



Genomic integration of ERR γ -HNF1 β regulates renal bioenergetics and prevents chronic kidney disease

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Edited by Bert W. O'Malley, Baylor College of Medicine, Houston, TX, and approved April 11, 2018 (received for review March 21, 2018)

Mitochondrial dysfunction is increasingly recognized as a critical determinant of both hereditary and acquired kidney diseases. However, it remains poorly understood how mitochondrial metabolism is regulated to support normal kidney function and how its dysregulation contributes to kidney disease. Here, we show that the nuclear receptor estrogen-related receptor gamma (ERR γ) and hepatocyte nuclear factor 1 beta (HNF1 β) link renal mitochondrial and reabsorptive functions through coordinated epigenomic programs. ERR γ directly regulates mitochondrial metabolism but cooperatively controls renal reabsorption via convergent binding with HNF1 β . Deletion of ERR γ in renal epithelial cells (RECs), in which it is highly and specifically expressed, results in severe renal energetic and reabsorptive dysfunction and progressive renal failure that recapitulates phenotypes of animals and patients with HNF1 β loss-of-function gene mutations. Moreover, ERR γ expression positively correlates with renal function and is decreased in patients with chronic kidney disease (CKD). REC-ERR γ KO mice share highly overlapping renal transcriptional signatures with human patients with CKD. Together these findings reveal a role for ERR γ in directing independent and HNF1 β -integrated programs for energy production and use essential for normal renal function and the prevention of kidney disease.

nuclear receptor | ERR γ | mitochondria | kidney | renal reabsorption

Mitochondria are organelles that generate the majority of cellular energy through oxidative phosphorylation (OxPhos) and fatty acid oxidation (FAO). Their optimal function is central to health. Mutations of mitochondrial DNA and proteins directly cause mitochondrial disease, with severe defects often observed in organs of high mitochondrial content and energetic demand, including the kidney (1–3). Mitochondrial dysfunction in general has been recognized to broadly contribute to heart disease, obesity, diabetes, neurodegeneration, aging, and many kidney diseases including acute kidney injury, polycystic kidney disease, and chronic kidney disease (CKD) (1, 4–8). CKD is characterized by gradual loss of kidney function with various etiologies, incompletely understood pathophysiology, and no cure. CKD features both glomerular and tubular cell dysfunction as well as metabolic dysregulation (9). Transcriptomic and metabolomic studies have implicated impaired mitochondrial OxPhos in many kidney diseases including CKD (10, 11). A recent study also shows that renal mitochondrial OxPhos and FAO are among the top dysregulated cellular pathways in both patients with CKD and animal models (12).

Among all kidney cells, the tubule and collecting duct renal epithelial cells (RECs) have a very high density of mitochondria. The main physiological function of RECs is to maintain whole-body homeostasis of osmolality, acid-base balance, and extracellular fluid volume through reabsorption of water, critical nutrients, and electrolytes. Mechanistically, these reabsorptive functions are achieved by the work of many membrane transporters and channels

specific for water, glucose, amino acids, sodium, chloride, bicarbonate, and other biomolecules on the REC apical and basolateral surfaces, using cellular ATP as the ultimate energy source. Accordingly, RECs are densely packed with mitochondria and depend on mitochondrial OxPhos and FAO to generate energy to support their reabsorptive functions. However, it remains poorly understood how mitochondrial metabolism is coordinated with kidney reabsorptive functions, and how mitochondrial dysfunction contributes to kidney disease.

Recent work has revealed nuclear receptor estrogen-related receptor gamma (ERR γ) as a critical transcriptional regulator of mitochondrial OxPhos and FAO (13–15). Studies using cell type-specific ERR γ KO mice bypassed the perinatal lethality of whole-body ERR γ KO mice and provided definitive evidence for an essential role of ERR γ in neuronal metabolism and learning/memory, cardiac metabolism and contraction/conduction, functional maturation of pancreatic β cells, brown adipocyte thermogenic function, and skeletal muscle fiber type determination and function (16–22). Genomic studies show that ERR γ directly

Significance

Renal epithelial cells (RECs) contain abundant mitochondria that are essential to support renal reabsorption of electrolytes, glucose, and amino acids. However, it remains poorly understood how mitochondrial metabolism is coordinated with kidney reabsorptive functions. Here we show that deletion of estrogen-related receptor gamma (ERR γ) in RECs results in severe renal mitochondrial and reabsorptive dysfunction with fluid-filled cysts. ERR γ directly regulates mitochondrial metabolism and cooperates in regulating renal reabsorption genes with hepatic nuclear factor 1 beta (HNF1 β), mutations of which cause strikingly similar renal dysfunction and cysts in animals and humans. These findings reveal a role for ERR γ in simultaneously coordinating a transcriptional program of renal energy-generating mitochondrial and energy-consuming reabsorptive functions relevant to kidney disease.

Author contributions: J.Z. and L.P. designed research; J.Z., K.L., B.J.W., J.L., Y.O., A.L.A., C.M., G.D.B., and L.P. performed research; C.Q. and K.S. contributed new reagents/analytic tools; J.Z., K.L., B.J.W., C.Q., J.L., C.M., K.S., G.D.B., and L.P. analyzed data; and J.Z., G.D.B., and L.P. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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Data deposition: Microarray data were deposited in ArrayExpress database (E-MTAB-2502). RNA-Seq, ChIP-Seq, and ChIP-reChIP-Seq data were deposited in the GEO database (GSE104907).

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1804965115/-DCSupplemental.

Published online May 7, 2018.

binds to and activates the transcription of hundreds of genes important in mitochondrial OxPhos in neurons, and OxPhos, FAO, and cardiac contraction genes in heart cells (17, 23). In addition, ERR γ is highly expressed in the kidney and is essential for normal embryonic kidney development (24). Notably, human genetic studies reveal that a de novo reciprocal translocation at t(1, 2)(q41; p25.3) involving the ERR γ locus is associated with bilateral renal agenesis/hypoplasia/dysplasia (25).

Here we generated REC-ERR γ KO mice and show that they develop renal mitochondrial and reabsorption dysfunction. Genomic studies suggest that ERR γ regulates mitochondrial metabolism and renal reabsorption through distinct mechanisms: although ERR γ alone regulates mitochondrial OxPhos/FAO functions, ERR γ cooperates with HNF1 β to activate the expression of renal reabsorption genes. In addition, human patients with CKD show decreased kidney ERR γ expression and share highly overlapping renal transcriptional signatures with REC-ERR γ KO mice. Together, these results uncover a mechanism for coordinated regulation of renal mitochondrial and reabsorption functions and identify ERR γ signaling as an important link to mitochondrial dysfunction associated with kidney disease.

Results

Loss of ERR γ in RECs Results in Kidney Disease with Cysts. We first determined where ERR γ is expressed in postnatal kidneys by analyzing an ERR γ heterozygous mouse strain in which LacZ was knocked into the ERR γ locus (16). X-gal staining revealed that ERR γ was highly and specifically expressed in the RECs of all of the segments of tubules and collecting ducts, but barely detectable in any other cells, including those in the glomeruli (SI Appendix, Fig. S1A). Furthermore, ERR γ protein was found exclusively in the nucleus but not in mitochondria or cytosol, in line with its function as a nuclear transcription factor (SI Appendix, Fig. S1B).

