Functional interaction between autophagy and ciliogenesis

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Nutrient deprivation is a stimulus shared by both autophagy and the formation of primary cilia. The recently discovered role of primary cilia in nutrient sensing and signalling motivated us to explore the possible functional interactions between this signalling hub and autophagy. Here we show that part of the molecular machinery involved in ciliogenesis also participates in the early steps of the autophagic process. Signalling from the cilia, such as that from the Hedgehog pathway, induces autophagy by acting directly on essential autophagy–related proteins strategically located in the base of the cilium by ciliary trafficking proteins. Whereas abrogation of ciliogenesis partially inhibits autophagy, blockage of autophagy enhances primary cilia growth and cilia–associated signalling during normal nutritional conditions. We propose that basal autophagy regulates ciliary growth through the degradation of proteins required for intraflagellar transport. Compromised ability to activate the autophagic response may underlie some common ciliopathies.

Autophagy is a cellular catabolic process that contributes to quality control and maintenance of the cellular energetic balance through the turnover of proteins and organelles in lysosomes. Induction of autophagy recruits proteins and lipids from different intracellular membranes to initiate the formation of autophagosomes, double-membrane vesicles that sequester cytoplasmic material and deliver it through vesicular fusion to lysosomes for degradation.

The primary cilium is a non-motile signalling organelle that grows in a specific region of the plasma membrane and senses, among other things, changes in the enrichment of nutrients in the environment. Cargo trafficking along the ciliary axoneme (intraflagellar transport; IFT) is maintained through motor proteins (kinesins and dyneins) and two large multiprotein complexes (IFT particles A and B). Some subunits of these complexes can be found in other cellular compartments such as Golgi, from where they facilitate mobilization of specific cargo to the basal body and cilium for ciliogenesis and ciliary signalling. The primary cilium coordinates a variety of signalling pathways including the Hedgehog (Hh) pathway, which requires IFT-mediated recruitment to the basal body and axoneme of smoothened (SMO) and the transcription factors GLI1 and GLI2.

In many types of cultured cells, deprivation of serum from the media induces almost linear growth of primary cilia for up to 2 days. Induction of autophagy also occurs in the first hours that follow serum removal, and can be sustained for the full duration of the starvation period. Despite the temporal coincidence of the formation of autophagosomes and primary cilia, any possible relation between the autophagic and ciliogenesis machineries remains unknown. Likewise, the possible involvement of autophagy in ciliary dynamics has not been analysed.

In this study we investigate the functional interaction between primary cilia and autophagic induction. We have found that maximum activation of autophagy in response to nutrient deprivation requires the strategic location of components of the autophagic machinery at the ciliary base in an IFT and Hh signalling-dependent manner. In contrast, when autophagy is compromised, ciliogenesis is enhanced and cilia grow longer. We conclude that ciliary signalling pathways, such as Hh, may specify a cilia-mediated autophagy, closely related to autophagosome assembly at the plasma membrane and that, in turn, basal autophagy negatively modulates ciliary growth through turnover of essential ciliogenesis proteins (Extended Data Fig. 1).

Disruption of IFT compromises autophagy

To determine the role of primary cilia on autophagy, we used two cellular models with compromised ciliogenesis: mouse embryonic fibroblasts (MEFs) stably knocked down for IFT20 (IFT20−/−) and kidney epithelial cells (KECs) from mice with a hypomorphic mutant IFT88 (IFT88−/−), two IFT-B complex components required for ciliogenesis (Extended Data Fig. 2a). After 24 h of serum removal, cells with detectable primary cilia were reduced by 60% in IFT20−/− MEFs and almost absent in the IFT88−/− KECs (Fig. 1a).

Rates of lysosomal protein degradation after serum deprivation, a stimulus that activates autophagy, were reduced in the two ciliogenesis-impaired models (Extended Data Fig. 2b). To test whether autophagy was defective we analysed flux through the autophagic pathway using lysosomal proteolysis inhibitors and measured changes in levels of LC3-II, an autophagosome component degraded with the cargo in lysosomes. The characteristic upregulation of the autophagic flux after serum removal was reduced in IFT20−/− MEFs whereas basal autophagic flux (rich media) was only discretely reduced (Fig. 1b). Although a high percentage of KECs are ciliated under basal conditions and serum removal has small effect on ciliogenesis (Extended Data Fig. 2c), we still observed a reduction in autophagic flux upon serum removal in IFT88−/− KECs when compared to wild type (Fig. 1b), indicating that primary cilia are not necessary to maintain the high basal autophagic flux of these cells (Extended Data Fig. 2d), but are essential to sustain proper autophagic flux during nutrient deprivation.

To directly analyse autophagosome formation and their clearance by lysosomes, we transfected cells with a pH-sensitive reporter (mCherry–GFP–LC3; fusion of mCherry, green fluorescent protein and LC3) that highlights autophagosomes as yellow puncta and

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autophagolysosomes (post-lysosomal fusion) as red puncta. Basal levels of autophagic vacuoles were comparable in wild-type and Ift88−/− KECs, but upon removal of serum Ift88−/− KECs showed a significantly lower content of autophagolysosomes (Fig. 1c and Extended Data Fig. 2e). This reduction in autophagolysosomes was not paralleled by an increase in autophagosomes, and the overall content of LC3-positive vesicles in serum-deprived Ift88−/− KECs was lower than in wild-type cells (Extended Data Fig. 2f), indicating that reduced autophagic flux was mainly owing to lower autophagosome synthesis rather than blockage in their degradation. Autophagosome formation (assessed as the increase in LC3-II levels at two time points after inhibition of lysosomal proteolysis) was also reduced in IFT20−/− MEFs (Fig. 1d). Ultrastructural analysis confirmed that despite normal autophagosome morphology, IFT20−/− and Ift88−/− cells failed to expand the autophagic compartment in response to serum deprivation (Fig. 1e).

