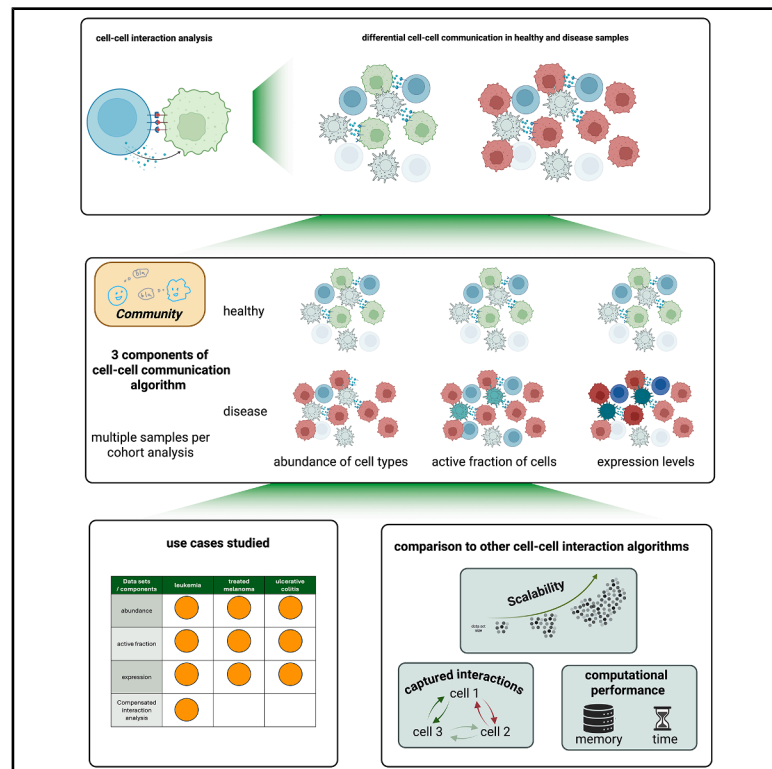


Community: Component based differential cell communication analysis in large multi-sample case-control scRNAseq datasets

Graphical abstract



Authors

Maria Solovey, Muhammet A. Celik, Felix R. Salcher, ..., Antonio Scialdone, Frank Ziemann, Maria Colomé-Tatché

Correspondence

maria.colome@bmc.med.lmu.de

In brief

Immunology; Cancer; Transcriptomics

Highlights

- Dissects communication into abundance, active fraction, and expression
- Identifies differential and compensated cell-cell interactions
- Captures UC, melanoma, and AML communication changes in scRNAseq cohorts
- Scales efficiently and is robust to outlier-driven signals



Article

Community: Component based differential cell communication analysis in large multi-sample case-control scRNAseq datasets

Maria Solovey,^{1,11} Muhammet A. Celik,^{1,2,11} Felix R. Salcher,^{1,2,3} Cem Güleç,² Mohmed Abdalfattah,² Mostafa I.H. Ali,² Matthias Heinig,^{2,9} Antonio Scialdone,^{2,7,8} Frank Ziemann,^{3,4,5,6} and Maria Colomé-Tatché^{1,2,10,12,*}

¹Department of Physiological Chemistry, Biomedical Center (BMC), Faculty of Medicine, LMU, Munich, Germany

²Institute of Computational Biology, Helmholtz Zentrum München, Munich, Germany

³Laboratory for Experimental Leukemia and Lymphoma Research (ELLF), LMU University Hospital, Munich, Germany

⁴Laboratory for Leukemia Diagnostics (Lfd), LMU University Hospital, Munich, Germany

⁵Department of Medicine III, LMU University Hospital, Munich, Germany

⁶German Cancer Consortium (DKTK), Partner Sites Munich, Germany

⁷Institute of Epigenetics and Stem Cells, Helmholtz Zentrum München – German Research Center for Environmental Health, Munich, Germany

⁸Institute of Functional Epigenetics, Helmholtz Zentrum München – German Research, Munich, Germany

⁹School of Computation, Information and Technology, Technical University MunichCenter for Environmental Health, Neuherberg, Germany

¹⁰Hospital del Mar Research Institute (HMRIB), Barcelona, Spain

¹¹These authors contributed equally

¹²Lead contact

*Correspondence: maria.colome@bmc.med.lmu.de

<https://doi.org/10.1016/j.isci.2026.117314>

SUMMARY

Changes in cell-cell communication during disease can result from shifts in tissue composition, variations in the proportion of cells engaged in signaling, and differences in ligand-receptor expression. To address this, we developed *community*, an R package designed for differential communication analysis in multi-sample case-control scRNAseq datasets. *Community* reconstructs interactions by evaluating cell type abundance, the active fraction of cells, and their expression levels. This method identifies communication patterns that are upregulated, downregulated, unchanged, or compensated. Applied to ulcerative colitis, melanoma under immune checkpoint inhibitor treatment, and acute myeloid leukemia, *community* captured disease- and response-associated communication changes, including increased communication in ulcerative colitis, reduced immunosuppressive signaling in melanoma responders, and decreased immune communication in AML. Comparisons with existing tools showed improved robustness to outlier-driven signals and better scalability. These component-level analyses help connect altered communication patterns to biological mechanisms in healthy and disease states.

INTRODUCTION

Cell-cell communication is crucial for maintaining healthy physiological functions in tissues. In disease, the immune and non-immune niches, as well as the resident cell types, change their abundance and function, which results in an altered interaction among them.^{1–4} Discovering these changes is important to better understand disease and identify new treatment strategies. Single-cell RNA sequencing (scRNAseq) techniques allow us to capture shifts in tissue composition, as well as in the expression of ligands, receptors, and adhesion molecules within individual cell types. ScRNAseq has become increasingly used in case-control studies where several cohorts with multiple samples are analyzed. This strategy allows better accounting for biological heterogeneity, which is especially relevant when working with human samples.

For the study of cell-cell communication changes between cases and controls, a differential communication analysis is needed. We previously published COMUNET, the first tool able to perform differential communication analysis.⁵ However, COMUNET performs differential communication analysis of one control versus one case sample, while often many samples are analyzed in cohorts. An algorithm that is able to scale to multiple samples easily is thus needed. Several other cell-cell interaction tools are able to perform differential analysis.^{6–9} The major limitation of these tools is that the disease-driven shifts in cell type abundance are not considered while analyzing differential communication. These shifts can, though, be massive and can vastly affect the overall communication dynamics within the diseased tissue. To maintain homeostasis and proper tissue function, several mechanisms adapt at the cellular level. First, cells (especially immune cells) can infiltrate a tissue, resulting



in an increase in their cell-type abundance.^{10,11} Second, cell types can decrease in their abundance due to, e.g., damage, aging, or clearance via the immune system.^{12–14} The remaining cells may adapt their ligand and receptor expression levels in an attempt to keep the physiological levels of interaction stable and compensate for the shifts in the tissue composition.^{15–18} It is of great importance to take these processes into consideration in the cell-cell communication analysis.

Here we present *community*, an R package and web server that performs differential communication analysis in large scRNAseq case-control studies. *Community* is a fast and user-friendly tool that is not limited by the number of samples in the case and control cohorts that can be processed. *Community* does not rely on prior differential expression analysis, but captures changes in cell type abundance, fraction of active cells, and ligand-receptor and adhesion molecule expression. *Community* can thus recognize not only interactions that are differential between cases and controls, but also distinguish between unchanged interactions and those that are being compensated through the interaction of several components.

We exemplify the differential communication analysis by applying *community* analysis to three very distinct disease entities: ulcerative colitis (UC), melanoma, and acute myeloid leukemia (AML). We show that UC undergoes an upregulation in communication intensity, including epithelial, vascular, and immune compartments, which is in line with the physiology of the disease manifesting increased inflammation. In melanoma, we were able to capture the drop in immunosuppressive signals in the tumors that responded to anti-PD1 antibody therapy, compared to the resistant ones. For the AML datasets, we show a decrease in communication activity, especially within the immune compartment. This observation is in line with the described effect of immune silencing via cancer cells. *Community* identifies differential interactions even in very small populations of cells, and it can also detect complex interactions. We compare *community* to four other communication algorithms: CellPhoneDB (v3.1),⁶ MultiNicheNet (v2.1),⁹ CellChat (v2.2)¹⁹ and scSeqCommDiff (v2.0)²⁰ using their differential communication pipelines and show that *community* is faster, more robust to outlier signals, and able to capture hidden compensatory effects.

The intuitive output of *community* makes it possible to track shifts in communication among cell types in diseased tissues compared to controls, visualize and investigate individual top differential interactions, as well as identify the driving components underlying the changes in communication. The large-scale studies of shifts in cellular interactions will help understand biological mechanisms driving disease progression at a new level of complexity.

RESULTS

Theoretical basis

To assess communication between case and control samples using scRNAseq, *community* uses ligand-receptor and adhesion molecule interactions between cell types in a tissue (Figure 1A). It works with a flexible built-in database of known ligand-receptor and adhesion molecule interactions adapted from *OmniPath*,²¹

which can easily be expanded by the user (Figure S1). To construct the database, we utilize two main interaction databases from *OmniPath*: “*ligrec extra*” and “*curated ligand-receptor interactions*”.²¹ We break down all complex interactions into their individual components, such that all interacting pairs consist of only one ligand and one receptor. The components of adhesion molecule pairs or other non-directional interactions are randomly labeled as “ligand” or “receptor”, but this directionality can be ignored in these cases. The generated database comprises 6,941 entries, of which 4,157 are ligand-receptor pairs, while 2,784 are between adhesion molecules. Each ligand, receptor, and adhesion molecule in the database has a functional annotation obtained from *OmniPath*.

Community is unique in its definition of the communication weights, which are defined by three major contributors to the communication intensity: cell type abundance ρ , active fraction ϕ , and mean expression level within the active fraction π (Figure 1B). Cell type abundance is frequently affected in disease due to shifts in the cell type composition. If one or both communicating cell types are shrinking or expanding, this will affect the tissue-level intensity of the interaction between these cell types. *Community* takes this into account by including changes in cell type abundance ρ in the differential communication analysis. The active fraction ϕ is defined as the fraction of cells in a particular cell type that have a non-zero expression of the ligand or the receptor of interest. In a disease, if the active fraction of a cell type expands or shrinks, the communication with this cell type may be affected, even if the cell type abundance and the expression level of the individual cells remain unchanged. Finally, the mean expression level π of ligands and receptors within the active fraction reflects the intensity of the interaction, and it is the most intuitive and well-studied component that affects communication intensity. Mainly, the communication tools designed to date calculate communication strength based only on average expression per cell type (pseudobulk), which is a product of ϕ and π . However, these pseudobulk expression changes do not capture the disease-driven change in cell type abundance, nor do they give specific information on whether the alteration in pseudobulk-level expression was due to a change in the fraction of cells expressing a gene or in the expression levels themselves.

Based on these three components, the weight of an interaction between two cell types (sending S and receiving R), mediated by a particular ligand-receptor pair $(l, r)_k$ (or adhesion molecule pair $(l, r)_k$) in a particular sample i is defined as the product of the three components:

$$\omega_{(S, R)_i}^{(l, r)_k} = (\rho_{S_i} \times \rho_{R_i}) \times (\phi_{S_i}^{l_k} \times \phi_{R_i}^{r_k}) \times (\pi_{S_i}^{l_k} \times \pi_{R_i}^{r_k})$$

ρ_{S_i} is the relative abundance of the sending cell type, ρ_{R_i} is the relative abundance of the receiving cell type, $\phi_{S_i}^{l_k}$ and $\phi_{R_i}^{r_k}$ are the relative active fractions of the ligand l_k in the sending cell type and the receptor r_k in the receiving cell type respectively, and $\pi_{S_i}^{l_k}$ and $\pi_{R_i}^{r_k}$ are the relative mean expression of the ligand l_k in the active fraction of the sending cell type and the receptor r_k in the active fraction of the receiving cell type, respectively (Figure 1C; Figure S2, method details). The interaction weight $\omega_{(S, R)_i}^{l_k r_k}$ can have a value between 0 and 1.

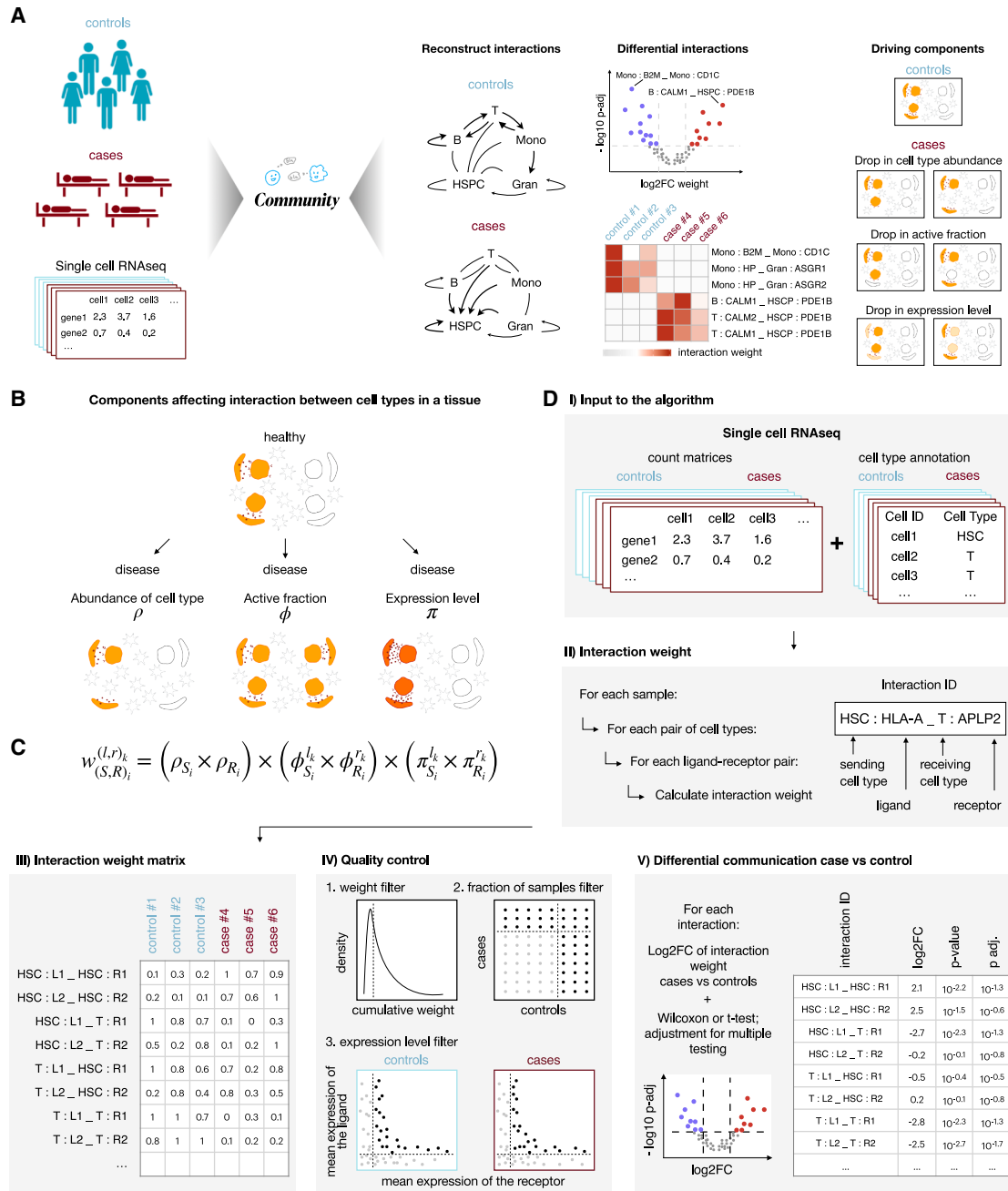


Figure 1. Overview of the community tool

(A) *Community* overview: the input is a set of scRNAseq data from a case-control study; the outputs are reconstructed interactions for the case and control cohorts, differential interactions, and driving component analysis.

