Cell-cell interaction (CCI)
Old mystery and new opportunities

Technical Journal Club

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PhD student

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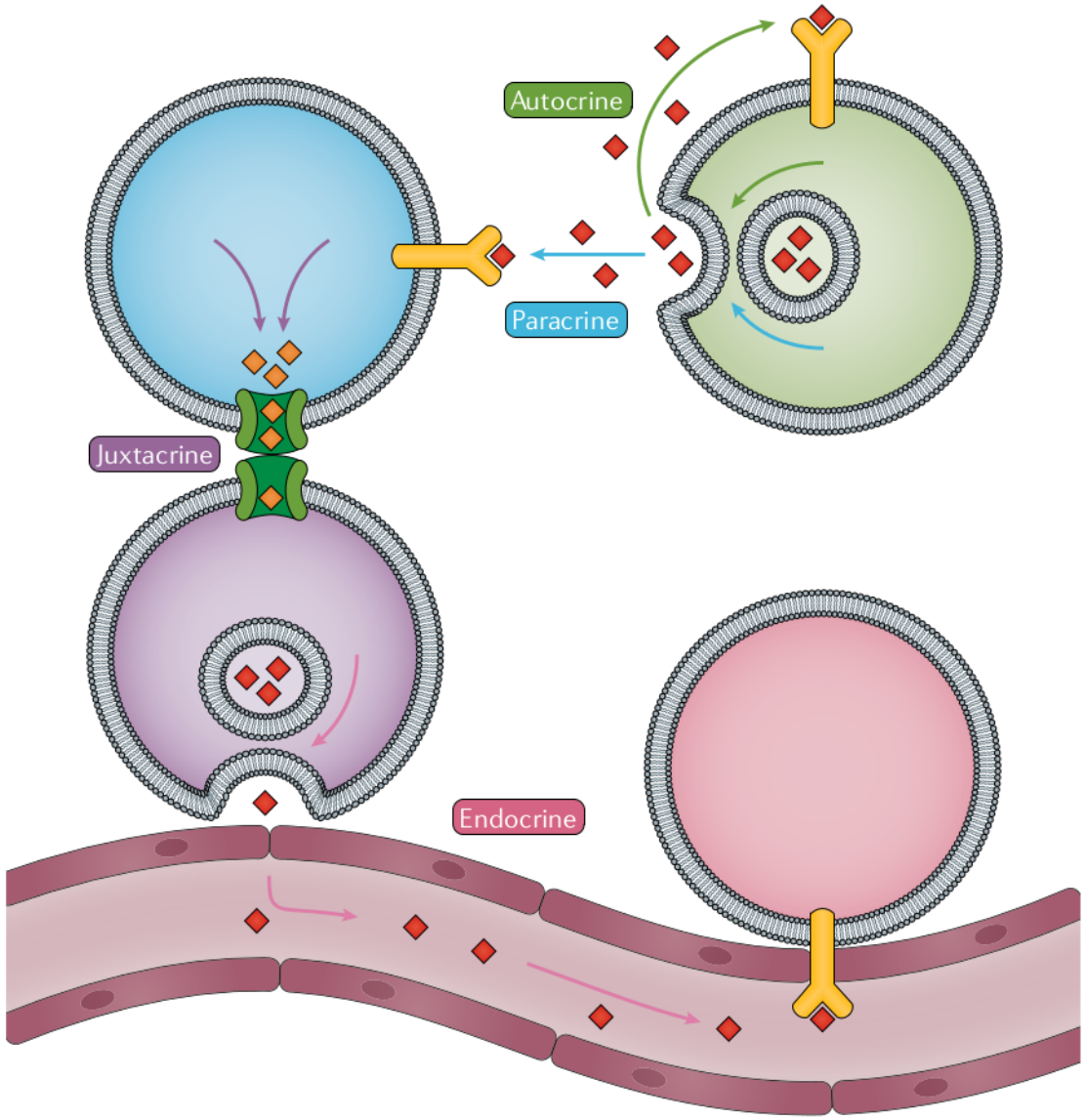
Multicellular life relies on the coordination of cellular activities



Autocrine: intracellular communication. Cells secrete ligands that are used to induce a cellular response through cognate receptors for those molecules expressed on the same cell

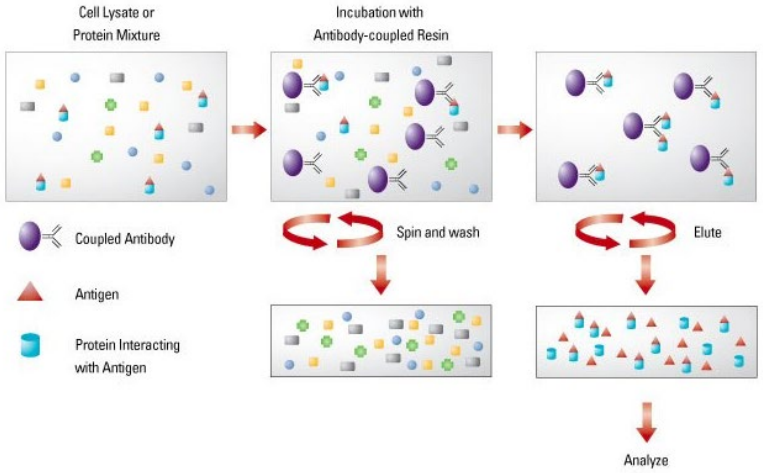
Paracrine: no cell–cell contact

Juxtacrine: contact-dependent cell–cell communication relies on gap junctions or other structures

Endocrine: intercellular communication whereby signalling molecules are secreted and travel long distances through extracellular fluids such as the blood plasma; typical mediators of this communication are hormones.

Direct measurements of proteins mediating CCIs

Co-immunoprecipitation



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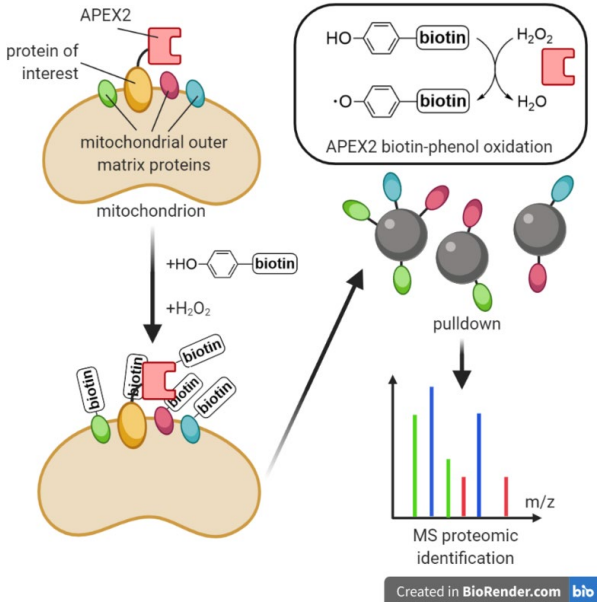
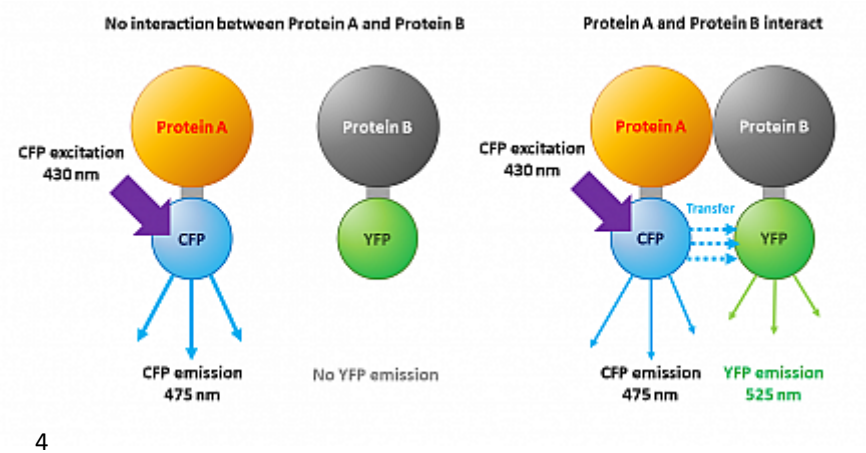
Proximity labelling proteomics



3

Fluorescence resonance energy transfer imaging



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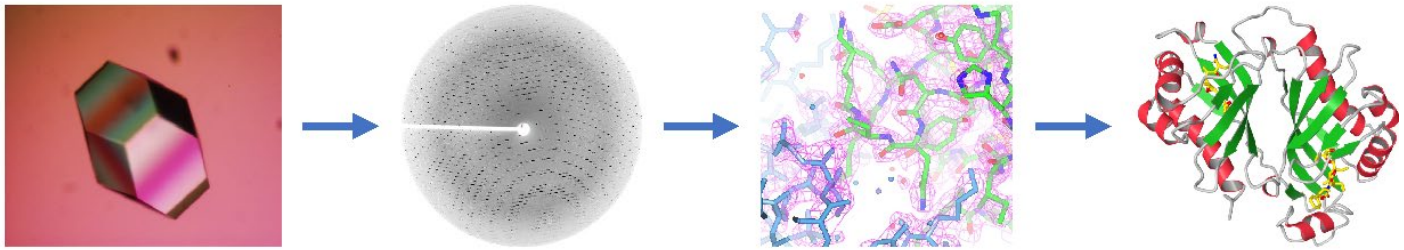
X-ray crystallography



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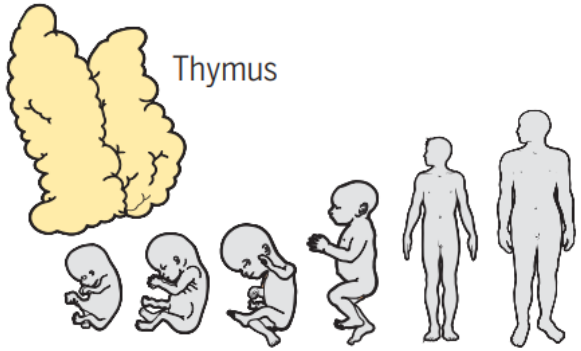
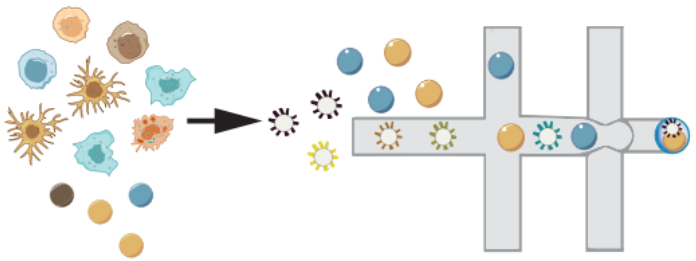
Identifying the cell type of origin of proteins mediating CCIs

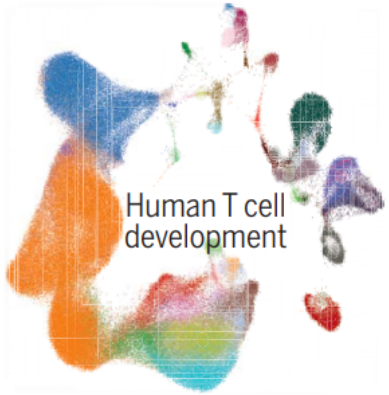
Development & Aging



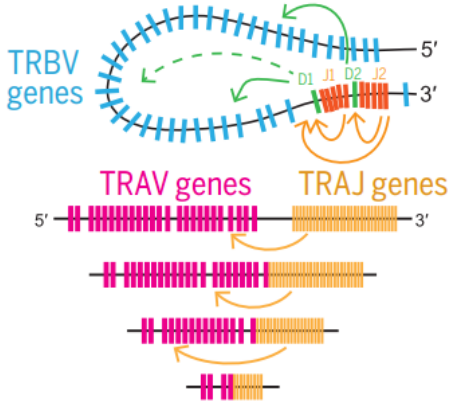
Single-cell RNA-seq + TCR



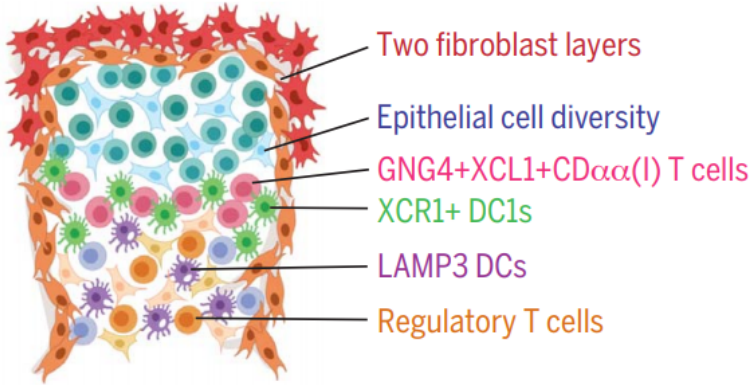
Atlas of the human thymus



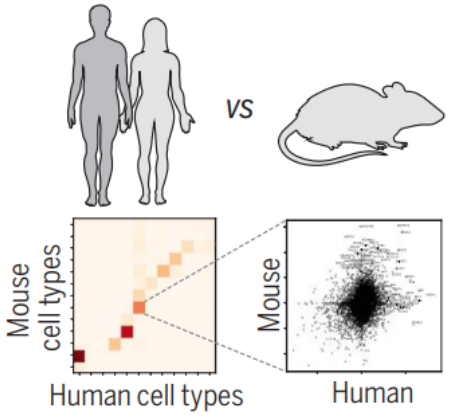
VDJ recombination and selection bias



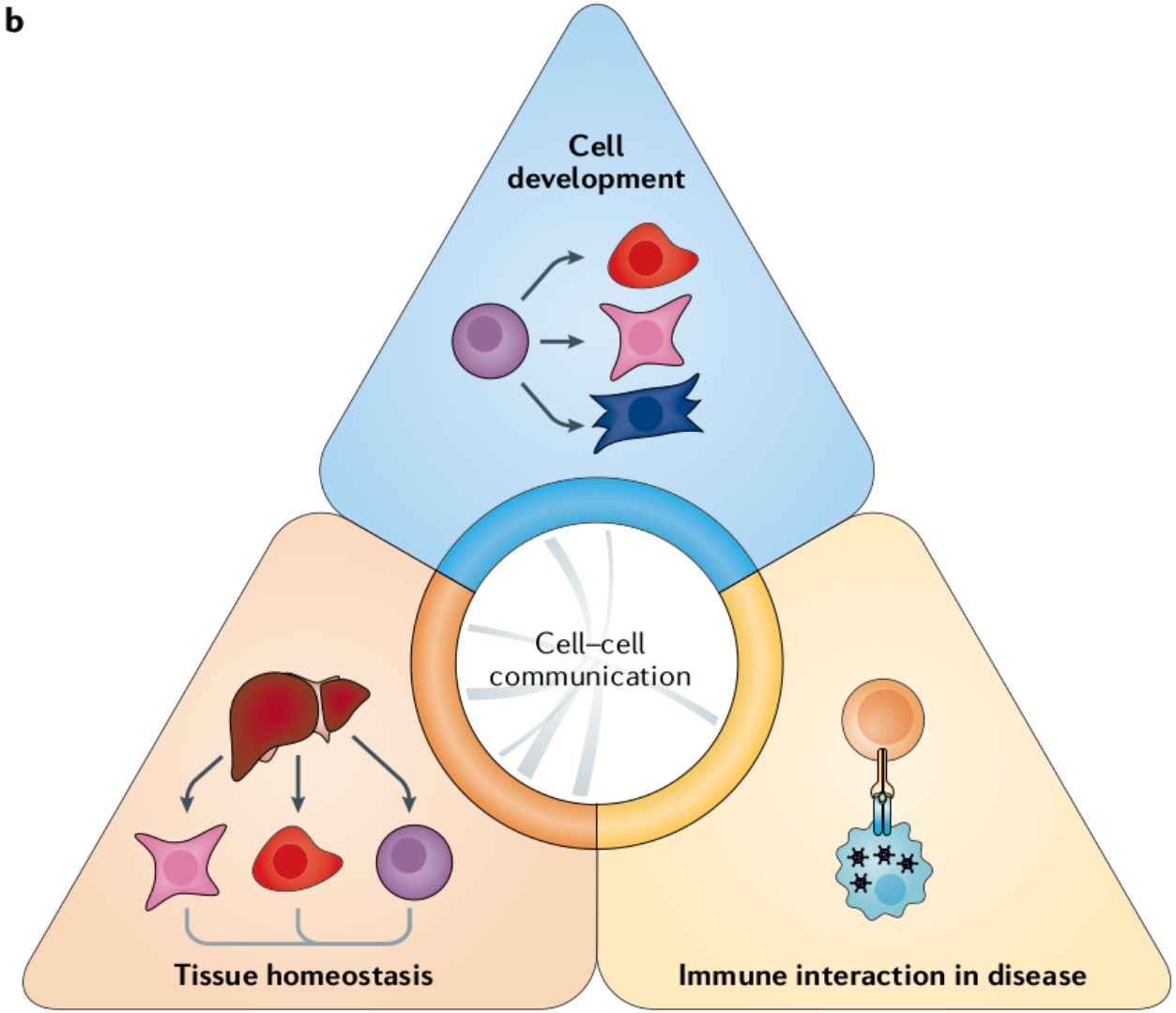
Mapping cell types *in situ*



Human-mouse comparison



Insights from RNA-based CCI analyses

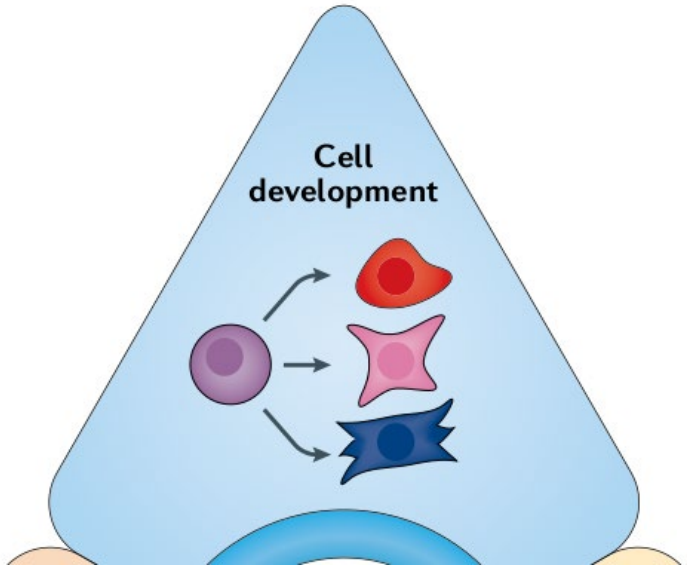


Use transcriptional profiling to decipher CCCs at any stage of development and in any multicellular community

Many studies focus on:

- signals mediating cellular differentiation
- interactions of cell types within tissues and organs
- immune responses

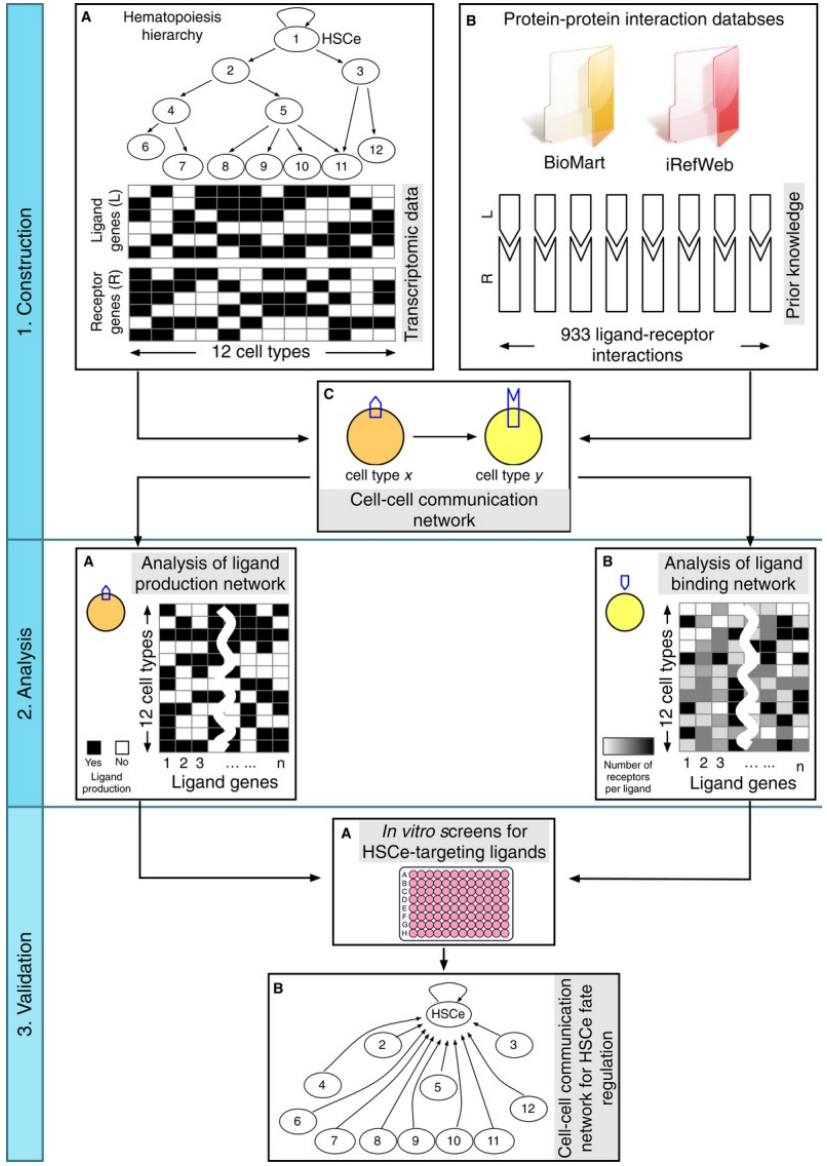
Interactions drive cellular differentiation and organ development



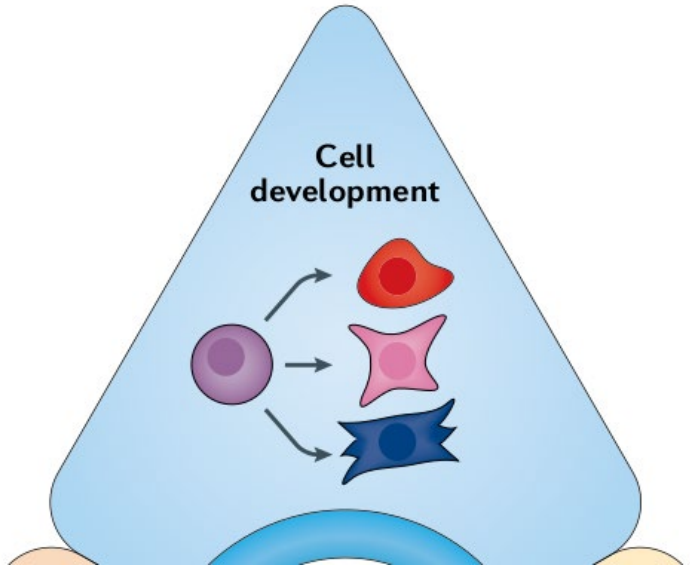
An example of CCC networks interrogating how differentiated cells influence haematopoietic stem cell fate revealed that ligand production is cell type specific

This work reports the construction of a CCC network that enables the discovery of cellular properties associated with the production of ligands and receptors.

