

HPVTIMER: A Shiny web application for tumor immune estimation in human papillomavirus-associated cancers

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<http://www.hpvtimer.com/>

Liyong Liu, Yanan Xie, Hong Yang, Anqi Lin, Minjun Dong, Haitao Wang, Cangang Zhang, Zaoqu Liu, Quan Cheng, Jian Zhang, Shuofeng Yuan, Peng Luo. 2023. HPVTIMER: A Shiny web application for tumor immune estimation in human papillomavirus-associated cancers. *iMeta* 1: e1. <https://doi.org/10.1002/imt2.130>



Highlights

- **HPVTIMER** provides **4** analysis modules for exploring differences between HPV-positive and HPV-negative samples based on transcriptome data such as; **differential expression analysis, correlation analysis, immune infiltration analysis, and pathway enrichment analysis.**
- The immune infiltration analysis module provides users with **7 immune infiltration algorithms.**
- **HPVTIMER** provides users with **meta analysis** which can be used to explore correlations between gene expression/pathway, ssGSEA scores/ immune infiltration scores, and differences in immune infiltration scores between HPV-positive/negative samples.

Introduction

● About HPV-associated cancers and immunotherapies

- HPV-associated cancers refer to cancers that are associated with high-risk HPV infections occurring in various sites but **share similar biological and immunological properties**.
- Despite numerous clinical investigations, the response rates to these therapies observed to date have been modest and resistance has emerged in some cases.
- The immune microenvironments of HPV-associated cancers share similarities, however, HPV-associated cancers at different primary sites have **distinct clinical features**.

● About data and web tools

Although a vast amount of transcriptomic data of HPV-associated cancers is stored in public databases. But it can be challenging for **users to extract relevant information without expertise in immune infiltration analysis**.

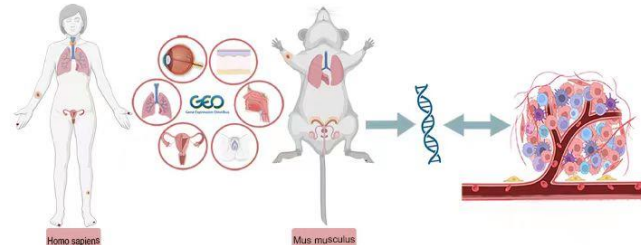
Limitations of existing relevant analytics platforms and web tools;

- TME research tools: CancerImmunityQTL, TIP and TIMER2.0
- Web-based tools focus on single cancer types: HNCDB
- Viral research tools : VISDB

HPVTIMER: HPV-associated Tumor Immune Micro-Environment ExploreR

Data preparing

Overall summary

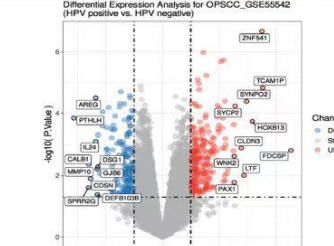


Data

- 2290 Samples
- 65 Datasets
- 8 Cancer Types
- 7 Immune Infiltration Estimation Algorithms

DE analysis


Differential Expression Analysis for OPSCC_GSE55542 (HPV positive vs. HPV negative)



read counts (edgeR package) → TPM & R/G (limma package) → log2(CPM) (limma package) → log2(TPM) / log2(R/G) → Result of differential expression analysis (limma package)

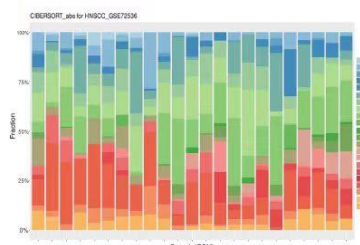
Correlation analysis

Gene co-expression in All sample



log2(CPM) / log2(TPM) / log2(R/G) → ssGSEA enrichment score (GSVA package) → Immune cell infiltration scores (IOBR package) → Co-expression (Gene vs Gene (root package) / Gene vs Pathways vs immune cell) → Meta analysis (metafor package)

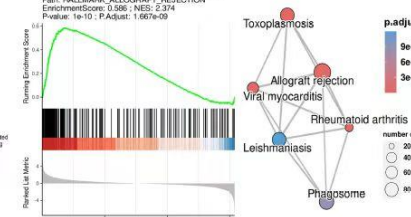
Immune infiltration analysis



human data / mouse data (biomaRt package) → Result (IOBR&metafor package)

Pathway analysis

HNSCC_GSE74927: HPV-Positive vs HPV-Negative
Path: HAL1MARK_ALLOGRAFT_REJECTION
EnrichmentScore: 0.59; NES: 2.214
Pvalue: 1e-10; P.adjust: 1.667e-09



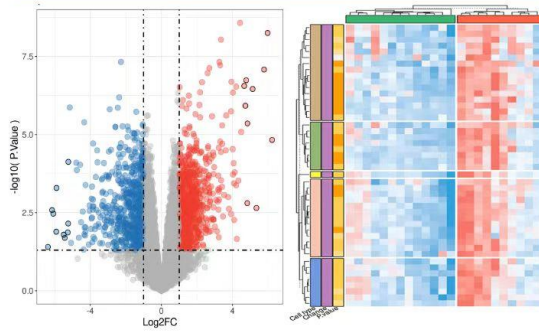
DEGs (log2(CPM) / log2(TPM) / log2(R/G)) → ES(ORA) (clusterProfiler) / ES(GSEA) (clusterProfiler) / ES(GSEA ES) (GSVA package) → Result (pathview&GSVA & clusterProfiler packages)

