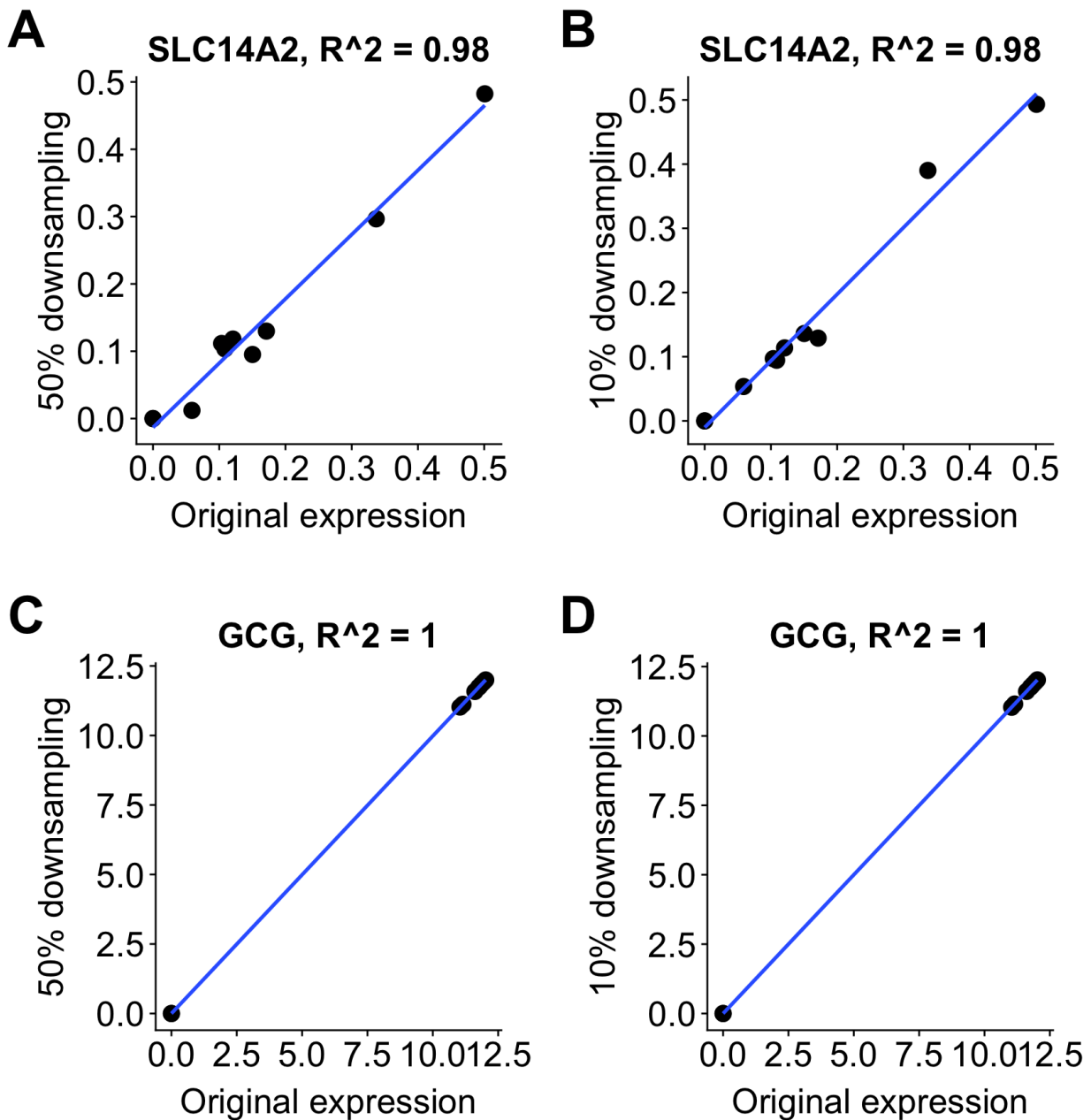
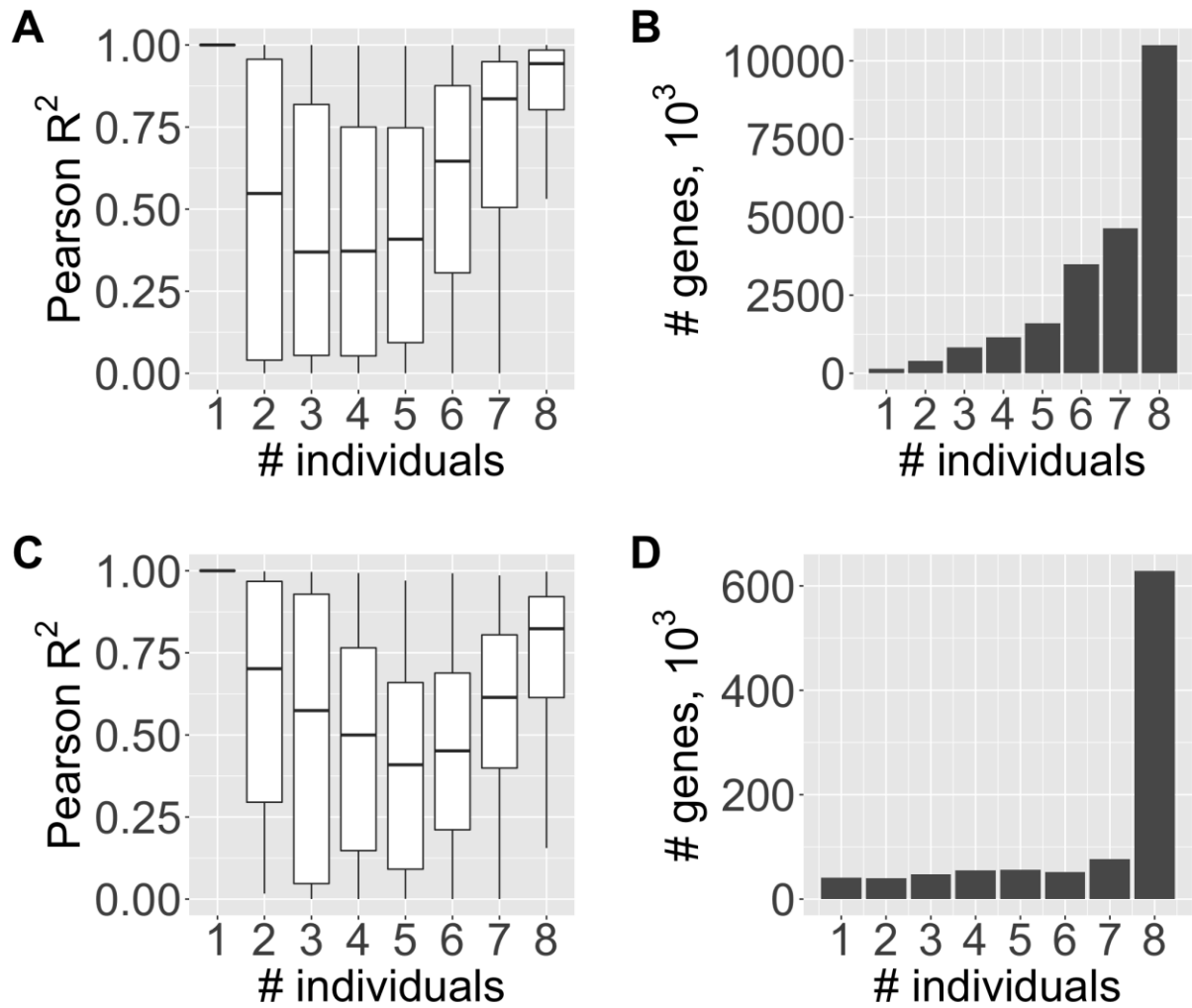