Intercellular network structure and regulatory motifs in the human hematopoietic system



Interactions drive cellular differentiation and organ development

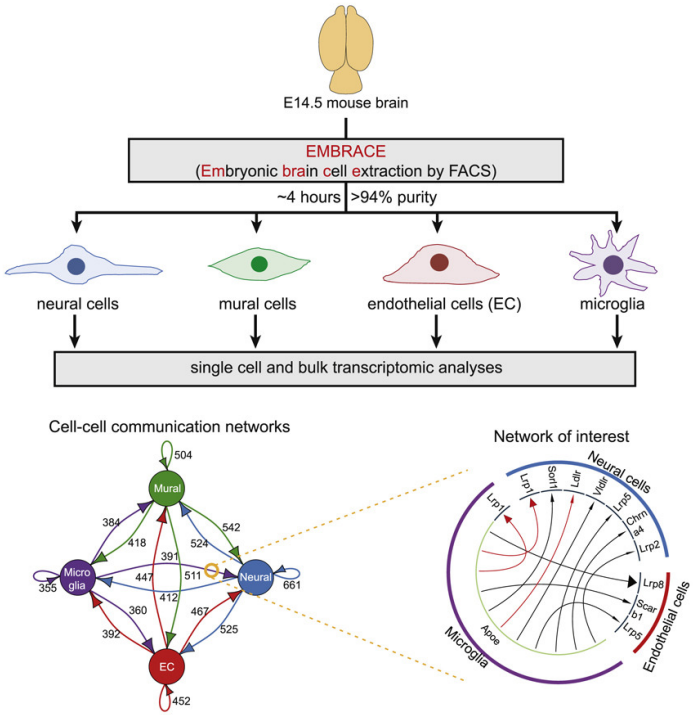


Utilizing EMBRACE they built a cell-cell communication map of the developing mouse brain

They identified 1,710 unique ligand-receptor interactions between neural, endothelial, mural, and microglial cells in silico

They confirmed the APOE-LDLR, APOE-LRP1, VTN-KDR, and LAMA4-ITGB1 interactions in the E14.5 brain

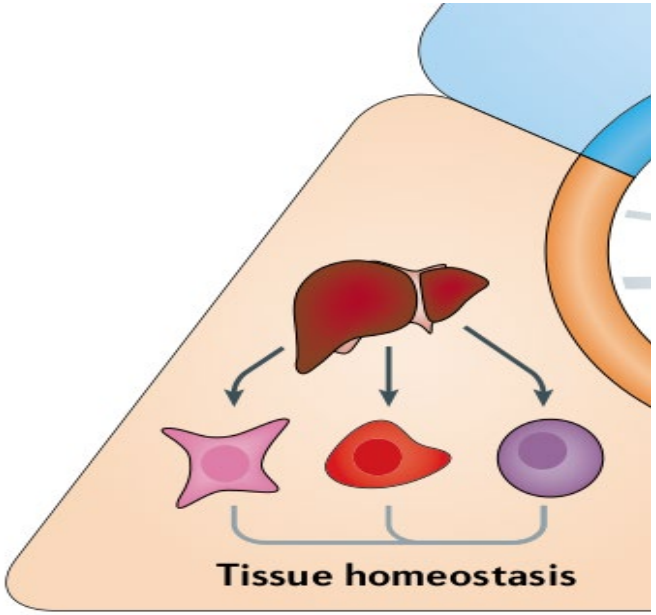
The analysis of brain CCC showed crosstalk involved in neurogenesis and identified novel mediators, such as apolipoprotein E (APOE), a protein associated with Alzheimer disease.



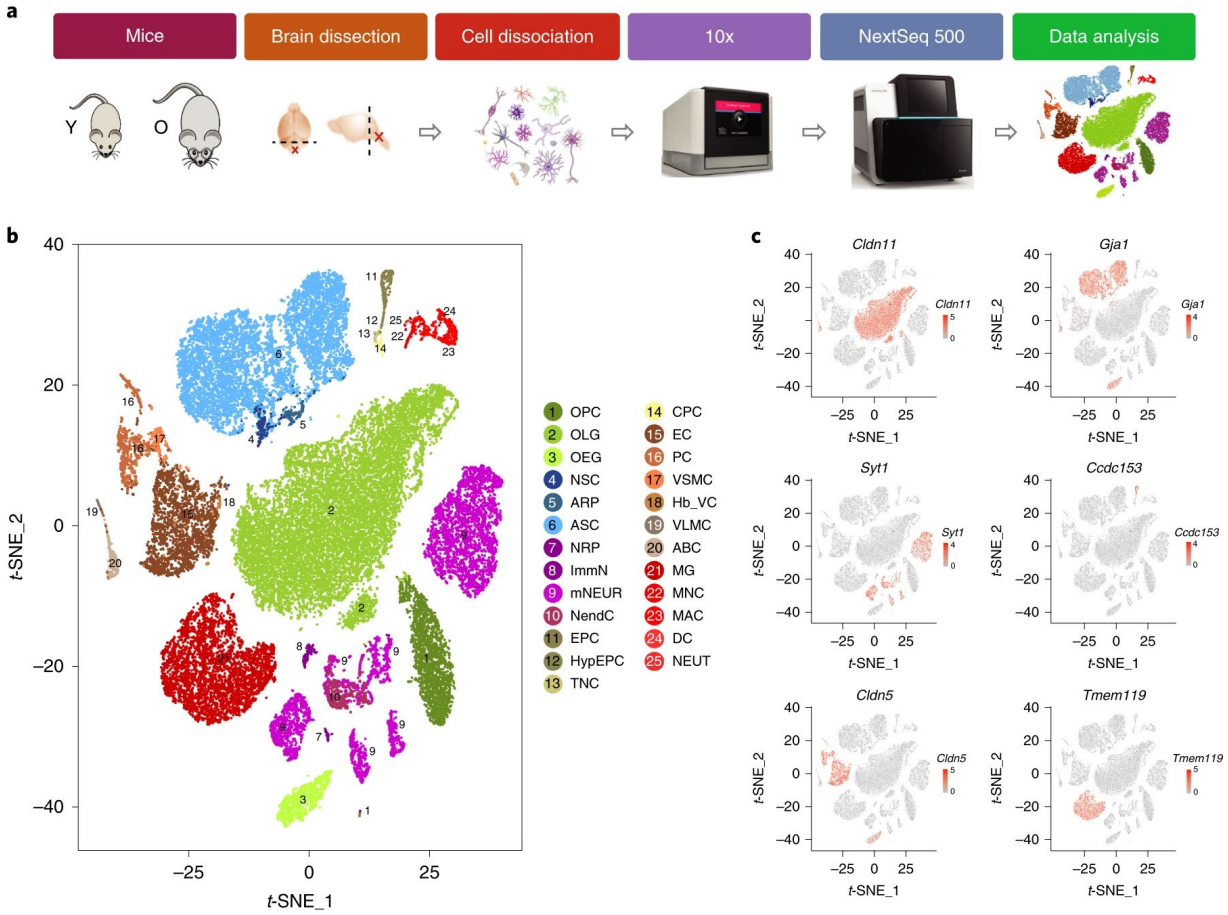
Brain interactome explorer
https://mpi-ie.shinyapps.io/braininteractomeexplorer/

iScience 2019

Cell-type communication in tissue and organ homeostasis



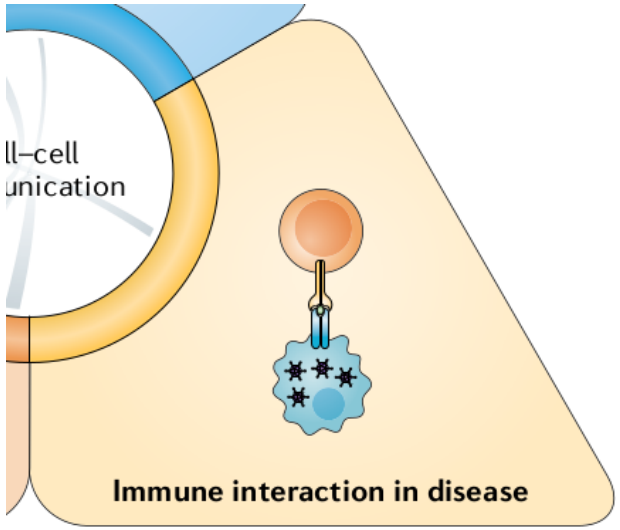
New roles of cells within a tissue and helped explain how ageing shape multicellular organization.



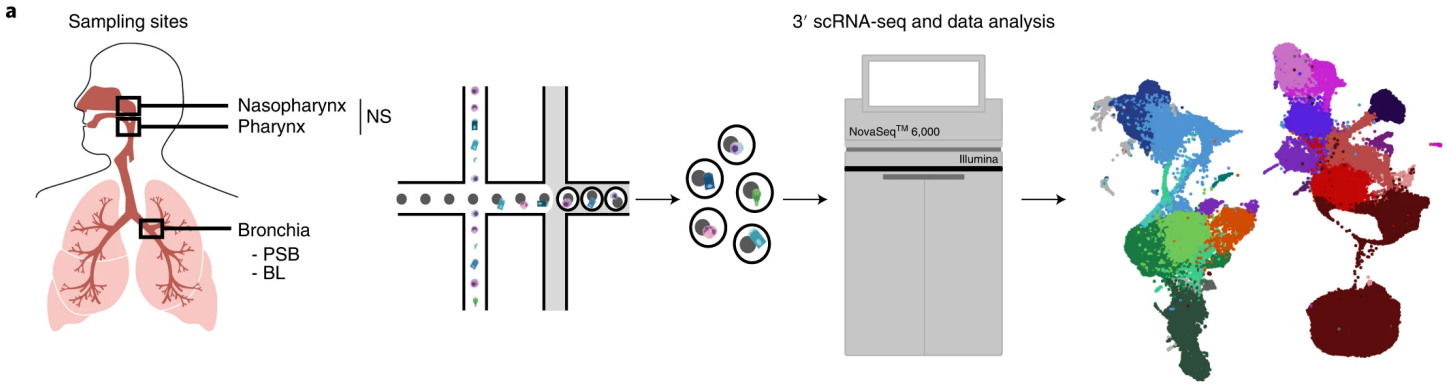
This revealed that CST3 and CXCL12 are mediators that differentiate intercellular interactions in young and old brains and
may modulate ageing-related processes

Nature Neuroscience

Immune interactions in disease



COVID-19 severity correlates with airway **epithelium–immune cell interactions** identified by single-cell analysis



The immune system receives signals from multiple tissues, but only specific signals allow it to coordinate healthy immune responses

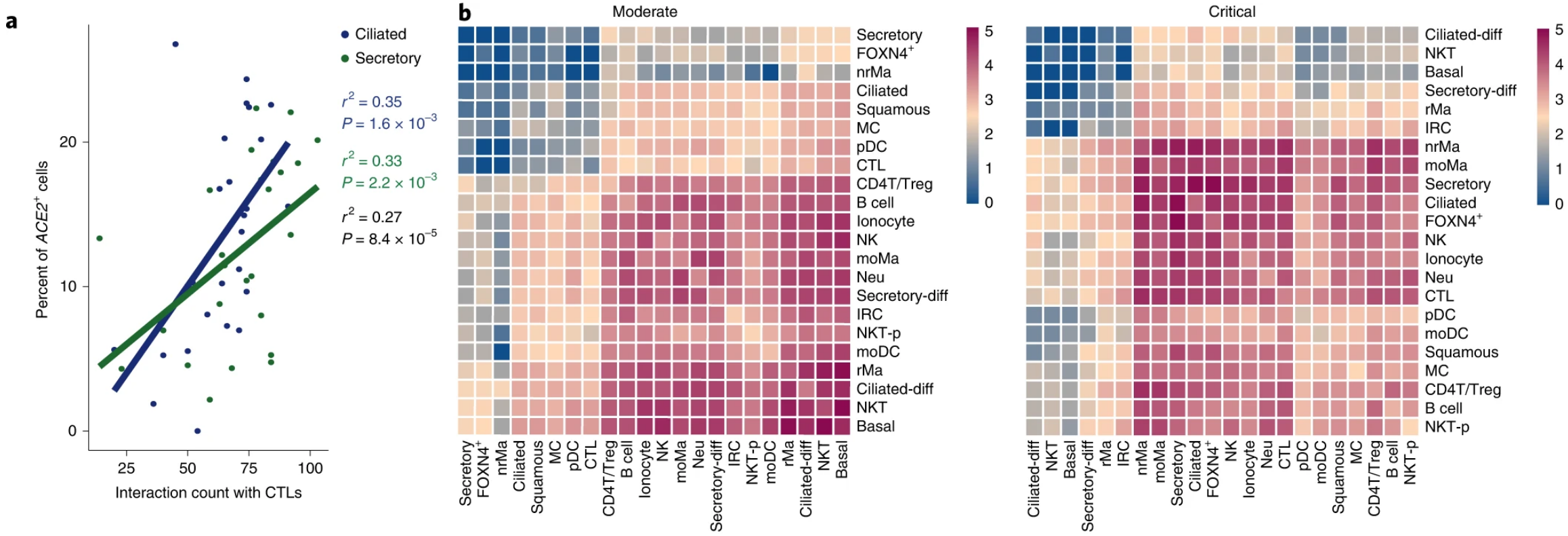
-> CCL2- and CX3CL1-mediated communication coordinate the recruitment and positioning of immune cells.

CCC is also involved in the response to viral infections

Crosstalk between lung and T cells in CCC associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection

Interactions between immune and epithelial cells correlated with COVID-19 severity.

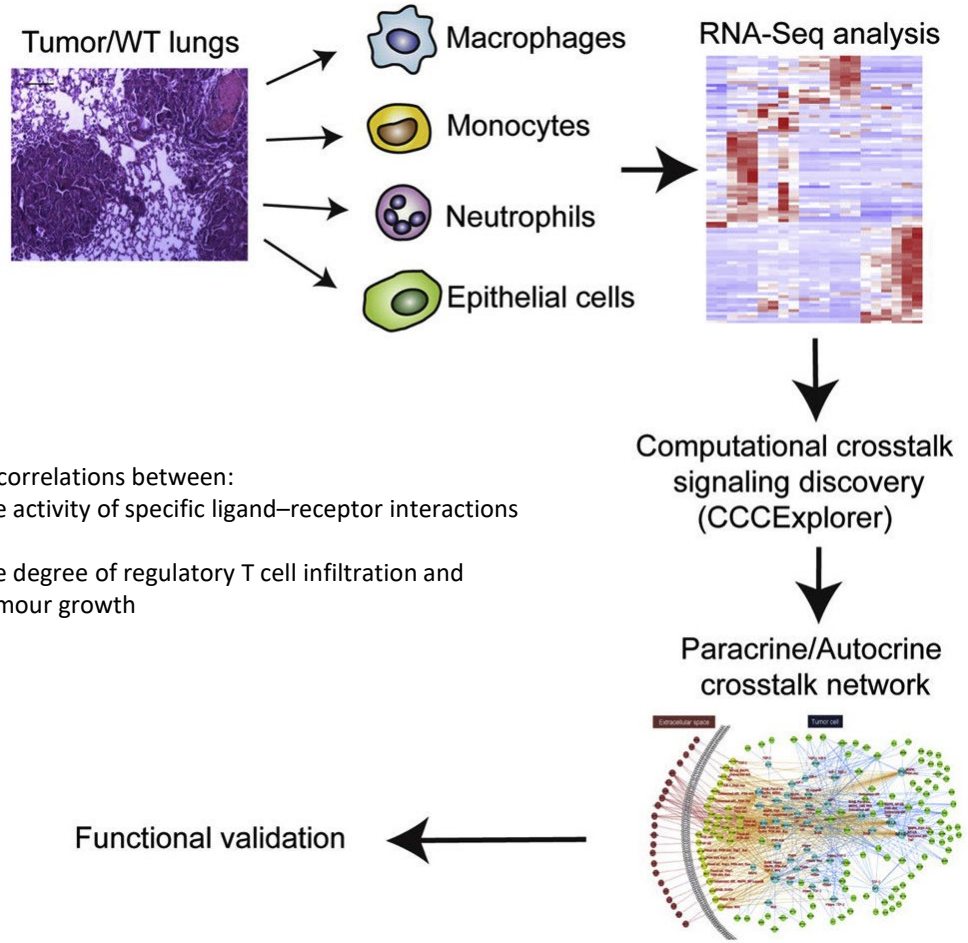
Correlation between the percentage of both ACE2+ ciliated and secretory cells in a given sample and the number of ligand–receptor interactions of those cells with cytotoxic T lymphocytes



Immune interactions in disease

Studying CCC within the tumour microenvironment provides opportunities to identify druggable pathways and develop new cancer therapeutics

