Roberson et al., http://www.jcb.org/cgi/content/full/jcb.201411087/DC1



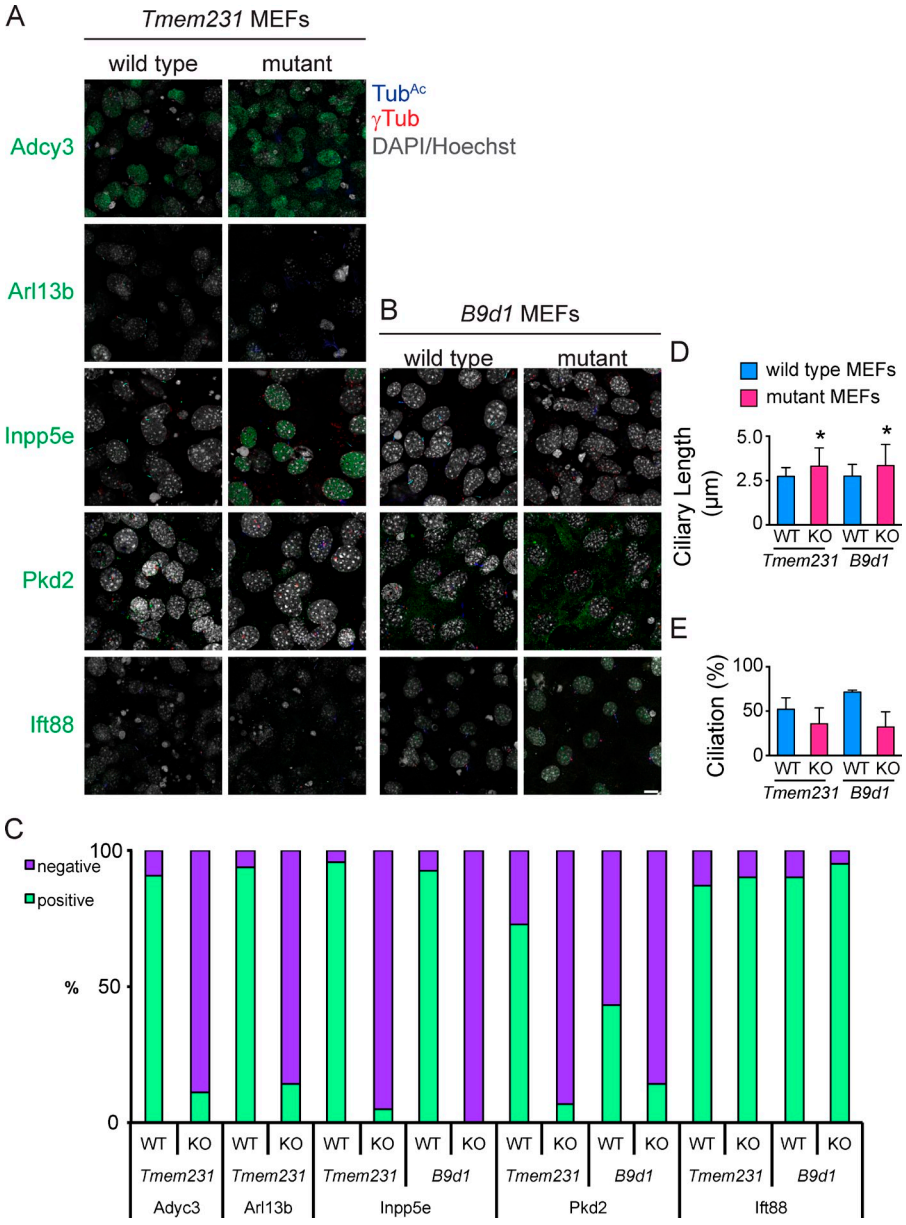


Figure S1. **Tmem231 and B9d1 are required for ciliary localization of membrane proteins.** *Tmem231* (A) and *B9d1* (B) wild-type and mutant MEFs stained for Tub$^{Ac}$ (blue), γ-tubulin (red), DNA (gray), and either Adcy3, Arl13b, Inpp5e, or Ift88 (green). Bar, 10 µm. Adcy3, Arl13b, and Inpp5e fail to localize to cilia in *Tmem231* and *B9d1* mutant MEFs, whereas Ift88 remains unaffected. (C) Quantitation of the percentage of positive (green) and negative (purple) cilia from A and B. At least 10 cilia were scored per sample. (D) Cilia in the mutant MEFs are modestly longer than those of wild-type MEFs. (E) *Tmem231* and *B9d1* mutant MEFs ciliate less frequently than *Tmem231* and *B9d1* wild-type MEFs. Error bars represent the 95% confidence interval. *, $P < 0.05$, as measured by Student's *t* test with Welch's correction.

