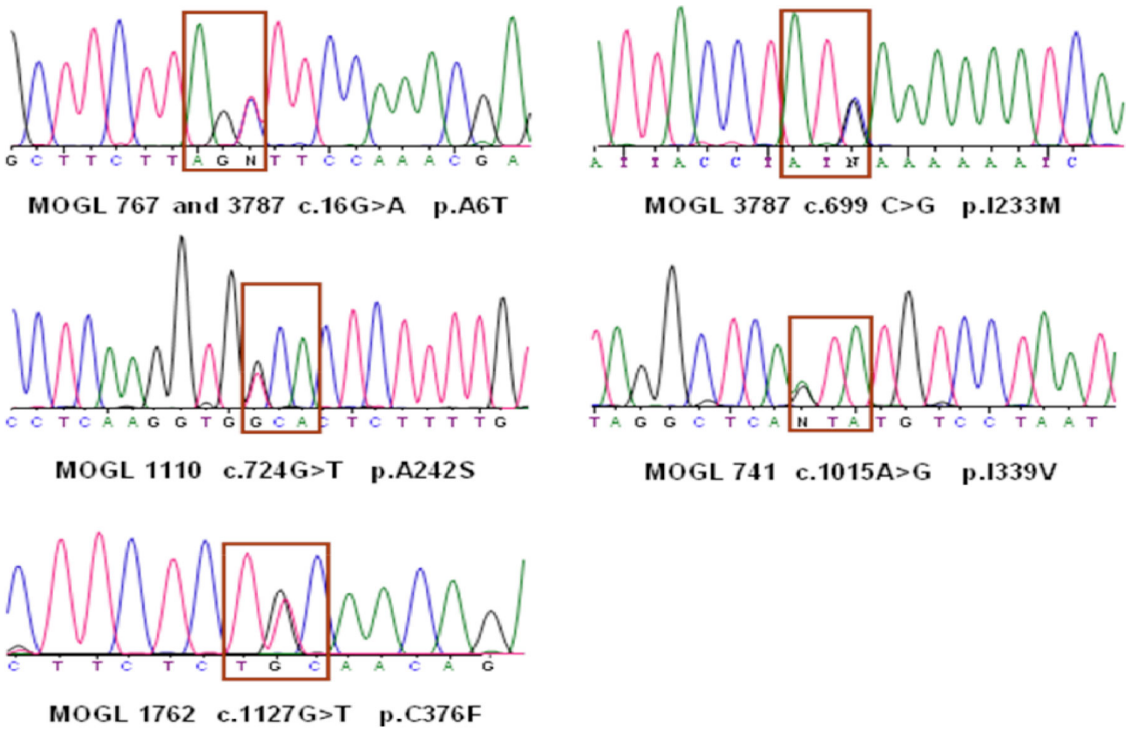
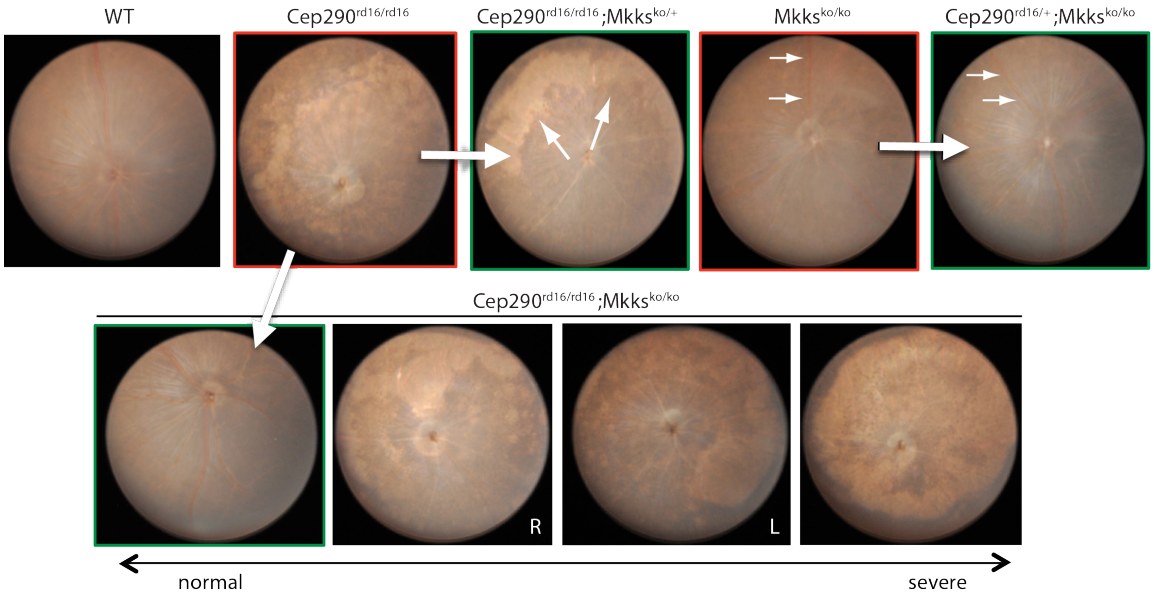


