Investigation of the heterogeneity of cancer cells using single cell Ca^{2+} profiling.

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Abstract

Calcium (Ca^{2+}) is an essential and ubiquitous second messenger that controls numerous cellular functions. The regulation of intracellular Ca^{2+} oscillations defines a Ca^{2+} signature that is representative of the cellular identity and the phenotype of a cell. In cancers, aberrant Ca^{2+} fluxes have been associated with characteristics such as migration, proliferation or resistance to chemotherapy. However, it remains unclear whether cancer cells have specific Ca^{2+} signatures that are representative of their oncogenic properties. To reveal the existence of oncogenic Ca^{2+} signatures, we develop a method combining single cell calcium imaging and unsupervised and supervised machine learning approaches. Briefly, we generated a database of 27,439 single-cell agonist-induced Ca^{2+} responses obtained from 16 prostate and colon cancer cell lines. Graph-based unsupervised clustering was used to define and compare Ca^{2+} signatures of cancer cell lines. A supervised neural network model trained on the collection of agonist-induced Ca^{2+} responses successfully identify individual cancer cells and predicted some of their characteristics. We extend these methods to observe Ca^{2+} signatures of docetaxel-resistant cancer cells and in a co-culture model mimicking the interaction of cancer cells with cancer-associated fibroblasts. Our models successfully highlight subtle changes in those Ca^{2+} signatures and are able to distinguish individual cancer cells from fibroblasts and predict their sensitivity to Docetaxel. Taken together, this study provides a proof of concept that cancer cells can be defined by their Ca^{2+} signature and conversely that Ca^{2+} signaling can serve as a predictor of cancer cell characteristics.

Introduction

Calcium (Ca^{2+}) is an essential and ubiquitous second messenger that controls numerous cellular functions (proliferation, apoptosis, migration, metabolism, etc.) and has been implicated in many pathologies including cancer. Ca^{2+} signaling relies on the finely tuned oscillations of the cytosolic Ca^{2+} concentration induced by components of the Ca^{2+} signaling toolkit (ion channels, pumps and ion exchangers, ...) in response to input signals (Brodskiy & Zartman, 2018; Berridge *et al*, 2003).

The spatio-temporal regulation of these cytosolic Ca^{2+} oscillations, the so-called "Ca^{2+} signature", is essential to define the appropriate cellular response (Berridge *et al*, 2000). Different phenotypes, cell types or tissues will exhibit different characteristics of cytosolic Ca^{2+} oscillations (duration, frequency, amplitude, …) (Smedler & Uhlén, 2014; Boulware & Marchant, 2008; Evans & Blackwell, 2015; Gryshchenko *et al*, 2018). For example, hippocampal neurons and cortical / cerebellar astrocytes show specific profiles of agonist-induced Ca^{2+} responses (James *et al*, 2011; Swain *et al*, 2018). Proliferating or migrating endothelial cells show distinct Ca^{2+} responses to extracellular concentration of VEGF (Noren *et al*, 2016). Subtypes of dorsal root ganglion (DRG) neurons, defined by their response to Veratridine, exhibit different profiles of Ca^{2+} oscillations (Mohammed *et al*, 2017). This heterogeneity of Ca^{2+} responses allows for the complexity of patterns associated with Ca^{2+} signaling, contributing to the diversification of cellular responses (Yokota *et al*, 2015; Scheuss *et al*, 2006).

Dysregulation of proteins that regulate Ca^{2+} signaling has been associated with various pathologies, including cancer (Marchi *et al*, 2020; Terrié *et al*, 2019; Guéguinou *et al*, 2014; Romito *et al*, 2022). Ca^{2+} fluxes have been linked to cancer hallmarks such as migration, proliferation or chemotherapy resistance (Marchi *et al*, 2020; Monteith *et al*, 2017; Chen *et al*, 2019; Figiel *et al*, 2019). Interestingly, the aberrant expression of ion channels is heterogeneous and depends on the type of cancer or the aggressiveness of the tumor (Chen *et al*, 2019; Bruce &

James, 2020; Perrouin-Verbe *et al*, 2019). However, if the Ca^{2+} signature of cancer cells reflects this heterogeneous expression of Ca^{2+} signaling toolkit remains to be investigated.

Defining a Ca^{2+} signature is a complex task that requires identifying patterns of Ca^{2+} responses that are able to discriminate different clusters of responses. Different parameters (amplitude, duration, number of oscillations, latency, …) have been proposed to quantify and classify Ca^{2+} oscillations (Bootman *et al*, 2013). Several studies have proposed unsupervised machine learning (kMeans) approaches to cluster neurons according to their Ca^{2+} oscillations (Swain *et al*, 2018; Wang *et al*, 2024). More advanced deep learning algorithms have been used to identify patterns of Ca^{2+} oscillations in the cortex and suprachiasmatic nucleus that are associated with locomotor function and circadian rythms, respectively, in rodents (Wang *et al*, 2024; Ajioka *et al*, 2024). In cancer cells, molecular studies have documented the contribution of store-Operated Ca^{2+} entry (SOCE), transient receptor potential (TRP) channels, and voltage-gated Ca^{2+} channels in the regulation of Ca^{2+} signaling (Chen *et al*, 2019). However, the heterogeneity of Ca^{2+} signaling and the definition of Ca^{2+} signatures associated with cancer development remain to be defined.

To identify Ca^{2+} signature in cancer cells and investigate their heterogeneity according the oncogenic properties of cancer cells, we collected agonist-induced Ca^{2+} responses of 27,439 single cancer cells originating from a panel of 16 prostate and colorectal cancer cell lines. From these Ca^{2+} responses, an unbiased unsupervised clustering inspired by single-cell transcriptomic workflows (Satija *et al*, 2015) generates Ca^{2+} signatures for each cancer models. In parallel, we develop a supervised neural network that predicts characteristics of a single cancer cell based on its profile of Ca^{2+} responses. These approaches allow to investigate the remodeling of Ca^{2+} signatures in various pathological contexts. Here, we applied both unsupervised and supervised approaches to described a remodeling of Ca^{2+} signatures associated with acquired docetaxel resistance or in the course of the interaction of cancer cells with fibroblasts. At single cell level,

our supervised neural network succeeded to identify docetaxel-resistant cancer cells and to diistinguish fibroblasts from cancer cells.

Taken together, our study proposes a new method to functionally investigate the cancer cell heterogeneity at the single cell level. Our study demonstrates the potential of Ca^{2+} profiling for discriminating cancer cells and predict their phenotypic characteristics, and provides a framework for researchers to investigate the remodeling of the Ca^{2+} signature during cancer development.

Results

Calcium (Ca^{2+}) signaling pathway is aberrantly regulated in multiple cancers.

Aberrant expression of proteins associated with Ca^{2+} signaling pathways has been reported in several cancers (Marchi *et al*, 2020; Monteith *et al*, 2017). Here, we performed a survey of 16 different cancers using transcriptomic data from The Cancer Genome Atlas (TCGA) consortium (Data ref: The Cancer Genome Atlas, https://portal.gdc.cancer.gov/). Compared to non-cancerous tissues, the gene set associated to the "KEGG Ca^{2+} signaling pathway" is significantly misregulated in primary tumors derived from 10 cancer types **(Fig. S1a)**. The differential expression of genes associated with the Ca^{2+} signaling toolkit in tumors is highly heterogeneous **(Fig. S1b)**. In colon and prostate cancers, both characterized by a downregulation of the Ca^{2+} signaling pathway, we observed distinct expression patterns of genes associated with this pathway (Data ref: Abeshouse *et al*, 2015; Muzny *et al*, 2012) (**Fig. S1c**). Taken together, this suggests that despite the common misregulation of Ca^{2+} signaling pathways across cancer types, cancer cells may exhibit heterogeneous expression profiles of the Ca^{2+} signaling toolkit, which may be translated into heterogeneous functional Ca^{2+} signals representative of their origin or characteristics.

Single cell Ca^{2+} imaging of cancer cell lines.

To test this hypothesis, we proposed to define Ca^{2+} signatures of prostate (PCa) and colorectal (CRC) cancer cell lines using an unbiased approach combining single cell Ca^{2+}

imaging, unsupervised and supervised machine learning algorithms (**Fig 1.**). Following the Depmap project, we selected cancer cell lines representative of the transcriptomic profiles of prostate and colon adenocarcinomas (**Fig. S1d**) (Data ref: Warren *et al*, 2021). Each cell line was described by the cancer of origins (CRC or PCa), the site of isolation (non-cancerous tissues, primary tumor, or metastatic tumors) and the EMT score (epithelial or mesenchymal) computed using the Kolmogorov-smirnov method (Mandal *et al*, 2021). Cancer cells are non-excitable cells also it is not expected to observe spontaneous Ca^{2+} transients in these models. To have an overview of the variety of Ca^{2+} responses elicited by cancer cells, we propose to evaluate agonist-induced Ca^{2+} responses. We assayed for a panel of agonists whose receptors interact with proteins associated with the Ca^{2+} signaling toolkit. This allows the Ca^{2+} signaling toolkit to be stimulated in a variety of ways (**Fig. S1e**). Selected agonists included Adenosine Tri-phosphate (ATP), Lysophosphatidic Acid (LPA), Acetylcholine (Ach.), Platelet-Aggregating Factor (PAF), Histamine (Hist.), Epidermal Growth Factor (EGF), and Prostaglandin E_2 (PGE_2). Thapsigargin (Tg) and the vehicle (Veh.) were used as positive and negative controls, respectively, for the Ca^{2+} response. Aside from Thapsigargin, all of the agonists have been shown in various models to influence Ca^{2+} signaling and impact key tumor phenotypes, such as proliferation and migration [ATP: (Shukla *et al*, 2024), Ach: (Bele *et al*, 2024), LPA: (Takai *et al*, 2024), PFA: (Tsoupras *et al*, 2009), EGF: (Crottès *et al*, 2019), PGE2: (Santiso *et al*, 2024), Histamine: (Azimi *et al*, 2024)]. Some of these agonists are also implicated in inflammatory cancers and/or play central roles in tumor development. All 16 cell lines were stimulated with each of 8 agonists. A total of 27,439 single cell Ca^{2+} responses were acquired and processed (**Fig S2**).

