

# Xenopus Cell Landscape - MafB

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## Introduction

To investigate the conservedness of MafB to imprint Mac identity across species we quantified the correlation between MafB expression and the expression of macrophage signature genes and MafB target genes in single cell atlases of mouse (Tabula Muris Senis), human (Tabula Sapiens), lemur (Tabula Microcebus), pig (Pig Cell Atlas), African clawed frog (Xenopus Cell Landscape) and zebrafish (Zebrafish Cell Landscape, ZCL).

## Load packages

```
suppressMessages({  
  library(SeuratObject)  
  library(Seurat)  
  library(reticulate)  
  library(sceasy)  
  library(ggplot2)  
  library(ggrastr)  
  library(readr)  
})
```

## Download meta.data, counts and cellInfo from Figshare

Extract meta.data from scanpy object:

[https://figshare.com/articles/dataset/Cell\\_Atlas\\_of\\_the\\_Xenopus\\_Laevis\\_at\\_Single-Cell\\_Resolution/191528](https://figshare.com/articles/dataset/Cell_Atlas_of_the_Xenopus_Laevis_at_Single-Cell_Resolution/191528)

Download counts from:

[https://figshare.com/articles/dataset/Cell\\_Atlas\\_of\\_the\\_Xenopus\\_Laevis\\_at\\_Single-Cell\\_Resolution/191528](https://figshare.com/articles/dataset/Cell_Atlas_of_the_Xenopus_Laevis_at_Single-Cell_Resolution/191528)

cellInfo:

[https://figshare.com/articles/dataset/Cell\\_Atlas\\_of\\_the\\_Xenopus\\_Laevis\\_at\\_Single-Cell\\_Resolution/191528](https://figshare.com/articles/dataset/Cell_Atlas_of_the_Xenopus_Laevis_at_Single-Cell_Resolution/191528)

## Reconstruct Seurat Object

```
XCA <- CreateSeuratObject(counts = counts, assay = "RNA", min.cells = 3,  
  meta.data = meta.data)  
  
cellInfo$...1 <- NULL  
  
rownames(cellInfo) <- cellInfo$cellID  
  
mat <- cellInfo[, c(1, 2)]  
rownames(mat) <- rownames(cellInfo)  
  
colnames(mat) <- c("tsne_1", "tsne_2")  
  
mat <- as.matrix(mat)
```

```
XCA[["tsne"]] <- CreateDimReducObject(embeddings = mat, key = "tsne_",
  assay = "RNA")

XCA <- NormalizeData(XCA)

saveRDS(XCA, file = "XCA_full.rds")
```

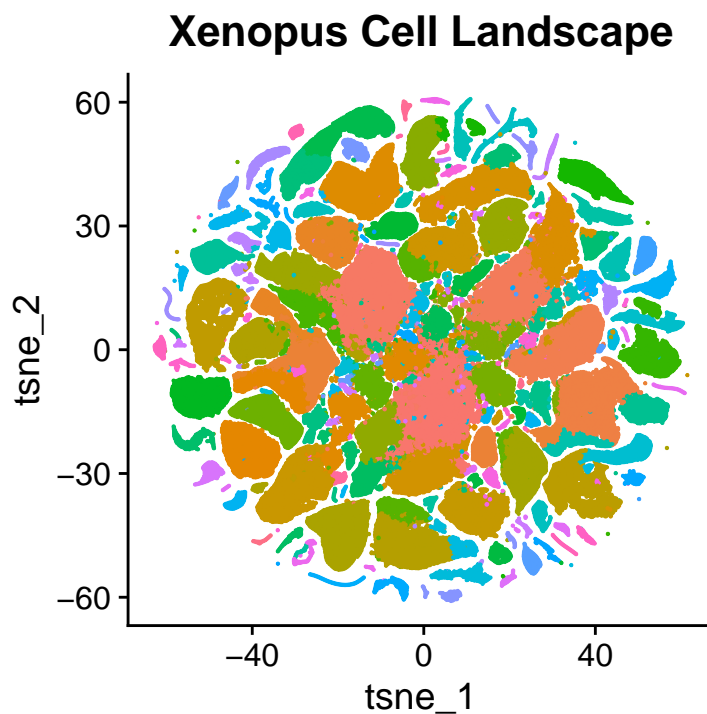
## Load Xenopus Cell Landscape Seurat object

```
XCA <- readRDS("XCA_full.rds")
```

