

17-hydroxy wortmannin restored TRAIL's response by ameliorating increased beclin 1 level and autophagy function in TRAIL resistant colon cancer cells

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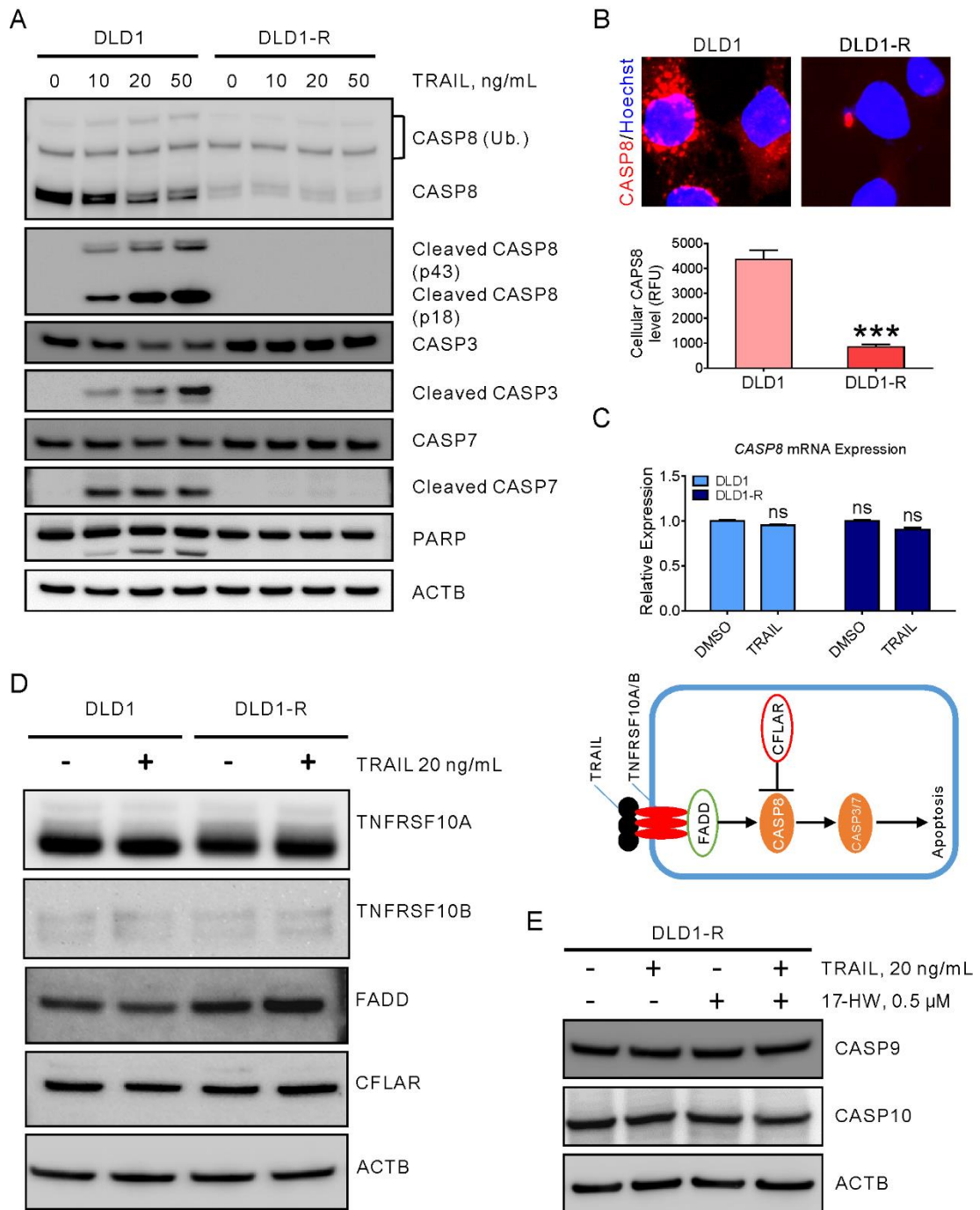
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#These authors contributed equally to this work.

Running title: 17-HW restored TRAIL response in resistant cancer cells.

