

Supplementary Figures

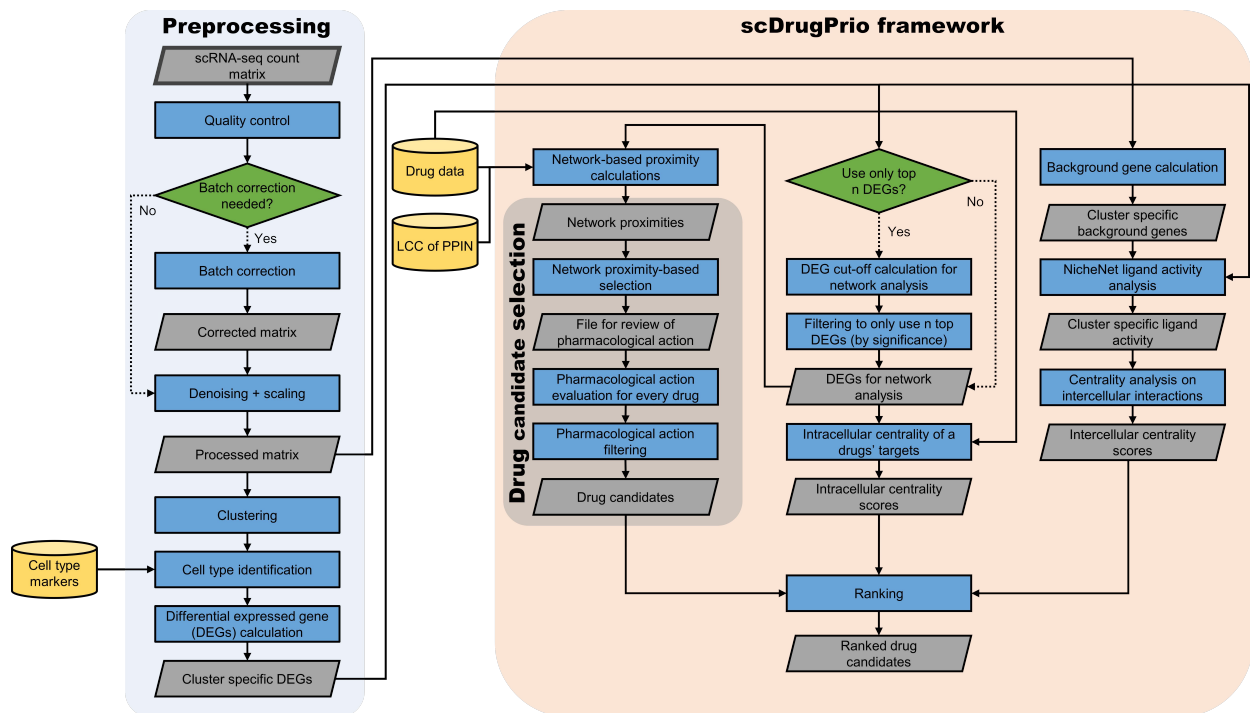


Fig. S1. scDrugPrio computational workflow, input and output data. Initially, data are preprocessed following a standard approach, resulting in the calculation of cell type-specific differentially expressed genes (DEGs) between sick and healthy cells. The need for batch correction was evaluated through heterogeneity analyses as described in **Additional file 2**. The DEG calculation utilized either paired samples (e.g., one healthy and one sick sample) from an individual or pooled samples from several healthy controls and patients. The scDrugPrio framework then performs drug selection using DEGs and drug data for network proximity calculation. The cell type-specific drug candidates are aggregated into a final drug ranking using intracellular and intercellular centrality. Gray parallelograms represent data files, yellow cylinders represent external data and green rombs indicate decision points.

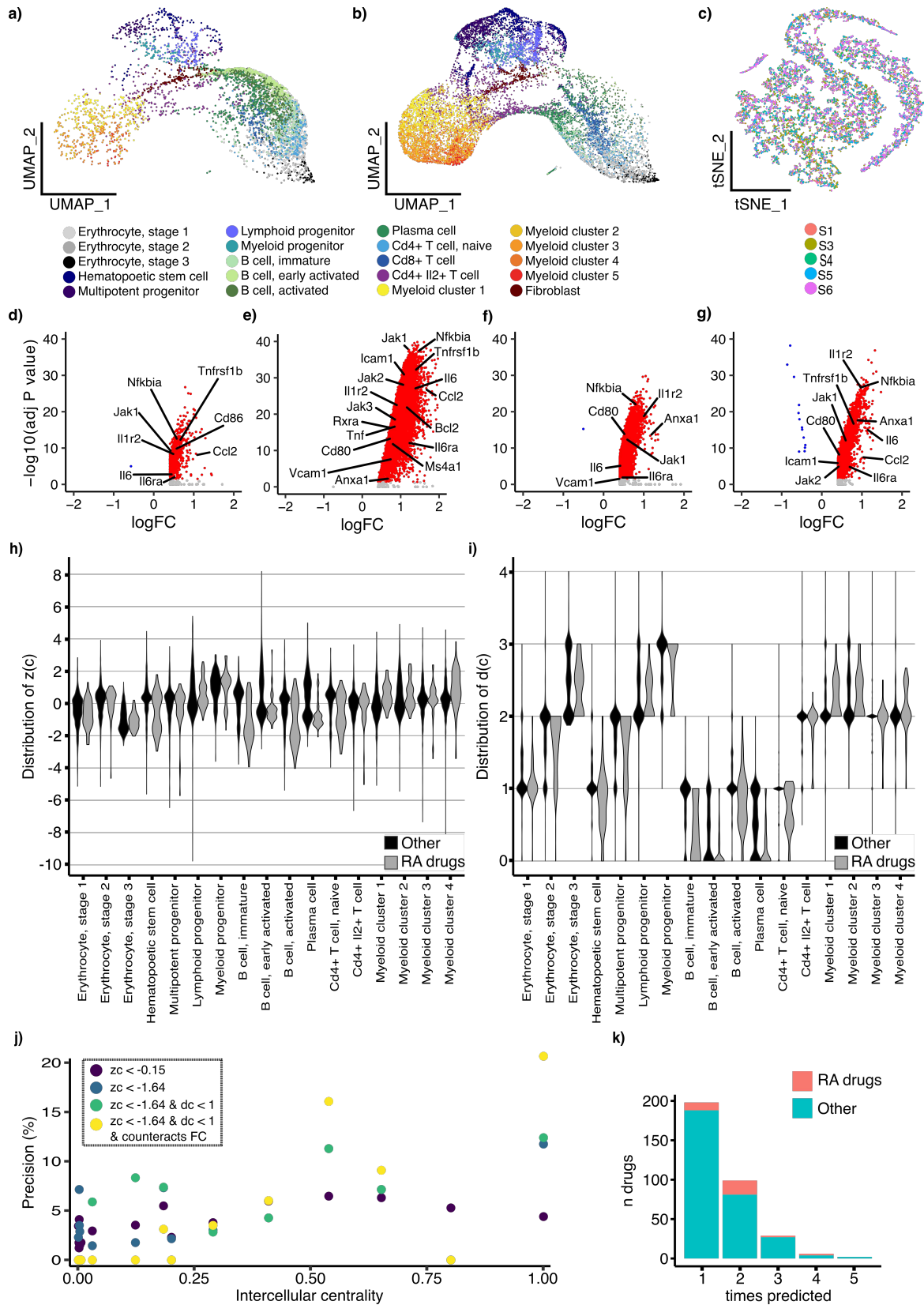


Fig. S2. Additional cluster information as well as network proximities between rheumatoid arthritis (RA) drugs and DEGs of AIA mice. UMAP plots including only cells from **a)** control mice or **b)** AIA mice. **c)** tSNE representation of cells' latent features of AIA mice to explore interindividual heterogeneity. Cells were colored based on the AIA mouse from which they originated (S1-S6; as indicated by legend). As cells from different AIA mice overlap nicely, no heterogeneity is apparent. **d–g)** Volcano plots of DEGs ($FC > 1.5$ and FDR-adjusted $P < 0.05$) that are upregulated (red) or downregulated (blue) in B-cell clusters. DEGs that were targeted by approved RA drugs are indicated by arrows. Plots present the DEGs for **d)** activated B cells, **e)** early activated B cells, **f)** plasma cells, and **g)** immature B cells. **h&i)** Violin plots showing z_c and d_c distributions, respectively, for each cell type. **j)** Precision among cell type drug candidates correlated with the cell type eigenvector centrality in the MCDM. Drug candidates were selected using four different sets of criteria, namely, 1) the basic z_c cut-off of $z_c < -0.15$ suggested by Guney et al.(1), 2) $z_c < -1.64$ (corresponding to $P < 0.05$), 3) drugs that fulfil both $z_c < -1.64$ and $d_c < 1$ and 4) drugs fulfilling our final selection criteria ($z_c < -1.64$, $d_c < 1$ and counteracting the fold change (FC) of at least one targeted DEG). Pearson correlation was calculated based on drugs passing the final selection criteria (Pearson's r [95% CI] = 0.77 [0.46 – 0.91], $P < 10^{-3}$). **g)** The proportion of known RA drugs was increased among final candidates that reoccurred in several cell types.

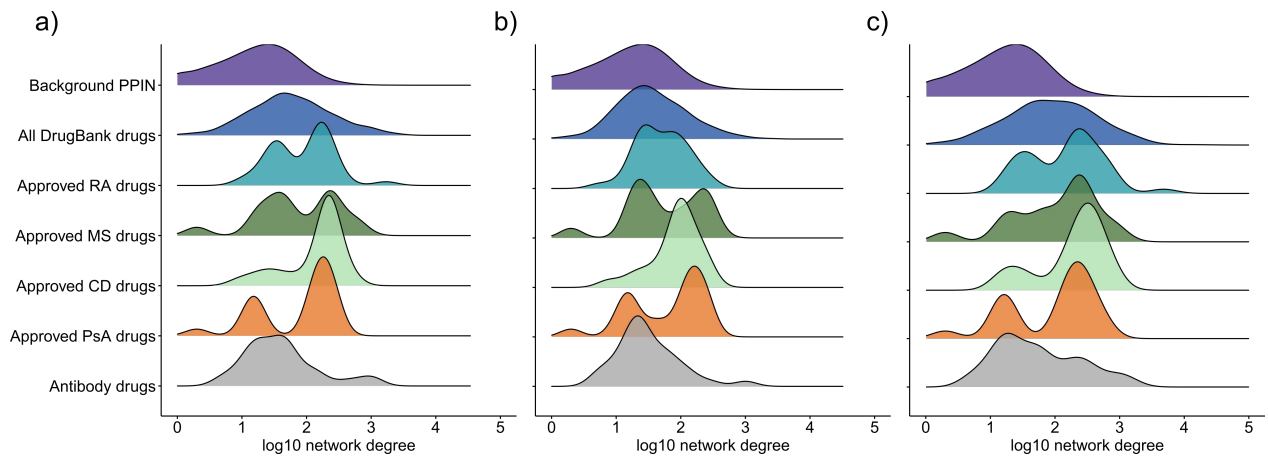


Fig. S3 Distribution of network degrees. In this figure, we visualize the distributions for **a)** max, **b)** mean and **c)** sum of drug targets' network degrees compared to the distribution of network degrees in the LCC of the literature-derived PPIN. As updated treatment regimens for inflammatory diseases often have antibody-based drugs as cornerstones, we visualised their network degrees separately. A total of 316 unique antibody drugs were identified by screening DrugBank for drug names ending with -ab.

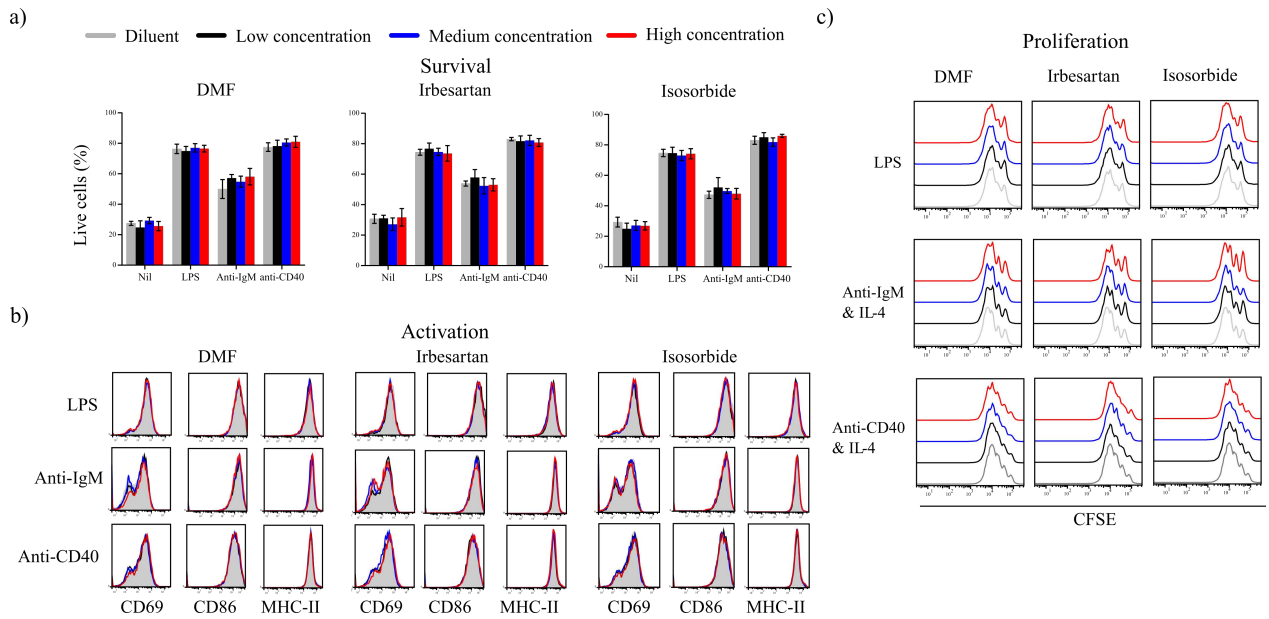


Fig. S4. Dimethyl fumarate (DMF), irbesartan and isosorbide have no effect on murine B-cell viability, activation or proliferation. Drug effect of the selected drugs on *in vitro* murine (a) B-cell survival, (b) activation, and (c) proliferation. Purified murine B cells were stimulated with the indicated B-cell modulators in the presence of dimethyl fumarate (DMF), irbesartan, and isosorbide at different concentrations. Drug concentrations can be found in Table S2.

