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 difficulties6–8. 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/Arl611–14. 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 coats16. 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 zone17,18 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)17,18,20. 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 & Neuauss 24). 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 OSs 25. Given this constant renewal, the retina is among the most metabolically active tissues 26,27 and highly sensitive to metabolic flux 28. 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 OSs 36. 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-outter 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 & Datta 39). In support of this hypothesis, accumulation of the IS protein syntaxin3 in the OS of Bbs3/- mice and bbs2/- zebrafish was recently described 40,41.

To further investigate the role of the BBSome in photoreceptor function, we turned to 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 45,46. 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 (zgbbst 1). Zebrafish zgbbst 1-/- 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 zgbbst 1-/- 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 (mzbbs1 1/-) by crossing zgbbst 1-/- females with heterozygous or homozygous mutant males. Like zgbbst 1-/-, mzbbs1 1/- 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<sup>-/-</sup> for larval analysis and zgbbs1<sup>-/-</sup> at adult stages.

**Functional visual deficit in bbs<sup>1-/-</sup> larvae despite normal photoreceptor development and morphology**

We next examined the retina of mzbbs1<sup>-/-</sup> 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<sup>-/-</sup> 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<sup>-/-</sup> 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 bbs<sup>1-/-</sup> mutants**

To determine if progressive morphological changes appear in the retina of mzbbs1<sup>-/-</sup> 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 cones<sup>47</sup>. Starting at 7 dpf, we observed subtle changes in this OS organization in mzbbs1<sup>-/-</sup> 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 organization 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<sup>-/-</sup> K390RM390R mouse retina<sup>48</sup>. 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-Opsin (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<sup>-/-</sup> 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 mutant<sup>41</sup>. The structured organization of the PR layer with distinct short and long cone OSs was lost in zgbbs1<sup>-/-</sup> (Suppl. Fig. 5B and B'). ERG data of adult fish confirmed a milder phenotype in zgbbs1<sup>-/-</sup> mutants (Suppl. Fig. 6): at 3 months, we measured only a slight reduction in the ERG response in zgbbs1<sup>-/-</sup>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 cells<sup>48</sup> and play a key role in transducing and regulating developmental signalling pathways such as hedgehog or Wnt signalling<sup>49</sup>. Moreover, it has been proposed that some BBS proteins can regulate the mRNA expression levels of other BBSome components by interaction with RNF2<sup>49,50</sup>. 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<sup>-/-</sup> larvae, where only a functional but no morphological phenotype was present, and of 10 dpf (±1h) mzbbs1<sup>-/-</sup> larvae, were both function and morphological abnormalities 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, fox12 and foxq1 (Suppl. Table 1). The variance between mutant and control samples was substantially smaller than between the biological replicates (Fig. 4b), indicating
Figure 2 | Functional abnormalities in mzbbs1-/- mutants at 5 dpf despite normal eye development and retinal morphology: (a) Representative images of semi-thin plastic sections stained with Richardson solution in wildtype (top) and maternal zygotic mutants (bottom). Note comparable lamination and layer thickness for OS, ONL, OPL and INL. (b-e) Immunostaining on cryosections with zpr1 marking red/green cones (b), DIO labelling membranes (c), 4D2 recognizing opsins (d) and SV2 overlay with DIO to identify the OPL (e), indicates normal differentiation of retinal cellular subtypes and unaffected organization of the retinal layers in mutants. Staining with 4D2 (d) does not show opsin mislocalization (Insert: magnification of central area). (f, g) Transmission electron microscopy shows the normal retinal organization and photoreceptor ultrastructure with neat stacking of membrane discs in mzbbs1-/- mutants (right image), similar to their sibling controls (left image). (h) Bar plots of the maximum b-wave amplitude by electroretinography (ERG) shows a significantly decreased response to light in mzbbs1-/- mutants for all light intensities (log0 to log-4). Unpaired two-tailed multiple T-test; Sig: ***= FDR(q-value)<0.001; ****= FDR(q-value)<0.0001; Sample size (n=10 WT, n=28 Mut larvae); Error bars show standard deviation. For more detailed statistics, please see suppl. Table 5. (i) Average ERG response curve at log-2 intensity for mutant and wildtype. Scale Bars: (a) 10µm, (b-e) 50µm (insert: 10µm), (f-g) 5µm. Abbreviations: RPE retinal pigment epithelium, OS outer segment, ONL outer nuclear layer, OPL outer plexiform layer, INL inner nuclear layer, M mitochondria.
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-Opsin (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 > ±2 & padj. ≤ 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 necrosis 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 assembly restricting the entry of the complex into the cilium in the absence of BBS1. However, a recent publication suggested that photoreceptor cilia, in contrast to primary cilia, grant entry to a partially assembled BBSome. 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., we observed accumulation of non-OS proteins including Spx3, Sxbp1, chaperonin containing TCP1 Subunits (Tcp/Cct) and proteasome subunits (Psma/b/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, Opn1w1 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 Nxn1. 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, Apoa1b), sterol-binding proteins (Erlin1/2, Scp2b, Npc2/zgc:193725), fatty acid-binding proteins (Fabp1a, Fabp7a, Fabp11b) and phosphatidylinositol-associated enzymes (Pitpnaa, Lpp5ka, 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 Apoe:lynGFP transgenic line (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 >±2.0 and p-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
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. Benjamin-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 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).
controlled cholesterol composition of OS membranes is known to impact photoreceptor function, and given the early functional deficit of mzbbs1−/− 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 mzbbs1−/− (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 BBosome 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 BBosome in retinal photoreceptors. Based on bbs1 mRNA decay and depletion of all BBosome subunits from mutant outer segments (OSs) on proteomics, we consider this to be a model for BBosome dysfunction, which recapitulates the retinal dysfunction and dystrophy described in human patients57, mice33,40 and zebrafish41 models. Our findings identify a novel role for BBS1/BBosome 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 mzbbs1−/− 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 with controls.
levels show converse enrichment, being high at the tip
and low at the base, thus creating a polyunsaturated fatty
acid (PUFA) environment that is unfavourable for cho- 
esterol integration into the membrane. Therefore, PUFA
and phospholipid compositions are thought to determine
the cholesterol distribution along the OS. The local
cholesterol content in turn has profound conse-
quences on the activity and stability of rhodopsin
and high cholesterol levels are known to reduce rhodopsin
activation and to promote PR degeneration. Likewise, altered
phosphoinositide 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 membranes. DHA-
containing phospholipids (PL-DHA) are thought to
preserve membrane disc shape, thereby maintaining
visual function. 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 BBS1, while over 90% of patients develop retinal dystrophy. 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
and cholesterol from the photoreceptor. 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 mice, 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 mutant. 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 blindness. 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 DHA. 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 Datta. 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
transport of enzymes involved in lipid 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 DHA. 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.
targeted protein trafficking\textsuperscript{16}. DRM have been described in PR OSs\textsuperscript{48} and the importance of lipid rafts in cilia-dependent signalling was shown in a recent publication about adipogenesis\textsuperscript{70}. The BBSome was initially introduced as a coat system-like complex that is capable to form planar coats\textsuperscript{15}. Moreover, selective PIP binding is described for BBS5 but also for a subcomplex composed of BBS4, 8, 9 and 18\textsuperscript{16}, 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\textsuperscript{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\textsuperscript{41} zebrafish mutant similarly showed normal retinal lamination, normal PR cilia architecture and tightly stacked disc membranes, although shortened OSs were described from the beginning\textsuperscript{41}. 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)\textsuperscript{41}. In contrast to mice, zebrafish possess a cone-dominated retina\textsuperscript{72}, 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.