CCC analyses have also elucidated cross-talk between tumour and stroma



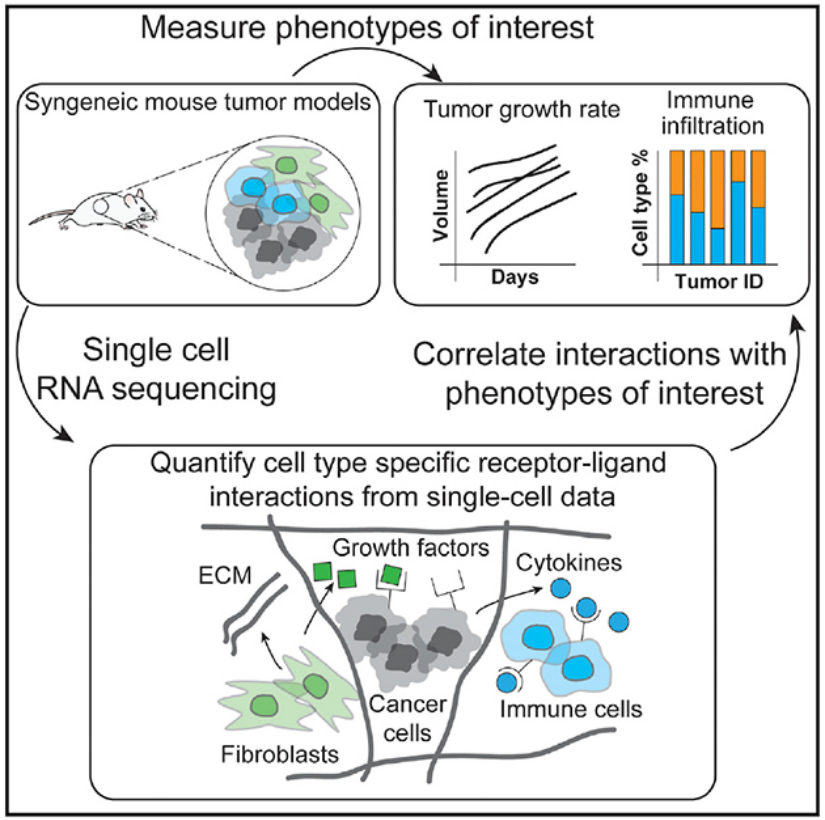
Clear correlations between:

- the activity of specific ligand–receptor interactions

and

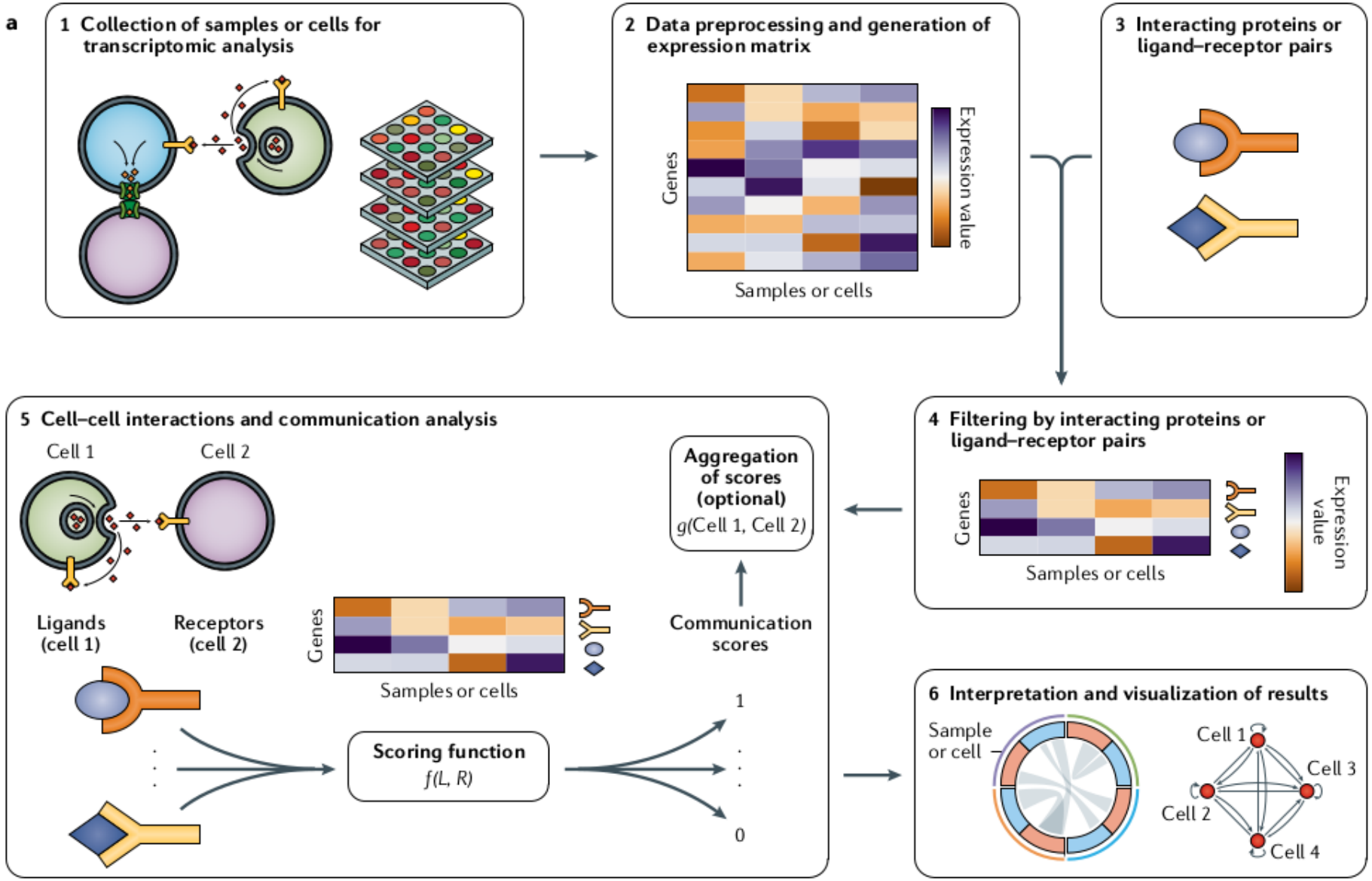
- the degree of regulatory T cell infiltration and tumour growth

Statistical models to connect inferred CCC mechanisms to cancer phenotypes



Example of using expression products for measuring intercellular communication and for finding relationships between ligand–receptor pairs and tumour phenotypes.

Deciphering CCC: Analysis workflow for inferring cell–cell interactions and communication from gene expression



Paper #1

eLife

TOOLS AND RESOURCES

A versatile system to record cell-cell interactions

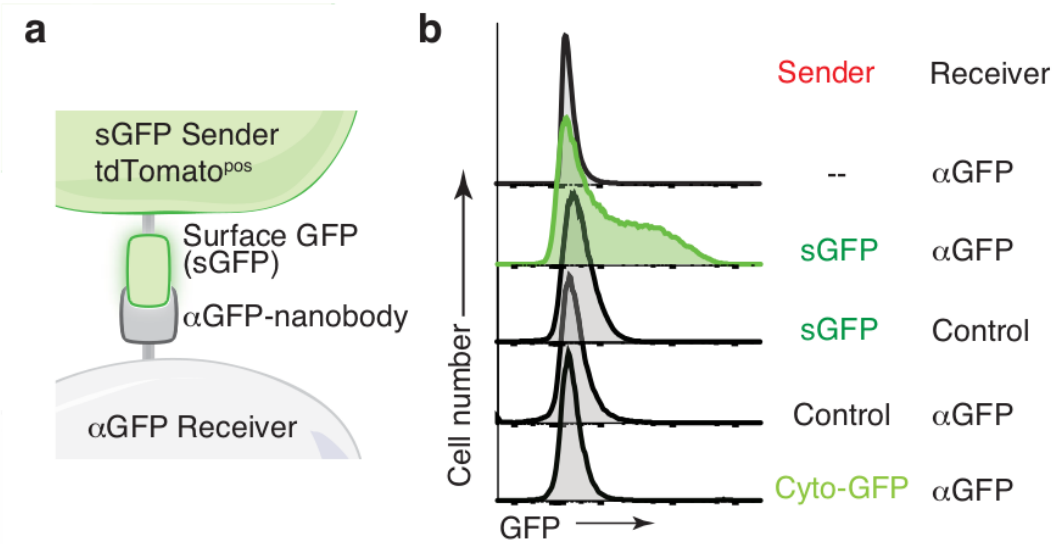
Rui Tang[1]*, Christopher W Murray[2], Ian L Linde[3], Nicholas J Kramer[1,4], Zhonglin Lyu[5], Min K Tsai[1], Leo C Chen[1], Hongchen Cai[1], Aaron D Gitler[1], Edgar Engleman[2,3,6], Wonjae Lee[5], Monte M Winslow[1,2,6]*

[1]Department of Genetics, Stanford University School of Medicine, Stanford, United States; [2]Cancer Biology Program, Stanford University School of Medicine, Stanford, United States; [3]Immunology Program, Stanford University School of Medicine, Stanford, United States; [4]Neuroscience Program, Stanford University School of Medicine, Stanford, United States; [5]Department of Neurosurgery, Stanford University School of Medicine, Stanford, United States; [6]Department of Pathology, Stanford University School of Medicine, Stanford, United States

- Cell-cell interactions influence all aspects of development, homeostasis, and disease.
- In cancer, interactions between cancer cells and stromal cells play a major role in nearly every step of carcinogenesis.
- The ability to record cell-cell interactions would facilitate mechanistic delineation of the role of the cancer microenvironment.
- They describe GFP-based Touching Nexus (G-baToN) which relies upon nanobody-directed fluorescent protein transfer to enable sensitive and specific labeling of cells after cell-cell interactions.
- G-baToN is a generalizable system that enables physical contact-based labeling between various human and mouse cell types, including endothelial cell-pericyte, neuron-astrocyte, and diverse cancer-stromal cell pairs.

The ability to track physically interacting cells with these simple and sensitive systems will greatly accelerate our understanding of the outputs of cell-cell interactions in cancer as well as across many biological processes.

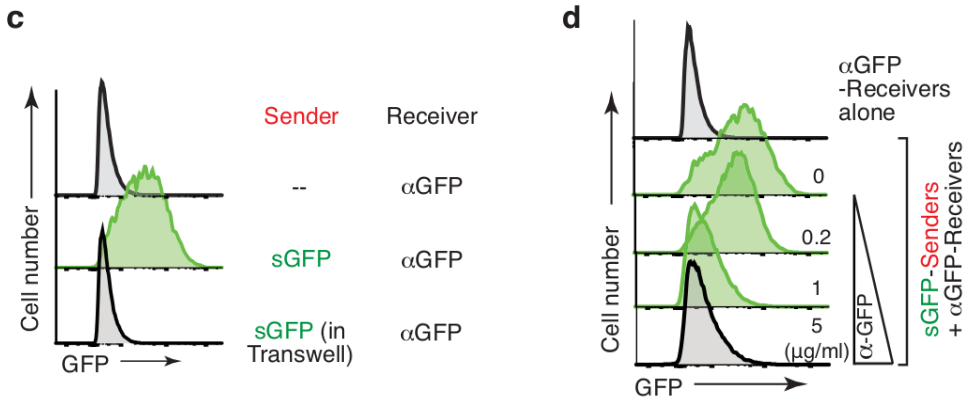
G-baToN enables cell-cell interaction-dependent labeling



Fluorescent signal could be transferred between neighboring cells

Synthetic ligand-receptor system based on the expression of surface GFP (sGFP) on sender cells and a cell surface anti-GFP (aGFP) nanobody on receiver cells.

Co-culturing sGFP sender cells with aGFP receiver cells led to GFP transfer and labeling of the receiver cells



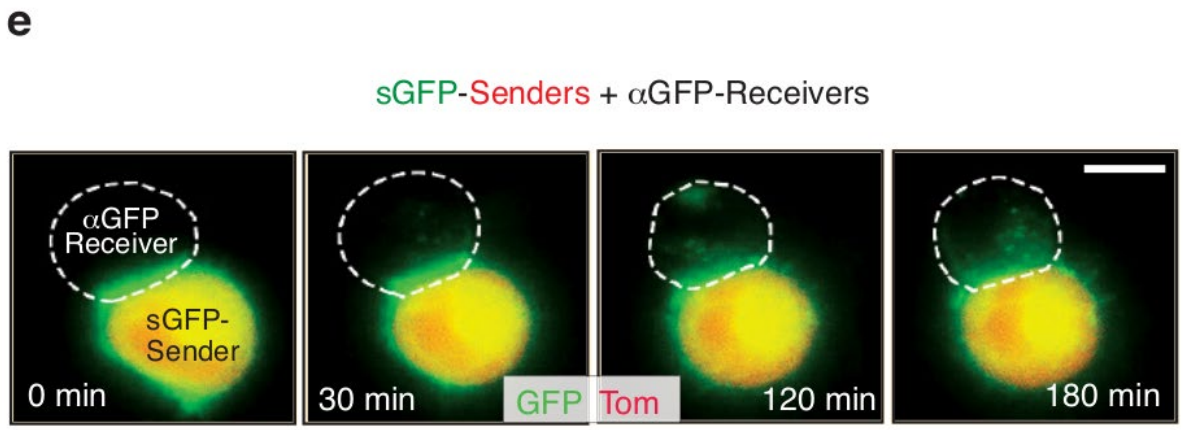
Receiver cell labeling required:

- direct cell-cell contact
- active membrane dynamics
- pairing between sGFP and its cognate aGFP receptor

Figure 1

G-baToN enables cell-cell interaction-dependent labeling

Time-lapse imaging showed rapid transfer and internalization of GFP by receiver cells



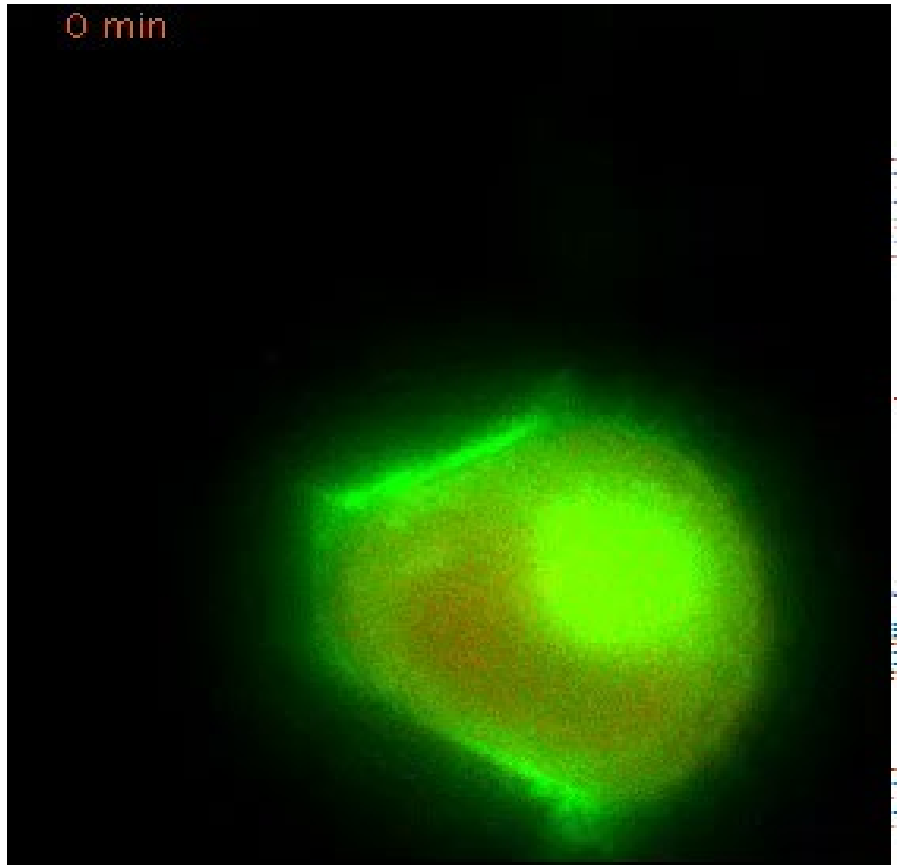
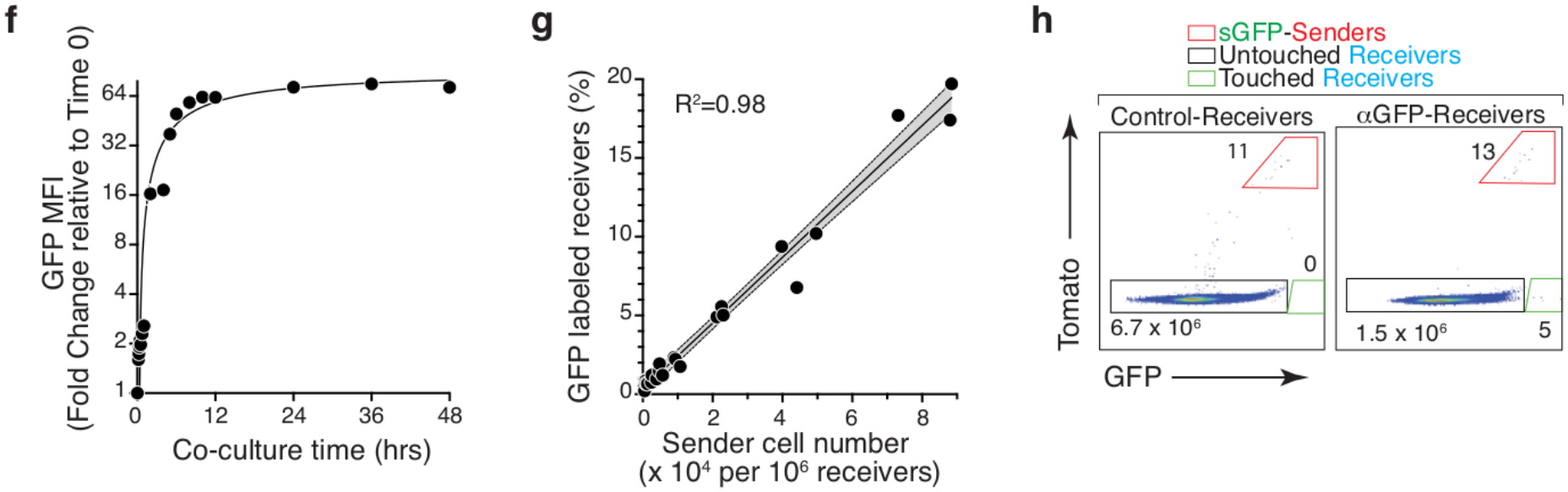


Figure 1

G-baToN enables cell-cell interaction-dependent labeling



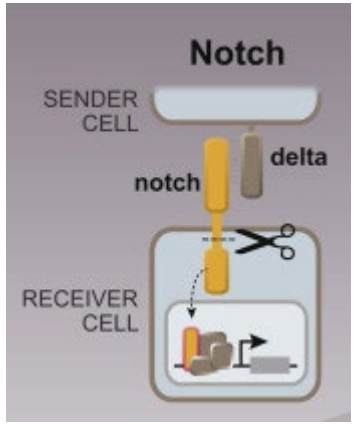
- GFP transfer could be detected within five minutes of co-culture and was half-maximal after 6 hr
- GFP fluorescence in receiver cells decayed rapidly after isolation of touched receiver cells from sender cells-> transient labeling of receiver cells
- The fraction of labeled receiver cells was proportional to the number of sender cells

The transfer of GFP to aGFP-expressing cells is a rapid and sensitive method to mark cells that have physically interacted with a predefined sender population

Figure 1

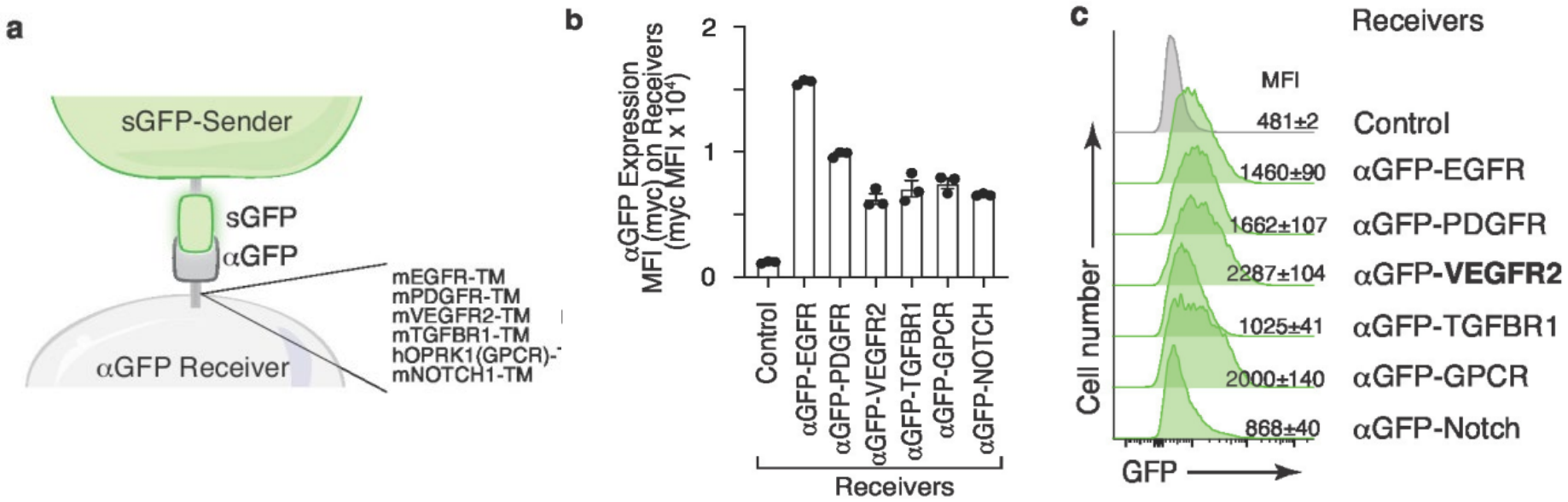
Fluorescence transfer efficiency is modulated by transmembrane domains and nanobody affinity