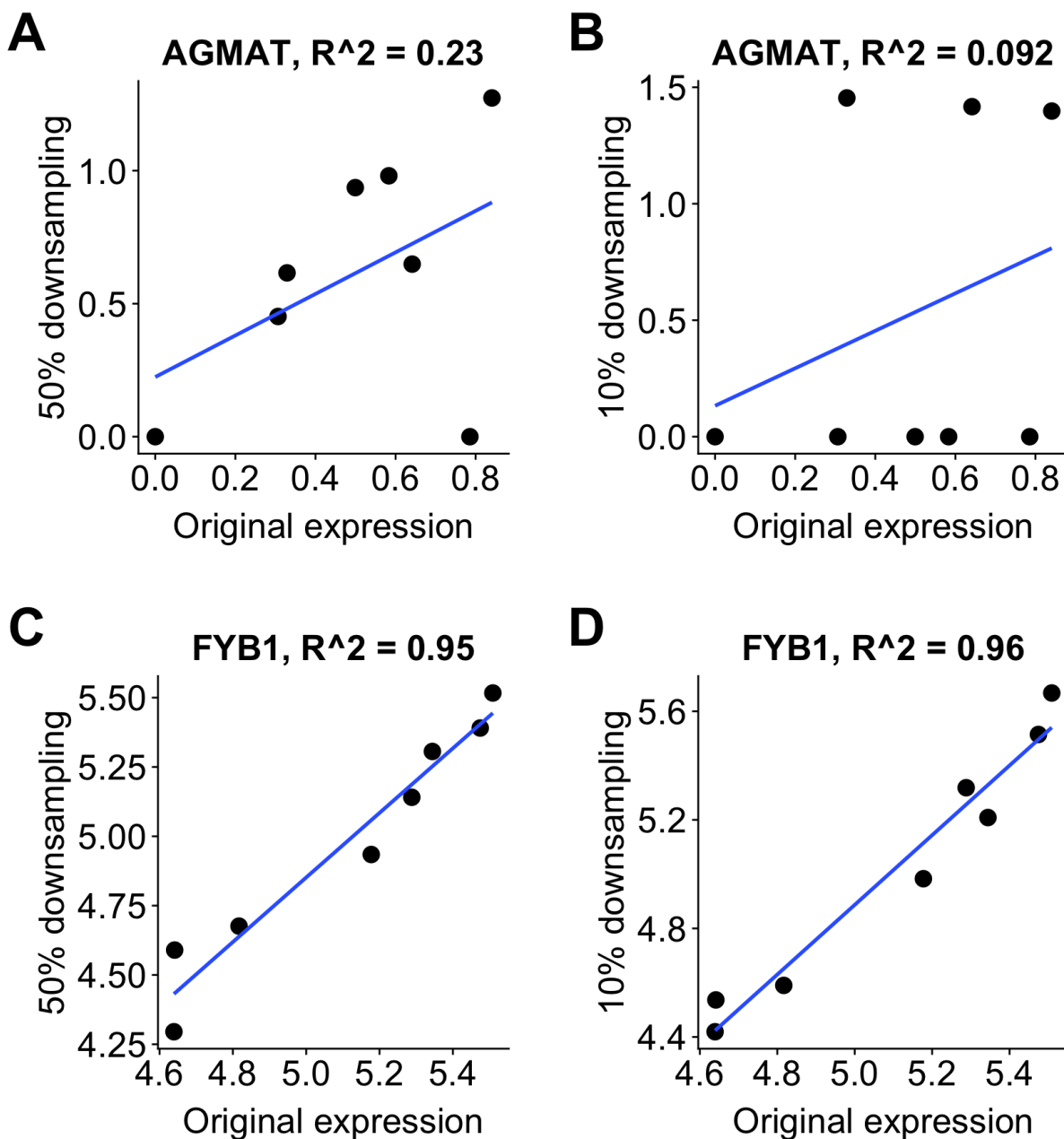
Supplementary Information



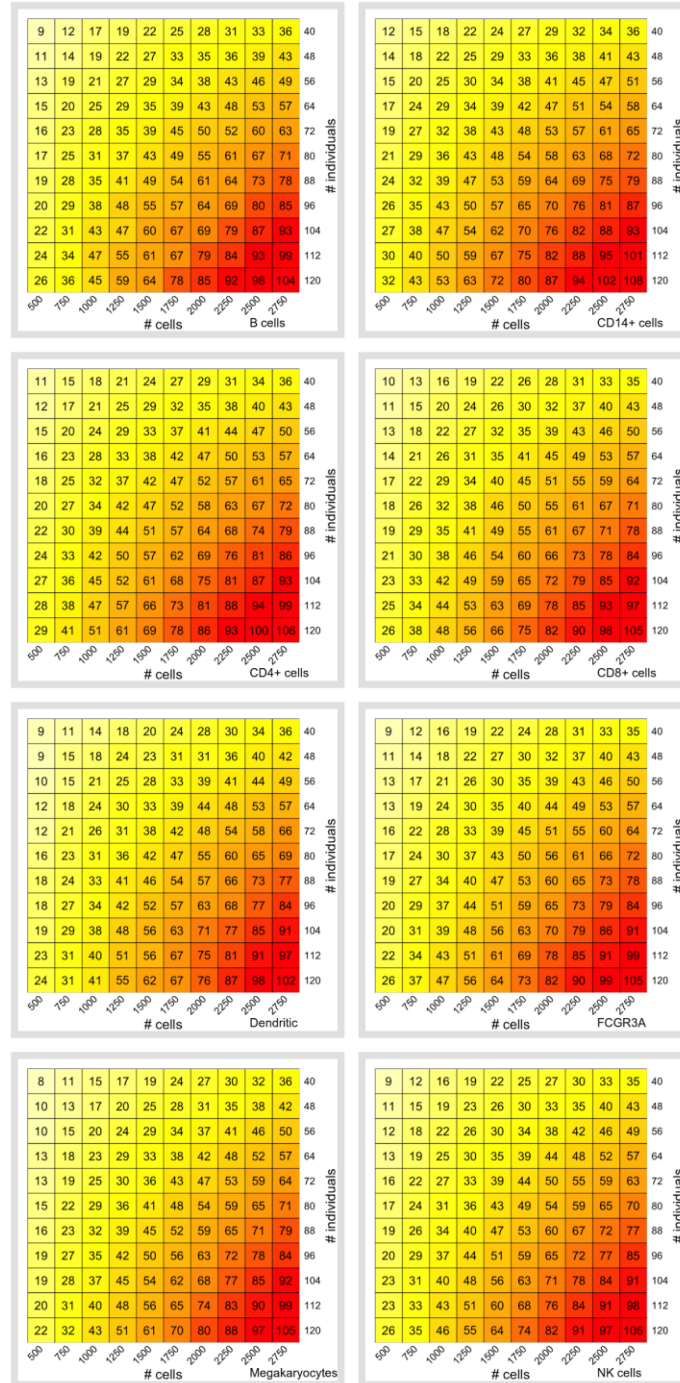
Supplementary Figure 1: Pearson R^2 between low-coverage estimates and the high-coverage gene expression in Smart-Seq2 dataset. A) *SLC14A2* gene, 50% downsampling ($\approx 375,000$ reads per cell); B) *SLC14A2* gene, 10% downsampling ($\approx 75,000$ reads per cell); C) *GCG* gene, 50% downsampling ($\approx 375,000$ reads per cell); D) *GCG* gene, 10% downsampling ($\approx 75,000$ reads per cell).



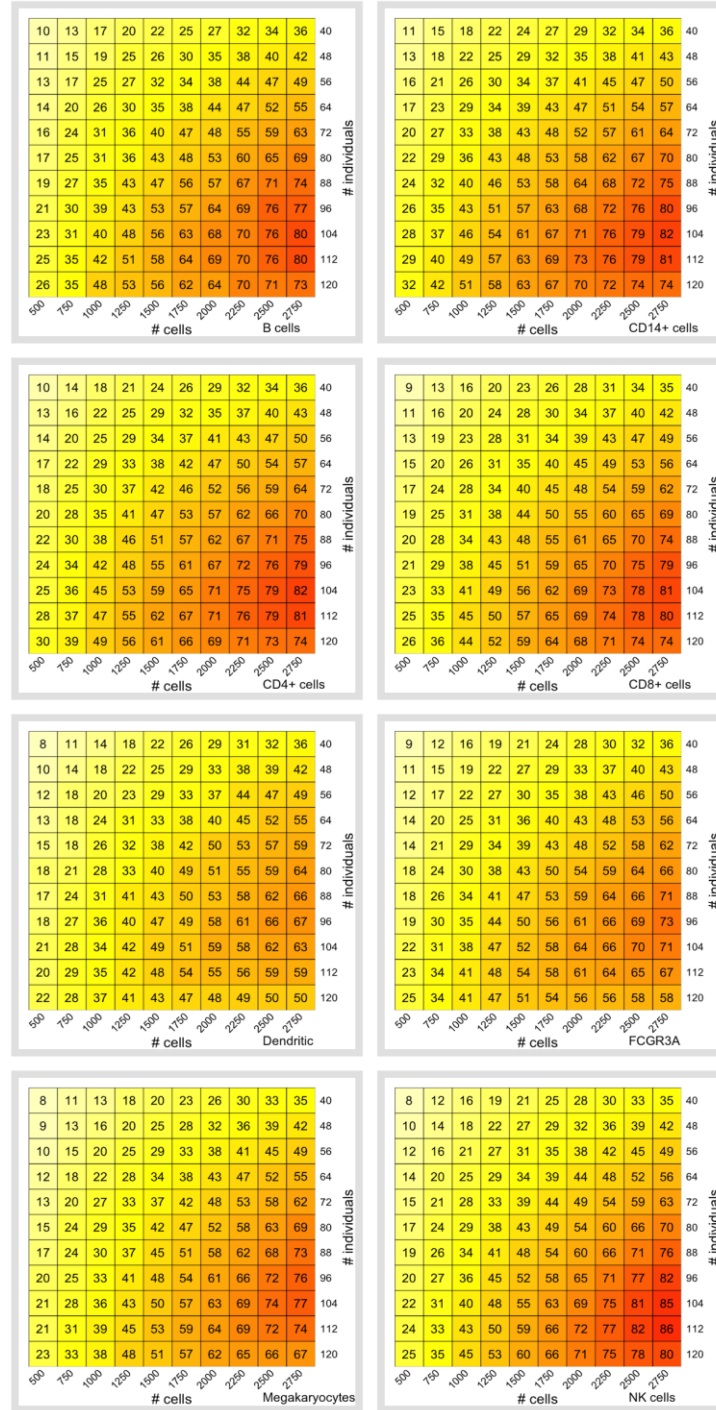
Supplementary Figure 2: Stratification of genes based on the number of individuals they are expressed in (Smart-Seq2 dataset). A) Distribution of genes by the number of individuals they are expressed in, Smart-Seq2 dataset. B) Average Pearson R^2 at 75,000 reads per cell (alpha cells) stratified by the number of individuals they are expressed in, Smart-Seq2 dataset (vertical bars indicate interquartile range). C) Distribution of genes by the number of individuals they are expressed in, 10X dataset (The Census of Immune Cells). D) Average Pearson R^2 at 4,000 reads per cell (erythroblast cells) stratified by the number of individuals they are expressed in, 10X dataset (The Census of Immune Cells) (vertical bars indicate interquartile range).



