The nuclear receptor HNF4 drives a brush border gene program conserved across murine intestine, kidney, and embryonic yolk sac

Lei Chen1,2✉, Shirley Luo1, Abigail Dupre1, Roshan P. Vasoya1, Aditya Parthasarathy1, Rohit Aita1, Raj Malhotra1, Joseph Hur1, Natalie H. Toke1, Eric Chiles2, Min Yang1, Weihuan Cao1, Juan Flores3, Christopher E. Ellison1, Nan Gao3, Amrik Sahota1, Xiaoyang Su2,4, Edward M. Bonder3 & Michael P. Verzi1,2,5✉

The brush border is comprised of microvilli surface protrusions on the apical surface of epithelia. This specialized structure greatly increases absorptive surface area and plays crucial roles in human health. However, transcriptional regulatory networks controlling brush border genes are not fully understood. Here, we identify that hepatocyte nuclear factor 4 (HNF4) transcription factor is a conserved and important regulator of brush border gene program in multiple organs, such as intestine, kidney and yolk sac. Compromised brush border gene signatures and impaired transport were observed in these tissues upon HNF4 loss. By ChIP-seq, we find HNF4 binds and activates brush border genes in the intestine and kidney. H3K4me3 HiChIP-seq identifies that HNF4 loss results in impaired chromatin looping between enhancers and promoters at gene loci of brush border genes, and instead enhanced chromatin looping at gene loci of stress fiber genes in the intestine. This study provides comprehensive transcriptional regulatory mechanisms and a functional demonstration of a critical role for HNF4 in brush border gene regulation across multiple murine epithelial tissues.

1 Department of Genetics, Human Genetics Institute of New Jersey, Rutgers University, Piscataway, NJ, USA. 2 Rutgers Cancer Institute of New Jersey, Rutgers University, New Brunswick, NJ, USA. 3 Department of Biological Sciences, Rutgers University, Newark, NJ, USA. 4 Department of Medicine, Rutgers-Robert Wood Johnson Medical School, New Brunswick, NJ, USA. 5 Rutgers Center for Lipid Research, New Jersey Institute for Food, Nutrition & Health, Rutgers University, New Brunswick, NJ, USA. ✉email: lchen@dls.rutgers.edu; verzi@biology.rutgers.edu
The brush border is a characteristic of epithelia functioning in transport and absorption. It is composed of tightly packed microvilli; an exquisite, dense, finger-like array of apical cell surface protrusions that dramatically expand surface area of the epithelium. Brush borders line the surface of simple cuboidal and simple columnar epithelium found in different organs such as the intestine, kidney, and yolk sac[1–3]. Structurally, microvilli are composed of actin filament bundles, situated on a terminal web (also known as actin roots)[4–5]. Microvilli actin filaments are bundled by Villin[4], Espin[5], and Filamin (also known as Plastin-1)[6], and then attached to the plasma membrane by class I myosin motor proteins[7]. Anchoring microvilli into the terminal web increases stability of the brush border[8]. When the terminal web contracts, the microvilli spread apart and increase absorptive surface area[9].

Brush border anomalies are reported in many human diseases, such as microvillus inclusion disease, Crohn's disease, celiac disease, congenital tufting enteropathy, and congenital sodium diarrhea[2]. Understanding the regulatory mechanisms controlling brush border physiology is of great importance to human health. Most current brush border studies focus on genes encoding brush border structural proteins or cell biological characteristics of brush border assembly and function. For example, loss of actin-bundling proteins such as Villin, Espin, and/or Plastin-1 does not impair microvilli or only results in mild brush border anomalies, but can increase sensitivity to induced colitis[6,8–10]. Interruption of Eps8, an actin remodeler, caused reduced intestinal microvillus length and compromised intestinal fat absorption[11]. Disruption of Ezrin, a membrane-cytoskeleton crosslinking protein, leads to a disorganized terminal actin web[12]. However, it remains unclear how the transcriptional regulatory networks of brush border genes are established and whether they are common across brush border-containing tissues. We previously found that HNF4 paralogs activate enterocyte genes and are required for stabilization of enterocyte identity[13] and maturation of the fetal intestine[16]. HNF4 paralogs also regulate fatty acid oxidation and are required for renewal of intestinal stem cells[17]. In this study, using genetics and epigenetics approaches, we explore the transcriptional regulatory mechanisms of the brush border gene expression program and find that HNF4 binds and activates brush border genes. The brush border is severely disrupted upon HNF4 loss, suggesting HNF4 is a key regulator of the brush border in tissues of organs as diverse as the intestine, kidney, and embryonic yolk sac.

**Results**

**Profiling of accessible chromatin points to a cross-tissue role for HNF4 in activating the brush border gene expression program.** In enterocytes (Fig. 1a), digestive enzymes and transporters are trafficked and inserted into the apical brush border to facilitate terminal digestion and absorption of nutrients[18]. Similarly, the cuboidal epithelial cells of the renal proximal tubules (Fig. 1a) also produce a brush border facing the lumen of the tubule to facilitate the renal functions of reabsorption and secretion[19]. Interestingly, a brush border also lines cells of the intestinal and renal proximal tubules (Supplementary Fig. 1a), which helps transport maternal nutrients during embryonic development[3,20]. We sought to investigate similarities and differences of brush border transcripts across these three tissues.

We examined the overlap of annotated brush border transcript expression between the intestine, kidney, and yolk sac (the brush border-related gene list, Supplementary Data 1, is composed of brush border genes (GO:0005903), brush border membrane genes (GO:0031526), and microvillus genes (GO:0005902) from the Gene Ontology knowledgebase[21,22]). We performed RNA-seq in adult intestine and kidney, and curated public RNA-seq data of embryonic yolk sac[23], to find the vast majority of brush border genes are commonly expressed (FPKM (fragments per kilobase of transcript per million mapped reads) or RPKM (reads per kilobase of transcript per million mapped reads) > 1) across all 3 tissues (Fig. 1b and Supplementary Fig. 1a). Genes known to contribute to documented brush border characteristics such as F-actin bundling, membrane-cytoskeleton crosslinking, intermicrovillar adhesion genes[1], and membrane/transport functions were commonly expressed in all three tissues (Fig. 1c), indicating similar features between brush borders of different tissues.

Given the similarities in brush border transcriptomes between these tissues, we sought to identify whether brush border gene regulatory programs could be conserved between the tissues. We employed DNase I hypersensitive sites sequencing (DNase-seq) and RNA-seq to identify transcription factors operating at these accessible chromatin regions of brush border genes (see strategy in Fig. 1d). We first collected presumed regulatory regions within 50 kb of annotated brush border gene promoters using DNase-seq data of the intestine[23] and kidney[24]. Model-based Analysis of ChIP-seq (MACS), P < 10−5, and compared these regions between tissues. We found that intestine and kidney exhibited a strikingly similar chromatin accessibility at brush border genes (Figs. 1e, f). Subsequent mining of these regions for transcription factor-binding motifs revealed that the HNF4 DNA-binding site was the top transcription factor motifs enriched in these regions (Fig. 1g and Supplementary Data 2, the architectural protein, CTCF, motif was the top overall motif). Of the transcription factor-binding motifs revealed that the HNF4 DNA-binding site (Figs. 1e, f). Subsequent mining of these regions for transcription factor-binding motifs revealed that the HNF4 DNA-binding site was the top transcription factor motifs enriched in these regions (Fig. 1g and Supplementary Data 2, the architectural protein, CTCF, motif was the top overall motif). Of the transcription factor-binding motifs enriched at these presumed brush border regulatory regions, HNF4A is highly expressed in all three brush border-containing tissues (Figs. 1b, i). Together, these epigenomic analyses suggest that HNF4 could be a fundamental regulator of brush border gene expression across tissues.

