

Supplementary Material 11

Pseudotime-based gene co-expression modules and functional enrichment (Suppl. Figure S15–S24)

Laser microdissection-based multi-omics integration unveils the pathological atlas and tumor differentiation network of adamantinomatous craniopharyngioma

Bowen Wu¹ #, MM, Chunming Xu¹ #, MD, Chenxing Yang¹ #, MM, Shenhao Xie¹ #, MM, Suping Duan², BMed, Jiye Ye³, MD, Jinshi Zhang⁴, MD, Shaoyang Li⁵, MD, Furong Liang¹, BMed, Zhicheng Ye¹, BMed, Hai Luo¹, MM, Rilege Su¹, BMed, Laisheng Pan¹, MM, Zhizheng Liu¹, MM, Shizhou Xing¹, BMed, Tianming Dong¹, BMed, Yiguang Chen⁶, MD, Like Chen¹, MM, Suyue Zheng¹, MM, Jie Wu¹ *, MD, Tao Hong¹ *, MD

¹ Department of Neurosurgery, Jiangxi Key Laboratory of Neurological Diseases, the First Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang, 330006, China

² Department of Neurology, the First Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang, 330006, China

³ Department of Neurosurgery, Hunan University of Medicine General Hospital, 418000, Huaihua, China

⁴ Department of Neurosurgery, Ganzhou Hospital-Nanfang Hospital, Southern Medical University (Ganzhou People's Hospital), Ganzhou, 341000, China

⁵ School of public health, Jiangxi medical college, Nanchang University, Nanchang, 330006, China

⁶ Department of Neurosurgery/Neuro-oncology, State Key Laboratory of Oncology in South China, Guangdong Provincial Clinical Research Center for Cancer, SunYat-sen University Cancer Center, 510060, Guangzhou, China

Bowen Wu, Chunming Xu, Chenxing Yang and Shenhao Xie contributed equally to this work.

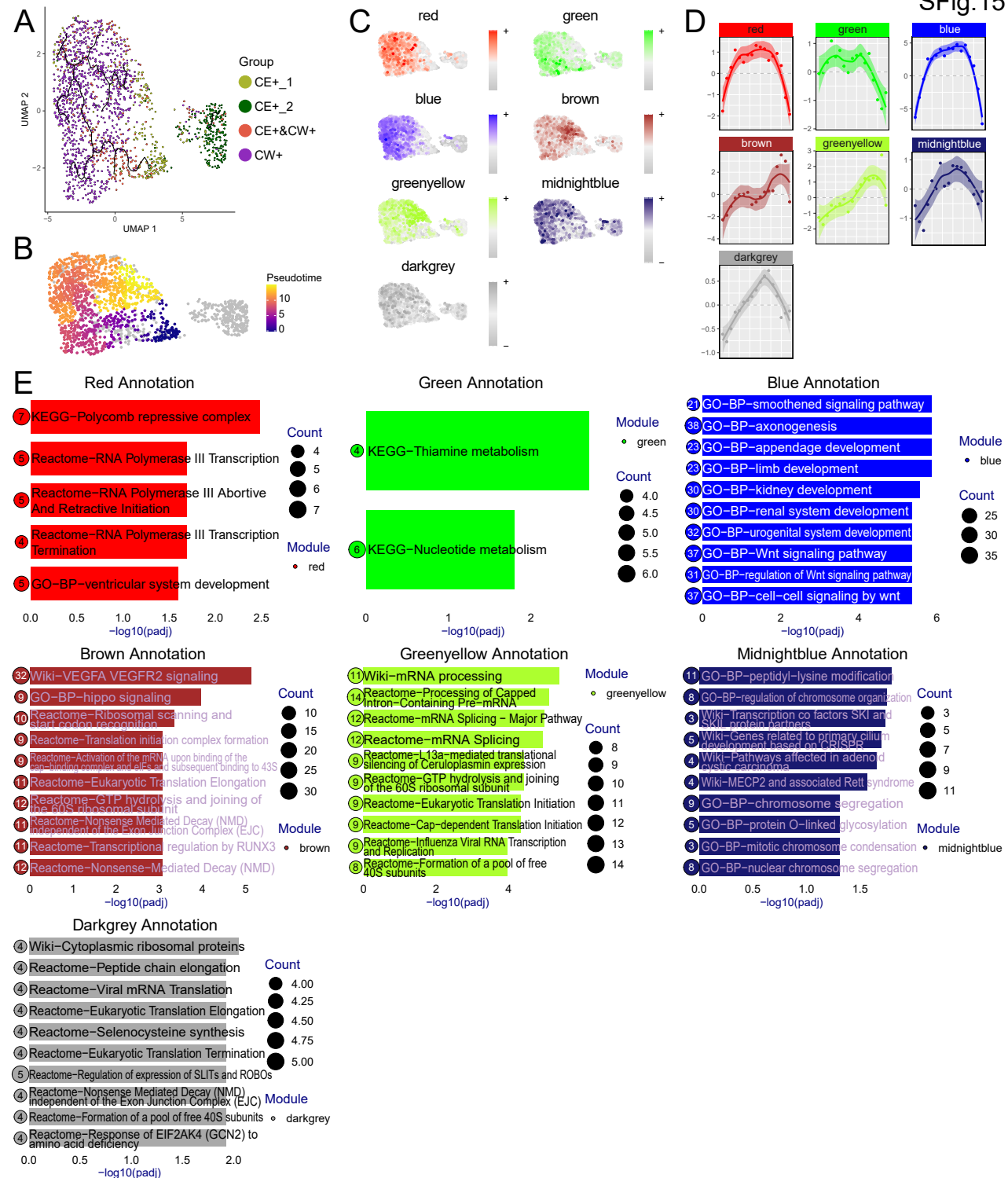
* Corresponding author:

Jie Wu: Department of Neurosurgery, Jiangxi Key Laboratory of Neurological Diseases, the First Affiliated Hospital, Jiangxi Medical College, Nanchang University, 17 Yong Wai Zheng Street, Nanchang, 330006, China, E-mail: ndyfy10128@ncu.edu.cn

Tao Hong: Department of Neurosurgery, Jiangxi Key Laboratory of Neurological Diseases, the First Affiliated Hospital, Jiangxi Medical College, Nanchang University, 17 Yong Wai Zheng Street, Nanchang, 330006, China, E-mail: ndyfy00567@ncu.edu.cn

Abbreviations of pathological structure cells

CE ⁺	Constitutive epithelium positive cells
CW ⁺	Cyst-wall epithelium positive cells
KRT ⁺	Wet keratin positive cells
PE ⁺	Palisading epithelium positive cells
SR ⁺	Stellate reticulum positive cells
WC ⁺	Whorl-like cell clusters positive cells



Supplementary Figure S15. The pseudotime modules for the differentiation of CE⁺_1 to CW⁺ cells.

