

SUPPLEMENTARY FIGURES

Figure S1. (A) UMAP of merged scRNA-seq profiles of the 6 cell mixtures with 21 clusters predicted using default parameters revealed groups of adjacent clusters. (B) The expression profiles of some clusters were significantly correlated and supervised bi-clustering using a 2000-marker gene set identified cliques of correlated clusters that were also adjacent in the UMAP. Merging 3 cluster cliques, shown as regrouped clusters (top), reduced the total number of clusters to 6. (C) Deconvolution using the original 21 clusters, and (D) the reduced set of 6 clusters produced markedly different deconvolution accuracy evaluations. Here, the gold standard was estimated from scRNA-seq analysis for fairness since there is no 1-to-1 mapping between 21 clusters and 6 cell types. Accuracy was evaluated using Pearson correlations (P) and RMSE (E).

Figure S2. Matched breast cancer samples were profiled fresh or after cryopreservation by scRNA-seq. Confirming our observations from the analysis of kidney samples (Figure 4C), the accuracy of tested deconvolution methods was lower when using profiles of cryopreserved tissues. However, SQUID composition estimates were the most accurate based on fresh profiles and were nearly unaffected by tissue preservation.

Figure S3. Analogous to Figure 4A, deconvolution accuracy on concurrently profiled tissues suggested that DWLS outperforms other published methods irrespective of normalization. However, SQUID estimates were the most accurate on every dataset. Note that accuracy estimates reported in Figure 5 were based on cross-validation test errors, while estimates reported here and in Figure 4 did not use cross validation.

Figure S4. Analogous to Figure 4B, normalization strategies altered deconvolution accuracy estimates, but the top-performing methods outperformed other methods in nearly all tests, irrespective of normalization. In particular, the accuracy of SQUID estimates was nearly unaffected by the normalization strategy used.

Figure S5. Analogous to Figure 4C, deconvolution accuracies were estimated for fresh, methanol-fixed, and cryopreservation after warm and cold dissociation of kidney tissues. Deconvolution accuracy was lower for profiles of cryopreserved kidney tissues than for profiles of fresh or methanol-preserved tissues. However, SQUID's accuracy was high irrespective of the tissue preservation technique used.

Figure S6. Analogous to Figures 3B and 4, we present a detailed comparison between predicted abundance estimates by deconvolution and gold standard abundance estimates for the normalization strategy with the lowest RMSE for each dataset. In addition, we included a comparison of Bisque when using all genes and after marker gene selection. These were excluded from other plots because the implementation of Bisque doesn't allow for optimizing data normalization methods; axes are in $\log_{10}$ scale.

Figure S7. Our strategy for counting cell types using flow. All flow samples were prepared and analyzed in triplicate. For each of the six mixtures (1–6), three flow samples (1A–1C, 2A–2C, etc.) containing one Hoechst-stained cell line (either T47D, BT474, or MCF7), one Syto 82-stained cell line (either Jurkat, THP1, or hMSC), and four unstained cell lines at identical proportions were generated. Single-stained cells from these samples represented the proportion of that cell line in the corresponding mixture. This strategy was developed to avoid spectral overlap and to increase our ability to accurately quantify positive cells.

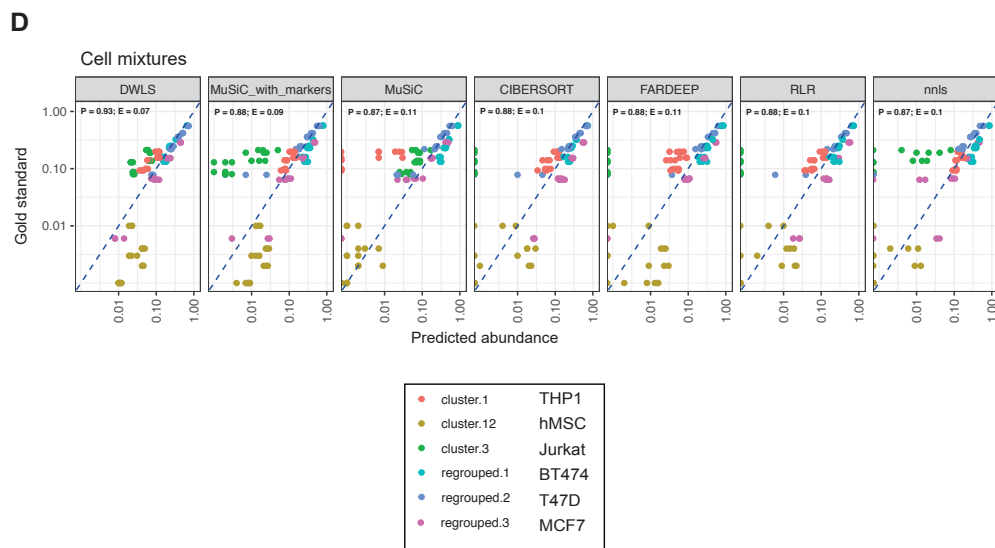
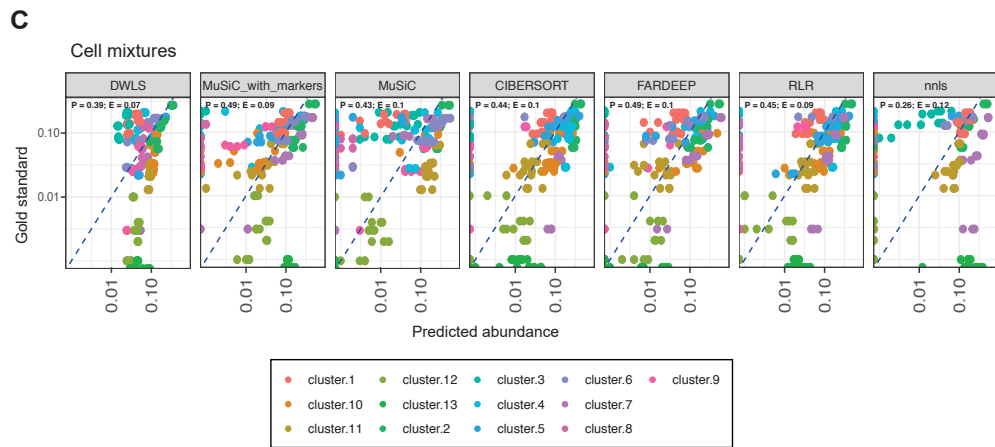
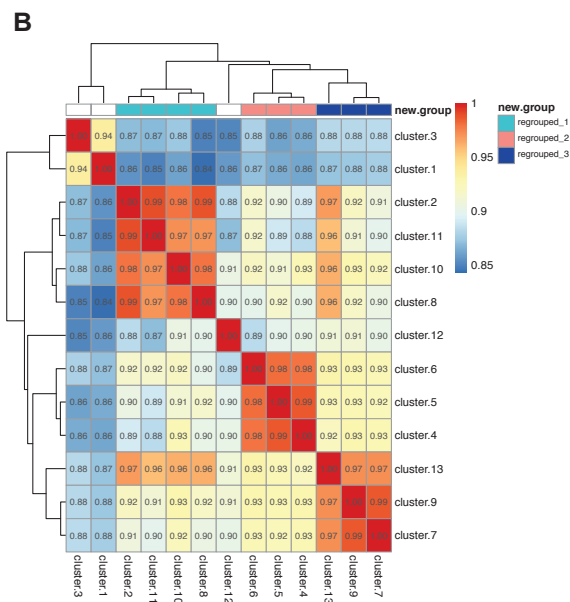
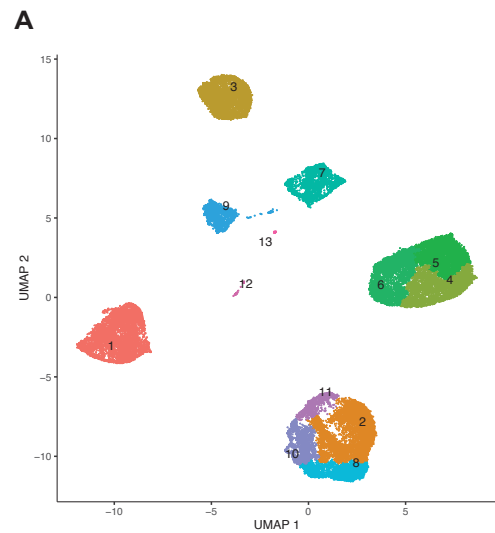


