

SI Materials and Methods

Animal experiments. All mouse studies were approved by and performed under the guidelines of the Institutional Animal Care and Use Committee of the Children's Hospital of Philadelphia (CHOP). The REC-ERR γ knockout mice were generated by crossing ERR γ -flox mice (1) with Sim1-Cre mice in the C57BL/6J background (JAX 006451). tdTomato-eGFP reporter mice in the C57BL6 background were obtained from the Jackson Laboratory (JAX 007676). Mice were maintained in temperature- and light-controlled environments. The weaned mice received a standard diet (LabDiet 5L0D); breeding mice and pups before weaning received a breeder diet (LabDiet 5058). Both male and female mice exhibited the described kidney phenotype and therefore were both included in the study. The number of mice used in each experiment and number of times experiments were replicated are described in figure legends or related Materials and Methods sections. All tissues were collected and weighed between 12 and 4 pm to avoid the impact of circadian rhythm.

Cell culture. HKC-8 human renal proximal tubule cells (2) were cultured in DMEM/F12 with 5% FBS (Gemini) and 1 \times antibiotic-antimycotic (ThermoFisher 15240062). HEK293FT cells were cultured in DMEM with 10% FBS and 1 \times antibiotic-antimycotic. Mouse renal tubule cells prepared as previously described (2) were cultured in RPMI 1640 with 10% FBS, 20 ng/ml EGF and 1 \times antibiotic-antimycotic. ERR γ knockdown was performed by transfecting ERR γ siRNA (Origene, SR301465).

Subcellular localization of ERR γ . Kidneys from 1-month old male and female C57BL/6J mice were dissected out and ground in homogenization buffer (10 mM Tris-HCl, pH 8.0, 5 mM CaCl₂,

3 mM Mg(Ac)₂, 0.1 mM EDTA, 1% BSA, 0.32M Sucrose, 0.5% Triton X-100, 1 mM DTT and complete protease inhibitor cocktail) using a Dounce homogenizer. Following passing through a 70 μm cell strainer, the homogenates were spun down at 800g for 10 min, and the pellets were taken as nuclear extracts. The supernatants were spun down at 15,000g for 15 min to separate mitochondrial and cytosolic fractions. The supernatants were designated as cytosolic fractions, and the pellets were kept as mitochondrial extracts. The extracts of nuclei and mitochondria were washed once with the homogenization buffer and lysed in a same volume (as cytosolic fraction) of RIPA buffer containing complete protease inhibitor cocktail on ice for 10 min, followed by a 10-min centrifugation at 12,000g. The supernatants were taken as nuclear and mitochondrial fractions, respectively. The western blot was performed as previously described (3) with antibody against ERRγ (1), Acetyl H3 (EMD Millipore 06-599), GAPDH (Abcam Ab8245) or HSP60 (Calbiochem 386028).

Adenovirus infection. The ERRγ and HNF1β adenoviruses were produced as previously described (4, 5). Briefly, cDNA of mouse ERRγ or HNF1β was cloned and inserted into pShuttle-CMV and then recombined in pAdEasy-1 BJ5183-AD-1 competent cells (Agilent). Adenovirus was generated by transfecting Pac I digested recombined vectors into HEK293FT cells. Control GFP or LacZ adenovirus was previously described (5, 6). Cells were spin-infected at 2,000 rpm for 15 min at room temperature to increase efficiency, with MOI of 75 for HKC-8 cells and MOI of 25 for mouse renal tubular cells. Cells were collected 72 hrs later for gene expression and mitochondrial respiration analysis, and 96 hrs later for glucose uptake assay.

Glucose uptake measured by 2-NBDG. 2-NBDG assay was performed following a previously published protocol with modifications (7). HKC-8 cells were plated in 96-well black wall plates (Greiner) pre-coated with 0.1% gelatin for at least 3 hrs. Briefly, HKC-8 cells were first incubated with 2 μ M glucose transporter (GLUT) inhibitor cytochalasin B (Cayman Chemical) in growth media for 2 hrs. Cells were then washed twice and incubated for 6 hrs in glucose-free incubation solution (140 mM NaCl, 5 mM KCl, 5 mM CaCl₂, 1 mM MgSO₄, 1 mM KH₂PO₄, 10 mM HEPES, 2 mM Sodium pyruvate, pH 7.4) with 50 μ M 2-NBDG (Cayman Chemical) and 2 μ M cytochalasin B. The plates were then rinsed three times with the incubation solution before 2-NBDG amount was measured using Spectramax Paradigm (Molecular Devices). Glucose uptake was calculated using 2-NBDG fluorescence intensity normalized to cell number in each well.

Histological analysis and X-gal staining. For histological analysis, kidneys were fixed with 4% paraformaldehyde and embedded in paraffin. The sections were stained with hematoxylin and eosin (H&E) or periodic acid Schiff (PAS). For X-gal staining, 1-month-old ERR γ heterozygous mice were perfused with 2 ml PBS and then 5 ml 2% paraformaldehyde in PBS (1 ml/min). Whole kidneys were embedded in OCT compound (Tissue-Tek) for cryosections of 30-40 μ m thickness. The sections were stained in staining solution (1 mg/ml X-gal, 5 mM K₃[Fe(CN)₆], 5 mM K₄[Fe(CN)₆], 2 mM MgCl₂, 0.01% sodium deoxycholate and 0.02% Nonidet P-40 in PBS) at 37 °C overnight. The sections were then post-fixed with 4% paraformaldehyde and counterstained with nuclear fast red for 5 min. Individual, overlapping parts of the kidney section were imaged and merged by Photoshop to generate the final picture.

