Autophagy promotes primary ciliogenesis by removing OFD1 from centriolar satellites

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The primary cilia is a microtubule-based organelle that functions in sensory and signalling pathways. Defects in ciliogenesis can lead to a group of genetic syndromes known as ciliopathies1–3. However, the regulatory mechanisms of primary ciliogenesis in normal and cancer cells are incompletely understood. Here we demonstrate that autophagic degradation of a ciliopathy protein, OFD1 (oral-facial-digital syndrome 1), at centriolar satellites promotes primary cilium biogenesis. Autophagy is a catabolic pathway in which cytosol, damaged organelles and protein aggregates are engulfed in autophagosomes and delivered to lysosomes for degradation4. We show that the population of OFD1 at the centriolar satellites is rapidly degraded by autophagy upon serum starvation. In autophagy-deficient Atg5 or Atg3 null mouse embryonic fibroblasts, OFD1 accumulates at centriolar satellites, leading to fewer and shorter primary cilia and a defective recruitment of BBS4 (Barde–Biedl syndrome 4) to cilia. These defects are fully rescued by OFD1 partial knockdown that reduces the population of OFD1 at centriolar satellites. More strikingly, OFD1 depletion at centriolar satellites promotes cilia formation in both cycling cells and transformed breast cancer MCF7 cells that normally do not form cilia. This work reveals that removal of OFD1 by autophagy at centriolar satellites represents a general mechanism to promote ciliogenesis in mammalian cells. These findings define a newly recognized role of autophagy in organelle biogenesis.

During autophagy, the membrane anchored LC3 (microtubule-associated protein 1 light chain 3, also known as MAP1LC3B) interacts with cargo and cargo-adaptor proteins, recruiting cargoes to the autophagosome for subsequent degradation upon fusion of the autophagosome with the lysosome2–4. We carried out a tandem-affinity purification using tagged LC3 as bait to search for its interacting proteins (Fig. 1a). In addition to known LC3-interacting proteins (MAP1B, Fyc01, p62 (also known as SQSTM1) and KEAP1)5–7, we identified a set of centriolar satellite proteins, including PCM1, OFD1 and CEP131 (also known as AZI2), that had not previously been shown to associate with LC3. PCM1 was also pulled down by LC3 orthologues, GATE16 (also known as GABARAPL2) and GABARAP (Extended Data Fig. 1a). PCM1, OFD1 and LC3 co-immunoprecipitated with each other, indicating that they are in the same complex (Fig. 1b–d). PCM1 probably enhances the interaction between LC3 and OFD1, as the OFD1–LC3 interaction is compromised in PCM1-depleted cells (Fig. 1e, f).

Depletion of PCM1 by RNA interference had no significant effect on autophagy activity as determined by LC3 lipidation and p62 degradation (Extended Data Fig. 1b). We then examined if any of these centriolar satellite proteins are an autophagic substrate. OFD1 protein levels were reduced by serum starvation and this reduction was compromised in autophagy-deficient Atg5−/− mouse embryonic fibroblasts (MEFs) compared to Atg5+/+ MEFs, whereas PCM1, IFT88 and BBS4 protein levels were not altered by serum starvation or in Atg5−/− MEFs (Fig. 1g). The messenger RNA levels of OFD1 were not significantly changed upon serum starvation in Atg5+/+ and Atg5−/− MEFs (Extended Data Fig. 1c), indicating that OFD1 protein level reduction upon serum starvation is through protein degradation rather than transcriptional downregulation. Blocking autophagic flux by lysosomal inhibitors bafilomycin A1 (Baf) or chloroquine resulted in increased OFD1 accumulation upon serum starvation (Fig. 1h). Taken together, these data indicate that OFD1 is degraded via the autophagy–lysosome pathway upon serum starvation.

OFD1 is the gene underlying the human disease oral-facial-digital syndrome type 1 (OFD1), an X-linked ciliopathy characterized by morphological abnormalities and renal cysts, as well as Joubert syndrome and Simpson–Golabi–Behmel syndrome type 21,12. OFD1 localizes to the distal ends of centrioles and is necessary for distal appendage formation, IFT88 recruitment and primary cilium formation12,19. OFD1 also localizes to centriolar satellites, interacting with proteins associated with human ciliary disease, PCMI, CEP290 and BBS420. However, the function of this OFD1 population remains unclear.

