

Supplemental information

**Direct androgen receptor control of sexually
dimorphic gene expression in the mammalian kidney**

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Figure S1

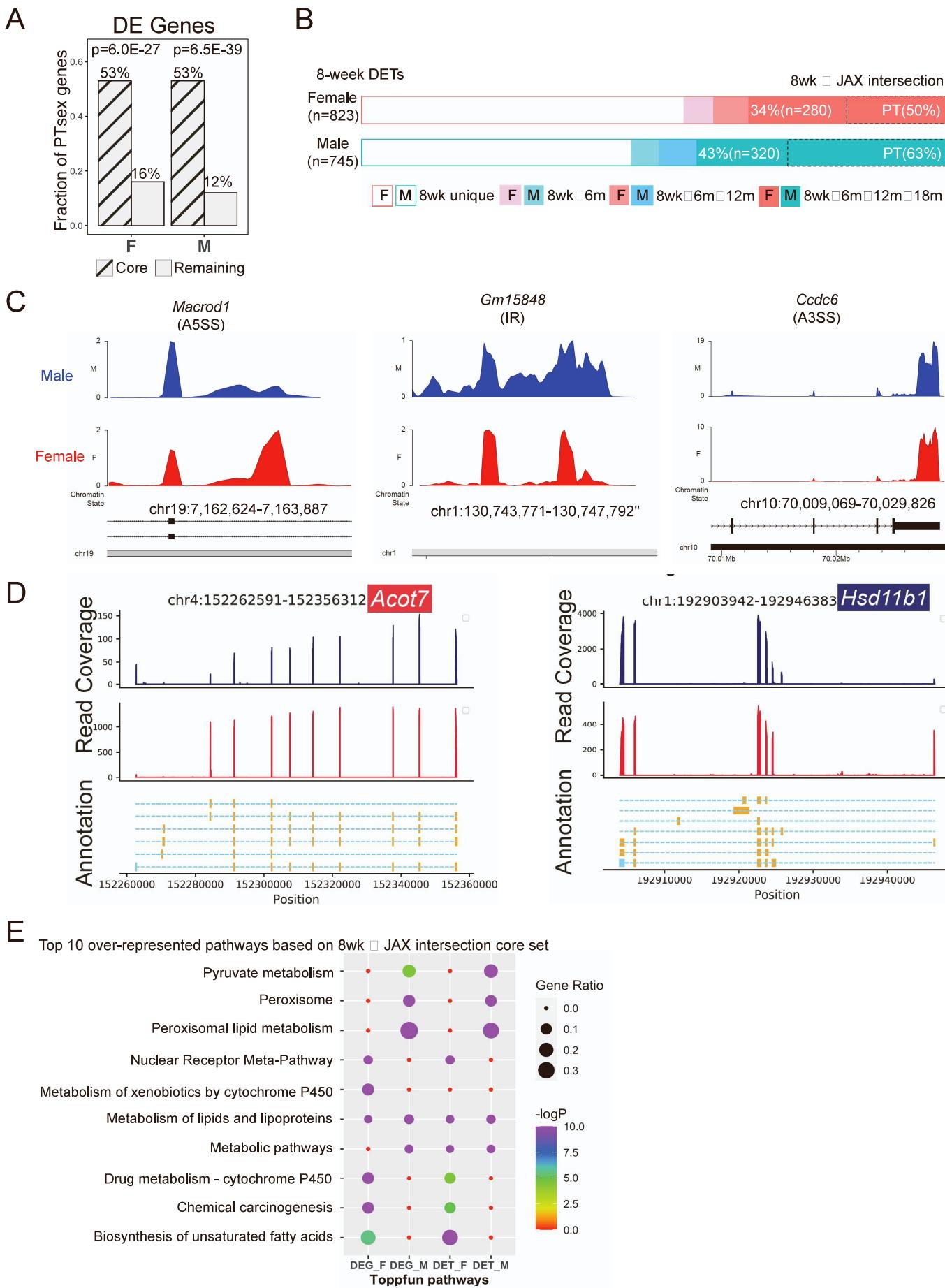


Figure S1. Transcript-level renal sex differences in adult mice. Related to Fig. 1.

(A) Bar plot shows the fraction of sex-biased genes previously identified in the PT segments from our previous single-cell RNA-seq experiment¹⁹ (PTsex genes) that are covered in the core set of sex-biased genes identified by whole-kidney bulk RNA-seq experiment in the current study. Enrichment analysis was based on Chi-square test.

(B) Stacked bar plot shows the proportion of sex-biased transcripts identified in 8-week C57BL/6 mice that continue to show dimorphic expression in the aging kidney in the diversity outbred (DO) mice (the JAX data⁴⁵) measured at 6, 12, and 18 months. The degree of overlap between 8-week C57BL/6 and JAX data is indicated by the progressive shading of the bars as age advances. The core set of sex-biased transcripts is defined as those that are shared by all four time points. Dashed boxes indicate the number and percentage of core sex-biased transcripts that are shared with PTsex genes.

(C) Read coverages of bulk RNA-seq at representative genomic regions demonstrate alternative splicing events.