Transmission electron microscopy (TEM). TEM samples were prepared and the images were taken using a Jeol-1010 transmission electron microscope as previously described (3).

Gene expression analysis. We isolated total RNA from mouse tissues or cells using RNazol RT (Molecular Research Center) following the manufacturer's instructions. We synthesized cDNA from 1 µg total RNA using iScript cDNA synthesis kit (Bio-Rad) and quantified mRNA levels by real-time qPCR using SYBR Green (Bio-Rad). We ran samples in technical triplicates and calculated relative mRNA levels using a standard curve and normalized to 36b4 mRNA level in the same sample. The primer sequences for qPCR were listed in Table S1.

RNA-Seq. We performed RNA-Seq largely as previously described (8). Total RNA from littermate mouse kidneys were purified. RNA quality was assessed using a Bioanalyzer (Agilent). Sequencing libraries were prepared using a TruSeq Stranded mRNA sample prep kit (Illumina). Libraries were assessed using a Bioanalyzer, quantified using qPCR (Kapa Biosystems) and sequenced on a NextSeq 500 (Illumina) to a depth of >35 million paired-end reads with read lengths of 42 bp. Sequenced reads were aligned to the mm10 reference mouse genome using STAR(9) and differentially expressed RNAs were identified using DESeq2 (10) (FDR-adjusted p-value <0.01, fold change>±1.5). STAR alignment and DESeq2 analysis were executed through the RNA Express BaseSpace application (Illumina). Pathway analysis was performed using Metascape (<http://metascape.org/gp/index.html#/main/step1>, performed in September 2017 and March 2018) (11). The raw and processed data are deposited in the GEO data base (accession number GSE104907).

Microarray. Transcript levels in human kidney tubule samples were analyzed by Affymetrix U133A and 1.0ST arrays. Probes were prepared using the Affymetrix 3' IVT kit. After hybridization and scanning, raw data files were imported into Genespring GX software (Agilent Technologies, USA). Raw expression levels were normalized using the RMA16 summarization algorithm. The raw and processed data are deposited in the ArrayExpress database (accession number E-MTAB-2502).

Mitochondrial enzyme activity. Kidneys of 3-week-old Cre- and Cre+ littermate mice were collected, and the activities of Complex II (succinate dehydrogenase) and Complex IV (cytochrome c oxidase) were determined as previously described (3).

Mitochondrial DNA analysis. The ratio of mitochondrial DNA to nuclear DNA (mtDNA/nDNA) was determined by quantifying two mitochondrial genes (Cytb and Cox1) and two nuclear genes (Glucagon and β -Globin) using qPCR as described previously (3). The primer sequences are listed in Table S1.

Oxygen consumption rate (OCR). The measurement of OCR in lacZ or ERR γ adenovirus infected HKC-8 cells was performed using XF24 or XFe96 extracellular flux analyzer (Seahorse Bioscience) as previously described (1). Briefly, HKC-8 cells were plated at density of 20,000 cells/well in a Seahorse cell culture microplate, and infected with lacZ or ERR γ adenovirus. Cellular OCR was measured 3 days later and normalized to protein quantity in each well. The final concentration for oligomycin, DNP, FCCP, rotenone and antimycin used was 1 μ M, 600 μ M, 8 μ M, 2 μ M and 4 μ M, respectively.

STZ induced diabetes mellitus model. 10-13 week-old mice were injected intraperitoneally with 50 mg/kg STZ (freshly dissolved in citrate buffer, pH 4.5) daily for 5 consecutive days. Two weeks after the last injection, blood, urine and tissues were collected for analysis after overnight fast.

Measurement of plasma glucose, insulin, urine glucose and creatinine. The glucose levels in plasma and urine were measured with a Glucose Colorimetric Assay Kit (Cayman Chemical). Plasma insulin level was measured with an ELISA kit (Crystal Chem). Urine creatinine level was measured with a Creatinine (urinary) Colorimetric Assay Kit (Cayman Chemical).

CoIP and western blot analysis. HEK 293 cells were transfected with pcDNA3.1D-ERR γ and pShuttle-CMV-HNF1 β using Fugene 6 (Promega). Cells were lysed 48 hrs later in non-denaturing buffer (20 mM Tris-HCl, pH 8, 150 mM NaCl, 0.5% Nonidet P-40, 10% glycerol, 1 mM EDTA and complete protease inhibitor cocktail). The lysates were pre-cleared with protein A agarose beads (Millipore) before incubation with IgG (Santa Cruz sc-2027) or ERR γ antibody (1) at 4 °C overnight. The antibody-protein mixture was then incubated with protein A agarose beads for 3 hrs at 4 °C and washed 4 times with wash buffer (20 mM Tris-HCl, pH 8, 150 mM NaCl, 0.5% Nonidet P-40, 1 mM EDTA and complete protease inhibitor cocktail) before being eluted with Laemmli sample buffer. The immunoprecipitates were analyzed by western blotting performed as previously described (3) with HNF1 β antibody (Santa Cruz sc-22840).