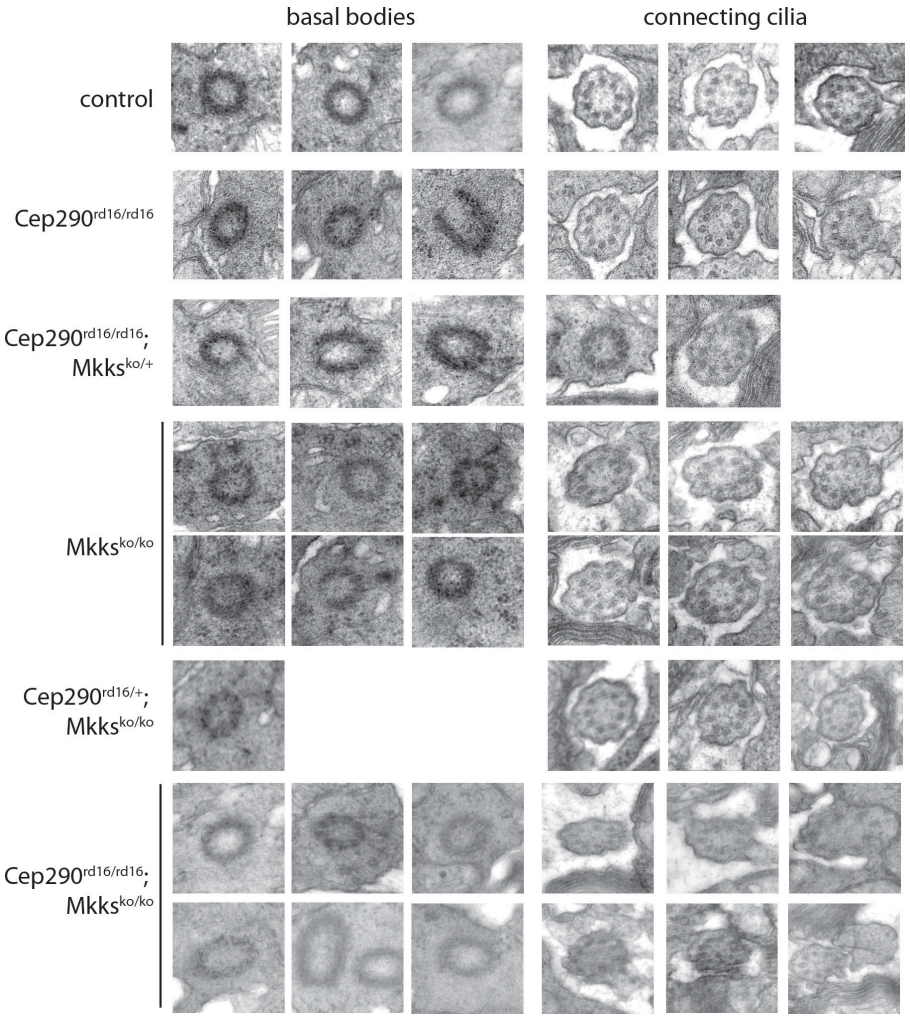
Supplemental Figure 1. New *MKKS* sequence variations in patients with LCA

Chromatograms showing nucleotide variations in *MKKS* coding sequence and corresponding amino acid changes. These variants have either been previously identified in BBS patients[1,2] or predicted to be pathogenic by *in silico* analysis (see Supplementary Methods). Newly identified DNA variants are in the same regions of the gene as previously reported mutations (see Figure 1A).

