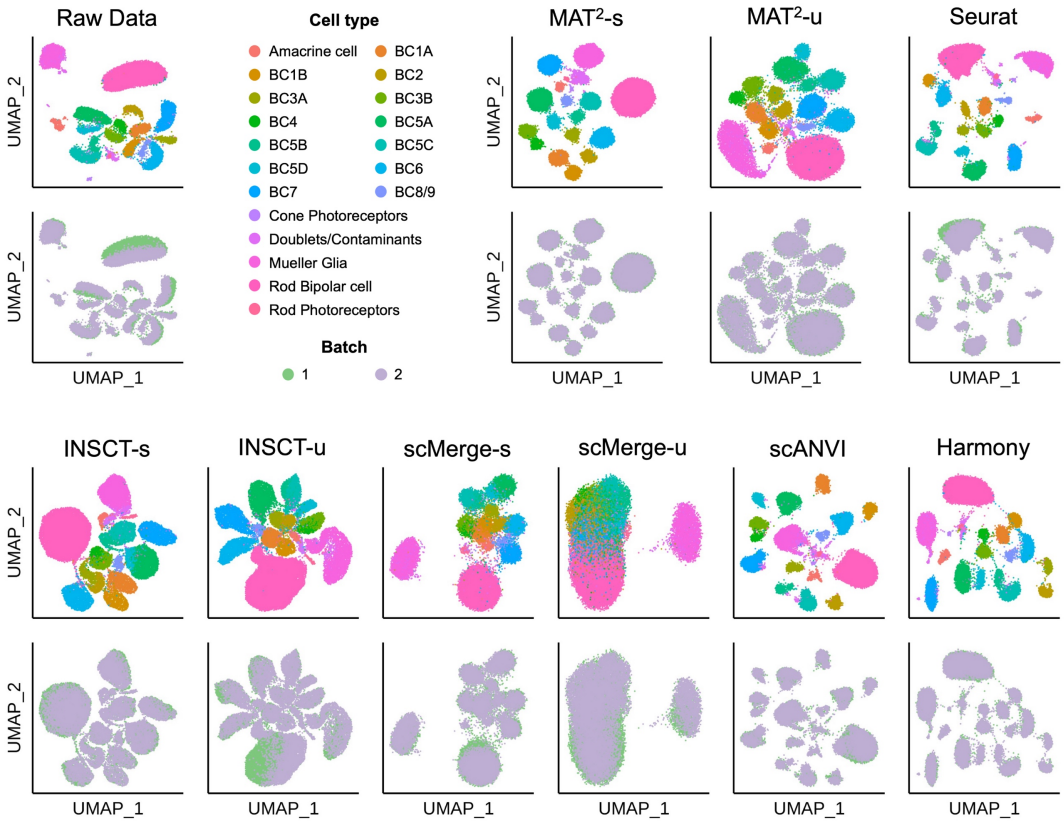

Fig. S1. UMAP visualization results of retina dataset without or with alignment. Each panel is colored according to cell type (upper) and batch (lower). There is no significant difference between the two batches in the retina dataset. Except scMerge-u, all methods can well remove batch effects in the datasets.