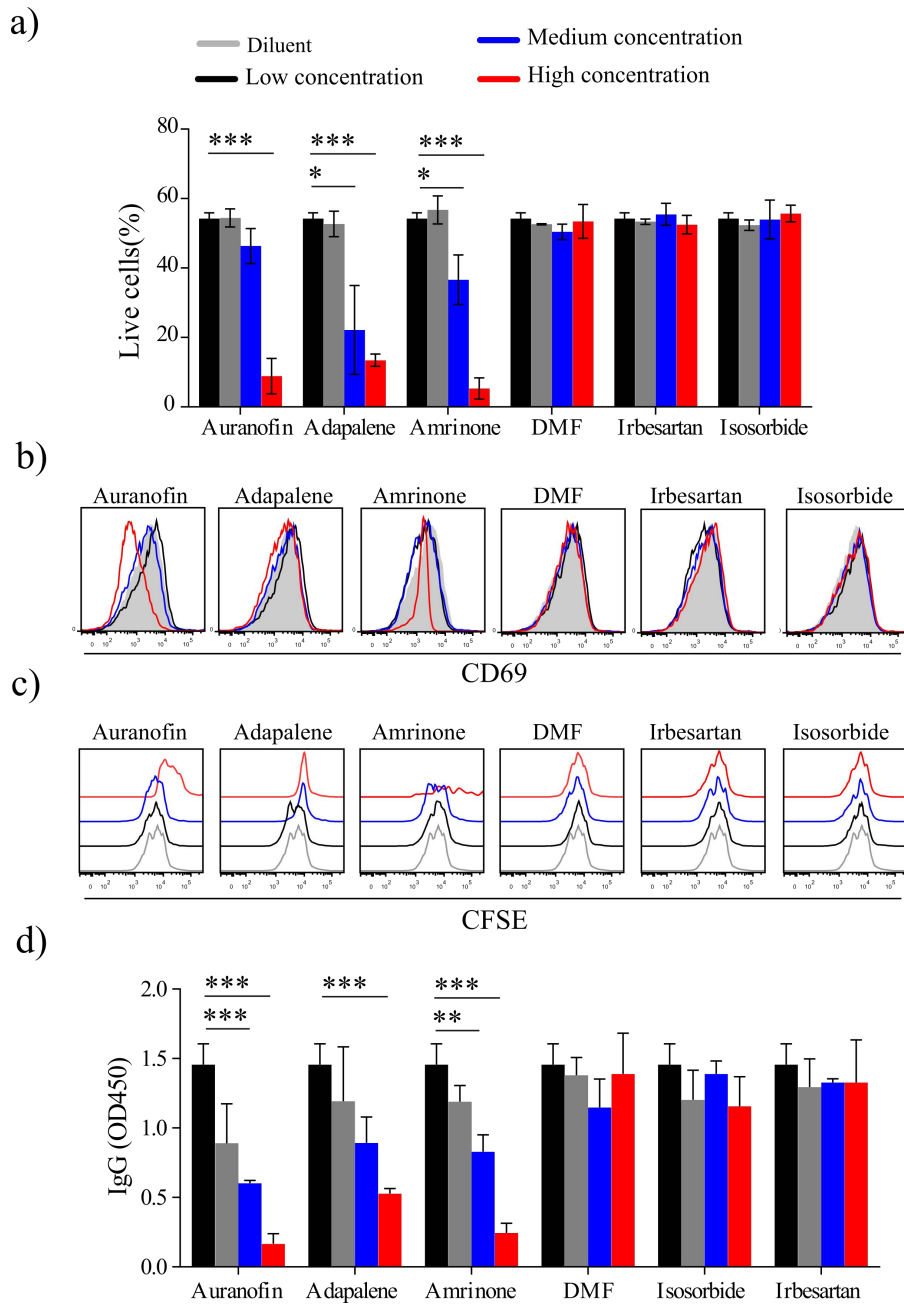


Fig. S5. *In vitro* analysis of selected drugs on human B-cell (a) viability, (b) activation, (c) proliferation and (d) IgG production. Purified human naïve B cells were activated with goat anti-human IgG + IgM (5 µg/mL), anti-human CD40 (5 µg/mL) and IL-21 (10 ng/mL) in the presence of selected drugs at the indicated concentrations. Drug concentrations can be found in **Table S2**. DMF, dimethyl furamate. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

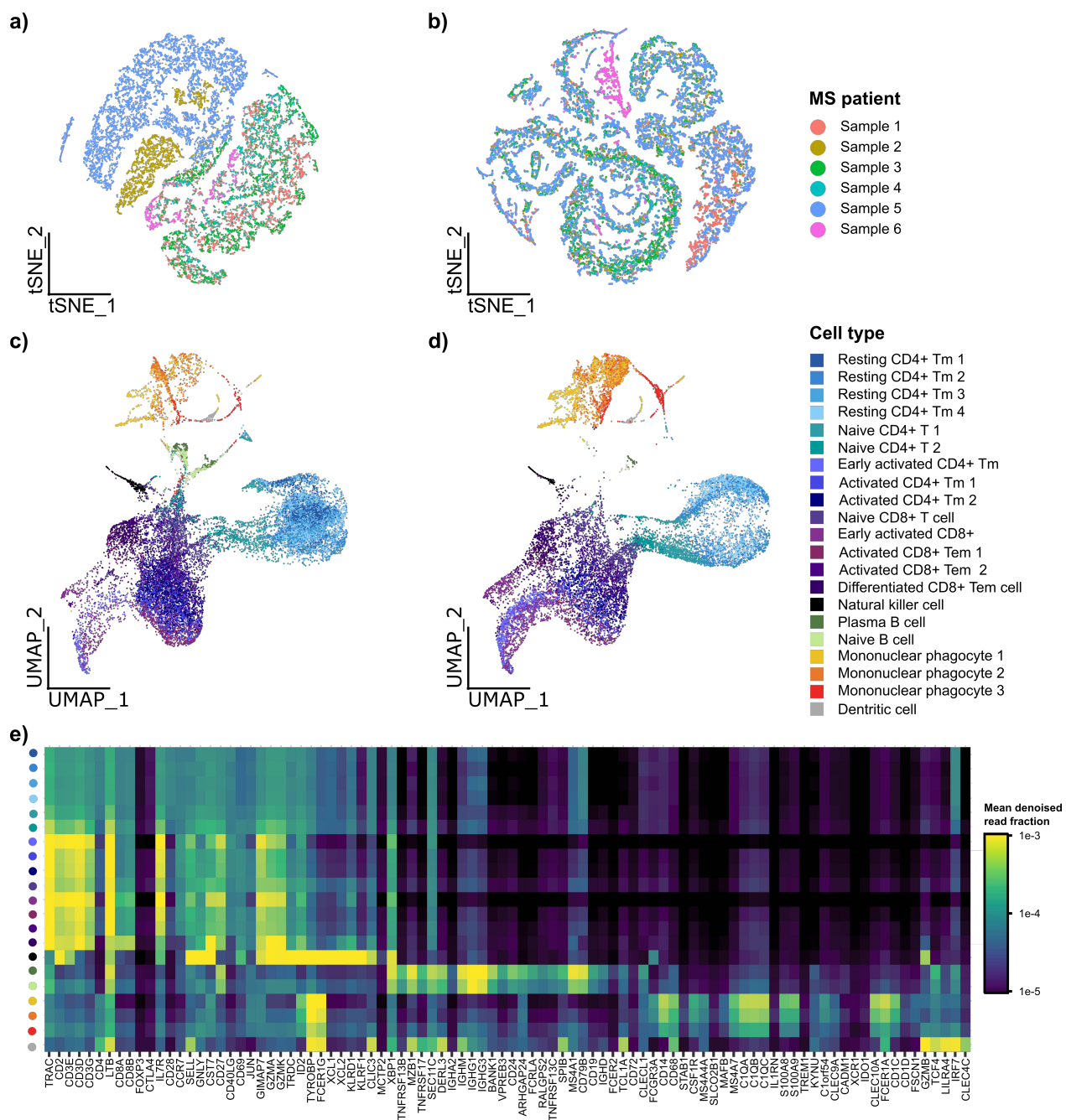


Fig. S6. Cell typing and selection of the number of DEGs for network-based drug screening in scRNA-seq data from human multiple sclerosis (MS) patients. tSNE representation of latent features for MS patients **a)** before and **b)** after batch correction. Cells were coloured based on which MS patient they originated from (patient 1 to 6). In contrast to **Fig. S1c**, we can see that cells from different patients do not mix well before batch correction (**a**), indicating transcriptomic heterogeneity between patients. UMAP clustering plots corresponding to **Fig. 5a** including only cerebrospinal fluid cells from **c)** MS patients or **d)** controls (idiopathic intracranial hypertension patients). **e)** Cell typing by cluster means of DCA-denoised, log10 adjusted gene expression fractions for known marker genes. Abbreviations: Tm, memory T cell; Tem, effector memory T cell.

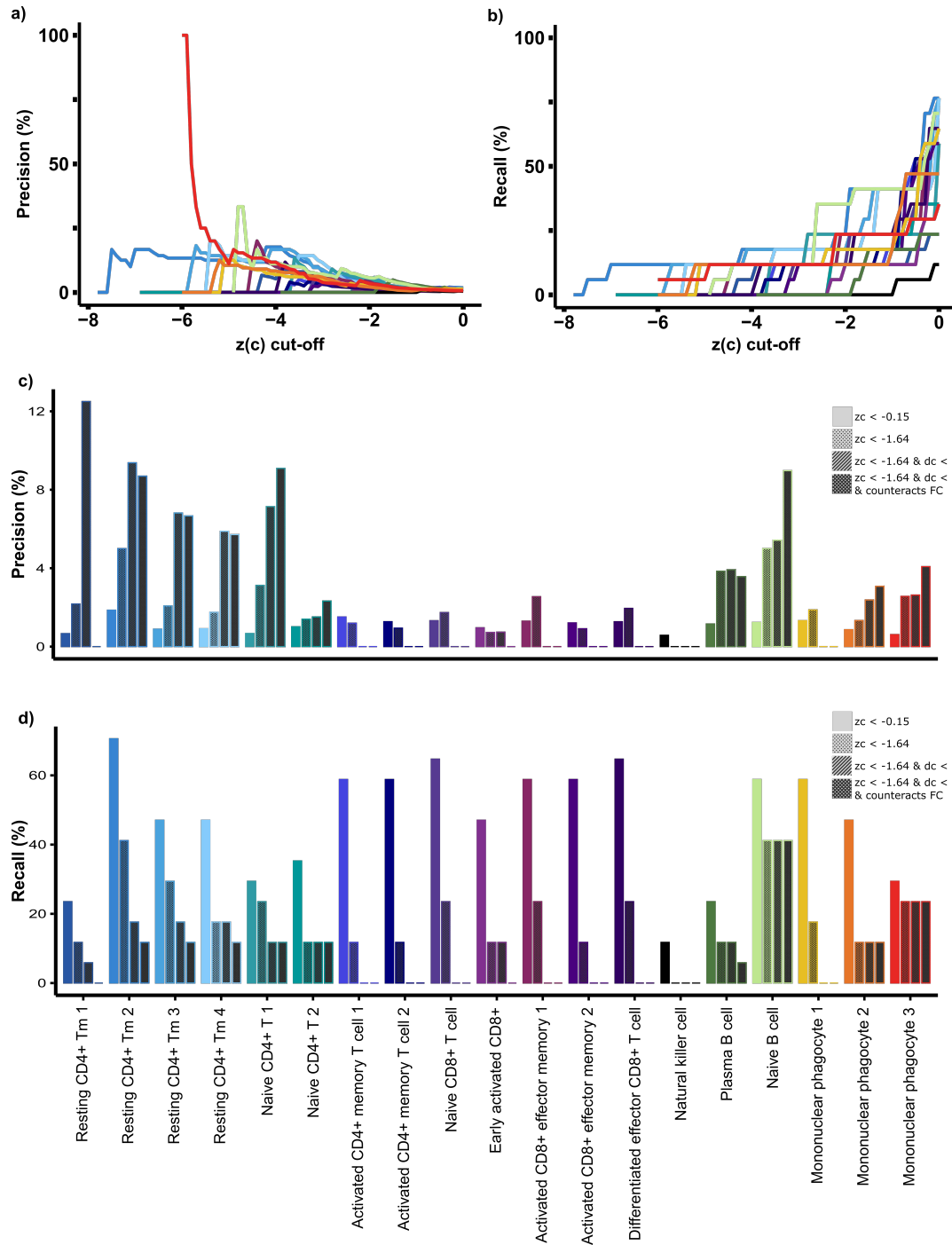


Fig. S7. Drug candidate selection for multiple sclerosis (MS) patients. a) Precision and b) recall curves for approved drugs among candidates at different z_c cut-offs indicate that decreasing z_c increases precision among central cell types in the MCDM. c&d) Precision (c) and recall (d) after stepwise application of drug selection criteria to candidates selected based on network distances to the top 3,000 significant DEGs.

