The Bardet-Biedl protein Bbs1 controls photoreceptor outer segment protein and lipid composition

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Summary

Primary cilia are key sensory organelles whose dysfunction leads to ciliopathy disorders such as Bardet-Biedl syndrome (BBS). Retinal degeneration is common in ciliopathies, since the outer segments (OSs) of photoreceptors are highly specialized primary cilia. BBS1, encoded by the most commonly mutated BBS-associated gene, is part of the BBSome protein complex. Using a new *bbs1* zebrafish mutant, we show that retinal development and photoreceptor differentiation are unaffected by Bbs1-loss, supported by an initially unaffected transcriptome. Quantitative proteomics and lipidomics on isolated OSs show that Bbs1 is required for BBSome-entry into OSs and that Bbs1-loss leads to accumulation of membrane-associated proteins in OSs, with enrichment in proteins involved in lipid homeostasis. Disruption of the tightly regulated OS lipid composition with increased OS cholesterol content are paralleled by early functional visual deficits, which precede progressive OS morphological anomalies. Our findings identify a new role for Bbs1/BBSome in OS lipid homeostasis and suggest a new pathomechanism underlying retinal degeneration in BBS.

Introduction

Primary cilia are microtubule-based sensory organelles protruding from the surface of most differentiated cells, where they transmit and regulate extracellular signals into the cell. Signal transmission is cell-type specific, including light sensation in photoreceptors, mechanical stimuli in various tissues¹ and developmental signalling pathways such as hedgehog², Wnt³ and Notch⁴. Given the multiple roles of primary cilia during development and cell homeostasis, their dysfunction leads to a group of pleiotropic human disorders called ciliopathies⁵. Bardet-Biedl Syndrome (BBS) (OMIM 209900) is an iconic ciliopathy characterized primarily by retinal dystrophy, polydactyly, obesity, genital abnormalities, renal defects and learning difficulties^{6–8}. To date, twenty-one disease-causing genes have been associated with BBS and the localisation and function of the encoded proteins identifies BBS as a ciliopathy.

BBS1 and *BBS10* are the most frequently mutated genes, accounting for ~23% and ~20%, respectively, of patients with BBS in European and North American populations⁹. Both genes are crucial for the formation of the octameric protein complex called BBSome. The BBSome is composed of BBS1, BBS2, BBS4, BBS5, BBS7, BBS8, BBS9, and BBS18, also known as BBIP10, and interacts with the small GTPase BBS3/Arl6^{10–14}. The assembly of the BBSome requires the activity of three chaperonin-like BBS proteins (BBS6, BBS10, and BBS12) and of CCT/TRiC (TriC: T-complex protein-1

ring complex) family chaperonins¹⁵. Based on sequence analysis, which revealed similarities of BBS4 and BBS8 to the COP-ε subunits of vesicle-coating complexes, a role for the BBSome in vesicle trafficking was proposed, but the complex was shown to form planar coat structures rather than typical smaller spherical vesical coats¹¹. In-depth structural studies revealed that BBS5 and a BBSome sub-complex containing BBS4, 8, 9 and 18 possess phosphoinositide-binding properties typical of membrane-associated proteins¹⁶.

The BBSome is enriched at the transition zone^{17,18} of primary cilia, a region at the base of the ciliary axoneme which was shown to act as a gatekeeper for the ciliary compartment, and is able to migrate bidirectionally using the intraflagellar transport machinery (IFT)^{17,19,20}. Initial studies showed that the BBSome facilitates protein transport towards the ciliary compartment by direct interaction with the cytosolic ciliary targeting sequences of ciliary-directed transmembrane proteins²¹ and that it plays an important role in ciliogenesis and cilia maintenance. More recent publications however focused on a role for the BBSome in retrograde trafficking of proteins out of the ciliary compartment. Ye and colleagues showed that the BBSome/Arl6 enables the lateral transport of activated ciliary G protein-coupled receptors (GPCRs) through the transition zone in order to remove them from the cilium²² and reintroduction of WT BBSome removed accumulated phospholipase D from cilia of *Chlamydomonas reinhardtii bbs4* mutants²³.

Despite the above-mentioned progress in understanding the role of the BBSome in ciliary biology in various cell types, its function in retinal photoreceptors remains unclear with contradicting evidence. Rod and cone photoreceptor cells are sensory neurons designed to convert light stimuli into neural responses. This process, called phototransduction, takes place in the outer segments (OSs) of rod and cone photoreceptors (PRs), which are highly modified primary cilia containing tightly packed stacks of photopigment-filled membrane discs organized around the microtubule-based axoneme (reviewed by Bachmann-Gagescu & Neuhauss²⁴). This microtubule structure anchors through the connecting cilium (the equivalent of the transition zone) with the basal body localized at the apical part of the inner segment (IS). The OS membrane discs are continuously replenished from their base, as the older apical discs are phagocytosed from the tip by retinal pigment epithelial (RPE) cells whose protrusions cover the OSs²⁵. Given this constant renewal, the retina is among the most metabolically active tissues^{26,27} and highly sensitive to metabolic flux²⁸. The on-going regeneration of the shed membrane discs places a very high biochemical burden on these cells since proteins and lipids are continuously required to form new membrane discs. Beyond its importance for the renewal of discs, lipid/cholesterol homeostasis of the retina is known to play a crucial role in photoreceptor function/ survival and disruption promotes photoreceptor neurodegeneration^{29,30}, as illustrated in age-related macular degeneration (AMD)³¹ or in a mouse model for Niemann-Pick disease type C (NPC)³².

Early studies of several BBS mouse models with retinal phenotypes described rhodopsin localization defects, with opsin accumulation in the IS^{33–35} and decreased opsin levels in the OSs³⁶. This opsin mislocalization was thought to disturb the cellular homeostasis and induce a slow process of degeneration eventually causing apoptosis of photoreceptors^{34,37}. However, more recent studies on mice defective in *Lztf1/Bbs17* failed to find such opsin mislocalization. On the contrary, Datta et al.³⁸ observed accumulation of non-outer segment proteins in OSs of *Lztf1*^{-/-} mice, suggesting a role for the BBSome in the removal of non-OS resident proteins that have aberrantly entered this compartment. Their observations led to the hypothesis that PR OSs act as a “sink for membrane proteins”, whereby the high membrane content of OSs makes them a default destination for membrane proteins, with the BBSome responsible for removing those proteins that should not reside in the OSs (reviewed by Seo & Datta³⁹). In support of this hypothesis, accumulation of the IS protein syntaxin3 in the OS of *Bbs8*^{-/-} mice and *bbs2*^{-/-} zebrafish was recently described^{40,41}.

