

Monocytes can Proliferate in Vacant Tissue Niches prior to Differentiation into Macrophages

10-DE genes across pseudotime

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Abstract

Resident tissue macrophages (RTM) are differentiated immune cells populating distinct niches and exhibiting important tissue-supportive functions. RTM maintenance is thought to depend either on monocyte engraftment and differentiation, or on the self-renewal of mature RTM. Here, we discovered that monocytes can re-enter cell cycle and proliferate locally before their differentiation into RTM. We developed a mouse model of inducible lung interstitial macrophage (IM) depletion in which the vacant niche is repopulated by BM-derived monocytes giving rise to fully differentiated IM subsets. By performing time-course single-cell RNA-sequencing analyses of myeloid cells during niche refilling, we found that few Ly6C+ classical monocytes could self-renew locally in a CSF1R-dependent manner. We further showed that the transcription factor MafB restricted such proliferation and was essential to mediate RTM specification and identity in our model. Our data provide evidence that, in the mononuclear phagocyte system, self-renewal is not merely restricted to myeloid progenitor cells and mature macrophages, but is also a tightly regulated capability of mature monocytes developing into RTM *in vivo*.

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1 Description

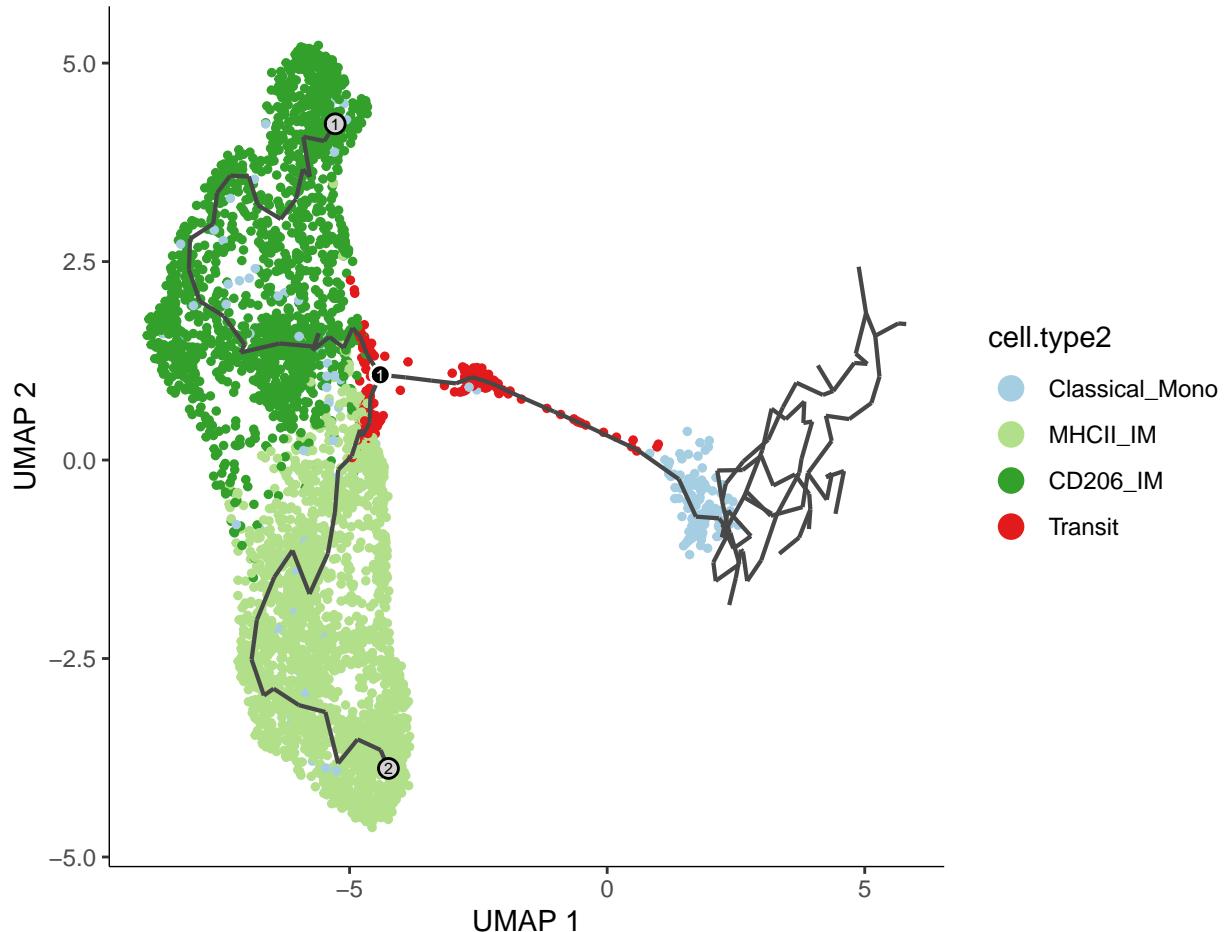
To compare the expression patterns of DE genes across pseudotime, the counts matrix, pseudotime and cell weights calculated above were then used as input in fitGAM function (TradeSeq package) (Berge et al., 2020). The association of average expression of each gene with pseudotime was tested using associationTest and the DE genes between CD206+ and CD206- IM trajectories were calculated with diffEndTest function. The value of the estimated smoother on a grid of pseudotimes was estimated for each of DE gene using predictSmooth. The DE genes with waldStat > 70 and |logFC| > 2 were annotated as “changed genes,” meaning that their expression patterns were different in CD206+ and CD206- IM trajectories, while the rest of DE genes were considered as “unchanged genes,” meaning that the expression patterns were similar in both trajectories. Finally, the scaled estimated smoothers calculated by predictSmooth were used to build heatmap with ComplexHeatmap package (Gu et al., 2016).

2 Prepare data

```
suppressMessages(  
{  
  library(Seurat)  
  library(ComplexHeatmap)  
  library(ggplot2)  
  library(dplyr)  
  library(RColorBrewer)  
  library(circlize)  
  library(monocle3)  
}  
  
mo <- readRDS(file = ".../9-Monocle_analysis_and_pseudotime_estimation/Mono  
_to_IM.cds")
```

Show trajectory in UMAP plot:

```
pal2 <- c(`Classical_Mono`="#A6CEE3",  
           `MHCII_IM`="#B2DF8A",  
           `CD206_IM`="#33A02C",  
           `Transit` = "#E31A1C")  
  
plot_cells(cds = mo, color_cells_by = "cell.type2",  
           cell_size = 1, label_cell_groups = FALSE,  
           label_branch_points = TRUE, label_leaves = TRUE,  
           label_roots = FALSE, alpha = 1) +  
           scale_color_manual(values = pal2)
```



```
ggsave(filename = ".../Figures/UMAPplot_trajectory.pdf", width = 6.5,
       height = 5)
```

3 DE gene expression across IM-differentiation

DE genes across pseudotime of IM differentiation ## Across pseudotime of IM differentiation

Prepare matrix with z-scores, smoothed and scaled data across pseudotime for heatmap.

```
pt.matrix <- exprs(mo)[match(genes, rownames(rowData(mo))), order(pseudotime
  (mo))]
cellnames <- colnames(pt.matrix)
#Can also use "normalized_counts" instead of "exprs" to use various
  normalization methods, for example:
#normalized_counts(cds, norm_method = "log")

pt.matrix <- t(apply(pt.matrix, 1, function(x){smooth.spline(x, df=3)$y}))
pt.matrix <- t(apply(pt.matrix, 1, function(x){(x-mean(x))/sd(x)}))
rownames(pt.matrix) <- genes
colnames(pt.matrix) <- cellnames
```

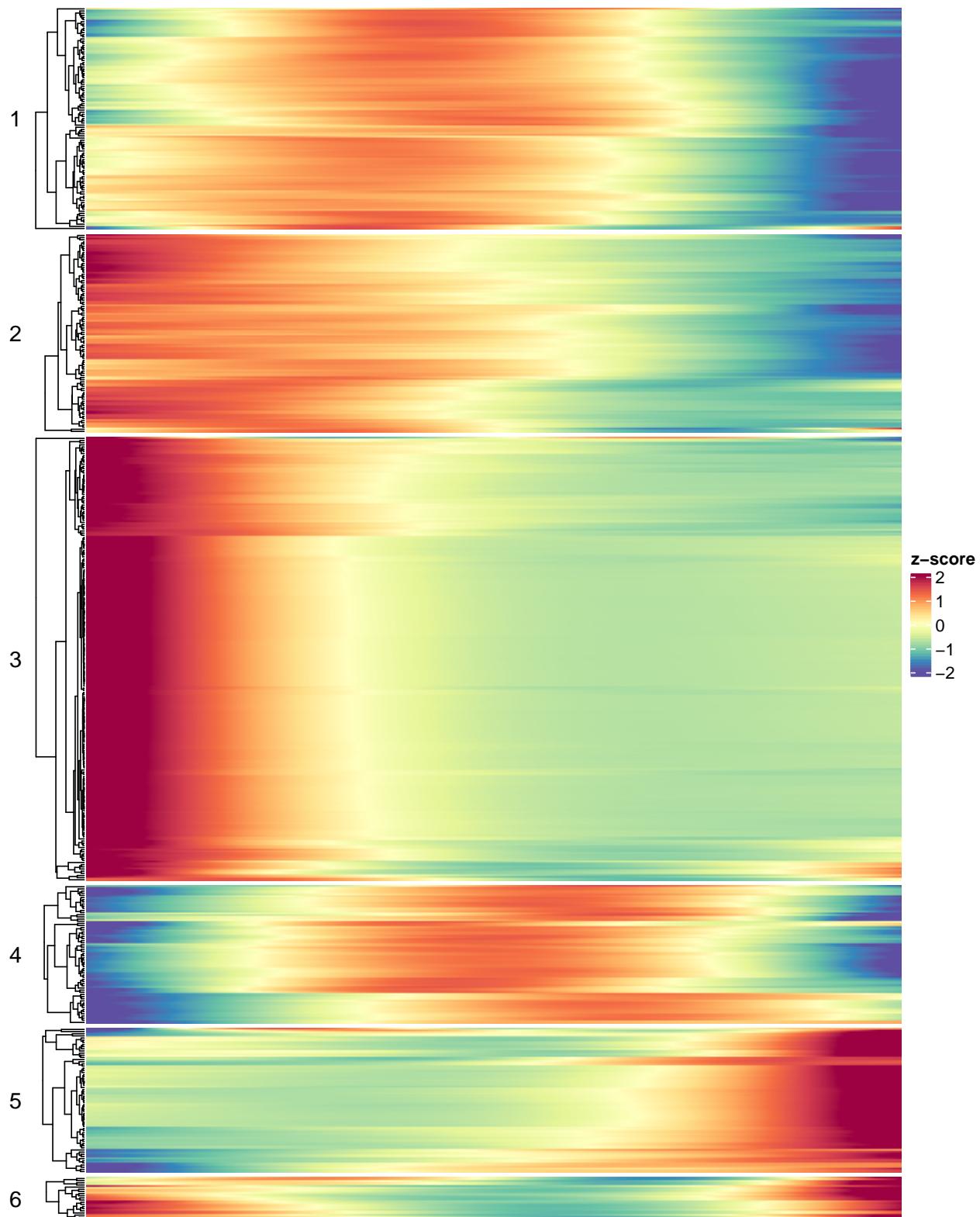
Show DE genes in unsupervised heatmap.

```
#K means with 6 groups
```

```

htkm <- Heatmap(
  pt.matrix,
  # use_raster = FALSE, # use FALSE to export to vector image.
  name                = "z-score",
  col                 = colorRamp2(seq(from=-2,to=2,length=11),
    rev(brewer.pal(11, "Spectral"))),
  show_row_names       = FALSE,
  show_column_names    = FALSE,
  row_names_gp         = gpar(fontsize = 3),
  row_km = 6,
  row_km_repeats = 31,
  row_dend_reorder = TRUE,
  row_title_rot        = 0,
  cluster_rows          = TRUE,
  cluster_row_slices    = FALSE,
  cluster_columns        = FALSE,
)
htkm <- draw(htkm)

```



In this heatmap, the x axis is pseudotime, which represents differentiation state from monocytes (left) to IMs (right).

3.1 Annotate the cells associated to either differentiation of CD206+ IMs or CD206- IMs

```

library(magrittr)                                     1
# Get the closest vertice for every cell           2
y_to_cells <- mo@principal_graph_aux$UMAP$pr_graph_cell_proj_closest_ 3
  vertex%>%as.data.frame()

y_to_cells$cells <- rownames(y_to_cells)           4
y_to_cells$Y <- y_to_cells$V1                     5

# Get the root vertices                           6
# It is the same node as above                  7
root <- mo@principal_graph_aux$UMAP$root_pr_nodes 8

principalgraph <- mo@principal_graph$UMAP          9

# Get the other endpoints                         10
endpoints <- names(which(igraph::degree(principalgraph) == 1))    11
endpoints <- endpoints[!endpoints %in% root]        12

# For each endpoint                            13
cellWeights <- lapply(endpoints, function(endpoint) { 14
  # We find the path between the endpoint and the root 15
  path <- igraph::shortest_paths(principalgraph, root, endpoint)$vpath 16
  [[1]]
  path <- as.character(path)                      17
  # We find the cells that map along that path 18
  df <- y_to_cells[y_to_cells$Y %in% path, ]      19
  df <- data.frame(weights = as.numeric(colnames(mo) %in% df$cells)) 20
  colnames(df) <- endpoint                       21
  return(df)
}) %>% do.call(what = 'cbind', args = .) %>%
  as.matrix()                                     22
rownames(cellWeights) <- colnames(mo)             23
colnames(cellWeights) <- c("CD206_IM_branch", "MHCII_IM_branch") 24
pseudotime <- matrix(mo@principal_graph_aux$UMAP$pseudotime, ncol = ncol( 25
  cellWeights),                                26
  nrow = ncol(mo), byrow = FALSE)                27
rownames(pseudotime) <- colnames(mo)              28

```

4 TradeSeq analysis for the differentiation of monocytes to either of IM subsets

4.1 Construct sce object for TradeSeq

```

suppressMessages(library(tradeSeq))               1

# this step is VERY time consuming               2
sce <- fitGAM(counts = mo@assays$data$counts,   3
  pseudotime = pseudotime,
  cellWeights = cellWeights)                   4

```

```
saveRDS(sce, file = "./sce.4339cells.newversion.Rds")
```

5
6

Between-lineage comparisons (CD206+ IM vs CD206- IM differentiation) ## Between-lineage comparisons (CD206+ IM vs CD206- IM differentiation)

Association of gene expression with pseudotime (find significant DE genes along pseudotime).

```
assoRes <- associationTest(sce)
endRes <- diffEndTest(sce)
head(assoRes)
```