Visualization

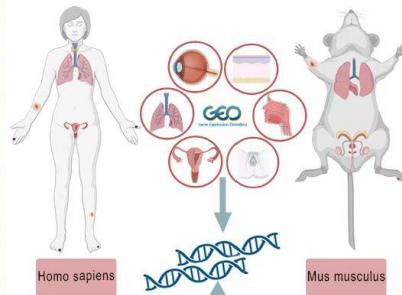
Results

HPVTIMER: HPV-associated Tumor Immune MicroEnvironment ExploreR

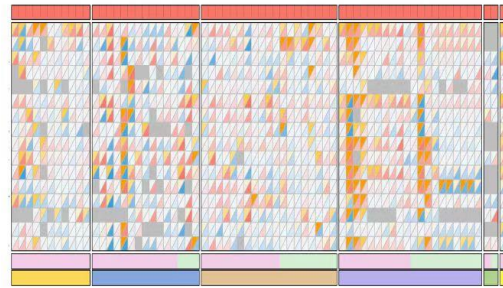
DE analysis



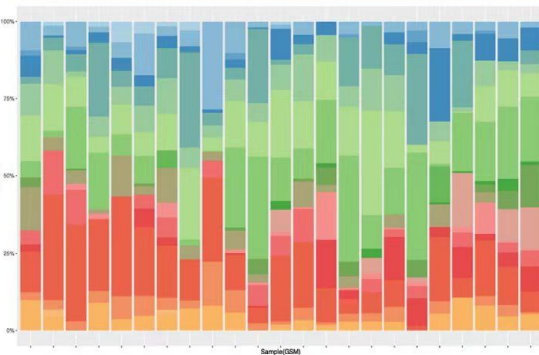
Overall summary



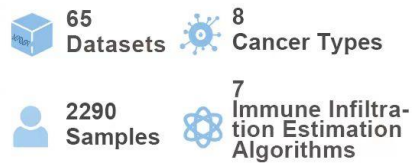
Correlation analysis



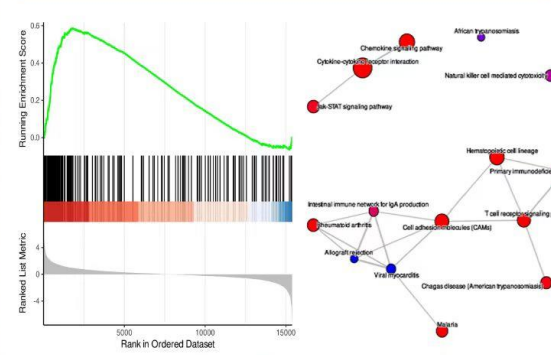
Immune infiltration analysis



Data information



Pathway analysis



● Web link:

<http://www.hpvtimer.com/>

- HPVTIMER comprises 65 transcriptomic datasets of HPV-associated tumors, covering 2 species and 8 cancer species.
- It provides users with four analysis modules including; differential expression analysis module, correlation analysis module, immune infiltration analysis module, and pathway analysis module.