Over the years, a variety of parameters have been proposed to analyze and quantify Ca^{2+} signals (Bootman *et al*, 2013). Considering the diversity and complexity of the acquired signals, we proposed here to limit our analysis to the description of the peak amplitude, the latency, the altered basal level, the number of oscillations and the proportions of responding cells (Bootman *et al*, 2013). The values obtained showed a large variability but a relatively uniform distribution (**Fig S3**). When comparing cancer cells according the cancer of origins (CRC vs PCa), the site of

isolation (Primary, Metastatic or non-cancerous tissues) or the EMT Score (Epithelial vs Mesenchymal), we observed similar distributions of values of each parameter **(Fig S4 and S5)**. This large heterogeneity of agonist-induced Ca^{2+} responses makes it difficult to define a Ca^{2+} signature based on a few parameters. To circumvent these problems, we proposed to analyze the whole signal using unsupervised machine learning tools and propose an unbiased clustering of the profiles of Ca^{2+} responses.

Unsupervised clustering of heterogeneous agonist-induced Ca^{2+} responses identified Ca^{2+} signature of cancer cell lines.

In analogy to the clustering workflow used in single-cell RNA-Seq (Satija *et al*, 2015; Patterson-Cross *et al*, 2021), we developed an approach that sequentially reduced the number of dimensions by principal component analysis (PCA), built a shared-nearest-neighbor network on the first 20 components, and clustered the nodes of the network using a Louvain algorithm (Blondel *et al*, 2008) (**Fig 2a and Fig S6**). This approach identified 5 groups of Ca^{2+} responses subdivided into 26 clusters (**Fig 2b-c** and **Fig S7**). Each cluster and group of clusters gathers single cell Ca^{2+} responses with particular characteristics. Thus, clusters of Ca^{2+} responses associated with groups A and B are from unresponsive cells (**Fig 2d-e**). Clusters associated with group E gather single cell Ca^{2+} response with the highest peak amplitude and number of oscillations. Clusters of the group C and D are associated with the highest value of the altered basal level and of the latency respectively (**Fig 2d-e**).

We defined Ca^{2+} signatures by observing the distribution of single cell Ca^{2+} responses elicited by each agonist in each cluster for each cancer models (**Fig 3a**). We examined the similarity of these Ca^{2+} signatures across cell lines by principal component analysis and computing the Wasserstein distance (also called Earth Mover's distance or EMD, a pairwise measure of the dissimilarity of two-dimensional objects) (**Fig 3b-c**). We observed that the Ca^{2+} signature of a cell line is not significantly modified by the cell density or the number of passages (**Fig S8** and **Fig S9c-e**). Some significant modifications of the Ca^{2+} signature are observed

according to the media used for culture, in particular keratinocytes-SFM supplemented with EGF or bovine pituitary extract (BPE) (**Fig S9**). This could be explained by the different composition of these media and the presence of growth factors that could affect the Ca^{2+} signaling of the cell lines.

Comparison of the average Ca^{2+} signature for CRC and PCa cell lines revealed a relatively similar pattern (**Fig 3a**). This was confirmed by principal component analysis of the Ca^{2+} signatures of each cell line, which showed a mixed spatial distribution of cell lines according to their cancer of origin (**Fig 3b**). Dissimilarity matrix analysis revealed that cell lines clustered into three groups with distinct Ca^{2+} signatures (**Fig 3c**). None of these groups are associated with a single cancer type suggesting a redundancy in the profile of Ca^{2+} responses across cancers. However, it is interesting to note that cell lines with similar genetics such as C4-2, C4-2b and their parent cell line, LNCap are grouped together and show similar Ca^{2+} signatures. We can also note that the SW480 and SW620 cancer cell lines have different Ca^{2+} signatures, even though these cell lines were isolated from primary and metastatic colon tumors, respectively, from the same individual. This may suggest that primary tumors evolving as metastasis will be subject to a remodeling of their Ca^{2+} signatures. However, we did not observe a significant dissimilarity when comparing Ca^{2+} signatures of cancer cell lines isolated from primary or metastatic sites (**Fig S10**). No significant difference was observed either between Ca^{2+} signatures of epithelial and mesenchymal cancer cells or between cancer cells isolated from primary / metastatic tumors and non-cancerous tissues (**Fig S10**).

Taken together, graph-based clustering of agonist-induced Ca^{2+} responses defines Ca^{2+} signatures of cancer cell lines. While we did not identify significant difference between Ca^{2+} signatures of cancer cells according their cancer type, their site of isolation or their EMT score, this approach allows to classify cancer cell lines according their Ca^{2+} signatures and to highlight some similarity or dissimilarity between cancer models. This may indicate a certain degree of redundancy in the profile of Ca^{2+} responses induced in different cancers.

The agonist-induced Ca^{2+} response of a single cell is predictive of its phenotype.

Ca^{2+} fingerprints show that each cancer cell line has a specific Ca^{2+} signature. We then wondered whether the Ca^{2+} response profile elicited by a single cell might reflect its characteristics and be associated with its lineage or cancer of origin. To verify this hypothesis, we developed a three-layer artificial neural network (ANN) that was trained to predict the cell lineage associated with a single cell agonist-induced Ca^{2+} response (**Fig 4a** and **Fig S11a,d**). We obtained different results depending on the class (cell lineage) predicted. The model had the best performance for cells derived from the PC3 cell line (F1-score: 0.722±0.017) and the worst for those derived from the LNCap cell line (F1-score: 0.211±0.068) (**Fig 4b and Fig S11b**). We then trained the ANN to predict the cancer type (2 classes: CRC or PCa) (**Fig 4c**). Similar values were obtained for the F1-score, with 0.814±0.003 and 0.805±0.01 for the prediction of CRC and PCa cells respectively (**Fig 4d**). The AUROC values for both classes were close to 0.9, indicating good class discrimination by our model (**Fig S11c**). Recall of the model for predicting of each class (CRC or PCa) as a function of the agonist-induced Ca^{2+} responses showed that ATP and Tg-induced Ca^{2+} responses had the best predictive performance for both classes. We can also note that Ach., LPA and PAF-induced Ca^{2+} responses have significantly different predictive performance for each cancer type (**Fig S11e**). Calculation of Shapley values (SHAP) shows that the first and last timepoints, and the timepoints immediately after agonist addition (timepoint 20 corresponding to 1 minute of the recording) have the highest weight in predictive performance (**Fig S11f**). Similarly, this supervised approach generate a high F1 score when trained to identify the EMT score or the site of isolation (primary / metastatic tumors) of a single cell according its agonist-induced Ca^{2+} response (**Fig 4d-e**).

Thus, we demonstrated that supervised machine learning models can successfully predict the cancer type or the lineage associated with a single cell using its agonist-induced Ca^{2+} response as input.

Acquired chemoresistance induces a remodeling of Ca^{2+} signature.

Under therapeutic pressure, tumors often develop molecular mechanisms of resistance to treatment. Proteins associated with Ca^{2+} signaling have been implicated in the development of these features (Romito *et al*, 2022). Here, we investigate whether acquired chemoresistance induces a remodeling of the Ca^{2+} signature of cancer cells and whether we can identify resistant cancer cells at the single cell level.

We developed docetaxel-resistant 22RV1 and PC3 cell lines. For PC3 (IC50 for docetaxel: 0.66±0.18 nM), we used two different protocols to generate 3- and 30-fold more resistant cell lines (designated $PC3R^{Low}$ and $PC3R^{High}$, respectively, with IC50 for Docetaxel of 2.33±0.317 nM and 19.2±8.42 nM). Docetaxel-resistant 22RV1 (referred to as 22RV1R) is 10-fold more resistant than its parental cell line (IC50 of 22RV1 and 22RV1R for docetaxel are respectively of: 1.99±0.431 nM and 20.7±4.22 nM, respectively) (**Fig 5a**). We observed that the distribution of single cell Ca^{2+} responses associated with docetaxel-resistant cell lines into clusters previously identified by our unbiased approach was slightly modified, generating distinct Ca^{2+} signatures (**Fig 5b, c** and **Fig S12a-c**). Pairwise dissimilarity analysis revealed that the Ca^{2+} signature of $PC3R^{High}$ is significantly modified compared to its sensitive counterpart (**Fig 5d**). $PC3R^{Low}$ has a similar Ca^{2+} fingerprint to the docetaxel-sensitive PC3, suggesting that moderate (3-fold) acquired resistance does not strongly alter the Ca^{2+} signature. Despite a high EMD value, the Ca^{2+} fingerprint of 22RV1R is not significantly different from that of 22RV1 (p value = 0.068).

