

## Supplemental material

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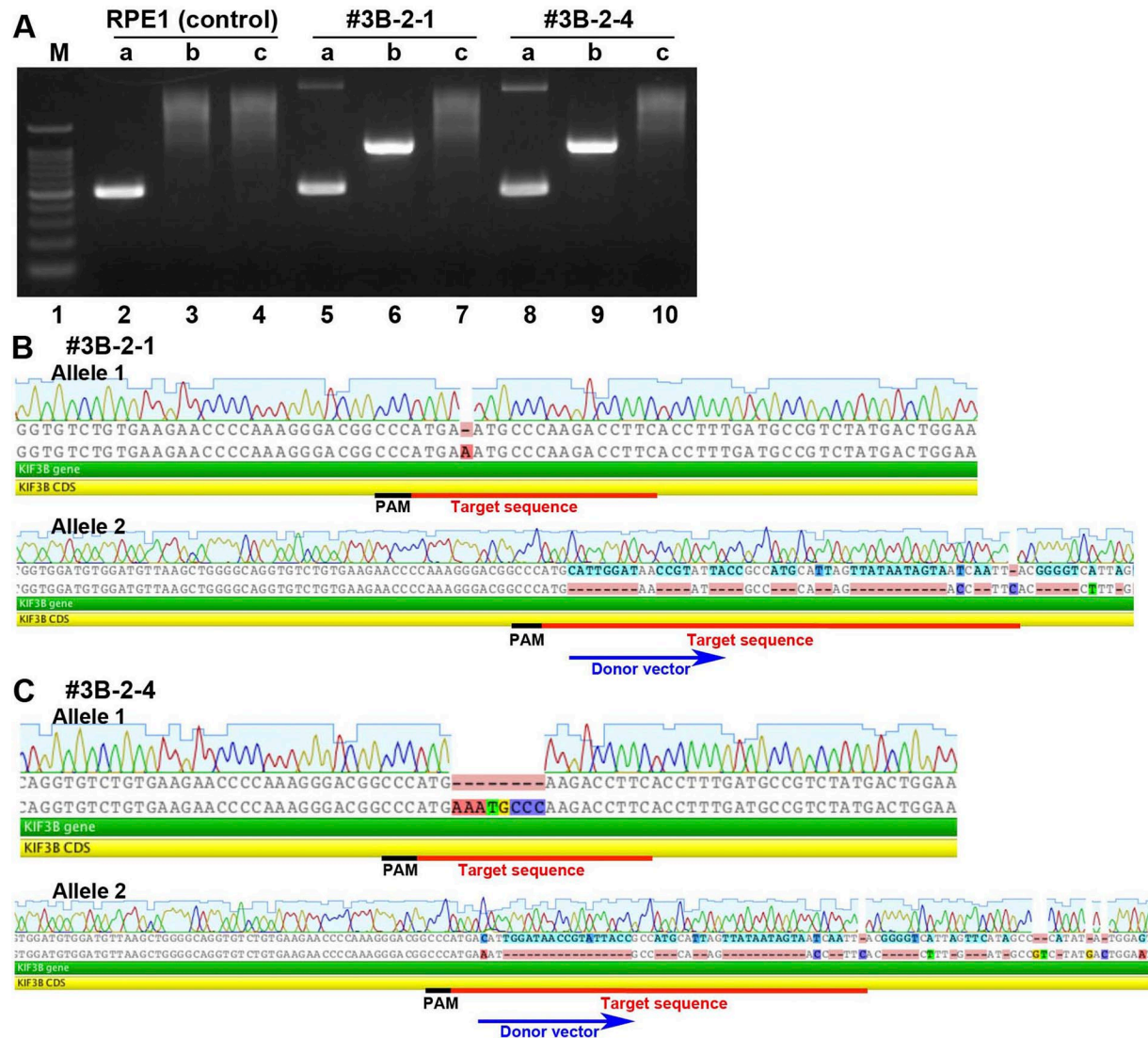


Figure S1. **Genomic PCR and sequence analyses of the *KIF3B*-KO cell lines.** (A) Genomic DNA was extracted from control hTERT-RPE1 cells and from the *KIF3B*-KO cell lines 3B-2-1 and 3B-2-4, which were established using a donor knock-in vector containing the target sequence, and subjected to PCR using the indicated primer sets (Table S2) to detect alleles with a small indel or no insertion (a), or with forward (b) or reverse (c) integration of the donor knock-in vector. Lane 1 (M), 100-bp ladder marker (the most intense band, 500 bp). (B and C) Alignments of allele sequences of the 3B-2-1 (B) and 3B-2-4 (C) cell lines determined by direct sequencing of the genomic PCR products. Red and black lines indicate the target sequence and protospacer-adjacent motif (PAM) sequence, respectively, and blue arrows indicate the direction of donor vector integration. CDS, coding sequence.