(B) Components affecting interactions between cell types in a tissue: abundance of the cell type ρ , active fraction of communication cells ϕ , and expression level of the ligands and receptors π .

(C) Formula to calculate an individual interaction.

(D) *Community* workflow. (I) The input to the algorithm is a set of scRNAseq count matrices and cell type annotation. (II) The interaction weight is calculated for each sample, each pair of cell types, and each ligand-receptor pair. The interaction ID contains the sending cell type, the ligand, the receiving cell type, and the receptor. (III) The resulting interaction weight matrix has interaction IDs in the rows and sample names in the columns. (IV) The quality control has three filters: the cumulative interaction weight, the fraction of samples where an interaction is present, and the mean expression levels of the ligand and the receptor in controls and cases. (V) For the differential interaction, the log2FC and the adjusted p value is calculated for each interaction between cases and controls.

Community is designed to work with scRNAseq data from case-control experiments. Its input is a pre-processed (i.e., normalized and batch-corrected, if needed) scRNAseq dataset with cell types already annotated (Figure 1D Input to the algorithm; Figure S2). As in all cell-cell interaction analyses, cell types have to be present in both cases and controls; thus, merging fine-grained cell type annotations might be needed. For diseases such as cancer, the malignant cell types should be annotated to the cell type category of their healthy counterparts.

In the first step of the algorithm, for each sample, we calculate the interaction weight between each pair of cell types for each ligand-receptor pair (LRP) from the database (Figure 1D Interaction weight; Figure S2). Three thresholds are applied: a cell type is considered as captured in a particular sample if there is a minimum number of cells in that cell type; this cell type is considered to have a big enough active fraction if there are a minimum number of cells in that cell type expressing a particular ligand or receptor; a cell is considered as active if it reaches a minimum expression level of the ligand or receptor. Every interaction gets an ID constructed as *sending cell type: ligand _ receiving cell type: receptor*. This step is repeated for all samples, which results in an interaction matrix with sample IDs in the columns and interaction IDs in the rows (Figure 1D Interaction weight matrix).

In the quality control (QC) step, the quality of the interactions is evaluated to filter interactions that are either too weak or are not present in a sufficient number of samples (Figure 1D Quality control). For this, we apply three filters: cumulative interaction weight over all samples, fraction of samples in which an interaction is present, and mean expression of the ligand and receptor per cohort (see STAR Methods section).

In the final step of our analysis, we calculate differential interactions between the case and the control samples (Figure 1D Differential communication case vs. control). For each interaction, we first calculate the log2 fold change between cases and controls and the respective *p* value. To calculate the *p* value, we use either the *t* test²² on the log2-transformed weight values, recommended for smaller cohorts, or the Wilcoxon test²³ on the actual weight values, recommended for bigger cohorts. We correct for multiple testing using the FDR method.²⁴ After this, significantly differential interactions can be visualized and further studied.

The output of *community* contains, most importantly, the interaction weight matrix and the interaction annotation matrix with information on the individual components used for the weight calculation, QC metrics, as well as statistics for differential communication analysis. *Community* also implements intuitive options for visualization, such as, for example, a volcano plot of differential interactions, a heatmap of top up- and down-regulated interactions, and forest plots for analysis of changes in the individual components (Figure 1A). The versatile output of *community* makes it possible to easily navigate from the top-level analysis of global changes in cellular communication between cases and controls, down to the detailed investigation of individual interactions, allowing to identify the components driving the change.

Changes in communication in ulcerative colitis vs. healthy colon

To demonstrate the capabilities of *community*, we applied it to a previously published scRNAseq dataset of primary human ulcerative colitis (UC) patients and healthy controls²⁵ (Figure 2A; Figures S3A–S3D). After filtering, the dataset contained 17 UC and 11 healthy control samples, as well as 13 cell types (Figure 2A; Figures S3A–S3D).

Using *community*, we identified 15,675 interactions that passed quality control, with 2,612 being significantly upregulated and 758 downregulated in UC (Figure 2B; Figure S3E). In UC, as an inflammatory bowel disease, we expect to observe overall increased levels of communication due to the influx of immune cells and inflammatory cytokine production. Our analysis revealed a predominance of non-immune cell types in upregulated interactions, specifically stem and multipotent cells, followed by goblet cells, vascular cells, enterocytes, and fibroblasts (Figure 2C; Figure S4A). Of those, for stem and multipotent cells, goblet cells, and fibroblasts, the change in communication was driven by an increase in active fraction and mean expression, whereas for enterocytes and vascular cells the shift was additionally driven by a strong change in cell type abundance (Figure S5). This is consistent with the vascularization of the inflamed tissue and the loss of enterocytes.^{26,27} Among immune cell types, Tregs and mast cells showed the highest number of upregulated interactions (Figure 2C). These were driven by all three components (Figure S5), consistent with immune activation. For the rest of the immune cell types, changes in communication were not caused by cell type abundance, and only infrequently driven by changes in active fraction or mean expression (Figure S5). Instead, the changes were driven by the interacting counterparts of those cell types (Figure S5). Using both more coarse-grained and more fine-grained cell type annotations (3 or 43 cell types) allowed us to identify the same trend, but with a number of new interactions that were only identified in the fine annotation, showcasing how the user can select the level of granularity (Supplemental Methods 2.2 and Figure S6).

Next, we explored individual ligands and receptors in the differential interactions (Figure 2D), identifying *LDLR*, *CD44*, *ITGB1*, and *EGFR*, which are known to play critical roles in UC and other related pathologies of the intestine by driving inflammation.^{28–31} Most of the top ligands and receptors were used by both non-immune and immune compartments. Others, such as *CD4* or *KIT*, were immune compartment-specific (Figure 2D). We observed that for different cell types, individual ligands and receptors can contribute to the change in the interaction strength via different components. For example, *CD44* is used as a receptor by 11 cell types (Figure S4B). Its contribution to the increased interactions in regulatory T cells, mast cells, and B cells is driven solely by an increased abundance of these cell types. In contrast, goblet cells, macrophages, and stem and multipotent cells increased their active fraction expressing *CD44*. For the other cell types, all three components were predominantly stable, and the change in interactions was driven by the sending counterpart cell types (Figure S4B). These results illustrate how various shifts in communication are regulated, and that it is important to consider all aspects of cell-cell interaction to gain biologically relevant analysis.

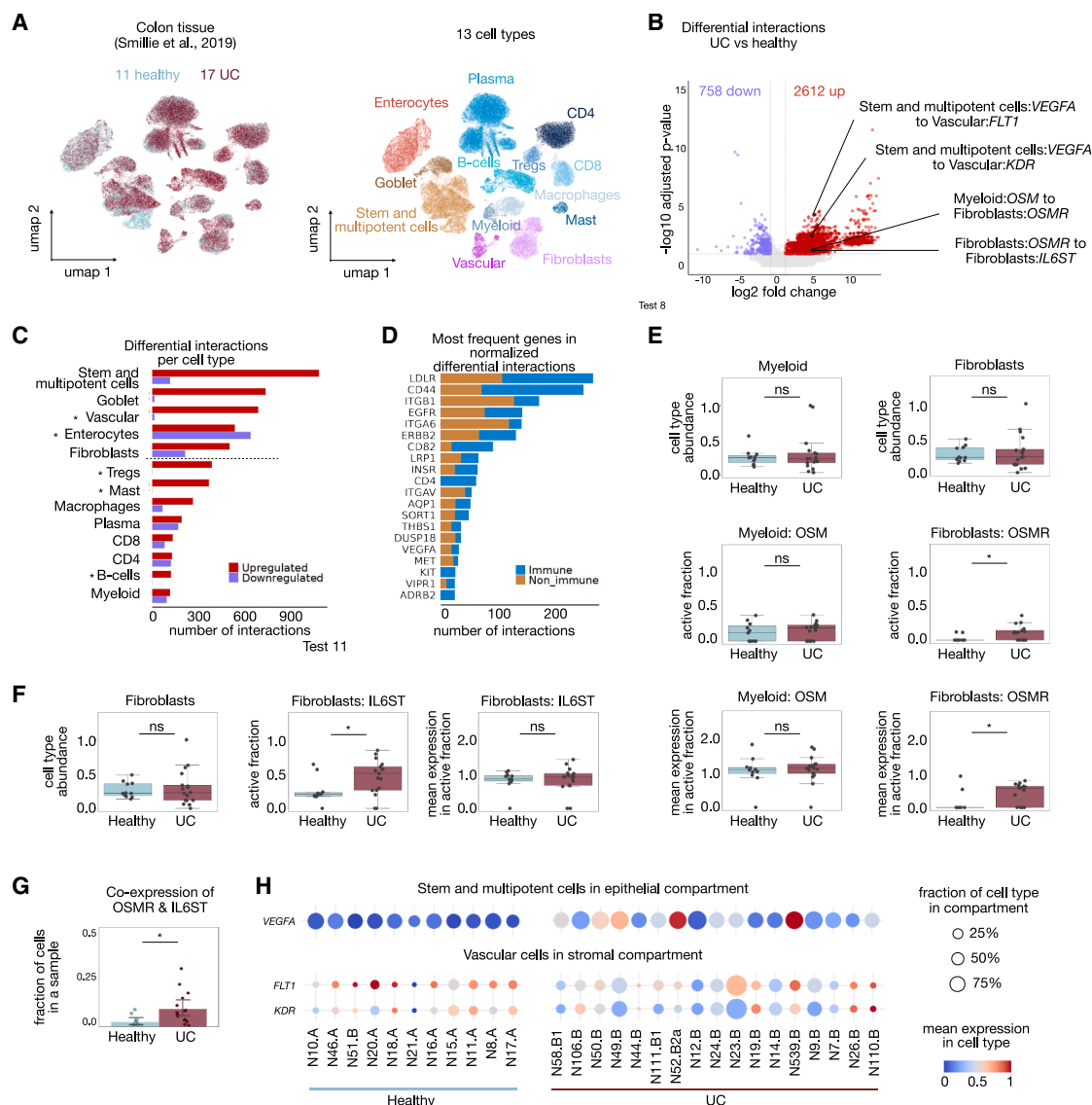


Figure 2. Communication in inflamed ulcerative colitis (UC) and healthy colon tissue

(A) UMAPs colored by health status (left image) and cell types (right image).
 (B) Volcano plot of differential interactions with highlighted relevant interactions.
 (C) Number of differential interactions per cell type. Asterisk indicates cell types with altered cell type abundance. Dashed line separates non-immune and immune compartments.
 (D) Ranking of most frequently occurring genes in significant interactions. The box plots in (E) and (F) represent the cell type abundance, active fraction, and mean expression in the active fraction for relevant genes in the selected cell types.
 (G) Co-expression of OSMR and IL6ST across all Fibroblasts.
 (H) Dot plot of *VEGFA* expression in stem and multipoint cells and *FLT1* and *KDR* expression in the vascular cells. The color represents mean gene expression for a cell type per sample. Dot size represents the fraction of the respective cell type in the cell-specific compartment. Statistics were performed using *t* tests. Significant findings (*p*-val. <0.05) are labeled with an asterisk.

As proof of concept, we explored the upregulated interaction *Myeloid:OSMR_Fibroblasts:OSMR* between myeloid cells expressing Oncostatin M (*OSM*) and fibroblasts expressing the Oncostatin M receptor (*OSMR*) (Figures 2B–2E and 2F, *p*.adj = 0.044, $\log_2$ FC = 4.798). This aligns with the findings of the original publication, which have been confirmed with confocal microscopy.²⁵ Community identified that the overall

increase in communication was driven by the expansion of an *OSMR*-expressing subpopulation of fibroblasts, as the fraction of fibroblasts expressing *OSMR* increased from 0% (healthy) to 17% (UC) (Figure 2E). Numerous publications show that *OSMR* activation is accompanied by a co-activation of its heterodimeric counterpart IL6ST.³² To test if this is the case, we compared co-expression levels of these two genes

and observed a higher fraction of co-expression in fibroblasts in UC compared to healthy controls ($p_{\text{val}} = 0.006$) (Figure 2G; Figure S4C). Also, the *community* database marks the *OSMR-IL6ST* pair as a complex, and the *community* captured the *Fibroblasts:OSMR_Fibroblasts:IL6ST* interaction as significantly upregulated ($p_{\text{adj}} = 0.049$, $\log_2\text{FC} = 4.747$), which is likely a consequence of the co-expression (Figures 2F and 2G; Figure S4C).

Besides exploring overall changes in communication and identifying components driving shifts in interactions already described in the initial publication, we also discovered novel interactions. Among them, we found the significantly upregulated interactions between *stem and multipotent cells:VEGFA_vascular:FLT1* and *stem and multipotent cells:VEGFA_vascular:KDR* (Figure 2B). *Community* identified these interactions to be driven by the increased active fraction and mean expression of *VEGFA* in stem and multipotent cells, as well as the increased cell type abundance of vascular cells (Figure 2H; Figure S4D). *VEGFA* is a known driver of vascular growth and pathological angiogenesis, which is often overexpressed in response to inflammatory hypoxia.²⁶ Our analysis shows increased mean expression of the PHD family (HRE complex members), which are upstream of *VEGFA*, in the stem and multipotent cells in the UC cohort. (Figure S4E).

These results showcase that *community* can be applied to case-control datasets of inflammatory disease. It allows for the identification of known interactions and enables the user to gain a deeper understanding of shifts in interactions. It is also capable of identifying complex interactions such as *OSM-OSMR/IL6ST*. Additionally, it can detect previously missed interactions that provide insights into disease-specific communication patterns, which could aid in identifying novel therapeutic targets.

Changes in communication in melanoma under immune checkpoint inhibitor treatment

We next aimed to explore interactions within the immune tumor niche under checkpoint inhibitor therapy. For this, we investigated a human melanoma scRNAseq dataset consisting of 19 samples before therapy, of which 8 responded and 11 did not respond, and 28 samples after therapy, of which 6 responded and 22 did not respond (Figure 3A).³³ In this publication, the immune tumor environment was isolated (Figure 3A).

We performed two separate communication analyses: non-responders vs. responders before therapy, and non-responders vs. responders after therapy. We ran *community* separately in these two scenarios, and after filtering out interactions that did not pass quality control, we identified 49,986 and 67,554 good-quality interactions, and 375 and 5,438 differential interactions in each comparison, respectively. In particular, we observed stronger differences in communication between the non-responders and responders after therapy compared to those before therapy (Figure 3B). Specifically, we observed significantly upregulated signaling from the antigen-presenting cells toward exhausted CD8 T cells via the following immunosuppressive pathways: PD-L1/PD-L2 – PD-1, CD80/CD86 – CTLA-4, MHC-II – LAG-3, Galectin-9/CEACAM-1 – TIM3, CD155 – TIGIT (Figure 3B). The exhausted CD8 T cells received this signaling predominantly from macrophages, dendritic cells (DCs), activated regulatory

T cells, memory CD8 T cells, as well as cycling T cells (Figure 3C). Other cell types within the immune niche also showed inhibitory signaling, yet to a lower level (Figure 3C). While responders showed active inhibitory signaling and PD-1 and CTLA-4 pathway activation in the exhausted CD8 T cells before therapy, this signal was abrogated after therapy (Figures 3C–3E). This was not the case for the non-responders, where the level of inhibition remained similar despite the therapy (Figures 3C–3E). Interestingly, non-responders had intrinsically higher levels of inhibition before therapy than the responders, which may have promoted therapy failure (Figures 3C–3E).

