

Supplemental Information

***In vivo* base editing reduces liver cysts in autosomal dominant polycystic kidney disease**

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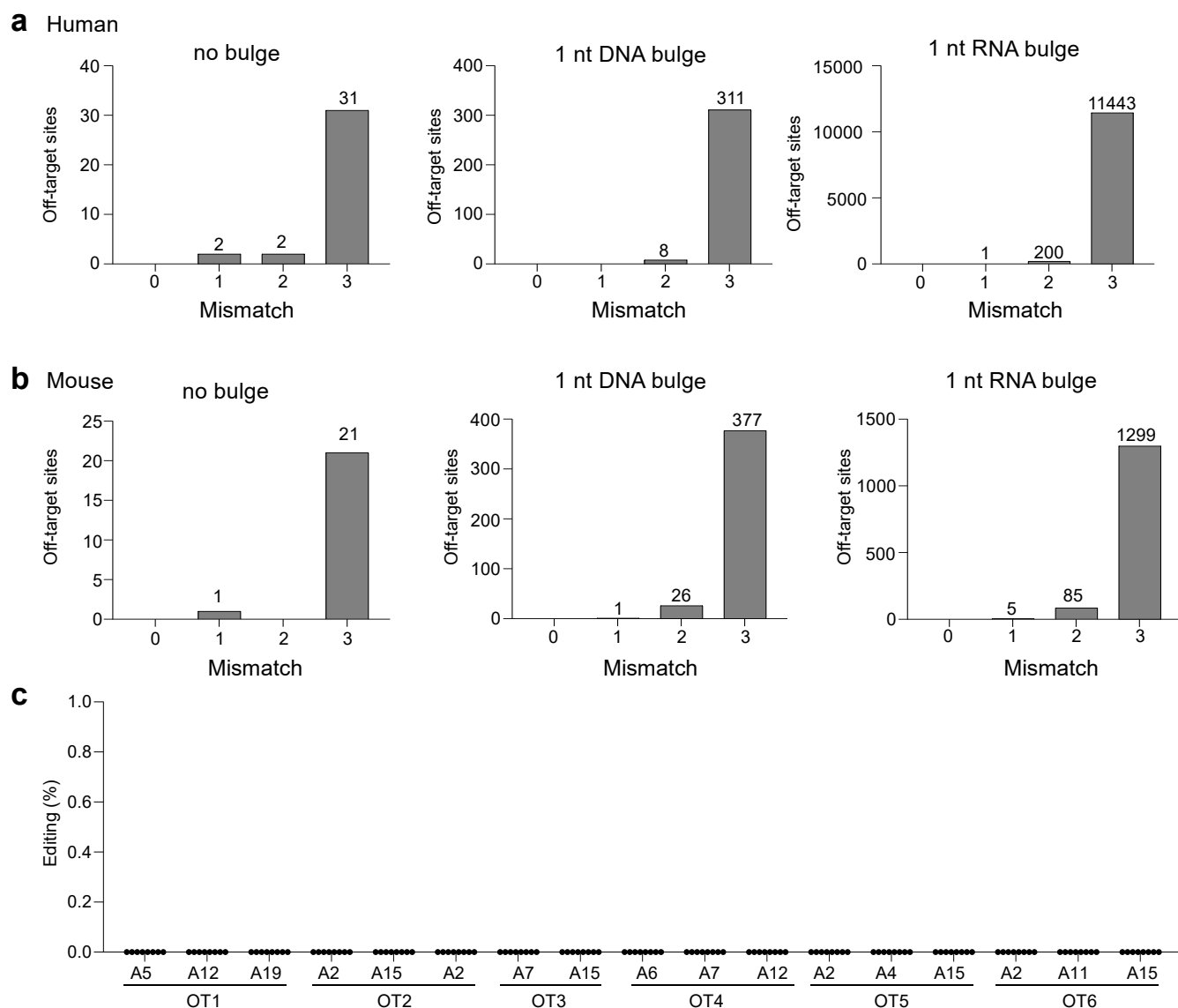


