Supplementary Information

Interstitial microRNA miR-214 attenuates inflammation and polycystic kidney disease progression

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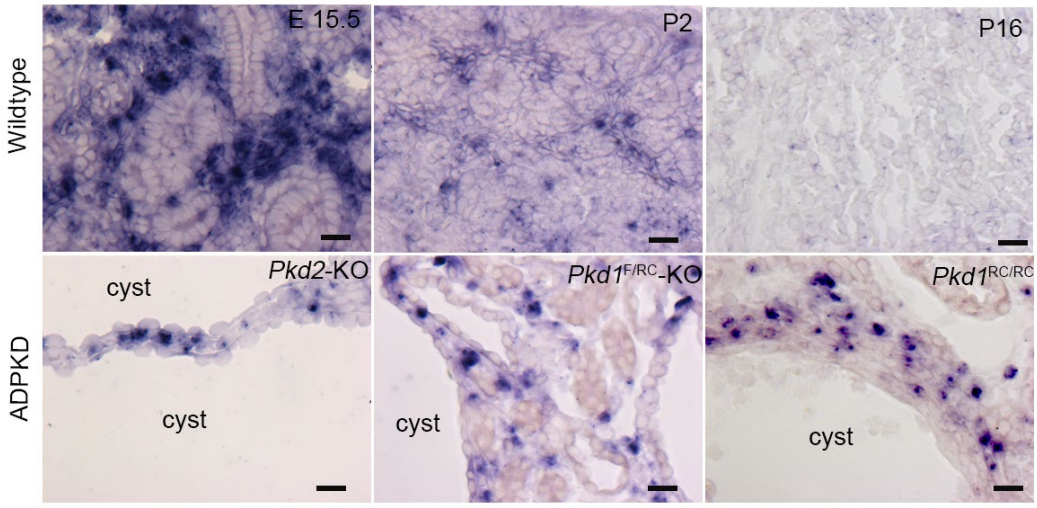
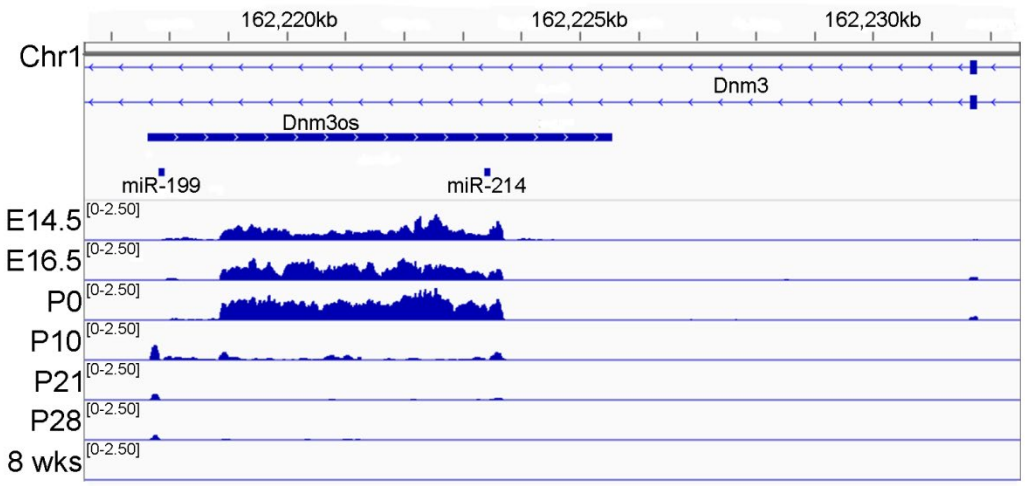
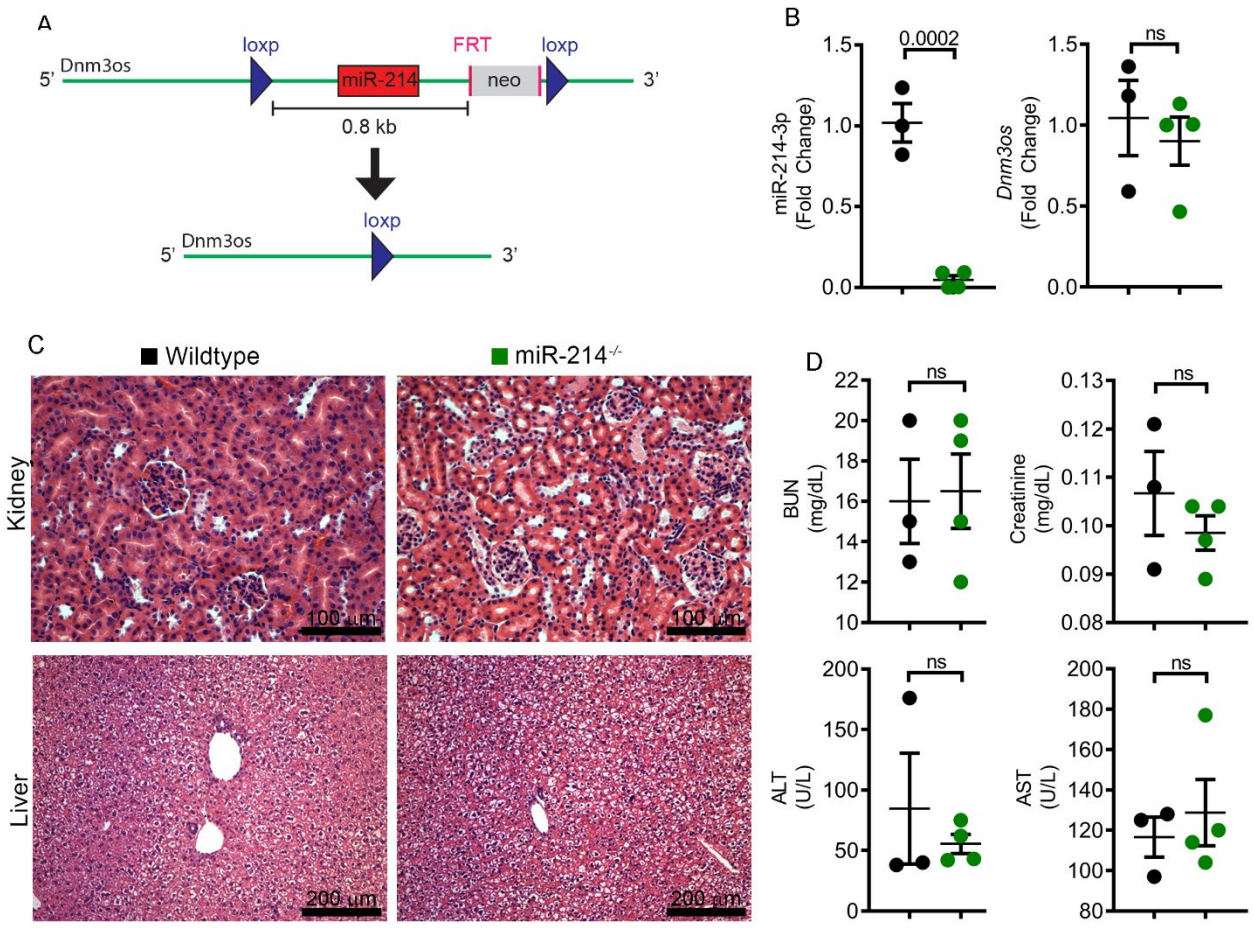