We then investigated whether single cell Ca^{2+} responses could predict the resistance status of these cell lines. We trained the ANN with 12,911 single cell Ca^{2+} responses elicited in 22RV1, 22RV1R, PC3, $PC3R^{Low}$ and $PC3R^{High}$ (**Fig S12a-b**). The model successfully predicted the resistance status associated with each single cell (**Fig 5e**). We noticed a drop in performance when predicting resistant cells (**Fig 5f**), which we attributed to the poor prediction performance when confronted with single cell Ca^{2+} responses of $PC3R^{Low}$ (**Fig 5g**). This may be explained by the similarity of the Ca^{2+} signatures of PC3 and $PC3R^{Low}$. We trained our ANN to predict the cell line associated with each single cell Ca^{2+} response. As expected, our model showed poor

performance in predicting $PC3R^{Low}$, misattributing most of the $PC3R^{Low}$ samples to PC3 (**Fig 5h** and **5i**).

Thus, we have identified a change in the Ca^{2+} signature of cancer cells that acquire resistance to chemotherapy. Our model has some limitations in identifying moderately resistant cell lines compared to sensitive cells. However, the Ca^{2+} response profile of a single cell seems to be a good indicator to classify sensitive and resistant cancer cells.

Direct co-culture with Fibroblasts induces a remodeling of the Ca^{2+} signature of cancer cells.

The interaction of cancer cells with the microenvironment is fundamental for tumorigenesis. Surrounding non-cancer cells such as fibroblasts, adipocytes, or immune cells can influence the phenotype of cancer cells. It has been observed that interactions between cancer cells and cancer-associated fibroblasts can modify the Ca^{2+} signaling pathway of cancer cells (Sadras *et al*, 2021).

Here, we tested whether our supervised model is able to discriminate between non-cancerous and cancerous cells in a mixed environment. We co-cultured cancer cells with fibroblasts (cancer-associated fibroblast cell line PF179T). Cancer cells were preloaded with a red fluorescent cell tracker (Red CMPTX). After 72h, we acquired agonist-induced Ca^{2+} responses (**Fig 6a** and **Fig S13a-c**). We evaluated the performance of the ANN to discriminate cancer cells from fibroblasts using single cell Ca^{2+} responses from both cell types cultured alone or in co-culture. The model was trained with 34676 single Ca^{2+} responses. We observed high F1 scores for both cell types (fibroblasts: 0.830±0.004, cancer cells: 0.864±0.01) (**Fig 6b-c**). We observed that the recall of cancer cell prediction is significantly lower when the model is confronted with single cell Ca^{2+} responses originated from cancer cell lines co-cultured with fibroblasts (**Fig 6d**). Regarding the prediction of fibroblasts, PF179 co-cultured with HCT116 and PC3 shows a lower recall of prediction than PF179T culture alone (**Fig 6e**). These results suggest that while our model successfully distinguishes cancer cells from fibroblasts, the co-culture

conditions affect the Ca^{2+} signature of cancer cells, resulting in a decrease in performance for their prediction. We confirmed this by comparing the Ca^{2+} signatures of cancer cells cultured alone or in the presence of fibroblasts (**Fig 6f**). Pairwise dissimilarity analysis revealed that PC3 Ca^{2+} signatures were significantly altered by the presence of PF179T (**Fig 6g**). HCT116 and HT29 Ca^{2+} signatures are not significantly modified by their co-culture with PF179T. Similarly, no significant differences were observed between PF179T cultured alone or in the presence of cancer cells (**Fig 6g**).

Taken together, these data suggest that single cell Ca^{2+} response can be an indicator to distinguish cancer cells from surrounding non-cancerous cells. The Ca^{2+} signatures are modulated by the interaction of cancer cells with their microenvironment, providing a functional window to study the crosstalk between intra-tumoral compartments.

Discussion

The aim of our study was to investigate the heterogeneity of cancer cells and address a classification of cancer cell lines according to their functional Ca^{2+} signatures through an original/innovative method. In this study, we proposed an original method to investigate the heterogeneity of cancer cells and to classify cancer cell lines according of their functional Ca^{2+} signatures. We collected thousands of single cell agonist-induced Ca^{2+} responses in several cancer cell lines and applied an unbiased graph-based unsupervised clustering to generate a Ca^{2+} signature of each cell line. Significant remodeling of the Ca^{2+} signature was observed during the development of chemoresistance or during the interaction of cancer cells with fibroblasts. In parallel, we developed a supervised neural network to predict characteristics of a single cell (identity, origin, resistance status, cell type) based on its agonist-induced Ca^{2+} responses.

Ca^{2+} signaling has been extensively studied in cancers (Marchi *et al*, 2020; Monteith *et al*, 2017). Aberrant expression of ion channels, transporters, or proteins involved in Ca^{2+} signaling has been observed in several cancers and has been associated with virtually all hallmarks of

cancer (Monteith *et al*, 2017). Importantly, alterations in Ca^{2+} signaling have been implicated in the development of chemoresistance (Romito *et al*, 2022) and in the interaction of cancer cells with their microenvironment (Sadras *et al*, 2021). To our knowledge, the definition of the functional Ca^{2+} signature of cancer cells has not been previously investigated.

To capture the heterogeneity of Ca^{2+} signaling in cancer cells, we proposed a graph-based unsupervised clustering approach inspired by single-cell transcriptomic workflows (Satija *et al*, 2015), which allows the definition of a 2D Ca^{2+} fingerprint. The advantages of this approach are that 1) it reduces the introduction of analysis bias by processing the entire signal instead of extracting a few descriptors, 2) it facilitates the scalability by using normalized and scaled signals as input, 3) it provides a 2-dimensional Ca^{2+} fingerprint (or signature) that can be qualitatively compared across conditions independently of the number of cells acquired.

Using this approach, we processed 27,439 single cell agonist-induced Ca^{2+} responses from 16 colon or prostate cancer cell lines stimulated with 8 agonists. To our knowledge, this study surpasses previous similar investigations by the amount of Ca^{2+} responses collected (James *et al*, 2011; Swain *et al*, 2018; Mohammed *et al*, 2017). Within this database, we identified 26 clusters of agonist-induced Ca^{2+} responses organized into 5 distinct communities (Fig 2b-c). This clustering highlights the diversity of patterns of Ca^{2+} responses elicited by cancer cells. It should be noted that this number of clusters is not absolute and likely depends of the type of agonist selected, its concentration and the cell line studied. Further studies involving additional agonists may verify this clustering of agonist-induced Ca^{2+} responses and permit the identification of additional clusters.

Although not being exhaustive, we have here examined a panel of single-dose agonists whose receptors interact with the Ca^{2+} signaling toolkit in a variety of ways (Fig S1c). From this clustering, we did not identify selective Ca^{2+} fingerprints significantly associated with either cancer type, the stage (primary or metastatic), or the aggressiveness (EMT score) of cancer cell lines.

This may suggest that our panel of selected cancer cell lines is heterogeneous and do not fully recapitulate features associated to each of these oncogenic markers.

However, it is interesting to note that our approach provides a classification of cancer cell lines according their Ca^{2+} signatures and thus identifying groups of cancer models with similar functional properties. By comprehensively documenting the heterogeneity of Ca^{2+} responses, our approach may help to understand the extent to which Ca^{2+} signatures define cellular identity and how information is encoded by Ca^{2+} signaling. Further studies of these groups, in particular defining their transcriptomic and genomic profiles, could identify molecular mechanisms involved in the heterogeneous oncogenic remodeling of Ca^{2+} signaling.

Interestingly, our data highlight that the Ca^{2+} signature of cancer cells is modified in the course of the resistance to chemotherapy or after direct interaction with fibroblasts. These two examples illustrate the ability of our approach to capture subtle changes in the Ca^{2+} signature of cancer cells associated with the microenvironment or the therapeutic pressure, and to provide a qualitative and quantitative way to study them. In terms of the co-culture model, these changes may reflect the communication between cancer cells and their microenvironment. Further investigation of these Ca^{2+} modifications may provide insight into disrupting this stroma / cancer cell crosstalk.

Tumor markers are critical to the diagnosis and prognosis of cancer. Whether structural, genetic or molecular, these markers make it possible to detect tumor cells, identify their characteristics, predict their evolution and thus guide the choice of therapeutic strategies. In recent years, supervised machine learning algorithms have been applied to MRI / CT images, sequencing data or histological H&E slides of various cancers and have demonstrated good accuracy for tumor cell detection and prediction of the aggressiveness (Zhong *et al*, 2024; Tran *et al*, 2021; Chuang *et al*, 2021; Cuocolo *et al*, 2019; Xu *et al*, 2024).

