SUPPLEMENTARY RESEARCH DATA

Supplemental Figure 1. PC1 knockdown induces changes in ploidy independently of the siRNA delivery system and in different cell lines. (**A**) The pVIRHD/P/siLuc and pVIRHD/P/siPKD1$_{3297}$ plasmids were transfected into IMCD3 using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). Twenty-four hours following transfection, cells were selected in puromycin (15μg/ml) for 48 hours and on day 4, cell cycle analysis was performed. Anti-*Pkd1* but not control anti-Luc siRNA-expressing plasmid induced peaks of abnormal ploidy similar to those observed upon delivery of the same siRNAs by viral transduction (compare with Fig. 1). (**B**) Cell cycle analysis was performed in Madin-Darby canine kidney (MDCK) cells at the indicated days (numbers on top panel) following transduction with the control VIRHD/P/siLuc or VIRHD/P/siPKD1$_{3211}$ lentivectors, as described in Materials and Methods. The normal cell cycle peaks and fraction of cells with abnormal ploidy (>4n) are indicated. Similarly to what observed in IMCD3 cells, massive changes of cell ploidy were clearly visible immediately after PC1 knockdown in MDCK cells but became undetectable at later time points despite the stable suppression of PC1 expression (1).

Supplemental Figure 2. The expression of an exogenous human *PKD1* gene rescues the normal phenotype during PC1 knockdown in 293T cells. (**A**) 293T cells were transfected with the control VVE/BBW empty vector or with the VVE/BBW/hPKD1 vector expressing a transcript comprising only the *PKD1* coding region in a dicistronic cassette that allows the co-expression of the blasticidin resistance gene (see Supplementary Methods). Sixteen hours post-transfection, the cells were transduced with the anti mouse *Pkd1* VIRHD/P/siPKD1$_{12632}$ lentivector (which we have shown in figure 2 to have no effects on the human *PKD1* gene) as a control or with the anti-human *PKD1* VIRHD/P/siPKD1$_{13333}$ lentivector. VIRHD/P/siPKD1$_{13333}$ carries a siRNA that targets the 3'

untranslated region present on the endogenous but not on the exogenous human *PKD1* mRNA (see Supplementary Methods). (**B**) The expression of PC1 was analyzed three days post-transduction relatively to untransfected control cells. Transduction with the VIRHD/P/siPKD1$_{13333}$ lentivector decreased PC1 levels, whereas the control mouse-specific VIRHD/P/siPKD1$_{12632}$ vector had no effect on the human PC1 expression. Tubulin expression was determined as control of sample loading. Molecular weight standards are indicated (kDa). The numbers under each lane represent the densitometric PC1:Tubulin signal ratio and are reported as percentage of the control untransfected/untransduced cells. (**C**) The parallel analysis of the cell cycle demonstrated that the suppression of endogenous PC1 in 293T cells caused ploidy abnormalities with DNA content >4n. However, in the presence of exogenously expressed PC1, the cycling profiles were unchanged by the VIRHD/P/siPKD1$_{13333}$ as compared to untransduced or control transduced cells, indicating the rescue of the parental phenotype. It is not unexpected that despite the common causative stimulus and the characteristic >4n DNA content, genomic instability may assume variable profiles according to the different cell types, particularly if these are adapted to grow in culture (compare IMCD3, MDCK and 293T cells) (2).

Supplemental Figure 3. Specificity of PC1 knockdown on centrosome amplification. (**A**) Knockdown of PC1 following transfection of IMCD3 with a plasmid expressing siPKD1$_{3297}$ but not control siLuc siRNAs induced centrosomal amplification. Cells were treated as described in Supplemental Fig. 1 and cells count was performed on day 4. The bars indicate the mean ± SD from four randomly chosen fields (n>100 per field); **p* <0.0001 by ANOVA. (**B**) Following initial transduction cells were cultured for 3 days and then immunostained with γ-tubulin specific antibody (green) and counterstained with DAPI (blue) 3 to determine the centrosomes (open arrows) number

