

# IOSig User Guide

# Introduction

- Immune Signature Explorer (IOSig) is a web interface that allows the user to query and explore immune checkpoint blockage treated cohorts with gene expression signatures for the purpose of predicting response to treatment.
- Users can upload their own signature, expression and clinical dataset, or simply query the built-in signatures and data.
- Note: Only datasets with included survival information will appear in the survival cohort drop down

# Outline

- This guide will walk through the 3 analysis methods within IOSig
1. Dataset Analysis
    - User uploads RNA-seq expression data with respective clinical data.
  2. User Signature Analysis
    - User uploads a list of genes to query the built-in datasets.
  3. Published Signature Analysis
    - User queries the built-in gene signatures and datasets.

# About Page

## Page Selection Tabs

The screenshot shows the 'About' tab highlighted with a red box. Below the tabs, the page content is titled 'About IOSig'. The main text describes the application as an interactive Shiny application for investigating immune checkpoint inhibitor (ICI) treated datasets with gene expression biomarker signatures for prediction of responder / non-responder. It also mentions previously published gene expression signatures and the ability to explore data without providing your own.

The About page for IOSig provides a written in-depth description of the analysis methods. These methods include Area Under Receiver Operating Characteristic Curve (AUROC), Survival analysis, Mann-Whitney U-test, and Gene Set Enrichment Analysis (GSEA).

## IOSig Methods

### Dataset Analysis

The Dataset Analysis tab is meant for users to upload their own RNA-seq expression datasets coupled with the necessary clinical data. The RNA-seq data is expected to be in a comma or tab separated format with the columns containing the sample ids and rows containing genes. The genes should be in HUGO format. Additionally the samples cannot start with a number. If your samples start with a numerical value, add a character, like 'X' at the start.

It is also necessary for the uploaded clinical data to have specific column names. These column names are: 'Sample\_ID' (sample name), 'response' (ICI response), 'os' (overall survival time), and 'os\_event' (overall survival event). 'response' and 'os\_event' should be binary columns where 1 represents responder to ICI treatment and censoring in os\_event.

Example RNA-seq and Clinical files can be downloaded from the left side panel on the Dataset Analysis page. Additionally, the datasets used for comparison in the AUROC portion can be filtered and selected in the left side panel.

### User Signature Analysis

The User Signature Analysis tab allows a user to upload a list of genes in HUGO format to query against the datasets within IOSig. The genes should be separated by a new line and pasted into the left side panel. A unique name for the uploaded gene signature is also required. An example gene signature file can be downloaded from the left side panel as well.

### Published Signature Analysis

The 'Published Signature Analysis' tab is similar to the 'User Signature Analysis' tab except that you can select from a variety of previously published gene signatures to query the IOSig datasets.

## AUROC Analysis

Area under the receiver operating characteristic curve (AUROC) is a performance measurement metric for a binary classifier. In IOSig, AUROC is utilized to assess the ability of gene signature to distinguish responder and non-responder based on the gene expression values for a sample. The curve is plotted with the true positive rate on the y-axis and false positive rate on the x-axis. A higher area under the

Genes	Sample_1	Sample_2	Sample_3	Sample_4	Sample_5	Sample_6
PLXNC1	0.1380254	1.5219420	0.1875000	0.1875000	0.1875000	0.1875000
TSPYK2	0.2202059	0.2210942	0.26239172	0.26239172	0.26239172	0.26239172
TNN	0.05076419	0.00839250	0.20097367	0.2119807	0.65675926	0.02024172
MIR553	0	30.153936	8.02314899	0	21.0635426	3.99707263
TAF1L	3.1017282	5.31203403	5.51203403	4.10181216	4.10181216	4.10181216
TAS1R4P2B	0.06127172	0.24320572	0.21539487	0.20204172	0.1875000	0.11158566
MIR3671	0	7.74066557	18.5362408	6.25030364	8.31067445	3.07820536
CPRH1	0	0	2.46464012	0.34992047	0	0.15079047
TMEM234	0	0	0	0	0	0

Sample_ID	os	os_event	response
Sample_1	1300	0	1
Sample_2	977	0	1
Sample_3	977	0	1
Sample_4	163	1	0
Sample_5	163	1	0
Sample_6	163	1	0

# Dataset Overview

- The “Expression Data Overview” tab in the “Dataset Overview” contains a table which details the datasets within IOSig

Page Selection {

The expression data overview page details the sequence and clinical datasets included in IOSig. Various information including the PubMed ID, cancer type, treatment drug, is listed among other information. This table can be referenced to help users query IOSig with the datasets that best fit their interest and needs.

**IOSig: Immuno-Oncology Signature Explorer**

Expression data included in this application:

Data Set ID	N	Cancer Type	Drug	Pre On Post	Biosources	Clinical Trials ID	Platform	PMID
1 GSE67501	11	Kidney	Nivolumab	Pre	Tissue	NCT00441337, NCT00730639, NCT01354431, NCT01358721	Microarray	<a href="#">27491898</a>
2 GSE99070	10	Malignant Pleural Mesothelioma	anti-PD1	Pre	Tissue		Microarray	<a href="#">29618661</a>
3 GSE111414	20	NSCLC	Nivolumab	Pre/On	PBMC		RNA-seq	<a href="#">30765392</a>
4 GSE154538	26	GI cancer	Nivolumab	Pre/Post	Tissue		RNA-seq	<a href="#">34014607</a>
5 GSE158403	81	Melanoma	Durvalumab	Pre/On	Tissue	NCT02027961	RNA-seq	<a href="#">33288749</a>
6 GSE165252	71	Esophageal Cancer	Atezolizumab	Pre/On/Post	Tissue	NCT03087864	RNA-seq	<a href="#">33504550</a>
7 GSE165278	22	Melanoma	Ipilimumab	Pre/Post	Tissue	NCT00796991, NCT00495066, NCT00920907, NCT00324155, NCT00162123, NCT0140045 and NCT00289640, NCT00495066, NCT00636168, NCT01515189, NCT00086489, NCT00471887	RNA-seq	<a href="#">33588426</a>
8 GSE173839	71	Breast	Durvalumab	Pre	Tissue	NCT01042379	Microarray	<a href="#">34143979</a>
9 GSE176307	87	Bladder	anti-PD1 or anti-PDL1	Pre	Tissue		RNA-seq	<a href="#">34294892</a>
10 GSE179351	52	GI cancer (CRC & PDAC)	Ipi + Nivo	Pre/Post	Tissue	NCT03104439	RNA-seq	<a href="#">35122060</a>

Showing 1 to 10 of 36 entries

Previous 1 2 3 4 Next

# Signature Overview

The “Signature Overview” page details the previously published gene expression signatures included in IOSig. Various information including the PubMed ID, a brief description of the signature, and the number of genes within a signature are found in the table. Additionally, the genes within a signature can be viewed in the left panel.

**Page Selection**

[About](#) **Dataset Overview** [Dataset Analysis](#) [User Signature Analysis](#) [Published Signature Analysis](#)

[Expression Data Overview](#) **Signature Overview**

**View Genes Within Signature**

angio genesis

Show 5 entries Search:

	angio genesis
1	VEGFA
2	CD34
3	ANGPTL4
4	KDR
5	TEK

Showing 1 to 5 of 16 entries Previous **1** 2 3 4 Next

**IOSig: Immuno-Oncology Signature Explorer**

**View genes within a signature**

**Signature data included in this application:**

Gene Signature Name	Description	Number of Genes	PMID
1 Angiogenesis	This signature is constructed from genes which are highly coexpressed in the angiogenesis pathway.	16	<a href="#">34965943</a>
2 Auslander - Impres	IMPRES score based on logical comparison of quantile-normalized expression of pre-defined checkpoint gene pairs.	15	<a href="#">30127394</a>
3 Chaurio	A set of genes that reflect T and B cell responses in human cancer. CXCL13 is the crucial marker	7	<a href="#">35021053</a>
4 Chemokine	Chemokine gene set	12	<a href="#">21703392: 23097687</a>
5 Davoli Cytotoxic Immune Signature	A signature based on scoring the immune infiltration based on gene expression profile.	7	<a href="#">28104840</a>

Showing 1 to 5 of 30 entries Previous **1** 2 3 4 5 6 Next

# Dataset Analysis

- The dataset analysis method in IOSig allows the user to import a tab or comma separated RNA-sequencing file and clinical data file.

