SPACEDX: A BAYESIAN TEST FOR LOCALIZED DIFFERENTIAL EXPRESSION IN POPULATION-LEVEL SPATIAL TRANSCRIPTOMICS DATASETS
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ABSTRACT

Spatial transcriptomics allows for the study of gene expression within its spatial context, yet current spatial methods for differential expression require the definition of specific regions of interest across the analyzed sections, which limits their applicability. To address this limitation, we introduce SpaceDX, the first framework for spatial differential expression that automatically localizes regions of interest without requiring tissue registration or manual annotations. SpaceDX employs an attention mechanism to detect tissue contexts exhibiting differential gene expression and uses a hierarchical Bayesian framework to overcome the typical challenge of low sample sizes in spatial datasets. We first applied SpaceDX to a structured mouse brain dataset consisting of Visium sections from 38 animals, comparing stressed and control groups. Since the brain has well-defined anatomical regions, we could benchmark SpaceDX against traditional differential expression methods that rely on predefined regions, showing a 110% increase in significant gene detection and the automatic localization of regions exhibiting these differences. Next, we tested SpaceDX on a less structured dataset, specifically using sections from patients with inflammatory skin disease, where it successfully identified regions of interest exhibiting differential gene expression, demonstrating its broad applicability.

1 INTRODUCTION

Spatial transcriptomics captures the gene expression of thousands of genes along with their spatial location within a tissue (Ståhl et al., 2016), providing an unprecedented opportunity to explore molecular features in their spatial context (Chen et al., 2020; Kuppe et al., 2022). Among the most widely used technologies, 10X Visium uses a grid of approximately 5000 spots, each capturing mRNA from multiple cells within the tissue, representing distinct microenvironments (Moses & Pachter, 2022).

As the technology becomes more established, datasets have grown to include dozens of sections across multiple subjects and conditions (Schäbitz et al., 2022), enabling population-level analysis of spatial expression. However, current methods for spatial differential expression (DE) still rely on tissue registration or manual annotation of regions of interest (Batiuk et al., 2022; Vanrobaeys et al., 2023; Otten et al., 2024; Teo et al., 2024), a labor-intensive process that is impractical for tissues with less-defined structures. As a result, these methods may miss opportunities to detect gene expression changes associated with less structured or novel spatial patterns.

We here introduce SpaceDX, the first method for spatial DE across multiple sections that does not require any prior tissue registration or annotation. Leveraging an attention mechanism, SpaceDX automatically localizes regions exhibiting DE. Moreover, it addresses the challenge of low sample size inherent to spatial datasets by employing a fully hierarchical Bayesian model and allowing for robust hypothesis testing via Bayes factors. After benchmarking SpaceDX on a well-structured mouse brain dataset—where it uncovered novel biology and identified more DE genes than annotation-based methods—we applied it to a spatial dataset of inflammatory skin disease, demonstrating its effectiveness in less structured tissues.

2 RELATED WORK

Spatially variable genes. A closely related area in spatial transcriptomics is the detection of spatially variable genes—namely, genes that exhibit distinct expression patterns within a single tissue sample. To date, at least 31 peer-reviewed methods have been developed to address this challenge (Yan et al., 2024), employing a variety of modeling approaches (Svensson et al., 2018; Sun et al., 2020; Edsgärd et al., 2018; Zhu et al., 2021; Hao et al., 2021; Cable et al., 2022; Mason et al., 2024). While these methods may seem similar to SpaceDX, the focus of SpaceDX is fundamentally different: it aims to identify genes that are differentially expressed between samples in the same spatial context, rather than within a single section across space.

Differential gene expression. SpaceDX is designed for DE analysis between subject groups or conditions, a well-established task in gene expression research, originally developed for bulk-RNA data (Love et al., 2014; Robinson et al., 2009) and later extended to single-cell datasets (Kharchenko et al., 2014; Finak et al., 2015). Recent benchmarks have shown that bulk DE methods applied to derived pseudobulk data from specific cell types or states often outperform newer single-cell DE methods (Murphy & Skene, 2022), primarily due to biases from subject-level pseudoreplication, which inflate type I error rates (Zimmerman et al., 2021). To address these challenges, SpaceDX follows a "bulk method" approach by performing DE on average expression levels in relevant spots identified by an attention mechanism. This approach avoids pseudoreplication and therefore ensures robust control of type I error and improves accuracy in detecting DE genes. We compare SpaceDX against region-based and whole-sample pseudobulk approaches, excluding single-cell DE methods applied directly to spots, to ensure fair and consistent comparisons and due to their discussed lack of superiority over pseudobulk methods.

Other methods for spatial differential expression. Current approaches for spatial differential expression typically require manual annotation of regions (Batiuk et al., 2022; Otten et al., 2024) or registration to a common coordinate framework (Vanrobaeys et al., 2023; Teo et al., 2024). Once these regions are mapped across sections, standard differential expression (DE) methods from single-cell or bulk RNA-seq are applied (Batiuk et al., 2022; Vanrobaeys et al., 2023; Otten et al., 2024). In contrast, SpaceDX bypasses the need for annotations or tissue registration, using an attention mechanism to automatically localize regions exhibiting differential expression, making it more suited to unstructured or novel spatial patterns.

Attention-based multiple instance learning. SpaceDX utilizes attention-based Multiple Instance Learning (MIL) (Ilse et al., 2018) to localize regions with differential expression of specific genes. MIL (Dietterich et al., 1997) is a supervised learning approach where data is organized into labeled bags, each containing multiple instances, with only a few instances contributing to the overall label prediction. Recent advances in MIL integrate attention mechanisms, allowing the model to focus on the most relevant instances in each bag for the task at hand. Attention-based MIL has proven effective in various computational biology problems, starting with histopathology (Ilse et al., 2018; Wagner et al., 2023; Zhao et al., 2020) and more recently in single-cell transcriptomics and imaging (Engelmann et al., 2023; Litinetskaya et al., 2024; Sadafi et al., 2020; Kraus et al., 2016). In SpaceDX, sections represent bags, spots are instances, and patient features serve as labels. This structure enables to link patient labels with gene expression patterns in specific spots, providing evidence for spatial differential expression.