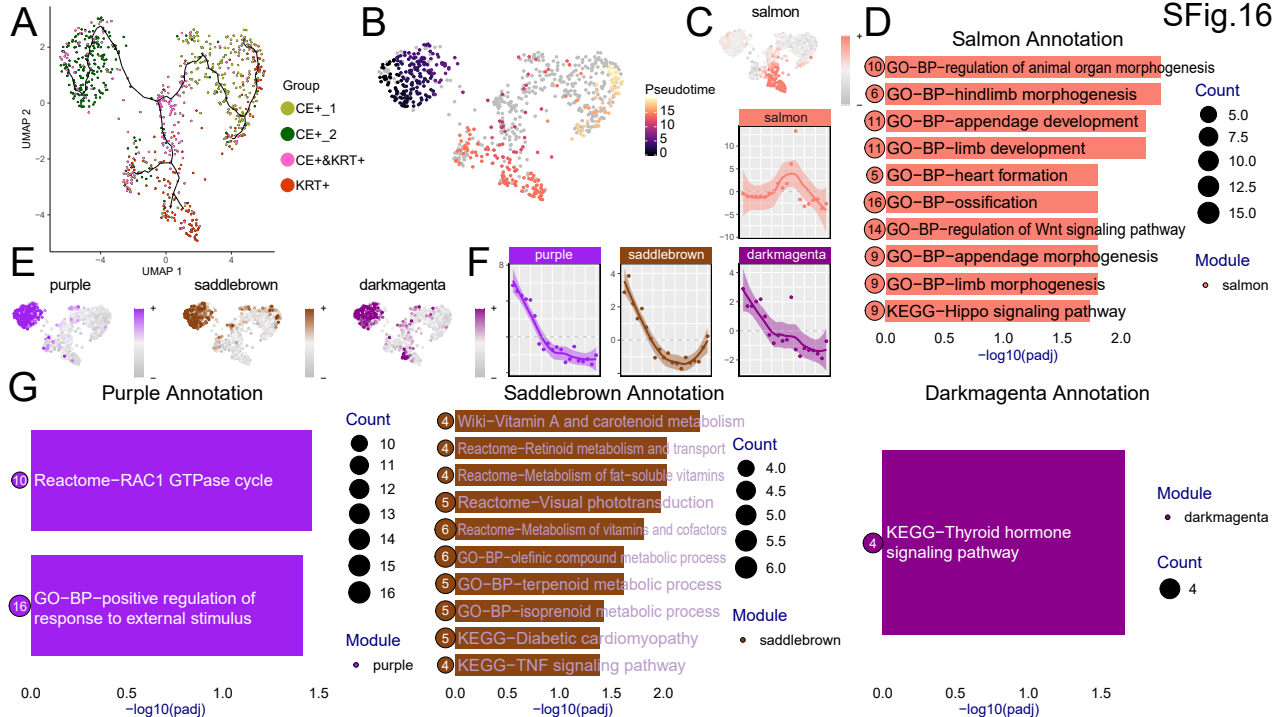
A The pseudotime axis derived from Monocle3.

B The pseudotime axis from CE⁺_1 to CW⁺ cells.

C UMAP visualization of pseudotime-upregulated co-expression modules identified by high dimensional weighted gene co-expression network analysis (hdWGCNA). Each subplot represents a module, and the color depth is proportional to the expression level of the module eigengene (the darker the color, the higher the expression).

D Plots of upregulated gene modules showing their dynamic expression along the pseudotime axis from CE⁺_1 to CW⁺ cell states.

E GO, KEGG, WikiPathways, and Reactome functional enrichment analysis of genes from the upregulated modules (top 10 terms by adjusted *p* value). The size of the circles on the left side of the figure and the numbers inside represent the number of genes involved in the pathway, and the bar length corresponds to the adjusted *p* value of enrichment. The longer the length is, the more significant the enrichment of the pathway.



Supplementary Figure S16. The pseudotime modules for the differentiation of CE⁺_2 to KRT⁺ cells.

A The pseudotime axis derived from Monocle3.

B The pseudotime axis from CE⁺_2 to KRT⁺ cells.