These results show how *community* can be used not only to study differences in communication between cases and controls, but also between disease subtypes. In this case, applying it to compare responder versus non-responder patients upon checkpoint inhibitor therapy in melanoma, we point at an increased communication using particular immunosuppressive pathways, which could potentially be targets for chemical modulation.

Changes in communication in AML vs. healthy bone marrow

To assess the versatility of *community*, we also applied it to a cancer case-control dataset, where, by nature, malignant cells are not present in the healthy tissue. This published human bone marrow (BM) scRNAseq dataset comprised 7 individuals with acute myeloid leukemia (AML) at diagnosis and 6 healthy controls.³⁴ We defined 8 cell types: hematopoietic stem and progenitor cells (HSPC), monocytes, granulocytes, dendritic cells (DC), erythrocytes, B-cells, T-cells and natural killer cells (NK) (Figure 4A; Figures S7A–S7D).

After running *community* and filtering out interactions that did not pass quality control, we identified 22,645 good quality interactions. First, to get a general impression of the communication results, we grouped the interactions per cell type. To do that, we aggregated the interaction weights of all communication edges between every pair of cell types, and then summarized them into three broad interaction categories: “engaging HSPC”, “among immune cell types”, and “engaging erythrocytes” (Figure 4B). The engaging HSPC category included classes that had HSPC either as a sending or as a receiving cell type, or both. The category “among immune cell types” included classes where both sending and receiving cell types were immune cells. The “engaging erythrocytes” category had erythrocytes either as a sending or as a receiving cell type or both. In the healthy cohort, we observed that the class of interactions in the “among immune cell types” category had higher average weights compared to the AML, whereas classes engaging HSPC or erythrocytes tended to have lower weights. In the AML cohort instead, we observed a drop in the average weights of immune interactions, indicating less immune-driven communication in these patients (Figure 4B). In AML, the HSPC-engaging classes had higher interaction weights compared to the healthy control (Figure 4B). These results align with known biology, as the immune compartment is most represented in healthy BM; therefore, we expect it to contribute most to the overall communication in the tissue. In AML, though, due to the differentiation block³⁵ and immune suppression,³⁶ we expect to observe

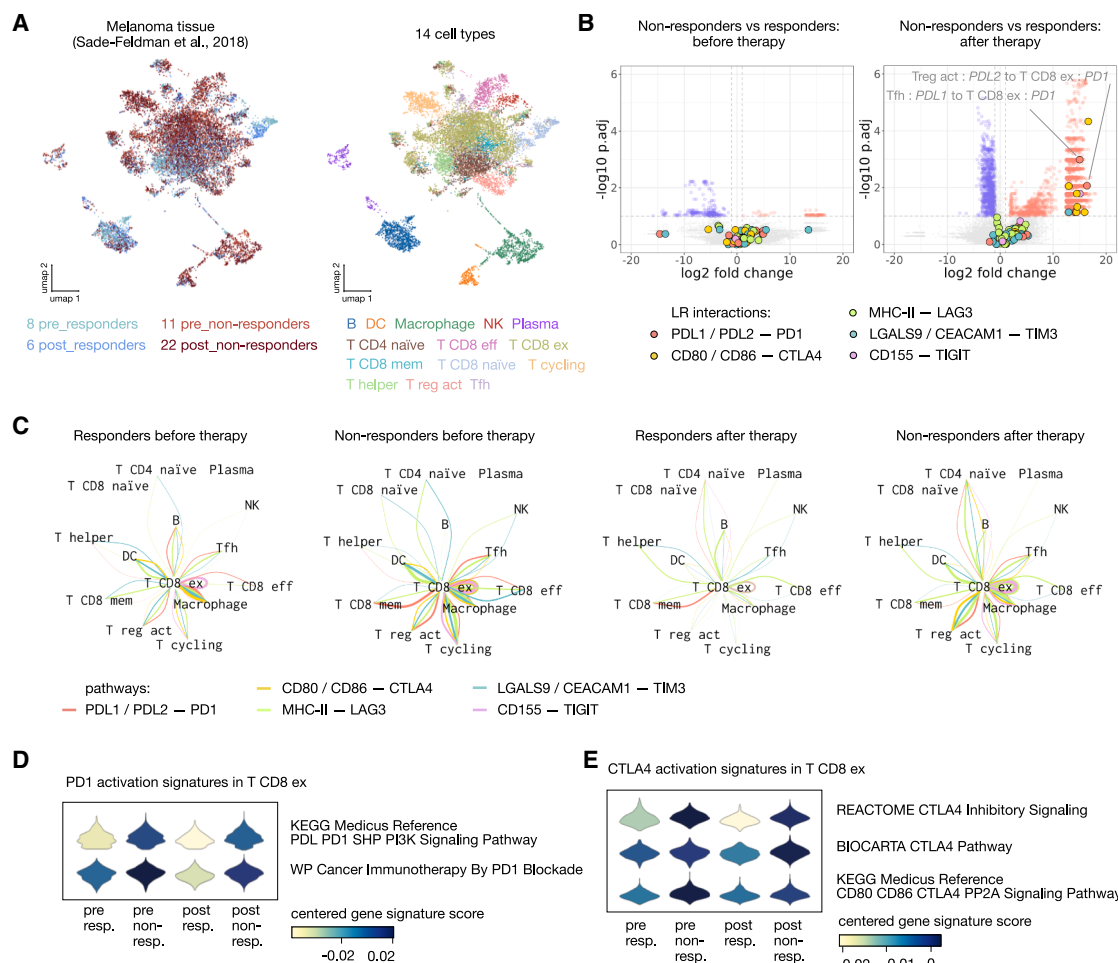


Figure 3. Communication in melanoma under immune checkpoint inhibitor treatment

(A) UMAPs colored by treatment timepoints and therapy response (left image) and cell types (right image).

(B) Volcano plot of differential interactions with highlighted inhibitory interactions.

(C) Network plots for the inhibitory interactions from the immune niche toward the exhausted CD8 T cells per cohort. Color represents individual inhibitory pathways. Edge width represents the mean weight of the interactions belonging to a particular inhibitory signaling between a sending cell type and the exhausted CD8 T cells.

(D) Scores for PD1 activation signatures in the exhausted CD8 T cells.

(E) Scores for CTLA4 activation signatures in the exhausted CD8 T cells.

silencing of the immune-driven communication. Additionally, due to the expansion of the blasts and their reported suppressive interaction activity toward other compartments, we expect to observe increased interactions engaging HSPCs in AML.

We next proceeded to the differential communication analysis. We identified 2,664 differential interactions, 109 upregulated in AML and 2,555 downregulated (Figure 4C). The majority of the downregulated interactions involved interactions of immune cell types among each other, while the upregulated interactions predominantly involved interactions between HSPCs and immune cell types (Figure 4D). Like many cancers, leukemic cells avoid immune destruction as a cancer hallmark by impairing immune response due to several mechanisms (e.g., T-cell exhaustion and HLA loss); therefore, it was to be expected to see an overall decrease in communication, especially involving

immune cells.^{36,37} To further study mechanisms of decreased immune communication, we checked which components are most prominent in each cell type. In this dataset, DCs most frequently showed a drop in all three components: cell type abundance, active fraction, and the mean expression (Figure S8). HSPC and erythrocyte precursors showed upregulated cell type abundance and downregulated active fraction, while B-cells, monocytes, and granulocytes showed changes in active fraction and occasionally in mean expression. The T- and NK-cells showed only occasional changes in active fraction and mean expression (Figure S8). Among the top downregulated interactions, we found those engaging monocytes expressing *TNFSF14*, *NRG1*, *HP*, *TNFRSF14*, and *SLGEC7*, predominantly driven by the reduction in the active fraction of these genes in the monocytes. (Figures 4E and 4F;

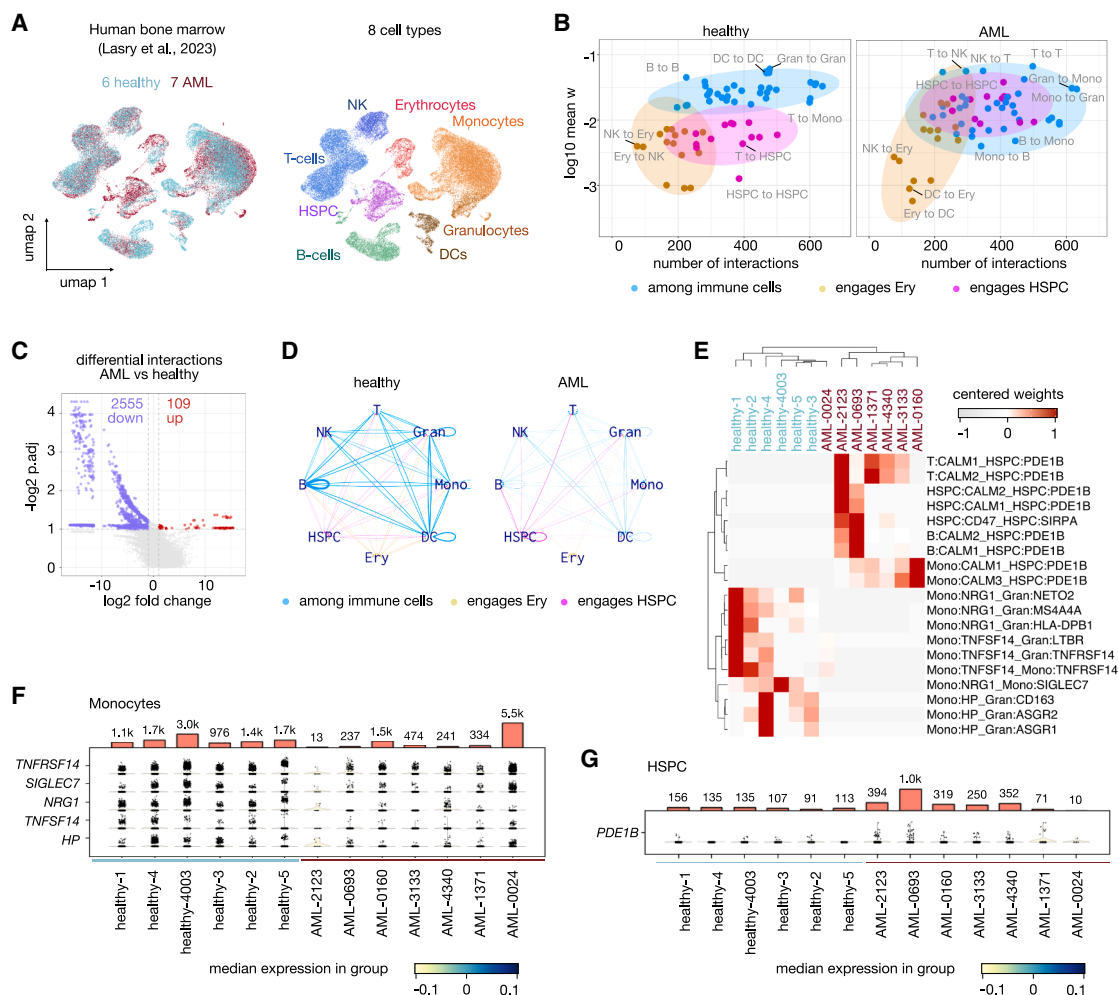


Figure 4. Communication in healthy and AML bone marrow

(A) UMAP of the human bone marrow scRNAseq dataset colored by health condition (left image) and cell type (right image).

(B) Good-quality interaction groups by cell type for healthy (left image) and AML (right image) cohorts.

(C) Volcano plot of differential interactions (AML vs. healthy).

(D) Network graph of differential interactions in the healthy (left image) and AML (right image) cohorts. Edge width represents the mean weight of all differential interactions between the two connected cell types; (E) heatmap of top up- and downregulated interactions.

(F) Stacked-violin plots of the ligands engaged in the top downregulated interactions of panel (E).

(G) Stacked-violin plot of the receptor engaged in the top upregulated interactions of panel (E).

Figure S7F). These genes are immune-activating, and loss of monocytes expressing these genes is an indication of an immunosuppressive milieu within the leukemic bone marrow.^{38–42} Similar results were obtained through more focused analysis on tumor immune interaction (Figure S9). Our communication results therefore point to reduced communication within the immune compartment, potentially as a consequence of the immune suppression caused by the tumor.

The top upregulated interactions included interactions between *PDE1B*-positive HSPC and three cell types: B-cells, T-cells, and monocytes, all expressing the calmodulin gene family (CALM1-3). *PDE1B* positive HSPC have been characterized as leukemia initiating cell populations.⁴³ Although calmodulin expression was unchanged in the immune cells, the change in communi-

cation was driven by the increase in all three components of HSPC: the cell type abundance, the active fraction of HSPC expressing *PDE1B*, as well as the mean expression level of *PDE1B* within the active fraction (Figures 4E and 4G; Figure S7F).