## Visualize clusters

```
p1 <- DimPlot(XCA, group.by = "leiden", raster = F) + theme(legend.position = "none") +
  ggtitle("Xenopus Cell Landscape")

rasterize(p1, layers = "Point", dpi = 1200)
```

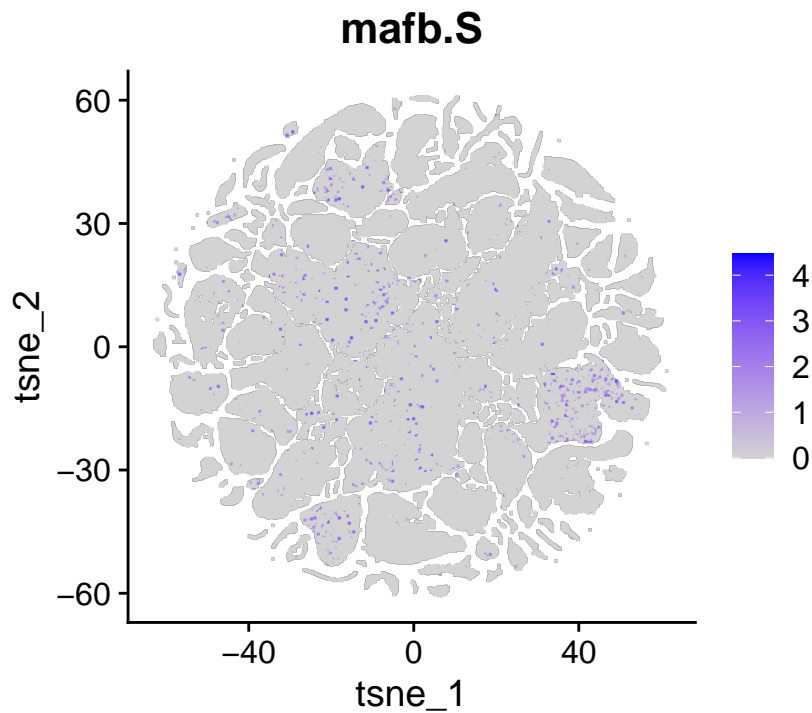


## Calculate correlation between mafb expression and Mac signature score

Note: *Xenopus laevis* has two MafB paralogs: mafb.S and mafb.L

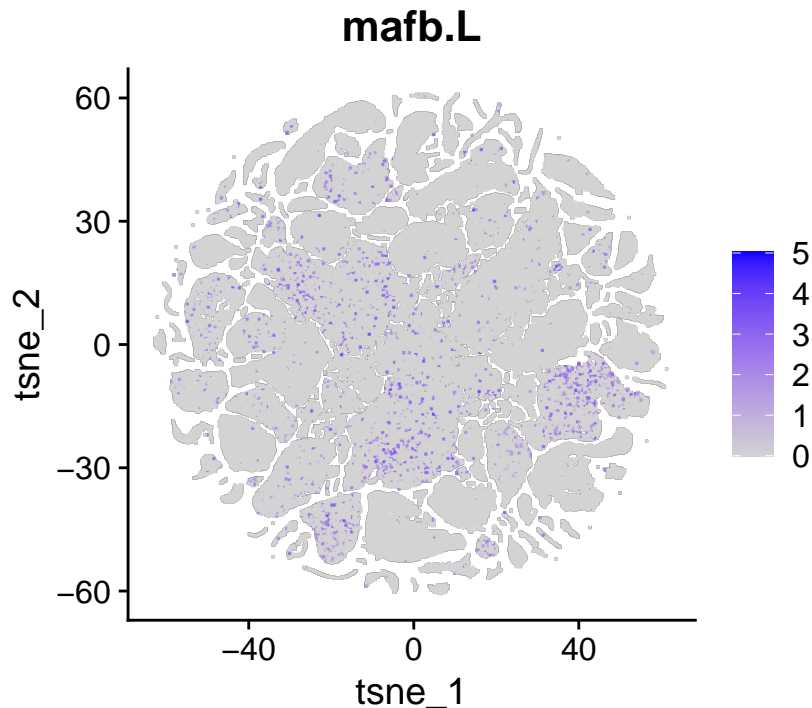
```
FeaturePlot(XCA, features = "mafb.S", raster = T)
```

```
## Rasterizing points since number of points exceeds 100,000.  
## To disable this behavior set 'raster=FALSE'
```



```
FeaturePlot(XCA, features = "mafb.L", raster = T)
```

```
## Rasterizing points since number of points exceeds 100,000.  
## To disable this behavior set 'raster=FALSE'
```



```
Mac_sign <- read_csv("Mac_sign_Xlaevis.csv")
```

```
## New names:
## Rows: 1166 Columns: 14
## -- Column specification
## ----- Delimiter: "," chr
## (11): Gene1ID, Gene1Symbol, Gene1SpeciesTaxonID, Gene1SpeciesName, Gene2... dbl
## (3): ...1, AlgorithmsMatch, OutOfAlgorithms
## 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.
## * ' ' -> '...1'
```

```
Mac_sign <- Mac_sign$Gene2Symbol
```

