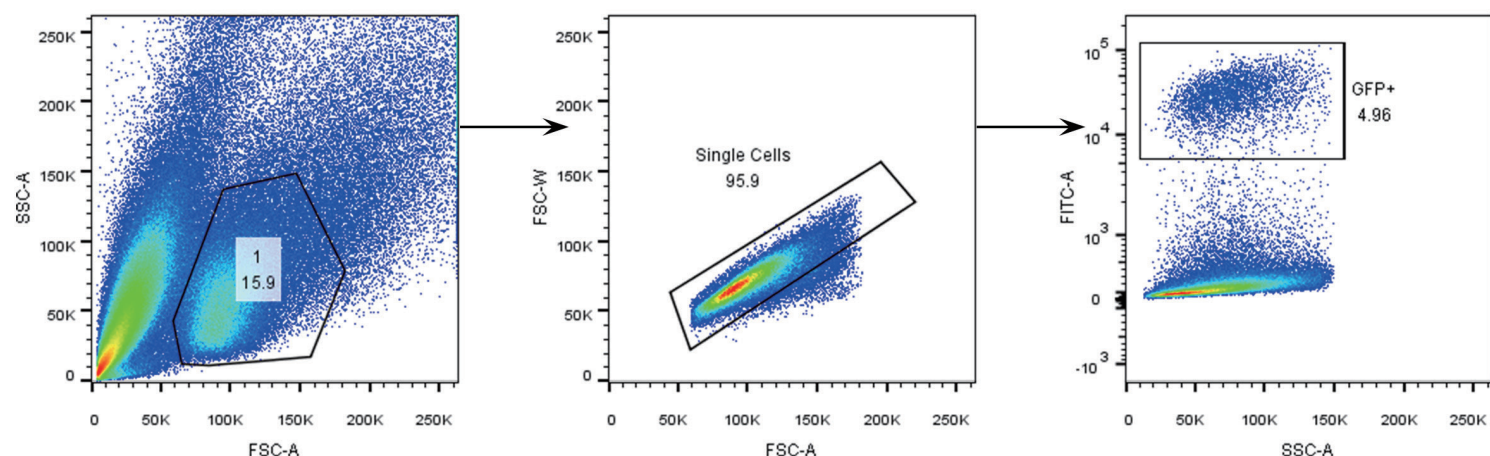


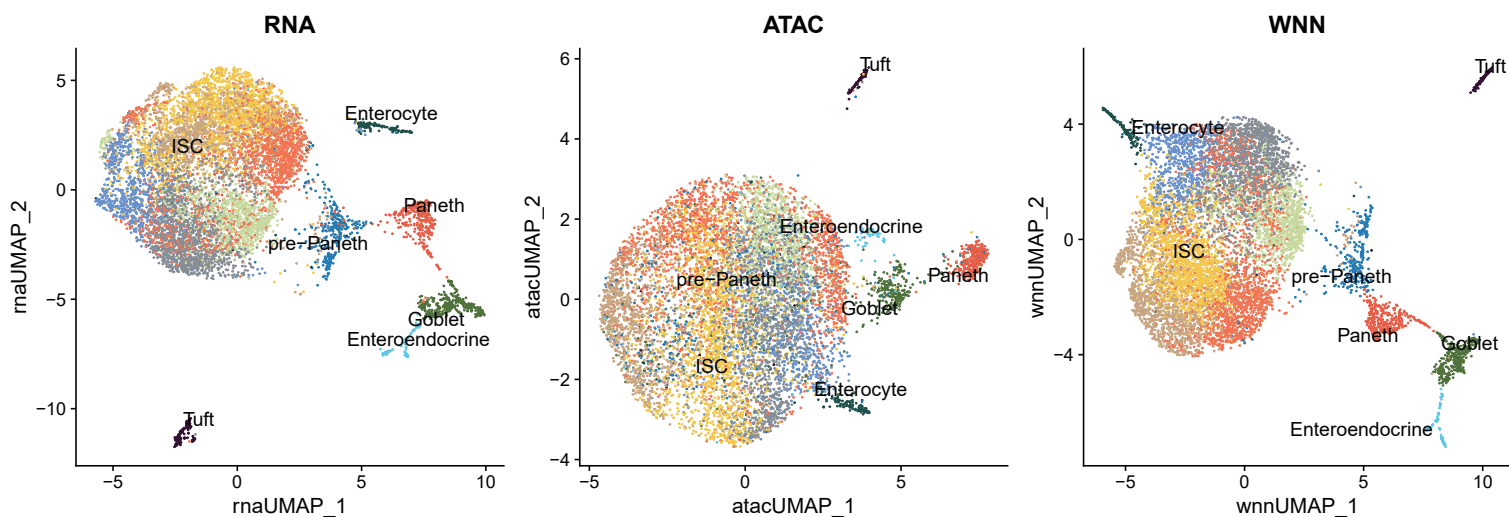
Supplementary Information for

**Single-nucleus multi-omics analysis of mouse small-intestinal
Lgr5⁺ cell populations reveals Foxa3-induced Paneth cell-lineage
differentiation**

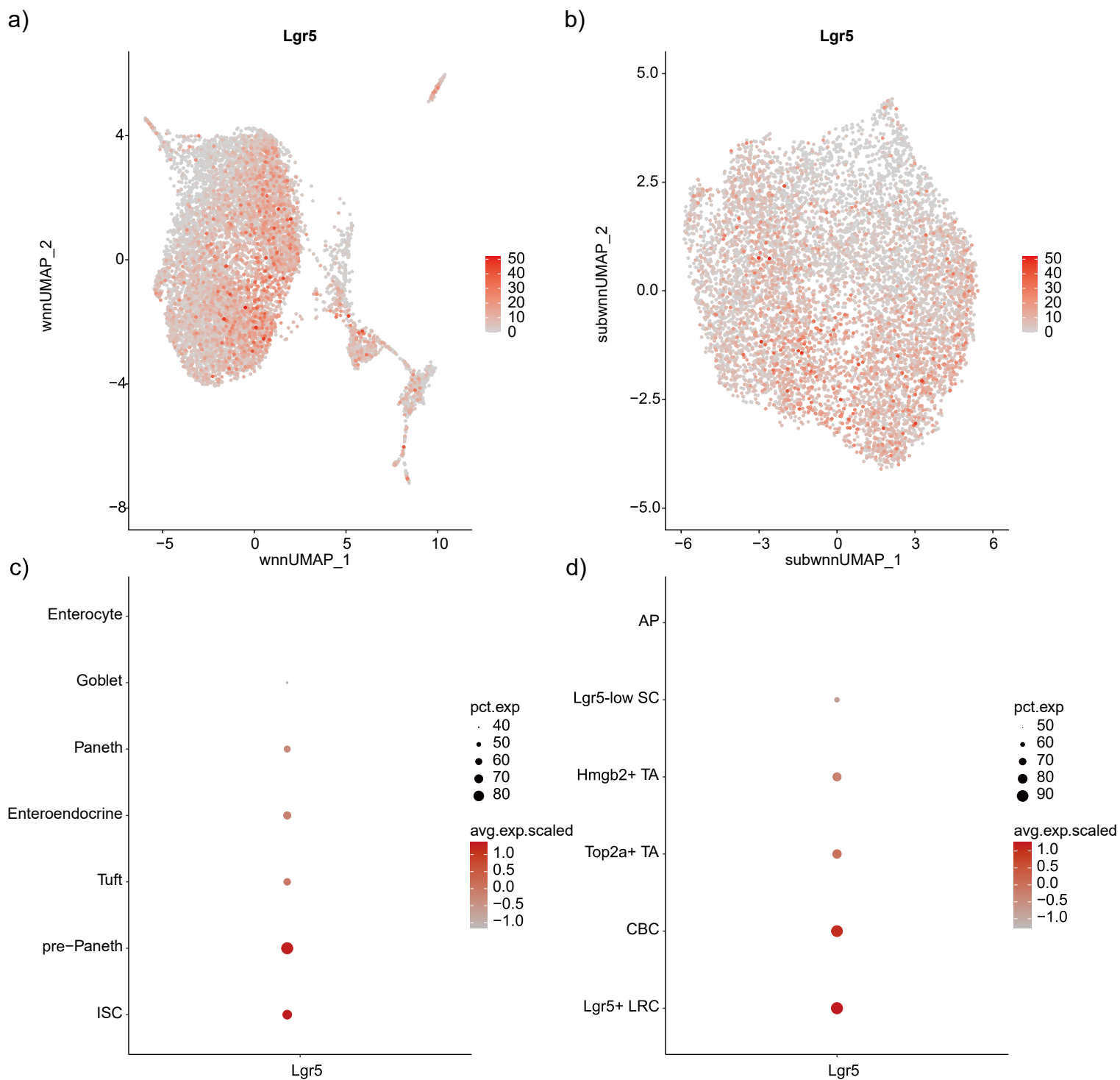
a)