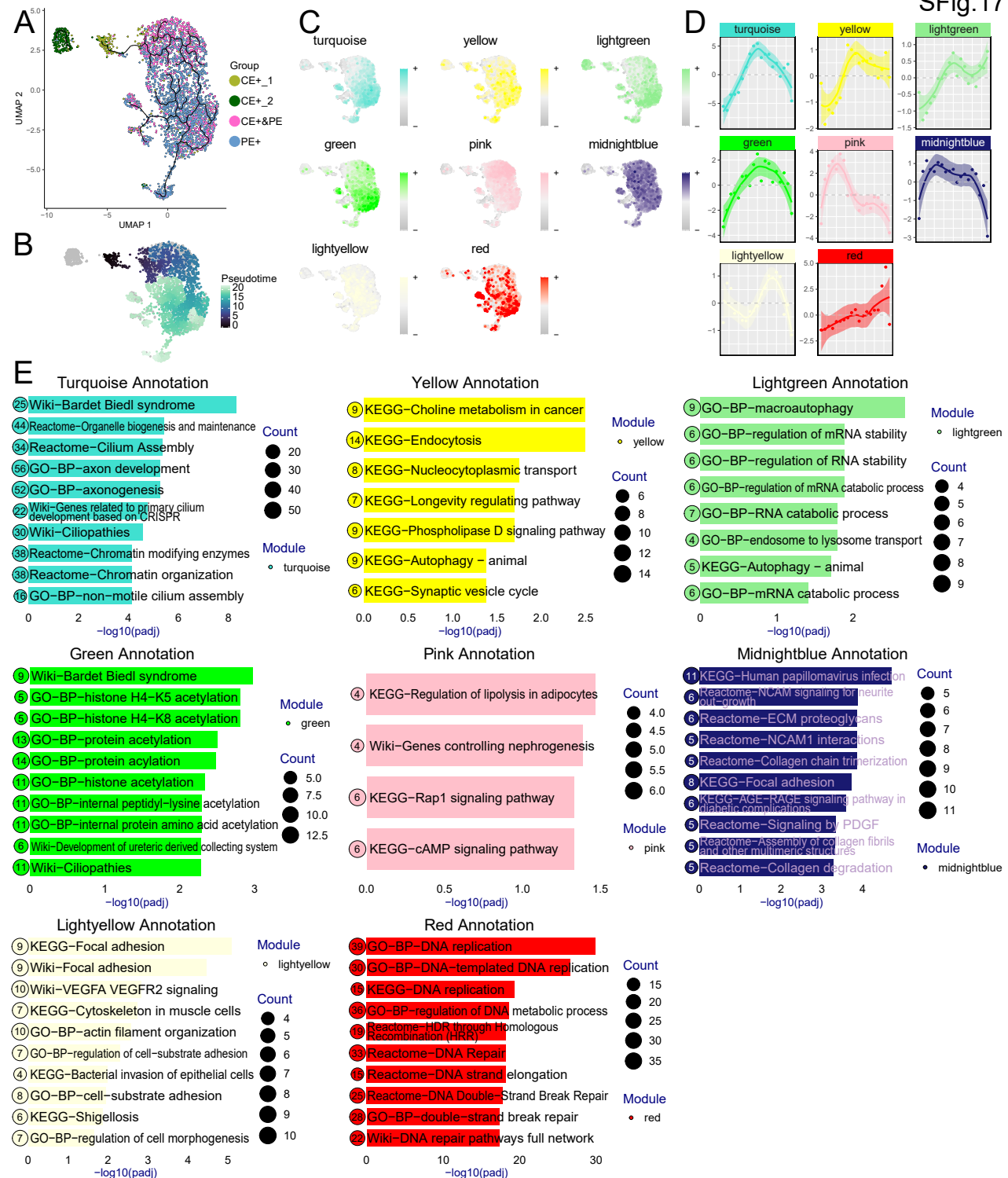
C Upper: UMAP visualization of pseudotime-upregulated co-expression module identified by hdWGCNA. The color depth is proportional to the expression level of the module eigengene (the darker the color, the higher the expression). Lower: Plot of upregulated gene module showing their dynamic expression along the pseudotime axis from CE⁺_2 to KRT⁺ cell states.

D GO, KEGG, WikiPathways, and Reactome functional enrichment analysis of genes from the upregulated module (top 10 terms by adjusted *p* value). The size of the circles on the left side of the figure and the numbers inside represent the number of genes involved in the pathway, and the bar length corresponds to the adjusted *p* value of enrichment. The longer the length is, the more significant the enrichment of the pathway.

E UMAP visualization of pseudotime-downregulated co-expression modules identified by hdWGCNA. Each subplot represents a module.

F Plots of downregulated gene modules showing their dynamic expression along the pseudotime axis from CE⁺_2 to KRT⁺ cell states.

G GO, KEGG, WikiPathways, and Reactome functional enrichment analysis of genes from the downregulated modules (top 10 terms by adjusted *p* value).



Supplementary Figure S17. The pseudotime modules for the differentiation of CE⁺_1 to PE⁺ cells.

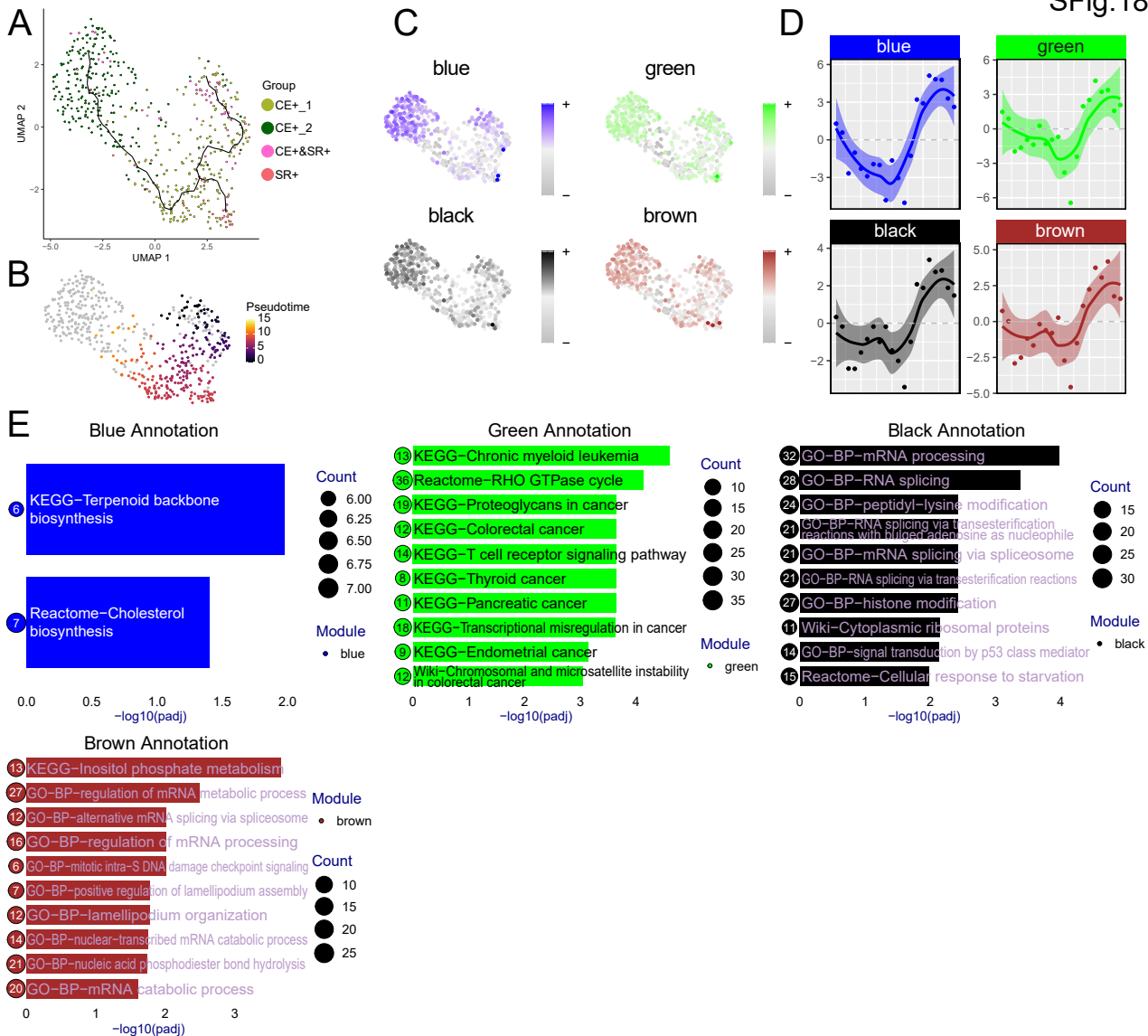
A The pseudotime axis derived from Monocle3.

