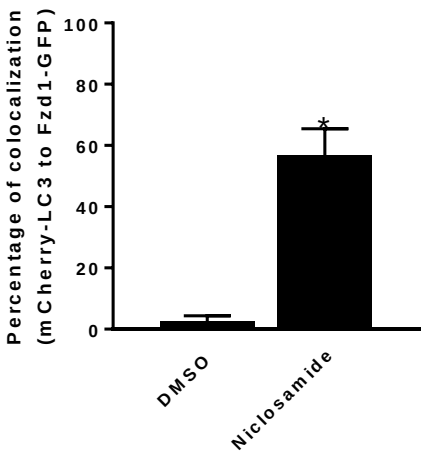
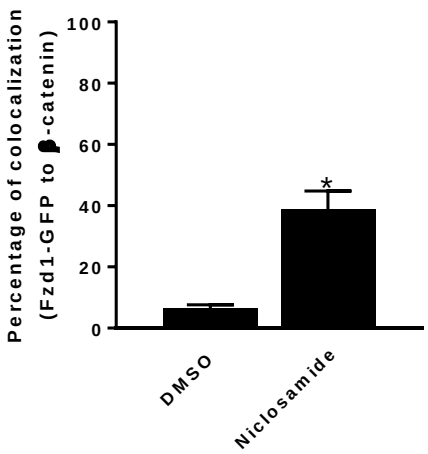
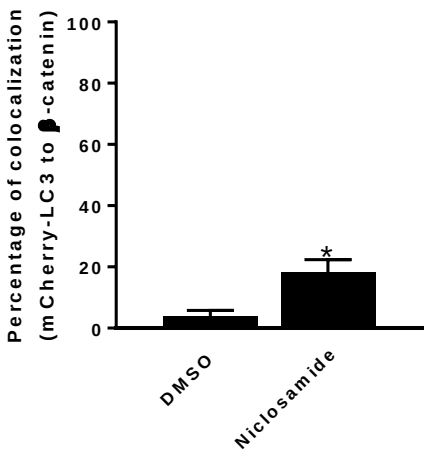


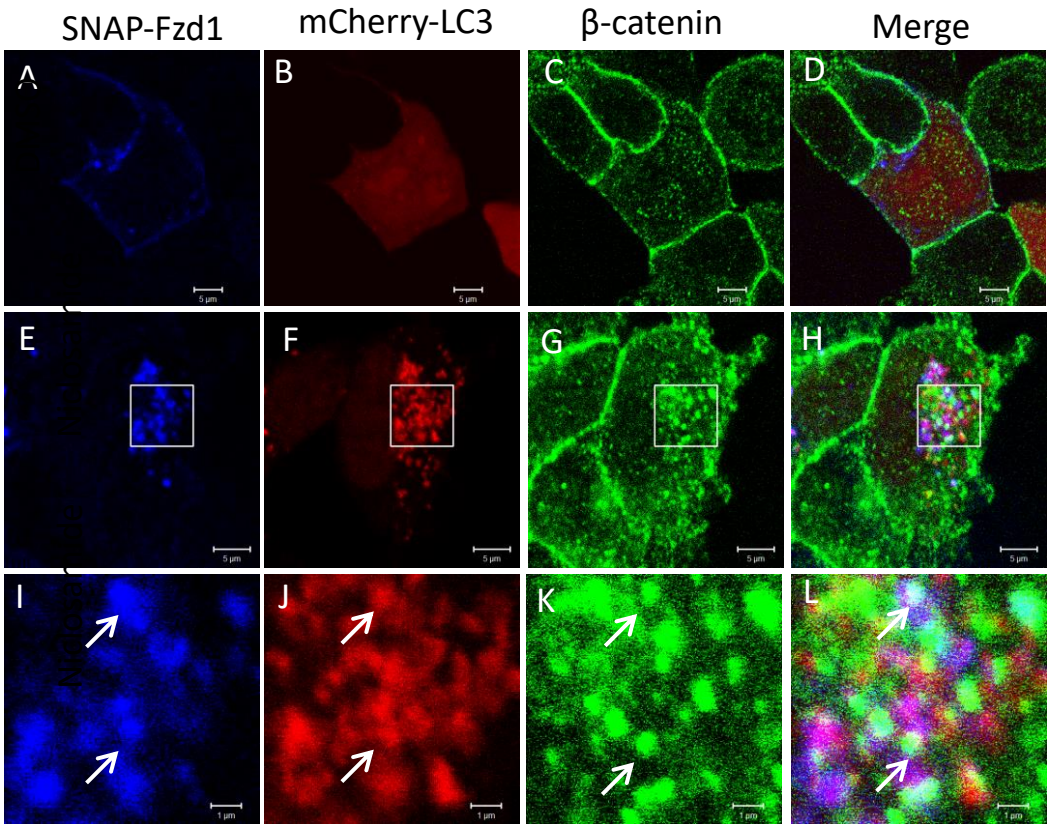
Supplementary Fig. 1. Niclosamide treatment significantly increased the co-localization of mCherry-LC3 and Fzd1-GFP. U2OS cells stably expressing Fzd1-GFP were transfected with mCherry-LC3. The cells were then treated with DMSO or 10 μM niclosamide for 4h. The images of the treated cells were analyzed by using Imaris 8.1 to quantify the numbers of mCherry-LC3 puncta (red), Fzd1-GFP puncta (green), and co-localized mCherry-LC3/Fzd1-GFP puncta in the cytosol of the cells. The percentage of mCherry-LC3 co-localizing to internalized Fzd1-GFP was calculated by normalizing the number of Fzd1-GFP puncta as 100% in the cells treated with DMSO or niclosamide, respectively. Results are mean ± SEM. (n = 4; asterisk, P < 0.05 of student's t test).



