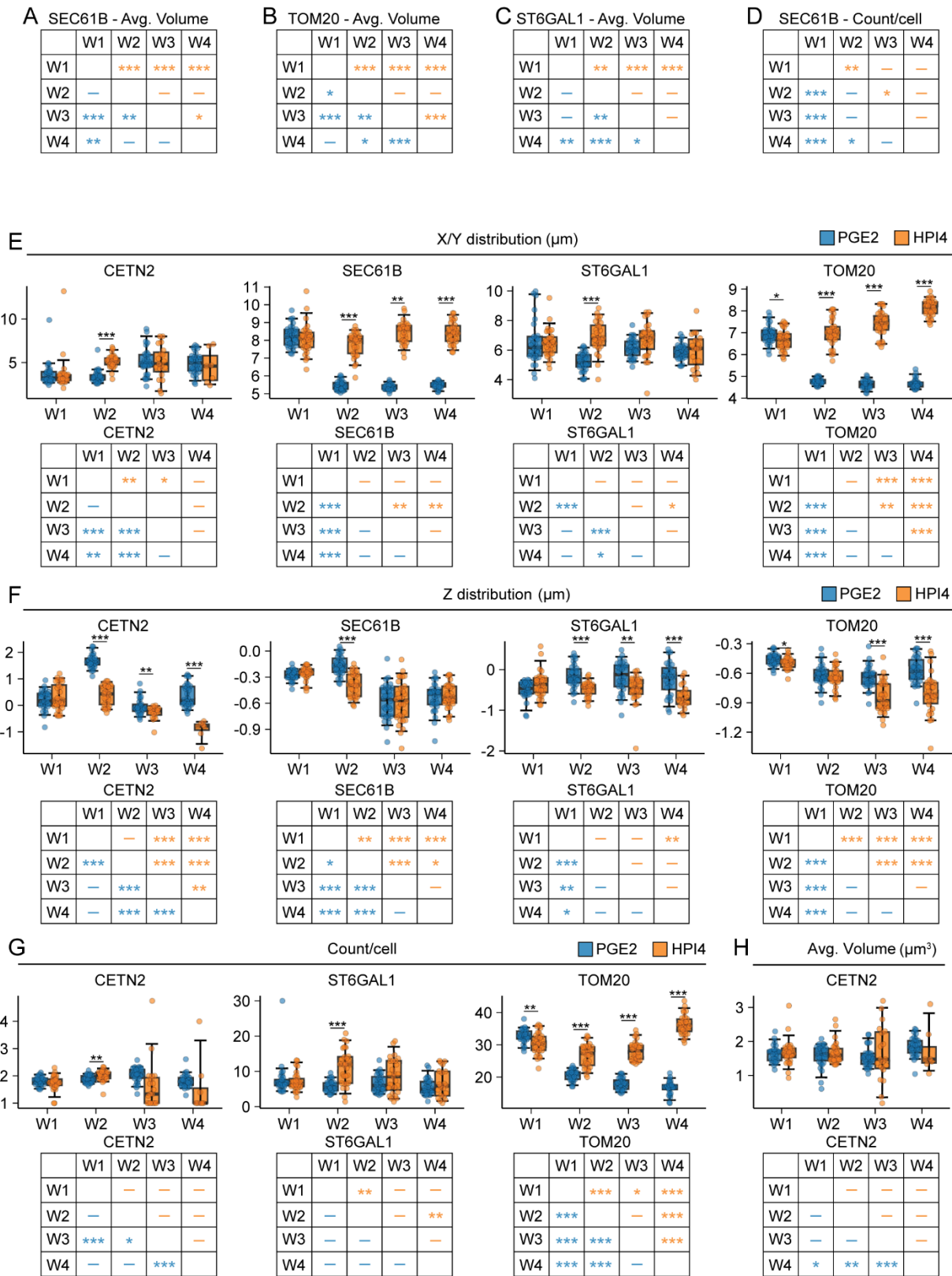


Figure S5: Shape metrics and multiple comparisons for centrioles and large organelles. (A-D) Results of Tukey's HSD pairwise comparison related to the graphs of **Figure 6D-G**. (N = 30; ***P < 0.005, **P < 0.01, *P < 0.05). (E) Boxplots showing the changes in organelle location on the X/Y plane. Distances from organelles and cell centroids were used. The tables indicate Tukey's HSD pairwise comparison. (F) Boxplots showing the changes in organelle location on the Z axis. Distances from organelles and cell centroids were used. The tables indicate Tukey's HSD pairwise comparison. (G) Boxplots showing the number of objects per cell and tables indicating Tukey's HSD pairwise comparison. (H) Boxplot showing centrioles average volume and its relative table of Tukey's HSD pairwise comparison. (Box limits represent the first and third quartile, the central line shows the median and the whiskers indicate the 5th and 95th percentile. N = 30; ***P < 0.005, **P < 0.01, *P < 0.05).

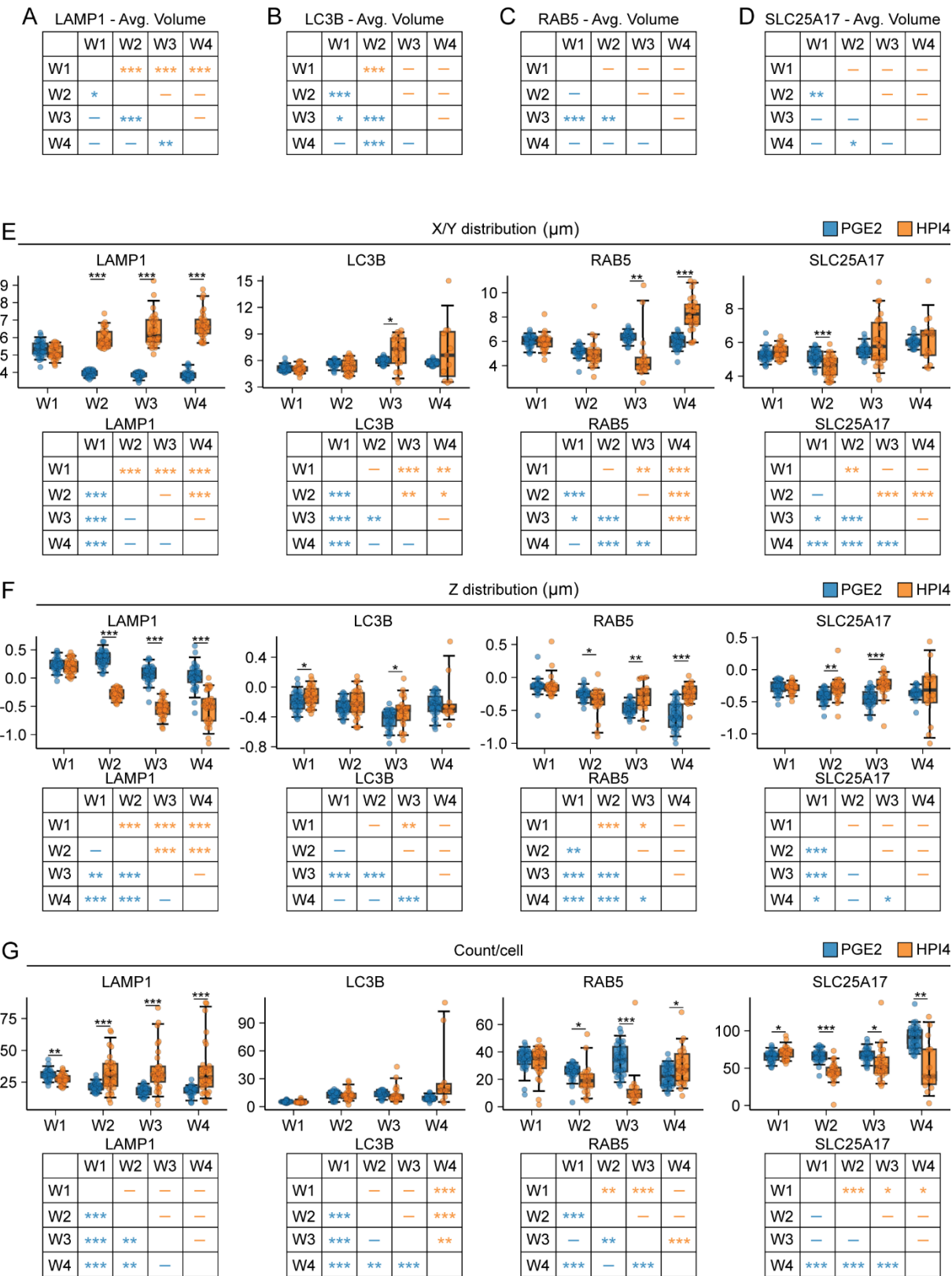


Figure S6: Shape metrics and multiple comparisons for vesicular organelles. (A-D)

Results of Tukey's HSD pairwise comparison related to the graphs of **Figure 7D-G**. (N = 30; ***P < 0.005, **P < 0.01, *P < 0.05). (E) Boxplots showing the changes in organelle location on the X/Y plane. Distances from organelles and cell centroids were used. The tables indicate Tukey's HSD pairwise comparison. (F) Boxplots showing the changes in organelle location on the Z axis. Distances from organelles and cell centroids were used. The tables indicate Tukey's HSD pairwise comparison. (G) Boxplots showing the number of objects per cell and tables indicating Tukey's HSD pairwise comparison. (Box limits represent the first and third quartile, the central line shows the median and the whiskers indicate the 5th and 95th percentile. N = 30; ***P < 0.005, **P < 0.01, *P < 0.05).