and the presence of micronuclei (closed arrows), respectively (top panel; original magnification: 1000X). A split of the same cultured was maintained for 35 days and then similarly analyzed. The bar graph at the bottom shows the mean ± SD of the percentage of cells with amplified centrosomes and/or micronucleation at 3 and 35 days post-transduction in control IMCD/P/siLuc or PC1 knockdown IMCD/P/siPKD$_{3297}$ cells (from five randomly chosen fields; n>500). The unpaired two-tailed t test with Welch correction was applied for statistical analysis. (**C**) Control of *Pkd1* knockdown specificity was performed on IMCD3 cells were transduced with the control VIRHD/P/siLuc or *Pkd1*-specific VIRHD/P/siPKD1$_{10071}$ lentivectors. Three days post-transduction, immunodetection analysis showed inhibition of PC1 expression by PKD$_{10071}$ siRNA comparable to what observed with the VIRHD/P/siPKD1$_{3297}$ lentivector (Fig 1). Following filter stripping, detection of actin expression was used as control of gel loading. The bands corresponding to the PC1 expression and sample loading control actin are shown along with the position of the molecular weight markers (kDa). The lower bands likely represent PC1 proteolytic products. The numbers under each lane indicate the densitometric PC1:Actin expression ratio reported as percentage of the control transduced cells. (**D**) Multiple cells with abnormal centrosome number as well as aberrant multinucleated cells with supernumerary centrosomes were visible following PC1 knockdown with the PKD$_{10071}$ siRNA (a representative cell is shown). (**E**) The number of the cells with centrosomal amplification was significantly higher in the IMCD/P/siPKD$_{10071}$ knockdown cells than in control IMCD/P/siLuc cells (n=100 for each population). The unpaired two-tailed t test with Welch correction was applied for statistical analysis. (**F**) Expression of exogenous PC1 rescues centrosome integrity. 293T cells were transfected and transduced as described in Supplemental Fig. 2. Following γ-tubulin staining, centrosome number per cell was scored in five randomly chosen fields (n>300 cells). Bars indicate mean ± SD; *p<0.0001 by ANOVA.

