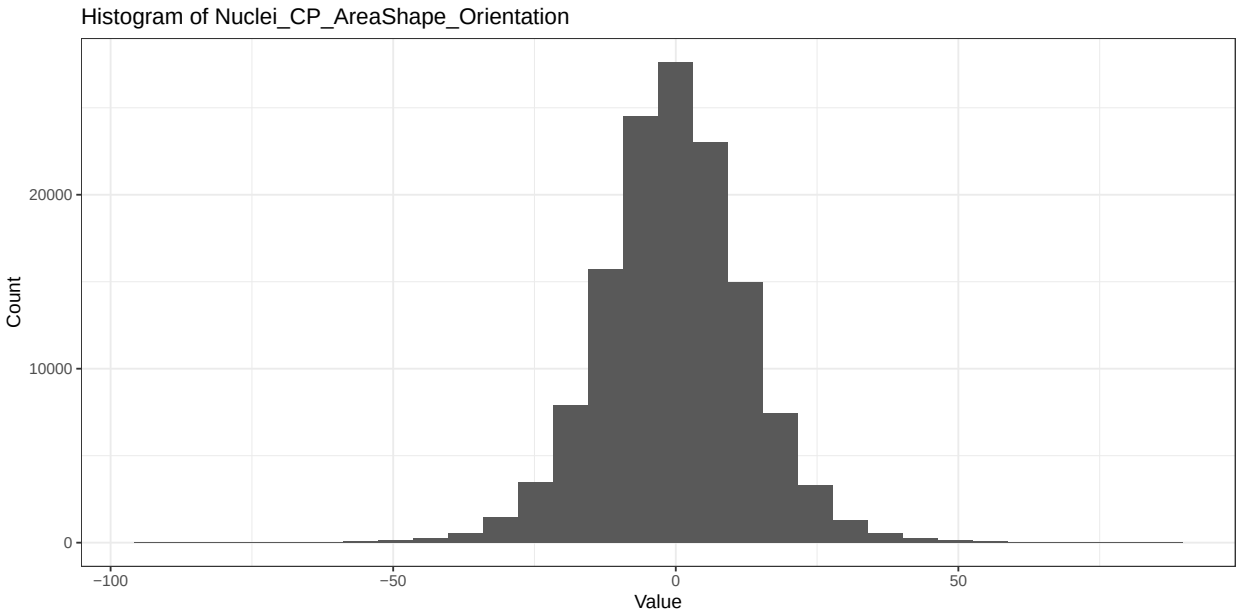

Figure 1: Histogram of nuclei orientation across all wells and spots.

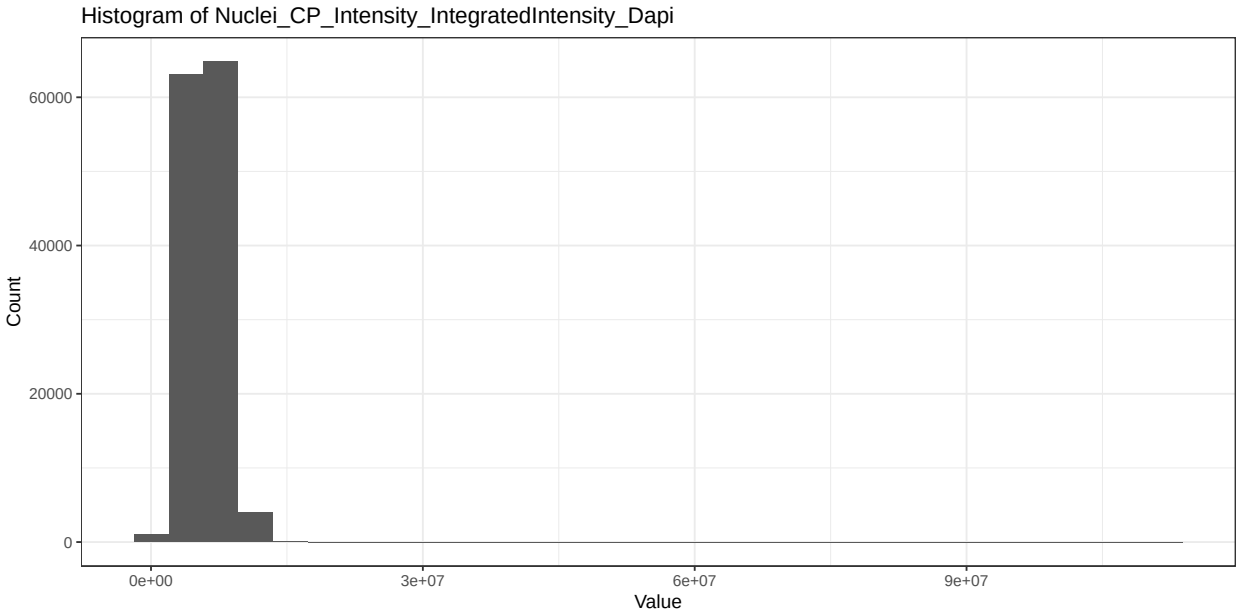

Figure 2: Histogram of total nuclei DAPI intensity across all wells and spots.

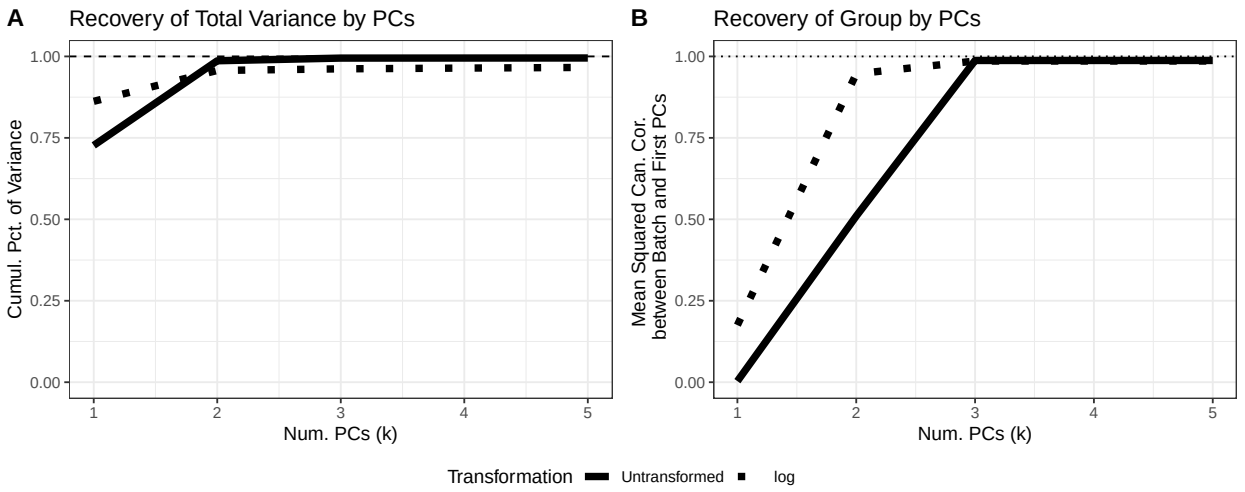

Figure 3: (A) The percentage of cumulative variance captured by first k principal components for both un-transformed data and log-transformed data. (B) The mean squared canonical correlations between the grouping factor and the first k principal components.

For clarity, we reproduce the explanation of (B) from main text.
We assess the transformations by measuring the percentage of the batch (group difference) captured by the first k singular vectors of the transformed feature matrix. Let $U = [u_1, \ldots, u_N] \in \mathbb{R}^{M \times N}$ be the (complete) left singular vectors of a feature matrix and $B \in \mathbb{R}^{M \times D}$ be the batch indicator matrix so that $B_{ij} = 1$ if well i is in batch j for $j = 1, \ldots, D$. Here we have $D = 2$ for the two groups. For $k = 1, \ldots, N$ and $t = 1 \ldots \min(k, D)$ define $C_k^{(t)}$ to be the t^{th} canonical correlation between the first k left singular vectors $U_k = [u_1, \ldots, u_k]$ and the batch B. Then let

$$C_k^2 = \frac{1}{D} \sum_{t=1}^{\min(k,D)} \left(C_k^{(t)} \right)^2$$

to be the average of these squared canonical correlations. We can interpret C_k^2 as the percentage of the batch B that is captured by these first k singular vectors.

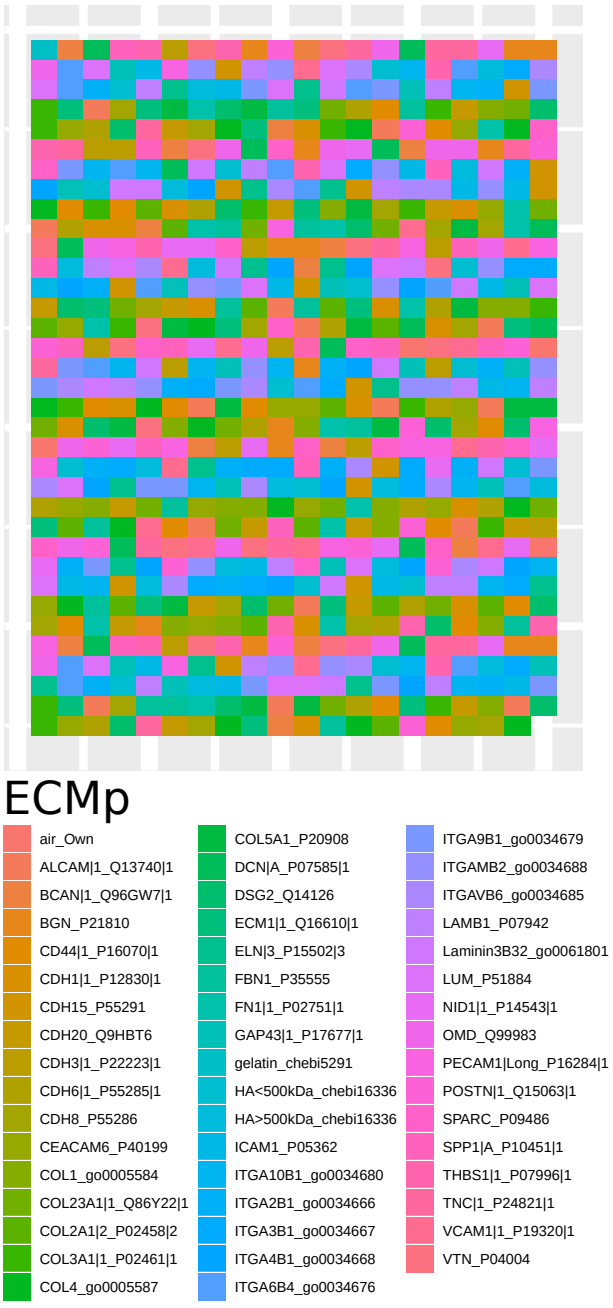

Figure 4: MEMA layout design of ECMps for each well. ECMp layout is identical across all wells.

