Nutrient-sensing nuclear receptors coordinate autophagy

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Autophagy is an evolutionarily conserved catabolic process that recycles nutrients upon starvation and maintains cellular energy homeostasis4,5. Its acute regulation by nutrient-sensing signalling pathways is well described, but its longer-term transcriptional regulation is not. The nuclear receptors peroxisome proliferator-activated receptor-α (PPARα) and farnesoid X receptor (FXR) are activated in the fasted and fed liver, respectively6,7. Here we show that both PPARα and FXR regulate hepatic autophagy in mice. Pharmacological activation of PPARα reverses the normal suppression of autophagy in the fed state, inducing autophagic lipid degradation, or lipophagy. In the fasted state, FXR is activated by bile acids described8.

This response is lost in PPARα knockout mice. Presumably, FXR strongly suppresses the induction of autophagy in the fasting state, whereas PPARα controls autophagy at the transcriptional level, activation of the basic helix-loop-helix transcription factor EB (TFEB) by fasting is the best described9. Overlapping networks govern both acute and longer-term responses to nutrients. In the fasted liver, glucagon induces both rapid glycogen breakdown and transcriptional activation of gluconeogenesis5. Nutrient deprivation also acutely regulates nutrient reclamation by autophagy7–9. Overlapping networks govern both acute and longer-term responses to nutrients. In the fasted liver, glucagon induces both rapid glycogen breakdown and transcriptional activation of gluconeogenesis5. Nutrient deprivation also acutely regulates nutrient reclamation by autophagy7–9.

Nutrient-sensing nuclear receptors are key integrators of metabolic responses. PPARα is activated by fatty acids in the fasted state, promoting fatty acid oxidation4,11,12. In the fed state, FXR is activated by bile acids returning to the liver along with nutrients, suppressing gluconeogenesis13. We proposed that PPARα and FXR would directly regulate autophagy, and initially tested the effect of their pharmacological activation in the mouse hepatocyte cell line AML12 expressing a triple fusion protein of monomeric red fluorescent protein (mRFP), green fluorescent protein (GFP) and the autophagosome marker LC3 (mRFP–GFP–LC3)14–16. In autophagosomes, the combination of both RFP and GFP in the triple fusion yields yellow fluorescence, whereas autolysosomal delivery results in red. As expected, the yellow fluorescence in normally cultured cells was converted to red by acute nutrient deprivation (Fig. 1 and Extended Data Fig. 1). The FXR agonist GW4064 (ref. 17) prevented this process in the nutrient-deprived cells, whereas the PPARα agonist GW7647 (ref. 18) mimicked it in non-starved cells (Fig. 1a and Extended Data Fig. 1f).

Recent extensive cell-based studies of levels of LC3 conjugated to phosphatidyethanolamine (LC3-II) and other indicators of autophagy confirmed that these responses are dose and time dependent, and indicated that they are not dependent on altered mammalian target of rapamycin complex 1 (mTORC1) activity, but are associated with a decrease in the inhibitory phosphorylation of ULK1 (ref.19) in response to PPARα activation, and an increase in response to FXR activation (Fig. 1b, c and Extended Data Fig. 2a–d). Indeed, GW7647 induces autophagic flux even in non-starved cells, whereas GW4064 suppresses it even in starved cells, or cells treated with the mTOR inhibitor Torin1 (ref. 20) (Extended Data Fig. 2a). The FXR agonist GW4064 (ref. 17) prevented this process in the nutrient-deprived cells, whereas the PPARα agonist GW7647 (ref. 18) mimicked it in non-starved cells (Fig. 1a and Extended Data Fig. 1f). The FXR agonist GW4064 (ref. 17) prevented this process in the nutrient-deprived cells, whereas the PPARα agonist GW7647 (ref. 18) mimicked it in non-starved cells (Fig. 1a and Extended Data Fig. 1f).

In livers of chow-fed or fasted wild-type C57BL/6j mice, LC3-II levels were increased in the fasted state (Fig. 1a, b and Extended Data Fig. 2e), and increased in response to both PPARα and FXR agonists (Extended Data Figs 2f and 3a–c). PPARα agonist treatment strongly increased levels of both non-lipidated LC3 (LC3-I) and LC3-II in the fed state in wild-type but not Ppara−/− mice21, and both were further increased in the fasted state (Fig. 2a and Extended Data Fig. 3b). GW7647 also induced LC3a mRNA expression in the fed and fasted states, and these responses were lost in Ppara−/− mice (Extended Data Fig. 3a).

Figure 1 | Activation of PPARα or FXR controls autophagic flux in mouse hepatocytes. a, Representative confocal images (out of 30 cells per condition) of AML12 cells transiently expressing mRFP–GFP–LC3 plasmids followed by treatment of vehicle (Veh), GW7647 (100 nM) or GW4064 (10 μM) for 24 h. Cells were starved for 2 h (bottom). DNA was counterstained with 4’,6-diamidino-2-phenylindole (DAPI) (blue). Scale bars, 20 μm. b, c, Immunoblotting of autophagy (LC3-I and p-ULK1 [Ser 757]) or mTORC1 activity (p-S6 [Ser 240/244]) in AML12 cells treated with indicated doses of Wy-14,643 (Wy) or GW4064 for 24 h or 48 h. GW4064-treated cells were starved for 1 h. kDa, kilodaltons.