To determine the importance of ERR γ in postnatal kidney biology and disease, we generated REC-ERR γ KO mice by crossing mice harboring a floxed allele of ERR γ (17) with Sim1-Cre strain. Strong renal Cre expression occurred at birth and resulted in rapid postnatal loss of kidney ERR γ expression with only 15% ERR γ mRNA remaining by 1 wk of age (SI Appendix, Fig. S1C). Using a double fluorescent Cre reporter mouse strain (26), we further found that this Sim1-Cre line mediated recombination specifically in all segments of the tubule and collecting duct epithelium within the kidney (SI Appendix, Fig. S1D), exactly where ERR γ is normally expressed (SI Appendix, Fig. S1A). We used both male and female REC-ERR γ KO mice for all subsequent studies.

Both male and female REC-ERR γ KO mice (Cre⁺) were born and survived perinatally at the expected Mendelian ratio. Their kidneys were of normal weight and histological appearance at birth and at 3 wk of age, suggesting no developmental defects (Fig. 1 A–C). Consistent with the histological observations, electron microscopy showed that renal ultrastructure, including mitochondria in the glomeruli, proximal tubules, and distal tubules, was comparable to controls at 3 wk of age (SI Appendix, Fig. S2). Subsequently, however, both male and female REC-ERR γ KO mice developed progressive renal abnormalities (Fig. 1A). Kidneys of REC-ERR γ KO mice were significantly heavier than controls by 1 mo of age, and threefold heavier by 3 mo of age (Fig. 1 B and D). Histological examination showed that REC-ERR γ KO mouse kidneys contained multiple fluid-filled cysts of variable sizes (Fig. 1E), resulting in the disruption of overall kidney organization and structure. Glomeruli were present and appeared histologically normal based on H&E staining. The carbohydrate-rich basement membranes of glomeruli including capillary loops responsible for renal filtration function also remained intact, as evaluated by periodic acid-Schiff staining

(Fig. 1E). These findings suggest that ERR γ is critical for maintaining normal kidney structure and function in vivo.

ERR γ Directly Regulates Mitochondrial Metabolic and Renal Reabsorptive Function.

To further understand the molecular mechanisms underlying the observed kidney disease phenotype, we used RNA-Seq to quantify the transcriptomic changes between control and REC-ERR γ KO mouse kidneys ($n = 4$ each) at 3 wk of age, just before morphological abnormalities became observable. We found that expression levels of 1,018 genes were significantly decreased and 1,406 genes were significantly increased in REC-ERR γ KO mouse kidneys (Fig. 2A; $P < 0.01$; fold change $> \pm 1.5$). Pathway analysis revealed that the down-regulated 1,018 genes were enriched for two cellular functions: mitochondrial metabolism (OxPhos, FAO, etc.) and transmembrane transport associated with renal reabsorption (Fig. 2B). Decreased mitochondrial metabolism (OxPhos, FAO and amino acid metabolism) was recently identified as a genetic signature of human CKD (12). Pathways enriched in the up-regulated 1,406 genes included inflammatory response (SI Appendix, Fig. S3A), which has also been implicated in human CKD (12). We validated expression changes for many of these genes, using qRT-PCR. For example, renal expression of genes important in OxPhos (*CS*, *Ndufa4*, *Sdhb*, *Cox5b*, and *Atp5b*) and FAO (*Cpt1b*, *Cpt2*, *Acadm*, and *Hadha*) were reduced in REC-ERR γ KO mice (Fig. 2C). Further, transcripts implicated in renal reabsorption were significantly decreased in REC-ERR γ KO kidneys, including *Slc5a2/Sglt2* (Na⁺/glucose cotransporter), *Atp1a1*, and *Atp1b1* (Na⁺/K⁺ ATPases, genetic variations of which are associated with hypertension), *Slc34a1* (Na⁺/phosphate cotransporter, mutations of which cause Fanconi Renotubular Syndrome 2 in humans), *Slc12a1* (Na⁺/K⁺/Cl⁻ cotransporter, mutations of which cause Bartter syndrome type 1 featuring renal tubular hypokalemic alkalosis), *Slc6a19* (neutral amino acid transporter, mutations of which cause Hartnup Disease because of blocked reabsorption of neutral amino acids in humans), *Sgk2* (a regulator of renal transport), and *Pkd2* (mutations of which cause autosomal dominant polycystic kidney disease in humans; Fig. 2C).

We next investigate whether the renal transcriptome changes in REC-ERR γ KO mice are gender-dependent. Both male and female REC-ERR γ KO mice exhibited similar pathway changes for both down- and up-regulated genes (SI Appendix, Fig. S3B). This is consistent with the fact that both sexes of REC-ERR γ KO mice develop kidney disease. To determine whether these molecular defects of mitochondrial and renal functions persisted in the later stage of kidney disease, we performed RNA-Seq analysis in 3-mo-old male control and REC-ERR γ KO mice and compared it with RNA-Seq data of 3-wk-old male mice. All the down-regulated and most of the up-regulated cellular pathways were maintained in 3-mo-old REC-ERR γ KO mice (SI Appendix, Fig. S3C). A few additional up-regulated cellular pathways were found at 3 mo of age, likely reflecting secondary changes at the later stage of kidney disease.

To determine whether expression of these genes was directly regulated by ERR γ , we performed chromatin immunoprecipitation sequencing (ChIP-Seq) of ERR γ , using two biological replicates of WT mouse kidney samples. Because ERR γ is specifically expressed in RECs, this effectively mapped the ERR γ cistrome in RECs. ERR γ binding peaks were distributed mainly in introns, intergenic and promoter regions (SI Appendix, Fig. S4A). Eighty-six percent (7,463/8,702) of the ERR γ peaks contain the consensus ERR binding motif (AAGGTCA). Pathway analysis revealed that mitochondrial metabolism, including OxPhos pathway genes that comprise most of the lists for Huntington's disease and Alzheimer disease, and renal reabsorption are among the most enriched pathways of genes annotated to the kidney ERR γ cistrome (Fig. 2D). This is nearly the same pattern seen among down-regulated RNA-Seq pathways in REC-ERR γ KO kidneys (Fig. 2B). We validated these ERR γ ChIP-Seq results