In this study, we demonstrated that a supervised neural network can accurately identify individual cancer cells based on the agonist-induced Ca^{2+} response. Similarly, docetaxel

resistance status and cancer type can be predicted. These results support the idea that Ca^{2+} signaling can define a cellular identity and be representative of its origin and its characteristics. It also demonstrates the potential of our approach to identify specific biomarkers related to cancer cell response to treatment, thereby strengthening its utility for precision oncology.

Cell lines may not recapitulate the full spectrum of tumor characteristics observed in the clinic, although they may retain similar genomic and transcriptomic profiles (Warren *et al*, 2021; Tang *et al*, 2022; Berg *et al*, 2017). Investigation of Ca^{2+} signatures in tumors *in vivo* (or *ex vivo* using organotypic culture or patient-derived organoid models) will be interesting to 1) compare *in vivo* Ca^{2+} signatures with those observed *in vitro* and 2) provide a functional overview of the organization of different cellular compartments in and around the tumor. However, these experiments will be challenging due to possible changes in imaging parameters constrained by the sample (thickening, autofluorescence, etc.). Cell segmentation used *in vitro* may need to be replaced by zoning approaches using tools such as convolutional neural networks. Recent studies have used such advanced deep learning models to decode Ca^{2+} signals from murine cortex and suprachiasmatic nucleus and correlate respectively to the walk function and circadian rythms (Wang *et al*, 2024; Ajioka *et al*, 2024).

Taken together, this study provides a proof of concept that cancer cells can be defined by their Ca^{2+} signature and conversely that Ca^{2+} signaling can serve as a predictor of cancer cell characteristics. Further studies will be required to evaluate Ca^{2+} signatures *in vivo*, to investigate their remodeling during tumor development and to determine whether targeting these functional signatures can influence cancer cell phenotype.

Materials and Methods

Cell culture: The colorectal cancer (CRC) cell lines (HT29, DLD1, HCT116, Lovo, SW48, SW480, and SW620) were cultured in McCoy media supplemented with GlutaMax (Gibco, Thermo Fisher, Illkirch, France), 10% fetal bovine serum (FBS; Eurobio, Les Ulis, France) and

1% penicillin and streptomycin (Eurobio). NCM356 cells (Incell Corporation, LLC, San Antonio, TX, USA) were cultured in high-glucose Dulbecco's Modifed Eagle Medium (DMEM) (Sigma-Aldrich, Missouri, USA) supplemented with 10% FBS and 1% penicillin and streptomycin (Eurobio). Prostate cancer cell lines 22RV1, DU-145, C4-2 and C4-2b were cultured in RPMI-1640 media + GlutaMax (Gibco, Thermo Fisher, Illkirch, France) supplemented with 10% fetal bovine serum (FBS; Eurobio, Les Ulis, France) and 1% penicillin and streptomycin (Eurobio). PC3 were cultured in RPMI-1640 media + GlutaMax (Gibco, Thermo Fisher, Illkirch, France) supplemented with 5% fetal bovine serum (FBS; Eurobio, Les Ulis, France) and 1% penicillin and streptomycin (Eurobio). VCap cell line was cultured in DMEM supplemented with 2 mM Glutamine (Eurobio), 10% fetal bovine serum (FBS; Eurobio, Les Ulis, France) and 1% penicillin and streptomycin (Eurobio). Fibroblast cell line PF179T was cultured in α-MEM supplemented with 10% FBS and 1% penicillin and streptomycin. All cells were cultured in an incubator at 37°C with 5% CO_2.

Generation of Docetaxel-resistance cell lines: Docetaxel-resistant 22RV1R and $PC3R^{High}$ cell lines were generated by stepwise increased concentrations of docetaxel (Sigma-Aldrich, Missouri, USA) over a period of 6 months (starting from 0.5 nM to 4 nM). Docetaxel moderately resistant $PC3R^{Low}$ cell lines were generated by repeated intermittent treatment of docetaxel (2 nM) (72h of treatment followed by 72h without treatment) over a period of 8 weeks.

Single cell calcium Imaging: Single cell calcium imaging was performed as described previously (Crottès *et al*, 2019). Each day of experiments include one positive control (such as Tg or LPA) per cell lines tested. Briefly, 48 h before the experiment, 5×10^4 cells were plated on 35-mm dishes (Fluorodish, WPI, USA). For cell density experiments, 5×10^4, 15×10^4, or 30×10^4 cells were plated on 35-mm dishes. For co-culture experiments, $5x10^4$ of PC3, HCT116, HT29 or NCM356 pre-loaded with red cell tracker dye (RedCMPTX, Invitrogen, USA) at 5 µM for 45 min were mixed with $4x\ 10^5$ PF179T cells per 35 mm dish. Cells were then cultured for 72h in alpha-MEM supplemented with 10% FBS and 1% penicillin and streptomycin. On the day of the

experiment, cells were loaded with Cal520-AM (4.5 µM, AAT Bioquest, Pleasanton, USA) at 37°C for 30 min in physiological saline solution (PSS) containing: 150 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM Hepes, and 10 mM D-glucose. Cells were then washed twice in PSS and incubate in PSS solution for 3 min before acquisition. For stimulation of cells, an equal volume of a 2× concentrated compound of interest (prepared in PSS) was added to the dish after the first minute of acquisition. Fluorescence was acquired using either a Nikon TiEclipse inverted scope equipped with a DS-Qi2 camera (Nikon, Tokyo, Japan), and a SOLA SE-5-LCR-SA Lamp (Photometrics, Germany), or a Nikon Ti2 inverted scope equipped with a Fusion BT camera (Hamamatsu) and a pE-800 (CoolLed, United Kingdom). A combination of dichroic cube, excitation and emission filters at 488 and 515 nm (SemRock, USA) filter fluorescence emitted by Cal520-AM, Images were acquired every 3s for 15 min.

Calcium imaging analysis: All videos were processed using a custom macro on Fiji / ImageJ software (NIH, Bethesda, USA). Images were subjected to rolling background substraction. Automated cell segmentation was performed using Cellpose plugin (Pachitariu & Stringer, 2022) on maximal projection of timeseries and manually checked. Fluorescence intensity of individual cells was then measure and normalizing to the fluorescent intensity measured in the first image (F/F_0).

Processing of calcium profiling: All profiles of calcium measurement were processed using R 4.4.1 and Rstudio 2023.03.0 Build 386. Multiple cell batches for the majority of cell type-agonist pair have been used. No major difference in the distribution of patterns of Ca^{2+} responses were observed for each batch. For those that were tested only once, the absence of multiple batches represents a limitation that as to be taken in consideration. For each single cell, maximal amplitude, time to reach the maximal amplitude and AUC were calculated after the first minute of acquisition. Cells are considered as positively responsive for the tested compounds if the fluorescence is superior to 110% of the initial fluorescence (F_0). Oscillations were determined

using *find_peaks* functions from *GCalcium* R package (n.points = 3). Only oscillations with a fluorescence above 110 % of the initial fluorescence (F_0) are counted.

Dimensionality reduction (UMAP): For visualization in two dimensional spaces, single cell Ca^{2+} responses are normalized and subjected to Uniform Manifold Approximation and Projection (UMAP) using *umap* R package. n_neighbors and min_dist parameters were modulated to assay for the best visualization. Unless otherwise is stated, min_dist = 0,1 and n_neighbors = 15 were used for UMAP representation.

Graph-based Unsupervised clustering: Each single cell calcium response are normalized and scaled. Principal component analysis (PCA) was applied on scaled dataset and first 20 components are extracted for clustering. Unsupervised clustering was performed using *bluster* and *igraph* R packages. Briefly, a shared-Nearest Neighbor (SNN) graph network is built by applying a *J*accard weighting scheme on reduced scaled dataset. Louvain algorithm for community detection identified clusters on the SNN graph. Pairwise modularity associated to Louvain clustering highlights group of clusters sharing similar values. Optimal values (k=15 and resolution = 1.2) were defined empirically by evaluating the segregation of calcium responses in each cluster. For further analysis of additional single cell Ca^{2+} responses, a k-Nearest-Neighbors (kNN) classifier is trained using the initial clustering provided for 27,439 single cell Ca^{2+} responses to predict the cluster associated to each additional single cell Ca^{2+} responses.

Supervised Machine learning and Neural Networks: Artificial Neural network (ANN) was built using *tensorflow, keras, scikit-learn* and *numpy* Python packages. Architecture of ANN models is composed of three layers of neurons and use Softmax activation function and Adam optimiser. The first layer is the *input* layer containing normalized and scaled fluorescence intensity recorded at each timepoints and binary values for each agonist, 2) *C1 "hidden"* layer of half-sized the input layer followed by a dropout layer (coefficient: 0.4) to reduce overfitting and 3) *output* layer composed of different possibles labels. Unless otherwise stated, default training:validation ratio of 0.8:0.2 was applied on the dataset. Mean squared error was used as a loss function. Learning

rate and batch size were set at 9e−6 and 32 respectively. ANN were trained for 500 epochs and for each models, 5 different runs were performed. Performance were evaluated by calculating F1 score, AUROC recall and accuracy. For evaluating performance of the model for predicting the cancer type of an "unknown" cell line, train / validation were performed on data obtained from 15 cell lines. Testing were performed using calcium responses measured on the 16th cell line.