ChIP and ChIP-Seq. Kidneys were dissected from 2-3-month-old WT mice and chopped into fine pieces. After digestion with 0.5 mg/ml collagenase I (Worthington) for 15 min at 37 °C, the kidney/collagenase mixture was pushed through a 70 µm cell strainer. ACK lysis buffer was then used to remove red blood cells. Typical yield was around 8 million cells per mouse (2 kidneys). ChIP was performed in duplicate as previously described (1), using around 2 million cells per antibody with the following antibodies: IgG (Santa Cruz sc-2027), HNF1β (Santa Cruz sc-22840), ERRγ (1), and acetyl-Histone H3 (Millipore 06-599). ERRγ or HNF1β ChIP-Seq was performed in duplicate using around 5-10 million cells per antibody. DNA libraries were prepared using KAPA Hyper Prep kits (Kapa Biosystems), size selected using a PippenHT (Sage Science), and sequenced using 75 bp single-end reads on an Illumina NextSeq 500 instrument. Raw sequence reads were aligned to the mm10 reference genome using bowtie (version 1.2) with parameters “—best” and “-m 1” to ensure reporting of uniquely mapping reads (tags). ChIP-Seq analysis was performed using HOMER. To begin, tag directories were generated using the “-tbp 1” command to eliminate clonal reads. Peak calling for ChIP-Seq was performed using fold enrichment over input chromatin > 4, FDR = 0.01 and Poisson p-value < 1.00e-04. Consensus ChIP-Seq peaks were identified as sites present in biological replicate datasets for ERRγ and HNF1β. Peaks were annotated to the gene with the nearest transcription start site. Colocalization analysis was performed using a 200 bp distance to determine co-bound peaks. *De novo* motif enrichment analysis was performed by scanning a 200 bp window around binding sites using standard background (random genomic sequences sampled according to GC content of peak sequences). Browser tracks were generated and displayed using the UCSC genome browser. Pathway analysis was performed using GREAT (performed in September 2017) (12). The raw and processed data are deposited in the GEO data base (accession number GSE104907).

ChIP-reChIP and ChIP-reChIP-Seq. We started with around 40 million cells for ChIP-reChIP-qPCR and 80-100 million cells for ChIP-reChIP-Seq. After the first round of ChIP with ERR γ or HNF1 β antibody, the immunoprecipitate was released in elution buffer (100 mM NaHCO₃ and 1% SDS) containing 10 mM DTT (to disrupt 1st antibody) for 30 min at 37 °C, and then diluted 60 times using dilution buffer (16.7 mM Tris, pH 8.0, 167 mM NaCl, 1.2 mM EDTA, 1.1% Triton X-100 and 0.01% SDS). This was followed by a second round of ChIP using IgG, HNF1 β or ERR γ antibody. DNA library preparation, sequencing and bioinformatic analysis are the same as described for ChIP-Seq, except that: a) peak calling used Poisson p-value < 1.00e-03; b) peaks were further filtered with fold enrichment over reChIP performed with pre-immune IgG (negative control antibodies) of >2-fold as an additional criterion for peak identification. The raw and processed data are deposited in the GEO data base (accession number GSE104907).

Statistical analysis. Two-tailed, unpaired, unequal variance Student's *t* test was used for comparison between two groups, and data were presented as mean \pm SEM. Pearson's correlation was used in Fig. 6A. Fisher's exact test was used in Fig. 6C, D. *P*<0.05 was considered to be statistically significant.

References

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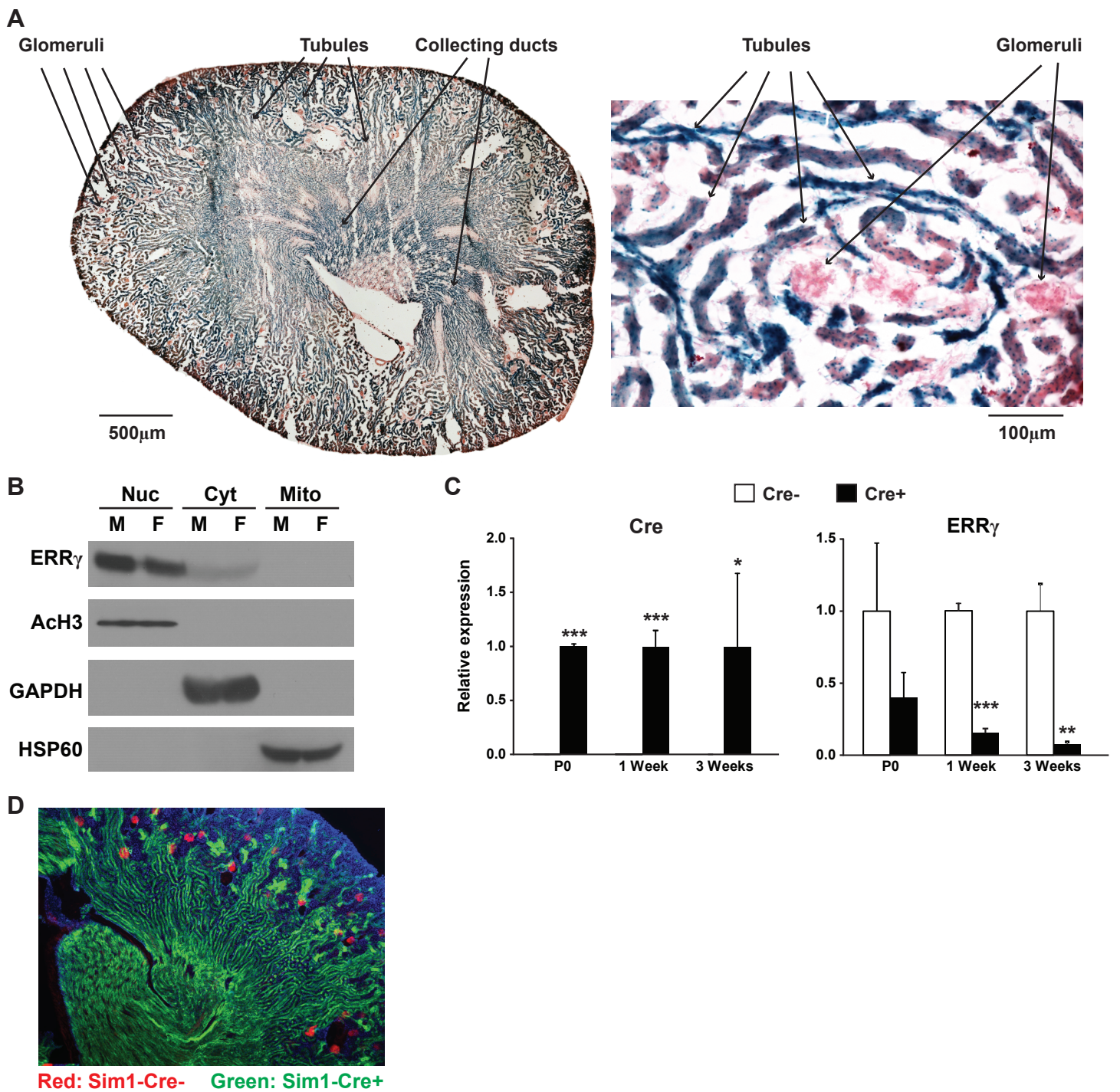


