Supplementary materials

Materials and Methods

Serum biochemical analysis

After 2h at room temperature, whole-blood samples were centrifuged at 3,000rpm for 15min at 4°C. Supernatant serum was collected. An automatic chemical analyzer (Shenzhen Rayto Life Technology) and standard diagnostic kits (Changchun Huili) were used to determine serum levels of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activity and total concentrations of bile acids (TBA) and bilirubin (TBIL).

RNA isolation, cDNA synthesis, and quantitative real-time PCR (RT-qPCR)

Total RNA was extracted from liver tissue using the FastPure Cell/Tissue Total RNA Isolation Kit (Vazyme) according to manufacturer's instructions. Quality of RNA was verified by evaluating absorbance at 260 nm and 280 nm. Qualified RNA was reverse-transcribed into cDNA with PrimeScript™ RT Master Mix (TaKaRa). RT-qPCR was conducted on a 7300 Fast Real-Time PCR System (Applied Biosystems) using SYBR Green PCR Master Mix (TaKaRa) with *Gapdh* as a housekeeping gene. Relative expression of target genes at the mRNA level was calculated using the $2^{-\Delta\Delta Ct}$ method, with data presented as mean ± SD. Supplementary Table 1 lists primers.

RNA sequencing (RNA-seq), human and mouse liver, and data analysis

Quality and concentration of RNA were determined using an Agilent 2100 Bioanalyzer (Agilent Technologies). The qualified RNA was polyA-selected and fragmented before cDNA synthesis. Constructed libraries were pooled and sequenced on a HiSeq 2500 platform (Illumina). The sequenced reads were aligned to the human/mouse reference genomes hg19/mm10 using HISAT v2.2.1. Differential gene expression was assessed with DESeq2v1.10.1. Differentially expressed genes (DEGs) were defined using the criteria of p values <0.05 and log2 (fold of change) > 1 or < -1, where log2 > 1 and log2 < -1 respectively represent up-regulated genes (Up) or

down-regulated genes (Down). Transcriptome sequencing data were deposited in the NCBI Gene Expression Omnibus as GSE169737.

Western blotting

Liver and cell samples were lysed in RIPA buffer (Beyotime) supplemented with a protease inhibitor cocktail. Protein concentration was determined by PierceTM BCA Protein Assay Kit (Thermo). Briefly, equal amounts of proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 10% gels and electrotransferred onto polyvinylidene fluoride (PVDF) membranes (Millipore). The blots were blocked by 5% nonfat milk, followed by incubation with primary antibodies at 4°C overnight and then incubation with horseradish peroxidase-conjugated secondary antibodies at room temperature for 1h. Immunoreactive bands were visualized using a Bio-Rad ChemiDoc MP System (Bio-Rad). Supplementary Table 2 and Supplementary Table 3 respectively list primary antibodies and secondary antibodies.

Histopathological staining

Liver samples were fixed in 4% paraformaldehyde overnight, dehydrated in an ethanol-xylene series, and embedded in paraffin. Sections at 4μm were used for hematoxylin and eosin (H&E) and Sirius Red staining and for immunofluorescence (IF) staining. Fibrosis (area marking with Sirius Red : total liver area) was assessed in 5 randomly selected fields. The results were analyzed using imaging software (Image J, NIH).

IF staining

Paraffin-embedded sections (4 μm) were deparaffinized and rehydrated in standard fashion. Antigen retrieval was carried out with epitope retrieval solution (Zytomed Systems) at pH 9.0. The sections were incubated with primary antibodies at 4°C overnight, washed with Tris-buffered saline and Tween 20 3 times, and incubated with secondary antibody at 37°C for 30min. Cells for IF assay, grown on coverglasses to suitable confluence, were fixed with 4% paraformaldehyde, permeabilized with carbinol, incubated with primary antibodies at 4°C overnight, washed with phosphate-buffered saline 3

times, and incubated with secondary antibody at room temperature for 30-60min. Supplementary Table 2 and Supplementary Table 3 list respectively primary antibodies and secondary antibodies.

