Supplemental information

The BBSome assembly is spatially controlled by BBS1 and BBS4 in human cells

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Supplemental Figure Legends

Supplemental Figures S1-S5

Supplemental Tables S1-S5

Supplemental Figure Legends

Figure S1. Localization of the YFP-tagged BBSome subunits in the WT and *BBS* KO RPE1 cell lines.

Micrographs of the YFP-tagged BBSome subunits in the *BBS* KO RPE1 cells. YFP-tagged BBSome subunits localize to primary cilia (Ac-tub) in WT, but not in the *BBS* KO cell lines. Note that YFP-BBS1 localizes to the ciliary base and YFP-BBS4 localizes to the PS in all the cell lines. DAPI was used to mark the cell nucleus. Scale bar, 5µm.

Figure S2. Functionality and relative expression of the YFP-tagged BBSome subunits in the WT and *BBS* KO RPE1 cell lines.

(A) Micrographs of the endogenous BBS9 localization in WT, *BBS* KO and *BBS* KO cell line reconstituted with the respective YFP-tagged BBSome subunit. Endogenous BBS9 localizes to the primary cilia in WT and reconstituted *BBS* KO cell lines but is absent from the primary cilia in *BBS* KO cells. Scale bar, 5µm. (B) Analysis of the relative expression levels of the YFP-tagged BBSome subunits compared to expression of the endogenous proteins in WT and *BBS* KO cells. Representative western blots out of two independent experiments are shown. Actin was used as a loading control. '*' marks the position of the signal of the endogenous BBSome subunits in WT cells. $BBS1^{HIGH}$ and $BBS5^{HIGH}$ depict higher exposure for better visualization. Bar graphs depict the quantification of the relative expression levels of the endogenous and YFP-tagged BBSome subunits in WT and *BBS* KO cells. Protein amounts were quantified using the Fiji ImageJ. Protein expression was first normalized to actin and then to endogenous expression levels. Means and SD of two experiments are shown. (C) Bar graphs depicting relative expression of the YFP-tagged BBSome subunits in WT and *BBS* KO cell lines analyzed by flow cytometry. Geometric means of the fluorescence intensity were obtained from the YFP positive histograms and normalized to WT. Means and SD from three independent experiments are shown.

Figure S3. The BBSome subunits are interdependent.

(A) Expression levels of endogenous BBSome subunits in the parental RPE1 cells and in the cell lines deficient in BBS1, BBS2, BBS4, BBS7, BBS8, BBS9, or BBS18 (see Table S1). Equal protein amounts were loaded into each lane. Actin is used as the loading control. Representative blots out of three independent experiment are shown. (B) Bar graphs depicting the relative expression levels of the endogenous BBSome subunits in the WT and *BBS* deficient cell lines. Protein amounts were quantified using the Fiji ImageJ. Protein expression was first normalized to actin and then to WT levels. Average and SD of three independent experiments is shown.

Figure S4. FCS measures the *in vivo* mobility of the BBSome subcomplexes in the cytoplasm.

(A) Bar graphs depicting abundance of primary cilia in YFP-BBS4 WT cell line either not starved and/or starved for 24 h in media without FBS. Average and SD of three independent experiments is shown. (B) Plots show the diffusion time, τ, obtained by fitting the ACFs acquired from FCS measurements in YFP-BBS8, YFP-BBS5 and BBS7-YFP WT and *BBS* KO cell lines. Statistical significance was calculated using two-tailed Mann-Whitney test. Medians with interquartile range of $n>10$ are shown. $p<0.05$. (C, D, E) Plots (left) show the diffusion time, τ, obtained by fitting the ACFs acquired from FCS measurements (right) of YFP-BBS1 (C), YFP-BBS18 (D) and BBS9-YFP (E) in WT and *BBS* KO cell lines. Statistical significance was calculated using two-tailed Mann-Whitney test. Medians with interquartile range of $n>10$ are shown. *$p<0.05$, ****$p<0.0001$. Plots on the right show the ACFs in case $p<0.05$. The ACFs were fitted with one-component anomalous 3D diffusion model. The means of $n>10$ measurements are shown. Arrows indicate possible presence of a second component.

Figure S5. BBS1 is required for completion of the BBSome at the ciliary base.

(A) Micrographs of YFP-BBS4 in non-ciliated and ciliated (Ac-tub) cells showing the localization of YFP-BBS4 at the PS (PCM-1) in WT RPE1 cells. Scale bar, 5µm. (B) Endogenous BBS9 and BBS2 localize to the cilia in WT RPE1 cells but are accumulated at the PS in *BBS1* KO RPE1 cells. DAPI was used to stain the cell nucleus. Scale bar, 5µm. (C) Micrographs (left) and quantification (right) of the abundance of the YFP-tagged BBSome subunits at the centrosome in non-ciliated WT cells. Ninein and acetylated tubulin staining visualizes the centrosome and tubular meshwork. Scale bar, 5µm. Bar graph shows average and SD of three independent experiments and the total number of counted cells

for each BBSome subunit. (D, E) Bar graphs depict the recovery halftimes (s) of the YFP-tagged BBSome subunits in the ciliary tip and base (Fig. 5 B and C). YFP-BBS1 shows a significantly faster recovery at the ciliary base when compared to the other BBSome subunits. Means of 20-30 measurements from three independent experiments are shown. Error bars represent the 90% confidence interval.

