Manuscript table1

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Table 1

```
# Load TIR1/AFB1, Aux/IAAs, and ARFs so we can bind all together.
TIR <- read.csv("../final_trees/AFB_input/2023_0919_AFB_Gm_ortholog.csv")


IAA <- read.csv("../final_trees/IAA_input/IAA_Gm_ortholog.csv")
IAA2 <- read.csv("../final_trees/IAA_input/LABELS2_20230917.csv") %>% .[30:90, ]
IAA2 <- IAA2 %>% select(tair_locus, ensembl_gene_id, Class)

IAA_ortho <- merge(IAA, IAA2, by = "tair_locus", all = TRUE)
IAA_ortho <- IAA_ortho %>% dplyr::rename(`Gene ID` = `ensembl_gene_id.x`,
                     `Transcript ID` = tair_locus,
                     Orthology = ortholog_name,
                     `Class/Clade` = Class) %>%
  select(`Transcript ID`, Orthology, `Class/Clade`)
IAA_ortho$Family <- "IAA"


expression_data <- read_csv("FINAL_manuscript_gene_INFO.csv") %>% select(-c(Average_Expression, `...22`)
```

```
## New names:
## Rows: 221 Columns: 22
## -- Column specification
## -------------------------------------------------------- Delimiter: "," chr
## (6): ensembl_gene_id, ortholog_number, tair_locus, class, name, Family dbl
## (16): AM, OF, IAM, IBM, RootTip, Cotyledon, Hypocotyl, SAM6D, SAM17D, SA...
## i Use `spec()` to retrieve the full column specification for this data. i
## Specify the column types or set `show_col_types = FALSE` to quiet this message.
## * `` -> `...22`
```

```
comb_expr_df <- merge(IAA_ortho, expression_data, by.x = "Transcript ID", by.y = "tair_locus", all =TRU

comb_expr_df <- comb_expr_df %>% mutate(Class = coalesce(`Class/Clade`, class))

comb_expr_df <- comb_expr_df %>%
  select(-c(class, `Class/Clade`, Orthology, Family.x))

# populate NA's in class in accordance to their ensembl gene ID. if the same ID they belong to the same
comb_expr_df2 <- comb_expr_df %>%
  group_by(ensembl_gene_id) %>%
  dplyr::mutate(Class = if (all(is.na(Class))) NA else na.omit(Class)) %>%
```

```
  ungroup()


# separate Class into Class and Clade
comb_expr_df2 <- comb_expr_df2 %>% separate(Class, c('Class', 'Clade'),sep = " - ")
```

```
## Warning: Expected 2 pieces. Missing pieces filled with `NA` in 120 rows [1, 2, 9, 10,
## 13, 17, 18, 19, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 38, 39, ...].
```

```
# Now combine TIR df to get their new clades.
df <- merge(comb_expr_df2, TIR, by.x = "ensembl_gene_id", by.y = "ensembl_gene_id", all = TRUE)

df <- df %>% dplyr::mutate(Clade = coalesce(Clade.x, Clade.y))

df <- df %>%
  dplyr::select(-c(Clade.x, Clade.y, class, tair_locus, ortholog_name, name.x, name.y)) %>%
  dplyr::rename(Orthology = ortholog_number)

df <- df %>%
  group_by(ensembl_gene_id) %>%
  dplyr::mutate(Clade = if(all(is.na(Clade))) NA else na.omit(Clade)) %>%
  ungroup()

# I can add clades to ARFs as I have not saved that as csv file when I built trees.
df <- df %>% dplyr::mutate(Clade = ifelse(grepl("ARF", .$Family.y) & grepl("A", .$Class), "II",
                           ifelse(grepl("ARF", .$Family.y) & grepl("B", .$Class), "I",
                                  ifelse(grepl("ARF", .$Family.y) & grepl("C", .$Class), "III", Clade))




df2 <-
  df %>% dplyr::mutate(Class = ifelse(grepl("Glyma.02G218100", .$ensembl_gene_id), "A", Class),
                Clade =
                  ifelse(grepl("Glyma.02G218100", .$ensembl_gene_id), "I", Clade))


df2 <- df2 %>% dplyr::rename(Family = Family.y, `Gene ID` = ensembl_gene_id) %>% drop_na()
```

Table 1

```
# save Table 1 for manuscript
df2 %>% dplyr::select(`Gene ID`, Orthology, `Transcript ID`, Class, Clade, Family) %>%
  arrange(Family, Clade, Class, Orthology) #%>% write_csv("Table1.csv")
```

```
## # A tibble: 221 x 6
##    `Gene ID`       Orthology       `Transcript ID`   Class     Clade Family
##    <chr>           <chr>           <chr>             <chr>     <chr> <chr>
##  1 Glyma.19G206800 GmTIR1/AFB1_A.1 Glyma.19G206800.1 TIR1/AFB1 I     AFB/TIR1
##  2 Glyma.03G209400 GmTIR1/AFB1_B.1 Glyma.03G209400.1 TIR1/AFB1 I     AFB/TIR1
##  3 Glyma.10G021500 GmTIR1/AFB1_C.1 Glyma.10G021500.1 TIR1/AFB1 I     AFB/TIR1
##  4 Glyma.10G021500 GmTIR1/AFB1_C.2 Glyma.10G021500.2 TIR1/AFB1 I     AFB/TIR1
##  5 Glyma.10G021500 GmTIR1/AFB1_C.3 Glyma.10G021500.3 TIR1/AFB1 I     AFB/TIR1
##  6 Glyma.02G152800 GmTIR1/AFB1_D.1 Glyma.02G152800.1 TIR1/AFB1 I     AFB/TIR1
```

```
##  7 Glyma.02G152800 GmTIR1/AFB1_D.2 Glyma.02G152800.2 TIR1/AFB1 I     AFB/TIR1
##  8 Glyma.19G100200 GmAFB2/3_A.1    Glyma.19G100200.1 AFB2/3    II    AFB/TIR1
##  9 Glyma.16G050500 GmAFB2/3_B.1    Glyma.16G050500.1 AFB2/3    II    AFB/TIR1
## 10 Glyma.02G065300 GmAFB2/3_C.1    Glyma.02G065300.1 AFB2/3    II    AFB/TIR1
## # i 211 more rows
```

```
# write_csv(df2, "20230919_expression_heatmap.csv")
```

Heatmap

```
# Source:
# https://stackoverflow.com/questions/43051525/how-to-draw-pheatmap-plot-to-screen-and-also-save-to-fil
save_pheatmap_pdf <- function(x, filename, width=7, height=4) {
   stopifnot(!missing(x))
   stopifnot(!missing(filename))
   pdf(filename, width=width, height=height)
   grid::grid.newpage()
   grid::grid.draw(x$gtable)
   dev.off()
}

# https://davetang.org/muse/2018/05/15/making-a-heatmap-in-r-with-the-pheatmap-package/
```

```
# Make a heatmap label by uniting orthology column and gene ID column
heatmap_df <- df2 %>%
  relocate(`Gene ID`, .after = `Transcript ID`) %>%
  unite("heatmap_label", `Gene ID`:Orthology, sep = "|", remove = TRUE)


# Genes with an median expression across tissues that are less than 2 TPM, will be excluded from downst
heatmap_df2 <- heatmap_df %>%
  mutate(expr_median = round(apply(heatmap_df[,c(4:17)], 1, median), digits = 4)) %>%
  subset(., expr_median>= 2) %>% dplyr::filter(., Class != "COI1")

 # we kept 133 genes for downstream normalization
    # I will save this as a csv file so we can perform a tau (Tissue specificity index analysis).
#write_csv(heatmap_df2, "Tau_133_genes_with_expression_across_tissues_are_greater_than_2_SoyARCs.csv")
```

table 2 with tau values for the 133 genes.

```
heatmap_df2
```

```
## # A tibble: 133 x 20
##    `Transcript ID`   heatmap_label       Family    AM     OF   IAM   IBM RootTip
##    <chr>             <chr>               <chr>  <dbl>  <dbl> <dbl> <dbl>   <dbl>
##  1 Glyma.01G002100.1 Glyma.01G002100|Gm~ ARF    10.2   11.1   9.94 11.8     6.06
##  2 Glyma.01G019400.1 Glyma.01G019400|Gm~ IAA     4.02   7.57  0      1.48    3.68
##  3 Glyma.01G019400.2 Glyma.01G019400|Gm~ IAA     3.95   0     6.01   7.65    2.22
##  4 Glyma.01G019400.3 Glyma.01G019400|Gm~ IAA    22.5   35.9  20.7   26.7    16.1
##  5 Glyma.01G098000.3 Glyma.01G098000|Gm~ IAA    68.2   51.4  53.6   96.3    32.6
##  6 Glyma.01G103500.1 Glyma.01G103500|Gm~ ARF    23.4    7.20 14.1   28.2     4.92
##  7 Glyma.02G000500.1 Glyma.02G000500|Gm~ IAA    26.5   33.1  14.3   12.0    28.4
##  8 Glyma.02G065300.1 Glyma.02G065300|Gm~ AFB/T~  8.00   6.50  7.76  11.8     3.85
```

```
##  9 Glyma.02G142500.3 Glyma.02G142500|Gm~ IAA    14.7  530.   73.1  28.5    43.2
## 10 Glyma.02G142600.1 Glyma.02G142600|Gm~ IAA     4.37  80.7  19.6  21.4    19.2
## # i 123 more rows
## # i 12 more variables: Cotyledon <dbl>, Hypocotyl <dbl>, SAM6D <dbl>,
## #   SAM17D <dbl>, SAM38D <dbl>, Callus <dbl>, Leaf <dbl>, Root <dbl>,
## #   Nodule <dbl>, Class <chr>, Clade <chr>, expr_median <dbl>
```

```
#read tau analysis in so we can incorporate later into our PCA analysis
tau_df <- read_csv("tau_df_contains_maximal_component_values.csv")
```

```
## Rows: 133 Columns: 18
## -- Column specification --------------------------------------------------------
## Delimiter: ","
## chr  (3): Transcript ID, heatmap_label, Family
## dbl (15): AM, OF, IAM, IBM, RootTip, Cotyledon, Hypocotyl, SAM6D, SAM17D, SA...
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
# Write tau as table 2 for sharing in supplemental material
tau_df %>% dplyr::select(-c(Family, `Transcript ID`)) %>%
  dplyr::rename("GeneID & Orthology" = heatmap_label)# %>%
```

```
## # A tibble: 133 x 16
##    `GeneID & Orthology`        AM    OF   IAM    IBM RootTip Cotyledon Hypocotyl
##    <chr>                    <dbl> <dbl> <dbl>  <dbl>   <dbl>     <dbl>     <dbl>
##  1 Glyma.01G002100|GmARF7~ 0.816  0.890 0.797 0.945   0.486   0.505       1
##  2 Glyma.01G019400|GmIAA8~ 0.382  0.718 0     0.140   0.349   0.0952      0.841
##  3 Glyma.01G019400|GmIAA8~ 0.175  0     0.266 0.339   0.0983  0.0930      0.499
##  4 Glyma.01G019400|GmIAA8~ 0.254  0.404 0.234 0.301   0.182   0.0454      1
##  5 Glyma.01G098000|GmIAA8~ 0.396  0.298 0.311 0.559   0.190   0.455       1
##  6 Glyma.01G103500|GmARF9~ 0.694  0.214 0.420 0.839   0.146   0.0621      0.0699
##  7 Glyma.02G000500|GmIAA1~ 0.252  0.315 0.137 0.114   0.271   0.0604      1
##  8 Glyma.02G065300|GmAFB2~ 0.522  0.424 0.506 0.772   0.251   0.0816      0.303
##  9 Glyma.02G142500|GmIAA1~ 0.0277 1     0.138 0.0538  0.0816  0.000382    0.216
## 10 Glyma.02G142600|GmIAA1~ 0.0541 1     0.243 0.266   0.238   0.0734      0.168
## # i 123 more rows
## # i 8 more variables: SAM6D <dbl>, SAM17D <dbl>, SAM38D <dbl>, Callus <dbl>,
## #   Leaf <dbl>, Root <dbl>, Nodule <dbl>, tau <dbl>
```

```
  # write_csv(., "Table2_tau_and_maximal_component_value.csv")
```

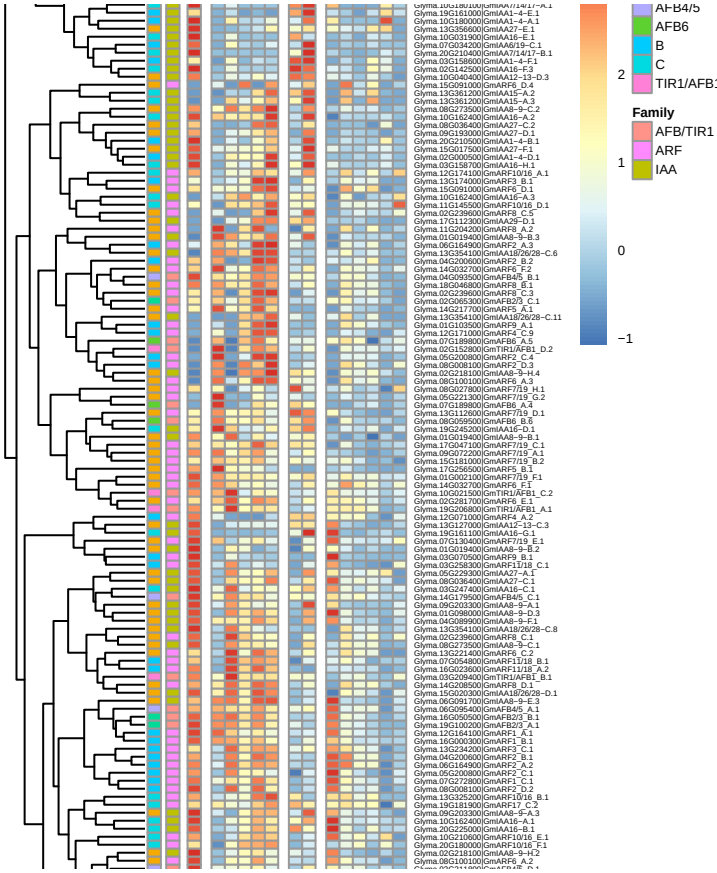
Normalization by transcript

```
Expr_data_Norm <- heatmap_df2 %>% relocate(c(Class, Clade), .after = Family) %>% select(-expr_median)

# Normalize data by performing a z-score transformation on selected columns.

# Iterate through each row of the 'Expr_data_Norm' data frame.
for (j in 1:nrow(Expr_data_Norm)) {
  # Iterate through columns starting from the 6th column to the last column.
for (i in 6:ncol(Expr_data_Norm)) {

  # Calculate the z-score normalization for each cell in the data frame.
    # 1. Subtract the mean of the selected row (columns 6 to the last column).
```

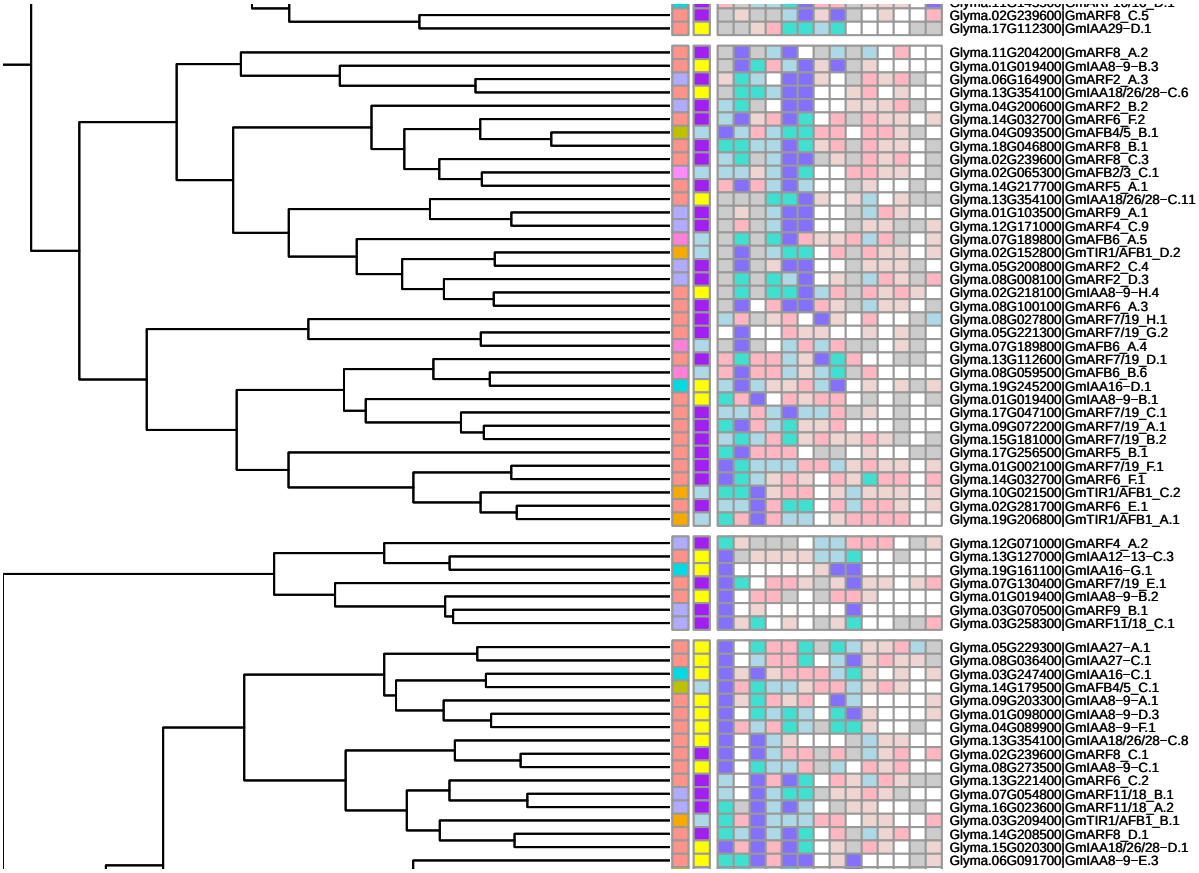
```
    # 2. Divide by the standard deviation of the selected row.
  Expr_data_Norm[j,i]<-
    (Expr_data_Norm[j,i]-rowMeans(Expr_data_Norm[j,6:ncol(Expr_data_Norm)]))/
    sd(Expr_data_Norm[j,6:ncol(Expr_data_Norm)])
}
}

Expr_data_Norm <-Expr_data_Norm %>% drop_na() %>% column_to_rownames(., var = "heatmap_label")
```

Build heatmap with normalized data

```
# Specify colors
Class_df1_colors = list(Family = c(ARF = "Purple", IAA = "yellow", `AFB/TIR1`="lightblue"), Class = c(A=


pheatmap::pheatmap(mat = Expr_data_Norm[,5:18],  annotation_row = Expr_data_Norm[,c(2,3)],
         fontsize = 5,
         fontsize_row = 3,
         cellwidth = 5,
         cutree_cols = 4,
         cellheight = 3,  na_col = "black",
         clustering_distance_rows = "euclidean",
         main = "Expression analysis")
```



```
(expr_analysis <- pheatmap::pheatmap(mat = Expr_data_Norm[,5:18],  annotation_row = Expr_data_Norm[,c(2
         fontsize = 6,
         fontsize_row = 5,
         cellwidth = 6,
         cellheight = 5,
         clustering_distance_rows = "euclidean",
         treeheight_row = 300,
         cutree_rows = 6,
         # main = "Expression analysis - normalized by transcripts",
        color = c("grey80", "white", "mistyrose2", "lightpink", "lightblue", "turquoise", "lightsteblu
```




```
save_pheatmap_pdf(expr_analysis, "20230926_FINAL_NORM_by_Transcript.pdf", height = 12, width = 9)
```

```
## pdf
##   2
```

Z-score normalization standardizes the data such that the mean of each row becomes 0 and the standard deviation becomes 1. The resulting values can be positive or negative and represent how many standard deviations a data point is from the mean of its row.

In the context of z-score normalization:

Values close to 0 represent gene expression levels similar to the mean of their respective rows. Negative values represent gene expression levels below the mean of their respective rows. Positive values represent gene expression levels above the mean of their respective rows.

So, in your heatmap, the -1 represents genes that have expression levels approximately 1 standard deviation below the mean of their respective rows.

These genes are relatively lower in expression compared to the mean expression of those genes across the samples or conditions under analysis.

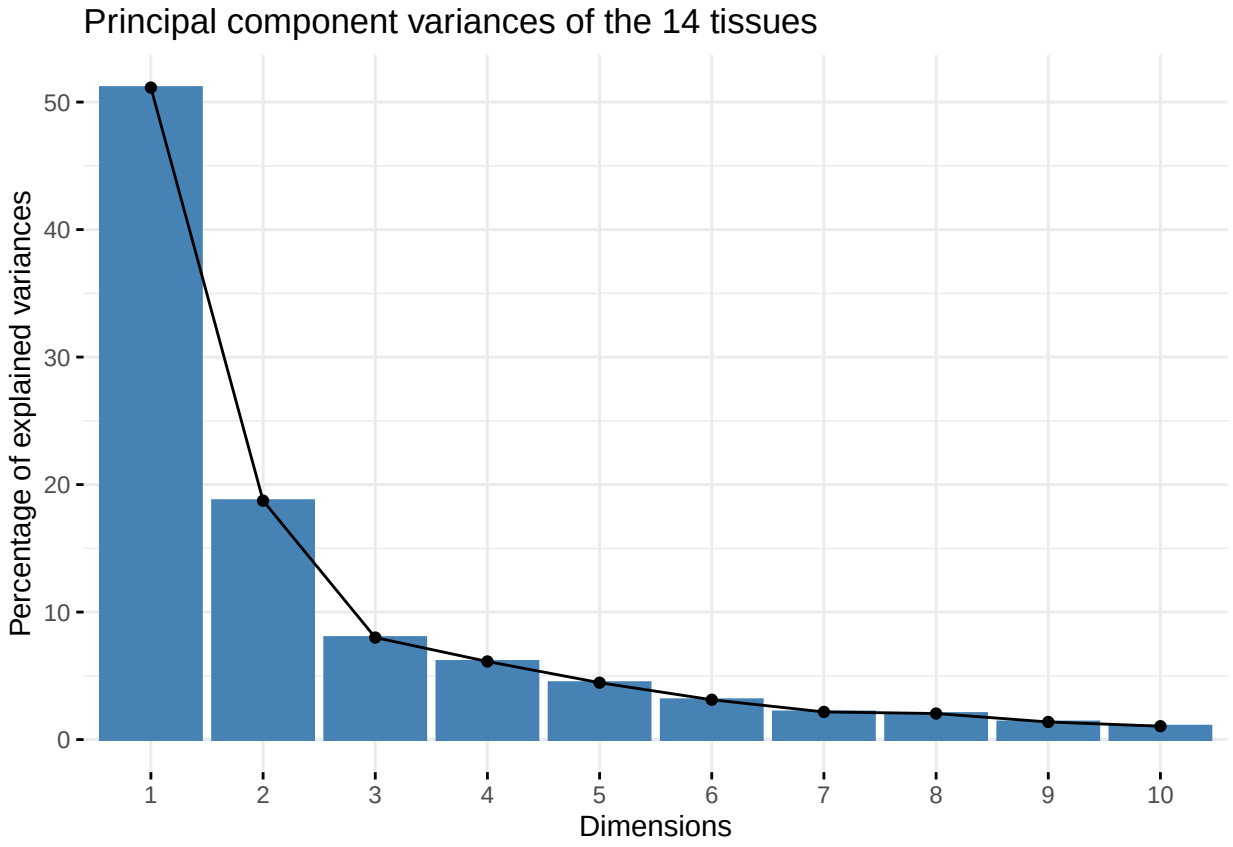
Principal component analysis

Using the full data set in which data median expression was greater than 2. In our PCA we are not using the normalized data. That is beacause we are already transforming our data once by using scale = true, which is known to change the range of the data whereas normalized changes its shape distribution.

```
pca_data <- heatmap_df2 %>% relocate(c(Class, Clade), .after = Family) %>% select(-expr_median)

# for analysis with all tissues
pca <- prcomp(pca_data[, -(1:5)],
              scale. = T, center = T)
summary(pca)
```

```
## Importance of components:
##                           PC1    PC2     PC3     PC4     PC5     PC6     PC7
## Standard deviation     2.6757 1.6196 1.05841 0.92601 0.79038 0.66102 0.55083
## Proportion of Variance 0.5114 0.1874 0.08002 0.06125 0.04462 0.03121 0.02167
## Cumulative Proportion  0.5114 0.6988 0.77878 0.84003 0.88465 0.91586 0.93753
##                           PC8     PC9    PC10    PC11    PC12   PC13    PC14
## Standard deviation     0.53473 0.43946 0.38317 0.32336 0.26775 0.2275 0.14373
## Proportion of Variance 0.02042 0.01379 0.01049 0.00747 0.00512 0.0037 0.00148
## Cumulative Proportion  0.95796 0.97175 0.98224 0.98971 0.99483 0.9985 1.00000
```

```
factoextra::fviz_eig(pca, main = "Principal component variances of the 14 tissues")
```



```
# ggsave("20230927_PCscreePlot_allTissues.png", dpi = 1000, width = 10, height = 8)
```

PC1 and PC2 for all 14 tissues

```
Family <- pca_data$Family

# extract the loading so we can change the arrows more easily
PCA_loadings <- data.frame(Variables=rownames(pca$rotation), pca$rotation)


comb_pca_df <- cbind(pca_data, pca$x[, 1:4])
```

PC1 and PC2 outside ellipse labels for the 14 tissues

```
PC1_2all <- ggbiplot::ggbiplot(pca, obs.scale = 1, var.scale = .5,
                               varname.adjust = 3, alpha = .1,
                              groups = Family, var.axes = F, varname.size = 2,
                    ellipse = TRUE,  circle = FALSE, ellipse.prob = .70)
```

```
## ------------------------------------------------------------------------------
## You have loaded plyr after dplyr - this is likely to cause problems.
## If you need functions from both plyr and dplyr, please load plyr first, then dplyr:
## library(plyr); library(dplyr)
## ------------------------------------------------------------------------------
##
## Attaching package: 'plyr'
## The following objects are masked from 'package:plotly':
##
##     arrange, mutate, rename, summarise
## The following objects are masked from 'package:dplyr':
##
##     arrange, count, desc, failwith, id, mutate, rename, summarise,
##     summarize
## The following object is masked from 'package:purrr':
##
##     compact
##
## Attaching package: 'scales'
## The following object is masked from 'package:viridis':
##
##     viridis_pal
## The following object is masked from 'package:purrr':
##
##     discard
## The following object is masked from 'package:readr':
##
##     col_factor
```

