PKD1 and PKD2 mRNA cis-inhibition drives polycystic kidney disease progression

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Autosomal dominant polycystic kidney disease (ADPKD), among the most common human genetic conditions and a frequent etiology of kidney failure, is primarily caused by heterozygous PKD1 mutations. Kidney cyst formation occurs when PKD1 dosage falls below a critical threshold. However, no framework exists to harness the remaining allele or reverse PKD1 decline. Here, we show that mRNAs produced by the noninactivated PKD1 allele are repressed via their 3′-UTR miR-17 binding element. Eliminating this motif (Pkd1Δ17) improves mRNA stability, raises Polycystin-1 levels, and alleviates cyst growth in cellular, ex vivo, and mouse PKD models. Remarkably, Pkd2 is also inhibited via its 3′-UTR miR-17 motif, and Pkd2Δ17-induced Polycystin-2 derepression retards cyst growth in Pkd1-mutant models. Moreover, acutely blocking Pkd1/2 cis-inhibition, including after cyst onset, attenuates murine PKD. Finally, modeling PKD1Δ17 or PKD2Δ17 alleles in patient-derived primary ADPKD cultures leads to smaller cysts, reduced proliferation, lower pCreb1 expression, and improved mitochondrial membrane potential. Thus, evading 3′-UTR cis-interference and enhancing PKD1/2 mRNA translation is a potentially mutation-agnostic ADPKD-arresting approach.

An estimated 12.5 million people worldwide suffer from autosomal dominant polycystic kidney disease (ADPKD), making it among the most common monogenic conditions known to humankind. A clinical hallmark of ADPKD is the relentless growth of innumerable fluid-filled cysts in the kidneys, which replace the normal parenchyma and, over decades, cause massive bilateral kidney enlargement and renal failure. ADPKD occurs because of heterozygous, loss-of-function mutations in PKD1 (~78% of cases) or PKD2 (~15% of cases). The classical hypothesis for cyst initiation is that in addition to a germline inactivating mutation in one allele of the PKD gene, there is somatic inactivation (referred to as the second hit) in the other allele, causing a complete loss of polycystin expression in the cell. However, in recent years, several lines of evidence support the gene dose threshold as a mechanism involved in cystogenesis. This hypothesis posits that complete PKD1 loss is not necessary, but rather cystogenesis ensues if the functional PKD1 dosage falls below a critical threshold. Supporting the gene dosage model, inactivating second hit mutation is not a universal feature, especially in smaller ADPKD cysts. Importantly, many individuals with ADPKD continue to have residual PC1 expression because they carry missense (rather than inactivating) germline PKD1 mutations. As proof of principle, lowering the Pkd1 dose is sufficient to produce PKD in mice, pigs, and monkeys. Thus, if reduced dosage causes ADPKD, increasing the expression of the normal PKD1 allele could arrest the disorder. However, despite this transformative potential, the factors governing PKD1 dosage in ADPKD are mostly unknown, and currently, there are no mechanisms to activate the normal PKD1 allele. The 3′-untranslated region (3′-UTR), the mRNA portion that lies immediately downstream of the translation termination codon, protects the mRNA from degradation and facilitates translation through
its poly(A) tail. Paradoxically, the 3′-UTR, via interaction with micro-RNAs (miRNAs), can also mediate mRNA translation repression or deadenylation26–28. Most mRNA 3′-UTRs harbor evolutionarily conserved miRNA-binding elements (MBEs), implying that cis-inhibition of translation is a pervasive mode of gene output regulation29. However, this intriguing aspect of the 3′-UTR function is poorly delineated. The prediction is that individual MBEs have a minor impact on host mRNA function, considering that miRNAs mostly act as rheostats and modestly repress mRNA targets. Counter to this prevailing logic, we reasoned that under certain circumstances, such as when gene dosage is already reduced due to haploinsufficiency, MBE-mediated cis-inhibition of the remaining allele could have a disease-modifying effect by governing the final protein output.

Our goal was to determine whether monoallelic Pkd1 derepression is possible and how it influences preclinical ADPKD progression. Pkd1 contains a miR-17 binding motif in its 3′-UTR, and miR-17 expression and activity are higher in ADPKD models4,23–26. Therefore, we tested the idea that Pkd1 mRNA is cis-inhibited by its 3′-UTR miR-17 motif, and blocking this inhibition reverses Pkd1 decline. We use CripsR/Cas9 editing to delete the miR-17 motif from the Pkd1 gene in monoallelic ADPKD models. We find that eliminating the miR-17 motif is sufficient to improve Pkd1 mRNA stability, raise Polycystin-1 (PC1) expression, and arrest cyst growth in cellular and ex vivo and mouse ADPKD models. The other ADPKD gene, PKD2, also contains a 3′-UTR miR-17 binding motif; remarkably, deleting this miR-17 motif increases Polycystin-2 (PC2) levels and attenuates cyst growth in Pkd1-mutant models. Furthermore, acute pharmaceutical blockade of Pkd1/2 cis-inhibition prevents cyst onset and stabilizes established PKD in mice. Finally, we demonstrate that Pkd1 or Pkd2 derepression reverses cystic pathogenic events in primary kidney cyst epithelia derived from individuals with ADPKD.

**Results**

**Pkd1 is cis-repressed via its 3′-UTR miR-17 binding motif**

The impact of individual 3′-UTR MBEs on host gene regulation is mostly unstudied. We explored the idea that cis-repression via its 3′-UTR miR-17 binding motif is a novel mechanism governing Pkd1 dosage. We began by examining the effect of deleting this MBE in normal mouse kidneys. We designed sgRNAs that bind to Pkd1 exon 46, flanking the DNA segment that encodes the miR-17 motif, and used CripsR/Cas9 editing to generate Pkd1 alleles (Pkd1Δ17) lacking the miR-17 binding site (Fig. 1a). First, we validated the motif deletion using DNA PCR followed by direct Sanger sequencing (Fig. 1b, c). Our editing approach did not inadvertently inactivate Pkd1 since we observed normal kidney histology and renal function in 6-week-old and 18-week-old Pkd1Δ17/+ mice (Fig. 1d–f and Supplementary Fig. 1). To assess Pkd1 levels, we performed western blot analysis using the 7E12 Pkd1 antibody in wildtype and Pkd1Δ17/+ cells (Fig. 2a and Supplementary Fig. 7a). Next, we validated the motif deletion using qRT-PCR revealed that in Pkd1Δ17/+ kidney cultures. This difference became even more pronounced in the presence of cAMP (Supplementary Fig. 4a-c). These observations further imply inhibition of wildtype Pkd1 mRNA by miR-17 but an evasion of repression and improved stability of Pkd1Δ17+ mRNAs in embryonic kidneys.