Supplementary Figure S1

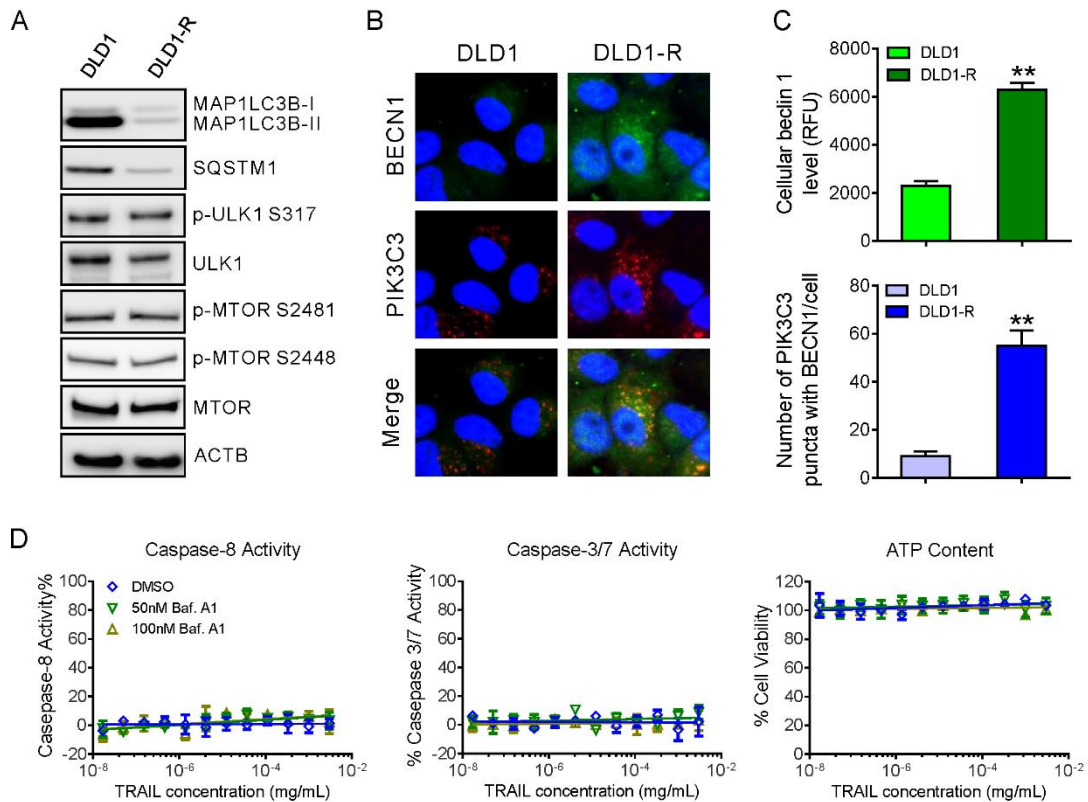


Supplementary Figure S1. 17-hydroxy wortmannin (17-HW) overcomes TRAIL resistance in DLD1-R cells.

(A) Western blot of CASP8 (Ubiquitinated), CASP8, cleaved CASP8, CASP3, cleaved CASP3, CASP7, cleaved CASP7, and PARP after treatment of DLD1 and DLD1-R cells with 0, 10, 20, or 50 ng/mL TRAIL for 8 hours. ACTB (beta-actin) acts as a loading control. (B) Immunofluorescent images of DLD1 and DLD1-R cells stained for CASP8

(red) and nuclei (blue) (top panel). Scale bar, 10 μ m. Quantification of the average CASP8 intensity in the top panel (bottom panel). (C) Real-time PCR showing the mRNA expression of *CASP8* of DLD1 and DLD1-R cells treated with 50 ng/mL TRAIL or DMSO for 8 hours. The data normalized to the *GAPDH* mRNA level of each sample. (D) Western blot of TNFRSF10A, TNFRSF10B, FADD and CFLAR after treatment of DLD1 and DLD1-R cells with 20 ng/mL TRAIL or DMSO for 8 hours. It showed no difference of these proteins after TRAIL treatment. ACTB acts as a loading control. (E) Western blot of CASP9 and CASP10 after treatment of DLD1-R cells with 20 ng/mL TRAIL and/or 0.5 μ M 17-HW for 8 hours. It showed alternate intrinsic apoptosis pathway was not affected. ACTB acts as a loading control. All values represent the mean \pm SEM (n = 3 replicates); a representative western blot is shown. Statistical analysis by two-tailed t-tests. No significance (ns), $p > 0.05$, ***, $p < 0.001$.

