Demo

```
library(rnaGinesis)
library(gridExtra)
```

```
##
## Attaching package: 'gridExtra'
```

```
## The following object is masked from 'package:Biobase':
##
##     combine
```

```
## The following object is masked from 'package:BiocGenerics':
##
##     combine
```

```
library(grid)
library(ggplot2)
library(lattice)
library(reshape)
library(GGally)
library(NMF)
```

```
## Loading required package: pkgmaker
```

```
## Loading required package: registry
```

```
##
## Attaching package: 'pkgmaker'
```

```
## The following object is masked from 'package:base':
##
##     isNamespaceLoaded
```

```
## Loading required package: rngtools
```

```
## Loading required package: cluster
```

```
## NMF - BioConductor layer [OK] | Shared memory capabilities [NO: bigmemory] | Cores 23/24
```

```
##   To enable shared memory capabilities, try: install.extras('
## NMF
## ')
```

```
library(nnls)
library(moments)
mu <- rnaGinesis::mu
A <- rnaGinesis::A
```

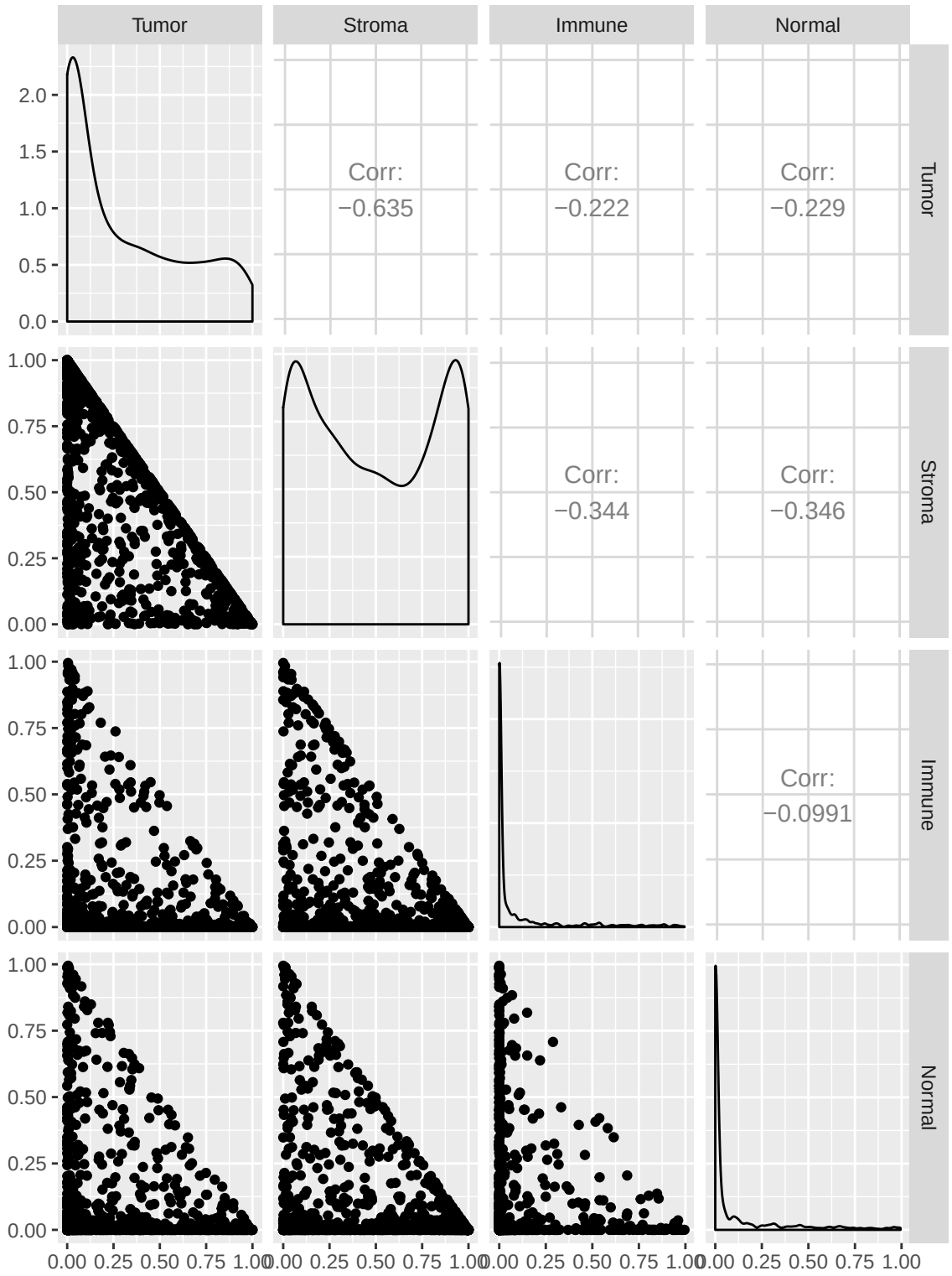
Generate simulated data

```
ngenes <- 500;
nsamples = 1000;
simresult <- Complete_simulation(A_tumor  = A[1:ngenes,1:ngenes],
                                 mu_tumor = mu[1:ngenes],
                                 Samplesize    = nsamples,
```

