STEpUP OA: Script used to process 'minimal' dataset required to generate figures in our Quality-Control (QC) Manuscript

Contents

Purpose of this vignette:

The full STEpUP OA dataset may be made available by application to the Data Access and Publication Group of STEpUP OA (stepupoa@kennedy.ox.ac.uk) once the primary analysis manuscript is published, in accordance with what is stipulated in our Consortium Agreement. The minimal datasets necessary for replicating figures along with the required R code are provided here.

Directory structure and the minimal data:

```
##                                     levelName
## 1 minimal datasets
## 2  ¦--PC1 Driver - Standardisation
## 3  ¦--PC1 Driver - Intracellular Protein Score
## 4  ¦--PC2 Driver - Bimodal Signal
## 5  ¦--Compare to Immunoassay
## 6  °--Disease Group After Filtering
```

Required R packages and file path:

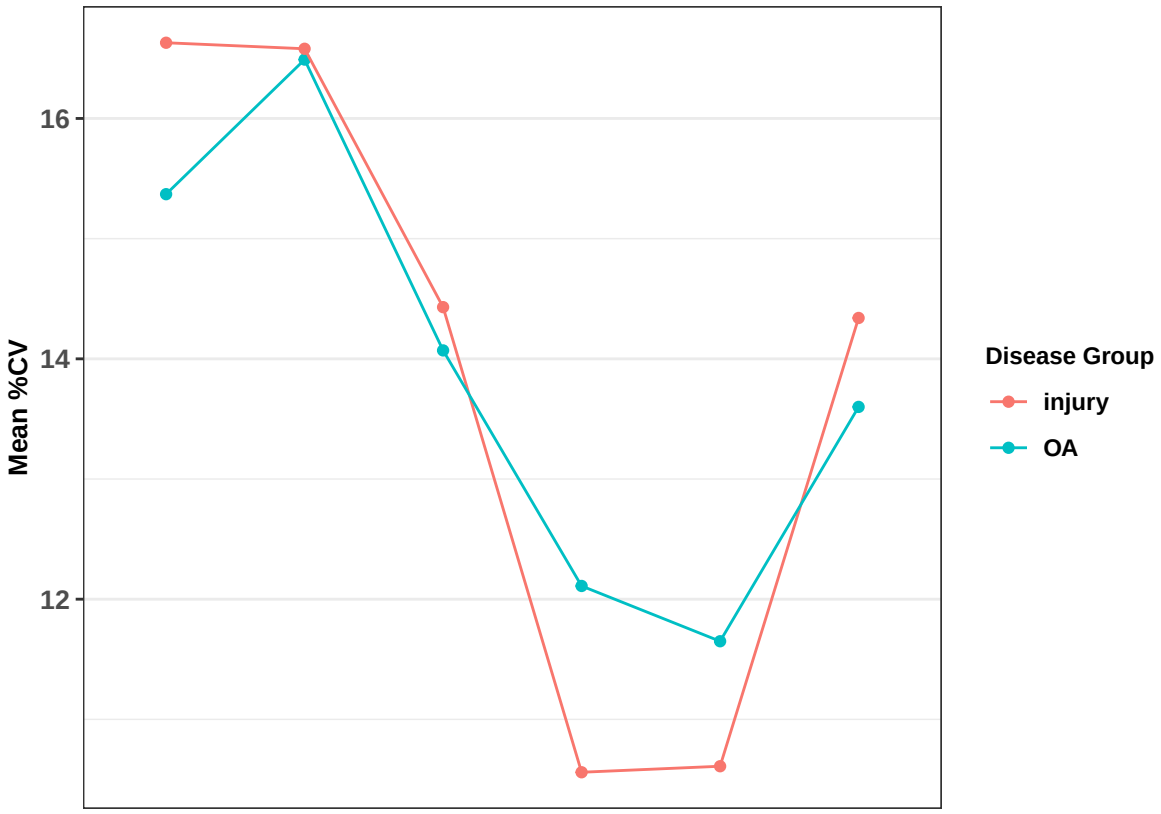
```
### load in required R packages used to generate plots in the manuscript
library(ggplot2)
library(cowplot)
library(GGally)
```

```
library(ggpubr)
library(factoextra)
library(ggforce)
library(scales)

### Set the file path to where the minimal data are downloaded on your personal machine
intermidiate.out <- "/Users/ydeng/Documents/QCpaper.Code/minimal datasets/"
```

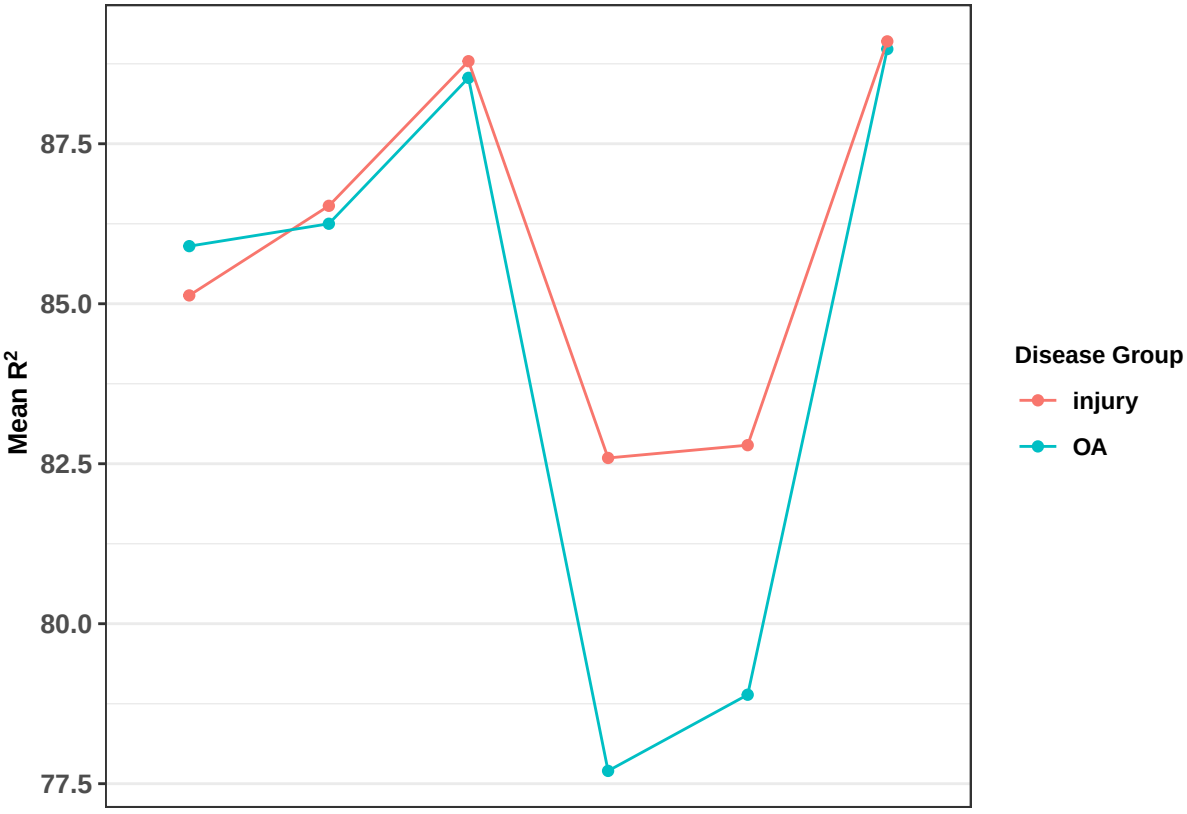
Assessment of Synovial Fluid Standardisation Procedures:

% CV and non-technical variation of each protein, correlation coefficient between SomaScan measure and immunoassay were investigated, and the comparisons across different standardization procedures were displayed as below.

