

Supplementary Materials for
**ETV4 mediates dosage-dependent prostate tumor initiation and cooperates
with p53 loss to generate prostate cancer**

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The PDF file includes:

Figs. S1 to S8
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Other Supplementary Material for this manuscript includes the following:

Tables S1 to S9

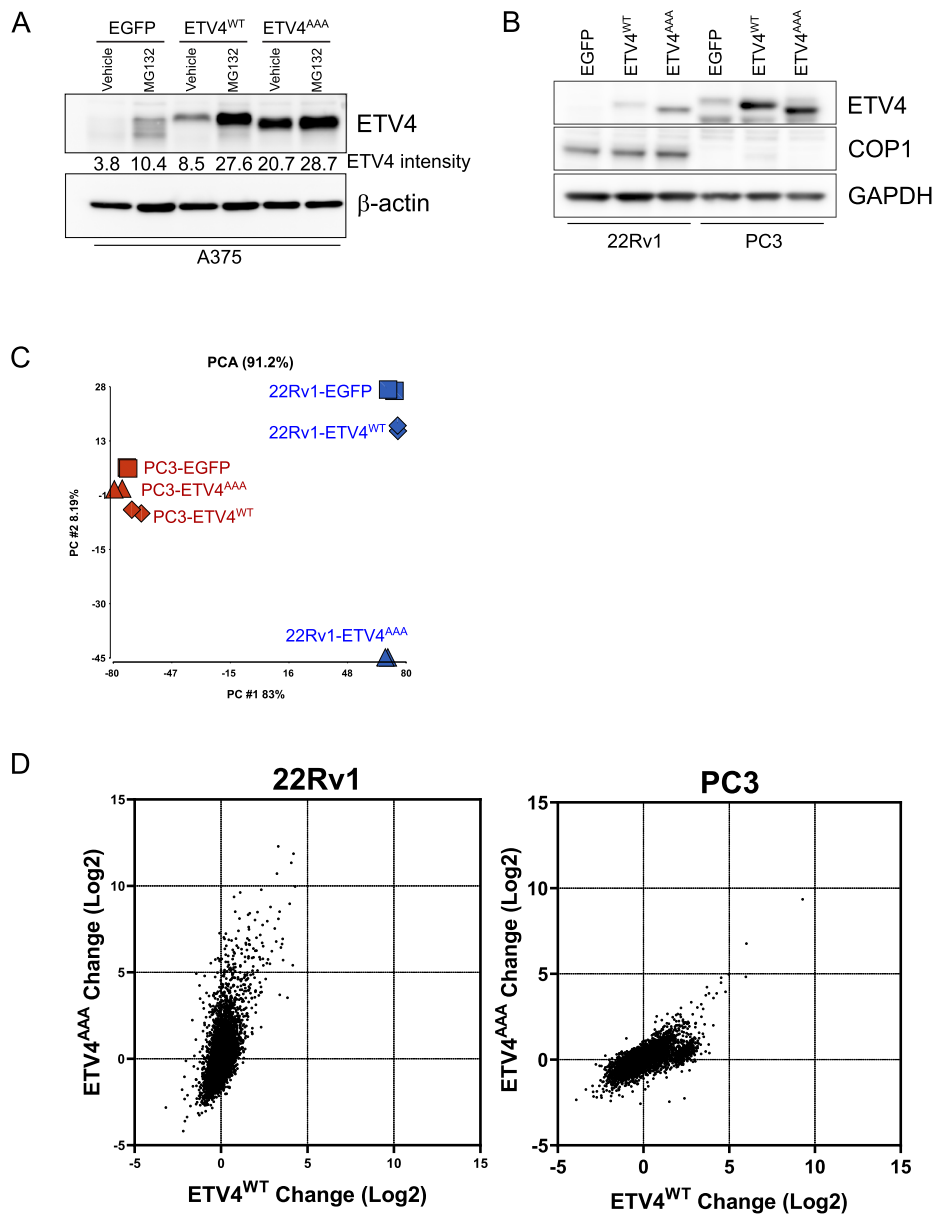


Figure S1

Fig. S1. ETV4^{AAA} shows increased stability and transcription activity *in vitro*

(A) Western blot of ETV4 expressed in A375 melanoma cells which are transduced with an pMSCV-IRES-EGFP vector expressing control EGFP, wild type human ETV4 (ETV4^{WT}) or ExxVPD to ExxAAA mutated ETV4 (ETV4^{AAA}), and treated with or without MG132 for 8 hours. The intensity of ETV4 bands is quantified using ImageJ. (B) Western blot of ETV4 and COP1 expressed in 22Rv1 and PC3 prostate cells which are transduced with an pMSCV-IRES-EGFP vector expressing control EGFP, ETV4^{WT} or ETV4^{AAA}. (C) PCA analysis of RNA-seq data of COP1 intact 22Rv1 and COP1-loss PC3 prostate cells which are transduced with an pMSCV-IRES-EGFP vector expressing control EGFP, ETV4^{WT} or ETV4^{AAA}. (D) Differentially expressed genes of ETV4^{AAA} compared with ETV4^{WT} in 22Rv1 and PC3 cells.