\section*{Material & Methods}

\subsection*{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).

\subsection*{Animal Husbandry:}
Zebrafish (Danio rerio) were maintained as described by Aleström et al.\textsuperscript{72}. Embryos were raised at 28°C in embryo medium and staged according to development in days post fertilization (dpf)\textsuperscript{15}.

\subsection*{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 described\textsuperscript{14}. The CRISPR efficiency was assessed as previously described\textsuperscript{75}. 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.

\subsection*{Synteny and phylogenetic analysis:}
Synteny analysis of the zebrafish locus was done using the synteny database setting Homo Sapiens as outgroup (Variant: Ens70) (http://syntenydb.uoregon.edu/synteny_db/) as previously described\textsuperscript{47}. 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 described \(^76\). 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 μW/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 15 h. 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 ddH\(2\)O 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 protocol as previously described \(^41\). 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 Columbia \(^41\)), mouse-anti-Zpr-1 (1:200, Zebrafish International Resource Center, Eugene), rabbit-anti-UV-opsin (1:300, gift from D. Hyde \(^41\)), mouse-anti-SV2 (1:100, Developmental Studies Hybridoma Bank), rabbit anti-Aril3b (1:100, gift from Z. Sun, Yale University \(^41\)), mouse-anti-acetylated-Tubulin (1:500, clone 61B-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 (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 Stellari 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% OsO\(4\) in 0.1M sodium cacodylate buffer at room temperature for 1 h, and 1% aqueous uranyl acetate at 4°C for 2 h. Samples were then dehydrated in an ethanol series, finally treated with propyleneoxide 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 5-month-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 120 hpf or 240 hpf. 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.
Library preparation was performed 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 aligner. The output BAM files of the second sequencing run were merged with the first file and sorted using SAMtools pipeline. The ensemble reference sequence (Refsseq) 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 DEseq2 [83]. 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 techniques [87]. 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=15 Ctrl) 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 limma [88]. 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 R [89] 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) [90] 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 described [90] 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 controls [93] 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 methods [84]. 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/ Cholestereryl Ester
Assay Kit (Abcam, Cambridge, MA, USA) following manufacturer 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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