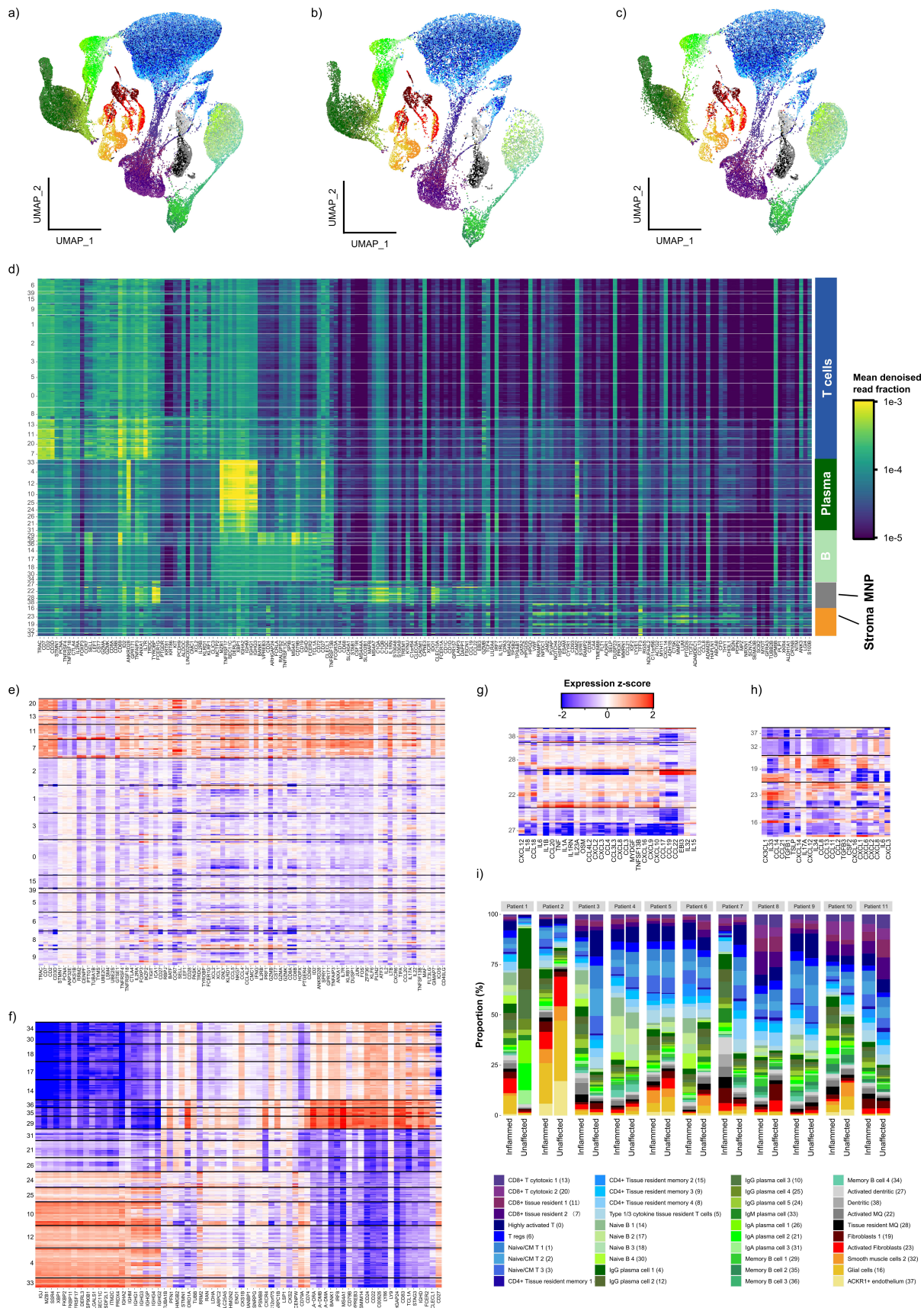


Fig. S8. Clustering and cell typing of Crohn's disease patient data. **a)** tSNE plot including all cells from inflamed and unaffected intestinal lesions of all patients. Clusters are colour coded by cell types in the colour legend of **i)**. **b&c)** are tSNE plots that include only cells from unaffected and inflamed intestinal biopsies. **d)** Heatmap representing individual cell expression of major cell type marker genes. Gene expression values correspond to DCA(2)-adjusted gene expression value fractions of a cell's total DCA-adjusted gene expression values. Individual cells are grouped by the cluster that is represented by a number on the y-axis corresponding to the number in the colour legend of **i)**. White lines separate clusters for visibility. Bars on the right side indicate the major cell type. **e-g)** Heatmaps of single-cell gene expression fraction-based z-score for further stratification of T cells (**e**), B cells (**f**), MNPs (**g**) and stroma/glia cells (**h**). **i)** Stacked bar plots representing the fractions of cells per cell type and sample. Abbreviations: ILC, innate lymphocyte cells; MNP, mononuclear phagocytes. Heatmap x labels can be found in **Additional file 5**.

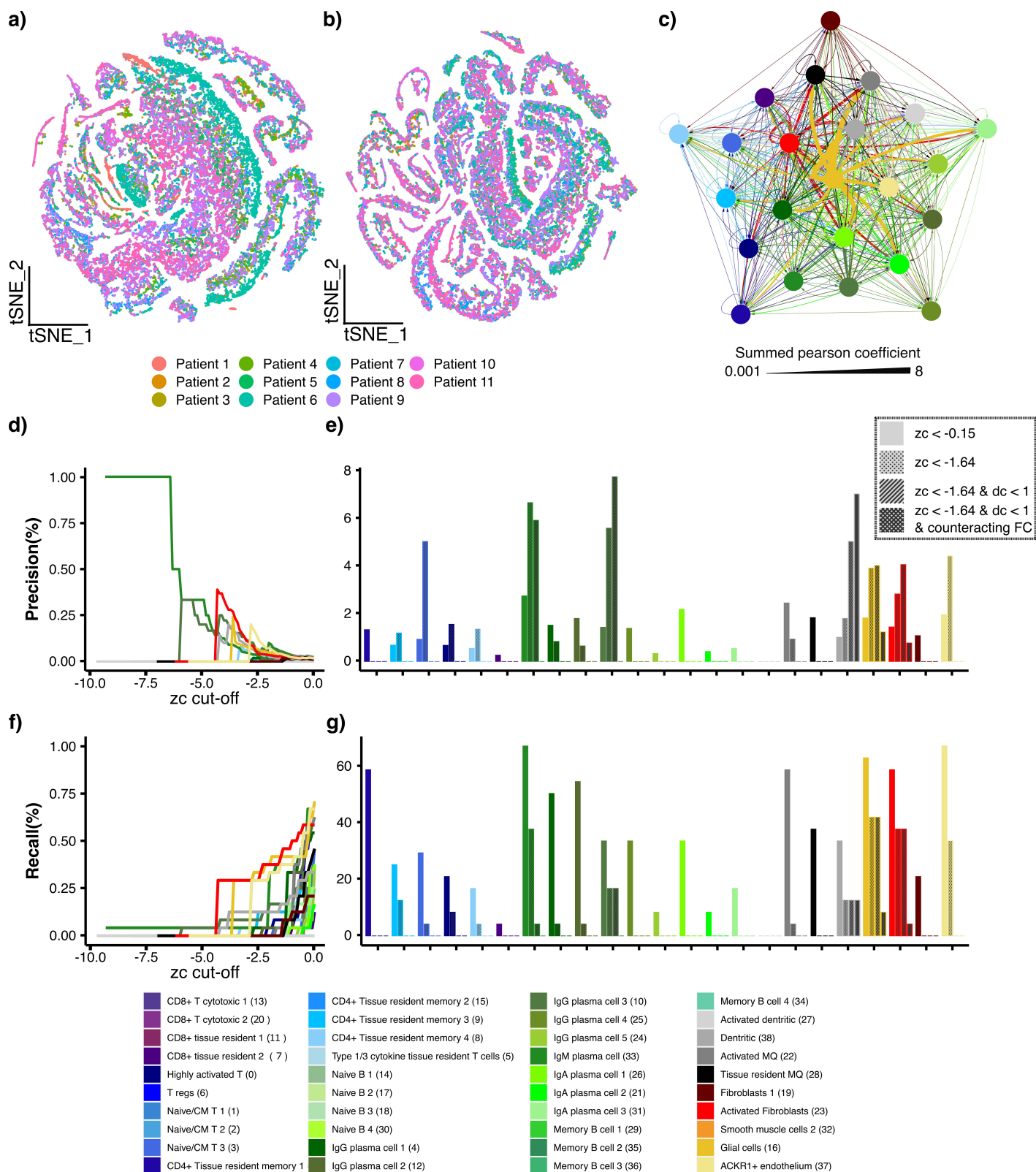


Fig. S9. Batch correction and drug candidate selection for Crohn's disease patient data.

tSNE representation of cell latent features for CD patients **a)** before and **b)** after batch correction. Cells were coloured based on which CD patient they originated from (patient 1 to 11, see **Additional file 5** for more information). Before batch correction, **(a)** cells from patients do not mix well, indicating underlying differences in gene expression profiles. **c)** MCDM based on NicheNet (3)-derived cellular interactions among cell types. Nodes represent cell types, and directed edges indicate ligand interactions from NicheNet. Edge width corresponds to the summed Pearson correlation coefficient of all ligands in the upstream cell type having a potential effect on the downstream cell type. **d&f)** Precision & recall curves at different z_c cut-offs when entering only the top 1,800 DEGs into network distance calculations. **e&g)** Precision and recall for known CD drugs depending on selection criteria (indicated by pattern) using only the top 1,800 significant DEGs for network calculations. Colours in **c-g)** match the colour legend in the bottom.