Contrary to the restricted location of IFT88 to the ciliary base, IFT20 is also present in other cellular compartments. However, the effect of IFT20 on autophagy seems to be primarily related to its function in ciliogenesis because additional knockdown for IFT20 in Ift88 knockdown cells did not result in further reduction of autophagy (Extended Data Fig. 3a–d), supporting that blockage of anterograde IFT (aIFT) compromises activation of inducible autophagy. Consistent with this notion, cilia resorption induced by treatment with platelet-derived growth factor (PDGF) treatment (**P = 0.001, ***P = 0.0009, 121, 230 and 162 cells, n = 5). g. LC3 immunoblot (top) for autophagic flux quantification (bottom) upon PDGF treatment (**P = 0.005, n = 3). Scale bars in a, 10 μm. n.s., statistically non-significant. Mean ± s.d. in b, d and e and mean ± s.e.m. in other panels.

**Ciliary Hh signalling induces autophagy**

Altered aIFT blocks ciliogenesis and prevents the recruitment of essential signalling molecules to the ciliary region. We proposed that malfunctioning of ciliary signalling may be behind the defective starvation-induced autophagy in cells with compromised IFT. Although primary cilia downregulate signalling through the mammalian target of rapamycin (mTOR) pathway, a well-known negative regulator of autophagy, treatment of IFT20−/− and Ift88−/− cells with the mTOR inhibitor
rapamycin failed to restore normal autophagic activity in these cells (data not shown); thereby reduced autophagy in ciliogenesis-defective cells was not due to enhanced mTOR signalling.

We next focused on the Hedgehog (Hh) signalling pathway because of its dependence on the primary cilium and intact IFT<sup>2–4</sup>. Treatment of serum-supplemented MEFs or KECs with purmorphamine, an SMO agonist that activates expression of Hh downstream factors (Extended Data Fig. 4a, c), returned content of LC3-positive compartments and autophagic flux to values observed upon serum removal (Fig. 2a–d and Extended Data Fig. 4d–f). Purmorphamine failed to induce autophagy in IFT20<sup>−</sup> or IFT88<sup>−</sup> MEFs and in Ift88<sup>−/−</sup> KECs, supporting dependence on IFT (Fig. 2b, d and Extended Data Fig. 4f) and in SMO<sup>−/−</sup> cells (Fig. 2c), which as expected showed reduced purmorphamine-mediated upregulation of GLI1 and GLI2 (Extended Data Fig. 4c). Two other conditions that activate Hh signalling, Patched-1 receptor knockout (Ptc<sup>−/−</sup>, also known as Pchtl1; constitutive activation of Hh signalling and ciliogenesis<sup>5</sup>) and overexpression of GLI1 also upregulated autophagy (Fig. 2a and Extended Data Fig. 4d, g–j), and in fact, overexpression of GLI1 is sufficient to partially rescue the autophagic defect in Ift88<sup>−/−</sup> cells (Fig. 2e). Conversely, two interventions that reduce Hh signalling, knockdown of SMO and treatment with Hh antagonist cyclopamine, reduced starvation-induced autophagy (Fig. 2c, f and Extended Data Fig. 4k).

Altogether, these data reveal a positive regulatory effect of Hh signalling on autophagy, and support that the inability to activate autophagy in cells with defective IFT originates, at least in part, from the loss of Hh signalling.

**Autophagic machinery localizes at the cilia**

Besides ciliogenesis, IFT also participates in recruitment of non-ciliary proteins to the plasma membrane<sup>16</sup>, a site of autophagosome formation<sup>17</sup>, which made us propose an involvement of αIFT in the delivery of the autophagic machinery to this membrane.

Co-immunostaining for different autophagy-related proteins (ATGs, green) and acetylated tubulin (red) to highlight the ciliary axoneme in serum-deprived KECs revealed that of the twelve ATGs analysed, five ATGs<sup>2,3</sup> (ATG16L, AMBRA1, LC3, GABARAP and VPS15) localized as discrete puncta along the ciliary axoneme, clearly distinguishable from the surrounding cytosol signal with deconvolution immunofluorescence and three-dimensional (3D) wire modelling (Fig. 3a, Extended Data Fig. 5a, b and Supplementary Videos 1–5). Similar colocalization was observed using fluorescent-tagged versions of the ciliary protein invesin<sup>18</sup> or of ATGs (Extended Data Fig. 5c, d). Other more abundant ATGs, such as ATG14, were not detectable in the axoneme, and knockdown against cilia-associate ATGs abolished their staining (Extended Data Fig. 5b, d) supporting specificity of the staining. These ATGs were also detectable in primary cilia isolated by the 'peeling off' procedure (Fig. 3b).

A larger number of ATGs associated with the basal body (highlighted with anti-gamma tubulin). In addition to the five cilia-associated ATGs, we also found ATG14, VPS34, ATG7 and ATG5, but not Beclin 1 or ULK-1, at the base of the axoneme (Fig. 3c; LAMP-2 is used a negative control). Similar colocalization experiments in serum-supplemented cells, to repress autophagy, and in cells with compromised ciliogenesis (Ift88<sup>−/−</sup> cells) revealed three types of association of ATGs with the basal body: serum- and αIFT-dependent, such as VPS34 and ATG16L; serum-independent but αIFT-dependent, as ATG7 and ATG14; and serum- and αIFT-independent, as ATG5 and LC3 (Fig. 3d–f and Extended Data Fig. 6).

Disruption of IFT also altered the overall intracellular distribution of ATGs that localize at the basal body in an αIFT-dependent manner. Thus, ATG7 was normally observed in KECs as discrete cytosolic puncta...
that markedly increased upon starvation, but it adopted a diffuse reticu-
lar pattern unchanged by starvation in Ift88−/− KECs (Extended Data
Fig. 7a). Disruption of IFT also reduced the basal association of ATG14,
key for autophagy initiation4, with the Golgi and its mobilization into
discrete cytosolic puncta upon serum removal (Extended Data Fig. 7a).