Figure S1

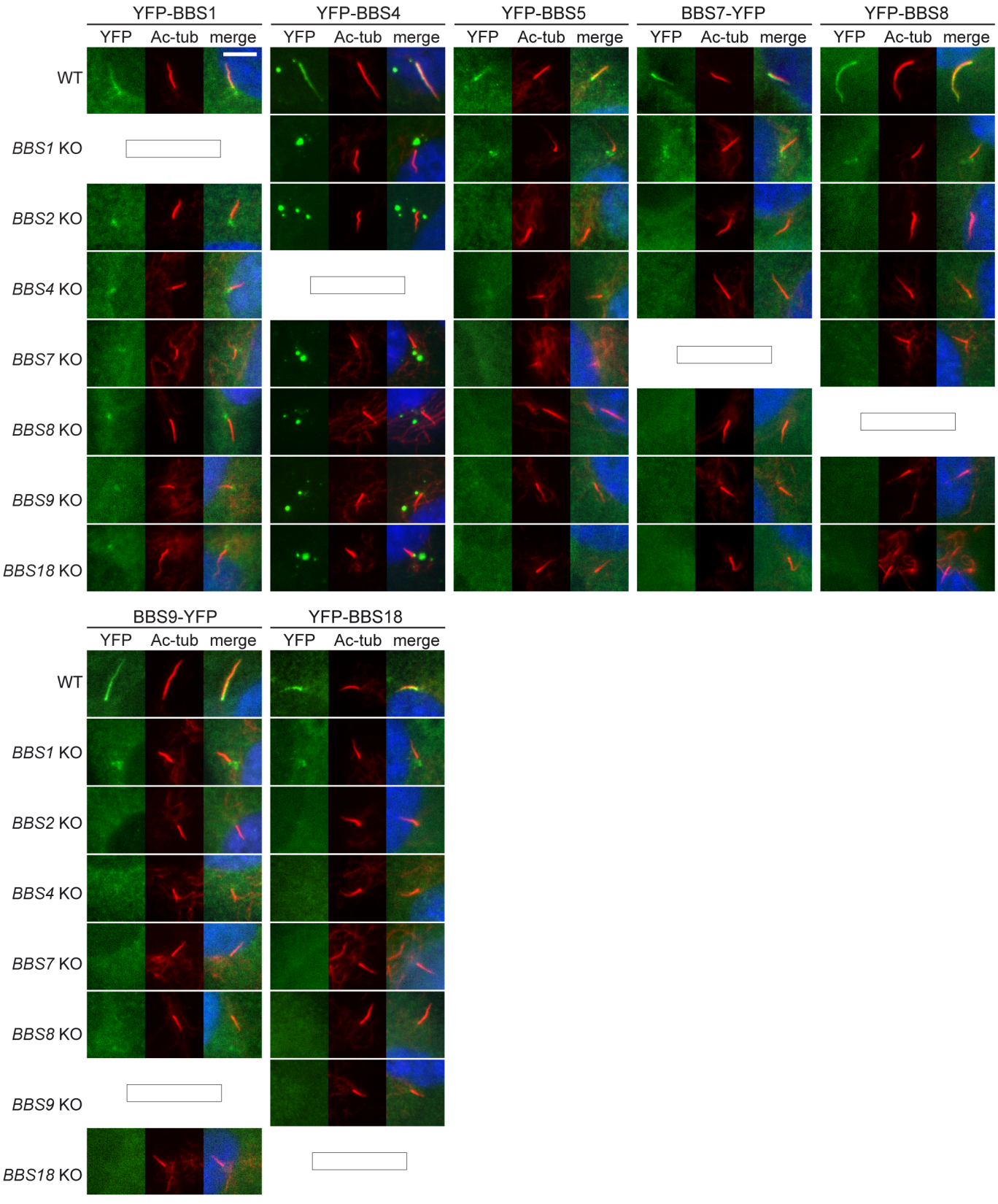

YFP-BBS1
YFP-BBS4
YFP-BBS5
BBS7-YFP
YFP-BBS8
YFP
Ac-tub
merge
WT
BBS1 KO
BBS2 KO
BBS4 KO
BBS7 KO
BBS8 KO
BBS9 KO
BBS18 KO
BBS9-YFP
YFP-BBS18