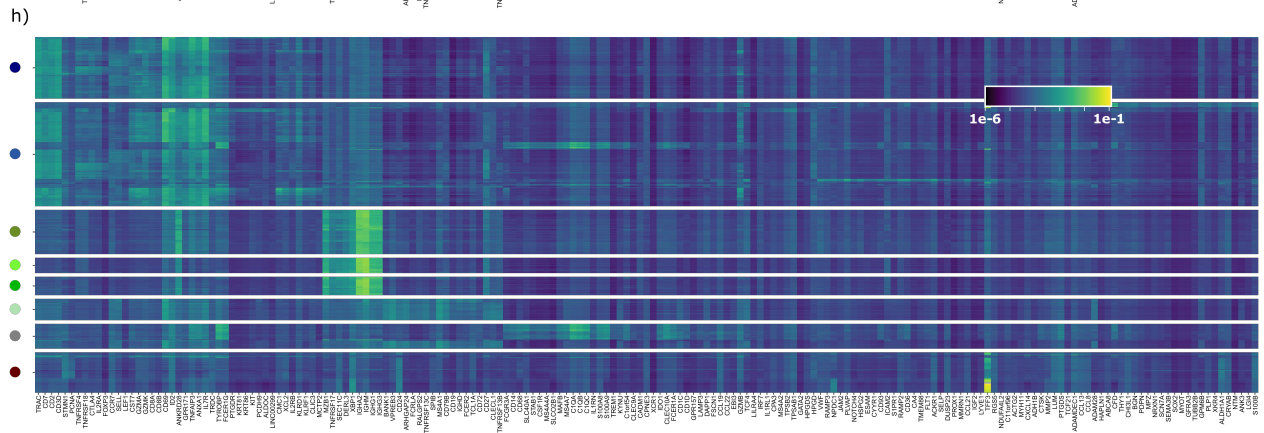
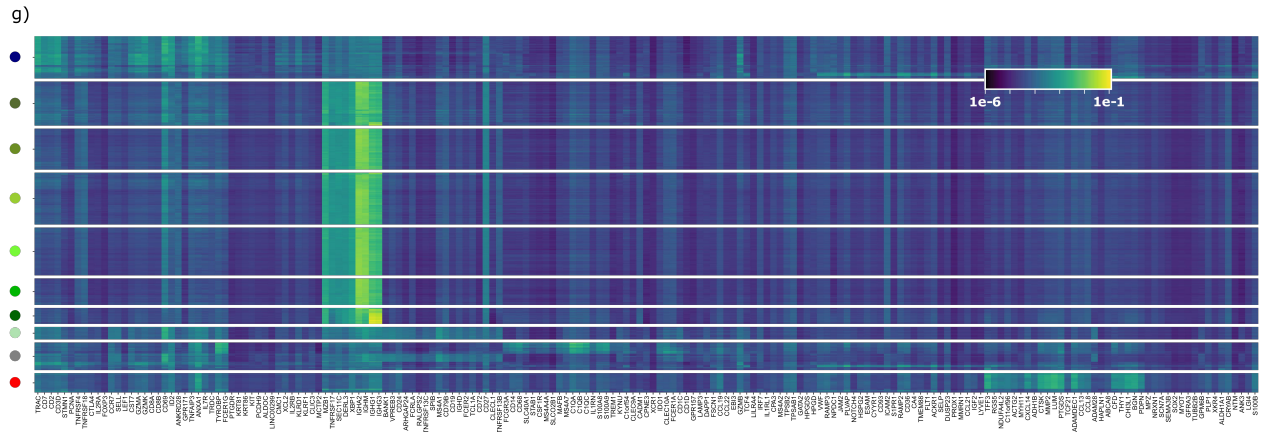
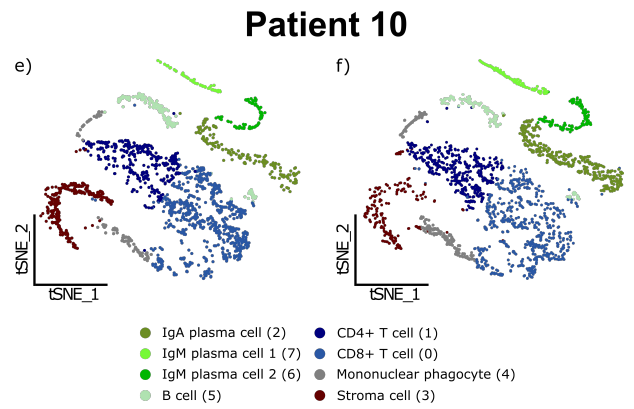
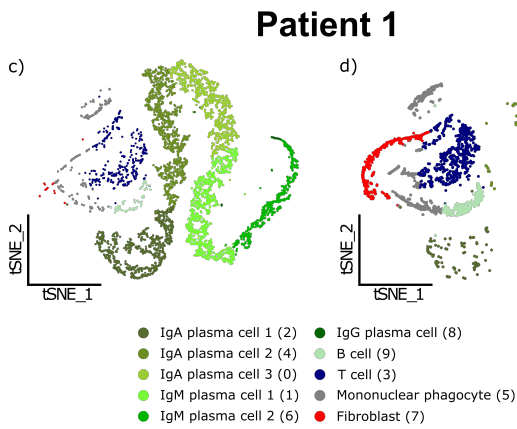
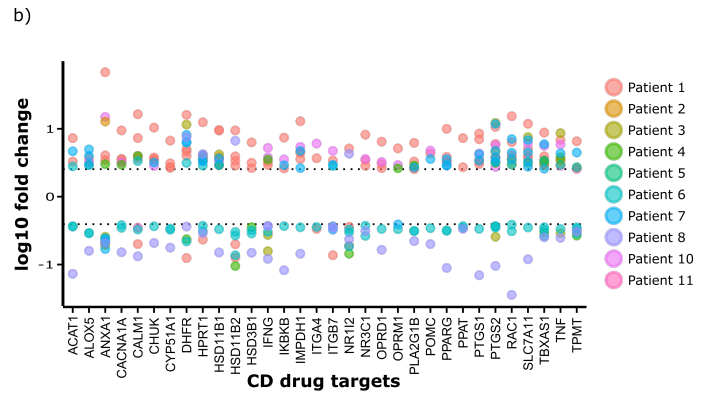
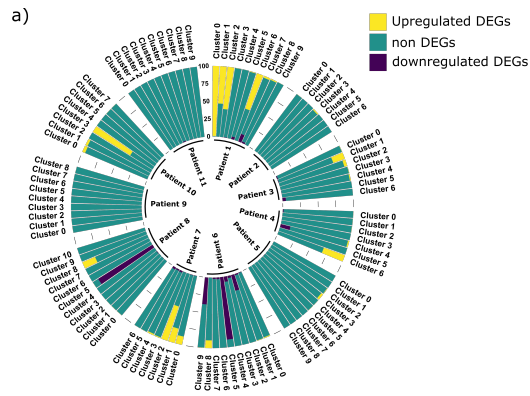
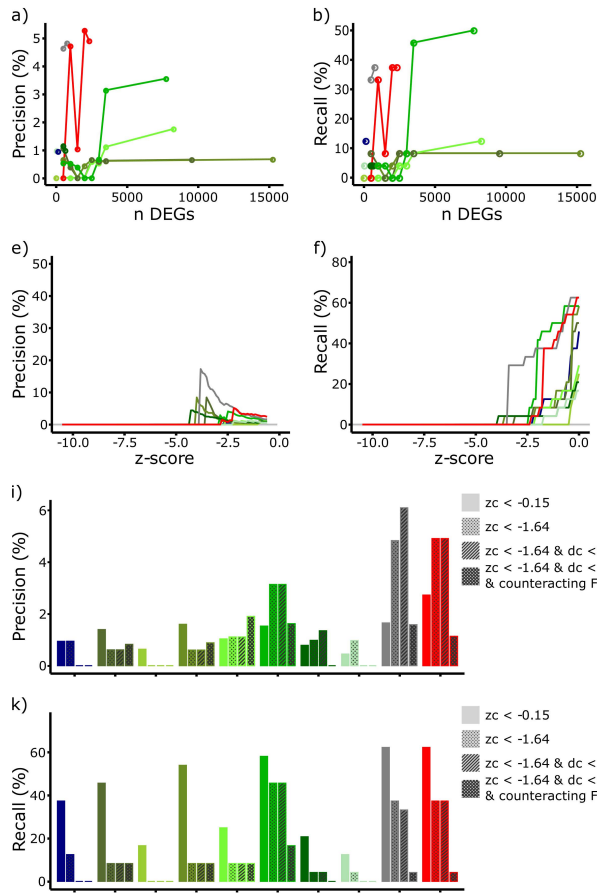


Fig. S10. Clustering and cell typing of individual Crohn's disease patients. **a)** Fraction of differentially expressed genes out of all genes. For this, data were divided by patient and clustered separately. For patient 1 and 10, clusters were cell typed, and cluster ID in a) corresponds to the number in parentheses in the figure legend of c-d) and e-f), respectively. **b)** Fold change of known CD drug targets for all patient-specific DEGs in all clusters. The colour represents the patients; in some instances, several clusters of a patient differentially expressed a certain drug target. **c-d)** tSNE plot showing only cells from the uninfamed (c) and inflamed (d) biopsy of patient 1. **e-f)** Same as c-d) for patient 10. **g)** Heatmap representing individual cell expression of major cell type marker genes used for cell typing the clusters of patient 1. Gene expression values correspond to DCA(2) adjusted gene expression value fractions of a cell's total DCA adjusted gene expression values. Individual cells are grouped by cluster. Colours on the y-axis correspond to the colour legend of c-d). Comparable to the heatmap in **Fig. S4d**. **h)** Same as g) for patient 10; colours on the Y-axis correspond to colour legend of e-f).

Patient 1



Patient 10

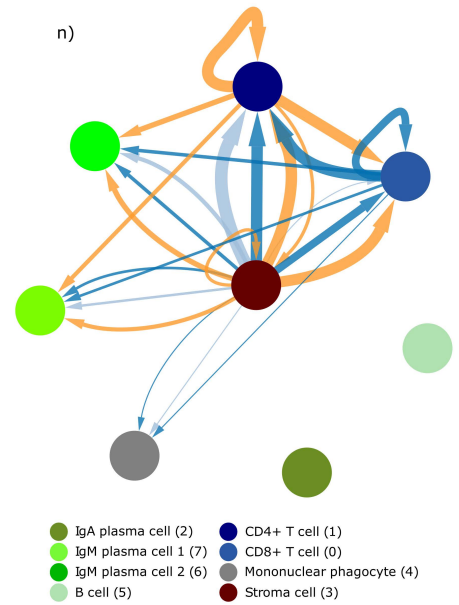
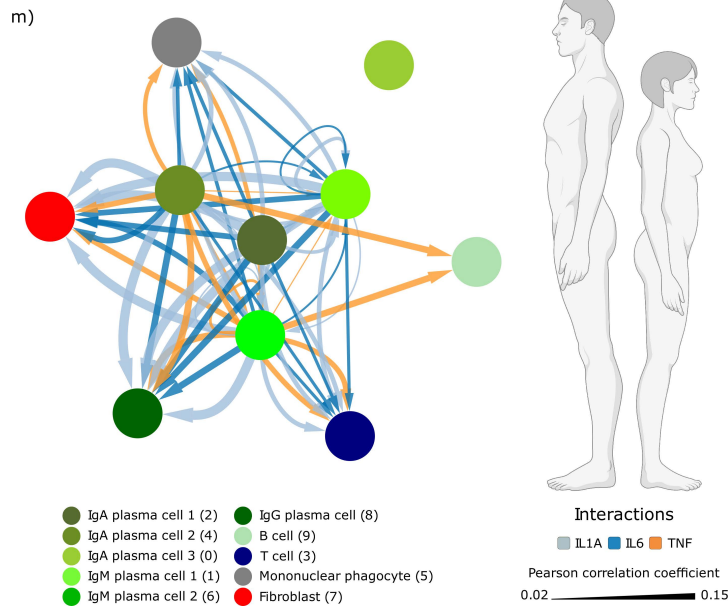
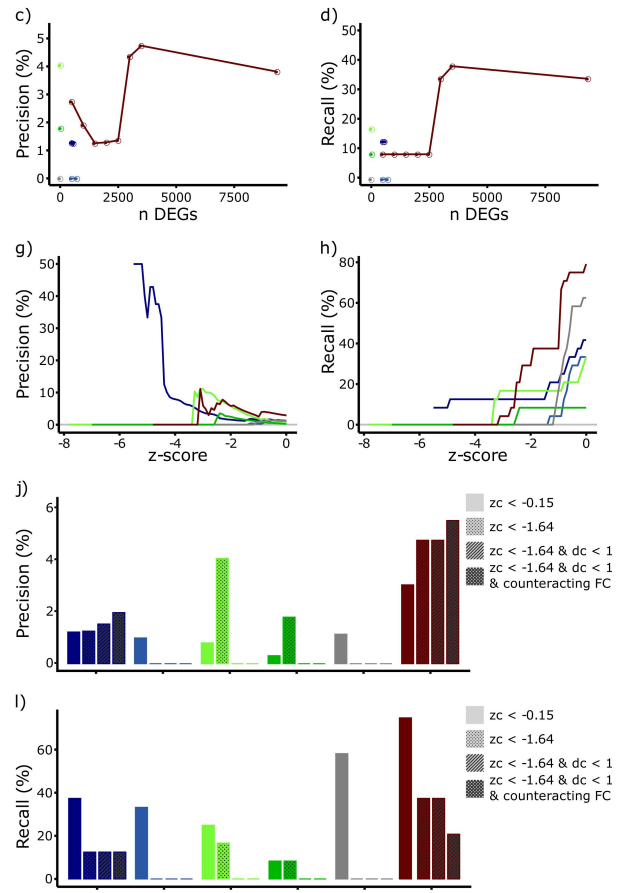
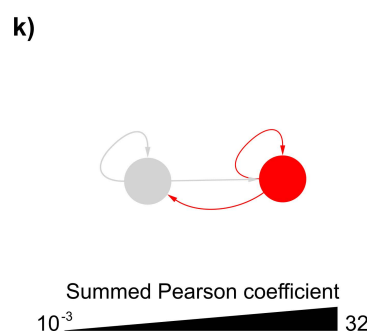
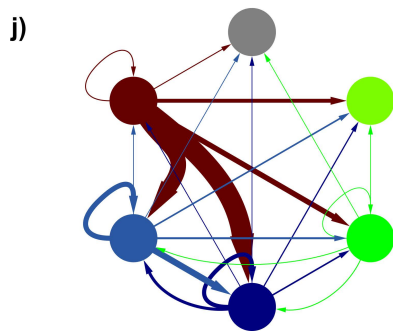
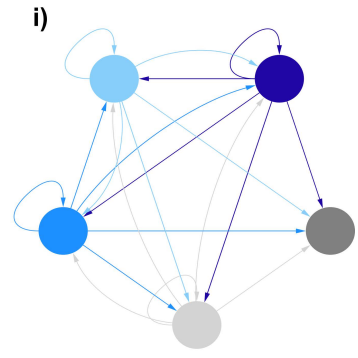
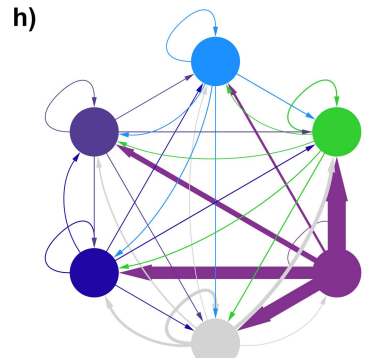
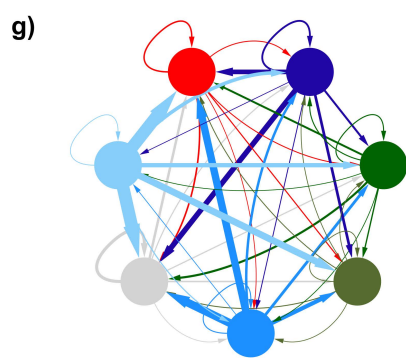
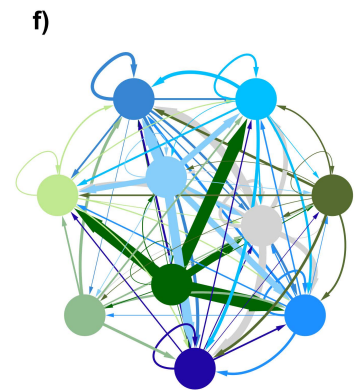
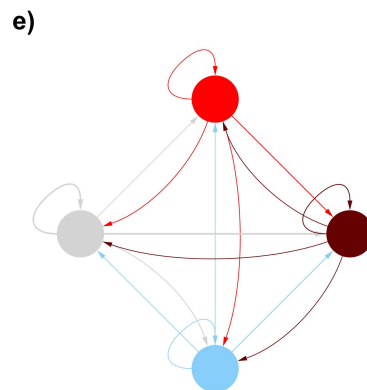
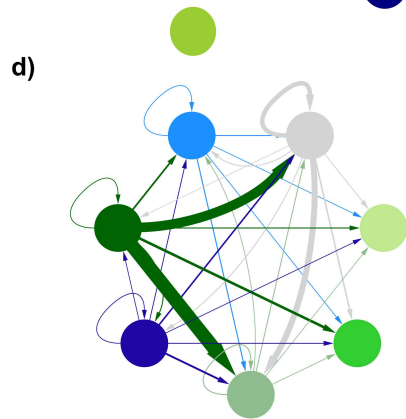
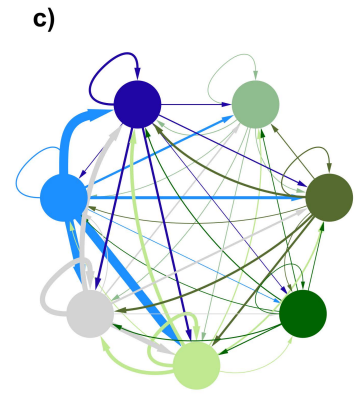
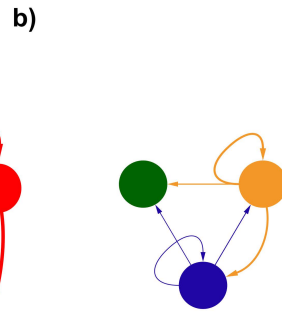
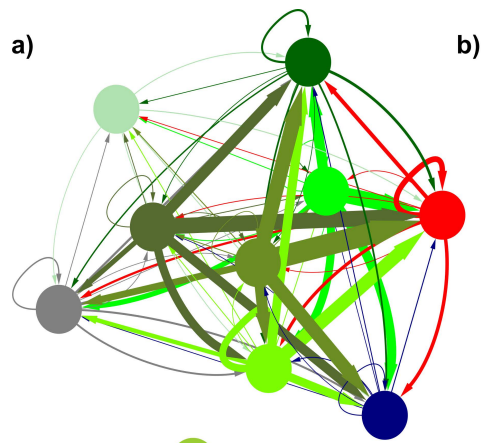