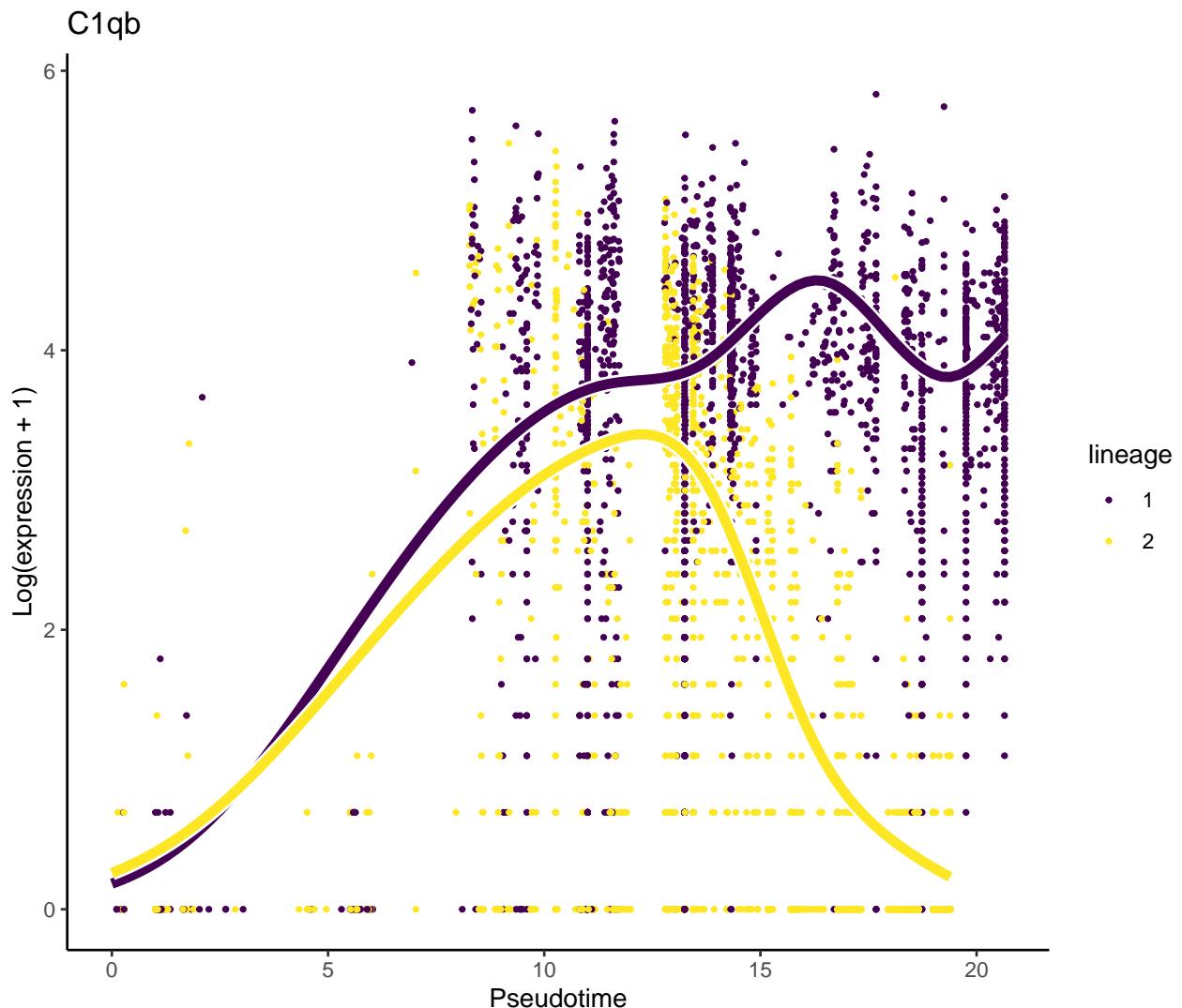
```
## # A tibble: 6 x 4
##   waldStat     df      pvalue  meanLogFC
##       <dbl>    <dbl>      <dbl>      <dbl>
## 1     210.      9     0.222
## 2     28.4      9    0.000815
## 3       NA      NA     NA     0.121
## 4     41.8      9    0.00000360
## 5     36.4      9    0.0000330
## 6     45.1      9    0.000000880
```

1
2
3
4
5
6
7
8
9

Plot the most sig gene:

```
library(ggplot2)
o <- order(endRes$waldStat, decreasing = TRUE)
sigGene <- names(sce)[o[1]]
plotSmoothers(sce, counts = counts(sce), gene = sigGene
               #, curvesCol = c("#33A02C", "#B2DF8A")
               ) + ggtitle(sigGene)
```

1
2
3
4
5
6



What's the top genes?

```

names(sce)[o[1:20]]                                1
## [1] "C1qb"      "Ctsb"       "C1qa"       "Selenop"   "Csf1r"     "Timp2"     "Pf4"        1
## [8] "C1qc"       "Serinc3"    "Cd209a"    "Lsp1"      "Lgmn"      "Apoe"      "Blvrb"     2
##
## [15] "Olfm1"     "Tnip3"     "Rpl13"     "Ninj1"     "Rpl28"     "H2-DMb1"   3

```

4.2 Clustering using RSEC, clusterExperiment

tradeSeq provides the functionality to cluster genes according to their expression pattern along the lineages with the clusterExpressionPatterns function. A number of equally spaced points for every lineage are selected to perform the clustering, and the number of points can be selected with the nPoints argument. (from vignette("tradeSeq"))

```

library(clusterExperiment)
nPointsClus <- 20 # The number of points to use for clustering the
# expression patterns..
clusPat <- clusterExpressionPatterns(sce ,                               1
                                         2
                                         3

```

```

nPoints = nPointsClus,
genes = genes,
random.seed = 43,
beta = 0.2
)

## 36 parameter combinations, 36 use sequential method, 36 use subsampling
## Running Clustering on Parameter Combinations...
## done.

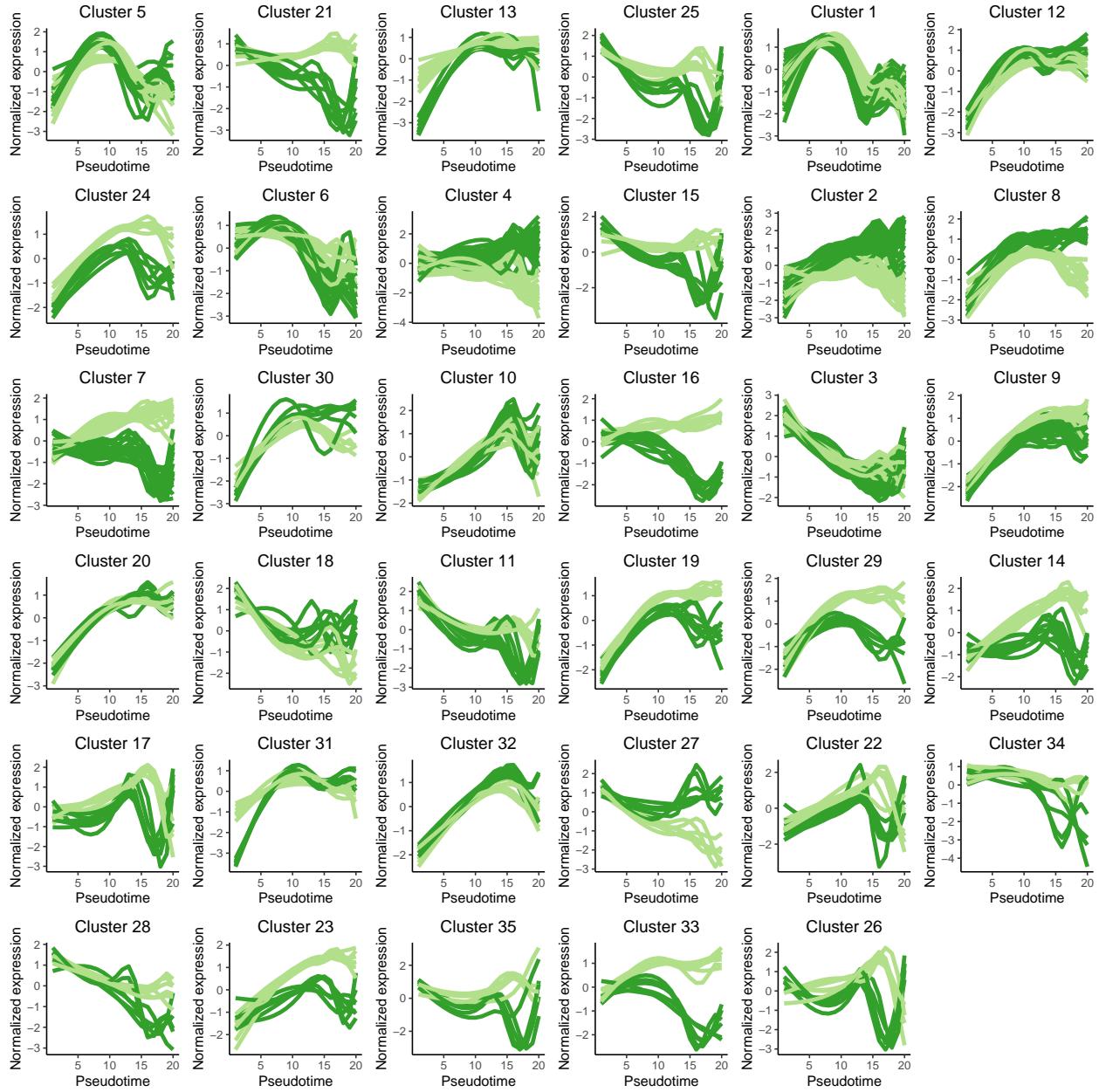
clusterLabels <- primaryCluster(clusPat$rsec)

cUniq <- unique(clusterLabels) #
cUniq <- cUniq[!cUniq == -1] # remove unclustered genes

if (exists("p.total")) { rm(p.total)}

for (xx in cUniq) {
  cId <- which(clusterLabels == xx)
  p <- ggplot(data = data.frame(x = 1:nPointsClus,
                                 y = rep(range(clusPat$yhatScaled[cId, ]),
                                         nPointsClus / 2)),
               aes(x = x, y = y)) +
    geom_point(alpha = 0) +
    labs(title = paste0("Cluster", xx), x = "Pseudotime", y = "Normalized expression") +
    theme_classic() +
    theme(plot.title = element_text(hjust = 0.5))
  for (ii in 1:length(cId)) {
    geneId <- rownames(clusPat$yhatScaled)[cId[ii]]
    p <- p +
      geom_line(data = data.frame(x = rep(1:nPointsClus, 2),
                                   y = clusPat$yhatScaled[geneId, ],
                                   lineage = rep(0:1, each = nPointsClus)),
                 aes(col = as.character(lineage), group = lineage), lwd =
                   1.5)
  }
  p <- p + guides(color = "none") +
    scale_color_manual(values = c("#33A02C", "#B2DF8A"),
                       breaks = c("0", "1"))
  if (exists("p.total")) { p.total <- p.total + p} else {p.total <- p}
}
print(p.total)

```



5 Show gene expression pattern calculated by TradeSeq in heatmap

5.1 Data preparation

Here we use the DE genes calculated in DE genes across pseudotime of IM differentiation.

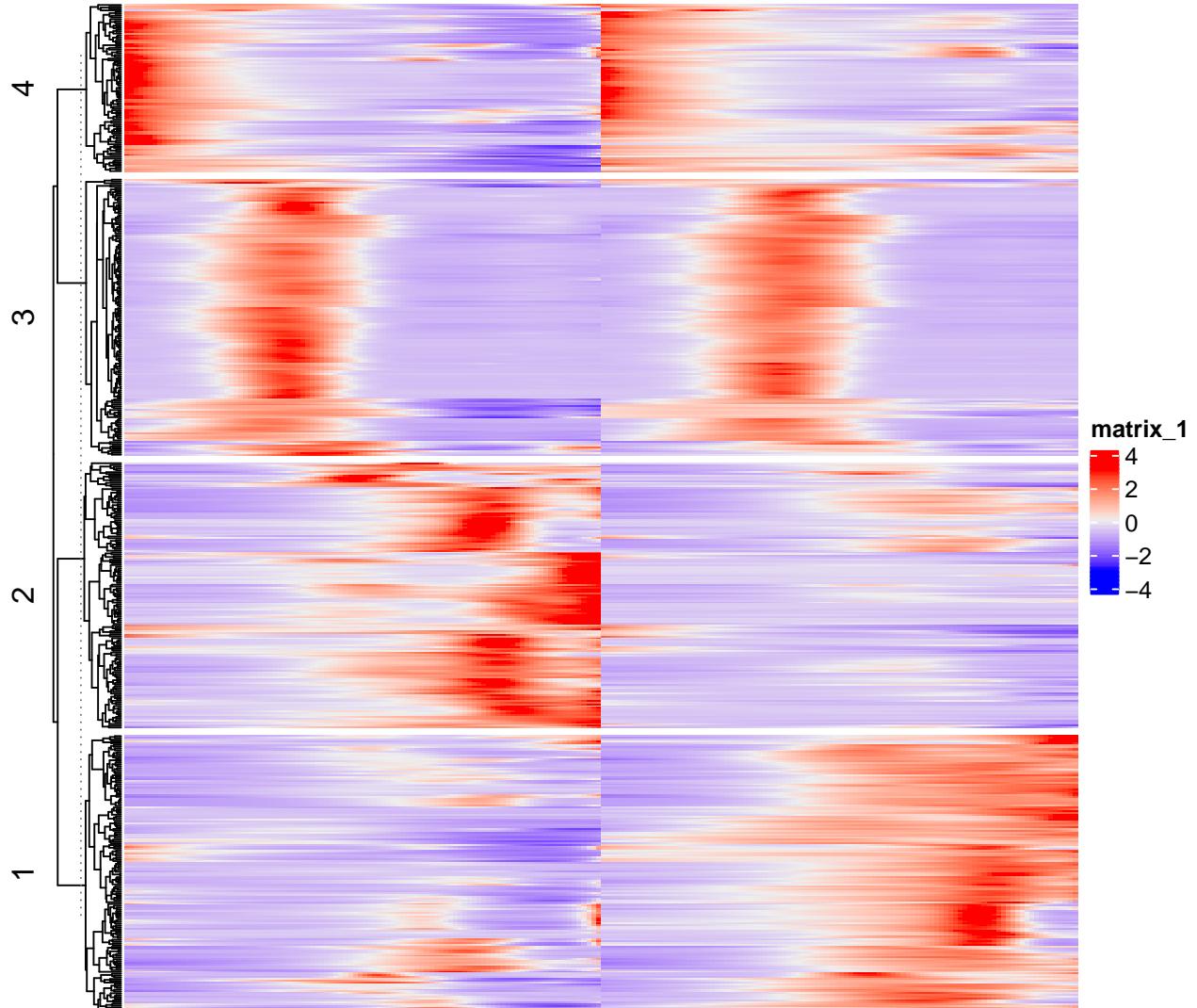
```
yhatSmooth <- predictSmooth(sce, gene = genes, nPoints = 100, tidy = FALSE) 1
      )
yhatSmoothScaled <- t(scale(t(yhatSmooth))) 2
```

5.2 Draw heatmap

```

heatSmooth <- Heatmap(yhatSmoothScaled, cluster_columns = FALSE, show_row_ 1
  names = FALSE, show_column_names = FALSE, row_km = 4)
heatSmooth <- draw(heatSmooth) 2

```



Two IM differentiation show similar patterns but some genes (especially cluster 2 and 1) are different in CD206+ and CD206-.

5.3 Annotate DE genes as CD206+/CD206- IM differentiation specific or common genes

According to the heatmap above, some of DE genes should remain unchanged (common) and half of them are specific to one of two IM differentiation.

We use wald statistic calculated in diffEndTest to annotate the “common” genes and “specific” genes. (in Between-lineage comparisons (CD206+ IM vs CD206- IM differentiation))

```

endRes.DE <- endRes[rownames(yhatSmooth), ]
summary(endRes.DE$waldStat) 1
 2

```

##	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.	1
##	0.0027	3.8599	41.4571	123.7353	168.7434	1263.9587	2

Let's use waldStat > 40 and logFC > 2 as cut threshold.

```
genes.changed <- rownames(filter(endRes.DE, waldStat > 70 & (logFC1_2 > 2
| logFC1_2 < -2) ))
genes.noChange <- setdiff(rownames(endRes.DE) , genes.changed)
```

