GLARE: Discovering hidden patterns in spaceflight transcriptome using representation learning

A bioRχiv Preprint

DongHyeon Seo[1], **Hunter F. Strickland**[2,3], **Mingqi Zhou**[3], **Richard Barker**[4], **Robert J Ferl**[3,5], **Anna-Lisa Paul**[3,6], **Simon Gilroy**[7]

[1]Information Sciences Institute, University of Southern California, Marina del Rey, CA 90292
[2]Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL 32611
[3]Department of Horticultural Sciences, University of Florida, Gainesville, FL 32611
[4]Blue Marble Space Institute of Science, Seattle, WA 98104
[5]Office of Research, University of Florida, Gainesville, FL 32611
[6]Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL 32610
[7]Department of Botany, University of Wisconsin-Madison, Madison, WI 53706

Abstract

Spaceflight studies present novel insights into biological processes through exposure to stressors outside the evolutionary path of terrestrial organisms. Despite limited access to space environments, numerous transcriptomic datasets from spaceflight experiments are now available through NASA's GeneLab data repository, which allows public access to these datasets, encouraging further analysis. While various computational pipelines and methods have been used to process these transcriptomic datasets, learning-model-driven analyses have yet to be applied to a broad array of such spaceflight-related datasets. In this study, we propose an open-source framework, GLARE: GeneLAb Representation learning pipelinE, which consists of training different representation learning approaches from manifold learning to self-supervised learning that enhances the performance of downstream analytical tasks such as pattern recognition. We illustrate the utility of GLARE by applying it to gene-level transcriptional values from the results of the CARA spaceflight experiment, an Arabidopsis root tip transcriptome dataset that spanned light, dark, and microgravity treatments. We show that GLARE not only substantiated the findings of the original study concerning cell wall remodeling but also revealed additional patterns of gene expression affected by the treatments, including evidence of hypoxia. This work suggests there is great potential to supplement the insights drawn from initial studies on spaceflight omics-level data through further machine-learning-enabled analyses.

Keywords Machine Learning, Representation Learning, Spaceflight, RNA-seq, Transcriptomics

1 Introduction

Spaceflight studies present unprecedented insights into biological processes through exposure to unique environmental stressors that have not been experienced by any form of life on Earth. In response to the spaceflight environment, organisms initiate specific transcriptional responses to novel conditions. Thus, one key to understanding how biology

responds to spaceflight stressors like microgravity, radiation, and hypoxia is through transcriptomic analysis to study the gene expression profiles that drive physiological adaptation triggered by the spaceflight environment (Mustroph et al., 2010). Space-related transcriptional studies have now also broadened into multi-omic spaceflight investigations that are well-suited to multiple rounds of analysis facilitated by the publicly available datasets in the NASA GeneLab database.

The importance of studying plant biology specifically in space has been identified both for exploring the fundamental responses of biology to the spaceflight environment and at a very practical level for developing bio-regenerative life support systems for long-term space exploration (Rutter et al., 2020; Fu et al., 2016). Understanding of transcriptomic and physiological changes elicited in plants by spaceflight conditions through analyzing transcriptional and other –omic patterns is therefore a focus of much current plant space biology experimentation (e.g., Paul et al. (2013); Villacampa et al. (2021)). For example, the CARA(Characterizing Arabidopsis Root Attraction) experiment was designed to compare the spaceflight transcriptome responses between different genotypes of *Arabidopsis thaliana's* root tips under various conditions (Paul et al., 2017). This experiment explored the patterns of gene expression from root tip cells in the spaceflight environment on the International Space Station (ISS), with comparable ground controls and the lighting sub-environments among three different genotypes. While these kinds of experiments in plant space biology have provided many key insights, they have so far largely relied upon the primary transcriptomic analysis of the original research team. To provide a framework that can be applied to increase the depth of transcriptomic analyses for previous and future spaceflight experiments, we introduce GLARE: GeneLab Representation learning pipelinE. We show the utility of the GLARE pipeline by applying it to the CARA dataset to illustrate how applying novel machine-learning methods to transcriptomic datasets extends insights beyond the original transcriptomic analysis of this data, adding new perspectives.

Our analysis pipeline applies state-of-the-art representation learning models to find underlying patterns in the FPKM values(fragments per kilobase of transcript per million mapped fragments) that are proportional to the abundance of each loci's transcript. These representation learning models allow for better data point representation and clustering using unsupervised learning methods. These methods allow for further investigation of the effects of spaceflight on, e.g., phytohormone signaling and associated physiological phenotypes (Abts et al., 2017; Ferl and Paul, 2016; Iqbal et al., 2017). Moreover, considering that the CARA experiment also utilized lighting sub-environments, we can shine further light on the potential spaceflight effects that were neglected in past studies. Overall, the GLARE method will provide insights to better understand plant behavior in the spaceflight environment based on its endogenous and exogenous cues.

2 Materials and Methods

2.1 GeneLab Data System and Data Entries

The Genelab Data System (GLDS) is a public, space-related -omics data repository, which curates data from a wide variety of species and experimental spaceflight conditions (Ray et al., 2018). GLDS obtains spaceflight-related –omics datasets from multiple locations such as the Gene Expression Omnibus (GEO), European Bioinformatics Institute (EBI), publications directly, and others (Ray et al., 2018). This data is then cataloged with the relevant metadata, such as protocols, payload numbers, and experimental variables, and made available as an Open Science Dataset (OSD) in NASA's Open Science Data Repository (OSDR).

The CARA dataset (OSD-120; `https://osdr.nasa.gov/bio/repo/data/studies/OSD-120`) was chosen from Genelab for use with GLARE due to its many experimental conditions. The CARA experiments were conducted with three ecotypes/genotypes of *Arabidopsis thaliana*: wild-type Wassilewskija (WS), wild-type Columbia-0 (Col-0), and a mutant in the *PHYTOCHROME D* gene in the Col-0 background (*PHYD*) (Paul et al., 2017). Briefly, these genotypes were planted on gel media in Petri dishes and grown in either ambient light conditions or in the dark on the ISS for 11 days; Parallel controls were performed on the ground. After the 11 days, germinated seedlings were photographed and collected into Kennedy Space Center Fixation Tubes (KFTs;Ferl et al. (2011)) containing RNAlater.

Seedlings preserved in RNAlater were returned to Earth frozen, and then the roots were dissected into the last 2 mm of the tip for the light-grown plants and the last 1 mm for the dark-grown plants. RNA was extracted and sent to the Interdisciplinary Center for Biotechnology Research (ICBR), University of Florida, for RNA sequencing using a NextSeq 500 system, producing ∼40 million paired-end reads per sample. Finally, these pair-end reads were mapped to the TAIR10 *A. thaliana* reference genome using Spliced Transcripts Alignment to a Reference (STAR) software, and differential expression was performed using the Cufflinks tool (Dobin et al., 2013; Trapnell et al., 2012).

2.2 High-dimensional Data Analysis

Overview: Statistical methods have been widely integrated into the bioinformatics pipeline in multi-omics studies for analyzing the data as well as preprocessing the data. Specifically, due to multi-omics datasets having complex data topology, dimension reduction and clustering are two commonly used techniques for further investigation (Rappoport and Shamir, 2018). GLARE capitalizes upon such approaches. For example, Principal Component Analysis (PCA) and Factor Analysis are fundamental methods with widespread application for dimensionality reduction (Zeng and Lumley, 2018). After achieving a statistical representation of the dataset with these dimensionality reduction techniques, clustering methods are utilized to group similar representations to uncover underlying patterns within the dataset. Among these, K-means and hierarchical clustering are featured as two of the most favored methodologies (Hulot et al., 2020).

2.2.1 Learning Data Representations

While PCA is popularly used for its simplicity, it has its limits for losing essential features through linear embedding, which often degrades the clustering quality (Gan et al., 2020). Several alternative methods that do not only rely on data point distribution but also leverage latent data structures via learned representations have shown advantages in handling biological data, thereby enhancing clustering precision (Karim et al., 2021). GLARE also uses these approaches in its analyses. These alternatives to PCA include t-distributed Stochastic Neighbour Embedding (t-SNE), a non-linear dimensionality reduction technique particularly adept at preserving local structures within high-dimensional data, and Uniform Manifold APproximation (UMAP) (Van der Maaten and Hinton, 2008; McInnes et al., 2018), a manifold learning approach that efficiently captures complex relationships within the data. However, alternative deep-learning-based approaches for obtaining data representations have been largely neglected in the field of plant biology, despite the advantage of their ability to capture contextual information from the non-linear mappings. Specifically, this approach of capturing contextual information through complex, higher-level features is known as representation learning or feature extraction (Aljalbout et al., 2018). Therefore, along with PCA, t-SNE, and UMAP, we have investigated the application of Sparse Autoencoder (SAE) as one of the representation learning methods in the GLARE pipeline. SAE is an unsupervised learning algorithm based on a neural network that aims to learn an approximation of the identity function that represents the data. The model is trained by encoding the data from its feedforward phase but with sparsity constraints that only activate neurons with the largest activation, allowing the discovery of the unique structure in the data (Makhzani and Frey, 2013). While autoencoders are more commonly used for reconstructing the original input data, prior studies show autoencoder as a representation learning approach that works favorably in the context of multi-omics datasets (Chaudhary et al., 2018).

