

1 **SUPPLEMENTAL MATERIAL**

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3 Supplemental Table 1. ADPKD patient information.

4 Supplemental Table 2. Annotation of p-CREB binding peaks in DBA+ cells from
5 ADPKD kidney.

6 Supplemental Table 3. Comparison of differentially expressed genes in DBA+ cells
7 from WT and ADPKD mice treated with or without 666-15.

8 Supplemental Figure 1. Phosphorylation of CREB increases steadily in ADPKD
9 kidneys.

10 Supplemental Figure 2. Comparison of p-CREB CUT&RUN and ChIP-seq.

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12 Supplemental Figure 4. Function enrichment analysis of p-CREB binding genes.

13 Supplemental Figure 5. Inhibition of CREB transactivity by 666-15 treatment.

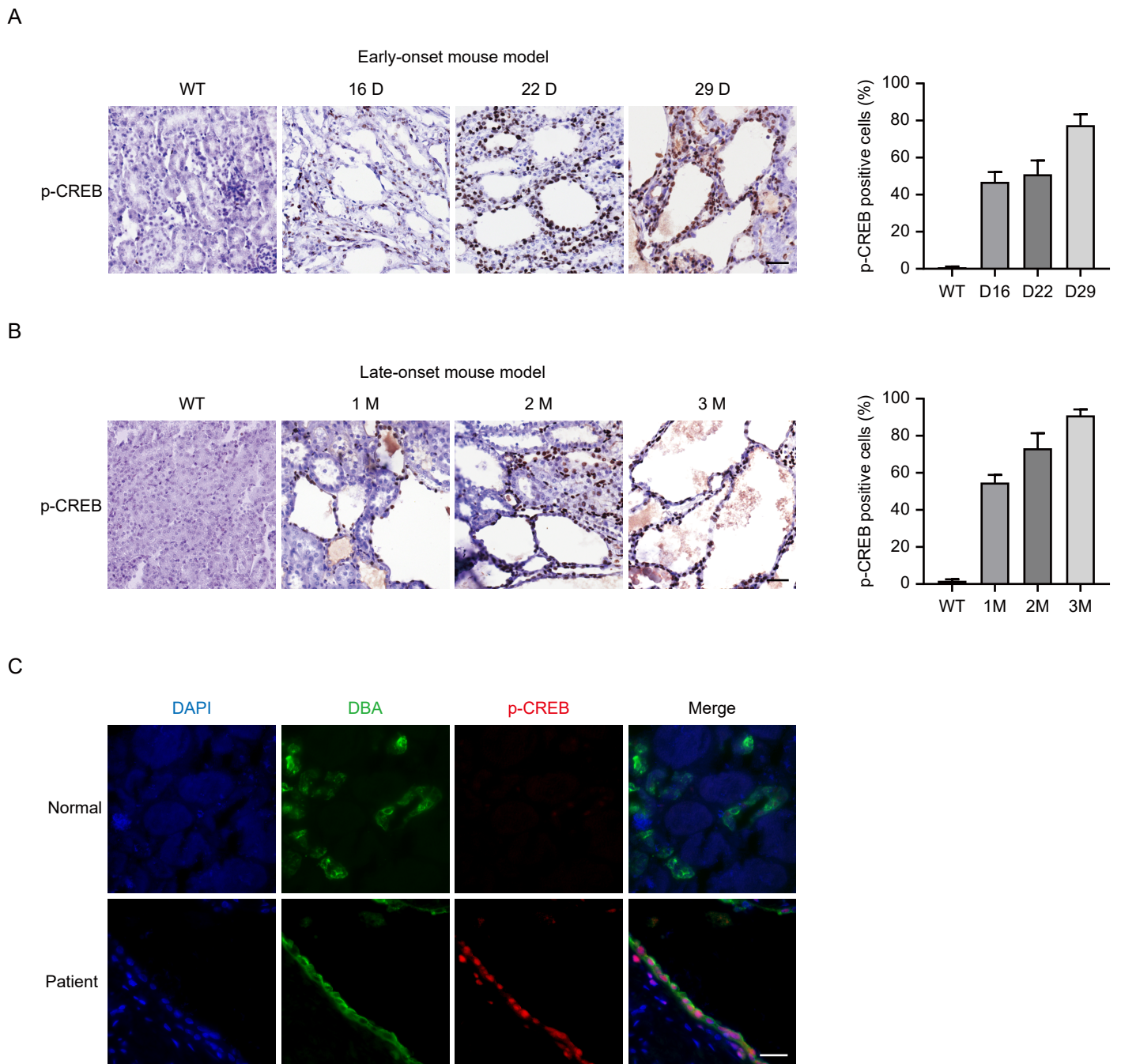
14 Supplemental Figure 6. p-CREB target genes previously reported as cystogenesis-
15 associated genes.