Make heatmap with unchanged/common genes.

```
heatSmooth_cd206.unchanged <- Heatmap(yhatSmoothScaled[genes.noChange ,
100:1], cluster_columns = FALSE, show_row_names = FALSE, show_column_
names = FALSE, column_title = "CD206+IM")  

1  

heatSmooth_mhcii.unchanged <- Heatmap(yhatSmoothScaled[genes.noChange ,
101:200], cluster_columns = FALSE, show_row_names = TRUE, row_names_gp
= gpar(fontsize = 3), show_column_names = FALSE, column_title = "MHCII
+IM")  

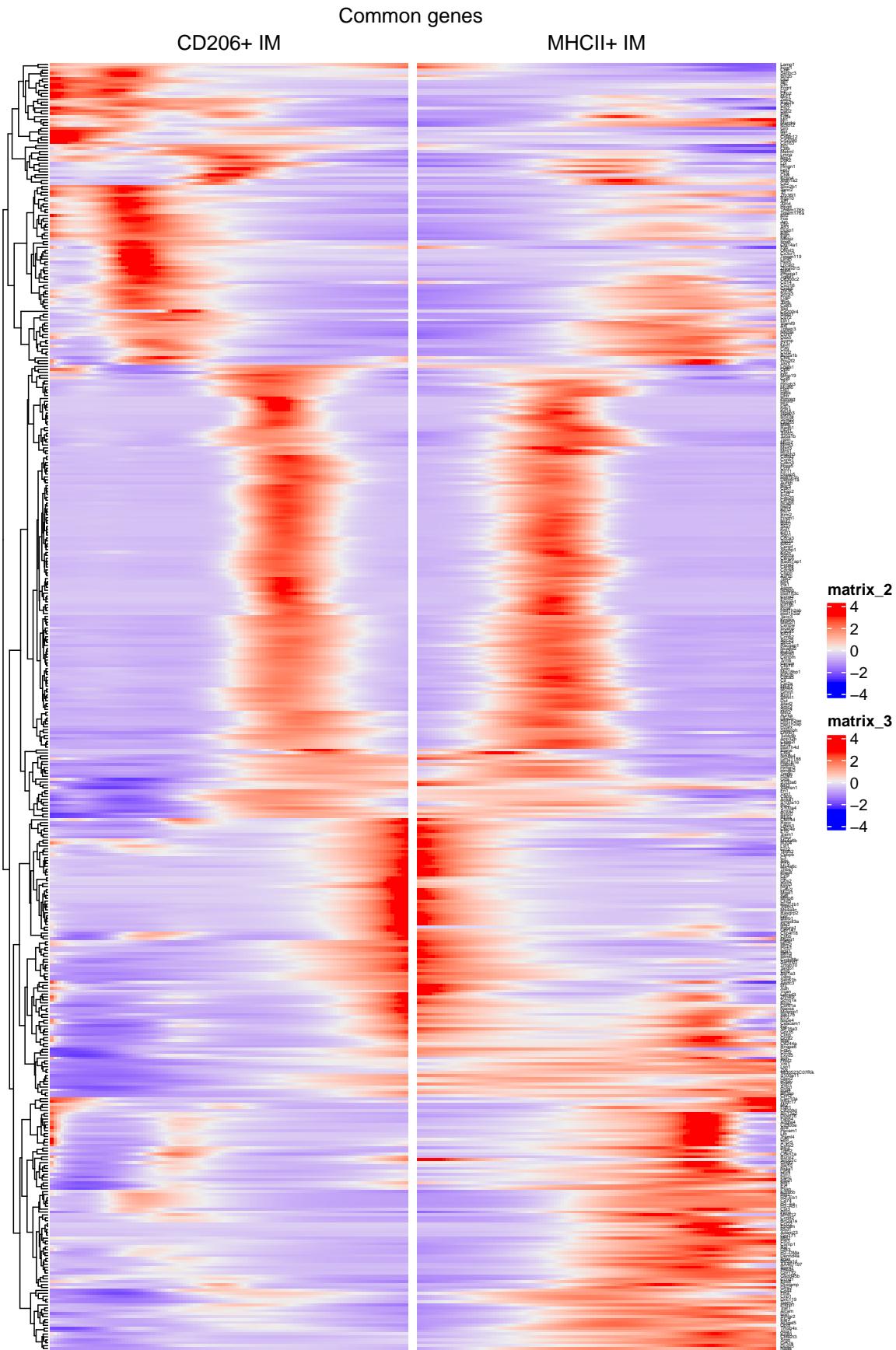
2  

heatSmooth_combined.unchanged <- draw ( heatSmooth_cd206.unchanged +
heatSmooth_mhcii.unchanged, column_title = "Common genes", auto_adjust
= FALSE)  

3  

4  

5
```



5.4 Draw heatmap with expression patterns of unchanged/common genes in the order of pseudotime

Let's find the expression peak of each gene:

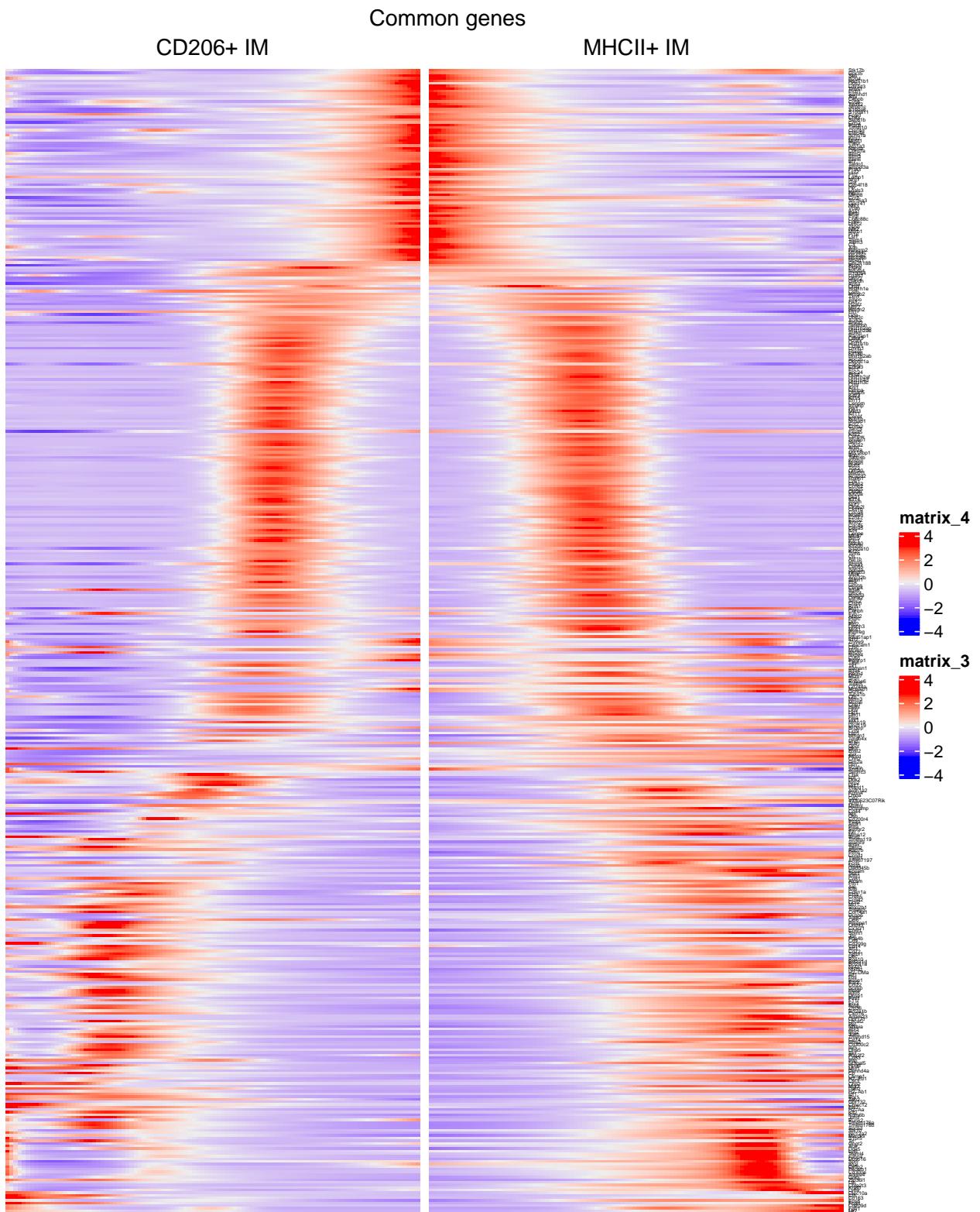
```

1  orderbyExpressionPeak <- function(x, # matrix
2      decreasing = FALSE,
3      output.position = FALSE # if true, give
4      relative position 0 - 1, or output
5      order.
6      )
7
8  indx.peak <- apply(x, 1 , which.max)
9
10 if(output.position) {
11   po <- indx.peak/nrow(x)
12   if (! length(rownames(x)) == 0) {names(po) <- rownames(x)}
13   return(po)
14 } else {
15   o <- order(indx.peak)
16   if (! length(rownames(x)) == 0) {names(o) <- rownames(x)[o]}
17   return (o)
18 }
```

Make average peak pseudotime peak for each gene:

```

1  po.cd206 <- orderbyExpressionPeak(yhatSmoothScaled[genes.noChange , 1:100] ,
2      output.position = TRUE)
3  po.mhcii <- orderbyExpressionPeak(yhatSmoothScaled[genes.noChange ,
4      101:200] , output.position = TRUE)
5  order.mean <- order ( ( po.cd206 + po.mhcii ) /2)
6
7  heatSmooth_cd206.unchanged.ordered <- Heatmap(yhatSmoothScaled[genes.
8      noChange , 100:1] , cluster_columns = FALSE, show_row_names = FALSE,
9      show_column_names = FALSE, row_order = order.mean, column_title =
10      "CD206+IM")
11
12  heatSmooth_combined.unchanged.ordered <- draw ( heatSmooth_cd206.unchanged
13      .ordered + heatSmooth_mhcii.unchanged, column_title = "Common genes",
14      auto_adjust = FALSE)
```



```

pdf(file = ".../Figures/Heatmap_common_genes_IMs_diff_across_pseudotime.pdf" 1
  , width = 8, height = 10)
heatSmooth_combined.unchanged.ordered
dev.off() 2
3

```

5.5 Make with changed/specific genes

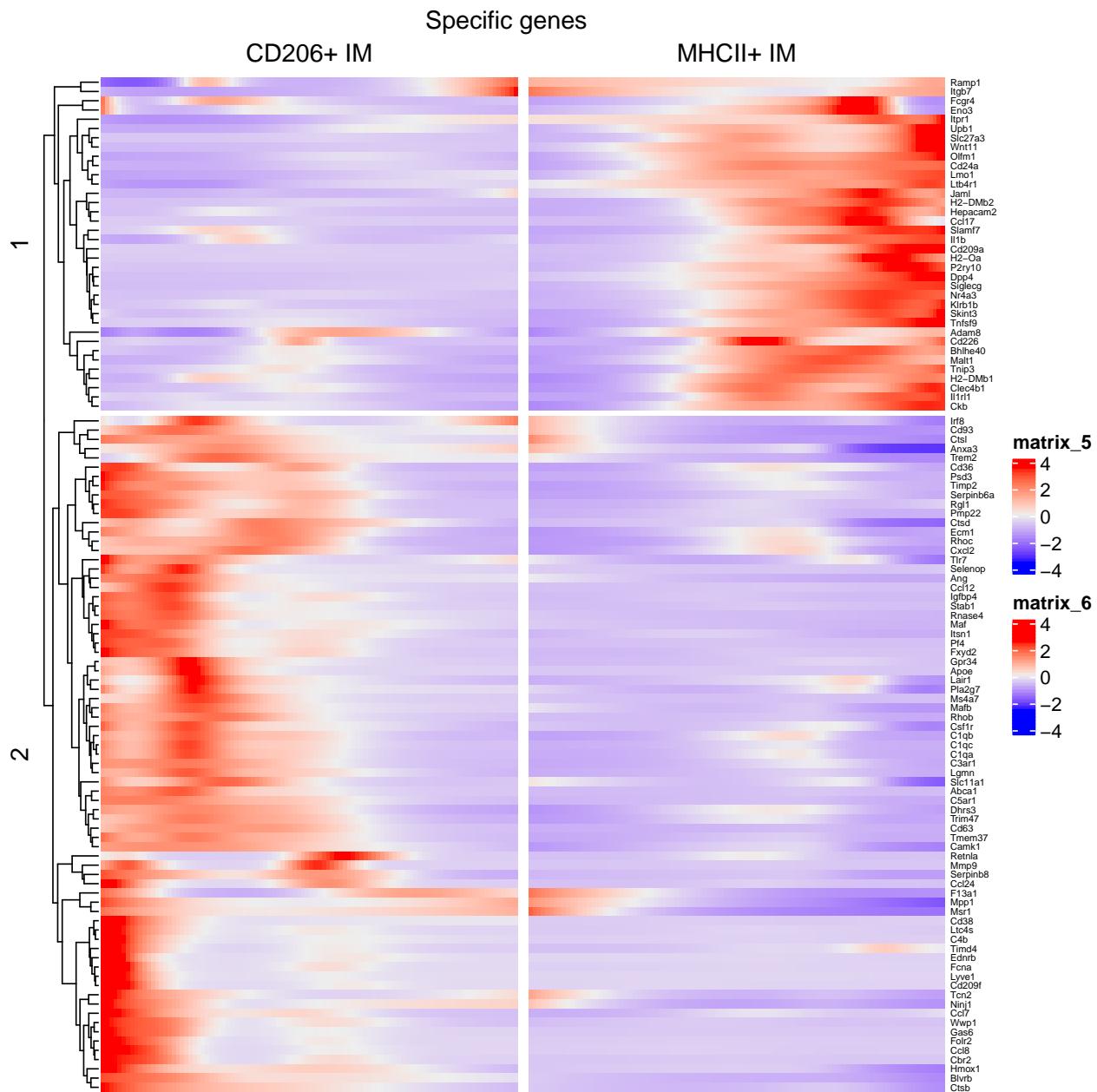
```

heatSmooth_cd206.changed <- Heatmap(yhatSmoothScaled[genes.changed,
  100:1], cluster_columns = FALSE, show_row_names = FALSE, cluster_rows
= hclust(dist(yhatSmoothScaled[genes.changed, ])), show_column_names =
FALSE, column_title = "CD206+ IM")

heatSmooth_mhcii.changed <- Heatmap(yhatSmoothScaled[genes.changed,
  101:200], cluster_columns = FALSE, show_row_names = TRUE, row_names_gp
= gpar(fontsize = 5), show_column_names = FALSE, column_title = "MHCII+
IM")

heatSmooth_combined.changed <- draw ( heatSmooth_cd206.changed +
heatSmooth_mhcii.changed, column_title = "Specific genes", split = 2)

```



```

pdf(file = "../Figures/Heatmap_specific_CD206_IM_genes_IMs_diff_across_
    pseudotime.pdf", width = 8, height = 8)
heatSmooth_combined.changed
dev.off()

geneSymbol.changed.mhcii <- read.csv("./geneSymbol_changed_mhcii_IM.csv",
    col.names = FALSE)[[1]]
geneSymbol.changed.cd206 <- read.csv("./geneSymbol_changed_cd206_IM.csv",
    col.names = FALSE)[[1]]

```

MHCII IM high genes:

geneSymbol.changed.mhcii	1
## [1] "Ramp1" "Itgb7" "Fcgr4" "Eno3" "Itpr1" "Upb1"	1
## [7] "Slc27a3" "Wnt11" "Olfm1" "Cd24a" "Lmo1" "Ltb4r1"	2
## [13] "Jaml" "H2-DMb2" "Hepacam2" "Ccl17" "Slamf7" "Il1b"	3
## [19] "Cd209a" "H2-Oa" "P2ry10" "Dpp4" "Siglecg" "Nr4a3"	4
## [25] "Klrb1b" "Skint3" "Tnfsf9" "Adam8" "Cd226" "Bhlhe40"	5
## [31] "Malt1" "Tnip3" "H2-DMb1" "Clec4b1" "Il1rl1" "Ckb"	6

geneSymbol.changed.cd206	1
## [1] "Irf8" "Cd93" "Ctsl" "Anxa3" "Trem2" "Cd36"	1
## [7] "Psd3" "Timp2" "Serpina6a" "Rgl1" "Pmp22" "Ctsd"	2
## [13] "Ecm1" "Rhoc" "Cxcl2" "Tlr7" "Selenop" "Ang"	3
## [19] "Ccl12" "Igfbp4" "Stab1" "Rnase4" "Maf" "Itsn1"	4
"	
## [25] "Pf4" "Fxyd2" "Gpr34" "Apoe" "Lair1" "	5
Pla2g7"	
## [31] "Ms4a7" "Mafb" "Rhob" "Csf1r" "C1qb" "C1qc"	6
## [37] "C1qaa" "C3ar1" "Lgmn" "Slc11a1" "Abca1" "C5ar1"	7
"	
## [43] "Dhrs3" "Trim47" "Cd63" "Tmem37" "Camk1" "	8
Retnla"	
## [49] "Mmp9" "Serpina8" "Ccl24" "F13a1" "Mpp1" "Msrl"	9
## [55] "Cd38" "Ltc4s" "C4b" "Timd4" "Ednrb" "Fcna"	10
## [61] "Lyve1" "Cd209f" "Tcn2" "Ninj1" "Ccl7" "Wwp1"	11
## [67] "Gas6" "Folr2" "Ccl8" "Cbr2" "Hmox1" "Blvrb"	12
"	
## [73] "Ctsb"	13

write.csv(geneSymbol.changed.cd206, file = "./geneSymbol_changed_cd206_IM. csv", quote = FALSE, row.names = FALSE)	1
write.csv(geneSymbol.changed.mhcii, file = "./geneSymbol_changed_mhcii_IM. csv", quote = FALSE, row.names = FALSE)	2

5.6 GO enrichment on the changed/specific genes

suppressMessages(library(clusterProfiler))	1
source("../R/entrez2symbol.R")	2
source("../R/replaceEntrezID.R")	3

5.6.1 GO enrichment on MHCII IM

```

symb <- geneSymbol.changed.mhcii
de_entrez <- bitr( geneID = symb, fromType = "SYMBOL", toType = "ENTREZID"
  , OrgDb = "org.Mm.eg.db", drop = TRUE ) $ ENTREZID
result.enrichGO <- enrichGO(de_entrez, OrgDb = "org.Mm.eg.db", ont = "BP")
result.enrichGO <- replaceEntrezID(result.enrichGO, organism = "mmu")
# write.csv(result.enrichGO@result, file = "./Results_enrichment/enrichGO_
  MHCII_IM_specific_genes.csv")
result.enrichGO@result

