Proteomics data analysis: heart

Lieven Clement

statOmics, Ghent University (https://statomics.github.io)

Contents

1 Background

Researchers have assessed the proteome in different regions of the heart for 3 patients (identifiers 3, 4, and 8). For each patient they sampled the left atrium (LA), right atrium (RA), left ventricle (LV) and the right ventricle (RV). The data are a small subset of the public dataset PXD006675 on PRIDE.

Suppose that researchers are mainly interested in comparing the ventricular to the atrial proteome. Particularly, they would like to compare the left atrium to the left ventricle, the right atrium to the right ventricle, the average ventricular vs atrial proteome and if ventricular vs atrial proteome shifts differ between left and right heart region.
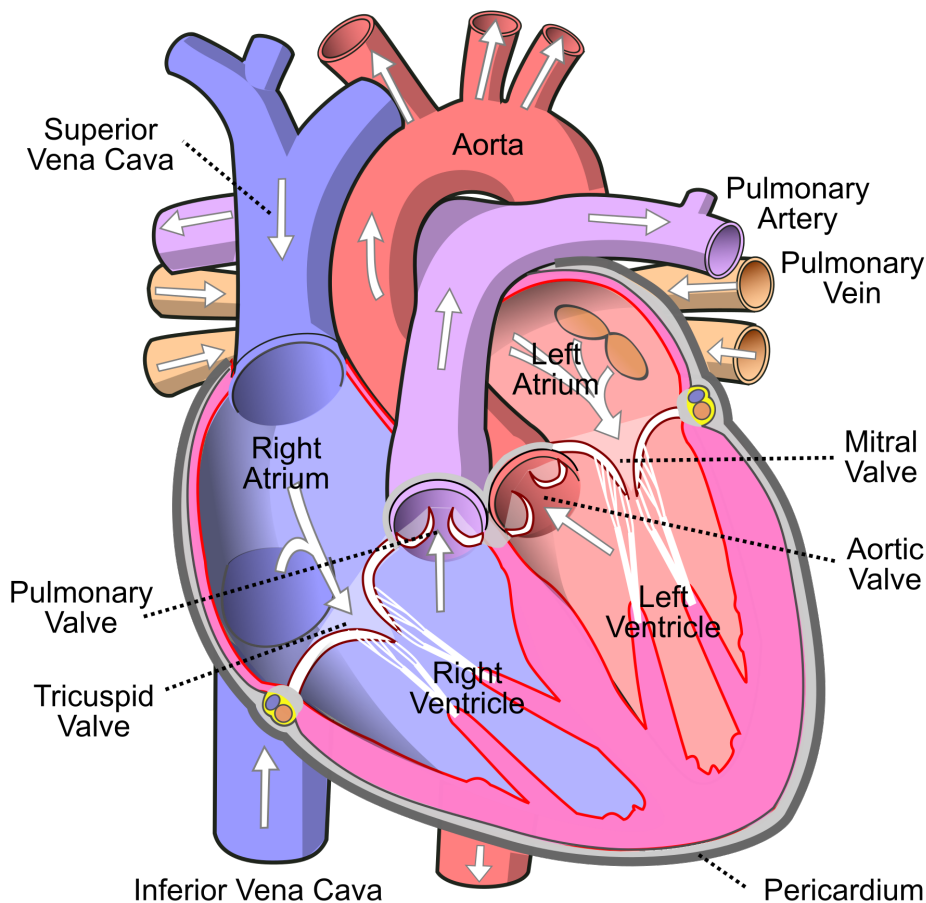
Figure 1: Representation of the heart

2 Data

We first import the peptides.txt file. This is the file that contains your peptide-level intensities. For a MaxQuant search [6], this peptides.txt file can be found by default in the "path_to_raw_files/combined/txt/" folder from the MaxQuant output, with "path_to_raw_files" the folder where raw files were saved. In this tutorial, we will use a MaxQuant peptides file from MaxQuant that can be found in the data tree of the SGA2020 github repository https://github.com/statOmics/SGA2020/tree/data/quantification/heart .

2.1 Data import

Click to see background and code

To import the data we use the `QFeatures` package.

We generate the object peptideRawFile with the path to the peptideRaws.txt file. Using the `grepEcols` function, we find the columns that contain the expression data of the peptideRaws in the peptideRaws.txt file.

```
library(tidyverse)
library(limma)
library(QFeatures)
library(msqrob2)
library(plotly)

peptidesFile <- "https://raw.githubusercontent.com/statOmics/PDA21/data/quantification/heart/peptides.t

ecols <- grep("Intensity\\.", names(read.delim(peptidesFile)))

pe <- readQFeatures(
  table = peptidesFile,
  fnames = 1,
  ecol = ecols,
  name = "peptideRaw", sep="\t")

pe
```

```
## An instance of class QFeatures containing 1 assays:
##  [1] peptideRaw: SummarizedExperiment with 31319 rows and 12 columns
```

```
pe[["peptideRaw"]]
```

```
## class: SummarizedExperiment
## dim: 31319 12
## metadata(0):
## assays(1): ''
## rownames(31319): AAAAAAAAAK AAAAAAAAEQQSSNGPVK ... YYTPVPCESATAK
##   YYTYLIMNK
## rowData names(91): Sequence N.term.cleavage.window ...
##   Oxidation..M..site.IDs MS.MS.Count
## colnames(12): Intensity.LA3 Intensity.LA4 ... Intensity.RV4
##   Intensity.RV8
## colData names(0):
```

We will make use from data wrangling functionalities from the tidyverse package. The %>% operator allows us to pipe the output of one function to the next function.

```
colData(pe)$location <- substr(
  colnames(pe[["peptideRaw"]]),
  11,
  11) %>%
  unlist %>%
```

```
    as.factor

colData(pe)$tissue <- substr(
    colnames(pe[["peptideRaw"]]),
    12,
    12) %>%
    unlist %>%
    as.factor

colData(pe)$patient <- substr(
  colnames(pe[["peptideRaw"]]),
  13,
  13) %>%
  unlist %>%
  as.factor
```

We calculate how many non zero intensities we have per peptide and this will be useful for filtering.

```
rowData(pe[["peptideRaw"]])$nNonZero <- rowSums(assay(pe[["peptideRaw"]]) > 0)
```

Peptides with zero intensities are missing peptides and should be represent with a `NA` value rather than `0`.

```
pe <- zeroIsNA(pe, "peptideRaw") # convert 0 to NA
```

2.2   Data exploration

Click to see background and code

63% of all peptide intensities are missing and for some peptides we do not even measure a signal in any sample. The missingness is similar across samples.

3   Preprocessing

Click to see background and code

This section preforms standard preprocessing for the peptide data. This include log transformation, filtering and summarisation of the data.

3.1   Log transform the data

```
pe <- logTransform(pe, base = 2, i = "peptideRaw", name = "peptideLog")
limma::plotDensities(assay(pe[["peptideLog"]]))
```
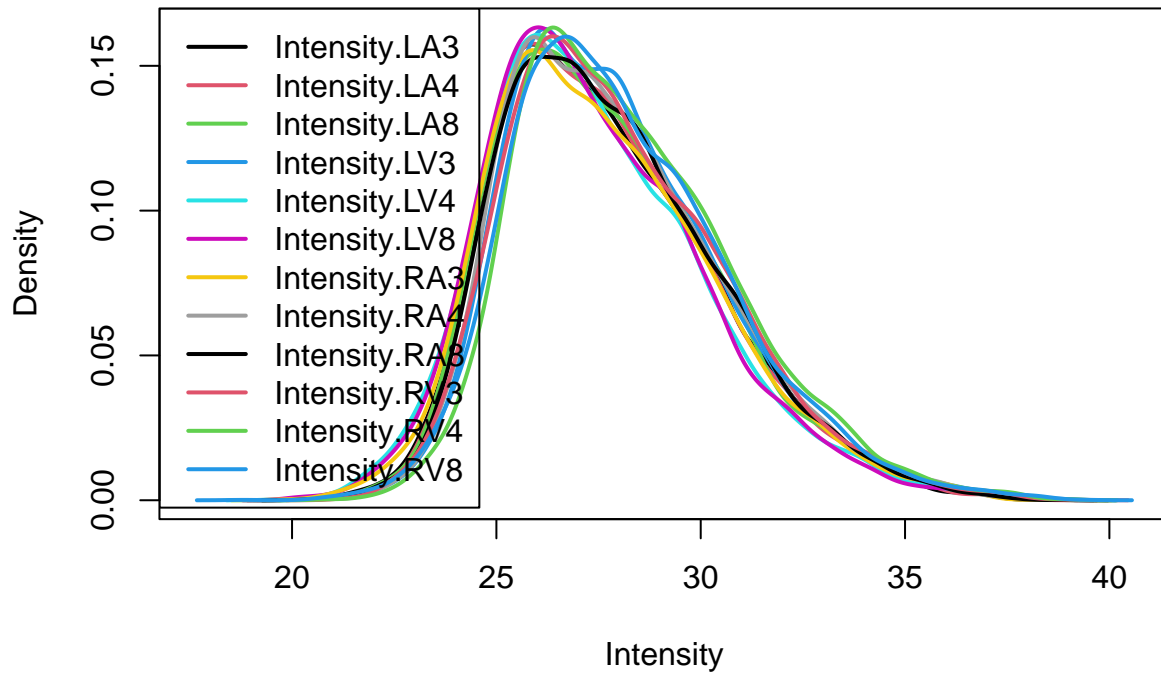
3.2 Filtering

Click to see background and code

3.2.1 Handling overlapping protein groups

In our approach a peptide can map to multiple proteins, as long as there is none of these proteins present in a smaller subgroup.

```
pe <- filterFeatures(pe, ~ Proteins %in% smallestUniqueGroups(rowData(pe[["peptideLog"]])$Proteins))
```

3.2.2 Remove reverse sequences (decoys) and contaminants

We now remove the contaminants, peptides that map to decoy sequences, and proteins which were only identified by peptides with modifications.

First look to the names of the variables for the peptide features

```
pe[["peptideLog"]] %>%
  rowData %>%
  names
```

```
##  [1] "Sequence"               "N.term.cleavage.window"
```

```
##  [3] "C.term.cleavage.window"   "Amino.acid.before"
##  [5] "First.amino.acid"         "Second.amino.acid"
##  [7] "Second.last.amino.acid"   "Last.amino.acid"
##  [9] "Amino.acid.after"         "A.Count"
## [11] "R.Count"                  "N.Count"
## [13] "D.Count"                  "C.Count"
## [15] "Q.Count"                  "E.Count"
## [17] "G.Count"                  "H.Count"
## [19] "I.Count"                  "L.Count"
## [21] "K.Count"                  "M.Count"
## [23] "F.Count"                  "P.Count"
## [25] "S.Count"                  "T.Count"
## [27] "W.Count"                  "Y.Count"
## [29] "V.Count"                  "U.Count"
## [31] "O.Count"                  "Length"
## [33] "Missed.cleavages"         "Mass"
## [35] "Proteins"                 "Leading.razor.protein"
## [37] "Start.position"           "End.position"
## [39] "Gene.names"               "Protein.names"
## [41] "Unique..Groups."          "Unique..Proteins."
## [43] "Charges"                  "PEP"
## [45] "Score"                    "Identification.type.LA3"
## [47] "Identification.type.LA4"  "Identification.type.LA8"
## [49] "Identification.type.LV3"  "Identification.type.LV4"
## [51] "Identification.type.LV8"  "Identification.type.RA3"
## [53] "Identification.type.RA4"  "Identification.type.RA8"
## [55] "Identification.type.RV3"  "Identification.type.RV4"
## [57] "Identification.type.RV8"  "Fraction.Average"
## [59] "Fraction.Std..Dev."       "Fraction.1"
## [61] "Fraction.2"               "Fraction.3"
## [63] "Fraction.4"               "Fraction.5"
## [65] "Fraction.6"               "Fraction.7"
## [67] "Fraction.8"               "Fraction.100"
## [69] "Experiment.LA3"           "Experiment.LA4"
## [71] "Experiment.LA8"           "Experiment.LV3"
## [73] "Experiment.LV4"           "Experiment.LV8"
## [75] "Experiment.RA3"           "Experiment.RA4"
## [77] "Experiment.RA8"           "Experiment.RV3"
## [79] "Experiment.RV4"           "Experiment.RV8"
## [81] "Intensity"                "Reverse"
## [83] "Potential.contaminant"    "id"
## [85] "Protein.group.IDs"        "Mod..peptide.IDs"
## [87] "Evidence.IDs"             "MS.MS.IDs"
## [89] "Best.MS.MS"               "Oxidation..M..site.IDs"
## [91] "MS.MS.Count"              "nNonZero"
```

No information on decoys.

```
pe <- filterFeatures(pe,~ Potential.contaminant != "+")
```

3.2.3 Drop peptides that were only identified in one sample

We keep peptides that were observed at last twice.

```
pe <- filterFeatures(pe,~nNonZero >= 2)
nrow(pe[["peptideLog"]])
```

```
## [1] 17432
```

We keep 17432 peptides after filtering.

3.3 Normalize the data

Click to see background and code

```
pe <- normalize(pe,
                i = "peptideLog",
                name = "peptideNorm",
                method = "center.median")
```

3.4 Explore normalized data

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After normalisation the density curves for all samples are comparable.

```
limma::plotDensities(assay(pe[["peptideNorm"]]))
```
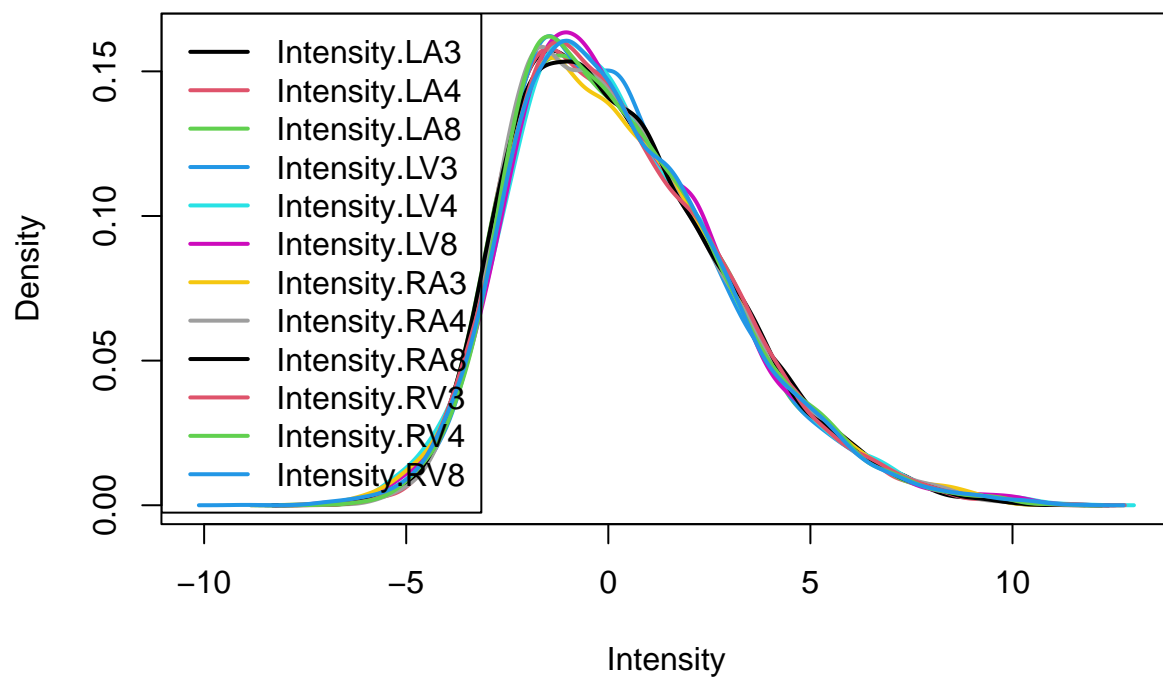
This is more clearly seen is a boxplot.

```
boxplot(assay(pe[["peptideNorm"]]), col = palette()[-1],
        main = "Peptide distribtutions after normalisation", ylab = "intensity")
```

Peptide distribtutions after normalisation
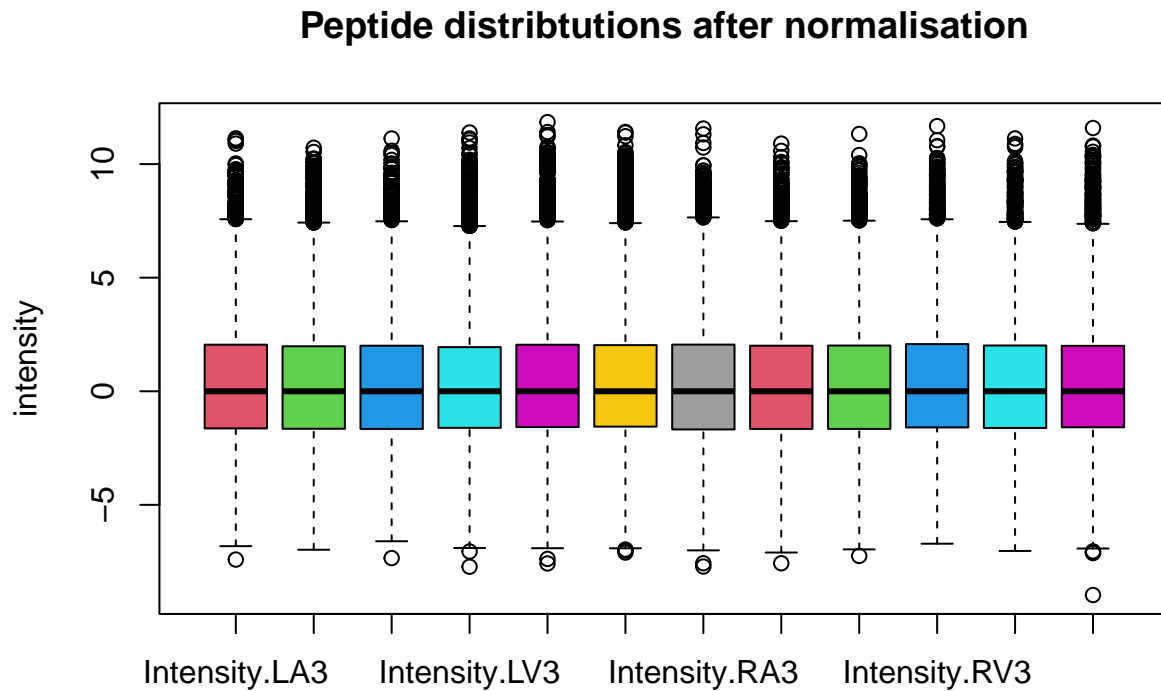
We can visualize our data using a Multi Dimensional Scaling plot, eg. as provided by the limma package.

```
limma::plotMDS(assay(pe[["peptideNorm"]]),
  col = colData(pe)$location:colData(pe)$tissue %>%
    as.numeric,
  labels = colData(pe) %>%
    rownames %>%
    substr(start = 11, stop = 13)
  )
```
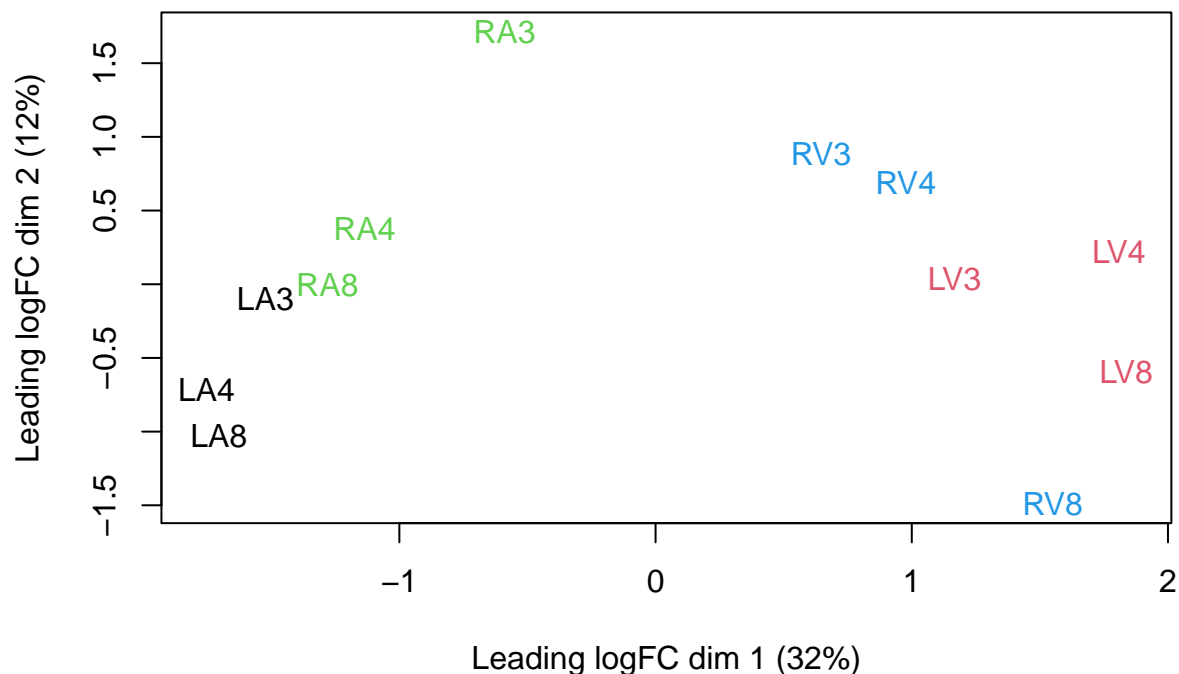
The first axis in the plot is showing the leading log fold changes (differences on the log scale) between the samples.