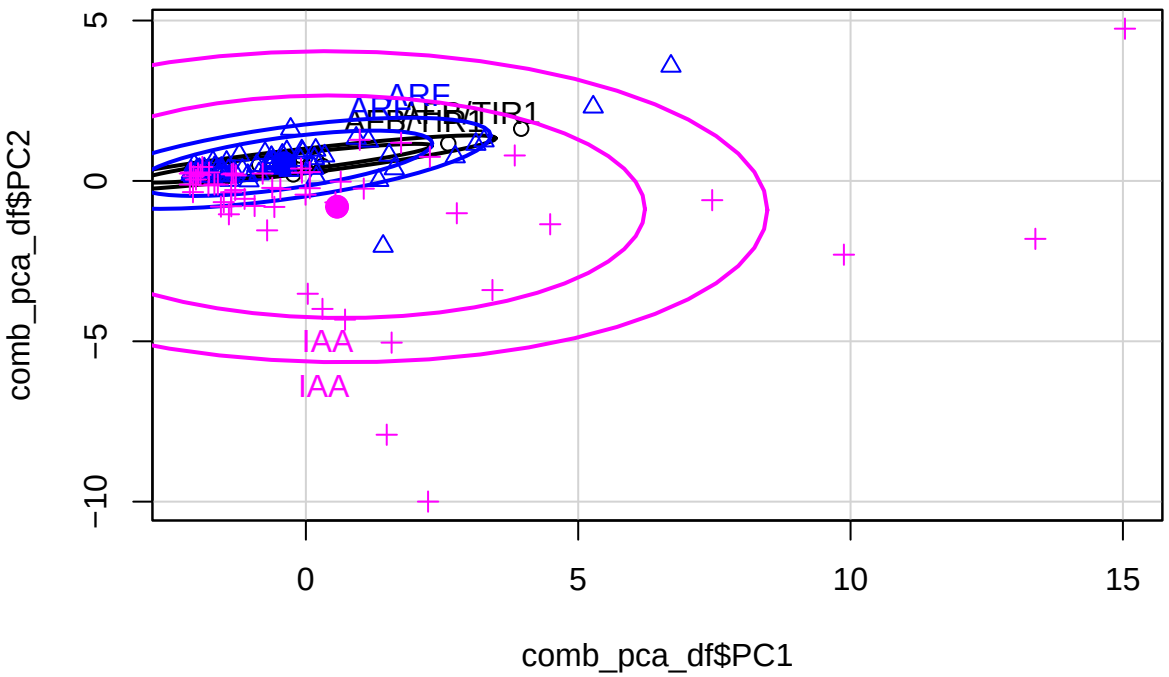
```
# Extract components so we can select out
PC1_2all$data
```

```
##             xvar         yvar   groups
## 1   -1.304753081  0.253967120      ARF
## 2   -1.934745295  0.185928231      IAA
## 3   -1.616343442 -0.113511655      IAA
## 4    0.071187241  0.274717011      IAA
## 5    7.459612561 -0.600002016      IAA
## 6   -0.749858791  0.859463281      ARF
## 7   -0.005956317 -0.427960267      IAA
## 8   -1.524808613  0.431662229 AFB/TIR1
## 9    1.574336185 -5.041777156      IAA
## 10  -1.125575851 -0.558512920      IAA
## 11  -0.578750074 -0.811787304      IAA
## 12  -0.102457801  0.788703488 AFB/TIR1
## 13  -1.734416069  0.129024379 AFB/TIR1
## 14   0.542400680 -0.670223439      IAA
## 15   2.276729634  0.750445550      IAA
## 16  -0.307178253  0.330600703      ARF
## 17   0.923944787  1.334152162      ARF
## 18  -1.417357564  0.157370925      ARF
## 19  -0.428112834  0.500704876      ARF
## 20   1.418306200 -2.036229235      ARF
## 21   1.623282025  0.380380191      ARF
## 22  -2.129452835 -0.114441199      IAA
## 23   3.425337748 -3.402760865      IAA
## 24   0.306505583  0.466557952 AFB/TIR1
## 25  13.392512855 -1.806412389      IAA
## 26  -1.395820127  0.019944454      ARF
## 27  -0.071996269  0.256945271      IAA
## 28  -0.636996614  0.476207228 AFB/TIR1
## 29   3.120519585  1.142973848      ARF
## 30  -0.431602510  0.803815826      ARF
## 31  -1.866388546  0.375426589      ARF
## 32   1.337994430  0.015559355      ARF
## 33   6.702648045  3.579346703      ARF
## 34  -2.050727705  0.494196522      ARF
## 35  -0.617206008 -0.218288936      IAA
## 36  15.037892920  4.746237323      IAA
## 37  -0.488627623  0.398718486 AFB/TIR1
## 38   3.271439778  1.250223968      ARF
## 39   5.275584046  2.305407189      ARF
## 40  -1.369724965 -0.788772964      IAA
## 41  -0.667462730  0.526307555      ARF
## 42  -0.350550067  0.913360463      ARF
## 43   0.191018481  0.098979610      ARF
## 44  -0.450954600  0.394826074      ARF
## 45  -1.955375052  0.413220346 AFB/TIR1
## 46  -1.668697519  0.421500106 AFB/TIR1
## 47  -1.422813511  0.323815463      ARF
## 48   0.144182276  0.393931829      ARF
## 49   2.741786531  0.753157423      ARF
## 50   1.148171748  1.245116561      ARF
```

```
## 51  -2.105944003  0.202653740      ARF
## 52   0.639274022 -0.023380766      IAA
## 53  -2.063221418  0.141276678      IAA
## 54  -1.837152604  0.261934275 AFB/TIR1
## 55  -0.632126448  0.732872508      ARF
## 56  -1.068012274  0.168806405      ARF
## 57  -1.789096929 -0.140394034      IAA
## 58  -1.329711750  0.231182561      IAA
## 59   3.834394420  0.793449297      IAA
## 60  -1.552833562  0.395883644      ARF
## 61  -2.130228985  0.229001671      IAA
## 62   1.062351513 -0.241185818      IAA
## 63  -0.468794376 -0.249922455      IAA
## 64   0.077758331  0.524357821 AFB/TIR1
## 65  -2.084695899  0.369234056 AFB/TIR1
## 66  -0.856921604  0.507222423 AFB/TIR1
## 67  -1.508225563 -0.662268126      IAA
## 68  -1.559775037 -0.800512633      IAA
## 69  -2.018657725  0.043427973      IAA
## 70  -1.909610168  0.277764356      ARF
## 71   4.485099923 -1.351421276      IAA
## 72   2.771186411 -1.008109821      IAA
## 73  -1.778294112  0.262406297      IAA
## 74  -0.711451800 -1.541788959      IAA
## 75   0.304514187 -3.991108850      IAA
## 76   2.243284561 -9.997776235      IAA
## 77  -1.044218657  0.004571344      ARF
## 78  -0.860081508  0.223425301      ARF
## 79  -1.540300976  0.174794131      ARF
## 80  -0.075389131  0.937109194      ARF
## 81  -1.455953728  0.582823128      ARF
## 82  -2.063880601  0.149412964      ARF
## 83  -0.902465346  0.479261947      ARF
## 84   1.526821368  0.804159673      ARF
## 85  -1.705462800  0.589012162      ARF
## 86  -1.600893255  0.192216165      ARF
## 87  -1.791100183  0.339552665      ARF
## 88  -1.737520983  0.145743103      ARF
## 89  -1.679057092 -0.108332600      IAA
## 90  -1.994650072  0.350064536      ARF
## 91  -0.056763350  0.882006175      ARF
## 92   0.344926556  0.780242803      ARF
## 93  -0.446664212  0.458750897      ARF
## 94  -0.564238437  0.443802208      ARF
## 95   0.988840347  1.281462617      IAA
## 96  -0.793439779  0.229054413      IAA
## 97  -1.900821570  0.435235820      IAA
## 98  -1.372216366 -0.353257233      IAA
## 99  -1.413117840 -1.040984678      IAA
## 100 -1.362338626  0.207864794      IAA
## 101 -1.285762155  0.171175760      IAA
## 102 -1.166597927  0.338183452      ARF
## 103 -0.098539817  0.642577969      ARF
## 104 -0.230981637  0.201050662 AFB/TIR1
```

```
## 105  0.071596867 -0.228022083      IAA
## 106  0.156497195  0.696034794      ARF
## 107 -1.219875713  0.814553891      ARF
## 108 -1.297431165 -0.285475152      IAA
## 109 -1.979546144  0.237586347      IAA
## 110  1.748018509  1.187868663      IAA
## 111 -0.603884550  0.638749151      ARF
## 112 -1.676009054  0.418512190      ARF
## 113 -1.955130698  0.355458289      ARF
## 114 -1.994415698  0.311126791      ARF
## 115 -0.353407486  0.428375254      ARF
## 116  0.180816068  0.960752443      ARF
## 117  3.952871915  1.625931704 AFB/TIR1
## 118 -1.649072111  0.285122668      ARF
## 119 -2.107793036  0.262895090      IAA
## 120 -0.279830679  1.613426920      ARF
## 121 -0.598906053  0.577784192      ARF
## 122 -2.099471731  0.273681105      ARF
## 123  2.615232113  1.164031145 AFB/TIR1
## 124 -2.073025165 -0.349419050      IAA
## 125  0.719480304 -4.324426296      IAA
## 126  0.034697877 -3.518281284      IAA
## 127 -1.545395901  0.285127917      ARF
## 128  0.193178086  0.633239300 AFB/TIR1
## 129 -0.088423804  0.385551512      IAA
## 130 -1.883285723  0.267885408      ARF
## 131  1.484478940 -7.913429916      IAA
## 132 -0.941227933 -0.781215430      IAA
## 133  9.877340758 -2.300225618      IAA
```

```
build <- ggplot_build(PC1_2all)$data
points <- build[[1]]

# co-ordinates of the ellipses
ell_points <- car::dataEllipse(comb_pca_df$PC1,
                               comb_pca_df$PC2,
                               as.factor(comb_pca_df$Family), levels=c(.7, .9))
```




```
# add geom_point with ellipses point

ell_ARF <- as.data.frame(ell_points$ARF$`0.7`)

ell_IAA <- as.data.frame(ell_points$IAA$`0.7`)

ell_TIR <- as.data.frame(ell_points$`AFB/TIR1`$`0.7`)

# Find which points are outside (!) the ellipse, and add this to the data
library(sp)
dat_TIR <- data.frame(
  points[2:3],
  in.ell_TIR = as.logical(point.in.polygon(points$x, points$y, ell_TIR$x, ell_TIR$y))
)

dat_IAA <- data.frame(
  points[2:3],
  in.ell_IAA = as.logical(point.in.polygon(points$x, points$y, ell_IAA$x, ell_IAA$y))
)

dat_ARF <- data.frame(
  points[2:3],
  in.ell_ARF = as.logical(point.in.polygon(points$x, points$y, ell_ARF$x, ell_ARF$y))
)
# as.logical(point..) equals to TRUE indicated points are inside ellipses

# Combining data points for labeling
transcript_expr_logic <- cbind(comb_pca_df, dat_TIR, dat_IAA, dat_ARF) %>% select(., - c(x, y))


expr_logic <- transcript_expr_logic %>% mutate(., in_ell = case_when(Family=="ARF" & `in.ell_ARF` == TRU
                                                Family=="IAA" & `in.ell_IAA` == TRUE ~ "TRUE",
```

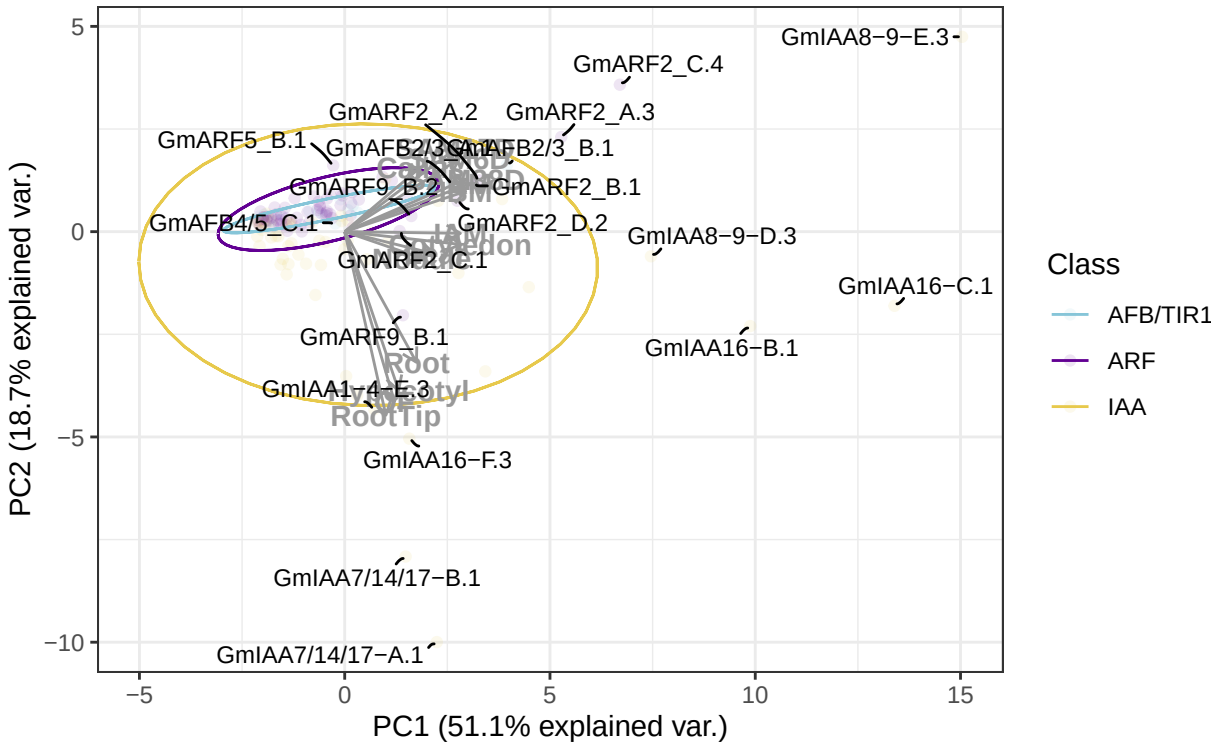
```
                                        Family=="AFB/TIR1" & `in.ell_TIR` == TRUE ~ "TRUE")) %>%
  mutate(in_ell = coalesce(in_ell, "FALSE"))

expr_logic[which(expr_logic$in_ell == FALSE),]
```

```
##         Transcript ID                    heatmap_label   Family  Class Clade
## 5   Glyma.01G098000.3     Glyma.01G098000|GmIAA8-9-D.3      IAA      A     I
## 9   Glyma.02G142500.3      Glyma.02G142500|GmIAA16-F.3      IAA      C   III
## 20  Glyma.03G070500.1       Glyma.03G070500|GmARF9_B.1      ARF      B     I
## 21  Glyma.03G070500.2       Glyma.03G070500|GmARF9_B.2      ARF      B     I
## 25  Glyma.03G247400.1      Glyma.03G247400|GmIAA16-C.1      IAA      C   III
## 29  Glyma.04G200600.1       Glyma.04G200600|GmARF2_B.1      ARF      B     I
## 32  Glyma.05G200800.1       Glyma.05G200800|GmARF2_C.1      ARF      B     I
## 33  Glyma.05G200800.4       Glyma.05G200800|GmARF2_C.4      ARF      B     I
## 36  Glyma.06G091700.3     Glyma.06G091700|GmIAA8-9-E.3      IAA      A     I
## 38  Glyma.06G164900.2       Glyma.06G164900|GmARF2_A.2      ARF      B     I
## 39  Glyma.06G164900.3       Glyma.06G164900|GmARF2_A.3      ARF      B     I
## 49  Glyma.08G008100.2       Glyma.08G008100|GmARF2_D.2      ARF      B     I
## 76  Glyma.10G180100.1 Glyma.10G180100|GmIAA7/14/17-A.1      IAA      C   III
## 104 Glyma.14G179500.1     Glyma.14G179500|GmAFB4/5_C.1 AFB/TIR1 AFB4/5    IV
## 117 Glyma.16G050500.1     Glyma.16G050500|GmAFB2/3_B.1 AFB/TIR1 AFB2/3    II
## 120 Glyma.17G256500.1       Glyma.17G256500|GmARF5_B.1      ARF      A    II
## 123 Glyma.19G100200.1     Glyma.19G100200|GmAFB2/3_A.1 AFB/TIR1 AFB2/3    II
## 125 Glyma.19G161000.3     Glyma.19G161000|GmIAA1-4-E.3      IAA      B    II
## 131 Glyma.20G210400.1 Glyma.20G210400|GmIAA7/14/17-B.1      IAA      C   III
## 133 Glyma.20G225000.1      Glyma.20G225000|GmIAA16-B.1      IAA      C   III
##             AM         OF        IAM        IBM    RootTip  Cotyledon
## 5    68.216252  51.350688  53.553189  96.275020  32.628226  78.301786
## 9    14.688070 529.887081  73.110411  28.516509  43.238091   0.202364
## 20   14.963834   9.149436  10.014337  18.303006   6.361899   6.335987
## 21   45.008611  32.497606  40.924358  42.192675   8.439588   8.282666
## 25  123.752976 182.226043 143.672394 136.882838  68.294552 100.483878
## 29   59.132821  34.776950  54.023677 104.014185   5.893899  15.036259
## 32   23.736932   7.300337  13.387625  33.516112   4.197497  21.693739
## 33  106.794815  41.848269  90.206750  89.149798   3.924654  25.580965
## 36  120.096829  73.349658  80.928913 112.799969  14.116324 123.198735
## 38   62.736767  31.362845  56.712867 104.850125   1.621706  14.695636
## 39   78.161251  91.333242 102.461124  95.846621   1.569549  29.653419
## 49   41.952212  22.684099  48.464588  54.600720   2.166574  14.976262
## 76    7.941917 536.895072   6.035997   7.033665 214.246604  30.729826
## 104  21.750232  21.417969  15.854160  17.435871  11.207461   8.506090
## 117  55.698420  35.061948  32.831068  58.586254  13.107811  27.899216
## 120   7.845772   2.581028   3.311743  17.662479   0.302999   1.222648
## 123  42.087683  43.031580  27.129734  42.689001  13.615904  12.545145
## 125   4.478037 404.803343  44.077715  17.846549  72.730750  41.028328
## 131   4.160975 265.136463  15.528614  13.540402 130.804062  41.664671
## 133  83.900583 259.425512 113.461714 100.588102  53.025576  53.477803
##      Hypocotyl      SAM6D     SAM17D     SAM38D    Callus       Leaf       Root
## 5   172.112449 101.538840 108.035003  57.568952  3.602672  72.181052  53.728970
## 9   114.709942   7.356839   6.637677   7.455989  1.051705  20.562404  67.383610
## 20    4.159165  21.230577  13.709403   9.639602  2.603449  42.803542 126.598709
## 21    7.071054  47.672295  55.806546  37.757871  0.685890   8.647167  43.277837
## 25  215.724837  75.229555 108.926253  87.081914 65.420159 178.440543  98.055423
## 29   13.614824  40.036100  67.500984  33.565553  5.088175  22.636836  12.527492
```

```
## 32   20.950298  31.857638  31.063118  16.461606  4.368482  44.030052  25.338768
## 33   38.494199  75.216825 290.336670 100.526085 25.977418   0.000000   0.000000
## 36  118.328990 229.139072 393.546142 181.391747 71.756006  45.788590  20.983248
## 38   13.618860  48.102339  51.456817  31.907033  8.665198  27.396630  14.501406
## 39   18.983798  38.298793 237.526834  76.153152  6.048278   3.674760   1.606524
## 49   26.934453  45.899996 104.119336  30.814124  3.509392  48.206033  26.688307
## 76  350.297556   2.600212   4.426392   5.209396  2.744771  20.850299  94.160633
## 104  17.456177  22.806197  19.605122   9.950014 12.500697  19.094595  14.355242
## 117  27.176499  82.133333  87.939197  38.297183 47.396469  32.138566  17.503017
## 120   1.046225  28.072725  24.136925   7.085973 69.182090   2.954026   4.491654
## 123  19.247151  60.919431  75.317808  31.290701 44.433814  27.410816  21.526502
## 125 177.214792   3.197441   2.314000   2.924909  0.843634   6.919914   6.338750
## 131 633.844120   1.661192   0.821472   1.444420  0.244966   0.972353  62.263900
## 133 102.248657  92.557209 144.677356  86.120903 10.711991  21.058177 104.233361
##        Nodule        PC1         PC2        PC3        PC4 in.ell_TIR
## 5   22.341412  7.4596126 -0.60000202  2.0307657  0.4458205      FALSE
## 9    1.533396  1.5743362 -5.04177716 -0.6172365  0.6633938      FALSE
## 20  27.491901  1.4183062 -2.03622924  5.7800703  1.3629861      FALSE
## 21   3.279099  1.6232820  0.38038019  0.1987720  1.1554167      FALSE
## 25   5.415049 13.3925129 -1.80641239  2.1698302 -5.4199946      FALSE
## 29  10.758737  3.1205196  1.14297385  0.1671277  1.3202221      FALSE
## 32  21.300657  1.3379944  0.01555935  2.7843694  0.4682530      FALSE
## 33   0.000000  6.7026480  3.57934670 -3.3730284  1.3243790      FALSE
## 36  22.794609 15.0378929  4.74623732 -1.3006126 -0.9309800      FALSE
## 38   9.673077  3.2714398  1.25022397  0.2925031  0.9717274      FALSE
## 39   0.000000  5.2755840  2.30540719 -2.8806692  1.9555757      FALSE
## 49   8.836310  2.7417865  0.75315742  0.9842749  0.4962433      FALSE
## 76   5.269519  2.2432846 -9.99777623 -1.0514431 -0.9241382      FALSE
## 104  3.394860 -0.2309816  0.20105066  0.5268626 -0.5560466      FALSE
## 117  8.945171  3.9528719  1.62593170  0.5054186 -1.6929049      FALSE
## 120  0.392173 -0.2798307  1.61342692  0.2055221 -3.4292754      FALSE
## 123  7.313215  2.6152321  1.16403114  0.5868563 -1.5858479      FALSE
## 125  0.000000  0.7194803 -4.32442630 -2.0960569 -0.4665481      FALSE
## 131  0.495348  1.4844789 -7.91342992 -2.4573024 -0.8906494      FALSE
## 133 53.539749  9.8773408 -2.30022562  3.5022307  4.6373748      FALSE
##     in.ell_IAA in.ell_ARF in_ell
## 5        FALSE      FALSE  FALSE
## 9        FALSE      FALSE  FALSE
## 20        TRUE      FALSE  FALSE
## 21        TRUE      FALSE  FALSE
## 25       FALSE      FALSE  FALSE
## 29        TRUE      FALSE  FALSE
## 32        TRUE      FALSE  FALSE
## 33       FALSE      FALSE  FALSE
## 36       FALSE      FALSE  FALSE
## 38        TRUE      FALSE  FALSE
## 39       FALSE      FALSE  FALSE
## 49        TRUE      FALSE  FALSE
## 76       FALSE      FALSE  FALSE
## 104       TRUE       TRUE  FALSE
## 117       TRUE      FALSE  FALSE
## 120       TRUE      FALSE  FALSE
## 123       TRUE      FALSE  FALSE
## 125      FALSE      FALSE  FALSE
```

```
## 131      FALSE      FALSE  FALSE
## 133      FALSE      FALSE  FALSE
```

```
PC1_2all +
    geom_segment(PCA_loadings, mapping=aes(x=0, y=0, # Change the size of arrows
                                           xend=(PC1*8.75), yend=(PC2*8.75)),
                 arrow = arrow(length = unit(1/2, "picas")), color="gray60") +
    annotate("text", x=(PCA_loadings$PC1*8.75), #add the tissue names to it manually
             y=(PCA_loadings$PC2*8.75),
             label=PCA_loadings$Variables, size=4, color="gray60", fontface="bold") +
    theme(panel.background = element_rect(fill = "white", linewidth = 1))+
    theme_bw()+
   scale_color_manual(values=c("#86C5D8", "#620093", "#E7C94C"))+
  ggrepel::geom_text_repel(data = expr_logic %>%
                           as_tibble(rownames = "name") %>%
                           filter(as.logical(in_ell == FALSE)),
                           aes(PC1, PC2, label=sub(".*\\|", "", heatmap_label)),
                         size=3, max.overlaps = 100,  min.segment.length = 0,
                         segment.curvature = -0.1) +
  labs(color = "Class") +
  theme_bw()
```



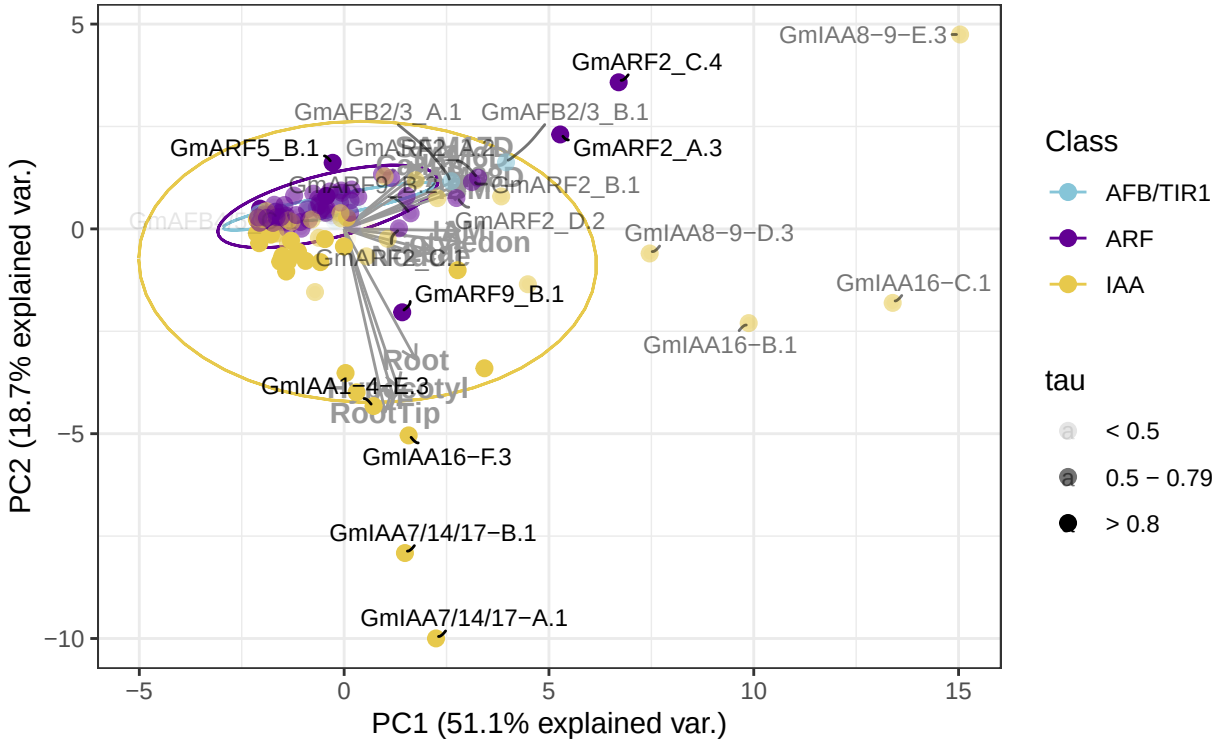

```
# ggsave("20230927_PC1_2_allTissues.png", dpi = 1000, width = 10, height = 8)
# ggsave("20230927_PC1_2_allTissues.pdf", dpi = 1000, width = 10, height = 8)
```

Lets now repeat with the tau values

```
# combine data frames expr_logic with tau values
expr_logic2 <- cbind(expr_logic, tau_df$tau)

# Define the intervals for expr_logic2$tau
expr_logic2$tau_interval <- cut(expr_logic2$tau,
```

```
                                breaks = c(-Inf, 0.5, 0.8, Inf),
                                labels = c("< 0.5", "0.5 - 0.79", "> 0.8"))

PC1_2all +
    geom_segment(PCA_loadings, mapping=aes(x=0, y=0, # Change the size of arrows
                                           xend=(PC1*8.75), yend=(PC2*8.75)),
                 arrow = arrow(length = unit(1/2, "picas")), color="gray60") +
    annotate("text", x=(PCA_loadings$PC1*8.75), #add the tissue names to it manually
             y=(PCA_loadings$PC2*8.75),
             label=PCA_loadings$Variables, size=4, color="gray60", fontface="bold") +
    theme(panel.background = element_rect(fill = "white", linewidth = 1))+
    theme_bw() +
  geom_point(data = expr_logic2 %>% as_tibble(rownames = "name"),
                                  aes(PC1, PC2, alpha = tau_interval, color = Family), size=2.5) +
  ggrepel::geom_text_repel(data = expr_logic2 %>%
                           as_tibble(rownames = "name") %>%
                           filter(as.logical(in_ell == FALSE)),
                           aes(PC1, PC2, label=sub(".*\\|", "", heatmap_label),
                               alpha = tau_interval),
                         size=3, max.overlaps = 100,  min.segment.length = 0,
                         segment.curvature = -0.1) +

  scale_color_manual(values=c("#86C5D8", "#620093", "#E7C94C"))+
  labs(color = "Class", shape = "tau", alpha ="tau") +
  theme_bw()
```

```
## Warning: Using alpha for a discrete variable is not advised.
```

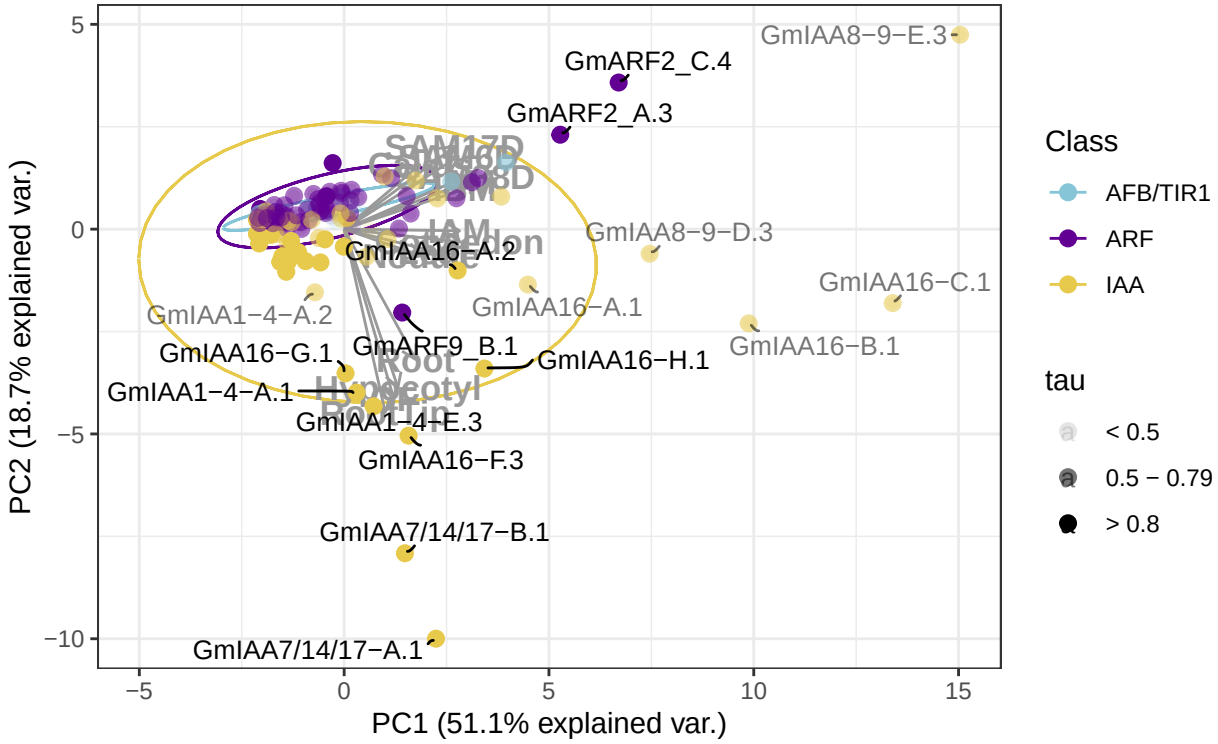



```
# ggsave("20240117_PC1_2_allTissues.png", dpi = 1000, width = 10, height = 8)
# ggsave("20240117_PC1_2_allTissues.pdf", dpi = 1000, width = 10, height = 8)
```

```
# How if we label all that has tau greater than 0.8

PC1_2all +
    geom_segment(PCA_loadings, mapping=aes(x=0, y=0, # Change the size of arrows
                                           xend=(PC1*8.75), yend=(PC2*8.75)),
                 arrow = arrow(length = unit(1/2, "picas")), color="gray60") +
    annotate("text", x=(PCA_loadings$PC1*8.75), #add the tissue names to it manually
             y=(PCA_loadings$PC2*8.75),
             label=PCA_loadings$Variables, size=4.5, color="gray60", fontface="bold") +
    theme(panel.background = element_rect(fill = "white", linewidth = 1))+
    theme_bw() +
  geom_point(data = expr_logic2 %>% as_tibble(rownames = "name"),
                                    aes(PC1, PC2, alpha = tau_interval, color = Family), size=2.5) +
  ggrepel::geom_text_repel(data = expr_logic2 %>%
                           as_tibble(rownames = "name") %>%
                           filter(as.logical(tau_interval != "< 0.5")),
                           aes(PC1, PC2, label=sub(".*\\|", "", heatmap_label),
                               alpha = tau_interval),
                         size=3.5, max.overlaps = 30,  min.segment.length = 0,
                         segment.curvature = -0.1) +

  scale_color_manual(values=c("#86C5D8", "#620093", "#E7C94C"))+
  labs(color = "Class", shape = "tau", alpha ="tau") +
  theme_bw()
```

```
## Warning: Using alpha for a discrete variable is not advised.
```

```
## Warning: ggrepel: 102 unlabeled data points (too many overlaps). Consider
## increasing max.overlaps
```



```
# ggsave("20240119_PC1_2_allTissues_tau_GREATER-5.pdf", dpi = 1000, width = 10, height = 8)
```