Explainability of neural network : Shap values were determined by using shap python packages. Briefly, 1000 randomly selected samples were used as background dataset to use for integrating out features. Then, shap values were estimated for 500 samples for each prediction class and representing the contribution of each features to the model output. Shap values are then represented as relative absolute values for quantitative features (timepoints) and absolute values for qualitative features (agonists). Importance of each features in the predicted labels of the validation test were alternatively evaluated by permutation and F1 score were re-calculated.

Transcriptomic analysis: Transcriptomic data from 16 different cancer types were generated by The Cancer Genome Atlas Research Network (Data ref: The Cancer Genome Atlas, https://portal.gdc.cancer.gov/). Read counts were downloaded from GDC using the *tcgaworkflow* package and used for differential expression analysis (DEA) using the package *edgeR*. DEA compared non-cancerous and primary tumors for each cancer types. The ranked list of genes differentially expressed according their log2(fold change) was submitted to Gene Set Enrichment Analysis (GSEA) using the fgsea package and gene set collections from MSigDB (https://www.gsea-msigdb.org/gsea/msigdb-c2.5.go.bp.v7.5.1.symbols.gmt). Alignement of transcriptomic profiles of cancer cell lines with primary and metastatic tumors were obtained from DepMap project (https://depmap.org/portal/) (Data ref: Warren et al. 2021). EMT score of each cell line were computed using the two-sample Kolmogorov Smirnov test as described previously (Mandal *et al*, 2021).

Viability assay: Cell viability of Docetaxel-sensitive and resistant 22RV1 and PC3 cell lines were evaluated using standard MTT assay after 7 days of treatment. Briefly, cells were treated with

MTT for 1h at 37°C and then formazan crystals were dissolved using DMSO over 10 min. Absorbance at 570 nm was determined with a BioTek (Vermont, United States) Spectrophotometer.

Statistical analysis and illustration: Figures are generated using either *ggplot2*, *alluvial*, *ComplexHeatmap* R packages and assembled using Inkscape software. Statistical test (Kruskall-Wallis and post-Dunn test, Wilcoxon test and ANOVA) were performed using *ggpubr* and *rstatix* R packages. Dissimilarity index for matrices Earth Mover's distance (also called as Wassernstein distance) was computed using *emddist* package.

Datasets and code availability: The microscopy calcium imaging data reported in this paper will be shared by the lead contact upon request. Datasets used in this paper are deposited on Figshare (doi: 10.6084/m9.figshare.28202924). R and Python scripts used for unsupervised clustering and neural networks are added as supplemental files.

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Figures and Tables

Figure 1. Heterogenous dys-regulation of Ca^{2+} signaling pathways in cancers. Schematic representation depicting the workflow of single cell Ca^{2+} profiling. Cells are loaded with fluorescent Ca^{2+} dye Cal-520™. Agonist-induced Ca^{2+} responses of different cancer models are captured using time-lapse fluorescence microscopy. From each recording, individual cell fluorescence intensity are extracted by applying background correction and cell segmentation. Analysis of Ca^{2+} profiling is defined by computing 1) individual descriptors of Ca^{2+} responses are computed (peak amplitude, latency of responses, altered basal level, number of oscillations, percentage of responsive cells) from single cell Ca^{2+} response, 2) defining Ca^{2+} signatures of each cancer model by using an unsupervised clustering approach, 3) Assaying the prediction of oncogenic characteristics at single cell level using supervised classifier.

Figure 2. Unsupervised clustering of agonist-induced single cell Ca^{2+} responses. a. Schematic workflow representing the unsupervised clustering of agonist-induced single cell Ca^{2+} response. **b.** Uniform Manifold Approximation and Projection (UMAP) representation of 27439 agonist-induced single cell Ca^{2+} responses. Each dot represents a single cell and colored

according 26 clusters defined by Louvain community detection algorithm applied on shared nearest neighbor graphs (SNN). **c.** Force-directed layout graph representing cluster modularity highlighting five communities of clusters. **d.** UMAP representation of single cell Ca^{2+} responses color-coded according values of Altered basal level, peak amplitude, responsiveness, latency, and numbers of oscillations. **e.** Heatmap representing the relative value of responsiveness, peak amplitude, altered basal level, latency, and number of oscillations of single cell Ca^{2+} responses averaged by clusters.

Figure 3. Definition of Ca2+ fingerprints of individual cancer cell lines. a. "Ca^{2+} fingerprint" of each cell line represented by the relative proportion of agonist-induced Ca^{2+} responses associated to each cluster. **b.** Principal components (PC) analysis of cell lines according the Ca^{2+} fingerprint. **c.** Pairwise measure of the Wasserstein distance (Earth mover's distance or EMD) representing the dissimilarity between Ca^{2+} fingerprints. (Significant p values are calculated by permutation test: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$). Agonists are: 1- vehicle (Veh.), 2- Thapsigargin (Tg), 3- Adenosine Tri-phosphate (ATP), 4- Acetylcholine (Ach.), 5- Lysophosphatidic Acid (LPA), 6- Platelet-Aggregating Factor (PAF), 7- Epidermal Growth Factor (EGF), 8- Prostaglandin E_2 (PGE_2), 9- Histamine (Hist.).

Figure 4. Predicting single cell identity using artificial neural network trained with Ca^{2+} responses. Artificial neural network (ANN) model was trained for multi-class classification of single cell Ca^{2+} responses according their lineage or their cancer type. Input are the values of each timepoint of the measure of Ca^{2+} response and the label of agonist used. Each model are trained with 80% of the dataset for training and 20% for validation. **a.** Average confusion matrix of 5 train / validation settings representing the recall of prediction of lineage. **b.** Boxplot representing the F1 score of the prediction of each lineage. **c.** Average confusion matrix of 5 train / validation settings of the ANN representing the recall of prediction of the cancer of origin of each cell. Boxplot representing the F1 score of the prediction of each cancer type. **d.** Prediction of the site of isolation of cancer cell lines (Non-cancerous tissues, Primary or Metastatic tumors) by ANN.

Average confusion matrix (Left) and F1 score (right) of 5 train / validation settings representing the recall of prediction of site of isolation.. e. Average confusion matrix (Left) and F1 score (right) of 5 train / validation settings representing the recall of prediction of the EMT score of each cancer cell. (Significant p values are calculated by Kruskal-wallis (**b.**) or Mann-Whitney test to compared "validation" and "test" set (**c-e**): * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$)

Figure 5. Investigating Docetaxel induced resistance in prostate cancer cell lines using unsupervised Ca^{2+} profiling. a. Cell viability of Docetaxel resistant $PC3R^{High}$, 22RV1R and moderately resistant $PC3R^{Low}$ cell lines and respective parent PC3 and 22RV1 cell lines. **b.** UMAP projection of single cell agonist-induced Ca^{2+} responses of PC3, $PC3R^{Low}$, $PC3R^{High}$, 22RV1 and 22RV1R. Grey points represent single cell Ca^{2+} responses from 16 different cell lines depicted in Figure 2a. **c.** PC projection of Ca^{2+} footprints of PC3, $PC3R^{Low}$, $PC3R^{High}$, 22RV1 and 22RV1R. Grey points represent Ca^{2+} footprints of 16 different cell lines depicted in Figure 2d. **d.** Pairwise measure of EMD between global Ca^{2+} footprints of PC3, $PC3R^{Low}$, $PC3R^{High}$, 22RV1 and 22RV1R. (Significant p values are calculated by permutation test: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$). **e.** Average confusion matrix of 5 train / validation settings of the ANN representing the recall of prediction to the resistance to Docetaxel. **f-g.** Boxplot showing the F1 score (f) or the recall of each cell line (**g**) for predicting resistance to Docetaxel. **h.** Average confusion matrix showing the recall of ANN trained to predict sensitive and Docetaxel resistant sub-cell lines. **i.** Boxplot showing the F1 score for predicting sensitive and Docetaxel-resistant subcellular lines. (Significant p values are calculated by Kruskal-wallis (**g-i.**) or Mann-Whitney test (**f.**): * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$)

Figure 6. Interaction of cancer cells and fibroblasts induces a remodeling of Ca^{2+} signature. a. Model of co-culture between cancer cells / fibroblasts. Cancer cells are loaded with RedCMPTX are cultured with PF179T for 72h prior single cell Ca^{2+} imaging. On the left, representative fluorescent images of cancer cell / fibroblasts cultured. **b.** ANN trained with 34676 single cell Ca^{2+} responses of PF179T, HT29, HCT116 and PC3 cultured alone or with cancer

cells / fibroblasts. Confusion matrix representing the recall of prediction of cancer cells / fibroblasts. **c.** Boxplot representing the F1 score of the prediction of Cancer cells / Fibroblasts. **d.** Boxplot representing the performance (recall) for the classification of cancer cells of single cell Ca^{2+} responses of each cancer cell lines cultured alone or in presence of PF179T. **e.** Boxplot representing the performance (recall) for the identification of fibroblasts using single cell Ca^{2+} responses of PF179T cultured alone or in presence of cancer cells. **f.** "Ca^{2+} fingerprint" of cancer cell line cultured alone or in presence of PF179T represented by the relative proportion of agonist-induced Ca^{2+} responses associated to each cluster. **g.** Pairwise measure of the Wasserstein distance (Earth mover's distance or EMD) representing the dissimilarity between Ca^{2+} fingerprints. The name in parenthesis indicate the cell line used for cocultured with the cell line compared. (Significant p values are calculated by Man-Whitney test (**c-e**) permutation test (**g**): * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).