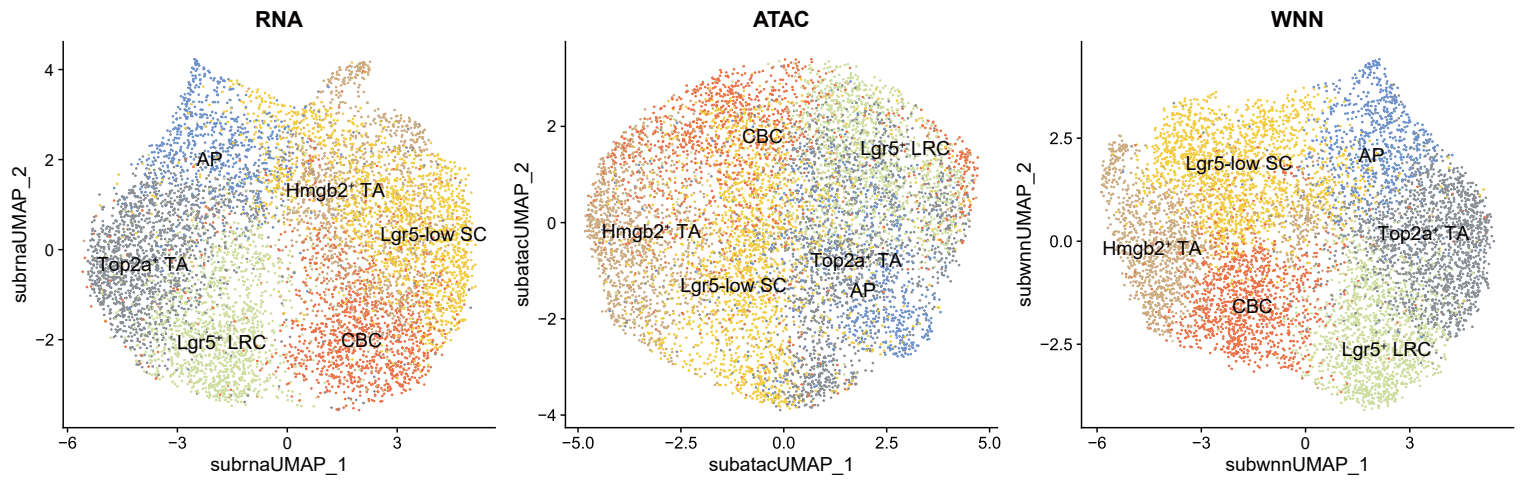
b)



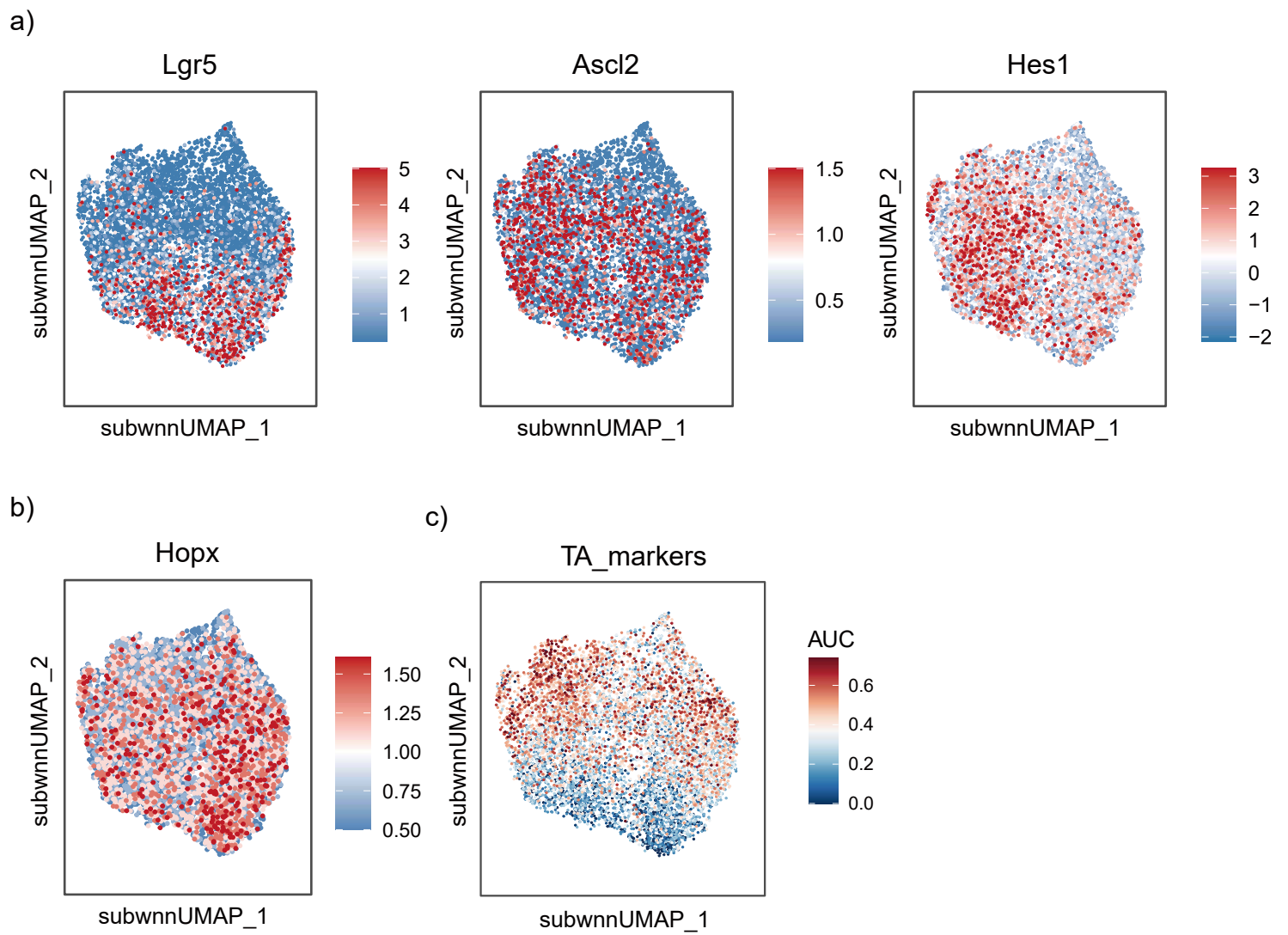
Supplementary Figure 1. Supplementary Figures for multi-omics profiling of mouse small intestinal epithelial cells. a) Flow cytometry plots of crypt cells showed the strategy of Lgr5+ small intestinal cell sorting. **b)** UMAPs showing identified cell types in mouse small intestine epithelial cells using both and either gene (mRNA) or chromatin accessibility (ATAC) information.



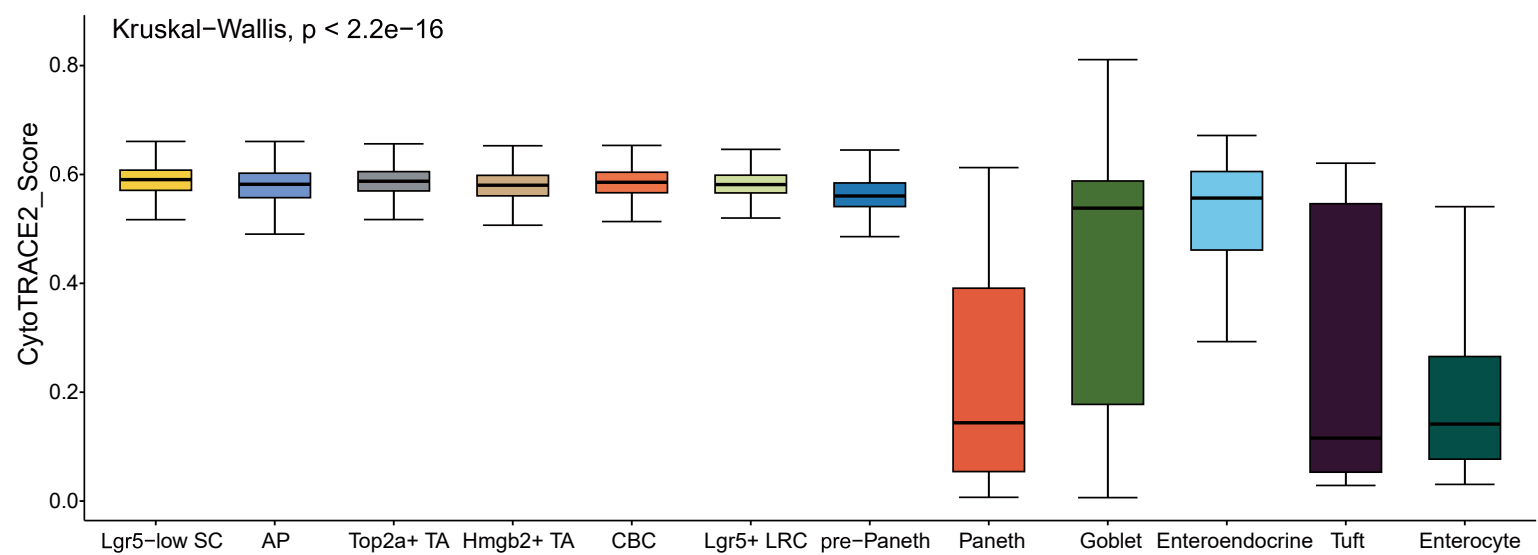
Supplementary Figure 2. FeaturePlot and DotPlot of Lgr5 expression in small intestine epithelial cells (a,c) and ISC subtypes (b,d).



