
TREMSUCS

Release 1.0

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“TREMSUCS” a tool to choose, harvest and analyse expression and methylation data of the TCGA-projects for revealing Biomarkers which indicate treatment success.

EXAMPLE REPORT:

An example report can be downloaded [here](https://media.githubusercontent.com/media/dendemayer/TREMSUCS-TCGA/main/suppl/report.html?download=true). [https://media.githubusercontent.com/media/dendemayer/TREMSUCS-TCGA/main/suppl/report.html?download=true]

Be aware that this report has a size of about 300 MB.

View the report directly within your browser: [there](#).

INSTALLING FROM GITHUB.COM:

```
$ git clone https://github.com/dendemayer/TREMSUCS-TCGA.git  
$ cd TREMSUCS-TCGA  
$ pip install .
```

To start the analysis with help of the interactive mode, call the pipeline without any argument:

```
$ TREMSUCS
```

Calling the help or the manual page:

```
$ TREMSUCS --help  
$ man TREMSUCS
```


HELP PAGE OF THE PIPELINE:

3.1 TREMSUCS

“TREMSUCS” a tool to choose, harvest and analyse expression and methylation data of the TCGA-projects for revealing Biomarkers which indicate therapy specific treatment success predictions.

Calling the pipeline without any argument starts the interactive mode to help setting all needed parameters for the analysis.

To recreate the analysis published you can run: `TREMSUCS -p TCGA-CESC -p TCGA-HNSC -p TCGA-LUSC -d carboplatin -d carboplatin,paclitaxel -d cisplatin -C 0 -C 5 -C 8 -t 0 -t 5 -t 10 -t 20 -o /your_output_path -c 40`

Note that `-c` gives the number of cores which should fit your environment

TREMSUCS [OPTIONS]

Options

-o, --out_path <out_path>
path to save the result files

Default
'/home/dende/TREMSUCS'

-p, --project <project>
TCGA project(s) to be applied. Any TCGA project can be chosen, like: `-p TCGA-CESC -p TCGA-HNSC ...`

-d, --drugs <drugs>
drug(s), like: `-d drug1 -d drug2` or drugcombination(s), like: `-d drug1,drug2`

-c, --cores <cores>
number of cores provided to snakemake

Default
1

-C, --cutoff <cutoff>
Cut-off parameter. Set minimum survival for positive outcome. If 0, compares dead to alive patients. Enter none, one or several like: `-C 5 -C 8`

You can estimate an appropriate cutoff value by running your analysis with default cutoff and checking out the created report html for the survival time distribution. See man TREMSUCS for further clarification of the Cutoff parameter

Default

0

-t, --threshold <threshold>

Threshold parameter. Exclude all patients within threshold % of the pivot from analysis to get better group separation. Enter none, one or several like: -t 5 -t 10

It is advised for the user not to exceed a threshold value of 20 since it is unlikely to gain any significance for the survival analysis with an exaggerated exclusion of patients. See man TREMSUCS for further clarification of the threshold parameter

Default

0

-e, --execute <execute>

choose which pipeline shall be executed

Default

'DESeq2', 'metilene'

-N, --dryrun

snakemake dryrun

Default

False

-D, --download

if set, just download raw and meta data for given projects and analysis types, revise them, link them, but do not run any analysis

Default

False

-u, --unlock

in case the analysis crashes, snakemake locks the output directory, run with -u to unlock, then repeat the analysis

Default

False

-v, --version

printing out version information: Version 1.0

SHORT TUTORIAL:

4.1 Usage of the interactive mode:

The following example composition of projects, drugs and parameters creates the configuration given in the example report [here](#).