**Endogenous monoallelic Pkd1 derepression alleviates polycystic kidney disease**

Kidney cyst formation ensues when Pkd1 dosage falls below a critical threshold. However, no approach exists to reverse the Pkd1 decline. Our observations in normal embryonic kidneys prompted us to ask whether Pkd1 is cis-repressed in ADPKD and if preventing this inhibition has a disease-modifying impact. We first examined the Pkd1Δ17/+ cellular ADPKD model. This is a collecting duct-derived mouse cell line that harbors the missense RC mutation on one Pkd1 allele, whereas the other allele is inactivated30. The RC mutation results in arginine to cysteine substitution two amino acids before the second transmembrane domain and reduces mature (functional) PC1 protein levels. The RC mutation maps to Pkd1 exon 30 and is significantly upstream of the miR-17 motif, which is encoded by Pkd1 exon 46. This allowed us to use CripsR/Cas9 editing to remove the 3′-UTR miR-17 motif from the RC allele (Pkd1Δ17/+) (Supplementary Fig. 5). We generated two independent Pkd1Δ17/+ clonal cell lines and characterized them both in relation to the unedited parental Pkd1Δ17/+ and Pkd1Δ17/+ cells. We previously reported that Pkd1 expression was reduced in Pkd1Δ17/+ cells compared to Pkd1Δ17/+ cells. Remarkably, western blot analysis using the 7E12 antibody revealed that eliminating the miR-17 motif restored Pkd1 expression in Pkd1Δ17/+ cells (Fig. 2a and Supplementary Fig. 7a). Next, employing several independent assays, we demonstrated that this degree of Pkd1 derepression was sufficient to reverse several well-known pathogenic events linked to cyst growth. First, we noted a higher proliferation rate and 3D cyst size in Pkd1Δ17/+ cells than in Pkd1Δ17/+ cells, which were normalized after Pkd1 derepression in Pkd1Δ17/Δ17 cells (Fig. 2b, c). Second, we observed that while cAMP, glucose, and SAM increased the already elevated proliferation of Pkd1Δ17/+ cells, Pkd1Δ17/Δ17 cells were resistant to these pro-proliferative stimuli (Fig. 2d and Supplementary Table 1). Third, we used MitoTracker to assess mitochondrial membrane potential as a proxy of oxidative phosphorylation and anti-pCreb1 antibody immunofluorescence as a readout of cAMP signaling. Compared to Pkd1Δ17/+ cells, we observed a reduced MitoTracker signal and higher pCreb1 expression in Pkd1Δ17/+ cells. The opposite was true for Pkd1Δ17/Δ17 cells, which exhibited restored MitoTracker signal and lowered pCreb1 expression (Fig. 2e and Supplementary Fig. 6b). Finally, immunoblot analysis revealed elevated Yap1, pCreb1, and c-Myc expression in Pkd1Δ17/+ cells compared to Pkd1Δ17/+ cells, which returned to baseline in Pkd1Δ17/Δ17 cells (Supplementary Fig. 6c).

Based on these encouraging results, we next modeled the 3′-UTR Δ17 deletions in vivo. We CripsR-edited KspΔ17/+; Pkd1Δ17/+ fertilized...
eggs to eliminate the miR-17 motif from the Pkd1 RC allele. We implanted these eggs into pseudopregnant surrogate female mice and eventually obtained three germline-transmitting heterozygous KspCre+; RC/Δ double heterozygous mice from one RC allele, whereas the other RC allele still contained the Δ17 deletion. We used DNA PCR and Sanger sequencing to validate that the miR-17 motif was indeed deleted in all three founder mice.

For each founder progeny, we observed reduced PC1 to-body-weight (KW/BW) ratio, and higher serum blood urea nitrogen (BUN) levels in 8-week-old Pkd1Δ17/+ and Pkd1Δ17/Δ17 mice. e–f Normal kidney weight-to-body weight (KW/BW) and serum blood urea nitrogen (BUN) levels in 8-week-old Pkd1Δ17/+ and Pkd1Δ17/Δ17 mice. g–h Images and cyst index quantification of E13.5 Pkd1Δ17/+ and Pkd1Δ17/Δ17 kidneys grown for four days in culture media containing vehicle, 100 μM cAMP, or 100 μM cAMP plus 250 μM SAM. i Immunoblots depicting PC1 expression in Pkd1Δ17/+ and Pkd1Δ17/Δ17 ex-vivo kidneys treated with vehicle, cAMP, or cAMP plus SAM. Actin was used as the loading control. j Allele-specific qRT-PCR showing the quantity of Pkd1 miRNAs produced by the wildtype (+) and Δ17 alleles in E15.5 Pkd1Δ17/+ kidneys (n = 5). Error bars indicate SEM. Statistical analysis: Two-tailed Student’s t-test (e, f); One-way ANOVA, Tukey’s multiple-comparisons test (h); Two-tailed paired t-test (j). Source data are provided as a Source Data file.

deletion caused PCI derepression, as assessed using the 7E12 antibody, in Pkd1Δ17/+ kidneys compared to Pkd1Δ17/Δ17 kidneys (Fig. 2g). We verified PCI derepression in cell lines and mice using a second independent antibody generated by the U Maryland PKD center (see methods for details) that detects the PCI c-terminus (Supplementary Fig. 7). Moreover, using paired-end RNA-seq, we noted higher RC allele usage in Pkd1Δ17/+ kidneys compared to Pkd1Δ17/Δ17 kidneys, further indicating Pkd1 derepression (Fig. 2j). Strikingly, the cystic disease was almost completely alleviated, and KW/BW and serum BUN were nearly normalized in Pkd1Δ17/+ kidneys (Fig. 2f–h). Data from the 18-day-old progeny of all three founders are shown in Fig. 2f–h. First, we noted normal kidney histology and function in Pkd1Δ17/Δ17 mice, again indicating that miR-17 motif deletion does not disrupt Pkd1 or produce PKD (Fig. 2f). For each founder progeny, we observed reduced PCI expression, severe cystic kidney disease, an increased kidney-weight-to-body-weight (KW/BW) ratio, and higher serum BUN levels in Pkd1Δ17/+ mice than in Pkd1Δ17/Δ17 mice (Fig. 2f–j). As with the cell lines, miR-17 motif deletion caused PCI derepression, as assessed using the 7E12 antibody, in Pkd1Δ17/+ kidneys compared to Pkd1Δ17/Δ17 kidneys (Fig. 2g). We verified PCI derepression in cell lines and mice using a second independent antibody generated by the U Maryland PKD center (see methods for details) that detects the PCI c-terminus (Supplementary Fig. 7). Moreover, using paired-end RNA-seq, we noted higher RC allele usage in Pkd1Δ17/+ kidneys compared to Pkd1Δ17/Δ17 kidneys, further indicating Pkd1 derepression (Fig. 2j). Strikingly, the cystic disease was almost completely alleviated, and KW/BW and serum BUN were nearly normalized in Pkd1Δ17/+ kidneys (Fig. 2f–h). Data from the 18-day-old progeny of all three founders are shown in Fig. 2f–h. First, we noted normal kidney histology and function in Pkd1Δ17/Δ17 mice, again indicating that miR-17 motif deletion does not disrupt Pkd1 or produce PKD (Fig. 2f). For each founder progeny, we observed reduced PCI expression, severe cystic kidney disease, an increased kidney-weight-to-body-weight (KW/BW) ratio, and higher serum BUN levels in Pkd1Δ17/+ mice than in Pkd1Δ17/Δ17 mice (Fig. 2f–j). As with the cell lines, miR-17 motif deletion caused PCI derepression, as assessed using the 7E12 antibody, in Pkd1Δ17/+ kidneys compared to Pkd1Δ17/Δ17 kidneys (Fig. 2g). We verified PCI derepression in cell lines and mice using a second independent antibody generated by the U Maryland PKD center (see methods for details) that detects the PCI c-terminus (Supplementary Fig. 7). Moreover, using paired-end RNA-seq, we noted higher RC allele usage in Pkd1Δ17/+ kidneys compared to Pkd1Δ17/Δ17 kidneys, further indicating Pkd1 derepression (Fig. 2j). Strikingly, the cystic disease was almost completely alleviated, and KW/BW and serum BUN were nearly normalized in Pkd1Δ17/+ kidneys (Fig. 2f–h). Data from the 18-day-old progeny of all three founders are shown in Fig. 2f–h. First, we noted normal kidney histology and function in Pkd1Δ17/Δ17 mice, again indicating that miR-17 motif deletion does not disrupt Pkd1 or produce PKD (Fig. 2f). For each founder progeny, we observed reduced PCI expression, severe cystic kidney disease, an increased kidney-weight-to-body-weight (KW/BW) ratio, and higher serum BUN levels in Pkd1Δ17/+ mice than in Pkd1Δ17/Δ17 mice (Fig. 2f–j). As with the cell lines, miR-17 motif deletion caused PCI derepression, as assessed using the 7E12 antibody, in Pkd1Δ17/+ kidneys compared to Pkd1Δ17/Δ17 kidneys (Fig. 2g). We verified PCI derepression in cell lines and mice using a second independent antibody generated by the U Maryland PKD center (see methods for details) that detects the PCI c-terminus (Supplementary Fig. 7).
All founder #3 Pkd1RC progeny survived until 18 weeks of age. However, they developed progressive kidney failure, as evidenced by an average blood urea nitrogen (BUN) of >100 mg/dl and serum creatinine of >0.4 mg/dl (Fig. 3b). Founder #3 Pkd1RCΔ mice exhibited minimal disease progression with average BUN < 30 mg/dl and serum creatinine <0.2 mg/dl (Fig. 3a, b).