Upon employing multiple approaches to obtain data representation, evaluating these data representations is critical to understanding the strengths and limitations of various data representation techniques. Prior research has used several evaluation techniques to assess the fidelity between data representations and the original dataset and the quality of the data representation structure, so we have used these methods in the development of the GLARE approach. Reconstruction error analysis, often conducted through linear regression, and trustworthiness scores that measure faithfulness, are widely applied to test the fidelity by comparing the original data and learned representation (Hinton and Salakhutdinov, 2006; Van Der Maaten, 2009). To test the quality of the data structure, the K-Nearest Neighbors (KNN) classifier can be utilized to assess the neighborhood preservation, showing the ability of the representation to

maintain local structure and inherent relationships (Liao and Vemuri, 2002). Furthermore, the Silhouette score measured via k-means clustering is widely used to check the insights into clustering performance and compactness of the data representation (Rousseeuw, 1987).

2.2.2 Clustering Data Representations

Within the clustering paradigm, several alternative methods to K-means exist for the effective organization of these representations, and we have explored their application as part of the GLARE pipeline. Among these, Gaussian Mixture Models (GMM) with the Expectation-Maximization (EM) algorithm offer a probabilistic framework, wherein each cluster is represented by a Gaussian distribution, facilitating more nuanced cluster assignments (Reynolds et al., 2009). Density-based clustering methods have gained considerable attention with respect to their ability to detect clusters of arbitrary shapes and sizes, thus overcoming some of the limitations associated with distance-based methods (Ester et al., 1996). Notably, an extension of this approach, Hierarchical Density-Based Spatial Clustering of Applications with Noise (HDBSCAN), utilizes a hierarchical approach to density-based clustering to robustly identify clusters at multiple levels with varying densities (Campello et al., 2013). Additionally, spectral clustering presents an alternative approach, leveraging the eigenstructure of the similarity matrix to partition the data into clusters, thereby offering an effective means of characterizing complex structures within the dataset (Ng et al., 2001).

Ensemble clustering is an additional powerful technique that combines these multiple clustering solutions to obtain consensus clusters that are more robust and accurate. Several ensemble clustering methods have been proposed, including Evidence Accumulation Clustering (EAC) (Fred and Jain, 2005), which accumulates evidence from different base clustering algorithms to build a co-association matrix. Applying hierarchical clustering to this matrix derives a final consensus clustering result. Other notable examples include HyperGraph-Partitioning Algorithm (HGPA) (Strehl and Ghosh, 2002), which derives consensus clustering through a partitioning hypergraph where each base clustering set is a hyperedge in a hypergraph, with vertices representing data points. These ensemble techniques have demonstrated their utility in various domains, such as bioinformatics, text mining, and computer vision, where data is often high-dimensional, noisy, and complex (Vega-Pons and Ruiz-Shulcloper, 2011). Therefore, they are strong candidates for equivalent analyses of the often highly complex structures that make up plant transcriptomics datasets.

3 Results

3.1 GLARE: GeneLAb Representation learning pipelinE

We introduce GLARE, a representation learning pipeline designed to empower researchers to move beyond conventional dimensionality reduction techniques in their omics-focused research, such as reliance on PCA or tSNE. The GLARE framework enables the extraction of data representations using a trained learning-based model, thereby allowing the exploration of latent structures to unveil the hidden patterns inherent within the dataset. We first report a verification study by training a classification model on the CARA study's spaceflight and ground control data, followed by the full analytical pipeline to highlight GLARE's ability to both confirm patterns revealed in the published primary analyses and reveal novel patterns within the data.

3.1.1 Verification study

Prior to applying the full end-to-end pipeline of GLARE, we perform a verification study through a prediction task to ensure that learnable patterns indeed exist within spaceflight transcriptome datasets. We focused this analysis on the CARA dataset (OSD-120). To enable this analysis, we first had to reorder the dataset in OSD-120 to be indexed by each experimental feature (analogous to resorting the data table to have column headings/labels) for each factor). We, therefore, restructured the unlabeled original data by extracting the feature vectors that represent each experiment (such as genotype, spaceflight versus ground control, and lighting regime) and performing data discretization (i.e., reordering

the numerical FPKM data within the data table to be indexed, not simply by gene locus versus sample but by gene locus versus each individual experiment factor, Figure 1(a)). This approach produced discrete labels for each instance of a feature, creating a pseudo multi-view dataset X'. Essentially, X' contains a reindexed version of the information in the original data, which has 36 continuous features as it contains results from two locations (spaceflight versus ground), under two different light conditions (light versus dark), and for three genotypes (Ws, Col-0, and *PHYD* mutants), with three replicate samples of each.

This restructuring allows the experiment environment to be explicitly indicated through the labels (Xu et al., 2013). We then trained the classification model using XGBoost (Chen and Guestrin, 2016), on the restructured and discretized data X' using the concatenated feature vectors as the input matrix and the discretized labels that represent the experiment environment as target labels. High predictive performance would indicate that learnable patterns are indeed present in the original data, thus motivating the use of unsupervised representation learning techniques from GLARE. Figure 1 shows an illustration of how we reconstructed our dataset through data discretization and the prediction performance of the best-performing data model. We compared prediction performances across multiple data discretization models on a held-out test set of X' is presented in Table 1. This test set was set aside during training to be used for evaluating the model performance on unseen data. Data models that were tested include our 'base' discretization model, where we have location labels indicating if the experiments were performed in space (ISS) or on the ground (KSC), having 18 continuous features and one flight versus ground label. We also discretize other experiment settings such as 'Genotype' and 'Light condition', adding these additional discretized labels to act as further categorical predictors. We found that our base data model that only discretizes the location variable yields the highest performance with $\sim$91% test accuracy on predicting if the experiments were done in space or the ground based on the normalized counts of FPKM values.

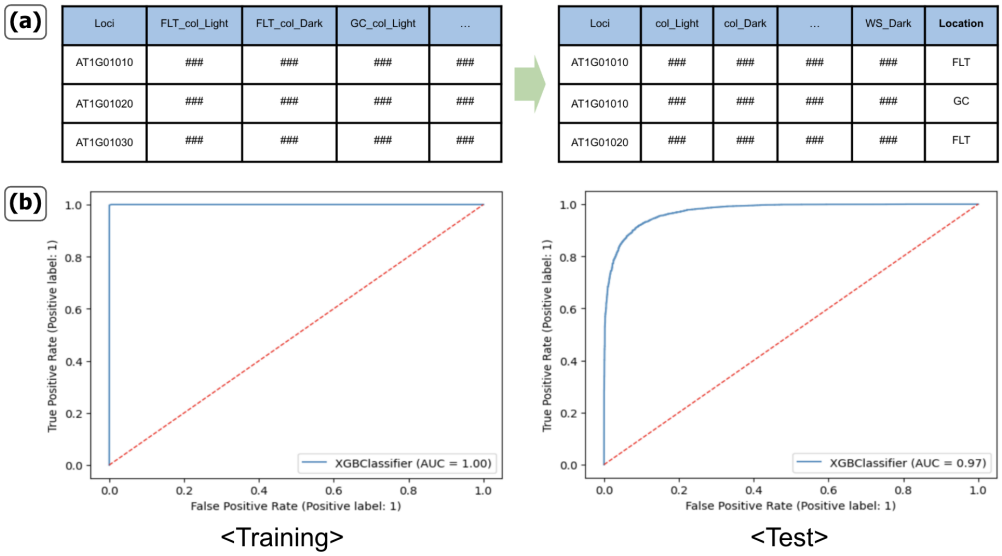


Figure 1: **Illustration of data discretization for data reconstruction and prediction performances.** (a) Illustration showing the restructuring process of our base data model where we discretize the experiment location. Raw FPKM numerical data (denoted as ### in tables) from the OSDR record is organized as reads per locus (e.g., AT1G01010, AT1G01020 across all $\sim$25,000 genes in the Arabidopsis genome) for each experimental sample (e.g., Flight sample of Columbia ecotype grown in the light, FLT_col_Light, or Ground control sample of Columbia ecotype grown in the light, GC_col_Light) as shown left. After discretization (right table), each gene has two instances, one from space and one from the ground sample separately. (b) ROC curves using the training and test dataset on the best-performing data model, which is the base model. Blue line represents XGBoost classifier, showing the ratio of true positives to false positives in the model predictions from the training data (left) and the test set (right). Red line is the random chance baseline. FLT, spaceflight; GC, ground control.

Data Model	Test Accuracy ↑	F1-score ↑	ROC-AUC ↑
Light & Genotype Discretization	68.69 ± 0.14	0.686 ± 0.001	0.772 ± 0.001
Genotype Discretization	77.09 ± 0.14	0.770 ± 0.002	0.865 ± 0.001
Light Discretization	83.18 ± 0.07	0.831 ± 0.001	0.919 ± 0.001
No additional Discretization ('base model')	$\mathbf{91.29 \pm 0.26}$	$\mathbf{0.913 \pm 0.002}$	$\mathbf{0.975 \pm 0.001}$

Table 1: **Classification performances on held-out test set using XGBoost on data from different data models (with $\pm$ standard deviation).** F1-score: The harmonic mean of precision (avoidance of false positives) and recall (avoidance of false negatives), ROC-AUC: Area under the Receiver Operating Characteristic curve, summarizing true positive vs. false positive trade-off. Test Accuracy is the % correctness of predictions for classifying a sample as spaceflight or ground control in the test set using each data model.