Figure S2

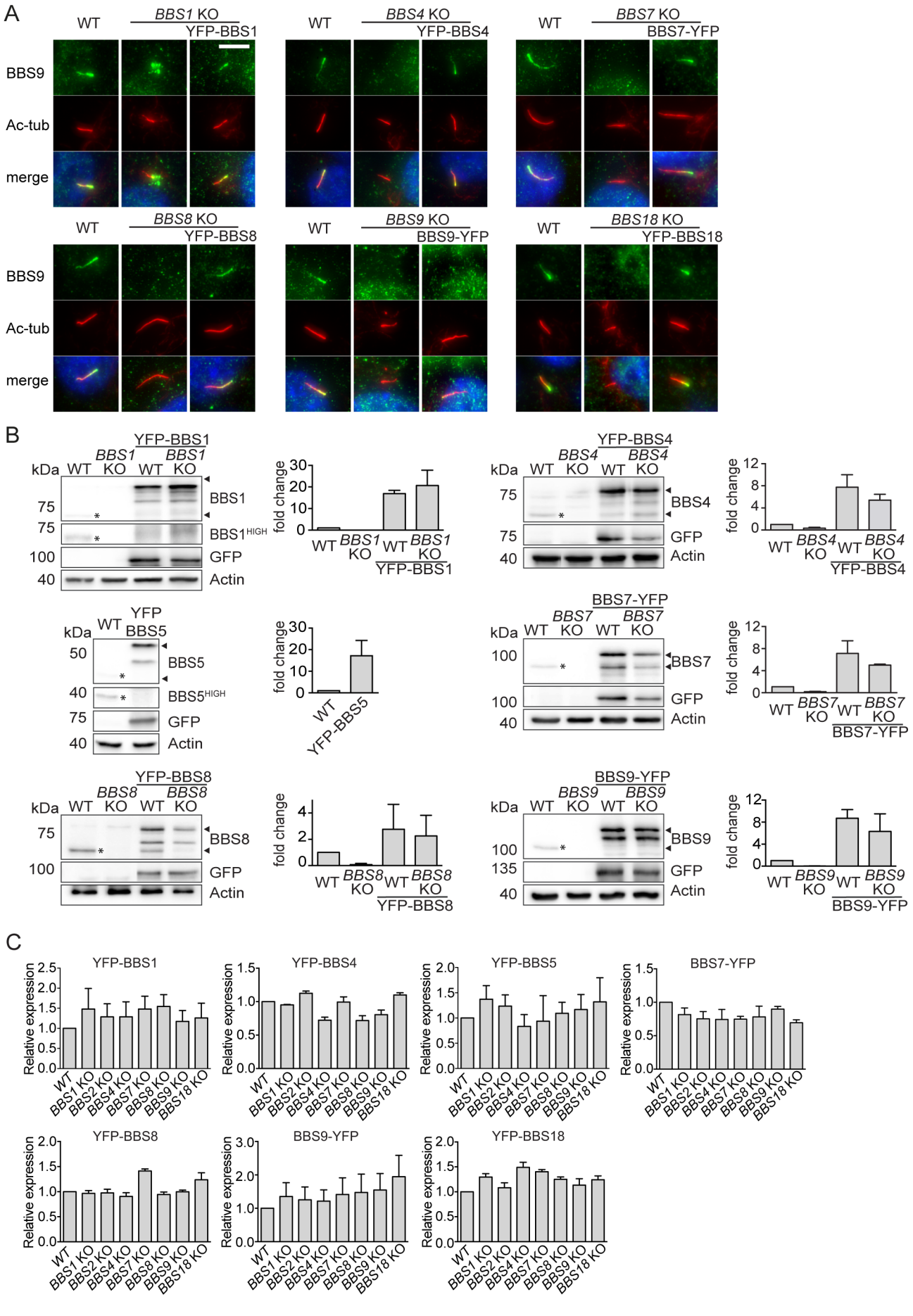
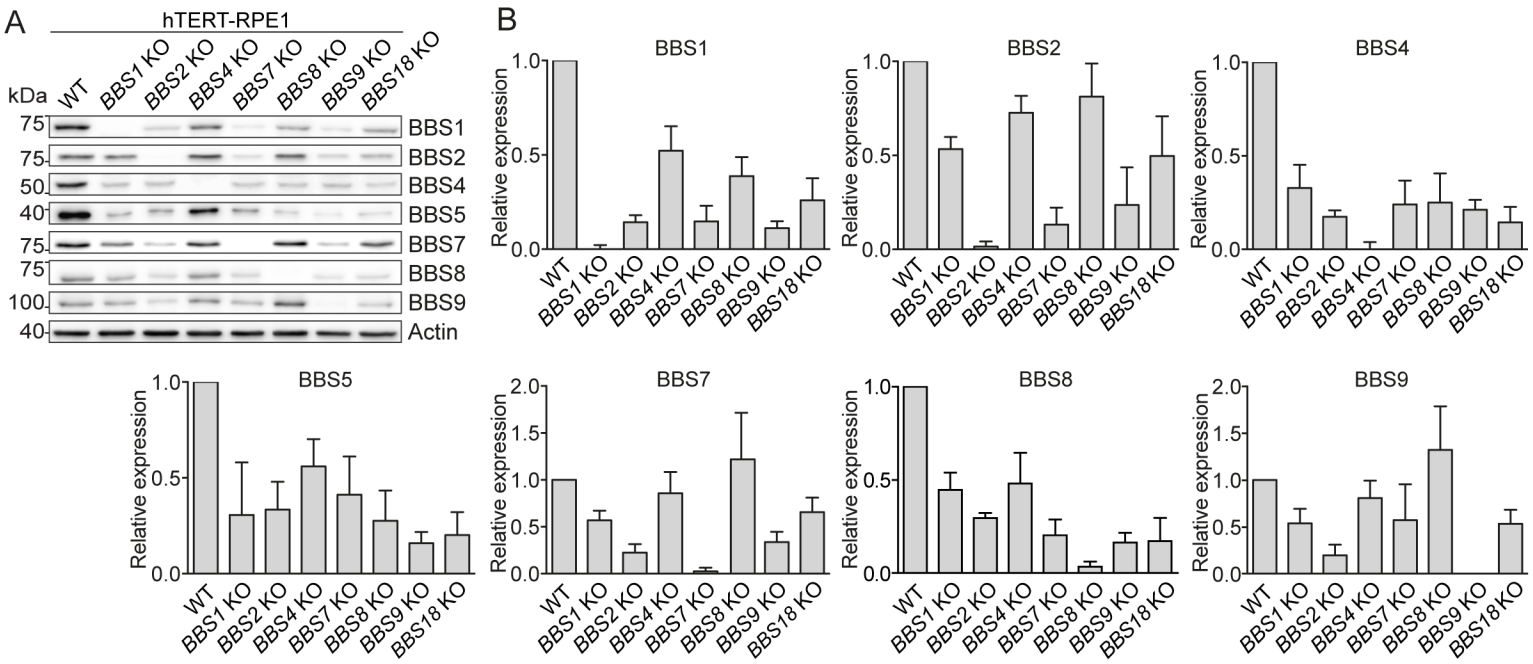

