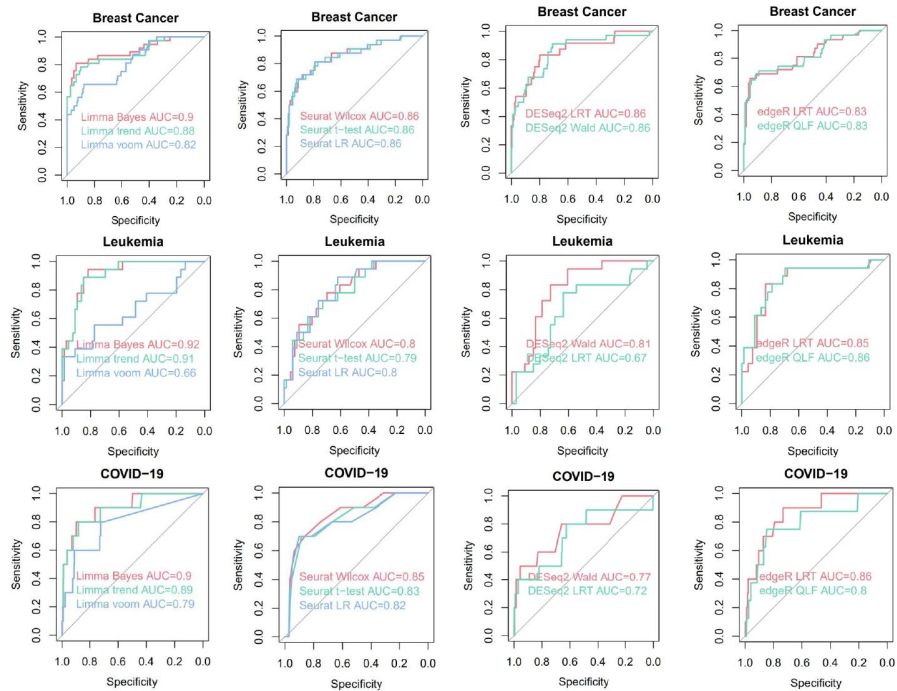
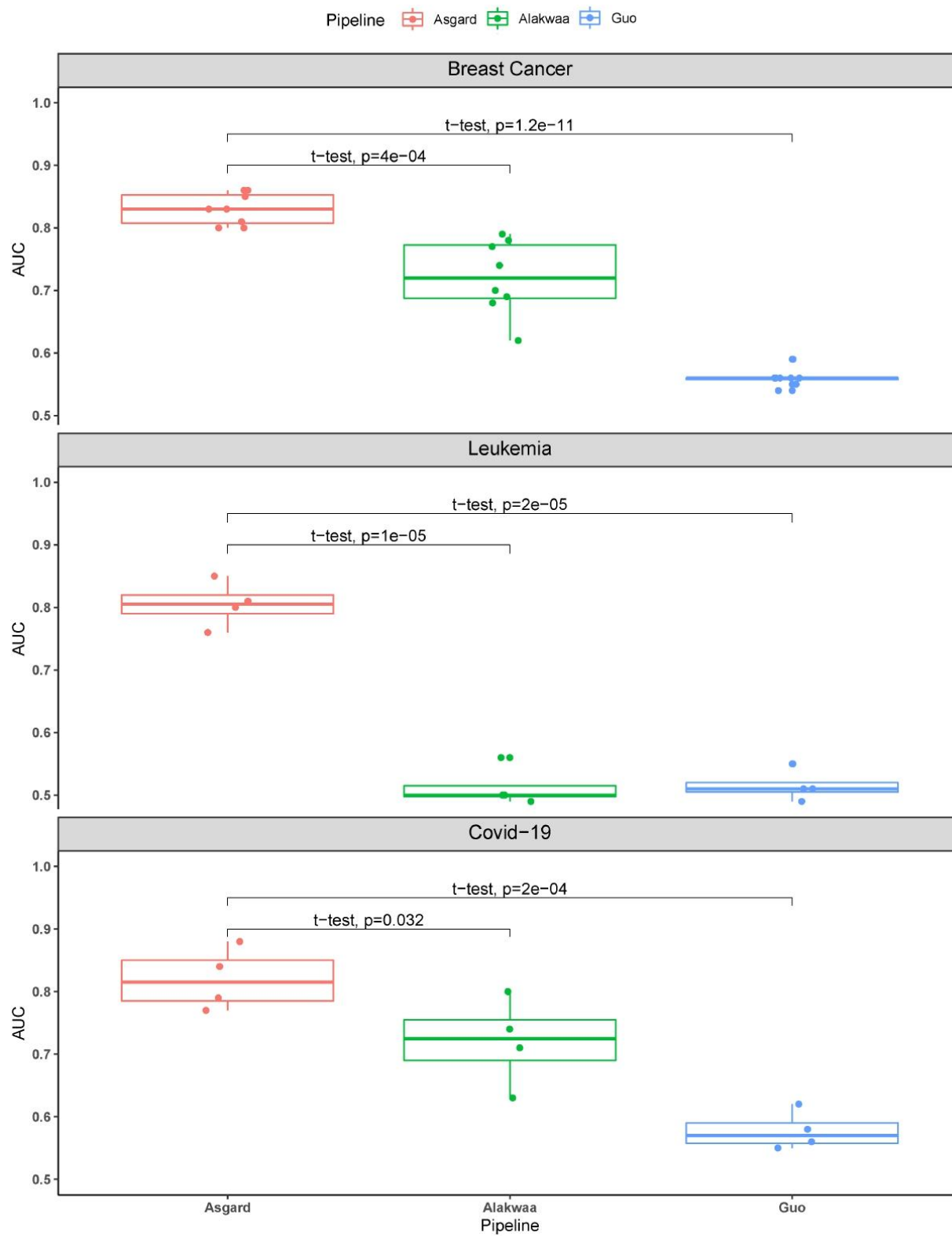


