

**Fig. S1. Analyses of *IFT54* genomic sequences of the *IFT54*-KO cell line**

(A) Genomic DNA extracted from the *IFT54*-KO cell line #IFT54-4-3 was subjected to PCR using primer sets (a, b, and c; Table S3) to detect alleles with forward (b) or reverse (c) integration of the donor knock-in vector or no insertion/small indel (a). (B) The amplified DNAs with no insertion/small indel (allele 1) and reverse integration of the donor vector (allele 2) were subjected to sequence analysis. Note that for allele 2, as an unrelated long sequence from human chromosome 1 (LOC126805873), in addition to the knock-in vector sequence, was inserted, we could not determine the exact insertion site of the knock-in vector. However, as the abnormal cilia-lacking phenotype of the cell line #IFT54-4-3 was rescued by exogenous expression of *IFT54*(WT) (see Fig. 1D, E), the abnormal phenotype is not likely to result from an off-target effect. (C, D) Control RPE1 cells (C) and the #IFT54-4-3 cell line (D) were cultured under serum-starved conditions for 24 h to induce ciliogenesis, and doubly immunostained for FOP and ARL13B. Enlarged (2.5-fold) images of the boxed regions are shown on the right. Scale bars, 5  $\mu$ m. The #IFT54-4-3 cell line could not form cilia, consistent with the disruption of both *IFT54* alleles.