A
WT
BBS1 KO
YFP-BBS1
BBS4 KO
YFP-BBS4
BBS7 KO
BBS7-YFP
BBS8 KO
YFP-BBS8
BBS9 KO
BBS9-YFP
BBS18 KO
YFP-BBS18
BBS9
Ac-tub
merge
B
kDa
BBS1
BBS1HIGH
GFP
Actin
fold change
YFP-BBS1
BBS4
YFP-BBS4
YFP
BBS5
BBS5HIGH
YFP-BBS5
BBS7
BBS7-YFP
BBS8
YFP-BBS8
BBS9
BBS9-YFP
C
Relative expression
YFP-BBS1
YFP-BBS4
YFP-BBS5
BBS7-YFP
YFP-BBS8
BBS9-YFP
YFP-BBS18
WT
BBS1 KO
BBS2 KO
BBS4 KO
BBS7 KO
BBS8 KO
BBS9 KO
BBS18 KO

Figure S3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hTERT-RPE1
kDa
WT
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75
75
50
40
75
75
100
40
BBS1
BBS2
BBS4
BBS5
BBS7
BBS8
BBS9
Actin
B
BBS1
BBS2
BBS4
BBS5
BBS7
BBS8
BBS9
Relative expression
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BBS2 KO
BBS4 KO
BBS7 KO
BBS8 KO
BBS9 KO
BBS18 KO

