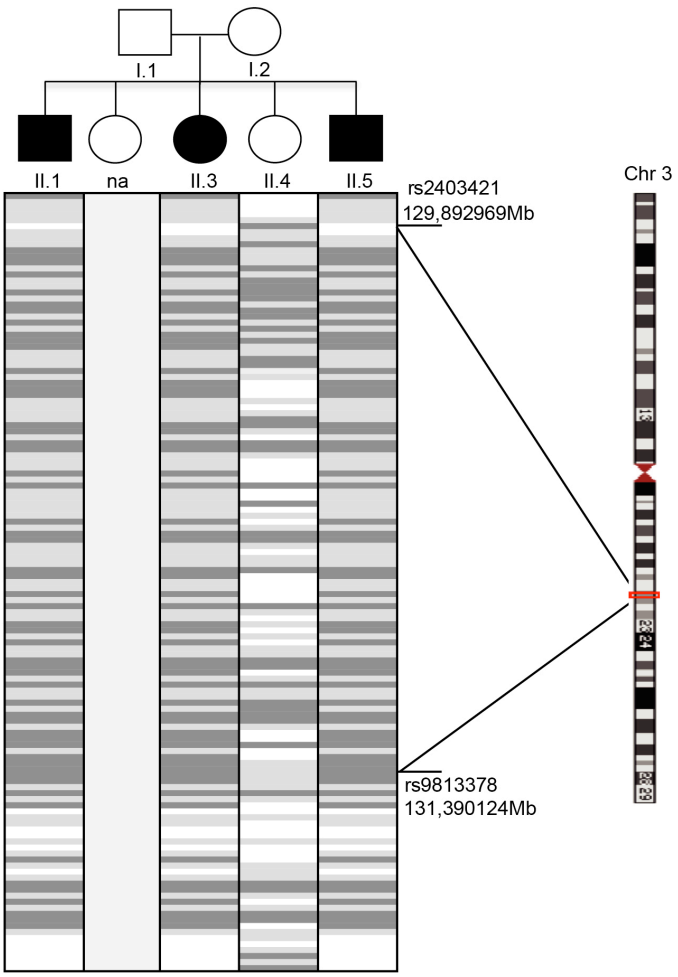
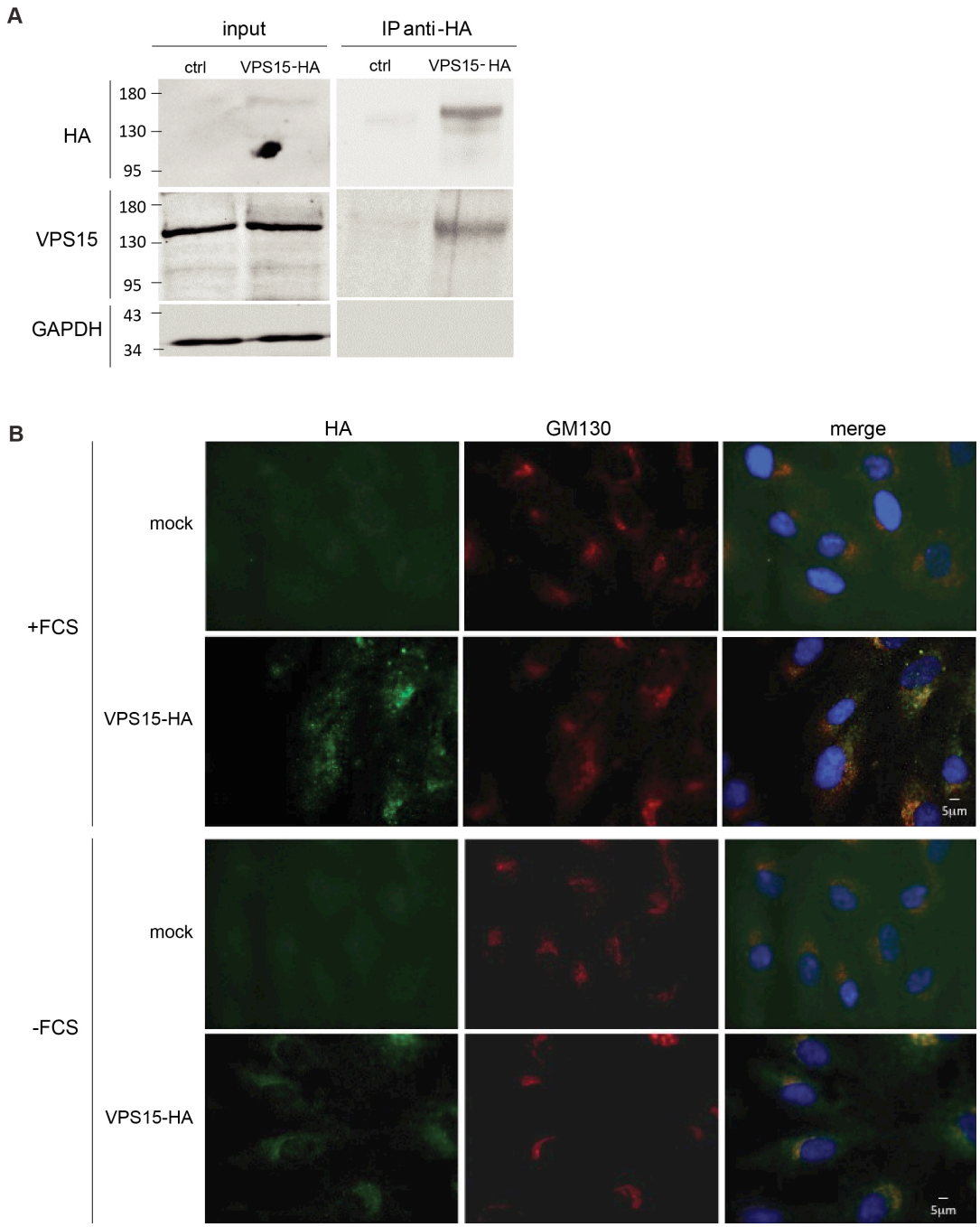
A