Suppl. Table 1 Primers for RT-qPCR

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
α-Sma	AAACAGGAATACGACGAAG	CAGGAATGATTTGGAAAGGA
α-SMA	GCCTTGGTGTGTGACAATGG	CCATCACCCCCTGATGTCTG
β-catenin	GTGTACCCATATCCCAGCCC	TCCTGCCCCACATCTCTCAG
Ccl2	AGGTCCCTATGGTGCCAATGT	CGGCAGGATTTTGAGGTCCA
Col1a1	GCTCCTCTTAGGGGCCACT	CCACGTCTCACCATTGGGG
COL1A1	GAGGGCCAAGACGAAGACATC	CAGATCACGTCATCGCACAAC
Ctgf	GGGCCTCTTCTGCGATTTC	ATCCAGGCAAGTGCATTGGTA
CTGF	ACCGACTGGAAGACACGTTTG	CCAGGTCAGCTTCGCAAGG
Cxcl1	GGCTTCCTTATGTTCAAACAGGG	GCCGTTACTCGGGTAAATTACA
Cxcl10	CCAAGTGCTGCCGTCATTTTC	GGCTCGCAGGGATGATTTCAA
Cxcl12	TGCATCAGTGACGGTAAACCA	TTCTTCAGCCGTGCAACAATC
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
GAPDH	GCACCGTCAAGGCTGAGAAC	TGGTGAAGACGCCAGTGGA
Il-1β	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG
IL-1β	ATGATGGCTTATTACAGTGGCAA	GTCGGAGATTCGTAGCTGGA
Il-6	GACAACTTTGGCATTGTGG	ATGCAGGGATGATGTTCTG
Itgb6	GCAGAACGCTCTAAGGCCAA	AAAGTGCTGGTGGAACCTCG
ITGB6	TCCATCTGGAGTTGGCGAAAG	TCTGTCTGCCTACACTGAGAG
Itgb8	ACTGGGCCAAAGTGAACACA	TCTTGAACACACCATCCGCA
ITGB8	ACCAGGAGAAGTGTCTATCCAG	CCAAGACGAAAGTCACGGGA
Jnk	ATGGCTGTCGATATTCAACCAG	CCTCTTGGGCATACCCCAC
Pcna	TTTGAGGCACGCCTGATCC	GGAGACGTGAGACGAGTCCAT
Pdgfα	GACGGTCATTTACGAGATACCTC	CTACGCCTTCCTGTCTCCTC
Rock2	TTGGTTCGTCATAAGGCATCAC	TGTTGGCAAAGGCCATAATATCT
Tgfb1	ATGTCACGGTTAGGGGCTC	GGCTTGCATACTGTGCTGTATAG
Tgfb2	ATGTCACGGTTAGGGGCTC	GGCTTGCATACTGTGCTGTATAG
TGFβ1	TGCAAGTGGACATCAACGGG	GCACGATCATGTTGGACAGC
Tnf-α	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
Yap	CTGCGTGCAGAAATGCTACTG	AGCCGTAGAGTAATGGTGGATAG
Zfyve19	GGCTTCGTCAGGAAAACAAG	GTATCTGGTGCCTGGTGTG
ZFYVE19	GGTCACCACCTCAGAACTATAA	AATCATCTGGTCTTGTCGTGTC