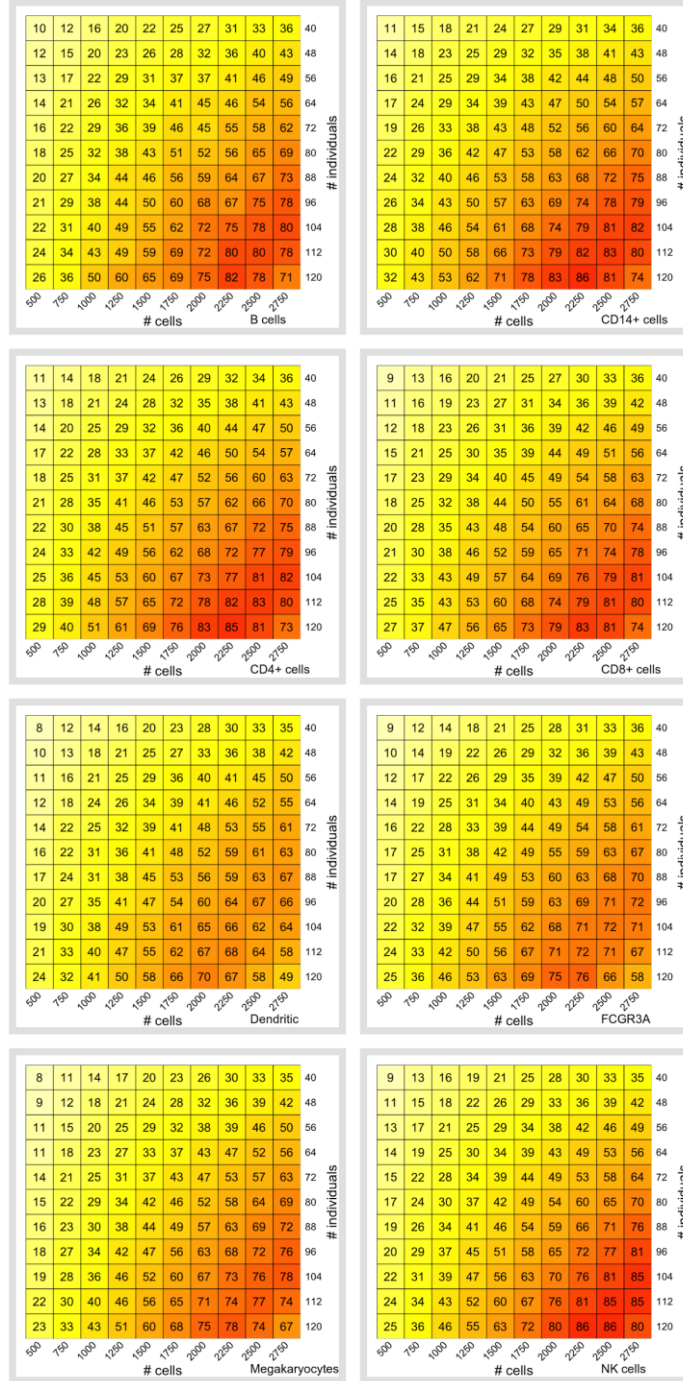
Supplementary Figure 3: Pearson R^2 between low-coverage estimates and the high-coverage gene expression in a 10X dataset (subset of the Census of Immune cells). A) AGMAT gene, 50% downsampling ($\approx 10,000$ reads per cell); B) AGMAT gene, 10% downsampling ($\approx 2,000$ reads per cell); C) FYB1 gene, 50% downsampling ($\approx 10,000$ reads per cell); D) FYB1 gene, 10% downsampling ($\approx 2,000$ reads per cell).



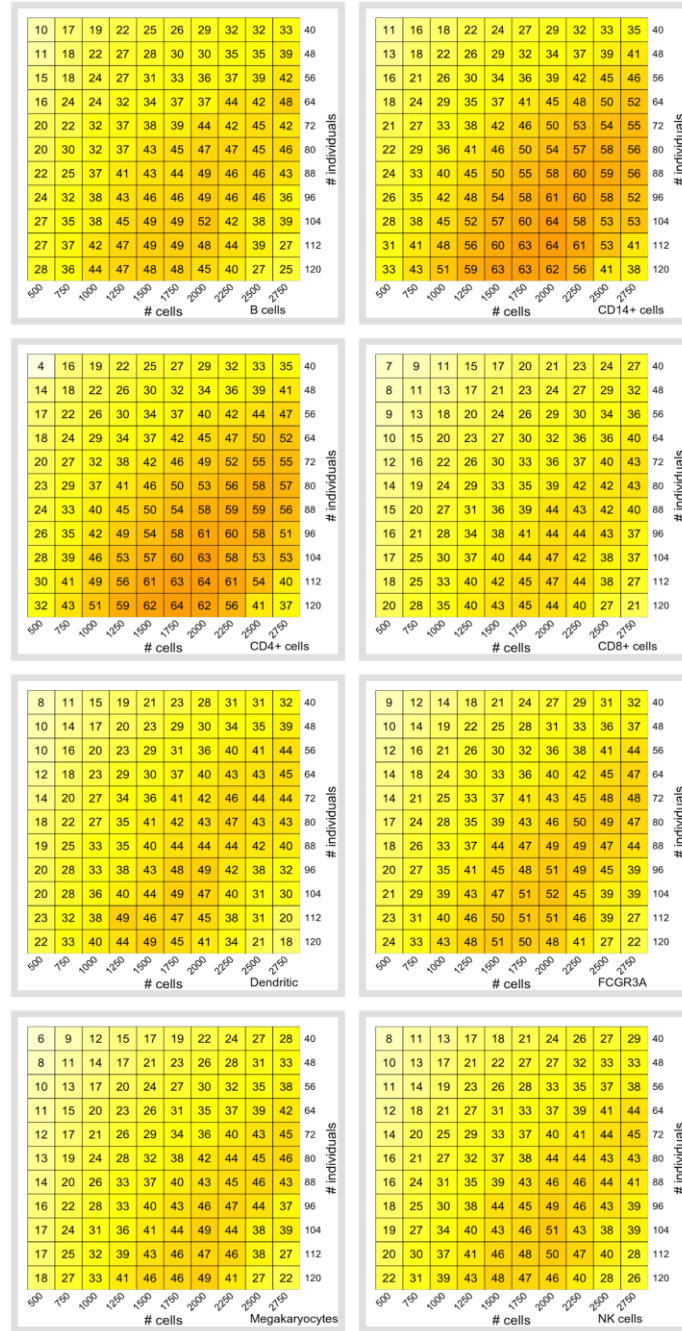
Supplementary Figure 4: Effective sample size as a function of number of individuals and number of cells per individual at budget \$35,000 assuming no library preparation cost, multiplexing of 8 individuals per reaction, and known cell types. The dependence on read coverage is implicit. The maximum effective size N_{eff} is 105 for B cells ($N = 120, M = 2,750, r = 14,500$), 108 for CD14+ cells ($N = 120, M = 2,750, r = 14,500$), 107 for CD4+ cells ($N = 120, M = 2,750, r = 14,500$), 105 for CD8+ cells ($N = 120, M = 2,750, r = 14,500$), 102 for dendritic cells ($N = 120, M = 2,750, r = 14,500$), 106 for Fcgr3a cells ($N = 120, M = 2,750, r = 14,500$), 105 for megakaryocytes ($N = 120, M = 2,750, r = 14,500$), 106 for NK cells ($N = 120, M = 2,750, r = 14,500$). Colour scales correspond to the magnitude of the values in each cell of the heatmap.



