

Supplemental Figure Legends

Supplemental Figure 1. SIRT1 expression was increased in *Pkd1* mutant kidney tissues as examined by immunohistochemical staining with anti-SIRT1 antibody in postnatal day 7 kidney sections from *Pkd1*^{+/+}:*Ksp-Cre* and *Pkd1*^{flox/flox}:*Ksp-Cre* neonates (A) as well as in kidney sections from normal human kidney and ADPKD kidney (B). Scale bar, 20 μm.

Supplemental Figure 2. TNF-α slightly induces the expression of SIRT1 in *Pkd1* wild type MEK cells, but not that in *Pkd1* heterozygous PH2 cells and mouse IMCD3 cells. Cells were treated with TNF-α (100 ng/ml) for indicated time periods and the expression of SIRT1 was determined by Western blot.

Supplemental Figure 3. Immunohistochemical staining of SIRT1 in kidney sections from *Pkd1*^{flox/flox}:*SIRT1*^{+/+}:*Ksp-Cre* and *Pkd1*^{flox/flox}:*SIRT1*^{flox/flox}:*Ksp-Cre* neonates. Scale bar, 20 μm.

Supplemental Figure 4. (A) Nicotinamide or EX-527 treatment induced cyst lining epithelial cell death in postnatal day 7 (PN7) kidney sections from *Pkd1*^{flox/flox}:*Ksp-Cre* neonates, while apoptosis was rare in PN7 kidney sections from DMSO treated *Pkd1*^{flox/flox}:*Ksp-Cre* neonates as detected with TUNEL assay. Scale bar, 20 μm. (B-D) Treatment with nicotinamide did not further delay cyst growth in *Pkd1*^{flox/flox}:*SIRT1*^{flox/flox}:*Ksp-Cre* mice. Histologic examination (B) of PN7 kidneys from *Pkd1*^{flox/flox}:*SIRT1*^{flox/flox}:*Ksp-Cre* neonates treated with DMSO (*n* = 10) or nicotinamide (*n* = 10), respectively. Scale bar, 2 mm. No significant difference was found on cystic index (C) and kidney weight/body weight (KW/BW) ratios (D) from *Pkd1*^{flox/flox}:*SIRT1*^{flox/flox}:*Ksp-Cre* neonates treated with DMSO (*n* = 10) and nicotinamide (*n* = 10), respectively.

Supplemental Figure 5. Nicotinamide or EX-527 treatment induced cyst lining epithelial cell death in postnatal 28 (PN28) kidney sections from *Pkd1^{nl/nl}* mice, while apoptosis was rare in PN28 kidney sections from DMSO treated *Pkd1^{nl/nl}* mice as detected with TUNEL assay. Scale bar, 20 μ m.

Supplemental Figure 6. Nicotinamide induced apoptosis in *Pkd1* null MEK cells and in PN24 cells but not that in *Pkd1* wild type MEK cells and PH2 cells. **(A)** *Pkd1* wild type and null MEK cells were treated with 10 mM nicotinamide for 24 hours and apoptotic cells were detected by TUNEL assay. **(B)** PH2 and PN24 cells were treated with 40 mM nicotinamide for 40 hours and apoptotic cells were detected by TUNEL assay. Scale bar, 50 μ m.

Supplemental Figure 7. The expression of DHFR, cyclin D3 and cyclin E, which were the downstream targets of E2F1, was upregulated in *Pkd1* mutant PN24 cells compared to that in *Pkd1* heterozygous PH2 cells. After treatment with nicotinamide for 24 hours, the expression of these proteins was downregulated in PN24 cells compared to that in PN24 cells without nicotinamide treatment.

Supplemental Figure 8. Western blot analysis of the expression of p53 and acetyl-p53 in SIRT1 overexpressing mouse IMCD3 cells transfected with wild type SIRT1, mutant SIRT1 H355A or empty vector, respectively, for 48 hours. We found that overexpressing wild type SIRT1 but not mutant SIRT1 H355A decreased the acetylation of p53 but had no effect on the expression of p53.