B The pseudotime axis from CE⁺_1 to PE⁺ cells.

C UMAP visualization of pseudotime-upregulated co-expression modules identified by hdWGCNA. Each subplot represents a module, and the color depth is proportional to the expression level of the module eigengene (the darker the color, the higher the expression).

D Plots of upregulated gene modules showing their dynamic expression along the pseudotime axis from CE⁺_1 to PE⁺ cell states.

E GO, KEGG, WikiPathways, and Reactome functional enrichment analysis of genes from the upregulated modules (top 10 terms by adjusted *p* value). The size of the circles on the left side of the figure and the numbers inside represent the number of genes involved in the pathway, and the bar length corresponds to the adjusted *p* value of enrichment. The longer the length is, the more significant the enrichment of the pathway.



Supplementary Figure S18. The pseudotime modules for the differentiation of CE⁺_1 to SR⁺ cells.

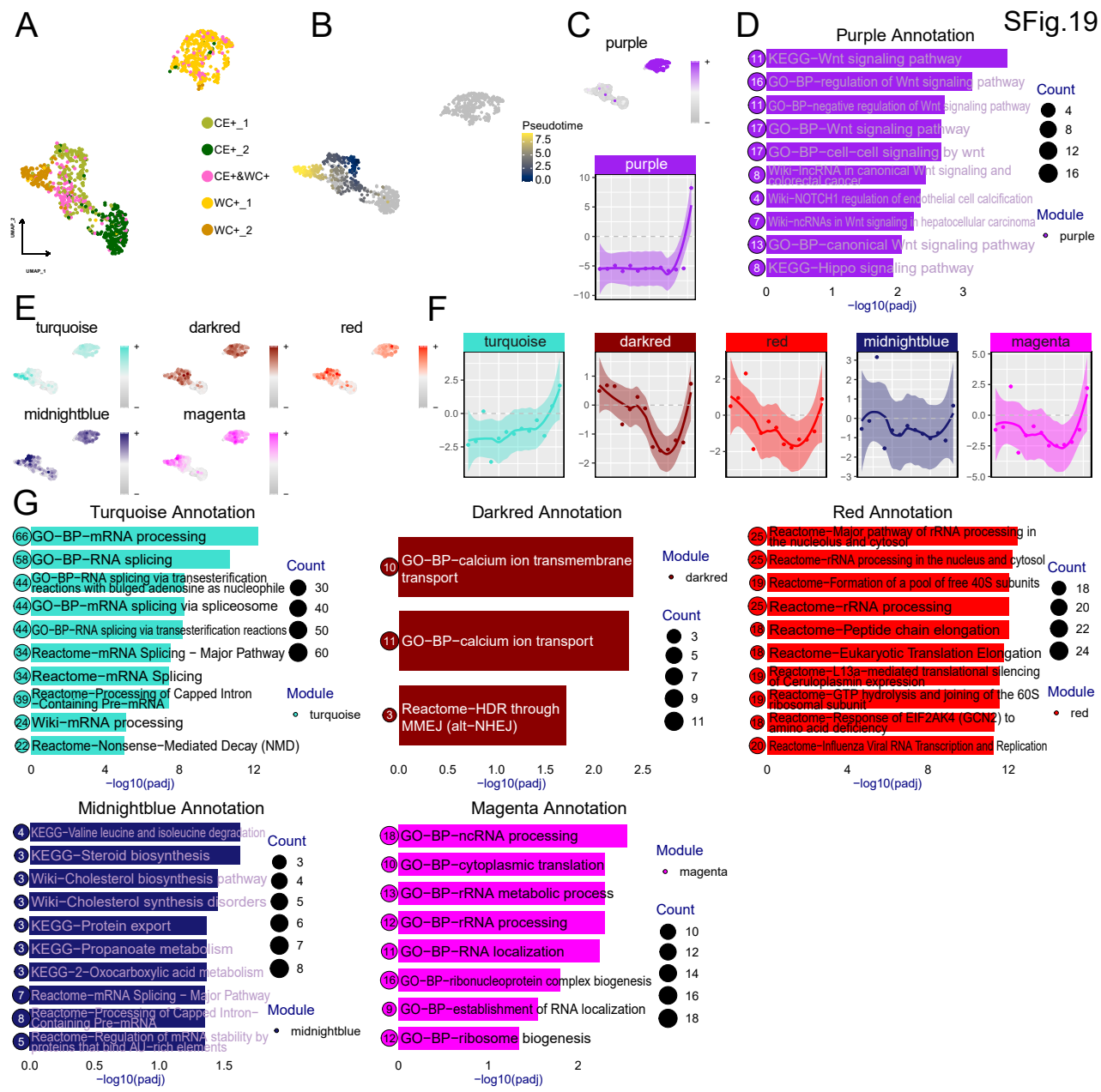
A The pseudotime axis derived from Monocle3.

B The pseudotime axis from CE⁺_1 to SR⁺ cells.