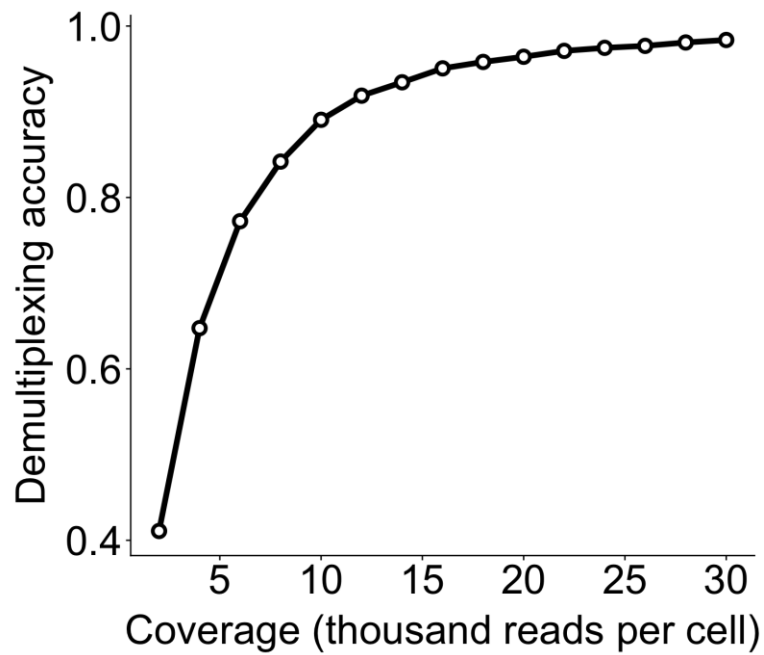
Supplementary Figure 5: Effective sample size as a function of number of individuals and number of cells per individual at budget \$35,000 assuming library preparation costs of \$2,000 per reaction, multiplexing of 8 individuals per reaction, known cell types. The maximum effective size N_{eff} is 81 for B cells ($N = 104, M = 2,750, r = 4,300$), 82 for CD14+ cells ($N = 104, M = 2,750, r = 4,300$), 82 for CD4+ cells ($N = 104, M = 2,750, r = 4,300$), 82 for CD8+ cells ($N = 104, M = 2,750, r = 4,300$), 67 for dendritic cells ($N = 96, M = 2,750, r = 5,700$), 73 for Fcgr3a cells ($N = 96, M = 2,750, r = 5,700$), 77 for megakaryocytes ($N = 104, M = 2,750, r = 4,300$), 86 for NK cells ($N = 112, M = 2,750, r = 3,100$). Colour scales correspond to the magnitude of the values in each cell of the heatmap.



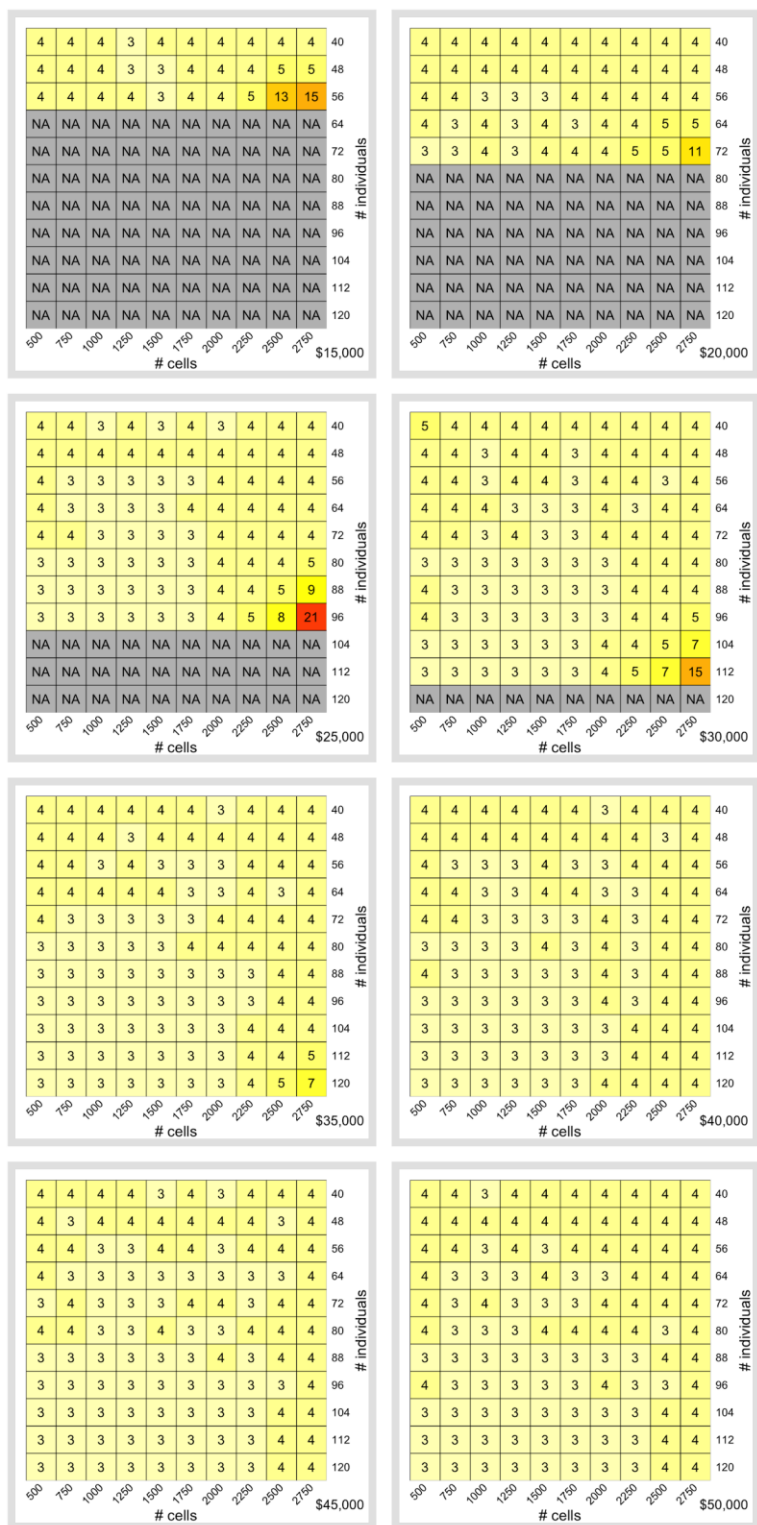
Supplementary Figure 6: Effective sample size as a function of number of individuals and number of cells per individual at budget \$35,000 assuming library preparation costs of \$2,000 per reaction, greedy multiplexing (up to 16 individuals per reaction) and known cell types. The level of multiplexing takes on values from 8 to 16. The maximum effective size N_{eff} is 83 for B cells ($N = 120, M = 2,250, r = 5,400$), 86 for CD14+ cells ($N = 120, M = 2,250, r = 5,400$), 86 for CD4+ cells ($N = 120, M = 2,250, r = 5,400$), 83 for CD8+ cells ($N = 120, M = 2,250, r = 5,400$), 70 for dendritic cells ($N = 120, M = 2,000, r = 8,000$), 76 for Fcgr3a cells ($N = 120, M = 2,250, r = 5,400$), 79 for megakaryocytes ($N = 120, M = 2,250, r = 5,400$), 86 for NK cells ($N = 120, M = 2,250, r = 5,400$). Colour scales correspond to the magnitude of the values in each cell of the heatmap.