The verification study serves dual purposes: 1) As a validity check for the approach prior to deploying the full GLARE pipeline. If the data did not exhibit any learnable and distinctive pattern between the experiment setting that we wanted to compare against, then applying unsupervised methods on that data of interest would be ineffective as the extracted representations would not capture meaningful latent information and make poor predictions from the test set. 2) The prediction task from the verification study can serve as the foundation for post-pipeline analysis, enabling the incorporation of feature importance explanation schemes, such as SHapley Additive exPlanations (SHAP) (Lundberg and Lee, 2017). The feature importance values can reveal, e.g., within CARA, which genotypes and light conditions contributed the most to the predictions overall, as well as provide more insights into specific genes of interest. Combining these insights with the clustering results from the GLARE pipeline should substantially empower researchers in general to see new patterns in their omics-level data.

Encouraged by the outcome of this verification study that a machine learning approach should be able to extract potentially novel features from spaceflight datasets, we implemented a full GLARE pipeline, as described in the following sections.

3.1.2 Preprocessing

GLARE starts with initiating an investigation of the dataset by employing the conventional dimensionality reduction approach of PCA to achieve initial data representation. Then, we utilize the PCA representations to conduct clustering using the k-means algorithm. Figure 2, shows the distribution of the principal components and clustering results on them for the CARA data. Notably, results of both spaceflight (FLT) and ground control (GC) experiments exhibit similar distributions and clustering patterns, characterized by a concentration of data points within a single cluster.

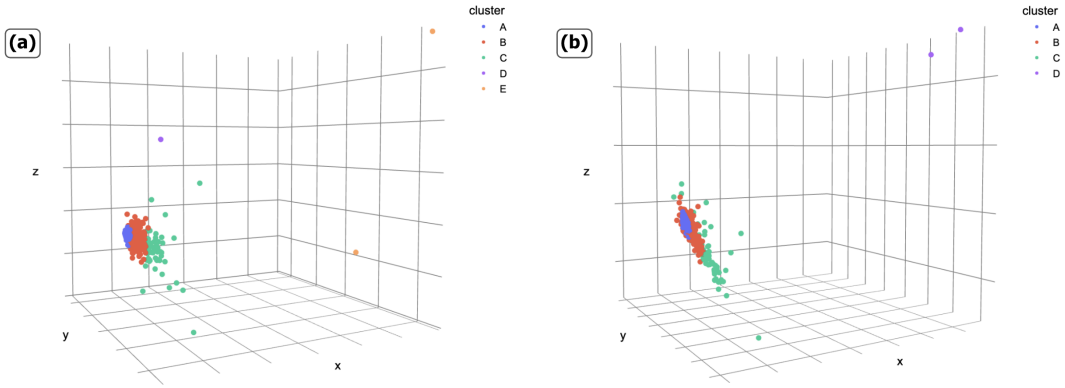


Figure 2: **Outlier detection via PCA and k-means.** (a) Clustering result on spaceflight (FLT) data (without any discretization). (b) Clustering results on ground control (GC) data (without any discretization). ∼98% of the data is clustered on cluster A (blue) for both FLT and GC. In this study, from the >25,000 genes in the datasets, we only discard three genes (cluster D and E in (a) and cluster D in (b)) for both FLT and GC that are separated from concentrated clusters A, B, and C. These genes are: AT1G0759, AT3G41768, ATMG00020.

Leveraging this outcome, GLARE employs initial investigation using PCA and k-means clustering as a means of outlier detection (Lei et al., 2012), discarding out-of-distribution clusters and keeping the concentrated clusters. For both FLT and GC data, we take the three most concentrated clusters to the next step of the pipeline.

3.1.3 Representation Learning

Taking the preprocessed data from the prior step, GLARE offers a range of widely applied representation learning techniques, including classic dimension reduction methods like PCA, t-SNE, and UMAP. However, GLARE also incorporates Sparse Autoencoder (SAE), a deep learning-based model that enables efficient data compression while preserving salient features. In this way, it can capture intricate hierarchical structures within the data by simultaneously learning both compressed data representation and the features necessary for reconstruction (Ng et al., 2011; Ranzato et al., 2007). The illustration of the overall pipeline and details of GLARE are shown in Figure 3.

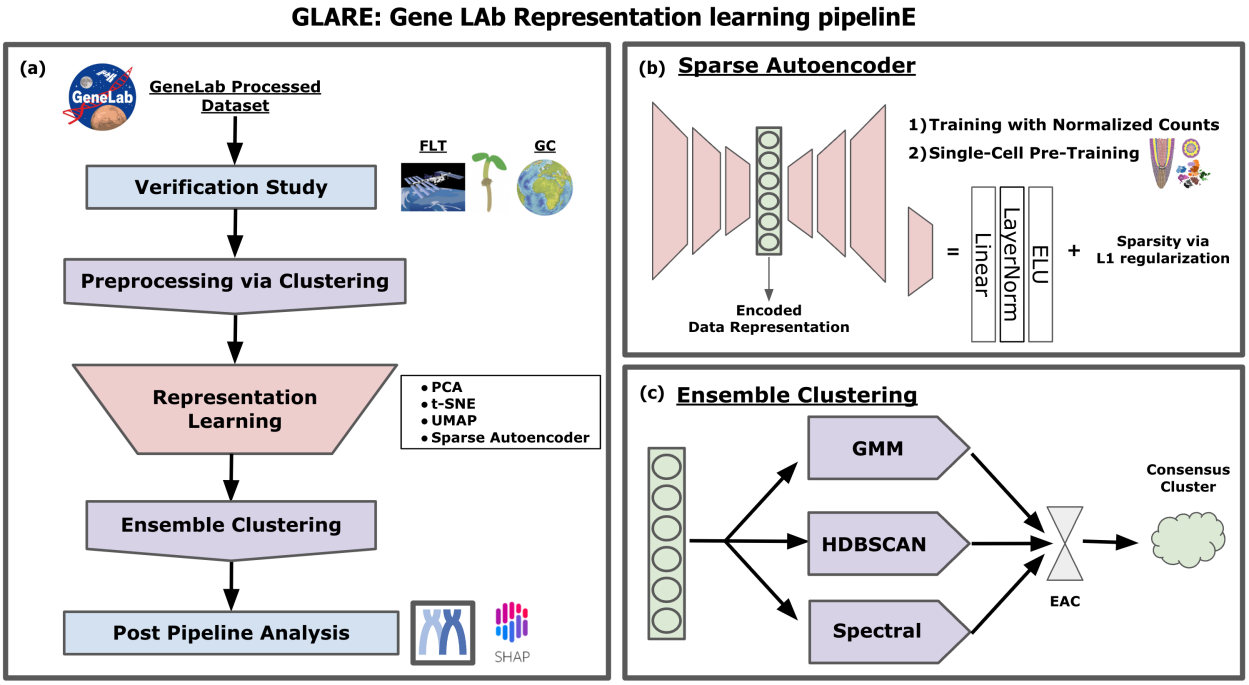


Figure 3: **Overall pipeline of GLARE: Gene LAb Representation learning pipelinE.** (a) Illustration of GLARE, starting with a verification study followed by preprocessing through detecting outliers using k-means clustering. Using the clean dataset, GLARE provides options for representation learning from PCA to state-of-the-art SAE pre-trained with high-throughput single-cell data. Retrieved data representation is then processed through ensemble clustering to find the hidden patterns within the data. Results from the verification study and ensemble clustering are then used for post-pipeline analysis. (b) Model architecture illustration of employed SAE for both training with and without pre-training. (c) Ensemble clustering using three base clustering algorithms based on different statistical methodologies. Evidence accumulation clustering is used to derive consensus clusters from these algorithms.

Our implementation of SAE is constructed with a sequence of building blocks, each comprising a Linear layer followed by LayerNorm and Exponential Linear Unit (ELU) activation (Ba et al., 2016; Clevert et al., 2015). We chose to add the LayerNorm block to improve convergence and stable optimization, considering that our data consists of multiple experimental results from different environment settings. Towards this matter, we employ ELU activation as well. We use three of these building blocks for the encoder and three blocks for the decoder to make the SAE. The sparsity is induced via L1-regularization to deal with the sparse and heterogeneous nature of normalized counts of FPKM values. The model training is optimized using mean squared error loss, Adam optimizer (Kingma and Ba, 2014) with weight decay, early stopping, and gradient clipping to address exploding gradients and ensure stable training. Numbers

of hyperparameters were tested to find optimal parameter sets, which are described in our shared code repository (`https://github.com/OpenScienceDataRepo/Plants_AWG/tree/main/Manuscript_Code/glare`). Finally, after the model training, we extract data representation from the bottleneck layer between the encoder and decoder using this optimized model.

To further enhance the utility of these representations for downstream tasks such as clustering, we introduce an additional self-supervised learning step. We leveraged the pre-training step for the SAE with the addition of high-throughput single-cell data, specifically a single-cell root transcriptome dataset from Shulse et al. (2019), as the CARA dataset is drawn from root tip samples. This pre-training step complements the representations from the model by incorporating detailed single-cell transcriptome profiling of plant root cell types. We then take the pre-trained weights to fine-tune SAE using our normalized counts data to build Fine-Tuned SAE (FT-SAE). We maintain the original model structure and introduce adapter layers atop the main model to adjust varying dimensions between the single-cell matrix and our data appropriately, ensuring seamless integration into our SAE framework. We take the same procedure for the model optimization. The suggested self-supervised learning step offers several advantages, including the augmentation of feature granularity and the incorporation of cellular-level insights, thereby enhancing the fidelity and relevance of the learned representations for downstream analyses (Kiselev et al., 2018). Similar to our approach, such building of foundation models pre-trained with high-throughput single-cell data has demonstrated great utility in a diverse array of tasks in the life science field, including pattern recognition by incorporating foundational knowledge of the data (Hao et al., 2023).

