Figure S1. Can induces autophagy and sensitizes cells to tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis. DU145 cells were treated with Can (0, 0.25, 0.50 and 1 μM) for 18 h. The levels of LC3-II and p62 were assessed by western blot analysis and quantification of (A) LC3-II and (B) p62 levels was performed with Image J software. (C) DU145 cells were treated with Can (0, 0.50 and 1 μM) for 18 h, and cells were then immunostained with p62 (green) and evaluated for fluorescence; the fluorescence intensity was quantified with Image J software. *P<0.05, **P<0.01 and ***P<0.0001 vs. control. Can, cantharidin; LC3-II, lipid-modified microtubule-associated proteins 1A/1B light chain 3B; p62, sequestosome 1.

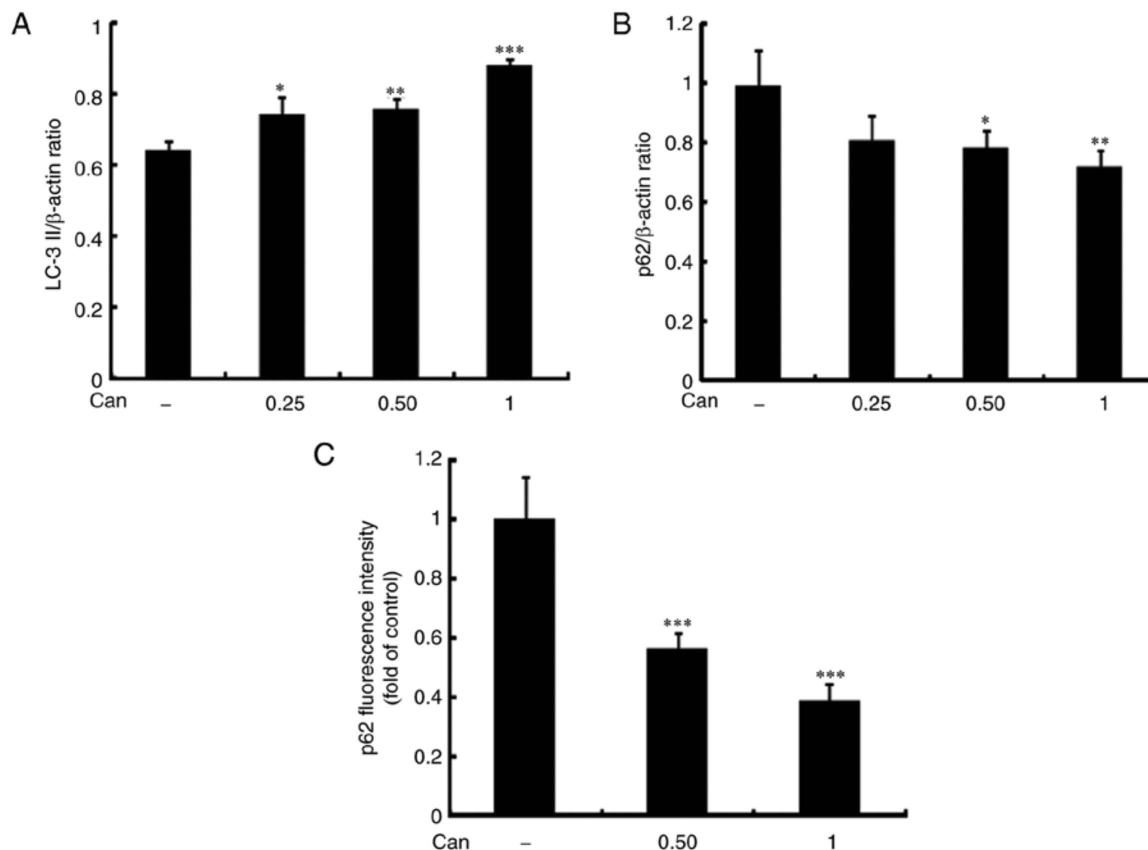

Figure S2. Autophagy inhibition encloses tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis sensitization by Can by promoting autophagy flux. DU145 cells were pretreated with CQ (10 μM) for 1 h prior to their exposure to Can (1 μM) for 18 h. The levels of LC3-II and p62 were assessed by western blot analysis and quantification of (A) LC3-II and (B) p62 was performed with Image J software. (C) Cells were immunostained with p62 (green) and evaluated for fluorescence; the fluorescence intensity was quantified using Image J software. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.0001$ vs. control. Can, cantharidin; CQ, chloroquine; LC3-II, lipid-modified microtubule-associated proteins 1A/1B light chain 3B; p62, sequestosome 1.