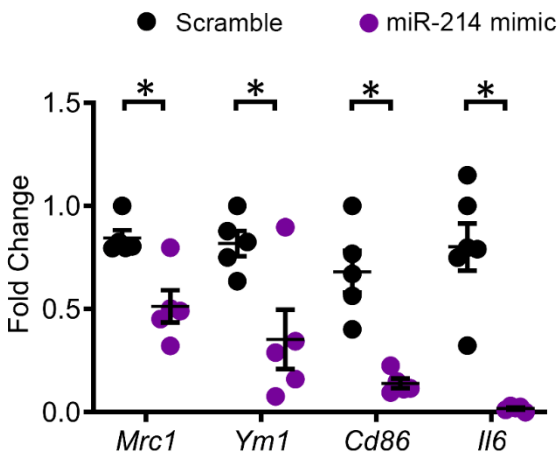
Supplementary Figure 1: *Dnm3os* expression in wildtype and cystic kidneys. *In situ* hybridization showed that *Dnm3os* is expressed in interstitial cells of embryonic mouse kidney (E.15.5) and 2-day-old post-natal kidney (P2). *Dnm3os* signal reduced with kidney maturation in P16 kidneys. *Dnm3os* expression in kidneys of P16 *Pkd2*-KO, P18 *Pkd1*$^{F/RC}$, and 6-month-old *Pkd1*$^{RC/RC}$ mice is shown in the bottom panel. In cystic kidneys, *Dnm3os* expression was observed in cells surrounding the cyst epithelia. Scale bar = 20 um.



