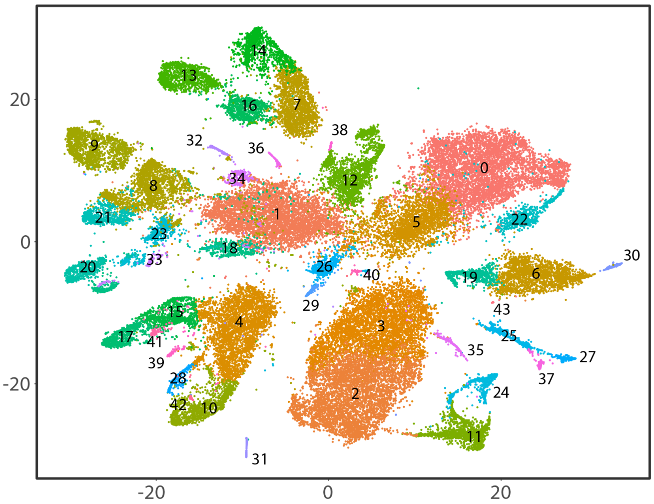
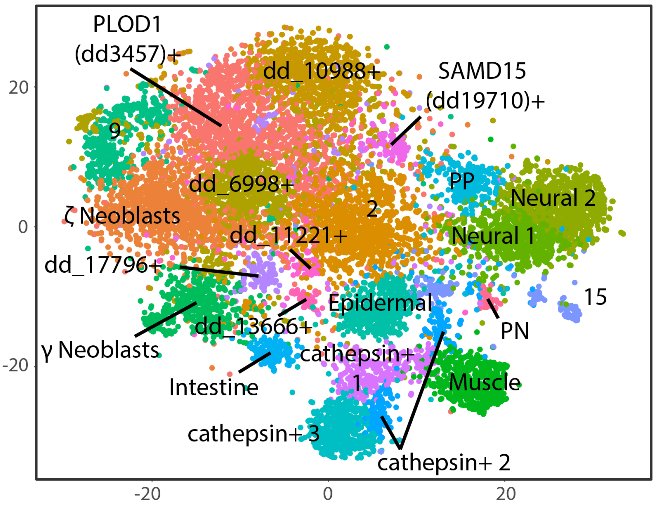
Supplementary Figure 9: Single-cell RNA-seq profiles of highly enriched X2 genes marked by bivalent H3K4me3 and H3K27me3 histones. We predicted the cell lineage involvement of X2 genes plotted in Figure 5 using single cell Drop-seq data from Fincher et al. (2018) and generated a pseudotime lineage tree using the database from Plass, Solana et al. (2018). For all genes we observe an enrichment in post-mitotic populations, and low expression in the *smedwi-1*+ X1 compartment. For *egr-1* and *SoxP-3* we note an enrichment also in *smedwi-1*+ stem cells, but the expression within cells taken as a whole increases in the post-mitotic compartment consistent with our FACS proportional analysis.

From [**http://digiworm.wi.mit.edu**](http://digiworm.wi.mit.edu)

