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Supplemental information

Single-cell sequencing identifies differentiation-related markers for molecular classification and recurrence prediction of PitNET

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A

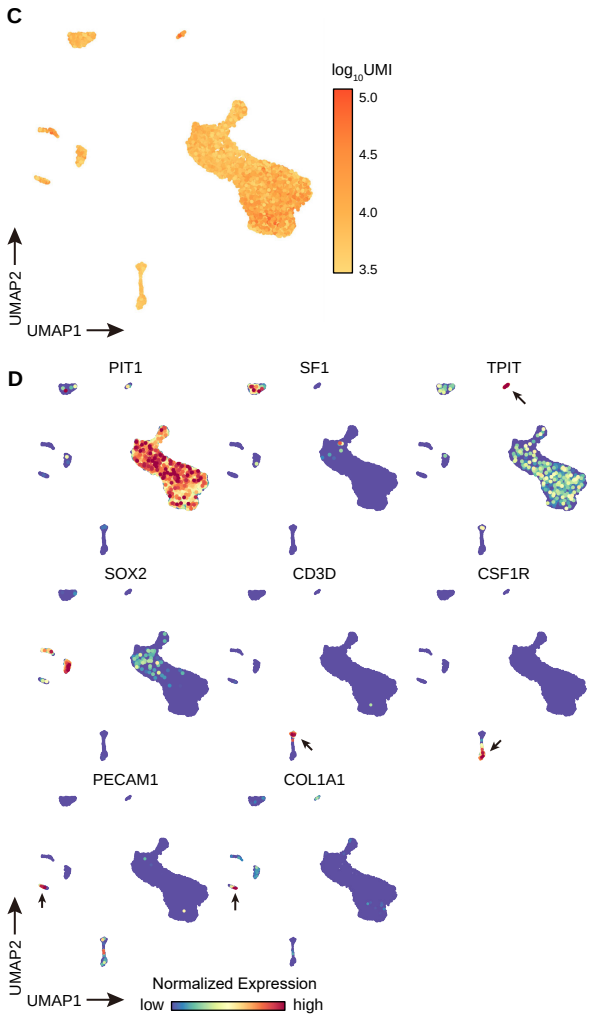
Classification of hormone-secreting cells in APG

TF lineage	Cell name	Hormone	Gene
PIT1	Lactotrope	Prolactin (PRL)	PRL
	Somatotrope	Growth hormone (GH)	GH1,GH2
	Thyrotrope	Thyroid stimulating hormone (TSH)	TSHB+CGA
TPIT	Corticotrope	Adrenocorticotropic Hormone (ACTH)	POMC
SF1	Gonadotrope	Follicle Stimulating Hormone (FSH)	FSHB+CGA
		Luteinizing Hormone (LH)	LHB+CGA

B

Clinicopathological classification of PitNET

TF lineage	Tumor name	Hormone	Gene
PIT1	Lactotroph tumor	PRL	PRL
	Somatotroph tumor	GH	GH1, GH2
	Thyrotroph tumor	TSH	TSHB, CGA
	Silent PIT1 tumor	None	-
TPIT	Corticotroph tumor	ACTH	POMC
	Silent TPIT tumor	None	-
SF1	Gonadotroph tumor	FSH, and/or LH	FSHB, LHB, CGA
	Silent SF1 tumor	None	-
Null	Null cell tumor	None	-
Pluri	Plurihormonal tumor	Multiple hormones	-



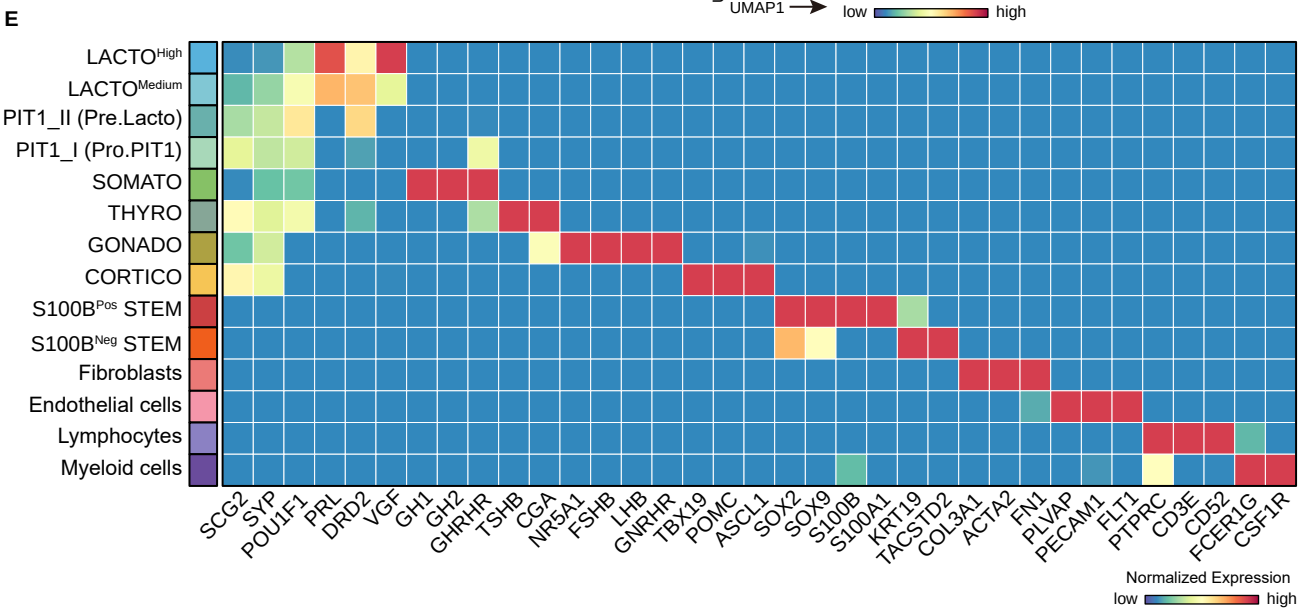


Figure S1. Characterization of major clusters in human APG. Related to Figure 1.

(A) Classification of hormone-secreting cells in APG with corresponding hormones and hormone expressing genes.

(B) Clinicopathological classification of PitNET with corresponding hormones and hormone expressing genes.

(C) Integrated UMAP plot of the UMI counts in APGs.

(D) Signature gene expression of selected marker genes for the cell type definition.

(E) Heatmap shows canonical marker genes of conserved cell types in APG.