Interestingly, IFT88 deficiency also blunted the starvation-induced
changes in the intracellular location of ATGs that localize to the basal
body independently of IFT88, such as LC3 and ATG5 (Fig. 3f). The
association with the plasma membrane of LC3 and GABARAP
(both involved in autophagosome membrane formation/elongation2),
clearly noticeable in wild-type cells upon serum removal, was no
longer observed in the IFT88-deficient cells (Extended Data Fig. 7a).
Likewise, the often ‘hook-like’ organization of ATG5 (involved in
autophagosome membrane elongation) at the basal body was also
abrogated in Ift88−/− KECs (Extended Data Fig. 7b, c). The abundance
of ATG5 hook-like structures under basal conditions in cells with
constitutive Hh signalling (Ptc−/−; Extended Data Fig. 7d) indicates
the need of a functional cilium for ATG5 clustering in this location.

These findings support a role for αIFT in the cellular relocation of
the autophagic machinery to sites of autophagosome formation.

**IFT-dependent trafficking of ATG16L**

To gain a better understanding of the mechanisms behind the ciliary
regulation of starvation-induced autophagy, we focused on ATGs that
associated preferentially with ciliary structures upon serum removal.
We focused on ATG16L because it was almost undetectable in the
basal body in serum-supplemented cells (Fig. 3d), starvation induced
its IFT-dependent association with the basal body and its presence in
this location increased in two models with enhanced ciliary Hh sig-
nalling (Extended Data Fig. 8a, b). We proposed that active recruit-
ment of ATG16L because IFT20 also participates in trafficking of ciliary mem-
brane proteins from the Golgi to the base of the cilium. To analyse
separately both functions of IFT20, we compared its association with
ATG16L in cells with intact or disrupted αIFT (Ift88−/− KECs). We
found that IFT20 and ATG16L colocalized in small cytosolic vesicles
that become more abundant upon serum removal and that this coloca-
lization was only partially reduced when αIFT was disrupted (Extended
Data Fig. 8a, b). We proposed that active recruitment of ATG16L to the ciliary base during starvation could be the
trigger for ciliary-induced autophagy.

We first investigated the contribution of IFT20 to ciliary delivery of
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that become more abundant upon serum removal and that this coloca-
lization was only partially reduced when αIFT was disrupted (Extended
Data Fig. 8c). Both proteins co-immunoprecipitated independently of
the presence of IFT88 (Fig. 4a), and IFT20, but not IFT88, could be pulled-down with ATG16L (Fig. 4b). Starvation did not change the association between ATG16L and IFT20, but changed the location of the IFT20- and ATG16L-positive vesicles from the proximity of the Golgi towards a more cytosolic location (Extended Data Fig. 8c), indicating that the same signal that enhances IFT20-mediated trafficking of proteins from the Golgi to the cilium may also be responsible for ciliary delivery of ATG16L. Other ATGs (VPS15, ATG7) have minimal colocalization with IFT20, or colocalize only at the Golgi (ATG14) (Extended Data Fig. 8d), and none of them binds directly to IFT20 (Extended Data Fig. 8e–g).

The amount of ATG16L in isolated clathrin-enriched vesicles was very low under basal conditions, but it increased markedly upon serum removal (Fig. 4c). Although the vesicular content of IFT20 did not change with starvation (Fig. 4d), knockdown of IFT20 reduced starvation-induced loading of ATG16L in the vesicles (Fig. 4d). Interestingly, ATG16L content was also reduced in vesicles isolated from serum-deprived cells knocked down for IFT88 (Fig. 4d), explaining the reduced colocalization between IFT20 and ATG16L in Ift88–/– cells. Electron microscopy and double immunogold labelling for IFT20 and ATG16L confirmed the vesicle nature of the fractions and the coincidence of both proteins in more than 60% of the labelled vesicles (Fig. 4e and Extended Data Fig. 8h).

We propose that, during starvation, ATG16L reaches the base of the cilium through the Golgi-to-cilia shuttling function of IFT20, and then it accesses the ciliary region by IFT88-dependent mechanisms. Overall, our results support a novel role for the centrioles as organelle centres for the autophagic machinery and for IFT proteins as common components shared by both ciliogenesis and starvation-induced autophagosome formation.

Autophagy activation reduces cilia growth

Although inducible autophagy relies on IFT and cilia function, we found that autophagy is not required for cilia formation. In fact, Atg5−/− MEFs formed cilia both longer and faster than wild-type MEFs upon serum removal, and a higher percentage of them grew cilia even in basal conditions (Fig. 5a–d). Knockdown of two other ATGs and even chemical inhibition of autophagy were sufficient to induce ciliogenesis in different cell types under basal conditions, whereas upregulation of autophagic activity with rapamycin did not affect either basal or inducible ciliary growth (Extended Data Fig. 9).

Scanning electron microscopy revealed that in Atg5−/− MEFs starvation induced formation of long and narrow cilia that come out of smaller ciliary pockets, and confirmed that Atg5−/− MEFs grow primary cilia in serum-supplemented media, although shorter and with fewer surface-adhered vesicles (presumably exosomes) than the cilia grown during starvation (Fig. 5c and Extended Data Fig. 10). Activation of ciliary Hh signalling by starvation or treatment with purmorphamine showed efficient recruitment of SMO to the cilia in Atg5−/− cells and increased expression of the downstream transcription factors GLI1 and GLI2 in these cells even under basal conditions, supporting full functionality of the cilia induced by autophagic blockage (Fig. 5e, f and Extended Data Fig. 11a).