Figure S1: Off-target analysis for RW correcting human and mouse sgRNAs. a, Number of CasOFFinder annotated putative off-target sites in the human genome for sgRNA_20 (for SpCas9 and a NGG PAM). Predicted off-target sites for one and two mismatch sites (without bulges) were identified within *PKD1* pseudogenes. **b,** Number of CasOFFinder annotated putative off-target sites in the mouse genome for sgRNA_45 (for SpCas9 and a NGG PAM). The one site predicted for one mismatch (no bulge) marks the target site. **c,** *In vivo* editing efficiency (y-axis) for the top 6 predicted mouse off-target sites (OT) as determined by targeted amplicon sequencing of gDNA isolated from liver tissue at all possible adenines (A).

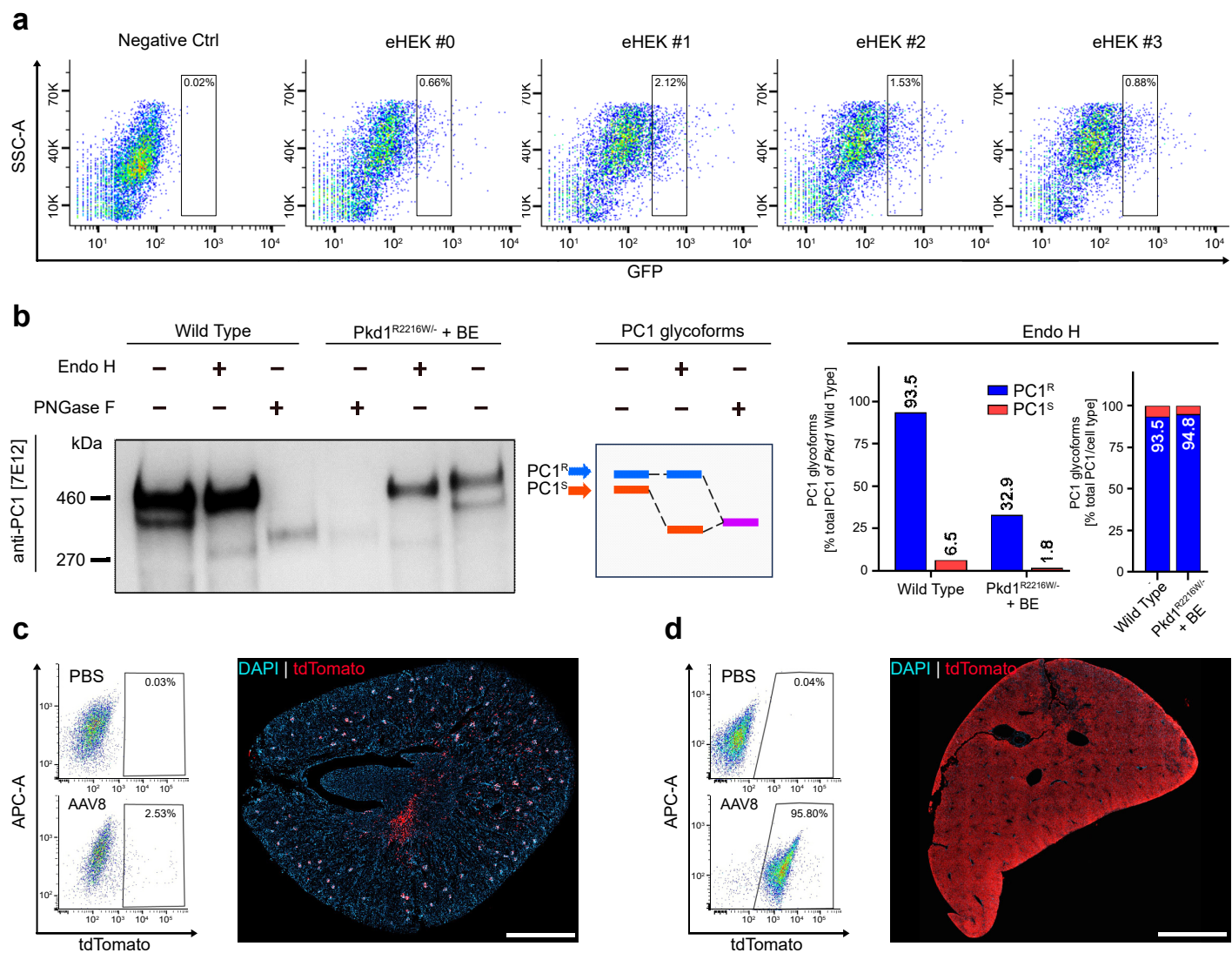


Figure S2: Quality control for engineered HEK293T cell lines and RTEC editing and assessment of *in vivo* AAV8 transduction efficiency in the kidney and liver. **a**, FACS plots illustrating the gating strategy used for sorting the four engineered HEK293T cell lines (eHEK #0-#3). Transfection and sorting were performed on the same day, applying identical gates to select for cells with medium-to-low expression levels to ensure consistent and low copy number integration across all four lines. **b**, Deglycosylation analysis of PC1 in base edited and control