Three functional modules:
(1) the transmembrane domain of aGFP on the receiver cells
(2) the pairing between GFP and aGFP
(3) the transmembrane domain of sGFP on the sender cells



- The Notch1 transmembrane domain links the **LaG17-aGFP nanobody** onto the receiver cell surface

- The PDGFR transmembrane domain links sGFP onto the sender cell surface



The VEGFR2 transmembrane domain enabled the highest transfer efficiency, resulting in about a threefold increase relative to the original design

Figure 2

Fluorescence transfer efficiency is modulated by transmembrane domains and nanobody affinity

- LaG17-aGFP nanobody replaced with aGFP nanobodies with varying affinity for GFP
- Minimal affinity required for GFP transfer

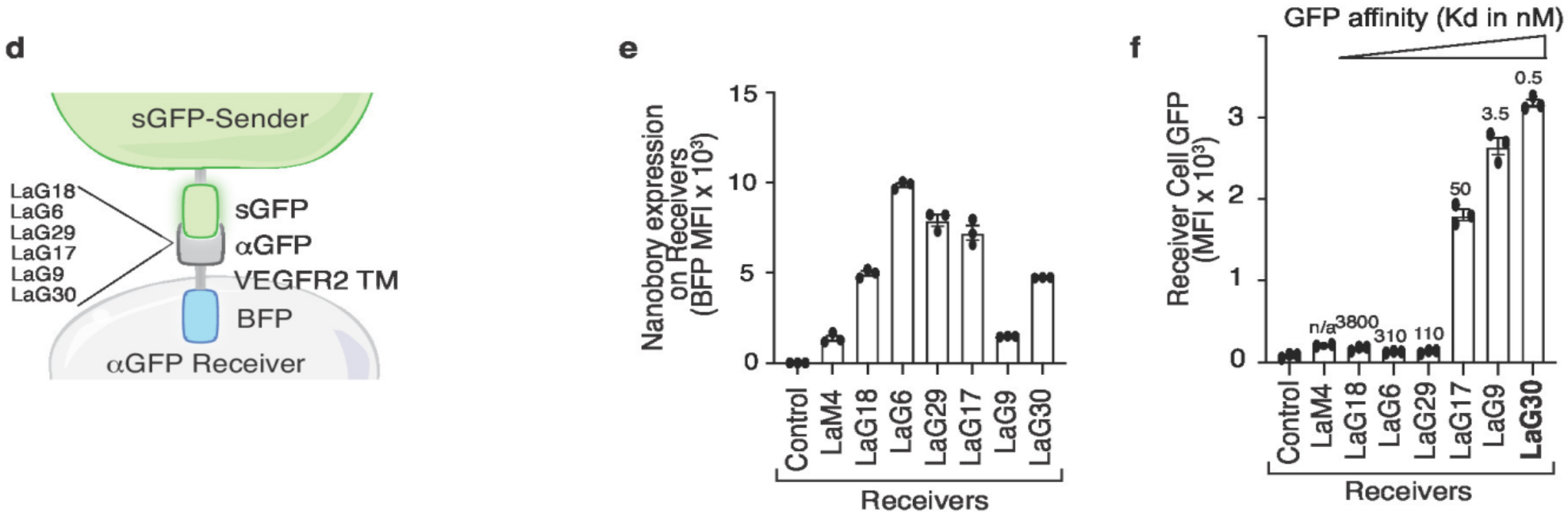


Figure 2

Fluorescence transfer efficiency is modulated by transmembrane domains and nanobody affinity

- The efficiency of GFP transfer correlated with GFP affinity
- Permutation of the transmembrane domain of sGFP on the sender cell revealed that the rate of retrograde transfer of aGFP-VEGFR2-BFP from receiver to sender cells was influenced by the sGFP transmembrane domain
- The PDGFR transmembrane domain minimized bidirectional transfer and thus was the optimal transferring GFP into a aGFP receiver cell

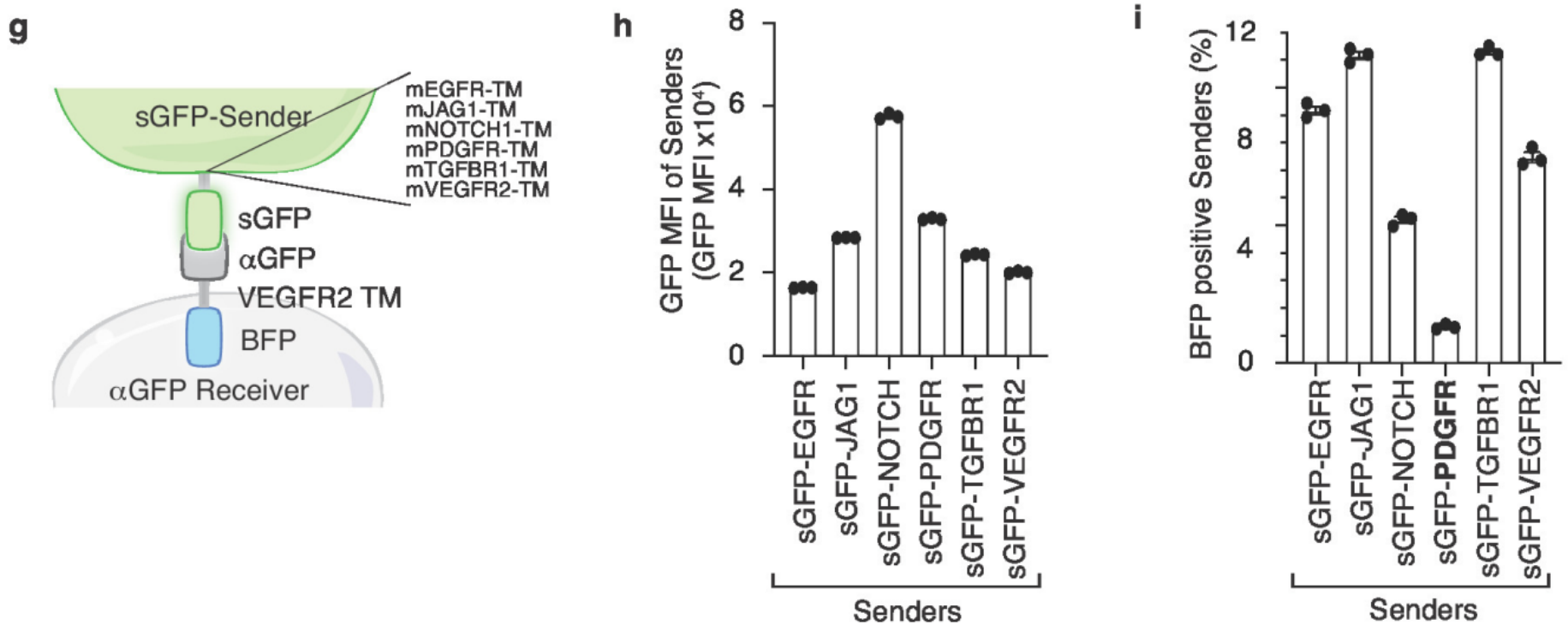
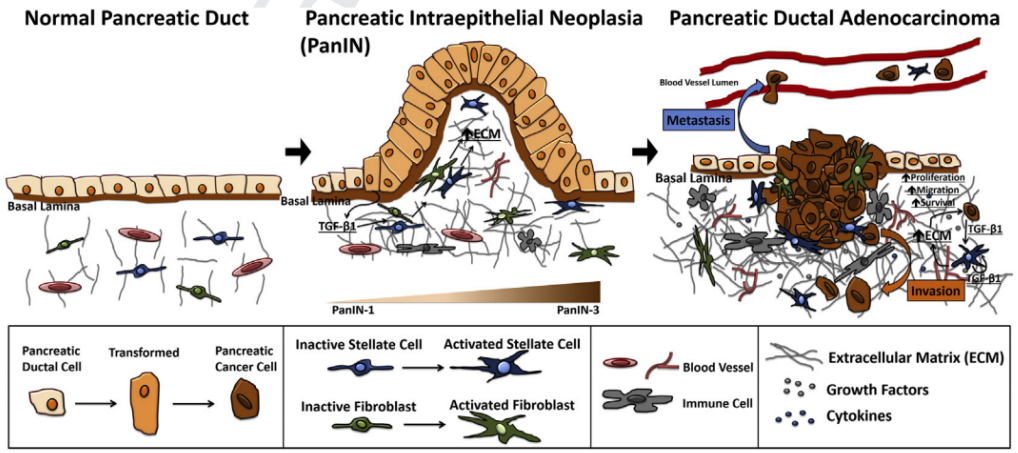


Figure 2

Tracking cancer-stroma interactions using G-baToN

Cancer cells interact with a variety of stromal cells
at both the primary and metastatic sites



Cancer Letters 2017

Tracking cancer-stroma interactions using G-baToN

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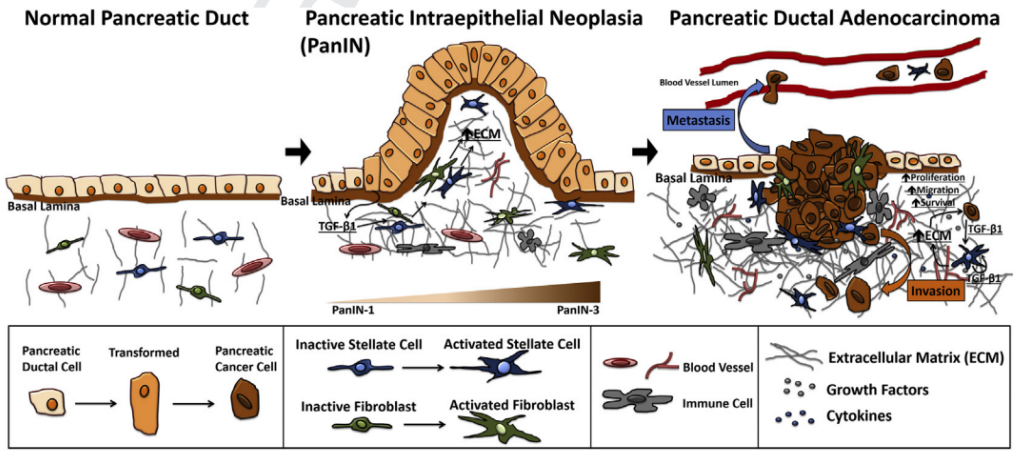
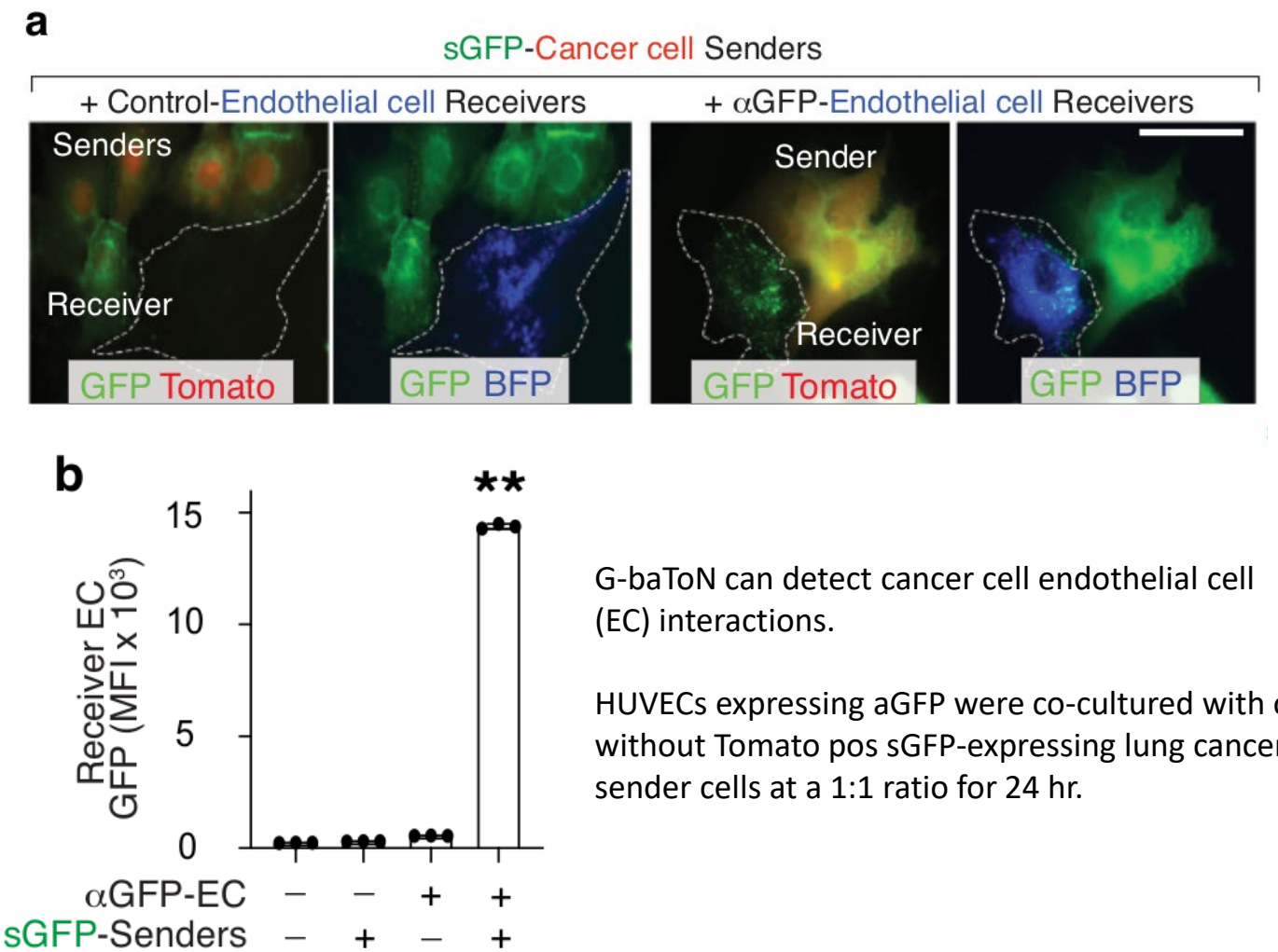


Cancer Letters 2017

Co-culturing sGFP-expressing lung adenocarcinoma cells with primary human umbilical vein endothelial cells (HUVECs) in a 2D format led to robust endothelial cell labeling



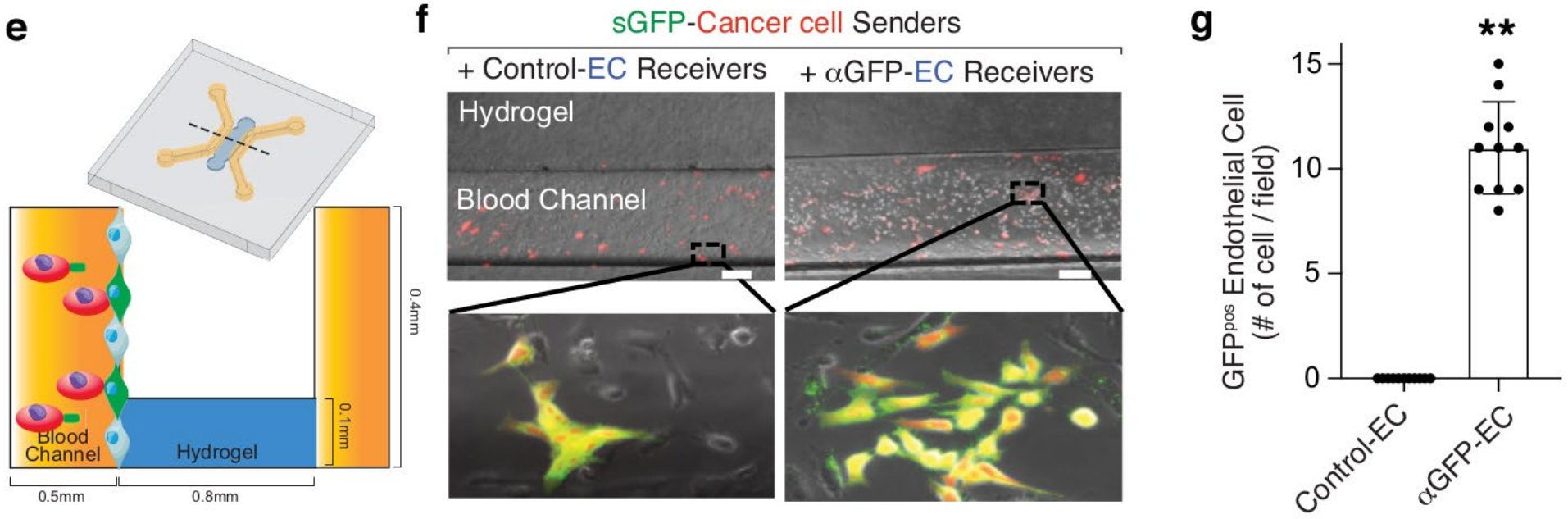
G-baToN can detect cancer cell endothelial cell (EC) interactions.

HUVECs expressing aGFP were co-cultured with or without Tomato pos sGFP-expressing lung cancer sender cells at a 1:1 ratio for 24 hr.

Figure 3

Tracking cancer-stroma interactions using G-baToN

Within 3D microfluidic chips, pre-seeded HUVECs expressing aGFP were robustly labeled following co-incubation with sGFP-expressing lung adenocarcinoma cells.

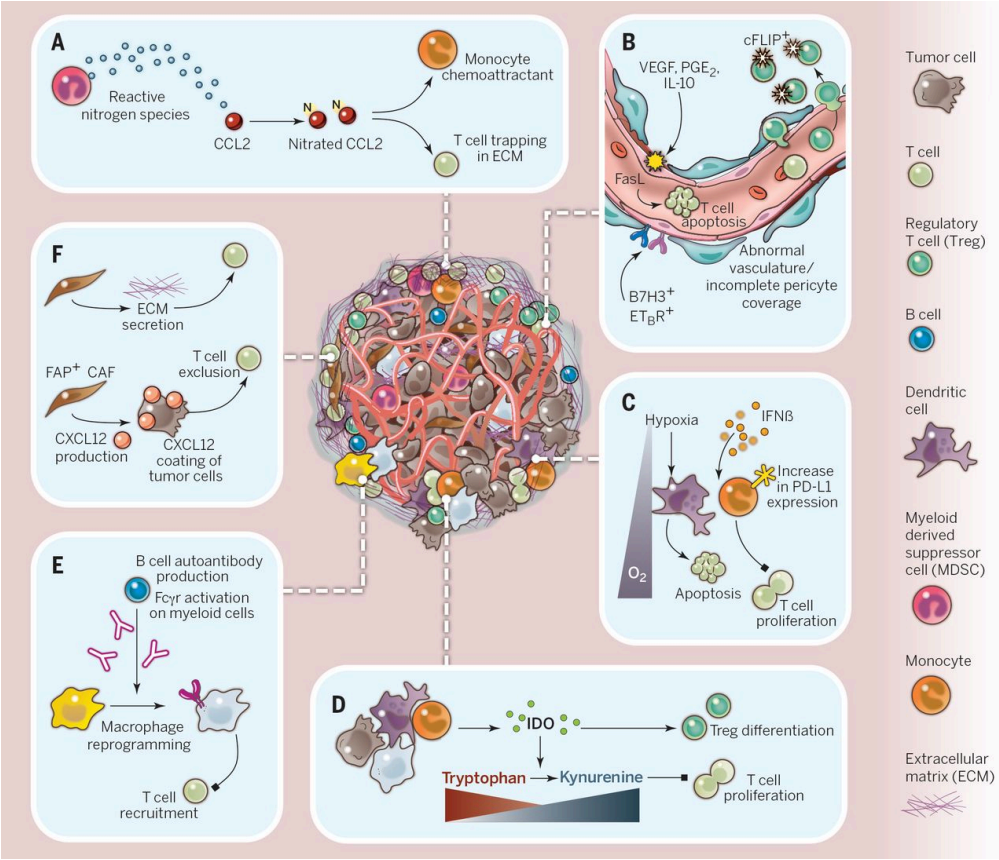


The G-baToN system is able to efficiently record cancer cell-endothelial cell interactions across multiple culture conditions.