Figure S4

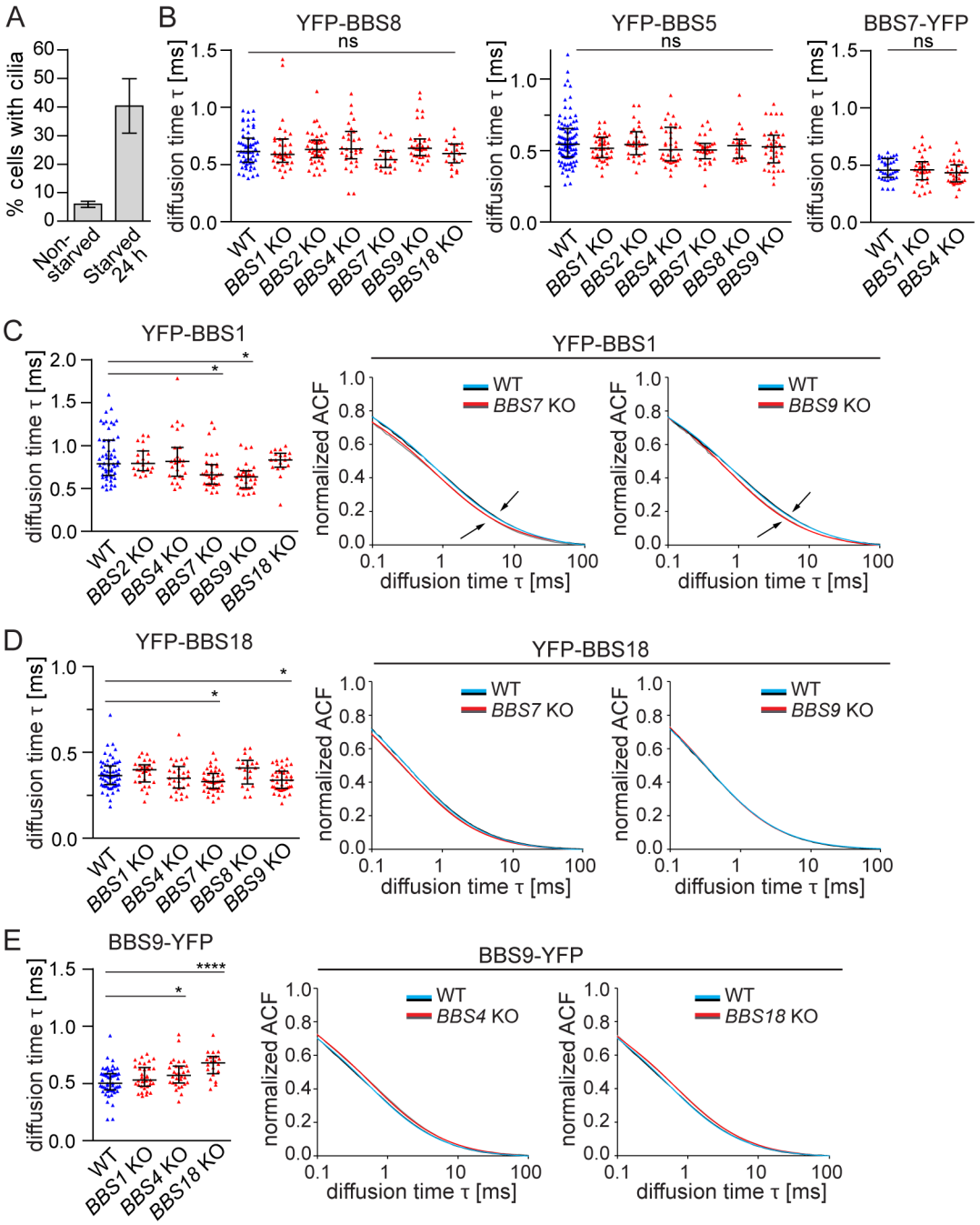
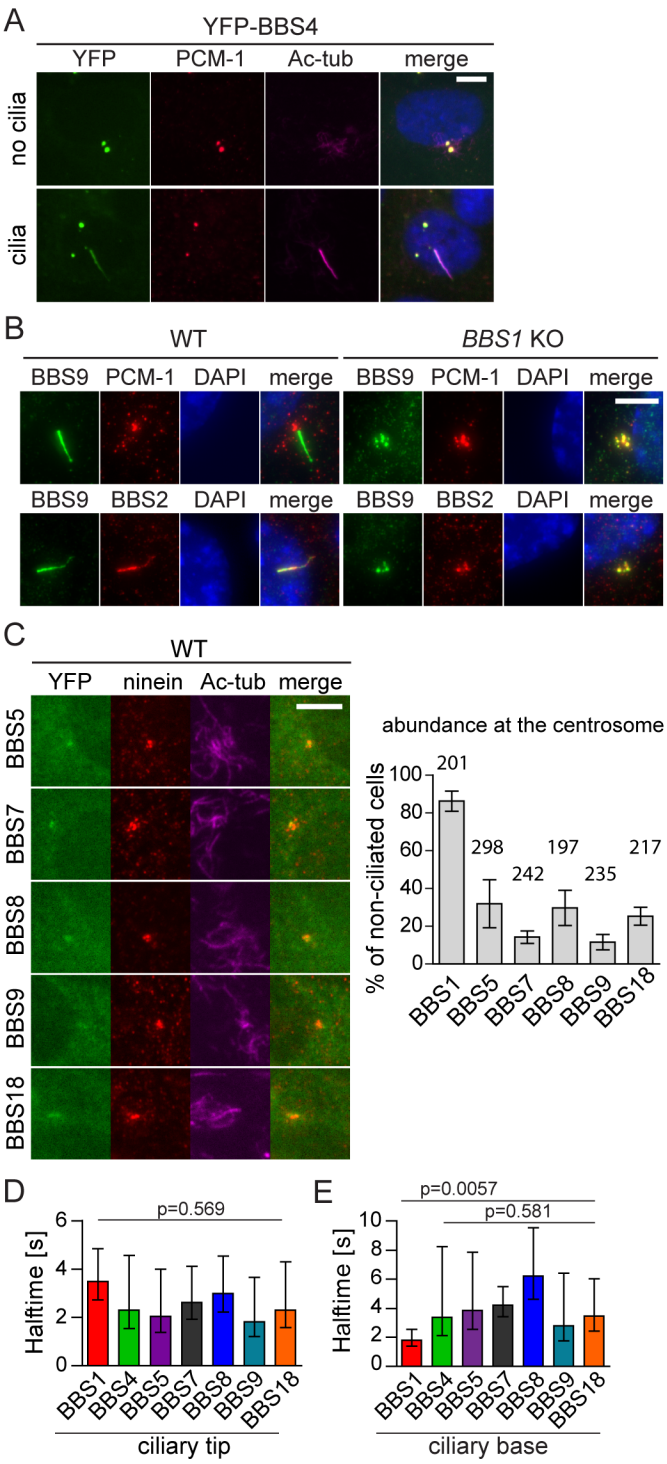

