а				b
	wt	hm /+	hm/hm	Rapamycin (hours):
Atg16L HM1	21 (18.4%)	64 (56.3%)	30 (25.2%)	p62
Atg16L HM2	40 (26,7%)	67 (41.2%)	43 (32.1%)	
		(2 / 0)		Actin





Supplementary Figure 1. Allelic inheritance in Atg16L1^{HM} mice and rescue of autophagy deficiency in Atg16L1 deficient fibroblasts. a. Mice heterozygous for the gene trap mutation were bred to each other and produced wild-type (wt), heterozygous (hm/+) and homozygous (hm/hm) progeny which were separated by genotype ~4 weeks after birth. Both gene trap alleles displayed Mendelian inheritance (no significant deviation by Fisher's chi-square test, p>0.2 for ATG16L1^{HM1} and p>0.3 for ATG16L1^{HM2}) b-c, Atg16L1 cDNA or vector control were reintroduced into Atg16L1^{HM1} MEFs via retroviral transduction. MEFs were selected for successful transduction with 5µg/ml puromycin, then grown in the presence of the chemical inducer of autophagy rapamycin $(50 \mu g/ml)$ and cyclohexamide $(5 \mu g/ml)$ for 0, 4, and 8 h. Western blot analysis of cell lysates show a restoration of p62 degradation in MEFs selected for Atg16L1 expression but not vector control (b) which was quantified by densitometry and normalized to actin (n = 3) (c).



Supplementary Figure 2. MEFs derived from Atg16L1^{HM1} and Atg16L1^{HM2} lines show reduced endogenous LC3 dot formation. a, Representative immunofluorescence images of primary low-passage MEFs grown in 50µg/ml rapamycin for 4h or in amino acid and serum replete EBSS media for 2h to induce autophagy and stained with LC3. LC3-positive dots >0.5µm in diameter are indicated by arrow heads. Scale bar, 20µm. b-e, Quantification of LC3 dots revealed that both the accumulation of LC3 dots per cell (b and d) or % cells with LC3 dots (c and e) after autophagy induction were compromised in Atg16L1^{HM} cells, indicating that autophagosome formation was aberrant under these conditions (n = 3, at least 70 cells were analyzed per sample). The increase in LC3 dots was statistically significant in all WT samples. There was no statistically significant increase in Atg16L1^{HM} cells (note: Atg16L1^{HM1} cells display a statistically significant decrease in dots per cell under starvation conditions). *P* values were calculated using two-tailed student's t test. Error bars represent SEM.



Supplementary Figure 3. GFP-LC3 dot formation is reduced in Atg16L1^{HM1} MEFs. a, Representative fluorescence images of in immortalized Atg16L1^{HM} MEFs stably expressing GFP-LC3. Cells were cultured in DMEM with 10% FBS or DMEM without amino acids and serum for 2 h. Scale bar, 10 µm. b, Quantification of the number of GFP-LC3 dots per cell (counted in at least 5 different images) show a significant reduction in dot formation in Atg16L1^{HM1} cells. Atg16L1^{HM2} cells did not show a statistically significant reduction in dot formation, consistent with the higher expression of Atg16L1 in these cells. *P* values were calculated using two-tailed student's t test. Error bars represent SEM.



Supplementary Figure 4. Atg16L1 is expressed throughout the ileal crypt-villus axis. RNA was procured by LCM from the villus tip, villus base, and crypt base of the distal ileum from Atg16L1^{HM} mice. qRT-PCR analysis shows detectable Atg16L1 transcripts in all three compartments (n = 3). There was a statistically significant difference between the villus tip and the villus base (p<0.1) or crypt (p<0.05) indicating that Atg16L1 transcripts are enriched in the villus base and crypt. *P* values were calculated using two-tailed student's t test. Error bars represent SEM.



Supplementary Figure 5. Conditional deletion of *Atg5* in the intestinal epithelium leads to reduced LC3 conversion and accumulation of p62. a, Western blot analysis of ileal lysates from *Atg5^{flox/flox} villin-Cre* mice reveal decreased Atg5 expression and an increase in LC3-I to LC3-II ratio similar to Atg16L1^{HM} mice suggesting a critical role for these proteins in intestinal autophagy (n = 3 of each genotype, 2 of each shown). b-c, *Atg5^{flox/flox} villin-Cre* mice also display an increase in p62 protein expression in the ileal epithelium (b) similar to Atg16L1^{HM} mice. Quantification of p62 levels by densitometry normalized to actin revealed ~7-fold increase in *Atg5^{flox/flox} villin-Cre+* mice (n = 3) (c). *P* values were calculated using two-tailed student's t test. Error bars represent SEM.



Supplementary Figure 6. Abnormal Paneth granule exocytosis in Atg16L1 deficient mice. a-c, Whole mounts of the small intestines from control (a) and Atg16L1^{HM} (b, c) mice stained with FITC-conjugated *Helix pomatia* lectin that labels goblet cell mucus (green) and antisera directed against lysozyme (red). Lectin positive goblet cells stud the surface of the villi. No accumulated mucin is shown in these fields. Strikingly, the lysozyme staining in the Atg16L1^{HM} mice is concentrated in small clusters of spherical aggregates (white arrow in b) that are present in the crypt lumen. High power view of the aggregate in (c) is 40µm in its greatest dimension. d-e, EM analysis of the Atg16L1^{HM} ileum reveals diminished microvilli on Paneth cells (d) and the adjacent crypt lumen (indicated by arrow heads) contains intact Paneth granules and cytoplasm (e). Scale bars: a, b, 200 µm; d, e, 2 µm.



Supplementary Figure 7. Atg16L1 mutant mice do not display increased susceptibility to oral *L. monocytogenes* infection. Littermate WT (n = 10) and Atg16L1^{HM} (n = 5 for HM1 and n = 4 for HM2) mice were infected orally with $10^9 L$. *monocytogenes* re-suspended in 200µl of 5% sodium bicarbonate by gastric gavage. The number of bacteria in liver, spleen, and mesenteric lymph nodes were determined 72 h after infection and displayed no significant differences with WT. *P* values were calculated using two-tailed Mann-Whitney test. Horizontal bars represent the geometric mean.