RTEC cells reveals comparable amounts of mature Endo H-resistant (PC1^R; blue) and immature Endo H-sensitive (PC1^S, red) glycoforms. PNGase F served as a positive control for complete deglycosylation (violet). **c**, Systemic AAV8-Cre delivery (1x10¹³ VG/kg) in Ai14 reporter mice show limited transduction in the kidney. Left panel: flow cytometry analysis of tdTomato expression in dissociated kidney cells. Top left plot shows kidney cells from a PBS-injected control mouse, while the bottom left plot shows kidney cells from an AAV8-Cre-injected mouse. Right panel: representative immunofluorescence image of a coronal kidney section from an AAV8-Cre-injected mouse. tdTomato expression is shown in red; nuclei are counterstained with DAPI (turquoise). Scale bar = 1000 μm. **d**, Systemic AAV8-Cre delivery (1x10¹³ VG/kg) in Ai14 reporter mice show successful transduction in the liver. Left panel: flow cytometry analysis of tdTomato expression in dissociated liver cells. Top left plot shows liver cells from a PBS-injected control mouse; the bottom left plot shows liver cells from an AAV8-Cre-injected mouse. Right panel: representative immunofluorescence image of a liver section from an AAV8-Cre-injected mouse. tdTomato expression is shown in red; nuclei are counterstained with DAPI (turquoise). Scale bar = 1000 μm.