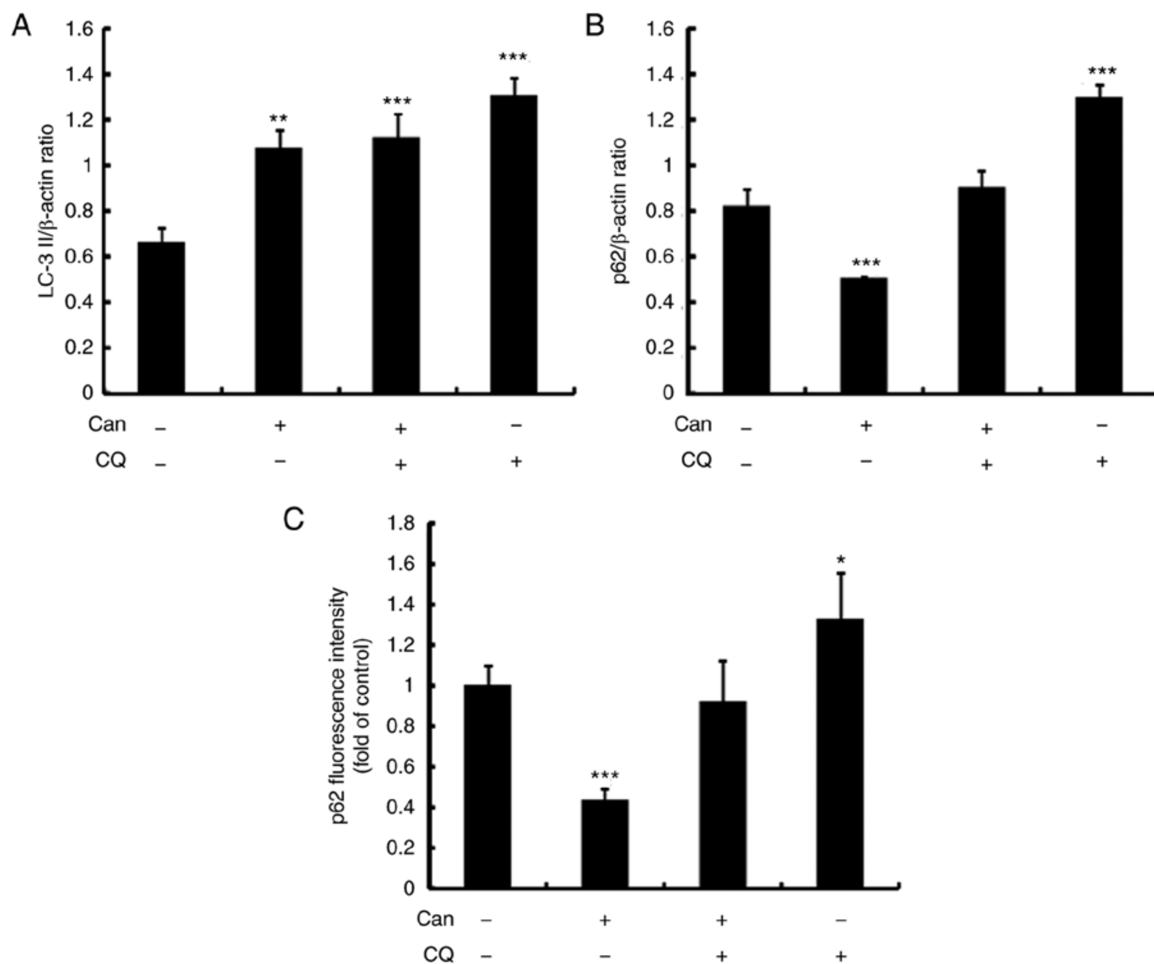

Figure S3. Genetic attenuation of autophagy encloses Can-sensitized tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis through activation of autophagy flux. DU145 cells were pretreated with ATG5 siRNA or negative control siRNA for 24 h prior to their exposure to Can (1 μM) for 18 h; (A) The level of p62 was assessed by western blot analysis and quantification of p62 was performed using Image J software. (B) Cells were immunostained with p62 antibody (green) and evaluated for fluorescence; the fluorescent intensity was quantified using Image J software. *P<0.01 and ***P<0.0001 vs. control. Can, cantharidin; ATG5, autophagy related 5; siRNA, small interfering RNA; p62, sequestosome 1; NC, negative control.