**HNF4A binds chromatin in a strikingly similar pattern in the intestine and kidney.** Analysis of our RNA-seq data indicated that 71% of brush border genes are commonly expressed in both the intestine and kidney of adult mice (Fig. 1b). For example, Espn (encodes actin-bundling protein) and Anks4b (functions in intermicrovillar adhesion and brush border assembly) are both expressed in the intestine and kidney (Supplementary Fig. 1a). In addition, there are 20 brush border genes only expressed in the intestine, whereas 33 brush border genes are only expressed in the kidney (Fig. 1b). Myo1a exemplifies a gene expressed in the intestine, whereas Myo1c is expressed in the kidney (Supplementary Fig. 1b, c). Besides these structure-based brush border genes, we also examined function-based brush border genes in these different tissues. We found Npc1l1, a cholesterol transporter, is only expressed in the intestine, whereas Slco2a1, a urate transporter, is only expressed in the kidney (Supplementary Fig. 1b, c).

We wondered whether brush border gene regulatory mechanisms were similar or divergent in these tissues and hypothesized that HNF4 could play a critical and conserved role in brush border gene regulation. We therefore compared HNF4A binding to the intestinal and renal genomes using chromatin immunoprecipitation sequencing (ChIP-seq) (Supplementary Fig. 2a). Strikingly similar HNF4A-binding profiles were observed between the intestine and kidney, with the vast majority of ChIP-seq–binding sites (90%) identified as common between the two tissues (11,017 sites, false discovery rate (FDR) < 0.01, DiffBind[25], Fig. 2a). DNA-binding sequence motif analysis of intestinal and renal HNF4A ChIP-seq binding regions revealed similar enrichment of transcription factor-binding motifs (Supplementary Fig. 2b and Supplementary Data 3). Quantitatively, relatively few HNF4A-binding regions were identified as
differentially bound in the intestine vs. kidney; differentially bound regions were referred to as intestine-enriched sites (732 sites, FDR < 0.01, DiffBind25, Supplementary Fig. 2c and Supplementary Data 4) and kidney-enriched sites (473 sites, FDR < 0.01, DiffBind25, Supplementary Fig. 2c and Supplementary Data 4). HNF4A-binding signals are more robust in the intestine than in the kidney, in the regions that are identified as intestine-enriched sites. Similarly, HNF4A-binding signals are more robust in the kidney than in the intestine, in the regions that are identified as kidney-enriched sites (Supplementary Fig. 2d). Consistent with functional regulatory elements, tissue-enriched HNF4A-binding sites coincide with tissue-specific chromatin
HNF4 directly activates the majority of the brush border gene expression program. In the intestine, HNF4 acts redundantly with HNF4G, an intestine-restricted HNF4 paralog, and these two paralogs activate enhancer chromatin and stabilize enterocyte cell identity. Unlike HNF4G, HNF4A is expressed in both the intestine and kidney (Fig. 1b). The molecular consequences of HNF4 dependency in the intestine and kidney were appreciated through transciptome analysis by a tamoxifen-inducible intestine-specific knockout Villin-CreERT2; Hnf4αf/f; Hnf4γCrispr/Crispr mouse model (hereafter referred to Hnf4αγKO). A tamoxifen-inducible knockout in the kidney using a UBC-CreERT2; Hnf4αf/f mouse model (hereafter referred to Hnf4αKO), respectively. In all, 410 genes were significantly downregulated in both the intestine (Villin-CreERT2, Hnf4αγKO) and kidney (UBC-CreERT2, Hnf4αKO) knockouts of HNF4 (log2 fold-change < −1, FDR < 0.05, Fig. 2b), and these common downregulated genes were much more enriched for ontologies associated with brush border and transport functions than genes that were only downregulated in one of the two tissues (Fig. 2c and Supplementary Data 5). Gene set enrichment analysis (GSEA) confirmed that brush border genes were disproportionately downregulated in both the intestine and kidney upon HNF4 loss (Fig. 2d and Supplementary Fig. 3). To understand how HNF4 factors are impacting the transcriptome of these brush border genes, we next investigated the relationship between HNF4 chromatin-binding events and brush border gene expression. Seventy-eight brush border genes were significantly downregulated in RNA-seq analysis of the intestines of Villin-CreERT2, Hnf4αγKO (FDR < 0.05, Supplementary Fig. 3) and 50 of them are directly bound by HNF4 in the intestine via ChIP-seq (genes with underline, MACS P ≤ 10−5, Supplementary Fig. 3). Similarly, 85 brush border genes were significantly downregulated in the kidneys of UBC-CreERT2, Hnf4αKO (FDR < 0.05, Supplementary Fig. 3) and 49 of them are directly bound by HNF4 in the kidney (genes with underline, MACS P ≤ 10−5, Supplementary Fig. 3). For example, HNF4 can directly bind and activate Myo7b (involved in intermicrovillar adhesion) and Espn in both the intestine and kidney (Fig. 2c). These findings suggest that HNF4 factors bind to brush border gene chromatin and directly activate an entire catalog of brush border gene expression.

We next sought to more specifically associate HNF4 binding to active enhancer chromatin structure and chromatin looping, to target brush border genes. We first profiled enhancer chromatin structures of brush border genes in the intestinal epithelial cells of control and Hnf4αγDKO using H3K27ac MNase ChIP-seq. Decreased H3K27ac signals (an active chromatin marker) were observed in Hnf4αγDKO compared to wild-type (WT) control (Supplementary Fig. 4a, b). The levels of the remaining H3K27ac signal were observed at the center of the regions in Hnf4αγKO, suggesting more restricted chromatin accessibility of these brush border genes upon loss of HNF4 paralogs (Supplementary Fig. 4b). We also investigated whether HNF4 binding impacted chromatin looping between presumed enhancers and transcriptional promoters of brush border genes using H3K4me3 HiChIP to capture three-dimensional (3D) chromatin configurations at brush border gene loci. There are 152 brush border genes with FPKM > 1 in the intestinal epithelium and 44 of these 152 genes show disrupted looping events in Hnf4αγDKO (DSeq2 P < 0.05, Genomic Regions Enrichment ofAnnotations Tool [GREAT] 10 kb). Examples of genes critical to brush border structure and function illustrate the contribution of HNF4 transcription factors to chromatin looping at these brush border genes (Fig. 2f). In each example of brush border-related genes, including F-actin bundling genes (e.g., Espn and Psl1), membrane-cytoskeleton crosslinking genes (e.g., Ezr and Myo1a), intermicrovillar adhesion genes (e.g., Myo7b and Cdh5r5), and brush border membrane/transport genes (e.g., Slc6a19 and Npc1l1), HNF4 directly binds (HNF4 ChIP-seq tracks, Fig. 2g and Supplementary Fig. 4c) and is required for maintaining active chromatin (H3K27ac MNase ChIP-seq tracks, Fig. 2g and Supplementary Fig. 4c) and transcript levels (RNA-seq bar charts, Fig. 2g and Supplementary Fig. 4c, d). In each case, reduced chromatin looping events were observed upon HNF4 loss in the intestinal epithelium (differential loops of H3K4me3 HiChIP-seq, DSeq2 P < 0.05, Fig. 2g and Supplementary Fig. 4c). Taken together, multiple-omics approaches demonstrate a mechanism through which HNF4 binds to brush border gene loci, maintains active and accessible nucleosome structures, and promotes 3D chromatin looping between regulatory elements and brush border gene promoters to activate their transcription.