```

	ID	Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue
	geneID	Count	<chr>	<chr>	<dbl>	<dbl>	<dbl>
	>	<int>	<chr>	<chr>	<dbl>	<dbl>	<chr>
## 1	GO:00~ regulation o~	9/36	Cd24a/~/	9	321/23~	1.07e-9	1.35e-6 8.17e-7
## 2	GO:00~ positive reg~	9/36	Cd24a/~/	9	479/23~	3.46e-8	1.17e-5 7.11e-6
## 3	GO:19~ positive reg~	7/36	Cd24a/~/	7	221/23~	4.12e-8	1.17e-5 7.11e-6
## 4	GO:00~ leukocyte ce~	8/36	Itgb7/~/	8	345/23~	4.45e-8	1.17e-5 7.11e-6
## 5	GO:00~ positive reg~	9/36	Cd24a/~/	9	496/23~	4.67e-8	1.17e-5 7.11e-6
## 6	GO:00~ positive reg~	7/36	Cd24a/~/	7	265/23~	1.42e-7	2.98e-5 1.80e-5
## 7	GO:00~ regulation o~	8/36	Cd24a/~/	8	440/23~	2.86e-7	4.76e-5 2.88e-5
## 8	GO:00~ positive reg~	8/36	Wnt11/~/	8	449/23~	3.34e-7	4.76e-5 2.88e-5
## 9	GO:19~ regulation o~	7/36	Cd24a/~/	7	309/23~	4.01e-7	4.76e-5 2.88e-5
## 10	GO:00~ regulation o~	6/36	Cd24a/~/	6	192/23~	4.56e-7	4.76e-5 2.88e-5
## # ... with 1,247 more rows							

5.6.2 GO enrichment on CD206+ IM

```

symb <- geneSymbol.changed.cd206
de_entrez <- bitr( geneID = symb, fromType = "SYMBOL", toType = "ENTREZID"
  , OrgDb = "org.Mm.eg.db", drop = TRUE ) $ ENTREZID
result.enrichGO <- enrichGO(de_entrez, OrgDb = "org.Mm.eg.db", ont = "BP")
result.enrichGO <- replaceEntrezID(result.enrichGO, organism = "mmu")
# write.csv(result.enrichGO@result, file = "./Results_enrichment/enrichGO_
  CD206_IM_specific_genes.csv")
result.enrichGO@result

```

	ID	Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue
	geneID	Count	<chr>	<chr>	<chr>	<dbl>	<dbl>
	>	<int>	<chr>	<chr>	<chr>	<dbl>	<dbl>
## # 1	## # A tibble: 1,842 x 9						
## # 2	## ID	Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue
## # 3	## geneID	Count	<chr>	<chr>	<chr>	<dbl>	<dbl>
## # 4	## >	<int>	<chr>	<chr>	<chr>	<dbl>	<dbl>

## 1	GO:00~ leukocyte ~ 17/70	360/23~ 4.11e-16 7.56e-13 4.90e-13	4
Trem2/~/	17		
## 2	GO:00~ leukocyte ~ 14/70	219/23~ 3.30e-15 2.03e-12 1.31e-12	5
Cxcl2/~/	14		
## 3	GO:00~ myeloid le~ 14/70	219/23~ 3.30e-15 2.03e-12 1.31e-12	6
Trem2/~/	14		
## 4	GO:00~ cell chemo~ 14/70	303/23~ 2.90e-13 1.34e-10 8.65e-11	7
Cxcl2/~/	14		
## 5	GO:00~ regulation~ 12/70	217/23~ 2.05e-12 7.55e-10 4.89e-10	8
Trem2/~/	12		
## 6	GO:00~ granulocyt~ 10/70	126/23~ 4.44e-12 1.36e- 9 8.82e-10	9
Cxcl2/~/	10		
## 7	GO:00~ mononuclea~ 9/70	89/233~ 6.08e-12 1.60e- 9 1.04e- 9	10
Ccl12/~/	9		
## 8	GO:00~ neutrophil~ 9/70	99/233~ 1.62e-11 3.72e- 9 2.41e- 9	11
Cxcl2/~/	9		
## 9	GO:00~ granulocyt~ 10/70	155/23~ 3.53e-11 6.93e- 9 4.48e- 9	12
Cxcl2/~/	10		
## 10	GO:00~ positive r~ 10/70	156/23~ 3.76e-11 6.93e- 9 4.48e- 9	13
Trem2/~/	10		
## # ... with 1,832 more rows			14

6 Functionality analysis of DE genes across pseudotime (common genes)

```
common.genes <- heatSmooth_cd206.unchanged.ordered@row_names_param$labels[ 1
heatSmooth_cd206.unchanged.ordered@row_order]
```

6.1 Manually classify genes by expression timing peak

Class-1 genes are the genes expressed in monocytes but turned off in the very early phase:

```
genes.class1 <- common.genes[1:which(common.genes == "Gm21188")]
length(genes.class1) 1
2
```

```
## [1] 75 1
```

Class-2 genes are the genes up-regulated in early phase of differentiation and turned off during transit phase.

```
genes.class2 <- common.genes[(which(common.genes == "Gm21188") + 1) : which(
common.genes == "Diaph3")]
length(genes.class2) 1
2
```

```
## [1] 140 1
```

Class-3 genes are the late upregulated genes during IM differentiation.

```
genes.class3 <- common.genes[(which(common.genes == "Diaph3") + 1) : length(
common.genes)]
length(genes.class3) 1
2
```

```
## [1] 228
```

Save gene lists:

```
write.csv(genes.class1, file = "./common_genes_class1.csv", quote = FALSE) 1
write.csv(genes.class2, file = "./common_genes_class2.csv", quote = FALSE) 2
write.csv(genes.class3, file = "./common_genes_class3.csv", quote = FALSE) 3
```

6.2 GO/KEGG enrichment analysis with 3 classes of common genes

6.2.1 KEGG enrichment for common genes class 1

```
symb <- genes.class1
de_entrez <- bitr( geneID = symb, fromType = "SYMBOL", toType = "ENTREZID"
  , OrgDb = "org.Mm.eg.db", drop = TRUE ) $ ENTREZID
result.enrichKEGG <- enrichKEGG(de_entrez, organism = "mmu", keyType = "
  ncbi-geneid")
result.enrichKEGG <- replaceEntrezID(result.enrichKEGG, organism = "mmu")
# write.csv(result.enrichKEGG@result, file = "./Results_enrichment/
  enrichKEGG_common_genes_class1.csv")
result.enrichKEGG@result
```

```
## # A tibble: 149 x 9
##       ID      Description  GeneRatio BgRatio    pvalue p.adjust    qvalue
##   <chr>    <chr>     <chr>     <chr>     <dbl>     <dbl>     <dbl>    <
## 1 mmu04145 Phagosome    7/36     182/89~  6.53e-6  0.000973  9.00e-4
## 2 Thbs1~        7
## 3 mmu05152 Tuberculosis 6/36     180/89~  7.21e-5  0.00537   4.97e-3
## 4 Cebpb~        6
## 5 mmu05140 Leishmanias~ 3/36     70/8943  2.73e-3  0.119    1.10e-1
## 6 Cybb/~        3
## 7 mmu04918 Thyroid hor~ 3/36     74/8943  3.19e-3  0.119    1.10e-1
## 8 Plcb1~        3
## 9 mmu04970 Salivary se~ 3/36     86/8943  4.88e-3  0.145    1.34e-1
## 10 Plcb1~       3
## 11 mmu04610 Complement ~ 3/36     93/8943  6.07e-3  0.151    1.39e-1
## 12 Plaur~        3
## 13 mmu04613 Neutrophil ~ 4/36     207/89~  9.20e-3  0.167    1.55e-1
## 14 Plcb1~       4
## 15 mmu04621 NOD-like re~ 4/36     211/89~  9.82e-3  0.167    1.55e-1
## 16 Ifi20~        4
## 17 mmu04960 Aldosterone~ 2/36     38/8943  1.01e-2  0.167    1.55e-1
## 18 Scnn1~        2
## 19 mmu04973 Carbohydrat~ 2/36     48/8943  1.58e-2  0.236    2.18e-1
## 20 Plcb1~       2
## # ... with 139 more rows
```

6.2.2 GO enrichment for common genes class 1

```
result.enrichGO <- enrichGO(de_entrez, OrgDb = "org.Mm.eg.db", ont = "BP") 1
```

```

result.enrichGO <- replaceEntrezID(result.enrichGO, organism = "mmu")          2
# write.csv(result.enrichGO@result, file = "./Results_enrichment/enrichGO_      3
  common_genes_class1.csv")
result.enrichGO@result                                         4

```

```

## # A tibble: 1,740 x 9
##   ID      Description  GeneRatio BgRatio    pvalue p.adjust    qvalue
##   <chr>    <chr>       <chr>     <dbl>      <dbl>      <dbl>      <chr>
##   geneID  Count
##   <chr>   <int>
##   1 GO:00~ myeloid leu~ 10/72      219/23~ 1.40e-9  2.16e-6 1.65e-6 Gpr35
##   /S~      10
##   2 GO:00~ cellular ex~ 7/72       72/233~ 2.48e-9  2.16e-6 1.65e-6 Sell/
##   Pl~      7
##   3 GO:00~ leukocyte m~ 11/72      360/23~ 1.32e-8  7.68e-6 5.86e-6 Gpr35
##   /S~      11
##   4 GO:00~ leukocyte c~ 9/72       219/23~ 2.45e-8  1.07e-5 8.14e-6 Gpr35
##   /S~      9
##   5 GO:00~ positive re~ 11/72      418/23~ 6.08e-8  2.12e-5 1.61e-5 Sell/
##   If~      11
##   6 GO:00~ type I inte~ 5/72       40/233~ 1.47e-7  3.64e-5 2.78e-5
##   Samhd1/~  5
##   7 GO:00~ cellular re~ 5/72       40/233~ 1.47e-7  3.64e-5 2.78e-5
##   Samhd1/~  5
##   8 GO:00~ defense res~ 11/72      464/23~ 1.74e-7  3.64e-5 2.78e-5 Slpi/
##   Ce~      11
##   9 GO:00~ positive re~ 9/72       278/23~ 1.88e-7  3.64e-5 2.78e-5
##   Ifi204/~  9
##   10 GO:00~ response to~ 5/72      45/233~ 2.70e-7  4.70e-5 3.59e-5
##   Samhd1/~  5
##   # ... with 1,730 more rows

```

6.2.3 KEGG enrichment for common genes class 2

```

symb <- genes.class2
de_entrez <- bitr( geneID = symb, fromType = "SYMBOL", toType = "ENTREZID" 1
  , OrgDb = "org.Mm.eg.db", drop = TRUE ) $ ENTREZID
result.enrichKEGG <- enrichKEGG(de_entrez, organism = "mmu", keyType = "2
  ncbi-geneid")
result.enrichKEGG <- replaceEntrezID(result.enrichKEGG, organism = "mmu") 3
# write.csv(result.enrichKEGG@result, file = "./Results_enrichment/
  enrichKEGG_common_genes_class2.csv")
result.enrichKEGG@result                                         4

```

```

## # A tibble: 88 x 9
##   ID      Description  GeneRatio BgRatio    pvalue p.adjust    qvalue
##   <chr>    <chr>       <chr>     <dbl>      <dbl>      <dbl>      <chr>
##   geneID  Count
##   <chr>   <int>
##   1 mmu04114 Oocyte mei~ 11/50      121/89~ 4.20e-11  2.64e-9 2.28e-9
##   Aurka~   11
##   2 mmu04110 Cell cycle 11/50      125/89~ 6.01e-11  2.64e-9 2.28e-9
##   Ccnb1~   11

```

## 3 mmu04914 Progesterone~	9/50	92/8943	1.54e- 9	4.51e-8	3.88e-8	6
Aurka~	9					
## 4 mmu04115 p53 signal~	5/50	72/8943	4.70e- 5	1.03e-3	8.90e-4	7
Ccnb1~	5					
## 5 mmu00240 Pyrimidine~	4/50	58/8943	2.94e- 4	5.17e-3	4.45e-3	8
Rrm2/~	4					
## 6 mmu04218 Cellular s~	6/50	184/89~	5.24e- 4	7.68e-3	6.62e-3	9
Ccnb1~	6					
## 7 mmu05222 Small cell~	4/50	93/8943	1.75e- 3	2.20e-2	1.90e-2	Fn1 10
/C~	4					
## 8 mmu05166 Human T-ce~	6/50	250/89~	2.55e- 3	2.64e-2	2.28e-2	11
Bub1b~	6					
## 9 mmu05132 Salmonella~	6/50	253/89~	2.70e- 3	2.64e-2	2.28e-2	12
Gapdh~	6					
## 10 mmu04512 ECM-recept~	3/50	88/8943	1.29e- 2	1.14e-1	9.80e-2	Fn1 13
/H~	3					
## # ... with 78 more rows						14

6.2.4 GO enrichment for common genes class 2

```
result.enrichGO <- enrichGO(de_entrez, OrgDb = "org.Mm.eg.db", ont = "BP")
result.enrichGO <- replaceEntrezID(result.enrichGO, organism = "mmu")
# write.csv(result.enrichGO@result, file = "./Results_enrichment/enrichGO_
common_genes_class2.csv")
result.enrichGO@result
```

## # A tibble: 1,780 x 9									1
## ID Description GeneRatio BgRatio pvalue p.adjust qvalue									2
## geneID Count									3
## <chr> <chr> <chr> <chr> <dbl> <dbl> <dbl> <chr>									4
## > <int>									5
## 1 GO:00~ chromosome~	50/127	324/23~	7.39e-60	1.32e-56	1.08e-56				6
Ube2c/~	50								7
## 2 GO:00~ nuclear ch~	43/127	262/23~	2.75e-52	1.75e-49	1.44e-49				8
Ube2c/~	43								9
## 3 GO:00~ sister chr~	39/127	181/23~	2.95e-52	1.75e-49	1.44e-49				10
Ube2c/~	39								11
## 4 GO:01~ mitotic nu~	43/127	268/23~	7.78e-52	2.91e-49	2.39e-49				12
Ube2c/~	43								13
## 5 GO:00~ mitotic si~	37/127	151/23~	8.18e-52	2.91e-49	2.39e-49				14
Ube2c/~	37								15
## 6 GO:00~ nuclear di~	46/127	418/23~	9.43e-48	2.80e-45	2.30e-45				16
Ube2c/~	46								17
## 7 GO:00~ organelle ~	47/127	472/23~	9.12e-47	2.32e-44	1.91e-44				18
Mtfr2/~	47								19
## 8 GO:00~ spindle or~	27/127	179/23~	1.59e-31	3.54e-29	2.91e-29				20
Aurka/~	27								21
## 9 GO:19~ microtubul~	24/127	142/23~	2.56e-29	5.06e-27	4.16e-27				22
Aurka/~	24								23
## 10 GO:00~ regulation~	21/127	103/23~	1.42e-27	2.53e-25	2.08e-25				24
Ube2c/~	21								25
## # ... with 1,770 more rows									26

6.2.5 KEGG enrichment for common genes class 3

```

symb <- genes.class3
de_entrez <- bitr( geneID = symb, fromType = "SYMBOL", toType = "ENTREZID"
  , OrgDb = "org.Mm.eg.db", drop = TRUE ) $ ENTREZID
result.enrichKEGG <- enrichKEGG(de_entrez, organism = "mmu", keyType = "
  ncbi-geneid")
result.enrichKEGG <- replaceEntrezID(result.enrichKEGG, organism = "mmu")
# write.csv(result.enrichKEGG@result, file = "./Results_enrichment/
  enrichKEGG_common_genes_class3.csv")
result.enrichKEGG@result

