Supplementary Figures

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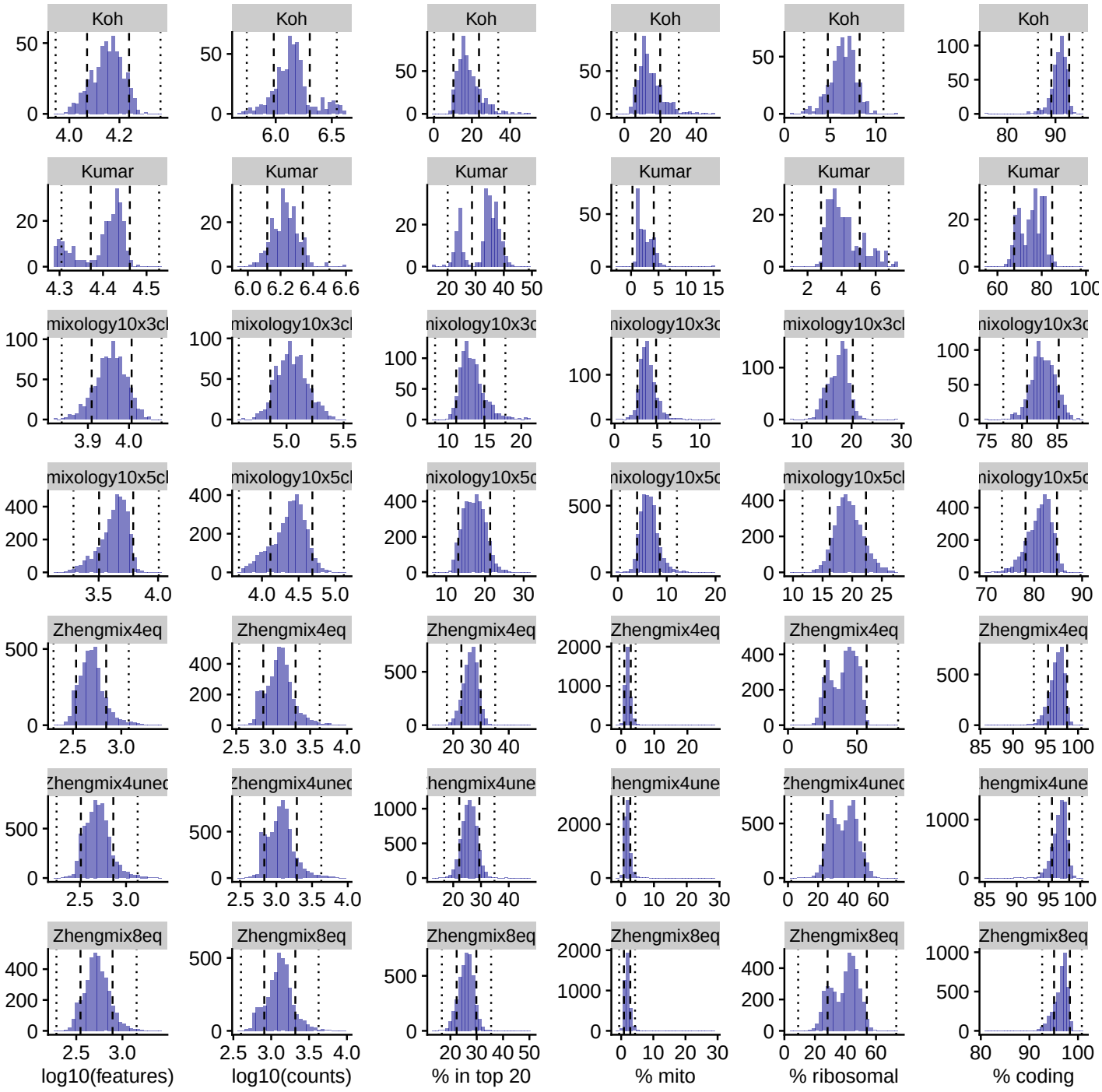
Contents

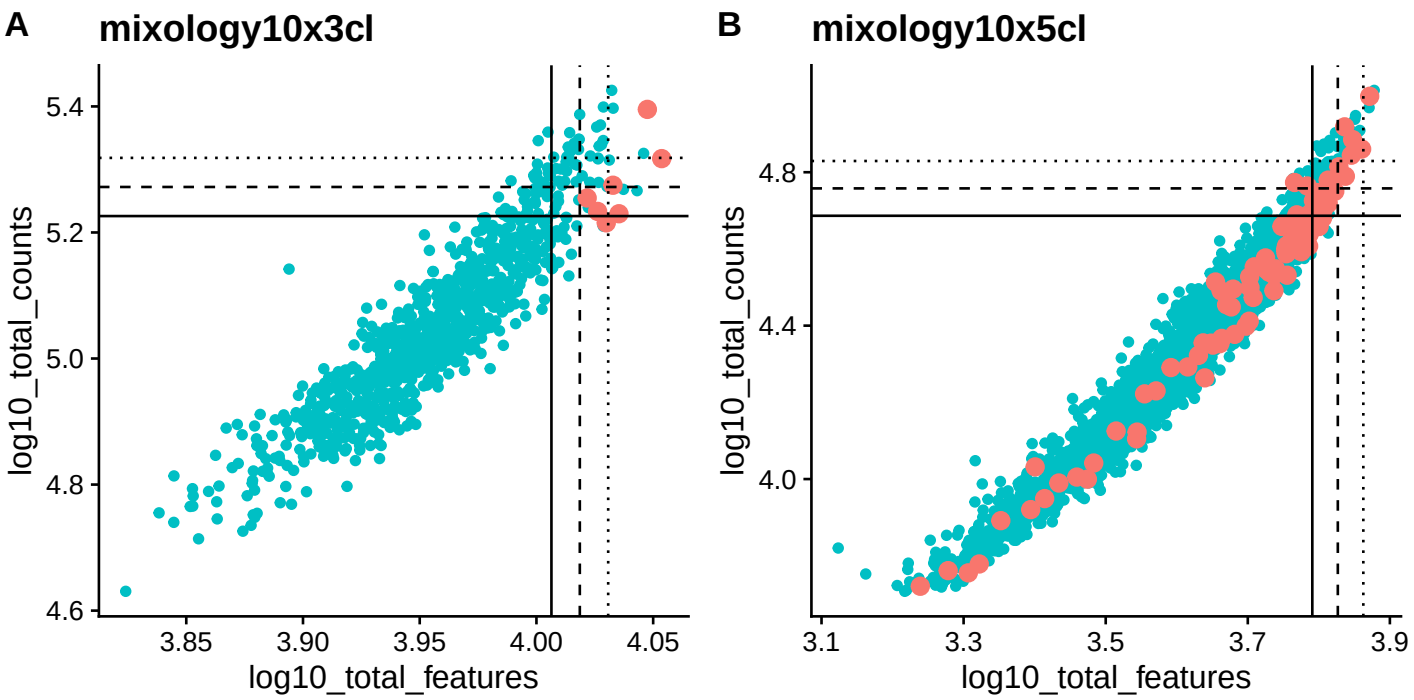
Supplementary Figure 1



Supplementary Figure 1

Distribution across cells of various control properties in the different datasets. The lines indicate respectively 2 and 5 median absolute deviations (MADs).