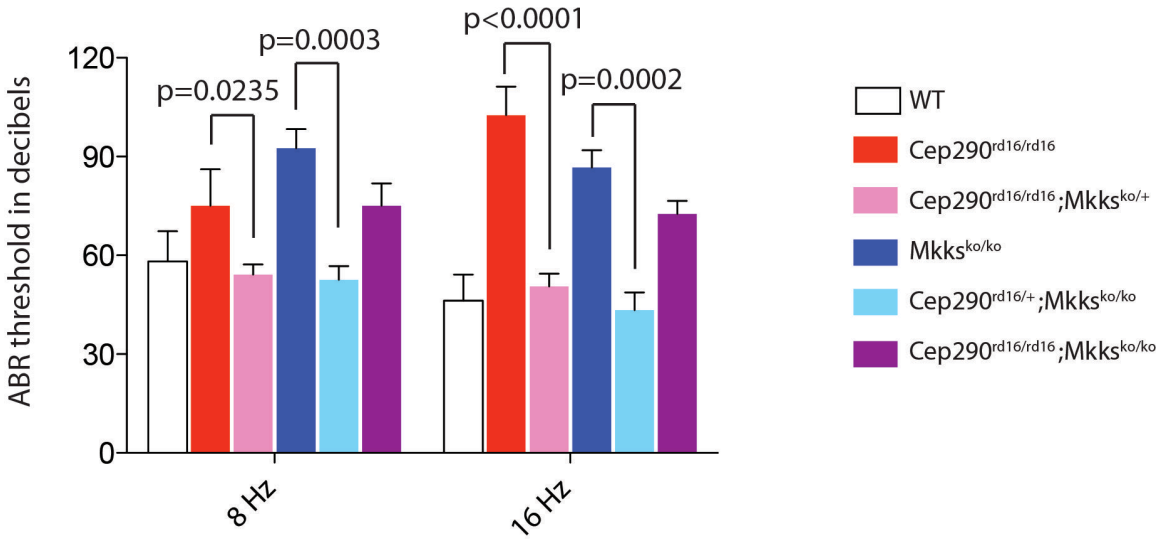


Supplemental Figure 2. Fundoscopy results show rescue in combination mutants

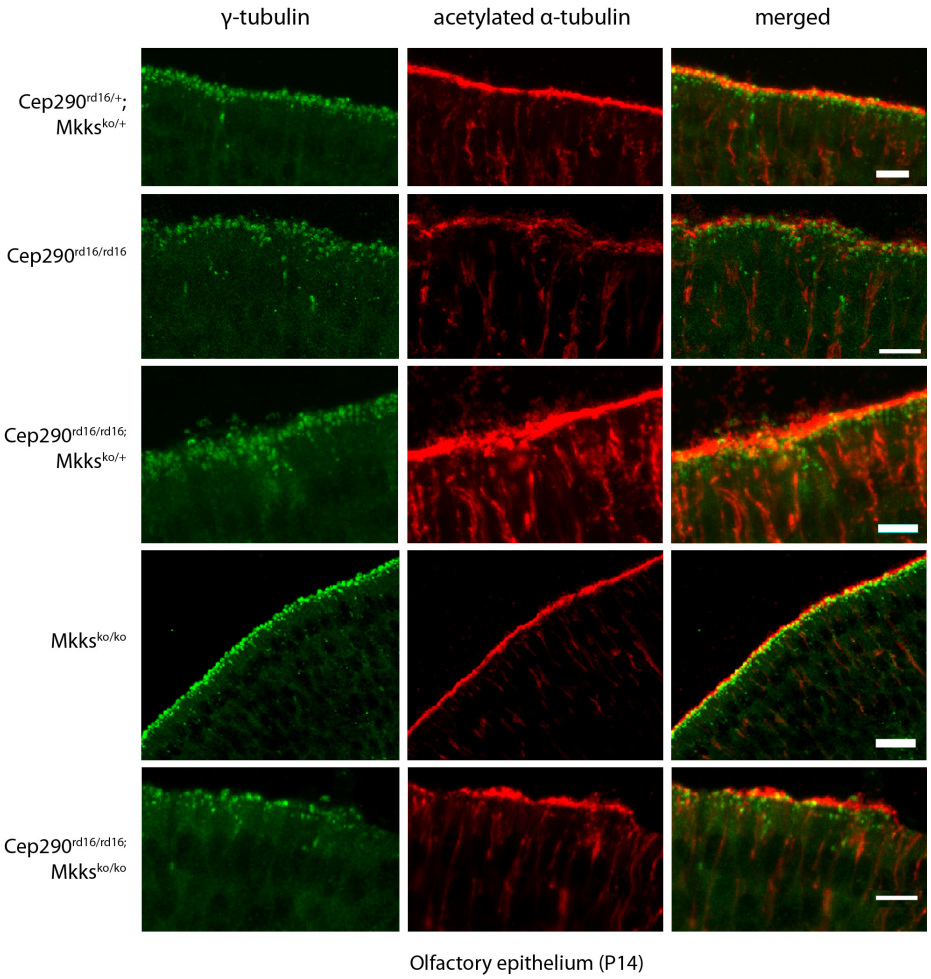
Fundus photographs of 3-4 month old mice carrying various combinations of *Mkks*^{ko} and *Cep290*^{rd16} alleles. **Upper panel:** *Cep290*$^{rd16/rd16}$ mice show extensive pathology, whereas in the *Cep290*$^{rd16/rd16}$;*Mkks*$^{ko/+}$ mouse, pathological retinal pigment epithelium (RPE) changes are restricted to the peripheral retina (see arrows). Note the blood vessel attenuation in *Mkks*$^{ko/ko}$ (arrows), but minimal fundus changes in the *Cep290*$^{rd16/+}$;*Mkks*$^{ko/ko}$ mouse. **Lower panel:** The *Cep290*$^{rd16/rd16}$;*Mkks*$^{ko/ko}$ double homozygous mutant mice show variability ranging from near normal fundus (left), to differences between eyes of the same individual (R and L images in the center), or within a single retina.