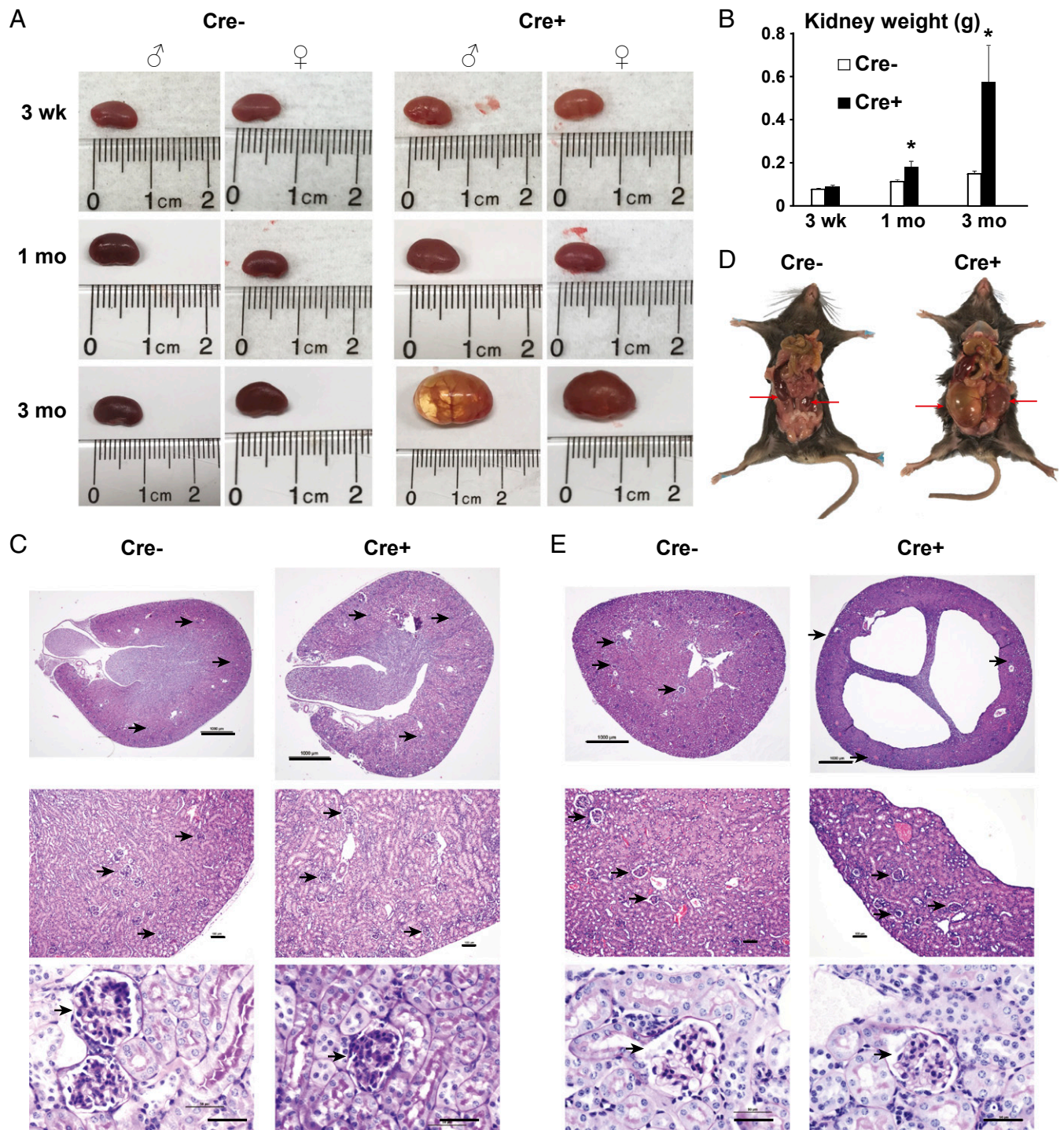


Fig. 1. REC-ERR γ KO mice develop kidney disease with fluid-filled cysts. (A and B) Representative pictures (A) and weight (B) of control and REC-ERR γ KO kidneys at different ages ($n = 12-19$). (C) Three-week-old control and REC-ERR γ KO kidney morphology. (Top) H&E stain. (Scale bar, 1,000 μm .) (Middle) H&E stain. (Scale bar, 100 μm .) (Bottom) Periodic acid-Schiff stain (Scale bar, 100 μm .) Arrowheads point to glomeruli. (D) Kidneys (red arrows) of 3-mo-old control and REC-ERR γ KO mice, illustrating their relative sizes to the body. (E) Three-month-old control and REC-ERR γ KO kidney morphology. Staining and scale bars are the same as in C. Error bar indicates SEM. * $P < 0.05$ between Cre $^-$ and Cre $^+$ by t test. Both male and female mice were included.

using ChIP-qPCR which showed that ERR γ directly bound to the same set of OxPhos, FAO, and renal reabsorption genes (Fig. 2E). By comparing the ChIP-Seq and RNA-Seq datasets, we found that 53% (537 of 1,018) of the genes down-regulated in REC-ERR γ KO mice were bound by ERR γ (Fig. 2F). These genes are again highly enriched for mitochondrial metabolism (OxPhos, FAO, etc.) and renal reabsorption pathways (Fig. 2G). In contrast, only

19% (271 of 1,406) of the up-regulated genes were bound by ERR γ (SI Appendix, Fig. S4B). Inflammatory pathway was again enriched in this list, but to a lesser extent than was observed in the ERR γ transcriptome as a whole (SI Appendix, Fig. S4C). These results suggest that the major biological function of ERR γ in RECs is transcriptional activation of mitochondrial metabolism and renal reabsorption.

