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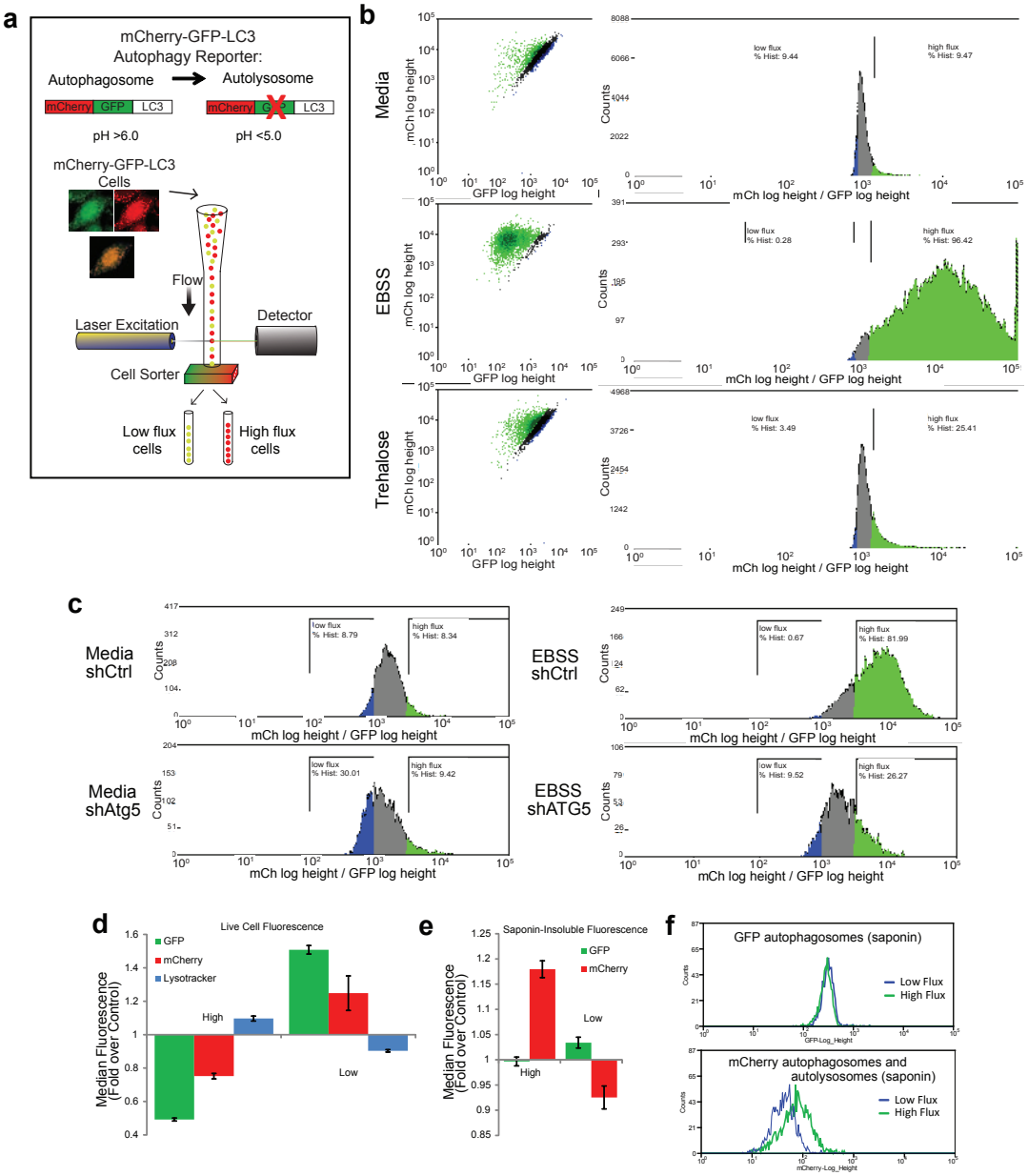
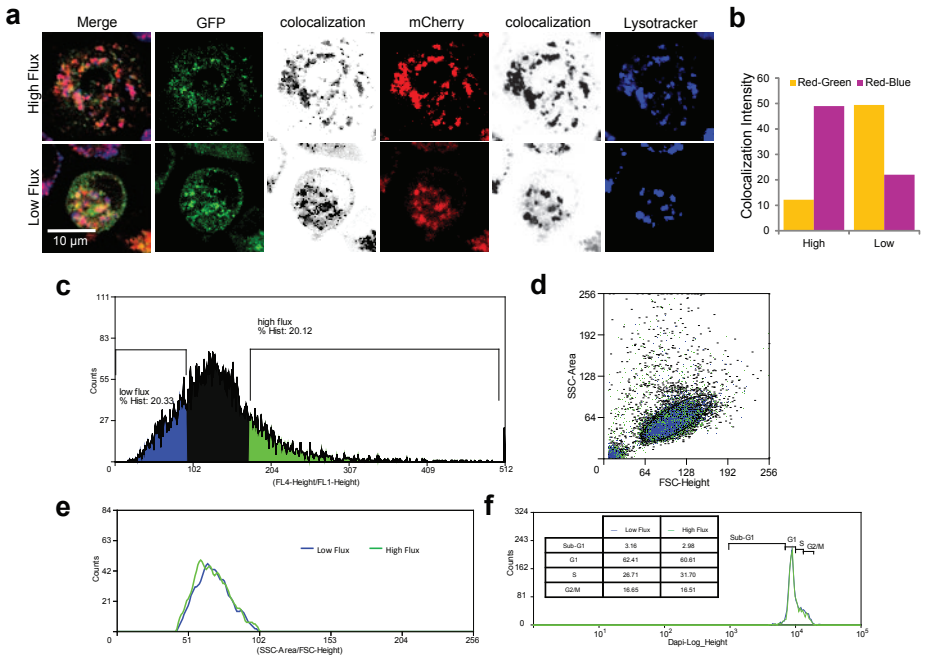
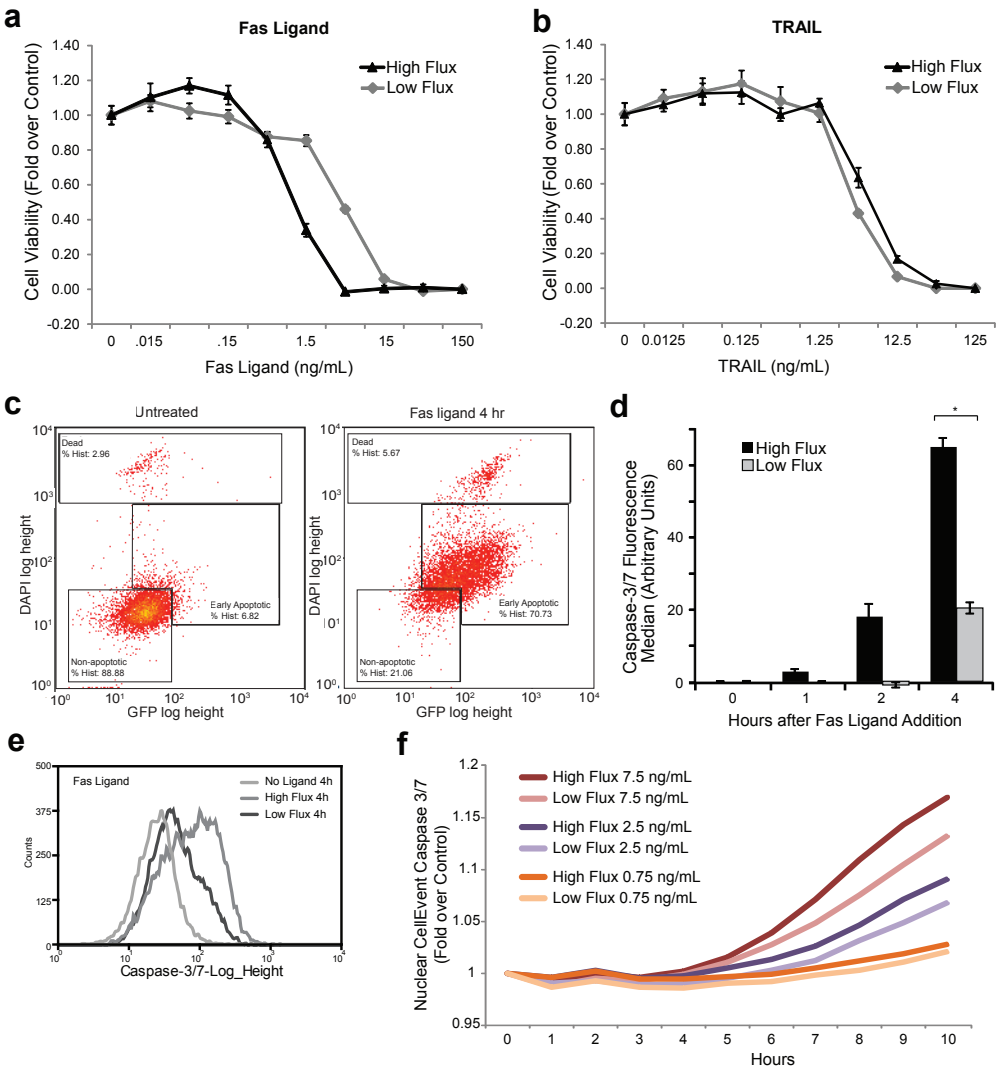


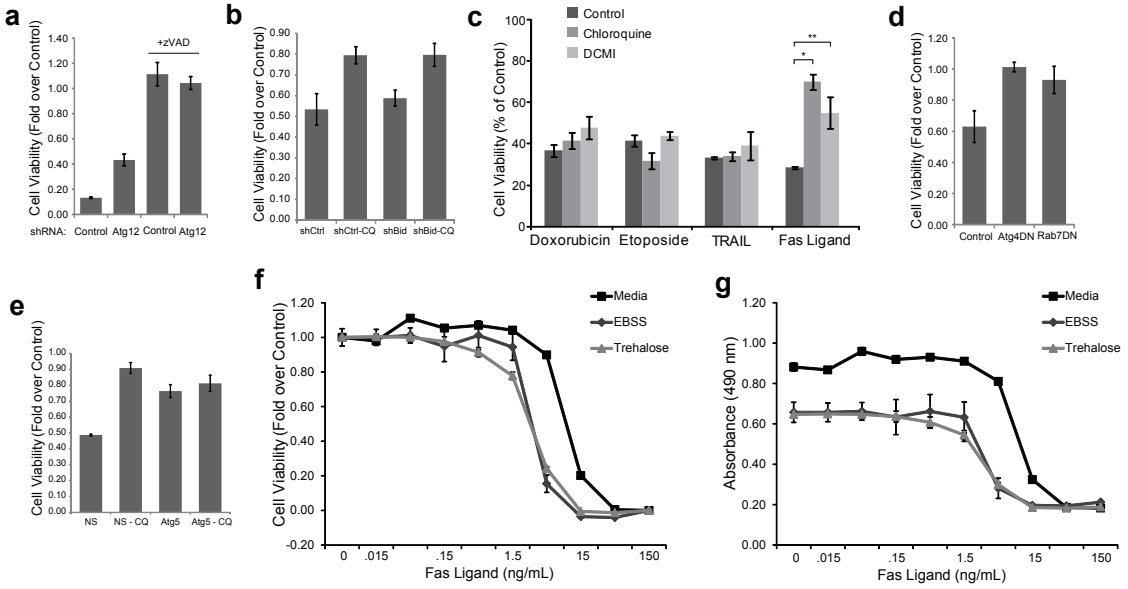
Supplementary Figure 1 Method to sort cells with high and low autophagic flux by flow cytometry accurately measures basal and induced autophagic flux. **a**, Cells constitutively expressing mCherry-GFP-LC3 are sorted based on the relative ratio of mCherry/EGFP fluorescence which changes in response to the pH gradient as autophagosomes fuse with lysosomes to form autolysosomes (autophagic flux). **b**, BJAB mCherry-GFP-LC3 cells were treated with EBSS (100%) or trehalose (75 mM) for 4 hours followed by flow cytometry for autophagic flux. Data are representative of at least 3 independent experiments. **c,** HeLa mCherry-GFP-LC3 cells expressing control or Atg5 shRNA were treated with EBSS for 4 hours followed by flow cytometry for autophagic flux. Data are representative of at least 3 independent experiments. **d**, BJAB mCherry-GFP-LC3 cells were sorted by flow cytometry for high or low autophagic flux, then treated with Lysotracker Blue for 30 min. at 37 °C, followed by flow cytometry (median GFP, mCherry or Lysotracker Blue fluorescence normalized to fold over unsorted control, mean ± s.e.m., n=3 wells). **e,** BJAB mCherry-GFP-LC3 cells were sorted as in (**d**) followed by cytoplasmic extraction with 0.1 % saponin to eliminate non-lipidated LC3[1] and flow cytometry to quantitate autophagosome/autolysosome number (normalized fold over unsorted control median GFP or mCherry fluorescence, mean ± s.e.m., n=3 wells). **f,** Flow cytometry histograms of representative data from (**e**).