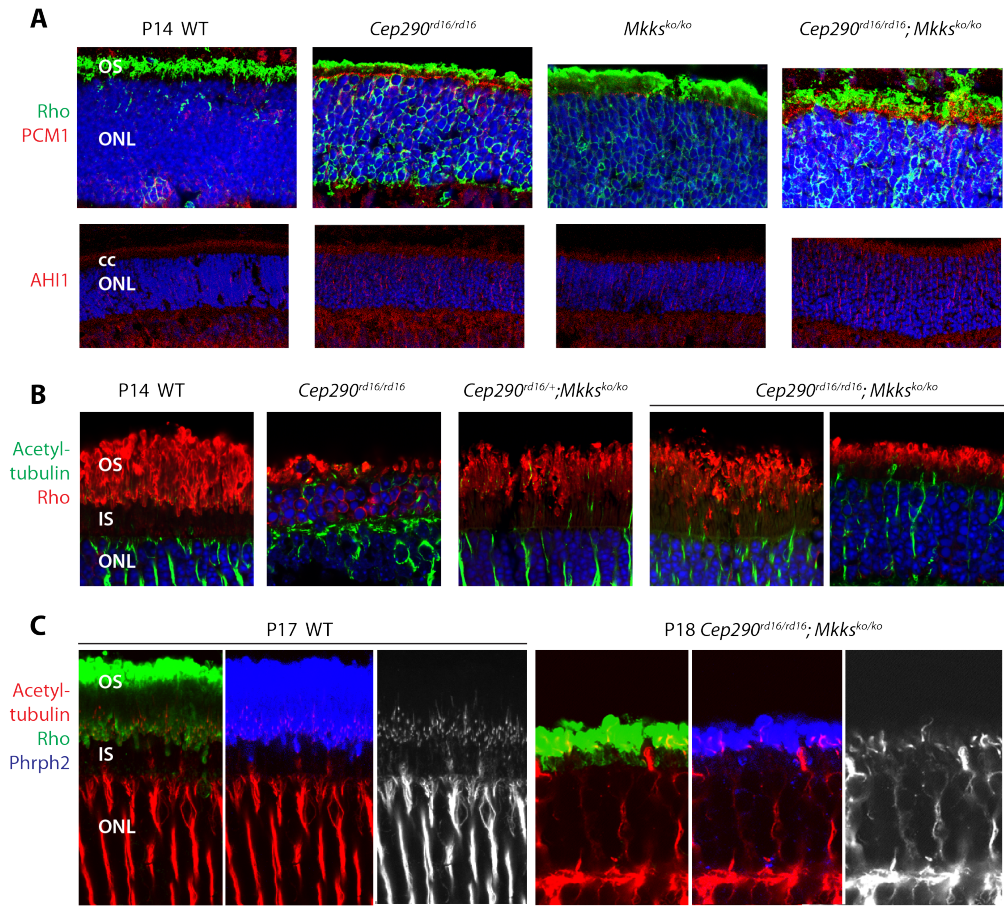
Supplemental Figure 3. Additional EM examples of basal bodies (left) and connecting cilia (right) of P14 photoreceptors. In *Cep290*$^{rd16/rd16}$ photoreceptors, basal bodies and connecting cilia appear structurally normal (as in wild type), whereas the basal bodies in *Mkks*$^{ko/ko}$ photoreceptors show extra electron dense material distributed beyond the microtubule ring, with cilia exhibiting an oblong, irregular shape. In *Cep290*$^{rd16/+}$*;Mkks*$^{ko/ko}$ photoreceptors, less extra electron-dense material is present compared to *Mkks*$^{ko/ko}$, and a circular profile of connecting cilia is restored. Photoreceptors of double homozygotes reveal a range of hazy circular structures, which rarely produce a separate membrane-bound cilium at this age.



Supplemental Figure 4. Rescue of ABR thresholds in mice carrying different combinations of *Cep290*^{rd16} or *Mkks*^{ko} alleles. Additional stimulus wavelengths are completely consistent with data shown in Figure 6D.