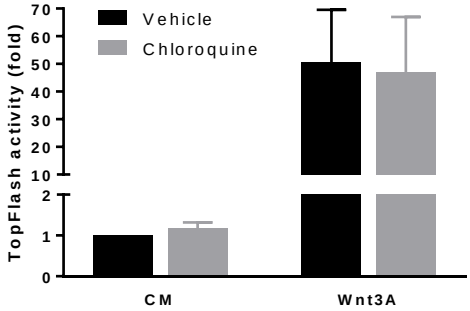
Supplementary Fig. 2. Niclosamide treatment significantly increased the co-localization of Fzd1-GFP and β-catenin. U2OS cells stably expressing Fzd1-GFP were treated with DMSO or 10 μM niclosamide for 4h. Endogenous β-catenin was visualized by immunostaining. The images of the treated cells were analyzed by using Imaris 8.1 to quantify the numbers of Fzd1-GFP puncta (green), β-catenin puncta (red), and co-localized Fzd1-GFP/β-catenin puncta in the cytosol of the cells. The percentage of Fzd1-GFP co-localizing to internalized β-catenin was calculated by normalizing the number of β-catenin puncta as 100% in the cells treated with DMSO or niclosamide, respectively. Results are mean ± SEM. (*n* = 4; asterisk, *P* < 0.05 of student's t test).



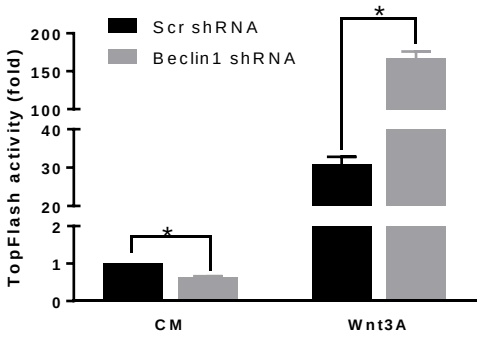
Supplementary Fig. 3. Niclosamide treatment significantly increased the co-localization of mCherry-LC3 and β-catenin. U2OS cells transfected with mCherry-LC3 were treated with DMSO or 10 μM niclosamide for 4h. Endogenous β-catenin was visualized by immunostaining. The images of the treated cells were analyzed by using Imaris 8.1 to quantify the numbers of mCherry-LC3 puncta (red), β-catenin puncta (green), and co-localized Fzd1-GFP/β-catenin puncta in the cytosol of the cells. The percentage of mCherry-LC3 co-localizing to internalized β-catenin was calculated by normalizing the number of β-catenin puncta as 100% in the cells treated with DMSO or niclosamide, respectively. Results are mean ± SEM. (*n* = 3; asterisk, $P < 0.05$ of student's t test).