PC2 and PC3 outside ellipse labels for the 14 tissues

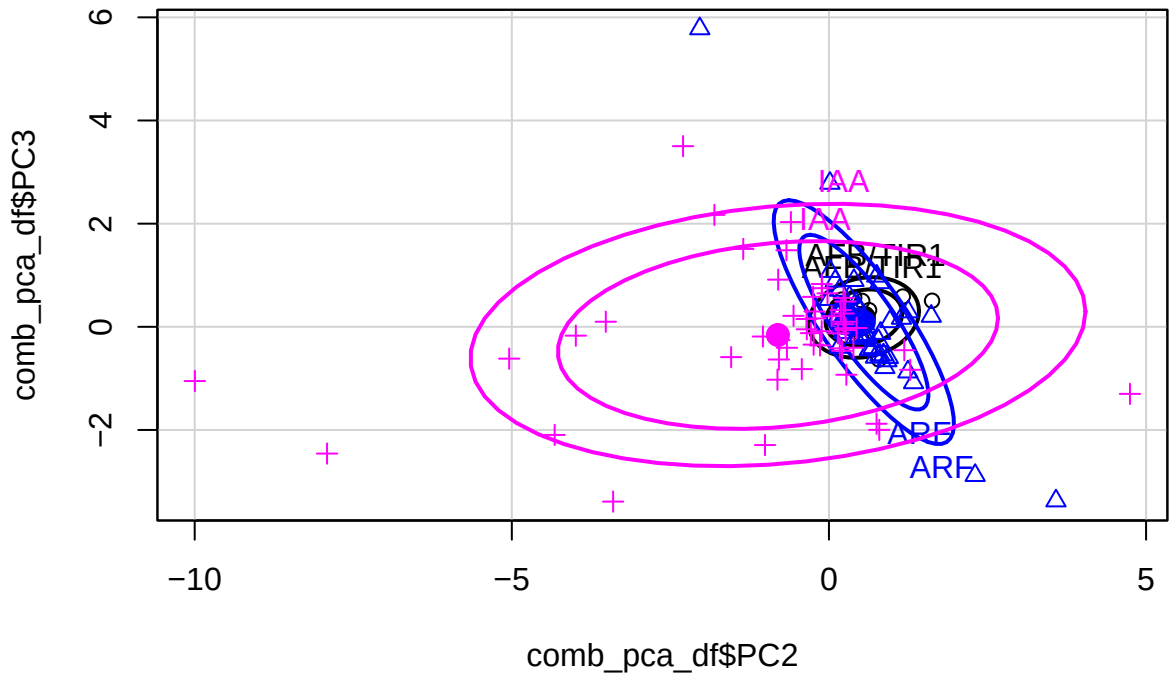
```
PC2_3all <- ggbiplot::ggbiplot(pca, obs.scale = 1, var.scale = .5, choices = c(2, 3),
                               varname.adjust = 3, alpha = .1,
                              groups = Family, var.axes = F, varname.size = 2,
                    ellipse = TRUE,  circle = FALSE, ellipse.prob = .70)


# Extract components so we can select out
PC2_3all$data
```

```
##             xvar         yvar   groups
## 1    0.253967120  0.192765997      ARF
## 2    0.185928231  0.323910990      IAA
## 3   -0.113511655  0.829138289      IAA
## 4    0.274717011 -0.929827851      IAA
## 5   -0.600002016  2.030765709      IAA
## 6    0.859463281 -0.543671987      ARF
## 7   -0.427960267 -0.820157717      IAA
## 8    0.431662229  0.097838721 AFB/TIR1
## 9   -5.041777156 -0.617236529      IAA
## 10  -0.558512920  0.212488063      IAA
## 11  -0.811787304 -1.025141950      IAA
## 12   0.788703488 -0.633363301 AFB/TIR1
## 13   0.129024379  0.388439486 AFB/TIR1
## 14  -0.670223439  1.484374427      IAA
## 15   0.750445550 -1.880148999      IAA
## 16   0.330600703  0.213426698      ARF
## 17   1.334152162 -1.082153419      ARF
## 18   0.157370925 -0.395001161      ARF
## 19   0.500704876  0.080303779      ARF
## 20  -2.036229235  5.780070252      ARF
## 21   0.380380191  0.198771991      ARF
## 22  -0.114441199 -0.085693178      IAA
## 23  -3.402760865 -3.388444330      IAA
## 24   0.466557952  0.166835894 AFB/TIR1
## 25  -1.806412389  2.169830163      IAA
## 26   0.019944454  1.074396091      ARF
## 27   0.256945271  0.489833024      IAA
## 28   0.476207228 -0.051671359 AFB/TIR1
## 29   1.142973848  0.167127745      ARF
## 30   0.803815826 -0.548952095      ARF
## 31   0.375426589  0.157488574      ARF
## 32   0.015559355  2.784369433      ARF
## 33   3.579346703 -3.373028396      ARF
## 34   0.494196522  0.018029747      ARF
## 35  -0.218288936  0.162561278      IAA
## 36   4.746237323 -1.300612613      IAA
## 37   0.398718486  0.569404529 AFB/TIR1
## 38   1.250223968  0.292503137      ARF
## 39   2.305407189 -2.880669155      ARF
```

```
## 40  -0.788772964 -0.633847587      IAA
## 41   0.526307555  0.141808591      ARF
## 42   0.913360463 -0.656456898      ARF
## 43   0.098979610  0.889037817      ARF
## 44   0.394826074  0.286525824      ARF
## 45   0.413220346 -0.086074243 AFB/TIR1
## 46   0.421500106  0.078045033 AFB/TIR1
## 47   0.323815463  0.576287661      ARF
## 48   0.393931829  0.899632729      ARF
## 49   0.753157423  0.984274929      ARF
## 50   1.245116561 -0.873135543      ARF
## 51   0.202653740  0.140445647      ARF
## 52  -0.023380766  0.649133026      IAA
## 53   0.141276678 -0.125741994      IAA
## 54   0.261934275  0.016357767 AFB/TIR1
## 55   0.732872508 -0.587549057      ARF
## 56   0.168806405  0.710504593      ARF
## 57  -0.140394034 -0.360521593      IAA
## 58   0.231182561  0.257918902      IAA
## 59   0.793449297 -1.998468838      IAA
## 60   0.395883644  0.150013982      ARF
## 61   0.229001671  0.049754903      IAA
## 62  -0.241185818 -0.345426570      IAA
## 63  -0.249922455  0.580533544      IAA
## 64   0.524357821  0.502244955 AFB/TIR1
## 65   0.369234056  0.114997043 AFB/TIR1
## 66   0.507222423 -0.214148722 AFB/TIR1
## 67  -0.662268126 -0.406399893      IAA
## 68  -0.800512633  0.914386871      IAA
## 69   0.043427973  0.206015394      IAA
## 70   0.277764356  0.074614796      ARF
## 71  -1.351421276  1.509715283      IAA
## 72  -1.008109821 -2.292586475      IAA
## 73   0.262406297 -0.029598759      IAA
## 74  -1.541788959 -0.587753821      IAA
## 75  -3.991108850 -0.171191334      IAA
## 76  -9.997776235 -1.051443078      IAA
## 77   0.004571344  0.531597276      ARF
## 78   0.223425301 -0.109188343      ARF
## 79   0.174794131  0.317995304      ARF
## 80   0.937109194 -0.594875791      ARF
## 81   0.582823128  0.004808883      ARF
## 82   0.149412964  0.324427333      ARF
## 83   0.479261947 -0.173718424      ARF
## 84   0.804159673  0.857615531      ARF
## 85   0.589012162 -0.146999936      ARF
## 86   0.192216165  0.092837656      ARF
## 87   0.339552665  0.275671087      ARF
## 88   0.145743103  0.204583749      ARF
## 89  -0.108332600  0.749279943      IAA
## 90   0.350064536 -0.025807891      ARF
## 91   0.882006175 -0.788482703      ARF
## 92   0.780242803 -0.264669941      ARF
## 93   0.458750897  0.352511287      ARF
```

```
## 94   0.443802208 -0.049101215      ARF
## 95   1.281462617 -0.833524403      IAA
## 96   0.229054413  0.559131560      IAA
## 97   0.435235820 -0.022773811      IAA
## 98  -0.353257233  0.152202391      IAA
## 99  -1.040984678 -0.188391551      IAA
## 100  0.207864794 -0.465096502      IAA
## 101  0.171175760 -0.417437561      IAA
## 102  0.338183452  0.239714138      ARF
## 103  0.642577969 -0.433940124      ARF
## 104  0.201050662  0.526862594 AFB/TIR1
## 105 -0.228022083  0.297046621      IAA
## 106  0.696034794 -0.401907770      ARF
## 107  0.814553891 -0.125386206      ARF
## 108 -0.285475152 -0.122534533      IAA
## 109  0.237586347  0.097778388      IAA
## 110  1.187868663 -0.453351398      IAA
## 111  0.638749151 -0.442597992      ARF
## 112  0.418512190 -0.204069007      ARF
## 113  0.355458289  0.229584716      ARF
## 114  0.311126791  0.219278723      ARF
## 115  0.428375254  0.457407776      ARF
## 116  0.960752443  0.101790408      ARF
## 117  1.625931704  0.505418632 AFB/TIR1
## 118  0.285122668 -0.017733146      ARF
## 119  0.262895090 -0.064799773      IAA
## 120  1.613426920  0.205522094      ARF
## 121  0.577784192 -0.221521206      ARF
## 122  0.273681105  0.113040052      ARF
## 123  1.164031145  0.586856325 AFB/TIR1
## 124 -0.349419050 -0.048212877      IAA
## 125 -4.324426296 -2.096056941      IAA
## 126 -3.518281284  0.098701172      IAA
## 127  0.285127917  0.193201295      ARF
## 128  0.633239300  0.321861245 AFB/TIR1
## 129  0.385551512 -0.405549799      IAA
## 130  0.267885408  0.074216762      ARF
## 131 -7.913429916 -2.457302433      IAA
## 132 -0.781215430 -0.258947266      IAA
## 133 -2.300225618  3.502230742      IAA
```

```
build_PC2_3all <- ggplot_build(PC2_3all)$data
points_PC2_3all <- build_PC2_3all[[1]]

# co-ordinates of the ellipses
ell_points_PC2_3all <- car::dataEllipse(comb_pca_df$PC2,
                                comb_pca_df$PC3,
                                as.factor(comb_pca_df$Family), levels=c(.7, .9))
```




```
# add geom_point with ellipses point

ell_ARF_PC2_3all <- as.data.frame(ell_points_PC2_3all$ARF$`0.7`)

ell_IAA_PC2_3all <- as.data.frame(ell_points_PC2_3all$IAA$`0.7`)

ell_TIR_PC2_3all <- as.data.frame(ell_points_PC2_3all$`AFB/TIR1`$`0.7`)

# Find which points are outside (!) the ellipse, and add this to the data

dat_TIR_PC2_3all <- data.frame(
  points_PC2_3all[2:3],
  in.ell_TIR = as.logical(point.in.polygon(points_PC2_3all$x, points_PC2_3all$y, ell_TIR_PC2_3all$x, ell
)

dat_IAA_PC2_3all <- data.frame(
  points_PC2_3all[2:3],
  in.ell_IAA = as.logical(point.in.polygon(points_PC2_3all$x, points_PC2_3all$y, ell_IAA_PC2_3all$x, ell
)

dat_ARF_PC2_3all <- data.frame(
  points_PC2_3all[2:3],
  in.ell_ARF = as.logical(point.in.polygon(points_PC2_3all$x, points_PC2_3all$y, ell_ARF_PC2_3all$x, ell
)
# as.logical(point..) equals to TRUE indicated points are inside ellipses

# Combining data points for labeling
transcript_expr_logic_PC2_3all <- cbind(comb_pca_df, dat_TIR_PC2_3all, dat_IAA_PC2_3all, dat_ARF_PC2_3al


expr_logic_PC2_3all <- transcript_expr_logic_PC2_3all %>% mutate(., in_ell = case_when(Family=="ARF" &
                                          Family=="IAA" & `in.ell_IAA` == TRUE ~ "TRUE",
```

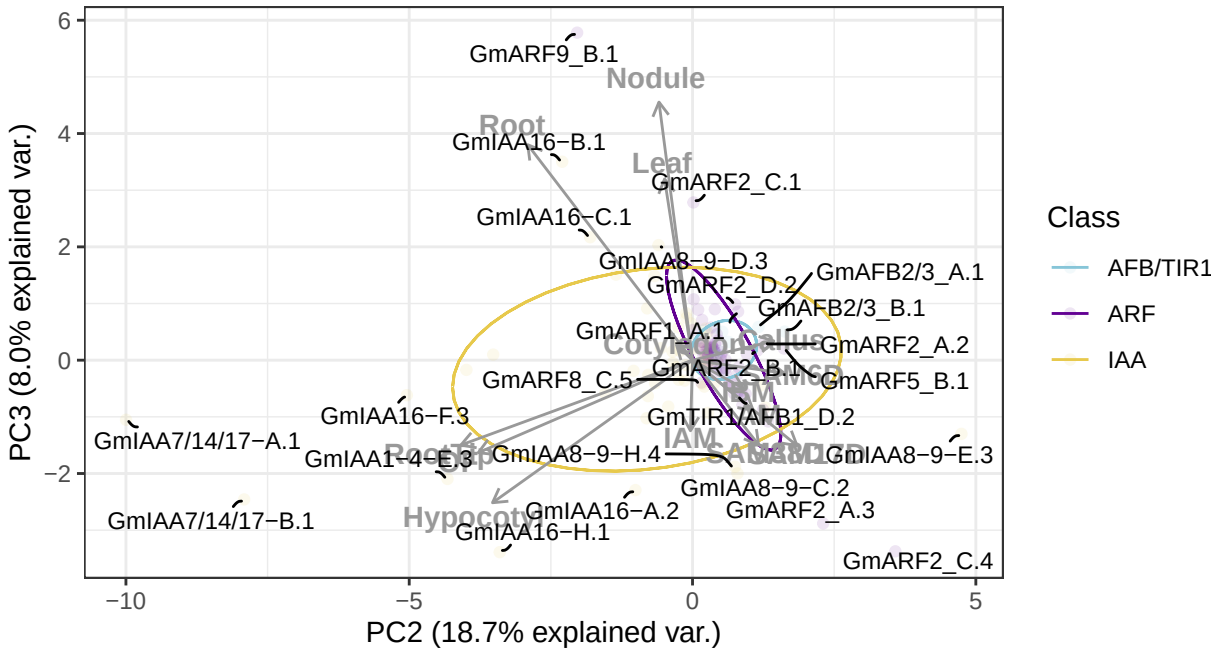
```
                                          Family=="AFB/TIR1" & `in.ell_TIR` == TRUE ~ "TRUE")) %>%
  mutate(in_ell = coalesce(in_ell, "FALSE"))

expr_logic_PC2_3all[which(expr_logic_PC2_3all$in_ell == FALSE),]
```

```
##         Transcript ID                    heatmap_label   Family     Class Clade
## 5   Glyma.01G098000.3     Glyma.01G098000|GmIAA8-9-D.3      IAA         A     I
## 9   Glyma.02G142500.3      Glyma.02G142500|GmIAA16-F.3      IAA         C   III
## 12  Glyma.02G152800.2  Glyma.02G152800|GmTIR1/AFB1_D.2 AFB/TIR1 TIR1/AFB1     I
## 15  Glyma.02G218100.4     Glyma.02G218100|GmIAA8-9-H.4      IAA         A     I
## 18  Glyma.02G239600.5       Glyma.02G239600|GmARF8_C.5      ARF         A    II
## 20  Glyma.03G070500.1       Glyma.03G070500|GmARF9_B.1      ARF         B     I
## 23  Glyma.03G158700.1      Glyma.03G158700|GmIAA16-H.1      IAA         C   III
## 25  Glyma.03G247400.1      Glyma.03G247400|GmIAA16-C.1      IAA         C   III
## 29  Glyma.04G200600.1       Glyma.04G200600|GmARF2_B.1      ARF         B     I
## 32  Glyma.05G200800.1       Glyma.05G200800|GmARF2_C.1      ARF         B     I
## 33  Glyma.05G200800.4       Glyma.05G200800|GmARF2_C.4      ARF         B     I
## 36  Glyma.06G091700.3     Glyma.06G091700|GmIAA8-9-E.3      IAA         A     I
## 38  Glyma.06G164900.2       Glyma.06G164900|GmARF2_A.2      ARF         B     I
## 39  Glyma.06G164900.3       Glyma.06G164900|GmARF2_A.3      ARF         B     I
## 49  Glyma.08G008100.2       Glyma.08G008100|GmARF2_D.2      ARF         B     I
## 59  Glyma.08G273500.2     Glyma.08G273500|GmIAA8-9-C.2      IAA         A     I
## 72  Glyma.10G162400.2      Glyma.10G162400|GmIAA16-A.2      IAA         C   III
## 76  Glyma.10G180100.1 Glyma.10G180100|GmIAA7/14/17-A.1      IAA         C   III
## 84  Glyma.12G164100.1       Glyma.12G164100|GmARF1_A.1      ARF         B     I
## 117 Glyma.16G050500.1     Glyma.16G050500|GmAFB2/3_B.1 AFB/TIR1    AFB2/3    II
## 120 Glyma.17G256500.1       Glyma.17G256500|GmARF5_B.1      ARF         A    II
## 123 Glyma.19G100200.1     Glyma.19G100200|GmAFB2/3_A.1 AFB/TIR1    AFB2/3    II
## 125 Glyma.19G161000.3     Glyma.19G161000|GmIAA1-4-E.3      IAA         B    II
## 131 Glyma.20G210400.1 Glyma.20G210400|GmIAA7/14/17-B.1      IAA         C   III
## 133 Glyma.20G225000.1      Glyma.20G225000|GmIAA16-B.1      IAA         C   III
##             AM         OF        IAM        IBM    RootTip  Cotyledon
## 5    68.216252  51.350688  53.553189  96.275020  32.628226  78.301786
## 9    14.688070 529.887081  73.110411  28.516509  43.238091   0.202364
## 12   22.069192  16.181094  21.004047  22.849022  12.164769  15.200032
## 15   58.683503  91.015780  54.923525  50.038109  25.887821   8.588162
## 18   13.757865  13.859804   8.771196   7.354321   6.099923  14.166313
## 20   14.963834   9.149436  10.014337  18.303006   6.361899   6.335987
## 23   62.978354 188.282417  63.415971  50.273803  93.913232  11.797675
## 25  123.752976 182.226043 143.672394 136.882838  68.294552 100.483878
## 29   59.132821  34.776950  54.023677 104.014185   5.893899  15.036259
## 32   23.736932   7.300337  13.387625  33.516112   4.197497  21.693739
## 33  106.794815  41.848269  90.206750  89.149798   3.924654  25.580965
## 36  120.096829  73.349658  80.928913 112.799969  14.116324 123.198735
## 38   62.736767  31.362845  56.712867 104.850125   1.621706  14.695636
## 39   78.161251  91.333242 102.461124  95.846621   1.569549  29.653419
## 49   41.952212  22.684099  48.464588  54.600720   2.166574  14.976262
## 59   60.235552  42.618679  62.597901  70.694115  15.151090  25.020319
## 72   50.429036  62.648515  44.799041  49.047824  54.886130  14.549869
## 76    7.941917 536.895072   6.035997   7.033665 214.246604  30.729826
## 84   29.907851  25.658848  21.848792  33.978092  12.158648  15.573356
## 117  55.698420  35.061948  32.831068  58.586254  13.107811  27.899216
## 120   7.845772   2.581028   3.311743  17.662479   0.302999   1.222648
## 123  42.087683  43.031580  27.129734  42.689001  13.615904  12.545145
```

```
## 125   4.478037 404.803343  44.077715  17.846549  72.730750  41.028328
## 131   4.160975 265.136463  15.528614  13.540402 130.804062  41.664671
## 133  83.900583 259.425512 113.461714 100.588102  53.025576  53.477803
##      Hypocotyl      SAM6D     SAM17D     SAM38D    Callus       Leaf       Root
## 5   172.112449 101.538840 108.035003  57.568952  3.602672  72.181052  53.728970
## 9   114.709942   7.356839   6.637677   7.455989  1.051705  20.562404  67.383610
## 12   20.202937  29.352679  33.917530  17.176745 17.838179   0.000000   0.000000
## 15   51.413131  79.581114  54.407495  42.568039  6.383128   0.000000   0.000000
## 18   33.826894   0.000000  11.829332  13.561473  1.551810   0.000000   0.000000
## 20    4.159165  21.230577  13.709403   9.639602  2.603449  42.803542 126.598709
## 23  382.933284  20.163323  39.919056  73.535631  0.583159   6.250057  14.892682
## 25  215.724837  75.229555 108.926253  87.081914 65.420159 178.440543  98.055423
## 29   13.614824  40.036100  67.500984  33.565553  5.088175  22.636836  12.527492
## 32   20.950298  31.857638  31.063118  16.461606  4.368482  44.030052  25.338768
## 33   38.494199  75.216825 290.336670 100.526085 25.977418   0.000000   0.000000
## 36  118.328990 229.139072 393.546142 181.391747 71.756006  45.788590  20.983248
## 38   13.618860  48.102339  51.456817  31.907033  8.665198  27.396630  14.501406
## 39   18.983798  38.298793 237.526834  76.153152  6.048278   3.674760   1.606524
## 49   26.934453  45.899996 104.119336  30.814124  3.509392  48.206033  26.688307
## 59  166.602217  77.910628  90.255018  63.645599  6.462795  11.030950   6.618096
## 72  258.563372  52.919869  43.482386  62.182188 12.401001   7.425099  12.077243
## 76  350.297556   2.600212   4.426392   5.209396  2.744771  20.850299  94.160633
## 84   22.344234  27.195518  37.963940  21.692550 51.460680  19.141297  21.871309
## 117  27.176499  82.133333  87.939197  38.297183 47.396469  32.138566  17.503017
## 120   1.046225  28.072725  24.136925   7.085973 69.182090   2.954026   4.491654
## 123  19.247151  60.919431  75.317808  31.290701 44.433814  27.410816  21.526502
## 125 177.214792   3.197441   2.314000   2.924909  0.843634   6.919914   6.338750
## 131 633.844120   1.661192   0.821472   1.444420  0.244966   0.972353  62.263900
## 133 102.248657  92.557209 144.677356  86.120903 10.711991  21.058177 104.233361
##        Nodule        PC1         PC2        PC3          PC4 in.ell_TIR
## 5   22.341412  7.4596126 -0.60000202  2.0307657  0.445820518      FALSE
## 9    1.533396  1.5743362 -5.04177716 -0.6172365  0.663393838      FALSE
## 12   0.000000 -0.1024578  0.78870349 -0.6333633 -0.606570751      FALSE
## 15   0.000000  2.2767296  0.75044555 -1.8801490  0.882776840      FALSE
## 18   0.000000 -1.4173576  0.15737092 -0.3950012 -0.059740809      FALSE
## 20  27.491901  1.4183062 -2.03622924  5.7800703  1.362986140      FALSE
## 23   2.016265  3.4253377 -3.40276087 -3.3884443  0.846071762      FALSE
## 25   5.415049 13.3925129 -1.80641239  2.1698302 -5.419994579      FALSE
## 29  10.758737  3.1205196  1.14297385  0.1671277  1.320222051       TRUE
## 32  21.300657  1.3379944  0.01555935  2.7843694  0.468252980      FALSE
## 33   0.000000  6.7026480  3.57934670 -3.3730284  1.324379018      FALSE
## 36  22.794609 15.0378929  4.74623732 -1.3006126 -0.930979975      FALSE
## 38   9.673077  3.2714398  1.25022397  0.2925031  0.971727378      FALSE
## 39   0.000000  5.2755840  2.30540719 -2.8806692  1.955575677      FALSE
## 49   8.836310  2.7417865  0.75315742  0.9842749  0.496243282      FALSE
## 59   1.380951  3.8343944  0.79344930 -1.9984688  0.825223890      FALSE
## 72   0.000000  2.7711864 -1.00810982 -2.2925865 -0.002501458      FALSE
## 76   5.269519  2.2432846 -9.99777623 -1.0514431 -0.924138217      FALSE
## 84   7.347936  1.5268214  0.80415967  0.8576155 -2.169356529      FALSE
## 117  8.945171  3.9528719  1.62593170  0.5054186 -1.692904946      FALSE
## 120  0.392173 -0.2798307  1.61342692  0.2055221 -3.429275402      FALSE
## 123  7.313215  2.6152321  1.16403114  0.5868563 -1.585847922      FALSE
## 125  0.000000  0.7194803 -4.32442630 -2.0960569 -0.466548138      FALSE
## 131  0.495348  1.4844789 -7.91342992 -2.4573024 -0.890649441      FALSE
```

```
## 133 53.539749  9.8773408 -2.30022562  3.5022307  4.637374786      FALSE
##     in.ell_IAA in.ell_ARF in_ell
## 5        FALSE      FALSE  FALSE
## 9        FALSE      FALSE  FALSE
## 12        TRUE       TRUE  FALSE
## 15       FALSE      FALSE  FALSE
## 18        TRUE      FALSE  FALSE
## 20       FALSE      FALSE  FALSE
## 23       FALSE      FALSE  FALSE
## 25       FALSE      FALSE  FALSE
## 29        TRUE      FALSE  FALSE
## 32       FALSE      FALSE  FALSE
## 33       FALSE      FALSE  FALSE
## 36       FALSE      FALSE  FALSE
## 38        TRUE      FALSE  FALSE
## 39       FALSE      FALSE  FALSE
## 49        TRUE      FALSE  FALSE
## 59       FALSE      FALSE  FALSE
## 72       FALSE      FALSE  FALSE
## 76       FALSE      FALSE  FALSE
## 84        TRUE      FALSE  FALSE
## 117       TRUE      FALSE  FALSE
## 120       TRUE      FALSE  FALSE
## 123       TRUE      FALSE  FALSE
## 125      FALSE      FALSE  FALSE
## 131      FALSE      FALSE  FALSE
## 133      FALSE      FALSE  FALSE
```

```
PC2_3all +
    geom_segment(PCA_loadings, mapping=aes(x=0, y=0, # Change the size of arrows
                                           xend=(PC2*8), yend=(PC3*8)),
                 arrow = arrow(length = unit(1/2, "picas")), color="gray60") +
    annotate("text", x=(PCA_loadings$PC2*8.75), #add the tissue names to it manually
             y=(PCA_loadings$PC3*8.75),
             label=PCA_loadings$Variables, size=4, color="gray60", fontface="bold") +
    theme(panel.background = element_rect(fill = "white", linewidth = 1))+
    theme_bw()+
   scale_color_manual(values=c("#86C5D8", "#620093", "#E7C94C"))+
   ggrepel::geom_text_repel(data = expr_logic_PC2_3all %>%
                            as_tibble(rownames = "name") %>%
                            filter(as.logical(in_ell == FALSE)),
                            aes(PC2, PC3, label=sub(".*\\|", "", heatmap_label)),
                           size=3, max.overlaps = 100,  min.segment.length = 0,
                           segment.curvature = -0.1) +
  labs(color = "Class") +
  theme_bw()
```



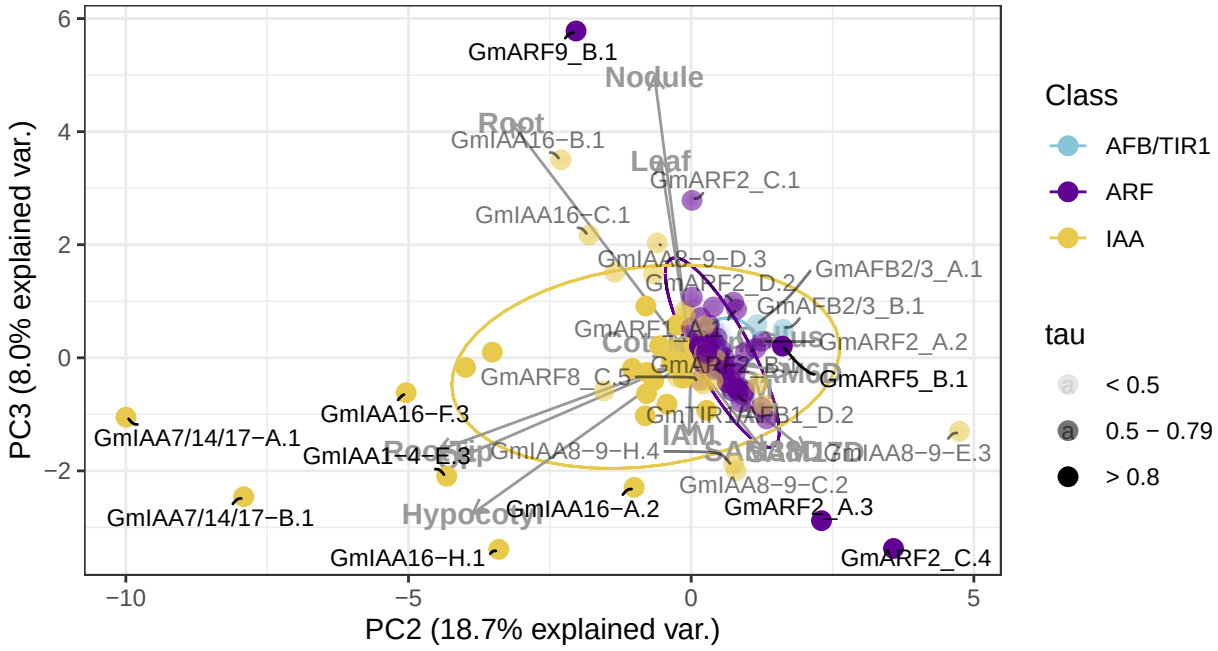

```
# ggsave("20230927_PC2_3_allTissues.png", dpi = 1000, width = 10, height = 8)
# ggsave("20230927_PC2_3_allTissues.pdf", dpi = 1000, width = 10, height = 8)
```