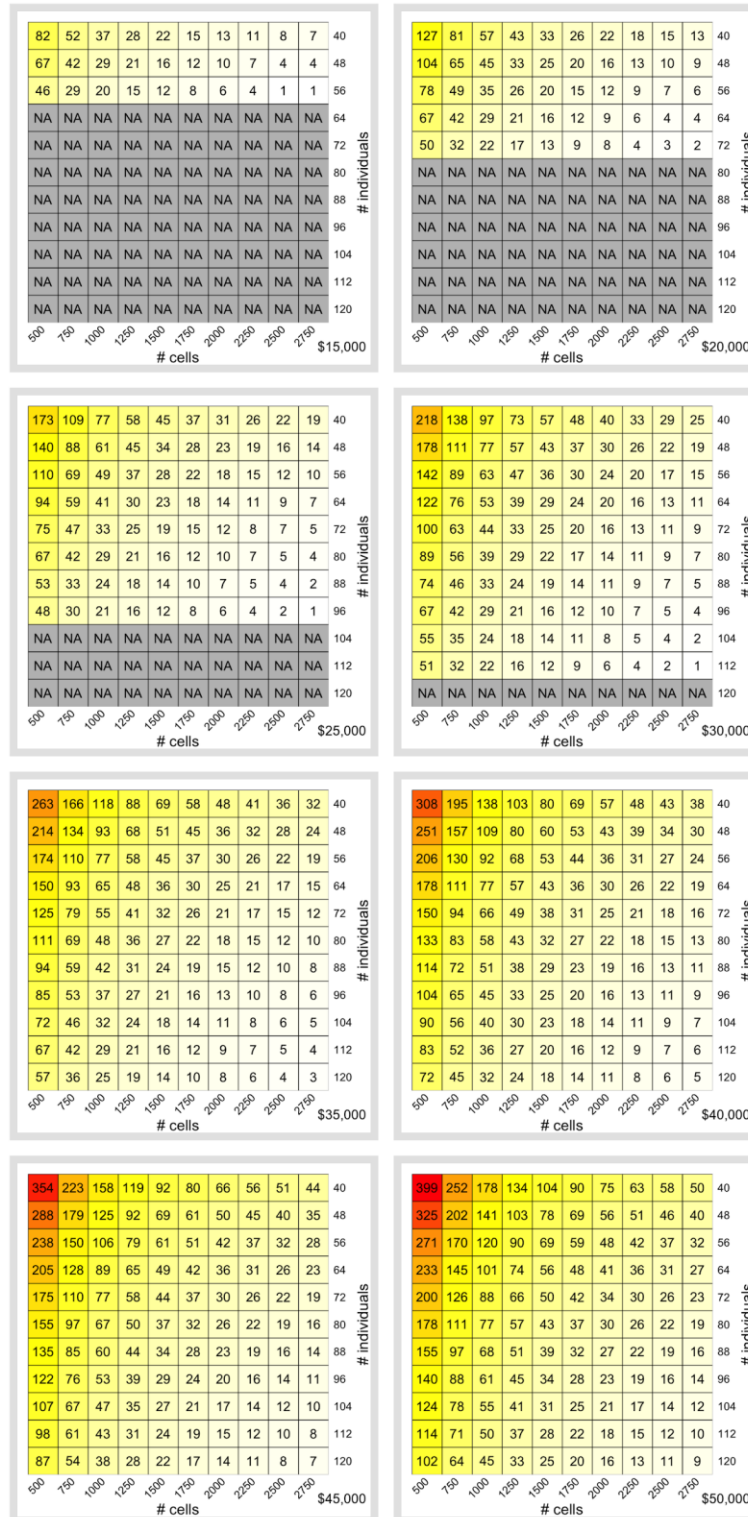
Supplementary Figure 7: Effective sample size as a function of number of individuals and number of cells per individual at budget \$35,000 assuming library preparation costs of \$2,000 per reaction, greedy multiplexing (up to 16 individuals per reaction) and unknown cell types. Demultiplexing inaccuracy at low coverage is taken into account. Cell types are inferred using Seurat's label transfer procedure. The maximum effective size N_{eff} is 52 for B cells ($N = 104, M = 2,000, r = 10,500$), 64 for CD14+ cells ($N = 112, M = 2,000, r = 8,500$), 64 for CD4+ cells ($N = 120, M = 1,750, r = 9,500$), 47 for CD8+ cells ($N = 112, M = 2,000, r = 8,500$), 49 for dendritic cells ($N = 96, M = 2,000, r = 12,500$), 52 for Fcgr3a cells ($N = 104, M = 2,000, r = 10,500$), 49 for megakaryocytes ($N = 120, M = 2,000, r = 7,500$), 51 for NK cells ($N = 104, M = 2,000, r = 10,500$). Colour scales correspond to the magnitude of the values in each cell of the heatmap.



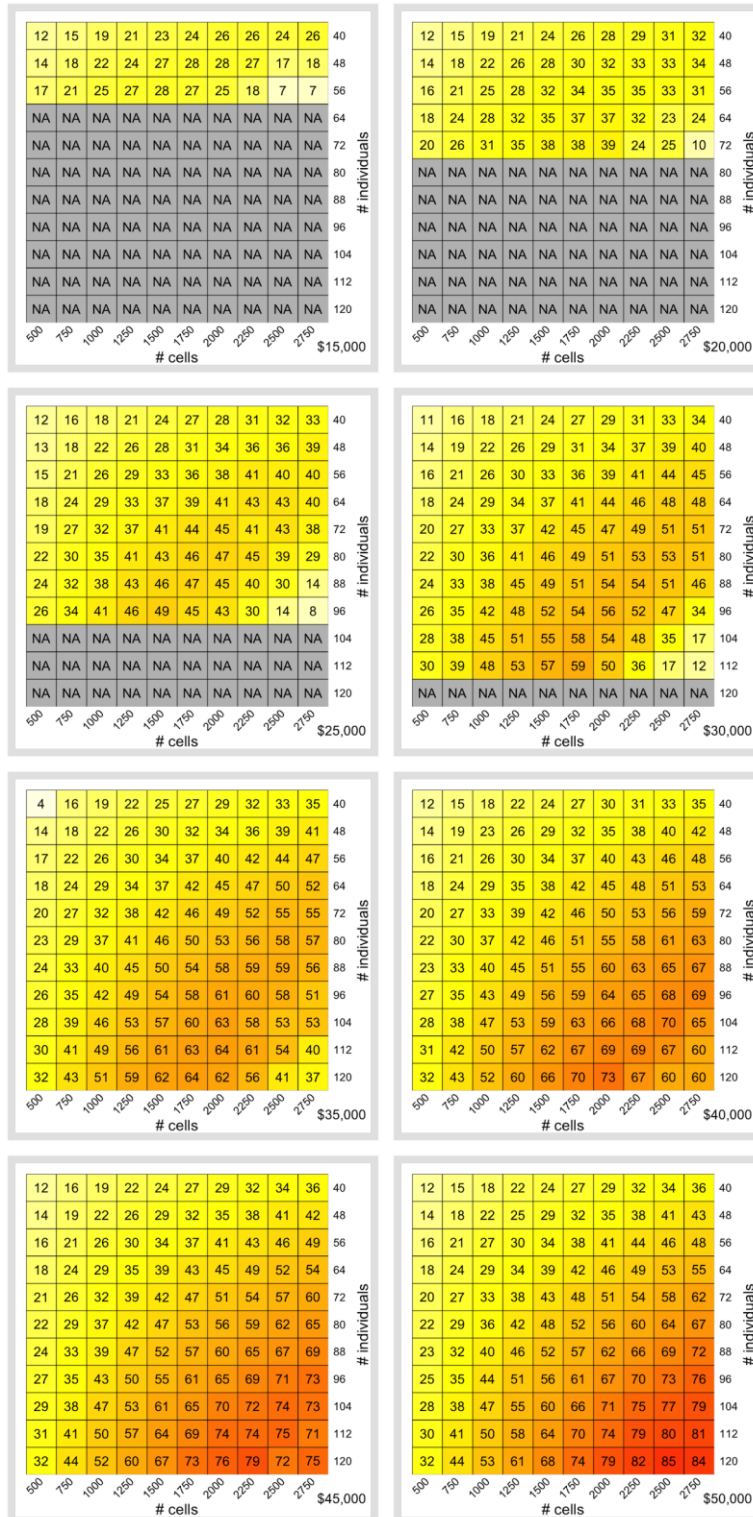
Supplementary Figure 8: The impact of read coverage on the accuracy of demultiplexing (10X dataset). Shown here is the percentage of correctly assigned cells (to the corresponding sample) as a function of average number of reads per cell.



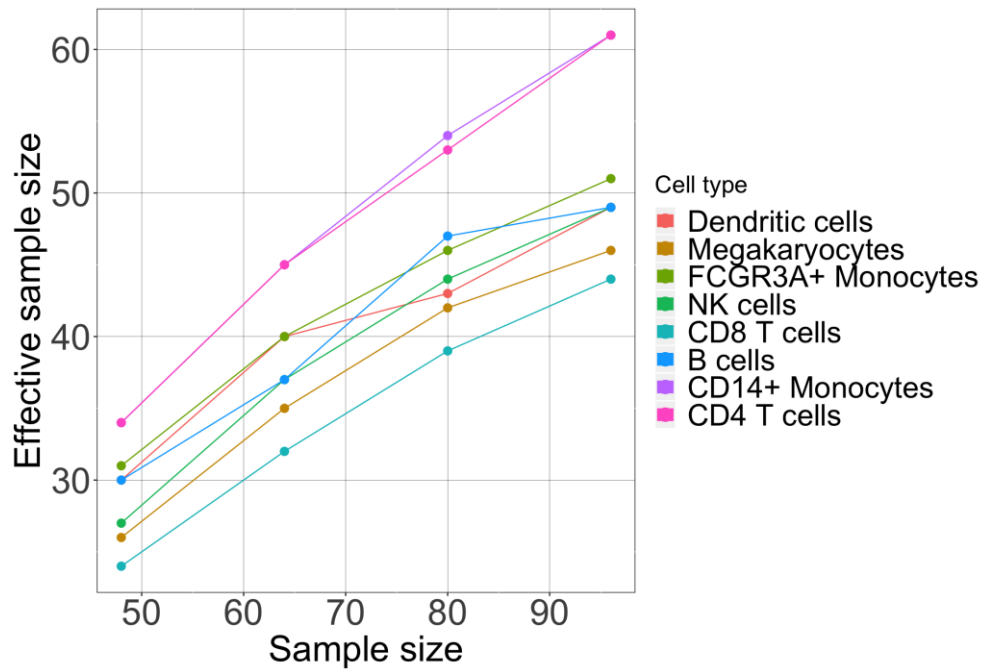
Supplementary Figure 9: Cell-type misclassification rate (in %) across different budgets. Library preparation costs of \$2,000 per reaction and greedy multiplexing are assumed. Demultiplexing inaccuracy at low coverage is taken into account. Colour scales correspond to the magnitude of the values in each cell of the heatmap.



