S2: MicroRNA associated to single drug components of R-CHOP identifies diffuse large B-cell lymphoma patients with poor outcome and adds prognostic value to the international prognostic index

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Load Libraries

```
library(affy)
library(limma)
library(knitr)
library(ggbiplot)
library(venn)
library(readxl)
library(tidyr)
library(sva)
library(survival)
library(survminer)
library(MASS)
library(randomForestSRC)
library(pec)
library(timeROC)
library(gridExtra)
library(VennDiagram)
library(devtools)
library(prodlim)
```

```
session_info()
```

```
## - Session info -------------------------------------------------------------
##  setting  value
##  version  R version 3.6.1 (2019-07-05)
##  os       Windows 10 x64
##  system   x86_64, mingw32
##  ui       RTerm
##  language (EN)
##  collate  Danish_Denmark.1252
##  ctype    Danish_Denmark.1252
##  tz       Europe/Paris
##  date     2020-01-06
##
## - Packages -----------------------------------------------------------------
##  ! package         * version    date       lib
##    affy            * 1.63.0     2019-05-14 [1]
##    affyio            1.55.0     2019-05-14 [1]
##    annotate          1.63.0     2019-05-14 [1]
```

```
##    AnnotationDbi     1.47.1    2019-08-20 [1]
##    assertthat        0.2.1     2019-03-21 [1]
##    backports         1.1.4     2019-04-10 [1]
##    Biobase         * 2.45.0    2019-05-14 [1]
##    BiocGenerics    * 0.31.5    2019-07-03 [1]
##    BiocManager       1.30.4    2018-11-13 [1]
##    BiocParallel    * 1.19.0    2019-05-02 [1]
##    bit               1.1-14    2018-05-29 [1]
##    bit64             0.9-7     2017-05-08 [1]
##    bitops            1.0-6     2013-08-17 [1]
##    blob              1.2.0     2019-07-09 [1]
##    broom             0.5.2     2019-04-07 [1]
##    callr             3.3.1     2019-07-18 [1]
##    cellranger        1.1.0     2016-07-27 [1]
##    cli               1.1.0     2019-03-19 [1]
##    codetools         0.2-16    2018-12-24 [1]
##    colorspace        1.4-1     2019-03-18 [1]
##    crayon            1.3.4     2017-09-16 [1]
##    data.table        1.12.2    2019-04-07 [1]
##    DBI               1.0.0     2018-05-02 [1]
##    desc              1.2.0     2018-05-01 [1]
##    devtools        * 2.1.0     2019-07-06 [1]
##    digest            0.6.20    2019-07-04 [1]
##    dplyr             0.8.3     2019-07-04 [1]
##    evaluate          0.14      2019-05-28 [1]
##    foreach           1.4.7     2019-07-27 [1]
##    formatR           1.7       2019-06-11 [1]
##    fs                1.3.1     2019-05-06 [1]
##    futile.logger   * 1.4.3     2016-07-10 [1]
##    futile.options    1.0.1     2018-04-20 [1]
##    genefilter      * 1.67.1    2019-05-14 [1]
##    generics          0.0.2     2018-11-29 [1]
##    ggbiplot        * 0.55      2018-08-31 [1]
##    ggplot2         * 3.2.1     2019-08-10 [1]
##    ggpubr          * 0.2.3     2019-09-03 [1]
##    ggsignif          0.6.0     2019-08-08 [1]
##    glue              1.3.1     2019-03-12 [1]
##    gridExtra       * 2.3       2017-09-09 [1]
##    gtable            0.3.0     2019-03-25 [1]
##    htmltools         0.3.6     2017-04-28 [1]
##    IRanges           2.19.14   2019-08-25 [1]
##    iterators         1.0.12    2019-07-26 [1]
##    km.ci             0.5-2     2009-08-30 [1]
##    KMsurv            0.1-5     2012-12-03 [1]
##    knitr           * 1.24      2019-08-08 [1]
##    lambda.r          1.2.3     2018-05-17 [1]
##  D lattice           0.20-38   2018-11-04 [1]
##    lava              1.6.6     2019-08-01 [1]
##    lazyeval          0.2.2     2019-03-15 [1]
##    limma           * 3.41.15   2019-07-24 [1]
##    magrittr        * 1.5       2014-11-22 [1]
##    MASS            * 7.3-51.4  2019-03-31 [1]
##  D Matrix            1.2-17    2019-03-22 [1]
##    matrixStats       0.54.0    2018-07-23 [1]
```

```
##    memoise           1.1.0      2017-04-21 [1]
##  D mgcv            * 1.8-28     2019-03-21 [1]
##    munsell           0.5.0      2018-06-12 [1]
##    mvtnorm           1.0-11     2019-06-19 [1]
##    nlme              3.1-141    2019-08-01 [1]
##    numDeriv          2016.8-1.1 2019-06-06 [1]
##    pec               2018.07.26 2018-07-26 [1]
##    pillar            1.4.2      2019-06-29 [1]
##    pkgbuild          1.0.5      2019-08-26 [1]
##    pkgconfig         2.0.2      2018-08-16 [1]
##    pkgload           1.0.2      2018-10-29 [1]
##    plyr              1.8.4      2016-06-08 [1]
##    preprocessCore    1.47.1     2019-05-14 [1]
##    prettyunits       1.0.2      2015-07-13 [1]
##    processx          3.4.1      2019-07-18 [1]
##    prodlim           2018.04.18 2018-04-18 [1]
##    ps                1.3.0      2018-12-21 [1]
##    purrr             0.3.3      2019-10-18 [1]
##    R6                2.4.0      2019-02-14 [1]
##    randomForestSRC   2.9.1      2019-07-08 [1]
##    Rcpp              1.0.2      2019-07-25 [1]
##    RCurl             1.95-4.12  2019-03-04 [1]
##    readxl            1.3.1      2019-03-13 [1]
##    remotes           2.1.0      2019-06-24 [1]
##    rlang             0.4.1      2019-10-24 [1]
##    rmarkdown         1.15       2019-08-21 [1]
##    rprojroot         1.3-2      2018-01-03 [1]
##    RSQLite           2.1.2      2019-07-24 [1]
##    S4Vectors         0.23.19    2019-08-21 [1]
##    scales            1.0.0      2018-08-09 [1]
##    sessioninfo       1.1.1      2018-11-05 [1]
##    stringi           1.4.3      2019-03-12 [1]
##    stringr           1.4.0      2019-02-10 [1]
##    survival          2.44-1.1   2019-04-01 [1]
##    survminer         0.4.6      2019-09-03 [1]
##    survMisc          0.5.5      2018-07-05 [1]
##    sva               3.33.1     2019-05-22 [1]
##    testthat          2.2.1      2019-07-25 [1]
##    tibble            2.1.3      2019-06-06 [1]
##    tidyr             0.8.3      2019-03-01 [1]
##    tidyselect        0.2.5      2018-10-11 [1]
##    timereg           1.9.4      2019-07-30 [1]
##    timeROC           0.3        2015-03-25 [1]
##    usethis           1.5.1      2019-07-04 [1]
##    vctrs             0.2.0      2019-07-05 [1]
##    venn              1.7        2018-07-31 [1]
##    VennDiagram       1.6.20     2018-03-28 [1]
##    withr             2.1.2      2018-03-15 [1]
##    xfun              0.9        2019-08-21 [1]
##    XML               3.98-1.20  2019-06-06 [1]
##    xtable            1.8-4      2019-04-21 [1]
##    yaml              2.2.0      2018-07-25 [1]
##    zeallot           0.1.0      2018-01-28 [1]
##    zlibbioc          1.31.0     2019-05-14 [1]
```

```
##    zoo               1.8-6      2019-05-28 [1]
##  source
##  Bioconductor
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##  CRAN (R 3.6.1)
```

```
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##  CRAN (R 3.6.0)
##  CRAN (R 3.6.0)
##  CRAN (R 3.6.1)
##  Bioconductor
##  CRAN (R 3.6.0)
##
## [1] C:/Users/y12c/Documents/R/win-library/3.6
## [2] C:/Program Files/R/R-3.6.1/library
##
##  D -- DLL MD5 mismatch, broken installation.
```

Set GGPLOT2 colors

```
#options(
#  ggplot2.continuous.colour = "viridis",
#  ggplot2.continuous.fill = "viridis"
#)
```

Custom functions

```
microarrayScale <- function(x, center = "median", scale = "sd") {
  if (class(x) == "ExpressionSet") {
    x.m <- affy::exprs(x)
  } else {
    x.m <- x
  }

  if(center == "median") {
    x.m <-  x.m - matrixStats::rowMedians(x.m,  na.rm = TRUE)
  }
  if(center == "mean") {
    x.m <-  x.m - rowMeans(x.m,  na.rm = TRUE)
  }
  if (scale == "sd") {
    x.m <- x.m / rowSds(x.m, na.rm = TRUE)
  }
  if (class(x) == "ExpressionSet") {
    Biobase::exprs(x)  <- x.m
  } else {
    x <- x.m
  }

  return(x)
}

cutFun <- function(x) {
cut(x,
breaks=c(-Inf, quantile(x, c(1/3, 2/3), na.rm = TRUE), Inf),
labels = c("Low", "Medium","High"))
}
```

Differential Expression Analysis

Load CEL files

```
cel.files <- just.rma(list.files("../ExternalData/Cell lines/CEL/All/", full.names = TRUE))
```

```
## Warning in just.rma(list.files("../ExternalData/Cell lines/CEL/All/", full.names = TRUE)): Incompatil
```

```
## Warning: replacing previous import 'AnnotationDbi::tail' by 'utils::tail'
## when loading 'mirna102xgaincdf'
```

```
## Warning: replacing previous import 'AnnotationDbi::head' by 'utils::head'
## when loading 'mirna102xgaincdf'
```

```
##
```

```
cel.files
```

```
## ExpressionSet (storageMode: lockedEnvironment)
## assayData: 7815 features, 15 samples
##   element names: exprs, se.exprs
## protocolData
##   sampleNames: DB_(miRNA-1_0_2Xgain).CEL
##     FARAGE_M_(miRNA-1_0_2Xgain).CEL ...
##     U2932_M_(miRNA-1_0_2Xgain).CEL (15 total)
##   varLabels: ScanDate
##   varMetadata: labelDescription
## phenoData
##   sampleNames: DB_(miRNA-1_0_2Xgain).CEL
##     FARAGE_M_(miRNA-1_0_2Xgain).CEL ...
##     U2932_M_(miRNA-1_0_2Xgain).CEL (15 total)
##   varLabels: sample
##   varMetadata: labelDescription
## featureData: none
## experimentData: use 'experimentData(object)'
## Annotation: mirna102xgain
```

Extract expression matrix and rename samples

```
#Expression
expr <- exprs(cel.files)

#Select human miRNAs
hsa.miRNA <- expr[grep("hsa-", rownames(expr)), ]
nrow(hsa.miRNA)
```

```
## [1] 847
```

```
colnames(hsa.miRNA) <- sapply(strsplit(colnames(hsa.miRNA), "_"), function(x) x[1])
colnames(hsa.miRNA)
```

```
##  [1] "DB"       "FARAGE"   "HBL-1"    "MC-116"   "NU-DHL-1" "NU-DUL-1"
##  [7] "OCI-Ly19" "OCI-Ly3"  "OCI-Ly7"  "OCI-Ly8"  "RIVA"     "SU-DHL-4"
## [13] "SU-DHL-5" "SU-DHL-8" "U2932"
```

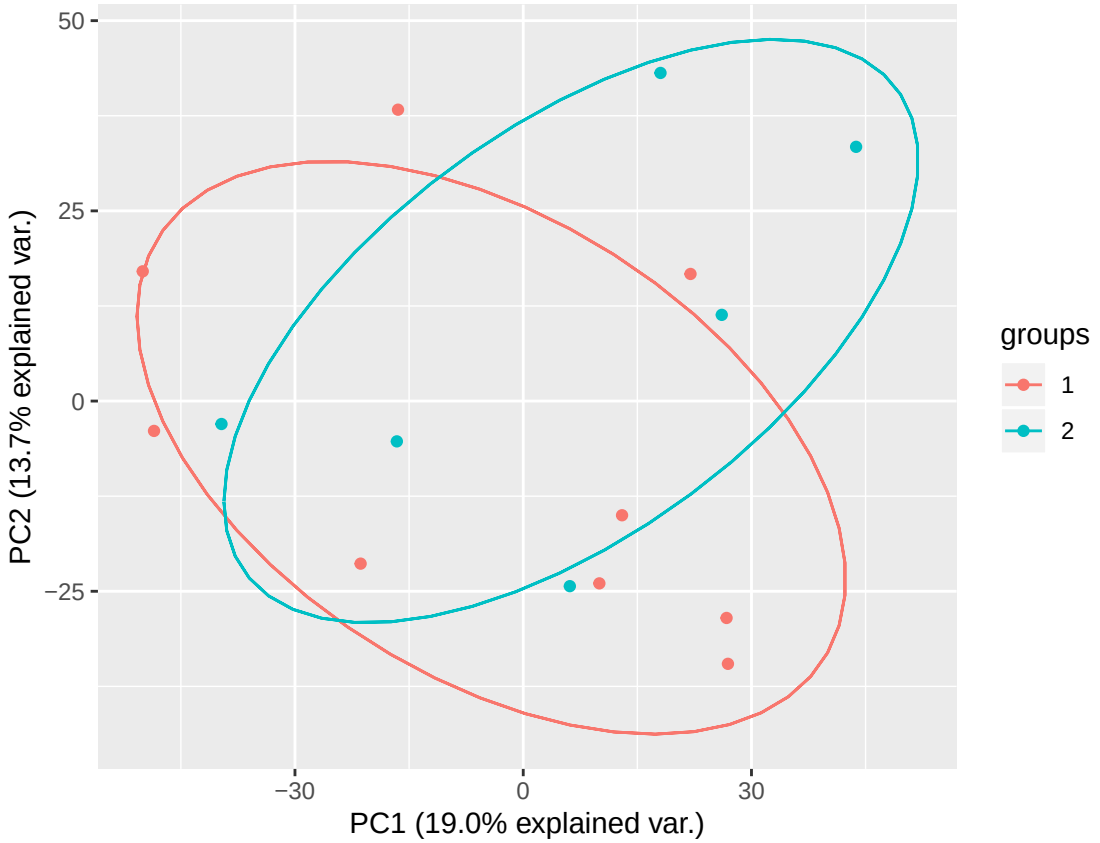
Load resistance classes

```
hsa.meta <- read.table("../ExternalData/Cell lines/Meta-data/Cell_line_division.csv",
                       sep = ";", header = TRUE)
kable(hsa.meta)
```

Cell_line	kit	VIN	RTX	DOX	CYC
OCI-Ly19	1	S	R	S	S
FARAGE	1	S	I	S	R
SU-DHL-5	2	S	S	S	S
MC-116	2	NA	NA	I	NA
OCI-Ly3	1	I	R	I	I
HBL-1	2	I	R	I	I
U2932	1	I	I	R	R
NU-DUL-1	2	I	S	I	S
RIVA	1	R	I	R	R
OCI-Ly7	1	R	S	R	I
SU-DHL-8	2	R	R	S	I
DB	1	R	I	R	R
SU-DHL-4	2	NA	S	R	NA
NU-DHL-1	1	NA	I	S	S
OCI-Ly8	1	NA	R	NA	NA

PCA and plot to check for batch effects

```
hsa.miRNA.pca <- prcomp(t(expr))
kit <- as.factor(hsa.meta$kit[order(hsa.meta$Cell_line)])

ggbiplot(hsa.miRNA.pca,
         obs.scale = 1,
         var.scale = 1,
         ellipse = T,
         var.axes = FALSE,
         choices = 1:2,
         groups = kit)
```



Vincristine

Extract subset for Vincristine

```
vinClass <- subset(hsa.meta, VIN %in% c("S", "R"), select = c("Cell_line", "kit", "VIN"))
vinClass
```

```
##    Cell_line kit VIN
## 1   OCI-Ly19   1   S
## 2     FARAGE   1   S
## 3   SU-DHL-5   2   S
## 9       RIVA   1   R
## 10   OCI-Ly7   1   R
## 11  SU-DHL-8   2   R
## 12        DB   1   R
```

Perform DE analysis for Vincristine Resistance

```
vinModel <- model.matrix(~ (VIN=="R")+kit, vinClass)
vinFit   <- lmFit(hsa.miRNA[, vinClass$Cell_line], vinModel)
vinFit.eb <- eBayes(vinFit)
vinResults <- topTable(vinFit.eb, coef = 2, number = 1000)
vinResults <- subset(vinResults, logFC > 2 | logFC < -2)
round(vinResults, 3)
```

```
##                    logFC AveExpr      t P.Value adj.P.Val      B
## hsa-miR-21_st     -3.217   4.197 -3.414   0.014     0.949 -4.429
```

```
## hsa-miR-21-star_st -2.282   4.204 -3.371   0.015     0.949 -4.431
## hsa-miR-155_st     -3.953  10.245 -2.566   0.043     0.949 -4.474
## hsa-miR-886-3p_st  -2.322   3.868 -2.281   0.063     0.949 -4.493
## hsa-miR-27a_st     -2.083   6.606 -1.839   0.116     0.949 -4.527
## hsa-miR-34a_st     -3.147   3.558 -1.658   0.149     0.949 -4.542
## hsa-miR-886-5p_st  -2.032   4.061 -1.627   0.155     0.949 -4.545
## hsa-let-7b_st      -2.346   9.878 -1.591   0.163     0.949 -4.548
## hsa-miR-148a_st    -2.603   4.726 -1.551   0.172     0.949 -4.551
## hsa-miR-222_st     -3.859   5.283 -1.209   0.272     0.949 -4.580
## hsa-miR-146a_st    -2.109   9.790 -1.127   0.303     0.949 -4.587
## hsa-miR-221_st     -2.819   5.788 -1.004   0.354     0.949 -4.597
## hsa-miR-708_st     -2.094   4.135 -0.976   0.367     0.949 -4.599
```

