Expanded View Figures

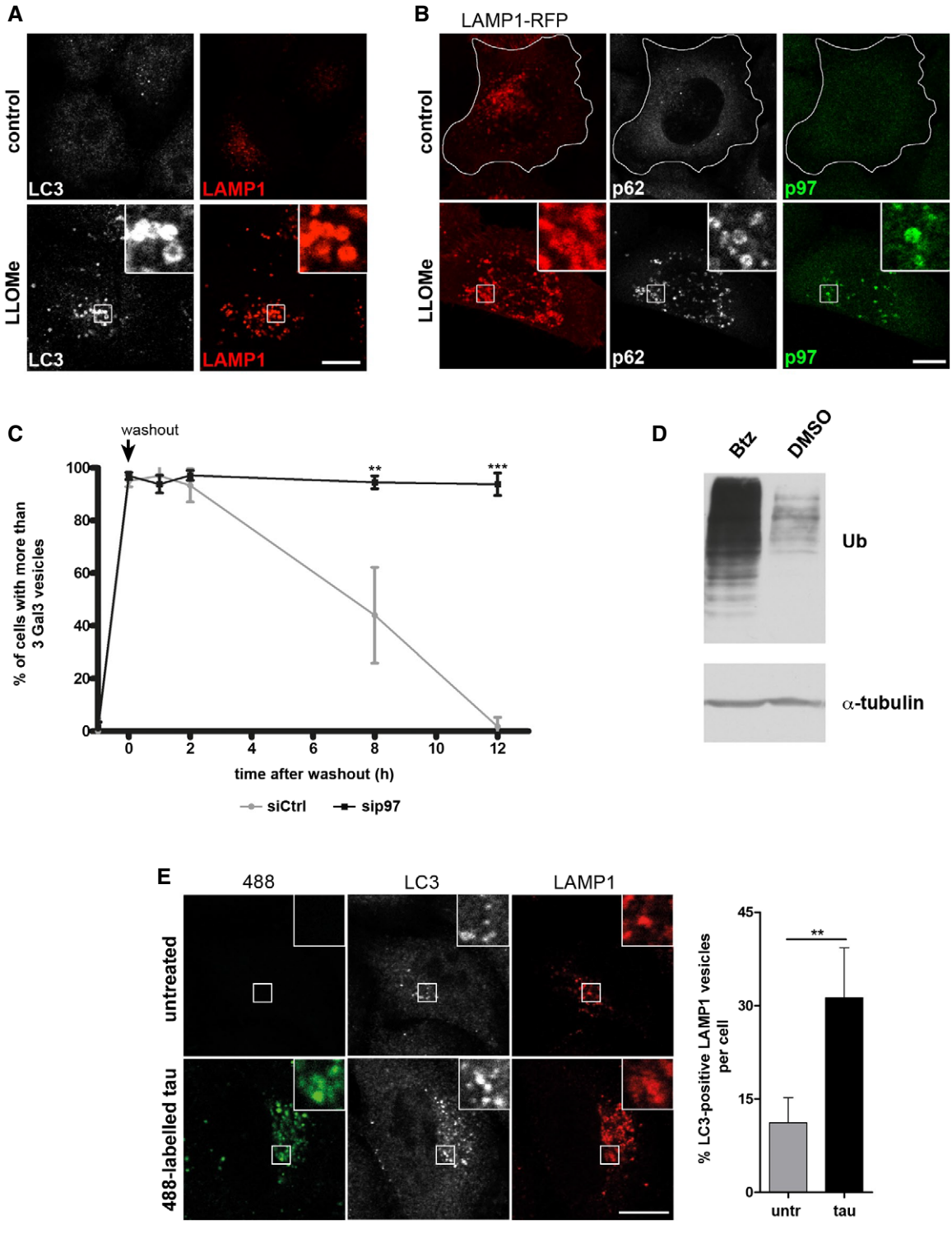

Figure EV1.

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Figure EV1. p97 is a direct component of the lysosome damage response (related to Fig 1).

A LLOMe ruptures lysosomes and induces an autophagic response. HeLa cells were treated with vehicle alone (control) or LLOMe for 1 h to induce lysosome damage. Cells were fixed 2 h after washout, stained with LAMP1 and LC3 antibodies, and analyzed by confocal immunofluorescence microscopy.