## RNA-Seq File

- Prior cleaning and normalization recommended
- HUGO format for genes
- Column 1 must contain the genes
- Sample IDs cannot begin with a number

## Clinical File

- 4 columns required: 'Sample\_ID', 'response', 'os', 'os\_event'
- Sample IDs cannot begin with a number
- Response must be 1 (responder) or 0 (non-responder)
- 'os' must be in numerical format
- os\_event must be 1 (event occurred) or 0 (no event occurred)

Genes	Sample_1	Sample_2	Sample_3	Sample_4	Sample_5	Sample_6
HHAT	0.13825056	1.53826279	0.15290465	0.51870962	0.3466875	0.10772357
TSPAN2	0.58290595	0.42199425	1.06235372	0.98291794	2.19382289	0.31626312
TNN	0.05076419	0.00839259	0.20097367	0.1219807	0.60676926	0.02002472
MIR553	0	30.153936	8.02314899	0	21.0635426	3.99707263
TAF12	3.1017382	5.31114303	5.58928237	3.79294216	4.10813913	4.19424231
SRGAP2B	0.06112194	0.24251952	0.21939487	0.20104764	0.15246727	0.11315856
MIR3671	0	7.74066557	18.5362408	6.25030364	8.11067445	3.07820536
CFHR1	0	0	2.46464012	0.34992047	0	0.15079047
TMEM234	0	0	0	0	0	0

Sample_ID	os	os_event	response
Sample_1	1300	0	1
Sample_2	977	0	1
Sample_3	977	0	1
Sample_4	163	1	0
Sample_5	163	1	0
Sample_6	163	1	0

# Dataset Analysis - Import

**IOSig: Immuno-Oncology Signature Explorer**

Link to download example RNA-seq file

Button to upload RNA-seq file

Link to download example clinical file

Button to upload clinical file

Button to confirm file upload and cohort selection

Optional cancer type cohort filter

Individual cohort filter

**Dataset Analysis**

[Click for example sequence file](#)

Please upload an expression dataset

[Browse...](#) `example_seq.f`

[Upload complete](#)

[Click for example clinical data](#)

Please upload relevant clinical data

[Browse...](#) `example_clin_f`

[Upload complete](#)

[Run analysis on dataset](#)

Filter datasets by cancer type

Melanoma

Select datasets to use for AUROC Comparison and Correlation

- GSE158403\_Pre
- GSE158403\_On
- GSE165278\_Pre
- GSE165278\_On
- GSE186143
- Hintzsche
- Du\_on
- Du\_pre
- GSE158403\_CTLA4

Import AUROC Overview AUROC Comparison AUROC Correlation Mann Whitney Survival GSEA

Show 10 entries Search:

	Invalid_Genes
141	WDR60
142	C7orf26
143	C7orf77
144	TWISTNB
145	FAM71F1
146	KIAA0895
147	C7orf69
148	MPP6
149	FAM71F2
150	ZBED6CL

Showing 141 to 150 of [Previous](#) [1](#) ... [14](#) [15](#) [16](#) ... [19](#) [Next](#) entries

**Uploaded Expression Data**

RNA-seq and Clinical data, uploaded by user, is displayed below after upload confirmation

Show 10 entries Search:

Genes	Sample_1	Sample_2	Sample_3	Sample_4	Sample_5	Sample_6
1 HHAT	0.138250563	1.53826279	0.152904653	0.518709618	0.346687499	0.107723567
2 TSPAN2	0.582905954	0.421994253	1.062353719	0.982917943	2.193822889	0.316263122
3 TNN	0.050764194	0.008392586	0.200973673	0.121980702	0.606769257	0.020024715
4 MIR553	0	30.15393604	8.023148985	0	21.06354259	3.997072629

# Dataset Analysis – AUROC Overview

**IOSig: Immuno-Oncology Signature Explorer**

The screenshot shows the 'Dataset Analysis' section of the IOSig platform. The 'Dataset Analysis' tab is selected. A red box highlights the 'AUROC Overview' button in the top navigation bar. Another red box highlights the 'Average AUC is: 0.4926' text. A red bracket points to the table below, which displays the AUC values for various signatures against the uploaded dataset. A red arrow points to the 'Select signature to view' dropdown menu, which is set to 'ipi\_neo'. A red arrow also points to the 'Download AUROC Plot' button. A red bracket points to the AUROC plot itself, showing the ROC curve for the 'ipi\_neo' signature.

Average AUC value of all signatures in IOSig on uploaded data

AUC	Cohort	Signature
0.67	example_seq_file	gMDSC
0.67	example_seq_file	ifng18
0.67	example_seq_file	impres
0.67	example_seq_file	ipi_neo
0.67	example_seq_file	mMDSC
0.67	example_seq_file	Ock
0.67	example_seq_file	tip_hot
0.56	example_seq_file	chemokines
0.56	example_seq_file	glycolysis
0.56	example_seq_file	pan

Showing 1 to 10 of 30 entries

1 2 3 Next

Plot download button

Select signature to view AUROC plot

Table displaying the AUC value of uploaded data against all signatures in IOSig

The AUROC plot shows the relationship between the true positive fraction (Y-axis) and the false positive fraction (X-axis). The X-axis ranges from 0.00 to 1.00, and the Y-axis ranges from 0.00 to 1.00. The plot area is mostly empty, with a small black line segment visible near the bottom-left corner.

# Dataset Analysis – AUROC Comparison

**IOSig: Immuno-Oncology Signature Explorer**

The screenshot shows the 'Dataset Analysis' section of the IOSig platform. The 'Dataset Analysis' tab is selected, and within it, the 'AUROC Comparison' tab is also selected. A red box highlights the 'AUROC Comparison' tab in both the main navigation bar and the sub-navigation bar.

A red arrow points from the text 'Download the AUROC Comparison plot' to the 'Download Plot' button, which is located below the sub-navigation bar.

