Supporting Information

SI Materials and Methods

Immunofluorescence imaging of human kidney sections

Immunofluorescence labelling of human kidney was performed on 4 µm thick paraffin 4% paraformaldehyde fixed sections, de-paraffinized and then boiled in citrate buffer (for antigen retrieval), pH 6.0 (2x5 min microwave at 900W). Non-specific binding sites were blocked with 1% BSA (PBS–0.1% Tween 20–1% BSA) followed by incubation in the same buffer overnight at 4°C with appropriate antibodies. Confocal images were taken using a spinning disc scanning microscope system (Yokogawa CSU-X1 spinning disk scanner coupled to a Zeiss Observer Z1 inverted microscope and controlled by Zen Blue software) with an X63.2 objective.

Cell isolation and culture

Human urine-derived renal epithelial cells (hURECs) were isolated from urine collected from patient JBTS-AA and healthy age-matched donors and cultured as previously described (9, 28). WT and *Cep290*^{Gt} HOM cells were isolated from kidneys of 1-mo-old transgenic mice and cultured as previously described (8). All cells were tested for mycoplasma every 2 weeks and were negative.

In vitro ASO treatment of hURECs and murine cells

To induce skipping of exon 41 from *CEP290* transcript, an antisense morpholino oligonucleotide (ASO) (GeneTools LLC, USA) was designed to target the intron-exon boundary of human

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CEP290 (exon 41-intron 41) 5'-ATGTTTCTTCACATACCTTTTCTTT-3'. ASO was dissolved in RNase free water to make a 1 mM stock solution. 80%-90% confluent hURECs were treated with 6µl/ml of Endo-Porter (GeneTools LLC, USA) and with ASO at the final concentration of 1 µM in serum-free medium for 48 h unless otherwise stated. Cells were then fixed prior to immunocytochemistry or lysed for RNA or protein extraction.

To induce exon skipping of the intronic splice acceptor/β-galactosidase/neomycin phosphotransferase (SA-IRES-βGEO) gene trap cassette in *Cep290*^{Gt} HOM cells *in vitro*, an octaguanidine dendrimer-modified antisense oligonucleotide morpholino (vivo-ASO^{Gt}) was designed and manufactured (GeneTools LLC, USA) to target the gene trap using the following sequence: 5'ACCTGGTTGTCATGGAGGAGAAA 3'. Vivo-ASO^{Gt} was dissolved in PBS to make a 1 mM stock solution. *Cep290*^{Gt} HOM cells at 70%-80% confluency were treated with vivo-ASO^{Gt} at the final concentration of 10 µM in serum-free medium for 48 h. Cells were then lysed for RNA or protein extraction.

Immunofluorescence imaging of hURECs

hURECs were fixed in ice-cold methanol for 10 min. After 30 min saturation with 5% BSA in PBS, cells were incubated for 1 h at room temperature with the following primary antibodies: rabbit anti-ARL13B (Proteintech, 17711-1-AP); rabbit anti-CEP290 (Abcam, ab 85728); mouse Anti-Pericentrin (Abcam ab28144). Following washes in PBS, cells were incubated at room temperature for 1 h with the following secondary antibodies: donkey anti-rabbit Alexa Fluor 488 (Thermo Fisher Scientific); goat anti-mouse Alexa Fluor 647 (Thermo Fisher Scientific). Alternatively, cells were incubated overnight at 4 °C with primary rabbit anti-ARL13B antibody directly labelled using Zenon Alexa Fluor 555 rabbit IgG labelling kit (Thermo Fisher Scientific,

Z25305), washed with 0.1% PBST and post-fixed with 4% PFA for 15 min. Following final washes in PBS, cells were mounted in Vectashield (Vector Laboratories Ltd, H-1200). Images and z-stacks were captured in a blinded fashion, using a Nikon (A1) confocal inverted microscope.

Scanning electron microscopy imaging

For SEM, samples were fixed overnight in 2% glutaraldehyde in 0.1 M Sorenson's phosphate buffer, dehydrated through a graded series of ethanol and then critical-point dried (Baltec dryer). They were coated with 10nm of gold (Polaron coating unit) and viewed on a Tescan Vega LMU SEM operated at 8–10 kV. Images were captured and measured in a blinded fashion.

In vivo ASO treatment of Cep290$^{Gt/Gt}$ animals

Vivo-ASO^{Gt} dissolved in PBS was injected into the tail vein of 28 day old Cep290$^{Gt/Gt}$ animals at a dose of 12.5 mg/Kg. 3 subsequent doses were administered at 3-4 day intervals over a period of 10 days, and tissues were collected 24 h after the final dose. Efficiency of the vivo-ASO^{Gt} was confirmed via RT-PCR and western blot.

Tissue fixation and processing

Murine kidneys were fixed in 4% paraformaldehyde (PBS) overnight at 4°C, then washed in PBS and dehydrated in 70% Ethanol. The tissue was processed into paraffin wax using a Shandon™ Pathcentre™ automatic processor (Thermo Fisher Scientific). Tissues were sectioned on a RM2235 microtome (Leica), at a thickness of 8 µm on to Polysine™ adhesion slides (Thermo Fisher Scientific).

Histological staining

Slides were dewaxed in HistoclearII (National diagnostics), then stained with Haematoxylin/Eosin (H+E) according to the following protocol. Slides were hydrated through a series of ethanol washes (100%, 90% 70% and 50%), rinsed in water, and then in Harris's haematoxylin for 1 min. Slides were transferred into running tap water and then counterstained with 1% eosin for 1 min, rinsed in tap water, and then dehydrated. Slides were mounted in DPX mounting medium (Sigma-Aldrich) and visualised using a SCN400 Slide Scanner (Leica).

