Package 'TCGAimmunosurv'

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Description
'TCGAimmunosurv', for the integrated analysis of bulk and single-cell RNA-Seq data across parameter studies. This package facilitates a deeper understanding of cancer immune dynamics in the context of specific oncogene mutations. Key features of this package include mutation specific survival analysis, pseudotime trajectory analysis, identification of differentially expressed genes, and comprehensive insights into mutations within cancers or specific genes.
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Contents

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assign_	cdQ	labels																												2
assigii_	_cuo_	_iabeis	•	•	•	•	•	•		•	•	•	•	٠	٠	•	•	 	•	٠	•	•	•	•	 					4

2 assign_cd8_labels

calculate_DEGs	3
cluster_and_umap	4
extract_genes_hr_gt1	4
filter_rna_data	5
filter_seurat_object	5
generate_mutation_summary	6
get_mutated_samples	
get_top_combined_genes	
identify_markers	
load cancer data	
load_seurat_object	
mapping_genes	
perform_pca	
perform_pseudotime	
perform_survival_analysis	
plot_cd8_trajectory	
plot_DEG_results	
plot_km_curves	
plot_mutation_counts	
plot_results	
plot_unique_mutation_types	
preprocess_data	
run_graph_test	
standardize tcga ids	
style_mutation_table	
visualize_cd8_markers	
visualize_cd8_subtypes	
visualize_pca	
visualize_umap	23
	24

assign_cd8_labels

 $Assign\ CD8+T\ cell\ Labels$

Description

This function assigns labels to CD8+ T cells based on marker gene expression (e.g., naive, cytotoxic, exhausted).

Usage

Index

```
assign_cd8_labels(
  seurat_obj,
  all_markers,
  naive_markers,
  cytotoxic_markers,
  exhausted_markers
)
```

calculate_DEGs 3

Arguments

seurat_obj A Seurat object with CD8+ T cell expression data.

all_markers A data frame of all markers identified across clusters.

naive_markers A vector of naive CD8+ T cell markers.

cytotoxic_markers

A vector of cytotoxic CD8+ T cell markers.

exhausted_markers

A vector of exhausted CD8+ T cell markers.

Value

A list containing the updated Seurat object with CD8+ labels and a subset of CD8+ T cells.

calculate_DEGs Perform Differential Expression Analysis

Description

This function calculates differentially expressed genes (DEGs) from RNA-Seq data using the DE-Seq2 package. It applies thresholds for statistical significance (p-value) and fold change to classify genes as upregulated, downregulated, or not significant.

Usage

```
calculate_DEGs(rna_data, condition_column, alpha, log2FC_threshold)
```

Arguments

rna_data A DESeqDataSet object containing the RNA-Seq count matrix and metadata. condition_column

A string specifying the name of the column in the metadata that defines the experimental conditions/groups.

alpha A numeric value specifying the significance threshold for the p-value (e.g.,

log2FC_threshold

A numeric value specifying the log2 fold change threshold to determine upregulated or downregulated genes.

Value

A data frame containing the DESeq2 results with an additional column, significance, indicating whether each gene is upregulated, downregulated, or not significant.

cluster_a	nd umap
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Cluster and Perform UMAP

Description

This function clusters the cells and performs UMAP dimensionality reduction for visualization.

Usage

```
cluster_and_umap(seurat_obj, dims = 20, resolution = 0.1)
```

Arguments

seurat_obj A Seurat object containing single-cell RNA-seq data.

dims Number of dimensions to use for clustering (default is 20).

Resolution parameter for clustering (default is 0.1).

Value

A Seurat object with clustering and UMAP results added.

Description

This function filters significant genes with a hazard ratio (HR) greater than 1 from a given results DataFrame. It returns a DataFrame of the filtered genes and their hazard ratios and saves the results to a .csv file.

Usage

```
extract_genes_hr_gt1(results, output_file = "significant_genes.csv")
```

Arguments

results A DataFrame containing gene analysis results with at least the following columns:

significant, hazard_ratio, and gene_name.

output_file Character. The name of the output .csv file to save the results. Default is

"significant_genes.csv".

Value

A DataFrame with two columns:

- gene_name: Names of significant genes with HR > 1.
- hazard_ratio: The hazard ratios of the significant genes.

filter_rna_data 5

filter_rna_data

Filter RNA Data

Description

This function filters RNA-Seq data stored in a SummarizedExperiment object. It allows for filtering samples based on specific conditions, removing rows with high NA values, replacing remaining NAs with the median, and removing low-count rows.