Large-scale transcriptomic dysregulation, activation of tubular proliferation and oncogenic signaling, and interstitial inflammation are some of the key pathological hallmarks of ADPKD. We next addressed whether these changes were blunted by PC1 derepression. We performed RNA-seq analysis using kidney samples from 18-day-old Pkd1RC/+, Pkd1RCΔ/+ , Pkd1RC/-, and Pkd1RCΔ/- mice. We observed dysregulation of an extensive network of gene transcripts with upregulation of 4157 and downregulation of 2067 mRNAs in cystic Pkd1RC-/- kidneys compared to noncystic Pkd1RC+/- control kidneys (Fig. 3c).

Mirroring kidney histology, Pkd1RCΔ/+ exhibited a nearly identical gene expression pattern as Pkd1RC+/- kidney. Impressively, >95% of dysregulated mRNAs in Pkd1RC-/- kidneys showed improved (or
while keeping the analysis, we observed higher Pkd2 Yap1 in the kidneys of 18-day-old mice compared to their unedited parental Pkd2 Rc/− cells (Fig. 4a). PC1 expression remained unchanged between the edited and unedited cells, indicating the specificity of miR-17 motif deletion from Pkd2 3′-UTRs (Supplementary Fig. 11b). Interestingly, as the result of the PC2 derepression, we noted that preventing Pkd2 cis-inhibition and improving PC2 expression in Pkd1RC/− kidneys was associated with sustained benefit (Supplementary Fig. 12 for details). These mice were then bred with Pkd1Fl/fl, KspCre; Pkd1Δ17/Δ17 mice to generate KspCre; Pkd1Δ17/Δ17 mice (Fig. 4e, Supplementary Fig. 11b-c). As was the case with the Pkd1 3′-UTR deletions, while cAMP, glucose, and SAM promoted proliferation of Pkd2Rc/− cells, we noted that this stimulatory effect was lost in Pkd1Fl/fl; Pkd2Δ17Δ17 cells (Fig. 4e, Supplementary Table 2).

Our observations in Pkd1Rc/− cells suggest a tantalizing possibility that preventing Pkd2 cis-inhibition and improving PC2 expression compensates and retards disease progression in Pkd1-mutant models. We tested this notion in vivo by deleting the Pkd2 3′-UTR miR-17 motif (Pkd2Δ17Δ17) in Pkd1Rc/− mice. Briefly, we CRISPR/Cas9-edited KspCre, Pkd1RcRc/− mice to generate KspCre; Pkd1RcRc/−; Pkd2Δ17Δ17 mice (see Supplementary Fig. 12 for details). These mice were then bred with Pkd1Rc/− mice to eventually generate the following four genotypes: (i) Pkd1RcRc; Pkd2Rc/−, (ii) Pkd1RcRc; Pkd2Δ17Δ17, (iii) KspCre; Pkd1RcRc, Pkd2Rc/−, and (iv) KspCre; Pkd1RcRc, Pkd2Δ17Δ17. Characterization of these mice revealed that Pkd2 miR-17 motif deletion in the noncystic setting did not cause PC2 upregulation, and both Pkd1RcRc; Pkd2Rc/− and Pkd1RcRc; Pkd2Δ17Δ17 mice exhibited normal kidney histology and function (Fig. 4f, g). In contrast, Pkd2 miR-17 motif deletion in cystic Pkd1Rc/−...
mice was associated with higher PC2 expression. Moreover, KW/BW and serum creatinine levels were reduced by 34.8 and 25%, respectively, in Pkd1Δ17/Δ17 compared to Pkd1Δ17/Δ2; Pkd2Δ/Δ mice (Fig. 4h, i). Consistently, we observed that compared to Pkd1ΔΔ/ΔΔ; Pkd2Δ/Δ kidneys, Pkd1ΔΔ/ΔΔ; Pkd2ΔΔ kidneys exhibited reduced c-Myc and Yap1 expression (Fig. 4G) and lower cyst proliferation and interstitial inflammation (Fig. 4F). As an additional phenotypic characterization, we performed RNA-seq analysis to compare the kidney transcriptomic profile in the four groups of mice (Fig. 4K). The mRNA expression patterns were nearly identical in Pkd1ΔΔ/ΔΔ; Pkd2ΔΔ and Pkd1ΔΔ/ΔΔ; Pkd1ΔΔ ΔΔ kidneys, further implying that Pkd2 miR-17 motif elimination has minimal impact in noncystic kidneys. As expected, the cystic Pkd1ΔΔ/ΔΔ; Pkd2ΔΔ kidneys exhibited widespread mRNA dysregulation compared to noncystic Pkd1ΔΔ/ΔΔ; Pkd2ΔΔ kidneys control kidneys. We found that Pkd2 miR-17 motif deletion was associated with improved expression of nearly 50% of these dysregulated mRNAs in Pkd1ΔΔ/ΔΔ; Pkd2ΔΔ kidneys (Fig. 4K).