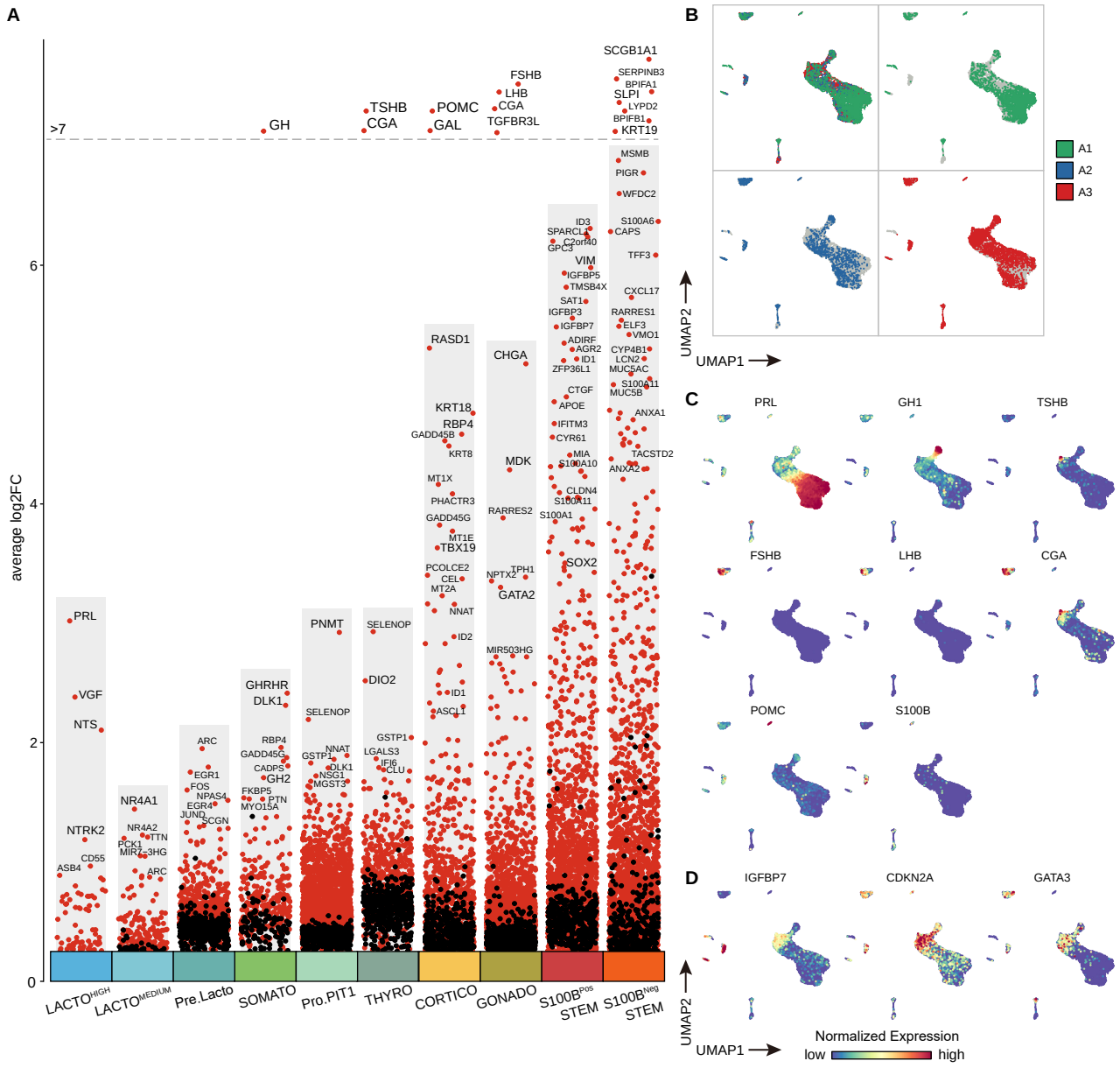


Figure S2. Characterization of endocrine and stem clusters in human APG. Related to Figure 1.

(A) Differential gene expression analysis showing upregulated genes across differentiated endocrine clusters. An adjusted p value <1E-6 is indicated in red, while an adjusted p value > 1E-6 is indicated in black.

(B) Integrated UMAP plot of the distribution of three APG samples.

(C) Classic hormone genes of differentiated endocrine cells in APGs.

(D) Signature gene expression of selected marker genes of PIT1_I (Pro.PIT1).

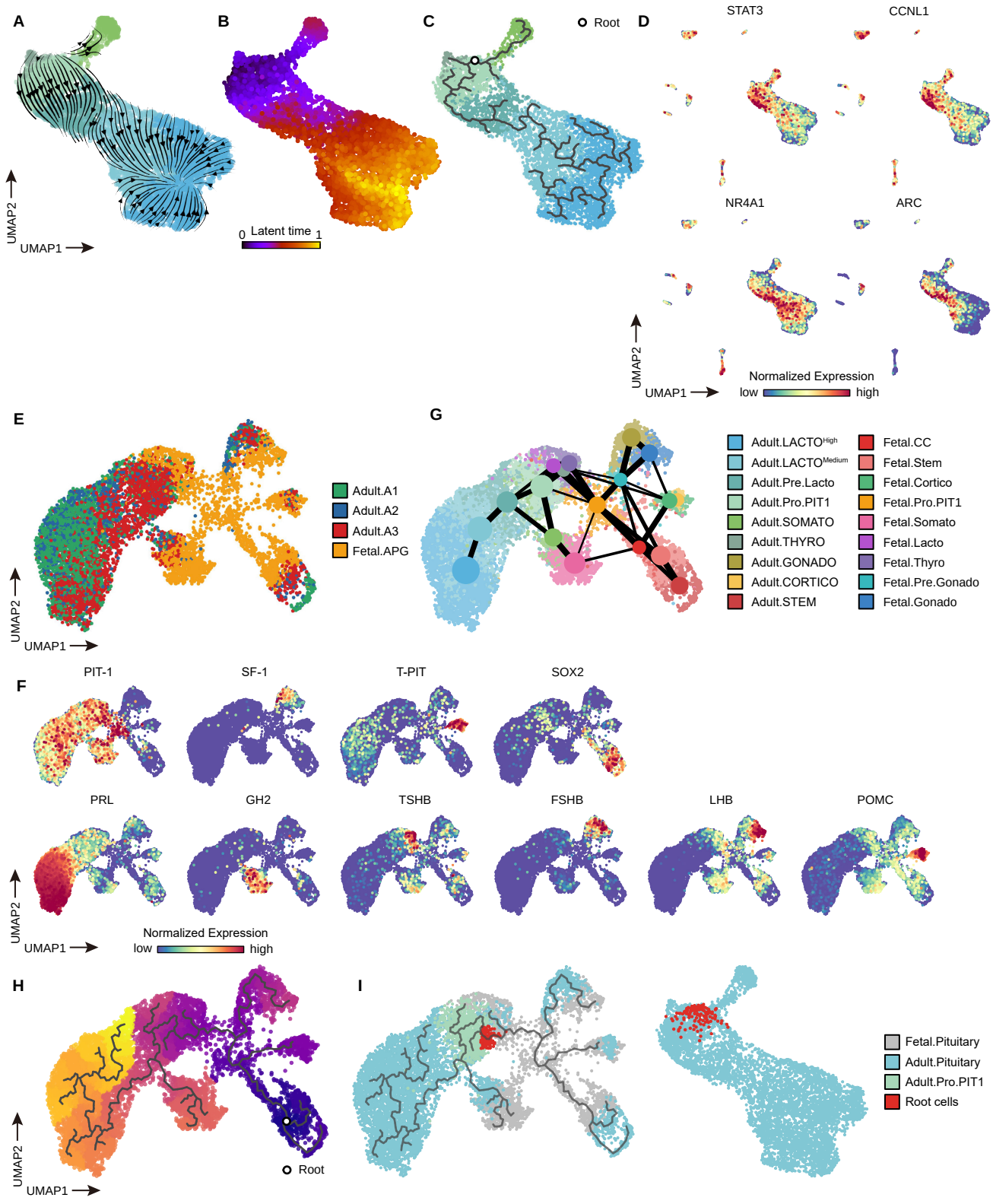


Figure S3. The cellular diversity within adult and fetal APGs. Related to Figure 2.