Immunofluorescence imaging of murine kidney sections

Slides were dewaxed in HistoclearII (National diagnostics), rehydrated then boiled in Citrate buffer (for antigen retrieval), pH 6.0 (2x5 min microwave at 900W). The slides were washed and subsequently blocked in 5% foetal calf serum for 1 h. Slides were incubated overnight at 4°C in the following antibodies: rabbit anti-ARL13B (Proteintech, 17711-1-AP), goat anti-Aquaporin2 (Santa Cruz Biotechnology, sc-9882). Following washes in PBS, slides were incubated at room temperature for 90 min in the following secondary antibodies: donkey anti-goat Alexa Fluor 647, donkey anti-rabbit Alexa Fluor 488 (Thermo Fisher Scientific, 1: 300). Following final washes in PBS, slides were mounted in Vectashield (Vector Laboratories Ltd, H-1200). Images were captured in a blinded fashion, using an Axio Imager Z1 fluorescent microscope (Zeiss).

Image analysis

Following capture, images were analysed using FIJI (ImageJ) software. To measure the size of cysts, 3 H+E images from each sample were set a threshold using the colour threshold function and the size of cysts calculated using the measure particles function. The resulting sum of all of

the cysts was then used to calculate the cystic index, correcting for the total size of the kidney. The length of cilia was measured using the segmented line tool on a maximum intensity projection of a z-stack.

Western blotting

Kidneys were snap frozen in liquid nitrogen and stored at -80°C. A lysis buffer solution containing 4 M urea, 125 mM Tris pH 6.8, 4% SDS, 10% glycerol, 5% β-mercaptoethanol and 0.02% bromophenol blue was used to homogenize kidney tissue or lyse hURECs and murine cells.

Protein samples were then heated to 95 °C for 5 min, spun at 16 000 rcf for 5 min and resolved by SDS-PAGE on a 4-20% gradient gel (Bio-Rad). Proteins were transferred to a 0.4 µm nitrocellulose membrane (Thermo Fisher Scientific). The membranes were blocked in TBST (Tris-buffered saline, 0.1% Tween-20) containing 1-5% low fat milk or 1% BSA for 1 h then incubated with the following primary antibodies in block overnight at 4 °C: rabbit anti-CEP290 (Abcam, ab 85728); rabbit anti-CEP290, (Covalab, (8)); rabbit anti-Gli3, (ab69838); mouse anti-β-Actin (Sigma, AC-74); rabbit anti-GAPDH, (Cell Signaling technology, 2118). After washing in TBST, membranes were incubated with fluorescently labelled secondary antibodies (LI-COR) for 90 min at room temperature, washed again in TBST and visualized with an Odyssey CLx imaging system (LI-COR).

RNA extraction and cDNA synthesis

RNA was extracted from hURECs and murine tissue using Trizol (Thermo Fisher Scientific) according to the manufacturer's instructions, and quantified using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific). 1 ug of RNA was reverse-transcribed using an

Oligo-dT primer and SuperScript III Reverse Transcriptase (Thermo Fisher Scientific). The resulting cDNA was diluted 10-fold in nuclease-free water.

Reverse transcriptase-PCR and quantitative RT-PCR

Reverse transcriptase PCR was carried out in a 20 µl reaction volume containing the following: 1µl of each primer (10µM stock, IDT), 0.2µl dNTPs (10mM stock, Thermo Fisher Scientific), 4 µl 5X Green GoTaq® Reaction Buffer (Promega), 0.1 µl GoTaq® DNA Polymerase, 2 µl of cDNA and 11.7 µl of nuclease-free water. The following primers were used for the reactions:
F1 5'-AAGTCCCTCATCCGGCAGTA-3'; F2 5'-AATGATGATTTCAGCCGCGC-3';
R1 5'-TGACAGTGTTCAGCCCTCTG-3'; R2 5'-TTGGGTAACGCCAGGGTTTT-3';
R3 5'-GCGCGGCTGAAATCATCATT-3'; R4 5'-GTATCGCCAAAATCACCGCC-3';
R5 5'-CTGTTGACTGTAGCGGCTGA-3'; R6 5'-AAAAGCGGCCATTTTCCACC-3'
Primers used for detecting exon skipping in human *CEP290* were:
FOR 5'-TTTTAGAACTCCGGGCAGAA-3'; REV 5'-TTGGCTTGCCACTTTTTACC-3'.

A prime time assay to determine *CEP290* expression was carried out using PrimeTime® Gene Expression Master Mix (IDT) and the PrimeTime® qPCR probe-based assay (IDT) CEP290-Hs.PT.58.2324908 (IDT) according to manufacturer's instructions. Expression levels were normalised to the expression of housekeeping genes *HPRT1* and *GAPDH*.

Quantitative analysis of *SHH* expression was carried out using SYBR Green PCR Master Mix (Applied Biosystems) according to manufacturer's instructions and the following primers: SHH-F 5'-ATCTCCAGAAACTCCGAGCGATTTAAG-3' and R 5'-AAGCGTTCAACTTGTCCTTACACCTC-3'. The PCR was run using a QuantStudio™ 7 Flex

Real-Time PCR System (Applied Biosystems). Expression levels were normalised to the expression of housekeeping genes *HPRT1* and *GAPDH*.