**Cohorts are columns, signatures are rows**

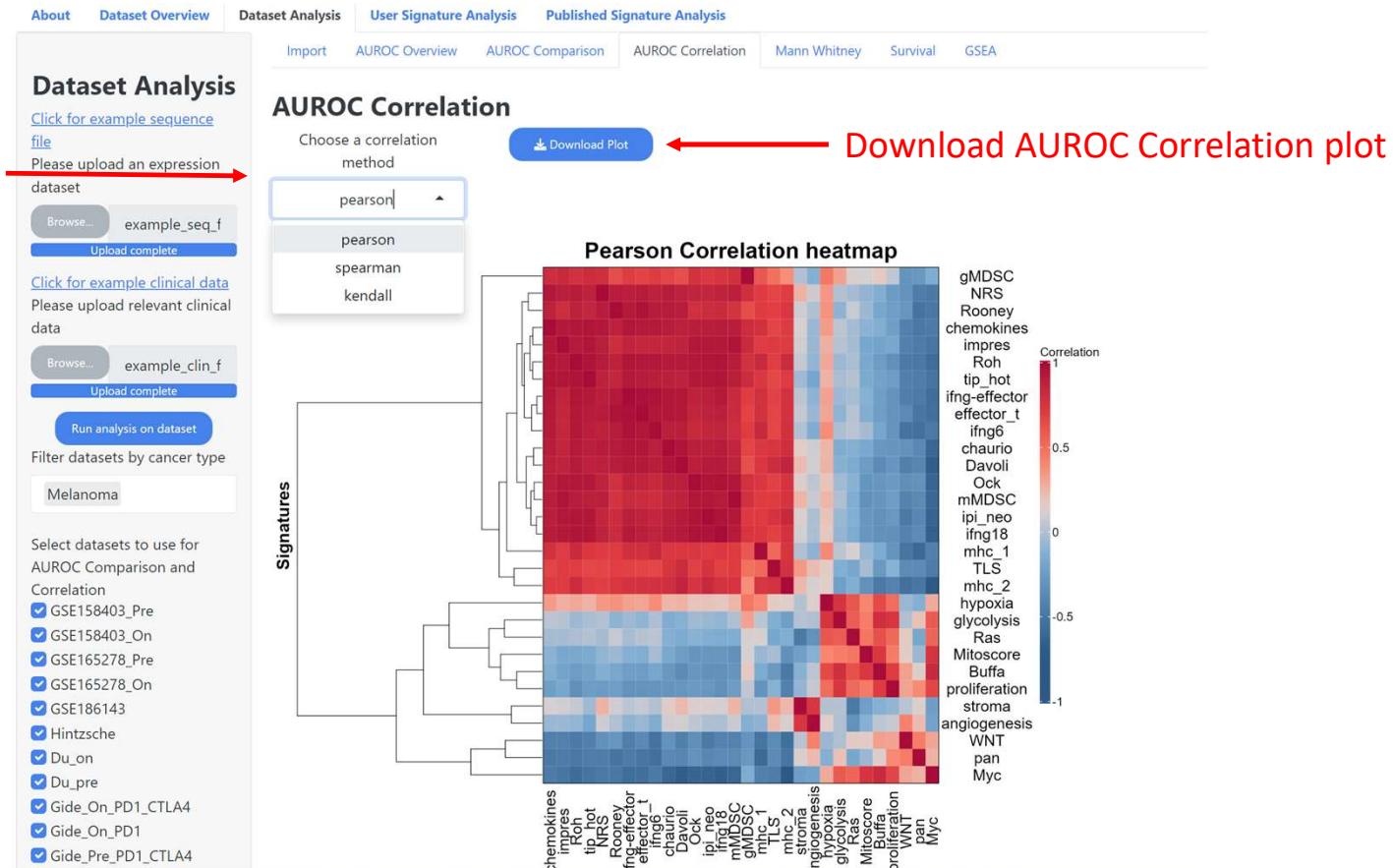
**Gene Signature AUROC Performance**

**Cohorts**

	Average	Du_on	Du_pre	example_seq_file	Glide_On_PD1	Glide_On_PDL1_CTL4	Glide_Pre_PD1	Glide_Pre_PDL1_CTL4	GSE158403_On	GSE158403_Pre	GSE165278_On	GSE165278_Pre	GSE186143	Hintsche	HugoLo_IPRES_2016	Lauss	Lee_on	Lee_pre	Liu_naive	Liu_prog	Riaz_on	Riaz_pre	.VanAllen_antCTLA4_2015
ifng18-	0.68	0.77	0.63	0.67	0.9	0.95	0.83	0.77	0.48	0.47	0.87	0.9	0.6	0.48	0.52	0.72	0.83	0.55	0.46	0.66	0.66	0.66	0.5
effector_t-	0.67	0.67	0.56	0.44	0.95	0.95	0.84	0.78	0.53	0.48	0.81	1	0.61	0.6	0.48	0.7	0.79	0.61	0.44	0.64	0.67	0.64	0.53
ifng-effector-	0.67	0.68	0.56	0.44	0.95	0.95	0.86	0.81	0.43	0.48	0.85	1	0.62	0.5	0.5	0.69	0.8	0.62	0.47	0.64	0.63	0.65	0.5
ipi_neo-	0.66	0.66	0.6	0.67	0.9	0.95	0.83	0.75	0.47	0.5	0.83	0.8	0.6	0.5	0.49	0.74	0.82	0.52	0.47	0.7	0.7	0.66	0.45
Davoll-	0.66	0.73	0.55	0.44	0.95	0.95	0.81	0.74	0.5	0.49	0.85	0.8	0.6	0.5	0.47	0.71	0.84	0.56	0.47	0.66	0.71	0.67	0.52
tip_hot-	0.66	0.61	0.53	0.67	0.9	0.95	0.83	0.78	0.44	0.49	0.83	1	0.58	0.5	0.47	0.7	0.82	0.56	0.47	0.63	0.66	0.66	0.43
chaurio-	0.66	0.76	0.55	0.44	0.85	0.9	0.85	0.72	0.51	0.48	0.85	0.9	0.53	0.56	0.5	0.7	0.79	0.58	0.46	0.7	0.72	0.68	0.47
Rooney-	0.66	0.65	0.49	0.33	0.9	0.95	0.81	0.76	0.58	0.52	0.81	1	0.63	0.6	0.41	0.69	0.76	0.56	0.47	0.65	0.72	0.65	0.52
Roh-	0.66	0.62	0.62	0.56	0.85	0.95	0.83	0.78	0.52	0.48	0.81	0.9	0.57	0.5	0.52	0.73	0.8	0.55	0.49	0.64	0.65	0.63	0.47
mhc_2-	0.65	0.62	0.65	0.44	0.95	0.95	0.74	0.76	0.49	0.52	0.81	0.6	0.57	0.62	0.61	0.77	0.75	0.48	0.53	0.68	0.69	0.61	0.48
chemokines-	0.65	0.6	0.58	0.56	0.9	0.85	0.82	0.77	0.47	0.51	0.83	1	0.49	0.5	0.39	0.73	0.83	0.57	0.48	0.7	0.61	0.66	0.49
mMDSC-	0.64	0.68	0.59	0.67	0.85	0.9	0.7	0.75	0.54	0.52	0.8	0.8	0.45	0.46	0.42	0.71	0.86	0.48	0.49	0.69	0.67	0.64	0.47

# Dataset Analysis – AUROC Correlation

Select a correlation method



# Dataset Analysis – Mann Whitney

Select a signature to view the Mann Whitney U-test boxplot

IOSig: Immuno-Oncology Signature Explorer

Dataset Analysis      User Signature Analysis      Published Signature Analysis

Mann Whitney

Download Mann Whitney plot

Dataset Analysis

Click for example sequence file

Please upload an expression dataset

Browse... example\_seq\_f Upload complete

Click for example clinical data

Please upload relevant clinical data

Browse... example\_clin\_f Upload complete

Run analysis on dataset

Filter datasets by cancer type

Melanoma

Select datasets to use for AUROC Comparison and Correlation

GSE158403\_Pre  
 GSE158403\_On  
 GSE165278\_Pre  
 GSE165278\_On  
 GSE186143  
 Hintzsche  
 Du\_on  
 Du\_pre  
 GSE158403\_GSEA

Mann Whitney U Test

Select a Mann Whitney plot to view

ipi\_neo

p value: 0.7

Average Z Score

Response Type

Non-Responder   Responder

Non-Responder   Responder Status

Method: Z Score

Show 10 entries

Search:

Signature	P_Value	n	Responders	Non_Responders
1 Mitoscore	0.4	6	3	3
2 angiogenesis	0.7	6	3	3
3 gMDSC	0.7	6	3	3
4 hypoxia	0.7	6	3	3
5 ifng18	0.7	6	3	3
6 ifng6	0.7	6	3	3
7 impres	0.7	6	3	3
8 ipi_neo	0.7	6	3	3
9 mMDS	0.7	6	3	3
10 Ock	0.7	6	3	3

Showing 1 to 10 of 30 entries

Previous 1 2 3 Next

A data table showing the p-values for each signature tested against the user uploaded dataset

# Dataset Analysis – Survival

Select a signature to view the survival plot for

**IOSig: Immuno-Oncology Signature Explorer**

**Dataset Analysis** **Survival** **Download the survival plot**

chaurio

Method: Z Score

High Low

Survival probability

p = 0.46

Time

0 250 500 750 1000 1250

Signature P\_Value n High.Category Low.Category

Signature	P_Value	n	High.Category	Low.Category
stroma	0.1138	6	2	4
TLS	0.3173	6	1	5
chaurio	0.4561	6	3	3
Davoli	0.4561	6	3	3
ifng18	0.4561	6	3	3
ifng6	0.4561	6	3	3
impres	0.4561	6	3	3
mMDSC	0.4561	6	3	3
Ock	0.4561	6	3	3
pan	0.4561	6	3	3

Showing 1 to 10 of 30 entries

A table that displays the p-value for the signatures queried against the upload data

# Dataset Analysis - GSEA

**Select a gene set to use**

The screenshot shows the 'Dataset Analysis' section of a web application. The 'Dataset Analysis' tab is selected. The 'GSEA' tab is also selected. A red box highlights the 'GSEA' tab. Another red box highlights the 'GSEA' button in the top navigation bar.

**GSEA**

Select a gene set to use for GSEA

Download GSEA plot

example\_seq\_file Cohort example\_seq\_file

Pathway enrichment results:

pathway	pval	padj	ES	NES	nMoreExtreme	size	le
HALLMARK_IL6_JAK_STAT3_SIGNALING	0.0321	0.0891	0.34	1.45	15	85	T
HALLMARK_KRAS_SIGNALING_DN	0.0142	0.0592	0.29	1.42	6	190	IF
HALLMARK_ALLOGRAFT_REJECTION	0.0209	0.0679	0.29	1.39	9	193	IL
HALLMARK_PANCREAS_BETA_CELLS	0.0817	0.1945	0.38	1.37	40	38	S
HALLMARK_SPERMATOGENESIS	0.048	0.1263	0.29	1.32	23	125	D
HALLMARK_INTERFERON_ALPHA_RESPONSE	0.1568	0.3135	0.27	1.18	78	95	IL
HALLMARK_APICAL_SURFACE	0.3333	0.5556	0.29	1.08	165	43	M
HALLMARK_COMPLEMENT	0.4242	0.6629	0.21	1.01	209	198	K
HALLMARK_IL2_STAT5_SIGNALING	0.5592	0.7812	0.2	0.95	268	196	T
HALLMARK_APICAL_JUNCTION	0.6577	0.8888	0.19	0.92	316	192	F

Showing 1 to 10 of 50 entries

**Hallmark Gene Sets**

The result table for the gene set enrichment analysis

# Dataset Analysis— GSEA Enrichment

Screenshot of the Dataset Analysis interface showing the GSEA tab selected.