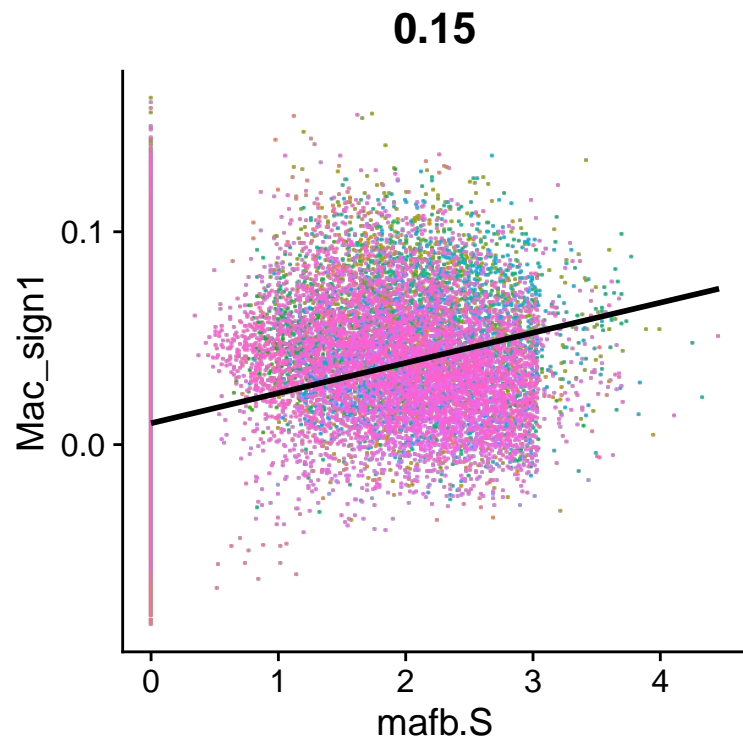
```
Mac_sign <- intersect(Mac_sign, rownames(XCA))
```

```
XCA <- AddModuleScore(XCA, features = list(c(Mac_sign)), name = "Mac_sign")
```

```
FeatureScatter(XCA, feature1 = "mafb.S", feature2 = "Mac_sign1",
  raster = T) + geom_smooth(method = "lm", colour = "black") +
  theme(legend.position = "none")
```

```
## Rasterizing points since number of points exceeds 100,000.
## To disable this behavior set 'raster=FALSE'
```