Feature
Cells_CP_AreaShape_Area
Cells_CP_AreaShape_Compactness
Cells_CP_AreaShape_Eccentricity
Cells_CP_AreaShape_Extent
Cells_CP_AreaShape_FormFactor
Cells_CP_AreaShape_MajorAxisLength
Cells_CP_AreaShape_MaxFeretDiameter
Cells_CP_AreaShape_MaximumRadius
Cells_CP_AreaShape_MeanRadius
Cells_CP_AreaShape_MedianRadius
Cells_CP_AreaShape_MinFeretDiameter
Cells_CP_AreaShape_MinorAxisLength
Cells_CP_AreaShape_Perimeter
Cells_CP_AreaShape_Solidity
Cytoplasm_CP_AreaShape_Area
Cytoplasm_CP_AreaShape_Compactness
Cytoplasm_CP_AreaShape_Eccentricity
Cytoplasm_CP_AreaShape_Extent
Cytoplasm_CP_AreaShape_FormFactor
Cytoplasm_CP_AreaShape_MajorAxisLength
Cytoplasm_CP_AreaShape_MaxFeretDiameter
Cytoplasm_CP_AreaShape_MaximumRadius
Cytoplasm_CP_AreaShape_MeanRadius
Cytoplasm_CP_AreaShape_MedianRadius
Cytoplasm_CP_AreaShape_MinFeretDiameter
Cytoplasm_CP_AreaShape_MinorAxisLength
Cytoplasm_CP_AreaShape_Perimeter
Cytoplasm_CP_AreaShape_Solidity
Nuclei_CP_AreaShape_Area
Nuclei_CP_AreaShape_Compactness
Nuclei_CP_AreaShape_Eccentricity
Nuclei_CP_AreaShape_Extent
Nuclei_CP_AreaShape_FormFactor
Nuclei_CP_AreaShape_MajorAxisLength
Nuclei_CP_AreaShape_MaxFeretDiameter
Nuclei_CP_AreaShape_MaximumRadius
Nuclei_CP_AreaShape_MeanRadius
Nuclei_CP_AreaShape_MedianRadius
Nuclei_CP_AreaShape_MinFeretDiameter
Nuclei_CP_AreaShape_MinorAxisLength
Nuclei_CP_AreaShape_Orientation
Nuclei_CP_AreaShape_Perimeter
Nuclei_CP_AreaShape_Solidity
Cells_CP_Intensity_IntegratedIntensity_CellMask
Cells_CP_Intensity_IntegratedIntensity_KRT19
Cells_CP_Intensity_IntegratedIntensity_KRT5
Cells_CP_Intensity_MedianIntensity_CellMask
Cells_CP_Intensity_MedianIntensity_KRT19
Cells_CP_Intensity_MedianIntensity_KRT5
Cytoplasm_CP_Intensity_IntegratedIntensity_CellMask
Cytoplasm_CP_Intensity_IntegratedIntensity_Dapi
Cytoplasm_CP_Intensity_IntegratedIntensity_KRT19
Cytoplasm_CP_Intensity_IntegratedIntensity_KRT5
Cytoplasm_CP_Intensity_MedianIntensity_CellMask
Cytoplasm_CP_Intensity_MedianIntensity_Dapi
Cytoplasm_CP_Intensity_MedianIntensity_KRT19
Cytoplasm_CP_Intensity_MedianIntensity_KRT5
Nuclei_CP_Intensity_IntegratedIntensity_Dapi
Nuclei_CP_Intensity_IntegratedIntensity_KRT19
Nuclei_CP_Intensity_IntegratedIntensity_KRT5
Nuclei_CP_Intensity_MedianIntensity_Dapi
Nuclei_CP_Intensity_MedianIntensity_KRT19
Nuclei_CP_Intensity_MedianIntensity_KRT5
Cytoplasm_PA_Intensity_LineageRatio
Spot_PA_SpotCellCount
Cells_CP_Intensity_IntegratedIntensity_Actin
Cells_CP_Intensity_IntegratedIntensity_MitoTracker
Cells_CP_Intensity_MedianIntensity_Actin
Cells_CP_Intensity_MedianIntensity_MitoTracker
Cytoplasm_CP_Intensity_IntegratedIntensity_Actin
Cytoplasm_CP_Intensity_IntegratedIntensity_MitoTracker
Cytoplasm_CP_Intensity_MedianIntensity_Actin
Cytoplasm_CP_Intensity_MedianIntensity_MitoTracker
Nuclei_CP_Texture_AngularSecondMoment_Fibrillarin_3_0
Nuclei_CP_Texture_AngularSecondMoment_Fibrillarin_3_90
Nuclei_CP_Texture_Contrast_Fibrillarin_3_0
Nuclei_CP_Texture_Contrast_Fibrillarin_3_90
Nuclei_CP_Texture_Correlation_Fibrillarin_3_0
Nuclei_CP_Texture_Correlation_Fibrillarin_3_90
Nuclei_CP_Texture_DifferenceEntropy_Fibrillarin_3_0
Nuclei_CP_Texture_DifferenceEntropy_Fibrillarin_3_90
Nuclei_CP_Texture_DifferenceVariance_Fibrillarin_3_0
Nuclei_CP_Texture_DifferenceVariance_Fibrillarin_3_90
Nuclei_CP_Texture_Entropy_Fibrillarin_3_0
Nuclei_CP_Texture_Entropy_Fibrillarin_3_90
Nuclei_CP_Texture_InfoMeas1_Fibrillarin_3_0
Nuclei_CP_Texture_InfoMeas1_Fibrillarin_3_90
Nuclei_CP_Texture_InfoMeas2_Fibrillarin_3_0
Nuclei_CP_Texture_InfoMeas2_Fibrillarin_3_90
Nuclei_CP_Texture_InverseDifferenceMoment_Fibrillarin_3_0
Nuclei_CP_Texture_InverseDifferenceMoment_Fibrillarin_3_90
Nuclei_CP_Texture_SumAverage_Fibrillarin_3_0
Nuclei_CP_Texture_SumAverage_Fibrillarin_3_90
Nuclei_CP_Texture_SumEntropy_Fibrillarin_3_0
Nuclei_CP_Texture_SumEntropy_Fibrillarin_3_90
Nuclei_CP_Texture_SumVariance_Fibrillarin_3_0
Nuclei_CP_Texture_SumVariance_Fibrillarin_3_90
Nuclei_CP_Texture_Variance_Fibrillarin_3_0
Nuclei_CP_Texture_Variance_Fibrillarin_3_90
Nuclei_CP_Intensity_IntegratedIntensity_EdU
Nuclei_CP_Intensity_IntegratedIntensity_Fibrillarin
Nuclei_CP_Intensity_MedianIntensity_EdU
Nuclei_CP_Intensity_MedianIntensity_Fibrillarin

Table 1: List of all features extracted from at least one MEMA plate.

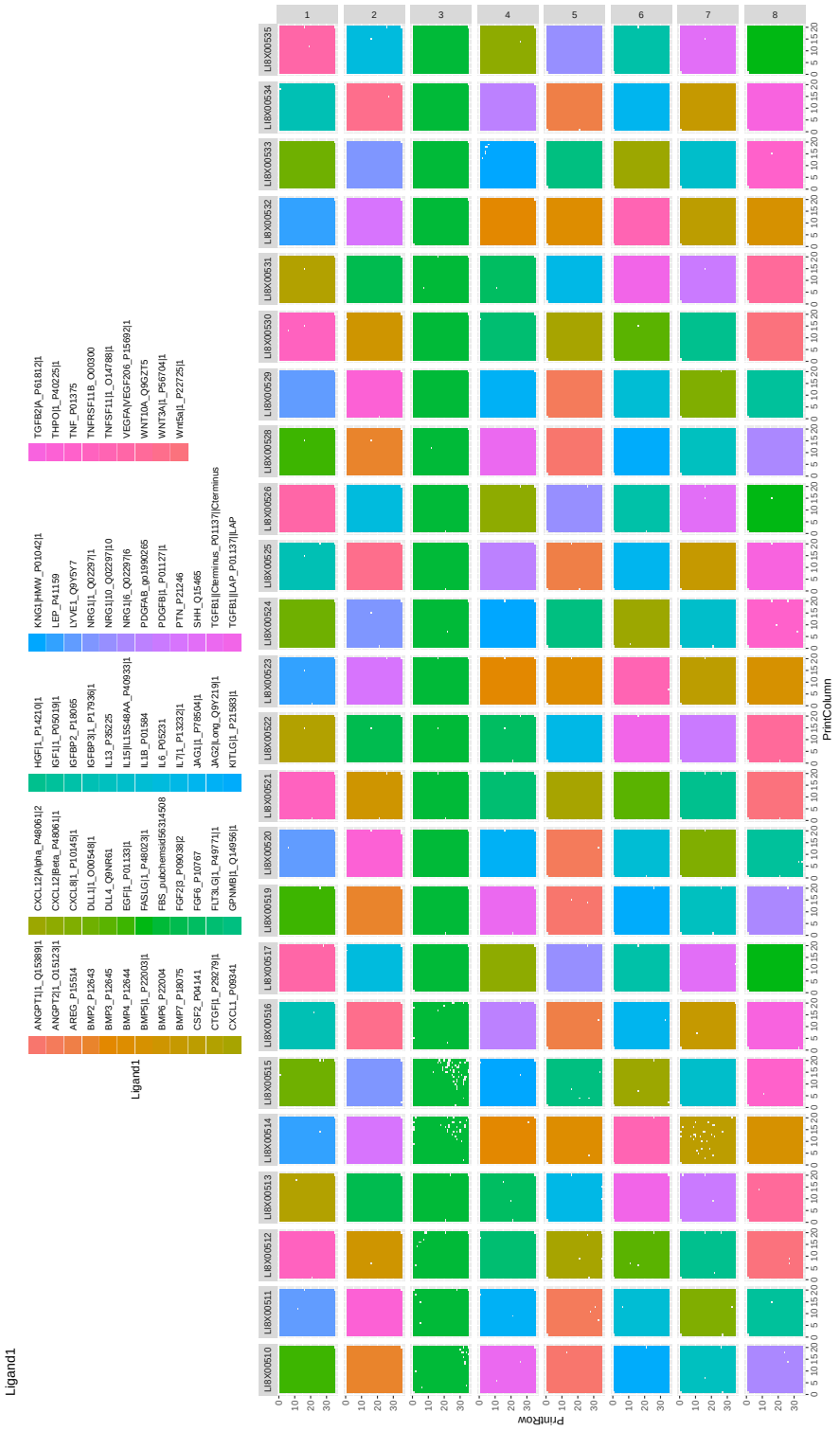

Figure 5: MEMA layout of ligands across plates and wells. Each row is a plate consisting of eight wells (columns). Color indicates ligand added to buffer solution of the well.

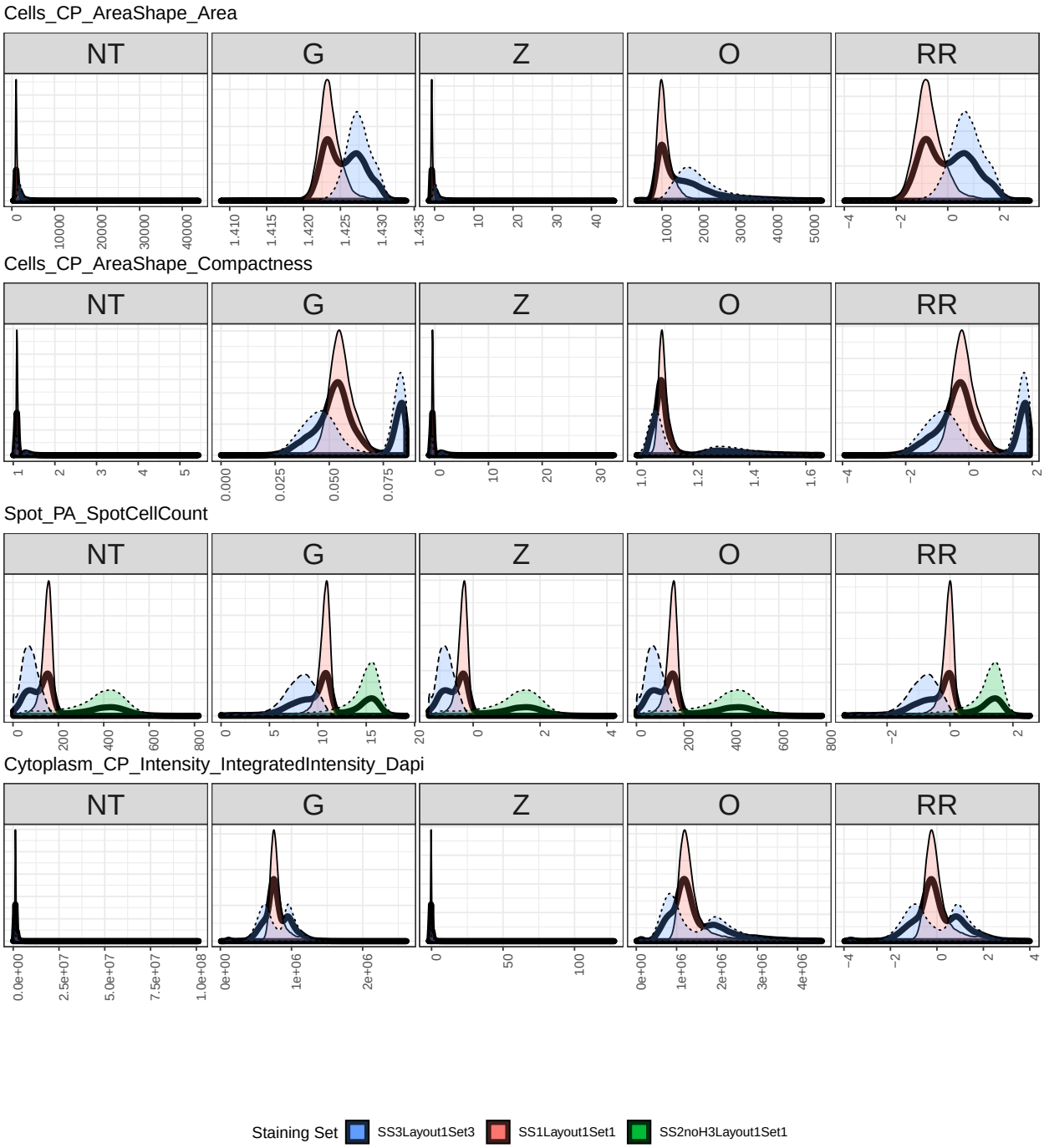

Figure 6: Density of elements of feature matrices. Black density is all elements combined. Colored densities are the densities denote staining batch. Subplots are for five processing transformations of this matrix: (NT) no transformation, (G) Gaussianization, (Z) z-score, (O) outlier removal, (RR) the three-step (G), (Z), and (O), robust re-scaling.