Visualization

Data preparing

Visualization

Results

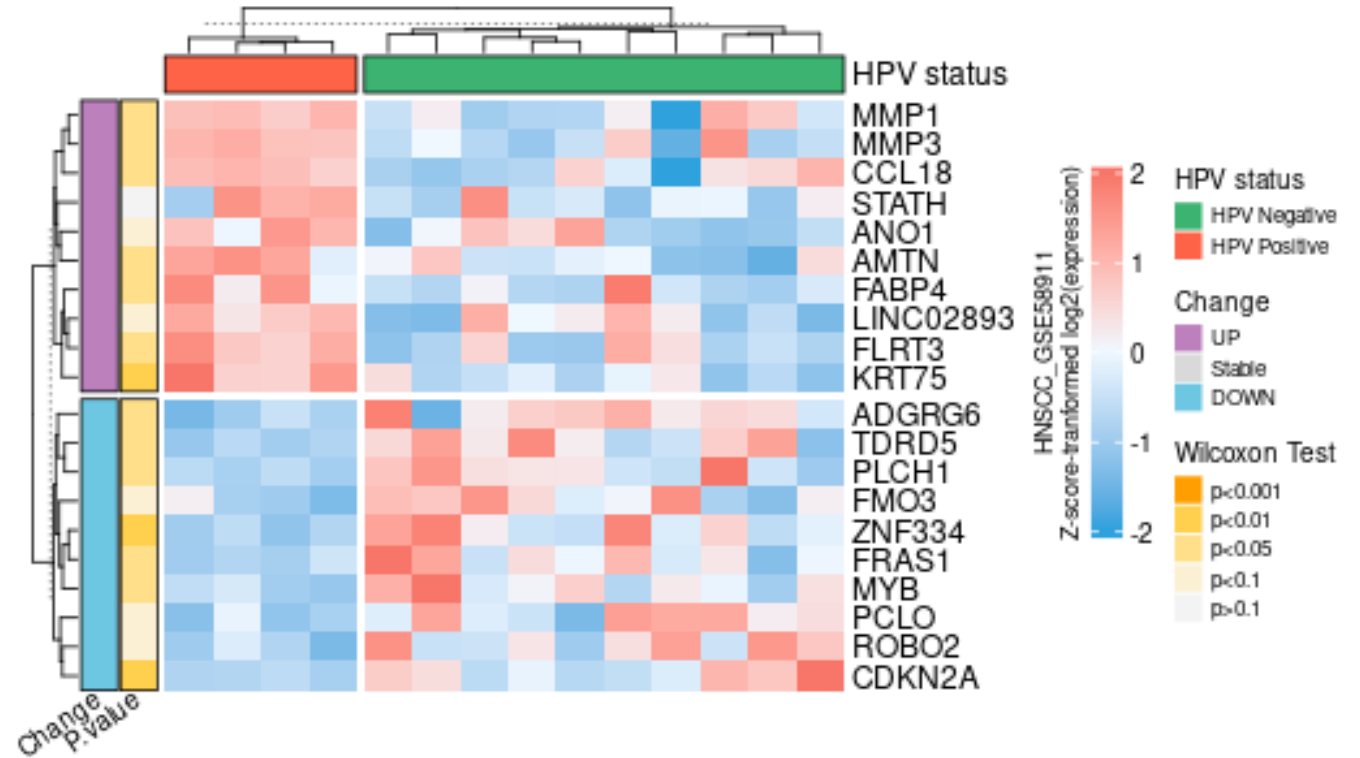
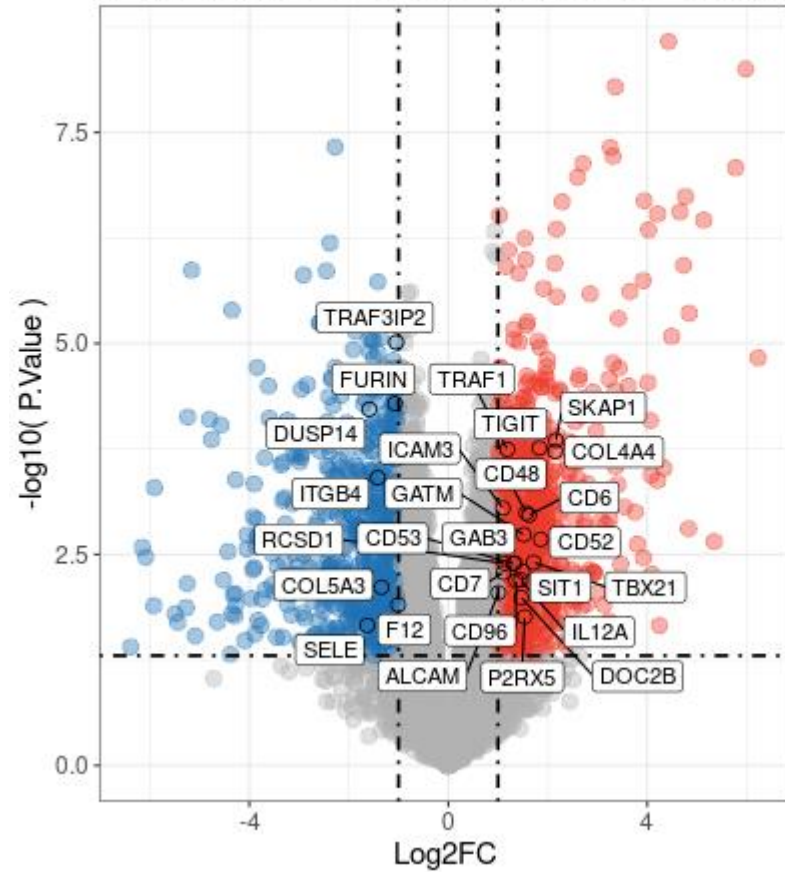
- **DE analysis**
- 3 submodules: **Overall, Immune cells, Pathways**
- 3 types of visualizations: volcano, heatmap, boxplot
- Customization of analysis parameters and plotting parameters

The screenshot displays the HPVTIMER web application interface. The top navigation bar includes the logo 'HPVTIMER' and a hamburger menu icon. A left sidebar contains navigation options: Introduction, DE analysis (expanded), Overall, Immune cells, Pathways, Correlation, Immune infiltration, Pathway analysis, Data, and About. The main content area is titled 'Differential Expression Analysis for Immune Cell' and features three visualization tabs: Volcano (selected), Heatmap, and Boxplot. The interface includes several input fields and controls: 'Search, select a dataset:' with a dropdown menu showing 'HNSCC_GSE72536 (n=23)'; 'Select, search an immune cell:' with a dropdown menu showing 'Type 1 T helper cell'; a 'Visualize' button; a 'Customize' section with a 'Threshold of P.Value or adj.P.Val:' slider set to 0.05 (with radio buttons for P.Value and adj.P.Val); a 'Threshold of |log2FC|:' input field set to 1; and 'Volcano Color' options for UP (red, #EF3B2C), Stable (grey, #B2B2B2), and DOWN (blue, #2171B5). The footer contains the text 'Copyright © 2023. All rights reserved.'