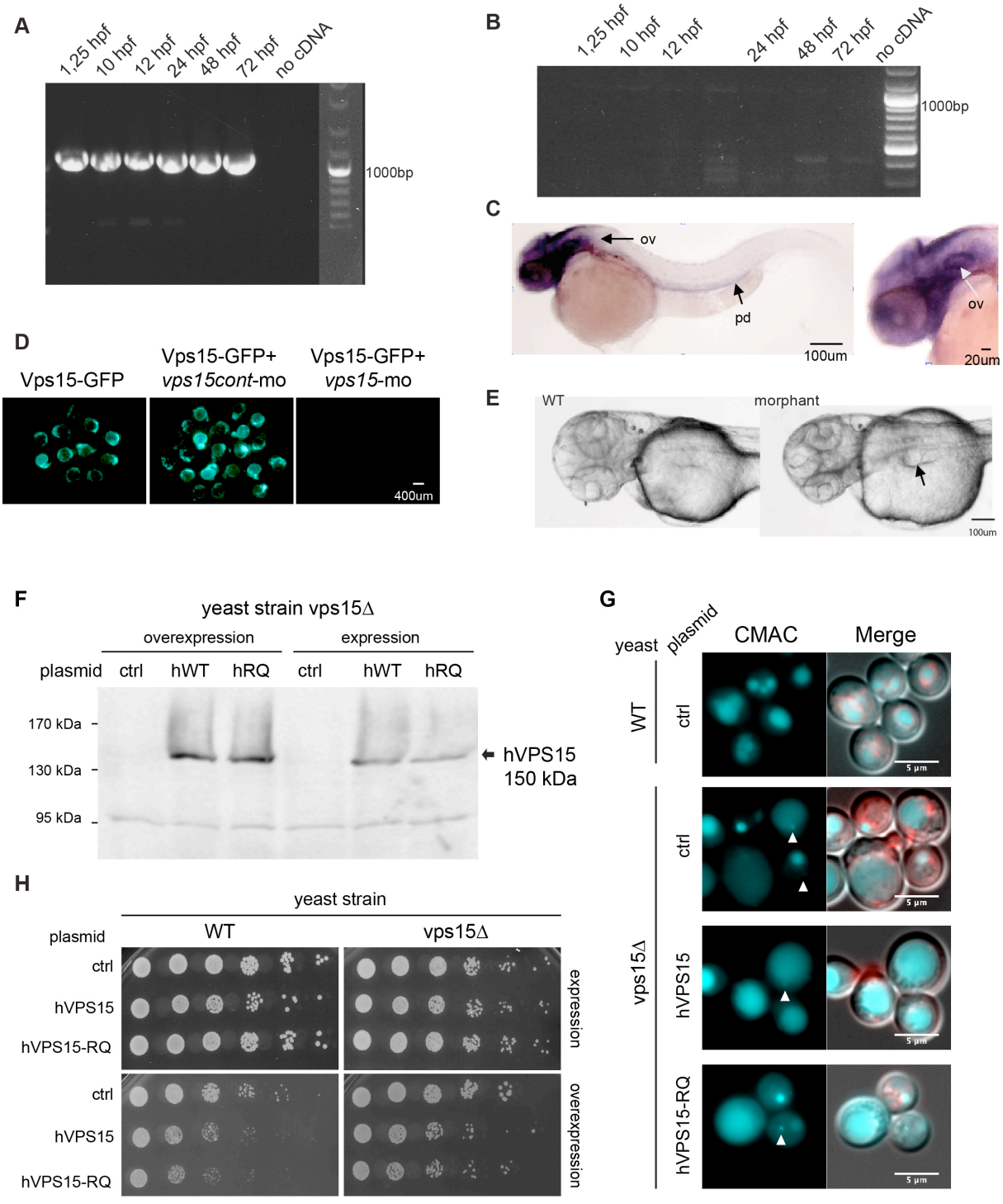

B

| Chromosome | 250K Affymetrix Array | genomic position (hg19) |
|---|---|---|
| Chr 3 | rs2403421-rs9813378 | 129,892969 to 131,390124 Mb |
| | rs1907861-rs2126806 | 163,606756 to 164,286477 Mb |
| Chr 6 | rs10944288-rs13202853 | 87,651742 to 88,505654 Mb |
| Chr 20 | rs6032171-rs6104453 | 44,044003 to 44,712840 Mb |

**Supplementary Figure 1: Pedigree of the family with the homozygosity mapping. A.** Pedigree of the family, underlined by corresponding schematic representation of the homozygosity mapping results that has been performed on 3 affected individuals and 1 unaffected individual using the Affymetrix 250K SNP array. This representation shows the chromosome 3 homozygous region that is common for the three affected individuals and the position of the *PIK3R4* gene. Gray shading indicates homozygous SNPs (light gray for AA and darker for BB) and white zones indicate heterozygous alleles (AB). **B.** Supplemental homozygous regions specific to the three affected individuals. Positions are given following GRCh37 hg19 reference genome.