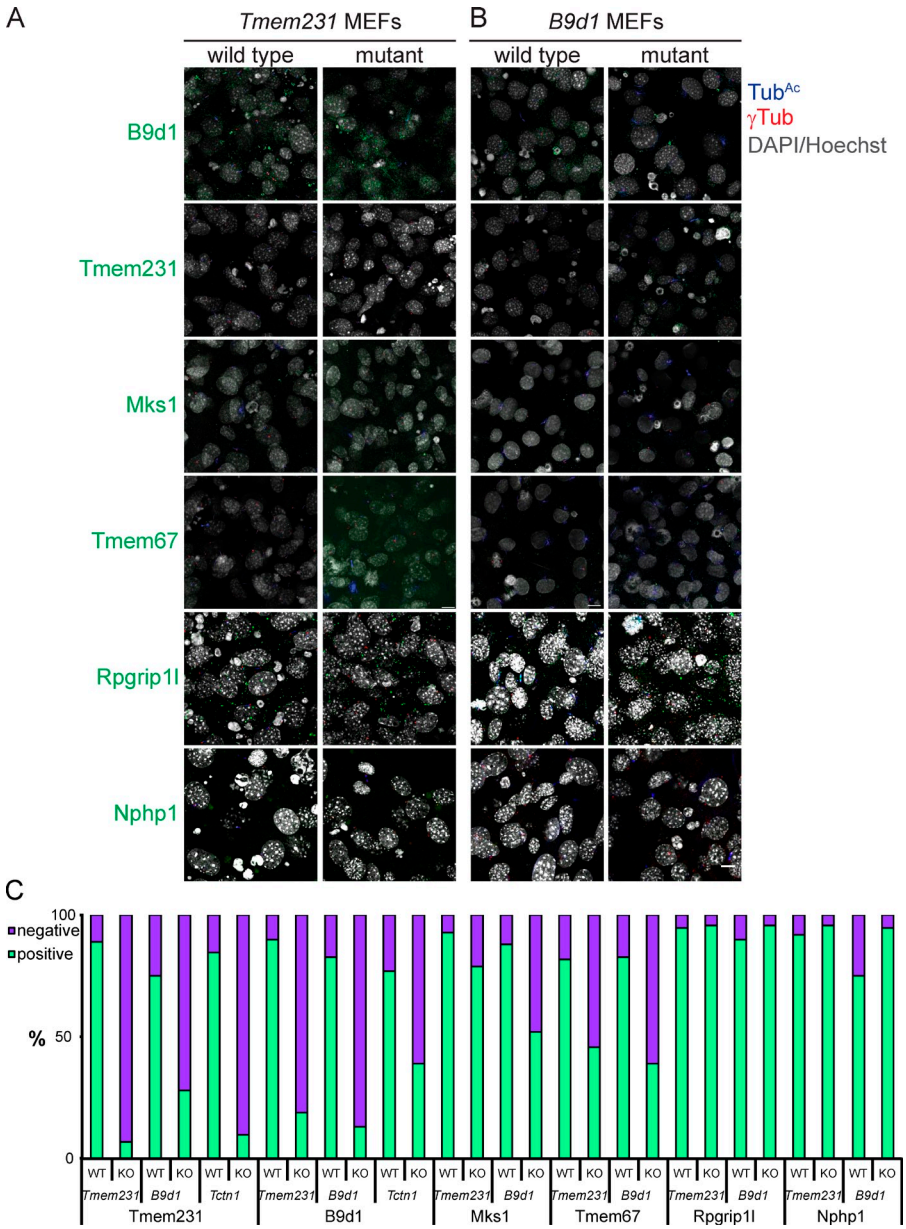


Figure S2. **B9d1 and Tmem231 are required for MKS complex assembly.** Ciliary TZ in *B9d1* (A) and *Tmem231* (B) wild-type and mutant MEFs stained for $Tub^{Ac}$ (blue), γ-tubulin (red), DNA (gray), and either B9d1, Tmem231, Mks1, Tmem67, Rpgrip1l, or Nphp1 (green). Bar, 10 µm. Tmem231 and B9d1 require each other for TZ localization. Mks1 and Tmem67 fail to localize to the ciliary TZ in the absence of *B9d1* or *Tmem231*. (C) Quantitation of the percentage of positive (green) and negative (purple) cilia from A and B. At least 10 cilia were scored per sample.