Table S1. **Plasmid vectors used in this study**

<b>Vector</b>	<b>Insert</b>	<b>Reference</b>
pCAG2-EGFP-C	Human KIF3A	This study
pCAG2-mCherry-C	Human KIF3A	This study
pCAG2-EGFP-C	Human KIF3B	This study
pCAG2-mCherry-C	Human KIF3B	This study
pCAG2-EGFP-C	Human KAP3	This study
pCAG2-mCherry-C	Human KAP3	This study
pCAG2-EGFP-C	Human KIF17	<a href="#">Funabashi et al. (2017)</a>
pCAG2-EGFP-C	Human KIF3B (1–704)	This study
pCAG2-EGFP-C	Human KIF3B (1–662)	This study
pCAG2-EGFP-C	Human KIF3B (1–625)	This study
pCAG2-EGFP-C	Human KIF3B (1–579)	This study
pCAG2-EGFP-C	Human KIF3B ( $\Delta$ CC: $\Delta$ 346–579)	This study
pRRLsinPPT-EGFP-N	Human KIF3B	This study
pRRLsinPPT-EGFP-N	Human KIF3B (1–704)	This study
pRRLsinPPT-EGFP-N	Human KIF3B (1–662)	This study
pRRLsinPPT-EGFP-N	Human KIF3B (1–625)	This study
pRRLsinPPT-EGFP-N	Human KIF3B (1–579)	This study
pTagRFP-T-N	Human IFT20	<a href="#">Kato et al. (2016)</a>
pTagRFP-T-C	Human IFT22	<a href="#">Kato et al. (2016)</a>
pTagRFP-T-C	Human IFT25	<a href="#">Kato et al. (2016)</a>
pTagRFP-T-C	Human IFT27	<a href="#">Kato et al. (2016)</a>
pTagRFP-T-C	Human IFT38	<a href="#">Kato et al. (2016)</a>
pCAG-mCherry-C	Human IFT46	<a href="#">Kato et al. (2016)</a>
pCAG-mCherry-C	Human IFT52	<a href="#">Kato et al. (2016)</a>
pTagRFP-T-C	Human IFT54	<a href="#">Kato et al. (2016)</a>
pCAG-mCherry-C	Human IFT56	<a href="#">Funabashi et al. (2017)</a>
pCAG-TagRFP-T-C	Human IFT56	<a href="#">Kato et al. (2016)</a>
pTagRFP-T-C	Human IFT57	<a href="#">Kato et al. (2016)</a>
pCAG2-mCherry-C	Human IFT70B	<a href="#">Kato et al. (2016)</a>
pCAG-mCherry-C	Human IFT74	<a href="#">Kato et al. (2016)</a>
pCAG-mCherry-N	Human IFT80	<a href="#">Kato et al. (2016)</a>
pCAG-mCherry-C	Human IFT81	<a href="#">Kato et al. (2016)</a>
pCAG-mCherry-C	Human IFT88	<a href="#">Kato et al. (2016)</a>
pCAG2-mCherry-C	Human IFT172	<a href="#">Kato et al. (2016)</a>
pmCherry-C1	IFT43	<a href="#">Hirano et al. (2017)</a>
pCAG2-mCherry-C	IFT121	<a href="#">Hirano et al. (2017)</a>
pCAG2-mCherry-C	IFT122	<a href="#">Hirano et al. (2017)</a>
pCAG2-mCherry-C	IFT139	<a href="#">Hirano et al. (2017)</a>
pCAG2-mCherry-C	IFT140	<a href="#">Hirano et al. (2017)</a>
pCAG2-mCherry-C	IFT144	<a href="#">Hirano et al. (2017)</a>
pmCherry-C1	TULP3	<a href="#">Hirano et al. (2017)</a>

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

<b>Name</b>	<b>Sequence (5'-3')</b>
pTagBFP-N-RV2	CGTAGAGGAAGCTAGTAGCCAGG
KIF3B-genome-FW	GCTGCAATGACAACGGTACTG
KIF3B-genome-RV	GATGTGGGTGAAGATATGGTC
KIF3B-gRNA 2-S	CACCGAAGGTCTTGGGCATTTTCAT
KIF3B-gRNA 2-AS	AAACATGAAATGCCCAAGACCTTC
KIF3B-Donor 2-AS	TCCAATGAAATGCCCAAGACCTTC

## References

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- Hirano, T., Y. Katoh, and K. Nakayama. 2017. Intraflagellar transport-A complex mediates ciliary entry and retrograde trafficking of ciliary G protein-coupled receptors. *Mol. Biol. Cell.* 28:429–439. <https://doi.org/10.1091/mbc.e16-11-0813>
- Katoh, Y., M. Terada, Y. Nishijima, R. Takei, S. Nozaki, H. Hamada, and K. Nakayama. 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. <https://doi.org/10.1074/jbc.M116.713883>