**Acute blockade of Pkd1 and Pkd2 cis-inhibition ameliorates PKD**

Our CRISPR-edited clonal cellular or mouse ADPKD models lead to chronic Pkd1 and Pkd2 derepression. Therefore, we could not assess whether acute derepression of Pkd1/2 just as the cysts are forming, will prevent disease onset or if restoring Pkd1/2 can reign in established PKD. To answer these questions, we employed the anti-miR-17 oligonucleotide RGLS4326 as a tool to acutely block Pkd1 and Pkd2 cis-inhibition. First, we confirmed that compared to vehicle (PBS) or control oligonucleotide, RGLS4326 increased Pkd1ΔΔ/ΔΔ and Pkd1ΔΔ/ΔΔ expression in Pkd1ΔΔ/ΔΔ cells (Fig. 5A, B). The Pkd1/2-blocking effect of RGLS4326 was apparent within three days after treatment. Importantly, RGLS4326 treatment did not lead to higher PC1 and PC2 levels and serum creatinine levels in 18-day-old mice with the indicated genotypes (n = 3 for each group). Representative images showing phH3 and MRC1 immunostaining in kidney sections from 18-day-old mice with the indicated genotypes (n = 3 for each group). Heatmap showing differential mRNA expression in kidneys of 18-day-old mice with the indicated genotypes (n = 3). miRNAs that were dysregulated in Pkd1ΔΔ/ΔΔ compared to Pkd1ΔΔ/ΔΔ kidneys but exhibited improved expression in Pkd1ΔΔ/ΔΔ; Pkd2ΔΔ kidney were chosen for heatmap visualization. Error bars indicate SEM. Statistical analysis: One-way ANOVA, Tukey’s multiple-comparisons test (b, h, and i). Source data are provided as a Source Data file.
marked kidney enlargement with a >10-fold higher KW/BW ratio and elevated BUN and serum creatinine in PBS and control oligonucleotide-treated mice compared to age-matched wildtype mice (Fig. 5d–g). Strikingly, PKD was virtually prevented, and renal function remained normal in P18 RGLS4326-treated Pkd1RC/- mice (Fig. 5d–g). In the second study, we began treatment at P16 when Pkd1RC/- mice had already developed cystic disease. By P26, one out of 15 control oligonucleotide-treated mice had died, and the surviving mice had developed progressive kidney enlargement and near-fatal kidney failure. In contrast, we observed attenuation of PKD progression and
stabilization of kidney function in RGLS4326-treated Pkd1Δ/Δ-KO mice (Fig. 5h–k). Finally, in a third study, we assessed the long-term effects of Pkd1/2 derepression in mice that had already developed PKD. We treated Pkd1Δ/Δ mice on P16 and P17 with the vehicle, 20 mg/kg RGLS4326, or 20 mg/kg control oligonucleotide. These mice then received their respective treatment regimens every week until 18 weeks of age. A fourth group of Pkd1Δ/Δ mice received 20 mg/kg RGLS4326 treatment on P16 and P17 and every other week thereafter. 85.7% (12 out of 14) of PBS-treated and 100% (14 out of 14) of control oligonucleotide-treated Pkd1Δ/Δ-KO mice succumbed to their disease before 18 weeks of age. In contrast, 70% (7 out of 10) and 50% (5 out of 10) of Pkd1Δ/Δ mice treated with RGLS4326 bi-monthly or weekly, respectively, survived until 18 weeks of age (Fig. 5m). Furthermore, we noted substantially preserved kidney parenchyma (Fig. 5i and Supplementary Fig. 15) and reduced KW/BW (Fig. 5n) among the surviving mice in the RGLS4326 group. Thus, acute pharmaceutical Pkd1/2 derepression, including after cyst onset, attenuates murine PKD.

PKD1Δ17 or PKD2Δ17 alleles reduce cyst growth of patient-derived primary ADPKD cultures

A logical but crucial question is whether PKD1/2 cis-inhibition is a feature of human ADPKD. We derived these cells from cysts of freshly discarded ADPKD nephrectomy samples from four affected individuals (three males aged 41, 48, and 52 years and one 57-year-old female). We performed PKD1 and PKD2 mutation analysis using DNA from cyst cells (Supplementary Fig. 16). Cell lines #1, #3, and #4 harbor heterozygous PKD1 mutations, whereas cell line #2 harbors heterozygous truncating PKD2 mutation and a missense heterozygous PKD2 mutation. To assess the translational potential of our findings in mice, we used CRISPR/Cas9 editing to eliminate the PKD1 or PKD2 Δ17 motif in these primary ADPKD cultures (Supplementary Fig. 17). We designed human-specific sgRNAs targeting the miR-17 motif in the PKD1 or PKD2 3′-UTRs. We then transfected primary ADPKD cultures from all four donors with Cas9 and either PKD1 or PKD2 3′-UTR sgRNAs. Mock-transfected cells from each donor served as unedited parental controls. We noted higher PCL levels within three days of Cas9 transfection compared to their respective mock-transfected parental controls (Fig. 6a). Similarly, modeling PKD2Δ17 alleles led to higher PCL expression in CRISPR-transfected cultures than their respective mock-transfected parental controls (Fig. 6b). We next assessed the functional significance of PKD1 or PKD2 derepression by performing Matrigel 3D cystogenesis, alamarBlue proliferation assays, live-cell MitoTracker labeling, and anti-pCREB1 immunofluorescence. The CRISPR-transfected cultures containing PKD1Δ17 or PKD2Δ17 cells formed smaller cysts (Fig. 6c–f) and exhibited lower proliferation rates (Supplementary Fig. 18), higher MitoTracker signal, and lower pCREB1 fluorescence. The CRISPR-transfected cultures containing PKD1Δ17 or PKD2Δ17 expression compared to their respective mock-transfected, unedited controls (Fig. 6g, h). These data imply ongoing PKD1/2 cis-inhibition and demonstrate the significant benefit of derepressing PKD1/2 in human ADPKD cells.

Discussion

Heterozygous PKD1 loss-of-function as the genetic cause of ADPKD was discovered over 25 years ago34,35, but approaches to restore PKD1 expression have remained elusive. In this work, we provide a feasible framework for increasing endogenous PKD1 levels and show for the first time that monoallelic Pkd1 derepression is sufficient to alleviate preclinical PKD.

A unifying and parsimonious explanation for ADPKD onset is that cystogenesis ensues when the functional Pkd1 dosage falls by 70–80%, dipping below a critical threshold1. Thus, germline inactivation of one Pkd1 allele alone cannot account for this magnitude of dose reduction. Additional stochastic events that repress the remaining allele are required and play a critical role in determining disease onset. In this regard, we discovered that miR-17-mediated inefficient translation of mRNAs transcribed by the non-inactivated Pkd1 allele represents a targetable, somatic inhibitory ADPKD onset mechanism. As an attractive safety feature, Pkd1 inhibition by miR-17 appears to be an ADPKD-specific phenomenon since we observed that the miR-17 level is low in normal adult mouse kidneys, and thus, it has no impact on Pkd1 mRNA stability in the non-cystic setting. In contrast, the miR-17 miRNA family becomes activated in PKD models, where it appears to mediate Pkd1 repression well into adulthood, as evidenced by the attenuation of cyst growth by the anti-miR-17 drug RGLS4326, even if the treatment is initiated at later stages of the disease. A noteworthy caveat here is that while RGLS4326 raises PCL levels, its benefits in later stages of disease could be derived from simultaneous derepression of other miR-17 targets, including PC2 and Ppara. Another insight from our work is that potentially restoring hypomorphic Pkd1 mutants may be a beneficial therapeutic approach. On a cautionary note, particularly for modalities employing exogenous PKD1 supplementation, raising Pkd1 above wildtype levels produces cystic disease in mice34,35. However, the uniqueness of our method is that, rather than transactivation, it relies on preventing inhibition, making it unlikely that PKD1 will rise to the suprapharmacological range. As a sign that the miR-17-mediated PKD1 inhibition may even be relevant in individuals with ADPKD, we noted that deleting the PKD1 miR-17 miRNA in primary human ADPKD cultures increases PCL and reduces 3D cyst growth and proliferation. Similarly, inhibiting miR-17 raises PCL levels and inhibits the cyst growth and proliferation of primary human ADPKD cultures36,37. As further proof, a recent phase 1b clinical trial showed that RGLS4326 treatment is associated with higher urinary PCL levels in ADPKD patients38. Additional clinical studies are planned using the next-generation anti-miR-17 oligo RGLS8429.

