TREMSUCS

Release 1.0

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CONTENTS:

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

CHAPTER

ONE

EXAMPLE REPORT:

An example report can be downloaded here. [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.

CHAPTER

TWO

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
```

CHAPTER

THREE

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 threapy 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. Enter none, one or several like: -C 5 -C 8

you can estimate an appropriate cutoff value by running your analysis wtih 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. 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 crashs, 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

CHAPTER

FOUR

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 Cystadenocarcinaoma
  7:     TCGA-LUAD           Lung Adenocarcinoma
  8:     TCGA-UCEC           Uterine Corpus Endometrial Carinoma
  9:     TCGA-KIRC           kindney 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":
```

Here are therapies listed where the maximum of a row is greater than 1. 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.

CHAPTER

FIVE

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 survivaltime of one year shall be categorized as treatment success makes little sense and would not enhance the significance of the final results.

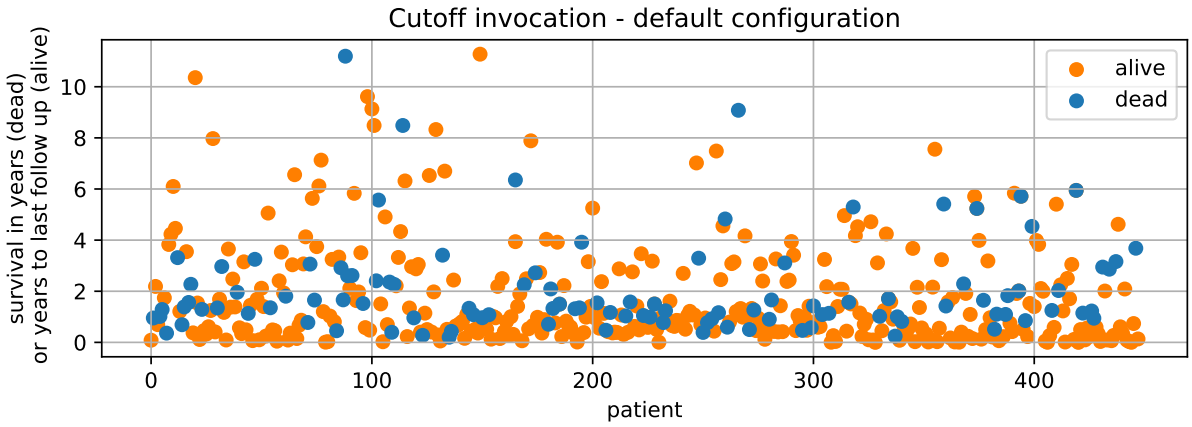


Fig. 1: Dead and alive grouping without a cutoff (default).

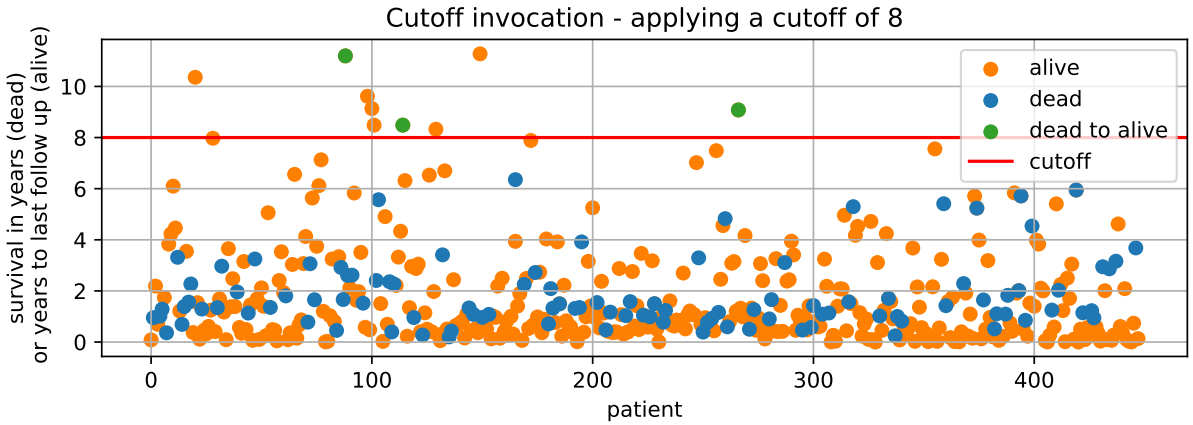


Fig. 2: Dead and alive grouping with a cutoff of 8.