3 METHOD

3.1 PROBLEM FORMULATION

A spatial transcriptomics section consists of expression measurements for $G(\approx 18000)$ genes across up to $S(\approx 5000)$ spots and is associated with a binary patient label $y \in \{0, 1\}$. The goal is to test the association between y and the expression of a single gene, $\mathbf{e} \in \mathbb{R}^S$. If region annotations are available, for a given region $\mathcal{R}$, we compute the pseudobulk expression level as:

$$p = \frac{1}{S_{\mathcal{R}}} \sum_{s \in \mathcal{R}} \boldsymbol{e}_s, \tag{1}$$

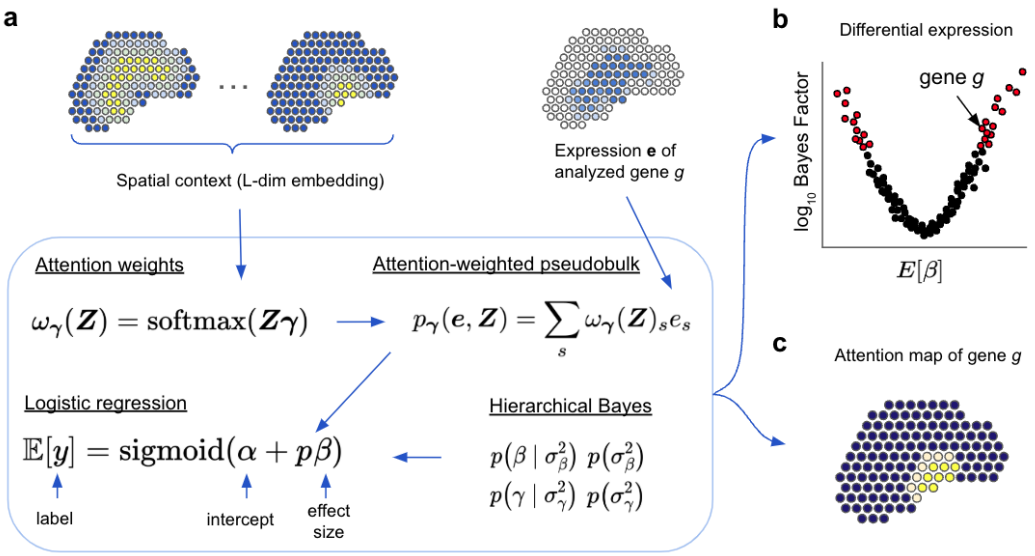

Figure 1: **Overview of the SpaceDX model.** (**a**) Schematic illustration of the individual parts of the SpaceDX model. (**b-c**) Outputs from SpaceDX. For each gene, our method provides the effect size (expected value of β, **b**), the significance of association (Bayes factor) (**b**), and an attention map highlighting the spots exhibiting differential expression (**c**).

where $S_{\mathcal{R}}$ denotes the number of spots in the region. Differential expression of a single gene within the defined region with respect to the binary label y can then be assessed using the logistic regression model:

$$\mathbb{E}[y] = \text{sigmoid}(\alpha + p\beta), \tag{2}$$

where α is the bias term and β represents the pseudobulk effect size, testing for $\beta \neq 0$. The challenge arises when region annotations are unavailable or unknown. In such cases, how can we reliably identify the regions exhibiting differential expression of the analyzed gene? SpaceDX addresses this by introducing an attention module, which can select the most relevant spots in which expression changes align with label y.

3.2 Our model: SpaceDX

To enable spatial DE without regional annotations, SpaceDX leverages spot-level context embeddings that describe the spot's local context (**Figure 1**). Indicating with $\boldsymbol{Z} \in \mathbb{R}^{S \times L}$ such spatial embeddings for S spots and L dimensions, we replace the traditional regional pseudobulk in Eq 2 with the attention-based MIL module

$$p_\gamma(\boldsymbol{e}, \boldsymbol{Z}) = \sum_s \omega_\gamma(\boldsymbol{Z})_s \boldsymbol{e}_s, \tag{3}$$

where attention weights $\omega_\gamma(\boldsymbol{Z})$ depend on context embeddings $\boldsymbol{Z}$ and are parameterized by $\boldsymbol{\gamma}$. Following Engelmann et al. (2023), we employ a shallow function for $\omega_\gamma(\boldsymbol{Z})$, consisting of a linear layer followed by softmax activation function, $\omega_\gamma(\boldsymbol{Z}) = \text{softmax}(\boldsymbol{Z}\boldsymbol{\gamma})$, which ensures that the weights sum up to 1. This simple model has been proven effective in identifying important instances in expression datasets (Engelmann et al., 2023). With these modeling choices, the likelihood for the SpaceDX model for an individual's gene expression $\boldsymbol{e}$ can be written as:

$$p(y \mid \boldsymbol{e}, \boldsymbol{Z}, \alpha, \beta, \boldsymbol{\gamma}) = \text{Bernoulli}\left(y, \text{rate} = \text{sigmoid}(\alpha + \omega_\gamma(\boldsymbol{Z})^T \boldsymbol{e}\ \beta)\right), \tag{4}$$

where we model the binary label y using a Bernoulli likelihood.

Context embeddings. To ensure that context embeddings reflect biological variation rather than technical artifacts, we use state-of-the-art single-cell batch correction method such as Scanorama (Hie et al., 2019) or Harmony (Korsunsky et al., 2019), which we select in a dataset-specific fashion based on established benchmarks (Luecken et al., 2021). Once the batch-corrected

latent variables are computed, we apply an averaging pooling kernel with a radius of 100 μm to capture local spatial contexts of the direct neighbors for each spot (Liu et al., 2022).