361 **Figure S7**

Cell				
Cross-Sectional Area				
	W1	W2	W3	W4
W1		—	***	***
W2	***		***	***
W3	***	***		***
W4	***	***	*	

Aspect Ratio				
	W1	W2	W3	W4
W1		—	***	***
W2	***		***	***
W3	***	**		***
W4	***	***	—	

Volume				
	W1	W2	W3	W4
W1		**	*	***
W2	***		***	***
W3	***	***		***
W4	***	***	—	

Nucleus				
Cross-Sectional Area				
	W1	W2	W3	W4
W1		***	***	*
W2	***		***	***
W3	***	—		—
W4	***	***	***	

Aspect Ratio				
	W1	W2	W3	W4
W1		—	***	***
W2	***		***	***
W3	***	—		—
W4	***	—	—	

Volume				
	W1	W2	W3	W4
W1		***	***	***
W2	***		***	***
W3	***	***		—
W4	***	***	**	

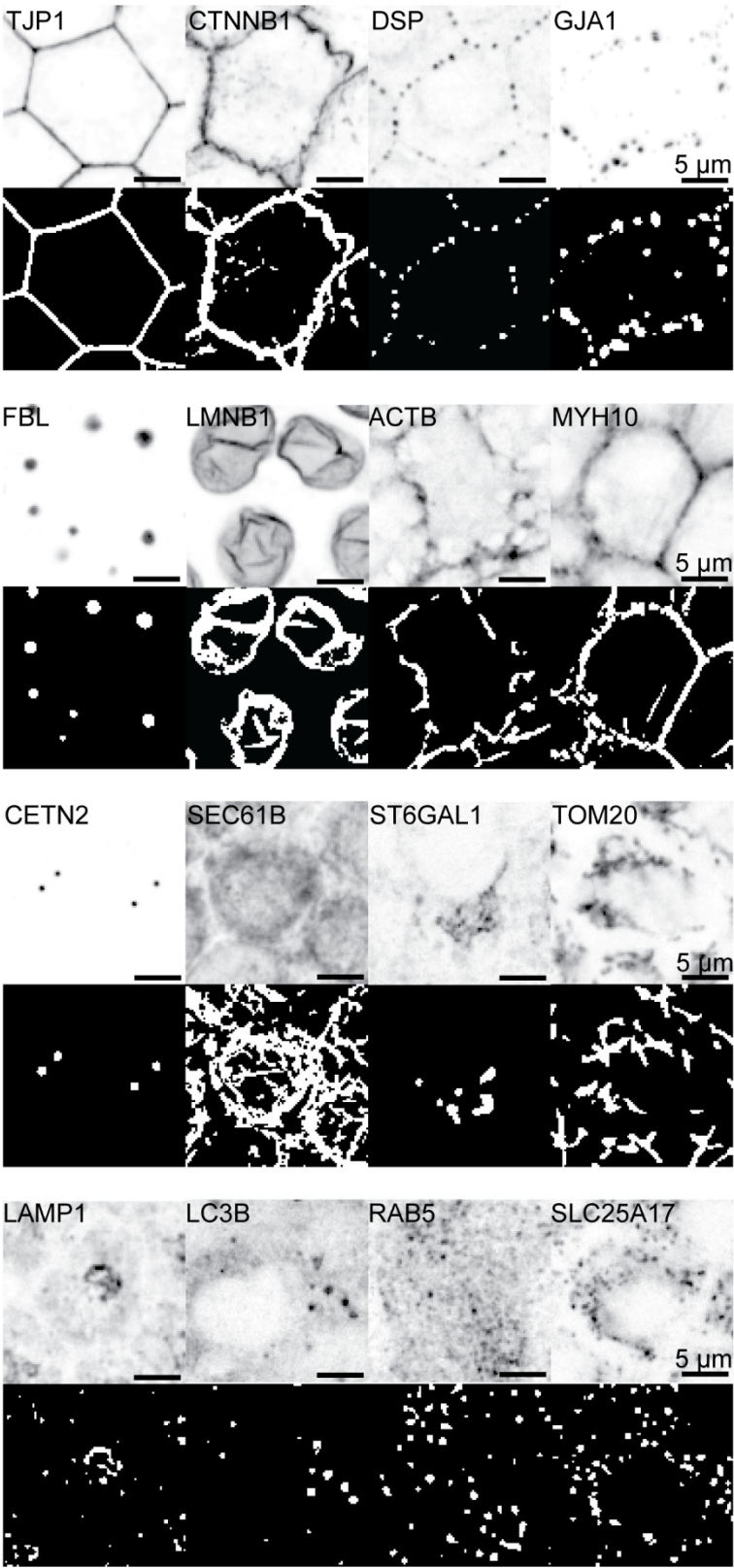
362

363 **Figure S7: Tukey’s HSD test for cell and nucleus metrics.** Results of Tukey’s HSD

364 pairwise comparison for cells and nuclei for three shape metrics: cross-sectional area,

365 aspect ratio, and volume (N = 30; ***P < 0.005, **P < 0.01, *P < 0.05).

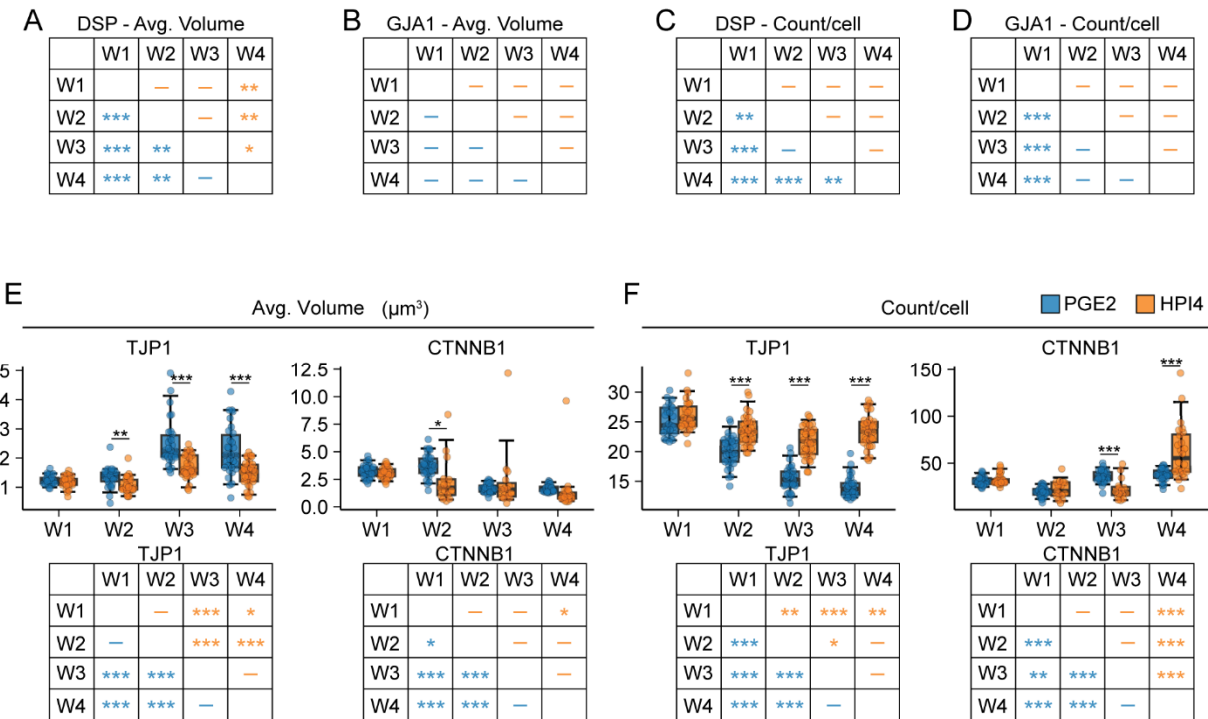
366



369 **Figure S8: Representative fluorescence images of intracellular structures and their**
370 **corresponding segmentation.** Panel showing single X/Y planes of the proteins of
371 interest and their corresponding binary segmentation (scale bars = 5 μm).

372

373 **Figure S9**



374

375 **Figure S9: Shape metrics and multiple comparisons for cell junctions.** (A-D) Results

376 of Tukey's HSD pairwise comparison related to the graphs of **Figure 5D-G**. (N = 30; ***P

377 < 0.005, **P < 0.01, *P < 0.05). (E) Boxplots indicating the changes over time and between

378 treatments of TJP1 and CTNNB1 volume and tables showing the results of Tukey's HSD

379 pairwise comparison. (F) Boxplots indicating the changes over time and between

380 treatments of TJP1 and CTNNB1 count/cell and tables showing the results of Tukey's

381 HSD pairwise comparison. (Box limits represent the first and third quartile, the central line

382 shows the median and the whiskers indicate the 5th and 95th percentile. N = 30; ***P <

383 0.005, **P < 0.01, *P < 0.05).

384

Figure S10

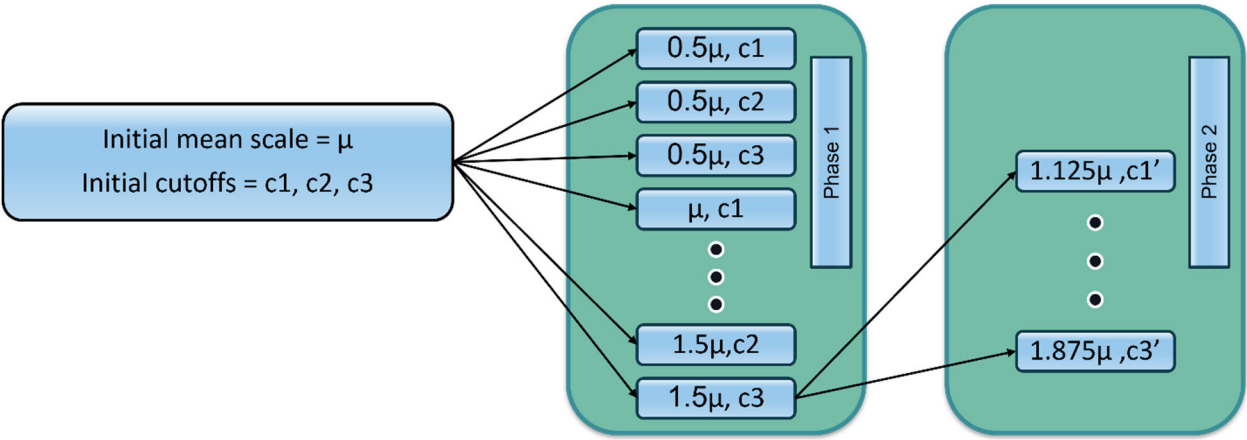


Figure S10: Parameter selection process for organelle segmentation. Schematic representation of the parameter selection process used for organelle segmentation with a human-in-the-loop approach.