A
% cells with cilia
Non-starved
Starved 24 h
B
YFP-BBS8
ns
diffusion time τ [ms]
WT
BBS1 KO
BBS2 KO
BBS4 KO
BBS7 KO
BBS9 KO
BBS18 KO
YFP-BBS5
ns
BBS8 KO
BBS7-YFP
ns
C
YFP-BBS1
YFP-BBS1
normalized ACF
WT
BBS7 KO
BBS9 KO
diffusion time τ [ms]
D
YFP-BBS18
YFP-BBS18
E
BBS9-YFP
BBS9-YFP
BBS4 KO
BBS18 KO

Figure S5


A
YFP-BBS4
YFP
PCM-1
Ac-tub
merge
no cilia
cilia
B
WT
BBS1 KO
BBS9
PCM-1
DAPI
merge
BBS2
C
WT
ninein
BBS5
BBS7
BBS8
BBS9
BBS18
abundance at the centrosome
% of non-ciliated cells
201
298
242
197
235
217
BBS1
BBS4
D
p=0.569
Halftime [s]
ciliary tip
E
p=0.0057
p=0.581
ciliary base

Supplemental Tables

Table S1. Genetic modifications in RPE-1 cell line obtained by utilizing CRISPR/Cas9 to knockout the BBSome subunits. Table comprises the DNA modifications in ORFs of the individual *BBS* genes in the established *BBS* KO cell lines and the derived protein sequences. ORF – open reading frame, KO – knockout, A1 – first allele, A2 – second allele, bold - change in aminoacid sequence, ***** - stop codon.

RPE-1	GENE ID	ORF nucleotide modification 5' to 3'	protein sequence from the START to STOP codon
BBS1 KO	NM_024649	A1: deletion A (65)	MAAASSSDSDACGAESNEAN**SGWMRTTTQWPISTPFLPA***
		A2: deletion ATTCGAAGTGGTTGGA (59-75)	MAAASSSDSDACGAESNEA**MRTTTQWPISTPFLPA***
BBS2 KO	NM_031885	A1: insertion A (72)	MLLPVFTLKLRHKISPRMVAIGR*
		A2: deletion CG (72-73)	
BBS4 KO	NM_033028	A1, A2: deletion GC (19-20)	MAEERV**DENSISCIY***
BBS7 KO	NM_176824	A1, A2: deletion TTTAC (180-184)	MDLILNRMDYLQVGVTSQKTMKLIPASRHRATQKVVIGDHDGVVM CFGMKKGEAAAVFKT**RAEDCKAGTGRGYQHTSGENFYCCSI***
BBS8 KO	NM_001288781	A1, A2: insertion A (107)	MSSEMEPLLLAWSYFRRRKFQLCADLCTQMLEKSP*
BBS9 KO	NM_198428	A1: insertion T (40)	MSLFKARDWWSTI**SGR***
		A2: deletion CTACTATTCT (32-41)	MSLFKARDWW**WEIKKNLIKAVCVWLMLTIVEMDKIK***
BBS18 KO	NM_001195305	A1: deletion AGTCAATGTTCCGGGA (80-95)	MLKAAAKRPELSGKNTISNNSDMAEVK**FFQSKGHCLWKI***
		A2: deletion AGTCAATGTTCCGGGAAGTTCTTC CAAAGCAAG (80-112)	Splicing defect – deletion of the intron3-4 5'-splice site – nucleotides GTA

Table S2. List of RPE1 cell lines used in this study. YFP position in name of the YFP-tagged BBSome subunit indicates either the N-terminal (YFP-BBS) or C-terminal tagging (BBS-YFP), KO – knockout.