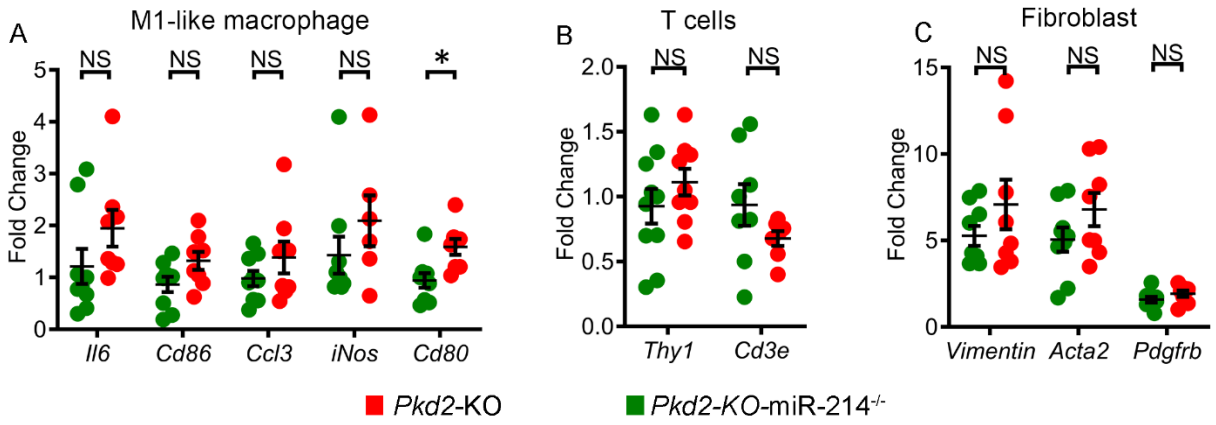
Supplementary Figure 2: *Dnm3os* expression in embryonic and postnatal kidney. RNA-Seq tracks of kidneys from embryonic (E14.5 and E.16.5) and postnatal (P0, P10, P21, P21, and 8 weeks) mice showing that *Dnm3os* expression declines with kidney maturation.