Hierarchical Bayesian Model. Given the relatively low sample sizes in current population-level spatial transcriptomics datasets, we adopt a hierarchical Bayesian approach, introducing normal priors on both β and $\boldsymbol{\gamma}$, $\beta \sim \mathcal{N}(0, \sigma_\beta^2)$ and $\boldsymbol{\gamma} \sim \mathcal{N}(\mathbf{0}, \sigma_\gamma^2 I_{L\times L})$, with hyper-priors on the variance parameters σ_β^2 and σ_γ^2 (**Supplementary Information**, **Supplementary Figure 1**). The resulting hierarchical Bayesian model for SpaceDX is:

$$\mathrm{p}(y \mid \boldsymbol{e}, \boldsymbol{Z}, \alpha) = \int \underbrace{\mathrm{p}(y \mid \boldsymbol{e}, \boldsymbol{Z}, \alpha, \beta, \boldsymbol{\gamma})}_{\text{likelihood}} \underbrace{\mathrm{p}(\beta \mid \sigma_\beta^2)\mathrm{p}(\boldsymbol{\gamma} \mid \sigma_\gamma^2)}_{\text{priors}} \underbrace{\mathrm{p}(\sigma_\beta^2)\mathrm{p}(\sigma_\gamma^2)}_{\text{hyperpriors}} \mathrm{d}\beta \mathrm{d}\boldsymbol{\gamma} \mathrm{d}\sigma_\beta^2 \mathrm{d}\sigma_\gamma^2. \tag{5}$$

3.3 Localized differential expression

We introduce a formal statistical hypothesis test to assess association between the expression of the analyzed gene $\mathbf{e}$ and disease label y, comparing the two following models

- the full model $\mathcal{M}_1$ in Eq equation 5, where the patient label y depends on the attention-weighted pseudobulk expression of the gene

$$\mathcal{M}_1: \quad \mathrm{p}(y \mid \boldsymbol{e}, \boldsymbol{Z}, \alpha) = \int \text{Bernoulli}\left(y, \text{rate} = \text{sigmoid}(\alpha + \omega_{\boldsymbol{\gamma}}(\boldsymbol{Z})^T \boldsymbol{e}\, \beta)\right) \tag{6}$$

$$\mathrm{p}(\beta \mid \sigma_\beta^2)\mathrm{p}(\boldsymbol{\gamma} \mid \sigma_\gamma^2)\mathrm{p}(\sigma_\beta)\mathrm{p}(\sigma_\gamma)\, \mathrm{d}\beta\, \mathrm{d}\boldsymbol{\gamma}\, \mathrm{d}\sigma_\beta\, \mathrm{d}\sigma_\gamma; \tag{7}$$

- and the null model $\mathcal{M}_0$, where y depends only on the intercept (no gene effect)

$$\mathcal{M}_0: \quad \mathrm{p}(y \mid \alpha_0) = \text{Bernoulli}\left(y, \text{rate} = \text{sigmoid}(\alpha_0)\right). \tag{8}$$

Variational Inference. The exact posterior distribution for the $\mathcal{M}_1$ model parameters $\boldsymbol{\theta} = \{\beta, \boldsymbol{\gamma}, \sigma_\beta, \sigma_\gamma\}$ is intractable due to the complexity of integrating over the priors and hyperpriors. Therefore, we resort to variational inference (Blei et al., 2017), which approximates the true posterior $\mathrm{p}(\boldsymbol{\theta} \mid \mathcal{D})$ with a variational distribution $\mathrm{q}_{\boldsymbol{\phi}}(\boldsymbol{\theta})$. This transforms the inference problem into an optimization problem, where we maximize the Evidence Lower Bound (ELBO) to estimate the posterior distributions. The ELBO is defined as:

$$\text{ELBO}(\alpha, \boldsymbol{\theta}, \boldsymbol{\phi}; \mathcal{D}) = \mathbb{E}_{\mathrm{q}_{\boldsymbol{\phi}}(\boldsymbol{\theta})}\left[\log \mathrm{p}(\mathcal{D}|\alpha, \boldsymbol{\theta})\right] - D_{\mathrm{KL}}(\mathrm{q}_{\boldsymbol{\phi}}(\boldsymbol{\theta})||\mathrm{p}(\boldsymbol{\theta})), \tag{9}$$

where D_{KL} is the Kullback-Leibler divergence between the variational approximation $\mathrm{q}_{\boldsymbol{\phi}}(\boldsymbol{\theta})$ and the true posterior $\mathrm{p}(\boldsymbol{\theta})$. For the variational distribution, we consider a fully-factorized Gaussian distribution, i.e. $\mathrm{q}_{\boldsymbol{\phi}}(\boldsymbol{\theta}) = \mathcal{N}(\boldsymbol{\theta}_i|\mu_i, \sigma_i^2)$, where $\boldsymbol{\phi} = \{\mu_i, \sigma_i^2\}_i$. As standard practice in gradient-based variational inference, we considered a Monte Carlo (MC) approximation of the expectation term in the ELBO using the reparameterization trick to enable gradient backpropagation (Ranganath et al., 2014). For full information on the employed factorized posterior, we refer to **Supplementary Information**.

Approximate Bayes factors. To formally compare the full and null model, we considered Bayes Factors (BF). BFs are defined as the ratio of the models' evidences (Kass & Raftery, 1995):

$$BF = \frac{\text{evidence of } \mathcal{M}_1}{\text{evidence of } \mathcal{M}_0} = \frac{p\left(y \mid \boldsymbol{e}, \boldsymbol{Z}, \alpha\right)}{p\left(y \mid \alpha_0\right)}. \tag{10}$$

While the evidence for $\mathcal{M}_0$ is tractable, the one for $\mathcal{M}_1$ is intractable due to the integration over the priors and hyperpriors. To address this, we approximate the Bayes Factor using the Importance Weighted Evidence Lower Bound (IW-ELBO) (Burda et al., 2015), which is a tighter bound on the evidence compared to the standard ELBO. Since the variational posterior distributions $\mathrm{q}_{\boldsymbol{\phi}}(\boldsymbol{\theta})$ were already estimated by optimizing the ELBO, we use these distributions to compute the IW-ELBO, which is defined as:

$$\text{IW-ELBO}\left(\alpha, \boldsymbol{\theta}, \boldsymbol{\phi}; y, \boldsymbol{e}, \boldsymbol{Z}\right) = \mathbb{E}_{\boldsymbol{\theta} \sim q_{\boldsymbol{\phi}}}\left[\ln\left(\frac{1}{k}\sum_{i=1}^{k} p(y \mid \boldsymbol{e}, \boldsymbol{Z}, \alpha, \boldsymbol{\theta}_i)\frac{p(\boldsymbol{\theta}_i)}{q_{\boldsymbol{\phi}}(\boldsymbol{\theta}_i)}\right)\right]. \tag{11}$$

3.4 Sign of effect and attention maps

Using the approximated posteriors $q(\beta, \boldsymbol{\gamma})$, we can get additional biological insights. First, we can examine the association effect size by calculating $\mathbb{E}[q(\beta)]$, which tell us whether specific genes are over- or underexpressed in the diseased tissue. Second, the expected values of the spot-level importance weights, i.e. $\mathbb{E}_{q(\boldsymbol{\gamma})}[\omega_{\gamma}(\boldsymbol{Z})]$, provide an attention map, which highlights spots exhibiting DE.