```

	ID	Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue
	geneID	Count	<chr>	<chr>	<dbl>	<dbl>	<dbl>
	chr	chr	<int>				
## 1	mmu04210	Apoptosis	15/141	136/89~	2.97e-9	6.77e-7	4.69e-7
	Ctsc	/~	15				
## 2	mmu04145	Phagosome	15/141	182/89~	1.56e-7	1.78e-5	1.23e-5
	Tubb5	/~	15				
## 3	mmu05166	Human T-cel~	17/141	250/89~	3.62e-7	2.27e-5	1.57e-5
	Il1r2	/~	17				
## 4	mmu05202	Transcripti~	16/141	223/89~	3.97e-7	2.27e-5	1.57e-5
	Il1r2	/~	16				
## 5	mmu04640	Hematopoiet~	10/141	94/8943	2.02e-6	9.20e-5	6.37e-5
	Il1r2	/~	10				
## 6	mmu04380	Osteoclast ~	11/141	128/89~	5.10e-6	1.94e-4	1.34e-4
	Fosl2	/~	11				
## 7	mmu05323	Rheumatoid ~	9/141	87/8943	8.42e-6	2.74e-4	1.90e-4
	Ctsk	/~	9				
## 8	mmu05140	Leishmanias~	8/141	70/8943	1.30e-5	3.69e-4	2.56e-4
	Itga4	/~	8				
## 9	mmu05152	Tuberculosis	12/141	180/89~	2.55e-5	6.46e-4	4.48e-4
	Lsp1	/~	12				
## 10	mmu04064	NF-kappa B ~	9/141	105/89~	3.88e-5	8.85e-4	6.13e-4
	Gadd4	/~	9				
## # ... with 218 more rows							

6.2.6 GO enrichment for common genes class 3

```

result.enrichGO <- enrichGO(de_entrez, OrgDb = "org.Mm.eg.db", ont = "BP")
result.enrichGO <- replaceEntrezID(result.enrichGO, organism = "mmu")
# write.csv(result.enrichGO@result, file = "./Results_enrichment/enrichGO_
  common_genes_class3.csv")
result.enrichGO@result

```

	ID	Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue
	geneID	Count	<chr>	<chr>	<dbl>	<dbl>	<dbl>
	>	<int>					
## 1	GO:00~	regulation ~	23/224	372/23~	1.72e-12	5.89e-9	4.30e-9
	Il1r2	/~	23				

## 2 GO:00~ negative re~ 22/224	462/23~	8.04e-10	7.67e-7	5.61e-7	Fgr/	5
Ce~ 22						
## 3 GO:00~ positive re~ 17/224	265/23~	8.72e-10	7.67e-7	5.61e-7		6
Ceacam~ 17						
## 4 GO:19~ regulation ~ 21/224	424/23~	9.85e-10	7.67e-7	5.61e-7		7
Ceacam~ 21						
## 5 GO:00~ leukocyte c~ 19/224	345/23~	1.12e- 9	7.67e-7	5.61e-7		8
Ceacam~ 19						
## 6 GO:00~ leukocyte m~ 19/224	360/23~	2.27e- 9	1.29e-6	9.44e-7		9
Itga4/~ 19						
## 7 GO:00~ regulation ~ 19/224	372/23~	3.88e- 9	1.75e-6	1.28e-6		10
Ceacam~ 19						
## 8 GO:19~ positive re~ 15/224	221/23~	4.10e- 9	1.75e-6	1.28e-6		11
Ceacam~ 15						
## 9 GO:00~ lymphocyte ~ 11/224	103/23~	4.60e- 9	1.75e-6	1.28e-6		12
Itga4/~ 11						
## 10 GO:00~ antigen pro~ 6/224	16/233~	5.42e- 9	1.85e-6	1.35e-6	Ctss	13
/H~ 6						
## # ... with 3,409 more rows						14

6.2.7 GO enrichment for MHCII IM specific genes + common genes class 3

As the specific genes have limited number. We try add the class 3 genes, which are also terminated differentiation genes. But we have to pay attention that the class 3 gene number is huge compare to the specific genes, so enrichment results will be diluted.

```
symb <- c(geneSymbol.changed.mhcii, genes.class3)
de_entrez <- bitr( geneID = symb, fromType = "SYMBOL", toType = "ENTREZID"
  , OrgDb = "org.Mm.eg.db", drop = TRUE ) $ ENTREZID
result.enrichGO <- enrichGO(de_entrez, OrgDb = "org.Mm.eg.db", ont = "BP")
result.enrichGO <- replaceEntrezID(result.enrichGO, organism = "mmu")
# write.csv(result.enrichGO@result, file = "./Results_enrichment/enrichGO_
  MHCIIISpecific_plus_Class3_common.csv")
result.enrichGO@result
```

## # A tibble: 3,607 x 9	1						
## ID Description GeneRatio BgRatio pvalue p.adjust qvalue	2						
geneID Count	3						
<chr> <chr> <chr> <dbl> <dbl> <dbl> <	3						
chr> <int>							
## 1 GO:00~ regulation ~ 28/260	372/23~	1.81e-15	4.10e-12	2.84e-12			4
Cd24a~ 28							
## 2 GO:00~ leukocyte c~ 27/260	345/23~	2.28e-15	4.10e-12	2.84e-12			5
Itgb7~ 27							
## 3 GO:00~ positive re~ 24/260	265/23~	3.41e-15	4.10e-12	2.84e-12			6
Cd24a~ 24							
## 4 GO:19~ positive re~ 22/260	221/23~	7.08e-15	6.38e-12	4.41e-12			7
Cd24a~ 22							
## 5 GO:00~ antigen pro~ 9/260	16/233~	2.47e-14	1.78e-11	1.23e-11	H2-		8
DM~ 9							
## 6 GO:00~ leukocyte m~ 26/260	360/23~	5.13e-14	3.08e-11	2.13e-11			9
Itgb7~ 26							
## 7 GO:00~ positive re~ 28/260	449/23~	1.99e-13	1.03e-10	7.09e-11			10
Wnt11~ 28							

```

## 8 GO:00~ regulation ~ 24/260      321/23~ 2.38e-13 1.07e-10 7.40e-11 11
Cd24a~ 24
## 9 GO:19~ regulation ~ 27/260      424/23~ 3.35e-13 1.20e-10 8.30e-11 12
Cd24a~ 27
## 10 GO:00~ positive re~ 29/260     496/23~ 3.65e-13 1.20e-10 8.30e-11 13
Cd24a~ 29
## # ... with 3,597 more rows          14

```

6.2.8 GO enrichment for CD206 IM specific genes + common genes class 3

```

symb <- c(geneSymbol.changed.cd206, genes.class3)
de_entrez <- bitr( geneID = symb, fromType = "SYMBOL", toType = "ENTREZID"
  , OrgDb = "org.Mm.eg.db", drop = TRUE ) $ ENTREZID
result.enrichGO <- enrichGO(de_entrez, OrgDb = "org.Mm.eg.db", ont = "BP")
result.enrichGO <- replaceEntrezID(result.enrichGO, organism = "mmu")
# write.csv(result.enrichGO@result, file = "./Results_enrichment/enrichGO_
  CD206Specific_plus_Class3_common.csv")
result.enrichGO@result

```

```

## # A tibble: 3,810 x 9
##   ID      Description GeneRatio BgRatio    pvalue p.adjust    qvalue
##   <chr>  <chr>       <chr>      <dbl>    <dbl>    <dbl>    <dbl> <chr>
##   >      <int>
##   1 GO:00~ leukocyte ~ 36/294     360/23~ 6.24e-22 2.38e-18 1.60e-18 1
Trem2/~ 36
##   2 GO:00~ myeloid le~ 25/294     219/23~ 6.62e-17 1.26e-13 8.50e-14 2
Trem2/~ 25
##   3 GO:00~ cell chemo~ 28/294     303/23~ 2.25e-16 2.86e-13 1.93e-13 3
Cxcl2/~ 28
##   4 GO:00~ leukocyte ~ 24/294     219/23~ 7.21e-16 6.87e-13 4.63e-13 4
Cxcl2/~ 24
##   5 GO:00~ lymphocyte~ 17/294     103/23~ 1.39e-14 1.06e-11 7.16e-12 Ecm1 5
/C~ 17
##   6 GO:00~ regulation~ 28/294     372/23~ 4.15e-14 2.26e-11 1.52e-11 Ctsl 6
/E~ 28
##   7 GO:00~ regulation~ 28/294     372/23~ 4.15e-14 2.26e-11 1.52e-11 7
Trem2/~ 28
##   8 GO:00~ regulation~ 22/294     217/23~ 6.10e-14 2.91e-11 1.96e-11 8
Trem2/~ 22
##   9 GO:00~ ERK1 and E~ 26/294     325/23~ 8.50e-14 3.60e-11 2.42e-11 9
Trem2/~ 26
##  10 GO:00~ response t~ 18/294     137/23~ 1.40e-13 5.32e-11 3.58e-11 Irf8 10
/C~ 18
## # ... with 3,800 more rows          11

```

7 Show gene expression pattern with TradeSeq results

7.1 Class 1 common genes

```

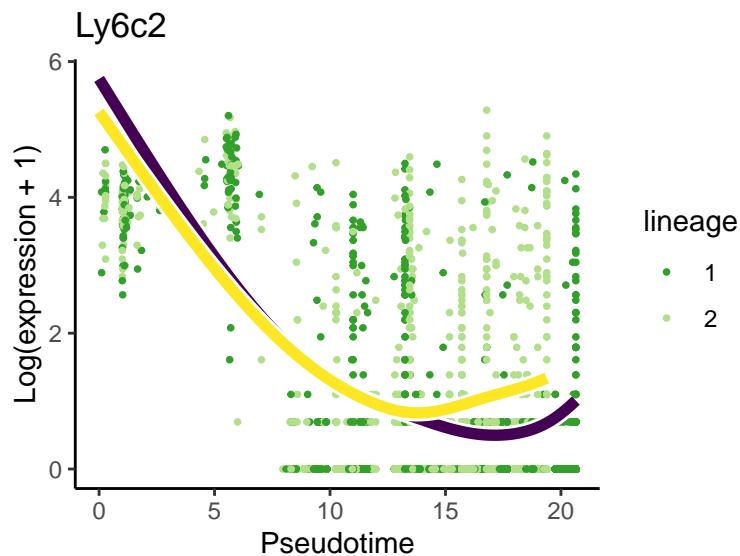
require(ggplot2)
sigGene <- "Ly6c2"

```

```

plotSmoothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene)
  + scale_color_manual(values =c("#33A02C", "#B2DF8A"))

```



```

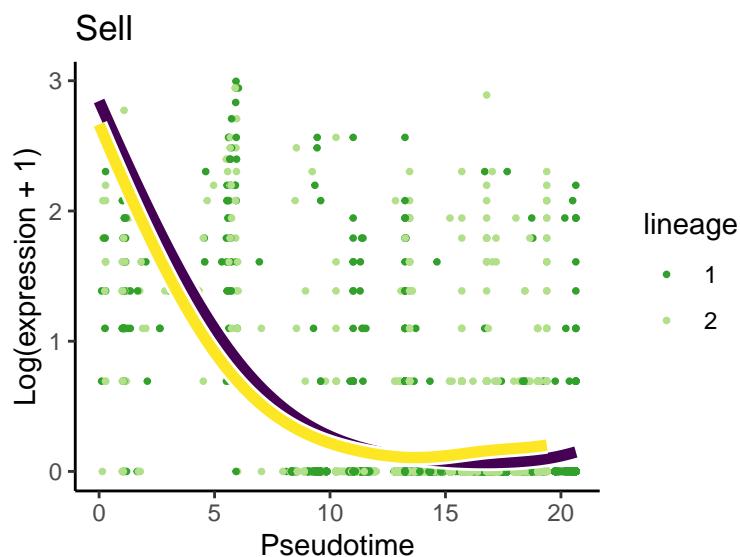
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
#   ".pdf"),
#         width = 4, height = 3)

```

```

sigGene <- "Sell"
plotSmoothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene)
  + scale_color_manual(values =c("#33A02C", "#B2DF8A"))

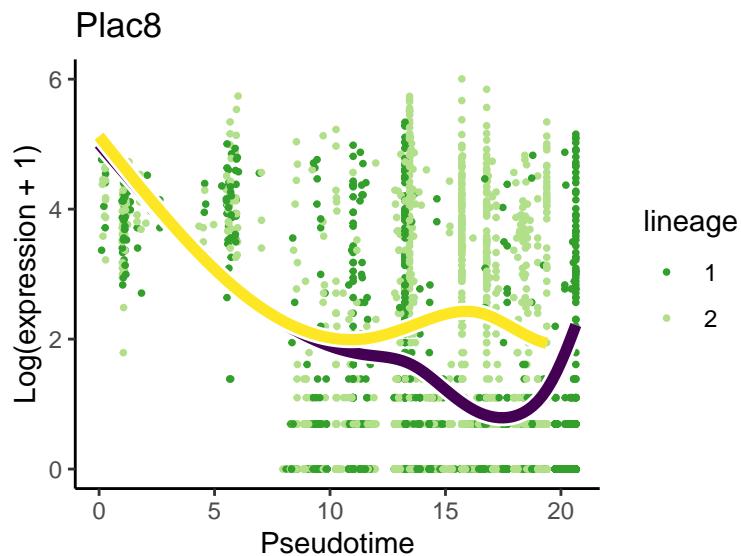
```



```

sigGene <- "Plac8"
plotSmoothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene)
  + scale_color_manual(values =c("#33A02C", "#B2DF8A"))

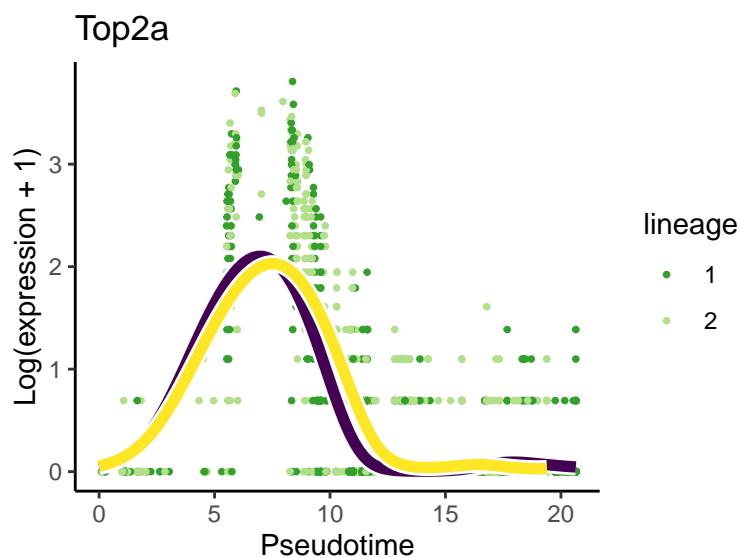
```



```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
#                           sigGene,
#                           ".pdf"),
#         width = 4, height = 3)
```

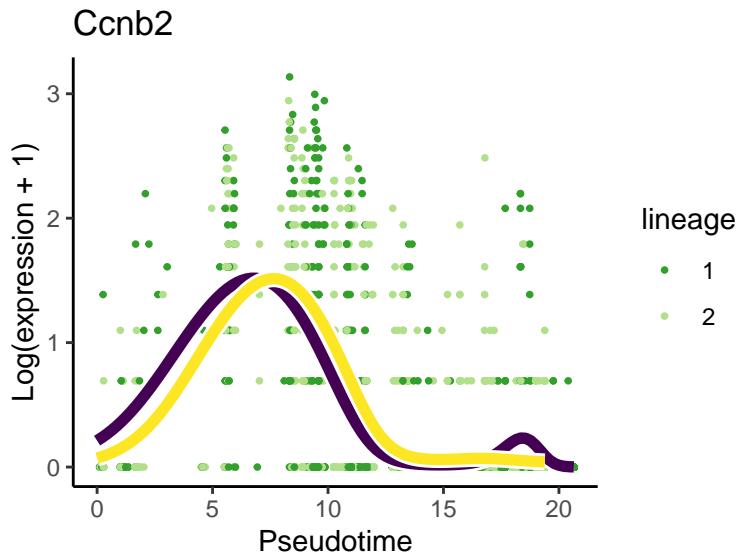
7.2 Class 2 common genes

```
sigGene <- "Top2a"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene)
  + scale_color_manual(values =c("#33A02C", "#B2DF8A"))
```



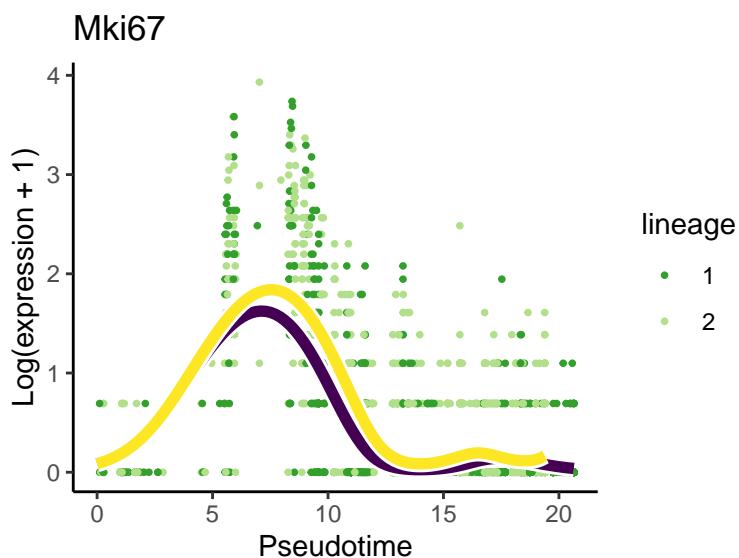
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
#                           sigGene,
#                           ".pdf"),
#         width = 4, height = 3)
```