To independently reproduce our results on a different dataset, we applied the same analysis to an integrated AML and healthy dataset with a higher number of samples^{44,45} (Figures S10A–S10E). This cohort consisted of 9 AML and 26 healthy samples, with 6 broad cell types (HSPCs, monocytes, erythrocytes, B-cells, T-cells, and DCs). Again, we found more downregulated than upregulated interactions in AML, and the downregulated interactions included interactions among the immune cells themselves, whereas the upregulated interactions included interactions with HSPCs

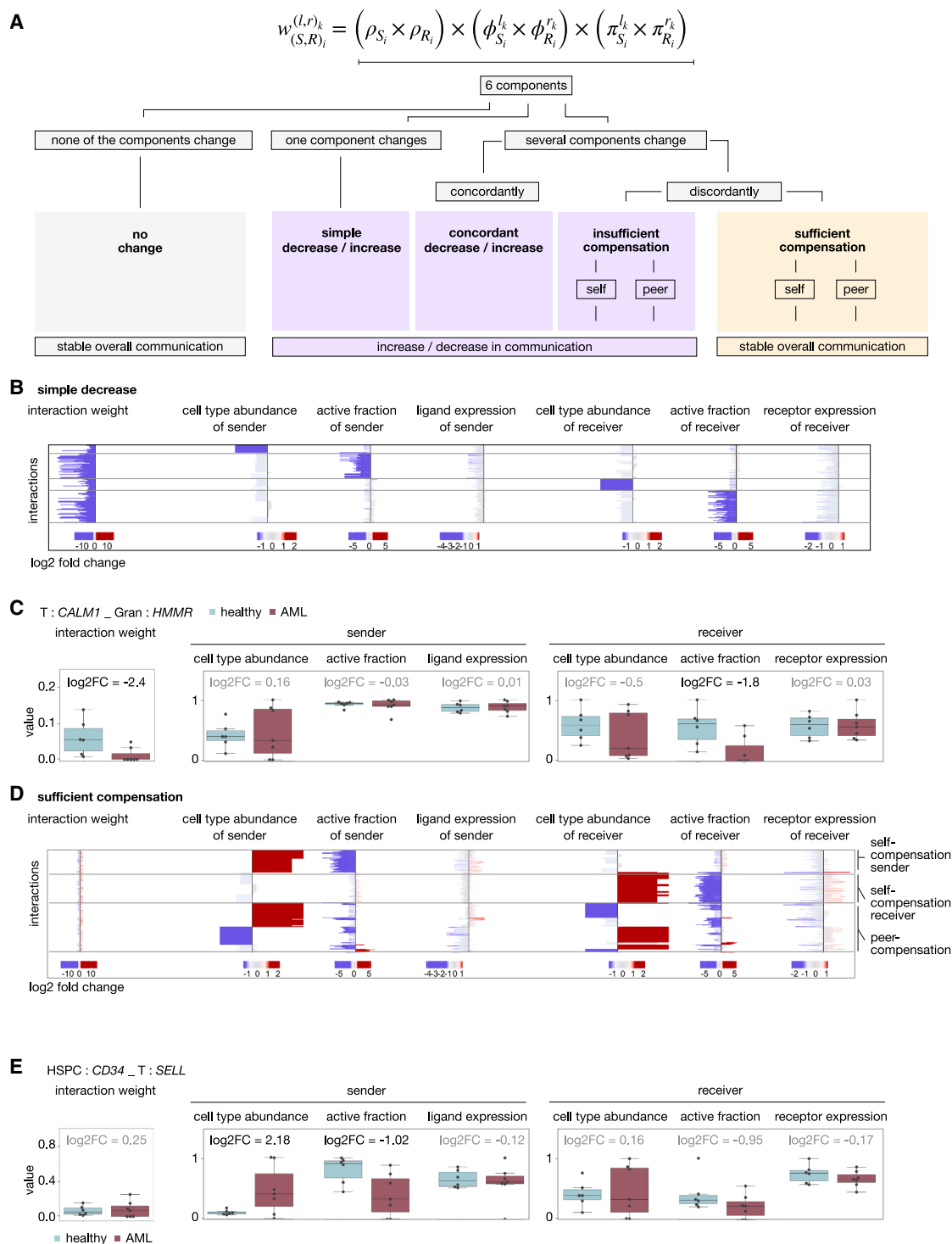


Figure 5. Interplay of individual components shapes the resulting communication shift

(A) Six types of changes in communication, driven by the interplay of the individual components.

(B) Forest plot representation to visualize driving components in Lasry et al. interactions that fall into the category of simple decrease. The y axis represents interactions; the x axis represents the log2 fold change of the following parameters: the interaction weight, the three components (cell type abundance, active

(legend continued on next page)

(Figures S10F–S10H). Using only the shared cell types between the two datasets, we compared the overlap between the interactions in the immune compartment and found 345 mutually differential interactions (Figure S11A). The mutually downregulated interactions among immune cell types showed a reduction of immune-activating signals in the bone marrow, specifically driven by the drop in monocytes expressing *NRG1* and *HP* (Figure S11B).

In summary, using *community*, we show that in AML there is a general downregulation of communication within the immune compartment, indicating immunosuppression, whereas the HSPC compartment containing the tumor cells shows upregulation of communication.

Interplay of the individual components during communication change

Community interaction strength calculation is purposely built in a modular manner to allow the exploration of the mode of action that leads to a communication change. Namely, for each interaction, *community* considers three components: the cell type abundance of the sending and the receiving cell types (ρ_S , ρ_R), the active fraction of the sending and the receiving cell types for the specific ligand and receptor (or adhesion molecule pair) (ϕ'_S , ϕ'_R), and the expression level of the sending and the receiving cell types for the ligand and the receptor pair in the active cells (alternatively, the adhesion molecule pair) (π'_S , π'_R). For any given interaction, there are several possible scenarios which can lead to differential communication between cases and controls, which can easily be disentangled with *community* (Figure 5A; Figure S12). First, there is a scenario with no change in any of the six components, in which case we speak of a stable interaction. Second, only one of the six components can change (decrease or increase). If strong enough, this change can lead to a statistically significant change of the whole interaction weight, which we address as a simple decrease or increase. Third, several of the six components can change. This change can either be concordant or discordant. In concordant changes, all affected components change in the same direction. Here, if the combined change is strong enough, it can also lead to a significant change in the overall interaction strength. Alternatively, the change in the several components can be discordant, i.e., the components change in opposite directions. In this case we can speak of a compensation scenario. If the overall interaction strength changes significantly, we can interpret it as an insufficient compensation. If the overall interaction strength stays stable, we can regard it as a sufficient compensation (Figure 5A; Figure S12). For both insufficient and sufficient compensation, if the changes are happening only within the sending or only within the receiving population, we speak of self-compensation. If the changes are happening in both the sending and the receiving population, we speak of peer-compensation.

We analyzed the behavior of the individual components in the AML-healthy dataset³⁴ and found cases for all categories described above (Figure 5B; Figure S12). In the dataset, 3,405 interactions were unchanged (Figure S12). Furthermore, 517 interactions were driven by a change in only one component, as in the case of *T:CALM1_Gran:HMMR*, where the change was driven by the drop in the active fraction of the receiver (Figures 5B and 5C; Figure S12). We observed a concordant change in 1,139 interactions, such as in *T:CALM1_HSPC:PDE1B* driven by the increase of all three components of the receiver (Figure S12). Further, 695 interactions showed discordant changes in the individual components, leading to insufficient compensation, as in the case of *HSPC:FBN1_T:ITGB1*, where, despite the increased cell type abundance of the HSPC, the drop in their active fraction expressing FBN1 led to a decrease in the intensity of this interaction (Figure S12). Then, very interestingly, we could identify 1,570 instances of compensated interactions. For example, in the interaction *HSPC:CD34_T:SELL*, there was an increase in the cell type abundance of the HSPC and a decrease in the active fraction of the HSPC expressing *CD34*, as well as the active fraction of the T cells expressing *SELL*. The overall communication in this interaction was not significantly affected due to the peer-compensation (Figures 5D and 5E; Figure S12).

The ability to distinguish between the situation of true stable communication and the sufficiently compensated communication is an important and unique feature of *community*. Compensated interactions can, for example, be detectable alterations observed in the early stages of a disease, which can potentially become decompensated during disease progression.

Method comparison

We compared *community* to four cell-cell communication tools: CellPhoneDB^{6,46} (v3.1.0), MultiNicheNet^{9,47} (v2.2), CellChat^{19,48} (v2.2) and scSeqCommDiff (v2.0).²⁰ Although initially CellPhoneDB, NicheNet, and CellChat were developed to study static communication in a sample, they have incorporated new pipelines for differential communication analysis across conditions or samples. However, their approach to identify differential signals differ (Table S2). CellPhoneDB relies on user-provided differential gene expression (DE) results from tools such as Seurat (v4.3.0),⁴⁹ to only consider ligand-receptor pairs that are differentially expressed between conditions/samples. In contrast, MultiNicheNet integrates muscat⁵⁰ for sample-level pseudo-bulk DE analysis between cohorts (e.g., case vs. control), focusing on DE target genes of ligands in receiver cells to prioritize differential ligand-receptor interactions, which mitigates limitations of cell-level methods (e.g., bias toward samples with more cells of a given cluster). Meanwhile, CellChat uses an internal Wilcoxon test to detect over-expressed ligand receptors (not case vs. control, but per gene) within each sample via one-vs-all DE per cluster (cell type), highlighting globally prevalent

fraction, and expression level) for the sender, and also for the receiver. The length of the line is equivalent to the value of the log2 fold change, and the color indicates whether the log2 fold change is above the threshold (black) or below (gray), also indicated by the legends at the bottom.

(C) An example interaction from this category (*T:CALM1_Gran:HMMR*) represented as boxplots with the values for the individual components.

(D and E) The forest plot for the category sufficient compensation (D) and an example interaction (*HSPC:CD34_T:SELL*) from this category represented as boxplots with the values for the individual components (E).

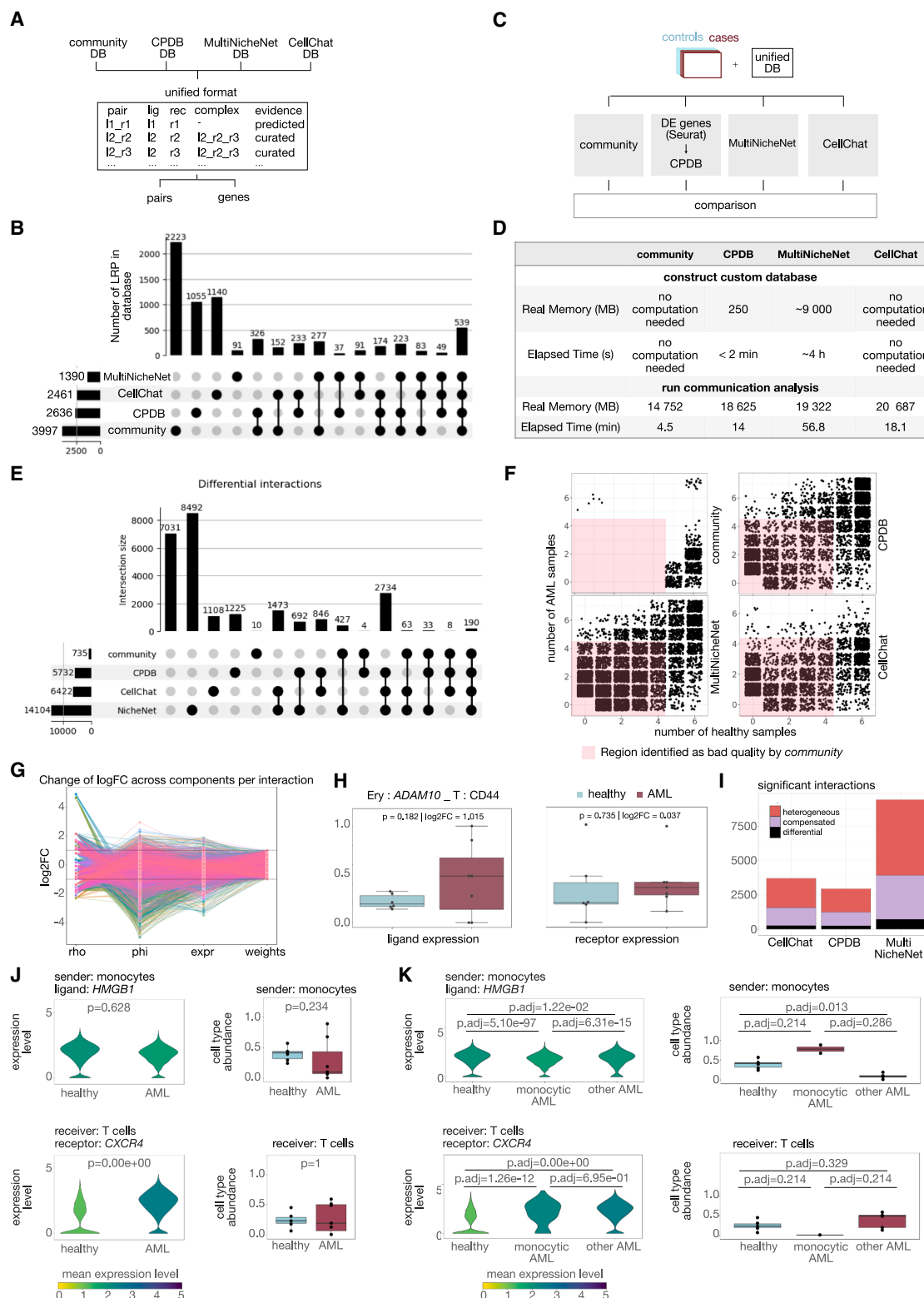


Figure 6. Comparison to existing pipelines

(A) Pipeline to compare the ligand-receptor databases of *community*, CellPhoneDB, MultiNicheNet and CellChat.

(B) Comparison of the number of ligand-receptor pairs in the curated databases of *community*, CellPhoneDB, MultiNicheNet, and CellChat.

(legend continued on next page)

interactions across samples via manifold learning and visualization without direct integration of external DE tools such as Seurat/muscat. ScSeqCommDiff calculates a reliability score for each gene by comparing the observed expression against a background null distribution approximated using the Central Limit Theorem. This approach assigns higher scores to genes that stand out significantly compared to this background noise. The method assigns a communication intensity score defined by the minimum value between the ligand and receptor scores, assuming the interaction strength is constrained by its limiting component. These scores are calculated independently for each sample, following differential communication analysis across conditions via Wilcoxon rank-sum tests and log-fold change estimation. These variations impact the resolution, granularity, and robustness of differential insights, as detailed in Table S2. For instance, only *community* and scSeqCommDiff (v2.0) perform explicit condition-aware DE directly at the communication interaction intensity, without relying on differentially expressed genes. However, scSeqCommDiff yielded no significant interactions when tested on our dataset. A similar lack of significant results was observed when applied to the reference dataset provided by the developers (as noted in Table S2), we therefore did not consider the comparison to scSeqCommDiff any further.

To assess the performance of the tools, we first compared the ligand-receptor pair databases that each of them provides. We unified the database formats by splitting complexes into individual components (see STAR Methods) and enforced the directionality of ligand-receptor pairs, with ligand being the first component of the pair and receptor being the second (Figure 6A). Both *community* and MultiNicheNet included two levels of confidence: curated, that is cited in the literature, or predicted, that is suggested by protein-protein interaction databases. CellPhoneDB and CellChat only included curated pairs. We compared the full-mode databases, i.e., curated and predicted together, as well as the curated-only mode. We performed the comparison on genes, as well as on pairs. *Community* showed the highest number of genes in both curated-only and full modes, as well as the highest number of pairs in curated-only mode. MultiNicheNet showed the highest number of pairs in the full mode, this large number was driven by the

predicted pairs. *Community* shows the second highest number of pairs in the full mode (Figure 6B; Figure S13).

We next compared the differential communication results of *community* to the Seurat-based application of CellPhoneDB, MultiNicheNet, and CellChat using the Lasry et al. dataset³⁴ (Figure 4A). To ensure that the differences in the results are driven by the algorithm behavior and not by the differences in the original ligand-receptor databases, we used the *community* database for all tools, which we converted to the tool-specific formats (Figure 6C). As customizing the database, e.g., adding new ligand-receptor pairs, might be important for some studies, we measured the time and memory consumption of this step for CellPhoneDB and MultiNicheNet, using their provided pipelines. The running time and the memory consumption for custom database construction took 2 min and 250 MB of memory for CellPhoneDB, and 5 h and 9 GB of memory for CellPhoneDB and MultiNicheNet (Figure 6D). In contrast, *community* and CellChat do not require a database to be built; instead, they require a simple dataframe with specific columns.

CellChat, CellPhoneDB and MultiNicheNet primarily infer differential communication from individual gene expression levels averaged within cell types, applying thresholds on pooled data from all samples, without per-sample filtering. For example, a default threshold in the three methods is >10% “active” cells (i.e., expressing the gene) per cell type, even if the gene is expressed by cells that only belong to one sample. As a result, an interaction can sometimes pass the filters even if expression changes come from cells belonging to a single sample. In contrast, *community* requires that an interaction be observed in multiple samples and filters out under-represented calls (Figure 6F). Because of that, the number of interactions discovered by CellChat, CellPhoneDB and MultiNicheNet is substantially higher than that discovered by *community* (6,422, 5,732 and 14,104 compared to 735, respectively) (Figure 6E). As shown in Figure 6F, *community* only retains interactions that were found in at least five healthy or AML samples, whereas CellPhoneDB, MultiNicheNet and CellChat captured many interactions that were present even in just one sample. When we removed these under-represented interactions, CellPhoneDB, MultiNicheNet and CellChat showed only 2,941, 9,429 and 3,700 differential interactions, respectively (Figure S13D).