A

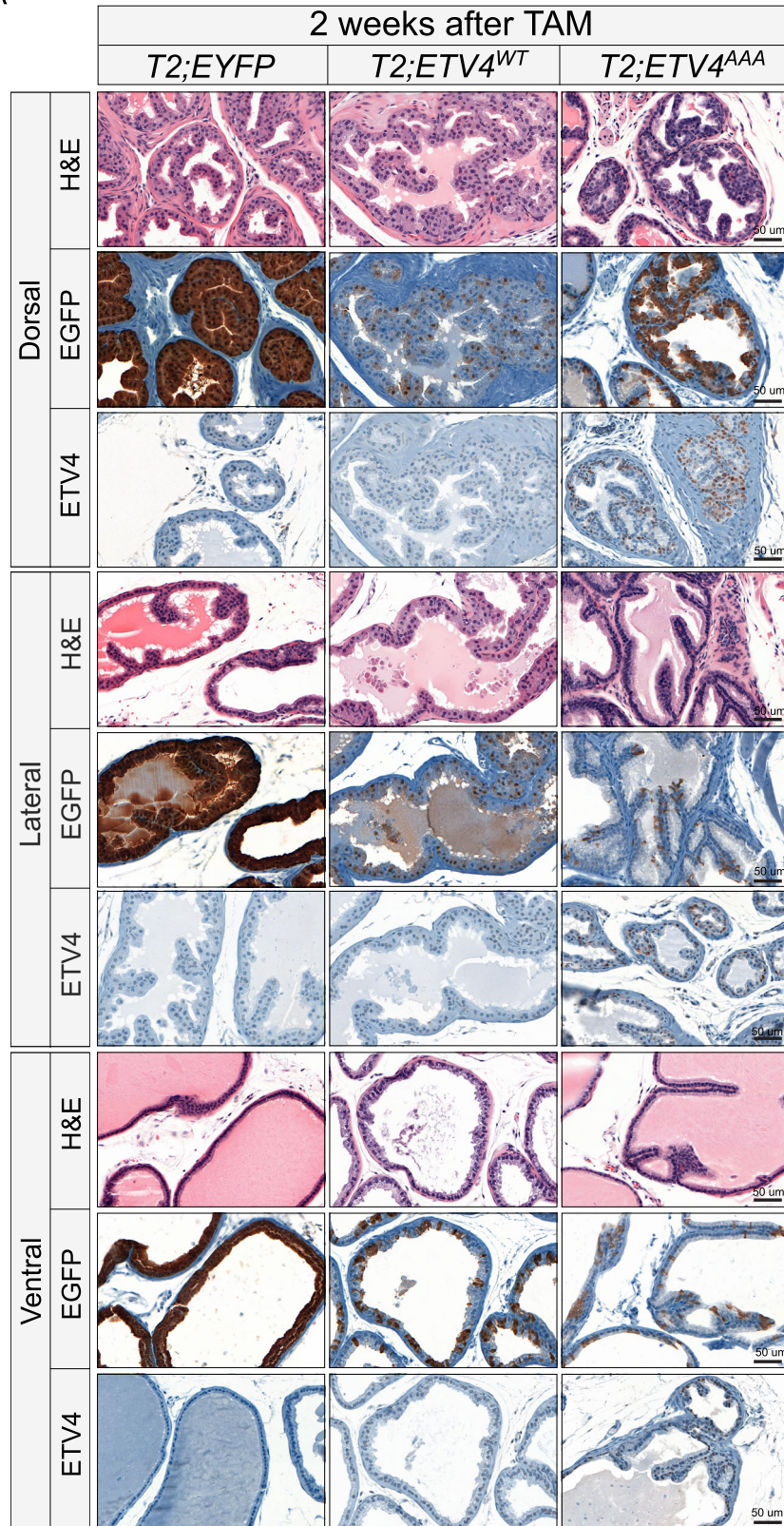


Figure S2

Fig. S2. Expression of Stabilized ETV4 (ETV4^{AAA}) induces prevalent mPin in dorsal prostate

(A) 2 weeks after tamoxifen treatment, histological changes in dorsal, lateral and ventral prostates in *T2; EYFP*, *T2; ETV4^{WT}* and *T2; ETV4^{AAA}* mice are shown by H&E. The expression of EGFP and ETV4 is analyzed with IHC staining. Scale bar 50 um.