3.5 Implementation and optimization.

We implemented SpaceDX in PyTorch (Paszke et al., 2019) and optimized the ELBO using BFGS (Fletcher, 2000) with full-batch optimization. Since BFGS requires stable and consistent gradients across iterations, we fixed the noise of the MC samples used to approximate the expectation—considering 128 MC samples. Finally, to obtain the IW-ELBO, we sampled from the posteriors after optimization was completed, using 32 importance weights for each of 128 MC samples.

4 Experiments

We validated SpaceDX using two Visium datasets from tissues with different levels of structural organization. First, we applied SpaceDX to a chronic social defeat stress dataset of mouse brain, where the tissue is highly structured with well-defined regions of interest. This allowed us to benchmark SpaceDX against traditional differential expression methods that rely on predefined regions. Next, we analyzed a non-communicable inflammatory human skin disease dataset (Schäbitz et al., 2022), which exhibits less distinct structural organization and lacks annotations, showcasing SpaceDX's ability to uncover novel biological insights in less structured tissues.

4.1 Application to Mouse Brain Dataset

Dataset and task. The data comprises 70 brain sections from 38 animals, each sampled from corresponding anatomical regions. 19 out of the 38 animals have undergone chronic social defeat stress while the other animals constitute a control group. See Supplementary Information for more details on data collection. We here focus on the task of identifying DE genes across the stress and control groups and localizing the regions in which such changes occur[1].

Data preprocessing. We first filtered for genes expressed in at least 10 spots per sample. To correct for potential batch effects and define context embeddings, we applied Scanorama (Hie et al., 2019) to the leading 10 principal components across sections, computed on the top 2000 common highly variable genes [2]. We checked that batch-corrected embeddings were consistent across sections, with no strong sample-specific effects (**Supplementary Figure 2**). Next, we applied the averaging pooling kernel to capture local spatial contexts for each spot (Liu et al., 2022). SpaceDX's input was normalized on a spot level using PFlog1pPF normalization (Booeshaghi et al., 2022). To annotate anatomical brain regions, we registered the sections using the QuickNII tool (Puchades et al., 2019), and small regions were merged while uninformative regions were dropped, resulting in 19 regions for analysis (**Supplementary Figure 3**). For more details on the preprocessing steps for the mouse brain dataset, refer to **Supplementary Table 1**.

Methods considered. Given the well-defined region labels in the brain dataset, we compared SpaceDX to the popular pseudobulk-based DESeq2 (Love et al., 2014) analysis performed within these predefined anatomical regions, using the Python implementation PyDESeq2 (Muzellec et al., 2023). Additionally, we tested a DESeq2 pseudobulk approach based on clusters identified via unsupervised analysis (Otten et al., 2024), with clustering hyperparameters chosen to achieve consistency across most samples[3]. Finally, we also included a DESeq2 pseudobulk analysis applied

[1] When multiple sections from the same mouse were available, the section-level predictions where averaged into a single value for each mouse in the SpaceDX model.

[2] Identified using the 'highly_variable_genes' function from scanpy (Wolf et al., 2018)

[3] Standard Scanpy Leiden clustering pipeline on the top 30 Scanorama-integrated PCs at a resolution of 0.25 (Heumos et al., 2023).

to the entire tissue, which can detect overall gene expression changes but may miss localized expression differences. For each DESeq2 analysis, pseudobulks were created on an animal level by aggregating spot-level gene counts within the regions or whole samples, as recommended in recent benchmarks (Murphy & Skene, 2022).

Comparison of frequentist and Bayesian frameworks. Bayesian methods like SpaceDX and frequentist methods like DESeq2 represent evidence in different ways (Held & Ott, 2018). To ensure a fair comparison, we used a permutation-based procedure to calibrate the significance thresholds of both methods to correspond to an empirical FDR of 5% (Xie et al., 2005). For example, we estimated the BF threshold by inverting the empirical FDR function:

$$\text{FDR}(x) = \frac{1}{P}\sum_{p=1}^{P}\frac{(\text{number of BFs} > x \text{ in permutation } p)}{\text{number of BFs} > x \text{ in real data}}, \tag{12}$$

where the average is computed over $P = 100$ permutations of biological replicate labels. This metric provides an empirical measure of FDR, as all significant instances in the numerator are false positives (as identified in permuted data), while significant instances in the denominator include both true and false positives (as identified in real data). We applied the same strategy to compute thresholds for DESeq2 p-values. For regional and cluster-based tests, single gene-level p-values were obtained by integrating DESeq2 results for the same gene across multiple regions using the aggregated Cauchy association test (Liu et al., 2019).

Results. When comparing the significant genes identified by SpaceDX with those found using region-based and cluster-based DESeq2 approaches, we observe that SpaceDX detects a higher number of differentially expressed genes (**Figure 2a**). Specifically, SpaceDX identifies 110% more genes than the annotation-based approach (99 vs. 47) and 13.8% more genes than the cluster-based approach (99 vs. 87). Although DESeq2 applied to whole-sample pseudobulks detected a greater overall number of genes (**Figure 2a**), SpaceDX identifies 18 genes that are not detected by any of the DESeq2-based approaches, many of which were previously linked to chronic social stress and inflammation[4]. Moreover, SpaceDX localized regions where specific genes exhibit DE across groups. To identify regions of interest for multiple genes simultaneously, we decomposed the attention weight matrix $\boldsymbol{W} \in \mathbb{R}^{G_\text{S} \times S}$ for G_S significant genes using singular value decomposition. We then generated a clustermap (using Ward clustering) of the top 5 left and right singular vectors to group genes with similar attention patterns (**Figure 2b**) and to define regions of interest across spots (**Figure 2c**). These clusters allowed us to rank genes based on their attention weights within each individual cluster (**Figure 2c**). For example, *Pmp22* displayed the highest attention weights in cluster 4, which corresponds to fiber tracts. This gene is down-regulated in demyelination (Jean Harry & Toews, 1998), a process linked to chronic stress and reduced nerve fiber density (Antontseva et al., 2020). Notably, attention maps for *Pmp22* and other significant genes consistently highlighted the same regions across sections from different animals, confirming the reliability of our latent context embeddings and attention mechanism (**Supplementary Figure 4**).