(D) Read coverages of bulk RNA-seq at representative genomic regions demonstrate alternative isoform usage.

(E) Dot plot shows the enrichment of ToppFun pathways for both the core set of sex-biased genes and transcripts. Gene count of individual pathways is shown by the relative size of the dot, and the color of the dot indicates the statistical significance.

Figure S2

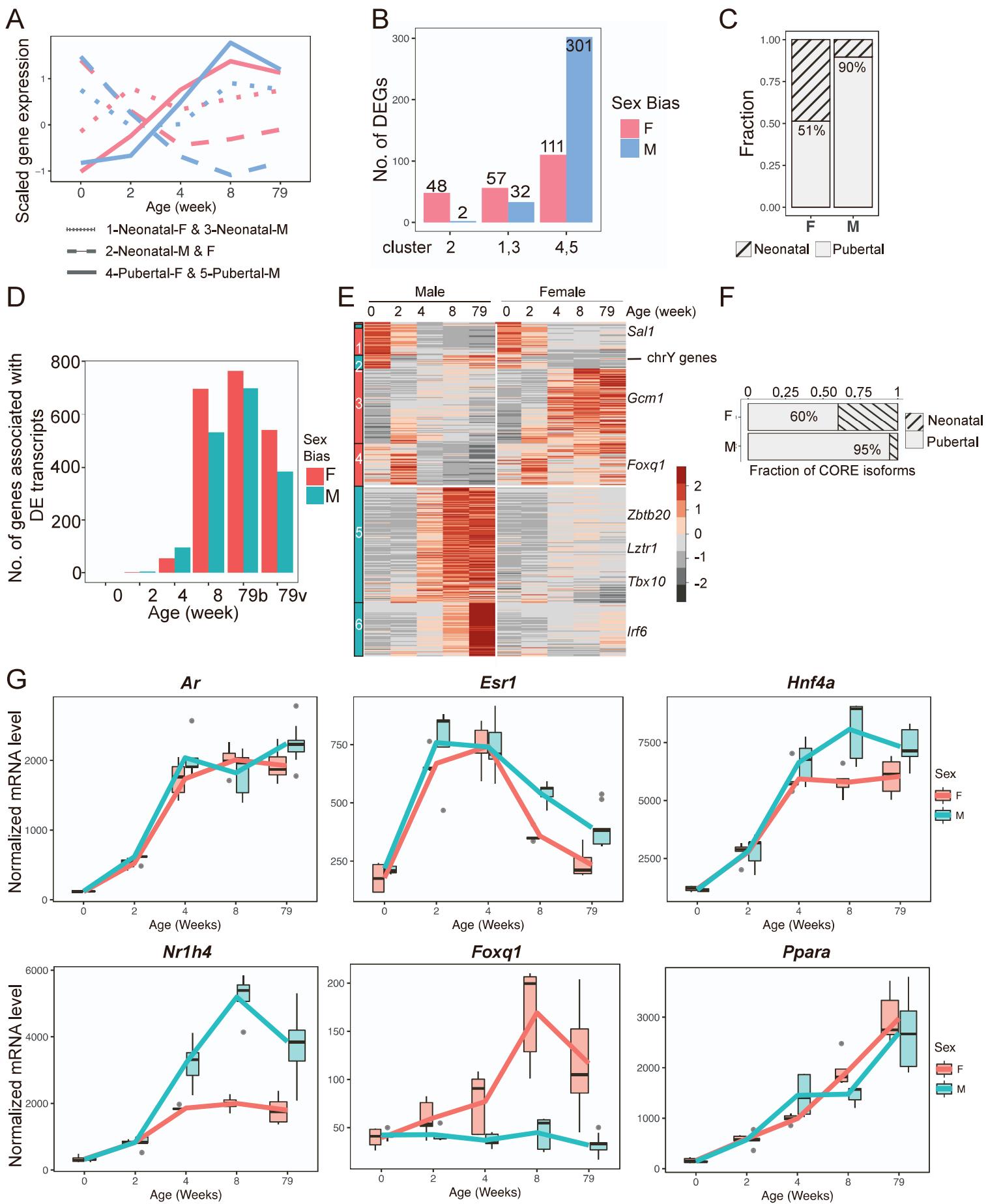


Figure S2. Dynamical features of male and female renal transcriptomes during postnatal development. Related to Fig. 2.

- (A) Scaled average expression of core sex-biased genes over the five timepoints we measured, grouped by clusters shown in Fig. 2D. Together with the heatmap, we can see that neonatal genes are already expressed at high levels at early timepoints (0 and 2 weeks); pubertal genes exhibit increased expression over time (4 weeks onwards).
- (B) Bar plot shows the number of core sex-biased genes in each cluster.
- (C) Stacked bar plot indicates the fraction of neonatal and pubertal genes in the core set.
- (D) Bar plot demonstrates the number of sex-biased transcripts identified at individual timepoints. Bars are colored by sex bias: M-biased genes are in green and F-biased genes in salmon.
- (E) Heatmap shows the scaled average expression levels of the core sex-biased transcripts in male and female samples at individual timepoints. Genes were clustered via hierarchical clustering.
- (F) Stacked bar plot indicates the fraction of neonatal and pubertal transcripts in the core set.
- (G) Normalized read count for *Ar*, *Esr1* and *Hnf4a* over the five timepoints we measured. Lines indicate average expression levels. Boxes show the variation among samples. Data from male samples are in green and data from female samples are in salmon.