3.5   Summarization to protein level

Click to see background and code

We use robust summarization in aggregateFeatures. This is the default workflow of aggregateFeatures so you do not have to specifiy the argument `fun`. However, because we compare methods we have included the `fun` argument to show the summarization method explicitely.

```
pe <- aggregateFeatures(pe,
 i = "peptideNorm",
 fcol = "Proteins",
 na.rm = TRUE,
 name = "proteinRobust",
 fun = MsCoreUtils::robustSummary)
```

```
## Your quantitative and row data contain missing values. Please read the
## relevant section(s) in the aggregateFeatures manual page regarding the
## effects of missing values on data aggregation.
```

```
plotMDS(assay(pe[["proteinRobust"]]),
  col = colData(pe)$location:colData(pe)$tissue %>%
    as.numeric,
```

9

```
    labels = colData(pe) %>%
      rownames %>%
      substr(start = 11, stop = 13)
)
```


4 Data Analysis

4.1 Estimation

We model the protein level expression values using `msqrob`. By default `msqrob2` estimates the model parameters using robust regression.

```
pe <- msqrob(
  object = pe,
  i = "proteinRobust",
  formula = ~ location*tissue + patient)
```

4.2 Inference

Explore Design

```
library(ExploreModelMatrix)
VisualizeDesign(colData(pe),~ location*tissue + patient)$plotlist
```

$`location = L`


```
##
## $`location = R`
```
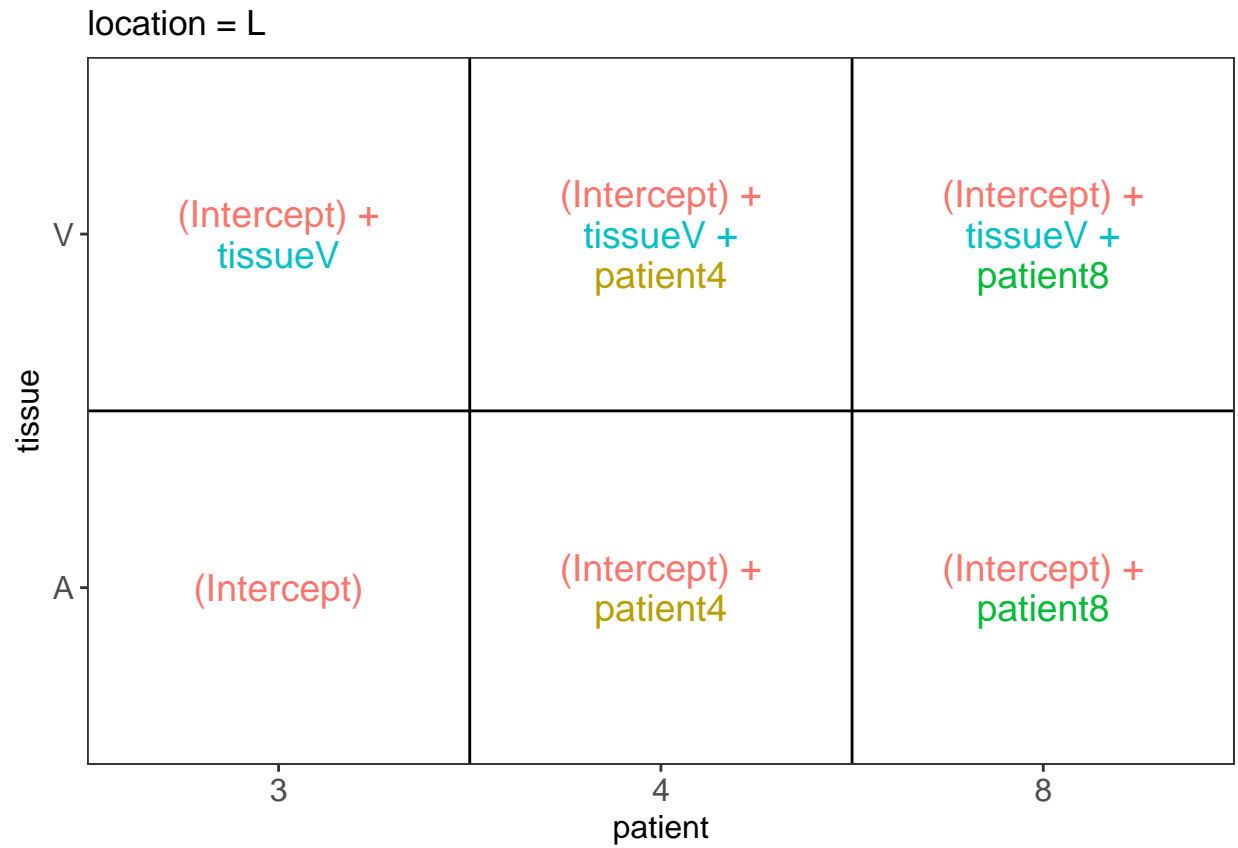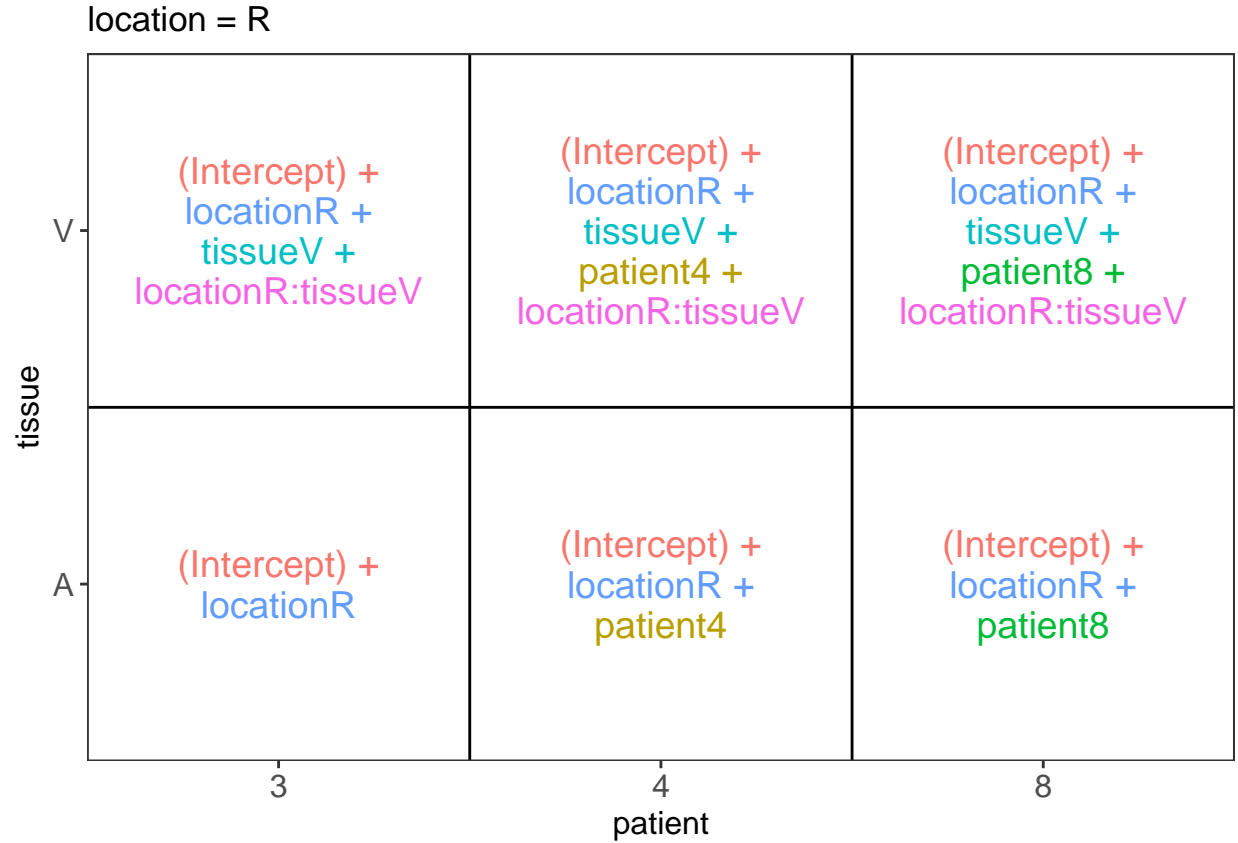
```r
design <- model.matrix(~location*tissue + patient, data = colData(pe))
L <- makeContrast(
  c(
    "tissueV = 0",
    "tissueV + locationR:tissueV = 0",
    "tissueV + 0.5*locationR:tissueV = 0","locationR:tissueV = 0"),
  parameterNames = colnames(design)
  )


pe <- hypothesisTest(object = pe, i = "proteinRobust", contrast = L, overwrite=TRUE)
```

4.3 Evaluate results contrast $\log_2 FC_{V-A}^{L}$

4.3.1 Volcano-plot

```r
volcanoLeft <- ggplot(rowData(pe[["proteinRobust"]])$"tissueV",
              aes(x = logFC, y = -log10(pval), color = adjPval < 0.05)) +
 geom_point(cex = 2.5) +
 scale_color_manual(values = alpha(c("black", "red"), 0.5)) + theme_minimal()
volcanoLeft
```
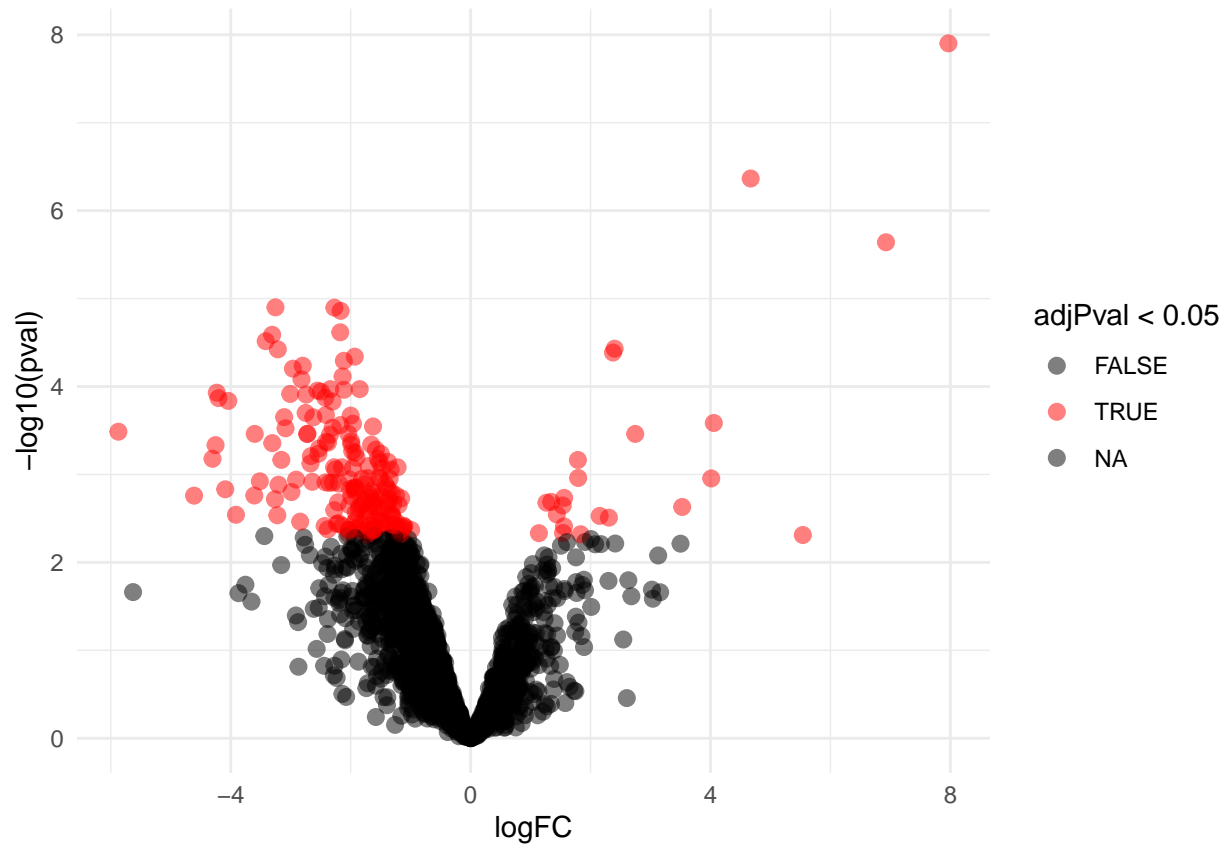
4.3.2   Heatmap

We first select the names of the proteins that were declared signficant.

```
sigNamesLeft <- rowData(pe[["proteinRobust"]])$tissueV %>%
 rownames_to_column("proteinRobust") %>%
 filter(adjPval<0.05) %>%
 pull(proteinRobust)
heatmap(assay(pe[["proteinRobust"]])[sigNamesLeft, ])
```
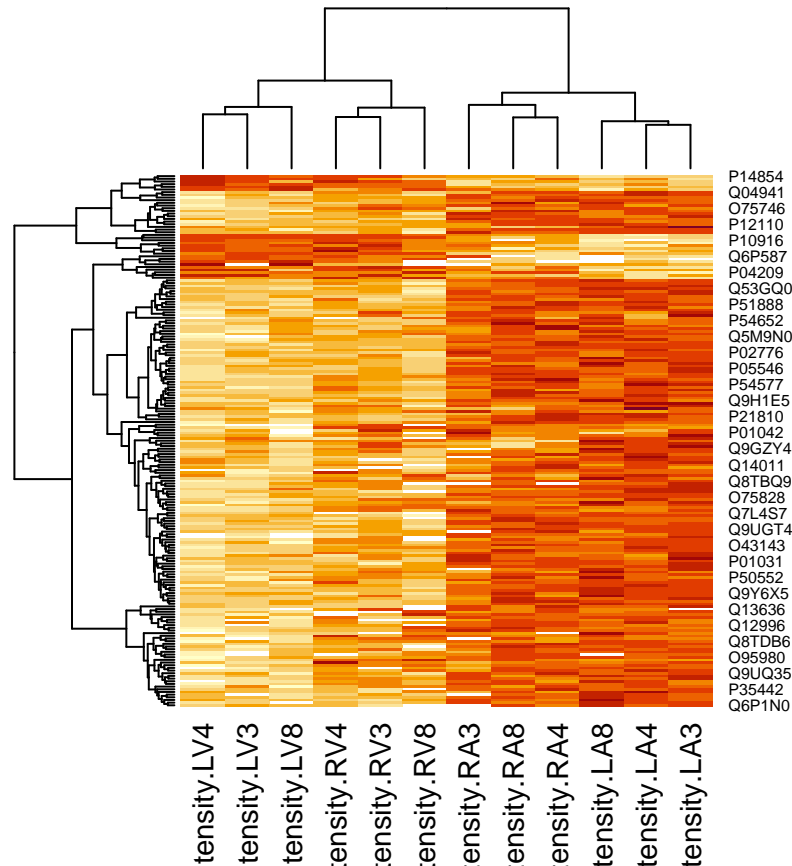
There are 199 proteins significantly differentially expressed at the 5% FDR level.

```r
rowData(pe[["proteinRobust"]])$tissueV %>%
  cbind(.,rowData(pe[["proteinRobust"]])$Protein.names) %>%
  na.exclude %>%
  filter(adjPval<0.05) %>%
  arrange(pval)  %>%
  knitr::kable(.)
```

	logFC	se	df	t	pval	adjPval	rowData(pe[["proteinRobust"]])$Protein.names
P08590	0.5707054	0.0437918	42	12.9370	0.0000	0.0000	Myosin light chain 3
P12883	0.3669083	0.0271601	28	13.5648	0.0000	0.0000	Myosin-7
P10916	0.6026005	0.0547413	47	11.0892	0.0000	0.0023	Myosin regulatory light chain 2, ventricular/cardiac muscle isoform
Q6UW-Y5	0.3829893	3.2536428	60	8.497596	0.0000	0.0246705	Olfactomedin-like protein 1
O75368	0.2719289	2.2715945	60	8.365932	0.0000	0.0246705	SH3 domain-binding glutamic acid-rich-like protein
P46821	0.2639267	2.1668803	47	8.230871	0.0000	0.0346705	Microtubule-associated protein 1B;MAP1B heavy chain;MAP1 light chain LC1
O95865	0.2826267	2.1732192	47	7.689536	0.0000	0.0465473	N(G),N(G)-dimethylarginine dimethylaminohydrolase 2
Q8N474	0.3807497	3.3090235	81	8.697787	0.0000	0.0565478	Secreted frizzled-related protein 1
Q9ULL5-3	0.3998746	3.4165405	27	8.543546	0.0000	0.0565882	Proline-rich protein 12

	logFC	se	df	t	pval	adjP	rowData(pe[["proteinRobust"]])$Protein.names

P148544010.12065786307.1654.8.6400.370.37060621 Cytochrome c oxidase subunit 6B1

P21810   0.4218.756796 0.00003.76B6glycan
      3.2145420   7.623194

O948757400.18088.189041.123001.00.00169621 Sorbin and SH3 domain-containing protein 2
10

P05546   0.2699.727.2661 0.000040.5871H533parin cofactor 2
      1.9318824   7.155698

P29622   0.3013.4357147 0.00005.10790044statin
      2.1134793   7.013527

Q16647   0.3962.4038244 0.00005.807B583tacyclin synthase
      2.7991521   7.064217

P02452   0.3999.2522268 0.000062.70961lagen alpha-1(I) chain
      2.9630153   7.409110

P51884   0.3169.757371 0.00007.68L803ican
      2.1343314   6.752566

Q8TBQ9 0.40686.6228892 0.00008.82B870ein kish-A
      2.8189960   6.942232

P07451   0.2896.2367147 0.0000107.96523onic anhydrase 3
      1.8489373   6.383889

P36955   0.3358.4913555 0.0000107.9B523ment epithelium-derived factor
      2.3377901   6.964984

P00325   0.3236.2874001 0.0000109.94523hol dehydrogenase 1B
      2.1119462   6.525931

Q9UBG0 0.3974.702396 0.0000109.6523pe mannose receptor 2
      2.5547510   6.427531