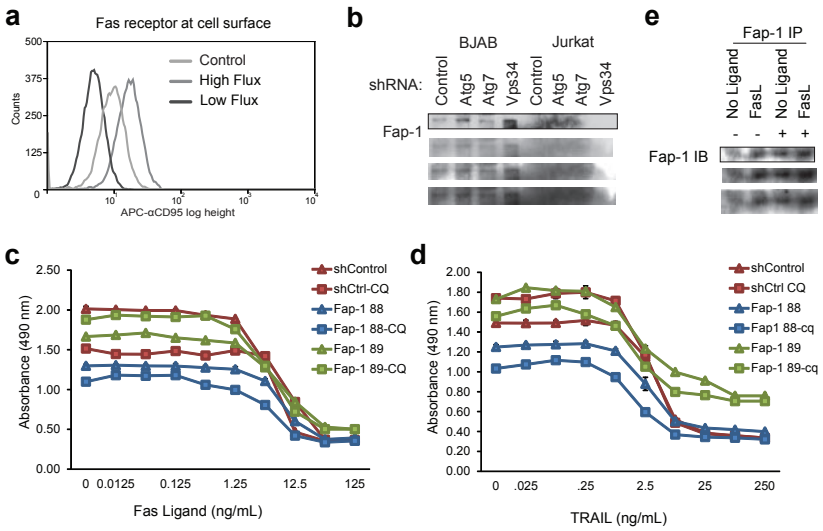
Supplementary Figure 2 Confocal microscopy reveals differences in the number of autophagosomes and autolysosomes in flux sorted cells; High and low flux sorted cells exhibit similar size, cell cycle and apoptotic profile. **a**, HeLa mCherry-GFP-LC3 cells were sorted for autophagic flux, treated with Lysotracker Blue for 30 min. at 37 °C, adhered to slides by cytospin centrifugation, fixed with formaldehyde and visualized by spinning disc confocal microscopy; these are the same fields depicted in Fig. 1d. Colocalization images were produced using ImageJ (see methods). **b**, Quantification of the colocalization images in (**a**). Data are representative of 20 separate fields and have been repeated 5 times. **c**, BJAB mCherry-GFP-LC3 cells were sorted for autophagic flux by flow cytometry. **d, e,** Forward and side scatter of sorted cells in (**c**). **f,** Cell cycle profiles of sorted cells in (**c**) stained with Hoechst 33342.