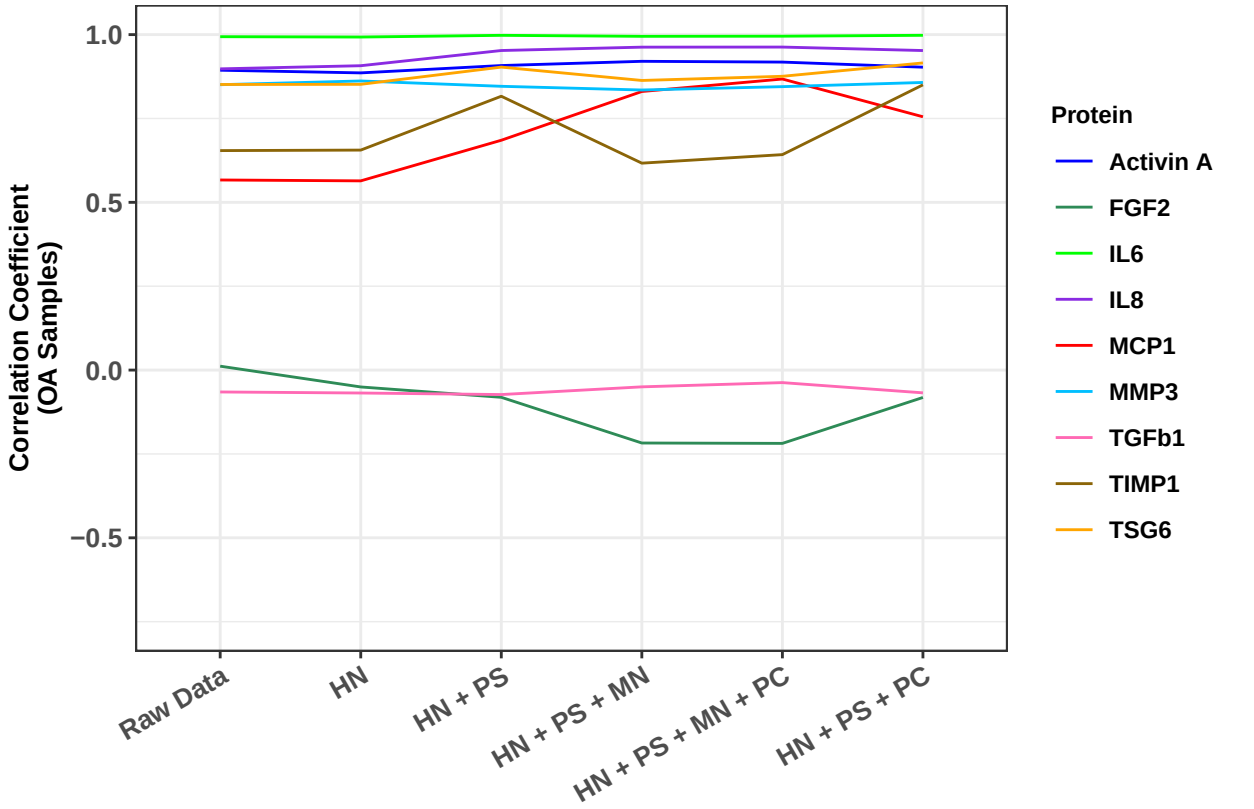
```
### read in mean % CV values and  mean R2 values for each protein and correlation coefficients assessin
meanCV <- read.csv(paste0(intermidiate.out,"Standardisation/meanCV.csv"), row.names = 1)
meanR2 <- read.csv(paste0(intermidiate.out,"Standardisation/meanR2.csv"), row.names = 1)
CorDatP.OA <- read.csv(paste0(intermidiate.out,"Standardisation/CorDatP.OA.csv"), row.names = 1)
CorDatP.INJ <- read.csv(paste0(intermidiate.out,"Standardisation/CorDatP.INJ.csv"), row.names = 1)


### Figure 1:

StandardisationLabel = c("Raw Data","HN","HN + PS","HN + PS + MN","HN + PS + MN + PC","HN + PS + PC") #

p.meanCV <- ggplot(data = meanCV) + geom_point(aes(x=NormalisationSteps,y=100*as.numeric(meanCV),group=
  xlab("") + ylab("\n\nMean %CV") + labs(color = "Disease Group") + scale_x_discrete(breaks=seq(1:lengt
  theme(axis.text.x = element_text(size=10, angle=30,face="bold",hjust=1),axis.text.y = element_text(si
        legend.title =element_text(size = 9,face="bold"), legend.text = element_text(size = 9,face="bol
        axis.title.y =element_text(size=10,face="bold"),axis.title.x =element_text(size=10))

p.meanR2 <- ggplot(data = meanR2) + geom_point(aes(x=NormalisationSteps,y=100*as.numeric(meanR2),group=
  xlab("") + ylab(bquote(atop("\n","" ~ bold("Mean R") ^ bold("2"))))) + labs(color = "Disease Group") +
  theme(axis.text.x = element_text(size=10, angle=30,face="bold",hjust=1),axis.text.y = element_text(si
        legend.title =element_text(size = 9,face="bold"), legend.text = element_text(size = 9,face="bol
        axis.title.y =element_text(size=10,face="bold"),axis.title.x =element_text(size=10))

p.immunoassay.OA <- ggplot(data = CorDatP.OA) + geom_line(aes(x=as.character(CorC),y=as.numeric(CorDatY
  xlab("") + ylab("Correlation Coefficient\n(OA Samples)") +labs(color = "Protein") + scale_x_discrete(
  theme(axis.text.x = element_text(size=10, angle=30,face="bold",hjust=1),axis.text.y = element_text(si
        legend.title =element_text(size = 9,face="bold"), legend.text = element_text(size = 9,face="bol
        axis.title.y =element_text(size=10,face="bold"),axis.title.x =element_text(size=10))

p.immunoassay.INJ <- ggplot(data = CorDatP.INJ) + geom_line(aes(x=as.character(CorC),y=as.numeric(CorDa
  xlab("") + ylab("Correlation Coefficient\n(Injury Samples)") +labs(color = "Protein") + scale_x_discr
  theme(axis.text.x = element_text(size=10, angle=30,face="bold",hjust=1),axis.text.y = element_text(si
        legend.title =element_text(size = 9,face="bold"), legend.text = element_text(size = 9,face="bol
        axis.title.y =element_text(size=10,face="bold"),axis.title.x =element_text(size=10))

### Figure 1:
p.meanCV
```


Mean %CV
16
14
12
Disease Group
injury
OA

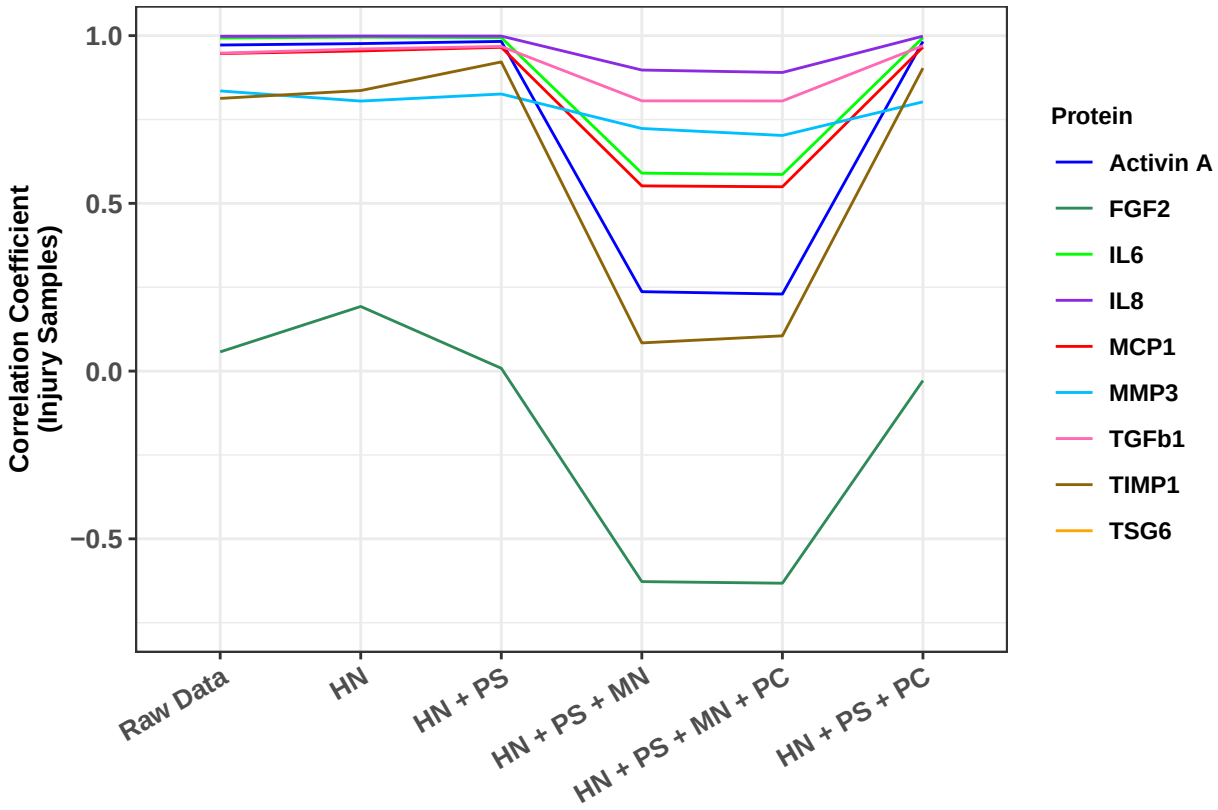
```
p.meanR2
```


Mean R²
87.5
85.0
82.5
80.0
77.5
Disease Group
injury
OA

```
p.immunoassay.OA
```




```
p.immunoassay.INJ
```

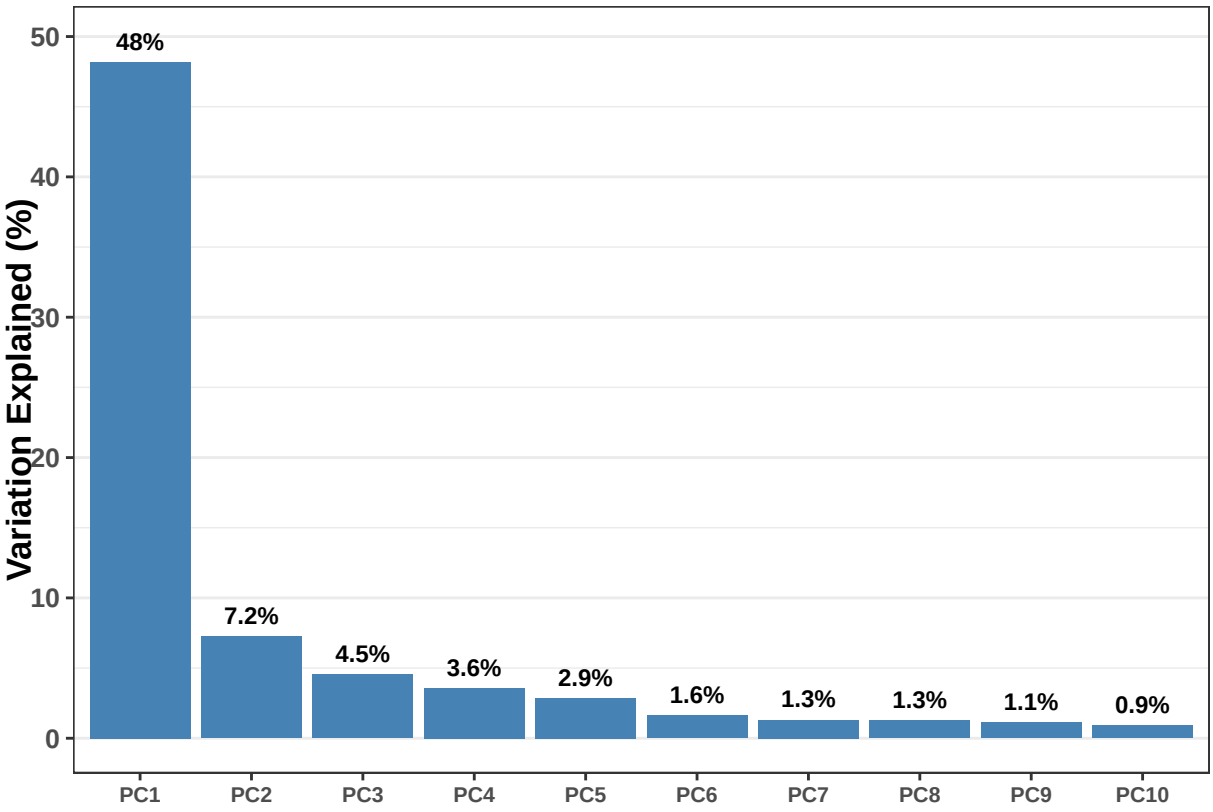


Investigate the drivers of PC1 – Intracellular Protein Score (IPS)