4.2 Application to Human Skin Dataset

Dataset and task. As a second application, we considered the Visium skin dataset from (Schäbitz et al., 2022), which contains skin samples from 29 patients with non-communicable inflammatory disease, each with two replicate sections per sample. For 12 of these 29 patients, matching control samples from healthy skin were also included, resulting in a total of 82 sections (29 diseased samples with 2 replicates, 12 healthy control samples with 2 replicates). We here focused on the task to identify and localize differentially expressed (DE) genes between inflamed and healthy skin samples, treating each pair of replicates as a single observation for analysis[5]. In contrast to the structured mouse brain dataset, this skin dataset lacks clear anatomical annotations and has a more heterogeneous tissue architecture, providing a benchmark for SpaceDX in less-structured tissues.

Preprocessing and experimental setup. We filtered for genes expressed in at least 20 spots across all samples. Context embeddings were generated by taking the top 10 principal components from the

[4]Notable examples are: *Pmp22* (Cathomas et al., 2018), *Lgals8* (Pardo et al., 2017), *Lta4h* (Adams et al., 2023), *Sall1* (Buttgereit et al., 2016), and *Zfp36* (Cook et al., 2022; Stein et al., 2017).

[5]This was achieved by averaging section-level predictions across replicates.

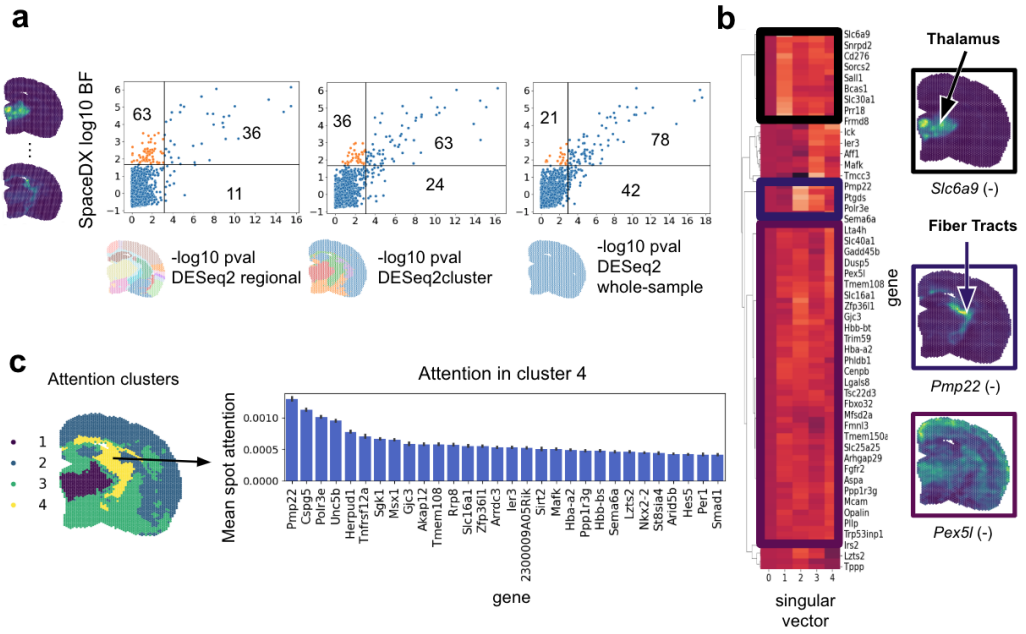

Figure 2: **Application of SpaceDX to the mouse brain dataset.** (**a**) Comparison of SpaceDX's Bayes factors with DESeq2 p-values from three approaches (region-based, cluster-based, and whole-sample), with permutation-based significance thresholds corresponding to 5% FDR. (**b**) Ward clustering of significant genes based on the top 5 singular vectors of their attention weights. Attention maps for three representative genes, uniquely detected by SpaceDX, are shown for each gene cluster, displaying distinct spatial patterns. The (-) symbol indicates underexpression in stressed mice. (**c**) The four spot clusters resulting from Ward clustering based on the top 5 singular vectors of attention weights reveal known brain regions, such as the Thalamus (dark blue) and Fiber Tracts (yellow). Genes within each spot cluster can be ranked and inspected according to their mean attention weights.

spots, after section batch correction using Harmony (Korsunsky et al., 2019). The input for SpaceDX was normalized at the spot level using PFlog1pPF normalization (Booeshaghi et al., 2022). Since no regional annotations were available for this dataset and unsupervised clustering does not yield consistent clusters across samples, we compared SpaceDX to a whole-sample pseudobulk approach. For more details on preprocessing, see **Supplementary Table 1**.

Results. SpaceDX BFs were consistent with DESeq2 pseudobulk p-values (**Supplementary Figure 5**), though DESeq2 identified more DE genes at the same significance level (932 genes for SpaceDX vs 1208 for DESeq2 pseudobulk; FDR $< 5\%$). However, SpaceDX provided unique insights by localizing specific regions where differential expression occurred, including several genes previously linked to skin disease. For instance, SpaceDX identified *PTPN13*, a gene associated with immune regulation in inflammatory skin conditions (Liu et al., 2022). Notably, SpaceDX's attention mechanism highlighted regions enriched with immune response pathways [6] (**Figure 3a**, **Supplementary Table 2**). Another notable gene identified solely by SpaceDX was *TPM1*, previously implicated in psoriasis (Gao & Si, 2018), whose attention weights also highlighted inflamed regions (**Figure 3b**, **Supplementary Table 3**).

[6]Gene set enrichment analysis was conducted using the top 100 genes differentiating the top 5% attention spots from the rest (across all slides), assessed via the Wilcoxon rank-sum test. Enrichr (Chen et al., 2013) was used through the GSEApy wrapper (Fang et al., 2022), with 'GO Biological Process 2023' annotations.

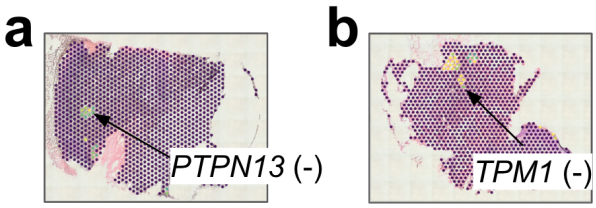

Figure 3: **Identification and localization of inflammation-associated genes in skin samples using SpaceDX.** SpaceDX attention map for *PTPN13* (**a**) and *TPM1* (**b**).