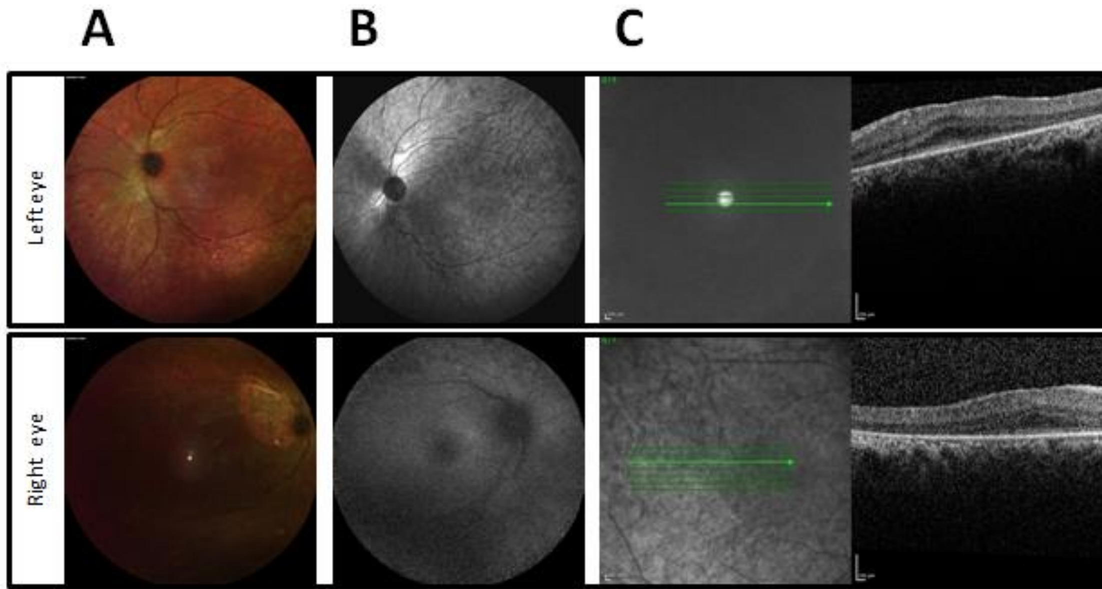

Fig. S1. Retinal phenotype of patient JBTS-AA

(A) Colour fundal images, (B) autofluorescence (AF) and (C) horizontal spectral domain optical coherence tomography (OCT) scans are shown from patient JBTS-AA, aged 14 years. The colour fundal images show subtle pigment spicules and coarseness of the retinal pigment epithelium. The AF images from the right eye show a mottled pattern of hypoautofluorescence peripherally with pericentric foveal hyperautofluorescence typical of a hereditary retinal degeneration. The OCT images show loss of definition of the ellipsoid and external limiting membrane lines centrally, with thinning of the outer nuclear layer.

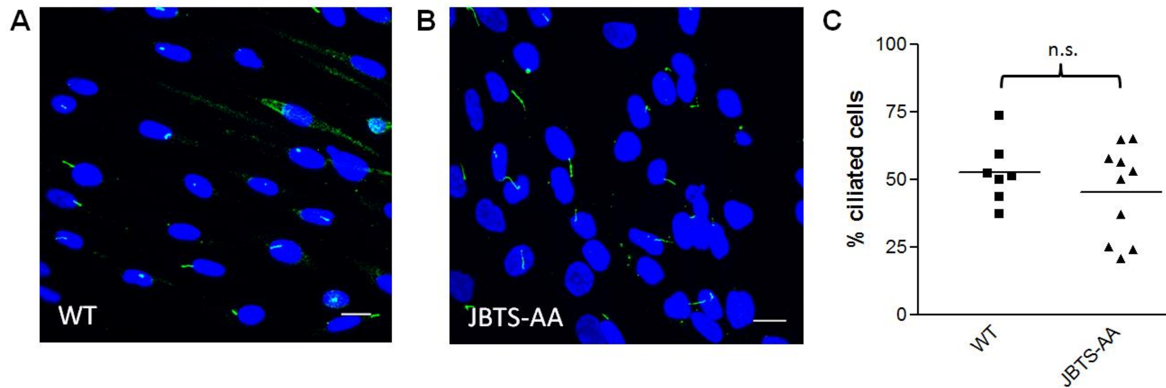

Figure S2: Ciliation rates of wild type and patient hURECs

Percentage rates of ciliation of hURECs in (A) 48 h serum starved wild type (WT) and (B) JBTS patient (JBTS-AA). Scale bar 15 μm. (C) A comparison of WT and JBTS-AA ciliation rates was not significant (n.s. P=0.35 Unpaired t test).