(A) RNA velocities of PIT1 lineage cells of APGs.

(B) Integrated UMAP plot of PIT1 lineage cells from APGs with cells colored by the latent time of scVelo.

(C) Integrated UMAP plot and Monocle3 developmental trajectories of PIT1 lineages of APGs. The root region was labeled by the circle.

(D) Common expressed genes among Pro.PIT1, Pre.Lacto and $LACTO^{Medium}$ cells.

(E) Integrated UMAP plot of the distribution of adult and fetal APG samples.

(F) Lineage and hormone gene expression of integrated adult and fetal APG endocrine cells.

(G) Partition-based graph abstraction (PAGA) connectivity among clusters of integrated adult and fetal APGs based on scRNA-seq data.

(H) Integrated UMAP plot and Monocle3 developmental trajectories of adult and fetal APG samples colored by the pseudo-time of Monocle3. The root region was labeled by the circle.

(I) Integrated Monocle3 analysis reveals the root cells in Adult.Pro.PIT1. left, integrated UMAP plot and Monocle3 developmental trajectories of adult and fetal APG samples, with root cells manually selected in Adult.Pro.PIT1 according to the entrance region of trajectories. Right, integrated UMAP plot of adult PIT1 lineage with root cells mapping from the left panel.

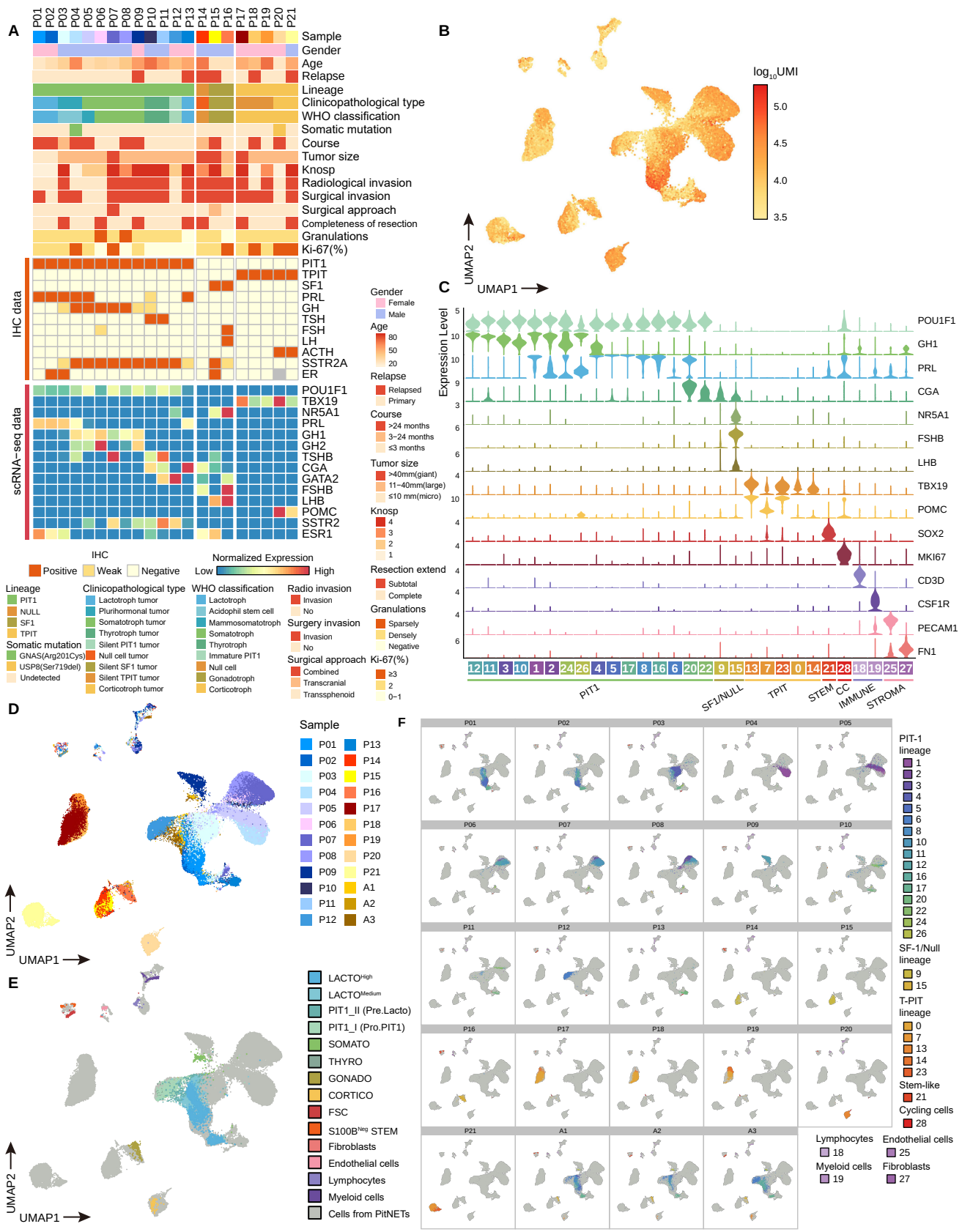

A
P01 P02 P03 P04 P05 P06 P07 P08 P09 P10 P11 P12 P13 P14 P15 P16 P17 P18 P19 P20 P21
Sample
Gender
Age
Relapse
Lineage
Clinicopathological type
WHO classification
Somatic mutation
Course
Tumor size
Knosp
Radiological invasion
Surgical invasion
Surgical approach
Completeness of resection
Granulations
Ki-67(%)
IHC data
PIT1
TPIT
SF1
PRL
GH
TSH
FSH
LH
ACTH
SSTR2A
ER
scRNA-seq data
POU1F1
TBX19
NR5A1
PRL
GH1
GH2
TSHB
CGA
GATA2
FSHB
LHB
POMC
SSTR2
ESR1
Gender
Female
Male
Age
80
50
20
Relapse
Relapsed
Primary
Course
>24 months
3–24 months
≤3 months
Tumor size
>40mm(giant)
11–40mm(large)
≤10 mm(micro)
Knosp
4
3
2
1
Resection extend
Subtotal
Complete
Granulations
Sparsely
Densely
Negative
Ki-67(%)
≥3
2
0–1
IHC
Positive
Weak
Negative
Normalized Expression
Low
High
Lineage
PIT1
NULL
SF1
TPIT
Somatic mutation
GNAS(Arg201Cys)
USP8(Ser719del)
Undetected
Clinicopathological type
Lactotroph tumor
Plurihormonal tumor
Somatotroph tumor
Thyrotroph tumor
Silent PIT1 tumor
Null cell tumor
Silent SF1 tumor
Silent TPIT tumor
Corticotroph tumor
WHO classification
Lactotroph
Acidophil stem cell
Mammosomatotroph
Somatotroph
Thyrotroph
Immature PIT1
Null cell
Gonadotroph
Corticotroph
Radio invasion
Invasion
No
Surgery invasion
Invasion
No
Surgical approach