```
sigGene <- "Ccnb2"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene)
  + scale_color_manual(values =c("#33A02C", "#B2DF8A"))
```



```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
#                           sigGene,
#                           ".pdf"),
#         width = 4, height = 3)
```

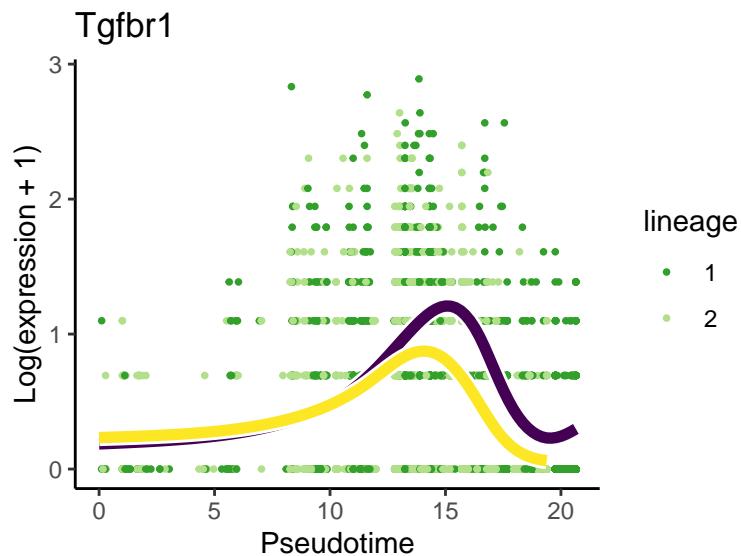
```
sigGene <- "Mki67"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene)
  + scale_color_manual(values = c("#33A02C", "#B2DF8A"))
```



```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
#                           sigGene,
#                           ".pdf"),
#         width = 4, height = 3)
```

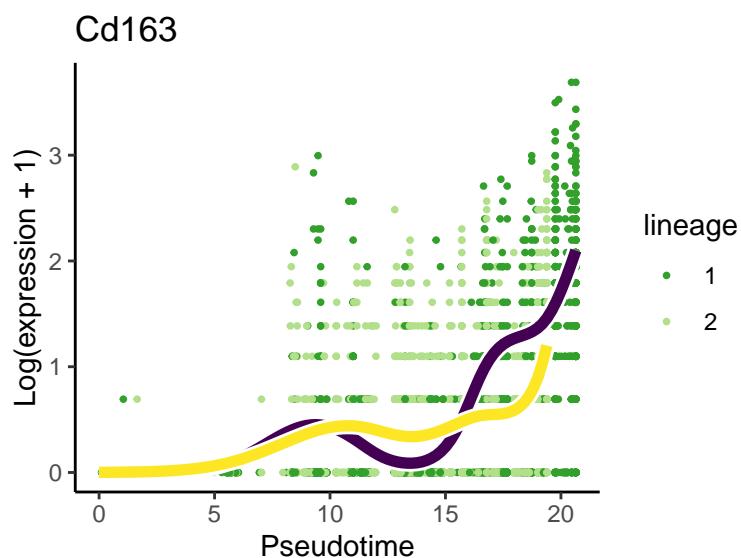
7.3 Class 3 common genes

```
sigGene <- "Tgfbri1"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene)
  + scale_color_manual(values = c("#33A02C", "#B2DF8A"))
```



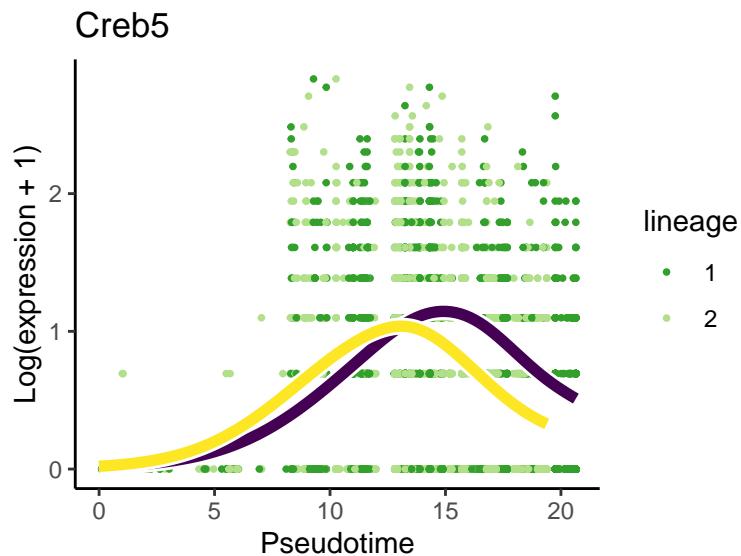
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
#                           ".pdf"),
#         width = 4, height = 3) 1
# 2
```

```
sigGene <- "Cd163"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



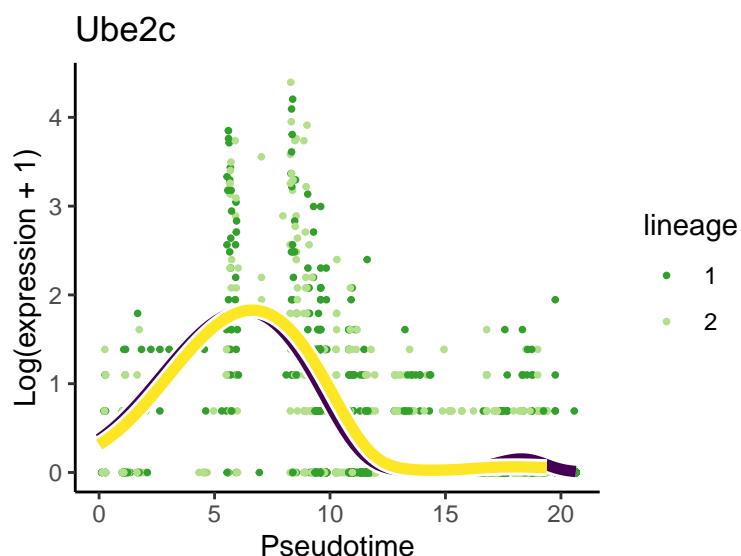
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
#                           ".pdf"),
#         width = 4, height = 3) 1
# 2
```

```
sigGene <- "Creb5"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
#                   ".pdf"),
#       width = 4, height = 3) 1
# 2
```

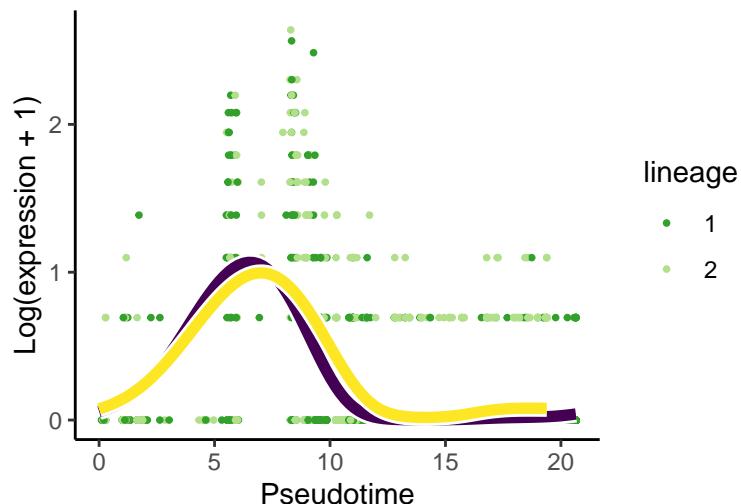
```
sigGene <- "Ube2c"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene) 1
  + scale_color_manual(values = c("#33A02C", "#B2DF8A")) 2
```



```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
#                   ".pdf"),
#       width = 4, height = 3) 1
# 2
```

```
sigGene <- "Aurkb"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene) 1
  + scale_color_manual(values = c("#33A02C", "#B2DF8A")) 2
```

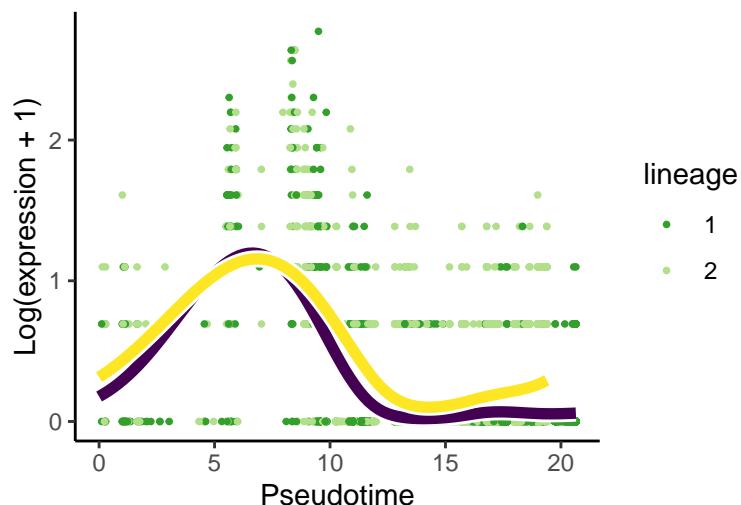
Aurkb



```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
#                           sigGene,
#                           ".pdf"),
#         width = 4, height = 3)
```

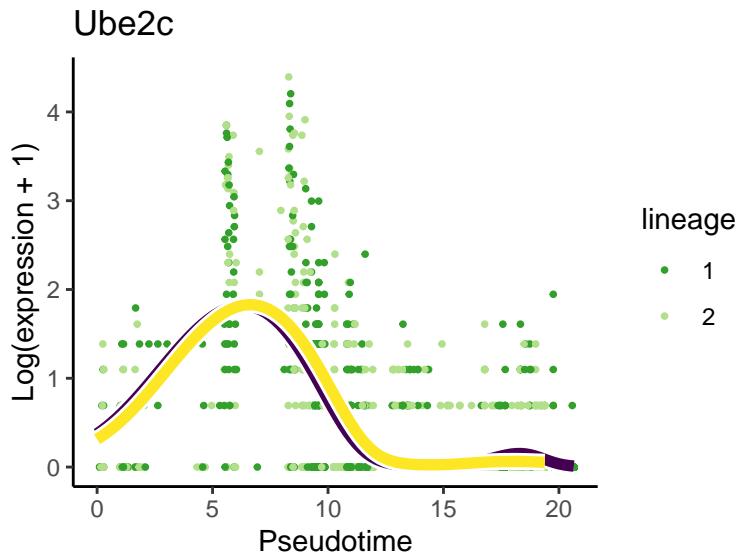
```
sigGene <- "Racgap1"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene)
  + scale_color_manual(values =c("#33A02C", "#B2DF8A"))
```

Racgap1



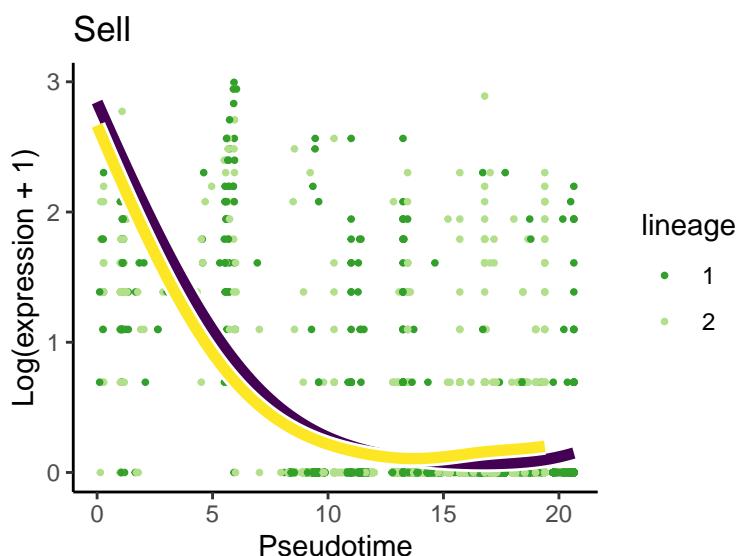
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
#                           sigGene,
#                           ".pdf"),
#         width = 4, height = 3)
```

```
sigGene <- "Ube2c"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene)
  + scale_color_manual(values =c("#33A02C", "#B2DF8A"))
```



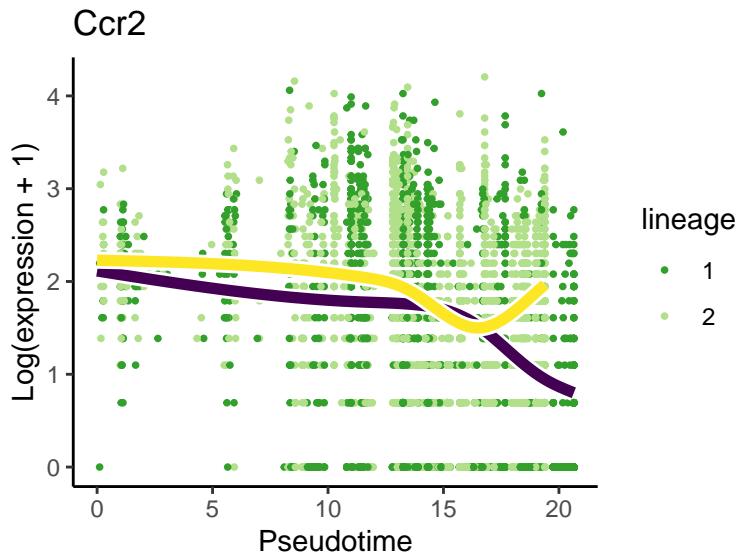
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
# , ".pdf"),
#        width = 4, height = 3) 1
# 2
```

```
sigGene <- "Sell"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```

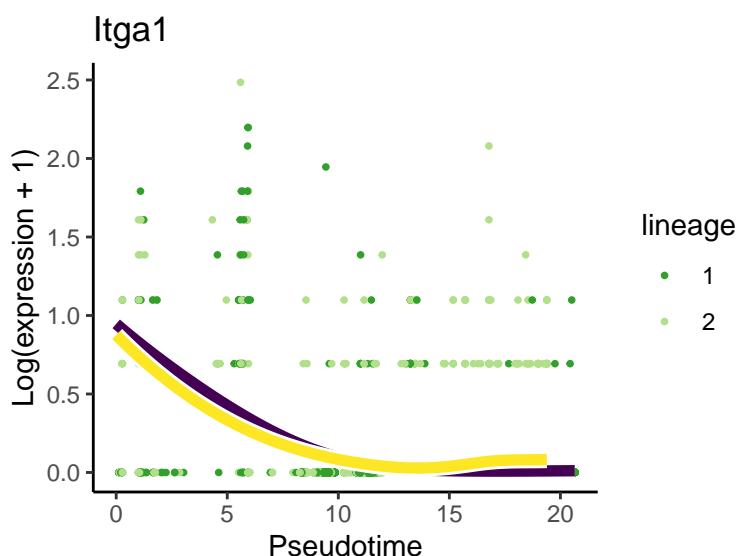


