Figure S1

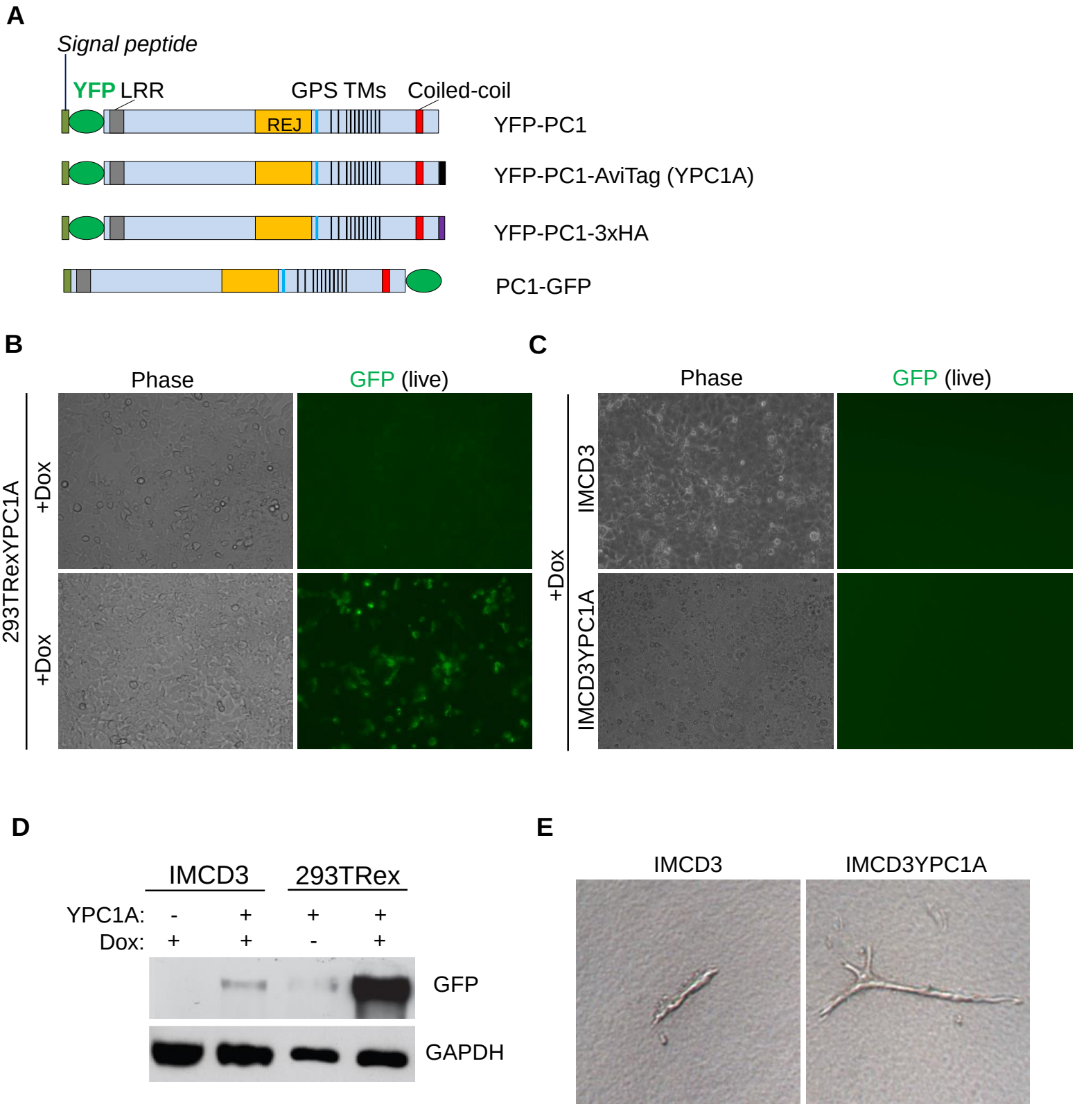
A
Signal peptide
YFP LRR
GPS TMs
Coiled-coil
REJ
YFP-PC1
YFP-PC1-AviTag (YPC1A)
YFP-PC1-3xHA
PC1-GFP
B
Phase
GFP (live)
293TRexYPC1A
+Dox
+Dox
C
Phase
GFP (live)
+Dox
IMCD3
IMCD3YPC1A
D
IMCD3
293TRex
YPC1A: - + + +
Dox: + + - +
GFP
GAPDH
E
IMCD3
IMCD3YPC1A