Figure 3

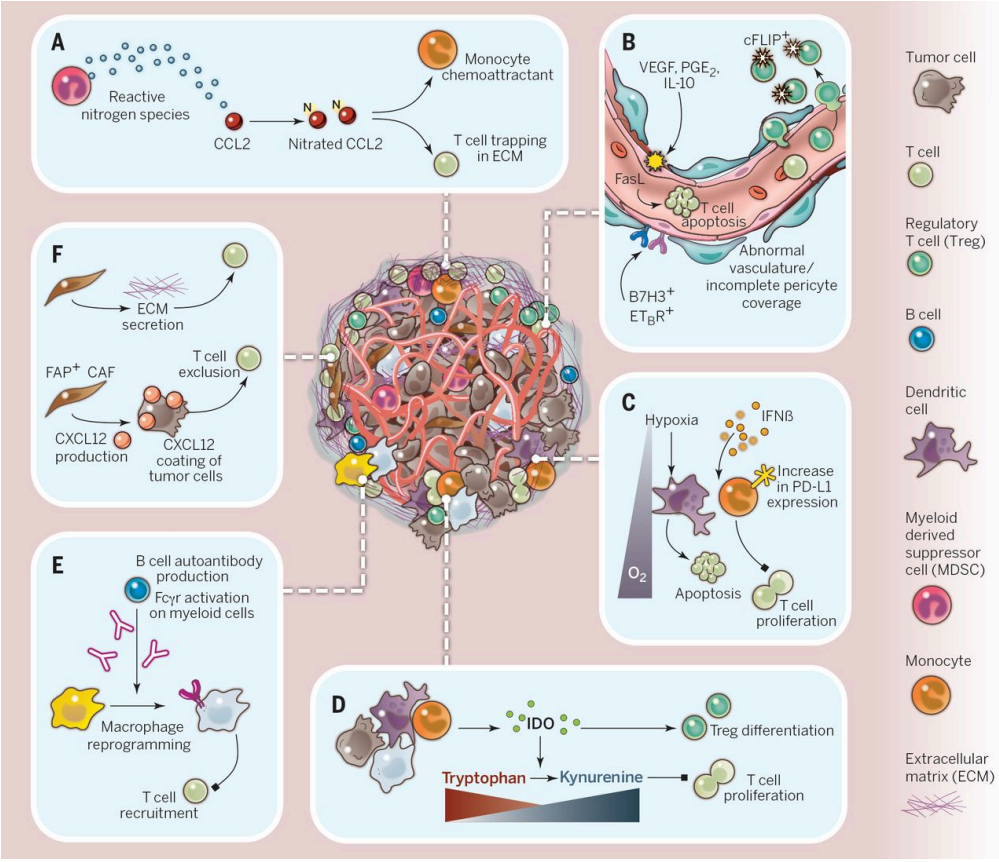
Tracking cancer-stroma interactions using G-baToN

Importance of interactions with adaptive immune cells during carcinogenesis

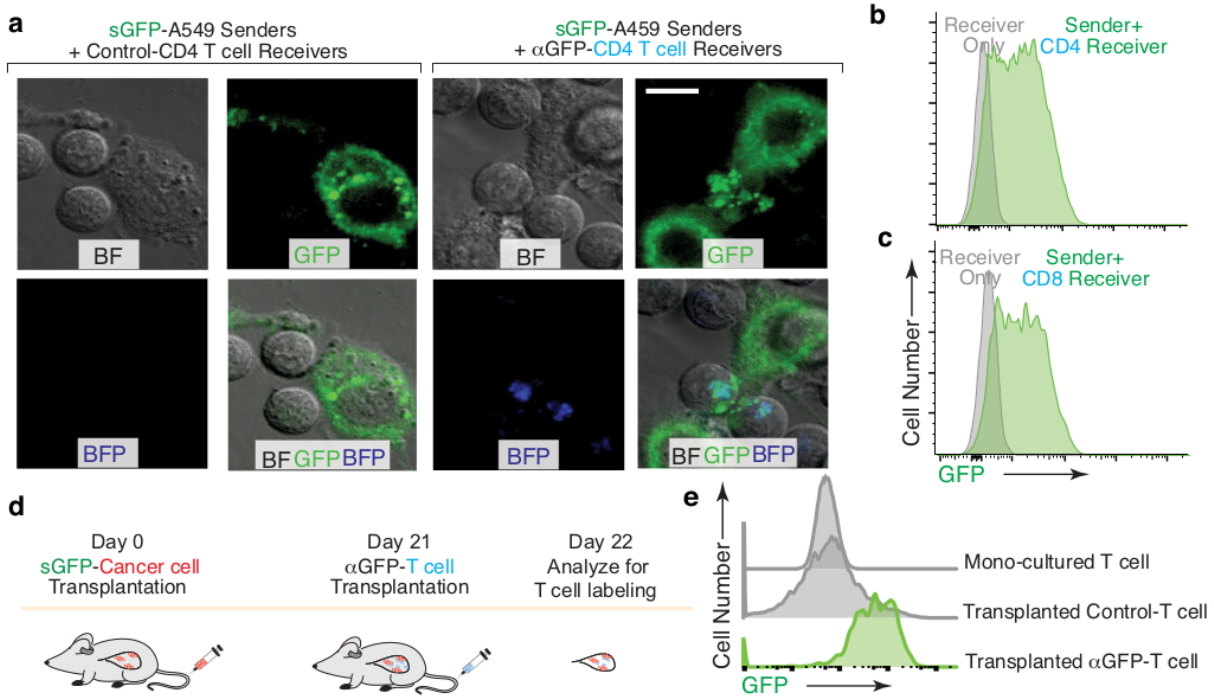


Tracking cancer-stroma interactions using G-baToN

Importance of interactions with adaptive immune cells during carcinogenesis



Assessment of the ability of the G-baToN system to track the interaction of **primary human CD4 and CD8 T cells with lung cancer cells**



aGFP-expressing CD4 and CD8 T cells that interacted with sGFP-expressing lung cancer cells in culture were specifically labeled

Figure 4

The G-baToN system is capable of recording cancer cell-T cell interactions both in vitro and in vivo

G-baToN can be applied in a wide range of cell types

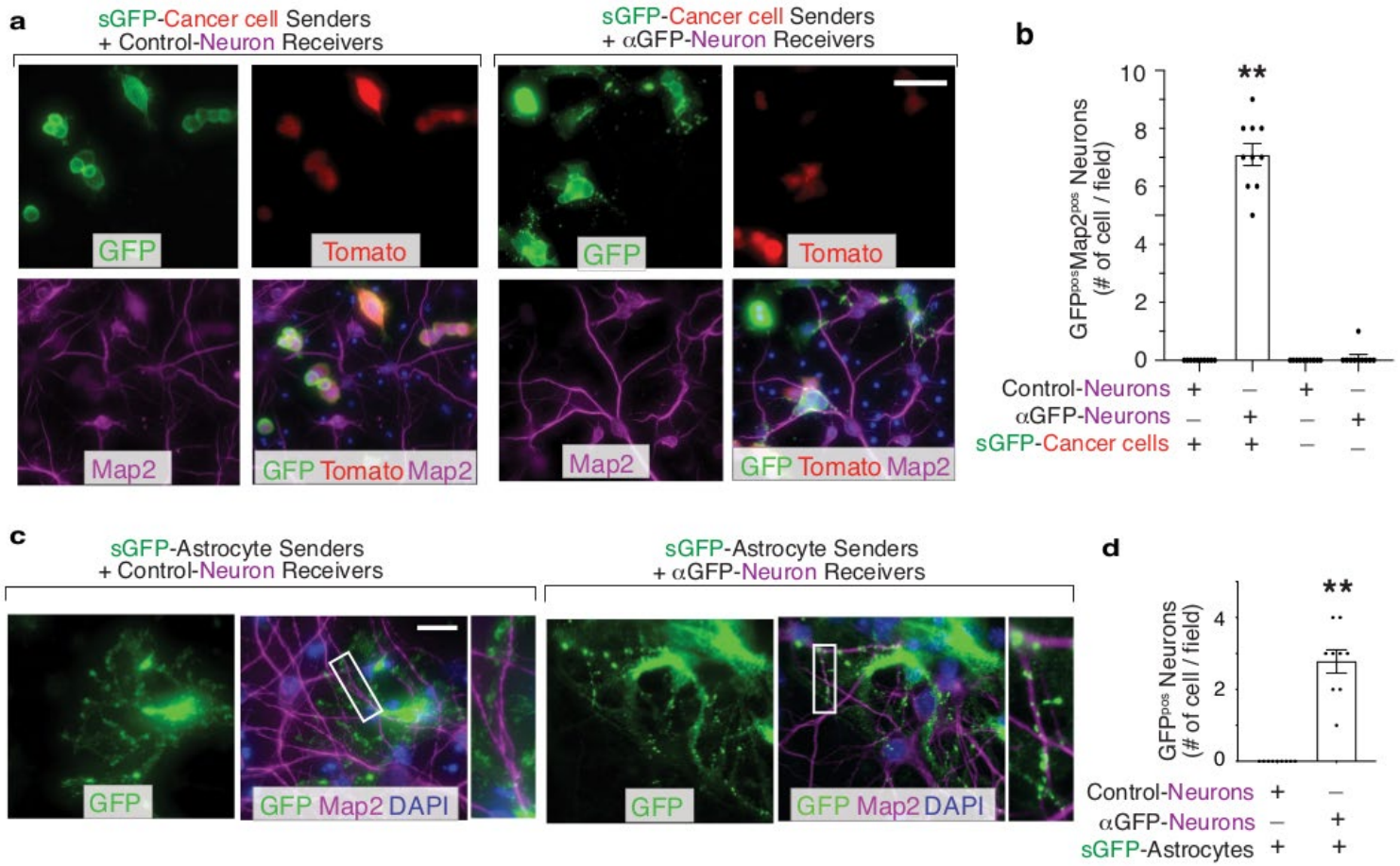


Figure 5

- To assess the generalizability of the G-baToN system across cell types, they expressed aGFP in a panel of cell lines and primary cells.
- Each receiver cell type was able to uptake GFP from sGFP-expressing lung cancer sender cells upon cell-cell contact.
- Diverse cancer cell lines and primary cell types expressing sGFP were able to transfer GFP to aGFP-expressing HEK293 receiver cells.
- Receiver cell labeling required sGFP-expression on the sender cell and aGFP expression on the receiver cells.

G-baToN-based labeling extends beyond transformed cell types and can label diverse primary cell types in co-culture

G-baToN can be applied in a wide range of cell types

Can primary cells serve as both sender and receiver cells?

Assessment of GFP transfer between interacting primary cells:
- endothelial cells interacting with smooth muscle cells

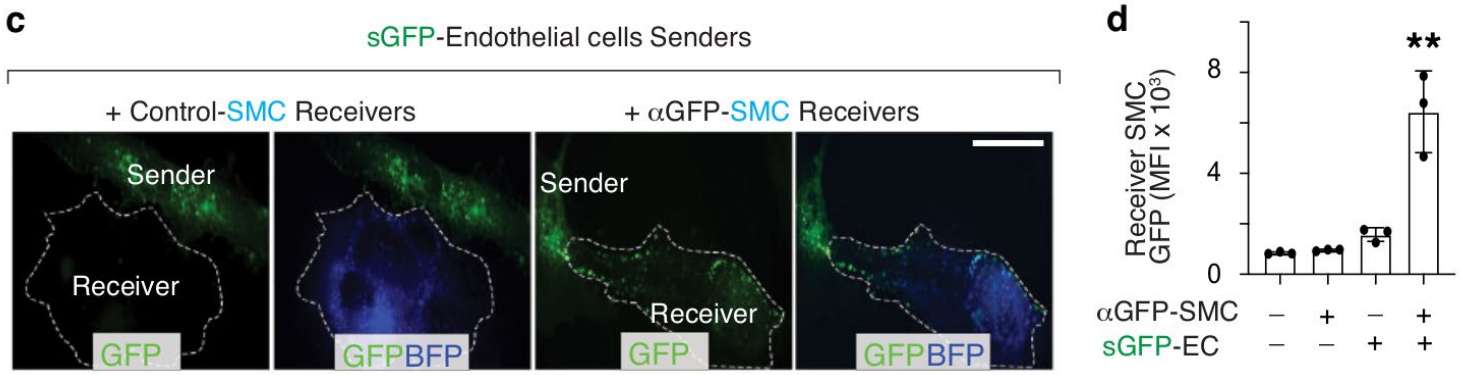


Figure 3

Co-culturing sGFP-expressing HUVEC and aGFP-expressing primary human umbilical vein smooth muscle cells (HUVSMC) resulted in efficient receiver smooth muscle cell labeling

sGFP-expressing astrocytes
were able to transfer GFP to aGFP-expressing cortical neurons

These results document the efficiency of G-baToN-based cell labeling across diverse cell types

Multicolor labeling enables recording of reciprocal and higher-order interactions

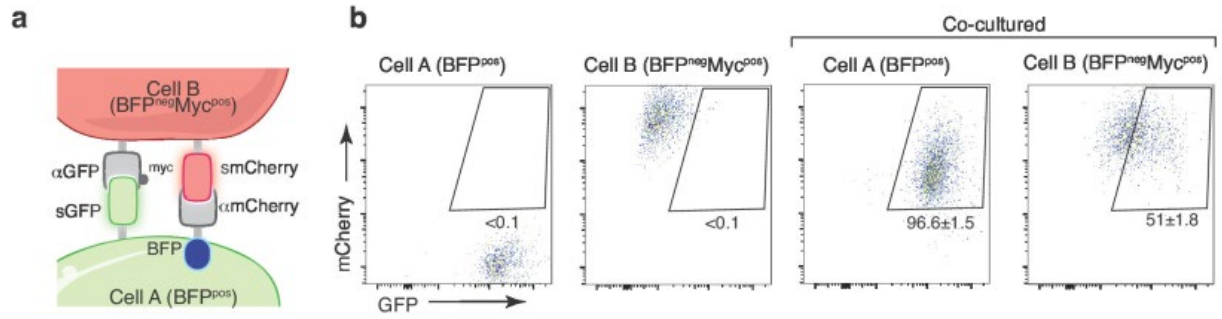
Could other surface antigen/antibody pairs lead to protein transfer and labeling?

Orthogonal systems consisting of:

- surface-mCherry/amCherry (LaM4)
- surface-GCN4-GFP/aGCN4 (single-chain variable fragment, scFV)

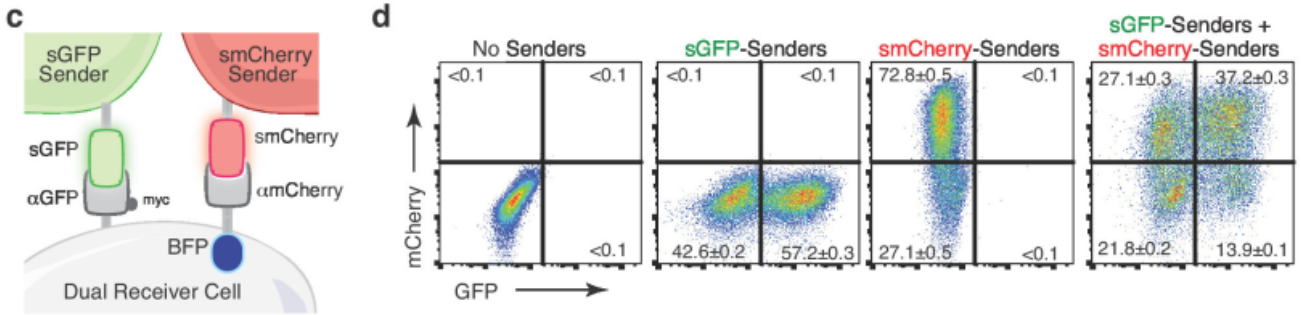
led to efficient and specific receiver cell labeling

Multicolor labeling enables recording of reciprocal and higher-order interactions

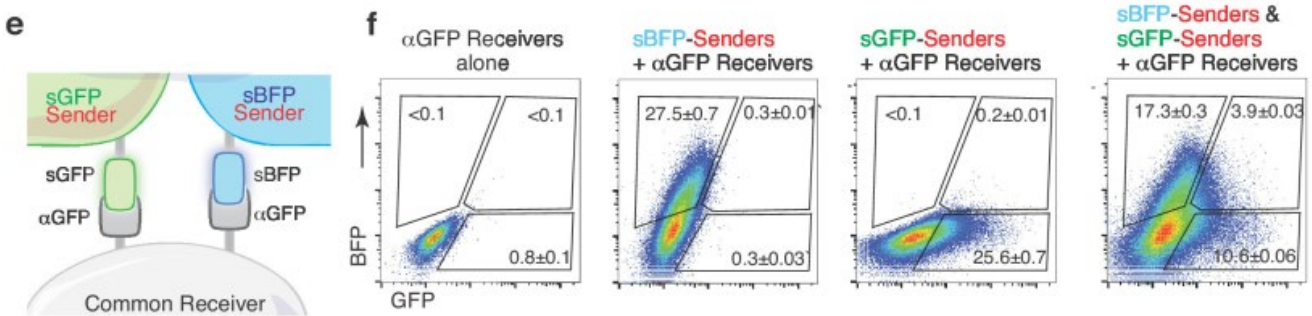


Reciprocal labeling of both interacting cell types

Using orthogonal ligand-receptor pairs, we also created an AND gate dual labeling strategy



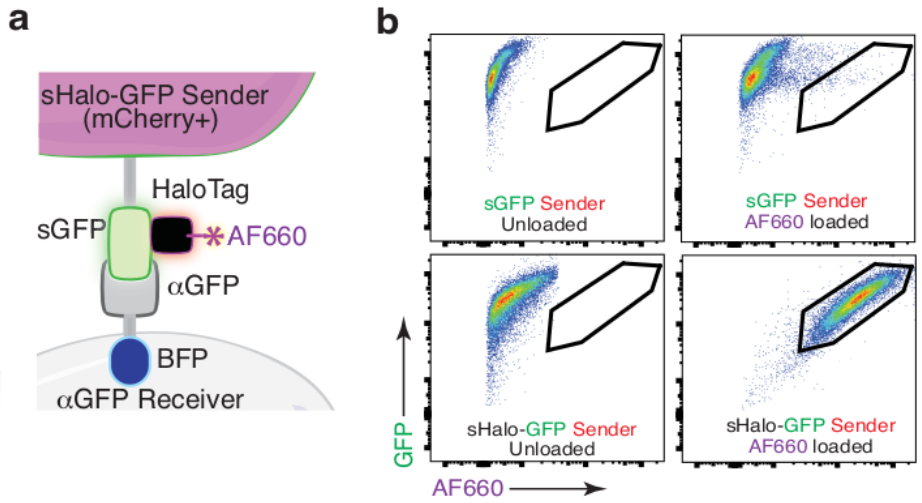
Co-expression of amCherry and aGFP on receiver cells enabled dual color labeling of receiver cells that had interacted with smCherry-expressing, sGFP-expressing, or both sender cell types



They achieved dual-color labeling of receiver cells by leveraging the ability of aGFP to bind to both sGFP and sBFP

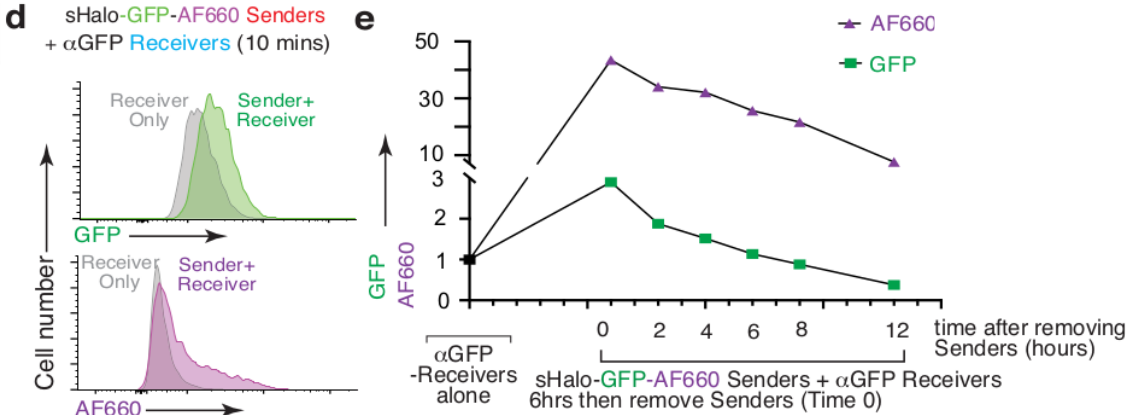
Derivatives of the G-baToN system allow for additional degrees of resolution of complex cell-cell interactions

Labeling with HaloTag-conjugated fluorophores enhances sensitivity and signal persistence



Generation of sender cells expressing the HaloTag protein fused to sGFP

Covalent attachment of a synthetic fluorophore to sHalo-GFP enabled specific loading onto sender cells



- Compared to GFP, transfer of the chemical fluorophore using sHalo-GFP-based labeling of receiver cells led to increased signal-to-noise ratio and higher sensitivity
- Changing from a protein (GFP) to a chemical fluorophore also extended the half-life of labeling, thus enabling partially tunable persistence of labeling after touching

Figure 7

The G-baToN system can function as a vehicle for molecular cargo

Can cargo molecules be co-transferred with GFP from sender cells to receiver cells?