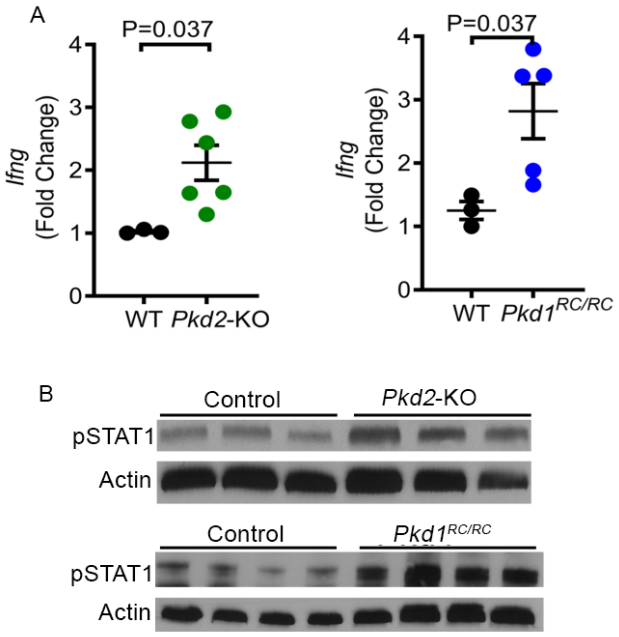
Supplementary Figure 3: miR-214$^{-/-}$ mice are phenotypically normal. **A.** The strategy employed to delete miR-214 from the *Dnm3os* locus is shown. **B.** Q-PCR analysis demonstrating the absence of miR-214 expression in kidneys of miR-214$^{-/-}$ mice (green circles, n=4). miR-214 deletion did not affect *Dnm3os* expression. Black circles denote wildtype mice (n=3). **C.** Hematoxylin and eosin (H&E)-stained sections of wildtype and miR-214$^{-/-}$ kidneys and livers revealed normal histology. **D.** Blood urea nitrogen (BUN), serum creatinine, ALT and AST were unaffected by miR-214 deletion. NS = P>0.05; Error bars indicate SEM.



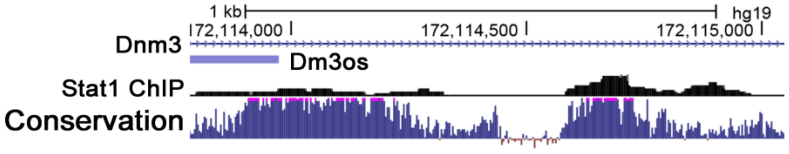
Supplementary Figure 4: miR-214 inhibits the expression of M2-like macrophage markers. RAW 264.7 cells were treated with miR-214 mimics (n=5) or scramble sequence control (n=5-6). Q-PCR analysis showed reduced expression of M2-like macrophage markers in RAW 264.7 cells treated with miR-214 mimics compared to scramble control. * indicates $P<0.05$; Error bars indicate SEM.



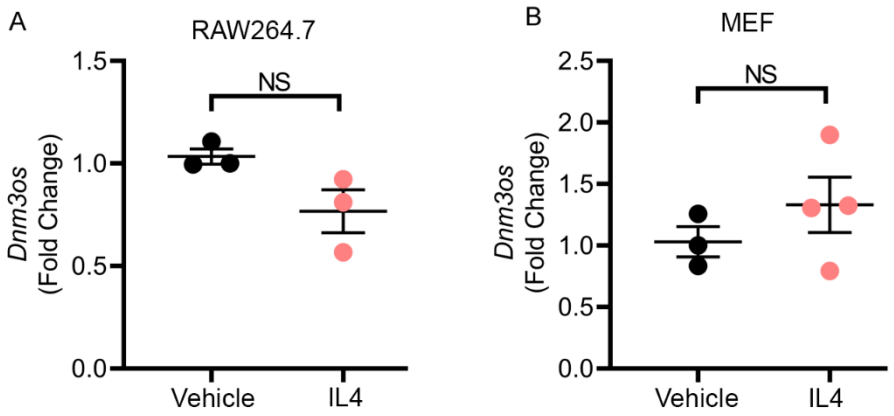
Supplementary Figure 5: Q-PCR analysis showed that the expression of markers of **(A)** M1-like macrophages, **(B)** T-cells, or **(C)** fibroblasts was not different between *Pkd2*-KO kidneys and *Pkd2*-KO;miR-214$^{-/-}$ kidneys. NS = P>0.05; * indicates P<0.05; Error bars indicate SEM.