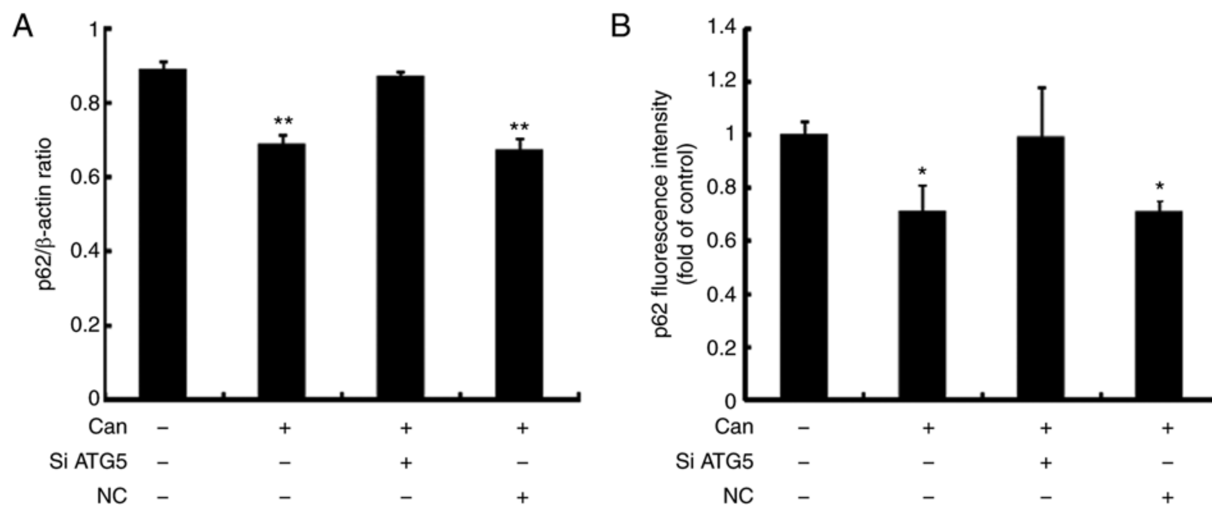

Figure S4. Downregulation of c-FLIP and upregulation of DR-5 by Can attenuates TRAIL resistance. DU145 cells were treated with Can (0, 0.25, 0.50 and 1 μM) for 18 h. The levels of (A) c-FLIP and (B) DR-5 were assessed by western blot analysis and quantified using Image J software. DU145 cells were treated with Can (1 μM) for 18 h and then with TRAIL (200 ng/ml) for an additional 1 h. The levels of (C) c-FLIP and (D) DR-5 were assessed by western blot analysis and quantified using Image J software. *P<0.05, **P<0.01 and ***P<0.0001 vs. control. c-FLIP, cellular FLICE-like inhibitory protein; DR-5, death receptor 5; Can, cantharidin; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.