Could other cargo be transferred to receiver cells?

Loading of:
- sGCN4-HaloTag sender cells with HaloTag conjugated
- AF647-coupled ssDNA prior to co-culture with aGCN4 receiver cells

revealed successful co-transfer of fluorescently labeled ssDNA to receiver cells

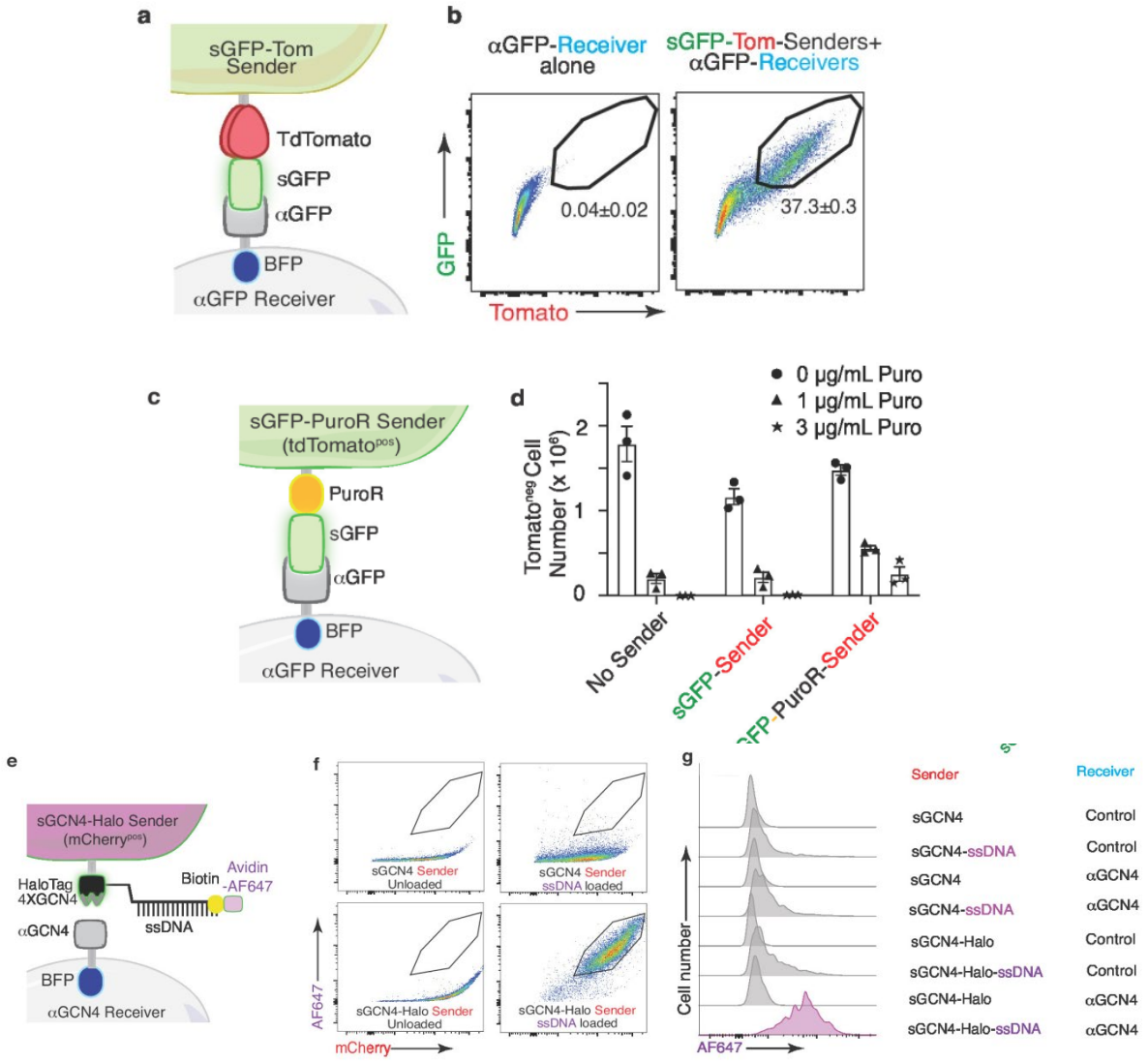


Figure 8

G-baToN systems enable contact-dependent transport of different macromolecules between cells

Discussion

- Novel cell-cell interaction reporter system
- G-baToN system can record diverse cell-cell interactions in a specific and sensitive manner
- Ability of diverse primary cell types to serve as both sender and receiver cells (suggesting that the G-baToN system is generalizable)
- Multicolor derivatives of G-baToN enable qualitative and quantitative analyses of higher order interactions involving more than two cell types
- <u>The ability to co-transfer protein, DNA and chemical cargo suggests that this platform could be leveraged to manipulate target cell function</u>

Discussion

The G-baToN system labels receiver cells through transfer of cell surface GFP which, due to its lability, ensures **only transient labeling**

Transient labeling is sufficient to label stable **cancer cell-stromal cell** interactions and many other diverse cell-cell interactions when sender cells consistently express GFP

This transient labeling should allow **dynamic interactions to be detected**, ensuring that the labeled receiver cells either are in contact with, or have recently interacted with sender cells

Discussion

-> G-baToN system is able to mediate cargo transfer

- Feasibility of transferring small molecules (HaloTag ligand), functional proteins (puromycin resistant protein, and non-protein macromolecules (ssDNA)
- **Transferred cargo proteins may be able to modify receiver cell signaling or promote cell death**
- In the future, additional design features could allow cancer cell-stromal **cell interaction dependent drug delivery** or **cell-cell interaction facilitated sgRNA transfer between interacting cells**
- Simplicity of its components, generalizability across cell types, excellent foreground to background ratio, and rapid labeling, should enable facile analysis of the dynamics of cellular interaction
- These types of approaches have the potential to have a broad impact on our ability to understand the outputs of cell-cell interactions in cancer and various other biological systems

Paper #2

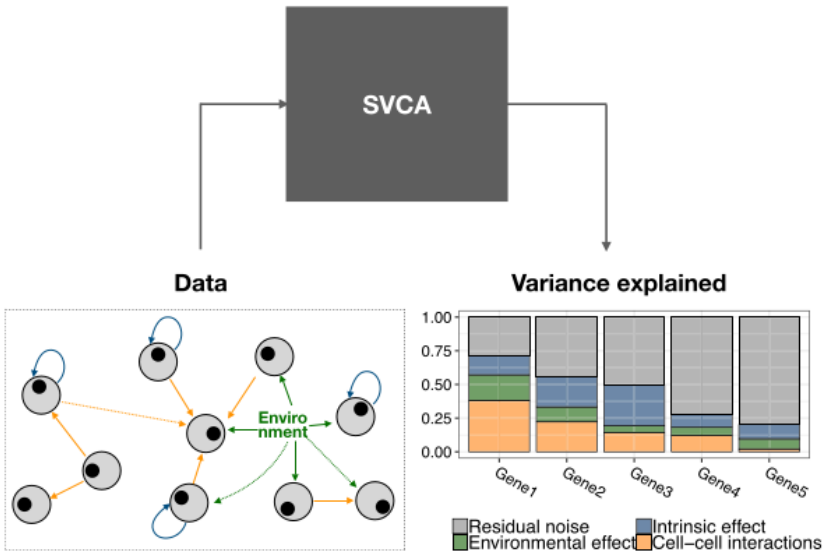
Cell Reports

Article

Modeling Cell-Cell Interactions from Spatial Molecular Data with Spatial Variance Component Analysis

Authors

Damien Arnol, Denis Schapiro,
Bernd Bodenmiller,
Julio Saez-Rodriguez, Oliver Stegle



- Statistical method for analyzing single-cell expression data in a spatial context
- Sources of gene expression variability by decomposing it into different components
- The components come from different sources
- These sources include aspects of spatial variation, in particular cell-cell interactions

Main points of the paper

- Capture molecular variations in physiological contexts: multiplexed spatially resolved RNA and protein expression profiling of individual cells
- Need of computational approaches for studying the interplay of the spatial structure of tissues and cell-cell heterogeneity
- Spatial variance component analysis (SVCA), a computational framework for the analysis of spatial molecular data

SVCA

√ Enables quantifying different dimensions of spatial variation

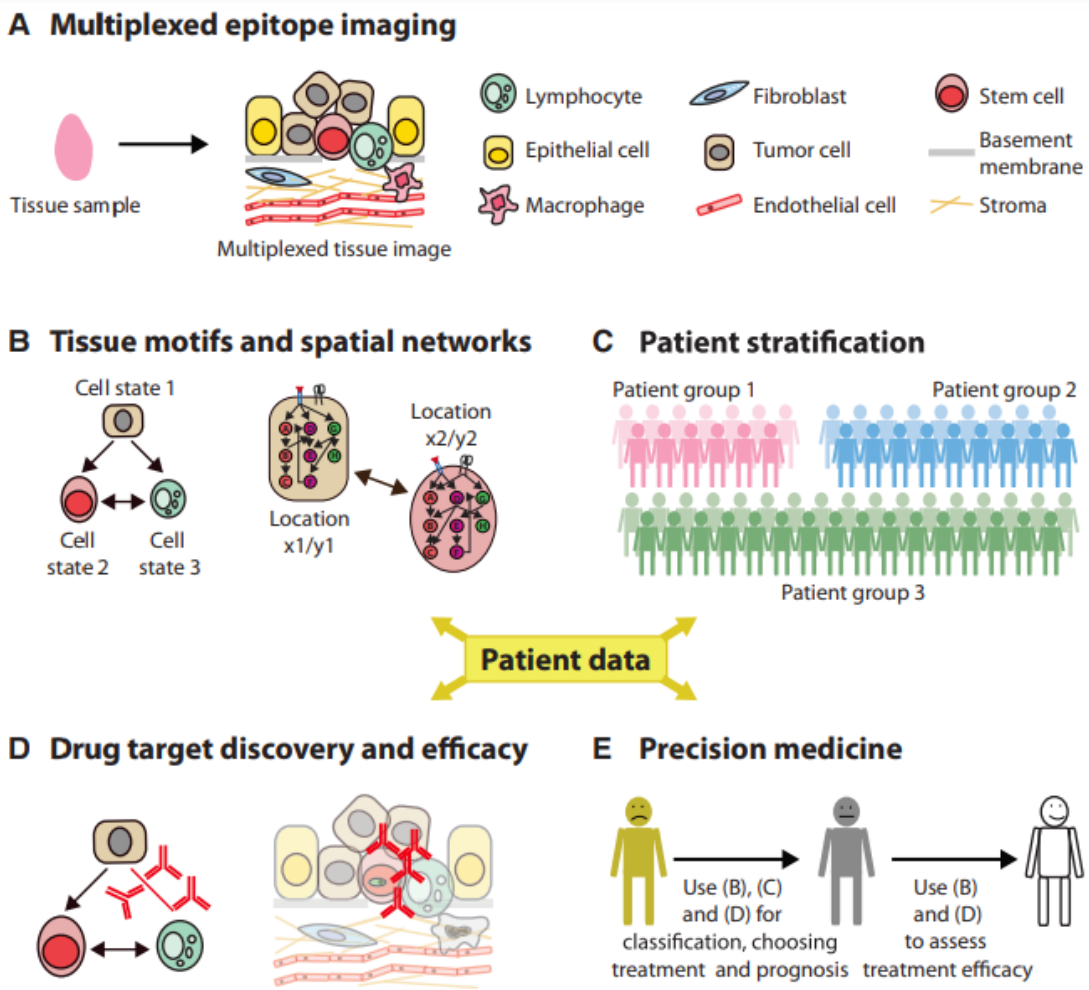
√ Quantifies the effect of cell-cell interactions on gene expression

√ In a breast cancer IMC dataset, the model yields interpretable spatial variance signatures, which reveal cell-cell interactions as a major driver of protein expression heterogeneity

√ Applied to high-dimensional imaging-derived RNA data, SVCA identifies plausible gene families that are linked to cell-cell interactions

√ SVCA is available as a free software tool that can be widely applied to spatial data from different technologies

New dimensions of gene expression variation also have the potential to deliver biomarkers in health and disease



(A) Multiplexed imaging provides a comprehensive, spatially resolved view on cell types and their state in tissues

(B) Statistical and machine learning approaches reveal tissue motifs and enable development of spatial network models

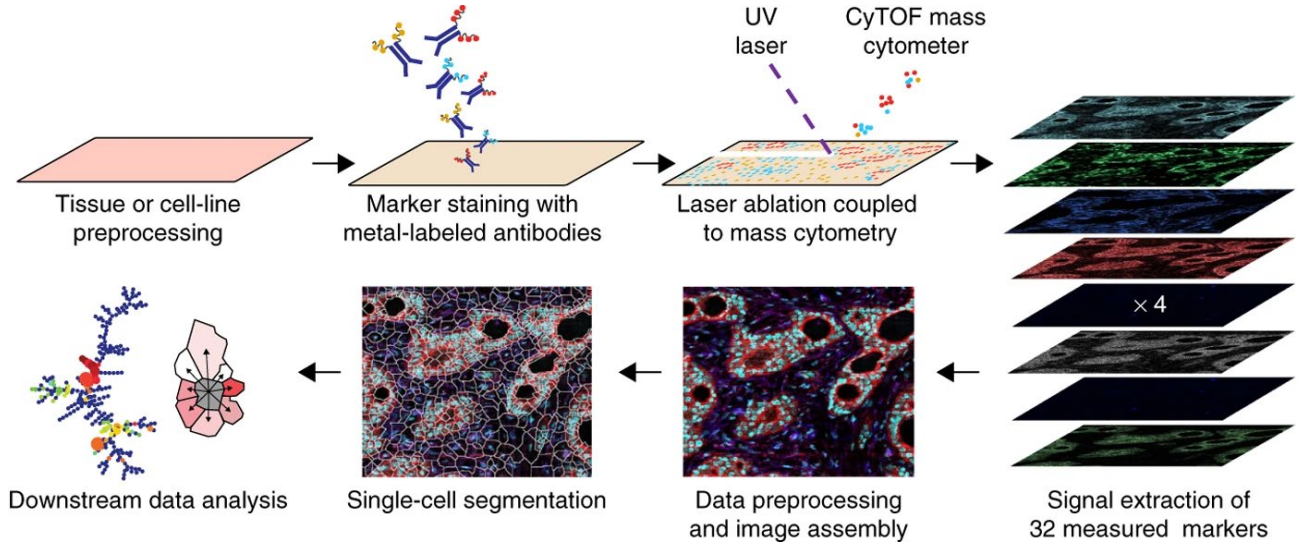
(C) Correlation of highly multiplexed epitope imaging with clinical data supports biomarker discovery

(D) Highly multiplexed epitope imaging can be used to identify drug targets and to assess how a drug is distributed in a tissue and the effects it elicits

(E) Highly multiplexed epitope imaging could be a core tool for future precision medicine applications

Technologies for profiling spatially resolved expression profiles (sources of variation)

Imaging Mass Cytometry (IMC)



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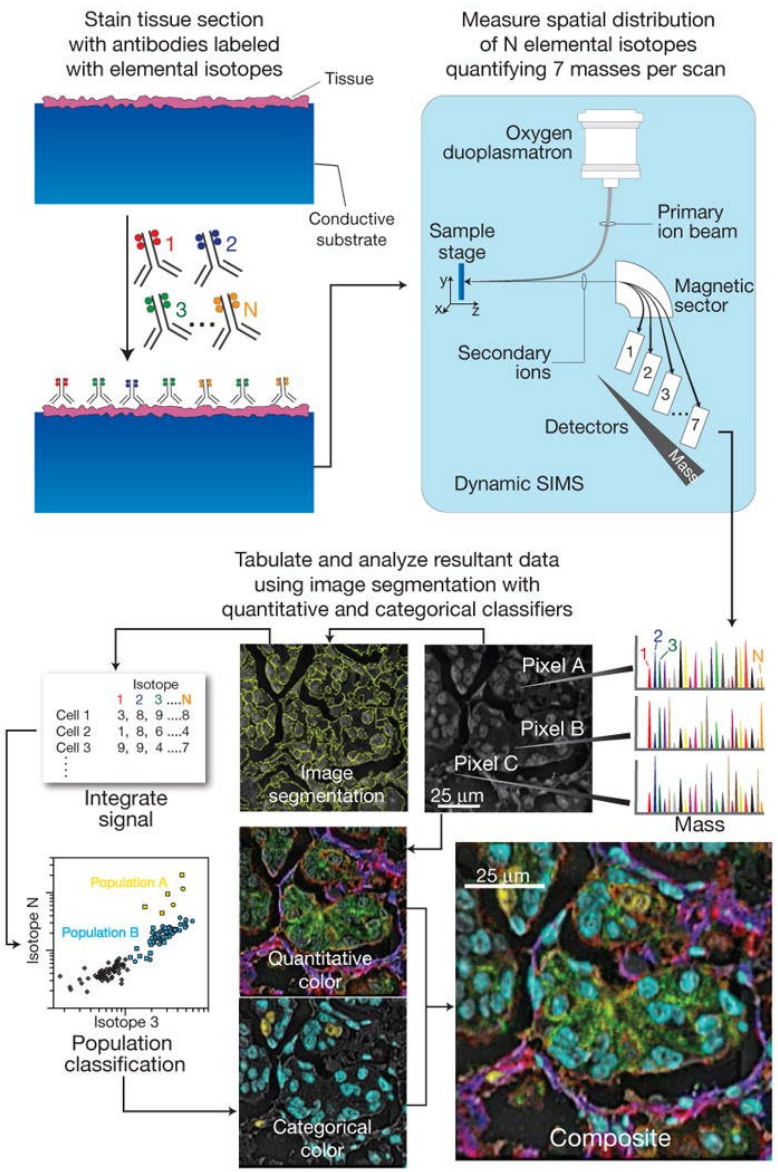
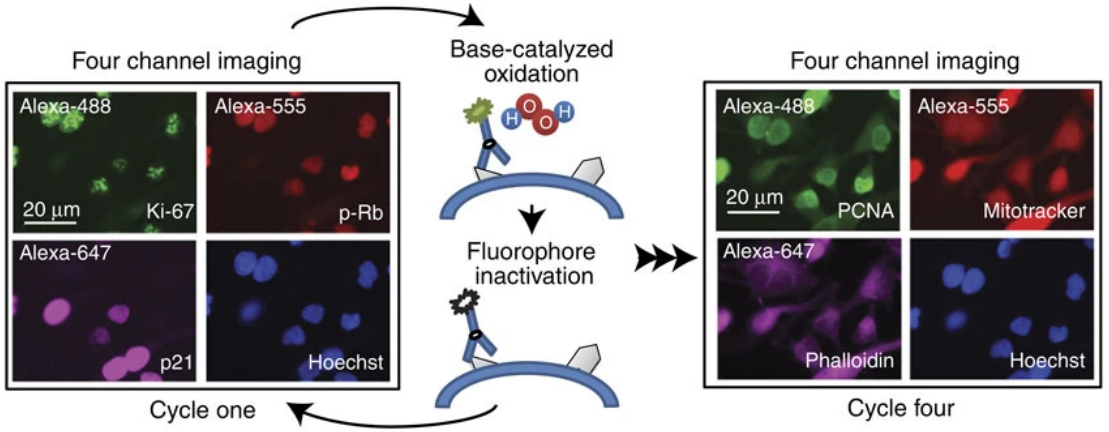
Multiplexed Ion Beam Imaging (MIBI)