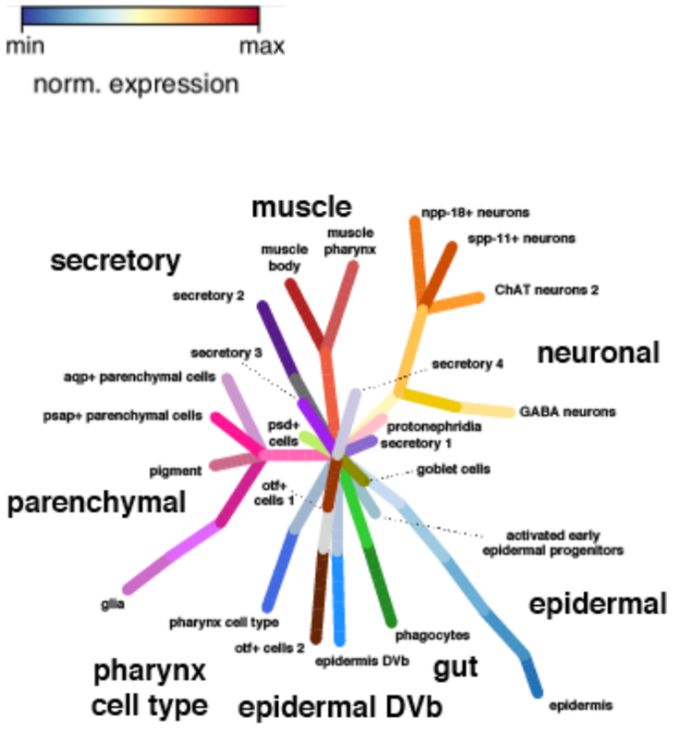
Overall clusters generated in dataset

Stem cell (*Smedwi-1)* sub-clustering





From https://shiny.mdc-berlin.de/psca/

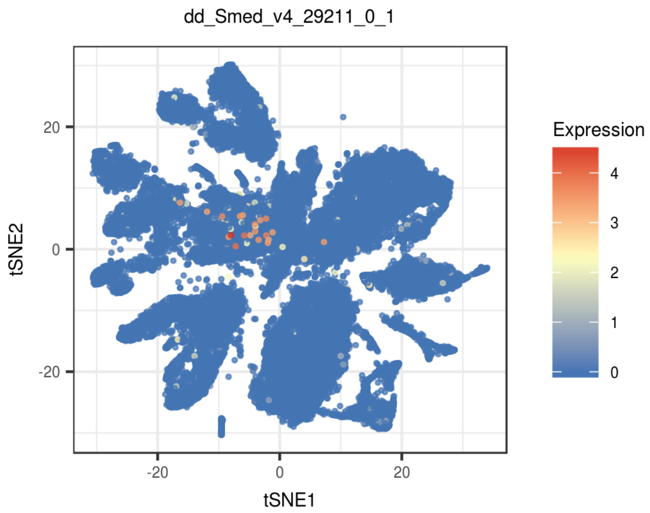


phox 2a:

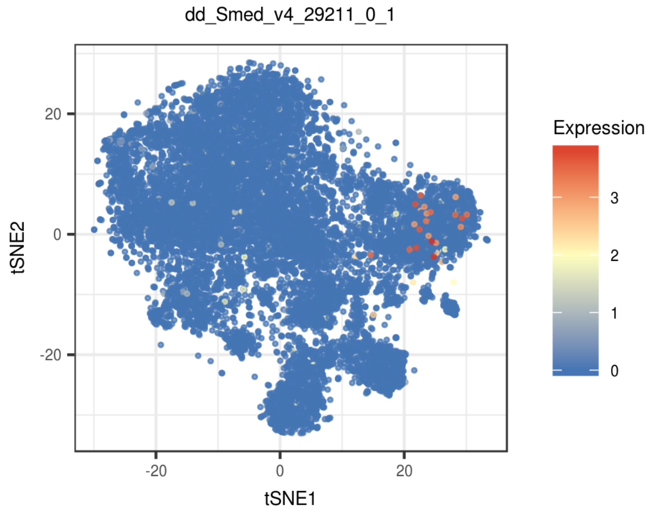
<u>enrichment in neural, non-cilliated neurons and parenchymal</u>

<u>no significant enrichment in smedwi-1 neoblasts; but a few ‘neural’ NBs have expression</u>

Overall expression:

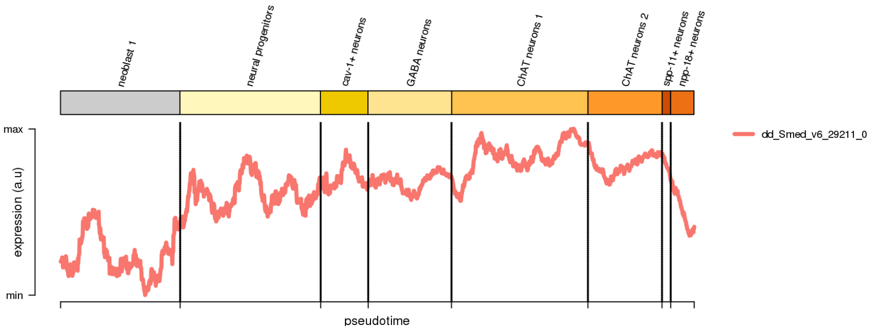


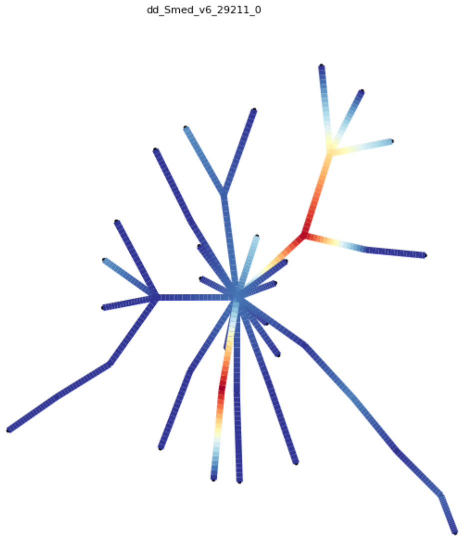
Stem Cell expression:



```
Contig is enriched in the following clusters
Based on main clustering analysis:
---------------------------------
Parenchymal: 38
Based on sub-clustering:
----------------------
Neural: 35
Non-ciliated neurons: 12, 25
Parenchymal: 7
```

Pseudotime Analysis for neuronal lineage:



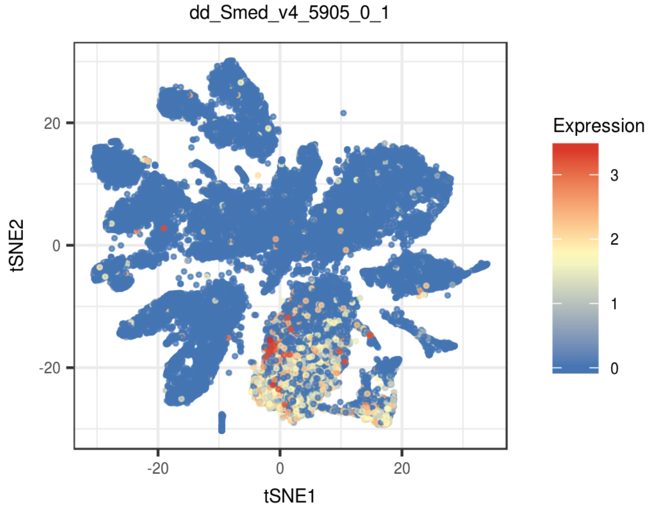


reticulocalbin-1

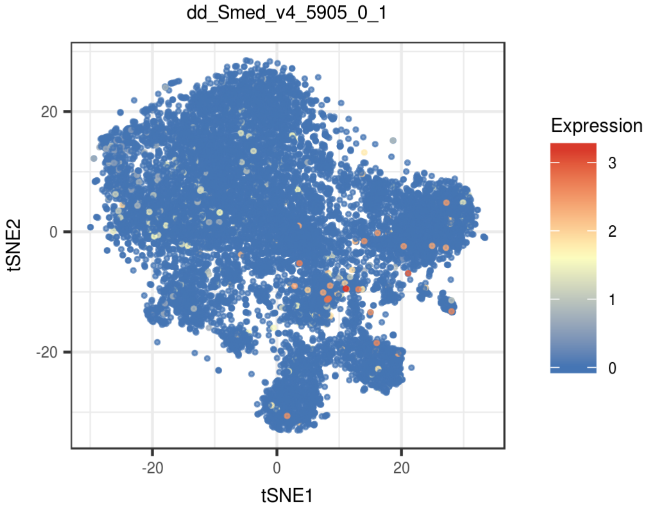
enrichment epidermis

no significant enrichment in smedwi-1 neoblasts; but a few ‘epidermal’ NBs have expression

Overall expression:

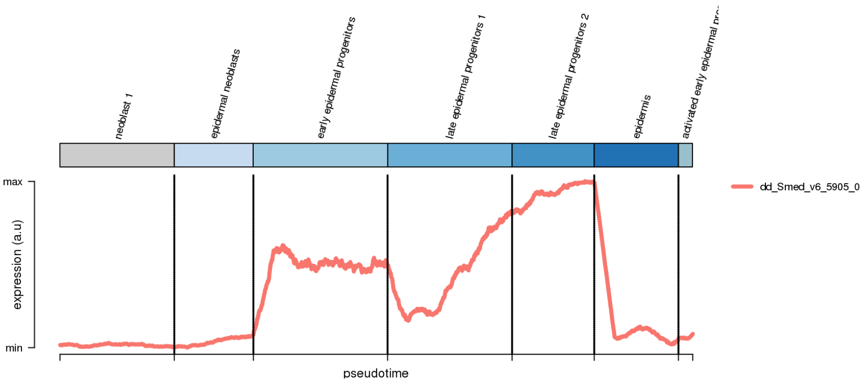


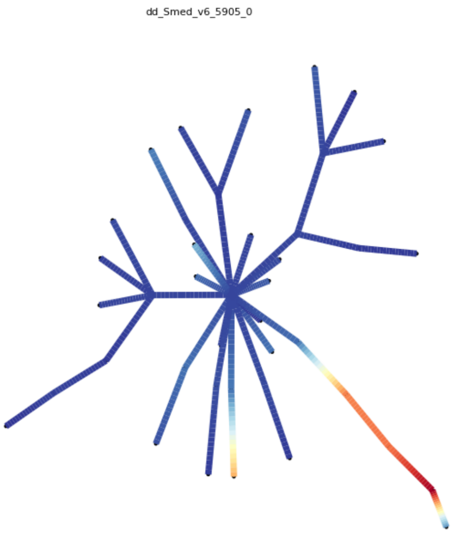
Stem Cell expression:



```
Contig is enriched in the following clusters
Based on main clustering analysis:
---------------------------------
Epidermal: 2, 3, 11, 35
Based on sub-clustering:
----------------------
Epidermal: 1
Pharynx: 0
```

Pseudotime Analysis for epidermal lineage:



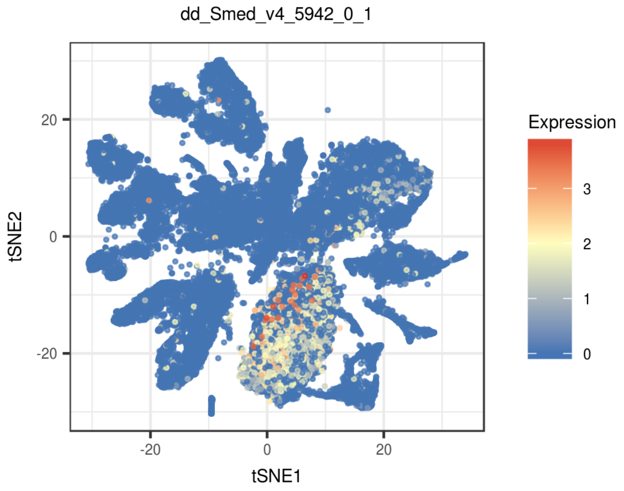


soxP-3

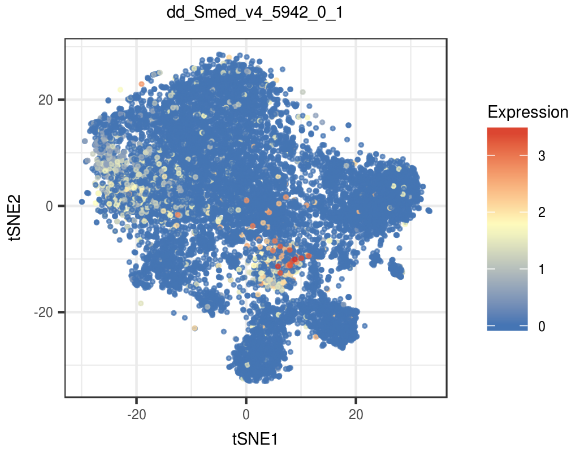
enrichment in epidermis

statistical enrichment in neoblasts, but expressed in epidermal and zeta class

Overall expression:

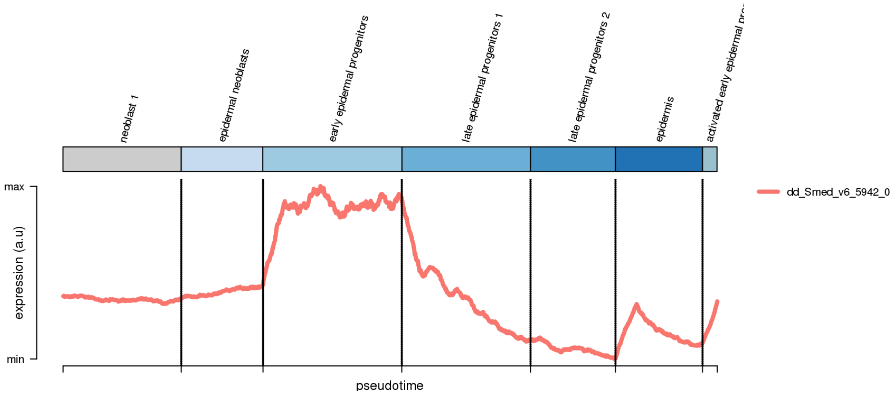


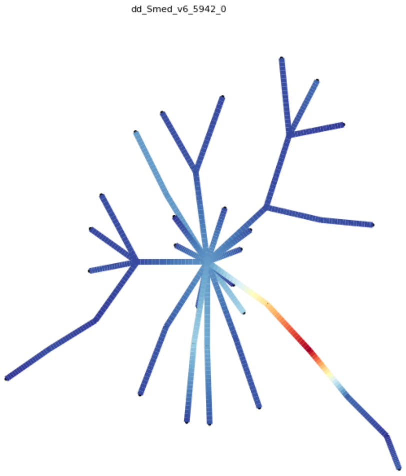
Stem Cell expression:



```
Contig is enriched in the following clusters
Based on main clustering analysis:
----------------------------------
Epidermal: 2, 3
Based on subclustering:
----------------------
Epidermal: 0, 1
Smedwi+1 cells: 1, 10
```

Pseudotime Analysis for epidermal lineage



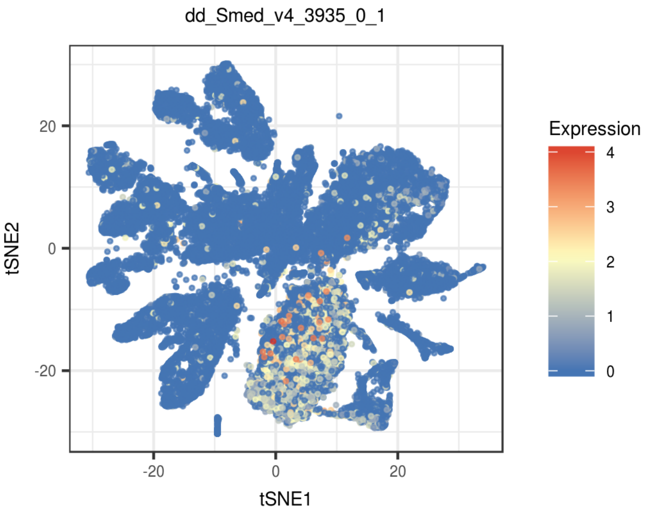


egr-1

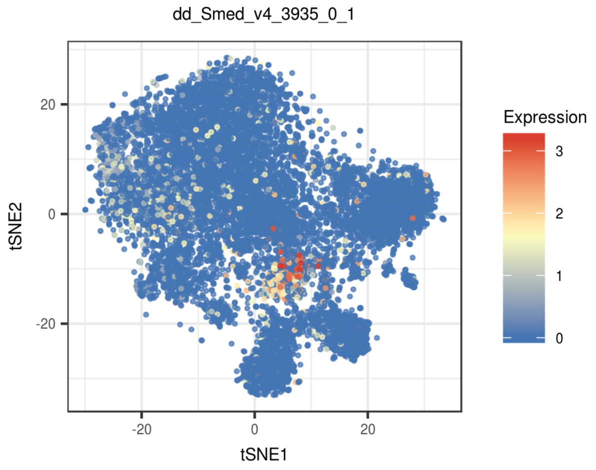
enrichment in epidermis

statistical enrichment in neoblasts, but expressed in epidermal and zeta class

Overall expression:

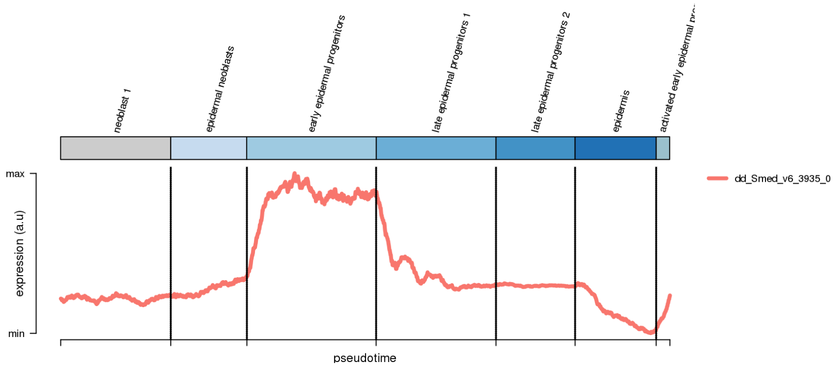


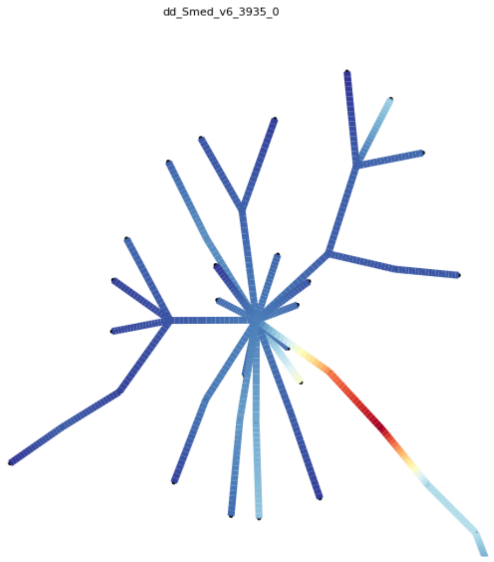
Stem Cell expression:



```
Contig is enriched in the following clusters
Based on main clustering analysis:
---------------------------------
Epidermal: 2, 3
Based on sub−clustering:
----------------------
Epidermal: 2
Smedwi+1 cells: 10
```

Pseudotime Analysis for epidermal lineage



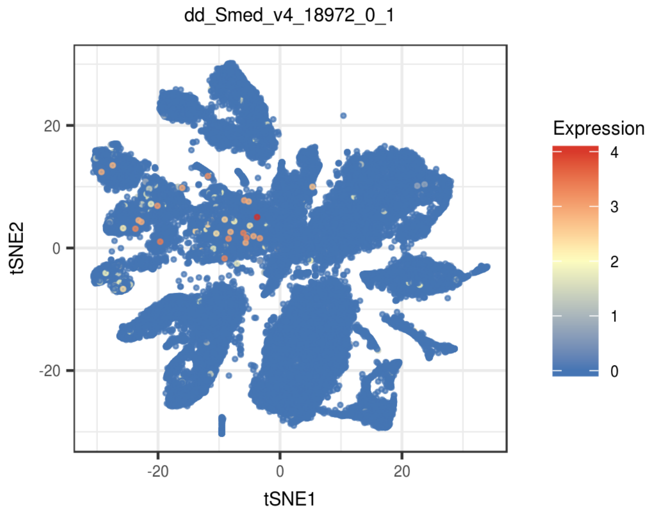


nkx1.2

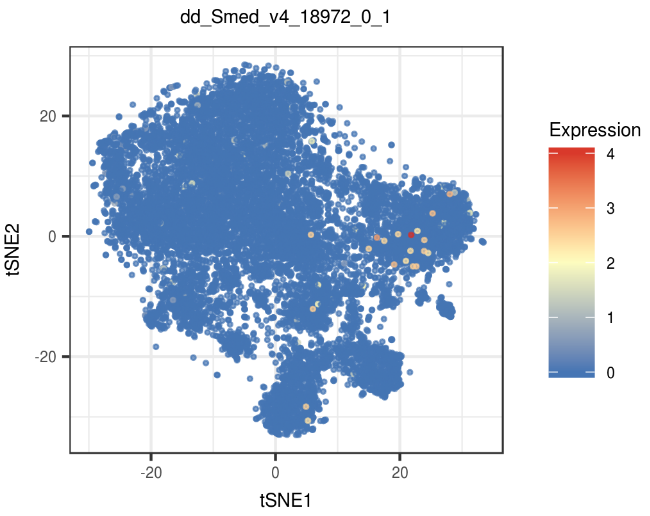
enrichment in neuron and non-cilliated neurons

some expression in neuronal subset of X1 neoblasts but no enrichment

Overall expression:



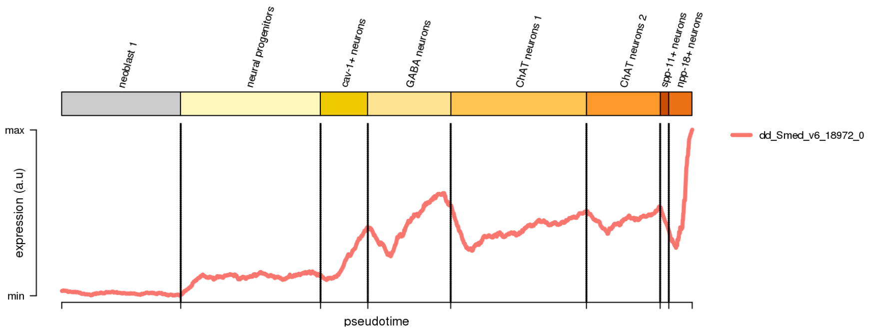
Stem Cell expression:

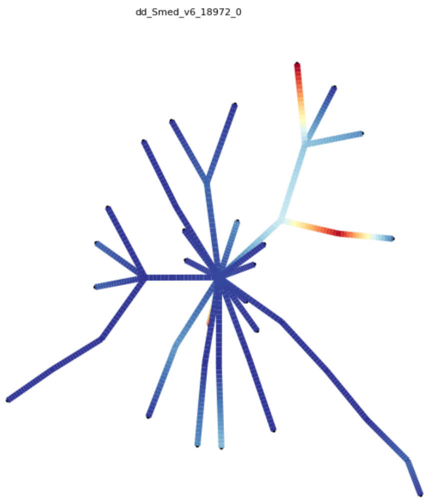


```
Contig is enriched in the following clusters
Based on main clustering analysis:
--------------------------------


Based on subclustering:
---------------------
Neural: 46
Non-ciliated neurons: 14
```

Pseudotime Analysis for neuronal lineage



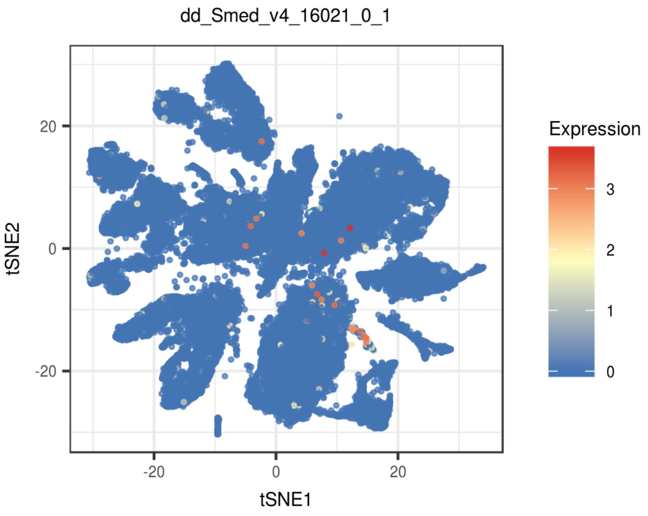


hoxb9 (post2a)

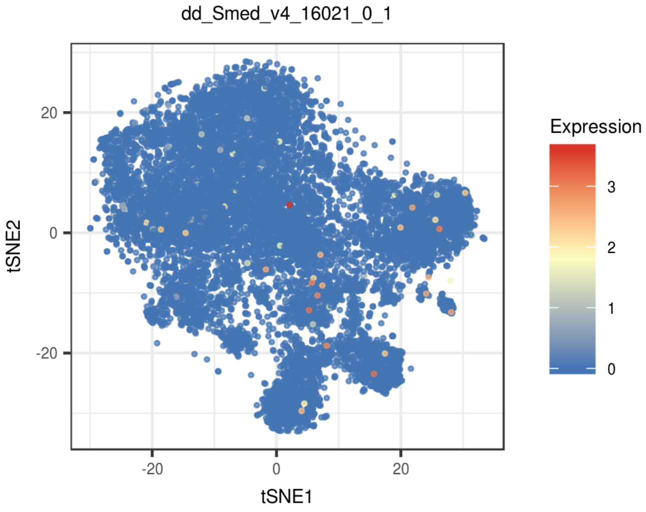
enrichment in epidermis

some expression in epidermal subset of X1 neoblasts but no enrichment

Overall expression:

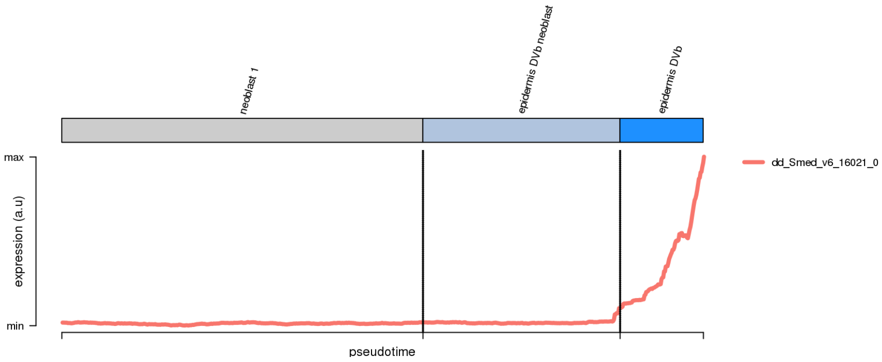


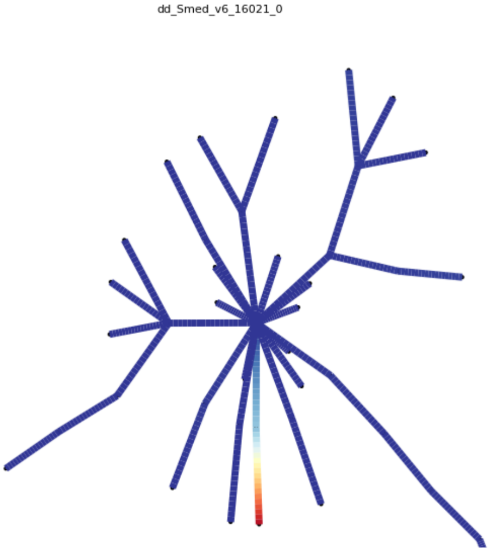
Stem Cell expression:



```
Contig is enriched in the following clusters
Based on main clustering analysis:
---------------------------------
Epidermal: 35
Based on subclustering:
----------------------
Epidermal: 10
```

Pseudotime Analysis for epidermal (DVb) lineage





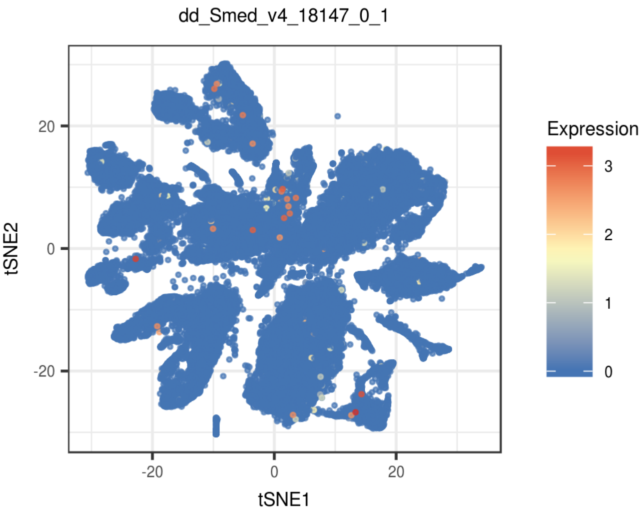
evx-1

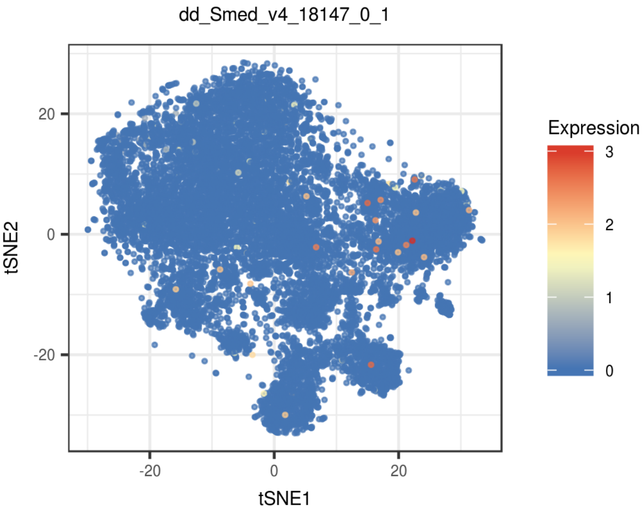
enrichment in parenchyma

some expression in subset of X1 neoblasts but no enrichment

Overall expression:

Stem Cell expression:

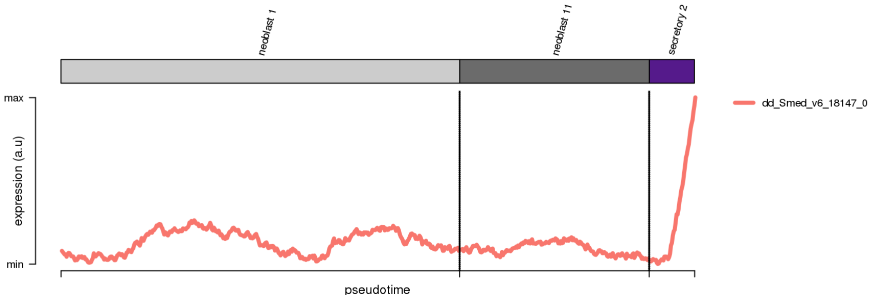


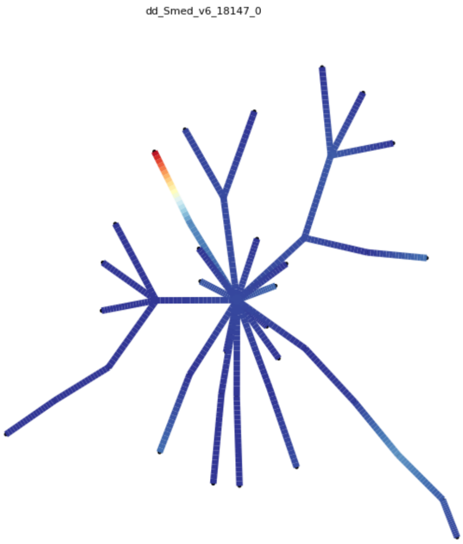


```
Contig is enriched in the following clusters
Based on main clustering analysis:
---------------------------------


Based on subclustering:
----------------------
Parenchymal: 6
```