HNF4 transcription factors are required for intestinal brush border. The strong regulatory relationship between HNF4 and brush border gene regulation prompted us to characterize the consequences of HNF4 loss on brush border function in each of these brush border-containing tissues. We recently found impaired intestinal function upon HNF4 loss by using Villin-CreERT2 and Shh-Cre mouse models, and one of the top Gene Ontology categories of HNF4-dependent genes in the intestine is associated with brush border. However, the extensive regulatory mechanisms of HNF4 transcription factors in the intestine and other brush border-containing tissues have not been dissected yet. Given the important roles of HNF4 in brush border gene expression, we further investigated whether HNF4 paralogs are required for intestinal brush border by a tamoxifen-inducible, intestine-specific knockout (Fig. 3a). HNF4A and HNF4G
transcription factors are expressed throughout the intestinal epithelium (top panel, Supplementary Fig. 5a). Phalloidin and β-actin were used to mark the brush border at the apical surface of intestinal epithelial cells and was clearly found in WT, but was compromised in Hnf4αγDKO (Fig. 3b, c and Supplementary Fig. 5). Expression of brush border markers, such as alkaline phosphatase (AP, a brush border membrane-bound enzyme), β-actin (localized to the apical cytoskeleton), Villin (actin-bundling protein of the brush border), and Ezrin (linking the cytoskeleton to the cell plasma membrane of the brush border) were all severely disrupted upon HNF4 loss in the intestine (Fig. 3d). In addition, the intestinal cells were nicely aligned in WT, but this
HNF4A in the intestine and kidney, followed by DiffBind analysis (FDR < 0.01, GSE112946, and GSE47192, n = 2) shows that 11,017 sites are shared, and only 732 and 473 sites are differentially bound by HNF4A in the intestinal epithelium (MACS P ≤ 10⁻³) and kidney (MACS P ≤ 10⁻³), respectively. b Venn diagram shows the numbers of downregulated genes in the intestine (Villin-CreERT²; Hnf4αKO, n = 3 biologically independent mice) and kidney (UBC-CreERT²; Hnf4αKO, n = 4 biologically independent mice) knockouts of HNF4 with log2 fold-change < -1, FDR < 0.05, as evidenced by RNA-seq. c In HNF4 mutants, brush border-related functions are enriched in the common downregulated genes of the intestine and kidney (410 genes, purple bar) compared to genes only downregulated in Hnf4αKO intestine (1489 genes, red bar) or Hnf4αKO kidney (876 genes, blue bar). Benjamini P-values were calculated using DAVID. d GSEA of RNA-seq data reveals that the brush border gene transcripts are compromised upon HNF4 loss in both intestine and kidney knockouts (Kolmogorov-Smirnov test, one-sided for positive and negative enrichment scores, P < 0.001). e Examples of HNF4 ChIP-seq (n = 2 in each tissue) and RNA-seq tracks (Intestine; n = 3; Kidney; n = 4) at brush border-related gene loci. f Working model of how HNF4 factors are impacting brush border genes. Brush border genes can be classified into F-actin bundling genes, membrane-cytoskeleton crosslinking genes, intermicrovillar adhesion genes, and brush border membrane/transport genes. HNF4 can bind and facilitate long-range chromatin interactions at genes from each category. g Examples of compromised chromatin looping and compromised active chromatin markers at loci of brush border genes. H3K4me3 HiChIP-seq was done in villus cells of Hnf4αKO and their littermate controls. Differential loops (DEseq2 P < 0.05) are visualized by Sushi package for loops with q < 0.0001 and counts ≥ 8 (combined 2 replicates). Bar charts show transcript levels of brush border genes. Statistical tests were embedded in Cuffdiff. The data are presented as mean ± SEM (n = 3 biological replicates, **Cuffdiff FDR < 0.001, and *FDR < 0.05). H3K4me3 HChiP-seq (GSE148691): n = 2 biological replicates; H3K27ac ChIP-seq (WT vs αγKO; GSE112946): n = 2 biological replicates; HNF4 ChIP-seq (GSE112946): n = 2 biological replicates; RNA-seq (WT vs Hnf4αKO; GSE112946): n = 3 biological replicates. More examples are shown in Supplementary Fig. 4c.

HNF4A is required for renal brush border. Besides lining the intestinal epithelium, a brush border is also found in the proximal tubules of the kidney. Deletion of Hnf4a by different embryonic-onset Cre drivers in the nephrons leads to defects in the development of proximal tubules26,27, but little is known about the adult-onset disruption of Hnf4a in the kidney and its impact on brush border. To study the function of HNF4 in the renal brush border (Fig. 4a), we took advantage of the fact that HNF4A is expressed in both the intestine and kidney, whereas HNF4G is not expressed in the kidney (Fig. 4b, c). We therefore created both developmental- and adult-onset knockout of Hnf4a in a tamoxifen-inducible UBC-CreERT²; Hnf4αKO mouse model (hereafter referred to Hnf4αKO), which deletes all kidney HNF4 without compromising intestinal function. By labeling renal proximal and distal tubules with ABCG2 (an ATP-binding cassette transporter that also functions as a uric acid transporter) and NCC (a sodium chloride cotransporter), respectively, we confirmed that HNF4A is only expressed in the proximal tubules within the kidney (Fig. 4f and Supplementary Fig. 6a), a finding confirmed by re-analysis of public single-cell RNA-seq data28 (Supplementary Fig. 7). AP marks the brush border of proximal tubules and its activity gets stronger over developmental time, from birth to adulthood (Supplementary Fig. 6b). AP activity was lost in the kidney of Hnf4αKO but was not affected in Hnf4γKO (Fig. 4d), indicating that HNF4A is required for renal brush border but HNF4G is not required. AP and HNF4A are localized in the proximal tubules of the kidney (Supplementary Fig. 6c), and AP activity is positively correlated with the expression level of HNF4A (Fig. 4e), suggesting that HNF4A works cell-autonomously in the proximal tubule epithelium. In addition, HNF4 can directly bind and activate Abcg2 in both the intestine and kidney (Supplementary Fig. 8a). ABCG2 is normally expressed in the brush border of both the intestine and kidney, and is dramatically compromised upon HNF4 loss in both tissues at the transcript and protein level (Supplementary Fig. 8a, b).

To broaden the scope of HNF4 gene regulation in the kidney, RNA-seq was conducted and found a robust shift in the the Hnf4αKO kidney transcriptome away from proximal tubule transcripts and towards distal tubule transcripts (Fig. 4g, Supplementary Fig. 9a and Supplementary Data 6. Gene lists deﬁned from ref. 28). Adult-onset knockout of HNF4A also resulted in decreased expression levels of brush border markers (Supplementary Fig. 9b) and reduced kidney weight (Supplementary Fig. 9c). Electron micrographs confirmed that both adult (Fig. 4h) and developing kidneys of Hnf4αKO (Supplementary Fig. 9d) exhibited diminished and sparse brush border microvilli in proximal tubules; distal tubules were unaffected upon HNF4A loss (Fig. 4h).