Figure S1

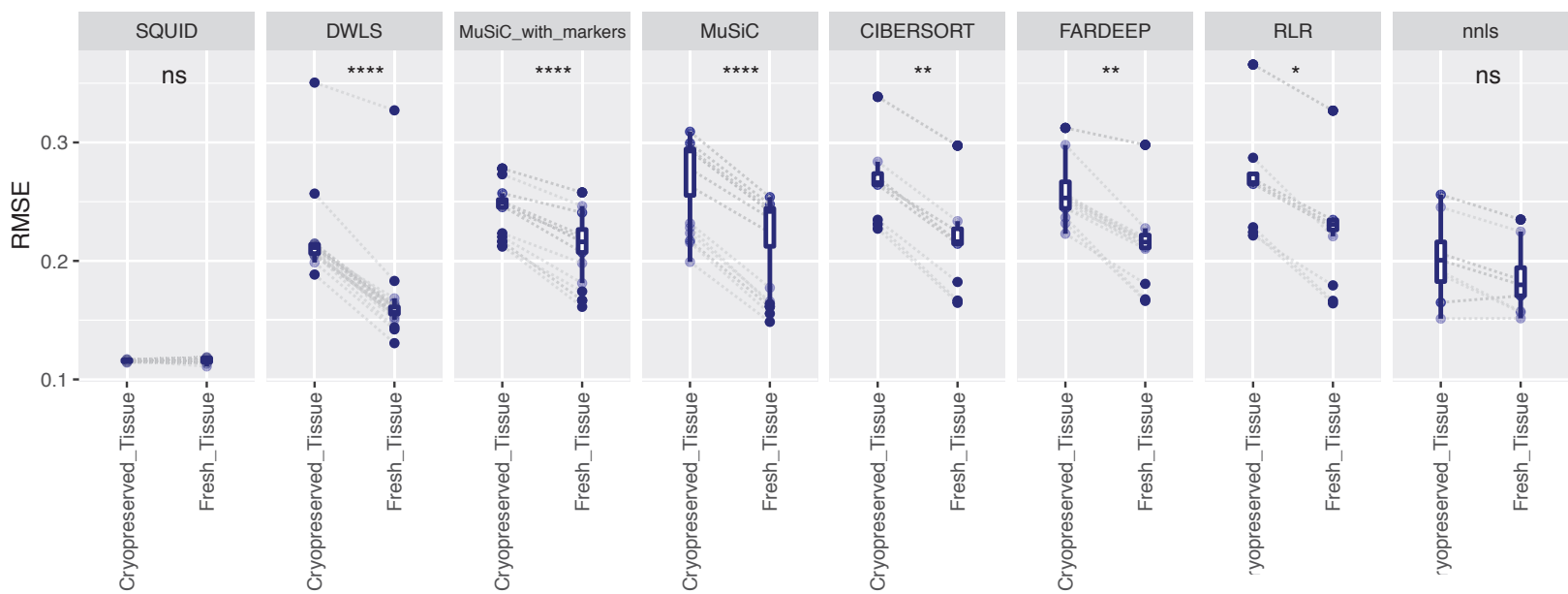
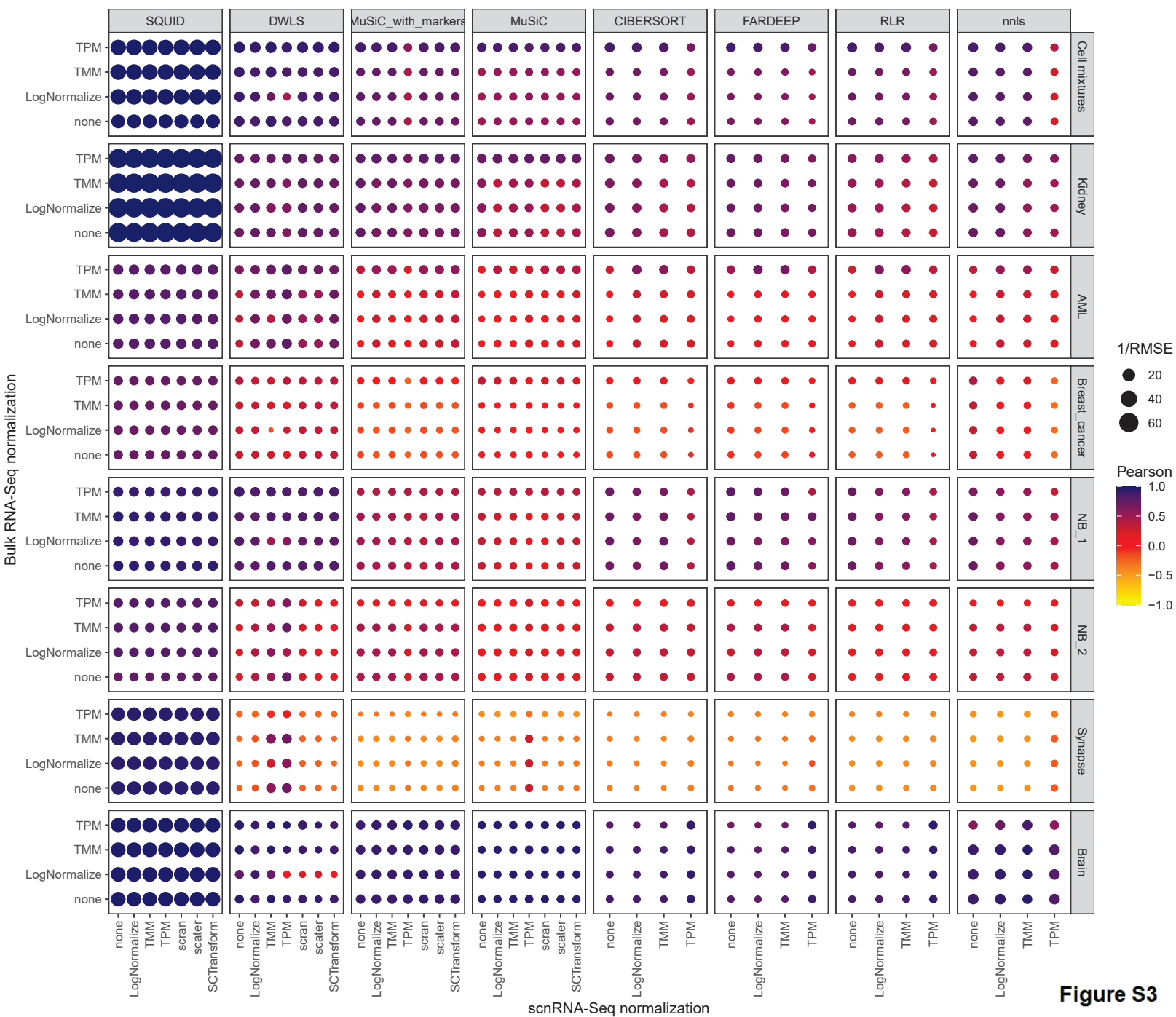