To start investigating the basis for this inhibitory effect of autophagy on ciliogenesis, we compared levels and distribution of IFT proteins in wild-type and Atg5−/− MEFs subjected to co-immunoprecipitation of IFT20 (a) and ATG16L (b). Inp, 1/10 input; IP, immunoprecipitate; FT, 1/10 flow-through.

c. Immunoblot for ATG16L and clathrin in MEF homogenate (Hom), and the pellets from 1 h centrifugation at 100,000g (organelles (Org)), 300,000g (300k) and 500,000g (vesicles, 500k).

d. Immunoblot for IFT20 and ATG16L in the same fractions isolated from Ctrl, IFT20− and IFT88− MEFs in the presence (top) and absence (bottom) of serum.

e. Immunogold electron microscopy for ATG16L (15-nm particles) and IFT20 (10-nm particles) in vesicles from MEFs isolated by the same method as in c. Right, quantification of IFT20 and ATG16L presence in the vesicles (11 fields). Mean ± s.e.m.
with lysosomal proteolysis inhibitors in wild-type MEFs confirmed absence of lysosomal degradation of IFT20 during the first 12 h of serum removal, when inducible autophagy is maximally activated (Extended Data Fig. 11c, d). In light of these findings, we propose that basal autophagy contributes to the regulation of ciliogenesis and the length of an already formed cilium, at least in part through the degradation of IFT20. The switch from basal to induced autophagy seems to spare cytosolic IFT20 from degradation and allow its active engagement in the vesicular trafficking required for ciliogenesis.

**Discussion**

In this work, we identify a previously unknown reciprocal relationship between primary cilia and autophagy whereby components of the ciliary machinery are shared with autophagy for starvation-induced autophagosome biogenesis. In contrast, basal autophagy inhibits ciliogenesis by limiting trafficking to the cilium of components required for ciliary growth. The temporal coincidence of activation of ciliogenesis and autophagy at the early times of nutritional deprivation may thus serve as a self-regulatory brake for each of these processes (Extended Data Fig. 1).

The localization of autophagy initiating ATGs at the ciliary base and the active recruitment of ATG16L to this location upon starvation suggests that sensing of nutrient deficiency and activation of signaling from the cilium could initiate a cilia-mediated autophagic program (see additional Discussion 1 in Supplementary Information). The presence along the cilium axoneme of the pre-autophagosomal ATG16L and integral autophagosome membrane ATGs—for example, LC3 and GABARAP—and their IFT-dependent enrichment at the plasma membrane suggest that cilia-mediated autophagy may induce autophagosomes formation from this location, making the ciliary pocket, characterized by high vesicular activity, an attractive place for autophagosome formation (see additional Discussion 2 in Supplementary Information).

The negative regulatory role of basal autophagy on ciliogenesis identified here may take place through different mechanisms. We have found that basal autophagy controls trafficking of ciliary proteins by limiting the amount of IFT20 accessible for shuttling between the Golgi and the ciliary base (see additional Discussion 3 in Supplementary Information). As induction of autophagy depends on the same IFT20 protein that autophagy degrades, this could represent a novel mechanism for self-containment of the autophagic process.

The primary cilium is constitutively present in most tissues, but the fact that ciliogenesis blockage in cells with permanent cilia compromises their starvation-induced autophagy supports that it is not the mere presence of a cilium but the activation of ciliary signalling by starvation that contributes to autophagic induction. We have also identified the regulatory effect of the ciliary Hedgehog signalling pathway, for which the large axoneme of the pre-autophagosomal ATG16L and integral autophagosome membrane ATGs—for example, LC3 and GABARAP—and their IFT-dependent enrichment at the plasma membrane suggest that cilia-mediated autophagy may induce autophagosomes formation from this location, making the ciliary pocket, characterized by high vesicular activity, an attractive place for autophagosome formation (see additional Discussion 4 in Supplementary Information). The possible participation of the cilium in the integration of other autophagy-inducing signals and the effect of autophagy in IFT20 functions beyond ciliogenesis require further investigation.

**METHODS SUMMARY**

MEFs defective in autophagy (Atg5−/−) were from N. Mizushima, Ifit8−/− (also known as Tg237−/−) mouse kidney epithelial cells were from G. Pazour, and the RGC-5 cell line from P. Boya. Cell culture, removal of serum and treatment with chemical modulators of autophagy were performed as described before. Intracellular protein degradation was measured as described before. Autophagic flux was measured as changes in levels of LC3-III upon inhibition of lysosomal proteolysis. Lentivirus-mediated short hairpin RNA silencing was performed as described before. Cilia were isolated by the peel-off technique and autophagosomes and autophagosomes by floatation in metrizamide gradients as previously described. Cells grown on coverslips were processed for immunofluorescence following standard procedures and imaged in an Apotome.2 system using an Axiovert 200 fluorescence microscope (Carl Zeiss). Transmission electron microscopy was performed as described before and scanning electron microscopy following standard procedures.

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**Figure 5 | Ciliogenesis is enhanced in autophagy-defective cells.** a, b, Ciliated Atg5−/− MEFs in serum (**P = 0.038, 25 cells each per experiment, n = 3) (a) or serum (***P = 0.006, nonlinear fit regression, 25 cells each per experiment, n = 3) (b). c, Scanning electron microscopy (SEM) of primary cilia. Arrows, cilia-associated vesicles; arrowheads, ciliary pocket. d, Cilia length quantification from SEM (**P = 0.027 n = 28; ***P = 0.001, ††P = 0.001, n = 23). e, f, GLI1 (***P = 0.04, n = 3) (e) and GLI2 (***P = 0.0005, **P = 0.0046, †P = 0.0132, n = 3) (f) messenger RNA expression in Atg5−/− MEFs. g, h, IFT20 immunoblot in Atg5−/− (g) and WT MEFs with lysosomal inhibitors (NL) (h), I, IFT20 immunoblot in cytosol (Cyt), homogenate (Hom) and autophagosomes (APG). j, IFT20 protein levels in Atg5−/− MEFs (**P = 0.038, n = 6). k, IFT20 immunoblot in autophagy-defective MEFs. n.s., statistically non-significant. Mean ± s.d. in d and j and mean ± s.e.m. in other panels.
procedures. Gel densitometry, morphometric analysis and quantification of colocalization was done using Image J (NIH). Protein quantification, co-immunoprecipitation, electrophoresis and immunoblot and semi-quantitative real-time PCR were performed as previously described. Immunoblot membranes were developed using the LAS-3000 Imaging System (Fujifilm). Results are shown as mean ± s.e.m. or mean ± s.d., and Student’s t-test for unpaired data was used for statistical analysis and one-way analysis of variance (ANOVA) was used for multiple comparisons. A value of P < 0.05 was considered statistically significant.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions O.P. designed and performed most of the experiments, analysed the data and contributed to writing the paper; I.O. and I.B. performed and analysed the experiments related to Hh signalling; B.P. performed the electron microscopy studies and morphometric analysis; S.S. performed the cystolic vesicle experiments; A.D.-C. assisted with cell culture; P.C. conceived the part of the study related to Hh signalling, provided interpretation to the data and contributed to the writing and revision of the paper; B.H.S. and P.S. set the bases for the rationale of the study, provided feedback in the interpretation of the data and revised the written manuscript; A.M.C. coordinated the study, designed experiments, analysed data and contributed to the writing and revision of the paper.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to A.M.C. (ana-maria.cuervo@einstein.yu.edu) or P.S. (peter.satir@einstein.yu.edu).
METHODS