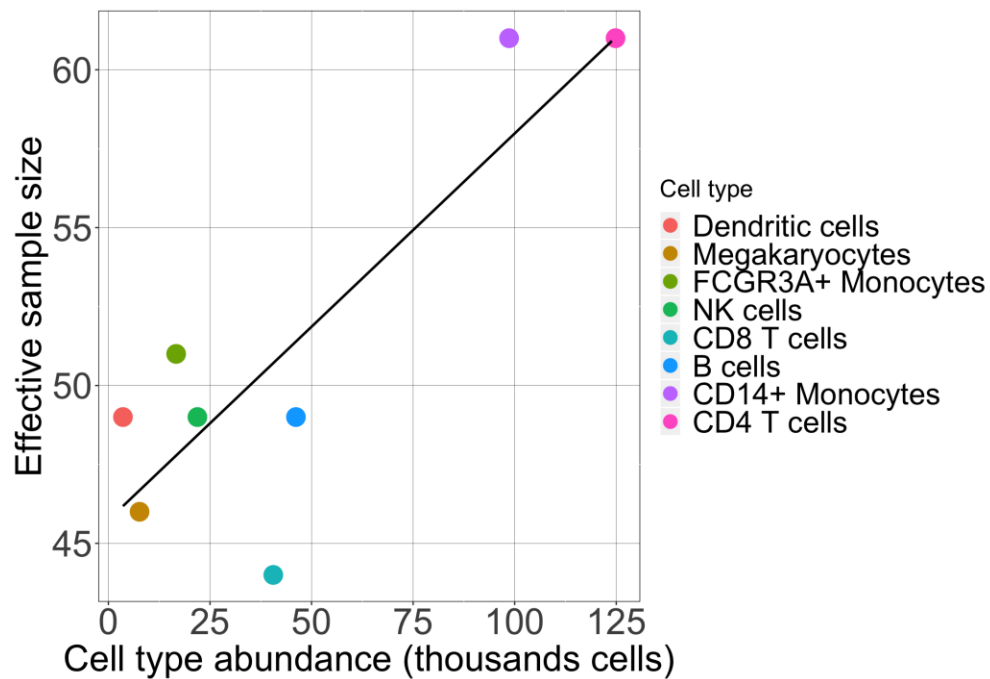
Supplementary Figure 10: Coverage (thousands of reads per cell) across different budgets. Library preparation costs of \$2,000 per reaction and greedy multiplexing (with at most 24,000 cells per reaction) are assumed. Colour scales correspond to the magnitude of the values in each cell of the heatmap.



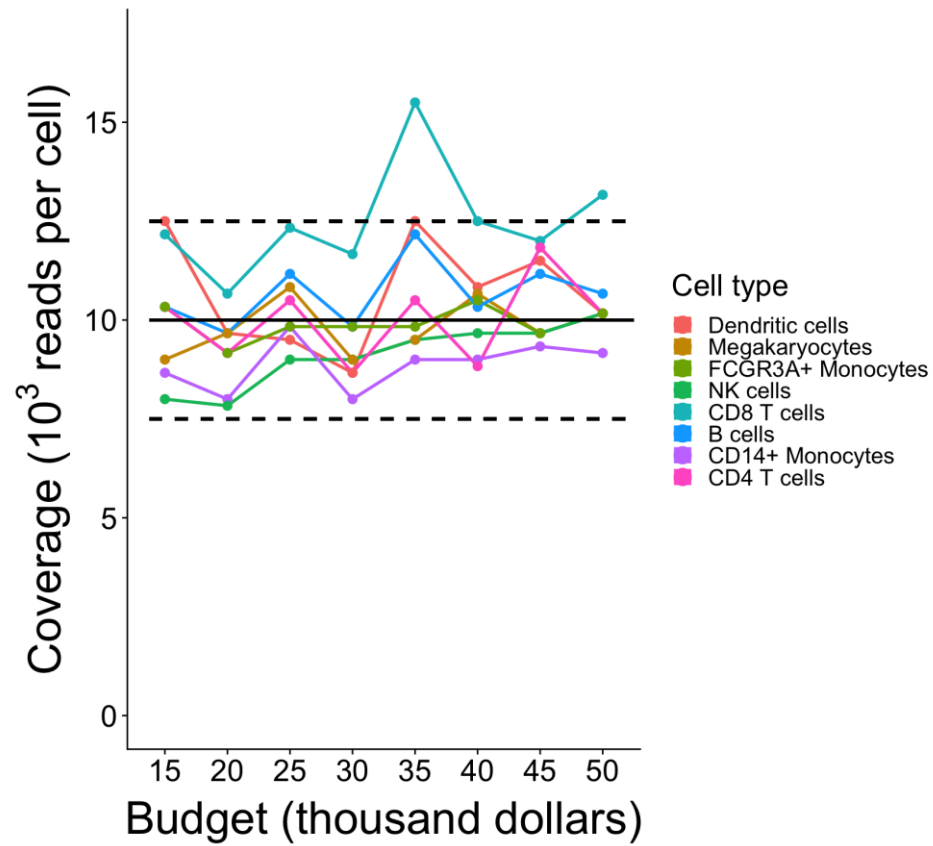
Supplementary Figure 11: Effective sample size for CD4 T cells across different budgets assuming library preparation costs of \$2,000 per reaction and greedy multiplexing. Demultiplexing inaccuracy at low coverage is taken into account. Colour scales correspond to the magnitude of the values in each cell of the heatmap.



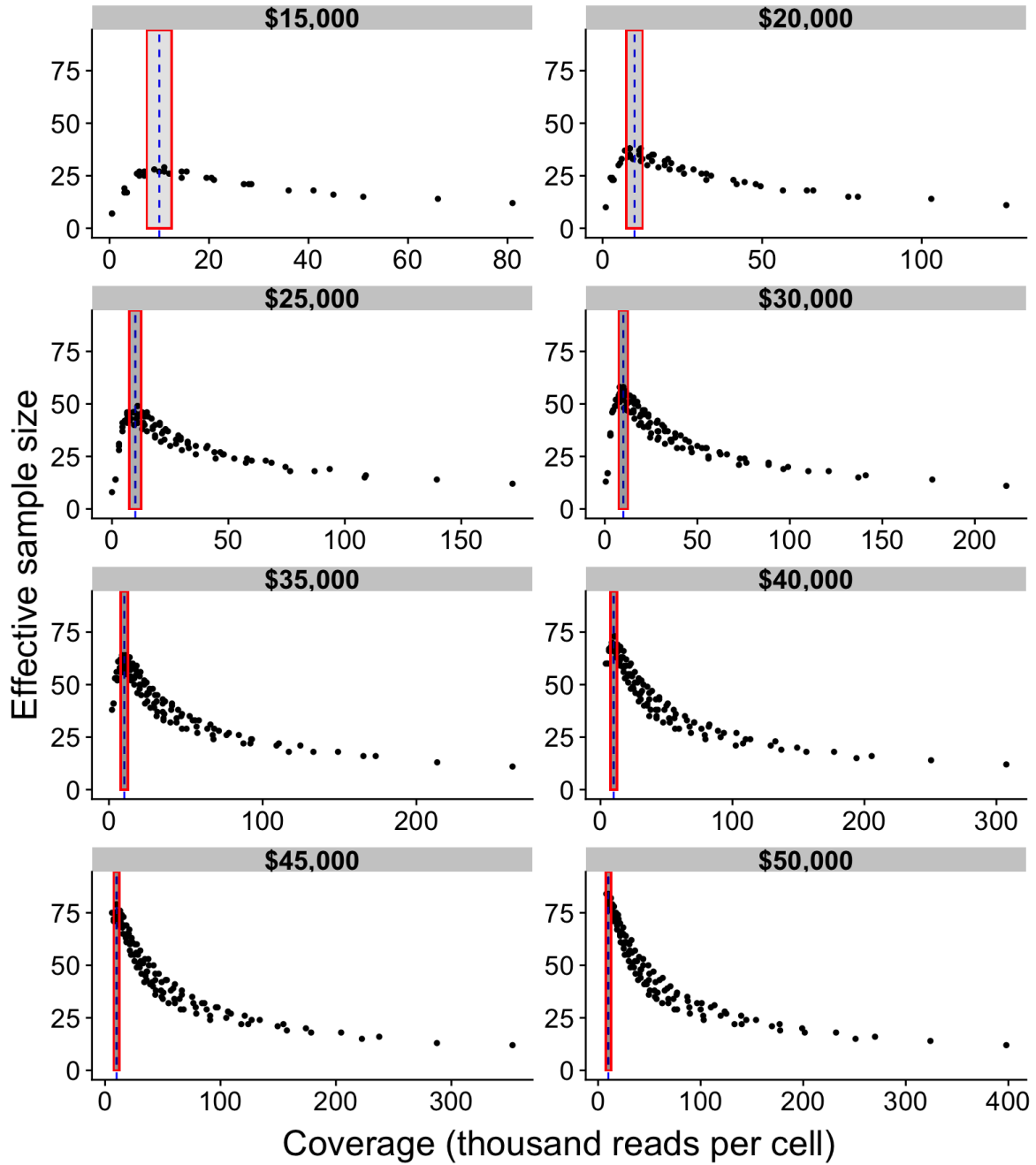
Supplementary Figure 12: Effective sample size for CD4 T cells as a function of sample size across different cell types at \$35,000.