Supplementary Figure 6: IFN-γ →Stat1 signaling is activated in ADPKD mouse models: A. Q-PCR analysis showing that, compared to kidneys of age-matched wildtype mice (n=3), *Ifng* expression was increased in kidneys of 21-day-old *Pkd2*-KO (n=6) and 6-month-old *Pkd1*$^{RC/RC}$ mice (n=5). **B.** Western blot analysis demonstrated that pStat1 was upregulated in *Pkd2*-KO (n=3) and *Pkd1*$^{RC/RC}$ kidneys (n=4). Error bars indicate SEM.

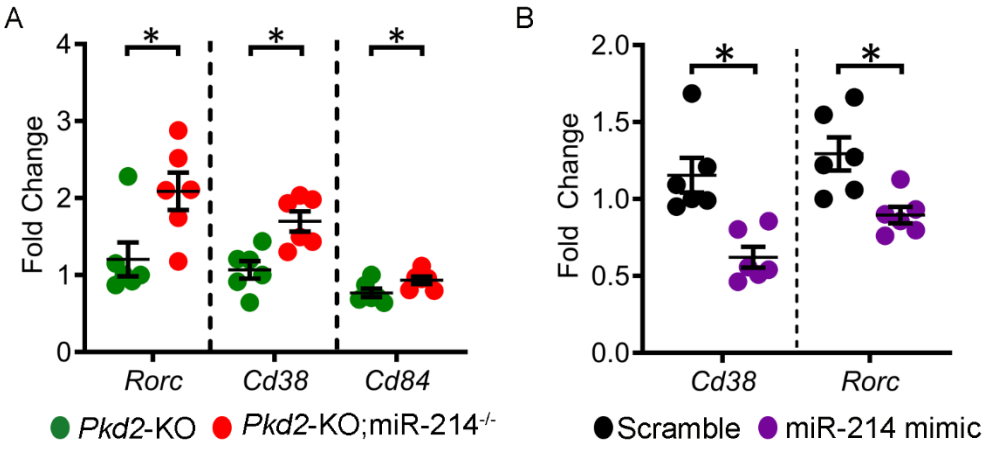


Supplementary Figure 7: Stat1 binds to *Dnm3os* promoter. ChIP-Seq analysis from the ENCODE database showed that Stat1 binds to the promoter region of *Dnm3os* in K562 cells 6 hours after treatment with IFN-γ.



Supplementary Figure 8: IL-4 does not induce *Dnm3os* expression. Q-PCR analysis showing that, compared to vehicle, IL-4 treatment did not promote *Dnm3os* expression in **(A)** RAW264.7 (n=3) or **(B)** MEF cells (n=3). Thus, IL4→STAT3/6 pathway does not regulate *Dnm3os* transcription. NS= $P>0.05$; Error bars indicate SEM.

Supplementary Figure 9: *Tlr4* 3'-UTR targeting. To validate *Tlr4* as a direct miR-214 target, we used CRISPR/Cas9 to generate a monoclonal cell line with targeted deletion of the miR-214 binding site in *Tlr4* 3'-UTR (*Tlr*$^{\Delta miR\text{-}214}$). sgRNAs were designed to induce Cas9 mediated DNA cleavage within the miR-214 binding site in the *Tlr4* 3'UTR. sgRNAs were cloned into pX330mCherry plasmid, which was then transfected into mIMCD3 cells. After 48 hours, mCherry positive cells were FACS sorted and plated individually in a 96 well plate. Colonies were screened for mutation of the *Tlr4* miR-214 binding site using PCR and subsequent sequencing. The miR-214 seed sequence, sgRNA PAM site, and primer locations used to amplify *Tlr4* 3'-UTR is shown. Nucleotides highlighted in red, which included the miR-214 binding site, were deleted in *Tlr4* 3'-UTR mutant cells.