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In fed or fasted mice treated with the Fxr agonist, induction of LC3-II protein in the fasted state was suppressed in wild-type, but not Fxr−/− mice (Extended Data Fig. 3c). As expected, GW4064 treatment also suppressed CYP7A1, but induced SHP expression in both the fasted and fed states in wild-type, but not Fxr−/− mice (Extended Data Figs 2g and 3c).

These results were confirmed using GFP–LC3 transgenic mice (GFP–LC3F/F; 15,16,23 and GFP–LC3F/F or Fxr−/− double mutants). Green puncta indicating autophagosome formation were increased in fasted GFP–LC3F/F mice (Fig. 2c). The PPARα agonist also significantly increased puncta in the fed GFP–LC3F/F mice, but not in the Ppara−/−/GFP–LC3F/F double mutants (Fig. 2c and Extended Data Fig. 3e). In the opposite direction, the FXR agonist strongly suppressed induction of puncta in the fasted GFP–LC3F/F mice, but not in the fed state, and had no effect in the Fxr−/−/GFP–LC3F/F double mutants (Fig. 2c and Extended Data Fig. 3e). The induction of puncta by fasting was significantly lower in the Ppara−/−/GFP–LC3F/F double mutants, whereas their number in the fed state was significantly increased in the Fxr−/−/GFP–LC3F/F mutants (Fig. 2c and Extended Data Fig. 3e). These partially defective responses in the double mutants demonstrate that PPARα is required for the full induction of autophagy by fasting, and FXR is required for its full suppression by feeding.

In agonist- or vehicle-treated fed and fasted livers, quantification of autophagic vesicles by transmission electron microscopy confirmed an increase in response to GW7647 in the fed state, and a strong decrease in response to GW4064 in the fasted state (Fig. 3a). Moreover, fasted livers of Ppara−/− mice show compromised formation of autophagic vesicles but increased numbers and size of lipid droplets. Conversely, livers of fed Fxr−/− mice show enhanced formation of autophagic vesicles (Extended Data Fig. 4a, c). Autophagosomes induced by the PPARα agonist frequently contained lipid droplets, suggesting an increase in lipophagy consistent with the role of this receptor in fatty acid oxidation (Extended Data Fig. 4b). This was confirmed in AML12 cells treated with or without oleate to induce lipid droplet formation, and then either starved or not and also treated with vehicle, the PPARα agonist Wy-14,643 or GW4064. Visualization of LC3 (red) revealed colocalization with lipid droplets labelled with the fluorophore diypyrromethene boron difluoride (BODIPY 493/503; green) in the starved cells, as expected, and also in the non-starved, Wy-14,643-treated cells (Fig. 3b and Extended Data Fig. 3f). In accord with a functional role for the induction of lipophagy, specific knockdown of either Atg5 or Atg7 significantly blunted the increase in fatty acid oxidation in response to Wy-14,643 in basal and oleate-treated AML12 cells, as indicated by ketone body production (Extended Data Fig. 3g). Similarly, both fasting and GW7647 induced serum β-hydroxybutyrate levels in control homozygous floxed Atg7 (Atg7F/F) mice, and both responses were decreased in liver specific Atg7 knockouts (Extended Data Fig. 3h).

Direct transcripational effects are the most likely explanation for the effect of both PPARα and FXR on autophagy. Initial results confirmed that total LC3 protein levels are increased and decreased in response to fasting and refeeding, and mRNA expression of LC3a and LC3b (also known as Map1lc3a and several other autophagic genes is increased in the fasted state and decreased in the refed state (Extended Data Fig. 2e, f). Among a core panel of 63 autophagy-related genes (Supplementary Table 1), 13 were both induced by GW7647 and repressed by GW4064 in wild-type mice, with both responses lost in relevant knockout mice (Fig. 4a and Extended data Fig. 5c), 11 more were responsive only to GW7647, whereas 4 responded only to GW4064 (Extended Data Fig. 5a, b).}

To define the basis for PPARα induction of autophagy-related genes, we determined the mouse liver PPARα cistrome with or without GW7647. The most significantly enriched binding motif among identified peaks was the known PPARα–retinoid X receptor (RXR)-binding site (direct repeat 1, or DR1), as expected, and nearly all of the peaks were absent in the Ppara−/− cistromes (Extended Data Figs 6a and 7a). PPARα-binding sites on core autophagy machinery gene loci as well as many regulatory and effector genes were confirmed by standard PPARα chromatin immunoprecipitation.
transcriptional coordination of hepatic autophagy by nutrient-sensing nuclear receptors in vivo. a, Hepatic autophagy-related expression of genes controlled by both PPARα and FXR in wild-type mouse liver. Fed or fasted mice treated with vehicle, GW7647 or GW4064 (n = 3 per group). Vps34 is also known as Pik3c3. b, PPARα and FXR ChIP followed by high-throughput sequencing (ChIP-seq) tracks for LC3a in wild-type mouse liver. Fed wild-type mice treated with vehicle or GW764 (n = 4 per group). Boxed peaks contain DR1 motif. c, PPARα/FXR binding to LC3a/b DR1 region determined by ChIP-qPCR in wild-type mouse liver (n = 3 per group). In a and c, *P < 0.05, **P < 0.01 versus vehicle-treated fasted wild-type mice; #P < 0.05, ##P < 0.01 versus vehicle-treated fasted wild-type mice (statistics by two-tailed t-test). Data represent mean ± s.e.m.
for this residual response, which cannot be correlated with direct FXR or FXR–RXR binding in vitro, is unclear. The ability of GW4064 to inhibit PPARγ-dependent transactivation of the LC3b DR1 motif is dependent on corepressors, because it was blocked by short interfering RNA (siRNA) knockdown of either SMRT or SHP (Extended Data Fig. 8e). Consistent with these functional results, GW7647 treatment increased p300 co-activator recruitment to the DR1 region of LC3a and LC3b enhancers and increased histone H4 acetylation, an active chromatin mark. By contrast, GW4064 treatment increased binding of the corepressors NCoR and SMRT, and increased levels of the repressive histone H3 trimethyl Lys 27 (H3K27me3) mark at the same DR1 regulatory region, particularly in the fasted state (Extended Data Fig. 9). Overall, we conclude that PPARγ and FXR directly compete for binding to the same DR1 elements at autophagic, and probably additional promoters, with opposite transcriptional outputs.