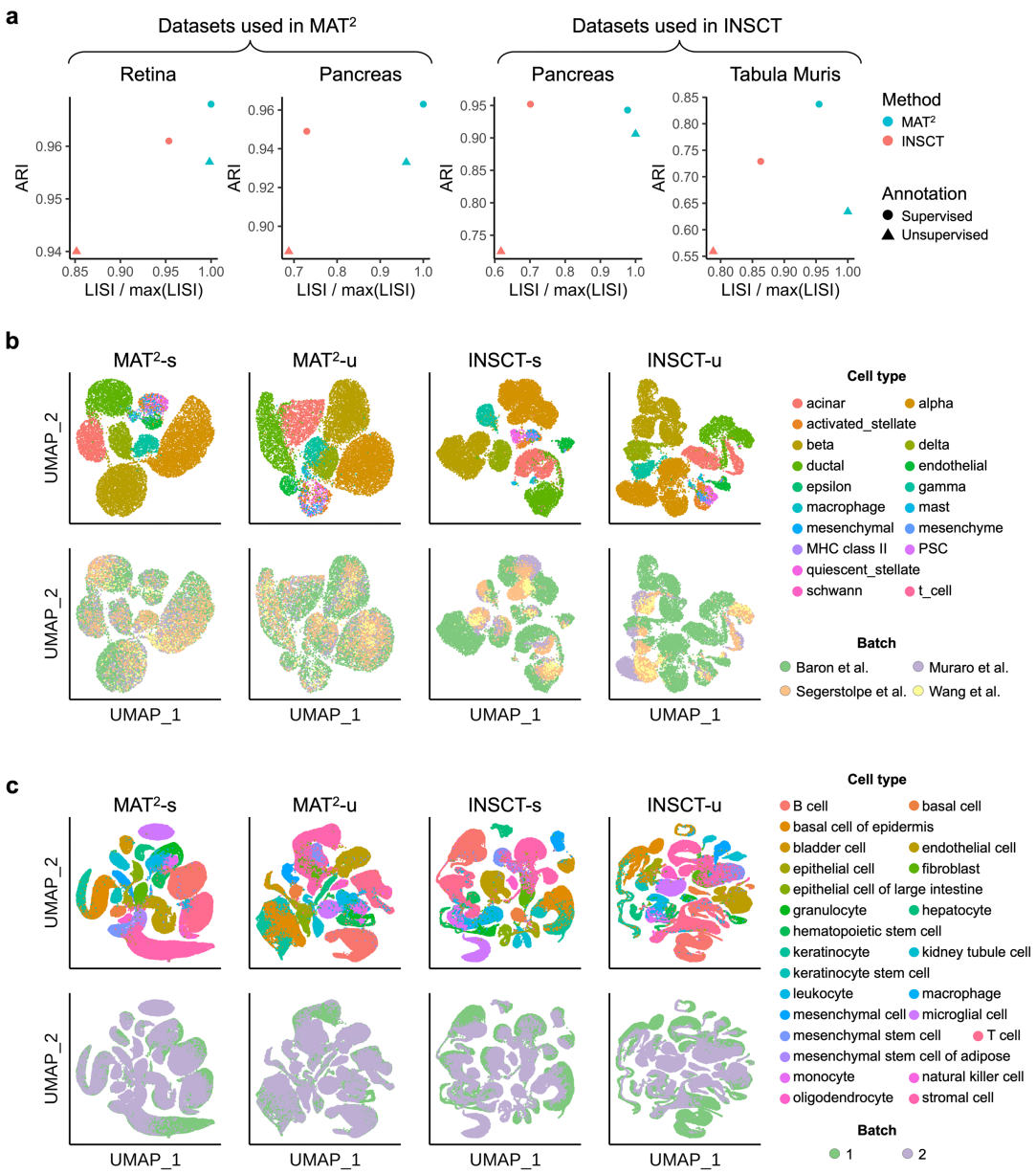

Fig. S2. Benchmarking of MAT² and INSCT on the dataset used in the article of both MAT² and INSCT. (**a**) The results of cell type assignment (ARI) and dataset mixing (LISI) on the dataset used in MAT² and INSCT articles. Regardless of whether ARI or LISI is used as a metric, MAT2 shows obvious superiority. (**b, c**) Visualize the results of MAT² and INSCT by UMAP on pancreas (**b**) and Tabula Muris (**c**) datasets in the article of INSCT. For clarity of visualization, only cell types with cell numbers greater than 1000 are used for alignment in the Tabula Muris dataset. MAT² shows better results of dataset mixing and more accurate cell type assignment in the two datasets.