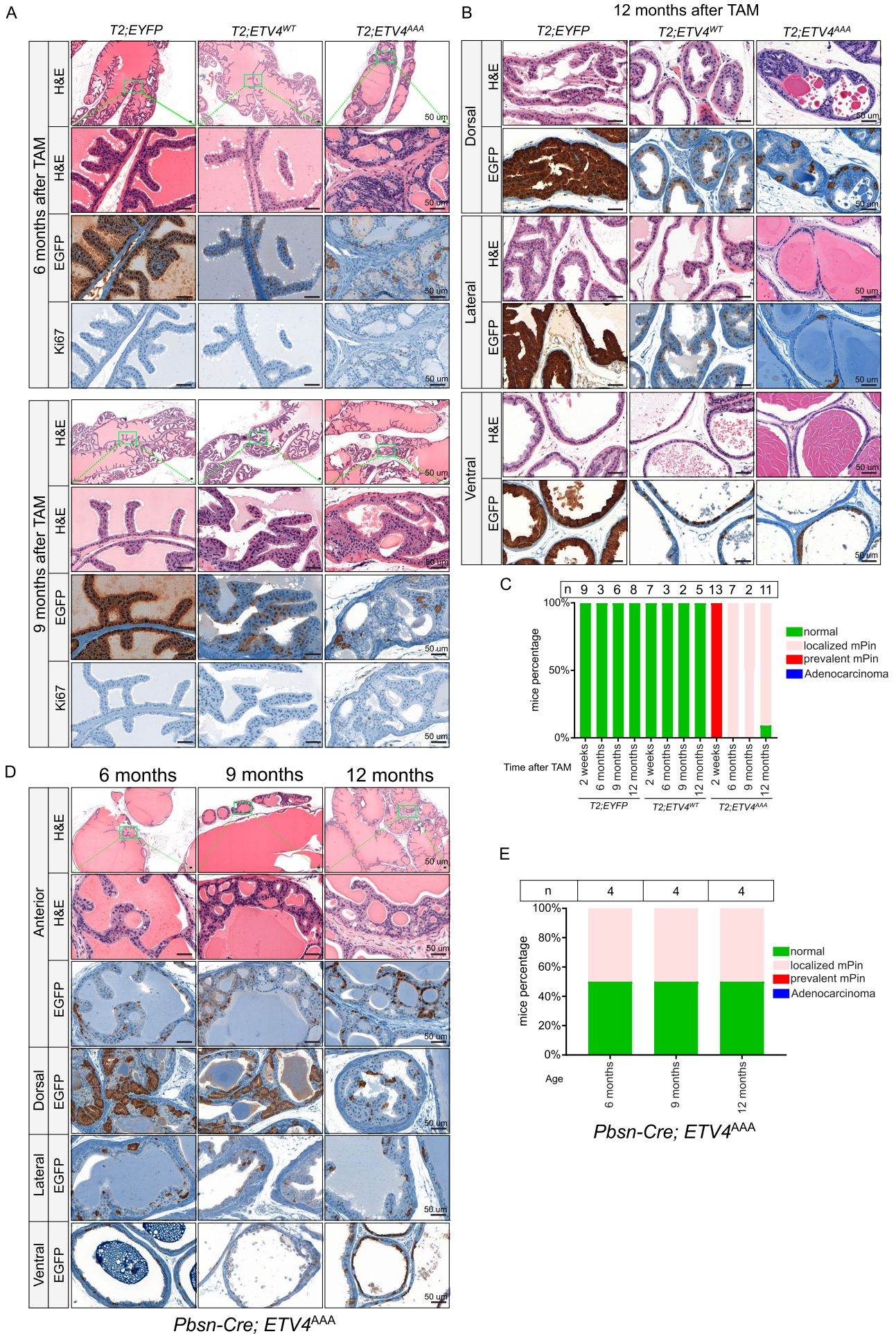


Figure S3

Fig. S3. Prevalent mPin regresses to localized mPin in aged ETV4^{AAA} mice

(A) Representative H&E (low magnification and high magnification of indicated area) and IHC of GFP (antibody recognizes both EGFP and EYFP) of anterior prostate 6 and 9 months after tamoxifen treatment in *T2; EYFP*, *T2; ETV4^{WT}* and *T2; ETV4^{AAA}* mice. (B) 12 months after tamoxifen treatment, histological changes in dorsal, lateral and ventral prostates in *T2; EYFP*, *T2; ETV4^{WT}* and *T2; ETV4^{AAA}* mice are shown by H&E. The expression of EGFP is analyzed with IHC staining. (C) The mice number of different genotypes and phenotypes are quantified. Scale bar 50 um. (D) Representative H&E (low magnification and high magnification of indicated area) and IHC of GFP (antibody recognizes both EGFP and EYFP) of dorsal, lateral and ventral prostate 6-month, 9-month and 12-month-old *Pbsn-Cre; ETV4^{AAA}* mice. (E) Quantification of mouse prostate histology categorized as normal, localized mPIN, prevalent mPIN and adenocarcinoma in 6-month, 9-month and 12-month-old *Pbsn-Cre; ETV4^{AAA}* mice. Scale bar 50 um.

