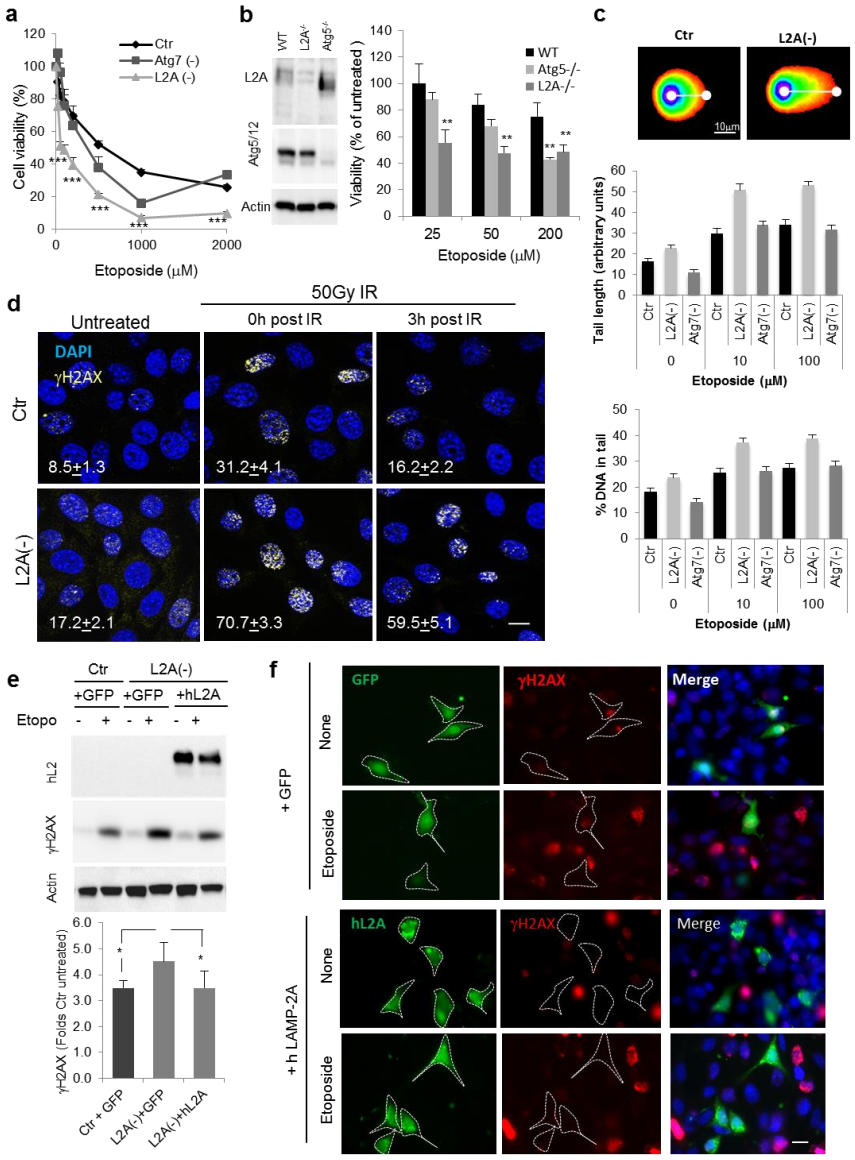
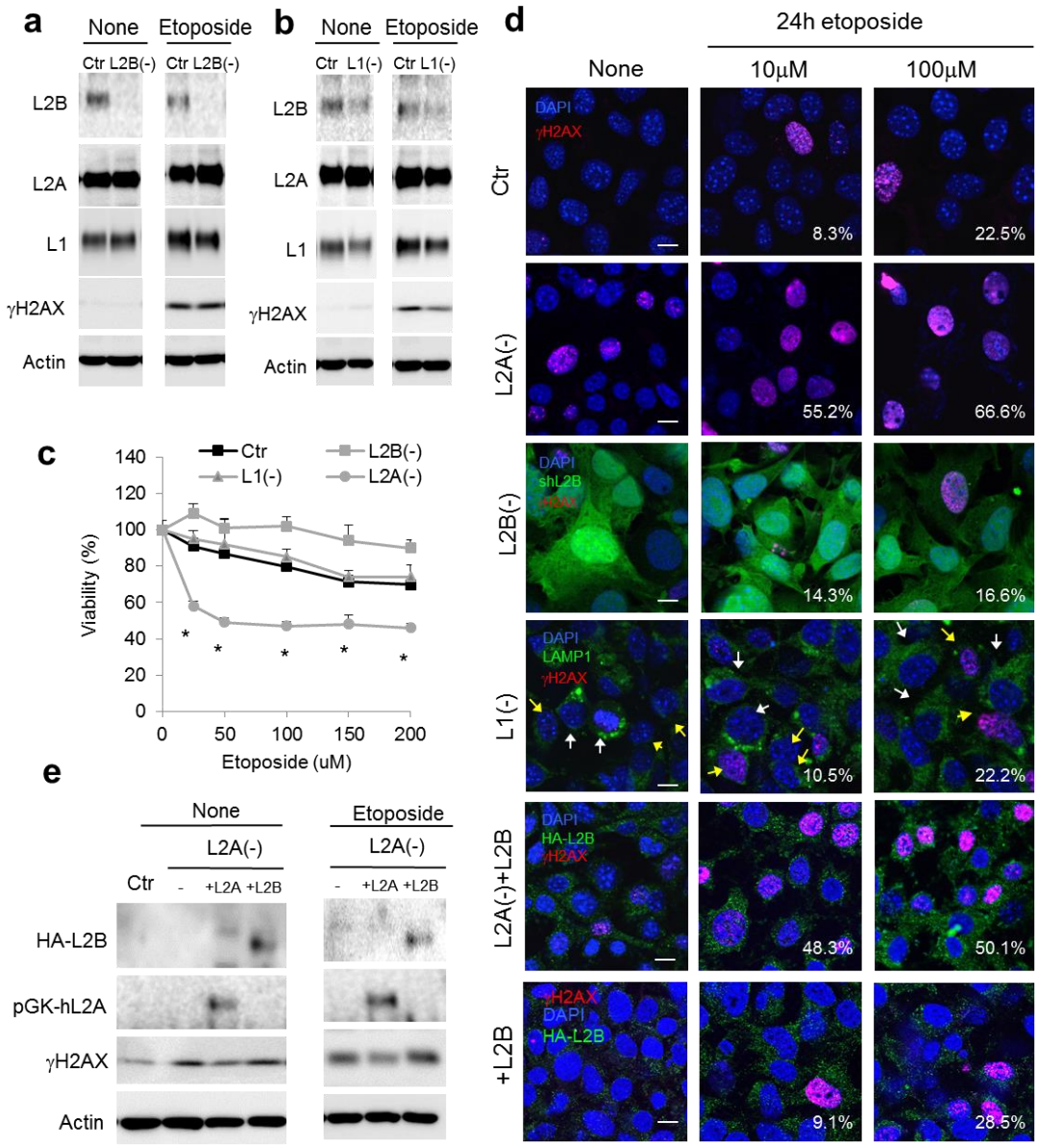
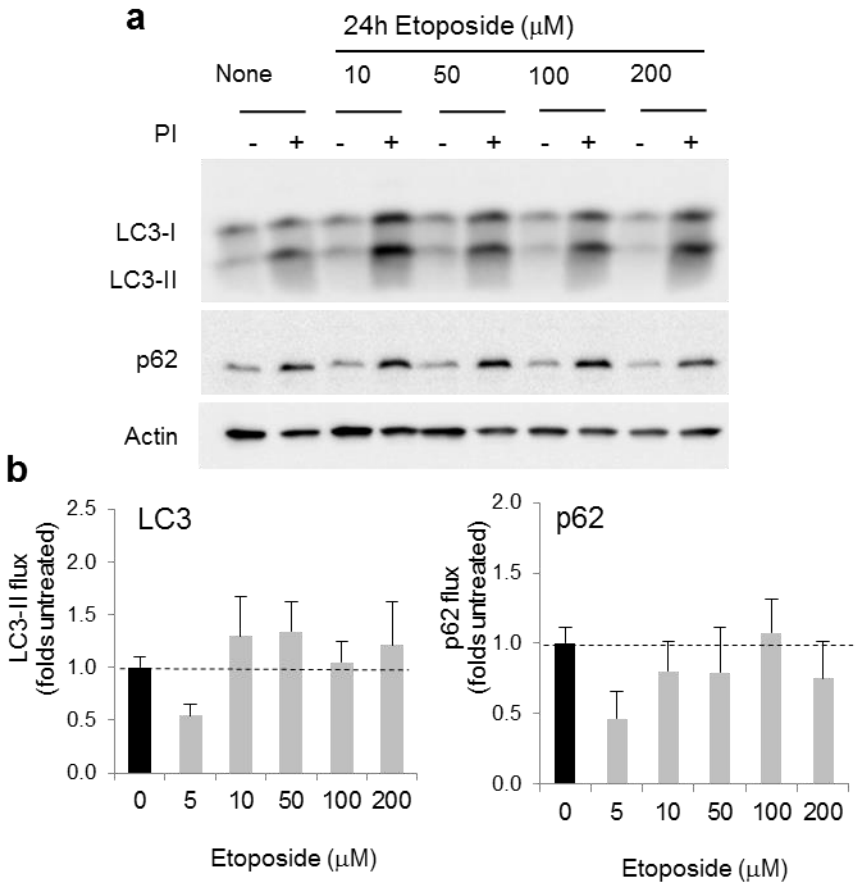


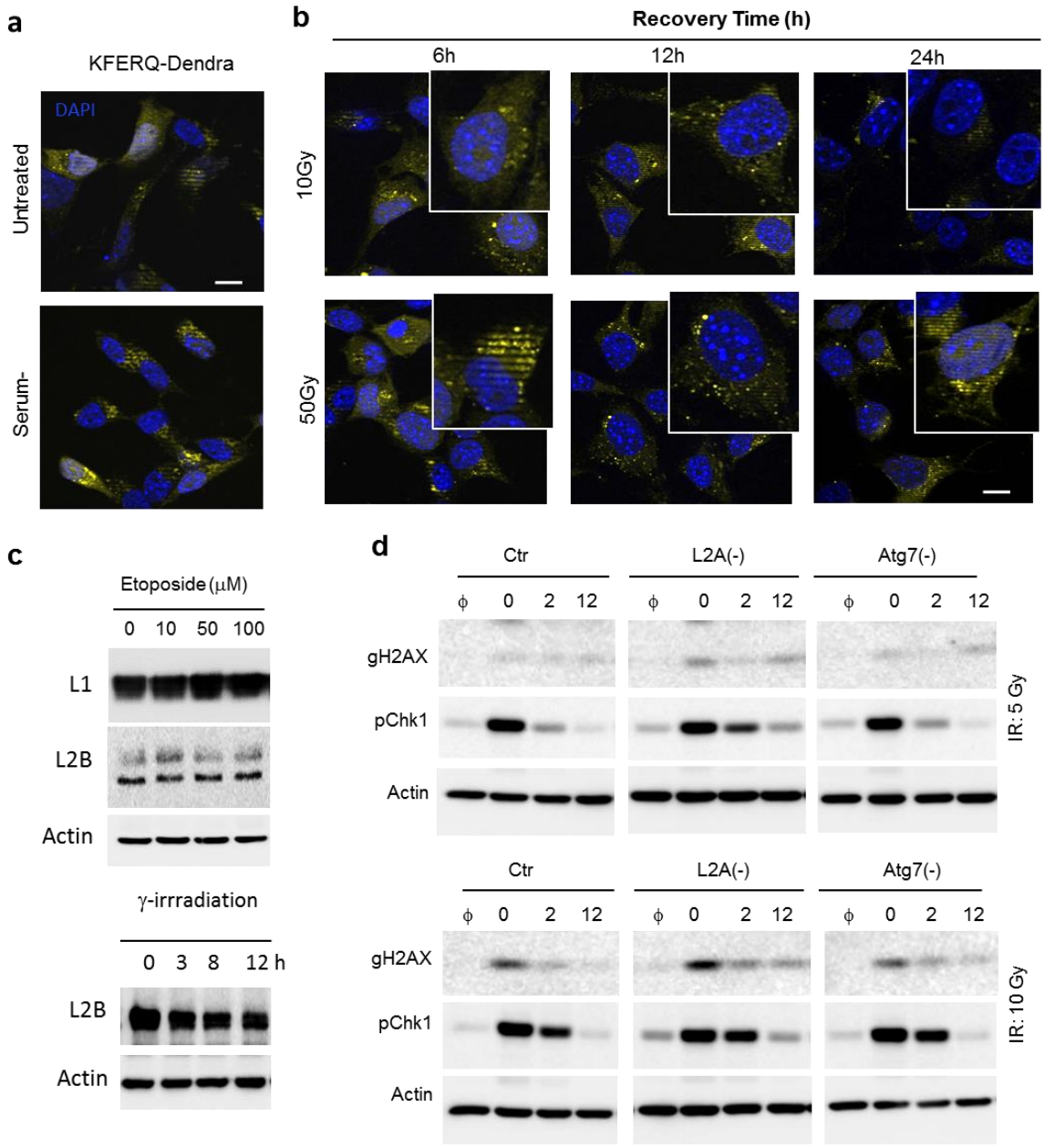
Supplementary Fig. 1| Increased DNA damage load in CMA-defective cells. a,b. Cellular viability (in percentage) 24h upon addition of the indicated concentrations of etoposide to mouse fibroblasts (NIH3T3 cells) Ctr, L2A(-) or Atg7(-) (**a**) or mouse embryonic fibroblasts (MEFs) from mice wild-type (WT), and knockout for LAMP-2A or Atg5 (**b**). Left: immunoblot for KO proteins. Concentrations below 200μM of the graph in A can be appreciated better in **Fig. 1a**. n=6 wells in 3 independent experiments. **c.** Neutral comet assay in the same mouse fibroblasts as in **a**. Images of representative cells to illustrate measurement of the length of the tail (with connector). Graphs in lower panel are the quantification of the tail length percentage of DNA in the tail calculated from >40 cells counted across 5 fields in 3 independent experiments. **d.** Immunofluorescence for γH2AX in the same cells untreated or at the indicated times after exposure to γ-irradiation. Percentage of cells with positive foci is shown in each image (n >75 cells/condition in 3 independent experiments). **e, f.** Representative immunoblot (of n = 3) (**e**) and immunofluorescence (**f**) for γH2AX in Ctr and L2A(-) cells transfected with GFP or hL2A and exposed to etoposide for 24h. Quantification of changes in γH2AX levels is shown at the bottom. Single channel images of representative fields and merged fields are show. Dashed lines mark cellular profiles. Values are mean+s.e.m. (unpaired two-tailed t-test) *p<0.01, ***p<0.0005. Scale bar: 10μm. Full gels are shown in Supplementary Fig. 8.