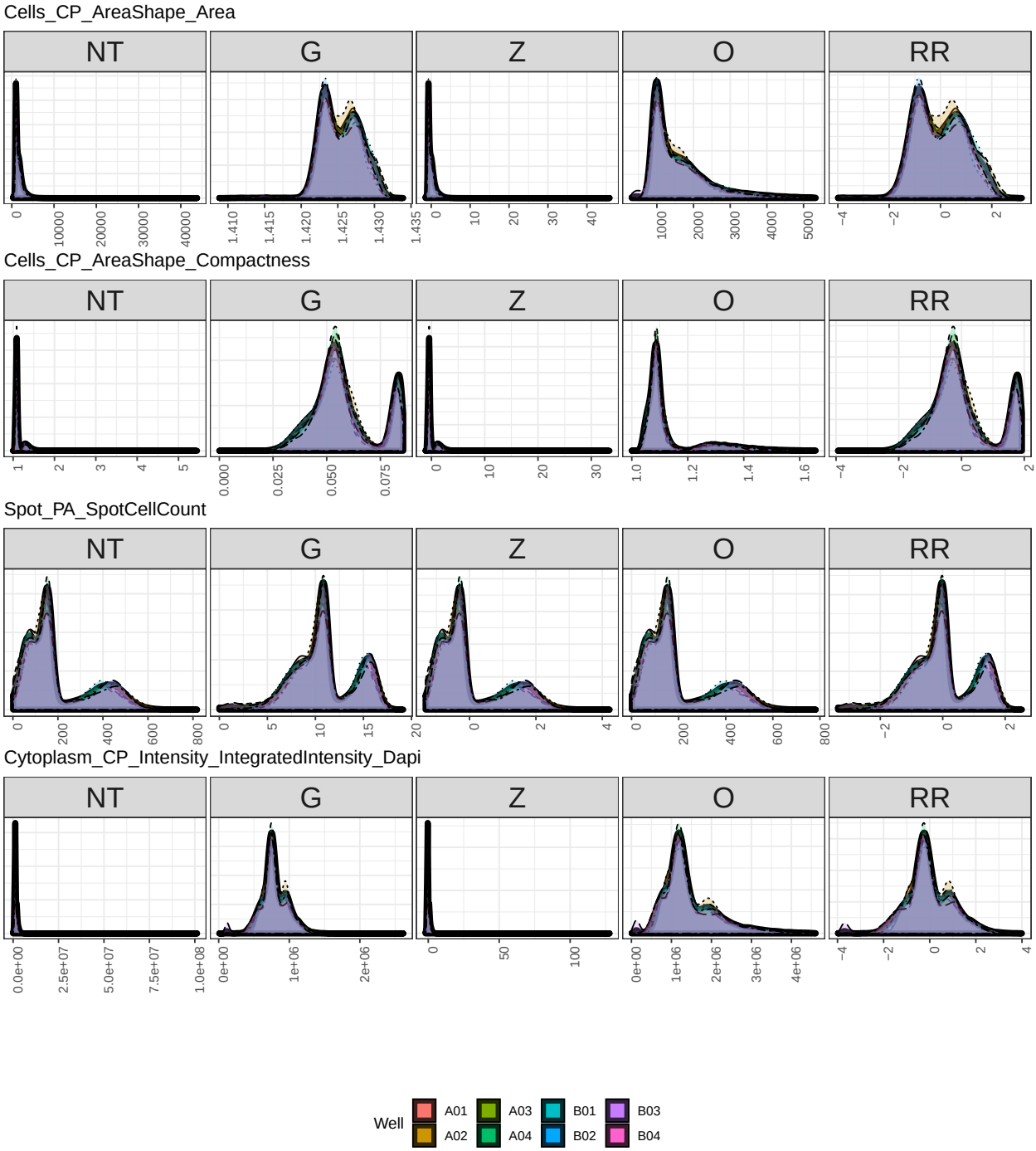

Figure 7: Similar to Figure 6 except colors indicate well.

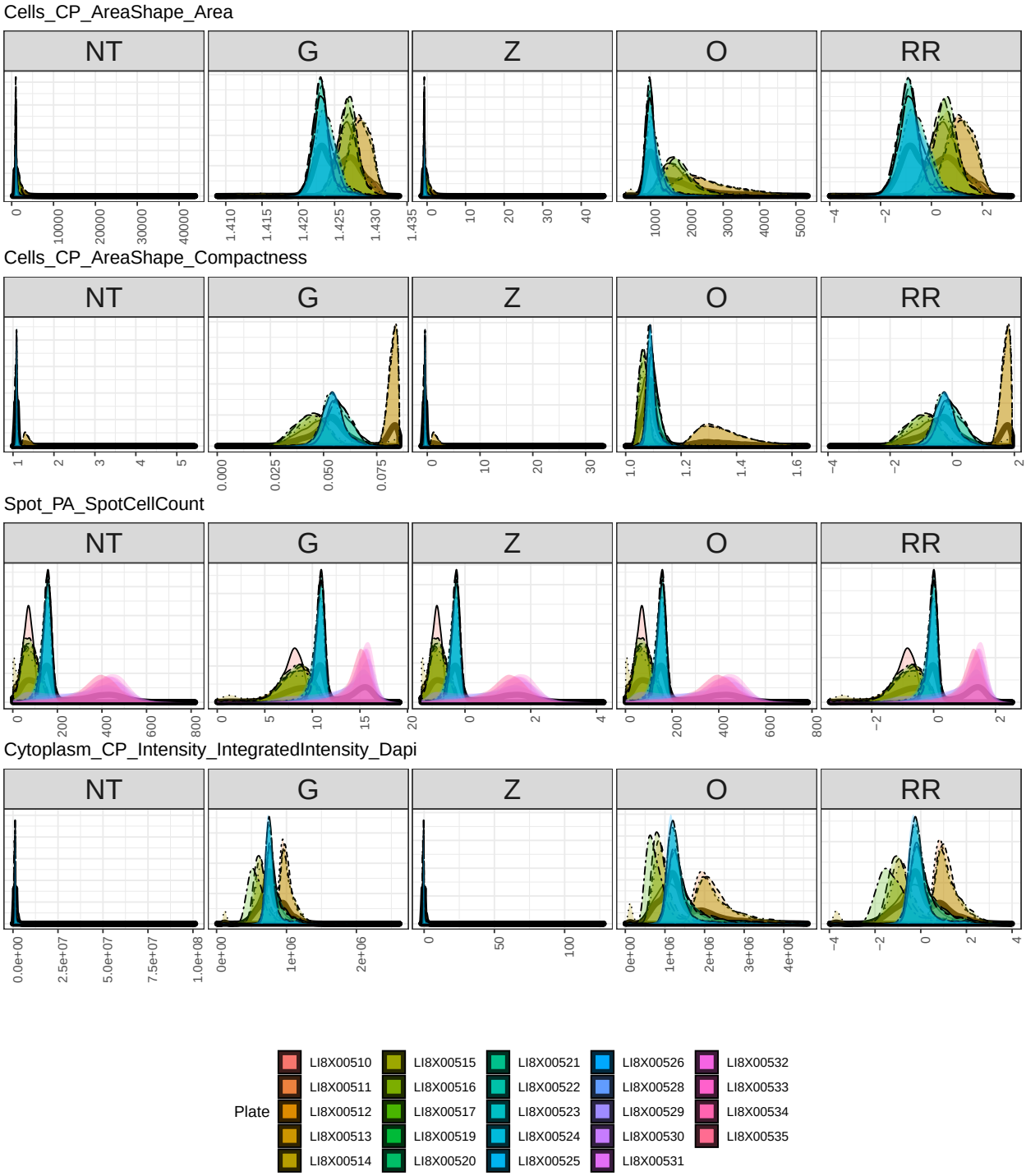

Figure 8: Similar to Figure 6 except colors indicate plate.

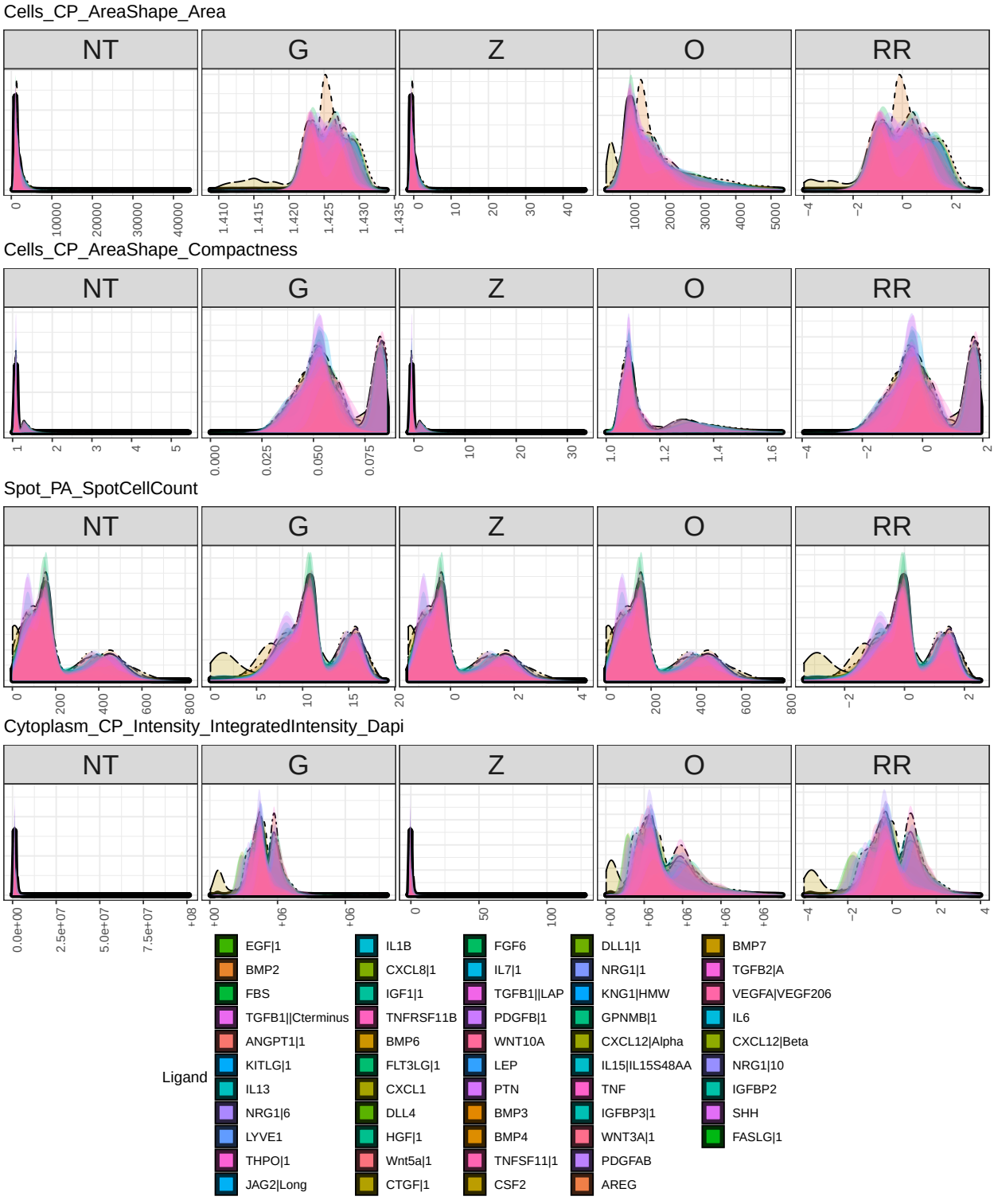

Figure 9: Similar to Figure 6 except colors indicate ligand.