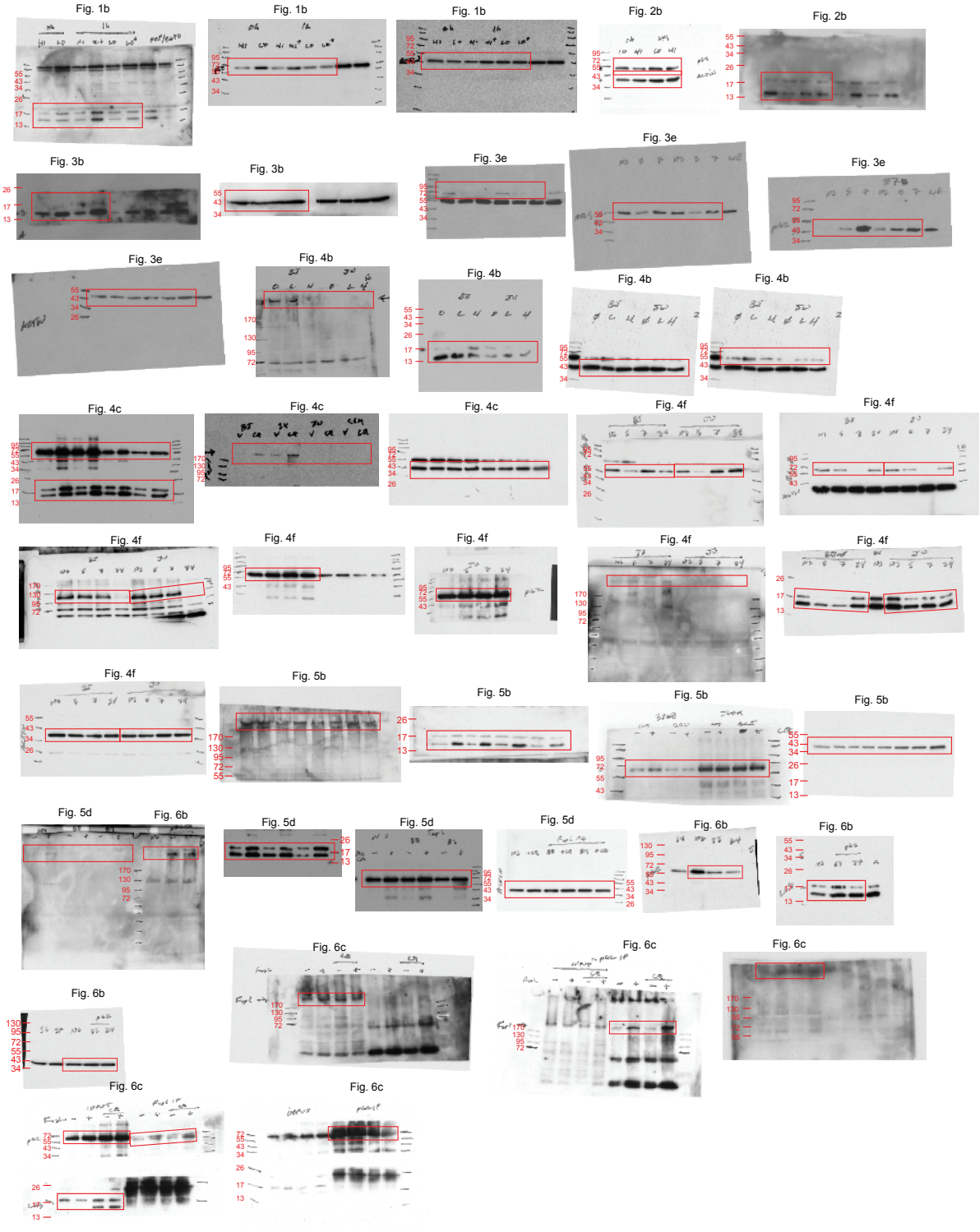
Supplementary Figure 3 Autophagy modulation of Fas ligand-induced apoptosis and cell killing. **a, b**, BJAB mCherry-GFP-LC3 cells were sorted for autophagic flux by flow cytometry followed by treatment with Fas ligand (**a**) or TRAIL (**b**) for 24 hours and MTS assay for viability (fold over no ligand control, mean ± s.e.m., n=3 wells). These are the full data sets from the same experiment depicted in Figure 2d. **c-e**, BJAB mCherry-GFP-LC3 cells were sorted as in (**a**) followed by treatment with Fas ligand for the indicated times in the presence of fluorogenic Caspase-3/7 substrate CellEvent Green (5 μM) and analyzed by flow cytometry. **c**, Dot plot of Caspase-3/7 activity vs. DAPI fluorescence in unsorted cells. **d**, Quantitation of Caspase-3/7 activity in sorted cells at the indicated time points (median CellEvent Green fluorescence, mean ± s.e.m., n=3 wells, *p=0.0091). **e**, Representative flow cytometry histograms of Caspase-3/7 activity in (**c, d**). **f**, BJAB mCherry-GFP-LC3 cells were sorted for autophagic flux as above, followed by treatment with Fas ligand at the indicated concentrations in the presence of fluorogenic CellEvent Caspase-3/7 substrate (5 μM) and analyzed on an IncuCyte ZOOM for the indicated times (mean CellEvent Green fluorescence per field, mean, n=12: 4 fields for each of 3 wells).