Lets now repeat with the tau values

```
# combine data frames expr_logic with tau values
expr_logic_PC2_3 <- cbind(expr_logic_PC2_3all, tau_df$tau)

# Define the intervals for expr_logic2$tau
expr_logic_PC2_3$tau_interval <- cut(expr_logic_PC2_3$tau,
                                breaks = c(-Inf, 0.5, 0.8, Inf),
                                labels = c("< 0.5", "0.5 - 0.79", "> 0.8"))



PC2_3all +
    geom_segment(PCA_loadings, mapping=aes(x=0, y=0, # Change the size of arrows
                                           xend=(PC2*8.75), yend=(PC3*8.75)),
                 arrow = arrow(length = unit(1/2, "picas")), color="gray60") +
    annotate("text", x=(PCA_loadings$PC2*8.75), #add the tissue names to it manually
             y=(PCA_loadings$PC3*8.75),
             label=PCA_loadings$Variables, size=4, color="gray60", fontface="bold") +
    theme(panel.background = element_rect(fill = "white", linewidth = 1))+
    theme_bw() +
  geom_point(data = expr_logic_PC2_3 %>% as_tibble(rownames = "name"),
                                aes(PC2, PC3, alpha = tau_interval, color = Family),
             size=3) +
  ggrepel::geom_text_repel(data = expr_logic_PC2_3 %>%
                           as_tibble(rownames = "name") %>%
                           filter(as.logical(in_ell == FALSE)),
                           aes(PC2, PC3, label=sub(".*\\|", "", heatmap_label),
                               alpha = tau_interval),
                         size=3, max.overlaps = 100,  min.segment.length = 0,
                         segment.curvature = -0.1) +
```

```
  scale_color_manual(values=c("#86C5D8", "#620093", "#E7C94C"))+
  labs(color = "Class", shape = "tau", alpha ="tau") +
  theme_bw()
```

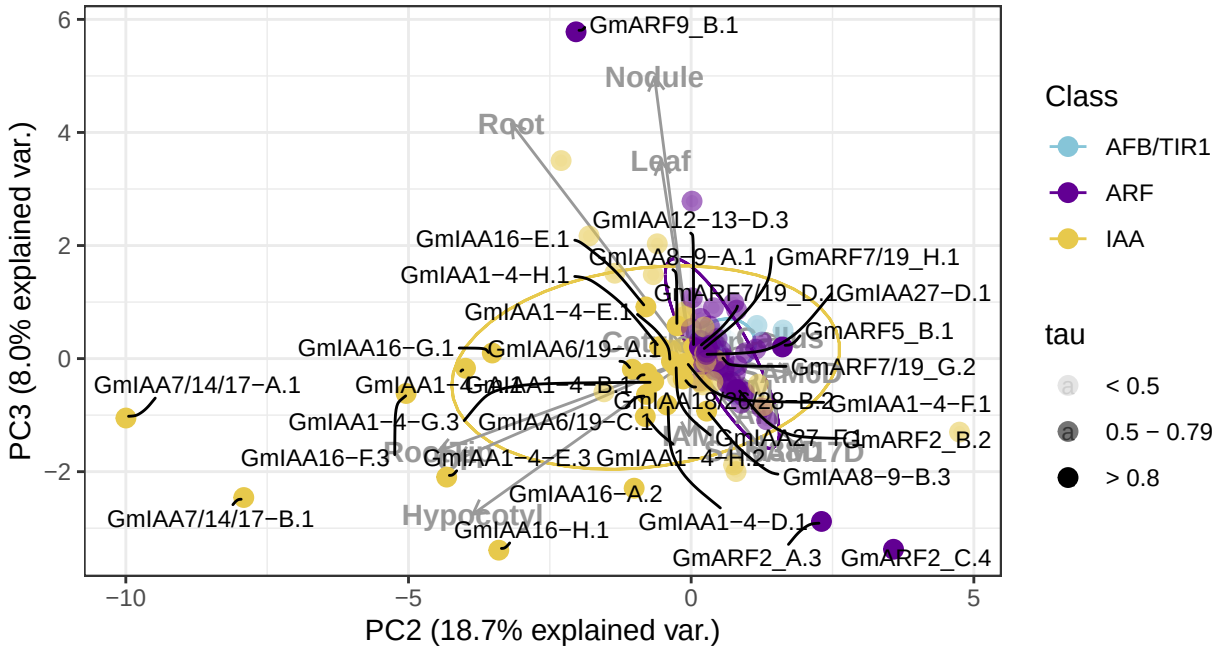
```
## Warning: Using alpha for a discrete variable is not advised.
```




```
# ggsave("20240117_PC2_3_allTissues.png", dpi = 1000, width = 10, height = 8)
# ggsave("20240117_PC2_3_allTissues.pdf", dpi = 1000, width = 10, height = 8)


# Now with tau lables
PC2_3all +
    geom_segment(PCA_loadings, mapping=aes(x=0, y=0, # Change the size of arrows
                                           xend=(PC2*8.75), yend=(PC3*8.75)),
                 arrow = arrow(length = unit(1/2, "picas")), color="gray60") +
    annotate("text", x=(PCA_loadings$PC2*8.75), #add the tissue names to it manually
             y=(PCA_loadings$PC3*8.75),
             label=PCA_loadings$Variables, size=4, color="gray60", fontface="bold") +
    theme(panel.background = element_rect(fill = "white", linewidth = 1))+
    theme_bw() +
  geom_point(data = expr_logic_PC2_3 %>% as_tibble(rownames = "name"),
                                  aes(PC2, PC3, alpha = tau_interval, color = Family),
             size=3) +
  ggrepel::geom_text_repel(data = expr_logic_PC2_3 %>%
                           as_tibble(rownames = "name") %>%
                           filter(as.logical(tau_interval == "> 0.8")),
                           aes(PC2, PC3, label=sub(".*\\|", "", heatmap_label),
                               alpha = tau_interval),
                         size=3, max.overlaps = 100,  min.segment.length = 0,
                         segment.curvature = -0.1) +

  scale_color_manual(values=c("#86C5D8", "#620093", "#E7C94C"))+
  labs(color = "Class", shape = "tau", alpha ="tau") +
```

```
  theme_bw()
```

```
## Warning: Using alpha for a discrete variable is not advised.
```



PC3 and PC4 for all 14 tissues

```
PC3_4all <- ggbiplot::ggbiplot(pca, obs.scale = 1, var.scale = .5, choices = c(3,4),
                               varname.adjust = 3, alpha = .1,
                              groups = Family, var.axes = F, varname.size = 2,
                    ellipse = TRUE,  circle = FALSE, ellipse.prob = .70)


# Extract components so we can select out
PC3_4all$data
```

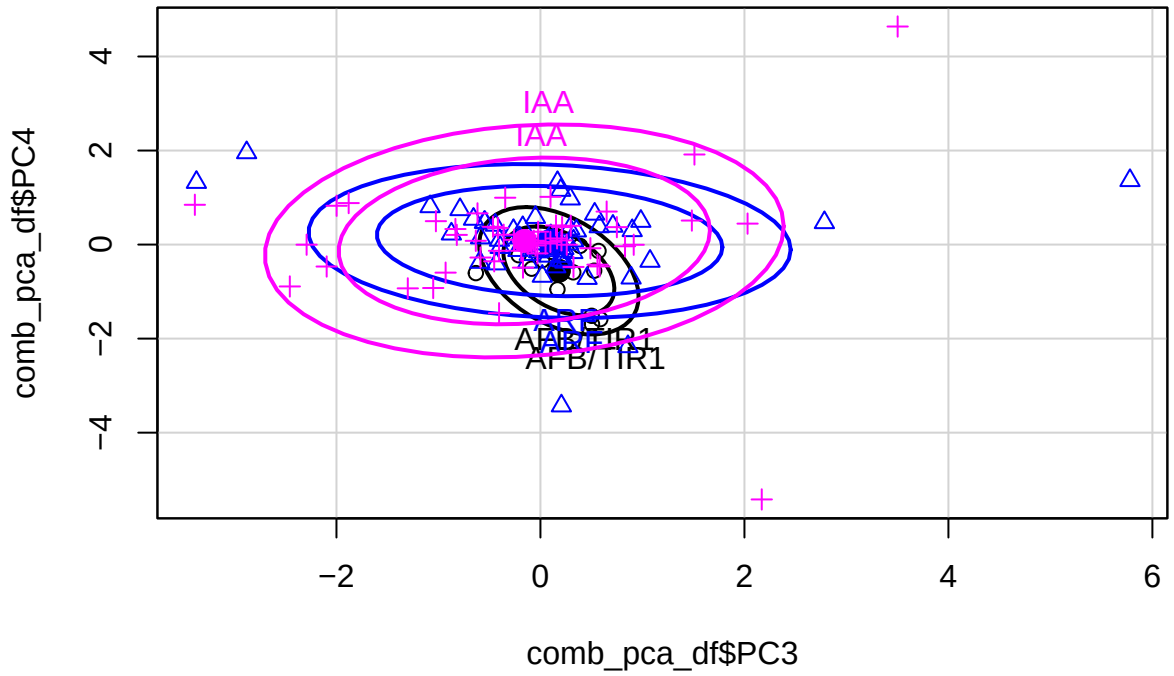
```
##             xvar         yvar   groups
## 1    0.192765997 -0.329157357      ARF
## 2    0.323910990 -0.475687016      IAA
## 3    0.829138289 -0.036476522      IAA
## 4   -0.929827851 -0.596814655      IAA
## 5    2.030765709  0.445820518      IAA
## 6   -0.543671987  0.467432769      ARF
## 7   -0.820157717  0.205971511      IAA
## 8    0.097838721  0.071591356 AFB/TIR1
## 9   -0.617236529  0.663393838      IAA
## 10   0.212488063  0.372205118      IAA
## 11  -1.025141950  0.497830190      IAA
## 12  -0.633363301 -0.606570751 AFB/TIR1
## 13   0.388439486 -0.027544084 AFB/TIR1
## 14   1.484374427  0.510884280      IAA
## 15  -1.880148999  0.882776840      IAA
## 16   0.213426698 -0.342539454      ARF
## 17  -1.082153419  0.807398759      ARF
```

```
## 18  -0.395001161 -0.059740809      ARF
## 19   0.080303779 -0.243991322      ARF
## 20   5.780070252  1.362986140      ARF
## 21   0.198771991  1.155416668      ARF
## 22  -0.085693178  0.034427672      IAA
## 23  -3.388444330  0.846071762      IAA
## 24   0.166835894 -0.952600938 AFB/TIR1
## 25   2.169830163 -5.419994579      IAA
## 26   1.074396091 -0.353111432      ARF
## 27   0.489833024 -0.078333073      IAA
## 28  -0.051671359 -0.075470037 AFB/TIR1
## 29   0.167127745  1.320222051      ARF
## 30  -0.548952095  0.475445406      ARF
## 31   0.157488574  0.046630592      ARF
## 32   2.784369433  0.468252980      ARF
## 33  -3.373028396  1.324379018      ARF
## 34   0.018029747 -0.672254946      ARF
## 35   0.162561278  0.130306707      IAA
## 36  -1.300612613 -0.930979975      IAA
## 37   0.569404529 -0.135589979 AFB/TIR1
## 38   0.292503137  0.971727378      ARF
## 39  -2.880669155  1.955575677      ARF
## 40  -0.633847587  0.080138588      IAA
## 41   0.141808591  0.005907838      ARF
## 42  -0.656456898  0.540261125      ARF
## 43   0.889037817 -0.709455025      ARF
## 44   0.286525824  0.333019125      ARF
## 45  -0.086074243 -0.512682037 AFB/TIR1
## 46   0.078045033 -0.027875326 AFB/TIR1
## 47   0.576287661  0.380679666      ARF
## 48   0.899632729  0.298409644      ARF
## 49   0.984274929  0.496243282      ARF
## 50  -0.873135543  0.226144958      ARF
## 51   0.140445647 -0.084054512      ARF
## 52   0.649133026  0.701903529      IAA
## 53  -0.125741994  0.095581276      IAA
## 54   0.016357767 -0.335554133 AFB/TIR1
## 55  -0.587549057  0.144105430      ARF
## 56   0.710504593  0.391861533      ARF
## 57  -0.360521593  0.209868199      IAA
## 58   0.257918902 -0.003108430      IAA
## 59  -1.998468838  0.825223890      IAA
## 60   0.150013982 -0.471664728      ARF
## 61   0.049754903  0.022310438      IAA
## 62  -0.345426570  0.996380369      IAA
## 63   0.580533544 -0.463754306      IAA
## 64   0.502244955 -1.514606539 AFB/TIR1
## 65   0.114997043 -0.155428110 AFB/TIR1
## 66  -0.214148722 -0.225376445 AFB/TIR1
## 67  -0.406399893 -0.132299390      IAA
## 68   0.914386871 -0.003733875      IAA
## 69   0.206015394  0.088858545      IAA
## 70   0.074614796 -0.126598662      ARF
## 71   1.509715283  1.915691304      IAA
```

```
## 72  -2.292586475 -0.002501458      IAA
## 73  -0.029598759 -0.187635626      IAA
## 74  -0.587753821 -0.275831866      IAA
## 75  -0.171191334 -0.490329128      IAA
## 76  -1.051443078 -0.924138217      IAA
## 77   0.531597276  0.648999766      ARF
## 78  -0.109188343 -0.208434682      ARF
## 79   0.317995304 -0.168331019      ARF
## 80  -0.594875791 -0.375545404      ARF
## 81   0.004808883  0.018108365      ARF
## 82   0.324427333  0.078558501      ARF
## 83  -0.173718424  0.362553950      ARF
## 84   0.857615531 -2.169356529      ARF
## 85  -0.146999936  0.007800111      ARF
## 86   0.092837656 -0.001694144      ARF
## 87   0.275671087  0.014778960      ARF
## 88   0.204583749 -0.247017881      ARF
## 89   0.749279943  0.370701122      IAA
## 90  -0.025807891 -0.090310742      ARF
## 91  -0.788482703  0.744876265      ARF
## 92  -0.264669941  0.300848696      ARF
## 93   0.352511287  0.291391237      ARF
## 94  -0.049101215  0.579170451      ARF
## 95  -0.833524403  0.330486790      IAA
## 96   0.559131560 -0.426860938      IAA
## 97  -0.022773811  0.273444399      IAA
## 98   0.152202391  0.406918506      IAA
## 99  -0.188391551 -0.132620836      IAA
## 100 -0.465096502  0.378210247      IAA
## 101 -0.417437561  0.354610124      IAA
## 102  0.239714138 -0.372805353      ARF
## 103 -0.433940124 -0.408984924      ARF
## 104  0.526862594 -0.556046556 AFB/TIR1
## 105  0.297046621  0.399860511      IAA
## 106 -0.401907770  0.093790074      ARF
## 107 -0.125386206 -0.371265410      ARF
## 108 -0.122534533  0.019319698      IAA
## 109  0.097778388  0.057486747      IAA
## 110 -0.453351398 -0.355495023      IAA
## 111 -0.442597992  0.413317924      ARF
## 112 -0.204069007  0.144534631      ARF
## 113  0.229584716 -0.081833467      ARF
## 114  0.219278723 -0.136465251      ARF
## 115  0.457407776 -0.717654679      ARF
## 116  0.101790408  0.189752408      ARF
## 117  0.505418632 -1.692904946 AFB/TIR1
## 118 -0.017733146 -0.152751925      ARF
## 119 -0.064799773  0.081077732      IAA
## 120  0.205522094 -3.429275402      ARF
## 121 -0.221521206 -0.123086248      ARF
## 122  0.113040052 -0.006051997      ARF
## 123  0.586856325 -1.585847922 AFB/TIR1
## 124 -0.048212877 -0.018020025      IAA
## 125 -2.096056941 -0.466548138      IAA
```

```
## 126  0.098701172  1.015184517      IAA
## 127  0.193201295  0.035834145      ARF
## 128  0.321861245 -0.590150626 AFB/TIR1
## 129 -0.405549799 -1.459565623      IAA
## 130  0.074216762  0.099160724      ARF
## 131 -2.457302433 -0.890649441      IAA
## 132 -0.258947266  0.101572514      IAA
## 133  3.502230742  4.637374786      IAA
```

```
build_PC3_4all <- ggplot_build(PC3_4all)$data
points_PC3_4all <- build_PC3_4all[[1]]

# co-ordinates of the ellipses
ell_points_PC3_4all <- car::dataEllipse(comb_pca_df$PC3,
                                comb_pca_df$PC4,
                                as.factor(comb_pca_df$Family), levels=c(.7, .9))
```




```
# add geom_point with ellipses point

ell_ARF_PC3_4all <- as.data.frame(ell_points_PC3_4all$ARF$`0.7`)

ell_IAA_PC3_4all <- as.data.frame(ell_points_PC3_4all$IAA$`0.7`)

ell_TIR_PC3_4all <- as.data.frame(ell_points_PC3_4all$`AFB/TIR1`$`0.7`)

# Find which points are outside (!) the ellipse, and add this to the data

dat_TIR_PC3_4all <- data.frame(
  points_PC3_4all[2:3],
  in.ell_TIR = as.logical(point.in.polygon(points_PC3_4all$x, points_PC3_4all$y, ell_TIR_PC3_4all$x, ell
)

dat_IAA_PC3_4all <- data.frame(
```

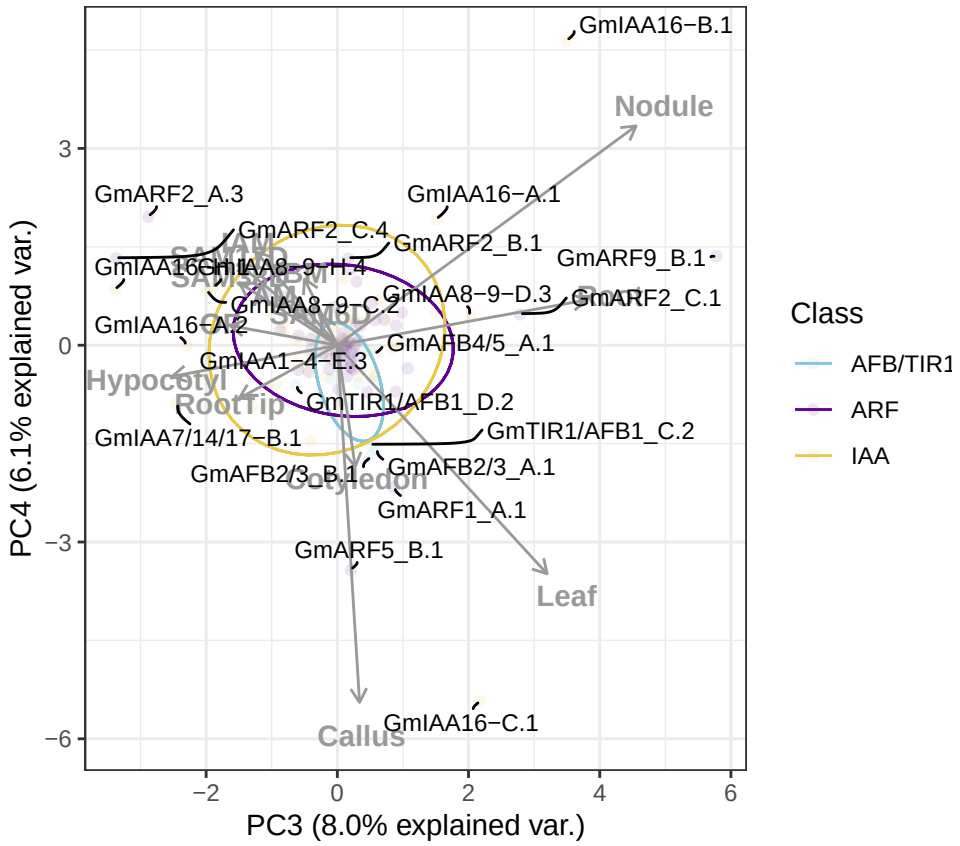
```
  points_PC3_4all[2:3],
  in.ell_IAA = as.logical(point.in.polygon(points_PC3_4all$x, points_PC3_4all$y, ell_IAA_PC3_4all$x, ell
)

dat_ARF_PC3_4all <- data.frame(
  points_PC3_4all[2:3],
  in.ell_ARF = as.logical(point.in.polygon(points_PC3_4all$x, points_PC3_4all$y, ell_ARF_PC3_4all$x, ell
)
# as.logical(point..) equals to TRUE indicated points are inside ellipses

# Combining data points for labeling
transcript_expr_logic_PC3_4all <- cbind(comb_pca_df, dat_TIR_PC3_4all, dat_IAA_PC3_4all, dat_ARF_PC3_4al


expr_logic_PC3_4all <- transcript_expr_logic_PC3_4all %>% mutate(., in_ell = case_when(Family=="ARF" &
                                          Family=="IAA" & `in.ell_IAA` == TRUE ~ "TRUE",
                                          Family=="AFB/TIR1" & `in.ell_TIR` == TRUE ~ "TRUE")) %>%
  mutate(in_ell = coalesce(in_ell, "FALSE"))

expr_logic_PC3_4all[which(expr_logic_PC3_4all$in_ell == FALSE),]
```

```
##         Transcript ID                    heatmap_label   Family     Class Clade
## 5   Glyma.01G098000.3     Glyma.01G098000|GmIAA8-9-D.3      IAA         A     I
## 12  Glyma.02G152800.2  Glyma.02G152800|GmTIR1/AFB1_D.2 AFB/TIR1 TIR1/AFB1     I
## 15  Glyma.02G218100.4     Glyma.02G218100|GmIAA8-9-H.4      IAA         A     I
## 20  Glyma.03G070500.1       Glyma.03G070500|GmARF9_B.1      ARF         B     I
## 23  Glyma.03G158700.1      Glyma.03G158700|GmIAA16-H.1      IAA         C   III
## 25  Glyma.03G247400.1      Glyma.03G247400|GmIAA16-C.1      IAA         C   III
## 29  Glyma.04G200600.1       Glyma.04G200600|GmARF2_B.1      ARF         B     I
## 32  Glyma.05G200800.1       Glyma.05G200800|GmARF2_C.1      ARF         B     I
## 33  Glyma.05G200800.4       Glyma.05G200800|GmARF2_C.4      ARF         B     I
## 37  Glyma.06G095400.1     Glyma.06G095400|GmAFB4/5_A.1 AFB/TIR1    AFB4/5    IV
## 39  Glyma.06G164900.3       Glyma.06G164900|GmARF2_A.3      ARF         B     I
## 59  Glyma.08G273500.2     Glyma.08G273500|GmIAA8-9-C.2      IAA         A     I
## 64  Glyma.10G021500.2  Glyma.10G021500|GmTIR1/AFB1_C.2 AFB/TIR1 TIR1/AFB1     I
## 71  Glyma.10G162400.1      Glyma.10G162400|GmIAA16-A.1      IAA         C   III
## 72  Glyma.10G162400.2      Glyma.10G162400|GmIAA16-A.2      IAA         C   III
## 84  Glyma.12G164100.1       Glyma.12G164100|GmARF1_A.1      ARF         B     I
## 117 Glyma.16G050500.1     Glyma.16G050500|GmAFB2/3_B.1 AFB/TIR1    AFB2/3    II
## 120 Glyma.17G256500.1       Glyma.17G256500|GmARF5_B.1      ARF         A    II
## 123 Glyma.19G100200.1     Glyma.19G100200|GmAFB2/3_A.1 AFB/TIR1    AFB2/3    II
## 125 Glyma.19G161000.3     Glyma.19G161000|GmIAA1-4-E.3      IAA         B    II
## 131 Glyma.20G210400.1 Glyma.20G210400|GmIAA7/14/17-B.1      IAA         C   III
## 133 Glyma.20G225000.1      Glyma.20G225000|GmIAA16-B.1      IAA         C   III
##             AM         OF        IAM       IBM    RootTip  Cotyledon  Hypocotyl
## 5    68.216252  51.350688  53.553189  96.27502  32.628226  78.301786 172.112449
## 12   22.069192  16.181094  21.004047  22.84902  12.164769  15.200032  20.202937
## 15   58.683503  91.015780  54.923525  50.03811  25.887821   8.588162  51.413131
## 20   14.963834   9.149436  10.014337  18.30301   6.361899   6.335987   4.159165
## 23   62.978354 188.282417  63.415971  50.27380  93.913232  11.797675 382.933284
## 25  123.752976 182.226043 143.672394 136.88284  68.294552 100.483878 215.724837
## 29   59.132821  34.776950  54.023677 104.01418   5.893899  15.036259  13.614824
## 32   23.736932   7.300337  13.387625  33.51611   4.197497  21.693739  20.950298
## 33  106.794815  41.848269  90.206750  89.14980   3.924654  25.580965  38.494199
```

```
## 37   16.624289  16.028732  15.517072  13.11757   3.675868   9.842971  17.361197
## 39   78.161251  91.333242 102.461124  95.84662   1.569549  29.653419  18.983798
## 59   60.235552  42.618679  62.597901  70.69411  15.151090  25.020319 166.602217
## 64   19.724328  16.170619  18.096732  18.50347  10.239295  14.506518  14.635121
## 71   60.897385  87.148645  58.213890  60.41856  39.516020  28.827155 119.352820
## 72   50.429036  62.648515  44.799041  49.04782  54.886130  14.549869 258.563372
## 84   29.907851  25.658848  21.848792  33.97809  12.158648  15.573356  22.344234
## 117  55.698420  35.061948  32.831068  58.58625  13.107811  27.899216  27.176499
## 120   7.845772   2.581028   3.311743  17.66248   0.302999   1.222648   1.046225
## 123  42.087683  43.031580  27.129734  42.68900  13.615904  12.545145  19.247151
## 125   4.478037 404.803343  44.077715  17.84655  72.730750  41.028328 177.214792
## 131   4.160975 265.136463  15.528614  13.54040 130.804062  41.664671 633.844120
## 133  83.900583 259.425512 113.461714 100.58810  53.025576  53.477803 102.248657
##          SAM6D     SAM17D     SAM38D    Callus       Leaf       Root    Nodule
## 5   101.538840 108.035003  57.568952  3.602672  72.181052  53.728970 22.341412
## 12   29.352679  33.917530  17.176745 17.838179   0.000000   0.000000  0.000000
## 15   79.581114  54.407495  42.568039  6.383128   0.000000   0.000000  0.000000
## 20   21.230577  13.709403   9.639602  2.603449  42.803542 126.598709 27.491901
## 23   20.163323  39.919056  73.535631  0.583159   6.250057  14.892682  2.016265
## 25   75.229555 108.926253  87.081914 65.420159 178.440543  98.055423  5.415049
## 29   40.036100  67.500984  33.565553  5.088175  22.636836  12.527492 10.758737
## 32   31.857638  31.063118  16.461606  4.368482  44.030052  25.338768 21.300657
## 33   75.216825 290.336670 100.526085 25.977418   0.000000   0.000000  0.000000
## 37   17.568729  22.163578  11.157726 12.838756   8.101368   7.946719  7.208545
## 39   38.298793 237.526834  76.153152  6.048278   3.674760   1.606524  0.000000
## 59   77.910628  90.255018  63.645599  6.462795  11.030950   6.618096  1.380951
## 64   12.243954  22.316362  15.443247 25.621399  26.106051   5.641934  3.205496
## 71   55.456636  78.452259  42.917887  2.247079  23.957562  65.117084 21.527670
## 72   52.919869  43.482386  62.182188 12.401001   7.425099  12.077243  0.000000
## 84   27.195518  37.963940  21.692550 51.460680  19.141297  21.871309  7.347936
## 117  82.133333  87.939197  38.297183 47.396469  32.138566  17.503017  8.945171
## 120  28.072725  24.136925   7.085973 69.182090   2.954026   4.491654  0.392173
## 123  60.919431  75.317808  31.290701 44.433814  27.410816  21.526502  7.313215
## 125   3.197441   2.314000   2.924909  0.843634   6.919914   6.338750  0.000000
## 131   1.661192   0.821472   1.444420  0.244966   0.972353  62.263900  0.495348
## 133  92.557209 144.677356  86.120903 10.711991  21.058177 104.233361 53.539749
##             PC1         PC2        PC3          PC4 in.ell_TIR in.ell_IAA
## 5    7.45961256 -0.60000202  2.0307657  0.445820518      FALSE      FALSE
## 12  -0.10245780  0.78870349 -0.6333633 -0.606570751      FALSE       TRUE
## 15   2.27672963  0.75044555 -1.8801490  0.882776840      FALSE      FALSE
## 20   1.41830620 -2.03622924  5.7800703  1.362986140      FALSE      FALSE
## 23   3.42533775 -3.40276087 -3.3884443  0.846071762      FALSE      FALSE
## 25  13.39251285 -1.80641239  2.1698302 -5.419994579      FALSE      FALSE
## 29   3.12051959  1.14297385  0.1671277  1.320222051      FALSE       TRUE
## 32   1.33799443  0.01555935  2.7843694  0.468252980      FALSE      FALSE
## 33   6.70264804  3.57934670 -3.3730284  1.324379018      FALSE      FALSE
## 37  -0.48862762  0.39871849  0.5694045 -0.135589979      FALSE       TRUE
## 39   5.27558405  2.30540719 -2.8806692  1.955575677      FALSE      FALSE
## 59   3.83439442  0.79344930 -1.9984688  0.825223890      FALSE      FALSE
## 64   0.07775833  0.52435782  0.5022450 -1.514606539      FALSE      FALSE
## 71   4.48509992 -1.35142128  1.5097153  1.915691304      FALSE      FALSE
## 72   2.77118641 -1.00810982 -2.2925865 -0.002501458      FALSE      FALSE
## 84   1.52682137  0.80415967  0.8576155 -2.169356529      FALSE      FALSE
## 117  3.95287191  1.62593170  0.5054186 -1.692904946      FALSE      FALSE
```

```
## 120 -0.27983068  1.61342692  0.2055221 -3.429275402      FALSE      FALSE
## 123  2.61523211  1.16403114  0.5868563 -1.585847922      FALSE      FALSE
## 125  0.71948030 -4.32442630 -2.0960569 -0.466548138      FALSE      FALSE
## 131  1.48447894 -7.91342992 -2.4573024 -0.890649441      FALSE      FALSE
## 133  9.87734076 -2.30022562  3.5022307  4.637374786      FALSE      FALSE
##     in.ell_ARF in_ell
## 5        FALSE  FALSE
## 12        TRUE  FALSE
## 15       FALSE  FALSE
## 20       FALSE  FALSE
## 23       FALSE  FALSE
## 25       FALSE  FALSE
## 29       FALSE  FALSE
## 32       FALSE  FALSE
## 33       FALSE  FALSE
## 37        TRUE  FALSE
## 39       FALSE  FALSE
## 59       FALSE  FALSE
## 64       FALSE  FALSE
## 71       FALSE  FALSE
## 72       FALSE  FALSE
## 84       FALSE  FALSE
## 117      FALSE  FALSE
## 120      FALSE  FALSE
## 123      FALSE  FALSE
## 125      FALSE  FALSE
## 131      FALSE  FALSE
## 133      FALSE  FALSE
```

```
PC3_4all +
    geom_segment(PCA_loadings, mapping=aes(x=0, y=0, # Change the size of arrows
                                           xend=(PC3*8), yend=(PC4*8)),
                 arrow = arrow(length = unit(1/2, "picas")), color="gray60") +
    annotate("text", x=(PCA_loadings$PC3*8.75), #add the tissue names to it manually
             y=(PCA_loadings$PC4*8.75),
             label=PCA_loadings$Variables, size=4, color="gray60", fontface="bold") +
    theme(panel.background = element_rect(fill = "white", linewidth = 1))+
    theme_bw()+
   scale_color_manual(values=c("#86C5D8", "#620093", "#E7C94C")) +
  ggrepel::geom_text_repel(data = expr_logic_PC3_4all %>%
                           as_tibble(rownames = "name") %>%
                           filter(as.logical(in_ell == FALSE)),
                           aes(PC3, PC4, label=sub(".*\\|", "", heatmap_label)),
                         size=3, max.overlaps = 100,  min.segment.length = 0,
                         segment.curvature = -0.1) +
   labs(color = "Class") +
  theme_bw()
```



