# Supplemental Materials

***Molecular Biology of the Cell***

Okazaki *et al*.

**Supplemental materials**

# Formation of the B9-domain protein complex MKS1–B9D2–B9D1 is essential as a diffusion barrier for ciliary membrane proteins

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**Video S1. TIRF microscopy of RPE1 cells expressing tRFP-IFT88**

**Video S2. TIRF microscopy of #MKS1-3-13 cells expressing tRFP-IFT88**

**Video S3. TIRF microscopy of #B9D2-3-6 cells expressing tRFP-IFT88**

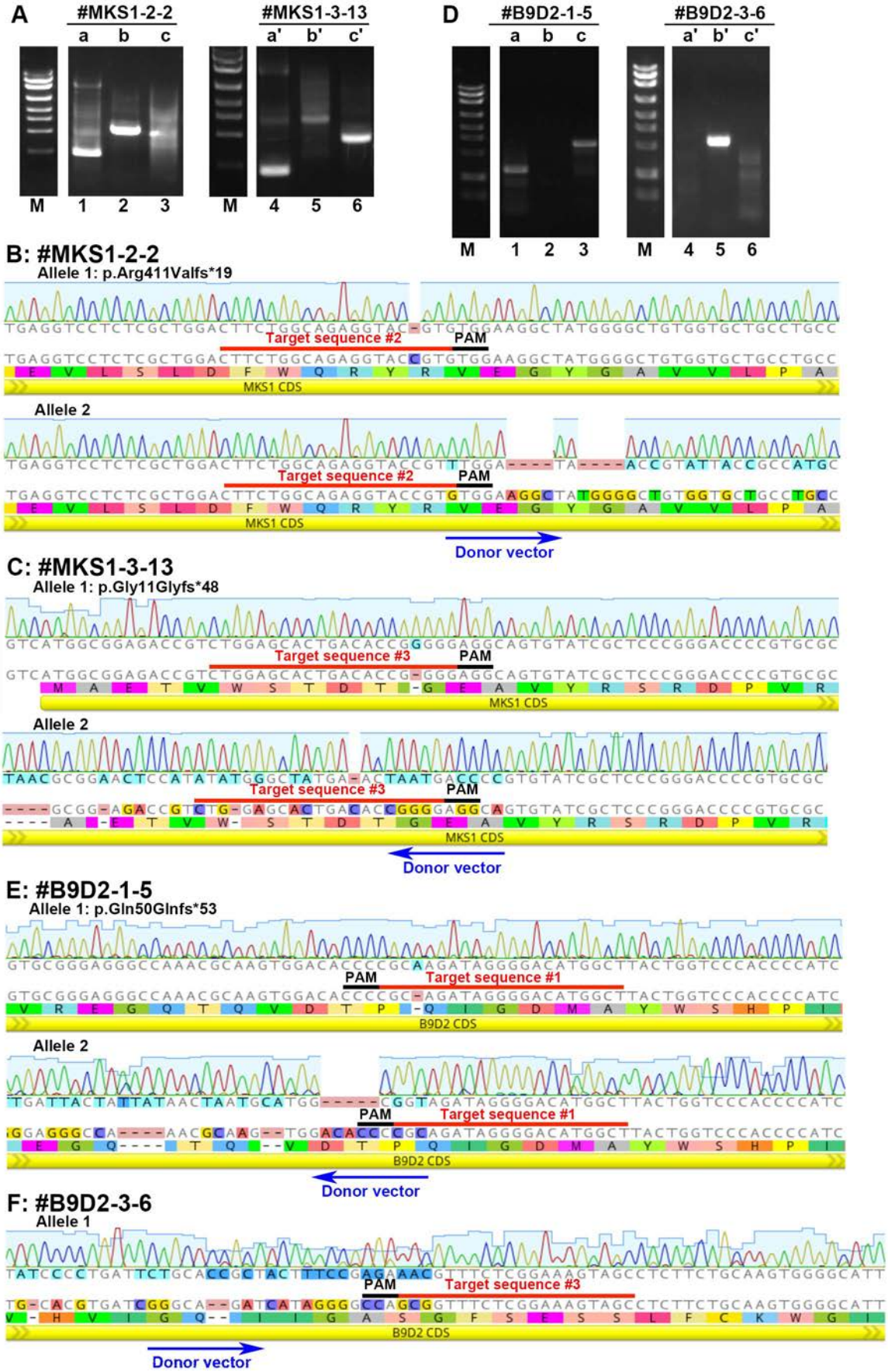
A
#MKS1-2-2
a b c
#MKS1-3-13
a' b' c'
M 1 2 3 M 4 5 6
D
#B9D2-1-5
a b c
#B9D2-3-6
a' b' c'
M 1 2 3 M 4 5 6
B: #MKS1-2-2
Allele 1: p.Arg411Valfs*19
Target sequence #2
PAM
MKS1 CDS
Allele 2
Target sequence #2
PAM
MKS1 CDS
Donor vector
C: #MKS1-3-13
Allele 1: p.Gly11Glyfs*48
Target sequence #3
PAM
MKS1 CDS
Allele 2
Target sequence #3
PAM
MKS1 CDS
Donor vector
E: #B9D2-1-5
Allele 1: p.Gln50Glnfs*53
PAM
Target sequence #1
B9D2 CDS
Allele 2
PAM
Target sequence #1
B9D2 CDS
Donor vector
F: #B9D2-3-6
Allele 1
PAM
Target sequence #3
B9D2 CDS
Donor vector