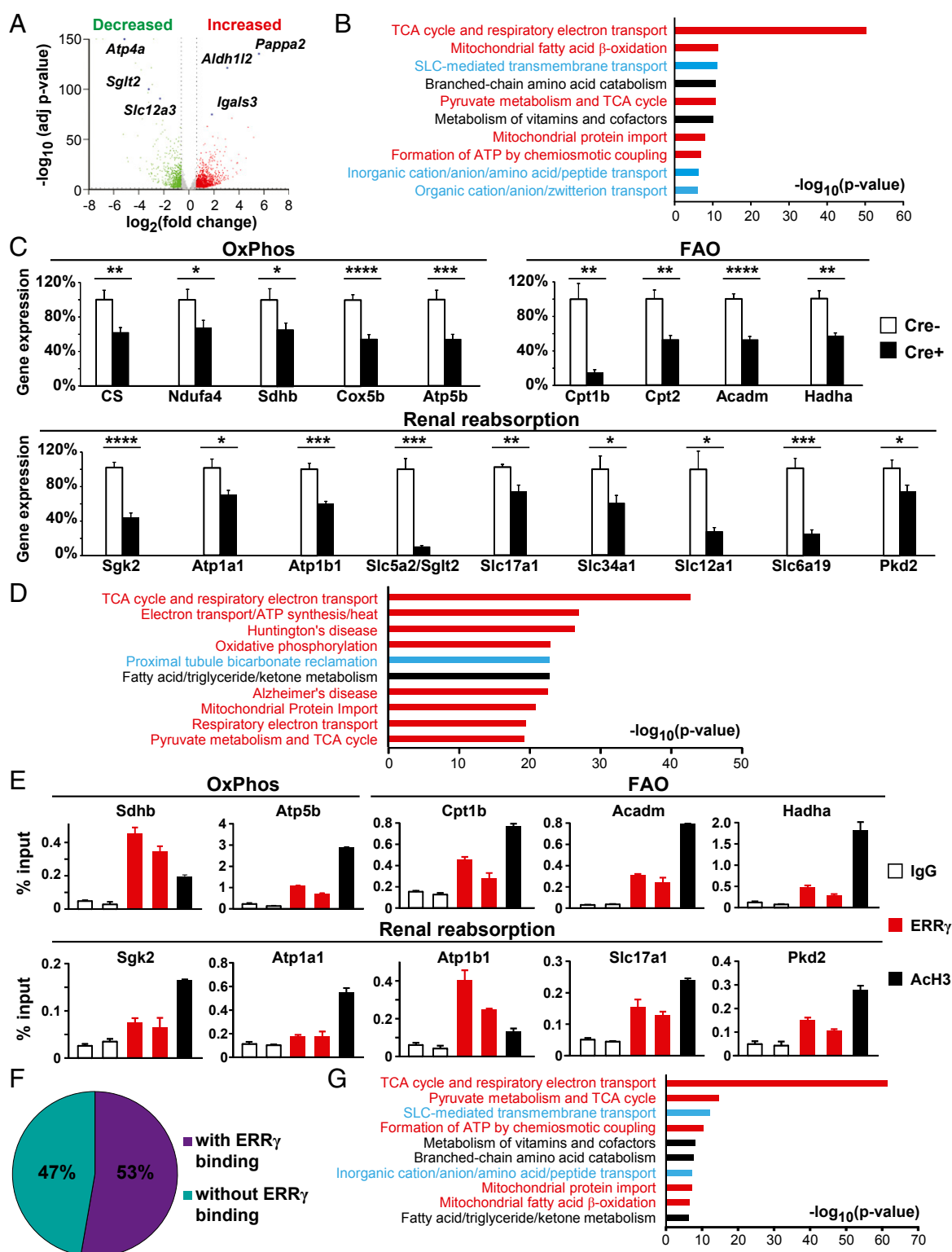


Fig. 2. ERR γ is essential for maintaining normal expression of REC mitochondrial metabolic and renal reabsorptive genes. (A) Volcano plot showing genes differentially expressed in 3-wk-old control and REC-ERR γ KO kidneys by RNA-Seq ($n = 4$). Filtering criteria is fold change $> \pm 1.5$ and $P < 0.01$ (dotted lines). (B) Top 10 enriched pathways of down-regulated genes. (C) Gene expression in 3-wk-old control ($n = 7$) and REC-ERR γ KO ($n = 10$) kidneys by qPCR. (D) Top 10 ranked pathways of genes with nearby ERR γ ChIP-Seq peaks. (E) ChIP-qPCR shows that ERR γ binds to OxPhos, FAO, and renal reabsorption genes. (F) Pie graph of down-regulated genes with or without annotated ERR γ binding peaks. (G) Top enriched pathways of down-regulated genes with annotated ERR γ binding peaks. Error bar indicates SEM. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$ between Cre $^{-}$ and Cre $^{+}$ by t test. Both male and female mice were included.

Next we investigated whether mitochondrial metabolism and renal reabsorption function were impaired in REC-ERR γ KO mice. Enzymatic activities of multiple mitochondrial electron transport chain complexes were found decreased in 3-wk-old REC-ERR γ KO kidneys (Fig. 3A), consistent with their reduced RNA expression. In addition, mtDNA content was significantly reduced (Fig. 3B), despite normal mitochondrial ultrastructures, indicating mitochondrial dysfunction. Importantly, all these mitochondrial metabolic defects were present at 3 wk of age, before any morphological or functional renal defects were observed, suggesting that mitochondrial dysfunction is a genesis for renal defects in REC-ERR γ KO mice.

The Sim1-Cre line also mediates recombination in nonrenal tissues such as the hypothalamus (27). We next studied whether ERR γ regulates REC mitochondrial functions in a cell-autonomous manner. We found that adenoviral-mediated ERR γ overexpression increased expression of mitochondrial metabolic genes (Fig. 3C), leading to increase in both basic and stimulated mitochondrial respiration in human tubular REC HKC-8 cells (Fig. 3D). In addition, siRNA-mediated knockdown of ERR γ resulted in decreased mitochondrial respiration, which was rescued by reintroduction of ERR γ (SI Appendix, Fig. S4D). These results indicate that the mitochondrial defects in REC-ERR γ KO mice occur as a result of kidney intrinsic functions of ERR γ .

A central function of the kidney is the reabsorption of water, essential nutrients, and electrolytes. For example, in normal health, almost all urine glucose is reabsorbed, mostly through the high-capacity sodium-glucose cotransporter 2 (SGLT2/Slc5a2) on the apical membrane of the proximal tubule RECs. Loss or inhibition of SGLT2 in humans leads to significant loss of glucose reabsorption and glucosuria (28). The almost complete dependence of renal SglT2 expression on ERR γ is particularly interesting because a new class of SGLT2 inhibitors (Gliflozins) have become the latest class of potent, FDA-approved medications to treat type 2 diabetes (29, 30). We found that ERR γ increased SglT2 expression (Fig. 3E) and significantly elevated cellular glucose uptake in human RECs (Fig. 3F).

SGLT2 inhibitors are clinically used to treat diabetes through inhibiting renal glucose reabsorption, resulting in glucosuria. Although REC-ERR γ KO mice showed similar, very low urine glucose levels compared with controls under normal conditions (SI Appendix, Fig. S4E), likely because of a small amount of remaining SGLT2, they decompensated in the setting of streptozotocin (STZ)-induced diabetes. In this model, STZ induced acute diabetes with very high plasma glucose and low plasma insulin levels in both control and REC-ERR γ KO mice (Fig. 3G). However, as seen in patients with diabetes treated with SGLT2 inhibitors, REC-ERR γ KO mice exhibited strong glucosuria with urine glucose levels soaring more than 100-fold above controls because of the loss of SGLT2 and renal glucose reabsorption function. Together, these results demonstrate that ERR γ is an essential regulator of renal glucose reabsorption through control of SglT2 transcription.

ERR γ Regulates Mitochondrial and Renal Reabsorption Genes Through Distinct Mechanisms. Genomic approaches including ChIP-Seq and ChIP-on-chip have been used to unambiguously demonstrate that ERR γ activates mitochondrial metabolic genes by directly binding to their promoters or enhancers in neurons and hearts (17, 23). It is therefore not surprising that ERR γ binds to and regulates mitochondrial metabolic genes in the kidney, another organ with abundant mitochondria and high energetic demand. It is, however, unexpected that ERR γ also directly binds to and activates the transcription of reabsorption genes, which was not the case in neurons or heart cells (17, 23). To understand whether these two sets of molecular signatures are regulated by ERR γ via similar mechanisms, we further examined the ERR γ binding patterns around mitochondrial and renal reabsorption genes. Notably, de

novo motif analysis of kidney ERR γ binding peaks revealed that the second most enriched motif after ERR is HNF1 β (Fig. 4A), a homeodomain-containing transcription factor known to cause maturity onset diabetes of the young (MODY5) (31). HNF1 β is highly expressed in both tubule and collecting duct RECs and is critical for kidney development and function (32–34). Mutations of HNF1 β are the most frequent monogenic cause of developmental kidney disease. The most common features of HNF1 β -associated kidney disease include fluid-filled renal cysts, renal hypoplasia, and electrolyte abnormalities (34), the very same symptoms seen in humans with chromosome translocation mapped to ERR γ locus (25) or in our REC-ERR γ KO mice. In addition, perinatal deletion of HNF1 β in mouse kidneys results in similar cystic renal disease (33, 35). The common pathological features of HNF1 β and ERR γ loss-of-function prompted us to investigate further a possible role for HNF1 β in ERR γ 's regulation of kidney metabolism and function.