Figure S1. Renal ERR γ expression pattern and generation of REC-ERR γ KO mice.

- (A) ERR γ is highly expressed in renal tubule and collecting duct RECs but barely in glomeruli. ERR γ expression pattern is revealed by X-gal staining of 1-month-old ERR γ Het (LacZ⁺) mouse kidney sections. Left: whole kidney cross section. Right: enlarged cortex area showing ERR γ ⁺ tubules and ERR γ ⁻ glomeruli. Some of the glomeruli, tubules and collecting ducts are labeled with arrows.
- (B) Nuclear localization of ERR γ in 1-month-old mouse kidneys detected by western blot. AcH3, GAPDH and HSP60 were used as controls for nuclear (Nuc), cytosolic (Cyt) and mitochondrial (Mito) proteins, respectively. M - male; F - female.
- (C) Expression of Cre and ERR γ in the kidneys of REC-ERR γ KO mice of different ages measured by qRT-PCR: P0 (n=2 for Cre-, n=3 for Cre⁺), 1 week (n=4 for Cre-, n=7 for Cre⁺) and 3 weeks (n=7 for Cre-, n=10 for Cre⁺). Both male and female mice are included. Error bar is s.e.m. *p<0.05, **p<0.01 and ***p<0.001 by t-test.
- (D) Sim1-Cre mediates recombination in both tubule and collecting duct RECs. Sim1-Cre mice were bred with a Tomato-eGFP reporter mouse strain. In this 1-month-old mouse kidney section, green indicates cells that express Cre thus the deletion of ERR γ . Red indicates areas without recombination, including the glomeruli.