Combined
Transcranial
Transsphenoid
B
log10UMI
5.0
4.5
4.0
3.5
UMAP2
UMAP1
C
Expression Level
POU1F1
GH1
PRL
CGA
NR5A1
FSHB
LHB
TBX19
POMC
SOX2
MKI67
CD3D
CSF1R
PECAM1
FN1
12 11 3 10 1 2 24 26 4 5 17 8 16 6 20 22 9 15 13 7 23 0 14 21 28 18 19 25 27
PIT1
SF1/NULL
TPIT
STEM
CC
IMMUNE
STROMA
D
Sample
P01 P02 P03 P04 P05 P06 P07 P08 P09 P10 P11 P12
P13 P14 P15 P16 P17 P18 P19 P20 P21 A1 A2 A3
UMAP2
UMAP1
E
LACTOHigh
LACTOMedium
PIT1_II (Pre.Lacto)
PIT1_I (Pro.PIT1)
SOMATO
THYRO
GONADO
CORTICO
FSC
S100BNeg STEM
Fibroblasts
Endothelial cells
Lymphocytes
Myeloid cells
Cells from PitNETs
UMAP2
UMAP1
F
P01 P02 P03 P04 P05
P06 P07 P08 P09 P10
P11 P12 P13 P14 P15
P16 P17 P18 P19 P20
P21 A1 A2 A3
PIT-1 lineage
1 2 3 4 5 6 8 10 11 12 16 17 20 22 24 26
SF-1/Null lineage
9 15
T-PIT lineage
0 7 13 14 23
Stem-like
21
Cycling cells
28
Lymphocytes
18
Endothelial cells
25
Myeloid cells
19
Fibroblasts
27

Figure S4. Characteristics of PitNET clusters. Related to Figure 3.

(A) Heatmap showing the detailed clinical characteristics, IHC intensities, and corresponding normalized gene expression in scRNA-seq data of all 21 patients. These clinical characteristics data include gender, age, relapse status, tumor lineage, tumor clinicopathological type, tumor WHO classification, course of disease, tumor size, Knosp grade, radiological invasion status, surgical invasion status, surgical approach, completeness of resection, and pathological results of granulations and Ki-67, which were indicated as different colors.

(B) Integrated UMAP plot of the UMI counts in 21 PitNETs.

(C) Violin plot showing the expression of marker genes in PitNETs. CC, cycling cells.

(D) Integrated UMAP visualization of cells from 21 PitNETs (P01-P21) and three APGs (A1-A3) samples colored by sample origins.

(E) Integrated UMAP visualization of cells from 21 PitNETs (P01-P21) and three APGs (A1-A3) samples. Cells from APGs were colored by APG cell clusters in **Figure 1B**.

(F) Each UMAP visualization of cells from one of 21 PitNETs (P01-P21) and three APGs (A1-A3) samples colored by cluster in **Figure 3A**.

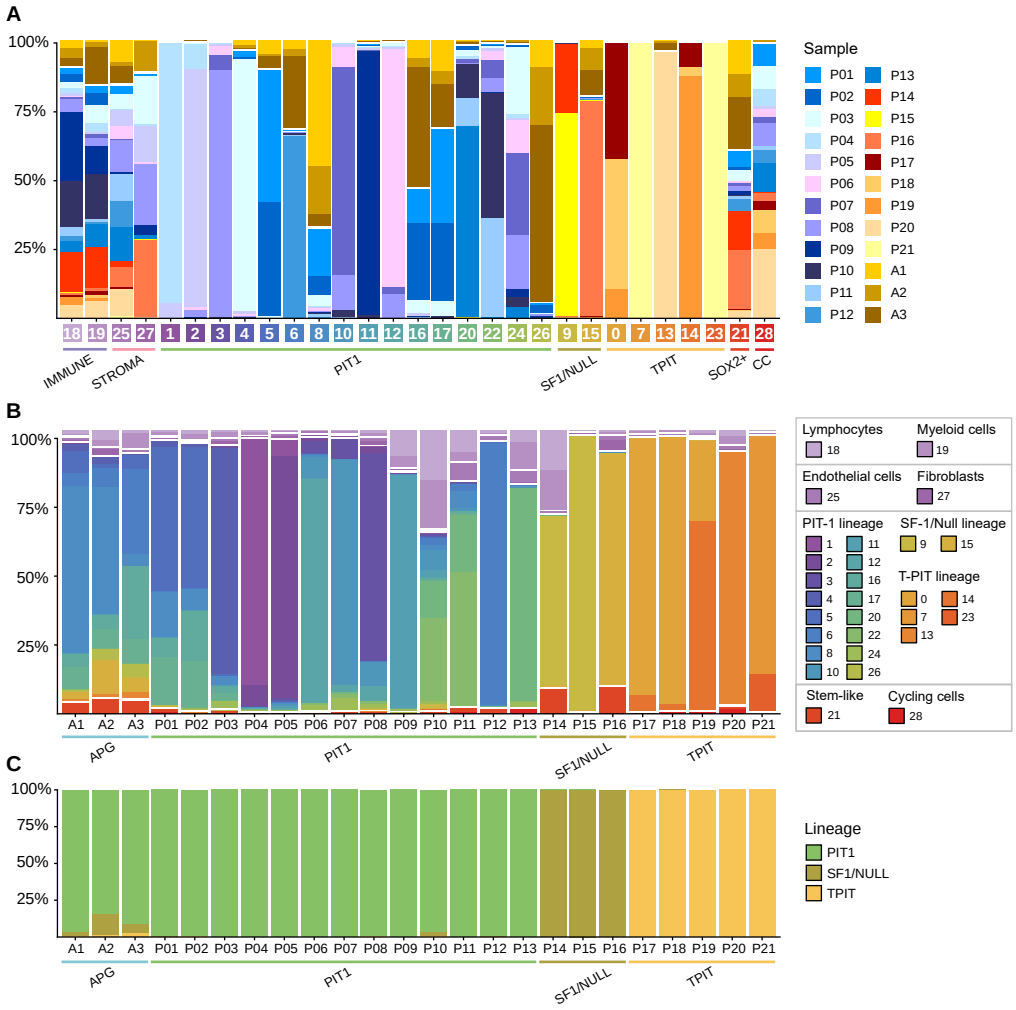


Figure S5. Composition of samples and clusters in the integrated UMAP of APGs and PitNETs, related to Figure 3.

(A) Bar plot visualization of sample composition within each cluster. The cells from APG (A1-A3) and cells from tumor samples (P01-P21) were sequentially arranged with intervals. The general cell class/lineage of each cluster was annotated below. CC, cycling cells.

