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## Input files from a directory
NeuN <- "raw/NeuN-Overlap"; Nuclei <- "raw/NucleiOverlap"
UpperQ <- "raw/UpperQ"; GFP <- "raw/GFPonly"

## Output directory
output_dir <- "analysis/NeuN/"

## List files using full file name
files_neun <- list.files(NeuN, full.names = T); files_nuclei <- list.files(Nuclei, full.names = T)
files_upperq <- list.files(UpperQ, full.names = T); files_gfp <- list.files(GFP, full.names = T)

stat1 <- vector(); stat2 <- vector(); stat3 <- vector()

## Define functions in script
upper <- function(data){
  data <- sort(data, na.last = NA)
  h1 <- ((length(data)-1)*0.99)+1
  upper <- data[floor(h1)]+((h1-floor(h1))*(data[floor(h1)+1]- data[floor(h1)]))
  return(upper)
}
lower <- function(data){
  data <- sort(data, na.last = NA)
  h2 <- ((length(data)-1)*0.75)+1
  lower <- data[floor(h2)]+((h2-floor(h2))*(data[floor(h2)+1]- data[floor(h2)]))
  return(lower)
}
zfactor <- function(data1,data2){
  z <- 1 - ((3*(sd(data1, na.rm = T) + sd(data2, na.rm = T)))/(abs(mean(data1, na.rm = T) - mean(data2,
na.rm = T))))
  return(z)
}
ssmd <- function(data1,data2){
  beta <- (median(data1, na.rm = T) - median(data2, na.rm = T))/(sqrt(mad(data1, constant = 1, na.rm = T)
+ mad(data2, constant = 1, na.rm = T)))
  return(beta)
}

## Beginning of script
for (i in seq_along(files_neun)){
  file <- read.csv(files_neun[i])
  colnames(file)[2] <- "Well.R"
  txt <- subset(file, Well.R == "2" | Well.R == "23")[,3]
  ntxt <- subset(file, Well.R == "1" | Well.R == "24")[,3]
  nam <- paste("Plate", i, "_NeuN", sep="")
  data <- list(ntxt, txt)
  for (j in 1:2){
    treatment <- c("ntxt","txt")
    output1 <- mean(data[[j]], na.rm = T)
    output2 <- sd(data[[j]], na.rm = T)
    output3 <- lower(data[[j]])
    output4 <- median(data[[j]], na.rm = T)
    output5 <- upper(data[[j]])
    plate <- paste(nam, treatment[j], sep="_")
    analysis <- data.frame(Mean = output1, Std = output2, Lower = output3, Median = output4, Upper =
output5)
    write.table(analysis, file = paste(output_dir, plate, ".csv", sep = ""), sep = ",", append = F,
row.names = F, col.names = T)
  }
  ## Statistical analysis
  stat1 <- c(stat1, t.test(txt,ntxt)$p.value); stat2 <- c(stat2, zfactor(txt,ntxt)); stat3 <- c(stat3,
ssmd(txt,ntxt))
}

pvalues <- data.frame(Ttest = stat1, Zfactor = stat2, SSMD = stat3)
write.table(pvalues, file = paste(output_dir, "pvalues_NeuN_analysis.csv", sep = ""), sep = ",", append =
F, row.names = F, col.names = T)

```