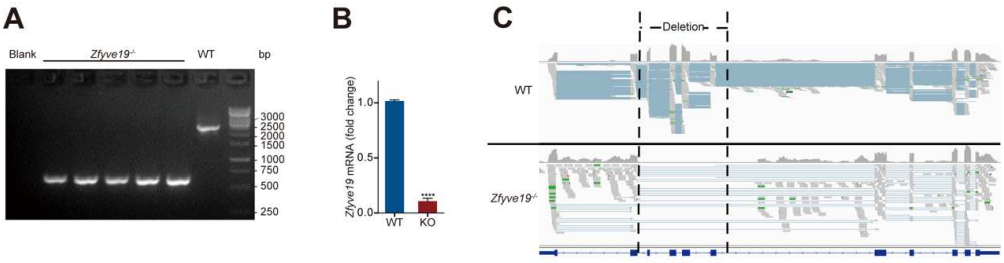
Suppl. Table 2. Primary antibodies

Target	Species	Source	Catalog #	Dilution	Application
ac-α-Tubulin	Mouse	SantaCruz	sc-23950	1:800	IF
γ-TUB	Mouse	sigma	T6557	1:2500	IF
ARL13B	Rabbit	Proteintech	17711-1-AP	1:2000	IF
ARL13B	Mouse	neuromab	75-287	1:1000	IF
CEP170	Mouse	Life Technologies	72-413-1	1:1500	IF
CK7	Mouse	Abcam	ab9021	1:400	IF
CK19	Rabbit	Abcam	ab52625	1:800	IF
CP110	Rabbit	Proteintech	12780-1-AP	1:1500	IF
GAPDH	Mouse	Proteintech	HRP-60004	1:6000	WB
HNF-4α	Mouse	SantaCruz	sc-374229	1:200	IF
Laminin	Rabbit	sigma	L9393	1:200	IF
p-SMAD2	Rabbit	CST	3108	1:1000	WB
PCNA	Mouse	Biossci	PA1037	1:800	IF
SOX9	Rabbit	Millipore	AB5535	1:800	IF
SMAD2	Mouse	CST	3103	1:1000	WB
ZFYVE19	Rabbit	Proteintech	23163-1-AP	1:3000	WB
ZFYVE19	Mouse	Sigma	WH0084936M1	1:50	IF

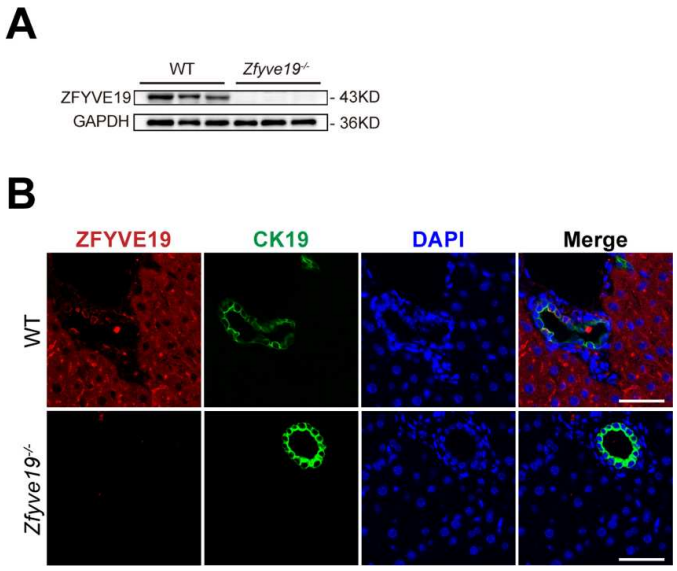
Suppl. Table 3. Secondary antibodies

Secondary antibody	Source	Catalog #	Dilution
Goat anti-mouse IgG (H+L) Highly Cross-Adsorbed, Alexa Fluor 594	Invitrogen	A-11032	1:50
Goat anti-rabbit IgG (H+L) Highly Cross-Adsorbed, Alexa Fluor 488	Invitrogen	A-11034	1:300
Horseradish peroxidase (HRP) – conjugated Affinipure goat anti-mouse IgG (H+L)	Proteintech	SA00001-1	1:5000
HRP-conjugated Affinipure goat anti-rabbit IgG (H+L)	Proteintech	SA00001-2	1:5000

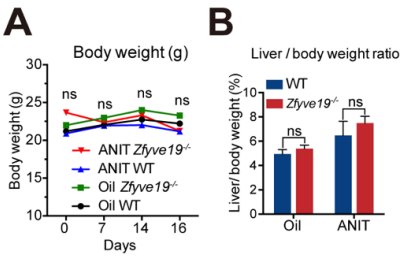
Supplementary figures and figure legends:



Suppl. Fig. 1. Depletion of *Zfyve19* at DNA and mRNA levels.
(A) *Zfyve19*$^{-/-}$ mice were genotyped by PCR, using primer 1 (mouse *Zfyve19*-F: 5′-CAA AATCTGGTCACAACTTCACTTCC-3′) and primer 2 (mouse *Zfyve19*-R: 5′-GTAGCAATT CAGCTCTACATCGCAC-3′) to obtain 2,643-bp and 620-bp fragments for the wild-type (WT) and mutant alleles, respectively. (B) Histogram, expression of *Zfyve19* mRNA as assessed by RT-qPCR (n=3). (C) Visualized expression of *Zfyve19* mRNA in *Zfyve19*$^{-/-}$ and WT mice by RNA-Seq mRNA reads in the Intergrative Genomics Viewer. Statistical significance is displayed as ****$p < 0.0001$.

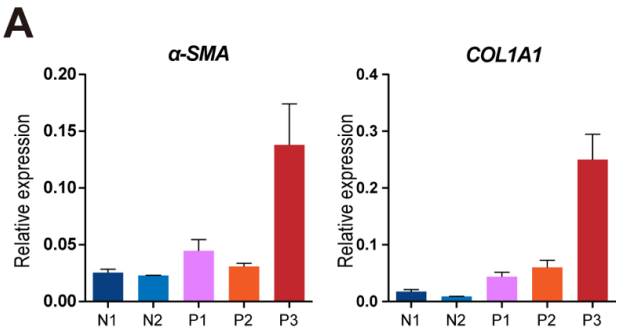


Suppl. Fig. 2. Depletion of Zfyve19. (A) Western blot of Zfyve19, mouse liver. (B) Immunofluorescence photomicrographs, Zfyve19 (red) in liver; cytoplasmic expression, hepatocytes and cholangiocytes of wild-type (WT) mice; absent expression, *Zfyve19*-knockout mice. Ck19 (green) and 4′,6-diamidino-2-phenylindole (DAPI, blue) as control. Scale bar: 50 µm.

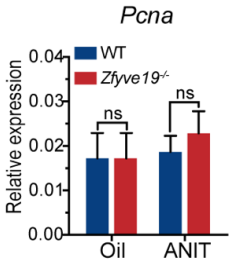


Suppl. Fig. 3. Changes in body weight and liver weight in *Zfyve19*$^{-/-}$ mice and wild-type (WT) littermate gavaged with alpha-naphthyl isothiocyanate (ANIT) or oil vehicle only.

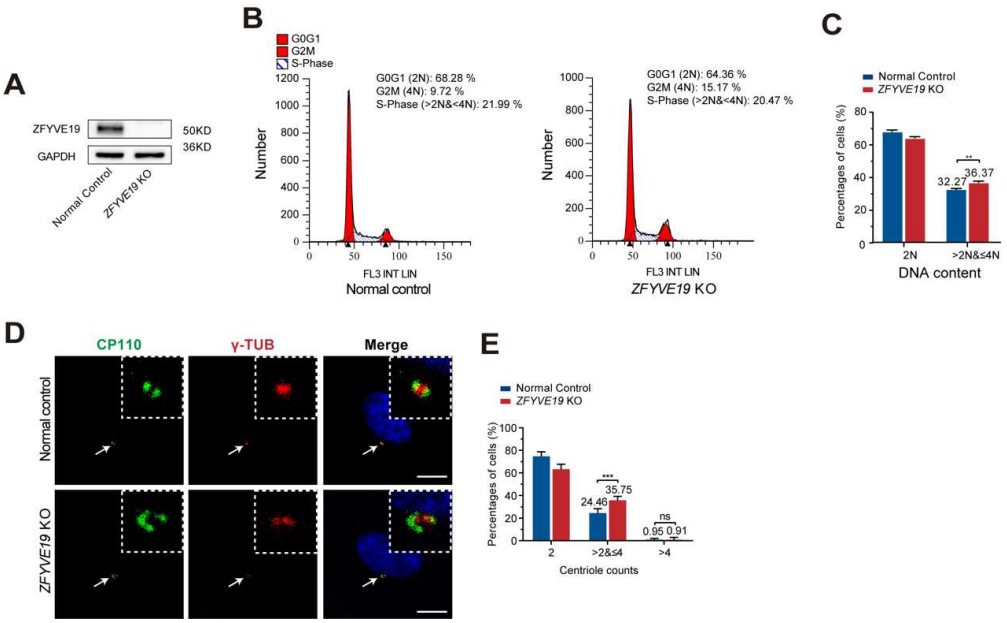
(A) Body weight over 16 days of treatment. (B) Liver weight / body weight ratios. ns, not significant.