Supplemental Figure 9. Nicotinamide induces cystic epithelial cell death through p53. **(A)** Western blot analysis of the expression of p53 and active caspase 3 in *Pkd1* mutant PN24 cells transfected with or without p53 siRNA for 24 hours and then treated with or without 10 mM nicotinamide (NIC) for another 24 hours. Active caspase-3 was strikingly decreased in p53 knockdown cells with nicotinamide treatment compared to that in cells with nicotinamide treatment alone. **(B)** Knockdown of p53 with siRNA prevented nicotinamide induced apoptosis as detected by TUNEL assay in *Pkd1* mutant PN24

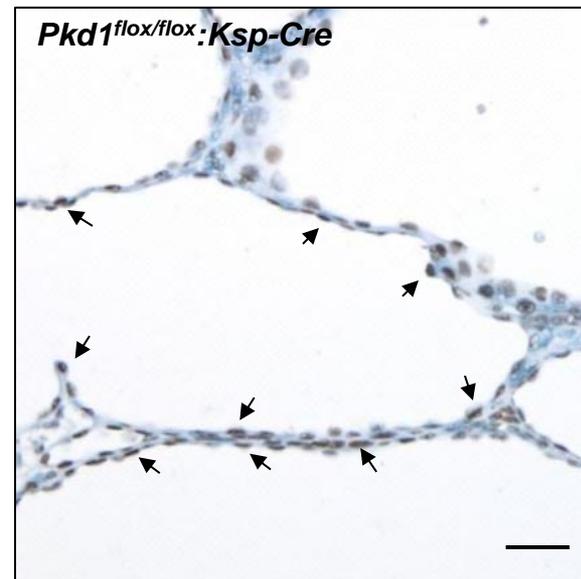
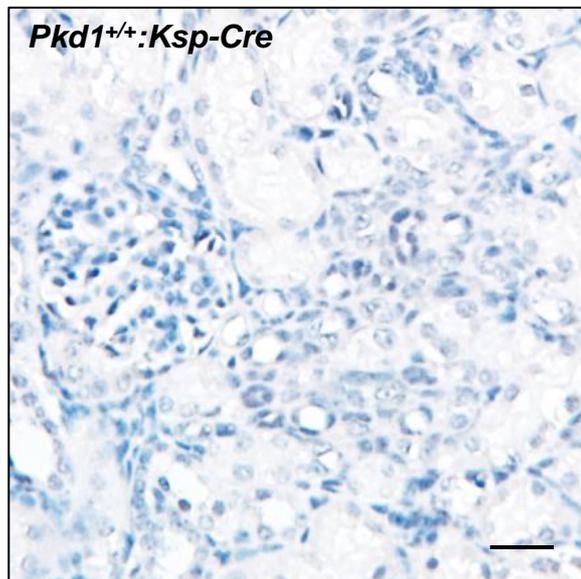
cells, which were transfected with p53 siRNA for 24 hours and then treated with 40 mM nicotinamide for 40 hours. ** $p < 0.01$. Scale bar, 50 μm .

Supplemental Table 1. The survival rate of E18.5 *Pkd1*^{-/-} embryos treated with or without nicotinamide.