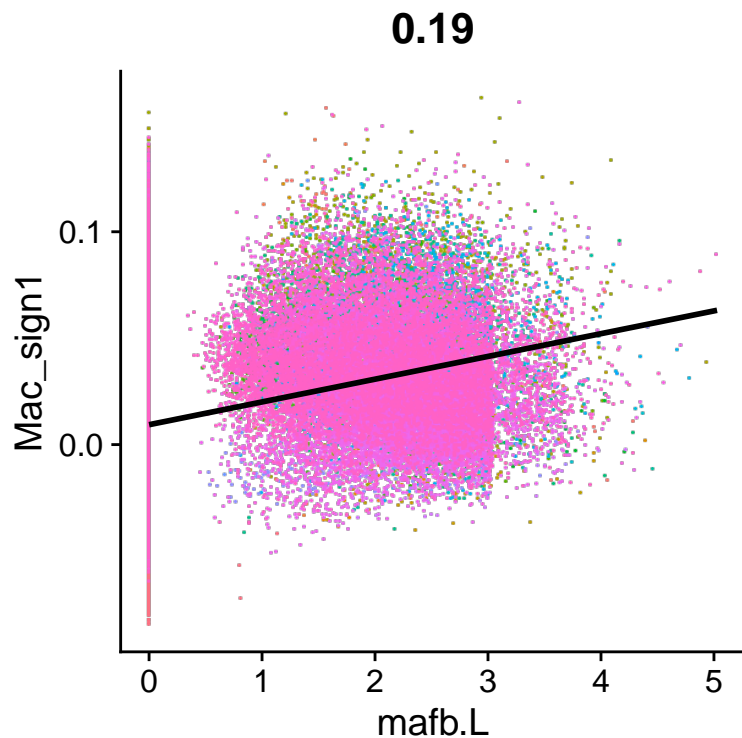
```
## 'geom_smooth()' using formula = 'y ~ x'
```



```
FeatureScatter(XCA, feature1 = "mafb.L", feature2 = "Mac_sign1",
  raster = T) + geom_smooth(method = "lm", colour = "black") +
  theme(legend.position = "none")
```

```
## Rasterizing points since number of points exceeds 100,000.
## To disable this behavior set 'raster=FALSE'
```

```
## 'geom_smooth()' using formula = 'y ~ x'
```



```
corr <- FetchData(XCA, vars = "mafb.S")
corr <- cbind(corr, FetchData(XCA, vars = "mafb.L"))
corr <- cbind(corr, FetchData(XCA, vars = "Mac_sign1"))

cor.test(corr$mafb.S, corr$Mac_sign1, method = "pearson")
```

```
##
## Pearson's product-moment correlation
##
## data:  corr$mafb.S and corr$Mac_sign1
## t = 105.27, df = 501356, p-value < 2.2e-16
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.1443415 0.1497579
## sample estimates:
##      cor
## 0.1470508
```

```
cor.test(corr$mafb.L, corr$Mac_sign1, method = "pearson")
```

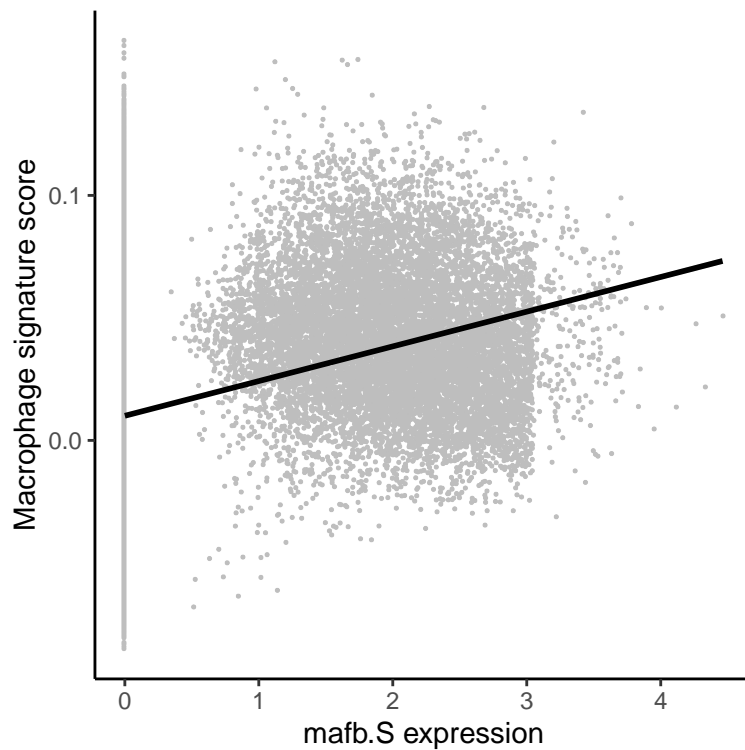
```
##
## Pearson's product-moment correlation
##
## data:  corr$mafb.L and corr$Mac_sign1
## t = 135.39, df = 501356, p-value < 2.2e-16
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.1851375 0.1904784
```

```
## sample estimates:
##      cor
## 0.1878094
```

```
p2 <- ggplot(corr, aes(x = mafb.S, y = Mac_sign1)) + geom_point(size = 0.1,
  colour = "grey") + geom_smooth(method = "lm", colour = "black") +
  xlab("mafb.S expression") + ylab("Macrophage signature score") +
  theme_classic()

rasterize(p2, layers = "Point", dpi = 600)
```

```
## 'geom_smooth()' using formula = 'y ~ x'
```

