Supplemental Materials

Molecular Biology of the Cell

Funabashi et al.

Supplementary materials

Ciliary entry of KIF17 is dependent on its binding to the IFT-B complex via IFT46-IFT56 as well as on its nuclear localization signal

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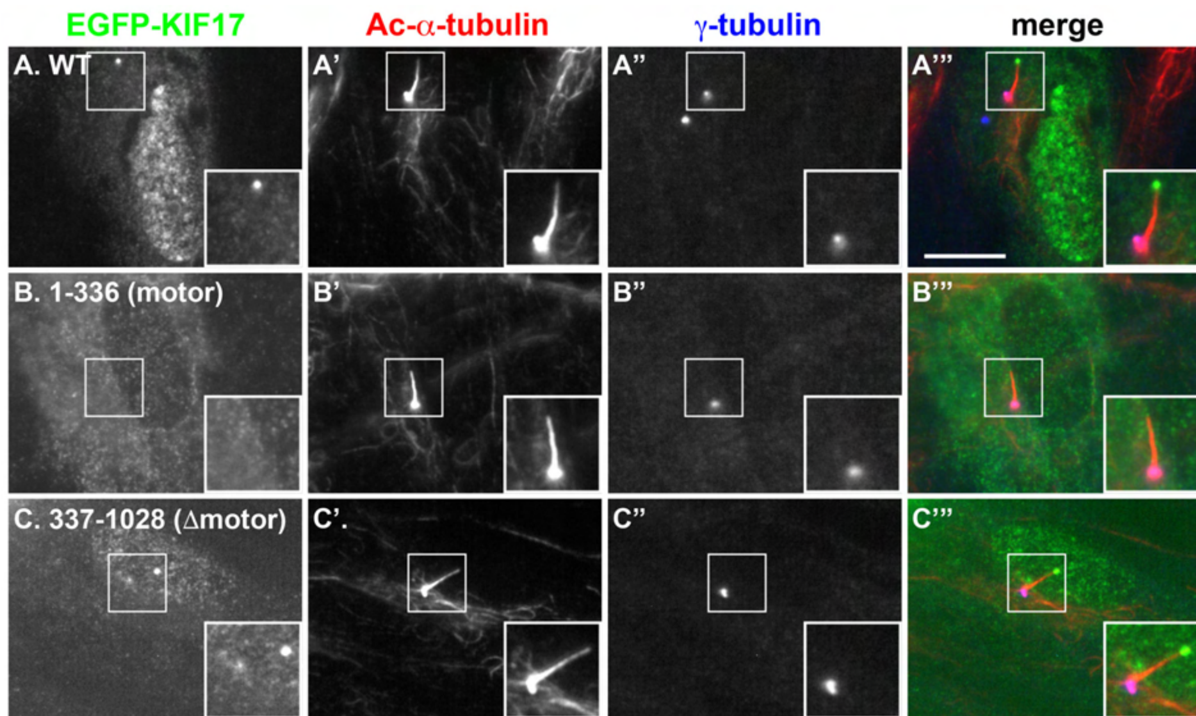

Fig. S1. Localization of motor-less KIF17 at the ciliary tip

hTERT-RPE1 cells transfected with an expression vector for EGFP-KIF17(WT) (A), EGFP-KIF17(1-336) (B), or EGFP-KIF17(337-1,028) (C) were processed as described in the legend for Fig. 3. Scale bar, 10 μm. The motor-less construct, KIF17(337-1,028) (see Fig. 2A), but not the motor-domain construct, KIF17(1-336), was found at the ciliary tip.