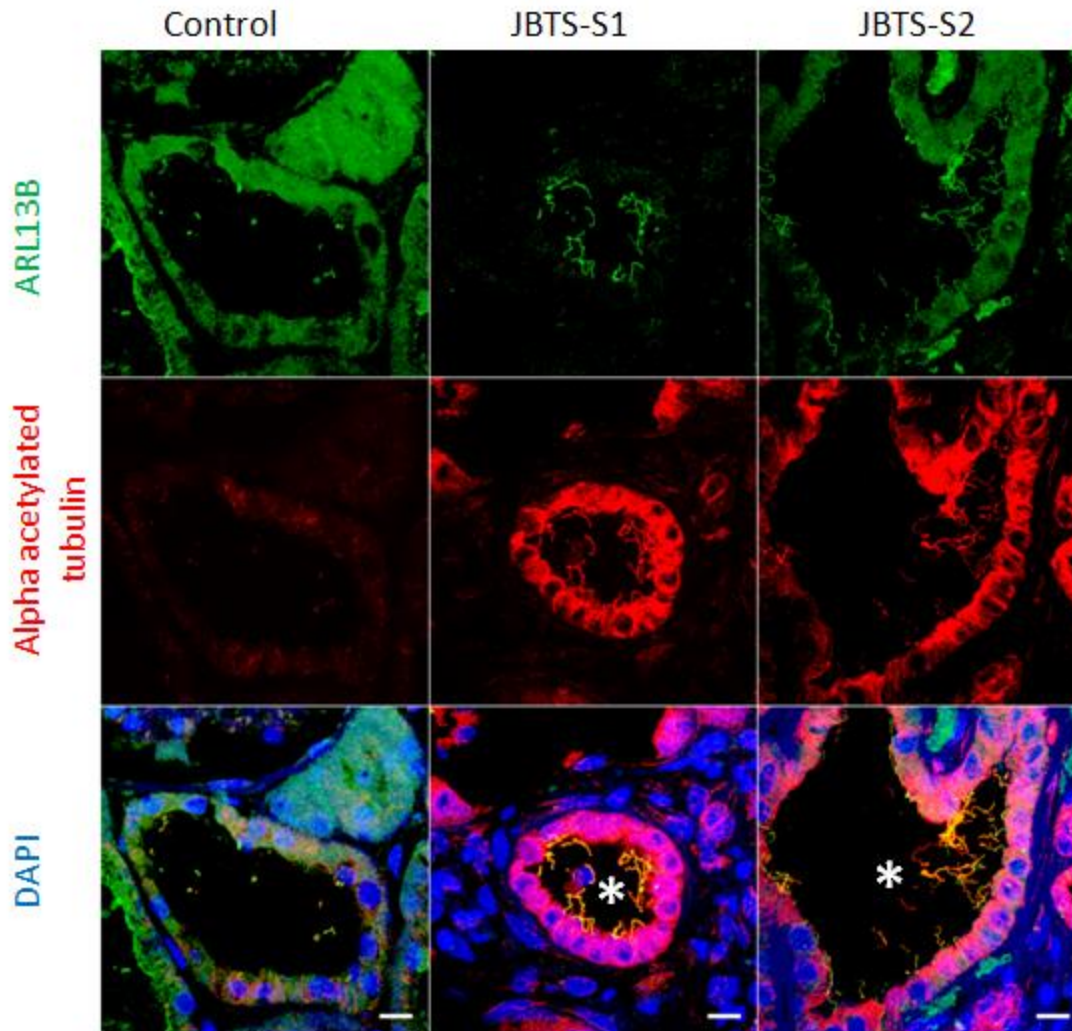

Fig. S3. Human renal kidney sections from JBTS patients with *CEP290* mutations show cilia defects

Renal biopsies from JBTS patients with cystic kidney disease secondary to *CEP290* mutations. Sections stained with antibodies directed towards cilia proteins (ARL13B, green; Alpha-acetylated tubulin, red) and DAPI (blue) to identify nuclei. Defects in cilia length, with elongated and tortuous cilia (marked with*), are seen in JBTS-S1 and JBTS-S2 when compared to control. Scale bar 10 µm. Molecular genetics confirmed *CEP290* mutations in both patients: JBTS-S1 has compound heterozygous frameshift mutations c.5649-5650insA, (p.1884fsx23) and c.5850delT, (p.F1950fsx15); JBT-S2 has a homozygous missense mutation c.2915T>C, (p.L972P).