5 Discussion

With SpaceDX, we introduced the first differential expression (DE) test for comparative spatial transcriptomics that does not rely on regional annotation or tissue registration and does not suffer from multiple hypothesis testing, making it adaptable across a range of tissues and conditions. On the structured mouse brain dataset, SpaceDX outperformed traditional annotation-based methods, highlighting the value of a data-driven attention mechanism (Ilse et al., 2018). Our approach enabled the identification and localization of inflammation-related genes, such as *Lgals8* and *Zfp36*, aligning with known links between inflammation and chronic stress (Miller & Raison, 2015; Golden et al., 2011). In the less-structured human skin dataset, SpaceDX successfully identified DE genes and localized regions associated with inflammation, even in the absence of annotation labels. Beyond *PTPN13* and *TPM1*, other known skin inflammation genes identified by SpaceDX include *FLG2* (Pellerin et al., 2013), *UBE2B* (Rácz et al., 2011), *CCL27* (Nedoszytko et al., 2014), *IGFBP5* (He et al., 2022), and *ZDHHC13* (Chen et al., 2017). While SpaceDX offers valuable spatial insights, it detected less genes compared to whole-sample pseudobulk DE, which does not account for localized expression differences. Practitioners should consider this trade-off between gene discovery and spatial context insights, based on the specific goals of their study.

A limitation of SpaceDX is its reliance on the quality of latent embeddings, which can be particularly challenging in heterogeneous tissues. Although tools for batch correction and embedding generation are available (Hu et al., 2024), they lack robustness and are an ongoing area of research. To address this, the rise of foundation models holds promise for the generation of batch-corrected context embeddings (Szałata et al., 2024), which will be integrated into future versions of SpaceDX. This improvement aims to streamline preprocessing and enhance robustness, especially in complex datasets.

Looking forward, we envision broad applications for SpaceDX in large-scale comparative spatial transcriptomics datasets. While our initial focus was on 10X Visium data, the introduced DE attention framework is compatible with many increasingly popular spatial modalities (Bressan et al., 2023; Cornett et al., 2007; Giesen et al., 2014; Angelo et al., 2014). This flexibility positions SpaceDX as a tool with the potential to unlock new insights in population-level spatial data analysis, advancing our understanding of both health and disease.

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APPENDIX

A1 SUPPLEMENTARY INFORMATION

A1.1 CHOICE OF PRIORS AND POSTERIORS

For SpaceDX, we followed a hierarchical Bayesian modeling approach. Gaussian priors were placed on the parameters β and $\boldsymbol{\gamma}$, specifically $\beta \sim \mathcal{N}(0, \sigma_\beta^2)$ and $\boldsymbol{\gamma} \sim \mathcal{N}(\mathbf{0}, \sigma_\gamma^2 I_{L\times L})$. To ensure that the variance components σ_β^2 and σ_γ^2 are positive, we applied a softplus transformation to their Gaussian-distributed hyperpriors. The softplus scale was set to 1, with normal means and standard deviations for σ_β and σ_γ set as $\mu_{\beta-hyp} = 2$, $\sigma_{\beta-hyp} = 0.5$, $\mu_{\gamma-hyp} = 1$, and $\sigma_{\gamma-hyp} = 0.2$. A visual representation of these prior distributions is provided in **Supplementary Figure 1**. For posterior inference, we used a mean-field Gaussian approximation, applied to parameters prior to the softplus transformation, ensuring that variance components remain positive throughout optimization.

A1.2 STRESSED MICE: DATASET GENERATION

FOSTRAP2 mice (n = 6 per group) were randomized into control (Ctrl) and stress groups. Mice were subjected to chronic social defeat stress (CSDS) or remained in their home cages as controls. To capture transcriptomic data following stress, mice were sacrificed at four different time points: D0, D7, D14, and D21. Brain tissues were harvested, snap-frozen in isobutane, and stored at -80°C until further use. For spatial transcriptomics, half-hemispheres of 4 brain slices (10 µm) from the respective groups were placed in capture zones of 10x Visium Spatial capture slides. The manufacturer's protocol was followed for all downstream processes. In brief, the Visium slides were primed at 37°C for 1 minute and post-fixed in methanol (-20°C) for 30 minutes. Tissues were stained for NeuN and DAPI with fluorescence used for imaging. After imaging, cDNA synthesis, amplification, and quality control (QC) were performed. Samples that passed the cDNA QC proceeded to library construction. Libraries were sequenced using S4 flow cells on an Illumina HiSeq 6000, targeting 150M paired-end reads per sample.

A2 SUPPLEMENTARY FIGURES

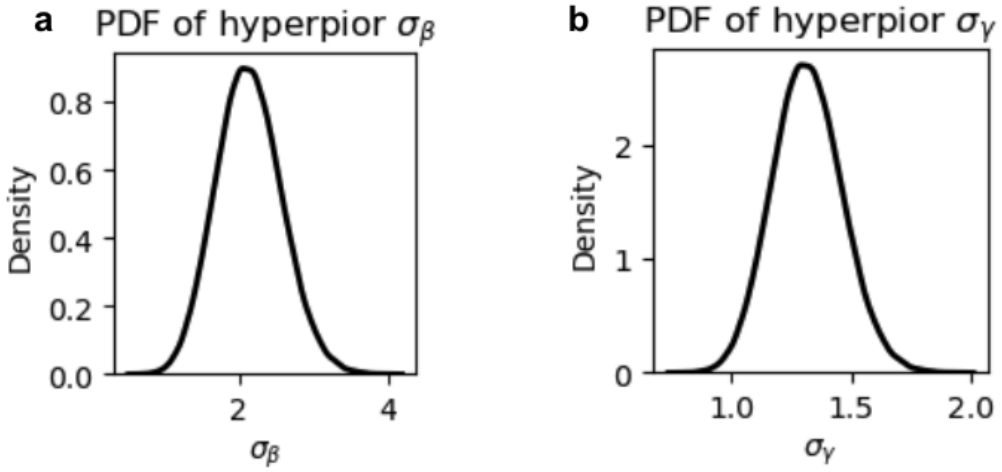

Figure A4: **Probability density functions of selected hyperpriors**. Probability density functions of the hyperpriors for (**a**) σ_β and (**b**) σ_γ, the standard deviation parameters for β and $\boldsymbol{\gamma}$, respectively. A softplus transformation is applied to these Gaussian-distributed hyperpriors to ensure positivity.