Rituximab

Extract subset for Rituximab

```
rtxClass <- subset(hsa.meta, RTX %in% c("S", "R"), select = c("Cell_line", "kit", "RTX"))
rtxClass
```

```
##    Cell_line kit RTX
## 1   OCI-Ly19   1   R
## 3   SU-DHL-5   2   S
## 5    OCI-Ly3   1   R
## 6      HBL-1   2   R
## 8   NU-DUL-1   2   S
## 10   OCI-Ly7   1   S
## 11  SU-DHL-8   2   R
## 13  SU-DHL-4   2   S
## 15   OCI-Ly8   1   R
```

Perform DE analysis for Rituximab Resistance

```
rtxModel <- model.matrix(~ (RTX=="R")+kit, rtxClass)
rtxFit   <- lmFit(hsa.miRNA[, rtxClass$Cell_line], rtxModel)
rtxFit.eb <- eBayes(rtxFit)
rtxResults <- topTable(rtxFit.eb, coef = 2, number = 1000)
rtxResults <- subset(rtxResults, logFC > 2 | logFC < -2)
round(rtxResults, 3)
```

```
##                       logFC AveExpr      t P.Value adj.P.Val      B
## hsa-miR-193b_st      -4.037   7.624 -3.915   0.004     0.995 -4.345
## hsa-miR-193b-star_st -3.765   4.193 -3.835   0.005     0.995 -4.349
## hsa-miR-151-5p_st    -2.737   9.110 -3.106   0.014     0.995 -4.398
## hsa-miR-138_st       -4.166   6.176 -3.073   0.015     0.995 -4.400
## hsa-miR-9-star_st     2.442   2.590  3.016   0.016     0.995 -4.405
## hsa-miR-183_st        2.526   5.651  3.008   0.016     0.995 -4.405
## hsa-miR-151-3p_st    -2.468   6.288 -2.635   0.029     0.995 -4.437
## hsa-miR-30a_st        2.787   4.874  2.434   0.040     0.995 -4.455
## hsa-miR-1303_st      -2.538   2.775 -2.417   0.041     0.995 -4.457
## hsa-miR-222_st        6.128   6.747  2.336   0.047     0.995 -4.464
## hsa-miR-503_st        2.518   3.586  2.300   0.049     0.995 -4.468
## hsa-miR-629-star_st  -2.252   3.931 -2.168   0.061     0.995 -4.481
## hsa-miR-27b_st        2.295   5.771  2.155   0.062     0.995 -4.482
## hsa-miR-155_st        3.043  10.724  1.965   0.084     0.995 -4.501
```

```
## hsa-miR-221_st        5.053   6.674  1.887   0.094     0.995 -4.509
## hsa-miR-424-star_st   2.293   3.464  1.858   0.099     0.995 -4.512
## hsa-miR-30a-star_st   2.039   1.707  1.795   0.109     0.995 -4.519
## hsa-miR-551b_st       2.399   2.332  1.782   0.111     0.995 -4.520
## hsa-miR-345_st        2.866   4.784  1.723   0.122     0.995 -4.526
## hsa-miR-886-3p_st     2.060   3.926  1.354   0.211     0.995 -4.564
## hsa-miR-708_st        2.108   4.108  1.156   0.280     0.995 -4.583
## hsa-miR-125b_st       2.598   4.129  1.049   0.324     0.995 -4.593
## hsa-miR-886-5p_st     2.070   4.041  0.988   0.351     0.997 -4.598
## hsa-miR-99a_st        2.121   3.714  0.905   0.391     0.997 -4.605
```

Doxorubicin

Extract subset for Doxorubicin

```
doxClass <- subset(hsa.meta, DOX %in% c("S", "R"), select = c("Cell_line", "kit", "DOX"))
doxClass
```

```
##    Cell_line kit DOX
## 1   OCI-Ly19   1   S
## 2     FARAGE   1   S
## 3   SU-DHL-5   2   S
## 7      U2932   1   R
## 9       RIVA   1   R
## 10   OCI-Ly7   1   R
## 11  SU-DHL-8   2   S
## 12        DB   1   R
## 13  SU-DHL-4   2   R
## 14  NU-DHL-1   1   S
```

Perform DE analysis for Doxorubicin Resistance

```
doxModel <- model.matrix(~ (DOX=="R")+kit, doxClass)
doxFit   <- lmFit(hsa.miRNA[, doxClass$Cell_line], doxModel)
doxFit.eb <- eBayes(doxFit)
doxResults <- topTable(doxFit.eb, coef = 2, number = 1000)
doxResults <- subset(doxResults, logFC > 2 | logFC < -2)
round(doxResults, 3)
```

```
##                         logFC AveExpr      t P.Value adj.P.Val      B
## hsa-miR-27a_st         -2.848   6.522 -3.529   0.006     0.955 -4.291
## hsa-miR-23a_st         -2.604   9.105 -3.117   0.012     0.955 -4.332
## hsa-miR-221_st         -5.511   6.382 -2.953   0.016     0.955 -4.349
## hsa-miR-222_st         -6.373   6.110 -2.868   0.018     0.955 -4.359
## hsa-miR-129-5p_st       2.009   2.524  2.751   0.022     0.955 -4.373
## hsa-miR-1295_st         2.241   2.367  2.596   0.029     0.955 -4.391
## hsa-miR-34a_st         -3.281   3.920 -2.130   0.062     0.955 -4.451
## hsa-miR-125a-5p_st      2.477   3.493  2.067   0.068     0.955 -4.460
## hsa-miR-708_st         -2.798   4.150 -1.920   0.086     0.955 -4.479
## hsa-miR-193b-star_st    2.192   4.766  1.660   0.131     0.955 -4.515
## hsa-miR-200c_st        -2.626   5.557 -1.636   0.136     0.955 -4.518
## hsa-miR-181a-2-star_st  2.467   4.282  1.526   0.161     0.955 -4.533
## hsa-miR-155_st         -2.172  10.326 -1.516   0.163     0.955 -4.534
```

Cyclophosphamide

Extract subset for Cyclophosphamide

```
cycClass <- subset(hsa.meta, CYC %in% c("S", "R"), select = c("Cell_line", "kit", "CYC"))
cycClass
```

```
##    Cell_line kit CYC
## 1   OCI-Ly19   1   S
## 2     FARAGE   1   R
## 3   SU-DHL-5   2   S
## 7      U2932   1   R
## 8   NU-DUL-1   2   S
## 9       RIVA   1   R
## 12        DB   1   R
## 14  NU-DHL-1   1   S
```

Perform DE analysis for Cyclophosphamide Resistance

```
cycModel <- model.matrix(~ (CYC=="R")+kit, cycClass)
cycFit   <- lmFit(hsa.miRNA[, cycClass$Cell_line], cycModel)
cycFit.eb <- eBayes(cycFit)
cycResults <- topTable(cycFit.eb, coef = 2, number = 1000)
cycResults <- subset(cycResults, logFC > 2 | logFC < -2)
round(cycResults, 3)
```

```
##                       logFC AveExpr      t P.Value adj.P.Val      B
## hsa-miR-486-5p_st    -4.419   4.465 -7.287   0.000     0.075  0.976
## hsa-miR-486-3p_st    -2.306   2.473 -7.081   0.000     0.075  0.853
## hsa-miR-708_st       -6.073   4.649 -6.339   0.000     0.101  0.368
## hsa-miR-30a_st        2.492   3.816  2.717   0.029     0.918 -3.300
## hsa-miR-151-3p_st    -2.236   5.757 -2.315   0.053     0.957 -3.829
## hsa-miR-664-star_st   2.152   3.392  2.160   0.067     0.957 -4.033
## hsa-miR-152_st        2.024   4.543  2.095   0.074     0.957 -4.118
## hsa-miR-99b_st        2.397   2.209  2.001   0.085     0.957 -4.240
## hsa-miR-148a-star_st  2.321   2.688  1.890   0.100     0.957 -4.383
## hsa-miR-146a_st       4.703   9.475  1.863   0.104     0.957 -4.417
## hsa-miR-193b-star_st  2.552   4.381  1.831   0.109     0.957 -4.458
## hsa-miR-221_st       -5.315   7.620 -1.829   0.109     0.957 -4.460
## hsa-miR-148a_st       2.946   5.477  1.729   0.127     0.957 -4.586
## hsa-miR-222_st       -5.986   7.443 -1.724   0.127     0.957 -4.592
## hsa-miR-138_st       -3.663   6.356 -1.667   0.138     0.957 -4.662
## hsa-miR-551b_st       2.351   2.460  1.485   0.180     0.971 -4.881
## hsa-miR-151-5p_st    -2.710   7.973 -1.351   0.218     0.984 -5.034
```

Dump DE results

```
write.csv2(vinResults, file = "../Output/vinResults.csv")
write.csv2(rtxResults, file = "../Output/rtxResults.csv")
write.csv2(doxResults, file = "../Output/doxResults.csv")
write.csv2(cycResults, file = "../Output/cycResults.csv")
```

Match MIRs to u133+2 probes

Make list with selected MIRs from DE analysis

```
vennResults <- list("Vincristine"      = rownames(vinResults),
                    "Rituximab"        = rownames(rtxResults),
                    "Doxorubicin"      = rownames(doxResults),
                    "Cyclophosphamide" = rownames(cycResults))
```

Function to rename MIRS to match u133+2 array

```
renameMir <- function(x){
  x <- sub("hsa-", "", x)
  x <- sub("_st", "", x)
  x <- sub("-", "", x)
  x <- toupper(x)
  x[x == "LET7B"] <- "hsa-let-7b"
  return(x)
}
```

Combined list of DE genes

```
comb.de.genes       <- data.frame("deMIR" = unique(unlist(vennResults)))
comb.de.genes$miRNA <- renameMir(comb.de.genes$deMIR)
comb.de.genes$miRNA
```

```
##  [1] "MIR21"          "MIR21-STAR"     "MIR155"         "MIR886-3P"
##  [5] "MIR27A"         "MIR34A"         "MIR886-5P"      "hsa-let-7b"
##  [9] "MIR148A"        "MIR222"         "MIR146A"        "MIR221"
## [13] "MIR708"         "MIR193B"        "MIR193B-STAR"   "MIR151-5P"
## [17] "MIR138"         "MIR9-STAR"      "MIR183"         "MIR151-3P"
## [21] "MIR30A"         "MIR1303"        "MIR503"         "MIR629-STAR"
## [25] "MIR27B"         "MIR424-STAR"    "MIR30A-STAR"    "MIR551B"
## [29] "MIR345"         "MIR125B"        "MIR99A"         "MIR23A"
## [33] "MIR129-5P"      "MIR1295"        "MIR125A-5P"     "MIR200C"
## [37] "MIR181A-2-STAR" "MIR486-5P"      "MIR486-3P"      "MIR664-STAR"
## [41] "MIR152"         "MIR99B"         "MIR148A-STAR"
```

Match MIRs to u133+2 probes

```
MIRu133 <- read_xlsx("../ExternalData/Cell lines/Meta-data/miRNAs_U133.xlsx", sheet = 1)
MIRu133 <- separate(MIRu133, "miRNA", sep =" /// ", into=letters[1:7])
```

```
## Warning: Expected 7 pieces. Missing pieces filled with `NA` in 298 rows [1,
## 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, ...].
```

```
comb.de.genes.u133 <- merge(comb.de.genes, MIRu133, by.x = "miRNA", by.y = "a")
comb.de.genes.u133 <- comb.de.genes.u133[, c("miRNA", "deMIR", "Probe")]
for(column in letters[2:7]){
  temp <- merge(comb.de.genes, MIRu133, by.x = "miRNA", by.y = column)
  comb.de.genes.u133 <- rbind(comb.de.genes.u133, temp[, c("miRNA", "deMIR", "Probe")])
}
probes <- comb.de.genes.u133$Probe
names(probes) <- comb.de.genes.u133$miRNA
probes
```

```
##        MIR146A        MIR146A         MIR155          MIR21          MIR21
##    "238225_at"    "232504_at"    "229437_at"  "220990_s_at"    "229417_at"
```

```
##         MIR23A         MIR23A         MIR34A     hsa-let-7b     hsa-let-7b
##    "235317_at" "1555847_a_at"    "235571_at" "1557342_a_at"  "241464_s_at"
##         MIR503
##    "227488_at"
```

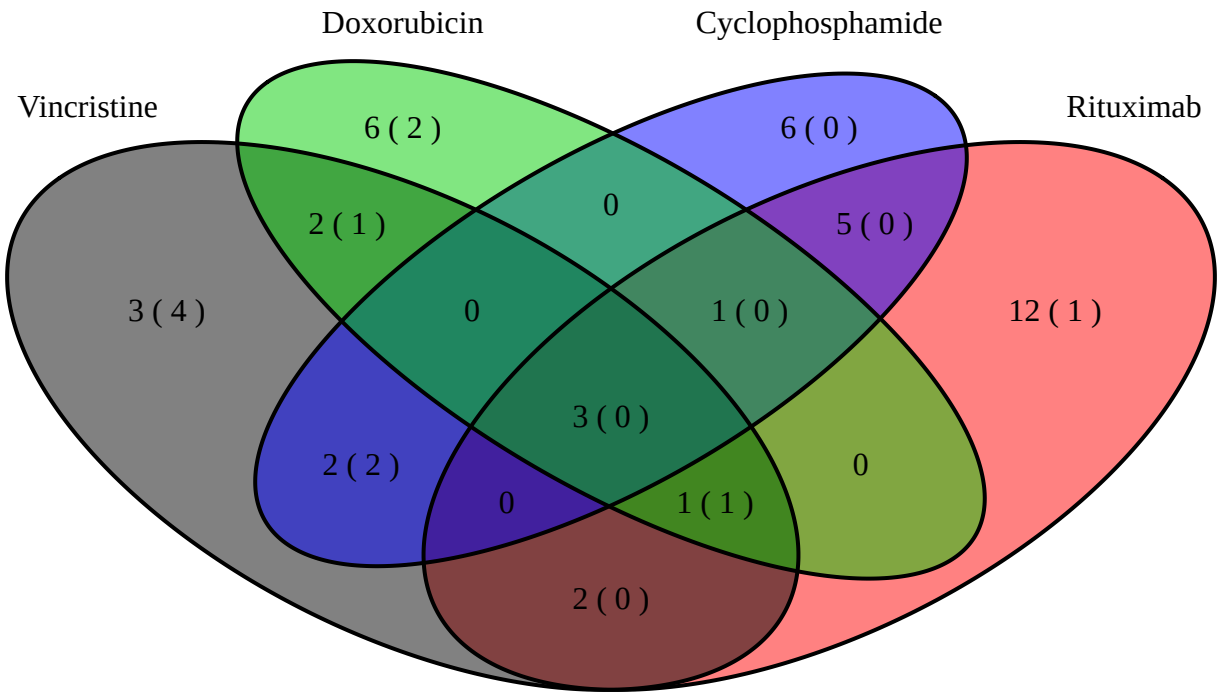
Match selected probes to lists from drugs too see how many of the selected probes correspond to DE Mirs in specific drugs

```
vennResults2 <- lapply(vennResults, renameMir)
probeInDrug <- function(x){
  DrugProbes    <- probes[names(probes) %in% x]
  numProbes     <- length(DrugProbes)
  return(list("nProbes" = numProbes,
              "probes"  = DrugProbes)
  )
}
drugProbes <- lapply(vennResults2, probeInDrug)
drugProbes
```

```
## $Vincristine
## $Vincristine$nProbes
## [1] 8
##
## $Vincristine$probes
##        MIR146A        MIR146A         MIR155          MIR21          MIR21
##    "238225_at"    "232504_at"    "229437_at"  "220990_s_at"    "229417_at"
##         MIR34A     hsa-let-7b     hsa-let-7b
##    "235571_at" "1557342_a_at"  "241464_s_at"
##
##
## $Rituximab
## $Rituximab$nProbes
## [1] 2
##
## $Rituximab$probes
##      MIR155      MIR503
## "229437_at" "227488_at"
##
##
## $Doxorubicin
## $Doxorubicin$nProbes
## [1] 4
##
## $Doxorubicin$probes
##         MIR155         MIR23A         MIR23A         MIR34A
##    "229437_at"    "235317_at" "1555847_a_at"    "235571_at"
##
##
## $Cyclophosphamide
## $Cyclophosphamide$nProbes
## [1] 2
##
## $Cyclophosphamide$probes
##     MIR146A     MIR146A
## "238225_at" "232504_at"
```

Construct Venn-diagram with selected MIRS and probes

```
v1 <- venn.diagram(vennResults, filename = NULL, fill = 1:4)
v2 <- venn.diagram(lapply(drugProbes, function(x) x$probes), filename = NULL)

# Combine Labels
for(i in 9:23){
  if(v1[[i]]$label != "0"){
     v1[[i]]$label = paste(v1[[i]]$label, "(", v2[[i]]$label, ")")
  }
}

grid.newpage()
grid.draw(v1)
```



Check correlation of MIR and U133+2 array

load U133+2 data for cell-lines and adjust column names

```
bCellu133 <- just.rma(list.files("../ExternalData/Cell lines/CEL U133", full.names = T))
```

```
## Warning in just.rma(list.files("../ExternalData/Cell lines/CEL U133", full.names = T)): Incompatible
```

```
## Warning: replacing previous import 'AnnotationDbi::tail' by 'utils::tail'
## when loading 'hgu133plus2cdf'
```

```
## Warning: replacing previous import 'AnnotationDbi::head' by 'utils::head'
```

```
## when loading 'hgu133plus2cdf'
##
##
## Attaching package: 'hgu133plus2cdf'
## The following objects are masked from 'package:mirna102xgaincdf':
##
##     i2xy, xy2i
```