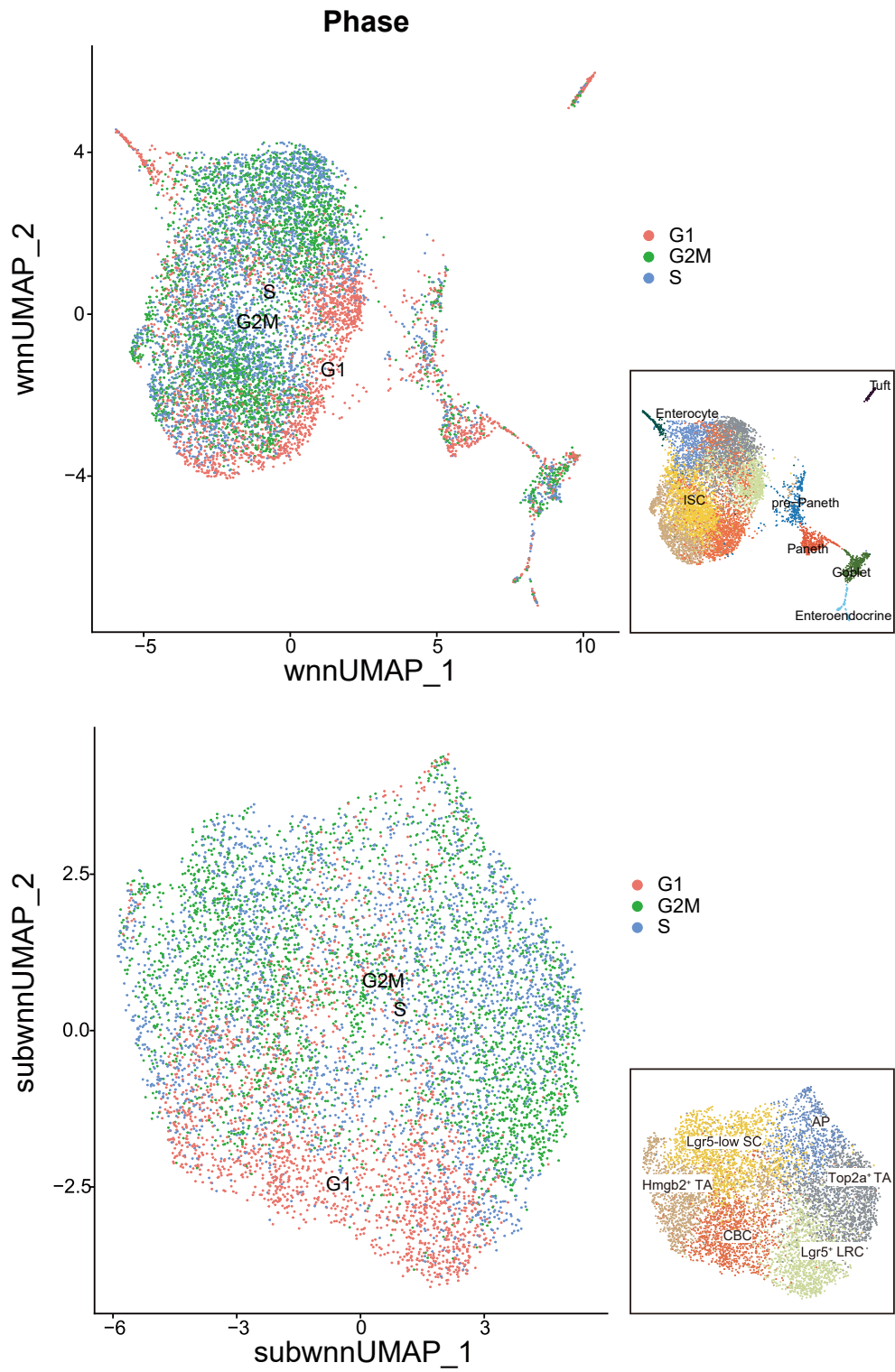
Supplementary Figure 3. UMAPs showing identified cell types in Lgr5⁺ ISCs using both and either gene (mRNA) or chromatin accessibility (ATAC) information.



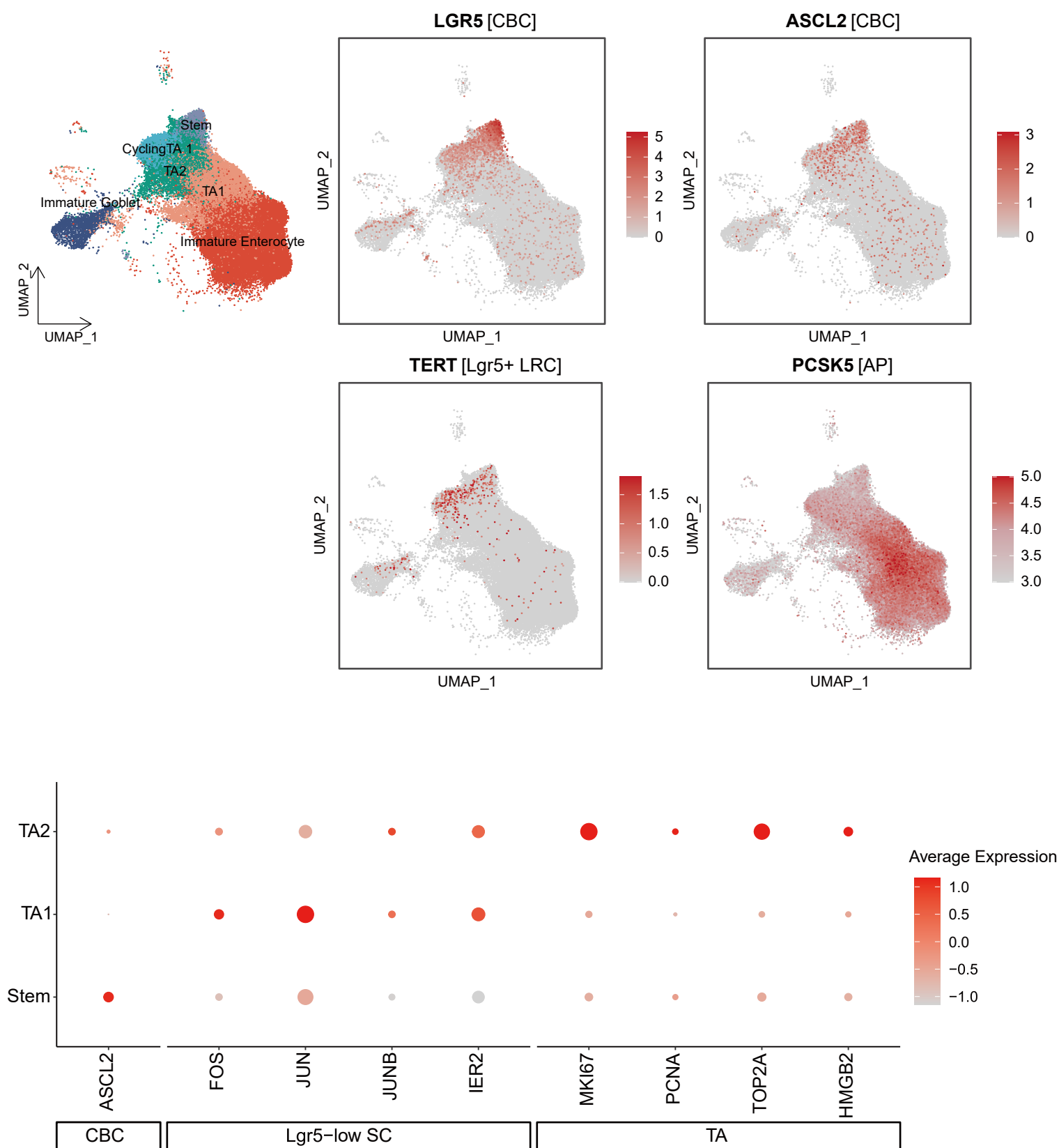
Supplementary Figure 4. FeaturePlot of marker genes expression in CBCs (a) ,LRCs (b) and the AUC score of marker genes (Mki67,Pcna,Ccna2,Mcm5) in TA cells (c).