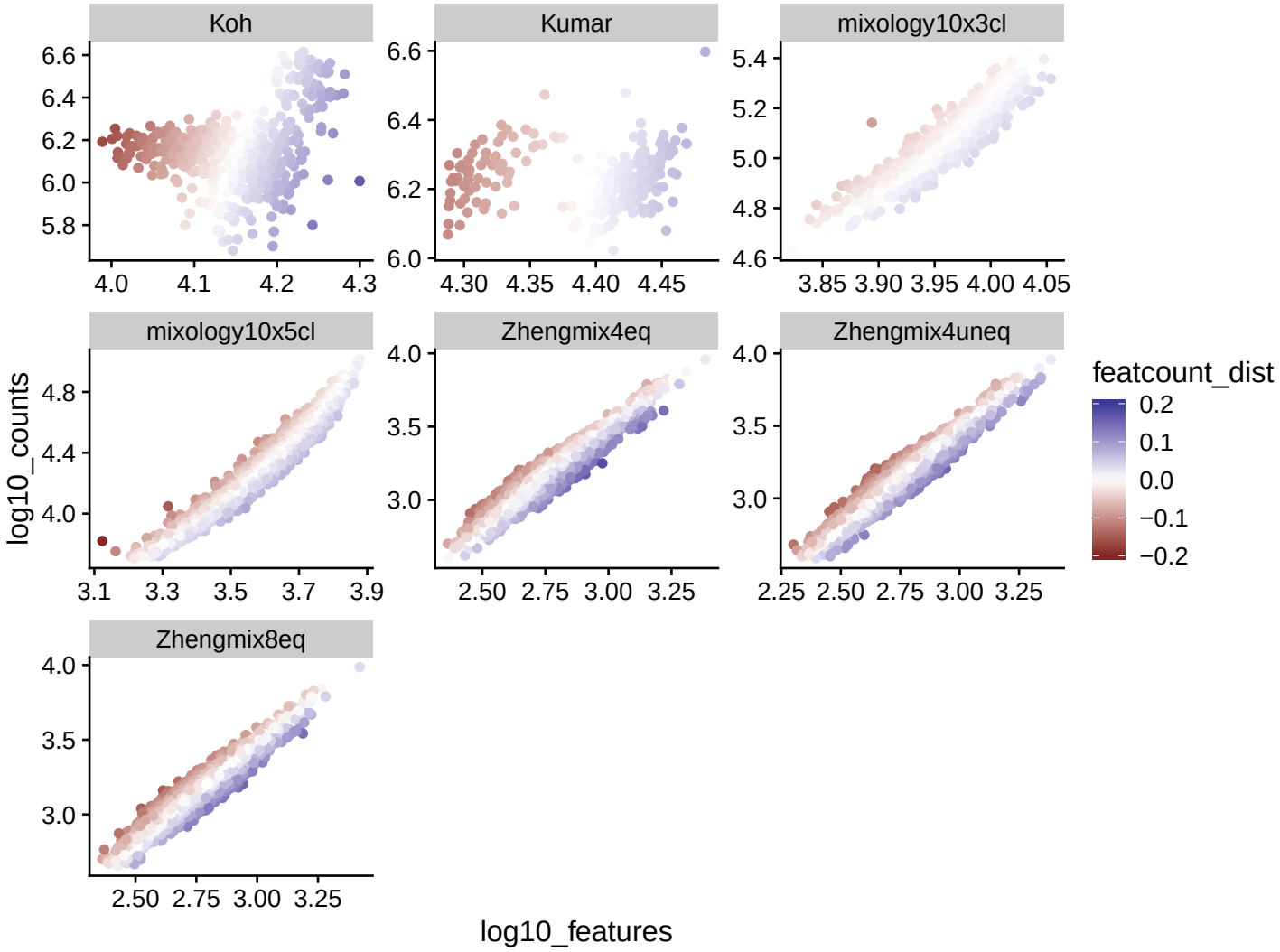
Supplementary Figure 2



Supplementary Figure 2

The total counts and total features per cell of doublets (red) versus other cells. We used the demuxlet annotation of doublets (based on SNPs) made available through CellBench. The lines indicate, respectively, 2, 2.5, and 3 median absolute deviations. While doublets tend to have a higher total count and especially number of detected features, these features alone are not always sufficient for their identification.