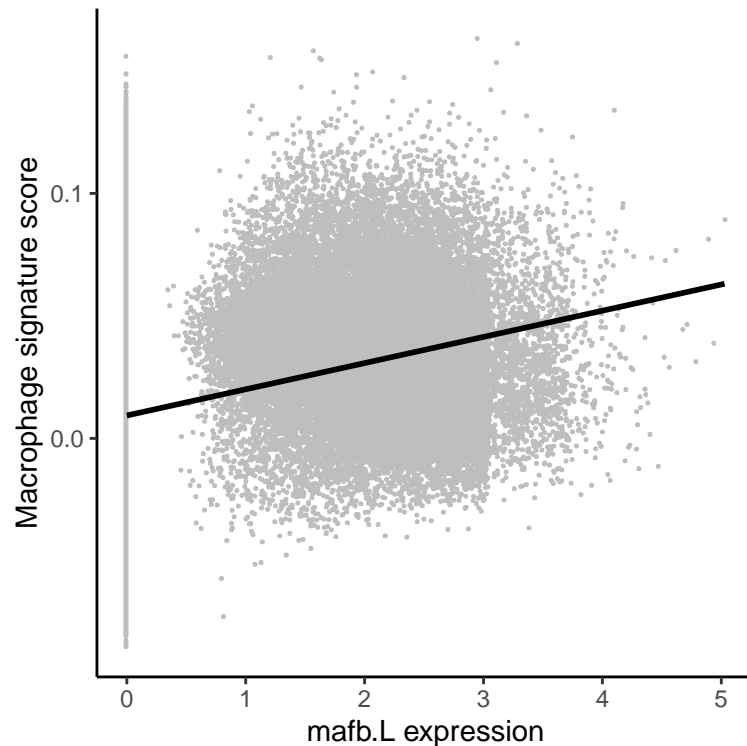


```
p3 <- ggplot(corr, aes(x = mafb.L, y = Mac_sign1)) + geom_point(size = 0.1,
  colour = "grey") + geom_smooth(method = "lm", colour = "black") +
  xlab("mafb.L expression") + ylab("Macrophage signature score") +
  theme_classic()

rasterize(p3, layers = "Point", dpi = 600)
```

```
## 'geom_smooth()' using formula = 'y ~ x'
```





Calculate correlation between MAFB expression and MafB target gene signature score

```
MafB_target_sign <- read_csv("MafB_target_Xlaevis.csv")

## New names:
## Rows: 1807 Columns: 14
## -- Column specification
## ----- Delimiter: "," chr
## (11): Gene1ID, Gene1Symbol, Gene1SpeciesTaxonID, Gene1SpeciesName, Gene2... dbl
## (3): ...1, AlgorithmsMatch, OutOfAlgorithms
## 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.
## * ' -> '...1'
```

```
MafB_target_sign <- MafB_target_sign$Gene2Symbol

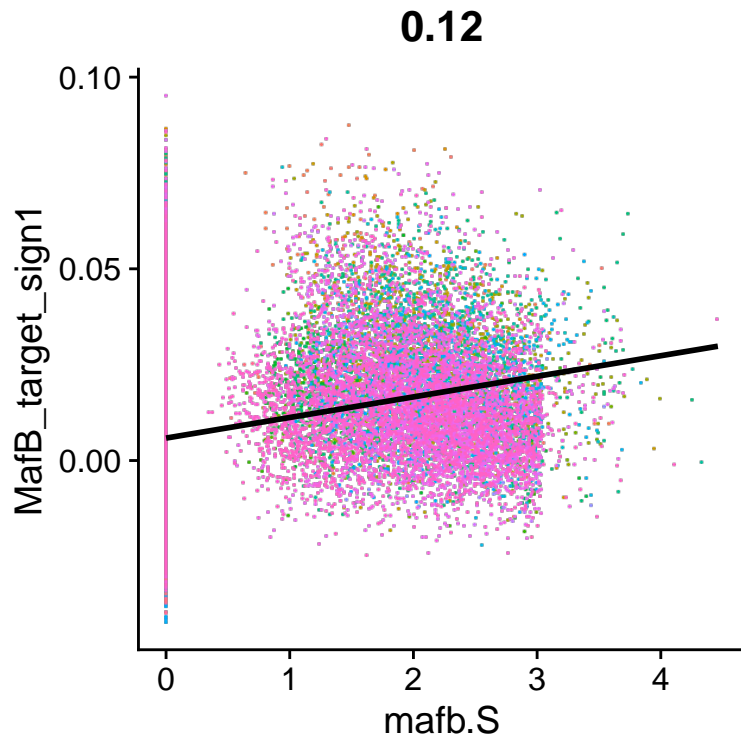
MafB_target_sign <- intersect(MafB_target_sign, rownames(XCA))

XCA <- AddModuleScore(XCA, features = list(c(MafB_target_sign)),
  name = "MafB_target_sign")
```

```
FeatureScatter(XCA, feature1 = "mafb.S", feature2 = "MafB_target_sign1",
  raster = T) + geom_smooth(method = "lm", colour = "black") +
  theme(legend.position = "none")
```

```
## Rasterizing points since number of points exceeds 100,000.
## To disable this behavior set 'raster=FALSE'
```