The centriolar satellite localization of OFD1 is determined by PCMI, because OFD1 was lost from satellites when PCMI was depleted (Extended Data Fig. 2a, b). LC3 partially colocalized with PCMI upon serum starvation in a majority of U2OS cells expression Myc-LC3, but rarely in unstimulated cells (Extended Data Fig. 2c, d). LC3 also partially colocalized with endogenous OFD1 when lysosome activity is blocked by Baf or chloroquine treatment (Extended Data Fig. 3a, b). This colocalization was limited to centriolar satellites, as LC3 did not colocalize with the centriole marker γ-tubulin (Extended Data Fig. 3c, d).

OFD1 was present at both centrioles and centriolar satellites in untreated retinal pigment epithelial (RPE) cells. Remarkably, the centriolar satellite pool of OFD1 was much reduced upon serum starvation, whereas the population of OFD1 at centrioles remained unchanged (Fig. 2a). This serum starvation-induced OFD1 degradation from centriolar satellites was blocked in Atg5−/− MEFs treated with the lysosome inhibitor chloroquine (Extended Data Fig. 4a, b). Notably, PCMI protein levels are not controlled by autophagy and the centriolar satellite distribution of PCMI is not altered upon serum starvation (Fig. 1g and Extended Data Fig. 4c–e), indicating that the autophagic degradation is specific to OFD1 at centriolar satellites rather than centriolar satellites as a whole. This notion is further supported by our observation that OFD1 remained at centriolar satellites upon serum starvation in Atg5−/− MEFs but was lost from centriolar satellites in Atg5+/+ MEFs (Fig. 2b).

The loss of OFD1 from centriolar satellites was significantly faster than the loss of OFD1 from centrioles, and the rate of loss of OFD1 in Atg5−/− MEFs was slower than that in Atg5+/+ MEFs (Extended Data

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OFD1 is an autophagic substrate. a, Silver staining of LC3 complexes purified from U2OS cells expressing ZZ–Flag–LC3 in normal medium or Earle’s balanced salt solution (EBSS) for 2 h. Asterisks mark centriolar satellite proteins. b–d, Co-immunoprecipitation (IP) of OFD1 with LC3, LC3 with PCM1, or OFD1 with PCM1 in HEK293T cells. HA, haemagglutinin. e, Western blotting analysis of PCM1 and OFD1 protein levels in control or PCM1 knockdown (KD) HEK293T cells. f, Co-immunoprecipitation of OFD1 with LC3 in control or PCM1 KD HEK293T cells. IP efficiency is calculated as the ratio of immunoprecipitated OFD1/LC3. g, h, Western blotting analysis of protein levels of indicated proteins in MEFs with indicated genotypes in normal medium or subjected to 24 h serum starvation (SS), 50 nM bafilomycin A1 (Baf), 20 μM chloroquine (CQ) or 1 μM MG132. Quantified OFD1 level was normalized with β-tubulin. Similar results were observed in three independent experiments.

Figure 1 | OFD1 is an autophagic substrate. a, Silver staining of LC3 complexes purified from U2OS cells expressing ZZ–Flag–LC3 in normal medium or Earle’s balanced salt solution (EBSS) for 2 h. Asterisks mark centriolar satellite proteins. b–d, Co-immunoprecipitation (IP) of OFD1 with LC3, LC3 with PCM1, or OFD1 with PCM1 in HEK293T cells. HA, haemagglutinin. e, Western blotting analysis of PCM1 and OFD1 protein levels in control or PCM1 knockdown (KD) HEK293T cells. f, Co-immunoprecipitation of OFD1 with LC3 in control or PCM1 KD HEK293T cells. IP efficiency is calculated as the ratio of immunoprecipitated OFD1/LC3. g, h, Western blotting analysis of protein levels of indicated proteins in MEFs with indicated genotypes in normal medium or subjected to 24 h serum starvation (SS), 50 nM bafilomycin A1 (Baf), 20 μM chloroquine (CQ) or 1 μM MG132. Quantified OFD1 level was normalized with β-tubulin. Similar results were observed in three independent experiments.