Our studies also clarify the role of PKD1 in the continual expansion and growth of kidney cysts. Along with cyst initiation, PKD1 inhibition unleashes large-scale transcriptomic and metabolic dysregulation and activates numerous oncogenic pathways, such as cAMP and c-Myc/Yap39,40. In turn, this downstream cyst-pathogenic signaling is thought to fuel cyst expansion. Despite such widespread dysregulation, a recent elegant study reported that transgenic Pkd1 or Pkd2 reconstitution rapidly reverts established cystic disease in mice41. Consistently, we found that acute Pkd1/2 derepression reigns in established cystic disease and makes Pkd1-mutant cells resistant to pro-cystogenic stimuli such as cAMP and SAM. These observations collectively point to PKD1 as the primary, if not the sole, factor governing cyst onset and growth.

We report an unexpected finding that Pkd2 influences the cystic phenotype of Pkd1-mutant models. PCI1 and PC2 physically interact and are coexpressed at multiple subcellular locations, indicating that the two proteins function in the same physiological pathway39,42–44. We add a new dimension by extending this relationship into the pathological context. Perhaps, enhancing Pkd2 expression in Pkd1-mutant cells may improve PCI trafficking and/or form more heteromorphic PCI-PC2 protein complexes.

Finally, our work provides new insights into miRNA biology. miRNAs are well known to simultaneously but subtly repress large miRNA networks. Our approach decouples and disentangles this pleiotropy in the context of PKD. We devised a system where miR-17 is employed exogenous miRNA family becomes activated in PKD models, where it appears to mediate Pkd1 derepression. In this regard, we discovered that miR-17-mediated inefficient translation of mRNAs transcribed by the non-inactivated Pkd1 allele represents a targetable, somatic inhibitory ADPKD onset mechanism. As an attractive safety feature, Pkd1 inhibition by miR-17 appears to be an ADPKD-specific phenomenon since we observed that the miR-17 level is low in normal adult mouse kidneys, and thus, it has no impact on Pkd1 mRNA stability in the non-cystic setting. In contrast, the miR-17 miRNA family becomes activated in PKD models, where it appears to mediate Pkd1 derepression well into adulthood, as evidenced by the attenuation of cyst growth by the anti-miR-17 drug RGLS4326, even if the treatment is initiated at later stages of the disease. A noteworthy caveat here is that while RGLS4326 raises PCL levels, its benefits in later stages of disease could be derived from simultaneous derepression of other miR-17 targets, including PC2 and Ppara. Another insight from our work is that potentially restoring hypomorphic Pkd1 mutants may be a beneficial therapeutic approach. On a cautionary note, particularly for modalities employing exogenous PKD1 supplementation, raising Pkd1 above wildtype levels produces cystic disease in mice34,35. However, the uniqueness of our method is that, rather than transactivation, it relies on preventing inhibition, making it unlikely that PKD1 will rise to the suprapharmacological range. As a sign that the miR-17-mediated PKD1 inhibition may even be relevant in individuals with ADPKD, we noted that deleting the PKD1 miR-17 miRNA in primary human ADPKD cultures increases PCL and reduces 3D cyst growth and proliferation. Similarly, inhibiting miR-17 raises PCL levels and inhibits the cyst growth and proliferation of primary human ADPKD cultures36,37. As further proof, a recent phase 1b clinical trial showed that RGLS4326 treatment is associated with higher urinary PCL levels in ADPKD patients38. Additional clinical studies are planned using the next-generation anti-miR-17 oligo RGLS8429.
because it targets the foreign HCV RNA genome, binds the 5′-UTR, and aids in HCV accumulation51,52.

Most miRNAs are dispensable for homeostatic tissue functions and are pharmaceutically inhibited with relative ease. Despite these favorable characteristics, miRNA-based drug development has languished compared to other forms of RNA therapeutics53. This is partly because the pleiotropic molecular mechanism of numerous downstream mRNA targets makes it difficult to validate the miRNA biological effect or develop pharmacodynamic readouts of anti-miRNA drugs. We argue that prioritizing miRNAs that function as tonic inhibitors of a handful of disease-central mRNAs is likely to be a fruitful drug development strategy. Importantly, our insights are transferable, and we speculate that similar modes of therapeutically targetable cis-inhibitory regulation exist in other disorders, especially haploinsufficient monogenetic conditions.

Methods

Generation of 3′-UTR cell lines via CRISPR/Cas9

We deleted the miR-17 binding site from Pkd1 or Pkd2 3′-UTR in primary ADPKD cultures from four human donors (#1 through #4). a, b, Immunoblots showing higher PCI expression in Pkd1Δ17 and higher PC2 expression in Pkd2Δ17 ADPKD cultures compared to their respective unedited (UE) parental ADPKD cultures. Protein bands are 460 kDa (a) and 110–120 kDa size (b). Actin serves as the loading control. c–f Images and quantification showing reduced cyst size of Pkd1Δ17 and Pkd2Δ17 compared to their respective unedited (UE) parental ADPKD cultures. g, h, Images showing higher mitotracker labeling (red) and reduced pCREB1 immunostaining (green) in Pkd1Δ17 and Pkd2Δ17 ADPKD cultures compared to their respective unedited parental ADPKD cultures. n = 3 biologically independent experiments for each cell line. Errors bars represent SEM. Statistical analysis: Two-tailed Students t-test (e, f). Source data are provided as a Source Data file.
we transected Pkd1<sup>flox</sup> cells with 0.6 µg of the SpCas9-2A-GFP plasmid carrying the upstream or the downstream sgRNA using Lipofectamine 3000. After 72 h, we performed FACS to select GFP-positive cells with the top 5% intensity. These cells underwent clonal expansion in 96-well plates. Well-formed colonies were screened for the absence of the miR-17 binding site by DNA PCR of the targeted Pkd1 genomic sequence. Clones with expected deletion bands were confirmed by Sanger sequencing. Two Pkd1<sup>flox/Δ17</sup> clonal cell lines with confirmed deletions were further characterized and analyzed along with their parental control cell lines, as shown in Figs 2 and Supplementary Fig. 4. We used the same strategy and experimental approach for generating the two Pkd1<sup>flox/Δ17</sup>; Pkd1<sup>flox/Δ17</sup> cell lines (Figs 4 and Supplementary Fig. 8). The sgRNA sequences and genotyping primers are provided in Supplementary Table 3.