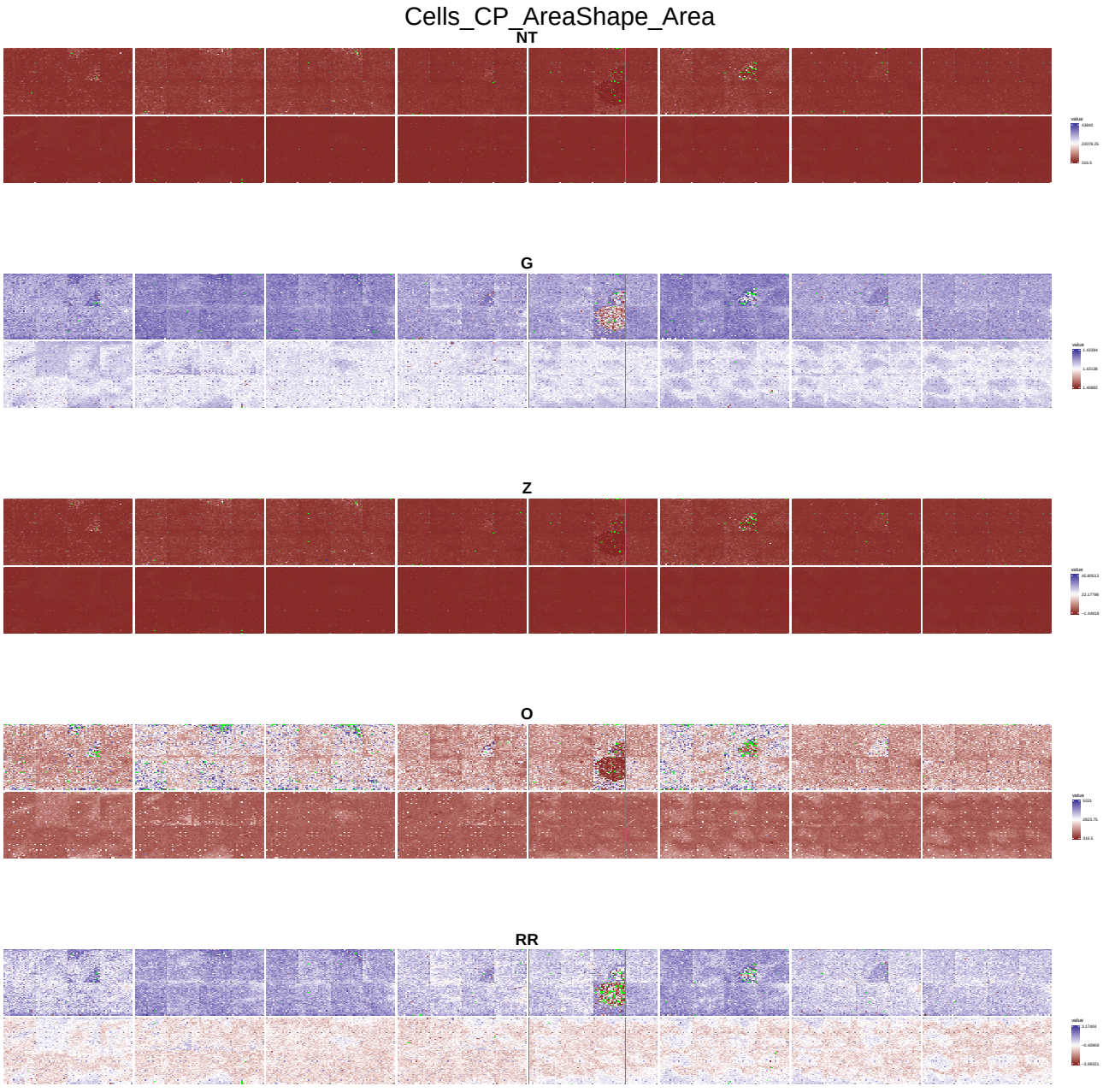

Figure 10: The next series of plots are heat-maps of MEMA plates across the five transformations (NT), (G), (Z), (O), (RR). Rows of each plot are the staining three batches. Colors are more blue if they are close to the minimum, red if they are close to the maximum, and white if they are close to half-way between. Green spots are missing. Dark grey spots are omitted according to the MEMA design.

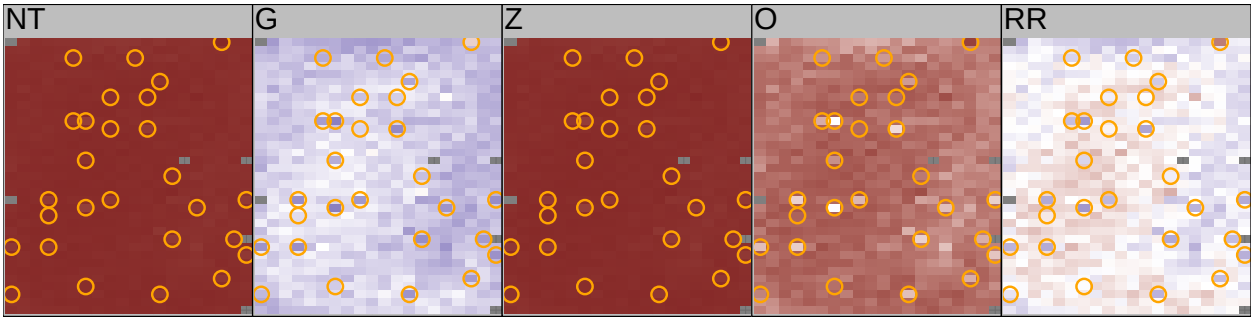

Figure 11: Heat map of a single well across the five transformations (NT), (G), (Z), (O), (RR). This is a sub-plot of Supplementary Figure 10. Color scaled is determined globally over all spots, wells, and plates in the dataset to reflect the fact that the transformation is similarly calculated over this data. Thus we see no blue in this (NT) sub-plot as we see almost no blue in Supplementary Figure 10. This plot is a representative microcosm of the larger plot. Orange circles highlight the ELN and NID1 spots.

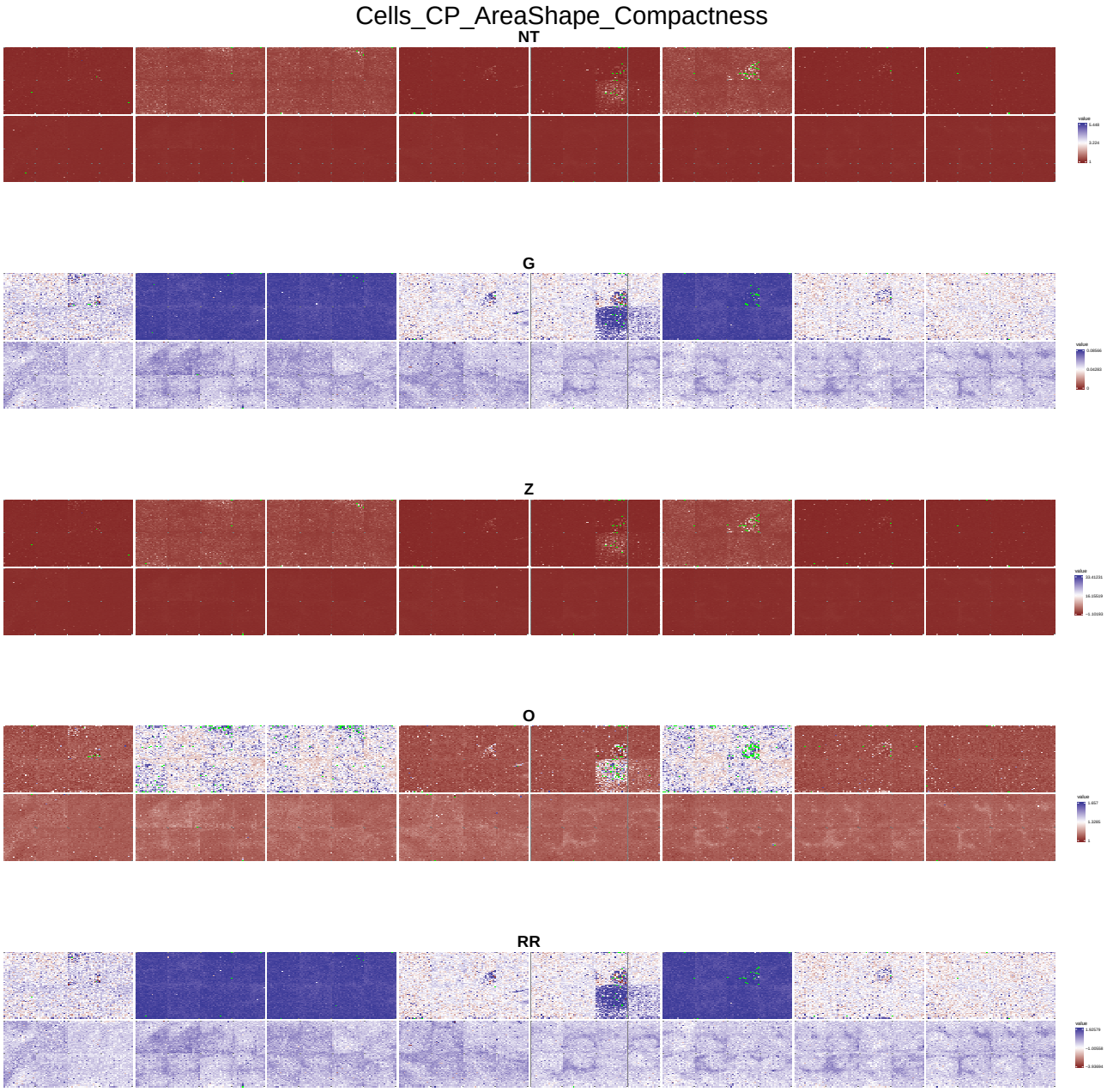

Figure 12: Similar to Figure 10 but for compactness.

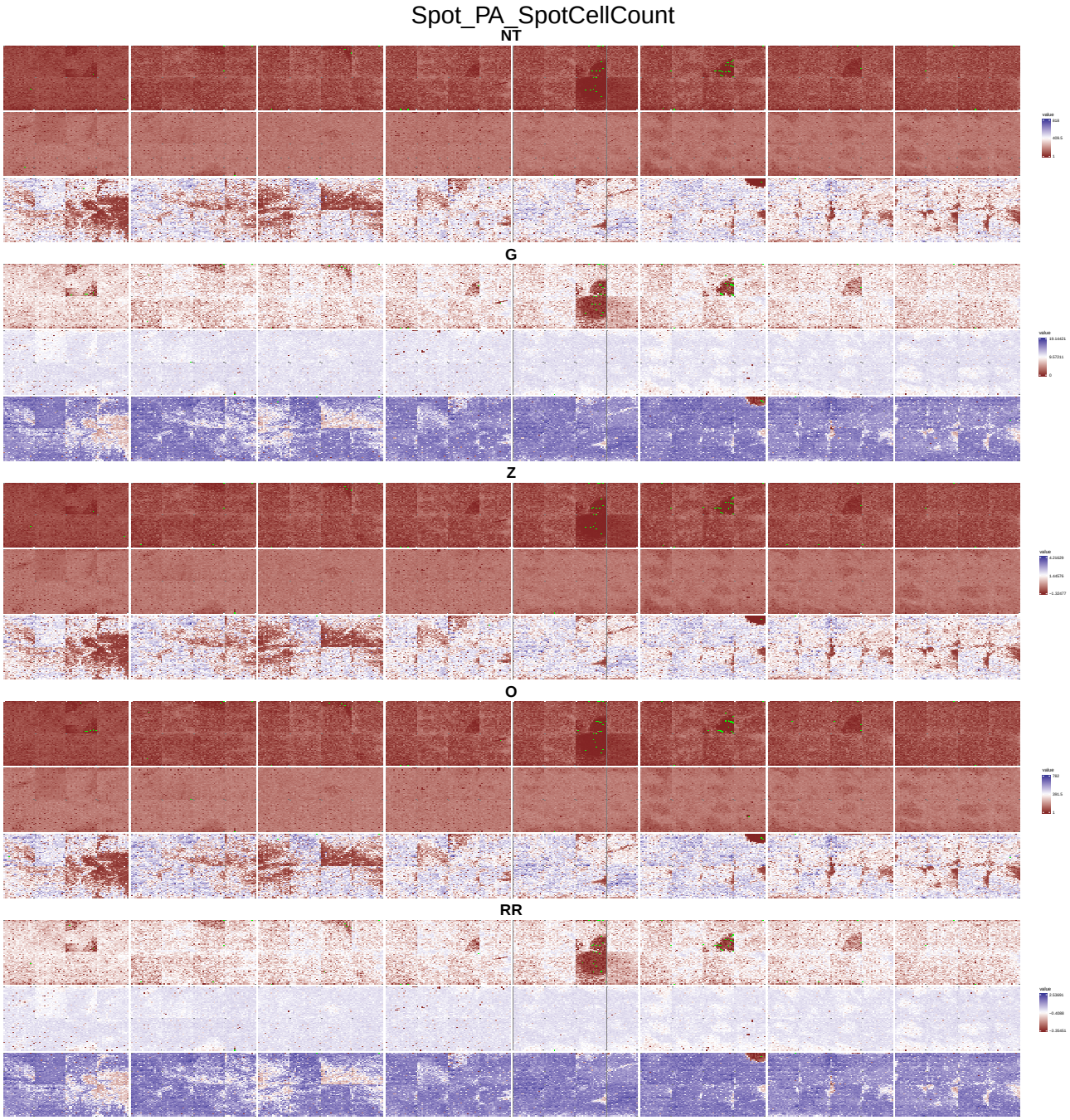

Figure 13: Similar to Figure 10 but for cell count.