391 **Supplementary tables**

392 **Table S1**

Catalog number	Clone	Gene	Gene symbol	Protein	Fluorophore	Tagged alleles	Abbreviation	Structure
AICS-0010	55	Sec61 translocon beta subunit	<i>SEC61B</i>	Sec61 beta	mEGFP	mono	SEC61B	Endoplasmic reticulum
AICS-0011	27	Translocase of outer mitochondrial membrane 20	<i>TOMM20</i>	Tom20	mEGFP	mono	TOM20	Mitochondria
AICS-0013	210	Lamin B1	<i>LMNB1</i>	Lamin B1	mEGFP	mono	LMNB1	Nuclear envelope
AICS-0014	6	Fibrillarin	<i>FBL</i>	Fibrillarin	mEGFP	mono	FBL	Nucleolus (Dense Fibrillar Component)
AICS-0016	184	Actin beta	<i>ACTB</i>	Beta-actin	mEGFP	mono	ACTB	Actin filaments
AICS-0017	65	Desmoplakin	<i>DSP</i>	Desmoplakin	mEGFP	mono	DSP	Desmosomes
AICS-0022	37	Lysosomal associated membrane protein mono 1	<i>LAMP1</i>	LAMP-1	mEGFP	mono	LAMP1	Lysosome
AICS-0023	20	Tight junction protein 1	<i>TJP1</i>	Tight junction protein ZO-1	mEGFP	mono	TJP1	Tight junctions
AICS-0024	80	Myosin heavy chain 10	<i>MYH10</i>	Non-muscle myosin heavy chain IIB	mEGFP	mono	MYH10	Actomyosin bundles
AICS-0025	44	ST6 beta-galactoside alpha-2,6-sialyltransferase 1	<i>ST6GAL1</i>	Sialyltransferase 1	mEGFP	bi	ST6GAL1	Golgi apparatus
AICS-0030	22	Microtubule-associated protein 1, light chain 3 beta	<i>LC3B</i>	Autophagy-related protein LC3 B	mEGFP	mono	LC3B	Autophagosomes
AICS-0032	19	Centrin 2	<i>CETN2</i>	Centrin-2	mTagRFP-T	mono	CETN2	Centrioles
AICS-0033	115	Solute carrier family 25 (mitochondrial carrier) member 17	<i>SLC25A17</i>	Peroxisomal membrane protein PMP34	mEGFP	mono	SLC25A17	Peroxisomes
AICS-0040	35	RAS-associated protein RAB5A	<i>RAB5</i>	Ras-related protein Rab-5A	mEGFP	bi	RAB5	Endosomes
AICS-0053	16	Gap junction protein alpha 1	<i>GJA1</i>	Connexin-43	mEGFP	mono	GJA1	Gap junctions
AICS-0058	67	Catenin beta 1	<i>CTNNB1</i>	Beta-catenin	mEGFP	mono	CTNNB1	Adherens junctions

393

394 **Table S1:** Complete list of the 16 cell lines used in this study.

395

396 **Table S2**

Channel		phase 0		phase 1		phase 2		phase 3		extra phase - for selected organelles	
		initial μ	σ	selected μ	selected σ	selected μ	selected σ	selected μ	selected σ	selected μ	selected σ
LMNB1	pixel	3		NA		NA		NA			
	parameter	1		NA	NA	NA	NA	NA	NA		
LAMP1	pixel	3.5		3.5		3.5		3.0625			
	parameter	1.166666667		1.166666667	0.05	1.166666667	0.07	1.020833333	0.05		
SEC61B	pixel	3		1.5		1.875		1.875			
	parameter	1		0.5	0.05	0.625	0.075	0.625	0.075		
TOM20	pixel	2.75		2.75		3.4375		3.4375			
	parameter	0.916666667		0.916666667	0.1	1.145833333	0.1	1.145833333	0.12		
ST6GAL1	pixel	3.75		5.625		5.625		5.625			
	parameter	1.25		1.875	0.1	1.875	0.075	1.875	0.0875		
FBL	pixel	7.5		7.5		7.5		6.5625			
	parameter	2.5		2.5	0.05	2.5	0.03	2.1875	0.05		
MYH10	pixel	5		2.5		1.875		1.640625			
	parameter	1.666666667		0.833333333	0.01	0.625	0.01	0.546875	0.01		
RAB5	pixel	3.25		4.875		6.09375		5.33203125			
	parameter	1.083333333		1.625	0.1	2.03125	0.05	1.77734375	0.07		
DSP	pixel	4.5		2.25		1.6875		1.6875		1.8984375	
	parameter	1.5		0.75	0.01	0.5625	0.01	0.5625	0.03	0.6328125	0.01
SLC25A1 7	pixel	2.75		2.75		2.75		2.75		2.40625	
	parameter	0.916666667		0.916666667	0.05	0.916666667	0.05	0.916666667	0.05	0.802083333	0.03
GJA1	pixel	8.5		4.25		4.25		4.78125		3.1875	
	parameter	2.833333333		1.416666667	0.05	1.416666667	0.03	1.59375	0.03	1.0625	0.01
CTNNB1	pixel	3.25		1.625		2.03125		1.77734375			
	parameter	1.083333333		0.541666667	0.05	0.677083333	0.05	0.592447917	0.05		
ACTB	pixel	5		2.5		2.5		2.1875			
	parameter	1.666666667		0.833333333	0.05	0.833333333	0.05	0.729166667	0.05		
CETN2	pixel	4.5		6.75		5.0625		5.0625			
	parameter	1.5		2.25	0.1	1.6875	0.1	1.6875	0.075		
LC3B	pixel	4.5		4.5		3.375		3.375			
	parameter	1.5		1.5	0.1	1.125	0.1	1.125	0.075		
TJP1	pixel	3		1.5		1.5		1.5			
	parameter	1		0.5	0.1	0.5	0.1	0.5	0.1		

398 **Table S2:** Summary of the parameters used in the selection process for the segmentation
399 of fluorophore-tagged organelles..

400

401 **Table S3**

Week	Treatment	mean	median	sd	Metric
W1	HPI4	246.6472	245.8339	11.34097	Cell Area
W1	PGE2	250.7003	249.3649	10.68075	Cell Area
W2	HPI4	244.4281	242.8142	16.34732	Cell Area
W2	PGE2	172.8955	172.357	7.178978	Cell Area
W3	HPI4	292.0758	295.4343	19.09591	Cell Area
W3	PGE2	191.6926	191.68	6.676774	Cell Area
W4	HPI4	319.6179	315.2461	25.19483	Cell Area
W4	PGE2	198.326	198.9845	7.699555	Cell Area
W1	HPI4	1.679921	1.681907	0.018349	Cell AR
W1	PGE2	1.685288	1.687944	0.025577	Cell AR
W2	HPI4	1.678297	1.682012	0.029985	Cell AR
W2	PGE2	1.567948	1.564439	0.024423	Cell AR
W3	HPI4	1.766743	1.768786	0.033366	Cell AR
W3	PGE2	1.59376	1.588844	0.027571	Cell AR
W4	HPI4	1.807365	1.805635	0.040745	Cell AR
W4	PGE2	1.602266	1.606304	0.032263	Cell AR
W1	HPI4	797.8425	795.0863	33.73205	Cell Volume
W1	PGE2	829.2331	831.0227	26.10755	Cell Volume
W2	HPI4	746.1454	749.1143	49.77023	Cell Volume
W2	PGE2	720.3617	718.8938	27.40783	Cell Volume
W3	HPI4	838.633	837.6534	56.09944	Cell Volume
W3	PGE2	776.6818	781.1513	29.12124	Cell Volume
W4	HPI4	925.0055	932.3818	76.98063	Cell Volume
W4	PGE2	792.0652	799.0138	38.17704	Cell Volume
W1	HPI4	4.526433	4.53479	0.186622	Cell Height
W1	PGE2	4.647321	4.621119	0.135903	Cell Height
W2	HPI4	3.99131	4.033761	0.1513	Cell Height
W2	PGE2	5.890957	5.891883	0.226267	Cell Height
W3	HPI4	3.570576	3.615438	0.206586	Cell Height

W3	PGE2	5.664749	5.653717	0.239069	Cell Height
W4	HPI4	3.510862	3.530555	0.165295	Cell Height
W4	PGE2	5.347095	5.354152	0.205777	Cell Height
W1	HPI4	19.00265	18.97801	0.439202	Cell Width
W1	PGE2	19.1883	19.24135	0.421734	Cell Width
W2	HPI4	18.85436	18.80384	0.651762	Cell Width
W2	PGE2	15.88731	15.87316	0.341789	Cell Width
W3	HPI4	20.72633	20.75613	0.692645	Cell Width
W3	PGE2	16.75183	16.72643	0.309419	Cell Width
W4	HPI4	21.78827	21.79297	0.830107	Cell Width
W4	PGE2	17.08859	17.10526	0.337246	Cell Width
W1	HPI4	101.5993	101.6037	3.238789	Nucleus Area
W1	PGE2	102.3619	102.2659	3.345728	Nucleus Area
W2	HPI4	92.18443	92.9629	4.199631	Nucleus Area
W2	PGE2	71.33851	71.41983	2.003204	Nucleus Area
W3	HPI4	96.97971	97.20465	4.104461	Nucleus Area
W3	PGE2	70.49165	70.7332	1.808936	Nucleus Area
W4	HPI4	98.45421	96.90384	6.000127	Nucleus Area
W4	PGE2	68.02148	68.36978	1.992393	Nucleus Area
W1	HPI4	1.629125	1.63155	0.033965	Nucleus AR
W1	PGE2	1.619778	1.618706	0.025149	Nucleus AR
W2	HPI4	1.651916	1.65225	0.025768	Nucleus AR
W2	PGE2	1.498438	1.495292	0.024674	Nucleus AR
W3	HPI4	1.781539	1.777162	0.05473	Nucleus AR
W3	PGE2	1.510796	1.507347	0.026322	Nucleus AR
W4	HPI4	1.764641	1.759558	0.061678	Nucleus AR
W4	PGE2	1.514803	1.508481	0.034067	Nucleus AR
W1	HPI4	372.7557	372.872	11.02692	Nucleus Volume
W1	PGE2	383.1021	385.4627	10.17176	Nucleus Volume
W2	HPI4	298.4583	298.8461	15.55892	Nucleus Volume
W2	PGE2	297.8123	298.7622	8.394385	Nucleus Volume

W3	HPI4	272.3898	274.0259	14.12415	Nucleus Volume
W3	PGE2	265.3363	264.4524	6.465467	Nucleus Volume
W4	HPI4	279.1706	276.1707	20.3864	Nucleus Volume
W4	PGE2	257.1566	255.465	9.841911	Nucleus Volume
W1	HPI4	4.533628	4.525423	0.191695	Nucleus Height
W1	PGE2	4.612521	4.603486	0.148627	Nucleus Height
W2	HPI4	3.81835	3.840905	0.167647	Nucleus Height
W2	PGE2	5.342801	5.335938	0.177721	Nucleus Height
W3	HPI4	3.182476	3.210514	0.187634	Nucleus Height
W3	PGE2	4.750954	4.750512	0.159101	Nucleus Height
W4	HPI4	3.191963	3.179871	0.14795	Nucleus Height
W4	PGE2	4.629422	4.64574	0.134717	Nucleus Height
W1	HPI4	11.58391	11.58974	0.182939	Nucleus Width
W1	PGE2	11.63386	11.65692	0.190566	Nucleus Width
W2	HPI4	10.94718	10.98229	0.272187	Nucleus Width
W2	PGE2	9.708055	9.716487	0.14012	Nucleus Width
W3	HPI4	11.18941	11.22528	0.239738	Nucleus Width
W3	PGE2	9.642591	9.657397	0.125086	Nucleus Width
W4	HPI4	11.25569	11.23235	0.329308	Nucleus Width
W4	PGE2	9.48755	9.490372	0.141559	Nucleus Width

402

403 **Table S3:** Summary of shape metrics for cells and nuclei.