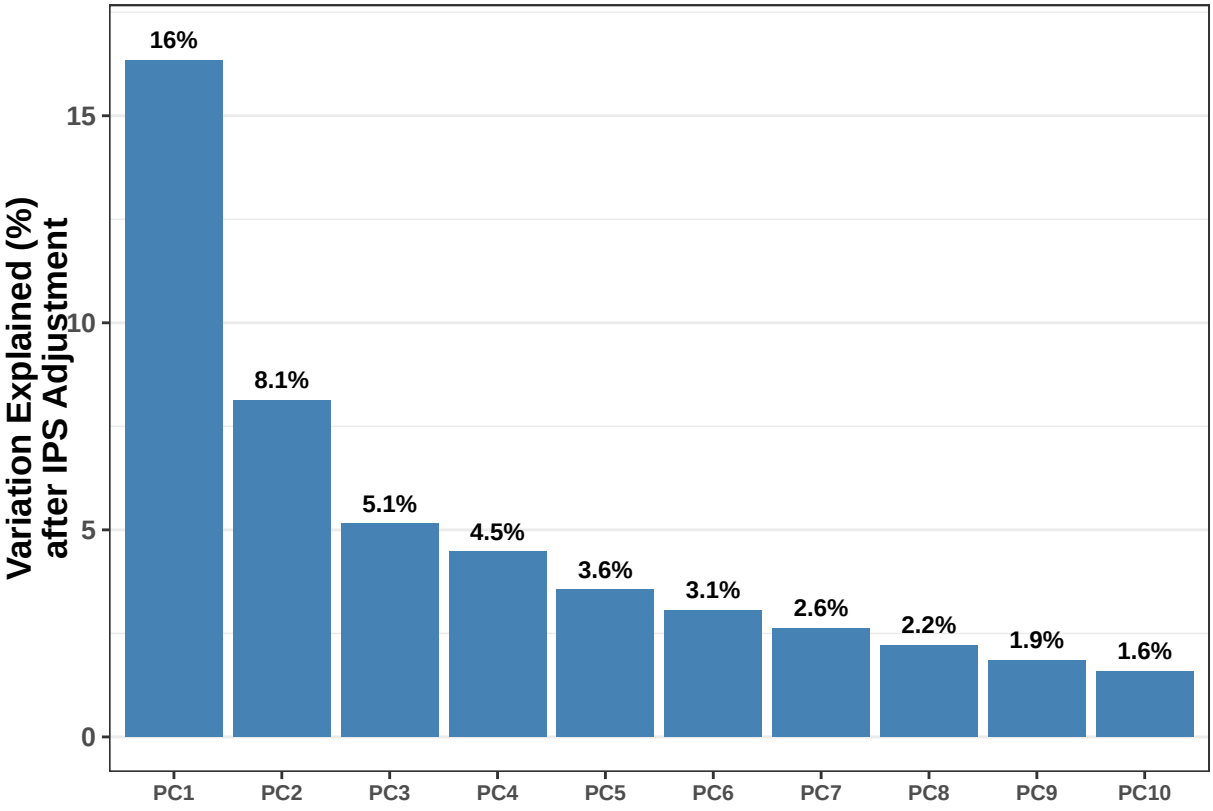
After data standardisation, PC1 explained 48% of data variance which was driven by intracellular protein. The intracellular protein signal can be effectively adjusted for using the limma package.

Variation explained by the top 10 PCs after standardisation and adjustment for IPS

```
### read in values that correspond to the variance explained by each PC
val.explained <- read.csv(paste0(intermidiate.out,"PC1 Driver - Intracellular Protein Score/variation.ex

p.variation.PC.before <- ggplot(val.explained[1:10,]) + geom_bar(aes(x=1:10,y=Standardised[1:10]),stat="
  xlab("") + ylab("Variation Explained (%)") +
  geom_text(aes(x=1:10,y=Standardised[1:10]+1.5,label=paste0(signif(Standardised[1:10],2),"%")),size=3,
  theme(axis.text.x = element_text(size=8,face="bold"),axis.text.y =element_text(size=10,face="bold"),ax
        panel.grid.major.x = element_blank())

p.variation.PC.after  <- ggplot(val.explained[1:10,]) + geom_bar(aes(x=1:10,y=IPS.Adjusted[1:10]),stat="
  xlab("") + ylab("Variation Explained (%)\nafter IPS Adjustment") + theme_bw() +
  geom_text(aes(x=1:10,y=IPS.Adjusted[1:10]+0.5,label=paste0(signif(IPS.Adjusted[1:10],2),"%")),size=3,
  theme(axis.text.x = element_text(size=8,face="bold"),axis.text.y =element_text(size=10,face="bold"),ax
        panel.grid.major.x = element_blank())

### Generate subplots of Figure 2:
p.variation.PC.before
```