Figure S2



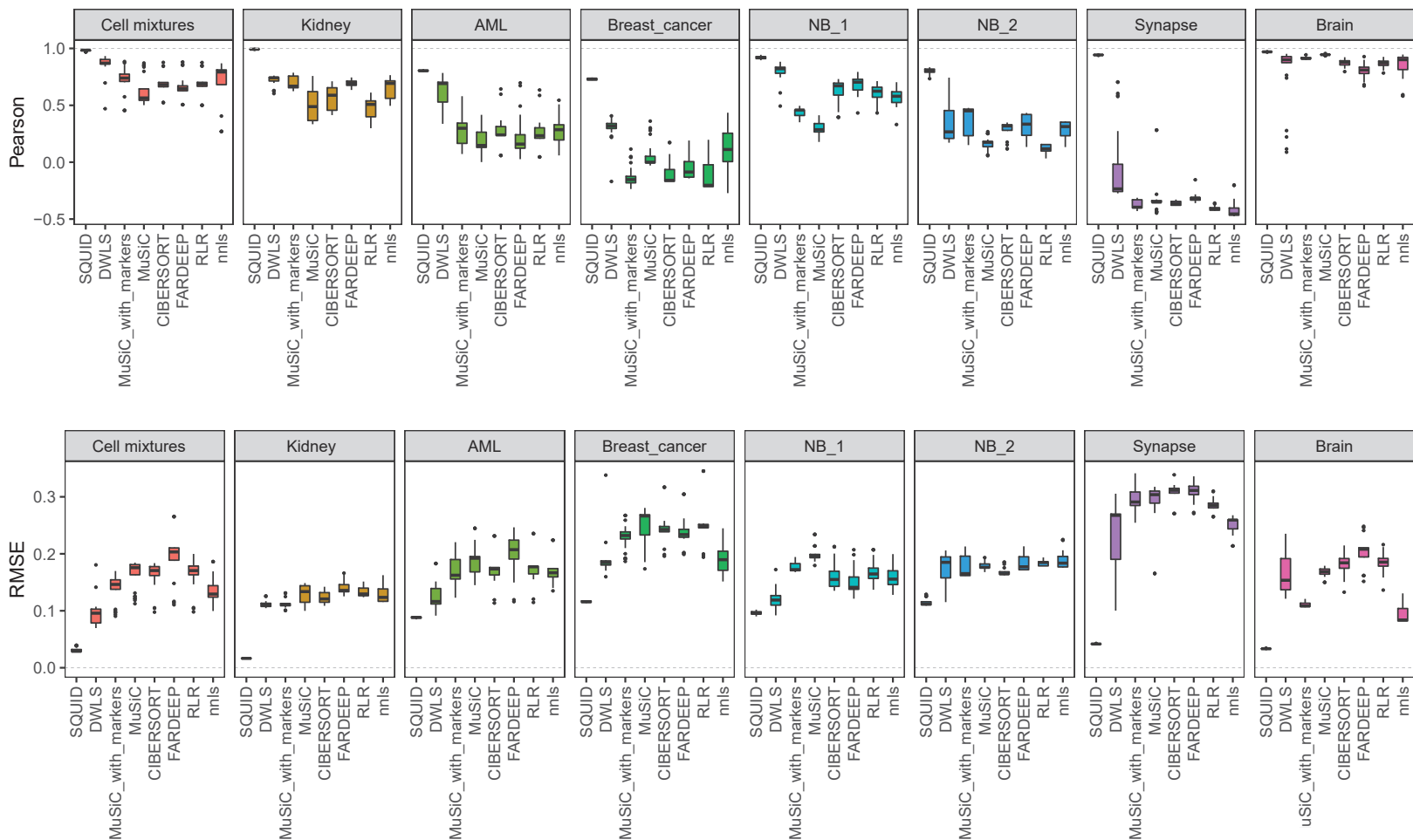