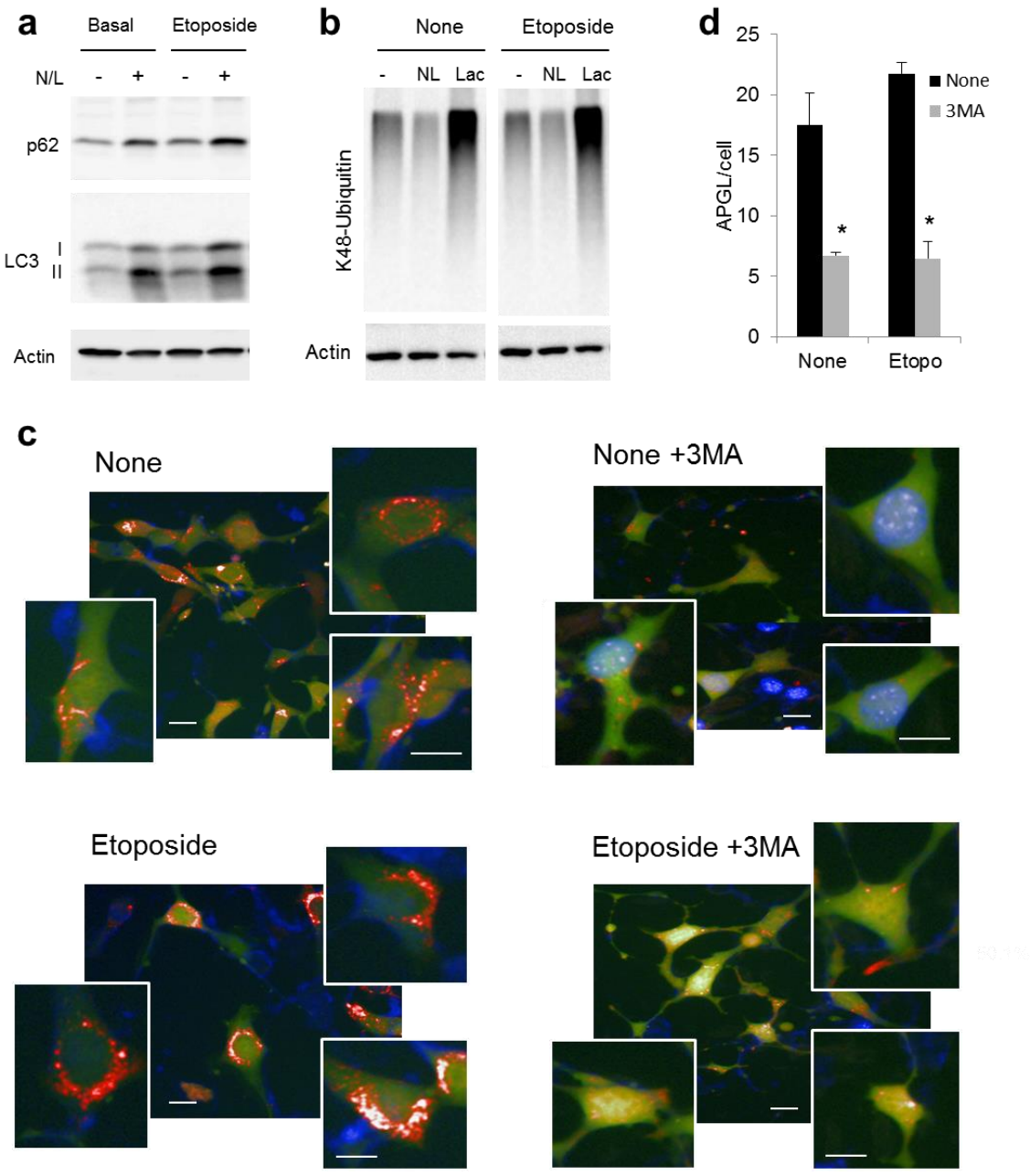
Supplementary Fig. 2| Effect of changes in other lysosomal membrane proteins in the cellular response to etoposide. a,b. Representative immunoblot for the indicated proteins in cells control (Ctr) and knock-down for LAMP-2B (L2B(-) **a**) or LAMP-1 (L1(-) **b**) without treatments (none) or treated with 50 μM etoposide for 24h. Levels of γH2AX are also shown as marker for DNA damage. Note: cells knock-down for LAMP1, even at levels <45% of those in control cells, show also a consistent decrease in levels of LAMP-2B. **c.** Cellular viability of fibroblasts (NIH3T3 cells) Ctr or knock-down for the indicated membrane proteins 24h upon addition of the indicated concentrations of etoposide. Note: L2B(-) cells present a discrete but consistent higher resistance to low doses of etoposide that we attribute to the compensatory upregulation of CMA in these cells in response to the reduced macroautophagy rates. **d**. Immunofluorescence for γH2AX in the same cells untreated (none) or 24h after adding the indicated concentrations of etoposide. Percentage of cells with positive foci upon exposure to etoposide is shown in each image (n >75 cells/condition from >3 independent experiments). **e**. Representative immunoblots in cells expressing the indicated recombinant proteins and treated as in **a**. Values are mean+s.e.m. (unpaired two-tailed t-test) *P<0.005. Scale bar: 10μm. Full gels are shown in Supplementary Fig. 8.