B Endogenous p97 translocates to ruptured lysosomes. Cells transiently expressing LAMP1-RFP were treated as in (A) and stained for endogenous p97 and p62.

C Quantification of the percentage of cells with more than three Gal3 vesicles per cell for each condition from Fig 1D.

D Cells were treated with 10 nM bortezomib (Btz) or with vehicle alone (DMSO) for 12 h and ubiquitin conjugates (Ub) in whole-cell lysates were detected by immunoblotting.

E Tau fibrils are endocytosed and induce autophagy. Tau fibrils were generated *in vitro*, fluorescently labeled by Dylight 488, and fragmented by sonication (488-labeled tau). HeLa cells were incubated with 300 nM tau fibrils for 24 h, fixed, and stained with LC3 and LAMP1 antibodies. Untreated cells served as a control. Quantification of the fraction of LC3-positive LAMP1 vesicles per cell.

Data information: Data represent mean ± SD from three independent experiments. $^{**}P < 0.01$ (one-sided Welch's *t*-tests). Scale bars, 10 μm.
Source data are available online for this figure.

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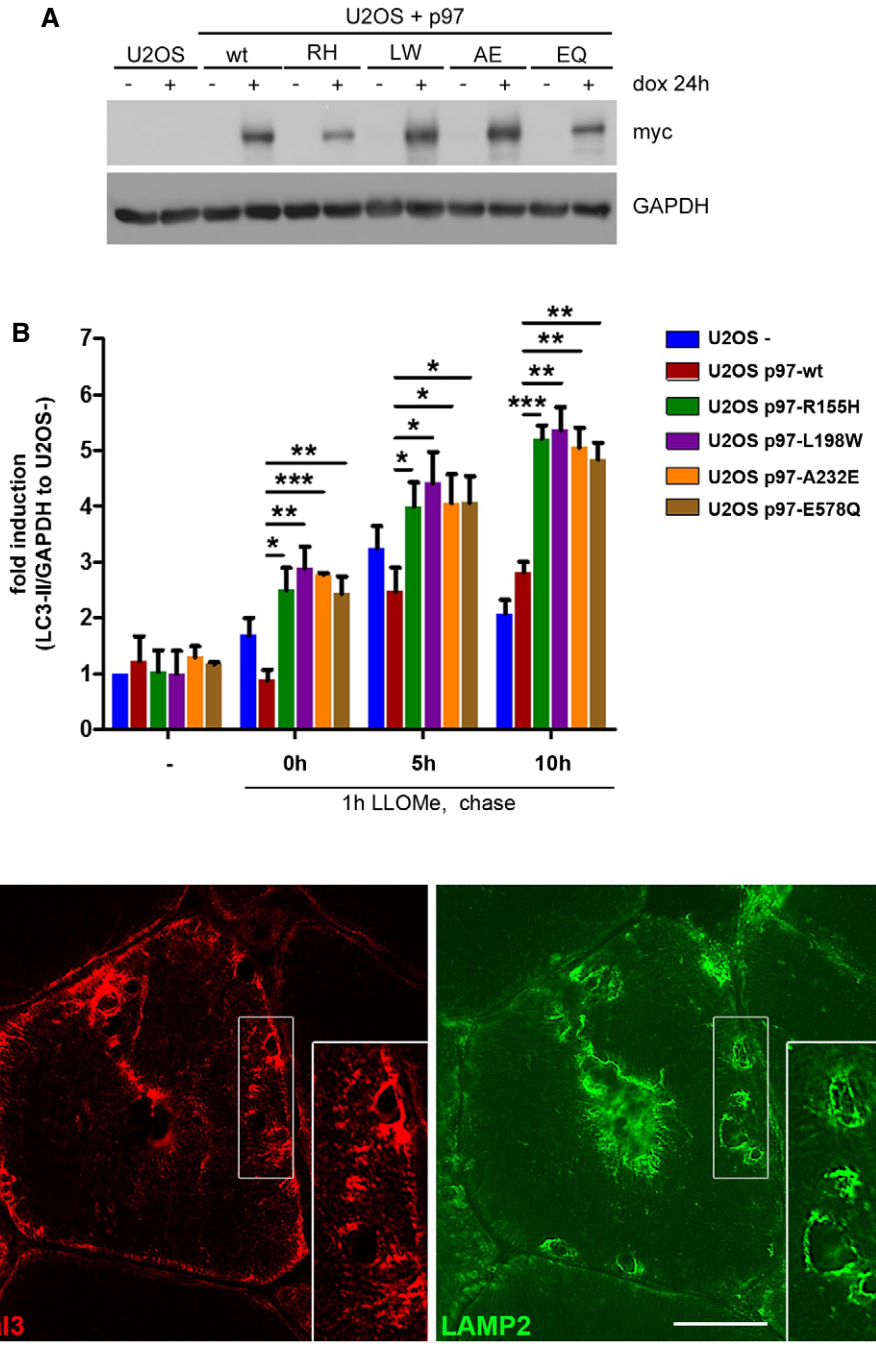

