Supplementary Materials and Methods

Cell Fractionation

MEFs were treated with the indicated concentrations of LLOMe for three hours, washed with ice-cold PBS, collected by centrifugation, and then homogenized in homogenization buffer (10 mM HEPES, 0.25 M sucrose, 10 mM Na₂EDTA, adjust to pH 7.0 with NaOH, 1 × protease inhibitor cocktail [Roche]; for centrifugation at 100,000 × g; homogenization buffer was supplemented with 1 mM KCl) by repeatedly shearing 25 times through a 25-gauge needle mounted on a 1 ml syringe. After centrifugation at 1,000 × g for 10 min, the post-nuclear supernatants were further centrifuged at 100,000 × g for 60 min to generate supernatant and pellet fractions. Equivalent volume of each fraction were examined by Western blot analysis.

Live-cell imaging

NIH3T3 cells were grown on glass bottom dishes (Matsunami Glass). After addition of
LLOMe with or without Lysotracker, the glass dish was mounted onto the microscope stage, which was equipped with a humidified environment chamber (MI-IBC, Olympus).

Images were acquired using an inverted microscope Olympus IX81 under the control of MetaMorph (MDS Analytical Technologies).

**MSU Preparation**

MSU crystals were prepared according to a previously described method (Nishimura et al., 1997). Crystals were dispersed into medium by repeatedly triturating through a 27-gauge needle before they were added in culture medium.
Supplementary Figure legends

Figure S1. Lysosomal Membrane Rupture Induces Lipidation of LC3

(A) MEFs were treated with the indicated concentrations of LLOMe for 3 h, and cell homogenates were fractionated. Equivalent volume of each fractions were subjected to Western blotting using the following antibodies: anti-Transferrin receptor (TfR; top), anti-GAPDH, anti-PDI, and anti-Cathepsin D (bottom). PNS: post-nuclear supernatant; S: supernatant; P: pellet. (B and C) J774 cells (B) or MEFs (C) were treated with the indicated concentrations of LLOMe or silica for the indicated time, and analyzed by Western blotting for LC3 and α-tubulin. (D) J774 cells were treated with or without 1,000 µM LLOMe or 250 µg/ml silica for 3 h, and subjected to immunocytochemistry for LC3 and Lamp1. Green: Lamp; Magenta: LC3. Bar: 10 µm.

Figure S2. The Recruitment of Gal3 to Lysosomes Under lysosomal damage conditions
(A) NIH3T3 cells stably expressing GFP-Gal3 were incubated with the indicated concentrations of LLOMe for 3 h. The number of GFP-Gal3 puncta positive cells was counted (Left Graph). The data represent means ± s.d. At least 300 cells were counted (n=3). The number of Gal3 puncta per Gal3 puncta positive cell was counted by G-Count (Right Graph). The data represent means ± s.d. At least 40 cells were counted (n=3). (B) MEFs stably expressing GFP-Gal3 were treated with or without 1,000 µM LLOMe for 3 h. Cells were incubated with 75 nM Lysotracker 1.5 h prior to fixation and subjected to immunocytochemistry for Lamp1. Bar: 20 µm. (C) NIH3T3 cells stably expressing CFP-Gal3 and YFP-LC3 were treated with or without 250 µM H₂O₂ for 10 h, and subjected to immunocytochemistry for Lamp1. Bar: 20 µm. (D) NIH3T3 cells stably expressing YFP-LC3 were incubated with 500 µM LLOMe for 1.5 h, and subjected to immunocytochemistry for Gal3. Bar: 10 µm.

Figure S3. GFP-Atg5 is Recruited to mStrawberry-Gal3 Puncta That is Not Stained
with Lysotracker

(A) NIH3T3 cells stably expressing GFP-Gal3 were incubated with 1,000 µM LLOMe and 75 µM Lysotracker, and images were then taken at 30 sec intervals using a fluorescence inverted microscope (See also Supplemental Movie1). Two different images (a and b) are shown. Green: GFP-Gal3; Magenta: Lysotracker. (B) NIH3T3 cells stably expressing GFP-Atg5 and mStrawberry-Gal3 were incubated with 1,000 µM LLOMe and images were taken at 30 sec intervals using a fluorescence inverted microscope (See also Supplemental Movie2). Three different images (a, b and c) are shown. Arrows indicate Gal3 puncta to which GFP-Atg5 is recruited. (C) NIH3T3 cells stably expressing mStrawberry-Gal3 along with GFP-tagged ULK1, Atg9L1, Atg14L, WIPI1, or Atg5 were treated with or without 1,000 µM LLOMe or 250 µg/ml silica for 2 h, and subjected to immunocytochemistry for Lamp1. Bar: 20 µm.