Supplemental Figure 5. Presence of olfactory cilia in P14 mice with different *Cep290^{rd16}* or *Mkks^{ko}* genotypes. Double immunolabeling with antibodies to acetylated α-tubulin (a marker of microtubules, red) and γ-tubulin (basal body marker, green) show that cilia are present in all mutant genotypes at this age. The loss of cilia in *Mkks$^{ko/ko}$* mice at later ages[9] is likely due to degeneration. Scale bar: 10 μm.



Supplemental Figure 6. Presence of olfactory cilia in P14-P18 mice with different combinations of *Cep290*^{rd16} or *Mkks*^{ko} genotypes. A. Immunolabeling of P14 retinas in the designated genotypes with the antibodies indicated. PCM1 appears to accumulate at the outer limiting membrane in both *Cep290*$^{rd16/rd16}$ and *Cep290*$^{rd16/rd16}$;*Mkks*$^{ko/ko}$ genotypes. **B.** Notice the large variation observed in the *Cep290*$^{rd16/rd16}$;*Mkks*$^{ko/ko}$ genotype. **C.** In the *Cep290*$^{rd16/rd16}$;*Mkks*$^{ko/ko}$ genotype, despite questionable development of the connecting cilium (see Figure 5B), rhodopsin and peripherin2 (Phrph2) accumulate in the outer segment region. Abbreviations: Rho, rhodopsin; OS, outer segments; cc, connecting cilia; IS, inner segments; ONL, outer nuclear layer.

Supplemental Tables

Supplemental Table 1. Oligonucleotide primer sequences for *MKKS* (NCBI accession # NM170784) mutation screening.

	Forward	**Reverse**
exon 3a	ATTTTATAGCCACAATGCTGC	AAAATGATGTGGCCTTCAGC
exon 3b	GATCCTCCTTTGTTTGGTGC	CAAGATGTTAACAGTGACACAAACC
exon 4	TTGTGGGGCTTTTATGTTGG	TTGCTTTCTATACTACCTAGGGAAGC
exon 5	TCTCTGTATTCACTATGATGCTTTG	TGCACCCCTGAACCTAAAAG
exon 6	CCAGACCCCAAATTAAATGAAAG	ACAGGGCTTTGGGAGAAAAC

Supplemental Table 2. Overview of *MKKS* sequence variants, including polymorphisms, likely pathological variants, and their allele frequencies.

		Total samples	**FREQUENCY**
Significant *MKKS* variants*		142	%
c.16 G>A	p.A6T	2	1.43
c. 699 C>G	p.I233M	1	0.71
c.724 G>T	p.A242S	1	0.71
c.1015 A>G	p.I339V	1	0.71
c.1127 G>T	p.C376F	1	0.71
	Total	**6**	**4.29**
Likely *MKKS* polymorphisms			
c.117 C>T	p.P39P	19	13.57
c.179 C>A	p.S60Y	13	9.29
c.534 T>C	p.I178I	29	20.71
c.556 T>C	p.L183L	1	0.71
c.1549 C>T	p.R517C	29	20.71
c.1595 G>T	p.G532V	29	20.71
c.IVS3+16 T>C		31	22.14
c.IVS3+33 G>C		64	45.71

* "Significant variants" are changes in conserved residues that are not detected in at least 100 normal controls and in 1000G database (see also Table S3 and S4).

Supplemental Table 3. Analysis of *MKKS* sequence variants, identified in LCA and juvenile RP patients.

MOGL Pt #	**primary retinal disease**	**primary gene**	**allele 1**	**allele 2**	***MKKS*/+ mutation**	**blosum62 score of *MKKS* variant**	**SIFT score of *MKKS* variant**	**POLYPHEN score of *MKKS* variant**	**conservation (see Table 4)**
767	Juv. onset RP	unknown			c.16G>A p.A6T	-1	Tolerated score 0.15	Benign PSIC score 1.378	Conserved
3787	LCA	unknown			c.16G>A p.A6T	-1	Tolerated score 0.15	Benign PSIC score 1.378	Conserved
3787	LCA	unknown			c.699 C>G p.I233M	1	Tolerated score 0.12	Benign PSIC score 1.239	Not conserved
1110	Juv. onset RP	unknown			c.724G>T p.A242S	1	**NOT** tolerated score **0.02**	Benign PSIC score 1.280	Conserved (1)
741	Juv. onset RP	unknown			c.1015A>G p.I339V	1	Tolerated score 1.00	Benign PSIC score .959	Conserved (2, 3)
1762	LCA	*CEP290*	p.Cys 998X	p.Arg 1508 X	c.1127G>T p.C376F	-2	**NOT** tolerated score **0.04**	Possibly damaging PSIC score 3.346	Highly Conserved

Supplemental Table 4. Alignments of MKKS and related proteins, showing sequences flanking the altered amino acid residue identified in patients.