(C) Pipeline to compare the communication results of *community*, CellPhoneDB, MultiNicheNet, and CellChat.

(D) Real memory and elapsed time comparison.

(E) Differential interactions.

(F) Differential interactions identified by *community*, CellPhoneDB, MultiNicheNet, and CellChat show in how many healthy or AML samples an interaction was found (each dot is an interaction).

(G) Log2FC variations per component for differential interactions identified as significant by other tools and classified as “compensated” by *community*, following the removal of under-represented interactions.

(H) An example interaction identified as significant by other tools (after removal of under-represented interactions), despite displaying heterogeneous variability. Per-sample expression values are displayed as boxplots for ligand and receptor, demonstrating substantial intra-group variability within the AML cohort.

(I) *Community*-based classification of significant interactions detected by CellChat, CellPhoneDB, and MultiNicheNet. The stacked bars show the proportion of interactions labeled as heterogeneous, compensated, or differential after filtering out under-represented interactions.

(J) An example of an interaction (Mono:HMGB1 to T:CXCR4) exhibiting a stabilizing compensatory effect via cell type abundance. Left images show expression levels of HMGB1 and CXCR4 in monocytes and T cells, respectively. Right images show cell type abundance of monocytes and T cells in healthy and AML samples.

(K) Mono:HMGB1 to T:CXCR4 interaction when regarding the two monocytic AML cases as separate subtypes. Left images show expression levels of HMGB1 and CXCR4 in monocytes and T cells, respectively. Right images show cell type abundance of monocytes and T cells in healthy and AML, split by subtype. For j, k *p* values were calculated using Wilcoxon testing. Bonferroni correction was used for adjusted *p*-values.

Critically, passing sample-level QC does not imply differential change between cohorts. *Community* tests interaction intensity per sample using a composite score (weights, see sections [theoretical basis](#) and [STAR Methods](#)) and then compares these scores across cohorts. This design resolves two failure modes. First, compensated interactions: opposing shifts in cellular composition or active fraction do offset expression changes, producing small overall $\log_2FC$ values despite large changes in individual components ([Figures 5D, 5E, and 6G](#)). Second, heterogeneous calls, where the $|\log_2FC(w)| > 1$ in the aggregated datasets, but there is high across-sample variance which renders the differences non-significant (e.g., $p\text{-value} > 0.05$), indicating outlier-driven patterns. This trend is exemplified by the Erythrocytes expressing ADAM10 and T cells expressing CD44 interaction ([Figure 6H](#)), where expression levels differ between cohorts but vary widely within the AML group for the cells expressing the ligand ADAM10 in Erythrocytes. The large $\log_2FC$ arises from a small subset of AML samples showing elevated expression, a skew that drives other tools to identify this interaction as significant, while distributions between cohorts overlap strongly. By evaluating composite scores per sample, *community* remains robust to both compensation and outlier-driven artifacts, while other methods output them as significant interactions ([Figure 6I](#)).

Both phenomena can co-occur within the same interaction, where one cohort displays outlier-driven expansion of a sender gene while another shows compensatory loss of the corresponding receiver. This dual pattern is illustrated between the monocytes expressing HMGB1 and the T-cells expressing CXCR4. HMGB1, together with CXCL12, has been shown to facilitate CXCR4-mediated recruitment of immune cells.⁵¹ Because CellPhoneDB, CellChat, and MultiNicheNet rely on mean gene expression within a cell type at pseudobulk level and disregard abundance changes, they fail to capture the compensation between sender and receiver fractions. In this case, HMGB1 expression in monocytes remained stable, whereas CXCR4 expression in T-cells increased significantly in the AML cohort ([Figure 6J](#)). Expression-based tools therefore predicted this interaction as upregulated ([Table S3](#)). However, *community* analysis revealed that the apparent upregulation is confounded by heterogeneity in cell-type composition ([Figure 6J](#)). Two AML samples of monocytic origin (AML-0024 and AML-0160) ([Figure S7C](#)) showed markedly expanded monocyte fractions but a depletion of T-cells, leading to inflated sender signal but reduced receiver availability. Conversely, in non-monocytic AML cases, monocytes decreased while T-cells were partially increased, resulting in the opposite compensatory trend ([Figure 6K](#)). As a result, the overall interaction weight remained stable across the cohort. *Community* thus correctly identified this interaction as unchanged, capturing the balance between outlier-driven and compensatory effects that expression-based approaches overlook.

While *community* and CellChat assign the differential interactions to up- or downregulated, CellPhoneDB and MultiNicheNet do not provide information on the directionality. To further compare the consistency of the results, we assigned the interactions of CellPhoneDB and MultiNicheNet as up- or downregulated if either one or both genes within the ligand-receptor pair

were differentially up- or downregulated, respectively. In cases where both partners were differentially expressed, but in discordant directions, the interaction was assigned to the class “ambiguous”. MultiNicheNet also identified some interactions as differential where neither the ligand nor the receptor were among differentially expressed genes. These interactions were also assigned as “ambiguous”. All four algorithms showed concordance in the assignment of direction for all overlapping interactions ([Figure S13E](#)).

Finally, we tested the resource usage for the four tools using all three datasets: the UC vs. healthy from Smillie et al.,²⁵ and the two AML vs. healthy datasets from Lasry et al.,³⁴ and the integrated one from van Galen et al.⁴⁴ and Oetjen et al.⁴⁵ We subsetted each into differing numbers of samples ($n = 2$ to $n = 40$) and measured the elapsed time, as well as peak VRAM usage. *Community* performed as the fastest algorithm in all testing conditions, and it had the best scalability with the increase in the number of samples. *Community* was also the most memory-efficient tool for the biggest dataset ([Figure 6D](#); [Figures S14A–S14D](#)).

DISCUSSION

Community is a fast and robust R package for differential communication analysis in large scRNAseq case-control studies. *Community* explores information on three different aspects of the dataset: the shrinkage or expansion of individual cell types (cell type abundance), the alteration in their active fraction, and the gene expression level. The overall change in communication for a particular pair of cell types using a particular ligand-receptor pair depends on the interplay of these components. Due to this feature, *community* can easily track back which components influenced the communication shift most, and by this gain a better understanding of the underlying biological processes.

Due to this detailed component analysis, *community* is able to distinguish between truly unchanged interactions, where none of the components changed, and compensated interactions, where components change in opposite directions compensating each other and keeping the overall communication intensity stable. These communication-stabilizing effects may play a particularly important role in the early stages of diseases where tissue homeostasis can be restored and preserved by such mechanisms.¹

The ability to detect compensated interactions is a completely novel feature that *community* offers. One of the key information that *community* uses in this case is the cell type abundance. Most conventional techniques omit this information, focusing only on the expression level of ligands and receptors within cell types. This causes a misleading assignment of differential interactions in cases of compensated communication. For example, if a sending cell type keeps the ligand expression at a stable level but becomes more abundant, it will result in an increased overall availability of a ligand in the tissue. The receiver cell type may sense this due to the overstimulation of the receptor-associated downstream pathway and activate a feedback mechanism to decrease the level of the receptor and bring the pathway activity back to the physiological level. The interaction between the

sender and the receiver will thus be compensated. In this case, it is crucial to regard the information about the sender cell type abundance increase together with the decrease in receptor expression by the receiver, because otherwise the interaction will be identified as downregulated, instead of compensated. This information may be crucial in studies focused on identifying novel disease targets, since disrupting a mechanism that is compensatory may lead to worsening of the disease.

Community is able to detect changes in the active fractions of cell types. This property is especially advantageous in cases where the fraction of cells within a cell type expressing a ligand or receptor is very small. The tools that rely on the differential expression analysis of the ligands and receptors may miss these interactions. This is because the DEG analysis pipeline may not detect these genes, as they are dominated by a large pool of non-expressing cells. For example, in UC, an interaction between the inflammatory monocytes expressing *OSM* and inflammatory fibroblasts expressing *OSMR* has been described.^{52,53} Being specific to a very small subtype of the monocytes and fibroblasts, these genes do not appear among the DEG and thus would be missed by DEG-based techniques. Yet the *community* was able to correctly identify this interaction as significantly upregulated.

The information on changes in expression levels within the active fraction of cells serves as an indication of potential shifts in the pathways upstream of the expressed ligands or receptors. This may be due to altered chromatin accessibility, transcription factor activity, or post-transcriptional modifications. *Community* makes it easy to screen for these interactions, providing a list of candidates for further analyses with orthogonal techniques.

Community provides a large database of curated and predicted ligand-receptor interactions and adhesion molecules. In contrast to other tools, *community* keeps the information about complex molecules in the database yet splits them for the calculation of the individual interaction. The tools that keep the composition of a complex fixed most often calculate the average expression over the complex components. If an interaction decreased or increased in disease, with this strategy it requires additional analysis to track back whether this change affected all components of a complex or just some of them. In contrast, with *community* keeping track of each component of the complex separately, it is easy to reconstruct the driving changes, as we show with the example of the *Myeloid:OSM_Fibroblasts:OSMR-IL6ST* interaction in the Smillie et al. dataset. Another phenomenon is that the composition of the complex may alter in disease compared to healthy^{54,55} or among different cell types.^{55,56} In this case, if by chance the average expression value of this complex stays similar, this compositional change will be missed by conventional methods, but captured by *community*.

Another strength of *community* is its robustness against outlier samples. Especially when dealing with human data, heterogeneity, and individual-specific signatures are frequently encountered issues, both due to sample preparation and interindividual differences. The conventional pipelines for DEG analysis in scRNAseq with several samples in cases and controls, such as Seurat⁴⁹ or scanpy,⁵⁷ do not distinguish between cells coming from different samples and are thus sensitive to outlier samples.

As a consequence, the communication analysis techniques discover putatively differential communication signatures that are driven by these outlier samples. In contrast, *community* does not merge all the cells from a cohort into one pool, but rather reconstructs communication in each sample separately. This makes it easy to discard outlier sample-driven interactions during the QC and differential communication analysis step, to robustly identify communication changes that are generally driven by the disease.

We compared *community* performance against the most popular tools in the field, such as CellPhoneDB,⁶ MultiNicheNet,^{9,47} scSeqCommDiff,²⁰ and CellChat.¹⁹ *Community* brings unique features by adding cell type abundance as one of the factors influencing cell-cell communication in the tissue, enabling the distinction between truly unchanged and compensated interactions, and is highly robust against outlier samples. It has the largest curated database of ligands, receptors, and adhesion molecules, which can be easily amended by the user. *Community* showed superiority in speed, scalability, and memory efficiency for large datasets. Other emerging tools with a cohort-based approach include DIALOGUE,⁵⁸ Tensor-cell2cell,⁵⁹ scITD,⁶⁰ and MOFAcell.⁶¹ DIALOGUE uses penalized matrix decomposition to infer multicellular programs, which corresponds to higher-order coordinated expression programs in different cell types. Tensor-cell2cell, scITD, and MOFAcell rely on factor analysis of co-varying expression patterns, reducing cell-cell communication into sets of factors or loadings, which can be difficult to interpret per ligand-receptor interaction. In contrast to *community*, these methods do not provide an option to distinguish truly unchanged interactions from the compensated ones. The component-based approach of *community* overcomes these hurdles, ensuring robust and interpretable differential communication analysis.

Collectively, *community* is a user-friendly and scalable R tool and an interactive web server to run differential communication analysis in scRNAseq-based case-control type of analyses. *Community* produces robust cell-cell interaction analysis, providing information on potential biological processes underlying the changes in communication in disease, such as expansion or shrinkage of certain cell types, the active fraction producing the ligand or the receptor, and their expression levels within the active cells. *Community* is fast, memory efficient and scalable, and provides intuitive visualization. In summary, *community* is a tool for analyzing large datasets and better understanding cell-cell communication processes in health and disease.

Limitations of the study

Community requires pre-processed scRNAseq data with cell types annotated, and cell types must be present in both case and control cohorts. In cancer datasets, malignant cells must be mapped to the corresponding healthy cell type categories. The results therefore depend on the quality of pre-processing, cell type annotation, and the level of cell type granularity chosen by the user. The analysis also depends on multiple thresholds such as cell type size, active cells, expression, quality control, log2FC, and FDR, and, as noted in the manuscript, small cohorts may require *t* tests that can produce false positive results needing downstream validation.

community infers communication from cell-type abundance, active fraction of signaling cells, and expression levels within those cells; thus, candidate interactions and inferred driving components should be interpreted as computational signals for follow-up analysis or orthogonal validation. Finally, this study was not powered to assess sex-based differences in the results because it is based on secondary analyses of heterogeneous publicly available datasets. Consequently, potential sex-specific effects on cell-cell communication were not evaluated and remain a limitation of the present study.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Maria Colomé-Tatché (maria.colome@bmc.med.lmu.de).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Data: Processed healthy and AML bone marrow datasets have been deposited at Zenodo and are publicly available under accession numbers Zenodo: 7962808 and Zenodo: 10013368. The processed ulcerative colitis and healthy colon dataset is publicly available at Zenodo under accession number Zenodo: 10013294. The publicly available datasets analyzed in this study are accessible through the following accession numbers: SCP259 for the ulcerative colitis and healthy colon dataset, GEO: GSE120575 for the melanoma immune checkpoint inhibitor dataset, GEO: GSE185381 and GEO: GSE116256 for the healthy and AML bone marrow datasets, and GEO: GSE120221 for the healthy bone marrow dataset. These accession numbers are also listed in the [key resources table](#).
- Code: The *community* R package and accompanying tutorials are publicly available on GitHub at [SoloveyMaria/community](https://github.com/SoloveyMaria/community). The data-preprocessing pipelines and scripts used to generate the figures are available at colomemaria.com-paper. The source code for the interactive web-based application is available at [macelik/community_rshiny](https://macelik.com/community_rshiny). Links to these repositories are provided in the [key resources table](#).
- Any additional information required to reproduce or reanalyze the results reported in this paper is available from the [lead contact](#) upon request.

ACKNOWLEDGMENTS

We acknowledge the Core Facility Statistical Consulting of Helmholtz Zentrum München, and in particular Dr. Marina Jimenez Munoz, Ronan Le Gleut, Raphael Meixner, and Prof. Dr. Christiane Fuchs, for the consulting on the statistical testing.

The present contribution is supported by the Helmholtz Association under the joint research school "HIDSS-006 - Munich School for Data Science@Helmholtz, TUM&LMU. This work was supported by the Impuls-und Vernetzungsfonds of the Helmholtz-Gemeinschaft (grant VH-NG-1219) for M.C.T. and M.S. M.C.T. acknowledges funding from the ATR2024-154840 grant financed by MICIU/AEI/10.13039/501100011033. F.Z. acknowledges funding from the Förderprogramm für Forschung und Lehre (grant FöFoLe-1048) of the LMU University as well as from the German Cancer Consortium (DKTK) and the Bavarian Cancer Research Center (BZKF). F.R.S. receives funding from the Förderprogramm für Forschung und Lehre (FöFoLe) of LMU Munich.