**Fig. S1. Genomic PCR and sequence analyses of the *MKS1*-KO and *B9D2*-KO cell lines**

(A, D) Genomic DNAs extracted from the *MKS1*-KO cell lines #MKS1-2-2 and #MKS1-3-13 (A), and those from the *B9D2*-KO cell lines #B9D2-1-5 and #B9D2-3-6 (D), were subjected to PCR using the indicated primer sets (see Table S3) to detect alleles with a small indel or no insertion (a, a′), or with forward (b, b′) or reverse (c, c′) integration of the donor knock-in vector. M, molecular weight marker (PSU1 DNA ladder). Note that we were unable to detect one of the two *B9D2* alleles in the #B9D2-3-6 cell line, possibly owing to the same knock-in site in both alleles or a relatively extensive deletion in one allele. (B, C, E, and F) Alignments of allele sequences of #MKS1-2-2 (B), #MKS1-3-13 (C), #B9D2-1-5 (E), #B9D2-3-6 (F) cell lines determined by sequencing of the PCR products shown in (A) and (D). Red and black lines indicate the target sequence and PAM sequence, respectively, and blue arrows indicate the direction of integration of the donor knock-in vector.

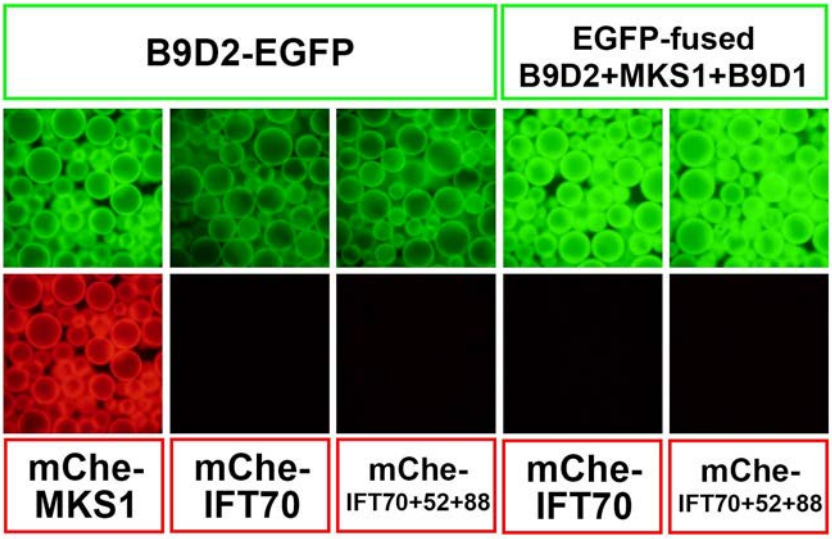


**Fig. S2. Interaction of B9D2 with IFT70 is not detectable**

Lysates of HEK293T cells coexpressing the indicated combinations of B9D2-EGFP or EGFP-fused B9D2+MKS1+B9D1 and either mChe-IFT70 or mChe-fused IFT70+IFT52+IFT88 were processed for the VIP assay. mChe-MKS1 was used as a positive control.

