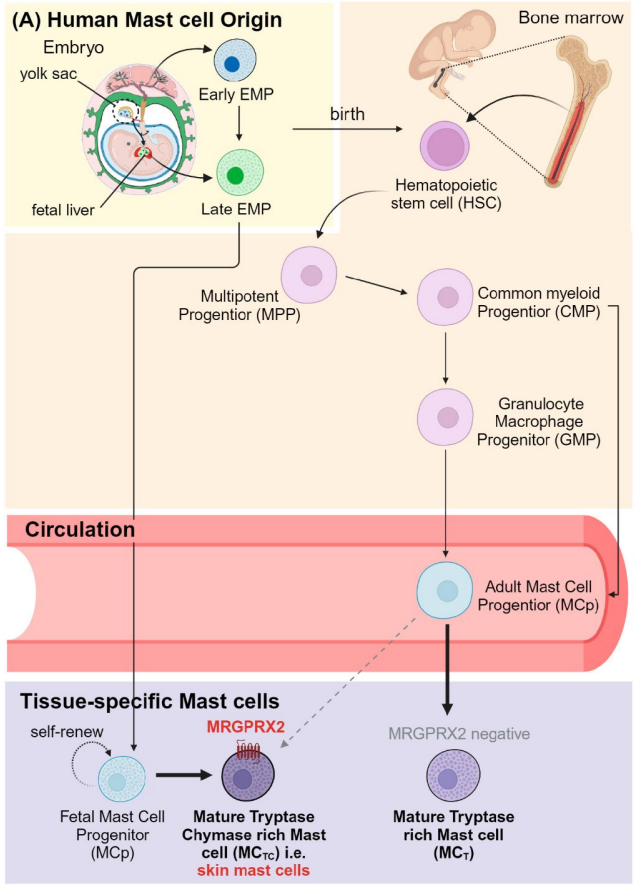
Exploring Myeloid Stem Cell Differentiation Pathways

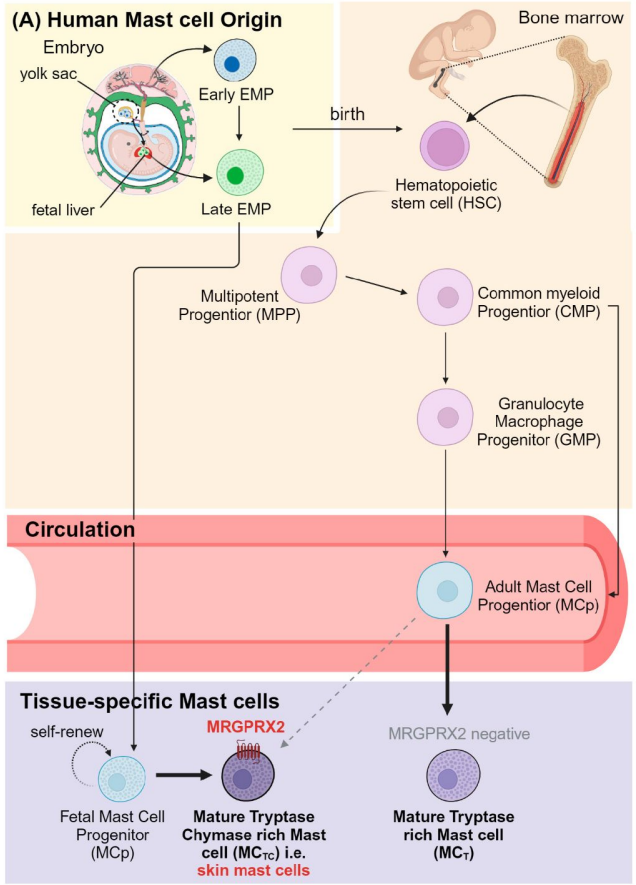
Ashley, Chase, Christian, Mariana, Paula
Group 11

Background: Mast Cell Development

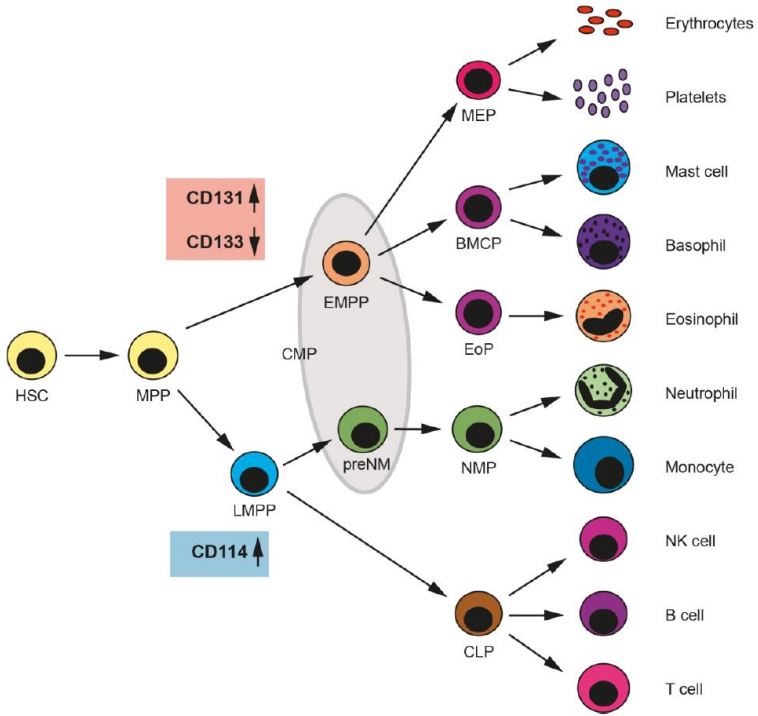


Bosveld, Guth, & Pundir., *Cells* 2023

Background: Mast Cell Development



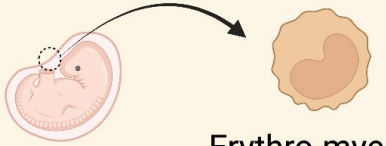
Bosveld, Guth, & Pundir., *Cells* 2023



Drissen, et al., *Science immunology* 2019

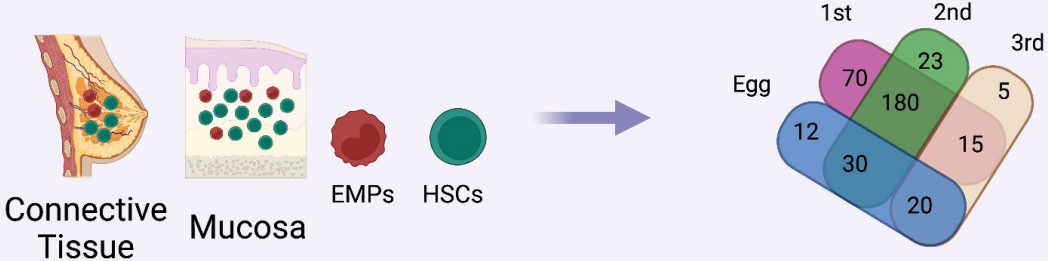
Hypothesis

Prenatal origin



Postnatal origin

Tissue-Specific Mast Cells



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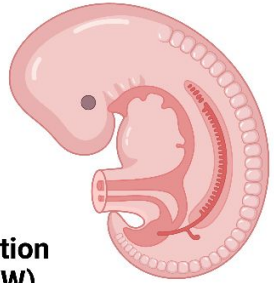
Data Overview

h5ad

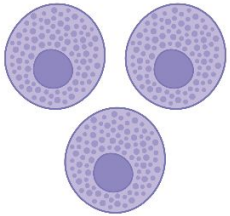
Embryonic

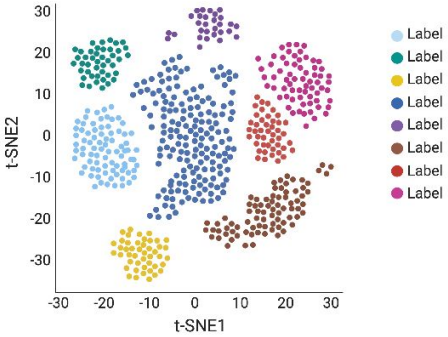


4-8 Postconception Weeks (PCW)

Seurat

Mast Cells





scRNA-Seq Data

h5ad

Adult

Dataset Information

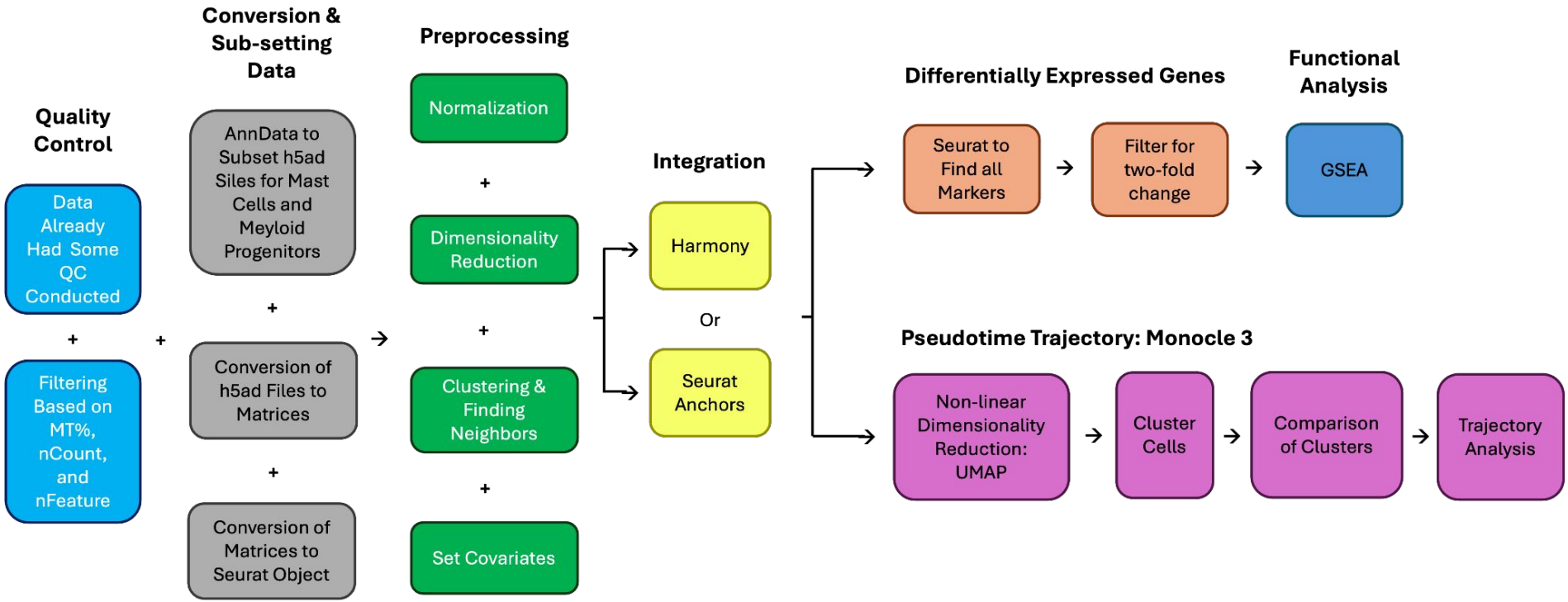
- 10 Samples
- 354 Mast Cells
- Goh, I., et al., Science. 2023.