Results

● DE analysis

Differential Expression Analysis for HNSCC_GSE72536
in Type 1 T helper cell (HPV positive vs. HPV negative)



Results

● Correlation

- 4 submodules: **Co-expression, Multi-genes, Scatter plot, Meta analysis**
- 5 types of visualizations: heatmap, scatter plot, correlation matrix, bubble matrix, forest plot
- Customization of analysis parameters and plotting parameters

The screenshot shows the HPVTIMER web application interface for the Co-Expression analysis. The left sidebar contains navigation links: Introduction, DE analysis, Correlation, Co-expression (highlighted), Scatter plot, Multi-genes, Meta analysis, Immune infiltration, Pathway analysis, Data, and About. The main content area is titled "Co-Expression" and contains the following configuration options:

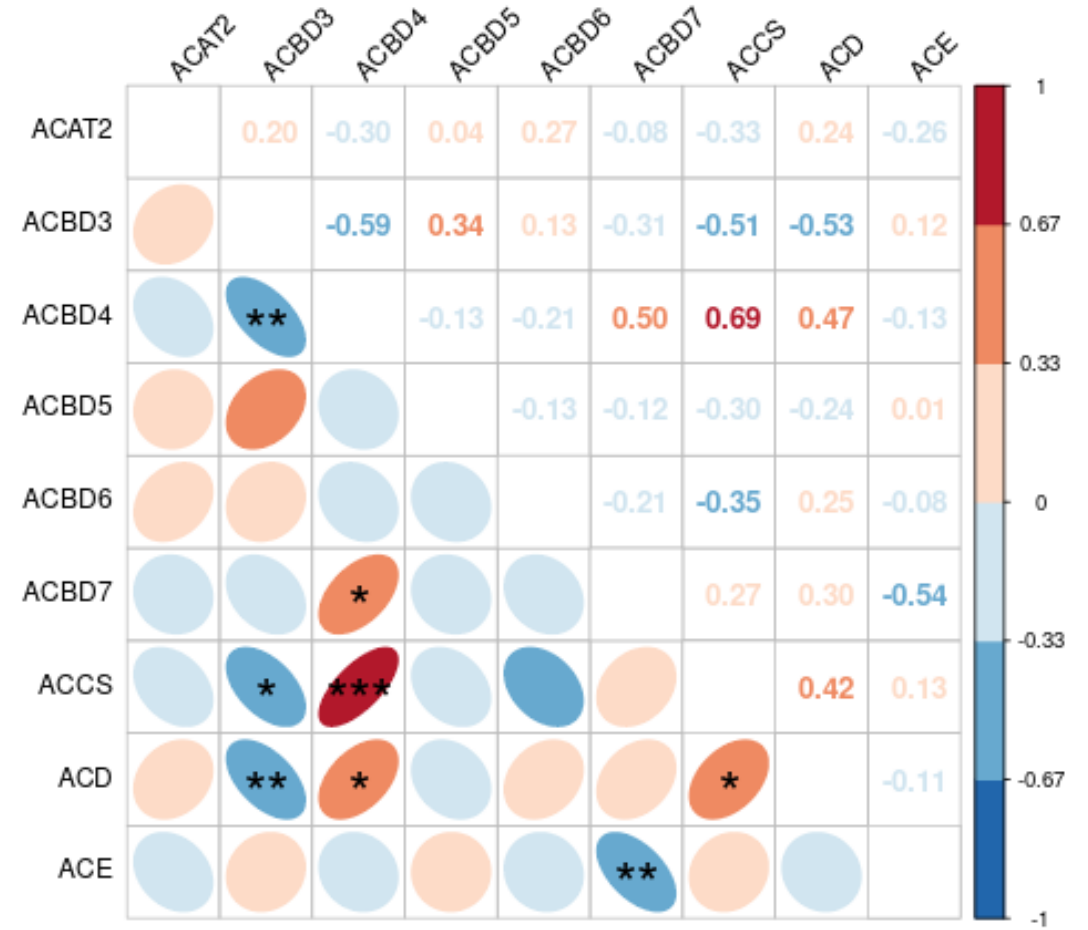
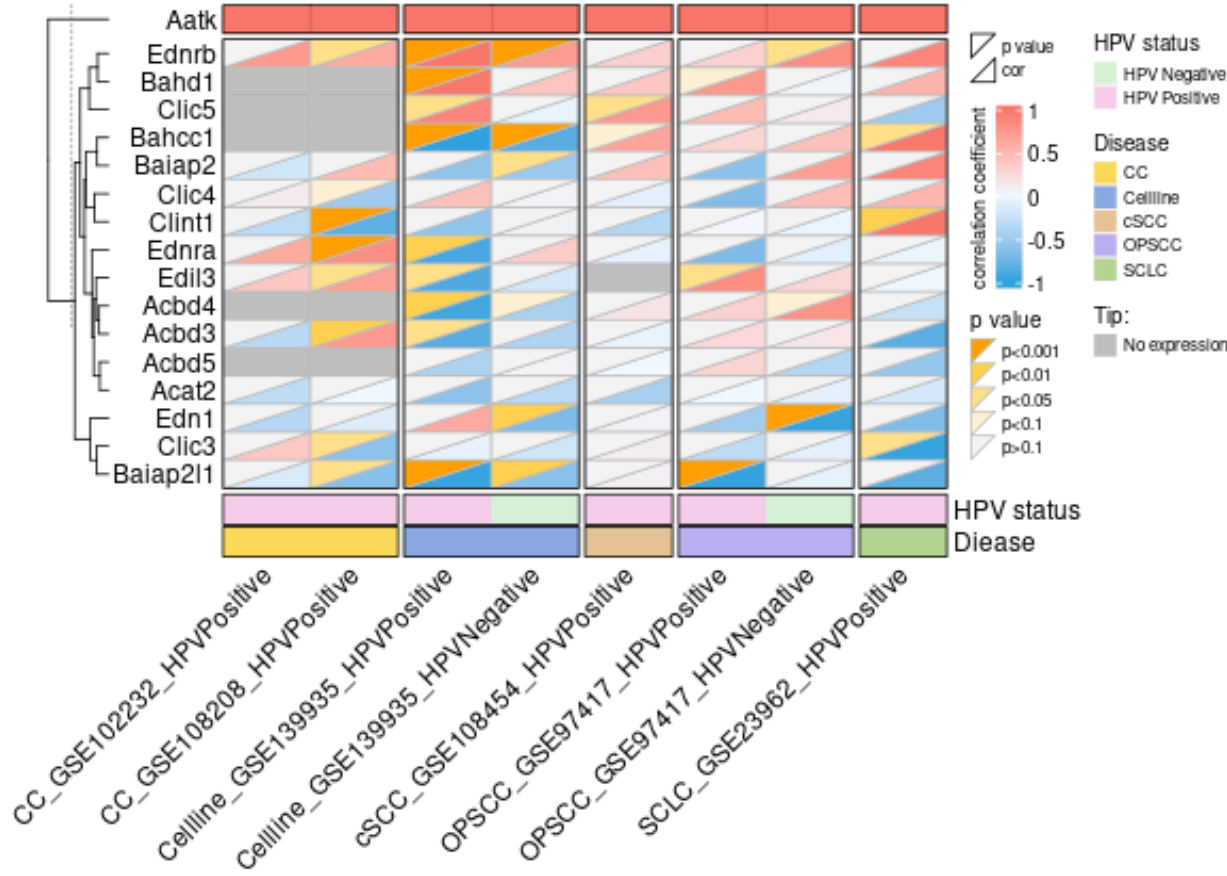
- Select the species:** Homo sapiens Mus musculus
- Select a method for correlation:** Pearson Spearman
- Search, select a key gene symbol:** ABCA1
- Correlation with the key gene:** A list of related genes including ACLY, ACO1, ACO2, ACOT11, BMPR1A, BMPR1B, BMPR2, BMS1, COPRS, COPS2, COPS3, COPS4, EPHA4, EPHB1, EPHB2, and EPHB3.
- Select the sample range for analysis:** HPV_Positive HPV_Negative All
- Color Option - Select colorscale:** light Red-Blue
- Visualize** button