Figure 2 | Autophagy specifically degrades OFD1 at centriolar satellites upon serum starvation. a, Representative confocal images of endogenous OFD1 puncta with cilia marker acetylated tubulin (Ac-tubulin), or centriole marker γ-tubulin in hTERT-RPE1 cells in normal medium (Un) or subjected to 24 h serum starvation (SS). b, Representative confocal images of endogenous OFD1 and acetylated tubulin in Atg5+/+ and Atg5−/− MEFs subjected to 24 h serum starvation. a, b, Data shown represent mean ± s.d. percentage of cells with centriolar satellite OFD1 for 100 cells per well in triplicate samples. ***P < 0.001, two-tailed unpaired student’s t-test. Similar results were observed in three independent experiments.

Fig. 5a). The centriolar satellite pool of OFD1 in Atg5+/+ cells was lost within 6 h of serum starvation, whereas this pool of OFD1 in Atg5−/− cells remained stable even after 24 h of serum starvation (Extended Data Fig. 5b). These data confirm that OFD1 at centrioles has a faster turnover rate than OFD1 at centrioles, and this serum-stimulation-induced accelerated degradation is controlled by autophagy.

We next sought to understand how OFD1 regulation might affect ciliogenesis. We observed that the percentage of Atg5−/− cells that form a primary cilia, as compared to Atg5+/+ cells, was significantly reduced and the cilia that did form were shorter (Fig. 3a). This difference was not due to cell cycle regulation (Extended Data Fig. 6a). Lysosome inhibition also compromised primary ciliogenesis in Atg5+/+ MEFs (Extended Data Fig. 6b–d). The defective ciliogenesis phenotypes were not limited to Atg5−/− MEFs, as similar phenotypes were also observed in MEFs lacking another essential autophagy gene, Atg3 (Extended Data Fig. 6e–g).

We next investigated if the ciliary recruitment of BBS4, a critical event for ciliogenesis23, is affected in autophagy-deficient cells. We observed that BBS4 accumulated at centriolar satellites in Atg5−/− MEFs; more than 50% of cilia had detectable BBS4 in Atg5−/− MEFs, whereas only about 10% of cilia were positive for BBS4 in Atg5+/+ MEFs (Extended Data Fig. 7). These data indicate that BBS4 recruitment to cilia is also defective in autophagy-deficient cells.

If OFD1 accumulation at centriolar satellites is responsible for the ciliary defects in Atg5−/− MEFs, we would expect that depletion of OFD1 by RNA interference might rescue these defects. In Atg5−/− MEFs stably expressing OFD1 short hairpin RNA (shRNA), with about 50% efficiency of overall depletion of OFD1 (Extended Data Fig. 8a), OFD1 remained at centrioles but was lost from centriolar satellites (Extended Data Fig. 8b–d). Primary cilia formation upon serum starvation was fully restored in these cells; nearly 70% of cells formed a cilia and the length of these cilia was comparable to those formed in Atg5+/+ MEFs (Fig. 3b). Hence, the centriolar satellite pool of OFD1 indeed plays a key role in suppressing primary ciliogenesis in Atg5−/− MEFs. Notably, with OFD1 knockdown, nearly 45% of the Atg5−/− cells formed cilia even without serum starvation (Fig. 3b), indicating that OFD1 degradation is likely to be required for serum-starvation-induced primary ciliogenesis. This notion is supported by
Figure 3 | Autophagy promotes primary cilia biogenesis by regulating OFD1 levels. a, b, Representative confocal images of ciliary marker acetylated tubulin from MEFs with indicated genotype subjected to 24 h serum starvation. Data shown represent mean ± s.d. percentage of cells with primary cilia or length of the cilia for 500 cells or 100 cells per well, respectively, in triplicate samples. ***P < 0.001, two-tailed unpaired student’s t-test. Similar results were observed in three independent experiments. Un, untreated; SS, serum-starved.

Figure 4 | Forced OFD1 reduction promotes ciliogenesis in human breast cancer MC7 cells. a, Western blotting analysis of OFD1 protein levels in control or OFD1 knockdown MC7 (C-19) cells, quantified OFD1 level was normalized with β-tubulin. b, Quantification of percentage of cells with primary cilia in MC7 or C19 cells subjected to 72 h serum starvation. Data shown represent mean ± s.d. percentage of cells with primary cilia for 500 cells per well in triplicate samples. ***P < 0.001, two-tailed unpaired student’s t-test. c, Quantification of length of primary cilia in C19 cells subject to 48 h or 72 h serum starvation. d, e, Representative confocal images of primary cilia with variable length formed in MC7 or C19 cells subject to 72 h serum starvation. Data shown represent 100 cells per well in triplicate samples. f, Scanning electron microscope analysis of primary cilia (marked by arrows) formed in C-19 cells subject to 72 h serum starvation and cycling Atg5+/+ MEFs in normal medium for 24 h. Similar results were observed in three independent experiments.
cilogenesis remains inefficient in C19 cells, indicating that addition factors are required to reach full capacity. Nevertheless, these data confirm that OFD1 at centriolar satellites functions as a crucial suppressor of primary cilogenesis in human cancer cells.