Cells and reagents. MEFs were prepared as previously described. Ago5−/− MEFs were a gift from N. Mizushima, WT (Tg[Cre−/−]), I688−/− (Tg[Cre−/−]) mouse kidney epithelial cells were from G. Paquot, Ptc−/− MEFs were from P. Scott and RGC-5 the cell line from P. Boya. Inversin–GFP 3T3s were from I. R. Veland and S. T. Christensen. All cells were cultured at 37 °C and 5% CO2 in Dulbecco’s modified Eagle’s medium (Sigma) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin/fungizone. Serum removal was performed by thoroughly washing the cells with Hanks’ balanced salt solution (Invitrogen) and placing them in serum-free medium. Where indicated, cells were treated with the macroautophagy inhibitor 3-methyladenine (10 mM) (Sigma) or with NH4Cl (20 mM) and leupeptin (100 μM) (Fisher Bioreagents) to inhibit lysosomal proteolysis or with rapamycin (100 μM) (Calbiochem) to autoactivate autophagy. For cilia resorption experiments cytocalasin D (0.5 μM) (Tocris) and PDGF (50 ng·mL−1) (R&D systems) were used. Pumorphamine was from Calbiochem and cyclopane from Sigma. The plasmid for EGFP–LC3 was from Addgene, and inversin–GFP was from I. R. Veland and S. T. Christensen. The antibodies against acetylated tubulin, beta-tubulin, gamma-tubulin (mouse) and U1K-1 were from Sigma; against IFT20 and IFT88 from Proteintech (rabbit) and Abcam (mouse); against AMBRA1, ATG5/6/12, beclin, IFT88 and IFT140 from Novus, against ATG7 (immunoblot) and LC3 from Cell Signalling; against ATG7 (immunofluorescence) from Serotec; against GABARAP and gamma-tubulin (rabbit) from Santa Cruz; against LC3 (immunogold), ATG14, and ATG16(L) from MBL; against actin and detyrosinated alpha-tubulin (Glu-tubulin) from AbCam; against VPS34 from Invitrogen; against SMO from AbCam, against LAMP-2 from the Iowa Hybridoma Bank and against clathrin heavy chain (mouse) from BD Biosciences. The rest of the chemicals were from Sigma.

Autophagic measurements. Intracellular protein degradation was measured by metabolic labelling and pulse-chase experiments as described before. Briefly, confluent cells labelled with [3H]leucine (2 μCi/mL) (NENPerkinElmer Life Sciences) for 4 h at 37 °C were extensively washed and maintained in medium supplemented or not with serum with an excess of unlabelled leucine (2.8 μM). Aliquots of the medium taken at different time points were precipitated in trichloroacetic acid and proteolysis was measured as the amount of acid-precipitable radioactivity (proteins) transformed in acid-soluble (amino acids and small peptides) at each time. This assay quantifies the degradation of the pool of long-lived proteins that become radiolabelled during the 48-h pulse. Lysosomal degradation was determined as the percentage of degradation sensitive to inhibition by ammonium chloride and leupeptin. Autophagic flux was measured by immunoblot as changes in levels of LC3-II upon inhibition of lysosomal proteolysis (net flux) and autophagosome formation as the increase in LC3-II levels at two consecutive times during lysosomal proteolysis inhibition. Autophagosome content was evaluated as the number of fluorescent puncta in cells transfected with GFP–LC3 or after immunostaining with antibodies against endogenous LC3. Autophagic flux was also tested by transient transfection of the mCherry–GFP–LC3 plasmid, which was a gift from T. Johansen. Constructs were transfected using either Lipofectamine 2000 or Optifect reagent (Invitrogen) according to manufacturer’s instructions. Quantification of the yellow and red puncta was performed by Green and Red Puncta Colocalization Macro for ImageJ (J. D. Swiwiarski modified by R. K. Dagda).

Lentivirus-mediated shRNA silencing. The transfer vector plasmids containing the I688/100k pellet was further subjected to two successive centrifugations; 300,000g and 500,000g, and the pellets from each of these centrifugations were collected as the 300k and 500k crude vesicular fractions, respectively.

Co-immunoprecipitation assays. Cytosol was resuspended in a buffer containing 0.5% NP-40, and incubated over-night with the desired antibody at 4 °C. The fraction bound to antibodies was precipitated using either protein A- or protein G-coated beads, eluted and subjected to SDS–PAGE as described in the general methods.