Usage

```
filter_rna_data(
    rna_data,
    condition_column,
    condition_levels,
    na_threshold = 0.8
)
```

Arguments

rna_data

A SummarizedExperiment object containing RNA-Seq count data.

condition_column

A character string specifying the column name in colData that contains the conditions for filtering.

condition_levels

A character vector of condition levels to retain in the data.

na_threshold

A numeric value (default = 0.8) specifying the maximum allowed fraction of NAs in a row. Rows with a higher fraction of NAs will be removed.

Value

A filtered SummarizedExperiment object with updated counts, rowRanges, and colData.

filter_seurat_object Filter Seurat Object Based on Quality Control Criteria

Description

This function filters a Seurat object based on the number of detected features (genes) and the percentage of mitochondrial genes. It ensures that only high-quality cells are kept for downstream analysis.

Usage

```
filter_seurat_object(
   seurat_obj,
   min_features = 200,
   max_features = 2500,
   max_percent_mt = 5
)
```

Arguments

A Seurat object containing single-cell RNA-seq data.

min_features
An integer specifying the minimum number of detected features (genes) required for a cell to be retained. Default is 200.

max_features
An integer specifying the maximum number of detected features (genes) allowed for a cell. Default is 2500.

max_percent_mt
A numeric value specifying the maximum percentage of mitochondrial genes allowed for a cell. Default is 5.

Details

The function adds a new metadata column to the Seurat object, percent.mt, which represents the percentage of mitochondrial genes expressed in each cell. The cells are then filtered based on the following criteria:

- The number of detected features (genes) must be between min_features and max_features.
- The percentage of mitochondrial gene expression must be below max_percent_mt.

This helps to remove cells with poor quality (e.g., low gene count or high mitochondrial gene expression) that could skew downstream analyses.

Value

A filtered Seurat object, where cells meeting the quality control criteria are retained.

```
generate_mutation_summary

Generate Mutation Summary for Genes of Interest
```

Description

This function generates a summary of mutation types and their counts for a given list of genes from a MAF (Mutation Annotation Format) dataset.

Usage

```
generate_mutation_summary(cancer_maf, genes_of_interest)
```

get_mutated_samples 7

Arguments

 ${\tt cancer_maf} \qquad A \ MAF \ object \ created \ using \ the \ maftools \ package, \ containing \ mutation \ data. \\ {\tt genes_of_interest}$

A character vector of gene names to summarize mutations for.

Details

- The function checks if each gene in genes_of_interest exists in the MAF data.
- If a gene is not found or has no non-synonymous variants, a warning is issued, and the gene is skipped.
- Mutation types and counts are extracted for each valid gene.

Value

A data table containing the mutation summary for the specified genes. The table includes:

- Gene: The gene name.
- Mutation_Type: The type of mutation.
- NumSamples: The number of samples with the specified mutation type.

get_mutated_samples Get Unique Mutated Sample IDs for Selected Genes

Description

This function extracts unique tumor sample barcodes for selected genes with mutations from a MAF (Mutation Annotation Format) dataset.

Usage

```
get_mutated_samples(cancer_maf, genes_of_interest)
```

Arguments

cancer_maf A MAF object created using the maftools package, containing mutation data. genes_of_interest

A character vector of gene names for which the mutated samples are to be extracted.

Details

- The function subsets the MAF data to include only the mutations for the specified genes.
- It then extracts the unique tumor sample barcodes where mutations for those genes are present.

Value

A character vector containing the unique tumor sample barcodes of samples where mutations in the selected genes are present.

```
get_top_combined_genes
```

Get Top Combined Ranked Genes and Save to CSV

Description

This function identifies the top genes based on a combined rank of q-values and Moran's I statistic from spatial transcriptomics analysis, and saves the results to a .csv file.

Usage

```
get_top_combined_genes(
  graph_test_res,
  q_value_threshold = 0.05,
  top_n = 10,
  output_file = "top_genes.csv"
)
```

Arguments

```
graph_test_res A data frame containing gene statistics, including q_value (adjusted p-value) and morans_I (Moran's I statistic).
```

q_value_threshold

Numeric. The threshold for significant q-values. Default is 0.05.

top_n Integer. The number of top genes to return based on the combined rank. Default

is 10.

output_file Character. The name of the output .csv file to save the results. Default is

"top_genes.csv".

Details

The function filters genes based on the q_value_threshold, then combines the ranks of q_value (ascending order) and morans_I (descending order). The combined rank is used to determine the top top_n genes. The top genes and their ranks are saved to a .csv file.