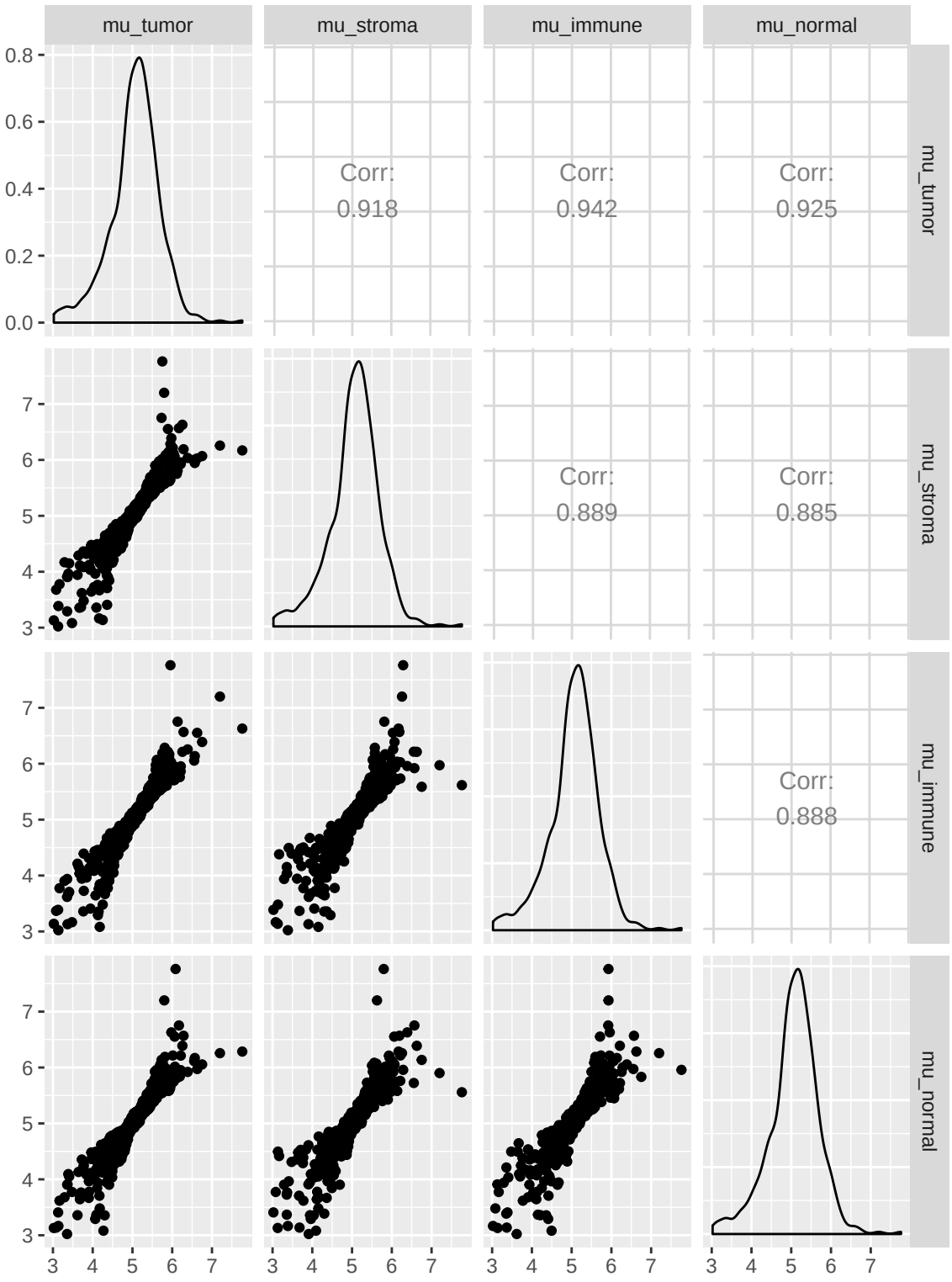
```
                                  scaleFactor   = 100,
                                  d.params      = c("Tumor"   = .3,
                                                    "Stroma" = .5,
                                                    "Immune"  = .1,
                                                    "Normal"  = .1),
                                  noise_setting = 1.5,
                                  seed          = 1234)
data   <- simresult[[1]]
W      <- simresult[[2]]
H      <- simresult[[3]]
rm("simresult")
rm("mu")
rm("A")
```

Display data properties

```
ggpairs(data = data.frame(t(H)))
```




```
ggpairs(data = as.data.frame(lapply(data.frame(W),log10)))
```



Define functions

```
my_nnls <- function(W,X){
  # X = WH
  # solve for H
  pinv <- (solve(t(W)%*%W)%*%t(W))
  H <- pinv%*%X
  H[H<0] <- 0
  residual <- X - (W %*% H)

  ngenes = dim(W)[1]

  skew <- skewness(residual)
  kurt <- kurtosis(residual)

  residual.scaled  <-
    sum((residual[residual>0])) /
    (ngenes * mean(mean(W)))

  return(list("H" = H,
              "residual" = residual.scaled,
              "skew" = skew,
              "kurt" = kurt))
}

fuzzy_nmf <- function(W,X){
  # X = WH
  # solve for H

  # start with nnls result, but don't let zeros happen
  nnls.result <- my_nnls(W,X)
  H <- nnls.result$H

  W.missing <- X - (W%*%H)
  W.missing[W.missing < 0] <- 0
  W.missing[W.missing < (mean(W.missing)*0.01)] <- (mean(W.missing)*0.01)

  H[H<mean(H)*0.01] <- mean(H)*0.01

  # add a dummy factor, populate it with garbage
  ngenes = dim(W)[1]
  nfactors = dim(W)[2]
  W <- cbind(W , W.missing )
  H <- rbind(H , nnls.result$residual)
  H.original <- H

  residual <- X - (W %*% H)
  exitcond <- TRUE
  # writeLines("------------")
  while(exitcond){
    # multiplicative NMF update, modified

    # stay close to known H
    H.new <- H *  ( t(W) %*% X ) / (t(W) %*% W %*% H )
    # H[nfactors + 1] <- H.new[nfactors + 1]
```

```
    # H[1:nfactors]   <- 0.5 * H.new[1:nfactors] + 0.5 * H.original[1:nfactors]
    H <- 0.5 * H.new + 0.5 * H.original

    # keep known W fixed
    W.new <- W *  ( X %*% t(H) ) / (W %*% H %*% t(H))
    W[nfactors + 1] <- W.new[nfactors + 1]

    new.residual <- X - (W %*% H)
    change = sum(abs(residual)) /sum(abs(new.residual))
    residual <- new.residual
    exitcond <- change > 1.001

    #print(H)
  }
  return(list("H" = H[1:nfactors],
              "residual" = H[nfactors+1]))
}
```