This mechanism is distinct from, but complementary to, the inhibition of CRTC2 recruitment described in the accompanying manuscript28. Thus, CRTC2 was not significantly recruited to the LC3a DR1 enhancer, as expected, but we confirmed the ability of GW4064 treatment to decrease CRTC2 occupancy to its proximal promoter site in the fasted state (Extended Data Fig. 10a).

These results define new mechanisms for regulation of autophagy by nutrient-sensing nuclear receptors (Extended Data Fig. 10b). Pharmacological activation of either PPARγ or FXR robustly affects hepatic autophagy. GW7647 activates it even in the fed state, which is associated with an increase in lipophagy. GW4064 potently suppresses the process in the fasted state. The significantly blunted autophagic responses of PPARγ and FXR knockouts to fasting or feeding, respectively, strongly confirm the physiological relevance of the pharmacologically defined responses.

Both nuclear receptors directly induce or repress extensive panels of autophagy-related genes in accord with the overall responses. However, the effect of the synthetic agonists seems greater than the magnitude of the transcriptional responses, suggesting a role for less direct effects. We did not observe effects on mTOR, the dominant upstream regulator of autophagy. However, there were alterations in the inhibitory phosphorylation of ULK1(Ser 757) that would be consistent with such effects. In addition, induction of the transcriptional repressor SHP is a well-recognized physiological nutrient response. They also establish PPARγ and FXR as potential targets for therapeutic modulation autophagy, which may be useful for liver or other metabolic tissues and could affect the pathogenesis of a wide range of human diseases.

Online Content Methods, along with any additional 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 J.M.L. conceived the project, designed and performed most experiments, interpreted results, and co-wrote the manuscript. M.W. performed animal experiments and participated in discussion of the results. R.X. analysed PPARs and FXR ChIP-seq data, and designed primers for PPARs ChIP-qPCR. K.H.K. performed ChIP assays and molecular cloning. D.F. performed PPARs ChIP-seq; M.A.L. supervised experimental designs. D.D.M. conceived the project, supervised experimental designs, interpreted results, and co-wrote the manuscript.

Author Information PPARs ChIP-seq data sets have been deposited in the NCBI Gene Expression Omnibus with the accession number GSE61817. 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 this paper. Correspondence and requests for materials should be addressed to D.D.M. (moore@bcm.edu).

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METHODOLOGY

C57BL/6J and Ppara−/− mice were from the Jackson Laboratory; For−/− and GFP-LC3 transgenic mice were previously described. Antibodies to LC3 (NB600-1384) were from Novus Biologicals; rabbit antibodies to phosphorylated Ser 240/244 S6 (36346), S6 (2217), phosphorylated Thr 37/46/4 E-BPI (2855), 4E-BPI (9452), anti-mouse IgG–horseradish peroxidase (HRP) (7076) and β-actin (5125) were from Cell Signaling Technology; antibodies to normal rabbit IgG (sc-2027), Parp-1 (sc-9121), Fxr1 (sc-7687), Ppara (sc-8322), TGT (sc-2884), CRTC2/ TORC2 (sc-6714x) and anti-rabbit IgG–HRP (sc-2337) were from Santa Cruz Biotechnology; antibody to histone H3K27me3 (39155) was from Active Motif. 

RT-qPCR. Total RNA was isolated from snap-frozen liver tissues using Trizol Reagent (Invitrogen, 15596-026) and prepared for the complementary DNA using Quantitect reverse transcription kit (Qiagen, 205311). The quantitation of any other primers was purchased from Qiagen.

RNA purification and qPCR analysis. Total RNA was isolated from snap-frozen liver tissues using Trizol Reagent (Invitrogen, 15596-026) and prepared for the complementary DNA using Quantitect reverse transcription kit (Qiagen, 205311). Liver tissues (50 mg) were homogenized in 1 ml RIPA buffer supplemented with protease and phosphatase inhibitors. Liver tissues (50 mg) were homogenized in 1 ml RIPA buffer supplemented with protease and phosphatase inhibitors, followed by brief sonication. Protein concentration was determined using Pierce BCA Protein Assay Kit. Total proteins (25–50 μg) were loaded on either 12% or 4–20% Mini-ProTEAN TGX Precast Gel (Bio-rad, 456-1043 or 456-1093), transferred onto Immuno-Blot PVDF (polyvinylidene difluoride) membrane (Bio-rad, 162-0177) and analyzed by immunoblot analysis using the ECL solution (Thermo Scientific, 34086, or Millipore, WBKLS0500).