```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
# , ".pdf"),
#        width = 4, height = 3) 1
# 2
```

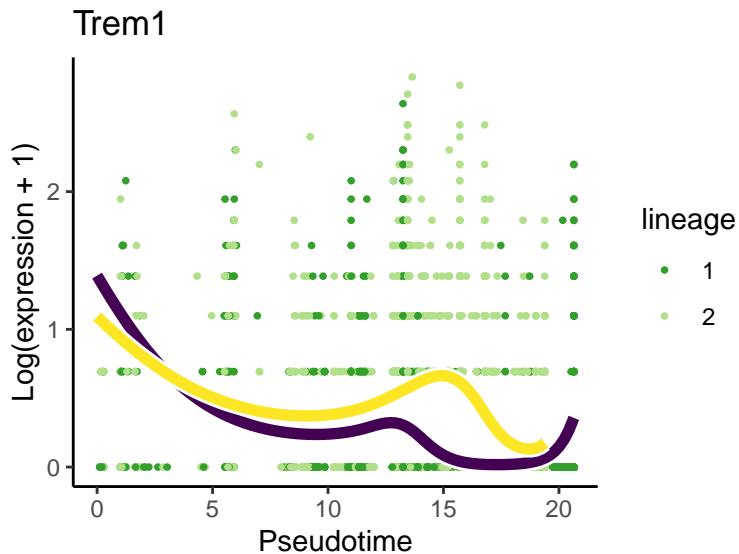
```
sigGene <- "Ccr2"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
#                           "sigGene",
#                           ".pdf"),
#         width = 4, height = 3) 1
#                           "Itga1"),
# plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 2
# ) + scale_color_manual(values =c("#33A02C", "#B2DF8A"))
```

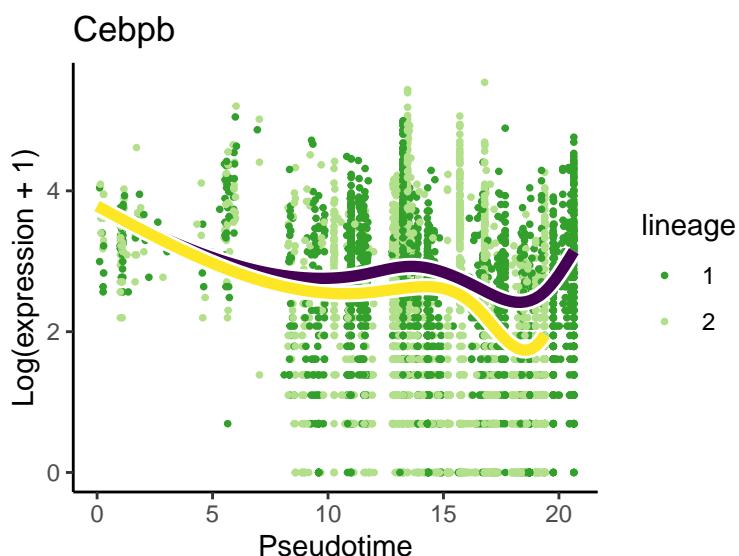


```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
#                           "sigGene",
#                           ".pdf"),
#         width = 4, height = 3) 1
#                           "Trem1"),
# plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 2
# ) + scale_color_manual(values =c("#33A02C", "#B2DF8A"))
```



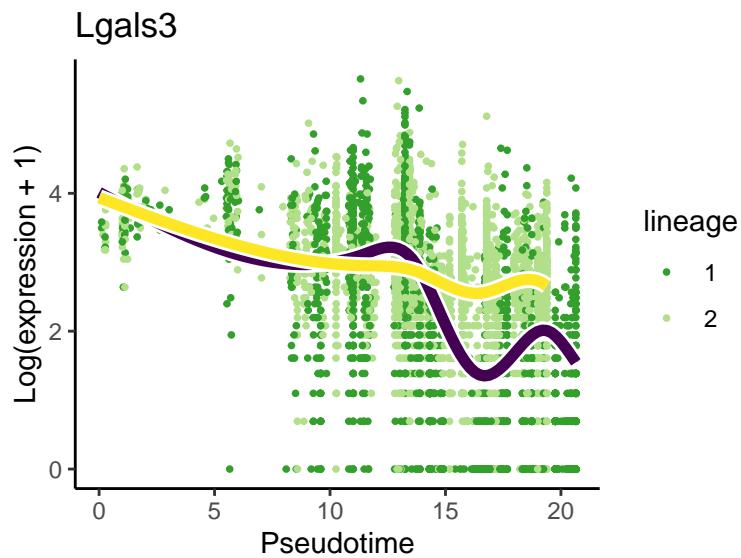
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
#                           ".pdf"),
#         width = 4, height = 3) 1
# 2
```

```
sigGene <- "Cebpb"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



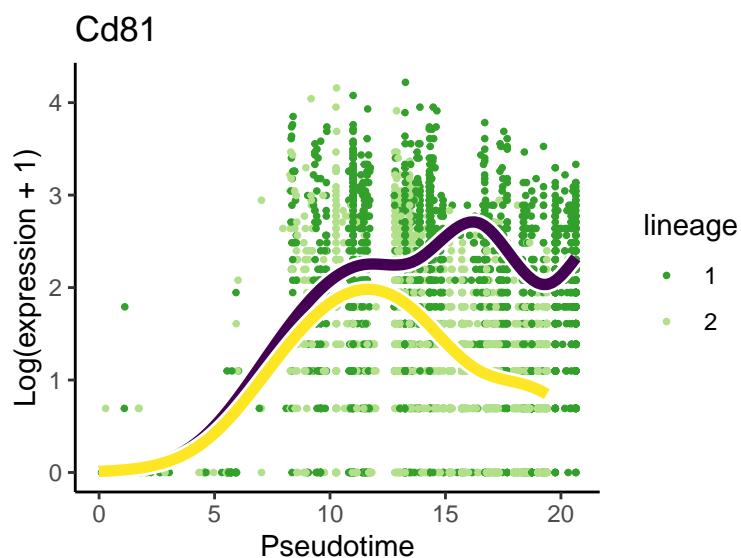
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
#                           ".pdf"),
#         width = 4, height = 3) 1
# 2
```

```
sigGene <- "Lgals3"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



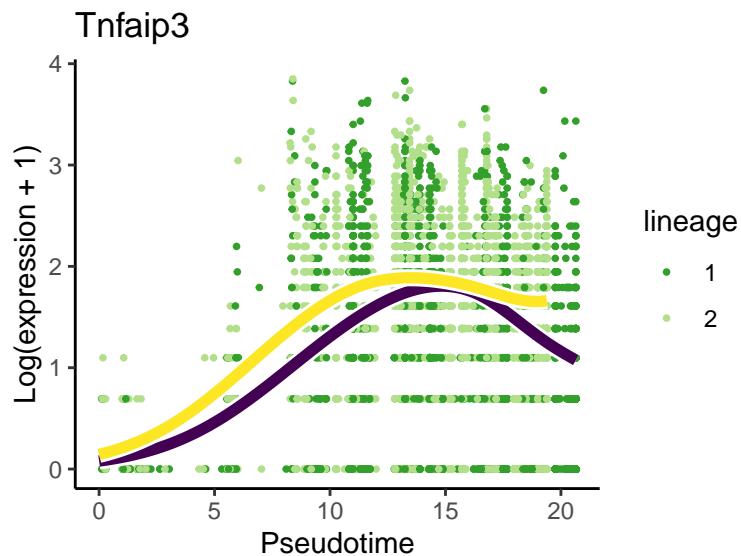
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
#                           sigGene,
#                           ".pdf"),
#         width = 4, height = 3) 1
#                           width = 4, height = 3) 2
```

```
sigGene <- "Cd81"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



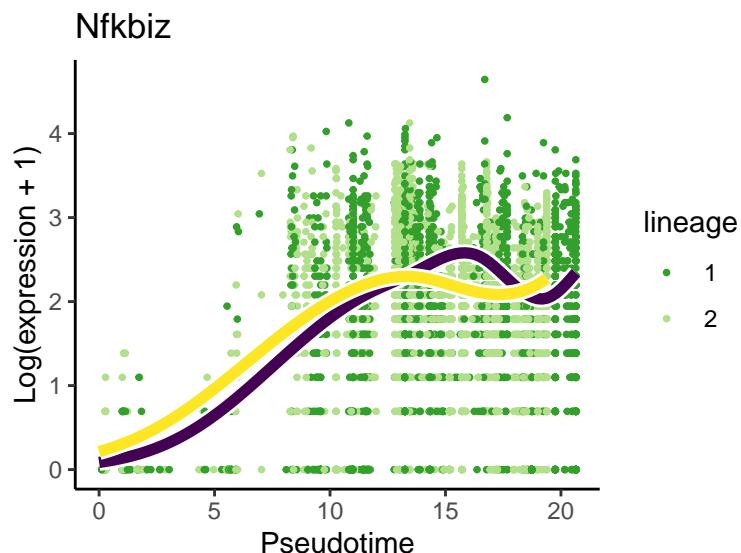
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
#                           sigGene,
#                           ".pdf"),
#         width = 4, height = 3) 1
#                           width = 4, height = 3) 2
```

```
sigGene <- "Tnfaip3"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



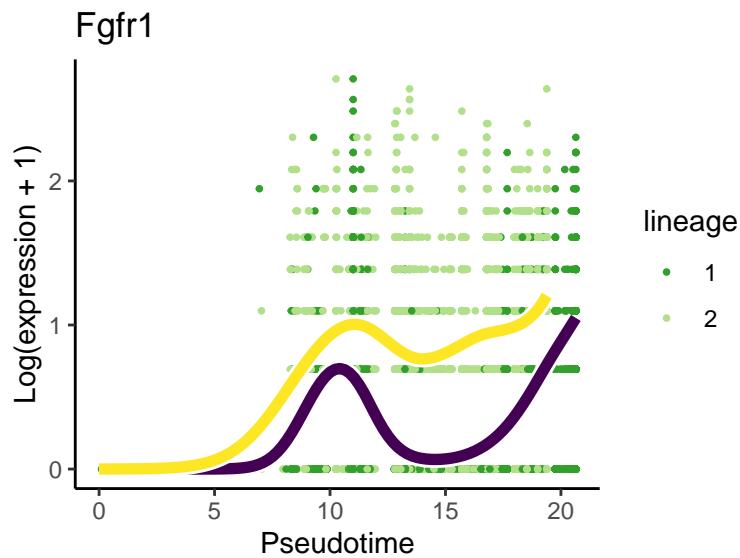
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
, sigGene
, ".pdf"), width = 4, height = 3) 1
```

```
sigGene <- "Nfkbiz"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



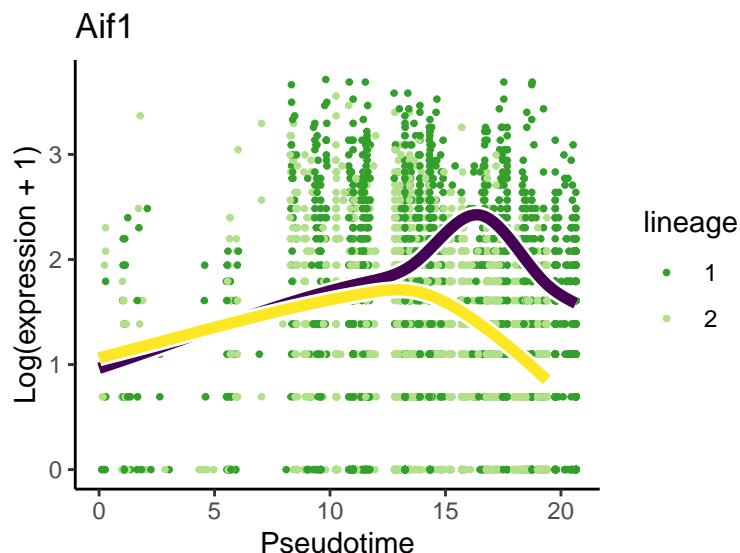
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
, sigGene
, ".pdf"), width = 4, height = 3) 1
```

```
sigGene <- "Fgfr1"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



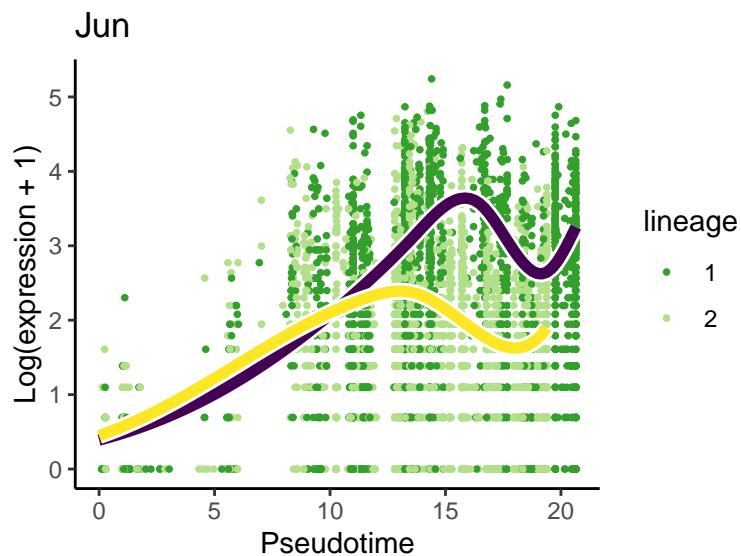
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
, sigGene 1
, ".pdf"), width = 4, height = 3)
```

```
sigGene <- "Aif1"
plotSmoothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



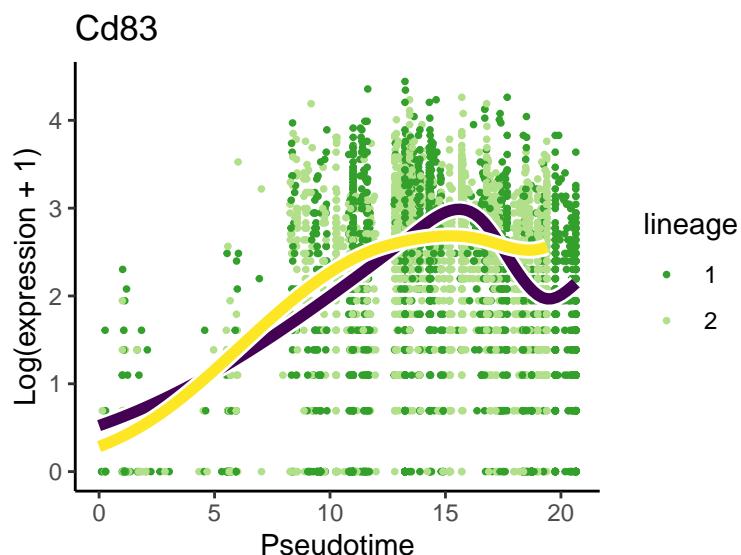
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
, sigGene 1
, ".pdf"), width = 4, height = 3)
```

```
sigGene <- "Jun"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



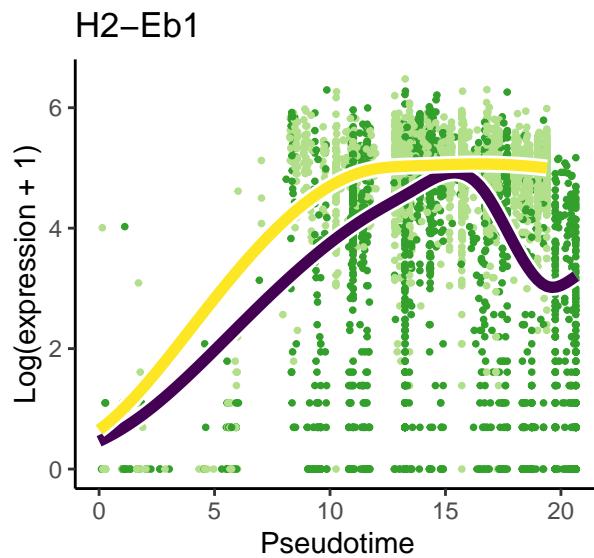
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
, sigGene
, ".pdf"), width = 4, height = 3) 1
```

```
sigGene <- "Cd83"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



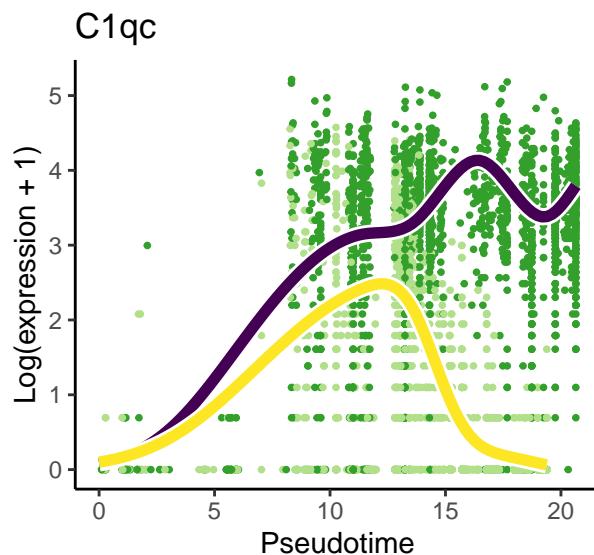
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
, sigGene
, ".pdf"), width = 4, height = 3) 1
```

```
sigGene <- "H2-Eb1"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



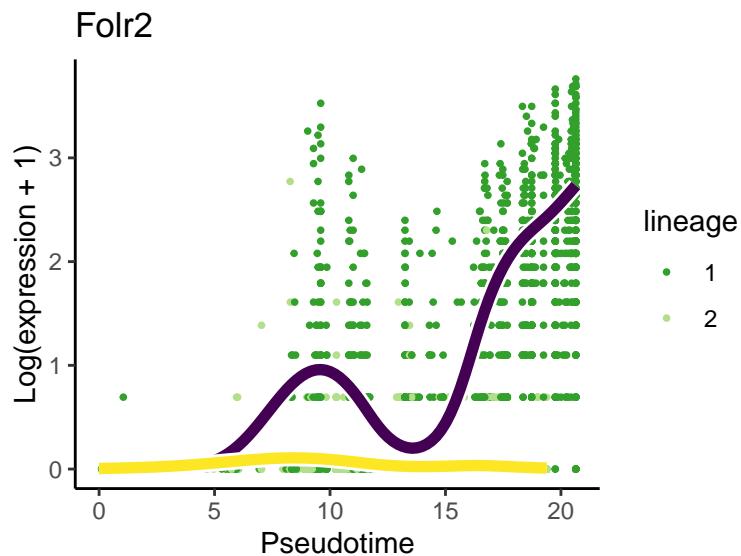
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
, sigGene 1
, ".pdf"), width = 4, height = 3)
```

```
sigGene <- "C1qc"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



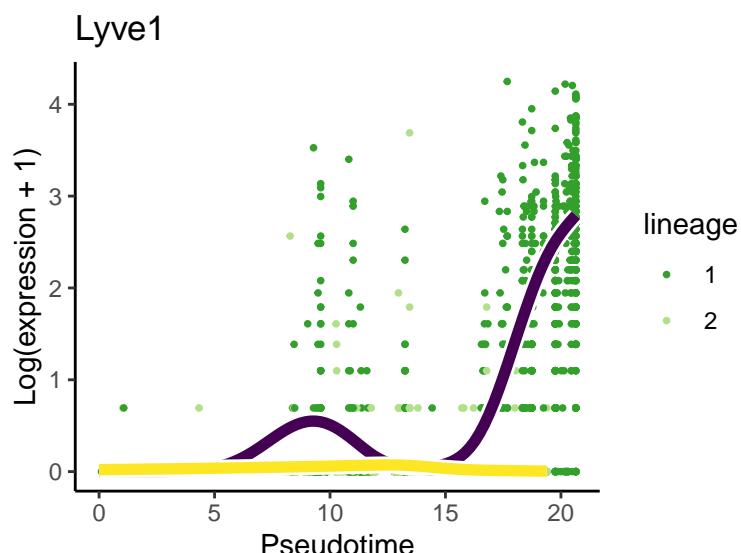
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
, sigGene 1
, ".pdf"), width = 4, height = 3)
```

```
sigGene <- "Folr2"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



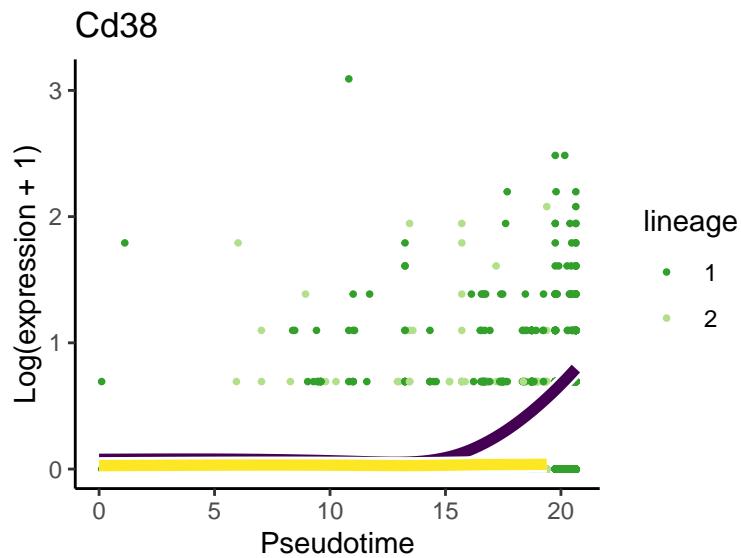
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
, sigGene
, ".pdf"), width = 4, height = 3) 1
```

```
sigGene <- "Lyve1"
plotSmoothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



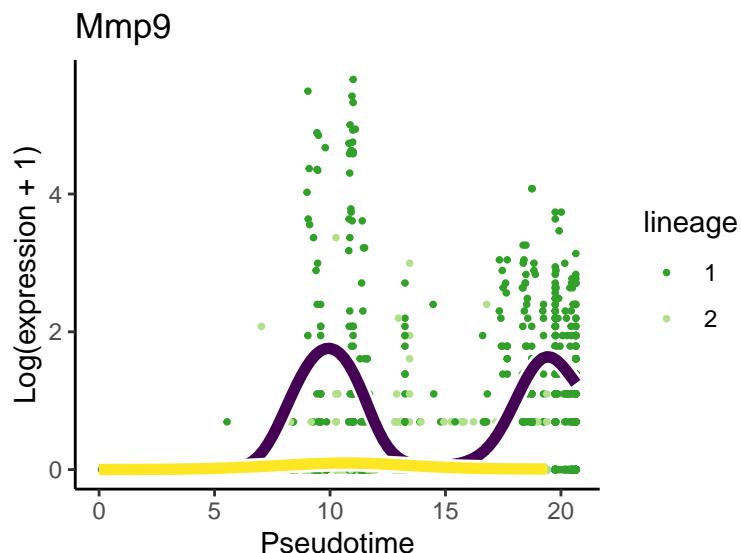
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
, sigGene
, ".pdf"), width = 4, height = 3) 1
```

```
sigGene <- "Cd38"
plotSmoothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



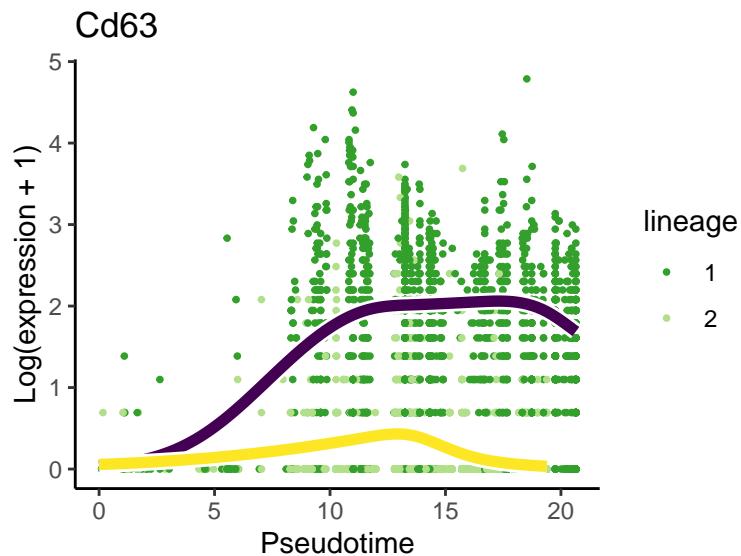
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
, sigGene
, ".pdf"), width = 4, height = 3) 1
```

```
sigGene <- "Mmp9"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



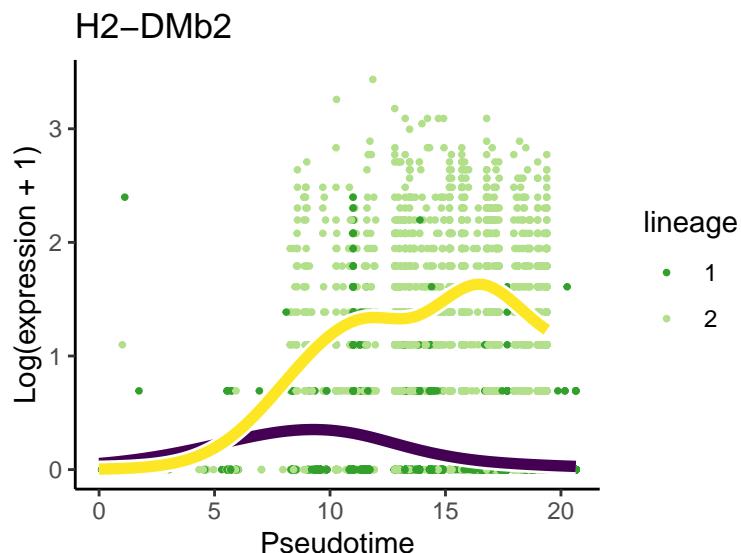
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
, sigGene
, ".pdf"), width = 4, height = 3) 1
```

```
sigGene <- "Cd63"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
, sigGene
, ".pdf"), width = 4, height = 3) 1
```