We show here that autophagy is linked to primary cilogenesis, by controlling the degradation of OFD1 at centriolar satellites. We propose that autophagy deficiency serves as a potential underlying mechanism of ciliopathies, and that autophagy modulation might provide a novel means of ciliopathy treatment. We demonstrate that OFD1 at centriolar satellites has a crucial role in suppressing primary cilogenesis. Removing OFD1 from centriolar satellites promotes ciliogenesis in autophagy-deficient cells, wild-type MEFs without serum starvation, and human breast cancer MCF7 cells that normally completely lack cilia, suggesting a general role of OFD1 in suppressing ciliogenesis. Primary cilogenesis is defective in human breast and pancreatic cancer cells, but activated in the corresponding normal tissues/cells3,23,25. The contribution of primary cilium function to tumorigenesis is complex26,27; however, our results suggest that dissecting the regulatory mechanisms of OFD1 will provide insight into these functions and potentially offer new therapeutic tools for treatment of ciliopathies and cancers.

METHODS SUMMARY

Primary cilia biogenesis assay. Cells were incubated in either fresh normal medium (10% FBS, 1% P/S DMEM) or serum-starved medium (0.5% FBS, 1% P/S DMEM) for 24 h. Cells were seeded at different numbers to ensure that they reached the same confluency 1 day after treatment.

Tandem affinity purification of LC3 complexes. This protocol was adapted from previously described methods28,29,30. Cell lysates from inducible cell lines expressing ZZ-Flag–LC3 treated with 10 ng ml

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METHODS

Reagents. Rabbit anti-OFD1 antibody, EGFP–OFD1 and Flag–OFD1 were described before12,20,31. Another rabbit anti-OFD1 antibody was a gift from J. F. Reiter. Rabbit anti-IFT88 antibody was a gift from B. Yoder. To generate BBS4–MycHAs (pTS1686), BBS4 was PCR-amplified, verified by sequencing and cloned into a pEGFP-N1-derived plasmid (Clontech Laboratories) in which GFP was replaced with a Myc–HA–His tag created as BBS4–MycHAs32. Primary antibodies used for western blotting and immunostaining are rabbit anti-PC1m (Cell signaling), mouse anti-PC1m (Sigma), mouse anti-β-tubulin (Sigma), rabbit anti-p62 (MLBL), rabbit anti-LS3 (C Sigma), mouse anti-acetylated tubulin (Sigma), mouse or rabbit anti-y tubulin (Sigma), Rabbit anti-BBS4 (Santa Cruz), chloroquine (Sigma), bafilomycin A1 and rapamycin (LC Laboratories), and MG132 (Alexis). Cell cycle and transfaction. HEK293T, MCF7 and MEFS were cultured in DMEM with 10% FBS and 1% penicillin and streptomycin in a 5% CO₂ incubator (Thermo). RPE1 cells were cultured in DMEM/Hams F12 with 10% FBS and 1% penicillin and streptomycin in a 5% CO₂ incubator (Thermo). Lipofectamine 2000 (Invitrogen) was used for mammalian cell transfection. Cells were incubated in OPTI medium (GIBCO 51985091) for 1 h before transfection. 10 μg or 5 μg plasmid was used for each 150-mm or 100-mm tissue culture dish, respectively. U2OS cells stably expressing Myc–LS3 were described before12. Atg5+/− and Atg6−/− MEFS were gifts from N. Mizushima33. Atg3−/− and Atg5−/− MEFS were gifts from M. Komatsu34. PC1m shRNA knockdown was carried out using a 19-mer shRNA targeting human PC1m with the sequence: 5’-GTATCACATCTGAACATGAAAA-3’ (pTS2063). PC1m shRNA oligonucleotides were designed using pSiCoOligomaker 1.5, annealed and subcloned into the lentiviral vector, pSiCoR-puro, which confers puromycin resistance. Stable depletion of PC1m was carried out by infection of cells with shRNA-expressing lentiviral particles and selection with puromycin (3 μg/ml); positive clones were screened by western blotting. Stable depletion of OFD1 was carried out by infection of cells with shRNA-expressing lentiviral particles (sc-91245-V, from Santa Cruz) and selection with puromycin (1 μg/ml or 10 μg/ml) for MCF7 cells or MEFS, positive clones were screened by western blotting. The OFD1 lentiviral particles are a pool of 3 different shRNA plasmids, targeting the OFD1 sequence: 5’-GAUGACUACAUCAUGAAGA-3’, 5’-CUACUCAGGUGGCCGAUUU-3’, 5’-GAACGAAGAGAACUAGAAA-3’.