Histology and immunofluorescence analysis. Liver tissues were collected and fixed in 4% PFA in PBS overnight at 4 °C. The tissues were treated with 15% sucrose in PBS at 4 °C for at least 4 h and then with 30% sucrose in PBS at 4°C overnight. Tissue samples were embedded in Tissue-Tek OCT (Sakura Finetek, 4583) and stored at −70°C. Cryosections were sectioned at 5–7 μm thickness, air-dried for 30 min, and stored at −20°C until use (Comparative Pathology Laboratory at Baylor College of Medicine). Images were taken on a confocal microscope (Nikon A1Rsi dual scanner). DNA, liver nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) and immunostained with antibodies against specific proteins.

ChIP assays. ChIP from mouse livers was performed as described previously. For PPARα ChIP-seq and ChIP-qPCR (Extended Data Fig. 7), livers were collected from fed wild-type or Ppara−/− mice treated with vehicle or GW7647 (5 mg kg−1, twice a day, n = 4 per group) after euthanasia. For PPARα, FXR, PII, p300, CRTC2 (TORC2), NCoR, SMRT, acetyl H4 and H3K27me3 ChIPs (Fig. 4c and Extended Data Fig. 9 and 10a), fed or fasted wild-type mice were treated with vehicle, GW7647 (5 mg kg−1) or GW4064 (100 mg kg−1) twice a day (n = 3 per group). Liver tissues (100 mg) were quickly minced using mortar and pestle with liquid nitrogen and cross-linked in 10 ml 1% formaldehyde/PBS for 15 min at room temperature, followed by quenching with 1/20 volume of 2.5 M glycine solution for 5 min. Minced liver tissues were washed twice with cold PBS (150 ml, 5 min, 4°C). Nuclear extracts were prepared by Dounce homogenization (30 strokes on ice, tight fitting pestle-type B) in cell lysis buffer (5 mM PIPES, 85 mM KCl, 0.5% Igepal and complete protease inhibitor tablet from Roche, pH 8.0). Chromatin fragmentation was performed by sonication in 300 μl ChIP SDS lysis buffer (50 mM HEPES, 1% SDS, 10 mM EDTA, pH 7.5), using the Bioruptor (Diagenode, 4′ × 5 min, 30 s on/30 s off). Proteins of interest were immunoprecipitated in ChIP dilution buffer (50 mM HEPES, 155 mM NaCl, 0.1% Triton X-100, 0.1% sodium deoxycholate, 1 mM phenylmethylsulphonyl fluoride (PMSF) and complete protease inhibitors tablet, pH 7.5) using 5 μl of each antibody. Cross-linking was reversed overnight at 65°C, and DNA was isolated using phenol/chloroform/isooamyl alcohol. Precipitated DNA was analysed by qPCR. Primer sequences used for ChIP-qPCR analysis are listed in Supplementary Table 2.
For ChIP, frozen liver tissues were ground in liquid nitrogen and cross-linked in 1% formaldehyde for 20 min, followed by quenching with 1/20 volume of 2.5 M glycine solution, and two washes with PBS. Cell lysis and chromatin fragmentation were performed by sonication in ChIP dilution buffer (50 mM HEPES, 155 mM NaCl, 1% Triton X-100, 1% SDS, 0.11% sodium deoxycholate, 1 mM EDTA, and complete protease inhibitors tablet, pH 7.5). PPARα proteins were immunoprecipitated using PPARα antibody, cross-linking was reversed over-night at 65 °C, and DNA was isolated using phenol/chloroform/isooamyl alcohol. Precipitated DNA was analysed by qPCR. ChIP was performed independently on liver samples from fed wild-type or Ppara−/− mice treated with vehicle or GW7647 (5 mg kg−1, twice a day), and the precipitated DNA or input DNA samples were pooled. Ten nanograms of the pooled DNA was then amplified according to ChIP Sequencing Sample Preparation Guide provided by Illumina, using adaptor oligonucleotides and primers from Illumina, enzymes from New England Biolabs and the PCR Purification Kit and MinElute Kit from Qiagen. Deep sequencing was performed by the Functional Genomics Core (J. Schug and K. Kaestner) of the Penn Diabetes Endocrinology Research Center using the Illumina Genome Analyzer Ix, and sequences were obtained using the Solexa Analysis Pipeline.

**ChIP-seq data analysis.** Solexa sequencing reads were mapped to reference genome mm9 using Bowtie3. Peak calling was performed with MACS1 using 200-bp regions centred at the summit of each peak. To analyse the enrichment of autophagy-related genes near PPARα-binding sites, all genes containing a PPARα-binding site (with or without the PPARα motif DR1) within 20 kb from the transcription start site were compared with a list of autophagy-related genes32–38,44–46 (Supplementary Table 1). Values were calculated with hypergeometric test.