3.1.4 Ensemble Clustering

GLARE provides an ensemble clustering scheme to improve upon the commonly used application of single clustering approaches. GLARE adopts Evidence Accumulation Clustering (EAC) (Fred and Jain, 2005) as its ensemble clustering method, integrating three base clustering algorithms: GMM, HDBSCAN, and Spectral clustering. Ensemble clustering offers several advantages over-relying on a single clustering algorithm. By merging the clustering outcomes from distinct statistical foundations through consensus voting, followed by hierarchical clustering with average linkage on the generated co-association matrix, we can mitigate the biases and noises inherent in each base clustering method to create more robust and reliable clustering results. Notably, when working with complex data such as representations retrieved from a fine-tuned sparse autoencoder, ensemble clustering can effectively address inherent complexities to capture hidden patterns and discover biologically meaningful clusters (Monti et al., 2003).

In addition to obtaining consensus cluster labels using EAC, researchers can leverage GLARE results from three base clustering algorithms to get unique clusters for each gene by retrieving the intersected cluster from its respective cluster assignments.

3.2 Data Representation Evaluation

In this section, we compare data representations from different algorithms that could be retrieved from GLARE. Figure 4, shows visualizations of each of the representations from FLT and GC using PCA, t-SNE, UMAP, SAE, and FT-SAE. Data representation from SAE and FT-SAE has n-dimensions depending on the number of neurons on the bottleneck layer. This value is determined through hyperparameter tuning and was set as $n = 16$. All other data representations from PCA, t-SNE, and UMAP have a 2-dimensional matrix. As we discovered from the preprocessing step of GLARE, the PCA representation data points are highly condensed in a single region of the map, while t-SNE and UMAP representation exhibit a more widespread distribution. On the other hand, SAE and FT-SAE representations show more cluster-forming shapes for their t-SNE coordinates where the locally condensed points are separated from others.

Table 2 shows the next element of the analysis, examining these data representations using multiple quantitative evaluation measures: reconstruction error through linear regression, trustworthiness score, neighborhood preservation through KNN classifier accuracy, and Silhouette Score through k-means. Among the data representations that could be

retrieved from GLARE, we compare all the methods that perform a non-linear transformation to the original dataset, leaving out PCA.

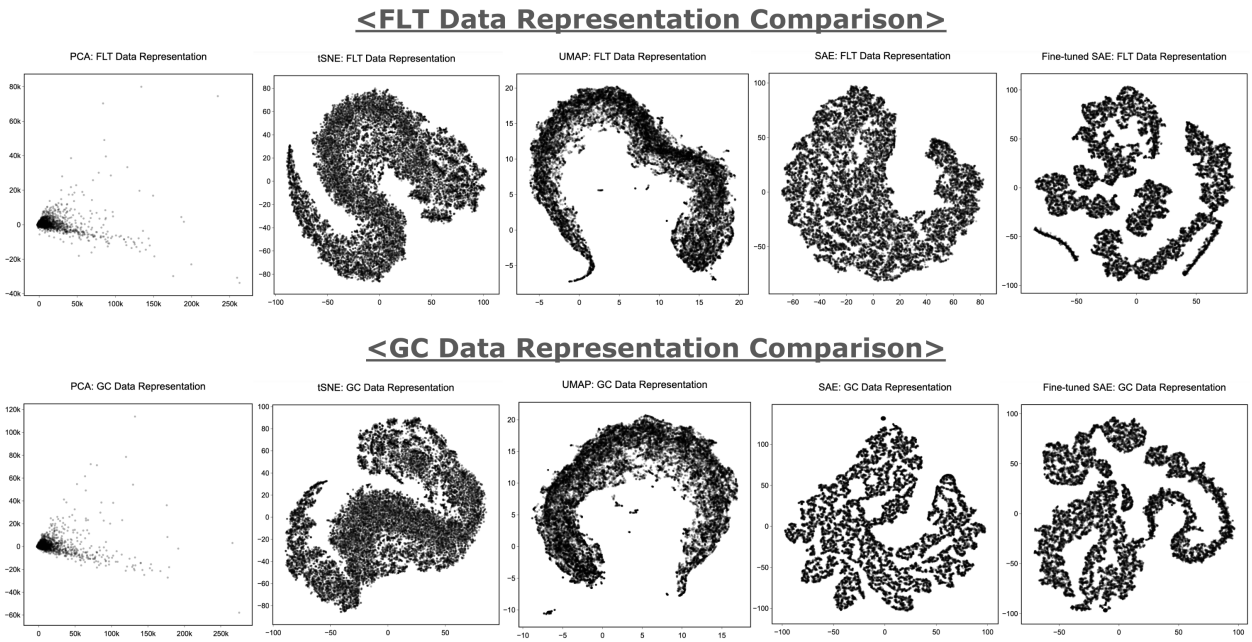


Figure 4: **Comparison of data representations retrieved from GLARE.** PCA, t-SNE, UMAP, SAE, and FT-SAE from left to right for both FLT and GC data. t-SNE was used for the visualization of n-dimensional data representation from SAE and FT-SAE.

Environment	Data Representations	Evaluation Metrics			
		Reconstruction Error ↓	Trustworthiness Score ↑	KNN Accuracy ↑	Silhouette Score ↑
FLT	t-SNE	2020.07	**0.964**	98.11	0.3638
	UMAP	1926.06	0.949	97.85	0.3772
	SAE	2033.51	0.951	97.39	0.3782
	FT-SAE	**1845.12**	0.884	**98.75**	**0.5323**
GC	t-SNE	2029.77	**0.967**	97.89	0.3584
	UMAP	1968.41	0.956	97.49	0.3756
	SAE	2066.24	0.946	97.99	0.3871
	FT-SAE	**1954.56**	0.864	**98.07**	**0.5397**

Table 2: **Comparison of various evaluation metrics on data representations.** FT-SAE shows the lowest linear reconstruction error, highest KNN accuracy, and highest Silhouette score while having a lower trustworthiness score compared to others for both FLT and GC.

Linear reconstruction provides an effective approach for these non-linear methods to see how well they preserve the global structure of the data. FT-SAE outperforms other methods on linear reconstruction, having the lowest error for both FLT and GC. Measuring the Silhouette score and performing the KNN classification on the labels from simple k-means clustering offers another perspective on the quality of data representation, specifically, their utility in downstream tasks and local neighborhood structure preservation. FT-SAE outperforms others on these measures as well for all cases. On the contrary, t-SNE shows the highest trustworthiness score for both FLT and GC. Although FT-SAE retains a fair score with > 0.8 (Lee et al., 2007), it has the lowest among others. This is likely due to the incorporated

transfer learning scheme and its emphasis on sparse representations, which may sacrifice fidelity. Overall, this analysis indicates FT-SAE has great promise in various metrics and its ability to learn sparse, nonlinear representations that effectively capture local and global structures in the data.

3.3 Clustering Results

Here, we present clustering results using GLARE on the CARA dataset. Figure 5 shows ensemble clustering results on the best-performing data representation, FT-SAE. We show individual clustering results from the base clustering algorithm we considered, GMM, HDBSCAN, and Spectral clustering, along with a final consensus cluster through evidence accumulation clustering. We note that GMM and spectral clustering require a user-defined cluster level. These were set to 20 and 25, respectively, for FLT and 25 and 20 for GC driven by results from previous studies (Shulse et al., 2019; Shahan et al., 2022). HDBSCAN defines its own cluster number.

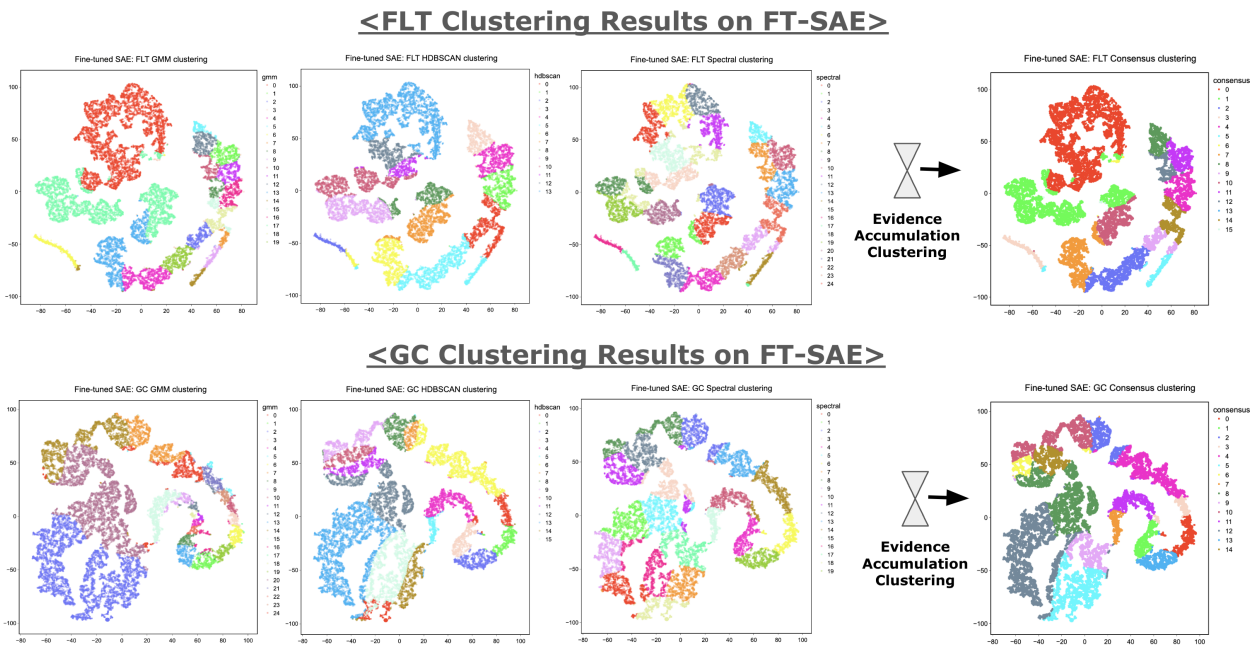


Figure 5: **Ensemble clustering via EAC.** Results from base clustering algorithms, GMM, HDBSCAN, and Spectral clustering, are shown starting from left to right for both FLT and GC. EAC results are shown at the right, with FLT having 16 consensus cluster labels and 15 consensus cluster labels for GC (depicted as different colors).