P24844   0.3939.3513934 0.0000109.6523sin regulatory light polypeptide 9
      2.4962161   6.351447

P23083   0.5732.3697147 0.0000109.5123avy chain V-I region V35
      4.2325442   7.383742

P51888   0.4007.8459338 0.0000209.52Bargin
      3.0081810   7.511353

Q15113   0.4329.0685821 0.0000209.B523ollagen C-endopeptidase enhancer 1
      2.7424988   6.336551

Q53GQ0 0.3909.2527570 0.0000309.8288-long-chain 3-oxoacyl-CoA reductase
      2.4232423   6.203561

Q06828   0.6622.5069004 0.0000309.B288omodulin
      4.2030186   6.346569

P13533   0.6482.4098373 0.0000409.M636sin-6
      4.0404076   6.232876

P35442   0.3523.307147 0.0000409.U636mbospondin-2
      2.3010500   6.535012

P08294   0.4255.964617 0.0000409.BX065acellular superoxide dismutase [Cu-Zn]
      2.7503241   6.463543

Q96LL9 0.3982.759147 0.0002.0163D065J homolog subfamily C member 30
      2.4127080   6.058855

P18428   0.3389.5378659 0.0002.0423D065polysaccharide-binding protein
      1.9996058   5.905830

P36021   0.5038.067147 0.0002.293M065ocarboxylate transporter 8
      3.1085951   6.170218

Q9UL18 0.4093.688346 0.0002.243D065ein argonaute-1
      2.6225313   6.406296

Q92508550.25737.3187.2787.00.0260114B405o-type mechanosensitive ion channel component 1

	logFC	se	df	t	pval	adjPval	rowData(pe[["proteinRobust"]])$Protein.names
P46060	0.3258	9.3567147		6.048	0.0000	R405	GTPase-activating protein 1
	1.9620904			6.019876			
P02743	0.3849	9.3027147			0.0002	0.04156795	Serum amyloid P-component;Serum amyloid P-component(1-203)
	2.1700557			5.639288			
Q14764	0.2898	9.3367147			0.0002	0.04781124	Major vault protein
	1.6267934			5.611689			
Q9UGT4	0.4078	6.031824			0.0002	0.04781124	Sushi domain-containing protein 2
	2.3006162			5.640659			
P05997	0.5408	6.2607173			0.0002	0.04896524	Collagen alpha-2(V) chain
	3.0856148			5.707478			
Q8WWA0	0.9875	5.032804			0.0003	0.05061420	Lectin-1
	5.8725166			5.946832			
Q9P2B2	0.3739	9.3367147			0.0003	0.04811420	Prostaglandin F2 receptor negative regulator
	2.0393915			5.467480			
O43677	0.4718	6.030596			0.0003	0.04811420	NADH dehydrogenase [ubiquinone] 1 subunit C1, mitochondrial
	2.7199107			5.769748			
O60760	0.6218	6.3707147			0.0003	0.04819420	Hematopoietic prostaglandin D synthase
	3.5990151			5.791158			
Q9UBB5	0.4507	9.3368761		4.925480	0.0003	0.04819420	Methyl-CpG-binding domain protein 2
Q9BW30	0.4898	9.299247			0.0003	0.04819420	Tubulin polymerization-promoting protein family member 3
	2.7245919			5.571480			
P40261	0.4269	9.220700			0.0003	0.04819420	Nicotinamide N-methyltransferase
	2.3445730			5.491570			
P00748	0.3694	4.783103			0.0004	0.05207497	Coagulation factor XII;Coagulation factor XIIa heavy chain;Beta-factor XIIa part 1;Coagulation factor XIIa light chain
	1.9914680			5.389946			
Q9NZ01	0.4362	6.032777			0.0004	0.05147497	Very-long-chain enoyl-CoA reductase
	2.4109216			5.527049			
O14967	0.4288	9.527297			0.0004	0.05147497	Calmegin
	2.3785961			5.544702			
Q92736-2	0.6048	6.673491			0.0004	0.05147497	Ryanodine receptor 2
	3.3064395			5.473615			
P12110	0.3538	9.619020			0.0004	0.05687497	Collagen alpha-2(VI) chain
	1.9881397			5.630590			
Q07954	0.3159	9.3707147			0.0004	0.05737497	Low-density lipoprotein receptor-related protein 1;Low-density lipoprotein receptor-related protein 1 85 kDa subunit;Low-density lipoprotein receptor-related protein 1 515 kDa subunit;Low-density lipoprotein receptor-related protein 1 intracellular domain
	1.6573532			5.258970			
O00180	0.7668	8.367147			0.0004	0.05427497	Potassium channel subfamily K member 1
	4.2526703			5.545857			
O95980	0.4553	7.846506			0.0005	0.06138950	Reversion-inducing cysteine-rich protein with Kazal motifs
	2.5277905			5.550964			
P31994-2	0.3019	4.879055			0.0005	0.05228576	Low affinity immunoglobulin gamma Fc region receptor II-b
	1.5795166			5.239074			
O00264	0.3789	4.054168			0.0005	0.05290097	Membrane-associated progesterone receptor component 1
	1.9731043			5.213653			
Q8TBP6	0.3698	9.078834			0.0005	0.05670302	Solute carrier family 25 member 40
	1.9233155			5.200951			
Q14195-2	0.4959	9.220176			0.0005	0.05819392	Dihydropyrimidinase-related protein 3
	2.5380012			5.117743			
Q8WZA9	0.2915	6.078039			0.0005	0.05219392	Immunity-related GTPase family Q protein
	1.4960398			5.130437			

logFC	se	df	t	pval	adjP	rowData(pe[["proteinRobust"]])$Protein.names
Q9BXN1	0.5178	9.959110	2.6634064		0.0006.0202775 5.141743	Sorcin
O43464	0.3759	9.443486	1.9028432		0.0006.0205653 5.071620	Serine protease HTRA2, mitochondrial
P01699	0.6888	9.228678	4.3035389		0.0006.6207537 6.251575	Ig lambda chain V-I region VOR
P41240	0.3048	9.267098	1.5466953		0.0006.6207537 5.079582	Tyrosine-protein kinase CSK
Q9GZY4	0.5785	9.712737	3.1557642		0.0006.8207601 5.454783	Cytochrome c oxidase assembly factor 1 homolog
P06858	0.5789	9.249207	2.6635		0.0006.8207601	Lipoprotein lipase
Q8NAT1	0.2787	9.207624	1.3792038		0.0007.0721514 4.948303	Protein O-linked-mannose beta-1,4-N-acetylglucosaminyltransferase 2
P04083	0.3089	9.367147	1.5201990		0.0007.2851454 4.924495	Annexin A1
P02747	0.4696	9.090471	2.6697776		0.0007.5217901 5.684373	Complement C1q subcomponent subunit C
Q92621	0.3507	9.624182	1.7036447		0.0008.0225580 4.857201	Nuclear pore complex protein Nup205
Q9NY15	0.4439	9.350476	2.1436460		0.0008.2825680 4.837723	Stabilin-1
A6NMZ7	0.4709	9.367147	2.2762344		0.0008.2922658 4.833535	Collagen alpha-6(VI) chain
O14980	0.2518	9.307147	1.2169774		0.0008.3022580 4.832729	Exportin-1
P02775	0.4079	9.367147	1.9622566		0.0008.4022580 4.819887	Platelet basic protein;Connective tissue-activating peptide III;TC-2;Connective tissue-activating peptide III(1-81);Beta-thromboglobulin;Neutrophil-activating peptide 2(74);Neutrophil-activating peptide 2(73);Neutrophil-activating peptide 2;TC-1;Neutrophil-activating peptide 2(1-66);Neutrophil-activating peptide 2(1-63)
Q96C86	0.3026	9.095986	1.4762676		0.0008.4022680 4.877936	m7GpppX diphosphatase
Q92604	0.4679	9.259173	2.2504519		0.0008.7522047 4.810036	Acyl-CoA:lysophosphatidylglycerol acyltransferase 1
P48681	0.2789	9.094182	1.3334013		0.0008.7922047 4.793288	Nestin
P50552	0.3238	9.053622	1.5288544		0.0010.7806764 4.730872	Vasodilator-stimulated phosphoprotein
P24311	0.1793	9.366321	4.6793		0.0010.9026764	Cytochrome c oxidase subunit 7B, mitochondrial
Q7L4S7	0.3243	9.409840	1.7124303		0.0010.9026764 5.279615	Protein ARMCX6
Q9UNW9	0.1203	9.595896	4.6874		0.0010.9026764	RNA-binding protein Nova-2
P49207	0.3206	9.094215	1.5105253		0.0010.9026634 4.711493	60S ribosomal protein L34
Q8WY22	0.3738	9.367147	1.8091979		0.0010.9026303 4.849894	BRI3-binding protein
P46063	0.2888	9.479952	1.3496984		0.0010.9026602 4.672705	ATP-dependent DNA helicase Q1
P56539	0.4208	9.375845	2.0161968		0.0010.9026602 4.790916	Caveolin-3

	logFC	se	df	t	pval	adjPval	rowData(pe[["proteinRobust"]])$Protein.names
Q53GG5-	0.601	8.565	632		0.001	0.027	Actin and LIM domain protein 3
2	2.9088697		4.834772				
Q5M9N0	0.7318	9.292	430		0.001	0.027	Coiled-coil domain-containing protein 158
	3.5146838		4.807438				
Q6SZW1	0.5187	6.967	147		0.001	0.027	Sterile alpha and TIR motif-containing protein 1
	2.6395602		5.088836				
Q9HAV4	0.4847	6.303	284		0.001	0.027	Exportin-5
	2.4188578		4.991140				
Q5NDL2	0.4878	8.362	509		0.001	0.027	EMILIN-3 domain-specific O-linked N-acetylglucosamine transferase
	2.2948242		4.704088				
Q9BXR6	0.5179	9.064	182		0.001	0.027	Complement factor H-related protein 5
	2.3571850		4.551516				
Q92681	0.4497	5.279	124		0.001	0.027	Regulatory solute carrier protein family 1 member 1
	2.1962163		4.886432				
O15230	0.3048	8.686	336		0.001	0.028	Laminin subunit alpha-5
	1.4246624		4.680464				
Q8TDB6	0.3837	7.048	834		0.001	0.029	E3 ubiquitin-protein ligase DTX3L
	1.7610737		4.589664				
Q96H79	0.5756	9.147	742		0.001	0.030	Zinc finger CCCH-type antiviral protein 1-like
	3.2031583		5.569827				
P14550	0.3048	8.302	749		0.001	0.039	Alcohol dehydrogenase [NADP(+)]
	1.3982496		4.586916				
Q15274	0.4007	3.862	886		0.001	0.039	Nicotinate-nucleotide pyrophosphorylase [carboxylating]
	1.9285076		4.814816				
Q9BUF5	0.4272	9.947	853		0.001	0.042	Tubulin beta-6 chain
	1.9157084		4.483389				
O15111	0.3816	5.863	146		0.001	0.044	Inhibitor of nuclear factor kappa-B kinase subunit alpha
	1.9484725		5.106218				
Q12996	0.3796	3.440	629		0.001	0.046	Cleavage stimulation factor subunit 3
	2.0094808		5.298775				
Q9Y5U8	0.9189	12.266	368		0.001	0.047	Mitochondrial pyruvate carrier 1
	4.0913780		4.456213				
P06727	0.3018	5.787	743		0.001	0.050	Apolipoprotein A-IV
	1.3683135		4.536079				
P04196	0.3967	5.453	131		0.001	0.052	Histidine-rich glycoprotein
	1.9303128		4.865279				
O75828	0.3759	6.507	147		0.001	0.053	Carbonyl reductase [NADPH] 3
	1.6544202		4.411081				
Q9UBI9	0.6065	7.378	007		0.001	0.058	Headcase protein homolog
	2.9866384		4.924355				
P01031	0.2898	4.031	718		0.001	0.061	Complement C5;Complement C5 beta chain;Complement C5 alpha chain;C5a anaphylatoxin;Complement C5 alpha chain
	1.3157564		4.546436				
Q9ULC3	0.3625	9.067	470		0.001	0.062	Ras-related protein Rab-23
	1.6333020		4.504536				
Q6ZSY5	0.3859	8.382	684		0.001	0.068	Protein phosphatase 1 regulatory subunit 3F
	1.6890696		4.381686				
P45877	0.2879	2.367	147		0.001	0.072	Peptidyl-prolyl cis-trans isomerase C
	1.2442932		4.332386				
P02461	0.7978	9.373	561		0.001	0.073	Collagen alpha-1(III) chain
	3.6062939		4.522579				
P04003	0.3412	3.069	835		0.001	0.073	C4b-binding protein alpha chain
	1.5009188		4.398549				

	logFC	se	df	t	pval	adjPval	rowData(pe[["proteinRobust"]])$Protein.names
P14555	-0.9304	4.863351 4.6109150			0.0017 4.955432	0.031	Phospholipase A2, membrane associated
Q8N5M1	0.4556	6.062735 1.9899041			0.0017 4.367233	0.031	ATP synthase mitochondrial F1 complex assembly factor 2
P08582	-0.3932	9.327606 1.6911943			0.0018 4.300091	0.032	Melanotransferrin
Q6YN16	0.5600...	3.2633858			0.0018 5.160293	0.032	Hydroxysteroid dehydrogenase-like protein 2
P34932	0.2699	9.338541 1.1578310			0.0018 4.302077	0.032	Heat shock 70 kDa protein 4
Q9UQ35	0.3179	9.207887 1.3570787			0.0019 4.268609	0.032	Serine/arginine repetitive matrix protein 2
O75746	0.3585	9.909528 1.5795706			0.0019 4.402415	0.032	Calcium-binding mitochondrial carrier protein Aralar1
Q13636	0.6320	4.080434 3.2633858			0.0019 5.160293	0.032	Ras-related protein Rab-31
Q5VIR6-4	-0.4123	9.261008 1.7638024			0.0020 4.272750	0.033	Vacuolar protein sorting-associated protein 53 homolog
P02776	-0.3927	8.375973 1.7615098			0.0020 4.491540	0.033	Platelet factor 4;Platelet factor 4, short form
P04004	0.3758	6.751887 1.6269790			0.0020 4.331499	0.033	Vitronectin;Vitronectin V65 subunit;Vitronectin V10 subunit;Somatomedin-B
Q9H1E5	0.3289	9.267147 1.3852786			0.0020 4.221707	0.033	Thioredoxin-related transmembrane protein 4
P04209	0.3430...				0.0020	0.033	Ig lambda chain V-II region NIG-84
P01042	-0.4729	9.753167 2.2103015			0.0020 4.673166	0.033	Kininogen-1;Kininogen-1 heavy chain;T-kinin;Bradykinin;Lysyl-bradykinin;Kininogen-1 light chain;Low molecular weight growth-promoting factor
Q8WWQ0	0.3855	9.367147 1.6224614			0.0020 4.208358	0.033	PH-interacting protein
P15924	2.64688...				0.0020	0.033	Desmoplakin
Q2TAA5	0.3067	9.651244 1.2916274			0.0020 4.210046	0.034	GDP-Man:Man(3)GlcNAc(2)-PP-Dol alpha-1,2-mannosyltransferase
Q9BTV4	0.4319	9.358605 1.8099873			0.0022 4.198158	0.034	Transmembrane protein 43
P05455	-0.2855	9.127952 1.2004499			0.0022 4.204543	0.034	Lupus La protein
Q08945	-0.4658	7.367147 2.0204841			0.0022 4.338444	0.034	FACT complex subunit SSRP1
Q04721	2.5358...				0.0022	0.034	Neurogenic locus notch homolog protein 2;Notch 2 extracellular truncation;Notch 2 intracellular domain
Q53T59	0.4187	9.624426 1.8461756			0.0022 4.412822	0.034	HCLS1-binding protein 3
Q9Y6X5	0.3248	8.651882 1.3875045			0.0022 4.271291	0.034	Bis(5-adenosyl)-triphosphatase ENPP4
P09619	-0.3859	9.563401 1.6085799			0.0022 4.177519	0.034	Platelet-derived growth factor receptor beta
Q5JPH6	0.28...				0.0023	0.034	Probable glutamate–tRNA ligase, mitochondrial
O60262	0.3668	9.875805 1.5577722			0.0023 4.244810	0.034	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-7
O75348	0.4525	9.367147 1.8591253			0.0024 4.108278	0.035	V-type proton ATPase subunit G 1

	logFC	se	df	t	pval	adjP	rowData(pe[["proteinRobust"]])$Protein.names
O00303	0.3498	8.358	7975		0.0024	4.005	Eukaryotic translation initiation factor 3 subunit F
	1.4561773		4.162459				
P01034	0.4078	8.285	5921		0.0025	3.766	Cystatin-C
	1.6978634		4.168290				
P49458	0.5037	8.198	623		0.0025	3.665	Signal recognition particle 9 kDa protein
	2.2735269		4.518307				
P25940	0.4259	9.272	407		0.0026	3.725	Collagen alpha-3(V) chain
	1.7454125		4.097911				
P62760	0.4698	8.586	554		0.0027	3.254	Visinin-like protein 1
	1.9146688		4.074988				
P08311	0.3218	8.729	026		0.0028	3.996	Cathepsin G
	1.3139510		4.092302				
Q9UKS6	0.4670	8.369	668		0.0028	3.710	Protein kinase C and casein kinase substrate in neurons protein 3
Q14019	0.8346	8.307	147		0.0028	3.670	Coactosin-like protein
	3.9129083		4.690111				
P36551	0.3266	6.525	556		0.0029	3.670	Oxygen-dependent coproporphyrinogen-III oxidase, mitochondrial
	1.3202398		4.041659				
O15116	0.6505	7.541	418		0.0029	3.676	U6 snRNA-associated Sm-like protein LSm1
	3.2231772		4.952445				
P30405	0.3806	6.367	147		0.0029	3.670	Peptidyl-prolyl cis-trans isomerase F, mitochondrial
	1.5177122		3.986967				
Q9UKX3	0.3105	7.932	200		0.0029	3.795	Myosin-13
Q9Y2Z0	0.4387	7.264	147		0.0030	3.823	Suppressor of G2 allele of SKP1 homolog
	1.7414076		3.969206				
P54577	0.3236	6.093	466		0.0030	3.810	Tyrosine–tRNA ligase, cytoplasmic;Tyrosine–tRNA ligase, cytoplasmic,
	1.3002728		4.017281			N-terminally processed	
P19429	0.6063	5.705	947		0.0030	4.229	Troponin I, cardiac muscle
P54652	0.4019	7.278	544		0.0030	3.741	Heat shock-related 70 kDa protein 2
	1.5932853		3.973066				
O75475	0.4838	8.259	788		0.0032	3.956	PC4 and SFRS1-interacting protein
	1.9038879		3.934575				
P25311	0.3733	9.003	030		0.0033	3.841	Zinc-alpha-2-glycoprotein
	1.5051007		4.030810				
P60468	0.3608	4.822	346		0.0034	3.568	Protein transport protein Sec61 subunit beta
	1.4647431		4.064796				
Q9BS26	0.3209	9.371	620		0.0034	3.568	Endoplasmic reticulum resident protein 44
	1.2463986		3.892639				
Q7LBR1	0.7319	8.267	147		0.0034	3.568	Charged multivesicular body protein 1b
	2.8413335		3.882531				
Q04941	0.4959	8.058	974		0.0035	4.065	Proteolipid protein 2
	1.9356392		3.908925				
P03950	0.5449	8.281	933		0.0035	4.536	Angiogenin
	2.2030636		4.042851				
Q1KMD3	0.3778	8.422	240		0.0035	4.536	Heterogeneous nuclear ribonucleoprotein U-like protein 2
	1.4744120		3.902186				
Q13641	0.5227	9.058	658		0.0036	4.220	Trophoblast glycoprotein
	2.2297004		4.263335				
P26447	0.4678	8.765	342		0.0036	4.218	Protein S100-A4
	1.8372154		3.933335				
Q96PK6	0.3379	7.405	474		0.0037	4.593	RNA-binding protein 14
	1.3089284		3.875547				