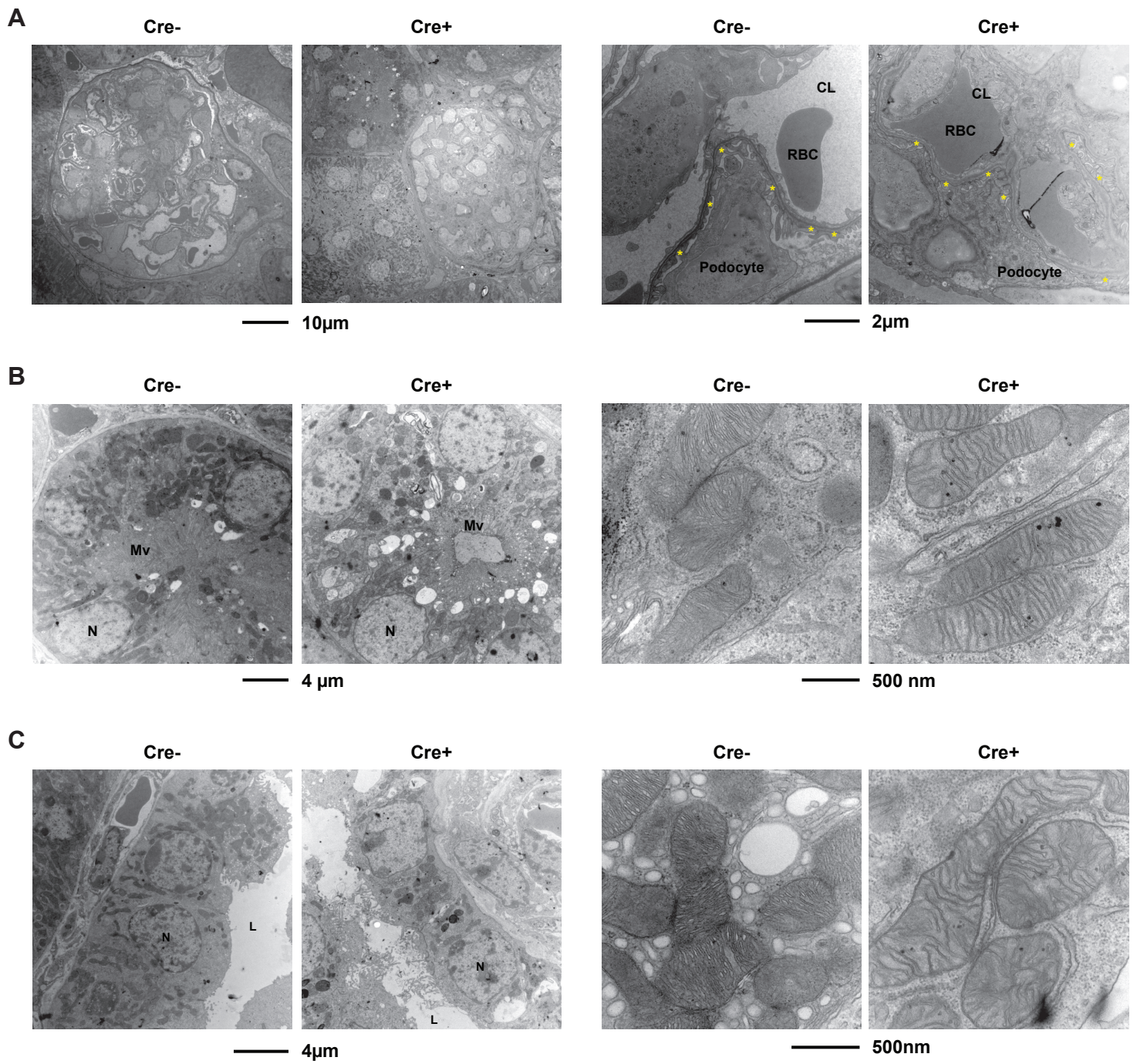


Figure S2. Renal epithelial cell ultrastructures in 3-week-old control and REC-ERR γ KO mice.

(A) EM pictures featuring glomeruli. CL - capillary lumen; RBC - red blood cell; * indicate pedicels of podocytes.

(B) EM pictures featuring proximal tubular epithelial cells (left) and their mitochondria (right). Mv - microvilli (brush border); N - nucleus.

(C) EM pictures featuring distal tubular epithelial cells (left) and their mitochondria (right). L - tubular lumen; N - nucleus.

In (A-C) left two pictures are of lower magnification; right two pictures are of higher magnification.

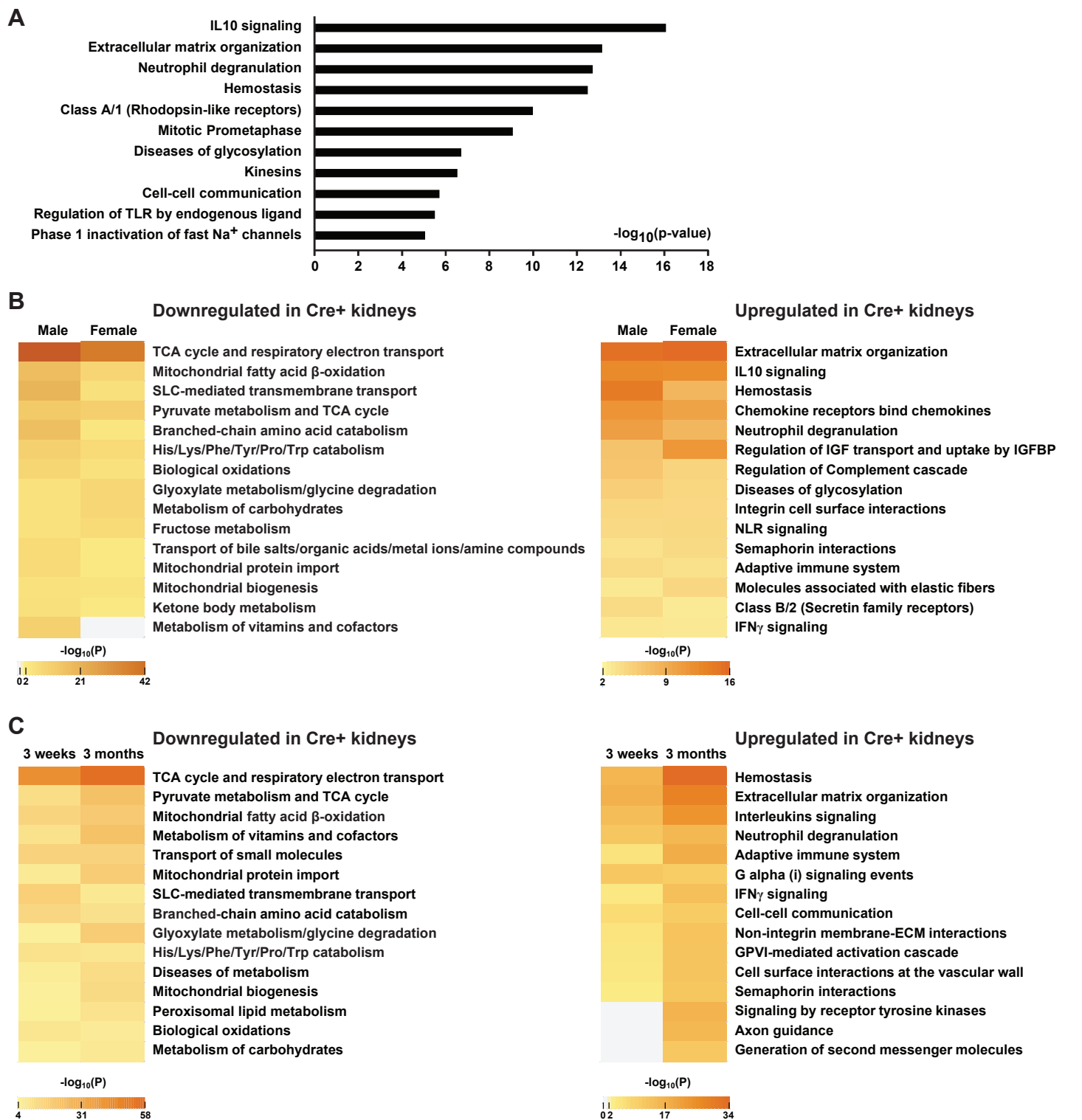


Figure S3. $ERR\gamma$ is essential for normal expression of REC mitochondrial and reabsorptive function genes.