**Generation of 3′-UTR mice via CRISPR/Cas9**

The following strains of mice were used: (1) for the mouse models shown in Fig. 1, wildtype C57BL/6 N female and male mice were used; (2) for the mouse models shown in Figs. 2 and 4, we used Ksp<sup>flox</sup>, Pkd1<sup>flox</sup> mice maintained on a C57BL/6<jmath>Δ</jmath> background by our laboratory. Prepubertal female mice underwent superovulation using a standard hormone regimen. The epididymis was collected from male mice for sperm harvest. After in vitro fertilization, one-cell fertilized eggs were isolated. CRISPR reagents (IDT) were delivered to the cytoplasm via electroporation using a Nepa21 Super Electroporator (NEPAGENE, Ichikawa, Japan). The eggs that survived the electroporation were washed and cultured in fresh M16 media in microdrop cultures. The eggs were then surgically transferred into the oviducts of day 1 pseudopregnant ICR females. At 21 days of age, founder mice were screened for deletion of the miR-17 binding site by genotyping, and confirmation of deletion was performed by Sanger sequencing.

**ADPKD mouse models**

Ksp<sup>flox</sup>, Pkd1<sup>flox</sup>, and Pkd1<sup>flox/Δ17</sup> mice were used in this study. All mice were maintained on C57BL/6<jmath>Δ</jmath> background. At prespecified time points, mice were anesthetized using an approved protocol, and blood was obtained via cardiac puncture. The right kidney was weighed to harvest. After in vitro fertilization, one-cell fertilized eggs were isolated. CRISPR reagents (IDT) were delivered to the cytoplasm via electroporation using a Nepa21 Super Electroporator (NEPAGENE, Ichikawa, Japan). The eggs that survived the electroporation were washed and cultured in fresh M16 media in micropop cultures. The eggs were then surgically transferred into the oviducts of day 1 pseudopregnant ICR females. At 21 days of age, founder mice were screened for deletion of the miR-17 binding site by genotyping, and confirmation of deletion was performed by Sanger sequencing.

**Pkd1<sup>flox</sup> and Pkd1<sup>flox/Δ17</sup> cell lines**

The Pkd1<sup>flox</sup> and Pkd1<sup>flox/Δ17</sup> are isogenic, collecting duct-derived epithelial cell lines. These cells were generated from the kidneys of a 14-day-old Pkd1<sup>flox/Δ17</sup> male mouse. A single-cell suspension was created by mincing the kidney tissue into 1 mm cubes followed by incubation for 40 min in DMEM containing 5% Collagenase (Sigma #C1639, USA) at 37 °C with intermittent agitation. The cells were then incubated with ice-cold 1X PBS and 4% (wt/vol) parafformaldehyde. The kidney was subsequently paraffin-embedded. All studies used equal numbers of males and females. The UT Southwestern Institutional Animal Care and Use Committee approved all experiments involving animals.

**Histology**

Tissue embedding in paraffin and subsequent sectioning were performed using standard protocols by the Histology core at UT Southwestern Medical Center. The tissues were cut into 5 µm sections and stained with hematoxylin-eosin (H&E) for histological analysis. The stained sections were imaged using a slide scanner.

**RNA**

A Qiagen miRNEASY kit was used for total RNA extraction. cDNA was prepared using an Invitrogen First Strand Superscript III cDNA synthesis kit. Q-PCR was performed using IQ SYBR Green Supermix (Bio-Rad). All samples were loaded in duplicate or triplicate on the CFX ConnectTM Real-time PCR detection system. 18s was used to normalize mRNA expression. The sequences of the primers are shown in Supplementary Table 4.

**PCL and other Western blots**

Total protein was isolated from kidneys or cells using a lysis buffer made by mixing T PER tissue protein extraction reagent (Invitrogen, catalog# 78510) with a protease phosphatase inhibitor tablet (Fisher, catalog# PIA32961) according to the manufacturer’s instructions. The lysis buffer was prepared and stored as one-time aliquots at −80 °C. The aliquots were thawed on ice immediately before protein isolation. Protein concentration was measured using the Bradford Assay reagent. Protein samples were prepared in 4X NuPAGE LDS Sample Buffer with 0.5% D-mercaptoethanol (Sigma, catalog# M6250) for all proteins except for PCL and PC2 and their loading control beta-actin, which were prepared with 0.1 M DTT (Sigma, catalog# D6032). The samples were always freshly prepared before gel electrophoresis. BME samples were boiled for 5 min at 98 °C before loading on gels. The DTT samples were incubated at 25 °C for 10 min before loading on gels.

For full-length PCL detection, the samples were run on the NuPAGE™ 3–8% Tris–Acetate Protein Gel (Invitrogen, EAO3785) at 160 V for 1.5 h on ice. A high molecular weight protein ladder (Invitrogen, catalog# LC5699) was used in each gel to track 460 kDa proteins. Electrophoretically separated proteins were transferred using the Invitrogen transfer system at 200 mAmps for 100 min on ice or at 4 °C. The samples containing 10 µg of protein were run on mini-PROTEAN...
SDS-polyacrylamide precast gels to detect other proteins. A standard molecular weight ladder was used in each gel to track protein sizes. The gels were run at 150 V until the dye ran out. The proteins were transferred to a nitrocellulose membrane using the Trans-Blot Semi-Dry Transfer system on the mixed MW program.

After completing the transfer, the membranes were blocked with 5% fat-free milk to avoid any non-specific binding for 1 h at 37 °C with primary antibodies. The membranes were washed three times with 1X TBS-Tween the next morning before and after probing for one hour with a secondary antibody. Goat-anti-rabbit or anti-mouse HRP-conjugated IgG was used as the secondary antibody. HRP-conjugated actin antibody (Sigma, catalog# a3854) was used to measure total protein. The blots were developed using the chemiluminescence substrate SuperSignal West Dura, ECL, or Femto from Pierce. The blots were developed using the Bio-Rad digital imager. The protein bands were quantified using ImageJ gel software from Bio-Rad. Each Western blot was repeated at least three times. Ten micrograms of protein from cells or kidneys were run on gels to detect <150 kDa proteins. 40–60 µg of protein was run on gels to detect heavy molecular weight (462 kDa) full-length PC1 protein. All the primary antibodies were used at a 1:1000 dilution, except for PC1 (used at 1:500), and the secondary antibodies were used at a 1:5000 dilution. The following primary antibodies were used: PC1 (7E12 Santa Cruz, catalog# sc-130554); PC1 E8-8C3C10 (Baltimore PKD core center), PC2 (gift from the Baltimore PKD Core); pCREB (Cell Signaling, catalog# 9198); and pHH3 (Cell Signaling, catalog# 9331). Mett3 (Invitrogen, catalog# MA5-27527).

**Immunofluorescence on tissue samples**

Paraffin sections of kidney tissues were used for immunofluorescence staining. Briefly, the slides were deparaffinized by first baking at 60 °C for 1 h and then washing in Histoclear (Fisher, HS-2001) three times for 5 min each. Next, the slides were re-hydrated through 100%, 95%, and 70% ethanol washes before incubation in IX PBS. The slides were then subjected to antigen retrieval with sodium citrate. The slides were treated with sodium borohydride to quench autofluorescence for 40 min. The slides were washed in IX PBS three times and then blocked in IX PBS + 10% goat serum +0.1% BSA (antibody block) for at least 1-2 h at RT. Sections were incubated with primary antibodies overnight. Primary antibodies were diluted with antibody block at a 1:500 dilution. Slides were washed in IX PBS three times for 5 min each, treated with Alexa Fluor secondary antibodies (diluted using the antibody block to a 1:500 dilution) for 1 h, and then washed three times for 5 min each. The slides were mounted using Vecta Shield containing Dapi. The slides were imaged using the Zeiss Compound Light microscope or the Zeiss Axioscan Z1 slide scanner. The following antibodies were used: DBA (Vector Labs, catalog# B-1033), THP (Biotechnological Technologies, catalog# BT-590), LTA (Vector Labs, catalog# B-1325), MRC1 (Abcam, catalog# ab64693), pCREB1 (Cell Signaling, catalog# 9198), and pHH3 (Sigma, catalog# #80412). Processing, immunostaining, and imaging of slides were performed simultaneously within each experiment.