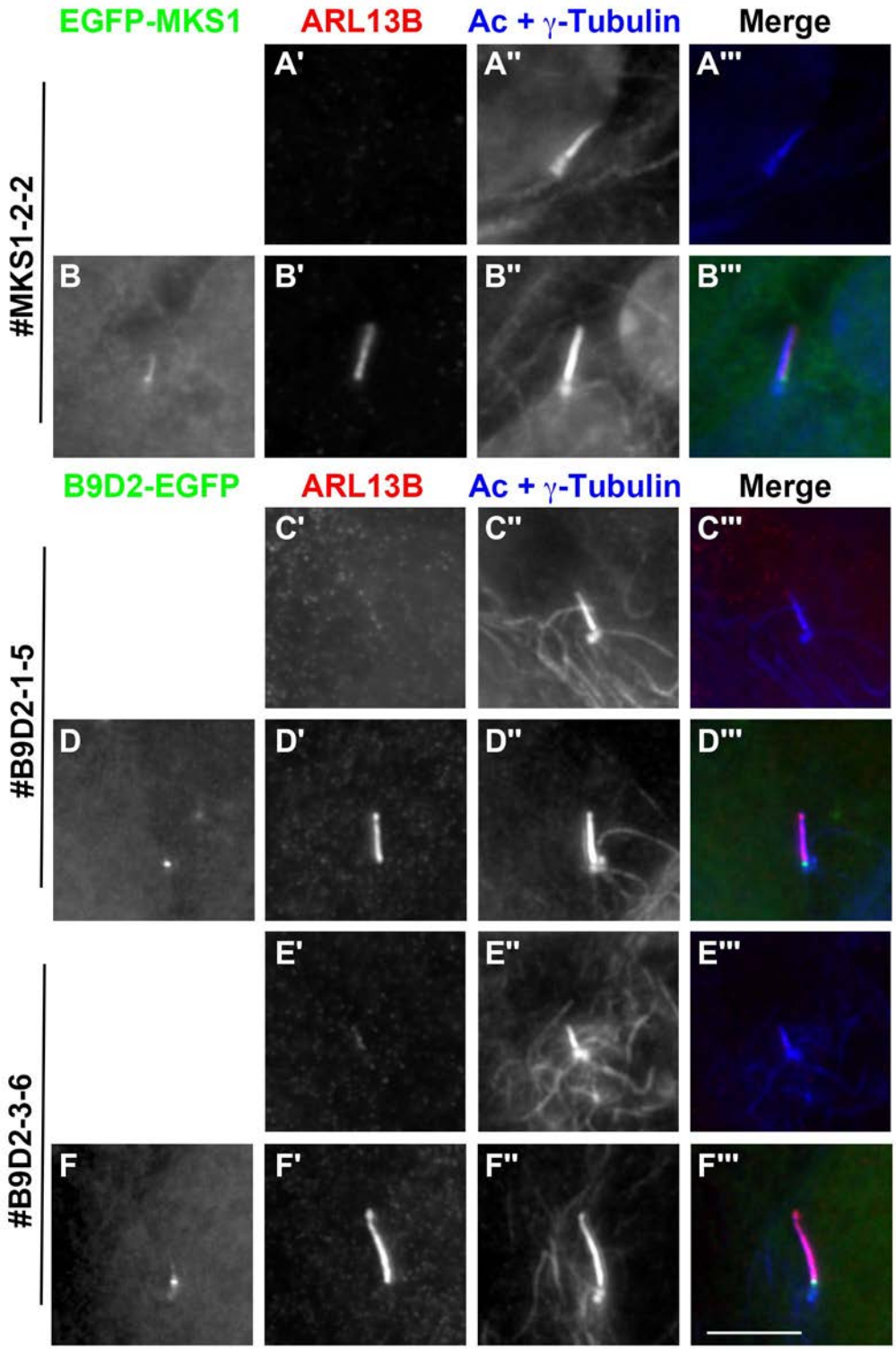


**Fig. S3. Rescue of *MKS1*-KO and *B9D2*-KO cell lines by the exogenous expression of MKS1 or B9D2**

The *MKS1*-KO cell line #MKS1-2-2 (A), or the *B9D2*-KO cell line #B9D2-1-5 (C) or #B9D2-3-6 (E), or cells stably expressing EGFP-MKS1 (B) or B9D2-EGFP (D, F), as indicated, were immunostained for ARL13B, and Ac-tubulin and γ-tubulin. Ciliary staining for ARL13B was restored by the exogenous expression of EGFP-MKS1 (B′) or B9D2-EGFP (D′, F′). Scale bar, 5 μm.

**Table S1. Plasmids used in this study**

| Vector | Insert | Reference |
|---|---|---|
| pCAG2-EGFP-C | MKS1 | This study |
| pCAG2-mCherry-C | MKS1 | This study |
| pEGFP-N | B9D1 | This study |
| pmCherry-N | B9D1 | This study |
| pEGFP-N | B9D2 | This study |
| pmCherry-N | B9D2 | This study |
| pTagBFP2-N | B9D2 | This study |
| pCAG2-EGFP-C | MKS1(110–559) | This study |
| pCAG2-EGFP-C | MKS1(290–559) | This study |
| pCAG2-EGFP-C | MKS1(311–559) | This study |
| pCAG2-EGFP-C | MKS1(1–492) | This study |
| pCAG2-EGFP-C | MKS1(1–310) | This study |
| pRRLsinPPT-EGFP-C-IRES-Blast | MKS1 | This study |
| pRRLsinPPT-EGFP-N-IRES-Blast | B9D1 | This study |
| pRRLsinPPT-EGFP-N-IRES-Blast | B9D2 | This study |
| pRRLsinPPT-EGFP-C-IRES-Blast | MKS1(110–559) | This study |
| pRRLsinPPT-EGFP-C-IRES-Blast | MKS1(1–492) | This study |
| pRRLsinPPT-tRFP-T-C-IRES-Zeo | IFT88 | This study |
| pCAG-mCherry-C | IFT70B | (Katoh et al., 2016) |
| pCAG-mCherry-C | IFT88 | (Katoh et al., 2016) |
| pCAG-mCherry-C | IFT52 | (Katoh et al., 2016) |
| pDonor-tBFP-NLS-Neo (Universal) | − | (Katoh et al., 2017) |
