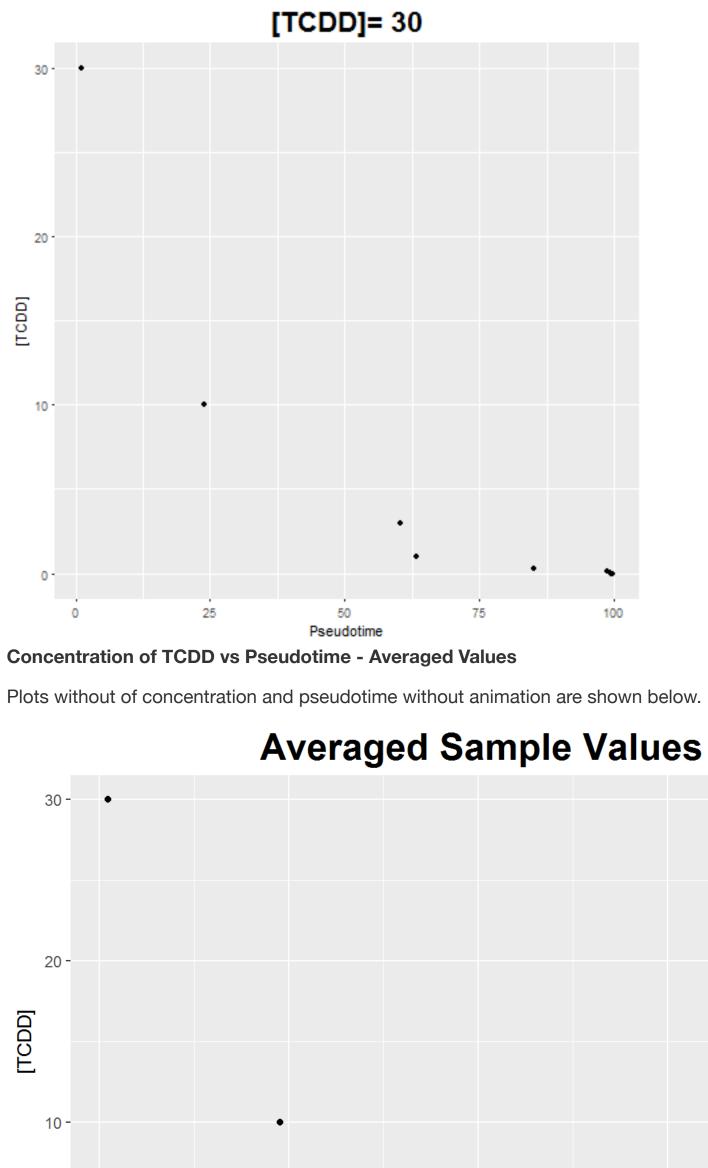
## Cellular Differentiation of Hepatic Cells Nick Wawee June 8, 2018 Below are representative plots of cell clusters using Rance's,[1], data and the single- cell data from the Yang, [2], article. Pseudotime is a scale that is used to indicate the direction of the differentiation process. In this case, as pseudotime increases the cells (or samples) become more differentiated. As dose increases, the samples approach a de-differentiated state. The best visual representations of this process are shown by the tSNE clustering produced the Monocle,[3], package and the PCA clustering from the Seurat,[4], package. All concentrations of TCDD are in $\mu$ g/kg. **Monocle tSNE** 10 -Sample Type tSNE2 Dosed Hepatoblast Hepatocyte -10 --20 --20 -10 10 tSNE1 **Monocle tSNE** 10 -Pseudotime 100 75 50 25 -10 --20 --20 -10 10 tSNE1 **Monocle tSNE** Dose or Cell Type 10 -• [0.01] • [0.03] • [0.1] • [0.3] • [0] • [1] • [10] • [3] • [30] -10 -Hepatoblast Hepatocyte -20 --20 -10 10 tSNE1 **Seurat tSNE** 10 -5 -Sample Type tSNE2 Dosed • Hepatoblast Hepatocyte -5 --10 --20 -10 10 20 tSNE1 **Seurat tSNE** 10 -5 -Pseudotime 100 tSNE2 75 25 -10 -10 -10 -20 20 tSNE1 **Seurat tSNE** 10 -Dose or Cell Type • [0.01] 5 -• [0.03] • [0.1] • [0.3] tSNE2 • [0] • [1] • [10] • [3] • [30] Hepatoblast Hepatocyte -10 --20 -10 20 10 tSNE1 **Seurat PCA** 20 -10 -Sample Type Dosed • Hepatoblast Hepatocyte -10 --20 --75 -50 -25 PC1 **Seurat PCA** 20 -10 -Pseudotime 75 50 25 -10 --20 --75 -50 PC1 **Seurat PCA** 20 -Cell Type or Dose • [0.01] 10 -• [0.03] • [0.1] • [0.3] • [0] • [1] • [10] • [3] -10 -• [30] Hepatoblast Hepatocyte -20 --75 -50 -25 PC1 Below are animations of the dosed samples as they progress through the sample trajectory. The animations show as [TCDD] increases, pseudotime decreases which indicates that de-differentiation is occuring. [TCDD]= 0.1 Pseudotime PC2 -90 -87 PC1 **PCA Clustering** [TCDD]= 0.032 -Pseudotime tSNE2 -21.0 -20.5 -22.0 -21.5 -20.0 tSNE1 tSNE Clustering [TCDD]= 0.01

20 -[TCDD] 10 -75 25 50 Pseudotime **Concentration of TCDD vs Pseudotime** The animations are further configured so the averaged values for each dose is depicted. [TCDD]= 30 Pseudotime -22.0 -21.5 -21.0 -20.5 -20.0 tSNE1 **PCA Clustering - Averaged Values** [TCDD]= 30 Pseudotime



-87.5

-85.0

-90.0

tSNE Clustering- Averaged Values

0 -25 75 50 100 Pseudotime **Sample Values** 30 -20 -[TCDD] 10 -0 -25 50 75 100 Pseudotime

[1] Nault R, Fader KA, Harkema JR, Zacharewski T (2017) Loss of liver-specific and sexually dimorphic gene expression by aryl hydrocarbon receptor activation in C57BL/6 mice. PLoS ONE 12(9): e0184842. https://doi.org/10.1371/journal.pone.0184842

[2] Yang, Li & Wang, Wei-Hua & Qiu, Wei-Lin & Guo, Zhen & Bi, Erfei & Xu, Cheng-Ran. (2017). A single-cell transcriptomic analysis reveals precise pathways and regulatory mechanisms underlying hepatoblast differentiation. Hepatology. 66. 10.1002/hep.29353.

[3] C. Trapnell and X. Qiu, "Monocle", Cole-trapnell-lab.github.io, 2018. [Online]. Available: http://cole-trapnell-lab.github.io/monocle-release/docs/#acknowledgements. [Accessed: 11- Jun- 2018].

[4] A. Butler, P. Hoffman, P. Smibert, E. Papalexi, and R. Satija, "Integrating single-cell transcriptomic data across different conditions, technologies, and species," Nature News, 02-Apr-2018. [Online]. Available: https://www.nature.com/articles/nbt.4096. [Accessed: 11-Jun-2018].