# Prioritized polycystic kidney disease drug targets and repurposing candidates from pre-cystic and cystic mouse model gene expression reversion

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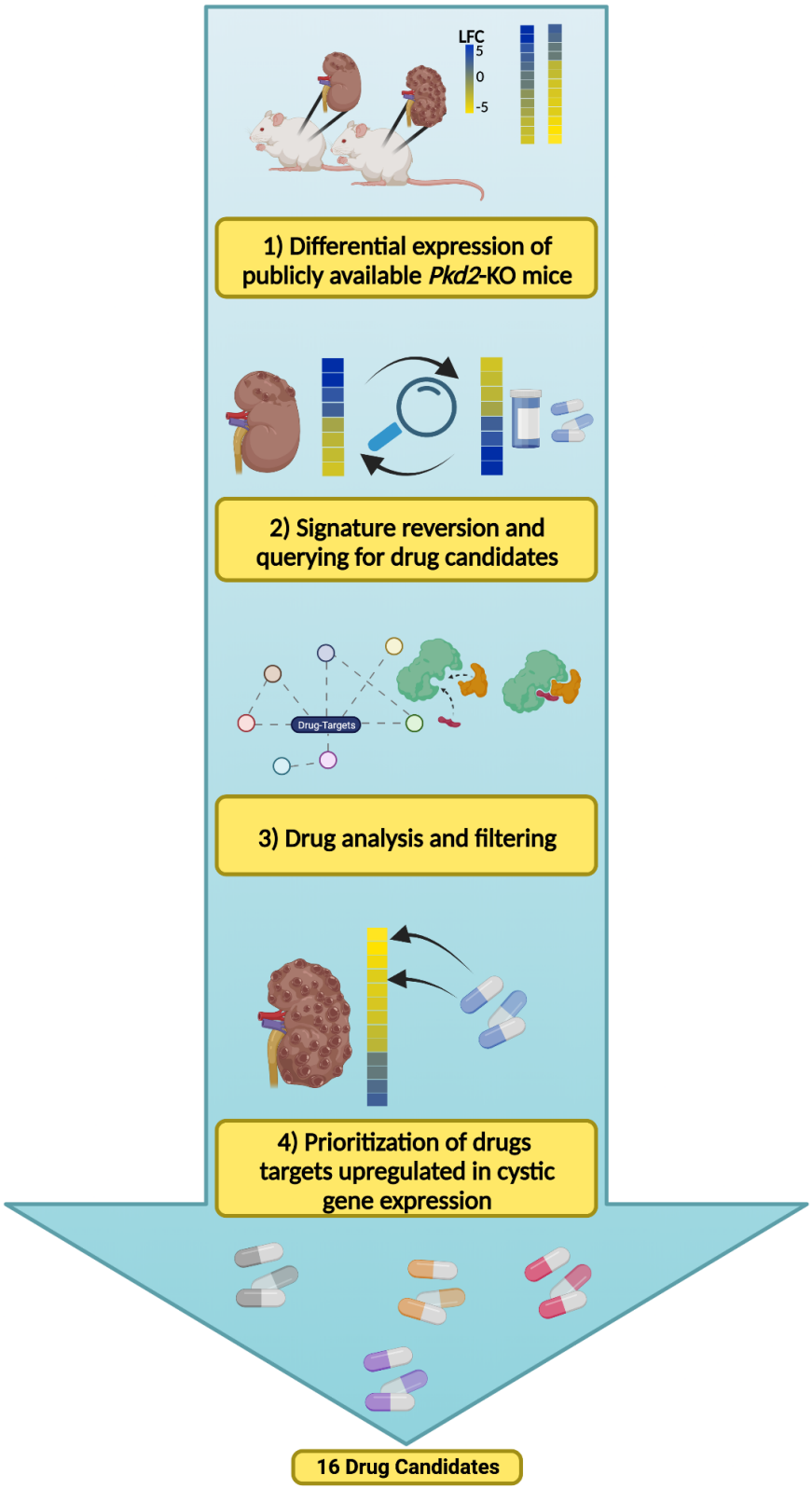
## Keywords

Polycystic kidney disease, PKD, ADPKD, drug repurposing, signature reversion, LINCS, drug prioritization




Graphical abstract of the study.

# Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most prevalent monogenic human diseases. It is mostly caused by pathogenic variants in *PKD1* or *PKD2* genes that encode interacting transmembrane proteins polycystin-1 (PC1) and polycystin-2 (PC2). Among many pathogenic processes described in ADPKD, those associated with cAMP signaling, inflammation, and metabolic reprogramming appear to regulate the disease manifestations. Tolvaptan, a vasopressin receptor-2 antagonist that regulates cAMP pathway, is the only FDA-approved ADPKD therapeutic. Tolvaptan reduces renal cyst growth and kidney function loss, but it is not tolerated by many patients and is associated with idiosyncratic liver toxicity. Therefore, additional therapeutic options for ADPKD treatment are needed. As drug repurposing of FDA-approved drug candidates can significantly decrease the time and cost associated with traditional drug discovery, we used the computational approach signature reversion to detect inversely related drug response gene expression signatures from the Library of Integrated Network-Based Cellular Signatures (LINCS) database and identified compounds predicted to reverse disease-associated transcriptomic signatures in three publicly available kidney transcriptomic data sets of mouse ADPKD models. We focused on a pre-cystic model for signature reversion, as it was less impacted by confounding secondary disease mechanisms in ADPKD, and then compared the resulting candidates' target differential expression in the two cystic mouse models. We further prioritized these drug candidates based on their known mechanism of action, FDA status, targets, and by functional enrichment analysis. With this approach, we prioritized 29 unique drug targets differentially expressed in ADPKD cystic models and 16 prioritized drug repurposing candidates that target them, including bromocriptine and mirtazapine. Collectively, these indicate drug targets and repurposing candidates that may effectively treat pre-cystic as well as cystic ADPKD.

# Background

Autosomal dominant polycystic kidney disease (ADPKD) is a potentially life-threatening disorder with renal and extrarenal manifestations. It is one of the most prevalent monogenic human diseases, impacting approximately 1 in 400 to 1 in 1,000 individuals.(1) In addition to end-stage renal disease (ESRD),

comorbidities include hypertension, extrarenal cysts, and intracranial aneurysms and hemorrhaging.(1) ADPKD is primarily caused by variants in either the *PKD1* or *PKD2* gene which encode the transmembrane proteins polycystin-1 (PC1) and polycystin-2 (PC2), respectively.(1) While many molecular processes have been associated with ADPKD, the interrelated processes of cyst expansion, inflammation, and metabolic reprogramming have all been identified as key molecular processes for regulating disease manifestation.(1,2) Variants in *PKD1* and *PKD2* disrupt extracellular sensing and calcium transport by the protein products, leading to decreased intracellular calcium and triggering upregulation of cyclic adenosine monophosphate (cAMP).(3)(4) This increased cAMP leads to increased fluid secretion and proliferation, resulting in fluid accumulation in the cyst lumen. Inflammation in ADPKD is initiated early in disease pathogenesis, as evidenced by cytokine and other proinflammatory signaling molecules in patients' urine, likely caused by cellular alterations from polycystin dysfunction.(5) Additionally, inflammation plays a role in disease progression through macrophage infiltration. Macrophage accumulation has been linked to cyst expansion, and macrophage depletion in mice causes decreased cyst growth, improved renal function, and reduced cell proliferation in cyst-lining cells.(5) PC1 and PC2 interact directly and indirectly with mitochondria, resulting in a "Warburg-like" aerobic glycolysis effect in ADPKD as well as dysregulation in the pentose phosphate pathway, fatty acid biosynthesis, oxidative phosphorylation, and other metabolic pathways.(2,6) Tolvaptan, the only current FDA-approved drug for ADPKD, is a vasopressin receptor-2 antagonist associated with cAMP regulation.(7) Vasopressin receptor antagonists (vaptans) have been the primary therapeutic focus to treat ADPKD.(7) While tolvaptan reduces cyst growth and formation, slowing disease progression, it is expensive and long-term treatment is associated with liver toxicity.(8) Therefore, additional therapeutic options are needed for ADPKD treatment.

Drug repurposing of FDA-approved drug candidates can significantly decrease the time and cost associated with traditional drug discovery by bypassing phase I and II clinical trials (the estimated average in the US is $300 million compared to ~$2-3 billion for a new drug).(9) Here, we use the computational approach signature reversion to identify compounds capable of reversing disease-associated transcriptomic signatures to prioritize drug repurposing candidates. As gene expression profiles from ADPKD patients and preclinical models have significantly altered transcriptomes, our approach presents an opportunity to compare disease signatures to drug response signatures from treated cell lines and identify drug candidates that may reverse

ADPKD-associated cellular phenotypes, ultimately slowing or reducing kidney cyst growth. Additionally, drug selection based on genetic evidence has become more common and is effective. For example, approximately 66% of FDA-approved drugs in 2021 were supported by demonstrated associations between genetic phenotype and drug targets.(10) This further emphasizes the utility of omics profiles to identify the right drug for the right patient at the right time.

Here, our focus was on *Pkd2*, due to public high-quality data set availability and similarity to *Pkd1* in leading to an ADPKD-like phenotype in preclinical mouse models. From those data sets, we prioritized drug repurposing candidates for ADPKD by detecting inversely related drug response gene expression signatures from the Library of Integrated Network-Based Cellular Signatures (LINCS) database compared to pre-cystic kidney gene expression. Drug candidates were further prioritized based on targets upregulated in cystic kidney gene expression as well as their known mechanism of action (MOA), FDA status, targets, side effects, and by functional enrichment analysis.

# Methods

Data Acquisition and Processing

Three previously published kidney C57BL6 mice RNA-Seq data sets that contained either *Pkd2*$^{fl/fl}$;*Pax8*rtTA;TetO-Cre (GSE149739(11)) or *Pkhd1*-cre;*Pkd2*$^{F/F}$ (GSE134719(12) and GSE69556(13)) (Additional file 1) with matched controls and sexes were downloaded from SRA using SRA-Toolkit version 2.10.7. We selected these data sets because they each were derived from *Pkd2* knock-out kidney mouse samples on C57BL6 backgrounds and had matched controls. GSE149739 samples (P70) were from mice induced at postnatal day 28 for 2 weeks and euthanized at postnatal day 70 before cysts developed (i.e., pre-cystic). For the P70 data set, 3 *Pkd2* knock-out (KO) samples and 3 matched wild-type samples were used in our study. *Pkhd1*/cre;*Pkd2*$^{F/F}$ mice rapidly develop renal cysts due to constitutive knockout of *Pkd2*. As a result, GSE134719 (P28) and GSE69556 (P21) kidneys were collected at postnatal day 28 and 21, respectively. For the analyses here, we used 11 *Pkd2* KO and 12 wild-type samples for the P28 data set, and 3 KO and 4 wild-type samples for the P21 data set.

In short, we processed FASTQ files for all samples on the UAB Cheaha supercomputer with the nf-core/rnaseq(14,15) pipeline version 3.6 using the STAR/Salmon route for alignment, adapter trimming, quality control (QC), and gene count calculations. We aligned reads to the GENCODE mouse reference genome (mm10 release M24). All other analyses were performed on a 2019 MacBook Pro (2.4 GHz Intel Core i9, 64 GB DDR memory, 2TB SSD storage) using R version 4.2.0 RStudio 2021.09.0+351 "Ghost Orchid" Release (2021-09-20) for macOS.

Differential Expression Analysis

For each of the three datasets, we performed differential expression analysis using DESeq2 (version 1.34.0)(16) on the STAR/Salmon merged gene counts from the nf-core pipeline outputs. Differentially expressed genes were selected based on a threshold of an absolute log2 fold change (LFC) greater than 1.1 (for pre-cystic P70 data set) or 2.0 (for cystic P21 and P28 data sets) and an adjusted p-value less than 0.05 after apeglm LFC shrinkage.(17) The LFC cutoff varied by data set to ensure a signature of 100-200 genes, which Yang et al. previously found to be the optimal gene signature size for signature reversion.(18) While a LFC threshold > 2 allowed for a total signature of 100-200 genes (126 genes for P21 and 189 genes for P28) for the cystic data sets; there were far fewer genes passing this stringent cutoff with the pre-cystic data set. The study by Yang et al. benchmarked transcriptomic signature compositions for drug repurposing and found the optimal signature to be dependent more on size (100-200 genes) than stringent LFC.(18) With this in mind, we lowered the pre-cystic LFC cutoff to > 1.1 and < -1.1 compared to cystic data sets, resulting in a signature of 130 genes for the P70 pre-cystic data set. We used BiomaRt to annotate gene descriptions for the differentially expressed genes and convert from Ensembl to Entrez and HGNC symbols (version 2.50.3).(19,20) The top differentially expressed genes by LFC were used to select rlog normalized counts and plotted as heatmaps using complete linkage hierarchical clustering.