(B) Bar plot visualization of cluster composition of all filtered cells within each sample. The immune cell cluster (Lymphocytes and Myeloid cells), stroma cell cluster (Endothelial cells and fibroblasts), tumor/APG endocrine cell clusters (PIT-1 lineage, SF-1/Null lineage, and T-PIT lineage), and Stem/CC cell clusters (Stem-like and Cycling cells) were sequentially arranged with interval. The pathological lineage of each tumor/APG was annotated below.

(C) Bar plot visualization of cell lineage composition of all tumor/APG endocrine cells within each sample. The pathological lineage of each tumor/APG was annotated below.

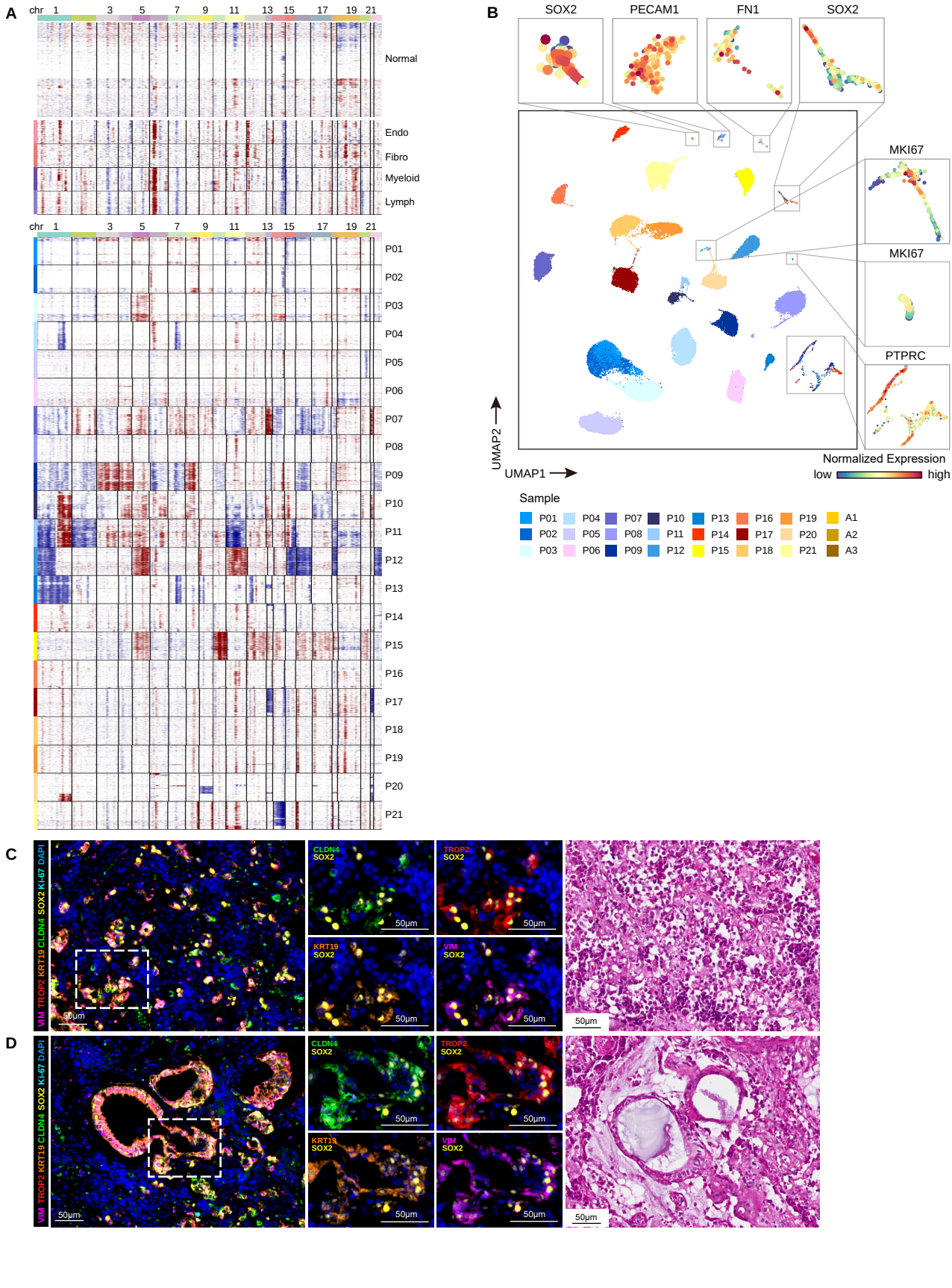

A
chr 1 3 5 7 9 11 13 15 17 19 21
Normal
Endo
Fibro
Myeloid
Lymph
P01
P02
P03
P04
P05
P06
P07
P08
P09
P10
P11
P12
P13
P14
P15
P16
P17
P18
P19
P20
P21
B
SOX2
PECAM1
FN1
SOX2
MKI67
MKI67
PTPRC
UMAP2
UMAP1
Normalized Expression
low
high
Sample
C
D
DAPI
Ki-67
SOX2
CLDN4
KRT19
TROP2
VIM
50μm

Figure S6. Characterization of inter-tumoral heterogeneity and tumor stem-like cells in PitNET, related to Figure3.

(A) Heatmap showing inferred large-scale CNVs of endocrine and stem cells from APGs (Normal), tumor endothelium (Endo), tumor fibroblast (Fibro), tumor myeloid cells (Myeloid), tumor lymphocytes (Lymph), and each patient's tumor cells (P01-P21).

(B) Merged UMAP plot of all cells merged from tumor samples after excluding suspicious APG cells. Clusters composed of multiple tumor samples were annotated with canonical markers representing their cell types.

(C-D) Left, mIHC staining of VIM, TROP2, KRT19, CLDN4, SOX2, and Ki-67 in sections from PitNETs. Tissues were counterstained with DAPI. Right, corresponding region stained by Hematoxylin and Eosin (HE). Scale bar: 50 μm.