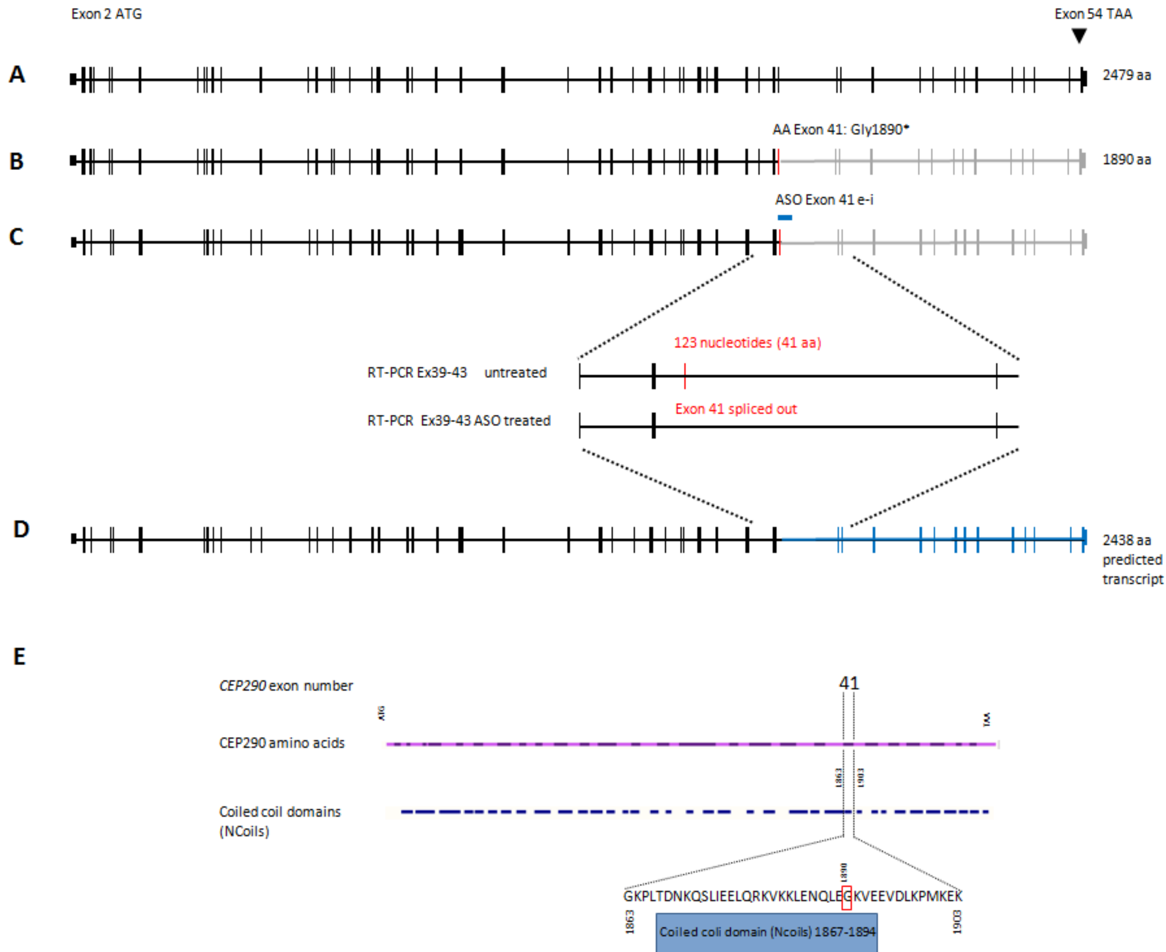

Fig. S4. Targeted exon skipping of exon 41 of CEP290

(A) Schematic of human CEP290 with exons shown as vertical bars. Human CEP290 is located on chromosome 12 at position 21.32. It consists of 54 coding exons and the full length mRNA transcript consists of 7,951 nucleotides encoding the CEP290 protein of 2479 amino acids
(B) Patient JBTS-AA has a homozygous non-sense mutation in the CEP290 gene (c.5668G>T; p.G1890*) leading to a premature stop codon and truncation of the reading frame within exon 41 as shown.
(C and D) Antisense oligonucleotide (ASO) mediated exon skipping of exon 41 leads to a predicted shorter mRNA transcript, by splicing out 123 nucleotides of exon 41, that encodes a protein consisting of 2438 amino acids
(E) Schematic of predicted CEP290 amino acid sequence encoded by exons 2 to 54 (in alternating colours pink and blue) and predicted coiled coil domains (NCoils, Ensembl) aligned to each exon. Exon 41 is predicted to encode a discrete coiled coil domain from amino acids 1867-1894. The JBTS-AA mutation G1890* is marked by a red square. Skipping of this exon would remove this single predicted coiled coil domain.

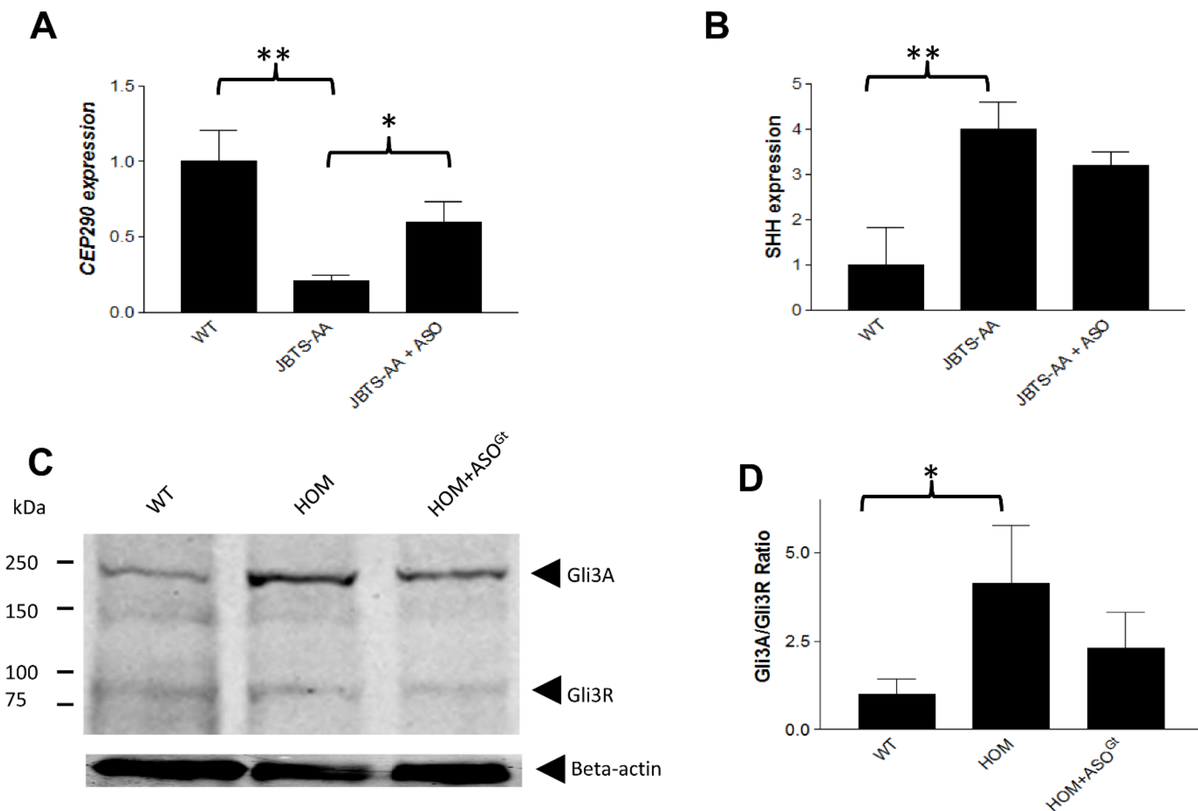

Fig. S5. Validation of ASO rescue of *CEP290* transcript and assessment of ciliary signalling pathway in hURECS and murine kidney

(A) Quantitative PCR assay for *CEP290* transcript abundance in hURECs from wild type controls (WT), JBTS-AA and JBTS-AA treated with antisense oligonucelotide (ASO) in serum-free medium for 48 h. In JBTS-AA hURECs, *CEP290* expression levels are reduced to approximately 20% of wild type levels. ASO treatment of JBTS-AA results in an approximately 3-fold increase of *CEP290* transcript levels (*, P<0.05,** P<0.001, one-way ANOVA). Each bar represents mean value from three replicates. Error bars represent SD from the mean.