Figure EV2. Compromised clearance of damaged lysosomes in p97 mutant-expressing cells, and vacuolated muscle fibers from a p97 patient are positive for Gal3 and LAMP2 (related to Fig 2).

A Induction and expression levels of myc-tagged p97 and variants in cells used in Fig 2B were determined via immunoblotting.

B Quantification of LC3-II level normalized to GAPDH from three independent experiments (mean ± SD, Student's *t*-test). LC3-II level in U2OS cells treated with doxycycline (U2OS-) were set to fold change = 1. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

C Skeletal muscle biopsy from a p97-R155H patient. Serial sections were immunostained for Gal3 (red) and LAMP2 (green) and demonstrate colocalization in areas of lysosomal membrane accumulation within vacuolated fibers. Scale bar, 25 μm.

Source data are available online for this figure.

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Figure EV3. Characterization of ELDR component depletion and overexpression (related to Fig 3).

A Depletion efficiency of siRNA oligos used in Fig 3 was confirmed in HeLa cells by immunoblotting of total lysates with specific antibodies as indicated.

B YOD1, UBXD1, and PLAA localize to lysosomes upon damage. HeLa cells co-transfected with mCherry-Gal3 and GFP-p97-EQ, YOD1-HA, UBXD1-HA, PLAA-HA, VCPIP1-HA, or p47-HA were treated with vehicle alone (control) or LLOMe for 1 h and chased for 2 h. After staining with anti-HA and anti-LAMP1 antibodies, the percentage of cells showing colocalization of ELDR components with LAMP1 or Gal3 was calculated from three independent experiments (mean ± SD).

C HeLa cells were transfected with indicated siRNA oligos and treated with 200 nM bafilomycin A_1 (Baf A1) for 4 h or left untreated (control). Lysates were analyzed for LC3 and α-tubulin. Quantification of LC3-II levels normalized to α-tubulin from three independent experiments. LC3-II level in control-depleted cells (siCtrl) treated with Baf A1 was set to fold change = 1 (mean ± SD, Student's *t*-test for control treated samples is shown, and one-sample *t*-test for Baf A1-treated samples revealed no significant changes). ns, not significant. $*P < 0.05$; $**P < 0.01$.

D HeLa cells transfected with indicated siRNA oligos were treated with LLOMe for 1 h and lysed directly or 10 h after LLOMe washout in the presence of Baf A1 or without. LC3-II levels were analyzed as in (C).

E The ATP-bound state of p97 stabilizes interaction with UBXD1 and PLAA. Stable HEK293 cell lines were doxycycline-induced to express p97 wild-type (wt) or the ATPase mutant E578Q (EQ), and transiently transfected with UBXD1-GFP. UBXD1-GFP was isolated and co-precipitating proteins were detected by immunoblotting with indicated antibodies. Arrowheads indicate endogenous and induced p97-myc-strep.

F Cells were processed for co-immunoprecipitation as in (E), but with GFP-PLAA instead of UBXD1-GFP. The asterisks indicate non-specific bands. Arrowheads indicate endogenous and induced p97-myc-strep.

G Trapping of ubiquitin conjugates using the catalytically inactive YOD1 mutant. Cells were transfected with empty vector (−), GFP-YOD1-wt, or the YOD1-C160S catalytic inactive mutant (CS). GFP or GFP-YOD1 was isolated (stained by Ponceau S) and co-precipitating ubiquitin conjugates (Ub) were detected by immunoblotting.