C UMAP visualization of pseudotime-upregulated co-expression modules identified by hdWGCNA. Each subplot represents a module, and the color depth is proportional to the expression level of the module eigengene (the darker the color, the higher the expression).

D Plots of upregulated gene modules showing their dynamic expression along the pseudotime axis from CE⁺_1 to SR⁺ cell states.

E GO, KEGG, WikiPathways, and Reactome functional enrichment analysis of genes from the upregulated modules (top 10 terms by adjusted *p* value). The size of the circles on the left side of the figure and the numbers inside represent the number of genes involved in the pathway, and the bar length corresponds to the adjusted *p* value of enrichment. The longer the length is, the more significant the enrichment of the pathway.



Supplementary Figure S19. The pseudotime modules for the differentiation of CE⁺_1 to WC⁺ cells.

A The pseudotime axis derived from Monocle3.

B The pseudotime axis from CE⁺_1 to WC⁺ cells.

C Upper: UMAP visualization of pseudotime-upregulated co-expression module identified by hdWGCNA (CE⁺_1 to WC⁺_1 cell states). The color depth is proportional to the expression level of the module eigengene (the darker the color, the higher the expression). Lower: Plot of upregulated gene module showing their dynamic expression along the pseudotime axis from CE⁺_1 to WC⁺_1 cell states.

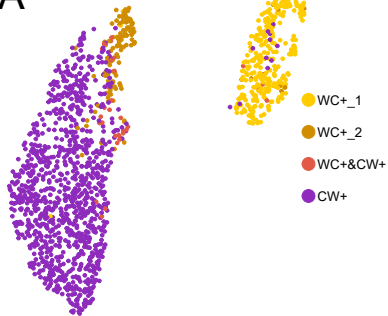
D GO, KEGG, WikiPathways, and Reactome functional enrichment analysis of genes from the upregulated module (CE⁺_1 to WC⁺_1 cell states) (top 10 terms by adjusted *p* value). The size of the circles on the left side of the figure and the numbers inside represent the number of genes involved in the pathway, and the bar length corresponds to the adjusted *p* value of enrichment. The longer the length is, the more significant the enrichment of the pathway.

E UMAP visualization of pseudotime-upregulated co-expression modules identified by hdWGCNA (CE⁺_1 to WC⁺_2 cell states). Each subplot represents a module.

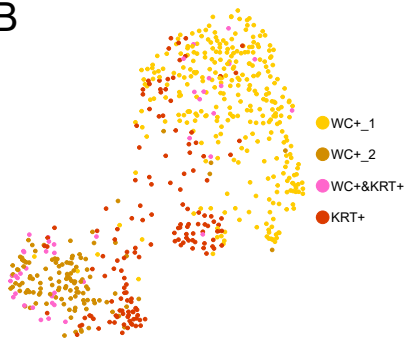
F Plots of upregulated gene modules showing their dynamic expression along the pseudotime axis from CE⁺_1 to WC⁺_2 cell states.

G GO, KEGG, WikiPathways, and Reactome functional enrichment analysis of genes from the upregulated modules (CE⁺_1 to WC⁺_2 cell states) (top 10 terms by adjusted *p* value).

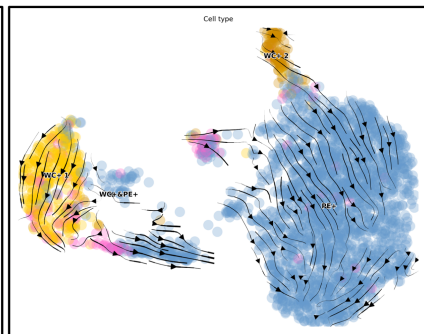
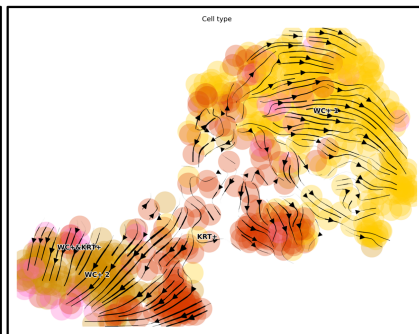
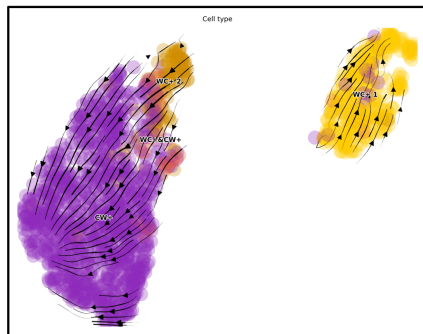
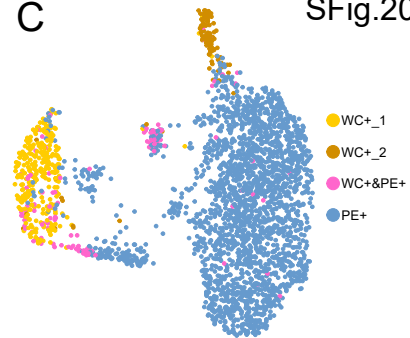
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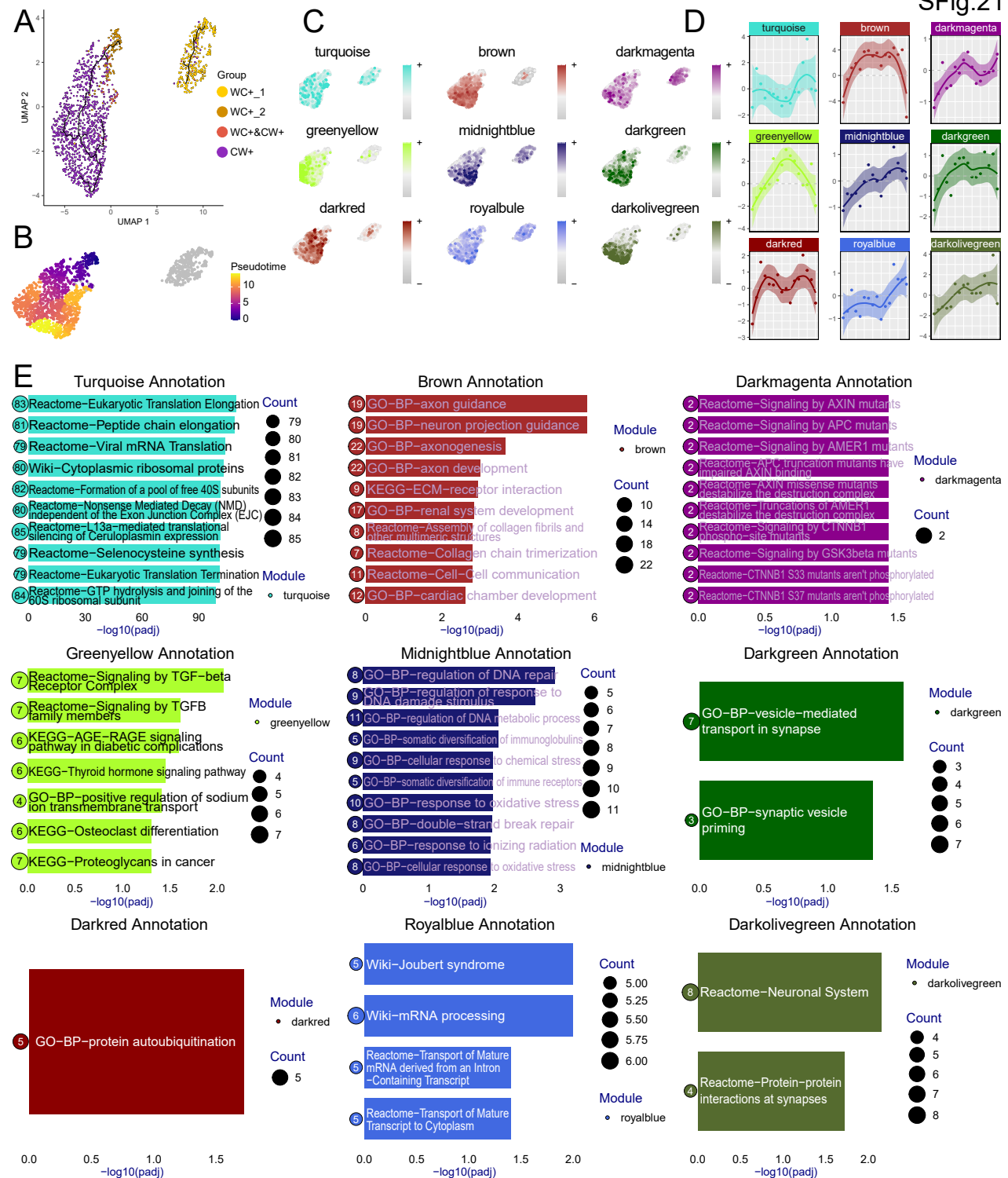
B