16 Supplemental Figure 7. 666-15 suppresses cystic cell proliferation in the early-onset
17 model.

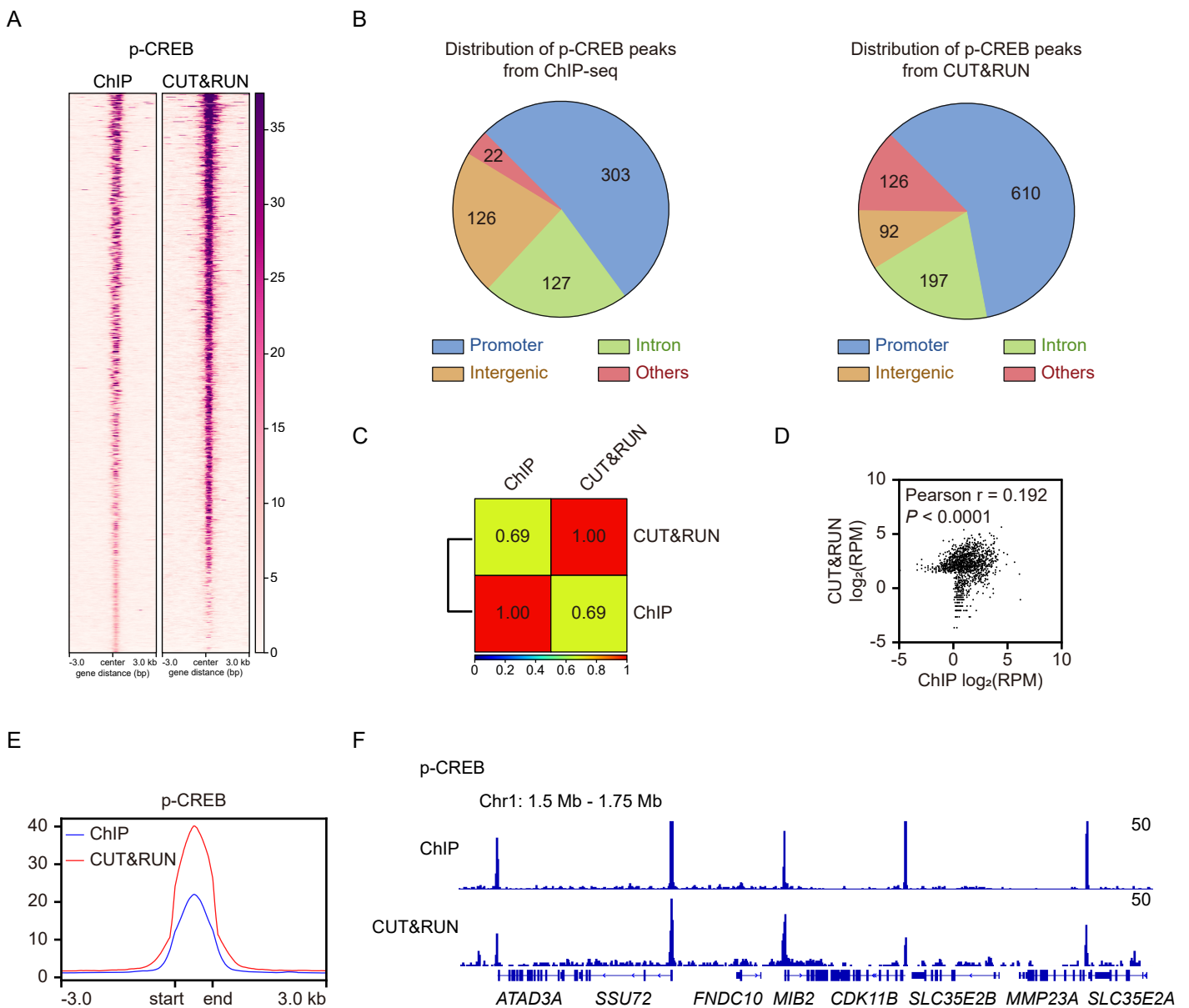
18 Supplemental Figure 8. Inhibition of CREB by overexpressing A-CREB.