Source data are available online for this figure.

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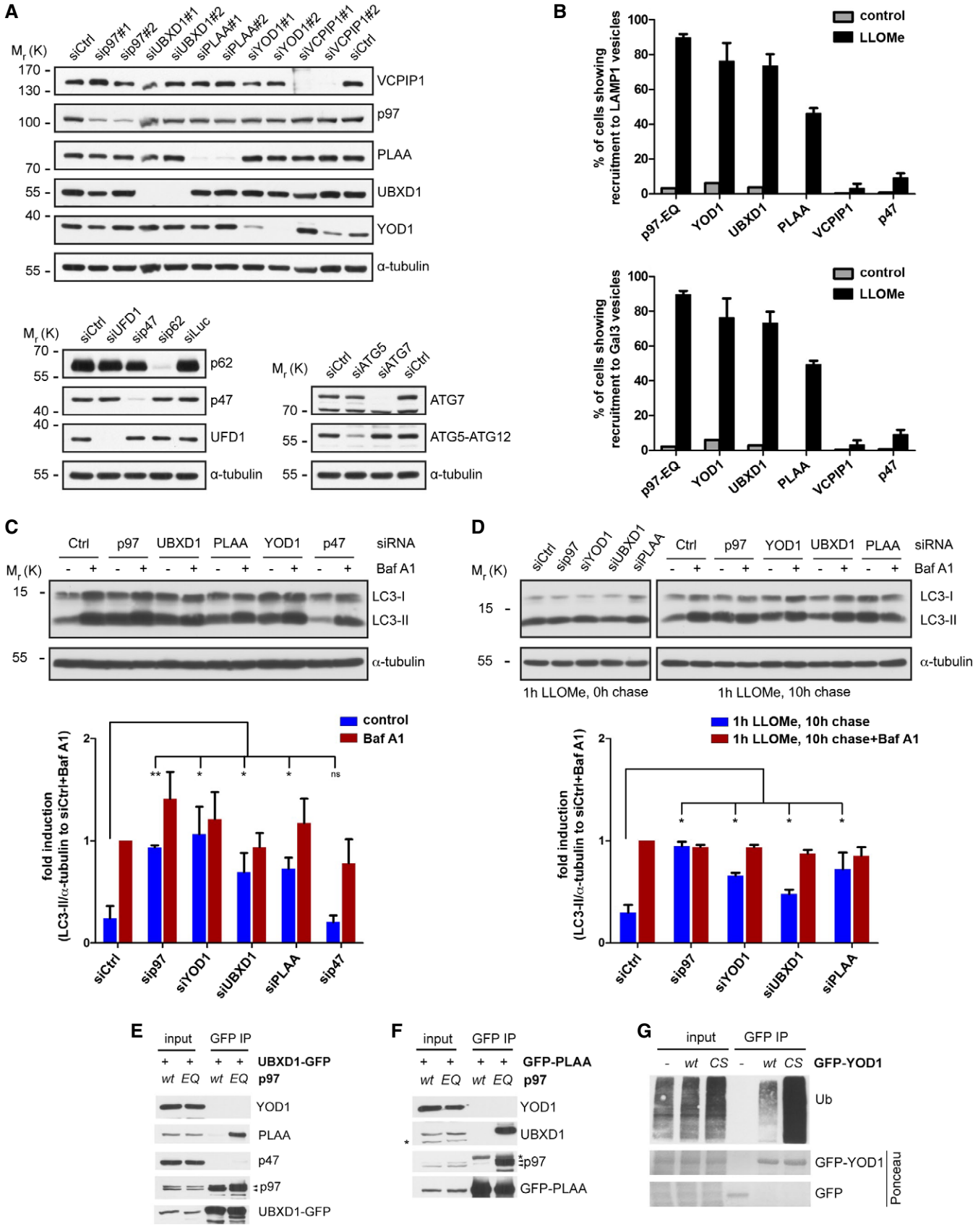

Figure EV3.

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Figure EV4. The ELDR components localize to damaged lysosomes (related to Fig 4).