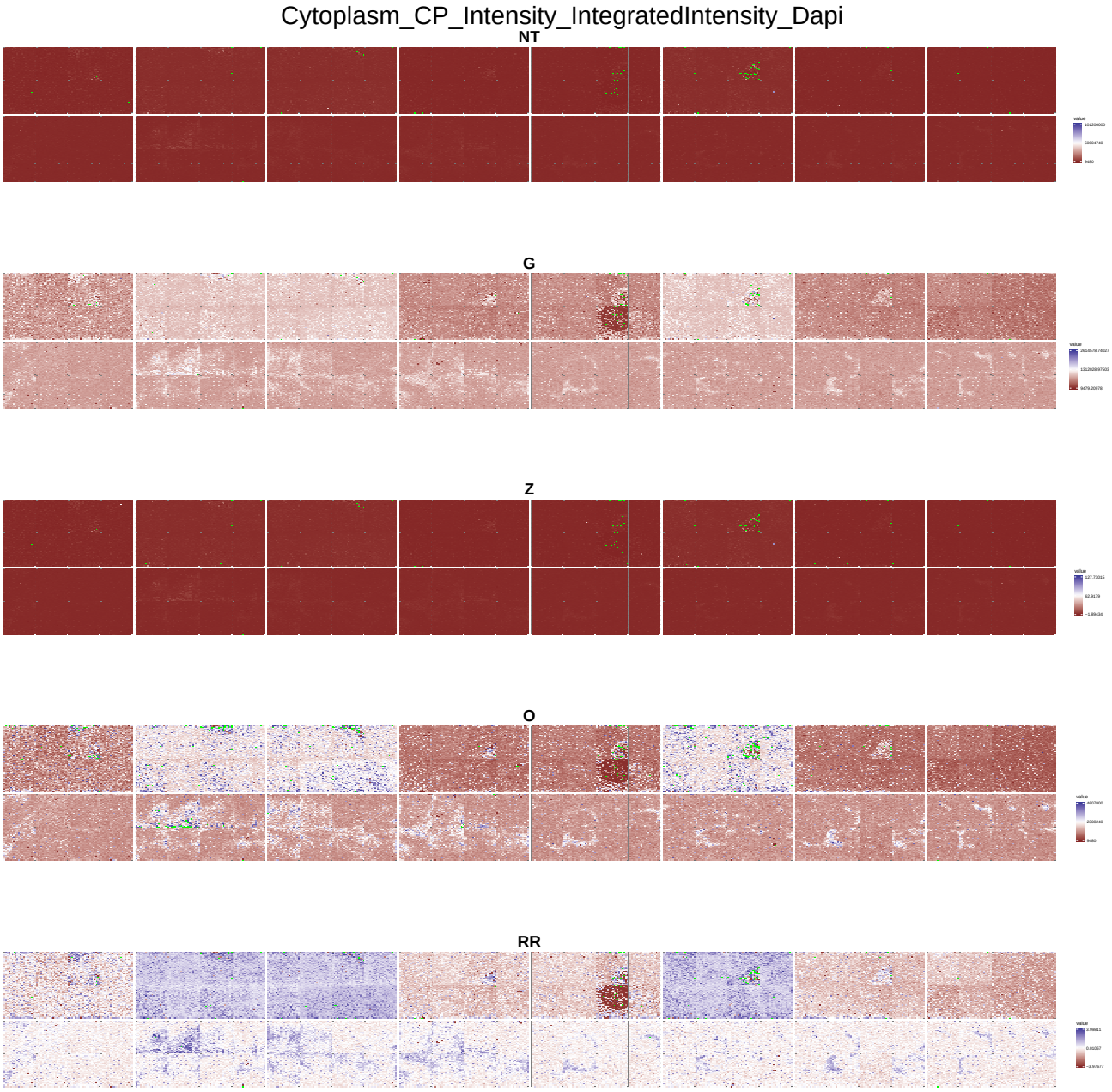

Figure 14: Similar to Figure 10 for for DAPI intensity.

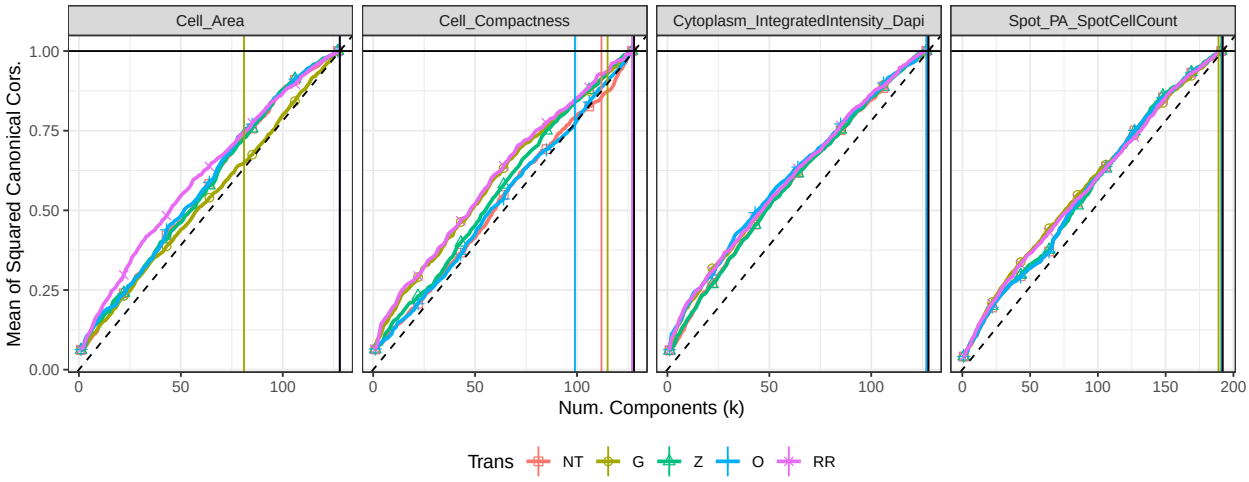

Figure 15: Mean of the squared canonical correlations between the first k principal components and the plate batch indicator variables.

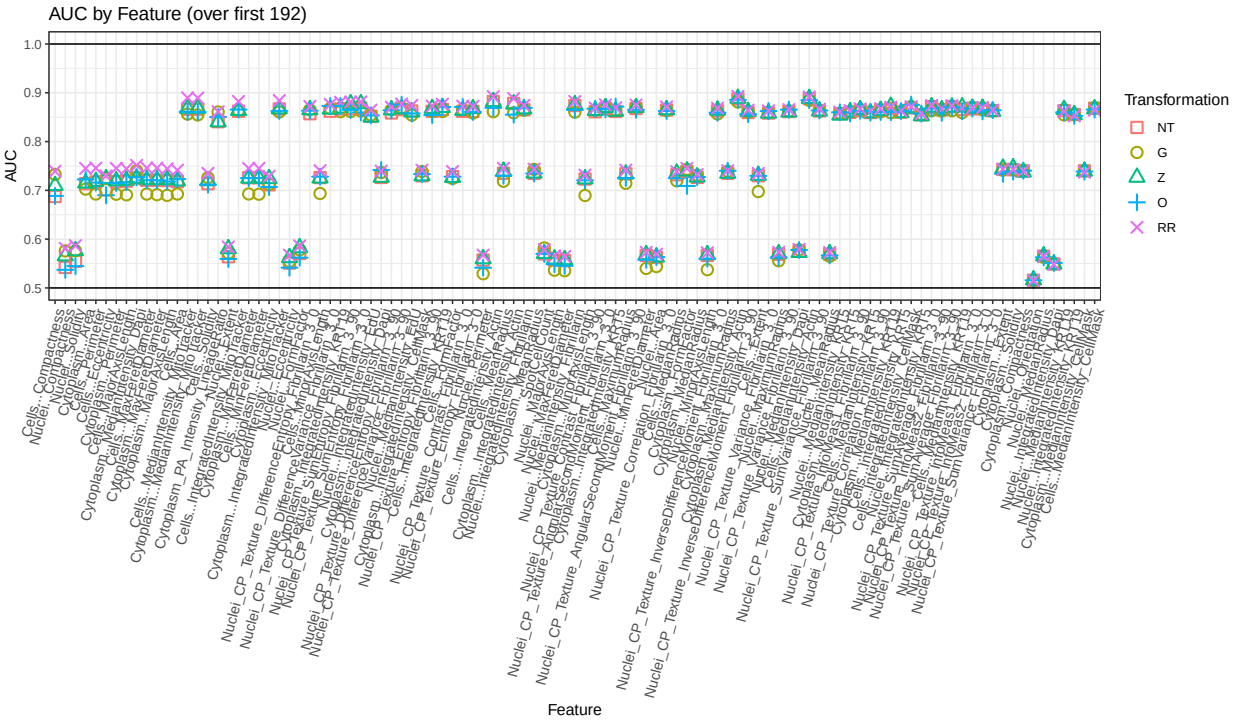

Figure 16: Grand mean of the squared canonical correlations across number of components (k). Canonical correlation is calculated between the first k principal components and the plate indicator variables.

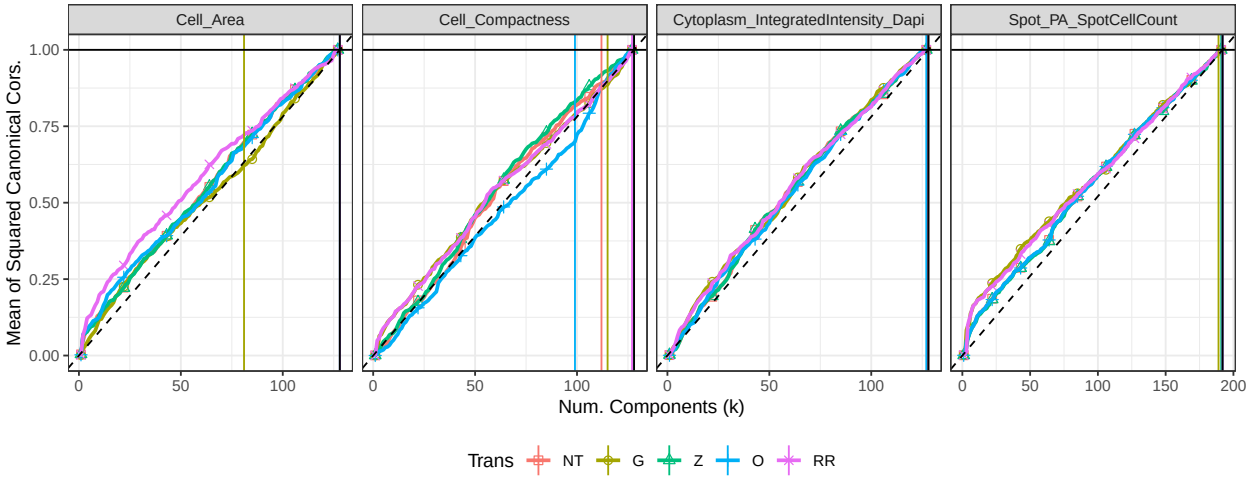

Figure 17: Similar to Figure 15 except correlation with well batch indicators.