404

405 **Table S4**

Line	Week	Treatment	mean	median	sd	Metric
ACTB	W1	PGE2	7.999321	7.993082	0.608366	X/Y distribution
ACTB	W1	HPI4	7.661851	7.645076	0.578039	X/Y distribution
ACTB	W2	PGE2	5.298201	5.229909	0.514801	X/Y distribution
ACTB	W2	HPI4	4.474	4.399867	1.624012	X/Y distribution
ACTB	W3	PGE2	7.150573	7.072148	0.403022	X/Y distribution
ACTB	W3	HPI4	7.858906	7.980864	0.860769	X/Y distribution
ACTB	W4	PGE2	7.357982	7.354374	0.378458	X/Y distribution
ACTB	W4	HPI4	7.101141	7.284834	1.265883	X/Y distribution
CETN2	W1	PGE2	3.718543	3.219556	1.415504	X/Y distribution
CETN2	W1	HPI4	3.680193	3.337471	1.946887	X/Y distribution
CETN2	W2	PGE2	3.395476	3.268633	0.738926	X/Y distribution
CETN2	W2	HPI4	5.153006	5.233947	0.75334	X/Y distribution
CETN2	W3	PGE2	5.110347	4.876212	1.570655	X/Y distribution
CETN2	W3	HPI4	4.999421	4.859668	1.926319	X/Y distribution
CETN2	W4	PGE2	4.916062	4.965908	1.223457	X/Y distribution
CETN2	W4	HPI4	4.51464	4.58562	1.92121	X/Y distribution
CTNNB1	W1	PGE2	6.986235	6.950445	0.436584	X/Y distribution
CTNNB1	W1	HPI4	7.023402	7.009501	0.421112	X/Y distribution
CTNNB1	W2	PGE2	5.992248	6.020617	0.777113	X/Y distribution
CTNNB1	W2	HPI4	4.581246	4.013463	1.499905	X/Y distribution
CTNNB1	W3	PGE2	7.270658	7.273669	0.444791	X/Y distribution
CTNNB1	W3	HPI4	4.848887	4.445379	1.435614	X/Y distribution
CTNNB1	W4	PGE2	7.158428	7.156523	0.391081	X/Y distribution
CTNNB1	W4	HPI4	9.76031	10.18562	2.586719	X/Y distribution
DSP	W1	PGE2	8.693746	8.642735	0.90515	X/Y distribution
DSP	W1	HPI4	8.689141	8.671229	0.722538	X/Y distribution
DSP	W2	PGE2	6.163519	6.06301	0.449289	X/Y distribution
DSP	W2	HPI4	7.976204	7.89295	0.951557	X/Y distribution
DSP	W3	PGE2	5.975327	6.059813	0.416205	X/Y distribution

DSP	W3	HPI4	8.620872	8.400218	1.61008	X/Y distribution
DSP	W4	PGE2	6.060471	6.064627	0.469211	X/Y distribution
DSP	W4	HPI4	8.444856	8.391807	0.980873	X/Y distribution
FBL	W1	PGE2	3.496021	3.499388	0.25881	X/Y distribution
FBL	W1	HPI4	3.471524	3.401697	0.382949	X/Y distribution
FBL	W2	PGE2	3.249522	3.223048	0.260309	X/Y distribution
FBL	W2	HPI4	2.768808	2.837889	0.569911	X/Y distribution
FBL	W3	PGE2	2.840976	2.827199	0.205732	X/Y distribution
FBL	W3	HPI4	3.445489	3.425931	0.683878	X/Y distribution
FBL	W4	PGE2	3.253748	3.283865	0.460977	X/Y distribution
FBL	W4	HPI4	5.248879	5.396442	1.263963	X/Y distribution
GJA1	W1	PGE2	7.853266	8.121644	1.52088	X/Y distribution
GJA1	W1	HPI4	7.643336	7.696331	1.066213	X/Y distribution
GJA1	W2	PGE2	6.763049	6.710809	0.853453	X/Y distribution
GJA1	W2	HPI4	6.69809	6.79079	1.666817	X/Y distribution
GJA1	W3	PGE2	7.376215	7.406686	0.856876	X/Y distribution
GJA1	W3	HPI4	6.314873	6.26627	1.610526	X/Y distribution
GJA1	W4	PGE2	7.023247	6.944946	0.872895	X/Y distribution
GJA1	W4	HPI4	8.681295	8.462218	1.477104	X/Y distribution
LAMP1	W1	PGE2	5.350178	5.347927	0.45091	X/Y distribution
LAMP1	W1	HPI4	5.155106	5.072539	0.393083	X/Y distribution
LAMP1	W2	PGE2	3.949721	3.946111	0.168478	X/Y distribution
LAMP1	W2	HPI4	5.970587	5.794298	0.537366	X/Y distribution
LAMP1	W3	PGE2	3.833359	3.853416	0.160305	X/Y distribution
LAMP1	W3	HPI4	6.401117	6.102867	0.944975	X/Y distribution
LAMP1	W4	PGE2	3.879483	3.819189	0.258595	X/Y distribution
LAMP1	W4	HPI4	6.739844	6.630087	0.78563	X/Y distribution
LC3B	W1	PGE2	5.158682	5.085295	0.374684	X/Y distribution
LC3B	W1	HPI4	5.086854	5.075382	0.447051	X/Y distribution
LC3B	W2	PGE2	5.620774	5.674705	0.378884	X/Y distribution
LC3B	W2	HPI4	5.372097	5.309214	0.780407	X/Y distribution

LC3B	W3	PGE2	5.951903	5.9956	0.323875	X/Y distribution
LC3B	W3	HPI4	6.983472	7.294795	1.807982	X/Y distribution
LC3B	W4	PGE2	5.743837	5.708257	0.245428	X/Y distribution
LC3B	W4	HPI4	7.09243	6.596253	3.494701	X/Y distribution
MYH10	W1	PGE2	8.288095	8.211405	0.726817	X/Y distribution
MYH10	W1	HPI4	8.42357	8.387502	0.560209	X/Y distribution
MYH10	W2	PGE2	5.759156	5.759465	0.275571	X/Y distribution
MYH10	W2	HPI4	7.114658	7.079687	0.607834	X/Y distribution
MYH10	W3	PGE2	5.799152	5.808292	0.254491	X/Y distribution
MYH10	W3	HPI4	7.716332	7.682444	0.702619	X/Y distribution
MYH10	W4	PGE2	5.88016	5.879538	0.288658	X/Y distribution
MYH10	W4	HPI4	8.126193	7.828607	1.050439	X/Y distribution
RAB5	W1	PGE2	6.020903	6.103036	0.48855	X/Y distribution
RAB5	W1	HPI4	5.967938	6.024844	0.674972	X/Y distribution
RAB5	W2	PGE2	5.181714	5.22884	0.470715	X/Y distribution
RAB5	W2	HPI4	5.06772	4.857977	1.210446	X/Y distribution
RAB5	W3	PGE2	6.390409	6.434056	0.419555	X/Y distribution
RAB5	W3	HPI4	4.704265	4.052152	1.975493	X/Y distribution
RAB5	W4	PGE2	5.960581	6.011931	0.570845	X/Y distribution
RAB5	W4	HPI4	8.279909	8.252202	1.439009	X/Y distribution
SEC61B	W1	PGE2	8.23142	8.198237	0.604541	X/Y distribution
SEC61B	W1	HPI4	8.102059	8.110551	0.856113	X/Y distribution
SEC61B	W2	PGE2	5.460078	5.434322	0.258745	X/Y distribution
SEC61B	W2	HPI4	7.779698	8.005598	0.689307	X/Y distribution
SEC61B	W3	PGE2	5.394098	5.394413	0.165452	X/Y distribution
SEC61B	W3	HPI4	8.426639	8.614289	0.642344	X/Y distribution
SEC61B	W4	PGE2	5.474878	5.502656	0.174207	X/Y distribution
SEC61B	W4	HPI4	8.412412	8.390734	0.620241	X/Y distribution
SLC25A17	W1	PGE2	5.282329	5.248673	0.401541	X/Y distribution
SLC25A17	W1	HPI4	5.466914	5.391687	0.406124	X/Y distribution
SLC25A17	W2	PGE2	5.134053	5.093752	0.400926	X/Y distribution

SLC25A17	W2	HPI4	4.582454	4.65351	0.615648	X/Y distribution
SLC25A17	W3	PGE2	5.546964	5.493785	0.356836	X/Y distribution
SLC25A17	W3	HPI4	6.01576	5.763907	1.479938	X/Y distribution
SLC25A17	W4	PGE2	6.019456	6.056123	0.27369	X/Y distribution
SLC25A17	W4	HPI4	6.222104	6.490793	1.409692	X/Y distribution
ST6GAL1	W1	PGE2	6.525392	6.180542	1.522937	X/Y distribution
ST6GAL1	W1	HPI4	6.492782	6.459179	0.945393	X/Y distribution
ST6GAL1	W2	PGE2	5.199753	5.168461	0.627976	X/Y distribution
ST6GAL1	W2	HPI4	6.927928	6.814449	1.07438	X/Y distribution
ST6GAL1	W3	PGE2	6.134848	6.076455	0.65112	X/Y distribution
ST6GAL1	W3	HPI4	6.705346	6.848704	1.213498	X/Y distribution
ST6GAL1	W4	PGE2	5.890911	5.869534	0.545531	X/Y distribution
ST6GAL1	W4	HPI4	5.938951	6.112371	1.203076	X/Y distribution
TJP1	W1	PGE2	7.59996	7.422312	0.589346	X/Y distribution
TJP1	W1	HPI4	7.377716	7.306474	0.619195	X/Y distribution
TJP1	W2	PGE2	6.689496	6.248021	1.109729	X/Y distribution
TJP1	W2	HPI4	7.627083	7.527967	0.49323	X/Y distribution
TJP1	W3	PGE2	6.145458	6.173138	0.198401	X/Y distribution
TJP1	W3	HPI4	8.703424	8.647357	0.709516	X/Y distribution
TJP1	W4	PGE2	6.708812	6.665043	0.520656	X/Y distribution
TJP1	W4	HPI4	9.361892	9.348104	0.752678	X/Y distribution
TOM20	W1	PGE2	6.925465	6.896542	0.435788	X/Y distribution
TOM20	W1	HPI4	6.672381	6.691665	0.478885	X/Y distribution
TOM20	W2	PGE2	4.765545	4.762037	0.139261	X/Y distribution
TOM20	W2	HPI4	6.998304	7.013266	0.62272	X/Y distribution
TOM20	W3	PGE2	4.623998	4.609516	0.204193	X/Y distribution
TOM20	W3	HPI4	7.428276	7.46013	0.562114	X/Y distribution
TOM20	W4	PGE2	4.671732	4.650626	0.222386	X/Y distribution
TOM20	W4	HPI4	8.126977	8.19049	0.380392	X/Y distribution
ACTB	W1	PGE2	1.143991	1.163942	0.158345	Avg. Volume
ACTB	W1	HPI4	1.200079	1.151524	0.180418	Avg. Volume