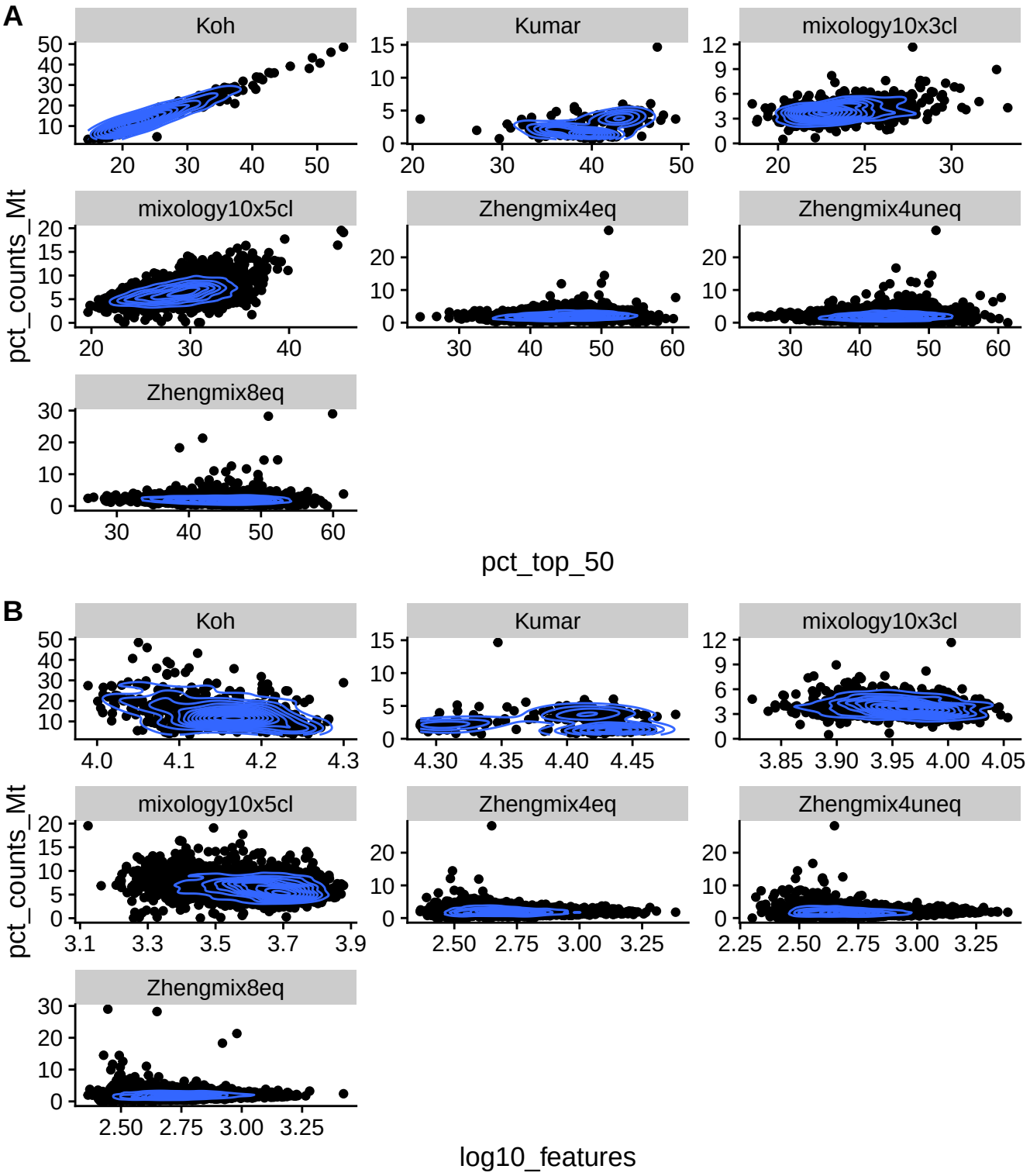
Supplementary Figure 3



Supplementary Figure 3

There is a tight relationship, in 10x datasets (i.e. not the `Koh` and `Kumar` datasets), between the total counts of a cell and its number of detected features. We therefore include, among control variables, deviation from this ratio.

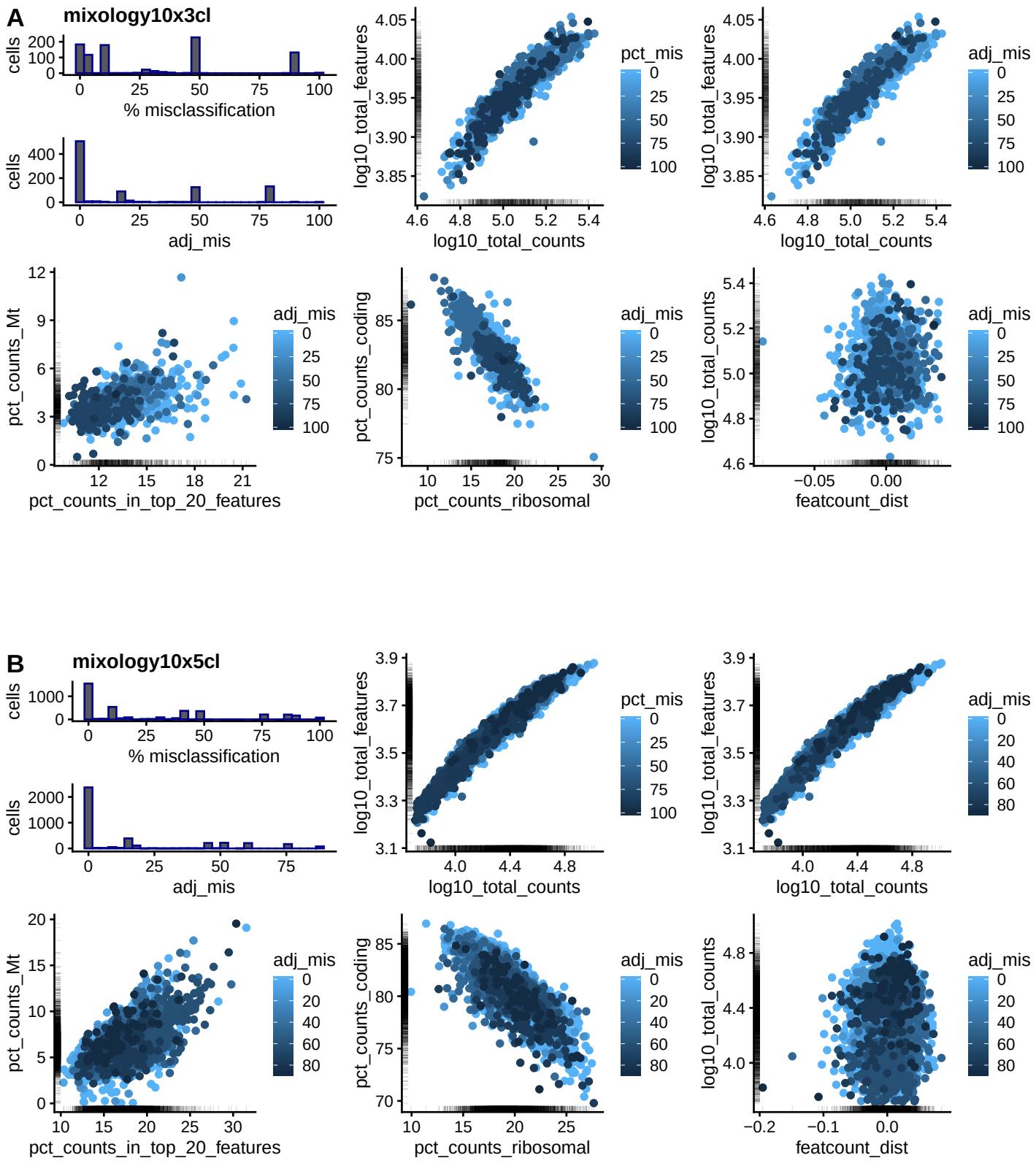
Supplementary Figure 4



Supplementary Figure 4

There is a tight relationship, in 10x datasets (i.e. not the `Koh` and `Kumar` datasets), between the total counts of a cell and its number of detected features. We therefore include, among control variables, deviation from this ratio.