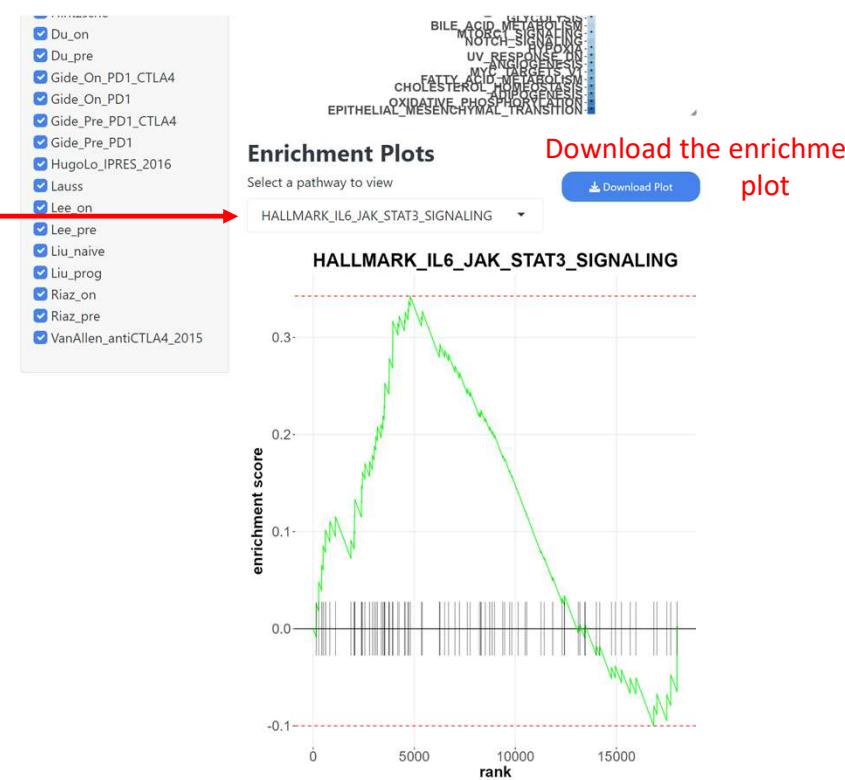
The top navigation bar includes: About, Dataset Overview, **Dataset Analysis**, User Signature Analysis, Published Signature Analysis.

The sub-navigation bar under Dataset Analysis includes: Import, AUROC Overview, AUROC Comparison, AUROC Correlation, Mann Whitney, Survival, **GSEA**.

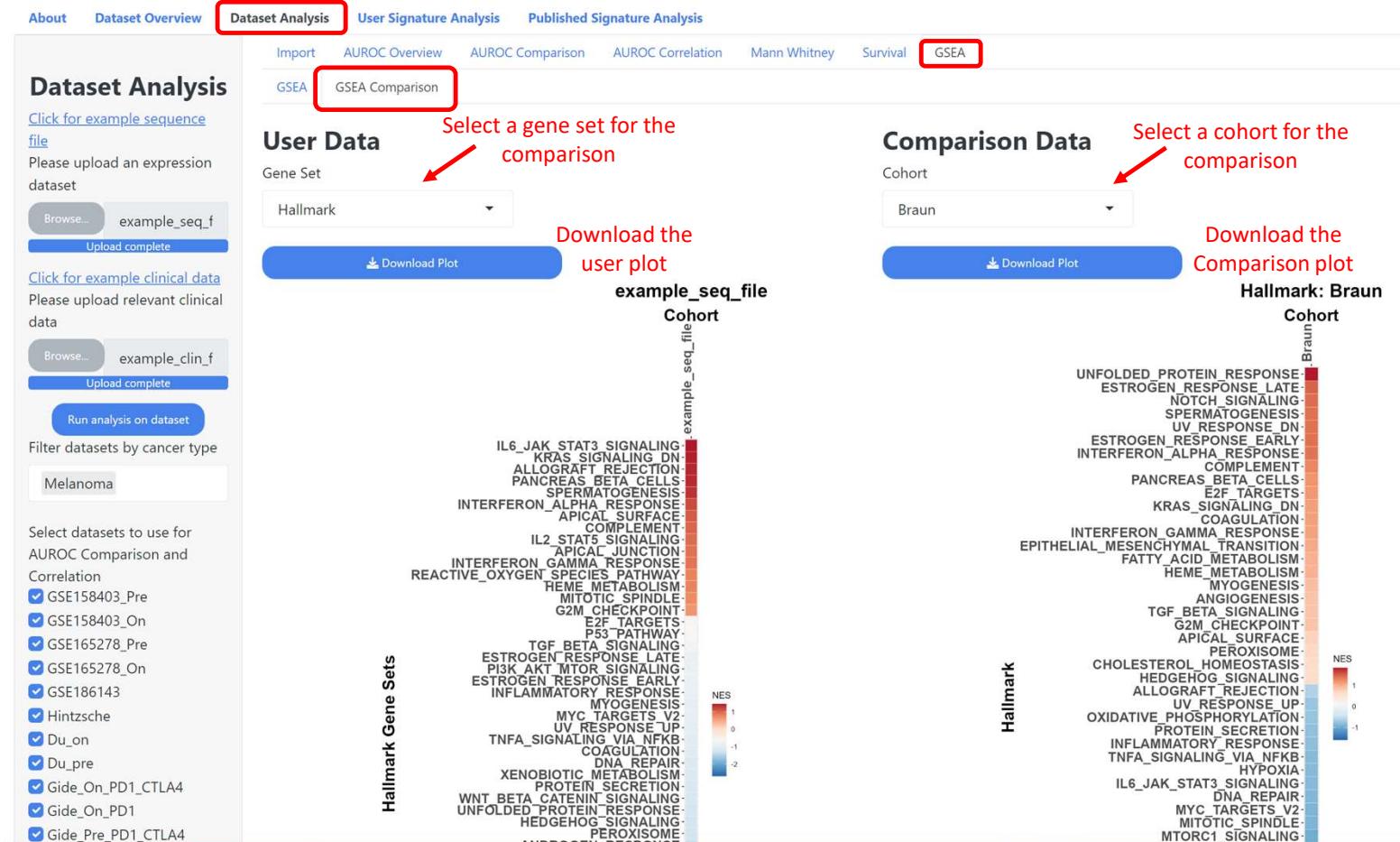
The main content area shows the **Dataset Analysis** section with tabs: GSEA (selected) and GSEA Comparison.

Scroll down on the page to view the specific enrichment plot output

Select an enrichment pathway to view



# Dataset Analysis – GSEA Comparison



# User Signature Analysis

The User Signature Analysis method in IOSig allows the user to upload a **newline separated list of HUGO formatted genes** and query their ability to predict responder / non-responder in the datasets included in IOSig.

Users must provide a name for their signature that does match with one of the signatures already included in IOSig.

The screenshot shows a web-based application titled "Signature Analysis". At the top, it says "Enter a unique signature name" followed by a text input field containing "New\_Signature". Below this, instructions say "Enter new line separated HGNC symbols. [Example Signature File](#)". A text input field contains "CD3E", "CD8A", and "IFNG".

**Signature Analysis**

Enter a unique signature name

New\_Signature

Enter new line separated HGNC symbols. [Example Signature File](#)

CD3E  
CD8A  
IFNG

# User Signature Analysis - Summary

**IOSig: Immuno-Oncology Signature Explorer**

**Signature Analysis**

Enter a unique signature name  
Custom\_Signature

Download example signature file  
[Example Signature File](#)

Paste newline separated HUGO genes  
Fake-Gene  
IDO1

Confirm gene signature upload and cohort selection  
**Confirm signature and datasets**

Optionally filter datasets by cancer type  
Breast Cancer NSCLC

Select individual cohorts to include / remove  
GSE111414\_Pre  
GSE111414\_On  
GSE173839  
GSE190265  
GSE194040

**Signature Summary**

Show 10 entries Search: Invalid\_Genes

Signature	Gene.Signature.Length	Gene_Count_Overlap	Signature_Overlap_Percentage
ifng6	6	4	66.667
ifng-effector	8	3	37.5
chemokines	12	2	16.667
effector_t	6	1	16.667
mhc_1	6	1	16.667
tip_hot	12	2	16.667
chaudio	7	1	14.286
ifng18	18	2	11.111
Roh	41	4	9.756
ipi_neo	32	3	9.375

Showing 1 to 10 of 30 entries

**Genes**

1	IDO1
2	CXCL10
3	CXCL9
4	IFNG

Select a signature to view gene overlap with input signature  
ifng6

Previous 1 Next

View overlapping genes between user uploaded signature and signatures included in IOSig

Gene overlap statistics between user uploaded signature and signatures included in IOSig