Proximal tubules play a prominent role in reabsorption of water, electrolytes, low-molecular-weight proteins, glucose, and amino acids. They also function in regulating acid–base balance by reabsorbing filtered bicarbonate. As an abnormal renal brush border can lead to malfunction of the proximal tubules, we collected urine and plasma for signs of renal malfunction. Compared to littermate controls, Hnf4αKO showed decreased urine volume and pH but increased urine-specific gravity and protein (Fig. 4i), consistent with diminished proximal tubule function. Fanconi syndrome is a disorder of the kidney proximal tubule function and results in decreased reabsorption of certain substances into the bloodstream29. Coincidentally, increased concentration of glucose was observed in the urine of Hnf4αKO (Fig. 4j), which is consistent with the glycosuria symptom of Fanconi syndrome. In addition, decreased concentrations of total carbon dioxide, potassium, calcium, and glucose were also observed in the plasma of Hnf4αKO (Fig. 4k). Taken together, these data indicate that HNF4A loss results in compromised proximal tubule brush border and dysfunction of tubular reabsorption.
HNF4A is required for embryonic yolk sac brush border. In addition to intestinal epithelium, the brush border can also be found in murine embryonic yolk sac epithelium. Unlike the intestinal epithelium, Hnf4αγ is not detectable in the yolk sac (Fig. 5a). HNF4A is expressed throughout the yolk sac epithelium, both in the villous tissue proximal to the placental disc and in smoother epithelial tissue in more distal areas (Fig. 5b). To study the function of HNF4 in the embryonic yolk sac, we crossed
female $Hnf4\alpha^{f/f}$ with male $UBC-Cre^{ERT2}; Hnf4\alpha^{f/f}$ and knocked out HNF4A in the embryonic yolk sac by gavaging tamoxifen in pregnant female mice at embryonic day (E) 14.5 (Fig. 5c). Yolk sac tissues were collected at E17.5 and exhibited a clear disruption of brush border markers in $Hnf4\alpha^{KO}$ tissues (Fig. 5d). The yolk sac brush border plays an important role in nutrient transport during embryonic development. To achieve a higher fraction of HNF4A-negative yolk sac tissues and assay for yolk sac functions, we treated pregnant mice with a mixture of tamoxifen and progesterone at E12.5 and E13.5 (Fig. 5e). We found that $Hnf4\alpha^{KO}$ embryos showed reduced size (Fig. 5f) and weight (Fig. 5g) compared to their Cre-negative littermate controls, suggesting a
disrupted brush border upon HNF4A loss (Fig. 5d) may contribute to these underdeveloped embryos. GSEA analysis confirmed that brush border genes were significantly downregulated in the yolk sac upon HNF4A loss (Fig. 5h and Supplementary Fig. 10a), which is consistent with the findings in the intestine and kidney upon HNF4 loss (Fig. 2d and Supplementary Fig. 3). The amniotic fluid is encased by the yolk sac and provides a supportive environment for the developing embryos. As brush border markers and gene expression are disrupted upon HNF4A loss in the yolk sac, we wondered whether there were corresponding deficiencies in the adjacent amniotic fluid. During pregnancy, amino acids represent one of the major nutrients for embryos and we found both essential and non-essential amino acids were all reduced in the amniotic fluid upon HNF4A loss, as evidenced by liquid chromatography–mass spectrometry (LC-MS) analysis (Fig. 5i and Supplementary Fig. 10b). Coincidentally, amino acid transport-related transcripts were also downregulated in Hnf4aKO yolk sac (Fig. 5j, k and Supplementary Fig. 10c), implying defective nutrient transport upon HNF4A loss. Furthermore, compared to Cre-negative littermate controls, we found increased uric acid in the amniotic fluid (Fig. 5l) and compromised expression of uric acid transporters in the yolk sac of Hnf4aKO (Fig. 5m, n and Supplementary Fig. 11), suggesting waste excretion might also be impaired in the yolk sac of Hnf4aKO. Together, these findings suggest that HNF4 is required for proper brush border assembly and functions of the yolk sac.

HNF4 loss leads to increased stress fiber formation. All together, we found disrupted brush borders of the intestine, kidney, and yolk sac upon HNF4A loss. Actin filaments provide microvilli structural support of brush border under normal conditions. Stress fibers are higher-order cytoskeletal structures consisting of actin filaments, crosslinking proteins, and myosin motors, and they counter membrane tension and stabilize cell structure by generating force and transducing mechanical signaling. In most cases, stress fibers connect to focal adhesions and play an important role in mechanotransduction. We wondered whether disrupted brush border could promote redirection of actin monomers away from the membrane and into stress fibers instead. In the yolk sac, we found upregulation of stress fiber and focal adhesion gene signatures upon HNF4A loss (Fig. 6a and Supplementary Fig. 12a–c). For example, the keratin network (Keratin 8/18) plays a crucial role in stress fiber reinforcement and mechanotransduction, whereas Laminin γ-2 functions in focal adhesion stability. In addition, calpains also play an important role in focal adhesion and stress fiber formation. The elevated protein levels of Keratin 18, Laminin γ-2, and Calpain 2 were all confirmed in the yolk sac epithelium upon HNF4A loss (Fig. 6b and Supplementary Fig. 12d). Similarly, in the intestinal epithelium, we found upregulated expression of many stress fiber and focal adhesion markers (Fig. 6c, e), as well as increased chromatin looping events and active chromatin marks at these gene loci upon HNF4 loss (Fig. 6d). The filamin family is named for its filamentous colocalization with actin stress fibers and increased filamin was also observed in the intestinal epithelium of Hnf4αKO (Fig. 6f–h), suggesting that alterations in architecture of brush border in Hnf4αKO might result in a cytoskeleton reorganization with increased stress fiber formation (Fig. 6i).

Discussion

HNF4A has been reported to regulate intestinal epithelial barrier function, with destabilized intercellular junctions in the absence of HNF4A. Chiba et al. previously reported that overexpression of HNF4A could induce the formation of brush border in the F9 cells in vitro. In this study, we show that HNF4 functions as a conserved and important regulator of brush border genes in multiple tissues in vivo, including the intestine, kidney, and yolk sac, and demonstrate comprehensive transcriptional regulatory mechanisms of HNF4 on brush border gene expression. We found that HNF4 factors activate brush border gene expression through binding to distal enhancer regions, maintaining enhancer chromatin activity and facilitating chromatin looping in the intestine. The brush border dysfunction is an important aspect of enterocyte abnormalities in Hnf4αKO, although this Villin-CreERT2 model could not exclude the effects of HNF4-deficient intestinal stem cells in this study. Mechanosignaling transduces signals much faster than chemical stimulation. When cells are under mechanical stress, actin stress fibers increase to reinforce their mechanical strength. Elevated membrane tension lowers actin-based-protrusion. Impaired brush border transcripts and enhanced stress fiber/local
adhesion transcripts were observed upon HNF4 loss, suggesting that HNF4 transcription factors may work as a mechanosignaling sensor, and future study is needed to test this idea. It is also of interest to note that there is a substantial increase of Filamin A upon HNF4 loss in the intestinal epithelium. Filamin A undergoes proteolysis and its proteolysed fragments can localize to the nucleus. It has been reported that Filamin A inhibits tumor growth and metastasis by interacting with transcription factors when the active cleaved form of Filamin A localizes to the nucleus. Rapid immunoprecipitation MS of endogenous proteins data show HNF4A can interact with Filamin A; it will be interesting to investigate whether active cleaved form of Filamin A localizes to the nuclear compartment.
Filamin A could activate HNF4 transcription factors to form a positive feedback of brush border gene expression in the future.