Fig. S2: Uncropped images

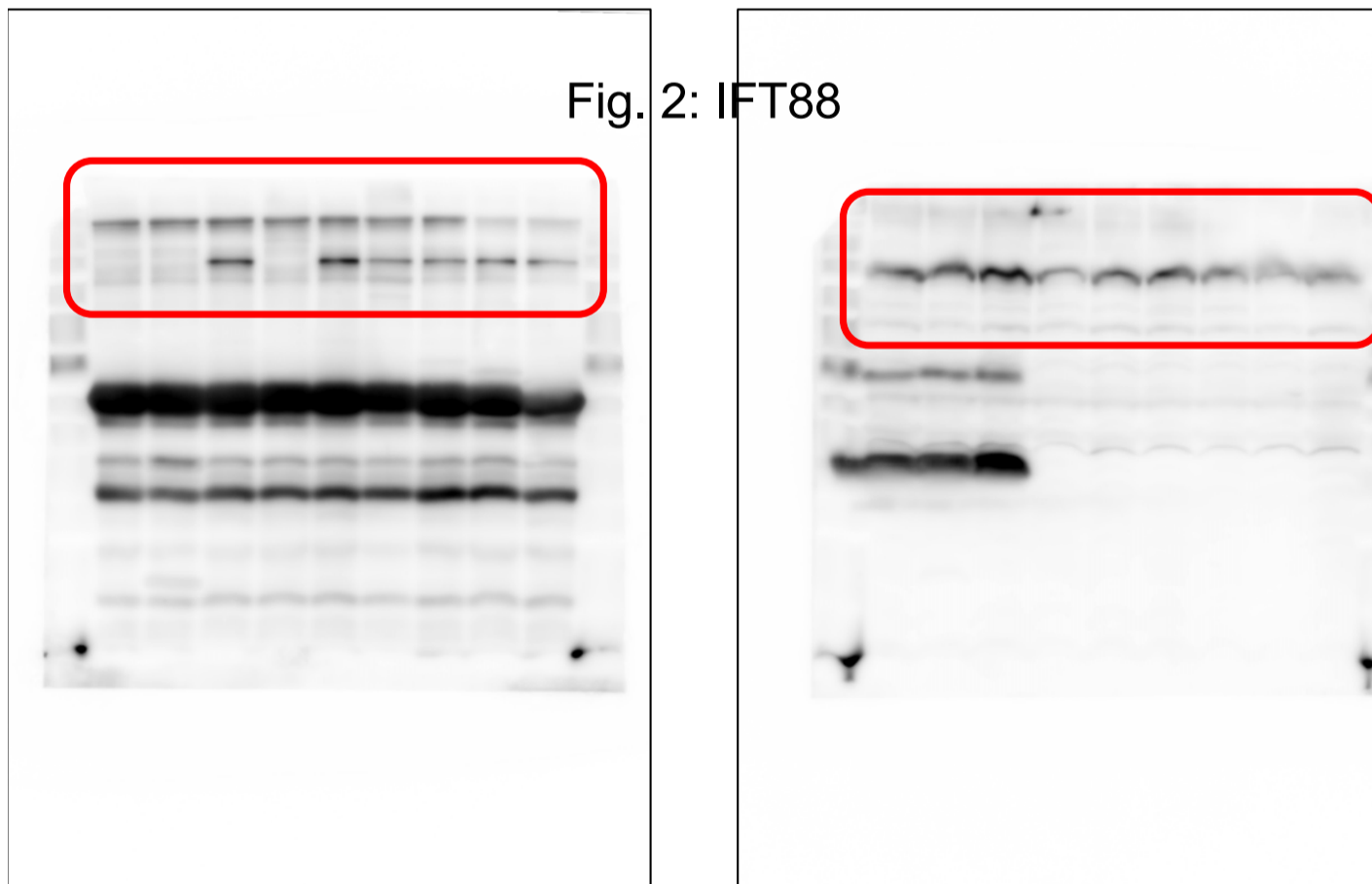
