5 Single Cell Data Exploration

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08/09/2019

Load required libraries

```
library(URD)
library(Seurat)
```

Single Cell Data Exploration

We analyzed the homeostatic expression patterns of the 36 high mapping gene transcripts upregulated in response to hywi knockdown by interrogating single-cell expression data (Siebert, 2019). To interrogate expression, we used URD spline objects for ectoderm and endoderm. These objects contain expression data for genes that are expressed in at least 1% of the ectodermal or endodermal epithelial cells. We found epithelial expression for 24 of the 36 putative gene targets: 1) nineteen gene transcripts are expressed in both the endodermal and ectodermal epithelial lineages, 2) one transcript is expressed only in the ectodermal epithelial lineage, and 3) four transcripts are expressed only in the endodermal epithelial lineage. No epithelial expression was found for 12 of the gene transcripts, which could indicate low expression in a homeostatic animal. Higher expression at the extremities in both the ectoderm and endoderm and lower expression in body regions would be consistent with Hywi-mediated repression. Putative targets could show high expression. The gene, t14391, shows such a pattern.

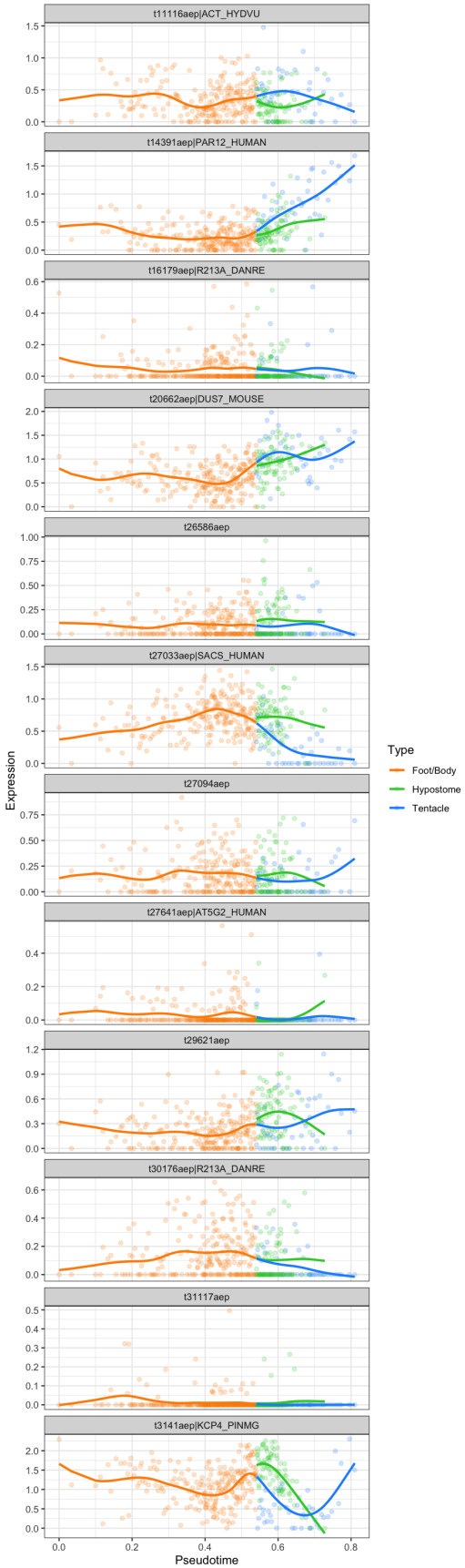
Load the Necessary Files

```
# Load the list of searchable high mapping genes for each lineage

endo_mappers <- read.table("objects/high_mappers_endo.txt", header = T)

ecto_mappers <- read.table("objects/high_mappers_ecto.txt", header = T)

# Load the seurat object to load the searchable transcript IDs The seurat object
# (file Hydra_Seurat_Whole_Transcriptome) can be downloaded from Dryad:
# https://doi.org/10.5061/dryad.v5r6077. After download, place in the 'objects'
# folder

Hydra_Object <- readRDS("objects/Hydra_Seurat_Whole_Transcriptome.rds")

# Load single cell pseudotime objects (spline objects) and High Mapping Gene List
# The spline objects (file Hydra_URD_analysis_objects) can be downloaded from
# Dryad: https://doi.org/10.5061/dryad.v5r6077. After download, place in the
# 'objects' folder

endoderm.splines <- readRDS("objects/Splines-Endoderm.rds")
ectoderm.splines <- readRDS("objects/Splines-Ectoderm.rds")
```