AUTHOR CONTRIBUTIONS

M.S., M.C.T., F.Z., and A.S. designed the study. M.S., M.A.C., F.R.S., M.I., and M.A.-F. performed the package development and data analysis. C.G. and

M.H. developed the web interface. M.S., M.C.T., F.Z., M.A.C., and F.R.S. interpreted the results. M.S., M.C.T., M.A.C., and F.Z. wrote the manuscript with input from all authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- [KEY RESOURCES TABLE](#)
- [EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS](#)
 - Human single-cell RNA sequencing datasets
- [METHOD DETAILS](#)
 - *Community* database
 - Interaction weight formula
 - Recommendations on study design and data preprocessing
 - *Community* pipeline
 - Interactive platform
 - Preprocessing of healthy - ulcerative colitis dataset
 - Run *community* on healthy - ulcerative colitis dataset
 - Processing of melanoma dataset
 - Run *community* on melanoma dataset
 - Preprocessing of healthy and AML bone marrow dataset
 - Run *community* on healthy and AML bone marrow dataset
 - Tumor-immune interaction analysis on healthy and AML bone marrow dataset
 - Preprocessing of integrated datasets from two human AML-healthy bone marrow studies
 - Run *community* on two integrated healthy and AML bone marrow datasets
 - Method comparison for cell-cell interaction tools
- [QUANTIFICATION AND STATISTICAL ANALYSIS](#)

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2026.117314>.

Received: May 6, 2026

Revised: July 4, 2026

Accepted: August 7, 2026

Published: August 26, 2026

REFERENCES

1. Armingol, E., Officer, A., Harismendy, O., and Lewis, N.E. (2021). Deciphering cell-cell interactions and communication from gene expression. *Nat. Rev. Genet.* 22, 71–88. <https://doi.org/10.1038/s41576-020-00292-x>.
2. Bechtel, T.J., Reyes-Robles, T., Fadeyi, O.O., and Oslund, R.C. (2021). Strategies for monitoring cell-cell interactions. *Nat. Chem. Biol.* 17, 641–652. <https://doi.org/10.1038/s41589-021-00790-x>.
3. Tanay, A., and Regev, A. (2017). Scaling single-cell genomics from phenomenology to mechanism. *Nature* 541, 331–338. <https://doi.org/10.1038/nature21350>.
4. Valls, P.O., and Esposito, A. (2022). Signalling dynamics, cell decisions, and homeostatic control in health and disease. *Curr. Opin. Cell Biol.* 75, 102066. <https://doi.org/10.1016/j.ceb.2022.01.011>.
5. Solovey, M., and Scialdone, A. (2020). COMUNET: a tool to explore and visualize intercellular communication. *Bioinformatics* 36, 4296–4300. <https://doi.org/10.1093/bioinformatics/btaa482>.
6. Efremova, M., Vento-Tormo, M., Teichmann, S.A., and Vento-Tormo, R. (2020). CellPhoneDB: inferring cell-cell communication from combined

- p>expression of multi-subunit ligand-receptor complexes.
- Nat. Protoc.*
- 15, 1484–1506.
- <https://doi.org/10.1038/s41596-020-0292-x>
- .
7. Raredon, M.S.B., Yang, J., Garritano, J., Wang, M., Kushnir, D., Schupp, J.C., Adams, T.S., Greaney, A.M., Leiby, K.L., Kaminski, N., et al. (2022). Computation and visualization of cell-cell signaling topologies in single-cell systems data using Connectome. *Sci. Rep.* 12, 4187. <https://doi.org/10.1038/s41598-022-07959-x>.
8. Wilk, A.J., Shalek, A.K., Holmes, S., and Blish, C.A. (2024). Comparative analysis of cell-cell communication at single-cell resolution. *Nat. Biotechnol.* 42, 470–483. <https://doi.org/10.1038/s41587-023-01782-z>.
9. Browaeys, R., Gillis, J., Sang-Aram, C., De Bleser, P., Hoste, L., Tavernier, S., Lambrechts, D., Seurinck, R., and Saeys, Y. (2023). MultiNicheNet: a flexible framework for differential cell-cell communication analysis from multi-sample multi-condition single-cell transcriptomics data. Preprint at bioRxiv. <https://doi.org/10.1101/2023.06.13.544751>.
10. Guzik, T.J., Skiba, D.S., Touyz, R.M., and Harrison, D.G. (2017). The role of infiltrating immune cells in dysfunctional adipose tissue. *Cardiovasc. Res.* 113, 1009–1023. <https://doi.org/10.1093/cvr/cvx108>.
11. Allam, M., Hu, T., Lee, J., Aldrich, J., Badve, S.S., Gökmen-Polar, Y., Bhawe, M., Ramalingam, S.S., Schneider, F., and Coskun, A.F. (2022). Spatially variant immune infiltration scoring in human cancer tissues. *npj Precis. Oncol.* 6, 60. <https://doi.org/10.1038/s41698-022-00305-4>.
12. Liu, L., and Rando, T.A. (2011). Manifestations and mechanisms of stem cell aging. *J. Cell Biol.* 193, 257–266. <https://doi.org/10.1083/jcb.201010131>.
13. López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., and Kroemer, G. (2023). Hallmarks of aging: An expanding universe. *Cell* 186, 243–278. <https://doi.org/10.1016/j.cell.2022.11.001>.
14. Guo, J., Huang, X., Dou, L., Yan, M., Shen, T., Tang, W., and Li, J. (2022). Aging and aging-related diseases: from molecular mechanisms to interventions and treatments. *Signal Transduct. Target. Ther.* 7, 391. <https://doi.org/10.1038/s41392-022-01251-0>.
15. Walesky, C.M., Kolb, K.E., Winston, C.L., Henderson, J., Kruff, B., Fleming, I., Ko, S., Monga, S.P., Mueller, F., Apte, U., et al. (2020). Functional compensation precedes recovery of tissue mass following acute liver injury. *Nat. Commun.* 11, 5785. <https://doi.org/10.1038/s41467-020-19558-3>.
16. Miller, M.A., and Zachary, J.F. (2017). Mechanisms and morphology of cellular injury, adaptation, and death. In *Pathologic Basis of Veterinary Disease*, J.F. Zachary, ed. (Elsevier), pp. 2–43.e19.
17. Barkaway, A., Rolas, L., Joulia, R., Bodkin, J., Lenn, T., Owen-Woods, C., Reglero-Real, N., Stein, M., Vázquez-Martínez, L., Gírbil, T., et al. (2021). Age-related changes in the local milieu of inflamed tissues cause aberrant neutrophil trafficking and subsequent remote organ damage. *Immunity* 54, 1494–1510.e7. <https://doi.org/10.1016/j.immuni.2021.04.025>.
18. Zimmerman, M.A., Huang, Q., Li, F., Liu, X., and Li, C.-Y. (2013). Cell death-stimulated cell proliferation: a tissue regeneration mechanism usurped by tumors during radiotherapy. *Semin. Radiat. Oncol.* 23, 288–295. <https://doi.org/10.1016/j.semradonc.2013.05.003>.
19. Jin, S., Guerrero-Juarez, C.F., Zhang, L., Chang, I., Ramos, R., Kuan, C.H., Myung, P., Pilius, M.V., and Nie, Q. (2021). Inference and analysis of cell-cell communication using CellChat. *Nat. Commun.* 12, 1088. <https://doi.org/10.1038/s41467-021-21246-9>.
20. Cesaro, G., Baruzzo, G., Tussardi, G., and Di Camillo, B. (2025). Differential cellular communication inference framework for large-scale single-cell RNA-sequencing data. *NAR Genom. Bioinform.* 7, lqaf084. <https://doi.org/10.1093/nargab/lqaf084>.
21. Túrei, D., Korcsmáros, T., and Saez-Rodriguez, J. (2016). OmniPath: guidelines and gateway for literature-curated signaling pathway resources. *Nat. Methods* 13, 966–967. <https://doi.org/10.1038/nmeth.4077>.
22. Student [Gosset WS]. (1908). The Probable Error of a Mean. *Biometrika* 6, 1–25. <https://doi.org/10.2307/2331554>.
23. Wilcoxon, F. (1945). Individual Comparisons by Ranking Methods. *Biom. Bull.* 1, 80–83. <https://doi.org/10.2307/3001968>.
24. Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. Royal Stat. Soc. Series B Stat. Methodol.* 57, 289–300. <https://doi.org/10.1111/j.2517-6161.1995.tb02031.x>.
25. Smillie, C.S., Biton, M., Ordovas-Montanes, J., Sullivan, K.M., Burgin, G., Graham, D.B., Herbst, R.H., Rogel, N., Slyper, M., Waldman, J., et al. (2019). Intra- and Inter-cellular Rewiring of the Human Colon during Ulcerative Colitis. *Cell* 178, 714–730.e22. <https://doi.org/10.1016/j.cell.2019.06.029>.
26. Alkim, C., Alkim, H., Koksai, A.R., Boga, S., and Sen, I. (2015). Angiogenesis in Inflammatory Bowel Disease. *Int. J. Inflam.* 2015, 970890. <https://doi.org/10.1155/2015/970890>.
27. Blander, J.M. (2016). Death in the intestinal epithelium-basic biology and implications for inflammatory bowel disease. *FEBS J.* 283, 2720–2730. <https://doi.org/10.1111/febs.13771>.
28. Tanaka, T., Oyama, T., Sugie, S., and Shimizu, M. (2016). Different Susceptibilities between Apoe- and Ldlr-Deficient Mice to Inflammation-Associated Colorectal Carcinogenesis. *Int. J. Mol. Sci.* 17, 1806. <https://doi.org/10.3390/ijms17111806>.
29. Franić, I., Rezić-Mužinić, N., Markotić, A., Živković, P.M., Vilović, M., Rušić, D., and Božić, J. (2022). Expression of CD44 in Leukocyte Subpopulations in Patients with Inflammatory Bowel Diseases. *Diagnostics* 12, 2014. <https://doi.org/10.3390/diagnostics12082014>.
30. Dotan, I., Allez, M., Danese, S., Keir, M., Tole, S., and McBride, J. (2020). The role of integrins in the pathogenesis of inflammatory bowel disease: Approved and investigational anti-integrin therapies. *Med. Res. Rev.* 40, 245–262. <https://doi.org/10.1002/med.21601>.
31. Hjortebjerg, R., Thomsen, K.L., Agnholt, J., and Frystyk, J. (2019). The IGF system in patients with inflammatory bowel disease treated with prednisolone or infliximab: potential role of the stanniocalcin-2/PAPP-A/IGFBP-4 axis. *BMC Gastroenterol.* 19, 83. <https://doi.org/10.1186/s12876-019-1000-6>.
32. Richards, C.D. (2013). The enigmatic cytokine oncostatin m and roles in disease. *ISRN Inflamm.* 2013, 512103. <https://doi.org/10.1155/2013/512103>.
33. Sade-Feldman, M., Yizhak, K., Bjorgaard, S.L., Ray, J.P., De Boer, C.G., Jenkins, R.W., Lieb, D.J., Chen, J.H., Frederick, D.T., Barzily-Rokni, M., et al. (2018). Defining T cell states associated with response to checkpoint immunotherapy in melanoma. *Cell* 175, 998–1013.e20.
34. Lasry, A., Nadorp, B., Fornerod, M., Nicolet, D., Wu, H., Walker, C.J., Sun, Z., Witkowski, M.T., Tikhonova, A.N., Guillamot-Ruano, M., et al. (2023). An inflammatory state remodels the immune microenvironment and improves risk stratification in acute myeloid leukemia. *Nat. Cancer* 4, 27–42. <https://doi.org/10.1038/s43018-022-00480-0>.
35. Stubbins, R.J., and Karsan, A. (2021). Differentiation therapy for myeloid malignancies: beyond cytotoxicity. *Blood Cancer J.* 11, 193. <https://doi.org/10.1038/s41408-021-00584-3>.
36. Tettamanti, S., Pievani, A., Biondi, A., Dotti, G., and Serafini, M. (2022). Catch me if you can: how AML and its niche escape immunotherapy. *Leukemia* 36, 13–22. <https://doi.org/10.1038/s41375-021-01350-x>.
37. Hanahan, D. (2022). Hallmarks of Cancer: New Dimensions. *Cancer Discov.* 12, 31–46. <https://doi.org/10.1158/2159-8290.CD-21-1059>.
38. Skeate, J.G., Otsmaa, M.E., Prins, R., Fernandez, D.J., Da Silva, D.M., and Kast, W.M. (2020). TNFSF14: LIGHTing the Way for Effective Cancer Immunotherapy. *Front. Immunol.* 11, 922. <https://doi.org/10.3389/fimmu.2020.00922>.
39. Berrocal-Rubio, M.Á., Pawer, Y.D.J., Dinevska, M., De Paoli-Iseppi, R., Widodo, S.S., Gleeson, J., Rajab, N., De Nardo, W., Hallab, J., Li, A., et al. (2024). Discovery of NRG1-VII: a novel myeloid-derived class of NRG1 isoforms. Preprint at bioRxiv. <https://doi.org/10.1101/2023.02.02.525781>.