Unmix single samples

perfect knowledge

```
known.set <- 1:4
nnls.H.perfect <- H[known.set,]
nnls.resids.perfect <- numeric(nsamples)
nnls.skew.perfect <- numeric(nsamples)
nnls.kurt.perfect <- numeric(nsamples)

nmf.H.perfect <- H[known.set,]
nmf.resids.perfect  <- numeric(nsamples)

for(i in 1:nsamples){
  this_sample <- data[,i]

  nnls.result <- my_nnls(W[,known.set],this_sample)
  nmf.result <- fuzzy_nmf(W[,known.set],this_sample)

  nnls.H.perfect[,i] <- nnls.result$H
  nnls.resids.perfect[i] <- nnls.result$residual
  nnls.skew.perfect[i] <- nnls.result$skew
  nnls.kurt.perfect[i] <- nnls.result$kurt

  nmf.H.perfect[,i] <- nmf.result$H
  nmf.resids.perfect[i] <- nmf.result$residual

}
```

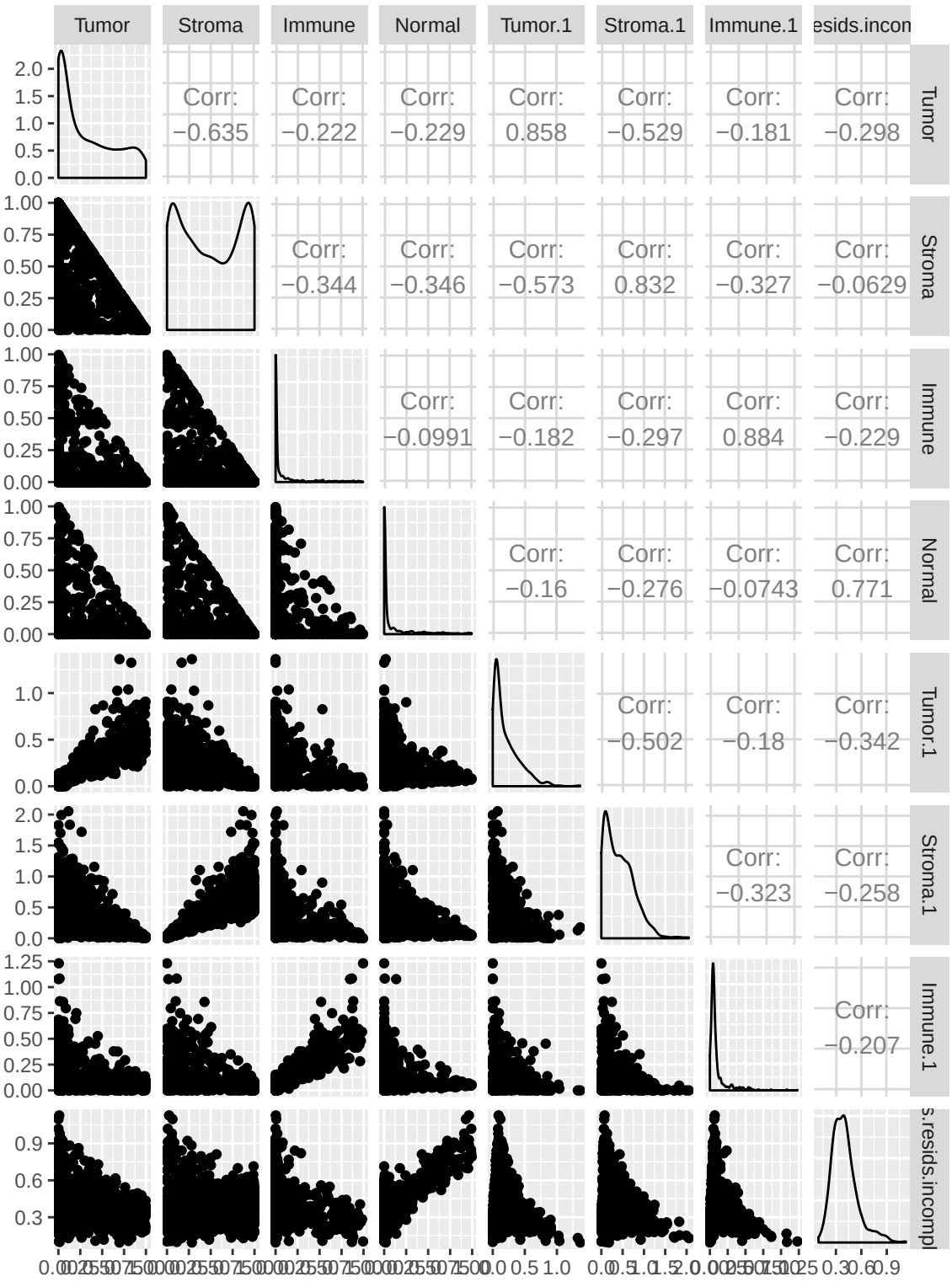
imperfect knowledge

```
known.set <- 1:3
nnls.H.incomplete <- H[known.set,]
nnls.resids.incomplete <- numeric(nsamples)
nnls.skew.incomplete <- numeric(nsamples)
nnls.kurt.incomplete <- numeric(nsamples)

nmf.H.incomplete <- H[known.set,]
nmf.resids.incomplete <- numeric(nsamples)

for(i in 1:nsamples){
  this_sample <- data[,i]

  nnls.result <- my_nnls(W[,known.set],this_sample)
  nmf.result <- fuzzy_nmf(W[,known.set],this_sample)

  nnls.H.incomplete[,i] <- nnls.result$H
  nnls.resids.incomplete[i] <- nnls.result$residual
  nnls.skew.incomplete[i] <- nnls.result$skew
  nnls.kurt.incomplete[i] <- nnls.result$kurt

  nmf.H.incomplete[,i] <- nmf.result$H
  nmf.resids.incomplete[i] <- nmf.result$residual

}
```