Figure S2

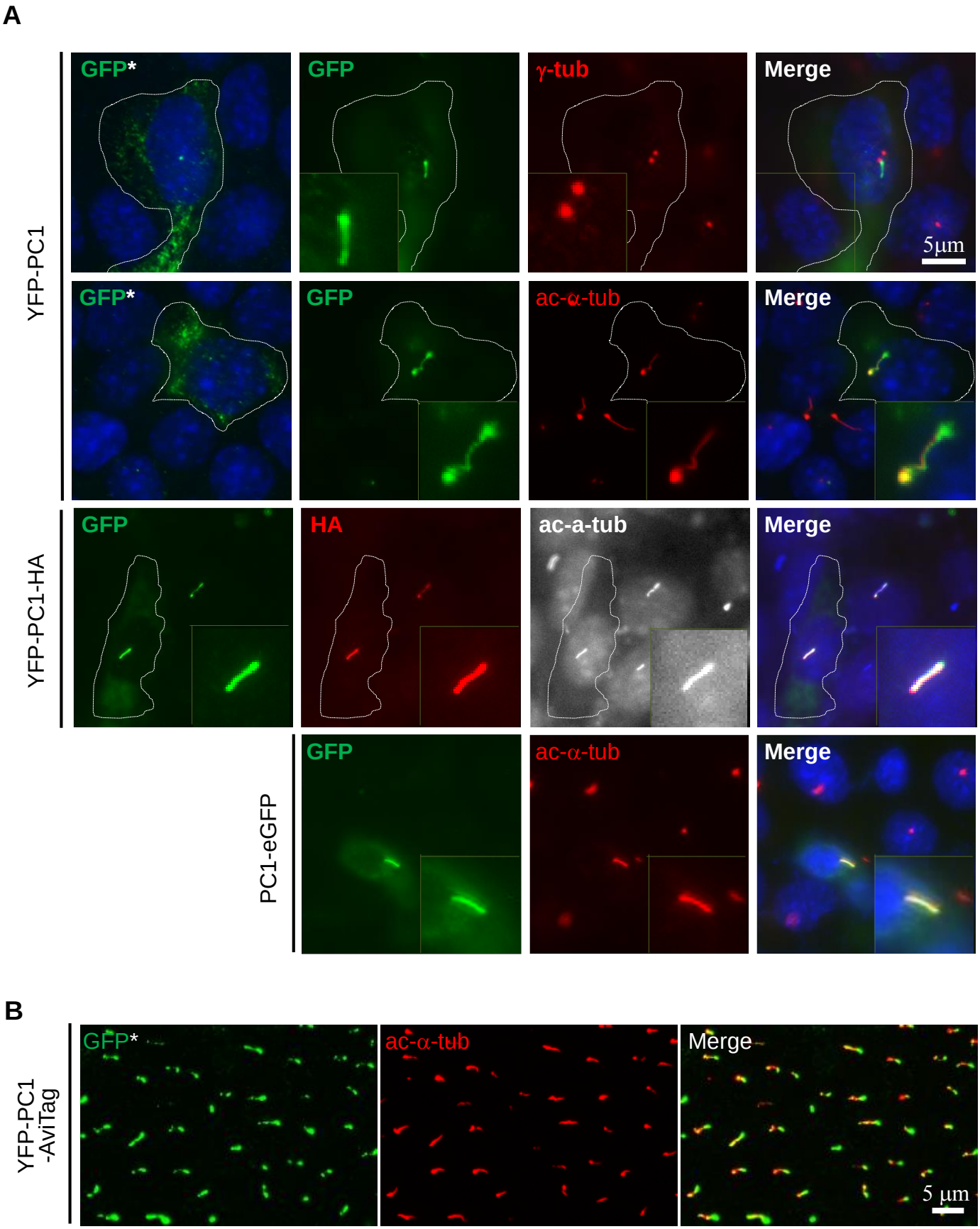
A
YFP-PC1
GFP*
GFP
γ-tub
Merge
5μm
GFP*
GFP
ac-α-tub
Merge
YFP-PC1-HA
GFP
HA
ac-a-tub
Merge
PC1-eGFP
GFP
ac-α-tub
Merge
B
YFP-PC1
-AviTag
GFP*
ac-α-tub
Merge
5 μm