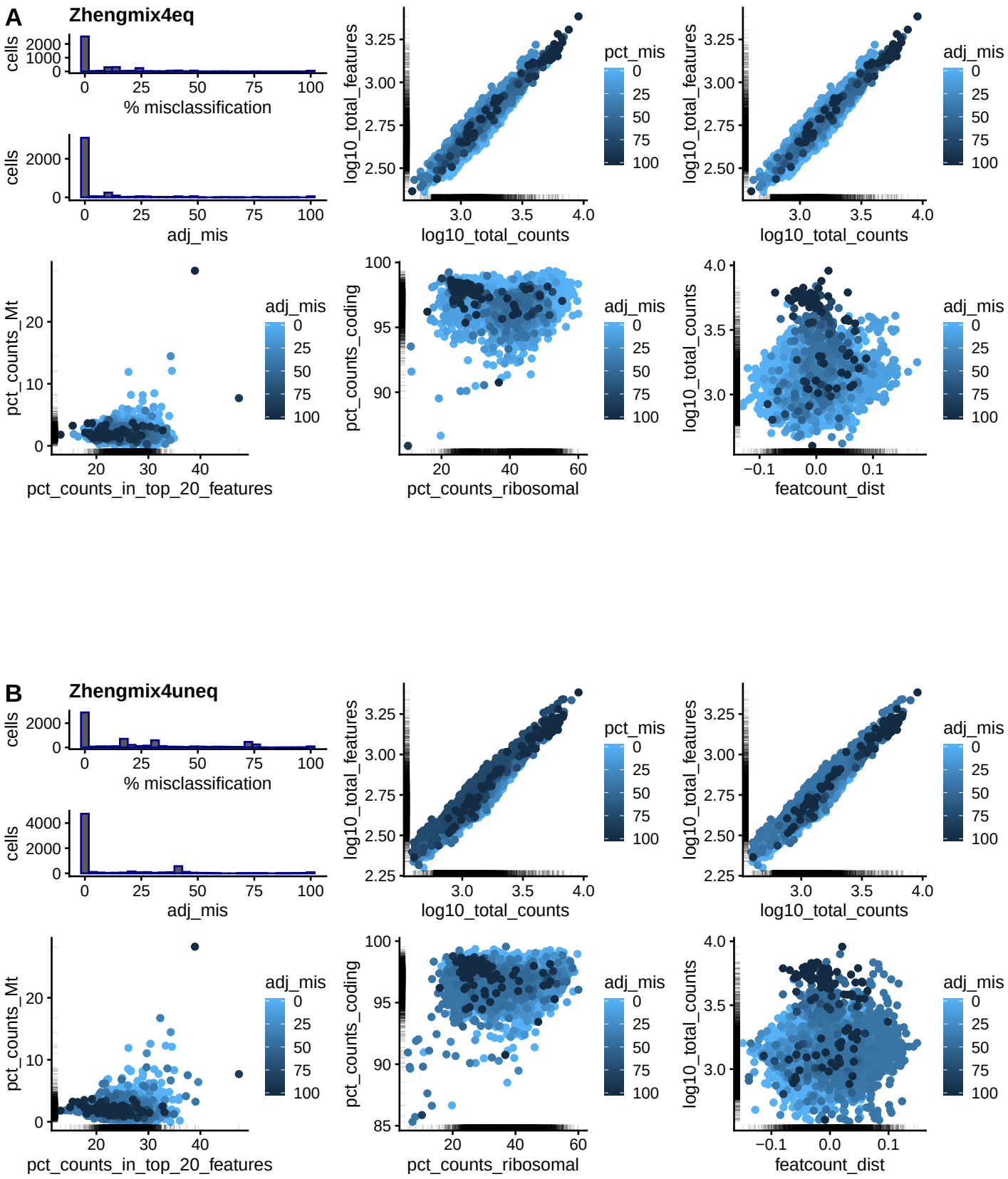
Supplementary Figure 5



Supplementary Figure 5

Relationship between various cellular properties and the frequency of cluster mis-assignment for the mixology10x3cl (A) and mixology10x5cl (B) datasets. The percentage of misclassification refers to the frequency with which a given cell is assigned the wrong cluster (using the Hungarian algorithm for cluster matching) across several hundred clustering runs with varying parameters. Since some subpopulations tend to be more misclassified than others, the adjusted rate of misclassification (`adj_mis`) is substracted for the subpopulation median misclassification rate.

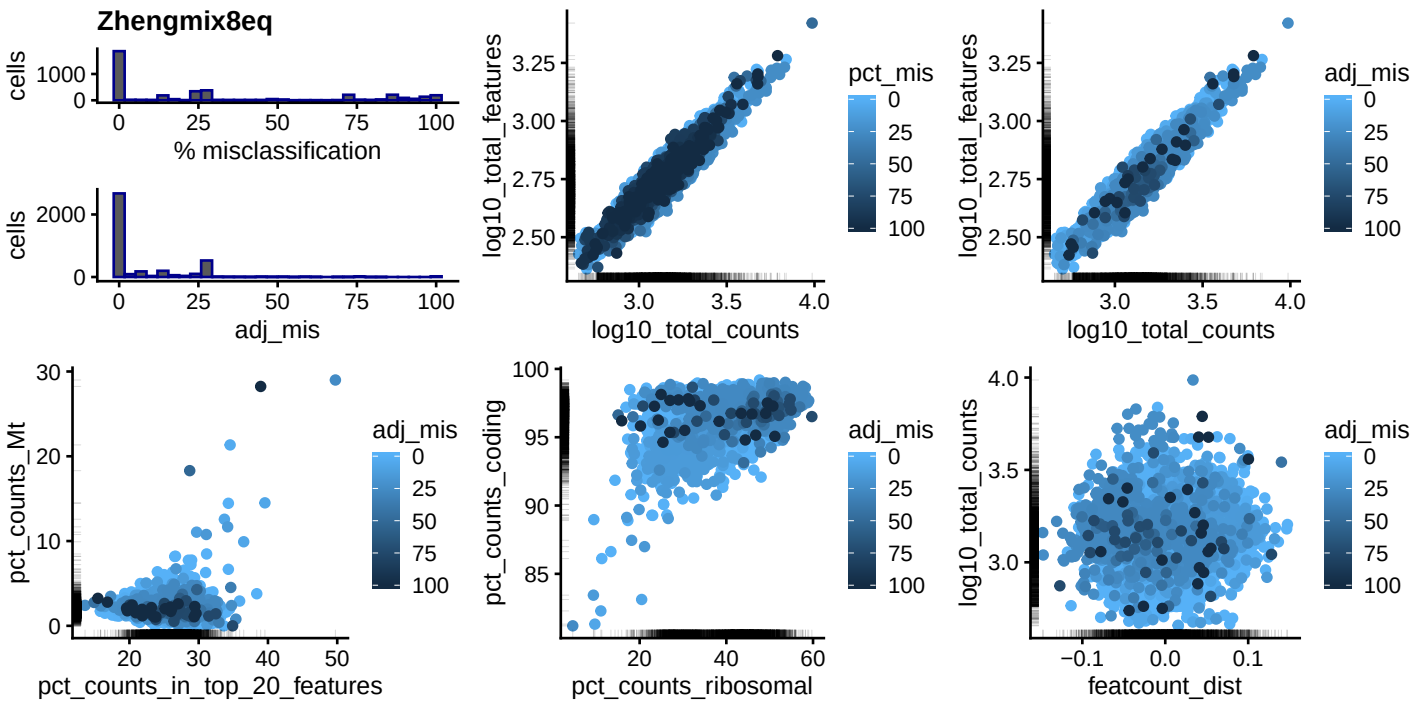
Supplementary Figure 6



Supplementary Figure 6

Relationship between various cellular properties and the frequency of cluster mis-assignment for the Zheng equal (A) or unequal (B) mixtures of four cell types. See Supplementary Figure 5 for more information. The only clear pattern is that cells with a high number of reads or features tend to have a higher misclassification rate.

Supplementary Figure 7



Supplementary Figure 7

Relationship between various cellular properties and the frequency of cluster mis-assignment for the Zheng mixture of 8 cell types. See Supplementary Figure 5 for more information.