Functional Enrichment Analysis (FEA)

We then analyzed Mouse Ensembl genes (C57BL_6NJ_v1) that met the differentially expressed LFC cutoff for pathway and functional enrichment, with upregulated and downregulated genes analyzed separately. We used the R package gprofiler2 (version 0.2.1)(21,22) to identify significantly enriched pathways using the Gene

Ontology (GO), Reactome, and WikiPathways gene set sources (term sizes filtered for > 5 and < 1,000). We applied the Bonferroni Procedure for multiple hypothesis correction and used a p-adjusted threshold of 0.05. The background gene list included all measured genes before filtering. For plotting, we used enrichment terms prioritized by recall value, the ratio of matching query genes/all query genes to enrichment term size. We aggregated the top 100 terms (up- and downregulated) across the results for all data sets and, using rrvgo (version 1.8.0), we used Wang term semantic similarity to calculate the similarity of terms to each other using the hierarchy of GO's graph for GO biological processes (GO:BP).(23–25) We plotted the term enrichment similarity across the data sets with a grouping similarity threshold set to 0.80 in order to select parent terms.

Acute Kidney Injury Signature Assessment

To assess differential expression of acute kidney injury (AKI)-associated genes across the data sets in our study, we obtained consistently differentially expressed genes associated with AKI from a previous study that aggregated 4 AKI data sets with 24-hour post-injury and time-matched controls.(26) A total of 157 and 88 genes were upregulated and downregulated, respectively, across all 4 data sets in that study. Using these genes, we subset the differential expression results of the pre-cystic and cystic data sets compared to controls in our three data sets.

Signature Reversion Searching for Drug Candidates

We mapped mouse Ensembl genes to orthologous human (GRCh38.p13) Ensembl genes and further converted them to Entrez gene IDs with BiomaRt (version 2.50.3).(19,20) The disease signatures from DESeq2 were mapped to Entrez genes available in the LINCS(27) drug perturbation data we procured from ExperimentHub version 2.2.1 data set EH3226. Then we selected the top 100 upregulated and downregulated genes based on DEseq2's log2 fold change as the gene signature for signature reversion. This cut-off was based on the previous work of Yang et al. as described above.(18) We used the LINCS bi-directional weighted Kolmogorov-Smirnov algorithm in the signatureSearch (1.8.2)(28) package for signature querying of the LINCS drug perturbation data (EH3226) from the signaturesearchData (1.8.4)(28) package.(27,29) Briefly, this algorithm uses as input the most upregulated genes and downregulated genes as mutually exclusive lists from an experiment (in our case) as a query to then compare against the reference database of rank-transformed

signatures. Similar to a weighted Kolmogorov-Smirnov statistic in gene set enrichment analysis (GSEA), the query is compared to reference lists to find if there is an overrepresentation of the reference genes at the "extreme ends" (top or bottom) of the query list.(29) The bi-directional method that computes a weighted connectivity score (WTCS), similar to an enrichment score (ES), calculates the representation (connectivity) by using this similarity metric to compare the degree of overrepresentation of the upregulated query genes to the top of the rank-ordered reference signature, and the overrepresentation of downregulated query genes to the bottom of the rank-ordered reference signature.(27) The WTCS is then scaled with values ranging from -1 (most inversely related) to 1 (most similarly related), where 0 is null (not related). Finally, the WTCS is normalized across cell lines and perturbagens within the reference database to give a normalized connectivity score (NCS).(27) To analyze only drugs inversely related to the ADPKD signature, we filtered results for negative NCS. Due to variability in the compound-induced expression being highly cell-line specific, we further analyzed only kidney-derived cell lines for compound results by filtering for HA1E and NKDBA compound-treated results.(18)

Drug Candidate Prioritization

We further annotated drugs with data regarding clinical phase status, mechanism of action, and drug targets using The Drug Repurposing Hub (release 3/24/2020 https://clue.io/repurposing#download-data).(30) The Drug Hub's "launched" annotation refers to the highest clinical status achieved by each compound and was manually curated across multiple sources worldwide for approval (The FDA Orange Book, prescribing labels, ClinicalTrials.gov, PubMed, EU Clinical Trials Register, Canada DPD, Japan PMDA, and more).(30) We used the "launched" status to filter potential drugs as well as the mechanism of action (MOA) and original disease indication annotations. We further prioritized by filtering for current FDA-approved drugs' active ingredients. For this, we downloaded the Drugs@FDA database as a compressed data file (drugsatfda.zip) from the FDA website.(31) Compounds were filtered for active ingredients and marketing status IDs (e.g., prescription, over-the-counter, or tentative approval) regardless of form (e.g., injectible, solution, tablet, etc) (accessed August 2022).(31) We compared drug targets from signatureSearch to differentially expressed genes from DESeq2 results. We compiled drug target data annotations using signatureSearch from DrugBank,(32) CLUE,(27) and STITCH.(33) We conducted Drug Set Enrichment Analysis (DSEA) using signatureSearch's

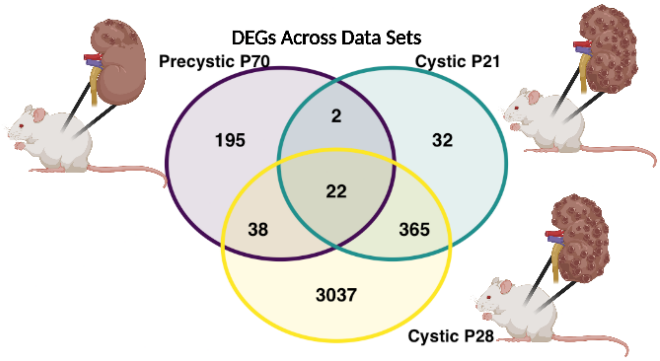
dsea_hyperG function with hypergeometric testing. We used the GO:MF ontology source for DSEA with gene set sizes > 10 and < 500 using the Benjamini-Hochberg procedure for multiple hypothesis correction (p-adjusted value cutoff 0.05). From the epocrates clinical database, we compiled the average monthly retail prices for drugs and black box warnings (accessed November 2022).(34)

# Results

## Genes and Pathways Driving Cyst Progression in Mouse Models of PKD

To identify disease-associated transcriptomic signatures for ADPKD, we acquired three publicly-available *Pkd2* KO mouse data sets (Additional file 1) and performed differential gene expression analysis using DESeq2 between the ADPKD model (condition) and wild-type (control) for each data set. We identified differentially expressed genes for each data set by comparing *Pkd2* KO samples to their wild type controls, resulting in the identification of 257 differentially expressed genes for pre-cystic P70(11), 421 for cystic P21(13), and 3,462 for cystic P28(12) (LFC > 1.1 or 2 and p-adjusted value < 0.05) (Figure 1A, Data files “deseq2_outputs”).(35) The P21 and P28 cystic data sets showed excellent agreement. A total of 387 genes (92%) of the P21 differentially expressed genes were also differentially expressed in the P28 analysis. There were many more differentially expressed genes in the P28 cystic data set than the other two data sets, likely due mostly to increased detection power given there were many more P28 samples (n = 23 in cystic P28, n = 7 in cystic P21, and n = 6 in pre-cystic P70) (Additional file 1).

A


DEGs Across Data Sets
Precystic P70
Cystic P21
Cystic P28
195
2
32
22
38
365
3037

B

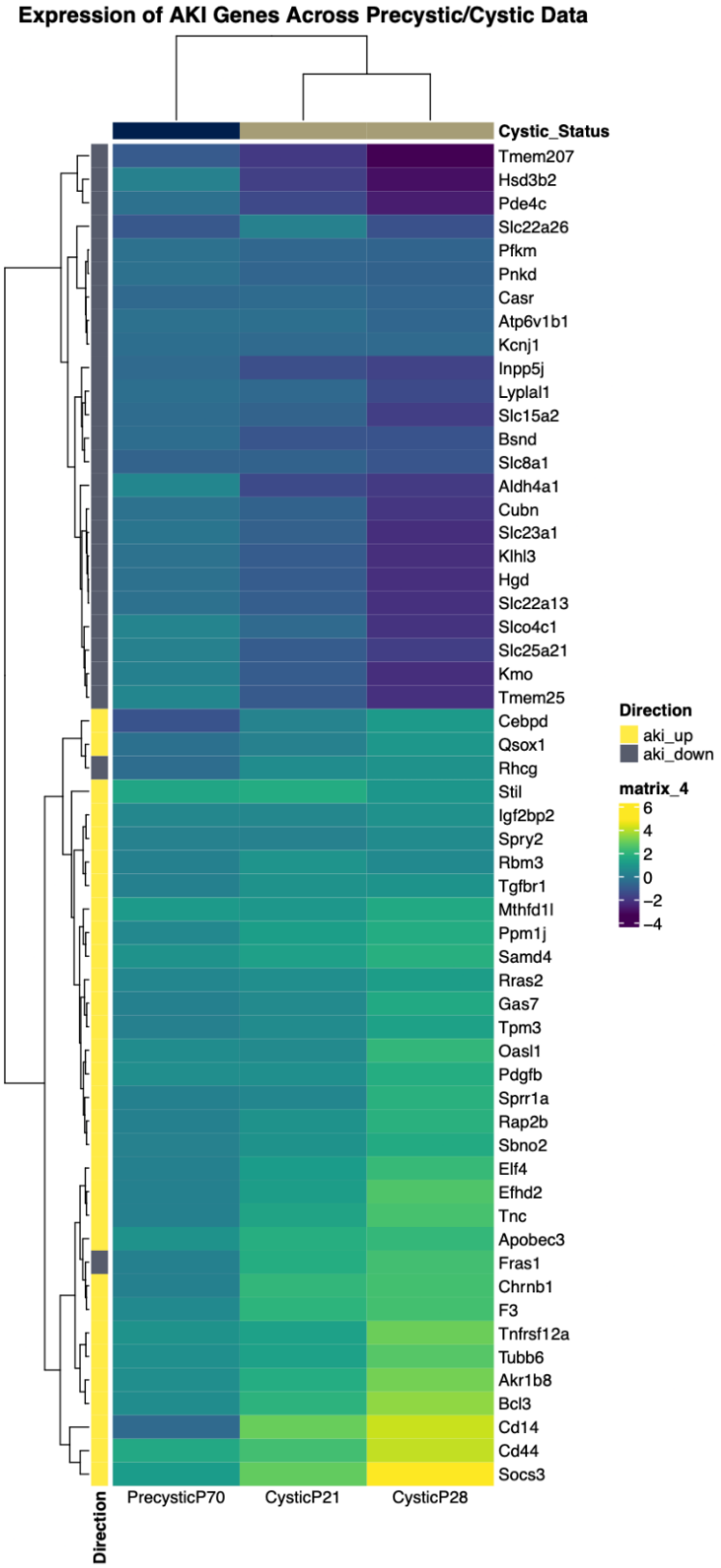

Expression of AKI Genes Across Precystic/Cystic Data
Cystic_Status
Tmem207
Hsd3b2
Pde4c
Slc22a26
Pfkm
Pnkd
Casr
Atp6v1b1
Kcnj1
Inpp5j
Lyplal1
Slc15a2
Bsnd
Slc8a1
Aldh4a1
Cubn
Slc23a1
Klhl3
Hgd
Slc22a13
Slco4c1
Slc25a21
Kmo
Tmem25
Cebpd
Qsox1
Rhcg
Stil
Igf2bp2
Spry2
Rbm3
Tgfbr1
Mthfd1l
Ppm1j
Samd4
Rras2
Gas7
Tpm3
Oasl1
Pdgfb
Sprr1a
Rap2b
Sbno2
Elf4
Efhd2
Tnc
Apobec3
Fras1
Chrnb1
F3
Tnfrsf12a
Tubb6
Akr1b8
Bcl3
Cd14
Cd44
Socs3
PrecysticP70
CysticP21
CysticP28
Direction
Direction
aki_up
aki_down
matrix_4
6
4
2
0
−2
−4