Supplementary Figure S2

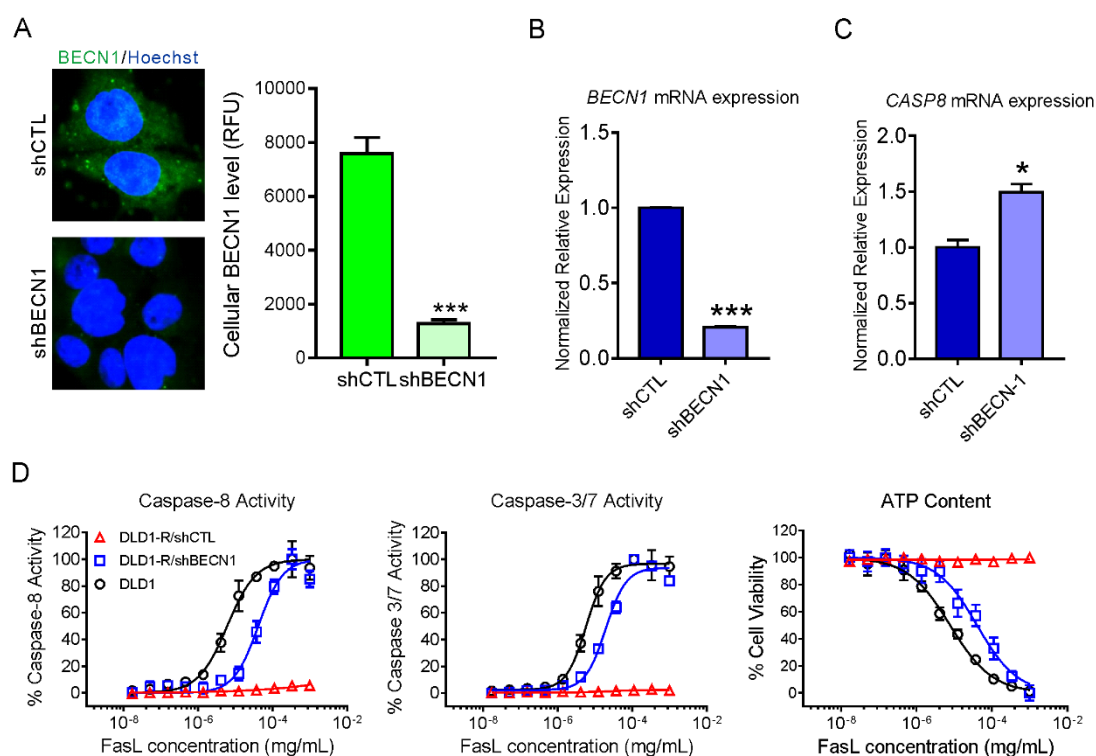


Supplementary Figure S2. Increased BECN1 lead to CASP8 degradation in TRAIL-resistant cells.

(A) Western blot of MAP1LC3B, SQSTM1, phosphorylated ULK1 ser317, ULK1, phosphorylated MTOR ser2481, phosphorylated MTOR ser2448, and MTOR of DLD1 and DLD1-R cells. ACTB acts as a loading control. It showed the significant decrease of MAP1LC3B and SQSTM1 in DLD1-R cells, indicating autophagy was over-activated autophagy. However, the protein expression and activity of ULK1 and MTOR was not changed, suggesting the activation of autophagy was not due to the effect of mTOR pathway. (B) Immunofluorescent images of DLD1 and DLD1-R cells stained for BECN1 (green), PIK3C3 (red), and nuclei (blue). Scale bar, 10 μ m. (C) Quantification of the average BECN1 intensity (top panel) and the number of PIK3C3 puncta merge with BECN1 (bottom panel) in B. (D) Dose-response curve showing the activity of caspase-8 (left panel), caspase-3/7 (middle panel) and ATP content (right panel) of DLD1-R cells treated with TRAIL, combined with 50 nM Baf.A1, 100 nM Baf.A1, or DMSO. All curves represent best fits for calculating the IC₅₀ values. All values represent the mean \pm SEM

(n = 3 replicates), a representative western blot is shown. Statistical analysis by two-tailed t-tests. **, $p < 0.01$.