(B) Expression of *SHH* RNA transcripts in hURECs from wild type controls (WT), JBTS-AA and JBTS-AA treated with antisense oligonucelotide (ASO) in serum-free medium for 48 h. In JBTS-AA hURECs, there is an approximately 4-fold upregulation of *SHH* expression levels. Upon ASO treatment, *SHH* levels are partially reduced to approximately 80% of *SHH* levels in untreated cells. Each bar represents mean value from three replicates. Error bars represent SD from the mean. **, P<0.001, ANOVA.

(C) Western blot analysis of protein extracts from whole murine kidney of wild type controls (WT), untreated *Cep290*$^{Gt/Gt}$ mice (HOM) and *Cep290*$^{Gt/Gt}$ mice treated with vivo-ASO^{Gt} (HOM+ASO^{Gt}) using Gli3 antibody. Bands at 190 kDa and 83 kDa correspond to full-length

(Gli3A) and processed repressor forms (Gli3R) respectively. Beta-actin (42 kDa) was used as a loading control.

(D) Quantification of relative intensity of Gli3A/Gli3R ratio following quantification by densitomery in murine kidney tissues from wild type controls (WT), untreated *Cep290*$^{Gt/Gt}$ mice (HOM) and *Cep290*$^{Gt/Gt}$ mice treated with vivo-ASO^{Gt} (HOM+ASO^{Gt}). Error bars indicate standard deviation; *, P<0.05, ANOVA . Experiments performed in triplicate.

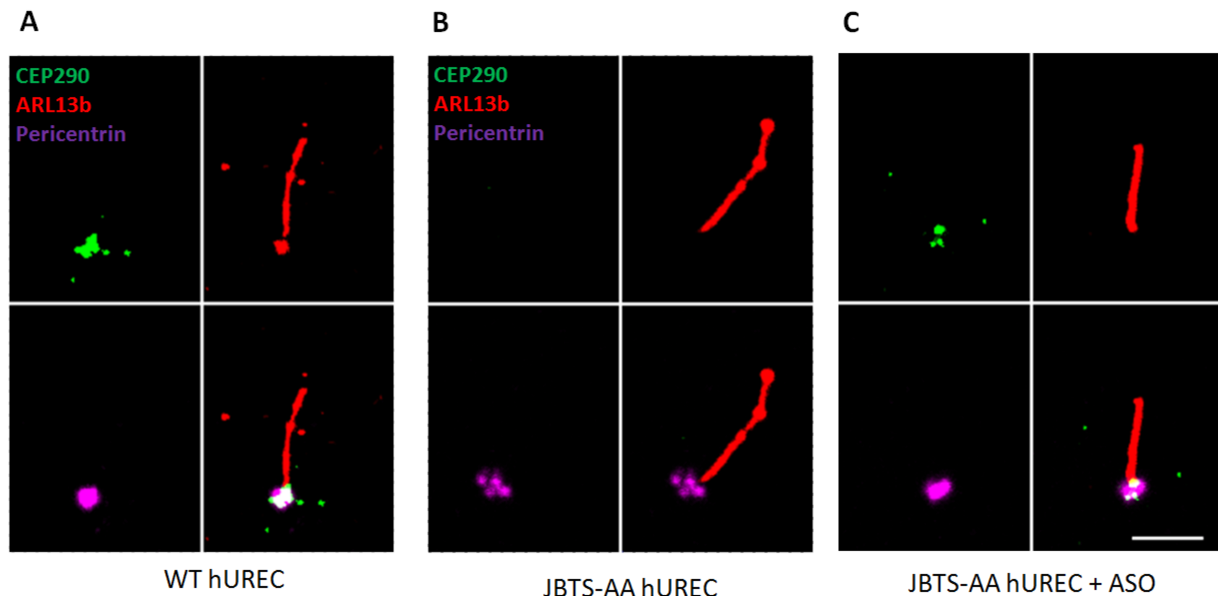

Fig. S6. ASO treatment of JBTS-AA hURECs restores basal body localisation of CEP290 protein

Immunofluorescence imaging of hURECs from (A) wild type (WT) controls, (B) patient JBTS-AA and (C) patient JBTS-AA treated with ASO directed at exon 41–intron41 junction. Cilia are stained with ARL13B (red), C-terminal CEP290 (green) and the basal body is identified by Pericentrin (magenta). Full length CEP290 (green) cannot be detected at the basal body of JBTS-AA hURECs. Following treatment of JBTS-AA hURECs with ASO, a near full length CEP290 protein is restored and localises to the basal body. Scale bar 5 µm.