Atg5^{flox/flox}

Atg5^{flox/flox}villin-Cre

Supplementary Figure 8. Mice with conditional deletion of *Atg5* in the intestinal epithelium have abnormal lysozyme distribution in Paneth cells similar to observations made in Atg16L1 deficient mice. Representative images of indirect immunofluorescence of sections stained for lysozyme (red) in $Atg5^{flox/flox}$ control and $Atg5^{flox/flox}$ villin-Cre mouse ileal crypts. Dotted lines denote crypt unit. Scale bars: 10 µm.



Supplementary Figure 9. Enterocytes in the ileal epithelium are similar between Atg16L1 mutant and wild-type controls by ultrastructural analysis. ab, EM of ileal villus enterocytes from WT (a, c, d) and Atg16L1^{HM} (b, e, f) mice show similar morphology with intact mitochondria. Scale bars: a, b, 2 μ m; c, e, 500 nm; d, f, 100 nm.

Gene	qRT-PCR Validation	Fold Change	Low Bound of FC
Selenoprotein P, plasma, 1	114.0 ± 10.8	1.10	1.03
Peripheral myelin protein 22	169.3 ± 10.2	1.40	1.27
Serine/cysteine peptidase inhibitor, clade B, member 1a	14.0 ± 1.1	1.38	1.20
Serum/glucocorticoid regulated kinase 1	2.8 ± 0.1	-2.05	-1.82
BTB and CNC homology 1	2.5 ± 0.4	-1.09	-0.99
Protein tyrosine phosphatase, non-receptor type 9	3.3 ± 0.7	-1.27	-1.04
Villin 1	Enterocyte marker*	1.12	1.04
Mucin 2	Goblet cell marker*	-1.13	-1.05
Chloride channel calcium activated 3	Goblet cell marker*	1.21	1.01
Chromogranin B	Enteroendocrine cell marker*	-1.25	-1.16

Supplementary Figure 10. Validation of lack of contamination of LCM procured Paneth cells with villus epithelial cells. a, We quantified 6 genes by qRT-PCR known to be enriched in villus epithelial cells¹ and not involved in fatty acid or cholesterol metabolism. We compared gene expression in LCM-procured Paneth cells versus villus epithelial cells. The fold-enrichment for villus epithelial cells is indicated for each gene (experiments were performed in triplicate). For each of these 6 genes and 4 well-characterized markers of villus epithelial cells (indicated by an asterisk), we compared the fold-change for Atg16L1^{HM} Paneth cells versus WT. Transcripts highly enriched in villus epithelial cells were not enriched in LCM procured Paneth cells.

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Sod't steanyl-Coenzyme A desaturase 1 Odec cell death-inducing DFFA-like effector c Sol't steanyl-Coenzyme A desaturase 1 LOC669888/// Lpc lipoprotein lipase /// similar to Lipoprotein lipase precursor (LPL) Car3: carbonic anhydrase 3 88144671: expressed sequence 88144871 Thrsp: thyroid hormone responsive SPOT14 hornolog (Rattus) Pidropalidin Cyp2e1: cytochrome P450, family 2, subfamily e, polypeptide 1 Fabp4: fatty acid binding protein 4, adipocyte Fabp1: fatty acid binding protein 1, liver Adipog: adiponectin, CIQ and collagen domain containing Sk5a12 solute carrier family 5 (soch an/glucose cottansporter), member 12 Fabp1: fatty acid binding protein 1, liver Hpchaptoclobin Saal: serum amyloid A1 Saal: seran amyoid A1 Lepcleptin Ofd: complement factor D (adipsin) Sk36a2 solute carrier family 36 (proton/amino acid symporter), member 2 Poc pyruvate carboxylase Retrika resistin like alpha Serpina10: serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 10 Dhir deserthedgehog Apoe4: apoli poprotein A-IV Cli /// LOC100047470: complement component factor i /// similar to complement component factor i Mm.2156961 \$3-12 plasma membrane associated protein, \$3-12 Krt12 keratin 12 Apoa4: apoli poprotein A-IV A481121 expressed sequence A481121 2010002M12Ric RIXEN cDNA 2010002M12 gene Acaa1b: acetyl-Coenzyme A acyltransferase 18 Cd3& CD36 antigen Fm2formin2 Socs3: suppressor of cytokine signaling 3 Bstl: bone manow stronal cell antigen 1 Hbb-b1 /// Hbb-b2 hemoglobin, beta adult major chain /// he moglobin, beta adult minor chain Neurod2:neurogenic differentiation 2 Socs3: suppressor of cytokine signaling 3 Ot doon Kif3b kinesinfamily member 38 Rhow as homolog gene family, member V LOC100047573 /// Rbp4: retinol binding protein 4, plasma /// similar to retinol binding protein LOC636537 /// 5sr1:signal sequence receptor, alpha /// similar to signal sequence receptor, alpha Lpt lipoprotein lipase Trim 15 bipartite motif protein 15 Mn 1735251 Mn 1618381 Chac 1: ChaC, cation transport regulator-like 1 (E. coli)