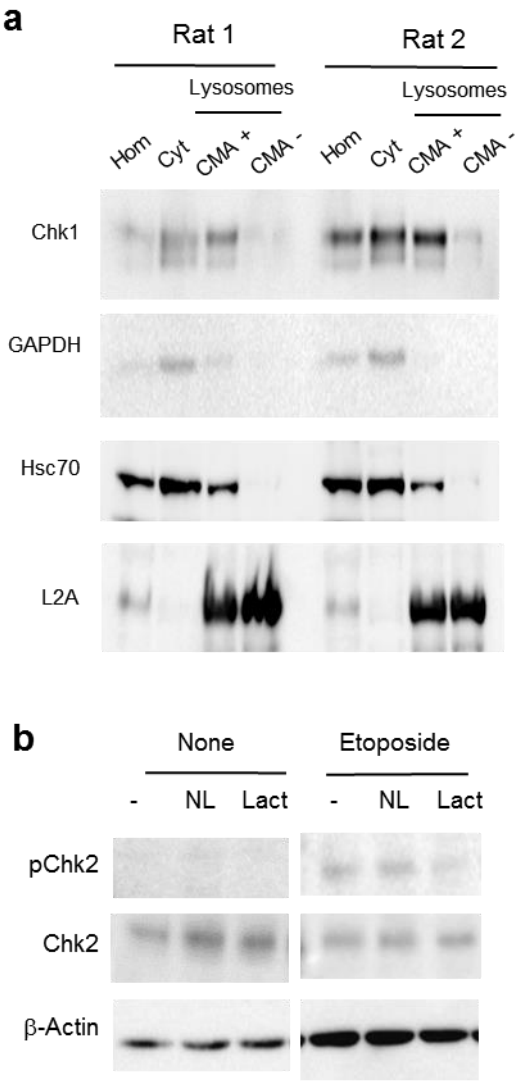
Supplementary Fig. 3| Effect of etoposide in macroautophagy flux. a. Representative immunoblot for LC3 and p62 in control cells treated with the indicated concentrations of etoposide and supplemented or not 22h before the end of the treatment with lysosomal proteolysis inhibitors (PI). **b.** Quantification of the flux of LC3 and p62 by densitometric analysis are expressed as folds of the values in untreated cells. Values are mean+s.e.m. n=3 independent experiments. No statistical significance was found using unpaired two-tailed t-test. Full gels are shown in Supplementary Fig. 8.



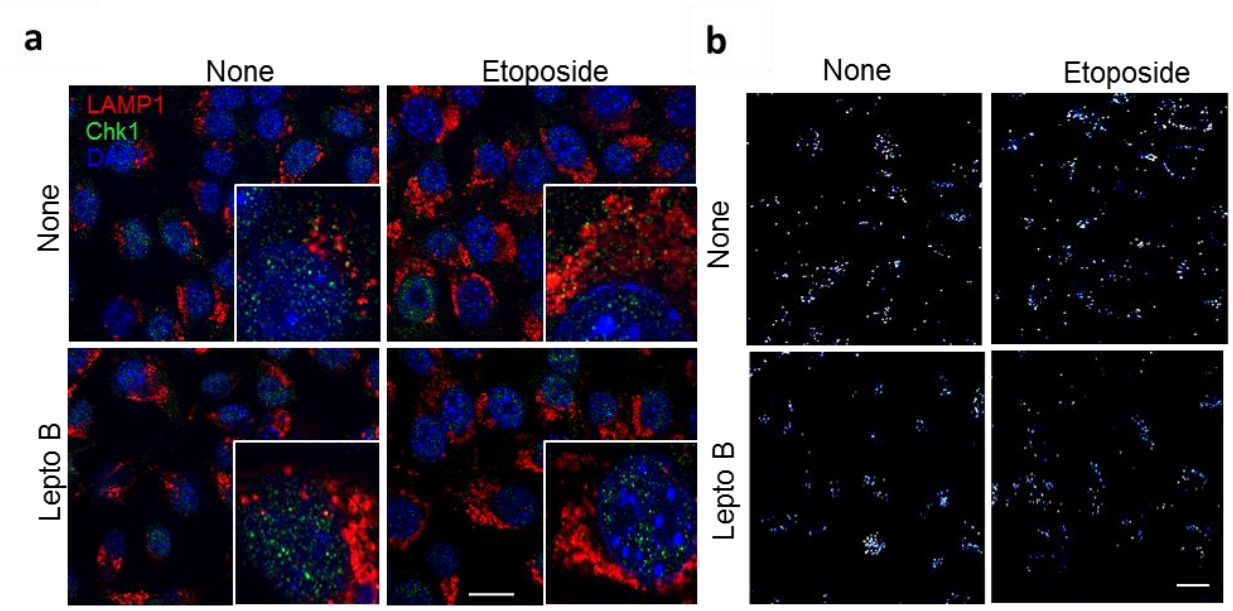
Supplementary Fig. 4| Activation of CMA in cells exposed to γ-irradiation. CMA activity in cells expressing a photoactivatable CMA reporter untreated (**a**) or at the indicated times after being exposed to two different γ-irradiation doses (**b**). Inserts show higher magnification. Nuclei are highlighted with DAPI. Cells maintained in absence of serum are shown as a positive control for CMA activation in **a.** (**c**) Immunoblot for different LAMPs in cells exposed to the indicated genotoxic treatments. **d**. Representative immunoblots for Chk1 at different times after exposure to 5 or 10 Gy γ-irradiation in the indicated cell types. The experiments were repeated 3 times (a-c) and 2 times (d). Scale bar: 10μm. Full gels are shown in Supplementary Fig. 8.