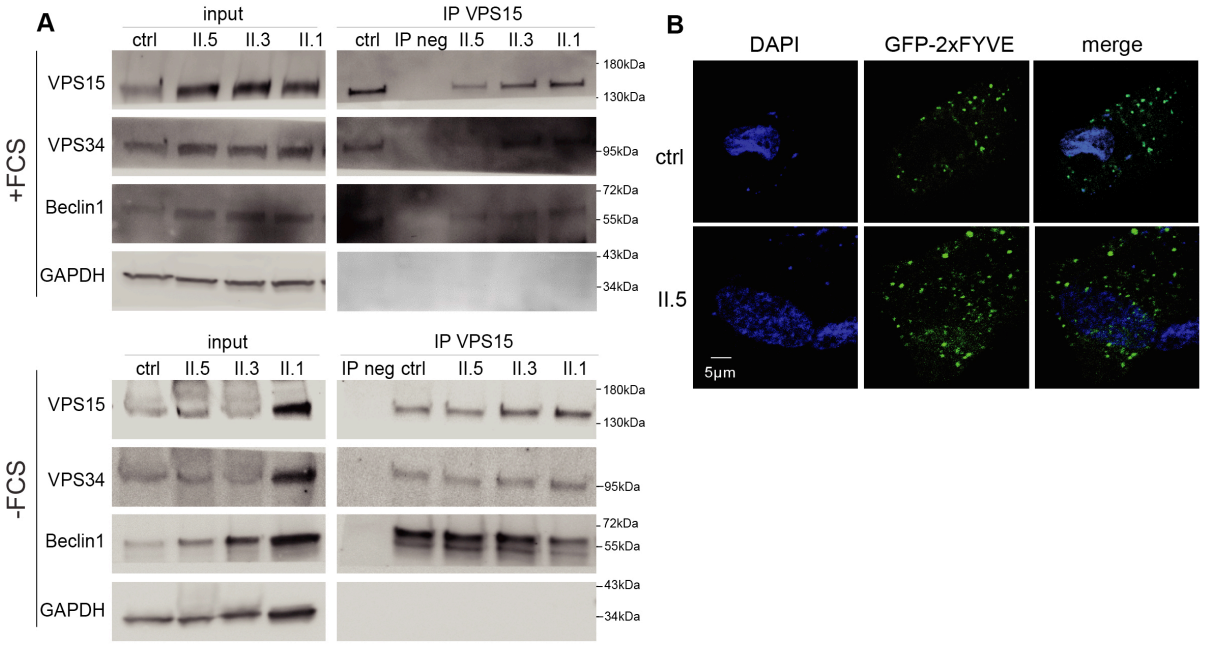



**Supplementary figure 2: VPS15-HA expression from the VPS15-HA construction in hTERT-RPE1 cells and primary fibroblasts. A**: hTERT-RPE1 cells were mock transfected (ctrl) or transfected with the VPS15-HA construct. 24h post transfection, cells were collected and an immunoprecipitation against HA performed. In WB HA was detected with an anti-HA antibody and VPS15 with the antibody directed against the WD40 region of VPS15. GAPDH was done as a control. **B:** Ctrl skin fibroblasts were either mock transfected or transfected with the VPS15-HA construct. 6h post transfection medium was changed and cells were either grown in complete medium (+FCS) or deprived of serum (-FCS) for 24h. Then DAPI staining as well as IFs against the VPS15-HA protein and the Golgi marker GM130 were performed.




**Supplementary Figure 3: Controls of zebrafish and yeast experiments. A-B:** RT-PCR with cDNA of 6 different developmental stages (from 1,25 to 72 hpf) amplifies a 1000 bp band (arrow) as expected. *vps15* mRNA is maternally expressed before midblastula transition (1,25 hpf) and mRNA is detectable until at least 72 hpf. PCR without prior reverse transcription did not yield a 1000 bp band (**B**) indicating that the amplified fragment is not due to contamination with cDNA or genomic DNA**. C**: *In situ* hybridization with *vps15* antisense probe in zebrafish embryos showing ubiquitous expression at 14 hpf and prominent localization in the head as well as in the pronephric duct (pd) at 48 hpf. Ov: otic vesicle.