We performed HNF1 β ChIP-Seq in biological duplicate WT mouse kidney samples and compared these results with our ERR γ genome-wide binding datasets. The renal ERR γ and HNF1 β cistromes shared 1,109 common peaks, representing about 13% of the total ERR γ peaks and 9% of the total HNF1 β peaks (Fig. 4B). Pathway analysis showed that ERR γ -only peaks annotate to mitochondrial metabolic pathways (OxPhos, FAO, and neurodegenerative diseases in which mitochondrial dysfunction is known to contribute), but are not associated with any renal reabsorption pathway (Fig. 4C). In sharp contrast, ERR γ /HNF1 β overlapping peaks had stronger representation of the renal absorption pathway compared with ERR γ - or HNF1 β -only peaks (Fig. 4C and SI Appendix, Fig. S5A), and importantly, lacked association with mitochondrial metabolic pathways (Fig. 4C). This complete separation of mitochondrial metabolism and renal reabsorption through pathway analysis was further confirmed by visualization of ChIP-Seq peaks near these genes (Fig. 4D and SI Appendix, Fig. S5B) and validated by ChIP-qPCR (Fig. 4E and SI Appendix, Fig. S5C). These results suggest that ERR γ regulates the transcription of mitochondrial and renal reabsorption genes through distinct mechanisms: mitochondrial metabolic genes are bound by ERR γ , as seen in neurons and heart cells (17, 23), whereas renal reabsorption genes are bound by both ERR γ and HNF1 β .

ERR γ and HNF1 β Functionally Cooperate to Regulate Renal Reabsorption Genes. Our ChIP-Seq results clearly suggest that ERR γ and HNF1 β bind to a common set of genes important in renal reabsorption. However, it could not yet be excluded that ERR γ and HNF1 β may bind to these genes in different cells or, rarely, occupy their common binding regions at the same time in the same cell because ChIP-Seq results represent an average binding signal from millions of cells. To address this, we next used HNF1 β ChIP-ERR γ reChIP-Seq to determine whether ERR γ and HNF1 β bind to any of these common peaks simultaneously in the same cells. By applying similar statistical and filtering parameters used in our ChIP-Seq studies, ChIP-reChIP-Seq identified a total of 1,892 peaks (Fig. 5A). Of these peaks, 1,725 (91%) colocalize with HNF1 β ChIP-Seq peaks (related to the first ChIP step of the ChIP-reChIP-Seq), validating the reproducibility of our ChIP and sequencing assay. Importantly, 20% (341) of these peaks colocalize to previously found ERR γ /HNF1 β overlapping peaks. This 20% is a significantly higher percentage than would be expected by the overlap of single ERR γ and HNF1 β ChIP-seq results (9%, $P = 5 \times 10^{-56}$ by hypergeometric test). These 341 peaks are highly likely to be simultaneously bound by both ERR γ and HNF1 β in the same cells, and they annotate to many renal reabsorption genes, including *Atp1b1*. These peaks are enriched for renal reabsorption-related pathways compared with the other 1,384 peaks, but not for any mitochondrial metabolic pathways (Fig. 5A). We validated this

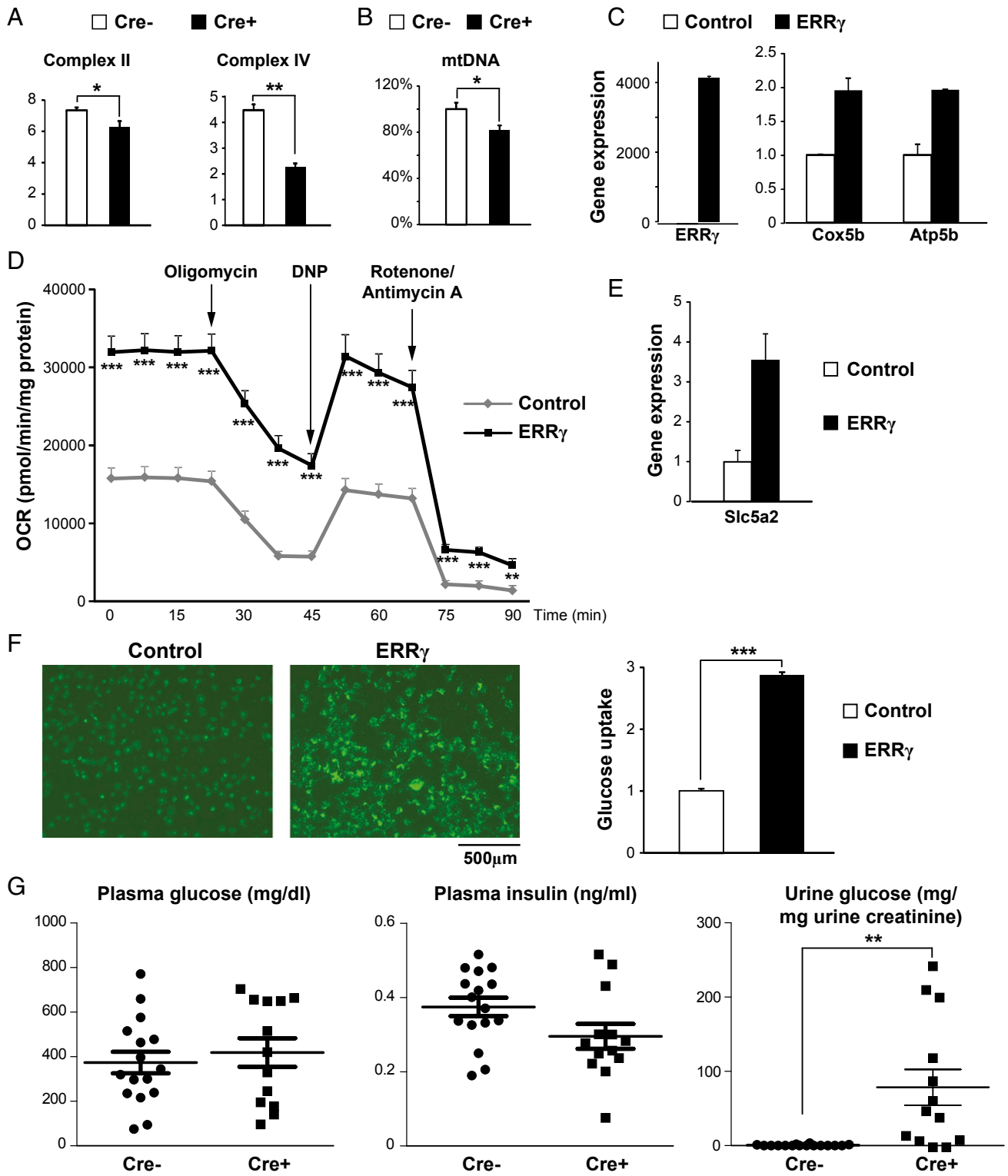


Fig. 3. ERR γ regulates REC mitochondrial and reabsorptive functions. (A) Enzymatic activities ($\mu\text{mol}/\text{min}/\text{mg}$ tissue) of mitochondrial electron transport chain complexes in 3-wk-old control ($n = 3$) and REC-ERR γ KO ($n = 6$) kidneys. (B) Relative mitochondrial DNA content in 3-wk-old control ($n = 13$) and REC-ERR γ KO ($n = 12$) kidneys. (C) ERR γ and mitochondrial OxPhos genes expression in HKC-8 cells by qPCR. (D) Oxygen consumption rate (OCR) in HKC-8 cells measured by Seahorse XF24 analyzer. $n = 7$ for control and $n = 8$ for ERR γ . (E) Slc5a2/Sglt2 expression in HKC-8 cells by qPCR. (F) Glucose transport into HKC-8 cells. (F, Left) Representative pictures visualizing fluorescent 2-NBDG uptake. (F, Right) Quantification ($n = 10$). (G) Plasma and urine glucose and plasma insulin levels in 10–13-wk-old control ($n = 16$) and REC-ERR γ KO ($n = 13$) mice 2 wk after STZ treatment. Error bar indicates SEM. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by t test. Both male and female mice were included.