| peSpCas9 (1.1)-2×gRNA | − | (Katoh et al., 2017) |
| pGEX-6P1 | Anti-GFP-nanobody | (Katoh et al., 2015) |

**Table S2. Antibodies used in this study**

| Antibody | Manufacturer or provider | Clone/catalog number or reference number | Dilution (purpose) |
|---|---|---|---|
| Polyclonal rabbit anti-IFT88 | Proteintech | 13967-1-AP | 1:500 (IF) |
| Polyclonal rabbit anti-IFT140 | Proteintech | 17460-1-AP | 1:500 (IF) |
| Polyclonal rabbit anti-ARL13B | Proteintech | 17711-1-AP | 1:1,000 (IF) |
| Polyclonal rabbit anti-INPP5E | Proteintech | 17797-1-AP | 1:500 (IF) |
| Polyclonal rabbit anti-GPR161 | Proteintech | 13398-1-AP | 1:500 (IF) |
| Polyclonal rabbit anti-TCTN1 | Proteintech | 15004-1-AP | 1:100 (IF) |
| Monoclonal mouse anti-MKS1 | Proteintech | 66518-1-Ig | 1:200 (IF) |
| Monoclonal mouse anti-SMO | Santa Cruz | sc-166685 | 1:100 (IF) |
| Monoclonal mouse anti-acetylated-α-tubulin | Sigma-Aldrich | 6-11B-1 | 1:1,000 (IF) |
| Monoclonal mouse anti-γ-tubulin | Sigma-Aldrich | GTU88 | 1:500 (IF) |
| Monoclonal mouse anti-polyglutamylation modification | AdipoGen | GT335 | 1:500 (IF) |
| Monoclonal mouse anti-FOP | Abnova | 2B1 | 1:10,000 (IF) |
| FluoTag-X4 anti-GFP, fluorophore-conjugated | NanoTag Biotechnologies | N0304-At488-L | 1:250 (IF) |
| Polyclonal rabbit anti-mCherry | Proteintech | 26765-1-AP | 1:10,000 (IB) |
| Monoclonal mouse anti-GFP | Proteintech | 66002-1-Ig | 1:10,000 (IB) |
| AlexaFluor-conjugated secondary | Molecular Probes | A11034, A27039, A21244, A11004, A21127, A21240, A21241, A21131, A21242 | 1:1,000 (IF) |
| Peroxidase-conjugated secondary | Jackson ImmunoResearch | 115-035-166, 111-035-144 | 1:3,000 (IB) |