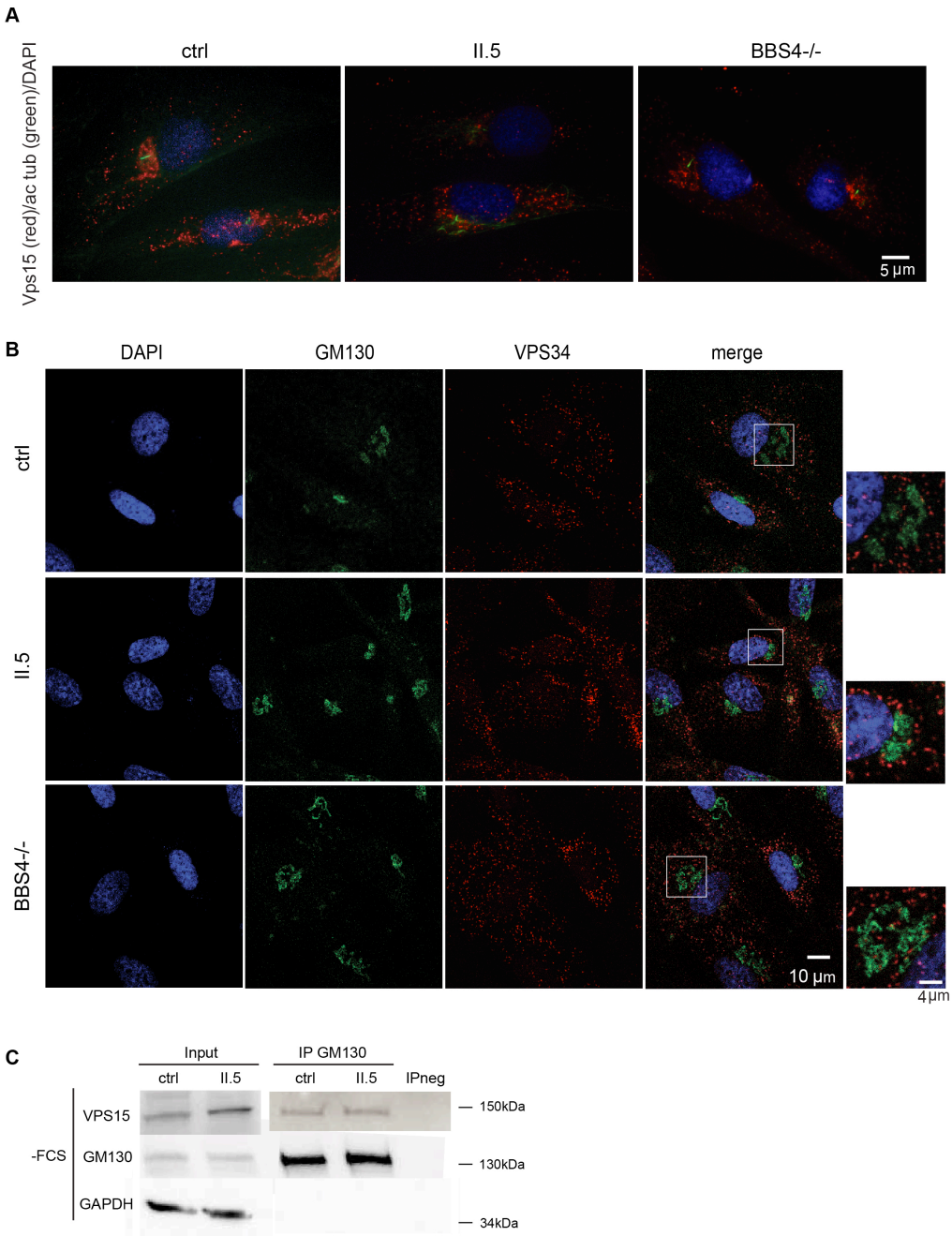
**D.** Co-injection of *vps15*-mo with zVps15-GFP plasmid completely blocked GFP expression. As expected, co-injection of the mismatch control *vps15cont*-mo with the zVps15-GFP reporter did not reduce the GFP expression as compared to controls injected with the zVps15-GFP plasmid. Embryos are 20 hpf old. **E** picture of WT and morphant zebrafish showing kidney cysts in the morphant (black arrow) but not the WT. **F.** *vps15*Δ yeast cells were transformed with a control empty plasmid (ctrl), an expression (CEN) or overexpression (2μ) plasmid bearing wild-type hVPS15 or mutant hVPS15-R998Q and a western blot against hVPS15 was performed. **G.** Wild-type (WT) and *vps15*Δ yeast cells transformed with a control (ctrl), hVPS15 or hVPS15-R998Q expression plasmid (CEN) were labeled with a soluble dye (CMAC) staining the lumen of the vacuole. White arrows show the additional CMAC positive compartment (CPC) in *vps15*Δ yeast cells. **H.** Drop test assays of wild-type WT and *vps15*Δ yeast cells expressing (CEN) or overexpressing (2μ) hVPS15 or hVPS15-R998Q. Plates were incubated at 30°C. Yeast strains transformed with an empty plasmid (ctrl) were used as controls.