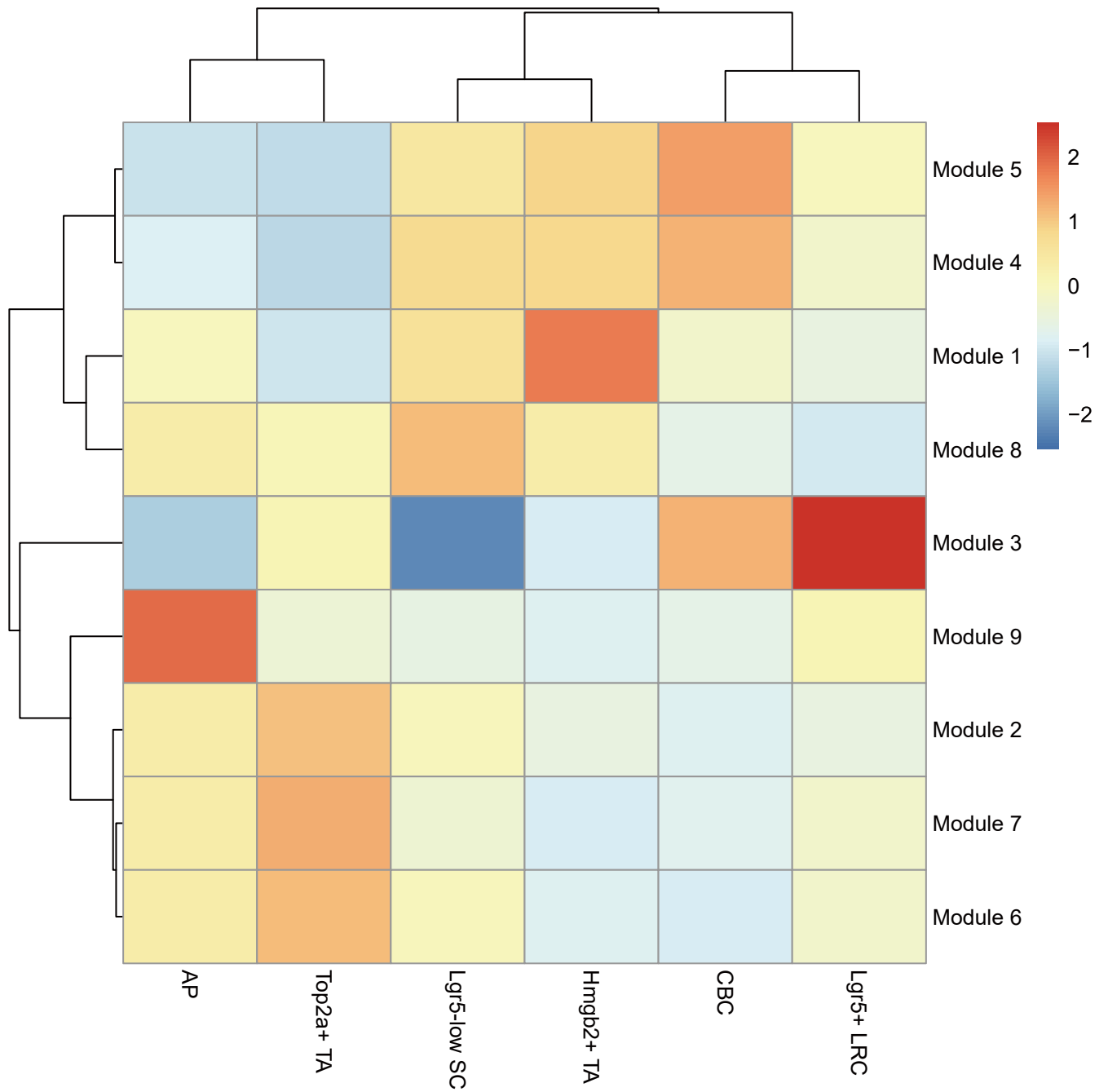
Supplementary Figure 5. CytoTrace2 prediction results for the differentiation potential of small intestinal epithelial cells.



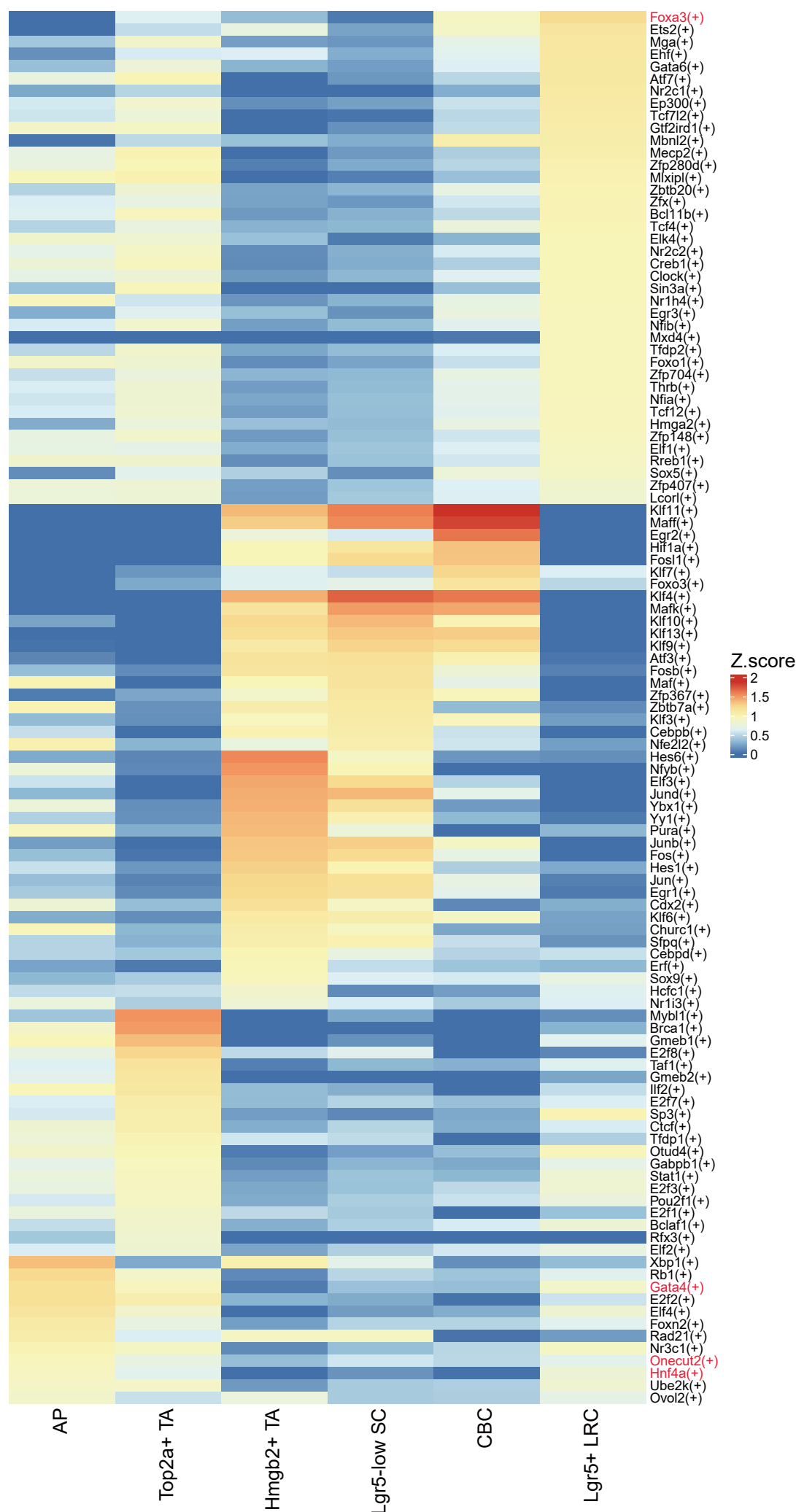
Supplementary Figure 6. Cell cycle scoring for each small intestinal epithelial cells (top) and stem cells (bottom).