Value

A character vector containing the names of the top-ranked genes.

identify_markers 9

identify_markers	Identify Cluster Markers	
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Description

This function identifies marker genes for each cluster.

Usage

```
identify_markers(seurat_obj, min_pct = 0.25, logfc_thresh = 0.25)
```

Arguments

seurat_obj A Seurat object with clustered cells.

min_pct Minimum percentage of cells expressing the gene to be considered as a marker

(default is 0.25).

logfc_thresh Minimum log fold change for a gene to be considered significant (default is

0.25).

Value

A data frame containing identified markers for each cluster.

load_cancer_data	Load Cancer Data for a Selected Cancer Type	

Description

This function loads TCGA data for a specified cancer type, including RNA-Seq counts, clinical data, and mutation data. If the data file exists, it is loaded directly.

Usage

```
load_cancer_data(selected_cancer_type)
```

Arguments

```
selected_cancer_type
```

A character string specifying the TCGA cancer type (e.g., "BRCA" for breast cancer, "LUAD" for lung adenocarcinoma).

10 load_seurat_object

Details

If the data file exists locally, this function will:

- Load the RNA-Seq count data.
- · Load the clinical data.
- Load the mutation data.

Value

None. This function loads the data into the global environment:

- tcga_count_data: RNA-Seq count data in a SummarizedExperiment object.
- clinical_data: Clinical data for survival analysis and metadata.
- mutation_data: Mutation data from TCGA.
- sample_type: A data frame of sample types.

load_seurat_object

Load Seurat Object for Single-Cell Analysis

Description

This function loads a Seurat object from a specified directory based on a given cancer type. The Seurat object will be used to perform further single-cell RNA-seq analysis.

Usage

```
load_seurat_object(
  directory,
  constant_prefix = "combined_seurat_",
  selected_cancer
)
```

Arguments

directory A character string specifying the directory where the Seurat object file is located. constant_prefix

A character string specifying the constant prefix for the Seurat object file name. The default is "combined_seurat_".

selected_cancer

A character string specifying the cancer type for which the Seurat object file will be loaded. The cancer type will be appended to the constant prefix.

Details

This function constructs the file name for the Seurat object based on the provided constant_prefix and selected_cancer. It checks if the file exists in the specified directory and loads the Seurat object if the file is found. If the file does not exist, an error message is displayed.

mapping_genes 11

Value

A Seurat object that has been loaded from the specified file.

mapping_genes

Map Differentially Expressed Genes (DEGs) with Gene Metadata

Description

This function takes in the results of differential gene expression analysis (deg_results), a gene metadata table (gene_metadata_dt), and filters for upregulated and downregulated genes based on user-defined thresholds for log fold change and adjusted p-value. It then returns a merged table with gene names and the associated differential expression data.

Usage

```
mapping_genes(deg_results, gene_metadata_dt, logFC_threshold, padj_threshold)
```

Arguments

deg_results

A data frame containing differential expression results. The data frame should include at least the following columns:

log2FoldChange The log2 fold change values for the genes.

padj The adjusted p-values for the genes.

ensemble_id A unique identifier for each gene (can be rownames if not present as a column).

gene_metadata_dt

A data frame containing gene metadata. This should include at least the following columns:

ensemble_id The unique identifier for the gene, which matches the ensemble_id in deg_results.

gene_name The gene names corresponding to the ensemble_id.

logFC_threshold

A numeric value specifying the threshold for log2 fold change. Genes with log2FoldChange > logFC_threshold are considered upregulated, and genes with log2FoldChange < -logFC_threshold are considered downregulated.

padj_threshold A numeric value specifying the adjusted p-value threshold. Only genes with padj < padj_threshold will be selected.

Value

A data frame containing the upregulated and downregulated genes based on the given thresholds, with columns:

ensemble_id The unique identifier for the gene.

gene_name The gene name associated with the ensemble_id.

log2FoldChange The log2 fold change values for the gene.

padj The adjusted p-values for the gene.

12 perform_pseudotime

perform_pca

Perform Principal Component Analysis (PCA) on Seurat Object

Description

This function performs PCA on a Seurat object after normalizing the data, identifying variable features, and scaling the data. The resulting PCA components can be used for dimensionality reduction and visualization in downstream analysis.

Usage

```
perform_pca(seurat_obj)
```

Arguments

seurat_obj

A Seurat object containing single-cell RNA-seq data.