Dataset Information

- 24 Organs of 15 Normal Human Subjects
- 2238 Mast Cells
- Tauber, M., et al., JEM 2023

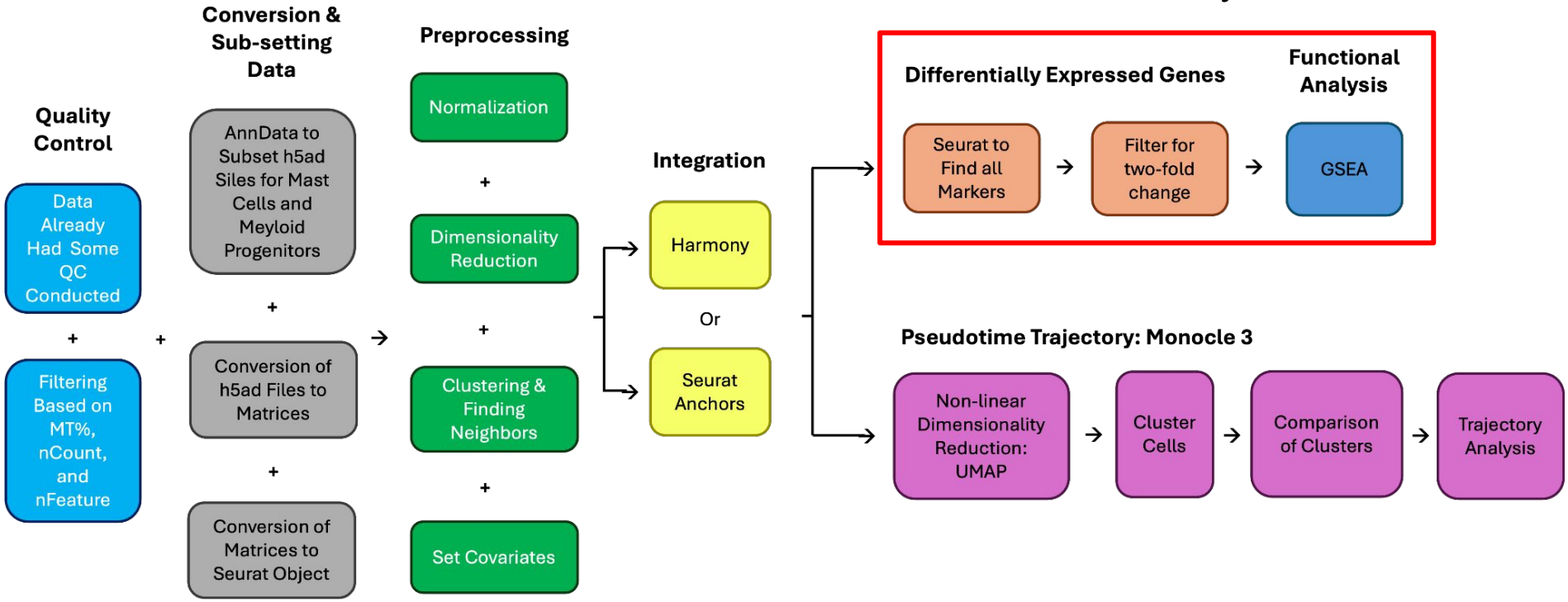
Created on BioRender

Analysis Workflow



Analysis Workflow

Purpose: To Study Differences in DEGs Between Adult and Embryonic Mast Cells



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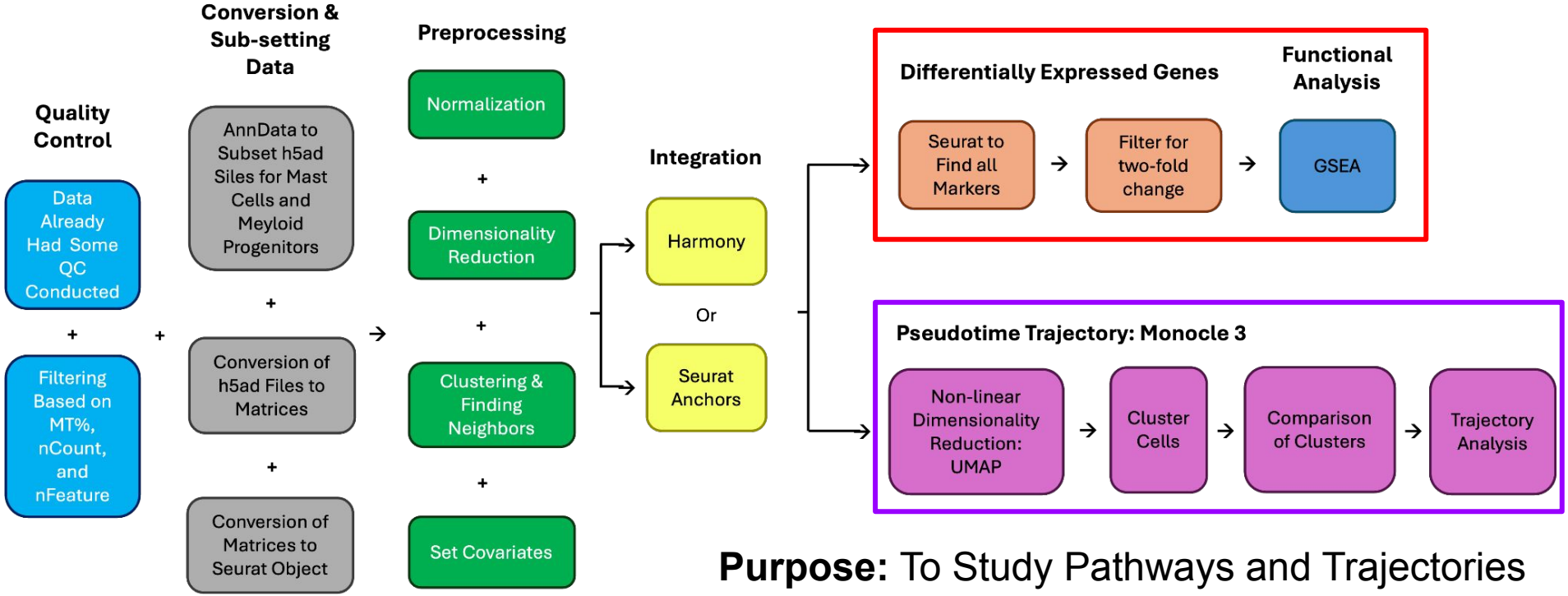
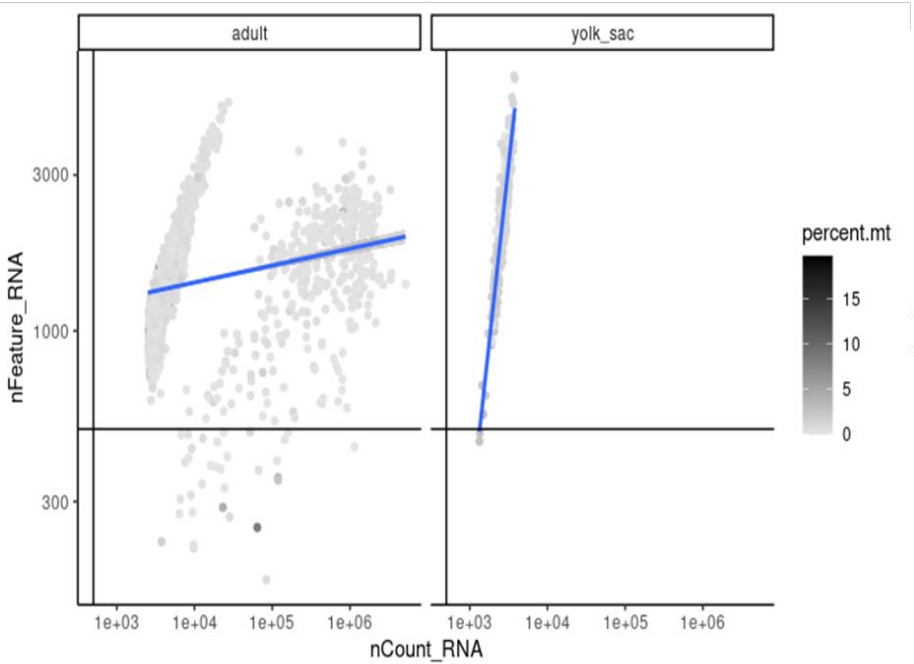
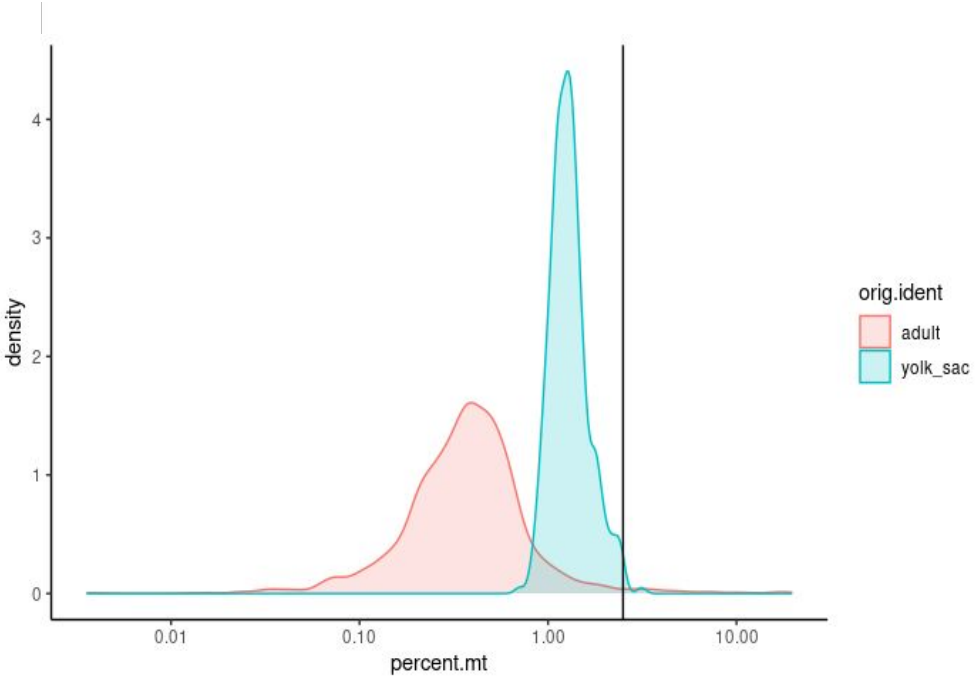


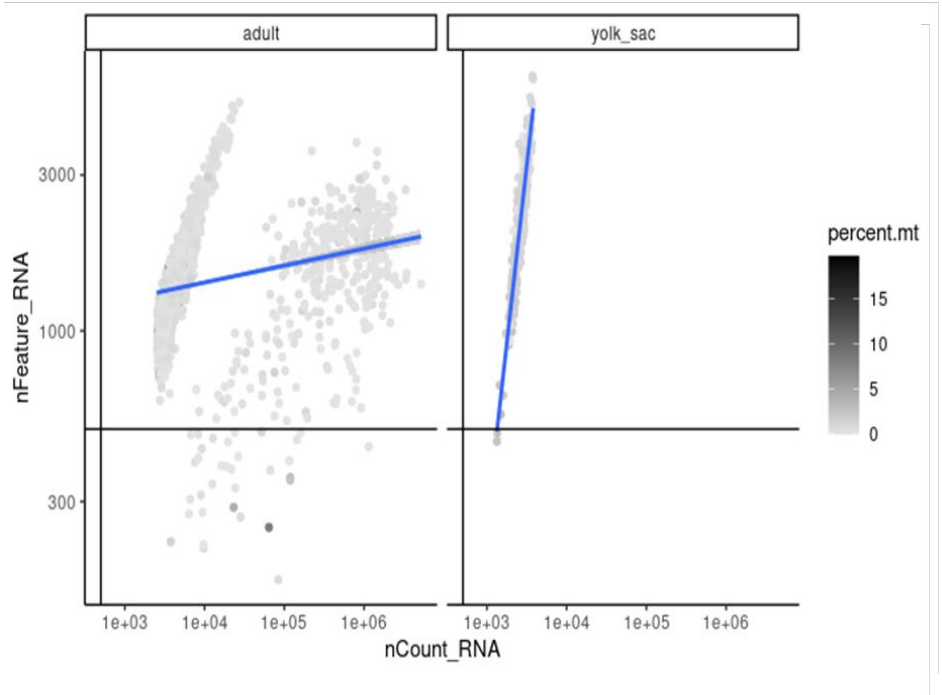
Purpose: To Study Pathways and Trajectories in Hematopoiesis Related to Mast Cells