Figure S3

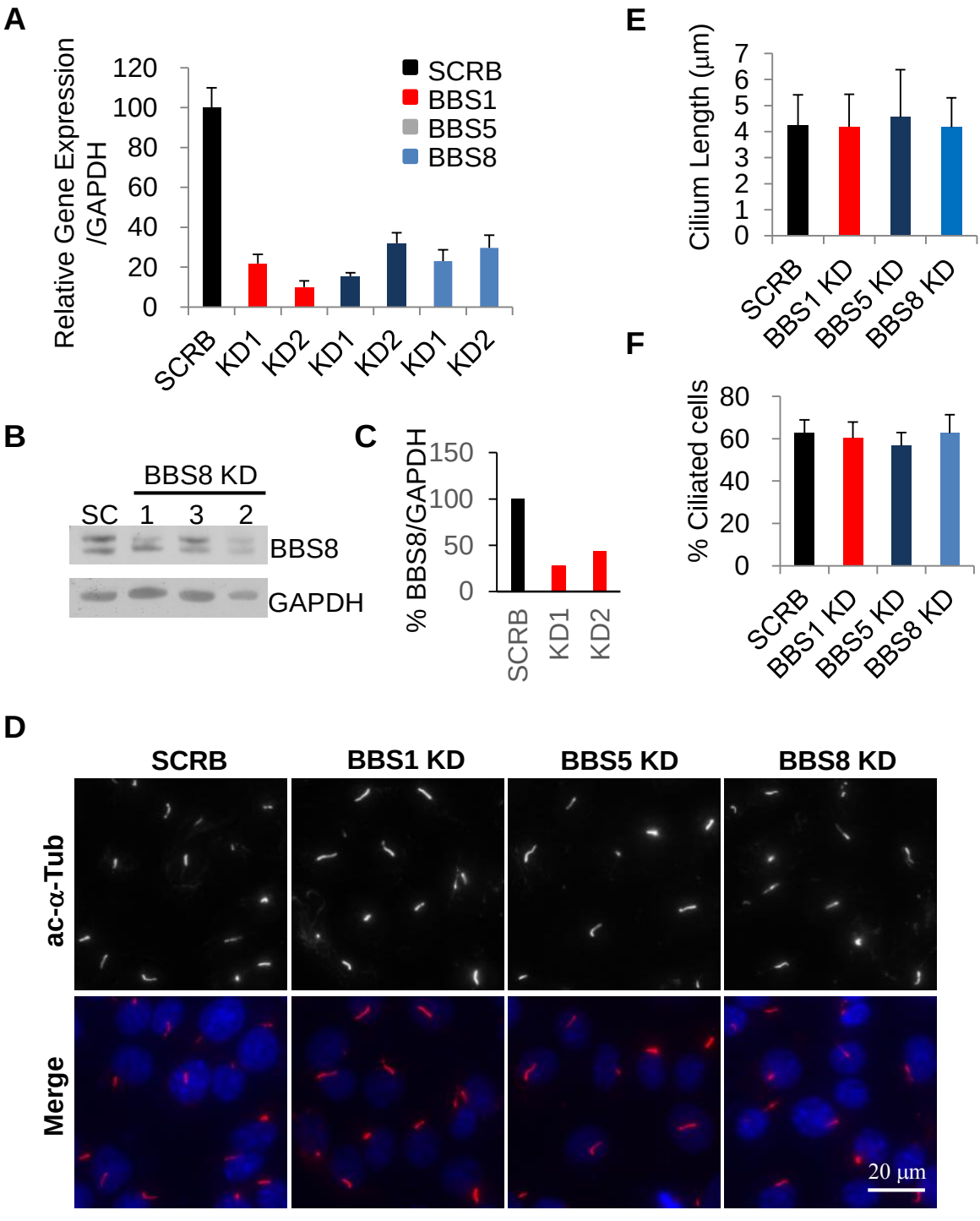
A
Relative Gene Expression /GAPDH
120
100
80
60
40
20
0
SCRB
BBS1
BBS5
BBS8
SCRB
KD1
KD2
KD1
KD2
KD1
KD2
B
BBS8 KD
SC
1
3
2
BBS8
GAPDH
C
% BBS8/GAPDH
150
100
50
0
SCRB
KD1
KD2
E
Cilium Length (μm)
7
6
5
4
3
2
1
0
SCRB
BBS1 KD
BBS5 KD
BBS8 KD
F
% Ciliated cells
80
60
40
20
0
SCRB
BBS1 KD
BBS5 KD
BBS8 KD
D
SCRB
BBS1 KD
BBS5 KD
BBS8 KD
ac-α-Tub
Merge
20 μm