To further investigate the role of the BBSome in photoreceptor function, we turned to the zebrafish, an established animal model for ciliopathies^{42–44}. The cone-rich retina of zebrafish larvae represents a particular asset to study this photoreceptor subtype compared to the rod-dominated mouse retina, as

diverging effects on cone and rod photoreceptors have been described^{38,41,45}. In this work, we generated a new zebrafish *bbs1* knock-out model and found that despite normal retinal development and photoreceptor differentiation, visual functional deficits were present prior to the appearance of any morphological anomaly in mutant fish. Subsequent disorganization of the OS membrane discs was followed by progressive retinal degeneration, recapitulating the retinal dystrophy observed in patients. Quantitative proteomics of isolated OSs showed a complete loss of all BBSome subunits from the OS compartment consecutive to *Bbs1* loss, an overall accumulation of proteins in mutant OSs with a predominance of membrane-associated proteins and an enrichment of proteins involved in lipid-homeostasis. These findings were paralleled by an altered OS lipid composition and an early accumulation of free (unesterified) cholesterol, providing a mechanism to explain the observed early functional deficit. Our findings indicate a role for BBS1/BBSome in controlling not only protein but also lipid composition of PR OSs and provide a new mechanism underlying the visual deficit caused by BBS1/BBSome dysfunction.

Results

Generation and characterization of a zebrafish *bbs1* mutant line

To generate a *bbs1* zebrafish mutant model, we first searched the zebrafish genome for the orthologue to the human gene. We found a single zebrafish *bbs1* gene and verified by synteny analysis that no unannotated paralog exists (Suppl. Fig. 1). The sequence similarity at the amino acid level between human and zebrafish *BBS1/bbs1* is 69% (Suppl. Fig. 2). We targeted Exon 4 of *bbs1* using the CRISPR/CAS9 gene editing system, to introduce a 5bp deletion resulting in a frameshift and premature stop codon at nucleotide position 421 (Fig. 1a). The theoretical truncated protein contains the first 24% of the protein but lacks important domains such as the b-propeller and GAE (Gamma-adaptin ear)¹⁶ domains. Heterozygous animals exhibit no obvious phenotypes and were incrossed to generate zygotic *bbs1* homozygous fish (*zgbbs1*^{-/-}). Zebrafish *zgbbs1*^{-/-} appeared indistinguishable from the wild-type counterpart and were present in Mendelian ratios at 5 days post-fertilization (dpf). The mutant larvae did not display typical ciliopathy phenotypes such as body curvature, kidney cysts or *situs inversus* until 5 dpf (Fig. 1b and c). However, we observed the development of a spinal curvature in mutant adult fish. Scoliosis is a commonly observed phenotype in zebrafish ciliopathy mutants^{41,46}. Given the lack of significant phenotypes in *zgbbs1*^{-/-} larvae, and since maternal contribution of transcript or protein in the egg can rescue early developmental defects in zebrafish, we next generated maternal zygotic *bbs1* homozygous mutants (*mzgbbs1*^{-/-}) by crossing *zgbbs1*^{-/-} females with heterozygous or homozygous mutant males. Like *zgbbs1*^{-/-}, *mzgbbs1*^{-/-} larvae did not exhibit most typical ciliopathy phenotypes such as

Figure 1 | Characterization of *bbs1* mutant zebrafish: (a) Deletion of 5 bp in exon 4 of *bbs1* causes a frameshift resulting in a premature termination codon (red letters). (b) Lateral view of 5 dpf larva and adult (>3 months post fertilization (mpf)) with wild-type in the top panel, zygotic mutant (*zgbbs1*) in the middle panel and maternal zygotic mutant (*mzbbs1*) in the bottom panel. (c) Summary of the phenotypic characterization with respect to typical cilia-associated phenotypes showing differences between zygotic and maternal zygotic mutants for larval body curvature. Note that scoliosis is present in nearly all zygotic and in all maternal-zygotic adult mutants. (d) Whole mount immunostaining of various ciliated tissues including kidney tubules, midline neuroepithelium and forebrain ventricle cilia at 30 hours post fertilization (hpf) and nose pit cilia at 3 dpf using anti-Arl13b or anti-acetylated tubulin antibodies to label cilia (green). No differences in abundance or morphology of cilia were noted between *mzbbs1* mutants (bottom) and their sibling controls (top). All images are dorsal views with rostral to the left and caudal to the right. The schematics at the bottom are for orientation. Scale bars: (b) 0.5mm (larvae), 1cm (adult), (d) 10µm.

kidney cysts, *situs inversus* or abnormal numbers of otoliths (Fig. 1c), and body curvature was present at 5 dpf in subsets of mutant larvae in proportions that varied by clutch. All *mzbbs1*^{-/-} larvae, even those that displayed a straight body at 5 dpf, developed scoliosis over time affecting 100% of *mzbbs1*^{-/-}

adults. Consistent with the lack of anticipated ciliopathy phenotypes in the mutants, cilia numbers and morphology were unaffected in larval kidney tubules, brain ventricles, midline neuroepithelium and nose pit (Fig. 1d), indicating that *Bbs1* is not required for ciliogenesis. For the following

experiments, we used *mzbbs1*^{-/-} for larval analysis and *zgbbs1*^{-/-} at adult stages.

Functional visual deficit in *bbs1*^{-/-} larvae despite normal photoreceptor development and morphology

We next examined the retina of *mzbbs1*^{-/-} mutants at 5 dpf and found normal retinal lamination, suggesting that retinal development does not require Bbs1. The organization and integrity of the outer segment (OS), outer nuclear (ONL) and outer plexiform layer (OPL) was unchanged in mutants compared to wild-type at 5 dpf (Fig. 2a and Suppl. Fig. 3A-A'). Apical-basal polarity of PRs was unaffected based on preservation of the characteristic PR cell body shape, highlighted by immunohistochemistry with the *zpr1* antibody, which marks *arr3a* in red/green cones (Fig. 2b). Outer segment organization and gross morphology was normal in *mzbbs1*^{-/-} larvae at 5 dpf based on fluorescence imaging using the membrane labelling dye DiO (Fig. 2c). We analysed the localization of opsins using the 4D2 antibody, but could not identify any increased mislocalization to the inner segment (IS) or cell body compared to controls (Fig. 2d and Suppl. Fig. 3E, E'). The outer plexiform layer revealed by the synaptic vesicle glycoprotein 2 (SV2) did not present any abnormalities (Fig. 2e). Ultrastructural analysis on transmission electron microscopy (TEM) of the 5 dpf retina showed perfectly developed outer segments in mutants with neatly organized membrane discs. No evidence for accumulation of vesicles in the IS was observed (Fig. 2f, g), which corroborates with lack of opsin mislocalization on immunofluorescence (Fig. 2d). When assessing the visual function of *mzbbs1*^{-/-} at 5 dpf using electroretinography (ERG), we were surprised to record a significantly decreased b-wave amplitude for all tested light intensities (Fig. 2h). Therefore, despite lack of any morphological defects, *mzbbs1* mutants demonstrated an early decreased visual response. Taken together, our results indicate that Bbs1 is not required for development and differentiation of photoreceptors but that its absence impacts phototransduction.