The same configuration can be applied by issuing the following command (the number of cores hereby can be adjusted and would also give the same results):

```
$ TREMSUCS -p TCGA-CESC -p TCGA-HNSC -p TCGA-LUSC -d cisplatin -d carboplatin,paclitaxel
↪ ↵
-d carboplatin -o /scr/TREMSUCS_out -c 40 -t 5 -t 10 -t 20 -C 5 -C 8
```

Calling the pipeline without any argument starts the interactive mode:

```
$ TREMSUCS

OUTPUT_PATH:          /homes/biertruck/gabor/TREMSUCS
SCRIPT_PATH:          /homes/biertruck/gabor/phd/test_git_doc/TREMSUCS/src/shared/
↪ modules
PIPELINES executed:   ['DESeq2', 'metilene']

which projects do you want to include in your analysis:

0:    TCGA-CESC          Cervical Squamous Cell Carcinoma and Endocervical
↪ Adenocarcinoma
1:    TCGA-HNSC          Head and Neck Squamous Cell Carcinoma
2:    TCGA-LUSC          Lung Squamous Cell Carcinoma
3:    TCGA-ESCA          Esophageal Carcinoma
4:    TCGA-BRCA          Breast Invasive Carcinoma
5:    TCGA-GBM           Glioblastoma Multiforme
6:    TCGA-OV            Ovarian Serous Cystadenocarcinoma
7:    TCGA-LUAD          Lung Adenocarcinoma
8:    TCGA-UCEC          Uterine Corpus Endometrial Carinoma
9:    TCGA-KIRC          kidney renal clear cell carcinoma
10:   TCGA-LGG           brain lower grade glioma
11:   TCGA-THCA          thyroid carcinoma
12:   TCGA-PRAD          prostate adenocarcinoma
13:   TCGA-SKCM          skin cutaneous melanoma
14:   TCGA-COAD          colon adenocarcinoma
15:   TCGA-STAD          stomach adenocarcinoma
```

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```

16:      TCGA-BLCA      bladder urothelial carcinoma
17:      TCGA-LIHC      liver hepatocellular carcinoma
18:      TCGA-KIRP      kidney renal papillary cell carcinoma
19:      TCGA-SARC      sarcoma
20:      TCGA-PAAD      pancreatic adenocarcinoma
21:      TCGA-PCPG      pheochromocytoma and paraganglioma
22:      TCGA-READ      rectum adenocarcinoma
23:      TCGA-TGCT      testicular germcelltumors
24:      TCGA-THYM      thymoma
25:      TCGA-KICH      kidney chromophobe
26:      TCGA-ACC      adrenochordical carcinoma
27:      TCGA-MESO      mesothelioma
28:      TCGA-UVM      uveal melanoma
29:      TCGA-DLBC      lymphoid neoplasm diffuse large b-cell lymphoma
30:      TCGA-UCS      uterine carcinoma
31:      TCGA-CHOL      cholangiocarcinoma
enter your choices one by one, when you are done, simply press "Enter":

```

As suggested, you can now, one by one include the projects you are interested in. A default OUTPUT_PATH is also already given together with the default analysis types “DESeq” and “metilene”. Those defaults can also be adjusted in next steps with help of the interactive mode.

To recreate the example set, the first three projects have to be selected, afterwards the following prompt is given:

```

you choose:
PROJECTS:      ['TCGA-CESC', 'TCGA-HNSC', 'TCGA-LUSC']

which therapy approach do you want to include in your analysis:

0: cisplatin                TCGA-CESC: 103 TCGA-HNSC: 64 TCGA-LUSC: 1
1: carboplatin,paclitaxel   TCGA-CESC: 5 TCGA-HNSC: 26 TCGA-LUSC: 14
2: 5-fluorouracil,cisplatin TCGA-CESC: 5 TCGA-HNSC: 2 TCGA-LUSC: 0
3: carboplatin              TCGA-CESC: 3 TCGA-HNSC: 6 TCGA-LUSC: 3
4: carboplatin,cisplatin,paclitaxel TCGA-CESC: 3 TCGA-HNSC: 0 TCGA-LUSC: 1
5: cisplatin,gemcitabine    TCGA-CESC: 3 TCGA-HNSC: 0 TCGA-LUSC: 9
6: paclitaxel               TCGA-CESC: 2 TCGA-HNSC: 1 TCGA-LUSC: 0
7: erbitux                  TCGA-CESC: 1 TCGA-HNSC: 9 TCGA-LUSC: 0
8: cisplatin,vectibix       TCGA-CESC: 0 TCGA-HNSC: 5 TCGA-LUSC: 0
9: carboplatin,erbitux,paclitaxel TCGA-CESC: 0 TCGA-HNSC: 4 TCGA-LUSC: 0
10: cisplatin,erbitux       TCGA-CESC: 0 TCGA-HNSC: 3 TCGA-LUSC: 0
11: carboplatin,cisplatin,erbitux,paclitaxel TCGA-CESC: 0 TCGA-HNSC: 3 TCGA-LUSC: 0
12: carboplatin,cisplatin   TCGA-CESC: 0 TCGA-HNSC: 2 TCGA-LUSC: 0
13: docetaxel,erbitux       TCGA-CESC: 0 TCGA-HNSC: 2 TCGA-LUSC: 0
14: cisplatin,docetaxel     TCGA-CESC: 0 TCGA-HNSC: 1 TCGA-LUSC: 10
15: carboplatin,docetaxel   TCGA-CESC: 0 TCGA-HNSC: 1 TCGA-LUSC: 3
16: cisplatin,vinorelbine    TCGA-CESC: 0 TCGA-HNSC: 0 TCGA-LUSC: 21
17: carboplatin,vinorelbine TCGA-CESC: 0 TCGA-HNSC: 0 TCGA-LUSC: 8
18: cisplatin,etoposide     TCGA-CESC: 0 TCGA-HNSC: 0 TCGA-LUSC: 7
19: carboplatin,gemcitabine TCGA-CESC: 0 TCGA-HNSC: 0 TCGA-LUSC: 5
20: cisplatin,pemetrexed    TCGA-CESC: 0 TCGA-HNSC: 0 TCGA-LUSC: 3
21: cisplatin,docetaxel,gemcitabine TCGA-CESC: 0 TCGA-HNSC: 0 TCGA-LUSC: 2
22: carboplatin,gemcitabine,paclitaxel TCGA-CESC: 0 TCGA-HNSC: 0 TCGA-LUSC: 2
23: carboplatin,cisplatin,vinorelbine TCGA-CESC: 0 TCGA-HNSC: 0 TCGA-LUSC: 2

```

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```

24: carboplatin,docetaxel,gemcitabine      TCGA-CESC: 0 TCGA-HNSC: 0 TCGA-LUSC: 2
25: carboplatin,docetaxel,paclitaxel      TCGA-CESC: 0 TCGA-HNSC: 0 TCGA-LUSC: 2
26: gemcitabine                           TCGA-CESC: 0 TCGA-HNSC: 0 TCGA-LUSC: 2

```

enter your choices one by one, when you are done, simply press "Enter":

List of therapies with at least 2 patients. We apply row 0, 1 and 3 to include cisplatin, the combination of carboplatin and paclitaxel and cases which got solely treated with carboplatin. In the following, every other parameter is requested.

With the next prompt, the default OUTPUT_PATH can be confirmed or replaced:

```

do you want to keep the default OUTPUT_PATH of:
/homes/biertruck/gabor/TREMSUCS
if so, press ENTER, if not, enter your custom output path:

```

In this example, we confirm the suggested OUTPUT_PATH and are asked to confirm or set the number of cores which shall be invoked into the analyses:

```

do you want to keep the default number of cores invoked of 1?
if so, press ENTER, if not, enter the number of cores:
40

```

We set the cores to 40 and then can decide which analysis approaches shall be triggered, per default, DESeq2 and metilene based biomarker predictions are produced:

```

which pipeline do you want to include into your analysis
press ENTER if DESeq2 and metilene (default) or
1 for DESeq2 or
2 for metilene

```

We confirm the default of those two analyses and can set the cutoff values, if we want to add those at all:

```

do you want to add one or multiple cutoffs?
it is recommend to choose cutoff values between 5 and 10 years
if not, just press ENTER, if so enter the coutoffs one by one:
5
8

```

Like the example set, we add here a cutoff of 5 and 8. Then the thresholds are requested:

```

do you want to add one or multiple thresholds?
it is recommend to choose threshold values which do not exceed a value of 50
if not, just press ENTER, if so enter the thresholds one by one:
5
10
20