FIGURE 1.

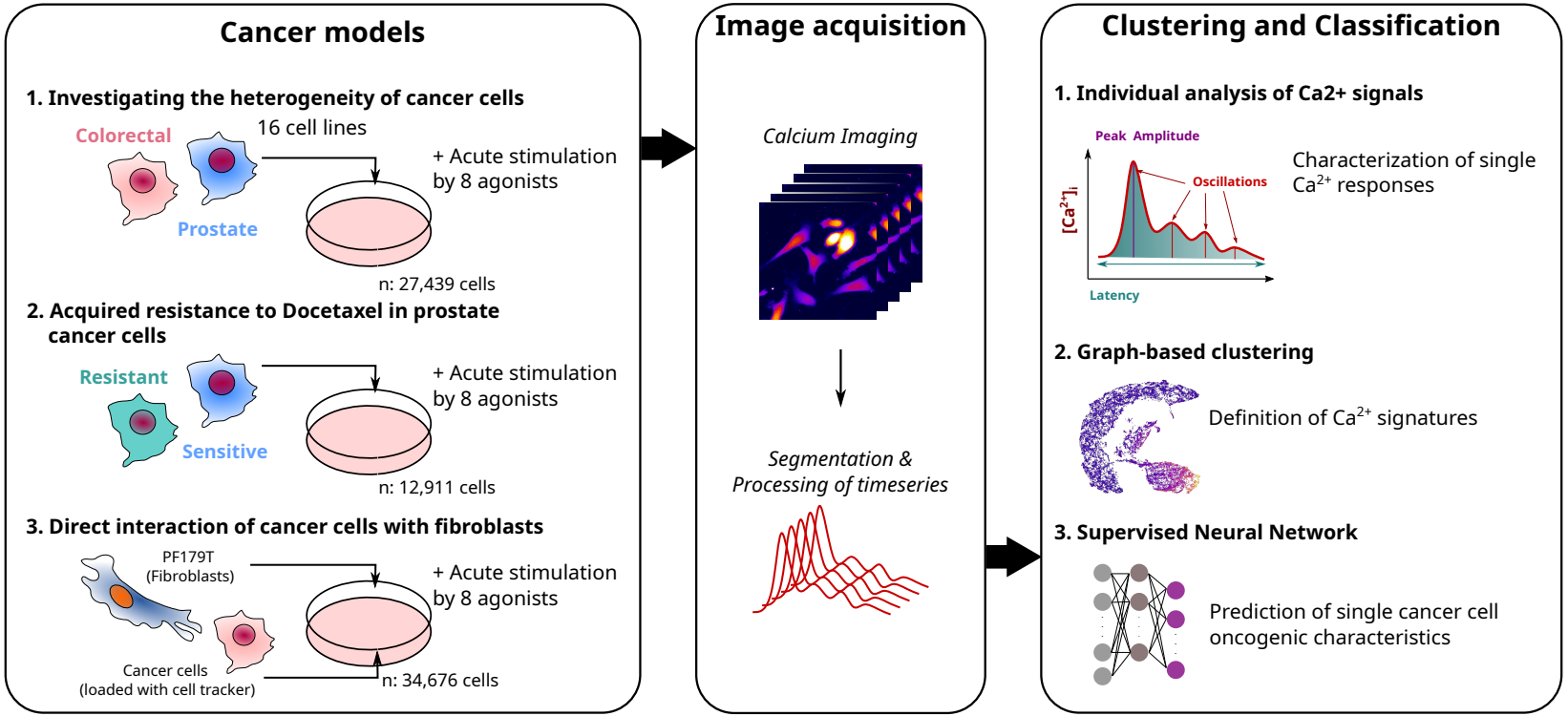

Cancer models
1. Investigating the heterogeneity of cancer cells
Colorectal
16 cell lines
Prostate
+ Acute stimulation by 8 agonists
n: 27,439 cells
2. Acquired resistance to Docetaxel in prostate cancer cells
Resistant
Sensitive
+ Acute stimulation by 8 agonists
n: 12,911 cells
3. Direct interaction of cancer cells with fibroblasts
PF179T (Fibroblasts)
Cancer cells (loaded with cell tracker)
+ Acute stimulation by 8 agonists
n: 34,676 cells
Image acquisition
Calcium Imaging
Segmentation & Processing of timeseries
Clustering and Classification
1. Individual analysis of Ca2+ signals
Peak Amplitude
Oscillations
[Ca2+]i
Latency
Characterization of single Ca2+ responses
2. Graph-based clustering
Definition of Ca2+ signatures
3. Supervised Neural Network
Prediction of single cancer cell oncogenic characteristics

FIGURE 2.

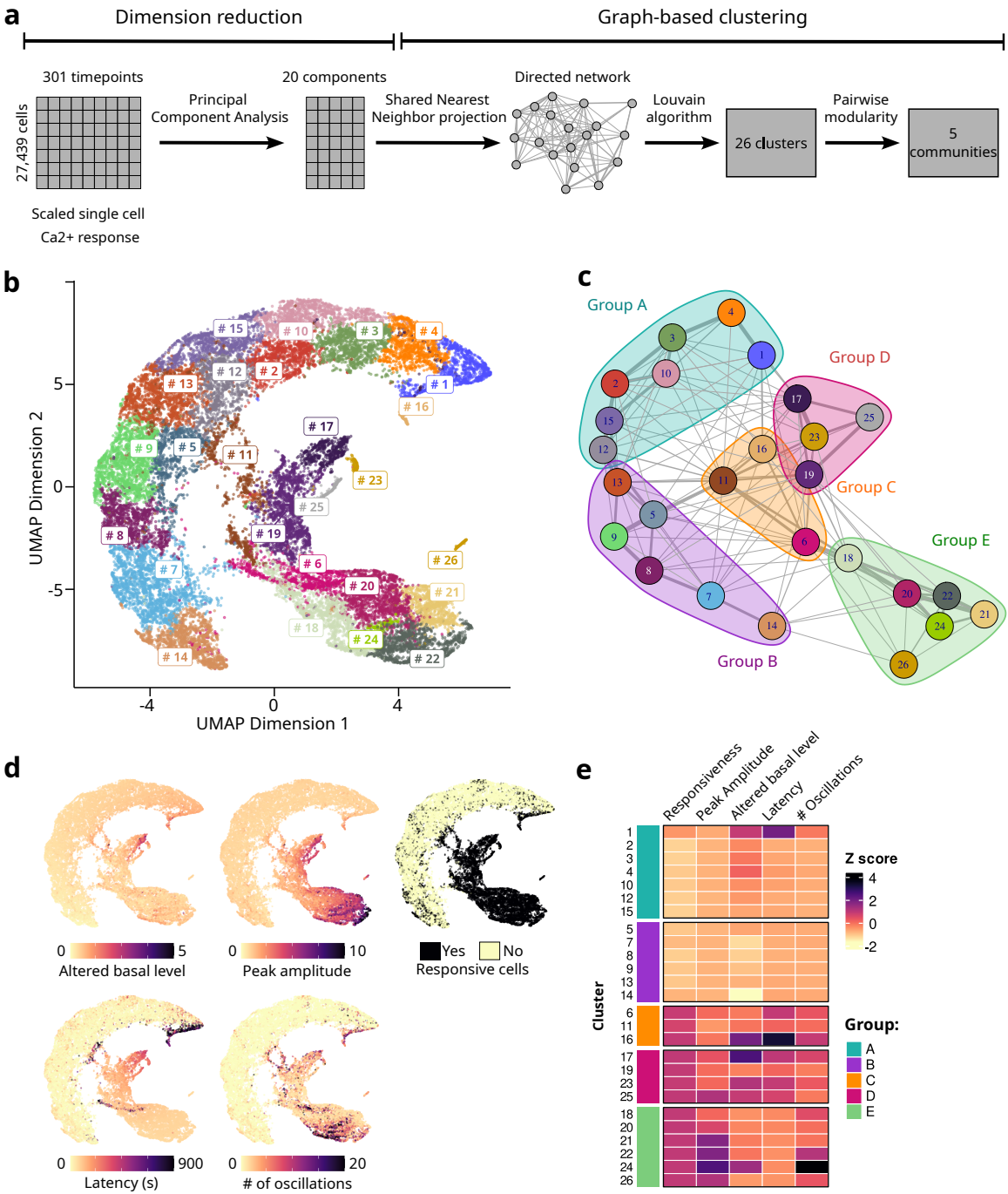

a
Dimension reduction
Graph-based clustering
301 timepoints
27,439 cells
Scaled single cell Ca2+ response
Principal Component Analysis
20 components
Shared Nearest Neighbor projection
Directed network
Louvain algorithm
26 clusters
Pairwise modularity
5 communities
b
UMAP Dimension 2
UMAP Dimension 1
c
Group A
Group B
Group C
Group D
Group E
d
Altered basal level
Peak amplitude
Responsive cells
Yes
No
Latency (s)
# of oscillations
e
Responsiveness
Peak Amplitude
Altered basal level
Latency
Oscillations
Cluster
Z score
Group:
A
B
C
D
E

FIGURE 3.