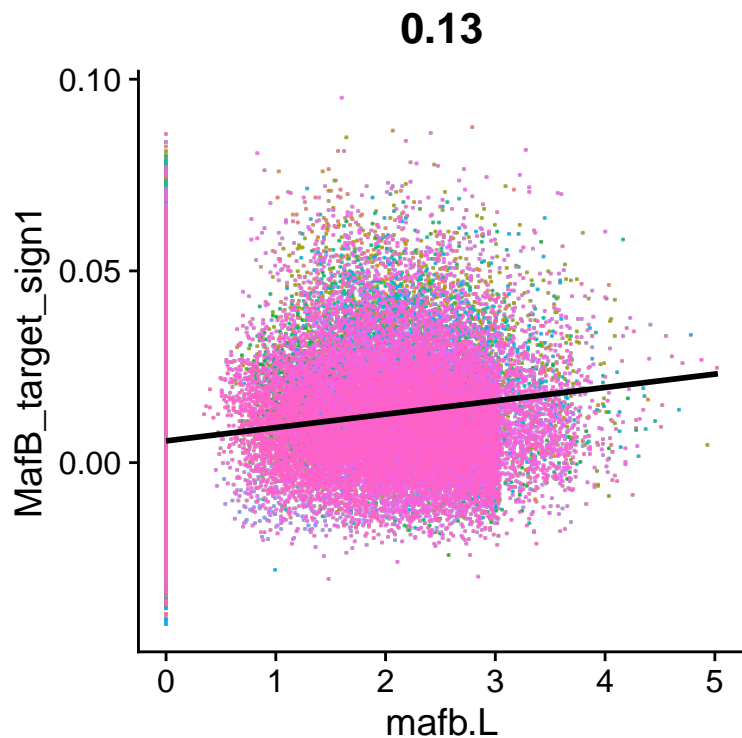
```
## 'geom_smooth()' using formula = 'y ~ x'
```



```
FeatureScatter(XCA, feature1 = "mafb.L", feature2 = "MafB_target_sign1",
  raster = T) + geom_smooth(method = "lm", colour = "black") +
  theme(legend.position = "none")
```

```
## Rasterizing points since number of points exceeds 100,000.
## To disable this behavior set 'raster=FALSE'
```

```
## 'geom_smooth()' using formula = 'y ~ x'
```



```
corr <- cbind(corr, FetchData(XCA, vars = "MafB_target_sign1"))
cor.test(corr$mafb.S, corr$MafB_target_sign1, method = "pearson")
```

```
##
## Pearson's product-moment correlation
##
## data:  corr$mafb.S and corr$MafB_target_sign1
## t = 86.628, df = 501356, p-value < 2.2e-16
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.1187108 0.1241652
## sample estimates:
##      cor
## 0.1214389
```