Pseudotime Analysis for secretory 2 lineage:



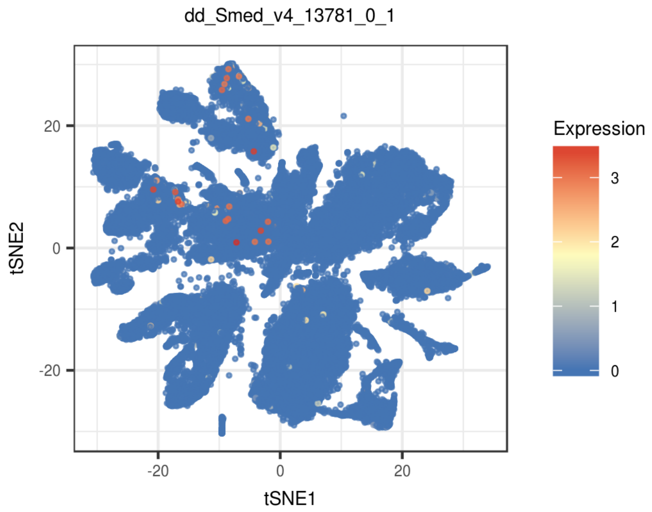


T-cell leukaemia homeobox 3 (annotated as tlx3)

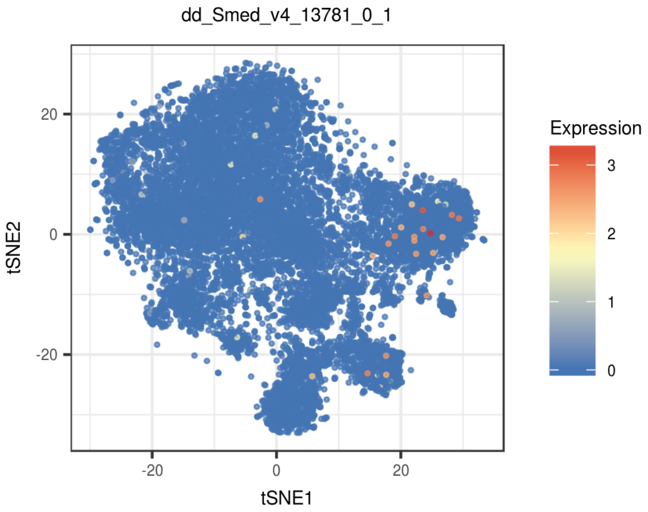
enrichment in neural and non-cilliated neurons

some expression in subset of neural X1 neoblasts but no enrichment

Overall expression:



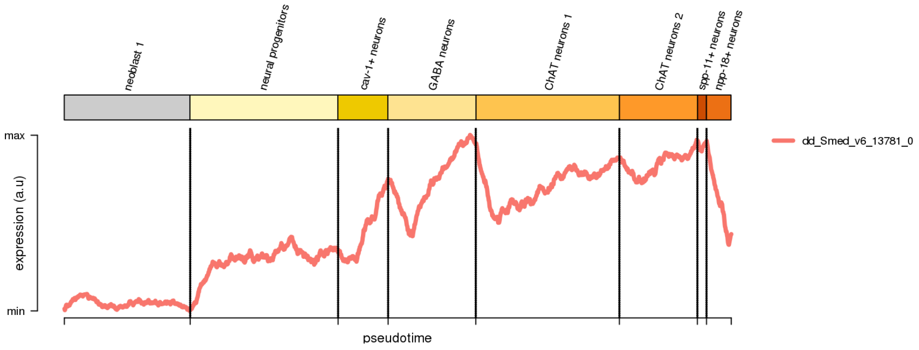
Stem Cell expression:

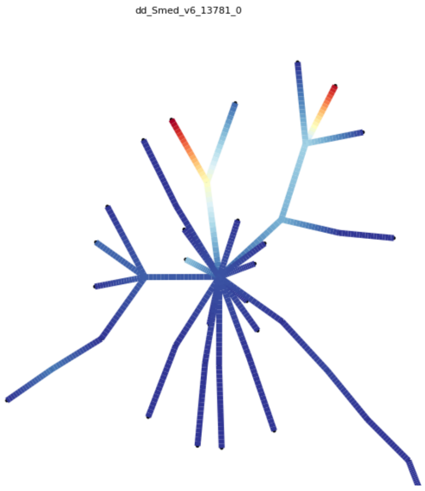


```
Contig is enriched in the following clusters
Based on main clustering analysis:
---------------------------------


Based on subclustering:
----------------------
Neural: 35
Non-ciliated neurons: 12, 25
```

Pseudotime Analysis for neuronal lineage:





N.B. Single Cell profiles for the Tbx2 gene in Figure 5 are not included in the Digiworm database, despite having a cognate Dresden ID (dd_Smed_v6_9460_0_2).