Amyl: amylase 1, salivary Npc3:nabiuretic peptide receptor 3 Hba-a1 ///Hba-a2:hemoglobin alpha, adult chain 1 /// hemoglobin alpha, adult chain 2 Sucraft: succinate receptor 1 Ketd14 potassium channel tetramerisation domain containing 14 Ext 1:Vexonuclease 3:5' domain-like 1 Ganhorargyne8 Ziscan1: zinc finger with KRAB and SCAN domains 1 Dbh: dopamine beta hydroxylase Scp2 Sterol carrier protein 2, liver Lhfpl2.lipomaHMGCfisionpartner-lice 2 Fbco41:F-boxprotein41 Mm.214071.1 LOC55290@hypothetical LOC552908 With: microsomal triglyceride transfer protein LOC100046877: similar to developmentally regulated RNA-binding protein 1 Phi21a: PHD finger protein 21 A Tmen 11% bansmembrane protein 116 Chatl: carbohydrate foeratan sulfate Gal-6) sulfotransferase 1 Mm.214584.1 Carit carbonic anhydrase 3 Arsic arybullatase K Hspa12a: heat shock protein 12A Upkib: unoplakin 18 Wildon?: WAP, folistatin/kazal, immunoglobulin, kunitz and netrin domain containing 2 Rtak1: PDZ domain containing 1 Alpit alkaline phosphatase, intestinal G530011006Rik: RIKEN dDNA G530011006 gene Timie transmembrane inner ear Hba-a1 /// Hba-a2:hen oglobin alpha, adult chain 1 /// he noglobin alpha, adult chain 2 Ap1m1: adaptor-related protein complex AP-1, mu subunit 1 Innic indolethylamine N-methyltransferase Mm.157620.1 Ggt1: gamma-glutarsylbarsferase 1 Theo: trombopoietin Gsdmc2///LOC100045250:gasdemin C2///hypothetical protein LOC100045250 Dgat2 diacylglycerol O-acyltransferase 2 Wdfy1:WD repeat and FYVE domain containing 1 Gedinc2/// Godinc4 gasdernin C4/// gasdernin C2 Code20: coiled-coil domain containing 80 Trf: transferrin Cd3& CD36 antioen Rad2 radical S-adenoryl methionine domain containing 2 Apt2l1: alanine-glycoylate aninotransferase 2-like 1 Apoal: apolipoprotein A-I Mm.207711.1 4931408014Rik: RIKEN (DNA 4931408014 gene Life le abocyte tyrosine kirase 1200013808Rik RIXEN cDWA 1200013808 gene Clec2h:C-type lectin domain family 2, member h



Apoal: apolipoprote in AH Apoal: apolipoprote in A-l Mn.46977.2 16 days neonate heart cDWA, RWEN full-length enriched library, cloneD830028N22 product unclassifiable, full insert sequence Nocili: NPCHite1 lit1:interferon-induced protein with tetratricopeptide repeats 1 Fogrt: Foreceptor, IgG, alpha chain transporter H47: histocompatibility 47 lsg15///LOC100088882 // LOC100044225 /// LOC677168:15G15 ubiguitin-like modifier /// similar to 15G15 ubiguitin-like modifi ORF9: open reading frame 9 0 day neurate cerebelium cDNA, RXEN full-length enriched library, clone C2300471.12 product und assifiable, full insert sequen LOC100041156/// LOC100041932: hypothetical protein LOC100041156 /// hypothetical protein LOC100041932 Vinn1: vanin 1 Akrib7:aldo-keto reductase family 1, member 87 Hao3: hydroxyacid oxidase (glycolate oxidase) 3 OTTMUSG00000 16644: predicted gene, OTTMUSG0000016644 Apoc2:apolipoprotein C-II 5730506809Rilc R KEN dDNA 5730508809 gene Fabp4 fatty acid binding protein 4, adipocyte That: freenine adobse 1 Piger3: prostaglandin Ereceptor 3 (subtype EP3) 2900026A0291k RIVEN cDNA 2900026A02 gene Cst&cystatinE/M Transcribed locus Pou#1:POU domain, class 4, transcription factor 1 Poch17:protocacherin 17 (d)63:00163 antigen Smyd1:SET and MYND domain containing 1 Exos: 10: exosome component 10 Fuz fuzzyhomolog (Drosophila) Mn 21669.1 Pleithm1: pleckstrin homology domain containing, family M (with RLM domain) member 1 Cocil: chemokine (C-X-Cmotif) ligand 1 Cherps caldium homeostasis endoplasmic reticulum protein Myo7a myosin Vila Trien140: transmembrane protein 140 Ace2 angiotensin I converting enzyme (peptidyl-dipeptidase A) 2 LOC100046740/// Span: secreted addic cysteine rich glycoprotein /// similar to Secreted acidic cysteine rich glycoprotein Gpt1: glutanic pynwic transaminase 1, soluble Tribit tribbles homolog 3 (Drosophila) Steap4: STEAP family member 4 Vinn1:vanin 1 Bola2:bolA-like 2 (E. col) Map6:micmfbrillarassociated protein 5 Acach /// LOC10004735& acetyl-Coenzyme A carboxylase beta // similar to acetyl-Coenzyme A carboxylase beta PikSap1: phosphoinositide-3-kinase adaptor protein 1 Code109b: collect-coil domain containing 1098 Cubre cubilin (intrinsic factor-cobalamin receptor) Apolt apoli poprotein B 64305976128 it: RKEN cCWA 6430597612 gene



Syt1:synaptotagmin I Osta: organic solute transporter alpha Mm.173854.1 Collal:collagen, type (alpha 1 4930539E08Rik: RIKEN cDNA 4930539E08 gene Box6: BOVL family member 6, elongation of long chain fatty acids (yeast) Sipic secretory leukocyte peptidase inhibitor Index indolearnine-pyrrole 2,3 dicoyogenase Encelastin Homer 2 homer homolog 2 (Drosophila) Centb1:centaurin, beta 1 Pyd: liver glycogen phosphorylate Solid synchecan 4 LOC100046081 /// Otub1: OTU domain, ubiquitin aldehyde binding 1/// similar to OTU domain, ubiquitin aldehyde binding 1 Mm.132077.1 1700011H14Ric RIKEN dDNA 1700011H14 gene ColSa3: collagen, type V, alpha 3 CiniScamello-like 5 this: interferon-induced protein with tetratricopeptide repeats 3 Adamts2: a disintegrin-like and metallopeptidase (reprohysin type) with thrombospondin type 1 motif, 2 SIc5a9: solute carrier family 5 (socium/glucose cotransporter), member 9 Tcea2 transcription elongation factor A (SIL 2 9430061123Rik RIKEN cDWA 9430061123 gene Lceli:late comfied envelope 11 Rab6b: RAB68, member RAS oncogene family Sc3a1: solute carrier family 3, member 1 Pcolce2: procollagen C-endopeptidase enhancer 2 Cod1: chemolone (C-X-C motif) ligand 1 TrimAC tripartite motif-containing 40 Stc2:stanniocalcin 2 Ttc3: tetratricopeptide repeat domain 3 181003001498k /// LOC100044832 RIKEN cONA 1810030014 gene /// similar to RIKEN cONA 1810030014 gene Apoal: apolicoprotein A-I Modil: MAX dimerization protein 1 Transcribed locus Arid4x AT ich interactive domain 44 (Rbp1 like) 2610019F08Rik: RIKEN cDNA 2610019F08 gene Mogat2 monoacylglycerol O-acyltransferase 2 16 days records cerebellum CONA, RXEN full-length en i ched library Lbh///LOC100048380: Imb-budandheart /// similar to Imb-budand heart Aldh 112 aldehyde dehydrogenase 1 family, member L2 Ace2 angiotensin I converting enzyme (peptidyl-dipeptidase A) 2 Znym5:zincfinger,WWHype5 Adax adenosine dearninase Cyp3a13: cytochrone P450, family 3, subfamily a, polypeptide 13 2010305C02Rik RIXEN cDWA 2010805C02 gene Apip1: anyloid beta (A4) precursor-like protein 1 Ubell: ubiquitin-activating enzyme ETHile Trim12 tripartite motif protein 12 Shibp4: SHB-domain binding protein 4