Figure S3

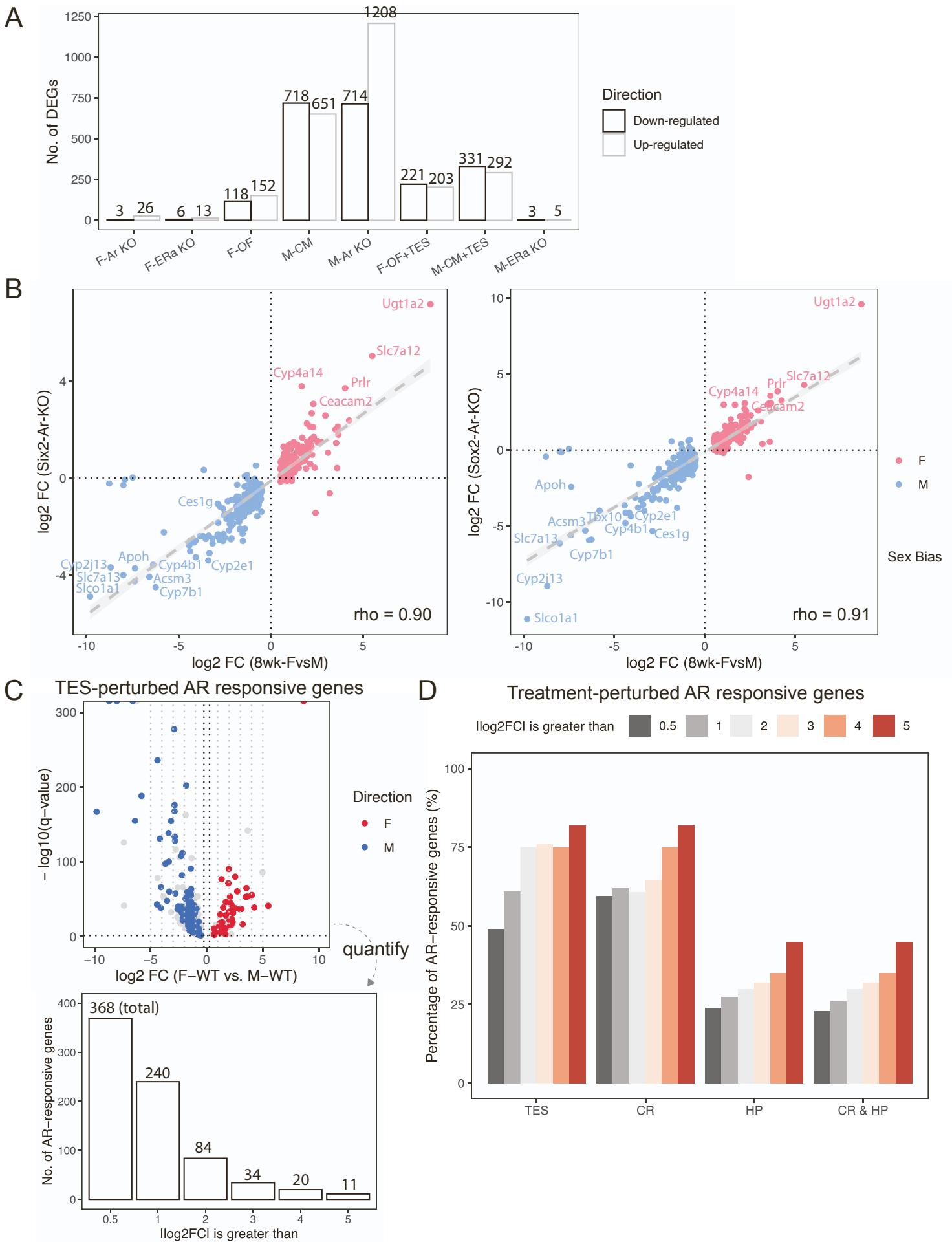


Figure S3. AR-mediated signaling plays an important role in renal sex differences.
Related to Fig. 3.

(A) Bar plot shows the total number of differentially expressed genes identified in each treatment when compared to controls. Direction of perturbation in gene expression is indicated by the shade of the bar: up-regulated genes are in gray and down-regulated genes are in black.

(B) Scatter plots compare difference in the expression of core sex-biased genes in either of the two types of AR knockout experiments (nephron-specific removal [Six2-Ar-KO] and whole-body removal [Sox2-Ar-KO]) versus male-female differences at 8 weeks. Each dot represents a sex-biased gene, where sex bias is indicated by colors. Changes in gene expression (KO vs. WT or F vs. M) are summarized by log₂ fold change (FC), which we used to evaluate the overall concordance of the two experiments by Spearman correlation.

(C) Volcano plot highlights AR-responsive genes that were perturbed by TES treatment. Bar plot quantifies the number of genes using different effect size cutoffs.

(D) Grouped bar plot shows percentage of AR-responsive genes that were perturbed by different treatments, using the gene sets identified in (C) as reference.