```

We apply thresholds of 5, 10 and 20. All mandatory and optional parameters are set with that and are finally listed before the whole approach is started:

```

OUTPUT_PATH:      /homes/biertruck/gabor/TREMSUCS
PROJECT:          ['TCGA-CESC', 'TCGA-HNSC', 'TCGA-LUSC']
DRUGS:            ['carboplatin', 'carboplatin,paclitaxel', 'cisplatin']
pipelines executed: ['DESeq2', 'metilene']

```

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```
cores:          40
cutoff:         [0, 5, 8]
threshold:      [0, 5, 10, 20]
press ENTER to start or q to quit:
```

If something went wrong, you can quit now and start over, or of course start the analysis.

THE CUTOFF AND THRESHOLD PARAMETER:

5.1 Cutoff:

The cutoff parameter can be used to replace the vital status classification with a classification based on a minimum survival time. If the parameter is set, patients are assigned to a group depending on whether or not they survived longer then the specified value. In figure 1 an example is given for patients out of CESC, HNSC and LUSC without any limitation to treatment. With a cutoff of 8 years, 3 dead patients are grouped with the alive cohort (Figure 2). Applying a cutoff of 5 groups an additional 7 dead cases to the alive cohort (Figure 3). This parameter is applied before the analysis steps. It is possible to apply multiple cutoff values to one run. The alteration of the survival data of just a few patients can have a noticeable impact on the overall outcomes, but it should not exceed the maximum value of the survivaltime of the dead patients