40. Varchetta, S., Brunetta, E., Roberto, A., Mikulak, J., Hudspeth, K.L., Mondelli, M.U., and Mavilio, D. (2012). Engagement of Siglec-7 receptor induces a pro-inflammatory response selectively in monocytes. *PLoS One* 7, e45821. <https://doi.org/10.1371/journal.pone.0045821>.
41. Huntoon, K.M., Wang, Y., Eppolito, C.A., Barbour, K.W., Berger, F.G., Shrikant, P.A., and Baumann, H. (2008). The acute phase protein haptoglobin regulates host immunity. *J. Leukoc. Biol.* 84, 170–181. <https://doi.org/10.1189/jlb.0208100>.
42. Godoy-Tena, G., Barmada, A., Morante-Palacios, O., de la Calle-Fabregat, C., Martins-Ferreira, R., Ferreté-Bonastre, A.G., Ciudad, L., Ruiz-Sanmartín, A., Martínez-Gallo, M., Ferrer, R., et al. (2022). Epigenetic and transcriptomic reprogramming in monocytes of severe COVID-19 patients reflects alterations in myeloid differentiation and the influence of inflammatory cytokines. *Genome Med.* 14, 134. <https://doi.org/10.1186/s13073-022-01137-4>.
43. Lerner, A., and Epstein, P.M. (2006). Cyclic nucleotide phosphodiesterases as targets for treatment of haematological malignancies. *Biochem. J.* 393, 21–41. <https://doi.org/10.1042/BJ20051368>.
44. van Galen, P., Hovestadt, V., Wadsworth II, M.H., Hughes, T.K., Griffin, G.K., Battaglia, S., Verga, J.A., Stephansky, J., Pastika, T.J., Lombardi Story, J., et al. (2019). Single-Cell RNA-Seq Reveals AML Hierarchies Relevant to Disease Progression and Immunity. *Cell* 176, 1265–1281.e24. <https://doi.org/10.1016/j.cell.2019.01.031>.
45. Oetjen, K.A., Lindblad, K.E., Goswami, M., Gui, G., Dagur, P.K., Lai, C., Dillon, L.W., McCoy, J.P., and Hourigan, C.S. (2018). Human bone marrow assessment by single-cell RNA sequencing, mass cytometry, and flow cytometry. *JCI Insight* 3, e124928. <https://doi.org/10.1172/jci.insight.124928>.
46. Garcia-Alonso, L., Handfield, L.F., Roberts, K., Nikolakopoulou, K., Fernando, R.C., Gardner, L., Woodhams, B., Arutyunyan, A., Polanski, K., Hoo, R., et al. (2021). Mapping the Temporal and Spatial Dynamics of the Human Endometrium in Vivo and in Vitro. *Nat. Genet.* 53, 1698–1711. <https://doi.org/10.1038/s41588-021-00972-2>.
47. Browaeys, R., Saelens, W., and Saeys, Y. (2020). NicheNet: modeling intercellular communication by linking ligands to target genes. *Nat. Methods* 17, 159–162. <https://doi.org/10.1038/s41592-019-0667-5>.
48. Jin, S., Plikus, M.V., and Nie, Q. (2024). CellChat for systematic analysis of cell-cell communication from single-cell transcriptomics. *Nat. Protoc.* 20, 180–219.
49. Hao, Y., Hao, S., Andersen-Nissen, E., Mauck, W.M., 3rd, Zheng, S., Butler, A., Lee, M.J., Wilk, A.J., Darby, C., Zager, M., et al. (2021). Integrated analysis of multimodal single-cell data. *Cell* 184, 3573–3587.e29. <https://doi.org/10.1016/j.cell.2021.04.048>.
50. Crowell, H.L., Soneson, C., Germain, P.L., Calini, D., Collin, L., Raposo, C., Malhotra, D., and Robinson, M.D. (2020). Muscat detects subpopulation-specific state transitions from multi-sample multi-condition single-cell transcriptomics data. *Nat. Commun.* 11, 6077. <https://doi.org/10.1038/s41467-020-19894-4>.
51. Haque, N., Fareez, I.M., Fong, L.F., Mandal, C., Abu Kasim, N.H., Kacharaju, K.R., and Soesilawati, P. (2020). Role of the CXCR4-SDF1-HMGB1 pathway in the directional migration of cells and regeneration of affected organs. *World J. Stem Cells* 12, 938–951. <https://doi.org/10.4252/wjsc.v12.i9.938>.
52. Liu, J.Z., van Sommeren, S., Huang, H., Ng, S.C., Alberts, R., Takahashi, A., Ripke, S., Lee, J.C., Jostins, L., Shah, T., et al. (2015). Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. *Nat. Genet.* 47, 979–986. <https://doi.org/10.1038/ng.3359>.
53. West, N.R., Hegazy, A.N., Owens, B.M.J., Bullers, S.J., Linggi, B., Buonocore, S., Coccia, M., Görtz, D., This, S., Stockenhuber, K., et al. (2017). Oncostatin M drives intestinal inflammation and predicts response to tumor necrosis factor-neutralizing therapy in patients with inflammatory bowel disease. *Nat. Med.* 23, 579–589. <https://doi.org/10.1038/nm.4307>.
54. Egbenya, D.L., Hussain, S., Lai, Y.C., Xia, J., Anderson, A.E., and Davanger, S. (2018). Changes in synaptic AMPA receptor concentration and composition in chronic temporal lobe epilepsy. *Mol. Cell. Neurosci.* 92, 93–103. <https://doi.org/10.1016/j.mcn.2018.07.004>.
55. Belfiore, A., Malaguarnera, R., Vella, V., Lawrence, M.C., Sciacca, L., Frasca, F., Morrione, A., and Vigneri, R. (2017). Insulin Receptor Isoforms in Physiology and Disease: An Updated View. *Endocr. Rev.* 38, 379–431. <https://doi.org/10.1210/er.2017-00073>.
56. Moruzzi, N., Lazzeri-Barcelo, F., Valladolid-Acebes, I., Moede, T., Paschen, M., Leibiger, B., Berggren, P.O., and Leibiger, I.B. (2021). Tissue-specific expression of insulin receptor isoforms in obesity/type 2 diabetes mouse models. *J. Cell Mol. Med.* 25, 4800–4813. <https://doi.org/10.1111/jcmm.16452>.
57. Wolf, F.A., Angerer, P., and Theis, F.J. (2018). SCANPY: large-scale single-cell gene expression data analysis. *Genome Biol.* 19, 15. <https://doi.org/10.1186/s13059-017-1382-0>.
58. Jerby-Arnon, L., and Regev, A. (2022). DIALOGUE maps multicellular programs in tissue from single-cell or spatial transcriptomics data. *Nat. Biotechnol.* 40, 1467–1477. <https://doi.org/10.1038/s41587-022-01288-0>.
59. Armingol, E., Baghdassarian, H.M., Martino, C., Perez-Lopez, A., Aamodt, C., Knight, R., and Lewis, N.E. (2022). Context-aware deconvolution of cell-cell communication with Tensor-cell2cell. *Nat. Commun.* 13, 3665. <https://doi.org/10.1038/s41467-022-31369-2>.
60. Mitchel, J., Gordon, M.G., Perez, R.K., Biederstedt, E., Bueno, R., Ye, C.J., and Kharchenko, P.V. (2024). Coordinated, multicellular patterns of transcriptional variation that stratify patient cohorts are revealed by tensor decomposition. *Nat. Biotechnol.* 43, 1192–1201.
61. Ramirez Flores, R.O., Lanzer, J.D., Dimitrov, D., Velten, B., and Saez-Rodriguez, J. (2023). Multicellular factor analysis of single-cell data for a tissue-centric understanding of disease. *eLife* 12, e93161. <https://doi.org/10.7554/eLife.93161>.
62. Mark, A., Thompson, R., Afrasiabi, C., and Wu, C. (2017). Mygene (Bioconductor). <https://doi.org/10.18129/B9.BIOC.MYGENE>.
63. Lun, A.T.L., McCarthy, D.J., and Marioni, J.C. (2016). A step-by-step workflow for low-level analysis of single-cell RNA-seq data with Bioconductor. *F1000Res.* 5, 2122. <https://doi.org/10.12688/f1000research.9501.2>.
64. Lotfollahi, M., Wolf, F.A., and Theis, F.J. (2019). scGen predicts single-cell perturbation responses. *Nat. Methods* 16, 715–721. <https://doi.org/10.1038/s41592-019-0494-8>.
65. Bischl, B., Richter, J., Bossek, J., Horn, D., Thomas, J., and Lang, M. (2017). mlrMBO: A modular framework for model-based optimization of expensive black-box functions. Preprint at arXiv, arXiv:1703.03373. <https://doi.org/10.48550/arXiv.1703.03373>.
66. Michielsen, L., Reinders, M.J.T., and Mahfouz, A. (2021). Hierarchical progressive learning of cell identities in single-cell data. *Nat. Commun.* 12, 2799. <https://doi.org/10.1038/s41467-021-23196-8>.
67. Garcia-Alonso, L., Iorio, F., Matchan, A., Fonseca, N., Jaaks, P., Peat, G., Pignatelli, M., Falcone, F., Benes, C.H., Dunham, I., et al. (2018). Transcription Factor Activities Enhance Markers of Drug Sensitivity in Cancer. *Cancer Res.* 78, 769–780. <https://doi.org/10.1158/0008-5472.CAN-17-1679>.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Processed healthy and AML bone marrow dataset	This paper	Zenodo: 7962808
Processed integrated healthy and AML bone marrow dataset	This paper	Zenodo: 10013368
Processed ulcerative colitis and healthy colon dataset	This paper	Zenodo: 10013294
Ulcerative colitis and healthy colon scRNAseq dataset	Smillie et al. ²⁵ ; Broad DUOS controlled-access repository	SCP259
Melanoma immune checkpoint inhibitor scRNAseq dataset	Sade-Feldman et al. ³³ ; ImmuCAN scDB	https://immucanscdb.vital-it.ch/api/scrnaseq/MEL_IMM_SS2_GSE120575/h5ad
Healthy and AML bone marrow scRNAseq dataset	Lasry et al. ³⁴	GSE185381
Healthy and AML bone marrow scRNAseq dataset	van Galen et al. ⁴⁴	GSE116256
Healthy bone marrow scRNAseq dataset	Oetjen et al. ⁴⁵	GSE120221
Software and algorithms		
community package and tutorials	This paper	https://github.com/SoloveyMaria/community
Data preprocessing pipelines and all the figures	This paper	https://github.com/colomemaria/community-paper
Interactive web-based application	This paper	https://github.com/macelik/community_rshiny
R (v4.3.3)	R Foundation	https://www.r-project.org/
Shiny (v1.8.0)	Posit/RStudio	https://shiny.posit.co/
MyGene (v1.38.0)	Mark et al. ⁶²	https://bioconductor.org/packages/mygene/
scran (v1.26.2; v1.30.2)	Lun et al. ⁶³	https://bioconductor.org/packages/scran/
scGen (v2.1.0)	Lotfollahi et al. ⁶⁴	https://github.com/theislab/scgen
scanpy (v1.11.0)	Wolf et al. ⁵⁷	https://scanpy.readthedocs.io/
CellPhoneDB (v3.1.0)	Efremova et al. ⁶	https://github.com/ventolab/CellphoneDB
MultiNicheNet (v2.1.0)	Browaeys et al. ^{9,47}	https://github.com/saeyslab/multinichenetr
NicheNet (v2.2.1)	Browaeys et al. ^{9,47}	https://github.com/saeyslab/nichenetr
CellChat (v2.2)	Jin et al. ^{19,48}	https://github.com/jinworks/CellChat
scSeqCommDiff (v2.0)	Cesaro et al. ²⁰	https://gitlab.com/sysbiobig/scseqcomm
mlrMBO (v1.1.5.1)	Bischi et al. ⁶⁵	https://cran.r-project.org/package=mlrMBO
Seurat (v4.3.0)	Hao et al. ⁴⁹	https://satijalab.org/seurat/
OmnipathR (v.3.10)	Türei et al. ²¹	https://bioconductor.org/packages/OmnipathR
Seurat (v4.3)	Hao et al. ⁴⁹	https://satijalab.org/seurat/
psrecord (v1.3)	psrecord	https://github.com/astrofrog/psrecord

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Human single-cell RNA sequencing datasets

This study is a computational methods study based exclusively on previously published human single-cell RNA sequencing (scRNA-seq) datasets. No new human participants were recruited, no biological specimens were collected, and no experimental procedures were performed as part of this work. The analyzed datasets comprise publicly available cohorts of patients with ulcerative colitis, melanoma undergoing immune checkpoint inhibitor therapy, and acute myeloid leukemia (AML), together with their respective healthy control cohorts, as described in the original publications and listed in the [key resources table](#). Sample sizes included 17 ulcerative colitis and 11 healthy control samples, 47 melanoma samples (19 pre-treatment and 28 post-treatment), and two independent AML cohorts comprising 7 AML and 6 healthy bone marrow samples, and 9 AML and 26 healthy bone marrow samples, respectively.

Sex, age, and other participant metadata were obtained from the original studies whenever available. This study was not powered to assess sex-based differences in the results, and this has been noted as a limitation.

METHOD DETAILS

Community database

To construct *community* database, two datasets from OmniPath²¹ were used: “*ligrec extra*”, containing ligand-receptor interactions without literature reference (total of 8350 interactions as of February 2024), “*curated ligand-receptor interactions*” (total of 5141 interactions as of February 2023). Both binary and complex interactions within the “*ligrec extra*” and “*curated ligand-receptor interactions*” datasets were considered. Complex interactions were broken down into binary interaction as follows. First, a complex interaction is broken down into all possible binary pairwise combinations. Next, these pairs were checked against the protein-protein interaction (PPI) network of OmniPath to ensure that the identified binary interactions are supported by existing PPI data. A gene was categorized as a true ligand or receptor, if it was classified as such by at least two resources. Genes that did not fall in the ligand or a receptor category were classified as adhesion molecules. The default order of genes in a ligand-receptor pair was set such that the ligand is first and the receptor is the second. If a ligand-receptor interaction was found in a swapped position, we correct the order accordingly. For adhesion molecule interactions, the consensus directionality among the resources was checked in “*ligrec extra*” and “*curated ligand-receptor interactions*”. In case of consensus agreement, the consensus directionality was retained (“*consensus_direction*” value of 1), otherwise, the directionality followed lexicographical order (“*consensus_direction*” value of 0). Additionally, functional annotation for each gene was also added to the dataframe using the MyGene library (v1.38.0),⁶² providing users with additional information. The final database included 6941 pairs.

Interaction weight formula

A weight of an interaction is calculated for a particular sample and a particular ligand-receptor (or adhesion molecule) pair between two cell types. The weight ω of an interaction for a sample i between the sending cell type S expressing a ligand l of a pair k and a receiving cell type R expressing a receptor r from the pair k is calculated as:

$$\omega_{(S,R)_i}^{(l,r)_k} = (\rho_{S_i} \times \rho_{R_i}) \times (\phi_{S_i}^k \times \phi_{R_i}^k) \times (\pi_{S_i}^k \times \pi_{R_i}^k)$$

where ρ_{S_i} and ρ_{R_i} are the relative cell type abundance of the sending cell type (ρ_{S_i}) and the receiving cell type (ρ_{R_i}); $\phi_{S_i}^k$ and $\phi_{R_i}^k$ are relative active fraction of the ligand in the sending cell type and the receptor in the receiving cell type; $\pi_{S_i}^k$ and $\pi_{R_i}^k$ are relative mean expression of the ligand in the active fraction of the sending cell type and the receptor in the active fraction of the receiving cell type. See supplemental methods for details.

Recommendations on study design and data preprocessing

Recommendations on the minimal sample size and analysis of the robustness of the results upon downsampling of the number of case-control samples or expression values are highlighted in the Supplemental Methods 2.1. Analyses and the recommendations on the effect of cell type granularity in case of hierarchical cell type structure⁶⁶ are elucidated in Supplemental Methods 2.2.

Community pipeline

To calculate communication, three thresholds were set: `threshold_celltype_size`, `threshold_nr_active_cells`, and `threshold_expr`. The `threshold_celltype_size` is a threshold for the minimum number of cells in a cell type in one sample. If the number of cells in the cell type of interest in a particular sample is less or equal to the `threshold_celltype_size`, this cell type is considered missing in this sample. The `threshold_nr_active_cells` is a threshold for the minimum number of active cells in a cell type in one sample. An active cell is defined as a cell expressing a gene over the `threshold_expr` (see below). If the number of active cells for a specific gene in a cell type in one sample is smaller or equal to the `threshold_nr_active_cells`, then the active fraction of this cell type for this gene in this sample is considered to be zero. The `threshold_expr` is a threshold for an expression value of a gene in a single cell. If an expression value of a gene in a cell is greater than `threshold_expr`, this cell is considered active. Otherwise, the expression of this gene in this cell is set to zero and the cell is considered inactive for this gene.

For the quality check, the following filters are used: the cumulative interaction weight filter, the presence per cohort filter and the ligand/receptor expression filter. An interaction is considered of good quality, if it passes all three filters. The cumulative interaction weight filter checks the log10 cumulative weight over all samples computed for each interaction and removes the low weight interactions. To pass this filter, the interaction needs to be greater than the `threshold_log10_cum_weight` threshold. This threshold is set at the elbow of probability distribution density of the log10 cumulative weights. The presence per cohort filter checks the fraction of samples in which an interaction was detected (i.e., has a non-zero value) in the control cohort and, separately, in the case cohort and removes interactions found in too few samples. To pass this filter, an interaction needs to have a greater value than the `threshold_frac_samples_per_condition` threshold either in the control cohort or in the case cohort or in both. The ligand/receptor expression filter checks the mean expression level of the ligand and the receptor of an interaction in the case and the control samples (separately) and removes the interactions with lowest expressed ligands or receptors or both. This filter uses a `threshold_log10meanexpr_per_condition` threshold. For each interaction, the following four log10 mean expression values are checked: i) the ligand in sending cells in control samples, ii) the receptor in receiving cells in control samples, iii) the ligand in sending cells in

case samples, and iv) the receptor in receiving cells in case samples. An interaction passes this filter if both its ligand and receptor pass the threshold either in control samples or in case samples or in both.