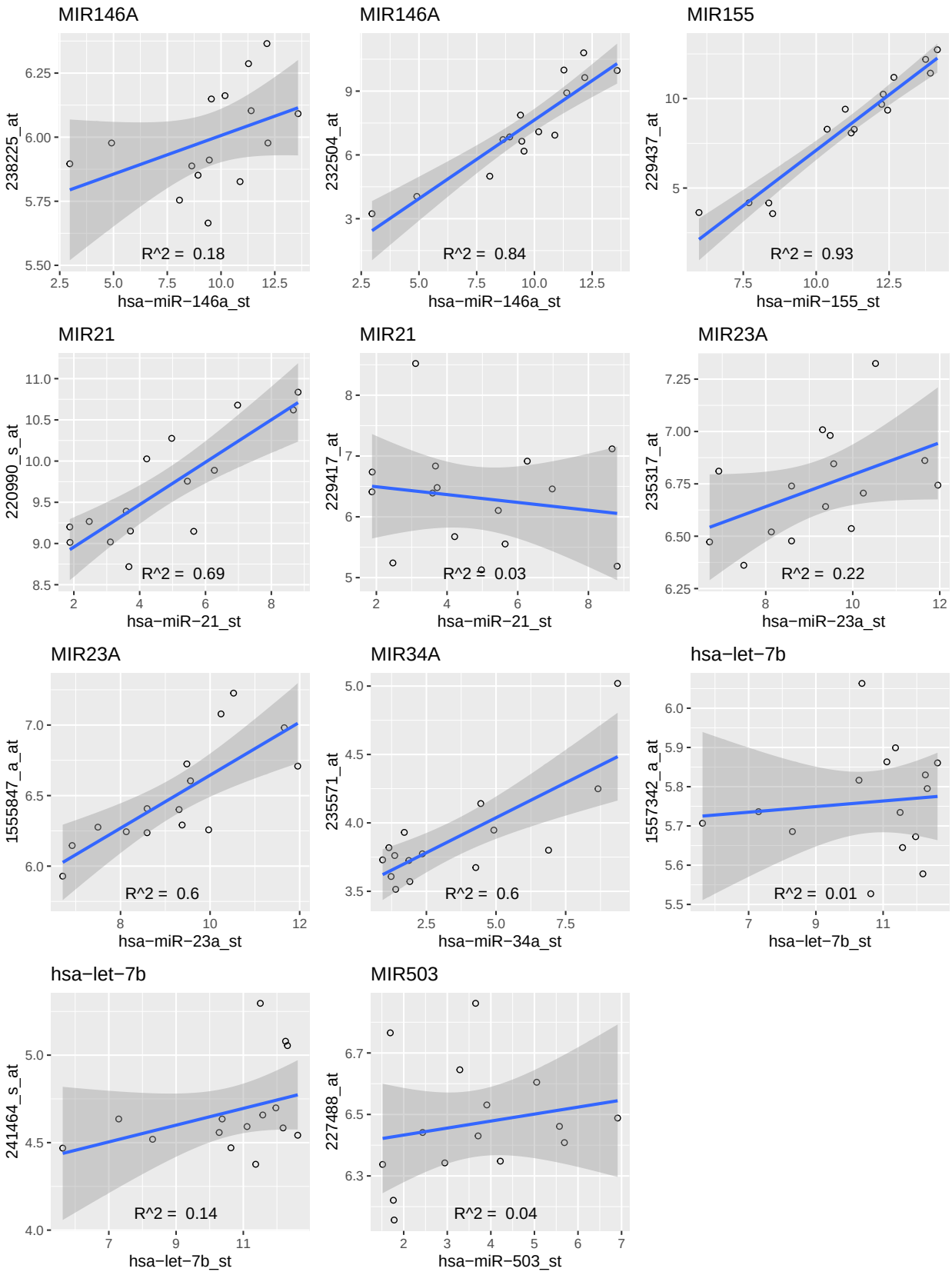
```
tempNames <- sapply(strsplit(colnames(bCellu133), "_"), function(x) x[3])
tempNames <- sub(".CEL", "", tempNames)
tempNames[15] <- substr(colnames(bCellu133)[15],1,8)
colnames(bCellu133) <- tempNames
bCellu133 <- bCellu133[, order(colnames(bCellu133))]
bCellu133 <- exprs(bCellu133)
```

Match columns in MIR and u133+2 datasets

```
table(colnames(bCellu133) == colnames(hsa.miRNA))
```

```
##
## TRUE
##   15
```

```
p <- list()
for(i in 1:nrow(comb.de.genes.u133)){
  mirName <- as.character(comb.de.genes.u133[i,2])
  u133Name <- as.character(comb.de.genes.u133[i,3])
  regression  <- lm(bCellu133[u133Name,] ~  hsa.miRNA[mirName,])

  tempPlot <- data.frame("mir" = hsa.miRNA[mirName,],
                         "u133" = bCellu133[u133Name,])

  p[[i]] <- ggplot(tempPlot, aes(x=mir, y=u133)) +
    geom_point(shape=1) +
    geom_smooth(method=lm) +
    xlab(mirName) +
    ylab(u133Name) +
    ggtitle(comb.de.genes.u133[i,1]) +
    annotate("text", x = -Inf, y = -Inf, hjust = -1, vjust = -1,
             label = paste("R^2 = ", round(summary(regression)$r.squared, 2)))

 }

grid.arrange(grobs = p, ncol = 3)
```


MIR146A
238225_at
hsa−miR−146a_st
R^2 = 0.18
MIR146A
232504_at
hsa−miR−146a_st
R^2 = 0.84
MIR155
229437_at
hsa−miR−155_st
R^2 = 0.93
MIR21
220990_s_at
hsa−miR−21_st
R^2 = 0.69
MIR21
229417_at
hsa−miR−21_st
R^2 = 0.03
MIR23A
235317_at
hsa−miR−23a_st
R^2 = 0.22
MIR23A
1555847_a_at
hsa−miR−23a_st
R^2 = 0.6
MIR34A
235571_at
hsa−miR−34a_st
R^2 = 0.6
hsa−let−7b
1557342_a_at
hsa−let−7b_st
R^2 = 0.01
hsa−let−7b
241464_s_at
hsa−let−7b_st
R^2 = 0.14
MIR503
227488_at
hsa−miR−503_st
R^2 = 0.04

Clinical Data

```
combined.data.combat.file <- "../GeneratedData/combined.data.combat.RData"
```

Load and pre-process GEP Data

Load pre-normalized u133+2 datasets

```
load("../ExternalData/Clinical data/IDRC_cohort/CelFiles/pre-processed/GEPIDRC_RMA_affy.RData")
load("../ExternalData/Clinical data/LLMPP_cohort/CelFiles/pre-processed/GEPLLMPPRCHOP_RMA_affy.RData")
```

Read and normalize new data

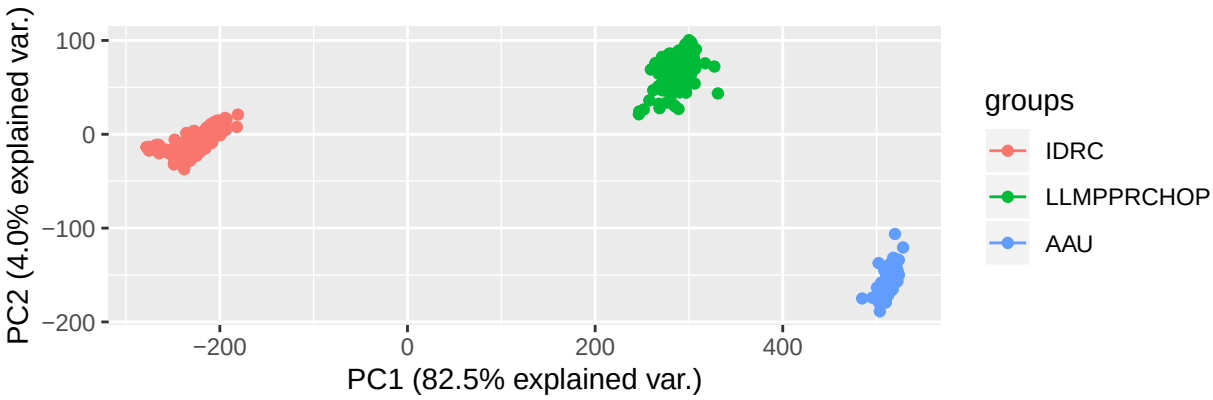
```
AAUGEP.file <- "../GeneratedData/AAUGEP.file.Rdata"
if(!file.exists(AAUGEP.file)){
  AAU <- just.rma(list.files("../ExternalData/Clinical data/AAU_cohort/CelFiles/All/",
                             full.names = TRUE))
  GEPAAU <- exprs(AAU)
  save(GEPAAU, file = AAUGEP.file)
} else{
  load(AAUGEP.file)
}
```

Combine data and remove probes with missing data

```
if(!file.exists(combined.data.combat.file)){
  combined.data <- cbind(exprs(GEPIDRC), exprs(GEPLLMPPRCHOP), GEPAAU)
  combined.data <- combined.data[rowSums(is.na(combined.data))==0, ]
}
```

PCA plot to check for batch effects

```
combined.data.pca.file <- "../GeneratedData/combined.data.pca.Rdata"

if(!file.exists(combined.data.pca.file)){
  combineddata.pca <- prcomp(t(combined.data))
  save(combineddata.pca, file = combined.data.pca.file)
} else{
  load(combined.data.pca.file)
}
batch <- c(rep("IDRC", ncol(GEPIDRC)),
           rep("LLMPPRCHOP", ncol(GEPLLMPPRCHOP)),
           rep("AAU", ncol(GEPAAU)))

ggbiplot(combineddata.pca,
         obs.scale = 1,
         var.scale = 1,
         ellipse = T,
         var.axes = FALSE,
         choices = 1:2,
         groups = batch
         )
```



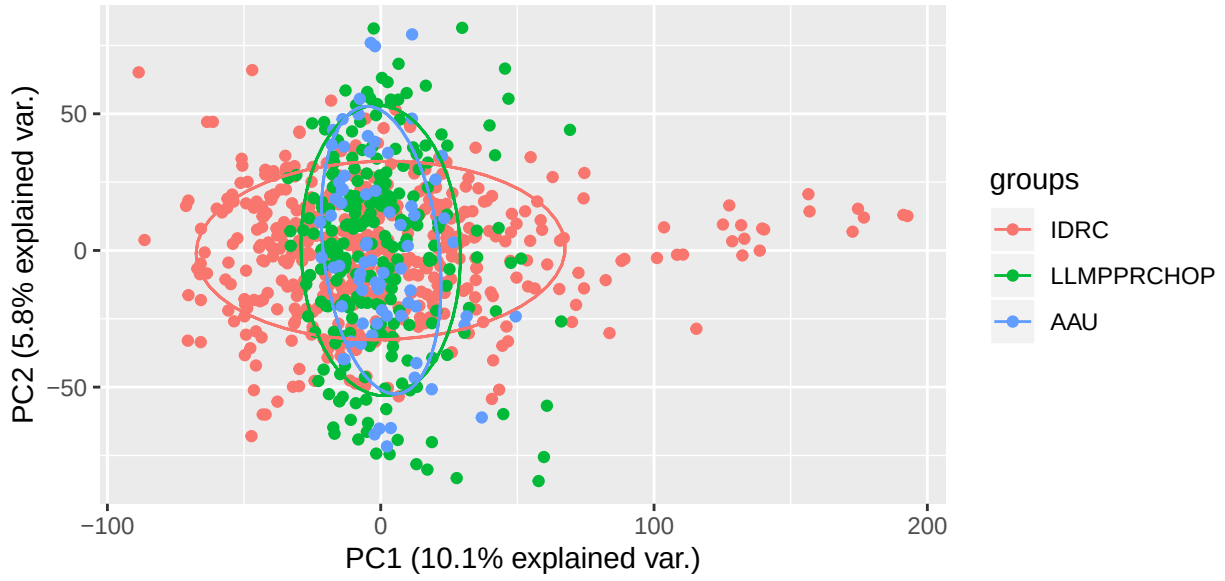
COMBAT adjust data

```
combined.data.combat.file <- "../GeneratedData/combined.data.combat.RData"
if(!file.exists(combined.data.combat.file)){
  combined.data.combat <- ComBat(combined.data,
                                 batch = batch)
  save(combined.data.combat, file = combined.data.combat.file)
} else{
  load(combined.data.combat.file)
}

colnames(combined.data.combat) <- sub(".CEL", "", colnames(combined.data.combat))
colnames(combined.data.combat) <- sub(".cel", "", colnames(combined.data.combat))
colnames(combined.data.combat) <- sub("_\\(HG-U133_Plus_2\\)", "", colnames(combined.data.combat))
```

Check for batch effect after removal

```
combined.data.combat.pca.file <- "../GeneratedData/combined.data.combat.pca.RData"
if(!file.exists(combined.data.combat.pca.file)){
  combineddata.combat.pca <- prcomp(t(combined.data.combat))
  save(combineddata.combat.pca, file = combined.data.combat.pca.file)
} else{
  load(combined.data.combat.pca.file)
}

ggbiplot(combineddata.combat.pca,
         obs.scale = 1,
```

```
         var.scale = 1,
         ellipse = T,
         var.axes = FALSE,
         choices = 1:2,
         groups = batch
         )
```



Combine metadata

Load raw data

```
load("../ExternalData/Clinical data/IDRC_cohort/Metadata/metadataIDRC.RData")
load("../ExternalData/Clinical data/LLMPP_cohort/Metadata/metadataLLMPPRCHOP.RData")
load("../ExternalData/Clinical data/AAU_cohort/Metadata/metadata_AAUcohort_RMA_BC_affy.RData")
metadataAAU <- tmp1
rm(tmp1)
```

Extract columns from IDRC

```
IDRC.tmp <- subset(metadataIDRC, select = c("Array.Data.File",
                                            "IPI.score",
                                            "WrightClass2",
                                            "OS",
                                            "PFS",
                                            "age",
                                            "Response",
```

```
                                                "Gender"))
IDRC.tmp$batch <- rep("IDRC", nrow(IDRC.tmp))
names(IDRC.tmp) <- c("ID", "IPI", "ABCGCB", "OS", "PFS", "age","Response", "Gender", "study")

IDRC.tmp$IPI[IDRC.tmp$IPI == "UNK"] <- NA
```

Extract columns from LLMPPRCHOP

```
LLMPRCHOP.tmp <- subset(metadataLLMPPRCHOP, select = c("GEO.ID",
                                                       "ipi",
                                                       "WrightClass2",
                                                       "OS",
                                                       "PFS",
                                                       "age",
                                                       "Response.to.Initial.Therapy",
                                                       "Gender"))
LLMPRCHOP.tmp$batch <- rep("LLMPPRCHOP", nrow(LLMPRCHOP.tmp))
names(LLMPRCHOP.tmp) <- c("ID", "IPI", "ABCGCB", "OS", "PFS","age", "Response", "Gender","study")
LLMPRCHOP.tmp$PFS[is.na(LLMPRCHOP.tmp$PFS)] <- LLMPRCHOP.tmp$OS[is.na(LLMPRCHOP.tmp$PFS)]


## Recode response
LLMPRCHOP.tmp$Response <- as.character(LLMPRCHOP.tmp$Response)
LLMPRCHOP.tmp$Response[LLMPRCHOP.tmp$Response == ""]             <- NA
LLMPRCHOP.tmp$Response[LLMPRCHOP.tmp$Response == "complete"]     <- "CR"
LLMPRCHOP.tmp$Response[LLMPRCHOP.tmp$Response == "partial"]      <- "PR"
LLMPRCHOP.tmp$Response[LLMPRCHOP.tmp$Response == "progression"] <- "PD"
LLMPRCHOP.tmp$Response[LLMPRCHOP.tmp$Response == "stable"]       <- "SD"
```

Extract columns from AAU

```
metadataAAU$OS <- with(metadataAAU, Surv(FU, OS.censor))
metadataAAU$PFS <- with(metadataAAU, Surv(PFS, PFScensor))
AAU.tmp <- subset(metadataAAU, select = c("H.nummer",
                                          "IPI",
                                          "ABC.GCB",
                                          "OS",
                                          "PFS",
                                          "Alder"))

AAU.tmp$ABC.GCB <- as.character(AAU.tmp$ABC.GCB)
AAU.tmp$ABC.GCB[AAU.tmp$ABC.GCB == "NC"] <- "UC"
AAU.tmp$Response <- rep(NA, nrow(AAU.tmp))
AAU.tmp$Gender <- ifelse(metadataAAU$Gender == "Kvinde", "F", "M")
AAU.tmp$batch <- rep("AAU", nrow(AAU.tmp))
names(AAU.tmp) <- c("ID", "IPI", "ABCGCB", "OS", "PFS","age", "Response", "Gender","study")
```