Progressive morphological OS abnormalities and retinal degeneration in *bbs1* mutants

To determine if progressive morphological changes appear in the retina of *mzbbs1*^{-/-} larvae, we assessed retinal structure beyond 5 dpf using semi-thin plastic sections, immunohistochemistry and TEM. In wild-type, two layers of OSs are observed, with the OSs of blue-sensitive and red/green double cones dwelling apically compared to those of UV-sensitive cones⁴⁷. Starting at 7 dpf, we observed subtle changes in this OS organization in *mzbbs1*^{-/-} larvae (Fig. 3a, b). At 10 dpf, the OSs of the four cone subtypes were completely intermingled in mutants (Fig. 3c, d) and the OSs became progressively shorter and bulkier. Ultrastructural analysis revealed that the tight organisation of the membrane discs was partially lost and became disorganised (Fig. 3e). In mutant OSs we observed membrane discs that were twisted and tilted vertically and some regions lost their tight and compact membrane disc stacking similar to what has been described in *Bbs1*^{M390R/M390R} mouse retina³⁸. Increased numbers of phagosomes

filled with membranous structures were visible and the melanosomes of the RPE migrated deeply into the mutant PR layer (Fig. 3c, e). At 10 dpf, *mzbbs1* mutant OSs were severely shortened and misshapen and a TUNEL assay showed a significant increase in apoptotic PR cells compared to controls (Suppl. Fig. 4). Significant retinal degeneration was also reflected in the strongly reduced ERG response at this stage (Fig. 3g). Despite advanced disruption of normal PR morphology, we did not observe mislocalized opsins as assessed by staining with 4D2 (Suppl. Fig. 3E-G') or UV-Op sin (Fig. 3f).

Of note, we observed substantial variability in the severity of the overall phenotype, with a correlation between severity of retinal degeneration and survival of the fish: larvae surviving beyond 14 dpf tended to have milder retinal degeneration. Moreover, we observed a similar but milder and more slowly progressive retinal degeneration in zygotic *bbs1* mutants (Suppl. Fig. 5 and 6). Zygotic *bbs1*^{-/-} adult fish retained a substantial number of long OSs, still lacking evidence for opsin mislocalization (Suppl. Fig. 5E, E'). The majority of OSs were misshapen, and the OS layer was invaded by nuclei either from disorganized RPE cells or from microglia as described in the *bbs2* zebrafish mutant⁴¹. The structured organization of the PR layer with distinct short and long cone OSs was lost in *zgbbs1*^{-/-} (Suppl. Fig. 5B and B'). ERG data of adult fish confirmed a milder phenotype in *zgbbs1*^{-/-} mutants (Suppl. Fig. 6): at 3 months, we measured only a slight reduction in the ERG response in *zgbbs1*^{-/-} fish, which progressed to a severe reduction at 10 months. Taken together, these data indicate that Bbs1 is important for photoreceptor homeostasis and maintenance and that maternal contribution is able to decelerate the progression of the degeneration.

The retinal transcriptome is not primarily affected in *bbs1* mutants

Primary cilia are signalling hubs of cells⁴⁸ and play a key role in transducing and regulating developmental signalling pathways such as hedgehog or Wnt signalling⁴⁸. Moreover, it has been proposed that some BBS proteins can regulate the mRNA expression levels of other BBSome components by interaction with RNF2^{49,50}. We therefore aimed at investigating the transcriptional consequences of Bbs1 loss-of-function in the eye by performing RNA sequencing on isolated eyes of 5 dpf (=120 hpf ±1h) *mzbbs1*^{-/-} larvae, where only a functional but no morphological phenotype was present, and of 10 dpf (±1h) *mzbbs1*^{-/-} larvae, where both function and morphology were affected. We first compared the normalized read counts of all the different BBSome subunits, to investigate a potential compensatory effect. We found that *bbs1* mRNA levels were significantly decreased in mutants (Fig. 4a) indicating nonsense-mediated mRNA decay. However, expression levels of all other BBSome subunits were unchanged. Moreover, we did not observe altered expression in the Rnf2 target genes *bcl11a*, *dlx2*, *hoxb8*, *lef1*, *pou3f2*, *foxl2* and *foxq1* (Suppl. Table 1). The variance between mutant and control samples was substantially smaller than between the biological replicates (Fig. 4b), indicating

Figure 3 | Morphological abnormalities and progressive retinal degeneration in *mzbbs1*^{-/-} mutants starting at 7 dpf: (a, c) Representative images of semi-thin plastic sections stained with Richardson solution in wildtype (top) and *mzbbs1*^{-/-} (bottom) retina at 7 dpf (a) and 10 dpf (c) showing slight morphological abnormalities at 7 dpf and more severe changes at 10 dpf with shorter and misshapen OSs. (b, d) DiO-staining highlighting OSs reveals progressive morphological alterations of the OSs between 7 dpf (b) and 10 dpf (d). (e) TEM reveals bulky OSs (*asterisk) with abnormal membrane disc stacking in mutant retina at 7 dpf. Disc membranes in mutants (middle & right image) are less compact in places or stacked vertically compared to controls (red arrowheads). Membrane-filled swirls are frequently observed in the RPE (yellow arrows) at that stage (middle & right image are two images of *mzbbs1*^{-/-} retinas showing various alterations seen in the mutants). (f) At 8 dpf, UV-Op sin (green) localizes normally to the OS in *mzbbs1*^{-/-} mutants and controls, despite abnormal ultrastructure and shortened OS revealed by the membrane dye BODIPY (magenta). (g) Top panel shows bar plots of the maximum b-wave amplitude by electroretinography at 10 dpf. Reduced b-wave intensity at various light intensities demonstrates severely decreased response to light. Unpaired two-tailed multiple T-test; Sig: ns= FDR(q-value)=0.089, ****= FDR(q-value)<0.0001; Sample size (n=13 WT, n=8 Mut larvae), Error bars show standard deviation. The bottom panel shows the average response curve at log-2 intensity for mutant (grey) compared to control (black). Scale Bars: (a, c) 12µm, (b, d, f) 50µm, (e) 5µm. Abbreviations: OS outer segment, ERG electroretinography, INL inner nuclear layer, M mitochondria, N nucleus, ONL outer nuclear layer, OPL outer plexiform layer, P pigment, TEM transmission electron microscopy.

only minor changes between the two conditions at 5 dpf. Indeed, the differential expression (DE) analysis revealed that at 5 dpf only two genes were significantly differentially expressed (cut off: $FC > \pm 2$ & $p_{adj} \leq 0.05$) (Fig. 4c). At 10 dpf we observed a total of 159 genes that were significantly differentially expressed, with 97 genes being down- and 62 upregulated respectively (Fig. 4d). Genes associated with oxidative phosphorylation, citrate cycle and ribosome were over-represented by KEGG-pathway analysis (Fig. 4e). Additionally, we found proteasome and necroptosis genes to be enriched in the data set. Likewise, we observed a significant upregulation of the proapoptotic factor *bbc3/puma* and *bmf2* indicating an increase in the apoptotic pathway activity in mutants, consistent with our findings in the TUNEL assay (Suppl. Fig. 4). Overall, the late transcriptional changes identified suggest that the loss of Bbs1 only indirectly affects the transcriptome as a consequence of retinal degeneration. Taken together, these results demonstrate that Bbs1 loss-of-function does not affect expression levels of other BBSome subunits or alter developmental pathways during eye formation, but rather leads to an upregulation of pro-apoptotic genes at later stages in response to retinal degeneration.