Multiple Alignments for:	**p. A6T**		
McKusick-Kaufman/Bardet-Biedl syndromes	**MSRLE**	**A**	**KKPSLCKSEPLTTERVRTTLSVLKR...**
McKusick-Kaufman/Bardet-Biedl (*Canis familiaris*)	MSRLE	A	KKPSLCKSEPLTSERVRATLCVLKG...
hypothetical protein (*Bos taurus*)	MSRLE	A	KKPSICRSDPLTGERARASLAALKG...
McKusick-Kaufman syndrome protein (*Mus musculus*)	MSRLE	A	KKPSLCKTEPLTSEKVRSTLSVLKG...
McKusick-Kaufman syndrome protein (*Rattus norvegicus*)	MSRLE	A	KKPSLCKTEPLTSERVRSTLSVLKG...
similar to McKusick-Kaufman/Bardet-Biedl (*Canis familiaris*)	MSRLE	A	KKPSLCKSEPLTSERVRATLCVLKG...
hypothetical protein (*Monodelphis domestica*)	MSRVK	A	KTPSLCTCELLTKEIVSKSLSGLRE...
hypothetical protein (*Gallus gallus*)	MSRLE	A	KKPSLFISEPLTT--VSQPLSLLIA...
McKusick-Kaufman syndrome (*Danio rerio*)	MSRIS	**K**	KKPALCTDEPLSNSTICQKITLLRN...
unnamed protein product (*Mus musculus*)	MSRLE	A	KKPSLCKTEPLTSEKVRSTLSVLKG...

Multiple Alignments for:	**p. I233M**		
McKusick-Kaufman/Bardet-Biedl syndromes	**SEVQLMRLLP**	**I**	**KKSTALKVALFCTTLSGDTSD**
McKusick-Kaufman/Bardet-Biedl (*Canis familiaris*)	SEIQLMKILP	I	KKSEAFKVALFCASLSGDLSD
hypothetical protein (*Bos taurus*)	SEVQLMKILP	I	KKSDSFKVALFCVSLSGDLSD
McKusick-Kaufman syndrome protein (*Mus musculus*)	SEVQLRRLLP	**T**	QKASGLRVALFCTSLSGDFSNA
McKusick-Kaufman syndrome protein (*Rattus norvegicus*)	SEVQLRRLLP	**T**	QKSSTLRVALFCASLSGDFSNA
similar to McKusick-Kaufman/Bardet-Biedl (*Canis familiaris*)	SEIQLMKILP	I	KKSEAFK----------...
hypothetical protein (*Monodelphis domestica*)	PEVQLMTF-P	I	KKSNALKVALFCISMSGEISDS
hypothetical protein (*Gallus gallus*)	PEIQFAKPFS	**V**	KRSDAVKVAVFCVSMSGDLFDP
McKusick-Kaufman syndrome (*Danio rerio*)	PEMLLPGDLE	**R**	LGDGPFKVVLFGVSLSGDISEVG
unnamed protein product (*Mus musculus*)	SEVQLRRLLP	T	QK----------...

Multiple Alignments for: p. A242S

McKusick-Kaufman/Bardet-Biedl syndromes	**QLMRLLPIKKSTALKV**	**A**	**LFCTTLSGDTSDTGEGT**
McKusick-Kaufman/Bardet-Biedl (*Canis familiaris*)	QLMKILPIKKSEAFKV	A	LFCASLSGDLSDTGEGT
hypothetical protein (*Bos taurus*)	QLMKILPIKKSDSFKV	A	LFCVSLSGDLSDTGEGT
McKusick-Kaufman syndrome protein (*Mus musculus*)	QLRRLLPTQKASGLRV	A	LFCTSLSGDFSNAGEGV
McKusick-Kaufman syndrome protein (*Rattus norvegicus*)	QLRRLLPTQKSSTLRV	A	LFCASLSGDFSNAGEGT
similar to McKusick-Kaufman/Bardet-Biedl (*Canis familiaris*)	QLMKILPIKKSEAFK-	-	----------...
hypothetical protein (*Monodelphis domestica*)	QLMTF-PIKKSNALKV	A	LFCISMSGEISDSGEGT
hypothetical protein (*Gallus gallus*)	QFAKPFSVKRSDAVKV	A	VFCVSMSGDLFDPEEGT
McKusick-Kaufman syndrome (*Danio rerio*)	MLLPGDLERLGDGPFKV	**V**	LFGVSLSGDISEVGDV
unnamed protein product (*Mus musculus*)	QLRRLLPTQK------	-	----------...