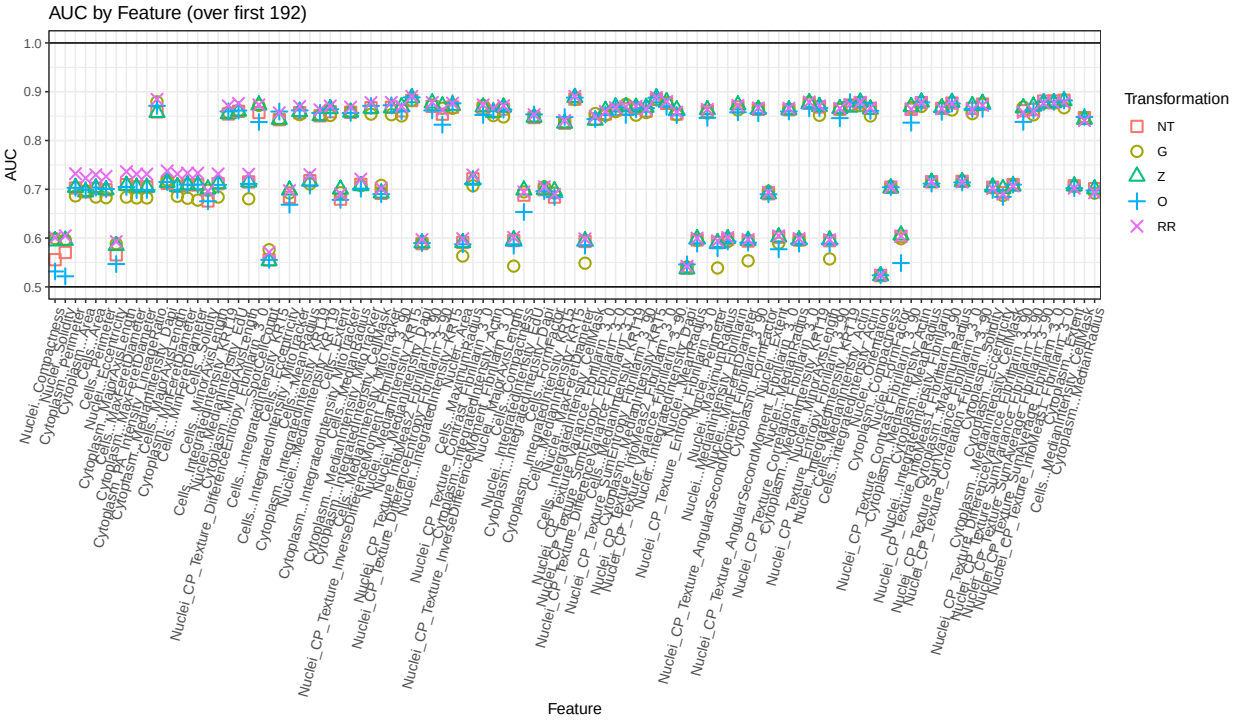

Figure 18: Similar to Figure 16 except correlation with well batch indicators.

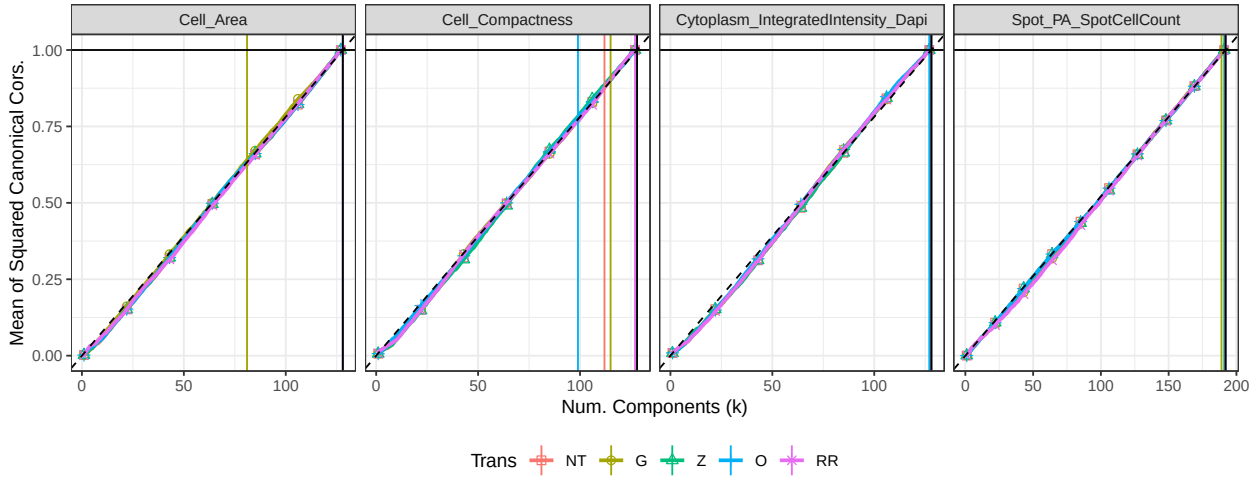

Figure 19: Similar to Figure 15 except correlation with ligand batch indicators.

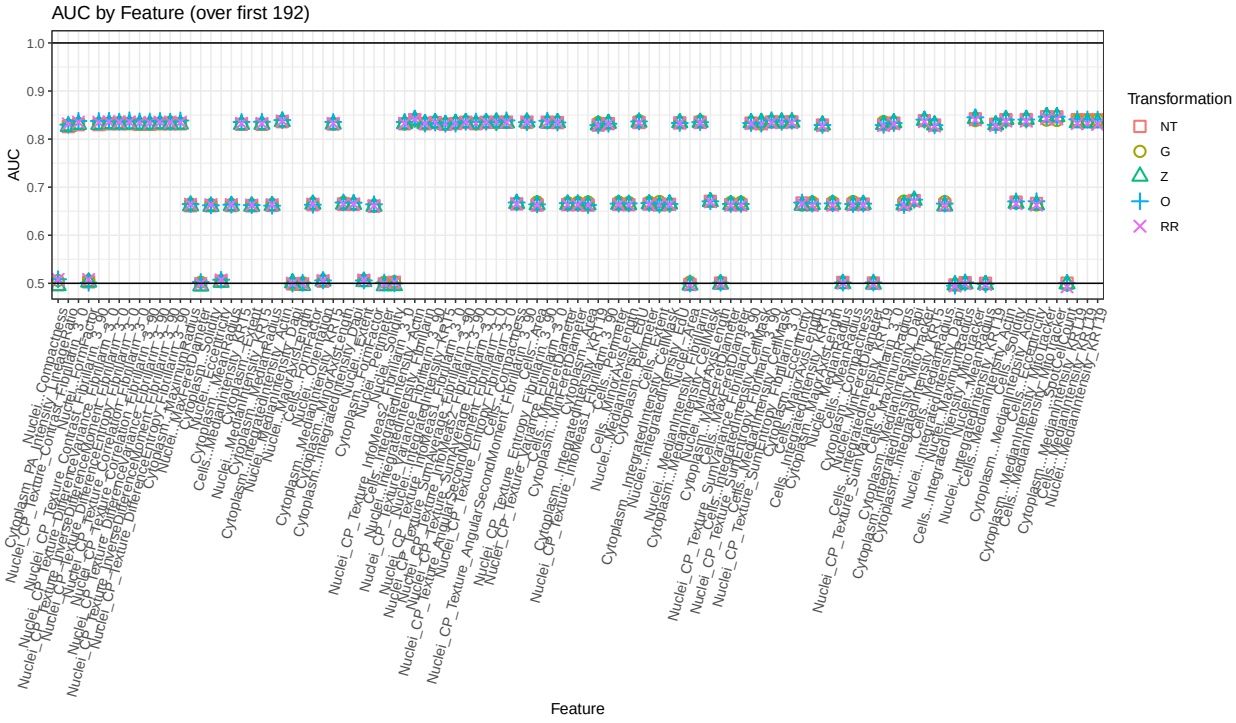

Figure 20: Similar to Figure 16 except correlation with well batch indicators.

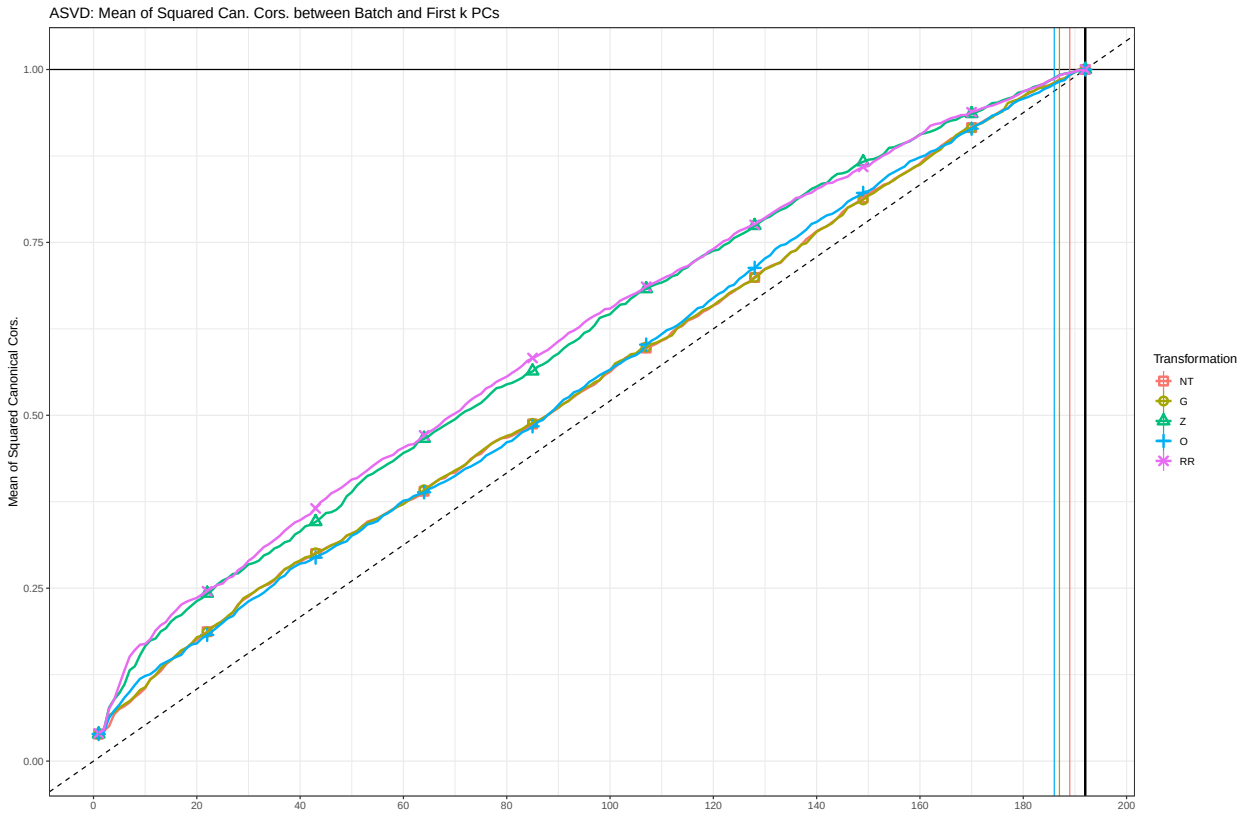

Figure 21: Mean of the squared canonical correlations between the first k principal components and the plate indicator variables. Principal components come from integration of the 21 features that are measured across all MEMAs.

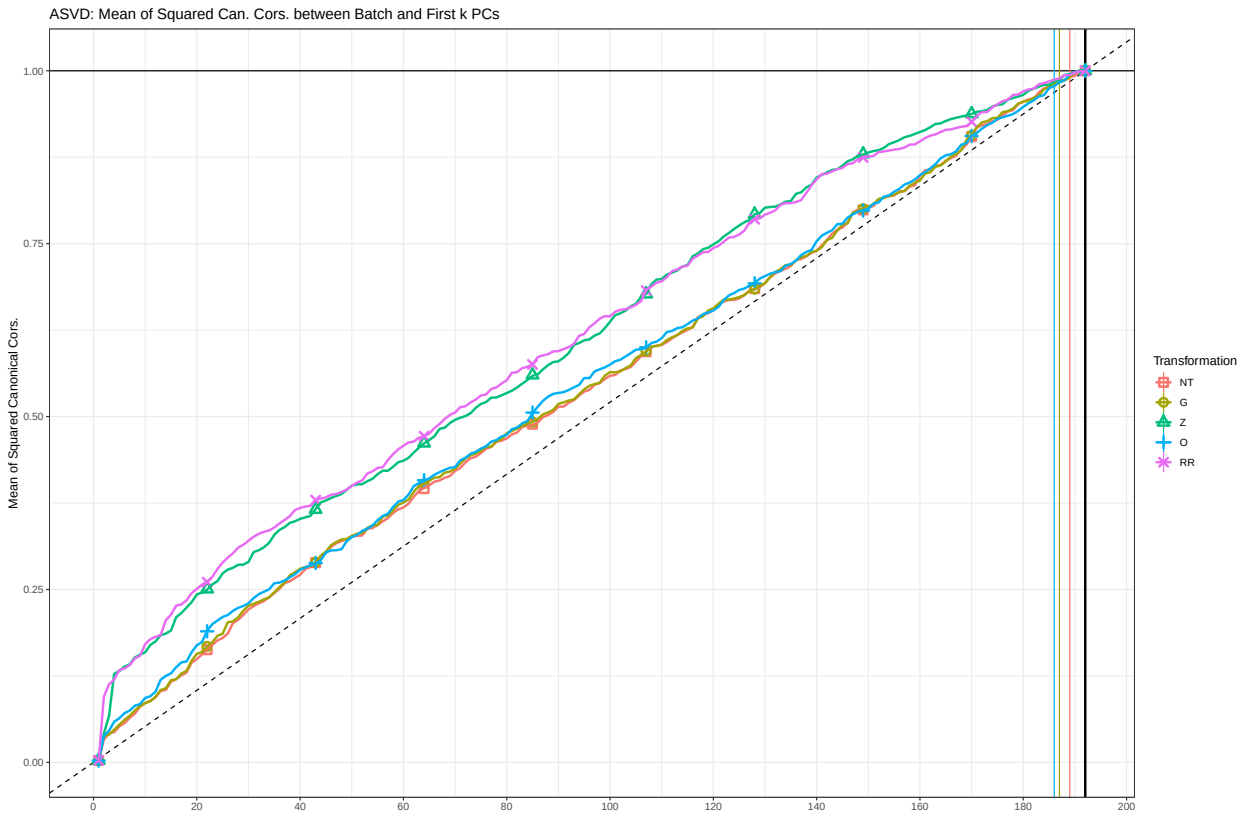

Figure 22: Similar to Figure 21 but calculating correlation with well indicators.