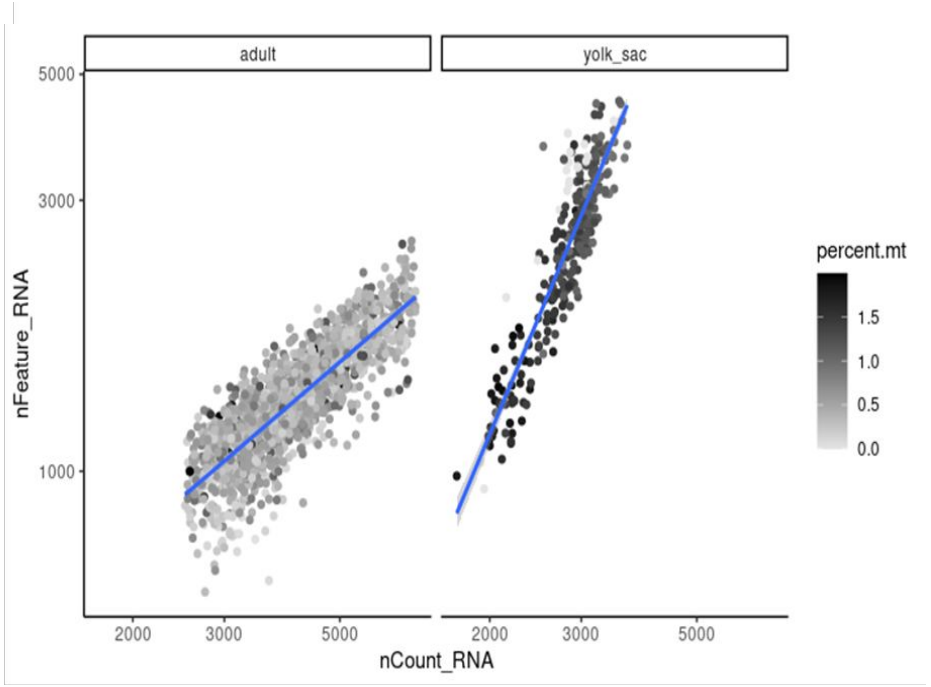
Pre-processing





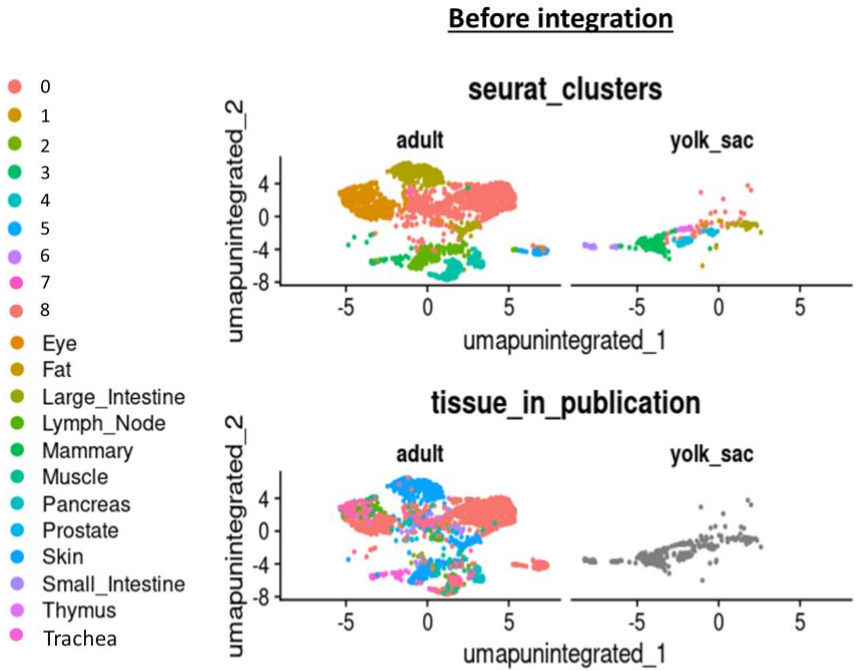
Pre-processing





<2~ % Mitochondrial reads & <7000 Counts.

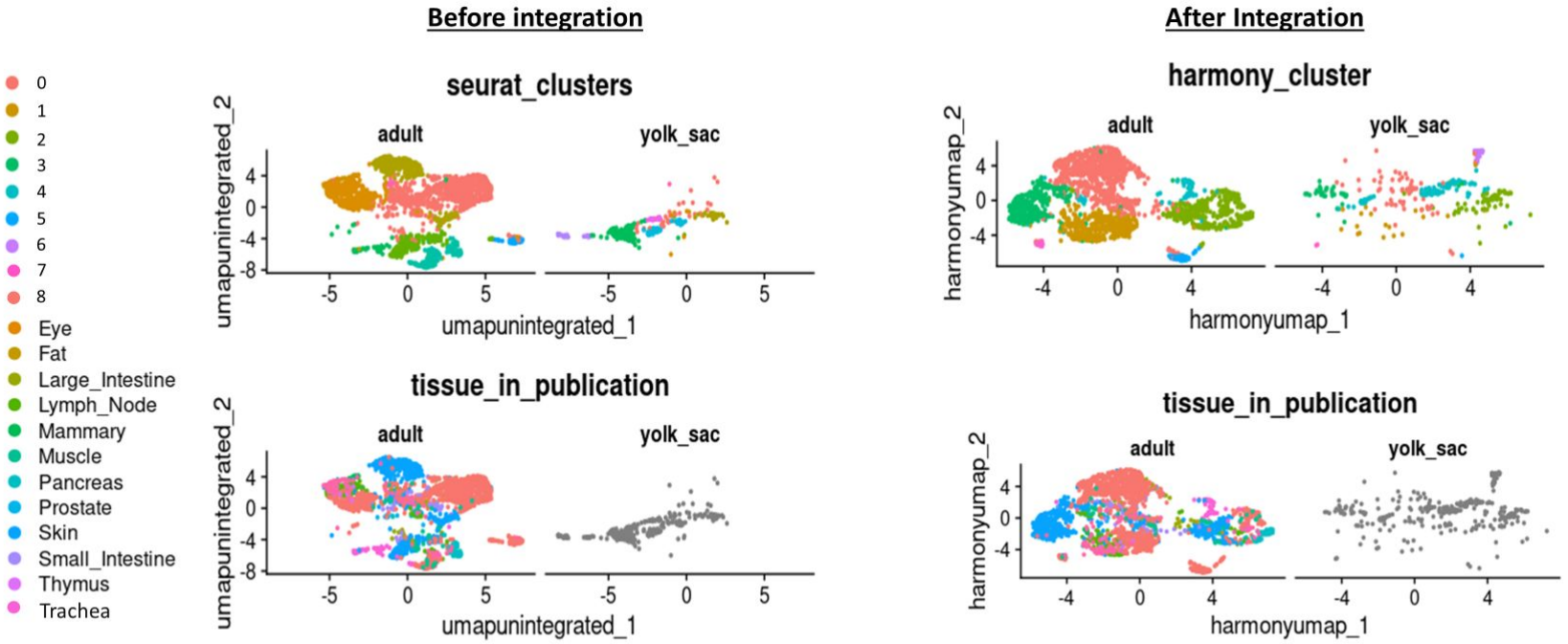
Normalization, Integration, and Clustering..



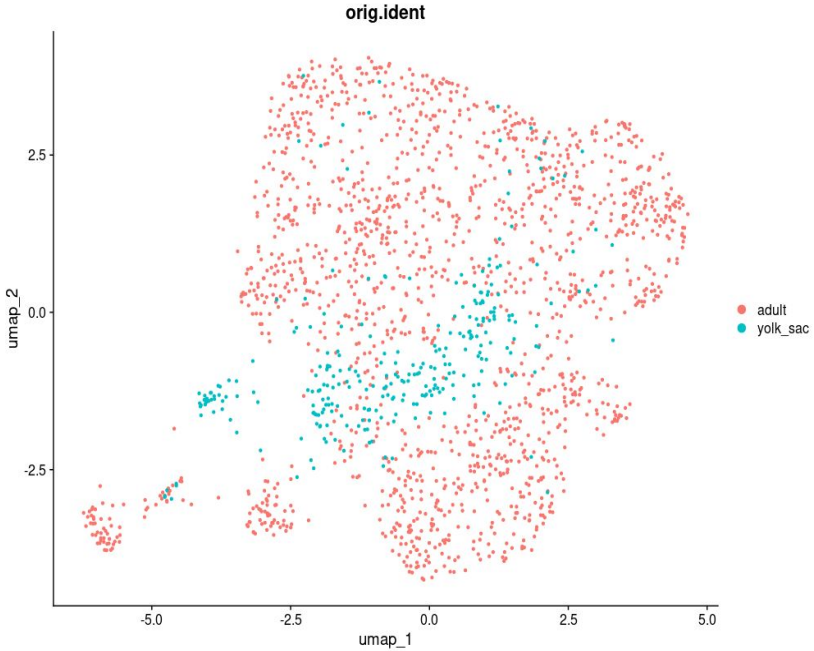
Iterated through several ways of integration…

We probably could have done our whole project on this step alone for these datasets

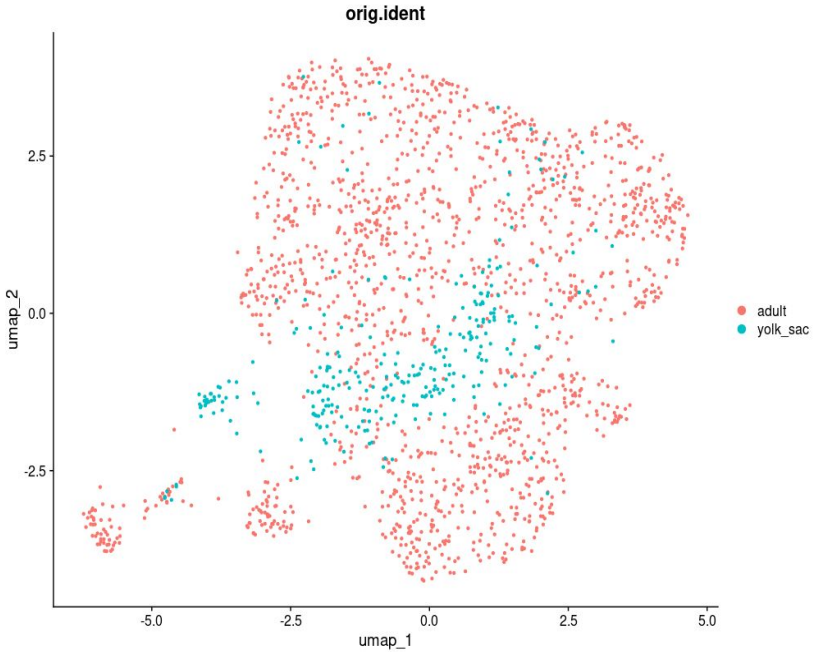
Clustering


Before integration
After Integration
0
1
2
3
4
5
6
7
8
Eye
Fat
Large_Intestine
Lymph_Node
Mammary
Muscle
Pancreas
Prostate
Skin
Small_Intestine
Thymus
Trachea
seurat_clusters
adult
yolk_sac
umapunintegrated_2
umapunintegrated_1
tissue_in_publication
harmony_cluster
harmonyumap_2
harmonyumap_1