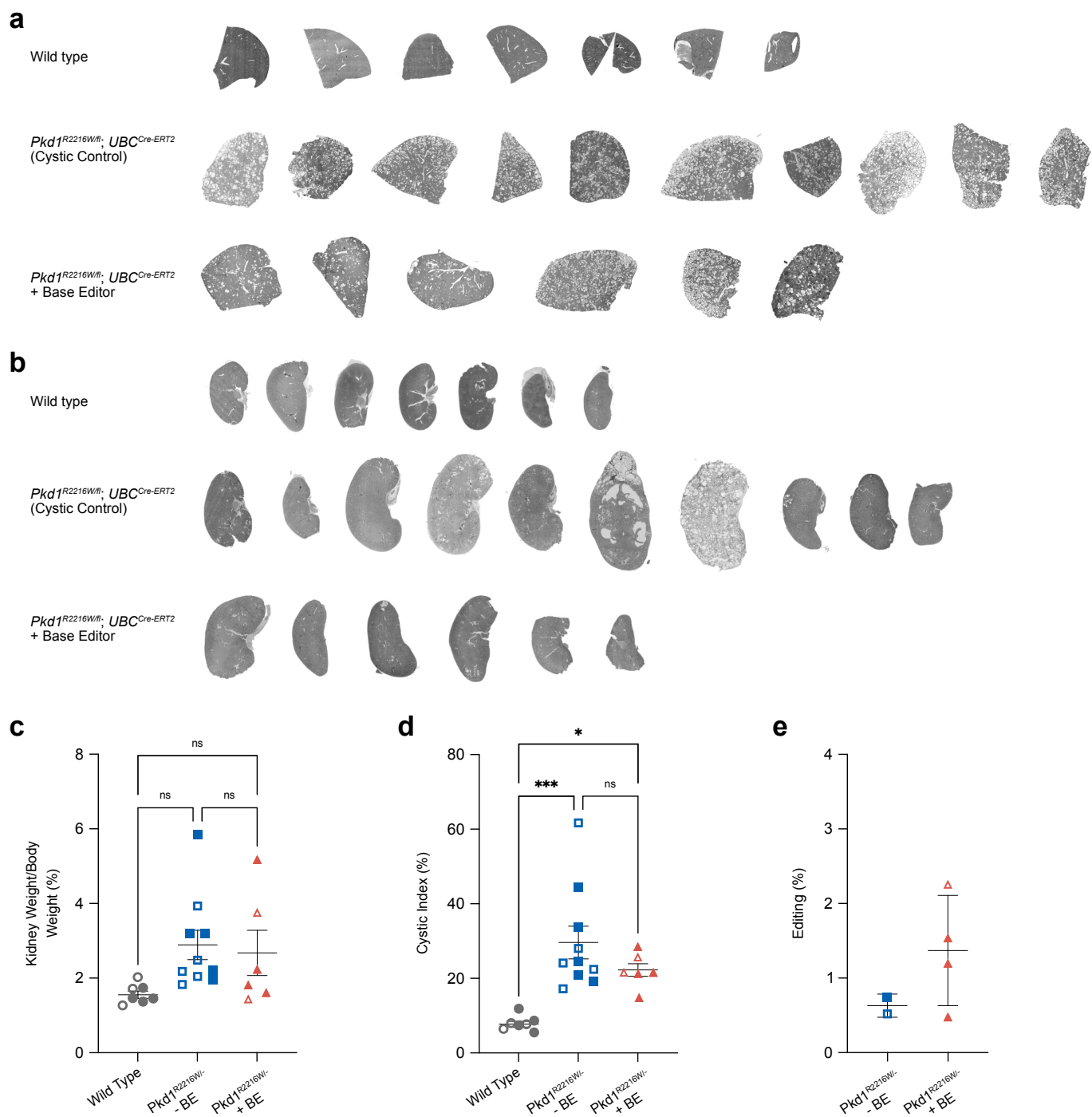


Figure S3: *In vivo* base editing shows no influence on kidney phenotype in the RW knock-in mouse model. **a**, Microscopic overview images of liver tissue from the indicated conditions from all animals. **b**, Microscopic overview images of kidney tissue from the indicated conditions from all animals. **c, d**, Quantification of kidney-to-body weight ratios (**c**) and kidney cystic indices (**d**) between wild-type mice (n=7), untreated RW knock-in mice (n=10) and base editor (BE) treated RW knock-in mice (n=6). **e**, Quantification of editing efficiency in gDNA isolated from the kidney of the untreated RW knock-in mice (n=2) and base editor (BE) treated RW knock-in mice (n=4). **c, d, e**, Data points represent independent biological replicates with means \pm SD, ***p < 0.001, *p < 0.05, ns: not significant. Filled data points indicate male mice, hollow once female mice.

Table S1: Primer sequences for targeted amplicon sequencing (TAS) and long-range PCR (LR-PCR). For TAS, PCR-1 was used for target locus-specific amplification and PCR-2 for addition of barcodes and Illumina adapter sequences. All sequences are shown in the 5'→3' orientation.