Cyclic immunofluorescence (CycIF)



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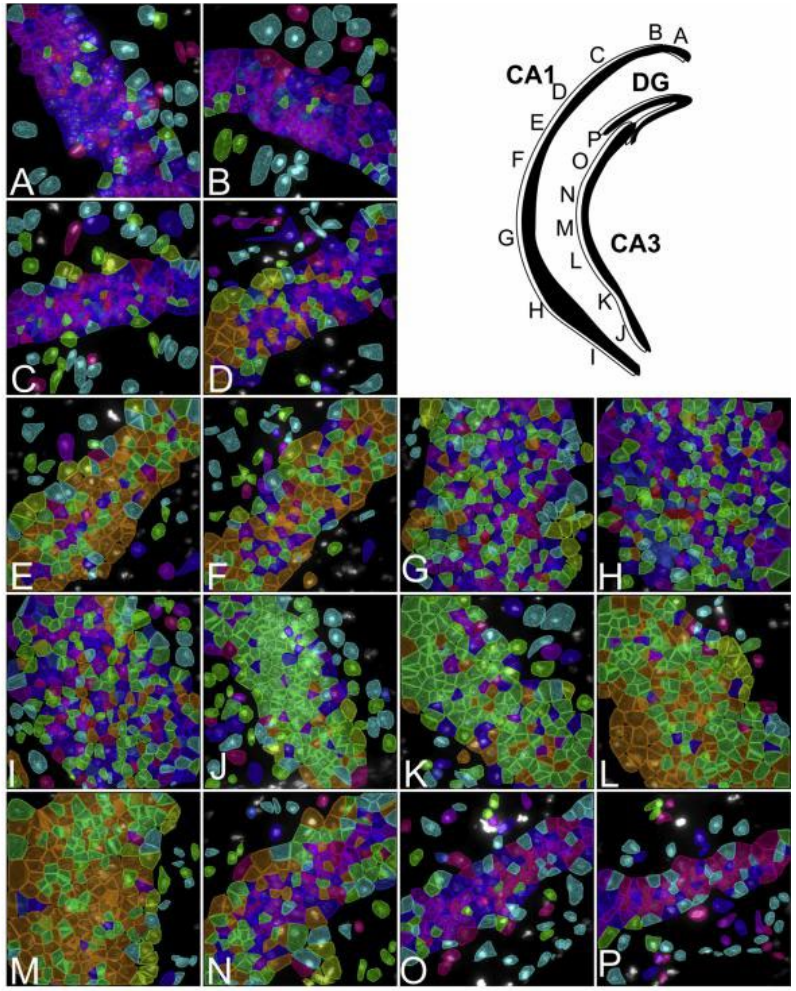
Fluorescence assays to measure single-cell RNA levels in spatial context

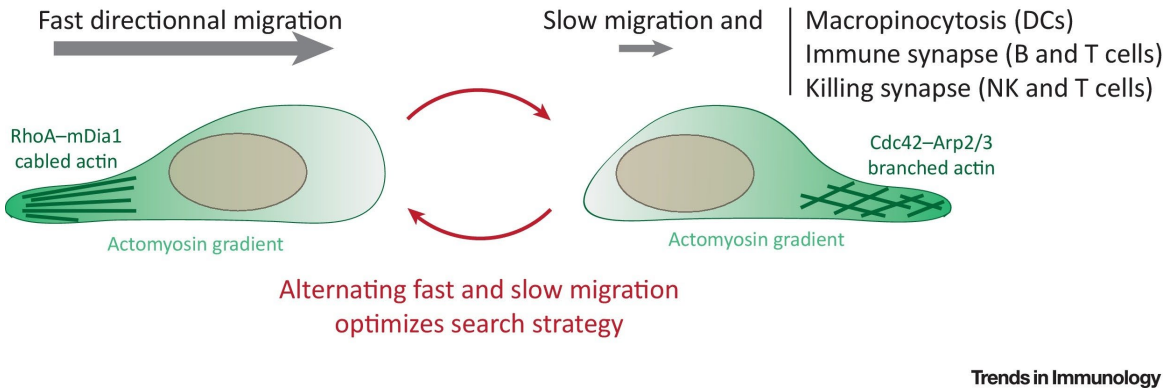
Multiplexederror robust-fluorescence in situ hybridization



Same Organization in CA1 Is
Observed Even with Only Barcoded Genes

Use of a combinatorial approach of fluorescence-labeled small RNA probes to identify and localize single RNA molecules

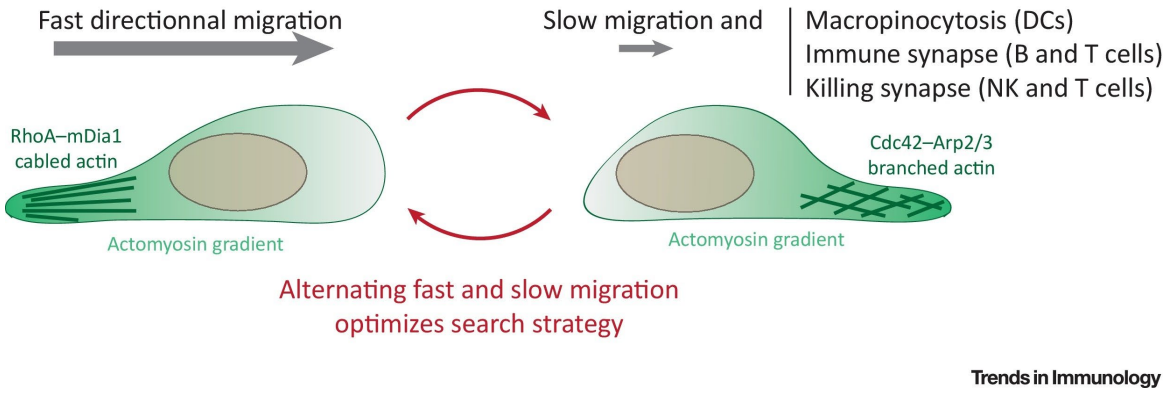
However, they currently do not offer single-cell resolution and are therefore not sufficient for studying cell-to-cell variations



Trends in Immunology

To perform their function, proximal cells need to interact via:
- direct molecular signals
- adhesion proteins
- physical contacts

Certain cell types such as immune cells may migrate to specific locations in a tissue to perform their function in tandem with local cells



To perform their function, proximal cells need to interact via:
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- adhesion proteins
- physical contacts

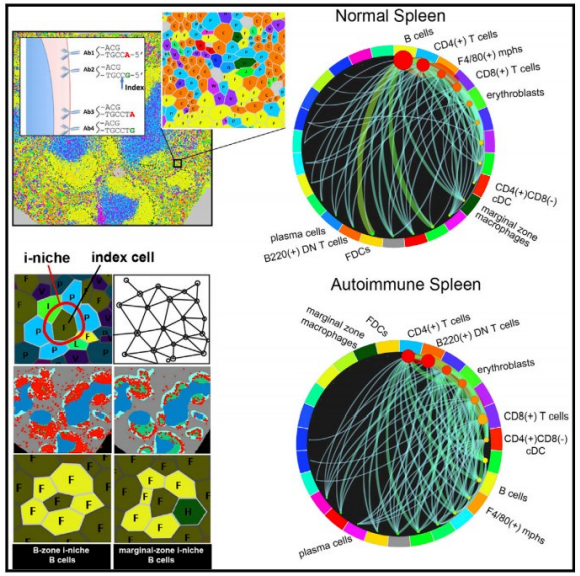
Certain cell types such as immune cells may migrate to specific locations in a tissue to perform their function in tandem with local cells

21

√ While intrinsic sources of variation have been extensively studied, cell-cell interactions are less well explored

√ Experimentally, the required spatial omics profiles can already be generated at high throughput

√ **Need of computational methods that allow for identifying and quantifying the impact of cell-cell interactions**



There exist regression-based models to assess interactions on gene expression profiles of genes based on pre-defined features that capture specific aspects of the cell neighborhood.

These models are conceptually closely related to their approach

However, they rely on the careful choice of relevant features

They tend to require specific discretization steps to define cell neighborhoods

22

Their approach

- SVCA models spatial sources of variation of individual genes
- SVCA allows for decomposing gene expression variation into **intrinsic effects**, **environmental effects** and an explicit **cell-cell interaction component**
- In contrast to previous methods, their model directly uses the spatial coordinates and the gene expression profile of each cell as input, thereby avoiding the need to define discrete cell types and other microenvironmental variables

They applied SVCA to two datasets from different technologies and biological domains:

- IMC proteomics profiles data from human breast cancer tissue

- Spatial single-cell RNA profiles from the mouse hippocampus generated using seqFISH

Across these domains, they find that the cell-cell interaction component in their model explains a **major share of expression variability**, thus facilitating the **identification of biologically relevant genes and pathways** that participate in cell-cell interactions

SVCA: A Statistical Framework for Decomposing Spatial and Non-spatial Sources of Variation

B

$$Y \sim \mathcal{N}(0, \mathbf{K}_{int} + \mathbf{K}_{env} + \mathbf{K}_{c-c} + \mathrm{I})$$

- Cell-cell covariance due to the intrinsic effect
- Cell-cell covariance due to environmental effects
- Cell-cell covariance due to cell-cell interactions
- Independent observational noise (identity matrix)

A

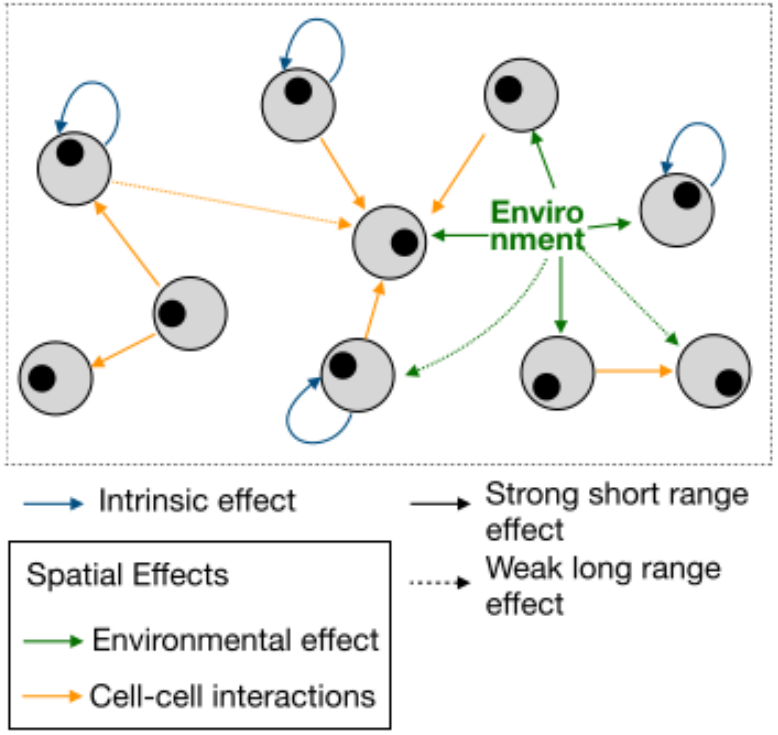


Figure 1

SVCA builds upon the random effect framework to model gene expression variation of individual genes as a function of:

U int : additive components of intrinsic cell state effects
U env: an environmental effect linked to the cell position
U c-c: an effect due to cell-cell interactions

Y = U int + U env + U c-c + e

Y denotes the vector of the expression levels of a gene of interest across all cells and **e** denotes Gaussian measurement noise

The intrinsic cell-state covariance K int is estimated based on the expression profiles of all genes except the focal gene:

$$(K_{int})_{i,j} = \sigma^2_{int} X_i \cdot X_j^T$$

The covariance for the environmental context K env is calculated based on the pairwise distance of all genes

$$K_{env} = \sigma^2_E exp(-d^2_{i,j}/2l^2)$$

This component captures differences in the (local) environment or technical drift in the measurement process

The cell-cell interaction covariance term K c-c quantifies the similarity of the cellular composition in the neighborhood of cells

SVCA: A Statistical Framework for Decomposing Spatial and Non-spatial Sources of Variation

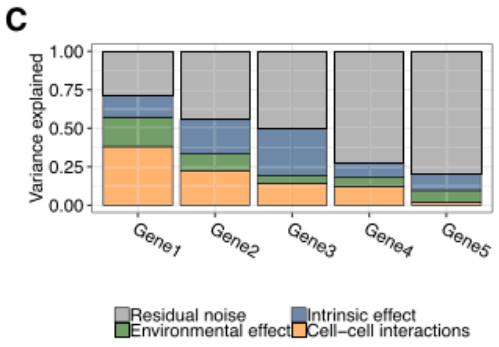


Figure 1

- Breakdown for each gene of the fraction of variance explainable by spatial and non-spatial variance components, yielding a compact representation of major drivers of gene expression variation.
- SVCA can be used to assess the statistical significance of individual variance terms, using model comparisons between the full SVCA model and reduced models in which individual covariance terms are omitted
- Finally, SVCA can also be used to predict expression profiles of held-out cells

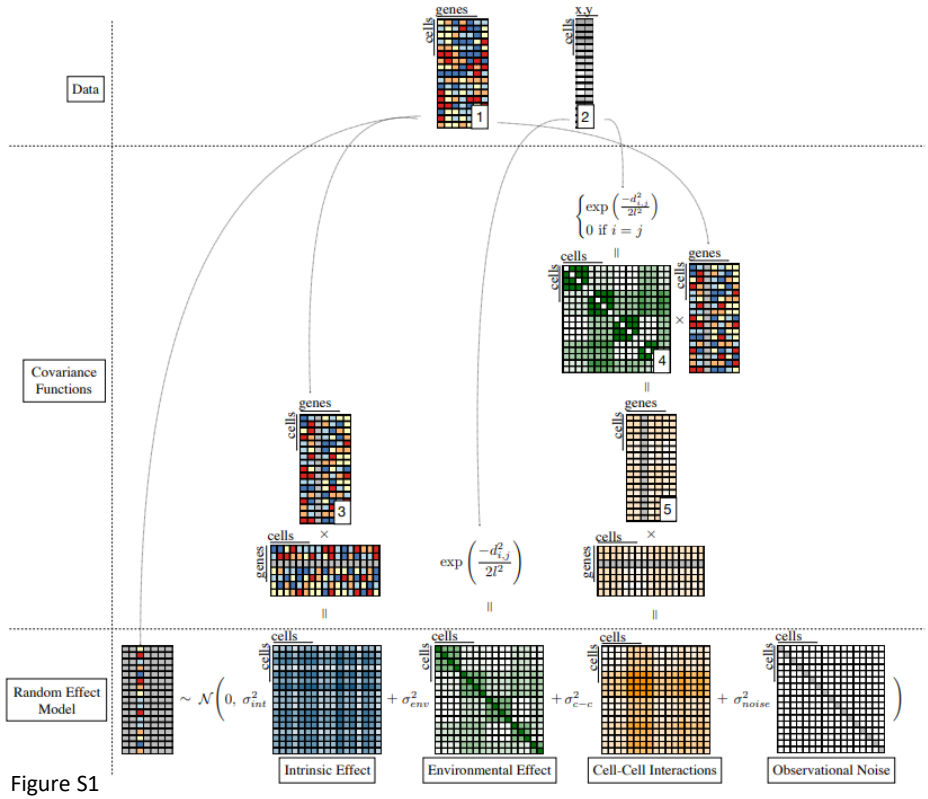


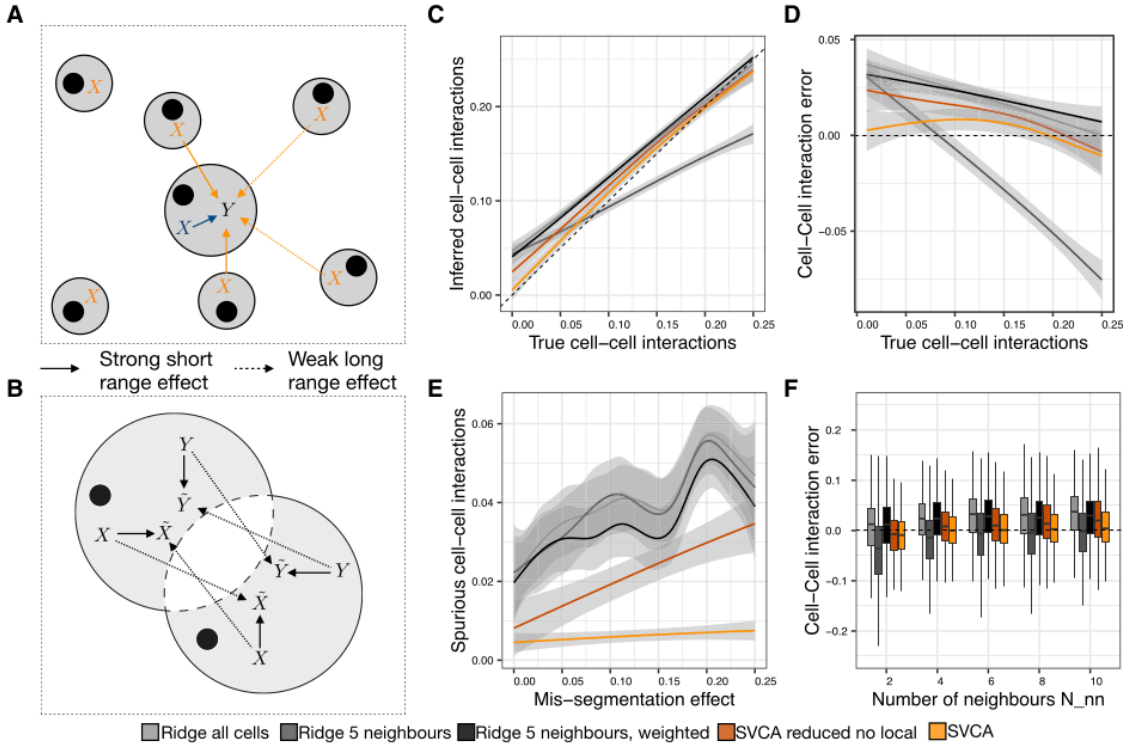
Figure S1

This covariance term corresponds to a linear regression that models the effect of the cell expression profile on the expression of the gene of interest

- SVCA does not require discrete cell-type assignments, but instead is based on continuous measures of cell-cell similarity that are directly estimated from cell expression profiles
- The model also circumvents the need to define local cell neighborhoods, but instead weights interactions between pairs of cells as a function of their distance

They demonstrate that SVCA can be used to estimate and test for spatial drivers of single-cell variability, in particular cell-cell interactions

SVCA Yields More Accurate Cell Interaction Estimates than Alternative Models

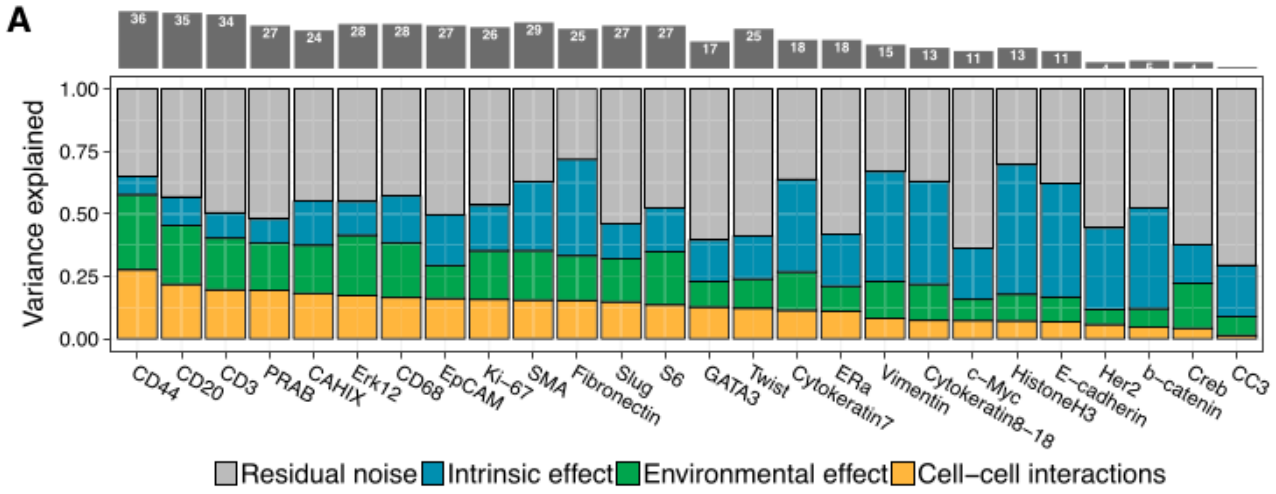


- They considered a more complex simulation using empirical parameters derived from 11 real datasets, to compare SVCA to alternative models
- They stimulated gene expression profiles based on a linear model that accounts for intrinsic effects and cell-cell interactions of variable size, as well as confounding effects due to cell mis-segmentation

(A) Simulation approach: the expression profile of a simulated target gene Y is generated as a linear combination of the empirically observed cell expression profile of all genes (X) and a linear combination of the N nn first neighbors expression profiles (X) (here, N nn = 4). The effect of the first neighbors is weighted by the function of their distance to the focal cell.