Clustering



Clustering


orig.ident
umap_2
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-2.5
-5.0
-2.5
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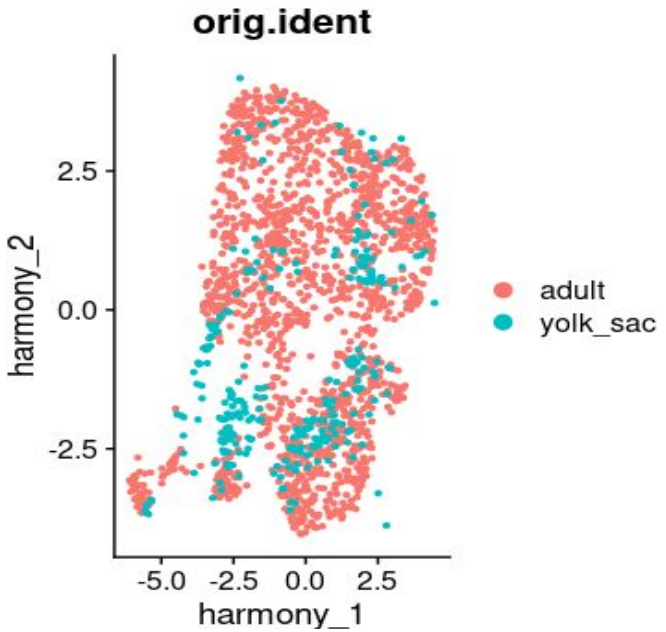
2.5
5.0
umap_1
adult
yolk_sac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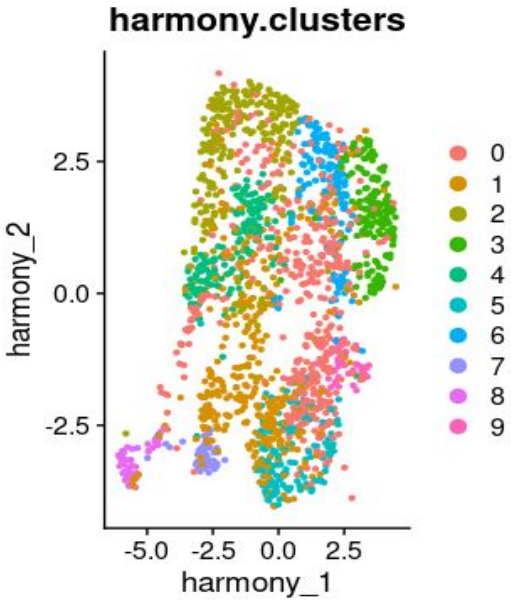
-2.5
-5.0
-2.5
0.0
2.5
harmony_1
adult
yolk_sac

Clustering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0.0
-2.5
-5.0
-2.5
0.0
2.5
harmony_1
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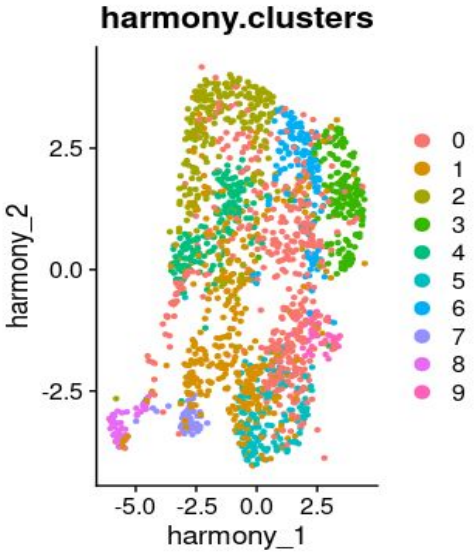
2
3
4
5
6
7
8
9

Clustering


harmony.clusters
harmony_2
2.5
0.0
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3
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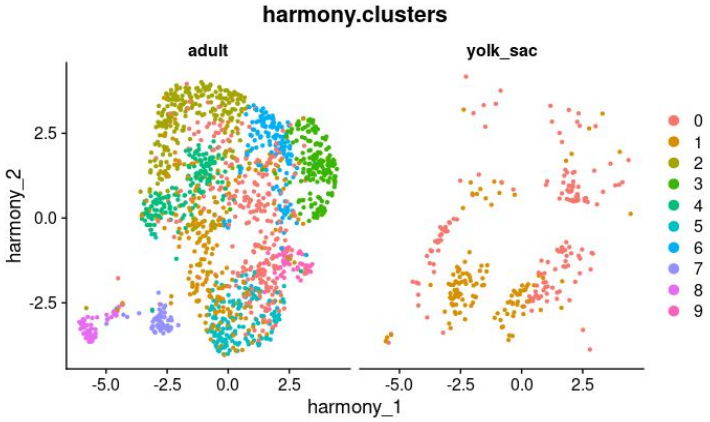
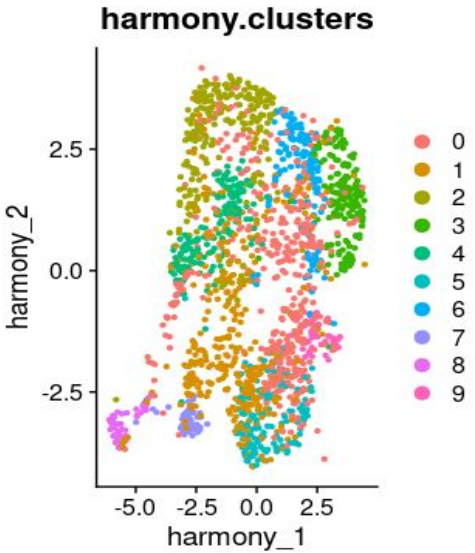

harmony.clusters
adult
yolk_sac
harmony_2
2.5
0.0
-2.5
-5.0
-2.5
0.0
2.5
harmony_1
0
1
2
3
4
5
6
7
8
9

Clustering


harmony.clusters
harmony_2
harmony_1
2.5
0.0
-2.5
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-2.5
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2
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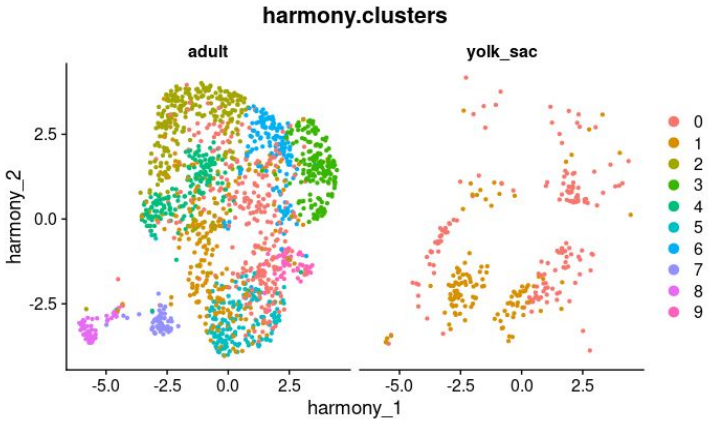
harmony.clusters
adult
yolk_sac
harmony_2
harmony_1
0
1
2
3
4
5
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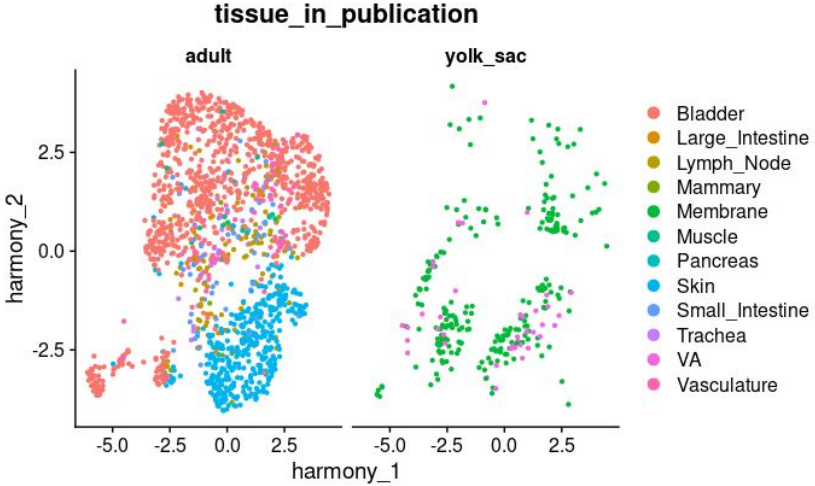
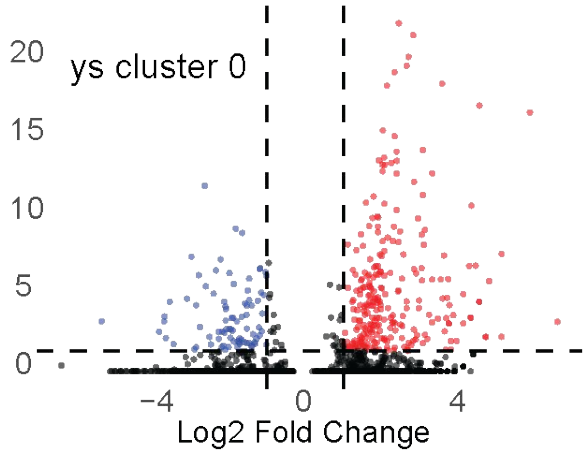

tissue_in_publication
adult
yolk_sac
harmony_2
harmony_1
Bladder
Large_Intestine
Lymph_Node
Mammary
Membrane
Muscle
Pancreas
Skin
Small_Intestine
Trachea
VA
Vasculature

Yolk Sac Cluster 0

Yolk sac cells from cluster 0 compared against all other cells

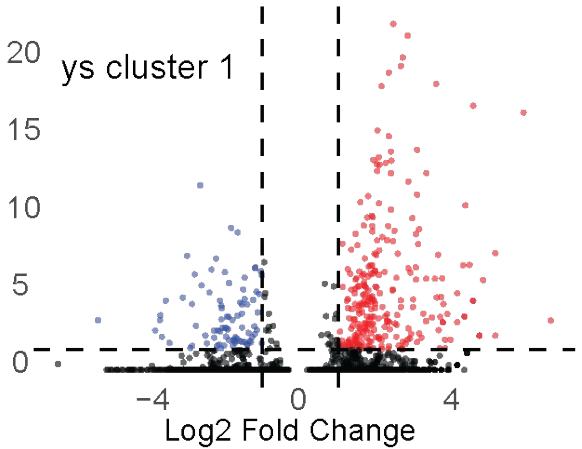
GO terms	TFs
Catalytic complex	**DIDO1**
Vesicle-mediated transport	ELF2
Microtubule cytoskeleton	**SUPT20H**
Endosome	**ZNF711**
Protein DNA complex organization	SKIL
Mitochondrion	ATF6
Vesicle membrane	CEBPZ
Post-translational protein modification	DACH1
Establishment of protein localization	
Nuclear protein containing complex	



Yolk Sac Cluster 1

Yolk sac cells from cluster 1 compared against all other cells

GO terms	TFs
Mitochondrion	**SUPT20H**
DNA metabolic processes	**DIDO1**
Catalytic complex	SKIL
Nuclear protein containing complex	ZNF711
Protein DNA complex organization	**ADA2**
Envelope	BRCA2
Organelle inner membrane	SETD1A
Chromosome	ZNF407
Chromatin remodeling	SALL4
Mitochondrial envelope	ZFHX3