Multiple Alignments for: p. I339V

McKusick-Kaufman/Bardet-Biedl syndromes	**KMTGTQPIGSLGS**	**I**	**CPNSYGSVKDVCTAKFGSK**
McKusick-Kaufman/Bardet-Biedl (*Canis familiaris*)	SKVTGTQPIGSIGS	I	SPSSYGSVKDLCPAKFGFK
hypothetical protein (*Bos taurus*)	SKVTGTWPIGSLGS	I	SPSSYGSVKDLCIAKFGCK
McKusick-Kaufman syndrome protein (*Mus musculus*)	SKVTGATPIGSLNP	I	VSTTYGSVKDVCSARFGS
McKusick-Kaufman syndrome protein (*Rattus norvegicus*)	SKVTGATPIGSLYP	I	VSTTYGSVKDVRSARFGSKY
similar to McKusick-Kaufman/Bardet-Biedl (*Canis familiaris*)	SKVTGTQPIGSIGS	I	SPSSYGSVKDLCPAKFGFK
hypothetical protein (*Monodelphis domestica*)	CEMTGTQPIGSLNF	I	SPTSYGYVKDLCYTNFGSKP
hypothetical protein (*Gallus gallus*)	SQVTGSKPIASIYS	**L**	SPSCYGSLKDVRAESFASK
McKusick-Kaufman syndrome (*Danio rerio*)	AKITGARAVASLFS	**L**	VPEAYGLVAGLCFQDCGSKK
unnamed protein product (*Mus musculus*)	SKVTGATPIGSLNP	I	VSTTYGSVKDVCSARFGSK

Multiple Alignments for:	**p. C376F**		
McKusick-Kaufman/Bardet-Biedl syndromes	**HLIPNEATICSLLL**	**C**	**NRNDTAWDELKLTCQ**
McKusick-Kaufman/Bardet-Biedl (*Canis familiaris*)	HLIPYEATICSLLL	**C**	NRNDTAWDELKLTCQ
hypothetical protein (*Bos taurus*)	HLIPNKTTICSLLL	**C**	NRNDTAWDELKLTCQ
McKusick-Kaufman syndrome protein (*Mus musculus*)	HLLPNEATVCTLLL	**C**	SRNDTAWEELKLTCQ
McKusick-Kaufman syndrome protein (*Rattus norvegicus*)	HLLPNEATICSLLL	**C**	SRNDTAWEELKLTCQ
similar to McKusick-Kaufman/Bardet-Biedl (*Canis familiaris*)	HLIPYEATICSLLL	**C**	NRNDTAWDELKLTCQ
hypothetical protein (*Monodelphis domestica*)	HLIPNDSTVCSLLL	**C**	NRNETSWNELKLTCQ
hypothetical protein (*Gallus gallus*)	HLIPNDTTVCSLIL	**C**	NRNETTWDELKRACE
McKusick-Kaufman syndrome (*Danio rerio*)	LLSSKAAISTMVL	**C**	HRNETMLEELKMTCQ
unnamed protein product (*Mus musculus*)	HLLPNEATVCTLLL	**C**	SRNDTAWEELKLTCQ

Supplemental methods

A summary of clinical and genetic characteristics of five patients with significant *MKKS* variants. Patient MOGL 767 is from a non-consanguineous pedigree from Trinidad and Montserrat. She presented at age 26 years with a lifelong history of nyctalopia, followed by color vision loss. Acuities were 20/80 in the right eye and 20/50 in the left with -3.50 D [correction]. She had a non-detectable ERG and 5° visual field by V4e target. One year later, her vision was only light perception, with an extinguished visual field. Her retinal vessels were attenuated, optic disc was normal color, and the retina had very mild salt and pepper changes with restricted pigmentary maculopathy. We have not determined the causal gene for this severe and rapidly progressive retinal dystrophy phenotype, but did identify a heterozygous *MKKS* variant, p.A6T, which changes the hydrophobic amino acid alanine to a hydrophilic threonine. The blosum62 score suggests a pathological change, while SIFT and Polyphen predictions do not (Supplemental Table 3). Ala6 is conserved in all mammals (see Supplemental Table 4).

Patient MOGL 1110 is from a non-consanguineous French-Canadian pedigree and presented at age 7 yr with a lifelong history of nyctalopia. Acuities were 20/70 in both eyes with -9.00D [correction], ERGs were non-detectable, and VFs were full to V4e and I4e targets. Fundus examination revealed diffuse pigmentary changes, narrow vessels and a prominent hypopigmented peri-foveal ring. At age 17 yr, acuities decreased to 20/80 (right) and 20/150 (left) and visual fields were decreased to 50° and 5° by V4e and I4e targets, respectively. Despite extensive mutation screening (APEX technology), the causal gene has not yet been determined in this patient; nonetheless,

MKKS analysis revealed a heterozygous p.A242S variant that changes the hydrophobic alanine to a hydrophilic serine. SIFT analysis suggests that this is a harmful change, while blosum62 and Polyphen analyses do not. Interestingly, this change has been reported in *MKS/BBS* and is considered a mutation (3). The Ala242 is well conserved (see Suppl. Table 4).