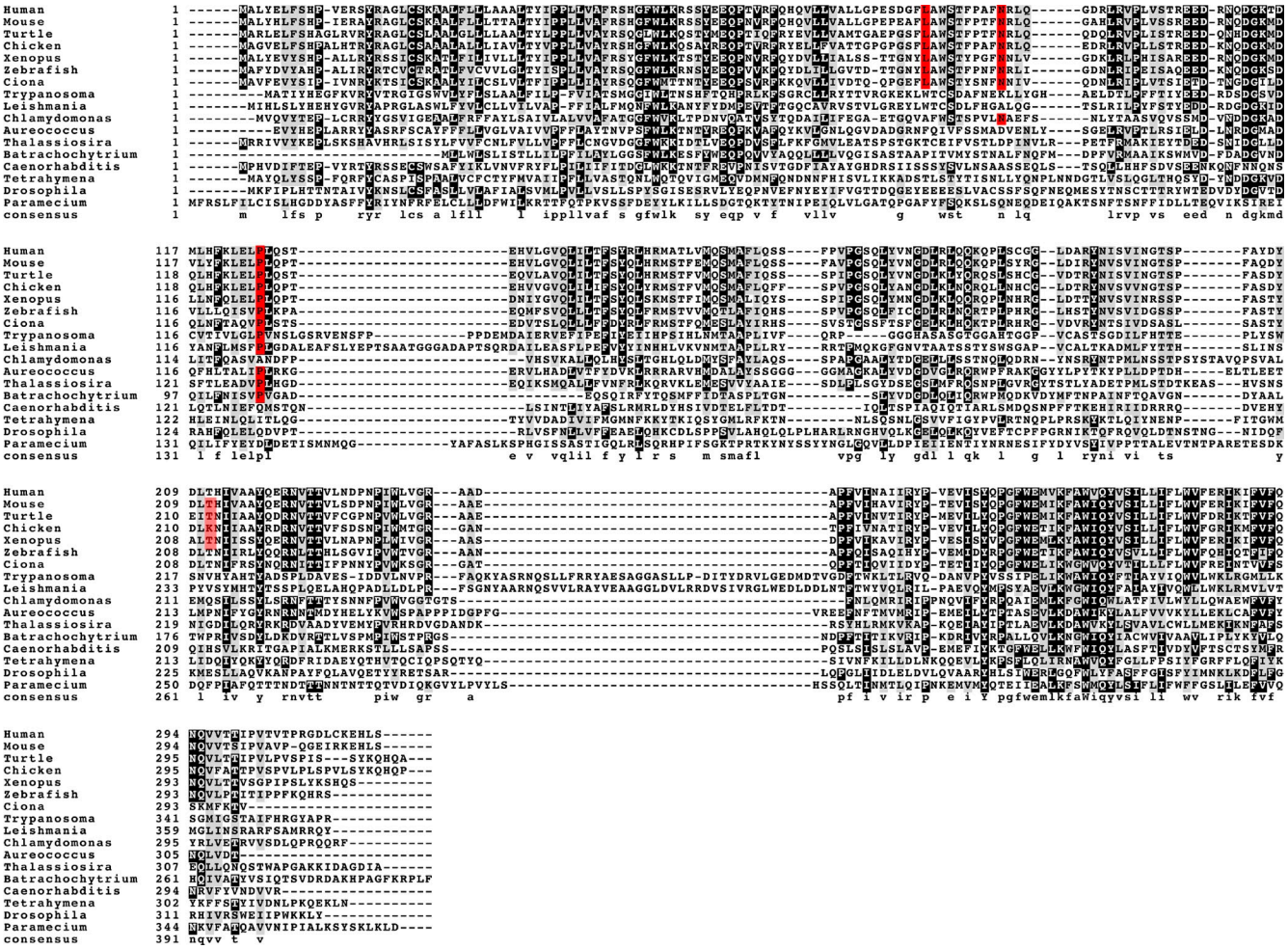

Figure S3. **TMEM231 is conserved in ciliated organisms.** ClustalW alignment of TMEM231 protein sequences reveals conservation of residues across diverse ciliated organisms. Residues highlighted in red indicate those mutated in OFD3 or MKS.