**Immunofluorescence on cells**

Immunofluorescence staining was performed on cells grown on 8-chambered slides (Fisher, catalog# 154534PK). The cells were fixed with 100% ice-cold methanol for 5 min at 4 °C. The slides were washed with 1X PBS 3 times for 5 min. The cells were then blocked in 1X PBS + 10% goat serum +0.1% BSA + 0.1 M glycine +0.1% Tween 20 (antibody block) for at least 30 min at room temperature. Primary antibodies were diluted with antibody block at a 1:1000 dilution and added to the slides for 2 h. Slides were washed in 1X PBS three times for 5 min each, treated with Alexa Fluor secondary antibodies (diluted using the antibody block to a 1:500 dilution) for 1 h, and then washed three times for 5 min each. The slides were counterstained in DAPI (Fisher, catalog# ICN15757410) diluted at 1:10000 in distilled water for 10 min before imaging under a Zeiss Compound Light microscope. For each experiment, the control and treatment cells or the control and the Δ17 cells were seeded simultaneously on different chambers of the same slide. Processing, immunostaining, and imaging of slides were also performed simultaneously.

**MitoTracker analysis**

MitoTracker Red CMXRos (Thermo Fisher, catalog# M7512) was used to analyze the mitochondrial membrane potential in live cells. The lipophilized MitoTracker® product was dissolved in dimethylsulfoxide (DMSO) to a final concentration of 1 mM and stored at −20 °C in small aliquots. Cells grown to 40–70% confluency were washed with sterile PBS and then treated with regular DMEM serum-free media containing 100 nM MitoTracker for 8 min. Immediately thereafter, the media was replaced with regular growth media and imaged under a Zeiss Compound Light microscope. The images were taken at the same exposure time for the samples of the same experiment. The intensity of fluorescence is directly proportional to mitochondrial membrane potential.

**3D cystogenesis assay**

25 µl of 100% Matrigel (Fisher, catalog# 354234) was spread onto each well of an 8-chambered slide with precooled 200 µl sterile pipette tips. The plate was then placed in a 37 °C incubator for 30 min for the Matrigel to set. In the interim, cells were trypsinized, washed once with PBS, filtered through a 40 µm cell strainer to create a single-cell suspension, and counted. Cells were seeded on the Matrigel-coated slide at a seeding density of 5000 cells/well in a 300 µl volume of growth media containing 2% Matrigel. For each cell line or treatment condition, cells were seeded in triplicate and incubated at 37 °C for 7 days to allow for the growth of 3D cysts in suspension. During this time, the wells were supplemented with growth medium 72 h after initial placement into Matrigel. On day 7, the chamber slides were imaged on a Leica DM 3000B light microscope. The images were analyzed using ImageJ software to obtain cyst size measurements. Each assay was repeated at least three times. Measurements from each experiment were combined and analyzed for statistical significance.

**Ex vivo organ culture**

Female mice bearing potential Pkd1+/−; Pkd1(17/−) and Pkd1Δ17/Δ17 embryos were dissected at embryonic day (E) 13.5 in PBS to harvest the kidneys and tail. The tail of each embryo was used for DNA extraction and subsequent genotyping. The kidneys were set up for culture on Whatman membranes (Sigma, catalog# WHA110409) in an air-medium interface as described. The kidneys were cultured in basal DMEM (Thermo Fisher, catalog# 12500) containing 10% fetal bovine serum (FBS), 2% PenStrep (Invitrogen, catalog# 154022), 5 µg/ml insulin, 5 µg/ml transferrin, 2.8 nM sodium selenite, 25 ng/ml prostaglandin E and 32 pg/ml T3. One kidney was grown in the above media, and the contralateral kidney was grown in 100 µM 8-Br-cAMP (Sigma, catalog# B7880)-supplemented media. Using a second cohort of mice, one kidney was grown in 100 µM 8-Br-c-AMP + 250 µM SAM. For all the cultures, the media was changed every 48 h. The cultures were imaged live using the Zeiss Stereo Lumar microscope on day 4. The cysts were measured and analyzed using ImageJ software. At the end of 6 days, the kidneys were flash-frozen and stored at −80 °C until further use for RNA or protein extraction.

**alaranBlue assay**

Pkd1+/− and Pkd1Δ17/Δ17 cells (3 × 103 density) were seeded on 96-well plates. The next morning, the medium was changed to contain 1× alaranBlue reagent (Invitrogen, catalog# DAL1025) and vehicle, 100 µM 8-Br-cAMP, 100 µM SAM, or 17 mM glucose. Colorimetric readings were taken at 570 nm and 600 nm in a microplate reader after 12 h. The redox reaction of alaranBlue was used to assess cell proliferation quantitatively. N × 8 was used for each condition. The values were plotted as a scaled heatmap using the Python MatplotlibLib.
package. The same experimental approach was used for the Pkd1RC/--; Pkd2Δ+/Δ cells and the control cells Pkd1RC/++; Pkd2+/+.

Serum electrolytes
Serum creatinine was measured by capillary electrophoresis by the UT Southwestern O'Brien Center. BUN was measured by Vitros250 Analyzer by the UT Southwestern Metabolic Phenotyping Core.

Microarray analysis of microRNAs
Total RNA was extracted from kidneys using miRNeasy mini kits (Qiagen). The small RNA fraction (~300 nucleotides) was hybridized on a μParaflo Microfluidic chip containing detection probes for all mouse microRNAs (miRNAs) in the miBase version-17 (miBase, http://microrna.sanger.uk/sequences). The hybridized microarray chips were labeled with fluorescent dyes and laser scanned to obtain fluorescent images. The signal values for each sample were derived by background subtraction and normalization. Microarray chip hybridization, fluorescent labeling, laser scanning, and background subtraction and normalization were performed by LC Sciences. The signal values from each of the five age groups (P2, P7, P14, P35) were averaged, and the P values using One-way ANOVA were calculated. The differentially detected signals are defined by P < 0.05.

RNA-seq preprocessing
Sequencing quality control was performed with FastQC v0.11.8. RNA-seq reads were trimmed, and low-quality reads were removed using Trimgalore v0.6.3_dev (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) with the “paired” parameter and length of 150 bps. Trimmed fastq sequences were aligned to the mouse reference genome GRCh38 using STAR aligner v2.5.3a with the produced bam files sorted by coordinate using the option “-outSAMtype BAM SortedByCoordinate.” Raw read gene counts were obtained using STAR aligner with the options “--quantMode GeneCounts” and “--sjdbGTFfile” with gene models in GTF format obtained from mouse Ensembl release 94. Alignment quality control and read mapping statistics were obtained from Picard tools v2.20.3 using the function “CollectMultipleMetrics” (https://broadinstitute.github.io/picard/).