Details

The function performs the following steps:

- Normalizes the data using Seurat::NormalizeData().
- Identifies highly variable features with Seurat::FindVariableFeatures().
- Scales the data using Seurat::ScaleData().
- Runs PCA using the identified variable features with Seurat::RunPCA().

These steps are essential for reducing the dimensionality of the data and identifying principal components that explain most of the variance in the dataset.

Value

A Seurat object with PCA results stored in the pca assay, ready for further analysis (e.g., visualization, clustering).

perform_pseudotime

Perform Pseudotime Analysis

Description

This function performs pseudotime analysis on CD8+ T cells using the Monocle3 package.

Usage

```
perform_pseudotime(seurat_obj, num_dim = 100, root_cluster = "Naive CD8+ T")
```

Arguments

seurat_obj A Seurat object with CD8+ T cells.

num_dim Number of dimensions for preprocessing (default is 100).

root_cluster The cluster to set as the root (default is "Naive CD8+ T").

Value

A Monocle3 CellDataSet with pseudotime trajectory learned.

```
perform_survival_analysis
```

Perform Survival Analysis on RNA-Seq Data

Description

This function performs survival analysis on RNA-Seq data by grouping samples into two strata (HIGH/LOW) based on the median expression of each gene. It then performs Cox regression analysis for each gene and returns the hazard ratios and p-values.

Usage

```
perform_survival_analysis(
  count_matrix_filtered,
  gene_metadata_dt,
  clinical_data_filtered
)
```

Arguments

count_matrix_filtered

A matrix of RNA-Seq count data, where rows represent genes and columns represent samples.

gene_metadata_dt

 $A \ data \ frame \ containing \ metadata \ for \ the \ genes, including \ a \ gene_name \ column. \\ clinical_data_filtered$

A data frame containing clinical data, including submitter_id, overall_survival, and deceased columns.

Value

A data frame with the results of the survival analysis for each gene, including:

- gene_name: The name of the gene.
- hazard_ratio: The hazard ratio for the gene.
- pvalue: The p-value from the Cox regression for the gene.
- significant: Whether the gene is significantly associated with survival (p-value \leq 0.05).

plot_DEG_results

Description

This function visualizes the pseudotime trajectory for CD8+ T cells.

Usage

```
plot_cd8_trajectory(
  cds,
  cd8_tcells,
  color_by_pseudotime = TRUE,
  label_size = 6
)
```

Arguments

Value

Pseudotime trajectory plot.

```
plot_DEG_results
```

Plot Differentially Expressed Genes (DEGs) Results

Description

This function generates two plots to visualize the results of differential expression analysis: a bar plot showing the sample distribution for different conditions and a volcano plot for visualizing the differentially expressed genes (DEGs).

Usage

```
plot_DEG_results(
  res_df,
  rna_data,
  condition_column,
  log2FC_threshold = 1,
  alpha = 0.05
)
```

plot_km_curves 15

Arguments

res_df A data frame containing the results of the differential expression analysis, in-

cluding columns for log2FoldChange, padj (adjusted p-values), and significance. The significance column should indicate whether a gene is "Upregulated",

"Downregulated", or "Not Significant".

rna_data A SummarizedExperiment object containing RNA-Seq data with sample meta-

data accessible via SummarizedExperiment::colData.

condition_column

A character string specifying the name of the column in SummarizedExperiment::colData(rna_data) that contains condition labels (e.g., "Control" and "Treatment").

log2FC_threshold

A numeric value specifying the threshold for log2FoldChange. Default is 1.

alpha A numeric value specifying the significance threshold for adjusted p-values. De-

fault is 0.05.

Details

• The bar plot displays the sample count for each condition in the dataset, with labels for the number of samples above each bar.

- The volcano plot uses log2FoldChange and -log10(padj) to visualize the significance and magnitude of gene expression changes. Points are colored based on their significance status.
- Both plots use customizable font styles for better readability.

Value

None. This function outputs two plots:

- A bar plot showing the distribution of samples across conditions.
- A volcano plot visualizing the DEGs.

plot_km_curves Plot Kaplan-Meier Curves for Significant Genes

Description

This function generates Kaplan-Meier survival curves for each significant gene based on its association with the clinical data. The function filters the strata_data for the significant genes, merges it with clinical data, and fits the Kaplan-Meier model for each gene. A survival plot is then created for each gene showing survival probability over time.