cohort, since then no change would be propagated. To figure out an appropriate custom value, you can first run the analysis with the default cutoff and refer to the created report. Within the patient_overview section, the survival data of the given cohort is shown. On the basis on the data plotted there, a second run can be started with a custom cutoff of interest. Already created results will not be overwritten but incorporated with the new ones based on the chosen cutoff. The final ranking gives then the same aggregation as if both, the default and the custom cutoff would have been started together, since the default is always calculated and incorporated within the analysis. The custom cutoff should also make medically sense, e.g., stating that an survialtime of one year shall be categorized as treatment success makes little sense and would not enhance the significance of the final results.

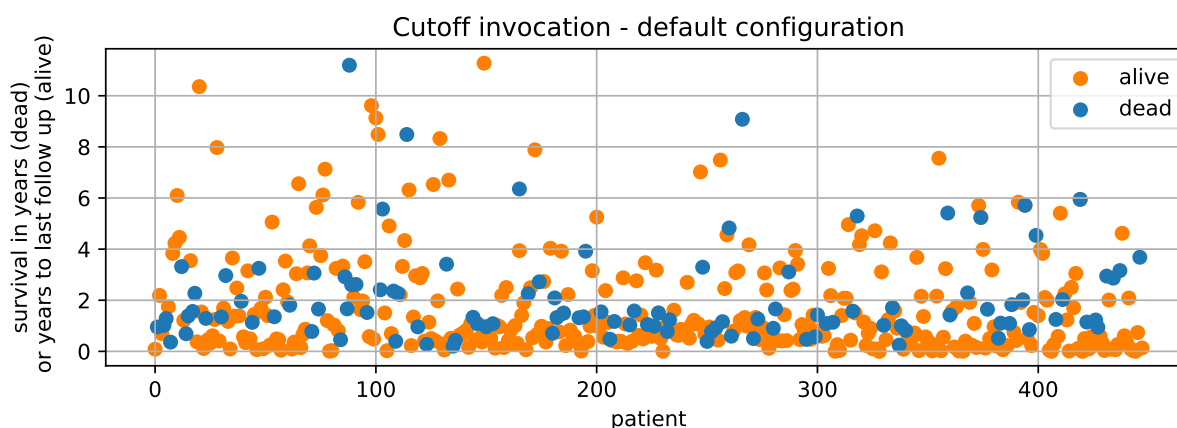


Fig. 1: Dead and alive grouping without a cutoff (default).

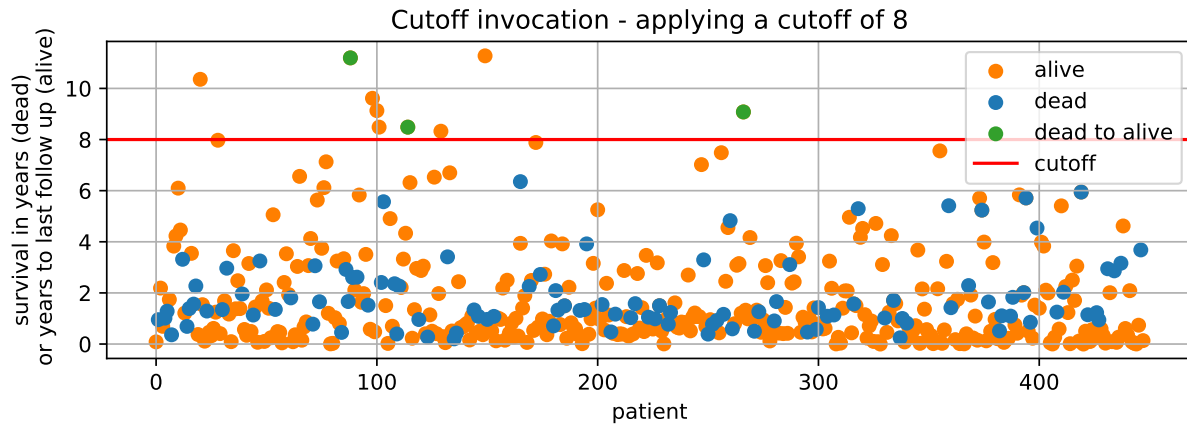


Fig. 2: Dead and alive grouping with a cutoff of 8.

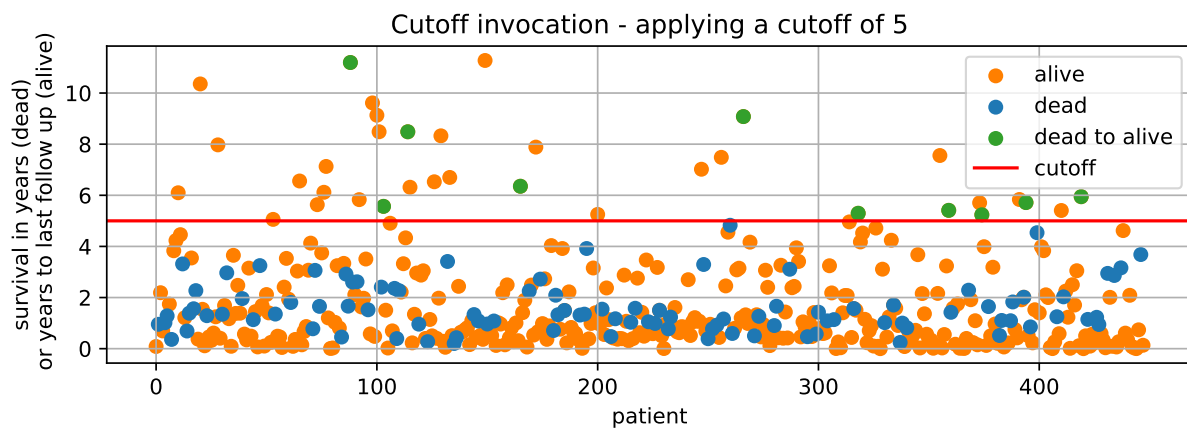


Fig. 3: Dead and alive grouping without a of 5.

5.2 Threshold:

The threshold parameter facilitates a modulation in the validation steps. Each previously identified marker, either a differentially methylated position or a differentially expressed gene of each patient, is grouped into the UP or DOWN regulated set depending on the mean of medians of all values. In the following, the Kaplan Meier estimations for each of these two