Kiki: kalilorein 1 Doep1: doeptidase 1 (renal) 8d215; 8cl2-like 15 Ghr. growth hormone receptor Sk26a6: solute carrier family 26, member 6 Pex19:perceisome biogenesis factor 19 Loak & lectin, galactose binding, soluble 1 Vip: vasoactive intestinal polypeptide Hid: headkinase 3 Fd1: fentin light chain 1 Aldh1a7: aldehyde dehydrogenase family 1, subfamily A7 1810007E14Rsic RIKEN cDWA 1810007E14 gene Gama: granzyme A Zeb2 zinc finger E-box binding homeobox 2 Nbk2:neurotrophic tyrosine kinase, receptor, type 2 AA409316: expressed sequence AA409816 1810010W01Rik:RKEN dDNA 1810010W01 gene Mpp1: membrane protein, painitoylated Loak & lectin, galactose binding, soluble 1 Tmem120a transmembrare protein 120A Gabarap: gamma-aminobatyric acid receptor associated protein Cni4;camelo-like 4 Omredesnusin 2310006M10Rik:RKEN dDWA 2310006M10 gene 2610204W08Rk/// LOC100047142:RKEN cDNA 2610204W06 gene/// similar to INF2 Mn.151642.1 Trim20: tripartite motif protein 30 Gadmc1:gasdeminC1 Fbow@F-box and WD-40 domain protein 8 Mm.44320.1 Mm.120603.1 Adam1a ///LOC100047833: a disintegrin and metallopeptidase domain 1a /// similar to WK-5 type 2 tfi27:interferon, alpha-inducible protein 27 Sk9a3rt: solute carrier family 9 (sodium /hydrogen exchanger), isoform 3 regulator 1 82mbeta-2microglobulin Contringuin Ttc22 tetratricopeptide repeat domain 22 AB56758: expressed sequence AB56758 Kiki: kalikeein 1 Dnajc2 DnaJ (Hsp40) homolog, subfamily C, member 2 Tmem66: transmembrane protein 66 2010003K15Rijc RIKEN dDNA 2010003K15 gene 3110021A11Ric RKEN cDNA 3110021A11 gene Acox1: acyl-Coenzyme A coxidase 1, palmitoyl Which we coinia related binase 3 CdS chemokine (C-Cmotif) ligand 5 Srp14 signal recognition particle 14 Casta 2-5 oligoadenylate synthetase 1A 2310084601Rik: RKEN dDWA 2310034601 gene Ddah1: dimethylargini re dimethylarni nohydrolase 1



Nup62nadeoporin 62 Prap1: proline-rich acidic protein 1 Desa 2-deoxynbose-5-phosphate aldolaxe homolog (C.elegans) Boldhb /// LOC100048676: branched chain ketoaoid dehydrogenase EI, beta polypeptide Col3a1: collagen, type II, alpha 1 G0s2G0/G1 switch gene 2 Ap1s3: adaptor-related protein complex AP-1, signa 3 For 13 fibroblast growth factor 13 Osth: organic solute transporter beta Gbp2 quarwhite nucleotide binding protein 2 Lpin1: lipin 1 Gbp2 quaryiste radeotide binding protein 2 Clight 2: Clig and tumor necrosis factor related protein 2 Apolita: apolipoprotein L. 7a BcB:B-cell leukenia/lymphona 3 2010106E10Rik: RIKEN cDWA 2010106E10 gene Wdfy1: WD repeat and FWE domain containing 1 Figh: C-fosinduced growth factor Lichtz lactate dehydrogenase & Cleafe chloride channel calcium activated 6 Tanantedhow Rtp4: receptor transporter protein 4 More matrix Glaprotein Gtpbp2 GTP binding protein 2 Slošait solute carrier family 6 (neurotransmitter transporter, creatine), member 8 Spr2a:small proline-rich protein 2A Cyp4F14: cytochrome P450, family 4, subfamily f, polypeptide 14 Rpg ip1: retinitis pigmentosa GTPase regulator interacting protein 1 Sk34a2 solute carrier family 34 (sodium phosphate), member 2 Fasic fatty acid synthese Trim 34 tripartite motif protein 34 Pla2g12b; phospholipase A2, group XIB Phicb1: piedestrin homology-like domain, family 8, member 1 Figh: C-fosinduced growth factor Mttp: microsomal triglyceride transfer protein Hisd3b3: hydroxy-delta-5-stexoid dehydrogenase, 3 beta- and steroid delta-isomerase 3 4833409A17Ric RIVEN (DNA 4833409A17 gene Krt4keratin4 LOC100047419/// Maf:avian musculoaponeurotic fibrosarcona (v-maf) AS42 oncogene homolog/// similar to c-Maflong form Gec7a: C-type lectin domain family 7, member a Rabl& RAB, member of RAS on cogene family-like 5 Ace2 angiotensin I converting enzyme (peptidyl-dipeptidase A) 2 Art's ADP-ribosyltransferase 3 1500011803Rik: RIKEN cDWA 1500011803 gene Gpd1: glycerol-3-phosphate dehydrogenase 1 (soluble) Transcribed locus, weakly similar to XP_001175112.1 similar to pol protein, partial (Pan troglodytes) S100a10: S100 calcium binding protein A10 (calpactin) D12Entd647e DNA segment, Chr 12, ERATO Doi 647, expressed Skáait solnte canter family 6 (neurotransmitter transporter, creatine), member 8 Mep1b: meprin 1 beta