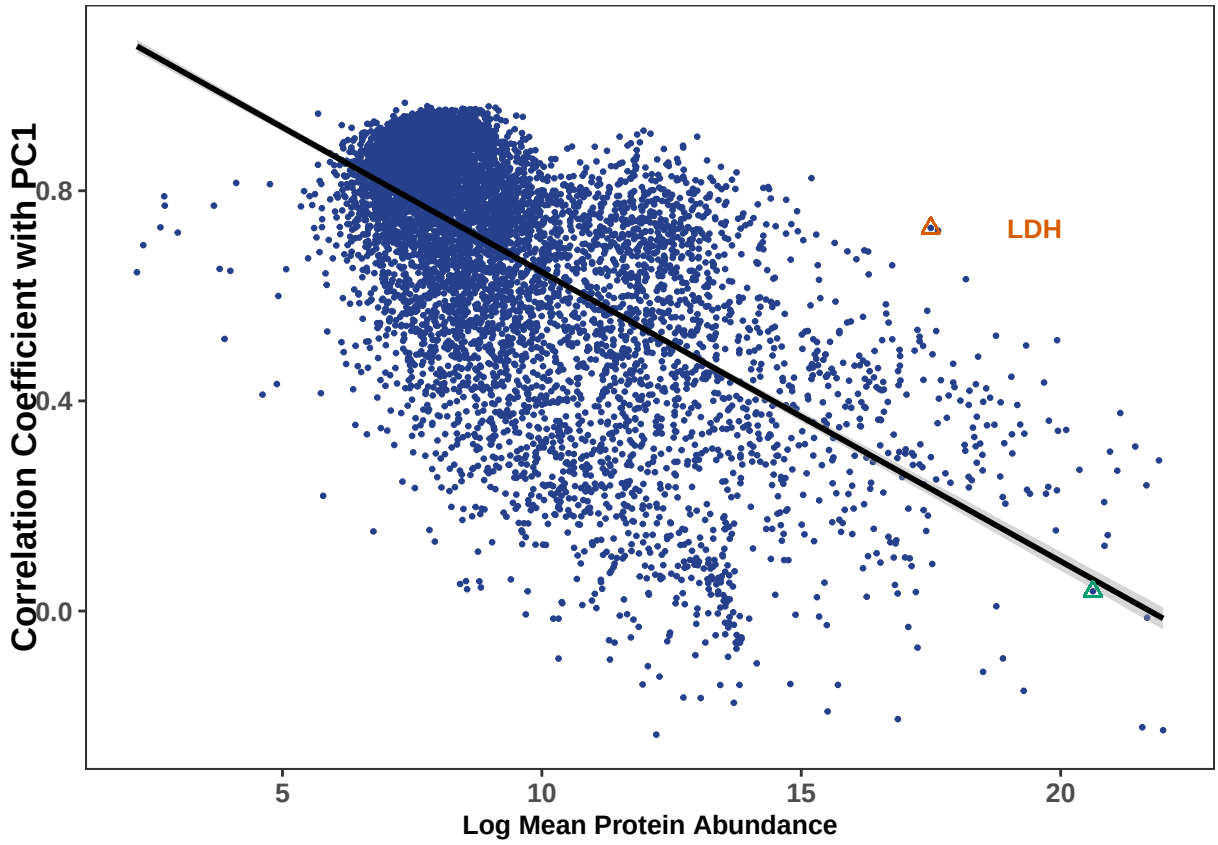



```
p.variation.PC.after
```



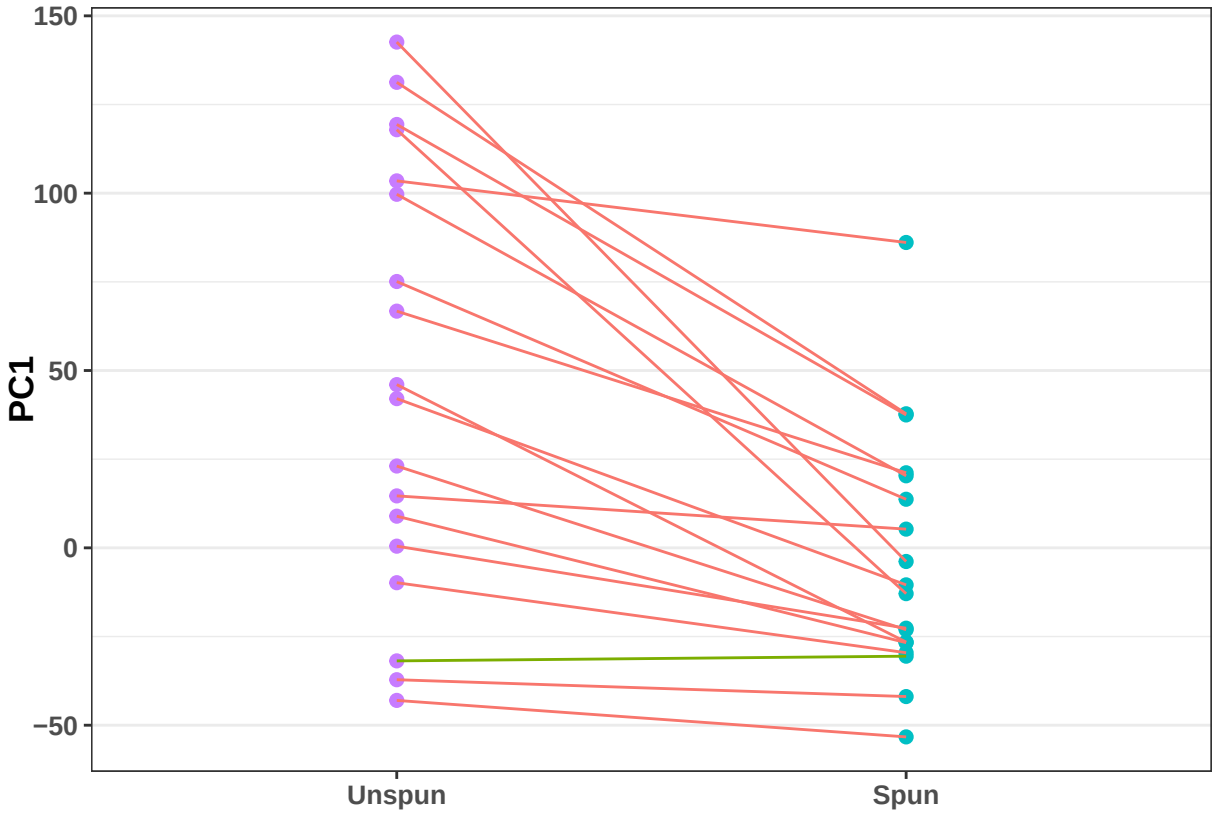
Visualization of PC1 driver – protein abundance

```
### read in protein abundance values and correlation with PC1 after standardisation
corPerPro.beforeIPS <- read.csv(paste0(intermidiate.out,"PC1 Driver - Intracellular Protein Score/corPe

albminSeq.LDHseq <- read.csv(paste0(intermidiate.out,"PC1 Driver - Intracellular Protein Score/albminSe
albminSeq <- albminSeq.LDHseq["ALBUMIN",1]
LDHseq <- albminSeq.LDHseq["LDH",1]

p.abundance.PC1 <- ggplot(data=corPerPro.beforeIPS[is.finite(corPerPro.beforeIPS$Abundance),],aes(x=log
  geom_point(aes(x=log(corPerPro.beforeIPS[albminSeq,"Abundance"]),y=corPerPro.beforeIPS[albminSeq,"Cor
  annotate(geom="text",x=log(corPerPro.beforeIPS[albminSeq,"Abundance"])-0.3, y=corPerPro.beforeIPS[albm
  geom_point(aes(x=log(corPerPro.beforeIPS[LDHseq,"Abundance"]),y=corPerPro.beforeIPS[LDHseq,"Correlati
  annotate(geom="text",x=log(corPerPro.beforeIPS[LDHseq,"Abundance"])+2, y=corPerPro.beforeIPS[LDHseq,"
  xlab("Log Mean Protein Abundance") + ylab("Correlation Coefficient with PC1") + theme_bw() +
  theme(axis.title.x = element_text(size=10,face="bold"),axis.text.x = element_text(size=10,face="bold"
        axis.title.y =element_text(size=12.5,face="bold",vjust=-1),axis.text.y = element_text(size=10,fa
        legend.position = c(0.8,0.1),legend.title =element_text(size = 11,face="bold"), legend.text = el
        panel.grid.major = element_blank(), panel.grid.minor = element_blank())

### Replicate subplot of Figure 2:
p.abundance.PC1
```



Correlation with PC1 for 18 paired spun/unspun samples

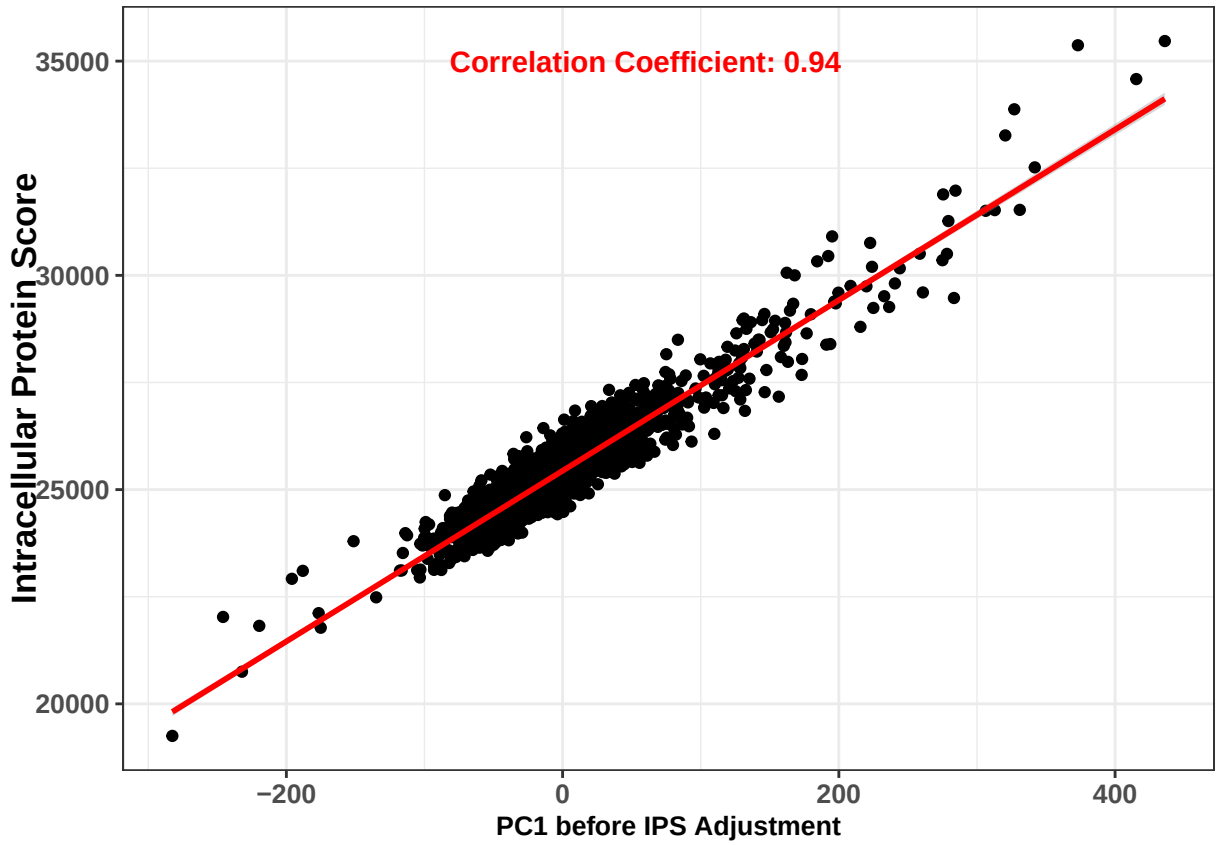
```
### read in correlation values (i.e. correlation with PC1) for the 18 paired spun/unspun samples
spinFrame <- read.csv(paste0(intermidiate.out,"PC1 Driver - Intracellular Protein Score/spinFrame.csv"))

### set the color of each line on the plot according to the direction of change in correlation with PC1
p.spin.PC1 <- ggplot(data=spinFrame,aes(x=Spin,y=as.numeric(PC1))) + geom_point(aes(color=Spin),size=2) +
  xlab("") + ylab("PC1") + scale_x_discrete(limits=c("Unspun","Spun")) + theme_bw() +
  theme(axis.title.x = element_text(size=10,face="bold"),axis.text.x = element_text(size=10,face="bold"),
        axis.title.y =element_text(size=12.5,face="bold",vjust=-1),panel.grid.major.x = element_blank())

### Generate subplot of Figure 2:
p.spin.PC1
```