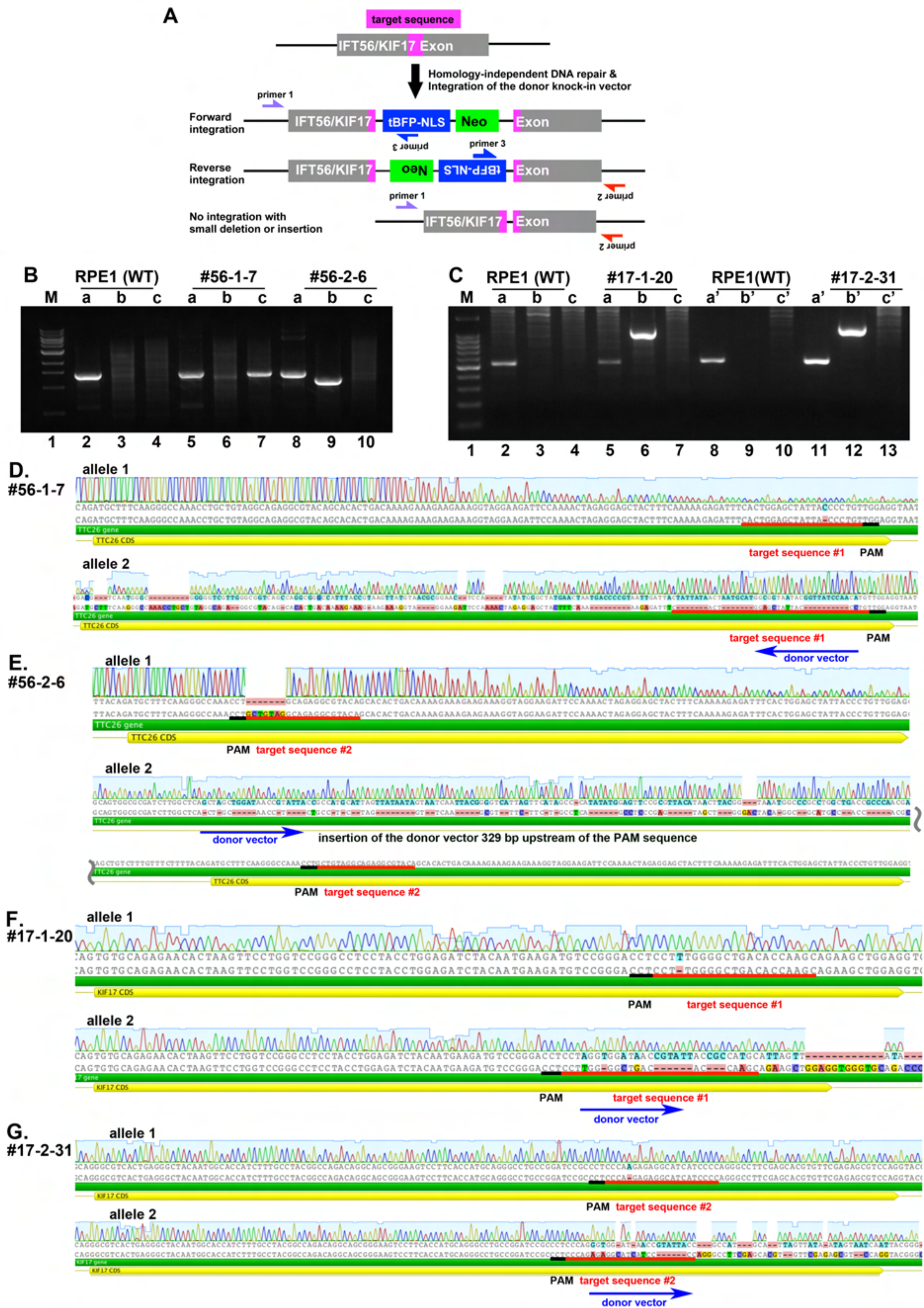
A
target sequence
IFT56/KIF17 Exon
Homology-independent DNA repair &
Integration of the donor knock-in vector
primer 1
Forward integration
tBFP-NLS
Neo
Exon
primer 3
Reverse integration
No integration with small deletion or insertion
primer 2
B
RPE1 (WT)
#56-1-7
#56-2-6
C
#17-1-20
RPE1(WT)
#17-2-31
D.
#56-1-7
allele 1
allele 2
target sequence #1
PAM
donor vector
E.
#56-2-6
target sequence #2
insertion of the donor vector 329 bp upstream of the PAM sequence
F.
#17-1-20
G.
#17-2-31

Fig. S2. Strategy for the establishment of KO cell lines and confirmation of knock-in donor vector integration or a small deletion/insertion in the genome of selected *IFT56*-KO and *KIF17*-KO cell lines