Patient MOGL 741 presented at age 20, with lifelong nyctalopia, nystagmus and high hyperopia since the age of eight months. Her visual acuities were 20/40 with +3.00 D [correction], with no detectable ERGs and constricted VFs and midperipheral scotomas. Her retinal exam was significant for a hypopigmented foveal change. At age 27 years, acuities were 20/50 with slightly decreased VFs sizes (still 60°) and unchanged retinal exam. Despite mutation analysis by APEX technology, we were unable to determine the causal gene. *MKKS* analysis revealed a heterozygous p. I339V variant that changes a hydrophobic isoleucine to a hydrophobic valine. Whilst not considered damaging by blosum62, SIFT or Polyphen analysis, the variant has been reported in *MKS/BBS* and is reasonably conserved(2).

Patient MOGL 3787, a 15 year old male patient, had severe vision loss and nystagmus since early childhood. There was parental consanguinity. Visual acuities were light perception in each eye (+1.00-1.75X90). Kinetic visual fields (V-4e target) indicated a small island of vision in the temporal field of each eye. ERGs were undetectable. There were small posterior subcapsular lens opacities and rare vitreous cells. On fundus examination, there was vessel attenuation, waxy pallor of the optic nerve head, small clumps of pigment with rare white dots throughout the mid- and far periphery and a

central ring of depigmentation. This patient has two *MKKS* variants p. A6T and p. I233M, the latter changes a hydrophobic isoleucine to a hydrophobic methionine. P. A6T has been described previously for patient MOGL767. The p. I233M change is not considered damaging by blosum62, SIFT or Polyphen and is not well conserved.

Patient MOGL 1762 has severe LCA and is from a non-consanguineous Mexican pedigree, but no further clinical data are available. She harbors two different *CEP290* mutations and one *MKKS* variant of high pathogenicity. In *CEP290*, we found p. C998X and p. R1508X, while in *MKKS* we discovered a heterozygous p. C376F variant that changes a hydrophilic cysteine to a hydrophobic phenylalanine. The *in silico* analyses (blossum62, SIFT, and Polyphen) suggest that this change is harmful to the MKKS protein function, and the conservation is complete amongst the nine species compared (Suppl. Table 4). The presence of potentially pathogenic mutations in two different ciliopathy genes (*CEP290* and *MKKS*) is consistent with recent reports of modifier loci (4-6) that influence clinical phenotypes. Unfortunately, we no longer have access to the patient for additional clinical studies.

References

1. den Hollander, A.I., Lopez, I., Yzer, S., Zonneveld, M.N., Janssen, I.M., Strom, T.M., Hehir-Kwa, J.Y., Veltman, J.A., Arends, M.L., Meitinger, T., et al. 2007. Identification of novel mutations in patients with Leber congenital amaurosis and juvenile RP by genome-wide homozygosity mapping with SNP microarrays. *Invest Ophthalmol Vis Sci* 48:5690-5698.
2. Slavotinek, A.M., Stone, E.M., Mykytyn, K., Heckenlively, J.R., Green, J.S., Heon, E., Musarella, M.A., Parfrey, P.S., Sheffield, V.C., and Biesecker, L.G. 2000. Mutations in MKKS cause Bardet-Biedl syndrome. *Nat Genet* 26:15-16.
3. Stone, D.L., Slavotinek, A., Bouffard, G.G., Banerjee-Basu, S., Baxevanis, A.D., Barr, M., and Biesecker, L.G. 2000. Mutation of a gene encoding a putative chaperonin causes McKusick-Kaufman syndrome. *Nat Genet* 25:79-82.
4. Wiszniewski, W., Lewis, R.A., Stockton, D.W., Peng, J., Mardon, G., Chen, R., and Lupski, J.R. 2011. Potential involvement of more than one locus in trait manifestation for individuals with Leber congenital amaurosis. *Hum Genet* 129:319-327.
5. Khanna, H., Davis, E.E., Murga-Zamalloa, C.A., Estrada-Cuzcano, A., Lopez, I., den Hollander, A.I., Zonneveld, M.N., Othman, M.I., Waseem, N., Chakarova, C.F., et al. 2009. A common allele in RPGRIP1L is a modifier of retinal degeneration in ciliopathies. *Nat Genet* 41:739-745.
6. Louie, C.M., Caridi, G., Lopes, V.S., Brancati, F., Kispert, A., Lancaster, M.A., Schlossman, A.M., Otto, E.A., Leitges, M., Grone, H.J., et al. 2010. AHI1 is required for photoreceptor outer segment development and is a modifier for retinal degeneration in nephronophthisis. *Nat Genet* 42:175-180.