A p97 localizes to non-acidified, damaged lysosomes. HeLa cells transfected with LAMP1-RFP, p97-wt-mCherry or EQ, or mCherry-Gal3 marker proteins were treated with LLOMe for 1 h and chased for 2 h. Cells were incubated with the pH-sensitive LysoTracker probe (100 nM, Invitrogen) for 1 h and imaged live.

B Cells transfected with GFP-p97-EQ or GFP-YOD1-CS were treated as in (A), incubated with the cathepsin B substrate MR-(RR)2 (Magic Red Cathepsin B detection kit, ImmunoChemistry Technologies, LLC), and imaged live by confocal spinning disk microscopy.

C Cells co-transfected with mCherry-Gal3 and GFP-p97-EQ or GFP-YOD1-CS were treated as in (A) and stained for LAMP1.

D Endogenous p97 localizes to K48-decorated lysosomes after damage. LLOMe-treated HeLa cells were chased for 2 h, fixed, and stained with antibodies specific for K48 chains and p97. Quantification of K48-positive p97 vesicles from three independent experiments (77.8% ± 9.6, mean ± SD).

E Lysosomes that recruit ELDR components are also positive for K63 chains. Detection of UBXD1-GFP alone, or PLAA-HA co-transfected with GFP-p97-EQ, 2 h after LLOMe washout and co-stained with indicated antibodies.

F Quantification of results in Fig 4F and panel (E). Percentage of YOD1, UBXD1, or PLAA vesicles positive for K63 or K48 chains was determined (mean ± SD).

Data information: Scale bars, 10 μm.

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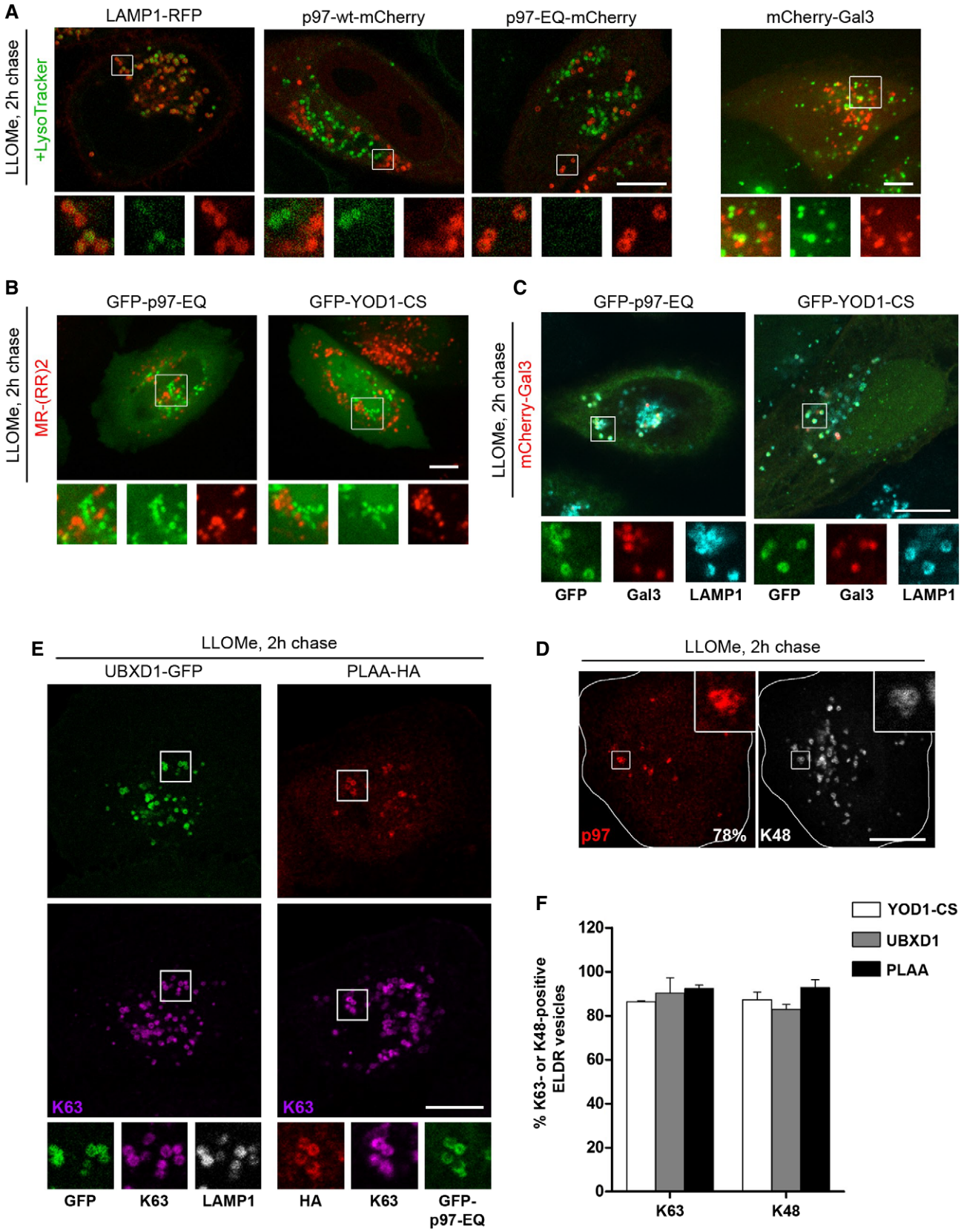
A
LAMP1-RFP
p97-wt-mCherry
p97-EQ-mCherry
mCherry-Gal3
LLOMe, 2h chase
+LysoTracker
B
GFP-p97-EQ
GFP-YOD1-CS
LLOMe, 2h chase
MR-(RR)2
C
GFP-p97-EQ
GFP-YOD1-CS
LLOMe, 2h chase
mCherry-Gal3
GFP
Gal3
LAMP1
GFP
Gal3
LAMP1
E
LLOMe, 2h chase
UBXD1-GFP
PLAA-HA
K63
K63
GFP
K63
LAMP1
HA
K63
GFP-p97-EQ
D
LLOMe, 2h chase
p97
78%
K48
F
YOD1-CS
UBXD1
PLAA
% K63- or K48-positive ELDR vesicles
120
100
80
60
40
20
0
K63
K48