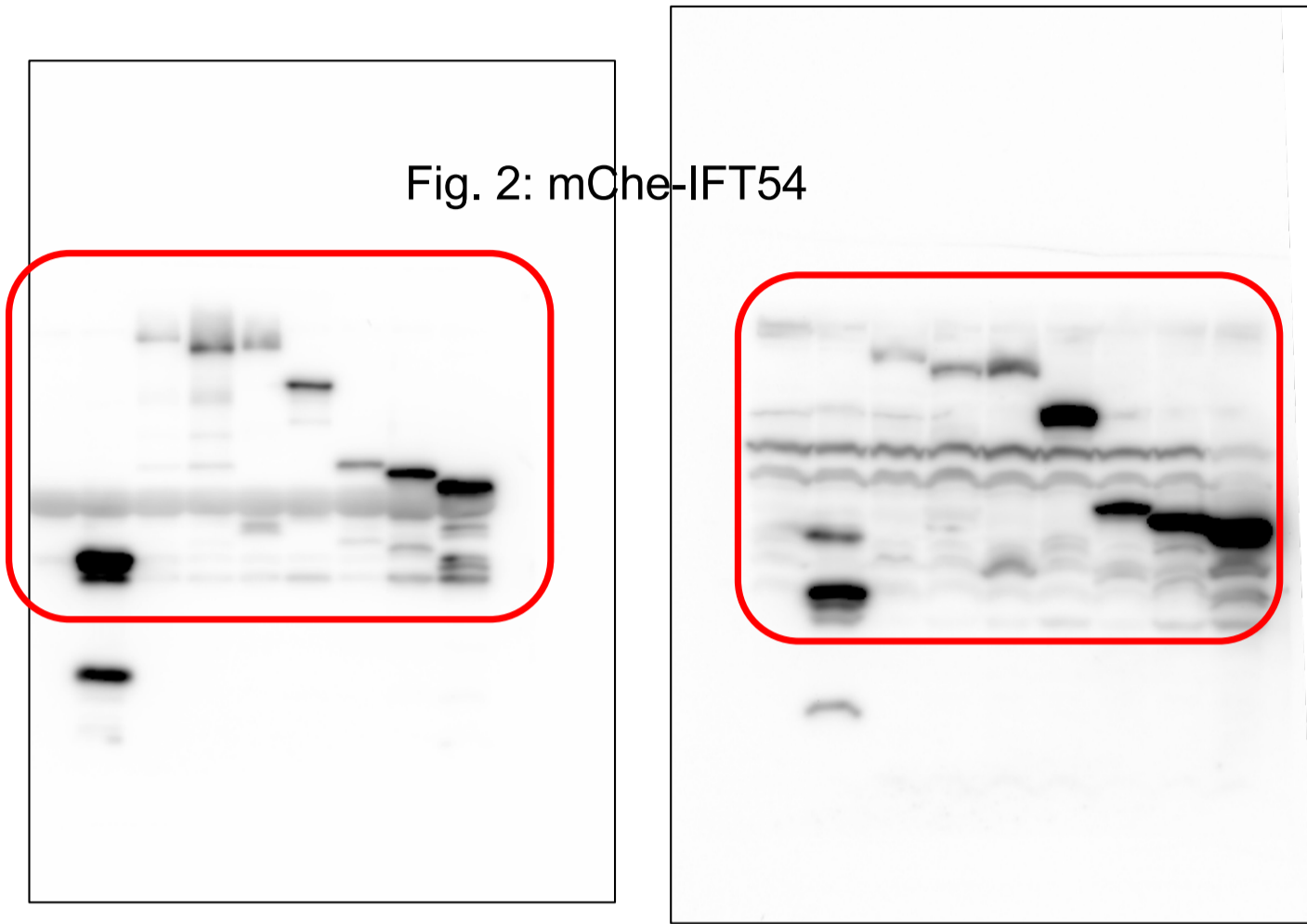