**Figure 1: Differential Expression Analysis** *A) Comparison of unique and overlapping differentially expressed genes between the 3 data sets. B) Heatmap of LFC values for AKI-associated genes for each data set.*

To further assess the difference in the pre-cystic versus cystic data sets, we used the overlapping differentially expressed genes in the cystic data sets as well as the genes uniquely differentially expressed in the pre-cystic data set. We tested them for enrichment using gprofiler2 (sources Reactome, Wikipathways, GO biological process (GO:BP), GO cellular component (GO:CC), and GO molecular function (GO:MF)). There were no enrichment results for overlapping downregulated cystic and pre-cystic genes, but the upregulated genes were enriched for chemokine, immune, and inflammation pathways (Additional file 2). We noted that NAD+ pathways were also enriched for the overlapped upregulated cystic genes. Due to NAD+ regeneration increase in response to high glycolysis, we hypothesize that this may be evidence of the Warburg-like effect previously seen in ADPKD.(2,36) Only those genes that were overexpressed compared to wild type in pre-cystic, but not in either cystic data sets, were largely involved in cell division and cell cycle pathways (Additional file 3). Furthermore, enrichment of differentially expressed genes unique to the pre-cystic data set were also *Cdk1* pathway members which, in particular, have been indicated as a critical driver of cyst proliferation in ADPKD (Additional file 2).(11)

Due to the cystic-associated differential expression being enriched for excessive immune, inflammatory, and keratin upregulation, we then sought to compare acute kidney injury (AKI)-associated genes across all 3 data sets to determine if this signature may reflect AKI response. For this, we obtained a list of consistently upregulated and downregulated genes from previous work by Chen et al., where 4 AKI data sets with 24 hour post-injury and time-matched controls were analyzed for consistent differential expression.(26) They identified a total of 157 upregulated genes and 88 downregulated genes across all 4 data sets. We then used these genes to subset the differential expression results of the pre-cystic and cystic data sets compared to controls (> 0.15 LFC and < 0.1 p-adjusted value). The P21 and P28 cystic gene expression clustered together and the known upregulated AKI genes had upregulated LFCs in the cystic data sets, and downregulated AKI genes had downregulated LFCs in the cystic data sets (Figure 1B). We conclude that the AKI molecular phenotype was much stronger in the cystic data compared to the pre-cystic data.

Comparing Pre-Cystic and Cystic Transcriptomic Signatures

We then asked how the rlog-normalized gene counts for the selected genes in our disease gene signatures compared across samples in each data set. These disease signatures consisted of the 100-200 differentially expressed genes from each data set which mapped to LINCS measured or inferred genes as Entrez gene IDs (see Methods, Data files " deseq2_outputs").(35) The expression of the signature genes for each data set for wild type and *Pkd2*-KO samples clearly clustered apart in each data set (Figure 2A-C).

We further compared the gene signatures from each data set to each other and found only 5 genes shared across the pre-cystic P70, cystic P21, and cystic P28 signatures (Figure 2D). Consistently upregulated genes in all 3 signatures included interleukin 1 receptor antagonist (*IL1RN*), serpin family A member 3 (*SERPINA3*), regulator of G protein signaling 16 (*RGS16*), and integrin subunit alpha M (*ITGAM*). The only consistently downregulated gene across all 3 signatures was parvalbumin, *PVALB,* which encodes a high-affinity calcium-binding protein found in the distal tubule.(37) We speculate that this downregulation is due to the decreased intracellular calcium availability resulting from polycystin dysfunction, as previously described (see Introduction).(3,37,38)

However, the two cystic transcriptomic signatures (P21 and P28) showed a high degree of similarity to each other, sharing 60 genes (47.6% and 32.2% of the P21 and P28 signatures in common with each other, respectively) (Figure 2D). Upregulated genes in both the P21 and P28 cystic signatures included 5 chemokine ligands/receptors *(CCR1, CCL8, CCL20, CXCL6,* and *CCN5*) and 5 interleukins (*IL1RN, IL6, LIF, IL18R1,* and *IL36A*). Chemokines and interleukins are commonly associated with PKD and, furthermore, as renal cysts progress in PKD and cystic vascularization increases, chemokine/immune responses increase and begin to reflect that of kidney injury.(39) Additionally, cancer-associated genes *VGF* and *WNT7A*, and the forkhead box genes *FOXJ1* and *FOXN1* were also upregulated in both cystic data sets, reflective of the well-described overlaps between PKD pathogenesis and the hallmarks of cancer.(6) Additionally, it has been hypothesized that *PKD2* regulates *WNT7A* expression, and *Wnt7a* inhibition in *Pkd2*-/- cells has been observed to have decreased β-catenin levels.(40)

The pre-cystic gene signature was much more unique, with 12 and 9 genes total shared with the cystic P21 and P28 signatures, respectively. Some of the upregulated genes that were found to be unique to the pre-cystic signature include cell division cycle genes *CDC25C* and *CDC20* and cyclin-family genes, including

*CDK1* which was previously suggested as a driver of cyst proliferation in ADPKD.(11,41) Kinases unique to the upregulated pre-cystic signature included *PBK, BUB1, TTK, MELK, FGR,* and aurora kinases A and B (*AURKA* and *AURKB*). Aurora kinase A is particularly well-studied for its involvement in PKD and has been researched as a potential target for PKD treatment.(38,42) Although chemokines were much more abundant in the cystic gene signatures (6 and 8 chemokine genes in P21 and P28 data sets, respectively, compared to 3 in the pre-cystic P70 signature)*, CCL18, CXCL10,* and *CCR5* were uniquely retained and specific to the pre-cystic signature after signature selection. Genes distinctly downregulated in the pre-cystic signature included DNA damage genes (*DDIT4* and *GADD45G*) and cytochrome P450 genes (*CYP2A13, CYP27B1,* and *CYP2A6*). Interestingly, *WNT11* was also downregulated only in the pre-cystic signature, and there is evidence that *WNT11* is critical for kidney tubules and renal function; C57Bl6 *Wnt11*−/− mice that survived to adulthood exhibited enlarged kidney tubules, altered kidney function, and prominent glomerular cysts.(43)

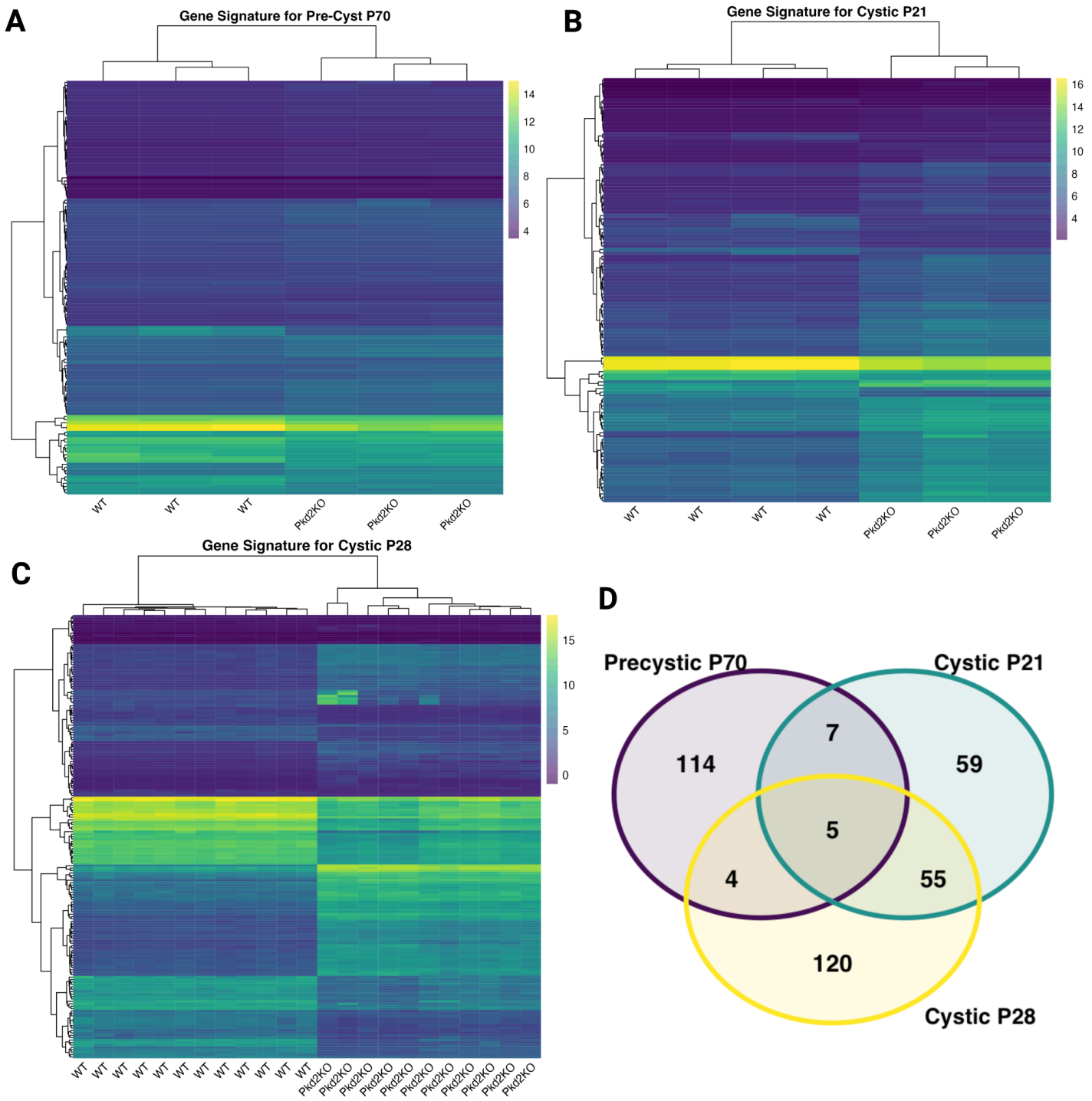



**Figure 2: Transcriptomic Signatures for Pre-cystic and Cystic Disease Signature Reversion.** *Heatmaps of rlog gene expression counts for genes selected for transcriptomic reversion signatures (filtered by genes available in LINCS perturbation data and then top 100-200 absolute LFC genes) showing complete linkage hierarchical clustering of gene expression and samples for A) pre-cystic P70, B) cystic P21, and C) cystic P28. D) Venn diagram of transcriptomic reversion signatures from the 3 data sets showing overlap and unique genes for each signature.*

Inflammatory Pathways Driving Variation in ADPKD Cystic Transcriptomic Signature

We then asked what gene pathways and modules were overrepresented in these pre-cystic and cystic disease signatures, and how they differ from the full differentially expressed gene list. We found our signatures to be consistent with previous ADPKD functional enrichment analyses, including chemokine activity/receptor binding, and dysregulation of multiple metabolic pathways (Figure 3A-C, Data files “fea”) (p-adjusted value < 0.05, sources GO, Reactome, and WikiPathways).(44,45)(35,44,45)(44,45) Consistent with the literature, both of the cystic signatures (P21 and P28) were enriched for multiple chemokine and interleukin pathways (Figure 3B-C), and the pre-cystic signature was enriched for multiple cell cycle and cell division pathways (Figure 3A). Pre-cystic transcriptomic signature resulted in NIMA Kinases (NEKs) as a top enrichment term. NEKs are characteristically dysregulated in PKD, which are involved in cilia assembly and function and may further play a role in PKD via cell cycle and mitotic spindle dysregulation.(46) Regulation of arterial blood pressure and blood circulation was downregulated in the pre-cystic signature, a previously noted indication of hypertension (Figure 3A).(47) Hypertension is a common comorbidity of ADPKD, likely due to the many complex overlapping roles between polycystin 1 and 2 and vascular structure and function. Impaired polycystin 1 and 2 function leads to decreased nitric oxide release and synthesis, which then leads to renin-angiotensin-aldosterone (RAAS) activation and cyst growth.(47) Furthermore, cyst growth further perpetuates hypertension via RAAS, accounting for some of the strong comorbidity between ADPKD and hypertension.(47) Hypertension is a driving factor in ADPKD for end-stage renal disease (ESRD), so it is intriguing that blood pressure regulation pathways were only identified in the pre-cystic signature and not the cystic signatures.