Spaceflight. Clustering of the FLT dataset resulted in the identification of 20, 13, and 25 clusters for GMM, HDBSCAN, and spectral respectively. GMM clusters had two large clusters, each containing 7,623 and 5,778 genes, with most of the other clusters having lesser sizes of 300 to 1,000 genes. HDBSCAN showed a smaller number of clusters, where most of the clusters had 1000 to 2500 genes. Spectral clusters had the most consistent cluster sizes compared to GMM and HDBSCAN, with most of the clusters having 1,000 to 1,300 genes. These results highlight how the precise nature of clusters is different depending on the clustering approach taken. Each clustering strategy has distinct strengths. GMM works well when the data does not have well-defined boundaries, where HDBSCAN is useful for datasets with noise and outliers, and spectral clustering is highly suited for data with non-linear manifold structures. In order to leverage all of these advantages to a robust and reliable analysis of CARA data representation, we combined all three approaches via ensemble clustering through consensus voting (Vega-Pons and Ruiz-Shulcloper, 2011). These ensemble clusters exhibited diverse characteristics, having clusters with a size of < 1000 genes to two large clusters finding patterns in local structure, each containing 7,627 and 4,715 genes, similar to clusters identified by GMM. The

number of clusters and size of remaining clusters, ranging from 1,000 to 2,500 genes, are suggestive of the outputs of HDBSCAN and spectral clusters finding patterns throughout the global structure (Jain, 2010).

Ground Control. Clustering of the GC dataset resulted in the identification of 25, 15, and 20 clusters for GMM, HDBSCAN, and spectral, respectively. Despite the slight change in the number of clusters, the qualitative characteristics of the results remained largely consistent with those obtained from FLT. Specifically, GMM results revealed two large clusters, each containing 7,445 and 6,157 genes for GC, along with most of the other clusters having lesser sizes of 200 to 900 genes highlighting local patterns. Similarly, HDBSCAN and spectral clusters had a comparable consistent number of genes as FLT clusters, finding patterns throughout the global structure. Ensemble clustering demonstrated similar outcomes to FLT as well, exhibiting a diverse range of gene counts within each cluster.

3.4 Post Pipeline Analysis

Lastly, we demonstrate the full utility of GLARE using the results derived from the ensemble clustering on learned data representation of the CARA data and applying feature explanation analysis from the prediction task that we undertook for the verification study.

3.4.1 Gene Ontology Analysis

Gene Ontology (GO) analysis, in conjunction with clustering results, is a widely used approach to find the functional significance of co-expressed genes in the clusters and provide a comprehensive understanding of the biological functions and processes underlying the observed gene expression patterns. We use the Metascape platform (`http://metascape.org`), which integrates various functional annotation databases (Zhou et al., 2019) to perform GO enrichment analysis. We take the clusters from EAC on FT-SAE and process them through Metascape after excluding clusters with extreme sizes, as this tool can only take gene lists of less than 3000 counts for the enrichment analysis. Specifically, two large clusters for both FLT and GC datasets, along with one small cluster comprising only 2 genes in the FLT dataset, which leaves us 13 significant clusters for both FLT and GC. GO analysis on these clusters revealed various groups of ontologies, including cellular metabolic processes, oxidative phosphorylation, light response and signaling, and vesicle-mediated transport. The prior study on the CARA dataset (Paul et al., 2017) found that genes associated with cell wall metabolism seemed most prevalent among the differentially expressed genes. We found that clusters associated with vesicle-mediated transport were the most prevalent group for both FLT and GC clusters. Specifically, these vesicle-mediated transport clusters were related to plant-specific metabolic and developmental pathways for GC, such as root morphogenesis and cell wall organization. In contrast, FLT clusters were more related to metabolic and catabolic processes, including protein processing and RNA processing (Supplementary Figure S1). Moreover, we found a unique hypoxia-related cluster that was only found in FLT results. Root zone hypoxia is predicted to occur in spaceflight as a loss of buoyancy-driven convection in microgravity should limit oxygen resupply to intensely respiring tissues (e.g., Porterfield (2002)). However, transcriptional fingerprints of hypoxia response in plants in spaceflight have often proven elusive. We therefore concentrated the focus of the rest of our analysis on this hypoxic cluster. In Figure 6, we show a heatmap for the FPKM values for the genes within the hypoxia cluster, GO analysis results for the hypoxia cluster using Metascape (Zhou et al., 2019) (Figure 6(b)), and Stress Knowledge Map (SKM) (Bleker et al., 2023) centered around the Transcription Factors (TFs) in the hypoxia cluster (Figure 6(c)).

The Stress Knowledge Map (SKM; `https://skm.nib.si/`) is a curated resource offering two types of knowledge graphs on plant molecular interactions and stress signaling (Bleker et al., 2023). We used the Comprehensive Knowledge Network (CKN) to gain insights into stress signaling and associated plant biological processes around our genes of interest. The map in Figure 6(c) was drawn with five transcription factors (TFs) that we found in the 43 gene hypoxia cluster: *'DREB2A'*, *'RHL41 / ZAT12'*, *'MYC2'*, *'RRTF1 / ERF109'*, and *'STZ / ZAT10'*. The CKN map shows an intricate network of TFs and their interactions in the context of stress response mechanisms and related signaling pathways with other genes such as *'HY5 / TED5'*, *'ABI1'*, and *'JAZ1'*. Inspection of this network reveals ethylene as a

likely important player in this response. GO analysis of the network for biological function (Supplementary Table S1) also indicates elements of defense, water stress and cold response may also be important elements for further study.

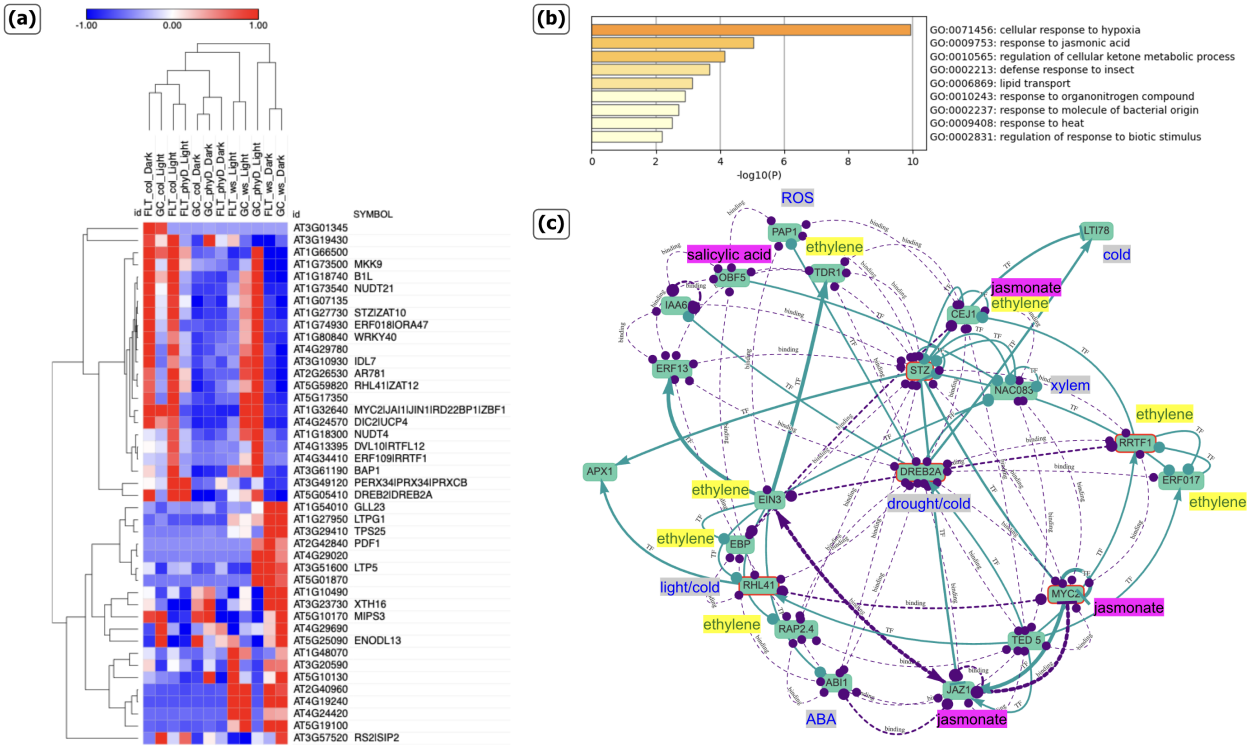
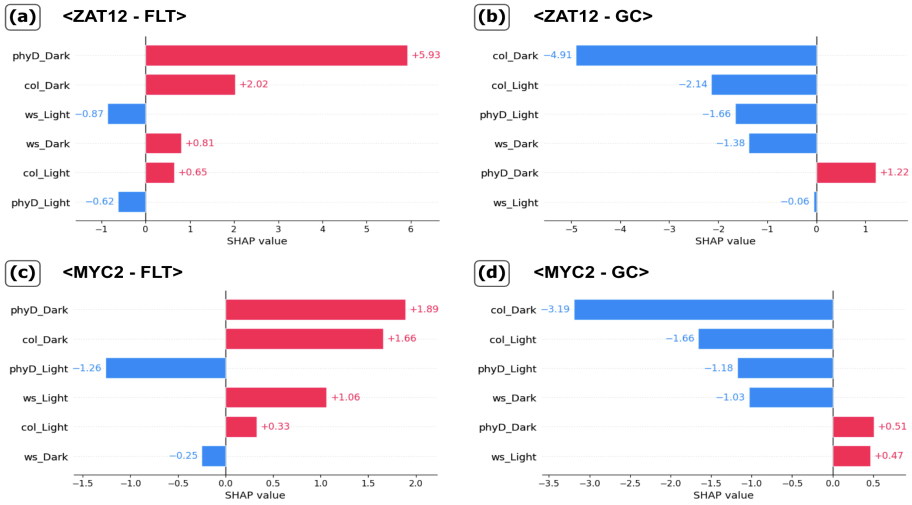