Deletion of HNF4A (embryonic-onset) in nephron progenitor cells by Six2GFPCre (mosaic expression) leads to a defect in the development of proximal tubules, as evidenced in the kidney of E18.5.26. Less clear is whether adult-onset disruption of HNF4A would compromise kidney function. Importantly, our study demonstrates compromised proximal tubules and impaired reabsorption in an adult-onset knockout of HNF4A, recapitulating Fanconi syndrome. Approximately 93% of genes that are significantly altered in the developing kidney upon HNF4A loss (Six2GFPCre, FDR < 0.05)26 also show significant changes in the adult kidney upon HNF4A loss (UBC-CreERT2, FDR < 0.05). Along with these findings, an R76W mutation in human HNF4A is also linked to Fanconi syndrome.47 Defective proximal tubule function causes Fanconi syndrome and the adult-onset knockout of Hnf4a could be used to study Fanconi syndrome. In addition, Wnt signaling patterns the proximal-distal axis of the nephron and bone morphogenetic protein (BMP) antagonizes Wnt gradient.48,49 The formation of the proximal tubules requires Notch signaling and an environment of low Wnt signaling.50–53 Recently, a reinforcing feed-forward module between HNF4 and BMP signaling was shown to promote enterocyte differentiation.15 Future investigation is needed to explore whether the interactions of BMP/Wnt/Notch signaling and HNF4 transcriptional regulatory networks are conserved in the development of the proximal tubules of kidney.

Methods

Mice and treatment. Laboratory mice (Mus musculus) were housed in a room with controlled temperature of 21–23°C and humidity of 30–70% under a 12 h light/12 h dark cycle. The Villin-CreERT2 transgene, UBC-CreERT2 transgene, Hnf4aflw/Hnf4aflw;Six2GFPCre15, alleles were generated at conditional compound mutants and controls. All mouse protocols and experiments conducted had the approval of the Rutgers Institutional Animal Care and Use Committee. To avoid circadian variability, we collected samples between noon to 2 pm.

To study intestinal brush border, experimental Villin-CreERT2 mice (8–12 weeks old) were administered with tamoxifen (Sigma T5648) at 50 mg/kg/day or vehicle, intraperitoneally (i.p.). Histologic and western blot samples were collected from mice after 4 consecutive days of tamoxifen to induce Cre recombination or vehicle treatment. H3K4me3 HiChIP-seq and H3K27ac MNase ChIP-seq samples were collected from mice after 3 consecutive days of tamoxifen or vehicle treatment. RNA-seq samples were collected from mice after 2 or 3 consecutive days of tamoxifen or vehicle treatment.

To study renal brush border in pups, experimental UBC-CreERT2-positive and -negative pups were all treated with tamoxifen at 0.1 mg/day by i.p. injection 2 days (P2) and 4 days (P4) after their birth, and kidney tissues were collected at day 4 after their birth (P8) for further analysis. See schematic of experimental design in Fig. 4a, left panel.

To study renal brush border in adults, experimental UBC-CreERT2-positive and -negative mice (5 weeks old) were all treated with tamoxifen at 100 mg/kg/day by i.p. injection for 4 consecutive days. Renal histologic analysis was conducted 7 days or 14 days after tamoxifen treatment. Kidney (RNA-seq), urine, and plasma were collected after 7 days after tamoxifen treatment. See schematic of experimental design in Fig. 1a, right panel.

To study brush border of yolk sac, pregnant UBC-CreERT2-negative female (crossed with UBC-CreERT2-positive male) were treated with (1) 5 mg tamoxifen by oral gavage at E14.5 or (2) treated with a mixture of tamoxifen and progesterone (Sigma P0130) at 5 mg by oral gavage at E12.5 and E13.5 or 3) treated with a mixture of tamoxifen and progesterone at 5 mg by oral gavage at E12.5, and followed by 2 mg via i.p. injection at E13.5. Here, progesterone was co-administered with tamoxifen (1:1 mix, 5 mg each) to prevent fetal abortions in pregnant mice.47. See schematic of experimental design in Fig. 5c, e and Supplementary Fig. 11b.

Intestinal epithelium isolation. Intestine was collected, flushed with cold phosphate-buffered saline (PBS), and opened longitudinally. After cutting the samples into 1 cm pieces, the samples were rotated in 3 mM EDTA/PBS at 4°C for 5 and 10 min. The EDTA/PBS was refreshed every time. After a light shake, the WT and mutant tissues were transferred to tubes with fresh 3 mM EDTA/PBS, respectively. The time of EDTA incubation was adjusted to release all the epithelial cells from the underlying muscular layer. WT tissues were incubated for 40 min, whereas mutant tissues were incubated for 20 min. The intestinal tissues were vigorously shaken and the whole epithelium fraction was collected from the supernatant. Villus and crypt cells were separated using a 70 μm cell strainer, where crypt cells passed through and villus cells were kept on the top of the strainer. Cells were pelleted by centrifugation at 4°C, 200 g x g for 3 min. After washing with cold PBS, cell pellets were used for experiments as described in later sections.

Histology and staining. Intestinal, renal, and yolk sac tissues were fixed in 4% paraformaldehyde at 4°C overnight, washed in PBS prior to dehydration and paraffin embedding. For cryo-embedding, after fixation and PFA wash, tissues were processed with 15% sucrose for 4 h and 30% sucrose until tissues sunk. Optimal cutting temperature (OCT) compound (Tissue-Tek 4583) was used for cryo-embedding. Five-micrometer paraffin sections were prepared for immunohistochemistry, immunofluorescence, and AP staining. Ten-micrometer cryosections of duodenal epithelium were prepared for phalloidin staining (Thermo Fisher Scientific A34055) according to the manufacturer’s instructions. AP is an enzyme colorized with brush border and its activity was detected using the AP Staining Kit II (Stemgent). Periodic Acid–Schiff staining was used to detect proximal tubule in the kidneys and slides were incubated in 0.5% periodic acid and stained with Schiff’s Reagent (612171, Alfa Aesar). Co-staining of AP and HNF4A was performed by AP staining and followed by HNF4A immunohistochemistry. Immunohistochemistry was performed using primary antibodies against Hnf4a (Santa Cruz sc-6558 X, 1 : 2000), Hnf4g (Santa Cruz sc-6558 X, 1 : 2000), β-actin (Abcam ab8227, 1 : 1000), Villin (Santa Cruz sc-7672, 1 : 500), Ezrin (Cell Signaling #3145, 1 : 1000), Aeg2 (Abcam ab21695, 1 : 1000), Kanuji Biochemicals, MC-980, 1 : 500), Cytokeratin18 (Santa Cruz sc-51582, 1 : 200), Calpain 2 (Santa Cruz sc-373966, 1 : 200), Laminin γ-2 (Santa sc-28330, 1 : 200), and Filamin A (Abcam ab76289, 1 : 500). Blocking reagents and secondary antibodies were used according to the manufacturer’s protocol of the Vectastain ABC HRP Kit (Vector Labs). DAB (0.05% Amresco, #9430) and 0.015% hydrogen peroxide in 0.1 M Tris were used for immunohistochemistry developing. The slides were counterstained with hematoxylin, mounted, and viewed on a Nikon Eclipse E800 microscope. The
staining was imaged with a Lumenera INFINITY3 camera and infinity capture imaging software (v6.5.6). A Zeiss Axiovert 200 M fluorescence microscope was used for imaging phalloidin staining using a Retiga-SRV CCD (QImaging) and QCapture imaging software (v2.0.8). A Zeiss LSM 510 Meta confocal microscope and Carl Zeiss ZEN 2009 (v5.5.0.451) software were used for imaging the immunofluorescence staining of Hnf4α (Abcam ab61898, 1 : 100), Hnf4γ (Santa Cruz sc-6558 X, 1 : 100), β-actin (Abcam ab8227, 1 : 100), Abcg2 (Kamiya Biochemical Company MC-980, 1 : 100), NCC (also known as Thiazide-Sensitive NaCl Cotransporter, Sigma AB3553, 1 : 100), Filamin A (Abcam ab76289, 1 : 100), and DAPI (Biotium 40043, 1 : 5000). ImageJ software (v1.52) was used for staining quantification.