Table S1		
Primer Name	Primer Sequence (5'-3')	Used for
16SrRNA_63f fwd/fwd_TAS	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCAGGCCTAACACATGCAAGTC	PCR-1
16SrRNA_1387f rev/rev_TAS	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCCTTGTAACCTCGCCCC	PCR-1
16SrRNA_1387r rev/fwd_TAS	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGCGGAGTGTAACAAGGC	PCR-1
AmpR rev_TAS	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTATGTGGCGCGTATTAT	PCR-1
AmpR fwd_TAS	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATAAATACCGGCCACATAGC	PCR-1
LucNrev rev_TAS	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGAGAGCAACTGCATAAGG	PCR-1
LucNrev fwd_TAS	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTTATGCAGTTGCTCTCC	PCR-1
Partial-P5 rev_TAS	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCTCGGTGTCGCCGTATCATT	PCR-1
Partial-P5 fwd_TAS	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAAATGATACGGCGACCCAGAGA	PCR-1
pUC19-fwd/rev_TAS	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTTAGACGTCAAGTGCGCACTT	PCR-1
pUC19-fwd/fwd_TAS	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAAAGTCCACCTGACGTCTAAG	PCR-1
TSO enrichment rev_TAS	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAAGCAGTGGTATCAACGCAGAGT	PCR-1
TSO enrichment fwd_TAS	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACTCTGCGTTGATACCACTGCTT	PCR-1
CMV Forward rev_TAS	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAGCCTACCGCCCATTTTGGC	PCR-1
CMV Forward fwd_TAS	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCGAAATGGGCGGTAGGCGTG	PCR-1
VR Primer rev_TAS	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTCACTCAAAGGCGGTAAT	PCR-1
VR Primer fwd_TAS	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATTACCGCCTTTGAGTAGGC	PCR-1
oAl411_LucNrev_fwd	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTATGCAGTTGCTCTCCGCACT	PCR-1
oAl411_AmpR_rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTATGTGGCGCGGTATTATCGTG	PCR-1
oAl411_AmpR_fwd	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCAGATAAATCCCGCCACATA	PCR-1
oAl411_16S rRNA Primer 1387r_rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTGTACACTCCGCCACCGT	PCR-1
oAl411_16S rRNA Primer 1387r_fwd	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACGGTGGGCGGAGTGTAACA	PCR-1
oAl411_16S rRNA Primer 63f_rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCATGTGTTAGGCCTGTCTCG	PCR-1
oAl412_Tso_Enrich_fwd	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTTGATACCACTGCTTCACG	PCR-1
oAl412_pUC19_rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTAGACGTCAAGTGCGCACTTCT	PCR-1
oAl412_LucNrev_fwd	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTGGCTGCCTTATGCAGTT	PCR-1
oAl412_AmpR_rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCAGCCGTGAGCTATGTGG	PCR-1
oAl412_AmpR_fwd	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCACATAGCTCACGGCTGA	PCR-1
oAl412_16S rRNA Primer 1387r_rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTGTGCCTTGTACACTCCG	PCR-1
oAl412_16S rRNA Primer 1387r_fwd	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCGGAGTGTAACAAGGCACAGC	PCR-1