C



Supplementary Figure S20. RNA velocity analysis results of WC⁺ to others.



Supplementary Figure S21. The pseudotime modules for the differentiation of WC⁺_2 to CW⁺ cells.

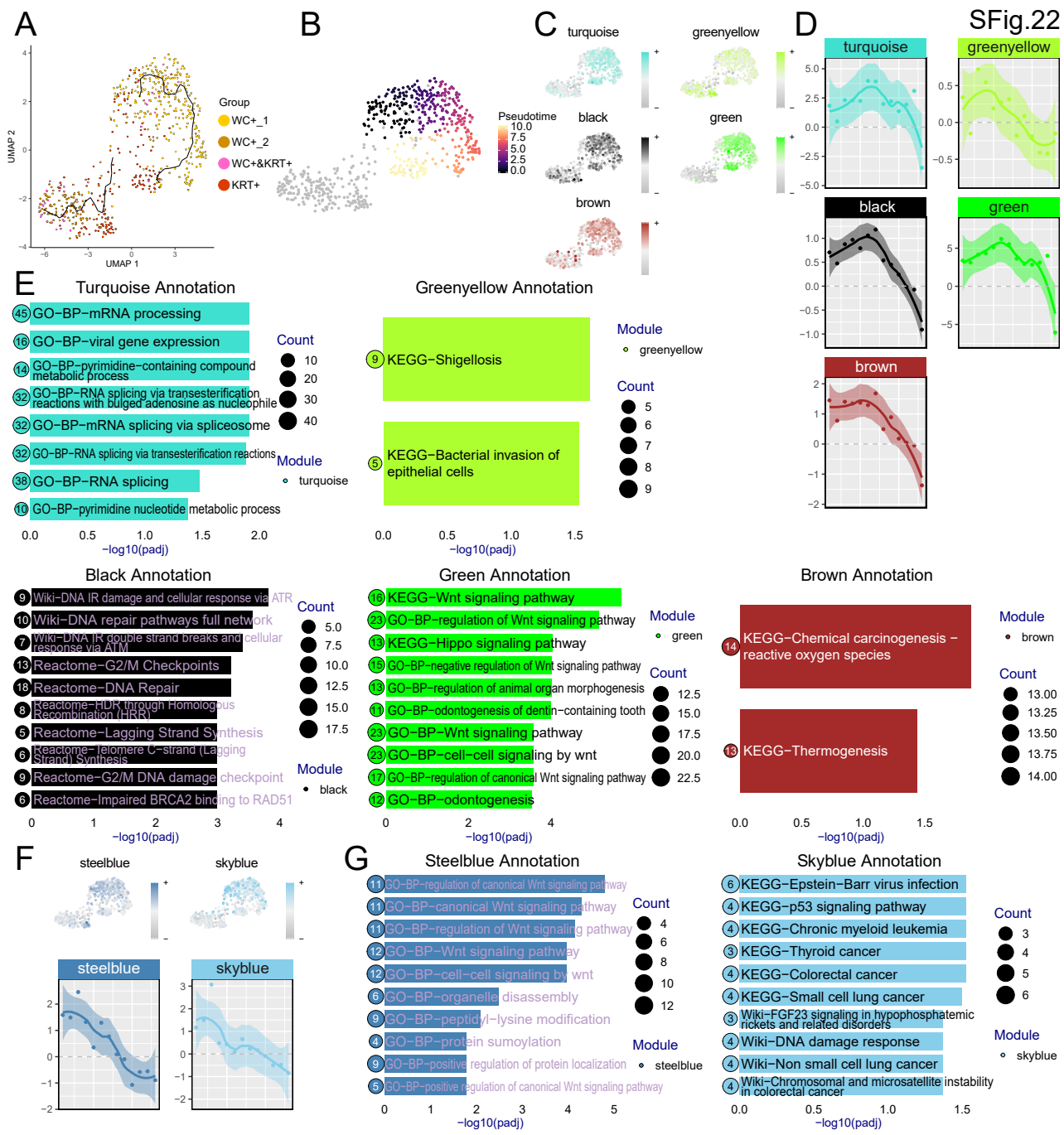
A The pseudotime axis derived from Monocle3.