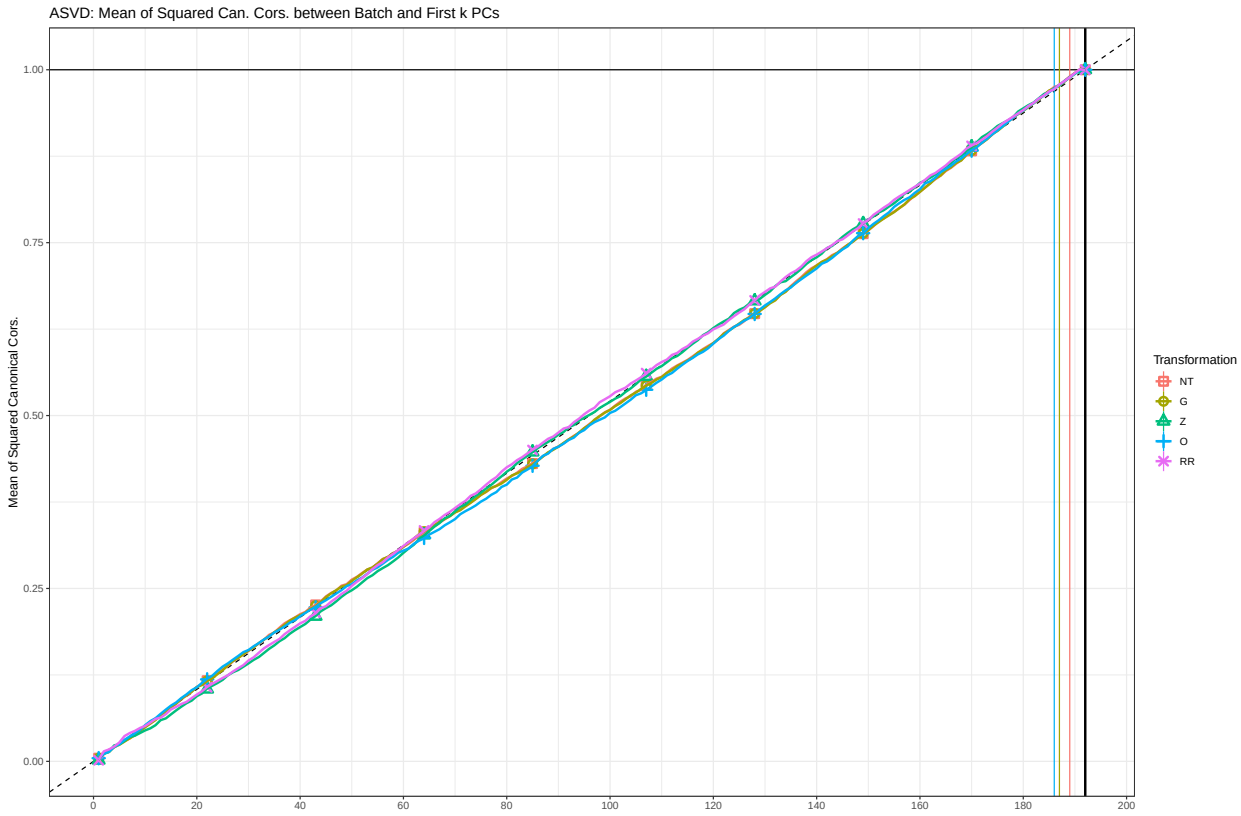

Figure 23: Similar to Figure 21 but calculating correlation with ligand indicators.

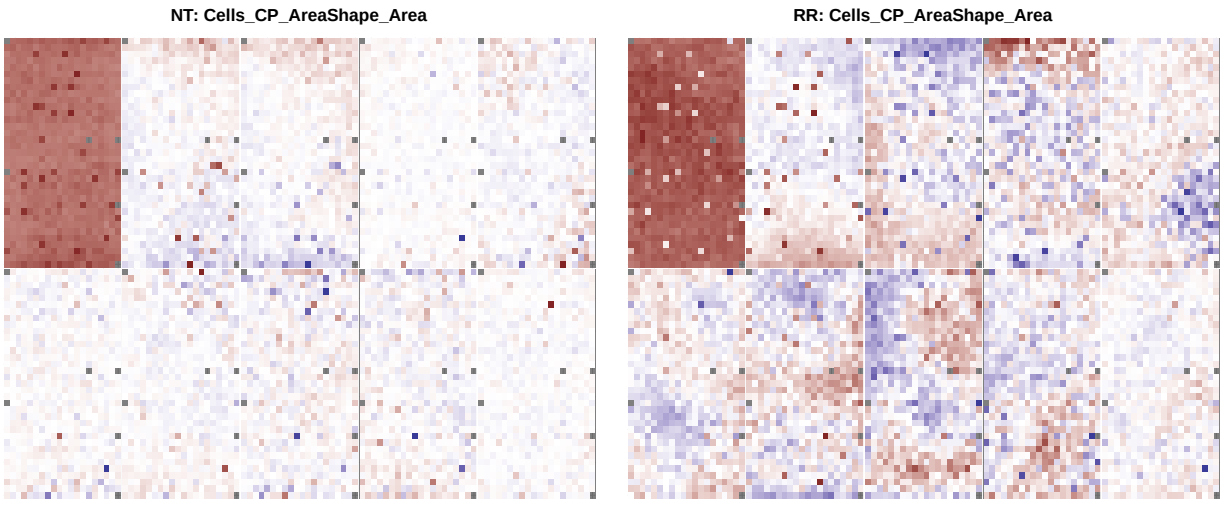

Figure 24: Heat map of elements of top 3 right singular vectors for the cell area feature.

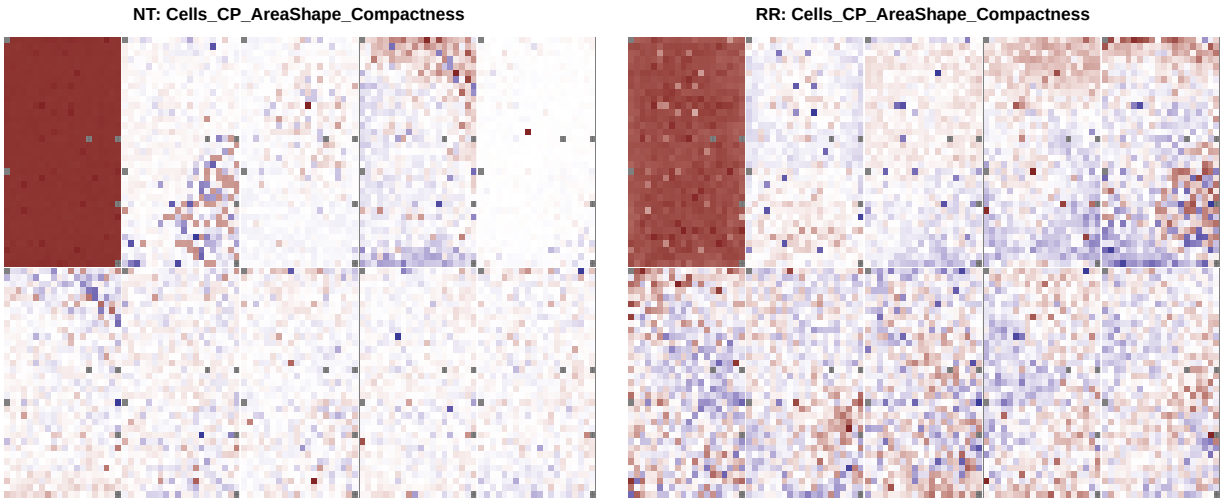

Figure 25: Similar to Figure 24 but for cell compactness feature.

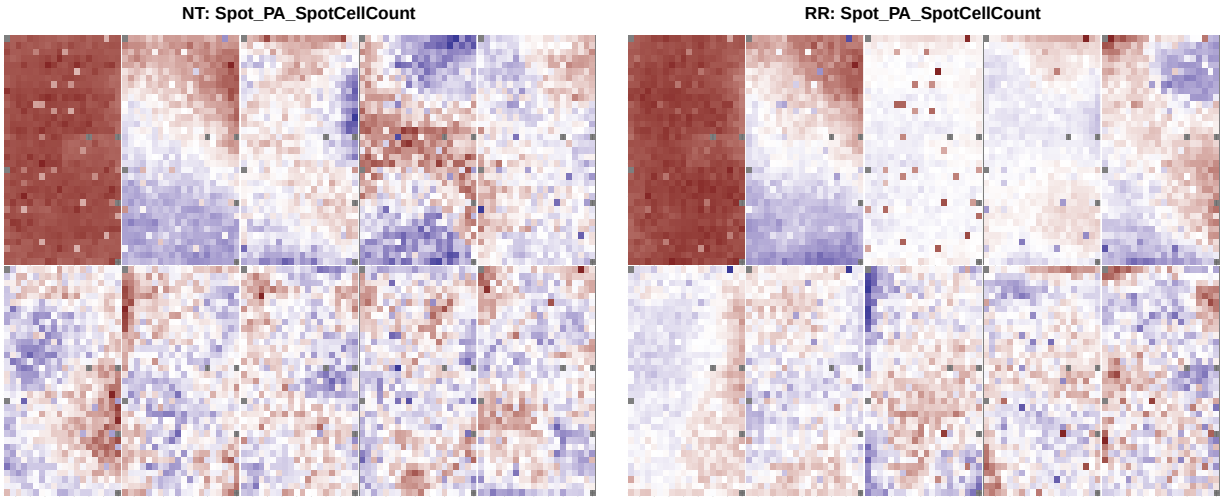

Figure 26: Similar to Figure 24 but for cell count feature.

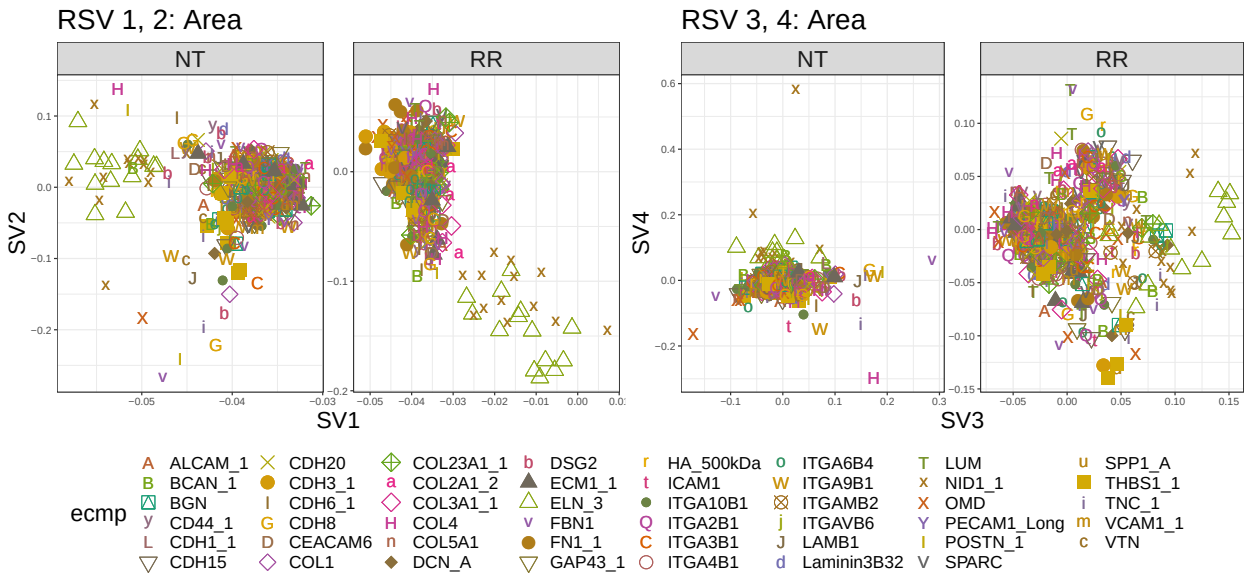

Figure 27: Scatter plot of elements of top two right singular vectors against each other for the cell area feature. Shape and color indicate ECMp of the spot corresponding to the elements of the singular vector.