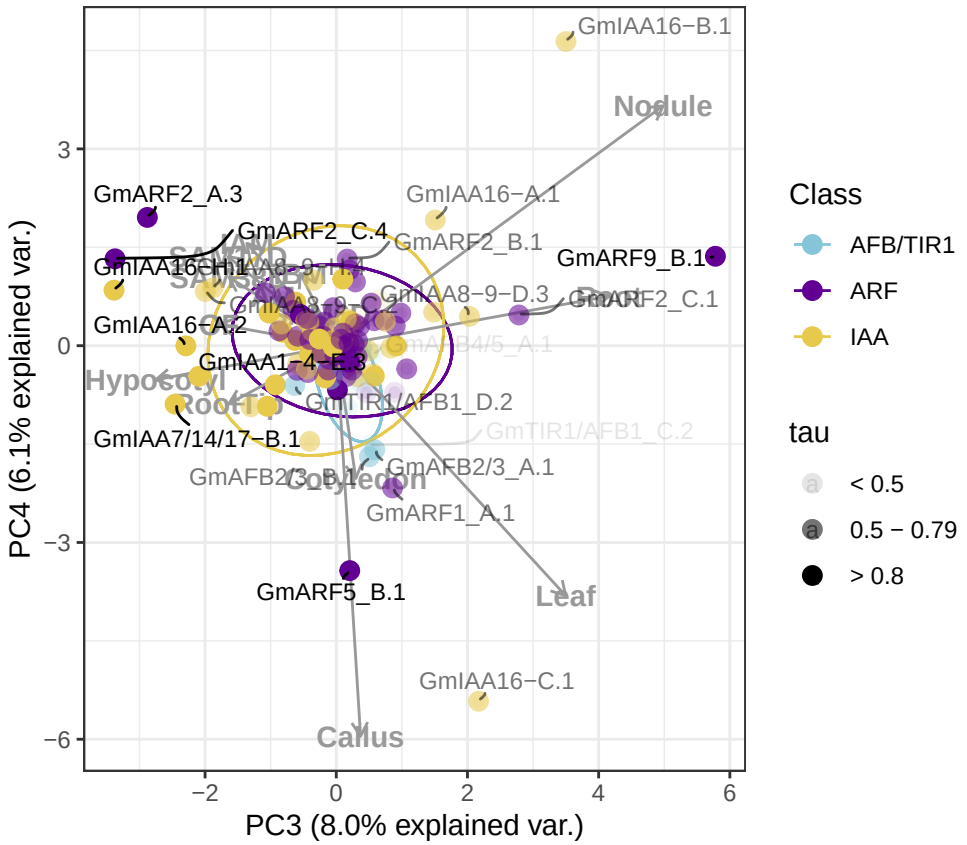

```
#ggsave("20230927_PC3_4_allTissues.png", dpi = 1000, width = 10, height = 8)
#ggsave("20230927_PC3_4_allTissues.pdf", dpi = 1000, width = 10, height = 8)
```

Lets now repeat with the tau values

```
# combine data frames expr_logic with tau values
expr_logic_PC3_4 <- cbind(expr_logic_PC3_4all, tau_df$tau)

# Define the intervals for expr_logic2$tau
expr_logic_PC3_4$tau_interval <- cut(expr_logic_PC3_4$tau,
                                     breaks = c(-Inf, 0.5, 0.8, Inf),
                                     labels = c("< 0.5", "0.5 - 0.79", "> 0.8"))



PC3_4all +
    geom_segment(PCA_loadings, mapping=aes(x=0, y=0, # Change the size of arrows
                                           xend=(PC3*8.75), yend=(PC4*8.75)),
                 arrow = arrow(length = unit(1/2, "picas")), color="gray60") +
    annotate("text", x=(PCA_loadings$PC3*8.75), #add the tissue names to it manually
             y=(PCA_loadings$PC4*8.75),
             label=PCA_loadings$Variables, size=4, color="gray60", fontface="bold") +
    theme(panel.background = element_rect(fill = "white", linewidth = 1))+
    theme_bw() +
  geom_point(data = expr_logic_PC3_4 %>% as_tibble(rownames = "name"),
                                     aes(PC3, PC4, alpha = tau_interval, color = Family),
             size=3) +
  ggrepel::geom_text_repel(data = expr_logic_PC3_4 %>%
```

```
                        as_tibble(rownames = "name") %>%
                        filter(as.logical(in_ell == FALSE)),
                        aes(PC3, PC4, label=sub(".*\\|", "", heatmap_label),
                            alpha = tau_interval),
                    size=3, max.overlaps = 100,  min.segment.length = 0,
                    segment.curvature = -0.1) +

  scale_color_manual(values=c("#86C5D8", "#620093", "#E7C94C"))+
  labs(color = "Class", shape = "tau", alpha ="tau") +
  theme_bw()
```

```
## Warning: Using alpha for a discrete variable is not advised.
```




```
#ggsave("20240117_PC3_4_allTissues.png", dpi = 1000, width = 10, height = 8)
#ggsave("20240117_PC3_4_allTissues.pdf", dpi = 1000, width = 10, height = 8)
```

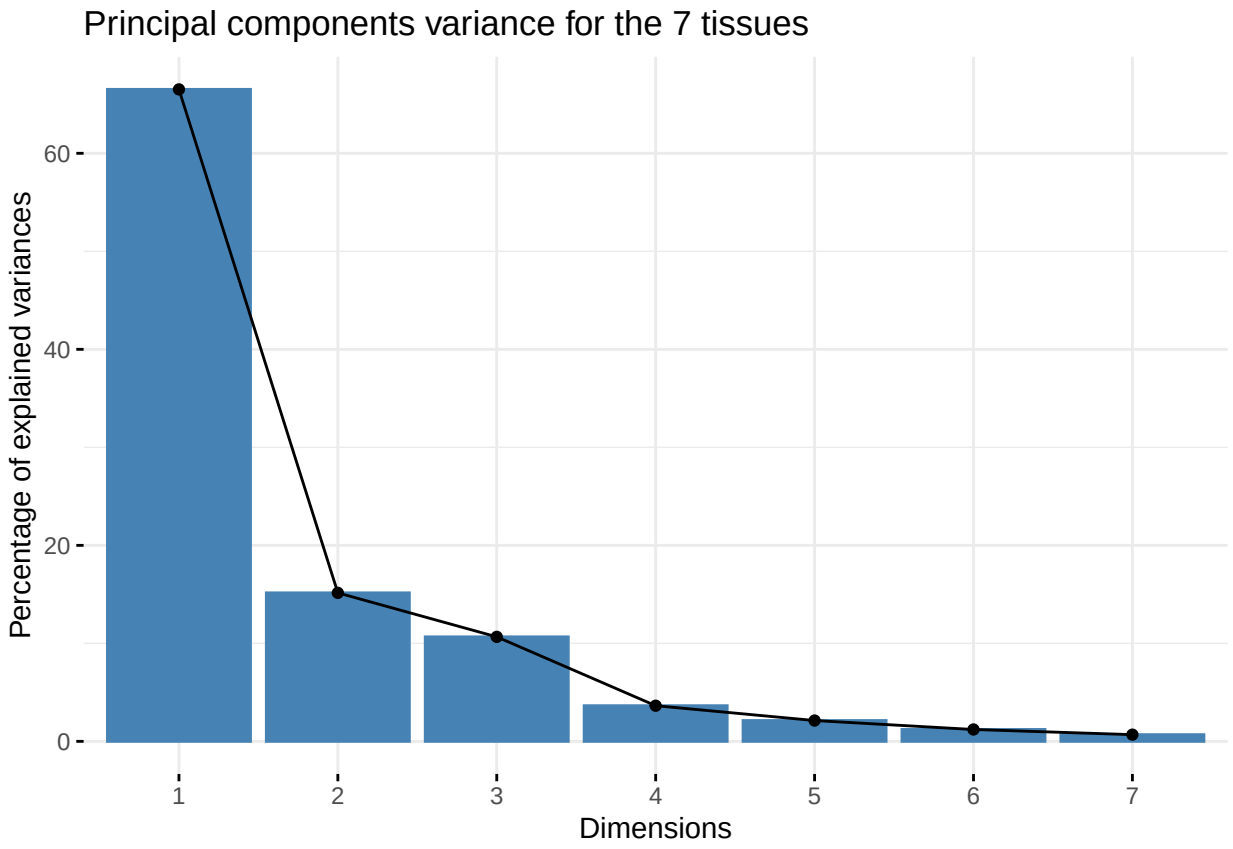
PCA with only tissues of interest

```
pca2 <- prcomp(pca_data[, -c(1:5, 7:10, 16, 18:19)],
               scale. = T, center = T)
summary(pca2)
```

```
## Importance of components:
##                           PC1    PC2    PC3     PC4     PC5     PC6     PC7
## Standard deviation     2.1579 1.0299 0.8640 0.50467 0.38576 0.29139 0.21916
## Proportion of Variance 0.6652 0.1515 0.1066 0.03638 0.02126 0.01213 0.00686
```

```
## Cumulative Proportion  0.6652 0.8167 0.9234 0.95975 0.98101 0.99314 1.00000
```

```
factoextra::fviz_eig(pca2, main = "Principal components variance for the 7 tissues")
```




```
# ggsave("20230927_PCscreePlot_7Tissues.png", dpi = 1000, width = 10, height = 8)
```

```
joint_pca_df <- cbind(pca_data, pca2$x[, 1:4])
head(joint_pca_df)
```

```
##       Transcript ID                 heatmap_label Family Class Clade        AM
## 1 Glyma.01G002100.1 Glyma.01G002100|GmARF7/19_F.1    ARF     A    II 10.166688
## 2 Glyma.01G019400.1  Glyma.01G019400|GmIAA8-9-B.1    IAA     A     I  4.023220
## 3 Glyma.01G019400.2  Glyma.01G019400|GmIAA8-9-B.2    IAA     A     I  3.945653
## 4 Glyma.01G019400.3  Glyma.01G019400|GmIAA8-9-B.3    IAA     A     I 22.514233
## 5 Glyma.01G098000.3  Glyma.01G098000|GmIAA8-9-D.3    IAA     A     I 68.216252
## 6 Glyma.01G103500.1    Glyma.01G103500|GmARF9_A.1    ARF     B     I 23.365836
##          OF       IAM       IBM   RootTip Cotyledon  Hypocotyl      SAM6D
## 1 11.093702  9.940766 11.778406  6.055558  6.293037  12.466244   8.948254
## 2  7.572444  0.000000  1.479181  3.679860  1.003818   8.866408   2.983280
## 3  0.000000  6.009114  7.650699  2.217478  2.098637  11.260883   9.399142
## 4 35.850834 20.735453 26.681081 16.122488  4.029864  88.717989  25.081359
## 5 51.350688 53.553189 96.275020 32.628226 78.301786 172.112449 101.538840
## 6  7.195187 14.135920 28.240802  4.918282  2.088168   2.353419  26.594544
##       SAM17D    SAM38D    Callus      Leaf      Root    Nodule        PC1
## 1   8.096833  5.745906  9.251965  4.882454  6.893253  1.926212 -1.0501429
## 2   7.873006  3.443632  6.039812 10.540945  5.246408  0.577947 -1.3485364
## 3   0.000000  2.491927  0.946290 11.828802 22.566715  1.890763 -1.2849928
## 4  26.514627 29.093086 17.719987  6.167892  0.000000  0.000000  0.1258125
```

```
## 5 108.035003 57.568952  3.602672 72.181052 53.728970 22.341412  6.2978744
## 6  33.652046 16.405528  1.091669  0.000000  0.000000  0.000000 -0.3140464
##           PC2        PC3         PC4
## 1  0.10515442  0.1357363 -0.16679473
## 2  0.03516704  0.3910160  0.02172556
## 3 -0.03259973  0.4674483 -0.06753696
## 4 -0.19841417 -0.6187942  0.41436903
## 5 -1.83724237  1.5164566 -1.17162708
## 6  0.76188043 -0.2984882  0.15505685
```

```
Family <- joint_pca_df$Family

# extract the loading so we can change the arrows more easily later while ploting
PCA_loadings2 <- data.frame(Variables=rownames(pca2$rotation), pca2$rotation)
```

```
# Plot
library(ggrepel)
PC1_2_7tissues <- ggbiplot::ggbiplot(pca2, obs.scale = 1, var.scale = .5, choices = c(1, 2),
                           varname.adjust = 3,alpha = 0.1,
                          groups = Family, var.axes = F, varname.size = 2,
                 ellipse = TRUE,  circle = FALSE, ellipse.prob = .70)
```

withdraw lables outside ellipses to label outliers (data points located outside the 70% ellipse interval)

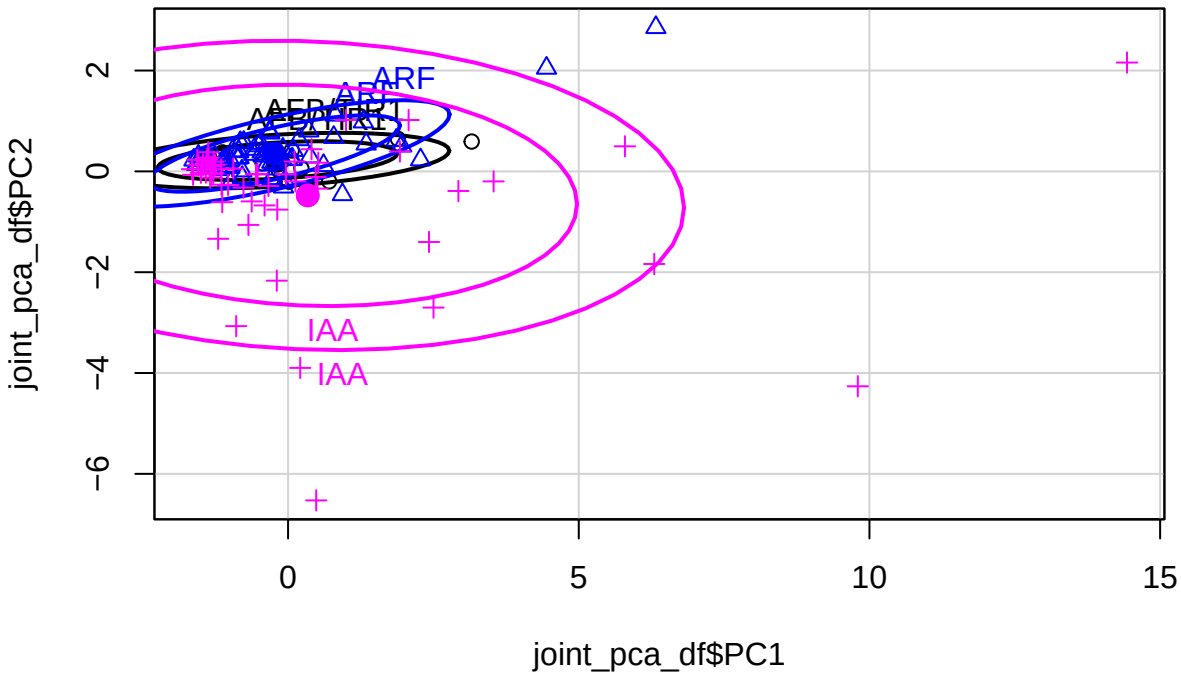
```
# Extract components so we can select out
PC1_2_7tissues$data
```

```
##            xvar        yvar   groups
## 1   -1.05014286  0.10515442      ARF
## 2   -1.34853637  0.03516704      IAA
## 3   -1.28499278 -0.03259973      IAA
## 4    0.12581250 -0.19841417      IAA
## 5    6.29787441 -1.83724237      IAA
## 6   -0.31404635  0.76188043      ARF
## 7    0.50539968 -0.34300647      IAA
## 8   -1.12869949  0.37674443 AFB/TIR1
## 9   -0.68221161 -1.06192064      IAA
## 10  -1.32267878 -0.05614018      IAA
## 11  -1.33942303 -0.12354575      IAA
## 12   0.06210206  0.41382610 AFB/TIR1
## 13  -1.32261153  0.16146909 AFB/TIR1
## 14   0.40306612  0.43976369      IAA
## 15   2.07329850  1.01893547      IAA
## 16  -0.05373907 -0.19219653      ARF
## 17   0.81044975  1.11700892      ARF
## 18  -0.78784060 -0.12083822      ARF
## 19  -0.24435712  0.14788376      ARF
## 20  -0.07853023 -0.30909424      ARF
## 21   1.29528965  0.98161007      ARF
## 22  -1.65539096  0.04406573      IAA
## 23   2.50142090 -2.70244068      IAA
## 24   0.70690012 -0.19818565 AFB/TIR1
## 25   9.79977762 -4.26352971      IAA
```

```
## 26  -0.99159124 -0.23057025      ARF
## 27  -0.03794509 -0.05712991      IAA
## 28  -0.35471399  0.37697535 AFB/TIR1
## 29   1.85976056  0.61742415      ARF
## 30  -0.31860769  0.79593666      ARF
## 31  -1.34000604  0.23346430      ARF
## 32   0.93454507 -0.45975448      ARF
## 33   6.32619943  2.85428889      ARF
## 34  -1.62029102  0.22580682      ARF
## 35  -0.52375557 -0.05145862      IAA
## 36  14.43049528  2.15899012      IAA
## 37  -0.43602126  0.15912727 AFB/TIR1
## 38   1.95569813  0.50580340      ARF
## 39   4.44562780  2.04813145      ARF
## 40  -1.20335584 -1.33745677      IAA
## 41  -0.10578553  0.24206119      ARF
## 42   0.35581526  0.80233487      ARF
## 43  -0.18778513  0.04913286      ARF
## 44  -0.08921436  0.44159097      ARF
## 45  -1.45080932  0.22207205 AFB/TIR1
## 46  -1.14906764  0.28259178 AFB/TIR1
## 47  -0.99845260  0.22602501      ARF
## 48   0.05595252  0.23517727      ARF
## 49   2.27842210  0.23014394      ARF
## 50   1.34307083  0.54381981      ARF
## 51  -1.55156504  0.16357844      ARF
## 52   0.51958352  0.17260396      IAA
## 53  -1.36883544  0.27331654      IAA
## 54  -1.38601667  0.12704175 AFB/TIR1
## 55  -0.47630116  0.55667830      ARF
## 56  -0.84258221  0.27501312      ARF
## 57  -1.56272647  0.22850724      IAA
## 58  -0.94247516  0.05578545      IAA
## 59   3.53507221 -0.19876183      IAA
## 60  -1.07587306  0.22563162      ARF
## 61  -1.46220344  0.13643709      IAA
## 62   0.47369476 -0.11275080      IAA
## 63  -0.18575199 -0.75842052      IAA
## 64   0.01141647 -0.20362431 AFB/TIR1
## 65  -1.42021154  0.20580527 AFB/TIR1
## 66  -0.17447944  0.17662980 AFB/TIR1
## 67  -1.14563503 -0.26383478      IAA
## 68  -1.49981620 -0.02775860      IAA
## 69  -1.54348979  0.12874603      IAA
## 70  -1.39507835  0.16168347      ARF
## 71   2.92945855 -0.38908091      IAA
## 72   2.42394340 -1.40080513      IAA
## 73  -1.15099418  0.04204640      IAA
## 74  -0.62584455 -0.59554073      IAA
## 75  -0.40410313 -0.67555222      IAA
## 76   0.20859279 -3.89840357      IAA
## 77  -0.84786884  0.39304286      ARF
## 78  -0.28107091 -0.14129573      ARF
## 79  -0.79489435  0.01134430      ARF
```

```
## 80  -0.42946116  0.30503427      ARF
## 81  -0.86565058  0.26459088      ARF
## 82  -1.57421557  0.13997225      ARF
## 83  -0.58850376  0.33976118      ARF
## 84   0.60982840  0.11755926      ARF
## 85  -0.95270455  0.41027661      ARF
## 86  -1.18400893  0.03642123      ARF
## 87  -1.35845402  0.18832650      ARF
## 88  -1.32330825  0.07623100      ARF
## 89  -1.32211008  0.09616199      IAA
## 90  -1.33857165  0.22631146      ARF
## 91   0.18759733  0.63062446      ARF
## 92   0.37773397  0.28877357      ARF
## 93  -0.28033129  0.18223673      ARF
## 94  -0.49772951  0.50936896      ARF
## 95   0.99582518  1.01924752      IAA
## 96  -0.55223302 -0.06070833      IAA
## 97  -1.37434561  0.37953170      IAA
## 98  -1.28152736  0.19095485      IAA
## 99  -1.13207935 -0.61127542      IAA
## 100 -1.25672736  0.12918250      IAA
## 101 -1.41443268 -0.02383471      IAA
## 102 -1.00237591  0.08053528      ARF
## 103  0.07271378  0.24466668      ARF
## 104 -0.16199629  0.02332871 AFB/TIR1
## 105  0.11133693  0.19479841      IAA
## 106  0.08787857  0.41799993      ARF
## 107 -0.76288272  0.57895723      ARF
## 108 -1.03198092 -0.27146457      IAA
## 109 -1.39231781  0.23943824      IAA
## 110  1.92371297  0.39438069      IAA
## 111 -0.58154082  0.43897284      ARF
## 112 -1.35296511  0.21805555      ARF
## 113 -1.39891628  0.16435444      ARF
## 114 -1.47279337  0.21819497      ARF
## 115 -0.37176747  0.12804991      ARF
## 116  0.78469153  0.67534859      ARF
## 117  3.15794243  0.59153383 AFB/TIR1
## 118 -1.10801710  0.21113642      ARF
## 119 -1.40473569  0.19705245      IAA
## 120 -0.82324378  0.57202079      ARF
## 121 -0.44612445  0.38556844      ARF
## 122 -1.54287005  0.30454921      ARF
## 123  1.88822437  0.64531129 AFB/TIR1
## 124 -1.63443659 -0.06758392      IAA
## 125 -0.19442276 -2.16841690      IAA
## 126 -0.89218238 -3.07007360      IAA
## 127 -1.14683163  0.16640283      ARF
## 128  0.22854712  0.06134928 AFB/TIR1
## 129 -0.33759214 -0.28989506      IAA
## 130 -1.26104706  0.28590134      ARF
## 131  0.48333718 -6.52671369      IAA
## 132 -0.76649931 -0.32242524      IAA
## 133  5.79424460  0.49697177      IAA
```

```
build_PC1_2_7tissues <- ggplot_build(PC1_2_7tissues)$data
points_PC1_2_7tissues <- build_PC1_2_7tissues[[1]]

# co-ordinates of the ellipses
ell_points_PC1_2_7tissues <- car::dataEllipse(joint_pca_df$PC1,
                                joint_pca_df$PC2,
                                as.factor(joint_pca_df$Family), levels=c(.7, .9))
```




```
# add geom_point with ellipses point

ell_ARF_PC1_2_7tissues <- as.data.frame(ell_points_PC1_2_7tissues$ARF$`0.7`)

ell_IAA_PC1_2_7tissues <- as.data.frame(ell_points_PC1_2_7tissues$IAA$`0.7`)

ell_TIR_PC1_2_7tissues <- as.data.frame(ell_points_PC1_2_7tissues$`AFB/TIR1`$`0.7`)

# Find which points are outside (!) the ellipse, and add this to the data
library(sp)
dat_TIR_PC1_2_7tissues <- data.frame(
  points_PC1_2_7tissues[2:3],
  in.ell_TIR = as.logical(point.in.polygon(points_PC1_2_7tissues$x, points_PC1_2_7tissues$y, ell_TIR_PC
)

dat_IAA_PC1_2_7tissues <- data.frame(
  points_PC1_2_7tissues[2:3],
  in.ell_IAA = as.logical(point.in.polygon(points_PC1_2_7tissues$x, points_PC1_2_7tissues$y, ell_IAA_PC
)

dat_ARF_PC1_2_7tissues <- data.frame(
  points_PC1_2_7tissues[2:3],
  in.ell_ARF = as.logical(point.in.polygon(points_PC1_2_7tissues$x, points_PC1_2_7tissues$y, ell_ARF_PC
)
```