groups are calculated. Incorporating values close to the mean of medians might be detrimental to the significance of the survival analyses. With the threshold, an upper and lower bound around the mean of medians is calculated (figure 4) and patient-data between those boundaries is excluded from the survival analysis. Here, the threshold gives the distance of the bounds from the mean of medians in percent of the mean of medians.

It is advised for the user not to exceed a threshold value of 20 since it is unlikely to gain any significance for the survival analysis with an exaggerated exclusion of patients.

In figure 5, the survival p-values of the 10 most significant genes for patients from the TCGA-CESC cohort with the therapeutic combination of carboplatin, carboplatin and paclitaxel (combined) and cisplatin are shown. With increasing threshold, incrementally improvement of the p-value for ENSG00000204187 (emphasized in red) is visible together with a higher difference of the life expectancies. Increasing the threshold will lower the size of the data base for p-value estimation, which can also result in increasing p-values. In figure 5, an example is the gene ENSG00000204832 emphasized in green.

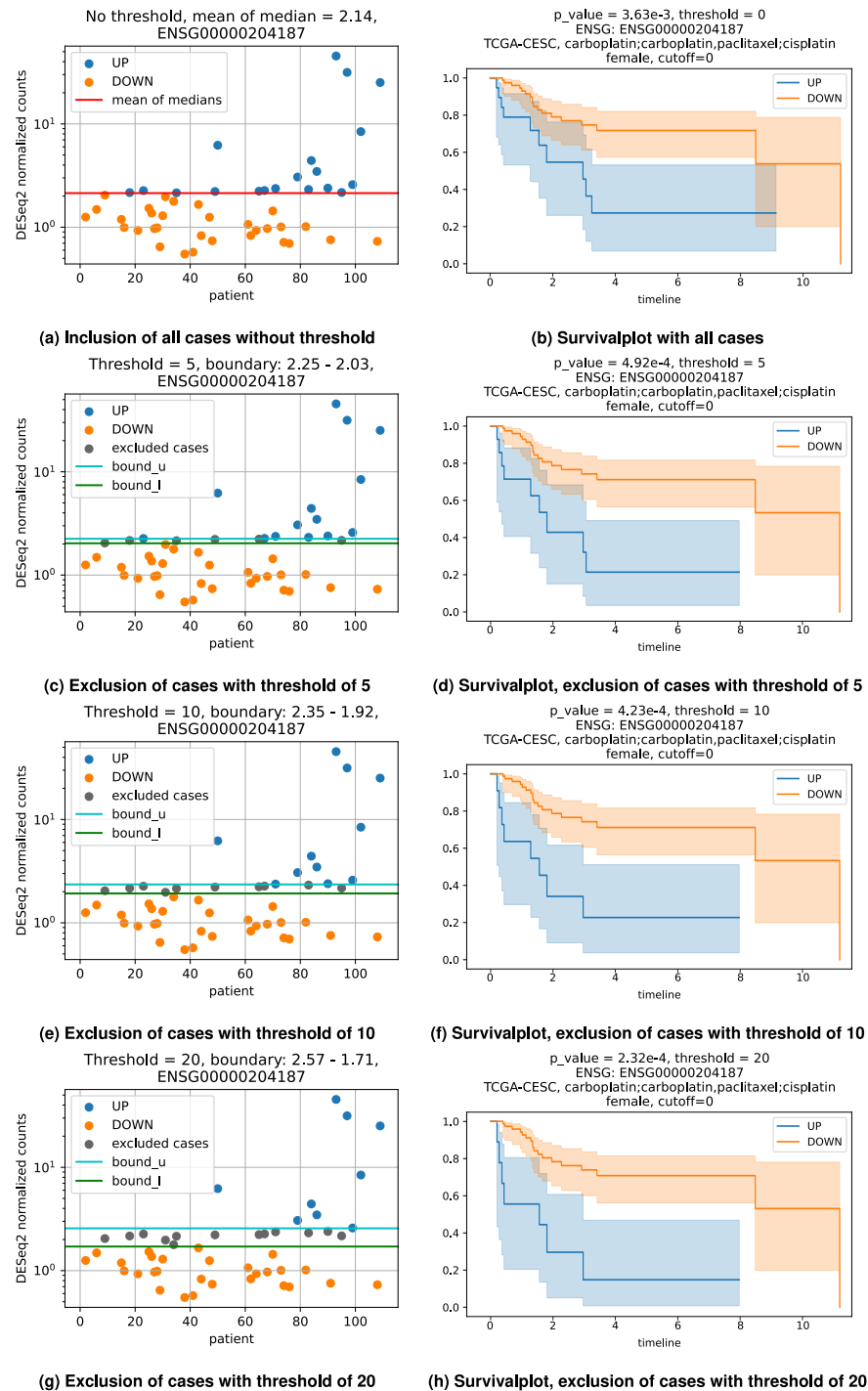


Fig. 4: Threshold example for ENSG00000204187. The panels on the left side show the exclusion of patients which are linked to the data in between the threshold bounds. On the right side the belonging Kaplan Meier plot is shown.

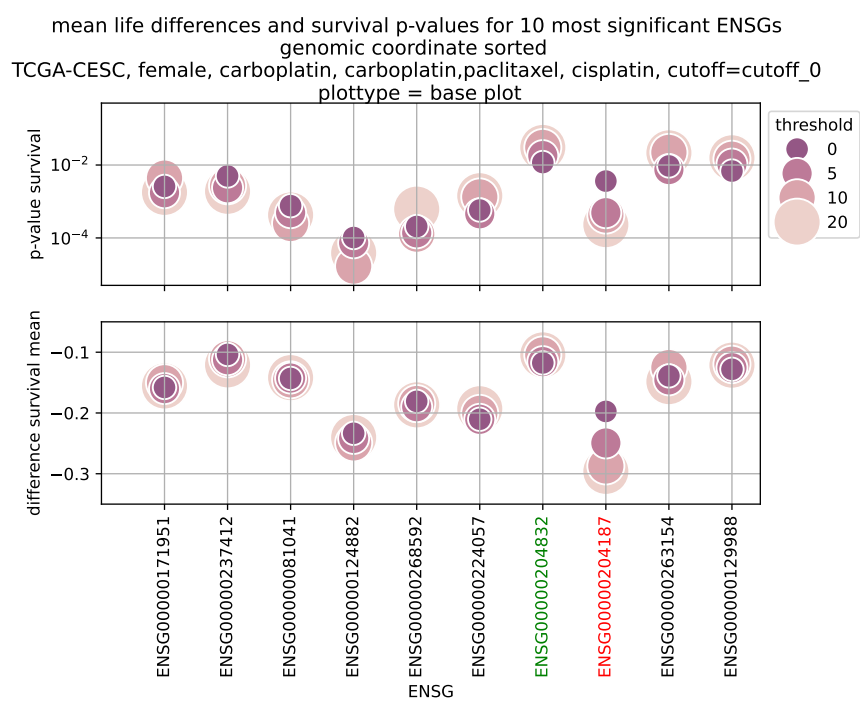


