Supplemental Figure 1. Quantification of siRNA efficiency and γH2AX protein levels on western blot. (A) mIMCD3 cells depleted for Ofd1, Mks1 and Cep290 by siRNA show increased γH2AX levels compared to control siRNA transfection (56 hours) after aphidicolin (APH) incubation (400 nM, 18 hours). Quantification is normalized to H2AX (loading control). Lanes were run on the same gel, but were noncontiguous. (B) Normalized CEP290 gene expression levels were measured by RT-qPCR of 20 nM control or CEP290 siRNA treated (48 hours) URECs and (72 hours) RPE cells (RPLP0 reference gene), and normalized Cep290, Mks1 and Ofd1 gene expression levels were measured of 20 nM control or Cep290, Mks1 and Ofd1 siRNA treated (48 hours) IMCD3 cells (RPL27 reference gene; n=3; T-test ***p<0.01, ***p<0.001). (C) Quantification of γH2AX protein expression of Western blots (see Figure 1A-C,E) of URECs, RPE and IMCD3 cells after Cep290-targeting siRNA transfection, URECs from a wild type and Joubert patient, and zebrafish injected with cep290 mo vs control clutchmates (48 hpf). Expression was normalized to H2AX or actin (n=3; T-test *p<0.05). (D) Representative phenotype of zebrafish embryos 36 hours post fertilization (hpf) of wild type zebrafish embryo and control morpholino (MO) injected embryo. Embryos injected with a morpholino against cep290 exhibit a range with phenotypes, in a dose-dependent manner: majority of 3.5 ng MO-injected embryos have milder phenotype with ventral body curvature, hydrocephalus, lack of pigmentation, underdeveloped eyes and pericardial edema, while 4 ng MO-injected embryos are more severely affected with poor survival rate beyond 48 hpf.
Supplemental 2. CEP290 expression and clonal Cep290\textsuperscript{LacZ/LacZ} cell lines. (A) Quantification of CEP290 protein expression of Western blots (see Figure 4B). Expression was normalized to β-actin (n=3; One-way ANOVA; *p<0.05). (B) Western blot of wild type, Cep290\textsuperscript{LacZ/LacZ} and seven clonal Cep290\textsuperscript{LacZ/LacZ} cell lines. Only wild type cells show CEP290 expression. β-actin is loading control. (C) BrdU FACS of wild type and clonal Cep290\textsuperscript{LacZ/LacZ} cell lines showing normal DNA content and cell cycle for wild type cells, however, Cep290\textsuperscript{LacZ/LacZ} cell lines have irregular DNA content (BrdU on y-axes, DAPI on x-axes; 10,000 events measured; n=3). (D) Immunofluorescent (IF) staining of Cep290 of wild type cells indicating centrosomal Cep290 expression (white), which is at the base of primary cilia (acetylated tubulin, green). Scale bar represents 10 μm.
Supplemental 3. Cell cycle S-phase progression and effects of CDK inhibition. (A) IMCD3 cells transfected with control or Cep290 siRNA were examined after BrdU pulse labeling by FACS after 18 hours of 30 min BrdU pulse labeling. Cep290 depleted IMCD3 cells lag in early S-phase, whereas control transfected IMCD3 cells progress normally. Early S-phase cells are relative to total number of cells in S-phase and normalized to siCtrl set to 1 (n=3 in duplicate, 10,000 events were measured. T-test **p<0.01). (B) Wild type and Cep290\textsuperscript{LacZ/LacZ} cells were incubated with 200 nM CDKi for 18 hours. Quantification of centrioles (immunofluorescence staining of pericentrin) revealed decreased supernumerary centriole number in the Cep290\textsuperscript{LacZ/LacZ} cells after CDKi treatment. Mean centriole numbers were 1.7 for Wild type, 2.3 for Cep290\textsuperscript{LacZ/LacZ} cells, and 2.1 for Cep290\textsuperscript{LacZ/LacZ} cells after CDKi treatment (n=3; >100 cells quantified; T-test *p<0.05). (C) FACS 7-AAD staining of wild type and Cep290\textsuperscript{LacZ/LacZ} cells revealed no enhanced apoptosis after CDKi (18 h 200 nM, n=3, 10,000 events measured in triplicate, no differences after 2-way ANOVA).
Supplemental Figure 4. Quantification of kinase activity. (A) Quantification of Cyclin A- and Cyclin B-associated CDK activity in wild type and Cep290LacZ/LacZ primary mouse kidney cells (see Figure 5B). Expression was normalized to activity in wild type cells (n=4). (B) Quantification of Cyclin A- and Cyclin B-associated CDK activity in wild type and Joubert patient URECs with CEP290 mutations (see Figure 5C). Expression was normalized to activity in wild type cells (n=1 due to lack of patient material).