Genes not detected as valid HUGO symbols  
Fake-Gene

Select a signature to view gene overlap with user uploaded signature

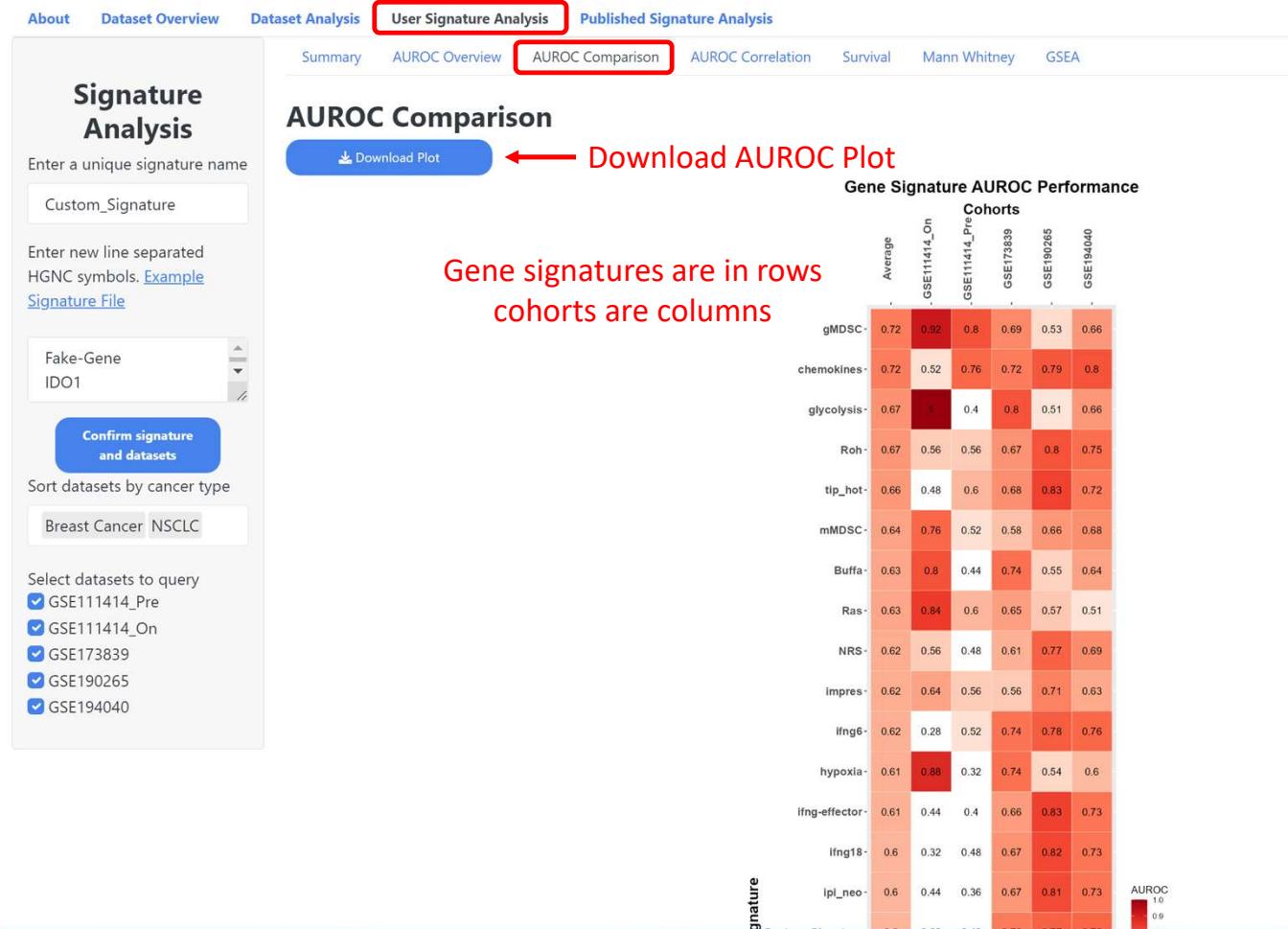
# User Signature Analysis – AUROC Overview

**IOSig: Immuno-Oncology Signature Explorer**

The screenshot shows the User Signature Analysis interface with several annotations:

- User Signature Analysis tab:** The "User Signature Analysis" tab is highlighted with a red box.
- AUROC Overview section:** The "AUROC Overview" section is highlighted with a red box. It displays the message "Average AUC is: 0.602" with a red arrow pointing to it. Below this, a table lists AUROC values for five cohorts: GSE190265 (AUC: 0.77), GSE194040 (AUC: 0.76), GSE173839 (AUC: 0.72), GSE111414\_Pre (AUC: 0.48), and GSE111414\_On (AUC: 0.28). A red bracket underlines the entire table area.
- Select a cohort plot to view:** A dropdown menu titled "Select an AUROC plot to view" contains the option "GSE111414\_Pre". A red arrow points from the text "Select a cohort plot to view" to this dropdown.
- Download AUROC plot:** A blue button labeled "Download Plot" is located next to the dropdown menu. A red arrow points from the text "Download AUROC plot" to this button.
- Plot area:** The plot shows the ROC curve for the GSE111414\_Pre cohort. The x-axis is "false\_positive\_fraction" (ranging from 0.00 to 1.00) and the y-axis is "true\_positive\_fraction" (ranging from 0.00 to 1.00). The AUC is labeled as 0.48. The plot title is "GSE111414\_Pre". A red arrow points from the text "plot" to the plot area.
- Annotations:** Several annotations are overlaid in red:
  - "The average AUC value of the signature across all selected cohorts"
  - "A table showing the user signature AUC values in each cohort selected"

# User Signature Analysis – AUROC Comparison



# User Signature Analysis – AUROC Correlation



# User Signature Analysis - Survival

Select a survival plot to view

**IOSig: Immuno-Oncology Signature Explorer**

Signature Analysis

Enter a unique signature name

Enter new line separated HGNC symbols. [Example Signature File](#)

Fake-Gene  
IDO1

Confirm signature and datasets

Sort datasets by cancer type  
Breast Cancer | NSCLC

Select datasets to query

- GSE111414\_Pre
- GSE111414\_On
- GSE173839
- GSE190265
- GSE194040

**Survival**

Select a survival plot to view

Download survival plot [Download Plot](#)

**GSE190265**  
Method: Z Score

High Low

Survival probability

Time

p = 0.21

Survival

Summary AUROC Overview AUROC Comparison AUROC Correlation Survival Mann Whitney GSEA

Show 10 entries Search:

Cohort	P_Value	n	High.Category	Low.Category
1 GSE190265	0.2072	43	22	21

Showing 1 to 1 of 1 entries

A table that shows the p-value and other stats for the cohort survival analysis with the user signature

# User Signature Analysis – Mann Whitney

**IOSig: Immuno-Oncology Signature Explorer**

Select Boxplot to view

**Signature Analysis**

Enter a unique signature name  
Custom\_Signature

Enter new line separated HGNC symbols. [Example Signature File](#)

Fake-Gene  
IDO1

Confirm signature and datasets

Sort datasets by cancer type  
Breast Cancer NSCLC

Select datasets to query  
 GSE111414\_Pre  
 GSE111414\_On  
 GSE173839  
 GSE190265  
 GSE194040

Mann Whitney U Test  
Select a Mann Whitney plot to view  
GSE194040

Download Boxplot

**GSE194040**

p value: 2e-04

Average Z Score

Non-Responder Responder

Responder Status

Method: Z Score

Response Type  
■ Non-Responder  
■ Responder

Show 10 entries

Cohort P\_Value n Responders Non\_Responders

1	GSE194040	0.0002	69	31	38
2	GSE173839	0.0011	71	29	42
3	GSE190265	0.0078	43	11	32
4	GSE111414_On	0.3095	10	5	5
5	GSE111414_Pre	1	10	5	5

Showing 1 to 5 of 5 entries

View p-value and other statistics

# User Signature Analysis - GSEA

Select a gene set to use

Signature Analysis

Enter a unique signature name  
Custom\_Signature

Enter new line separated HGNC symbols. [Example Signature File](#)

Fake-Gene  
IDO1

Confirm signature and datasets

Sort datasets by cancer type  
Breast Cancer NSCLC

Select datasets to query  
 GSE111414.Pre  
 GSE111414.On  
 GSE173839  
 GSE190265  
 GSE194040

GSEA

Summary AUROC Overview AUROC Comparison AUROC Correlation Survival Mann Whitney GSEA

GSEA Comparison Select a cohort to view

Select gene set to use Hallmark Select a set to use GSE111414.Pre Download Plot

Download Plot

Hallmark: GSE111414.Pre Cohort

ANGIOGENESIS  
UV\_RESPONSE\_DN  
MYC\_TARGETS\_V2  
IL2\_STAT5\_SIGNALING  
GLYCOLYSIS  
ANDROGEN\_RESPONSE  
PROTEIN\_SECRETION  
WNT\_BETA\_CATENIN\_SIGNALING  
XENOBIOTIC\_METABOLISM  
ADIPOGENESIS  
ESTROGEN\_RESPONSE\_LATE  
COMPLEMENT  
DNA\_REPAIR  
OXIDATIVE\_PHOSPHORYLATION  
MITOTIC\_SPINDLE  
BILE\_ACID\_METABOLISM  
INFLAMMATORY\_RESPONSE  
IL6\_JAK\_STAT3\_SIGNALING  
P53\_PATHWAY  
MYC\_TARGETS\_V1  
ALLOGRAFT\_REJECTION  
MTORC1\_SIGNALING  
COAGULATION  
KRAS\_SIGNALING\_DN  
HEDGEHOG\_SIGNALING