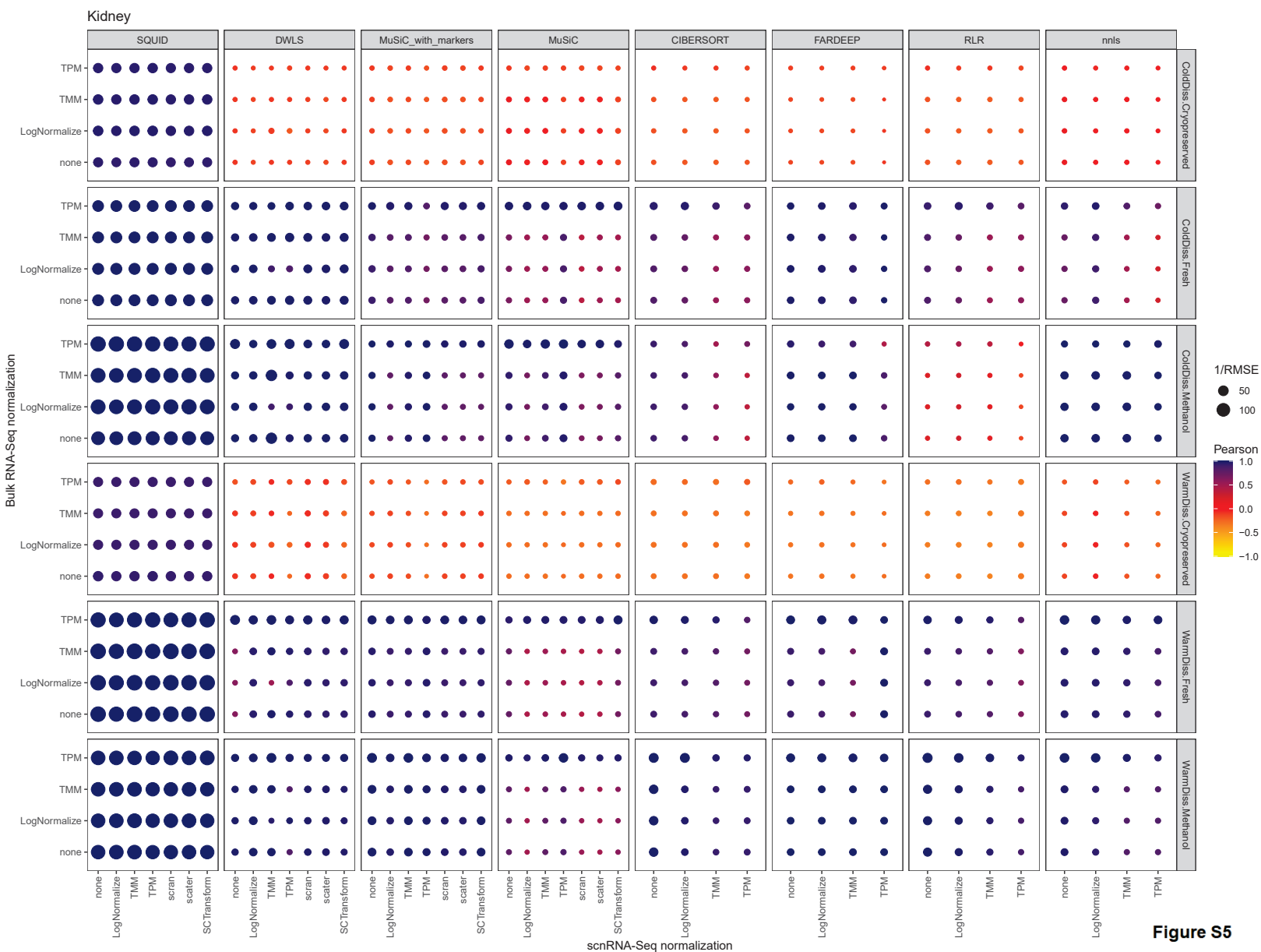
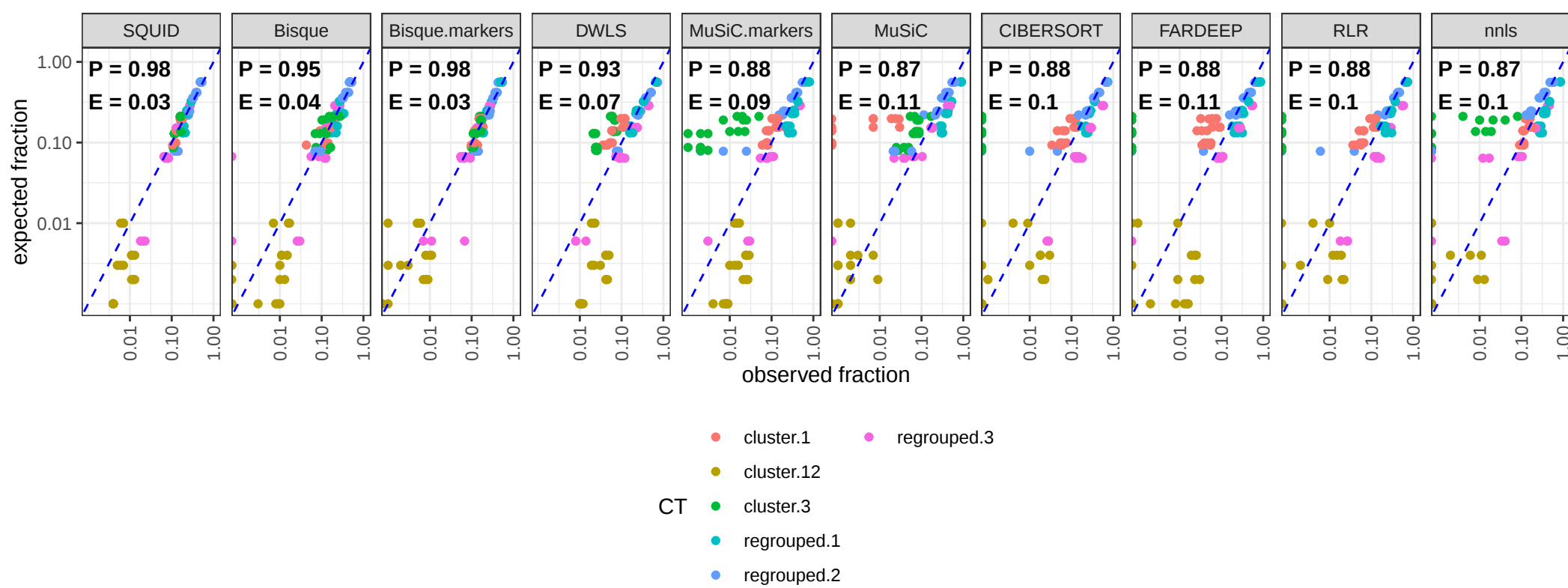