Usage

plot_km_curves(strata_data, clinical_data_filtered, significant_genes)

plot_mutation_counts

Arguments

strata_data

A data frame containing gene expression data for multiple cases. This data frame should include at least the columns:

gene_name The name of the gene.

case_id The identifier for the case/sample.

clinical_data_filtered

A data frame containing clinical data filtered for the relevant cases. This data frame should include at least the columns:

submitter_id The identifier for the case/sample.

overall_survival The survival time of the patient (in days).

deceased A binary indicator of whether the patient is deceased (1 = deceased, 0 = alive).

strata A factor or categorical variable used to stratify the survival analysis (e.g., treatment groups).

significant_genes

A character vector containing the names of significant genes to plot. The function will generate Kaplan-Meier curves for each gene in this vector.

Value

A list of Kaplan-Meier plots (ggsurvplot objects) for each significant gene. The plots are printed individually for each gene.

Description

This function generates separate bar plots showing the mutation counts for each gene, based on mutation types and sample counts.

Usage

plot_mutation_counts(mutation_counts)

Arguments

mutation_counts

A data.frame or data.table containing the mutation data, including columns for:

- Gene: The gene name.
- Mutation_Type: The type of mutation (e.g., missense, nonsense).
- NumSamples: The number of samples with that mutation type for the given gene.

plot_results 17

Details

The function iterates over each unique gene in the mutation_counts data and creates a bar plot for that gene. Each plot represents the count of mutations per mutation type for the gene, with the bars filled according to the mutation type.

Value

A series of bar plots, one for each gene, showing the count of mutations by mutation type.

plot_results

Plot Survival Analysis Results

Description

This function generates three types of plots to visualize the survival analysis results: a bar plot showing the number of significant vs. non-significant genes, a bar plot showing the count of significant genes categorized by hazard ratio, and a volcano plot representing the relationship between hazard ratio and p-value.

Usage

```
plot_results(results)
```

Arguments

results

A data frame containing the results of the survival analysis. The data frame should have the following columns:

gene_name Character string, the name of the gene.

hazard_ratio Numerical value representing the hazard ratio.

pvalue Numerical value representing the p-value of the gene.

significant Character string, "Yes" if the gene is statistically significant, otherwise "No".

Value

A list containing three ggplot objects:

SignificancePlot A bar plot showing the count of significant vs. non-significant genes.

HRPlot A bar plot showing the count of significant genes categorized by hazard ratio.

VolcanoPlot A volcano plot showing the relationship between log hazard ratio and -log10 p-value.

18 preprocess_data

```
plot_unique_mutation_types
```

Plot Unique Mutation Types Across Genes

Description

This function generates a bar plot to visualize the distribution of unique mutation types across genes for a given cancer type based on the MAF (Mutation Annotation Format) data.

Usage

```
plot_unique_mutation_types(cancer_maf)
```

Arguments

cancer_maf

A MAF object representing mutation data for a specific cancer type. This object is typically created using the maftools::read.maf function.

Details

- The function extracts the mutation type counts from the MAF object using the maftools::getGeneSummary function.
- Mutation types with zero counts are filtered out.
- The mutation counts are aggregated across all genes for each mutation type.
- The resulting plot displays mutation types on the x-axis and the total number of samples on the y-axis.

Value

A ggplot object showing the bar plot of unique mutation types and their total number of samples across all genes in the provided MAF object.

preprocess_data

Preprocess RNA-Seq and Clinical Data

Description

This function preprocesses RNA-Seq count data and clinical data, filtering both to retain only the samples that have mutation data and are present in both the RNA-Seq and clinical datasets. It also standardizes the sample IDs to a common format.

Usage

```
preprocess_data(rna_data, clinical_data, extracted_mutated_sample_ids)
```

19 run_graph_test

Arguments

A SummarizedExperiment or similar object containing RNA-Seq count data. It rna_data

must have a count matrix and associated gene metadata.

clinical_data A data frame containing clinical data with a column submitter_id that matches

sample IDs in rna_data.

extracted_mutated_sample_ids

A character vector of sample IDs that have mutations of interest.

Details

The function filters the RNA-Seq count matrix and the clinical data to include only the samples that are present in both the mutation data and clinical data. The sample IDs are standardized to the first three components (using hyphen delimiters) for consistency.

Value

A list containing two elements:

- · count_matrix_filtered: A matrix of RNA-Seq count data for samples that have mutation data and are present in both datasets.
- clinical_data_filtered: A data frame of clinical data for samples that have mutation data and are present in both datasets.

run_graph_test Run Graph Test

Description

This function performs a graph test on the Monocle3 CellDataSet.