B The pseudotime axis from WC⁺_2 to CW⁺ cells.

C UMAP visualization of pseudotime-upregulated co-expression modules identified by hdWGCNA. Each subplot represents a module, and the color depth is proportional to the expression level of the module eigengene (the darker the color, the higher the expression).

D Plots of upregulated gene modules showing their dynamic expression along the pseudotime axis from WC⁺_2 to CW⁺ cell states.

E GO, KEGG, WikiPathways, and Reactome functional enrichment analysis of genes from the upregulated modules (top 10 terms by adjusted *p* value). The size of the circles on the left side of the figure and the numbers inside represent the number of genes involved in the pathway, and the bar length corresponds to the adjusted *p* value of enrichment. The longer the length is, the more significant the enrichment of the pathway.



Supplementary Figure S22. The pseudotime modules for the differentiation of WC⁺_1 to KRT⁺ cells.

A The pseudotime axis derived from Monocle3.

B The pseudotime axis from WC⁺_1 to KRT⁺ cells.

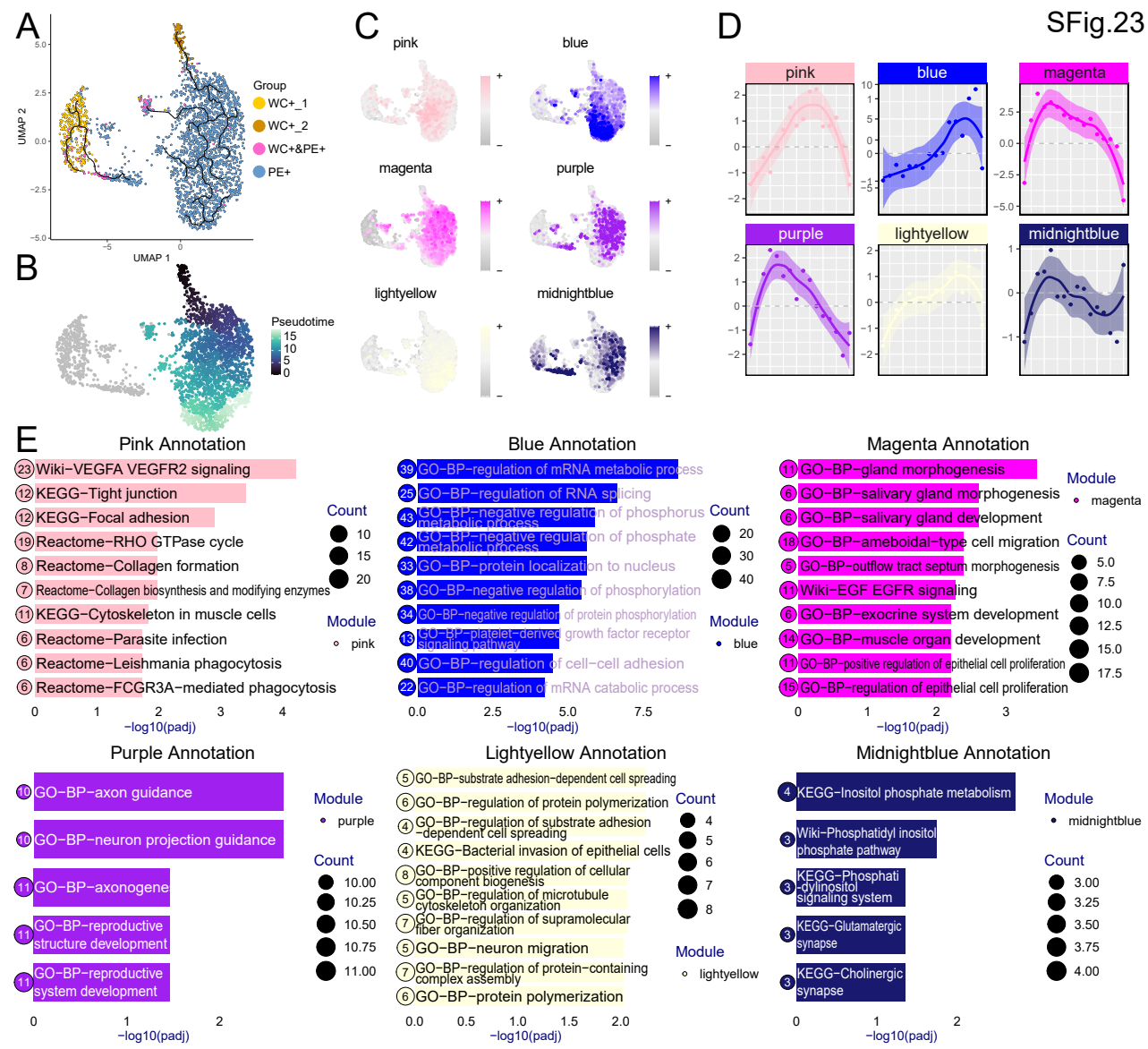
C UMAP visualization of pseudotime-upregulated co-expression modules identified by hdWGCNA. Each subplot represents a module, and the color depth is proportional to the expression level of the module eigengene (the darker the color, the higher the expression).

D Plots of upregulated gene modules showing their dynamic expression along the pseudotime axis from WC⁺_1 to KRT⁺ cell states.

E GO, KEGG, WikiPathways, and Reactome functional enrichment analysis of genes from the upregulated modules (top 10 terms by adjusted *p* value). The size of the circles on the left side of the figure and the numbers inside represent the number of genes involved in the pathway, and the bar length corresponds to the adjusted *p* value of enrichment. The longer the length is, the more significant the enrichment of the pathway.