Compare resutls

truth versus nnls incomplete

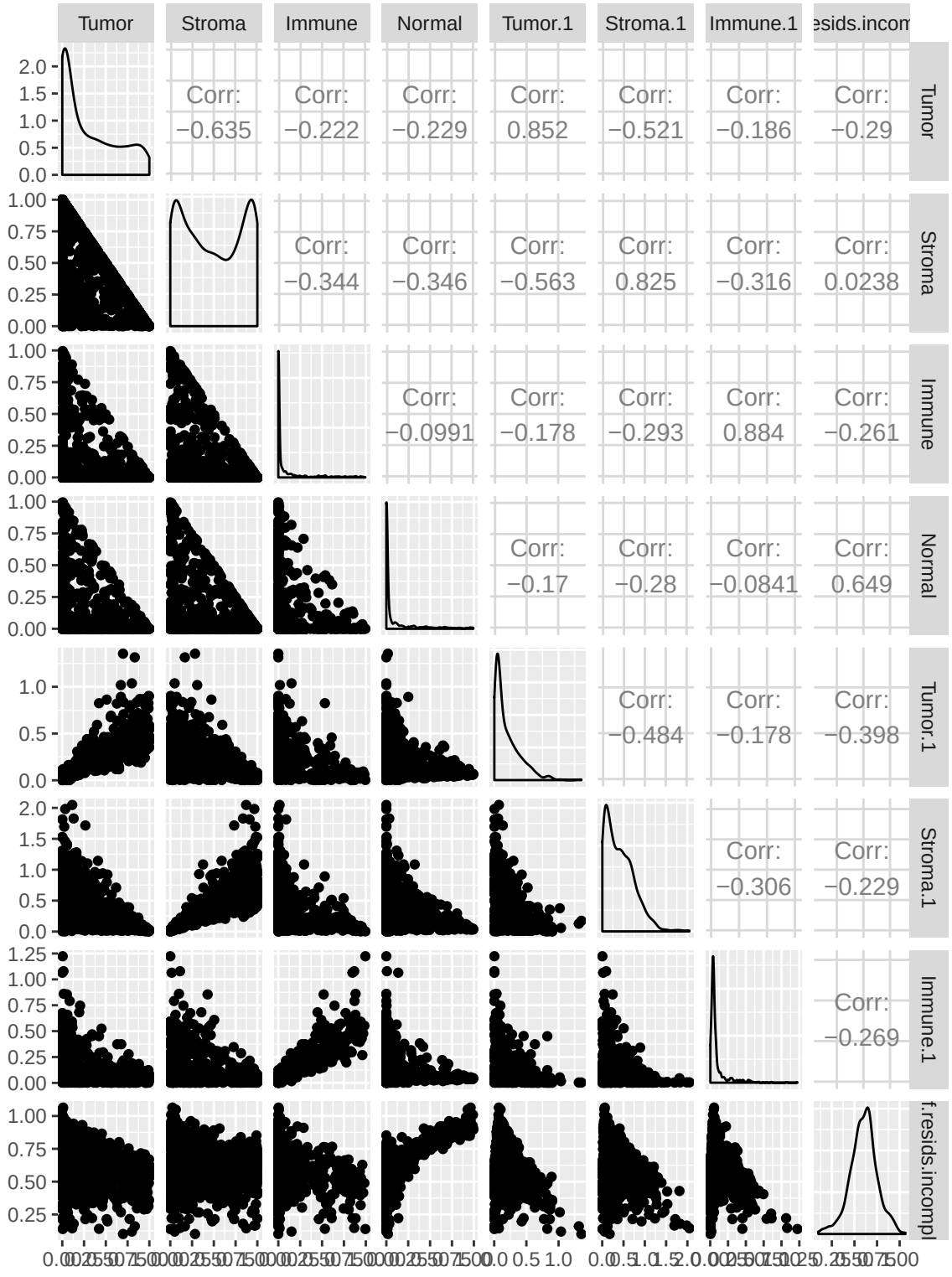
```
ggpairs(data = data.frame(t(rbind(H,
                                  nnls.H.incomplete,
                                  nnls.resids.incomplete))))
```