No.	Name	Genetic background	Transgene
1.	YFP-BBS1	RPE1	YFP-BBS1
2.	YFP-BBS4	RPE1	YFP-BBS4
3.	YFP-BBS5	RPE1	YFP-BBS5
4.	BBS7-YFP	RPE1	BBS7-YFP
5.	YFP-BBS8	RPE1	YFP-BBS8
6.	BBS9-YFP	RPE1	BBS9-YFP
7.	YFP-BBS18	RPE1	YFP-BBS18
8.	*BBS1* KO	RPE1 *BBS1* KO	-
9.	*BBS2* KO	RPE1 *BBS2* KO	-
10.	*BBS4* KO	RPE1 *BBS4* KO	-
11.	*BBS7* KO	RPE1 *BBS7* KO	-
12.	*BBS8* KO	RPE1 *BBS8* KO	-
13.	*BBS9* KO	RPE1 *BBS9* KO	-
14.	*BBS18* KO	RPE1 *BBS18* KO	-
15.	*BBS1 BBS4* dKO	RPE1 *BBS1* KO *BBS4* KO	-
16.	YFP-BBS1 *BBS1* KO	RPE1 *BBS1* KO	YFP-BBS1
17.	YFP-BBS4 *BBS1* KO	RPE1 *BBS1* KO	YFP-BBS4
18.	YFP-BBS5 *BBS1* KO	RPE1 *BBS1* KO	YFP-BBS5
19.	BBS7-YFP *BBS1* KO	RPE1 *BBS1* KO	BBS7-YFP
20.	YFP-BBS8 *BBS1* KO	RPE1 *BBS1* KO	YFP-BBS8
21.	BBS9-YFP *BBS1* KO	RPE1 *BBS1* KO	BBS9-YFP
22.	YFP-BBS18 *BBS1* KO	RPE1 *BBS1* KO	YFP-BBS18
23.	YFP-BBS1 *BBS2* KO	RPE1 *BBS2* KO	YFP-BBS1
24.	YFP-BBS4 *BBS2* KO	RPE1 *BBS2* KO	YFP-BBS4
25.	YFP-BBS5 *BBS2* KO	RPE1 *BBS2* KO	YFP-BBS5
26.	BBS7-YFP *BBS2* KO	RPE1 *BBS2* KO	BBS7-YFP
27.	YFP-BBS8 *BBS2* KO	RPE1 *BBS2* KO	YFP-BBS8
28.	BBS9-YFP *BBS2* KO	RPE1 *BBS2* KO	BBS9-YFP
29.	YFP-BBS18 *BBS2* KO	RPE1 *BBS2* KO	YFP-BBS18
30.	YFP-BBS1 *BBS4* KO	RPE1 *BBS4* KO	YFP-BBS1
31.	YFP-BBS4 *BBS4* KO	RPE1 *BBS4* KO	YFP-BBS4
32.	YFP-BBS5 *BBS4* KO	RPE1 *BBS4* KO	YFP-BBS5
33.	BBS7-YFP *BBS4* KO	RPE1 *BBS4* KO	BBS7-YFP
34.	YFP-BBS8 *BBS4* KO	RPE1 *BBS4* KO	YFP-BBS8
35.	BBS9-YFP *BBS4* KO	RPE1 *BBS4* KO	BBS9-YFP
36.	YFP-BBS18 *BBS4* KO	RPE1 *BBS4* KO	YFP-BBS18
37.	YFP-BBS1 *BBS7* KO	RPE1 *BBS7* KO	YFP-BBS1
38.	YFP-BBS4 *BBS7* KO	RPE1 *BBS7* KO	YFP-BBS4
39.	YFP-BBS5 *BBS7* KO	RPE1 *BBS7* KO	YFP-BBS5
40.	BBS7-YFP *BBS7* KO	RPE1 *BBS7* KO	BBS7-YFP
41.	YFP-BBS8 *BBS7* KO	RPE1 *BBS7* KO	YFP-BBS8
42.	BBS9-YFP *BBS7* KO	RPE1 *BBS7* KO	BBS9-YFP
43.	YFP-BBS18 *BBS7* KO	RPE1 *BBS7* KO	YFP-BBS18
44.	YFP-BBS1 *BBS8* KO	RPE1 *BBS8* KO	YFP-BBS1
45.	YFP-BBS4 *BBS8* KO	RPE1 *BBS8* KO	YFP-BBS4
46.	YFP-BBS5 *BBS8* KO	RPE1 *BBS8* KO	YFP-BBS5
47.	BBS7-YFP *BBS8* KO	RPE1 *BBS8* KO	BBS7-YFP
48.	YFP-BBS8 *BBS8* KO	RPE1 *BBS8* KO	YFP-BBS8
49.	BBS9-YFP *BBS8* KO	RPE1 *BBS8* KO	BBS9-YFP

50.	YFP-BBS18 *BBS8* KO	RPE1 *BBS8* KO	YFP-BBS18
51.	YFP-BBS1 *BBS9* KO	RPE1 *BBS9* KO	YFP-BBS1
52.	YFP-BBS4 *BBS9* KO	RPE1 *BBS9* KO	YFP-BBS4
53.	YFP-BBS5 *BBS9* KO	RPE1 *BBS9* KO	YFP-BBS5
54.	BBS7-YFP *BBS9* KO	RPE1 *BBS9* KO	BBS7-YFP
55.	YFP-BBS8 *BBS9* KO	RPE1 *BBS9* KO	YFP-BBS8
56.	BBS9-YFP *BBS9* KO	RPE1 *BBS9* KO	BBS9-YFP
57.	YFP-BBS18 *BBS9* KO	RPE1 *BBS9* KO	YFP-BBS18
58.	YFP-BBS1 *BBS18* KO	RPE1 *BBS18* KO	YFP-BBS1
59.	YFP-BBS4 *BBS18* KO	RPE1 *BBS18* KO	YFP-BBS4
60.	YFP-BBS5 *BBS18* KO	RPE1 *BBS18* KO	YFP-BBS5
61.	BBS7-YFP *BBS18* KO	RPE1 *BBS18* KO	BBS7-YFP
62.	YFP-BBS8 *BBS18* KO	RPE1 *BBS18* KO	YFP-BBS8
63.	BBS9-YFP *BBS18* KO	RPE1 *BBS18* KO	BBS9-YFP
64.	YFP-BBS18 *BBS18* KO	RPE1 *BBS18* KO	YFP-BBS18