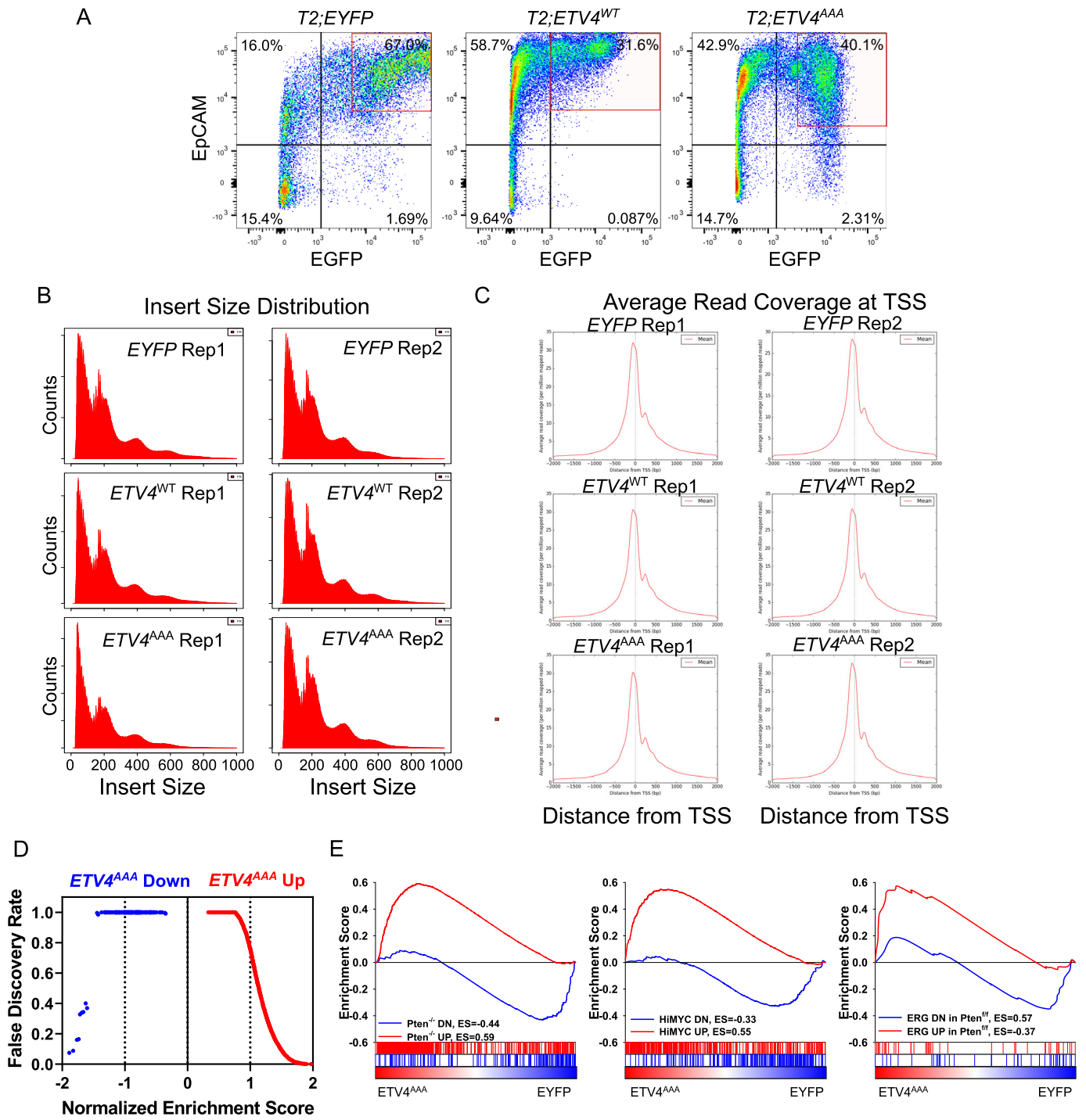


Figure S4

Fig. S4. Insert size distribution of open-chromatin and nucleosome peaks enriched around known promoters are expected

(A) EpCAM-positive and EGFP-positive prostate cells in *T2; EYFP*, *T2; ETV4^{WT}* and *T2; ETV4^{AAA}* mice 1 week after tamoxifen treatment are analyzed by FACS sorting. The mean cell ratios of 3 replicates are labeled. (B) ATAC sequencing reads from FACS sorted EYFP, ETV4^{WT} and ETV4^{AAA} cells exhibited expected insert size distribution of open-chromatin. (C) ATAC sequencing reads from FACS sorted EYFP, ETV4^{WT} and ETV4^{AAA} cells exhibited expected nucleosome peaks enriched around known promoters. (D) Normalized enrichment scores are shown from Gene Set Enrichment Analysis with ETV4^{AAA} expressing cells compared with EYFP expressing cells using ~3,000 curated genes sets and custom prostate cancer gene sets. (E) The up and down regulated genes in ETV4^{AAA} expressing cells compared with EYFP expressing cells are enriched in gene sets up-regulated in *Pten^{-/-}*, HiMYC and ERG models.