Figure S4

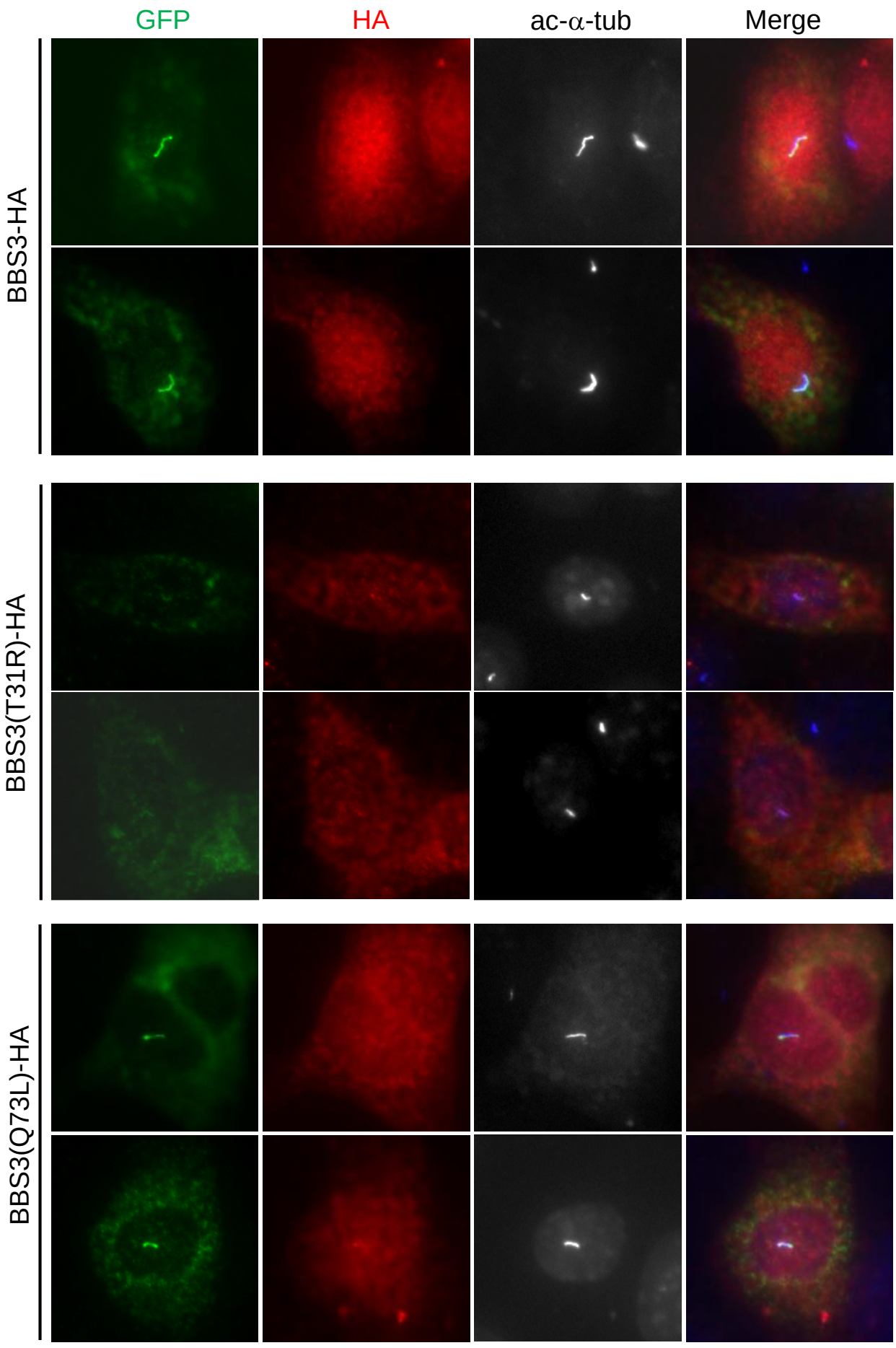

Figure S5

Full gels for figures

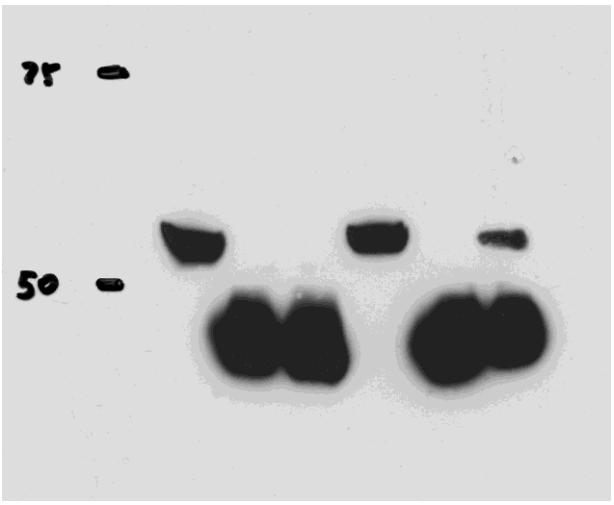

Figure 1A

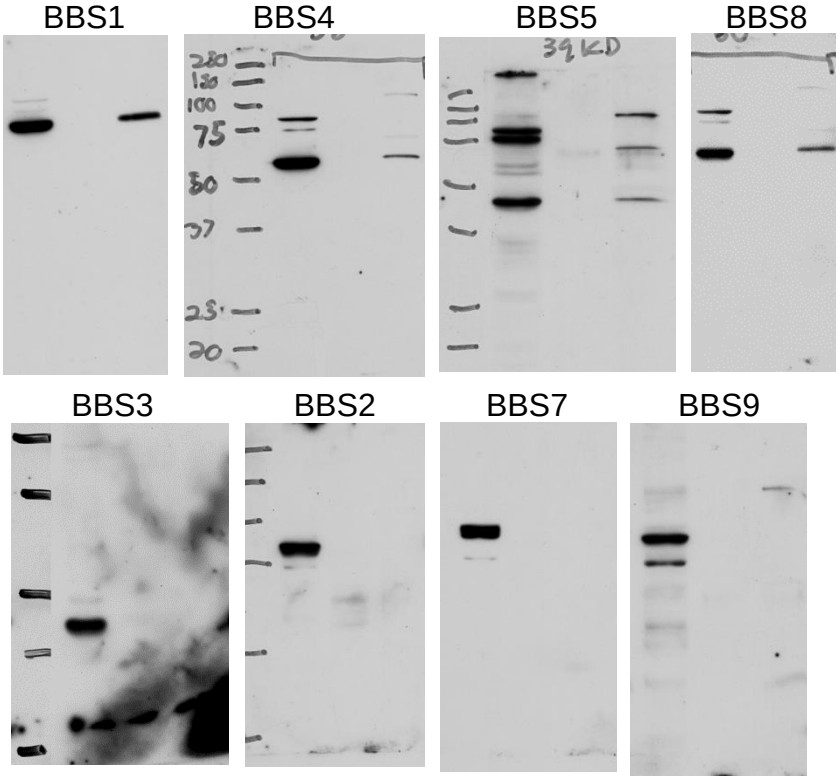

Figure 1B

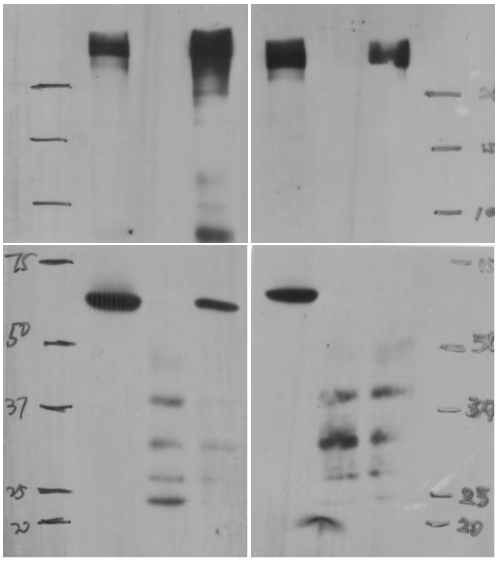

Figure 4B

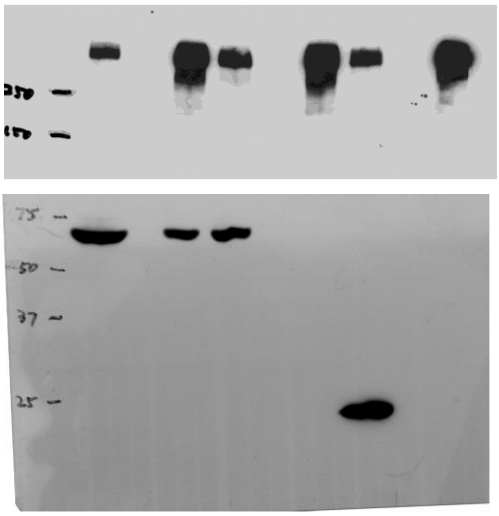

Figure 5E

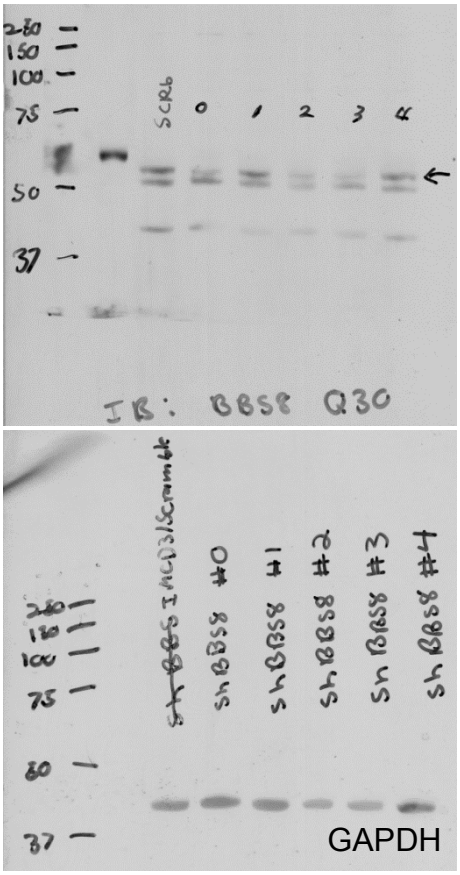

Figure S3B

Figure S1. Low expression level of YFP-PC1 in IMCD3 cells compared to 293TRex cells. (A) Diagram of a set of tagged full-length PC1 constructs generated in this study. Key domains in the PC1 proteins are indicated. LRR: leucine rich repeat. REJ: receptor for egg jelly. GPS: G protein-coupled receptor proteolytic site. TMs: transmembrane domains. (B) Live fluorescence of YFP-PC1-AviTag in 293TRex cells. YPC1A was transiently transfected to 293TRex cells. Cells were cultured to confluence and serum starved in the absence or presence of 1μg/ml doxycyclin for 24 hours. Live epifluorescence of GFP was observed from cells using a Nikon Eclipse