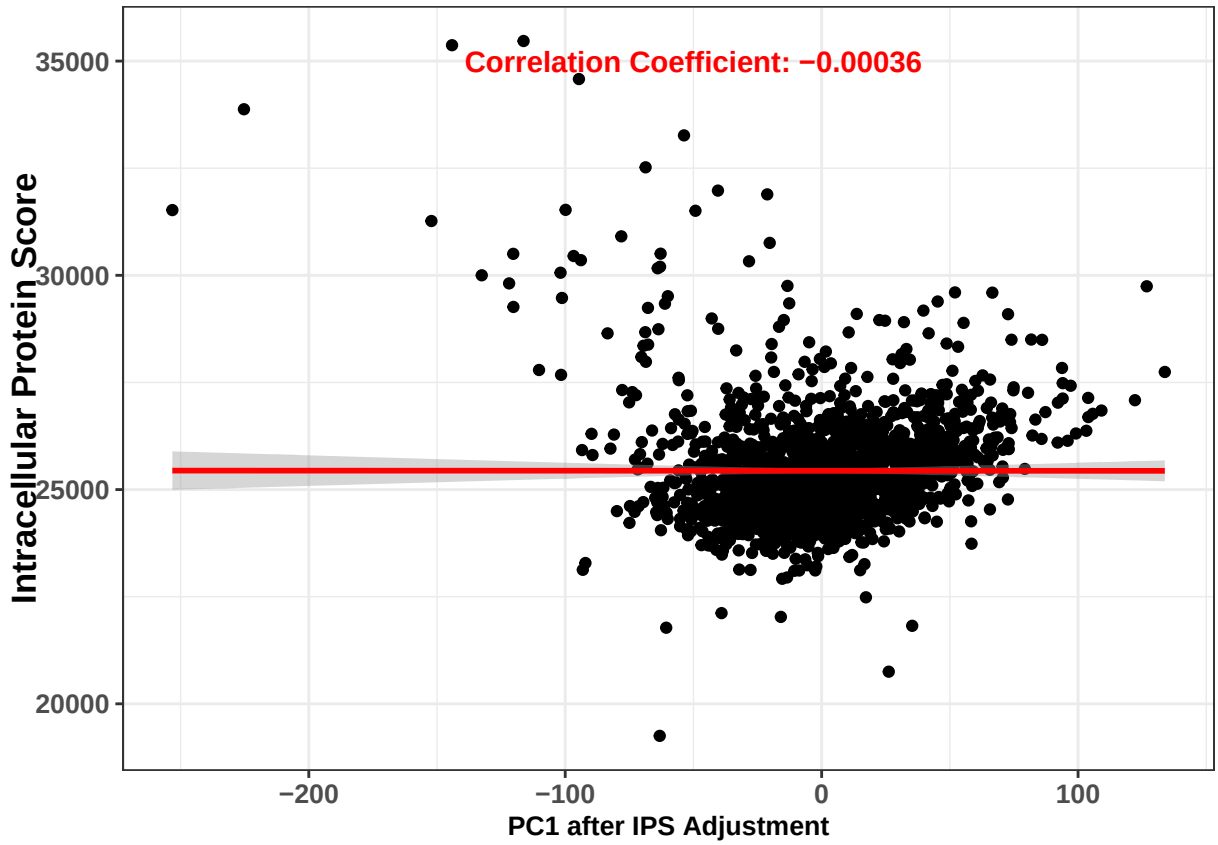
Intracellular protein score vs PC1 using either IPS adjusted and non-IPS adjusted data

```
### read in intracellular score and PC1 before and after IPS adjustment
IPSvsPC1 <- read.csv(paste0(intermidiate.out,"PC1 Driver - Intracellular Protein Score/IPSvsPC1.csv"))

### calculate the pearson correlation coefficients between PC1 and intracellular protein score
cor.IPS.PC1.before <- signif(cor(IPSvsPC1$non.IPS.adjusted,IPSvsPC1$Intracellular.Protein.Score),2)
p.RegProBefore <- ggplot(IPSvsPC1) + geom_point(aes(x=non.IPS.adjusted,y=Intracellular.Protein.Score)) +
  xlab("PC1 before IPS Adjustment") + ylab("Intracellular Protein Score") + annotate(geom="text",x=60, y
  theme(axis.text.x = element_text(size=10,face="bold"),axis.title.x =element_text(size = 10,face="bold
        axis.text.y =element_text(size=10,face="bold"),axis.title.y =element_text(size=12.5,face="bold"

cor.IPS.PC1.after <- signif(cor(IPSvsPC1$IPS.adjusted,IPSvsPC1$Intracellular.Protein.Score),2)
p.RegProAfter <- ggplot(IPSvsPC1) + geom_point(aes(x=IPS.adjusted,y=Intracellular.Protein.Score)) + geom
  xlab("PC1 after IPS Adjustment") + ylab("Intracellular Protein Score") + annotate(geom="text",x=-50, y
```

```
  theme(axis.text.x = element_text(size=10,face="bold"),axis.title.x =element_text(size = 10,face="bold
        axis.text.y =element_text(size=10,face="bold"),axis.title.y =element_text(size=12.5,face="bold"

### Generate subplot of Figure 2:
p.RegProBefore
```




```
p.RegProAfter
```



Investigation of drivers of PC1 (regression model)

```
### read in protein subcellular location information from Human Protein Atalas https://www.proteinatlas
SubLocation <- read.csv(paste0(intermidiate.out,"PC1 Driver - Intracellular Protein Score/subcellular_l
SubLocationDat = SubLocation[,c("Gene.name","Main.location")]

CytoplasmL <- read.csv(paste0(intermidiate.out,"PC1 Driver - Intracellular Protein Score/Cytoplasm.txt"
NucleusL <- read.csv(paste0(intermidiate.out,"PC1 Driver - Intracellular Protein Score/Nucleus.txt"),hea
EndomembraneL <- read.csv(paste0(intermidiate.out,"PC1 Driver - Intracellular Protein Score/Endomembran
secreted <- SubLocation$Gene.name[which(SubLocation$Extracellular.location=="Predicted to be secreted")]

# for each protein define whether it is a 'secreted nuclear protein' (here we excluded the multiple loc
for (indexCounter in 1:nrow(SubLocationDat)){
  x = SubLocationDat$Main.location[indexCounter]
  pro = SubLocationDat$Gene.name[indexCounter]
  mainLoc = strsplit(x,";")[[1]]
  if(any(CytoplasmL %in% mainLoc)){newLocation = "Cytoplasm"
  }else if(any(EndomembraneL %in% mainLoc)){newLocation = "Endomembrane"
  }else if(any(NucleusL %in% mainLoc) & !(any(pro %in% secreted))){newLocation = "Nucleus"
  }else{newLocation = NA}
  SubLocationDat$Broad.location[indexCounter]= newLocation
}

NucleusGenes <- SubLocationDat$Gene.name[which(SubLocationDat$Broad.location=="Nucleus")]

ProMeta <- read.csv(paste0(intermidiate.out,"PC1 Driver - Intracellular Protein Score/ProMeta.csv"))
```

```
keepseq <- which(ProMeta$Organism=="Human" & ProMeta$Type=="Protein")
Nucleus <- sapply(keepseq,function(x) {ifelse(any(ProMeta[x,"EntrezGeneSymbol"] %in% NucleusGenes),1,0)

# Visualize the significant drivers: nuclear protein and protein abundance
### non-IPS adjusted data
driverNuclear.beforeIPS <- summary(lm(corPerPro.beforeIPS$Correlation[keepseq] ~ as.factor(Nucleus) + l
driverNuclear.beforeIPS$coefficients
```

```
##                                              Estimate   Std. Error    t value
## (Intercept)                                1.19309121 0.0083427405 143.009508
## as.factor(Nucleus)1                        0.03277124 0.0054273648   6.038149
## log(corPerPro.beforeIPS$Abundance[keepseq]) -0.05517403 0.0008454898 -65.256883
##                                                 Pr(>|t|)
## (Intercept)                                 0.000000e+00
## as.factor(Nucleus)1                         1.635458e-09
## log(corPerPro.beforeIPS$Abundance[keepseq]) 0.000000e+00
```

```
### IPS adjusted data
corPerPro.afterIPS <- read.csv(paste0(intermidiate.out,"PC1 Driver - Intracellular Protein Score/corPerP
driverNuclear.afterIPS <- summary(lm(corPerPro.afterIPS$Correlation[keepseq] ~ as.factor(Nucleus) + log
driverNuclear.afterIPS$coefficients
```

```
##                                              Estimate   Std. Error    t value
## (Intercept)                                -0.165534813 0.0061358661 -26.978231
## as.factor(Nucleus)1                         0.012423007 0.0108146603   1.148719
## log(corPerPro.afterIPS$Abundance[keepseq]) -0.007812608 0.0009930221  -7.867506
##                                                 Pr(>|t|)
## (Intercept)                                7.086765e-153
## as.factor(Nucleus)1                         2.507095e-01
## log(corPerPro.afterIPS$Abundance[keepseq])  4.140385e-15
```

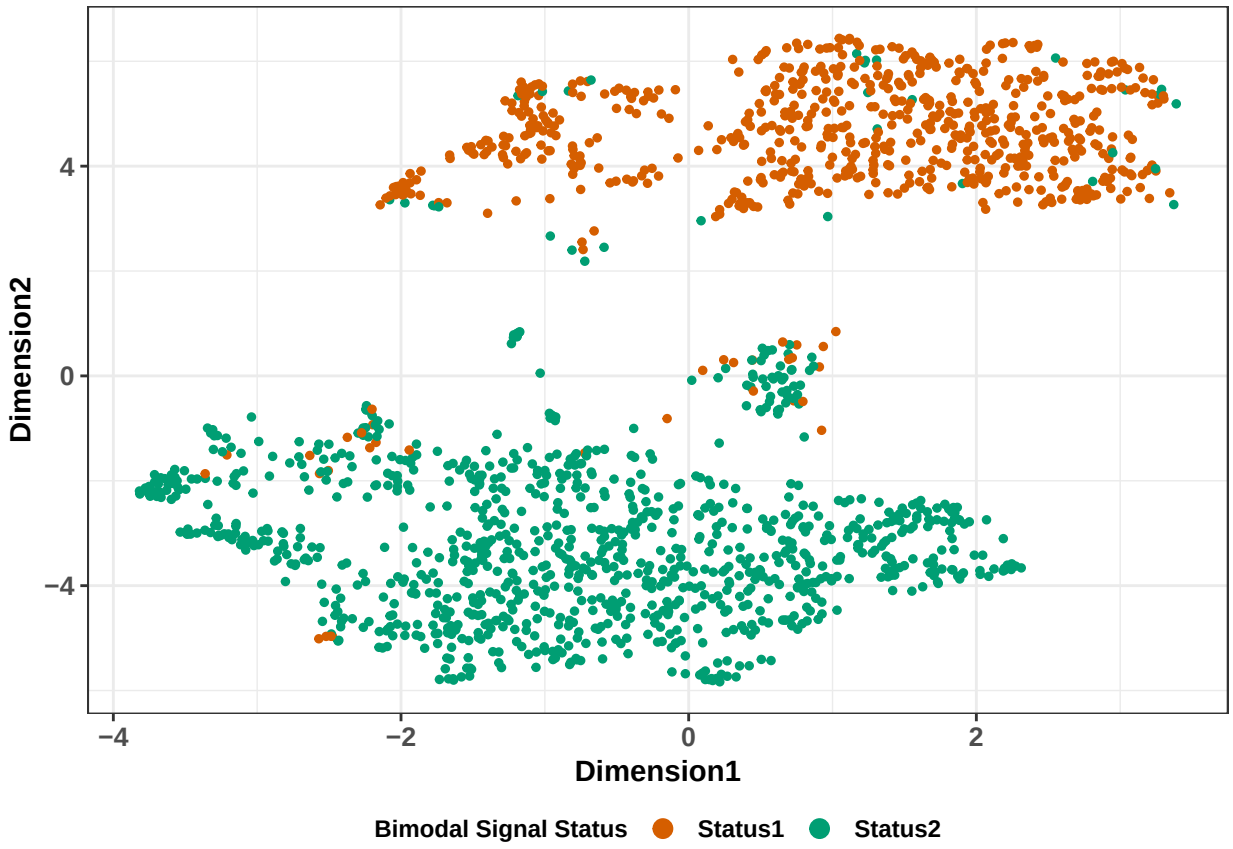
Investigate the drivers of PC2 – bimodal signal