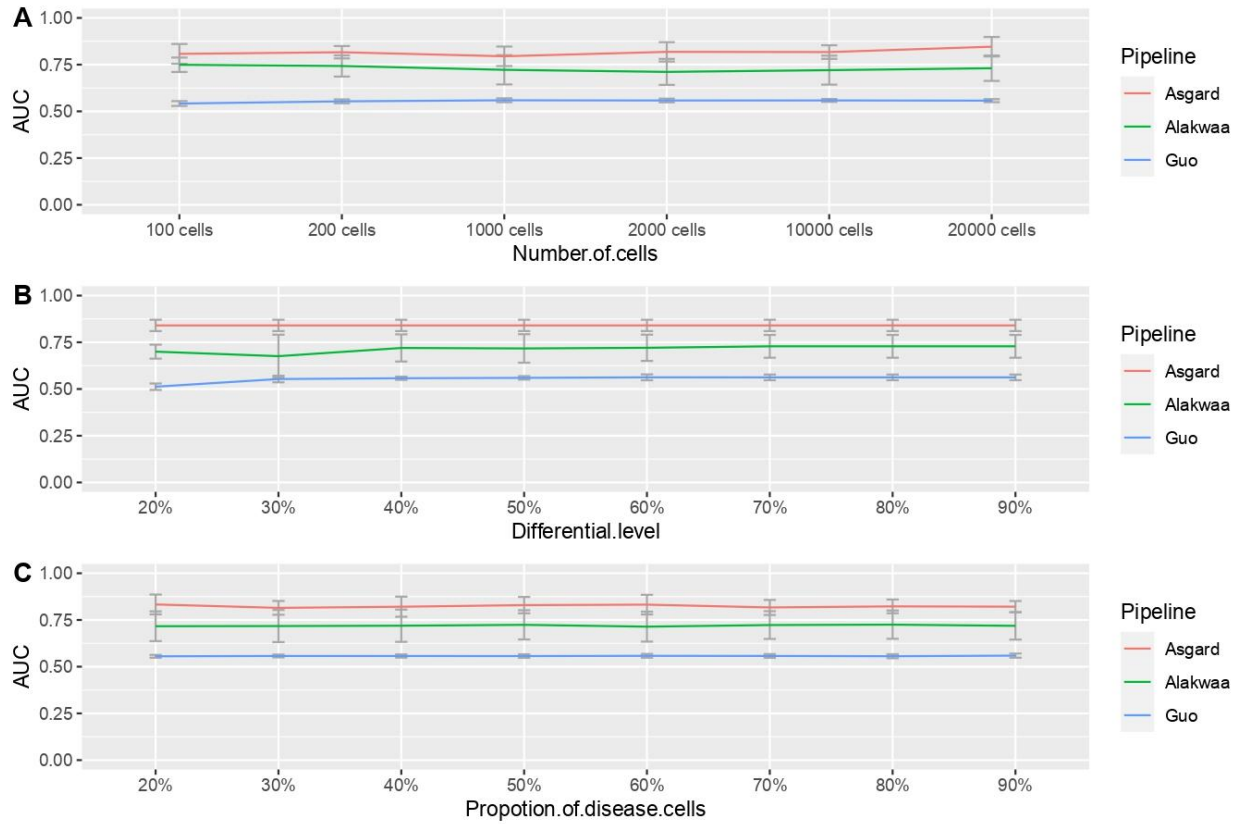
Supplementary Materials



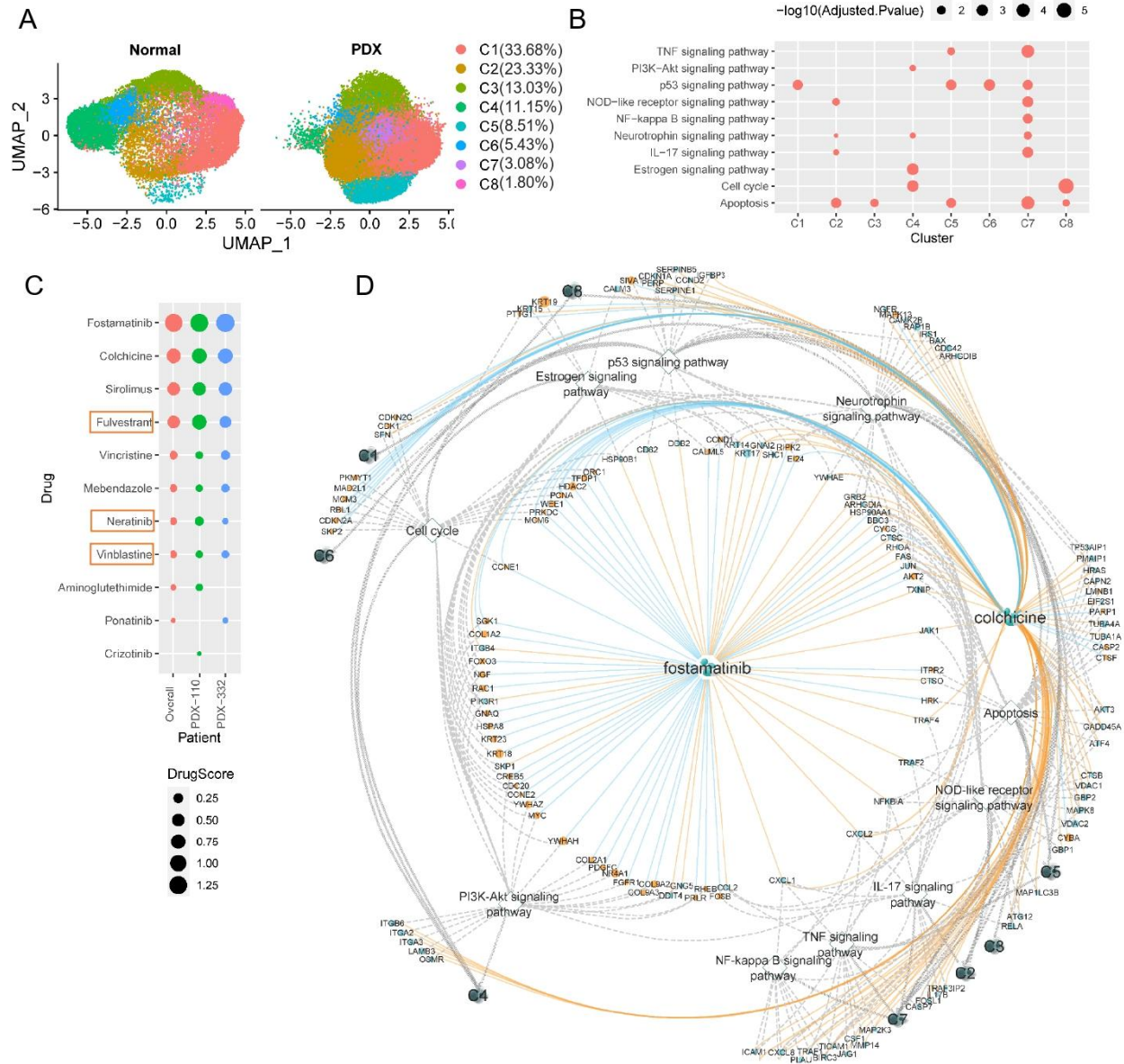
Supplementary Figure 1. The impact of single-cell differential expression methods on ASGARD performances. The receiver operating characteristic (ROC) curves and area under curve (AUC) scores of the ASGARD, using DE analysis methods (Limma, DESeq2, Seurat, and edgeR) with tuned parameters. The tests are done on advanced metastatic breast cancer, acute lymphoblastic leukemia, and coronavirus disease 2019 (COVID-19), respectively.