Figure S4

A

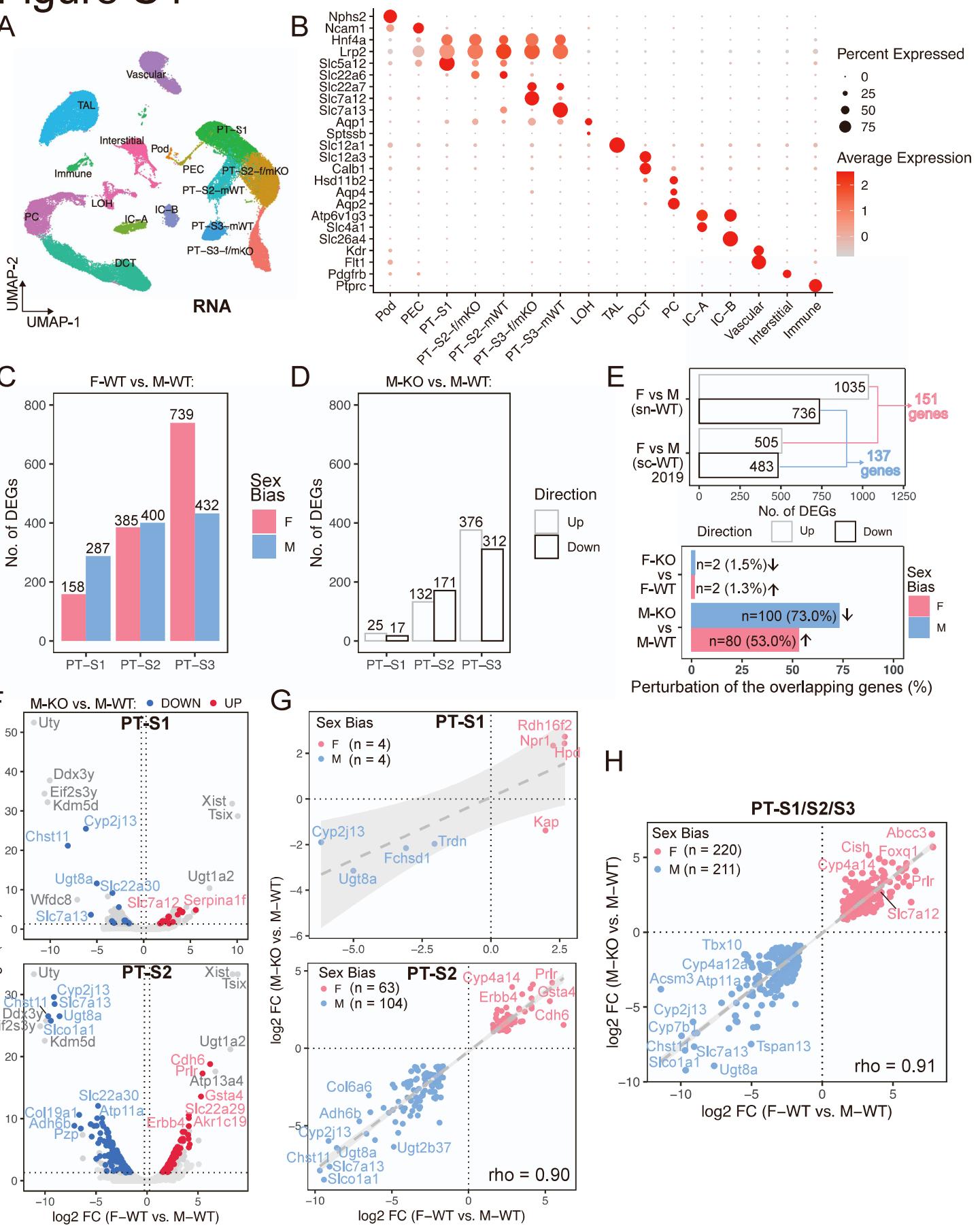


Figure S4. Nephron-specific AR removal reverses dimorphic gene expression in males. Related to Fig. 4.

- (A) UMAP plot shows clustering outcome the RNA modality of the multimodal data. Cluster nomenclature and color scheme are consistent with Fig. 4B.
- (B) Dot plot of established marker expression for all cell populations.
- (C) Bar plot shows the number of sex-biased genes identified in each PT segment.
- (D) Bar plot shows the number of differentially expressed genes comparing M-KO to M-WT in each PT segment.
- (E) Top bar plot shows the number of multimodal sex-biased genes and those identified from our previous single-cell RNA-seq experiment, with the number of overlapping sex-biased genes indicated on the right. Bottom bar plot shows the percentage of the overlapping sex-biased genes that were perturbed by nephron-specific AR removal. The number of genes that are perturbed in each treatment and the corresponding percentages are listed. Arrows indicate the direction of perturbation in gene expression as compared to controls.
- (F) Volcano plots show multimodal sex-biased genes identified in PT-S1 and PT-S2 separately, where genes that are perturbed by AR removal in male are highlighted: comparing M-KO to M-WT, up-regulated genes are in red and down-regulated genes are in blue.
- (G) Scatter plots contrast the effect of nephron-specific AR removal in male to the observed sex biases, among multimodal AR-responsive genes identified in PT-S1 and PT-S2, as shown in (E). Each dot represents a gene, where sex bias differs by color. Changes in gene expression (F-WT vs. M-WT or M-KO vs. M-WT) are summarized by log₂ FC. Spearman's rank correlation coefficient was calculated to assess the overall concordance of the two comparisons.
- (H) Scatter plot compares the effect of nephron-specific AR removal in male to the observed sex biases, among multimodal AR-responsive genes identified in PT-S1, PT-S2, and PT-S3 combined. Each dot represents a gene, where sex bias differs by color. Changes in gene expression (F-WT vs. M-WT or M-KO vs. M-WT) are summarized by log₂ FC. For genes that were perturbed in more than one segments, the maximum effect sizes are shown.