NES

Showing 1 to 10 of 50 entries

1 2 3 4 5 Next

Select a cohort to view

Download Plot

Hallmark: GSE111414.Pre Cohort

ANGIOGENESIS  
UV\_RESPONSE\_DN  
MYC\_TARGETS\_V2  
IL2\_STAT5\_SIGNALING  
GLYCOLYSIS  
ANDROGEN\_RESPONSE  
PROTEIN\_SECRETION  
WNT\_BETA\_CATENIN\_SIGNALING  
XENOBIOTIC\_METABOLISM  
ADIPOGENESIS  
ESTROGEN\_RESPONSE\_LATE  
COMPLEMENT  
DNA\_REPAIR  
OXIDATIVE\_PHOSPHORYLATION  
MITOTIC\_SPINDLE  
BILE\_ACID\_METABOLISM  
INFLAMMATORY\_RESPONSE  
IL6\_JAK\_STAT3\_SIGNALING  
P53\_PATHWAY  
MYC\_TARGETS\_V1  
ALLOGRAFT\_REJECTION  
MTORC1\_SIGNALING  
COAGULATION  
KRAS\_SIGNALING\_DN  
HEDGEHOG\_SIGNALING

NES

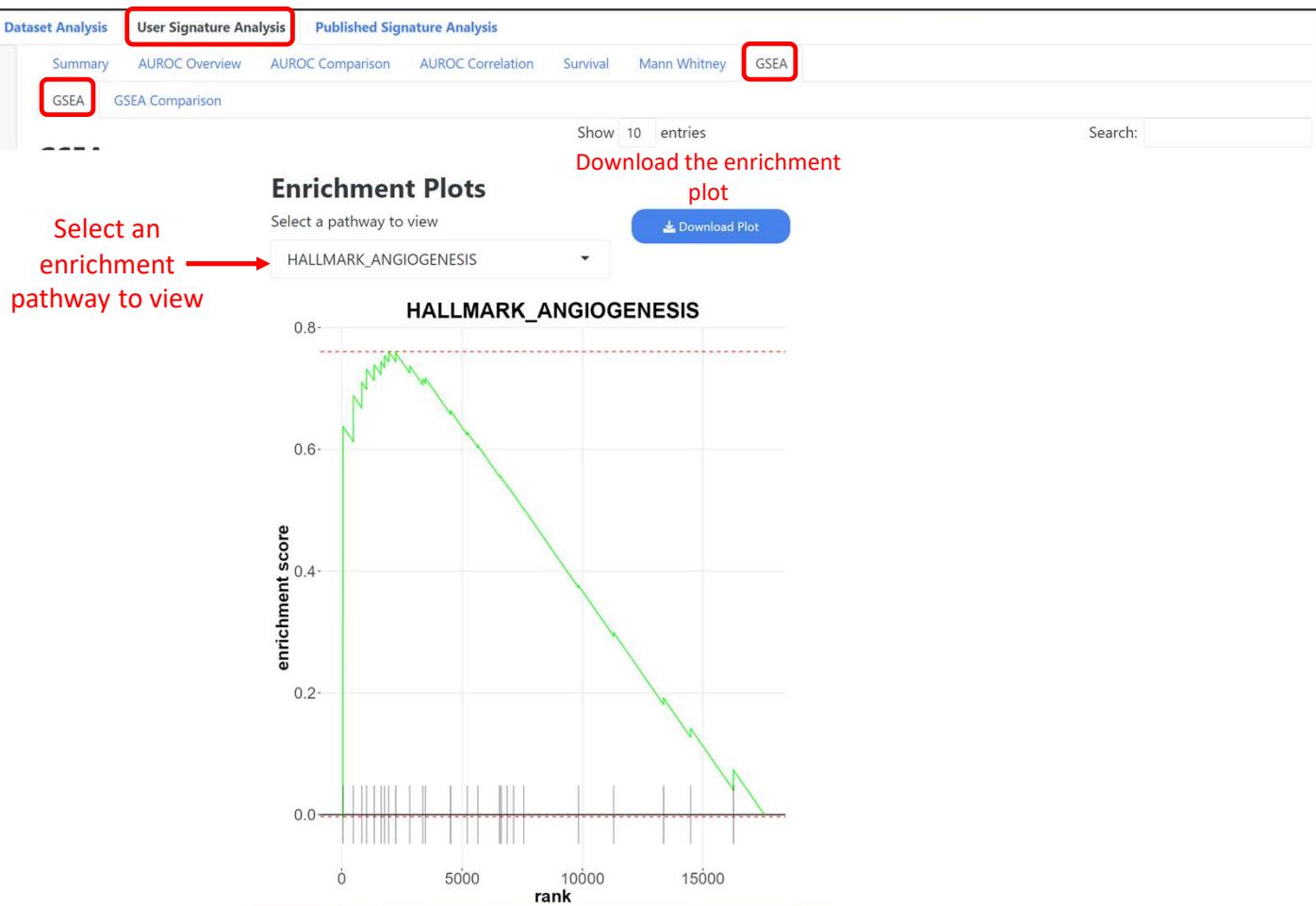
Showing 1 to 10 of 50 entries

1 2 3 4 5 Next

Resulting gene set enrichment data table

# User Signature Analysis – GSEA Enrichment

Scroll down on the User Signature GSEA page



# User Signature Analysis – GSEA Comparison

**Signature Analysis**

Enter a unique signature name  
Custom\_Signature

Enter new line separated HGNC symbols. [Example Signature File](#)

Fake-Gene  
IDO1

Confirm signature and datasets

Sort datasets by cancer type  
Breast Cancer NSCLC

Select datasets to query  
 GSE111414\_Pre  
 GSE111414\_On  
 GSE173839  
 GSE190265  
 GSE194040

**User Data**

Select a gene set for the comparison  
Hallmark

**Comparison Data**

Select a cohort to view  
GSE111414\_Pre

**GSEA**

GSEA Comparison

Select a gene set for the comparison  
Hallmark

Select a gene signature for the comparison  
angiogenesis

Select a cohort to view  
GSE111414\_Pre

Download Plot

Custom\_Signature: GSE111414\_Pre

Cohort: GSE111414\_Pre

UV\_RESPONSE\_DN  
ANGIOGENESIS  
MYC TARGETS\_V2  
IL2\_STAT5\_SIGNALING  
GLYCOLYSIS  
ANDROGEN\_RESPONSE  
PROTEIN\_SECRETION  
WNT\_BETA\_CATEININ\_SIGNALING  
ADIPOGENESIS  
XENOBIOTIC\_METABOLISM  
ESTROGEN\_RESPONSE\_LATE  
COMPLEMENT  
DNA\_REPAIR  
OXIDATIVE\_PHOSPHORYLATION  
MITOTIC\_SPINDLE  
P53\_PATHWAY  
INFLAMMATORY\_RESPONSE  
MYC\_TARGETS\_V1  
BILE\_ACID\_METABOLISM  
IL6\_JAK\_STAT3\_SIGNALING  
ALLOGRAFT\_REJECTION  
MTORC1\_SIGNALING  
COAGULATION  
KRAS\_SIGNALING\_DN  
HEDGEHOG\_SIGNALING  
PANCREAS\_BETA\_CELLS  
UNFOLDED\_PROTEIN\_RESPONSE  
MYOGENESIS  
PI3K\_AKT\_MTOR\_SIGNALING  
HEME\_METABOLISM  
REACTIVE\_OXYGEN\_SPECIES\_PATHWAY  
APOTOPSIS  
INTERFERON\_ALPHA\_RESPONSE  
KRAS\_SIGNALING\_UP  
APICAL\_JUNCTION  
ESTROGEN\_RESPONSE\_EARLY  
TGF\_BETA\_SIGNALING

NES

**GSEA**

Select a cohort to use for the comparison  
GSE111414\_Pre

Download Plot

angio genesis: GSE111414\_Pre

Cohort: GSE111414\_Pre

UNFOLDED\_PROTEIN\_RESPONSE  
TGF\_BETA\_SIGNALING  
NOTCH\_SIGNALING  
ADIPOGENESIS  
CHOLESTEROL\_HOMEOSTASIS  
HYPOXIA  
EPITHELIAL\_MESENCHYMAL\_TRANSITION  
PANCREAS\_BETA\_CELLS  
INFLAMMATORY\_RESPONSE  
TNFA\_SIGNALING  
COAGULATION  
G2M\_CHECKPOINT  
ANDROGEN\_RESPONSE  
GLYCOLYSIS  
APOPTOSIS  
ESTROGEN\_RESPONSE\_LATE  
ESTROGEN\_RESPONSE\_EARLY  
IL2\_STAT5\_SIGNALING  
KRAS\_SIGNALING\_UP  
REACTIVE\_OXYGEN\_SPECIES\_PATHWAY  
OXIDATIVE\_PHOSPHORYLATION  
P53\_PATHWAY  
PROTEIN\_SECRETION  
MYOGENESIS  
UV\_RESPONSE\_UP  
HEME\_METABOLISM  
MITOTIC\_SPINDLE  
ALLOGRAFT\_REJECTION  
ANGIOGENESIS  
APICAL\_SURF  
KRAS\_SIGNALING\_DN  
HEDGEHOG\_SIGNALING  
COMPLEMENT  
WNT\_BETA\_CATEININ\_SIGNALING  
PEROXISOME  
UV\_RESPONSE\_DN  
INTERFERON\_GAMMA\_RESPONSE

NES

# Previously Published Signature Analysis

The previously published signature analysis method is for users who want to query the data built into IOSig. IOSig contains RNA-seq, with accompanying clinical data, with previously published gene expression signatures.