Immunocytochemistry and morphometric analysis. Cells grown on coverslips were fixed either in 4% paraformaldehyde or 100% cold methanol, blocked and permeabilized, and incubated with primary antibodies (1:200 except for acetylated tubulin which was 1:1,000) and fluorophore-conjugated secondary antibodies (1:200) sequentially. Mounting medium contained DAPI (4',6-diamidino-2-phenylindole) to highlight the nucleus. Images were acquired with an ApoTome2 system using an Axiovert 200 fluorescence microscope (Carl Zeiss) equipped with a ×63 1.4 NA objective lens and red (excitation 570/30 nm, emission 615/30 nm), cyan (excitation 365/50 nm and emission 530/45 nm) and green (excitation 475/40 nm and emission 555/45 nm) filter sets (Chroma), and prepared using Adobe Photoshop 6.0 software (Adobe Systems). 3D reconstruction images were modelled as mixed renderings using the Inside-3D module for AxioVision Rel. 4.8, after applying the Nyquist sampling criteria. For quantification of the number of ciliated cells Z-stack sections were acquired and orthogonal views generated using AxioVision Rel. 4.8. Cilia length was measured using AxioVision Rel. 4.8 and number of fluorescent puncta using ImageJ (NIH) after thresholding of the images. Quantification of colocalization was performed using ImageJ (NIH) in individual frames after thresholding, and colocalization was calculated with the JACoP plugin of the same program in merged images.

Transmission and scanning electron microscopy and morphometric analysis. Cells grown to confluence were pelleted and fixed in 2.5% glutaraldehyde in 0.1M sodium cacodylate (SC) pH 7.43 at room temperature for 45 min. The pellet was then rinsed in SC, post-fixed in 1% osmium tetroxide in SC followed by 1% uranyl acetate, dehydrated through a graded series of ethanol, and embedded in LX112 resin (LADD Research Industries). Ultrathin sections were cut on a Reichert Ultracut E, stained with uranyl acetate fixed with lead citrate. Immunogold labelling was performed in ultrathin sections of isolated cysotic vesicles fixed in 4% paraformaldehyde/0.1% glutaraldehyde in sodium cacodylate, dehydrated and embedded in Lowicryl. Grids were washed in 50 mM glycine in PBS blocked, either single labelled with ATG16L and IFT20 or double labelled with ATG16L and IFT20 antibody for 2 h, washed extensively and incubated with the gold-conjugated secondary antibodies (1:100) for 2 h. Control grids were incubated with the secondary antibody alone or with an irrelevant immunoglobulin G. After extensive washing, samples were fixed a second time for 5 min in 2% glutaraldehyde, washed and negatively stained with 1% uranyl acetate for 15 min. All grids were viewed on a JEOL 1200EX transmission electron microscope at 80 kV. Scanning electron microscopy was performed by the Analytical Imaging facility at the Albert Einstein College of Medicine, following standard protocols. Images were acquired on a Zeiss Supra 40 Field Emission Scanning Electron Microscope at 5 kV. Morphometric analysis of transmitted light and scanning electron micrographs was done using ImageJ (NIH).

mRNA quantification. Semi-quantitative PCR was performed after extracting total RNA using the RNeasy Protect Mini Kit (Qiagen) following the manufacturer’s instructions. The first strand cDNA was synthesized from 0.5 μg of the total RNA with the SuperScript II RNase H Reverse Transcriptase (Invitrogen) and oligo(dT)12–18 primers. The expression levels were normalized to levels of β-actin in the same samples after amplification using the SYBR Green PCR kit (PE Biosystems) and the following primers from the QuantiTect Primer Assay (Qiagen): Gli1 (QT00173537); Gli2 (QT01062236); Ptch1 (QT00149135) and mouse β-actin 5′-GGCTGTATTCCCCTCCATCG-3′; 5′-GGCTGTATTCCCCTCCATCG-3′. Differences between samples were calculated using the 2−ΔΔCT method.

Statistical analysis. Results are shown as mean ± s.e.m. or mean ± s.d. and represent data from a minimum of three independent experiments unless otherwise stated. Student’s t-test for unpaired data was used for statistical analysis and one-way analysis of variance (ANOVA) was used for multiple comparisons. For cilia growing curves nonlinear regression was performed applying the least squares fitting method with Sigma Plot software. A value of P < 0.05 was considered statistically significant.

General methods. Cells were solubilized in RIPA buffer (150 mM NaCl, 0.5% Triton X-100, 0.5% NP-40, 0.5% NaDoc, 0.1% SDS) and protein concentration was determined using the Lowry method. After SDS–PAGE and immunoblotting, the proteins recognized by the specific antibodies were visualized by chemiluminescent HRP substrate from Pierce in a FujiFilm Las-300 Imager. Densitometric quantification of the bands was done using the square method with ImageJ (NIH), and all values were corrected by actin or tubulin as labelled in the figures.

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Extended Data Figure 1 | Schematic model of the interplay between autophagy and ciliogenesis. Starvation conditions (serum−) increase intralagellar transport (IFT) and ciliogenesis and Hedgehog (Hh) signalling. We show in this work that both events increase autophagosome formation interdependently. ATG16L is trafficked in IFT20-enriched vesicles to the base of the cilia where most ATG localize. Arrival of ATG16L to this ATG assembly and/or its transport along the axoneme seems to initiate the elongation of ATG5 structures and presence of lipid-binding ATGs (such as LC3 and GABARAP) in the cilia and at the plasma membrane. The cilium also contains under these conditions molecules of SMO that are mobilized to the ciliary membrane after release of the inhibitory effect of Ptc. Activated SMO induces the downstream transcription factors GLI1/GLI2 which further favours recruitment and assembly of ATGs in the base of the cilium. Normal nutritional conditions (serum+) lead to decreased Hh and IFT and suppression of starvation-induced autophagy. Basal autophagy contributes to maintain ciliogenesis to a minimum through the degradation of IFT20, which in turns reduces trafficking of ATG16L into the ATG assembly at the base of the cilium, preventing induction of ciliary autophagy.
Extended Data Figure 2 | Ciliogenesis and LC3 in different cell types.