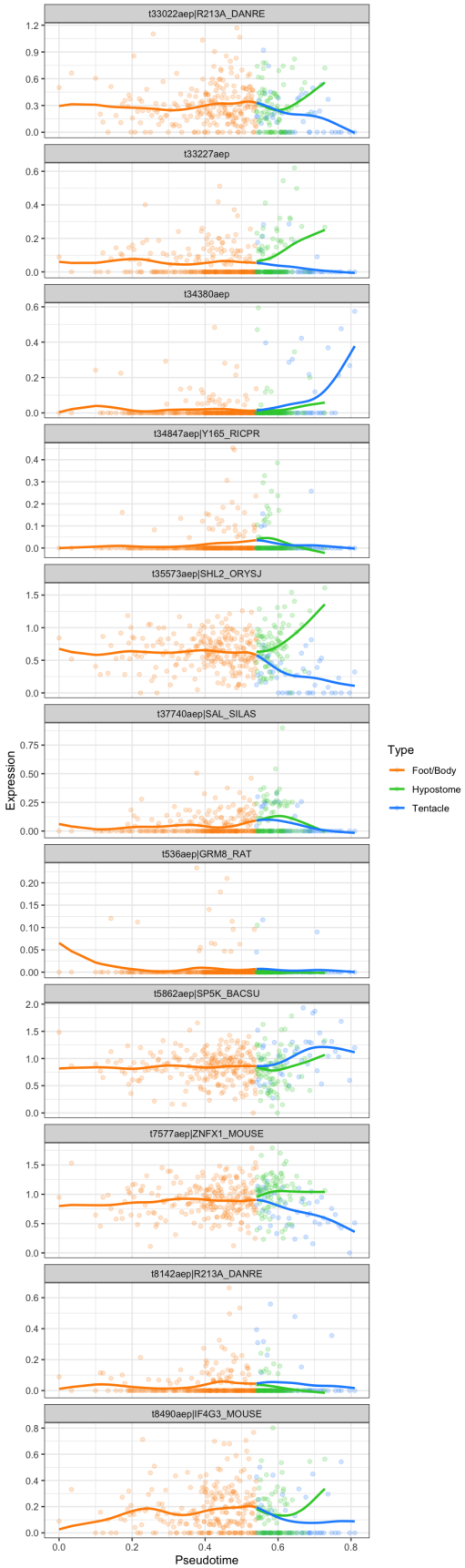
Data Exploration

```
# Establish hFind function to return the searchable transcript IDs

hFind <- function(x) {
    return(Hydra_Object@data@Dimnames[[1]][grep(x, Hydra_Object@data@Dimnames[[1]],
        ignore.case = T)])
}

# Run hFind function to convert to searchable transcript IDs

endo_mappers$ID <- sapply(endo_mappers$ID, FUN = hFind)

ecto_mappers$ID <- sapply(ecto_mappers$ID, FUN = hFind)
```

```
# Generate Endodermal Plots

plotSmoothFitMultiCascade(endoderm.splines, genes = endo_mappers$ID[1:12], scaled = F,
    colors = c(`Foot/Body` = "#FF8C00", Tentacle = "#1E90FF", Hypostome = "#32CD32"),
    ncol = 1)
```