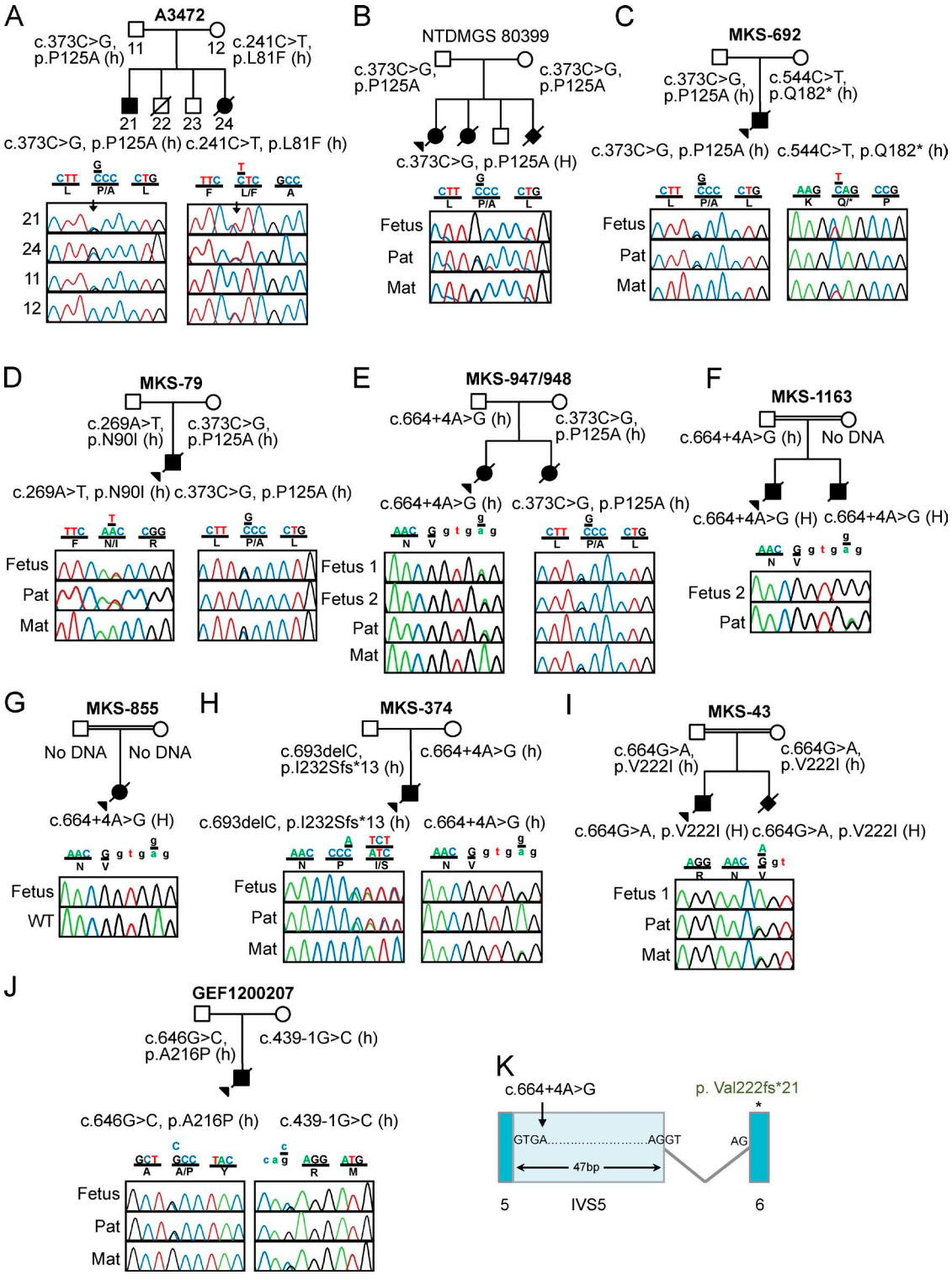


Figure S4. **Mutation of *TMEM231* is associated with OFD3 and MKS.** Pedigree and chromatogram of OFD3- (A) and MKS (B–J)-affected individuals with recessive mutations in *TMEM231* (GenBank accession no. NM_001077418.2). (K) Functional consequences of c.664+4A>G. RT-PCR analysis conducted on primary skin fibroblasts from MKS-374 shows that the intronic change abolishes the canonical exon 5 splice donor sequence and results in retention of 47 bp of intron 5–6 to produce a premature frameshift p.Val222fsX21. H, homozygous; h, heterozygous; Pat, paternal; Mat, maternal.

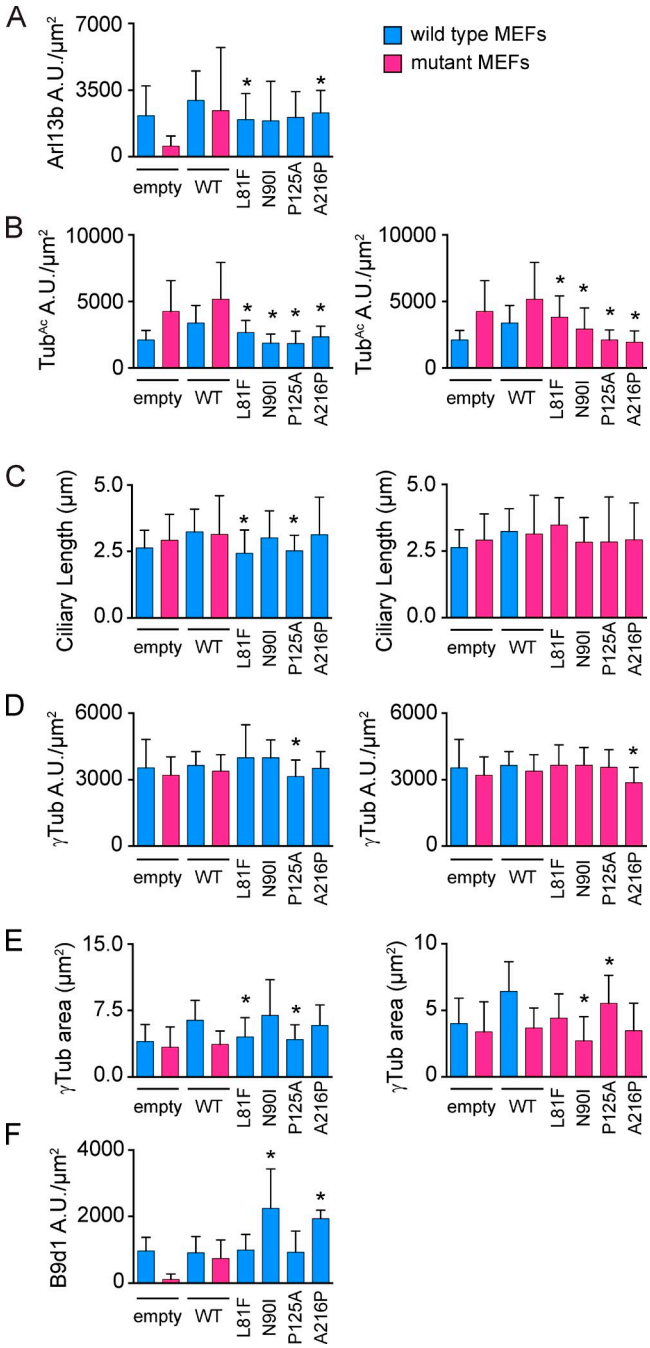


Figure S5. ***Tmem231* mutant alleles partially rescue Arl13b localization to the ciliary membrane.** (A–E) Quantitation of various aspects of the rescue assays in *Tmem231* wild-type and mutant MEFs, including fluorescence intensity of Arl13b, $Tub^{Ac}$, and γ-tubulin, ciliary length, and γ-tubulin area. $Tub^{Ac}$ intensity is decreased when mutant forms of Tmem231 are transfected, as compared with transfection of wild-type Tmem231. (F) Quantitation of fluorescence intensity of B9d1 at the TZ/basal body in *Tmem231* wild-type MEFs. Error bars represent the 95% confidence interval. *, $P < 0.05$, as measured by Student's *t* test with Welch's correction.