Accumulation of membrane-associated proteins in mutant outer segments

Given the suggested role for BBS1 and the BBSome in protein transport, we next investigated the protein composition of *bbs1* mutant OSs versus control OSs. For this experiment, we used mechanically isolated OSs of 5 month old zygotic *bbs1*^{-/-} fish and their wild-type siblings as controls (Fig. 5a), which we submitted to label-free quantitative MS-MS. BBS4 and BBS1 are thought to play a key role in the spatial regulation of the full BBSome complex assembly⁵¹ restricting the entry of the complex into the cilium in the absence of BBS1⁵². However, a recent publication suggested that photoreceptor cilia, in contrast to primary cilia, grant entry to a partially assembled BBSome⁵³. We found that in the absence of Bbs1, other BBSome subunits were lost from the outer segment (Fig 5b). Importantly, we confirmed by qPCR that mRNA levels of BBSome components were not decreased in *bbs1*^{-/-} eyes at this stage (Suppl. Fig. 7), indicating that the absence of BBSome components from mutant OSs is not caused by decreased transcription of the respective genes but rather by a direct role for Bbs1 in BBSome entry into the ciliary compartment of PRs.

Overall, our proteomics analysis revealed an accumulation of proteins in mutant OSs, with 169 proteins having a positive fold change ($FC > +2.0$) and only 59 proteins with a negative fold change ($FC < -2.0$) (Fig. 5c). Of these, 115 proteins had an adjusted p-value of < 0.05 and were called significantly enriched and 36 proteins were found significantly reduced (Fig. 5c). Similar to Datta et al.³⁸, we observed accumulation of non-OS proteins including Stx3, Stxbp1, chaperonin containing TCP1 Subunits (Tcpc/Cct) and proteasome subunits (Psmab/c) (Suppl. Table 2). The substantial overlap between our zebrafish dataset and the mouse dataset emphasises the conserved role of the

BBSome in controlling protein content of the OS (Suppl. Fig. 8A and Suppl. Table 3).

We next used gene ontology over-representation analysis and found that a majority of the significantly enriched proteins belonged to the *cellular component* terms “component of membrane” and “plasma membrane” (Fig. 5d and Suppl. Fig. 9). A majority of the decreased proteins were associated to cone-specific functions such as cone-specific transducin subunits (Gnat2, Gnb3b) or cone opsins (Opn1mw1, Opn1sw1, Opn1mw2, Opn1sw2, Opn1lw1 and Opn1mw4) (Suppl. Table 2). Some rod-specific proteins were also found to be absent from mutant OSs such as the rod derived cone viability factor Nxn11. Strikingly, proteins associated to lipid response, lipid-binding or lipid localization were overrepresented in the *biological process* and *molecular function* terms. We found apolipoproteins (Apoeb, Apoai1b), sterol-binding proteins (Erlin1/2, Scp2b, Npc2/zgc:193725), fatty acid-binding proteins (Fabp1a, Fabp7a, Fabp11b) and phosphatidylinositol-associated enzymes (Pitpnaa, Inpp5ka, Pip4k2cb) accumulated in mutant OSs (Fig. 5d and Suppl. Fig. 8B). Since the expression of Apoeb in the retina is not defined with certainty, we used an Apoeb:lynGFP transgenic line⁵⁴ (Suppl. Fig. 10) and found that this key apolipoprotein is expressed in PRs but not in RPE cells. The strong enrichment in proteins associated with lipid homeostasis in OSs lacking Bbs1 led to the hypothesis that the lipid composition of OSs might be impaired.

Loss of Bbs1 affects the lipid composition of outer segments

To investigate potential changes in lipid composition of OSs, we conducted a targeted LC-MS analysis on OSs isolated from 5 months old zygotic *bbs1* mutants and their wild-type sibling controls. A total of 14 lipids were significantly changed by loss of Bbs1 ($FC > \pm 2.0$ and $p\text{-value} < 0.05$) (Fig. 6a). Mutant OSs exhibited an increase in phosphatidylinositol PI(38:5), triglyceride TGA(52:4), ceramide Cer(m36:2) and various phospholipids with a phosphocholine (PC) head group. A majority of the 8 lipid specimens that were reduced in mutant OSs were PCs with a docosahexaenoic acid (DHA) (22:6) fatty acid. DHA and free unesterified cholesterol are mutually exclusive in OSs^{55,56} and we indeed observed that the reduction in DHA (22:6)-PCs in mutant OSs was accompanied by a significant, twofold increase in free cholesterol (Fig. 6a, b). Given the known global metabolic alterations observed in patients with Bardet-Biedl syndrome, we ruled out that the accumulation of cholesterol in mutant OSs is due to hypercholesterolemia by analysing the total serum cholesterol, which showed no significant increase in *bbs1* mutants compared to sibling controls (Suppl. Fig. 11). We assessed the potential impact of the impaired lipid homeostasis on the morphology of photoreceptors using electron microscopy on retinas of 5 month old fish (Fig. 6e). We observed dysmorphic OSs with abnormal disc stacking, including the presence of electro-lucent spherical structures within the OS layer that could resemble lipid droplets. Since disruption of the tightly

Figure 4 | The retinal transcriptome is not primarily affected in *bbs1* mutants: Eye-specific transcriptomic analysis in 5 dpf and 10 dpf maternal zygotic *bbs1*^{-/-} larvae and their sibling controls. **(a)** Bar plot of the normalized read counts of all BBSome components at 10 dpf shows a significant reduction in *bbs1* mRNA, indicating mutation-induced mRNA decay. No significant alteration in mRNA levels of other BBSome components was observed, indicating that no transcriptional compensation effect occurs. Benjamini-Hochberg (BH) adjusted p-values of Wald test; Sig: **= adj. P-value < 0.01.; Sample size (n=3 samples for each condition, composed of a pool of >10 larvae); Error bars show standard deviation. **(b)** Principle component analysis of the Deseq2 normalized, variance-stabilizing transformed top100 variable genes, shows large variability between sample pairs at 5 dpf and to a lesser extent at 10 dpf. To account for batch effects, a paired sample design was thus considered in the differential expression (DE) analysis. **(c-d)** DE genes are visualized in volcano plots by their fold change (FC) (mutant over control) and their adjusted p-value. In red are genes with an adjusted p-value < 0.05 and fold change > FC±2; in blue are genes with adj.p-value < 0.05 & FC between +2 and -2 and in green are genes with a FC > ±2 but an adj.p-value > 0.05. **(c)** DE-analysis at 5 dpf reveals only two genes that are significantly changed; neither are associated to a known developmental or BBSome compensation pathway. **(d)** At 10 dpf several genes are significantly DE (red dots). **(e)** Over-representation analysis was used to group the genes that were DE at 10 dpf into functional groups using Webgestalt KEGG-pathway analysis. Several KEGG pathway terms associated with metabolic change and necroptosis were overrepresented in our data set. Abbreviations: CTRL control, MUT mutant, DE differential expression, FC fold change (*mzbbs1*^{-/-} over control).