(A) Top enriched pathways of upregulated 1406 genes in 3-week-old REC- $ERR\gamma$ KO mouse kidneys.

(B) Comparison of top enriched pathways of down- (left) and upregulated (right) genes between male and female 3 weeks old REC- $ERR\gamma$ KO mouse kidneys.

(C) Comparison of top enriched pathways of down- (left) and upregulated (right) genes between 3 weeks and 3 months old male REC- $ERR\gamma$ KO mouse kidneys.

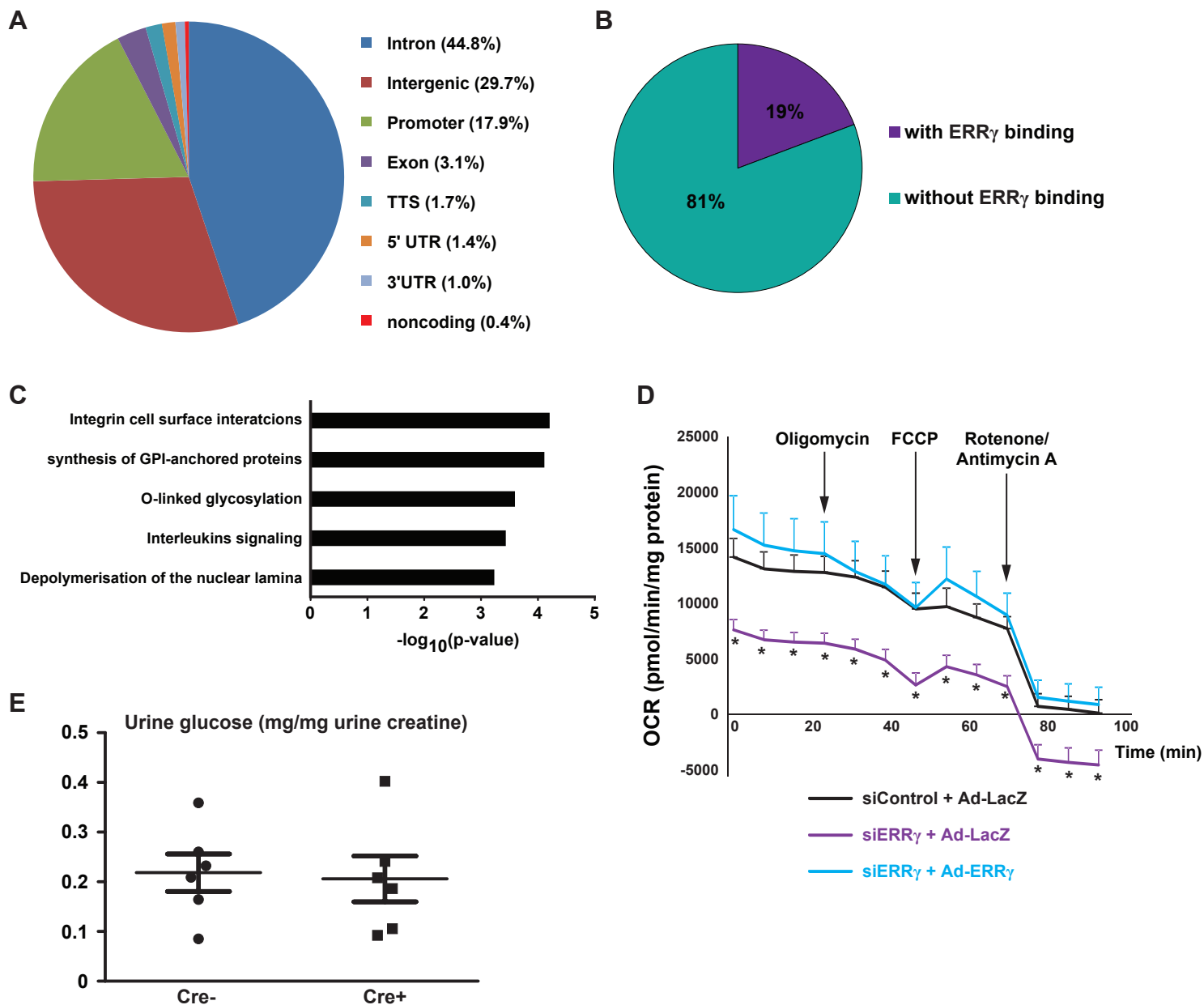


Figure S4. $ERR\gamma$ regulates REC mitochondrial and reabsorptive functions.

(A) Distribution of $ERR\gamma$ ChIP-Seq peaks shown in a pie chart.

(B) Pie graph showing upregulated genes in 3-week-old REC- $ERR\gamma$ KO mouse kidneys with or without annotated $ERR\gamma$ binding peaks based on ChIP-Seq annotations.

(C) Top enriched pathways of upregulated genes with annotated $ERR\gamma$ binding peaks.