Primary cilia biogenesis assay. Equal numbers (3 × 104) for serum rich medium and 4.5 × 104 for serum-starved medium) of Atg5+/− MEFS and Atg6−/− MEFS were seeded into 6-well dishes. After cells were attached on the coverslips for 8 h, they were incubated in either fresh normal medium (10% FBS, 1% P/S DMEM) or serum-starved medium (0.5% FBS, 1% P/S DMEM) for 24 h. Cells were seeded at different numbers at beginning to ensure that these cells reach the same confluence 1 day after treatment. Cells were then fixed for immunofluorescence staining. For western blotting, cells cultured in 10-cm dishes were serum-starved alone or in combination with 50 nM bafilomycin A1, 20 μM chloroquine or 1 μM MG132 treatment for 24 h and lysed with TAP buffer. Immunofluorescence staining. Cells were treated according to the protocol described above with or without serum starvation or compound treatment. Cells were fixed with cold methanol for five minutes at −20 °C. Cells were then washed three times with PBS and then blocked with blocking buffer (2.5% BSA + 0.1% Triton X-100 in PBS) at room temperature for 1 h. Cells were incubated with primary antibodies at 4 °C overnight, washed five times with PBS buffer and then incubated with appropriate secondary antibodies conjugated to Alexa 488-Alexa 549 (Molecular Probes) for 2 h at room temperature. DNA was stained with DAPI. Slides were examined using a laser scanning confocal microscope (Zeiss LSM 510 META UV/Vis).

Autophagy analysis. Autophagy was induced by serum starvation or rapamycin treatment, or blocked by lysosome inhibitors, chloroquine or bafilomycin A1. For rapamycin (LC Laboratories) treatment, cells were incubated with 500 nM rapamycin in complete medium for 16 h at 37 °C. To block autophagy flux, 50 nM bafilomycin A1 (LC Laboratories) or 20 μM chloroquine (Sigma) was added to normal complete medium or serum-starved medium and incubated for 24 h at 37 °C. Autophagy activity was assessed using two approaches, LC3-II formation and p62 degradation. For LC3-II and p62 detection, cell lysates were prepared as described above and subjected to standard western blotting protocols using 1:10,000 dilution of the antibody against LC3 (Sigma) and 1:1,000 dilution of the antibody against p62 (MLBL).

Establishment of stable RNA interference cell lines. Human MCF7 cells or Atg5+/− and Atg5−/− MEFS were seeded into 12-well dishes at 50% confluency before transfection. 24 h after seeding, cells were incubated with normal complete medium containing 5 μg/ml polybrene (Santa Cruz), and infected overnight with lentiviral particles carrying short hairpin RNA OFD1 (Santa Cruz). 24 h later, cells were selected with puromycin (Invitrogen) (1 μg/ml for MCF7 or 10 μg/ml for MEFS) until positive clones were identified. Single clones were picked and identified by western blotting or immunofluorescence analysis.

Cell cycle analysis. 103 cells were centrifuged at 485 g for 5 min, washed once with PBS, and pellets were resuspended in 1 ml PBS and fixed overnight in 75% ethanol at −20 °C. Fixed cells were washed three times with 10 ml PBS, resuspended in 200–400 μl PBS with 10 μl RNase (Qiagen) at 37 °C for 30 min in the dark. Cells were subjected to FACS analysis using DAKO-Cytomation MoFlo High Speed Sorter at the Flow Cytometry Facility at UC Berkeley.