Sk27a4 solute carrier family 27 (fatty acid transporter), member 4 Clics: chloride intracellular channel 5 Colon2bx cyclin-dependentikinase inhibitor 28 (p15, inhibits CDK4) Rad2Ba: RAD2Bahomolog (S. cerevisiae) Arhodic: Rho GDP dissociation in hibitor (GDI) gamma Fbn1:fbdlin1 Grifty glamaturation factor, gamma Fbd17:F-box and leucine-rich repeat protein 17 AlgR apparagine linked glycosylation 8 homolog (yeast, alpha-1,3-glucosyltansferase) Cank1d:calcium/calmodulin-dependent protein kinase ID Sorbs1: Sorbin and SH3 domain containing 1 Nrp1:neuropiin1 Trim31: tripartite motif-containing 31 Rablet RAB, member of RAS oncogene family-like 4 TriAf 1: transmembrane 4 superfamily member 1 9130409120Rik: RKEN cDNA 9130409120 gene Cd59a: CD59a antigen Fbow 17: F-box and WD-40 domain protein 17 Stodi: SI RNAbinding domain 1 Visual cortex cDNA RIKEN full-length enriched library Itpka: inositol 1,4,5-trisphosphate 3-kinase A Atp1a2 ATPase, Na+/K+ transporting, alpha 2 polypeptide Sk44a4 solute canterfamily 44, member 4 Chorde 1: cysteine and histidine-rich domain (CHORO)-containing, zinc-binding protein 1 Timed?: transmembrane emp24 protein transport domain containing? Nedd1: neural precursor cell expressed, developmentally down-regulated gene 1 Dock10: dedicator of cytokinesis 10 Zip326 zinc finger protein 326 Galract2 chondroitin sulfate GalWAcT-2 Bace2 beta-site APP-cleaving enzyme 2 Danal 2 Dana-like 2 Ari 14 AOP-ribosylation factor-like 14 Hado3:homeo bax83 Fos: FBJ osteosarcoma oncogene Zip106 zinc finger protein 106 Aebp2: AE binding protein 2 Cd44 CD44 antigen Gog ducadon Tub& tubby like protein 4 Mfr1:mitochondrial fission regulator 1 Bace2 beta-site APP-cleaving enzyme 2 TubgopS: tubulin, gamma complex associated protein 5 Ralgos2: Ral GEF with PH domain and SH3 binding motif 2 6430706D2291k/// A730006H23Rik: RKEN dDNA A730008H23 gene /// RKEN dDNA 6430706D22 gene Drajbt Dnai (Hzp40)honolog, subfanity 8, member 4 Zbfb41: zirc finger and BTB domain containing 41 homolog Higt1:hippocampes abundant gene transcript1 Totp21:transcription factor CP2-like 1 Kif 11: kinesin fan ily member 11 D630004K10Rik:RIKEN cDWA D630004K10 gene



10 days recease cerebelum CNA, RNEH full-length enriched library Cical: chloride channel culcium activated 1 Tsc22dit TSC22 domain family 3 Dachil: dackshund 1 (Drosophila) Cd28: chemokine (C-C motif) ligand 28 Dars: aspartyl-tXWA synthetase Cont: catechol-O-methyltransferace Wm.105231.1 Enfil: EIII receptor feedback inhibitor 1 CadlB:chemokine (C-X:C motif) ligand 13 Tmem46:transmembrane protein 46 Clu///LOC100046120: dusterin /// similar to clusterin EG665407 N EG668022 N LOCINON3225 N/LOCI00045256 N LOCINON47935 Nufip2: nuclear fragile X mental relandation protein interacting protein 2 AE06767: Expressed sequence AE06767 Hspall beat shock protein 4 like Defb1: defensin beta 1 22104170091it: IREN cONA 2210417009 gene Grm4 glutamate receptor, metabotropic 4 Tried?: transmembrane emp24 protein transport domain containing 7 Ner3: period homolog 3 (Drosophila) Mn 209616.1 4833420G17Rik 1KEN cDNA 4833420G17 gene Citr: cysic fibrosistranan enbrane conductance regulator homolog Andol: anextin domain containing 4 C230091D08Ril: RIKEN cDWA C230091D08 gene Ochidi: discoidin, CUB and LCCL domain containing 1 31100(3)0968; RIVEN cDNA 31100(3)09 gene AS principal and Comparison of the Astronomy Section 1 HA467197: expressed sequence HA467197 Wistd2 male sterility domain containing 2 Gbp1: quarylate nucleotide binding protein 1 Cull:Cullin 3 Usp31: ubiquitin specific peptidase 31 Syde2 synapse defective 1, No GTPase, homolog 2 (C elegans) 170003F191it: LIKEN cDNA 170003F19 gene LOC100047857: similar to Cachedomain containing 1 Cast calpastation Sypt synaptophysin-like protein Acth& protocachein & Hsp110; heat shock protein 110 4933431E2001: RIVEN cONA 4933431E20 gene Ints& integrator complex subunit 8 Clu///LOC100046120: dusterin/// similar to clusterin Cica2 chloride channel calcium activated 2 O day neonate cerebelium dONA, RNEH full-length enriched library KIN7: keich-like 7 (Drosophila) 493(634+1)(Fit: 1)(EN c)(MA 493(634+18)) gene Mn 215865.1 Raigps2:RailGEF with PH domain and 9+8 binding motif2