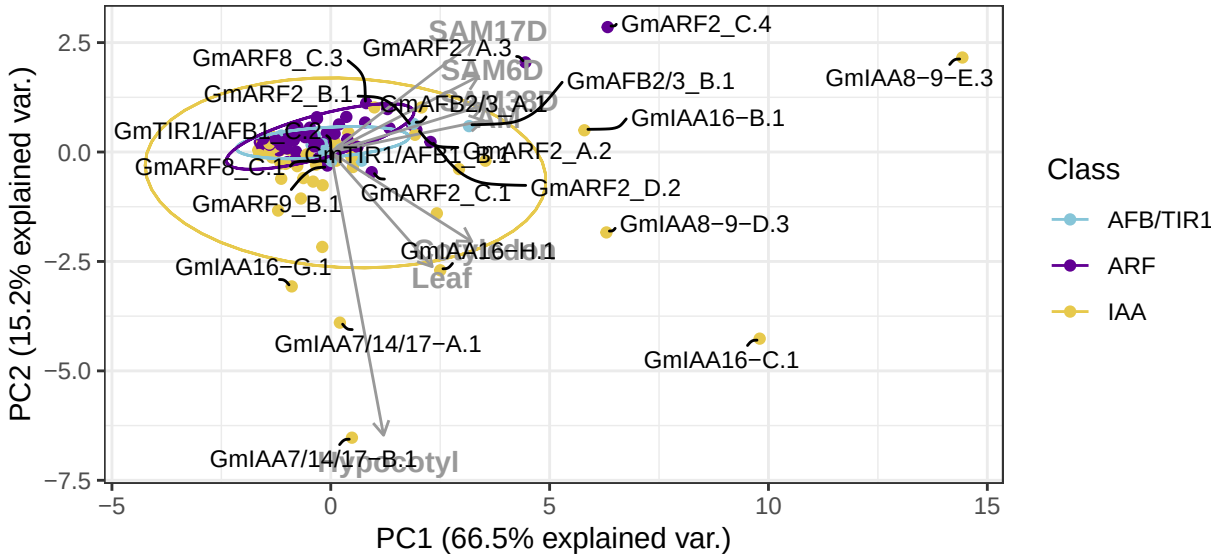
```r
# as.logical(point..) equals to TRUE indicated points are inside ellipses

# Combining data points for labeling
#Combine data points coordinates with PCs and expression data containing gene names will help us to hav
transcript_expr_logical_PC1_2_7tissues <- cbind(joint_pca_df, dat_TIR_PC1_2_7tissues, dat_IAA_PC1_2_7ti


expr_logical_PC1_2_7tissues <- transcript_expr_logical_PC1_2_7tissues %>% mutate(., in_ell = case_when(
                                          Family=="IAA" & `in.ell_IAA` == TRUE ~ "TRUE",
                                          Family=="AFB/TIR1" & `in.ell_TIR` == TRUE ~ "TRUE")) %>%
  mutate(in_ell = coalesce(in_ell, "FALSE"))

expr_logical_PC1_2_7tissues[which(expr_logical_PC1_2_7tissues$in_ell == FALSE),]
```

```
##         Transcript ID                    heatmap_label   Family     Class Clade
## 5   Glyma.01G098000.3     Glyma.01G098000|GmIAA8-9-D.3      IAA         A     I
## 16  Glyma.02G239600.1       Glyma.02G239600|GmARF8_C.1      ARF         A    II
## 17  Glyma.02G239600.3       Glyma.02G239600|GmARF8_C.3      ARF         A    II
## 20  Glyma.03G070500.1       Glyma.03G070500|GmARF9_B.1      ARF         B     I
## 23  Glyma.03G158700.1      Glyma.03G158700|GmIAA16-H.1      IAA         C   III
## 24  Glyma.03G209400.1  Glyma.03G209400|GmTIR1/AFB1_B.1 AFB/TIR1 TIR1/AFB1     I
## 25  Glyma.03G247400.1      Glyma.03G247400|GmIAA16-C.1      IAA         C   III
## 29  Glyma.04G200600.1       Glyma.04G200600|GmARF2_B.1      ARF         B     I
## 32  Glyma.05G200800.1       Glyma.05G200800|GmARF2_C.1      ARF         B     I
## 33  Glyma.05G200800.4       Glyma.05G200800|GmARF2_C.4      ARF         B     I
## 36  Glyma.06G091700.3     Glyma.06G091700|GmIAA8-9-E.3      IAA         A     I
## 38  Glyma.06G164900.2       Glyma.06G164900|GmARF2_A.2      ARF         B     I
## 39  Glyma.06G164900.3       Glyma.06G164900|GmARF2_A.3      ARF         B     I
## 49  Glyma.08G008100.2       Glyma.08G008100|GmARF2_D.2      ARF         B     I
## 64  Glyma.10G021500.2  Glyma.10G021500|GmTIR1/AFB1_C.2 AFB/TIR1 TIR1/AFB1     I
## 76  Glyma.10G180100.1 Glyma.10G180100|GmIAA7/14/17-A.1      IAA         C   III
## 117 Glyma.16G050500.1     Glyma.16G050500|GmAFB2/3_B.1 AFB/TIR1    AFB2/3    II
## 123 Glyma.19G100200.1     Glyma.19G100200|GmAFB2/3_A.1 AFB/TIR1    AFB2/3    II
## 126 Glyma.19G161100.1      Glyma.19G161100|GmIAA16-G.1      IAA         C   III
## 131 Glyma.20G210400.1 Glyma.20G210400|GmIAA7/14/17-B.1      IAA         C   III
## 133 Glyma.20G225000.1      Glyma.20G225000|GmIAA16-B.1      IAA         C   III
##             AM         OF        IAM        IBM    RootTip  Cotyledon
## 5    68.216252  51.350688  53.553189  96.275020  32.628226  78.301786
## 16   18.062407   8.153791  22.217284  25.144304   6.595787  17.054927
## 17   37.168779   5.439277  41.924483  51.089719   6.308382   0.000000
## 20   14.963834   9.149436  10.014337  18.303006   6.361899   6.335987
## 23   62.978354 188.282417  63.415971  50.273803  93.913232  11.797675
## 24   25.967032  26.377921  14.286116  20.719333   9.445604  22.549691
## 25  123.752976 182.226043 143.672394 136.882838  68.294552 100.483878
## 29   59.132821  34.776950  54.023677 104.014185   5.893899  15.036259
## 32   23.736932   7.300337  13.387625  33.516112   4.197497  21.693739
## 33  106.794815  41.848269  90.206750  89.149798   3.924654  25.580965
## 36  120.096829  73.349658  80.928913 112.799969  14.116324 123.198735
## 38   62.736767  31.362845  56.712867 104.850125   1.621706  14.695636
## 39   78.161251  91.333242 102.461124  95.846621   1.569549  29.653419
## 49   41.952212  22.684099  48.464588  54.600720   2.166574  14.976262
## 64   19.724328  16.170619  18.096732  18.503468  10.239295  14.506518
## 76    7.941917 536.895072   6.035997   7.033665 214.246604  30.729826
## 117  55.698420  35.061948  32.831068  58.586254  13.107811  27.899216
```

```
## 123  42.087683  43.031580  27.129734  42.689001  13.615904  12.545145
## 126   6.156592 108.404702  30.826540   8.523381  43.829775   4.519645
## 131   4.160975 265.136463  15.528614  13.540402 130.804062  41.664671
## 133  83.900583 259.425512 113.461714 100.588102  53.025576  53.477803
##      Hypocotyl      SAM6D     SAM17D     SAM38D    Callus       Leaf       Root
## 5   172.112449 101.538840 108.035003  57.568952  3.602672  72.181052  53.728970
## 16   16.326781  22.517324  12.659333  10.229414  2.716798  21.320212   7.310538
## 17   23.986700  52.872850  68.490459  26.447317  6.139986   0.000000   3.626608
## 20    4.159165  21.230577  13.709403   9.639602  2.603449  42.803542 126.598709
## 23  382.933284  20.163323  39.919056  73.535631  0.583159   6.250057  14.892682
## 24   20.867913  28.476275  25.128377  20.548182 11.542469  26.686917   5.465411
## 25  215.724837  75.229555 108.926253  87.081914 65.420159 178.440543  98.055423
## 29   13.614824  40.036100  67.500984  33.565553  5.088175  22.636836  12.527492
## 32   20.950298  31.857638  31.063118  16.461606  4.368482  44.030052  25.338768
## 33   38.494199  75.216825 290.336670 100.526085 25.977418   0.000000   0.000000
## 36  118.328990 229.139072 393.546142 181.391747 71.756006  45.788590  20.983248
## 38   13.618860  48.102339  51.456817  31.907033  8.665198  27.396630  14.501406
## 39   18.983798  38.298793 237.526834  76.153152  6.048278   3.674760   1.606524
## 49   26.934453  45.899996 104.119336  30.814124  3.509392  48.206033  26.688307
## 64   14.635121  12.243954  22.316362  15.443247 25.621399  26.106051   5.641934
## 76  350.297556   2.600212   4.426392   5.209396  2.744771  20.850299  94.160633
## 117  27.176499  82.133333  87.939197  38.297183 47.396469  32.138566  17.503017
## 123  19.247151  60.919431  75.317808  31.290701 44.433814  27.410816  21.526502
## 126 334.804651   0.817625   0.938549   2.175286  0.045598   1.253888  31.879730
## 131 633.844120   1.661192   0.821472   1.444420  0.244966   0.972353  62.263900
## 133 102.248657  92.557209 144.677356  86.120903 10.711991  21.058177 104.233361
##        Nodule         PC1        PC2        PC3         PC4 in.ell_TIR
## 5   22.341412  6.29787441 -1.8372424  1.5164566 -1.17162708      FALSE
## 16   0.657995 -0.05373907 -0.1921965  0.8084196 -0.30166116      FALSE
## 17   0.000000  0.81044975  1.1170089 -0.7546656  0.42743184      FALSE
## 20  27.491901 -0.07853023 -0.3090942  1.7082831  0.36298038      FALSE
## 23   2.016265  2.50142090 -2.7024407 -2.7850869  1.95425693      FALSE
## 24   1.007309  0.70690012 -0.1981856  0.8944149 -0.24184281      FALSE
## 25   5.415049  9.79977762 -4.2635297  5.7326293  1.19531413      FALSE
## 29  10.758737  1.85976056  0.6174242  0.3612860  0.76750320      FALSE
## 32  21.300657  0.93454507 -0.4597545  1.6269670 -0.09431274      FALSE
## 33   0.000000  6.32619943  2.8542889 -2.3102058  1.29013983      FALSE
## 36  22.794609 14.43049528  2.1589901 -1.5310904 -2.57494825      FALSE
## 38   9.673077  1.95569813  0.5058034  0.6227889  0.85277438      FALSE
## 39   0.000000  4.44562780  2.0481314 -1.4582800  0.62005161      FALSE
## 49   8.836310  2.27842210  0.2301439  1.2286204  0.63434759      FALSE
## 64   3.205496  0.01141647 -0.2036243  0.9369297  0.04368445      FALSE
## 76   5.269519  0.20859279 -3.8984036 -0.9846724 -0.43448129      FALSE
## 117  8.945171  3.15794243  0.5915338  0.5657640 -0.06986253      FALSE
## 123  7.313215  1.88822437  0.6453113  0.4537617  0.33399282      FALSE
## 126 15.878389 -0.89218238 -3.0700736 -1.9027957  0.34702454      FALSE
## 131  0.495348  0.48333718 -6.5267137 -3.4003668 -0.81527683      FALSE
## 133 53.539749  5.79424460  0.4969718 -0.8400912 -0.23361517      FALSE
##     in.ell_IAA in.ell_ARF in_ell
## 5        FALSE      FALSE  FALSE
## 16        TRUE      FALSE  FALSE
## 17        TRUE      FALSE  FALSE
## 20        TRUE      FALSE  FALSE
## 23       FALSE      FALSE  FALSE
```

```
## 24        TRUE      FALSE  FALSE
## 25       FALSE      FALSE  FALSE
## 29        TRUE      FALSE  FALSE
## 32        TRUE      FALSE  FALSE
## 33       FALSE      FALSE  FALSE
## 36       FALSE      FALSE  FALSE
## 38        TRUE      FALSE  FALSE
## 39       FALSE      FALSE  FALSE
## 49        TRUE      FALSE  FALSE
## 64        TRUE      FALSE  FALSE
## 76       FALSE      FALSE  FALSE
## 117       TRUE      FALSE  FALSE
## 123       TRUE      FALSE  FALSE
## 126      FALSE      FALSE  FALSE
## 131      FALSE      FALSE  FALSE
## 133      FALSE      FALSE  FALSE
```

```
ggbiplot::ggbiplot(pca2, obs.scale = 1, var.scale = .5, choices = c(1, 2),
                           varname.adjust = 3,
                          groups = Family, var.axes = F, varname.size = 2,
                 ellipse = TRUE,  circle = FALSE, ellipse.prob = .70) +
   geom_segment(PCA_loadings2, mapping=aes(x=0, y=0, # Change the size of arrows
                                           xend=(PC1*8), yend=(PC2*8)),
                arrow = arrow(length = unit(1/2, "picas")), color="gray60") +
   annotate("text", x=(PCA_loadings2$PC1*8.75), #add the tissue names to it manually
            y=(PCA_loadings2$PC2*8.75),
            label=PCA_loadings2$Variables, size=4, color="gray60", fontface="bold") +
   theme(panel.background = element_rect(fill = "white", linewidth = 1))+
   theme_bw()+
  scale_color_manual(values=c("#86C5D8", "#620093", "#E7C94C")) +
 ggrepel::geom_text_repel(data = expr_logical_PC1_2_7tissues %>%
                          as_tibble(rownames = "name") %>%
                          filter(as.logical(in_ell == FALSE)),
                          aes(PC1, PC2, label=sub(".*\\|", "", heatmap_label)),
                        size=3, max.overlaps = 100,  min.segment.length = 0,
                        segment.curvature = -0.1) +
  labs(color = "Class") +
 theme_bw()
```



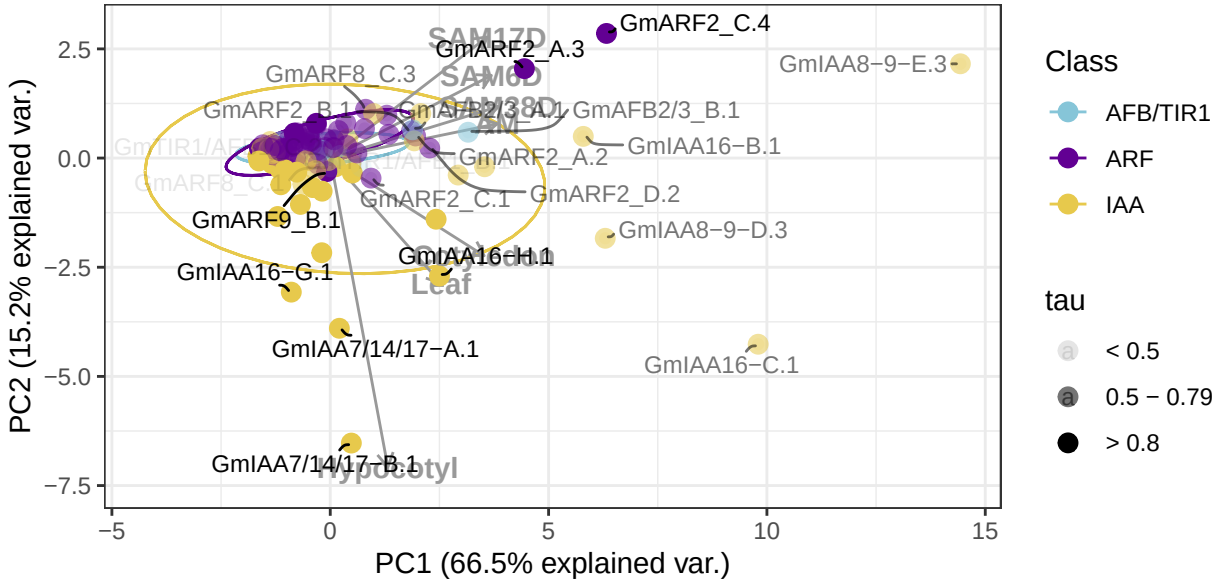

```
#ggsave("20230927_PC1_2_7Tissues.png", dpi = 1000, width = 10, height = 8)
#ggsave("20230927_PC1_2_7Tissues.pdf", dpi = 1000, width = 10, height = 8)
```

Lets now repeat with the tau values for 7 tissues kept

```
# combine data frames expr_logic with tau values
expr_logic_PC1_2_7tissues <- cbind(expr_logical_PC1_2_7tissues, tau_df$tau)

# Define the intervals for expr_logic2$tau
expr_logic_PC1_2_7tissues$tau_interval <- cut(expr_logic_PC1_2_7tissues$tau,
                                 breaks = c(-Inf, 0.5, 0.8, Inf),
                                 labels = c("< 0.5", "0.5 - 0.79", "> 0.8"))

PC1_2_7tissues +
    geom_segment(PCA_loadings2, mapping=aes(x=0, y=0, # Change the size of arrows
                                            xend=(PC1*8.75), yend=(PC2*8.75)),
                 arrow = arrow(length = unit(1/2, "picas")), color="gray60") +
    annotate("text", x=(PCA_loadings2$PC1*8.75), #add the tissue names to it manually
             y=(PCA_loadings2$PC2*8.75),
             label=PCA_loadings2$Variables, size=4, color="gray60", fontface="bold") +
    theme(panel.background = element_rect(fill = "white", linewidth = 1)) +

  geom_point(data = expr_logic_PC1_2_7tissues %>% as_tibble(rownames = "name"),
                                  aes(PC1, PC2, alpha = tau_interval, color = Family),
             size=3) +
  ggrepel::geom_text_repel(data = expr_logic_PC1_2_7tissues %>%
                           as_tibble(rownames = "name") %>%
                           filter(as.logical(in_ell == FALSE)),
                           aes(PC1, PC2, label=sub(".*\\|", "", heatmap_label),
                               alpha=tau_interval),
                         size=3, max.overlaps = 100,  min.segment.length = 0,
                         segment.curvature = -0.1) +

   scale_color_manual(values=c("#86C5D8", "#620093", "#E7C94C")) +
   labs(color = "Class") +
  labs(color = "Class", alpha ="tau") +
  ylim(-7.5, 3) +
```

```
  theme_bw()
```

```
## Warning: Using alpha for a discrete variable is not advised.
```




```
#ggsave("20240117_PC1_2_7Tissues.png", dpi = 1000, width = 10, height = 8)
#ggsave("20240117_PC1_2_7Tissues.pdf", dpi = 1000, width = 10, height = 8)
```

PC2 and PC3 outside ellipse labels

```
PC2_3_7tissues <- ggbiplot::ggbiplot(pca2, obs.scale = 1, var.scale = .5, choices = c(2, 3),
                            varname.adjust = 3, alpha = .1,
                           groups = Family, var.axes = F, varname.size = 2,
                  ellipse = TRUE,  circle = FALSE, ellipse.prob = .70)


# Extract components so we can select out
PC2_3_7tissues$data
```

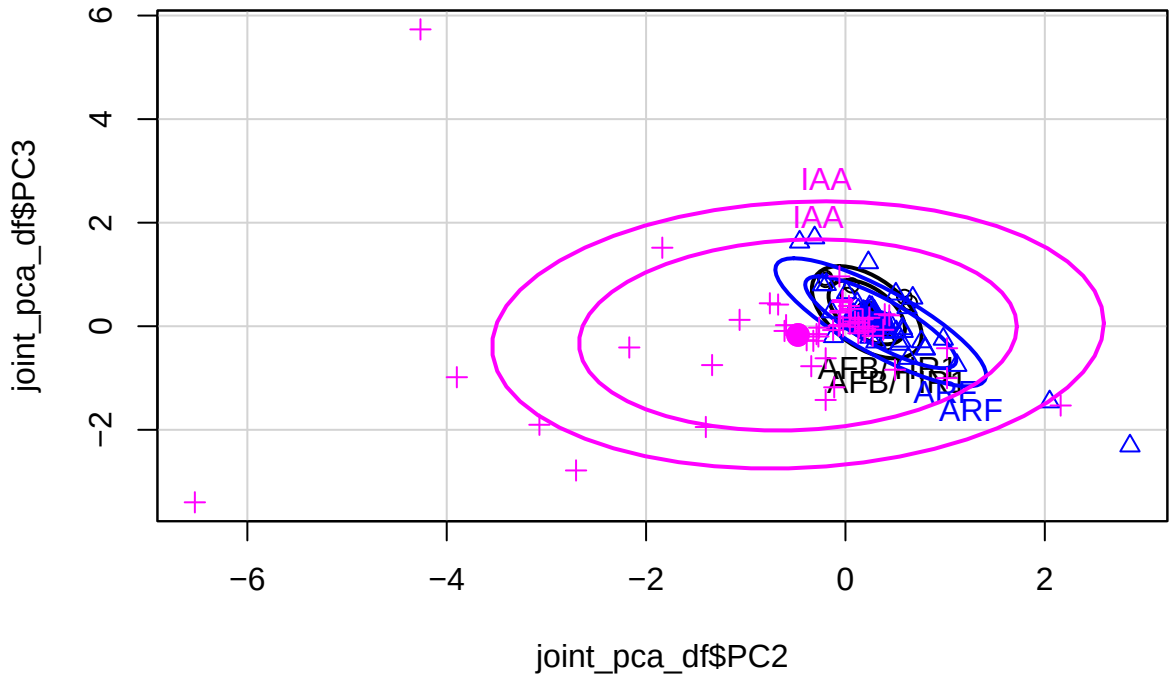
```
##            xvar         yvar   groups
## 1    0.10515442  0.135736327      ARF
## 2    0.03516704  0.391015981      IAA
## 3   -0.03259973  0.467448263      IAA
## 4   -0.19841417 -0.618794158      IAA
## 5   -1.83724237  1.516456612      IAA
## 6    0.76188043 -0.298488215      ARF
## 7   -0.34300647 -0.771942835      IAA
## 8    0.37674443 -0.019034277 AFB/TIR1
## 9   -1.06192064  0.123263847      IAA
## 10  -0.05614018  0.296718025      IAA
## 11  -0.12354575 -0.074216901      IAA
## 12   0.41382610 -0.307513791 AFB/TIR1
## 13   0.16146909  0.118751120 AFB/TIR1
## 14   0.43976369  0.210544795      IAA
## 15   1.01893547 -1.002175172      IAA
## 16  -0.19219653  0.808419635      ARF
```

```
## 17   1.11700892 -0.754665609      ARF
## 18  -0.12083822 -0.198053390      ARF
## 19   0.14788376  0.203702144      ARF
## 20  -0.30909424  1.708283097      ARF
## 21   0.98161007 -0.249254037      ARF
## 22   0.04406573  0.048238640      IAA
## 23  -2.70244068 -2.785086871      IAA
## 24  -0.19818565  0.894414856 AFB/TIR1
## 25  -4.26352971  5.732629313      IAA
## 26  -0.23057025  0.800391367      ARF
## 27  -0.05712991  0.024957986      IAA
## 28   0.37697535 -0.074310120 AFB/TIR1
## 29   0.61742415  0.361286016      ARF
## 30   0.79593666 -0.433613208      ARF
## 31   0.23346430  0.101766304      ARF
## 32  -0.45975448  1.626967022      ARF
## 33   2.85428889 -2.310205824      ARF
## 34   0.22580682  0.024556471      ARF
## 35  -0.05145862  0.264364967      IAA
## 36   2.15899012 -1.531090403      IAA
## 37   0.15912727  0.141906335 AFB/TIR1
## 38   0.50580340  0.622788860      ARF
## 39   2.04813145 -1.458279986      ARF
## 40  -1.33745677 -0.750021960      IAA
## 41   0.24206119  0.377262769      ARF
## 42   0.80233487 -0.439075999      ARF
## 43   0.04913286  0.051104600      ARF
## 44   0.44159097  0.071044809      ARF
## 45   0.22207205 -0.047741305 AFB/TIR1
## 46   0.28259178 -0.031576561 AFB/TIR1
## 47   0.22602501  0.161153854      ARF
## 48   0.23517727  0.291896516      ARF
## 49   0.23014394  1.228620351      ARF
## 50   0.54381981 -0.346616692      ARF
## 51   0.16357844  0.061513280      ARF
## 52   0.17260396  0.134356933      IAA
## 53   0.27331654 -0.185967868      IAA
## 54   0.12704175  0.051140263 AFB/TIR1
## 55   0.55667830 -0.286010626      ARF
## 56   0.27501312  0.222705920      ARF
## 57   0.22850724 -0.007314077      IAA
## 58   0.05578545  0.335059268      IAA
## 59  -0.19876183 -1.424143572      IAA
## 60   0.22563162  0.022943597      ARF
## 61   0.13643709 -0.009929315      IAA
## 62  -0.11275080 -1.179610247      IAA
## 63  -0.75842052  0.443021793      IAA
## 64  -0.20362431  0.936929695 AFB/TIR1
## 65   0.20580527  0.214269342 AFB/TIR1
## 66   0.17662980  0.040059182 AFB/TIR1
## 67  -0.26383478 -0.027750940      IAA
## 68  -0.02775860  0.504784255      IAA
## 69   0.12874603  0.067089129      IAA
## 70   0.16168347  0.144718515      ARF
```

```
## 71  -0.38908091 -0.270753178      IAA
## 72  -1.40080513 -1.946238594      IAA
## 73   0.04204640  0.165611508      IAA
## 74  -0.59554073  0.021079016      IAA
## 75  -0.67555222  0.418826607      IAA
## 76  -3.89840357 -0.984672374      IAA
## 77   0.39304286 -0.039468673      ARF
## 78  -0.14129573  0.280245925      ARF
## 79   0.01134430  0.650288391      ARF
## 80   0.30503427 -0.302086741      ARF
## 81   0.26459088  0.360448967      ARF
## 82   0.13997225  0.032561259      ARF
## 83   0.33976118  0.035765053      ARF
## 84   0.11755926  0.440653982      ARF
## 85   0.41027661 -0.100032400      ARF
## 86   0.03642123  0.131115667      ARF
## 87   0.18832650  0.112977921      ARF
## 88   0.07623100  0.060351273      ARF
## 89   0.09616199  0.093041365      IAA
## 90   0.22631146  0.075836951      ARF
## 91   0.63062446 -0.618197885      ARF
## 92   0.28877357  0.291349997      ARF
## 93   0.18223673  0.286799671      ARF
## 94   0.50936896 -0.107640571      ARF
## 95   1.01924752 -0.425150473      IAA
## 96  -0.06070833  0.962239600      IAA
## 97   0.37953170 -0.067668928      IAA
## 98   0.19095485 -0.106174638      IAA
## 99  -0.61127542 -0.090989128      IAA
## 100  0.12918250 -0.136450892      IAA
## 101 -0.02383471 -0.002678882      IAA
## 102  0.08053528  0.228044041      ARF
## 103  0.24466668 -0.211148378      ARF
## 104  0.02332871  0.603415593 AFB/TIR1
## 105  0.19479841 -0.088816512      IAA
## 106  0.41799993  0.024403946      ARF
## 107  0.57895723 -0.099986924      ARF
## 108 -0.27146457 -0.198514723      IAA
## 109  0.23943824  0.163541169      IAA
## 110  0.39438069  0.232333327      IAA
## 111  0.43897284 -0.116129991      ARF
## 112  0.21805555 -0.051162129      ARF
## 113  0.16435444  0.183674413      ARF
## 114  0.21819497  0.118174410      ARF
## 115  0.12804991  0.315839653      ARF
## 116  0.67534859  0.544740295      ARF
## 117  0.59153383  0.565764005 AFB/TIR1
## 118  0.21113642  0.022927865      ARF
## 119  0.19705245 -0.047540345      IAA
## 120  0.57202079 -0.045815482      ARF
## 121  0.38556844 -0.100377639      ARF
## 122  0.30454921 -0.025405134      ARF
## 123  0.64531129  0.453761721 AFB/TIR1
## 124 -0.06758392  0.007290830      IAA
```

```
## 125 -2.16841690 -0.410351711      IAA
## 126 -3.07007360 -1.902795711      IAA
## 127  0.16640283  0.079576472      ARF
## 128  0.06134928  0.790073247 AFB/TIR1
## 129 -0.28989506 -0.151673820      IAA
## 130  0.28590134  0.055831155      ARF
## 131 -6.52671369 -3.400366836      IAA
## 132 -0.32242524 -0.282003528      IAA
## 133  0.49697177 -0.840091171      IAA
```

```
build_PC2_3_7tissues <- ggplot_build(PC2_3_7tissues)$data
points_PC2_3_7tissues <- build_PC2_3_7tissues[[1]]

# co-ordinates of the ellipses
ell_points_PC2_3_7tissues <- car::dataEllipse(joint_pca_df$PC2,
                                joint_pca_df$PC3,
                                as.factor(joint_pca_df$Family), levels=c(.7, .9))
```




```
# add geom_point with ellipses point

ell_ARF_PC2_3_7tissues <- as.data.frame(ell_points_PC2_3_7tissues$ARF$`0.7`)

ell_IAA_PC2_3_7tissues <- as.data.frame(ell_points_PC2_3_7tissues$IAA$`0.7`)

ell_TIR_PC2_3_7tissues <- as.data.frame(ell_points_PC2_3_7tissues$`AFB/TIR1`$`0.7`)

# Find which points are outside (!) the ellipse, and add this to the data

dat_TIR_PC2_3_7tissues <- data.frame(
  points_PC2_3_7tissues[2:3],
  in.ell_TIR = as.logical(point.in.polygon(points_PC2_3_7tissues$x, points_PC2_3_7tissues$y, ell_TIR_PC
)
```