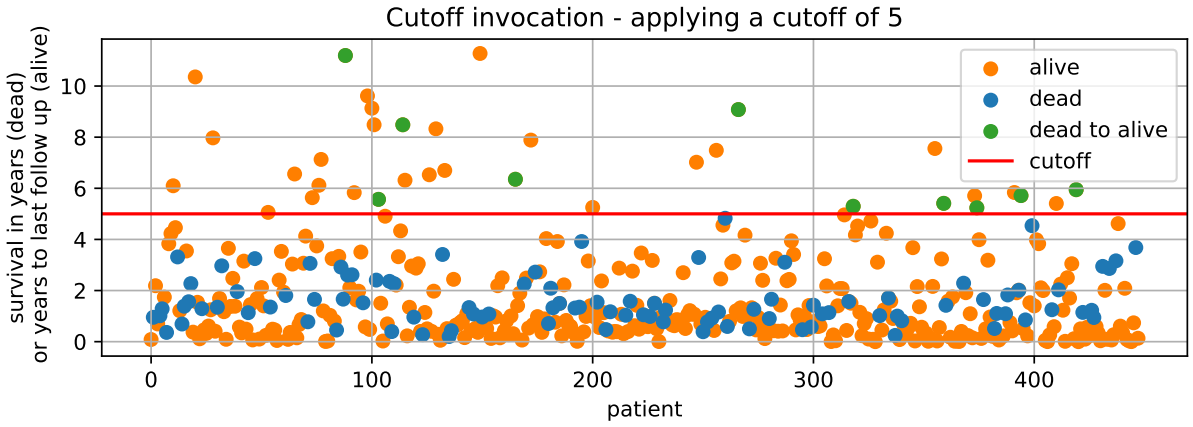


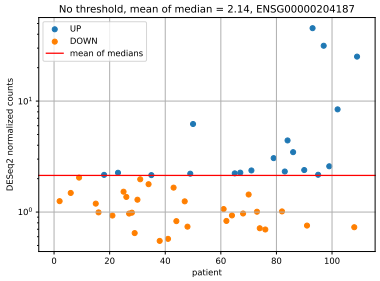
Fig. 3: Dead and alive grouping without a of 5.

5.2 Threshold:

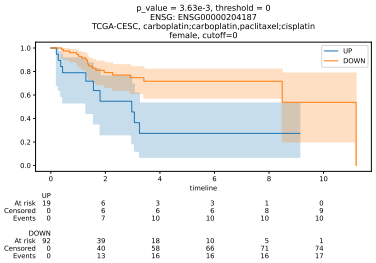
The threshold parameter facilitates a modulation in the validation steps. Each previously identified marker, either a differentially methylated position or a differentially expressed gene of each patient, is grouped into the UP or DOWN regulated set depending on the mean of medians of all values. In 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.

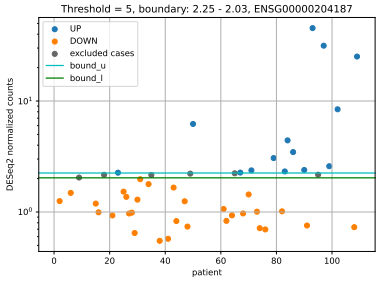
In figure 5, the survival p-values of the 10 most significant genes for patients from the TCGA-CESC cohort with the therapeutic combination of carboplatin, carboplatin and paclitaxel (combined) and cisplatin are shown. With increasing threshold, incrementally improvement of the p-value for ENSG00000204187 (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.