Fig1:FSHD region gene 1 Akric 14: aldo-beto reductase family 1, member C14 Setol4 SET domain containing 4 6720463 M244 ik: RIVEN cD NA 6720463 M24 gene Sits12ip1:SFIS12-interacting protein 1 Silc STE20Hitekinase (yeast) Hir4.5 hydroxytryptamine (serotonia) receptor 4 463241106Filt RKENcDNA 4632411.06 gene Defb1: defensin beta 1 Cox15: COX15 homolog, cytochromec axidase assembly protein (yeast) Sirs11: splicing factor, argining/sering-rich 11 Pho1: pre B-cell leukernia transcription factor 1 Ywhaz tyrodine 3-monocorygenase tryptophan 5-monocorygenase activation protein, zela polypeptide kd-V1: inmunodkbulin lambda chain, wiable 1 Jai d'Id: jumonii, AT ich interactive domain 10 (Rbp2 Rice) LOC100043062///LOC100044665////Wwtaz:tyrosine 3-monooxygenase C3006P091it 1KEN dNA C3006NB gare Apt aguatorin 4 Sk2SaVx solute carrier family 25 (mitochondrial carrier, Graves disease autoentigen), member 16 GIS: Glycerol kingse 5 (putative) Tool:TOX high mobility group box family member 3 Ros13: regulator of G-protein signaling 13 Mm.000231 Mm.84118.1 Enol:enolase 3, betamusde N467657: expressed sequence N467657 Sco3a1:solute carrier organic anion transporter family, member 3a1 Zipó 18 zinc finoer protein 518 Gpr1 77: G protein-coupled receptor 177 Septinb5:serine (or cysteine) peptidase inhibitor, clude 1, member 5 1700110KT7K8k: RKEN dDWA 1700110K17 gene Zotb16:zinc finger and BTB domain containing 16 25 1004911981: IBEN CONA 25 10049119 gene Ou///LOC100046120: clusterin // similar to dusterin Tubocp4: tubulin, gamma complex associated protein 4 Wark van Boncogene Linic lin-7 honolog C (C. elegand) 2610021A01111: RIVEN cD WA 2610021A01 gene Pphin1:periphilin 1 Bd2:B-cell extensis/tymphoma 2 4633414E09ABIc RINEM dDNA 4633414E09 gene Transcribed locus Nyta myelob lastos is oncogene Capp & centrosome and spindle pole associated protein 1 2210019511Ric RIVEN cDWA 2210019511 gene Oca2 chloride channel calcium activated 2 Mistd2:male sterility domain containing 2 Ocik1: double contin file kinase 1 0830012F22R8c RIVEN cCNA 0330012F22 gene Usp40: obiquitio specific peptidase 40



Syciesyncolin Nn 1222.1 BC003267; cDWA sequence BC009267 Sultidi: suffotansierase family 10, member 1 N12 netallothionein 2 Ant'IN: ring finger protein 180 Zdhhc14:zinc finger, DHHC domain containing 14 Greb 1///LOC XXXXV5413: gene regulated by estrogen in breast cancer protein Niph: netarophilin To: transitivetin Hspalk heat shock protein 4 like To: transtityretin Psip1:RC4 and SFRS1 interacting protein 1 Cescan Vt CEA-related cell adhesion molecule 10 Ky: kyphoscoliosis peptidase Fasted 3: FAST kinase domains 3 Floct FH2 domain containing 1 Ceacan 10: CEA-related cell adhesion molecule 10 5530402H23Rik: RKEN dDWA 5530402H23 gene 15 days embryo head cDNA, INEN full-length enriched library Gated glutathione S-transferaze, C-terminal domain containing Lonri?: LOW peptidase N-terminal domain and ring finger 3 Zdhhc14:zinc finger, DHHC domain containing 14 Stock storkhead box 2 Stefal4: solute carrier family 6 (beurotransmitter transporter), member 14 Nn.2158661 Andac: anylaceta mide deacetylase (esterase) Could (///LOCI00041521 // LOCI00047065 ///LOCI00048613: cytochrome could se, suburit Vic 49365580911: RIVEN (DWA 4936535809 gene Cd177: CD177 antigen Nosip:nitric oxide synthese interacting protein LOC677143/// Stor2 sideoflexin2 // similar to Sideoflexin-2 4833416E15Rik///Enpp1:ectonucleotide.pyrophospikatase/pikosphodiesterase 1 Ptov1:prostate tumor over expressed gene 1 Contratechol-O-methyltransferase Nn.45061.1 Snap23: synaptosomal-associated protein 23 PpageTa: peroxisome proliferative activated receptor, gamma, coactivator 1 alpha 4021509.1171 it: FINEH cD NA 4021509.117 gene Atp Bal: ATPace type 1343 Earroy estrogen-related receptor gamma A930005H10Nic RIVEN cCNA A930005H10 gene Spred 1: sprouty protein with EVH-1 domain 1, related sequence Regi: regeneral a gislet-derived family member 4 ALCOAD 13: expressed sequence ALCOAD 13 2810487A2250c RIKEH dDNA 2610487A22 gene A 330021E22Rik: RIVEN cDNA A 330021E22 gene Nsil: Nusashi homolog 1 (Drosophila) Gdap2: ganglioxide-induced differentiation-associated-protein 2 Subtrack sufficiences and support of the support of



trig3 leadine-rich repeats and immunoglobulin-file domains3 Yahdc2: YTH domain containing2 Sk22a 15: solute carrier family 22 (organic anion/cation transporter), member 15 Sgk1:serum/glucocorficoid regulated binase 1 Ref 187: ring finger protein 167 LOC675567 /// Rocd1: ROC1 domain containing 1/// similar to MYC binding protein 2 Transcribed locus Mm.1212.1 LOC633417: similar to histocompetibility 2, Tregion locus 3 Ppargela: Perodoone proliferative activated receptor, gamma, coactivator 1 alpha Code32: coiled-coil domain containing 32 Vcanil: vascular cell achesion molecule 1 LOC100044194 /// Micc: mintated in colorectal cancers /// hypothetical protein LOC100044194 Transcribed locus, moderately similar to XP 001117064.1 BTB (PCZ) domain containing 9 (Macaca mulatia) 26 1020410 490c RHEH dCMA 26 102041/14 gene Hspath heat shock protein 41 ke Diaja4 ///LOC100048331: Dna/(Hsp40) homolog, subfamily A, member 4 Stor@:Storkheadbox2 4833420G17lik:RIXEN cDNA 4833420G17 gene P4kal: procolages-proline; 2-outglutante 4-dioxygenate (proline 4-hydroxytaxe), alpha 1 polypeptide hydrisovaleryl coenzyme A dehydrogenase 6030422H211ik:RIXEN cDNA 6030422H21gene Poarocita Perodisome proliferative activated receptor, gamma, coactivator 1 alpha Refl: period homolog 1 (Drosophila) D7Entd715e:DWA segment, Chr7, BVATO Doi 715, expressed EG633640: predicted gene EG633640 5730437W04BitcRIKEN.cDWA.5730437W04gene Th: transthyretin Oip5: Opainteracting protein 5 Arsk: any suffature K LOC100046254 similar to Adil protein LOC100047788 similar to gamma-2a immunoglobulin heavy chain Mm.162870.1 Bonp1: ICMP1 homolog igh-6: immunoglobulis heavy chain 6 (heavy chain of IgM) D2hodh:D-2-hydroxyolutarate delydrogenase Neto2 neuropiin (NFP) and tolloid (TLL)-like 2 Hspalacheat shockprotein W 16 days neonate thymus cDWA, RIXEN full-length enriched library Satb2: special AT-rich sequence binding protein 2 CodeNet colled-coll domain containing 88C H3F3b: H3 histore, family 31 Usp52: abiquitin specific peptidase 52 05 10040 DOSRik: RHEN dOWA 06 10040009 gene 5730601R06R9ic RIMEN cDMA 5730601F06 gene 3110040M11Nic RMEN cDMA 3110040M11gene Gp: gastric inhibitory polypeptide Trp53: Transformation related protein 53 Hist2h2bbchistone cluster2, H2bb Cd16311:00163 molecule-like 1