Figure 2

Application of SVCA to a Breast Cancer Proteomics Dataset Identifies Cell-Cell Interactions as a Major Driver of Expression Variation



CAHIX, a marker of hypoxia, was also found among the top markers linked to cell-cell interaction effects

What they did:

Application of SVCA to an IMC dataset from human breast cancer

26 protein expression levels were quantified at the single-cell level in 46 breast cancer biopsies

What they found out:

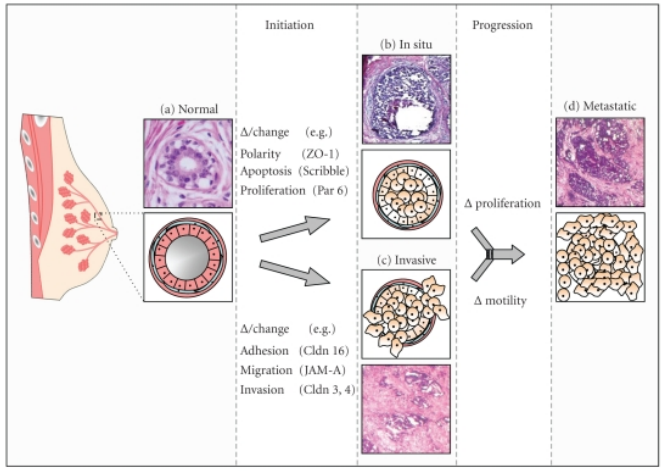
SVCA revealed substantial differences of the overall importance of cell-cell interaction components across proteins, explaining up to 25% of the total expression variance on average

Immune cell markers in particular were identified among the set of proteins with the largest cell-cell interaction effects: CD44, CD20, CD3, and CD68, for which cell-cell interaction explained more than 10% of the variance

Hypothesis: this effect could reflect the recruitment of immune cells by specific cellular environments

Figure 3

Application of SVCA to a Breast Cancer Proteomics Dataset Identifies Cell-Cell Interactions as a Major Driver of Expression Variation

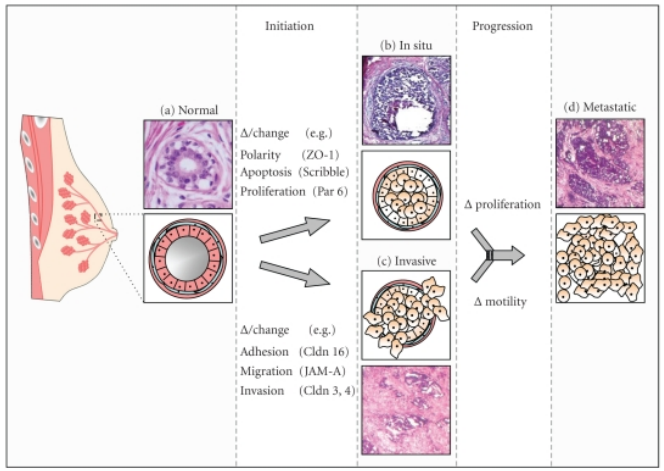


How SVCA signatures are affected by these environmental features?

- They discovered a significant correlation between <u>the average number of neighbors per cell and the average cell-cell interaction components across proteins</u> (using cellProfiler to estimate the number of cells)

- This relationship may in part explain the separation by tumor grade

Application of SVCA to a Breast Cancer Proteomics Dataset Identifies Cell-Cell Interactions as a Major Driver of Expression Variation



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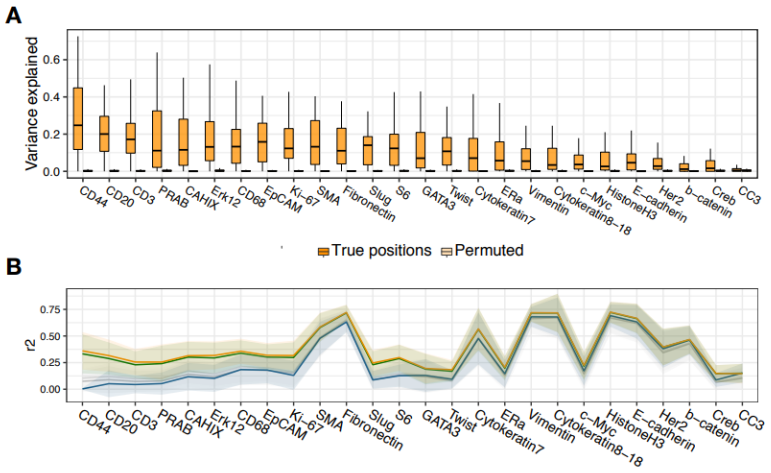


Figure S3

- <u>Substantial variation of the estimated spatial variance signatures between images</u>
- Motivating investigation of the relationship between spatial variance components and clinical covariates, including tumor grade

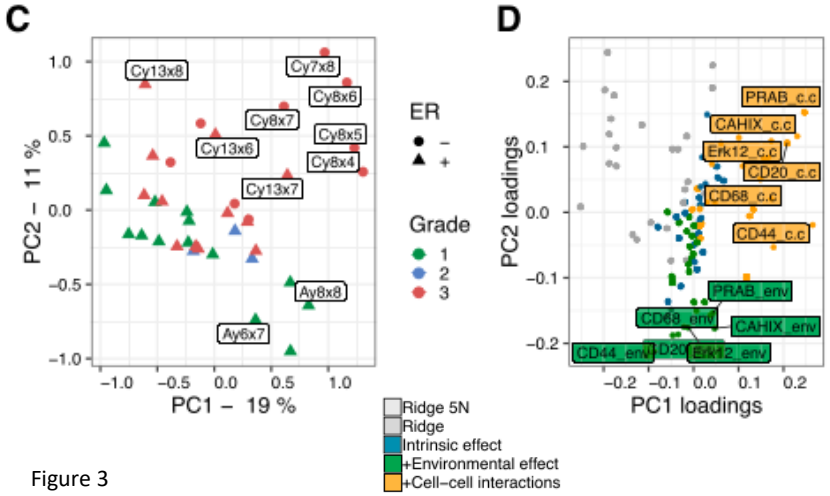
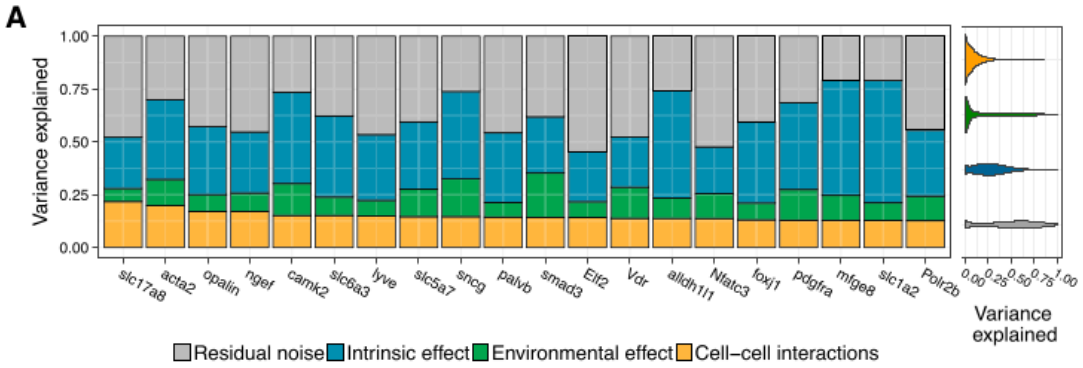


Figure 3

- Sub-structure between images that was significantly aligned with tumor grade
- Cell-cell interaction component and the environmental component for a subset of proteins (including CD20 and CD44) as the most informative SVCA features for PC1, which correlates with tumor grade.

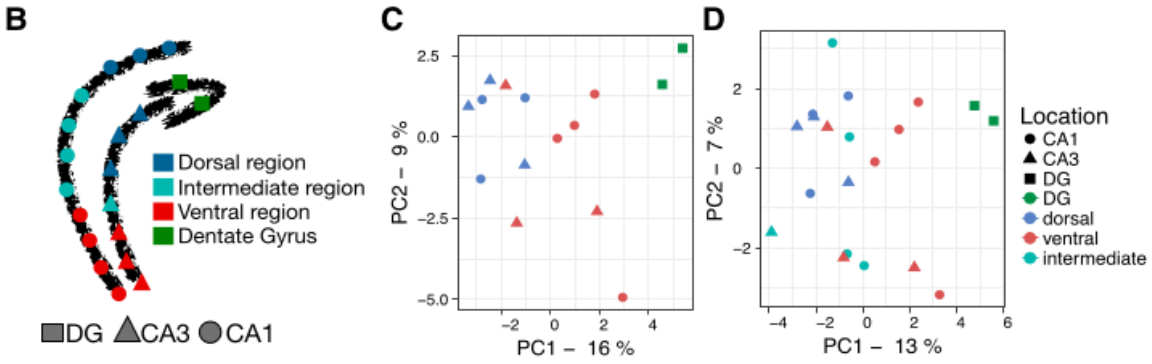
Application of SVCA to an Hippocampus RNA Dataset Identifies Relevant Gene Families Involved in Cell-Cell Interactions

Mouse hippocampus dataset profiled using seqFISH
249 RNA expression levels were assayed in 21 distinct brain regions of a single animal



Spatial variance signatures for the 20 genes with the largest cell-cell interaction component

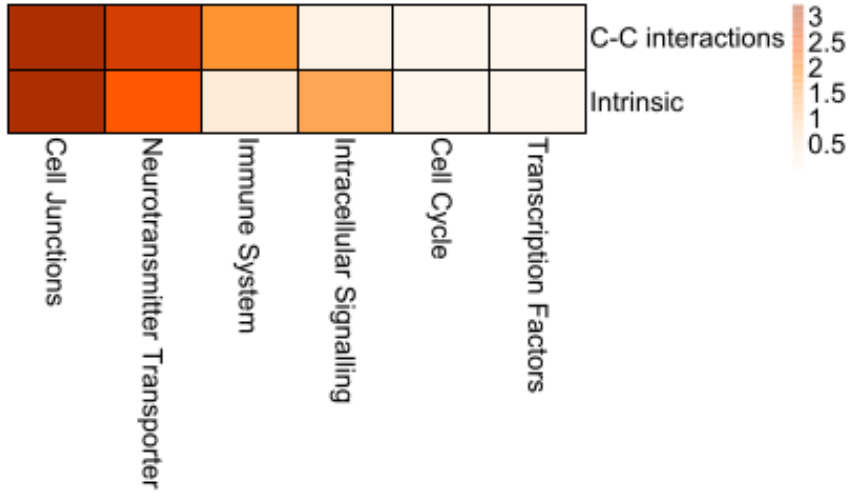
-> Differences in the spatial variance signatures across images, which were sampled from functionally distinct regions of the hippocampus.



- Principal components of the spatial variance signature for the dorsal region clustered together, irrespective of their CA1/CA3 location
- Similarly, images from the dentate gyrus (DG) also clustered together
- This is consistent with the observation by Shah et al. (2017) that the ventral and dorsal regions of the CA1 and CA3 mirror each other with respect to their cellular compositions and ventral regions are more heterogeneous in their cellular composition
- Spatial variance signatures for intermediate regions, however, did not show much resemblance

Application of SVCA to an Hippocampus RNA Dataset Identifies Relevant Gene Families Involved in Cell-Cell Interactions

Identification of gene families that participate in cell-cell interactions



Which of the selected genes categories are enriched for large cell-cell interaction components?

-> Cell junction genes and neurotransmitter transporters were the most enriched groups

- Individual cell junction genes, such as GJA1 (connexin), are involved in gap junction intercellular communication

- Actin skeleton has a known role in the adaptation of tissue structure and geometry to external stimulus

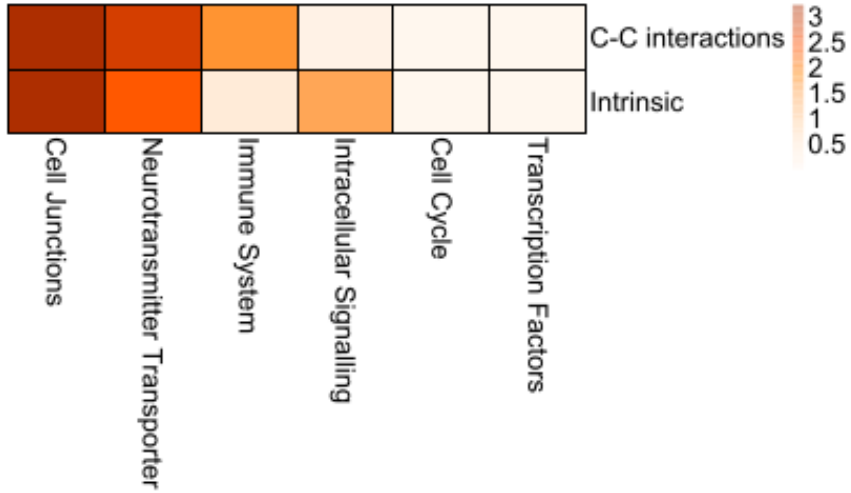
Single-cell expression levels of cell junction genes appeared to be regulated by cell-cell interactions.

The enrichment of glutamate transporters is also consistent with their involvement in the transport and (re)uptake of the neurotransmitter at the neuronal synapses, a critical cell-cell interaction in the brain.

In addition, Slc5a7 (CHT) was also found to be preferentially expressed in specific interneurons with a link to the spatial organization of the tissue

Application of SVCA to an Hippocampus RNA Dataset Identifies Relevant Gene Families Involved in Cell-Cell Interactions

Identification of gene families that participate in cell-cell interactions



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In addition, Slc5a7 (CHT) was also found to be preferentially expressed in specific interneurons with a link to the spatial organization of the tissue

Genes with large cell-cell interaction components, as identified using SVCA, have known implications in cell-cell communication between neurons or for regulating the spatial architecture of the tissue.

Discussion

- SVCA, a regression-based framework for the analysis of spatially resolved molecular expression data
- The model computes a spatial variance signature for individual mRNA or protein levels, decomposing their sources of variation into spatial and non-spatial components
- SVCA provides a quantitative assessment of the effect of cell-cell interactions on the expression profile of individual molecules
- SVCA tackles the problem of cellular classification and neighborhood definition using a continuous representation of space and cellular identity
- Application of SVCA to multiple datasets generated using alternative technologies, probing either RNA transcripts or proteins, demonstrating the broad applicability of the approach
- Cell-cell interactions can substantially contribute to gene expression variation,which is consistent with previous reports
- Studying single-cell expression in the native context is important for understanding the sources of these variations

Discussion

What are the causes of many variations in the SVCA signatures across images?

-> differences in SVCA signatures could result from differences in the spatial structure of tissue, different clinical and biological contexts, different tissue organizations between samples.

What are the spatial variance signatures of individual genes and pathways?

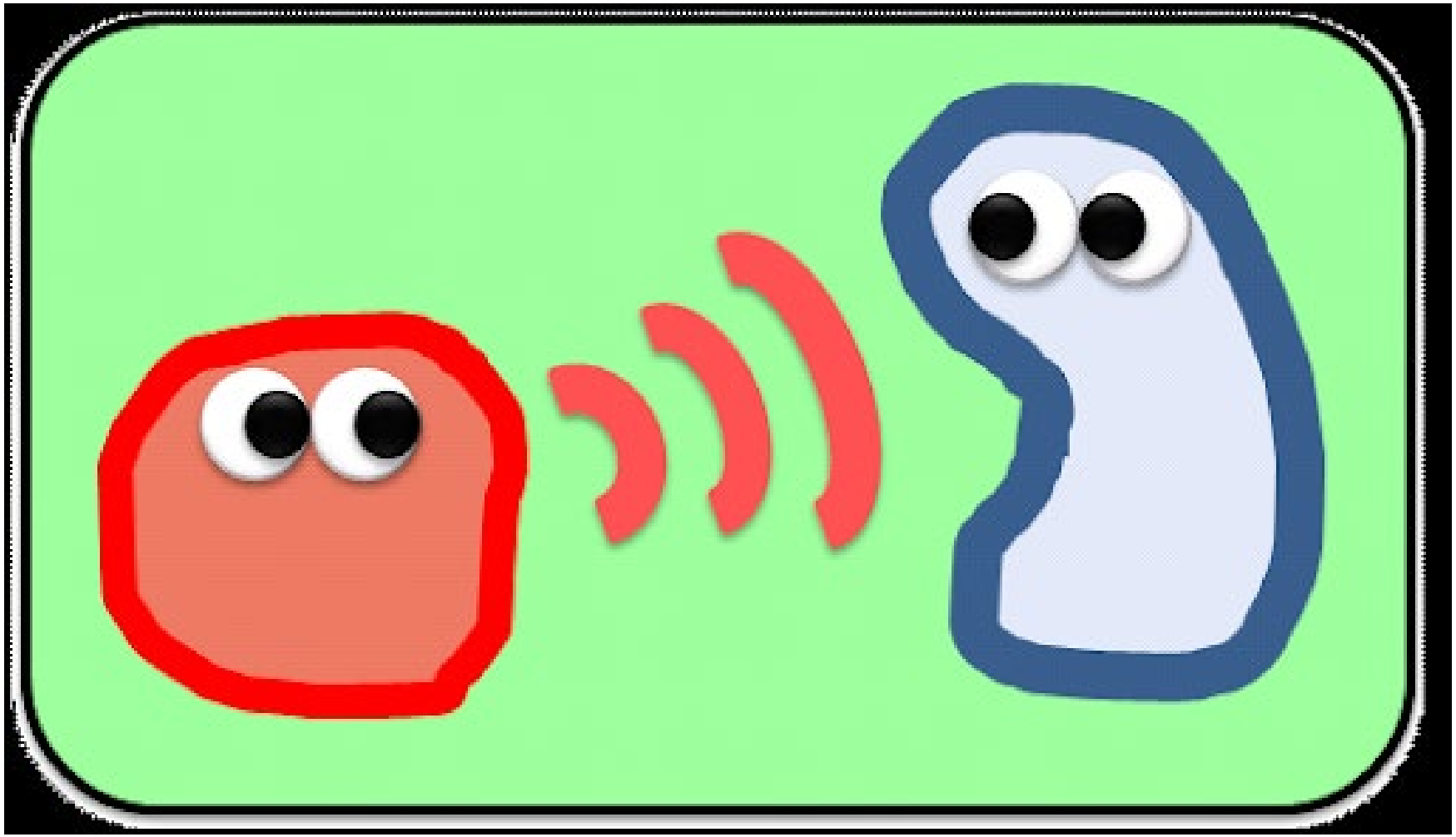
-> SVCA indentifies genes with known involvements in cellular interactions, even specific to the brain, such as SLCs, to be predominantly enriched in the corresponding terms of our models.

To confirming the biological relevance of SVCA signatures, these results suggest that spatial variance signatures can be utilized to study the involvement of individual genes in tissue-level functions.

Spatial distribution of proteins, transcripts, and other molecules is important in determining tissue functioning and its deregulation in disease

It has a potential value as predictors of clinical outcomes

Thank you for your attention!



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3. By Kbufdbqjubmjtn - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=89865457
4. https://www.berthold.com/en/bioanalytic/knowledge/glossary/fret/
5. https://www.creative-biostructure.com/x-ray-crystallography-platform_60.htm
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