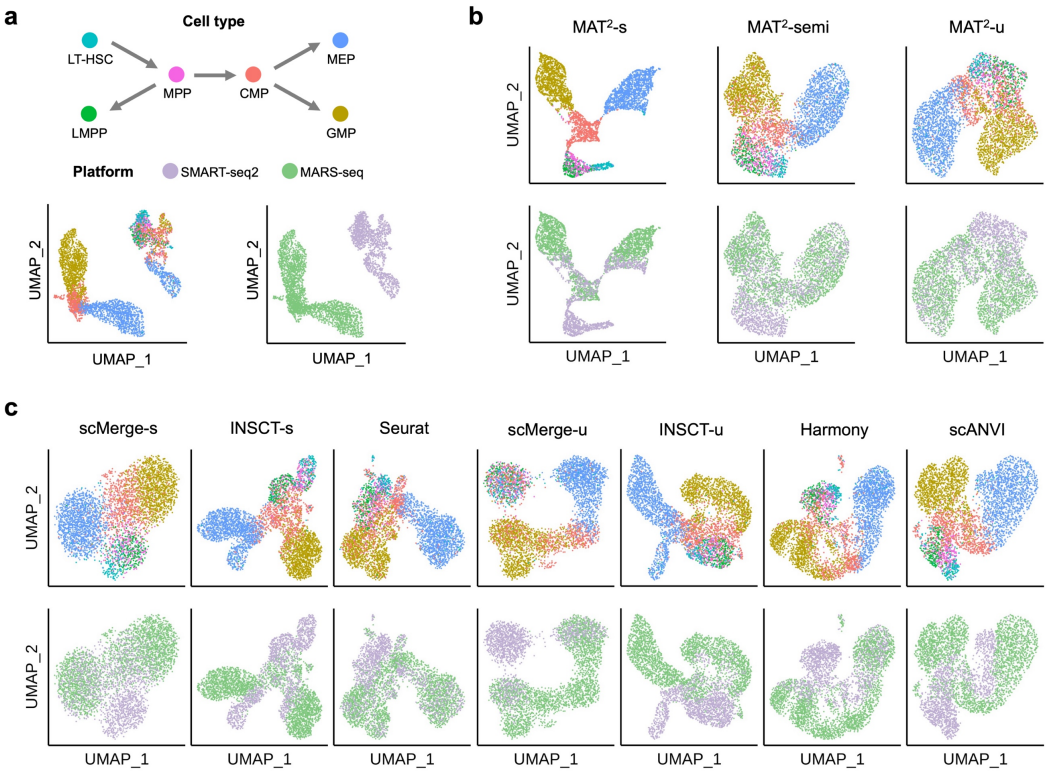

Fig. S3. (**a**) UMAP visualization of hematopoietic datasets generated by MARS-seq and SMART-seq2. The cells were colored according to their types and sequencing platforms. (**b, c**) UMAP visualization of the results by MAT², scMerge, INSCT, Seurat, Harmony and scANVI.The color mark is the same as (**a**).

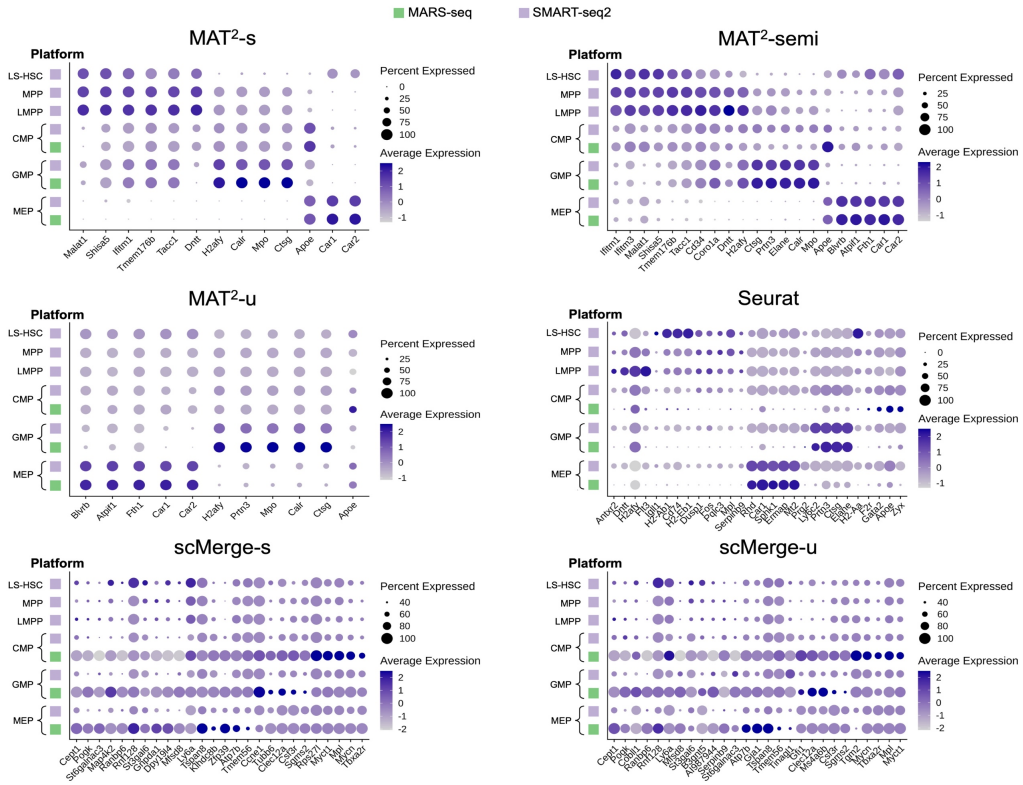

Fig. S4. The clustering results of cells with those genes differentially expressed between cell types defined with the alignment results by six methods, each of which can output the corrected gene expression matrix. All differential expression analysis was calculated through the same process in Seurat (min.pct = 0.15, logfc.threshold = 0.15). The expressions of DEGs for scMerge on different platforms were significantly different, which means that the results cannot be used for downstream analysis. In the results of MAT²-u and Seurat, the expression profiles of GMP and MEP were similar on different platforms, but no meaningful DEGs can be found in other cell types. In the result of MAT²-s, MEPs and CMPs were almost separated. And MAT²-semi alleviates this overfitting.