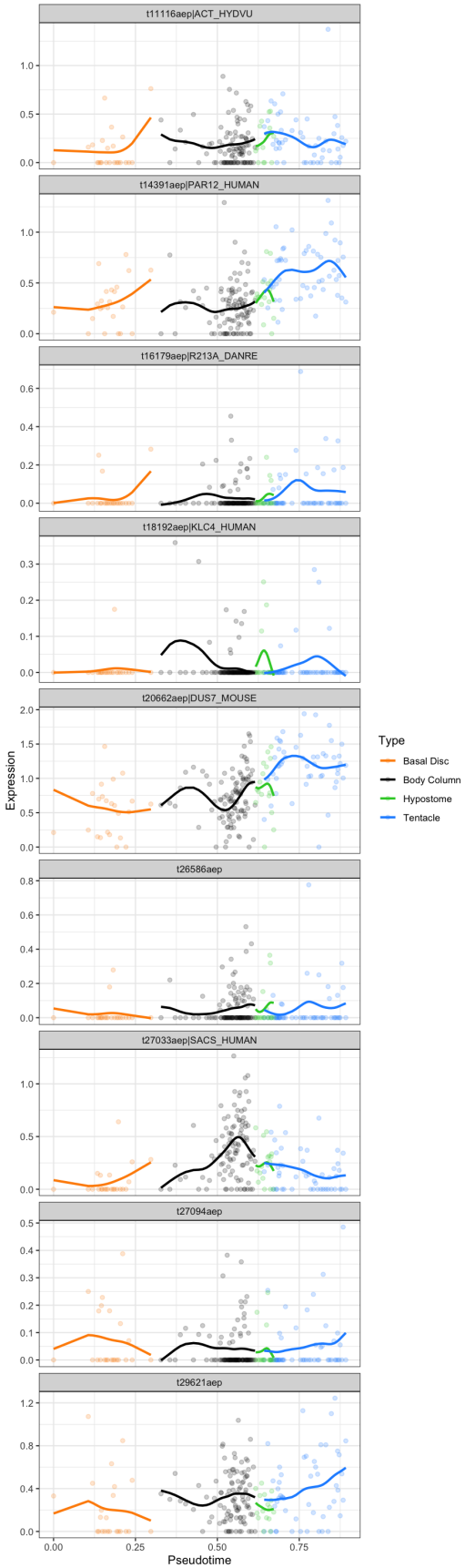

t11116aep|ACT_HYDVU
t14391aep|PAR12_HUMAN
t16179aep|R213A_DANRE
t20662aep|DUS7_MOUSE
t26586aep
t27033aep|SACS_HUMAN
t27094aep
t27641aep|AT5G2_HUMAN
t29621aep
t30176aep|R213A_DANRE
t31117aep
t3141aep|KCP4_PINMG
Expression
Pseudotime
Type
Foot/Body
Hypostome
Tentacle

```r
plotSmoothFitMultiCascade(endoderm.splines, genes = endo_mappers$ID[13:23], scaled = F,
    colors = c(`Foot/Body` = "#FF8C00", Tentacle = "#1E90FF", Hypostome = "#32CD32"),
    ncol = 1)
```