To further resolve terms and discover parent pathway terms, we sought to reduce GO:BP enrichment results by considering the GO term hierarchical system using Wang similarity. The -log10 of the p-values were used as scoring for terms with a similarity threshold of 0.8, allowing us to visualize the nested enrichment hierarchy (Figure 3D-F). We, therefore, identified the most representative parent terms for the pre-cystic signature as cell cycle and cell division (Figure 3D), and the most representative parent terms for the cystic signatures as inflammatory response, cytokine response, and leukocyte activity (Figure 3E-F). One particularly interesting parent term that was consistent between the cystic signatures was vascular development. As previously mentioned, as renal cysts progress in ADPKD, vascularization of cysts increases and further exacerbates inflammation and other immune responses, somewhat mimicking AKI.(39) This, as well as our

comparison of AKI genes in cystic differentially expressed genes (Figure 1B), supports the hypothesis that the already cystic disease signatures may be confounded by excessive inflammation and disease response, potentially masking some critical driving mechanisms of ADPKD pathogenesis.

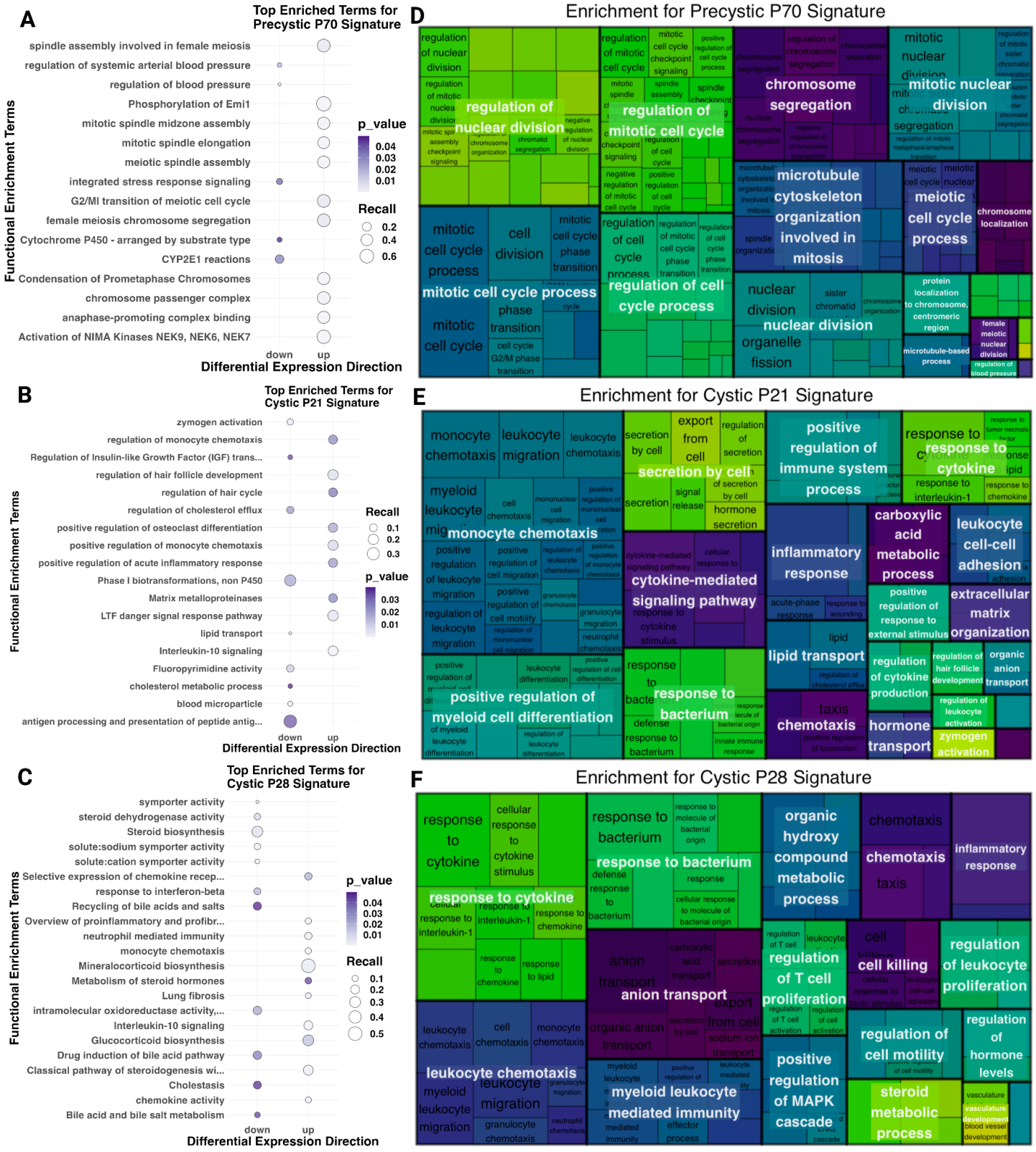



**Figure 3: Pathway Enrichment of Pre-cystic and Cystic Transcriptomic Signatures** *Bubbleplots of pathway enrichment using GO, Reactome, and WikiPathways of upregulated and downregulated genes from the A) pre-cystic P70 transcriptomic signature, B) cystic P21 transcriptomic signature, and C) cystic P28 transcriptomic signature, with point size dependent on the number of query genes matching to each term and color dependent on the p-value. Treemaps display*

*the enrichment hierarchy, using Wang similarity to reduce GO:BP term redundancy with parent terms consisting of largest boxes and enriched child terms within for each signature, and varying child terms reflecting term size for D) pre-cystic P70 signature E) cystic P21 transcriptomic signature, and F) cystic P28 transcriptomic signature.*

## Signature Reversion of the Pre-Cystic PKD Profile

We decided to focus on the pre-cystic P70 transcriptomic signature for signature reversion drug repurposing candidate prioritization, based on our findings that it may provide the strongest biological signals for ADPKD driving mechanisms, compared to the cystic signatures which may be confounded by inflammation and PKD pathogenic response (Figures 1-3). To identify drugs that might reverse the cellular PKD phenotype, we applied signature reversion analysis, filtering for drug signatures with negative normalized connectivity scores (NCS) (drug signatures inversely related to the pre-cystic signature) in kidney-derived cell lines. This analysis resulted in 730 drug candidates. We then filtered for “launched” drugs via the Drug Repurposing Hub, narrowing down to 178 drug candidates. Finally, we filtered for FDA-approval status of drug candidates by comparing the active ingredients with either over-the-counter, prescription, or tentative-approval statuses as of August 2022. This resulted in 109 candidates (Data file “sigsearch_outputs”).(35) The most frequent mechanisms of action (MOAs) were in line with previously studied drug MOAs for ADPKD (i.e., topoisomerase inhibitors, tubulin interfering agents, and dopamine receptor antagonists) (Figure 4A).(48–50) For the drugs with original disease area indication available, we compared the most frequent original indications for our 109 FDA-approved drug candidates (Figure 4B). While it was unsurprising to observe oncological/malignancy drugs as a frequent repurposing option for ADPKD due to the aforementioned overlap of ADPKD and cancer, the unexpected most frequent original disease indication was neurology/psychology.(6)

When further investigating the top individual drugs by sorting for the most inversely enriched NCS, we found multiple drugs previously investigated as repurposing candidates for ADPKD (red arrows in Figure 4C) and/or AKI or chronic kidney disease (CKD) (purple arrows in Figure 4C). For plotting purposes, we selected the top 35. Our top candidates by NCS that were previously studied in the literature for PKD included vincristine, clemastine, simvastatin, pyrimethamine, pimozide, crizotinib, and vinblastine, and previously studied treatments for chronic kidney diseases and AKI included vincristine and nilotinib.(49,51–57)

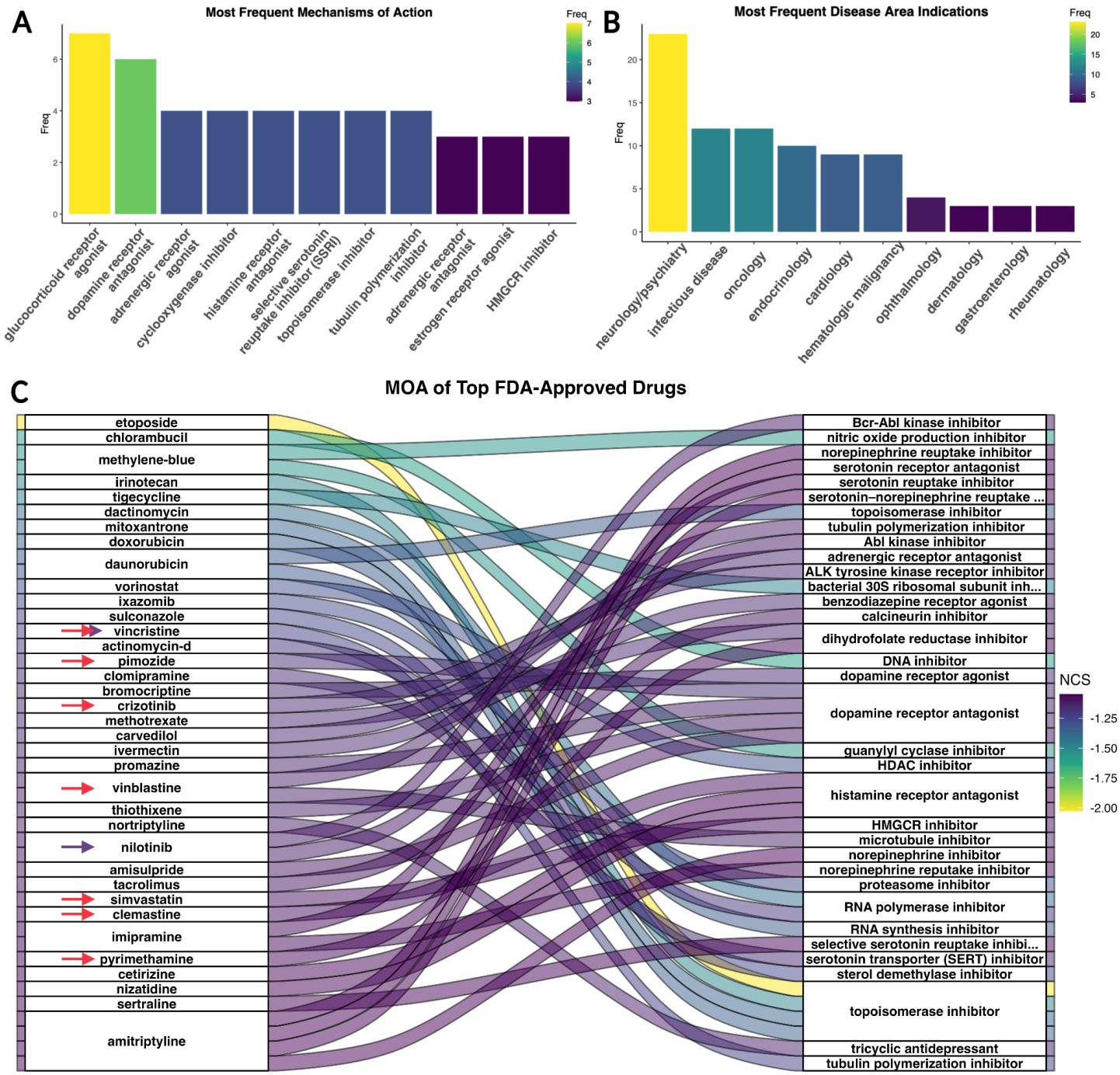



**Figure 4: FDA-Approved Drug Candidates MOA and Original Indications from Pre-Cystic Signature Reversion** *A) The most frequent MOA of all FDA-approved candidates, B) the most frequent disease areas for original indications of candidates, and C) alluvial plot of the top FDA approved candidates by NCS and their MOA, with drugs that have been previously explored for PKD treatment marked with red arrows, and drugs previously explored for CKD and/or AKI are marked with dark purple arrows.*