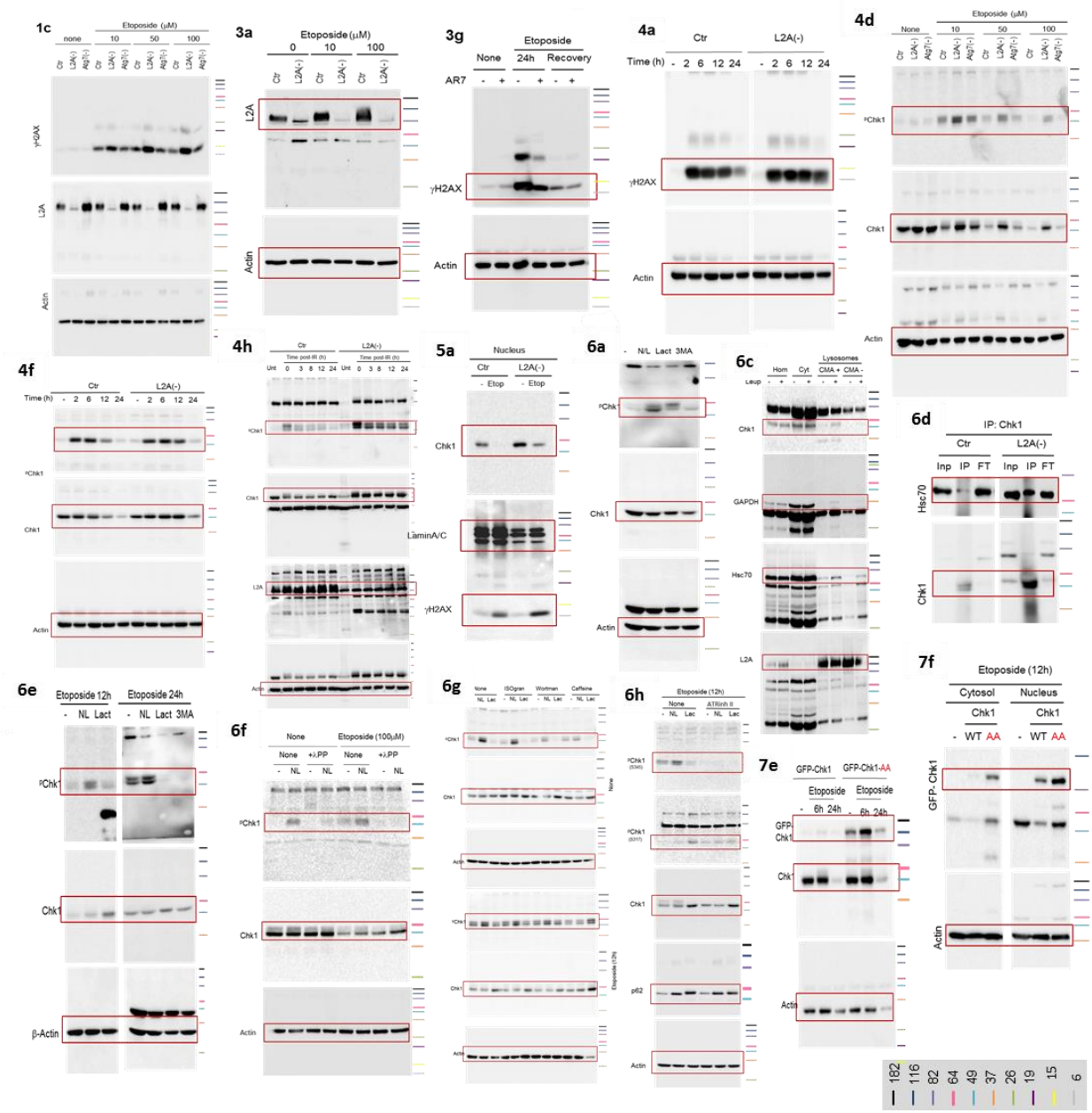
Supplementary Fig. 5| Efficacy of lysosome and proteasome inhibitors. a. Representative immunoblots for p62 and LC3 (**a**) or for K48-ubiquitinated proteins (**b**) in control cells exposed or not to etoposide for 12hin the absence (-) or presence (NL) of a combination of ammonium chloride and leupeptin to block lysosomal degradation or lactacystin (Lac). Experiments were repeated 3 times**.** Fluorescence images of control cells expressing the mCherry-GFP-LC3 reporter exposed or not to etoposide and allowed to recover in the absence or presence of 3-methyladenine (3MA). Merged channels of DAPI stained cells to highlight the nuclei are shown. Insets: higher magnification images. Blocking effect of 3MA is visualized as absence of fluorescent puncta. **d.** Quantification of the number of red puncta only (autophagolysosomes; APGL) per cell in images similar to the ones shown in c. n=3, >200 cells per experiment. Values are mean+s.e.m. (unpaired two-tailed t-test) *p<0.005. Scale bar: 10μm. Full gels are shown in Supplementary Fig. 8.