(A) Schematic outline of the establishment of *IFT56*-KO and *KIF17*-KO cell lines. Upon homology-independent DNA repair of the target sequence in the *IFT56* or *KIF17* gene, two types of integration of the donor knock-in vector containing tBFP-3×NLS (blue) and Neo (green) can occur; forward integration and reverse integration. In other cases, the target gene often undergoes error-prone repair with small insertions or deletions, causing a frameshift. Half-headed arrows indicate primers used for genomic PCR, with the same color representing identical primers. The target sequence is shown in magenta. (B) Genomic PCR of isolated *IFT56*-KO cell lines. Genomic DNAs extracted from WT hTERT-RPE1 cells (lanes 2–4), and from cell lines (#56-1-7, lanes 5–7; and #56-2-6, lanes 8–10) established using a knock-in vector containing target sequences 1 and 2, respectively (see Table S1), both of which target the coding region within exon 2 of the human *IFT56* gene, were subjected to PCR using the primer pair a (primers 1 + 2; lanes 2, 5, and 8), pair b (primers 1 + 3; lanes 3, 6, and 9), or pair c (primers 2 + 3; lanes 4, 7, and 10) (see Table S1) to detect alleles with a small deletion or insertion, with donor vector forward integration, and with reverse integration, respectively (see Table S1). (C) Genomic PCR of isolated *KIF17*-KO cell lines. Genomic DNAs extracted from WT hTERT-RPE1 cells (lanes 2–4, and 8–10), and from cell lines (#17-1-20, lanes 5–7; and #17-2-31, lanes 11–13) established using a knock-in vector containing target sequences 1 and 2, respectively (see Table S1), which target the coding region within exon 3 and exon 2, respectively, of the human *KIF17* gene, were subjected to PCR using the primer pair a (primers 1 + 2; lanes 2 and 5), pair b (primers 1 + 3; lanes 3 and 6), pair c (primers 2 + 3; lanes 4 and 7), pair a′ (primers 1′ + 2′; lanes 8 and 11), pair b′ (primers 1′ + 3; lanes 9 and 12), or pair c′ (primers 2′ + 3; lanes 10 and 13) (see Table S1). (D)–(G) Alignments of allele sequences of cell lines #56-1-7, #56-2-6, #17-1-20, and #17-2-31 determined by direct sequencing of the genomic PCR products with the reference sequence encompassing the coding sequence of the *IFT56* or *KIF17* gene. Red and black lines indicate the target sequences and protospacer adjacent motif (PAM) sequences, respectively, and blue arrows indicate the direction of donor vector integration. Note that in the #56-2-6 cell line, donor vector integration occurred in a region considerably upstream of the PAM sequence (329-bp upstream).