Figure S4. The Lipidated Level of LC3 by Temporal LLOMe Treatment

(A and B) The number of Lamp1 (A) or GFP-Gal3 (B) puncta was quantified by G-Count.
Then, the percent of GFP-Gal3 positive Lamp1 puncta per cell was determined (see in Figure 2A). The data represent means ± s.d. At least 80 cells were counted (n=3). (C and D) NIH3T3 cells expressing empty vector (control) were treated with or without 1,000 µM LLOMe for 1 h and cultured in the absence of LLOMe for the indicated time. Cell lysates were subjected to Western blotting for LC3 and α-tubulin (C). Band intensities were measured by ImageGauge (D). (E and F) NIH3T3 cells stably expressing tfLC3 were treated as (C) and subjected to immunocytochemistry for Gal3. Bar: 20 µm (E). Colocalization efficiency was measured by ImageJ software (F). The data represent means ± s.d. At least 30 cells were counted (n=3).

Figure S5. The kinetics of Damaged Lysosomes in Atg7-deficient MEFs.

(A, B and C) Atg7+/+ and Atg7−/− MEFs stably expressing GFP-Gal3 were treated as Figure 2A, and subjected to immunocytochemistry for Lamp1. The number of GFP-Gal3 (B) or Lamp1 (C) puncta per cell was quantified using G-Count. Then, the percent of GFP-Gal3–positive Lamp1 puncta was determined (A). The data represent means ± s.d. At least 70
cells were counted (n=3). (D) Wild type MEFs stably expressing GFP-Gal3 were treated with 1,000 µM LLOMe for 1 h. After LLOMe washout, cells were incubated with 75 nM Lysotracker 1.5 h prior to fixation and subjected to immunocytochemistry for Lamp1. Bar: 20 µm. (E) Atg7+/+ and Atg7−/− MEFs were treated with 1,000 µM LLOMe for 1 h. After LLOMe washout, cells were incubated with 75 nM Lysotracker 1.5 h prior to fixation and subjected to immunocytochemistry for Lamp1. The number of Lysotracker or Lamp1 puncta was counted by G-Count. Then, the percentage of Lysotracker-negative Lamp1 puncta was determined. The data represent means ± s.e. At least 80 cells were counted (n=3). Statistical analyses were performed using a two-tailed unpaired t test; p values < 0.01 were considered statistically significant. (F and G) Atg7+/+ and Atg7−/− MEFs were treated as Figure 2A, and subjected to immunocytochemistry for Gal3 and DAPI (F). Green: Gal3; Blue: DAPI. Bar: 20 µm. The number of Gal3 puncta per cell was quantified using G-Count (G). The data represent means ± s.d. At least 30 cells were counted (n=3). (H) NIH3T3 stably expressing empty vector (control) or mStrawberry-Atg4B<sup>C74A</sup> (Atg4B mutant) were treated with 1,000 µM LLOMe for 1 h. After LLOMe washout, cells were
cultured in the presence or absence of 25 μg/ml Cycloheximide (CHX) for the indicated time. Cell lysates were subjected to Western blotting for Lamp1, p62 and GAPDH. The protein level of p62 decreased by CHX.

Figure S6. The Kinetics of the Damaged Lysosome Under Long Exposure of LLOMe.

(A, B and C) NIH3T3 cells stably expressing GFP-Gal3 and either empty vector (control) or mStrawberry-Atg4B<sup>C74A</sup> (Atg4B mutant) were incubated with 1,000 μM LLOMe for the indicated time. Cells were fixed and subjected to immunocytochemistry for Lamp1 and DAPI. The number of GFP-Gal3 (B) or Lamp1 (C) puncta per cell was quantified using G-Count. Then, the percent of GFP-Gal3-positive lysosomes was determined (A). The data represent means ± s.d. At least 70 cells were counted (n=3).