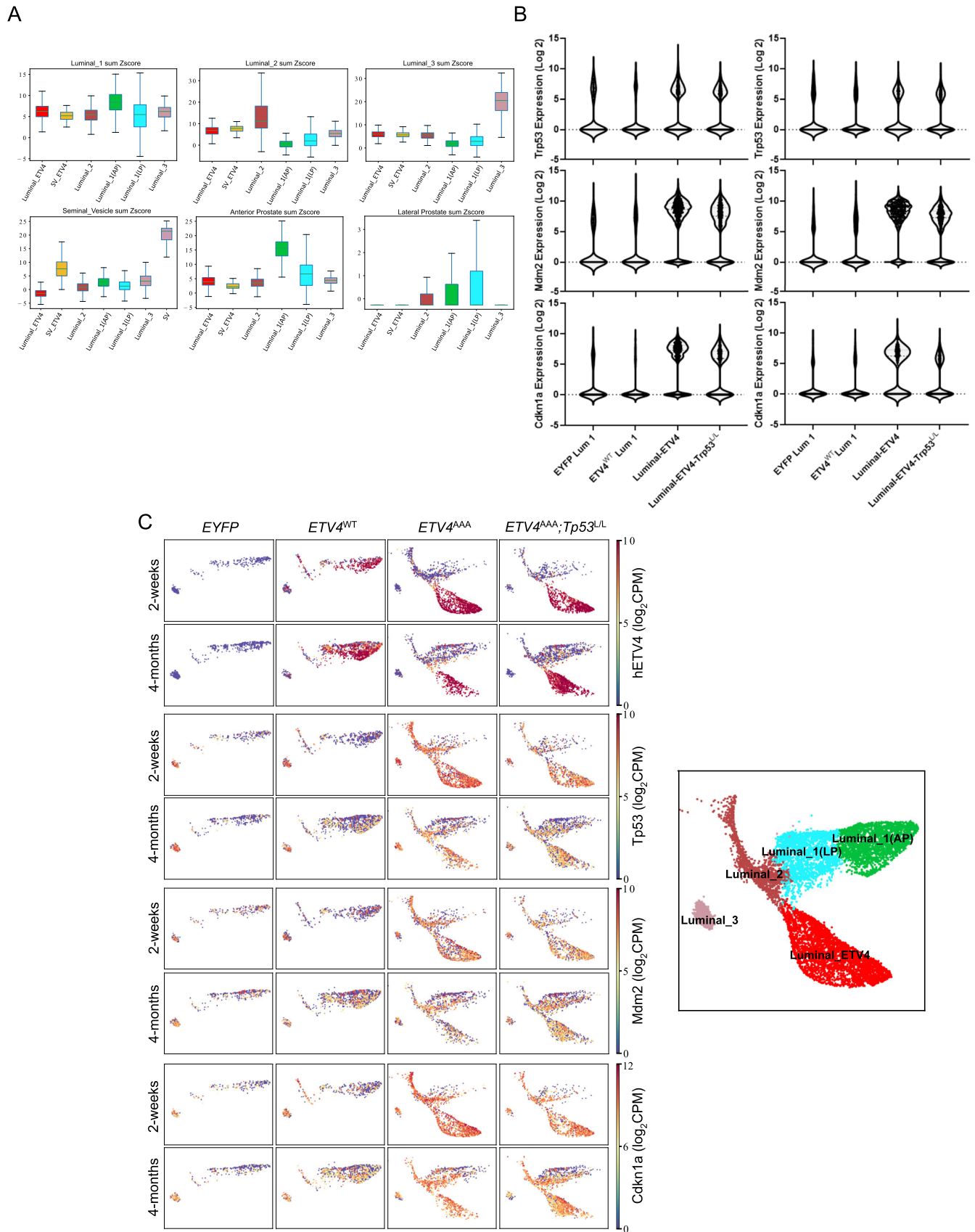


Figure S5

Fig. S5. The expression of Tp53 and its downstream genes in ETV4^{AAA};Tp53^{L/L} mice

(A) Sum of Z score of marker genes of luminal clusters. (B) Violin plot shows the mRNA expression of Tp53 and its downstream genes, Mdm2 and Cdkn1a, in Luminal_ETV4 and Luminal_1(AP+LP) clusters in *T2; EYFP*, *T2; ETV4^{WT}*, *T2; ETV4^{AAA}* and *T2; ETV4^{AAA};Tp53^{L/L}* mice. (C) Scatter plot shows the expression of ETV4, Tp53, Mdm2 and Cdkn1a in luminal clusters in *T2; EYFP*, *T2; ETV4^{WT}*, *T2; ETV4^{AAA}* and *T2; ETV4^{AAA};Tp53^{L/L}* mice.