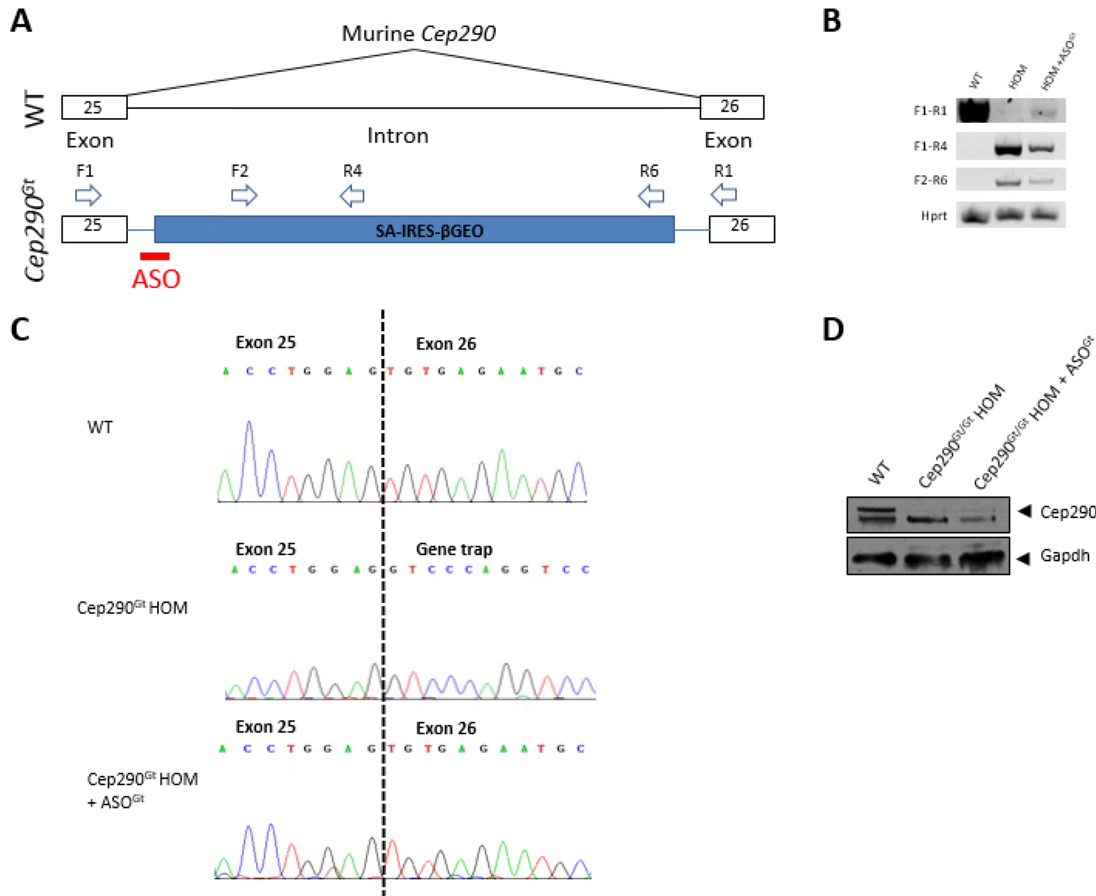

Fig. S7. Treatment of *Cep290$^{Gt/Gt}$* HOM murine kidney cell line with ASO^{Gt} induces exon skipping of SA-IRES-βGEO gene trap to restore normal Cep290 transcript and protein.

(A) Schematic of SA-IRES-βGEO gene trap (Gt) situated in intron 25 of *Cep290* and oligonucleotide primers to detect skipping and partial skipping and target site for vivo-ASO^{Gt} marked in red.
(B) RT-PCR of mRNA from *Cep290^{Gt}* HOM cell line to identify skipping and partial skipping of SA-IRES-βGEO gene trap in intron 25 of *Cep290^{Gt}* HOM cell line. RT-PCR products from primer pairs are shown across exons 25-26 and the SA-IRES-βGEO gene trap. Primer pairs F1-R1 amplify correctly spliced (exon 25 to 26) *Cep290* transcript in *Cep290^{Gt}* HOM cells treated with vivo-ASO^{Gt} along with a concomitant decrease in splicing into the SA-IRES-βGEO gene trap (primers F1-R4 and F2-R6). *Hprt* is used as a control (as shown in Fig.3).
(C) Chromatograms of RT-PCR products across exon 25-26 splice junction in wild type murine cells, *Cep290^{Gt}* HOM showing exon25-gene trap sequence and *Cep290^{Gt}* HOM cells treated with vivo-ASO^{Gt} to confirm restoration of correct splicing between exon 25 and 26.
(D) Western blot showing CEP290 (detected with C-terminal antibody) protein in wild type murine cells (WT), *Cep290^{Gt}* HOM untreated and *Cep290^{Gt}* HOM cells treated with 10 µM vivo-ASO^{Gt} showing a partial restoration of near-full molecular weight CEP290 protein following vivo-ASO^{Gt} treatment. A slightly lower non-specific band is also seen in all samples.

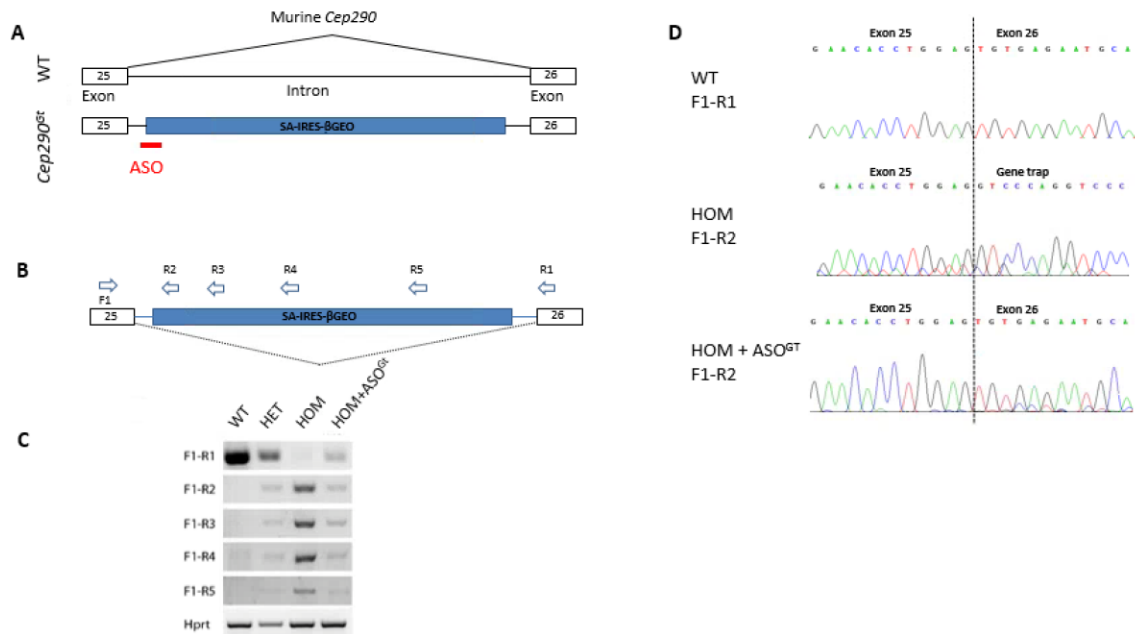

Fig. S8. Exon skipping of SA-IRES-βGEO gene trap following in vivo treatment of *Cep290^{Gt}* mice with vivo-ASO^{Gt} restores splicing between exons 25 and 26.