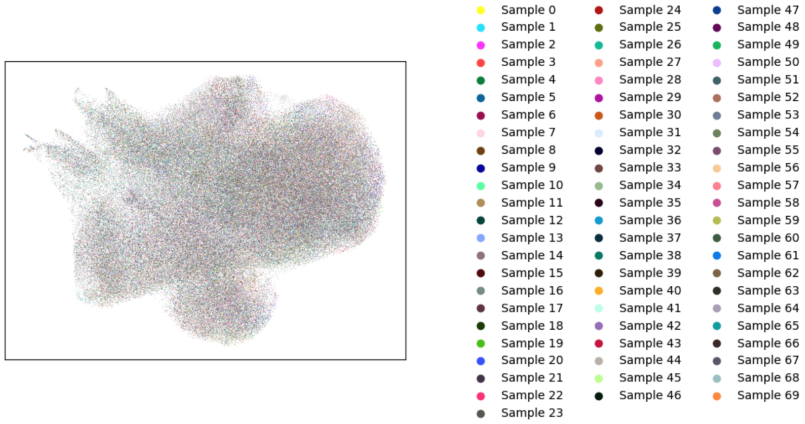

Figure A5: **Integration of slides in the mouse stress data.** UMAP of the top 10 Scanorama Hie et al. (2019) integrated PCs of the 70 sections does not show any batch effects.

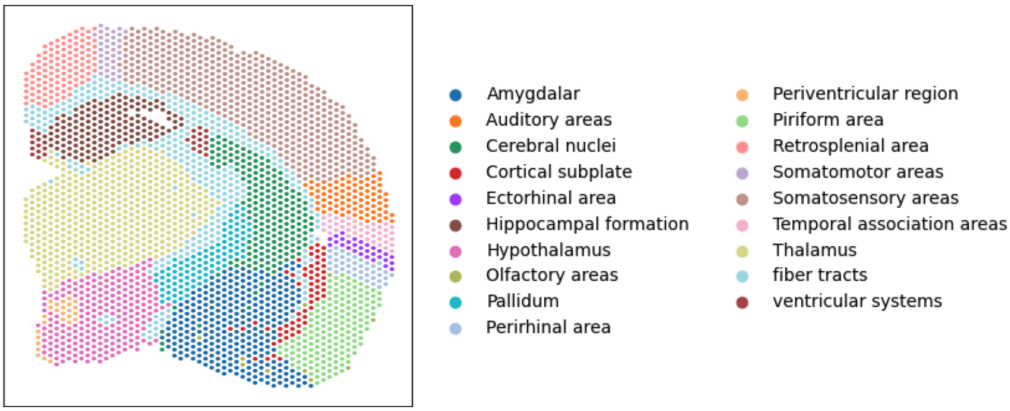

Figure A6: **Registered brain region with labels.** Annotations of 19 distinct anatomical brain regions of a sample after registration using the QuickNII Puchades et al. (2019) tool.