Fig. 5: Survival p-values and mean life differences for the first 10 most significant genes found by DESeq2, gathered from base plots, with a cutoff of 0. Succession of ENSGs is genomic coordinate wise.

Symbols

-C
TREMSUCS command line option, 7

-D
TREMSUCS command line option, 8

-N
TREMSUCS command line option, 8

--cores
TREMSUCS command line option, 7

--cutoff
TREMSUCS command line option, 7

--download
TREMSUCS command line option, 8

--drugs
TREMSUCS command line option, 7

--dryrun
TREMSUCS command line option, 8

--execute
TREMSUCS command line option, 8

--out_path
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--project
TREMSUCS command line option, 7

--threshold
TREMSUCS command line option, 8

--unlock
TREMSUCS command line option, 8

--version
TREMSUCS command line option, 8

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TREMSUCS command line option, 7

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TREMSUCS command line option, 7

-e
TREMSUCS command line option, 8

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-p
TREMSUCS command line option, 7

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TREMSUCS command line option, 8

-u

TREMSUCS command line option, 8

-v

TREMSUCS command line option, 8

T

TREMSUCS command line option

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-D, 8

-N, 8

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--drugs, 7

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--execute, 8

--out_path, 7

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-c, 7

-d, 7

-e, 8

-o, 7

-p, 7

-t, 8

-u, 8

-v, 8