RNA-seq data analysis
Raw gene counts were used for quality control and differential expression analysis. Raw counts were normalized to the total number of reads by calculating log2CPM (counts per million). We carefully examined the log2CPM distribution and its relationship to the standard deviation and determined the appropriate cutoff (average Log2CPM < -3) to eliminate lowly expressed genes before differential gene expression analysis. TPM (transcript per million) quantification was performed using RSEM v1.3.1, and differential gene expression analysis was performed using the limma-trend (version 3.40.6) in R(19).

Transcript quantification
Individual Pkd1 transcript quantification was performed using Salmon v1.3.0. The five different transcript versions for Pkd1 were added to the RefSeq mm9 fasta reference transcriptome to build a novel Salmon index. Then, the fastq files were directly mapped and read counts, and TPM values were quantified with the standard process.

In vitro RGLS4326 experiments
Pkd1RC/− cells were seeded at 2 × 10^5 confluence in 6-well plates. The next morning, cells were transfected using Lipofectamine 3000 with a vehicle, control oligonucleotide, or RGLS4326 at a final concentration of 100 μM. Forty-eight hours after transfection, the cells were collected for RNA extraction. Seventy-two hours after transfection, cells were harvested for protein or further seeded for alamarBlue assay and 3D cystogenesis assay. For the experiment shown in Fig. 5C, the 3D cystogenesis assay was performed with untreated Pkd1RC/− cells, as described in the methods section of the 3D cystogenesis assay with the following changes. On day 4 of Matrigel culture, the wells were imaged using the Leica light microscope DMI 3000B, and then the cultures were transfected with a vehicle, 100 μM control oligonucleotide, or 100 μM RGLS4326 and grown for 3 additional days. On day 7, the samples underwent imaging to assess cyst size.

RGLS4326 mouse experiments
The Ksp^{tcig}, Pkd1RC mouse line was used for the drug studies. Mice were randomly assigned and administered 20 mg·kg⁻¹ vehicle (PBS), control oligonucleotide, or RGLS4326 via subcutaneous injections. For the first cyst prevention study (Fig. 5D–G), mice were injected on postnatal days (P) 10, P11, P12, and P16 and sacrificed on P18. Non-transgenic strain-matched mice were also sacrificed on the same days. For the second disease stabilization study (Fig. 5H–K), mice were injected at P16 and P17 and sacrificed at P26. One mouse from the study succumbed to the disease and died earlier than 26 days of age. For the third long-term study (Fig. 5L–N), mice were injected on P16 and P17 and then every week until 18 weeks of age. Another cohort of mice received the same dose of RGLS4326 treatment on P16 and P17 and then semimonthly thereafter until 18 weeks of age. The mice were observed every day for 18 weeks to note death. At the end of 18 weeks, the surviving mice were sacrificed to harvest tissue. Equal numbers of males and females were used in all study groups.

Human ADPKD cell experiments
Primary human ADPKD cyst cells were obtained from PKD Research Biomarker and Biomaterial Core at the University of Kansas Medical Center (KUMC). The use of surgically discarded kidney tissues complied with federal regulations and was approved by the Institutional Review Board at the University of Kansas Medical Center. PKD1 and PKD2 mutation analysis of RNA from donor cyst cells was performed by Ambry Genetics (Aliso Viejo, CA). Each primary cell line was cultured in DMEM/F12 (Gibco, catalog# 10565-018) supplemented with 10% FBS, 5 μg·kg⁻¹ insulin, 5 μg·L⁻¹ transferrin, and 5 ng·L⁻¹ sodium selenite and incubated in an atmosphere of 95% air and 5% CO2 at 37°C until 80% confluence. At the 2nd passage, cells from each human donor underwent reverse transfection using CRISPRMAX reagent (Invitrogen) containing Cas9 protein (IDT) and synthetic sgRNAs (IDT) or were transfected with vehicle (lacking Cas9). The Cas9/sgRNA-transfected cultures are a mixed population of edited and unedited cells. Clonal propagation was not possible because these are primary cells that allow only a limited number of passages. Cas9- or vehicle-transfected cells (control) were then seeded into 6-well plates and chamber slides. After 72 h, the cells were harvested for genotyping. Western blot analysis, and immunofluorescence/MitoTracker staining. In addition, at 72 h posttransfection, cells were trypsinized and plated at 4000 cells/well/density in 200 μL of media plus Matrigel (Corning, catalog# 354234) in a 96-well plate (Corning, catalog# 333072). Media was replenished 72 h after the initial implantation into Matrigel. Cyst images were obtained on the 7th day of Matrigel culture (10th day after Cas9/sgRNA or vehicle transfection). One hundred cyst images were obtained for Cas9- or vehicle-transfected cells from each donor. Similarly, 72 h post-transfection, cells were seeded at 2000 cells/well/density in 96-well plates for the alamarBlue proliferation assay. The following day, the media was replaced with growth media containing 1X alamarBlue, and readings were taken 12 h later.

Statistics and reproducibility
All experiments were carried out with at least three biological replicates and showed successful reproducibility. For in vivo experiments, N is the number of mice analyzed. For in vitro experiments, N refers to the number of biological replicates. Two-tailed Student’s t-test was used for pairwise comparisons and analysis of variance (ANOVA), followed by Tukey’s post hoc test was used for multiple comparisons. The
Mantel-Cox test was used for the analysis of mouse survival. All data were analyzed using Prism software (GraphPad Software). P < 0.05 was considered statistically significant. The sample size and P values are mentioned in the figure graphs, the figure legends, or the results section. For the RGLS4326 studies, animals were randomly assigned to treatment arms. Investigators were not blinded to the treatment or the genotypes of the animals.

**Reporting summary**

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

**Data availability**

The data that support this study are available from the corresponding author upon request. The RNA-seq datasets have been deposited in the NCBI Gene Expression Omnibus repository under accession numbers GSE196237. The microarray dataset has been deposited in the NCBI Gene Expression Omnibus repository under accession number GSE208429. Source data are provided with this paper.

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Author contributions

R.L., H.R., C.C., and V.P. designed, performed experiments, and/or analyzed data involving miR-17 motif deletions. A.F., T.V., E.L., C.M., H.R., R.L., and V.P. designed, performed experiments, and/or analyzed data involving RGLS4326 studies. D.W. provided human ADPKD cells. P.C., J.A., H.R., R.L., and V.P. designed, performed experiments, and/or analyzed data involving human ADPKD cells. H.R., R.L., and V.P. prepared the figures; V.P. wrote the manuscript with contributions from H.R. and R.L.

Competing interests

V.P has patents involving anti-miR-17 for the treatment of ADPKD (16/466,752 and 15/753,865). V.P serves as a scientific consultant for Otsuka Pharmaceuticals, Maze Therapeutics, and Regulus Therapeutics. V.P. lab has a sponsored research agreement with Regulus Therapeutics. V.P. lab also has a sponsored research agreement with Sanofi SA, MyoKardia Therapeutics, and Vifor pharmaceuticals, which are unrelated to this work. T.V. and E.L. are employees of Regulus Therapeutics. The remaining authors declare no competing interests.

Additional information

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