Figure EV4.

Figure EV5. The ELDR components remove K48-linked ubiquitin conjugates from damaged lysosomes (related to Fig 5).

A Timing of p62, LC3, and p97 recruitment correlates with K63 and K48 ubiquitination, respectively. HeLa cells were transfected with GFP-p97 (lower panel) or left untransfected (upper and middle panel), treated with LLOMe, and chased for the indicated time points. Cells were fixed and stained for endogenous p62 or LC3. Percentage of cells showing recruitment of p62, LC3, or GFP-p97 to LAMP1 vesicles was determined (mean ± SD from three independent experiments).

B K48 conjugates persist upon depletion of ELDR components. HeLa cells were transfected with control (Ctrl), UBXD1, or PLAA siRNA oligos and treated with LLOMe, chased for 12 h, and then fixed and stained with antibodies specific for K48 chains. Percentage of cells with persistence of K48 vesicles was determined (mean ± SD from three independent experiments, Student's *t*-test).

C The catalytically inactive YOD1 mutant is trapped on K48-positive lysosomes. HeLa cells expressing GFP-YOD1-wt or C160S were LLOMe-treated, chased for 2 h, and stained with antibodies specific for K48 or K63 chains. Arrows indicate LAMP1-positive vesicles colocalizing with YOD1-wt or C160S. Note increased colocalization of the K48 signal, but not K63, with the catalytically inactive YOD1-C160S compared to wt.

D Number of K63-positive lysosomes 2 h after washout in cells treated as in (C) were quantified from three independent experiments. Values on K48-positive lysosomes 2 h after washout (taken from Fig 5F) were plotted for direct comparison.

Data information: $^{**}P < 0.01$; $^{***}P < 0.001$. ns, not significant. Scale bars, 10 μm.