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Results

● Correlation

Genes co-expression in all samples



Results

- **Immune infiltration**

- 7 immune infiltration estimation algorithms: **CIBERSORT, CIBERSORT absolute mode, MCP-counter (or mMCP-counter for mice), EPIC, xCell, quanTiseq, and TIMER**

- 2 submodules: **Immune cells, Meta analysis**

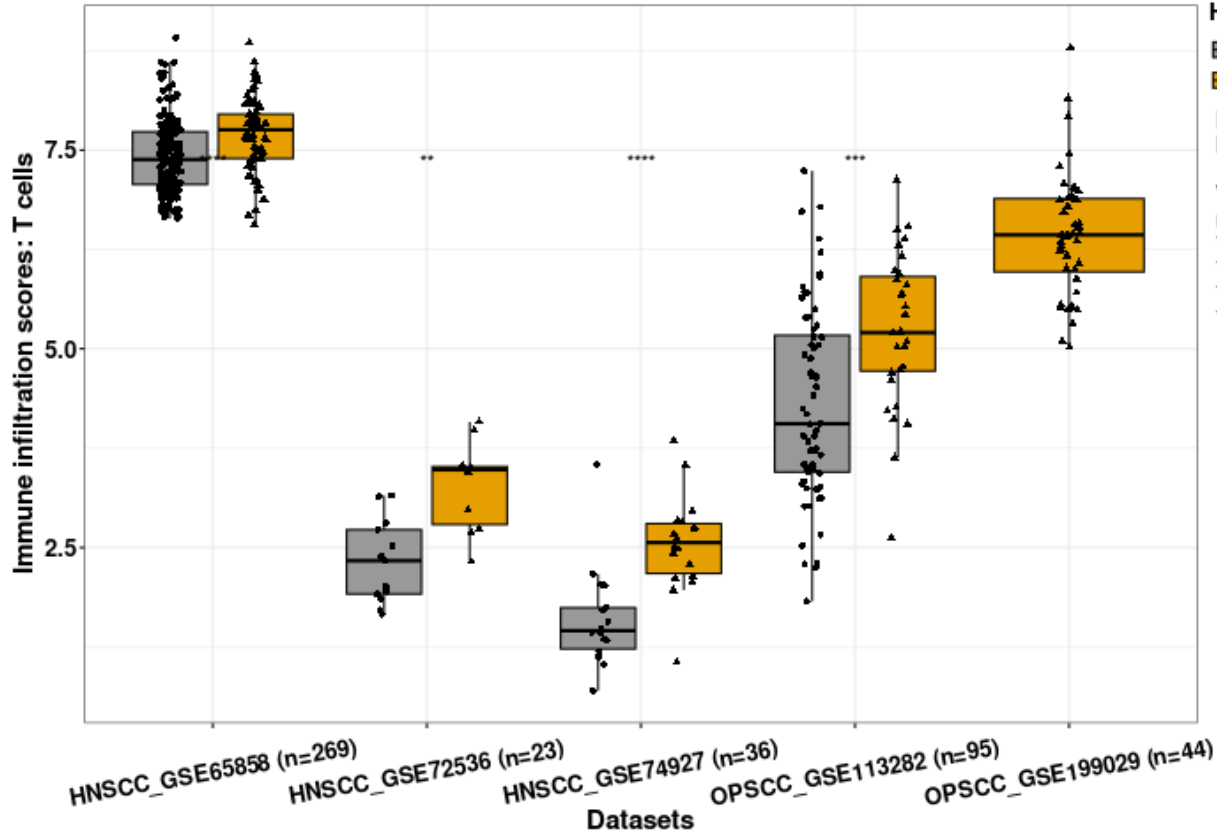
- 4 types of visualizations: stacked plot, heatmap, boxplot, forest plot

