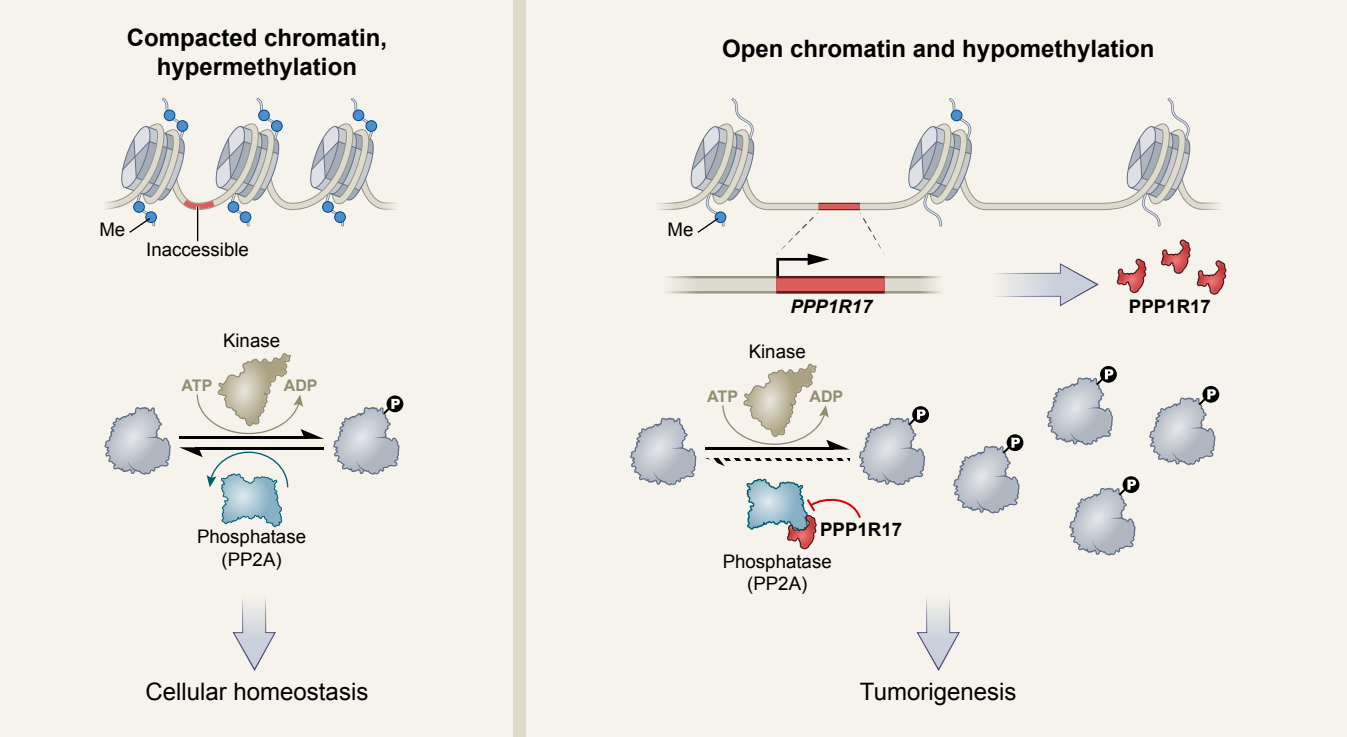
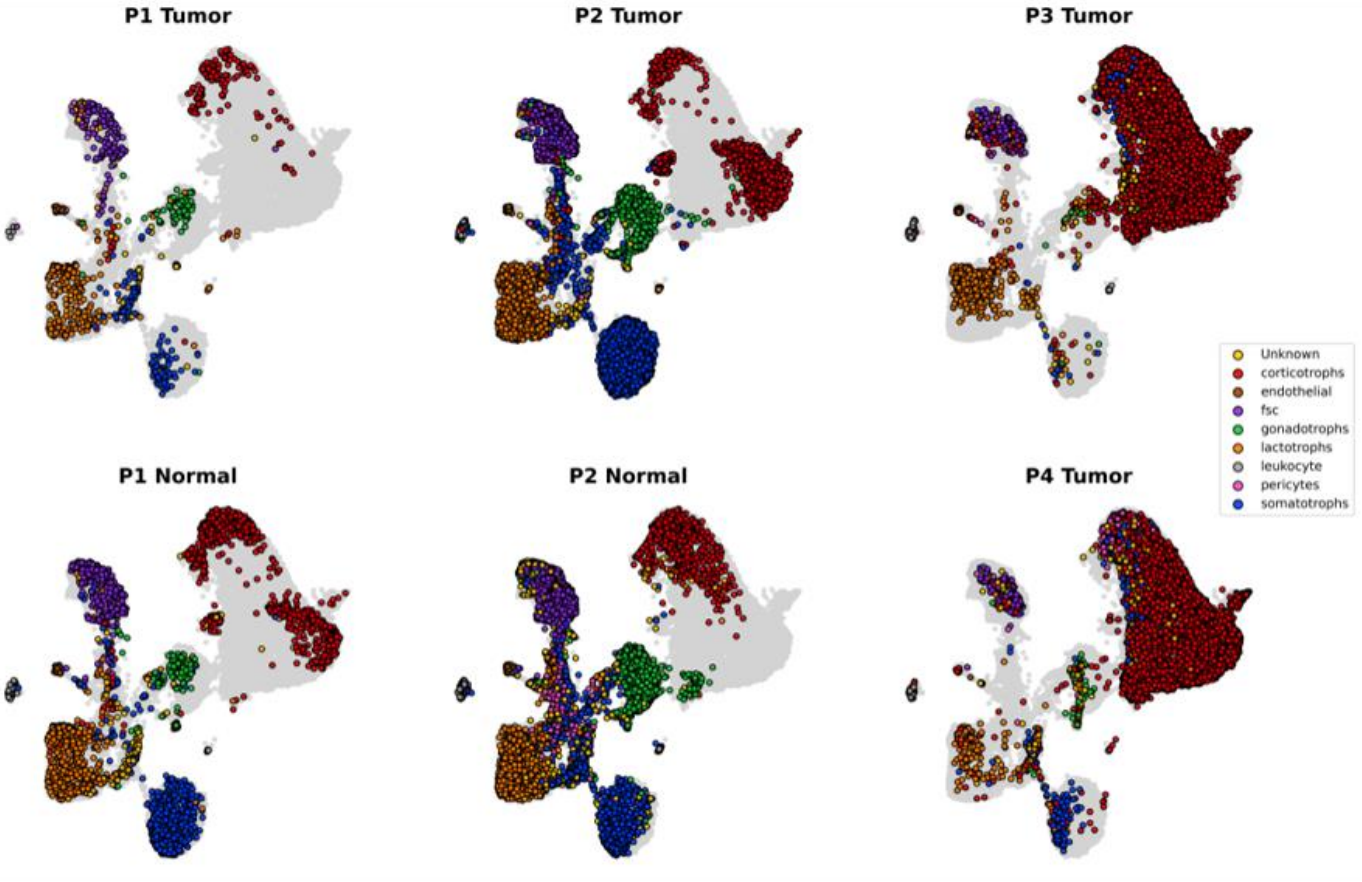
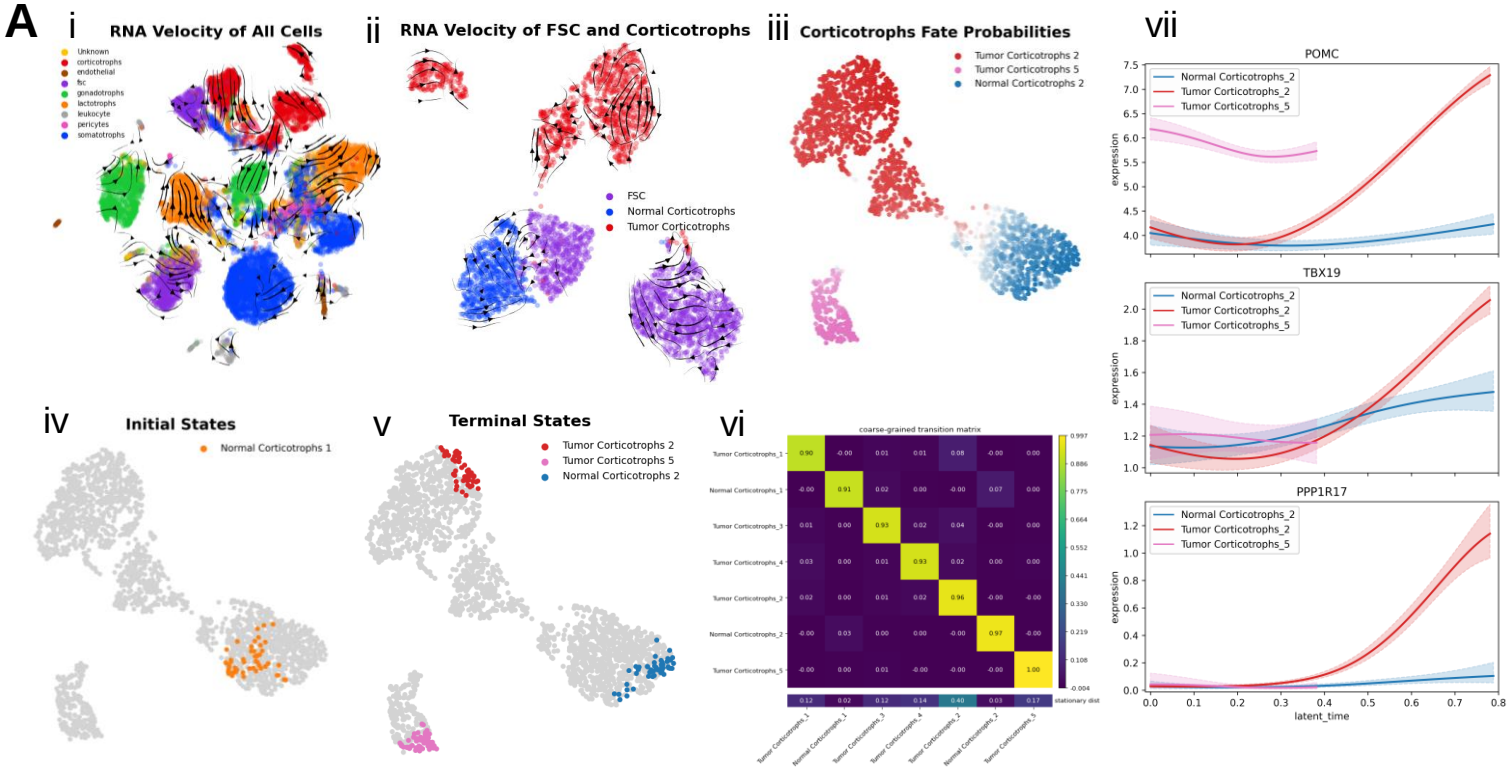