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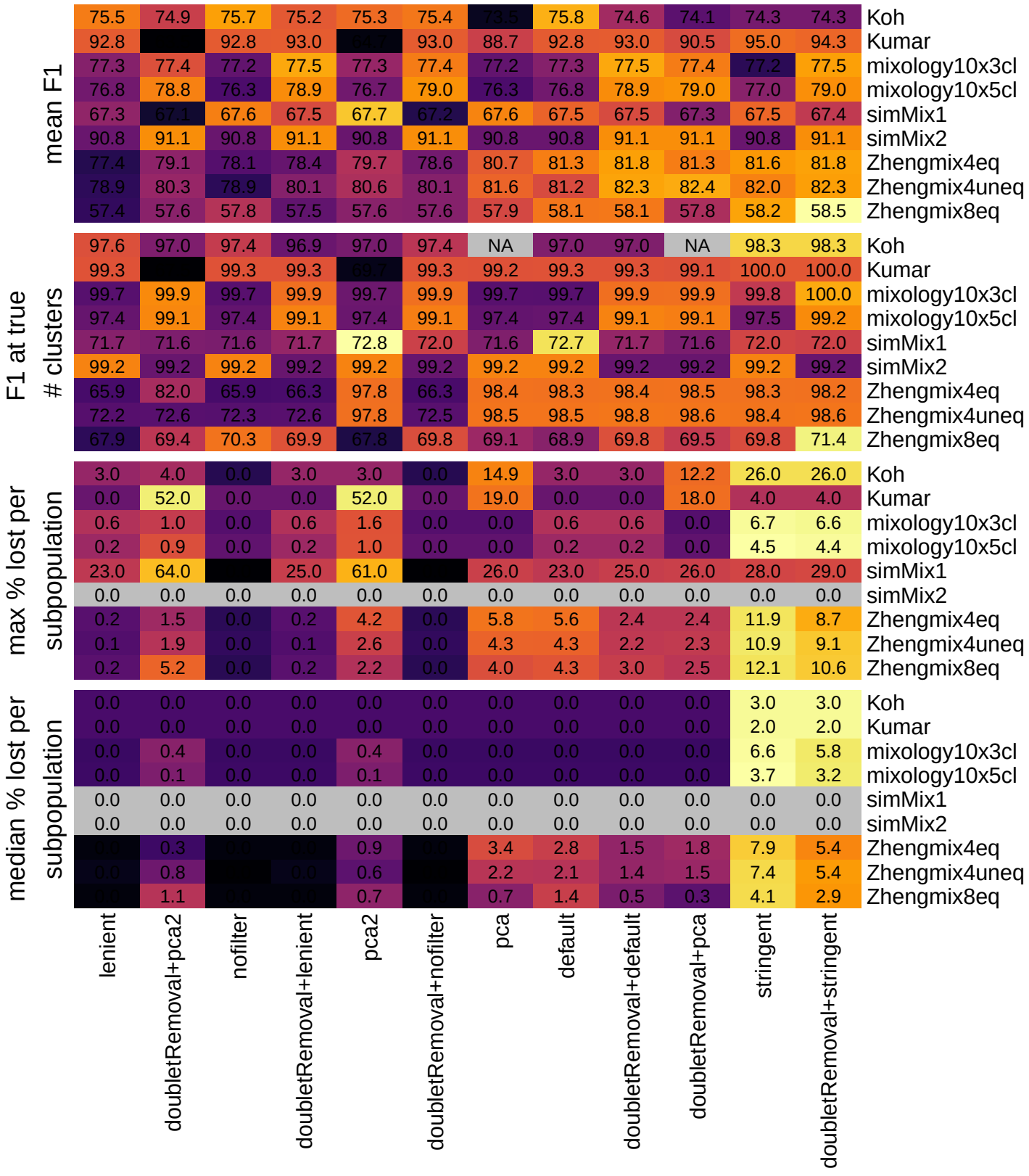
## Warning: replacing previous import 'SummarizedExperiment::shift' by
## 'data.table::shift' when loading 'pipeComp'