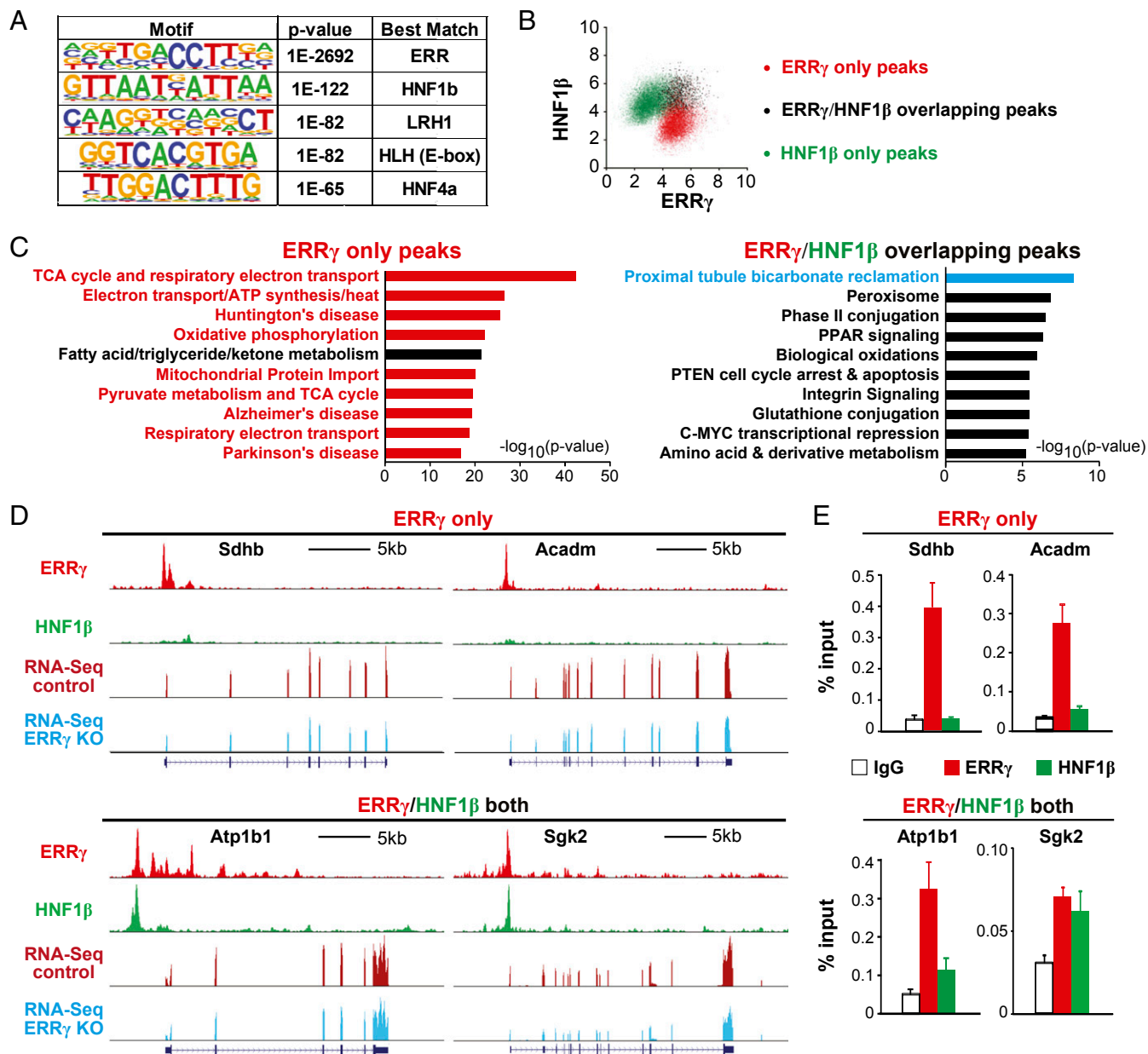


Fig. 4. ERR γ cooperates with HNF1 β to regulate the transcription of renal reabsorptive genes. (A) Top 5 de novo motifs near ERR γ ChIP-Seq peaks. (B) Overlap of renal ERR γ and HNF1 β ChIP-Seq peaks. Axis is \log_2 tags/ 10^7 reads. (C) Top 10 pathways of genes annotated to ERR γ and/or HNF1 β ChIP-Seq peaks. (D) Representative ERR γ and HNF1 β ChIP-Seq peaks. y axis is peak reads. Same scale for control and ERR γ KO RNA-Seq tracks. (E) Binding of ERR γ and/or HNF1 β near OxPhos, FAO and renal reabsorption genes by ChIP.

result on *Atp1b1* using reciprocal ERR γ and HNF1 β ChIP-reChIP-qPCR (Fig. 5B).

We next investigated whether ERR γ and HNF1 β functionally cooperate to regulate the transcription of renal reabsorption genes. We were unable to detect a physical interaction between ERR γ and HNF1 β in lysates from kidney cells or mouse kidneys, using coimmunoprecipitation followed by Western blot (*SI Appendix*, Fig. S5D). This suggests that they either do not interact directly or their interaction is not strong enough to be detected by this method, as previously reported in cases of transcription factor tethering (36). The expression of ERR γ and HNF1 β cobound genes such as *Atp1b1* was activated by either ERR γ or HNF1 β , and further increased with both ERR γ and HNF1 β (Fig. 5C). Together, these results suggest that ERR γ and HNF1 β functionally cooperate to regulate renal reabsorption genes.