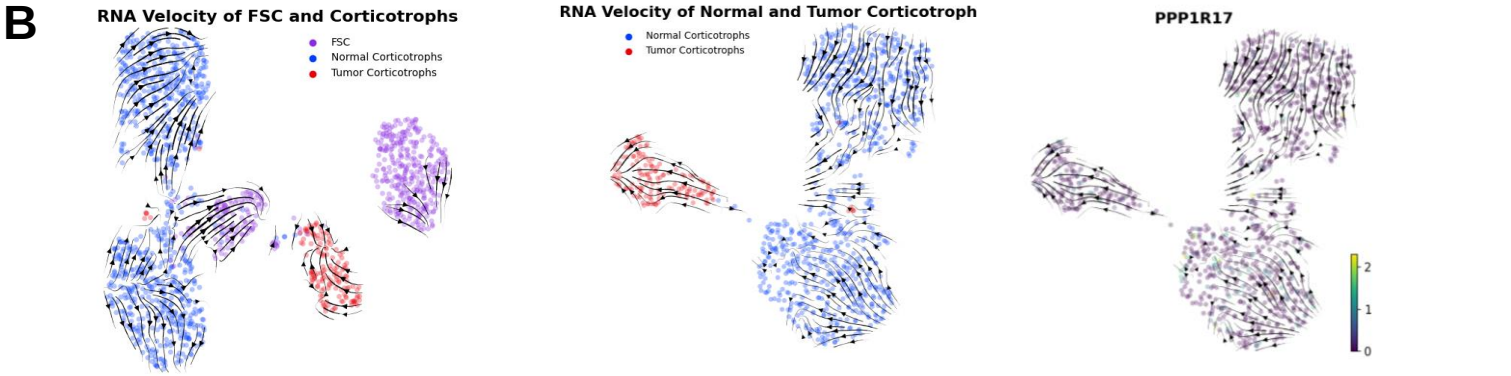
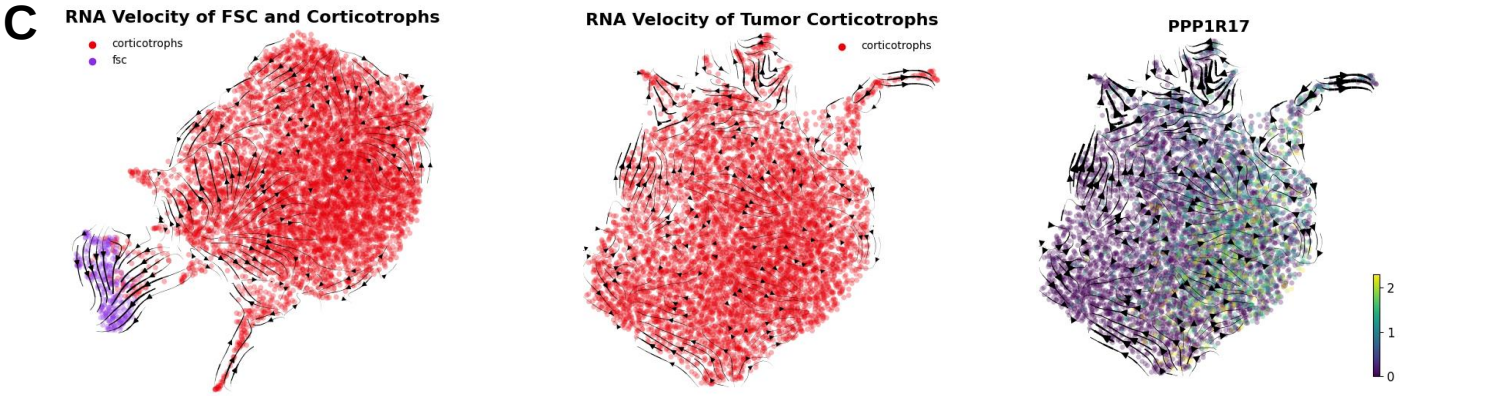