## Warning: replacing previous import 'SummarizedExperiment::rowRanges' by
## 'matrixStats::rowRanges' when loading 'pipeComp'
```

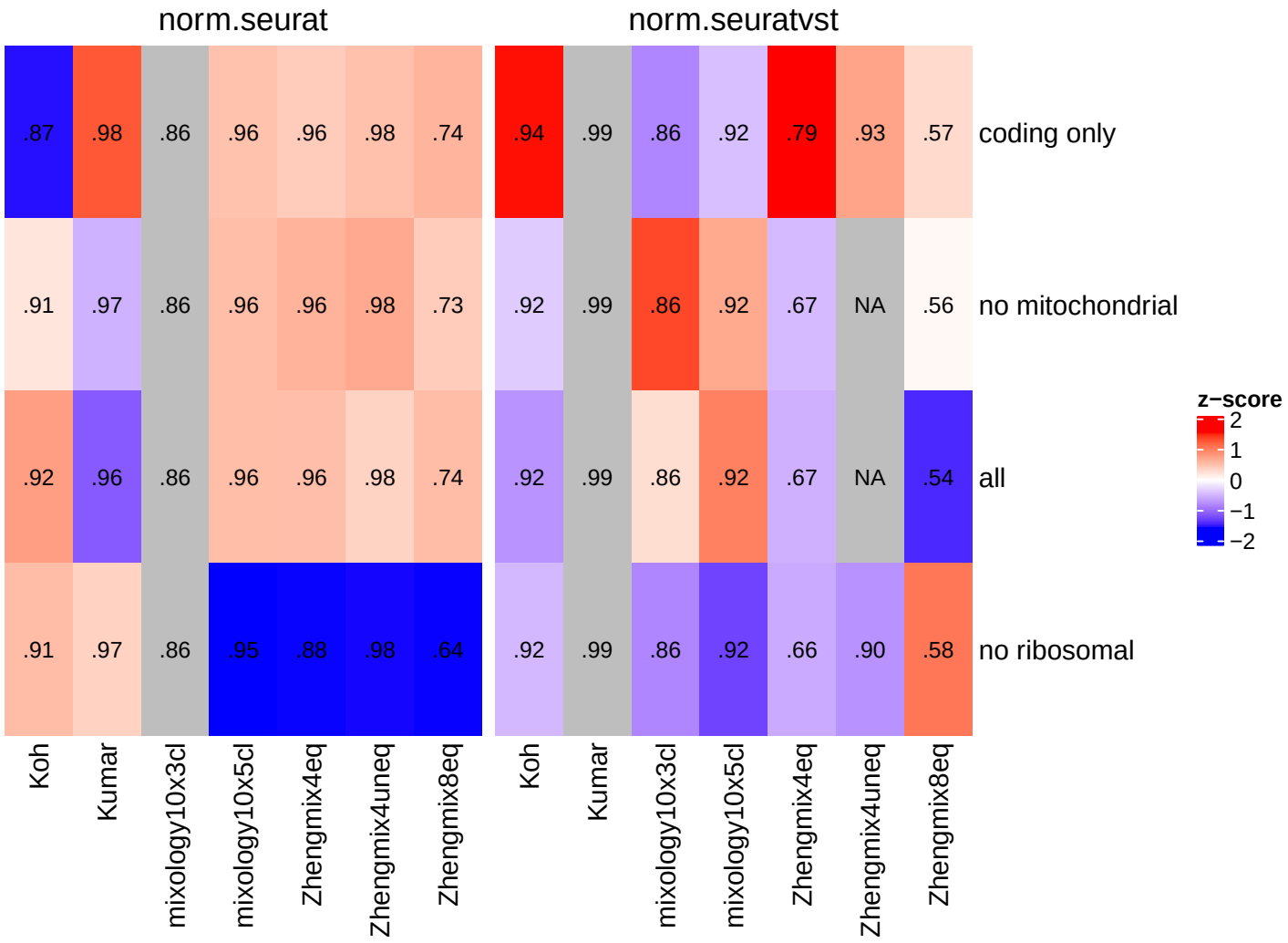
Supplementary Figure 8



Supplementary Figure 8

Mean clustering F1 score per subpopulation, mean F1 at true number of clusters, as well as maximum and median proportion of excluded cells per subpopulation across various filtering strategies. Doublet removal generally improves clustering accuracy with very mild exclusion rates, even in datasets that do not have heterotypic doublets. Stringent distribution-based filtering creates large cell type biases.

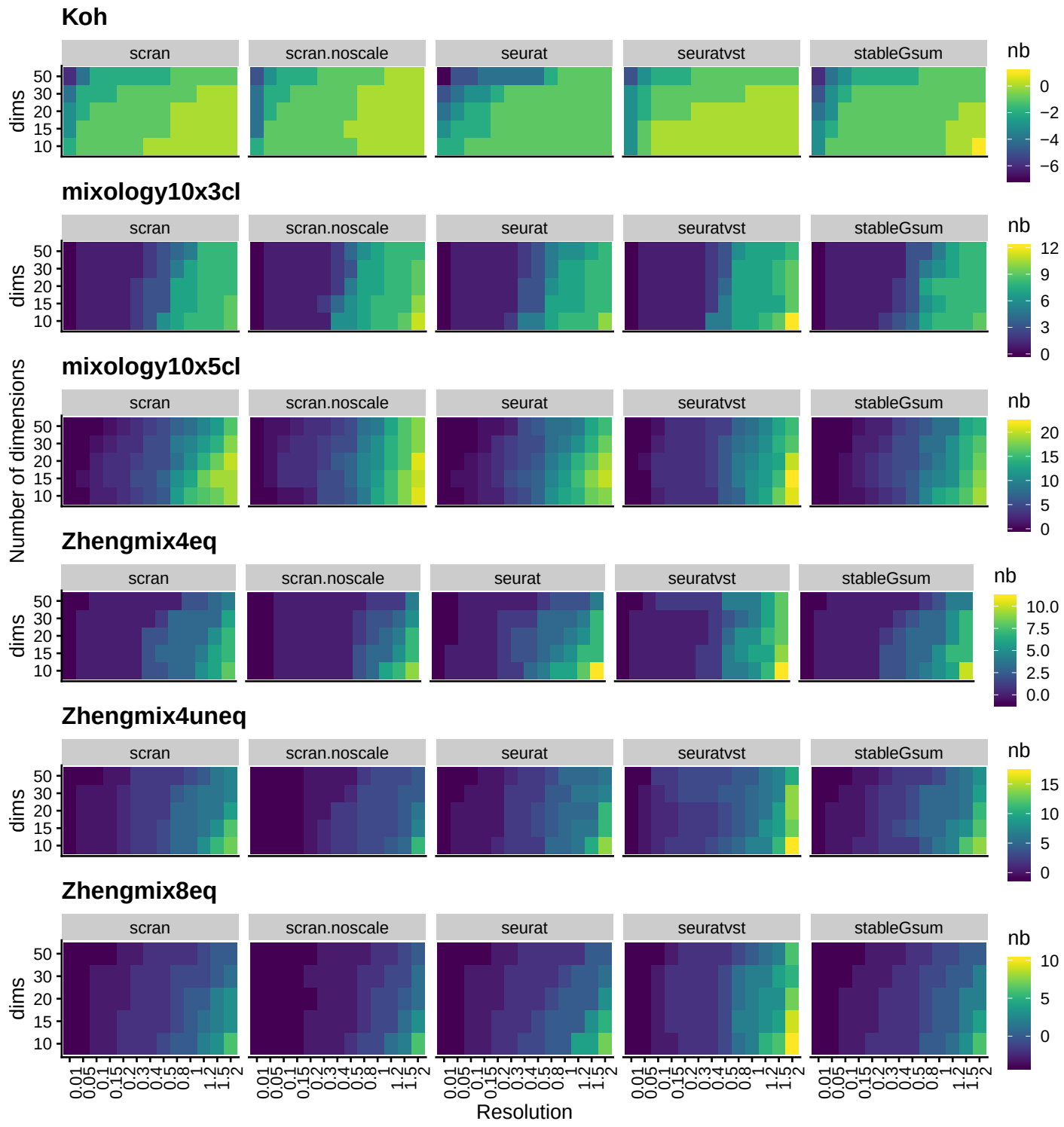
Supplementary Figure 9



Supplementary Figure 9

Impact of restricting the type of features used on the ARI of the clustering.

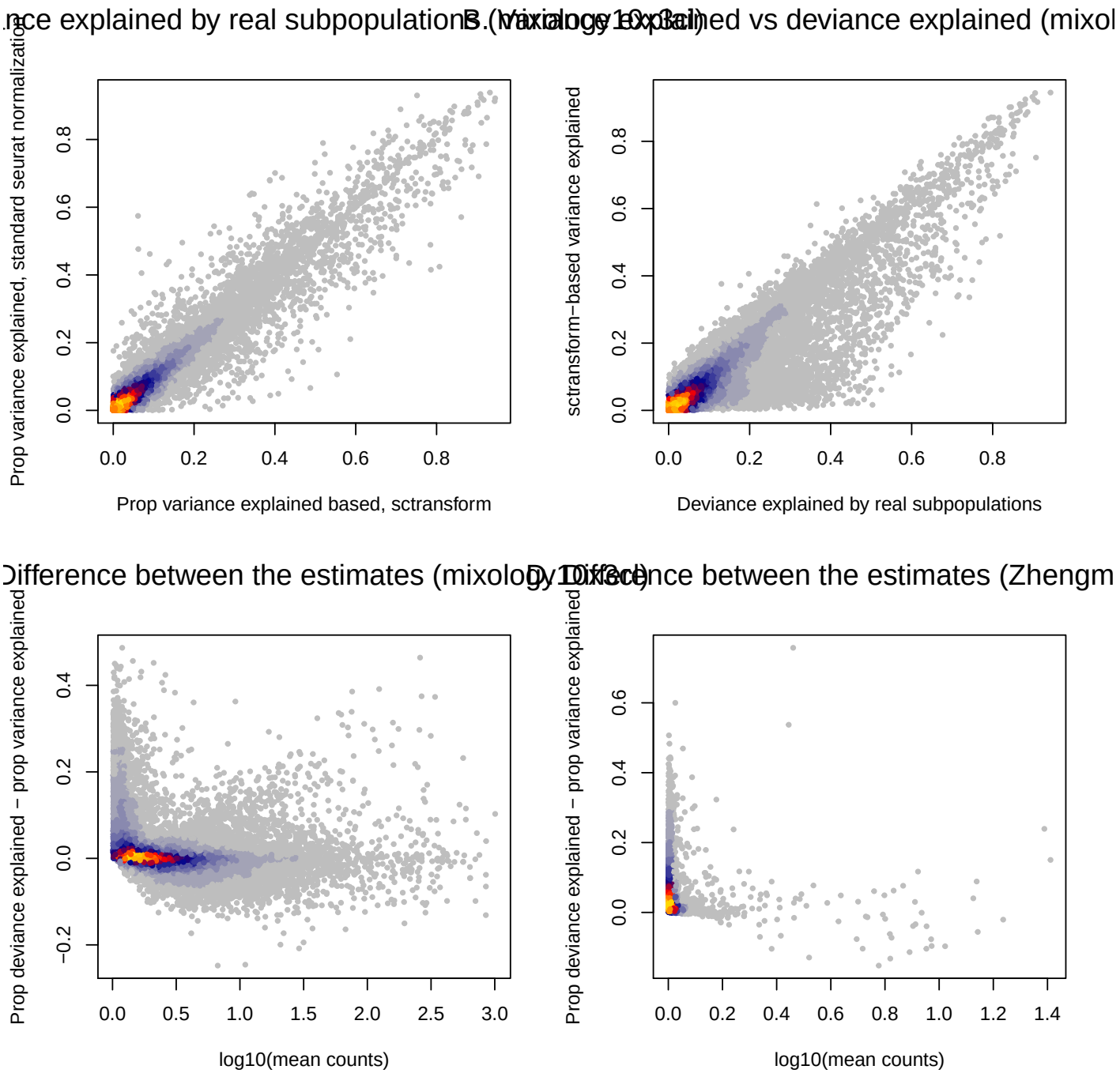
Supplementary Figure 10



Supplementary Figure 10

Mean difference between the number of detected clusters and the number of real subpopulations, depending on the normalization method, the resolution and the number of dimensions used. The Kumar dataset is not shown here due to a lack of