Usage

```
run_graph_test(cds, neighbor_graph = "knn", cores = 1)
```

Arguments

cds A Monocle3 CellDataSet to run the graph test on.

neighbor_graph The neighbor graph type to use ("knn" or "principal_graph").

cores Number of CPU cores to use for parallel processing.

Value

Data frame with graph test results.

20 style_mutation_table

Description

This function standardizes TCGA sample IDs by extracting the first three components of the ID before the first hyphen ("-"). If the ID format is invalid (does not contain "-"), it returns NA.

Usage

```
standardize_tcga_ids(sample_ids)
```

Arguments

sample_ids A character vector of TCGA sample IDs.

Details

The function ensures that all TCGA sample IDs are standardized to the format containing only the first three components separated by hyphens. If the input contains IDs in an incorrect format (i.e., not containing "-"), the function returns NA for those IDs.

Value

A character vector of standardized TCGA sample IDs, where each ID is truncated to the first three components, or NA if the format is invalid.

Description

This function applies styling to a mutation counts table, enhancing the readability and presentation of the data using the kable and kableExtra packages.

Usage

```
style_mutation_table(mutation_counts)
```

Arguments

mutation_counts

A data frame or data table containing the mutation counts, with columns for:

- Gene: The gene name.
- Mutation_Type: The type of mutation.
- NumSamples: The number of samples with that mutation type for the given gene.

visualize_cd8_markers

Details

The function uses kableExtra::kbl() to create a table and then applies various styling options:

21

- striped, hover, condensed, and responsive bootstrap options for table formatting.
- The first column (Gene) is made bold with a fixed width of 3 cm.
- The second column (Mutation Type) has a width of 4 cm.
- The third column (NumSamples) is colored blue.
- A footnote is added to summarize the content of the table.

Value

A styled table with mutation counts for selected genes, formatted for better presentation.

Description

This function visualizes CD8+ T cell markers using a FeaturePlot and identifies clusters enriched for these markers.

Usage

```
visualize_cd8_markers(
  seurat_obj,
  all_markers,
  cd8_markers,
  reduction_type = "umap",
  color_scheme = c("red", "blue")
)
```

Arguments

```
seurat_obj A Seurat object.

all_markers A data frame containing all identified markers.

cd8_markers A vector of CD8+ T cell marker genes.

reduction_type Type of dimensionality reduction to use for plotting (default is "umap").

color_scheme A vector of colors for plotting (default is c("red", "blue")).
```

Value

Feature plot of CD8+ T cell markers and printed list of enriched clusters.

22 visualize_pca

visualize_cd8_subtypes

Visualize CD8+ Subtypes on UMAP

Description

This function visualizes the CD8+ T cell subtypes on a UMAP plot.

Usage

```
visualize_cd8_subtypes(seurat_obj)
```

Arguments

seurat_obj

A Seurat object containing CD8+ T cells with assigned labels.

Value

UMAP plot of CD8+ subtypes with custom theme.

visualize_pca

Visualize PCA and Elbow Plot

Description

This function generates an Elbow plot for PCA variance and visualizes the PCA results using a DimPlot.

Usage

```
visualize_pca(seurat_obj)
```

Arguments

seurat_obj

A Seurat object containing single-cell RNA-seq data.

Value

The Elbow plot, PCA plot, and heatmap of the first 5 principal components.

visualize_umap 23

Description

This function visualizes the UMAP embedding of the Seurat object.

Usage

```
visualize_umap(seurat_obj)
```

Arguments

seurat_obj

A Seurat object containing UMAP results.

Value

UMAP plot with labels and custom themes.

Index

```
assign_cd8_labels, 2
calculate_DEGs, 3
cluster_and_umap, 4
extract_genes_hr_gt1, 4
filter_rna_data, 5
filter_seurat_object, 5
generate_mutation_summary, 6
get_mutated_samples, 7
get_top_combined_genes, 8
identify_markers, 9
load_cancer_data, 9
load_seurat_object, 10
mapping_genes, 11
perform_pca, 12
perform_pseudotime, 12
perform_survival_analysis, 13
plot_cd8_trajectory, 14
plot_DEG_results, 14
plot_km_curves, 15
plot_mutation_counts, 16
plot_results, 17
plot_unique_mutation_types, 18
preprocess_data, 18
run_graph_test, 19
standardize_tcga_ids, 20
style_mutation_table, 20
visualize_cd8_markers, 21
visualize_cd8_subtypes, 22
visualize_pca, 22
visualize_umap, 23
```