Table S3. Calculated parameters derived from FCS measurements in WT and *BBS* KO RPE1 cells expressing YFP-tagged BBSome subunits. Diffusional mobility of BBSome subunits was measured in the cytoplasm by FCS. Diffusion time τ was obtained by fitting the autocorrelation function with the one-component anomalous diffusion fit. The mean ± SD from n>10 is shown. n.a., not applicable, n.d., not determined

	Diffusion time $\tau \pm \tau_{SD}$ [µs]						
Cell line	**YFP-BBS1**	**YFP-BBS4**	**YFP-BBS5**	**BBS7-YFP**	**YFP-BBS8**	**BBS9-YFP**	**YFP-BBS18**
WT	867±272	850±299	602±280	467±92	641±158	516±123	372±84
BBS1 KO	n.a.	676±217	522±87	465±133	651±218	616±103	385±70
BBS2 KO	830±156	810±313	560±114	n.d.	646±139	n.d.	n.d.
BBS4 KO	902±401	n.a.	536±133	436±101	695±278	586±124	357±88
BBS7 KO	701±205	672±180	505±92	n.a.	564±109	n.d.	336±63
BBS8 KO	n.d.	n.d.	540±113	n.d.	n.a.	n.d.	395±86
BBS9 KO	636±150	610±466	523±138	n.d.	687±164	n.a.	341±66
BBS18 KO	815±152	917±258	568±85	n.d.	591±110	666±114	n.a.

Table S4. Calculated parameters derived from FRAP analysis of the BBSome subunits at the pericentriolar satellites and the centrosome/basal body. Dynamic turnover of the BBSome subunits was measured at the pericentriolar satellites and centrosome/basal body by FRAP. Halftime $T_{1/2}$ and mobile fraction F_m were obtained by fitting the recovery curves with the one component association fit. The mean and 95% confidence intervals (CI) of 20-30 measurements is shown.

RPE1	**Cell line**	**Protein analysed**	**Halftime $T_{1/2}$ [s]**		**Mobile fraction F_m [%]**	
			mean	95% CI	mean	95% CI
Pericentriolar satellites	WT	YFP BBS4	20.75	15.06 to 33.39	0.1606	0.1493 to 0.1718
	BBS1 KO	YFP BBS4	15.11	12.36 to 19.42	0.1783	0.1724 to 0.1843
	BBS1 KO	YFP BBS5	11.67	7.787 to 23.26	0.3041	0.2900 to 0.3182
	BBS1 KO	BBS7 YFP	14.34	9.264 to 31.73	0.3811	0.3518 to 0.4104
	BBS1 KO	YFP BBS8	11.19	8.819 to 14.75	0.2115	0.2046 to 0.2183
	BBS1 KO	BBS9 YFP	8.24	5.637 to 15.30	0.4335	0.4188 to 0.4482
	BBS1 KO	YFP BBS18	13.10	8.542 to 28.08	0.3474	0.3230 to 0.3717
Centrosome/ basal body	WT	YFP BBS1	4.812	4.078 to 5.869	0.7095	0.7025 to 0.7166
	BBS4 KO	YFP BBS1	4.028	3.657 to 4.483	0.8309	0.8261 to 0.8357

Table S5. Calculated parameters derived from FRAP analysis of the BBSome subunits in ciliary base and ciliary tip. Dynamic turnover of the BBSome subunits was measured in the base and tip of the primary cilia by FRAP. Halftime $T_{1/2}$ and mobile fraction F_m were obtained by fitting the recovery curves with the one component association fit. The mean and 90% confidence intervals (CI) of 20-30 measurements is shown.

RPE1 WT	Protein analysed	Halftime $T_{1/2}$ [s]		Mobile fraction F_m [%]	
		mean	90% CI	mean	90% CI
Ciliary BASE	YFP BBS1	1.806	1.399 to 2.548	0.4147	0.4122 to 0.4171
	YFP BBS4	3.373	2.120 to 8.260	0.3572	0.3537 to 0.3607
	YFP BBS5	3.843	2.544 to 7.856	0.3390	0.3363 to 0.3417
	BBS7 YFP	4.216	3.416 to 5.505	0.2974	0.2951 to 0.2998
	YFP BBS8	6.223	4.617 to 9.543	0.2437	0.2408 to 0.2466
	BBS9 YFP	2.783	1.776 to 6.433	0.2791	0.2764 to 0.2817
	YFP BBS18	3.454	2.421 to 6.024	0.3254	0.3230 to 0.3277
Ciliary TIP	YFP BBS1	3.488	2.728 to 4.836	0.5600	0.5566 to 0.5635
	YFP BBS4	2.308	1.545 to 4.564	0.5410	0.5376 to 0.5444
	YFP BBS5	2.045	1.373 to 4.002	0.5187	0.5157 to 0.5218
	BBS7 YFP	2.626	1.927 to 4.123	0.3995	0.3959 to 0.4031
	YFP BBS8	2.993	2.226 to 4.567	0.3850	0.3814 to 0.3885
	BBS9 YFP	1.823	1.215 to 3.649	0.4534	0.4500 to 0.4568
	YFP BBS18	2.310	1.578 to 4.305	0.5192	0.5162 to 0.5223