Transmission electron microscopy. Freshly dissected intestinal and renal tissues were cut into 1 mm fragments and immediately fixed in 0.1 M sodium cacodylate buffer (pH 7.4, Electron Microscopy Sciences 11653) containing 2%
Fig. 6 HNF4 loss results in an increased stress fiber formation. a, b Yolk sac data of WT vs Hnf4fl/fl. Schematic of experimental design is shown in Fig. 5e. a GSEA of RNA-seq data from E18.5 yolk sac reveals that stress fiber assembly (Kolmogorov-Smirnov test, one-sided for positive and negative enrichment scores, P = 0.0001) and focal adhesion assembly (Kolmogorov-Smirnov test, one-sided for positive and negative enrichment scores, P = 0.0039) gene signatures are all elevated upon HNF4A loss. b IHC staining of stress fiber/focal adhesion-related proteins (n = 3 biological replicates) in E17.5 yolk sac. c–i Intestinal epithelium data of WT vs. Hnf4fl/fl. c Heatmap of RNA-seq data shows upregulated stress fiber/focal adhesion transcripts upon HNF4 loss in the intestinal epithelium (FDR < 0.05, n = 3 biologically independent mice). d Increased chromatin loops were observed at stress fiber/focal adhesion gene loci upon HNF4 loss. H3K4me3 HiChIP-seq was done in villus cells of Hnf4fl/fl and their littermate controls. Differential interactions (DESeq2 P < 0.05) were visualized by Sushi package for the loops with q ≤ 0.0001 and counts ≥ 2 (combined 2 replicates). Bar charts show transcript levels of brush border genes. The data are presented as mean ± SEM (RNA-seq: n = 3 biological replicates). **Cuffdiff FDR < 0.001 and *FDR < 0.05). H3K4me3 HiChIP-seq; n = 2 biological replicates; H3K27ac ChIP-seq: n = 2 biological replicates; HNF4 ChIP-seq: n = 2 biological replicates. e IHC staining of stress fiber/focal adhesion-related proteins (n = 3 biological replicates). f Increased chromatin loops at Flnb locus were observed upon HNF4 loss. Elevated protein levels of Filamin A were observed by g western blotting (n = 2 biologically independent mice) and h immunofluorescence staining (n = 3 biologically independent mice). I Loss of HNF4 factors results in downregulated brush border genes and upregulated stress fiber genes.

paraformaldehyde (Electron Microscopy Sciences 15714-S) and 2.5% glutar- aldehyde (Electron Microscopy Sciences 16216) at 4 °C overnight. Tissue proces- sing, embedding, sectioning, and imaging were performed using standard procedures as described38.

Protein extraction and western blotting. RIPA buffer (50 mM Tris-HCl pH 8.0, 1% SDS, 150 mM NaCl, 0.5% Na-deoxycholate, 1% NP-40, phosphatase inhibi- tors, and protease inhibitor cocktails) was used to extract the total protein. The protein extraction from brush or plasmatic fractions was described as separated as described39. Bioruptor sonication was used to break cells from duodenal epithelium before (4 cycles, 30 s on, and 30 s off) and after (2 cycles, 30 s on, and 30 s off) rotating in lysis buffer at 4 °C for 30 min, respectively. Pierce BCA Protein Assay Kit (Thermo) was used to measure protein concentration. The following primary antibodies were used in this study: Hnf4α (Santa Cruz sc-6558 X, 1:1000), Villin (Santa Cruz sc-7672, 1:1000), Keratin 20 (Cell Signaling #13063, 1:2500), Filamin A (Abcam ab76289, 1:1000), Lamin B1 (Abcam ab6048, 1:1000), GAPDH (Santa Cruz sc-25778, 1:5000), and β-actin (Abcam ab8227, 1:5000). The blots with protein ladders can be found in the Source Data file.

RNA extraction and quantitative reverse transcription-PCR. Yolk sac tissues were processed for RNA extraction using Trizol (Invitrogen) according to the manufacturer's instructions. RNA was purified as described24. DNase I was used to remove contaminating DNA from total RNA with Oligo (dT)20 primers using SuperScript III First-Strand Synthesis SuperMix (Invitrogen). Quantitative reverse transcription-PCR was performed to measure changes in mRNA expression using Applied Biosytems 7900HT Sequence Detection System with Power SYBR Green PCR Master Mix. The amplification conditions were as follows: 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. The primer sequences are provided in Table 1. The quality of raw sequencing reads. Tophat2 (v2.1.0) was used to align the reads to the mouse (mm9) genome and generate bam files. The cxb files were generated from these bam files using Cuffquant (v2.2.1) and FPKM values were calculated with Cuffnorm (v2.2.1) using quartile normalization. Differentially expressed genes were identified between the controls and the mutant using Cuffdiff (v2.2.1) with quartile normalization and per-condition dispersion. Genes with FPKM > 1 were used for RNA-seq-related analysis. RNA-seq data (bam files) were visualized using the Integrative Genomics Viewer25 (IGV 2.4.13). The heatmaps were plotted using Heatmapper26 with normalized FPKM values of RNA-seq. GSEA (v4.0.3)20 was done on the proranked gene list. Gene Ontology analysis was done with DAVID27 (v6.8). Brush border gene list used in this study was combined from following Gene Ontology terms, including brush border genes (GO:0031526), and microvillus genes (GO:0005902). The scRNA-seq data were re-analyzed and visualized by Seurat package28,39 (v3.1.5).