Compacted chromatin, hypermethylation
Me
Inaccessible
Kinase
ATP
ADP
P
Phosphatase (PP2A)
Cellular homeostasis
Open chromatin and hypomethylation
Me
PPP1R17
PPP1R17
Kinase
ATP
ADP
P
PPP1R17
Phosphatase (PP2A)
Tumorigenesis

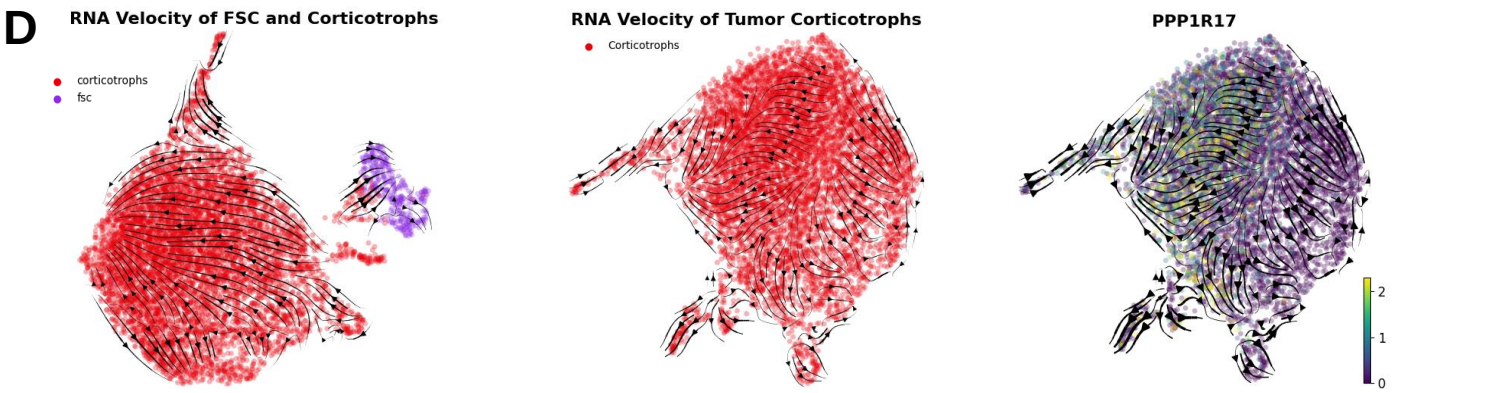


Supplemental Fig S1. UMAPs of canonical cell types in each individual tumor sample. Refer to Fig 1d for aggregate figure.

