Supplementary Figure 1 Nod1 ligands induce autophagy in vivo and in vitro. (a) Fluorescence microscopy of GFP-LC3 transduced HeLa cells stimulated by C12-ieDAP (2.5 µg/ml) for 4h or rapamycin (Rapa, 50 mg/ml) for 2 h in medium (−) or in the presence of chloroquine (+CQ, 50 µM) or bafilomycin A1 (+Baf A1 100 nM). Green, GFP-LC3; blue, DAPI. Scale bars, 5 µm. (b) Immunoblot analysis of LC3-II conversion in HEK293 cells transfected with GFP-LC3 and left unstimulated (Unstim) or treated with MDP (10 µg/ml), or M-triDAP (1 mM) for 8 h. As positive controls the cells were treated with rapamycin (50 µg/ml) or starved (Starv) for 2 h. (c) Immunofluorescence microscopy of macrophages recovered from the peritoneal cavity of thioglycolate elicitated wild-type, Nod1- or Nod2-deficient mice, injected i.p with PBS or Rapamycin (300 µg/ml) for 4 h, cytopun and stained for endogenous LC3. Red, LC3; blue, DAPI. Scale bars, 10 µm. (d) Quantification of LC3 signals in experiments performed as in c. Error bars, s.d. At least 100 cells were counted in each condition. (e) Electronmicrographs of BMDM from WT and Nod2-deficient mice, treated with MDP or rapamycin. Data is representative of one out of four (a), three (b) and two (c-e) experiments performed.

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Supplementary Figure 2 ATG5-deficient cells do not wrap *Shigella* in LC3\(^+\) compartments. Confocal fluorescence microscopy of GFP-LC3 transduced wild-type and ATG5-deficient MEFs left unstimulated (Unstim) or treated with rapamycin (Rapa, 50 µg/ml) for 2 h or infected with *S. flexneri* M90T-RFP strain for 0.5 h then incubated for 0.5 h in the presence of gentamicin. Green, GFP-LC3; red, RFP. Data represent one out of two independent experiments.
Supplementary Figure 3 Decreased autophagy in Nod1-deficient MEFs infected with *Shigella* (a) Immunoblot analysis of LC3-II conversion in GFP-LC3 transduced MEFs from wild-type and Nod1−/− mice infected with *S. flexneri* M90T for 0.5 h and the incubated in the presence of gentamicin for different time periods with (+CQ) or without (−) chloroquine to block lysosomal turnover of LC3. Data is representative of one out of two independent experiments.
Supplementary Figure 4 Nod1-deficient cells display normal basal- and stress-induced autophagy. (a) Confocal fluorescence microscopy of GFP-LC3 transduced MEFs from wild-type and Nod1-deficient mice left unstimulated (Unstim), treated with chloroquine (CQ, 50 µM) or starved (Starv) or for 2h. (b,c) Quantification of GFP-LC3 signals in GFP-LC3 transduced MEFs from wild-type and Nod1−/− mice left unstimulated and treated with CQ (b) or starved (c) for 2 h. (d) Immunoblot analysis of the LC3-II conversion in GFP-LC3 transduced MEFs left unstimulated, starved or treated with rapamycin (50 µg/ml) for 2 h in the presence or in the absence (−) of the V-ATPase inhibitor bafilomycin A1 (+Baf A1, 100 nM). Green. GFP-LC3. Scale bar, 10 µm. Error bars, s.d. At least 100 cells were counted in each condition. Data represent one out of three (a,b and c) and two (d) experiments.

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Supplementary Figure 5 Decreased autophagy in Nod1-deficient cells during *Listeria* infection. (a) Confocal fluorescence microscopy of GFP-LC3 transduced wild-type and Nod1<sup>−/−</sup> MEFs infected with *L. monocytogenes* for 1 h and then incubated in medium containing gentamicin for 1 h. (b) Quantification of GFP-LC3 signals co-localizing with *L. monocytogenes* in experiments performed as in a. (c) Immunoblot analysis of LC3-II conversion in GFP-LC3 transduced wild-type and Nod1-deficient infected with *L. monocytogenes* for 1 h then incubated with gentamicin for different time periods with or without CQ (50 µM) to block lysosomal turnover of LC3. (d) Confocal fluorescence microscopy of GFP-LC3 transduced wild-type and Nod1-deficient MEFs infected with *L. monocytogenes* Δ*hly* for 1 h and then incubated in medium containing gentamicin for 1 h. (e) Quantification of GFP-LC3 signals co-localizing with *L. monocytogenes* Δ*hly* in experiments performed as in d. Green, GFP-LC3; red, Texas Red-stained bacteria. Scale bar, 10 µm. Error bars, s.d. Data represent one out of three experiments. *, *P* < 0.05; ** *P* < 0.01 (t-test).