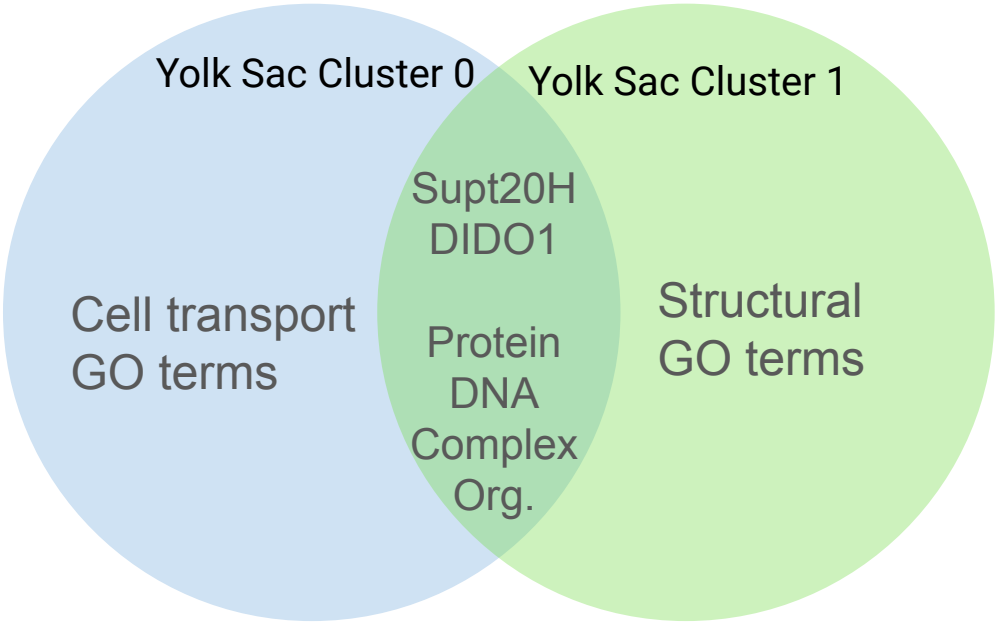


Transcriptional regulators

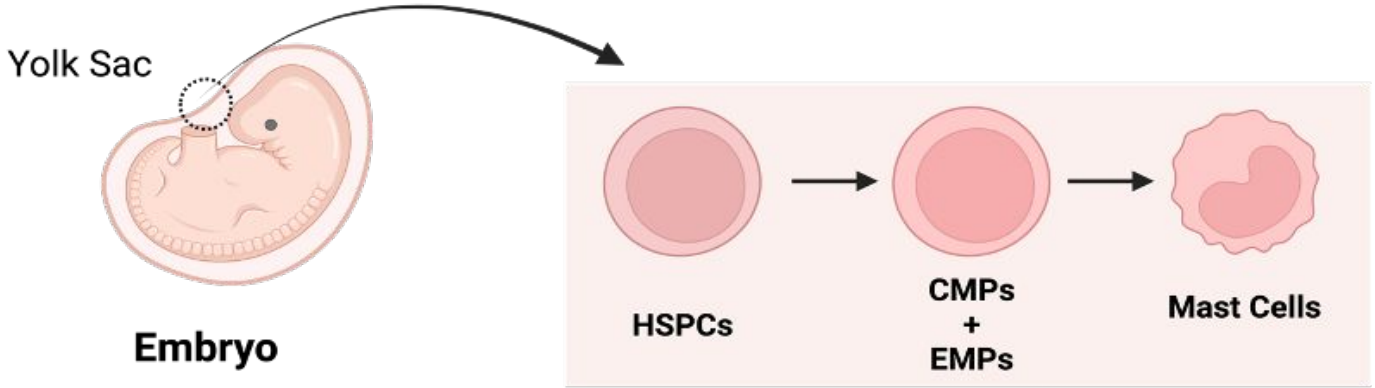
Supt20H: regulation of transcription by RNA polymerase 2

DIDO1 (Death-inducer obliterator): Promotes embryonic stem cell self-renewal

ADA2: growth factor

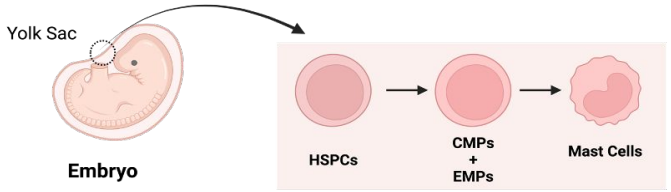


Embryonic Yolk Sac Data Annotation For Pseudotime Trajectory



HSPCs: Hematopoietic stem cells
CMPs: Common myeloid progenitor cells
EMPs: EO/Baso/Mast Precursors

Distribution of Cell Types in Yolk Sac Data Based on Carnegie Stage



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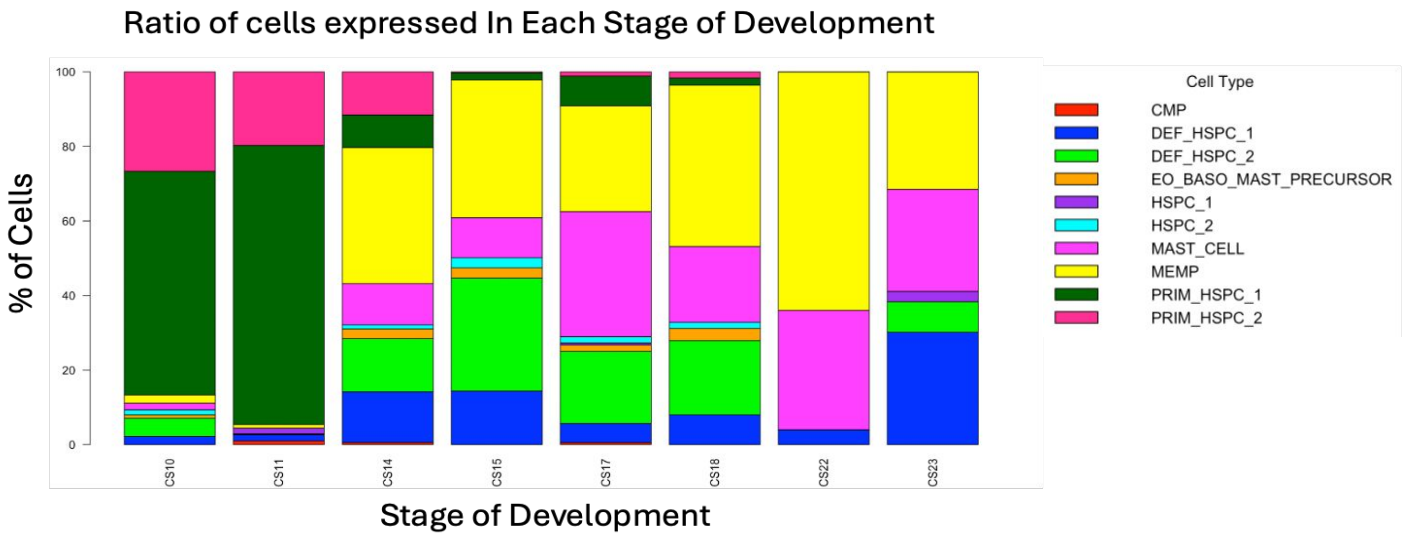
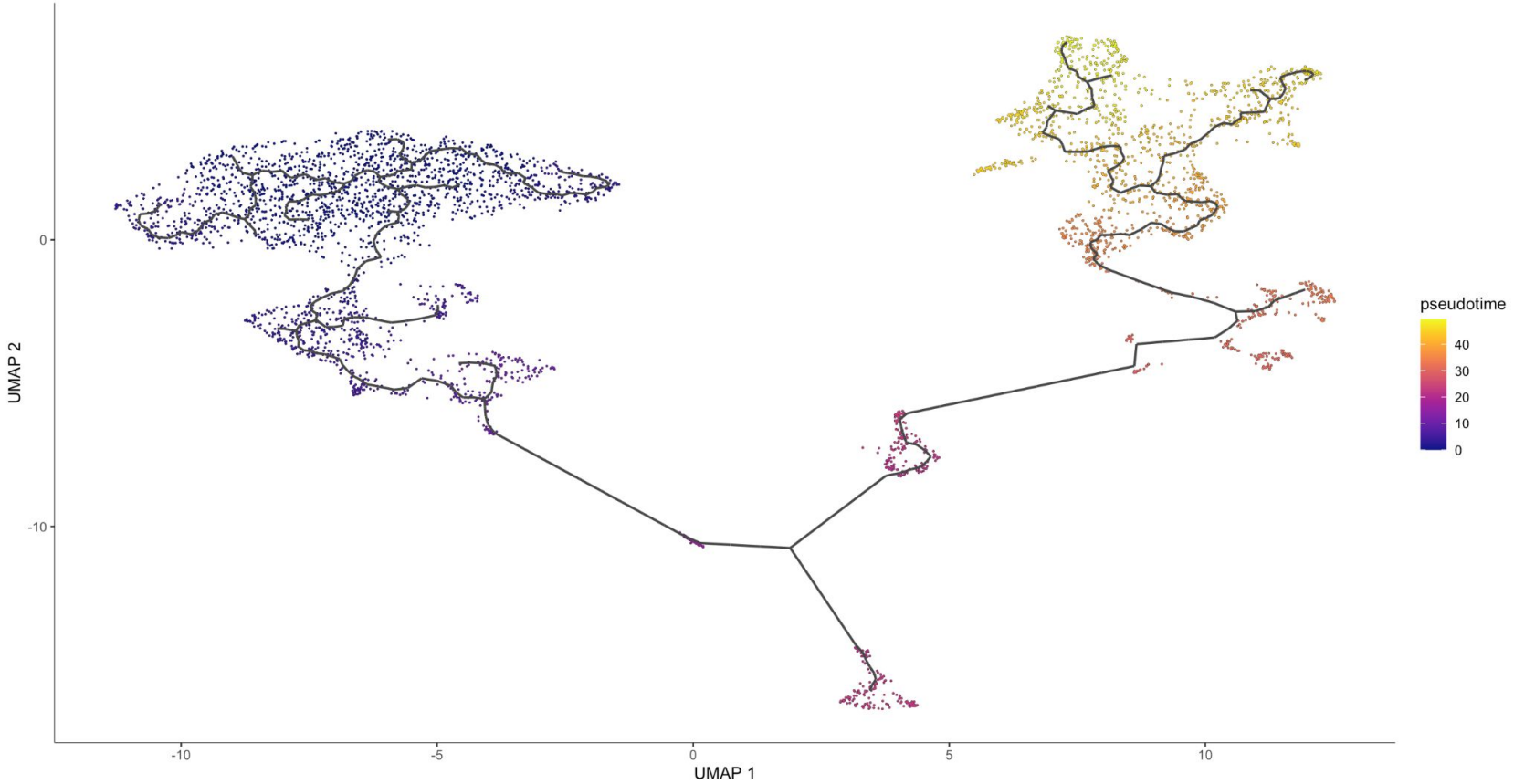
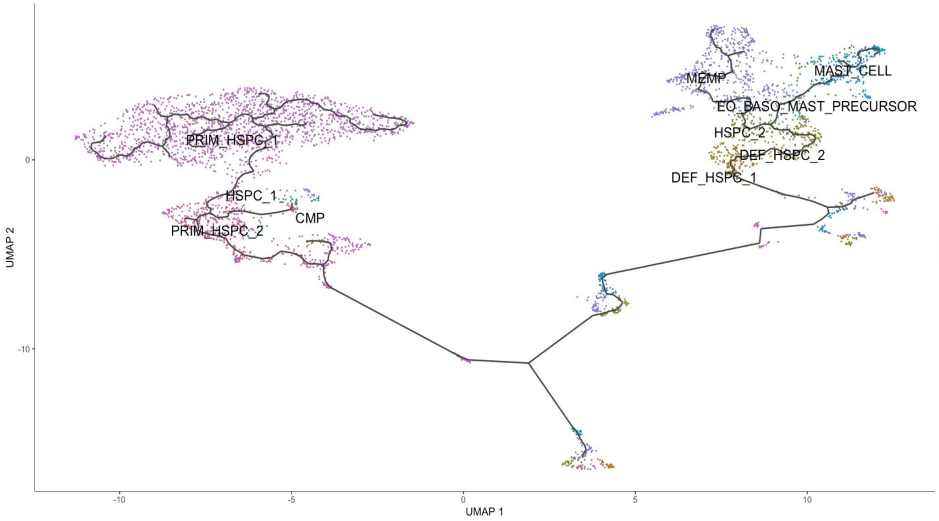
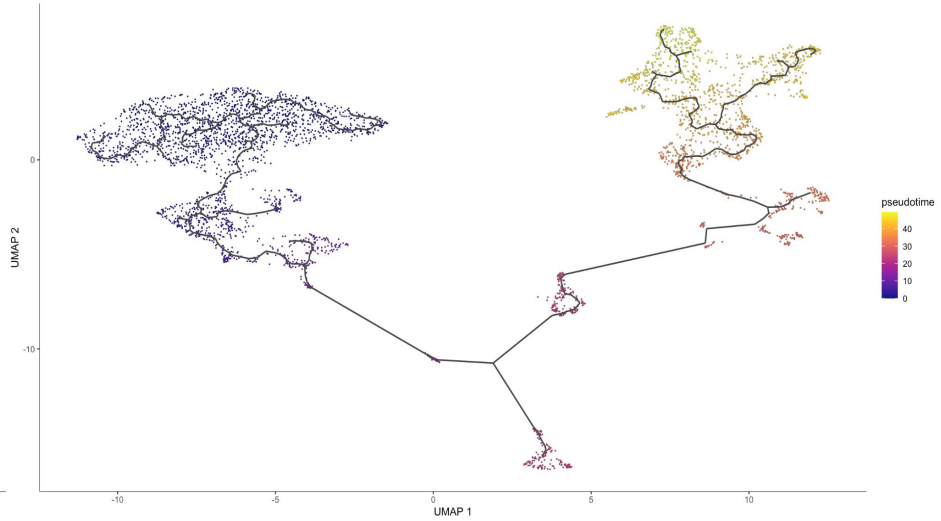


Does Pseudotime Trajectory Analysis Model Hematopoiesis?