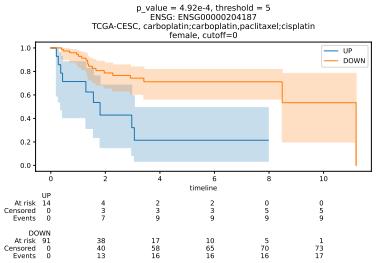
(a) Inclusion of all cases without threshold



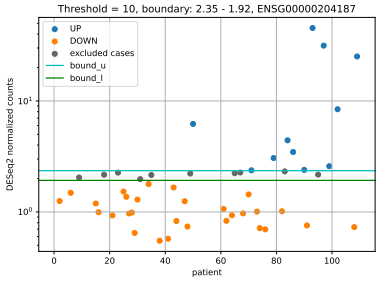
(b) Survivalplot with all cases



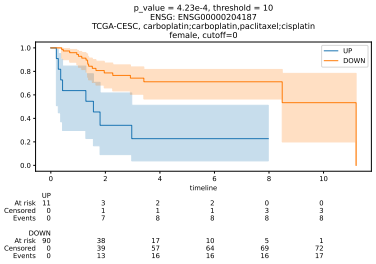
(c) Exclusion of cases with threshold of 5



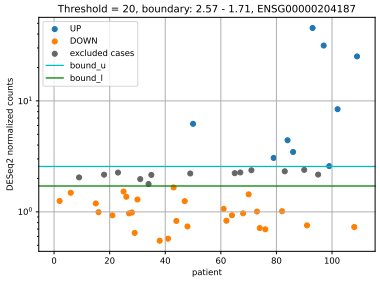
(d) Survivalplot, exclusion of cases with threshold of 5



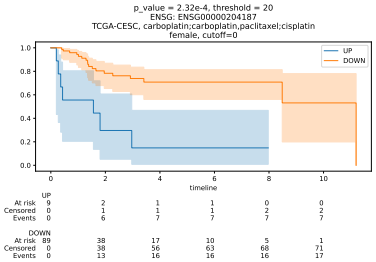
(e) Exclusion of cases with threshold of 10



(f) Survivalplot, exclusion of cases with threshold of 10



(g) Exclusion of cases with threshold of 20



(h) Survivalplot, exclusion of cases with threshold of 20

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.

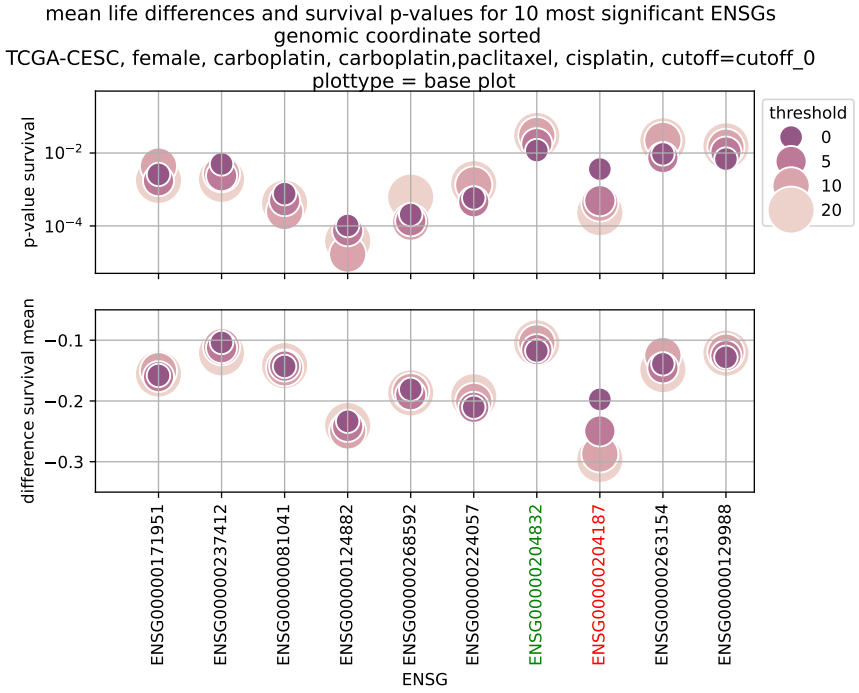


Fig. 5: Survival p-values and mean life differences for the first 10 most significant genes found by DESeq2, gathered from base plots, with a cutoff of 0. Succession of ENSGs is genomic coordinate wise.

INDEX

Symbols

T