Combine data

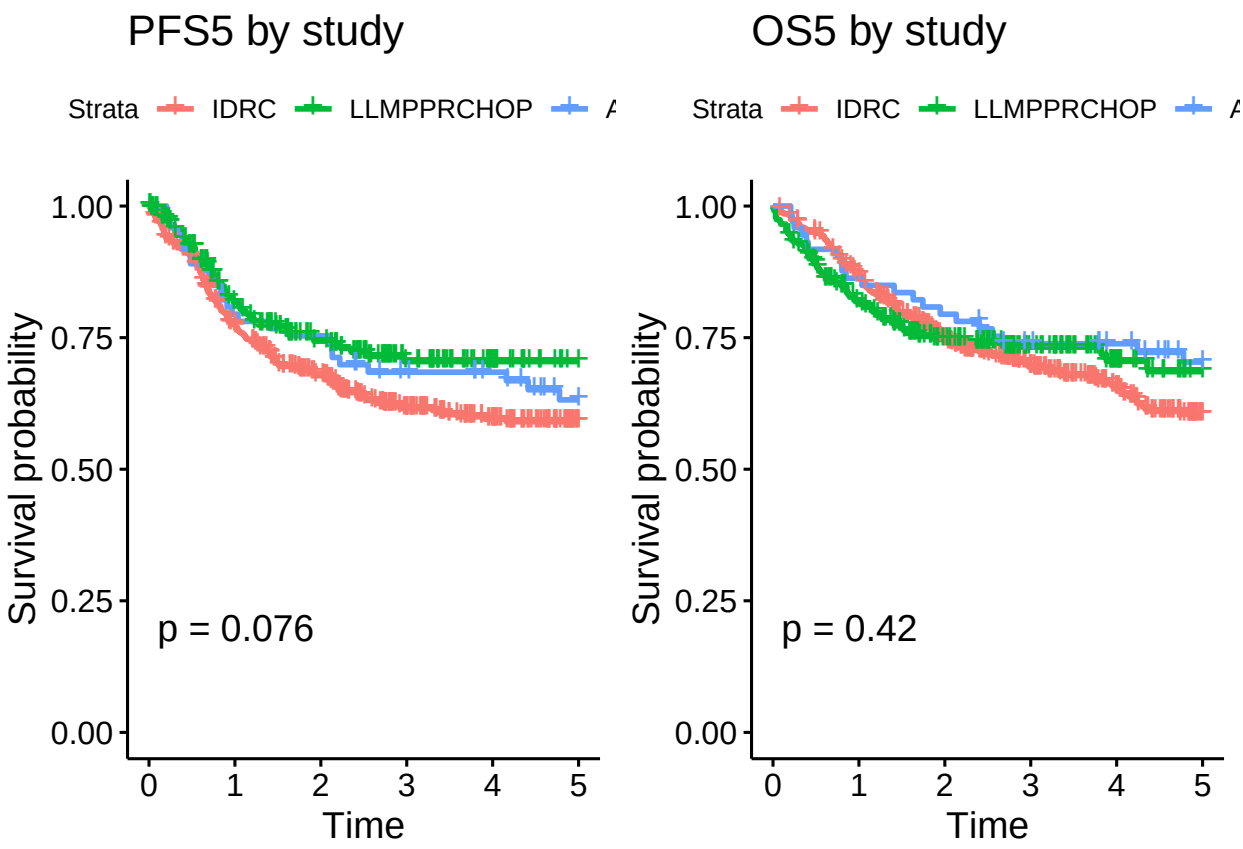
```
combined.metadata <- rbind(IDRC.tmp, LLMPRCHOP.tmp, AAU.tmp)
combined.metadata <- droplevels.data.frame(combined.metadata)
combined.metadata$ID <- sub(".CEL", "", combined.metadata$ID)
combined.metadata$OS <- Surv(combined.metadata$OS[,1], combined.metadata$OS[,2])
combined.metadata$PFS <- Surv(combined.metadata$PFS[,1], combined.metadata$PFS[,2])
```

Unwrap OS and PFS variables. This is needed for calcualtion of the Brier score and time varying AUC.

```
combined.metadata$OStime   <- combined.metadata$OS[,1]
combined.metadata$OSstatus <- combined.metadata$OS[,2]

combined.metadata$PFStime   <- combined.metadata$PFS[,1]
combined.metadata$PFSstatus <- combined.metadata$PFS[,2]

# 5 year OS / PFS
time5   <- pmin(combined.metadata$OStime, 5)
status5 <- as.numeric(time5 < 5 & combined.metadata$OSstatus == 1)
combined.metadata$OS5 <- Surv(time = time5, event = status5)

time5   <- pmin(combined.metadata$PFStime, 5)
status5 <- as.numeric(time5 < 5 & combined.metadata$PFSstatus == 1)
combined.metadata$PFS5 <- Surv(time = time5, event = status5)
```

Plot survival in different studies included in the combined data

```
pfs.survfit <- survfit(PFS5~study, data = combined.metadata)
names(pfs.survfit$strata) <- sub("study=", "", names(pfs.survfit$strata))
pfs.study.plot <- ggsurvplot(pfs.survfit,
           data = combined.metadata,
           title = "PFS5 by study",
           pval = TRUE)

os.survfit <- survfit(OS5~study, data = combined.metadata)
names(os.survfit$strata) <- sub("study=", "", names(os.survfit$strata))
os.study.plot <- ggsurvplot(os.survfit,
           data = combined.metadata,
           title = "OS5 by study",
           pval = TRUE)

arrange_ggsurvplots(list(pfs.study.plot, os.study.plot))
```



Get 5-year OS / PFS times

```
pfs.sum <- summary(pfs.survfit, times = 5)
os.sum <- summary(os.survfit, times = 5)
pfs.sum
```

```
## Call: survfit(formula = PFS5 ~ study, data = combined.metadata)
##
##                 IDRC
##         time       n.risk      n.event     survival      std.err
##        5.000       81.000      172.000        0.590        0.025
## lower 95% CI upper 95% CI
##        0.543        0.641
##
##                 LLMPPRCHOP
##         time       n.risk      n.event     survival      std.err
##       5.0000      22.0000      56.0000       0.7045       0.0343
## lower 95% CI upper 95% CI
##       0.6403       0.7751
##
##                 AAU
##         time       n.risk      n.event     survival      std.err
##       5.0000      33.0000      26.0000       0.6318       0.0582
## lower 95% CI upper 95% CI
##       0.5275       0.7568
```

```
os.sum
```

```
## Call: survfit(formula = OS5 ~ study, data = combined.metadata)
##
##                 IDRC
##         time       n.risk      n.event     survival      std.err
##       5.0000      98.0000     155.0000       0.6044       0.0266
## lower 95% CI upper 95% CI
##       0.5544       0.6589
##
##                 LLMPPRCHOP
##         time       n.risk      n.event     survival      std.err
##       5.0000      24.0000      60.0000       0.6854       0.0393
## lower 95% CI upper 95% CI
##       0.6125       0.7670
##
##                 AAU
##         time       n.risk      n.event     survival      std.err
##       5.0000      38.0000      21.0000       0.7036       0.0548
## lower 95% CI upper 95% CI
##       0.6040       0.8195
```

Create metadata summary table

```
# Survival summary
table1.pfs <- paste0(round(pfs.sum$surv, 2), " (", round(pfs.sum$lower, 2), ";", round(pfs.sum$upper, 2
table1.os <- paste0(round(os.sum$surv, 2), " (", round(os.sum$lower, 2), ";", round(os.sum$upper, 2), "


metaSummary <- rbind(table(combined.metadata$study),
t(table(combined.metadata$study, combined.metadata$Gender)),
tapply(combined.metadata$age, combined.metadata$study, median),
tapply(combined.metadata$age, combined.metadata$study,
       function(x) paste(range(x), collapse = "-")),
t(table(combined.metadata$study, as.numeric(as.character(combined.metadata$IPI)) < 2)),
t(tapply(combined.metadata$IPI, combined.metadata$study, function(x) sum(is.na(x)))),
t(table(combined.metadata$study, combined.metadata$ABCGCB)),
t(table1.pfs),
t(table1.os))
row.names(metaSummary) <- c("N", "Female", "Male", "Median age",
                            "Range age", "IPI 0-1", "IPI 2-5",
                            "IPI NA", "ABC", "GCB", "UC", "5-year PFS", "5-year OS")
kable(metaSummary)
```

	IDRC	LLMPPRCHOP	AAU
N	468	233	73
Female	198	99	30
Male	270	134	43
Median age	63	61	66
Range age	18-92	17-92	20-87
IPI 0-1	254	94	48
IPI 2-5	168	70	21
IPI NA	46	69	4
ABC	199	93	32
GCB	225	107	32

	IDRC	LLMPPRCHOP	AAU
UC	44	33	9
5-year PFS	0.59 (0.54;0.64)	0.7 (0.64;0.78)	0.63 (0.53;0.76)
5-year OS	0.6 (0.55;0.66)	0.69 (0.61;0.77)	0.7 (0.6;0.82)

Check if metadata match GEP data

```
table(colnames(combined.data.combat) %in% combined.metadata$ID)
```

```
##
## FALSE  TRUE
##     2   774
```

Two GEP files have no data, so they are removed

```
combined.data.combat <- combined.data.combat[, colnames(combined.data.combat) %in%
                                                combined.metadata$ID]
```

Function to do uni- and multivariate cox regressions and summarize results

```
uni.multi.cox <- function(cox.data, probes, outcome = "OS"){
  ## Univariate Cox for each probe
  uni.cox.results <- list()
  for(i in 1:length(probes)){
    uni.cox.formula <- formula(paste0(outcome,"~","study+", "`",probes[i],"`"))
    uni.cox.results[[probes[i]]] <- summary(coxph(uni.cox.formula, data = cox.data))
  }

  ## Summarize univariante results
  uni.coef <- round(sapply(uni.cox.results, function(x) x$coefficients[3,2]),2 )
  uni.conf <- round(t(sapply(uni.cox.results, function(x) x$conf.int[3,3:4])),2)
  uni.conf <- paste0("(", uni.conf[,1], ";", uni.conf[,2],")")
  uni.p    <- round(sapply(uni.cox.results, function(x) x$coefficients[3,5]),3)

  ## Multivariate Cox
  multi.cox.formula <- formula(paste0(outcome,"~","study+","`",
                                      paste(probes, collapse="`+`"),"`"))
  multi.cox  <- summary(coxph(multi.cox.formula, data = cox.data))

  ## Summarize multivariate results
  multi.coef <- round(multi.cox$coefficients[-c(1,2),2], 2)
  multi.conf <- round(multi.cox$conf.int[-c(1,2),3:4], 2)
  multi.conf <- paste0("(", multi.conf[,1], ";", multi.conf[,2],")")
  multi.p    <- round(multi.cox$coefficients[-c(1,2),5], 3)

  combined.results <- data.frame("MIR" = names(probes),
                                 "uni.HR"   = uni.coef,
                                 "uni.conf" = uni.conf,
                                 "uni.p"    = uni.p,
                                 "multi.HR" = multi.conf,
                                 "multi.conf" = multi.conf,
                                 "multi.p"    = multi.p)

  return(list("uni.cox.results"   = uni.cox.results,
              "multi.cox.results" = multi.cox,
```

```
              "combined.results"  = combined.results))
}
```

Analysis with Cross-validation

```
timeVaryingAUC <- function(survivalPrediction, timeVar, delta, evalTimes){
      timeAUC <- rep(NA, length(evalTimes))
      for(i in 1:length(evalTimes)){
        timeAUC[i] <- timeROC(T = timeVar,
                              marker = - survivalPrediction[, i],
                              delta = delta,
                              times = evalTimes[i],
                              cause = 1)$AUC[2]
      }
      timeAUC
    }
```

Function to plot a list of timeVarying AUCs

```
plottAUC <- function(x, times.interest = times.interest){
  nvar <- length(x)
  xx <- data.frame("time" = rep(times.interest, nvar),
                   "tAUC" = unlist(x),
                   "Model" = rep(names(x), each = length(times.interest)))
  ggplot(xx, aes(x = time, y = tAUC, col = Model)) +
    geom_line() +
    ylab("tAUC") +
    ylim(c(0.4,0.75))
}

plottAUCList <- function(x, times.interest = times.interest){
  nvar <- length(x[[1]])
  xx <- list()
  for(i in 1:length(x)){
    xx[[i]] <- data.frame("time" = rep(times.interest, nvar),
                     "tAUC" = unlist(x[[i]]),
                     "Model" = rep(names(x[[i]]), each = length(times.interest)))
  }
  p <- ggplot(xx[[i]], aes(x = time, y = tAUC, col = Model)) +
    geom_line() +
    ylab("tAUC") +
    ylim(c(0.4,0.8))

  for(i in 2:length(xx)){
    p <- p + geom_line(data = xx[[i]])
  }
  return(p)
}

plottAUCListMean <- function(x, times.interest = times.interest){
  models <- names(x[[1]])
  plotData <- data.frame("Model" = NULL,"time" =NULL, "Mean" = NULL, "SD" = NULL)
```

```
  for(m in models){
    y <- sapply(x, function(x) x[[m]])
    means <- rowMeans(y)
    sds   <- rowSds(y)
    temp <- data.frame("Model" = rep(m, length(means)),
                       "time" = times.interest,
                       "Mean" = means,
                       "SD" = sds)
    plotData = rbind(plotData, temp)
  }

  ggplot(plotData, aes(x = time, y = Mean, col = Model)) +
    geom_line(size  = 1.5) +
    ylab("tAUC") +
    ylim(c(0.4,0.8)) +
    geom_ribbon(aes(x = time, ymin = (Mean -2*SD),
                    ymax = (Mean+2*SD), fill = Model), alpha = 0.2)
}
```

Function to do plots for Brier Score

```
plotPec <- function(pecx){
  nvar <- length(pecx$AppErr)
  xx <- data.frame("time" = rep(pecx$time, nvar),
                   "AppErr" = unlist(pecx$AppErr),
                   "Model" = rep(names(pecx$AppErr), each = length(pecx$time)))
  ggplot(xx, aes(x = time, y = AppErr, col = Model)) +
    geom_line() +
    ylab("Prediction Error") +
    ylim(c(0,0.3))
}

plotPecList <- function(pecx){
  nvar <- length(pecx[[1]][["AppErr"]])
  xx <- list()
  for(i in 1:length(pecx)){
    xx[[i]] <- data.frame("time" = rep(pecx[[i]][["time"]], nvar),
                          "AppErr" = unlist(pecx[[i]][["AppErr"]]),
                          "Model" = rep(names(pecx[[i]][["AppErr"]]),
                          each = length(pecx[[i]][["time"]])))
  }
  p <- ggplot(xx[[i]], aes(x = time, y = AppErr, col = Model)) +
    geom_line() +
    ylab("Prediction Error") +
    ylim(c(0,0.3))

  for(i in 2:length(xx)){
    p <- p + geom_line(data = xx[[i]])
  }
  return(p)
}

plotPecListMean <- function(pecx){
  models   <- names(pecx[[1]][["AppErr"]])
```

```
  plotData <- data.frame("Model" = NULL,"time" =NULL, "Mean" = NULL, "SD" = NULL)

  for(m in models){
    y <- sapply(pecx, function(x) x[["AppErr"]][[m]])
    means <- rowMeans(y)
    sds   <- rowSds(y)
    temp <- data.frame("Model" = rep(m, length(means)),
                       "time"  = pecx[[1]][["time"]],
                       "Mean"  = means,
                       "SD"    = sds)
    plotData = rbind(plotData, temp)
  }
  ggplot(plotData, aes(x = time, y = Mean, col = Model)) +
    geom_line() +
    ylab("Prediction Error") +
    ylim(c(0.1,0.3)) + xlim(c(1,5)) +
    geom_ribbon(aes(x = time, ymin = (Mean -2*SD),
                    ymax = (Mean+2*SD), fill = Model), alpha = 0.2)
}
```

Specify times of interest

```
evalTimes <- seq(0,5, by = 0.5)
```

In this section we perform a more formal 10 fold cross validation of the different models. we randomly split the data into ten groups, and then iteratively train on 9/10 of the data and predict on the remaining 1/10. We can then evalulate the performance of the model in the entire dataset.

Set parameters for cross validation

```
nFolds <- 10
nRepsCV  <- 10
recompute.cv <- FALSE
```