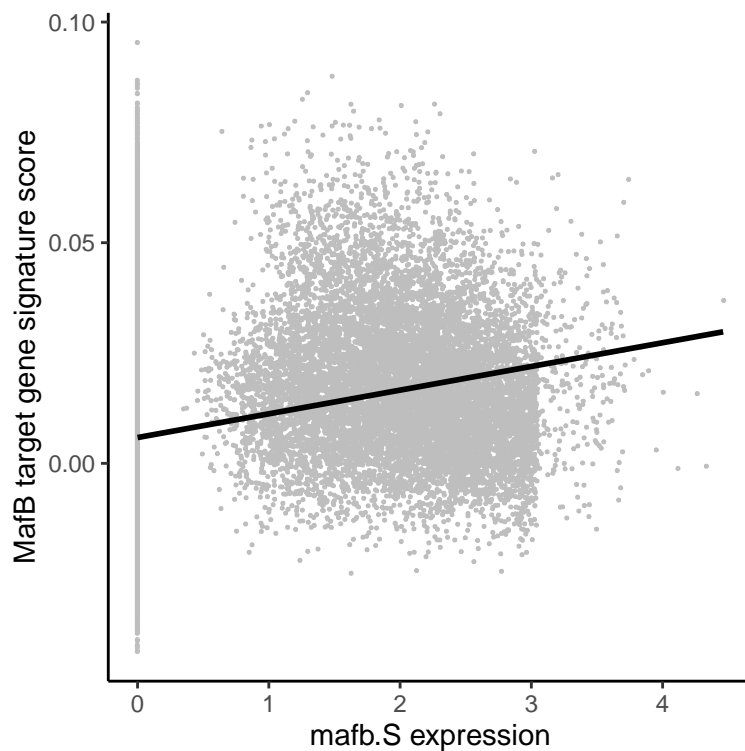
```
cor.test(corr$mafb.L, corr$MafB_target_sign1, method = "pearson")
```

```
##
## Pearson's product-moment correlation
##
## data:  corr$mafb.L and corr$MafB_target_sign1
## t = 95.262, df = 501356, p-value < 2.2e-16
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.1306168 0.1360545
## sample estimates:
##      cor
```

```
## 0.1333367
```