Immunoprecipitation and immunoblot. Transfected cells were lysed in TAP lysis buffer on ice for 30 min, and centrifuged at 13,000 g for 15 min. For immunoprecipitation, cell lysates were incubated with primary antibody overnight at 4 °C, and then incubated with IgG beads for 2 h at 4 °C. The beads were washed 5 times with 1 ml of lysis buffer and eluted with elution buffer containing 200 μg/ml 3× Flag peptide (Sigma) or resolved with SDS sample buffer. Co-immunoprecipitated proteins were resolved by SDS–PAGE, transferred to PVDF membranes (Bio-Rad), and detected with antigen-specific primary antibodies followed by horse-radish peroxidase-conjugated secondary antibodies.

Tandem affinity purification of LC3 complexes. This protocol is adapted from what was described before12,28,29. Stable cell lines expressing ZZ–Flag–LC3, ZZ–Flag–GATE16, or ZZ–Flag–GABARAP, upon doxycycline induction were treated with 10 ng/ml doxycycline for one day. The collected cell pellet was washed with chilled PBS three times and suspended in TAP lysis buffer. Resuspended cell pellets were incubated on ice for 30 min and then gently vortexed for one minute. Homogenates were centrifuged for 20 min at 10,000 g. The supernatants were transferred to fresh tubes containing 0.8 ml of packed IgG beads followed by gentle rotation overnight at 4 °C. Bound protein was eluted using TEV protease cleavage and further purified by anti-Flag M2 Affinity Gel (Sigma). Bound protein were eluted with elution buffer containing 200 μg/ml 3× Flag peptide (Sigma) and resolved by SDS–PAGE on a 4–12% gradient gel (Invitrogen), then visualized by silver staining (Invitrogen). Distinct bands were cut and subjected to mass spectrometry analysis.

Quantitative RT–PCR. Total RNAs were extracted with TRizol reagent (Invitrogen), from which 1 μg was used for reverse transcription in a 20-μl reaction system with the RNA PCR (AMV) kit (Promega). Quantitative RT–PCR was performed with SYBR-Green PCR Mastermix (Applied Biosystems), and amplification was real-time monitored on a MicorAmp optical 96-Well Reaction Plate (Applied Biosystems). The level of transcript abundance relative to reference gene (termed fold change) was determined according to the function ΔΔCt = (Ct test − Ct reference) − (ΔCt control) where control represented mock-treated cells. The induction ratio of treatment/control was then calculated by the equation 2−ΔΔCt. Gene expression levels were normalized with the Atg5+/− untreated sample.

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Extended Data Figure 1 | LC3-interacting protein PCM1 is not required for autophagy. **a**, PCM1 associates with LC3, GATE16 and GABARAP. Silver staining of LC3, GATE16 or GABARAP complexes purified from U2OS cells that stably express ZZ–Flag–LC3, ZZ–Flag–GATE16, or ZZ–Flag–GABARAP in normal medium or subjected to 2 h Earle’s balanced salt solution (EBSS) starvation. Both PCM1 and p62 were identified by mass spectrometry analysis. **b**, PCM1 is not required for autophagy. Western blotting analysis of p62, LC3-I/II, PCM1 levels in control or PCM1 shRNA knockdown U2OS cells in normal medium or subjected to rapamycin treatment; quantified LC3-II level was normalized with β-tubulin. **c**, OFD1 messenger RNA levels remain unchanged upon serum starvation. Quantitative analysis of messenger RNA levels of OFD1 in Atg5+/− and Atg5−/− MEFs in normal medium or subjected to 24 h serum starvation. OFD1 mRNA levels were detected by quantitative RT–PCR and plotted after normalization. Similar results were obtained in three independent experiments.
Extended Data Figure 2 | PCM1 is required for OFD1 centriolar satellite localization. a, Representative confocal images of OFD1 and PCM1 localization from control or PCM1 knockdown U2OS cells in normal medium. Data shown represent 100 cells per well in triplicate samples. b, Quantified percentage of cells with PCM1 positive centriolar satellite OFD1 in a. Data shown represent 100 cells per well in triplicate samples. ***P < 0.001, two-tailed unpaired student’s t-test. c, LC3 partially colocalizes with PCM1 upon serum starvation. Representative confocal images of Myc–LC3 and PCM1 colocalization in U2OS cells expressing Myc–LC3 in normal medium or subjected to 24 h serum starvation. Arrows denote colocalized LC3 (green) and PCM1 (red) puncta. Data shown represent 100 cells per well in triplicate samples. d, Quantified percentage of cells with colocalization of Myc–LC3 and PCM1 in c. ***P < 0.001, two-tailed unpaired student’s t-test. Similar results were observed in three independent experiments.
Extended Data Figure 3 | LC3 partially colocalizes with OFD1 but not with \(\gamma\)-tubulin.  