We also found a strong bimodal signal on PC2 which is highly correlated with laboratory processing batch

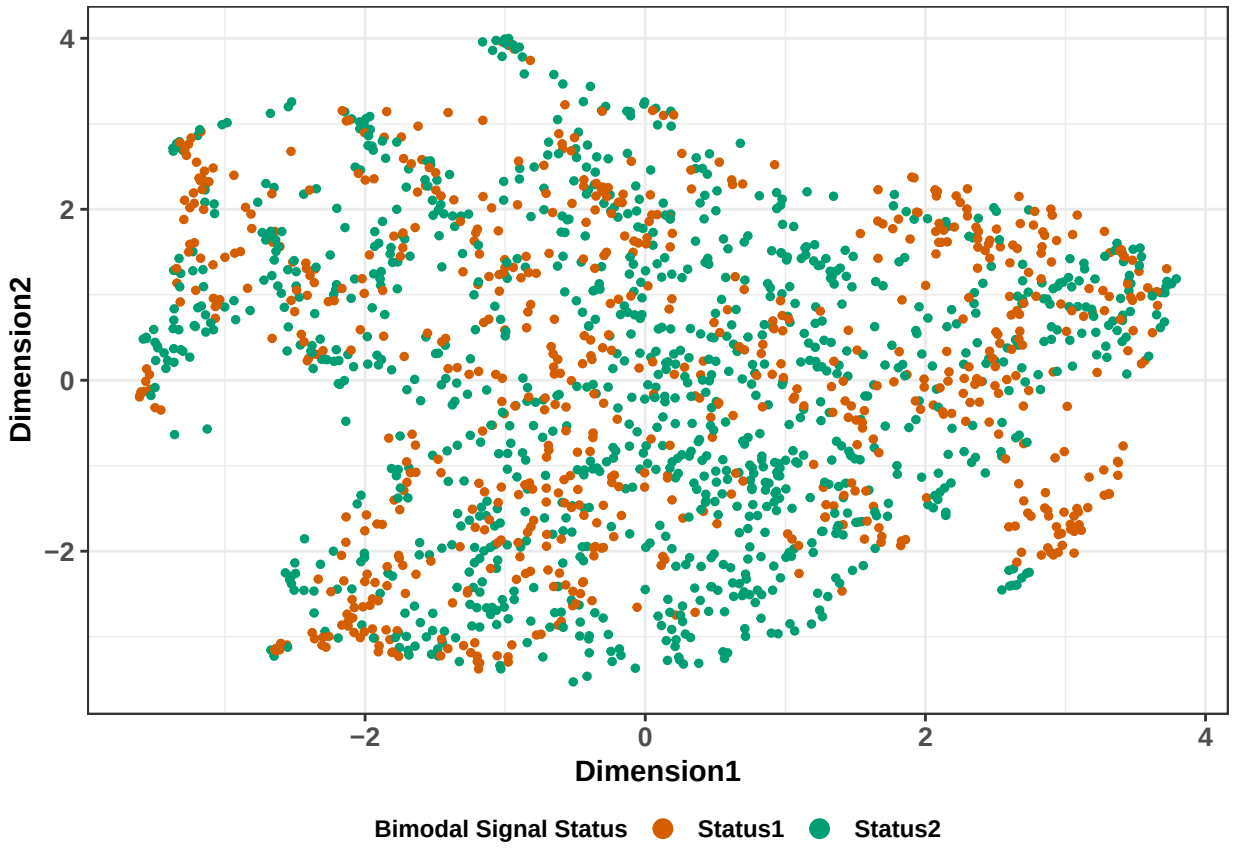
UMAP before and after batch correction

```
### read in data for the reduced dimension on Umap before bimodal signal batch correction
umap.BimodalBefore <- read.csv(paste0(intermidiate.out,"PC2 Driver - Bimodal Signal/umap.BimodalBefore.

### read in data for the reduced dimension on Umap after bimodal signal batch correction
umap.BimodalAfter <- read.csv(paste0(intermidiate.out,"PC2 Driver - Bimodal Signal/umap.BimodalAfter.csv

p.umap.BimodalBefore <- ggplot(umap.BimodalBefore) + geom_point(aes(x=D1,y=D2,color=as.factor(BimodalLab
  xlab("Dimension1") + ylab("Dimension2") + labs(color="Bimodal Signal Status") + scale_colour_manual(va
  guides(color = guide_legend(override.aes = list(size = 3))) + theme_bw() +
  theme(axis.title.x = element_text(size=11,face="bold"),axis.text.x = element_text(size=10,face="bold"
        axis.title.y =element_text(size=11,face="bold"), axis.text.y = element_text(size=10,face="bold"
        legend.position="bottom", legend.title =element_text(size = 9,face="bold"), legend.text = elemen
        legend.margin=margin(0,0,0,0),legend.box.margin=margin(-4,-4,-4,-4))

p.umap.BimodalAfter <- ggplot(umap.BimodalAfter) + geom_point(aes(x=D1,y=D2,color=as.factor(BimodalLabel
  xlab("Dimension1") + ylab("Dimension2") + labs(color="Bimodal Signal Status") + scale_colour_manual(va
  guides(color = guide_legend(override.aes = list(size = 3))) + theme_bw() +
  theme(axis.title.x = element_text(size=11,face="bold"),axis.text.x = element_text(size=10,face="bold"
```

```
        axis.title.y =element_text(size=11,face="bold"), axis.text.y = element_text(size=10,face="bold"
        legend.position="bottom", legend.title =element_text(size = 9,face="bold"), legend.text = eleme
        legend.margin=margin(0,0,0,0),legend.box.margin=margin(-4,-4,-4,-4))

### Generate subplots of Figure 3:
p.umap.BimodalBefore
```