**Transmission electron microscopy.** Anaesthetized mice were perfused with PBS for 3 min, followed immediately by perfusion with 2% PFA plus 2.5% glutaraldehyde in 0.1 M Millonig’s phosphate buffer, pH 7.4. Liver tissue was quickly removed and minced in a large drop of cold fixative, then transferred to vials of cold fix and fixed overnight in 4 °C. After washing three times in 0.1 M Millonig’s phosphate buffer, the tissue was post-fixed at room temperature in 0.1 M Millonig’s phosphate buffer containing 1% osmium tetroxide for 1 h. Post-fixation was followed by three rinses, 5 min each, of 0.1 M Millonig’s phosphate buffer, after which all tissues were dehydrated through a gradient series of ethanol, beginning with two 10 min changes of 50% ethanol and ending with three 20 min changes of 100% ethanol from a freshly opened bottle. Liver tissue was en bloc stained with saturated uranyl acetate in 50% ethanol for 1 h during the 50% ethanol dehydration stage. After complete dehydrated, the tissue was infiltrated over a period of 2 days with progressively concentrated mixtures of plastic resin and 100% ethanol. Infiltration continued until the tissue reached pure resin. The tissue was then given three changes of pure, freshly treated mixtures of plastic resin and 100% ethanol. Infiltration continued until the tissue was post-fixed at room temperature in 0.1 M Millonig’s phosphate buffer, the tissue was post-fixed at room temperature in 0.1 M Millonig’s phosphate buffer containing 1% osmium tetroxide for 1 h. Post-fixation was followed by three rinses, 5 min each, of 0.1 M Millonig’s phosphate buffer, after which all tissues were dehydrated through a gradient series of ethanol, beginning with two 10 min changes of 50% ethanol and ending with three 20 min changes of 100% ethanol from a freshly opened bottle. Liver tissue was en bloc stained with saturated uranyl acetate in 50% ethanol for 1 h during the 50% ethanol dehydration stage. After complete dehydrated, the tissue was infiltrated over a period of 2 days with progressively concentrated mixtures of plastic resin and 100% ethanol. Infiltration continued until the tissue reached pure resin. The tissue was then given three changes of pure, freshly made Spur’s plastic resin40 for 3 h each, after which the liver tissue was embedded in 80 BEEM capsules41 and placed in a 68 °C oven overnight. Thin sections of approximately 70 nm were obtained using an RMC MT6000-XL ultramicrotome (RMC) and a Diatome Ultra45 diamond knife (DIATOME), and collected on 150 hex-mesh copper grids. The sections were counterstained with Reynold’s lead citrate47 for 4 min. Transmission electron microscopy. Anaesthetized mice were perfused with PBS for 3 min, followed immediately by perfusion with 2% PFA plus 2.5% glutaraldehyde in 0.1 M Millonig’s phosphate buffer, pH 7.4. Liver tissue was quickly removed and minced in a large drop of cold fixative, then transferred to vials of cold fix and fixed overnight in 4 °C. After washing three times in 0.1 M Millonig’s phosphate buffer, the tissue was post-fixed at room temperature in 0.1 M Millonig’s phosphate buffer containing 1% osmium tetroxide for 1 h. Post-fixation was followed by three rinses, 5 min each, of 0.1 M Millonig’s phosphate buffer, after which all tissues were dehydrated through a gradient series of ethanol, beginning with two 10 min changes of 50% ethanol and ending with three 20 min changes of 100% ethanol from a freshly opened bottle. Liver tissue was en bloc stained with saturated uranyl acetate in 50% ethanol for 1 h during the 50% ethanol dehydration stage. After complete dehydrated, the tissue was infiltrated over a period of 2 days with progressively concentrated mixtures of plastic resin and 100% ethanol. Infiltration continued until the tissue reached pure resin. The tissue was then given three changes of pure, freshly made Spur’s plastic resin40 for 3 h each, after which the liver tissue was embedded in 80 BEEM capsules41 and placed in a 68 °C oven overnight. Thin sections of approximately 70 nm were obtained using an RMC MT6000-XL ultramicrotome (RMC) and a Diatome Ultra45 diamond knife (DIATOME), and collected on 150 hex-mesh copper grids. The sections were counterstained with Reynold’s lead citrate47 for 4 min. Dry samples were examined on a Hitachi H7500 transmission electron microscope and images were captured using a Gatan US1000 digital camera with Digital Micrograph (Gatan, v1.82.366) software.

**Molecular cloning and cell-based luciferase reporter assay.** The oligonucleotides encompassing three copies of DR1 motifs found in mouse LC3a or LC3b enhancer region were annealed and treated with Klenow. Purified DNAs were cloned into pT7-luc plasmid by a serial digesting with SacI and BglII. Successful cloning was confirmed by DNA sequencing analysis. Oligonucleotide sequences used for luciferase reporter assays are listed in Supplementary Table 3. For the luciferase assay, AML12 cells were cultured in 24-well plates. Transient transfections were performed using Lipofectamine 2000. Cell were transfected with 200 ng of reporter constructs, 100 ng of cytomegalovirus-promoter (CMX)-mouse PPARα, or CMX-human FXR, and 50 ng of CMX-β-galactosidase. pCDNA3.1 was added to prepare the total DNA to 500 ng per well. After 4 h transfection, cells were treated with or without vehicle (0.1% DMOSO), 10 μM Wy-14,643 or 1 μM GW4064 in media containing charcoal-stripped serum for 20 h before performing luciferase and β-galactosidase assay. Luciferase activity was normalized with β-galactosidase activity. Normalized values from vehicle-treated cells transfected with empty CMX plasmid were set as fold 1.

**Measurement of β-hydroxybutyrate.** AML12 cells were transfected with siControl (ON-TARGETplus Control pool, D-001810-10-20), siAtg5 (ON-TARGETplus SMARTpool siRNA Atg5, L-064838-00-0010), or siAtg7 (ON-TARGETplus SMARTpool siRNA Atg7, L-049953-00-0010) according to manufacturer’s instructions. siRNAs were purchased from Thermo Scientific Dharmacoen. Twenty-four hours after transfection using Lipofectamine 2000, cells were treated with vehicle (0.1% DMOSO) or Wy-14,643 (10 μM) in media containing charcoalstripped serum and 250 μM oleate for 48 h. Released β-hydroxybutyrate in the medium was measured using a β-hydroxybutyrate assay kit. Female Atg7F/F mice48 were bred with male Alb-Cre/+ mice to generate control littersmates (Atg7F/F) and hepatocyte-specific Atg7−/−null (Alb-Cre/+ +Atg7−/− mice). Eight-week-old male Atg7−/− and Alb-Cre/+ +Atg7−/− mice were orally gavaged with either vehicle (DMOSO) or 4:1 of PEG-400 and Tween 80) or GW7647 (5 mg kg−1 body weight) twice a day (first injection: 00:00, second injection: 12:00). Mice were fed ad libitum or fasted for 24 h from 18:00 (day 1) to 18:00 (day 2) and then starved to collect blood and livers. Serum β-hydroxybutyrate concentrations were normalized with mouse liver weights.