The differential communication is calculated on good quality interactions between the case and the control cohort using either t test²² on log2-transformed weights or Wilcoxon test²³ on non-transformed weights. The default adjustment method for multiple testing is FDR. If the dataset contains few samples per cohort, we recommend to use the t test, even if the data do not follow the normal distribution. With a small sample size and a large number of features to test and correct for multiple testing, using the Wilcoxon test may be too conservative and produce the result consisting solely of true negative and false negative values. The t test option may in this case produce statistically significant results, which contain a high number of false positive results, those can though be sorted out in the subsequent wet-lab validation steps. The log2FC of an interaction is calculated as log2 of the ratio of its mean weight in the cases over its mean weight in the controls. To identify significantly differential interactions, the threshold_log2FC and the threshold_fdr should be specified by the user. Recommendations on how to best choose thresholds used in the *community* pipeline are included in the Supplemental Methods 3.

Interactive platform

An interactive web-based application is available at https://github.com/macelik/community_rshiny and was built using Rshiny software (v.1.8.0), an open-source R framework, and can be executed locally to explore data and results.

Preprocessing of healthy - ulcerative colitis dataset

The dataset consists of 48 biopsies taken from the colon of 12 healthy and 18 ulcerative colitis (UC) individuals,²⁵ several biopsies were taken per individual, yet to exclude co-dependencies in the data, only one biopsy was kept per individual (see below) (accession number SCP259). For the UC individuals, only inflamed biopsies were considered. Epithelial and lamina propria layers were combined for each sample. The original 50 cell types were merged to 19 broader categories. (Table S1) During quality control, only cells with 1,000 to 30,000 reads and more than 500 genes were kept. For gene filtering, a pseudo bulk count threshold greater than 3 was set. Each cell type needed to have a minimum of 5 cells in each sample and be captured in at least 40 out of 48 samples. The sample filter was set to 12 out of 13 cell types. For each individual, only the biopsy having the highest number of cell types and cells was kept. This yielded 13,861 genes, as well as 93,593 cells, 13 cell types, and 11 healthy and 17 UC samples. The filtered data was normalized by cell type using scran⁶³ (v1.26.2). Batch correction was performed using scGen⁶⁴ (v2.1.0).

Run community on healthy - ulcerative colitis dataset

Community was run on log-transformed normalized counts. The threshold_celltype_size and threshold_nr_active_cells were both set to >6, and threshold_expr >0.1. The total number of interactions was 491,959. For the quality control the threshold_log10_cum_weight was selected to be > 0.03, the threshold_frac_samples_per_condition >0.5 and threshold_log10meanexpr_per_condition >0.015. The total number of good quality interactions was 15,675. Finally, the threshold_log2FC was set to >1 and threshold_fdr was set to <0.1, yielding a total of 758 downregulated and 2,612 upregulated interactions.

Processing of melanoma dataset

The.h5ad file containing the count matrix and their annotation files was obtained from https://immucanscdb.vital-it.ch/api/scrnaseq/MEL_IMM_SS2_GSE120575/h5ad. The dataset contained 47 samples. Cell type were adapted from the “annotation_minor” annotation available in the.h5ad object. “DC plas” and “DC conv” were merged into “DC”. The gene threshold for the log10 cumulative expression in pseudobulks was set to greater than 0.25, yielding 25,483 genes for sample before, and 29,399 genes for samples after therapy. The minimum number of cell types per samples was set to 7, all samples passed the filter.

Run community on melanoma dataset

Community was run separately on the samples before therapy (11 non-responders as cases vs. 8 responders as controls), and after therapy (22 non-responders as cases vs. 6 responders as controls). In both cases, *Community* was run on log-transformed normalized counts.

For samples before therapy, the threshold_celltype_size and threshold_nr_active_cells were both set to >0, and threshold_expr >0.1. The total number of interactions was 931,588. For the quality control the threshold_log10_cum_weight was selected to be > 0.02, the threshold_frac_samples_per_condition >0.3 and threshold_log10meanexpr_per_condition >0.02. The total number of good quality interactions was 49,986. Finally, the threshold_log2FC was set to >1 and threshold_fdr was set to <0.1, yielding a total of 375 differential interactions.

For samples after therapy, the threshold_celltype_size and threshold_nr_active_cells were both set to >0, and threshold_expr >0.1. The total number of interactions was 1,077,608. For the quality control the threshold_log10_cum_weight was selected to be > 0.02, the threshold_frac_samples_per_condition >0.3 and threshold_log10meanexpr_per_condition >0.02. The total number of good quality interactions was 67,554. Finally, the threshold_log2FC was set to >1 and threshold_fdr was set to <0.1, yielding a total of 5,438 differential interactions.

The PD1 and CTLA4 activation signatures were obtained from <https://www.gsea-msigdb.org/gsea/msigdb/human/genesets.jsp>. Gene scores were calculated using the “score_genes” function from scanpy.⁵⁷

Preprocessing of healthy and AML bone marrow dataset

ScRNAseq communication analysis of the bone marrow of healthy and AML individuals was performed on publicly available data.³⁴ The log-transformed normalized read counts and the annotation files were downloaded from GSE185381. In the AML cohort, only samples at initial diagnosis were kept. Duplicated samples from healthy individuals #4 and #5 were removed. This yielded 14 samples. In the first pre-processing step, we removed genes that had all-zero values, yielding 31,843 genes. Cell IDs that were not present in the cell annotation files were excluded, yielding 58,354 cells.

In the quality control step, filters on cells, cell types, genes, and samples were applied. To exclude bad quality cells, the library size per cell was set to be between 1,100 and 30,000 reads and the number of genes was set to greater than 500. To filter cell types, first original cell subtypes were pulled into 11 bigger classes (Table S1). A good quality cell type was defined as having at least 5 cells in each sample and being captured in at least 12 samples. Megakaryocytes, perivascular cells and lymphoid progenitor (lymP) cell types did not pass these thresholds and were excluded. Genes having a cumulative pseudobulk count less than 0.25 were excluded, yielding 15,770 genes. Samples having had less than 7 cell types were excluded, yielding 13 samples, 8 cell types and 46,702 total cells.

Run community on healthy and AML bone marrow dataset

Community was run on log-transformed normalized counts. For the communication analysis, the threshold_celltype_size and the threshold_nr_active_cells were both set to 6 cells, the threshold_expr was set to 0.1. The total number of interactions was 151,744. For the QC, the threshold_log10_cum_weight, the threshold_frac_samples_per_condition, and the threshold_log10meanexpr_per_condition were set to 0.01, 0.6, and 0.02 respectively. The total number of good quality interactions was 22,645. The differential communication analysis was run on good quality interactions using *t* test on log2-transformed weights and FDR adjustment. The threshold_log2FC was set to 1 and the threshold_fdr was set to 0.1, which yielded 2,555 downregulated and 109 upregulated interactions.

Tumor-immune interaction analysis on healthy and AML bone marrow dataset

The tumor-immune communication analysis of the Lasry et al. dataset was based on the analysis described above. First *community* was run on log-transformed normalized counts. Next, the cell types were divided into tumor (HSPC and Mono) or immune (T, B, NK, DC, Gran) compartments. The weights of the interaction engaging erythrocytes, as well as tumor-to-tumor or immune-to-immune interactions were set to zero, while the tumor-to-immune and immune-to-tumor interactions were kept. The differential testing and the visualization of the results on the subsetted interactions was performed using same threshold described above. This resulted in 421 down-regulated and 6 up-regulated interactions.

Preprocessing of integrated datasets from two human AML-healthy bone marrow studies

An integrated dataset containing the scRNAseq of the bone marrow of healthy individuals (GSE116256,⁴⁴ GSE120221⁴⁵) and AML patients at initial diagnosis (GSE116256⁴⁴) was created. From the GSE120221 dataset, the samples S1, Sk1, S2, Ck, C2 were removed as duplicates. From the dataset GSE116256, the BM5-34p, MUTZ3, and OCI-AML3 samples, as well as samples with less than 50% blasts were removed. In the first pre-processing step, the genes that were missing in one of the datasets, as well as a cumulative pseudobulk count less than 1 were removed. Cell IDs that were not found in the cell annotation files were excluded. The library size per cell was set to be between 1,000 and 30,000 reads and the number of genes was set to be greater than 500. Additionally, cells that formed a cluster of high library size and low gene number area were removed by applying a linear threshold of $3,000 \cdot \log_{10}(\text{library size} + 1) - 10,500$. Original cell types were pooled into 8 bigger classes (Table S1). The mutation bearing cells (marked as “-like”) and their healthy counterparts were merged into the same cell type categories. A well represented cell type was defined as the one having at least 5 cells in each sample and being captured in at least 30 samples. Samples which had less than 5 cell types were excluded. This yielded 12,485 genes, 74,583 total cells, 6 cell types and 33 samples. The data was normalized using *scran*⁶³ (v1.30.2) on each cell type separately, preserving natural cell type differences. The batch effect was corrected using *scGen*⁶⁴ (v2.1.0).

Run community on two integrated healthy and AML bone marrow datasets

Community was run on log-transformed normalized and batch corrected counts. For the communication analysis, the threshold_celltype_size and the threshold_nr_active_cells were both set to 6 cells, and the threshold_expr was set to 0.1. The total number of interactions was 62,064. For the QC, the threshold_log10_cum_weight, threshold_frac_samples_per_condition, and the threshold_log10meanexpr_per_condition were set to 0.01, 0.6, and 0.02 respectively. The total number of good quality interactions was 7,043. The differential communication analysis was run on good quality interactions using *t* test on log2-transformed weights with FDR adjustment. The threshold_log2FC was set to 1 and the threshold_fdr was set to 0.1, which yielded 3,280 downregulated and 229 upregulated interactions.

Method comparison for cell-cell interaction tools

To ensure that the results of different algorithms were not driven by the differences in the databases, the comparison was run on the *community* database, which was brought to the algorithm-specific format. For CellPhoneDB,⁶ three main CSV files were built:

gene_input, *protein_input*, and *interactions*. For MultiNicheNet,⁴⁷ three individual networks were built: *ligand-receptor interactions* was built using *community* database, *signaling interactions* was built using all the protein-protein interactions datasets from OmniPath while removing self-interactions, *gene regulatory network* was built using DoRothEA⁶⁷ regulons available through OmniPath, with confidence levels ranging from A to C. The optimization was done via mlrMBO (v1.1.5.1) as described in the NicheNet workflow.

Method comparison was performed on the log-transformed normalized counts of the Lasry et al. dataset. To identify differentially expressed genes (DEGs) per cell type between cases and controls, the FindMarkers module (min.pct = 0.10) from Seurat⁴⁹ library was run on each cell type separately (v4.3.0). To count as DEG, the average log₂-fold change (avg_log2FC) has to be greater than 0.25 or less than -0.25 and the adjusted *p*-value has to be <0.05 (Table S2).

CellPhoneDB was run on each sample separately with method *degs_analysis* flag along with the list of DEGs sourced from Seurat and a *-threshold* of cells expressing the ligand or receptor set to 0.10. The directionality of the output pairs was double checked and put according to the original database, if swapped. MultiNicheNet was executed for every combination of sender and receiver cell types. To ensure consistency, parameters shared with the *community* algorithm such as min_cells, min_sample_prop, were adjusted to match. Otherwise, default parameters were utilized as suggested by the documentation. CellChat was run on each sample separately. We used the liftCellChat function to align cell type compositions across datasets, as the samples contained different cell type compositions. The 'truncatedMean' parameter was set to 0.10, ensuring that the average gene expression was considered zero if the expression of signaling genes of interest was less than 10%. Subsequently, all CellChat objects were merged for joint analysis. CellChat is able to identify upregulated and downregulated signaling ligand-receptor pairs through differential expression analysis (DEA). We performed DEA between two biological conditions (healthy and AML) for each cell group with the thresh.pc parameter set to 0.10. Upregulated and downregulated signaling interactions were determined based on the fold change of ligands in sender cells and receptors in receiver cells. ScSeqCommDiff was run using default parameters in multi-sample scenario mode. Additionally, we also tested by varying the parameters across a range of higher and lower values such as count_thr, and min_cells. However, scSeqCommDiff did not provide any significant results, we did not consider it further.

Finally, we combined each cell type from each sample and unified the structure, where rows are corresponding to an interaction while columns are samples, and values are corresponding scores. *Community* was run with the following thresholds: the threshold_celltype_size and the threshold_nr_active_cells were both set to 6 cells, and the threshold_expr was set to 0.1. For the QC, the threshold_log10_cum_weight, the threshold_frac_samples_per_condition, and the threshold_log10meanexpr_per_condition were set to 0.01, 0.6, and 0.02 respectively. As for the differential communication analysis, threshold_log2FC was set to 1 and the threshold_fdr was set to 0.1. An additional threshold of genes being expressed in at least 10% of a cell type in a sample was used to mimic the CellPhoneDB and CellChat pipelines.

To measure the computational time and memory usage, "psrecord" (v1.3) was used. All algorithms were run on one computational node of the server, equipped with 36 Cores, 256 GB RAM and running CentOS Linux 7 (Core).

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical details for all analyses are provided in the corresponding Results, figure legends, figures, STAR methods, and supplementary materials. Unless otherwise stated, *n* represents human scRNA-seq samples. Individual cells were used to calculate cell-type abundance, active fraction, gene expression, and interaction weights for each sample, whereas differential communication was tested using sample-level interaction weights.

Community's differential communication was assessed using two-sided Welch two-sample *t*-tests on log₂-transformed interaction weights. The *community* package also implements a two-sided Wilcoxon rank-sum test on untransformed weights as an alternative. *p*-values were adjusted for multiple testing using the Benjamini-Hochberg false discovery rate (FDR) method. Interactions were considered differential at absolute log₂FC > 1 and FDR <0.1. Log₂FC was calculated from the arithmetic mean interaction weight in each cohort. The analyses included 17 UC and 11 healthy samples; 11 non-responder and 8 responder melanoma samples before therapy; 22 non-responder and 6 responder melanoma samples after therapy; and 7 AML and 6 healthy samples in the Lasry dataset. The integrated AML analysis used 9 AML and 24 healthy samples.

The Figure 2 legend reports *t*-tests with significance defined as *p* < 0.05. For Figures 6J and 6K, Wilcoxon tests with Bonferroni-adjusted *p*-values were used. Figure 6J included 7 AML and 6 healthy samples; Figure 6K included 2 monocytic AML, 5 other AML, and 6 healthy samples. All the boxplots show the median, first and third quartiles, and 1.5-IQR whiskers, with individual sample values overlaid.

Differential gene expression for the method comparison was performed with Seurat v4.3.0 using default parameters. CellPhoneDB, MultiNicheNet, CellChat, and scSeqCommDiff were used for communication-method comparisons as described in method details. scSeqCommDiff used Wilcoxon rank-sum testing but yielded no significant interactions. Computational time and memory were measured with psrecord v1.3 on a node with 36 cores and 256 GB RAM running CentOS Linux 7 (Core).

Software versions used in the analysis are provided in key resources table and analysis parameters are provided in method details and the accompanying code repositories.