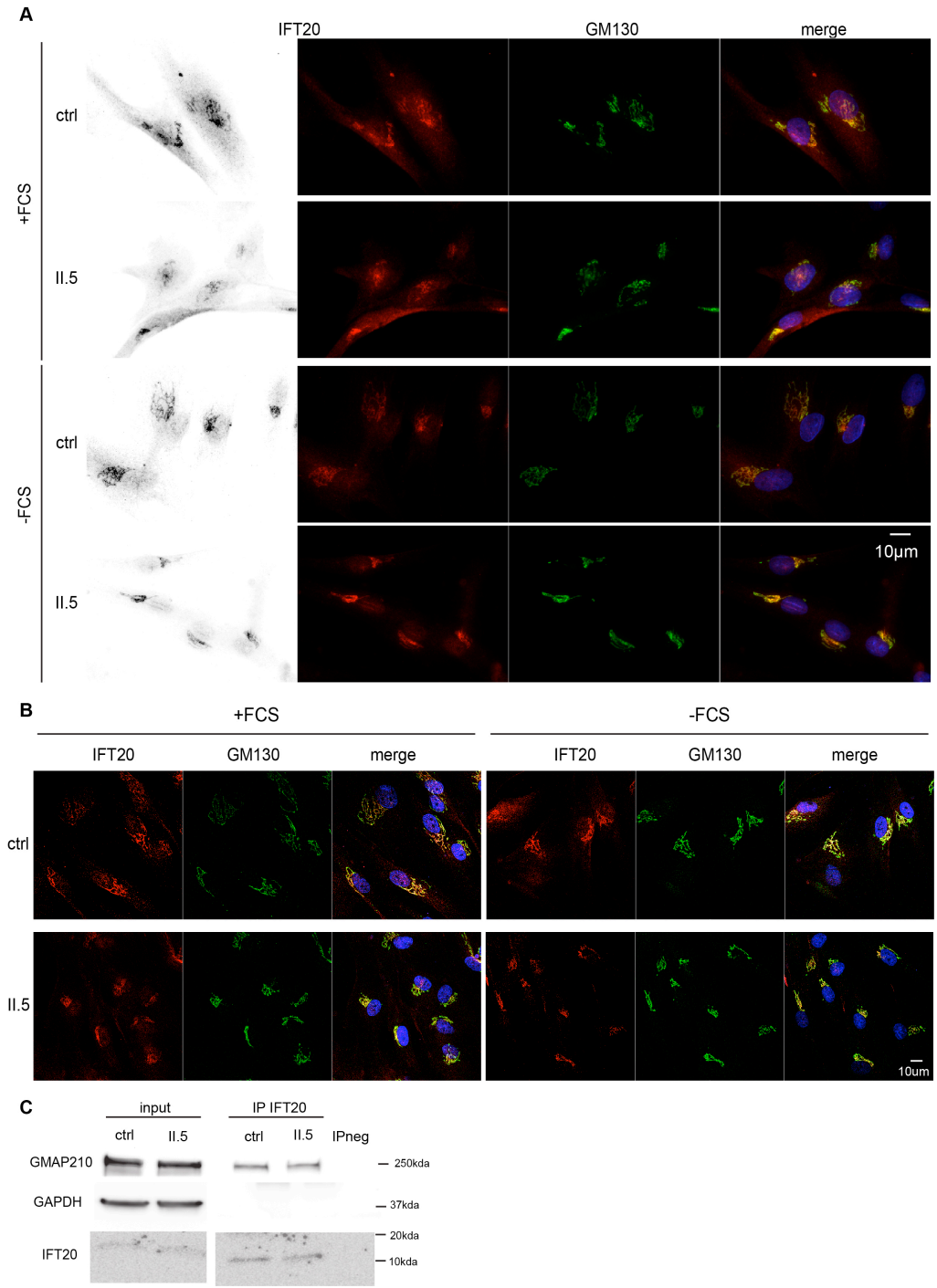

C

| | | | | | mean nb spectra | | ctrl 1 sample 26032015 | | | | | II.5 sample 26032015 | | | | | ctrl 2 sample 09062015 | | | | | II.1 sample 09062015 | | | | | II.3 sample 09062015 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | IPneg | IP | IP-VPS15_ctrl1 | | | | | IP-VPS15_2-5 | | | | | IP-VPS15_ctrl2 | | | | | IP-VPS15_2-1 | | | | | IP-VPS15_2-3 | | | | |
| Protein | ACCESSION Nb | MW(kDa) | pI | Gene ID | #Spectra | #Spectra | Score | #Peps | #Spectra | SC(%) | RMS(ppm) | Score | #Peps | #Spectra | SC(%) | RMS(ppm) | Score | #Peps | #Spectra | SC(%) | RMS(ppm) | Score | #Peps | #Spectra | SC(%) | RMS(ppm) | Score | #Peps | #Spectra | SC(%) | RMS(ppm) |
| VPS15 | PI3R4_HUMAN | 153.0 | 6.8 | 30849 | 0 | 140,8 | 1787 | 32 | 84 | 30 | 4,02 | 2595 | 40 | 112 | 37 | 7,82 | 3674 | 56 | 208 | 45,8 | 5,48 | 3880 | 58 | 163 | 48,5 | 6,55 | 3512 | 52 | 137 | 46,4 | 5,47 |
| VPS34 | PK3C3_HUMAN | 101.5 | 6.4 | 5289 | 0 | 93,6 | 1115 | 18 | 43 | 23,8 | 3,98 | 1432 | 20 | 68 | 29,2 | 8,3 | 2962 | 46 | 155 | 58,5 | 6,36 | 3096 | 44 | 121 | 59,9 | 8 | 2172 | 32 | 81 | 41,4 | 5,15 |
| GM130 | GOGA2_HUMAN | 113.0 | 4.9 | 2801 | 0 | 39,2 | 719,6 | 12 | 23 | 18 | 10,33 | 1035 | 14 | 31 | 21,3 | 9,79 | 1465 | 21 | 49 | 30,3 | 4,1 | 1593 | 22 | 51 | 33,4 | 5,47 | 1632 | 20 | 42 | 31,1 | 4,04 |
| UVRAG | UVRAG_HUMAN | 78.1 | 9.4 | 7405 | 0 | 31,8 | 475 | 8 | 14 | 14,2 | 4,17 | 505,6 | 8 | 16 | 14,4 | 6,61 | 1336 | 25 | 56 | 38,6 | 6,41 | 1218 | 20 | 37 | 36,2 | 5 | 1138 | 20 | 36 | 39,3 | 4,11 |
| Beclin1 | BECN1_HUMAN | 51.9 | 4.7 | 8678 | 0 | 31,4 | 484,6 | 7 | 17 | 22 | 4,04 | 571,6 | 7 | 18 | 21,1 | 6,71 | 1223 | 19 | 56 | 40,7 | 4,17 | 1162 | 17 | 33 | 37,8 | 6,61 | 1136 | 18 | 33 | 47,1 | 5,09 |
| Nrbf2 | NRBF2_HUMAN | 32.4 | 5.5 | 29982 | 0 | 21,6 | 416,5 | 7 | 10 | 27,9 | 5,84 | 366,5 | 5 | 10 | 34,1 | 5,02 | 585 | 7 | 33 | 41,8 | 9,28 | 687,4 | 12 | 32 | 64,1 | 11,78 | 524,8 | 8 | 23 | 40,4 | 5,69 |
| Atg14L | BAKOR_HUMAN | 55.3 | 6.7 | 22863 | 0 | 18,6 | 390,7 | 5 | 11 | 16,3 | 3,63 | 501,7 | 7 | 15 | 22,6 | 4,8 | 666 | 10 | 32 | 28,7 | 3,63 | 654 | 8 | 19 | 25,8 | 4,72 | 525,1 | 6 | 16 | 19,9 | 2,08 |