F Upper: UMAP visualization of pseudotime-downregulated co-expression modules identified by hdWGCNA (WC⁺_1 to KRT⁺ cell states). Lower: Plots of downregulated gene modules showing their dynamic expression along the pseudotime axis from WC⁺_1 to KRT⁺ cell states.

G GO, KEGG, WikiPathways, and Reactome functional enrichment analysis of genes from the downregulated modules (top 10 terms by adjusted *p* value).



Supplementary Figure S23. The pseudotime modules for the differentiation of WC⁺_2 to PE⁺ cells.

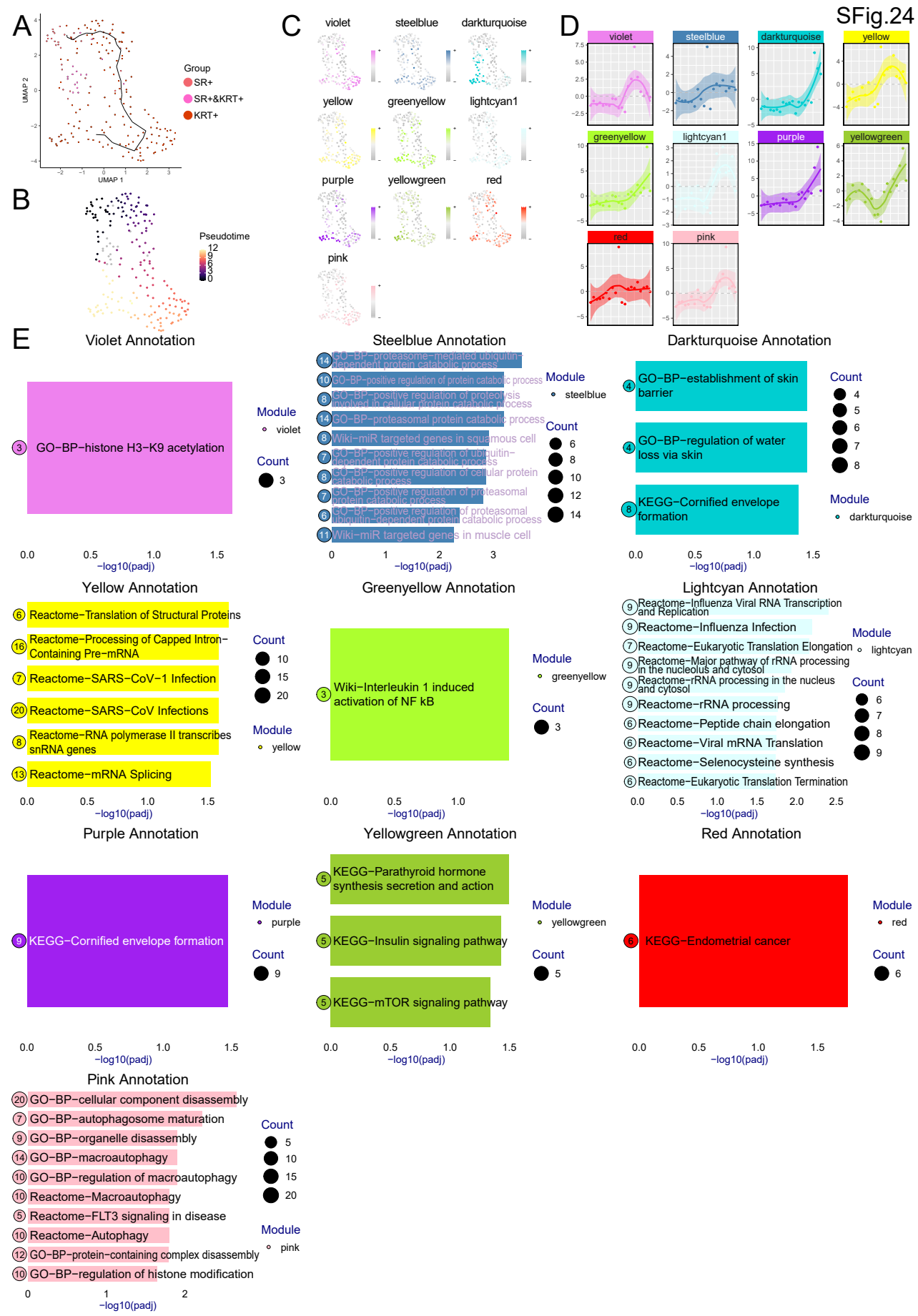
A The pseudotime axis derived from Monocle3.

B The pseudotime axis from WC⁺_2 to PE⁺ cells.

C UMAP visualization of pseudotime-upregulated co-expression modules identified by hdWGCNA. Each subplot represents a module, and the color depth is proportional to the expression level of the module eigengene (the darker the color, the higher the expression).

D Plots of upregulated gene modules showing their dynamic expression along the pseudotime axis from WC⁺_2 to PE⁺ cell states.

E GO, KEGG, WikiPathways, and Reactome functional enrichment analysis of genes from the upregulated modules (top 10 terms by adjusted *p* value). The size of the circles on the left side of the figure and the numbers inside represent the number of genes involved in the pathway, and the bar length corresponds to the adjusted *p* value of enrichment. The longer the length is, the more significant the enrichment of the pathway.



Supplementary Figure S24. The pseudotime modules for the differentiation of SR⁺ to KRT⁺ cells.

A The pseudotime axis derived from Monocle3.

B The pseudotime axis from SR⁺ to KRT⁺ cells.

C UMAP visualization of pseudotime-upregulated co-expression modules identified by high-dimensional weighted gene co-expression network analysis (hdWGCNA). Each subplot represents a module, and the color depth is proportional to the expression level of the module eigengene (the darker the color, the higher the expression).

D Plots of upregulated gene modules showing their dynamic expression along the pseudotime axis from SR⁺ to KRT⁺ cell states.

E GO, KEGG, WikiPathways, and Reactome functional enrichment analysis of genes from the upregulated modules (top 10 terms by adjusted *p* value). The size of the circles on the left side of the figure and the numbers inside represent the number of genes involved in the pathway, and the bar length corresponds to the adjusted *p* value of enrichment. The longer the length is, the more significant the enrichment of the pathway.