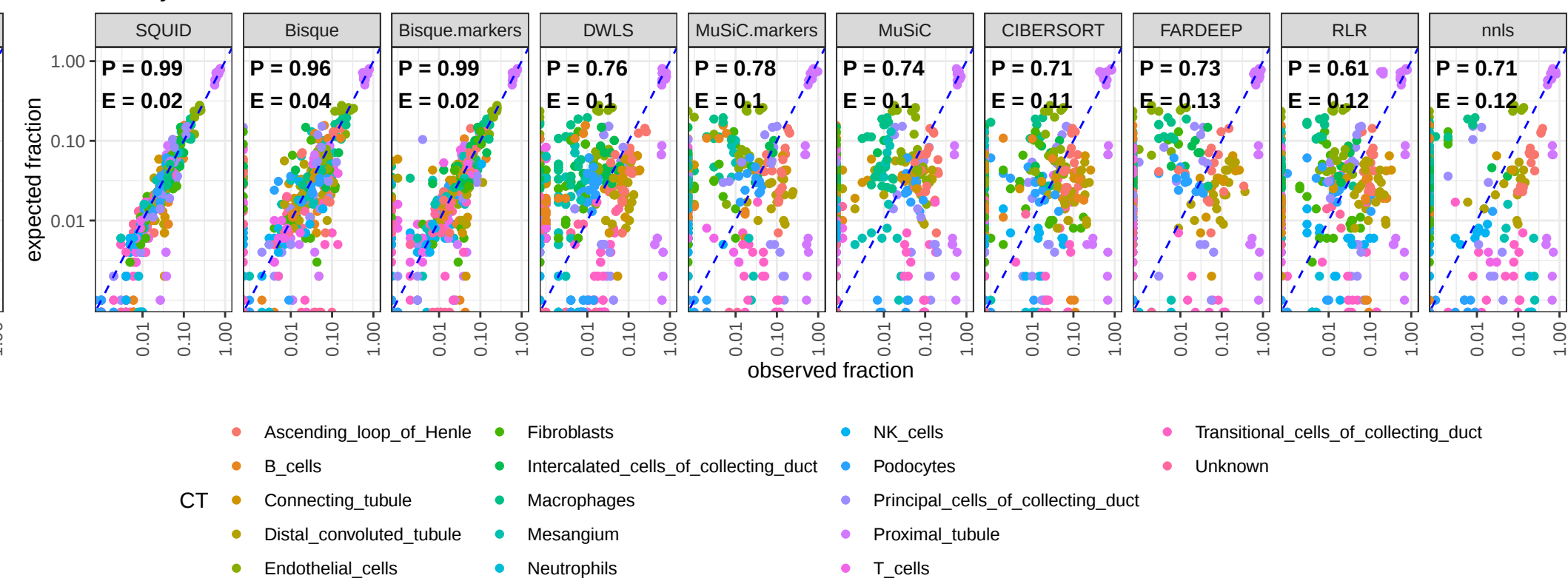
Figure S4



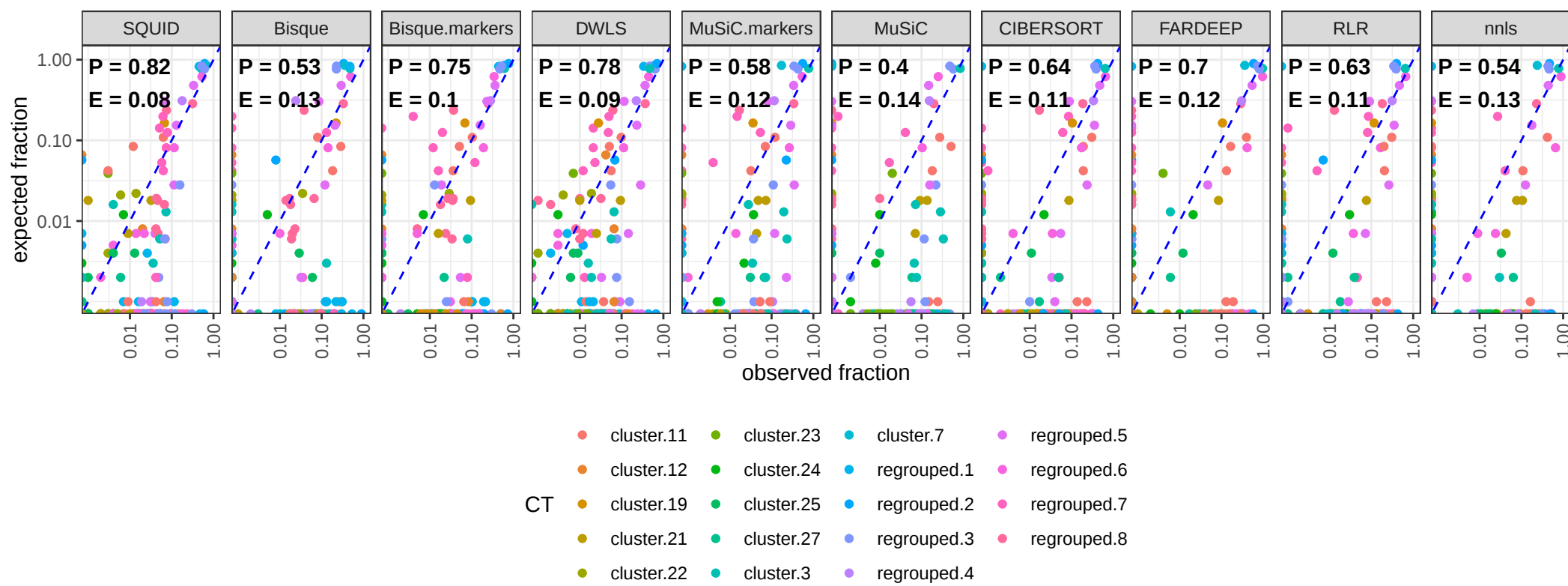
Cell Mixtures



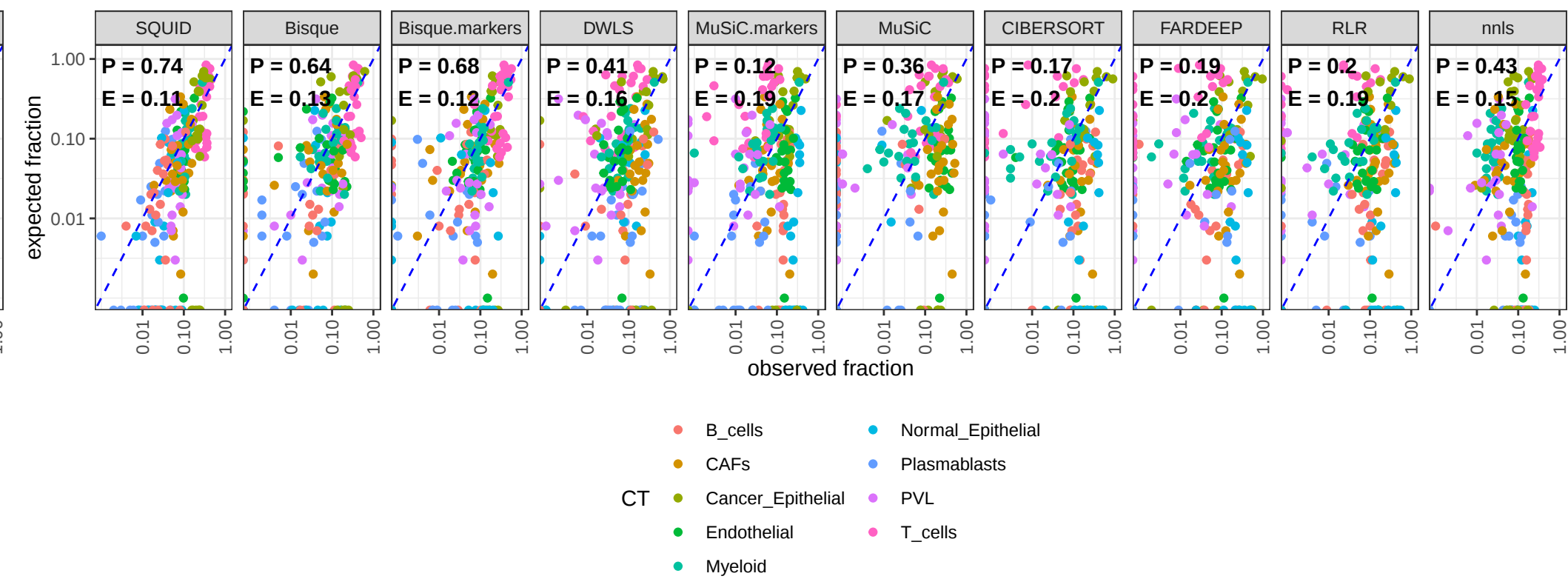