	logFC	se	df	t	pval	adjPval	rowData(pe[["proteinRobust"]])$Protein.names
Q9Y3B4	0.3269	0.6315209			0.0038	0.0355	Splicing factor 3B subunit 6
	1.2603707		3.865414				
Q9HB40	0.4806	5.7518048			0.0038	0.0645	Retinoid-inducible serine carboxypeptidase
	2.1393191		4.451581				
P01024	0.2909	6.4031573			0.0038	0.0456	Complement C3;Complement C3 beta chain;C3-beta-c;Complement C3 alpha chain;C3a anaphylatoxin;Acylation stimulating protein;Complement C3b alpha chain;Complement C3c alpha chain fragment 1;Complement C3dg fragment;Complement C3g fragment;Complement C3d fragment;Complement C3f fragment;Complement C3c alpha chain fragment 2
	1.1180307		3.846785				
O00567	0.6379	9.8962457			0.0038	0.0765	Nucleolar protein 56
	2.4335450		3.819803				
P12814	0.4222	2.1526643			0.0038	0.0745	Alpha-actinin-1
	1.6827515		3.985531				
Q13478	0.4166	9.9974897			0.0038	0.0375	Interleukin-18 receptor 1
	1.6083281		3.859681				
Q86VU5	0.5580	6.9873.8730.70			0.0038	0.0456	Catechol O-methyltransferase domain-containing protein 1
P07384	0.2899	4.5248204			0.0039	0.0456	Calpain-1 catalytic subunit
	1.1052065		3.818222				
P01008	0.4138	3.3223357			0.0040	0.0547	Antithrombin-III
	1.5692162		3.791914				
P14543	0.3769	2.9150089			0.0040	0.0463	Nidogen-1
	1.4365423		3.819276				
O94919	0.2959	9.9300892			0.0040	0.0446	Endonuclease domain-containing 1 protein
	1.1247209		3.810121				
Q9UK22	0.4297	6.3353956			0.0040	0.0836	F-box only protein 2
	1.8807802		4.376569				
P07357	0.3019	4.7389682			0.0040	0.0936	Complement component C8 alpha chain
	1.1425305		3.789753				
Q7Z3T8	0.4195	5.8329932			0.0040	0.0548	Zinc finger FYVE domain-containing protein 16
	1.6269924		3.876021				
O95486	0.4479	1.0251990			0.0040	0.0816	Protein transport protein Sec24A
	1.7001274		3.802477				
Q9BXY0	0.4366	0.0827155			0.0040	0.0546	Protein MAK16 homolog
	1.9170100		4.396722				
Q14011	0.6089	6.2383933			0.0042	0.0786	Cold-inducible RNA-binding protein
	2.3803571		3.914650				
Q9Y2D4	0.5287	4.6669332			0.0042	0.0572	Exocyst complex component 6B
	2.0300937		3.839431				
Q96ST3	0.4246	5.5853453			0.0042	0.0636	Paired amphipathic helix protein Sin3a
	1.6077298		3.785937				
O43143	0.2647	2.2647147			0.0042	0.0746	Pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15
	0.9916403		3.745907				
P56199	0.3308	0.7590476			0.0043	0.0446	Integrin alpha-1
	1.2716373		3.844058				
Q6IC98	0.3048	1.1002633			0.0043	0.0366	GRAM domain-containing protein 4
	1.1710653		3.841831				
P09467	0.4947	7.3720615			0.0043	0.0446	Fructose-1,6-bisphosphatase 1
	2.0219103		4.092623				
P51665	0.3459	4.3694310			0.0043	0.0346	26S proteasome non-ATPase regulatory subunit 7
	1.2958979		3.751475				

	logFC	se	df	t	pval	adjPval	rowData(pe[["proteinRobust"]])$Protein.names
Q9BVC6	0.4374	4.367147			0.0043		Transmembrane protein 109
	1.6329609		3.732978				
P50991	0.4658	6.151032			0.0044		T-complex protein 1 subunit delta
	1.7929240		3.850651				
Q96FJ2							Dynein light chain 2, cytoplasmic
P23434							Glycine cleavage system H protein, mitochondrial
Q8NBF2	0.3146	6.024232			0.0047		NHL repeat-containing protein 2
	1.1613746		3.691495				
P04843	0.5398	8.883713			0.0047		Dolichyl-diphosphooligosaccharide–protein glycosyltransferase subunit 1
	2.0344244		3.771026				
Q53FA7							Quinone oxidoreductase PIG3
Q6P1N0	0.4246	6.099225			0.0048		Coiled-coil and C2 domain-containing protein 1A
	1.7290768		4.075037				
Q6P587							Acylpyruvase FAHD1, mitochondrial

4.4 Evaluate results contrast $\log_2 FC_{V-A}^{R}$

4.4.1 Volcano-plot

```
volcanoRight <- ggplot(rowData(pe[["proteinRobust"]])$"tissueV + locationR:tissueV",
               aes(x = logFC, y = -log10(pval), color = adjPval < 0.05)) +
 geom_point(cex = 2.5) +
 scale_color_manual(values = alpha(c("black", "red"), 0.5)) + theme_minimal()
volcanoRight
```
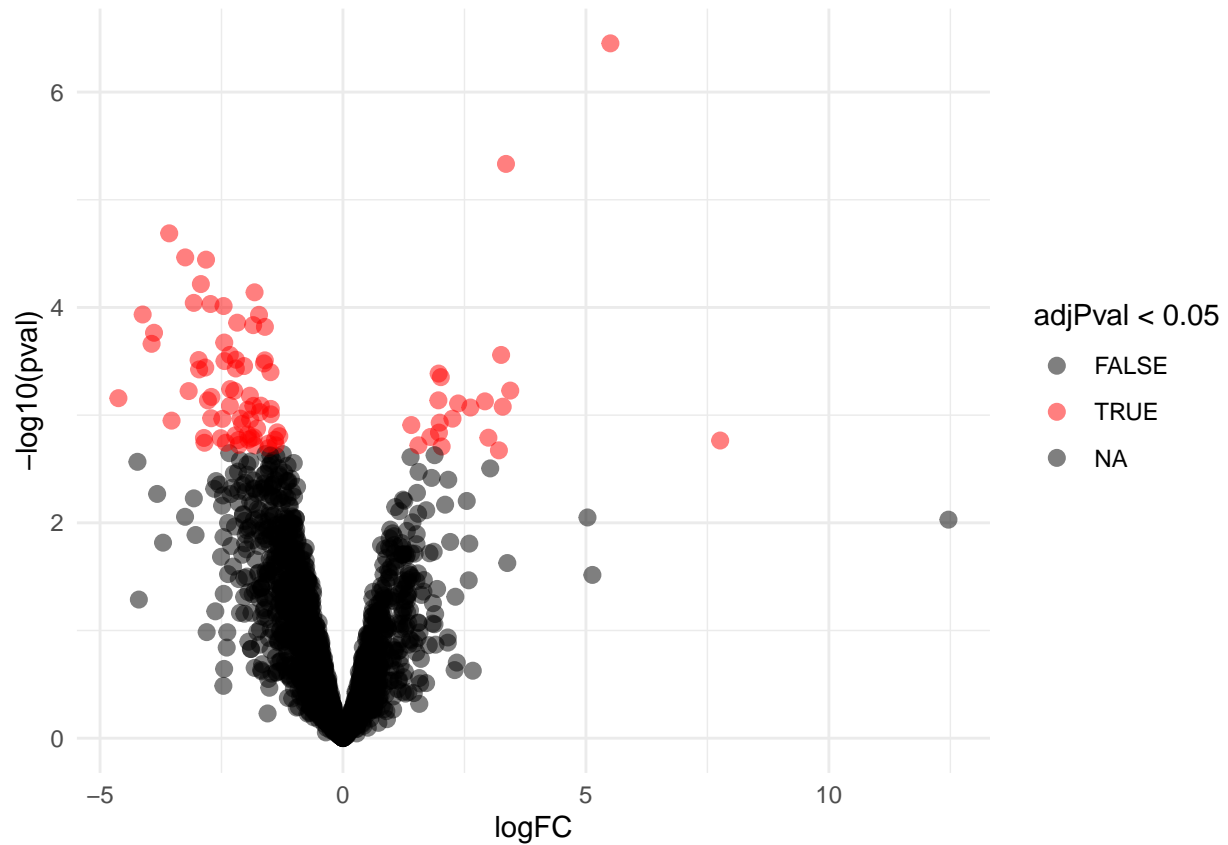
4.4.2 Heatmap

We first select the names of the proteins that were declared signficant.

```
sigNamesRight <- rowData(pe[["proteinRobust"]])$"tissueV + locationR:tissueV" %>%
 rownames_to_column("proteinRobust") %>%
 filter(adjPval<0.05) %>%
 pull(proteinRobust)
heatmap(assay(pe[["proteinRobust"]])[sigNamesRight, ])
```
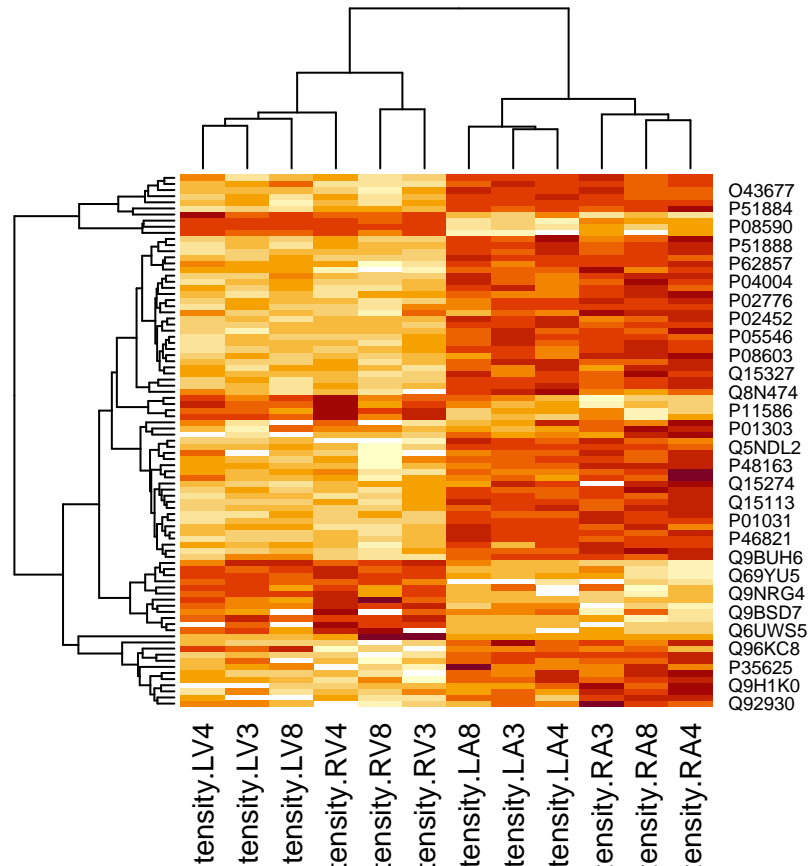
There are 87 proteins significantly differentially expressed at the 5% FDR level.

```
rowData(pe[["proteinRobust"]])$"tissueV + locationR:tissueV"  %>%
  cbind(.,rowData(pe[["proteinRobust"]])$Protein.names) %>%
  na.exclude %>%
  filter(adjPval<0.05) %>%
  arrange(pval) %>%
  knitr::kable(.)
```

	logFC	se	df	t	pval	adjPval	rowData(pe[["proteinRobust"]])$Protein.names
P08590	0.5950383	0.0383794	57.11475	15.503650	0.0000167		Myosin light chain 3
P06858	0.5854635	0.0449230	24.74012	13.032840	0.0000647		Lipoprotein lipase
Q9ULD0	0.3737327		57.147	0.000	0.0021538		2-oxoglutarate dehydrogenase-like, mitochondrial
	3.575906		9.568355				
P35442	0.4068328		57.147	0.000	0.0043765		Thrombospondin-2
	3.246460		7.984746				
P02776	0.3427597		15.973	0.000	0.0043265		Platelet factor 4;Platelet factor 4, short form
	2.818363		8.237189				
P21810	0.4081756		6.796	0.000	0.0061973		Biglycan
	2.924783		7.166550				
O75368	0.2697289		57.260	0.000	0.0072951		SH3 domain-binding glutamic acid-rich-like protein
	1.819326		6.745396				
A6NMZ7	0.4709267		57.147	0.000	0.0091797		Collagen alpha-6(VI) chain
	3.070848		6.520879				

	logFC	se	df	t	pval	adjPval	rowData(pe[["proteinRobust"]])$Protein.names
P54652	0.411	70.48544			0.0000	9297128	Heat shock-related 70 kDa protein 2
		2.726681	6.622904				
Q6UWY5	0.3739	3513260			0.0000	9707128	Olfactomedin-like protein 1
		2.456874	6.570320				
Q06828	0.636	1.919004			0.0000	1029286	Fibromodulin
		4.122091	6.477083				
P05546	0.272	2.243661			0.0000	1719286	Heparin cofactor 2
		1.727800	6.346960				
P28066	0.338	5.729782			0.0000	3205206	Proteasome subunit alpha type-5
		2.176824	6.429476				
P29622	0.301	3.467147			0.0000	4205216	Kallistatin
		1.847919	6.132273				
P46821	0.263	2.6267 3.467147			0.0000	5205216	Microtubule-associated protein 1B;MAP1B heavy chain;MAP1 light chain LC1
		1.606539	6.102419				
P13533	0.637	4.096373			0.0000	7081208	Myosin-6
		3.888317	6.097975				
Q9UGT4	0.414	3.431824			0.0002	1245753	Sushi domain-containing protein 2
		2.443145	5.896430				
P35625	0.616	7.086282			0.0002	1745763	Metalloproteinase inhibitor 3
		3.938312	6.392443				
Q6PCB0	0.403	3.934041			0.0002	7263652	von Willebrand factor A domain-containing protein 1
		2.328852	5.773148				
Q69YU5	0.547	5.635 5.63655			0.0002	7263652	Uncharacterized protein C12orf73
Q15327	0.393	3.236445			0.0003	5276452	Ankyrin repeat domain-containing protein 1
		2.203003	5.596523				
O43677	0.506	2.820596			0.0003	7263652	NADH dehydrogenase [ubiquinone] 1 subunit C1, mitochondrial
		2.969964	5.865758				
Q14764	0.289	3.467147			0.0003	9263652	Major vault protein
		1.608232	5.547661				
P60468	0.411	3.822346			0.0003	1026652	Protein transport protein Sec61 subunit beta
		2.438022	5.929209				
P01031	0.281	3.491718			0.0003	3026652	Complement C5;Complement C5 beta chain;Complement C5 alpha chain;C5a anaphylatoxin;Complement C5 alpha chain
		1.626524	5.781772				
Q9P2B2	0.373	3.467147			0.0003	5026652	Prostaglandin F2 receptor negative regulator
		2.035226	5.456312				
Q92930	0.466	7.026745			0.0003	6026652	Ras-related protein Rab-8B
		2.833666	6.079489				
P02775	0.407	3.467147			0.0003	6026652	Platelet basic protein;Connective tissue-activating peptide III;TC-2;Connective tissue-activating peptide III(1-81);Beta-thromboglobulin;Neutrophil-activating peptide 2(74);Neutrophil-activating peptide 2(73);Neutrophil-activating peptide 2;TC-1;Neutrophil-activating peptide 2(1-66);Neutrophil-activating peptide 2(1-63)
		2.205543	5.417470				
P48163	0.488	4.261840			0.0003	7026652	NADP-dependent malic enzyme
		2.959729	6.059718				
P48681	0.278	2.408182			0.0003	9025464	Nestin
		1.491288	5.359530				
P12889	0.690	0.6594 88315 6875889			0.0004	1026464	Myosin-7
P11586	0.133	3.5187 4995 6720			0.0004	4028859	C-1-tetrahydrofolate synthase, cytoplasmic;Methylenetetrahydrofolate dehydrogenase;Methenyltetrahydrofolate cyclohydrolase;Formyltetrahydrofolate synthetase;C-1-tetrahydrofolate synthase, cytoplasmic, N-terminally processed

logFC	se	df	t	pval	adjPval	rowData(pe[["proteinRobust"]])$Protein.names
P30711	-0.448					Glutathione S-transferase theta-1
2.321740	5.178408					
Q9BZH6						WD repeat-containing protein 11
Q9H1K0	0.390					Rabenosyn-5
2.237374	5.726736					
Q9UHG2	0.610					ProSAAS;KEP;Big SAAS;Little SAAS;Big PEN-LEN;PEN;Little
3.175806	5.198401					LEN;Big LEN
P00748	0.378					Coagulation factor XII;Coagulation factor XIIa heavy chain;Beta-factor
1.915326	5.057248					XIIa part 1;Coagulation factor XIIa light chain
Q5NDL2	0.525					EGF domain-specific O-linked N-acetylglucosamine transferase
2.708130	5.153345					
O00180	0.885					Potassium channel subfamily K member 1
4.621308	5.219183					
Q00G						Perilipin-5
P23142	0.508					Fibulin-1
2.779235	5.469018					
P10916						Myosin regulatory light chain 2, ventricular/cardiac muscle isoform
Q09161						Nuclear cap-binding protein subunit 1
P18428	0.345					Lipopolysaccharide-binding protein
1.690875	4.895012					
Q9BW30	0.472					Tubulin polymerization-promoting protein family member 3
2.326597	4.924870					
P30405	0.380					Peptidyl-prolyl cis-trans isomerase F, mitochondrial
1.842938	4.841321					
Q5JPH6						Probable glutamate–tRNA ligase, mitochondrial
Q9NRG4						N-lysine methyltransferase SMYD2
Q9BUH6	0.306					Protein PAXX
1.485484	4.844388					
P35052	0.377					Glypican-1;Secreted glypican-1
1.965366	5.200513					
O15061	0.360					Synemin
1.710970	4.747319					
P08603	0.314					Complement factor H
1.484314	4.715011					
Q96FN9	0.555					Probable D-tyrosyl-tRNA(Tyr) deacylase 2
2.712489	4.885119					
Q8N474	0.420					Secreted frizzled-related protein 1
2.108536	5.012244					
Q04760						Lactoylglutathione lyase
Q96KC8	0.505					DnaJ homolog subfamily C member 1
2.480944	4.905335					
P04004	0.399					Vitronectin;Vitronectin V65 subunit;Vitronectin V10
1.908509	4.773059					subunit;Somatomedin-B
Q99983	0.660					Osteomodulin
3.527403	5.343578					
P24298						Alanine aminotransferase 1
P62857	0.415					40S ribosomal protein S28
2.074531	4.994359					
P23434						Glycine cleavage system H protein, mitochondrial
P24844	0.391					Myosin regulatory light polypeptide 9
1.767461	4.518410					

	logFC	se	df	t	pval	adjPval	rowData(pe[["proteinRobust"]])$Protein.names
P04275	0.298	1.682806	1.352764	4.532424	0.0010445		von Willebrand factor;von Willebrand antigen 2
Q6PI78	0.375693	3.896297.12			0.0145245		Transmembrane protein 65
Q15113	0.43993821		1.959899	4.454929	0.0015075		Collagen C-endopeptidase enhancer 1
P01611	0.50198317147		2.213481	4.409491	0.0015405		Ig kappa chain V-I region Wes
Q86WV6	0.29692.232627		1.310297	4.422592	0.0015685		Stimulator of interferon genes protein
Q6UW78	0.58536722.73882985				0.0015975		Protein PET117 homolog, mitochondrial
P51888	0.37369059338		1.838614	4.920156	0.0016055		Prolargin
Q9BQ50	0.9732.109073.721432.9243.9				0.0016225		Cancer-related nucleoside-triphosphatase
P04350	0.57370939826		2.861432	4.987600	0.0016285		Tubulin beta-4A chain
Q7L4S7	0.51074099840		2.514619	4.924387	0.0016365		Protein ARMCX6
P08294	0.40473.981617		1.884866	4.661544	0.0016445		Extracellular superoxide dismutase [Cu-Zn]
P82663	0.44655.379706		1.944516	4.354262	0.0016935		28S ribosomal protein S25, mitochondrial
P50453	0.31682.925377		1.398216	4.420644	0.0016945		Serpin B9
P01303	0.48685.937242		2.151374	4.418962	0.0017025		Pro-neuropeptide Y;Neuropeptide Y;C-flanking peptide of NPY
Q9UL25	0.76300750862547.465.70				0.0017205		Ras-related protein Rab-22A
Q9HCB6	0.54389610884		2.412515	4.435619	0.0018052		Spondin-1
Q8TDB4	0.54867.232826		2.849906	5.193780	0.0018053		Protein MGARP
Q9Y6X5	0.34686251882		1.531154	4.420673	0.0018346		Bis(5-adenosyl)-triphosphatase ENPP4
Q15274	0.46871862886		2.142900	4.577796	0.0018896		Nicotinate-nucleotide pyrophosphorylase [carboxylating]
Q6YN16	0.530763936741.276.50				0.0019066		Hydroxysteroid dehydrogenase-like protein 2
P51884	0.32299097371		1.386041	4.301927	0.0019076		Lumican
P02452	0.40669292268		1.816507	4.466489	0.0019046		Collagen alpha-1(I) chain
P61925	1.14367.75324.500.50				0.0019556		cAMP-dependent protein kinase inhibitor alpha
P07360	0.35376025608		1.535545	4.340638	0.0020285		Complement component C8 gamma chain
Q96MM6	0.36626.376098.328.00				0.0022057		Heat shock 70 kDa protein 12B