Fig. S11. Drug prediction outcomes based on individual Crohn's disease patients. The panel is divided in the middle and represents outcomes for patients 1 and 10 on the left and right sides, respectively. **a-d)** Cell type precision and recall for known CD drugs at $z_c < -1.64$, shown as a function of the number of an individual patient's top significant DEGs that entered network distance calculations. **e-h)** Precision & recall curves at different z_c cut-offs when entering only the top 3,500 DEGs for each patient into network distance calculations. **i-l)** Precision and recall for known CD drugs depending on selection criteria (indicated by pattern), using only the top 3,500 significant DEGs of each patient for network calculations. **m-n)** MCDMs highlighting only *IL1A*, *IL6* and *TNF* ligand interactions between cell types from patients 1 and 10. Edge width is scaled by the NicheNet (3) Pearson correlation coefficient, which is a measure of each single ligand association with the observed differential gene expression in the downstream cell type.



Summed Pearson coefficient
 10^{-3} 32

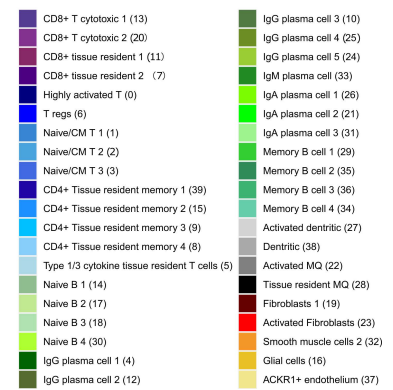


Fig. S12. MCDMs of all individual CD patients. a-k) Correspond to patients 1 to 11. Nodes correspond to cell types that expressed DEGs and are coloured according to the colour legend in the lower right corner. Patient 1 had one cell type that was not connected to the rest of the remaining MCDM. Except for patients 1 and 10, which were cell typed as described in **Fig. S8g&h**, cell types were derived from the overall cell typing for the pooled patient data (**Fig. S6d**). The directed edges are coloured by colour of the upstream cell type. Edge width corresponds to the summed Pearson correlation coefficient.

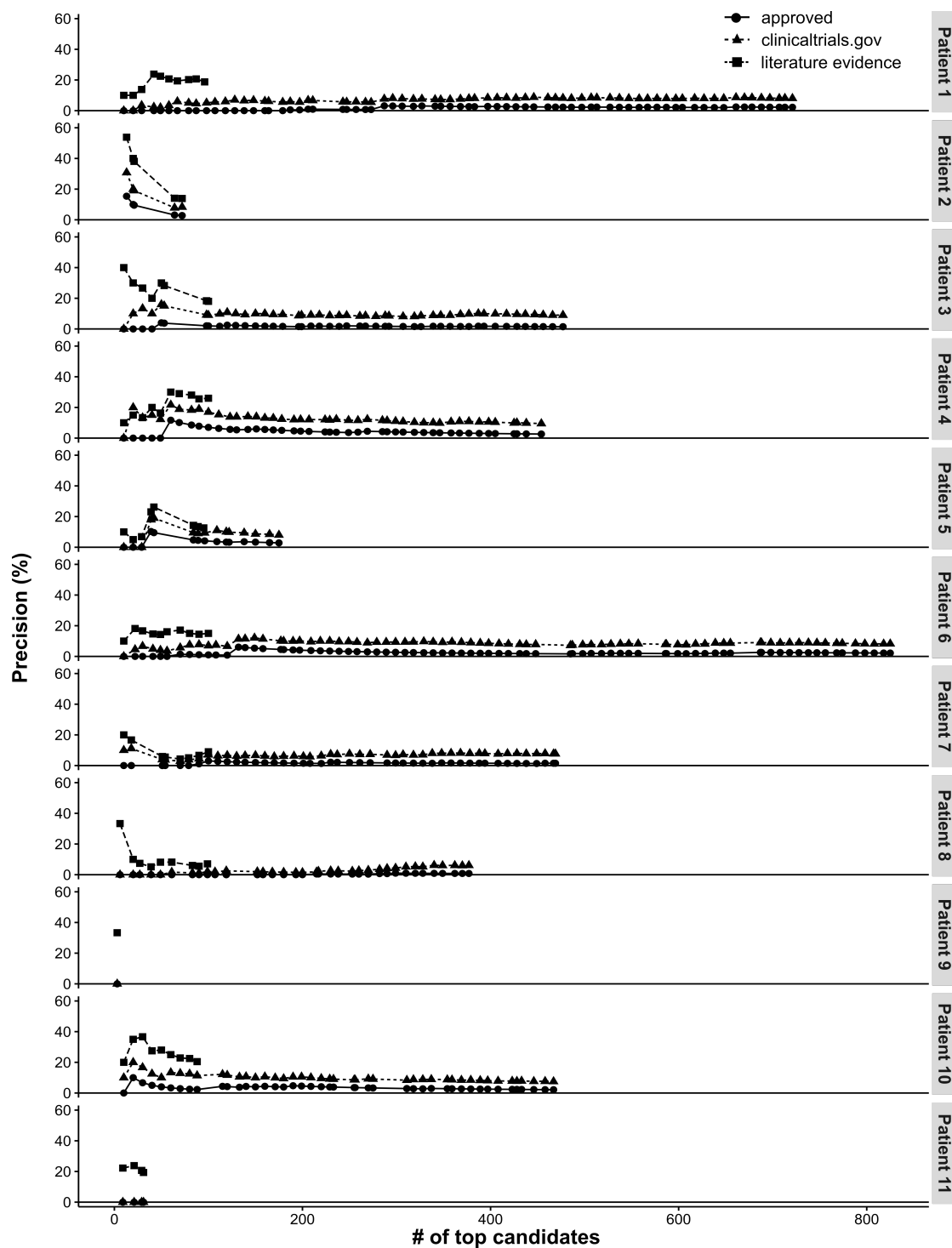


Fig. S13. Precision among ranked candidates of all individual CD patients. Drug rank on the x-axis and precision on the y-axis. Showing precision for approved CD drugs, drugs that were registered for clinical trials in CD as well as for drugs with literature evidence as indicated by shape and line type. Literature evidence was only collected for candidates with a rank up to 100.

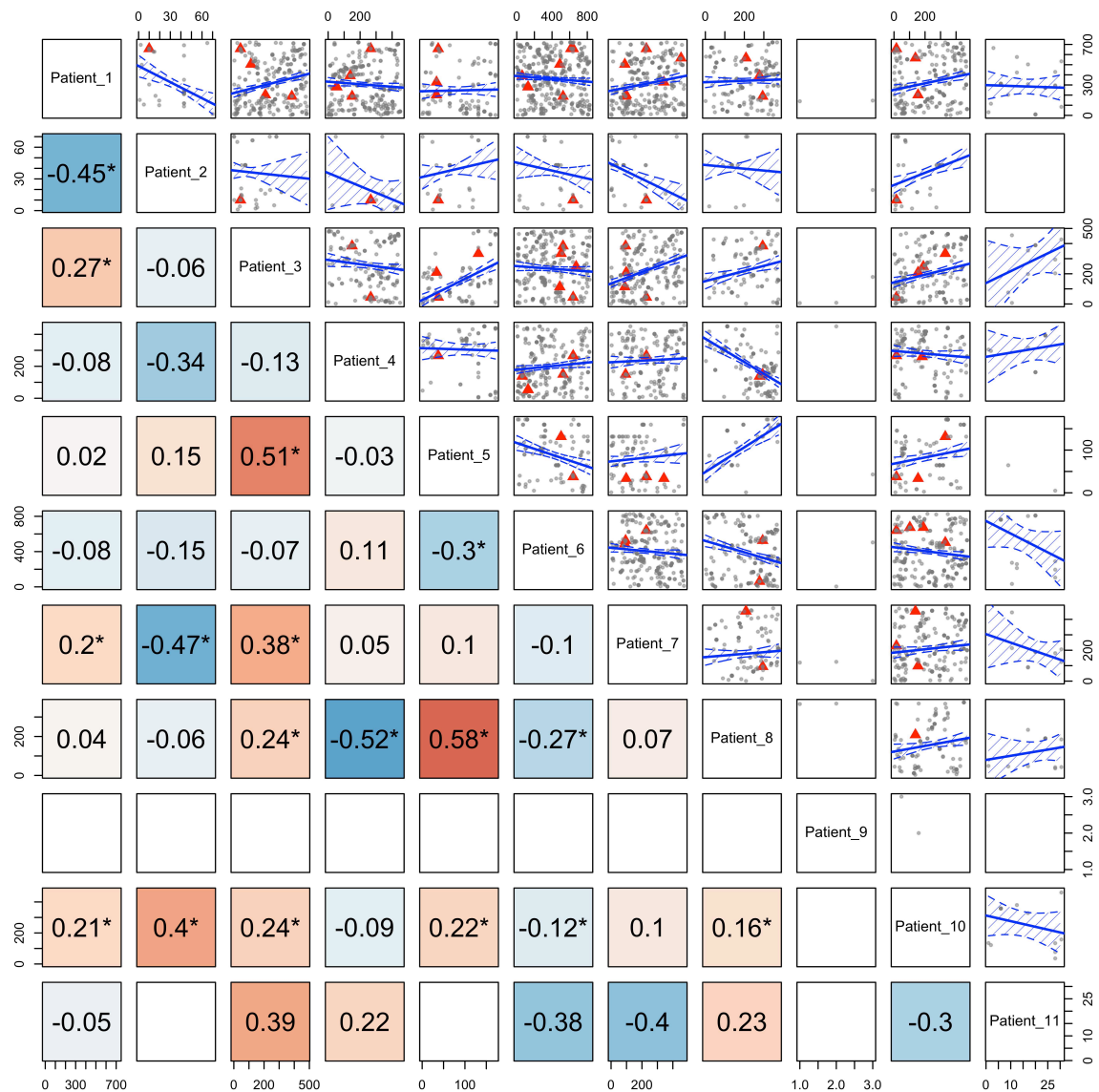


Fig. S14. Correlation between individual Crohn's disease patient drug ranks. Patients are indicated on the diagonal axis. The upper right portion of this panel presents scatter plots depicting the correlation of drug ranks in drug predictions from two individual patients. Known CD drugs are depicted by red triangles, and all other drugs are presented as gray dots. In cases where more than 5 drugs overlapped between patients, a Pearson correlation was calculated. The correlation coefficients are presented in the lower left panel and are coloured by correlation coefficient ($0 >$ blue and red > 0), and significant P values are indicated by asterisks. Blue lines in the upper right panel show the Pearson correlation coefficient and 95% confidence intervals.