Human Patients with CKD Show Decreased Kidney ERR γ Expression and Share Highly Overlapping Renal Transcriptional Signatures with REC-ERR γ KO Mice. To relate these findings of ERR γ to human kidney diseases beyond congenital disorders (25), we measured renal ERR γ expression in 95 human kidney tubule samples from patients with CKD and control cohorts. We found that ERR γ expression positively correlated with glomerular filtration rate (GFR), a well-established clinical index for kidney function (Fig. 6A). In addition, kidney ERR γ expression was significantly reduced in patients with CKD (GFR < 60) compared with healthy controls (GFR > 60; Fig. 6B). We next compared the renal transcriptomes between REC-ERR γ KO mice and human patients with CKD (12). About 25% of down-regulated and 19% of up-regulated genes in REC-ERR γ KO mice were shared in human patients with CKD (Fig. 6 C and D). There is a significant

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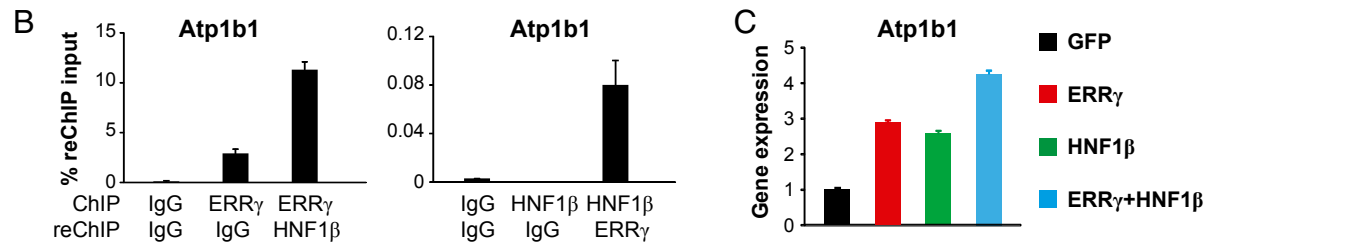
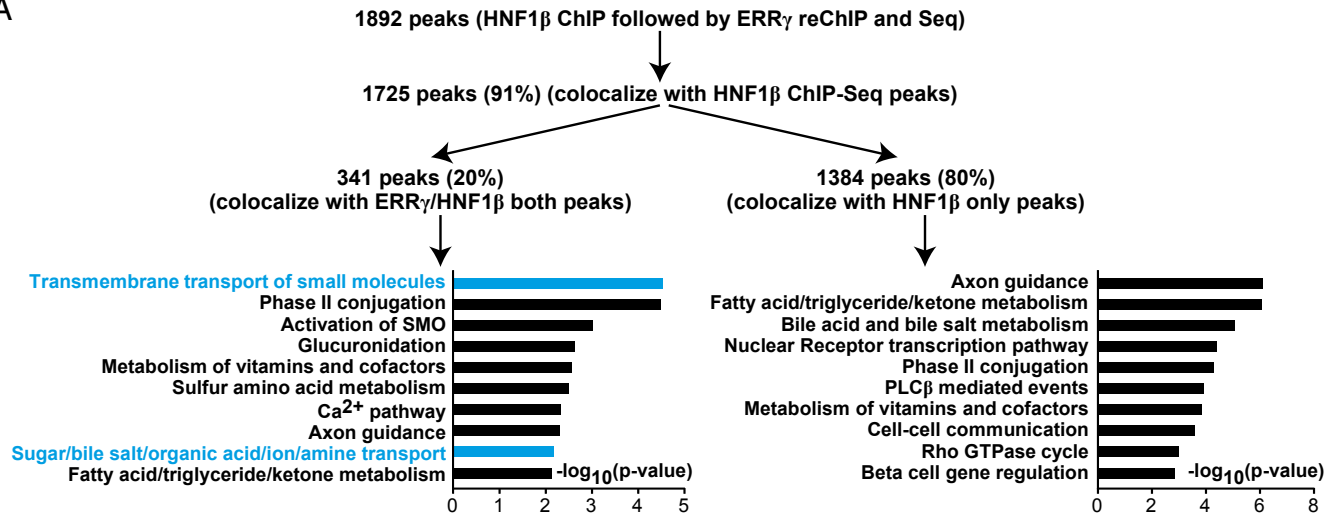


Fig. 5. Functional cooperation between ERR γ and HNF1 β in regulating renal function. (A) HNF1 β ChIP-ERR γ reChIP-Seq and data analysis. (B) Atp1b1 is cobound by ERR γ and HNF1 β in the same cells by ChIP-reChIP-qPCR. (C) Enhanced activation of Atp1b1 expression by both ERR γ and HNF1 β .

overlap of affected pathways between REC-ERR γ KO mice and human patients with CKD. In particular, pathways down-regulated are nearly identical in mouse and human and include many mitochondrial function and renal reabsorption pathways that are regulated by ERR γ . These results suggest a broad and critical role for ERR γ in human kidney disease.

Discussion

Mitochondrial dysfunction has been increasingly recognized as an important determinant of kidney diseases including CKD (4–7, 10, 11). ERR γ has recently emerged as a key transcriptional regulator of mitochondrial biogenesis and function, and human genetic studies have linked ERR γ to kidney disease (13–15, 25). However, definitive evidence linking regulation of mitochondrial function and kidney biology/disease is still missing. Here we show that ERR γ is an essential transcriptional coordinator of kidney mitochondrial and reabsorptive functions relevant to human kidney disease. Renal ERR γ expression correlates with kidney function in humans. Mice lacking REC ERR γ develop kidney mitochondrial and reabsorptive dysfunction with similar signs and molecular signatures as seen in human CKD. Mechanistically, we demonstrate that ERR γ directly regulates mitochondrial metabolism and renal reabsorption, but through distinct mechanisms. The functional cooperation between ERR γ and HNF1 β , another transcriptional regulator important in kidney biology and disease, further uncovers the transcriptional network optimized to coordinate kidney mitochondrial and reabsorptive functions. Together, these results improve our understanding of how mitochondrial function is coordinately regulated to support normal kidney function and how its dysregulation contributes to kidney disease (Fig. 6E).

The near complete dependence of SGLT2 expression and renal glucose reabsorption on ERR γ is particularly noteworthy because of the recent clinical use and efficacy of SGLT2 inhib-

itors in treating type 2 diabetes, and their therapeutic potential in treating obesity, kidney disease, and cardiovascular disease. We further demonstrated that ERR γ can increase SglT2 expression and REC glucose uptake in a cell-autonomous manner. Under STZ-induced diabetic conditions, REC-ERR γ KO mice faithfully recapitulated glucosuria seen in patients treated with SGLT2 inhibitors. However, this did not result in lower blood glucose, as observed clinically. One possible explanation for this is there remains a small amount of SGLT2 and intact SGLT1 in the REC-ERR γ KO animals. This incomplete glucose excretion phenomenon has also been observed in SglT2 KO mice (37), as well as in SGLT2 inhibitor-treated patients with diabetes in whom only 40–50% of the expected glucose reabsorption inhibition can be achieved with a full dose (30). Another possible explanation is that STZ destroys pancreatic β cells, causing type 1 diabetes and very high plasma glucose levels. Loss of insulin production and incomplete glucose excretion may explain the lack of blood glucose normalization in REC-ERR γ mice, similar to what was reported in SglT2 KO mice (37). Notably, SGLT2 inhibitors are not currently approved for type 1 diabetes.