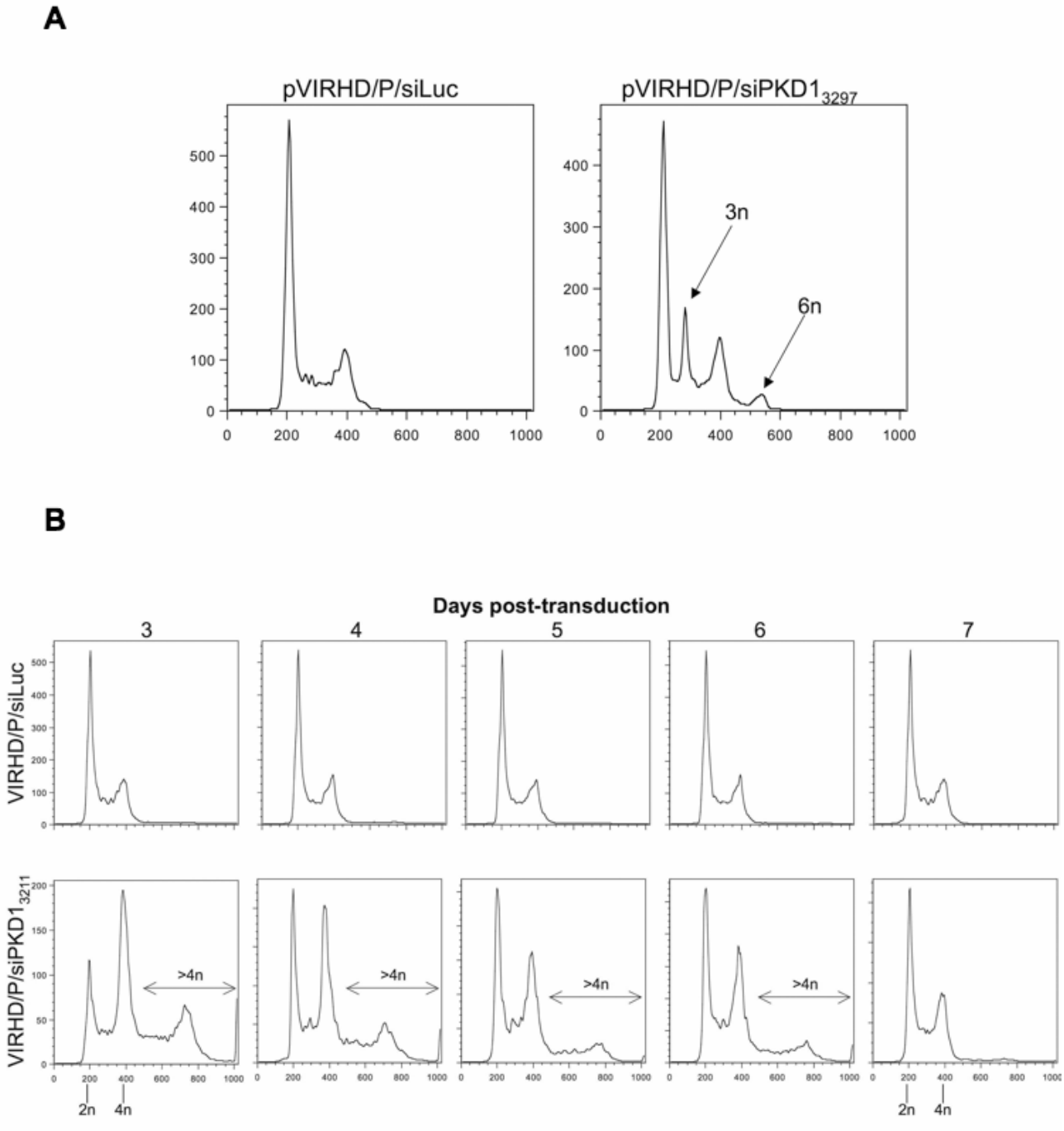
Supplementary Methods. For the construction of the VVE/BBW lentivector, the internal ribosome entry site from the immunoglobulin heavy chain binding protein was inserted between the NotI and MscI sites in pEF6/V5-HisA to obtain the pEF6/BB plasmid. This was then cut with BlpI, blunt ended with Klenow polymerase and then further digested with NotI to excide the BiP and the blasticidin resistance gene (BiP/Blas) cassette, which was then inserted between NotI and a blunt-ended XhoI site of the VVEW vector to generate VVE/BBW. The human *PKD1* gene was cloned following RT-PCR from 293T cells as described (3) except for the following modifications: the high fidelity PrimeSTAR polymerase (Takara Bio USA, Madison, WI) was used in the amplification step and three sets of primers were used to amplify 3 partially overlapping fragments: hPKD5X/F (5'-*tatctagaATGccgcccgccgcgcccgcccgcctggcgct*-3') and hPKD5289R (5'*ggaggtctcccagctcagcccctcctccaaggacca*-3'); hPKD5141F (5'*tcgtggagcctgtggggtggctgatggtgaccgcct*-3') and hPKD9445R (5'-tccggctgtccaccccatacagcatgatgcccacgt-3'); hPKD8796F (5'-*ctacctgtctgaggaacctgagccctacctggca*gt-3') and hKD12909R (5'-*ctgaattcTGAtcaagtgctgctggggtggaccttgttcttggcccga*-3'); starting and stop codons are shown in capital letters and the XbaI and EcoRI sites used for final cloning between the NheI and EcoRI sites of VVE/BBW, to generate VVE/BBW/hPKD1, are underlined. In the VVE/BBW/hPKD1, the PC1 is expressed from a from a dicistronic transcript that allows the co-expression of the blasticidin resistance gene under the control of the constitutive EF1 promoter. 293T cells were transfected with control VVE/BBW or VVE/BBW/hPKD1 using Effectene (Qiagen, Valencia, CA) following the manufacturer's instructions. Transfection efficiencies, as determined by cotransfection with an EGFP-expressing plasmid (1:20 of total DNA), were higher than 80%. Blasticidin (10 μg/ml) was added to the culture the day after the cells were transfected

and puromycin selection (10 μg/ml) was applied 16 hours post-transduction. The antibiotics were maintained until the cells were harvested for protein expression and cell cycle analyses. The VIRHD/P/siPKD1$_{10071}$ and VIRHD/P/siPKD1$_{13333}$ lentiviral vectors were generated by inserting between the BamHI and EcoRI sites of VIRHD/P the following annealed oligonucleotides: siPKD1/10071/S: 5'-gatccc*gcacatctggctctccata*tctagatca*tatggagagccagatgtgc*ttttgg-3' and PKD1/10071/AS 5'-aattccaaaaa*gcacatctggctctccata*tgatctaga*tatggagagccagatgtgc*gg-3'; siPKD1/13333/S: 5'-gatccc*ggacacagcagtattggac*ttcaagaga*gtccaatactgctgtgtcc*tttttcg-3' and siPKD1/13333/AS: 5'-aattcgaaaaa*ggacacagcagtattggac*tctcttgaa*gtccaatactgctgtgtcc*gg-3'. Lentiviral transductions were performed as described in Materials and Methods.