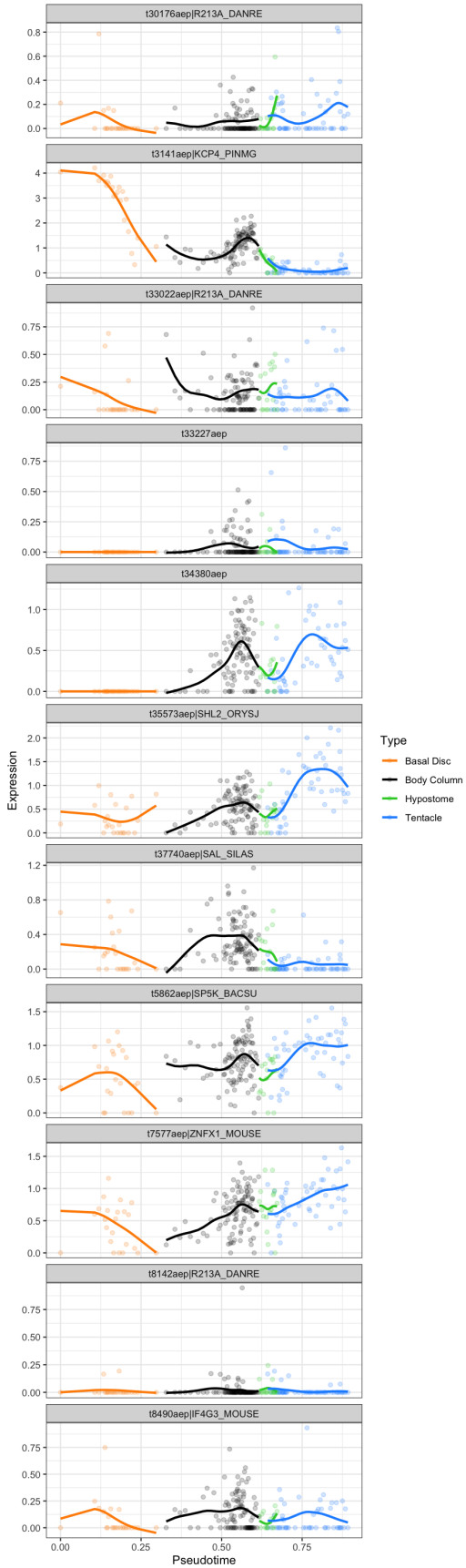

t33022aep|R213A_DANRE
t33227aep
t34380aep
t34847aep|Y165_RICPR
t35573aep|SHL2_ORYSJ
t37740aep|SAL_SILAS
t536aep|GRM8_RAT
t5862aep|SP5K_BACSU
t7577aep|ZNFX1_MOUSE
t8142aep|R213A_DANRE
t8490aep|IF4G3_MOUSE
Expression
Pseudotime
Type
Foot/Body
Hypostome
Tentacle

```r
# Generate Ectodermal Plots

plotSmoothFitMultiCascade(ectoderm.splines, genes = ecto_mappers$ID[1:9], scaled = F,
    colors = c(`Basal Disc` = "#FF8C00", `Body Column` = "#000000", Tentacle = "#1E90FF",
        Hypostome = "#32CD32"), ncol = 1)
```


t11116aep|ACT_HYDVU
t14391aep|PAR12_HUMAN
t16179aep|R213A_DANRE
t18192aep|KLC4_HUMAN
t20662aep|DUS7_MOUSE
t26586aep
t27033aep|SACS_HUMAN
t27094aep
t29621aep
Expression
Pseudotime
0.00
0.25
0.50
0.75
Type
Basal Disc
Body Column
Hypostome
Tentacle

```
plotSmoothFitMultiCascade(ectoderm.splines, genes = ecto_mappers$ID[10:20], scaled = F,
    colors = c(`Basal Disc` = "#FF8C00", `Body Column` = "#000000", Tentacle = "#1E90FF",
        Hypostome = "#32CD32"), ncol = 1)
```


t30176aep|R213A_DANRE
t3141aep|KCP4_PINMG
t33022aep|R213A_DANRE
t33227aep
t34380aep
t35573aep|SHL2_ORYSJ
t37740aep|SAL_SILAS
t5862aep|SP5K_BACSU
t7577aep|ZNFX1_MOUSE
t8142aep|R213A_DANRE
t8490aep|IF4G3_MOUSE
Expression
Pseudotime
Type
Basal Disc
Body Column
Hypostome
Tentacle

Software versions

This document was computed on Fri Aug 09 19:25:35 2019 with the following R package versions.

```
R version 3.5.3 (2019-03-11)
Platform: x86_64-apple-darwin15.6.0 (64-bit)
Running under: macOS Mojave 10.14.5

Matrix products: default
BLAS: /Library/Frameworks/R.framework/Versions/3.5/Resources/lib/libRblas.0.dylib
LAPACK: /Library/Frameworks/R.framework/Versions/3.5/Resources/lib/libRlapack.dylib

locale:
[1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8

attached base packages:
[1] stats     graphics  grDevices utils     datasets  methods   base

other attached packages:
[1] Seurat_2.3.4  cowplot_0.9.4 URD_1.0.3     Matrix_1.2-16 ggplot2_3.2.0
[6] knitr_1.22

loaded via a namespace (and not attached):
  [1] reticulate_1.11.1           R.utils_2.8.0
  [3] tidyselect_0.2.5            htmlwidgets_1.3
  [5] grid_3.5.3                  trimcluster_0.1-2.1
  [7] ranger_0.11.2               BiocParallel_1.14.2
  [9] Rtsne_0.15                  munsell_0.5.0
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 [15] colorspace_1.4-1            Biobase_2.40.0
 [17] rstudioapi_0.9.0            stats4_3.5.3
 [19] ROCR_1.0-7                  robustbase_0.93-3
 [21] dtw_1.20-1                  vcd_1.4-4
 [23] VIM_4.8.0                   TTR_0.23-4
 [25] gbRd_0.4-11                 labeling_0.3
 [27] Rdpack_0.10-1               lars_1.2
 [29] GenomeInfoDbData_1.1.0      polyclip_1.10-0
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```

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```