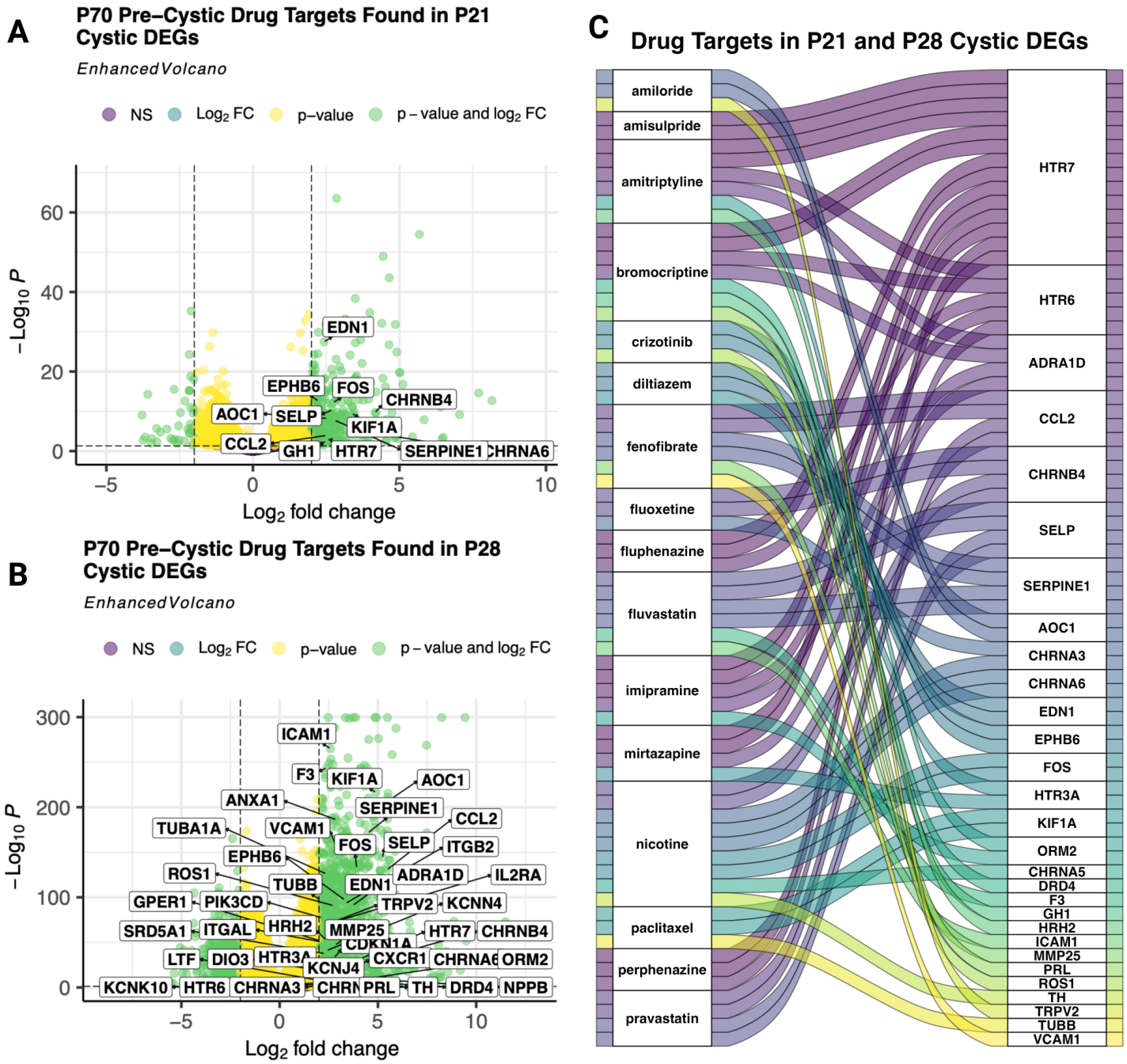



**Figure 5: Pre-cystic Signature Reversion Drug Candidates Target Upregulated Genes in Pre-cystic and Cystic Profiles** *Volcano plots of differentially expressed genes (p-adjusted < 0.05) with differential expression of candidate drug targets labeled in the A) P70 pre-cystic (LFC > 1.1), B) P21 cystic (LFC > 2.0), and C) P28 cystic (LFC > 2.0). D) Alluvial plot of the 16 drugs with upregulated drug targets in both cystic data sets*

*connected to their targets that were also found upregulated in the cystic differentially expressed genes (B and C).*

## Pre-cystic Signature Drug Candidate Target Expression in Cystic Kidney Tissue

We then asked if the drugs we uncovered by signature reversion of the pre-cystic data set have known drug targets with upregulated gene expression in the pre-cystic as well as the cystic data sets. These drugs would therefore be ideal candidates for patients with already cystic kidneys as well. Drug targets for the 109 FDA-approved drug candidates from pre-cystic signature reversion were compared against upregulated differentially expressed genes for each data set, revealing even more targets upregulated in the cystic data sets P21 and P28 (Figure 5B-C) than the pre-cystic data set from which the candidates were found (Figure 5A). Total upregulated genes matching to unique drug candidates were 12, 16, and 38 for data sets P70, P21, and P28, respectively. We further investigated the drugs with robust cystic target expression by prioritizing drugs with upregulated drug targets in both cystic data sets, leaving us with a total of 16 drugs targeting 29 unique cystic-upregulated genes (Figure 5D, Table 1).

Table 1: Summary of Prioritized Drug Candidates.

| **Drug** | **Drug Targets** | **Mechanism of Action** | **Original Indication** | **Approximate Retail Price** |
|---|---|---|---|---|
| amiloride | ADCY10; AOC1; ASIC1; ASIC2; ASIC3; ASIC4; PKD2; PKD2L1; PLAT; PLAU; SCNN1A; SCNN1B; SCNN1D; SCNN1G; SLC9A1; SLC9A2; SLC9A3; TRPC7; TRPV2 | sodium channel blocker | hypertension, congestive heart failure | $ |
| amisulpride | DRD3; HTR7 | dopamine receptor antagonist | psychosis, bipolar disorder, schizophrenia | N/A |
| amitriptyline | ADRA1A; ADRA1B; ADRA1D; ADRB1; ADRB2; ADRB3; CHRM1; CHRM4; CHRM5; CYP2C9; HRH1; HRH2; HRH4; HTR1B; HTR1D; HTR6; HTR7; KCNA1; KCND2; KCND3; KCNQ2; KCNQ3; NTRK1; NTRK2; OPRD1; OPRK1; OPRM1; ORM2; SIGMAR1; SLC6A2; SLC6A4 | norepinephrine inhibitor, norepinephrine reuptake inhibitor, serotonin receptor antagonist, serotonin–norepinephrine reuptake inhibitor (SNRI) | depression | $ |
| bromocriptine | ADCY10; ADRA1A; ADRA1B; ADRA1D; ADRA2B; ADRA2C; DRD1; DRD3; DRD4; GH1; HTR1B; HTR1D; HTR2B; HTR6; HTR7; PRL; PRLR | dopamine receptor agonist | hyperprolactinemia, Parkinson's Disease, acromegaly | $$$$ |
| crizotinib | ALK; AXL; CYP2B6; CYP3A5; EPHA2; EPHA6; EPHB6; IRAK1; IRAK3; LTK; MAP3K2; MAP4K1; MAP4K2; MAP4K3; MAP4K5; MERTK; MET; MST1R; NTRK2; NTRK3; NUDT1; PLK4; ROS1; SLK; STK10; TEK; TIE1 | anaplastic lymphoma kinase (ALK) receptor tyrosine kinase inhibitor | non-small cell lung cancer (NSCLC) | $$$$$ |
| diltiazem | CACNA1C; CACNA1S; CACNA2D1; CACNG1; CYP3A5; CYP3A7; EDN1; HTR3A; KCNA5 | calcium channel blocker | hypertension, angina pectoris | $$$$ |
| fenofibrate | ABCD2; ABCD3; ABCD4; APOA1; APOB; APOC3; APOE; CCL2; CETP; CRAT; CRP; CYP2C8; CYP2C9; | peroxisome proliferator-activated receptor | hypercholesterolemia, hypertriglyceridemia | $$$ |

| | | | | |
|---|---|---|---|---|
| | LPL; MMP25; NR1I2; PPARA; PPARD; PPARG; SERPINE1; SREBF2; VCAM1 | (PPAR) receptor agonist | | |
| fluoxetine | ANO1; CHRNA2; CHRNA3; CHRNB4; CKS1B; CYP2C9; HTR2B; KCNH2; SLC6A4 | selective serotonin reuptake inhibitor (SSRI) | depression, obsessive compulsive disorder (OCD), bulimia nervosa, panic disorders, bipolar disorder | $$ |
| fluphenazine | ADCY10; AR; CALM1; CALM2; CALM3; CHRNA7; DRD1; HRH1; HTR1E; HTR6; HTR7 | dopamine receptor antagonist | schizophrenia | $$$$ |
| fluvastatin | APOA1; APOB; APOE; CYBA; CYP2C8; CYP2C9; F3; HMGCR; ICAM1; LDLR; RHOA; SELP; SERPINE1 | hydroxymethylglutaryl-CoA reductase (HMGCR) inhibitor | hypercholesterolemia, congenital heart defects | $$$$ |
| imipramine | ADRA1A; ADRA1B; ADRA1D; CHRM1; CHRM4; CHRM5; DRD1; FMO1; HRH1; HTR6; HTR7; KCND2; KCND3; KCNH1; KCNH2; ORM2; SLC22A3; SLC6A2; SLC6A3; SLC6A4 | norepinephrine reuptake inhibitor, serotonin reuptake inhibitor | depression, nocturnal enuresis | $$$$ |
| mirtazapine | ADRA1A; ADRA1B; ADRA1D; ADRA2B; ADRA2C; ADRB1; ADRB2; DRD1; DRD3; HRH1; HRH3; HTR2B; HTR3A; HTR7; OPRK1; SLC6A2; SLC6A3; SLC6A4 | adrenergic receptor antagonist, serotonin receptor antagonist | depression | $ |
| nicotine | AOX1; CHAT; CHRNA1; CHRNA10; CHRNA2; CHRNA3; CHRNA4; CHRNA5; CHRNA6; CHRNA7; CHRNA9; CHRNB2; CHRNB3; CHRNB4; CHRND; CHRNE; CYP19A1; CYP2A6; CYP2B6; CYP2E1; CYP2U1; FMO1; FMO2; FMO3; FMO4; FMO5; FMO6P; FOS; INMT; PNMT; TBXAS1; TH; TRPA1; UGT1A4; UGT2B10; UGT2B11 | acetylcholine receptor agonist | smoking cessation | N/A |
| paclitaxel | ABCB1; BCL2; CYP2C8; KIF11; KIF1A; KIF1B; KIF1C; KIF5B; MAP2; MAP4; MAPT; NDC80; NR1I2; NUF2; TLR4; TUBB; TUBB1 | tubulin polymerization inhibitor | ovarian cancer, breast cancer, non-small cell lung cancer (NSCLC) | $$$ |
| perphenazine | AOX1; CALM1; CALM2; CALM3; DRD1; HRH1; HTR6; HTR7 | dopamine receptor antagonist | schizophrenia, nausea, vomiting | $$ |
| pravastatin | ABCC2; APOB; CCL2; CETP; CRP; HMGCR; LCAT; SELP; SLCO1B1 | hydroxymethylglutaryl-CoA reductase (HMGCR) inhibitor | hypercholesterolemia, myocardial infarction, hyperlipidemia | $ |

*For each drug, known drug targets, mechanism of action, original indication, and approximate retail price. Cost indicators are based on average prices by quartile after the removal of crizotinib as an outliner ($$$$$ = $17,955.50/month). $ is < $24/month, $$ is between $24 and $29/month, $$$ is between $29 and $38.69/month, $$$$ is between $38.69 and $82/month.*

## Pathway and Drug Set Enrichment Analysis of Top Drug Repurposing Candidates

We further considered our 16 prioritized drug candidates by investigating pathways for which they were enriched and the drug-target networks within those enriched pathways. For this, we used signatureSearch's drug set enrichment analysis (DSEA) with hypergeometric testing (p-adjusted value < 0.05) and plotted the top enriched GO:MF terms by p-adjusted value (Figure 6A). Several of the top resulting pathways are known to be involved in PKD, including oxidoreductase activity, G protein-coupled serotonin receptors, and histamine receptor activity.(58–60) Closer inspection of the protein interactions of our prioritized drugs for oxidoreductase

activity showed drug metabolism activity specifically with the genes encoding cytochrome P450 enzymes, *CYP2C8* and *CYP2C9* (Figure 6B). *CYP2C8* and *CYP2C9* P450 enzymes also metabolize fatty acids, a metabolic pathway well known to be altered in ADPKD disease progression.(2) *CYP2C8* and *CYP2C9* metabolize many other drugs and are highly polymorphic, heavily impacting pharmacokinetics.(61) As shown here, *CYP2C8* metabolizes paclitaxel, fluvastatin, and fenofibrate, and *CYP2C9* also metabolizes fluvastatin and fenofibrate as well as amitriptyline and fluoxetine (Figure 6B). It should be noted that these CYP interactions are not the only CYP interactions for our prioritized drugs, but are part of the drug-target network for this GO:MF oxidoreductase activity pathway. For example, fluoxetine also interacts with *CYP2C19* and *CYP3A4* but is most metabolized by *CYP2D6*, to which it is also a strong inhibitor.(62)