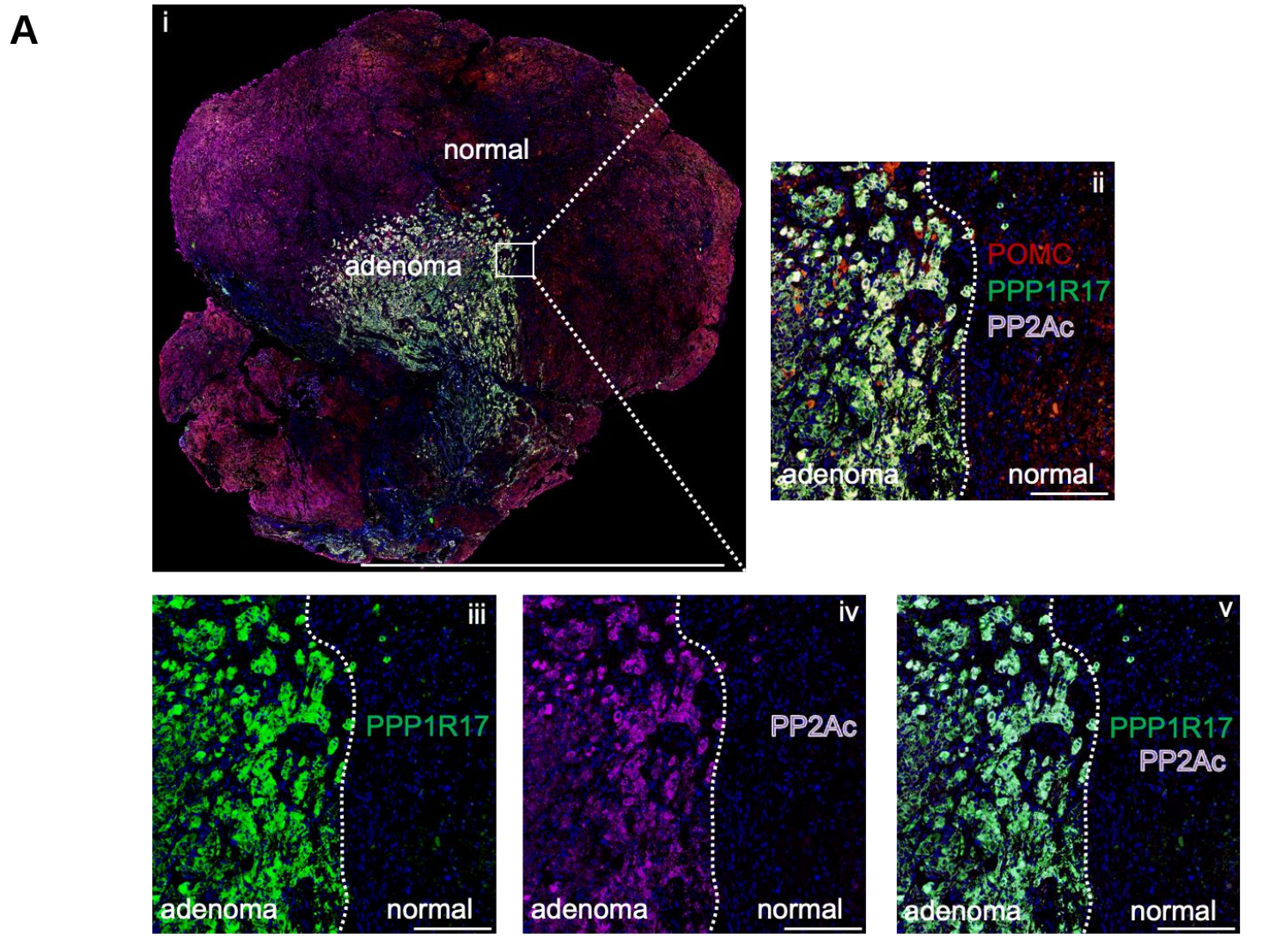

A
i
RNA Velocity of All Cells
Unknown
corticotrophs
endothelial
fsc
gonadotrophs
lactotrophs
leukocyte
pericytes
somatotrophs
ii
RNA Velocity of FSC and Corticotrophs
FSC
Normal Corticotrophs
Tumor Corticotrophs
iii
Corticotrophs Fate Probabilities
Tumor Corticotrophs 2
Tumor Corticotrophs 5
Normal Corticotrophs 2
vii
POMC
TBX19
PPP1R17
Normal Corticotrophs_2
Tumor Corticotrophs_2
Tumor Corticotrophs_5
expression
latent_time
iv
Initial States
Normal Corticotrophs 1
v
Terminal States
Tumor Corticotrophs 2
Tumor Corticotrophs 5
Normal Corticotrophs 2
vi
coarse-grained transition matrix
stationary dist