4.5 Evaluate results average contrast $\log_2 FC_{V-A}$

4.5.1 Volcano-plot

```
volcanoAvg <- ggplot(rowData(pe[["proteinRobust"]])$"tissueV + 0.5 * locationR:tissueV",
              aes(x = logFC, y = -log10(pval), color = adjPval < 0.05)) +
```

```
  geom_point(cex = 2.5) +
  scale_color_manual(values = alpha(c("black", "red"), 0.5)) + theme_minimal()
volcanoAvg
```
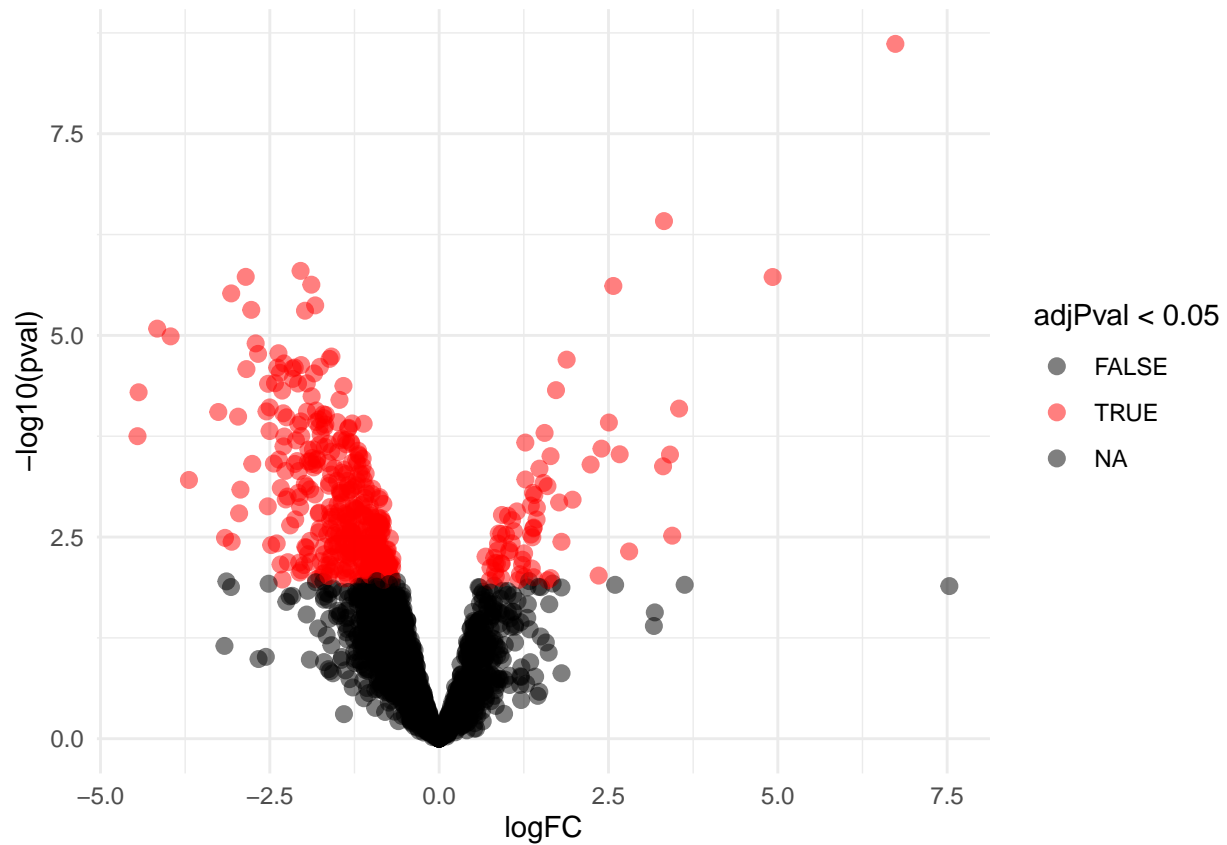

4.5.2   Heatmap

We first select the names of the proteins that were declared signficant.

```
sigNamesAvg <- rowData(pe[["proteinRobust"]])$"tissueV + 0.5 * locationR:tissueV" %>%
  rownames_to_column("proteinRobust") %>%
  filter(adjPval<0.05) %>%
  pull(proteinRobust)
heatmap(assay(pe[["proteinRobust"]])[sigNamesAvg, ])
```
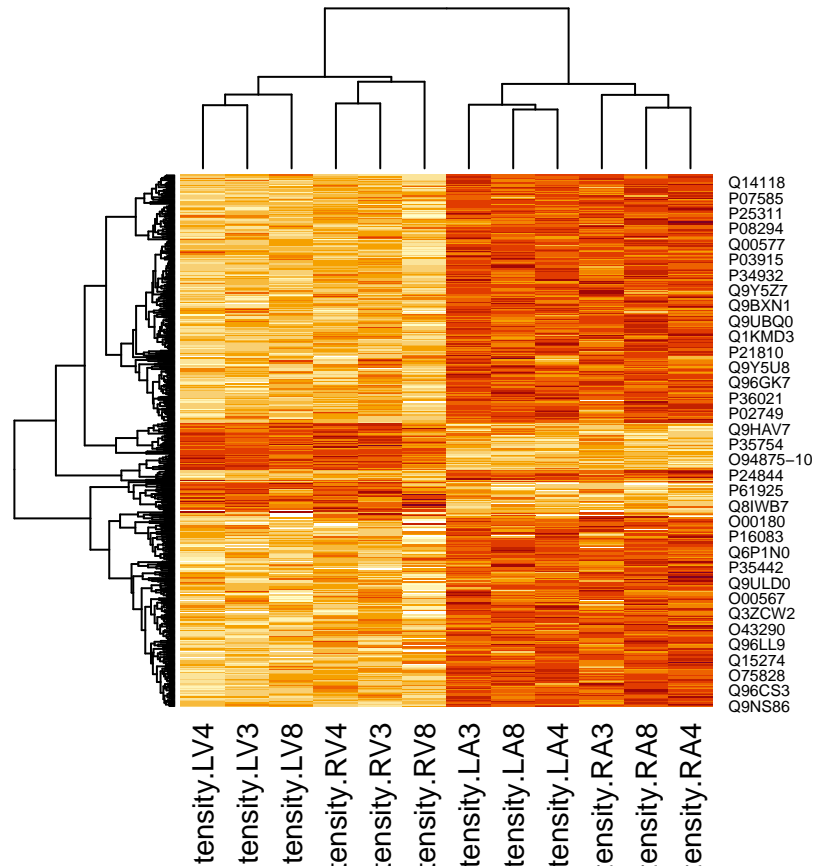
There are 449 proteins significantly differentially expressed at the 5% FDR level.

```
rowData(pe[["proteinRobust"]])$"tissueV + 0.5 * locationR:tissueV" %>%
  cbind(.,rowData(pe[["proteinRobust"]])$Protein.names) %>%
  na.exclude %>%
  filter(adjPval<0.05) %>%
  arrange(pval) %>%
  knitr::kable(.)
```

	logFC	se	df	t	pval	adjPval	rowData(pe[["proteinRobust"]])$Protein.names
P08590	0.7358945	0.0593967	211.77031	12.390999	0.0000000	0.0000049	Myosin light chain 3
P12883	0.3190926	0.0263461	263.03045	12.110000	0.0000000	0.0000891	Myosin-7
O75368	0.1913289	0.3529260	0.0000000	0.0065082			SH3 domain-binding glutamic acid-rich-like protein
	2.0454601		10.689106				
Q6UW-Y5	0.2675968	0.3529260	0.0000000	0.0097082			Olfactomedin-like protein 1
	2.8552584		10.670003				
P10916	0.4232043	0.6671350	113.44834	9.000000	0.0000000	0.0097082	Myosin regulatory light chain 2, ventricular/cardiac muscle isoform
P46821	0.1869367	0.9367147	0.0000000	0.0097082			Microtubule-associated protein 1B;MAP1B heavy chain;MAP1 light
	1.8867094		10.135167				chain LC1
P06858	0.3571032	0.5194892	16.72081	910.000000	0.0000000	0.0097082	Lipoprotein lipase
P21810	0.2938275	0.2757796	0.0000000	0.0030366			Biglycan
	3.0696624		10.472314				
P05546	0.1916273	0.9271661	0.0000000	0.0042Hepa			Heparin cofactor 2
	1.8298412		9.545370				

	logFC	se	df	t	pval	adjP	rowData(pe[["proteinRobust"]])$Protein.names
P35442	2.2689	0.2302	3067	147	0.0000	0.0089134	Thrombospondin-2
	2.7737551			10.314070			
P29622	2.2130	0.2307	3067	147	0.0000	0.0090634	Kallistatin
	1.9806994			9.295484			
Q06828	2.4598	2.3069	004		0.0000	0.0213965	Fibromodulin
	4.1625547			9.064061			
P13533	2.4545	0.7098	373		0.0000	0.0316072	Myosin-6
	3.9643622			8.721124			
Q8N474	2.2827	1.8207	481		0.0000	0.0618244	Secreted frizzled-related protein 1
	2.7087798			9.599391			
Q9UGT4	0.2907	0.7028	824		0.0000	0.0628451	Sushi domain-containing protein 2
	2.3718806			8.159126			
A6NMZ7	0.3329	0.3067	147		0.0000	0.0720451	Collagen alpha-6(VI) chain
	2.6735410			8.028783			
O95865	0.1998	0.8207	147		0.0000	0.0832461	N(G),N(G)-dimethylarginine dimethylaminohydrolase 2
	1.5898713			7.955611			
Q14764	0.2049	0.8807	147		0.0000	0.1002045	Major vault protein
	1.6175126			7.890852			
O94875	2.2650	0.0919	137039		0.0000	0.1002045	Sorbin and SH3 domain-containing protein 2
	5.826302308109910						
P02776	0.2607	2.2875	973		0.0000	0.1223451	Platelet factor 4;Platelet factor 4, short form
	2.2899367			8.799713			
Q9P2B2	0.2637	0.5367	147		0.0000	0.1232451	Prostaglandin F2 receptor negative regulator
	2.0373086			7.724288			
P51884	0.2259	0.7257	371		0.0000	0.1402451	Lumican
	1.7601864			7.799750			
P24844	0.2772	2.3117	934		0.0000	0.1502451	Myosin regulatory light polypeptide 9
	2.1318385			7.689181			
P02452	0.2858	2.2202	268		0.0000	0.1502451	Collagen alpha-1(I) chain
	2.3897612			8.384480			
Q16647	0.2769	0.0886	244		0.0000	0.1502451	Prostacyclin synthase
	2.1622988			7.833887			
O43677	0.3455	3.3137	596		0.0000	0.1622451	NADH dehydrogenase [ubiquinone] 1 subunit C1, mitochondrial
	2.8449373			8.228647			
Q15113	0.3089	0.7285	821		0.0000	0.1702404	Procollagen C-endopeptidase enhancer 1
	2.3511987			7.619599			
P18428	0.2419	0.4307	659		0.0000	0.1702494	Lipopolysaccharide-binding protein
	1.8452405			7.629891			
P54652	0.2879	3.0078	544		0.0000	0.1702451	Heat shock-related 70 kDa protein 2
	2.1599833			7.516383			
P51888	0.2713	1.5593	338		0.0000	0.1702451	Prolargin
	2.4233975			8.930701			
P00748	0.2645	0.5103	103		0.0000	0.1802519	Coagulation factor XII;Coagulation factor XIIa heavy chain;Beta-factor XIIa part 1;Coagulation factor XIIa light chain
	1.9533971			7.383809			
P02775	0.2879	0.7307	147		0.0000	0.1802519	Platelet basic protein;Connective tissue-activating peptide III;TC-2;Connective tissue-activating peptide III(1-81);Beta-thromboglobulin;Neutrophil-activating peptide 2(74);Neutrophil-activating peptide 2(73);Neutrophil-activating peptide 2;TC-1;Neutrophil-activating peptide 2(1-66);Neutrophil-activating peptide 2(1-63)
	2.0838997			7.238905			
Q9BW30	0.3399	0.7209	217		0.0000	0.1802451	Tubulin polymerization-promoting protein family member 3
	2.5255943			7.428830			

	logFC	se	df	t	pval	adjPval	rowData(pe[["proteinRobust"]])$Protein.names
P48681	-1.196...	0.196	7.204182		0.0000402257395		Nestin
	1.4123446		7.179175				
P14854	-1.4725082...	8.1817169508			0.0000407027396		Cytochrome c oxidase subunit 6B1
P08294	-0.2927.461617				0.0000482272296		Extracellular superoxide dismutase [Cu-Zn]
	2.3175953		7.916749				
O00180	-0.5856.707147				0.0000502278665		Potassium channel subfamily K member 1
	4.4369891		7.575947				
P02743	-0.2729.307147				0.0000563984166		Serum amyloid P-component;Serum amyloid P-component(1-203)
	1.8823835		6.917936				
P01031	-0.2028.631718				0.0000680328447		Complement C5;Complement C5 beta chain;Complement C5 alpha
	1.4711401		7.280573				chain;C5a anaphylatoxin;Complement C5 alpha chain
Q5NDL2	0.3585.1024509				0.0000782327419		EGF domain-specific O-linked N-acetylglucosamine transferase
	2.5014769		6.977247				
Q92508	-3.5430879827078.8350990				0.0000808D040		Piezo-type mechanosensitive ion channel component 1
Q53GQ0	0.2763.256570				0.0000850402212		Very-long-chain 3-oxoacyl-CoA reductase
	1.8169289		6.575413				
P05997	0.3783.581173				0.0000870402212		Collagen alpha-2(V) chain
	2.5486728		6.736140				
P60468	0.2748.222346				0.0000880D202		Protein transport protein Sec61 subunit beta
	1.9513827		7.111933				
P23083	0.4235.367147				0.0000890402212		Ig heavy chain V-I region V35
	3.2601200		7.700684				
Q9ULL5-3	-0.3157.296127				0.0000925D854		Proline-rich protein 12
	2.3006667		7.300700				
Q8TBP6	0.2569.573834				0.0000940450119		Solute carrier family 25 member 40
	1.7084465		6.648854				
P28066	0.2478.723782				0.0000969D069		Proteasome subunit alpha type-5
	1.6712466		6.747351				
P35625	0.4157.046282				0.0001082D030		Metalloproteinase inhibitor 3
	2.9679404		7.139594				
Q14195-2	-0.3485.650176				0.0001087D030		Dihydropyrimidinase-related protein 3
	2.2534620		6.464943				
P46060	0.2489.367147				0.0001062D030		Ran GTPase-activating protein 1
	1.7047689		6.848188				
Q15327	0.2769.236415				0.0001003D030		Ankyrin repeat domain-containing protein 1
	1.7740861		6.410333				
Q9ULD0	0.2760.367147				0.0001066D030		Oxoglutarate dehydrogenase-like, mitochondrial
	2.0406330		7.393273				
Q6PCB0	0.2808.656041				0.0001084D030		von Willebrand factor A domain-containing protein 1
	1.8163629		6.478527				
P00325	0.2338.584001				0.0001089D030		Alcohol dehydrogenase 1B
	1.5034066		6.450965				
Q69YU5	0.5050633818.795132906				0.0001203D030		Uncharacterized protein C12orf73
P07451	0.2049.367147				0.0001280D030		Carbonic anhydrase 3
	1.2833190		6.266322				
O14980	0.1780.367147				0.0001205D030		Exportin-1
	1.1146843		6.260035				
P30405	0.2699.367147				0.0001273D030		Peptidyl-prolyl cis-trans isomerase F, mitochondrial
	1.6803249		6.242542				
Q92604	0.3319.299173				0.0001280D030		1-acyl-sn-glycerol-3-CoA:lysophosphatidylglycerol acyltransferase 1
	2.0782169		6.263469				

	logFC	se	df	t	pval	adjPval	rowData(pe[["proteinRobust"]])$Protein.names
P04004	0.2748	3.0731887			0.0000	3.6045115	Vitronectin;Vitronectin V65 subunit;Vitronectin V10 subunit;Somatomedin-B
	1.7677440		6.444506				
P36955	0.2522	8.043555			0.0000	3.004B1g15	Pigment epithelium-derived factor
	1.6933164		6.712038				
P41240	0.2118	6.097098			0.0000	3.0945115	Tyrosine-protein kinase CSK
	1.3344948		6.307837				
P04083	0.2182	2.367147			0.0000	4.0804A388	Annexin A1
	1.3421955		6.148823				
P36021	0.3848	3.867147			0.0000	5.804M94	Monocarboxylate transporter 8
	2.5034435		6.506026				
Q6YN16	1.56712573	3.6076.0749081			0.0000	6.1094H854	Hydroxysteroid dehydrogenase-like protein 2
Q8TBQ9	0.27789.522892				0.0000	7.031P640	Protein kish-A
	1.7482778		6.295657				
Q15274	0.30689.862886				0.0000	7.052039	Nicotinate-nucleotide pyrophosphorylase [carboxylating]
	2.0357040		6.633155				
Q8WWA0	0.68493.392804				0.0000	7.052039	Intelectin-1
	4.4529908		6.501292				
Q9BXN1	0.37378.352110				0.0000	7.052039	Asporin
	2.2858485		6.115406				
P04003	0.2365	6.069835			0.0000	8.05C4263	C4b-binding protein alpha chain
	1.4359842		6.065061				
O15230	0.2238	4.536336			0.0000	9.805L021	Laminin subunit alpha-5
	1.3744676		6.159502				
Q9Y6X5	0.23784.292882				0.0000	9.005B02	Bis(5-adenosyl)-triphosphatase ENPP4
	1.4593292		6.146375				
Q7L4S7	0.30247.039840				0.0002	0.005P02	Protein ARMCX6
	2.1135247		6.987472				
P14550	0.2159	9.842749			0.0002	1.005A906	Alcohol dehydrogenase [NADP(+)]
	1.3048277		6.043546				
P23434	2.72078.489.3675.873641				0.0002	1.0256998	Glycine cleavage system H protein, mitochondrial
Q07954	0.2229.367147				0.0002	1.08P557	Low-density lipoprotein receptor-related protein 1;Low-density lipoprotein receptor-related protein 1 85 kDa subunit;Low-density lipoprotein receptor-related protein 1 515 kDa subunit;Low-density lipoprotein receptor-related protein 1 intracellular domain
	1.2959588		5.815564				
Q9UBG0	0.27866.472396				0.0002	2.05C705	C-type mannose receptor 2
	1.6345146		5.866800				
P49207	0.2318.976215				0.0002	2.0456705	60S ribosomal protein L34
	1.3710319		5.927053				
Q9BUF5	0.30373.227853				0.0002	2.205T61	Tubulin beta-6 chain
	1.7682813		5.821834				
P23142	0.35199.535834				0.0002	3.0595567	Fibulin-1
	2.2973847		6.526703				
P04196	0.2571.653131				0.0002	4.005H567	Histidine-rich glycoprotein
	1.6877176		6.562746				
Q9NRG4	1.347048058.9657.10377091				0.0002	5.36205	N-lysine methyltransferase SMYD2
Q9NZ01	0.3149.832777				0.0002	5.00Y235	Very-long-chain enoyl-CoA reductase
	1.8635396		5.933357				
O95980	0.3149.736506				0.0002	6.00B235	Reversion-inducing cysteine-rich protein with Kazal motifs
	1.9294212		6.129555				
P04275	0.2068.542806				0.0002	6.0863466	von Willebrand factor;von Willebrand antigen 2
	1.1968998		5.791793				