Supplementary Figure 2. Boxplots on AUCs of the three single-cell-based repurposing pipelines, based on all cell clusters, as shown in Figure 3.



Supplementary Figure 3. AUC scores of the three single-cell-based repurposing pipelines, using simulation data adapted from the real datasets. (A) The effect of varying total cell sizes from 100 cells to 20000 cells. (B) The effect of varying differential expression levels from 20% to 90%. (C) The effect of varying the proportions of diseased cells ranges from 20% to 90%.



Supplementary Figure 4. Drug repurposing in Patient-Derived Xenograft (PDX) models derived from advanced metastatic TNBC patients. (A) UMAP plots of single-cell data from 3 normal controls and 2 breast cancer PDX samples. **(B)** Pathway enrichment analysis (breast cancer vs normal) for each single-cell cluster. **(C)** The overall drug score combining both PDX models and drug score in each breast cancer PDX model, among top-ranked significant single drugs (FDR<0.05). Drugs approved for breast cancer treatment by the FDA are labeled in red

boxes. **(D)** The drug candidates fostamatinib and colchicine, their target genes, pathways, and single-cell clusters. All labels and their annotations are the same as Figure 4F.

Supplementary Note 1. Analysis of PDX breast cancer model.

We collected scRNA-seq data from 24,741 epithelial cells of advanced metastatic breast cancer Patient-Derived Xenografts (PDXs) models ¹¹ and 16,998 epithelial cells from normal breast tissues ²¹. After preprocessing, all cancer cells and 16,954 normal cells were paired and clustered into 8 populations (Supplementary Figure 3A). Cluster 1 (C1) is the largest one covering 33.68% of cells, while cluster 8 (C8) is the smallest one accounting for only 1.8% of cells (Supplementary Figure 3A). The differentially expressed genes (adjusted P-value <0.05, cancer vs normal) in the clusters are significantly enriched in 10 well-known breast cancer-related pathways, including apoptosis, cell cycle, estrogen signaling, IL-17 signaling, neurotrophin signaling, NF-kappa B signaling, NOD-like receptor signaling, p53 signaling, PI3K-Akt signaling and TNF signaling pathways (Supplementary Figure 3B). Cluster 7 (C7) has the largest number of 7 significant pathways, while C1 and C6 each have only 1 significant pathway.

We first applied ASGARD for multi-cluster drug repurposing prediction and predicted 11 drugs (FDR<0.05 and overall drug score >0.99 quantiles) for advanced metastatic breast cancer (Supplementary Figure 3C, Supplementary Table 4). Fostamatinib is the top 1 drug candidate (Supplementary Figure 3C). It is a tyrosine kinase inhibitor medication approved for the treatment of chronic immune thrombocytopenia ⁷⁷. Colchicine, the second best candidate, is an alkaloid approved for treating the inflammatory symptoms of familial Mediterranean fever ⁷⁸. Both fostamatinib and colchicine have shown antitumor and anti-metastasis effects in animal models of breast cancer ^{79,80}. Moreover, the 4th candidate fulvestrant and 7th candidate

neratinib have been approved by the Food and Drug Administration (FDA) for breast cancer treatment ^{58,59}.

To explore the potential molecular mechanisms of the top 2 candidates, we next investigated the target genes and pathways of fostamatinib and colchicine across the eight cell clusters (Supplementary Figure 3D). Fostamatinib and colchicine both target all the significant pathways in each cluster. Fostamatinib and colchicine are complementary in targeting genes of these pathways. Among the 143 target genes from these significant pathways, only 29 target genes are shared by fostamatinib and colchicine (Supplementary Figure 3D). The fostamatinib and colchicine also show biologically synergistic targeting of multiple genes on the same significant pathways. For example, fostamatinib inhibits Cyclin D1 (CCND1) to produce G1 arrest in the p53 signaling pathway, while colchicine inhibits Cyclin-dependent kinase 1 (CDK1) to produce G2 arrest in the p53 signaling pathway and cell cycle pathway ⁸¹ (Supplementary Figure 3D). Additionally, the drug scores of top drug candidates vary from one PDX model to another (Supplementary Figure 3D), demonstrating that ASGARD is a forward-looking precision medicine strategy *in silico*.

Supplementary Table 1. FDA-approved drugs and compounds used in advanced clinical trials or have been proven effective in animal models

Supplementary Table 2. Predicted drugs for leukemia.

Supplementary Table 3. Predicted drugs for COVID-19.

Supplementary Table 4. Predicted drugs for breast cancer PDX model.