**a**, LC3 colocalizes with OFD1 when the lysosome activity is blocked. Representative confocal images of Myc–LC3 and OFD1 colocalization in U2OS cells that stably express Myc–LC3 in normal medium or subjected to 2 h 50 nM bafilomycin A1 (Baf) or 100 \(\mu\)M CQ. Data shown represent 100 cells per well in triplicate samples. 

**b**, Quantified percentage of cells with colocalization of Myc–LC3 and OFD1 in **a**. 

**c**, LC3 does not colocalize with centrioles. Representative confocal images of LC3 and \(\gamma\)-tubulin colocalization in U2OS cells in normal medium or subjected to 24 h serum starvation. Data shown represent 100 cells per well in triplicate samples. 

**d**, Quantified percentage of cells with colocalization of LC3 and \(\gamma\)-tubulin in **c**. Similar results were obtained in three independent experiments.
Extended Data Figure 4 | OFD1 but not PCM1 at centriolar satellites was degraded by autophagy. **a**, OFD1 accumulates at centriolar satellites in CQ-treated cells. Representative confocal images of EGFP–OFD1 and PCM1 colocalization in Atg5−/− cells expressing EGFP–OFD1 subjected to 24 h serum starvation or 20 μM CQ. **b**, Quantified percentage of cells with centriolar satellite OFD1 in a. Data shown represent mean ± s.d. for 100 cells per well in triplicate samples. ***P < 0.001, two-tailed unpaired student’s t-test. **c**, PCM1 is not degraded upon serum starvation. Representative confocal images of PCM1 centriolar satellite staining in Atg5−/− cells in normal medium or subjected to 24 h serum starvation. Data shown represent mean ± s.d. for 200 cells per well in triplicate samples. **d**, Quantified percentage of cells with PCM1 centriolar satellite staining in c. e, OFD1 but not PCM1 is degraded from centriolar satellites upon serum starvation. Representative confocal images of PCM1 and OFD1 colocalization in Atg5−/− cells in normal medium or subjected to 24 h serum starvation. Data shown represent 200 cells per well in triplicated samples. Enlarged images were shown in the left bottom panels. Similar results were obtained in three independent experiments.
Extended Data Figure 5 | The turnover rate of centriolar satellite OFD1 is faster than OFD1 at centrioles. 

(a) Centriolar satellite OFD1 has a shorter half-life compared to OFD1 at centrioles. Quantified percentage of cells with OFD1 at centrioles or at centriolar satellites from Atg5+/+ and Atg5−/− MEFs in normal medium or subjected to 75 μM cycloheximide (CHX) with indicated time points. Data shown represent 200 cells per well in triplicate samples.