oAl412_16S rRNA Primer 63f_rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGTGTTAGGCCTGACCCAC	PCR-1
oAl412_16S rRNA Primer 63f_fwd	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGGGGTCAGGCCTAACACA	PCR-1
oAl412_mCherry_rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTCTGCATTACGGGGCGTCC	PCR-1
oAl413_LucNrev_fwd	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTATGCAGTTGCTCTCCGGAT	PCR-1
oAl413_AmpR_rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAAGGCTTCAACGACGTATGTG	PCR-1
oAl413_AmpR_fwd	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCACATAGCTGCGTGAAGCCTT	PCR-1
oAl413_16S rRNA 1387_rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTAAGACGCCTTGTACACTCCG	PCR-1
oAl413_16S rRNA 1387_fwd	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCGGAGTGTAACAAGGCGTCTTA	PCR-1
oAl413_16S rRNA 63f_rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGAAGACTTGATGTGTTAGGC	PCR-1
oAl413_16S rRNA 63f_fwd	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCCTAACACATGCAAGTCTCC	PCR-1
oAl413_mCherry_rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTCTTCTGCATTACGGGGCAC	PCR-1
oAl414_LucNrev_fwd	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACGGCCTTATGCAGTTGCT	PCR-1
oAl414_AmpR_rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACAGCTATGTGGCGCGGTAT	PCR-1
oAl414_AmpR_fwd	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATAACCGGCCACATAGCTGT	PCR-1
oAl414_16S rRNA 1387_rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGAAGCCTTGTACACTCCGC	PCR-1
oAl414_16S rRNA 1387_fwd	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCGGAGTGTAACAAGGCTTCA	PCR-1
oAl414_16S rRNA Primer 63f_rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCATGTGTTAGGCCTGACCGT	PCR-1
oAl414_16S rRNA Primer 63f_fwd	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCAGGTCAAGCCTAACACATG	PCR-1
oAl414_mCherry_rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCTTCTGCATTACGGGGCTC	PCR-1
Pkd1_TAS R2216W Fwd	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGTAGGAGCCTGAAGTGGA	PCR-1
Pkd1_TAS R2216W Rev	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCTGCGTGTCTGACCATAACC	PCR-1
N701	CAAGCAGAAGACGGCATAACGAGATTCGCCTTAGTCTCGTGGGCTC	PCR-2
N702	CAAGCAGAAGACGGCATAACGAGATCTAGTACGGTCTCGTGGGCTC	PCR-2
N703	CAAGCAGAAGACGGCATAACGAGATTTCTGCCT GTCTCGTGGGCTC	PCR-2
N704	CAAGCAGAAGACGGCATAACGAGATGCTCAGGAGTCTCGTGGGCTC	PCR-2
N705	CAAGCAGAAGACGGCATAACGAGATAGGAGTCCGTCTCGTGGGCTC	PCR-2
N706	CAAGCAGAAGACGGCATAACGAGATCATGCCTAGTCTCGTGGGCTC	PCR-2
N707	CAAGCAGAAGACGGCATAACGAGATGTAGAGAGGTCTCGTGGGCTC	PCR-2
N710	CAAGCAGAAGACGGCATAACGAGATCAGCCTCGGTCTCGTGGGCTC	PCR-2
N711	CAAGCAGAAGACGGCATAACGAGATTTGCCTTGTCTCGTGGGCTC	PCR-2
N712	CAAGCAGAAGACGGCATAACGAGATTCCTCTACGTCTCGTGGGCTC	PCR-2
N714	CAAGCAGAAGACGGCATAACGAGATTCATGAGCGTCTCGTGGGCTC	PCR-2
N715	CAAGCAGAAGACGGCATAACGAGATCCTGAGATGTCTCGTGGGCTC	PCR-2
S502	AATGATACGGCGACCAACCGAGATCTACACCTCTCTATTCTCGGCGAGCG	PCR-2
S503	AATGATACGGCGACCAACCGAGATCTACACTATCCTCTTCTCGGCGAGCG	PCR-2
S505	AATGATACGGCGACCAACCGAGATCTACACGTAAGGAGTCGTGGCGAGCG	PCR-2
S506	AATGATACGGCGACCAACCGAGATCTACACTGCAATATCGTCGGCAGCG	PCR-2
S507	AATGATACGGCGACCAACCGAGATCTACACAAGGAGTATCGTCGGCAGCG	PCR-2