ACTB	W2	PGE2	1.627259	1.661222	0.282735	Avg. Volume
ACTB	W2	HPI4	1.544752	0.711579	1.851111	Avg. Volume
ACTB	W3	PGE2	1.482323	1.425829	0.211961	Avg. Volume
ACTB	W3	HPI4	1.071215	1.065257	0.227639	Avg. Volume
ACTB	W4	PGE2	1.528482	1.587626	0.269415	Avg. Volume
ACTB	W4	HPI4	1.033385	0.907911	0.450973	Avg. Volume
CETN2	W1	PGE2	1.640048	1.665026	0.253088	Avg. Volume
CETN2	W1	HPI4	1.670975	1.637823	0.377122	Avg. Volume
CETN2	W2	PGE2	1.58092	1.63532	0.331474	Avg. Volume
CETN2	W2	HPI4	1.661021	1.59845	0.315696	Avg. Volume
CETN2	W3	PGE2	1.536347	1.503813	0.287	Avg. Volume
CETN2	W3	HPI4	1.643904	1.474205	0.825827	Avg. Volume
CETN2	W4	PGE2	1.847705	1.827503	0.288702	Avg. Volume
CETN2	W4	HPI4	1.723532	1.47875	0.699569	Avg. Volume
CTNNB1	W1	PGE2	3.24078	3.19628	0.598093	Avg. Volume
CTNNB1	W1	HPI4	3.07751	2.986283	0.500887	Avg. Volume
CTNNB1	W2	PGE2	3.711934	3.690175	1.044314	Avg. Volume
CTNNB1	W2	HPI4	2.387014	1.739552	2.115306	Avg. Volume
CTNNB1	W3	PGE2	1.709891	1.670674	0.398697	Avg. Volume
CTNNB1	W3	HPI4	2.278804	1.579592	2.863998	Avg. Volume
CTNNB1	W4	PGE2	1.676844	1.607074	0.308562	Avg. Volume
CTNNB1	W4	HPI4	1.373445	0.961524	1.792573	Avg. Volume
DSP	W1	PGE2	0.380548	0.388553	0.033427	Avg. Volume
DSP	W1	HPI4	0.388447	0.394896	0.034113	Avg. Volume
DSP	W2	PGE2	0.527068	0.524892	0.069748	Avg. Volume
DSP	W2	HPI4	0.386782	0.370741	0.066957	Avg. Volume
DSP	W3	PGE2	0.470532	0.471265	0.079654	Avg. Volume
DSP	W3	HPI4	0.397958	0.406742	0.070434	Avg. Volume
DSP	W4	PGE2	0.470327	0.475464	0.062219	Avg. Volume
DSP	W4	HPI4	0.447791	0.450188	0.073528	Avg. Volume
FBL	W1	PGE2	5.153083	5.128439	0.535695	Avg. Volume

FBL	W1	HPI4	5.154413	5.149073	0.493135	Avg. Volume
FBL	W2	PGE2	4.552862	4.59941	0.503223	Avg. Volume
FBL	W2	HPI4	6.339517	6.479428	1.310185	Avg. Volume
FBL	W3	PGE2	6.050995	6.016479	0.476648	Avg. Volume
FBL	W3	HPI4	8.738051	8.884743	1.955745	Avg. Volume
FBL	W4	PGE2	3.192355	3.208565	0.453001	Avg. Volume
FBL	W4	HPI4	4.027771	3.955832	1.14012	Avg. Volume
GJA1	W1	PGE2	0.785799	0.557067	1.258691	Avg. Volume
GJA1	W1	HPI4	0.481977	0.421473	0.278042	Avg. Volume
GJA1	W2	PGE2	0.538343	0.553887	0.102151	Avg. Volume
GJA1	W2	HPI4	0.569507	0.478849	0.47946	Avg. Volume
GJA1	W3	PGE2	0.495439	0.460741	0.160837	Avg. Volume
GJA1	W3	HPI4	0.546988	0.456217	0.268988	Avg. Volume
GJA1	W4	PGE2	0.500994	0.437234	0.177939	Avg. Volume
GJA1	W4	HPI4	0.566894	0.55081	0.144351	Avg. Volume
LAMP1	W1	PGE2	0.453987	0.453065	0.023674	Avg. Volume
LAMP1	W1	HPI4	0.442164	0.440845	0.023536	Avg. Volume
LAMP1	W2	PGE2	0.433268	0.438823	0.027206	Avg. Volume
LAMP1	W2	HPI4	0.227098	0.219531	0.040631	Avg. Volume
LAMP1	W3	PGE2	0.487202	0.488816	0.041029	Avg. Volume
LAMP1	W3	HPI4	0.217787	0.211166	0.039322	Avg. Volume
LAMP1	W4	PGE2	0.445859	0.44505	0.069043	Avg. Volume
LAMP1	W4	HPI4	0.227305	0.218704	0.046106	Avg. Volume
LC3B	W1	PGE2	0.465276	0.471513	0.052163	Avg. Volume
LC3B	W1	HPI4	0.481962	0.489373	0.048941	Avg. Volume
LC3B	W2	PGE2	0.33489	0.330926	0.039871	Avg. Volume
LC3B	W2	HPI4	0.363536	0.352632	0.065833	Avg. Volume
LC3B	W3	PGE2	0.427827	0.42995	0.04081	Avg. Volume
LC3B	W3	HPI4	0.419323	0.406852	0.172593	Avg. Volume
LC3B	W4	PGE2	0.436449	0.437993	0.048378	Avg. Volume
LC3B	W4	HPI4	0.417834	0.464624	0.146344	Avg. Volume

MYH10	W1	PGE2	1.271709	1.25704	0.146307	Avg. Volume
MYH10	W1	HPI4	1.277801	1.285359	0.145831	Avg. Volume
MYH10	W2	PGE2	1.992359	2.010487	0.270925	Avg. Volume
MYH10	W2	HPI4	1.161497	1.174101	0.138049	Avg. Volume
MYH10	W3	PGE2	2.169272	2.19618	0.172539	Avg. Volume
MYH10	W3	HPI4	1.08309	1.071898	0.173121	Avg. Volume
MYH10	W4	PGE2	2.207228	2.201543	0.253551	Avg. Volume
MYH10	W4	HPI4	1.248506	1.212858	0.286006	Avg. Volume
RAB5	W1	PGE2	0.573103	0.59056	0.099573	Avg. Volume
RAB5	W1	HPI4	0.551033	0.564772	0.136424	Avg. Volume
RAB5	W2	PGE2	0.560797	0.570557	0.082842	Avg. Volume
RAB5	W2	HPI4	0.837121	0.560803	0.785132	Avg. Volume
RAB5	W3	PGE2	0.480571	0.482202	0.059453	Avg. Volume
RAB5	W3	HPI4	0.720985	0.599778	0.525274	Avg. Volume
RAB5	W4	PGE2	0.516187	0.514985	0.102612	Avg. Volume
RAB5	W4	HPI4	0.546166	0.479829	0.244303	Avg. Volume
SEC61B	W1	PGE2	2.045515	2.105764	0.413579	Avg. Volume
SEC61B	W1	HPI4	1.97642	1.956465	0.371854	Avg. Volume
SEC61B	W2	PGE2	2.23993	2.2789	0.37174	Avg. Volume
SEC61B	W2	HPI4	1.424174	1.417242	0.239599	Avg. Volume
SEC61B	W3	PGE2	2.635173	2.563899	0.466075	Avg. Volume
SEC61B	W3	HPI4	1.476274	1.419498	0.431207	Avg. Volume
SEC61B	W4	PGE2	2.457829	2.50981	0.564193	Avg. Volume
SEC61B	W4	HPI4	1.195176	1.263405	0.308156	Avg. Volume
SLC25A17	W1	PGE2	0.414033	0.420085	0.036642	Avg. Volume
SLC25A17	W1	HPI4	0.407784	0.419535	0.047366	Avg. Volume
SLC25A17	W2	PGE2	0.450263	0.459874	0.051821	Avg. Volume
SLC25A17	W2	HPI4	0.463	0.412677	0.131497	Avg. Volume
SLC25A17	W3	PGE2	0.423998	0.434714	0.047569	Avg. Volume
SLC25A17	W3	HPI4	0.398216	0.374036	0.095567	Avg. Volume
SLC25A17	W4	PGE2	0.417567	0.422653	0.038048	Avg. Volume

SLC25A17	W4	HPI4	0.374223	0.311627	0.207255	Avg. Volume
ST6GAL1	W1	PGE2	1.09183	1.084123	0.748676	Avg. Volume
ST6GAL1	W1	HPI4	1.404694	1.296205	0.671341	Avg. Volume
ST6GAL1	W2	PGE2	1.392369	1.287421	0.444635	Avg. Volume
ST6GAL1	W2	HPI4	0.936131	0.941542	0.373967	Avg. Volume
ST6GAL1	W3	PGE2	0.953714	0.907448	0.328203	Avg. Volume
ST6GAL1	W3	HPI4	0.805659	0.77173	0.335654	Avg. Volume
ST6GAL1	W4	PGE2	0.605979	0.552034	0.238364	Avg. Volume
ST6GAL1	W4	HPI4	0.672341	0.60552	0.309142	Avg. Volume
TJP1	W1	PGE2	1.243816	1.23024	0.17098	Avg. Volume
TJP1	W1	HPI4	1.181825	1.185302	0.203893	Avg. Volume
TJP1	W2	PGE2	1.343131	1.383632	0.335916	Avg. Volume
TJP1	W2	HPI4	1.073935	0.977323	0.281245	Avg. Volume
TJP1	W3	PGE2	2.502049	2.241683	0.805917	Avg. Volume
TJP1	W3	HPI4	1.698175	1.594555	0.408371	Avg. Volume
TJP1	W4	PGE2	2.247562	2.09513	0.866373	Avg. Volume
TJP1	W4	HPI4	1.473042	1.539548	0.426649	Avg. Volume
TOM20	W1	PGE2	3.88246	3.854016	0.458433	Avg. Volume
TOM20	W1	HPI4	3.859425	3.90326	0.445906	Avg. Volume
TOM20	W2	PGE2	4.347876	4.202047	0.659689	Avg. Volume
TOM20	W2	HPI4	2.813705	2.756008	0.521648	Avg. Volume
TOM20	W3	PGE2	4.916073	4.936354	0.593053	Avg. Volume
TOM20	W3	HPI4	2.511965	2.43153	0.574445	Avg. Volume
TOM20	W4	PGE2	3.933182	4.010203	0.659698	Avg. Volume
TOM20	W4	HPI4	3.175705	3.091077	0.677279	Avg. Volume
ACTB	W1	PGE2	0.002333	0.010551	0.071647	Z distribution
ACTB	W1	HPI4	0.034671	0.042294	0.08425	Z distribution
ACTB	W2	PGE2	0.071911	0.080081	0.055381	Z distribution
ACTB	W2	HPI4	-0.02585	-0.03702	0.306863	Z distribution
ACTB	W3	PGE2	0.070705	0.080477	0.127209	Z distribution
ACTB	W3	HPI4	-0.08625	-0.0842	0.088621	Z distribution