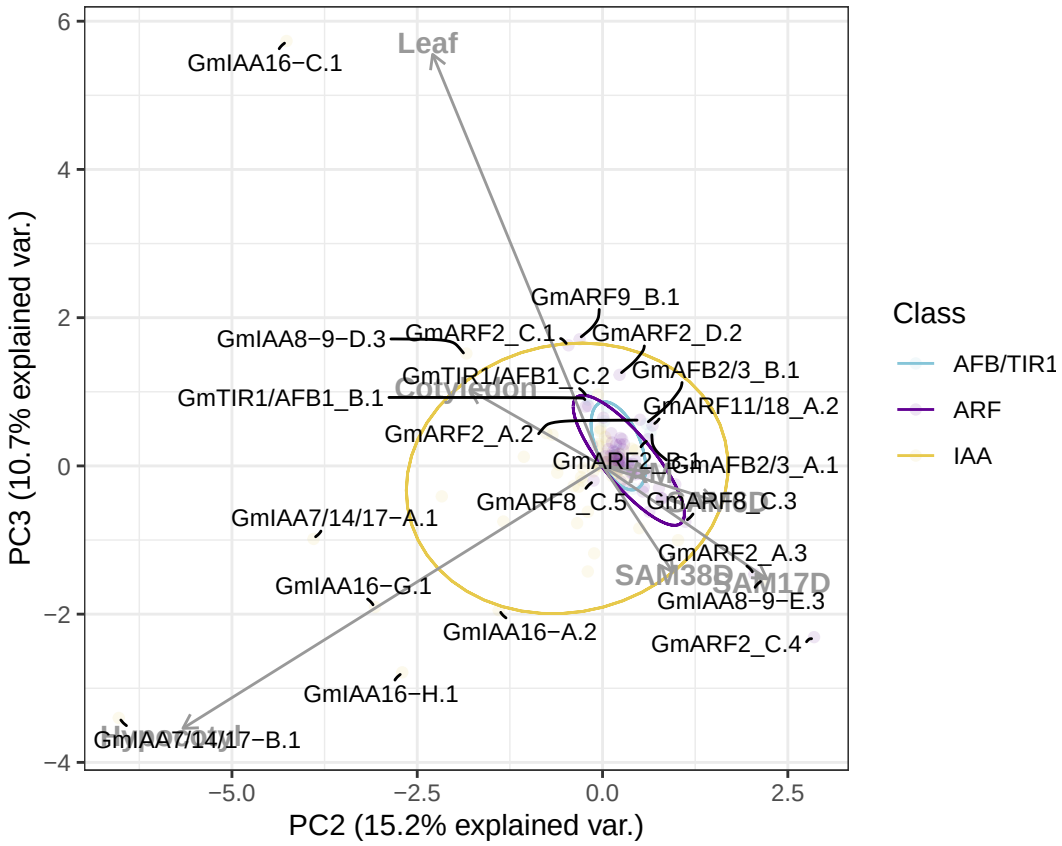
```r
dat_IAA_PC2_3_7tissues <- data.frame(
  points_PC2_3_7tissues[2:3],
  in.ell_IAA = as.logical(point.in.polygon(points_PC2_3_7tissues$x, points_PC2_3_7tissues$y, ell_IAA_PC2
)

dat_ARF_PC2_3_7tissues <- data.frame(
  points_PC2_3_7tissues[2:3],
  in.ell_ARF = as.logical(point.in.polygon(points_PC2_3_7tissues$x, points_PC2_3_7tissues$y, ell_ARF_PC2
)
# as.logical(point..) equals to TRUE indicated points are inside ellipses

# Combining data points for labeling
transcript_expr_log_PC2_3_7tissues <- cbind(joint_pca_df, dat_TIR_PC2_3_7tissues, dat_IAA_PC2_3_7tissues


expr_logical_PC2_3_7tissues <- transcript_expr_log_PC2_3_7tissues %>% mutate(., in_ell = case_when(Famil
                                          Family=="IAA" & `in.ell_IAA` == TRUE ~ "TRUE",
                                          Family=="AFB/TIR1" & `in.ell_TIR` == TRUE ~ "TRUE")) %>%
  mutate(in_ell = coalesce(in_ell, "FALSE"))

expr_logical_PC2_3_7tissues[which(expr_logical_PC2_3_7tissues$in_ell == FALSE),]
```

```
##         Transcript ID                    heatmap_label   Family     Class Clade
## 5   Glyma.01G098000.3     Glyma.01G098000|GmIAA8-9-D.3      IAA         A     I
## 17  Glyma.02G239600.3       Glyma.02G239600|GmARF8_C.3      ARF         A    II
## 18  Glyma.02G239600.5       Glyma.02G239600|GmARF8_C.5      ARF         A    II
## 20  Glyma.03G070500.1       Glyma.03G070500|GmARF9_B.1      ARF         B     I
## 23  Glyma.03G158700.1      Glyma.03G158700|GmIAA16-H.1      IAA         C   III
## 24  Glyma.03G209400.1  Glyma.03G209400|GmTIR1/AFB1_B.1 AFB/TIR1 TIR1/AFB1     I
## 25  Glyma.03G247400.1      Glyma.03G247400|GmIAA16-C.1      IAA         C   III
## 29  Glyma.04G200600.1       Glyma.04G200600|GmARF2_B.1      ARF         B     I
## 32  Glyma.05G200800.1       Glyma.05G200800|GmARF2_C.1      ARF         B     I
## 33  Glyma.05G200800.4       Glyma.05G200800|GmARF2_C.4      ARF         B     I
## 36  Glyma.06G091700.3     Glyma.06G091700|GmIAA8-9-E.3      IAA         A     I
## 38  Glyma.06G164900.2       Glyma.06G164900|GmARF2_A.2      ARF         B     I
## 39  Glyma.06G164900.3       Glyma.06G164900|GmARF2_A.3      ARF         B     I
## 49  Glyma.08G008100.2       Glyma.08G008100|GmARF2_D.2      ARF         B     I
## 64  Glyma.10G021500.2  Glyma.10G021500|GmTIR1/AFB1_C.2 AFB/TIR1 TIR1/AFB1     I
## 72  Glyma.10G162400.2      Glyma.10G162400|GmIAA16-A.2      IAA         C   III
## 76  Glyma.10G180100.1 Glyma.10G180100|GmIAA7/14/17-A.1      IAA         C   III
## 116 Glyma.16G023600.2   Glyma.16G023600|GmARF11/18_A.2      ARF         B     I
## 117 Glyma.16G050500.1     Glyma.16G050500|GmAFB2/3_B.1 AFB/TIR1    AFB2/3    II
## 123 Glyma.19G100200.1     Glyma.19G100200|GmAFB2/3_A.1 AFB/TIR1    AFB2/3    II
## 126 Glyma.19G161100.1      Glyma.19G161100|GmIAA16-G.1      IAA         C   III
## 131 Glyma.20G210400.1 Glyma.20G210400|GmIAA7/14/17-B.1      IAA         C   III
##             AM         OF        IAM        IBM    RootTip  Cotyledon
## 5    68.216252  51.350688  53.553189  96.275020  32.628226  78.301786
## 17   37.168779   5.439277  41.924483  51.089719   6.308382   0.000000
## 18   13.757865  13.859804   8.771196   7.354321   6.099923  14.166313
## 20   14.963834   9.149436  10.014337  18.303006   6.361899   6.335987
## 23   62.978354 188.282417  63.415971  50.273803  93.913232  11.797675
## 24   25.967032  26.377921  14.286116  20.719333   9.445604  22.549691
## 25  123.752976 182.226043 143.672394 136.882838  68.294552 100.483878
## 29   59.132821  34.776950  54.023677 104.014185   5.893899  15.036259
```

```
## 32   23.736932   7.300337  13.387625  33.516112   4.197497  21.693739
## 33  106.794815  41.848269  90.206750  89.149798   3.924654  25.580965
## 36  120.096829  73.349658  80.928913 112.799969  14.116324 123.198735
## 38   62.736767  31.362845  56.712867 104.850125   1.621706  14.695636
## 39   78.161251  91.333242 102.461124  95.846621   1.569549  29.653419
## 49   41.952212  22.684099  48.464588  54.600720   2.166574  14.976262
## 64   19.724328  16.170619  18.096732  18.503468  10.239295  14.506518
## 72   50.429036  62.648515  44.799041  49.047824  54.886130  14.549869
## 76    7.941917 536.895072   6.035997   7.033665 214.246604  30.729826
## 116  21.273921   9.543860  18.469447  22.065738   1.482088   5.689943
## 117  55.698420  35.061948  32.831068  58.586254  13.107811  27.899216
## 123  42.087683  43.031580  27.129734  42.689001  13.615904  12.545145
## 126   6.156592 108.404702  30.826540   8.523381  43.829775   4.519645
## 131   4.160975 265.136463  15.528614  13.540402 130.804062  41.664671
##      Hypocotyl      SAM6D     SAM17D     SAM38D    Callus       Leaf       Root
## 5   172.112449 101.538840 108.035003  57.568952  3.602672  72.181052  53.728970
## 17   23.986700  52.872850  68.490459  26.447317  6.139986   0.000000   3.626608
## 18   33.826894   0.000000  11.829332  13.561473  1.551810   0.000000   0.000000
## 20    4.159165  21.230577  13.709403   9.639602  2.603449  42.803542 126.598709
## 23  382.933284  20.163323  39.919056  73.535631  0.583159   6.250057  14.892682
## 24   20.867913  28.476275  25.128377  20.548182 11.542469  26.686917   5.465411
## 25  215.724837  75.229555 108.926253  87.081914 65.420159 178.440543  98.055423
## 29   13.614824  40.036100  67.500984  33.565553  5.088175  22.636836  12.527492
## 32   20.950298  31.857638  31.063118  16.461606  4.368482  44.030052  25.338768
## 33   38.494199  75.216825 290.336670 100.526085 25.977418   0.000000   0.000000
## 36  118.328990 229.139072 393.546142 181.391747 71.756006  45.788590  20.983248
## 38   13.618860  48.102339  51.456817  31.907033  8.665198  27.396630  14.501406
## 39   18.983798  38.298793 237.526834  76.153152  6.048278   3.674760   1.606524
## 49   26.934453  45.899996 104.119336  30.814124  3.509392  48.206033  26.688307
## 64   14.635121  12.243954  22.316362  15.443247 25.621399  26.106051   5.641934
## 72  258.563372  52.919869  43.482386  62.182188 12.401001   7.425099  12.077243
## 76  350.297556   2.600212   4.426392   5.209396  2.744771  20.850299  94.160633
## 116   0.823623  51.901613  58.246322  22.000399  0.000000  23.922628   7.371101
## 117  27.176499  82.133333  87.939197  38.297183 47.396469  32.138566  17.503017
## 123  19.247151  60.919431  75.317808  31.290701 44.433814  27.410816  21.526502
## 126 334.804651   0.817625   0.938549   2.175286  0.045598   1.253888  31.879730
## 131 633.844120   1.661192   0.821472   1.444420  0.244966   0.972353  62.263900
##        Nodule         PC1        PC2        PC3         PC4 in.ell_TIR
## 5   22.341412  6.29787441 -1.8372424  1.5164566 -1.17162708      FALSE
## 17   0.000000  0.81044975  1.1170089 -0.7546656  0.42743184      FALSE
## 18   0.000000 -0.78784060 -0.1208382 -0.1980534 -0.30047323      FALSE
## 20  27.491901 -0.07853023 -0.3090942  1.7082831  0.36298038      FALSE
## 23   2.016265  2.50142090 -2.7024407 -2.7850869  1.95425693      FALSE
## 24   1.007309  0.70690012 -0.1981856  0.8944149 -0.24184281      FALSE
## 25   5.415049  9.79977762 -4.2635297  5.7326293  1.19531413      FALSE
## 29  10.758737  1.85976056  0.6174242  0.3612860  0.76750320      FALSE
## 32  21.300657  0.93454507 -0.4597545  1.6269670 -0.09431274      FALSE
## 33   0.000000  6.32619943  2.8542889 -2.3102058  1.29013983      FALSE
## 36  22.794609 14.43049528  2.1589901 -1.5310904 -2.57494825      FALSE
## 38   9.673077  1.95569813  0.5058034  0.6227889  0.85277438      FALSE
## 39   0.000000  4.44562780  2.0481314 -1.4582800  0.62005161      FALSE
## 49   8.836310  2.27842210  0.2301439  1.2286204  0.63434759      FALSE
## 64   3.205496  0.01141647 -0.2036243  0.9369297  0.04368445      FALSE
## 72   0.000000  2.42394340 -1.4008051 -1.9462386  0.94710249      FALSE
```

```
## 76   5.269519  0.20859279 -3.8984036 -0.9846724 -0.43448129      FALSE
## 116  1.267483  0.78469153  0.6753486  0.5447403  0.07576599      FALSE
## 117  8.945171  3.15794243  0.5915338  0.5657640 -0.06986253      FALSE
## 123  7.313215  1.88822437  0.6453113  0.4537617  0.33399282      FALSE
## 126 15.878389 -0.89218238 -3.0700736 -1.9027957  0.34702454      FALSE
## 131  0.495348  0.48333718 -6.5267137 -3.4003668 -0.81527683      FALSE
##     in.ell_IAA in.ell_ARF in_ell
## 5        FALSE      FALSE  FALSE
## 17        TRUE      FALSE  FALSE
## 18        TRUE      FALSE  FALSE
## 20       FALSE      FALSE  FALSE
## 23       FALSE      FALSE  FALSE
## 24        TRUE       TRUE  FALSE
## 25       FALSE      FALSE  FALSE
## 29        TRUE      FALSE  FALSE
## 32        TRUE      FALSE  FALSE
## 33       FALSE      FALSE  FALSE
## 36       FALSE      FALSE  FALSE
## 38        TRUE      FALSE  FALSE
## 39       FALSE      FALSE  FALSE
## 49        TRUE      FALSE  FALSE
## 64        TRUE       TRUE  FALSE
## 72       FALSE      FALSE  FALSE
## 76       FALSE      FALSE  FALSE
## 116       TRUE      FALSE  FALSE
## 117       TRUE      FALSE  FALSE
## 123       TRUE      FALSE  FALSE
## 126      FALSE      FALSE  FALSE
## 131      FALSE      FALSE  FALSE
```

```
PC2_3_7tissues +
  geom_segment(PCA_loadings2, mapping=aes(x=0, y=0, # Change the size of arrows
                                          xend=(PC2*7), yend=(PC3*7)),
                  arrow = arrow(length = unit(1/2, "picas")), color="gray60") +
    annotate("text", x=(PCA_loadings2$PC2*7.2), #add the tissue names to it manually
             y=(PCA_loadings2$PC3*7.2),
             label=PCA_loadings2$Variables, size=4, color="gray60", fontface="bold") +
    theme(panel.background = element_rect(fill = "white", linewidth = 1))+
    theme_bw()+
   scale_color_manual(values=c("#86C5D8", "#620093", "#E7C94C")) +
  ggrepel::geom_text_repel(data = expr_logical_PC2_3_7tissues %>%
                           as_tibble(rownames = "name") %>%
                           filter(as.logical(in_ell == FALSE)),
                           aes(PC2, PC3, label=sub(".*\\|", "", heatmap_label)),
                         size=3, max.overlaps = 100,  min.segment.length = 0,
                         segment.curvature = -0.1) +
   labs(color = "Class") +
  theme_bw()
```



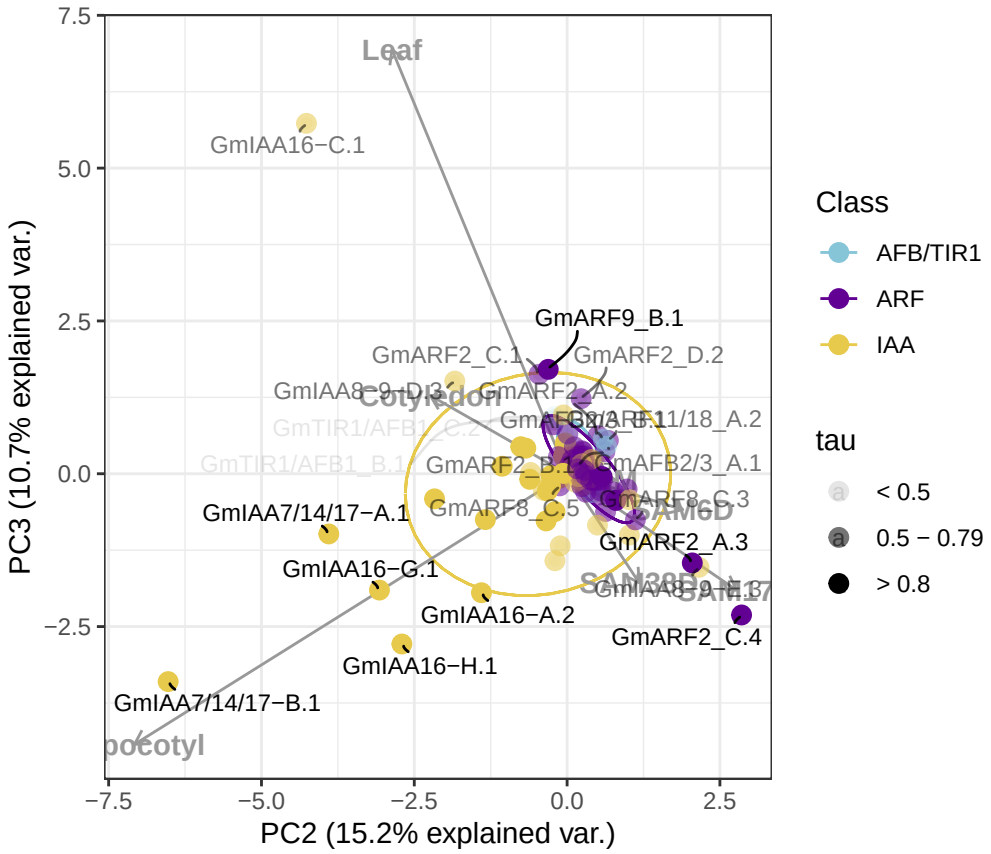

```
# ggsave("20230927_PC2_3_7Tissues.png", dpi = 1000, width = 10, height = 8)
# ggsave("20230927_PC2_3_7Tissues.pdf", dpi = 1000, width = 10, height = 8)
```

Lets now repeat with the tau values for 7 tissues kept

```
# combine data frames expr_logic with tau values
expr_logic_PC2_3_7tissues <- cbind(expr_logical_PC2_3_7tissues, tau_df$tau)

# Define the intervals for expr_logic2$tau
expr_logic_PC2_3_7tissues$tau_interval <- cut(expr_logic_PC2_3_7tissues$tau,
                                breaks = c(-Inf, 0.5, 0.8, Inf),
                                labels = c("< 0.5", "0.5 - 0.79", "> 0.8"))

PC2_3_7tissues +
    geom_segment(PCA_loadings2, mapping=aes(x=0, y=0, # Change the size of arrows
                                            xend=(PC2*8.75), yend=(PC3*8.75)),
                 arrow = arrow(length = unit(1/2, "picas")), color="gray60") +
    annotate("text", x=(PCA_loadings2$PC2*8.75), #add the tissue names to it manually
             y=(PCA_loadings2$PC3*8.75),
             label=PCA_loadings2$Variables, size=4, color="gray60", fontface="bold") +
    theme(panel.background = element_rect(fill = "white", linewidth = 1)) +

  geom_point(data = expr_logic_PC2_3_7tissues %>% as_tibble(rownames = "name"),
                                aes(PC2, PC3, alpha = tau_interval, color = Family),
             size=3) +
  ggrepel::geom_text_repel(data = expr_logic_PC2_3_7tissues %>%
                           as_tibble(rownames = "name") %>%
                           filter(as.logical(in_ell == FALSE)),
```

```
                        aes(PC2, PC3, label=sub(".*\\|", "", heatmap_label),
                            alpha=tau_interval),
                        size=3, max.overlaps = 100,  min.segment.length = 0,
                        segment.curvature = -0.1) +

  scale_color_manual(values=c("#86C5D8", "#620093", "#E7C94C")) +
  labs(color = "Class") +
 labs(color = "Class", alpha ="tau") +

 theme_bw()
```

```
## Warning: Using alpha for a discrete variable is not advised.
```




```
#ggsave("20240117_PC2_3_7Tissues.png", dpi = 1000, width = 10, height = 8)
#ggsave("20240117_PC2_3_7Tissues.pdf", dpi = 1000, width = 10, height = 8)
```

PC3 and PC4 outside ellipse labels

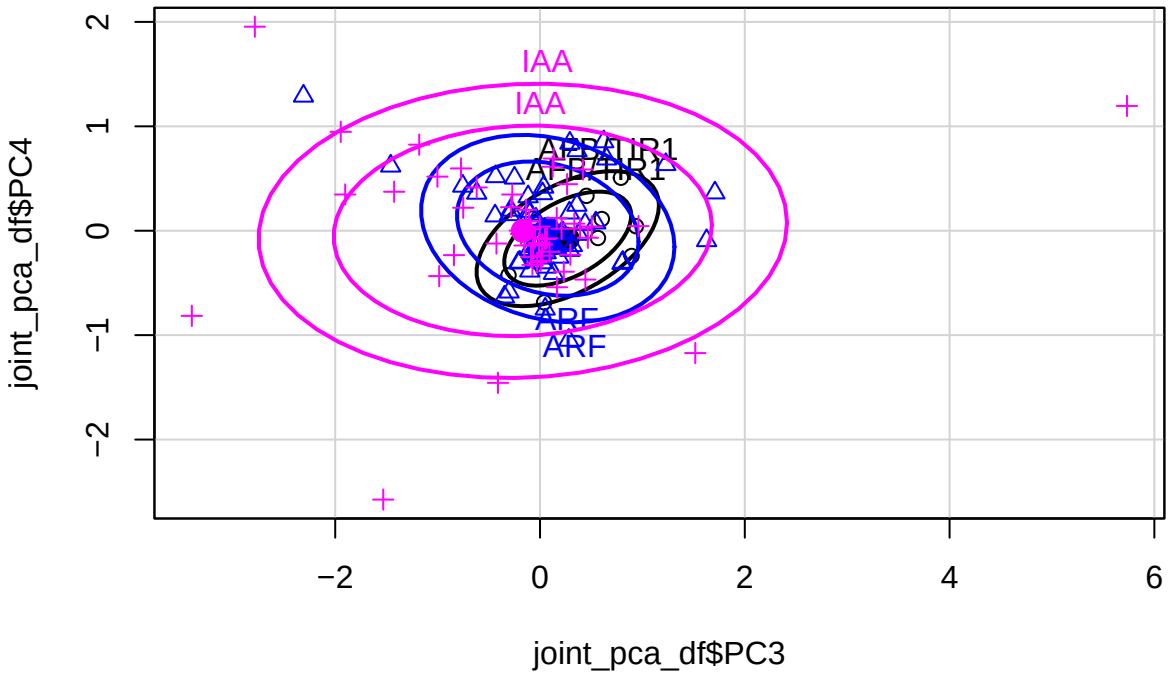
```
PC3_4_7tissues <- ggbiplot::ggbiplot(pca2, obs.scale = 1, var.scale = .5, choices = c(3,4),
                            varname.adjust = 3, alpha = .1,
                           groups = Family, var.axes = F, varname.size = 2,
                  ellipse = TRUE,  circle = FALSE, ellipse.prob = .70)


# Extract components so we can select out
PC3_4_7tissues$data
```

```
##             xvar         yvar   groups
## 1    0.135736327 -0.166794726      ARF
## 2    0.391015981  0.021725559      IAA
## 3    0.467448263 -0.067536958      IAA
## 4   -0.618794158  0.414369028      IAA
## 5    1.516456612 -1.171627082      IAA
## 6   -0.298488215  0.155056850      ARF
## 7   -0.771942835  0.596692174      IAA
## 8   -0.019034277 -0.085791352 AFB/TIR1
## 9    0.123263847  0.612851368      IAA
## 10   0.296718025 -0.229885278      IAA
## 11  -0.074216901 -0.327047811      IAA
## 12  -0.307513791 -0.426101201 AFB/TIR1
## 13   0.118751120 -0.092852907 AFB/TIR1
## 14   0.210544795  0.019512657      IAA
## 15  -1.002175172  0.518106927      IAA
## 16   0.808419635 -0.301661161      ARF
## 17  -0.754665609  0.427431840      ARF
## 18  -0.198053390 -0.300473230      ARF
## 19   0.203702144 -0.032318665      ARF
## 20   1.708283097  0.362980383      ARF
## 21  -0.249254037  0.504865466      ARF
## 22   0.048238640 -0.079805461      IAA
## 23  -2.785086871  1.954256930      IAA
## 24   0.894414856 -0.241842809 AFB/TIR1
## 25   5.732629313  1.195314126      IAA
## 26   0.800391367 -0.312065182      ARF
## 27   0.024957986 -0.239320843      IAA
## 28  -0.074310120  0.116710951 AFB/TIR1
## 29   0.361286016  0.767503195      ARF
## 30  -0.433613208  0.518572902      ARF
## 31   0.101766304 -0.077183564      ARF
## 32   1.626967022 -0.094312744      ARF
## 33  -2.310205824  1.290139828      ARF
## 34   0.024556471 -0.195978255      ARF
## 35   0.264364967  0.445650978      IAA
## 36  -1.531090403 -2.574948254      IAA
## 37   0.141906335 -0.156514601 AFB/TIR1
## 38   0.622788860  0.852774381      ARF
## 39  -1.458279986  0.620051612      ARF
## 40  -0.750021960  0.220037571      IAA
## 41   0.377262769 -0.033857714      ARF
## 42  -0.439075999  0.142648481      ARF
## 43   0.051104600 -0.749801388      ARF
## 44   0.071044809 -0.068042055      ARF
## 45  -0.047741305 -0.140250716 AFB/TIR1
## 46  -0.031576561 -0.264358663 AFB/TIR1
## 47   0.161153854 -0.163854323      ARF
## 48   0.291896516 -0.187321330      ARF
## 49   1.228620351  0.634347589      ARF
## 50  -0.346616692 -0.633762872      ARF
## 51   0.061513280 -0.348275075      ARF
## 52   0.134356933  0.691566165      IAA
## 53  -0.185967868 -0.030659800      IAA
```

```
## 54   0.051140263 -0.104071856 AFB/TIR1
## 55  -0.286010626  0.197392945      ARF
## 56   0.222705920 -0.018660135      ARF
## 57  -0.007314077 -0.117126136      IAA
## 58   0.335059268  0.067574118      IAA
## 59  -1.424143572  0.373460488      IAA
## 60   0.022943597 -0.306222560      ARF
## 61  -0.009929315 -0.125112574      IAA
## 62  -1.179610247  0.824300448      IAA
## 63   0.443021793 -0.467603321      IAA
## 64   0.936929695  0.043684449 AFB/TIR1
## 65   0.214269342 -0.060390329 AFB/TIR1
## 66   0.040059182 -0.686082095 AFB/TIR1
## 67  -0.027750940 -0.304216067      IAA
## 68   0.504784255  0.041205637      IAA
## 69   0.067089129 -0.073136425      IAA
## 70   0.144718515 -0.017263227      ARF
## 71  -0.270753178  0.346803341      IAA
## 72  -1.946238594  0.947102490      IAA
## 73   0.165611508 -0.541531009      IAA
## 74   0.021079016 -0.130540709      IAA
## 75   0.418826607  0.586608071      IAA
## 76  -0.984672374 -0.434481291      IAA
## 77  -0.039468673  0.111993163      ARF
## 78   0.280245925 -1.048339856      ARF
## 79   0.650288391  0.687757199      ARF
## 80  -0.302086741 -0.586239367      ARF
## 81   0.360448967  0.244664502      ARF
## 82   0.032561259 -0.154052291      ARF
## 83   0.035765053  0.418059786      ARF
## 84   0.440653982  0.061925950      ARF
## 85  -0.100032400 -0.385496367      ARF
## 86   0.131115667 -0.408555161      ARF
## 87   0.112977921 -0.153389597      ARF
## 88   0.060351273 -0.210476652      ARF
## 89   0.093041365 -0.201897919      IAA
## 90   0.075836951 -0.127619481      ARF
## 91  -0.618197885  0.360031756      ARF
## 92   0.291349997  0.839323391      ARF
## 93   0.286799671  0.170762865      ARF
## 94  -0.107640571  0.185923451      ARF
## 95  -0.425150473 -0.122584160      IAA
## 96   0.962239600  0.043951364      IAA
## 97  -0.067668928 -0.140894757      IAA
## 98  -0.106174638  0.088468230      IAA
## 99  -0.090989128  0.016103825      IAA
## 100 -0.136450892  0.200696132      IAA
## 101 -0.002678882  0.039279304      IAA
## 102  0.228044041 -0.121129223      ARF
## 103 -0.211148378 -0.307775202      ARF
## 104  0.603415593  0.112023704 AFB/TIR1
## 105 -0.088816512 -0.250014529      IAA
## 106  0.024403946  0.355643287      ARF
## 107 -0.099986924 -0.179283342      ARF
```

```
## 108 -0.198514723  0.001283642      IAA
## 109  0.163541169  0.122506596      IAA
## 110  0.232333327 -0.392657919      IAA
## 111 -0.116129991  0.326223430      ARF
## 112 -0.051162129 -0.151720388      ARF
## 113  0.183674413 -0.252281288      ARF
## 114  0.118174410 -0.100612169      ARF
## 115  0.315839653 -0.141738678      ARF
## 116  0.544740295  0.075765990      ARF
## 117  0.565764005 -0.069862526 AFB/TIR1
## 118  0.022927865 -0.260894094      ARF
## 119 -0.047540345 -0.066857327      IAA
## 120 -0.045815482 -0.223146203      ARF
## 121 -0.100377639  0.160113846      ARF
## 122 -0.025405134 -0.135980283      ARF
## 123  0.453761721  0.333992817 AFB/TIR1
## 124  0.007290830 -0.286482378      IAA
## 125 -0.410351711 -1.457000005      IAA
## 126 -1.902795711  0.347024541      IAA
## 127  0.079576472 -0.140137191      ARF
## 128  0.790073247  0.507465423 AFB/TIR1
## 129 -0.151673820 -0.141425041      IAA
## 130  0.055831155 -0.099674050      ARF
## 131 -3.400366836 -0.815276835      IAA
## 132 -0.282003528  0.225510060      IAA
## 133 -0.840091171 -0.233615166      IAA
```

```
build_PC3_4_7tissues <- ggplot_build(PC3_4_7tissues)$data
points_PC3_4_7tissues <- build_PC3_4_7tissues[[1]]

# co-ordinates of the ellipses
ell_points_PC3_4_7tissues <- car::dataEllipse(joint_pca_df$PC3,
                                joint_pca_df$PC4,
                                as.factor(joint_pca_df$Family), levels=c(.7, .9))
```




```
# add geom_point with ellipses point

ell_ARF_PC3_4_7tissues <- as.data.frame(ell_points_PC3_4_7tissues$ARF$`0.7`)

ell_IAA_PC3_4_7tissues <- as.data.frame(ell_points_PC3_4_7tissues$IAA$`0.7`)

ell_TIR_PC3_4_7tissues <- as.data.frame(ell_points_PC3_4_7tissues$`AFB/TIR1`$`0.7`)

# Find which points are outside (!) the ellipse, and add this to the data
library(sp)
dat_TIR_PC3_4_7tissues <- data.frame(
  points_PC3_4_7tissues[2:3],
  in.ell_TIR = as.logical(point.in.polygon(points_PC3_4_7tissues$x, points_PC3_4_7tissues$y, ell_TIR_PC3
)

dat_IAA_PC3_4_7tissues <- data.frame(
  points_PC3_4_7tissues[2:3],
  in.ell_IAA = as.logical(point.in.polygon(points_PC3_4_7tissues$x, points_PC3_4_7tissues$y, ell_IAA_PC3
)

dat_ARF_PC3_4_7tissues <- data.frame(
  points_PC3_4_7tissues[2:3],
  in.ell_ARF = as.logical(point.in.polygon(points_PC3_4_7tissues$x, points_PC3_4_7tissues$y, ell_ARF_PC3
)
# as.logical(point..) equals to TRUE indicated points are inside ellipses

# Combining data points for labeling
transcript_expr_logical_PC3_4_7tissues <- cbind(joint_pca_df, dat_TIR_PC3_4_7tissues, dat_IAA_PC3_4_7tis


expr_logical_PC3_4_7tissues <- transcript_expr_logical_PC3_4_7tissues %>% mutate(., in_ell = case_when(
                                          Family=="IAA" & `in.ell_IAA` == TRUE ~ "TRUE",
```

```
                                                Family=="AFB/TIR1" & `in.ell_TIR` == TRUE ~ "TRUE")) %>%
  mutate(in_ell = coalesce(in_ell, "FALSE"))

expr_logical_PC3_4_7tissues[which(expr_logical_PC3_4_7tissues$in_ell == FALSE),]
```