(D) Mitochondrial respiration in HKC-8 cells treated with $ERR\gamma$ siRNA and/or Ad- $ERR\gamma$. Oxygen consumption rate (OCR) was measured using a Seahorse XFe96 analyzer. $n=7$ for all treatment groups. Error bar indicates s.e.m. * $p<0.05$ between si $ERR\gamma$ +Ad-LacZ and the other two groups by t-test. No significant difference between siControl+Ad-LacZ and si $ERR\gamma$ +Ad- $ERR\gamma$ group.

(E) Urine glucose levels in 13 weeks old control ($n=6$) and REC- $ERR\gamma$ KO ($n=6$) mice.

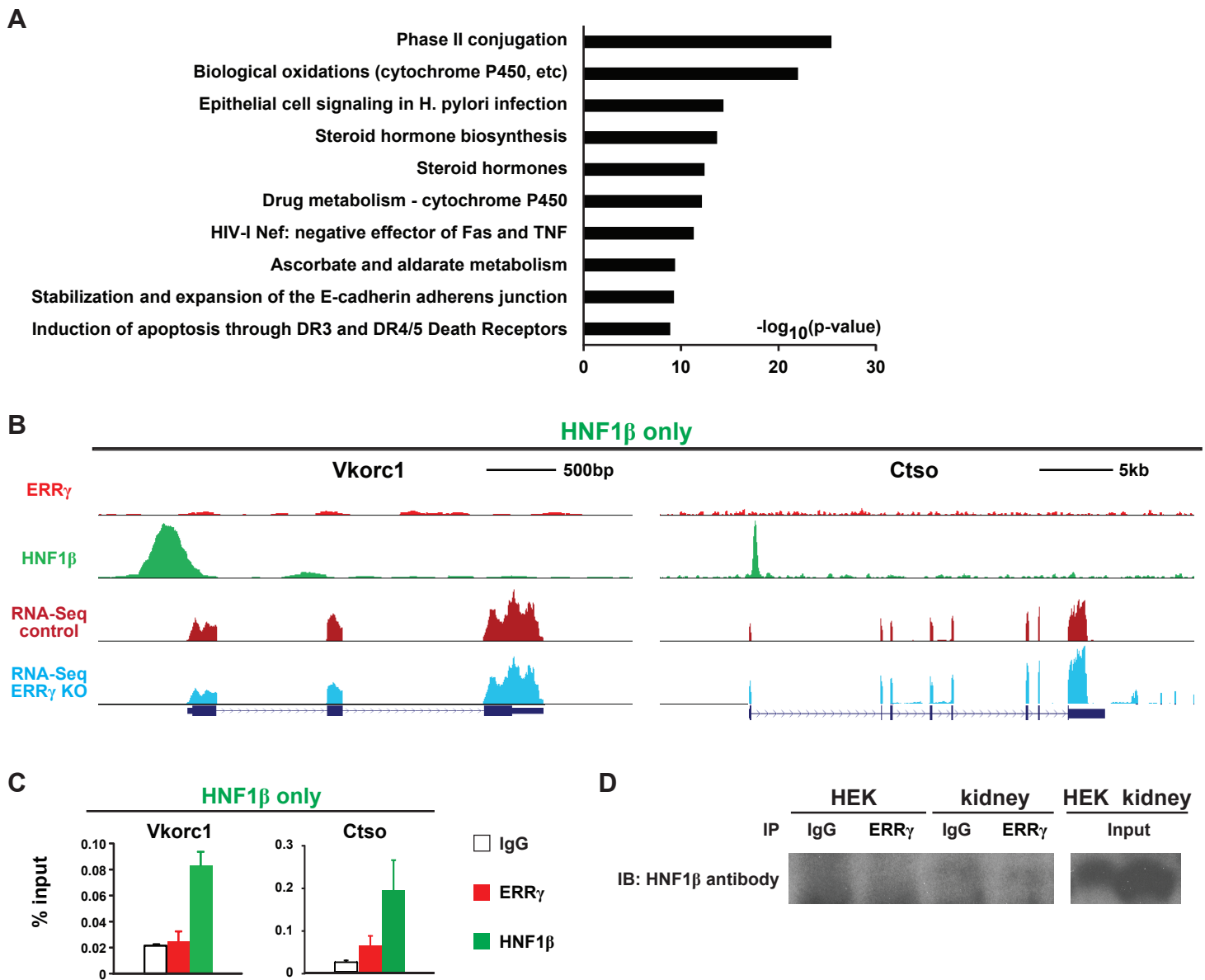


Figure S5. ERR γ cooperates with HNF1 β to regulate the transcription of renal reabsorptive genes.

(A) Top 10 ranked pathways of genes annotated to HNF1 β only ChIP-Seq peaks.

(B) Representative ERR γ and HNF1 β ChIP-Seq peaks. Y axis indicates peak reads. The scales of control and ERR γ KO RNA-Seq tracks are the same.

(C) Increased binding of HNF1 β to its targets.

(D) Interaction between ERR γ and HNF1 β is either indirect or not strong enough to be detected by coIP-Western blot.

Lysates from ERR γ and HNF1 β transfected HEK293 cells or from adult mouse kidneys were immunoprecipitated with IgG or ERR γ antibody, followed by Western blot using HNF1 β antibody.