truth versus nmf imperfect

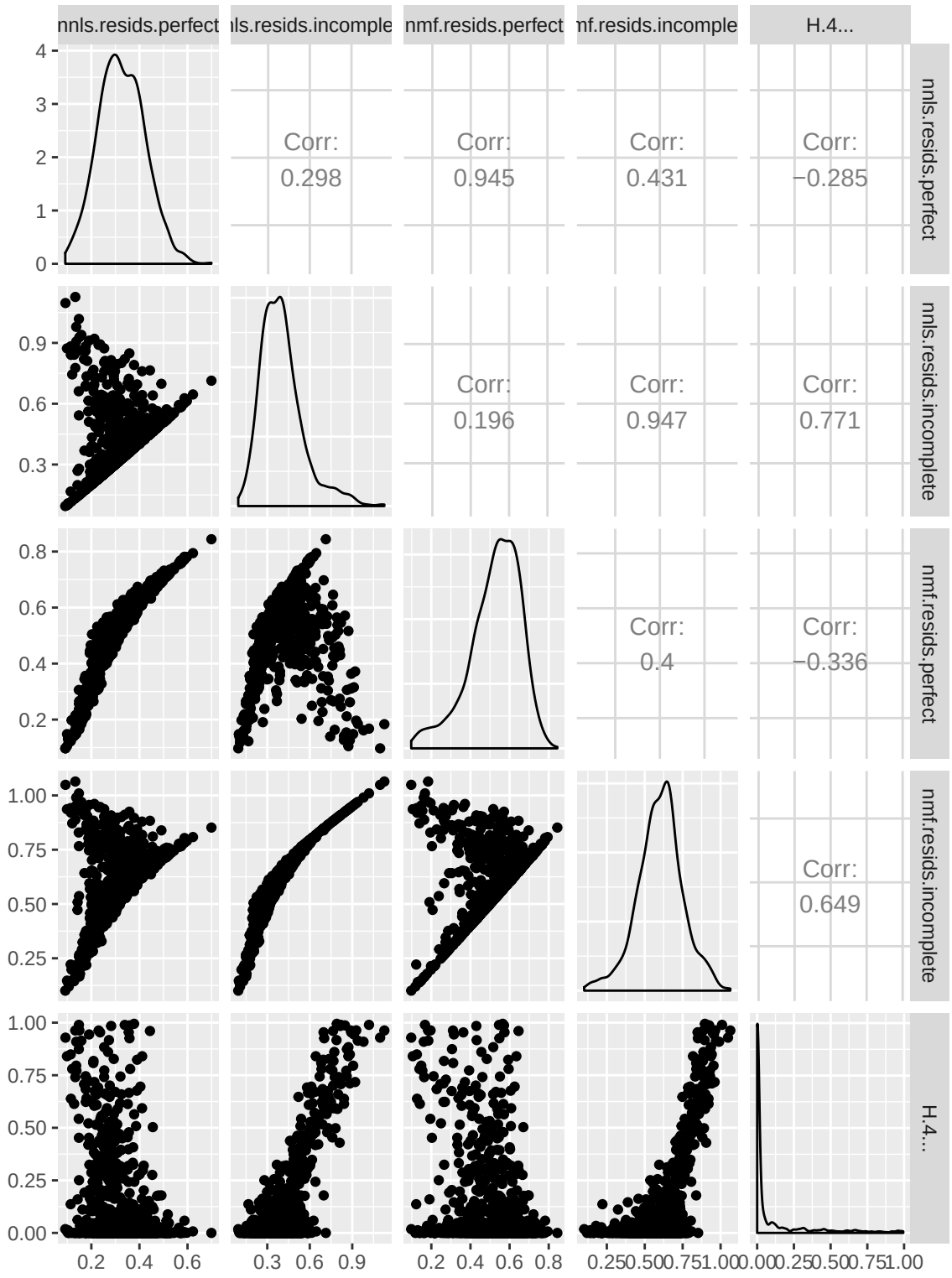
```
ggpairs(data = data.frame(t(rbind(H,
                                  nmf.H.incomplete,
```

```
nmf.resids.incomplete))))
```



comparison of residuals

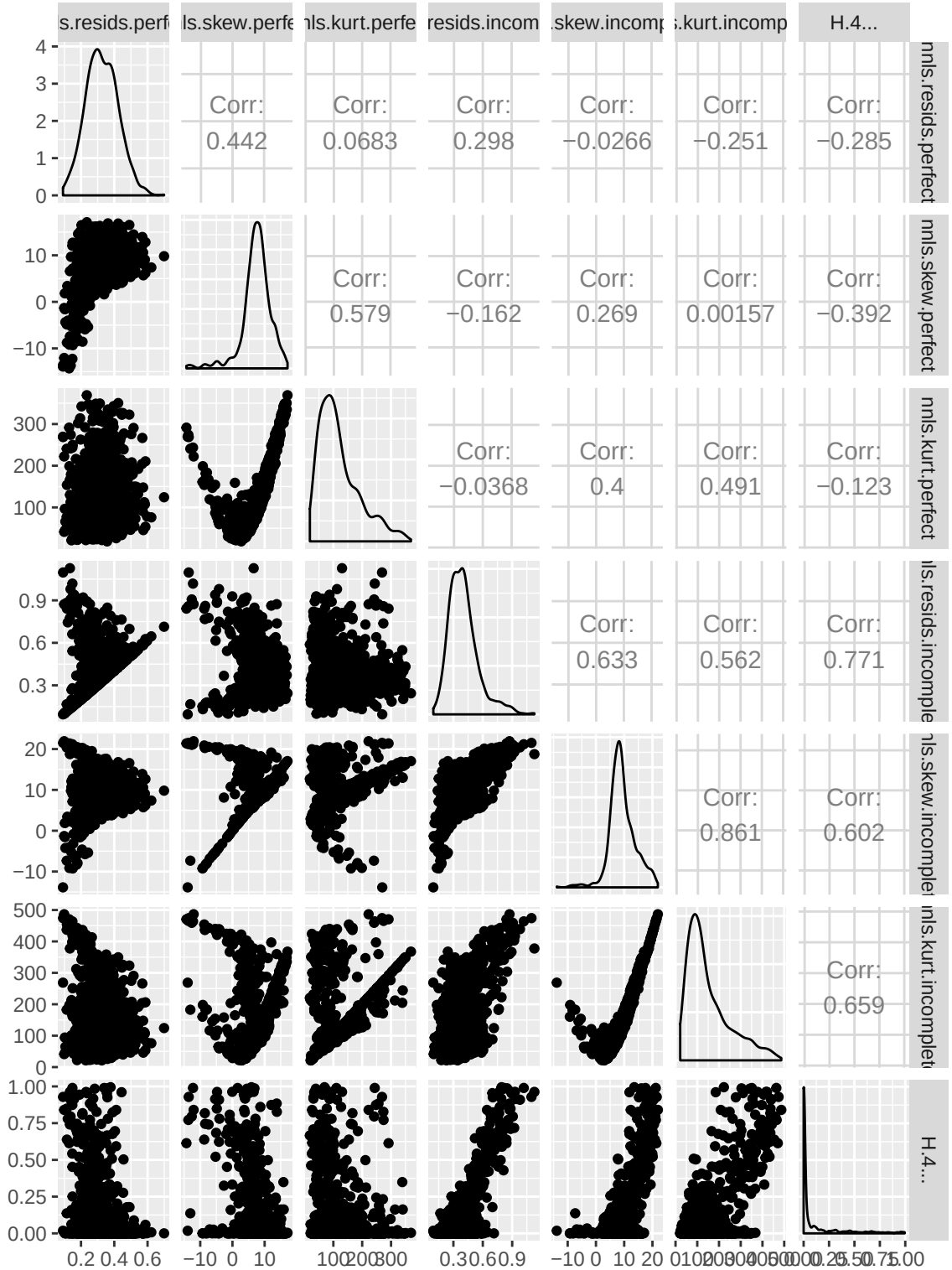
```
ggpairs(data = data.frame(nnls.resids.perfect,
                          nnls.resids.incomplete,
                          nmf.resids.perfect,
                          nmf.resids.incomplete,
                          H[4,]))
```