ACTB	W4	PGE2	-0.09894	-0.05195	0.150017	Z distribution
ACTB	W4	HPI4	-0.1135	0.011311	0.318321	Z distribution
CETN2	W1	PGE2	0.189742	0.161574	0.382012	Z distribution
CETN2	W1	HPI4	0.300507	0.190814	0.498935	Z distribution
CETN2	W2	PGE2	1.660531	1.633432	0.258869	Z distribution
CETN2	W2	HPI4	0.382778	0.431581	0.376923	Z distribution
CETN2	W3	PGE2	-0.05065	-0.10725	0.304101	Z distribution
CETN2	W3	HPI4	-0.27104	-0.20168	0.23754	Z distribution
CETN2	W4	PGE2	0.364738	0.182473	0.438683	Z distribution
CETN2	W4	HPI4	-0.89917	-0.78051	0.351101	Z distribution
CTNNB1	W1	PGE2	-0.63854	-0.6689	0.142516	Z distribution
CTNNB1	W1	HPI4	-0.68065	-0.689	0.118296	Z distribution
CTNNB1	W2	PGE2	-0.47742	-0.48768	0.154646	Z distribution
CTNNB1	W2	HPI4	-0.25863	-0.13854	0.380323	Z distribution
CTNNB1	W3	PGE2	-0.4253	-0.40115	0.133781	Z distribution
CTNNB1	W3	HPI4	-0.22485	-0.21772	0.191594	Z distribution
CTNNB1	W4	PGE2	-0.33386	-0.29833	0.099838	Z distribution
CTNNB1	W4	HPI4	-0.33565	-0.27694	0.158229	Z distribution
DSP	W1	PGE2	0.549585	0.505339	0.282926	Z distribution
DSP	W1	HPI4	0.569849	0.538081	0.201661	Z distribution
DSP	W2	PGE2	1.258493	1.289161	0.30926	Z distribution
DSP	W2	HPI4	0.074151	0.008122	0.33497	Z distribution
DSP	W3	PGE2	0.562705	0.538635	0.508644	Z distribution
DSP	W3	HPI4	-0.02792	-0.00585	0.13925	Z distribution
DSP	W4	PGE2	0.057681	-0.03502	0.439236	Z distribution
DSP	W4	HPI4	-0.06974	-0.0563	0.227127	Z distribution
FBL	W1	PGE2	-0.44908	-0.45788	0.050531	Z distribution
FBL	W1	HPI4	-0.46493	-0.46225	0.044405	Z distribution
FBL	W2	PGE2	-0.44495	-0.42313	0.078531	Z distribution
FBL	W2	HPI4	-0.2042	-0.17585	0.103965	Z distribution
FBL	W3	PGE2	-0.3834	-0.38929	0.052131	Z distribution

FBL	W3	HPI4	-0.21045	-0.20069	0.09534	Z distribution
FBL	W4	PGE2	-0.4466	-0.45675	0.153142	Z distribution
FBL	W4	HPI4	-0.3101	-0.28732	0.108525	Z distribution
GJA1	W1	PGE2	-0.38421	-0.34846	0.222045	Z distribution
GJA1	W1	HPI4	-0.43705	-0.418	0.27406	Z distribution
GJA1	W2	PGE2	-0.01528	-0.03591	0.132482	Z distribution
GJA1	W2	HPI4	-0.35599	-0.34026	0.199647	Z distribution
GJA1	W3	PGE2	-0.12067	-0.05012	0.300234	Z distribution
GJA1	W3	HPI4	-0.23836	-0.18817	0.223852	Z distribution
GJA1	W4	PGE2	-0.09677	-0.1312	0.191622	Z distribution
GJA1	W4	HPI4	-0.08172	-0.0481	0.128599	Z distribution
LAMP1	W1	PGE2	0.233997	0.225429	0.126273	Z distribution
LAMP1	W1	HPI4	0.205223	0.190746	0.128954	Z distribution
LAMP1	W2	PGE2	0.344092	0.349528	0.144763	Z distribution
LAMP1	W2	HPI4	-0.28635	-0.28903	0.093715	Z distribution
LAMP1	W3	PGE2	0.076347	0.08599	0.161622	Z distribution
LAMP1	W3	HPI4	-0.53724	-0.53041	0.16437	Z distribution
LAMP1	W4	PGE2	0.02099	0.018418	0.234206	Z distribution
LAMP1	W4	HPI4	-0.50963	-0.49534	0.289022	Z distribution
LC3B	W1	PGE2	-0.1928	-0.19985	0.132877	Z distribution
LC3B	W1	HPI4	-0.12183	-0.12764	0.130098	Z distribution
LC3B	W2	PGE2	-0.26204	-0.26038	0.106346	Z distribution
LC3B	W2	HPI4	-0.21961	-0.22759	0.183254	Z distribution
LC3B	W3	PGE2	-0.43467	-0.39772	0.128423	Z distribution
LC3B	W3	HPI4	-0.32975	-0.30625	0.204771	Z distribution
LC3B	W4	PGE2	-0.24195	-0.23195	0.150213	Z distribution
LC3B	W4	HPI4	-0.18653	-0.29412	0.320868	Z distribution
MYH10	W1	PGE2	-0.15354	-0.13567	0.109165	Z distribution
MYH10	W1	HPI4	-0.15645	-0.13214	0.113376	Z distribution
MYH10	W2	PGE2	0.25471	0.257161	0.0805	Z distribution
MYH10	W2	HPI4	0.060774	0.051954	0.092892	Z distribution

MYH10	W3	PGE2	0.014896	0.009792	0.162185	Z distribution
MYH10	W3	HPI4	0.128339	0.126206	0.067209	Z distribution
MYH10	W4	PGE2	-0.09068	-0.0725	0.126746	Z distribution
MYH10	W4	HPI4	0.128737	0.124864	0.08755	Z distribution
RAB5	W1	PGE2	-0.13488	-0.1271	0.128825	Z distribution
RAB5	W1	HPI4	-0.12707	-0.15225	0.155053	Z distribution
RAB5	W2	PGE2	-0.25083	-0.242	0.090862	Z distribution
RAB5	W2	HPI4	-0.36768	-0.32883	0.220094	Z distribution
RAB5	W3	PGE2	-0.47251	-0.48611	0.096584	Z distribution
RAB5	W3	HPI4	-0.29324	-0.25589	0.213357	Z distribution
RAB5	W4	PGE2	-0.57847	-0.60571	0.207854	Z distribution
RAB5	W4	HPI4	-0.24239	-0.23529	0.137132	Z distribution
SEC61B	W1	PGE2	-0.27755	-0.28126	0.060679	Z distribution
SEC61B	W1	HPI4	-0.25719	-0.24862	0.075728	Z distribution
SEC61B	W2	PGE2	-0.16701	-0.16936	0.114916	Z distribution
SEC61B	W2	HPI4	-0.39031	-0.38969	0.135964	Z distribution
SEC61B	W3	PGE2	-0.57907	-0.55999	0.203777	Z distribution
SEC61B	W3	HPI4	-0.57877	-0.56638	0.241061	Z distribution
SEC61B	W4	PGE2	-0.5356	-0.49364	0.167791	Z distribution
SEC61B	W4	HPI4	-0.51733	-0.53284	0.139926	Z distribution
SLC25A17	W1	PGE2	-0.27645	-0.26771	0.101331	Z distribution
SLC25A17	W1	HPI4	-0.27983	-0.27001	0.078605	Z distribution
SLC25A17	W2	PGE2	-0.41501	-0.41186	0.103661	Z distribution
SLC25A17	W2	HPI4	-0.29055	-0.2717	0.168758	Z distribution
SLC25A17	W3	PGE2	-0.44969	-0.43517	0.138067	Z distribution
SLC25A17	W3	HPI4	-0.24312	-0.2238	0.174741	Z distribution
SLC25A17	W4	PGE2	-0.36222	-0.35462	0.086832	Z distribution
SLC25A17	W4	HPI4	-0.34962	-0.31721	0.450703	Z distribution
ST6GAL1	W1	PGE2	-0.49656	-0.45994	0.23066	Z distribution
ST6GAL1	W1	HPI4	-0.35763	-0.36363	0.322819	Z distribution
ST6GAL1	W2	PGE2	-0.15298	-0.1864	0.292805	Z distribution