The screenshot shows the HPVTIMER web application interface. The top navigation bar includes the logo 'HPVTIMER' and a hamburger menu icon. A sidebar on the left contains navigation links: Introduction, DE analysis, Correlation, Immune infiltration (expanded), Immune cells, Meta analysis, Pathway analysis, Data, and About. The main content area is titled 'Immune Infiltration Analysis' and features four visualization tabs: Stacked plot, Heatmap, Boxplot, and Multi-datasets. The 'Heatmap' tab is selected. The interface includes several interactive elements: a dropdown menu for 'Which algorithm are you interested:' set to 'MCPcounter'; a dropdown menu for 'Search, select an immune cell:' set to 'T_cells'; a search box for 'Search, select 1~5 dataset(s):' containing 'HNSCC_GSE72536 (n=23)', 'HNSCC_GSE74927 (n=36)', 'CC_GSE113942 (n=7)', and 'CC_GSE122697 (n=11)'; a 'Color Option' section with 'HPV Positive' set to '#E69F00' and 'HPV Negative' set to '#999999'; and a 'Visualize' button. The footer contains the text 'Copyright © 2023. All rights reserved.'

Results

● Immune infiltration

MCPcounter for T cells



Study	HPV Positive		HPV Negative		Standardised Mean Difference	SMD	95%-CI	Weight	
	Total	Mean	Total	Mean					
Disease = HNSCC									
HNSCC_GSE159067	11	0.01	0.0189	49	0.02	0.0400	-0.11	[-0.76; 0.55]	7.0%
HNSCC_GSE181805	7	0.01	0.0212	18	0.00	0.0000	0.00	[-1.19; 1.13]	0.0%
HNSCC_GSE3292	8	0.00	0.0011	28	0.00	0.0003	0.55	[-0.24; 1.35]	5.3%
HNSCC_GSE58911	4	0.01	0.0240	10	0.01	0.0133	-0.03	[-0.33; 0.21]	2.9%
HNSCC_GSE65858	73	0.07	0.0441	196	0.07	0.0485	-0.06	[-0.43; 0.82]	15.4%
HNSCC_GSE6791	16	0.02	0.0192	26	0.02	0.0246	0.20	[0.31; 2.13]	7.4%
HNSCC_GSE72536	10	0.06	0.0519	13	0.02	0.0129	1.22	[0.37; 1.77]	4.3%
HNSCC_GSE74927	18	0.04	0.0347	18	0.01	0.0116	1.07	[-0.04; 0.76]	6.4%
Random effects model	147		358				0.36		48.8%
Heterogeneity: $I^2 = 62\%$, $\tau^2 = 0.1657$, $p = 0.01$									
Disease = OPSCC									
OPSCC_GSE113282	31	0.06	0.0310	64	0.05	0.0326	0.41	[-0.02; 0.85]	11.1%
OPSCC_GSE171898	262	0.03	0.0407	49	0.02	0.0273	0.48	[-1.97; 1.29]	14.3%
OPSCC_GSE190222	3	0.01	0.0231	3	0.03	0.0540	-0.34	[-0.16; 1.18]	1.6%
OPSCC_GSE55542	20	0.05	0.0768	16	0.02	0.0343	0.51	[-0.27; 1.49]	6.8%
OPSCC_GSE55544	15	0.06	0.0770	8	0.02	0.0190	0.61	[-0.45; 1.27]	4.6%
OPSCC_GSE55545	8	0.04	0.0931	16	0.02	0.0343	0.41	[-1.21; 1.06]	4.8%
OPSCC_GSE97417	6	0.00	0.0045	6	0.00	0.0059	-0.08		3.0%
Random effects model	345		162				0.44		46.2%
Heterogeneity: $I^2 = 0\%$, $\tau^2 = 0$, $p = 0.92$									
Disease = VSCC									
VSCC_GSE183454	12	0.04	0.0198	11	0.05	0.0202	-0.37	[-1.20; 0.46]	5.0%
Random effects model	504		531				0.32		100.0%
Heterogeneity: $I^2 = 40\%$, $\tau^2 = 0.0584$, $p = 0.05$									
Test for subgroup differences: $\chi^2 = 3.43$, $df = 2$ ($p = 0.18$)									