variation in the number of clusters detected. A rough ANOVA on `nbClusters~dataset+norm+dims+resolution` suggests that seuratvst (sctransform) is associated with a higher number of clusters (p~0.002).

Supplementary Figure 11



Supplementary Figure 11

A: Comparison of the gene-wise proportion of variance explained by real subpopulations based on Seurat's standard log normalization and on `sctransform` variance-stabilizing transformation. Across 10x datasets, there is a good agreement between the two, the correlation ranging between 0.92 and 0.97. **B:** There is also a good agreement between *variance* and *deviance* explained, with some genes having a higher deviance explained. **C-D:** Relationship between mean expression and the difference between the proportion of deviance explained and the proportion of variance explained in two datasets. Genes that have a higher proportion of the deviance explained than of the variance explained are generally the lowly-expressed ones.

Supplementary Figure 12

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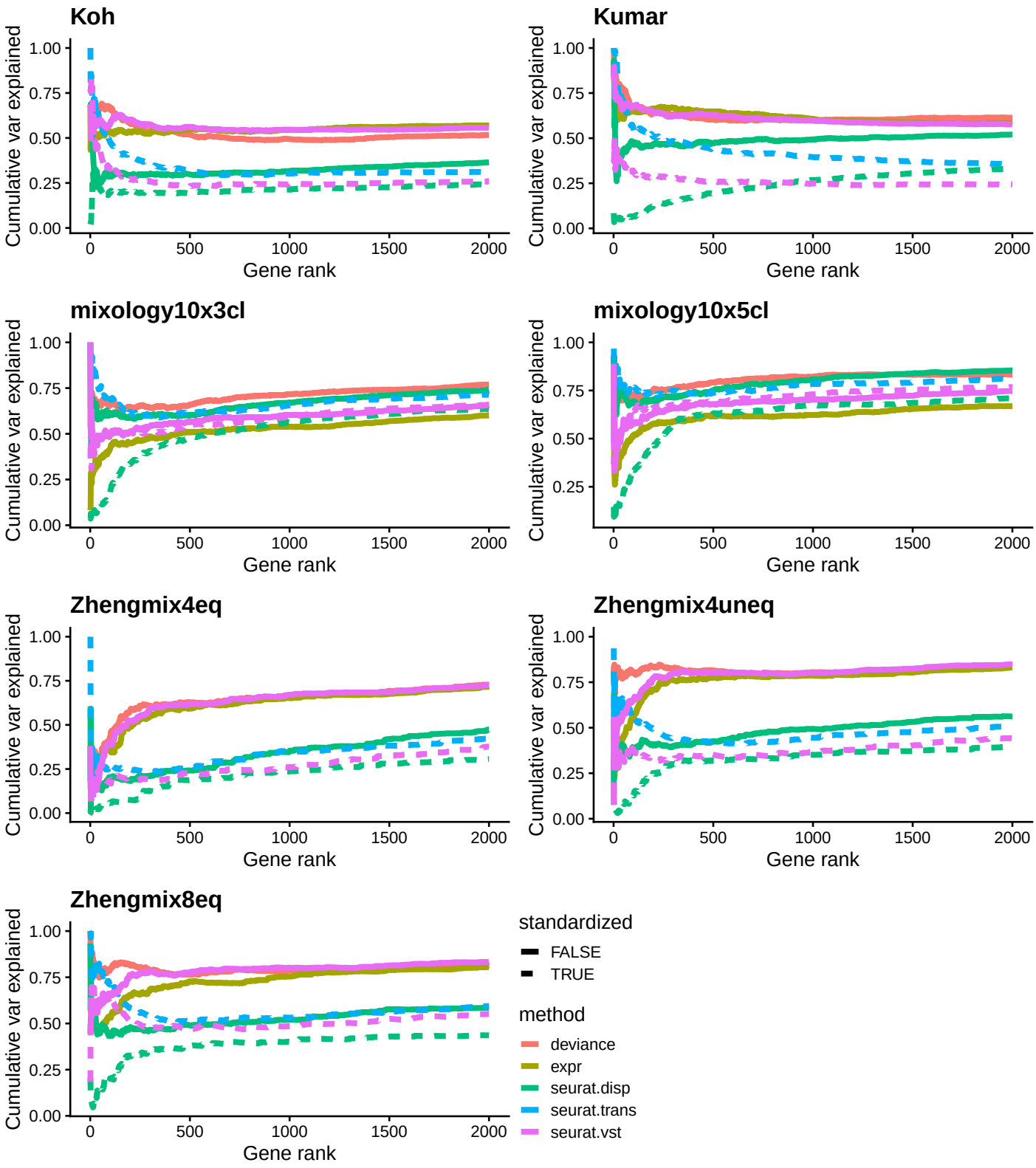

Supplementary Figure 12