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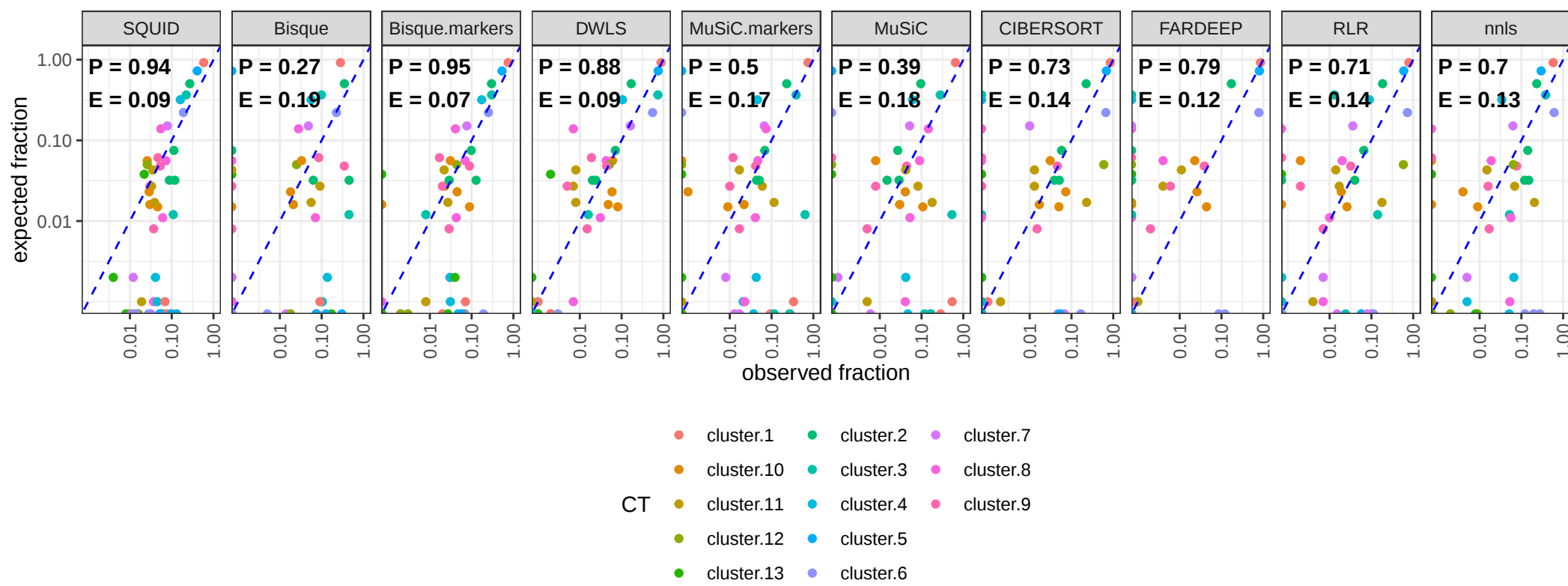
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