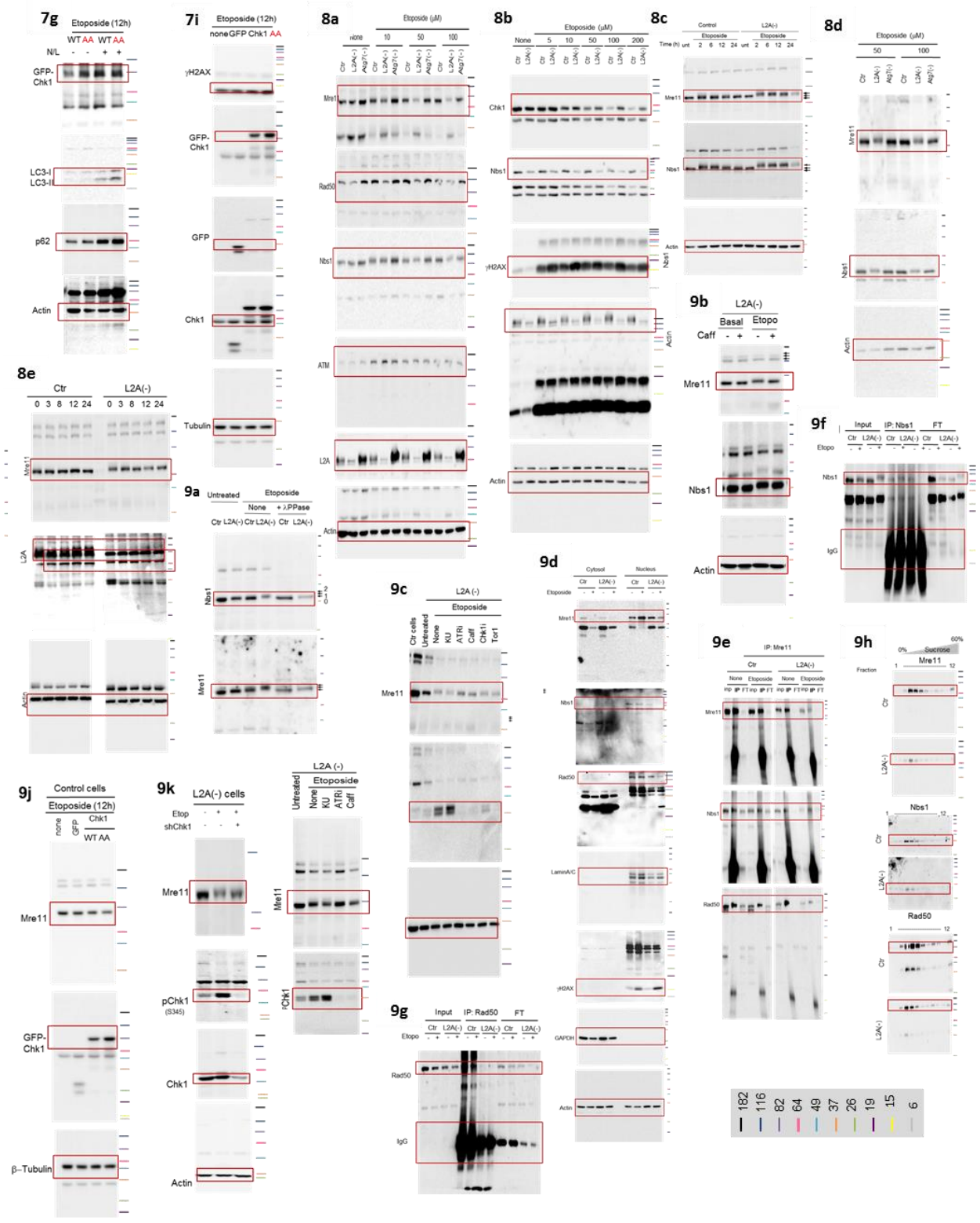
Supplementary Fig. 6| Lysosomal degradation of Chk proteins. a. Immunoblot for the indicated proteins in homogenates (Hom), cytosol (Cyt) and two populations of lysosomes with high (CMA+) and low (CMA-) CMA activity isolated from rat liver. Fractions purified from two different animals are shown. **b.** Immunoblot for phosphorylated and total Chk2 in mouse fibroblasts exposed or not to etoposide and treated with NH_4Cl and leupeptin (NL), lactacystin (Lact) or 3-methyladenine (3MA), as indicated. Experiment was repeated twice. Full gels are shown in Supplementary Fig. 8.