Results

- **Pathway analysis**

- 3 submodules: **Pathway enrichment, Pathview, and ssGSEA**

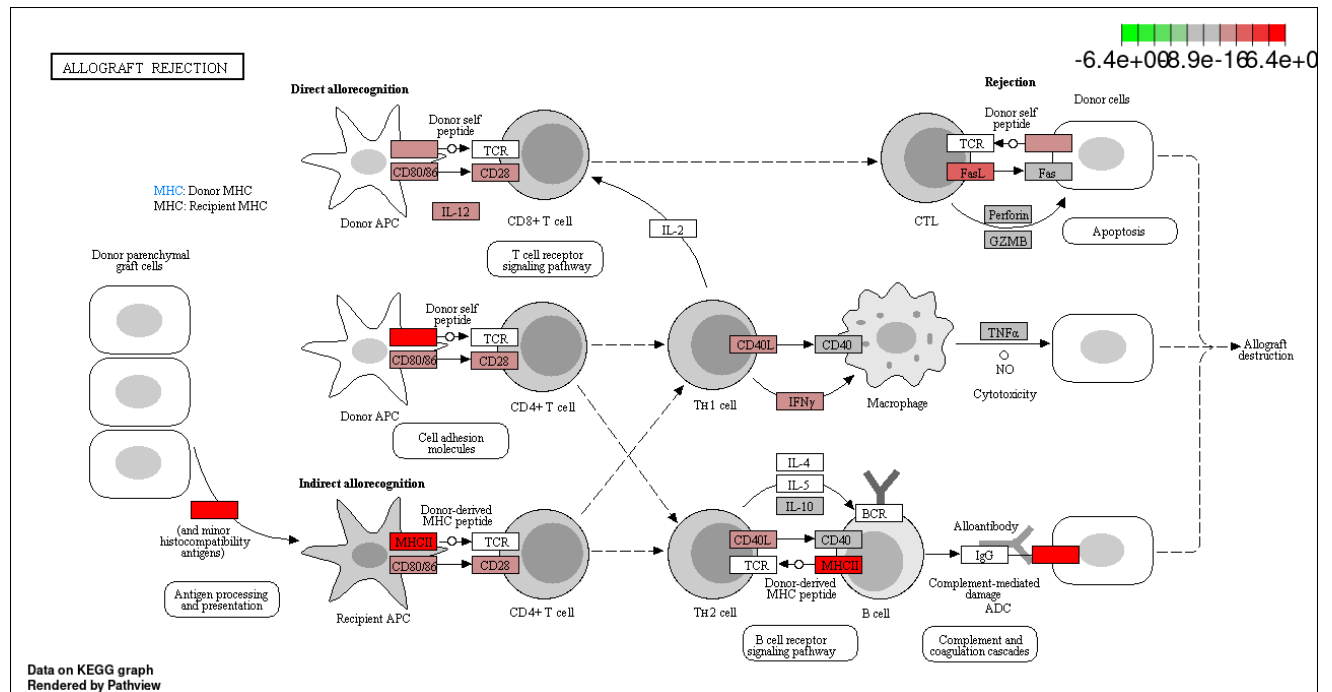
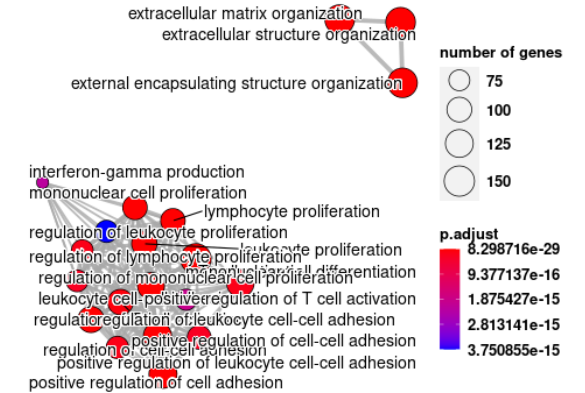
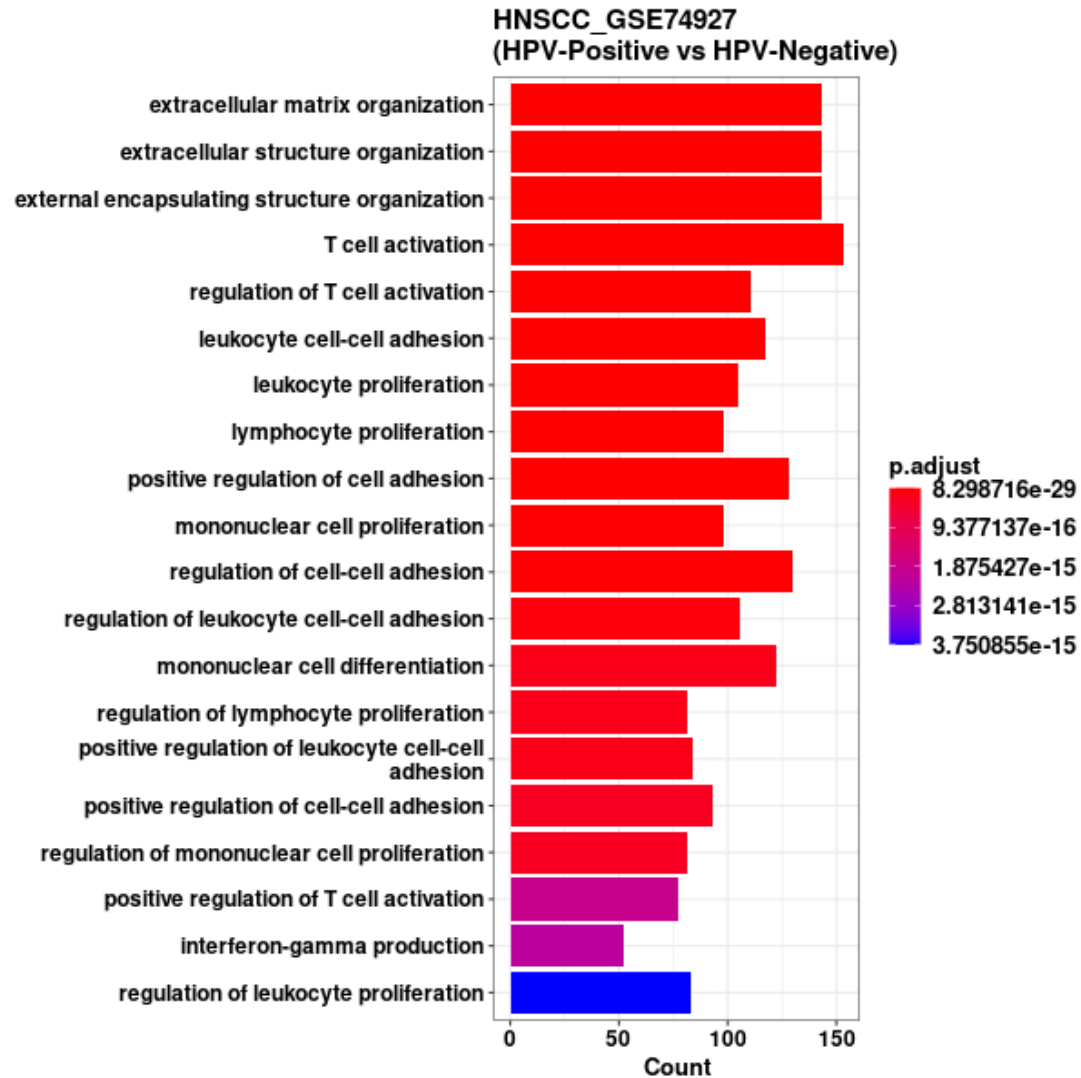
- 7 types of visualizations: bar plot, dot plot, Emap plot, ridgeline plot, GSEA plots, KEGG pathway maps, and heatmap