Figure 5 | Accumulation of membrane-associated proteins in *zgbbs1*^{-/-} adult mutant OS: Quantitative label free proteomics was applied to mechanically isolated OSs of 5 month old zygotic mutants and sibling controls. (a) Semi-thin plastic sections stained with Richardson's solution show persistent OSs with morphological abnormalities in *zgbbs1*^{-/-} mutants (right image) at 5month compared to controls (left image). The blow-up image shows examples of isolated OSs used for proteomic analysis. (b) Bar plot showing the sum of normalized peptide LFQ-intensities reported by MaxQuant of all detected BBSome components. Significant reduction or complete loss (#) suggest impaired entry of all BBSome subunits into the OS in the absence of Bbs1. Independent multiple T-test on LFQ-intensities; Sig.***= FDR(q-value) < 0.001; sample size (n=4 Mut and n=4 Ctrl Samples, each sample is a pool of 7 retinas from 4 animals); Error bars show standard deviation. (c) Binned frequency distribution plot of proteins based on their fold changes (*zgbbs1*^{-/-} over control) revealing an overall accumulation of proteins (FC ≥ 2). Out of the 169 enriched proteins, the abundance of 115 proteins was significantly increased (adj. p-value < 0.05) while 36 out of the 59 reduced proteins were significantly decreased. (d) GO-ORA analysis of all proteins (FC ≥ 2 & adj. p-value < 0.05) reveals an overrepresentation of "membrane-associated proteins" for the *cellular component* term. The majority of these proteins have a positive FC and are enriched in the mutant OSs. In the *biological process* and *molecular function* terms, we found an overrepresentation of proteins associated with lipid homeostasis that accumulate in mutant OSs. Complete plots are found in Suppl. Fig. 9. Scale bars: (a) 25 μ m, zoom: 10 μ m. Abbreviations: RPE retinal pigment epithelium, OS outer segment, ONL outer nuclear layer, GO-ORA Gene ontology over representation analysis, LFQ label free quantification.

controlled cholesterol composition of OS membranes is known to impact photoreceptor function, and given the early functional deficit of *mzbbbs1*^{-/-} larvae, we next investigated the content of cholesterol in mutant OSs at larval stages. We used the fluorescent cholesterol probe Filipin-III that specifically interacts with unesterified (free) but not esterified cholesterol on retinal sections of 5 dpf *mzbbbs1*^{-/-} (Fig. 6c and quantification in Fig. 6d). This analysis confirmed that free cholesterol is increased at 5 dpf when visual function is already affected but before morphological changes are visible in the mutant retina. These data suggest that the BBSome is not only required for controlling protein composition in the OS but also that it regulates lipid homeostasis of OSs, whose tight regulation is crucial for phototransduction.

Discussion

In this work, we use a novel zebrafish *bbs1* model to shed light onto the role of the BBSome in retinal

photoreceptors. Based on *bbs1* mRNA decay and depletion of all BBSome subunits from mutant outer segments (OSs) on proteomics, we consider this to be a model for BBSome dysfunction, which recapitulates the retinal dysfunction and dystrophy described in human patients⁵⁷, mice^{33,40} and zebrafish⁴¹ models. Our findings identify a novel role for BBS1/BBSome in controlling not only protein but also lipid composition of photoreceptor (PR) OSs and suggest that early disruption of OS lipid homeostasis can contribute to explain early functional deficits as well as subsequent progressive morphological defects (Fig. 6f).

Photoreceptor OSs display a highly specific lipid distribution, and even small changes in lipid structures and composition can have profound effects on fundamental biological functions. Previous work has shown that free (unesterified) cholesterol is distributed non-homogeneously along the OS-membrane discs, with decreasing levels from the base to the apical portion of the OS (reviewed in Albert et al.⁵⁸). Docosahexaenoic acid 22:6 (DHA)

Figure 6 | Loss of the BBSome affects the lipid composition of photoreceptor outer segments: (a) Volcano plot showing lipids that are significantly affected (p-value <0.05 & FC >±2) in *bbs1* mutant OSs, as identified by targeted lipidomics on isolated OSs of 5 month-old *zgbbs1*^{-/-} fish. (b) Box plot highlighting the roughly twofold enrichment of free cholesterol seen in (a). Independent t-test Benjamini-Hochberg corrected Significance: ****= p-value <0.0001; Error bars showing the min/max values. (c) Accumulation of free cholesterol in 5 dpf *mzbbbs1*^{-/-} mutants (right image) is verified by staining with the free cholesterol fluorescent reporter Filipin-III (grey), co-stained with the membrane binding dye BODIPY (red). (d) Violin plot of the mean Filipin fluorescence intensity of single OSs shows that free cholesterol significantly accumulates in mutant OS compared

levels show converse enrichment, being high at the tip and low at the base⁵⁹, thus creating a polyunsaturated fatty acid (PUFA) environment that is unfavourable for cholesterol integration into the membrane⁶⁰. Therefore, PUFA and phospholipid compositions are thought to determine the cholesterol distribution along the OS. The local cholesterol content in turn has profound consequences on the activity and stability of rhodopsin⁶¹ and high cholesterol levels are known to reduce rhodopsin activation and to promote PR degeneration^{29,58,60,62}. Likewise, altered phosphoinositide⁶³ distribution has been shown to cause functional abnormalities in photoreceptors. Therefore, we propose that the early accumulation of cholesterol at larval stages in *bbs1* mutant OSs contributes to the observed early decrease in visual function seen on electroretinography despite normal retinal and photoreceptor morphology at this stage.

The subsequent morphological OS abnormalities seen in *bbs1* mutants could also be explained, at least in part, by abnormal cholesterol and lipid composition. Indeed, membrane fluidity is lipid-dependent and cholesterol is well known to stabilize membranes, resulting in stiffer membranes⁶⁴. DHA-containing phospholipids (PL-DHA) are thought to preserve membrane disc shape, thereby maintaining visual function⁶⁵. The abnormal membrane disc shape/organization observed in *bbs1*^{-/-} mutants could therefore be a consequence of the reduced PL-DHA and increased cholesterol levels in the OS. Importantly, we found that cholesterol is increased in photoreceptor OSs but not in serum of *bbs1* mutant fish, indicating that the BBSome alters the local lipid distribution by affecting the intracellular distribution, rather than by a systemic effect on lipid metabolism. Consistent with our finding of a local role for BBS1 in controlling OS lipid composition, hypercholesterolemia is observed in only ~20% of patients with BBS1⁶⁶, while over 90% of patients develop retinal dystrophy⁶. The local cholesterol level in the retina of individuals with BBS has yet to be analysed, but disrupted lipid/cholesterol distribution now provides a new model for the pathomechanism contributing to the retinal dystrophy in BBS.