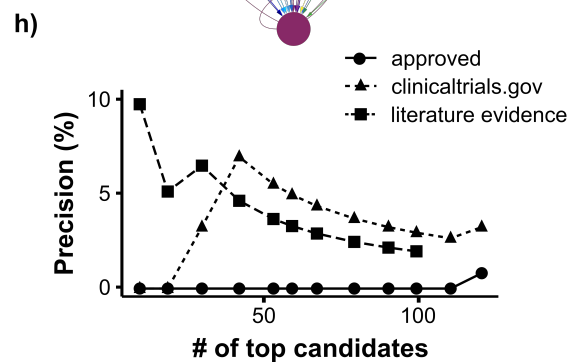
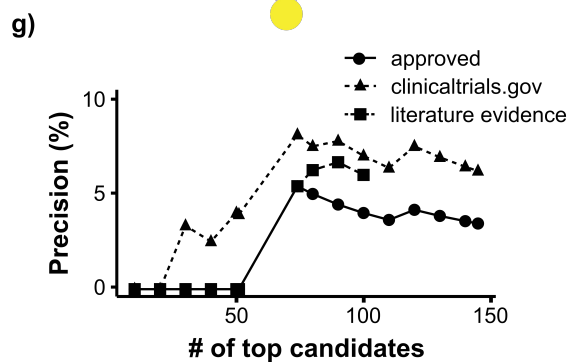
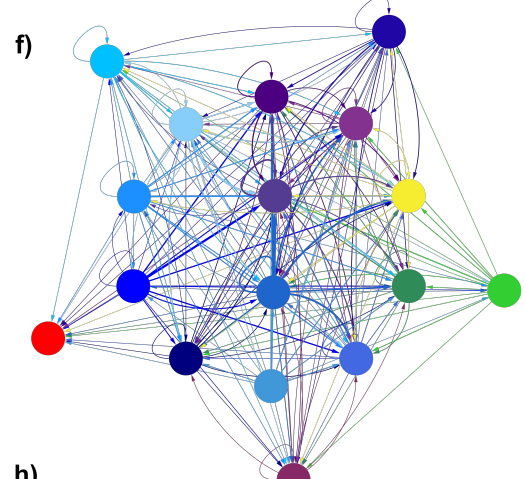
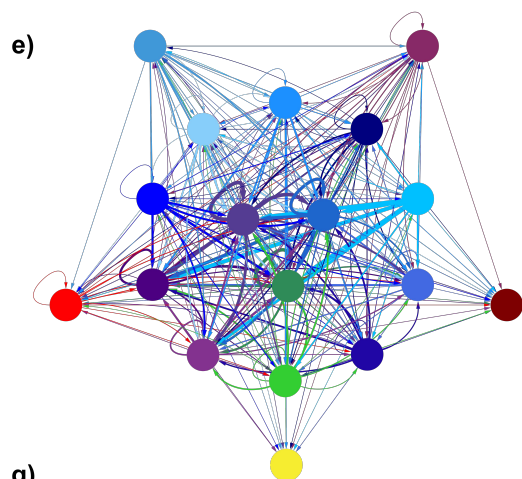
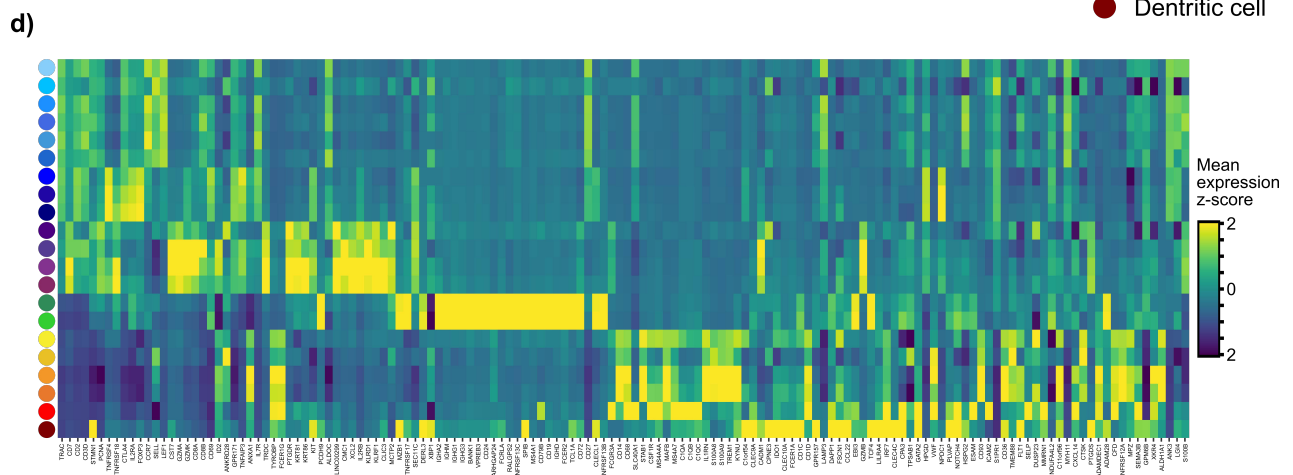
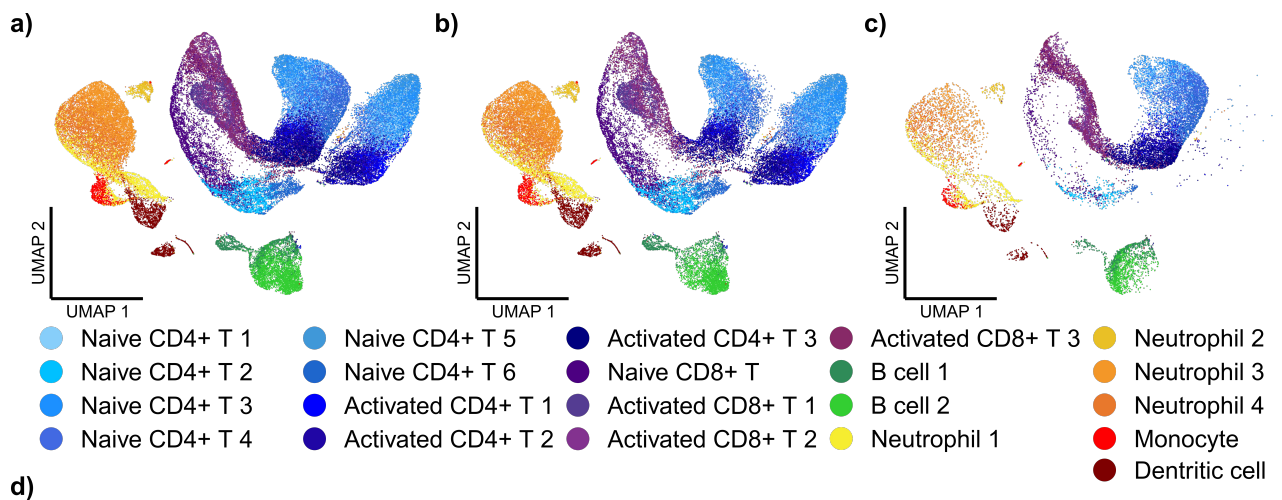


Fig. S15. scDrugPrio applied to psoriatic arthritis patients who were or were not anti-IL17 responders. UMAP visualisation of cells from **a)** controls and PsA patients (both responders and nonresponders to anti-IL17), **b)** only PsA patients, and **c)** only controls. **d)** Cell typing performed on marker genes; cell type is indicated by cluster colours to the left. MCDMs were created for **e)** anti-IL17 responders and **f)** nonresponders. Precision for approved PsA drugs, drugs for which clinical trials had been registered in PsA and drugs with literature evidence (only collected for top 100 ranking drugs) for **g)** responders and **h)** nonresponders were derived separately. Responders achieve a precision of 4% for approved PsA drugs and 6% for drugs with literature evidence among the top 100 ranking drugs. The approved PsA drugs are cortisone derivatives. Nonresponders have a precision of 0% for approved PsA drugs and 2% for drugs with literature evidence among the top 100 ranking drugs.

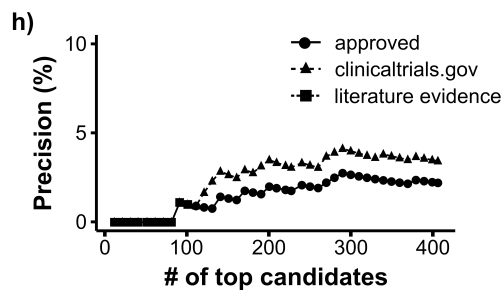
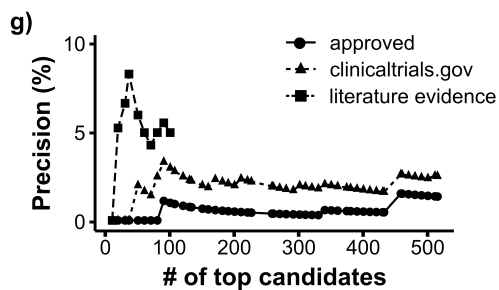
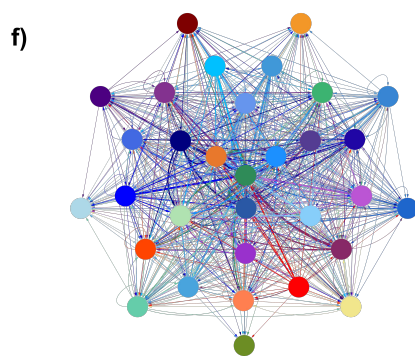
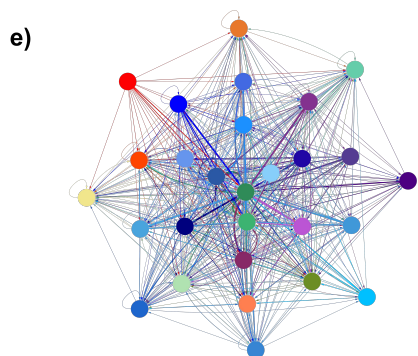
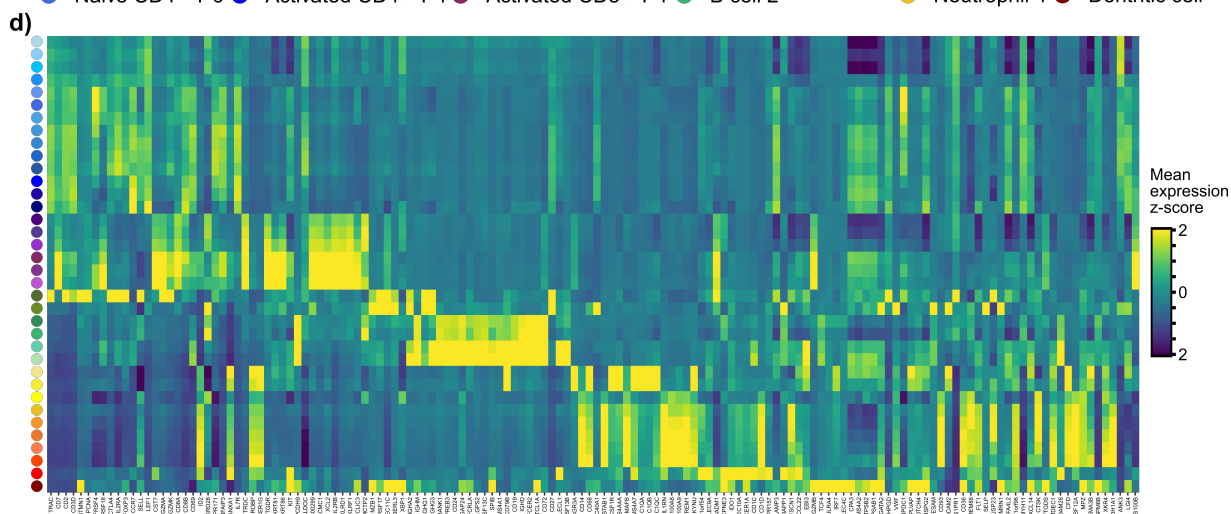
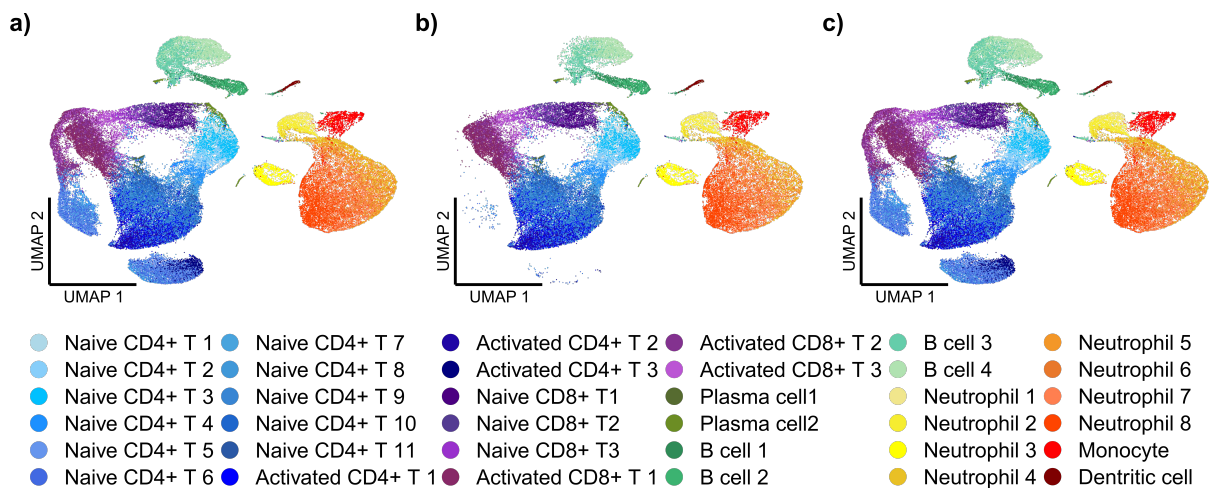


Fig. S16. scDrugPrio applied to psoriatic arthritis patients who were or were not anti-TNF responders. UMAP visualisation of cells from **a)** controls and PsA patients (both responders and nonresponders to anti-TNF), **b)** only PsA patients, and **c)** only controls. **d)** Cell typing performed on marker genes; cell type is indicated by cluster colours to the left. MCDMs were created for **e)** anti-TNF responders and **f)** nonresponders. Precision for approved PsA drugs, drugs for which clinical trials had been registered in PsA and drugs with literature evidence (only collected for top 100 ranking drugs) for **g)** responders and **h)** nonresponders were derived separately. Responders achieve a precision of 1% for approved PsA drugs and 4% for drugs with literature evidence among the top 100 ranking drugs. The approved PsA drugs are cortisone derivatives. Nonresponders have a precision of 1% for approved PsA drugs and 1% for drugs with literature evidence among the top 100 ranking drugs.