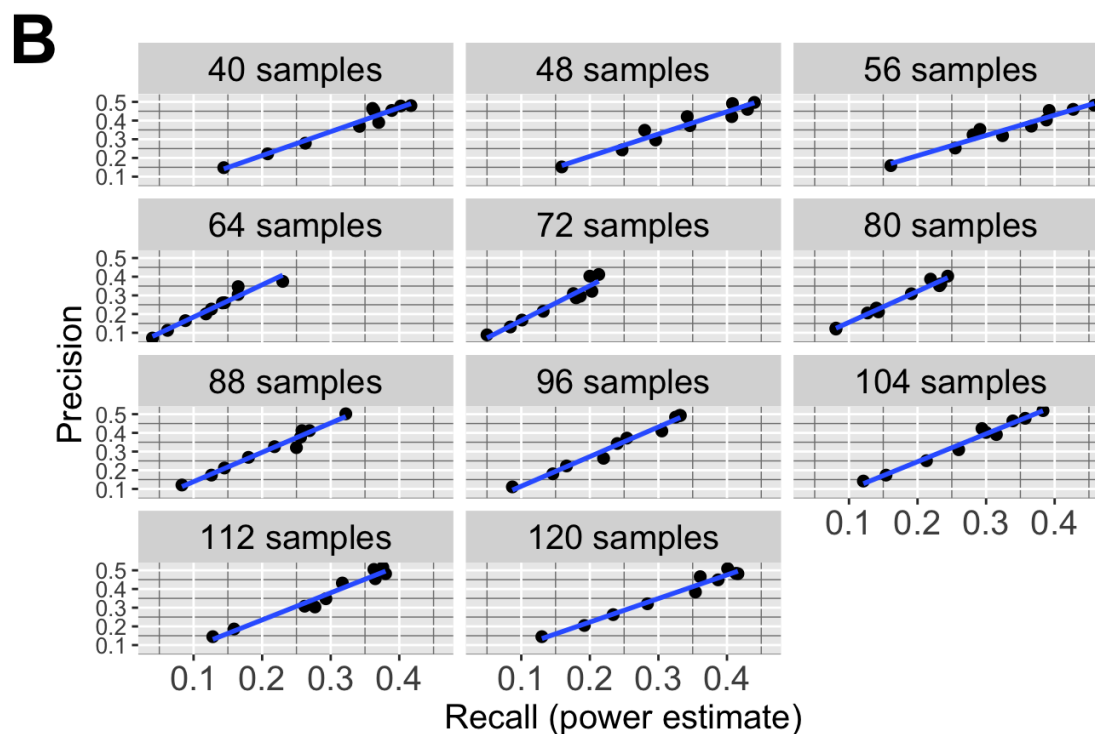
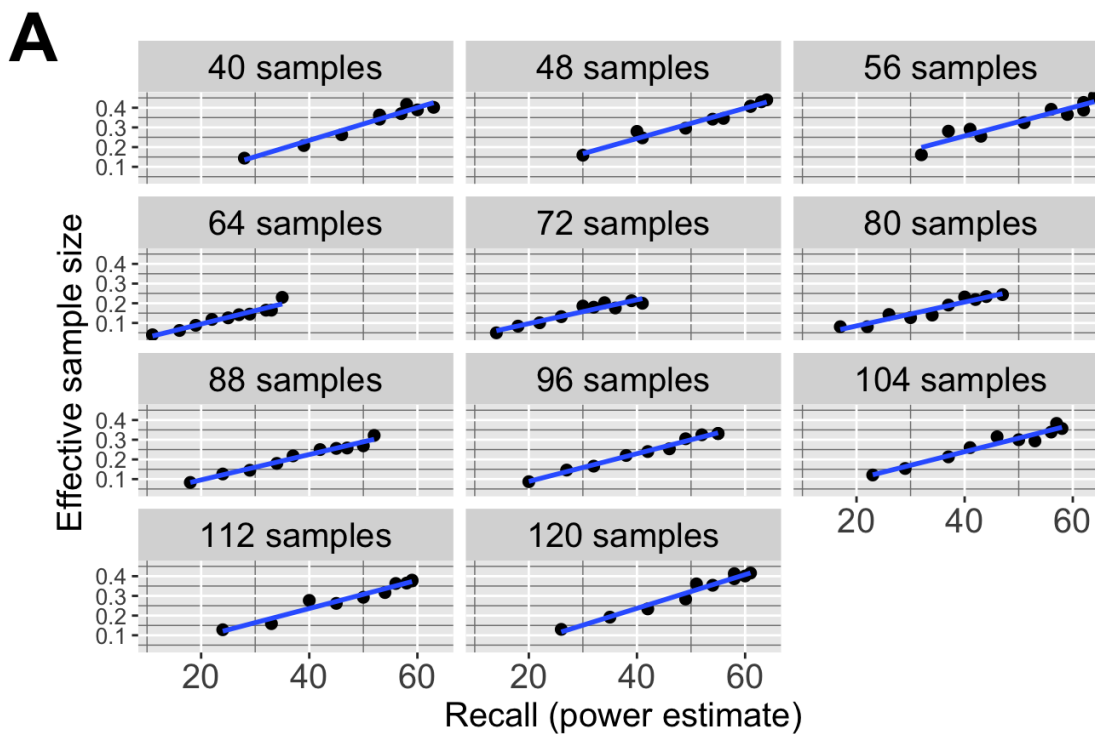
Supplementary Figure 13: R^2 between cell type abundances and the effective sample size is equal 0.72 ($N = 96$, $B = \$35,000$).



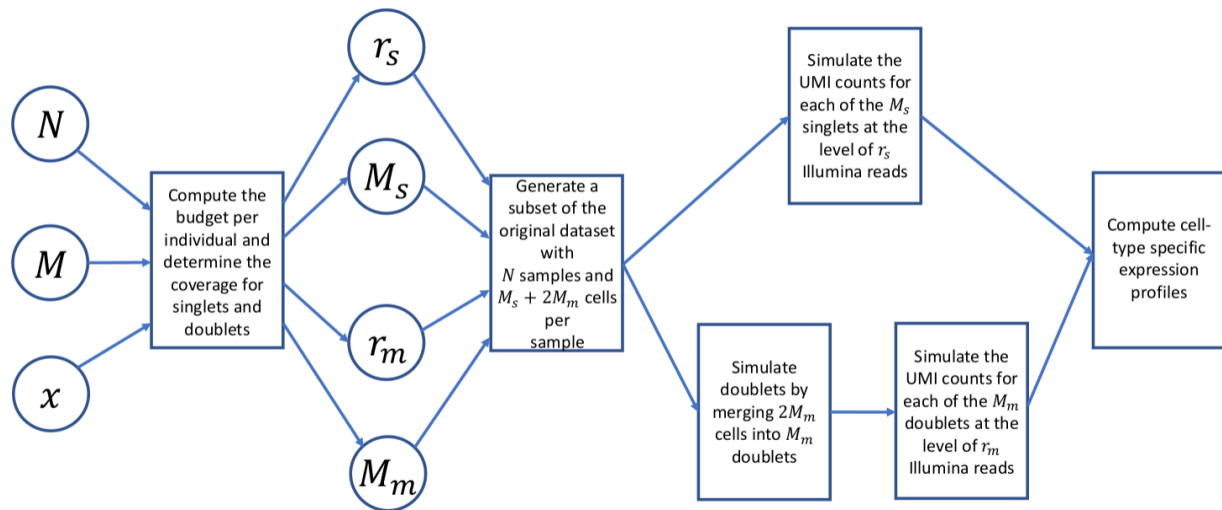
Supplementary Figure 14: Average coverage across top 3 most powerful ct-eQTL studies as a function of budget. Coverage corresponding to the experiments with the highest effective sample size (in the search space defined in the main text of the paper) is $10,000 \pm 2,500$ reads per cell.