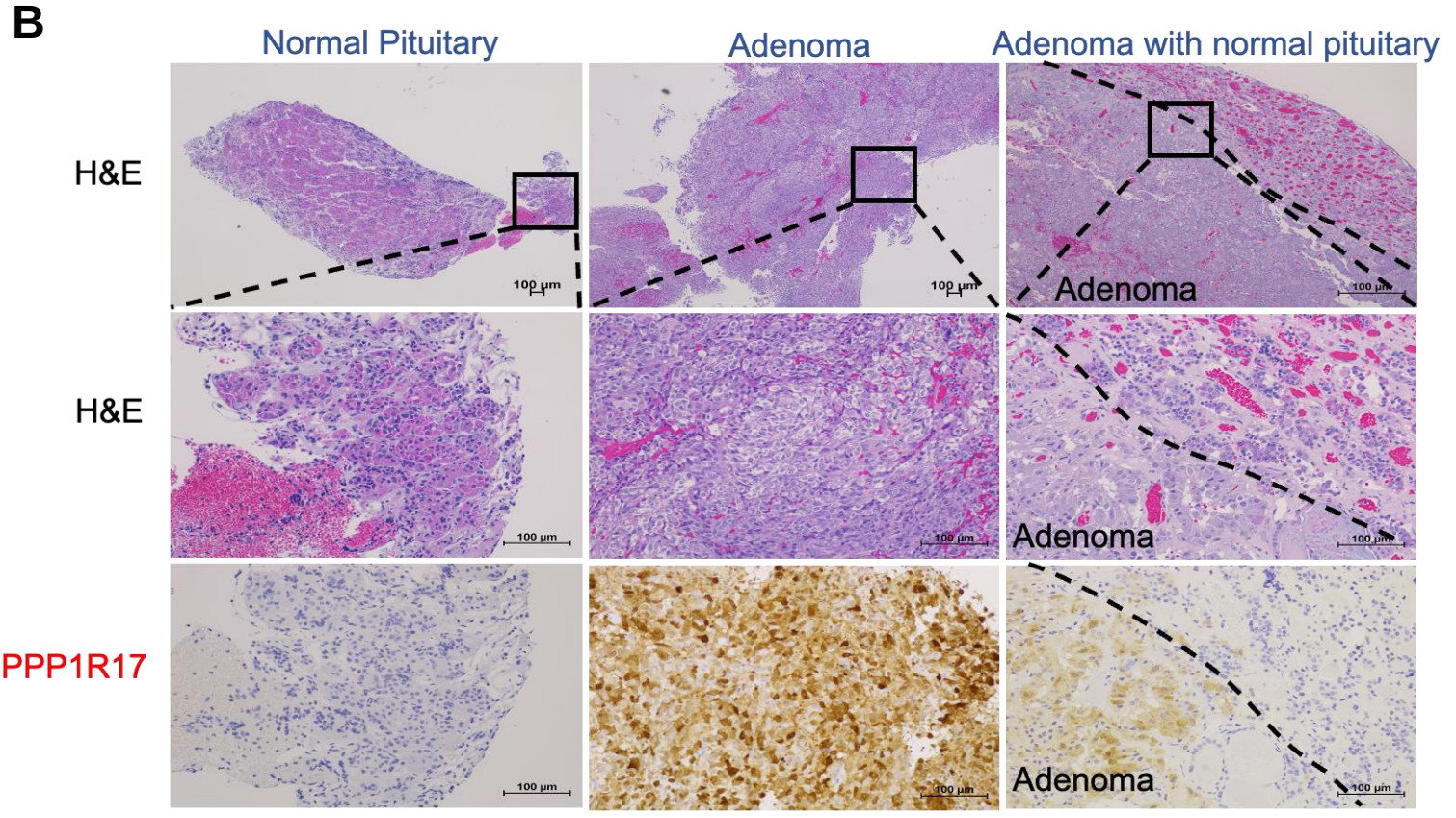

B
RNA Velocity of FSC and Corticotrophs
FSC
Normal Corticotrophs
Tumor Corticotrophs
RNA Velocity of Normal and Tumor Corticotroph
Normal Corticotrophs
Tumor Corticotrophs
PPP1R17


C
RNA Velocity of FSC and Corticotrophs
corticotrophs
fsc
RNA Velocity of Tumor Corticotrophs
corticotrophs
PPP1R17

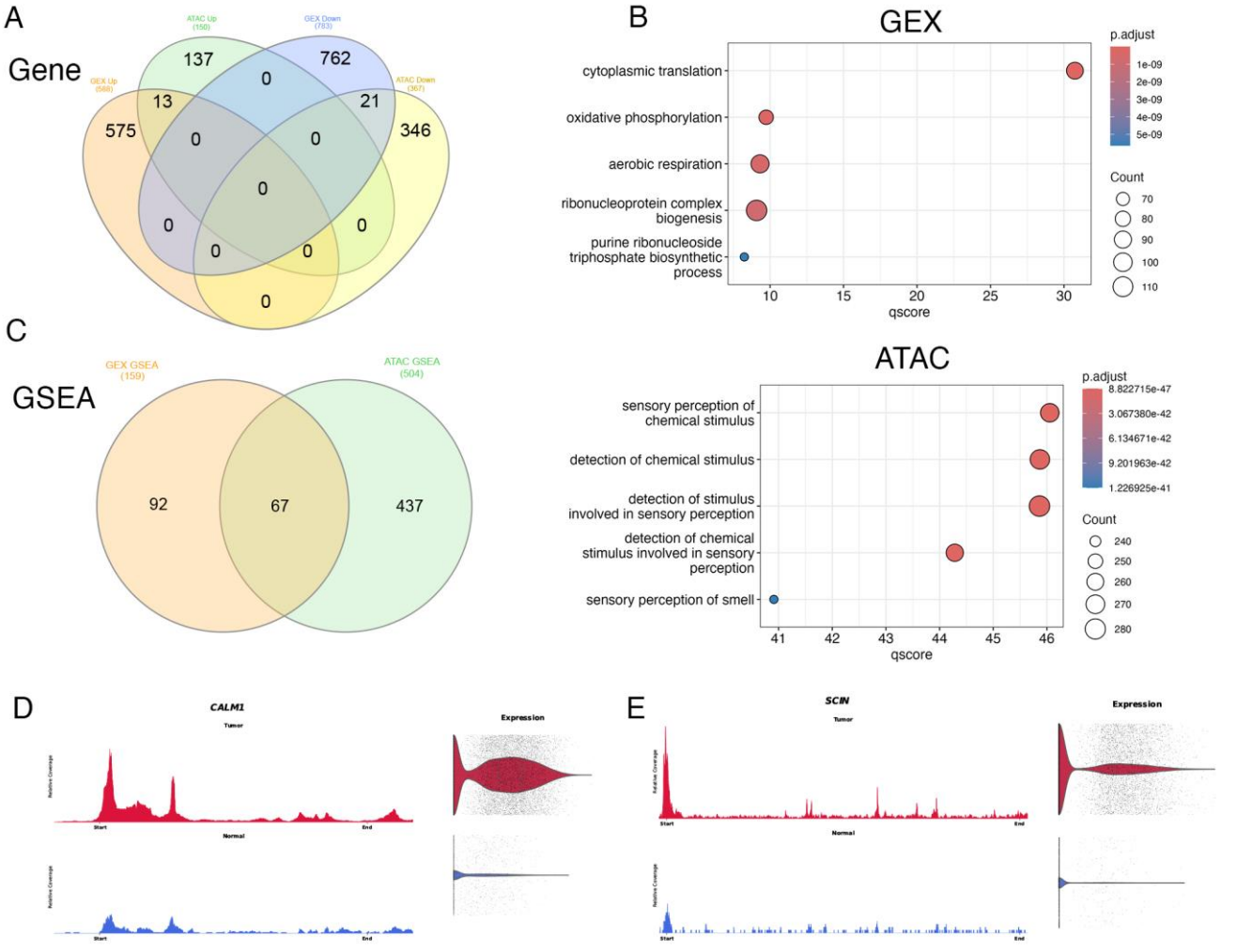

D
RNA Velocity of FSC and Corticotrophs
corticotrophs
fsc
RNA Velocity of Tumor Corticotrophs
Corticotrophs
PPP1R17