Figure S7. Ultrastructure of Lysosomes in LLOMe-untreated HeLa Cells

(A–D) mStrawberry-Gal3 transfected HeLa cells stably expressing GFP-LC3, not treated with LLOMe, were fixed and subjected to CLEM analysis.
Figure S8. Autophagy-Deficient Renal Tubules Exhibit Abnormal Distribution of Lysosomes and Accumulation of Ubiquitin Positive Structures Under Hyperuricemia

(A) GFP-LC3 transgenic mice treated with vehicle or UA+OA (n = 3). Kidney sections were subjected to immunocytochemistry for megalin (red), a marker of proximal tubules, and DAPI (blue). Bar: 10 µm. (B) Plasma uric acid in Atg5<sup>F/F</sup> and Atg5<sup>F/F;KAP</sup> mice treated with vehicle or UA+OA (n=4 to 7). Data represent means ± s.e. Statistically significant differences (*p < 0.05) are indicated. N.S.: not significant. F/F: Atg5<sup>F/F</sup> mice. F/F;KAP: Atg5<sup>F/F;KAP</sup> mice. (C) Kidney cortexes from Atg5<sup>F/F</sup> and Atg5<sup>F/F;KAP</sup> mice treated with vehicle or UA+OA were subjected to immunohistochemical analysis of Lamp2 and megalin. Brown: Lamp2; Blue: megalin. F/F: Atg5<sup>F/F</sup> mice; F/F;KAP: Atg5<sup>F/F;KAP</sup> mice. Bar: 40 µm. (D and E) Kidney cortexes from Atg5<sup>F/F</sup> and Atg5<sup>F/F;KAP</sup> mice treated with vehicle or UA+OA were subjected to immunohistochemical analysis of ubiquitin and DAPI (n = 4 to 7). Green: ubiquitin; Blue: DAPI. F/F: Atg5<sup>F/F</sup> mice; F/F;KAP: Atg5<sup>F/F;KAP</sup> mice. Bars: 20 µm (D). The number of ubiquitin positive puncta was counted (E). The data
represent means ± s.e. Statistically significant differences (*p < 0.05) are indicated. (F) Kidney cortaxes from Atg5^{F/F} and Atg5^{F/F};KAP mice treated with vehicle or UA+OA were subjected to immunohistochemical analysis of Lamp1 and ubiquitin. Green: ubiquitin; Magenta: Lamp1; Blue: DAPI. F/F: Atg5^{F/F} mice; F/F;KAP: Atg5^{F/F};KAP mice. Bars: 10 μm.

**Figure S9. The Recruitment of Ubiquitin and LC3 to Damaged Lysosomes by MSU crystals in Proximal Tubular Cells**

(A) Atg5-revertant (Atg5-positive) proximal tubular cells were treated with the indicated concentrations of MSU crystals for the indicated time, and analyzed by Western blotting for LC3 and α-tubulin. (B and C) Atg5-revertant (Atg5-positive) and Atg5-knockout (Atg5-negative) proximal tubular cells stably expressing GFP-Gal3 (B) or mStrawberry-Ub (C) were treated with 2 mg/ml MSU crystals for 4 h, fixed, and subjected to immunocytochemistry using the following antibodies: anti-LC3 and anti-Lamp1 (B) or anti-p62 and anti-Gal3 (C). Bar: 10 μm.
**Movie S1.** The LLOMe-induced GFP-Gal3 puncta was never stained with Lysotracker

NIH3T3 cells stably expressing GFP-Gal3 were incubated with 1,000 µM LLOMe for 30 min and images were taken in the presence of LLOMe and 75 nM Lysotracker Red. Green: GFP-Gal3; Magenta: Lysotracker.

**Movie S2. The recruitment of GFP-Atg5 to mStrawberry-Gal3 puncta**

NIH3T3 cells stably expressing GFP-Atg5 and mStrawberry-Gal3 were incubated with 1,000 µM LLOMe for 30 min and images were taken in the presence of LLOMe. Green: GFP-Atg5; Magenta: mSt-Gal3.
Supplementary Figure 5

A. GFP-Gal3 positive Lamp1 puncta (%)

B. GFP-Gal3 puncta/cell

C. Lamp1 puncta/cell

D. GFP-Gal3, Lysotracker, Lamp1, Merge

E. Lysotracker (-) Lysosomes / Total Lysosomes (%)

F. Time after LLOMe washout (h)

G. Endo-Gal3 puncta/cell

H. LLOMe CHX

<table>
<thead>
<tr>
<th>Control</th>
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<tr>
<td>Time after LLOMe washout (h)</td>
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Supplementary Figure 9

A

Untreatment MSU Untreatment MSU
Atg5 (+) Atg5 (-)

B

Untreatment MSU Untreatment MSU
Atg5 (+) Atg5 (-)
mSt-Ub Gal3
p62 Merge

C

Untreatment MSU Untreatment MSU
Atg5 (+) Atg5 (-)
p62 mSt-Ub Gal3 Merge