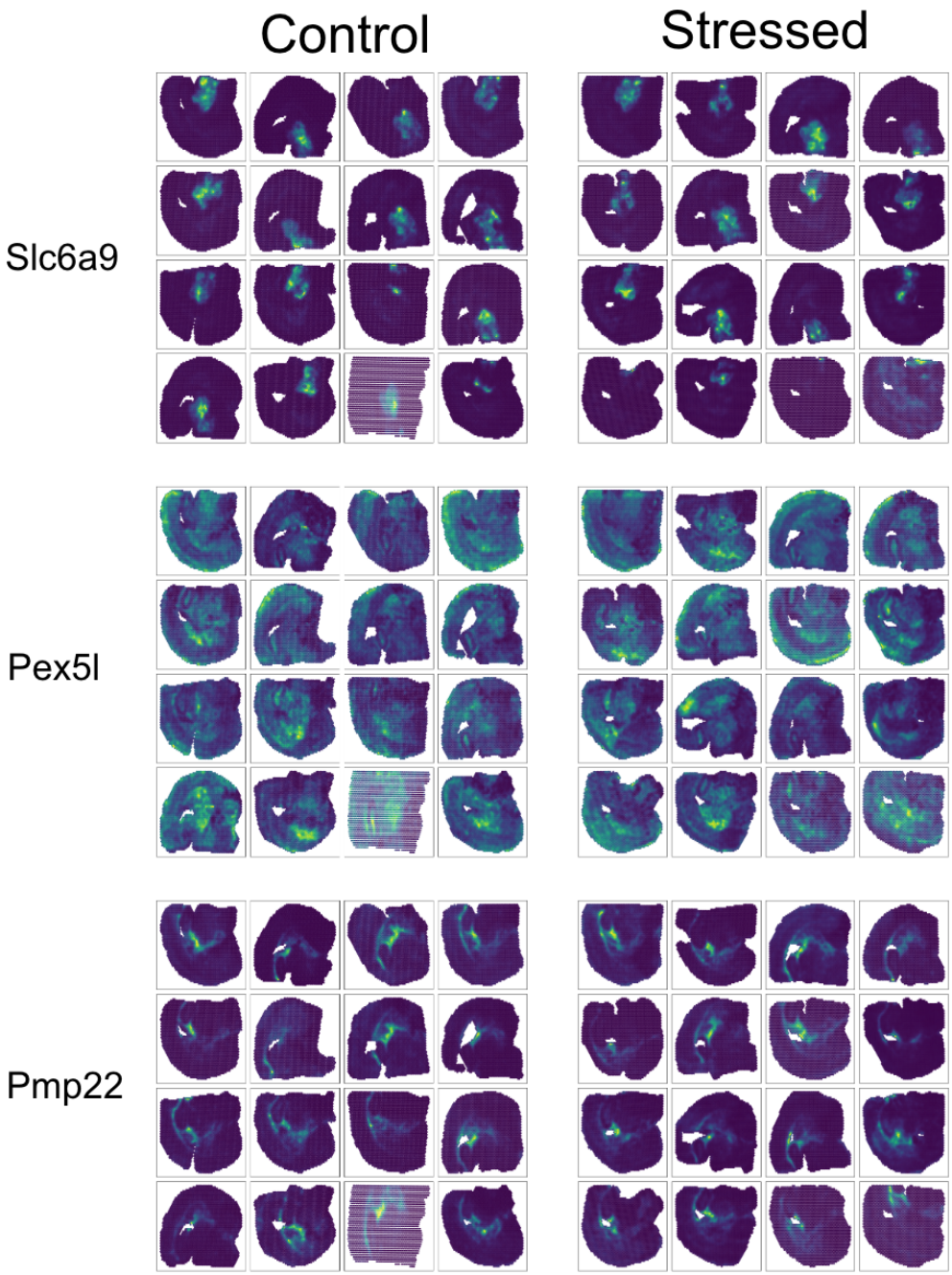

Figure A7: **Consistency of attention maps across animals for selected genes.** The attention maps of the three selected genes Slc6a9, Pex5l, Pmp22 show consistency across the different sections. Note that sections are not all oriented in the same way.

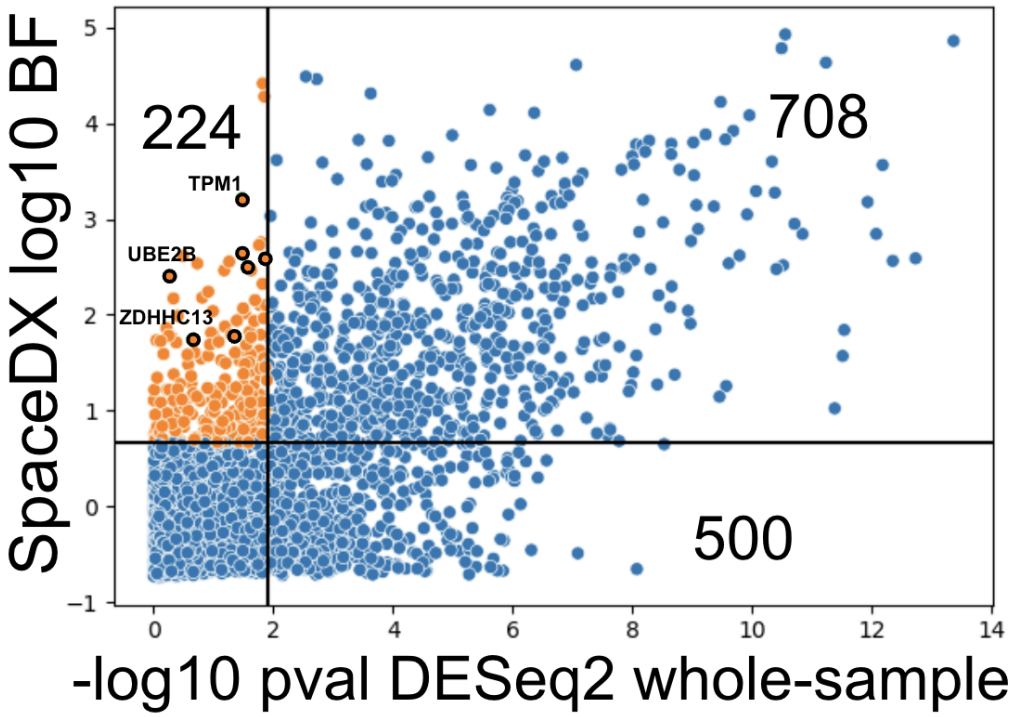

Figure A8: **Scatter of pseudobulk and brain in the skin dataset.** SpaceDX detects 224 additional genes compared to DESeq2. Among them, genes which have been previously linked to inflammatory skin disease (marked with a circle). Namely *TPM1* Gao & Si (2018), *FLG2* Pellerin et al. (2013), *UBE2B* Rácz et al. (2011), *CCL27* Nedoszytko et al. (2014), *OPTN*, *DSP*, *IGFBP5* He et al. (2022) and *ZDHHC13* Chen et al. (2017).

A3 SUPPLEMENTARY TABLES

Table A1: **Data preprocessing table**

(i) Mice Brain	**(ii) Human Skin**
General	
Select genes present in at least 10 spots per sample	Select genes at least present in at least 20 spots over all
Latent Embeddings	
1. Normalization (scanpy's Wolf et al. (2018) (sc) normalize_total, sc's log1p) 2. Select top 2000 (Mice) / 1000 (Human) common highly variable genes among sections (sc's highly_variable_genes(n_top_genes=2000 / 1000, batch_key=section)) 3. PCA (sc's pca)	
Integrate PCs using Scanorama Hie et al. (2019)	Integrate PCs using python port of harmony Korsunsky et al. (2019)
Take top 10 integrated PCs	
Selection of Genes To Test	
1. Normalization (sc's normalize_total, sc's log1p) 2. Select top 3000 highly variable genes (sc's highly_variable_genes)	

Table A2: **Top pathways of up-regulated genes in high attention spots of gene PTPN13**

Term	**Adjusted P-value**	**Genes**
Extracellular Matrix Organization (GO:0030198)	3.852617e-09	COL18A1; POSTN; COL14A1; ELN; COL1A1; SMOC2; COL3A1; COL1A2; CCDC80; CREB3L1; COL8A1; CTSG; MATN4
B Cell Receptor Signaling Pathway (GO:0050853)	2.843634e-08	IGHG3; IGHG4; IGHG1; IGHG2; IGKC; IGLC3; PLCG2; IGHA1
Antimicrobial Humoral Response (GO:0019730)	5.115050e-07	CXCL8; RARRES2; CTSG; IGHA1; CXCL14; S100A9; GAPDH; JCHAIN; LTF

Table A3: **Top pathways of up-regulated genes in high attention spots of gene TPM1**

Term	**Adjusted P-value**	**Genes**
B Cell Receptor Signaling Pathway (GO:0050853)	9.353878e-10	GHG3; CD79A; IGHG4; IGHG1; IGHG2; IGKC; IGLC3; PLCG2; IGHA1
Antigen Receptor-Mediated Signaling Pathway (GO:0050851)	8.630038e-06	IGHG3; IGHG4; CD79A; IGHG1; IGHG2; IGKC; PLCG2; IGLC3; IGHA1
Defense Response To Fungus (GO:0050832)	7.441316e-05	PLCG2; GAPDH; S100A9; S100A8; LTF

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