Supplemental Fig S2. Tumor corticotroph lineage tracing analysis. **A.** CellRank and scVelo analysis of patient sample P2. **i.** scVelo velocities projected onto UMAP embedding of all cells in both tumor and normal compartments colored by cell type. **ii.** scVelo velocities projected onto UMAP embedding of tumor corticotrophs, normal corticotrophs, and folliculostellate cells (FSC). **iii.** UMAP of corticotrophs from tumor and normal compartments. Cells are colored by CellRank fate probabilities, which is the likelihood that a given cell will transition toward the terminal population. **iv.** CellRank identified initial cell states. **v.** CellRank identified terminal cell states. **vi.** Transition matrix showing the fate probabilities of each macrostate. **vii.** Relative expression of relevant genes in each macrostate plotted over latent time. **B.** CellRank and scVelo analysis of patient sample P1. scVelo velocities projected onto UMAP embedding of tumor corticotrophs, normal corticotrophs, and FSCs (left), scVelo velocities projected onto UMAP embedding of tumor and normal corticotrophs (center), scVelo velocities projected onto UMAP embedding of tumor and normal corticotrophs with cells colored by relative *PPP1R17* expression (center). **C.** CellRank and scVelo analysis of patient sample P3. scVelo velocities projected onto UMAP embedding of tumor corticotrophs and FSCs (left), scVelo velocities projected onto UMAP embedding of only corticotrophs (center), scVelo velocities projected onto UMAP embedding of corticotrophs with cells colored by relative *PPP1R17* expression (center). **D.** CellRank and scVelo analysis of patient sample P3. scVelo velocities projected onto UMAP embedding of tumor corticotrophs and FSCs (left), scVelo velocities projected onto UMAP embedding of only corticotrophs (center), scVelo velocities projected onto UMAP embedding of corticotrophs with cells colored by relative *PPP1R17* expression (center).

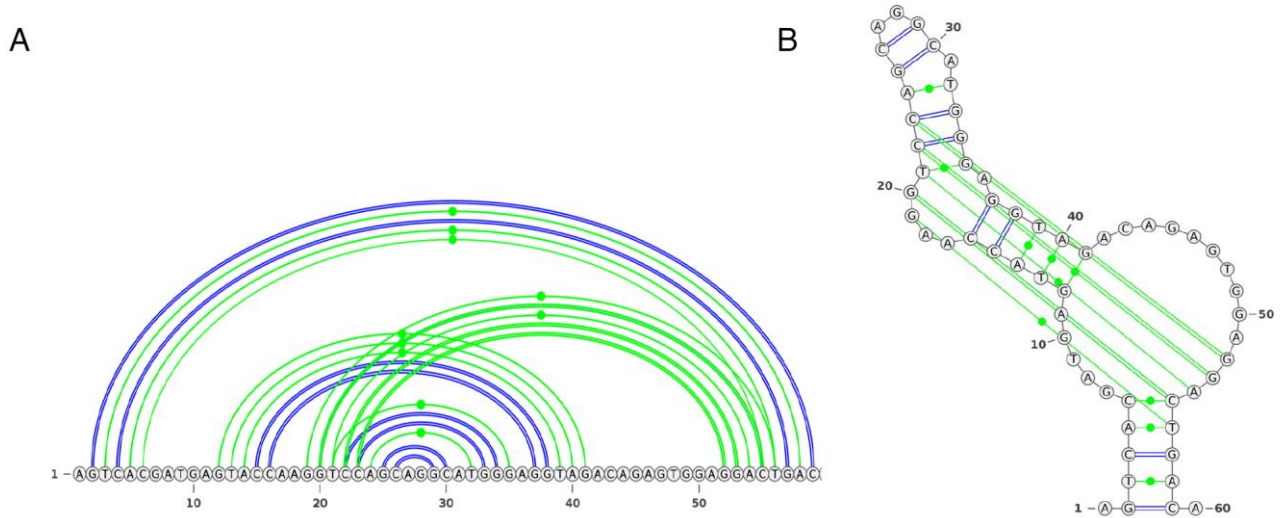

A
i
normal
adenoma
ii
POMC
PPP1R17
PP2Ac
adenoma
normal
iii
PPP1R17
adenoma
normal
iv
PP2Ac
adenoma
normal
v
PPP1R17
PP2Ac
adenoma
normal


B
Normal Pituitary
Adenoma
Adenoma with normal pituitary
H&E
H&E
PPP1R17
Adenoma
Adenoma
100 µm

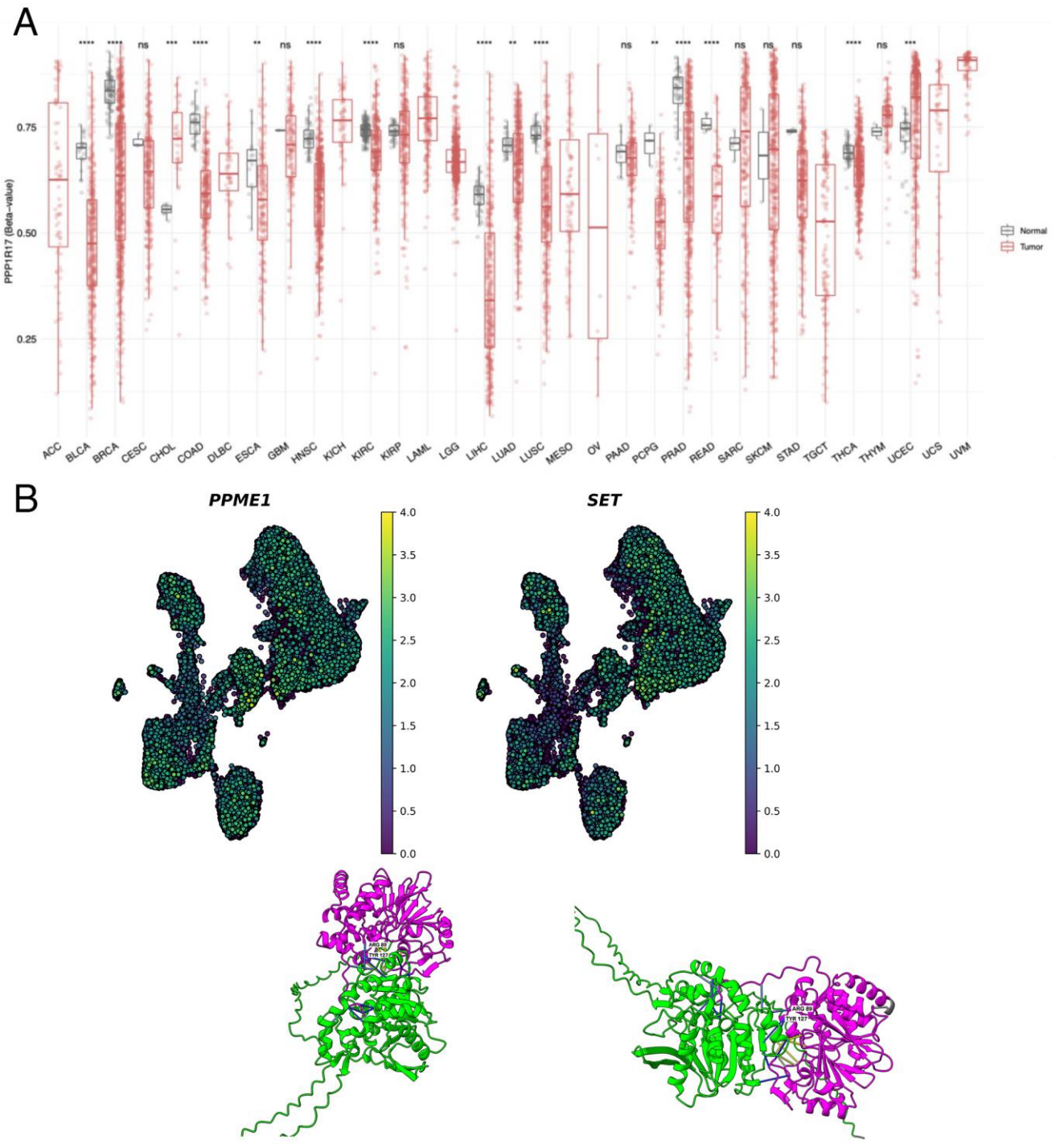
Supplemental Fig S3. PPP1R17 expression is confined to CD adenomas. **A.** Multiplex immunohistochemistry of CD adenoma and surrounding normal pituitary gland (i) showing PPP1R17 expression (green) compared to corticotroph-specific POMC expression (red). PP2Ac expression (purple) staining is compared to PPP1R17 staining within the adenoma (iii–v). Scale bar = 50μM. **B.** Representative H&E of normal pituitary gland and CD adenoma demonstrating PPP1R17 staining. CD = Cushing's disease. H&E = hematoxylin and eosin.