**Statistical analysis.** Sample size for experiments was determined empirically based on preliminary experiments to ensure appropriate statistical power. Age, sex and weight-matched mice were randomly assigned for the treatments. No animals were excluded from statistical analysis, and the investigators were not blinded in the studies. Values are expressed as mean ± s.e.m., and error bars for all results were derived from biological replicates rather than technical replicates. Significant differences between two groups were evaluated using a two-tailed, unpaired t-test, which was found to be appropriate as groups displayed a normal distribution and comparable variance. P < 0.05 was considered statistically significant.

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Extended Data Figure 1 | PPARα or FXR agonist affects autophagic flux in mouse hepatocytes. a, Autophagic flux was assessed by LC3 immunoblot analysis in AML12 cells treated with indicated dose of Wy-14,643, or co-treated with both Wy-14,643 and bafilomycin A1 (BafA1). b, c, Biochemical determination of autophagy (p62/SQSTM1 immunoblot) in AML12 cells treated with indicated doses of Wy-14,643 or GW4064 for 24 h. GW4064-treated cells were starved for 2 h. d, Primary hepatocytes from GFP-LC3Tg/+ mice were treated with indicated doses of Torin1 or GW7647. GFP–LC3 cleavage was assessed by GFP immunoblot analysis. e, LC3 immunoblot in AML12 cells treated with indicated doses of GW4064 or co-treated with GW4064 and Torin1. All drug treatments were done in complete media for 24 h unless otherwise indicated. β-actin is a loading control. f, Quantification of autophagic flux shown in Fig. 1a. Numbers of autolysosomes (ALs) induced by 2 h starvation plus vehicle were set as 100%. Numbers of autolysosomes = RFP-positive vesicles – GFP-positive vesicles. Thirty cells were counted per condition (**P < 0.01 versus no starvation plus vehicle; two-tailed t-test). Data represent mean ± s.e.m.
Extended Data Figure 2 | Nutrient availability regulates the expression of autophagy-related genes. a, b, Biochemical determination of autophagy (LC3 immunoblot; LC3-I and LC3-II denote non-lipidated and phosphatidylethanolamine-conjugated forms of MAP1LC3, respectively) or mTORC1 activity (p-S6(Ser 240/244) immunoblot) in AML12 cells treated for indicated time with 100 μM Wy-14,643 or 10 μM GW4064. GW4064-treated cells were starved in HBSS medium for 1 h.

b-actin is a loading control.

c, Bile acids suppress autophagosome formation. AML12 cells were treated with indicated doses of each bile acid for 24 h, followed by 1-h starvation in HBSS medium.

d, Immunoblot analysis of LC3-I/II and β-actin in AML12 cells treated with indicated dose of GW7647 or GW4064 for 24 h. GW4064-treated cells were starved in HBSS medium for 1 h.

e, Immunoblot analysis of LC3-I/II and β-actin in liver samples. Proteins (50 μg) from liver homogenates were separated by SDS–PAGE and probed with the indicated antibodies. β-actin is a loading control. A pooled sample was loaded onto the gel in duplicate (n = 5 per group).

g, Hepatic expression levels of autophagy-related genes, and PPARα and FXR target genes affected by nutrient availability (n = 5 per group, *P < 0.05, **P < 0.01 versus fed wild-type mice; two-tailed t-test).