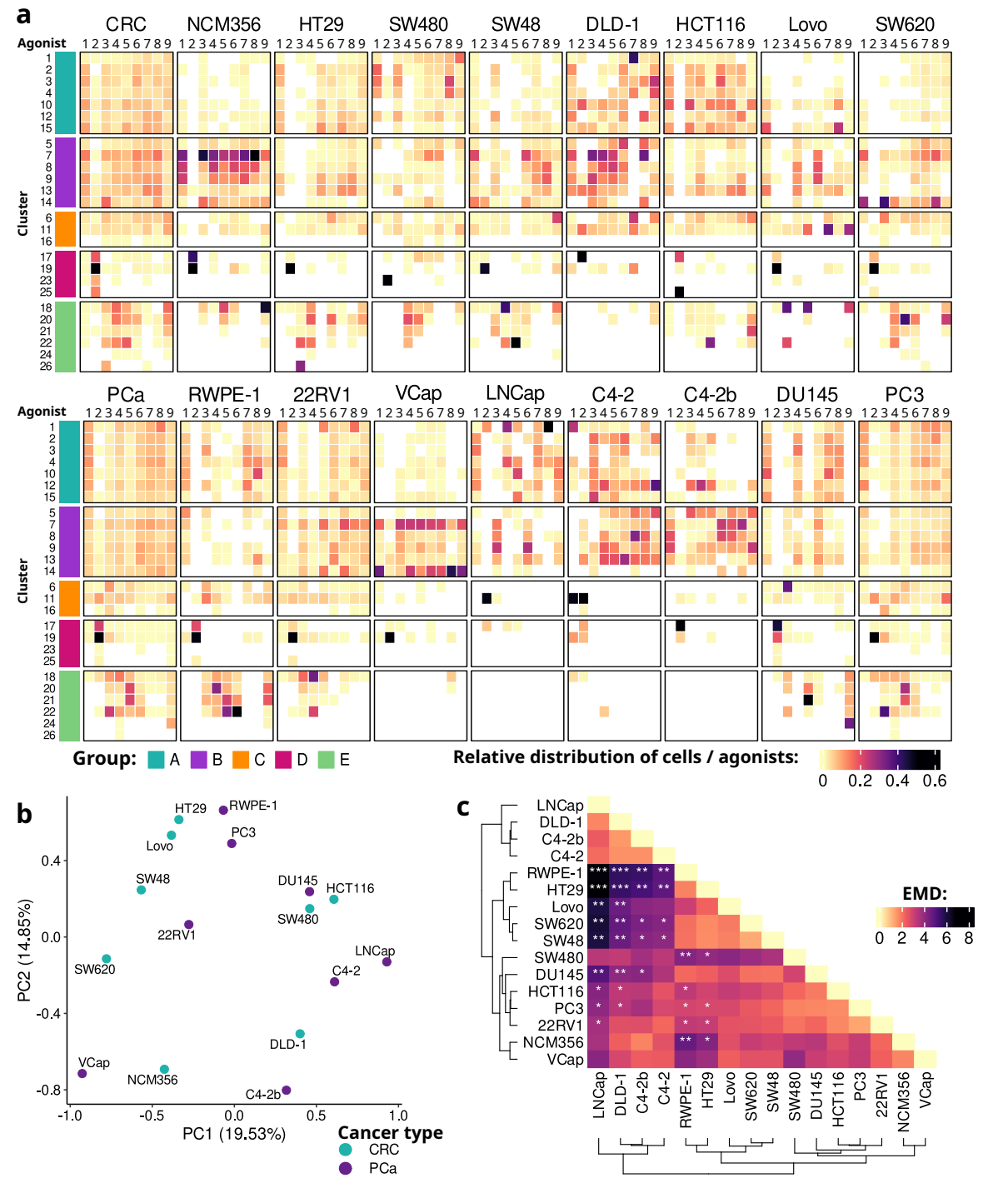

a
CRC
NCM356
HT29
SW480
SW48
DLD-1
HCT116
Lovo
SW620
PCa
RWPE-1
22RV1
VCap
LNCap
C4-2
C4-2b
DU145
PC3
Agonist
Cluster
Group: A B C D E
Relative distribution of cells / agonists:
0 0.2 0.4 0.6
b
PC1 (19.53%)
PC2 (14.85%)
Cancer type
CRC
PCa
c
EMD:
0 2 4 6 8

FIGURE 4.

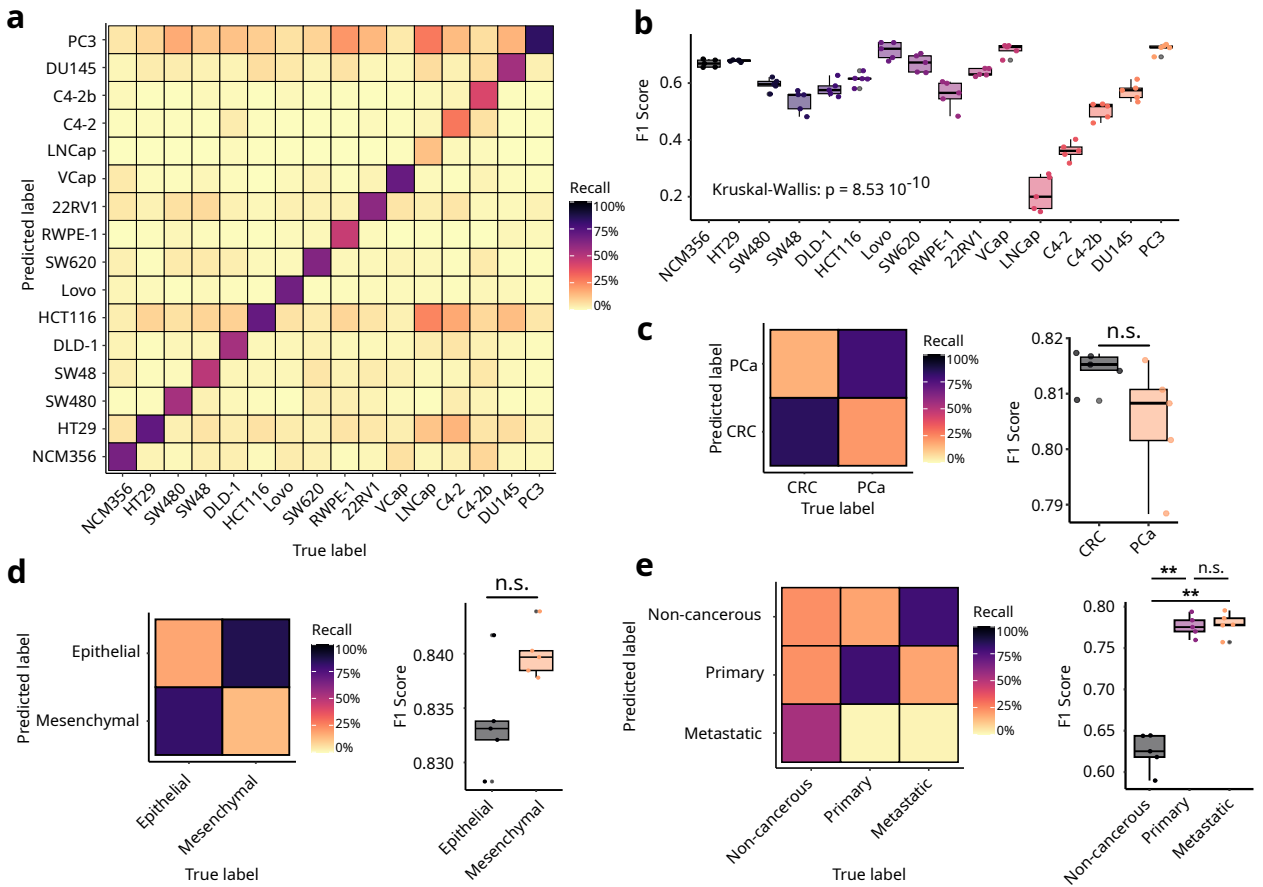

a
Predicted label
True label
PC3
DU145
C4-2b
C4-2
LNCap
VCap
22RV1
RWPE-1
SW620
Lovo
HCT116
DLD-1
SW48
SW480
HT29
NCM356
Recall
100%
75%
50%
25%
0%
b
F1 Score
Kruskal-Wallis: p = 8.53 10-10
c
Predicted label
True label
CRC
PCa
n.s.
d
Epithelial
Mesenchymal
n.s.
e
Non-cancerous
Primary
Metastatic
**
n.s.

FIGURE 5.