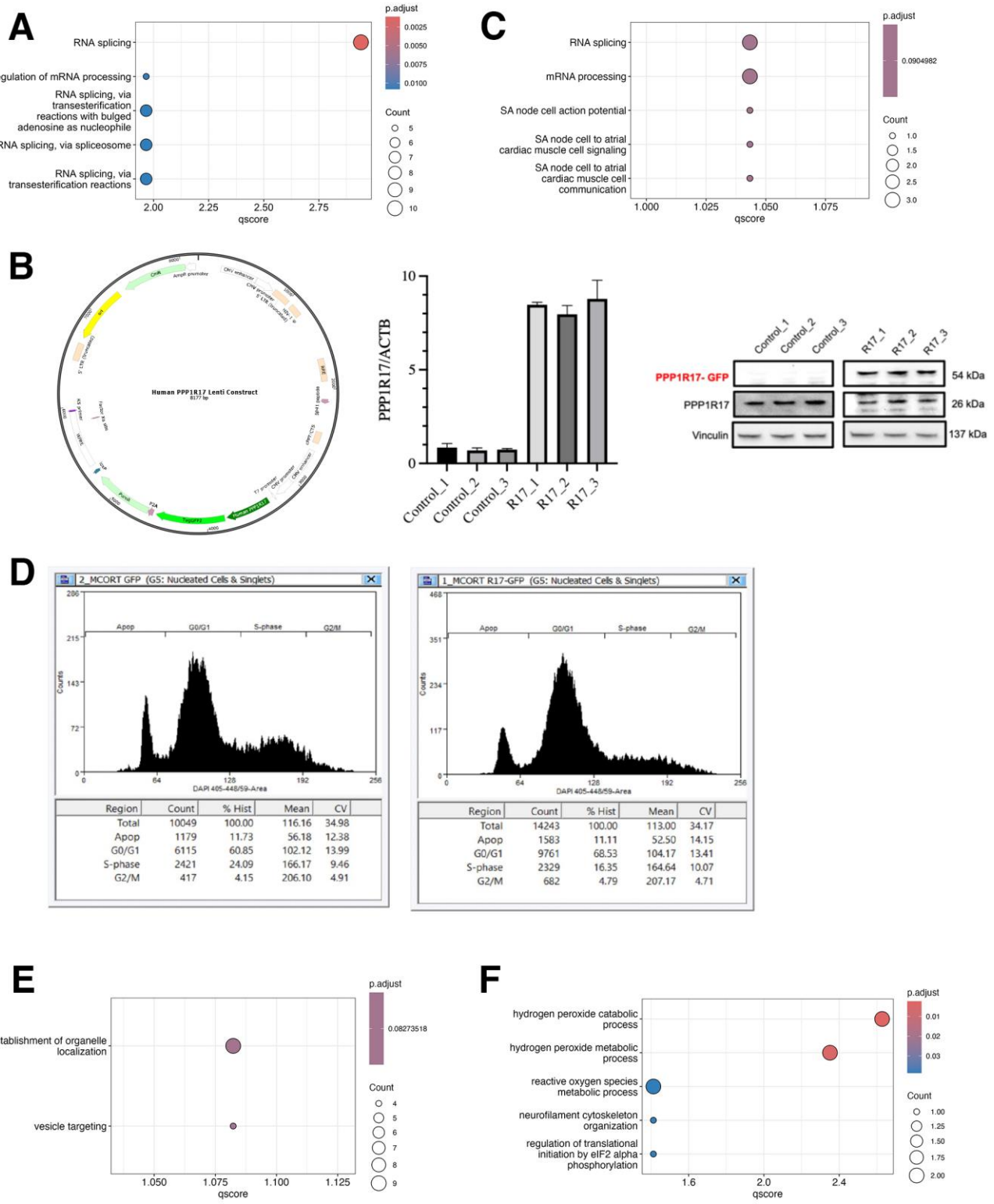
Supplemental Fig S4. Correlation between ATACseq accessibility and gene expression. **A-B.** Comparison of chromatin accessibility (left panels) and gene expression (right panels) of NF-Y target genes *CALM1* and *SPCS2* in CD corticotrophs (tumor) versus adjacent normal corticotrophs. **C.** Comparison of chromatin accessibility (left panels) and gene expression (right panels) of SP1 target gene *SCIN* in CD corticotrophs (tumor) versus adjacent normal corticotrophs. CD = Cushing's disease.