Fig. S2: Uncropped images

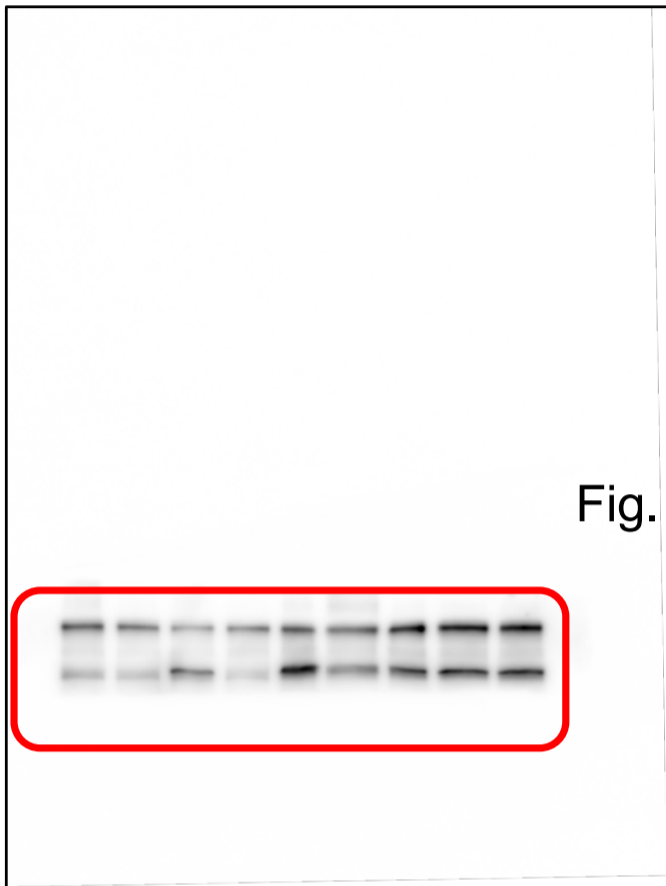


Fig. 2: IFT81

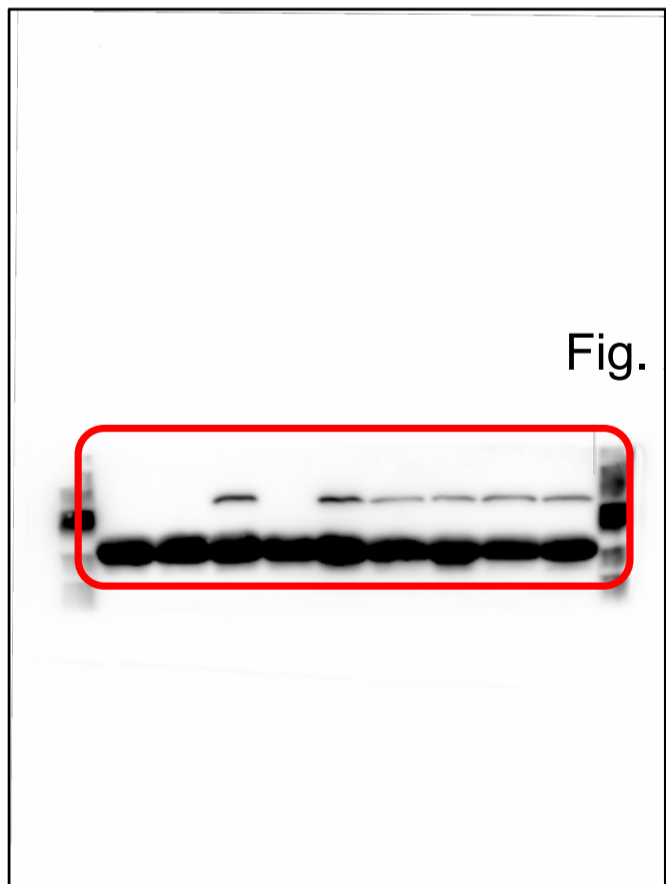
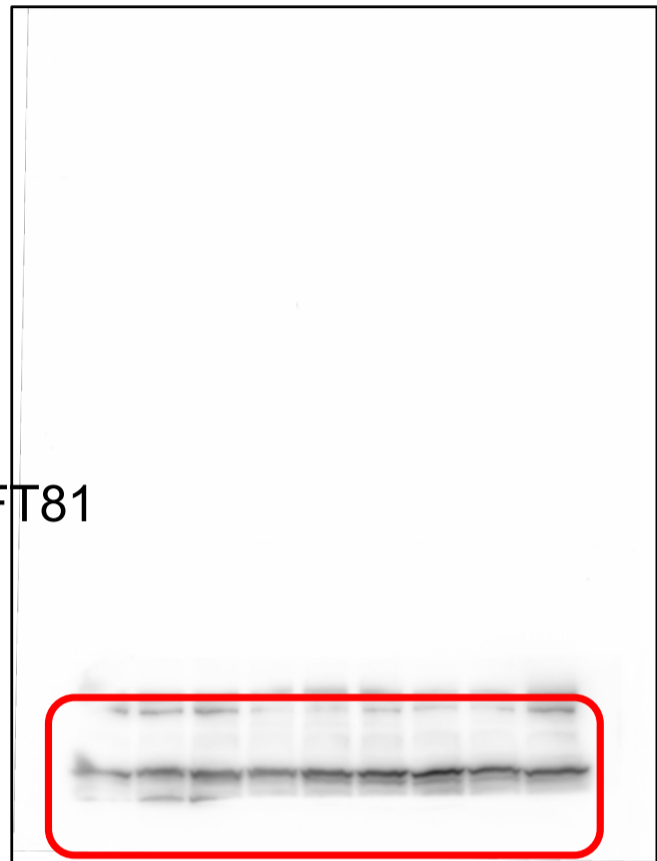