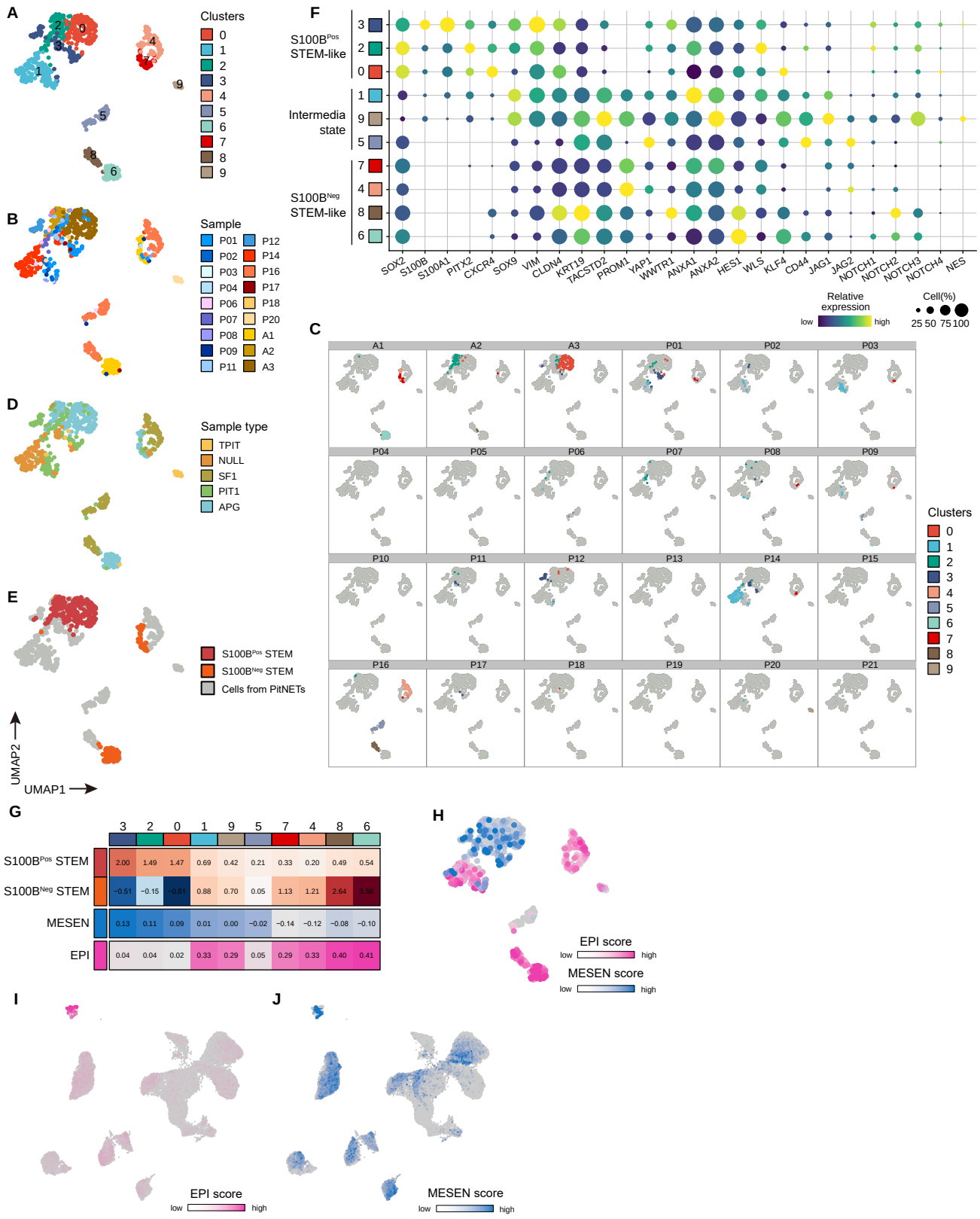


Figure S7. The EMT state of SOX2 expressing cells in PitNETs and APGs. Related to Figure 3.

(A) Merged UMAP plot of all 750 SOX2 expressing cells from three APGs and 21 PitNETs with cells colored by clusters.
(B) Merged UMAP plot of the distribution of SOX2 expressing cells from PitNETs and APGs samples. Six tumors (P05, P10, P13, P15, P19, and P21) were not shown due to not containing any tumor stem-like cells.
(C) Each UMAP visualized APGs stem cells or tumor stem-like cells from one of 21 PitNETs (P01-P21) and three APGs (A1-A3) samples colored by cluster in **Figure S7A**.
(D) Merged UMAP plot of SOX2 expressing cells colored by sample types.
(E) Merged UMAP plot of SOX2 expressing cells colored by APG cluster in **Figure 1B**.
(F) Dot plot of marker genes in re-grouped SOX2 expressing cells. The color represents the scaled relative expression level of the marker genes in each cell type, and the size indicates the proportion of cells expressing the marker genes.
(G) The heatmap shows each re-grouped SOX2-expressing cluster of PitNETs (columns) scored by $S100B^{Pos}$ STEM and $S100B^{Neg}$ STEM in the APGs, and epithelial (EPI) and mesenchymal (MESEN) scores (rows). The cells from APGs were excluded from re-grouped SOX2-expressing clusters.
(H) Merged UMAP plot of SOX2 expressing cells from PitNETs and APGs with cells colored by epithelial (EPI) and mesenchymal (MESEN) scores.
(I-J) UMAP plot of PitNETs and APGs. Cells were colored by EPI (I) and MESEN (J) scores.

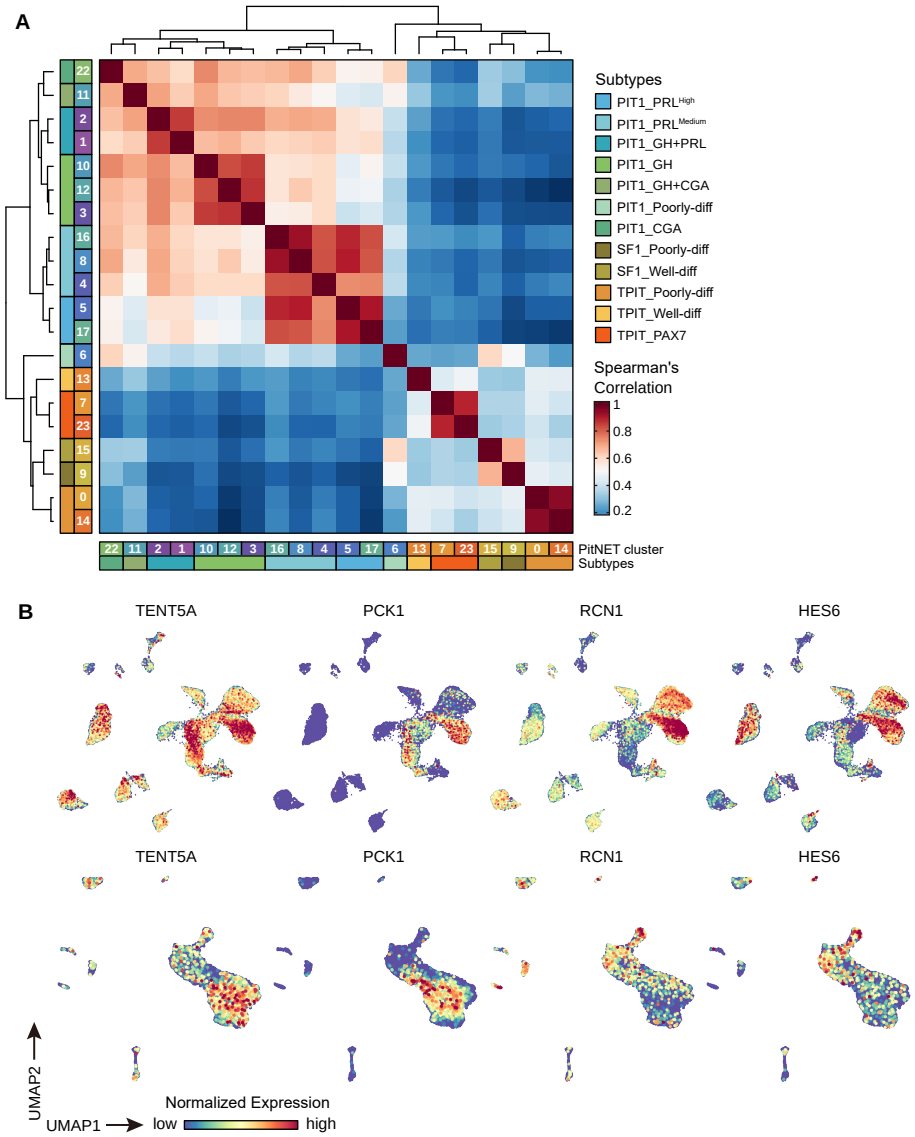


Figure S8. The clusters in each subtype according to tumor origin showed similar molecular features. Related to Figure 3.