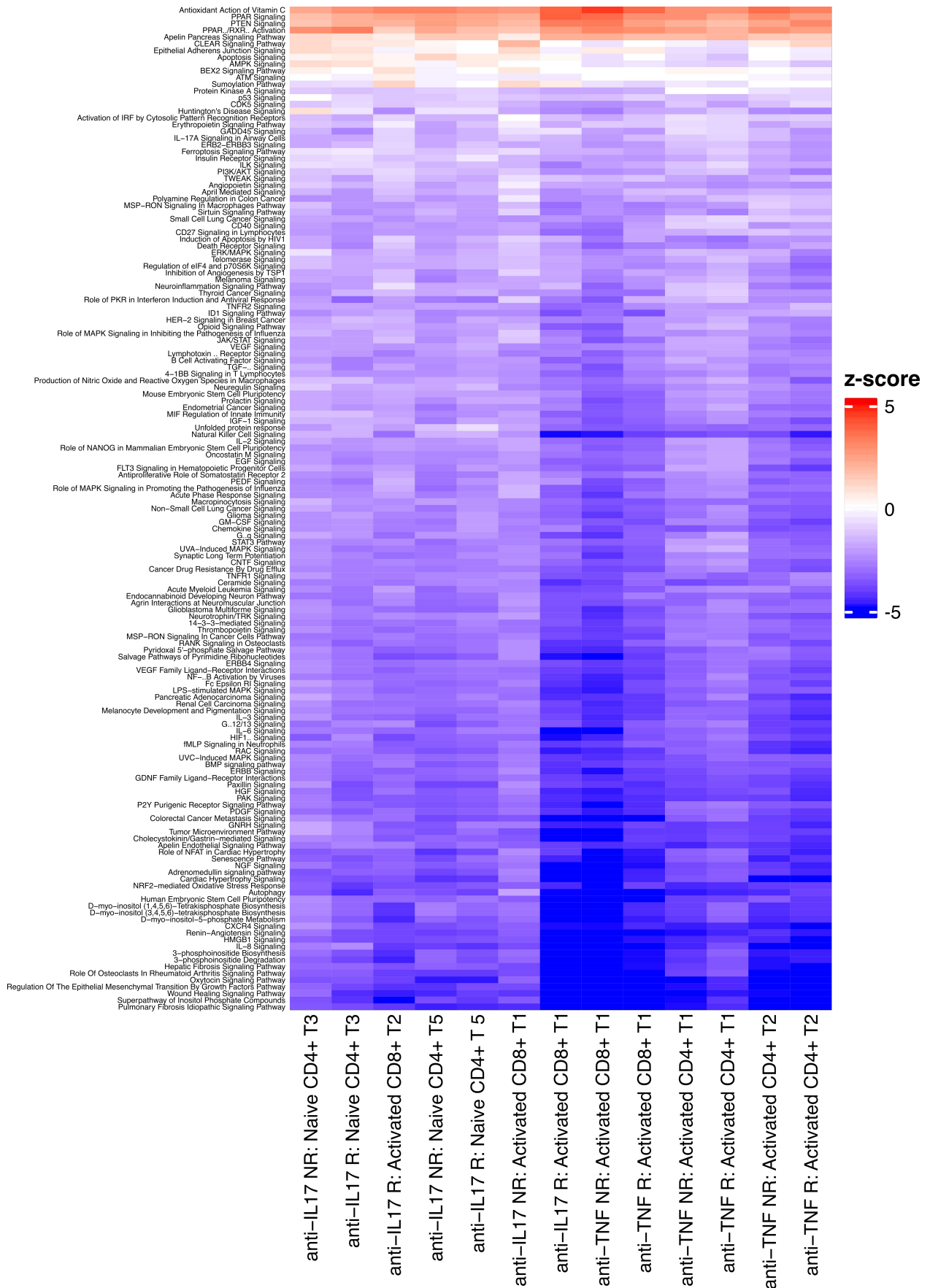


Fig. S17. Ingenuity pathway analysis of cell types enriching IL17 or TNF KEGG pathways. To investigate why scDrugPrio does not prioritize valid targets based on PBMC data from PsA patients, we checked whether treatment-relevant pathways were enriched among the DEGs of responders and nonresponders to the respective drug. We found that only a few cell types were enriched in the TNF- α and IL17 KEGG pathways. Since KEGG does not enable prediction of up- or downregulation, we redid pathway enrichment for the relevant cell types in ingenuity pathway analysis (IPA). In this heatmap, we present IPA-derived z-scores for up- (positive) or down (negative) pathway regulation. The IL17A signalling pathway and TNFR signalling pathway were either downregulated or insignificantly enriched, with a z-score trending towards downregulation.

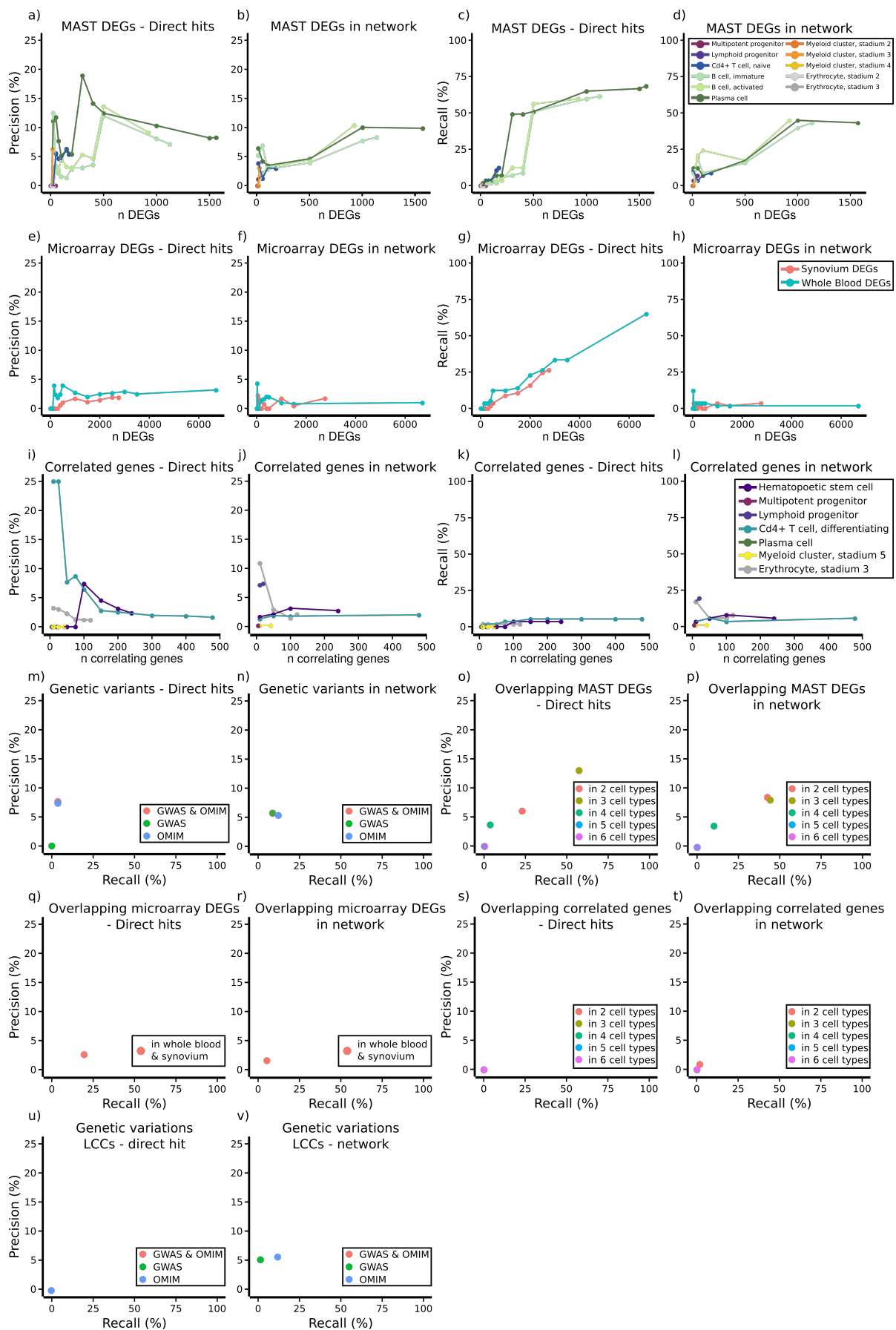


Fig. S18 Precision and recall as a function of different feature selection methods and different data sets. a-d) Precision (a, b) and recall (c, d) derived based on the top x most significant MAST DEGs. Selected genes were checked for known drug targets, and drugs that targeted at least one DEG (“Direct hits”) were selected as candidates for calculation of precision (a) and recall (c). Selected genes were also used as input to the previously described network-proximity screening, and drugs that showed $z_c < -1.64$ in the literature curated PPIN were selected as candidates for calculation of precision (b) and recall (d). **e-h)** correspond to a-b) for microarray-derived DEGs. **i-l)** corresponds to a-b) for genes derived by correlation of each cell type’s gene expression values with the arthritis score of the AIA mice. **m&n)** shows precision/recall plots for GWAS genes and OMIM genes (as previously defined) as well as a combined gene list. **o&p)** Precision/recall plot investigating the predictive capability of MAST DEGs that overlapped between x cell types. **q&r)** corresponding to o&p) for overlapping microarray-derived DEGs. **s&t)** corresponding to o&p) for cell type-specific genes correlated with arthritis score. **u&v)** Precision/recall plot for the LCCs formed by genetic variations.

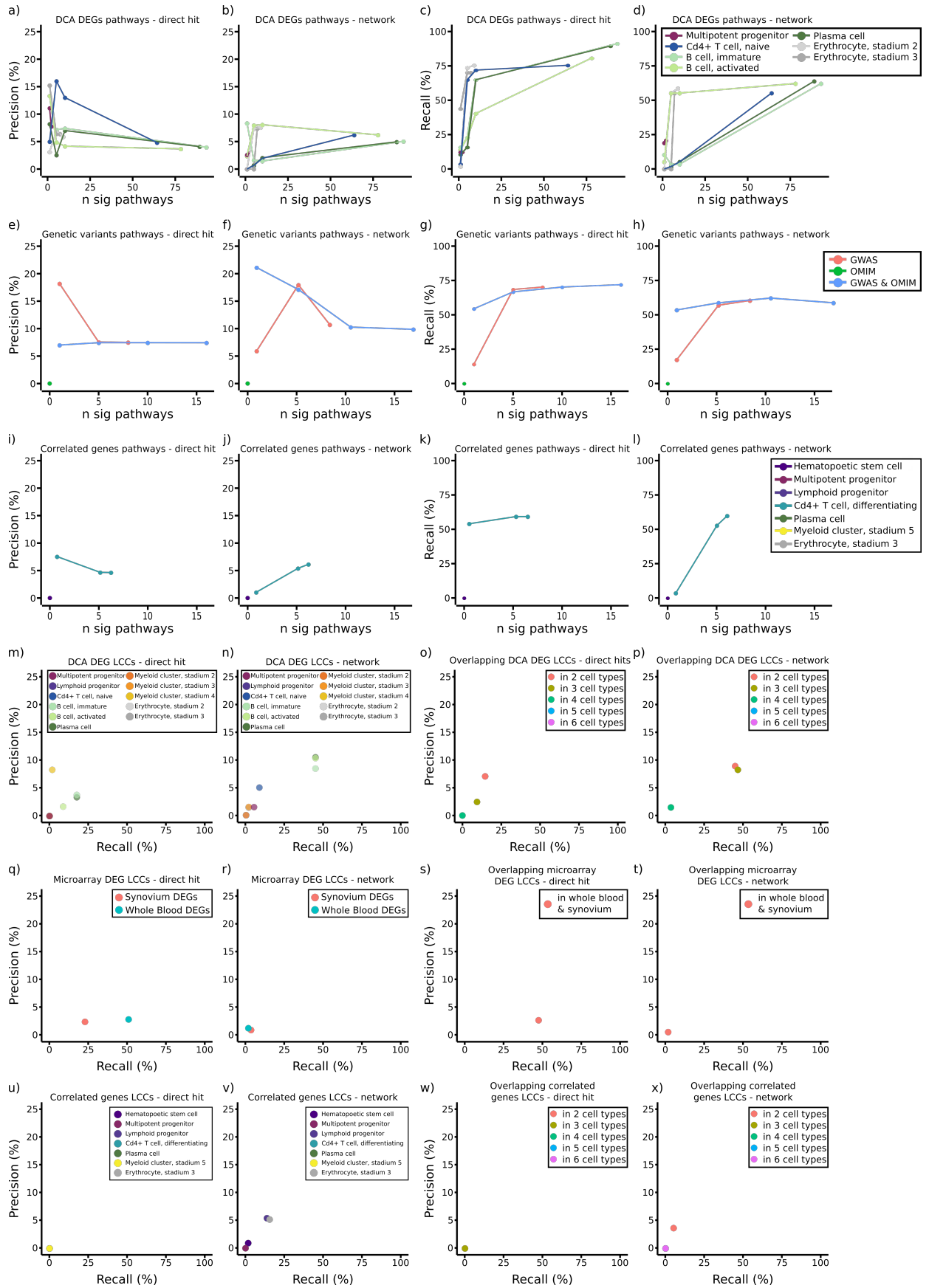


Fig S19. Precision and recall as a function of different feature selection methods and different data sets. Continuation of **Fig. S8**. **a-d)** Precision (a, b) and recall (c, d) derived based on the top significantly single-cell derived DEG-enriched KEGG pathways. Selected genes were checked for known drug targets, and drugs that targeted at least one DEG (“Direct hits”) were selected as candidates for calculation of precision (a) and recall (c). Selected genes were also used as input to the previously described network-proximity screening, and drugs that showed $z_c < -1.64$ in the literature curated PPIN were selected as candidates for calculation of precision (b) and recall (d). **e-h)** corresponds to a-b) for genetic variations. **i-l)** corresponds to a-b) for genes derived by correlation of each cell type’s gene expression values with the arthritis score of the AIA mice. **m&n)** Precision/recall plots for LCCs formed by single-cell derived DEGs. **o&p)** Precision/recall plot for the overlap between LCCs formed by single-cell derived cell type-specific DEGs. **q&r)** corresponding to m&n) for microarray-derived DEGs. **s&t)** corresponding to o&p) for LCCs formed by microarray-derived DEGs. **u&v)** corresponding to m&n) for LCCs formed by cell type-specific genes that correlated with arthritis severity score. **w&x)** corresponding to o&p) for genes overlapping between LCCs formed by correlated genes.