Fig. 2: IFT52

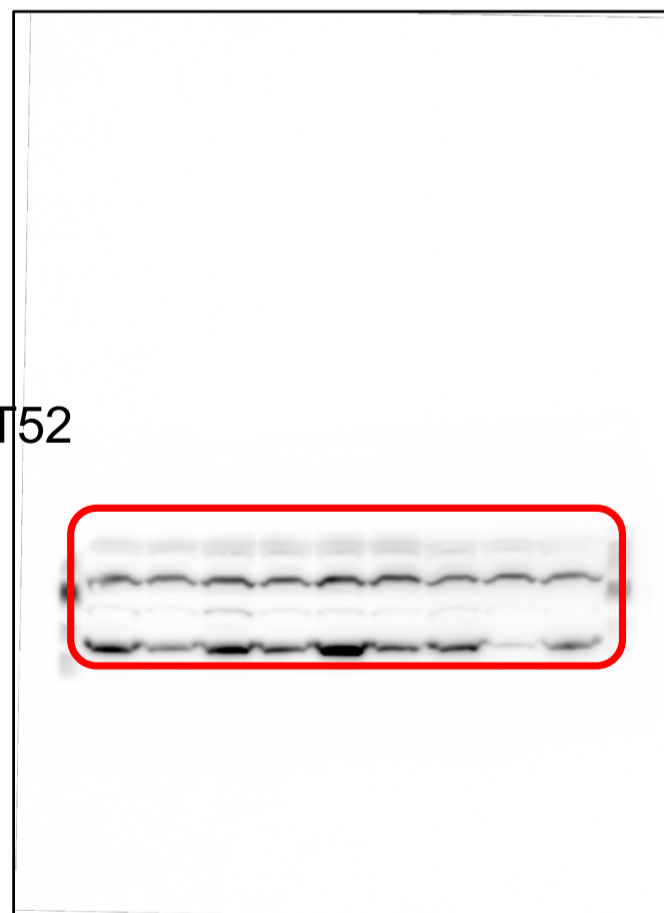


Fig. S2: Uncropped images

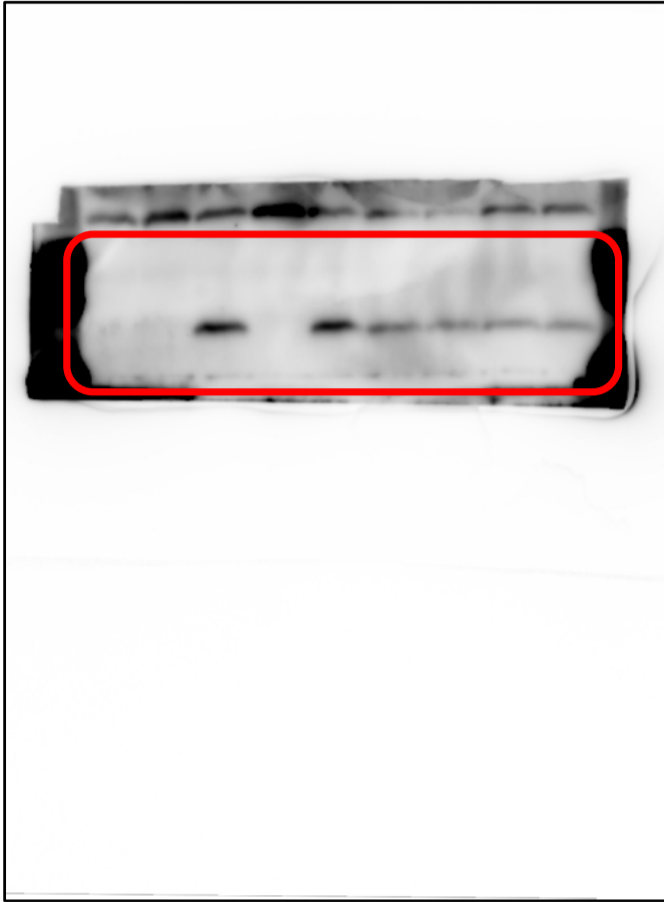
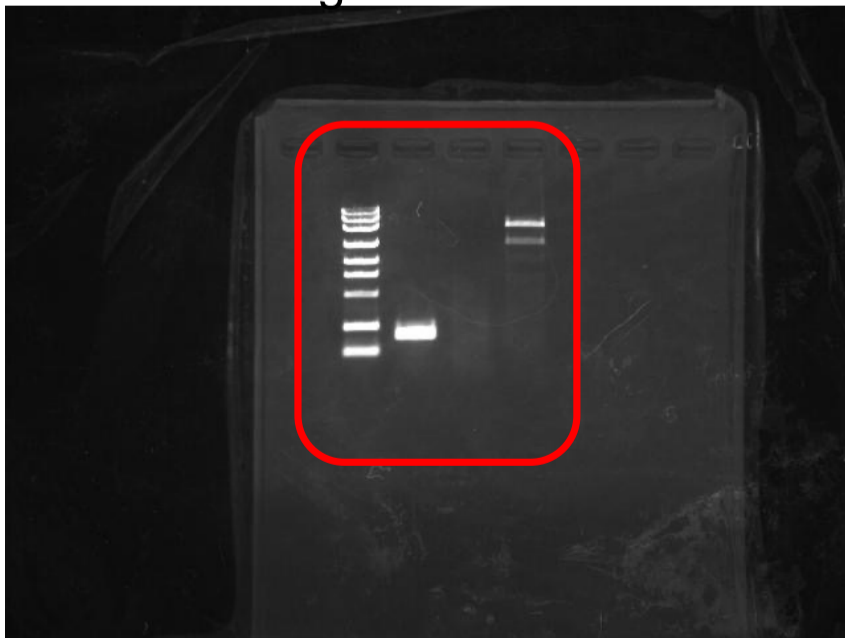