19 Supplemental Figure 9. 666-15 suppresses cystic cell proliferation in the kidney-
20 specific mouse model.

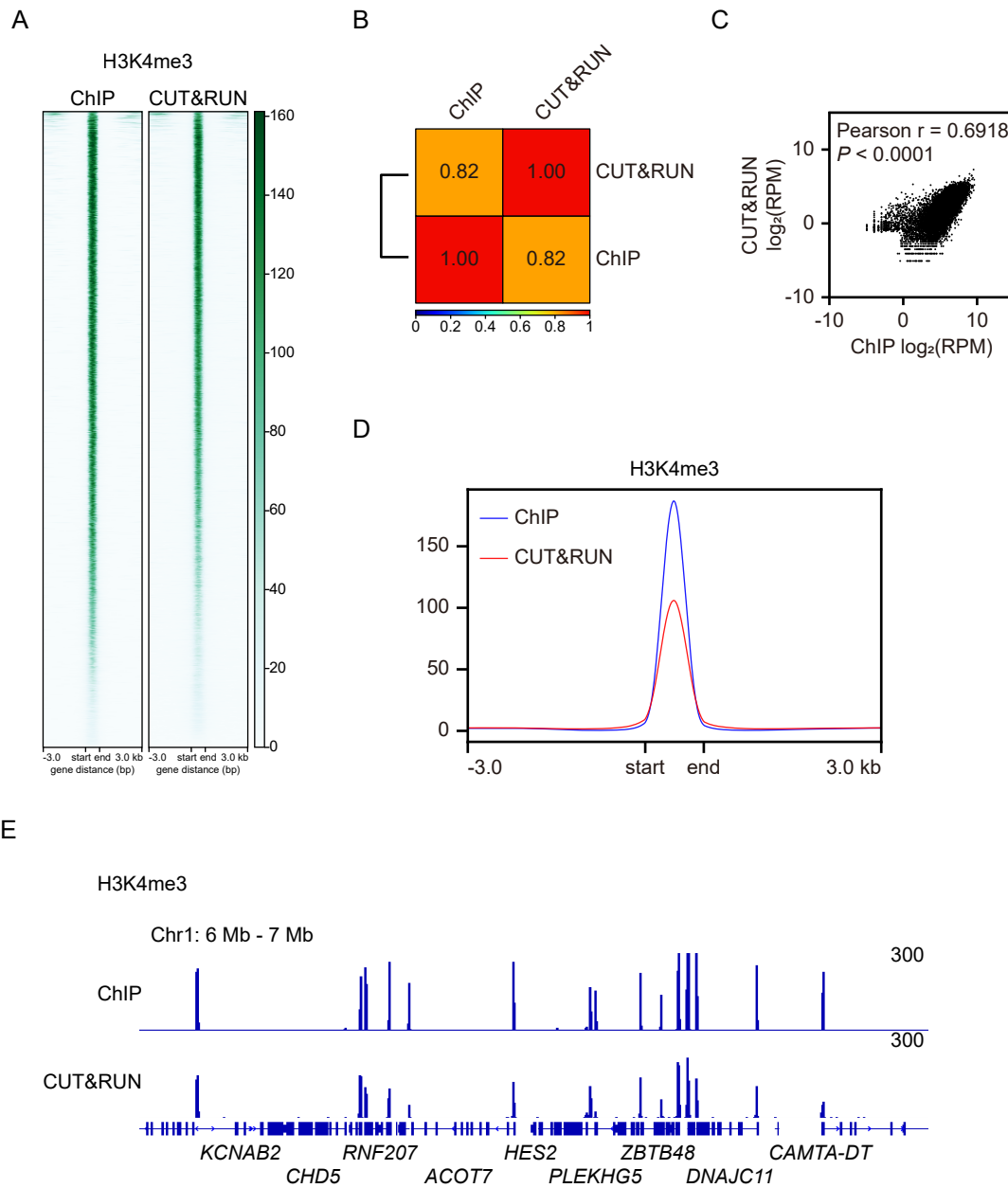
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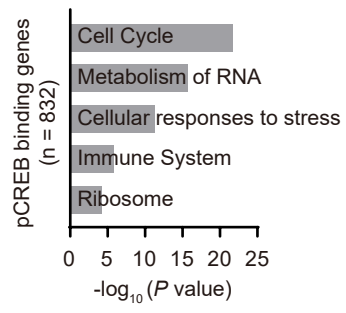
Supplemental Figure 1. Phosphorylation of CREB increases steadily in ADPKD kidneys. (A and B) Immunostaining of p-CREB in kidney sections from early-onset (A) or late-onset (B) ADPKD mouse model at the indicated times. The proportion of p-CREB-positive cells in normal tubular or cystic epithelial cells were counted. (C) Immunofluorescence analysis of p-CREB level in normal kidneys and kidneys from ADPKD patient. Scale bars represent 50 μm in A and B (200 \times magnification), 25 μm in C (650 \times magnification).