Data analysis of ChIP-seq and DNase-seq. FastQC (v0.11.3) was used to check the quality of raw sequencing reads (fastq), and bowtie2 (v2.2.6) was used to align the sequences to mouse (mm9) genome and generate bam files. MACS2 (v1.4.1) was used for peak calling and to generate bed files from aligned reads. Hm4 ChIP- seq (HNF4A, Santa Cruz sc-6556 X, lot B1015, 6 μg per ChIP; HNF4G, Santa Cruz sc-6538 X, lot F0310, 6 μg per ChIP) of mouse duodenal epithelium are at a p-value of 10−3; DNase-seq of the intestine and kidney, Hm4 ChIP-seq of kidney, and H3K27ac (Abcam ab7479, lot GR148332-2, 6 μg per ChIP) Mmase ChIP-seq of mouse duodenal epithelium are at a p-value of 10−5. The distribution of HNF4-binding sites was analyzed with the cis-regulatory element annotation system (CEAS, v1.0.7)39. BedTools31 (v2.17.0) was used to merge, intersect, or subtract the intervals of bed files. Peak2gene/BEATa-minus32 (v1.0.0) or GREAT analysis33 (v3.0.0) was used to identify genes within from genomic regions (bed file). DeepTools bamCoverage32 (v2.4.2, duplicate reads ignored, RPMK normalized, and extended reads) was used to generate bigwig files from bam files. BigWigMerge (UCSC, v2) was used to merge the bigwig files of different replicates. IGV was used to visualize normalized bigwig tracks, k-means clustering heatmaps of ChIP-seq/ DNase-seq were created with Haystack34 (v0.4.0) quantile normalized bigwigs using computeMatrix and plotHeatmap from deepTools32 (v2.4.2). Genomic regions of desired k-means clusters were extracted from bed files generated by plotHeatmap. Difflib35 (v2.4.7) was used to identify differential signals of Hm4 ChIP-seq between the intestinal and kidney tissues, and 0.01 was used as the cutoff for significance. SitePro70 (v1.0.2) was used to plot the average signal profiles of ChIP-seq/DNase-seq. Homer findMotifsGenome.pl (v4.8.3) was used to find transcription factor motifs. Promoter was defined as region from 2 kb upstream of the transcription start site (TSS) to 2 kb downstream of the TSS, whereas enhancer was defined by excluding the promoter region. HiChIP-seq and data analysis. Duodenal epithelial cells, kidney, and E18.5 yolk sac were processed for RNA extraction using Trizol (Invitrogen) according to the manufacturer’s instructions. Duodenal endothelial cells were sequenced using Illumina’s TrueSeq RNA Library Prep kit v2 at Sequencing Facility of the Rutgers Cancer Institute of New Jersey, whereas kidney and yolk sac tissues were sequenced using Illumina HiSeq 2000 at BGI Shenzhen, China. To control for the expression of brush border genes in different tissues, Kidallito (v0.4.40) was utilized to quantify the transcript abundances of the RNA-Seq samples through pseudocounting, using single-end or paired-end reads and a RefSeq mm9 transcriptome build index. Then, the tximport38 (v1.8.0) package was run in R (v3.5.2) to create gene-level counts, followed by DiffQuest (v1.20) by importing quantification data obtained from Kidallito. DESeq2 was then used to generate FPKM values per kilobase of gene length per million mapped fragments for the RNA-seq data of intestine tissues and kidney tissues. These FPKM values were further used to compare with the public RPMK values of embryonic yolk sac29. To compare transcript levels of mutants vs. controls, fastQC (v0.11.3) was used to check the quality of raw sequencing reads. Tophat2 (v2.1.0) was used to align the reads to the mouse (mm9) genomes and generate bam files. The cxb files were generated from these bam files using Cuffquant (v2.2.1) and FPKM values were calculated with Cuffnorm (v2.2.1) using quartile normalization. Differentially expressed genes were identified between the controls and the mutant using Cuffdiff (v2.2.1) with quartile normalization and per-condition dispersion. Genes with FPKM > 1 were used for RNA-seq-related analysis. RNA-seq data (bam files) were visualized using the Integrative Genomics Viewer25 (IGV 2.4.13). The heatmaps were plotted using Heatmapper26 with normalized FPKM values of RNA-seq. GSEA (v4.0.3)30 was done on the proranked gene list. Gene Ontology analysis was done with DAVID27 (v6.8). Brush border gene list used in this study was combined from following Gene Ontology terms, including brush border genes (GO:0005903), brush border membrane genes (GO:0031526), and microvillus genes (GO:0005902). The scRNA-seq data were re-analyzed and visualized by Seurat package28,39 (v3.1.5).
washed with ice-cold PBS twice by centrifugation at 300 g for 4 °C. The HiChIP protocol was conducted and HiK-meq (Millipore 05-745 R, lot 3158071, 9 g, per ChIP) and nuclear lysis buffer was used as described [2]. HiC-Pro pipeline [22] (v2.11.1) was used to process the HiChIP data as described [23]. Bowtie2 (v2.3.4.3) was used to align reads to the mouse (mm9) genome assembly. DESeq2 [24] (v1.20) was applied to identify differential loops using sequencing counts of HiK-meq HiChIP-seq data to identify. To identify the differential loops, we took hichipper [25] (v0.7.0) loops with raw counts ≥4 in both replicates and ≥0.0001 in at least one replicate, from at least one of the two conditions being compared. HiChIP loops were visualized using Sushi package [26] (v1.20). For simplicity, biological replicates were combined for Sushi looping visualization.

**Statistical analysis.** The data is presented as mean ± SEM (GraphPad Prism v8.4.3) and statistical comparisons were performed using Student’s t-test at ***P < 0.001, **P < 0.01, *P < 0.05. Source data are provided as a Source Data file. The exact P-values are also shown in the Source Data file. Kolmogorov–Smirnov test was used on GSEA. Mann–Whitney test was used as part of RNA-seq analysis. Bioinformatics-related statistical analysis was done with the embedded statistics in each package, including Cuffdiff, DiffBind, HOMER, GSEA, DESeq2, DAVID, and hicchipper. P < 0.05 (95% confidence interval) was considered statistically significant.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability.** All the kidney and yolk sac RNA-seq data have been deposited in the Gene Expression Omnibus (GEO) database under accession code: GSE11282826. The HiChIP-seq data of this study have been deposited in the GEO database under accession code: GSE148697 [27]. The villi ATAC-seq, Hnf4α mutants vs. WT transcriptome, Hnf4α ChIP-seq, and HESK2 Dnase1 ChIP-seq of mouse intestinal epithelial cells have been deposited in the GEO database under accession code: GSE129466 [28]. GSE47192 [29] was used to reanalyze renal Hnf4α ChIP-seq. GSE57914 [30] and GSE51336 [31] were used to reanalyze Dnase-seq of the duodenum and kidney. PRJEB1876720 was used to reanalyze DNase-seq of the duodenum and kidney. PRJEB1876720 was used to reanalyze DNase-seq of the duodenum and kidney. PRJEB1876720 was used to reanalyze DNase-seq of the duodenum and kidney. PRJEB1876720 was used to reanalyze DNase-seq of the duodenum and kidney. PRJEB1876720 was used to reanalyze DNase-seq of the duodenum and kidney. All the kidney and yolk sac RNA-seq data have been deposited in the Gene Expression Omnibus (GEO) database under accession code: GSE11282826. The yolk sacs of human, mouse, and chicken. Proc. Natl Acad. Sci. USA 114, E4753–E4761 (2017).

Ashburner, M. et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat. Genet. 25, 25–29 (2000).