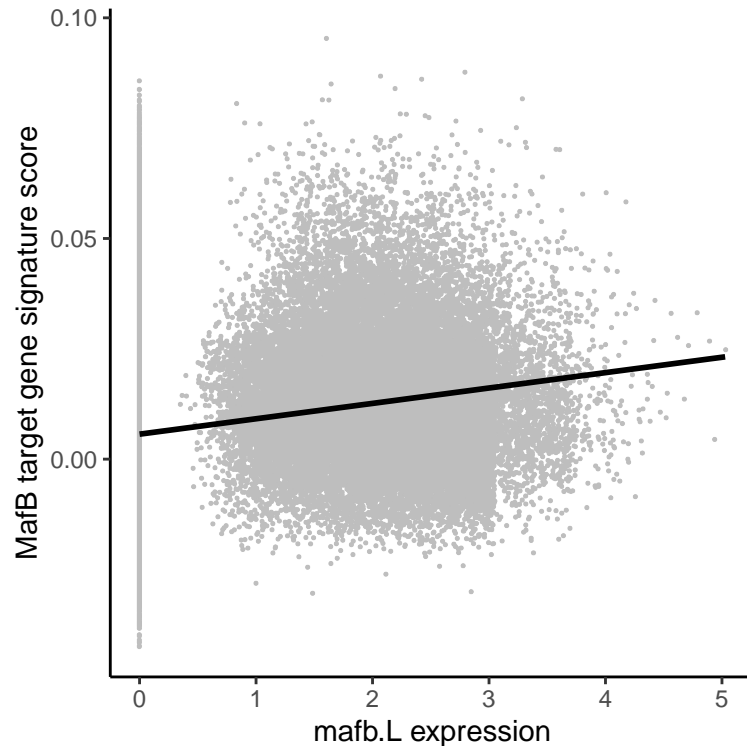
```
p4 <- ggplot(corr, aes(x = mafb.S, y = MafB_target_sign1)) +  
  geom_point(size = 0.1, colour = "grey") + geom_smooth(method = "lm",  
    colour = "black") + xlab("mafb.S expression") + ylab("MafB target gene signature score") +  
  theme_classic()  
  
rasterize(p4, layers = "Point", dpi = 600)
```

```
## 'geom_smooth()' using formula = 'y ~ x'
```



```
p5 <- ggplot(corr, aes(x = mafb.L, y = MafB_target_sign1)) +  
  geom_point(size = 0.1, colour = "grey") + geom_smooth(method = "lm",  
    colour = "black") + xlab("mafb.L expression") + ylab("MafB target gene signature score") +  
  theme_classic()  
  
rasterize(p5, layers = "Point", dpi = 600)
```

```
## 'geom_smooth()' using formula = 'y ~ x'
```



```
sessionInfo()
```

```
## R version 4.4.1 (2024-06-14)
## Platform: x86_64-pc-linux-gnu
## Running under: Ubuntu 20.04.6 LTS
##
## Matrix products: default
## BLAS: /usr/lib/x86_64-linux-gnu/blas/libblas.so.3.9.0
## LAPACK: /usr/lib/x86_64-linux-gnu/lapack/liblapack.so.3.9.0
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
##  [3] LC_TIME=fr_BE.UTF-8      LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=fr_BE.UTF-8  LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=fr_BE.UTF-8     LC_NAME=C
##  [9] LC_ADDRESS=C             LC_TELEPHONE=C
## [11] LC_MEASUREMENT=fr_BE.UTF-8 LC_IDENTIFICATION=C
##
## time zone: Europe/Brussels
## tzcode source: system (glibc)
##
## attached base packages:
## [1] stats      graphics  grDevices  utils      datasets  methods    base
##
## other attached packages:
## [1] readr_2.1.5      ggrastr_1.0.2    ggplot2_3.4.4    sceasy_0.0.7
## [5] reticulate_1.39.0 Seurat_4.3.0     SeuratObject_4.1.3 sp_2.1-4
##
## loaded via a namespace (and not attached):
```

```

## [1] RColorBrewer_1.1-3      rstudioapi_0.16.0      jsonlite_1.8.9
## [4] magrittr_2.0.3          spatstat.utils_3.1-0   ggbeeswarm_0.7.2
## [7] farver_2.1.2            rmarkdown_2.28         vctrs_0.6.5
## [10] ROCR_1.0-11             Cairo_1.6-2            spatstat.explore_3.3-2
## [13] htmltools_0.5.8.1       sctransform_0.4.1      parallelly_1.26.0
## [16] KernSmooth_2.23-24      htmlwidgets_1.5.3      ica_1.0-2
## [19] plyr_1.8.6              plotly_4.10.4          zoo_1.8-9
## [22] igraph_1.2.6            mime_0.11              lifecycle_1.0.4
## [25] pkgconfig_2.0.3         Matrix_1.6-1.1         R6_2.5.1
## [28] fastmap_1.2.0           fitdistrplus_1.1-5     future_1.21.0
## [31] shiny_1.9.1             digest_0.6.37          colorspace_2.1-1
## [34] patchwork_1.1.1         tensor_1.5             irlba_2.3.5.1
## [37] labeling_0.4.3          progressr_0.14.0       fansi_1.0.6
## [40] spatstat.sparse_3.1-0   mgcv_1.9-1            httr_1.4.7
## [43] polyclip_1.10-0         abind_1.4-5            compiler_4.4.1
## [46] bit64_4.5.2            withr_3.0.1           highr_0.11
## [49] MASS_7.3-61            tools_4.4.1           vipor_0.4.7
## [52] lmtest_0.9-38          beeswarm_0.4.0         httpuv_1.6.1
## [55] future.apply_1.7.0      goftest_1.2-2          glue_1.7.0
## [58] nlme_3.1-165           promises_1.2.0.1       grid_4.4.1
## [61] Rtsne_0.15             cluster_2.1.6          reshape2_1.4.4
## [64] generics_0.1.0         gtable_0.3.5           spatstat.data_3.1-2
## [67] tzdb_0.4.0            tidyr_1.3.1           data.table_1.14.0
## [70] hms_1.1.3             utf8_1.2.4            spatstat.geom_3.3-3
## [73] RcppAnnoy_0.0.18       ggrepel_0.9.6          RANN_2.6.1
## [76] pillar_1.9.0           stringr_1.5.1          vroom_1.6.5
## [79] later_1.2.0            splines_4.4.1         dplyr_1.1.4
## [82] lattice_0.22-5         bit_4.5.0             survival_3.7-0
## [85] deldir_2.0-4           tidyselect_1.2.1      miniUI_0.1.1.1
## [88] pbapply_1.4-3          knitr_1.48            gridExtra_2.3
## [91] scattermore_0.7        xfun_0.47             matrixStats_1.4.1
## [94] stringi_1.6.2          lazyeval_0.2.2         yaml_2.2.1
## [97] evaluate_1.0.0         codetools_0.2-19      tibble_3.2.1
## [100] cli_3.6.3             uwot_0.2.2           xtable_1.8-4
## [103] munsell_0.5.1          Rcpp_1.0.13           globals_0.14.0
## [106] spatstat.random_3.3-2  png_0.1-8            spatstat.univar_3.0-1
## [109] parallel_4.4.1         listenv_0.8.0         viridisLite_0.4.2
## [112] scales_1.3.0           ggridges_0.5.3        crayon_1.4.1
## [115] leiden_0.3.8           purrr_1.0.2           rlang_1.1.4
## [118] cowplot_1.1.1         formatR_1.14

```