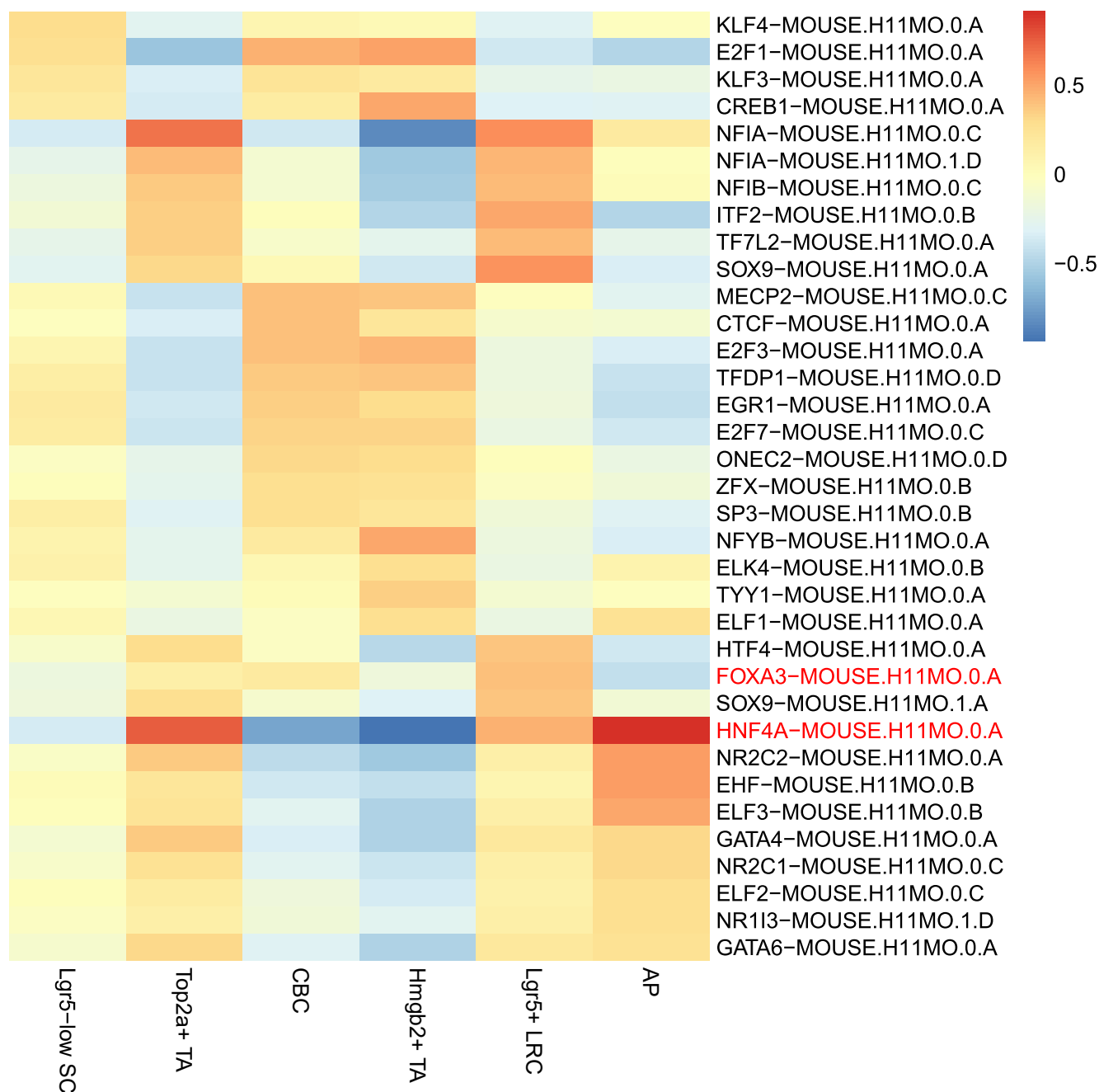
Supplementary Figure 7. FeaturePlot (top) and DotPlot (bottom) of small intestinal stem cell subtype markers expressed in the human jejunum. TERT is the marker for LRC and PCSK5 is the marker for AP.



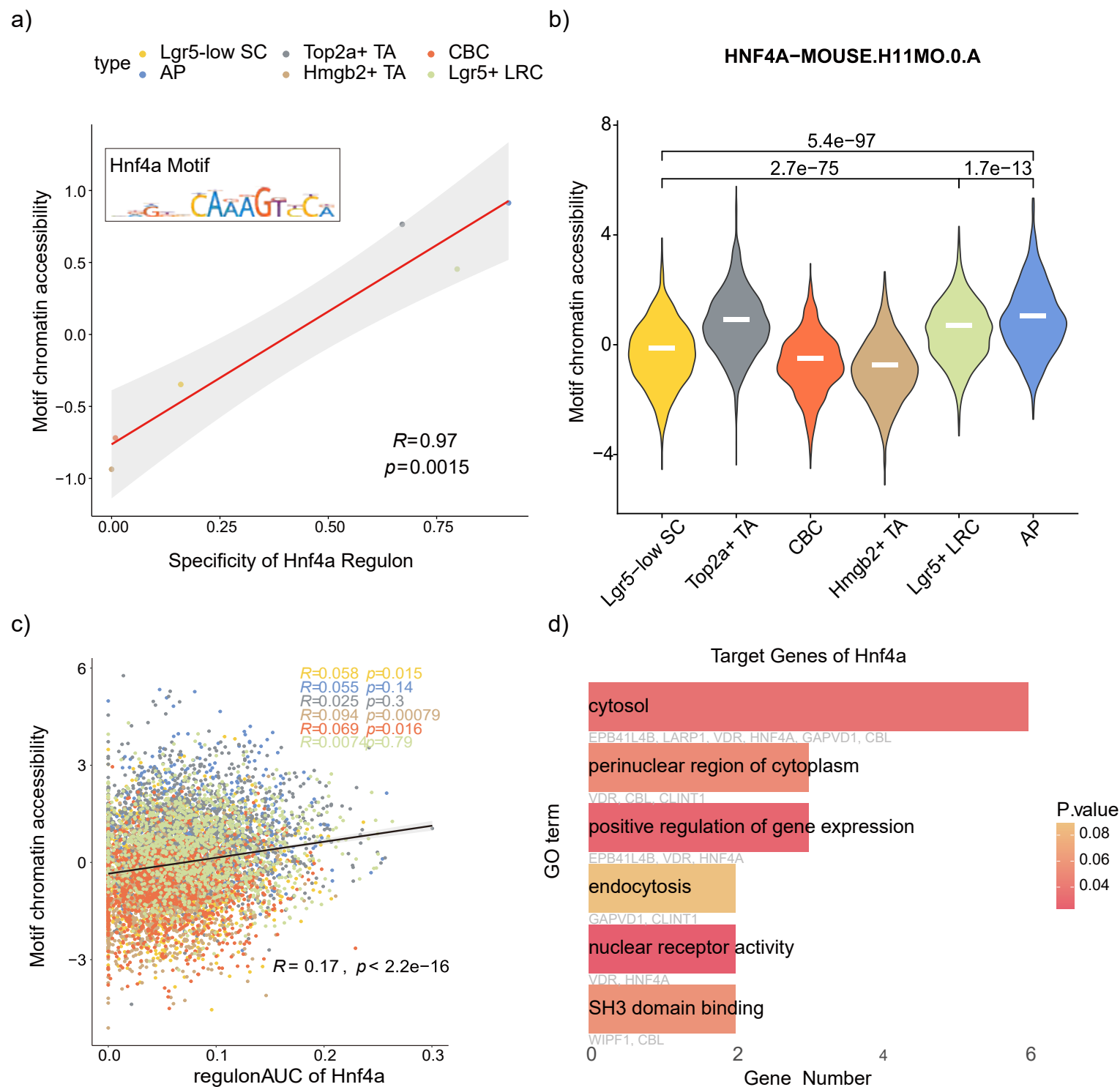
Supplementary Figure 8. Heat map of co-expression modules for driver genes. Driver genes are defined as genes whose differential expression is associated with trajectories. The colors of the heatmap represent the overall expression of the entire co-expression module in each cell type.



Supplementary Figure 9. Heat map of the top transcriptional factors inferred by the pySCENIC package within intestine stem cell populations. The Z-score is a SCENIC-calculated index of the specificity of regulon (TF and its regulatory genes) in each cell type.