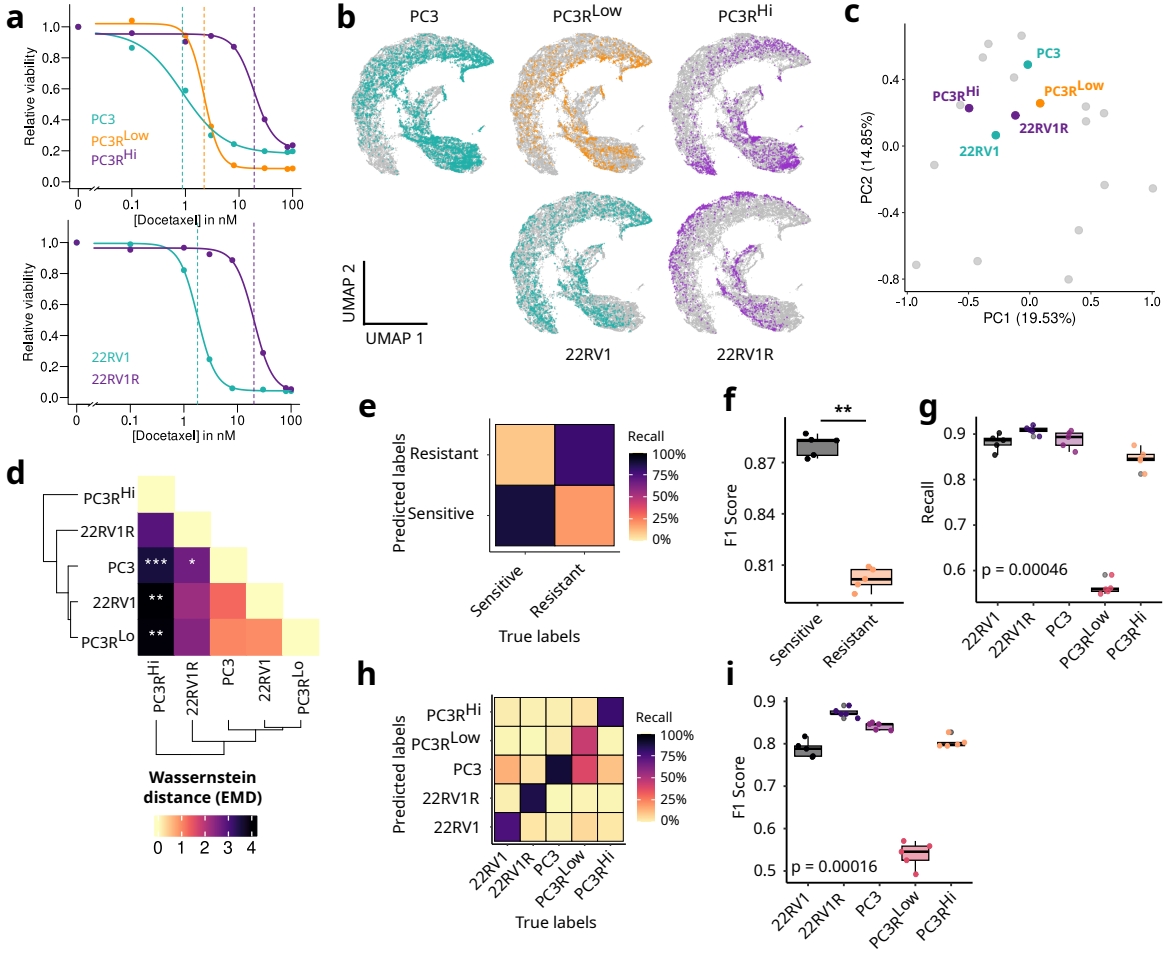
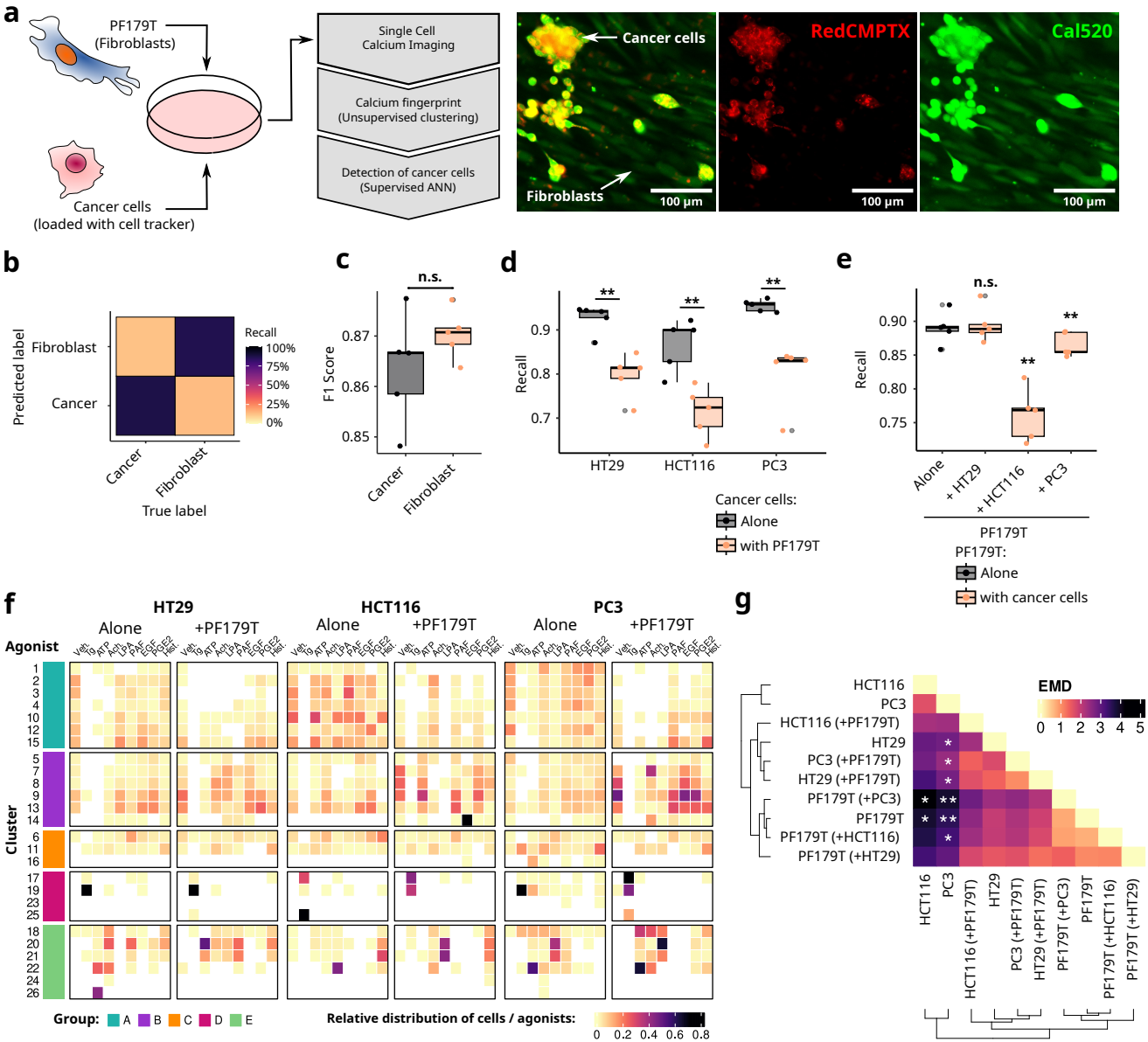

a
Relative viability
PC3
PC3R^Low
PC3R^Hi
[Docetaxel] in nM
22RV1
22RV1R
b
PC3
PC3R^Low
PC3R^Hi
UMAP 2
UMAP 1
22RV1
22RV1R
c
PC3
PC3R^Hi
PC3R^Low
22RV1R
22RV1
PC2 (14.85%)
PC1 (19.53%)
d
PC3R^Hi
22RV1R
PC3
22RV1
PC3R^Lo
Wasserstein distance (EMD)
e
Predicted labels
Resistant
Sensitive
True labels
Recall
f
F1 Score
Sensitive
Resistant
g
Recall
p = 0.00046
22RV1
22RV1R
PC3
PC3R^Low
PC3R^Hi
h
Predicted labels
PC3R^Hi
PC3R^Low
PC3
22RV1R
22RV1
True labels
Recall
i
F1 Score
p = 0.00016
22RV1
22RV1R
PC3
PC3R^Low
PC3R^Hi

FIGURE 6.


a
PF179T (Fibroblasts)
Cancer cells (loaded with cell tracker)
Single Cell Calcium Imaging
Calcium fingerprint (Unsupervised clustering)
Detection of cancer cells (Supervised ANN)
Cancer cells
Fibroblasts
RedCMPTX
Cal520
100 µm
b
Predicted label
Fibroblast
Cancer
True label
Recall
100%
75%
50%
25%
0%
c
n.s.
F1 Score
d
Recall
HT29
HCT116
PC3
Cancer cells:
Alone
with PF179T
e
n.s.
Alone
+ HT29
+ HCT116
+ PC3
PF179T
PF179T:
Alone
with cancer cells
f
HT29
HCT116
PC3
Alone
+PF179T
Agonist
Cluster
Group: A B C D E
Relative distribution of cells / agonists:
g
HCT116
PC3
HCT116 (+PF179T)
HT29
PC3 (+PF179T)
HT29 (+PF179T)
PF179T (+PC3)
PF179T
PF179T (+HCT116)
PF179T (+HT29)
EMD