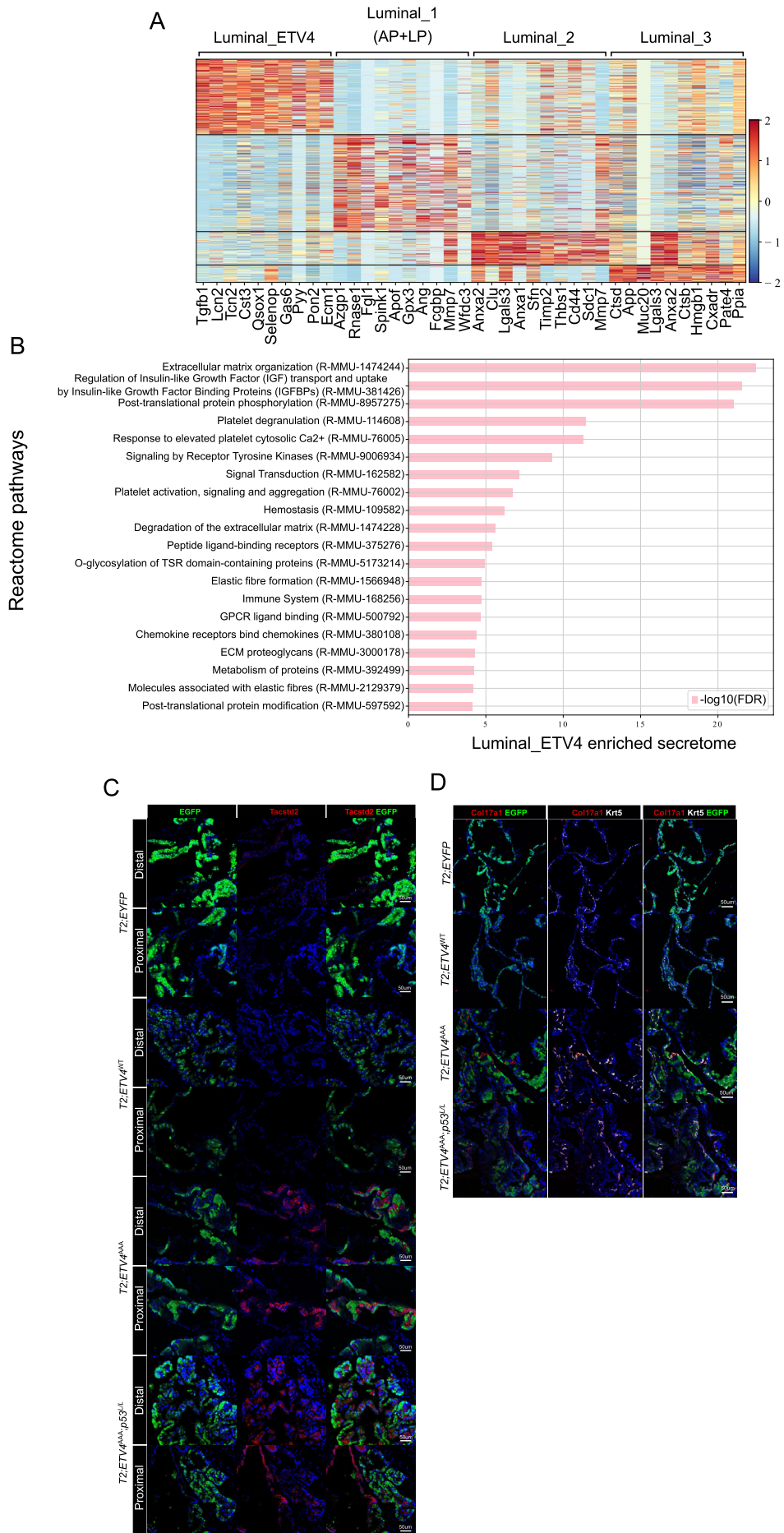


Figure S6

Fig. S6. Secretome and paracrine effect of Luminal_ETV4 cells

(A) Heatmap shows the highly expressed secretome genes of luminal clusters. (B) Bar graph shows the enriched secretome pathways in Luminal_ETV4 cluster found by GO analysis. (C, D) The protein level of Tacstd2/Trop2 (A) and Col17a1, Krt5 (B) are shown with IF staining. Scale bar 50 um.