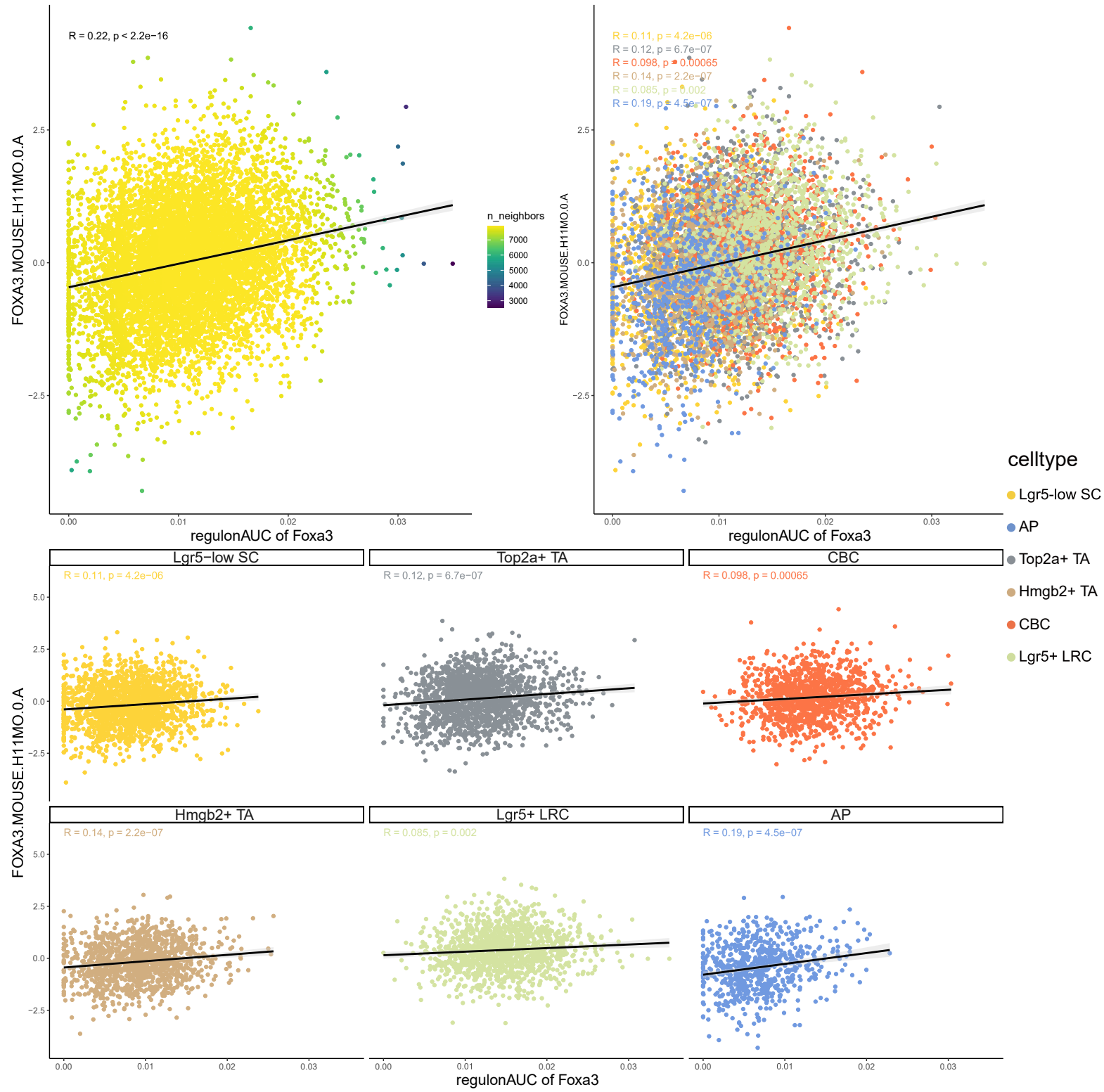


Supplementary Figure 10. Heat map of the top 10 variable DNA-binding motifs' accessibility within intestine stem cell populations.

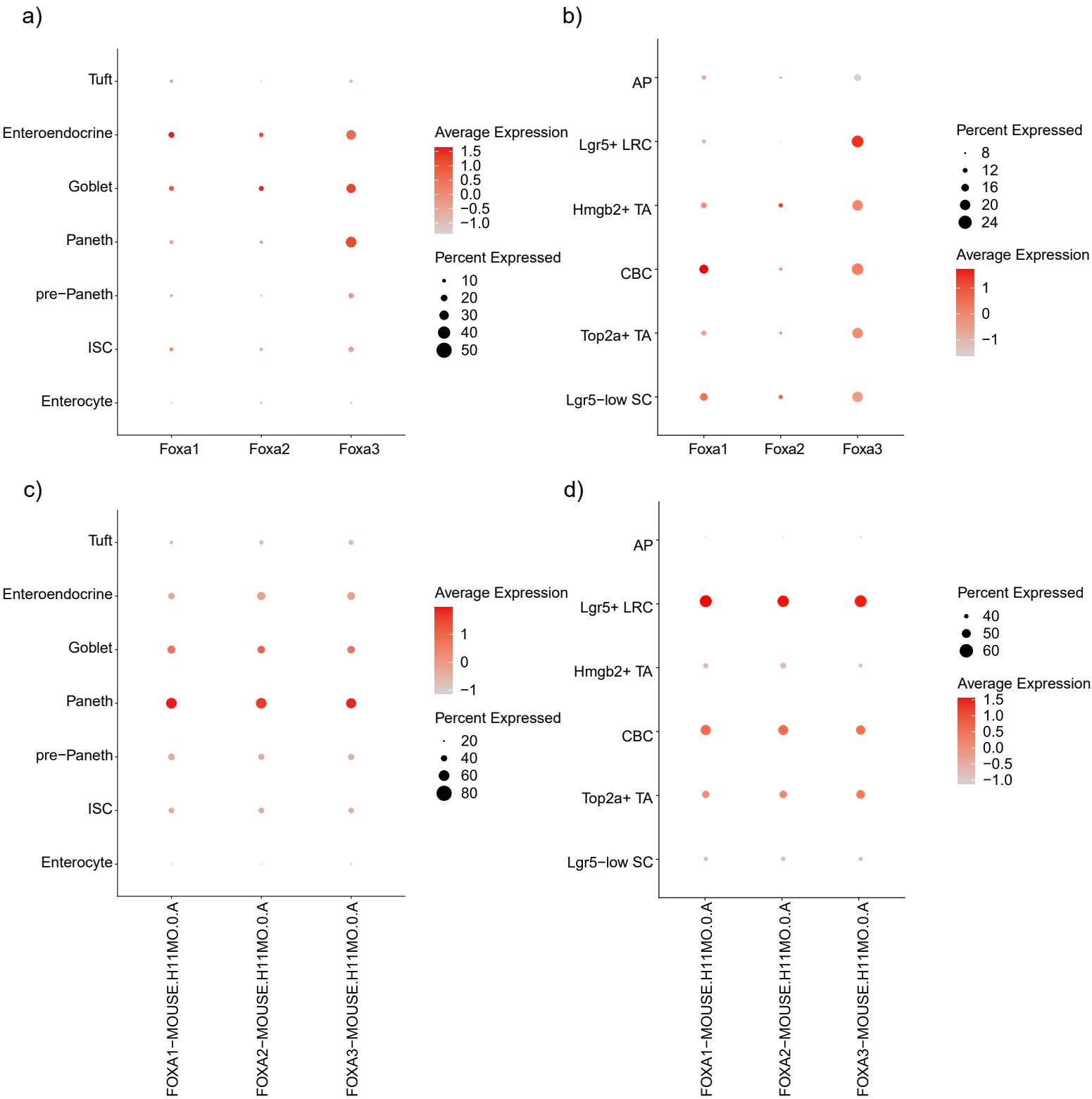


Supplementary Figure 11. Regulatory role of Hnf4a in the differentiation of Lgr5-low SC to absorbing lineage cells. **a)** Pearson's correlation analysis of Hnf4a (+) regulator specificity and the mean open scoring of the Hnf4a motif across ISC subpopulations. The sequence of the Hnf4a motif is shown at the top left of the figure. **b)** Violin plots of Hnf4a motif scoring across ISC subpopulations. The adjusted P-values for the significance test of differences were analyzed using the Wilcoxon test and corrected with the Benjamini-Hochberg method. **c)** Pearson correlation analysis of Hnf4a (+) regulator activity and Hnf4a motif chromatin openness in each cell. Cells were colored according to ISC subpopulations. **d)** Gene ontology enrichment analysis of Hnf4a direct target genes. P-values were analyzed using a hypergeometric distribution test with FDR correction (Benjamini-Hochberg method).