This method allows users to explore the data without needing to provide any of their own.

### Published Signature Analysis

Select a previously published signature.

**Confirm selections**

Optionally sort by cancer type

GI Cancer  
 Esophageal Cancer

Select datasets to query.

- GSE154538\_Post
- GSE154538\_Pre
- GSE165252\_On
- GSE165252\_Pre
- GSE165252\_Post
- GSE183924

# Previously Published Signature - Summary

IOSig: Immuno-Oncology Signature Explorer

Published Signature Analysis

Summary

AUROC Overview AUROC Comparison AUROC Correlation Survival Mann Whitney GSEA

Signature Overview

Show 10 entries Search:

Gene Signature Name	Description	Number of Genes	Reference	DOI
1 IFNg-6	6 gene IFNg associated gene set	6	Ayers et al (2017)	doi:10.1172/JCI91190

Showing 1 to 1 of 1 entries Previous 1 Next

Gene Overview

Show 10 entries Search:

Genes
1 IDO1
2 CXCL10
3 CXCL9
4 HLA-DRA
5 IFNG
6 STAT1

Showing 1 to 6 of 6 entries Previous 1 Next

Select a signature to view  
Confirm signature and dataset selections  
Optional filter datasets by cancer type  
Select individual datasets to use for the analysis

A table that displays various data points about the selected signature

A table of the genes within the selected signature

The screenshot shows the 'Published Signature Analysis' tab selected in the top navigation bar. The 'Signature Overview' section contains a table with one entry: IFNg-6, which is a 6 gene IFNg associated gene set from Ayers et al (2017) with DOI doi:10.1172/JCI91190. The 'Gene Overview' section contains a table with six entries: IDO1, CXCL10, CXCL9, HLA-DRA, IFNG, and STAT1. Red arrows and annotations point to the 'Summary' button, the 'IFNg-6' selection in the dropdown, the 'Confirm selections' button, the 'Kidney' filter, the dataset selection checkboxes, and the 'Previous' and 'Next' buttons in both tables.

# Previously Published Signature – AUROC Overview

**IOSig: Immuno-Oncology Signature Explorer**

The screenshot shows the 'Published Signature Analysis' tab selected in the top navigation bar. The 'AUROC Overview' section is highlighted with a red box.

**AUROC Overview**

The average AUC value of the signature across all cohorts is 0.66.

Table displaying the AUC values in each cohort:

Cohort	AUC
GSE158403_Pre	0.5
GSE158403_On	0.47
GSE165278_Pre	0.8
GSE165278_On	0.83
GSE186143	0.6
Hintzsche	0.5
Du_on	0.66
Du_pre	0.6
Gide_On_PD1_CTLA4	0.95
Gide_On_PD1	0.9