Hspallscheat shock protein 18 Nie202 nuclear factor, erythroid derived 2, like 2 Tch/ctricbolryalin Poliet pyruvate dehydrogenase kinase, isoenzyme 4 NupQ:nudeoporia 62 Theci: THO complex 1 Nn.23767.1 Cank2d: calcium/cain odulin-dependent protein linase II, delta Cap1: CAP, adenylate cyclase-associated protein 1 (yeast) Vps392 vacualar protein sorting 39 (yeast) Hspallscheat shock protein 18 A730016A17: hypotheticalLOC330538 Ralgps2: Rai GEF with PH domain and SH3b inding notif 2 Odn& daudin & D130051D11R8c RIVEN dDNA D130051D11 gene lgtbp?: Insulin-like growth factor binding protein 7 Pla2g4b: phospholipase A2, group I VII (cytosofic) Lgak&lectin, galactose binding, soluble 6 Hspallscheat shock protein 18 Ppfibp1:PTPRF interacting protein, binding protein 1 (lipin beta 1) Adult male corpora quadrigenina cDWA, RXEN full-length envicted library, Hisppd2x histidine add phosphatase domain containing 2A 1810012K16Filt INEN cDNA 1810012K16 gene Activated spleen CINA, INEN full-length enriched library Stip1: secreted frizzled-related protein 1 Nn.163127.1 Sip1: secreted frizzled-related protein 1 Dhrs1:dehydrogenase/reductase (SDR family) member 1 Th: 3 tetratricopeptide repeat domain 3 160002902100: FIKEN dDWA 1600029021 gene Transcribed locus Bublib: bodding uninhibited by benzimidazoles 1 homolog, beta (5 cerevisiae) Sk 15a2: solute carrier family 15 (H+/peptide transported, member 2 RgsScregulator of G-protein signaling 5 2700089E24Rik: RIKEH dDNA 2700089E24 gene Cep27:centrosomal protein 27 Stigal art 576 Hal: histódine annonia lyase Tubgop & tubulin, gamma complex associated protein 4 BC06407& cD1WA sequence IC064078 16 days embryo head cDNA, IIKEN full-length enriched library, clone C130044M19 SpataSII: spermatogenesis associated 5-like 1 Adult male urinary blad der cD NA, RMEH full-length enriched library, clones/530057N21 Stix22 supertillervisilicidic activity 24ile 2(S. cerevisiae)

Supplement Figure 11. Heat map for mRNAs that are significantly enriched or diminished in Atg16L1^{HM} thymocytes relative to control.

A 1.3-fold difference and a 90% lower bound cut-off were used.

Term	RT	Genes	Count	<u>%</u> \$	P-Value	Fold Enrichment	Bonferroni	<u>Benjamini</u>	<u>FDR</u>
PPAR signaling pathway	<u>RT</u>		8	6.0	2.0E-6	12.4	3.9E-4	3.9E-4	0.0
lipid metabolic process	<u>RT</u>		16	12.0	6.7E-6	4.1	3.4E-2	3.4E-2	0.0
lipid transporter activity	<u>RT</u>		6	4.5	1.4E-5	19.1	3.8E-2	3.8E-2	0.0
extracellular space	<u>RT</u>		29	21.8	1.9E-5	2.3	1.5E-2	1.5E-2	0.0
cellular lipid metabolic process	<u>RT</u>		14	10.5	3.0E-5	4.1	1.4E-1	7.4E-2	0.1
extracellular region part	<u>RT</u>		29	21.8	5.8E-5	2.2	4.4E-2	2.2E-2	0.1
inflammatory response	<u>RT</u>		9	6.8	6.1E-5	6.6	2.7E-1	9.9E-2	0.1
signal	<u>RT</u>	_	32	24.1	9.4E-5	2.0	7.8E-2	7.8E-2	0.1
response to wounding	<u>RT</u>		10	7.5	1.1E-4	5.2	4.4E-1	1.4E-1	0.2
acute inflammatory response	<u>RT</u>	=	6	4.5	1.3E-4	12.2	4.8E-1	1.2E-1	0.2
lipid binding	<u>RT</u>		11	8.3	1.5E-4	4.5	3.3E-1	1.8E-1	0.3
carboxylic acid metabolic process	<u>RT</u>	-	12	9.0	2.0E-4	3.9	6.4E-1	1.6E-1	0.4
organic acid metabolic process	<u>RT</u>		12	9.0	2.0E-4	3.9	6.5E-1	1.4E-1	0.4
regulation of cholesterol absorption	<u>RT</u>	-	3	2.3	2.1E-4	124.8	6.6E-1	1.3E-1	0.4
lipid transport	<u>RT</u>		6	4.5	2.2E-4	10.9	6.8E-1	1.2E-1	0.4
extracellular region	<u>RT</u>		29	21.8	2.3E-4	2.0	1.7E-1	5.9E-2	0.4
alcohol metabolic process	<u>RT</u>		9	6.8	2.7E-4	5.3	7.5E-1	1.3E-1	0.5
cholesterol absorption	<u>RT</u>	-	3	2.3	3.5E-4	99.8	8.3E-1	1.5E-1	0.7
Secreted	<u>RT</u>		19	14.3	3.5E-4	2.5	2.6E-1	1.4E-1	0.5
monocarboxylic acid metabolic process	<u>RT</u>	=	8	6.0	4.1E-4	5.8	8.8E-1	1.6E-1	0.8
signal peptide	<u>RT</u>		29	21.8	4.3E-4	1.9	1.0E0	1.0E0	0.9
lipid digestion	<u>RT</u>	-	З	2.3	5.2E-4	83.2	9.3E-1	1.9E-1	1.0
acute-phase response	<u>RT</u>	-	4	3.0	5.4E-4	24.7	9.4E-1	1.8E-1	1.0
lipoprotein	<u>RT</u>	-	12	9.0	6.1E-4	3.5	4.1E-1	1.6E-1	0.9
response to external stimulus	<u>RT</u>		11	8.3	7.1E-4	3.7	9.7E-1	2.2E-1	1.3