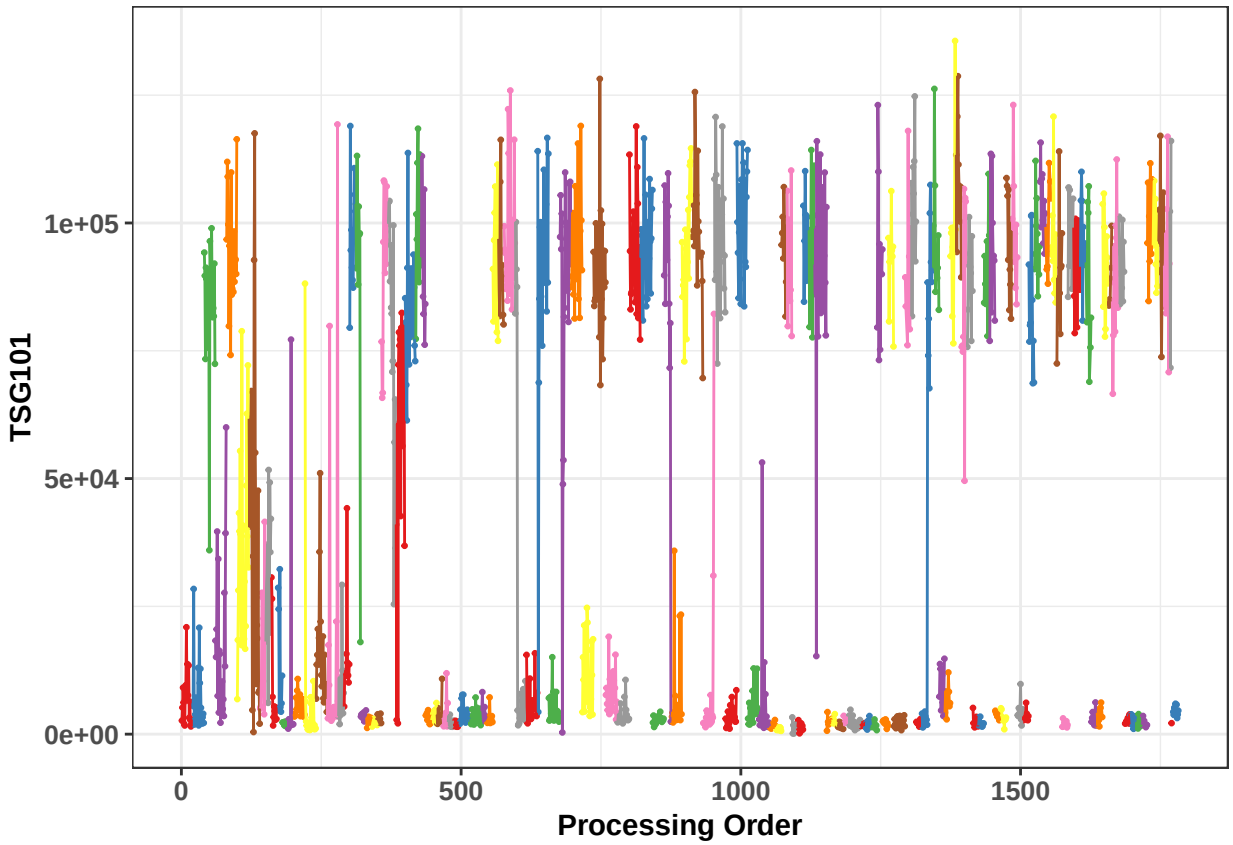



```
p.umap.BimodalAfter
```



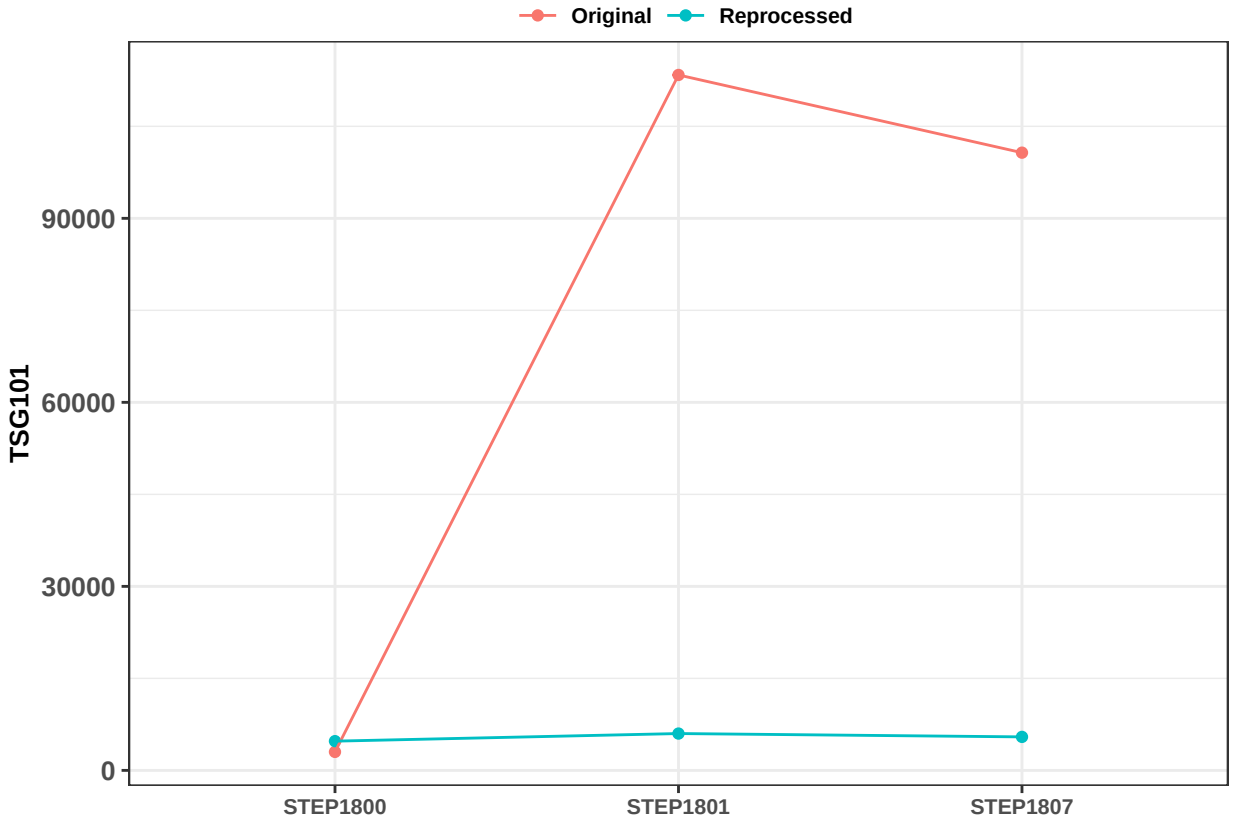
Investigate one of the strongest bimodal signal marker proteins, TSG101, against processing batch

```
### read in data for TSG101 vs processing order, processing batch; and for re-processed samples
plotData.TSG101 <- read.csv(paste0(intermidiate.out,"PC2 Driver - Bimodal Signal/plotData.TSG101.csv"),
plotData.TSG101$ProcessingBatch <- as.factor(plotData.TSG101$ProcessingBatch)

### generate a variable corresponding to the different processing batches
cols <- rep(RColorBrewer::brewer.pal(9, "Set1"),100)[1:length(levels(plotData.TSG101$ProcessingBatch))]
names(cols) <- sort(as.numeric(levels(plotData.TSG101$ProcessingBatch)))

p.TSGseq <- ggplot(plotData.TSG101,aes(x=ProcessingOrder,y=TSG101,color=ProcessingBatch),group=Processi
  geom_line() + scale_colour_manual(values = cols) +
  xlab("Processing Order") + ylab("TSG101") + theme_bw() +
  theme(legend.position="none",
        axis.title.x = element_text(size=11,face="bold"),axis.text.x = element_text(size=10,face="bold"
        axis.title.y =element_text(size=11,face="bold"), axis.text.y = element_text(size=10,face="bold"

### replication subplot of Figure 2:
p.TSGseq
```



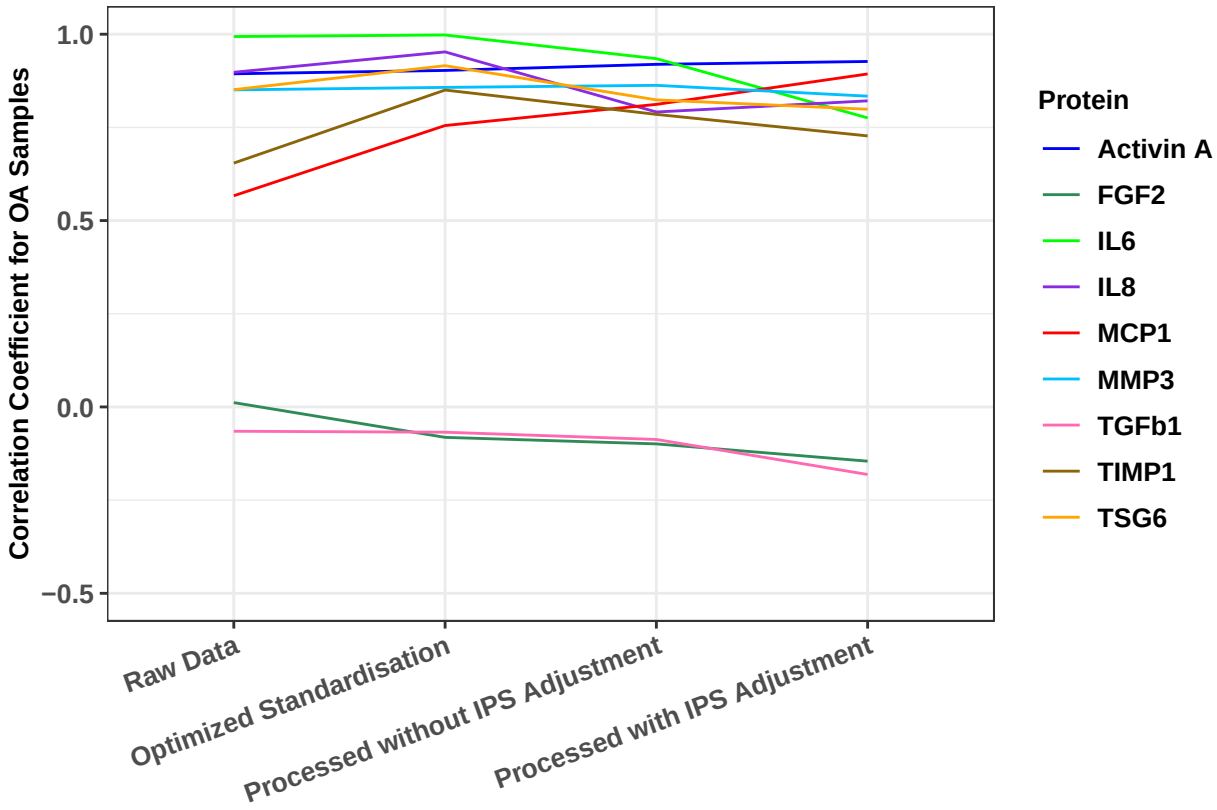
Invetigate one of the strongest bimodal signal marker proteins, TSG101, when re-processed

```
### read in TSG101 values for three samples which were reprocessed
reprocessFrame <- read.csv(paste0(intermidiate.out,"PC2 Driver - Bimodal Signal/reprocessFrame.csv"))

p.reprocess <- ggplot(data=reprocessFrame) + geom_point(aes(x=Sample,y=as.numeric(TSG101),group=Processi
  geom_line(aes(x=Sample,y=as.numeric(TSG101),group=Processing,color=Processing)) + labs(color="") + xla
  theme(axis.text.x = element_text(size=8,face="bold"),
        axis.title.y =element_text(size=10,face="bold"), axis.text.y = element_text(size=10,face="bold"
        legend.position="top",legend.title =element_text(size = 8,face="bold"), legend.text = element_te
        legend.margin=margin(0,0,0,0),legend.box.margin=margin(-10,-10,-10,-10))

### replication subplot of Figure 2:
p.reprocess
```