Figure 6: **Analysis of hypoxia cluster found in FLT clustering result.** (a) Heatmap of normalized FPKM values on hypoxia cluster. (b) Enriched ontology on hypoxia cluster from Metascape (c) Stress Knowledge Map (SKM) on five Transcription Factors (TFs) in hypoxia cluster: *'DREB2A'*, *'RHL41 / ZAT12'*, *'MYC2'*, *'RRTF1 / ERF109'*, and *'STZ / ZAT10'*.

3.4.2 SHAP Analysis

Up to this point, our analysis has been directed toward uncovering distinct patterns between FLT and GC by generating separate data representations for clustering and GO analysis. However, we chose CARA as a dataset to interrogate due to the multiple experimental factors within the experiment's design. Therefore, after identifying the patterns within the FLT data using GLARE, particularly a hypoxia cluster, we used this newly identified cluster to evaluate the effect of varying light conditions on different genotypes in each location. We took the found TFs within the hypoxia cluster and applied SHAP analysis to quantify feature contribution, thereby explaining which experimental conditions had the most effect in classifying this pattern within the data between FLT and GC. SHAP analysis provides a way to understand the impact of each feature on the model's predictions, enabling better model transparency and insights into the underlying relationships within the data (Lundberg and Lee, 2017). Higher positive SHAP scores reflect features contributing more to this discrimination within the dataset to designate a sample to FLT, while negative values reveal factors that have a negative impact on the FLT assignment, i.e., reveal the data as GC. In Figure 7, we show local bar plots explaining the feature importance among the five identified TFs in the FLT hypoxia cluster. Among these five TFs, *'ZAT12'* has the largest aggregate difference in SHAP values between FLT and GC and *MYC2* the smallest.

We see that *PHYD* mutants in the dark setting had the most contribution in model prediction in FLT for both *'ZAT12'* and *'MYC2'*, while *WS* genotype in the light setting for *'ZAT12'* and *PHYD* mutants in the light setting for *'MYC2'* had a notable negative effect towards FLT prediction. On the other hand, *col* genotype in the dark setting had the most contribution in model prediction in GC for both *'ZAT12'* and *'MYC2'*, indicating a strong differentiation between



(e)

TFs	Difference in SHAP value between FLT / GC
ZAT12	16.842
ZAT10	16.472
ERF109	13.964
DREB2A	9.606
MYC2	9.51

Figure 7: **SHAP analysis on Transcription Factors (TFs) in the hypoxia cluster.** A positive SHAP value (Red color) means that the feature value made a greater contribution than others in classifying the gene as FLT, while a negative SHAP value (Blue color) suggests they had more contribution in GC classification. (a) *ZAT12* - FLT (b) *ZAT12* - GC (c) *MYC2* - FLT (d) *MYC2* - GC (e) Summary of difference in SHAP value between FLT and GC for the 5 TFs in hypoxia.

conditions in a different location. The large difference in aggregated SHAP value between FLT and GC for *'ZAT12'* suggests that the relative importance and contributions of these features vary significantly between the FLT and GC. In contrast, the contributions for *'MYC2'* appear more consistent and stable across both FLT and GC classifications. Lastly, in Figure 8, we present summary SHAP plots on these features, varying light conditions on different genotypes, to offer a more comprehensive understanding of feature contribution across the entire dataset.

We can observe features with different degrees of impact on the model's prediction from the SHAP value scatterplot in Figure 8(a), for example, for the WS genotype: in a light setting, the majority of the data aligns with positive SHAP values, supporting FLT classification, whereas under dark conditions, the trend is reversed. The beeswarm plot (Figure 8(b)) illustrates the distribution of SHAP values for each feature. The color gradient from blue to red represents the feature value (FPKM values), with blue indicating low expression and red indicating high expression. Figure 8(b) illustrates that *PHYD* mutants in a dark setting have the highest effect on the classification with longer tails towards positive value, while most of the high FPKM values have negative SHAP value. Suggesting that high expression levels from *PHYD* mutants in dark settings decrease the likelihood of FLT classification. Similarly, the Col genotype in a dark setting has tails toward negative values, while most of the high FPKM values have positive SHAP values. These

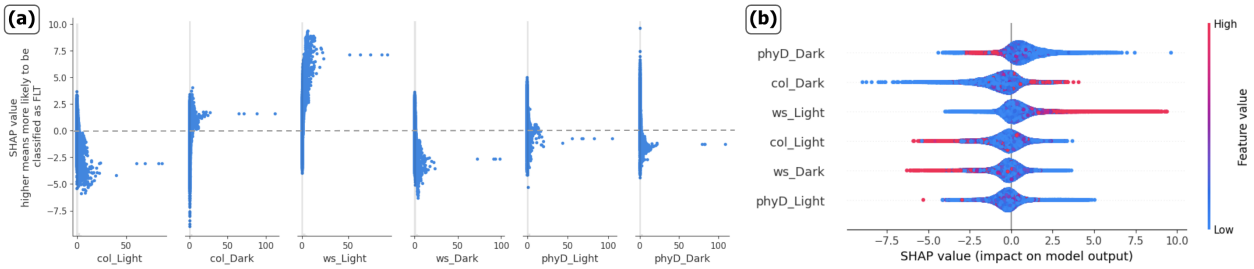


Figure 8: **SHAP value distribution for each treatment.** Comparing SHAP values from a classification using the XGBoost on the discretized CARA dataset. (a) The summary SHAP value scatterplot for each feature displays the distribution of SHAP values alongside raw feature values. (b) The summary SHAP beeswarm plots, where features are ordered by their importance (measured by mean absolute SHAP values), with the most impactful features appearing at the top. The color bar represents raw feature values. Both plots present the same information.

observations underscore the presence of intricate interactions between gene expressions, reflecting the complexity of the transcriptome data and the underlying biological mechanisms.

SHAP analysis provides a unique perspective on the patterns within the dataset, especially when the data comprises various environmental settings as features. Through analyzing the differences and similarities in SHAP values, researchers can identify genes that are sensitive to complex environment by genotype-dependent patterns in the data.

4 Discussion

In this study, we present an analysis pipeline, GLARE, that employs a state-of-the-art representation learning model with self-supervised learning. We chose a previously analyzed dataset, the CARA experiment (OSD-120), which allows for an investigation of the overall utility of the pipeline itself and a comparison with the prior findings. For analysis of the root samples in the CARA spaceflight data, we trained the system using high-throughput plant root single-cell data, along with ensemble clustering, to identify hidden patterns in the spaceflight transcriptome. For other spaceflight datasets, such as whole seedlings, shoot tissues, microbe, animal tissues, or cell types, matching training datasets to the particular experimental design would similarly add significant depth to these analyses. After the full pipeline, we present a recommended framework for post-pipeline analysis employing select bioinformatics tools and adding post hoc explainability to the deep learning approach by applying approaches such as SHAP analysis. Such analyses confirmed previous patterns found in the data, such as cell wall remodeling and vesicle-mediated transport, but critically revealed new features, notably a molecular signature of hypoxic stress in the spaceflight samples that is predicted from the lack of buoyancy-driven convection in spaceflight but that has proven complex to extract from many plant transcriptomic datasets. However, our analyses also revealed that this cryptic signature was dependent on experimental conditions such as plant genotype and lighting regime. For example, Figure 7 shows that SHAP analysis of the 5 signature spaceflight-related, hypoxia-response transcription factors identified in this study potentially help explain why these signals can be complex to identify in current spaceflight datasets without machine learning interrogation.