ST6GAL1	W2	HPI4	-0.47604	-0.43636	0.179348	Z distribution
ST6GAL1	W3	PGE2	-0.17409	-0.12284	0.382819	Z distribution
ST6GAL1	W3	HPI4	-0.48312	-0.45587	0.365579	Z distribution
ST6GAL1	W4	PGE2	-0.22891	-0.17752	0.412919	Z distribution
ST6GAL1	W4	HPI4	-0.66529	-0.72638	0.287806	Z distribution
TJP1	W1	PGE2	-0.05602	-0.07174	0.114496	Z distribution
TJP1	W1	HPI4	-0.04114	-0.03376	0.123749	Z distribution
TJP1	W2	PGE2	0.354251	0.377269	0.24981	Z distribution
TJP1	W2	HPI4	-0.07054	-0.05882	0.09263	Z distribution
TJP1	W3	PGE2	0.927932	0.90597	0.265617	Z distribution
TJP1	W3	HPI4	-0.09765	-0.10095	0.075318	Z distribution
TJP1	W4	PGE2	0.9519	0.969002	0.311131	Z distribution
TJP1	W4	HPI4	0.028209	0.011401	0.128634	Z distribution
TOM20	W1	PGE2	-0.45828	-0.45161	0.060977	Z distribution
TOM20	W1	HPI4	-0.49231	-0.48376	0.059451	Z distribution
TOM20	W2	PGE2	-0.62262	-0.62487	0.111237	Z distribution
TOM20	W2	HPI4	-0.63884	-0.64875	0.10707	Z distribution
TOM20	W3	PGE2	-0.63987	-0.63023	0.113684	Z distribution
TOM20	W3	HPI4	-0.84505	-0.87929	0.157785	Z distribution
TOM20	W4	PGE2	-0.57366	-0.58224	0.133682	Z distribution
TOM20	W4	HPI4	-0.80745	-0.81654	0.205356	Z distribution
ACTB	W1	PGE2	87.23362	86.56887	14.05822	Count/cell
ACTB	W1	HPI4	80.06511	78.88889	12.4295	Count/cell
ACTB	W2	PGE2	49.30337	50.25467	9.230974	Count/cell
ACTB	W2	HPI4	70.44414	62	57.58975	Count/cell
ACTB	W3	PGE2	51.99168	50.86075	7.218139	Count/cell
ACTB	W3	HPI4	61.68898	58.02372	24.04927	Count/cell
ACTB	W4	PGE2	48.46465	47.5716	7.10428	Count/cell
ACTB	W4	HPI4	59.19822	61.66667	16.56637	Count/cell
CETN2	W1	PGE2	1.807603	1.797101	0.166936	Count/cell
CETN2	W1	HPI4	1.744743	1.748265	0.273297	Count/cell

CETN2	W2	PGE2	1.884705	1.869191	0.136725	Count/cell
CETN2	W2	HPI4	2.009673	2.018717	0.191751	Count/cell
CETN2	W3	PGE2	2.041925	2.05719	0.270289	Count/cell
CETN2	W3	HPI4	1.657942	1.333333	0.918382	Count/cell
CETN2	W4	PGE2	1.812202	1.783198	0.239868	Count/cell
CETN2	W4	HPI4	1.55	1	1.051529	Count/cell
CTNNB1	W1	PGE2	31.23372	30.66318	4.489316	Count/cell
CTNNB1	W1	HPI4	32.77639	30.96461	5.571062	Count/cell
CTNNB1	W2	PGE2	19.66947	19.56006	5.16662	Count/cell
CTNNB1	W2	HPI4	22.27003	20.5	9.869678	Count/cell
CTNNB1	W3	PGE2	36.18025	35.68531	6.594703	Count/cell
CTNNB1	W3	HPI4	22.76287	19.61538	11.3811	Count/cell
CTNNB1	W4	PGE2	37.81126	37.76974	6.303202	Count/cell
CTNNB1	W4	HPI4	62.36833	55.5	29.5605	Count/cell
DSP	W1	PGE2	22.4279	22.16986	4.306592	Count/cell
DSP	W1	HPI4	22.96419	22.19681	4.13484	Count/cell
DSP	W2	PGE2	38.18739	36.4845	19.04854	Count/cell
DSP	W2	HPI4	25.81432	24.84809	8.809618	Count/cell
DSP	W3	PGE2	48.76091	46.54559	17.75294	Count/cell
DSP	W3	HPI4	26.92075	26.86005	12.04335	Count/cell
DSP	W4	PGE2	64.4891	66.2561	19.40443	Count/cell
DSP	W4	HPI4	26.78821	26.79248	8.47539	Count/cell
FBL	W1	PGE2	3.328321	3.319609	0.151966	Count/cell
FBL	W1	HPI4	3.287919	3.265517	0.19514	Count/cell
FBL	W2	PGE2	2.83646	2.809712	0.163528	Count/cell
FBL	W2	HPI4	2.132831	2.133019	0.199569	Count/cell
FBL	W3	PGE2	2.594694	2.627847	0.119541	Count/cell
FBL	W3	HPI4	2.25038	2.233459	0.32354	Count/cell
FBL	W4	PGE2	2.053592	2.017857	0.195164	Count/cell
FBL	W4	HPI4	1.921168	1.950617	0.222177	Count/cell
GJA1	W1	PGE2	35.02399	27	26.59997	Count/cell

GJA1	W1	HPI4	25.48729	23.6	19.78163	Count/cell
GJA1	W2	PGE2	17.85682	16.47123	6.153537	Count/cell
GJA1	W2	HPI4	21.07016	22.90244	6.55076	Count/cell
GJA1	W3	PGE2	15.57391	12.76003	9.399503	Count/cell
GJA1	W3	HPI4	23.63975	21.66667	15.29987	Count/cell
GJA1	W4	PGE2	13.82282	10.66856	9.319185	Count/cell
GJA1	W4	HPI4	29.27808	27.69231	10.88306	Count/cell
LAMP1	W1	PGE2	30.83033	30.39027	4.284323	Count/cell
LAMP1	W1	HPI4	27.45415	27.77545	3.869381	Count/cell
LAMP1	W2	PGE2	21.55159	21.50674	3.540155	Count/cell
LAMP1	W2	HPI4	31.58389	30.05895	14.45797	Count/cell
LAMP1	W3	PGE2	18.16013	18.4093	3.514018	Count/cell
LAMP1	W3	HPI4	34.83054	30.84806	17.7006	Count/cell
LAMP1	W4	PGE2	18.2536	19.19027	3.829334	Count/cell
LAMP1	W4	HPI4	34.92709	28.97793	21.39582	Count/cell
LC3B	W1	PGE2	5.367347	5.194995	1.01324	Count/cell
LC3B	W1	HPI4	5.221037	4.761529	1.252366	Count/cell
LC3B	W2	PGE2	12.18473	12.93609	4.064754	Count/cell
LC3B	W2	HPI4	12.46629	11.79167	5.539167	Count/cell
LC3B	W3	PGE2	13.2353	13.50359	3.365832	Count/cell
LC3B	W3	HPI4	13.23853	10.25	8.730159	Count/cell
LC3B	W4	PGE2	9.412286	8.649202	2.538833	Count/cell
LC3B	W4	HPI4	31.4	18	36.02166	Count/cell
MYH10	W1	PGE2	107.8578	111.6847	17.05805	Count/cell
MYH10	W1	HPI4	108.1583	110.3304	14.57664	Count/cell
MYH10	W2	PGE2	72.29838	72.51908	7.817025	Count/cell
MYH10	W2	HPI4	86.98671	86.176	26.04653	Count/cell
MYH10	W3	PGE2	75.23054	74.65337	6.728169	Count/cell
MYH10	W3	HPI4	105.1951	103.8493	20.81065	Count/cell
MYH10	W4	PGE2	75.40474	75.03264	10.10306	Count/cell
MYH10	W4	HPI4	118.8849	112.5983	40.34169	Count/cell

RAB5	W1	PGE2	34.65063	35.41157	8.276418	Count/cell
RAB5	W1	HPI4	32.81838	35.3284	10.86322	Count/cell
RAB5	W2	PGE2	25.75901	26.45047	6.008182	Count/cell
RAB5	W2	HPI4	20.10093	19	11.35283	Count/cell
RAB5	W3	PGE2	34.34977	34.2662	11.36197	Count/cell
RAB5	W3	HPI4	12.69899	8.666667	15.81981	Count/cell
RAB5	W4	PGE2	22.64071	22.2309	7.843066	Count/cell
RAB5	W4	HPI4	29.8052	26.97727	13.86868	Count/cell
SEC61B	W1	PGE2	88.85381	88.42036	12.73156	Count/cell
SEC61B	W1	HPI4	89.77024	90.77002	11.94917	Count/cell
SEC61B	W2	PGE2	40.94056	40.4002	3.90342	Count/cell
SEC61B	W2	HPI4	76.55362	77.67026	11.05403	Count/cell
SEC61B	W3	PGE2	39.14335	39.01614	4.511668	Count/cell
SEC61B	W3	HPI4	87.28651	85.50027	12.31061	Count/cell
SEC61B	W4	PGE2	35.32954	34.20253	4.426611	Count/cell
SEC61B	W4	HPI4	85.20879	86.26874	17.23908	Count/cell
SLC25A17	W1	PGE2	65.56802	66.84302	7.410307	Count/cell
SLC25A17	W1	HPI4	70.78701	71.50346	8.270478	Count/cell
SLC25A17	W2	PGE2	66.84068	67.3736	8.795055	Count/cell
SLC25A17	W2	HPI4	44.91907	43.42118	12.35468	Count/cell
SLC25A17	W3	PGE2	67.6567	67.39285	8.904761	Count/cell
SLC25A17	W3	HPI4	56.28552	52.08333	22.80802	Count/cell
SLC25A17	W4	PGE2	90.76213	91.60691	16.47659	Count/cell
SLC25A17	W4	HPI4	53.0461	39	35.46659	Count/cell
ST6GAL1	W1	PGE2	7.651994	6.8	4.633276	Count/cell
ST6GAL1	W1	HPI4	7.130191	6.52197	2.576658	Count/cell
ST6GAL1	W2	PGE2	5.760102	5.373271	1.569807	Count/cell
ST6GAL1	W2	HPI4	11.19277	11.90909	4.946318	Count/cell
ST6GAL1	W3	PGE2	6.590594	6.02737	2.525899	Count/cell
ST6GAL1	W3	HPI4	8.633952	8.536232	4.942221	Count/cell
ST6GAL1	W4	PGE2	5.609627	4.926732	2.406982	Count/cell

ST6GAL1	W4	HPI4	6.583755	5.904762	4.054568	Count/cell
TJP1	W1	PGE2	25.12116	24.39857	2.571314	Count/cell
TJP1	W1	HPI4	26.07301	25.58716	2.579621	Count/cell
TJP1	W2	PGE2	20.00504	19.95988	2.681085	Count/cell
TJP1	W2	HPI4	23.64381	23.38156	2.614834	Count/cell
TJP1	W3	PGE2	15.39179	15.29952	2.172855	Count/cell
TJP1	W3	HPI4	21.69841	21.7694	2.678675	Count/cell
TJP1	W4	PGE2	14.12039	13.72738	1.826884	Count/cell
TJP1	W4	HPI4	23.41669	23.41759	2.770092	Count/cell
TOM20	W1	PGE2	32.74479	32.73789	2.125116	Count/cell
TOM20	W1	HPI4	30.41775	30.37972	3.33696	Count/cell
TOM20	W2	PGE2	20.59847	20.92666	1.680753	Count/cell
TOM20	W2	HPI4	26.69942	27.37056	3.571065	Count/cell
TOM20	W3	PGE2	17.86991	17.81696	1.768584	Count/cell
TOM20	W3	HPI4	28.14307	28.16973	2.842487	Count/cell
TOM20	W4	PGE2	16.75126	16.93805	2.067822	Count/cell
TOM20	W4	HPI4	36.23014	36.01703	3.089113	Count/cell

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407 **Table S4:** Summary of shape metrics for fluorophore-tagged organelles.