Intra-retinal lipid homeostasis is a complex process which is thought to be driven by a tightly balanced interplay between phagocytosis and lipid transport via HDL (high-density lipoprotein), in which HDL particles that cycle between PR and RPE could transport DHA to, and cholesterol from the photoreceptor⁶⁷. In comparison, the regulation of intracellular cholesterol transport within PRs between OS and inner segment (IS) remains poorly understood but is likely tightly linked to protein transport between these two subcellular compartments. Consistent with findings in *Bbs17* mutant mice³⁸, we found numerous proteins to be enriched in OSs lacking the BBSome. Among these,

an unbiased overrepresentation analysis based on GO terms highlighted multiple proteins involved in lipid transport or metabolism, in particular different sterol and fatty acid-binding proteins, lipid metabolism enzymes and apolipoproteins. Secondary changes of ciliary lipid composition, due to accumulation of enzymes involved in lipid metabolism, have previously been shown in a *Chlamydomonas reinhardtii bbs4* mutant²³. Here, we hypothesize that accumulation of proteins such as the phosphatidylinositol phosphate (PIPs) interconversion enzymes Pitpnaa, Inpp5ka and Pip4k2cb leads to a change in the PIP profile of the OS. Despite their low abundance, PIPs play an important role in PR homeostasis and their dysregulation can lead to blindness⁶³. Unfortunately, the low abundance of PIPs and their charge make them hard to detect by LC-MS, such that an altered profile in our data set remains speculative. On the other hand, the observed aberrant cholesterol and PL-DHA levels point towards a defect in intracellular cholesterol transport. In healthy tissue, cholesterol from basal membrane discs is thought to be removed from photoreceptors and transported towards the RPE, in order to achieve the normal basal-to-apical cholesterol gradient in OSs. Apolipoproteins (ApoE and ApoB) are potential candidates for controlling this shuttling between the PR and RPE, removing cholesterol and delivering DHA⁶⁷. However, components and route of the intracellular cholesterol transport in PRs remain largely unknown. We show that ApoE, which is found in HDL particles, is expressed in PRs and not in RPE cells, suggesting a role for ApoE in the removal of cholesterol from the OS prior to its secretion. Our proteomics results demonstrate an enrichment of apolipoproteins (ApoEb and ApoA1b) in BBSome-deficient OSs, suggesting a model whereby this accumulation of ApoE is secondary to impaired Bbs1/BBSome function and in turn leads to increased cholesterol and decreased DHA levels. Further work will determine if the BBSome controls apolipoprotein localisation.

This model and the overall accumulation of proteins observed in *bbs1* mutant OSs are consistent with the proposed role for the BBSome complex in mediating retrograde transport to remove non-OS resident proteins as suggested by Seo and Datta³⁹. In this case, the observed disruption of lipid composition would be secondary to abnormal accumulation of lipid-associated proteins such as ApoE in the OS. An alternative model can however be proposed, whereby the BBSome simultaneously controls the sorting of proteins and lipids before entrance into the OS by regulating specific lipid microdomains. Detergent-resistant membranes (DRM) or so-called lipid rafts form such microdomains, creating specific protein and lipid environments that are essential in various signalling processes and form platforms for

Figure 6 (continued) | to controls at 5 dpf. Mann-Whitney-Test on mean OS intensities; Sig: ****= p-value <0.0001; (n=525 Mut OSs, n=733 Ctrl OSs) (e) TEM images of 5 month old zygotic mutants (**right image**) reveals dysmorphic OSs with loss of compact membrane stacking, vertical membrane discs (yellow arrowheads) and presence of electro-lucent spherical “bubbles” in the OS (red arrowheads). (f) Schematic summary of findings in this work, illustrating the altered protein and lipid content caused by Bbs1 loss. In the physiological situation (**left image**) the OSs have a tightly regulated protein and lipid composition, including a non-homogenous lipid distribution (blue-red gradient). In the *bbs1*^{-/-} mutant OS (**right image**) accumulation of non-OS proteins is observed with altered lipid composition, including early accumulation of cholesterol. Morphological anomalies include abnormal OS membrane disc stacking resulting in bulky OSs and electro-lucent spherical “bubbles”. Scale Bars: (c) 5µm; (e) 5µm. Abbreviations: TEM transmission electron microscopy, M mitochondria, N nucleus, P pigment, OS outer segment.

targeted protein trafficking⁶⁸. DRM have been described in PR OSs⁶⁹ and the importance of lipid rafts in cilia-dependent signalling was shown in a recent publication about adipogenesis⁷⁰. The BBSome was initially introduced as a coat system-like complex that is capable to form planar coats¹¹. Moreover, selective PIP binding is described for BBS5 but also for a subcomplex composed of BBS4, 8, 9 and 18¹⁶, further suggesting direct interactions between the BBSome and lipid membranes. One can thus speculate that in photoreceptors the BBSome helps to form microdomains that are able to enrich specific lipids and proteins simultaneously, constituting a rudimentary sorting mechanism. Impaired BBSome function would in this case simultaneously disturb the lipid and protein composition and especially affect transmembrane and membrane-associated proteins as observed in *bbs1* mutant OSs. This model is further supported by the early accumulation of free cholesterol in *bbs1* larval OSs, which speaks for a primary consequence of BBSome dysfunction rather than a secondary effect, which would require a time-lag to appear. The lack of opsin mislocalization in our model does not exclude the possibility of the BBSome as ciliary sorting/entry regulator, since depletion of the BBSome could impair the selective sorting of proteins without affecting the global entry mechanism itself. Additional studies would be required to investigate if the BBSome co-localizes with lipid raft-associated proteins and thus is able to contribute to the formation of lipid microdomains. Reconciling both models, the BBSome could be involved both in initial sorting of OS-bound proteins and lipids by acting as a membrane-coat on microdomains and also act in the removal of non-OS resident proteins that escaped this sorting mechanism to avoid their accumulation in the OS.

Numerous BBSome mutant studies highlight the crucial role of the BBSome complex for photoreceptor homeostasis and morphogenesis^{40,41,45}. The *bbs1* mutant zebrafish presented here recapitulates the progressive retinal dysfunction and degeneration seen in humans and in all published models. In accordance with recent reports, we find no evidence that the BBSome is required for ciliogenesis. Likewise, we find that Bbs1 is dispensable for development of the retina or for differentiation of PRs and initial formation of OSs. This is in line with the absence of alterations in the retinal transcriptome, which further indicates that Bbs1 plays no role in transduction of developmental pathways during formation of the retina. A role for Bbs1/BBSome in OS maintenance is however clear, based on the subsequent appearance of morphological anomalies, first of OSs, then of photoreceptors, with slowly progressive retinal degeneration. A recent description of a *bbs2*^{-/-} zebrafish mutant similarly showed normal retinal lamination, normal PR cilia architecture and tightly stacked disc membranes, although shortened OSs were described from the beginning⁴¹. Normal ciliary architecture and membrane stacking in these two zebrafish mutants stand in contrast with several BBSome mutant mice that show abnormal OSs already at early stages (P15)⁷¹. In contrast to mice,

zebrafish possess a cone-dominated retina⁷², especially at larval stages, possibly accounting for some of the observed differences. Despite these differences, all mutant models display progressive retinal degeneration, similar to patients with BBS who show an early involvement of the cone-rich macula.

In summary, our work using a zebrafish *bbs1* mutant model expands the knowledge of the molecular consequences of Bbs1/BBSome dysfunction in PRs, confirming its key role in the fine control of OS protein content and identifying a new role in regulating OS lipid composition. This could potentially open the way for selective therapies to improve the locally disrupted lipid homeostasis. Given our findings that PR differentiation and OS formation are unaffected by loss of Bbs1/BBSome, which is consistent with the appearance of retinal dystrophy in later childhood in patients with BBS, this indicates a window of opportunity for applying neuroprotective treatments and retaining visual function for affected individuals.