Fig. 2: IFT25



Fig. 2: GAPDH

Fig. S1A





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

| Antibody                         | Manufacturer           | Clone/catalog number or reference number               | Dilution (purpose)       |
|----------------------------------|------------------------|--|--------------------------|
| Polyclonal rabbit anti-IFT25     | Proteintech            | 15732-1-AP   | 1:1,000 (IB)             |
| Polyclonal rabbit anti-IFT52     | Proteintech            | 17534-1-AP   | 1:1,000 (IB)             |
| Polyclonal rabbit anti-IFT81     | Proteintech            | 11744-1-AP   | 1:1,000 (IB)             |
| Polyclonal rabbit anti-IFT88     | Proteintech            | 13967-1-AP   | 1:500 (IF), 1:1,000 (IB) |
| Polyclonal rabbit anti-IFT140    | Proteintech            | 17460-1-AP   | 1:500 (IF)               |
| Polyclonal rabbit anti-GPR161    | Proteintech            | 13398-1-AP   | 1:200 (IF)               |
| Polyclonal rabbit anti-ARL13B    | Proteintech            | 17711-1-AP   | 1:500 (IF)               |
| Monoclonal mouse anti-ARL13B     | Abcam                  | N295B/66   | 1:500 (IF)               |
| Monoclonal mouse anti-FOP        | Abnova                 | 2B1  | 1:10,000 (IF)            |
| Monoclonal mouse anti-Smoothened | Santa Cruz             | sc-166685  | 1:100 (IF)               |
| Monoclonal mouse anti-RFP        | MBL                    | 3G5  | 1:1,000 (IF)             |
| Monoclonal mouse anti-GAPDH      | Ambion                 | 6C5  | 1:10,000 (IB)            |
| Polyclonal rabbit anti-mCherry   | Proteintech            | 26765-1-AP   | 1:10,000 (IB)            |
| AlexaFluor-conjugated secondary  | Molecular Probes       | A11034, A21127, A21131, A21147, A21241, A21242, A21245 | 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. Oligodeoxyribonucleotides used in this study**

| Name               | Sequence                       |
|--------------------|--------------------------------|
| IFT54-gRNA#4-S     | 5'-CACCGCAAGAGCGCACTTCCCCTG-3' |
| IFT54-gRNA#4-AS    | 5'-AAACCAGGGGAAGTGCGCTCTTGC-3' |
| IFT54-Genome-#4-FW | 5'-GCAGTGCTGTGTCCTCTGAT-3'     |
| IFT54-Genome-#4-RV | 5'-TGCCACATCTGCAGCTCATT-3'     |
| pTagBFP-N-RV2      | 5'-CGTAGAGGAAGCTAGTAGCCAGG-3'  |

S, sense; AS, antisense; FW, forward; RV, reverse