Download the AUROC plot

Select and AUROC to view

Select a cohort to view

Download

AUC: 0.49825

GSE158403\_Pre: ipi\_neo

true\_positive\_fraction

false\_positive\_fraction

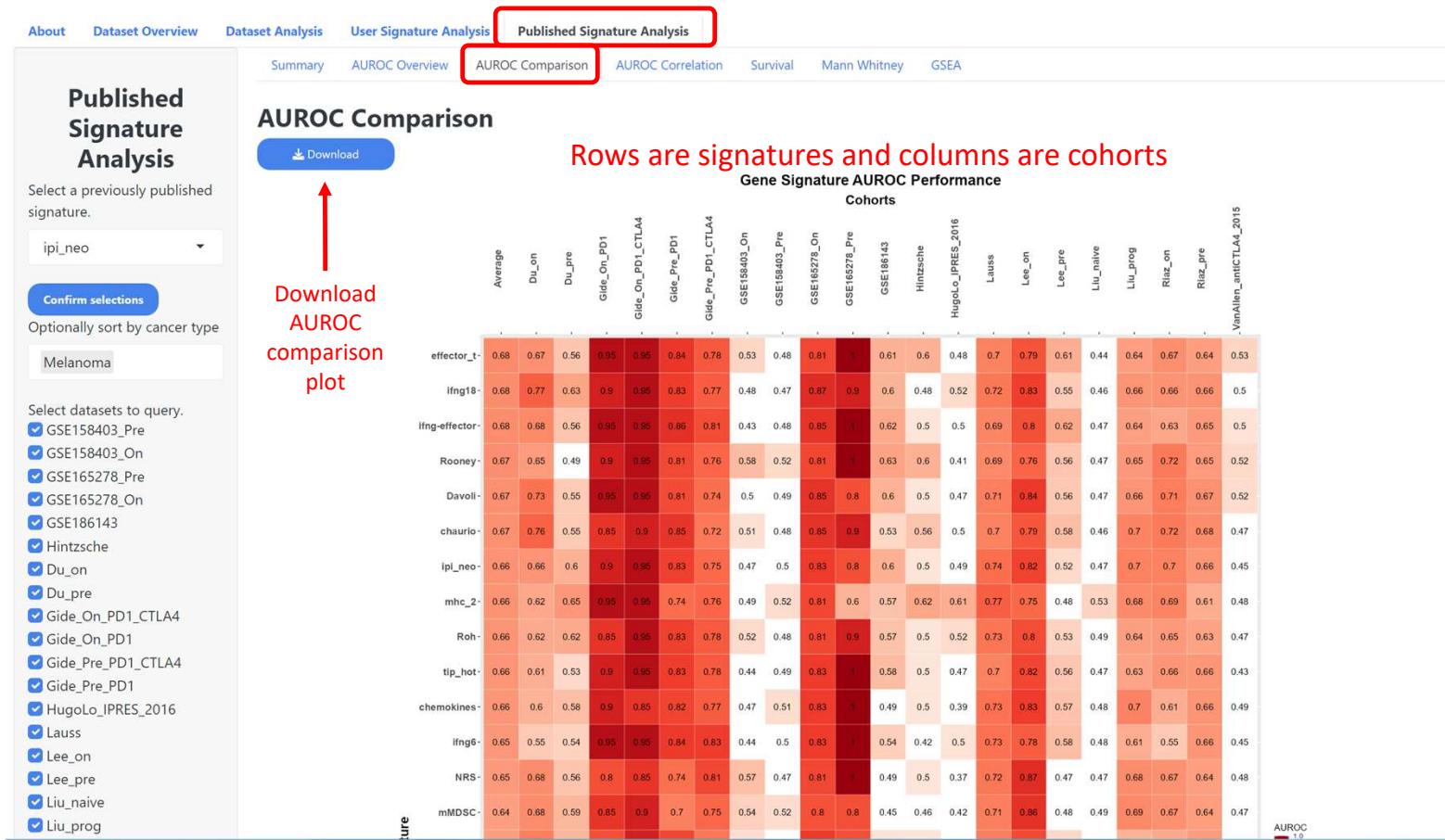
Z Score method

Showing 1 to 10 of 21 entries

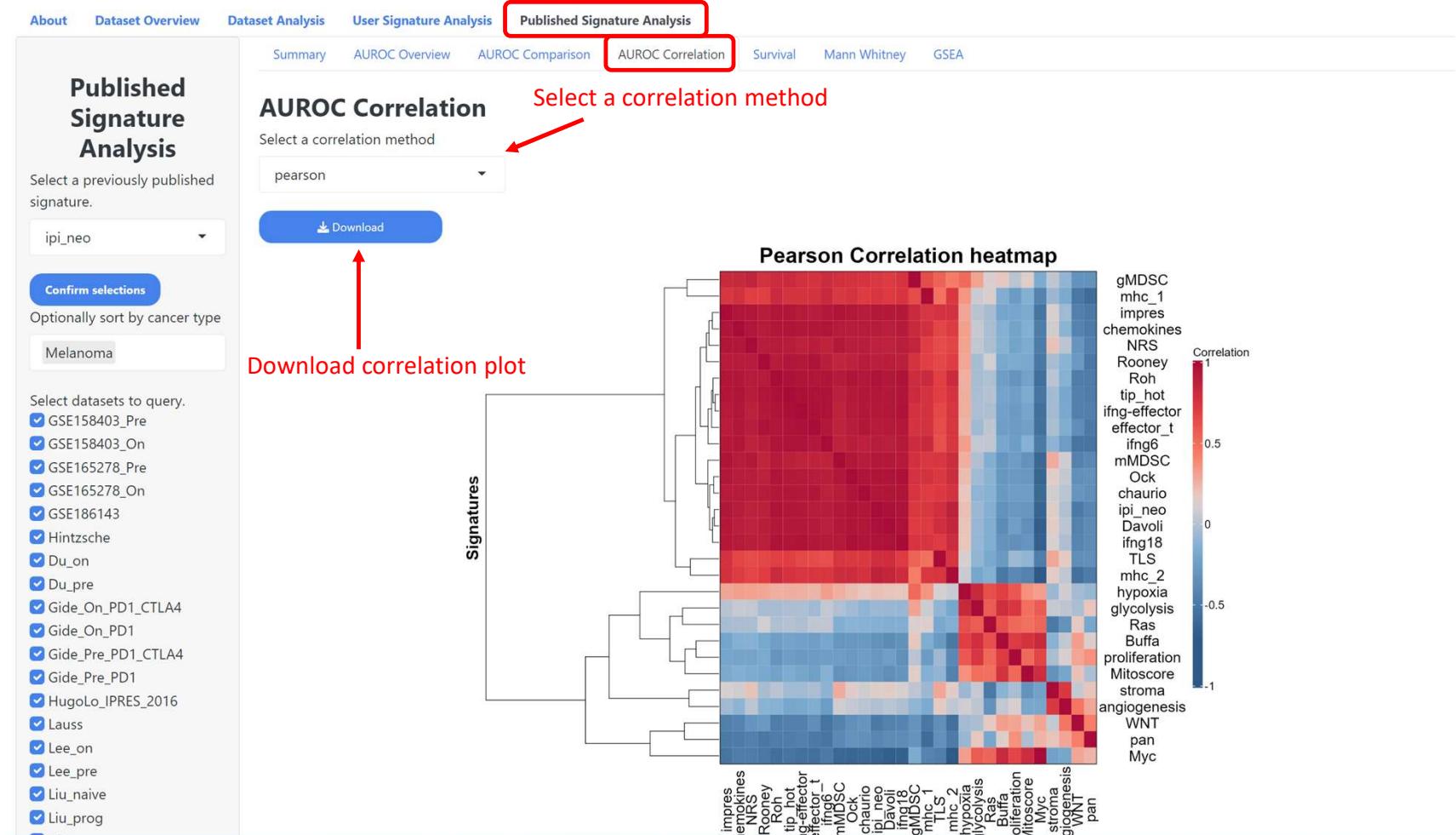
Previous 1 2 3 Next

Table displaying the AUC values in each cohort

# Previously Published Signature – AUROC Comparison



# Previously Published Signatures – AUROC Correlation



# Previously Published Signature - Survival

About   Dataset Overview   Dataset Analysis   User Signature Analysis   **Published Signature Analysis**

Summary   AUROC Overview   AUROC Comparison   AUROC Correlation   **Survival**   Mann Whitney   GSEA

**Published Signature Analysis**

Select a previously published signature. [Download Survival Plot](#)

ipi\_neo

Confirm selections

Optional sort by cancer type

Melanoma

Select datasets to query.

- GSE158403\_Pre
- GSE158403\_On
- GSE165278\_Pre
- GSE165278\_On
- GSE186143
- Hintzsche
- Du\_on
- Du\_pre
- Gide\_On\_PD1\_CTLA4
- Gide\_On\_PD1
- Gide\_Pre\_PD1\_CTLA4
- Gide\_Pre\_PD1
- HugoLo\_IPRES\_2016
- Lauss
- Lee\_on
- Lee\_pre
- Liu\_naive
- Liu\_prog

Select a cohort to view

GSE165278\_On

Method: Z Score

High   Low

Survival probability

Time

p = 0.0011

Survival

Show 10 entries

Cohort   P\_Value   n   High.Category   Low.Category

Cohort	P_Value	n	High.Category	Low.Category
1 GSE165278_On	0.0011	15	6	9
2 Gide_Pre_PD1	0.0278	41	21	20
3 VanAllen_antiCTLA4_2015	0.0442	41	22	19
4 Riaz_on	0.0604	49	25	24
5 Liu_prog	0.0617	48	25	23
6 Du_pre	0.0638	19	7	12
7 Gide_On_PD1	0.0653	9	5	4
8 Lee_pre	0.1055	43	15	28
9 Du_on	0.1905	31	17	14
10 Gide_Pre_PD1_CTLA4	0.2026	32	16	16

Showing 1 to 10 of 19 entries

Table showing p-value and other survival analysis stats

# Previously Published Signature – Mann Whitney

Published Signature Analysis

Select a previously published signature.

ipi\_neo

Confirm selections

Optional sort by cancer type

Melanoma

Select datasets to query.

- GSE158403\_Pre
- GSE158403\_On
- GSE165278\_Pre
- GSE165278\_On
- GSE186143
- Hintzsche
- Du\_on
- Du\_pre
- Gide\_On\_PD1\_CTLA4
- Gide\_On\_PD1
- Gide\_Pre\_PD1\_CTLA4
- Gide\_Pre\_PD1
- HugoLo\_IPRES\_2016
- Lauss
- Lee\_on
- Lee\_pre
- Liu\_naive
- Liu\_prog

Mann Whitney U Test

Select a cohort

Gide\_Pre\_PD1

Download

Gide\_Pre\_PD1

p value: 0.00022

Average Z Score

Non-Responder Responder

Responder Status

Method: Z Score

Response Type

- Non-Responder
- Responder

Summary AUROC Overview AUROC Comparison AUROC Correlation Survival Mann Whitney GSEA

Show 10 entries Search:

Cohort	P_Value	n	Responders	Non_Responders
1 Gide_Pre_PD1	0.0002	41	19	22
2 Lee_on	0.0122	35	6	29
3 Liu_prog	0.0212	48	17	31
4 Gide_Pre_PD1_CTLA4	0.022	32	21	11
5 Gide_On_PD1_CTLA4	0.0317	9	5	4
6 GSE165278_On	0.036	15	6	9
7 Lauss	0.0475	25	10	15
8 Riaz_on	0.0536	49	10	39
9 Gide_On_PD1	0.0635	9	4	5
10 Riaz_pre	0.135	49	10	39

Showing 1 to 10 of 21 entries

Previous 1 2 3 Next

Select a cohort to view

Download Plot

Data table containing Mann Whitney p-value and other statistics

# Previously Published Signature - GSEA

**Published Signature Analysis**

Select a previously published signature.

ipi\_neo

Confirm selections

Optionally sort by cancer type

Melanoma

Select datasets to query.

- GSE158403\_Pre
- GSE158403\_On
- GSE165278\_Pre
- GSE165278\_On
- GSE186143
- Hintsche
- Du\_on
- Du\_pre
- Gide\_On\_PD1\_CTL4
- Gide\_On\_PD1
- Gide\_Pre\_PD1\_CTL4
- Gide\_Pre\_PD1
- HugoLo\_IPRES\_2016
- Lauss
- Lee\_on
- Lee\_pre
- Liu\_naive
- Liu\_prog

**GSEA**

Select a gene set

Hallmark

Select a cohort

GSE158403\_Pre

Download

ipi\_neo: GSE158403\_Pre

Cohort

ALLOGRAFT\_REJECTION  
GLYCOLYSIS  
BILE\_ACID\_METABOLISM  
KRAS\_SIGNALING\_DN  
EPITHELIAL\_MESENCHYMAL\_TRANSITION  
MITOTIC\_SPINDLE  
SPERMATOGENESIS  
APICAL\_SURFACE  
HYPOXIA  
IL6\_JAK\_STAT3\_SIGNALING  
COMPLEMENT  
ESTROGEN\_RESPONSE\_LATE  
UV\_RESPONSE\_UP  
MYOGENESIS  
PANCREAS\_BETA\_CELLS  
ANDROGEN\_RESPONSE  
NOTCH\_SIGNALING  
TGF\_BETA\_SIGNALING  
MTORC1\_SIGNALING  
UV\_RESPONSE\_DN  
PI3K\_AKT\_MTOR\_SIGNALING  
HEME\_METABOLISM  
APOPTOSIS  
G2M\_CHECKPOINT  
UNFOLDED\_PROTEIN\_RESPONSE  
PROTEIN\_SECRETION  
DNA\_REPAIR

NES

**Published Signature Analysis**

Summary AUROC Overview AUROC Comparison AUROC Correlation Survival Mann Whitney GSEA

Show 10 entries

Search:

pathway	T1	pval	T1	padj	T1	ES	T1	NES	T1	nMoreExtreme	T1	si
1 HALLMARK_ALLOGRAFT_REJECTION		0.0363		0.7426		0.9		1.88		13		
2 HALLMARK_GLYCOLYSIS		0.1362		0.7426		0.71		1.47		52		
3 HALLMARK_BILE_ACID_METABOLISM		0.1418		0.7426		0.7		1.45		55		
4 HALLMARK_KRAS_SIGNALING_DN		0.2031		0.7426		0.62		1.31		77		
5 HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION		0.259		0.7426		0.57		1.19		100		
6 HALLMARK_MITOTIC_SPINDLE		0.2674		0.7426		0.56		1.17		103		
7 HALLMARK_SPERMATOGENESIS		0.3282		0.7923		0.53		1.1		126		
8 HALLMARK_APICAL_SURFACE		0.4247		0.7951		0.48		0.99		157		
9 HALLMARK_HYPOXIA		0.4611		0.7951		0.45		0.94		177		
10 HALLMARK_IL6_JAK_STAT3_SIGNALING		0.5183		0.8318		0.43		0.89		197		

Showing 1 to 10 of 50 entries

Previous 1 2 3 4 5 Next

A data table showing the results of the GSEA analysis