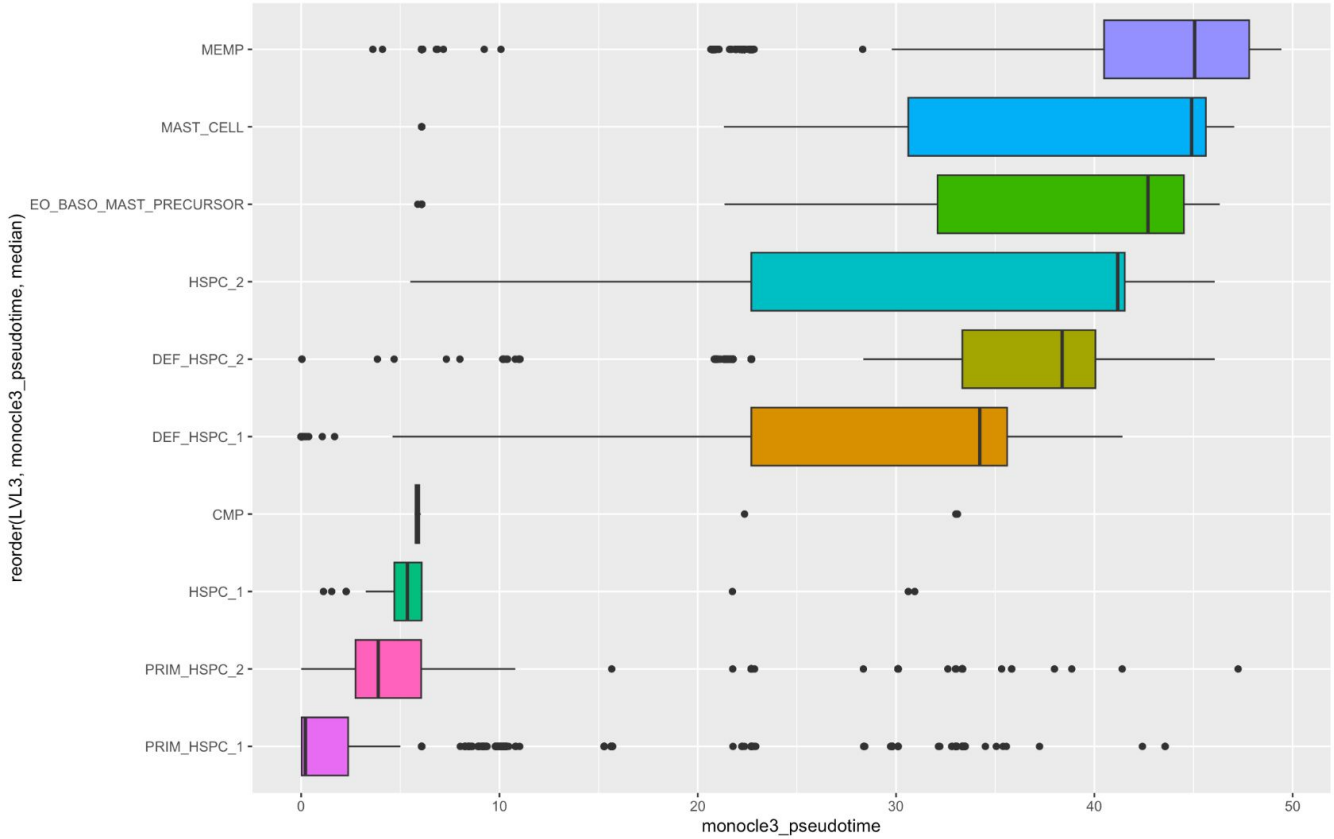


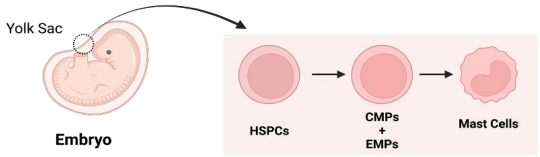
Does Pseudotime Trajectory Is Able to Capture Expected Mast Differentiation Pathway





Cells Within Yolk Sac Data Follow Expected Trends in Pseudotime Analysis





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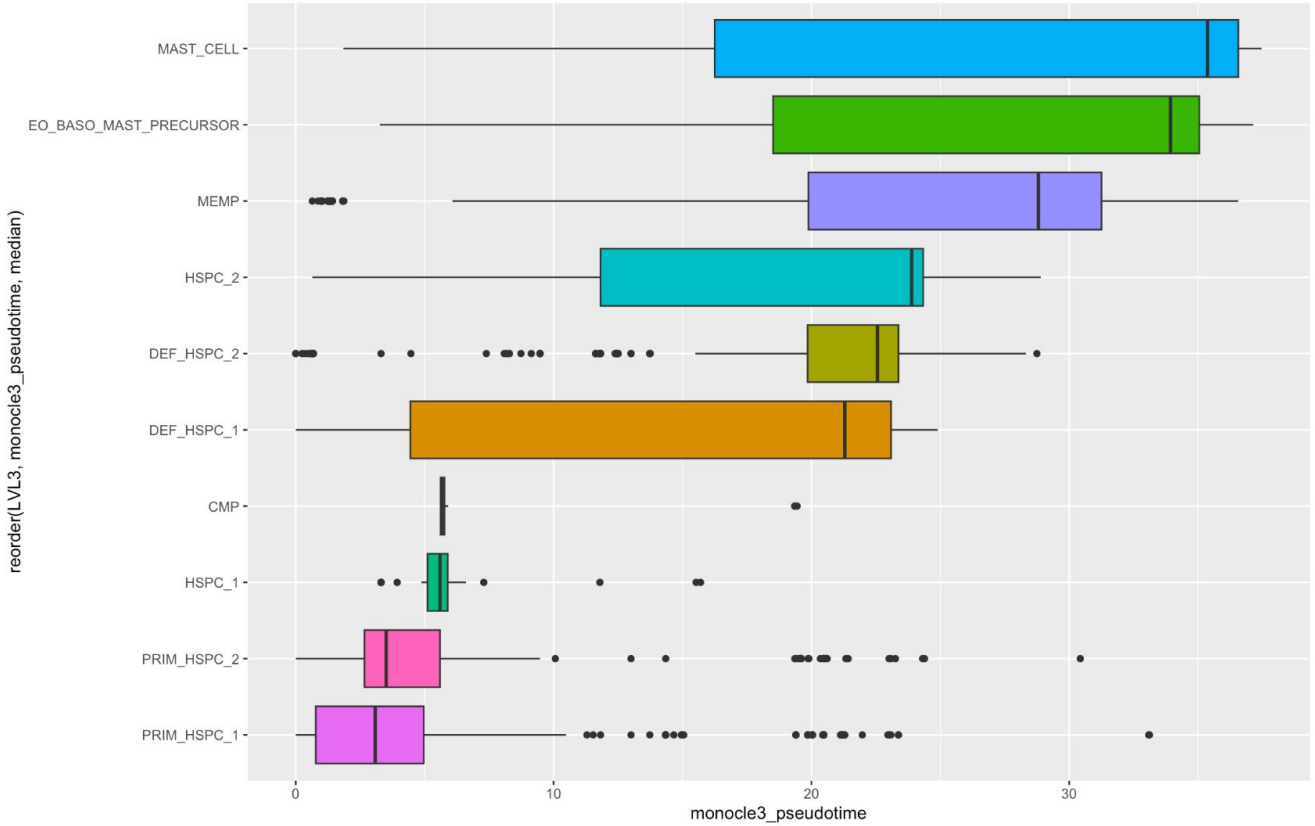
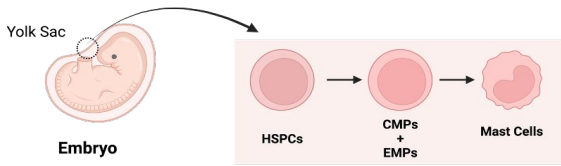
LVL3
- CMP
- DEF_HSPC_1
- DEF_HSPC_2
- EO_BASO_MAST_PRECURSOR
- HSPC_1
- HSPC_2
- MAST_CELL
- MEMP
- PRIM_HSPC_1
- PRIM_HSPC_2