(b) Centriolar satellite OFD1 but not centriole OFD1 degrades upon serum starvation. Quantified percentage of cells with OFD1 at centrioles or at centriolar satellites from Atg5+/+ and Atg5−/− MEFs in normal medium or subjected to serum starvation with indicated time points. Data shown represent 200 cells per well in triplicate samples. Similar results were obtained in three independent experiments.
Extended Data Figure 6 | Autophagy regulates primary ciliogenesis in a cell cycle independent manner. a, FACS analysis of Atg5+/- and Atg5−/− MEFs in normal medium or subjected to 24 h serum starvation. Data shown represent 10^6 cells per well in triplicate samples. b, Primary ciliogenesis is less efficient when the lysosome activity is blocked in MEFs. Representative confocal images of primary cilia formed in Atg5+/- MEFs subjected to 24 h serum starvation alone or combined with 20 μM CQ treatment. c, Quantified percentage of cells with primary cilia in b. d, Quantified length of primary cilia in b. e, Degradation of OFD1 is also blocked in Atg3+/- MEFs. Western blot analysis of OFD1, p62, LC3-I/II and BBS4 protein levels in MEFs with indicated genotypes in normal medium or subjected to 24 h serum starvation; quantified OFD1 levels were normalized with β-tubulin. f, g, Primary ciliogenesis is also defective in Atg3+/- MEFs. Quantified percentage of cells with primary cilia in Atg3+/- and Atg3−/− MEFs in normal medium or subjected to 24 h serum starvation. g, Quantified length of primary cilia formed in Atg3+/- and Atg3−/− MEFs as described in f. c, d, f, g. Data shown represent mean ± s.d. for 100 cells per well in triplicate samples. ***P < 0.001, two-tailed unpaired student’s t-test. Similar results were obtained in three independent experiments.
Extended Data Figure 7 | BBS4 recruitment to primary cilia is defective in Atg5⁻/⁻ MEFs. a, Representative confocal images of Atg5⁺/+ and Atg5⁻/⁻ MEFs expressing Myc–BBS4 subjected to 24 h serum starvation. Scale bar 5 μm. b, Quantified percentage of cells with Myc–BBS4 translocation into primary cilia in Atg5⁺/+ and Atg5⁻/⁻ MEFs. Data shown represent mean ± s.d. for 100 cells per well in triplicate samples. ***P < 0.001, two-tailed unpaired student’s t-test. Similar results were obtained in three independent experiments.
Extended Data Figure 8 | Partial shRNA knockdown of OFD1 leads to depletion of OFD1 from centriolar satellites in Atg5+/+ and Atg5−/− MEFs.

a, Western blot analysis of OFD1 in MEFs with indicated genotypes in normal medium. Quantified OFD1 levels were normalized with β-tubulin. KD, knockdown. b, Quantified percentage of cells with centriolar satellite OFD1 in MEFs with indicated genotypes in normal medium. Data shown represent mean ± s.d. percentage of cells with centriolar satellite OFD1 for 100 cells per well in triplicate samples. ***P < 0.001, two-tailed unpaired student’s t-test.  
c, d, OFD1 was depleted from centriolar satellites but not centrioles in OFD1 knockdown MEFs. Representative confocal images of OFD1 and axoneme marker acetylated tubulin in c or centriole marker γ-tubulin in d in MEFs with indicated genotypes in normal medium. Data shown represent 100 cells per well in triplicate samples. Similar results were obtained in three independent experiments.
Extended Data Figure 9 | Knockdown of OFD1 in wild-type MEFs promotes primary ciliogenesis. a, Representative confocal images of primary cilia formed in MEFs with indicated genotypes in normal medium (Un) or subjected to 24 h serum starvation (SS). Quantified percentage of cells with primary cilia and the length of primary cilia from MEFs with indicated genotypes were shown in the bottom panels. Data shown represent mean ± s.d. for 100 cells per well in triplicate samples. b, Representative confocal images of primary cilia formed in MEFs with indicated genotypes subjected to 24 h serum starvation. The primary cilia formed are positive for both axoneme marker acetylated tubulin and ciliary membrane marker ARL13B. Data shown represent 100 cells per well in triplicate samples. Similar results were obtained in three independent experiments.
Extended Data Figure 10 | Partial knockdown OFD1 in MCF7 cells depletes OFD1 from centriolar satellites and promotes primary ciliogenesis.

a, OFD1 was depleted from centriolar satellites in OFD1 shRNA knockdown MCF7 cells. **a**, Representative confocal images of relative localization of OFD1 with axoneme marker acetylated tubulin in MCF7 OFD1 knockdown clone (C19). Data shown represent 100 cells per well in triplicate samples. **b**, Representative confocal images of OFD1 and centriole marker γ-tubulin from C19. Data shown represent 100 cells per well in triplicate samples. **c**, Quantified percentage of parental MCF7 and C19 cells with centriolar satellite OFD1. Data shown represent mean ± s.d. for 100 cells per well in triplicate samples. ***P < 0.001, two-tailed unpaired student’s t-test. d–f, Primary cilia formed in OFD1 knockdown C19 MCF7 cells are positive for ciliary markers. Representative confocal images of primary cilia formed in C19 subjected to 72 h serum starvation. Cilia were positive for ciliary membrane marker ARL13B, axoneme marker acetylated tubulin and intraflagellar transport protein IFT88. Similar results were obtained in three independent experiments.