Figure S5

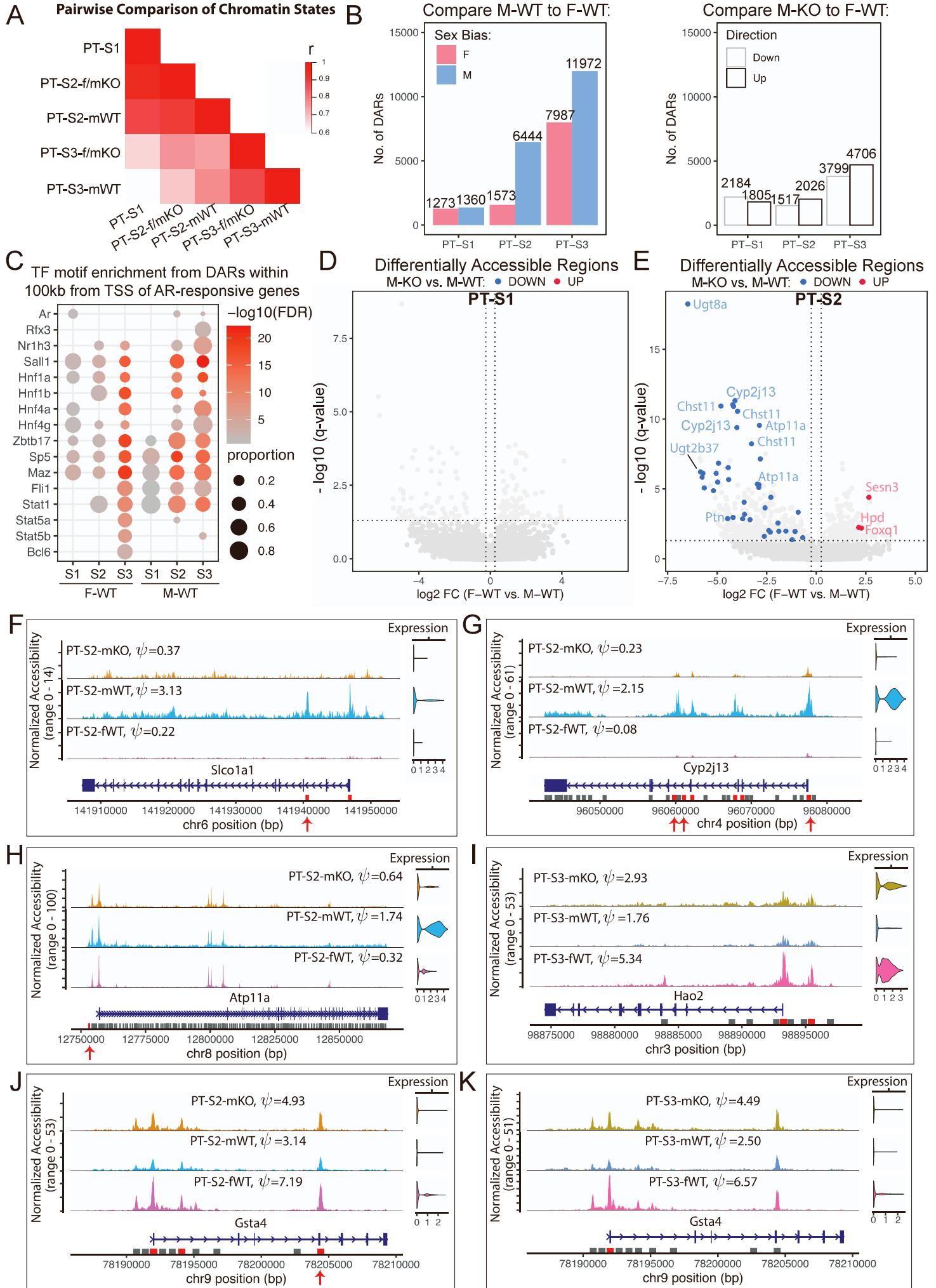
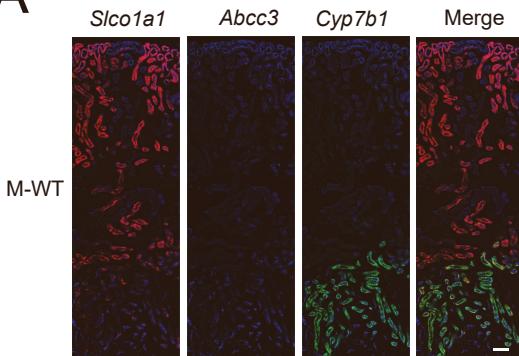
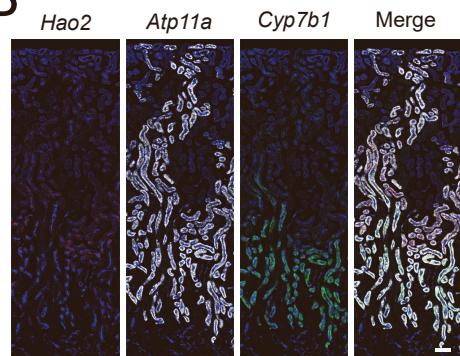
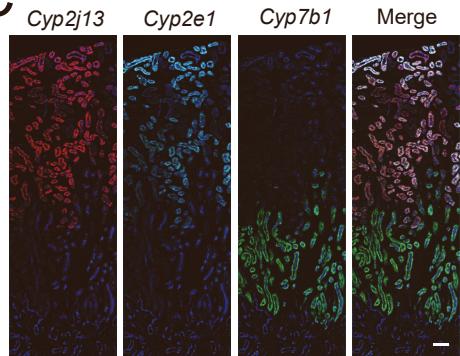
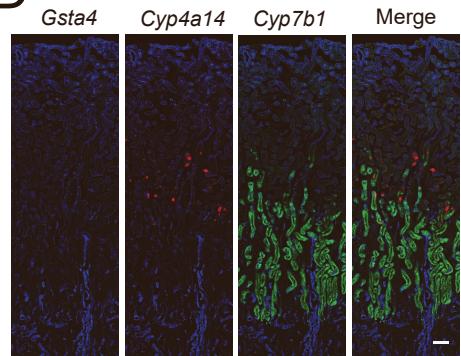