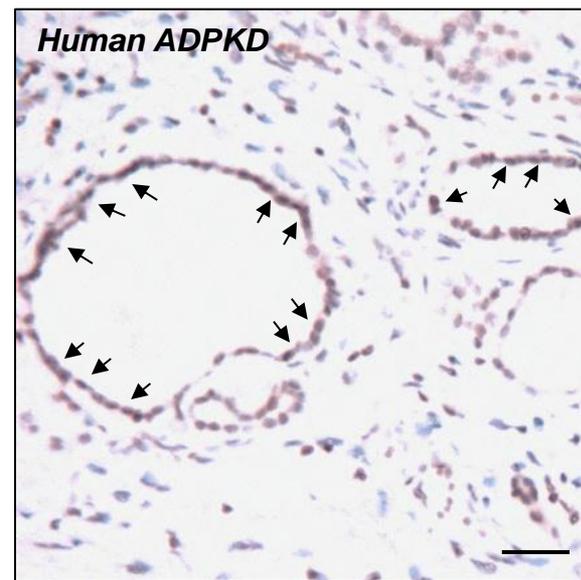
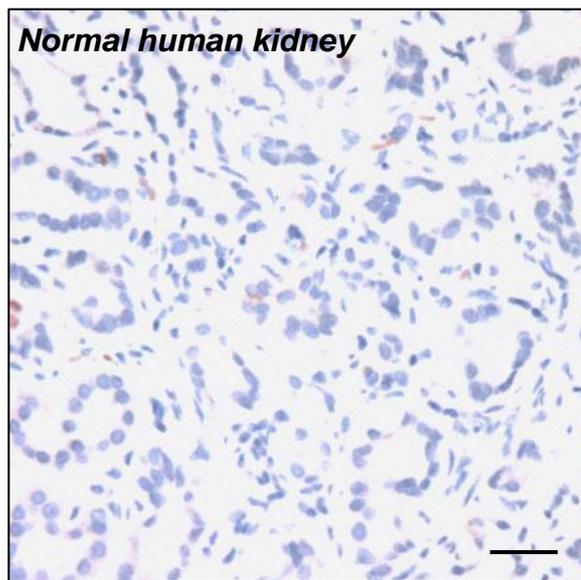
E18.5 Embryos treated with	Total	<i>Pkd1</i> ^{-/-}	<i>Pkd1</i> ^{-/-} (Dead)	<i>Pkd1</i> ^{-/-} (Live)	Survival rate
DMSO	179	45	35	10	22.22%
Nicotinamide	93	22	10	12	54.50%

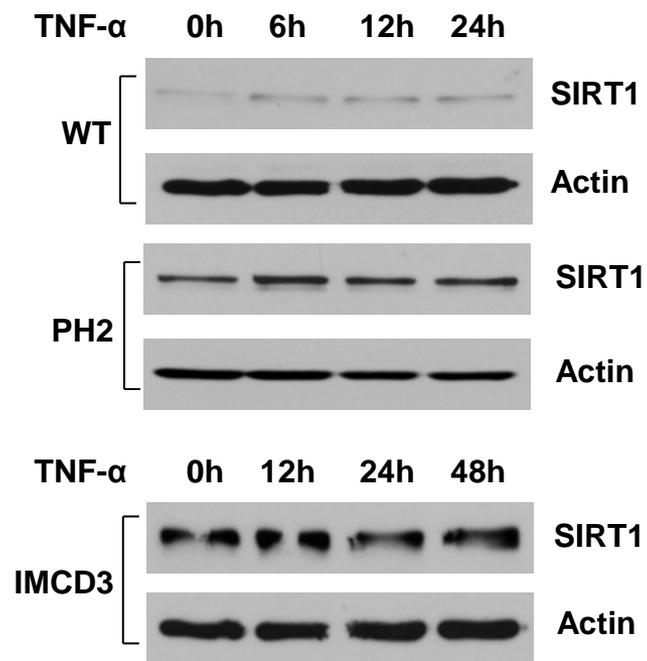
P was determined by the chi-square test. $\chi^2 = 7$; $p < 0.01$.