Table S1 (continued)

Primer Name	Primer Sequence (5'-3')	Used for
S508	AATGATACGGCGACCACCGAGATCTACACCTAAGCCTTCGTGGGCAGCG	PCR-2
S510	AATGATACGGCGACCACCGAGATCTACACCGTCTAATTCGTGGGCAGCG	PCR-2
S511	AATGATACGGCGACCACCGAGATCTACACTCTCTCCGTCTCGTGGGCAGCG	PCR-2
N716	CAAGCAGAAGACGGCATACGAGATTAGCGAGTGTCTCGTGGGCTCGG	PCR-2
N718	CAAGCAGAAGACGGCATACGAGATGTAGTCTCCGTCTCGTGGGCTCGG	PCR-2
N719	CAAGCAGAAGACGGCATACGAGATTACTACGCGTCTCGTGGGCTCGG	PCR-2
N720	CAAGCAGAAGACGGCATACGAGATAGGCTCCGGTCTCGTGGGCTCGG	PCR-2
N721	CAAGCAGAAGACGGCATACGAGATGCAGCGTAGTCTCGTGGGCTCGG	PCR-2
N722	CAAGCAGAAGACGGCATACGAGATCTGCGCATGTCTCGTGGGCTCGG	PCR-2
N723	CAAGCAGAAGACGGCATACGAGATGAGCGTAGTCTCGTGGGCTCGG	PCR-2
N724	CAAGCAGAAGACGGCATACGAGATCGCTCAGTGTCTCGTGGGCTCGG	PCR-2
N726	CAAGCAGAAGACGGCATACGAGATGTCTTAGGGTCTCGTGGGCTCGG	PCR-2
N727	CAAGCAGAAGACGGCATACGAGATACTGATCGGTCTCGTGGGCTCGG	PCR-2
N728	CAAGCAGAAGACGGCATACGAGATTAGCTGCGTCTCGTGGGCTCGG	PCR-2
N729	CAAGCAGAAGACGGCATACGAGATGACGTGAGTCTCGTGGGCTCGG	PCR-2
S513	AATGATACGGCGACCACCGAGATCTACACTCGACTAGTCTGGCAGCGTC	PCR-2
S515	AATGATACGGCGACCACCGAGATCTACACTTCTAGCTTCGTGGCAGCGTC	PCR-2
S516	AATGATACGGCGACCACCGAGATCTACACCCTAGAGTTCGTGGCAGCGTC	PCR-2
S517	AATGATACGGCGACCACCGAGATCTACACGCGTAAGATCGTCGGCAGCGTC	PCR-2
S518	AATGATACGGCGACCACCGAGATCTACACCTATTAAGTCGTGGCAGCGTC	PCR-2
S520	AATGATACGGCGACCACCGAGATCTACACAAGGCTATTCGTGGCAGCGTC	PCR-2
S521	AATGATACGGCGACCACCGAGATCTACACGAGCCTTATCGTCGGCAGCGTC	PCR-2
S522	AATGATACGGCGACCACCGAGATCTACACTTATGCGATCGTCGGCAGCGTC	PCR-2
PKD1_c.9320_Exon25_fwd	TCGTGCCCCAAGCCATGT	LR-PCR
PKD1_c.9320_exon34_rev	AGCCGGACACTCACAGGCT	LR-PCR

Table S2: Guide RNA (gRNA) sequences used for correction of the indicated variants via base editing.

Sequences are shown in the 5'→3' orientation, excluding the PAM sequence.