(A) Heatmap showing similarity based on Spearman's correlation between PitNET clusters.

(B) Common gene expression of PIT1_GH+PRL with LACTOHigh (TENT5A and PCK1) and SOMATO (HES6 and RCN1) clusters.

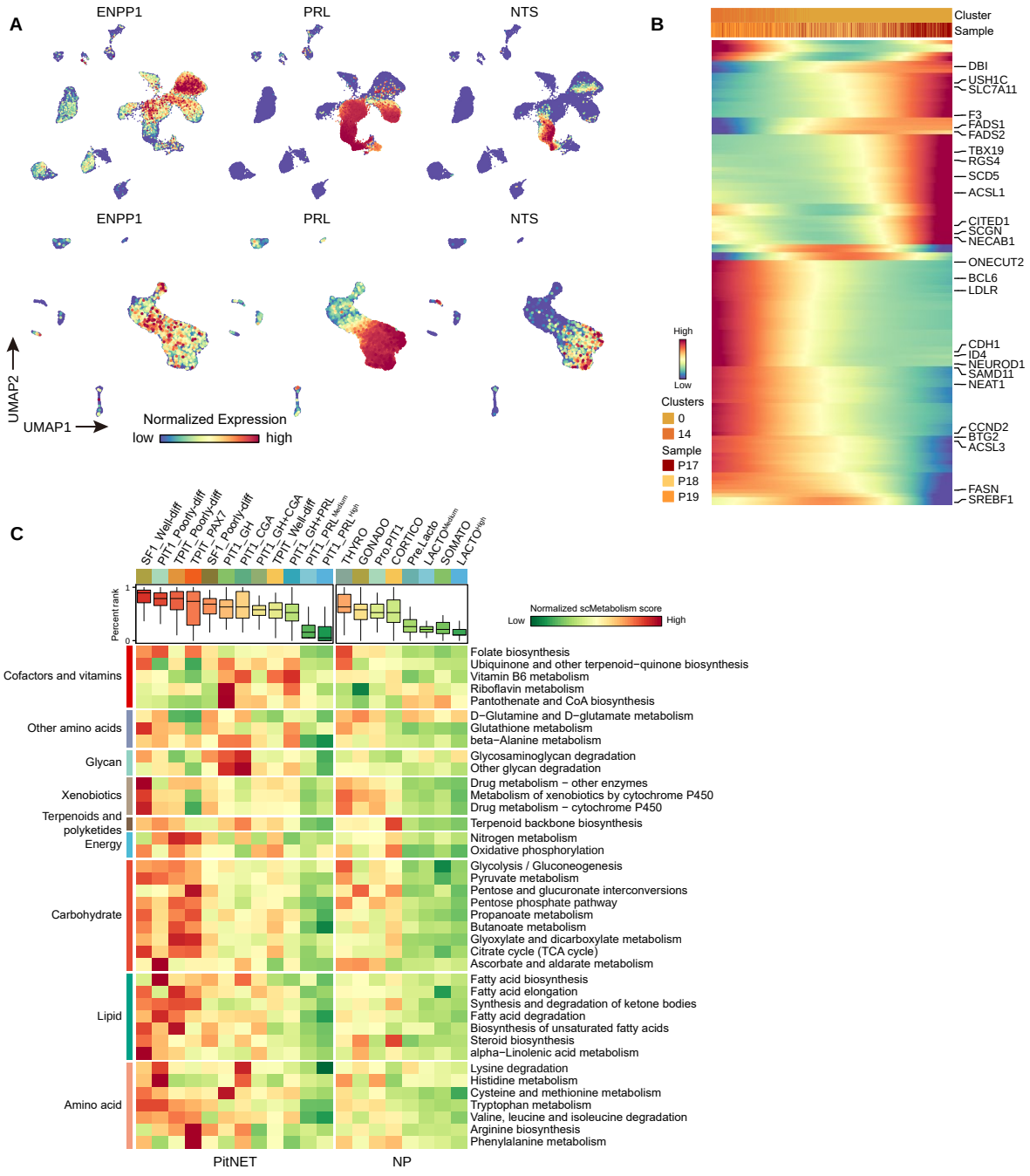


Figure S9. Molecular features of PIT1_PRL^{High},TPIT_Poorly-diff, and metabolic alterations in PitNETs and APGs. Related to Figure 4.

(A) Gene expression of DLK1, PRL, and NTS in PitNETs.

(B)A heatmap showing the relative expression levels of the marker genes of TPIT_Poorly-diff along the latent time axis.

(C)The metabolic activity analysis of PitNETs and APGs. The top 50% of metabolic pathways based on the standard error of the enrichment score are plotted.