A

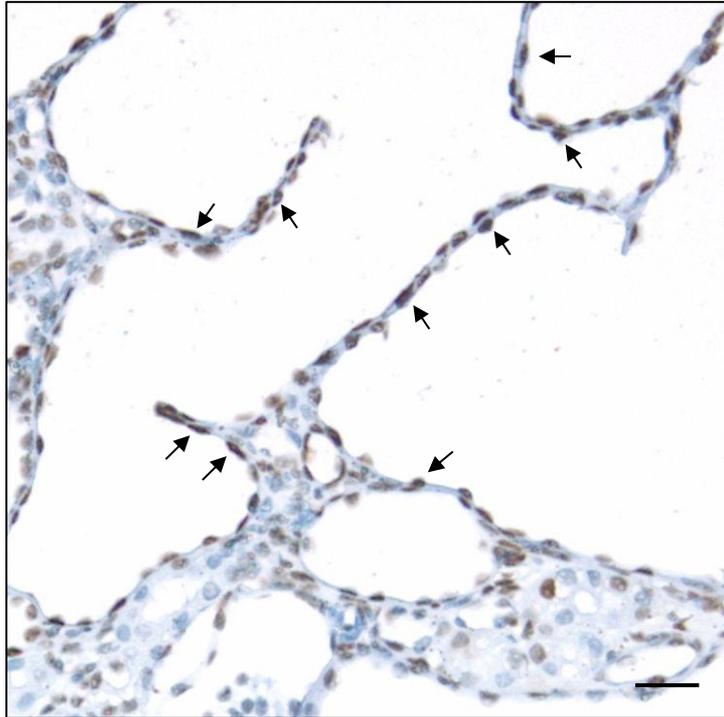


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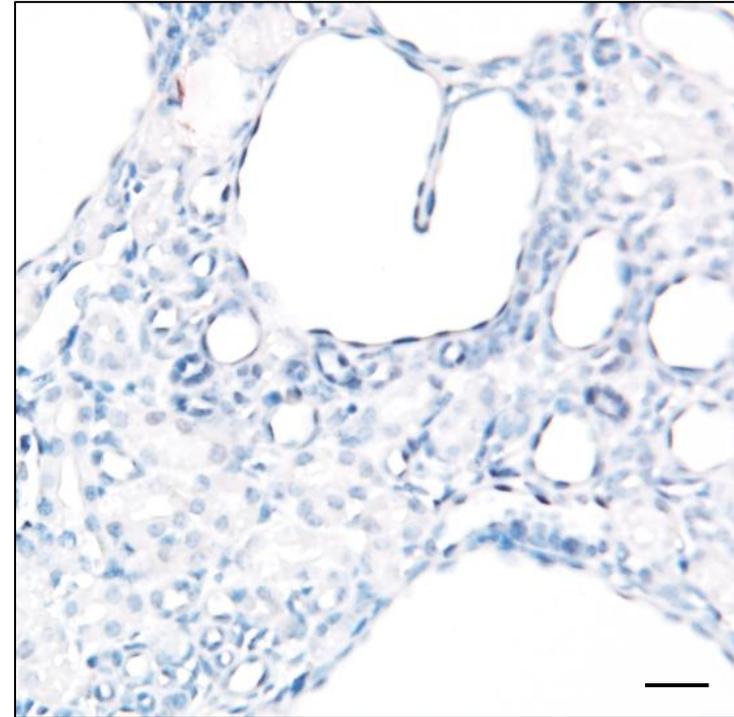


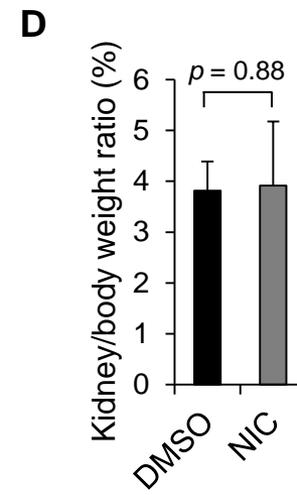
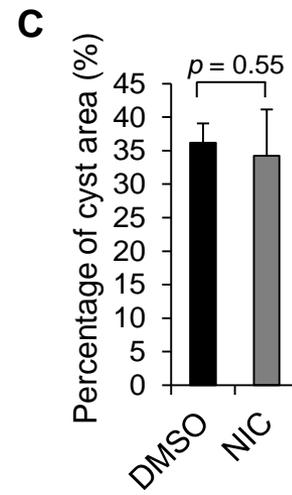
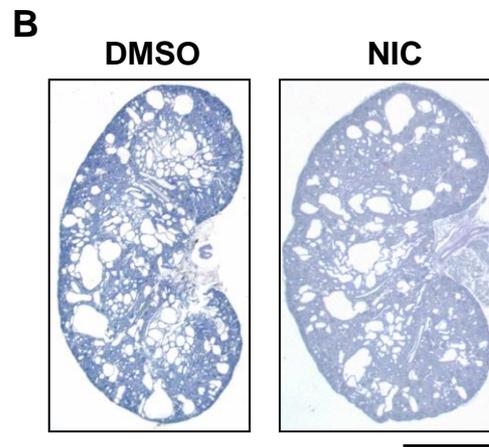
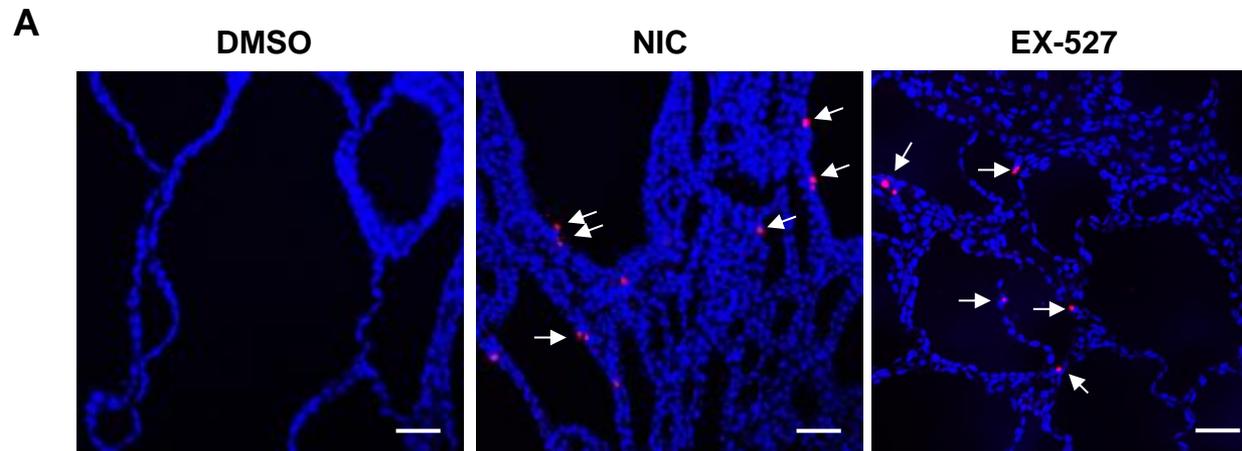