a, Immunoblot for IFT20 and IFT88 and quantification in MEFs control (Ctrl) or knocked down (−) for IFT20 (*P = 0.007, n = 7), or kidney epithelial cells (KECs) wild-type (WT) or knockout for IFT88 (Ift88−/−; **P = 0.0003, n = 3). ADU, arbitrary densitometric units (n = 3).
b, Rates of degradation of long-lived proteins in the same cells expressed as a percentage of total proteolysis (top) or as a percentage of lysosomal degradation (sensitive to inhibition of lysosomal proteases) (*P = 0.021, n = 3).
c, Ciliated cells in MEFs (**P = 0.003, n = 5), KECs (*P = 0.011, *P = 0.021, n = 5) and retinal ganglion cells (RGC-5; **P = 0.004, ##P = 0.0002, n = 5) maintained in the presence or absence of serum.
d, Immunoblot for LC3 in MEFs and KECs maintained in the presence of serum and treated or not with protease inhibitors (PI).
e, Single-channel images and merge of WT and Ift88−/− KECs transfected with the mCherry–GFP–LC3 reporter.
f, Immunofluorescence for LC3 in WT and Ift88−/− KECs upon serum removal. Quantification of LC3-positive puncta (*P = 0.04, n = 3). Scale bars, 10 µm. Mean ± s.e.m. unless otherwise stated.
Extended Data Figure 3 | Autophagy in IFT88 and IFT20 double-knockdown cells. a. Immunoblot for the indicated proteins of MEFs Ctrl or knockdown for IFT88 (IFT88−/−) or for IFT88 and IFT20 (IFT20−/−IFT88−/−). b. Percentage of ciliated cells after 24-h serum starvation and representative images of cilia (acetylated tubulin, green) and basal body (γ-tubulin, red) in the same cells (IFT88−/−, **P < 0.00001; IFT20−/−IFT88−/−, **P < 0.0006, n = 5). Arrows indicate cilia. c, LC3 flux immunoblot in the same cell lines maintained in presence or absence of serum. d, Quantification of LC3-II levels (S+, **P = 0.00008, **P = 0.0004, n = 4; S−, *P = 0.034, n = 5) and LC3-II flux (S+, n = 4; S−, *P = 0.016, n = 5) by densitometry of blots as the ones shown in c. Mean ± s.d. e, LC3 flux in a retinal ganglion cell line (RGC-5) and in KEC treated or not with platelet-derived growth factor (PDGF). Scale bars, 10 μm. Mean ± s.e.m. unless otherwise stated.
Extended Data Figure 4 | Ciliary hedgehog signalling modulates autophagy.

a, Scheme of chemical and genetic approaches to modulate hedgehog (Hh) signalling. Both the agonist purmorphamine (Purmo) and genetic ablation of the Patched-1 receptor (Ptc) result in the recruitment of SMO to the cilia and initiation of expression of GLI factors. Knockdown of SMO or treatment with the SMO antagonist cyclopamine (Cyclo) suppress activation of downstream effectors. b, mRNA expression of Hh downstream effector genes relative after PDGF-induced ciliary resorption relative to untreated Serum − cells (Gli1, **P = 0.0006, ***P = 0.007; Gli2, ***P = 0.0001; Ptc, ***P = 0.002; n = 3). c, mRNA levels for downstream target genes of Hh signalling measured by RT–PCR after the indicated treatments (Smo; Ctrl *P = 0.028, purmorphamine *P = 0.027; Gli1, ctrl ***P = 0.006, purmorphamine *P = 0.037; Bcl-2, Ctrl **P = 0.007; n = 3). d, Immunofluorescence for LC3 in control cells treated or not with purmorphamine, and in Ptc−/− cells. Right, quantification (n = 3). e, mCherry–GFP–LC3 reporter in cells treated with purmorphamine. f, LC3 flux in IFT88−/− MEFs treated with purmorphamine. Right, quantification of LC3-II flux (Mean ± s.e.m.; n = 3). g, Ptc mRNA levels in MEF WT and Ptc1−/− (n = 3). h, Immunofluorescence for acetylated tubulin in MEF WT and Ptc−/− MEFs (*P = 0.014, **P = 0.0009, n = 3). j, Relative mRNA expression by RT–PCR of Hh downstream effector genes in control and myc–GLI1 cells (Gli1, ***P = 0.0017, Bcl2 ***P = 0.004; Ptc ***P = 0.0006; Hhip *P = 0.03; Gli2 *P = 0.028; n = 3). k, LC3 flux immunoblot in MEFs treated or not with Cyclo and PI. Quantification of LC3-II flux (n = 3). Differences with Ctrl (*) or with Serum + (#) are significant for P < 0.05. n.s., statistically non-significant. Mean ± s.e.m. unless otherwise stated. Scale bars, 10 μm.
Extended Data Figure 5 | Presence of ATGs at the primary cilia. a. Co-immunostaining for the indicated autophagy-related proteins (ATGs; green) and acetylated tubulin (red) of mouse kidney epithelial cells maintained in the absence of serum for 24 h. b, 3D reconstruction of the co-staining for ATG16L and acetylated tubulin in the cilia. ATG14 is shown as an example of absence of colocalization. 0.2-μm Z-stack are shown from the surface to the bottom part of the cilium. c, Staining with ATG16L or acetylated tubulin, and 3D images in cells transiently transfected with either GFP–LC3 or GFP–inversin and in GFP–inversin 3T3 stable cell lines. d, Immunostaining for ATG16L in NRK Ctr or knockdown for ATG16L transiently transfected with GFP–inversin to highlight the primary cilia. Individual channels, merge and 3D reconstitution of the co-staining are shown. e, Immunoblot for ATG16L in ATG16L−/− NRK cells. Scale bars, 10 μm.
Extended Data Figure 6 | Autophagy-related proteins associate with the basal body in a serum-dependent manner. a, b, Co-immunostaining for ATGs and gamma tubulin of kidney epithelial cells (KECs) maintained in serum-free (a) or serum-supplemented media (b). Arrows, colocalization (yellow) or no colocalization (white) in the centriole. c, Co-immunostaining of ATG7 (green) with gamma tubulin (red) in WT and Ift88^-/- KECs maintained in the presence or absence of serum for 24 h. Scale bars, 10 μm.
Extended Data Figure 7 | Changes in intracellular distribution of ATGs in kidney epithelial cells with defective IFT.  