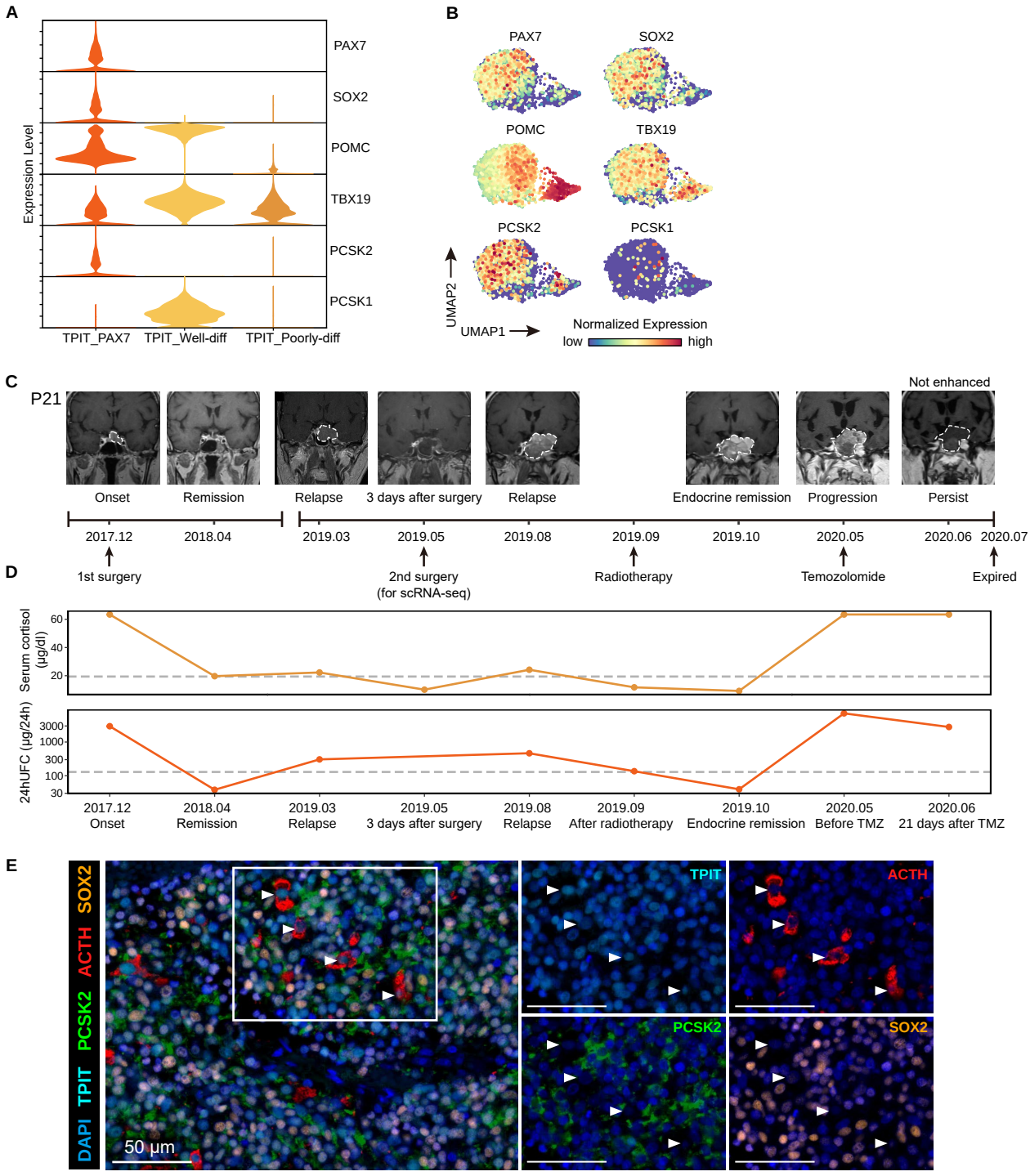


Figure S10. Molecular features and clinical characteristics of TPIT_PAX7. Related to Figure 4.

(A) Violin plot showing the expression of marker genes in TPIT lineage PitNETs.

(B) Gene expression of PAX7, SOX2, POMC, TBX19, PCSK2, and PCSK1 in TPIT_PAX7 subtype.

(C) Diagram of the diagnosis, treatment, and relapse timeline of P21. Magnetic resonance imaging (MRI) images of each key point were displayed above the timeline. Tumor borders were outlined by white dashed lines.

(D) Serum cortisol and 24h urinary free cortisol (24hUFC) along the treatment process of P21.

(E) mIHC staining of TPIT, PCSK2, ACTH and SOX2 in sections from P21. Tissues were counterstained with DAPI. Arrowheads indicate cells with ACTH-positive and SOX2-negative. Scale bar: 50 μm.

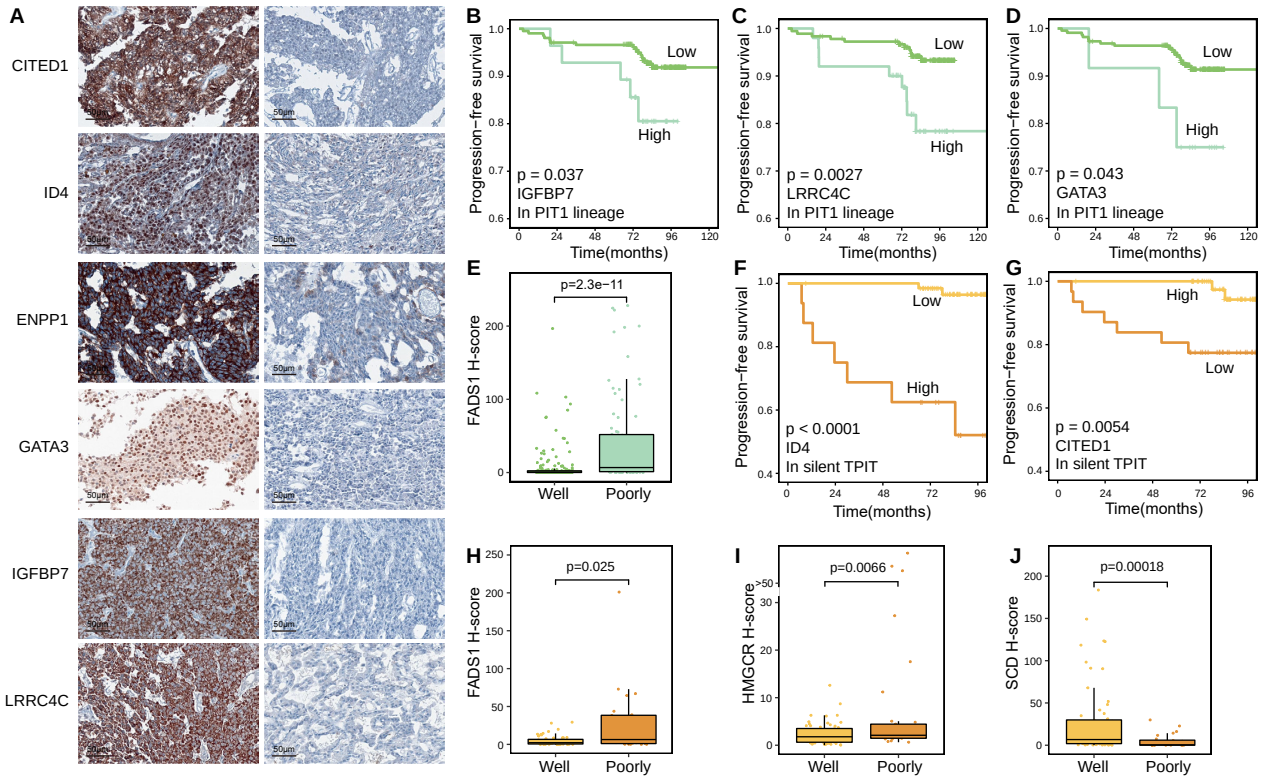


Figure S11. The clinical characterization, diagnostic markers, and molecular features of well and poorly differentiated PitNETs. Related to Figure 5.

(A) The IHC micrograph of novel markers for differentiation status classification.

(B-D) Kaplan–Meier PFS curves for patients of PIT1 lineage tumor stratified by the H-score of IGFBP7 (B), LRRC4C (C), and GATA3 (D). The p value was calculated by the log-rank test.

(E) Box plot of the FADS1 H-score in well and poorly differentiated PIT1 tumors. The p value was calculated by the Wilcoxon rank-sum test.

(F-G) Kaplan–Meier PFS curves for patients of silent TPIT tumor stratified by the H-score of ID4 (F), CITED1 (G). The p value was calculated by the log-rank test.

(H-J) Box plot of the FADS1 H-score (H), HMGCR H-score (I), and SCD H-score (J) in well and poorly differentiated silent TPIT tumors. The p values were calculated by the Wilcoxon rank-sum test.