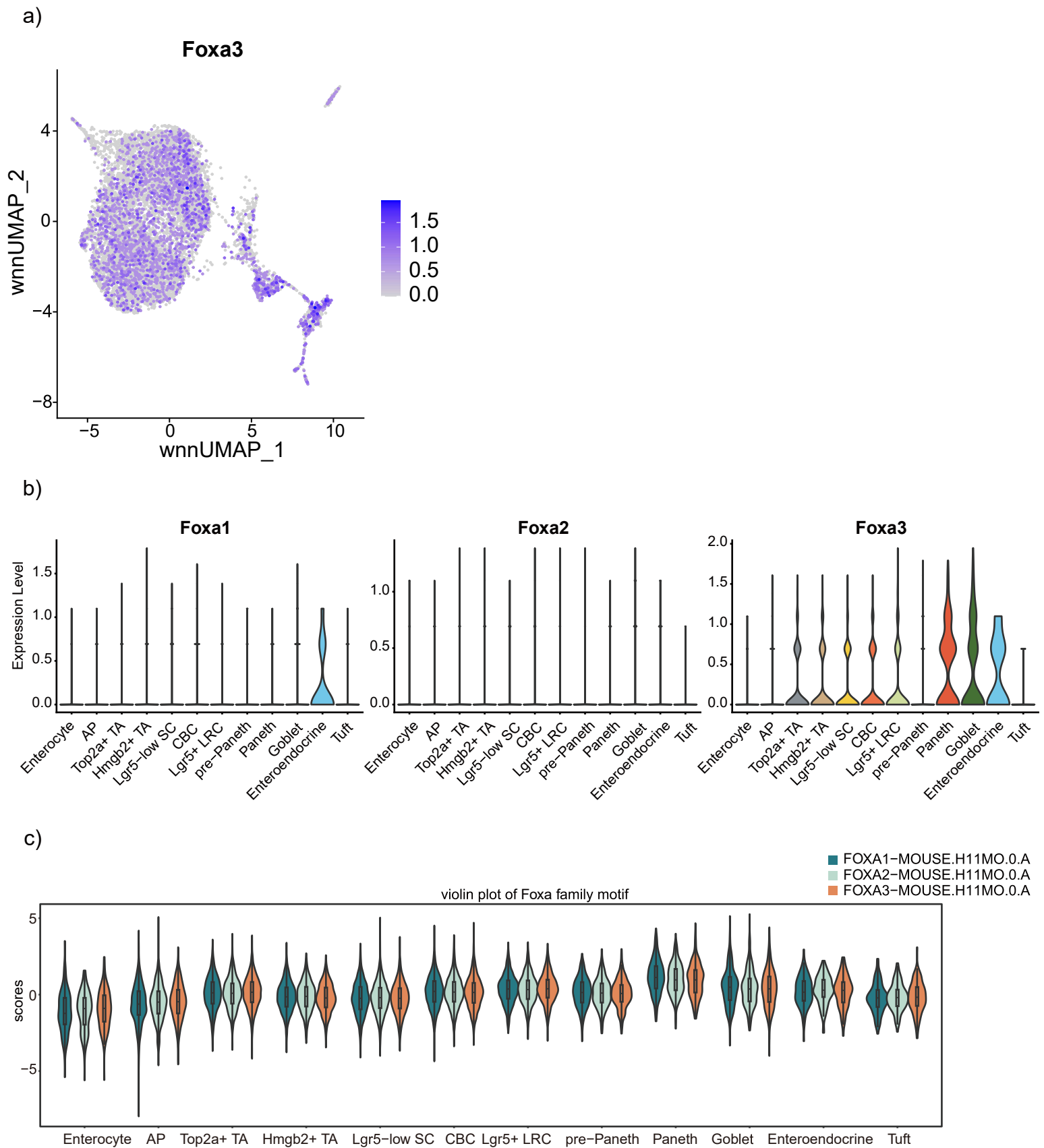
Foxa3



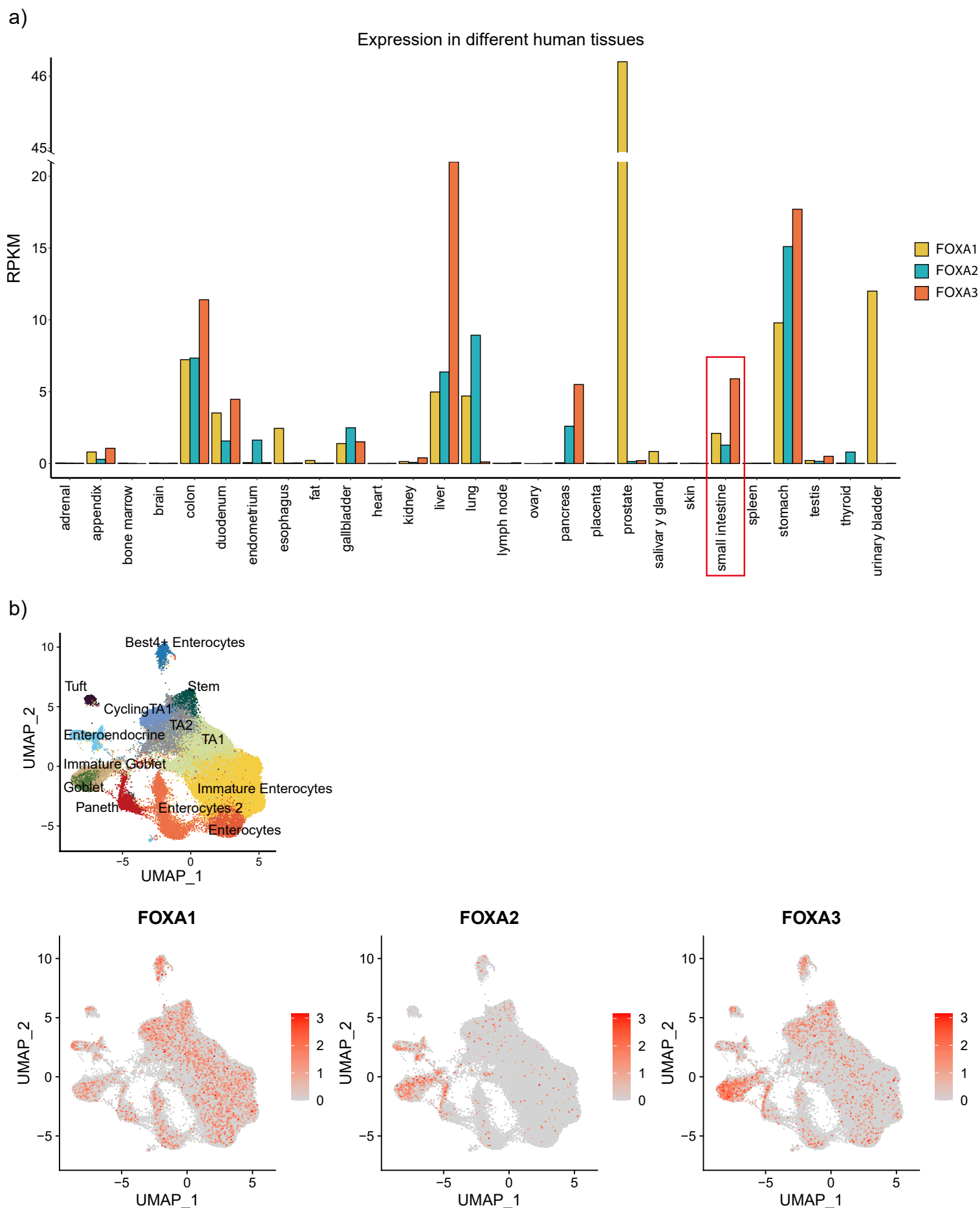
Supplementary Figure 12. Pearson correlation analysis of Foxa3 (+) regulator activity and Foxa3 motif chromatin openness in each cell. Cells were colored according to density (top left) and ISC subpopulations (top right).



Supplementary Figure 13. DotPlot of FOXA family gene expression and its motif score in small intestine epithelial cells (a,c) and ISC subtypes (b,d).

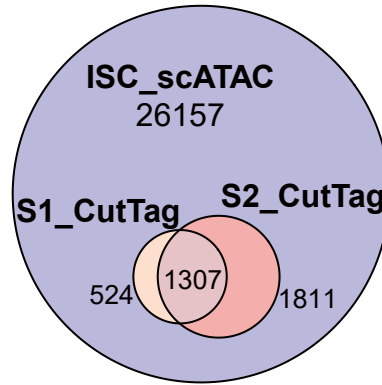


Supplementary Figure 14. Expression of FOXA family genes and their motif accessibility in small intestinal epithelial cells. a) FeaturePlot of Foxa3. **b)** VlnPlot of FOXA family gene expression. **c)** VlnPlot of motif scores for FOXA family genes.