Split data into training and validation. Train models and predict.

```
results.cv.file <- file.path("../GeneratedData/results.cvRep.file.Rdata")

if(file.exists(results.cv.file) & recompute.cv == FALSE){
  load(results.cv.file)
} else{
  results.cv.reps <- list()
  times.interest  <- seq(0,5, by = 0.5)
  batchSize       <- table(combined.metadata$study)
  set.seed(191919)

  for(rep in 1:nRepsCV){
    results.cv <- list()
    results.cv.scores <- list()

    combined.metadata$cv <- c(sample(1:nFolds, batchSize[1], replace = TRUE),
                              sample(1:nFolds, batchSize[2], replace = TRUE),
                              sample(1:nFolds, batchSize[3], replace = TRUE))


    ## Loop over different subsets of data
```

```
for(subData in c("ALL", "ABC", "GCB")){

  ## Select subset of metadata
  if(subData != "ALL"){
    combined.metadata2 <- subset(combined.metadata, ABCGCB == subData)
  } else{
    combined.metadata2 <- combined.metadata
  }

  ## Dicotomize IPI
  combined.metadata2$IPI  <- factor(ifelse(combined.metadata2$IPI %in% c("0", "1"), "0-1", "2-5"))

  ## Prepare matrices for output
  predictCoxIPI <- matrix(NA, ncol = length(times.interest), nrow = nrow(combined.metadata2),
                          dimnames = list(combined.metadata2$ID))
  predictCoxAge <- matrix(NA, ncol = length(times.interest), nrow = nrow(combined.metadata2),
                          dimnames = list(combined.metadata2$ID))
  predictCoxMIR <- matrix(NA, ncol = length(times.interest), nrow = nrow(combined.metadata2),
                          dimnames = list(combined.metadata2$ID))
  predictRSF <- matrix(NA, ncol = length(times.interest), nrow = nrow(combined.metadata2),
                          dimnames = list(combined.metadata2$ID))
  predictCoxMIRIPI <- matrix(NA, ncol = length(times.interest), nrow = nrow(combined.metadata2),
                          dimnames = list(combined.metadata2$ID))
  predictRSFMIRIPI <- matrix(NA, ncol = length(times.interest), nrow = nrow(combined.metadata2),
                          dimnames = list(combined.metadata2$ID))

  ## Matrices for linear predictions
  scoreCoxMIR <- matrix(NA, ncol = 1, nrow = nrow(combined.metadata2),
                          dimnames = list(combined.metadata2$ID))
  scoreCoxMIRIPI <- matrix(NA, ncol = 1, nrow = nrow(combined.metadata2),
                          dimnames = list(combined.metadata2$ID))

  ## Loop over folds
  for(i in 1:nFolds){

    ## Select meta and gep data for training and validation
    meta.train <- subset(combined.metadata2, cv != i)
    meta.val   <- subset(combined.metadata2, cv == i)
    gep.train.sc <- microarrayScale(combined.data.combat[probes, meta.train$ID], scale = FALSE)
    gep.val.sc   <- microarrayScale(combined.data.combat[probes, meta.val$ID])

    ## Fit cox models with age or IPI
    fit.cox.age <- coxph(PFS ~ age, data = meta.train)
    fit.cox.IPI <- coxph(PFS ~ IPI, data = meta.train)

    ## Fit Random survival forest w/ wo IPI
    trainInput <- data.frame("time"=meta.train$PFS[,1],
                             "status"=meta.train$PFS[,2], t(gep.train.sc))
    fit.rsf <- rfsrc(formula = Surv(time, status)~.,
                  data = trainInput,
                  ntree = 1000)

    trainInput$IPI <- meta.train$IPI
```

```
fit.rsf.IPI <- rfsrc(formula = Surv(time, status)~.,
                    data = trainInput,
                    ntree = 1000)

## Fit cox models with MIR by first doing univariate cox for each probe
## adjusted for effect of study, and then selecting the significant probes
cox.data <- data.frame(meta.train, t(gep.train.sc))
names(cox.data) <- c(names(meta.train), probes)

cox.temp   <- uni.multi.cox(cox.data, probes, outcome = "PFS")
sig.probes <- row.names(cox.temp$combined.results[cox.temp$combined.results$uni.p<0.05,])

trainInput.sig.probes <- data.frame(meta.train$PFS, t(gep.train.sc[sig.probes,,drop=FALSE]))
names(trainInput.sig.probes) <- c("PFS", sig.probes)
fit.cox.MIR <- coxph(PFS~., data = trainInput.sig.probes)

## Add IPI to MIR prediction
trainInput.sig.probes$IPI <- meta.train$IPI
fit.cox.MIRIPI <- coxph(PFS~., data = trainInput.sig.probes)

## Get standard deviation of probes in training data and build validation data
trainSDs <- rowSds(combined.data.combat[probes, meta.train$ID])
valInput <- data.frame(meta.val$age, meta.val$IPI, t(gep.val.sc*trainSDs))
names(valInput) <- c("age", "IPI", probes)
valInput.rsf <- data.frame(valInput[,probes])
valInput.rsf.ipi <- data.frame(valInput[,-1])

## Predict in validation data and save results
predictCoxAge[meta.val$ID,] <- predictSurvProb(fit.cox.age,
                                               newdata = valInput,
                                               times = times.interest)
predictCoxIPI[meta.val$ID,] <- predictSurvProb(fit.cox.IPI,
                                               newdata = valInput,
                                               times = times.interest)
if(!is.null(coef(fit.cox.MIR))){
  predictCoxMIR[meta.val$ID,] <- predictSurvProb(fit.cox.MIR,
                                                 newdata = valInput,
                                                 times = times.interest)
}
predictRSF[meta.val$ID,]    <- predictSurvProb(fit.rsf,
                                               newdata = valInput.rsf,
                                               times = times.interest)
predictCoxMIRIPI[meta.val$ID,] <- predictSurvProb(fit.cox.MIRIPI,
                                                  newdata = valInput,
                                                  times = times.interest)
predictRSFMIRIPI[meta.val$ID,] <- predictSurvProb(fit.rsf.IPI,
                                                  newdata = valInput.rsf.ipi,
                                                  times = times.interest)

## Linear predictions for coxMIR models
if(!is.null(coef(fit.cox.MIR))){
  scoreCoxMIR[meta.val$ID,1]    <- predict(fit.cox.MIR, newdata = valInput)
}
```

```
        scoreCoxMIRIPI[meta.val$ID,1] <- predict(fit.cox.MIRIPI, newdata = valInput)


      }
      results.cv[[subData]] <- list("CoxAge" = predictCoxAge,
                                    "CoxIPI" = predictCoxIPI,
                                    "CoxMIR" = predictCoxMIR,
                                    "RSFMIR"    = predictRSF,
                                    "CoxMIRIPI" = predictCoxMIRIPI,
                                    "RSFMIRIPI" = predictRSFMIRIPI)
      results.cv.scores[[subData]] <- list("scoreCoxMIR" = scoreCoxMIR,
                                           "scoreCoxMIRIPI" = scoreCoxMIRIPI)
    }
    results.cv.reps[[rep]] <- list("cv" = results.cv, "scores" = results.cv.scores)

  }
  save(results.cv.reps, times.interest, file = results.cv.file, nRepsCV)
}
```

Results of CV on OS

We first evaluate the predictive accuracy of the model on overall survival.

Calculate brier score for OS

```
error.all.OS <- list()
error.abc.OS <- list()
error.gcb.OS <- list()

for(i in 1:nRepsCV){
error.all.OS[[i]] <- pec(results.cv.reps[[i]][["cv"]][["ALL"]],
                         formula = Surv(OStime,OSstatus) ~ 1,
                         data = combined.metadata,
                         times = times.interest,
                         reference = F,
                         verbose = T, exact = FALSE)



error.abc.OS[[i]] <- pec(results.cv.reps[[i]][["cv"]][["ABC"]],
                         formula = Surv(OStime,OSstatus) ~ 1,
                         data = subset(combined.metadata, ABCGCB == "ABC"),
                         times = times.interest,
                         reference = F,
                         verbose = T, exact = FALSE)


error.gcb.OS[[i]] <- pec(results.cv.reps[[i]][["cv"]][["GCB"]],
                         formula = Surv(OStime,OSstatus) ~ 1,
                         data = subset(combined.metadata, ABCGCB == "GCB"),
                         times = times.interest,
                         reference = F,
                         verbose = T, exact = FALSE)
```

```
}
```

```
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
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## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
```
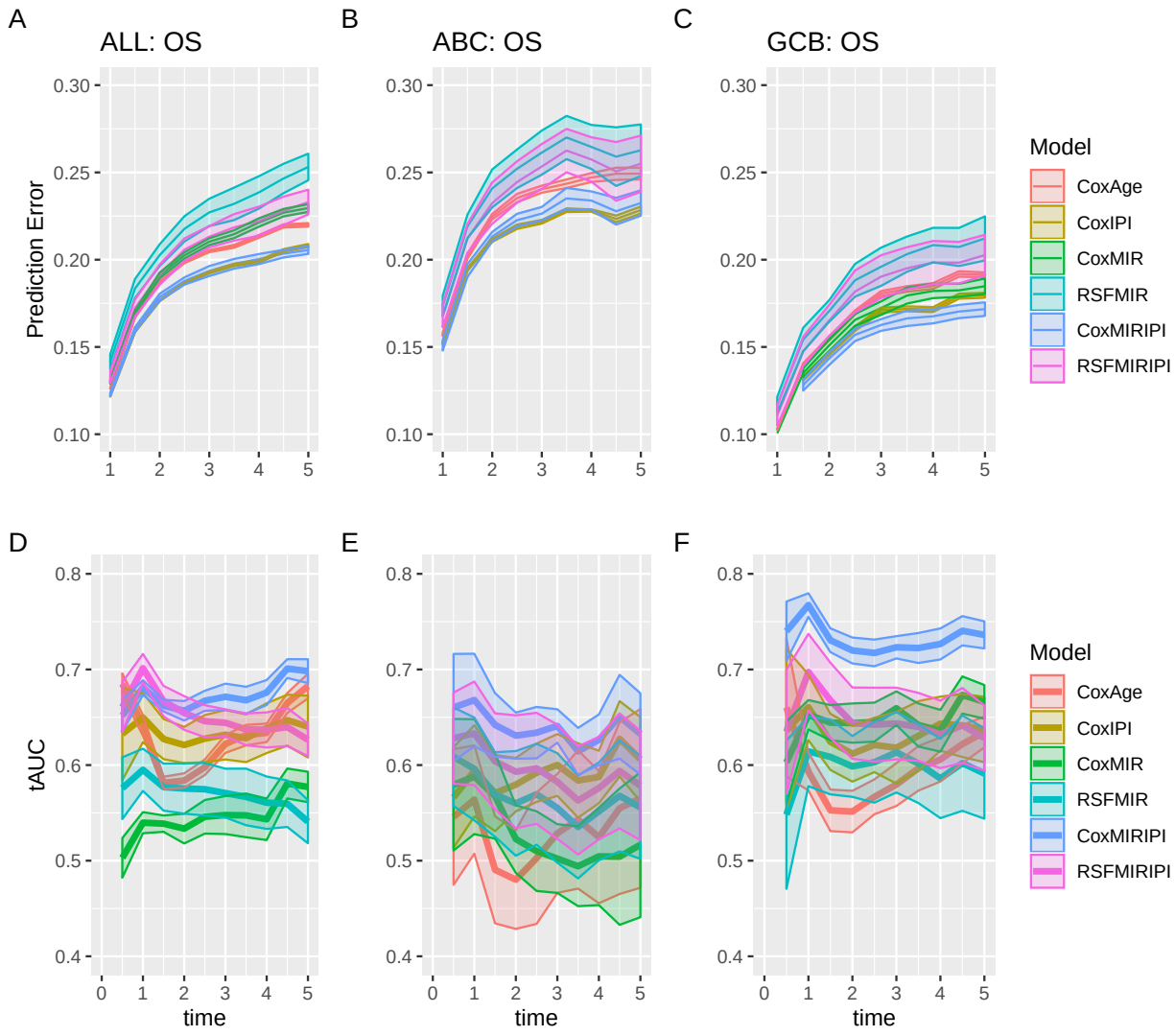

Calucate time varying AUCs

```
tAUC.all.OS <- list()
tAUC.abc.OS <- list()
tAUC.gcb.OS <- list()

tAUC.OS.ALL <- function(x) timeVaryingAUC(x, combined.metadata$OStime,
                                              combined.metadata$OSstatus, times.interest)
tAUC.OS.ABC <- function(x) timeVaryingAUC(x, subset(combined.metadata, ABCGCB == "ABC")$OStime,
                                          subset(combined.metadata, ABCGCB == "ABC")$OSstatus,
                                          times.interest)
tAUC.OS.GCB <- function(x) timeVaryingAUC(x, subset(combined.metadata, ABCGCB == "GCB")$OStime,
                                          subset(combined.metadata, ABCGCB == "GCB")$OSstatus,
                                          times.interest)

for(rep in 1:nRepsCV){
  tAUC.all.OS[[rep]] <- lapply(results.cv.reps[[rep]][["cv"]][["ALL"]], tAUC.OS.ALL)
  tAUC.abc.OS[[rep]] <- lapply(results.cv.reps[[rep]][["cv"]][["ABC"]], tAUC.OS.ABC)
  tAUC.gcb.OS[[rep]] <- lapply(results.cv.reps[[rep]][["cv"]][["GCB"]], tAUC.OS.GCB)
}
```

Plot Results

```r
# Brier Scores
p11 <- plotPecListMean(error.all.OS) + ggtitle("ALL: OS") +
  scale_color_discrete(guide = FALSE) +
  xlab("") + scale_fill_discrete(guide = FALSE) +
  labs(tag = "A")
p12 <- plotPecListMean(error.abc.OS) + ggtitle("ABC: OS") +
  scale_color_discrete(guide = FALSE) +
  xlab("") + scale_fill_discrete(guide = FALSE) +
  ylab("") + labs(tag = "B")
p13 <- plotPecListMean(error.gcb.OS) + ggtitle("GCB: OS") +
  xlab("") + ylab("") + labs(tag = "C")

#tAUC
p21 <- plottAUCListMean(tAUC.all.OS, times.interest)  +
  scale_color_discrete(guide = FALSE) +
  scale_fill_discrete(guide = FALSE) + labs(tag = "D")
p22 <- plottAUCListMean(tAUC.abc.OS, times.interest)  +
  scale_color_discrete(guide = FALSE)  +
  ylab("")  + scale_fill_discrete(guide = FALSE) + labs(tag = "E")
p23 <- plottAUCListMean(tAUC.gcb.OS, times.interest) +
  ylab("") + labs(tag = "F")

grid.arrange(p11,p12,p13,p21,p22,p23, ncol = 3, widths = c(2,2,3.2))
```



Results of CV on PFS

Next the predictive accuracy for progression free survival is evaluated.

Calculate brier score for PFS

```
error.all.PFS <- list()
error.abc.PFS <- list()
error.gcb.PFS <- list()

for(i in 1:nRepsCV){
error.all.PFS[[i]] <- pec(results.cv.reps[[i]][["cv"]][["ALL"]],
                          formula = Surv(PFStime,PFSstatus) ~ 1,
                          data = combined.metadata,
                          times = times.interest,
                          reference = F,
                          verbose = T, exact = FALSE)
```

```r
error.abc.PFS[[i]] <- pec(results.cv.reps[[i]][["cv"]][["ABC"]],
                          formula = Surv(PFStime,PFSstatus) ~ 1,
                          data = subset(combined.metadata, ABCGCB == "ABC"),
                          times = times.interest,
                          reference = F,
                          verbose = T, exact = FALSE)


error.gcb.PFS[[i]] <- pec(results.cv.reps[[i]][["cv"]][["GCB"]],
                          formula = Surv(PFStime,PFSstatus) ~ 1,
                          data = subset(combined.metadata, ABCGCB == "GCB"),
                          times = times.interest,
                          reference = F,
                          verbose = T, exact = FALSE)
}
```

```
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
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## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
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## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
## No covariates  specified: Kaplan-Meier for censoring times used for weighting.
```
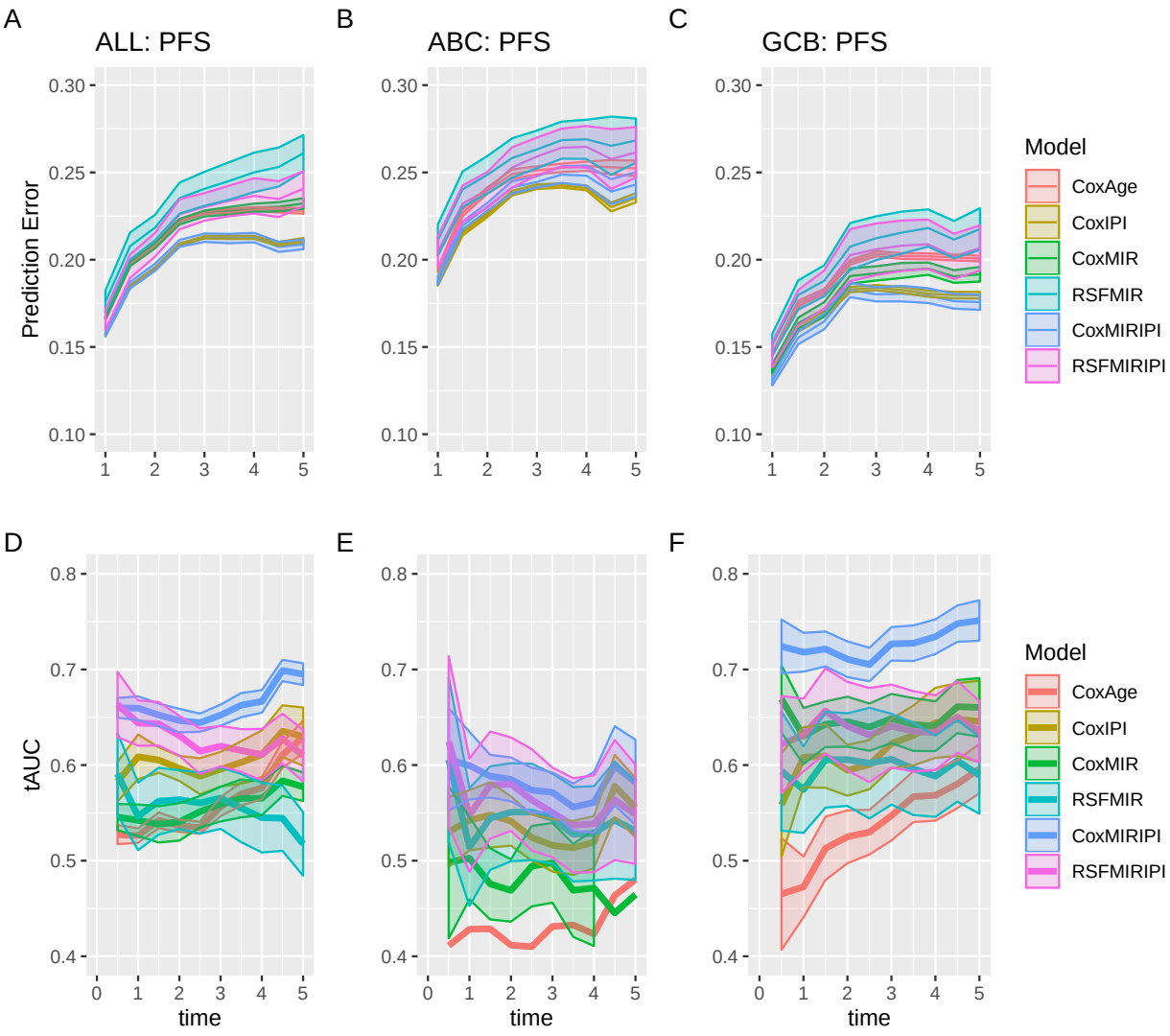

Calucate time varying AUCs

```r
tAUC.all.PFS <- list()
tAUC.abc.PFS <- list()
tAUC.gcb.PFS <- list()

tAUC.PFS.ALL <- function(x) timeVaryingAUC(x, combined.metadata$PFStime,
```