Supplementary Figure 4 Effect of autophagy inhibition or autophagy induction on Fas ligand-induced death. **a**, BJAB cells expressing control or Atg12 shRNA were treated with Fas ligand (15 ng/mL) for 24 hours in the presence or absence of zVAD-fmk (100 μM). Cell viability was then quantitated by MTS absorbance (fold over untreated control, mean ± s.e.m., n=3 wells). **b**, BJAB cells expressing control or Bid shRNA were treated with autophagy inhibitor chloroquine (25 μM) for 12 hours followed by Fas ligand (4 ng/mL) for 24 hours. Cell viability was then quantitated by MTS (fold over untreated control, mean ± s.e.m., n=3 wells). **c**, BJAB cells were treated with autophagy inhibitors chloroquine (20 μM) or desmethylclomipramine (DCMI, 10 μM) for 12 hours followed by treatment with the indicated cytotoxic agents for 24 hours. Cells were then assayed for viability by MTS (% of control (no cytotoxic drug), mean ± s.e.m., n=3 wells, *p=0.0024, **p=0.017). **d**, BJAB cells expressing vector control or the indicated autophagy dominant-negative constructs were treated with Fas ligand (4 ng/mL) for 24 hours; cell viability was then quantitated by MTS absorbance (fold over untreated control, mean ± s.e.m., n=3 wells). Data are representative of 2 independent experiments. **e**, BJAB cells expressing control or Atg5 shRNA were treated with chloroquine (20 μM) for 12 hours followed by Fas ligand (4 ng/mL) for 24 hours. Cell viability was then quantitated by MTS (fold over no ligand control, mean ± s.e.m., n=3 wells). Data are representative of 3 independent experiments. **f**, **g**, BJAB cells were treated with the indicated autophagy inducers overnight (EBSS, 100%; trehalose, 75 mM) followed by treatment with Fas ligand at the indicated concentrations for 24 hours. Cell viability was then quantitated by MTS absorbance. **f**, MTS data normalized (fold over untreated (no Fas ligand) control, mean ± s.e.m., n=3 wells). **g**, Raw MTS values (absorbance at 490 nm, mean ± s.e.m., n=3 wells).



Supplementary Figure 5 Autophagy controls cell surface expression of Fas receptor via Fap-1. **a**, BJAB mCherry-GFP-LC3 cells were sorted for autophagic flux by flow cytometry, and then stained with APC-conjugated anti-Fas antibody at 4 °C for 30 minutes. Cells were then washed and analyzed by flow cytometry. Data are representative of 3 replicates from 3 independent experiments. **b**, Additional exposures of immunoblots from experiment in Fig. 4f. **c-d**, BJAB cells expressing control or Fap-1 shRNAs were treated with 20 μM chloroquine for 12 hours followed by treatment with Fas ligand (**c**) or TRAIL (**d**) at the indicated concentrations for 24 hours. Cell viability was quantitated by MTS (absorbance at 490 nm, mean ± s.e.m., n=3 wells). These are the full dose response curves (without normalization) from the data in Figure 5c. **e**, Additional exposures from immunoprecipitation experiment depicted in Fig. 6c.



Supplementary Figure 6 Full immunoblot images. Red boxes indicate the cropped portion of each western blot presented in the corresponding main figures.