comparison of gaussianity

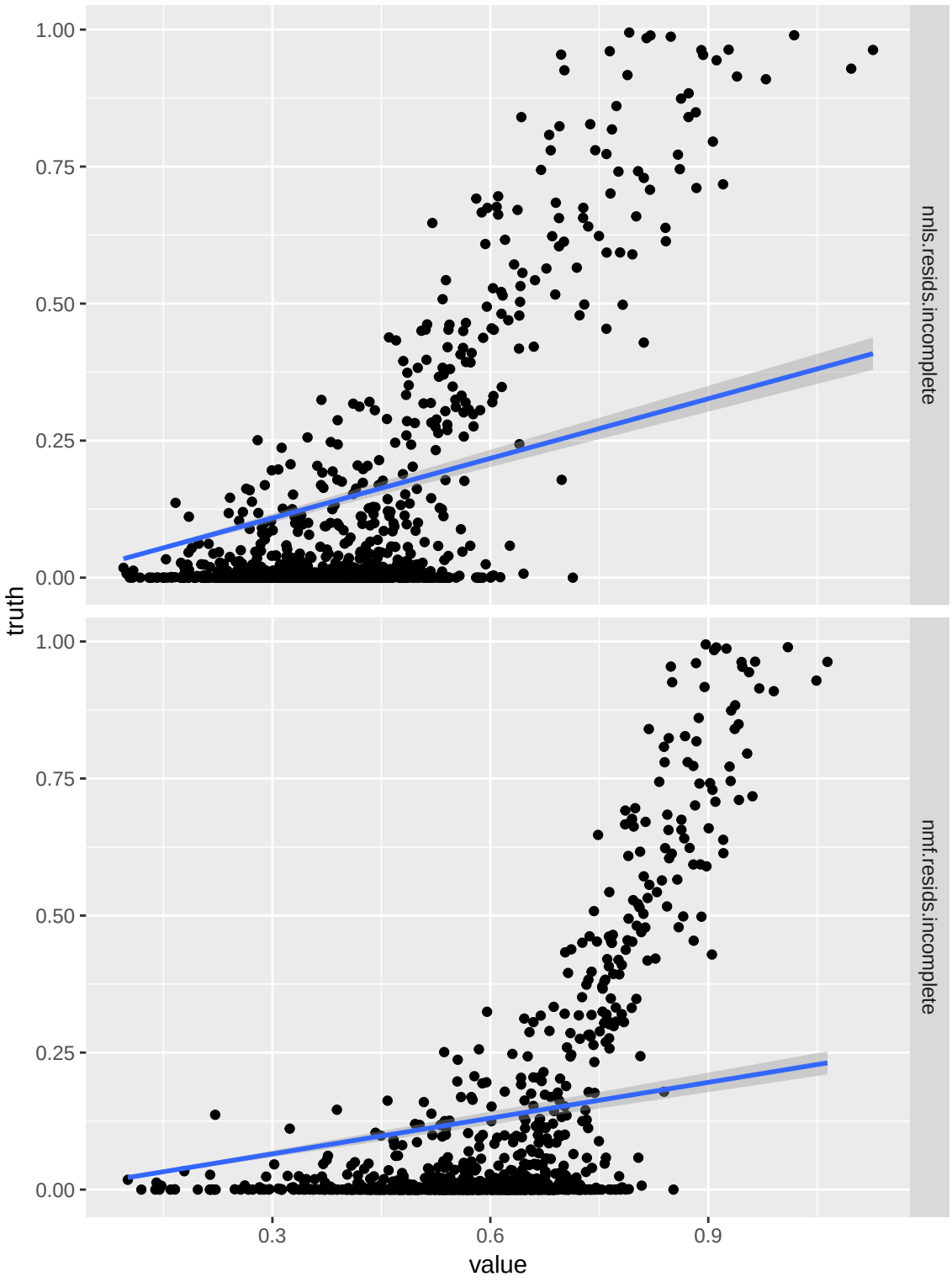
```
ggpairs(data = data.frame(nnls.resids.perfect,
                          nnls.skew.perfect,
```

```
                    nnls.kurt.perfect,
                    nnls.resids.incomplete,
                    nnls.skew.incomplete,
                    nnls.kurt.incomplete,
                    H[4,]))
```