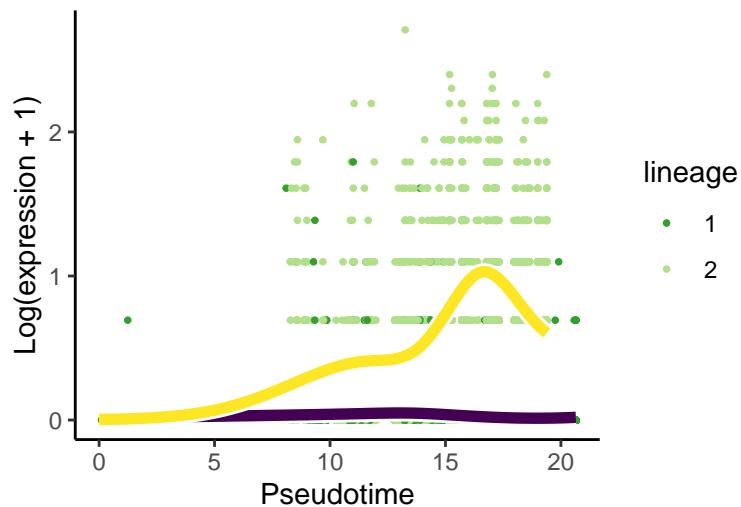
```
sigGene <- "H2-DMb2"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
, sigGene,
, ".pdf"), width = 4, height = 3) 1
```

```
sigGene <- "H2-0a"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```

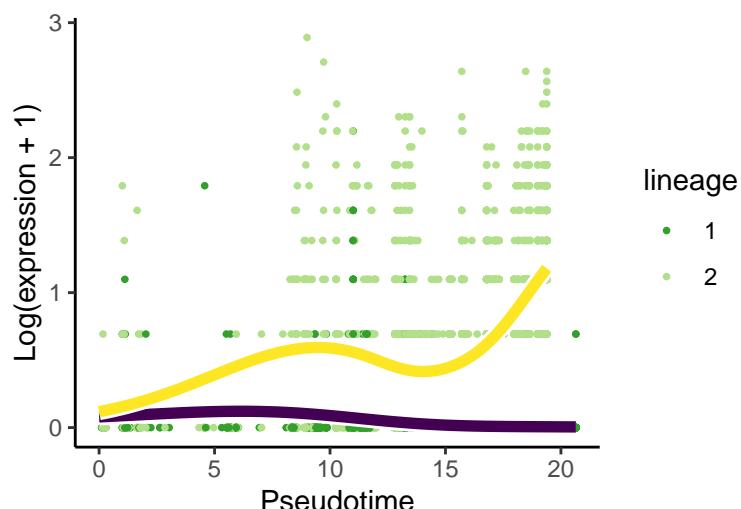
H2-Oa



```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
, sigGene
, ".pdf"), width = 4, height = 3) 1
```

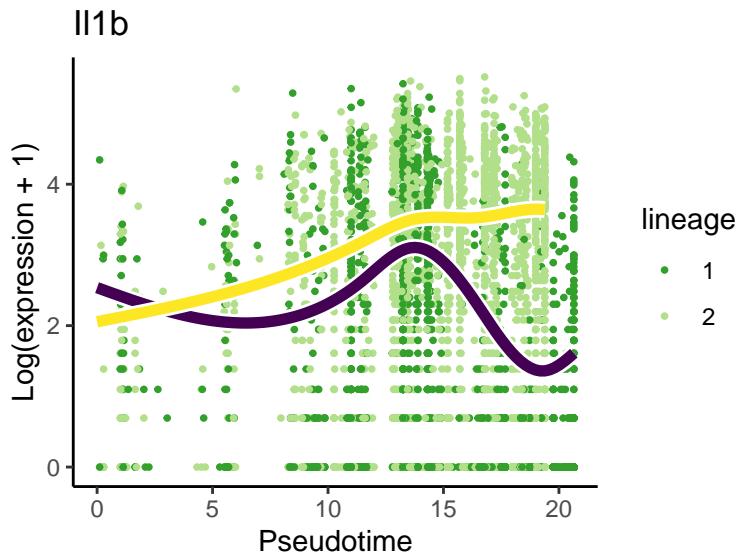
```
sigGene <- "Wnt11"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```

Wnt11



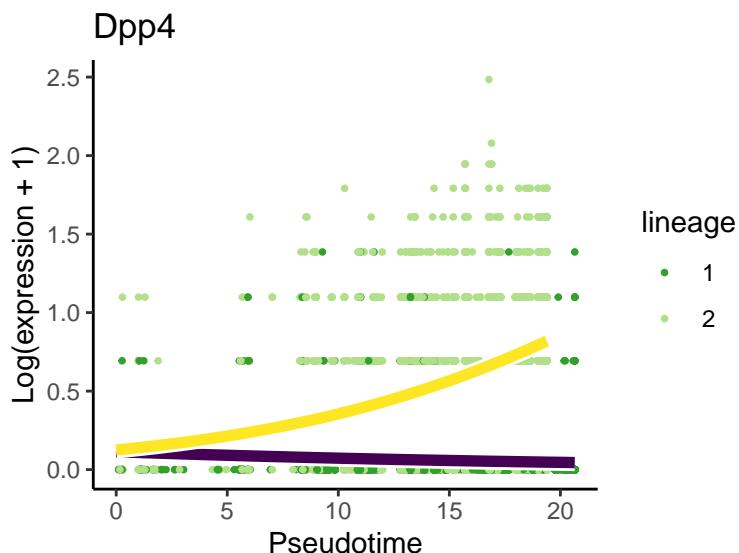
```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
, sigGene
, ".pdf"), width = 4, height = 3) 1
```

```
sigGene <- "Il1b"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
, sigGene 1
, ".pdf"), width = 4, height = 3)
```

```
sigGene <- "Dpp4"
plotSmothers(sce, counts = counts(sce), gene = sigGene) + ggtitle(sigGene 1
) + scale_color_manual(values =c("#33A02C", "#B2DF8A")) 2
```



```
# ggsave(filename = paste("../Figures/TradeSeq_plot_IM_lineages_",
, sigGene 1
, ".pdf"), width = 4, height = 3)
```

8 Session information

R session:

```
sessionInfo()
```

```

## R version 4.0.3 (2020-10-10)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 20.04.3 LTS
##
## Matrix products: default
## BLAS:    /usr/lib/x86_64-linux-gnu/openblas-pthread/libblas.so.3
## LAPACK:  /usr/lib/x86_64-linux-gnu/openblas-pthread/liblapack.so.3
##
## locale:
## [1] LC_CTYPE=en_US.UTF-8          LC_NUMERIC=C
## [3] LC_TIME=en_GB.UTF-8          LC_COLLATE=en_US.UTF-8
## [5] LC_MONETARY=en_GB.UTF-8      LC_MESSAGES=en_US.UTF-8
## [7] LC_PAPER=en_GB.UTF-8         LC_NAME=C
## [9] LC_ADDRESS=C                  LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_GB.UTF-8   LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats4     parallel   grid       stats      graphics  grDevices utils
## [8] datasets   methods    base
##
## other attached packages:
## [1] org.Mm.eg.db_3.12.0           AnnotationDbi_1.52.0
## [3] clusterProfiler_3.18.1        clusterExperiment_2.11.2
## [5] tradeSeq_1.4.0                magrittr_2.0.1
## [7] monocle3_1.0.0               SingleCellExperiment_1.12.0
## [9] SummarizedExperiment_1.20.0   GenomicRanges_1.42.0
## [11] GenomeInfoDb_1.26.7          IRanges_2.24.1
## [13] S4Vectors_0.28.1             MatrixGenerics_1.2.1
## [15] matrixStats_0.61.0            Biobase_2.50.0
## [17] BiocGenerics_0.36.1          circlize_0.4.13
## [19] RColorBrewer_1.1-2            dplyr_1.0.7
## [21] ggplot2_3.3.5                ComplexHeatmap_2.6.2
## [23] SeuratObject_4.0.4            Seurat_4.0.5
##
## loaded via a namespace (and not attached):
## [1] scattermore_0.7              prncurve_2.1.6      coda_0.19-4
## [4] pkgmaker_0.32.2              tidyR_1.1.4         bit64_4.0.5
## [7] knitr_1.36                   irlba_2.3.5         DelayedArray_0.16.3
## [10] data.table_1.14.2            rpart_4.1-15        RCurl_1.98-1.5
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