Material & Methods

Ethics statement:

All animal protocols were in compliance with internationally recognized and with Swiss legal ethical guidelines for the use of fish in biomedical research (Veterinäramt Zürich Tierhaltungsnummer 150). Zebrafish husbandry and experimental procedures were performed in accordance with German animal protection regulations (Regierungspräsidium, Karlsruhe, Germany, 35-9185.81/G-69/18).

Animal Husbandry:

Zebrafish (*Danio rerio*) were maintained as described by Aleström et al.⁷². Embryos were raised at 28°C in embryo medium and staged according to development in days post fertilization (dpf)⁷³.

CRISPR/CAS9 Gene Editing:

CRISPR guide RNAs were designed using the ChopChop web interference (<https://chopchop.cbu.uib.no/>). Bbs1 specific sgRNA was prepared and injected into one cell-stage embryos as previously described⁷⁴. The CRISPR efficiency was assessed as previously described⁷⁵. For details, please see supplementary methods. *bbs1* mutations were identified by Sanger sequencing and the founder fish was outcrossed to AB wild-type to balance potential off-target effects. All larvae and fish used for these experiment were of the third generation or more after mutagenesis.

Syntenic and phylogenetic analysis:

Syntenic analysis of the zebrafish locus was done using the syntenic database setting Homo Sapiens as outgroup (Variant: Ens70) (http://syntenydb.uoregon.edu/syntenic_db/) as previously described⁴¹. In brief: Parameters were adjusted to a sliding window size of 50 genes, and several genes in the vicinity of *bbs1* were used for additional syntenic

comparison. Colour and size of the final synteny graph were adjusted using affinity designer. The protein homology was calculated using Unipro UGENE (vers. 36.0) using standard methods from the Unipro UGENE Manual (vers. 36). For details, please see supplementary methods.

Electroretinography (ERG):

White-light ERG measurements were carried out as described⁷⁶. We dark-adapted the fish for at least 30 minutes prior to stimulation. Five 100-ms flashes of increasing light intensity (ranging from log-4 to log0, where log0 corresponds to 24,000 $\mu\text{W}/\text{cm}^2$) were applied with an inter-stimulus interval of 7000 ms. Statistical analysis of b-wave amplitudes was carried out using independent samples t-test for each light intensity (WT vs. Mut).

Histology:

Larvae and adult eyes were fixed in 4% formaldehyde at 4°C for at least 15h. The tissue was embedded in Technovit 7100 (Kulzer, Wehrheim, Germany) following manufacturer standard protocol. Samples were sectioned at 3 μm thickness on a LeicaRM2145 microtome (Leica Microsystems, Nussloch, Germany) and stained in Richardson solution (1% methylene blue, 1% borax, 1% Azure II) for 5 seconds, washed 3x 5 minutes in ddH₂O and cover-slipped with Entellan mounting medium (Merck, Darmstadt, Germany). Images were acquired on an Olympus BX61 microscope.

Immunohistochemistry and TUNEL assay:

Larvae were fixed in 4% PFA at room temperature (RT) for 30 min, embedded in tissue freezing medium (Electron Microscopy Sciences, Hatfield, PA, USA) and cryosectioned following standard protocols as previously described⁴¹. In brief, unspecific binding was blocked using PBDT for 30 minutes at RT and primary antibodies were added overnight at 4°C. Primary antibodies were mouse anti-4D2 (1:200, gift from R. Molday, University of British Columbia⁷⁷), mouse-anti-Zpr-1 (1:200, Zebrafish International Resource Center, Eugene), rabbit-anti-UV-opsin (1:300, gift from D. Hyde⁷⁸), mouse-anti-SV2 (1:100, Developmental Studies Hybridoma Bank), rabbit anti-Arl13b (1:100, gift from Z. Sun, Yale University⁷⁹), mouse-anti-acetylated-Tubulin (1:500, clone 611B-1, SIGMA). Secondary antibodies were Alexa Fluor-conjugated goat anti-rabbit or goat anti-mouse IgG (1:400, Life Technologies, Darmstadt, Germany). BODIPY TR methyl ester (1:300, Life Technologies, Darmstadt, Germany) or Vybrant DiO (1:200, Life Technologies, Darmstadt, Germany) were applied for 20 min and nuclei were counterstained with DAPI or Draq5. TUNEL assay was performed following manufacturer protocol for paraffin sections (ApopTag® Red In-Situ Apoptosis Detection Kit (S7165, millipore)), modifying the permeabilization step by using 1% triton for 20 min instead of the proteinase K treatment. Sections were cover-slipped with Mowiol (Polysciences, Warrington, PA, USA) containing DABCO (Sigma-Aldrich, Steinheim, Germany) and imaged on a confocal microscope Leica TCS LSI or Leica SP5.

Filipin staining:

Staining was performed according to manufacturer protocol using Cholesterol Assay Kit (ab133116, Abcam, Cambridge, MA, USA). In brief, cryosectioned zebrafish larvae were washed 3x in wash buffer for 5 min and subsequently stained with Filipin solution for 60 min in a humidified dark chamber and co-stained with BODIPY TR methyl ester (1:300, Life Technologies, Darmstadt, Germany). Staining solution was removed and slides were washed twice in wash buffer. Sections were cover-slipped with Mowiol (Polysciences, Warrington, PA, USA) containing DABCO (Sigma-Aldrich, Steinheim, Germany) and imaged on a confocal microscope Stellaris 5 (Leica microscopy system, Nussloch, Germany) with low laser intensity to minimize bleaching.

Transmission electron microscopy:

Zebrafish larvae were fixed overnight at 4°C in a freshly prepared mixture of 2.5% glutaraldehyde and 2% formaldehyde (FA) in 0.1M sodium cacodylate buffer (pH 7.4). Post fixation with 1% OsO₄ in 0.1 M sodium cacodylate buffer at room temperature for 1 hour, and 1% aqueous uranyl acetate at 4°C for 2 hours. Samples were then dehydrated in an ethanol series, finally treated with propylene oxide and embedded in Epon/Araldite (Sigma-Aldrich) followed by polymerization at 60°C for 28 hours. Ultrathin (70 nm) sections were post-stained with lead citrate and examined with a Talos 120 transmission electron microscope at an acceleration voltage of 120 kV using a Ceta digital camera and the MAPS software package (Thermo Fisher Scientific, Eindhoven, The Netherlands).

Real-Time PCR:

RNA of a dissected 5month old retina was isolated using the ReliaPrep RNA Tissue Miniprep System (Promega, Madison, WI). cDNA was created using the SuperScript-III First-Strand Synthesis SuperMix (Thermo Fisher Scientific, Waltham, MA) with oligo dT primers. Real-time quantitative PCR (qPCR) was performed using SsoAdvanced Universal SYBR Green Super-mix (Catalogue #172-5270, Bio-Rad, Hercules, CA) on a Bio-Rad CFX96 C1000 Touch Thermal Cycler (Bio-Rad Laboratories, Hercules, CA) using 0.1 ng cDNA. Differential expression was calculated by normalizing the CT-values to the housekeeping gene G6pd and comparing the levels to wild-type. Primers used for qPCR are found in Suppl. Fig. 11.