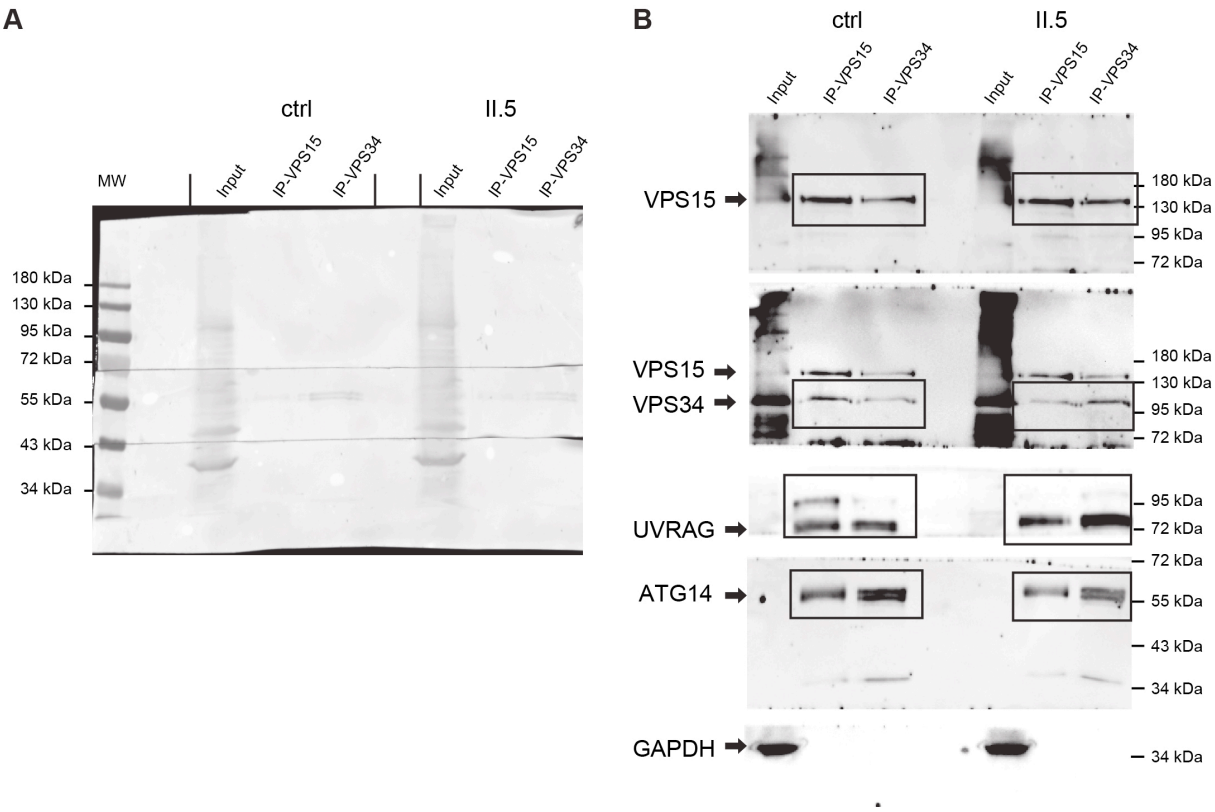
**Supplementary Figure 4: VPS15 and VPS15-R998Q protein complexes and PtdIns(3)P staining in human fibroblasts. A.** Control (ctrl) and patient (II.1, II.3 and II.5) fibroblasts were grown in complete medium (+FCS) or deprived of FCS for 24 hours. Immunoprecipitation with a VPS15 antibody was done on cell lysates (input) and interaction (IP VPS15) with the indicated proteins (VPS34, Beclin1 and GAPDH) was detected by western blot. IPneg represent the control immunoprecipitation done without VPS15 antibodies. **B.** Control and patient II.5 fibroblasts were grown in complete medium and transfected with a GFP-2XFYVE plasmid, 24 hrs post transfection cells were observed on a fluorescence microscope. **C.** VPS15 was immunoprecipitated from control (ctrl1 and ctrl2) and patient (II.5, II.1 and II.3) fibroblast protein lysates and samples prepared for and analysed by mass spectrometry. Interaction partners were determined by searching against the complete Human proteome set from the SwissProt database. All retained interaction partners were completely absent from the negative control immunoprecipitations (IPneg) omitting VPS15 antibodies (mean number of spectra "0" in the IPneg column) but present in all five VPS15 immunoprecipitation samples (ctrl1, ctrl2, II.5, II.1 and II.3).