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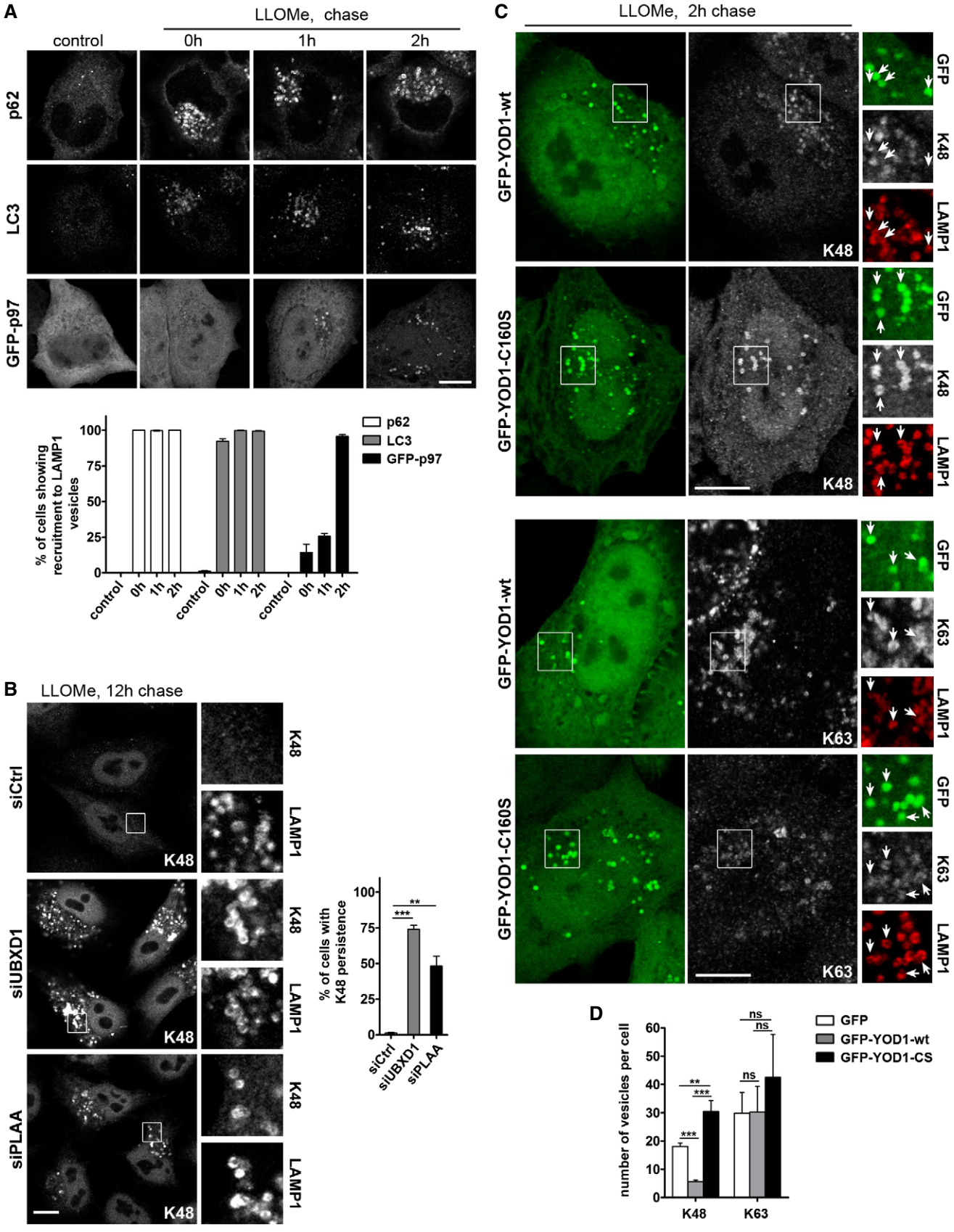
A
LLOMe, chase
control
0h
1h
2h
p62
LC3
GFP-p97
% of cells showing recruitment to LAMP1 vesicles
p62
LC3
GFP-p97
B
LLOMe, 12h chase
siCtrl
siUBXD1
siPLAA
K48
LAMP1
% of cells with K48 persistence
siCtrl
siUBXD1
siPLAA
C
LLOMe, 2h chase
GFP-YOD1-wt
GFP-YOD1-C160S
K48
K63
GFP
LAMP1
D
number of vesicles per cell
GFP
GFP-YOD1-wt
GFP-YOD1-CS
K48
K63
ns

Figure EV5.