Cells Within Yolk Sac Data Follow Expected Trends in Pesudotime Analysis





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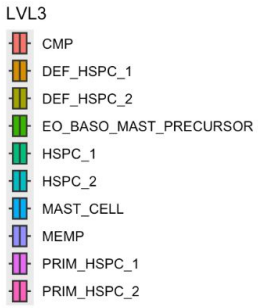
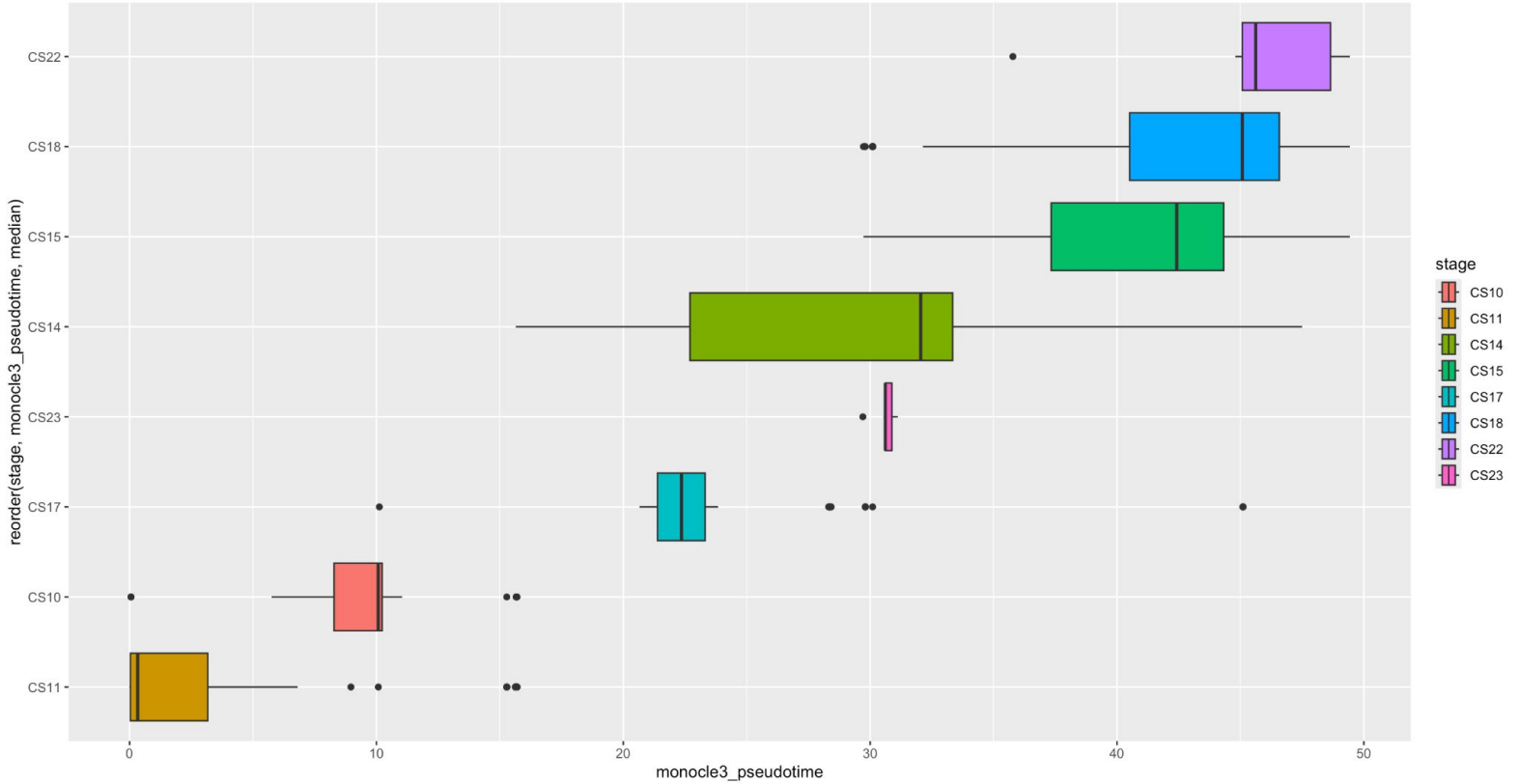
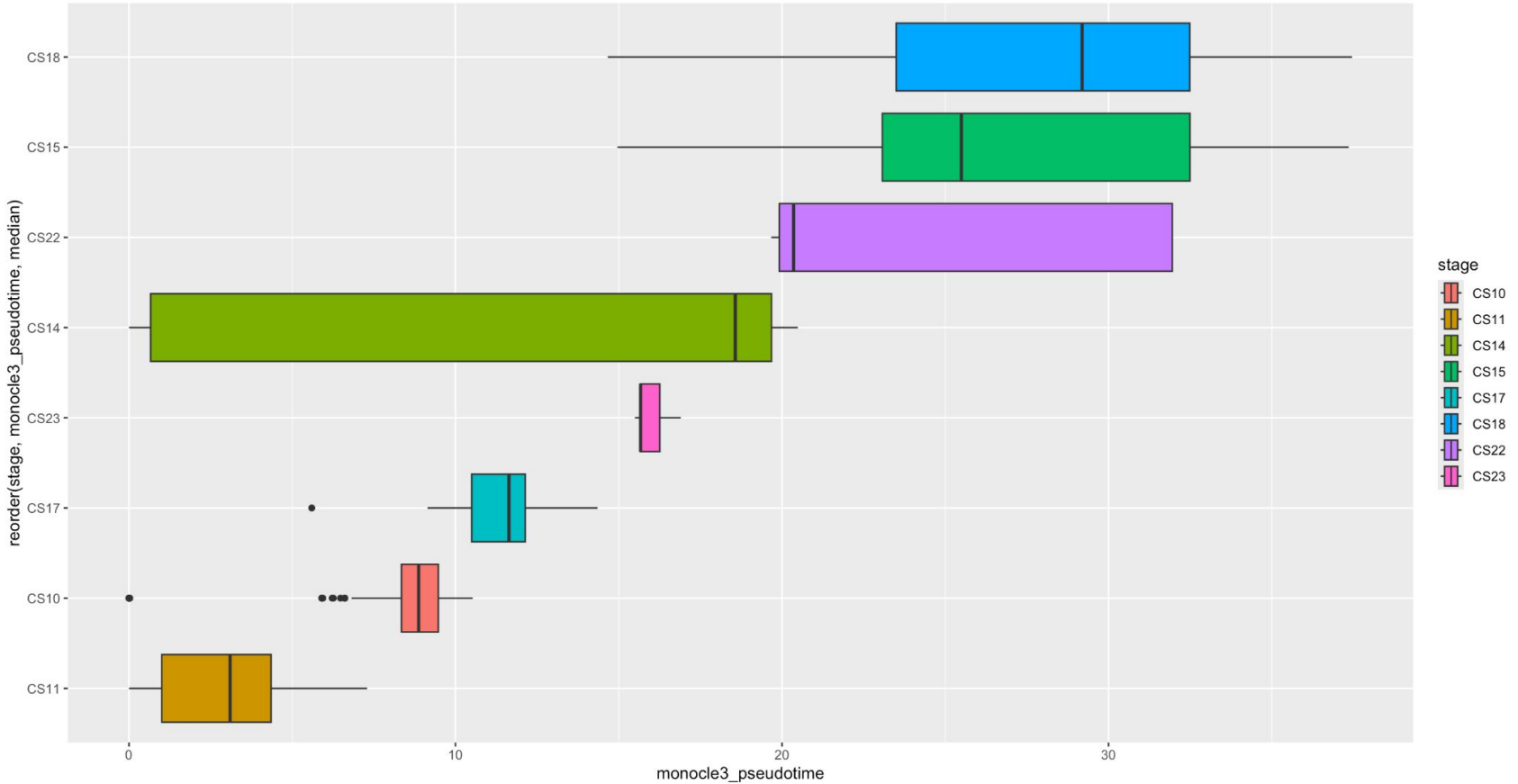


Samples Separate in Pseudotime Based on Carnegie Stage of Development But Do Not Completely Follow A Trend Based On Age



Samples separate in Pesudotime Based on Carnegie Stage of Development: But Do Not Completely Follow A Trend Based On Age



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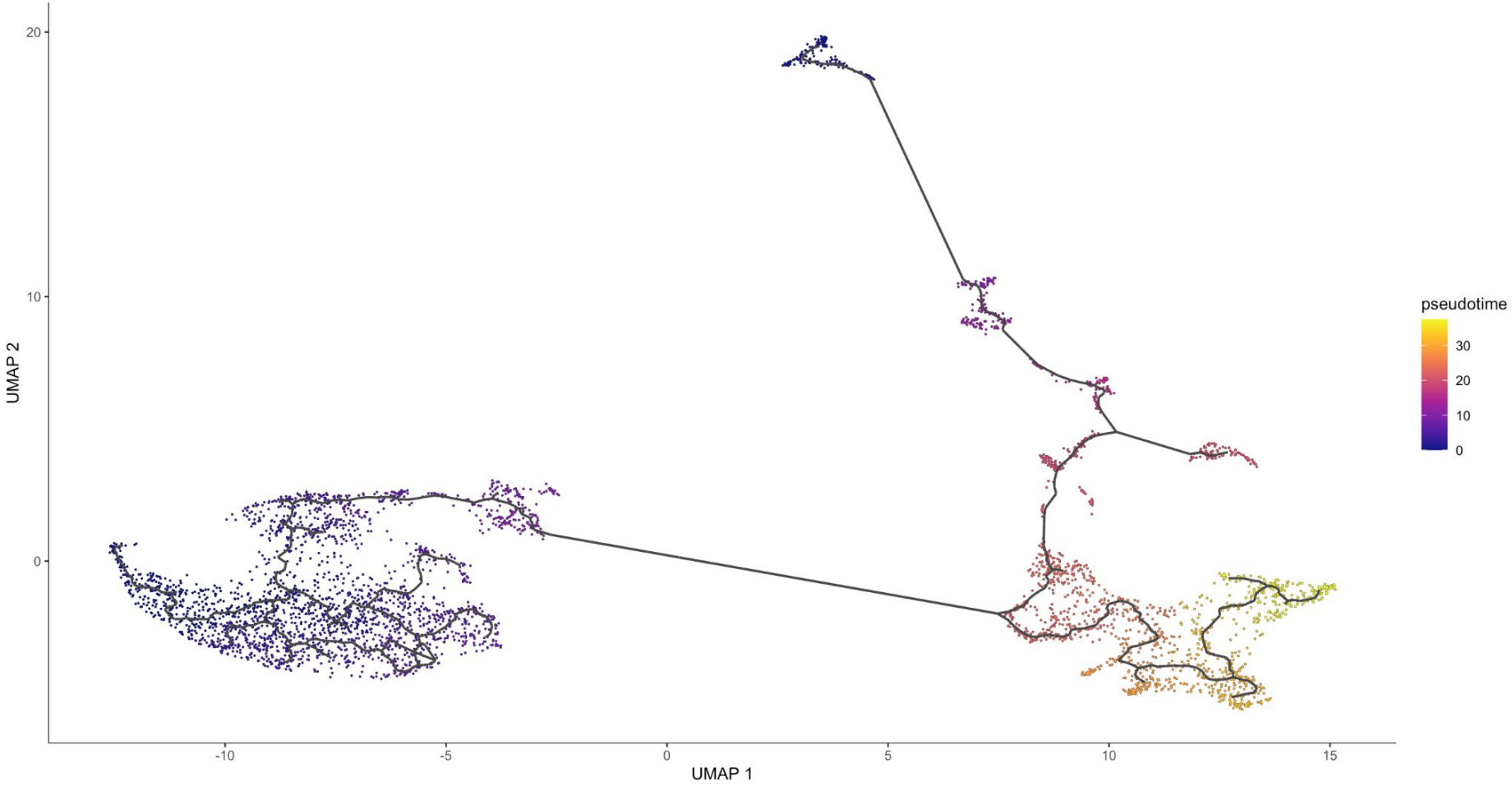
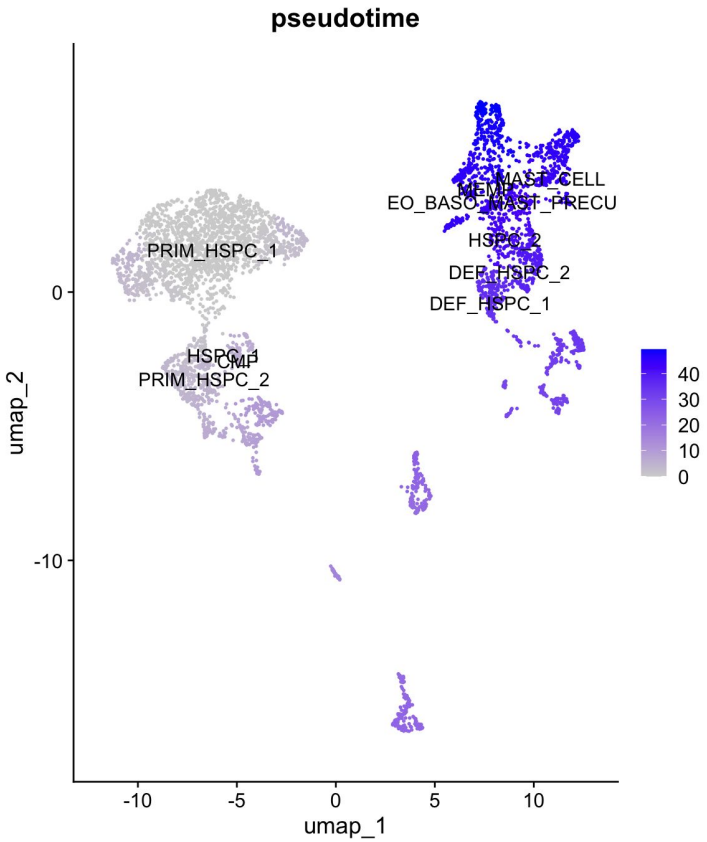
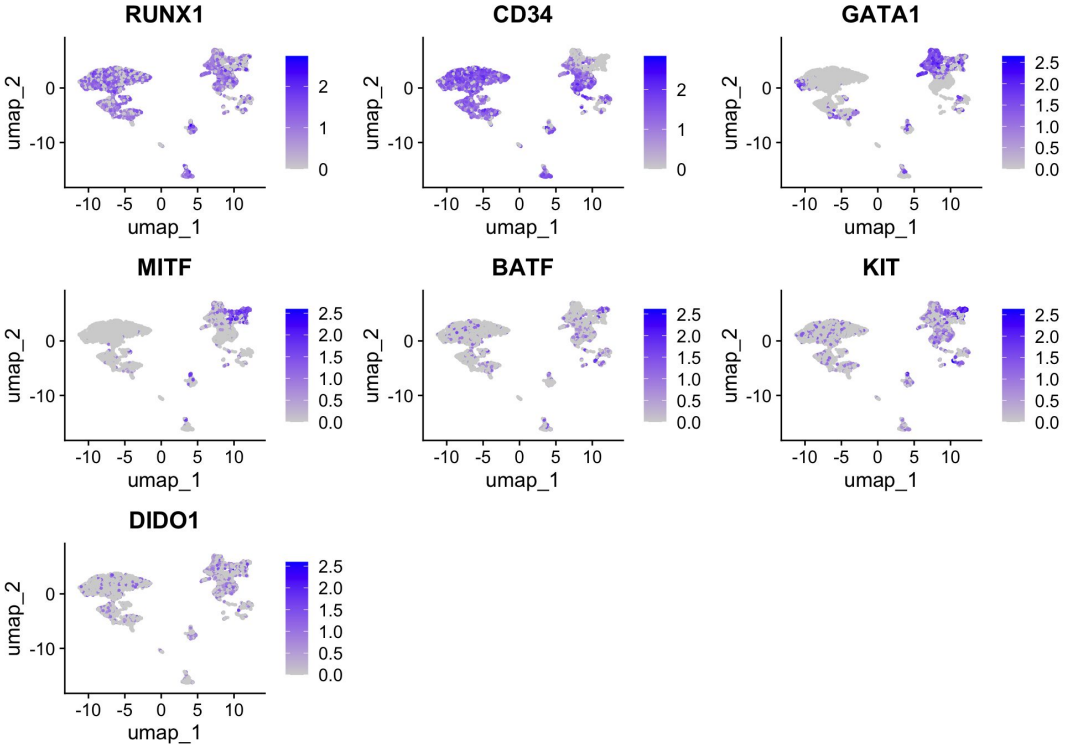
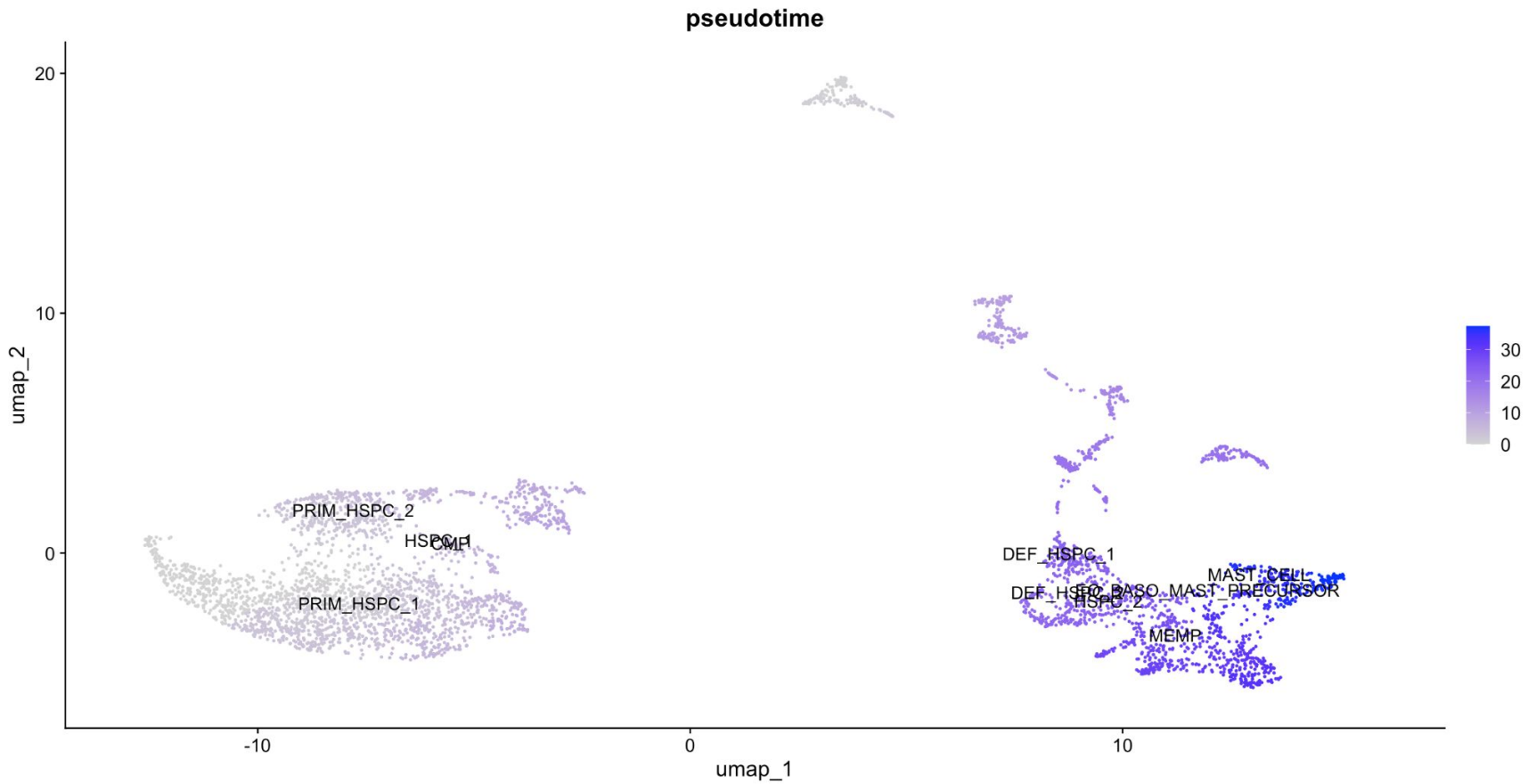