Term	RT	Genes	<u>Coun</u> t	<u>%</u> \$	<u>P-Value</u>	<u>Fold</u> Enrichment	Bonferron	<u>Benjamin</u> ‡	<u>FDR</u>
ubl conjugation	<u>RT</u>		7	7.5	1.1E-3	5.9	6.1E-1	6.1E-1	1.7
amino acid and derivative metabolic process	<u>RT</u>	=	6	6.5	5.1E-3	5.3	1.0E0	1.0E0	9.3
<u>cytoplasm</u>	<u>RT</u>		30	32.3	5.3E-3	1.5	9.8E-1	9.8E-1	7.8
regulation of catalytic activity	<u>RT</u>	-	6	6.5	7.4E-3	4.8	1.0E0	1.0E0	13.2
apoptosis	<u>RT</u>		8	8.6	8.3E-3	3.4	1.0E0	1.0E0	14.7
programmed cell death	<u>RT</u>	-	8	8.6	9.1E-3	3.3	1.0E0	1.0E0	16.0
alternative splicing	<u>RT</u>		21	22.6	9.8E-3	1.7	1.0E0	9.9E-1	14.2
amine metabolic process	<u>RT</u>	-	6	6.5	1.0E-2	4.5	1.0E0	1.0E0	17.6
chaperone	<u>RT</u>		4	4.3	1.0E-2	8.8	1.0E0	9.5E-1	14.6
metabolic process	<u>RT</u>		37	39.8	1.0E-2	1.3	1.0E0	1.0E0	17.7
<u>cell death</u>	<u>RT</u>		8	8.6	1.1E-2	3.2	1.0E0	1.0E0	19.0
<u>death</u>	<u>RT</u>	-	8	8.6	1.1E-2	3.2	1.0E0	1.0E0	19.2
regulation of a molecular function	<u>RT</u>		6	6.5	1.3E-2	4.2	1.0E0	1.0E0	22.4
nitrogen compound metabolic	<u>RT</u>	-	6	6.5	1.3E-2	4.2	1.0E0	1.0E0	22.4

Supplementary Figure 12. Functional analysis of enriched and diminished transcripts of Atg16L1-deficient Paneth cells. a-b, The Functional Classification Tool from the DAVID Bioinformatics Resource² (NIAID, NIH) was used to determine the enriched functional categories of the two gene lists in Supplementary Table 1 comparing the transcriptome of Atg16L1^{HM} versus WT Paneth cells (enriched (**a**) or diminished (**b**) in Atg16L1^{HM} mice). In both cases, the highest statistically significant gene ontology (GO) terms that were represented within the gene list are indicated. We used medium stringency to cluster the genes. Count = number of genes in the list with a given GO term; % = fractional representation of a GO term within the list. Various statistical methods are available to determine significance (p-value, fold-enrichment, Bonferoni, Benjamini, and false-discovery rate, FDR). Functional groups were sorted by p-value in these tables. The statistical significance of the clustering for the top rated GO terms is much higher in the gene list for the enriched transcripts.

WT HM1





Actin

LC3

Atg16L1

Supplementary Figure 13. Thymocytes from Atg16L1^{HM} mice have reduced Atg16L1 expression and conversion of LC3-I to LC3-II. Western blot analysis of whole cell thymocyte lysates from Atg16L1^{HM} mice reveal decreased Atg16L1 expression and an increase in LC3-I to LC3-II ratio compared to WT control.





15 days embryo male testis cDNA, RIKEN full-length enriched library, clone:8030447L24 6430706D22Rik: RIKEN cDNA 6430706D22 gene Cdk5rap1: CDK5 regulatory subunit associated protein 1 3 days neonate thymus cDNA, RIKEN full-length enriched library, clone:A630047A13 Mid1: midline 1 Gnb4: guanine nucleotide binding protein (G protein), beta 4 Phf17: PHD finger protein 17 Pou6f1: POU domain, class 6, transcription factor 1 D8Ertd82e: DNA segment, Chr 8, ERATO Doi 82, expressed 3 days neonate thymus cDNA, RIKEN full-length enriched library, clone:A630047A13 C77651: expressed sequence C77651 Atg16l1: autophagy-related 16-like 1 (yeast) Stk25: serine/threonine kinase 25 (yeast) Abca1: ATP-binding cassette, sub-family A (ABC1), member 1 Casp7: caspase 7 Pcbd2: pterin 4 alpha carbinolamine dehydratase Mgat5: mannoside acetylglucosaminyltransferase 5 Ccnb1: cyclin B1 Ctse: cathepsin E Hbb-b1 /// Hbb-b2: hemoglobin, beta adult major chain or minor chain Aurkb: aurora kinase B Bub1: budding uninhibited by benzimidazoles 1 homolog (S. cerevisiae) Cenpf: centromere protein F Hba-a1 /// Hba-a2: hemoglobin alpha, adult chain 1 /// hemoglobin alpha, adult chain 2 Dapk1: death associated protein kinase 1 Ramp1: receptor (calcitonin) activity modifying protein 1

Supplement Figure 14. Heat map for mRNAs that are significantly enriched or diminished in Atg16L1^{HM} Paneth cells relative to control.

A 1.3-fold difference and a 90% lower bound cut-off were used.