Hepatic expression levels of PPARα or FXR target gene (Acox1 and Cyp7a1, respectively) were determined by qPCR analysis. Fed or fasted wild-type mice were orally gavaged with vehicle, GW7647 or GW4064 twice a day (n = 5 per group, *P < 0.05, **P < 0.01 versus fed wild-type mice). Data represent mean ± s.e.m.
Extended Data Figure 3 | Pharmacological activation of PPARα or FXR in fed or fasted mouse liver. a, c, Hepatic expression levels of autophagy-related genes (LC3a, LC3b and Atg12), PPARα-target gene (Acox1) and FXR-target gene (SHP) were determined by qPCR analysis. Fed or fasted wild-type, Ppara<sup>−/−</sup> or Fxr<sup>−/−</sup> mice were orally treated with vehicle, GW7647 or GW4064 twice a day (n = 5 per group, *P < 0.05, **P < 0.01 versus fed wild-type mice treated with vehicle). b, d, Immunoblot analysis of LC3-I/II, β-actin, phosphorylated S6 (p-S6) and total S6 in liver samples. Fed or fasted wild-type mice were orally gavaged with vehicle, GW7647 or GW4064 twice a day. A pooled sample was loaded onto the gel in duplicates (n = 5 per group). β-actin is a loading control. e, Representative confocal images (out of nine tissue sections per condition) of GFP–LC3 puncta (green: autophagosomes) and DAPI (blue: DNA) staining in livers. Fed or fasted bigenic Ppara<sup>−/−</sup>GFP-LC3Tg<sup>/1</sup> or Fxr<sup>−/−</sup>GFP-LC3Tg<sup>/1</sup> mice were orally gavaged with vehicle, GW7647 or GW4064 twice a day. Liver samples were fixed and cryosections were analysed by confocal microscopy. Scale bars, 50 μm. f, Co-localization of BODIPY 493/503 (green) with LC3 (red) in AML12 cells treated with vehicle or 1 μM GW4064 for 24 h and simultaneously cultured with or without 125 μM oleate in complete medium. GW4064-treated cells were starved for 2 h. DNA was stained with DAPI (blue). Scale bars, 20 μm. Quantification of lipophagic vacuoles shown in f and Fig. 3b. Thirty cells were counted per condition (**P < 0.01). g, Measuring β-hydroxybutyrate. AML12 cells were transiently transfected with control siRNA (siControl), Atg5 siRNA (siAtg5) or Atg7 siRNA (siAtg7) for 24 h followed by indicated drug treatments for 48 h with or without 250 μM oleate (vehicle: 0.1% DMSO, Wy: 10 μM Wy-14,643). Released β-hydroxybutyrate in the medium was determined (**P < 0.01 versus siControl treated with vehicle; #P < 0.01 versus siControl treated with Wy-14,643; ##P < 0.01 versus siControl treated with oleate plus Wy-14,643). h, Serum β-hydroxybutyrate were normalized to liver weights. Fed or 24-h fasted control littermates (Atg7<sup>F/F</sup>) and hepatocyte-specific Atg7<sup>F/F</sup>–null (Alb-Cre/+ Atg7<sup>F/F</sup>) mice were treated with vehicle or GW7647 twice a day (n = 4 per group, *P < 0.05, **P < 0.01 versus fed Atg7<sup>F/F</sup> mice treated with vehicle; #P < 0.01 versus fasted Atg7<sup>F/F</sup> mice treated with vehicle; ##P < 0.01 versus fasted Atg7<sup>F/F</sup> mice treated with GW7647). Data are mean ± s.e.m. Statistics by two-tailed t-test.
Extended Data Figure 4 | PPARα activation or loss of FXR induces autophagy in liver. Magnification of representative transmission electron micrograph images (out of 30 cells per group) of livers. a–c, Fed or fasted wild-type, Ppara−/− or Fxr−/− mice were orally gavaged with vehicle, GW7647 or GW4064 twice a day. Lipophagy (yellow arrowheads), autophagosomes (blue arrowheads), autolysosomes (red arrowheads), microautophagy (black arrowheads) and multivesicular bodies (purple arrowheads). Scale bars, 0.5 μm.
Extended Data Figure 5 | Expression profiles of autophagy-related genes by PPARα or FXR activation in liver. a–c, Hepatic expression levels of autophagy-related genes were determined by qPCR analysis in wild-type (a, b) or Ppara<sup>−/−</sup> or Fxr<sup>−/−</sup> (c) mice. Eleven genes in a were induced by PPARα activation, but not affected by FXR activation. Four genes in b were suppressed by FXR activation, but not affected by PPARα activation (a and b, n = 5 per group, *P < 0.05, **P < 0.01 versus fed wild-type mice treated with vehicle; #P < 0.05, ##P < 0.01 versus fasted wild-type mice treated with vehicle). Altered expression levels of 13 genes shown in Fig. 4a were lost in Ppara<sup>−/−</sup> or Fxr<sup>−/−</sup> mice in c (n = 5 per group, *P < 0.05, **P < 0.01 versus fed Ppara<sup>−/−</sup> or Fxr<sup>−/−</sup> mice treated with vehicle). Fed or fasted wild-type, Ppara<sup>−/−</sup> or Fxr<sup>−/−</sup> mice were orally gavaged with vehicle, GW7647 or GW4064 twice a day. Data represent mean ± s.e.m. Statistics by two-tailed t-test.
Extended Data Figure 6 | Cistromic analysis of PPARα and FXR in mouse liver. a, De novo motif analysis of PPARα-bound genomic regions. Top PPARα peak regions (±150 base pairs (bp) from peak summits, ranked by enrichment fold) were subjected to de novo motif discovery by MEME. The best motif discovered by MEME (top, E value $= 4.7 \times 10^{-227}$) highly resembles the PPARγ/RXRA heterodimer binding motif from JASPAR (bottom, ID: MA0065.2) as a direct repeat 1 motif (DR1). b, Venn diagram depicting increasing PPARα cistrome upon PPARα agonism in vivo. PPARα highly confident (HC) binding peaks: peaks of wild-type mice treated with vehicle or GW7647 subtracted from peaks of $Ppara^{-/-}$ mice treated with vehicle or GW7647, respectively. c, Venn diagram showing overlapping binding peaks between PPARα ChIP-seq and FXR ChIP-seq from wild-type mice treated with synthetic agonists GW7647 or GW4064. d, Autophagy-related genes of PPARα and FXR cistrome. Within 20 kb from the transcription start site (TSS), PPARα ChIP-seq showed that 7,738 genes of total 28,661 genes (mm9) have highly confident peaks in wild-type mice treated with GW7647 (false discovery rate (FDR) < 0.0001, enrichment over $Ppara^{-/-}$ > 10), and that 124 genes out of 230 autophagy-related genes (HADb: Human Autophagy Database, http://autophagy.lu/) have at least one PPARα peak. FXR ChIP-seq showed that 3,835 genes have peaks in wild-type mice treated with GW4064, and 61 out of 231 autophagy-related genes have at least one FXR peak. e, PPARα ChIP-qPCR for known PPARα target genes ($n = 4$ per group, *$P < 0.05$, **$P < 0.01$ versus fed wild-type mice treated with vehicle; two-tailed t-test). Data represent mean ± s.e.m.
Extended Data Figure 7  |  PPARα ChIP-seq profiles at loci of autophagy-related genes. Fed wild-type or *Ppara*−/− mice were orally gavaged with vehicle or GW7647 twice a day. Mouse livers were taken out 6 h after the last injection of drugs to perform PPARα ChIP-seq and ChIP-qPCR.