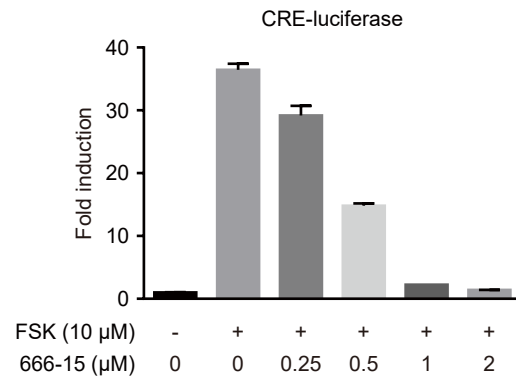
Supplemental Figure 2. Comparison of p-CREB CUT&RUN and ChIP-seq. (A) Heatmap of normalized p-CREB signals from ChIP-seq or CUT&RUN in WT 9-12 cells ($n = 1$). (B) Genomic distribution of p-CREB peaks from ChIP-seq or CUT&RUN. (C) Pearson correlation matrix between p-CREB ChIP-seq and CUT&RUN. (D) Dot plots of p-CREB signals from ChIP-seq and CUT&RUN. (E) Composite plots of normalized p-CREB signals from ChIP-seq or CUT&RUN in WT 9-12 cells. (F) Representative ChIP-seq and CUT&RUN tracks of p-CREB.



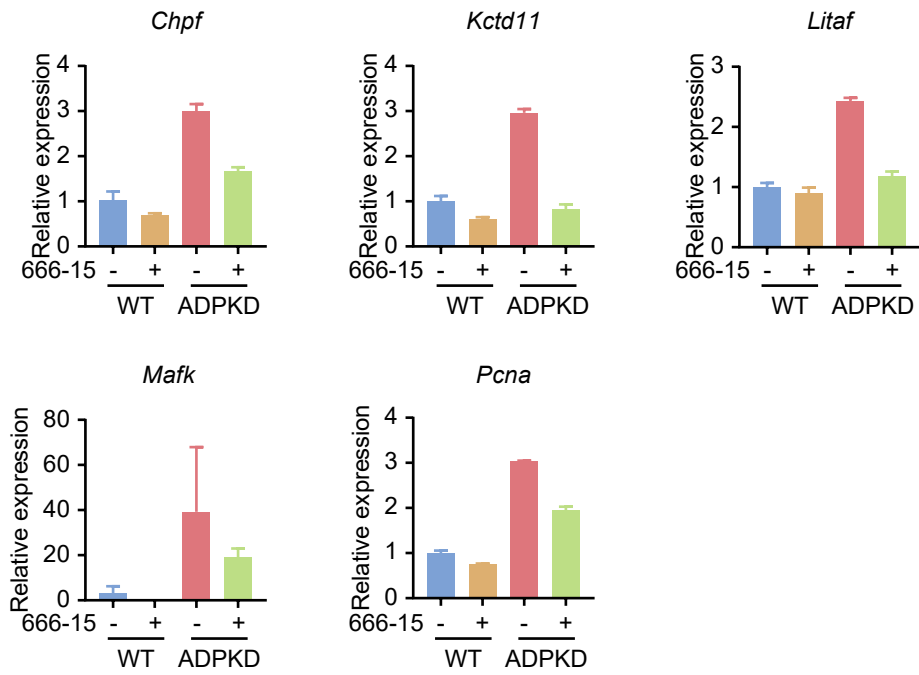
Supplemental Figure 3. Comparison of H3K4me3 CUT&RUN and ChIP-seq. (A) Heatmap of normalized H3K4me3 signals from ChIP-seq or CUT&RUN in WT 9-12 cells ($n = 1$). (B) Pearson correlation matrix between H3K4me3 ChIP-seq and CUT&RUN. (C) Dot plots of H3K4me3 signals from ChIP-seq and CUT&RUN. (D) Composite plots of normalized H3K4me3 signals from ChIP-seq or CUT&RUN in WT 9-12 cells. (E) Representative ChIP-seq and CUT&RUN tracks of H3K4me3.