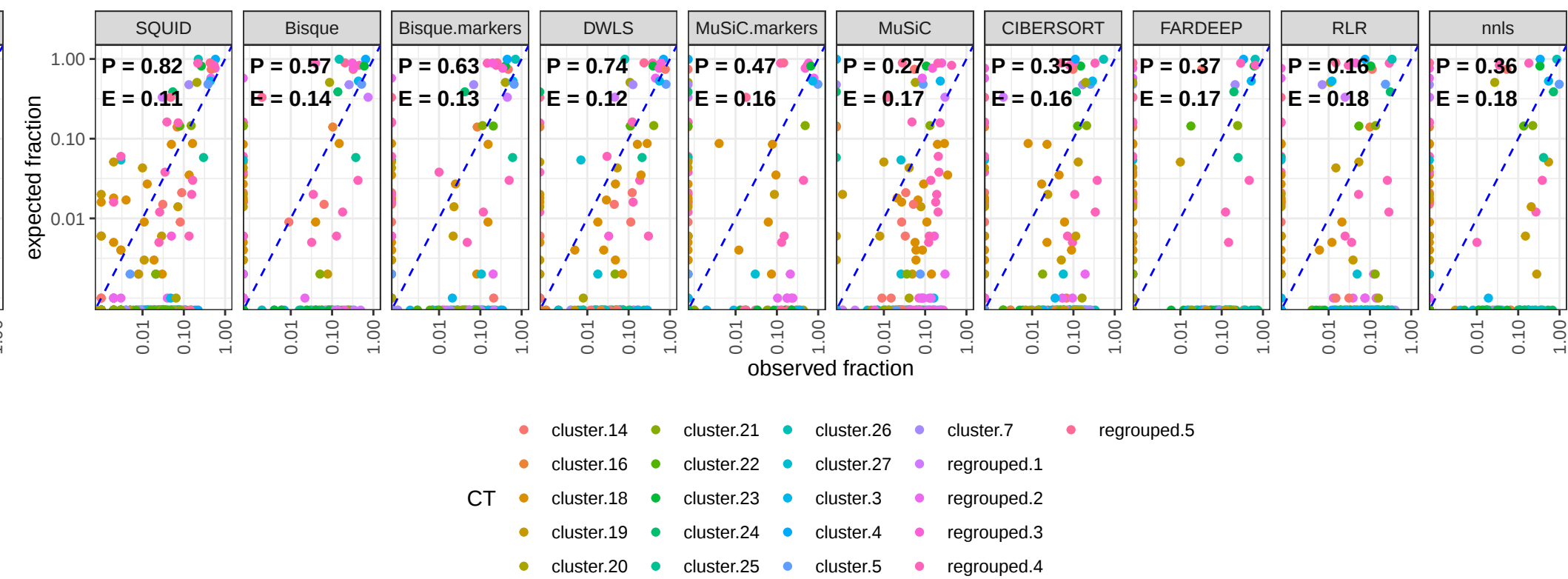
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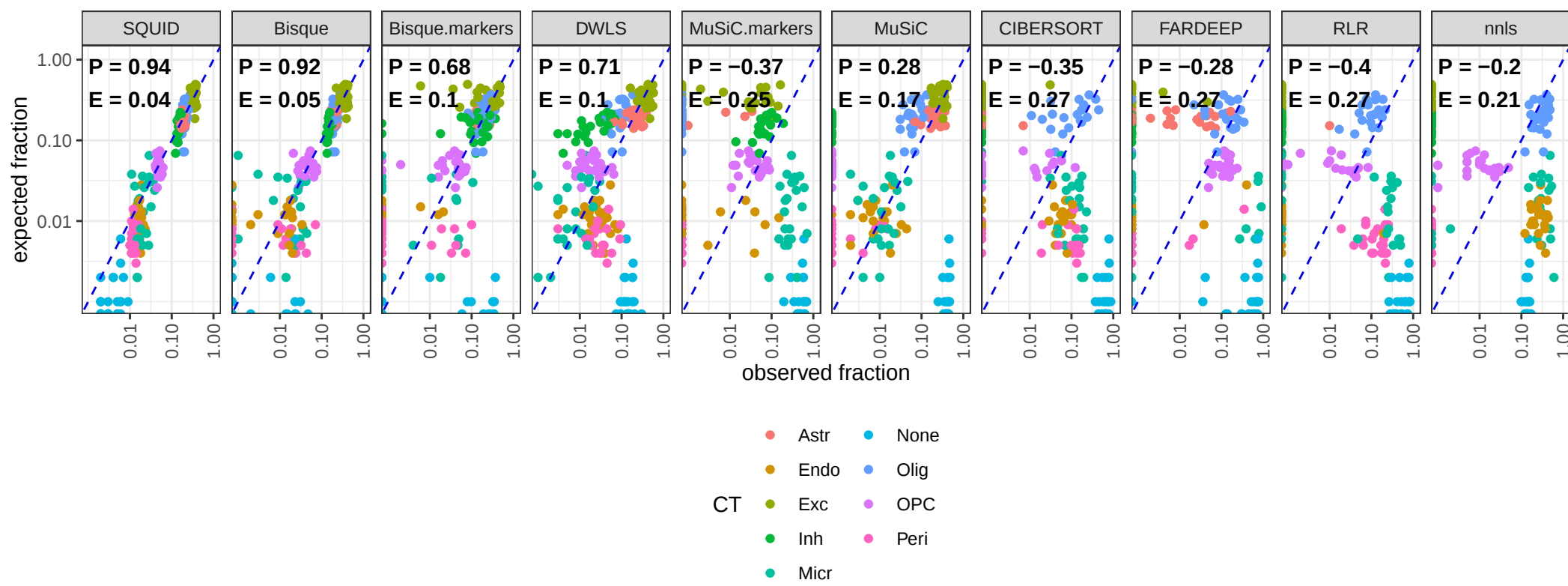
NB_1