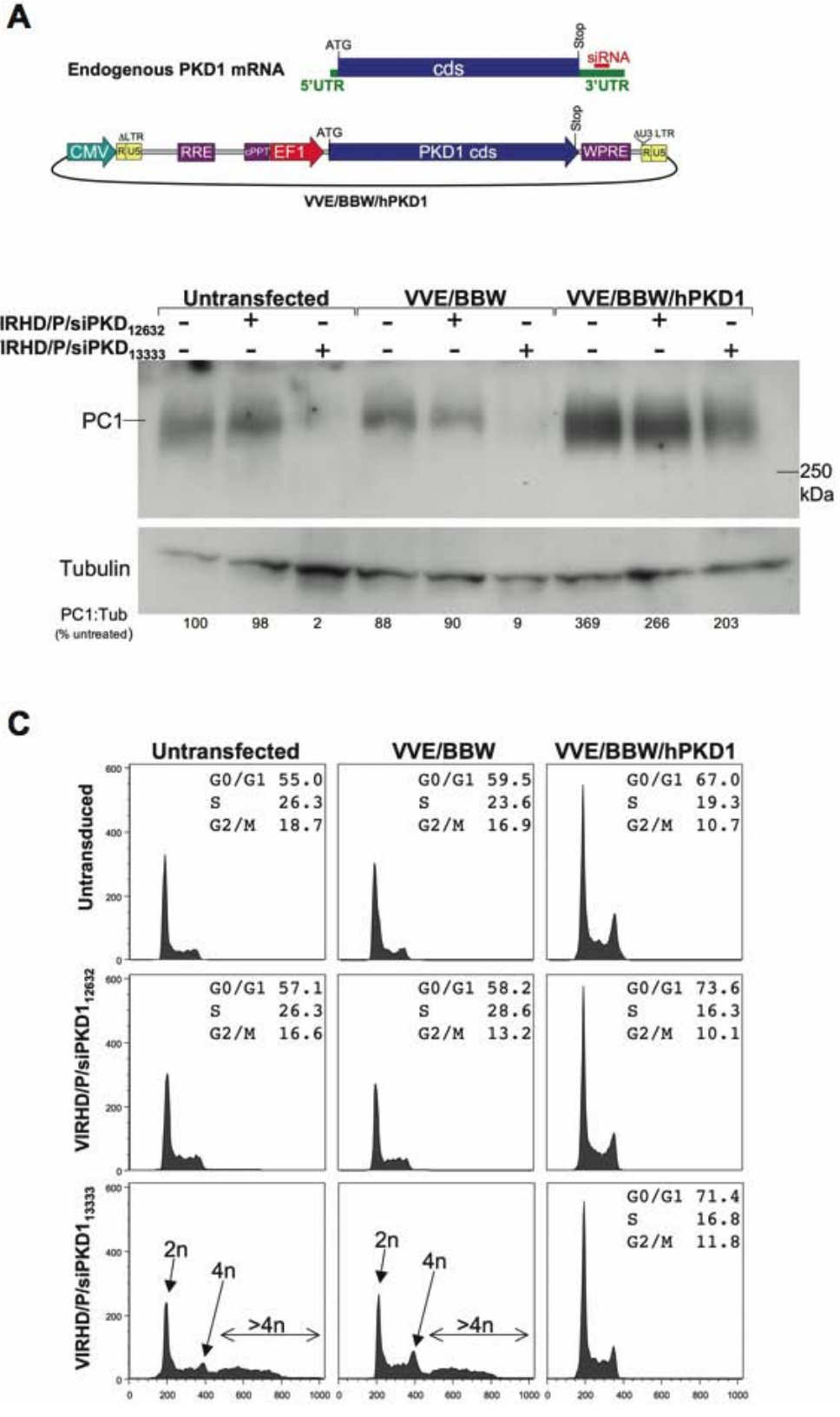
REFERENCES to Supplemental Data

1. Battini, L., Fedorova, E., Macip, S., Li, X., Wilson, P.D. Gusella, G.L. (2006) Stable Knockdown of Polycystin-1 Confers Integrin-α2β1-Mediated Anoikis Resistance. *J. Am. Soc. Nephrol.*, **17**, 3049-3058.
2. Weaver, B.A., Silk, A.D., Montagna, C., Verdier-Pinard, P. Cleveland, D.W. (2007) Aneuploidy acts both oncogenically and as a tumor suppressor. *Cancer Cell*, **11**, 25-36.
3. Thongnoppakhun, W., Wilairat, P., Vareesangthip, K. Yenchitsomanus, P.T. (1999) Long RT-PCR Amplification of the entire coding sequence of the polycystic kidney disease 1 (*PKD1*) gene. *Biotechniques*, **26**, 126-132.

Battini_Supplemental_FIG.1



Battini_Supplemental_FIG.2



Battini_Supplemental_FIG.3