a, Immunostaining for ATG7, ATG14, LC3 and GABARAP in WT and Ift88<sup>−/−</sup> kidney epithelial cells (KECs) maintained in the presence or absence of serum for 24 h.

b, ATG5 co-immunostaining (green) with gamma tubulin (red) in KECs. Arrows indicate clusters of ATG5.

c, Immunostaining for ATG5 in WT and Ift88<sup>−/−</sup> KECs maintained in the absence of serum for 24 h.

d, Immunostaining for ATG5 (green) and acetylated tubulin (red) in WT or Ptc<sup>−/−</sup> MEFs. Yellow arrow, ATG5 over ciliary structures. Scale bars, 10 μm.
Extended Data Figure 8 | Interaction of IFT20 with ATGs. a, Co-immunostaining for ATG16L and γ-tubulin in kidney epithelial cells (KECs) WT or Ift88−/− maintained in the presence or absence of serum for 24 h. Arrows, colocalization (yellow) or no colocalization (white) at the basal body (BB). b, Percentage of WT MEFs untreated (−), treated with purmorphamine (⁎P < 0.01, n = 4) or Ptc−/− (⁎⁎P < 0.005, n = 4) showing colocalization of ATG16L and BB in the absence of serum. c, Co-immunostaining for IFT20 and ATG16L in the same cells as in a. Insets show split channels of boxed areas at higher magnification. Arrows, colocalization. Right: quantification of the colocalization (##P < 0.004, **P < 0.004, n = 4). d, Co-immunostaining for IFT20 (red) with VPS15, ATG7 and ATG14 (green) in WT KECs during starvation. Insets show boxed areas at higher magnification. Yellow arrows, colocalization. Percentage of colocalization is indicated. e-g, Immunoblot for the indicated proteins after coimmunoprecipitation for IFT20 (e, f) or ATG14 (g) in the same cells. h, Immunogold electron microscopy for ATG16L and IFT20 in isolated cytosolic vesicles. Full-field images of double immunogold staining for IFT20 (10 nm) and ATG16L (15 nm) captured by transmission electron microscopy. ATG16L alone (yellow), IFT20 alone (blue), both proteins (red). Right image shows absence of gold particles in a region of vesicle-free film. Mean ± s.e.m. unless otherwise stated. Scale bars, 10 μm.
Extended Data Figure 9 | Effect of blockage of autophagy on ciliogenesis.
a, Co-immunostaining for acetylated tubulin and gamma tubulin in MEFs from WT or ATG5 null mice (Atg5^{−/−}) maintained in the absence of serum for the indicated periods of time. Arrows, cilia. 
b, Immunostaining for acetylated and gamma tubulin in MEFs control or knocked down (−) for ATG7, and maintained in the presence or absence of serum. Arrows, cilia. 
c, Quantification of the percentage of ciliated cells in ATG7^{−/−} (n = 5). d, Immunostaining for acetylated tubulin in KEC control or ATG14^{−/−}. e, Quantification of the percentage of ciliated cells in ATG14^{−/−} cells. Cells from Ift88 null mice are used as negative control in e, (n = 4). f, Percentage of ciliated MEFs upon treatment with 3-methyladenine (3MA) or rapamycin (Rapa) in the presence or absence of serum (n = 3). g, Percentage of ciliated retinal ganglionar cells (RGC-5) at the indicated times of treatment with 3MA or rapamycin in the presence of serum (n = 3). Scale bars, 10 μm. Arrows; cilia. All values are mean ± s.e.m. unless otherwise stated. Differences with Ctrl (*) or with Serum+ (#) are significant for P < 0.05. n.s., statistically non-significant.
Extended Data Figure 10 | Ultrastructure of the primary cilia in autophagy-deficient cells. Scanning electron microscopy images of embryonic fibroblasts from WT and ATG5-defective mice (Atg5$^{−/−}$) grown in the absence or presence of serum.

**a**, Surface of the different cells to highlight lower levels of villi in the Atg5$^{−/−}$ cells. Red arrow, primary cilia.

**b**, Details of primary cilia. Arrows, cilia-associated vesicles. Arrowhead, ciliary pocket.

**c–e**, Morphometric analysis of the cilia: cilia diameter (S−, **P = 0.0002, n = 28; S+, **P = 0.026, n = 24) (c), area of the ciliary pocket (S−, ***P = 0.009, n = 28; S+, ***P = 0.0001; n = 24) (d), and exosome diameter and number of exosomes per cilia (e) in WT and Atg5$^{−/−}$ MEFs. Mean ± s.d.
Extended Data Figure 11 | Enhanced ciliogenesis in autophagy-deficient cells. a, Co-immunostaining for SMO and acetylated tubulin in WT and Atg5\(^{-/-}\) MEFs treated or not with purmorphamine. Arrows, colocalization (yellow) or no colocalization (white). b, Immunofluorescence for IFT20 in MEF WT and Atg5\(^{-/-}\). Arrows, IFT20 cytosolic vesicles. Scale bars, 10 \(\mu\)m. All values are mean ± s.e.m. unless otherwise stated. c, Immunoblot for the indicated proteins in WT MEFs treated or not with ammonium chloride and leupeptin (N/L) and collected at different times after serum removal. d, Time-course of changes in IFT20 protein levels in WT and Atg5\(^{-/-}\) cells during serum removal relative to levels in serum supplemented WT MEFs. Time-course of changes in LC3-II flux is plotted as discontinuous line.