Supplementary Figure 10: Inflammation-related mRNAs are direct targets of miR-214. (A) Q-PCR analysis showing de-repression of miR-214 target genes *Rorc*, *Cd38*, and *Cd84* in *Pkd2*-KO;miR-214$^{-/-}$ (n=6) kidneys compared to *Pkd2*-KO kidneys (n=6). (B) Conversely, compared to cells treated with a mimic with scramble sequence, treatment with miR-214 mimic reduced *Cd38* and *Rorc* expression (n=5). * indicates $P<0.05$; Error bars indicate SEM

Gene		Primer Sequence
Dnm3os	Forward	5'-TGTTGCTGCTGTACCCATAAAG-3'
	Reverse	5'-GTTTGCACTCTCCTTTGGGTTA-3'
Pkd2	Forward	5'-GCGTGGTACCCTCTTGGCAGTT-3'
	Reverse	5'-CACGACAATCACAACATCC-3'
Mrc1	Forward	5'-CTCTGTTCAGCTATTGGACGC-3'
	Reverse	5'-CGGAATTTCTGGGATTCAGCTTC-3'
Ccl2	Forward	5'=TTAAAAACCTGGATCGGAACCAA-3'
	Reverse	5'-GCATTAGCTTCAGATTTACGGGT-3'
Arg1	Forward	5'-CTCCAAGCCAAAGTCCTTAGAG-3'
	Reverse	5'-AGGAGCTGTCATTAGGGACATC-3'
Ym1	Forward	5'-CAGGTCTGGCAATTCTTCTGAA-3'
	Reverse	5'-GTCTTGCTCATGTGTGTAAGTGA-3'
Tlr4	Forward	5'-ATGGCATGGCTTACACCACC-3'
	Reverse	5'-GAGGCCAATTTTGTCTCCACA-3'
Rorc	Forward	5'-GACCCACACCTCACAAATTGA-3'
	Reverse	5'-AGTAGGCCACATTACACTGCT-3'
Cd38	Forward	5'-TCCTCAGCACAGCTGATAACAT-3'
	Reverse	5'-CAGCACCTTCCCTATAATGACC-3'
Il6	Forward	5'-TAGTCCTTCCTACCCCAATTTCC-3'
	Reverse	5'-TTGGTCCTTAGCCACTCCTTC-3'
Cd86	Forward	5'-CTGGACTCTACGACTTCACAATG-3'
	Reverse	5'-AGTTGGCGATCACTGACAGTT-3'
Ccl3	Forward	5'-TTCTCTGTACCATGACACTCTGC-3'
	Reverse	5'-CGTGGAATCTTCCGGCTGTAG-3'
Inos	Forward	5'-GGAGTGACGGCAAACATGACT-3'
	Reverse	5'-TCGATGCACAACTGGGTGAAC-3'
Cd80	Forward	5'-ACCCCCAACATAACTGAGTCT-3'
	Reverse	5'-TTCCAACCAAGAGAAGCGAGG-3'
Thy1	Forward	5'-TGCTCTCAGTCTTGCAGGTG-3'
	Reverse	5'-TGGATGGAGTTATCCTGGTGTT-3'
Cd3e	Forward	5'-GCACGTCAACTCTACACTGGT-3'
	Reverse	5'-ATGCGGTGGAACACTTTCTGG-3'
Vimentin	Forward	5'-CGTCCACACGCACCTACAG-3'
	Reverse	5'-GGGGGATGAGGAATAGAGGCT-3'
Acta2	Forward	5'-GTCCCAGACATCAGGGAGTAA-3'
	Reverse	5'-TCGGATACTTCAGCGTCAGGA-3'
Pdgfrb	Forward	5'-TTCCAGGAGTGATACCAGCTT-3'
	Reverse	5'-AGGGGGCGTGATGACTAGG-3'
Ifng	Forward	5'-ATGAACGCTACACACTGCATC-3'
	Reverse	5'-CCATCCTTTTGCCAGTTCCTC-3'
hDNM3OS	Forward	5'-AACAGTGAGCATCTTCAACT-3'
	Reverse	5'-CAGTTAACTCTGCTCAATTA-3'

Supplementary Table 1: List of primers used in this study.