Suppl. Fig. 4. Histograms, expression of *α-SMA* and *COL1A1* as assessed by RT-qPCR in livers from *ZFYVE19*-disease patients (P1~3) and normal controls (N1~2).

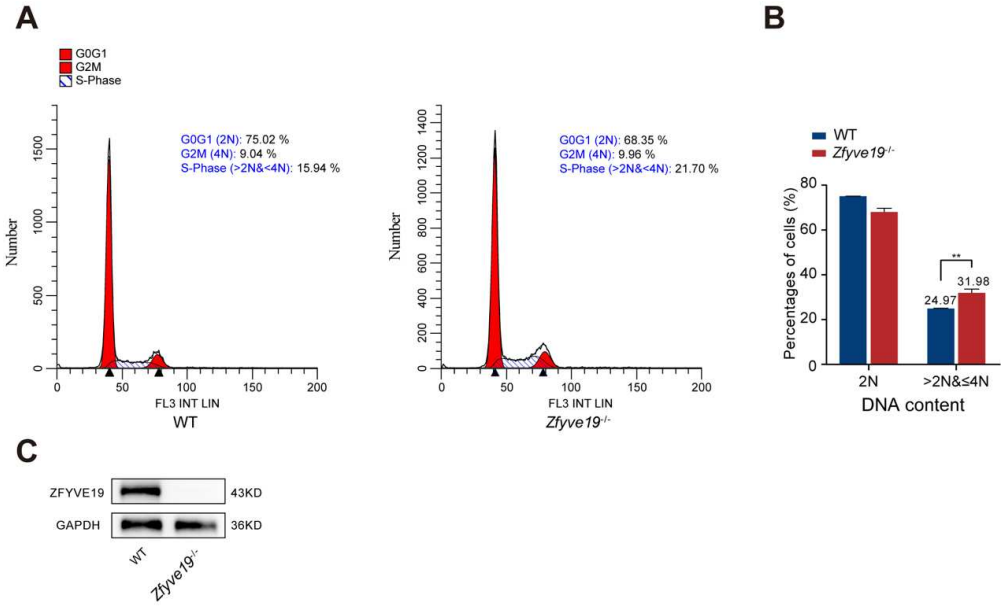


Suppl. Fig. 5. The expression of *Pcna* mRNA in *Zfyve19*$^{-/-}$ mice and wild-type (WT) littermate gavaged with ANIT or oil vehicle only. ns, not significant.