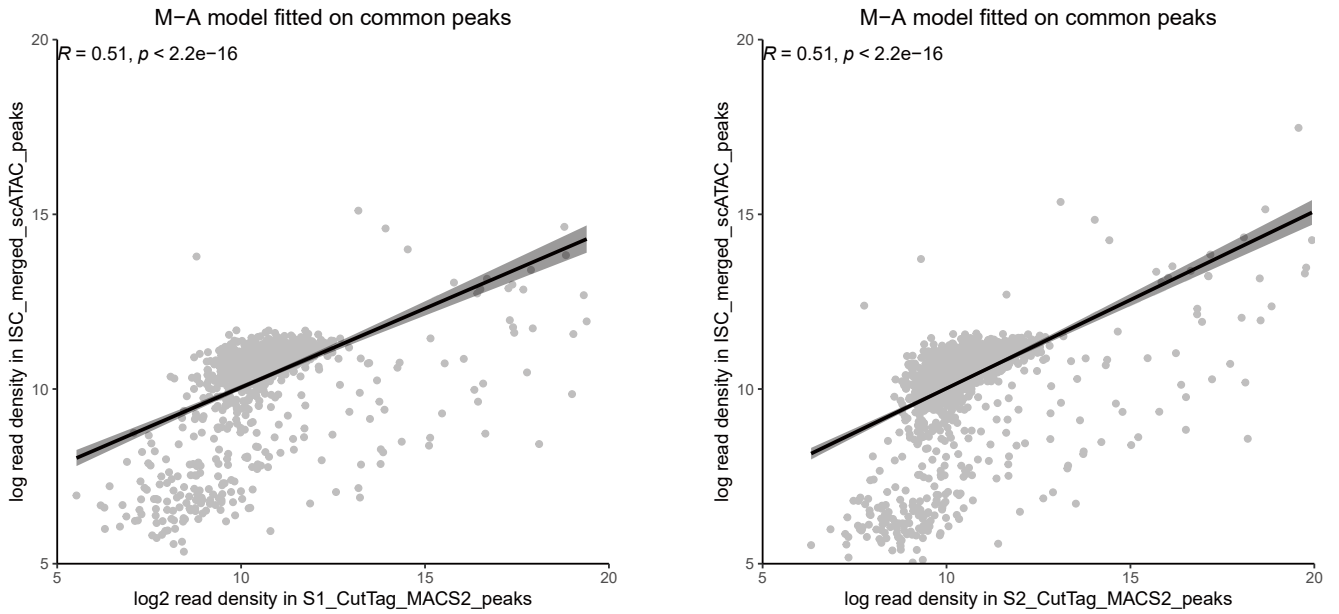


Supplementary Figure 15. Expression of FOXA family in human. a) Expression of the FOXA family in human tissues. **b)** Featureplot of FOXA family expression in human jejunum.

a)



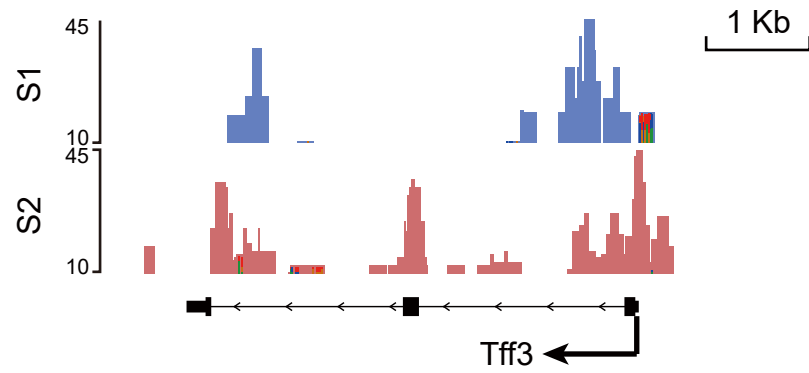
b)



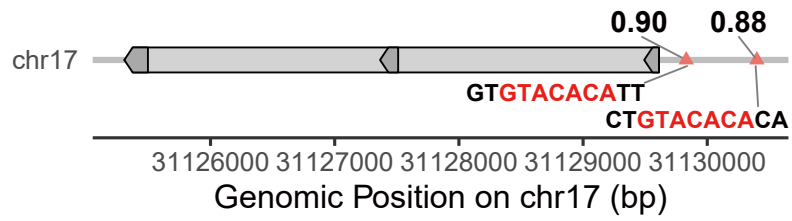
Supplementary Figure 16. The consistency of peaks between Cut&Tag-seq data and single-cell ATAC-seq data.

a) Statistical Venn diagram with peaks falling within genes and their regulatory regions.

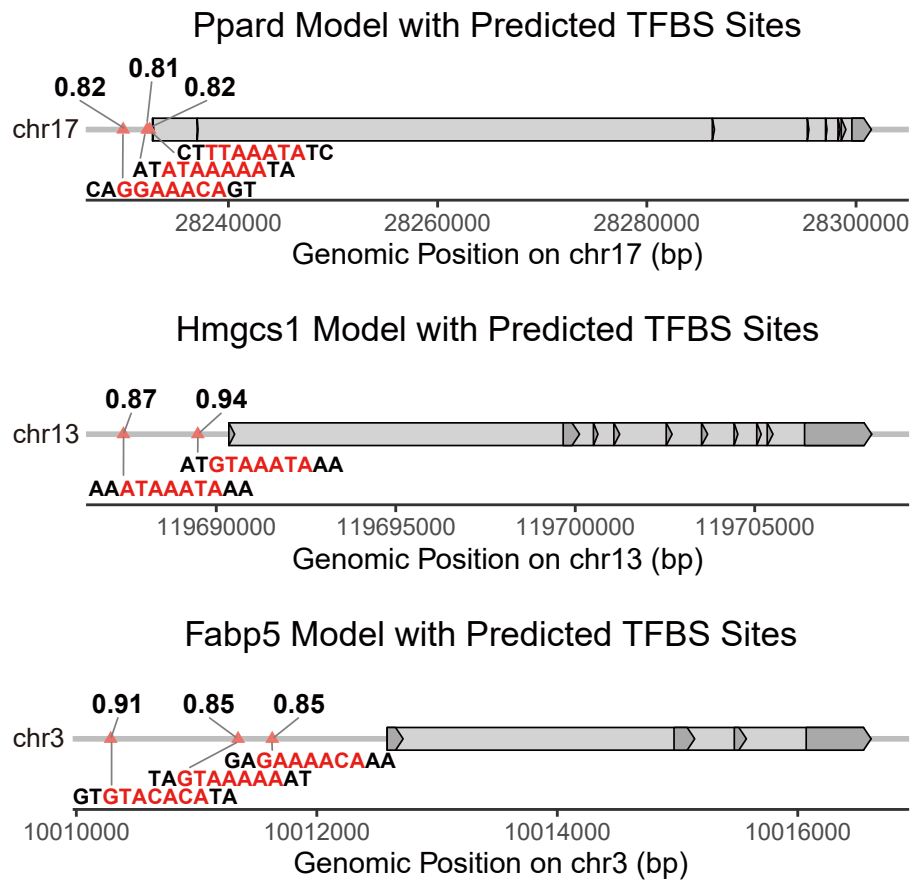
b) The correlation of copeak signal intensity between Cut&Tag-seq data and single-cell ATAC-seq data.



Tff3 Model with Predicted TFBS Sites



Supplementary Figure 17. Gene tracks showing Cut&Tag-seq data for marker genes of goblet cells (top). Model of predicted Transcription Factor Binding Sites in the upstream regions of Foxa3 (bottom).



Supplementary Figure 18. Model of Predicted Transcription Factor Binding Sites in the Upstream Regions of Genes in the PPAR Signaling Pathway.

TF	Motif	P-Value
Foxa3		1e-2
Foxa2		1e-8
Foxa1		1e-9

Supplementary Figure 19. Motifs in the upstream region of genes in the PPAR pathway obtained by Homer analysis.

Supplementary Table 1 | RT-PCR primers used in this study.

Primers	Forward (5'-3')	Reverse (5'-3')
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Foxa3	GCTGACCCTGAGTGAAATCTAC	ACGAAGCAGTCATTGAAGGAC
Reg4	CTGAGCTGGAGTGTGAGTCAT	GTCCACTGCCATAATTGCTTCT
Tff3	TTGCTGGGTCCTCTGGGATAG	TACACTGCTCCGATGTGACAG
Mmp7	AGGAAGCTGGAGATGTGAGC	TCTGCATTTCTTGAGGTTG