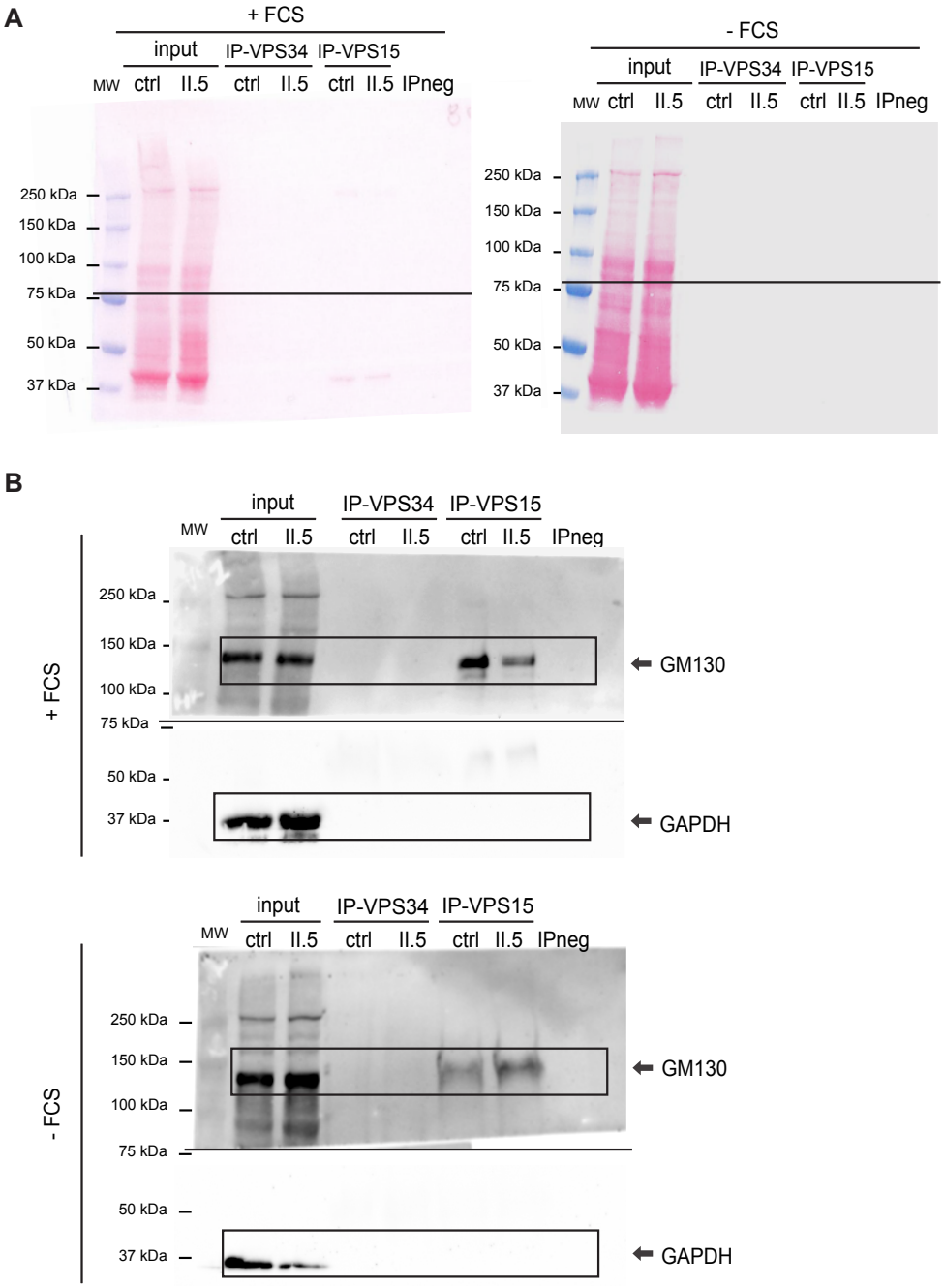

**Supplementary Figure 5: Primary cilia and VPS15 do not colocalize and VPS34 is not Golgi-localized. A.** Control (ctrl) and patient II.5 fibroblasts were deprived of serum for 24 hrs and fixed. Immunofluorescence against acetylated tubulin (green) and VPS15 (red) and DAPI staining were performed and pictures taken on a fluorescence microscope, only the merge is shown. **B.** Control (ctrl) and patient II.5 fibroblasts were deprived of FCS for 24 hrs and fixed. Immunofluorescence against GM130 (green) and VPS34 (red) and DAPI (blue) staining were performed and pictures taken on a confocal microscope. White square: zoomed area. **C.** Control (ctrl) and patient (II.5) fibroblasts were deprived of serum for 24 hrs. An immunoprecipitation against GM130 was performed on total cell lysates and VPS15 and GM130 detected by western blot. Negative immunoprecipitation without antibodies (IP neg) was used as a control.




**Supplementary Figure 6: Intracellular localization of IFT20 in control and patient fibroblasts. A.** Control (ctrl) and patient II.5 fibroblasts were grown in complete medium (+FCS) or deprived of serum for 24 hrs (-FCS) and fixed. Immunofluorescence against IFT20 (red and inverted black/white panel) and GM130 (green), and DAPI staining were performed and localization of IFT20 in the cells was observed on a fluorescence microscope. **B.** The same immunofluorescence stained slides were observed with a confocal microscope. **C.** Control (ctrl) and patient (II.5) fibroblasts were deprived of serum for 24 hrs. An immunoprecipitation against IFT20 was performed on total cell lysates and GMAP210 and IFT20 detected by western blot. Negative immunoprecipitation without antibodies (IP neg) was used as a control.




**Supplementary Figure 7: Uncropped images of western blots for Figure 4B. A.** Ponceau staining of the blot, the blot was cut in several pieces that were incubated with the indicated antibodies. **B.** Western blots were revealed with anti-VPS34, -VPS15, -UVRAG, -ATG14 and -GAPDH antibodies, as indicated.




**Supplementary Figure 8: Uncropped images for western blots for Figure 5A. A.** Ponceau staining of the two blots, the blots were cut in two pieces that were incubated with the indicated antibodies. **B.** Western blots were revealed with anti-GM130 and anti-GAPDH antibodies, as indicated.

| **Patients** | **II.1** | | **II.3** | | **II.5** | | **II.4** | |
|---|---|---|---|---|---|---|---|---|
| **Type of sequence variant** | SNV | Indel | SNV | Indel | SNV | Indel | SNV | Indel |
| Total number of variants | 52646 | 8185 | 52434 | 8178 | 52576 | 8253 | 55365 | 8522 |
| After exclusion of non-pathogenic variants (as determined from the ClinicalSignificance field in dbSNP) validated by at least 2 methods in dbSNP (as determined from the "Validation Status" field) | 8980 | 5296 | 8427 | 5294 | 8962 | 5378 | 10899 | 5578 |
| After exclusion of variants with an allele frequency > 1% (extracted from the dbSNP database, the Exome Variant Server, the 1000g and the ExAC database) | 7132 | 3790 | 6533 | 3813 | 7081 | 3883 | 8830 | 4084 |
| After exclusion of variants found in the homozygous state + exclusion of variants found more than once in the heterozygous state in 70 control exomes | 2193 | 844 | 1633 | 816 | 2199 | 892 | 4227 | 926 |
| After exclusion of 5'UTR, 3'UTR, downstream, upstream and intron locations without local splice effect prediction (from the "localSpliceEffect" field of Alamut-Batch) | 1120 | 204 | 778 | 196 | 1121 | 233 | 2190 | 245 |
| After exclusion of synonymous variants without local splice effect prediction (from the "localSpliceEffect" field of Alamut-Batch) | 881 | 204 | 597 | 196 | 891 | 233 | 1764 | 245 |
| After selection of variants consistent with recessive transmission (compound heterozygous, homozygous variants). | 1 homozygous variant in the *PIK3R4* gene<br>2 compound heterozygous variants in the *AK9* and *RECQL4/MFSD3* genes | | | | | | | |

**Supplementary Table 1:** Summary of the exome sequencing results from the three affected probands and the healthy sister.