	logFC	se	df	t	pval	adjPval	rowData(pe[["proteinRobust"]])$Protein.names
P12110	0.2625	1.5935296	7.169020		0.00002766	0.002466602	Collagen alpha-2(VI) chain
			6.065519				
O75828	0.2659	1.4912698	2.367147		0.00002805	0.002505760	Carbonyl reductase [NADPH] 3
			5.623030				
P07357	0.2117	1.1909118	7.289682		0.00002976	0.002976701	Complement component C8 alpha chain
			5.624991				
Q9BZH6	0.16650449	8.52053793	6080100		0.00002976	0.002976 WD	WD repeat-containing protein 11
Q5JPH6	0.7054425	9.73617708	0890030		0.0005P00l		Probable glutamate–tRNA ligase, mitochondrial
Q86WV6	0.2109	1.1787564	5.224627		0.000300068		Stimulator of interferon genes protein
			5.590434				
P24298	0.86440428748.4267953906		0700301425		0.000301425		Alanine aminotransferase 1
P62760	0.3363	1.8748180	6.027554		0.000330175		Visinin-like protein 1
			5.573799				
P01024	0.2030	1.1291448	1.474573		0.000337075		Complement C3;Complement C3 beta chain;C3-beta-c;Complement C3 alpha chain;C3a anaphylatoxin;Acylation stimulating protein;Complement C3b alpha chain;Complement C3c alpha chain fragment 1;Complement C3dg fragment;Complement C3g fragment;Complement C3d fragment;Complement C3f fragment;Complement C3c alpha chain fragment 2
			5.561340				
P30711	0.3229	1.7974916	1.237365		0.000337075		Glutathione S-transferase theta-1
			5.580063				
Q9UHG2	0.4208	2.3658698	6.644100		0.000346672308		ProSAAS;KEP;Big SAAS;Little SAAS;Big PEN-LEN;PEN;Little LEN;Big LEN
			5.621492				
Q92681	0.3015	1.8052155	5.879124		0.000348723		Regulatory solute carrier protein family 1 member 1
			5.985708				
Q9UQ35	0.2246	1.2247564	3.227887		0.000350373728		Serine/arginine repetitive matrix protein 2
			5.452518				
Q9HCB6	0.3708	2.0954145	1.574884		0.000360273089		Spondin-1
			5.656290				
P12814	0.3268	1.9016090	1.556643		0.000368774089		Alpha-actinin-1
			5.830451				
P48163	0.3187	1.9351889	0.961840		0.000369730089		NADP-dependent malic enzyme
			6.083652				
Q9Y3B4	0.2349	1.2875533	2.216209		0.000370373286		Splicing factor 3B subunit 6
			5.497136				
P25940	0.3009	1.6429098	7.512407		0.000386673286		Collagen alpha-3(V) chain
			5.462664				
P50453	0.2198	1.2078225	9.045377		0.000384673286		Serpin B9
			5.507626				
Q53GG5-2	0.4288	2.4399434	2.756632		0.000386673286		PDZ and LIM domain protein 3
			5.697097				
Q5M9N0	0.4839	2.7584485	9.562430		0.000390176286		Coiled-coil domain-containing protein 158
			5.699667				
P07585	0.3937	2.1323372	7.206541		0.000390227286		Decorin
			5.415987				
P49458	0.3022	1.8786710	8.358623		0.000390673286		Signal recognition particle 9 kDa protein
			6.212876				
P19429	0.86898238.2389749027		00390043776		0.0039004377		Troponin I, cardiac muscle
Q53T59	0.3112	1.8080103	2.984426		0.000408769001		HCLS1-binding protein 3
			5.809627				
Q9UNW9	0.6032624.6889697053		0041087 RNA		0.0041087 RNA		RNA-binding protein Nova-2

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O94919	0.2100645	0.0435892			0.0000420075276		Ribonuclease domain-containing 1 protein
	1.1297756		5.378231				
P02747	0.2976092	0.0462471			0.0000430077188		Complement C1q subcomponent subunit C
	1.8530999		6.234226				
P01008	0.2929823	0.0523357			0.0000440078553		Antithrombin-III
	1.5453986		5.290988				
Q00G26	0.1810523	0.0355605	9.504	0.004	0.0000460087610		Perilipin-5
O00264	0.2635374	0.0536168			0.0000450079851		Membrane-associated progesterone receptor component 1
	1.4077482		5.341736				
O60760	0.4118060	0.0530147			0.0000470082000		Hematopoietic prostaglandin D synthase
	2.2693850		5.520786				
Q6SZW1	0.3497050	0.0560147			0.0000480082004		Sterile alpha and TIR motif-containing protein 1
	2.0732831		5.928664				
Q9ULC3	0.2659160	0.0537470			0.0000500082209		Ras-related protein Rab-23
	1.4313739		5.382908				
Q9BS26	0.2279404	0.0431620			0.0000510085811		Endoplasmic reticulum resident protein 44
	1.1794250		5.194575				
Q8WZA9	0.2079197	0.0418039			0.0000510085196		Immunity-related GTPase family Q protein
	1.0848657		5.216213				
P14543	0.2620950	0.0500089			0.0000520085106		Nidogen-1
	1.3732462		5.227532				
P08603	0.2220367	0.0433147			0.0000520086106		Complement factor H
	1.1479994		5.157195				
Q9BTV4	0.3060148	0.0552605			0.0000530286531		Transmembrane protein 43
	1.5944595		5.201232				
Q2TAA5	0.2187826	0.0361244			0.0000600790337		GDP-Man:Man(3)GlcNAc(2)-PP-Dol alpha-1,2-mannosyltransferase
	1.1153927		5.098178				
P11586	0.2730427	0.0379955	9.647	0.020	0.0000610097837		C-1-tetrahydrofolate synthase, cytoplasmic;Methylenetetrahydrofolate dehydrogenase;Methenyltetrahydrofolate cyclohydrolase;Formyltetrahydrofolate synthetase;C-1-tetrahydrofolate synthase, cytoplasmic, N-terminally processed
Q9UKR5	0.7000734	0.0761780			0.0000610098388		Probable ergosterol biosynthetic protein 28
	3.6937409		5.271700				
Q9UJG5	0.2412327	0.0467075			0.0000630298543		C2 domain-binding glutamic acid-rich-like protein 2
	1.2143605		5.034295				
Q8WY22	0.2849360	0.0517147			0.0000630593643		BRI3-binding protein
	1.5074312		5.290848				
P13671	0.2709326	0.0467147			0.0000630598644		Complement component C6
	1.3576211		5.020926				
O14967	0.3123527	0.0558297			0.0000670103425		Calmegin
	1.6233066		5.197312				
Q6PI78	0.1542063	0.0298916	9.626795	0.006	0.0000670103425		Transmembrane protein 65
P01042	0.3517972	0.0763167			0.0000690820123		Kininogen-1;Kininogen-1 heavy chain;T-kinin;Bradykinin;Lysyl-bradykinin;Kininogen-1 light chain;Low molecular weight growth-promoting factor
	1.9829048		5.636499				
Q8WWQ0	0.2726367	0.0536147			0.0000720593831		PH-interacting protein
	1.3446280		4.932367				
Q92621	0.2480357	0.0434182			0.0000720408283		Nuclear pore complex protein Nup205
	1.2226648		4.929192				
Q12996	0.2706326	0.0400629			0.0000730108483		Cleavage stimulation factor subunit 3
	1.6262833		6.015988				
Q9HAT2	0.1590235	0.0732980	9.676069	0.007	0.0000740085638		Sialate O-acetylesterase

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P20774	0.3708	6.0125847			0.0007431086538		Biglycan
	1.9551933		5.277741				
Q9BUH6	0.2199	9.953080			0.0007631195617		Protein PAXX
	1.0828646		4.942437				
Q16082	0.2549	4.056163			0.0007669115117		Heat shock protein beta-2
	1.2629563		4.971383				
Q8TDB4	0.3846	5.732826			0.0007805116689		Protein MGARP
	2.3381087		6.079718				
P45877	0.2039	9.367147			0.0007918128803		Peptidyl-prolyl cis-trans isomerase C
	0.9878277		4.864079				
Q14314	0.3929	5.064182			0.0008013171		Fibroleukin
	1.9054905		4.850299				
P35052	0.2457	8.504113			0.0008040137		Glypican-1;Secreted glypican-1
	1.2972987		5.278154				
P02461	0.5758	8.373561			0.0008170107114		Collagen alpha-1(III) chain
	2.9334098		5.093632				
P02790	0.2719	9.373505			0.0008327510		Hemopexin
	1.3146905		4.833533				
P01034	0.2965	8.095921			0.0008441316936		Cystatin-C
	1.4676678		4.949824				
Q86VP6	0.2705	9.069745			0.0008861108		Cullin-associated NEDD8-dissociated protein 1
	1.3506010		4.992852				
P40261	0.2999	8.270700			0.0008926		Nicotinamide N-methyltransferase
	1.4439118		4.813234				
Q86VU5	0.3846	29.936174	4.778721		0.0008970108		Catechol O-methyltransferase domain-containing protein 1
P50479	0.4099	8.168814			0.0008989198		PDZ and LIM domain protein 4
	2.0822296		5.084608				
P28300	0.2816	6.741987			0.0009023198		Protein-lysine 6-oxidase
	1.4644405		5.199244				
P06727	0.2198	5.887743			0.0009128A437		Apolipoprotein A-IV
	1.0726685		4.896720				
Q15126	0.2099	2.367147			0.0009201120757		Phosphomevalonate kinase
	0.9960340		4.760285				
O95183	0.3055	6.367147			0.0009372V480		Vesicle-associated membrane protein 5
	1.4512639		4.747910				
Q9HAV4	0.3517	6.117284			0.0009382E480		Exportin-5
	1.8358132		5.219807				
Q9NRX4	0.5059	29.624827	2.768003		0.0009521244		14 kDa phosphohistidine phosphatase
Q96H79	0.3816	3.047712			0.0009452709		Zinc finger CCCH-type antiviral protein 1-like
	2.2370397		5.866853				
O43175	0.4349	7.168272			0.0010087633		D-3-phosphoglycerate dehydrogenase
	2.0597036		4.738201				
O43143	0.1879	9.807147			0.0010112764		Pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15
	0.8789725		4.695622				
P13667	0.2319	8.149006			0.0010332605		Protein disulfide-isomerase A4
	1.0953363		4.731772				
P34932	0.1892	2.738541			0.0010693298		Heat shock 70 kDa protein 4
	0.8861862		4.682936				
Q15582	0.4552	4.064872			0.0010813966		Transforming growth factor-beta-induced protein ig-h3
	2.2645249		4.974325				
Q09161	0.9700	34.116576	0.006010		0.0010883N056		Nuclear cap-binding protein subunit 1

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Q9NY15	0.313	1.4385610		4.594463	0.001	0.714	Stabilin-1
Q9UKX3	0.337				0.001	0.714	Myosin-13
P46940	0.240	1.1105871		4.616963	0.001		Ras GTPase-activating-like protein IQGAP1
P00747	0.180	0.8238672		4.564962	0.001		Plasminogen;Plasmin heavy chain A;Activation peptide;Angiostatin;Plasmin heavy chain A, short form;Plasmin light chain B
P26447	0.319	1.4990741		4.686469	0.001		Protein S100-A4
O60262	0.267	1.2559109		4.694045	0.001		Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-7
Q96CS3	0.249	1.1510089		4.605044	0.001		FAS-associated factor 2
Q6UW68					0.001		Protein PET117 homolog, mitochondrial
P01019	0.223	1.0239723		4.575714	0.001		Angiotensinogen;Angiotensin-1;Angiotensin-2;Angiotensin-3;Angiotensin-4;Angiotensin 1-9;Angiotensin 1-7;Angiotensin 1-5;Angiotensin 1-4
O95445	0.552	2.5298428		4.576159	0.001		Apolipoprotein M
P27658	0.358	1.6133702		4.500743	0.001		Collagen alpha-1(VIII) chain;Vastatin
Q96JB2	0.435	2.0512914		4.708473	0.001		Conserved oligomeric Golgi complex subunit 3
Q07507	0.259	1.2545210		4.829391	0.001		Dermatopontin
Q9BXW4					0.001		Uncharacterized protein C14orf142
P09619	0.274	1.2391198		4.510829	0.001		Platelet-derived growth factor receptor beta
P02748	0.268	1.2229145		4.562276	0.001		Complement component C9;Complement component C9a;Complement component C9b
Q96LL9	0.273	1.2440322		4.552625	0.001		DnaJ homolog subfamily C member 30
Q04721					0.001		Neurogenic locus notch homolog protein 2;Notch 2 extracellular truncation;Notch 2 intracellular domain
Q92930	0.306	1.4774889		4.815434	0.001		Ras-related protein Rab-8B
Q9UQR1	0.359	1.7724910		4.930831	0.001		Zinc finger protein 148
P54577	0.228	1.0184999		4.463276	0.001		Tyrosine–tRNA ligase, cytoplasmic;Tyrosine–tRNA ligase, cytoplasmic, N-terminally processed
O95486	0.311	1.3819063		4.443060	0.001		Protein transport protein Sec24A
Q99983	0.588	2.9528995		5.015589	0.001		Osteomodulin
P83916	0.306	1.6056296		5.243487	0.001		Chromobox protein homolog 1
P03950	0.384	1.7791248		4.628425	0.001		Angiogenin
Q9UBV8	0.255	1.1380629		4.455098	0.001		Peflin
P15924					0.001		Desmoplakin

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Q7Z3T8	0.3058					0.00169011775727	Zinc finger FYVE domain-containing protein 16
	1.3746348		4.504815				
P00352	0.2488					0.00170497R527	Retinal dehydrogenase 1
	1.1346512		4.574033				
Q9H1E5	0.2329					0.00170217T527	Thioredoxin-related transmembrane protein 4
	1.0067111		4.338812				
O75489						0.001723TN52DH	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial
O15061	0.2549					0.00172787527	Synemin
	1.1041834		4.332737				
P62857	0.2882					0.00172187527	40S ribosomal protein S28
	1.3551185		4.701559				
P80723	0.3279					0.00175407587	Brain acid soluble protein 1
	1.5107829		4.607079				
Q9NS69	0.2448					0.00175177587	Mitochondrial import receptor subunit TOM22 homolog
	1.0847694		4.434265				
P19447	0.3333					0.00178298T46	TFIIH basal transcription factor complex helicase XPB subunit
	1.5879548		4.763439				
Q96CX2	0.2093					0.00179065B72B	BTB/POZ domain-containing protein KCTD12
	0.9045513		4.319920				
Q9NS86	0.2292					0.00182918B09C	LanC-like protein 2
	0.9960486		4.345126				
P52907	0.1989					0.00186118F363	F-actin-capping protein subunit alpha-1
	0.8523732		4.283362				
Q08945	0.3088					0.00187185A63	FACT complex subunit SSRP1
	1.3761500		4.467405				
Q9BYN0	0.2979					0.00190187263	Sulfiredoxin-1
	1.2801043		4.307206				
O15118	0.4186					0.00190187263	Niemann-Pick C1 protein
	2.1247916		5.074379				
Q9UBB5						0.00190187263	Methyl-CpG-binding domain protein 2
P62328	0.2882					0.00193287340	Thymosin beta-4;Hematopoietic system regulatory peptide
	1.3455146		4.667628				
Q9Y287	0.3779					0.00193187340	Integral membrane protein 2B;BRI2, membrane form;BRI2 intracellular
	1.6105936		4.267253				domain;BRI2C, soluble form;Bri23 peptide
Q8N142						0.00195278A053	Adenylosuccinate synthetase isozyme 1
Q14118	0.1948					0.00196918B802	Dystroglycan;Alpha-dystroglycan;Beta-dystroglycan
	0.8487363		4.356875				
P82663	0.3163					0.00203192898	28S ribosomal protein S25, mitochondrial
	1.3399129		4.235714				
P31323	0.2175					0.00205709442	cAMP-dependent protein kinase type II-beta regulatory subunit
	0.9232942		4.244697				
Q8NAT1	0.1965					0.00205719P421	Protein O-linked-mannose beta-1,4-N-acetylglucosaminyltransferase 2
	0.8308927		4.226584				
P61009	0.2048					0.00209096584	Signal peptidase complex subunit 3
	0.8882212		4.348111				
Q07065	0.2218					0.00209913584	Cytoskeleton-associated protein 4
	0.9488623		4.280098				
P50991	0.3458					0.00211296878	T-complex protein 1 subunit delta
	1.5101244		4.369937				
P07384	0.2059					0.00217200526	Calpain-1 catalytic subunit
	0.8641607		4.200015				

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Q6IC98	0.2102	2.802633			0.0020872	0.2020204	SAM domain-containing protein 4
	0.9057366	4.307102					
A5D6W6	0.4938	3.725927			0.0022609	0.2050743	Fat storage-inducing transmembrane protein 1
	2.1994339	4.453538					
P05455	0.2049	2.847952			0.0022620	0.2090742	Lupus La protein
	0.8562036	4.191561					
Q96AG4	0.2559	2.796639			0.0029023	0.2303786	Leucine-rich repeat-containing protein 59
	1.0641384	4.157119					
Q12988	0.2428	2.862381			0.0023331	0.2311686	Heat shock protein beta-3
	1.0103210	4.159643					
Q04760	0.3958	3.3186729	82.760510	24.12017654			Lactoylglutathione lyase
P13796	0.2124	4.076454			0.0024322	0.2317898	Plastin-2
	0.8819572	4.151955					
Q8IYI6	0.2279	3.566285			0.0024322	0.2317898	Exocyst complex component 8
	0.9330152	4.108487					
P62277	0.3165	3.727670			0.0024502	0.2140455	40S ribosomal protein S13
	1.3005300	4.108183					
P61925	2.5391	1.311	7.2973224	24.02002460		0.2248A4	cAMP-dependent protein kinase inhibitor alpha
P50238	0.4218	4.017847			0.0024832	0.218598	Cysteine-rich protein 1
	1.7621993	4.182309					
Q9H1K0	0.3567	4.367147			0.0024852	0.2181598	Rabenosyn-5
	1.6022771	4.492591					
Q96A65	0.2036	3.061182			0.0025102	0.2182602	Exocyst complex component 4
	0.8321129	4.086862					
P05543	0.2627	2.095488			0.0025402	0.2227182	Thyroxine-binding globulin
	1.1801143	4.492224					
Q9UK22	0.2916	4.358956			0.0025702	0.2283752	F-box only protein 2
	1.3974278	4.794352					
Q9Y490	0.2099	4.335353			0.0026102	0.2273867	Talin-1
	0.8597753	4.095275					
P62745	0.2865	3.976677			0.0026502	0.2283325	Rho-related GTP-binding protein RhoB
	1.1929691	4.159700					
P24311	1.0563	2.793.9064	1.07440510	26.802290		0.2840	Cytochrome c oxidase subunit 7B, mitochondrial
O00625	0.2729	4.367147			0.0026902	0.23D026	Pirin
	1.1028338	4.040897					
P58546	0.3158	4.695643			0.0027052	0.23N028	Myotrophin
	1.2963604	4.109419					
O75348	0.3199	3.667147			0.0027402	0.23V122	V-type proton ATPase subunit G 1
	1.2891767	4.028827					
Q96FN9	0.4249	3.667147			0.0027502	0.23P182	Probable D-tyrosyl-tRNA(Tyr) deacylase 2
	1.7766077	4.189288					
P04843	0.3685	4.618713			0.0027902	0.23D873	Dolichyl-diphosphooligosaccharide–protein glycosyltransferase subunit 1
	1.5241453	4.132887					
P63316	0.7904	5.3159.73974	1.01637102	8.6237255			Troponin C, slow skeletal and cardiac muscles
Q9UL18	0.3097	3.369316			0.0028602	0.23D255	Protein argonaute-1
	1.3184619	4.261436					
Q9HB40	0.3126	6.302048			0.0028702	0.23R255	Retinoid-inducible serine carboxypeptidase
	1.4736038	4.713116					
Q9Y4W6	0.0568	3.2283.443625002	0.23A2F53				AFG3-like protein 2
Q96C86	0.2119	3.2955986			0.0028802	0.23627G	m7GpppX diphosphatase
	0.8529077	4.035995					