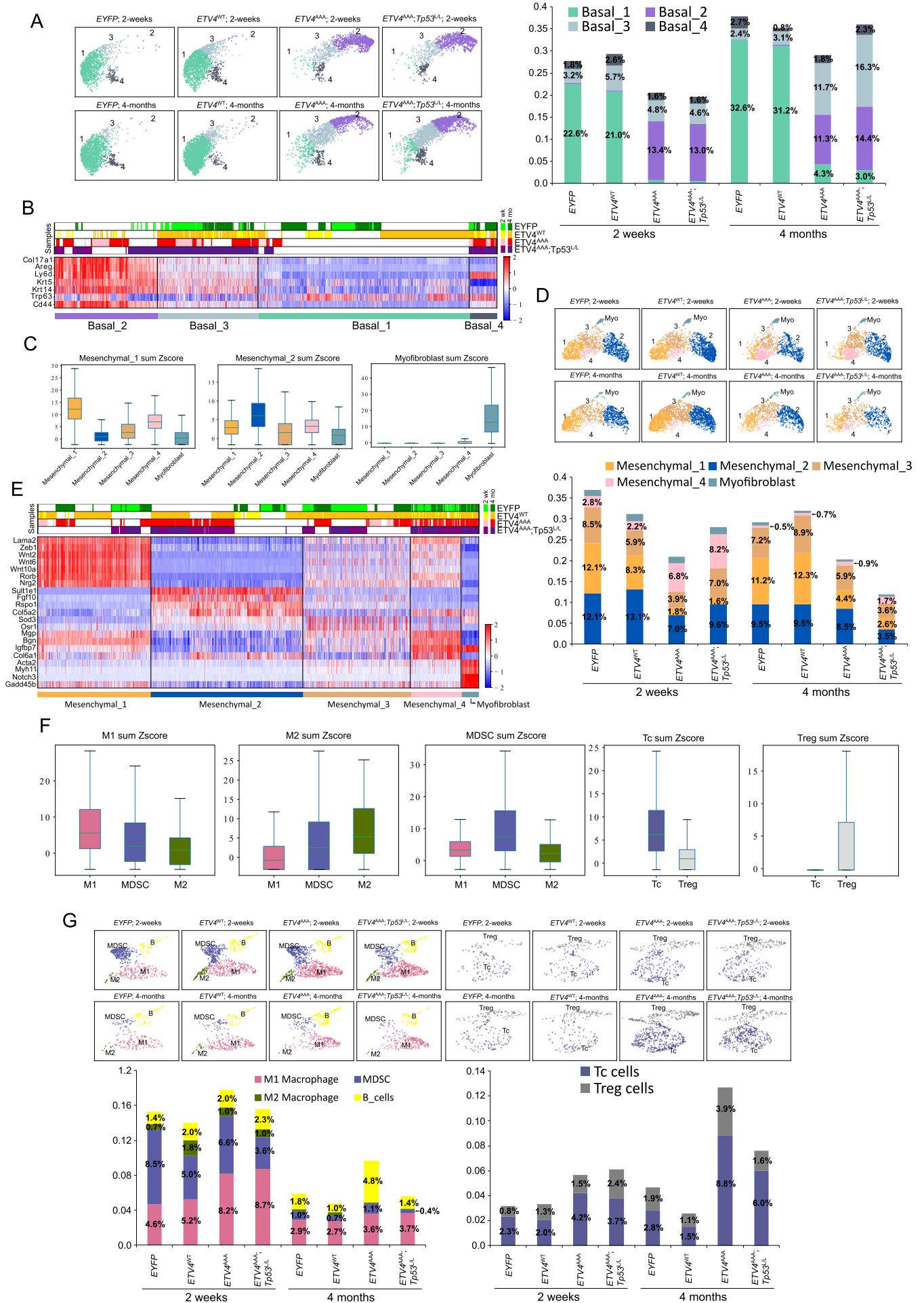


Figure S7

Fig. S7. A single-cell atlas of the mPin induced by ETV4^{AAA}

(A) Basal subsets and are shown separately for each sample and cell numbers are quantified. (B) Heatmap shows the highly differentially expressed genes for each Basal clusters. The gene expression matrix was normalized to CPM and $\log_2(\text{CPM}+1)$, gene imputation was performed. (C) Sum of Z score of marker genes of stroma clusters. (D) Stroma subsets and are shown separately for each sample and cell numbers are quantified. (E) Heatmap shows the highly differentially expressed genes for each stroma clusters. The gene expression matrix was normalized to CPM and $\log_2(\text{CPM}+1)$, gene imputation was performed. (F) Sum of Z score of marker genes of macrophage and T cell clusters. (G) Macrophage, B cell and T cell subsets and are shown separately for each sample and cell numbers are quantified.