Proportion of the cumulative *variance* explained by real subpulations that is retrieved through the selection. For each gene, we compute the proportion of the variance explained by real subpopulations. For each rank X, we sum this proportion for the X genes selected by a given method, and divide it by the sum when selecting the X genes with the highest variance explained. An ideal selection would therefore be a horizontal line at 1.

Supplementary Figure 13

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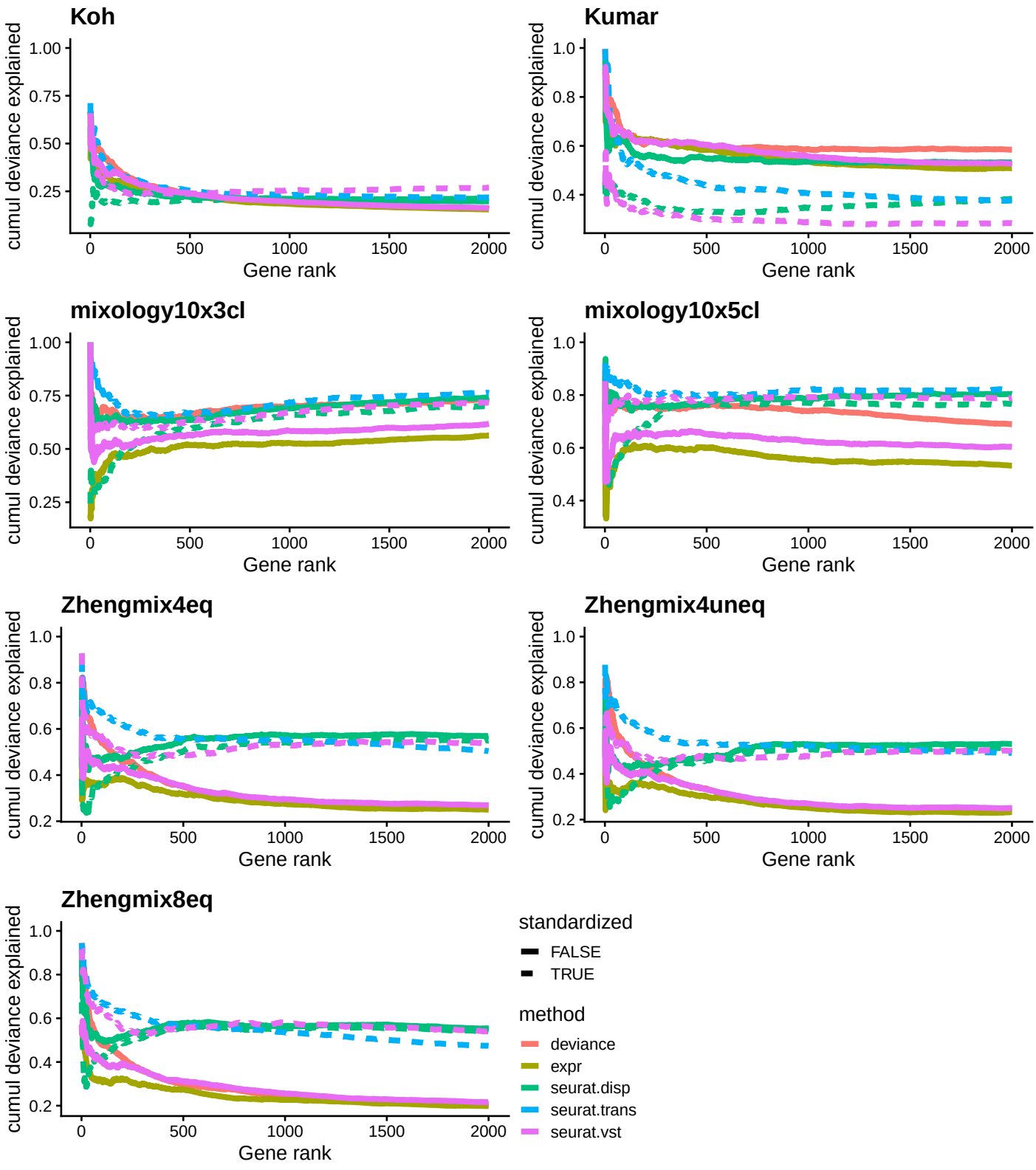
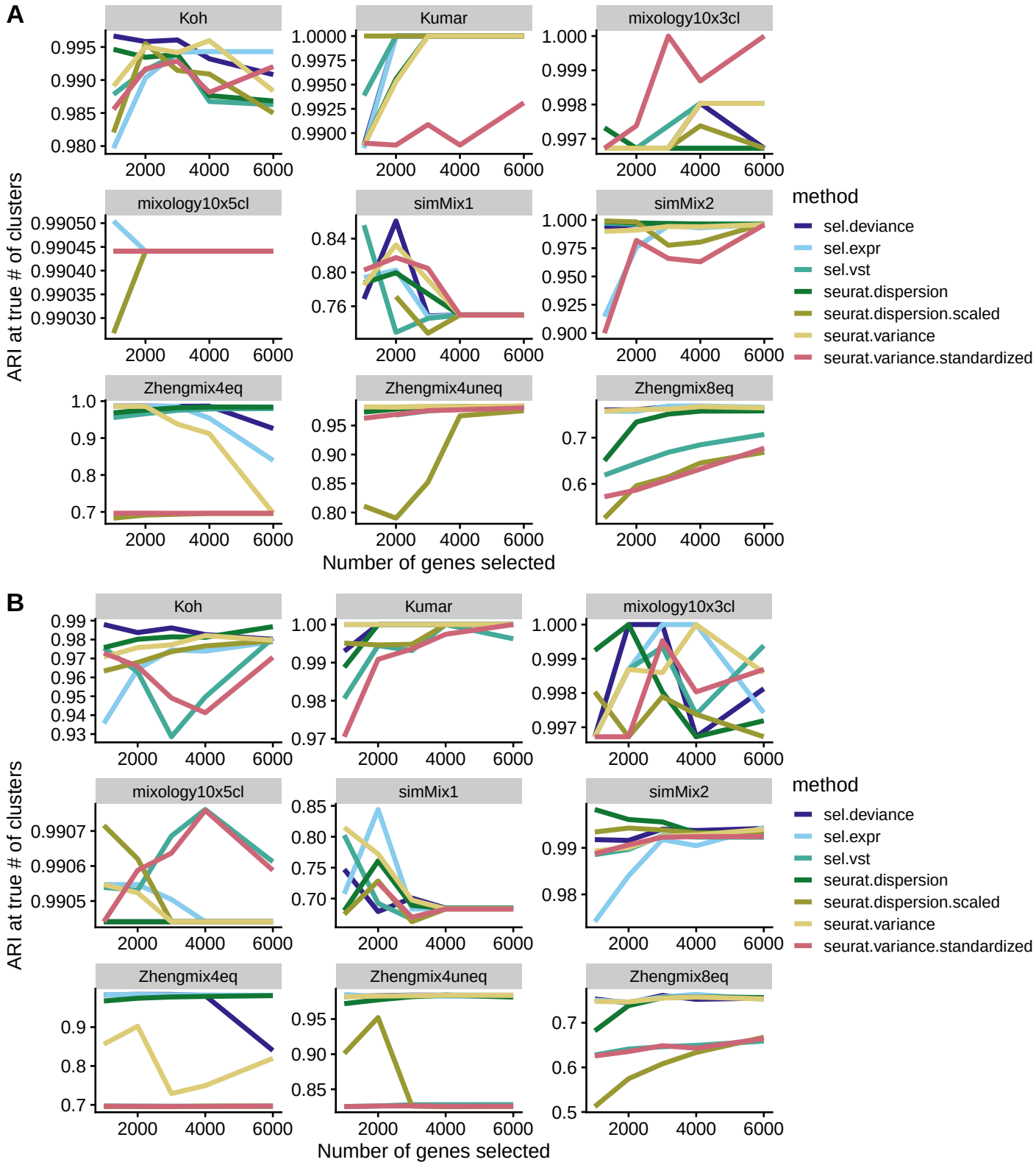


Supplementary Figure 13

Proportion of the cumulative *deviance* explained by real subpulations that is retrieved through the selection. For each gene, we compute the proportion of the variance explained by real subpopulations. As for Supplementary Figure 12, except using deviance explained.

Supplementary Figure 14



Supplementary Figure 14

Clustering accuracy according to the number of genes selected using various ranking/selection methods. **A:** Based on sctransform, **B:** Based on standard Seurat normalization.