Table S1. Oligonucleotide sequences

qRT-PCR	
mERRg For	GAATCTTTTTCCCTGCACTACGA
mERRg Rev	GCTGGAATCAATGTGTCGATCTT
mCS For	GGACAATTTTCCAACCAATCTGC
mCS Rev	TCGGTTCATTCCCTCTGCATA
mNdufa4 For	TCCCAGCTTGATTCTCTCTT
mNdufa4 Rev	GGGTTGTTCTTTCTGTCCCAG
mSdhb For	CTGAATAAGTGCGGACCTATGG
mSdhb Rev	AGTATTGCCTCCGTTGATGTTC
mCox5b For	GGAAGACCCTAATCTAGTCCCG
mCox5b Rev	GTTGGGGCATCGCTGACTC
hCox5b For	TGTGAAGAGGACAATACCAGCG
hCox5b Rev	CCAGCTTGTAATGGGCTCCAC
mAtp5b For	ACGTCCAGTTCGATGAGGGAT
mAtp5b Rev	TTTCTGGCCTCTAACCAAGCC
hAtp5b For	AAACAATTTGCTCCCATTTCATGC
hAtp5b Rev	GACAACCTTGATAACCAGTCACC
mCpt1b For	GCACACCAGGCAGTAGCTTT
mCpt1b Rev	CAGGAGTTGATTCCAGACAGGTA
mCpt2 For	CAGCACAGCATCGTACCCA
mCpt2 Rev	TCCCAATGCCGTTCTCAAAAT
mAcadm For	AGGGTTTAGTTTTGAGTTGACGG
mAcadm Rev	CCCCGCTTTTGTTCATATTCCG
mHadha For	TGCATTTGCCGCAGCTTTAC
mHadha Rev	GTTGGCCCAGATTTTCGTTCA
mSgk2 For	TCCAGCCCAGTTGGAGTTC

mSgk2 Rev	CCGTAGTTCCTTTGCCAATG
mAtp1a1 For	GTATCGGAGCATGGTGACAAA
mAtp1a1 Rev	TCGTCCATAGACACTTCCTTCT
mAtp1b1 For	TCGGGACCATCCAAGTAATGC
mAtp1b1 Rev	GGGAATCTGTGTCAATCCTG
mSlc5a2 For	ATGGAGCAACACGTAGAGGC
mSlc5a2 Rev	ATGACCAGCAGGAAATAGGCA
hSlc5a2 For	ACACGGTACAGACCTTCGTCA
hSlc5a2 Rev	GCTGCTCCCAGGTATTTGTC
mSlc17a1 For	TCTGTTCCCTCCGGTATGGAC
mSlc17a1 Rev	AGAACTGAGAATAAGCCCTTGGA
mSlc34a1 For	TGCCTCTGATGCTGGCTTTC
mSlc34a1 Rev	GATAGGATGGCATTGTCCTTGAA
mSlc12a1 For	TCATTGGCCTGAGCGTAGTTG
mSlc12a1 Rev	TTTGTGCAAATAGCCGACATAGA
mSlc6a19 For	CAGGTGCTCAGGTCTTCTACT
mSlc6a19 Rev	CGATCACAGAATCCATCTCACAA
mPkd2 For	GCAAGCTGACAACCGAAGC
mPkd2 Rev	CTCACTGCTGACGGAGTAGAC
mHNF1 β For	GAGTGTAACAGGGCAGAATGT
mHNF1 β Rev	GGTTTGCGAACCAGTTGTAG

ChIP/ChIP-reChIP qPCR

mSdhb For	CTTAGGAACATAGGAAGCCCAG
mSdhb Rev	TTTCTCCGAGCCAATGTACAG
mAtp5b For	CATGTTGAGTCTTGTGGGGCGTGTGGC
mAtp5b Rev	GAGAAGAACCGAACTAGCTCCTCCAGG
mCpt1b For	CCTTACTCTCAGCCAAGCTATC

mCpt1b Rev	AGCAATGGTGCAGGAATCT
mAcadm For	TCTCCAAGTAAAGGTCACAGC
mAcadm Rev	AAGGTCACGTTCTTTCCAGAG
mHadha For	GAGAAGAGTCGGGAGCAAAG
mHadha Rev	TCTGGACTTGACCTTGTTGG
mSgk2 For	GTGATTCAGCCCAGCAGTTA
mSgk2 Rev	TGACTCTCAAACCAGCACAG
mAtp1a1 For	GAGGGACAAAGAACAGGG
mAtp1a1 Rev	TGCTCCGATCATAACTGC
mAtp1b1 For	GGTCATAGAGCAGGAAGC
mAtp1b1 Rev	TAACTTGAGGCTATTGG
mSlc17a1 For	AGGTTCTAGTCCAGGAGAT
mSlc17a1 Rev	CCTACTTGCTGGTAAACTCT
mPkd2 For	ACACGGCAAGAAAGGACAC
mPkd2 Rev	CCTGGGCTGGA ACTCTTAAAG
mVkorc1 For	CCA ACTACAATTCCCATCATGC
mVkorc1 Rev	ATTCTTAGGCATGACAGCTAGG
mCtso For	CACCAAACCTGTCGCATAGA
mCtso Rev	GAGAGAACTTGATGGTGGTAGAC

mtDNA/nDNA analysis

mCytb For	CATTTATTATCGCGGCCCTA
mCytb Rev	TGTTGGGTTGTTTGATCCTG
mCox1 For	TGCTAGCCGCAGGCATTACT
mCox1 Rev	CGGGATCAAAGAAAGTTGTGT
mGlucagon For	CAGGGCCATCTCAGAACC
mGlucagon Rev	GCTATTGGAAAGCCTCTTGC
mGlobin For	GAAGCGATTCTAGGGAGCAG

mGlobin Rev

GGAGCAGCGATTCTGAGTAGA