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409 **Table S5**

Description	Metric	Treatment	Pval const	Pval Week	Pval Week squared	Coef const	Coef Week	Coef Week squared	Diff last- first week	Rate change	min/ max week
Cell	Volume	PGE2	0.00E+00	4.12E-16	4.43E-16	1044.9 0	-149.47	29.39	-7.48	ACC	2.54
Cell	Volume	HPI4	8.38E- 225	4.70E-07	9.74E-12	998.76	-143.41	38.17	142.26	ACC	1.88
Cell	Aspect ratio	PGE2	0.00E+00	4.39E-34	3.10E-27	1.78	-0.18	0.03	-0.07	ACC	2.86
Cell	Aspect ratio	HPI4	0.00E+00	7.64E-01	9.62E-04	1.63	0.00	0.01	0.14	ACC	0.23
Cell	Area	PGE2	0.00E+00	1.38E-69	1.61E-56	376.70	-127.36	22.53	-44.18	ACC	2.83
Cell	Area	HPI4	2.06E- 144	2.65E-01	4.66E-05	272.95	-10.84	7.80	84.49	ACC	0.69
Nuclei	Volume	PGE2	0.00E+00	1.85E- 228	1.28E- 115	506.56	-138.07	19.25	-125.43	ACC	3.59
Nuclei	Volume	HPI4	0.00E+00	6.84E-73	9.77E-45	489.86	-131.68	20.15	-92.84	ACC	3.27
Nuclei	Area	PGE2	0.00E+00	6.31E- 120	8.56E-75	140.73	-46.38	7.18	-31.51	ACC	3.23
Nuclei	Area	HPI4	0.00E+00	2.62E-09	3.54E-09	112.41	-13.54	2.64	-0.97	ACC	2.56
Nuclei	Aspect ratio	PGE2	0.00E+00	5.89E-41	2.32E-29	1.75	-0.18	0.03	-0.10	ACC	3.03
Nuclei	Aspect ratio	HPI4	0.00E+00	1.19E-03	2.84E-01	1.54	0.07	0.00	0.14	DEC	7.69
ACTB	Volume	PGE2	2.69E-06	2.27E-09	3.06E-07	0.62	0.72	-0.12	0.34	DEC	2.97
ACTB	Volume	HPI4	4.38E-02	1.52E-01	8.05E-02	0.88	0.59	-0.14	-0.37	DEC	2.06
MYH10	Volume	PGE2	3.65E-03	1.53E-26	1.49E-15	0.34	1.15	-0.17	0.91	DEC	3.39
MYH10	Volume	HPI4	1.07E-54	5.02E-04	8.80E-04	1.59	-0.32	0.06	-0.06	ACC	2.66
ACTB	Count/Cell	PGE2	0.00E+00	1.77E-46	3.68E-29	5.19	-0.84	0.13	-36.30	ACC	3.16
ACTB	Count/Cell	HPI4	0.00E+00	7.25E-08	2.31E-04	4.68	-0.31	0.04	-20.80	ACC	3.63
MYH10	Count/Cell	PGE2	0.00E+00	3.97E-34	4.18E-24	5.19	-0.61	0.10	-28.61	ACC	3.01
MYH10	Count/Cell	HPI4	0.00E+00	8.71E-14	3.70E-17	4.95	-0.34	0.07	12.36	ACC	2.26
FBL	Count/Cell	PGE2	4.11E-06	7.96E-01	7.57E-01	1.33	-0.07	-0.02	-1.29	DEC	-2.07
FBL	Count/Cell	HPI4	3.36E-08	7.24E-02	2.14E-01	1.66	-0.52	0.07	-1.26	ACC	3.58
FBL	Z distribution	PGE2	1.84E-30	2.09E-02	5.58E-02	-0.58	0.11	-0.02	0.06	DEC	3.06
FBL	Z distribution	HPI4	2.02E-71	1.14E-27	1.89E-23	-0.89	0.50	-0.09	0.14	DEC	2.77
FBL	X/Y distribution	PGE2	2.31E- 140	7.08E-09	4.59E-07	4.62	-0.97	0.17	-0.41	ACC	2.91
FBL	X/Y distribution	HPI4	4.16E-43	2.94E-11	5.38E-18	5.55	-2.45	0.63	2.07	ACC	1.95
DSP	Volume	PGE2	1.17E-10	6.48E-10	4.39E-08	0.23	0.20	-0.04	0.08	DEC	2.87
DSP	Volume	HPI4	1.35E-40	1.26E-01	2.68E-02	0.43	-0.05	0.01	0.06	ACC	1.76
GJA1	Volume	PGE2	1.94E-03	1.64E-01	2.48E-01	1.32	-0.54	0.09	-0.30	ACC	3.08
GJA1	Volume	HPI4	1.10E-01	1.65E-01	2.28E-01	0.32	0.25	-0.04	0.11	DEC	2.92
DSP	Count/Cell	PGE2	2.39E- 179	9.00E-17	4.06E-05	2.61	0.63	-0.06	42.93	DEC	5.43

DSP	Count/Cell	HPI4	8.30E-198	3.03E-02	8.56E-02	3.02	0.20	-0.03	3.42	DEC	3.22
GJA1	Count/Cell	PGE2	0.00E+00	2.38E-23	1.26E-12	4.42	-1.01	0.15	-19.78	ACC	3.43
GJA1	Count/Cell	HPI4	1.07E-273	8.81E-04	4.20E-05	3.50	-0.30	0.07	5.44	ACC	2.07
SEC61B	Volume	PGE2	2.29E-10	1.78E-03	2.04E-02	1.49	0.67	-0.10	0.54	DEC	3.42
SEC61B	Volume	HPI4	1.92E-40	1.72E-03	6.08E-02	2.45	-0.53	0.06	-0.65	ACC	4.25
ST6GAL1	Volume	PGE2	1.90E-02	2.08E-01	4.89E-02	1.21	0.60	-0.18	-0.98	DEC	1.62
ST6GAL1	Volume	HPI4	1.55E-09	4.91E-03	4.85E-02	3.19	-1.38	0.19	-1.24	ACC	3.57
TOM20	Volume	PGE2	1.06E-11	2.98E-11	2.05E-10	2.29	2.04	-0.38	0.36	DEC	2.66
TOM20	Volume	HPI4	5.58E-89	4.91E-18	2.58E-15	5.97	-2.36	0.42	-0.71	ACC	2.78
TOM20	Count/Cell	PGE2	0.00E+00	2.36E-75	2.65E-42	5.50	-1.19	0.18	-51.24	ACC	3.29
TOM20	Count/Cell	HPI4	0.00E+00	7.27E-04	7.38E-04	4.63	-0.17	0.03	-0.72	ACC	2.54
LAMP1	Volume	PGE2	1.47E-70	3.53E-01	4.70E-01	0.43	0.02	0.00	0.01	DEC	3.26
LAMP1	Volume	HPI4	5.78E-261	5.98E-71	3.10E-49	0.73	-0.34	0.06	-0.19	ACC	3.07
LC3B	Volume	PGE2	2.64E-101	4.78E-13	3.95E-13	0.63	-0.19	0.04	-0.01	ACC	2.53
LC3B	Volume	HPI4	3.11E-17	7.89E-02	1.77E-01	0.59	-0.11	0.02	-0.07	ACC	3.19
RAB5	Volume	PGE2	8.91E-51	1.95E-02	4.34E-02	0.67	-0.10	0.02	-0.04	ACC	2.94
RAB5	Volume	HPI4	6.42E-01	5.28E-03	4.61E-03	0.10	0.58	-0.12	-0.01	DEC	2.49
SLC25A17	Volume	PGE2	9.98E-63	1.12E-02	7.90E-03	0.38	0.05	-0.01	0.00	DEC	2.43
SLC25A17	Volume	HPI4	4.99E-06	1.16E-01	9.02E-02	0.33	0.11	-0.02	-0.03	DEC	2.28

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411 **Table S5:** Summary of results from GLM.

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Organelle	Pre-processing	Segmentation	Post-processing
TJP1	1) Auto contrast normalization 2) Edge-preserving smoothing	2D Frangi vesselness	Remove small objects
CTNNB1	1) Auto contrast normalization 2) 3D Gaussian smoothing	2D LoG based spot filter	-
DSP	1) Auto contrast normalization 2) 2D Gaussian smoothing	1) 3D LoG based spot filter 2) 3D Watershed	-
GJA1	1) Auto contrast normalization 2) 2D Gaussian smoothing	3D LoG based spot filter	-
CETN2	1) Auto contrast normalization 2) 2D Gaussian smoothing	1) 3D LoG based spot filter 2) 3D Watershed	Remove small objects
SEC61B	1) Auto contrast normalization 2) Edge-preserving smoothing	2D Frangi vesselness	Remove small objects
	Edge-preserving smoothing		
ST6GAL1	1) Auto contrast normalization 2) 3D Gaussian smoothing	3D LoG based spot filter	-
TOM20	1) Auto contrast normalization 2) 3D Gaussian smoothing	2D Frangi vesselness	Remove small objects
LAMP1	1) Auto contrast normalization 2) Edge-preserving smoothing	2D LoG based spot filter	1) Fill holes 2) Remove small objects
LC3B	1) Auto contrast normalization 2) 2D Gaussian smoothing	1) 3D LoG based spot filter 2) 3D Watershed	Remove small objects
RAB5	1) Auto contrast normalization 2) 2D Gaussian smoothing	2D LoG based spot filter	1) Fill holes 2) Remove small objects
SLC25A17	1) Auto contrast normalization 2) 2D Gaussian smoothing	3D LoG based spot filter	-
ACTB	1) Auto contrast normalization 2) Edge-preserving smoothing	2D Frangi vesselness	-
MYH10	1) Auto contrast normalization 2) Edge-preserving smoothing	2D Frangi vesselness	-
FBL	1) Auto contrast normalization 2) 3D Gaussian smoothing	3D LoG based spot filter	Remove small objects

LMNB1	1) Auto contrast normalization 2) 3D Gaussian smoothing	1) Obtain middle frame from intensity distribution 2) Obtain enclosing cell outline from middle frame with 2D Frangi vesselness. Find cell interior. 3) Seeded watershed and cell interior are used to obtain LMNB1 layer	-
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415 **Table S6:** Summary of image processing algorithms used to segment fluorophore-

416 tagged organelles.