IF, immunofluorescence; IB, immunoblotting

**Table S3. Oligo DNAs used in this study**

| Name | Sequence |
|---|---|
| pTagBFP-N-RV2 (primer 3) | 5'- CGTAGAGGAAGCTAGTAGCCAGG -3' |
| MKS1-genome-FW (primer 1) | 5'- CCTTGCCCACCCTATGACAC -3' |
| MKS1-genome-RV (primer 2) | 5'- TCAGGAAGTCAGTGCCAAGG -3' |
| MKS1-genome-FW (primer 4) | 5'- ACATGGTAAGCGGTGATCGG -3' |
| MKS1-genome-RV (primer 5) | 5'- AAGGGGAGAGGGTGTACTGG -3' |
| B9D2-genome-FW (primer 6) | 5'- GCAGGAAGAGGTAGTGCCAG -3' |
| B9D2-genome-RV (primer 7) | 5'- CTTGAATGCCACCTCCCCTC -3' |
| B9D2-genome-FW (primer 8) | 5'- TCCAGGCTTTCATGGGTTGG -3' |
| B9D2-genome-RV (primer 9) | 5'- CACACCACCTTACTCCAGCC -3' |
| MKS1-gRNA#2-S | 5'- CACCGCTTCTGGCAGAGGTACCGTG -3' |
| MKS1-gRNA#2-AS | 5'- AAACCACGGTACCTCTGCCAGAAGC -3' |
| MKS1-gRNA#3-S | 5'- CACCGCTGGAGCACTGACACCGGGG -3' |
| MKS1-gRNA#3-AS | 5'- AAACCCCCGGTGTCAGTGCTCCAGC -3' |
| B9D2-gRNA#1-S | 5'- CACCGAGCCATGTCCCCTATCTGCG -3' |
| B9D2-gRNA#1-AS | 5'- AAACCGCAGATAGGGGACATGGCTC -3' |
| B9D2-gRNA#3-S | 5'- CACCGCTACTTTCCGAGAAACCGC -3' |
| B9D2-gRNA#3-AS | 5'- AAACGCGGTTTCTCGGAAAGTAGC -3' |

**Supplemental References**

Katoh, Y., Michisaka, S., Nozaki, S., Funabashi, T., Hirano, T., Takei, R., and Nakayama, K. (2017). Practical method for targeted disruption of cilia-related genes by using CRISPR/Cas9-mediated homology-independent knock-in system. Mol. Biol. Cell *28*, 898–906.

Katoh, Y., Nozaki, S., Hartanto, D., Miyano, R., and Nakayama, K. (2015). Architectures of multisubunit complexes revealed by a visible immunoprecipitation assay using fluorescent fusion proteins. J. Cell Sci. *128*, 2351-2362.

Katoh, Y., Terada, M., Nishijima, Y., Takei, R., Nozaki, S., Hamada, H., and Nakayama, K. (2016). Overall architecture of the intraflagellar transport (IFT)-B complex containing Cluap1/IFT38 as an essential component of the IFT-B peripheral subcomplex. J. Biol. Chem. *291*, 10962-10975.