Supplementary Figure 6 Nod2 colocalization at plasma membrane is specific to ATG16L1. (a) Cross-line scans depicting fluorescence intensity in confocal immunofluorescence microscopy images of HeLa cells expressing HA-tagged Nod2 and FLAG-tagged DN85-ATG16L1. Red, HA-Nod2; green, Flag-ΔN85-ATG16L1. (b) Cross-line scans depicting fluorescence intensity in confocal immunofluorescence microscopy images of HeLa cells expressing HA-tagged Nod2 and FLAG-tagged unrelated control protein GFP. Red, HA-Nod2; green, GFP-Flag. Scale bars, 8 µm. Data are representative of two experiments.
**Supplementary Figure 7** Modek cells express high amounts of functional Nod2. (a) Expression of Nod2 mRNA in Modek cells or splenocytes (Spleen) was measured by RT-PCR. Arrows indicate the molecular sizes in base pairs (bp) of the amplified products. (b) Secretion of the chemokine KC from Modek cells left unstimulated (Unstim) or stimulated with MDP (5 µg/ml) for 18 h was measured by ELISA. (c) Induction of autophagosome formation by L18-MDP or rapamycin (Rapa) in GFP-LC3 transduced Modek cells incubated with or without chloroquine (CQ). Green, GFP-LC3; blue, DAPI. Scale bar, 10 µm. Error bars, s.d. Data are representative of two experiments.
Supplementary Figure 8 Nod2 colocalizes with ATG16L1 but not ATG5. (a) Cross-line scans of confocal immunofluorescence microscopy images of Modek cells, depicting fluorescence intensity related to endogenous Nod2 and ATG16L1 expression. Red, Nod2; Green, ATG16L1. (b) As a control for the ATG16L1 antibody specificity, we transduced Modek cells with GFP-LC3 and treated them with rapamycin for 1 h. Arrows indicate the colocalization of GFP-LC3 signals with ATG16L1. Green, GFP-LC3; red, ATG16L1; blue, DAPI. (c) Immunofluorescence microscopy images of HeLa cells stained for endogenous Nod2 and ATG5. Green, Nod2; red, ATG5; blue, DAPI. Arrows indicate lack of colocalization between endogenous Nod2 and ATG16L1. Scale bar, 10 µm. Data are representative of two experiments.
Supplementary Figure 9 The Crohn’s disease-associated ATG16L1 polymorphism colocalizes and interacts with Nod1 and Nod2. (a) Immunofluorescence microscopy of HeLa cells expressing the myc-tagged full-length ATG16L1 risk allele (FL-ATG16L1—*300A). The image on the right is an enlargement of the fields outlined on the left. Green, FL-ATG16-Myc; blue, DAPI. (b,c) Immunofluorescence microscopy of HeLa cells co-expressing HA-tagged Nod1 (b) or HA-tagged Nod2 (c) and Myc-tagged FL-ATG16L1 (*300A). The outlined field in the top left image is shown enlarged in the other images. Green, FL-ATG16L1-Myc (*300A); red, HA-Nod1 or HA-Nod2; blue, DAPI. Arrows indicate protein localization at the plasma membrane. (d) Immunofluorescence microscopy of HeLa cells expressing a FLAG-tagged truncated form of the ATG16L1 risk allele (ΔN85-ATG16L1—*300A). The image on the right is an enlargement of the field outlined on the left. Green, Flag-ΔN85-ATG16L1 (*300A); blue, DAPI. Scale bar, 10 µm. (e,f) HEK293 cells were transfected with Flag-tagged ΔN85-ATG16L1 (*300A) (e) or Myc-tagged FL-ATG16L1 (*300A) (f), together with HA-tagged Nod1 or HA-tagged Nod2. Twenty-four hours after transfection, cells lysates were prepared, immunoprecipitated with anti-Flag or anti-Myc and subjected to immunoblotting analysis with the indicated antibodies. Data are representative of three independent experiments.
**Supplementary Figure 10** Nod1 recruits ATG16L1 to the bacterial entry site to promote autophagy. Immunofluorescence microscopy of HeLa cells expressing Flag-tagged ΔN85-ATG16L1 and HA-tagged Nod1 and infected with *S. flexneri* M90T for 10-20 min. Green, Flag-ΔN85-ATG16L1; red, HA-Nod1, blue, DAPI. Scale bar, 5 µm. Data is representative of three experiments.