Drug binding with histamine receptors showed mirtazapine, a tetracyclic antidepressant, also targets *HRH3* and *HRH1* (Figure 6C). Mirtazapine has strong antihistamine effects and was found to be well-tolerated in CKD patients for the treatment of chronic kidney disease-associated pruritus (CKD-aP).(63) Another drug originally indicated for depression that was also shown to have antihistamine effects is amitriptyline. Amitriptyline has already been investigated for repurposing in osteoarthritis and gout for its anti-inflammatory properties.(64,65)

Fluphenazine and perphenazine, two other antidepressants, were both additionally shown to interact with histamine receptors and, further, bind to calmodulin (encoded by *CALM1* and *CALM3*) as calcium-dependent protein binding (Figure 6C-D). Calmodulin is a primary calcium sensor, likely playing a critical role in PKD pathogenesis in response to decreased calcium levels due to polycystin 1 or polycystin 2 defect, leading to increased levels of cAMP.(4) Furthermore, fluphenazine's calmodulin inhibition has been hypothesized to prevent fibrosis via calcium-saturated calmodulin-dependent myosin light chain kinase (CaM-MLCK) as well as cancer cell invasion and metastasis.(66,67)

The catecholamine binding pathway enriched in our prioritized drug targets includes binding to dopamine receptors Drd1 and Drd3 (Figure 6E). As we previously discussed, dopamine receptor antagonists are promising for ADPKD treatment, and have been shown to reduce cystic growth and cell proliferation in *Pkd1*-/- mice.(50) HDAC5 export maintains renal epithelial architecture via de-repression of MEF2C target genes and is dependent on polycystin calcium influx. Dopamine receptor antagonists, specifically for Drd3, have been shown to restore HDAC5 nuclear export in *PKD1loxP/loxP* cells.(50) Bromocriptine, amisulpride,

and mirtazapine were all shown to target Drd3 specifically, and therefore may be particularly effective in cyst reduction and cell proliferation by HDAC5 nuclear export. This shows that our prioritized candidates target pathways with known involvement in ADPKD pathogenesis.

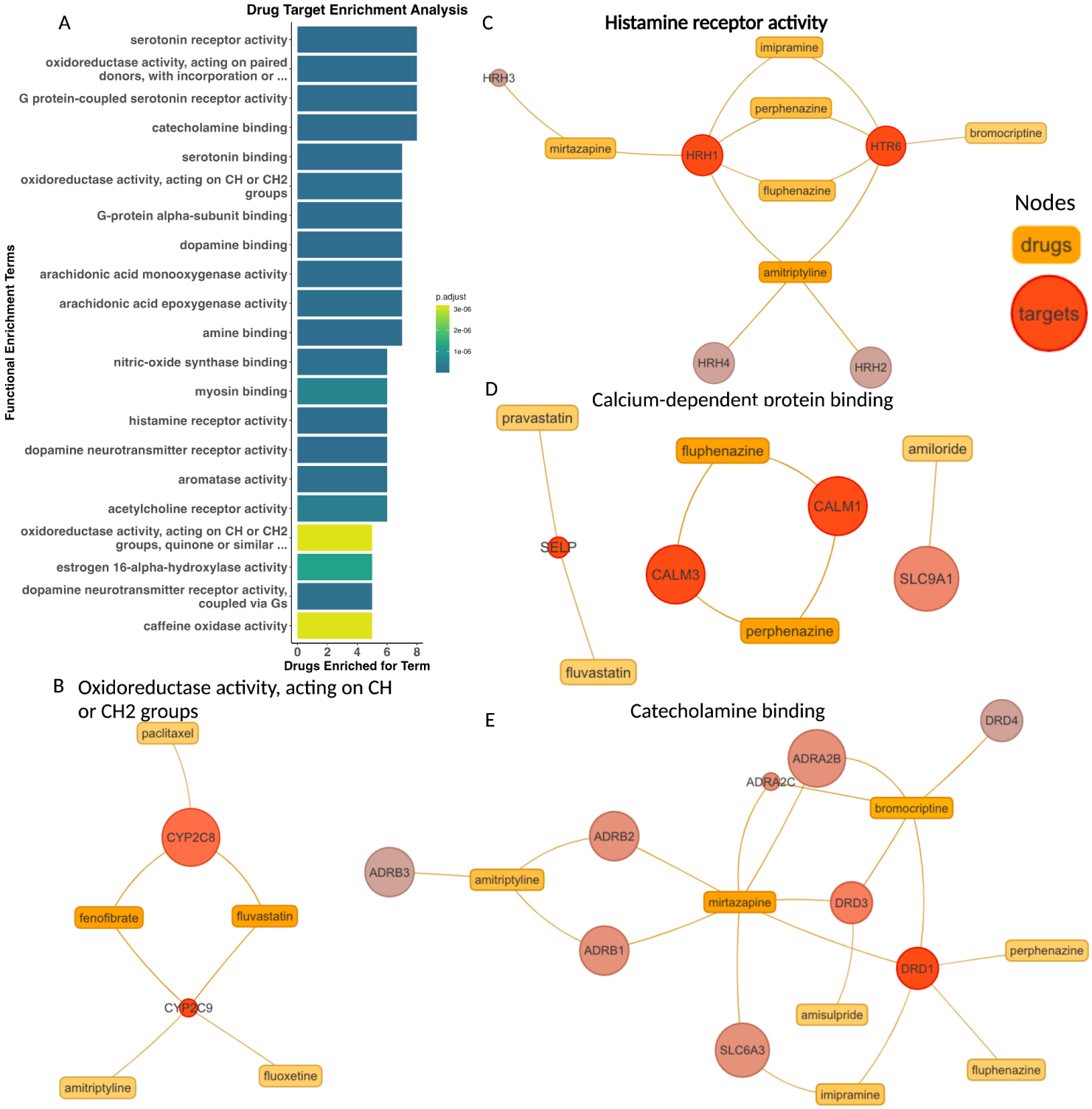


**Figure 6: Prioritized Drug Targets' Pathways and Binding** *Top enriched GO:MF pathways of targets from 16 prioritized drugs by p-adjusted value as A) a barplot and B-E) specific pathways shown as networks of prioritized drugs (orange rectangular nodes) and their targets (red circular nodes) with color shade defined by the number of connections to other nodes.*

# Discussion

We analyzed publicly available *Pkd2* KO mouse RNA-Seq data sets to assess differential gene expression, pathway enrichment, and transcriptomic signature reversion to identify potential drug repurposing candidates to identify prioritized therapeutics for the ADPKD community. Our work extends beyond the original analyses reported for these data sets and highlights the important role public data repositories can have in developing therapeutic paths for ADPKD. Additionally, as the P70 pre-cystic data set consisted of tissue collection at the pre-cystic/cyst-initiation stage, this presented an opportunity to identify early molecular changes associated with cystic kidney disease and potentially confounding signatures associated with full cystogenesis (i.e., fibrosis and inflammation).(11) This is ideal for prioritizing drug repurposing candidates that may be effective for early or preventative treatment. Further, by comparing the drug targets for the pre-cystic P70 candidates to differentially expressed genes of the P21 and P28 PKD mouse models (*Pkhd1*-cre;*Pkd2*$^{F/F}$), we identified 29 unique drug targets that were differentially expressed in both cystic data sets for 16 drug candidates. These indicate drug candidates that may effectively treat pre-cystic as well as cystic ADPKD.

Consistent with previous findings, we identified genes known to have a significant role in the pathogenesis of ADPKD including *WNT7A, LCN2,* and *MMP7* (Data files "deseq2_outputs").(35,40,68–70) The previously noted biomarkers of CKD progression *LCN2* and *MMP7* were upregulated in both P28 and P21 cystic data sets.(68–70) Additionally, we also found that *Wnt7a*, previously characterized for its role in WNT signaling in *Pkd2*-/- cells, was upregulated in both cystic datasets (P28 and P21).(40) Foreseeably, there were more similarities in the differential expression of the P28 and P21 cystic data sets compared to the P70 pre-cystic data set (Figure 1A). All three datasets shared a total of 22 differentially expressed genes and the cystic data sets shared 387 differentially expressed genes. Consistently upregulated genes across all the data sets included many involved in immune pathways characteristic of ADPKD (*Ccl2, Ccl7, Ccr5, Cd44,* and the colony

stimulating factor *Csf1*).(7) Our pathway enrichment analysis on all three data sets confirmed previously noted pathways involved in ADPKD pathogenesis, including inflammation, MAPK signaling, chemokine activity/receptor binding, and metabolic pathways.(2,71,72) In line with other studies, most of the upregulated pathways in the cystic data sets P21 and P28 were associated with inflammation, leukocyte activity, NAD+ activity, and cytokine response. Additionally, genes differentially expressed only in the P70 pre-cystic data set were enriched for other pathways characteristic of ADPKD pathogenesis, including upregulation of cell cycle and *Cdk1*, a known driver of cyst proliferation in ADPKD.(11) We further found that the P21 and P28 cystic differentially expressed genes were highly similar to consistently upregulated and downregulated genes in AKI compared to the P70 pre-cystic differentially expressed genes (Figure 1B). Finally, while gene expression changes do not always reflect disease etiology and may only be due to downstream disease responses, careful selection of both the data sets (such as the pre-cystic P70) and drug candidates is critical.(73) For example, some of our top candidates, such as phosphodiesterase inhibitors (Figure 4A), are predicted to further increase cellular cAMP, which may actually exacerbate ADPKD-associated cellular phenotypes due to decreased intracellular mechanisms from defective PC1/PC2 calcium-sensing and transport. This further underscores the need for placing such analyses in the biological context.

In this study, we prioritized drug repurposing candidates by selecting FDA-approved compounds (i.e., those that already have safety profiles for other diseases) predicted to reverse ADPKD transcriptomic signatures so they could be most greatly expedited as potential ADPKD treatments. We further investigated each drug's known mechanism of action and their expressed or differentially expressed drug targets in all 3 data sets (i.e., P70, P21, and P28). This approach identified drugs previously investigated for ADPKD treatment in clinical trials (pravastatin) as well as preclinical studies (e.g. fenofibrate, paclitaxel), and uncovered promising novel candidates. For example, topoisomerase inhibitors and tubulin interference drugs were previously identified for their ability to reduce cyst growth in a large screen of ~8,000 compounds in *Pkd1*-null 3D-grown cells.(48) Topoisomerase inhibitors, originally indicated for cancer, disrupt the ability of cells to repair single-stranded DNA breaks incurred during DNA replication.(74) As topoisomerase activity is drastically increased in cancer due to rapid division (as is likely the case in ADPKD given the overlapping molecular characteristics between cancer and ADPKD(6)), inhibiting the repair of single-stranded nicks from DNA replication leads to replication inhibition followed by cell death.(74) Notably, tubulin interference drugs

were previously found not only to reduce cyst growth in 3D-grown cells, but also to reduce growth without impacting cell viability.(48) Interference of tubulin dynamics has been previously studied in ADPKD mouse models, resulting in significantly increased survival time, renal function, adult body weight, and decreased cyst growth.(49) However, another class of drugs, dopamine receptor antagonists, are under-researched in ADPKD even though they increase *HDAC5* nuclear export.(50) In healthy kidney cells, the polycystin complex formed by the *PKD1* and *PKD2* proteins facilitates the nuclear export of *HDAC5*, resulting in the maintenance of renal epithelial architecture.(50) Furthermore, *Pkd1*-/- mice treated with dopamine receptor antagonists did exhibit cyst reduction and increased body weight and activity.(50)

However, the most frequent MOA of these FDA-approved drug candidates was glucocorticoid receptor agonists (Figure 4A). While, to our knowledge, these have not been previously investigated for ADPKD, there are multiple, intriguing potential connections between glucocorticoid receptor agonists and ADPKD. Glucocorticoids are steroid hormones that are often used for inflammation and immune suppression across multiple diseases by repressing the activity of immunologic transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), AP-1, and T-bet.(75) Additionally, glucocorticoids have more recently been investigated for potential pathway involvement in nephrological diseases and in pathways such as Wnt signaling and autophagy.(76) While neurology/psychology-indicated drugs do have the most compounds annotated in the Drug Repurposing Hub we used for annotation further investigation is warranted, especially due to the noted comorbidity between ADPKD and depression.(77)