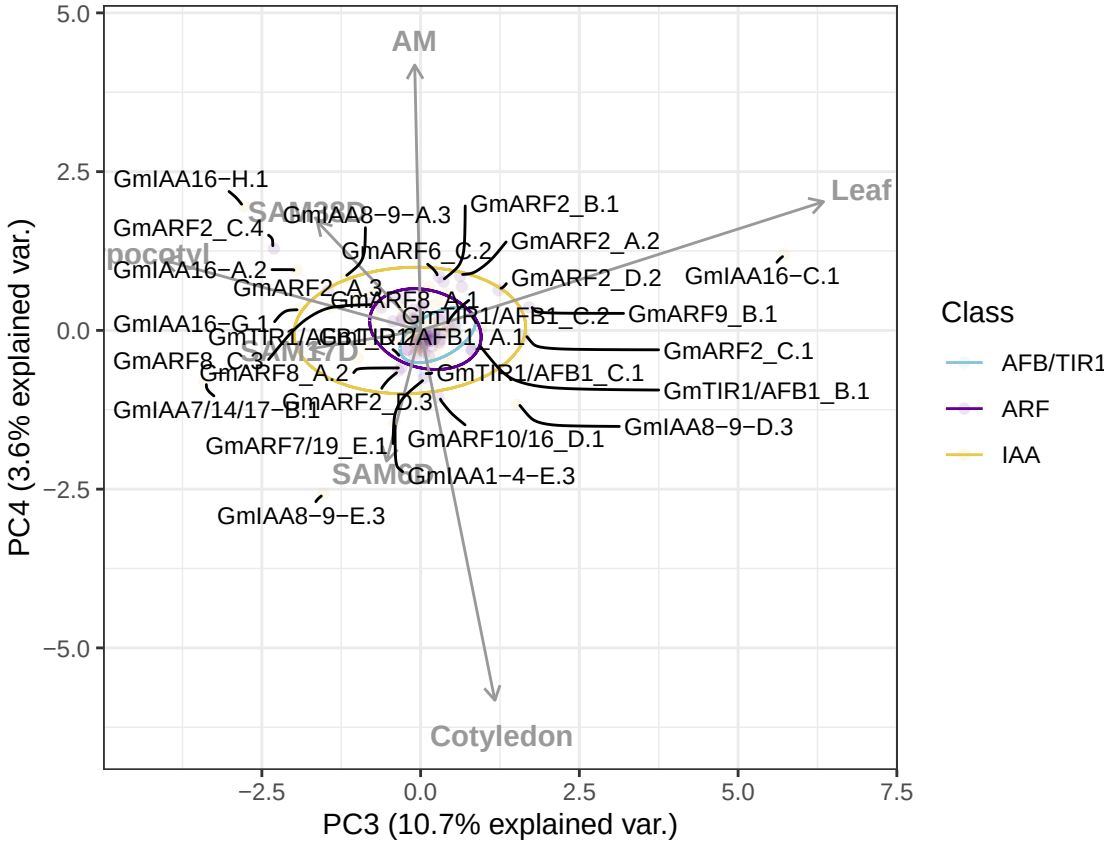
```
##         Transcript ID                    heatmap_label   Family     Class Clade
## 5   Glyma.01G098000.3     Glyma.01G098000|GmIAA8-9-D.3      IAA         A     I
## 12  Glyma.02G152800.2  Glyma.02G152800|GmTIR1/AFB1_D.2 AFB/TIR1 TIR1/AFB1     I
## 17  Glyma.02G239600.3       Glyma.02G239600|GmARF8_C.3      ARF         A    II
## 20  Glyma.03G070500.1       Glyma.03G070500|GmARF9_B.1      ARF         B     I
## 23  Glyma.03G158700.1      Glyma.03G158700|GmIAA16-H.1      IAA         C   III
## 24  Glyma.03G209400.1  Glyma.03G209400|GmTIR1/AFB1_B.1 AFB/TIR1 TIR1/AFB1     I
## 25  Glyma.03G247400.1      Glyma.03G247400|GmIAA16-C.1      IAA         C   III
## 29  Glyma.04G200600.1       Glyma.04G200600|GmARF2_B.1      ARF         B     I
## 32  Glyma.05G200800.1       Glyma.05G200800|GmARF2_C.1      ARF         B     I
## 33  Glyma.05G200800.4       Glyma.05G200800|GmARF2_C.4      ARF         B     I
## 36  Glyma.06G091700.3     Glyma.06G091700|GmIAA8-9-E.3      IAA         A     I
## 38  Glyma.06G164900.2       Glyma.06G164900|GmARF2_A.2      ARF         B     I
## 39  Glyma.06G164900.3       Glyma.06G164900|GmARF2_A.3      ARF         B     I
## 43  Glyma.07G130400.1    Glyma.07G130400|GmARF7/19_E.1      ARF         A    II
## 49  Glyma.08G008100.2       Glyma.08G008100|GmARF2_D.2      ARF         B     I
## 50  Glyma.08G008100.3       Glyma.08G008100|GmARF2_D.3      ARF         B     I
## 62  Glyma.09G203300.3     Glyma.09G203300|GmIAA8-9-A.3      IAA         A     I
## 64  Glyma.10G021500.2  Glyma.10G021500|GmTIR1/AFB1_C.2 AFB/TIR1 TIR1/AFB1     I
## 66  Glyma.10G021500.1  Glyma.10G021500|GmTIR1/AFB1_C.1 AFB/TIR1 TIR1/AFB1     I
## 72  Glyma.10G162400.2      Glyma.10G162400|GmIAA16-A.2      IAA         C   III
## 78  Glyma.11G145500.1   Glyma.11G145500|GmARF10/16_D.1      ARF         C   III
## 79  Glyma.11G204200.1       Glyma.11G204200|GmARF8_A.1      ARF         A    II
## 80  Glyma.11G204200.2       Glyma.11G204200|GmARF8_A.2      ARF         A    II
## 92  Glyma.13G221400.2       Glyma.13G221400|GmARF6_C.2      ARF         A    II
## 125 Glyma.19G161000.3     Glyma.19G161000|GmIAA1-4-E.3      IAA         B    II
## 126 Glyma.19G161100.1      Glyma.19G161100|GmIAA16-G.1      IAA         C   III
## 128 Glyma.19G206800.1  Glyma.19G206800|GmTIR1/AFB1_A.1 AFB/TIR1 TIR1/AFB1     I
## 131 Glyma.20G210400.1 Glyma.20G210400|GmIAA7/14/17-B.1      IAA         C   III
##             AM         OF       IAM        IBM    RootTip  Cotyledon  Hypocotyl
## 5    68.216252  51.350688  53.55319  96.275020  32.628226  78.301786 172.112449
## 12   22.069192  16.181094  21.00405  22.849022  12.164769  15.200032  20.202937
## 17   37.168779   5.439277  41.92448  51.089719   6.308382   0.000000  23.986700
## 20   14.963834   9.149436  10.01434  18.303006   6.361899   6.335987   4.159165
## 23   62.978354 188.282417  63.41597  50.273803  93.913232  11.797675 382.933284
## 24   25.967032  26.377921  14.28612  20.719333   9.445604  22.549691  20.867913
## 25  123.752976 182.226043 143.67239 136.882838  68.294552 100.483878 215.724837
## 29   59.132821  34.776950  54.02368 104.014185   5.893899  15.036259  13.614824
## 32   23.736932   7.300337  13.38762  33.516112   4.197497  21.693739  20.950298
## 33  106.794815  41.848269  90.20675  89.149798   3.924654  25.580965  38.494199
## 36  120.096829  73.349658  80.92891 112.799969  14.116324 123.198735 118.328990
## 38   62.736767  31.362845  56.71287 104.850125   1.621706  14.695636  13.618860
## 39   78.161251  91.333242 102.46112  95.846621   1.569549  29.653419  18.983798
## 43   18.550414   8.914698  15.95095  18.729053  15.689098  23.181993  16.710000
## 49   41.952212  22.684099  48.46459  54.600720   2.166574  14.976262  26.934453
## 50   41.903106  15.801812  23.41788  55.329663   4.236413  28.260708  18.724678
## 62   36.472556  37.373970  38.15859  43.541833  11.505870   0.000000 117.295863
## 64   19.724328  16.170619  18.09673  18.503468  10.239295  14.506518  14.635121
## 66   14.419899   9.741531   6.79071  12.735183   6.157738  17.688606  17.203871
```

```
## 72   50.429036  62.648515  44.79904  49.047824  54.886130  14.549869 258.563372
## 78   15.909573  17.106814  12.58611  12.520897   6.758486  30.401493   8.543969
## 79   19.241075  21.103199   0.00000   3.055660   3.172375   0.000000   5.721453
## 80   13.373647   0.000000  35.09998  44.159391   4.581945  10.261290  31.902014
## 92   34.943626  18.239549  27.73661  43.916594   1.658267   1.279513  28.990865
## 125   4.478037 404.803343  44.07771  17.846549  72.730750  41.028328 177.214792
## 126   6.156592 108.404702  30.82654   8.523381  43.829775   4.519645 334.804651
## 128  29.377480  13.907445  22.73580  26.728168   6.489989   7.571060  13.364493
## 131   4.160975 265.136463  15.52861  13.540402 130.804062  41.664671 633.844120
##          SAM6D     SAM17D     SAM38D    Callus       Leaf       Root    Nodule
## 5   101.538840 108.035003  57.568952  3.602672  72.181052  53.728970 22.341412
## 12   29.352679  33.917530  17.176745 17.838179   0.000000   0.000000  0.000000
## 17   52.872850  68.490459  26.447317  6.139986   0.000000   3.626608  0.000000
## 20   21.230577  13.709403   9.639602  2.603449  42.803542 126.598709 27.491901
## 23   20.163323  39.919056  73.535631  0.583159   6.250057  14.892682  2.016265
## 24   28.476275  25.128377  20.548182 11.542469  26.686917   5.465411  1.007309
## 25   75.229555 108.926253  87.081914 65.420159 178.440543  98.055423  5.415049
## 29   40.036100  67.500984  33.565553  5.088175  22.636836  12.527492 10.758737
## 32   31.857638  31.063118  16.461606  4.368482  44.030052  25.338768 21.300657
## 33   75.216825 290.336670 100.526085 25.977418   0.000000   0.000000  0.000000
## 36  229.139072 393.546142 181.391747 71.756006  45.788590  20.983248 22.794609
## 38   48.102339  51.456817  31.907033  8.665198  27.396630  14.501406  9.673077
## 39   38.298793 237.526834  76.153152  6.048278   3.674760   1.606524  0.000000
## 43   16.764956  21.488396  10.756881 24.155359   2.997797  25.928328  7.688516
## 49   45.899996 104.119336  30.814124  3.509392  48.206033  26.688307  8.836310
## 50   55.230644  26.660841  30.862812  6.303453   0.000000   0.000000  0.216233
## 62   30.426647  41.114157  30.034268  6.427166   0.000000  30.080385  5.435140
## 64   12.243954  22.316362  15.443247 25.621399  26.106051   5.641934  3.205496
## 66   28.663983  21.325627  11.737596  3.718783   4.726063   2.093318  0.000000
## 72   52.919869  43.482386  62.182188 12.401001   7.425099  12.077243  0.000000
## 78    9.310248  10.078082  10.215456  0.759723   4.047713   2.691333  0.596545
## 79    0.000000   0.000000  17.832069  0.000000  18.572984   3.488127  1.310676
## 80   32.214786  40.620313   0.000000 17.181705   0.000000   0.000000  0.000000
## 92   22.677736  46.861420  20.641988  2.961649  19.960475   4.115777  1.477684
## 125   3.197441   2.314000   2.924909  0.843634   6.919914   6.338750  0.000000
## 126   0.817625   0.938549   2.175286  0.045598   1.253888  31.879730 15.878389
## 128  20.598762  24.312187  18.931187 13.373494  25.380331   5.994108  3.091861
## 131   1.661192   0.821472   1.444420  0.244966   0.972353  62.263900  0.495348
##             PC1         PC2         PC3         PC4 in.ell_TIR in.ell_IAA
## 5    6.29787441 -1.83724237  1.51645661 -1.17162708      FALSE      FALSE
## 12   0.06210206  0.41382610 -0.30751379 -0.42610120      FALSE       TRUE
## 17   0.81044975  1.11700892 -0.75466561  0.42743184      FALSE       TRUE
## 20  -0.07853023 -0.30909424  1.70828310  0.36298038      FALSE      FALSE
## 23   2.50142090 -2.70244068 -2.78508687  1.95425693      FALSE      FALSE
## 24   0.70690012 -0.19818565  0.89441486 -0.24184281      FALSE       TRUE
## 25   9.79977762 -4.26352971  5.73262931  1.19531413      FALSE      FALSE
## 29   1.85976056  0.61742415  0.36128602  0.76750320      FALSE       TRUE
## 32   0.93454507 -0.45975448  1.62696702 -0.09431274      FALSE       TRUE
## 33   6.32619943  2.85428889 -2.31020582  1.29013983      FALSE      FALSE
## 36  14.43049528  2.15899012 -1.53109040 -2.57494825      FALSE      FALSE
## 38   1.95569813  0.50580340  0.62278886  0.85277438      FALSE       TRUE
## 39   4.44562780  2.04813145 -1.45827999  0.62005161      FALSE       TRUE
## 43  -0.18778513  0.04913286  0.05110460 -0.74980139      FALSE       TRUE
## 49   2.27842210  0.23014394  1.22862035  0.63434759      FALSE       TRUE
```

```
## 50   1.34307083  0.54381981 -0.34661669 -0.63376287      FALSE       TRUE
## 62   0.47369476 -0.11275080 -1.17961025  0.82430045      FALSE      FALSE
## 64   0.01141647 -0.20362431  0.93692969  0.04368445      FALSE       TRUE
## 66  -0.17447944  0.17662980  0.04005918 -0.68608210      FALSE       TRUE
## 72   2.42394340 -1.40080513 -1.94623859  0.94710249      FALSE      FALSE
## 78  -0.28107091 -0.14129573  0.28024593 -1.04833986      FALSE      FALSE
## 79  -0.79489435  0.01134430  0.65028839  0.68775720      FALSE       TRUE
## 80  -0.42946116  0.30503427 -0.30208674 -0.58623937      FALSE       TRUE
## 92   0.37773397  0.28877357  0.29135000  0.83932339      FALSE       TRUE
## 125 -0.19442276 -2.16841690 -0.41035171 -1.45700001      FALSE      FALSE
## 126 -0.89218238 -3.07007360 -1.90279571  0.34702454      FALSE      FALSE
## 128  0.22854712  0.06134928  0.79007325  0.50746542      FALSE       TRUE
## 131  0.48333718 -6.52671369 -3.40036684 -0.81527683      FALSE      FALSE
##     in.ell_ARF in_ell
## 5        FALSE  FALSE
## 12        TRUE  FALSE
## 17       FALSE  FALSE
## 20       FALSE  FALSE
## 23       FALSE  FALSE
## 24        TRUE  FALSE
## 25       FALSE  FALSE
## 29       FALSE  FALSE
## 32       FALSE  FALSE
## 33       FALSE  FALSE
## 36       FALSE  FALSE
## 38       FALSE  FALSE
## 39       FALSE  FALSE
## 43       FALSE  FALSE
## 49       FALSE  FALSE
## 50       FALSE  FALSE
## 62       FALSE  FALSE
## 64        TRUE  FALSE
## 66       FALSE  FALSE
## 72       FALSE  FALSE
## 78       FALSE  FALSE
## 79       FALSE  FALSE
## 80       FALSE  FALSE
## 92       FALSE  FALSE
## 125      FALSE  FALSE
## 126      FALSE  FALSE
## 128      FALSE  FALSE
## 131      FALSE  FALSE
```

```
PC3_4_7tissues +
    geom_segment(PCA_loadings2, mapping=aes(x=0, y=0, # Change the size of arrows
                                            xend=(PC3*8), yend=(PC4*8)),
                 arrow = arrow(length = unit(1/2, "picas")), color="gray60") +
    annotate("text", x=(PCA_loadings2$PC3*8.75), #add the tissue names to it manually
             y=(PCA_loadings2$PC4*8.75),
             label=PCA_loadings2$Variables, size=4, color="gray60", fontface="bold") +
    theme(panel.background = element_rect(fill = "white", linewidth = 1))+
    theme_bw()+
   scale_color_manual(values=c("#86C5D8", "#620093", "#E7C94C")) +
  ggrepel::geom_text_repel(data = expr_logical_PC3_4_7tissues %>%
```

```
                        as_tibble(rownames = "name") %>%
                        filter(as.logical(in_ell == FALSE)),
                        aes(PC3, PC4, label=sub(".*\\|", "", heatmap_label)),
                      size=3, max.overlaps = 100,  min.segment.length = 0,
                      segment.curvature = -0.1) +
  labs(color = "Class") +
 theme_bw()
```



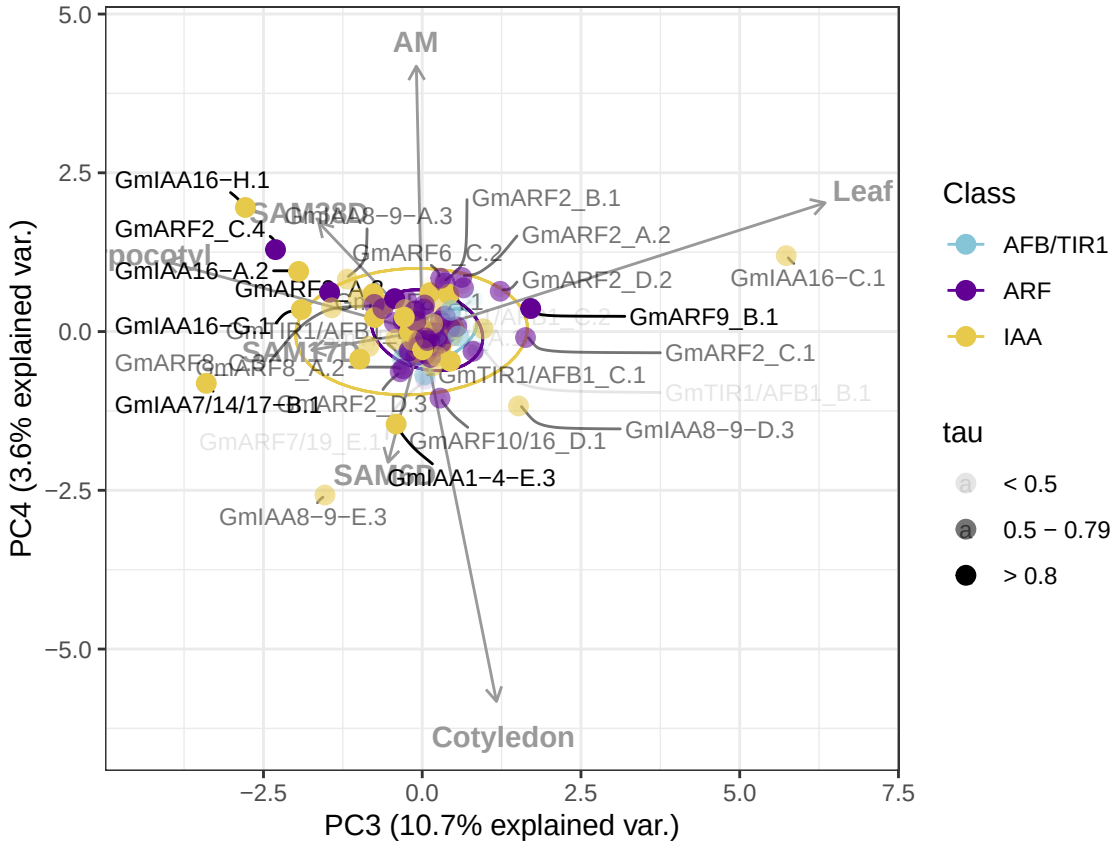

```
#ggsave("20230927_PC3_4_7Tissues.png", dpi = 1000, width = 10, height = 8)
#ggsave("20230927_PC3_4_7Tissues.pdf", dpi = 1000, width = 10, height = 8)
```

Repeat the same to add tau measurement

```
# combine data frames expr_logic with tau values
expr_logical_PC3_4_7tissues <- cbind(expr_logical_PC3_4_7tissues, tau_df$tau)

# Define the intervals for expr_logic2$tau
expr_logical_PC3_4_7tissues$tau_interval <- cut(expr_logical_PC3_4_7tissues$tau,
                                breaks = c(-Inf, 0.5, 0.8, Inf),
                                labels = c("< 0.5", "0.5 - 0.79", "> 0.8"))

PC3_4_7tissues +
    geom_segment(PCA_loadings2, mapping=aes(x=0, y=0, # Change the size of arrows
                                            xend=(PC3*8), yend=(PC4*8)),
                 arrow = arrow(length = unit(1/2, "picas")), color="gray60") +
    annotate("text", x=(PCA_loadings2$PC3*8.75), #add the tissue names to it manually
             y=(PCA_loadings2$PC4*8.75),
```

```
            label=PCA_loadings2$Variables, size=4, color="gray60", fontface="bold") +
   theme(panel.background = element_rect(fill = "white", linewidth = 1))+
   theme_bw()+

 ggrepel::geom_text_repel(data = expr_logical_PC3_4_7tissues %>%
                          as_tibble(rownames = "name") %>%
                          filter(as.logical(in_ell == FALSE)),
                          aes(PC3, PC4, label=sub(".*\\|", "", heatmap_label),
                              alpha = tau_interval),
                        size=3, max.overlaps = 100,  min.segment.length = 0,
                        segment.curvature = -0.1) +
  labs(color = "Class") +

  geom_point(data = expr_logical_PC3_4_7tissues %>% as_tibble(rownames = "name"),
                                  aes(PC3, PC4, alpha = tau_interval, color = Family),
            size=3) +
  scale_color_manual(values=c("#86C5D8", "#620093", "#E7C94C")) +
  labs(color = "Class") +
 labs(color = "Class", alpha ="tau") +
 theme_bw()
```

```
## Warning: Using alpha for a discrete variable is not advised.
```




```
#ggsave("20240117_PC3_4_7Tissues.png", dpi = 1000, width = 10, height = 8)
#ggsave("20240117_PC3_4_7Tissues.pdf", dpi = 1000, width = 10, height = 8)
```

Principal component comparisons.

We observe here the differences between our principal component analysis with all 14 tissues from databases, and with only the 7 tissues that are part of aerial architecture. It is visible that our principal component analysis is minimally affected by the exclusion of tissues herein analysed, hence we are confident to display only the aerial tissues we are interested in. We also observe that root, hypocotyl, nodule, and open flower fall along the same direction, implying that these tissues are correlated. As we move to principal components that accounts for smaller amounts of variation, we notice an improvement in the discrimination of the correlation between root, nodule and hypocotyl tissues. On the other hand root tips, and open flower correlation to hypocotyl shifts more when looking at PC3 and PC4, with a very small discrimination between these tissues. We can speculate that this correlation could be due to these tissues being at approximate similar developmental stages, and therefore exhibit similar patterns of gene expression as they respond to common developmental cues. It also shows the redundancy of auxin response genes as they have overlap in functional roles. Further investigation of these genes are important to gain more insightful information of which genes are important in growth and development of these tissues, and if different pair of auxin regulatory genes are important during this process.

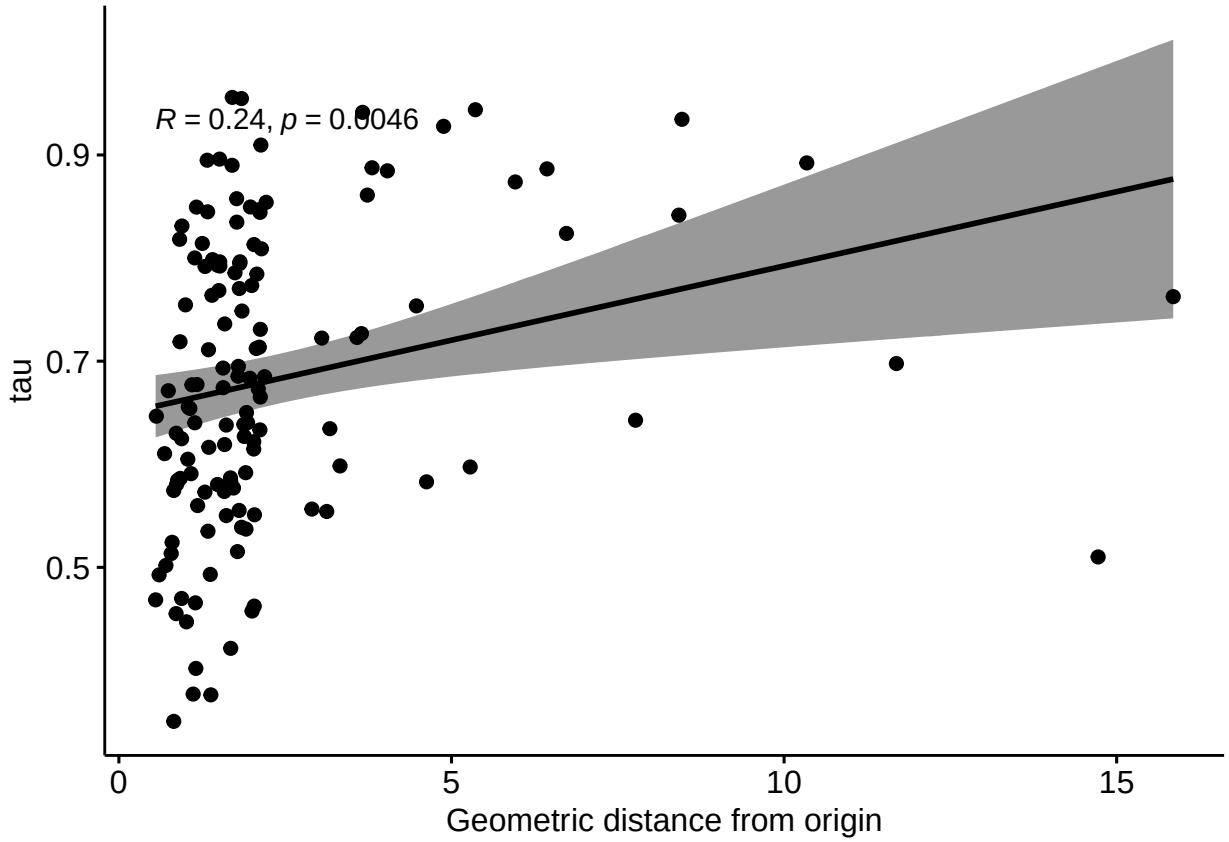
add tau correlation to Geom distance from origin

- Tau does provide some additional information
- highlight some original candidates with high | low tau identified in PCA
- family wise tau distribution: are ARFs or IAAs more tissue specific?
- Discussion:
  - other means of calculating tau, or better suited datasets
  - machine learning model of candidate genes

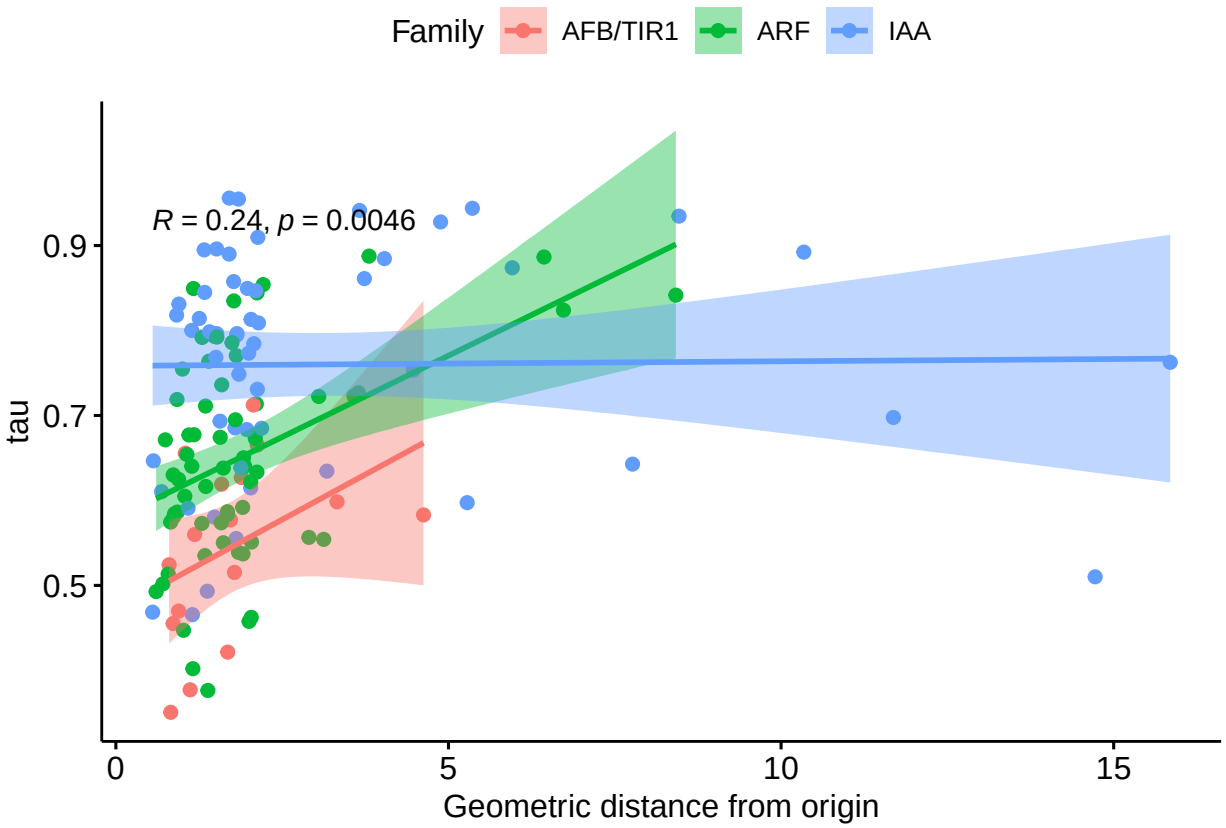
```
geom_dist_calc_df <- cbind(comb_pca_df, tau_df$tau)


geom_dist_calc_df2 <- geom_dist_calc_df %>% dplyr::select(c(heatmap_label, Family, PC1, PC2, PC3, PC4,

# square PC values
geom_dist_calc_df2[,3:6] <- (geom_dist_calc_df2[, 3:6])^2

# sum all PCs then take its square root
geom_dist_calc_df2$Geom_dist <- NA


for (i in 1:nrow(geom_dist_calc_df2)) {
  geom_dist_calc_df2[i, 8] <- sum(geom_dist_calc_df2[i, 3:6], na.rm = TRUE)
}

geom_dist_calc_df2$Geom_Dist_sqrt <-
  sqrt(geom_dist_calc_df2$Geom_dist)

library("ggpubr")
```

```
##
## Attaching package: 'ggpubr'
## The following object is masked from 'package:plyr':
##
##     mutate
```

```
ggscatter(geom_dist_calc_df2, x = "Geom_Dist_sqrt",
          y = "tau_df$tau",
         add = "reg.line", conf.int = TRUE,
          cor.coef = TRUE, cor.method = "pearson",
          xlab = "Geometric distance from origin", ylab = "tau")
```




```
ggscatter(geom_dist_calc_df2, x = "Geom_Dist_sqrt",
          y = "tau_df$tau",
          add = "reg.line", conf.int = TRUE,
          cor.coef = TRUE, cor.method = "pearson",
          color = "Family",
          xlab = "Geometric distance from origin", ylab = "tau")
```



From the correlation plots above we can see that there isn't a strong correlation between Geometric distance and and tau values, supporting the importance of tau values in this analysis. We believe a larger dataset may be important in order to in this calculations and we recommend that that should be pursued in future analysis.