Correlation to unknown

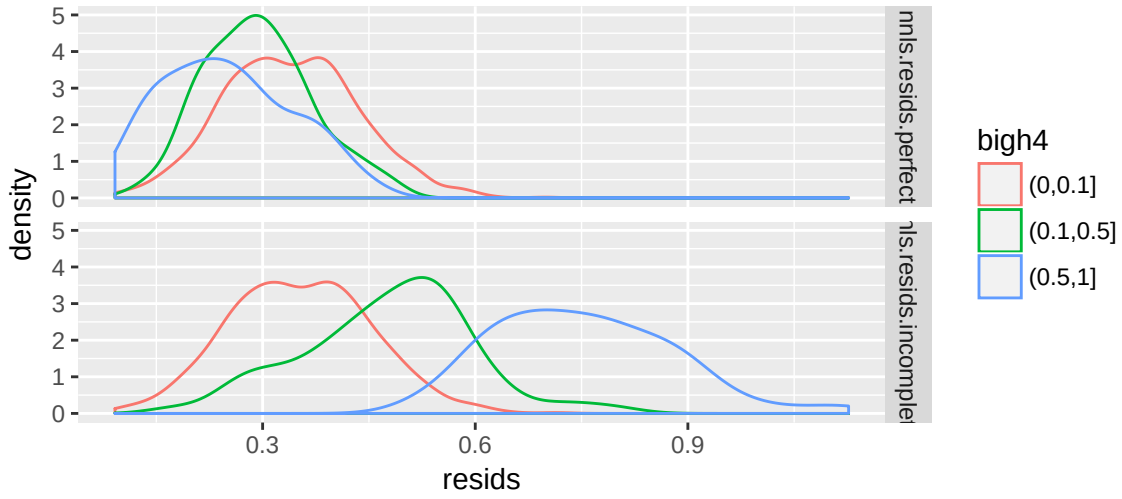
```
my_df <- data.frame(nnls.resids.incomplete,
                    nmf.resids.incomplete,
                    truth = H[4,])
my_df <- melt(my_df,id.vars = "truth")
p <- ggplot(data = my_df, aes(y = truth,x = value))
p <- p + facet_grid(facets = variable ~ .)
p <- p + geom_point()
p <- p + geom_smooth(method = "glm",formula = y ~ x -1)
print(p)
```

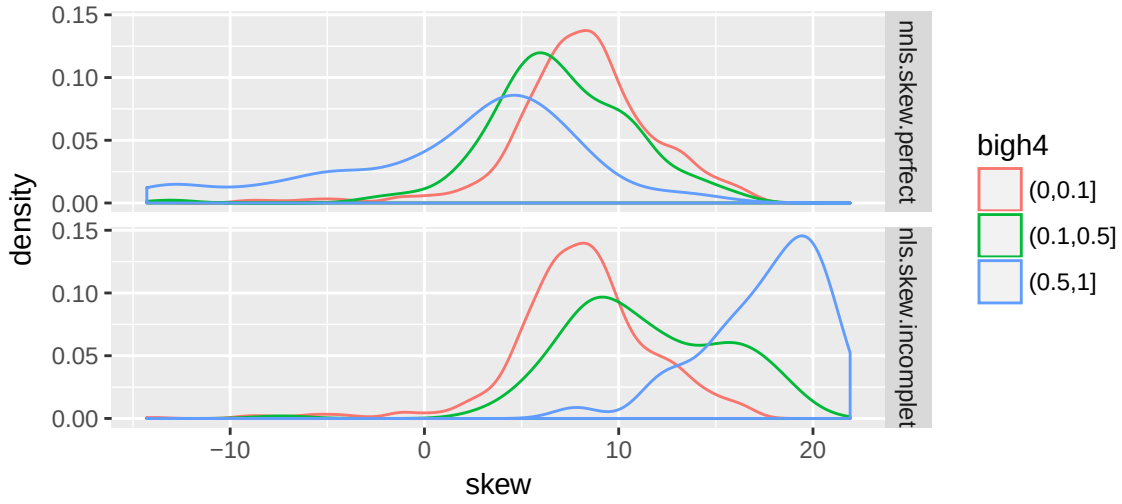


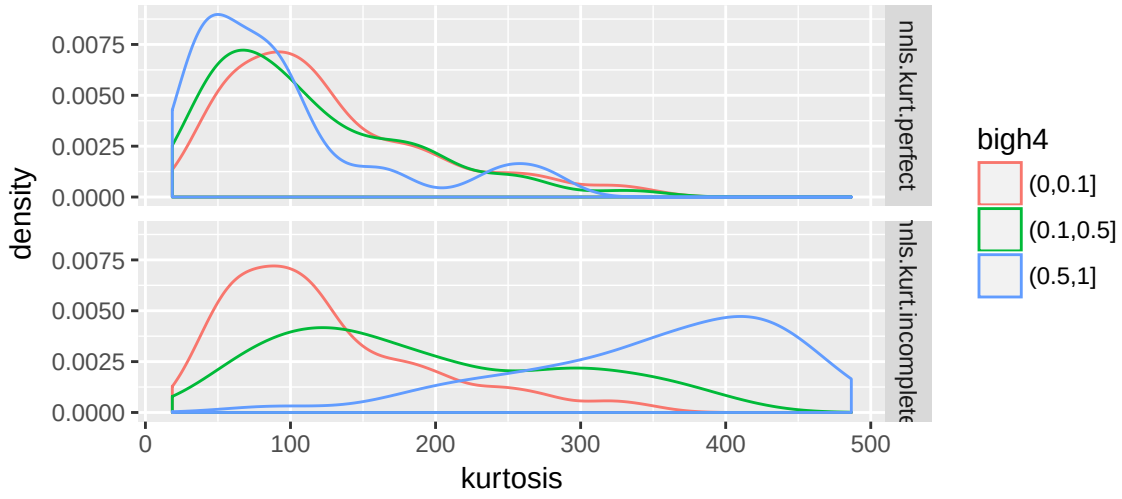
predictive power nnls metrics

```
my_df <- data.frame(nnls.resids.perfect,
                    nnls.resids.incomplete,
```

```
                    bigh4 = cut(H[4,],breaks = c(0, 0.1, 0.5 ,1)))
my_df <- melt(my_df,id.vars = "bigh4")
p1 <- ggplot(data = my_df, aes(x = value,color = bigh4))
p1 <- p1 + facet_grid(facets = variable ~ .)
p1 <- p1 + geom_density()
p1 <- p1 + labs(x = "resids")

my_df <- data.frame(nnls.skew.perfect,
                    nnls.skew.incomplete,
                    bigh4 = cut(H[4,],breaks = c(0, 0.1, 0.5 ,1)))
my_df <- melt(my_df,id.vars = "bigh4")
p2 <- ggplot(data = my_df, aes(x = value,color = bigh4))
p2 <- p2 + facet_grid(facets = variable ~ .)
p2 <- p2 + geom_density()
p2 <- p2 + labs(x = "skew")

my_df <- data.frame(nnls.kurt.perfect,
                    nnls.kurt.incomplete,
                    bigh4 = cut(H[4,],breaks = c(0, 0.1, 0.5 ,1)))
my_df <- melt(my_df,id.vars = "bigh4")
p3 <- ggplot(data = my_df, aes(x = value,color = bigh4))
p3 <- p3 + facet_grid(facets = variable ~ .)
p3 <- p3 + geom_density()
p3 <- p3 + labs(x = "kurtosis")

grid.arrange(p1,p2,p3)
```