Figure S5. Multimodal data reveals putative AR response elements near sex-biased genes. Related to Fig. 5.

- (A) Heatmap shows pairwise comparison of chromatin state between individual PT clusters. Scatter plot on the right exemplifies the correlation of normalized average peak counts for all PT marker peaks between PT-S3-mWT and PT-S3-f/mKO cluster.
- (B) Bar plot shows number of significantly open regions for each condition of each pairwise comparison (absolute Log2FC > 0.25, adjusted p-value < 0.05).
- (C) Dot plot summarizes TF motif enrichment in the DARs within 100KB to TSS of the AR-responsive sex-biased genes in M-WT compared to F-WT PT segments.
- (D) Volcano plot shows DARs within 100KB of sex-biased genes in PT-S1. 1,360 peaks were differentially open in male (left) and 1,273 peaks were differentially open in female (right). We colored F-biased peaks that are preferentially open in M-KO in red, and M-biased peaks that are preferentially closed in M-KO in blue, if any. Each dot represents a 500-bp region, where the nearest gene is annotated.
- (E) Volcano plot shows DARs within 100KB of sex-biased genes in PT-S2. 6,444 peaks were differentially open in male (left) and 1,573 peaks were differentially open in female (right). We colored F-biased peaks that are preferentially open in M-KO in red, and M-biased peaks that are preferentially closed in M-KO in blue. Each dot represents a 500-bp region, where the nearest gene is annotated.
- (F-K) Coverage plots of top sex-biased genes in M-WT, F-WT, and M-KO PT-S2/3: *Slco1a1*, *Cyp2j13*, and *Atp11a* (M-biased; F-H); *Hao2*, *Gsta4* (F-biased; I-K). Gene expression is also shown by violin plots adjacent to the tracks, with gene accessibility score ψ listed above each track. All peaks called in the region are shown in gray boxes, where DARs are highlighted in red. Peaks with potential AR binding site are indicated by red arrows.