Table S1. **Mutation of *TMEM231* is associated with OFD3 and MKS**

| Family | Ethnic origin | Nucleotide alteration[a] | Protein change | Exon/intron (zygosity, segregation) | AA conservation | Parental consanguinuity | PolyPhen2-Score/mutation taster | Additional mutations | Clinical features | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Ind. age | PD | CNS | Kidney | Liver | Other |
| A3472 (OFD3) | UK | c.241C>T[b] | p.Leu81Phe | 2 (het, m) | *C. intestinalis* | no | 0.976/DC | | −21, 24 yr | 1 upper limb | OMA; CVH | ESRD | | lingual hamartomas, ID |
| | | c.373C>G | p.Pro125Ala | 3 (het, p) | *C. intestinalis* | | 0.998/DC | | −24, 13 yr | 2 upper limbs | OMA; CVH; DWS | ESRD | | lingual hamartomas, ID |
| NTDMGS 80399 (MKS) | European American/ France | c.373C>G | p.Pro125Ala | 3 (hom) | *C. intestinalis* | no | 0.998/DC | 46,XX,inv(10) (p11.1q21.1) (het, p) | 40 wk | 4 limbs | DWS; HC | CK | HPF | |
| MKS-79 (MKS) | France | c.373C>G | p.Pro125Ala | 3 (het, m) | *C. intestinalis* | no | 0.998/DC | | 23 wk | 4 limbs | DWS; CVA | CK | HPF | CP, epidymal cysts, micropenis, accessory spleen/heterotaxia |
| | | c.269A>T | p.Asn90Ile | 2 (het, p) | *C. elegans* | | 0.987/DC | | | | | | | |
| MKS-692 (MKS) | Northern Europe | c.373C>G | p.Pro125Ala | 3 (het, p) | *C. intestinalis* | no | 0.998/DC | *CC2D2A:* c.834delG; p.Leu279Cysfx* (het) | 20 wk | 4 limbs | DWS; CVA | CK | HPF | accessory spleen/heterotaxia, skeletal dysplasia (ulnar bowing) |
| | | c.544C>T | p.Gln182* | 4 (het, m) | | | N/A | | | | | | | |
| MKS-374 (MKS) | Romania/ France/ Turkey/ Spain | c.363delC | p.Ile232Serfs* | 6 (het, p) | | no | N/A | | 13 wk | 2 upper limbs | AC | CK | | |
| | | c.664+4A>G | | IVS5 (het, m) | | | N/A | | | | | | | |
| MKS-1163 (MKS) | Turkey/ Kurd | c.664+4A>G | | IVS5 (hom) | | yes | N/A | | F1 14 wk | 4 limbs | MEN | CK | HPF | CP, single umbilical artery, epididymal cysts |
| | | | | | | | | | F2 13 wk | 4 limbs | BM | CK | | IUGR |
| MKS-855 (MKS) | Libya | c.664+4A>G | | IVS5 (hom) | | yes | N/A | | 22 wk | 2 upper limbs | MEN | CK | HPF | pancreatic fibrosis |
| MKS43 (MKS) | Tunisia | c.664G>A[c] | p.Val222Ile | 5 | *X. laevis* | yes | N/A | | F1 23 wk | 4 limbs | EN; MC | CK | | IUGR, absent uvula, micropenis, anomalous pulmonary venous connection |
| | | | | (hom) | | | | | F2 18 wk | | EN | CK | | bowing of long bones, malposition of the feet, TGA |
| MKS-947 (F1) MKS-948 (F2) (MKS) | France | c.373C>G | p.Pro125Ala | 3 (het, m) | *C. intestinalis* | no | 0.998/DC | | F1 25 wk | 2 upper; 1 lower | HC; HPE | CK | HPF | splenopancreatic fusion |
| | | c.664+4A>G | | IVS5 (het, p) | | | | | F2 16 wk | | HC; CVH; CD | CK | HPF | malposition of the hands with ulnar deviation |
| GEF 1200207 (MKS) | | c.439-1G>C | | IVS3 (het, m) | | no | 0.994/DC | | 17 wk | 4 limbs | MEN | CK | HPF | CP, short limbs; epididymal cysts; malposition of the left hand with ulnar deviation; bilateral malposition of the feet, single umbilical artery, common mesentery |
| | | c.646G>C | p.Ala216Pro | 5 (het, p) | G. gallus | | | | | | | | | |