Additionally, the HDAC inhibitor vorinostat has been found to treat mechanisms similar to ADPKD pathogenic mechanisms, including increased renal size, extracellular matrix accumulation, and increased cell proliferation.(6) Vorinostat treatment in diabetic neuropathy rat models have resulted in decreased renal size while similar treatment in a renal epithelial cell (NRK52-E) resulted in decreased extracellular matrix expression and cell proliferation.(53,78,79) In general, HDAC inhibitors have been of interest in treating ADPKD. Multiple studies in ADPKD pre-clinical models showed reduced cyst formation, cyst growth, improved renal function, and decreased cell proliferation following HDAC inhibition.(79)

Interestingly, our initial top FDA-approved drugs (by most negative NCS) included three drugs that have been found to inhibit STAT family genes (Figure 4C). One study investigating STAT-targeting drugs for ADPKD therapies suggested pimozide inhibits STAT5, crizotinib inhibits STAT3 phosphorylation, and pyrimethamine

inhibits STAT3 by dimerization.(57) Pyrimethamine, an antiparasitic compound, has been further tested in ADPKD preclinical models and was found to decrease cell proliferation in ADPKD patient-derived cultured epithelial cells and prevent renal cyst formation in *Pkd1*-KO mice.(54)

Another FDA-approved candidate, the tyrosine kinase inhibitor nilotinib, was previously named as a top candidate for the treatment of CKD in a study that combined gene expression data across 8 different glomerular diseases that lead to CKD using a different signature reversion method.(55) Additionally, when studied in rats for CKD, nilotinib-treated rats had preserved renal function, decreased profibrotic gene expression, and significantly prolonged survival.(56)

We further compared the targets of our FDA-approved drug candidates from pre-cystic P70 signature reversion to differentially expressed genes from the cystic data sets P21 and P28. Among the 16 FDA-approved candidates with known drug targets overexpressed in both cystic data sets, there were 3 drugs currently in clinical trials, or in the same class of drugs in clinical trials, for ADPKD. For example, pravastatin is already in phase 4 clinical trials for ADPKD and phase 2 clinical trials in combination with acidosis treatment for ADPKD patients with worsening CKD (ClinicalTrials.gov Identifiers: NCT03273413 and NCT04284657).(80) Another prioritized candidate, fluvastatin, belongs to the same drug class as pravastatin: cholesterol-lowering drugs 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitors, referred to as statins. Statins have been studied for the treatment of ADPKD since 1995 due to their anti-proliferative, anti-inflammatory, and antioxidant properties.(81–83) Finally, amiloride is being tested in clinical trials for the prevention of hypertension in ADPKD patients as hypertension is a common comorbidity of ADPKD (ClinicalTrials.gov Identifier: NCT05228574).(6,47)

Other prioritized candidates included previously studied ADPKD therapeutics in preclinical models. Fenofibrate is an agonist of peroxisome proliferator-activated receptor α (PPARα), which promotes fatty acid β-oxidation and oxidative phosphorylation, both pathways decreased in ADPKD.(84) Further, treatment of *Pkd1RC/RC* mice with fenofibrate resulted in reduced renal cyst volume, proliferation, and infiltration of inflammatory cells as well as reduced bile duct cysts and liver inflammation and fibrosis.(84) Additionally, paclitaxel has been studied as a potential repurposing candidate for ADPKD given its ability to arrest the cell cycle, and we identified multiple targets of paclitaxel upregulated in both cystic data sets (*KIF1A* and *TUBB*, Figure 5D).(85)

In addition to previously considered repurposing candidates for ADPKD, some of these results raise novel therapeutic options that may be advantageous to further consider for their strong gene expression reversion and impact on ADPKD-relevant pathways. For example, bromocriptine has been suggested for repurposing in cancer for mechanisms that additionally overlap with ADPKD.(6) Specifically, bromocriptine has a strong affinity for DNA-bound NF-κB, thereby inhibiting the expression of pro-inflammatory and proliferative interleukins, tumor necrosis factor, interferons, cyclooxygenases, and growth factors.(86) Interestingly, one study also tested paclitaxel and bromocriptine together as an anticancer combination therapy and found them to be synergistic using the Loewe isobologram calculation in CEM/ADR5000. (86)

Due to ADPKD being a chronic condition, it is also important to consider long-term adverse drug events as well as cost when further prioritizing candidates. For retail cost associations, we manually compared each drug price from the epocrates clinical database and found the average monthly cost was less than $83 (after removing one outlier, crizotinib, with an average monthly cost of $17,956) and listed the average monthly cost by quartile (Table 1). We can further de-prioritize other drugs due to their long-term adverse events and adverse events specifically unfit for patients with kidney disease, including paclitaxel, amiloride, and nicotine. Amiloride has a black box warning for increased risk of hyperkalemia in patients with renal impairment.(87) Nicotine is particularly unfit for kidney disease patients as it has been linked with increased renal injury severity in kidney disease preclinical models and potentially leads to increased reactive oxygen species in pro-fibrotic pathways.(88) Lastly, paclitaxel has multiple severe adverse events as black box warnings, and as such may not be practical long-term treatment options.(89)

Based on all of our findings presented here, we suggest bromocriptine and mirtazapine as highly favorable to further pursue as novel ADPKD treatments. Bromocriptine had one of the top most negative NCSs, suggesting the greatest anti-relation to pre-cystic gene expression (Figure 4C), had multiple targets upregulated in cystic data sets (P21 and P28) (Figure 5D), and also targets Drd3, which was previously found as a potential target for restoring HDAC5 nuclear transport in ADPKD (Figure 6E).(50) Bromocriptine directly impacts other ADPKD pathogenic pathways, specifically by decreasing cAMP by adenylyl cyclase, as well as MAPK inhibition by blocking phosphorylation of p42/p44 MAPK.(90) Furthermore, bromocriptine had fewer and less severe reported adverse drug events and, to our knowledge, no black box warnings.(91) We further highlight mirtazapine, which has an average monthly cost of less than $24 and, like bromocriptine, also has

antihistamine effects and targets Drd3 (Figure 6E). Mirtazapine, like most other antidepressants, has a black box warning for the risk of suicide, especially in children and young adults, but has generally been found to be well tolerated.(92,93)

Limitations of this current study include the use of mouse preclinical data, sample size, data availability, and comparisons of sex, age, and disease stage. As with other disease preclinical models, the ADPKD mouse models do not perfectly recapitulate the human disease; attempted full *Pkd1* and *Pkd2* null mouse is embryonic lethal, which has led to models such as the ones used here that are expressed in the kidney only or as an inducible model to closer mimic adult onset ADPKD.(94,95) While the *Pkd2*$^{fl/fl}$;*Pax8*rtTA;TetO-Cre mouse model may best recapitulate adult ADPKD, we could only find one kidney RNA-seq data set from this model, and only found two kidney RNA-seq data sets for *Pkhd1*-Cre; *Pkd2*$^{fl/fl}$. Lastly, we prioritized drugs with available drug target data, limiting this study to drugs with known targets and other annotations available in the Drug Repurposing Hub.

Future directions for this work include confirming the ability of these compounds to reverse ADPKD phenotypes in preclinical models and eventually, safety and efficacy for long-term treatment in patients. Additional drug repurposing approaches (such as combination therapy approaches) and a detailed assessment of how these drugs might differ across patients of different ages, sexes, and disease stages is also critical.(96) However, this study provides a proof of concept on how transcriptomic data from ADPKD mouse models can be leveraged to identify drug repurposing candidates and novel drug targets.

# Conclusions

We provide prioritized drug repurposing candidates predicted to reverse the ADPKD molecular phenotype that we identified by transcriptomic signature reversion of publicly available pre-cystic ADPKD mouse model RNA-seq data. We further prioritized these drugs by FDA status, pathway enrichment, and comparing their drug targets to the differentially expressed genes in additional cystic ADPKD mouse model RNA-seq profiles. We therefore determine the drug repurposing candidates that may be effective for early or preventative treatment, as well as cystic ADPKD.

# List of Abbreviations

Autosomal dominant polycystic kidney disease (ADPKD)
polycystin-1 (PC1) and polycystin-2 (PC2)
Library of Integrated Network-Based Cellular Signatures (LINCS)
cyclic adenosine monophosphate (cAMP)
mechanism of action (MOA)
knock-out (KO)
quality control (QC)
log2 fold change (LFC)
Functional Enrichment Analysis (FEA)
Gene Ontology (GO)
GO biological process (GO:BP), GO cellular component (GO:CC), and GO molecular function (GO:MF))
gene set enrichment analysis (GSEA)
weighted connectivity score (WTCS)
enrichment score (ES)
normalized connectivity score (NCS)
Drug Set Enrichment Analysis (DSEA)
NIMA Kinases (NEKs)
renin-angiotensin-aldosterone (RAAS)
end-stage renal disease (ESRD)
Acute kidney injury (AKI)
chronic kidney disease (CKD)
non-small cell lung cancer (NSCLC)
obsessive compulsive disorder (OCD)
serotonin–norepinephrine reuptake inhibitor (SNRI)
hydroxymethylglutaryl-CoA reductase (HMGCR)
chronic kidney disease-associated pruritus (CKD-aP)
calmodulin-dependent myosin light chain kinase (CaM-MLCK)
nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)
peroxisome proliferator-activated receptor α (PPARα)

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# Declarations

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Availability of data and materials

All data is available from public repositories as stated in the text. All code needed to reproduce the analyses in this study and resulting data files are available at https://doi.org/10.5281/zenodo.7384372.(35)

## Competing Interests

M. M. reports grants and consulting fees outside the submitted work from Otsuka Pharmaceuticals, Sanofi, Palladio Biosciences, Reata, Natera, Chinook Therapeutics, Goldilocks Therapeutics and Carraway Therapeutics.

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## Authors' contributions

EJW, MM, and BNL conceptualized the project and EJW, TCH, and JLF contributed to the methodology used. Mouse RNA-seq data was curated by TCH, KCM, and HLC and preliminary analyses conducted by KCM and HLC. Gene expression heatmap and volcano plot visualizations were contributed by RTB. Original code by JLF was adapted for FDA approval testing. All other analyses were coded and performed by EJW. Code was reviewed and validated for reproducibility by TCH, JLF, and VHO. BNL provided supervision, project administration, and funding acquisition. EJW, TCH, and BNL contributed to writing the first draft. EJW, TCH, JLF, VHO, RTB, KCM, HLC, BKY, JFG, MM, and BNL reviewed and edited writing. All authors read and approved the final manuscript.