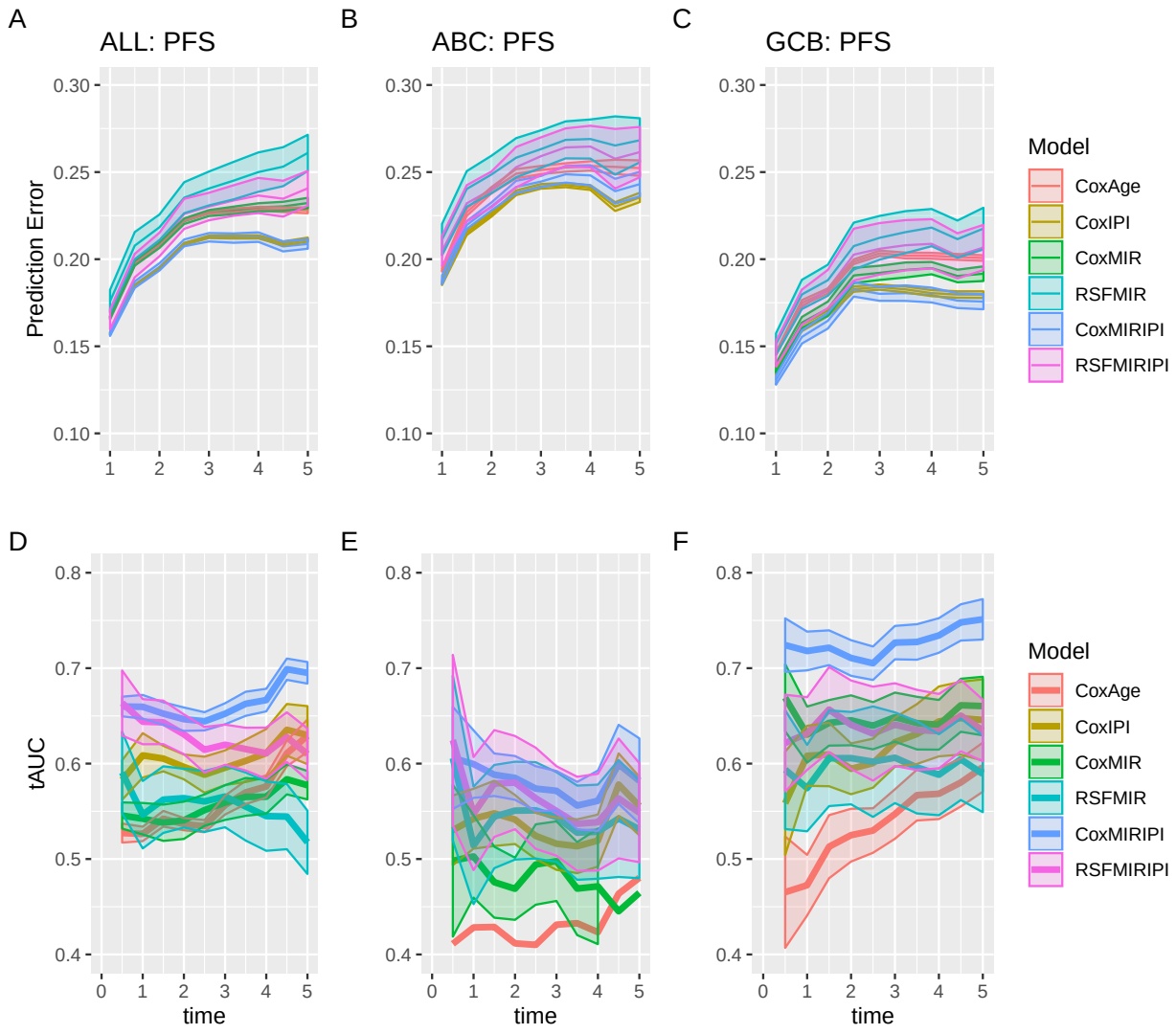
```
                                        combined.metadata$PFSstatus,
                                        times.interest)
tAUC.PFS.ABC <- function(x) timeVaryingAUC(x, subset(combined.metadata, ABCGCB == "ABC")$PFStime,
                                      subset(combined.metadata, ABCGCB == "ABC")$PFSstatus,
                                      times.interest)
tAUC.PFS.GCB <- function(x) timeVaryingAUC(x, subset(combined.metadata, ABCGCB == "GCB")$PFStime,
                                      subset(combined.metadata, ABCGCB == "GCB")$PFSstatus,
                                      times.interest)

for(rep in 1:nRepsCV){
  tAUC.all.PFS[[rep]] <- lapply(results.cv.reps[[rep]][["cv"]][["ALL"]], tAUC.PFS.ALL)
  tAUC.abc.PFS[[rep]] <- lapply(results.cv.reps[[rep]][["cv"]][["ABC"]], tAUC.PFS.ABC)
  tAUC.gcb.PFS[[rep]] <- lapply(results.cv.reps[[rep]][["cv"]][["GCB"]], tAUC.PFS.GCB)
}
```

Plot Results

```
# Brier Scores
p11 <- plotPecListMean(error.all.PFS) + ggtitle("ALL: PFS") +
  scale_color_discrete(guide = FALSE) +
  scale_fill_discrete(guide = FALSE) +
  xlab("") + labs(tag = "A")
p12 <- plotPecListMean(error.abc.PFS) + ggtitle("ABC: PFS") +
  scale_color_discrete(guide = FALSE) +
  scale_fill_discrete(guide = FALSE) +
  xlab("") +ylab("") + labs(tag = "B")
p13 <- plotPecListMean(error.gcb.PFS) + ggtitle("GCB: PFS") +
  xlab("") + ylab("") + labs(tag = "C")

#tAUC
p21 <- plottAUCListMean(tAUC.all.PFS, times.interest)  +
  scale_color_discrete(guide = FALSE) +
  scale_fill_discrete(guide = FALSE) + labs(tag = "D")
p22 <- plottAUCListMean(tAUC.abc.PFS, times.interest)  +
  scale_color_discrete(guide = FALSE) +
  scale_fill_discrete(guide = FALSE) + ylab("") + labs(tag = "E")
p23 <- plottAUCListMean(tAUC.gcb.PFS, times.interest) +
  ylab("") + labs(tag = "F")

fig2 <- grid.arrange(p11,p12,p13,p21,p22,p23, ncol = 3, widths = c(2,2,3.2))
```


A
ALL: PFS
B
ABC: PFS
C
GCB: PFS
Prediction Error
0.30
0.25
0.20
0.15
0.10
1
2
3
4
5
Model
CoxAge
CoxIPI
CoxMIR
RSFMIR
CoxMIRIPI
RSFMIRIPI
D
E
F
tAUC
0.8
0.7
0.6
0.5
0.4
0
time

```
plot(fig2)
```




```
scoresMIR <- do.call(cbind, lapply(results.cv.reps,
                                   function(x) x[["scores"]][["GCB"]][["scoreCoxMIR"]]))
scoresMIRIPI <- do.call(cbind, lapply(results.cv.reps,
                                      function(x) x[["scores"]][["GCB"]][["scoreCoxMIRIPI"]]))

scores.gcb <- data.frame("coxMIR" = rowMeans(scoresMIR),
                         "coxMIRIPI" = rowMeans(scoresMIRIPI))
```

Kaplan meier plots

We use the linear predictions from the Cox models in the crossvalidation to get a score for each individual. These are then split into tertiles and kaplan meier plots are made. Start by adding linear predictions to GCB subset and splitting into tertiles.

```
combined.metadata.gcb <- merge(combined.metadata, scores.gcb,by.x = "ID", by.y = 0)
combined.metadata.gcb$coxMIRClass <- cutFun(combined.metadata.gcb$coxMIR)
combined.metadata.gcb$coxMIRIPIClass <- cutFun(combined.metadata.gcb$coxMIRIPI)
combined.metadata.gcb$Response <- factor(combined.metadata.gcb$Response,
```

```
                                        levels =  levels(combined.metadata.gcb$Response)[c(1,3,4,2)])
```

Then do KM plots

```
fitMIROS  <- with(combined.metadata.gcb, survfit(OS5~coxMIRClass))
fitMIRPFS <- with(combined.metadata.gcb, survfit(PFS5~coxMIRClass))

kmMIROS <- ggsurvplot(fitMIROS,
                      risk.table = TRUE,
                      data = combined.metadata.gcb,
                      risk.table.y.text=FALSE,
                      pval = TRUE,
                      tables.height = 0.3,
                      legend.labs = c("Low", "Intermediate", "High")) +
           ggtitle("MIR: OS")

kmMIRPFS <- ggsurvplot(fitMIRPFS,
                      risk.table = TRUE,
                      data = combined.metadata.gcb,
                      risk.table.y.text=FALSE,
                      pval = TRUE,
                      tables.height = 0.3,
                      legend.labs = c("Low", "Intermediate", "High")) +
           ggtitle("MIR: PFS")
```

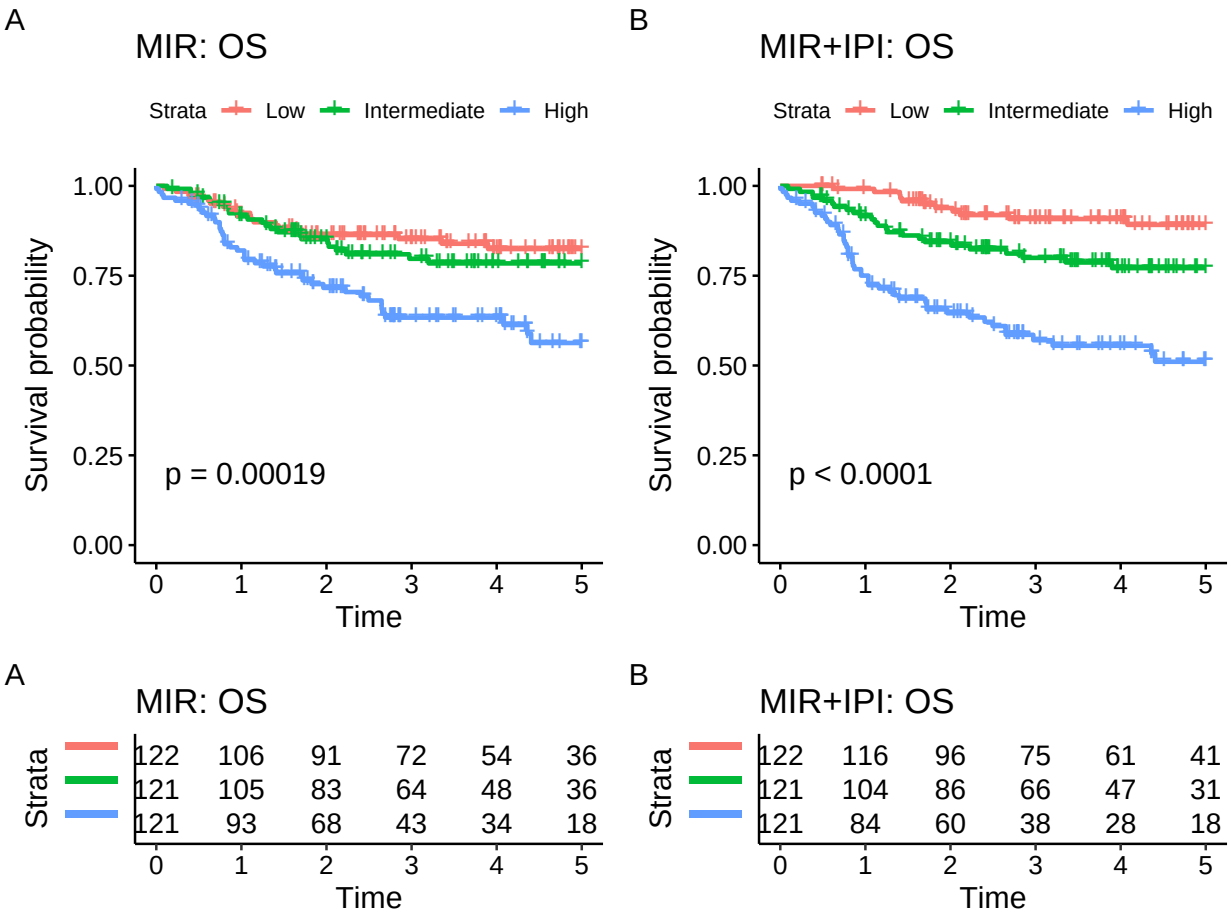
Try with coxMIRIPI

```
fitMIRIPIOS  <- with(combined.metadata.gcb, survfit(OS5~coxMIRIPIClass))
fitMIRIPIPFS <- with(combined.metadata.gcb, survfit(PFS5~coxMIRIPIClass))

kmMIRIPIOS <- ggsurvplot(fitMIRIPIOS,
                      risk.table = TRUE,
                      data = combined.metadata.gcb,
                      risk.table.y.text=FALSE,
                      pval = TRUE,
                      tables.height = 0.3,
                      legend.labs = c("Low", "Intermediate", "High")) +
                      ggtitle("MIR+IPI: OS")

kmMIRIPIPFS <- ggsurvplot(fitMIRIPIPFS,
                      data = combined.metadata.gcb,
                      risk.table = TRUE,
                      risk.table.y.text=FALSE,
                      pval = TRUE,
                      tables.height = 0.3,
                      legend.labs = c("Low", "Intermediate", "High")) +
                      ggtitle("MIR+IPI: PFS")
```

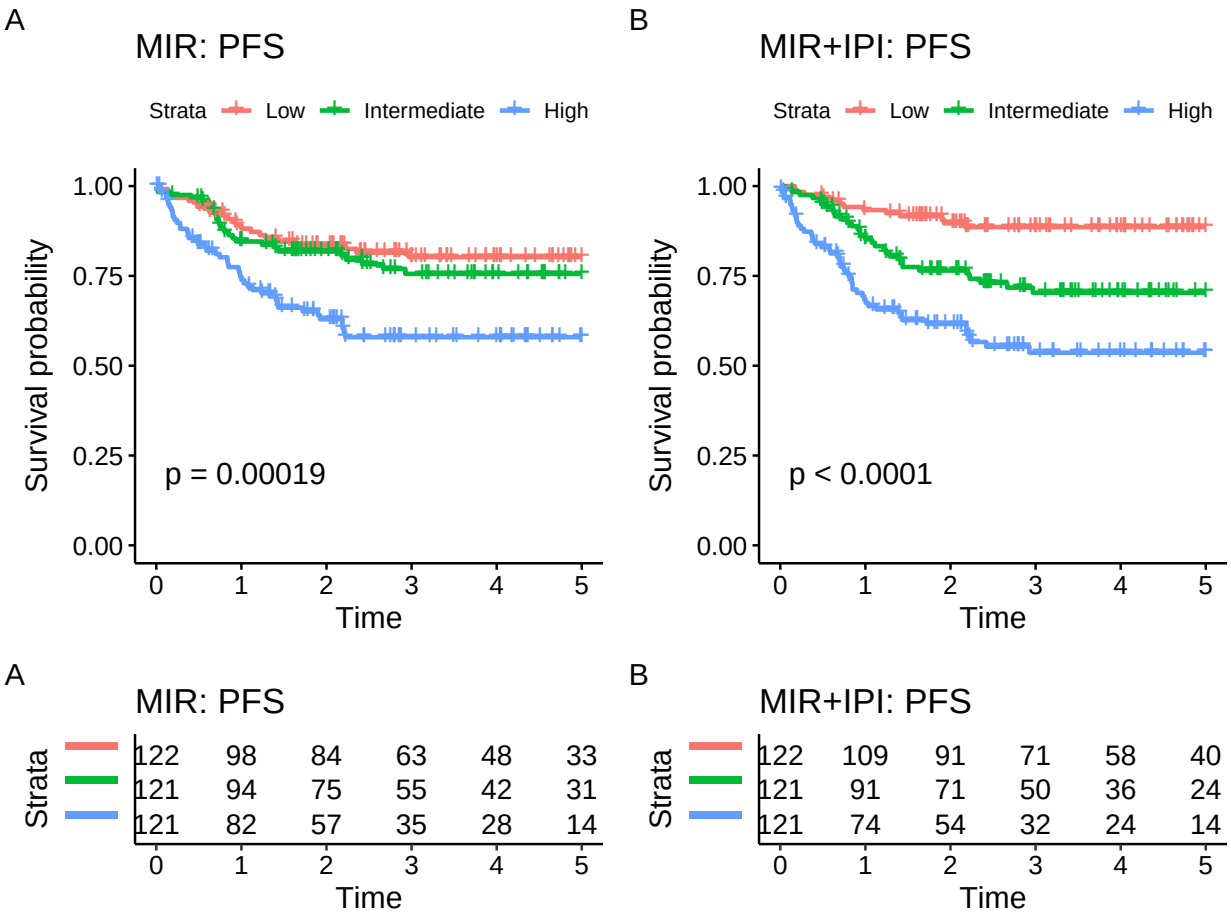
Create side-by-side plots

```
kmMIROS <- kmMIROS + labs(tag = "A")
kmMIRIPIOS <- kmMIRIPIOS + labs(tag = "B")
kmMIRPFS <- kmMIRPFS + labs(tag = "A")
kmMIRIPIPFS <- kmMIRIPIPFS + labs(tag = "B")
```

```
arrange_ggsurvplots(list(kmMIROS, kmMIRIPIOS))
```