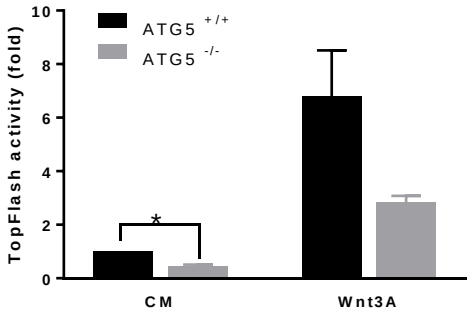
Supplementary Fig. 4. SNAP–Fzd1, β-catenin and mCherry-LC3 co-localize upon niclosamide treatment. HEK293T cells were transfected with SNAP-Fzd1 and mCherry-LC3. SNAP–Fzd1 was labelled for 15 min at room temperature with SNAP–Surface 649 prior to treating the cells with DMSO (A, B, C, D) or 10 µM niclosamide (E, F, G, H) for 4 hours at 37°C. I, J, K, L show a higher magnification of the boxed regions in panels D, E, and F, respectively. Endogenous β-catenin was visualized by immunostaining. Arrows in niclosamide treated cells indicate representative locations (white color) where co-localization of SNAP-Fzd1, mCherry-LC3, and β-catenin were observed (I, J, K, L). Scale bar: 5 µM (A-H); 1 µM (I-L).



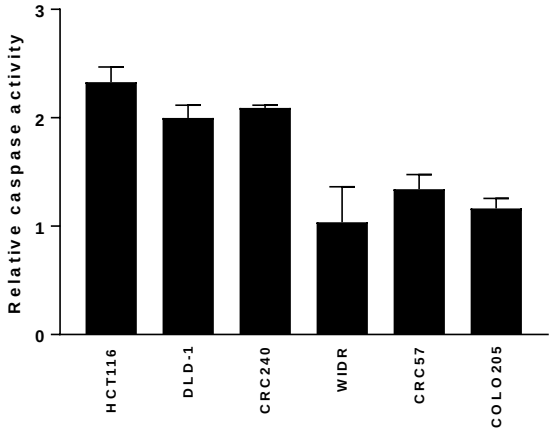
Supplementary Fig. 5. The effect of chloroquine on TopFlash reporter activity in TP6 cells. The TopFlash reporter activity of the cells treated with vehicle (H_2O) in control conditioned media plus DMSO (CM) was set as 1-fold (the first bar) and the fold numbers of the other columns were normalized to the first bar. In CM, TopFlash reporter activity was similar in vehicle treated cells or in 10μM chloroquine treated cells. In Wnt3A conditioned medium plus DMSO (Wnt3A), TopFlash reporter activity was also similar in vehicle treated cells or in 10μM chloroquine treated cells. Results are means ± SEM. (n = 3; asterisk, $P < 0.05$ of student's t test).



Supplementary Fig. 6. The effect of Beclin1 knockout on TopFlash reporter activity in TP6 cells. TP6 cells infected with either a scrambled shRNA (Scr shRNA) or Beclin1 shRNA lentiviruses were used in the experiment. The TopFlash reporter activity of the cells infected with Scr shRNA in control conditioned media plus DMSO (CM) was set as 1-fold (the first bar) and the fold numbers of the other columns were normalized to the first bar. In CM, TopFlash reporter activity was significantly less in Beclin1 shRNA infected cells than in Scr shRNA control cells. In Wnt3A conditioned media plus DMSO (Wnt3A), TopFlash reporter activity is significantly greater in Beclin1 shRNA infected cells than in Scr shRNA control cells. Results are means ± SEM. (n = 4; asterisk, $P < 0.05$ of student's t test).



Supplementary Fig. 7. The effect of ATG5 knockout on TopFlash reporter activity in MEF cells. The TopFlash reporter activity of ATG5 $^{+/+}$ cells in control conditioned media plus DMSO (CM) was set as 1-fold (the first bar) and the fold numbers of the other columns were normalized to the first bar. In CM, TopFlash reporter activity is significantly less in ATG5 $^{-/-}$ cells than in ATG5 $^{+/+}$ cells. In Wnt3A conditioned medium plus DMSO (Wnt3A), TopFlash reporter activity in ATG5 $^{-/-}$ cells is not significantly different from that in ATG5 $^{+/+}$ cells. Results are means ± SEM. (n = 4; asterisk, P < 0.05 of student's t test).



Supplementary Fig. 8. The effects of niclosamide on Caspase activity in niclosamide sensitive (HCT116, DLD-1, and CRC240) and resistant (WIDR, CRC57, and COLO205) colorectal cancer cell lines. Cells were plated in 100 µl growing medium/well in 96 well plates at 10000 cells per well and treated with DMSO or 2 µM niclosamide for 48h. Caspase 3/7 enzymatic activity was measured using an Apo-one Homogenous Kit. DMSO induced Caspase 3/7 enzymatic activity was set as 1 and the graphed data represents niclosamide induced Caspase 3/7 enzymatic relative to DMSO. Results are mean ± SEM, n=3. A student's t test was used to compare the relative niclosamide induced Caspase activity of the 3 sensitive cells vs the 3 resistant cells and $P<0.05$.