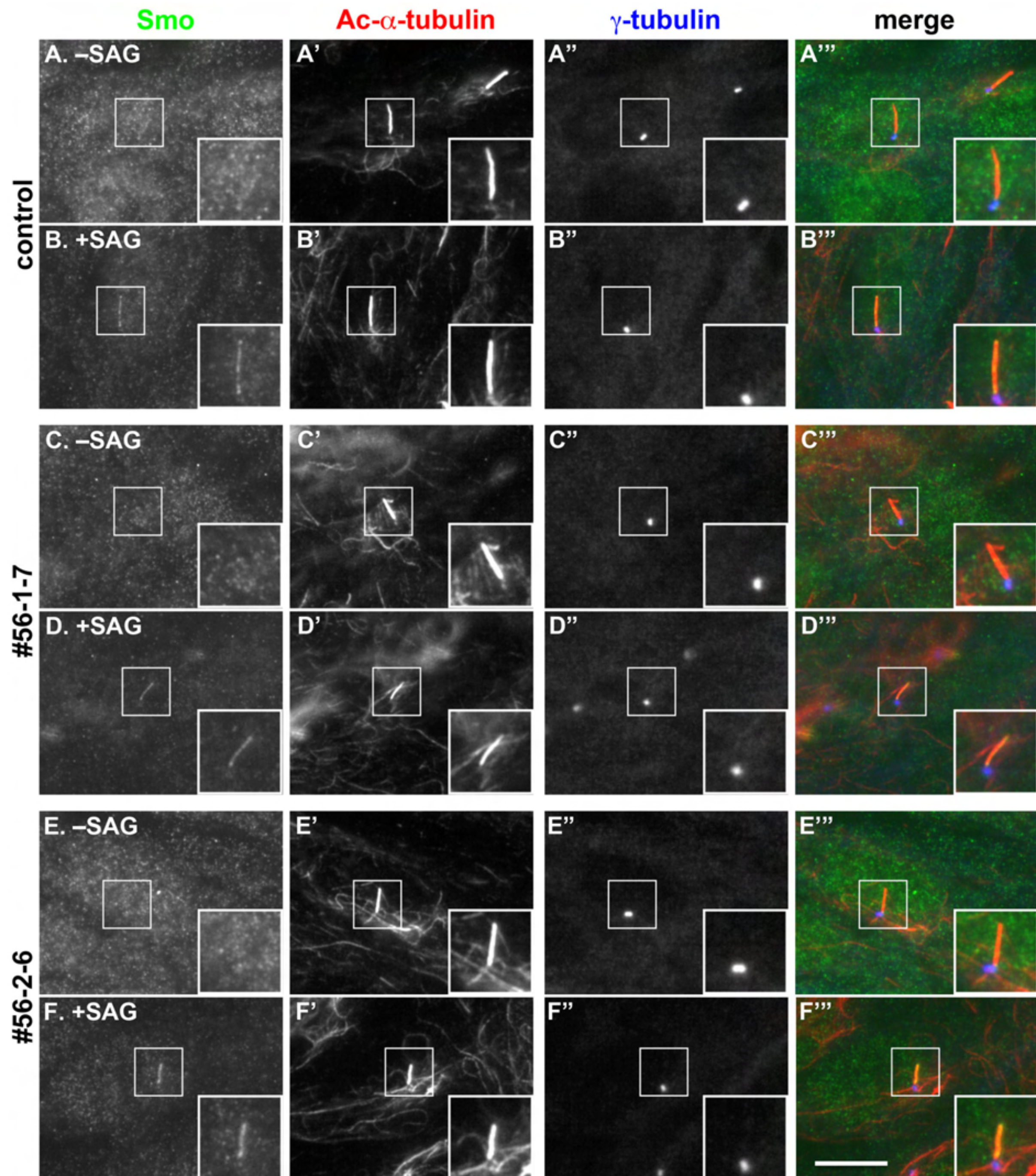

Fig. S3. Localization of Smo in *IFT56*-KO cells

(A)–(F) Control hTERT-RPE1 cells (A and B), and the *IFT56*-KO cell lines #56-1-7 (C and D) and #56-2-6 (E and F) were cultured under serum starvation conditions for 24 h to induce ciliogenesis. The cells were cultured for a further 24 h in Opti-MEM containing 0.2% bovine serum albumin with (B, D, and F) or without (A, C, and E) 200 nM SAG. The cells were then triple immunostained for Smo (A–F), Ac-α-tubulin (A′–F′), and γ-tubulin (A″–F″). Merged images are shown in A‴–F‴. Enlarged images of the boxed regions are shown in the insets. Scale bar, 10 µm.