| RefSeq Gene | Gene | Forward (5'-3') | Reverse (5'-3') |
|---|---|---|---|
| Human VPS15/PIK3R4 NM_014602.2 | PIK3R4-ex2 | TGGTGTTTCACAGCTTCTTTG | GCAGGAATGCTGCCTCTAAC |
| | PIK3R4-ex3 | GGTTTTGCATTTTGGGTTTT | TTAAAGCAAAAGTGGGAACTAGA |
| | PIK3R4-ex4 | GCCTTTGTAGCTTTCATCTGG | TGAGAGAACACACAAACGTTGA |
| | PIK3R4-ex5 | GACAGGGAGGAAAAGTTAATAAGC | GTTTTACAAGTTAGCATCAGCAATTC |
| | PIK3R4-ex6 | TGGGACTATCTGTATGGGGAAA | TGCATGGAAGTATTTGAGAGGA |
| | PIK3R4-ex7 | TCCTTACATGTCTTGAAATGACTTT | AGTAGGAACCCCACACAATCA |
| | PIK3R4-ex8 | TTTGTTTATCCCGATAGTTTGAA | CCTTCAAAGGTGCTTGGTTC |
| | PIK3R4-ex9 | CTTCTACACACTTACAGATGTTG | TGCCTAAGATGTTTGCCAGA |
| | PIK3R4-ex10 | TGGGTGATTTGTCCCTTCAG | TCTGTGAAATGGGGCTTTTT |
| | PIK3R4-ex11 | GGCAATAAAAGCACATAATGGTT | CGTTCAAAGACATTAGGAGGAC |
| | PIK3R4-ex12 | TGTCTTCAAATATACTTTGCTTACGAC | TTCTGGGTTTCCCAAATAACA |
| | PIK3R4-ex13 | TTGCCAAATTGATGTCTCAG | CACAGAAGAAGGATTTTTGTCCA |
| | PIK3R4-ex14 | TTTAACATTAGCAGTGACATTGGTT | CATTGCAGGGTAGAAAGGAGA |
| | PIK3R4-ex15 | TCGGCTTTCGTTTTCTTGAT | CAGGCATTAGTGGCATTCAA |
| | PIK3R4-ex16 | ATGCAGCTTGATGCACTTTG | CCAAGAAAATCTCCCAACACA |
| | PIK3R4-ex17-ex18 | TGTCTCTCCCCCTGAAAGAA | TGATTTTAAACATTACAGTGCCATC |
| | PIK3R4-ex18-ex19 | CGGATGTCTTTTATTTGACTCCA | CACTTTCTTTATGCTTATTTTAC |
| | PIK3R4-ex20 | CTGACGAGAGGAAAAATGTG | CCACCAAAACCACAAACTCTT |
| Zebrafish *zvps15/pik3r4* ZDB-GENE-050309-84 | pikwt | cttgaattcATGGGGAACCAACT GGCTGGAATTGCTCCATCG | cttctcgaggggACTTCCAGACTTT GACAATGCCATCTCTAGAG |
| | pikmut | GCACAAATCTGCTGTCAAC CaAATCCGAGTCTCAGATGAG | CTCATCTGAGACTCGGA TTtGGTTGACAGCAGATTTGTGC |
| | pik3r4WM | gtgggagaagcatctgtgatc | ctacacaggttgttgaggatg |

**Supplementary Table 2:** Primers used in this study

| List of antibodies used in this study | | | |
|---|---|---|---|
| **Name Primary Antibodies** | **Applications** | **Producer** | **Conditions** |
| Mouse Anti-Acetylated alpha Tubulin [6-B11-1] | western, IF | Abcam #ab24610 | IF: 1/200 |
| Rabbit Anti-PIK3R4 | western, IF, IP | Novus Biologicals #NBP1-30463 | WB 1/1000 IF: 1/200-1/300 IP: 1µl Ab/100µg prot |
| Mouse Anti-PIK3R4 | IF | Abnova #H00030849-M02 | IF:1/100 |
| Rabbit anti-Beclin-1 | Western | Cell Signaling #3495 | WB: 1/1000 |
| Anti-UVRAG | Western | Abcam #ab174550 | WB: 1/1000 |
| Rabbit Anti-GAPDH | Western | Abcam #ab181602 | WB: 1/5000 |
| Rabbit Anti-PI3 Kinase Class 3 (VPS34) | IF | Abcam #ab137784 | IF : 1/500 |
| Mouse Anti-PI3 Kinase Class 3 (D9A5) (VPS34) | IP, Western, IP | Cell Signaling #5289 | WB: 1/1000 IP: 200µg Prot+ 2µl Ab |
| Mouse Anti-GM130 | Western, IF | Abcam #ab169276 | western: 1/1000 IF:1/250 IP: 2µl/200µg Prot |
| Rabbit Anti-IFT20 | Western, IF, IP | Proteintech #13615-1-AP | WB:1/1000-2000 IF: 1/300 IP:300µg Prot + 3µl Ab |
| RabbitAnti-Atg14 | Western | Cell Signaling #5504 | WB:1/1000 |
| Mouse Anti- GMAP210 | Western | Thermo Scientific #MA1-23294 | WB:1/500 |
| Mouse anti-HA | Western, IF | Abcam #ab130275 | WB: 1/1000 IF: 1/200 |
| Rat monoclonal Anti-HA | Western , IF | Roche #1 867 423 | WB:1/1000 IF:1/200 |
| **Name Secondary Antibodies** | **Applications** | **Producer** | **Conditions** |
| Mouse Anti-rabbit IgG (Conformation Specific) (L27A9) mAb (HRP Conjugate) | Western | Cell Signaling #5127 | WB:1/1000 |
| Donkey anti rabbit IgG FITC | IF | Santa Cruz #sc-2090 | IF:1/200 |
| Donkey anti-Mouse IgG (H+L), Alexa Fluor 594 conjugate | IF | Thermo Scientific A-21203 | IF:1/500 |
| Chicken anti-Rabbbit IgG-HRP | Western | Santa Cruz #sc-2955 | WB: 1/5000 |
| Goat anti-mouse Alexa Fluor coupled (either 488 or 568) IgG (Invitrogen) | IF | Thermo Scientific #A-11001 #A-11031 | IF: 1/500 |
| Goat anti-Rabbit IgG (H+L) Alexa Fluor 568 conjugate | IF | Thermo Scientific # A-11011 | IF:1/400 |
| Goat anti-rat Alexa Fluor 488 coupled IgG | IF | Abcam #ab150157 | IF:1/600 |
| Donkey anti-Mouse IgG (H+L), FITC conjugate | IF | Thermo Scientific #A16012 | IF:1/500 |
| Goat anti mouse IgG-HRP | Western | Santa Cruz #sc-2060 | WB: 1/5000 |
| Goat anti mouse IgG FITC | IF | Thermo Scientific # A16085 | IF:1/400 |

**Supplementary Table 3:** Antibodies used in this study