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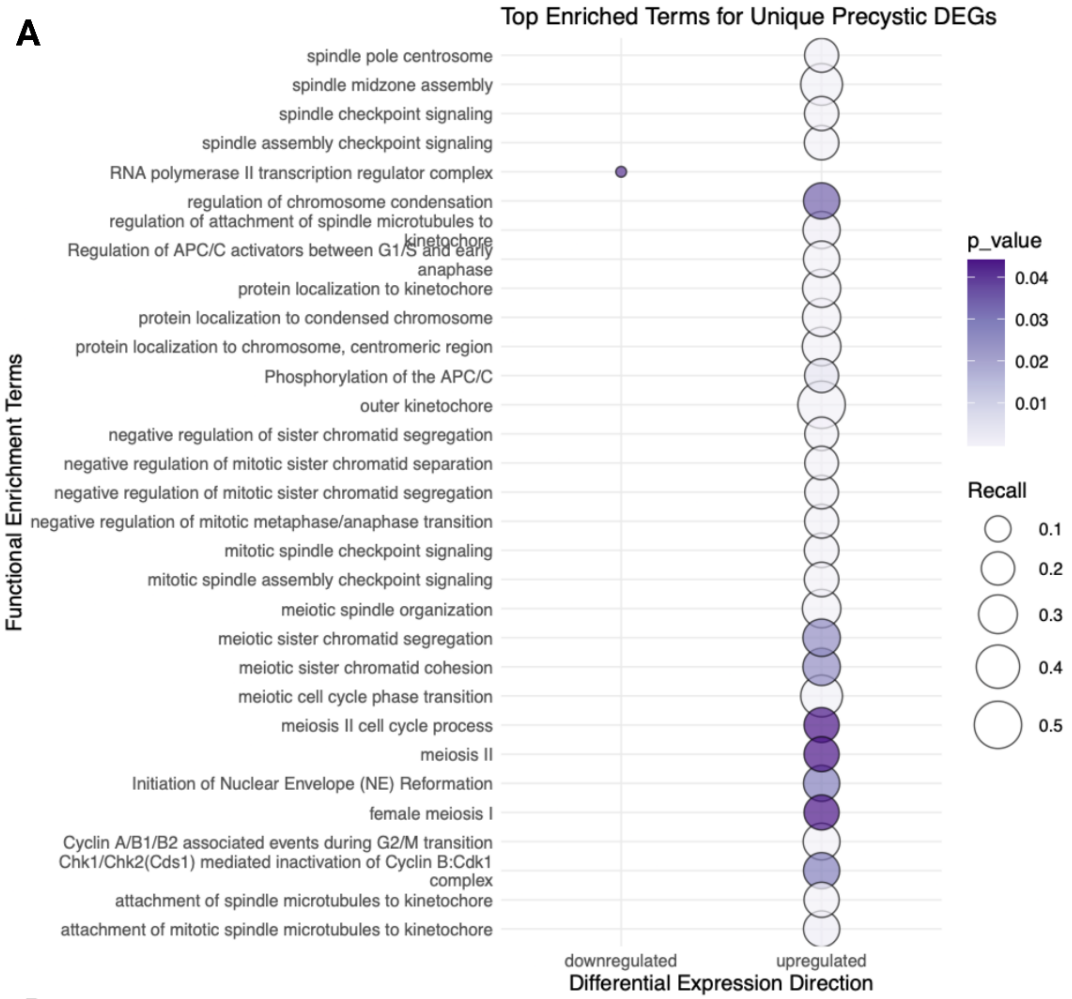
# Additional Files

**Additional file 1: Summary of Data Sets used in this Study.**

| Data Origination | Kidney Phenotype at Collection | Age at Collection (days) | Mouse Model | Sample Numbers (KO/WT) |
|---|---|---|---|---|
| GSE149739 (11) | Pre-cystic, mild tubule dilation but no overt cyst formation | 70 | *Pkd2*$^{fl/fl}$;*Pax8*rtTA;TetO-Cre | 3/3 |
| GSE134719 (12) | Cystic | 28 | *Pkhd1*-Cre; *Pkd2*$^{fl/fl}$ | 11/12 |
| GSE69556 (13) | Cystic | 21 | *Pkhd1*-Cre; *Pkd2*$^{fl/fl}$ | 3/4 |

GEO accession numbers for publicly available data used in this study along with kidney phenotype and age at collection, mouse model genotype, and number of samples used from each data set.

**Additional file 2: Enrichment of Unique Pre-Cystic DEGs and Overlapping Cystic Differentially Expressed Genes**


A
Top Enriched Terms for Unique Precystic DEGs
spindle pole centrosome
spindle midzone assembly
spindle checkpoint signaling
spindle assembly checkpoint signaling
RNA polymerase II transcription regulator complex
regulation of chromosome condensation
regulation of attachment of spindle microtubules to kinetochore
Regulation of APC/C activators between G1/S and early anaphase
protein localization to kinetochore
protein localization to condensed chromosome
protein localization to chromosome, centromeric region
Phosphorylation of the APC/C
outer kinetochore
negative regulation of sister chromatid segregation
negative regulation of mitotic sister chromatid separation
negative regulation of mitotic sister chromatid segregation
negative regulation of mitotic metaphase/anaphase transition
mitotic spindle checkpoint signaling
mitotic spindle assembly checkpoint signaling
meiotic spindle organization
meiotic sister chromatid segregation
meiotic sister chromatid cohesion
meiotic cell cycle phase transition
meiosis II cell cycle process
meiosis II
Initiation of Nuclear Envelope (NE) Reformation
female meiosis I
Cyclin A/B1/B2 associated events during G2/M transition
Chk1/Chk2(Cds1) mediated inactivation of Cyclin B:Cdk1 complex
attachment of spindle microtubules to kinetochore
attachment of mitotic spindle microtubules to kinetochore
Functional Enrichment Terms
downregulated
upregulated
Differential Expression Direction
p_value
0.04
0.03
0.02
0.01
Recall
0.1
0.2
0.3
0.4
0.5

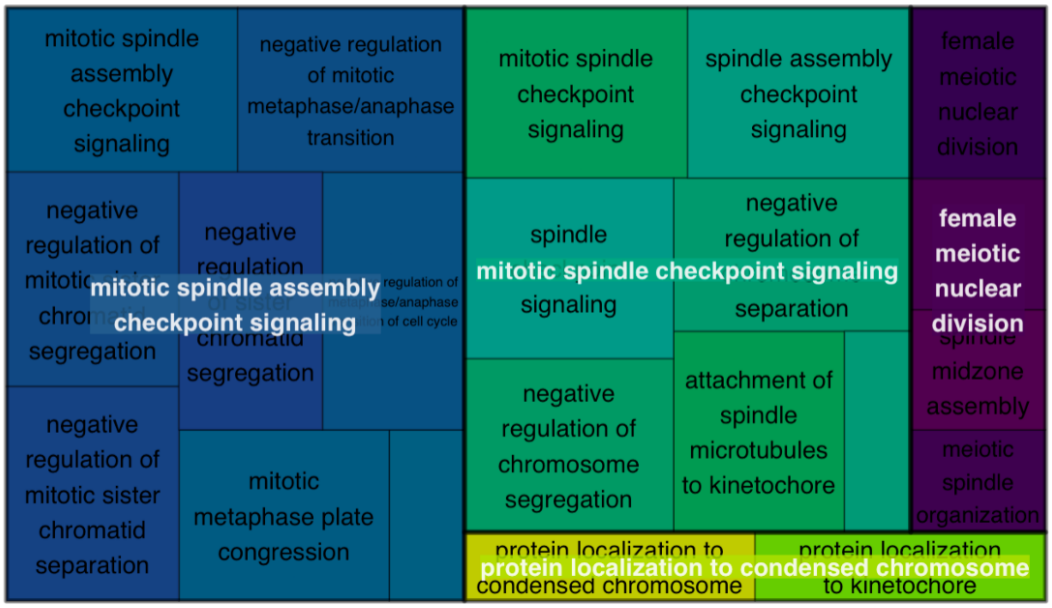
B
Top Enriched Terms for Overlapped Cystic DEGs
response to chemokine
regulation of monocyte chemotaxis
regulation of macrophage migration
regulation of macrophage chemotaxis
regulation of heterotypic cell–cell adhesion
regulation of hair follicle development
regulation of glial cell migration
positive regulation of monocyte chemotaxis
positive regulation of macrophage migration
positive regulation of macrophage chemotaxis
positive regulation of inflammatory response to antigenic stimulus
positive regulation of heat generation
positive regulation of hair follicle development
positive regulation of glial cell migration
positive regulation of cellular extravasation
positive regulation of acute inflammatory response
NAD+ nucleotidase, cyclic ADP–ribose generating
NAD+ nucleosidase activity
NAD(P)+ nucleosidase activity
monocyte chemotaxis
macrophage migration
interleukin–1–mediated signaling pathway
G protein–coupled purinergic nucleotide receptor activity
fever generation
CXCR chemokine receptor binding
chemokine–mediated signaling pathway
chemokine activity
cellular response to chemokine
cell junction disassembly
antifungal innate immune response
Functional Enrichment Terms
upregulated
Differential Expression Direction
p_value
0.04
0.03
0.02
0.01
Recall
0.20
0.25
0.30
0.35
0.40
0.45

**Additional file 2: Pathway Enrichment of Pre-cystic (P70) vs Cystic (P21 and P28) Kidney Differential Gene Expression** Bubbleplots showing enrichment for genes uniquely differentially expressed in the A) pre-cystic data set and B) both cystic data sets, where point size is the ratio of query genes to pathway gene set size. D) Heatmap of LFC values for AKI-associated genes for each data set.

**Additional file 3: Pathway Enrichment for Differentially Expressed Genes**

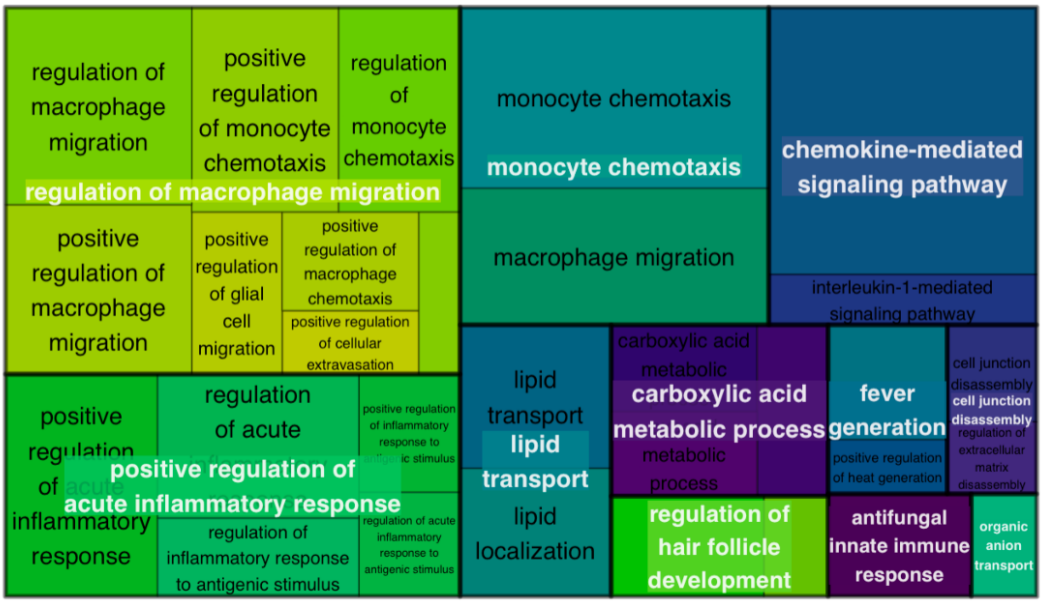
**A**

Enrichment for Pre-cystic P70 DEGs



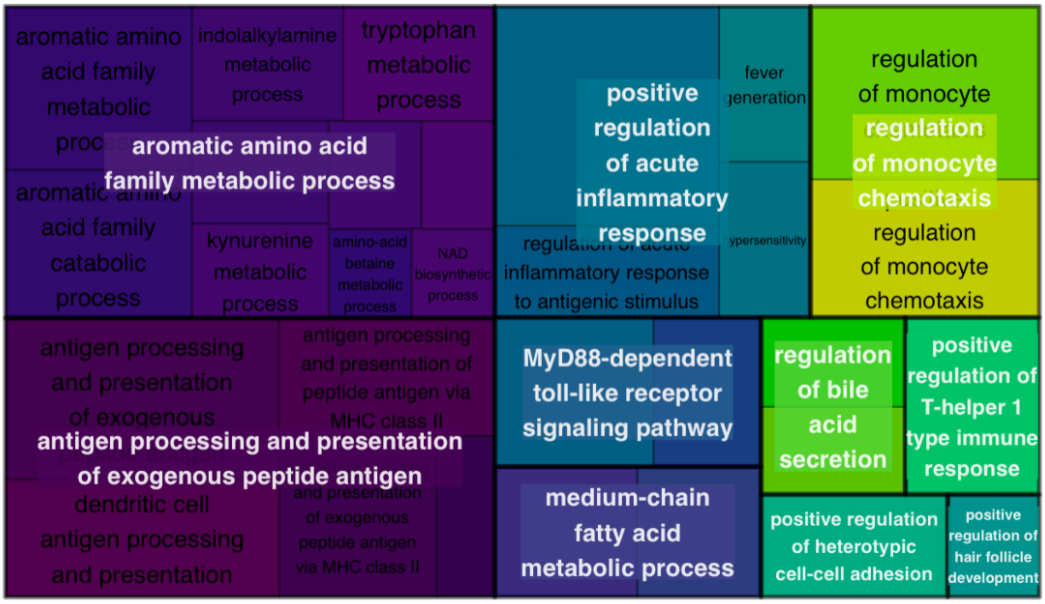

**B**

Enrichment for Cystic P21 DEGs




**C**

Enrichment for Cystic P28 DEGs



**Additional file 3: Differential Expression Analysis** Treemaps of A) pre-cystic P70, B) cystic P21, and C) cystic P28 differentially expressed genes display enrichment nesting, with GO:BP parent terms consisting of largest boxes and enriched child terms reflecting enrichment term size nested within, for each signature.

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