**a**, Representative ChIP-seq reads for PPARα aligned to the autophagy-related genes (LC3a, LC3b, Gabarapl1, Bnip3, Atg12, Pex14, Sesn2, Atg7 and Prkaa2).

**b**, PPARα ChIP-qPCR for autophagy-related genes shown in a (*n* = 4 per group, *P* < 0.05, **P** < 0.01 versus fed wild-type mice treated with vehicle; two-tailed *t*-test). Data represent mean ± s.e.m.
Extended Data Figure 8 | PPARα/FXR genomic competition for DR1 in Acox1 gene and autophagy-related genes. a, Representative ChIP-seq reads for FXR and PPARα aligned to the Acox1 and LC3b genes. The peaks in the box contain DR1 motif. Fed wild-type mice were orally gavaged with vehicle or GW764 twice a day (n = 4 per group). b, PPARα or FXR ChIP-qPCR in livers. Fed or fasted wild-type mice were orally gavaged with vehicle, GW764 or GW4064 twice a day (n = 3 per group, *P < 0.05, **P < 0.01 versus fed wild-type mice treated with vehicle; ##P < 0.01 versus fasted wild-type mice treated with vehicle). c, Cell-based luciferase reporter assays. AML12 cells were transiently transfected with a 3×PPRE luciferase reporter construct (3×PPRE-luc) and CMX-β-galactosidase in a combination of expression plasmids of PPARα, FXR or both, followed by drug treatment for 20 h (vehicle: 0.1% DMSO; Wy: 10 μM Wy-14,643; GW4064: 1 μM GW4064). Normalized values (luciferase activity/β-galactosidase activity) of vehicle-treated cells transfected with empty plasmid were set as fold 1 (**P < 0.01 versus empty treated with vehicle; ##P < 0.01). d, Functional role of DR1 motif in the regulatory region of mouse LC3a and LC3b for PPARα or FXR activity. Transiently transfecting three tandem copies of mouse LC3a/LC3b DR1 luciferase reporter construct (3×LC3a/LC3b DR1 WT-luc) or mutated version (3×LC3a/LC3b DR1 mutant-luc) and CMX-β-galactosidase in a combination of expression plasmids of PPARα, FXR or both, followed by drug treatment for 24 h (vehicle: 0.1% DMSO; Wy: 10 μM Wy-14,643; GW4064: 1 μM GW4064). Normalized values (luciferase activity/β-galactosidase activity) of vehicle-treated cells transfected with empty plasmid were set as fold 1 (**P < 0.01 versus empty treated with vehicle; ##P < 0.01). e, Cell-based luciferase reporter assays were performed in AML12 cells by transiently transfecting siControl, siNCoR, siSMRT or siSHP along with three tandem copies of mouse LC3b DR1 luciferase reporter construct (3×LC3b DR1 WT-luc), expression plasmids of PPARα and FXR, and CMX-β-galactosidase followed by drug treatment for 24 h (vehicle: 0.1% DMSO; Wy: 10 μM Wy-14,643; GW4064: 1 μM GW4064). Normalized values (luciferase activity/β-galactosidase activity) of vehicle-treated cells transfected with siControl were set as fold 1 (**P < 0.01 versus siControl treated with vehicle; ##P < 0.01). Data represent mean ± s.e.m. Statistics by two-tailed t-test.
Extended Data Figure 9 | PPARα or FXR activation controls recruitments of co-regulators and epigenetic marks in the enhancer regions of LC3a and LC3b genes. Fed or fasted wild-type mice were orally gavaged with vehicle, GW7647 or GW4064 twice a day. Hepatic ChIP-qPCR analysis with indicated antibodies (p300, NCoR1, SMRT, acetyl-H4 and H3K27me3) was used to determine recruitments of co-regulators and subsequent alterations of epigenetic marks induced by PPARα/FXR genomic competition for DR1 found in the enhancer region of LC3a and LC3b genes (n = 3 per group, *P < 0.05, **P < 0.01 versus fed wild-type mice treated with vehicle; ##P < 0.01 versus fasted wild-type mice treated with vehicle). Data represent mean ± s.e.m. Statistics by two-tailed t-test.
**Extended Data Figure 10 | Working model of the coordination of hepatic autophagy by nutrient-sensing nuclear receptors, PPARα and FXR.**

**a,** Fed or fasted wild-type mice were orally gavaged with vehicle, GW7647 or GW4064 twice a day. Hepatic CRTC2 ChIP-qPCR in the promoter and enhancer region of \( \text{LC3a} \) gene (\( n = 3 \) per group, **\( P < 0.01 \) versus fed wild-type mice treated with vehicle; ##\( P < 0.01 \) versus fasted wild-type mice treated with vehicle). Data represent mean ± s.e.m. Statistics by two-tailed \( t \)-test. **b,** Proposed model depicting transcriptionally activating or suppressive nutrient-sensing nuclear receptors, PPARα or FXR, respectively, which coordinate autophagy in liver. Activated PPARα or FXR compete with each other for binding to response elements found in autophagy-related genes.