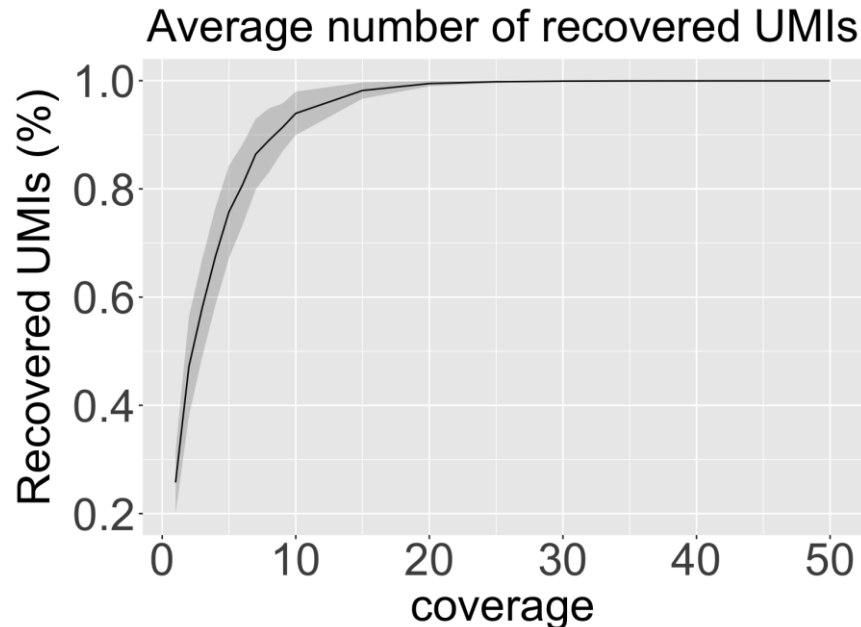
Supplementary Figure 15: Effective sample size as a function of read coverage (CD4 T cells). The optimal effective sample size is achieved at $10,000 \pm 2,500$ reads per cell. Each dot represents an experiment, the grey strip delimits the optimal coverage, i.e. the coverage at which the highest effective sample size is achieved.



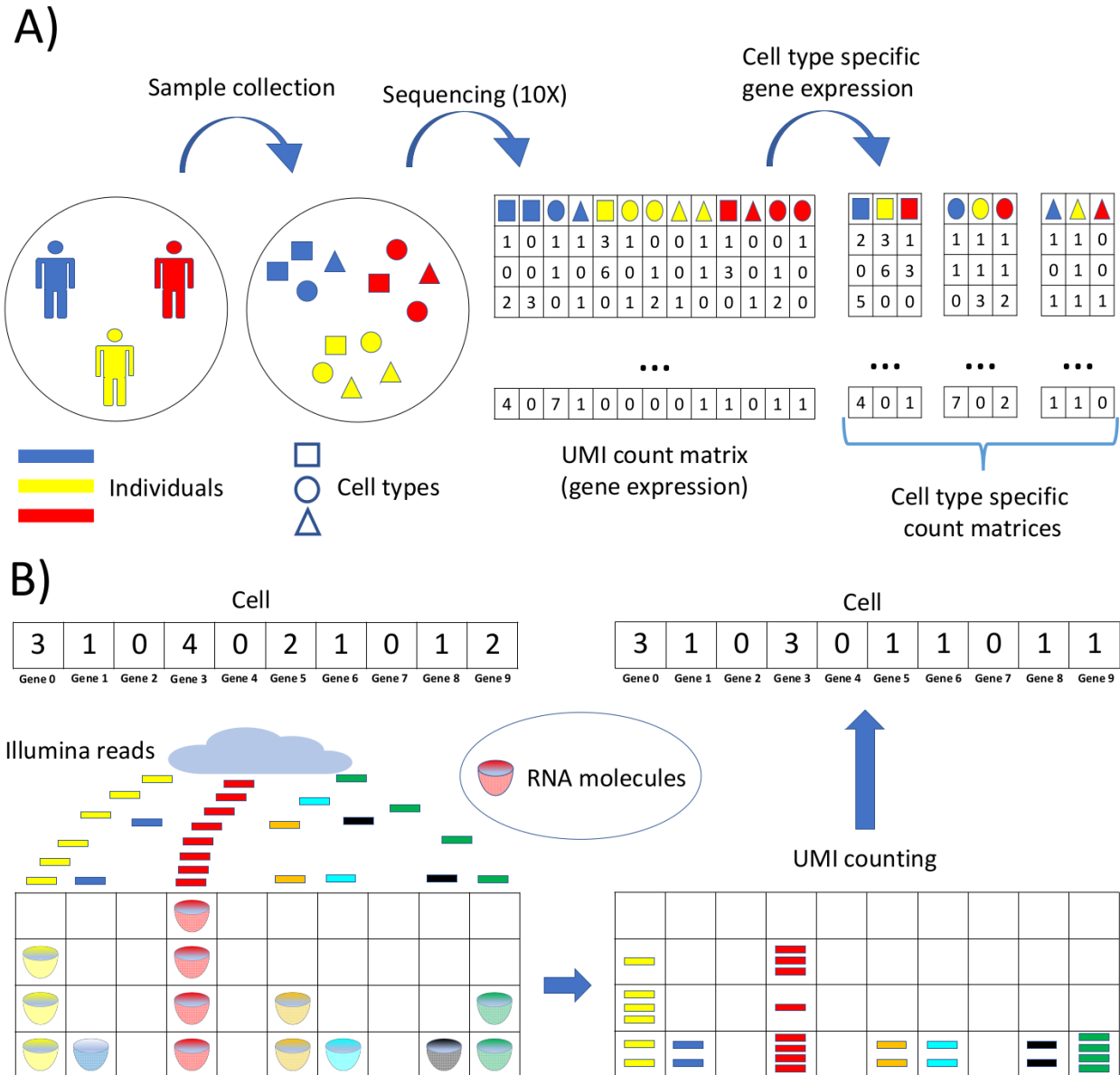
Supplementary Figure 16: Power estimates in ct-eQTL studies (CD4 T cells) stratified by sample size. A) Recall (power estimate) as a function of effective sample size. B) Precision-recall plot.



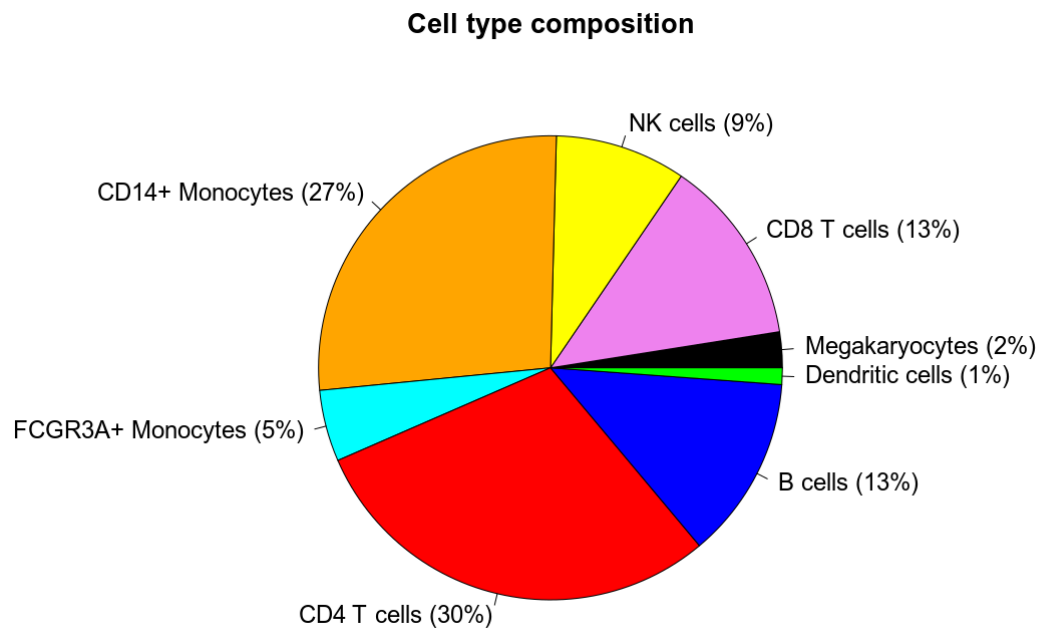
Supplementary Figure 17: The simulation workflow. The input to the simulation are the parameters N - the sample size, M - the number of cells per sample, x - multiplexing level. We first compute the budget per each individual. Then, by using the Satija lab single-cell cost calculator (<https://satijalab.org/costpercell>) we compute the number of singlets M_s with the coverage r_s and the number of multiplets M_m with the coverage r_m . We then randomly “merge” the expression profiles of $2M_m$ cells pairwise to obtain M_m doublets. Finally, the UMI counts are simulated from each cell and the cell-type specific expression is computed for each cell type across all of the samples.



Supplementary Figure 18: Percent of total detected UMIs at different levels of coverage. At each level of coverage r , we randomly sampled a cell from the 10X dataset and simulated r read counts. The average number of recovered UMIs per cell (± 1 standard deviation) is computed over 1000 iterations.



Supplementary Figure 19: Read count simulation for 10X. A) First, samples are collected, then sequenced by using 10X Genomics technology. Second, cell-type-specific gene expression is determined for each of the individuals. B) Each RNA molecule (or, equivalently, each UMI) regardless of the gene it was transcribed from is an urn, and each Illumina read is a ball which is randomly thrown into the urns. Given the high-coverage expression of a cell (on the left) as the number of RNA molecules in the high-coverage, the simulation of gene expression under a specified level of read coverage is performed as a random throwing of reads (“balls”) into the corresponding RNA molecules (“urns”). After all the “balls” are thrown into the “urns”, some urns remain empty (the gene received 0 reads, consequently, was not sequenced). In case a small number of reads is thrown into the “urns”, a considerable number of so-called “drop-out” events (i.e., missing all UMIs from a gene) will occur.



Supplementary Figure 20: Cell type composition of the 10X dataset.