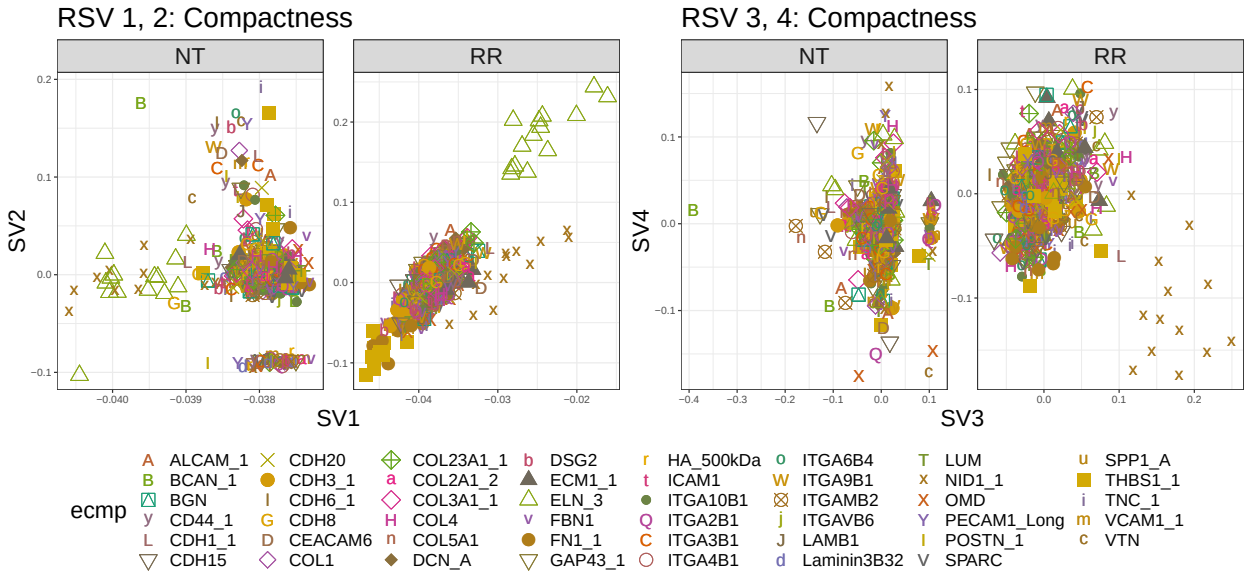

Figure 28: Similar to Figure 27 but for cell compactness feature.

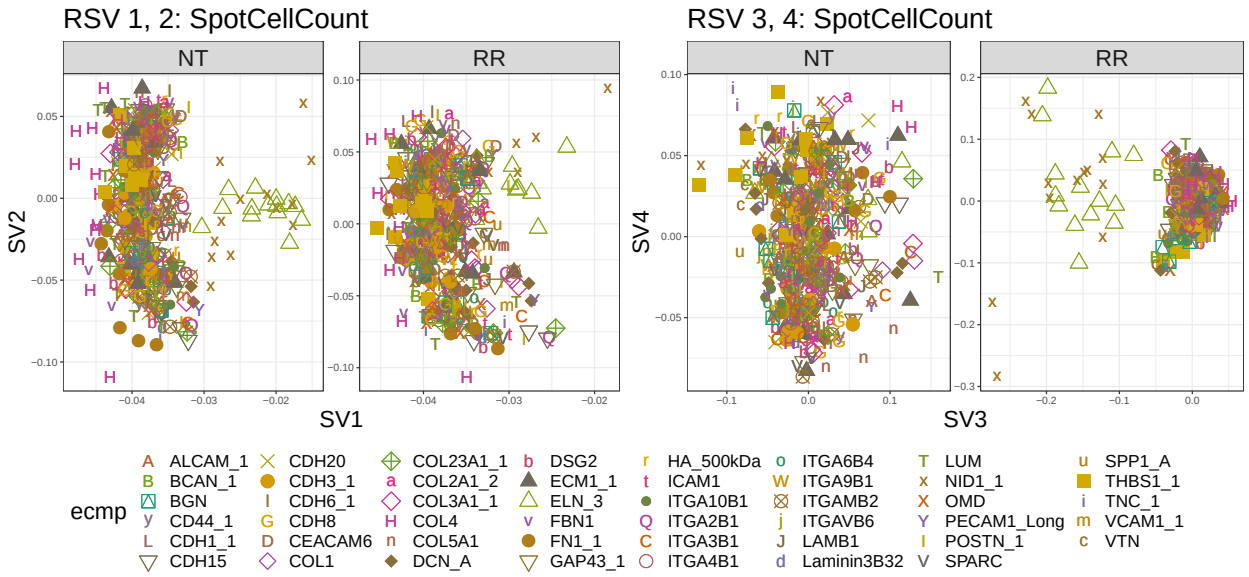

Figure 29: Similar to Figure 27 but for cell count feature.

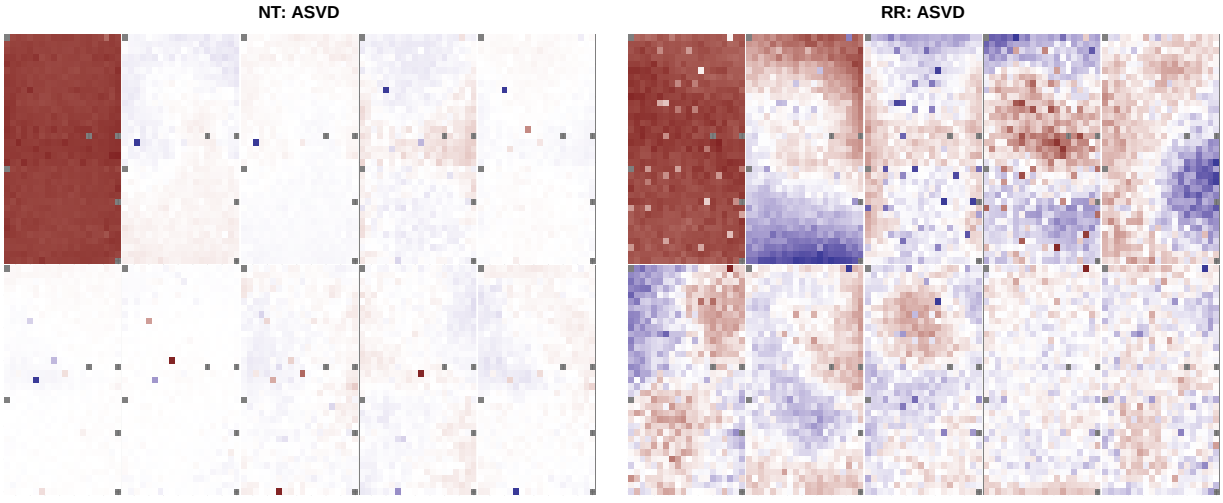

Figure 30: Heat-map of top 3 right ASVs calculated over 21 features measured on all MEMAs.

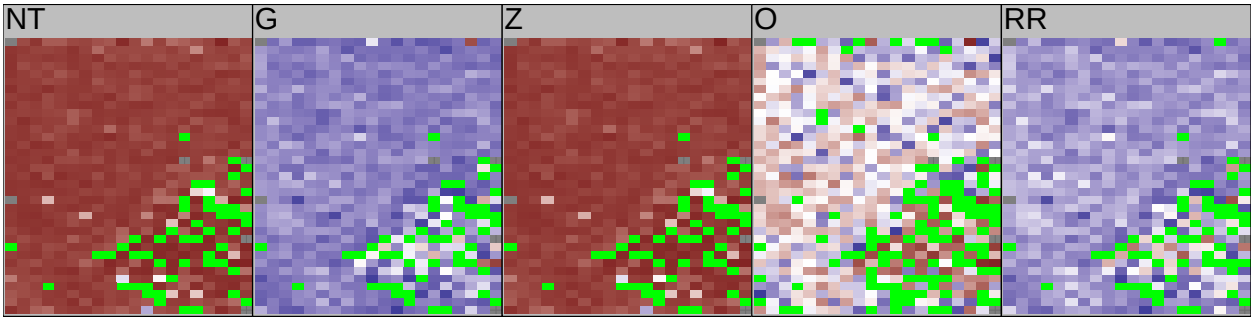

Figure 31: Missing values for well A03 on outlier plate LI8X00515. This plate is an outlier because it was processed using a different version of imaging processing software. Missing spots are indicated in green. Other colors indicate cell compactness feature. Notice that the missing values for (NT) are nearly identical to the the dark red spots in the second right singular vector in Supplementary Figure 25. This is what forms the group structure in Figure 11 as these missing spots are picked up on the outlying plate.

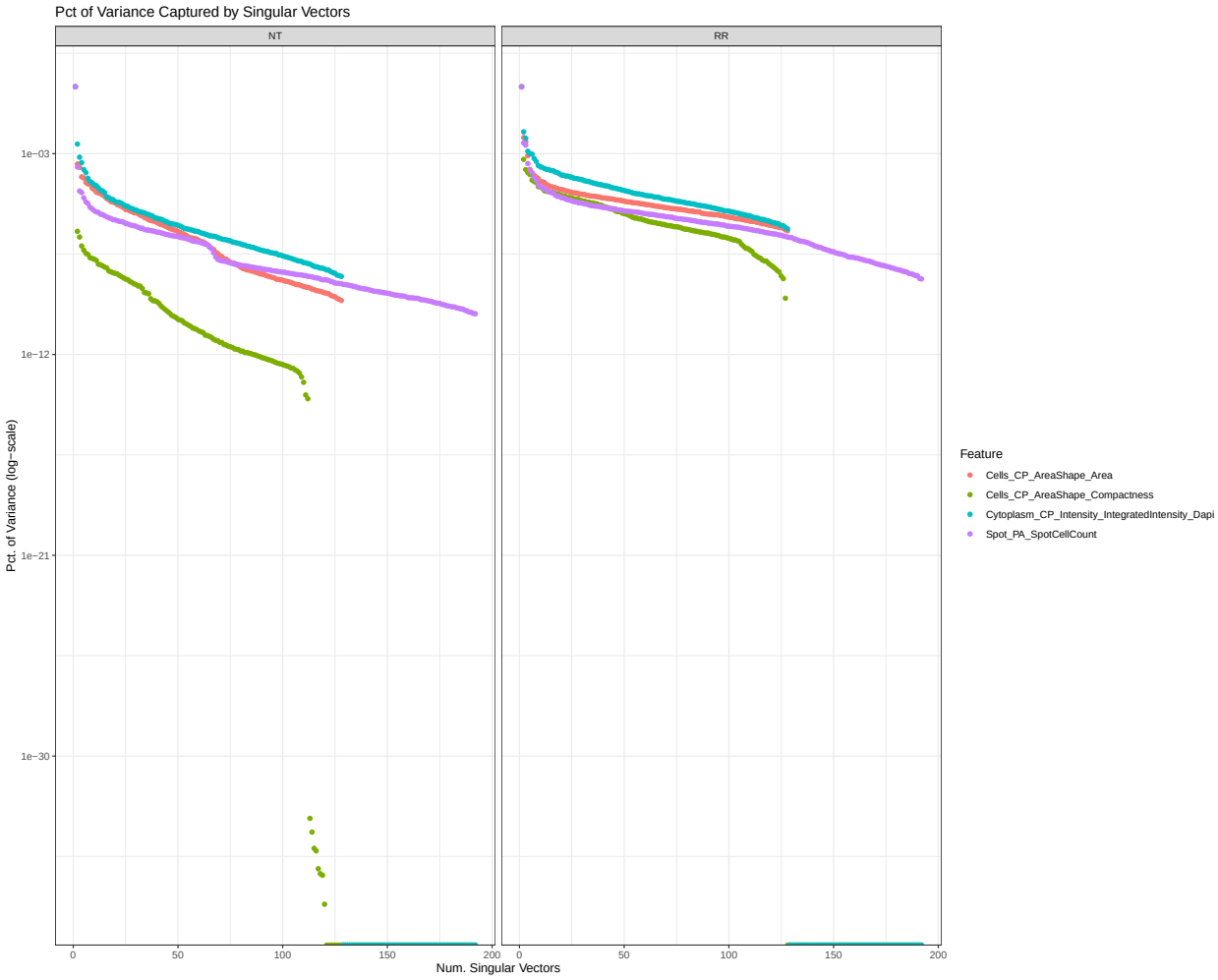

Figure 32: Pct. of variance captured by successive singular vectors for our four example features.