Figure S6

A**B****C****D**

M-WT

F-WT

M-KO

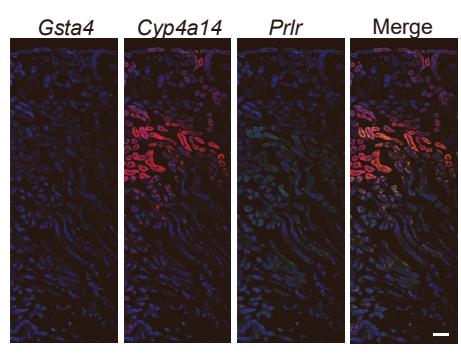
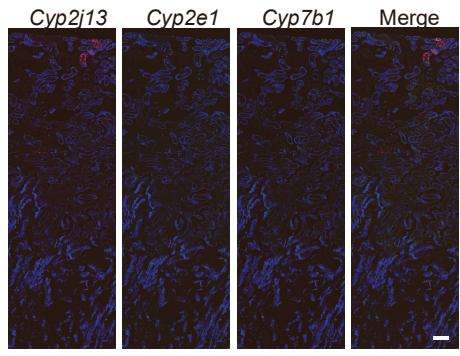
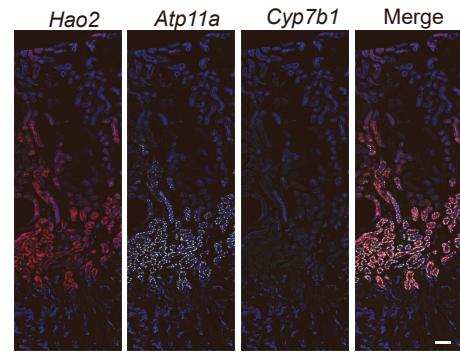
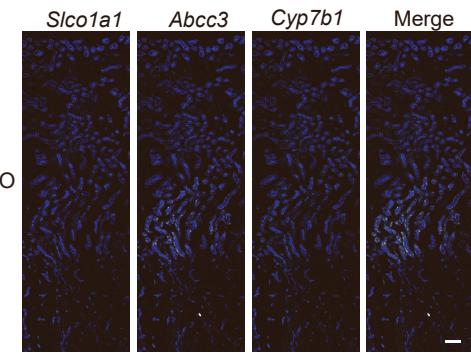
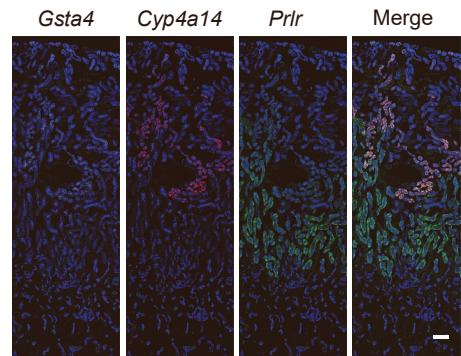
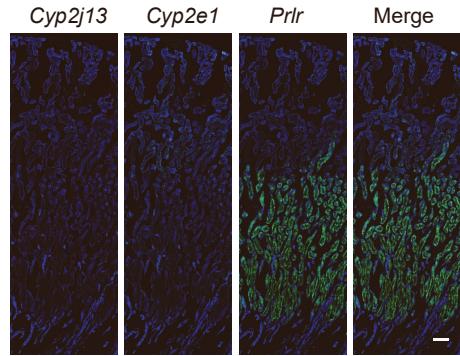
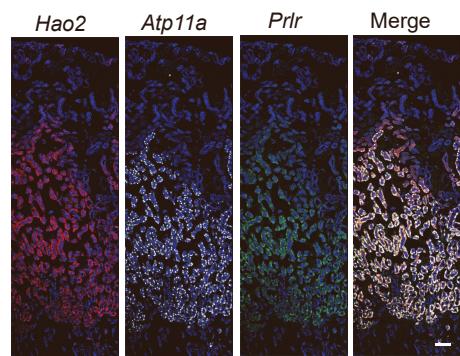
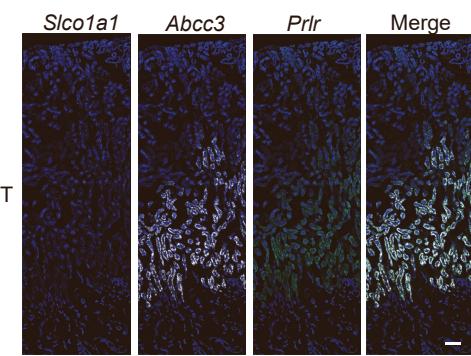


Figure S6. Fluorescent RNA *in situ* hybridization by RNAscope validates dimorphic gene expression in proximal tubule. Related to Fig. 6.

RNAscope assay directly visualized the expression levels of sex-biased genes in M-WT, F-WT, and M-KO across the renal cortex. Scale bars = 100 μ m.

(A) *Slco1a1* (red), *Abcc3* (white), *Cyp7b1* (green) co-stained with antibody against Aqp1 (blue).

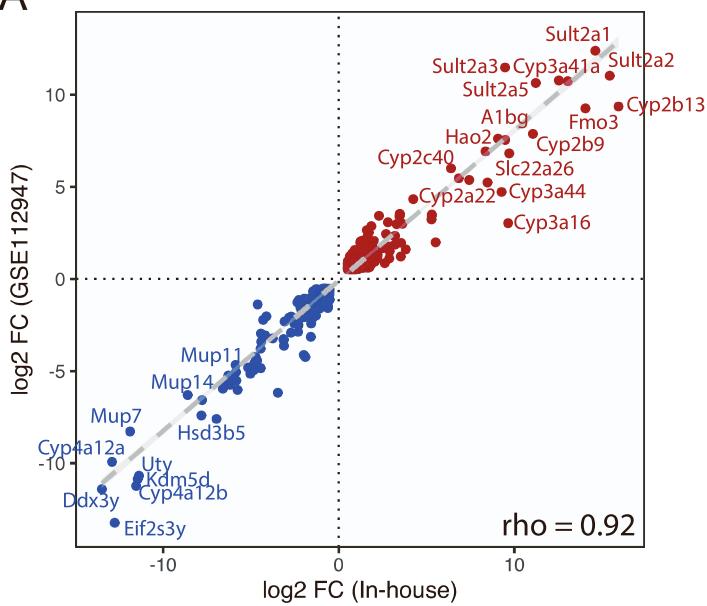
(B) *Hao2* (red), *Atp11a* (white), *Cyp7b1* (green) co-stained with antibody against Aqp1 (blue).