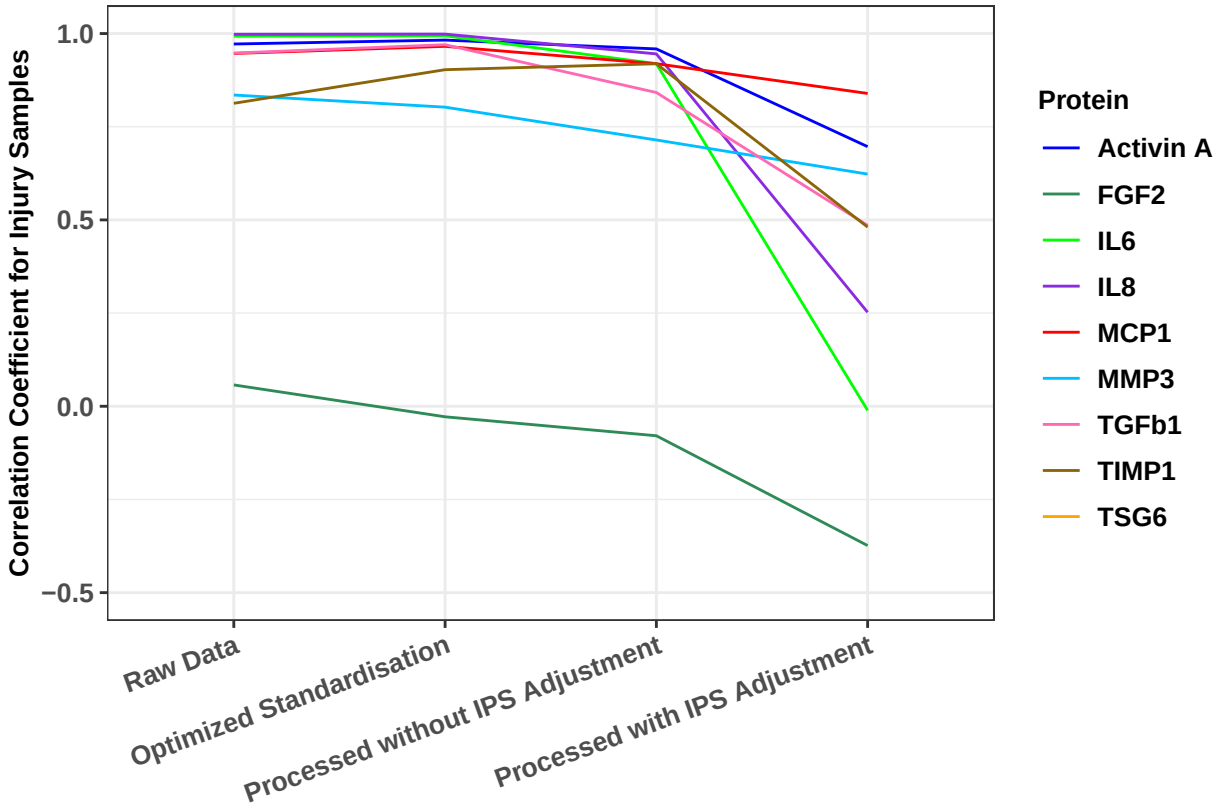


Agreement between SOMAscan and immunoassay: comparing correlation coefficient values for raw, standardized, non-IPS/IPS adjusted data

```
### read in correlation coefficient values assessing agreement between SomaScan and immunoassays for ea
CorDatP.OA.IPS <- read.csv(paste0(intermidiate.out,"Compare to Immunoassay/CorDatP.OA.IPS.csv"))
CorDatP.INJ.IPS <- read.csv(paste0(intermidiate.out,"Compare to Immunoassay/CorDatP.INJ.IPS.csv"))

NormalisationLabel = c("Raw Data","Optimized Standardisation","Processed without IPS Adjustment","Proces

p.immunoassay.OA.IPS <- ggplot(data = CorDatP.OA.IPS) + geom_line(aes(x=as.character(CorC),y=as.numeric
  xlab("") + ylab("Correlation Coefficient for OA Samples") +labs(color = "Protein") + scale_x_discrete
  theme(axis.text.x = element_text(size=10, angle=20,face="bold",hjust=1),axis.text.y = element_text(si
        legend.title =element_text(size = 10,face="bold"), legend.text = element_text(size = 10,face="b
        axis.title.y =element_text(size=10,face="bold"),axis.title.x =element_text(size=10))

p.immunoassay.INJ.IPS <- ggplot(data = CorDatP.INJ.IPS) + geom_line(aes(x=as.character(CorC),y=as.numer
  xlab("") + ylab("Correlation Coefficient for Injury Samples") +labs(color = "Protein") + scale_x_disc
  theme(axis.text.x = element_text(size=10, angle=20,face="bold",hjust=1),axis.text.y = element_text(si
        legend.title =element_text(size = 10,face="bold"), legend.text = element_text(size = 10,face="b
        axis.title.y =element_text(size=10,face="bold"),axis.title.x =element_text(size=10))

### replicate Figure 4:
p.immunoassay.OA.IPS
```

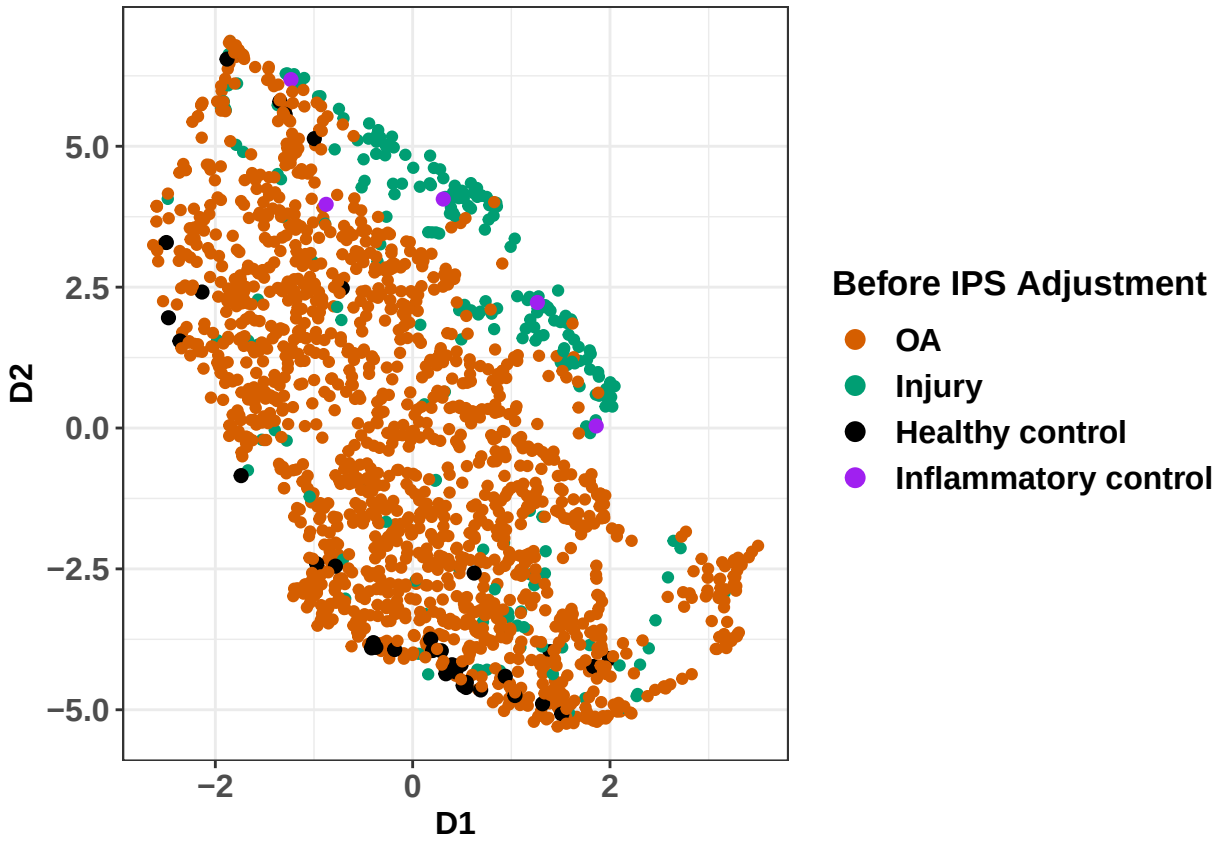



```
p.immunoassay.INJ.IPS
```



UMAP visualisation on filtered data for non-IPS adjusted and IPS adjusted data

```
### read in data for the reduced dimensions on Umap for non-IPS adjusted data
myUmap.BC.final.F <- read.csv(paste0(intermidiate.out,"Disease Group After Filtering/non-IPS adjusted.cs

### read in data for the reduced dimensions on Umap for IPS adjusted data
myUmap.IPS.final.F <- read.csv(paste0(intermidiate.out,"Disease Group After Filtering/IPS adjusted.csv"

DiseaseSize1=ifelse(myUmap.BC.final.F$DiseaseGroup==4,"Big",ifelse(myUmap.BC.final.F$DiseaseGroup==3,"M
p.umap.BC.Dis <- ggplot(data=myUmap.BC.final.F) + geom_point(aes(x=D1,y=D2,color=as.character(DiseaseGr
  xlab("D1") + ylab("D2") + scale_colour_manual(name="Before IPS Adjustment", values = c("#D55E00","#009
  scale_size_manual (values= c(2,2,1.5)) + guides(size=FALSE,color = guide_legend(override.aes = list(si
  theme(axis.title.x = element_text(size=12,face="bold"),axis.text.x = element_text(size=12,face="bold"
        axis.title.y =element_text(size=12,face="bold"), axis.text.y = element_text(size=12,face="bold"
        legend.title =element_text(size = 13,face="bold"), legend.text = element_text(size = 12,face="bo

DiseaseSize2=ifelse(myUmap.IPS.final.F$DiseaseGroup==4,"Big",ifelse(myUmap.IPS.final.F$DiseaseGroup==3,
p.umap.IPS.Dis <- ggplot(data=myUmap.IPS.final.F) + geom_point(aes(x=D1,y=D2,color=as.character(Disease
  xlab("D1") + ylab("D2") + scale_colour_manual(name="After IPS Adjustment", values = c("#D55E00","#009
  scale_size_manual (values= c(2,2,1.5)) + guides(size=FALSE,color = guide_legend(override.aes = list(si
  theme(axis.title.x = element_text(size=12,face="bold"),axis.text.x = element_text(size=12,face="bold"
        axis.title.y =element_text(size=12,face="bold"), axis.text.y = element_text(size=12,face="bold"
        legend.title =element_text(size = 13,face="bold"), legend.text = element_text(size = 12,face="bo

### plot Figure 7:
p.umap.BC.Dis
```



```
p.umap.IPS.Dis
```