AC, anencephaly; BM, brain malformation; CD, cortical dysplasia; CK, cystic kidneys; CP, cleft palate; CVH, cerebellar vermis hypoplasia; CVA, cerebellar vermis agenesis; DC, disease causing; DWS, Dandy-Walker Syndrome; EN, encephalocele; ESRD, end stage renal disease; HC, hydrocephalus; het, heterozygous; HPE, holoprosencephaly; HPF, hepatic portal fibrosis; ID, intellectual disability; IUGR, intrauterine growth restriction; m, maternal; MC, microcephaly; MEN, meningoencephalocele; N/A, not applicable; OMA, oculomotor apraxia, "metronome eye movements;" p, paternal; PD, polydactyly; TGA, transposition of the great arteries.

[a]Mutations are numbered according to human cDNA reference sequence NM_001077418.2, isoform 1 (*TMEM231*), where +1 corresponds to the A of ATG start translation codon.

[b]Variant is listed in Exome variant server database: TT = 0/TC = 1/CC = 6155.

[c]Reported previously by Shaheen et al. 2013. *J. Med. Genet.* http://dx.doi.org/10.1002/humu.21507 and shown experimentally to disrupt mRNA splicing and result in premature truncation.

**Table S1 shows mutations of *TMEM231* associated with OFD3 and MKS.**

Table S2. ***C. elegans* strains used**

| Strain | Genotype |
|---|---|
| PT709 | nphp-4(tm925) |
| MX1855 | tmem-231(tm5963) |
| MX1251 | mks-2(nx111) |
| MX754 | mks-5(tm3100) |
| MX1415 | N2; nxEx[*tmem-231*:gfp + P*osm-5*::*xbx-1*::tdTomato + *rol-6(su1006)*] |
| MX1450 | *nphp-4*; nxEx[*tmem-231*::gfp + P*osm-5*::*xbx-1*::tdTomato + *rol-6(su1006)*] |
| MX1470 | *mks-2*; nxEx[*tmem-231*::gfp + P*osm-5*::*xbx-1*::tdTomato + *rol-6(su1006)*] |
| MX1471 | *mks-5*; nxEx[*tmem-231*::gfp + P*osm-5*::*xbx-1*::tdTomato + *rol-6(su1006)*] |
| YH930 | N2; Ex[P*osm-5*::*mks-5*::tdTomato + P*osm-5*::*dyf-11*::gfp + *rol-6(su1006)*] |
| MX1811 | *tmem-231*; nxEx[P*osm-5*::*mks-5*::tdTomato + P*osm-5*::*dyf-11*::gfp + *rol-6(su1006)*] |
| MX1420 | N2; nxEx[P*bbs-8*::*tram-1*::tdTomato + P*bbs-8*::*mks-2*::gfp + *rol-6(su1006)*] |
| MX1813 | *tmem-231*; nxEx[P*bbs-8*::*tram-1*::tdTomato + P*bbs-8*::*mks-2*::gfp + *rol-6(su1006)*] |
| YH237 | N2; yhEx142[*nphp-1*::cfp + *che-13*::yfp + *rol-6(su1006)*] |
| MX1814 | *tmem-231*; nxEx[*nphp-1*::cfp + *che-13*::yfp + *rol-6(su1006)*] |
| MX1065 | N2; nxEx[*tmem-17*::gfp + P*osm-5*::*xbx-1*::tdTomato + *rol-6(su1006)*] |
| MX1815 | *tmem-231*; nxEx[*tmem-17*::gfp + P*osm-5*::*xbx-1*::tdTomato + *rol-6(su1006)*] |