Although we present one post hoc analysis pipeline for the output of GLARE, researchers can readily leverage their preferred analytics tools when applying GLARE to their datasets to uncover patterns. To this end, we actively encourage contributions and novel suggestions through our open science repository. Its open-source nature means researchers can readily adapt GLARE on other datasets from GeneLab and elsewhere to reinforce their initial studies and expand on these computational findings. The recent rapid advancement in the machine learning field warrants future work on GLARE. Similar to our approach, integrating single-cell datasets has been widely adopted for their advantage in providing nuanced insights to the cellular level. Indeed, transformer-based foundation models for single-cell multi-omics have been suggested (Cui et al., 2024), which offer the potential to generate synthetic data or for gene network inference. Our future vision for GLARE is to extend beyond autoencoder-based models to add more advanced self-supervised representation learning models, such as contrastive learning methods that are well-used in the field of computer vision and natural language processing (Chen et al., 2020), to enhance robustness for smaller datasets with fewer features. Additionally, causal representation learning methods can be employed to discover the causal relationship between related genes (Uelwer et al., 2023; Schölkopf et al., 2021).

Conflict of Interest Statement

The authors declare they have no conflicts of interest.

Author Contributions

DH.S. and R.B. conceived of the study and fundamental design. DH.S. and H.F.S. contributed to model testing, data analysis, and figure preparation. DH.S., H.F.S., M.Z., R.B., A.-L.P, R.J.F., and S.G. contributed to manuscript preparation. All authors contributed to the manuscript review and editing.

Funding

The CARA experiment was supported by grant number GA-2013-104, Center for Advancement of Science in Space to A.-L. Paul (PI) and R.J. Ferl (CoI). We gratefully acknowledge support from NASA 80NSSC19K0126 and 80NSSC21K0577 to S.G.

Acknowledgments

The authors would like to acknowledge the sequencing and bioinformatics services provided by the Interdisciplinary Center for Biotechnology Research's (ICBR) Gene Expression (RRID:SCR_019145), NextGen Sequencing (RRID:SCR_019152), and Bioinformatics (RRID:SCR_019120) cores.

Data Availability Statement

The dataset (OSD-120) utilized in this method can be found on the NASA GeneLab Data System (`https://genelab.nasa.gov/`). The code utilized for data analysis can be found on the publicly available GitHub repository (`https://github.com/OpenScienceDataRepo/Plants_AWG/tree/main/Manuscript_Code/glare`).

References

Abts, W., Vandenbussche, B., De Proft, M. P., and Van de Poel, B. (2017). The role of auxin-ethylene crosstalk in orchestrating primary root elongation in sugar beet. *Frontiers in Plant Science*, 8:444.

Aljalbout, E., Golkov, V., Siddiqui, Y., Strobel, M., and Cremers, D. (2018). Clustering with deep learning: Taxonomy and new methods. *arXiv preprint arXiv:1801.07648*.

Ba, J. L., Kiros, J. R., and Hinton, G. E. (2016). Layer normalization. *arXiv preprint arXiv:1607.06450*.

Bleker, C., Ramšak, Ž., Bittner, A., Podpečan, V., Zagorščak, M., Wurzinger, B., Baebler, Š., Petek, M., Križnik, M., van Dieren, A., et al. (2023). Stress knowledge map: A knowledge graph resource for systems biology analysis of plant stress responses. *bioRxiv*, pages 2023–11.

Campello, R. J., Moulavi, D., and Sander, J. (2013). Density-based clustering based on hierarchical density estimates. In *Pacific-Asia conference on knowledge discovery and data mining*, pages 160–172. Springer.

Chaudhary, K., Poirion, O. B., Lu, L., and Garmire, L. X. (2018). Deep learning–based multi-omics integration robustly predicts survival in liver cancer. *Clinical Cancer Research*, 24(6):1248–1259.

Chen, T. and Guestrin, C. (2016). Xgboost: A scalable tree boosting system. In *Proceedings of the 22nd acm sigkdd international conference on knowledge discovery and data mining*, pages 785–794.

Chen, T., Kornblith, S., Norouzi, M., and Hinton, G. (2020). A simple framework for contrastive learning of visual representations. In *International conference on machine learning*, pages 1597–1607. PMLR.

Clevert, D.-A., Unterthiner, T., and Hochreiter, S. (2015). Fast and accurate deep network learning by exponential linear units (elus). *arXiv preprint arXiv:1511.07289*.

Cui, H., Wang, C., Maan, H., Pang, K., Luo, F., Duan, N., and Wang, B. (2024). scgpt: toward building a foundation model for single-cell multi-omics using generative ai. *Nature Methods*, pages 1–11.

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., and Gingeras, T. R. (2013). Star: ultrafast universal rna-seq aligner. *Bioinformatics*, 29(1):15–21.

Ester, M., Kriegel, H.-P., Sander, J., Xu, X., et al. (1996). A density-based algorithm for discovering clusters in large spatial databases with noise. In *kdd*, volume 96, pages 226–231.

Ferl, R. J. and Paul, A.-L. (2016). The effect of spaceflight on the gravity-sensing auxin gradient of roots: Gfp reporter gene microscopy on orbit. *npj Microgravity*, 2(1):1–9.

Ferl, R. J., Zupanska, A., Spinale, A., Reed, D., Manning-Roach, S., Guerra, G., Cox, D. R., and Paul, A.-L. (2011). The performance of ksc fixation tubes with rnalater for orbital experiments: A case study in iss operations for molecular biology. *Advances in Space Research*, 48(1):199–206.

Fred, A. L. and Jain, A. K. (2005). Combining multiple clusterings using evidence accumulation. *IEEE transactions on pattern analysis and machine intelligence*, 27(6):835–850.

Fu, Y., Li, L., Xie, B., Dong, C., Wang, M., Jia, B., Shao, L., Dong, Y., Deng, S., Liu, H., et al. (2016). How to establish a bioregenerative life support system for long-term crewed missions to the moon or mars. *Astrobiology*, 16(12):925–936.

Gan, G., Ma, C., and Wu, J. (2020). *Data clustering: theory, algorithms, and applications*. SIAM.

Hao, M., Gong, J., Zeng, X., Liu, C., Guo, Y., Cheng, X., Wang, T., Ma, J., Song, L., and Zhang, X. (2023). Large scale foundation model on single-cell transcriptomics. *bioRxiv*, pages 2023–05.

Hinton, G. E. and Salakhutdinov, R. R. (2006). Reducing the dimensionality of data with neural networks. *science*, 313(5786):504–507.

Hulot, A., Chiquet, J., Jaffrézic, F., and Rigaill, G. (2020). Fast tree aggregation for consensus hierarchical clustering. *BMC bioinformatics*, 21(1):1–12.

Iqbal, N., Khan, N. A., Ferrante, A., Trivellini, A., Francini, A., and Khan, M. (2017). Ethylene role in plant growth, development and senescence: interaction with other phytohormones. *Frontiers in plant science*, 8:475.

Jain, A. K. (2010). Data clustering: 50 years beyond k-means. *Pattern recognition letters*, 31(8):651–666.

Karim, M. R., Beyan, O., Zappa, A., Costa, I. G., Rebholz-Schuhmann, D., Cochez, M., and Decker, S. (2021). Deep learning-based clustering approaches for bioinformatics. *Briefings in bioinformatics*, 22(1):393–415.

Kingma, D. P. and Ba, J. (2014). Adam: A method for stochastic optimization. *arXiv preprint arXiv:1412.6980*.

Kiselev, V. Y., Yiu, A., and Hemberg, M. (2018). scmap: projection of single-cell rna-seq data across data sets. *Nature methods*, 15(5):359–362.

Lee, J. A., Verleysen, M., et al. (2007). *Nonlinear dimensionality reduction*, volume 1. Springer.

Lei, D., Zhu, Q., Chen, J., Lin, H., and Yang, P. (2012). Automatic k-means clustering algorithm for outlier detection. In *Information Engineering and Applications: International Conference on Information Engineering and Applications (IEA 2011)*, pages 363–372. Springer.

Liao, Y. and Vemuri, V. R. (2002). Use of k-nearest neighbor classifier for intrusion detection. *Computers & security*, 21(5):439–448.

Lundberg, S. M. and Lee, S.-I. (2017). A unified approach to interpreting model predictions. In Guyon, I., Luxburg, U. V., Bengio, S., Wallach, H., Fergus, R., Vishwanathan, S., and Garnett, R., editors, *Advances in Neural Information Processing Systems 30*, pages 4765–4774. Curran Associates, Inc.

Makhzani, A. and Frey, B. (2013). K-sparse autoencoders. *arXiv preprint arXiv:1312.5663*.

McInnes, L., Healy, J., and Melville, J. (2018). Umap: Uniform manifold approximation and projection for dimension reduction. *arXiv preprint arXiv:1802.03426*.

Monti, S., Tamayo, P., Mesirov, J., and Golub, T. (2003). Consensus clustering: a resampling-based method for class discovery and visualization of gene expression microarray data. *Machine learning*, 52:91–118.

Mustroph, A., Lee, S. C., Oosumi, T., Zanetti, M. E., Yang, H., Ma, K., Yaghoubi-Masihi, A., Fukao, T., and Bailey-Serres, J. (2010). Cross-kingdom comparison of transcriptomic adjustments to low-oxygen stress highlights conserved and plant-specific responses. *Plant Physiology*, 152(3):1484–1500.

Ng, A. et al. (2011). Sparse autoencoder. *CS294A Lecture notes*, 72(2011):1–19.