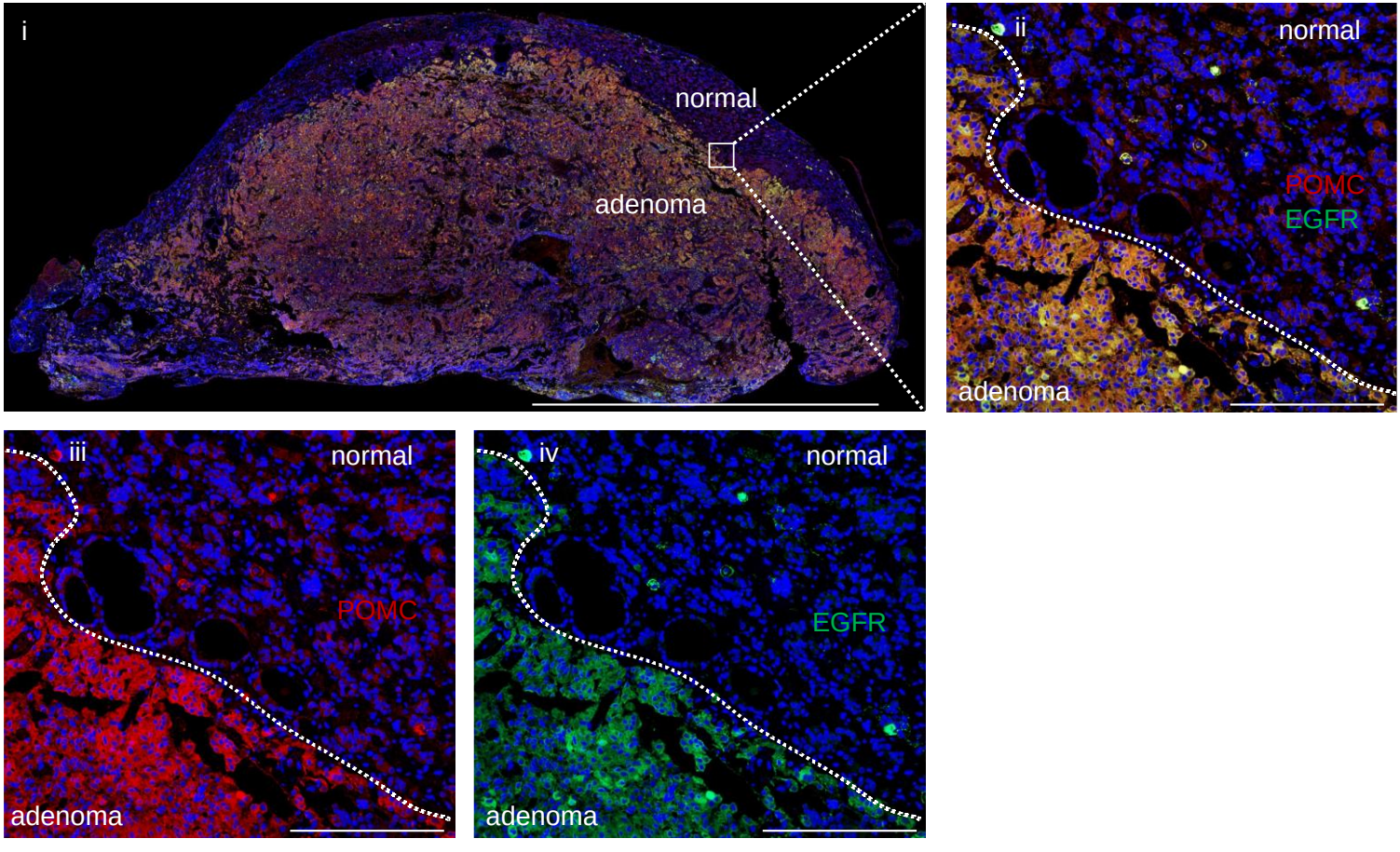
Supplemental Fig S5. The novel lncRNA PPP1R17-203 is transcribed from an internal *PPP1R17* site. **A.** Long-range interactions predicted for PPP1R17-203. **B.** Predicted 3D structure of PPP1R17-203. Source: https://bioinformaticslab.erc.monash.edu/linc2function



Supplemental Fig S6 Endogenous PP2A agonists are not transcriptionally overexpressed in CD. UMAP projections demonstrating expression patterns of *PPME1* and *SET* in *POMC*-overexpressing CD adenoma cells. UMAP = uniform manifold approximation and projection. CD = Cushing's disease.



Supplemental Fig S7. A. GSEA analysis of protein pathways with dysregulated phosphorylation in CD adenomas versus adjacent normal pituitary glands. Results of TNMT labeling and mass spectroscopy, n = 3/group. B. Lentiviral vector overexpressing *PPP1R17* tagged to *GFP*. Transient transfection in mCort cells was verified by qRT-PCR and by Western immunoblotting. C. GSEA analysis of protein pathways with dysregulated phosphorylation in stable mCort^{PPP1R17} versus mCort^{GFP} cells. Results of TNMT labeling and mass spectroscopy, n = 5/group. D. Cell cycle analysis of stable mCort^{PPP1R17} versus mCort^{GFP} cells. E GSEA analysis of protein pathways with differential protein abundance in CD adenomas versus adjacent normal pituitary glands. Results of TNMT labeling and mass spectroscopy, n = 3/group. F. GSEA analysis of protein pathways with differential protein abundance in stable mCort^{PPP1R17} versus mCort^{GFP} cells. Results of TNMT labeling and mass spectroscopy, n = 5/group.



Supplemental Fig S8. EGFR is overexpressed in CD. Representative mIHC image of whole-mounted CD adenoma and adjacent normal gland (i) with corticotroph-specific POMC expression. (ii-iv) EGFR expression (green) was compared to POMC expression (red). Scale bar = 200 µM.