RNA Sequencing:

For RNA isolation, mutant females were crossed with heterozygous males and the larvae were raised to exactly 120hpf or 240hpf. Each clutch represents one sample pair, consisting of one mutant sample and their siblings as heterozygous controls. Larvae were euthanized and a tail biopsy was taken for genotyping, during which the larvae were kept in RNAlater (Sigma life science, Darmstadt, Germany) at 4°C. Larvae with the same genotype were pooled

(min. n=10 larvae/sample) and RNA was extracted using the ReliaPrep RNA Miniprep System (Promega AG, Dübendorf, Switzerland) following manufacturer's user guide. Three biological triplicates per time point and genotype were collected and the total RNA was stored at -80°C for further use. Library preparation was performed using the TruSeq Stranded Total RNA Library Prep Gold (Illumina, Inc, California, USA) after ribosomal RNA depletion (see supplementary methods). The Novaseq 6000 (Illumina, Inc, California, USA) was used for cluster generation and sequencing according to standard protocol. Sequencing was single end 100 bp in two separate runs. The acquired sequencing reads were aligned to the zebrafish reference genome (GRCz11) using STAR aligner⁸⁰. The output BAM files of the second sequencing run were merged with the first file and sorted using SAMtools pipeline⁸¹. The ensemble reference sequence (Refseq) was used to assign sequence reads to genomic features using featureCount (vers. 2.0.1)⁸². Differential expression analysis between control and mutant samples were implemented using the R package DESeq2⁸³. To account for batch effects and minor differences in the genetic background, a paired term was added to the linear regression to account for the paired sample design. Default parameters of DESeq2 were used for the gene differential expression analysis reporting the Benjamini-Hochberg adjusted p-values from the Wald test. Over-representation analysis was performed using the WEB-based gene set analysis toolkit (WEB-Gestalt)^{84–86} to obtain the enriched KEGG pathway terms.

Isolation and enrichment of photoreceptor outer segments:

Photoreceptor outer segments of 5-month old zygotic adult zebrafish were mechanically isolated using a modified version of the established techniques⁸⁷. The OSs of 7 retinas were pooled for the proteomic analysis and of two retinas for the lipidomic analysis. A total of (n=4 Mut & n=4 Ctrl) samples were extracted for the proteomic investigation and (n=13 Mut & n=15Ctrl) for the lipidomic analysis. For details, see supplementary methods.

Label-free proteomics:

Isolated outer segment samples were prepared by using a commercial iST Kit (PreOmics, Germany) with an updated version of the protocol (see supplementary methods). Mass spectrometry analysis was performed on a Q Exactive HF-X mass spectrometer (Thermo Scientific) equipped with a Digital PicoView source (New Objective) and coupled to a M-Class UPLC (Waters). The acquired raw MS data were processed by MaxQuant (version 1.6.2.3), followed by protein identification using the integrated Andromeda search engine. Spectra were searched against a Uniprot zebrafish reference proteome (taxonomy 7955, canonical version from 2019-07-01), concatenated to its reversed decoyed

fasta database and common protein contaminants. The maximum false discovery rate (FDR) was set to 0.01 for peptides and 0.05 for proteins. Protein fold changes were computed based on peptide intensity values reported in the MaxQuant generated peptides.txt file, using linear mixed-effects models. Fold changes and p-values were estimated based on this model using the R-package lmerTest [lmerTest]. Next, p-values were adjusted using the Benjamini-Hochberg procedure to obtain the false discovery rates (FDR). Gene ontology over-representation analysis was performed on all the highly significant proteins (adj. P-value <0.05 & FC>±2), comparing them to all detected proteins to find terms that were overrepresented. ClusterProfiler (vers. 3.10.1) in R⁸⁸ was used for the analysis and the over-represented terms (BH adj. p-value <0.05) were simplified and visualized using the enrichplot package (vers. 1.2.0). The mass spectrometry proteomics data were handled using the local laboratory information management system (LIMS)⁸⁹ and all relevant data have been deposited to the ProteomeXchange Consortium via the PRIDE (<http://www.ebi.ac.uk/pride>) partner repository with the data set identifier PXD026646. For details, please see supplementary methods.

Lipidomics:

Lipid extraction was performed as previously described⁹⁰ with some modifications (please see supplementary methods). Mass spectrometry analysis was carried out using a QTRAP 6500+ mass spectrometer in MRM acquisition mode (Sciex Pte Ltd). Data integration and analysis was performed using the Skyline software package and the MetaboAnalyst Suite^{91,92}. Lipid quantification was done by calculating the ratios of the peak areas of each species with the areas of the peaks of the corresponding internal standards. Quality controls prepared as mixtures of all samples were used in five concentrations (1x, 0.8x, 0.5x, 0.2x and 0.1x). The quality controls were measured in triplicate. The CV% for each lipid was calculated, and values below 30% were reported. MetaboAnalyst (vers. 5.0) was used to compare the median normalized lipid profiles of mutants and controls⁹³ and the results are reported in suppl. Table 4. Significance was estimated using a parametric t-test and the Benjamini-Hochberg corrected false discovery rate was reported along the p-values.

Serum Cholesterol:

Blood was collected using a modified form of published methods⁹⁴. In brief, the adult zebrafish were euthanized using ice cold Tricain-Methansulfonat. An Incision between the anal and caudal fin was made and blood (4 - 9µL) was collected using a micropipette. The blood was left to coagulate in a 200µL tube and then centrifuged 2min at 6000rpm (Labnet PRISM, Edison, NJ, USA). The serum is at the top layer and was collected for subsequent colorimetric analysis of the total cholesterol using the Cholesterol/ Cholesteryl Ester

Assay Kit (Abcam, Cambridge, MA, USA) following manufactures protocol.

Statistic:

Statistics were performed using GraphPad Prism (vers. 9.0.0). For the ERG data and the comparison of the summed LFQ-intensities a multiple independent T-Test using the “two-stage step-up multiple test correction” (Benjamini, Krieger, and Yekutieli), was used with a Q-value of 1%. The pairwise comparisons between wild-type (wt) and *bbs1*^{-/-} were assessed and visualized in a bar graph indicating the standard deviation as error bars. Statistical analysis of the quantification for the Filipin-III intensity between wt and *bbs1*^{-/-} was performed using a Mann-Whitney U-test. All detailed p-value and degrees of freedom are reported in suppl. Table 5. The details on the statistical analyses and calculations of the significance levels for the RNAseq, proteomic and lipidomic datasets are described in detail in the respective material and methods section.

Data availability:

All data generated or analysed during this study are included in this published article (and its Supplementary Information files). All relevant protein data have been deposited to the ProteomeXchange Consortium via the PRIDE (<http://www.ebi.ac.uk/pride>) partner repository with the data set identifier PXD026646.

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Author contributions:

MM, CE, JZ, CH, AH, MT performed experiments and generated data; MM, CE, TH, MG, SN, US, RBG planned and designed experiments; MM, CE, RBG wrote the manuscript; all Authors edited the manuscript.

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