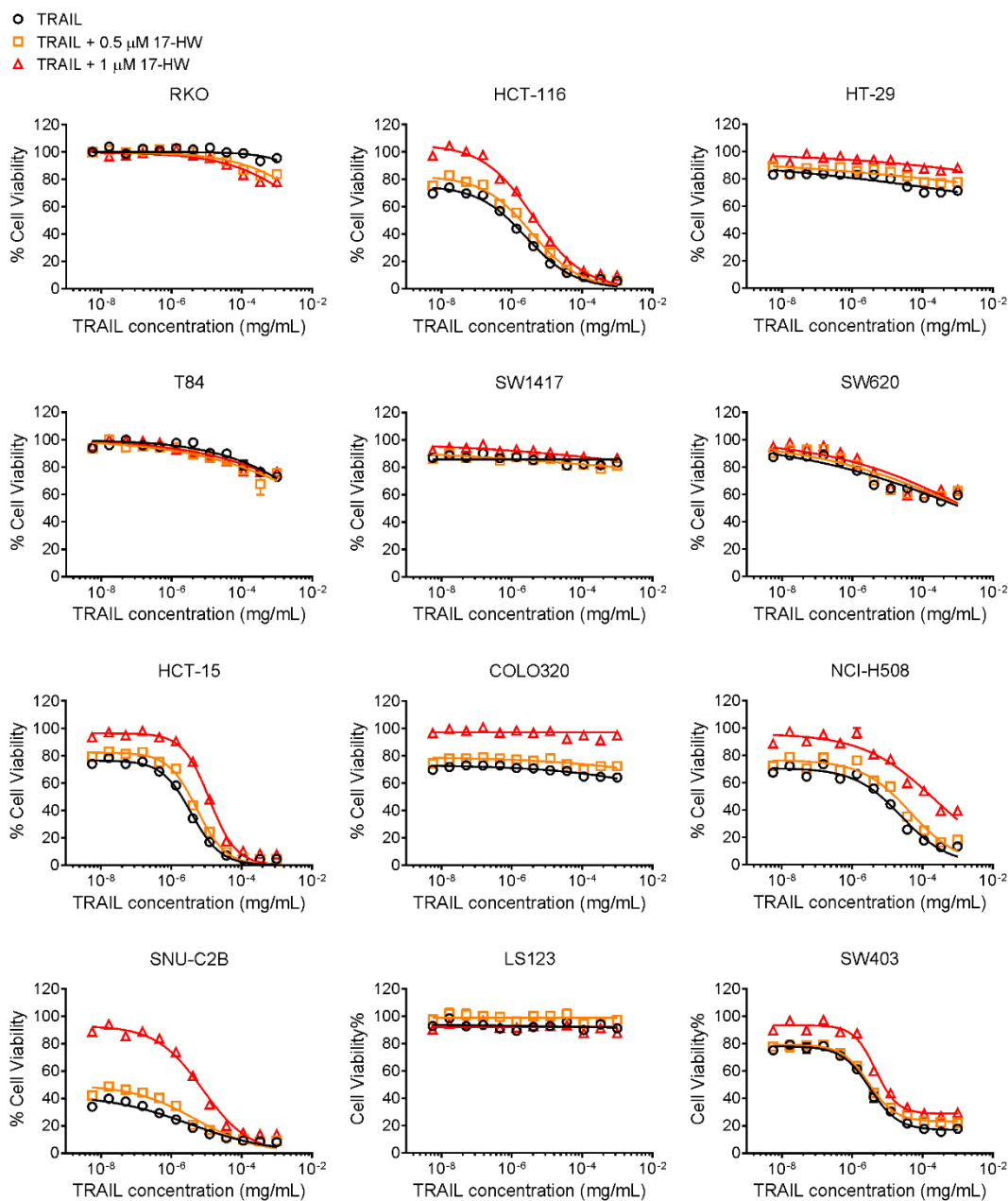
Supplementary Figure S3



Supplementary Figure S3. TRAIL response is restored by the shBECN1 knockdown.

(A) Immunofluorescent images of BECN1-knockdown DLD1-R cells and cells with control (CTL) shRNA. BECN1 is shown in green and nuclei in blue. (left panel). Scale bar, 10 μ m. Quantification of the average BECN1 intensity in the left panel (right panel). (B) Real-time PCR showing the mRNA expression of *BECN1* in DLD1-R cells treated with shBECN1 or shCTL. The data are normalized to the *GAPDH* mRNA level of each sample. (C) Real-time PCR showing the mRNA expression of *CASP8* of BECN1-knockdown DLD1-R cells and control cells. The data are normalized to the *GAPDH* mRNA level of each sample. (D) Dose-response curve showing the activity of caspase-8 (left panel), caspase-3/7 (middle panel) and ATP content (right panel) in DLD1, DLD1-R shCTL, and DLD1 shBECN1 cell lines treated with FasL. All curves represent best fits for calculating the IC_{50} values. All values represent the mean \pm SEM (n = 3 replicates). Statistical analysis by two-tailed t-test. *, $p < 0.05$, ***, $p < 0.001$.

Supplementary Figure S4



Supplementary Figure S4. Controls for the effect of 17-HW in other colon cancer cell lines.

Dose-response curve showing the cell viability of RKO, HCT-116, HT-29, T84, SW1417, SW620, HCT-15, COLO320, NCI-H508, SNU-C2B, LS123, and SW403 cells treated with TRAIL, combined with 0.5 μ M 17-HW, 1.0 μ M 17-HW or DMSO for 8 hours. RKO, HT-29, T84, SW1417, SW620, COLO320, NCI-H508 and LS123 cells were TRAIL-resistant cells. HCT-116, HCT-15, SNU-C2B, and SW403 were TRAIL-sensitive cells. All curves represent best fits for calculating the IC₅₀ values.