Pkd1^{flox/flox}: *SIRT1*^{+/+}:Ksp-Cre

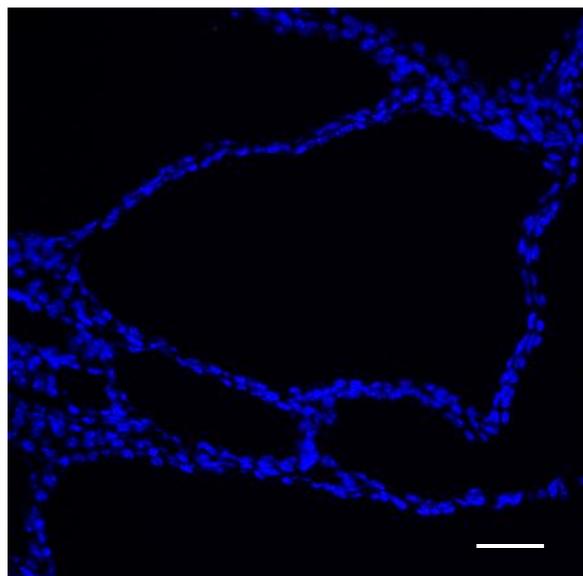


Pkd1^{flox/flox}: *SIRT1*^{flox/flox}:Ksp-Cre

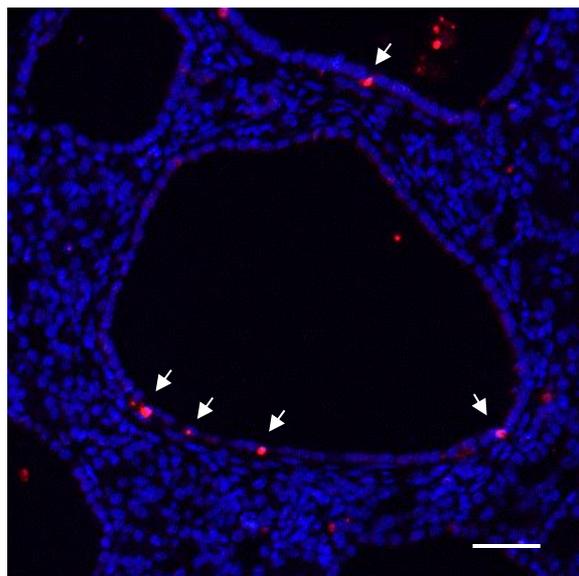




DMSO



NIC



EX-527