Ng, A., Jordan, M., and Weiss, Y. (2001). On spectral clustering: Analysis and an algorithm. *Advances in neural information processing systems*, 14.

Paul, A.-L., Sng, N. J., Zupanska, A. K., Krishnamurthy, A., Schultz, E. R., and Ferl, R. J. (2017). Genetic dissection of the arabidopsis spaceflight transcriptome: Are some responses dispensable for the physiological adaptation of plants to spaceflight? *PLoS One*, 12(6):e0180186.

Paul, A.-L., Zupanska, A. K., Schultz, E. R., and Ferl, R. J. (2013). Organ-specific remodeling of the arabidopsis transcriptome in response to spaceflight. *BMC Plant Biology*, 13(112).

Porterfield, D. M. (2002). The biophysical limitations in physiological transport and exchange in plants grown in microgravity. *Journal of Plant Growth Regulation*, 21(2).

Ranzato, M., Boureau, Y.-L., Cun, Y., et al. (2007). Sparse feature learning for deep belief networks. *Advances in neural information processing systems*, 20.

Rappoport, N. and Shamir, R. (2018). Multi-omic and multi-view clustering algorithms: review and cancer benchmark. *Nucleic acids research*, 46(20):10546–10562.

Ray, S., Gebre, S., Fogle, H., Berrios, D. C., Tran, P. B., Galazka, J. M., and Costes, S. V. (2018). GeneLab: Omics database for spaceflight experiments. *Bioinformatics*, 35(10):1753–1759. _eprint: https://academic.oup.com/bioinformatics/article-pdf/35/10/1753/48969335/bioinformatics_35_10_1753.pdf.

Reynolds, D. A. et al. (2009). Gaussian mixture models. *Encyclopedia of biometrics*, 741(659-663).

Rousseeuw, P. J. (1987). Silhouettes: a graphical aid to the interpretation and validation of cluster analysis. *Journal of computational and applied mathematics*, 20:53–65.

Rutter, L., Barker, R., Bezdan, D., Cope, H., Costes, S., Degoricija, L., Fisch, K., Gabitto, M., Gebre, S., Giacomello, S., et al. (2020). A new era for space life science: international standards for space omics processing (issop). patterns.

Schölkopf, B., Locatello, F., Bauer, S., Ke, N. R., Kalchbrenner, N., Goyal, A., and Bengio, Y. (2021). Toward causal representation learning. *Proceedings of the IEEE*, 109(5):612–634.

Shahan, R., Hsu, C.-W., Nolan, T. M., Cole, B. J., Taylor, I. W., Greenstreet, L., Zhang, S., Afanassiev, A., Vlot, A. H. C., Schiebinger, G., et al. (2022). A single-cell arabidopsis root atlas reveals developmental trajectories in wild-type and cell identity mutants. *Developmental cell*, 57(4):543–560.

Shulse, C. N., Cole, B. J., Ciobanu, D., Lin, J., Yoshinaga, Y., Gouran, M., Turco, G. M., Zhu, Y., O'Malley, R. C., Brady, S. M., et al. (2019). High-throughput single-cell transcriptome profiling of plant cell types. *Cell reports*, 27(7):2241–2247.

Strehl, A. and Ghosh, J. (2002). Cluster ensembles—a knowledge reuse framework for combining multiple partitions. *Journal of machine learning research*, 3(Dec):583–617.

Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D. R., Pimentel, H., Salzberg, S. L., Rinn, J. L., and Pachter, L. (2012). Differential gene and transcript expression analysis of rna-seq experiments with tophat and cufflinks. *Nature protocols*, 7(3):562–578.

Uelwer, T., Robine, J., Wagner, S. S., Höftmann, M., Upschulte, E., Konietzny, S., Behrendt, M., and Harmeling, S. (2023). A survey on self-supervised representation learning. *arXiv preprint arXiv:2308.11455*.

Van Der Maaten, L. (2009). Learning a parametric embedding by preserving local structure. In *Artificial intelligence and statistics*, pages 384–391. PMLR.

Van der Maaten, L. and Hinton, G. (2008). Visualizing data using t-sne. *Journal of machine learning research*, 9(11).

Vega-Pons, S. and Ruiz-Shulcloper, J. (2011). A survey of clustering ensemble algorithms. *International Journal of Pattern Recognition and Artificial Intelligence*, 25(03):337–372.

Villacampa, A., Ciska, M., Manzano, A., Vandenbrink, J. P., Kiss, J. Z., Herranz, R., and Medina, F. J. (2021). From spaceflight to mars g-levels: Adaptive response of a. thaliana seedlings in a reduced gravity environment is enhanced by red-light photostimulation. *International Journal of Molecular Sciences*, 22(2):899.

Xu, C., Tao, D., and Xu, C. (2013). A survey on multi-view learning. *arXiv preprint arXiv:1304.5634*.

Zeng, I. S. L. and Lumley, T. (2018). Review of statistical learning methods in integrated omics studies (an integrated information science). *Bioinformatics and biology insights*, 12:1177932218759292.

Zhou, Y., Zhou, B., Pache, L., Chang, M., Khodabakhshi, A. H., Tanaseichuk, O., Benner, C., and Chanda, S. K. (2019). Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nature communications*, 10(1):1523.

Supplementary Data and Table

We provide supplementary data at the OSDR GitHub repository (`https://github.com/OpenScienceDataRepo/Plants_AWG/tree/main/Manuscript_Code/glare`), including the codes for the method and reproducible results such as single-cell pre-trained model weights, data representations, ensemble clustering results, Gene Ontology analysis results for all clusters, and predicted SHAP values for both FLT and GC. Supplementary Table S1 is also included in the repository.

Supplementary Figure

The supplementary Figure S1 provides an enriched ontology analysis from Metascape (Zhou et al., 2019) on vesicle-mediated transport related clusters using FT-SAE data representations from FLT and GC datasets. FLT clusters emphasize metabolic and catabolic processes, such as “small molecule catabolic process" and “reactive oxygen species metabolic process". Regulatory processes, such as “regulation of programmed cell death" are also prominent in FLT. In contrast, GC clusters focus on cellular structure and developmental pathways, with terms like “cellular macromolecule localization" and “plant-type cell wall organization", as well as developmental terms like “embryo development". This analysis illustrates the distinct biological pathways captured by FLT and GC data representations, offering complementary insights to prior study (Paul et al., 2017) with plant-specific metabolic and developmental pathways.

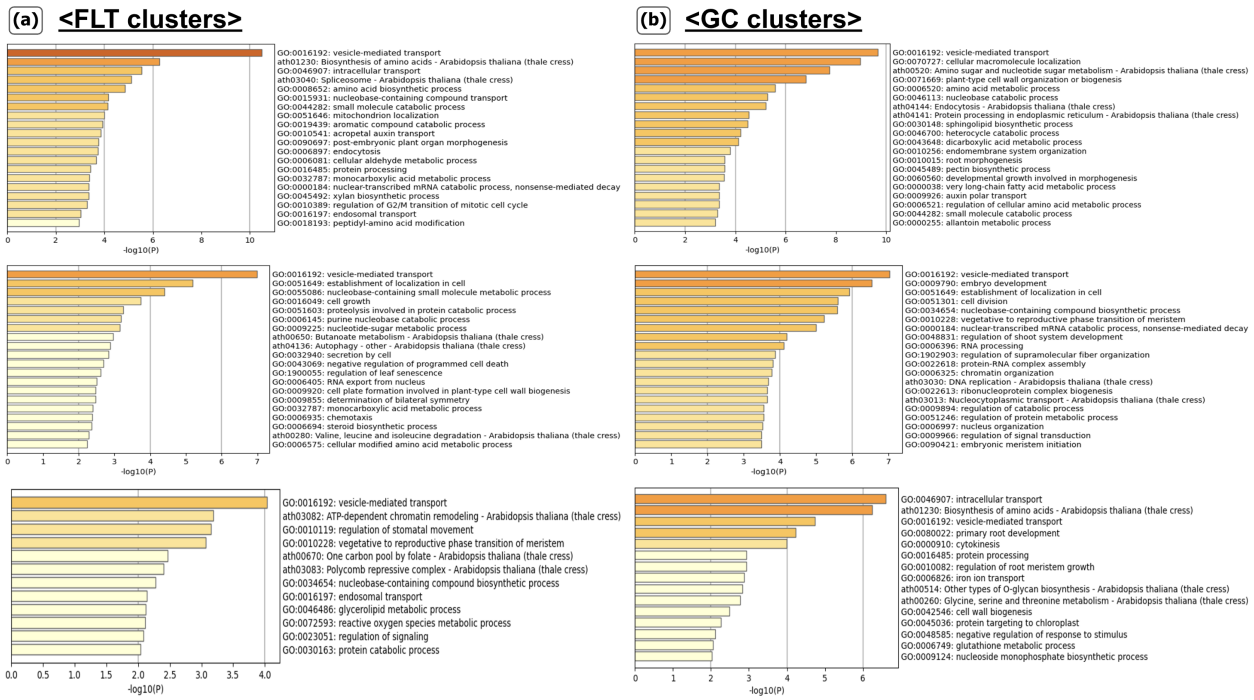


Figure S1: **Vesicle-mediated transport related clusters in FLT and GC.** (a) Enriched ontology analysis from Metascape on vesicle-mediated transport related clusters using FLT FT-SAE data representations. (b) Equivalent analysis using GC FT-SAE data representations.