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Q8N5M1	0.3179		72.2062735		0.00288		ATP synthase mitochondrial F1 complex assembly factor 2
	1.2833584		4.039196				
Q96GK7	0.2950		70.857234		0.00289		Fumarylacetoacetate hydrolase domain-containing protein 2A
	1.2557512		4.255426				
Q6P1N0	0.3683		62.029225		0.00293		Coiled-coil and C2 domain-containing protein 1A
	1.6508051		4.481980				
Q3ZCW2	0.3090		65.833204		0.00294		Galectin-related protein
	1.3933792		4.508585				
Q9NQ75	0.3550		69.992820780		0.00294		StAR-related lipid transfer protein 7, mitochondrial
Q9UKS6	0.8052		64.579386		0.00297		Protein kinase C and casein kinase substrate in neurons protein 3
P00492	0.2388		73.9664		0.00297		Hypoxanthine-guanine phosphoribosyltransferase
	0.9642312		4.036570				
Q15125	0.3858		50.578077		0.00299		3-beta-hydroxysteroid-Delta(8),Delta(7)-isomerase
	1.5522594		4.028644				
Q9NNX1	0.4109		73.671.63802		0.00301		Tomoregulin
Q00577	0.2548		76.94439		0.00304		Transcriptional activator protein Pur-alpha
	1.0249972		4.023306				
Q53FA7	0.7508		72.170136		0.00305		Quinone oxidoreductase PIG3
P14555	0.7156		54.863351		0.00323		Phospholipase A2, membrane associated
	3.1616763		4.418573				
Q9UHD9	0.2269		63.827970		0.00325		Ubiquilin-2
	0.8978150		3.965820				
P25311	0.2668		55.328030		0.00326		Zinc-alpha-2-glycoprotein
	1.0782056		4.045301				
O95810	0.1859		43.358276		0.00327		Serum deprivation-response protein
	0.7267426		3.919091				
P09467	0.3327		70.240615		0.00328		Fructose-1,6-bisphosphatase 1
	1.4304382		4.307195				
P50552	0.2329		63.63622		0.00329		Vasodilator-stimulated phosphoprotein
	0.9233883		3.963265				
P53618	0.2333		39.367147		0.00340		Coatomer subunit beta
	0.9076351		3.888800				
P01303	0.3518		76.637212		0.00341		Pro-neuropeptide Y;Neuropeptide Y;C-flanking peptide of NPY
	1.3886725		3.947708				
Q7L4E1	0.4899		69.050716		0.00352		Protein FAM73B
	1.9056994		3.895814				
O15031	0.2329		28.905474		0.00353		Plexin-B2
	0.9094327		3.915175				
Q9BVC6	0.3093		39.367147		0.00353		Transmembrane protein 109
	1.1958920		3.866221				
Q92575	0.2518		40.702687		0.00360		UBX domain-containing protein 4
	0.9922287		3.946701				
P28070	0.2267		50.878722		0.00360		Proteasome subunit beta type-4
	0.9263111		4.089535				
Q8IYU8	0.6834		39.367147		0.00361		Calcium uptake protein 2, mitochondrial
	3.0629900		4.481421				
Q96MM6	0.6084		112.960098754		0.00362		Heat shock 70 kDa protein 12B
Q7KZF4	0.2299		39.367147		0.00368		Staphylococcal nuclease domain-containing protein 1
	0.8794696		3.839895				
P21980	0.2358		42.073692		0.00368		Protein-glutamine gamma-glutamyltransferase 2
	0.9178411		3.898610				

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Q14258	0.2478	1.8575155			0.0037.0217		E3 ubiquitin/ISG15 ligase TRIM25
	0.9919848		4.008255				
A6ND01	0.0710627	79.742383548450			0.0037.82173467		Phosphoglycolate phosphatase
Q8IUX7	0.4997	5.624662			0.0037.9217A44		Adipocyte enhancer-binding protein 1
	2.3978246		4.797989				
P43121	0.2729	1.758685			0.0037.92376447		Cell surface glycoprotein MUC18
	1.0780079		3.949937				
P14625	0.2529	3.049955			0.0037.92575447		Endoplasmin
	0.9671286		3.822935				
P10109	0.708452691.87256490			1003.802734460			Adrenodoxin, mitochondrial
Q9Y2D4	0.3888	3.5259332			0.0038.02677660		Exocyst complex component 6B
	1.5165389		3.905057				
P27695	0.3917	0.367147			0.0038.62077066		DNA-(apurinic or apyrimidinic site) lyase;DNA-(apurinic or
	1.4919668		3.808921				apyrimidinic site) lyase, mitochondrial
E5RK69	0.2963	6.367147			0.0039.37817737		Annexin
	1.1254137		3.797417				
Q96PK6	0.2419	4.765474			0.0039.62482514		RNA-binding protein 14
	0.9252023		3.831903				
Q9Y5U8	0.6529	4.255368			0.0039.72826100		Mitochondrial pyruvate carrier 1
	2.4800568		3.803503				
Q15738	0.2856	3.827147			0.0040.01832890		Sterol-4-alpha-carboxylate 3-dehydrogenase, decarboxylating
	1.0818010		3.787259				
Q9BWJ5	0.3118	3.367147			0.0040.02857018		Splicing factor 3B subunit 5
	1.1786521		3.779657				
Q01581	0.3817	0.263324			0.0040.65285855		Hydroxymethylglutaryl-CoA synthase, cytoplasmic
	1.5736067		4.129965				
P62312	0.4058	7.531421			0.0040.93857286		U6 snRNA-associated Sm-like protein LSm6
	1.5682756		3.863930				
P31949	0.2579	9.019115			0.0041.02787286		Protein S100-A11;Protein S100-A11, N-terminally processed
	0.9710473		3.775501				
P04207	0.3679	0.020032			0.0041.02857286		Ig kappa chain V-III region CLL
	1.4064185		3.822804				
P78406	0.3860	0.267405			0.0041.02862786		mRNA export factor
	1.6990379		4.401581				
P49773	0.2499	3.267147			0.0041.02857286		Histidine triad nucleotide-binding protein 1
	0.9406572		3.765306				
P27169	0.2728	3.335189			0.0041.57286362		Serum paraoxonase/arylesterase 1
	1.0408608		3.824822				
Q13636	0.4476	7.530434			0.0041.90285057		Ras-related protein Rab-31
	1.9750968		4.412876				
Q96EM0	0.3139	3.647348			0.0042.02887057		trans-3-hydroxy-L-proline dehydratase
	1.2281296		3.912034				
Q2TAY7	0.2749	0.572883			0.0042.02887240		WD40 repeat-containing protein SMU1;WD40 repeat-containing protein
	1.0380001		3.787532				SMU1, N-terminally processed
Q9BT09	0.3179	2.852006			0.0042.50287120		Protein canopy homolog 3
	1.2017444		3.787537				
P01699	0.4496	2.048678			0.0042.97229062		Ig lambda chain V-I region VOR
	1.9617461		4.367970				
P00488	0.3059	6.287528			0.0043.01296460		Coagulation factor XIII A chain
	1.1450813		3.745784				
Q04941	0.3559	2.268974			0.0043.02297534		Proteolipid protein 2
	1.3354806		3.759546				

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P01611	-0.3549		147		0.0044		Ig kappa chain V-I region Wes
	1.3210912					3.721864	
P07360	0.2503				0.0044		Complement component C8 gamma chain
	0.9508577					3.797726	
O75165	0.2029				0.0045		DnaJ homolog subfamily C member 13
	0.7578962					3.734455	
Q9BXF6	0.2416		147		0.0045		Rab11 family-interacting protein 5
	0.8964783					3.709638	
Q02818	0.2686				0.0045		Nucleobindin-1
	1.1067621					4.119362	
Q9UNN8	0.3238				0.0045		Endothelial protein C receptor
	1.2379487					3.826899	
Q96KP1	0.2567				0.0045		Exocyst complex component 2
	1.0392328					4.053659	
P46063	0.2065				0.0045		ATP-dependent DNA helicase Q1
	0.7705141					3.730880	
Q9HB...					0.0045		...in-1
P20042	0.2360				0.0046		Eukaryotic translation initiation factor 2 subunit 2
	0.8792944					3.724821	
Q8IVD9	0.2438				0.0046		NUDC domain-containing protein 3
	0.9380220					3.858329	
Q13011					0.0046		Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial
Q5JU...					0.0047		Protein FAM78A
P10643	0.3105				0.0047		Complement component C7
	1.1503065					3.704147	
Q9Y5U9	0.2518				0.0048		Immediate early response 3-interacting protein 1
	0.9688010					3.846636	
P31689	0.3869				0.0048		DnaJ homolog subfamily A member 1
	1.4848105					3.837599	
Q53G...					0.0048		Actin and LIM domain protein 3
Q04837	0.3379				0.0048		Single-stranded DNA-binding protein, mitochondrial
	1.2706968					3.760378	
Q9BU61	0.3533		147		0.0048		NADH dehydrogenase [ubiquinone] 1 alpha subcomplex assembly factor 3
	1.2937160					3.661782	
Q9BXR6	0.3662				0.0048		Complement factor H-related protein 5
	1.3408193					3.660951	
Q08357	0.3308				0.0049		Sodium-dependent phosphate transporter 2
	1.2512681					3.781623	
Q9HCN8	0.2289				0.0049		Stromal cell-derived factor 2-like protein 1
	0.8376149					3.668688	
Q8N...					0.0050		[Protein ADP-ribosylarginine] hydrolase-like protein 1
P61020	0.2252				0.0050		Ras-related protein Rab-5B
	0.8349426					3.706218	
P00918	0.2609				0.0050		Carbonic anhydrase 2
	0.9493414					3.649846	
Q8TDB6	0.2672				0.0050		E3 ubiquitin-protein ligase DTX3L
	0.9819803					3.674197	
P31994	0.2103				0.0050		Low affinity immunoglobulin gamma Fc region receptor II-b
2	0.7713913					3.667677	
Q8NBF2	0.2239				0.0050		NHL repeat-containing protein 2
	0.8115466					3.636582	

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O00299	0.2519.4251612	0.9153236		3.640298	0.005	0.55160076	Chloride intracellular channel protein 1
Q9UBI9	0.4887.8078007	1.9350473		3.963798	0.005	0.66811036	Headcase protein homolog
O75663	0.4618.7036339	1.6975991		3.674689	0.005	0.79850741	TIP41-like protein
Q9Y5J7	0.3979.5307147	1.4394711		3.620850	0.005	2.09716947	Mitochondrial import inner membrane translocase subunit Tim9
Q8WUM0	0.2183.8223881	0.7928646		3.630508	0.005	2.62319115	Nuclear pore complex protein Nup133
O15121	0.3779.1175551	1.3734454		3.634300	0.005	2.76719...	Sphingolipid delta(4)-desaturase DES1
P00742	0.3246.7365924	1.3361965		4.123118	0.005	4.52928709	Coagulation factor X;Factor X light chain;Factor X heavy chain;Activated factor Xa heavy chain
P46777	0.2885.7503996	1.0578875		3.665643	0.005	4.93360263	60S ribosomal protein L5
Q6P1L8	0.685065390.5435407010				0.005	5.28533949	39S ribosomal protein L14, mitochondrial
P49756	0.2438.2607147	0.8733625		3.581909	0.005	5.08337349	RNA-binding protein 25
Q15493	0.4378.9452542	1.5865116		3.625528	0.005	5.723520...	Regucalcin
O00567	0.4490.2892457	1.6066654		3.577936	0.005	6.51335702	Nucleolar protein 56
Q9H4A6	0.2769.2579941	0.9863755		3.573364	0.005	7.05327999	Golgi phosphoprotein 3
Q6PCE3	0.3388.3017566	1.3068366		3.856780	0.005	7.43338422	Glucose 1,6-bisphosphate synthase
P24752	0.281460.17258.20625.876631057.4632A442				0.005		Acetyl-CoA acetyltransferase, mitochondrial
O15144	0.2838.0798089	1.0266458		3.622551	0.005	7.6253A42	Actin-related protein 2/3 complex subunit 2
Q9Y646	0.1939.0221032	0.6906706		3.561953	0.005	8.43942504	Carboxypeptidase Q
Q1KMD3	0.2678.7192210	0.9588418		3.579466	0.005	9.12347833	Heterogeneous nuclear ribonucleoprotein U-like protein 2
P06396	0.3168.5401875	1.1557324		3.651024	0.005	9.6842C206	Gelsolin
P56199	0.2438.6538476	0.8817502		3.623178	0.005	9.90617206	Integrin alpha-1
P62330	0.2229.4869563	0.7931556		3.564969	0.005	9.9734A206	ADP-ribosylation factor 6
Q9HAN9	0.390673213.52673.826070060.0324N73				0.006		Nicotinamide/nicotinic acid mononucleotide adenylyltransferase 1
Q96PE7	0.3259.3087147	1.1472298		3.528421	0.006	0.4384V73	Methylmalonyl-CoA epimerase, mitochondrial
P17050	0.2669.9182996	0.9454418		3.541005	0.006	0.9205A428	Alpha-N-acetylgalactosaminidase
Q9UBS4	0.4938.9281571	1.7470606		3.540916	0.006	3.036D080	DnaJ homolog subfamily B member 11
Q9H9B4	0.2178.4705504	0.7776690		3.573084	0.006	3.2505080	Sideroflexin-1
Q8TCJ2	0.2449.8667863	0.8698436		3.551625	0.006	3.4736D307	Dolichyl-diphosphooligosaccharide–protein glycosyltransferase subunit STT3B

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P62191	0.2849	5.2748632		0.00604036249			26S protease regulatory subunit 4
	1.0000517		3.514782				
Q9Y5Z7	0.4676	6.5163248		0.00641206496			Host cell factor 2
	1.9019574		4.071384				
P01597	0.6335	7.9289760		0.00648261908			Ig kappa chain V-I region DEE
	2.2333895		3.524349				
P23284	0.2198	5.9229246		0.00652087045			Peptidyl-prolyl cis-trans isomerase B
	0.7744408		3.527355				
P05062	0.5510	7.3514182		0.00655955200			Fructose-bisphosphate aldolase B
	2.0647894		3.747246				
P60903	0.2379	6.0048479		0.00659036880			Protein S100-A10
	0.8289441		3.496770				
O43920	0.28296	8.3798822	5.4690506	0.0066036800			NADH dehydrogenase [ubiquinone] iron-sulfur protein 5
Q68DH5	0.2738	7.291375		0.00662036880			LRR and PDZ domain-containing protein 2
	0.9839434		3.595361				
P08754	0.2979	6.357147		0.00668320621			Guanine nucleotide-binding protein G(k) subunit alpha
	1.0292580		3.465194				
O75643	0.2103	6.237147		0.00669806923			U5 small nuclear ribonucleoprotein 200 kDa helicase
	0.7286175		3.464284				
Q9HAV7	0.5303364	6.153200	5.706031062				GrpE protein homolog 1, mitochondrial
P15848	0.2005557	6.6073637003	0.01369461				Arylsulfatase B
P30050	0.3259	7.87400		0.00733698812			60S ribosomal protein L12
	1.1309945		3.479147				
P17540	0.2104867	7.2452502	0.0072870568				Creatine kinase S-type, mitochondrial
P27797	0.2533	6.384182		0.00679370568			Calreticulin
	0.8755252		3.455605				
O14807	0.2835	5.524869		0.00681317568			Ras-related protein M-Ras
	0.9860449		3.477921				
O43290	0.2153	5.367147		0.00682037568			U4/U6.U5 tri-snRNP-associated protein 1
	0.7436611		3.452549				
P98160	0.2009	8.252685		0.00683378568			Basement membrane-specific heparan sulfate proteoglycan core protein;Endorepellin;LG3 peptide
	0.6944147		3.468908				
Q9GZY4	0.4427	8.728737		0.00689340927			Cytochrome c oxidase assembly factor 1 homolog
	1.6172207		3.650753				
Q16204	0.2178	8.402720		0.00690340927			Coiled-coil domain-containing protein 6
	0.7607082		3.505328				
Q14019	0.5976	8.367147		0.00691096095			Coactosin-like protein
	2.3417241		3.921945				
O75190	0.22303569.3073.4346000	0.0702827561					DnaJ homolog subfamily B member 6
	3						
Q9Y623	0.5628	8.336159		0.00703787619			Myosin-4
	1.9955095		3.547614				
P30101	0.2143	3.348665		0.00704237600			Protein disulfide-isomerase A3
	0.7361350		3.435003				
Q12907	0.4678	2.280767		0.00709347356			Vesicular integral-membrane protein VIP36
	1.6602075		3.553352				
O75533	0.2189	9.028009		0.00723738511			Splicing factor 3B subunit 1
	0.7480960		3.429955				
P21281	0.3926	8.324422		0.00724738581			V-type proton ATPase subunit B, brain isoform
	1.3428216		3.419588				
Q6ZVF9	0.3683	4.157648		0.00731636145			G protein-regulated inducer of neurite outgrowth 3
	1.5316163		4.158478				