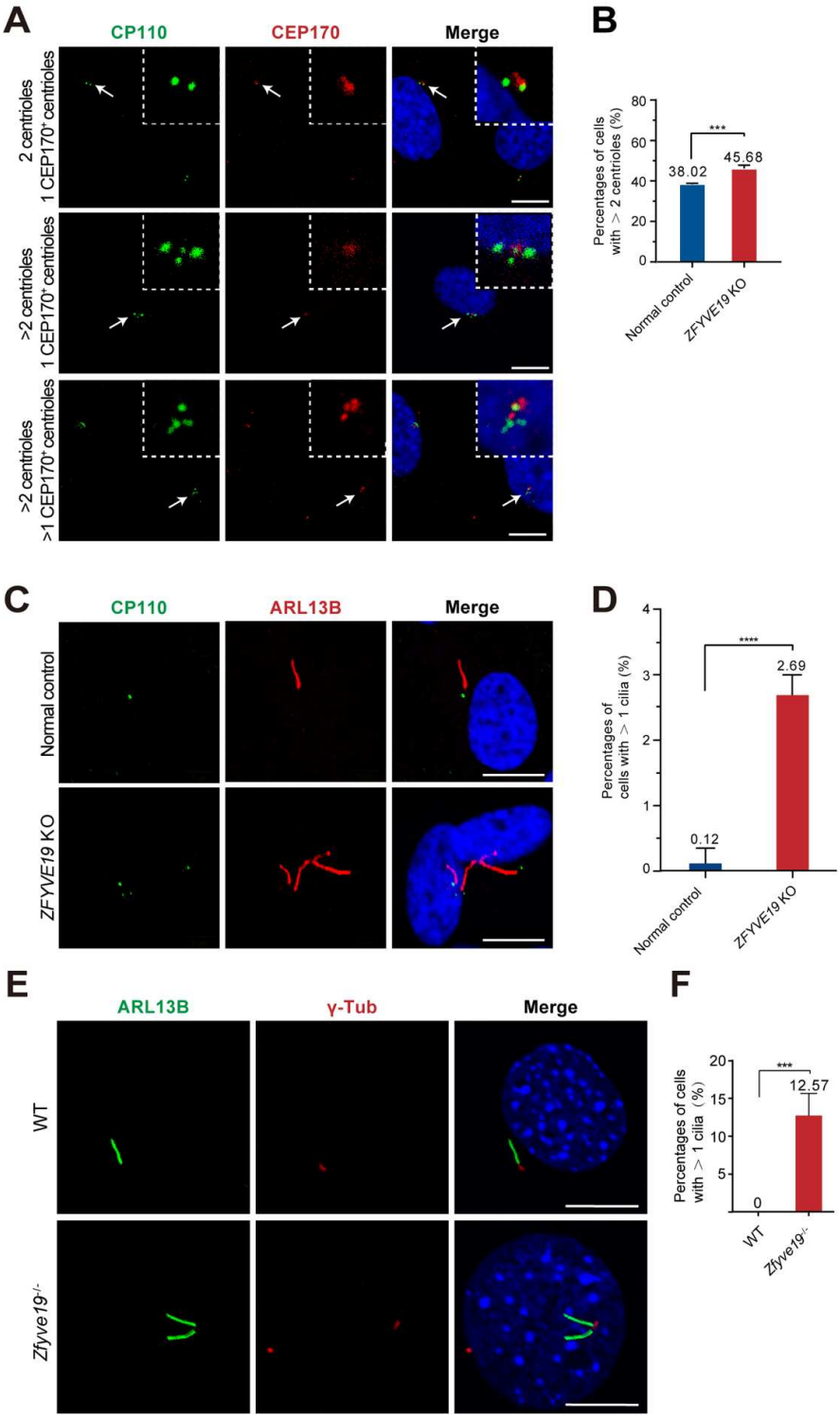
Suppl. Fig. 6. Changes in DNA content and centriole numbers in *ZFYVE19*-knockout RPE-1 cells.

(A) Western blot of ZFYVE19. (B) Cell cycle distributions of DNA content by flow cytometry analysis. (C) Histogram of DNA content (3 independent experiments). (D) Immunofluorescence photomicrographs of CP110 and γ-tubulin (γ-Tub). 4′,6-diamidino-2-phenylindole, DAPI. Scale bars: 10 μm. (E) Histogram, percentages of cells with different numbers of centrioles ($CP110^{+}$). At least 120 cells per group were counted in 3 independent experiments. Data are shown as mean ± standard deviation. ns, not significant; $^{**}p < 0.01$; $^{***}p < 0.001$.



Suppl. Fig. 7. Changes of DNA content in *Zfyve19*$^{-/-}$ mouse embryonic fibroblasts (MEFs).

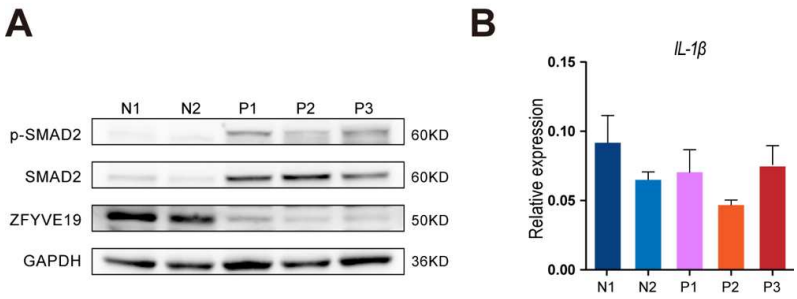
(A) Cell cycle distributions on DNA content, flow cytometry analysis. (B) Histogram of DNA content (3 independent experiments). (C) Western blot of Zfyve19. Data are shown as mean ± standard deviation. **p < 0.01.