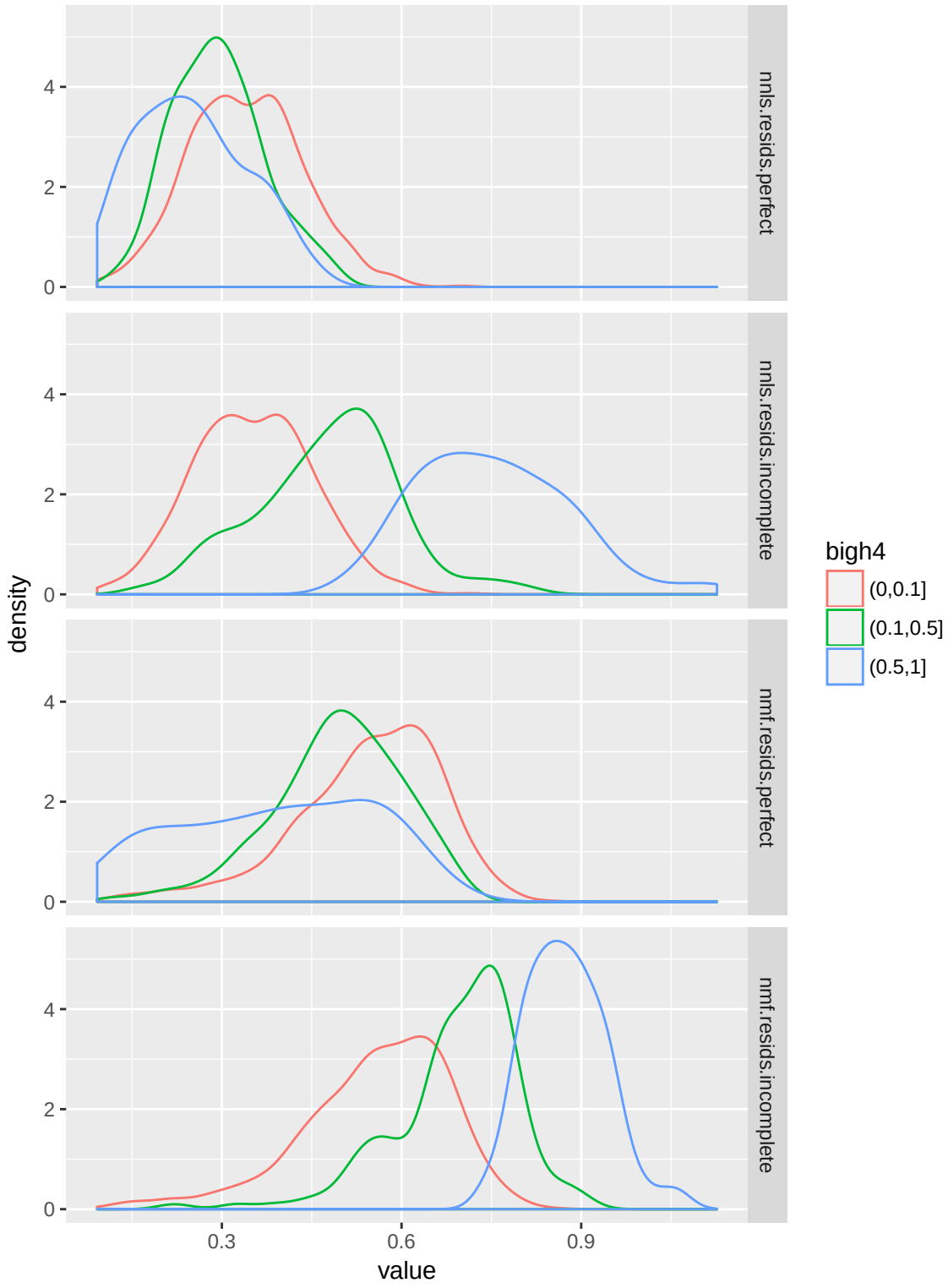




predictive power nmf

```
my_df <- data.frame(nnls.resids.perfect,
                    nnls.resids.incomplete,
```

```
                    nmf.resids.perfect,
                    nmf.resids.incomplete,
                    bigh4 = cut(H[4,],breaks = c(0, 0.1, 0.5 ,1)))
my_df <- melt(my_df,id.vars = "bigh4")
p <- ggplot(data = my_df, aes(x = value,color = bigh4))
p <- p + facet_grid(facets = variable ~ .)
p <- p + geom_density()
print(p)
```


nnls.resids.perfect
nnls.resids.incomplete
nmf.resids.perfect
nmf.resids.incomplete
density
value
0
2
4
0.3
0.6
0.9
bigh4
(0,0.1]
(0.1,0.5]
(0.5,1]