The Gene Ontology, C. The Gene Ontology Resource: 20 years and still GOing strong. Nucleic acids Res. 47, D330–D338 (2019).

Camp, J. G. et al. Microbiota modulate transcription in the intestinal epithelium without remodeling the accessible chromatin landscape. Genome Res. 25, 1504–1516 (2015).

Vierstra, J. et al. Mouse regulatory DNA landscapes reveal global principles of cis-regulatory evolution. Science 346, 1007–1012 (2014).

Stark, R. & Brown, G. D. DiffBind: differential binding analysis of ChIP-seq peak data. Bioconductor, http://bioconductor.org/packages/releases/bioconductor.html (2011).

Marable, S. S., Chang, E., Adam, M., Potter, S. S. & Park, J. S. Hnfp4a deletion in the mouse kidney phenocopies Fanconi renitubular syndrome. JCI Insight 3, e97497 (2018).

Marable, S. S., Chang, E. & Park, J. Hnf4a is required for the development of Cdh6-expressing progenitors into proximal tubules in the mouse kidney. J. Am. Soc. Nephrol. https://doi.org/10.1681/asn.2020020184 (2020).

Park, J. et al. Single-cell transcriptomics of the mouse kidney reveals potential cellular targets of kidney disease. Science 360, 758–763 (2018).

Yaxley, J. & Pirrone, C. Review of the diagnostic evaluation of renal tubular acidosis. Ochsner J. 16, 525–530 (2016).

Pendergrass, P. B., Ream, L. J. & Scott, J. N. Absorptive structures of the mouse yolk sac placenta and associated placental surfaces. A scanning electron microscope study. J. Submicrosc. Cytol. 14, 279–289 (1982).

Burridge, K. & Guillery, C. Focal adhesions, stress fibers and mechanical tension. Exp. Cell Res. 343, 14–20 (2016).

Tojkander, S., Gateva, G. & Lappalainen, P. Actin stress fibers—assembly, dynamics and biological roles. J. Cell Sci. 125, 1855–1864 (2012).

Fujisawa, S., Ohashi, K., Mashiko, T., Kondo, H. & Mizuno, K. Interplay between Solo and keratin filaments is crucial for mechanical force-induced stress fiber reinforcement. Mol. Biol. Cell 27, 954–966 (2016).

Cheah, J. S., Jacobs, K. A., Heinrich, V., Lo, S. H. & Yamada, S. Force-induced stress fiber recruitment of cten along keratin network in epithelial cells. Proc. Natl Acad. Sci. USA 116, 19799–19801 (2019).

Gagnon-Palacios, L. et al. Functional Re-expression of laminin-5 in laminin-gamma2-deficient human keratinocytes modifies cell morphology, motility, and adhesion. J. Biol. Chem. 271, 18437–18444 (1996).

Glading, A., Lauffenburger, D. A. & Wells, A. Cutting to the chase: calpain proteases in cell motility. Trends Cell Biol. 12, 46–54 (2002).

Shao, Q. Q. et al. Filamin A: insights into its exact role in cancers. Pathol. Oncol. Res. 22, 245–252 (2016).

Djidjaj, S. et al. Keratins are novel markers of renal epithelial cell injury. Kidney Int. 89, 792–808 (2016).

Cattin, A. L. et al. Hepatocyte nuclear factor alpha, a key factor for homeostasis, cell architecture, and barrier function of the adult intestinal epithelium. Mol. Cell. Biol. 29, 6294–6308 (2009).

Chiba, H. et al. The nuclear receptor hepatocyte nuclear factor 4alpha acts as a morphogen to induce the formation of microvilli. J. Cell Biol. 175, 971–980 (2006).

Na, S. et al. Rapid signal transduction in living cells is a unique feature of mechanotransduction. Proc. Natl Acad. Sci. USA 105, 6626–6631 (2008).

Keren, K. et al. Mechanism of shape determination in motile cells. Nature 453, 475–480 (2008).

Bedolla, R. G. et al. Nuclear versus cytoplasmic localization of HNF4 to regulate androgen receptor and coactivator functions. Proc. Natl Acad. Sci. USA 100, 4562–4567 (2003).
54. Wang, Y. et al. A 90 kDa fragment of filamin A promotes Casodex-induced growth inhibition in Casodex-resistant androgen receptor positive C4-2 prostate cancer cells. Oncogene 26, 6061–6070 (2007).

55. Chellappa, K. et al. Opposing roles of nuclear receptor HNF4α isoforms in colitis and colitis-associated colon cancer. elife 5, e19093 (2016).

56. Hamilton, A. J. et al. The HNF4A R76W mutation causes atypical dominant Fanconi syndrome in addition to a beta cell phenotype. J. Med. Genet. 51, 156–163 (2014).

57. Lindstrom, N. O. et al. Integrated beta-catenin, BMP, PTEN, and Notch signalling patterns the nephron. eLife 3, e04000 (2015).

58. Schneider, J., Arraf, A. A., Grinstein, M., Yelin, R. & Schultheiss, T. M. Wnt signaling orients the proximal-distal axis of chick kidney nephrons. Development 142, 2680–2695 (2015).

59. Chung, E., Deacon, P. & Park, J. S. Notch is required for the formation of all nephron segments and primed nephron progenitors for differentiation. Development 144, 4530–4539 (2017).

60. El Marjou, F. et al. Tissue-specific and inducible Cre-mediated recombination in the gut epithelium. Genesis 39, 186–193 (2004).

61. Perekatt, A. O. et al. YY1 is indispensable for Lgr5+ intestinal crypts and transcription programs. EMBO J. 33, 312–326 (2014).

62. Kandasamy, P., Gyimesi, G., Kanai, Y. & Hediger, M. A. Amino acid transporters revisited: New views in health and disease. Trends Biochem. Sci. 43, 752–789 (2018).

Acknowledgements

This research was funded by grants from the NIH (R01CA196558 and R01DK121915 to M.P.V. and R01DK121782 to A.S.). M.P.V. is also supported by the Intestine Stem Cell Consortium from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health under grant number 1U01 DK103141. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. L.C. was supported by New Jersey Commission on Cancer Research grant (DFHS18PPC051). Support was also received from the Sequencing Facility and Metabolomics Shared Resource of the Rutgers Cancer Institute of New Jersey (PC00072720) and imaging core facility of Human Genetics Institute of New Jersey. S.L., A.D., R.P.V., A.R.A., R.M., and N.H.T. were supported by MacMillan Summer Undergraduate Research Fellowships. We thank Eileen White for sharing the UBC-Cre;P20/J2 mice. We thank Lauren Alekxunes for helping us understand yolk sac biology. We thank Noriko Goldsmith for helpful imaging support. We also thank Anbo Zhou for his extra efforts, especially with helping us overcome difficulties during the pandemic.

Author contributions

L.C. conceived and designed the study, performed benchwork, sequencing data processing, and bioinformatics, collected and analyzed the data, and wrote the manuscript. S.L. supervised the study, and wrote the manuscript. A.D., R.P.V., A.P., R.A., R.M., and N.H.T. were supported by MacMillan Summer Undergraduate Research Fellowships. We thank Benjamin Humphreys and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-021-22761-5.

Correspondence and requests for materials should be addressed to L.C. or M.P.V.

Peer review information Nature Communications thanks Benjamin Humphreys and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.