gRNA Name	Mutation	gRNA Sequence 5'-3' without PAM	Species	Gene
gRNA_1	c.412C>T	CCCATCACGGCAGCCACGCC	human	<i>PKD1</i>
gRNA_2	c.1202-9G>A	TTCCACCAAGTCTCCAGCGG	human	<i>PKD1</i>
gRNA_3	c.1261C>T	CAGGCAGTAGCAGTGCCCGT	human	<i>PKD1</i>
gRNA_4	c.1816C>T	GCAGCCACAGCTGGGCGGGC	human	<i>PKD1</i>
gRNA_5	c.1831C>T	GGAGCCAGTACACCTGCAGC	human	<i>PKD1</i>
gRNA_6	c.2839C>T	TCCCTACAGTACACGGGCCT	human	<i>PKD1</i>
gRNA_7	c.3037G>A	TAACCATGGAGCGGATGAAC	human	<i>PKD1</i>
gRNA_8	c.3490G>A	GACTTCAGGGACGGCTCCCC	human	<i>PKD1</i>
gRNA_9	c.3955G>A	TCACCAGGAACCCGGCCAC	human	<i>PKD1</i>
gRNA_10	c.3984G>A	TTCGACTGAACCTTCGGGGA	human	<i>PKD1</i>
gRNA_11	c.4322A>G	CAAGACAGGAGCCTGGGTCT	human	<i>PKD1</i>
gRNA_12	c.4906C>T	CCACCTACAGCCCTCTATG	human	<i>PKD1</i>
gRNA_13	c.5086C>T	CAGCTACACATGGTAGGTGC	human	<i>PKD1</i>
gRNA_14	c.5477G>A	CTTTTAGGGGCAGCTGGCCA	human	<i>PKD1</i>
gRNA_15	c.6011A>G	CCAGGCGCAGGCGACCCGAG	human	<i>PKD1</i>
gRNA_16	c.6031C>T	CTTCTACAGCGAGAAGTACC	human	<i>PKD1</i>
gRNA_17	c.6406C>T	CGTGGCCTACGCCACGAAGA	human	<i>PKD1</i>
gRNA_18	c.6472C>T	CACCTACAGGGGCAGGACCA	human	<i>PKD1</i>
gRNA_19	c.6487C>T	ATCACCGCATCAGCACCTGC	human	<i>PKD1</i>
gRNA_20	c.6658C>T	AGCCACGGCAGCACCAGCCG	human	<i>PKD1</i>
gRNA_21	c.6743A>G	TCACACTGGCCTGGATGCTC	human	<i>PKD1</i>
gRNA_22	c.6916-9G>A	CTCAGCCTGCAGAGGGAGGC	human	<i>PKD1</i>
gRNA_23	c.7244T>C	TGCACGCACGTTACGAACA	human	<i>PKD1</i>
gRNA_24	c.7271C>T	GCCCATGGATGTGGTGGTCT	human	<i>PKD1</i>
gRNA_25	c.7915C>T	GGGCTCAGTGCTGCCGCTCG	human	<i>PKD1</i>
gRNA_26	c.7949T>C	GAGACTCCGGTGTCCTGAG	human	<i>PKD1</i>
gRNA_27	c.7984C>T	CTGCTAGATGTCATCCACAG	human	<i>PKD1</i>
gRNA_28	c.8311G>A	CAACAAGGAGCCCCTGACGC	human	<i>PKD1</i>
gRNA_29	c.8381T>C	AGCCTGCCGTGCTATGGCGG	human	<i>PKD1</i>
gRNA_30	c.8558T>C	ATGGCATCCCAGACACAGGC	human	<i>PKD1</i>
gRNA_31	c.8593C>T	CAGCCACTCGATGGGGATCT	human	<i>PKD1</i>
gRNA_32	c.8929A>G	AAGGCGTAGGGCCGGTGGTC	human	<i>PKD1</i>
gRNA_33	c.9335G>A	CTTTCTATGGGCAGCGGGGC	human	<i>PKD1</i>
gRNA_34	c.9340C>T	CCGCTACCCACAGAAAGGGA	human	<i>PKD1</i>
gRNA_35	c.9547C>T	CCACACTCAGATCTTCCACA	human	<i>PKD1</i>
gRNA_36	c.9851G>A	CCTGCTACGTTCTCCTCATC	human	<i>PKD1</i>
gRNA_37	c.10290G>A	CCATTGTAGGTAGCAATCTG	human	<i>PKD1</i>
gRNA_38	c.10945C>T	CCGTGGGATGGCCGTACGCG	human	<i>PKD1</i>
gRNA_39	c.10951G>A	CCACAGCTTTGCACTCTTCC	human	<i>PKD1</i>
gRNA_40	c.11156+3A>G	GCCCGCACCGCGTGATGGCC	human	<i>PKD1</i>
gRNA_41	c.11384G>A	GACGTAGGCCTATTACGCGC	human	<i>PKD1</i>
gRNA_42	c.12010C>T	TGCTAGGCAGCCTGCGGACG	human	<i>PKD1</i>
gRNA_43	c.12061C>T	CAGAGCTCAGCATAATGTCT	human	<i>PKD1</i>
gRNA_44	c.12124C>T	CAGCTAGGCGTAGGCTACCC	human	<i>PKD1</i>
gRNA_45	c.6646C>T_1	AGCCATGGCACCACCAGCTG	mouse	<i>Pkd1</i>
gRNA_46	c.6646C>T_2	CAGCCATGGCACCACCAGCT	mouse	<i>Pkd1</i>
gRNA_47	c.6646C>T_3	CCAGCCATGGCACCACCAGC	mouse	<i>Pkd1</i>
gRNA_48	c.6646C>T_4	GCCATGGCACCACCAGCTGG	mouse	<i>Pkd1</i>