A
CP110
CEP170
Merge
2 centrioles 1 CEP170+ centrioles
>2 centrioles 1 CEP170+ centrioles
>2 centrioles >1 CEP170+ centrioles
B
Percentages of cells with > 2 centrioles (%)
38.02
45.68
***
Normal control
ZFYVE19 KO
C
CP110
ARL13B
Merge
Normal control
ZFYVE19 KO
D
Percentages of cells with > 1 cilia (%)
0.12
2.69
****
Normal control
ZFYVE19 KO
E
ARL13B
γ-Tub
Merge
WT
Zfyve19-/-
F
Percentages of cells with > 1 cilia (%)
0
12.57
***
WT
Zfyve19-/-

Suppl. Fig. 8. Changes in numbers of mature mother centrioles and cilia in RPE-1 cells and mouse embryonic fibroblasts (MEFs).
(A) Immunofluorescence (IF) photomicrographs, representing pattern of CP110 and CEP170 in RPE-1 cells. (B) Histogram, percentages of RPE-1 cells with > 1 $CEP170^{+}$ centrioles, among cells with > 2 centrioles. At least 70 cells with > 2 centrioles per group were counted in each of 3 independent experiments. (C) IF photomicrographs, CP110 and ARL13B in RPE-1 cells. (D) Histogram, percentages of RPE-1 cells with more than 1 cilium, averaged over at least 3 view fields (40x) in each group. (E) IF photomicrographs, ARL13B and γ-tubulin (γ-Tub) in wild-type (WT) and *Zfyve19*$^{-/-}$ MEFs. (F) Histogram, percentages of cells with > 1 cilium, averaged over at least 3 view fields (40x) in each group. Data are shown as mean ± standard deviation. Scale bars: 10 μm. ***$p < 0.001$, ****$p < 0.0001$.

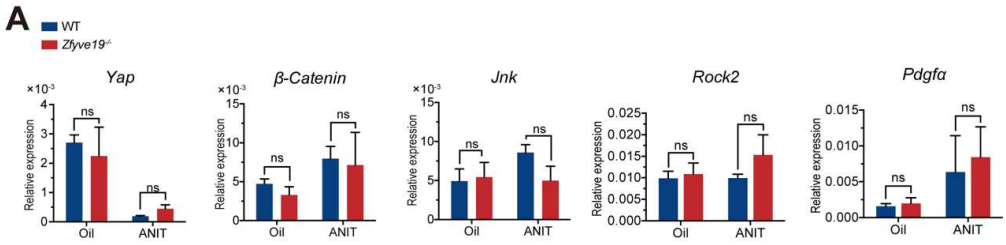
Suppl. Fig. 9. Gene set variation analysis of signaling pathways in patients with *ZFYVE19* defects (n=3) compared with normal data from The Cancer Gene Atlas database.

TGF-β signaling pathways are significantly up-regulated in patients with *ZFYVE19* defects.



Suppl. Fig. 10. Changes in inflammatory factor and signaling pathway associated with fibrosis in human livers.

(A) Western blot of p-SMAD2, SMAD2, and ZFYVE19. (B) *IL-1β* mRNA expression, measured by RT-qPCR. N: Normal controls. P: *ZFYVE19*-disease patients.



Suppl. Fig. 11. Changes in signaling pathways associated with liver fibrosis in mouse.

Expression of *Yap* (Hippo), *β-Catenin* (Wnt), *Jnk, Rock2* (Planar cell polarity pathway), and *Pdgfα* assayed by RT-qPCR in livers of *Zfyve19*$^{-/-}$ and WT mice treated with either ANIT or oil (n=5-6 each). Data are shown as mean ± standard deviation. ns, not significant.