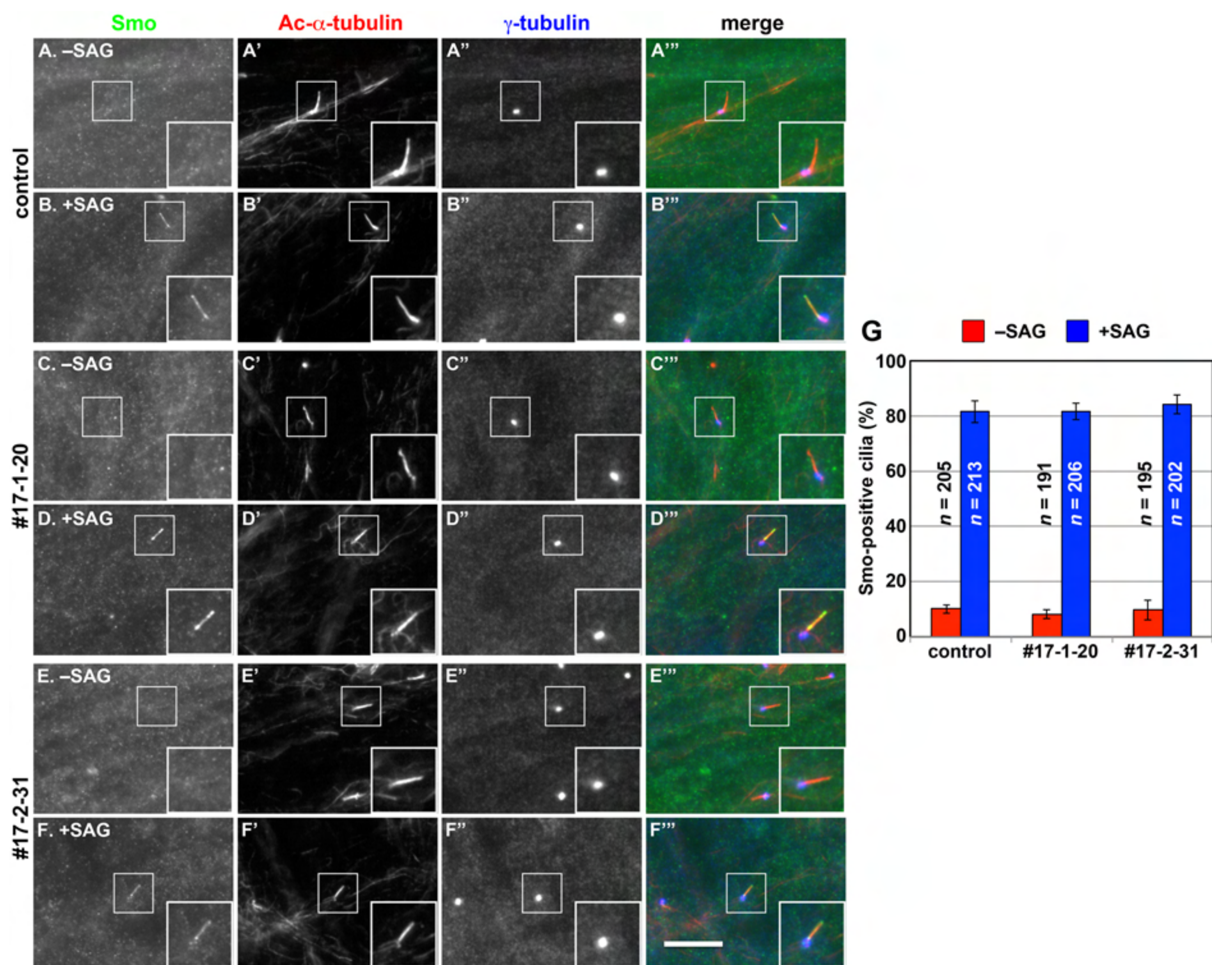

Fig. S4. Localization of Smo in *KIF17*-KO cells

(A)–(F) Control hTERT-RPE1 cells (A and B), and the *KIF17*-KO cell lines #17-1-20 (C and D) and #17-2-31 (E and F) were cultured under serum starvation conditions for 24 h to induce ciliogenesis. The cells were cultured for a further 24 h in Opti-MEM containing 0.2% bovine serum albumin with (B, D, and F) or without (A, C, and E) 200 nM SAG. The cells were then triple immunostained for Smo (A–F), Ac-α-tubulin (A′–F′), and γ-tubulin (A″–F″). Merged images are shown in A‴–F‴. Enlarged images of the boxed regions are shown in the insets. Scale bar, 10 µm. (G) Cells with Smo-positive cilia were counted, and the percentages of ciliated cells with Smo-positive cilia are represented as bar graphs. Values are means±S.E. (error bars) of three independent experiments. In each set of experiments, 53–81 cells were counted, and the total numbers of ciliated cells observed (*n*) are shown in the bar graphs.

Table S1. Oligo DNAs used in this study

IFT56-gRNA-#1-S	5′-CACCGCACTGGAGCTATTACCCTGT-3′
IFT56-gRNA-#1-AS	5′-AAACACAGGGTAATAGCTCCAGTGC-3′
IFT56-Donor#1-AS	5′-TCCAACAGGGTAATAGCTCCAGTGC-3′
IFT56-gRNA-#2-S	5′-CACCGTGTACGCCTCTGCCTACAGC-3′
IFT56-gRNA-#2-AS	5′-AAACGCTGTAGGCAGAGGCGTACAC-3′
IFT56-Donor#2-AS	5′-TCCAGCTGTAGGCAGAGGCGTACAC-3′
pTagBFP-N-RV2	5′-CGTAGAGGAAGCTAGTAGCCAGG-3′
IFT56-genome-FW	5′-GTCTAGGTGTGTCCGCAGAGAG-3′
IFT56-genome-RV	5′-GGATAAGCAATGTGTGAAATGAC-3′
KIF17-gRNA-#1-S	5′-CACCGCTTGGTGTCAGCCCCAAGG-3′
KIF17-gRNA-#1-AS	5′-AAACCCTTGGGGCTGACACCAAGC-3′
KIF17-Donor#1-AS	5′-TCCACCTTGGGGCTGACACCAAGC-3′
KIF17-gRNA-#2-S	5′-CACCGGGGATGATGCCTCTCTGGG-3′
KIF17-gRNA-#2-AS	5′-AAACCCCAGAGAGGCATCATCCCC-3′
KIF17-Donor#2-AS	5′-TCCACCCAGAGAGGCATCATCCCC-3′
KIF17-genome-#1-FW	5′-GAGAGAGCTTGCCAGGAAGG-3′
KIF17-genome-#1-RV	5′-CTCCTGACCTCAAGTGATCTG-3′
KIF17-genome-#2-FW	5′-CAGAGAGTCAACTCCTTGAGGAC-3′
KIF17-genome-#2-RV	5′-GTTAGAAGGCAAAAATGGTGAGAG-3′