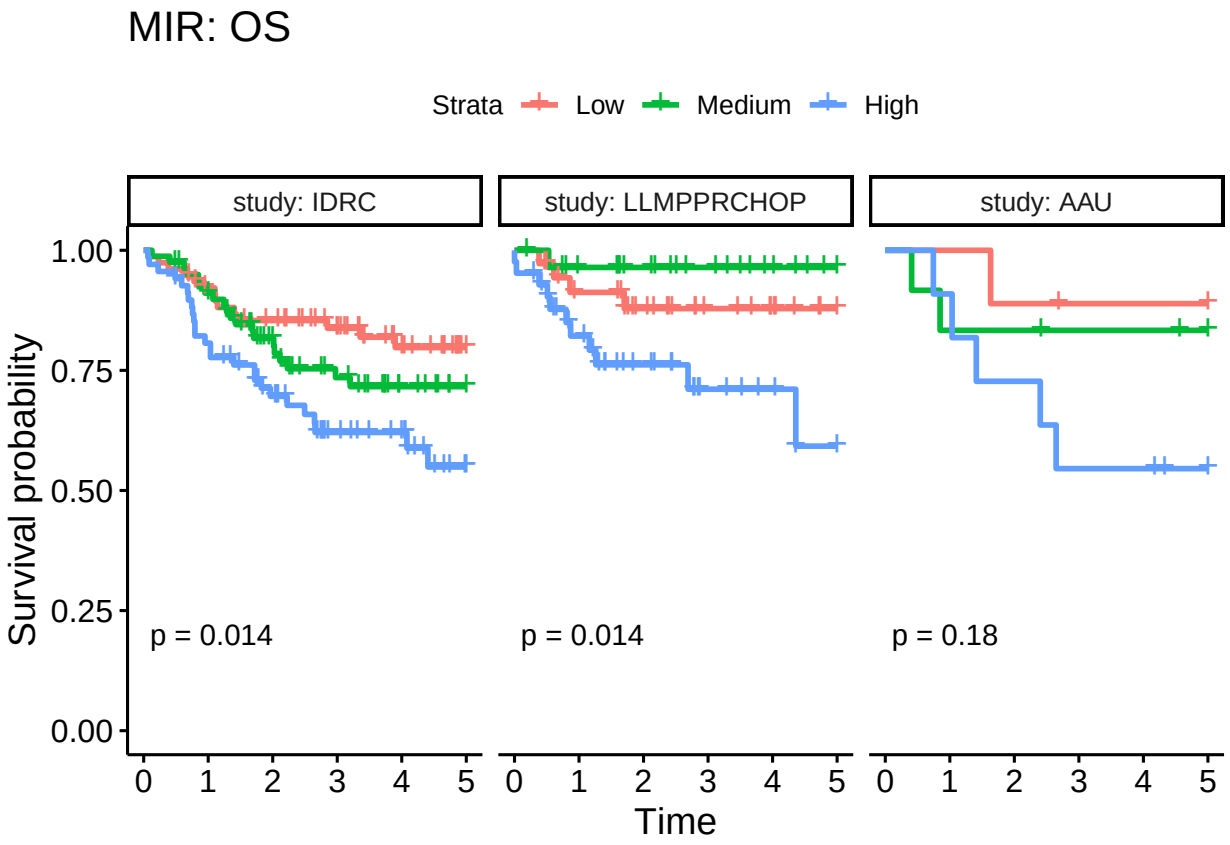



```
fig3 <- arrange_ggsurvplots(list(kmMIRPFS, kmMIRIPIPFS))
```

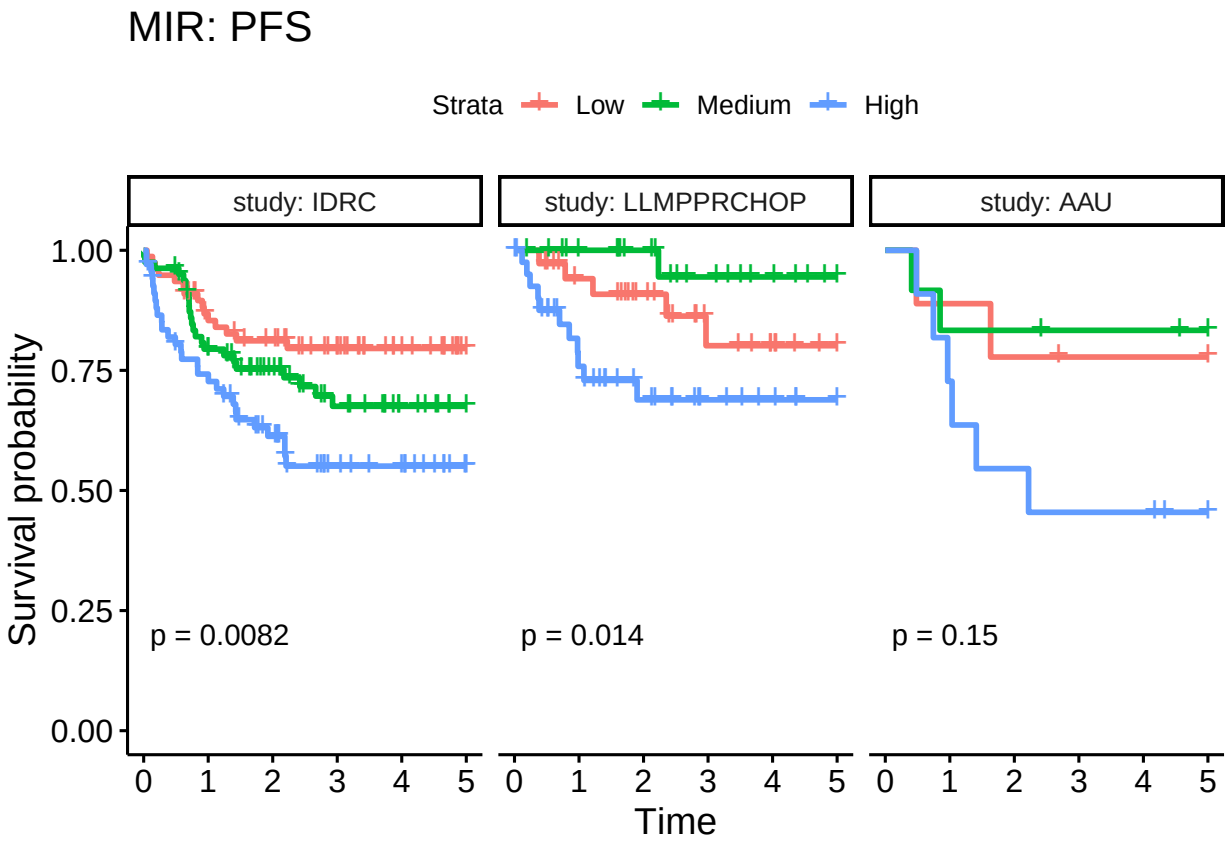


Stratify survival plots by study

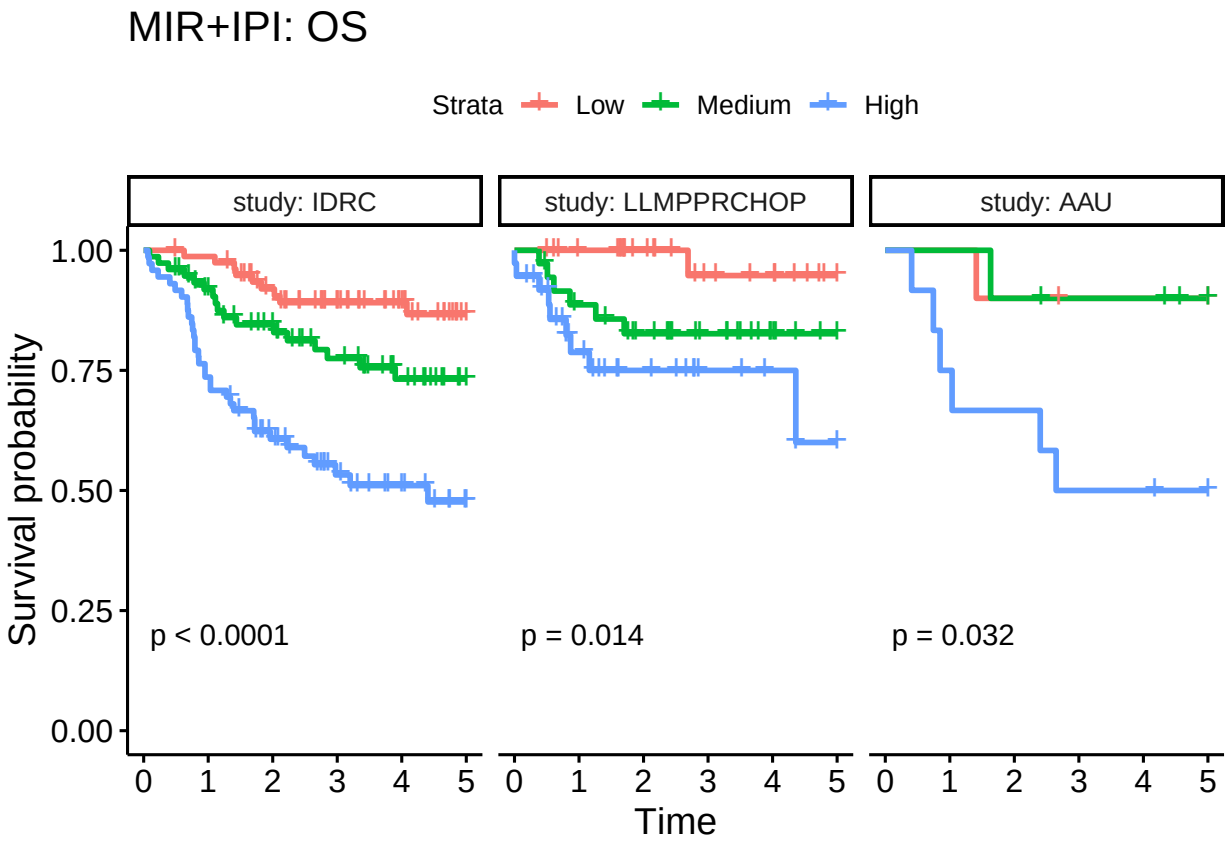
```
kmMIROS_study <- ggsurvplot_facet(fitMIROS,
                                  data = combined.metadata.gcb,
                                  pval = TRUE,
                                  facet.by = "study") + ggtitle("MIR: OS")

kmMIRPFS_study <- ggsurvplot_facet(fitMIRPFS,
                                  data = combined.metadata.gcb,
                                  pval = TRUE,
                                  facet.by = "study")  + ggtitle("MIR: PFS")
kmMIRIPIOS_study <- ggsurvplot_facet(fitMIRIPIOS,
                                  data = combined.metadata.gcb,
                                  pval = TRUE,
                                  facet.by = "study")  + ggtitle("MIR+IPI: OS")

kmMIRIPIPFS_study <- ggsurvplot_facet(fitMIRIPIPFS,
                                  data = combined.metadata.gcb,
                                  pval = TRUE,
                                  facet.by = "study") + ggtitle("MIR+IPI: PFS")

kmMIROS_study
```



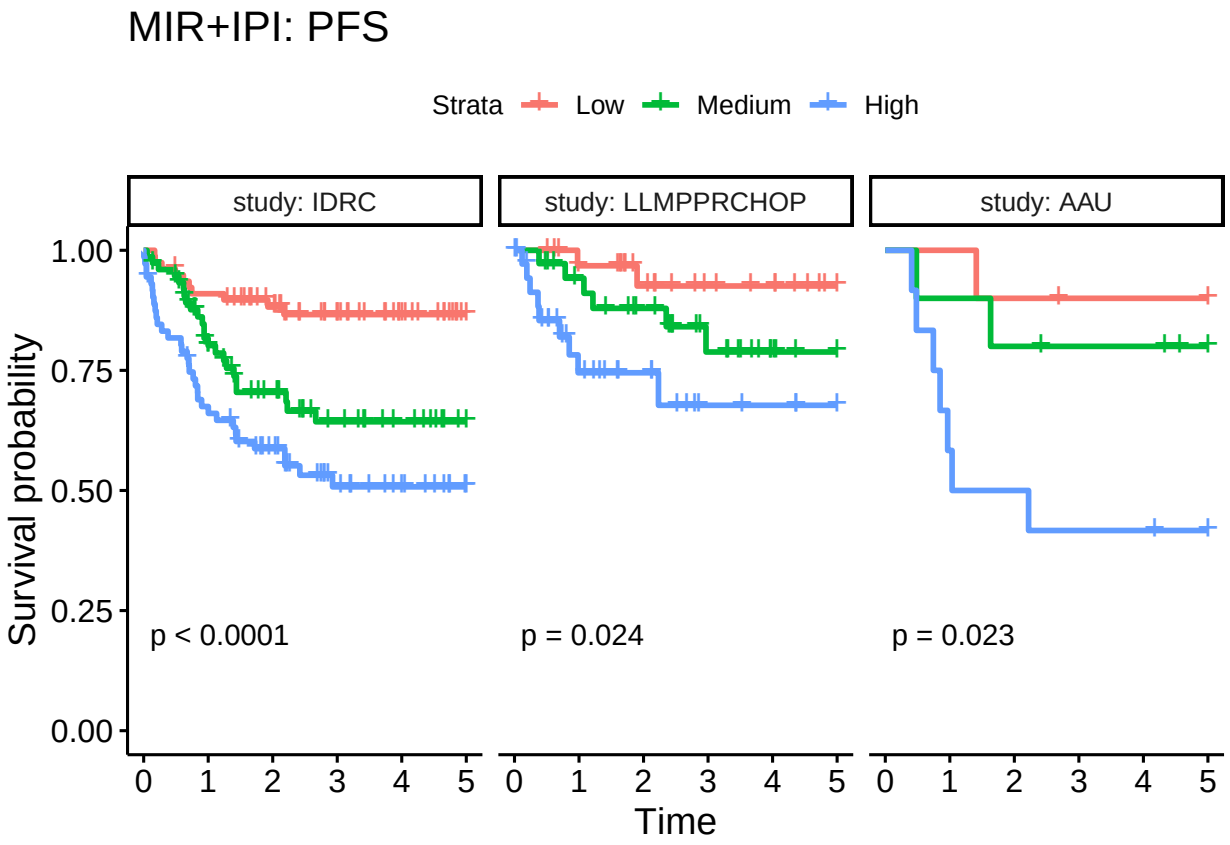

```
kmMIRPFS_study
```




```
kmMIRIPIOS_study
```




```
kmMIRIPIPFS_study
```



Compare with clinical response

Test for significant difference in prognostic score across clinical response groups and store p-values.

```
lmMIR    <- lm(coxMIR~Response, data = combined.metadata.gcb)
lmMIRIPI <- lm(coxMIRIPI~Response, data = combined.metadata.gcb)
summary(lmMIR)
```

```
##
## Call:
## lm(formula = coxMIR ~ Response, data = combined.metadata.gcb)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.28027 -0.36027 -0.03468  0.32147  1.95475
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) -0.07897    0.03293  -2.398  0.01703 *
## ResponsePR   0.25209    0.08176   3.083  0.00223 **
## ResponseSD   0.17395    0.15326   1.135  0.25723
## ResponsePD   0.49665    0.12999   3.821  0.00016 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.5185 on 321 degrees of freedom
```

```
##   (39 observations deleted due to missingness)
## Multiple R-squared:  0.06532,    Adjusted R-squared:  0.05658
## F-statistic: 7.477 on 3 and 321 DF,  p-value: 7.472e-05
```

```
summary(lmMIRIPI)
```

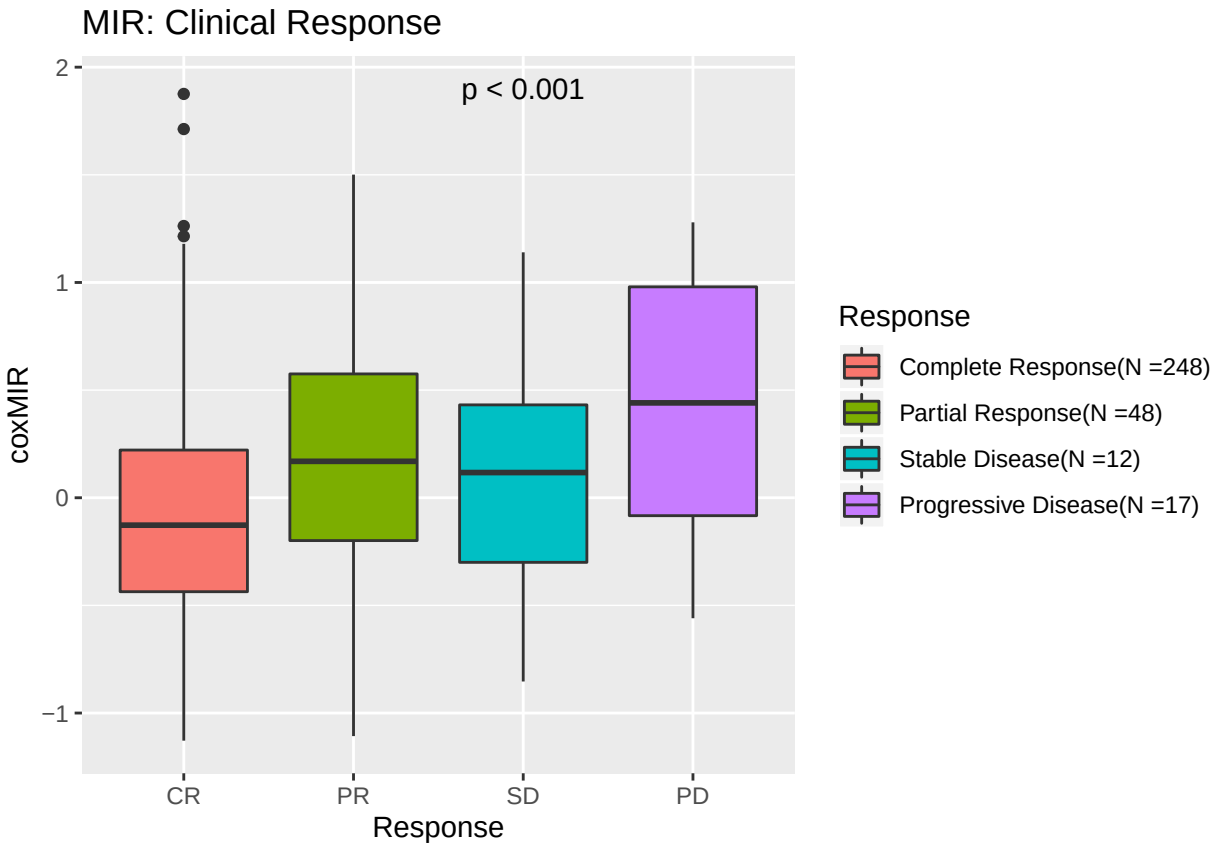
```
##
## Call:
## lm(formula = coxMIRIPI ~ Response, data = combined.metadata.gcb)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -1.67992 -0.65568  0.06101  0.57496  2.22702
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  -0.1719     0.0482  -3.567 0.000417 ***
## ResponsePR    0.5955     0.1197   4.975 1.06e-06 ***
## ResponseSD    0.5421     0.2244   2.416 0.016240 *
## ResponsePD    0.7858     0.1903   4.129 4.65e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.7591 on 321 degrees of freedom
##   (39 observations deleted due to missingness)
## Multiple R-squared:  0.1141, Adjusted R-squared:  0.1058
## F-statistic: 13.78 on 3 and 321 DF,  p-value: 1.775e-08
```

```
## Get p-values
pMIR <- ifelse(anova(lmMIR)$`Pr(>F)`[1] < 0.001,
               "p < 0.001",
               paste("p =", round(anova(lmMIR)$`Pr(>F)`[1]),3))


pMIRIPI <- ifelse(anova(lmMIRIPI)$`Pr(>F)`[1] < 0.001,
               "p < 0.001",
               paste("p =", round(anova(lmMIRIPI)$`Pr(>F)`[1]),3))
```