Pseudotime Trajectory of Yolk Sac Data Follows Expected Trajectory



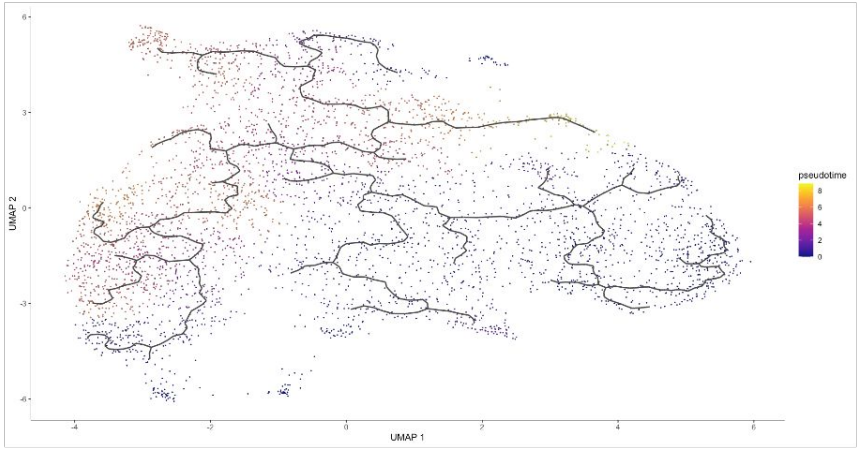


Pseudotime Trajectory of Yolk Sac Data Follows Expected Trajectory

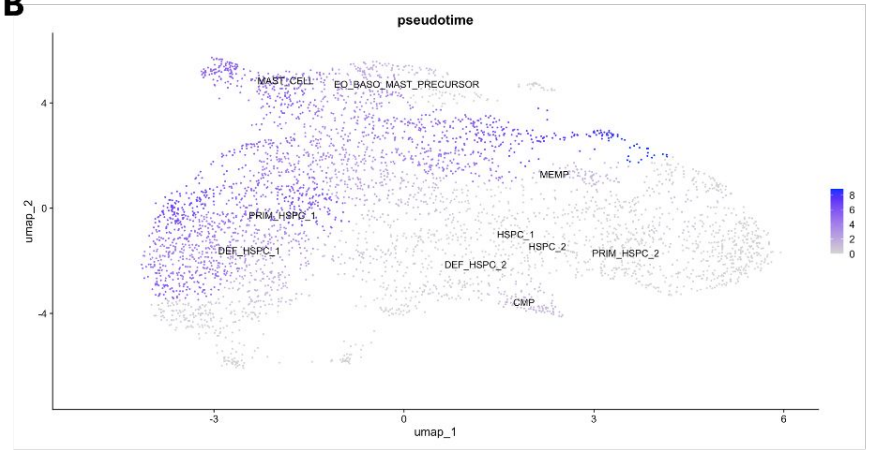


Interesting Finding: Seurat Anchors Clustering give a much different output.

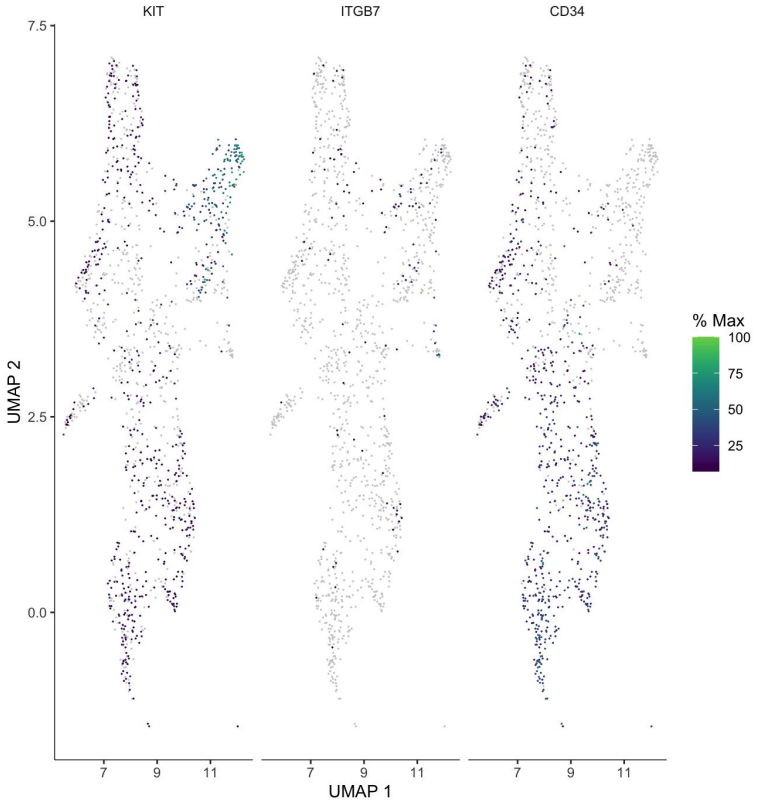
A

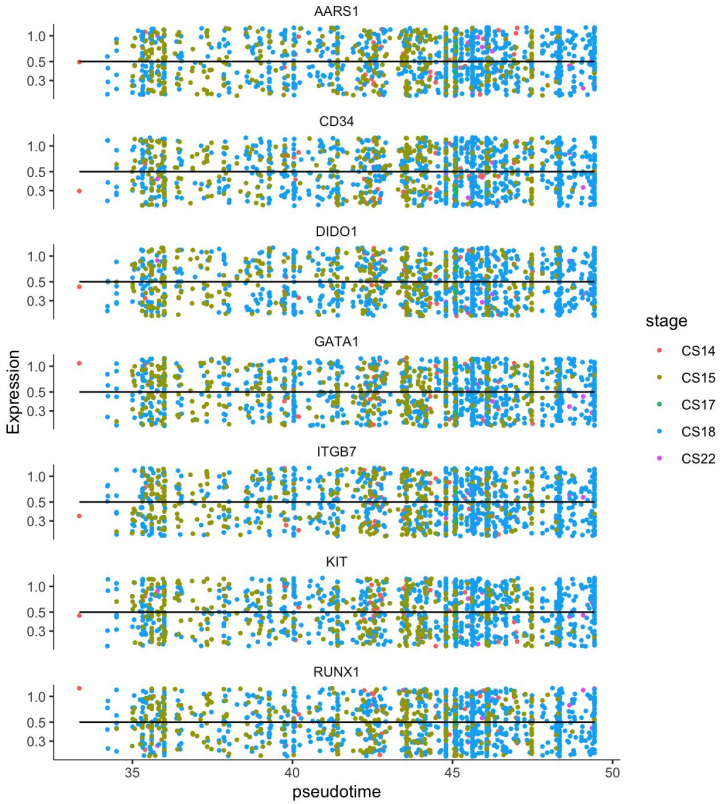


B



Subset of Mast Cells and Progenitors Show No Clear Difference Based on Stage of Development





Conclusions

- Chromatin associated proteins enriched in clusters 1 & 0 (DIDO1, SUPT, BRCA2, etc.)
  - Might suggest cells annotated as mast cells were progenitors / less differentiated in the embryonic dataset.
- We were able to do pseudotime analysis to map hematopoiesis of mast cells.
  - The more primitive cells did not seem to be closely related to MCs?
  - If primitive hspcs are connected to MC lineage fate there seems to be an intermediate state that is missing in the pseudotime plot.
- Small subsets of two separate data sets
  - Could have done a whole project on QC!

Thank You! Questions?