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Supplemental Data

Bi-allelic Mutations in *FAM149B1* Cause Abnormal Primary Cilium and a Range of Ciliopathy Phenotypes in Humans

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Supplement Materials and Methods

Transcriptome Expression Profiling

Skin fibroblasts cells of the affected individuals 17DG0722 and 17DG0168 and of age-matched control individuals (n = 2) were processed for transcriptome sequencing. The cells were treated with SAG (Smoothened Agonist) in Opti-MEM-reduced serum medium at a concentration of 100 nM for 16 h and the total RNA were extracted using the QIAamp RNA Mini Kit (Qiagen Inc., Germantown, MD) protocol. RNA and the NGS library quality (size and quantity) control (QC) were checked using Agilent Technologies 2100 Bioanalyzer using a DNA 1000 chip, Illumina qPCR Quantification Protocol Guide and Roche's Rapid library standard Quantification. TruSeq Stranded mRNA LT Sample Prep Kit was used and the library was prepared using TruSeq Stranded mRNA Sample Preparation Guide, Part # 15031047 Rev. E followed by sequencing using NovaSeq 6000 System. The reads were mapped to reference genome with HISAT2, splice-aware aligner and Transcript is assembled by StringTie with aligned reads. The FPKM (Fragments Per Kilobase of transcript per Million Mapped reads) value or the RPKM (Reads Per Kilobase of transcript per Million mapped reads) is used as a normalization value and the Expression profiles are represented as read count and normalization value which is based on transcript length and depth of coverage. Functional annotation and gene-set enrichment analysis were performed using GO and KEGG database on differentially expressed genes with known annotation.

Relative quantification RT-PCR

Total RNA from cell were extracted using the QIAamp RNA Mini Kit (Qiagen Inc., Germantown, MD) and DNase treated by the RNase-Free DNase Set (Qiagen), according to the manufacturer's recommendations. Preparation of the cDNA was carried out using the iScript™ cDNA synthesis kit and poly-T oligonucleotide primers (Applied Biosystems, Carlsbad, CA). The primer sequences for the desired genes are listed in the table *below.* The relative quantification (q) RT-PCR for the expression was performed using SYBR green and Applied Biosystems 7500 Fast Real-Time PCR System.

	Forward	Reverse
GLI1	AGAGAGACCAACAGCTGC	TCATCTGGGCTGGGAATC
PTCH1	ACGATGGAGTCCTTGCCTAC	TTCTCAGCCTTGTTTCAGG
SUFU	ACCCGCTCCAGGTTACC	GTGCCAGGACACATGGTC
HHIP	GGAGCCTTATTTGGACATTCAC	TCATCCCATCACCAAGAATG
SMO	GCAGGTGGATGGGGACTC	CATTGGCCTGACATAGCACA
GAPDH	GGTGAAGGTCGGAGTCAAC	ATGGGTGGAATCATATTGGA
FAM149B1	GACACAAGTTCCCAAAGCAAGT	GCCTACCTAGAATCCTGAGGTG

Figure S1

A) Immunofluorescent staining images of the affected individual 17DG0722 fibroblast cilia and control stained with the ciliary marker acetylated α-tubulin (Sigma-Aldrich, T7451) (green) and IFT-122 (Santa Cruz Biotechnology, sc-102612) (red) showing similar pattern of IFT-122 staining in the affected individual cilia as compared with control cilia. B) Confocal staining images of the affected individual 17DG0722 fibroblast cilia and control stained with the centrosome marker gamma tubulin (Sigma-Aldrich, T6557) (green) and retrograde marker IFT-88 (ProteinTech, 13967-1-AP) (red). Scale bars represent 1 μm

Figure S2

A) Bar Graph showing the relative quantification to the mRNA expression of *HHIP,* and *SMO and SUFU* which were markedly lower in fibroblasts from the affected individual cells 17DG0722 in family 1 than control fibroblasts. B) Bar graph showing the efficiency of *FAM149B1* siRNA in HEK293 cells compared to negative control scrambled siRNA (siScr) as quantified by qRT-PCR for the *FAM149B1* transcript. C) Bar Graph showing the relative quantification to the mRNA expression of *HHIP,* and *SMO and SUFU* which were significantly lower in *FAM149B1* siRNA in HEK293 cells than to negative control scrambled siRNA. Error bars represent the SEM, statistical significance represents results from two-tailed Student's t-test and two-way ANOVA are indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$. ns (non-significant).