- Customization of analysis parameters and plotting parameters

The screenshot shows the HPVTIMER web application interface. The top navigation bar is dark purple with the 'HPVTIMER' logo and a hamburger menu icon. A left sidebar contains navigation links: Introduction, DE analysis, Correlation, Immune infiltration, Pathway analysis, Pathway enrichment (highlighted), Pathview, and ssGSEA. The main content area is titled 'Pathway Enrichment' and features two tabs: 'Over Representation Analysis(ORA)' and 'Gene Set Enrichment Analysis(GSEA)'. The GSEA tab is active. Below the tabs, there are two input fields: 'Search, select a dataset:' with a dropdown menu showing 'HNSCC_GSE74927 (n=36)', and 'Pick genesets for enrichment:' with a dropdown menu showing 'GO(Gene Ontology gene sets)'. To the right, there are radio buttons for 'Choose the pathways to display:', with 'Top20' selected and 'Customize' unselected. A 'Visualize' button is located below these options. At the bottom of the page, a copyright notice reads 'Copyright © 2023. All rights reserved.'

Results

● Pathway analysis



Results

- **Data**

- 3 submodules: **Datasets, Clinical data, Immune cells**

- 4 forms: And the forms can be searched.

The screenshot shows the HPVTIMER Dataset Browser interface. The left sidebar contains navigation options: Introduction, DE analysis, Correlation, Immune infiltration, Pathway analysis, Data, Datasets, Clinical data, Immune cells, and About. The main content area is titled "Dataset Browser" and includes filters for Species, Classification, and Cancer Type, each with a dropdown menu. A "Download full data(CSV)" button is also present. Below the filters, it indicates "Showing 1 to 8 of 65 entries". A table displays the first three entries:

	Dataset Name	Species	Classification	Cancer Types	Platform	Publication	clinical information
1	GSE102232	Mus musculus	Tissue	CC	Affymetrix Mouse Genome 430 2.0 Array	PMID:29073104	NOT AVAILABLE
2	GSE108208	Mus musculus	Tissue	CC	Affymetrix Mouse Genome 430 2.1 Array	PMID:29458011	NOT AVAILABLE
3	GSE113942	Homo sapiens	Tissue	CC	Illumina HiSeq 2000	PMID:30696738	age

Summary

- HPVTIMER provides a comprehensive analysis platform that integrates over 10,000 genes and 2,290 tumor samples from 65 transcriptomic datasets across 8 cancer types sourced from the Gene Expression Omnibus (GEO) database.
- The tool features 4 built-in analysis modules; the differential expression analysis module, correlation analysis module, immune infiltration analysis module, and pathway analysis module. These modules enable users to perform systematic and vertical analyses. Meanwhile, we expect that HPVTIMER will help users to explore the immune microenvironment of HPV-associated cancers and uncover potential immune regulatory mechanisms and immunotherapeutic targets.
- **The source code for HPVTIMER is available at <https://github.com/SMULiuLY/HPVTIMER/>**
- **Web link: www.hpvtimer.com**
- **Contact: luopeng@smu.edu.cn**

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