(A) Schematic of exon structure of wild type (WT) and *Cep290^{Gt}* gene trap showing SA-IRES-βGEO situated in intron 25 of *Cep290* and target site for vivo-ASO^{Gt} marked in red.

(B) Schematic of *Cep290^{Gt}* gene trap showing location of RT-PCR primers to detect skipping and partial skipping of gene trap.

(C) RT-PCR of murine kidney mRNA showing restoration of correct splicing (skipping of SA-IRES-βGEO) with primers F1-R1 and concomitant decrease in splicing into the SA-IRES-βGEO gene trap construct (primers F1-R2, F1-R3, F1-R4, F1-R5). RT-PCR products from wild type (WT) mice, heterozygous *Cep290^{Gt}* (HET) mice, homozygous (HOM) *Cep290^{Gt}* mice and homozygous *Cep290^{Gt}* mice treated with vivo-ASO^{Gt} (HOM+ASO^{Gt}).

(D) Chromatograms showing wild type (WT) murine kidney sequence between exon 25 and 26, homozygous Cep290^{Gt} (HOM) murine kidney sequence showing exon 25 and gene trap sequence and homozygous Cep290^{Gt} + ASO^{Gt} murine kidney sequence (HOM + ASO^{Gt}) showing restoration of correct splicing (skipping of gene trap SA-IRES-βGEO).

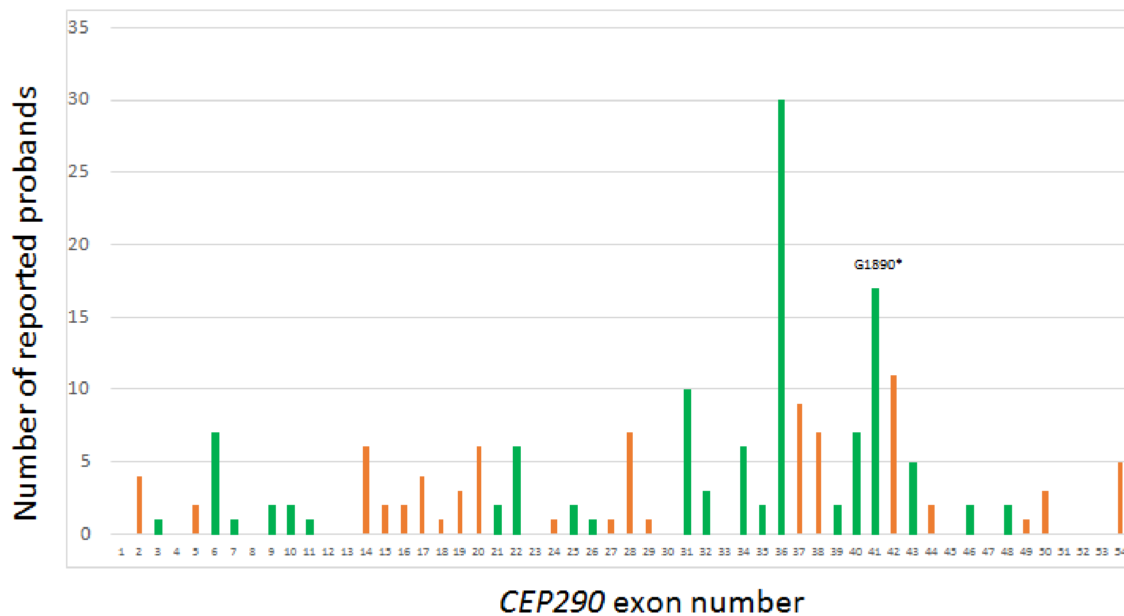

Fig. S9. Number of reported probands with mutations in each coding exon of *CEP290*

The *CEP290* mutation database was used to determine data (https://cep290base.cmgg.be). Bars coloured green indicating a potential skippable exon, in terms of no change in reading frame. JBTS-AA mutation G1890* located in exon 41 is shown.

Table S1. Molecular and clinical characteristics of affected Joubert syndrome patients (JBTS-S1 and JBTS-S2)

	JBTS-S1	JBTS-S2
Ethnicity and consanguinity	Caucasian, non-consanguineous family, segregation confirmed	Saudi Arabian, parents first cousins, segregation confirmed
Renal findings	No cysts, atrophic kidneys	Hyperechogenic kidneys, small medullary cysts, loss of corticomedullary differentiation, microdilated tubules, atrophic epithelium and thickening of the basement membrane
Kidney function	ESRD at 11 years and 9 months	ESRD at 13 years and 7 months
Ocular symptoms (age of onset, years)	Early onset retinal degeneration, blindness at 3 months	Early onset retinal degeneration, nystagmus, blindness at 2 years
Central nervous symptoms	Cerebellar ataxia and intellectual disability	No
Nucleotide alterations	Compound heterozygous c.5649dup (1, exon 41) c.5850delT (2, exon 42)	Homozygous c.2915T>C (exon 26)
Alteration in coding sequence	p.Leu1884Thrfsx23 p.Phe1950Leufsx15	p.Leu972Pro
Reference sequence	NM_025114	NM_025114
SNP ID	rs281865188 (1) rs386834159 (2)	Not found
ExAC allele frequency or gnomAD frequency	Not found (1) 0.00001820 (2) (gnomAD)	Not found