(C) *Cyp2j13* (red), *Cyp2e1* (Cyan), *Cyp7b1* (green) co-stained with antibody against Aqp1 (blue).

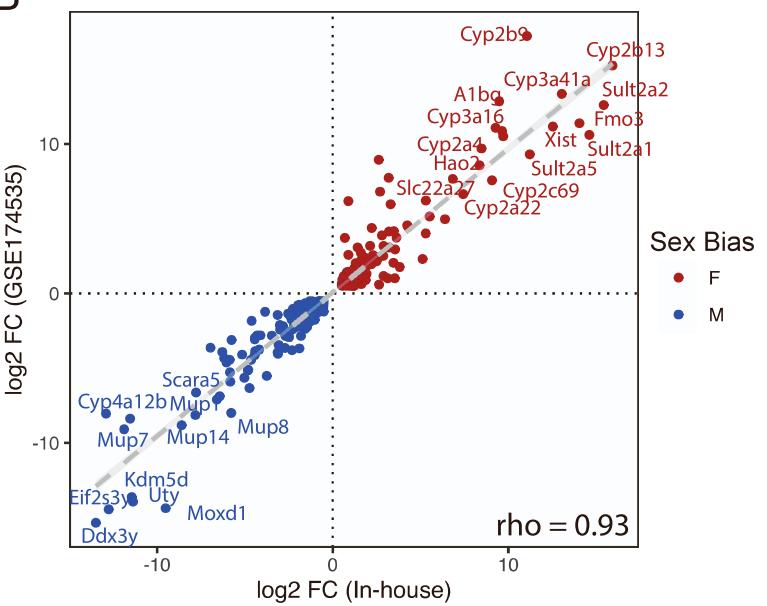
(D) *Gsta4* (white), *Cyp4a14* (red), *Cyp7b1* (green) co-stained with antibody against Aqp1 (blue).

Figure S7

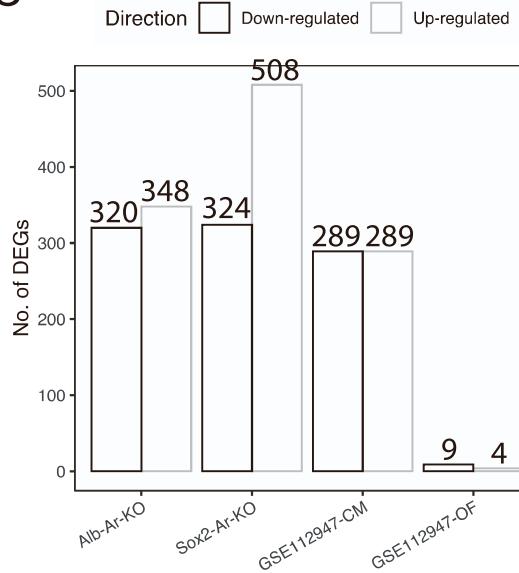
A



B



C



D

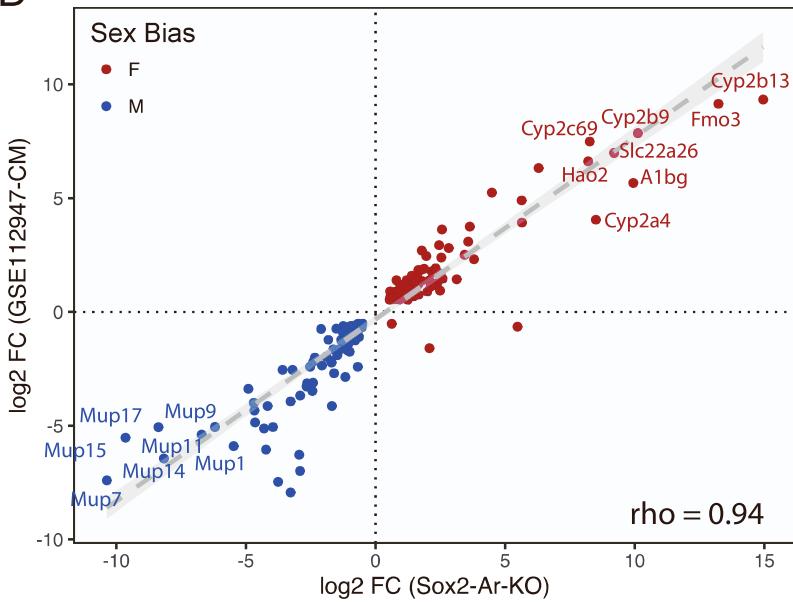


Figure S7. Comparative study on kidney chromatin and hepatic gene expression.
Related to Fig. 7.

(A-B) Scatter plot compares changes in the expression of liver sex-biased genes that are in common between the in-house list and those identified from GSE112947 (A) and GSE174535 (B).

(C) Bar plot compares the number of differentially expressed genes identified in different treatment experiments.

(D) Scatter plot compares changes in the expression of liver sex-biased genes that are commonly perturbed by whole-body AR removal and castration in male mice.