ERR γ has been shown to promote energy-generating mitochondrial functions in several energy-demanding cell types, including neurons, cardiomyocytes, skeletal myocytes, brown adipocytes, and now in RECs (17–23, 38). In three of these cell types (neurons, cardiomyocytes, and RECs) ChIP-Seq or ChIP-on-chip has been used to demonstrate that ERR γ directly binds near mitochondrial OxPhos genes (17, 23). However, ERR γ cistromes also exhibit cell-type-specific features that match the metabolic profile and energy-intensive cellular functions of individual cell types. Neurons depend on aerobic catabolism of glucose, including OxPhos as an energy source, and they cannot metabolize fat. Accordingly, the neuronal ERR γ cistrome includes many glycolysis and OxPhos genes but is depleted of FAO genes (17). Cardiomyocytes, in contrast, use both glucose and fat

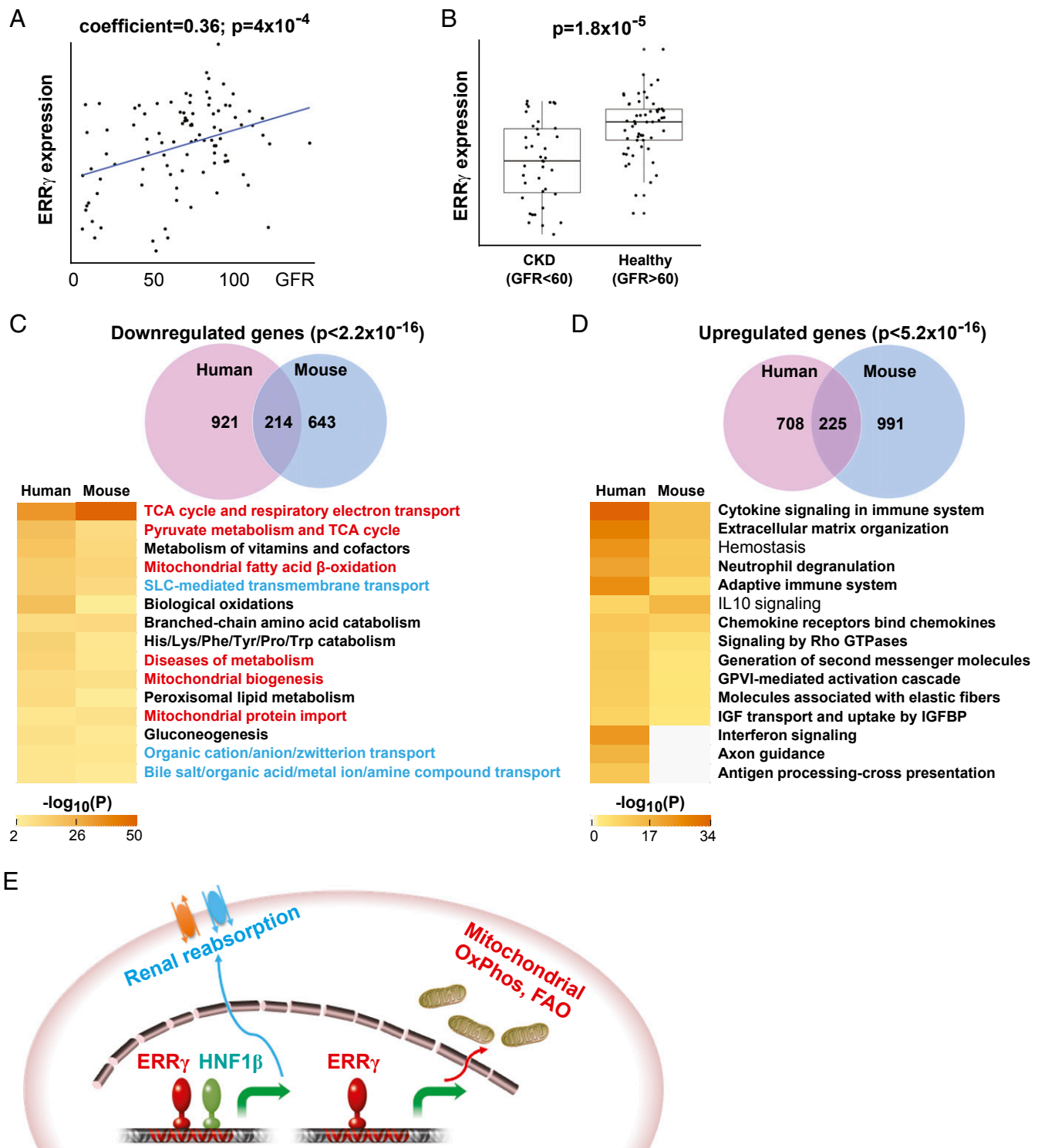


Fig. 6. Human patients with CKD show decreased kidney $ERR\gamma$ expression and share highly overlapping renal transcriptional signatures with REC- $ERR\gamma$ KO mice. (A) $ERR\gamma$ expression positively correlates with GFR across 95 human kidney tubule samples, by Pearson correlation. (B) $ERR\gamma$ expression is significantly reduced in human CKD kidney tubules, by *t* test. (C and D) Venn diagram and heat map comparison of top enriched pathways of down-regulated (C) or up-regulated (D) kidney genes between 3-wk-old REC- $ERR\gamma$ KO mice and human patients with CKD. Statistical analysis was performed using Fisher's exact test. (E) Model illustrating that $ERR\gamma$ coordinates energy-generating mitochondrial metabolism and energy-consuming renal reabsorption functions through distinct mechanisms.

as fuel. As a consequence, cardiac $ERR\gamma$ binds to and activates the transcription of FAO genes in addition to OxPhos genes (23). Here in RECs, we demonstrate that $ERR\gamma$ directly regulates both OxPhos and FAO, key metabolic pathways used by

these cells (12). Therefore, $ERR\gamma$ is a key transcriptional regulator of cell-type specific metabolic functions. In addition, $ERR\gamma$ has been shown to directly bind to and activate genes for cardiac contraction and conduction, two major cellular energy utilization

processes specific to the heart (18, 23). Here, we show that ERR γ directly regulates the major energy consumption process in another cell type; namely, reabsorption in RECs, via functional cooperation with the REC-related transcription factor HNF1 β . Together these studies reveal a common theme of cellular metabolic regulation: cellular energy production and consumption must be coordinated because most cells do not have an extensive capacity to fully store energy. Our studies thus demonstrate that ERR γ is a central transcriptional coordinator of cell-type specific energy production and utilization.

Materials and Methods

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. Adenoviruses were generated as previously described (39, 40). Transmission electron microscopy, gene expression, Western blot, cellular oxygen consumption measurement, mitochondrial DNA, and enzyme activity

analysis were performed as previously described (18, 41). Please see *SI Appendix, SI Materials and Methods* for detailed description of mouse studies, cell culture, glucose uptake, histological analysis, RNA-Seq, ChIP-Seq, and ChIP-reChIP-Seq experiments. Statistical analysis was performed using Student's *t* test, Pearson correlation, or Fisher's exact test. Microarray data were deposited in ArrayExpress database (E-MTAB-2502). RNA-Seq, ChIP-Seq, and ChIP-reChIP-Seq data were deposited in the GEO database (GSE104907).

ACKNOWLEDGMENTS. We thank Dr. Douglas Wallace, Dr. Mitchell Lazar, Dr. Matthew Weitzman, Dr. Amita Sehgal, Dr. Michael Marks, and Dr. Mark Kahn for critical discussion of the project. We thank Dr. Biao Zuo, Dr. Ray Meade, and the UPenn Electron Microscopy Resource Laboratory core facility for technical support. The authors and this work were supported by the Office of the Assistant Secretary of Defense for Health Affairs through the Peer Reviewed Medical Research Program under Award W81XWH-16-1-0400, and pilot awards from the Diabetes Research Center at the University of Pennsylvania from a grant sponsored by the NIH (DK19525; DK111495 to L.P.; DK099379 to B.J.W.; DK108987 to G.D.B.; and DK087635 and DP3 DK108220 to K.S.).

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