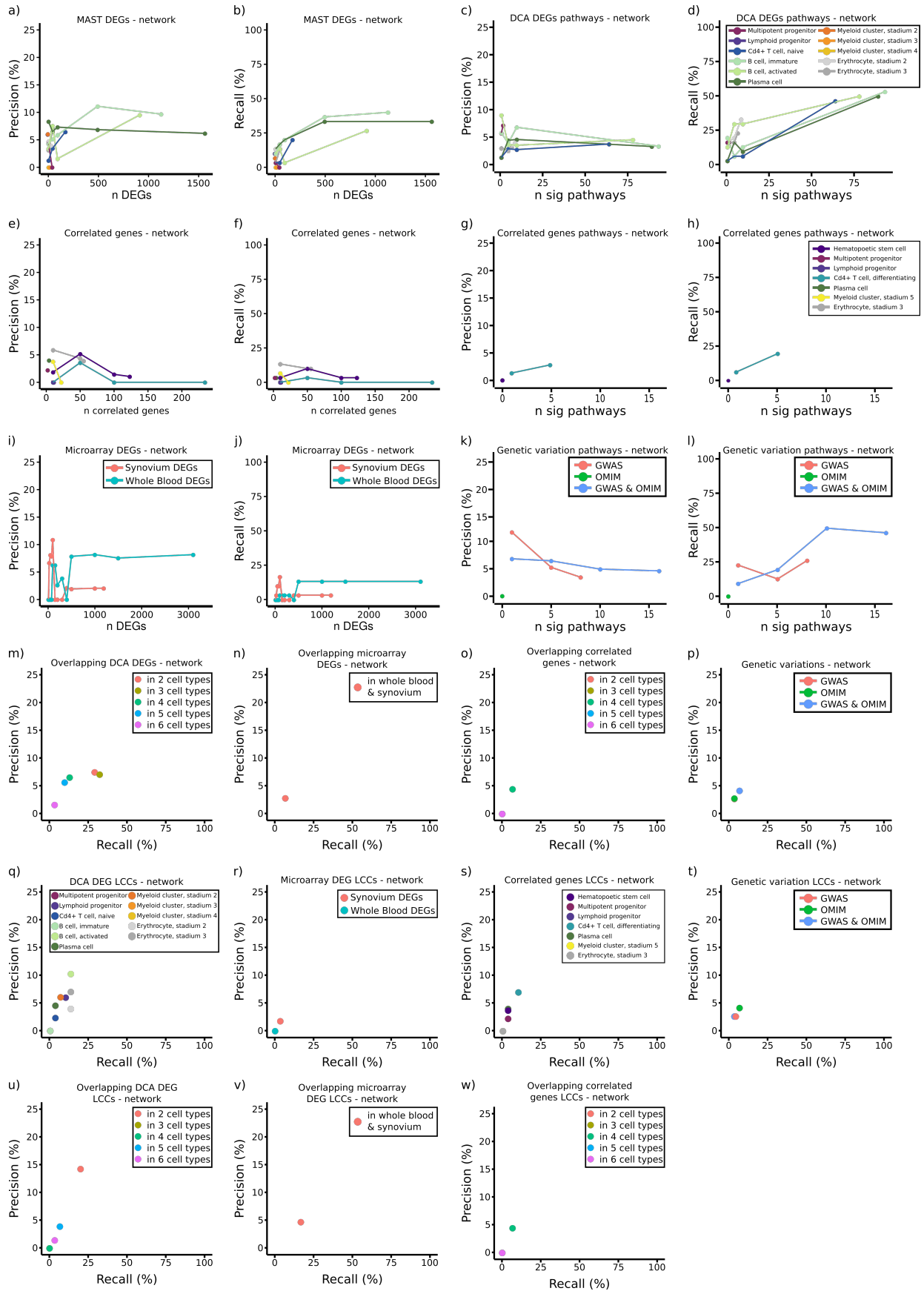


Fig S20. Precision and recall in the HuRI protein–protein interaction network (PPIN) as a function of different feature selection methods and different data sets. For calculation of the above plots, genes that were not found in the HuRI PPIN were removed prior to calculation. This included even the removal of drugs from the calculation if they did not target any gene included in HuRI. This panel exclusively shows the network-proximity screening outcomes in the HuRI PPIN, and drugs that showed $z_c < -1.64$ were selected as candidates for calculation of precision (b) and recall (d). **a&b)** Precision and recall for the top x most significantly single-cell derived DEGs. **c&d)** Precision and recall for DCA DEG-enriched KEGG pathways. **e&f)** corresponding to a&b) for correlated genes. **g&h)** corresponding to c&d) with correlated gene enriched KEGG pathways. **i&j)** corresponding to a&b) for microarray-derived DEGs. **k&l)** corresponding to c&d) for genetic variation-enriched KEGG pathways. **m-o)** Precision/recall plot for overlapping single-cell derived DEGs, microarray DEGs and correlated genes, respectively. **p)** Precision/recall plot for genetic variations. **q-t)** Precision/recall plots for LCCs formed by single-cell derived DEGs, microarray DEGs, genes correlated with arthritis score, and genetic variations. **u-w)** Precision/recall plots for LCCs overlapping between cell type-specific single-cell derived DEGs, microarray data sets, and cell type-specific genes correlated with arthritis severity score.

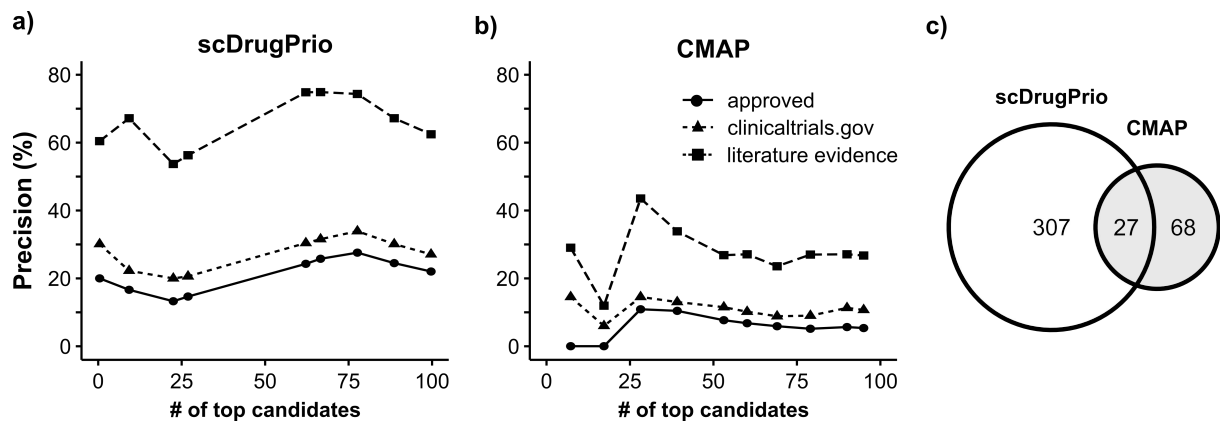


Fig. S21. Comparison of drug prediction using scDrugPrio and CMAP. Precision for **a)** the top 100 ranked candidates using scDrugPrio on DEGs derived from scRNA-seq AIA data. In a similar manner, **b)** CMAP prediction was conducted based on DEGs derived from pseudobulk RNA-seq of the AIA data. CMAP prediction was ranked by the CMAP effect measure. Precision is calculated for approved RA drugs, drugs for which clinical trials had been registered for RA and drugs with literature evidence among ranked drug candidates. Both drug predictions used the same DrugBank-derived drug target data for $n = 1,840$ drugs. **c)** shows the overlap between drug candidates.

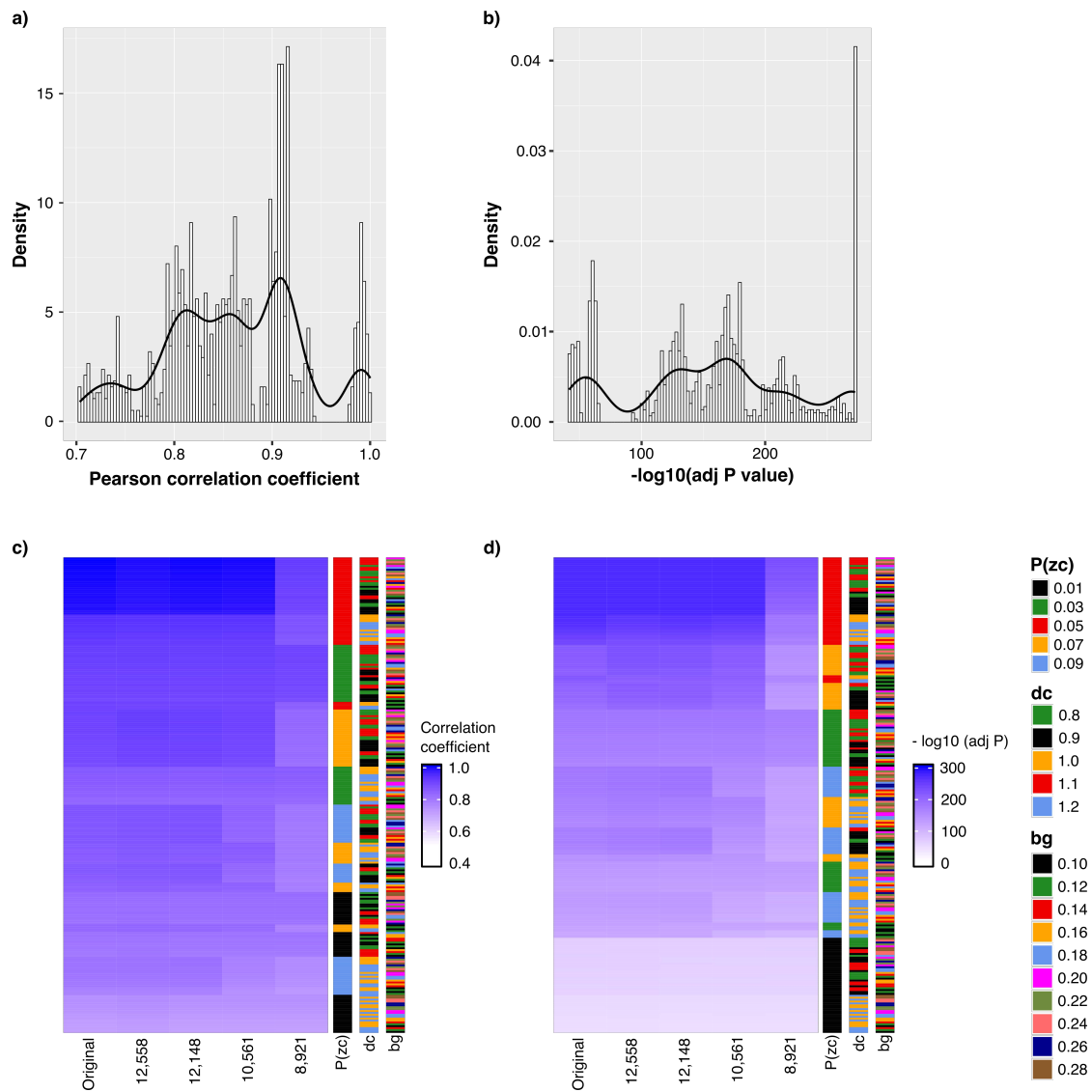


Fig. S22. Robustness analysis of scDrugPrio. Robustness was validated by variation of thresholds during recomputation of drug selection and drug ranking for the AIA data set. In this analysis, we varied 1) the number of DEGs that entered network calculations, 2) the P value cut-off for network proximity, derived from z_c , 3) network distance cut-off d_c , and 4) the cut-off for background gene calculation during NicheNet ligand activity analysis. Standard cut-offs for these included 1) all DEGs ($n = 12,769$), 2) $P < 0.05$, 3) $d_c < 1$, and 4) $Ea(i) \geq 0.2$. Collectively, 1250 drug rankings with varying thresholds were calculated, and the results were compared to the original drug ranking by Pearson correlation of drug ranks between computations. **a)** shows the distribution of Pearson correlation coefficients, **b)** shows the distribution of Bonferroni adjusted P values. Similarly, **c & d)** visualize the Pearson correlation coefficient and adjusted P value, respectively. Side bars indicate the combination of cut-offs used for results in each row. scDrugPrio returns overall returns stable and comparable results over a variety of thresholds.

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