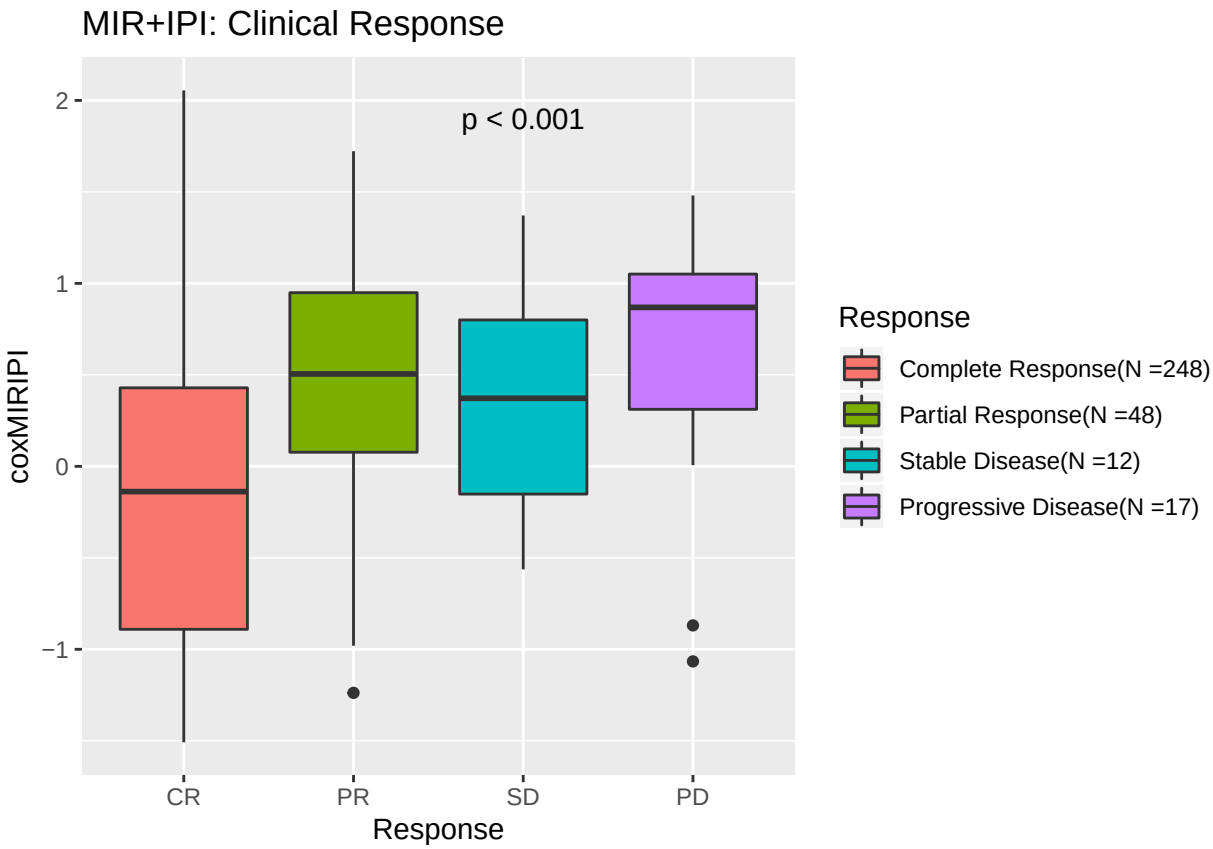
Create boxplots of clinical response vs prognostic score.

```
## Create Labels for x-axis
labls <-  c("Complete Response", "Partial Response", "Stable Disease", "Progressive Disease")
labls <-  paste0(labls, "(N =", table(combined.metadata.gcb$Response), ")")


crMIR <- ggplot(subset(combined.metadata.gcb, is.na(Response) == FALSE),
             aes(x = Response, y = coxMIR, fill = Response)) +
             geom_boxplot() +
             ggtitle("MIR: Clinical Response") +
             scale_fill_discrete(labels = labls) +
             annotate("text", label = pMIR, x = 3, y = 1.9)

crMIRIPI <- ggplot(subset(combined.metadata.gcb, is.na(Response) == FALSE),
               aes(x = Response, y = coxMIRIPI, fill = Response)) +
               geom_boxplot() +
               ggtitle("MIR+IPI: Clinical Response") +
```

```
              scale_fill_discrete(labels = labls)  +
              annotate("text", label = pMIRIPI, x = 3, y = 1.9)
crMIR
```




```
crMIRIPI
```



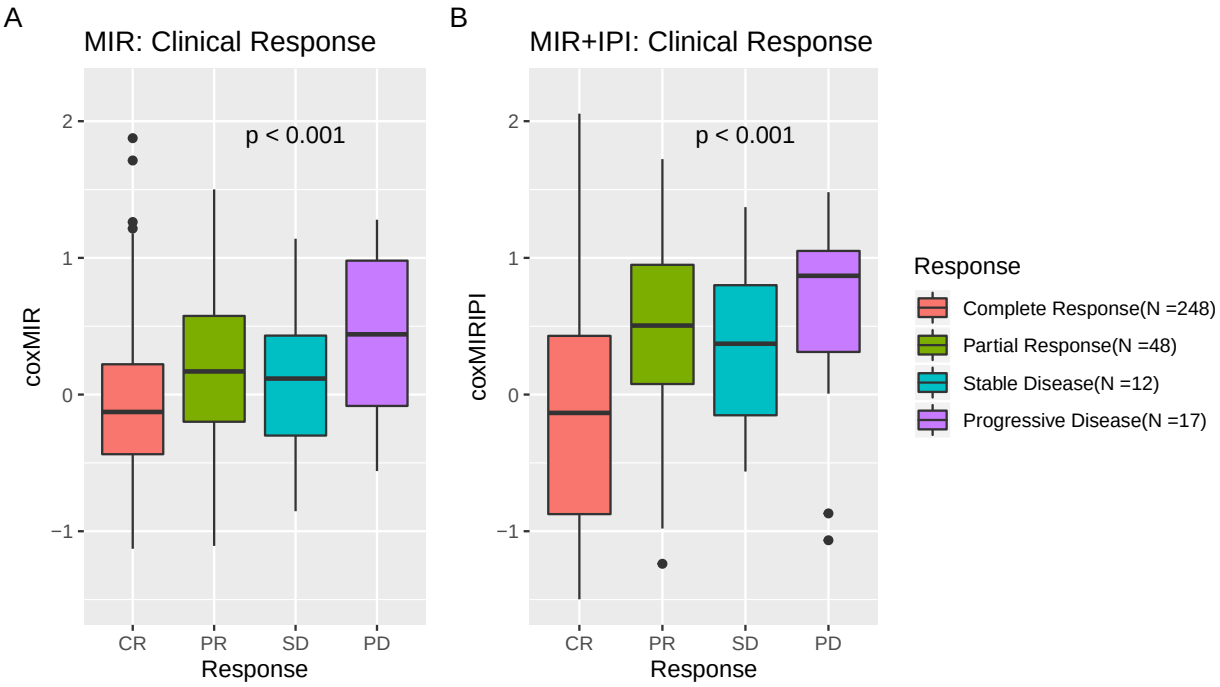
Combined side-by-side plot

```
crMIR <- crMIR + scale_fill_discrete(guide = FALSE) + ylim(c(-1.5, 2.2)) + labs(tag = "A")
```

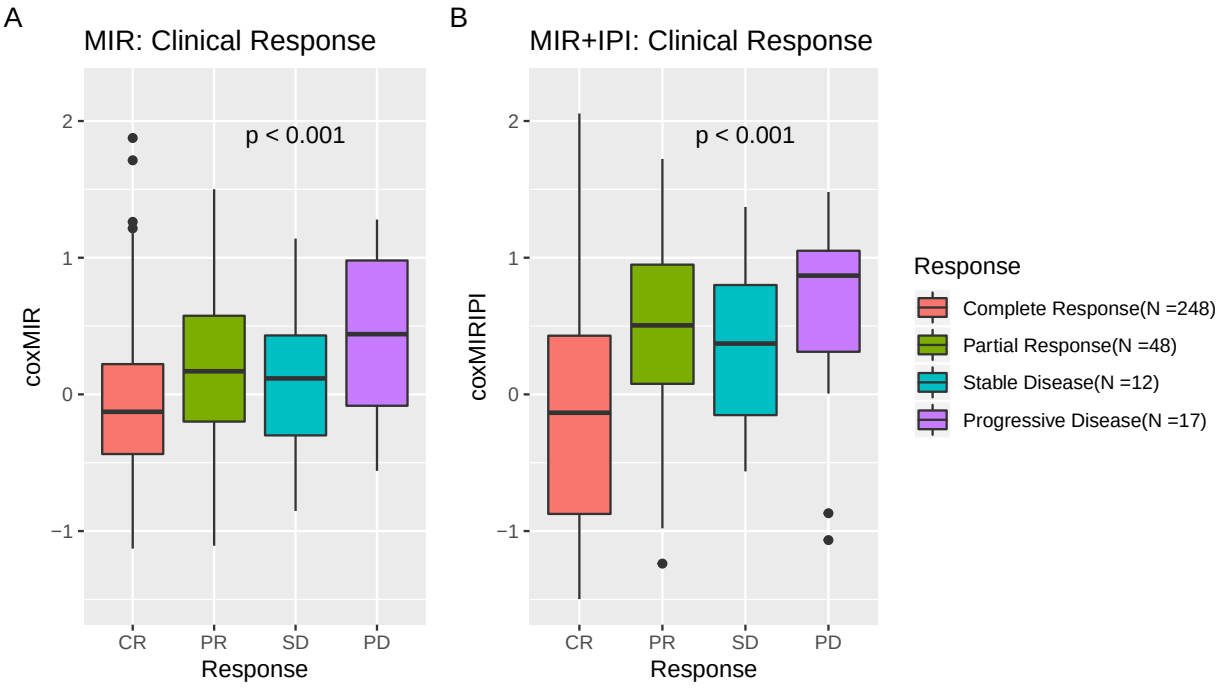
```
## Scale for 'fill' is already present. Adding another scale for 'fill',
## which will replace the existing scale.
```

```
crMIRIPI <- crMIRIPI + ylim(c(-1.5, 2.2)) + labs(tag = "B")
fig4 <- grid.arrange(crMIR, crMIRIPI, ncol = 2, widths = c(1,1.8))
```

```
## Warning: Removed 1 rows containing non-finite values (stat_boxplot).
```




```
plot(fig4)
```



Train final classifer

Based on results above we train a classifier with MIR probes and IPI in the full GCB subset of data Build dataset

```
cox.data <- data.frame(t(combined.data.combat[probes, ]))
names(cox.data) <- probes
cox.data <- cbind(combined.metadata, cox.data)
cox.data.gcb <- subset(cox.data, ABCGCB == "GCB")
```

Do the univariate cox regression for each probe

```
cox.gcb.PFS <- uni.multi.cox(cox.data.gcb, probes, outcome = "PFS")
kable(cox.gcb.PFS$combined.results)
```

	MIR	uni.HR	uni.conf	uni.p	multi.HR	multi.conf	multi.p
238225_at	MIR146A	0.92	(0.49;1.71)	0.792	(0.61;2.14)	(0.61;2.14)	0.669
232504_at	MIR146A	0.69	(0.58;0.81)	0.000	(0.65;1)	(0.65;1)	0.047
229437_at	MIR155	0.75	(0.66;0.85)	0.000	(0.73;1.04)	(0.73;1.04)	0.120
220990_s_at	MIR21	0.74	(0.62;0.88)	0.001	(0.79;1.39)	(0.79;1.39)	0.760
229417_at	MIR21	0.90	(0.55;1.46)	0.666	(0.54;1.42)	(0.54;1.42)	0.591
235317_at	MIR23A	0.98	(0.64;1.5)	0.932	(0.8;2.29)	(0.8;2.29)	0.257
1555847_a_at	MIR23A	0.76	(0.59;0.97)	0.025	(0.61;1.23)	(0.61;1.23)	0.418
235571_at	MIR34A	0.54	(0.38;0.77)	0.001	(0.44;1.02)	(0.44;1.02)	0.064
1557342_a_at	hsa-let-7b	1.02	(0.57;1.84)	0.945	(0.46;1.51)	(0.46;1.51)	0.549
241464_s_at	hsa-let-7b	0.73	(0.33;1.58)	0.422	(0.35;1.62)	(0.35;1.62)	0.464
227488_at	MIR503	1.11	(0.62;1.98)	0.726	(0.57;1.93)	(0.57;1.93)	0.875

```
## Probes that were significant in univariate cox for PFS
sig.probes <- row.names(cox.gcb.PFS$combined.results[cox.gcb.PFS$combined.results$uni.p<0.05,])

## Pick out necessary columns from GCB subset of data, and recode IPI
cox.final.data <- cox.data.gcb[, c("PFS", "IPI", sig.probes)]
cox.final.data$IPI <- factor(ifelse(cox.final.data$IPI %in% c("0", "1"), "0-1", "2-5"))

## Fit model and print results
cox.final <- coxph(PFS~., data = cox.final.data)
kable(summary(cox.final)$conf.int)
```

	exp(coef)	exp(-coef)	lower .95	upper .95
IPI2-5	3.3345365	0.2998918	1.9559374	5.684810
232504_at	0.8568170	1.1671104	0.6916290	1.061458
229437_at	0.8676394	1.1525525	0.7308004	1.030101
220990_s_at	0.9952559	1.0047667	0.7652018	1.294475
1555847_a_at	0.9578975	1.0439531	0.7233349	1.268524
235571_at	0.7759503	1.2887424	0.5220012	1.153443

Prepare figures for publication

```
## Single column 3.125" @ 600 dpi
## Double column 7"     @ 600 dpi

tiff("../Output/figures/fig1.tiff", width = 1875, height = 1875, pointsize = 50, compression = "lzw")
  grid.draw(v1)
dev.off()
```

```
## pdf
```

```
##   2
```

```
ggsave("../Output/figures/fig2.tiff", plot = fig2, dpi = 600, width = 7.5, height = 7, compression = "lz
ggsave("../Output/figures/fig3.tiff", plot = fig3, dpi = 600, width = 7.5, height = 7, compression = "lz
ggsave("../Output/figures/fig4.tiff", plot = fig4, dpi = 600, width = 7.5, height = 3.5, compression =
```

Validate MIR expression with ddPCR

Read ddPCR data

```
ddPCR <- read_xlsx("../ExternalData/Cell lines/MiR_exp_ddPCR_correlation_analysis.xlsx")
```

```
## New names:
## * `` -> ...7
```

```
### Extract actual data and probeset to mir names
ddPCRdata <- ddPCR[,1:6]
ddPCRnames <- ddPCR[1:5,8:9]
```

Correlate U133+2 data to ddPCR data

```
Cors <- c()
for(i in 1:nrow(ddPCRnames)){
  Cors[i] <- cor(ddPCRdata[,ddPCRnames$miRNA[i]],
                 bCellu133[ddPCRnames$Probeset[i],])
}
Cors <- data.frame("miR" = ddPCRnames$miRNA, "Correlation" = Cors)
kable(Cors, caption = "Correlation of ddPCR vs U133+2 expression")
```

Table 5: Correlation of ddPCR vs U133+2 expression

miR	Correlation
miR-146a	0.8969691
miR-155	0.9429651
miR-21	0.7759889
miR-27a	0.6526918
miR-34a	0.7322472

Create figure

```
p <- list()
ddPCRdata <- as.data.frame(ddPCRdata)

for(i in 1:nrow(ddPCRnames)){
  mirName <- as.character(ddPCRnames$miRNA[i])
  u133Name <- as.character(ddPCRnames$Probeset[i])
  regression  <- lm(bCellu133[u133Name,] ~ ddPCRdata[,mirName])

  tempPlot <- data.frame("mir" = ddPCRdata[,ddPCRnames$miRNA[i]],
                         "u133" = bCellu133[ddPCRnames$Probeset[i],])

  p[[i]] <- ggplot(tempPlot, aes(x=mir, y=u133)) +
    geom_point(shape=1) +
    geom_smooth(method=lm) +
```

```
    xlab(mirName) +
    ylab(u133Name) +
    ggtitle(mirName) +
    annotate("text", x = -Inf, y = -Inf, hjust = -1, vjust = -1,
             label = paste("R^2 = ", round(summary(regression)$r.squared, 2)))

}

grid.arrange(grobs = p, ncol = 3)
```