NB_2



Synapse



Brain

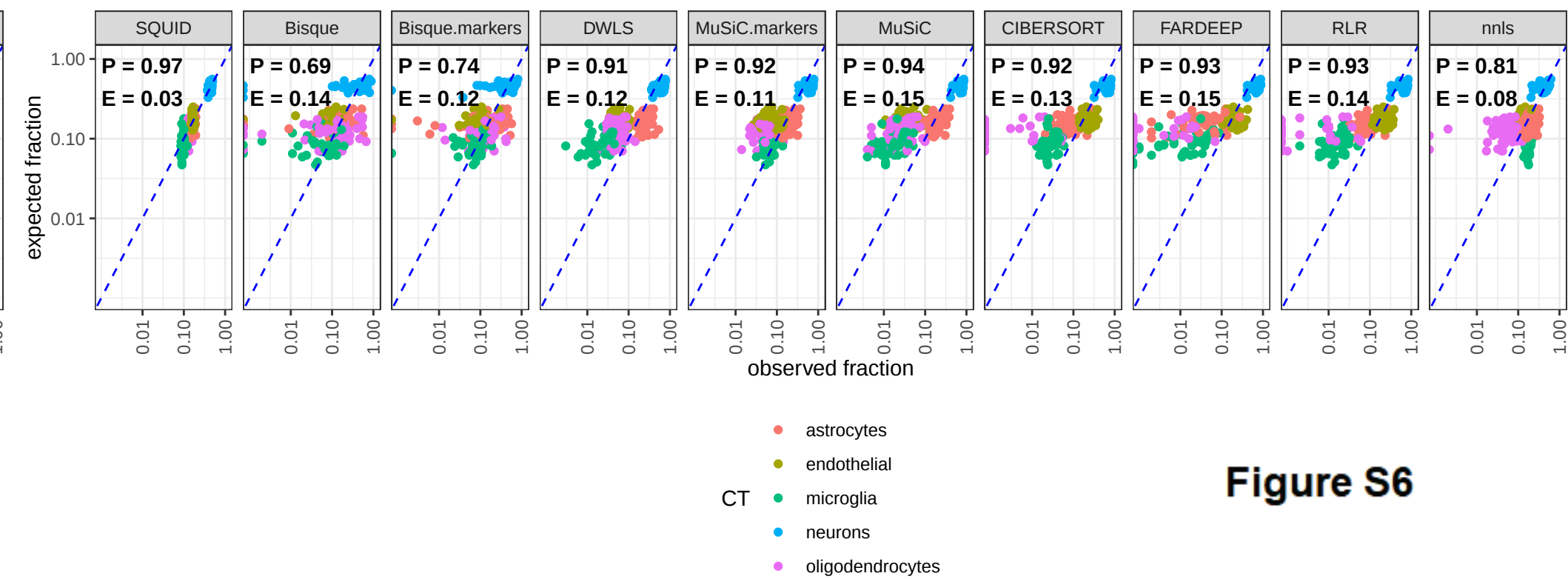


Figure S6

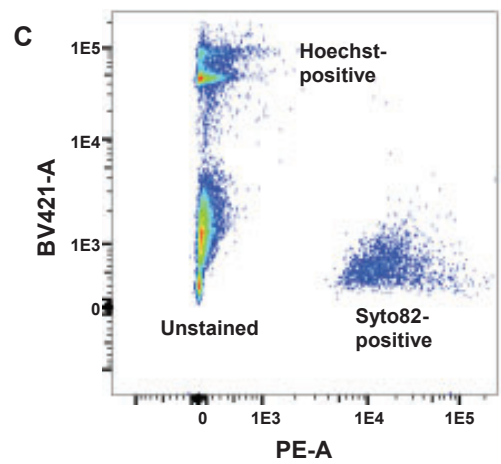
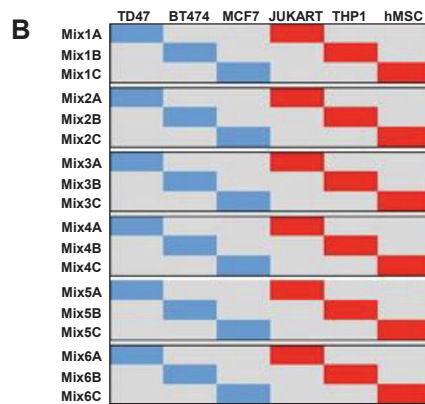
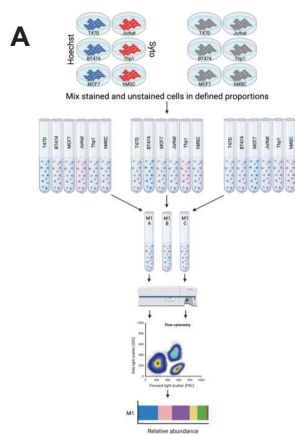


Figure S7