Ti fluorescence microscope equipped with a 40x long range lens. (C) Lack of YFP-PC1 epifluoresence in IMCD3 cells stably expressing YFP-PC1-AviTag (YPC1A). Tetracycline-inducible IMCD3 cells stably expressing YPC1A were cultured and YPC1A fluorescence was observed as described in (B). Parental IMCD3 cells were used as a control. (D) Western blot shows a much lower protein expression level for YPC1A in IMCD3 stable cells than that of 293TRex cells transiently transfected with YPC1A. YPC1A was probed using a mouse anti-GFP antibody mms-118. GAPDH was used as a loading control. (E) Tubulogenesis of IMCD3YPC1A and its parental cell line IMCD3 in 3D collagen gel. Dispersed cells were seeded into 2% collagen gel. 1μg/ml doxycyclin and HGF were added to cells and cells were allowed for growth for a week.

Figure S2. Tagged full-length PC1 traffics to the primary cilia in IMCD3 cells. (A) Expression of indicated constructs in the primary cilia of IMCD3 cells. YFP-PC1 was stained by an antibody against GFP (green). Antibodies to acetylated α-tubulin (ac-α-tub, red) or γ-tubulin (red) were used to mark cilia and basal bodies, respectively. *denotes images that are focused on the cell body (outlined with dashed lines) to indicate successful transfection of YFP-PC1. YFP-PC1-HA was also stained by an antibody against HA (rat, red) to show both the N- and C-termini of PC1 are present on cilia. 4',6-diamidino-2-phenylindole (DAPI) staining showing the nucleus in blue. (B) Live cell staining of surface YFP-PC1 stably expressed in IMCD3 cells. GFP (green) marks YFPPC1 and acetylated α-tubulin (ac-α-tub, red) marks cilia.

Figure S3. Depletion of BBS1, 5 or 8 does not affect ciliogenesis of IMCD3 cells. (A) Establishment of BBS1, 5 and 8 knockdown IMCD3 cells by lentiviral shRNA. Knockdown efficiency for each gene was assayed by real-time RT-PCR. A set of five sites was tested for each gene, two sites with a knockdown efficiency >70% were selected for further experiments to eliminate potential off-target effect of the shRNA. Each experiment was repeated for three times. (B) Western blot showing the depletion of BBS8 (arrow) at protein level. GAPDH was used as a loading control. (C) The upper bands from the blot in (B) was quantified and the ratio of BBS8 to GAPDH plotted. SCRB was set as 100%. (D) Ciliogenesis is not affected by BBS1, 5 or 8 knockdown. Representative images of ciliated IMCD3 cells with respective gene knockdown are shown. Cilia were visualized by staining with antibody to acetylated α-tubulin (ac-α-tub). (E) Quantification of cilium length in cells described in (D). At least 150 cells were counted from different fields of microscopic slides using Image Pro software. Error bars represent the standard deviations (SDs). (F) Quantification of ciliation rate similar to described in (E).

Figure S4. BBS3/Arl6 controls the ciliary trafficking of PC1. Additional cell images as in Fig. 4.

Figure S5. Full gel images for all Western blots shown in this study.