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P41208	0.3219	1.1048997	3.432766	8.6683594	0.0073928		Centrin-2
P32119	0.2447	0.8759792	3.580284	6.8743882	0.0074123		Peroxiredoxin-2
P55735	0.2648	0.9148870	3.458196	5.5912215	0.0075683		Protein SEC13 homolog
P56192	0.2978	1.0251690	3.446200	4.7817363	0.0075908		Methionine–tRNA ligase, cytoplasmic
Q5VUM1	0.5887				0.0075980		Succinate dehydrogenase assembly factor 4, mitochondrial
Q8NBJ5	0.2929	1.0090957	3.455259	9.4668730	0.0076039		Procollagen galactosyltransferase 1
O43707	0.3948	1.3709413	3.471934	8.5691412	0.0076653		Alpha-actinin-4
P11021	0.2026	0.6930674	3.419941	6.5963582	0.0076708		78 kDa glucose-regulated protein
P02749	0.2878	0.9821379	3.420999	9.9413313	0.0076870		Beta-2-glycoprotein 1
Q92804	0.5367				0.0077509		...ein subfamily A member 1
Q96G03	0.2599	0.8738839	3.373274	9.6317147	0.0077603		Phosphoglucomutase-2
Q9UK41	0.4609	1.5562781	3.377097	8.3215373	0.0078930		Vacuolar protein sorting-associated protein 28 homolog
P61026	0.2417	0.8659400	3.578803	6.5368968	0.0079264		Ras-related protein Rab-10
P43686	0.3872	1.2985795	3.353104	2.7368890	0.0080462		26S protease regulatory subunit 6B
Q6Y288	0.3177	1.0690665	3.365585	6.4665175	0.0080540		Beta-1,3-glucosyltransferase
Q9BVG4	0.4279	1.4282890	3.344717	9.2866464	0.0080305		Protein PBDC1
Q9UBQ0	0.3287	1.1043560	3.359625	7.1486452	0.0080549		Vacuolar protein sorting-associated protein 29
Q6ICB0	0.5476	2.0382992	3.722843	6.5142201	0.0080665		Desumoylating isopeptidase 1
O60568	0.3199	1.0635431	3.327439	9.2317147	0.0083045		Procollagen-lysine,2-oxoglutarate 5-dioxygenase 3
Q5T447	0.3034	1.0258262	3.380520	8.5226996	0.0083641		E3 ubiquitin-protein ligase HECTD3
P17174	0.1826				0.0083861		Aspartate aminotransferase, cytoplasmic
Q14011	0.4678	1.6060873	3.434499	8.2373933	0.0085106		Cold-inducible RNA-binding protein
Q9BSH5	0.2408	0.8065712	3.355692	8.5925093	0.0085472		Haloacid dehalogenase-like hydrolase domain-containing protein 3
Q9HCJ6	0.2629	0.8677868	3.310310	9.4674182	0.0086042		Synaptic vesicle membrane protein VAT-1 homolog-like
P16455	0.2335	0.7988054	3.417997	7.0298415	0.0086284		Methylated-DNA–protein-cysteine methyltransferase
Q9BX97	0.3968	1.3847012	3.489081	8.6704864	0.0087082		Plasmalemma vesicle-associated protein
Q96LD4	0.5325	2.0603233	3.871537	5.7894822	0.0087104		Tripartite motif-containing protein 47

	logFC	se	df	t	pval	adjP	rowData(pe[["proteinRobust"]])$Protein.names
P29992	-0.2948	7.358031			0.0087		Guanine nucleotide-binding protein subunit alpha-11
	1.0018710		3.399222				
P05387	-0.2249	8.268344			0.0087		60S acidic ribosomal protein P2
	0.7447264		3.311871				
P51692	-0.5109	9.016817			0.0088		Signal transducer and activator of transcription 5B
	1.6950637		3.323333				
O60927	-0.2647	7.292336			0.0089		Protein phosphatase 1 regulatory subunit 11
	0.9472893		3.578333				
A4D2B0	0.5058	8.913870			0.0089		Metallo-beta-lactamase domain-containing protein 1
	1.7140517		3.388464				
P16083	-0.2968	8.955055			0.0089		Ribosyldihydronicotinamide dehydrogenase [quinone]
	1.0028849		3.383609				
Q13425	-0.2314	9.254288			0.0090		Beta-2-syntrophin
	0.7615654		3.289806				
I3L505	1.9203073	7.208336			0.0090		Acyl carrier protein
Q9NX08	0.2206	8.245229			0.0093		COMM domain-containing protein 8
	0.7274661		3.297295				
Q96CN7	0.3099	9.038448			0.0093		Isochorismatase domain-containing protein 1
	1.0144660		3.278800				
O43592	0.2463	9.074135			0.0094		Exportin-T
	0.8026366		3.258736				
Q8N293	2.9550967	8.188960			0.0095		Rho GTPase-activating protein 18
P01621	-0.3618	7.997835			0.0095		Ig kappa chain V-III region NG9
	1.1890923		3.286787				
Q9UMR3	0.4717	7.357147			0.0096		T-box transcription factor TBX20
	1.6328959		3.465748				
P35754	1.8370725	9.723247			0.0097		Glutaredoxin-1
O60503	0.2570238	6.534368			0.0097		Adenylate cyclase type 9
Q13825	2.3940923	8.361720			0.0099		Methylglutaconyl-CoA hydratase, mitochondrial
O95159	0.5278	8.107934			0.0100		Zinc finger protein-like 1
	1.7663091		3.350868				
Q8IWU2	0.2118	9.016368			0.0100		Serine/threonine-protein kinase LMTK2
	0.7035098		3.333113				
Q6B0K9	0.3993	7.051037			0.0100		Hemoglobin subunit mu
	1.3448839		3.368015				
Q13541	4.9176128	8.367206			0.0100		Eukaryotic translation initiation factor 4E-binding protein 1
Q6UXG3	4.8704517	9.123796			0.0101		CMRF35-like molecule 9
P03915	-0.3488	8.364164			0.0102		NADH-ubiquinone oxidoreductase chain 5
	1.1507202		3.299231				
P14174	0.2608	9.071113			0.0103		Macrophage migration inhibitory factor
	0.8418646		3.232881				
P34947	0.4397	7.378356			0.0103		G protein-coupled receptor kinase 5
	1.4080509		3.202173				
O75436	0.3055	7.898092			0.0105		Vacuolar protein sorting-associated protein 26A
	0.9929865		3.247407				
P19474	0.3407	8.075271			0.0105		E3 ubiquitin-protein ligase TRIM21
	1.1365988		3.335584				
O14656	0.5548	7.367147			0.0105		Torsin-1A
	1.8183830		3.278015				
Q08AG7	0.3487	9.108164			0.0105		Mitotic-spindle organizing protein 1
	1.1984106		3.440703				

	logFC	se	df	t	pval	adjPval	rowData(pe[["proteinRobust"]])$Protein.names
P05060	-0.6787	0.2060747	2.3136323	0.010063948883	3.412414		Secretogranin-1;PE-11;GAWK peptide;CCB peptide
Q08211	-0.2199	0.2854182	0.6963521	0.010605743839	3.179276		ATP-dependent RNA helicase A
Q0VAK6	3.0906208.7305258791107.04421.581						Leiomodin-3
P51665	-0.2455	0.2942310	0.7816374	0.010768492639	3.182703		26S proteasome non-ATPase regulatory subunit 7
Q9H2J4	0.2609	0.3594182	0.8248510	0.010790749921	3.171334		Transducin-like protein 3
Q8IWB6	0.5099817.2457.331080108235W022						WD repeat and FYVE domain-containing protein 1
P51970	0.746092390543.1200056109.0143N19DH						NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8
Q15691	0.2778	0.3209540	0.9045387	0.010909891190	3.261696		Microtubule-associated protein RP/EB family member 1

## 4.6 Interaction

4.6.1 Volcano-plot

```r
volcanoInt <- ggplot(rowData(pe[["proteinRobust"]])$"locationR:tissueV",
               aes(x = logFC, y = -log10(pval), color = adjPval < 0.05)) +
 geom_point(cex = 2.5) +
 scale_color_manual(values = alpha(c("black", "red"), 0.5)) + theme_minimal()
volcanoInt
```
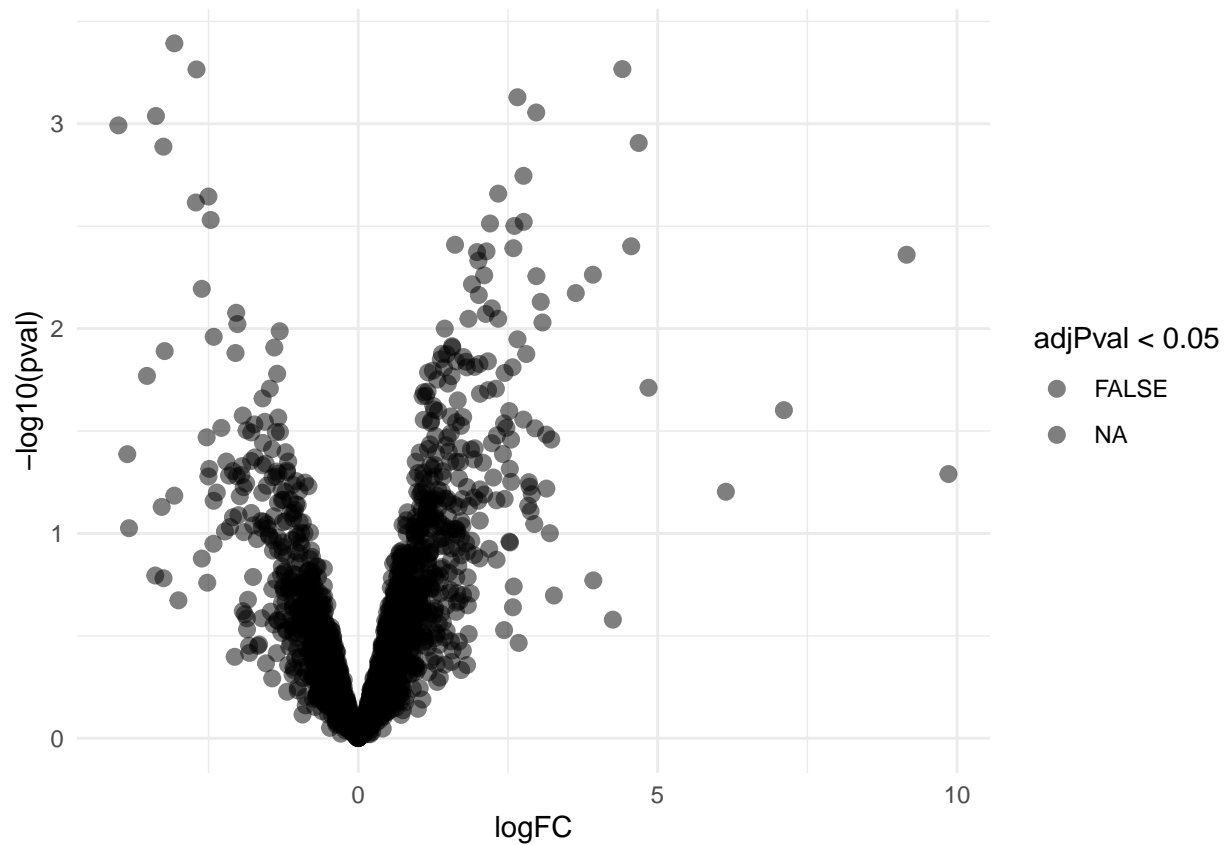
4.6.2 Heatmap

There were no genes significant at the 5% FDR level. We return the top 25 genes.

```
sigNamesInt <- rowData(pe[["proteinRobust"]])$"locationR:tissueV" %>%
 rownames_to_column("proteinRobust") %>%
 filter(adjPval<0.05) %>%
 pull(proteinRobust)
hlp <- order((rowData(pe[["proteinRobust"]])$"locationR:tissueV")[,"adjPval"])[1:25]
heatmap(assay(pe[["proteinRobust"]])[hlp, ])
```
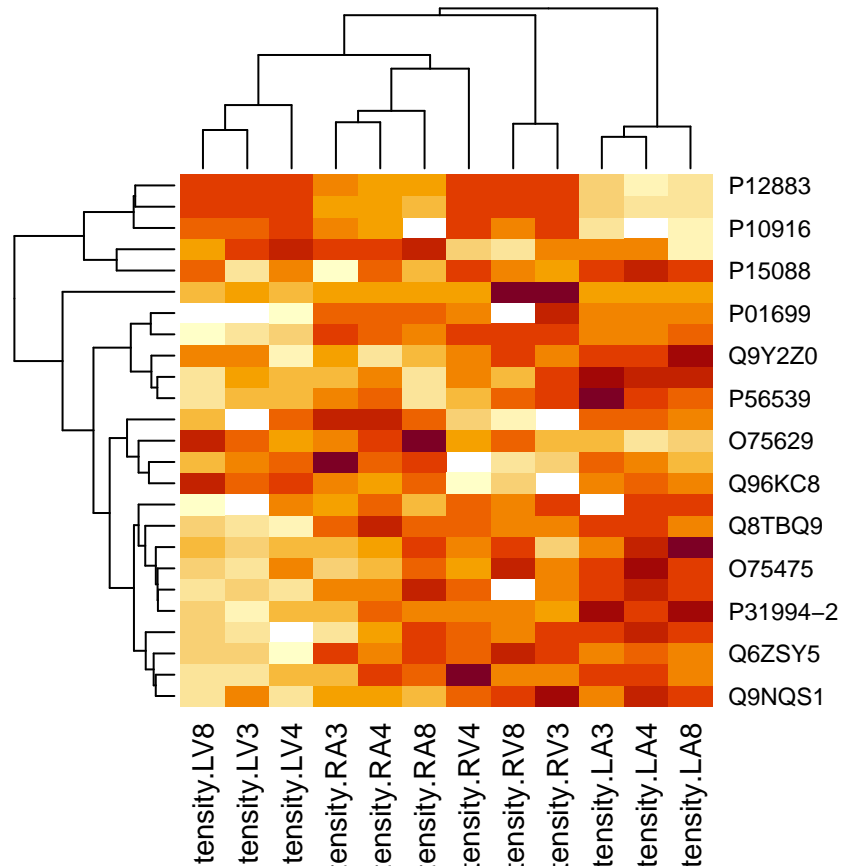
There are 0 proteins significantly differentially expressed at the 5% FDR level.

```
rowData(pe[["proteinRobust"]])$"locationR:tissueV" %>%
  cbind(.,rowData(pe[["proteinRobust"]])$Protein.names) %>%
  na.exclude %>%
  filter(adjPval<0.05) %>%
  arrange(pval)
```

```
## [1] logFC
## [2] se
## [3] df
## [4] t
## [5] pval
## [6] adjPval
## [7] rowData(pe[["proteinRobust"]])$Protein.names
## <0 rows> (or 0-length row.names)
```

5 Large difference in number of proteins that are returned

Note, that much more proteins are returned significant for average contrast ($\log_2 FC_{V-A}$) as compared to contrast for assessing the fold change between ventriculum and atrium left and right. The power for the average contrast is larger than for the contrast left or right because the log2 FC can be estimated with higher precision.

For none of the proteins the interaction was significant (change in log2 FC between ventriculum and atrium in the right vs the left heart region). The power for the interaction is typically low.

5.1 Reason

Part of variance covariance matrix of model parameters due to design:

```
X <- model.matrix(~ location*tissue + patient, colData(pe))
covarUnscaled <- solve(t(X) %*% X)
```

Variance of contrasts (diagonal elements) due to design

```
varContrasts <- t(L)%*%covarUnscaled%*%L %>%
  diag
varContrasts
```

```
##                              tissueV        tissueV + locationR:tissueV
##                            0.6666667                          0.6666667
## tissueV + 0.5 * locationR:tissueV                    locationR:tissueV
##                            0.3333333                          1.3333333
```

```
sqrt(varContrasts)
```

```
##                              tissueV        tissueV + locationR:tissueV
##                            0.8164966                          0.8164966
## tissueV + 0.5 * locationR:tissueV                    locationR:tissueV
##                            0.5773503                          1.1547005
```

So it is clear that the standard error of the log2 FC left and right is the same. That of the average contrast is a factor $\sqrt{2}$ smaller! Indeed, we use double the number of samples to estimate it!

```
varContrasts[3]/varContrasts[2]
```

```
## tissueV + 0.5 * locationR:tissueV
##                               0.5
```

```
sqrt(varContrasts)[3]/sqrt(varContrasts)[2]
```

```
## tissueV + 0.5 * locationR:tissueV
##                         0.7071068
```

```
1/sqrt(2)
```

```
## [1] 0.7071068
```

The standard error of the interaction is a factor $\sqrt{2}$ larger than that of the main effects!

```r
varContrasts[4]/varContrasts[2]
```

```
## locationR:tissueV
##                 2
```

```r
sqrt(varContrasts)[4]/sqrt(varContrasts)[2]
```

```
## locationR:tissueV
##          1.414214
```

```r
sqrt(2)
```

```
## [1] 1.414214
```

5.2 Msqrob

This is not the case for the standard errors of protein 2???

```r
rowData(pe[["proteinRobust"]])$"tissueV"[2,]
```

```
##           logFC       se       df        t     pval   adjPval
## A0PJW6 0.2355243 0.556499 7.924465 0.423225 0.683383 0.8078152
```

```r
rowData(pe[["proteinRobust"]])$"tissueV + locationR:tissueV"[2,]
```

```
##           logFC        se       df         t      pval   adjPval
## A0PJW6 0.1822338 0.6124218 7.924465 0.2975626 0.7736885 0.8763294
```

```r
rowData(pe[["proteinRobust"]])$"tissueV + 0.5 * locationR:tissueV"[2,]
```

```
##           logFC        se       df         t      pval   adjPval
## A0PJW6 0.2088791 0.4126595 7.924465 0.5061778 0.6265101 0.7376959
```

```r
rowData(pe[["proteinRobust"]])$"locationR:tissueV"[2,]
```

```
##             logFC        se       df           t     pval   adjPval
## A0PJW6 -0.05329048 0.8296697 7.924465 -0.06423095 0.950377 0.9949944
```

Because msqrob is using robust regression to assess DE!

```r
pe %>%
  colData %>%
  as_tibble %>%
  mutate(w=getModel(rowData(pe[["proteinRobust"]])$msqrobModels[[2]])$w)
```

```
## # A tibble: 12 x 4
##    location tissue patient     w
##    <fct>    <fct>  <fct>   <dbl>
##  1 L        A      3       0.966
##  2 L        A      4       1
##  3 L        A      8       0.667
##  4 L        V      3       1
##  5 L        V      4       1
##  6 L        V      8       1
##  7 R        A      3       1
##  8 R        A      4       1
##  9 R        A      8       1
## 10 R        V      3       0.485
## 11 R        V      4       1
## 12 R        V      8       0.440
```

For protein 2 the samples at the left and right side have different weights!

5.2.1 Part of standard error due to design:

```
covUnscaledRobust <- solve(
  t(X) %*%
  diag(
    getModel(rowData(pe[["proteinRobust"]])$msqrobModels[[2]])$w) %*% X)

varContrastsRobust <- t(L)%*%covUnscaledRobust%*%L %>%
  diag
varContrastsRobust
```

```
##                                tissueV    tissueV + locationR:tissueV
##                              0.7161526                      0.8673176
## tissueV + 0.5 * locationR:tissueV               locationR:tissueV
##                              0.3937862                      1.5917957
```

```
sqrt(varContrastsRobust)
```

```
##                                tissueV    tissueV + locationR:tissueV
##                              0.8462580                      0.9312989
## tissueV + 0.5 * locationR:tissueV               locationR:tissueV
##                              0.6275239                      1.2616639
```

5.2.2 Standard errors Contrasts

```
sqrt(varContrastsRobust) * getSigmaPosterior(rowData(pe[["proteinRobust"]])$msqrobModels[[2]])
```

```
##                                tissueV    tissueV + locationR:tissueV
##                              0.5564990                      0.6124218
## tissueV + 0.5 * locationR:tissueV               locationR:tissueV
##                              0.4126595                      0.8296697
```

```
rowData(pe[["proteinRobust"]])$"tissueV"[2,]
```

```
##            logFC       se       df        t     pval   adjPval
## A0PJW6 0.2355243 0.556499 7.924465 0.423225 0.683383 0.8078152
```

```
rowData(pe[["proteinRobust"]])$"tissueV + locationR:tissueV"[2,]
```

```
##            logFC        se       df         t      pval   adjPval
## A0PJW6 0.1822338 0.6124218 7.924465 0.2975626 0.7736885 0.8763294
```

```
rowData(pe[["proteinRobust"]])$"tissueV + 0.5 * locationR:tissueV"[2,]
```

```
##            logFC        se       df         t      pval   adjPval
## A0PJW6 0.2088791 0.4126595 7.924465 0.5061778 0.6265101 0.7376959
```

```
rowData(pe[["proteinRobust"]])$"locationR:tissueV"[2,]
```

```
##              logFC        se       df           t     pval   adjPval
## A0PJW6 -0.05329048 0.8296697 7.924465 -0.06423095 0.950377 0.9949944
```