Figure S1: *FAM149B1*-related ciliopathy is associated with impaired ciliary structure

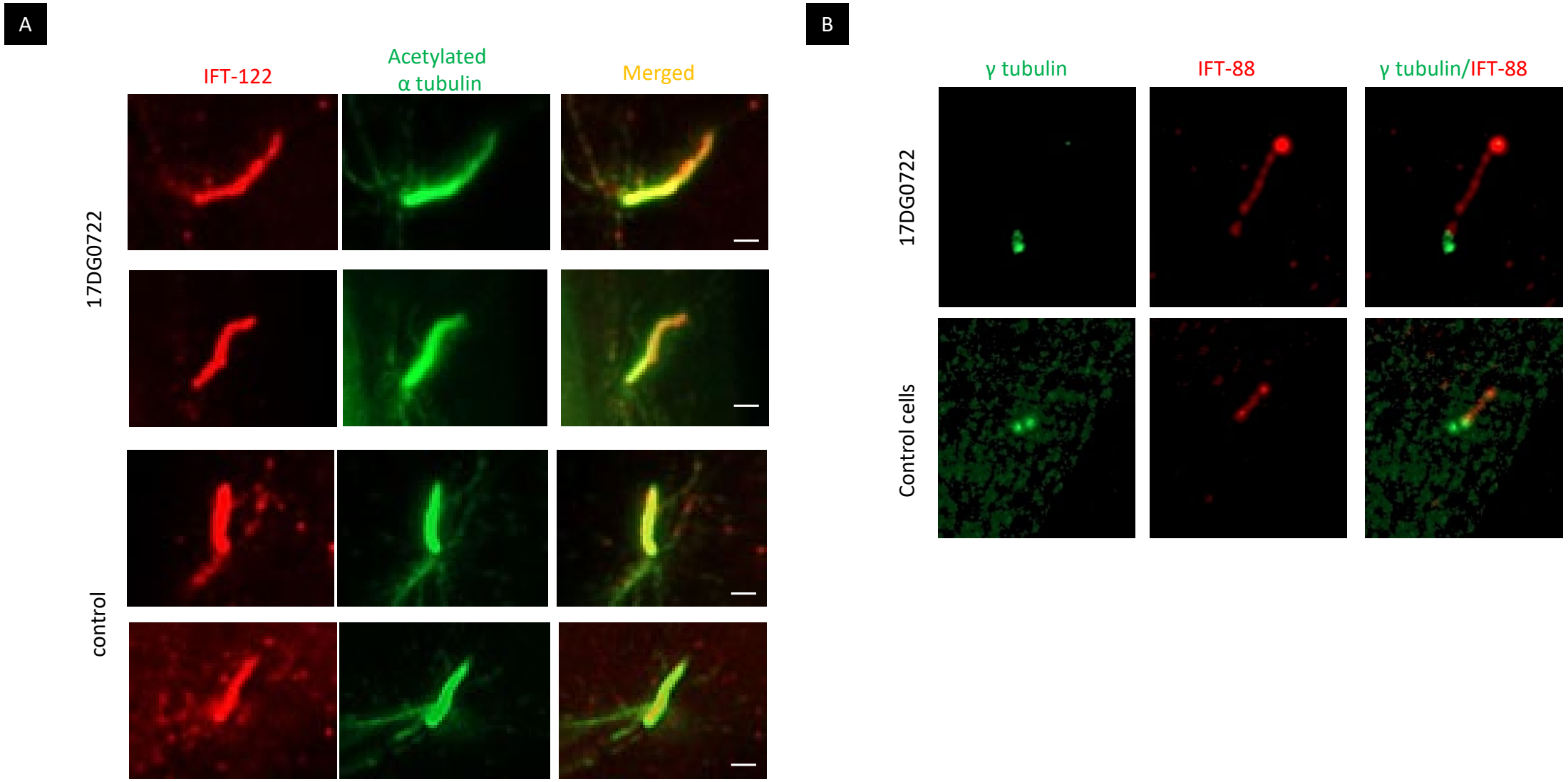

Figure S2: *FAM149B1*-related ciliopathy is associated with impaired ciliary function