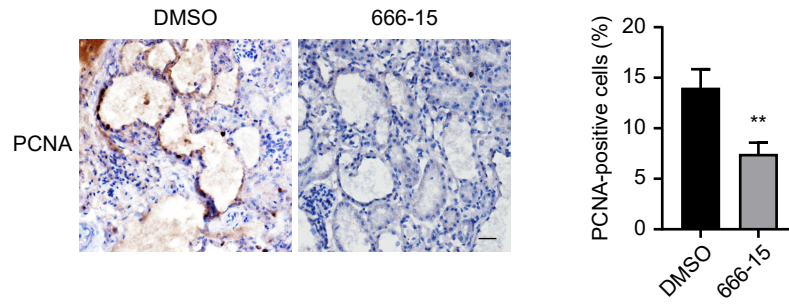
Supplemental Figure 4. Function enrichment analysis of p-CREB binding genes.



Supplemental Figure 5. Inhibition of CREB transactivity by 666-15 treatment. CRE-luciferase activity in 293T cells with 666-15 and forskolin (FSK) treatment.

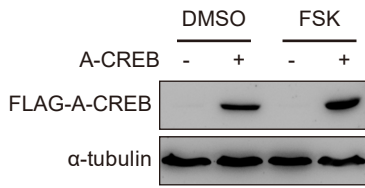


Supplemental Figure 6. p-CREB target genes previously reported as cystogenesis-associated genes. qPCR validation of previously reported cystogenesis-associated genes in DBA-positive cells from the indicated groups.

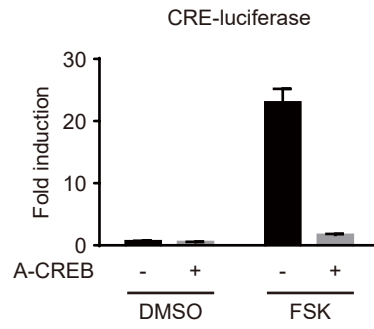


Supplemental Figure 7. 666-15 suppresses cystic cell proliferation in the early-onset model. Immunohistochemistry analysis of PCNA level in kidney sections from early-onset ADPKD mice treated with DMSO or 666-15. Proportion of PCNA-positive cells in cystic epithelial cells was determined. ** $P < 0.01$. Scale bar represents 50 μm (200 \times magnification).

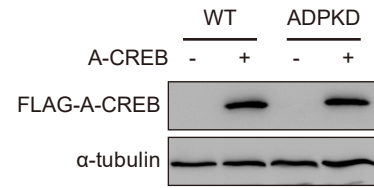
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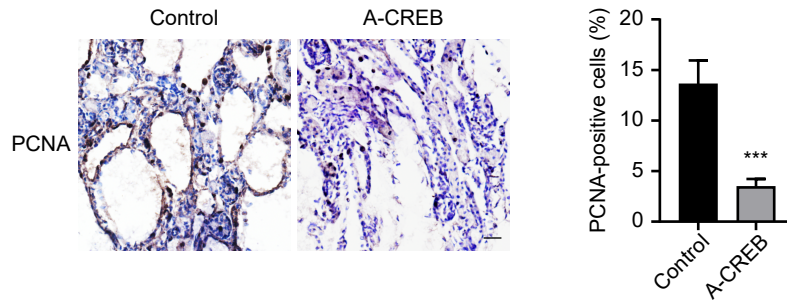
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Supplemental Figure 8. Inhibition of CREB by overexpressing A-CREB. (A) WB analysis of overexpressed FLAG-A-CREB in 293T cells. (B) CRE-luciferase activity in 293T cells from the indicated groups. (C) WB analysis of FLAG-A-CREB in mice from the indicated groups.



Supplemental Figure 9. 666-15 suppresses cystic cell proliferation in the kidney-specific mouse model. Immunohistochemistry analysis of PCNA level in kidney sections from ADPKD mice with or without A-CREB expression. Proportion of PCNA-positive cells in cystic epithelial cells was determined. *** $P < 0.001$. Scale bar represents 50 μm (200 \times magnification).