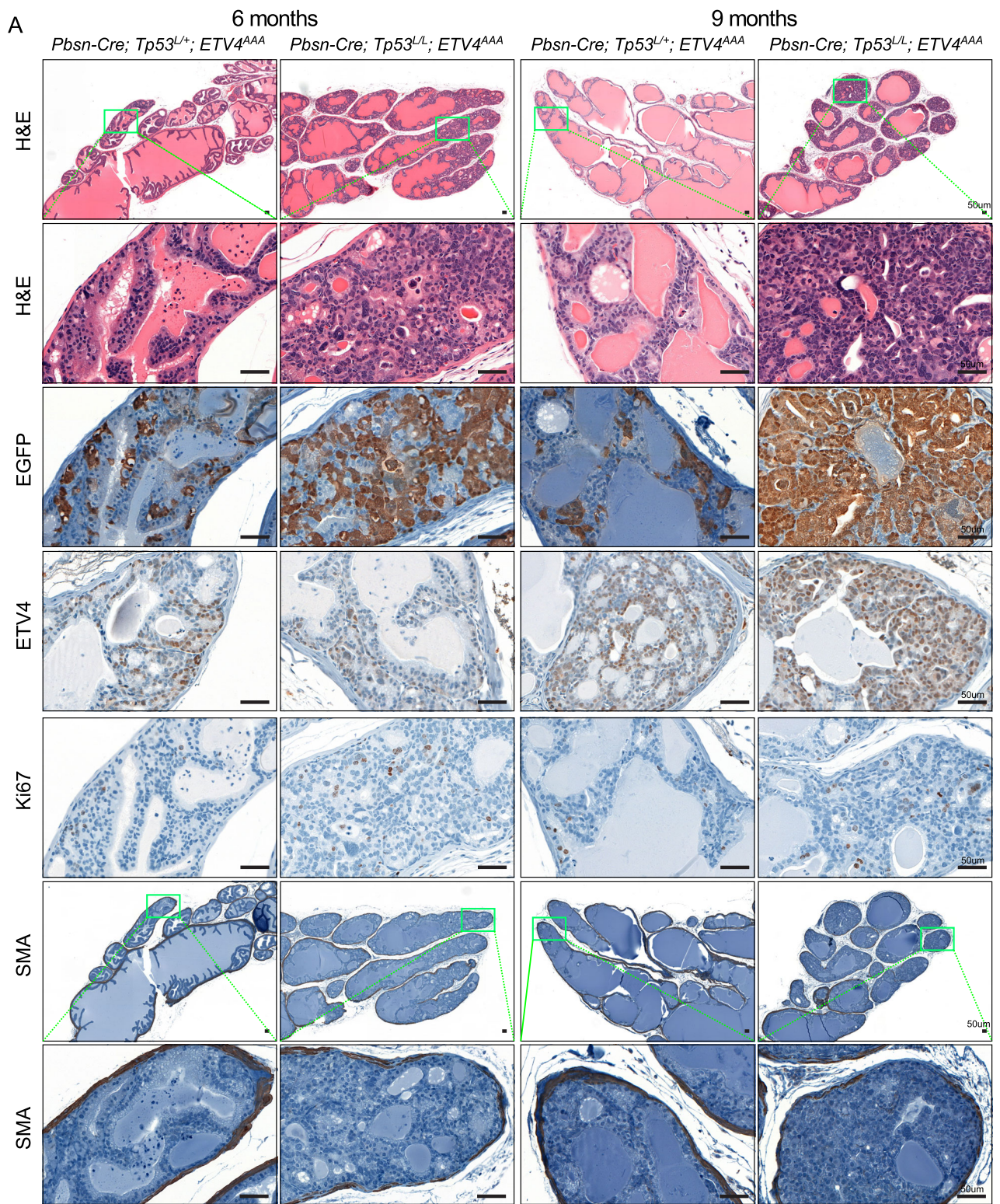


Figure S8

Fig. S8. loss Trp53 suppresses ETV4^{AAA} induced senescence and induces prostate cancer

(A) Representative H&E (low magnification and high magnification of indicated area) and IHC of GFP (antibody recognizes both EGFP and EYFP), ETV4, Ki67 and smooth muscle actin (SMA) in anterior prostate 6 months old and 9 months old *Pbsn-Cre; Tp53^{L/+}; ETV4^{AAA}* and *Pbsn-Cre; Tp53^{L/L}; ETV4^{AAA}* mice. Scale bar 50 um.

Additional files related to the supplementary materials:

Table S1. MEME-ChIP

Table S2. Bindetect_results

Table S3. Custom gene sets.

Table S4. Rank genes in clusters

Table S5. Cluster_cell_numbers

Table S6. Marker genes for Zscore

Table S7. Rank gene list Luminal_1_ETV4 vs Luminal_1(AP)

Table S8. Rank gene list ETV4AAAp53KO vs ETV4AAA 2 weeks

Table S9. Rank gene list ETV4AAAp53KO vs ETV4AAA 4 months