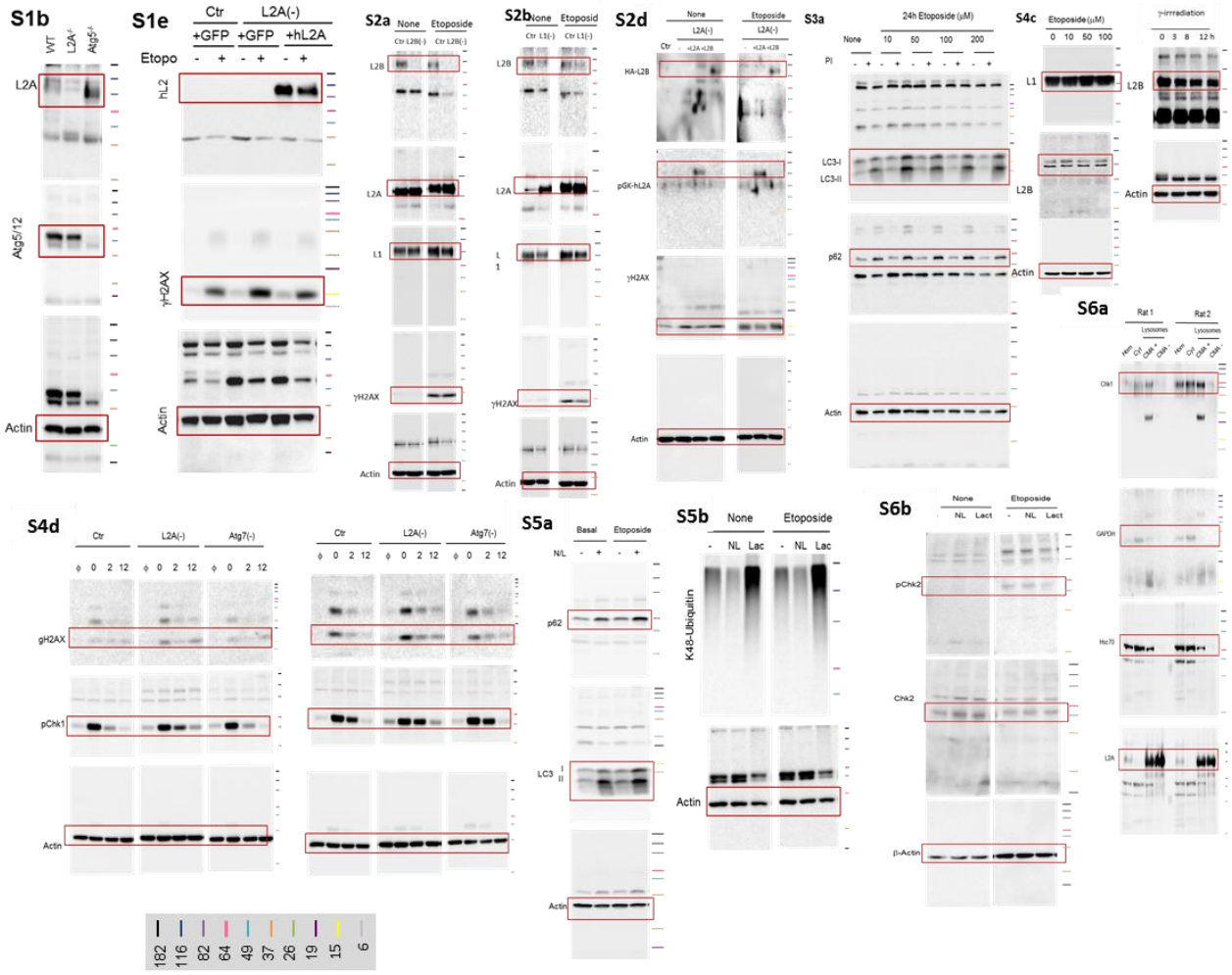
Supplementary Fig. 7 Association of Chk1 with lysosomal components. a. Immunofluorescence for pChk1 and LAMP1 in mouse fibroblasts untreated (none) or exposed to 100μM etoposide for 12h in the presence or not of 20nM leptomycin. Representative fields and higher magnification inserts are shown. Nuclei are highlighted with DAPI. Percentage of colocalization is indicated in images in Fig. 6A. **b.** Example of the colocalization mask applied for quantification. Bright white spots correspond to pixels where both fluorophores overlap. Scale bar: 10μm.



Supplementary Fig. 8| Full blots and gels shown in main figures 1-7 as cropped versions. Molecular weight markers are color coded and the values in KDa are shown in the bottom inset. Black (182), blue (116), purple (82), pink (64), cyan (49), orange (37), green (26), magenta (19).



Supplementary Fig. 8 (cont) | Full blots and gels shown in main figures 7-9 as cropped versions. Molecular weight markers are color coded and the values in KDa are shown in the bottom inset. Black (182), blue (116), purple (82), pink (64), cyan (49), orange (37), green (26), magenta (19).



Supplementary Fig. 8 (cont)| Full blots and gels shown in supplementary figures as cropped versions. Molecular weight markers are color coded and the values in KDa are shown in the bottom inset. Black (182), blue (116), purple (82), pink (64), cyan (49), orange (37), green (26), magenta (19).