Supplemental Figure Legends

Supplemental Figure 1. GST or GST-Cdc37 fusion protein were expressed in bacteria and purified; these proteins were then incubated with Ulk1 (translated in *vitro*). Western blotting was performed to detect the interaction of Ulk1 with GST-Cdc37.

Supplemental Figure 2. Flag-Ulk2(WT) or Flag-Ulk2(KI) (a mutant Ulk2 lacking kinase activity) proteins were immunopurified from transfected HEK293 cells. Then *in vitro* kinase assays were performed in the presence of GST-Cdc37(WT) or GST-Cdc37(3A). Phosphorylated proteins were visualized with autoradiography.

Supplemental Figure 3. (A-B) Wild-type and Ulk1-KO DLD1 cells were treated with 17-AAG (A) or AUY922 (B) at different concentration for 36 h, and cell proliferation was tested with the MTT assay. (C-D)Wild-type, Ulk1-KO, Atg3-KO Ulk1/Atg3-KO HCT116 cells were treated with 40 nM 17-AAG (C) or 20 nM AUY922 (D), and cell proliferation was tested with the MTT assay at different time point. (E-F) Wild-type and Ulk1-KO DLD1 cells were treated with 40 nM 17-AAG (E) or 20 nM AUY922 (F) combined with phenformin, and cell proliferation was tested with the MTT assay.

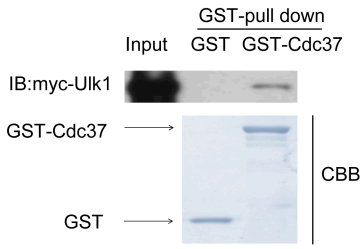
Supplemental Figure 4. HCT116 cells transfected with Flag-Cdc37(WT), Flag-Cdc37(3A) or Flag-Cdc37(3E) were subjected to Flag-IP.

Supplemental Figure 5. (A) Atg3 RNAi was performed in Ulk1(WT)- expressing HCT116 cells. Cells were then treated at different concentrations of 17-AAG for 12 h, proteins were extracted for Western blot. (B) Atg3-KO and Ulk1/Atg3-KO HCT116 cells were treated with the combination of 17-AAG and phenformin for 12 h. Proteins were then extracted for Western blot. (C) A set of RNAi-resistant rescue forms of Cdc37 plasmids were transfected into stable Cdc37-RNAi HCT116 cells. Cells were then treated with phenformin for 12 h.

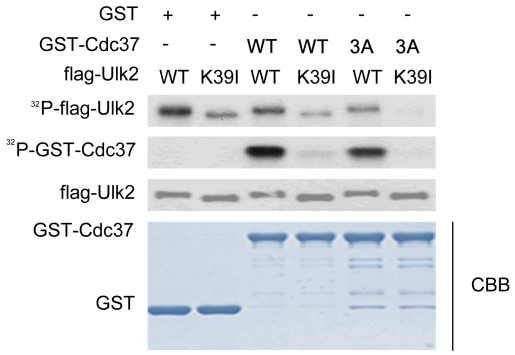
Supplemental Figure 6. (A) HCT116 cells were treated with 17-AAG at different concentration for 36 h. Proteins were then extracted for Western blot. (B) HCT116 cells were treated with 40 nM 17-AAG combined with phenformin. Proteins were then extracted for Western blot.

Supplemental Figure 7. (A) Different amount HA-Ulk1 plasmid was transfected into Ulk1-KO HCT116 cells. Western blotting was then performed. (B) Wild-type and Ulk1-KO HCT116 cells were treated with the combination of 17-AAG and phenformin for 12 h. Proteins were then extracted for Western blot.

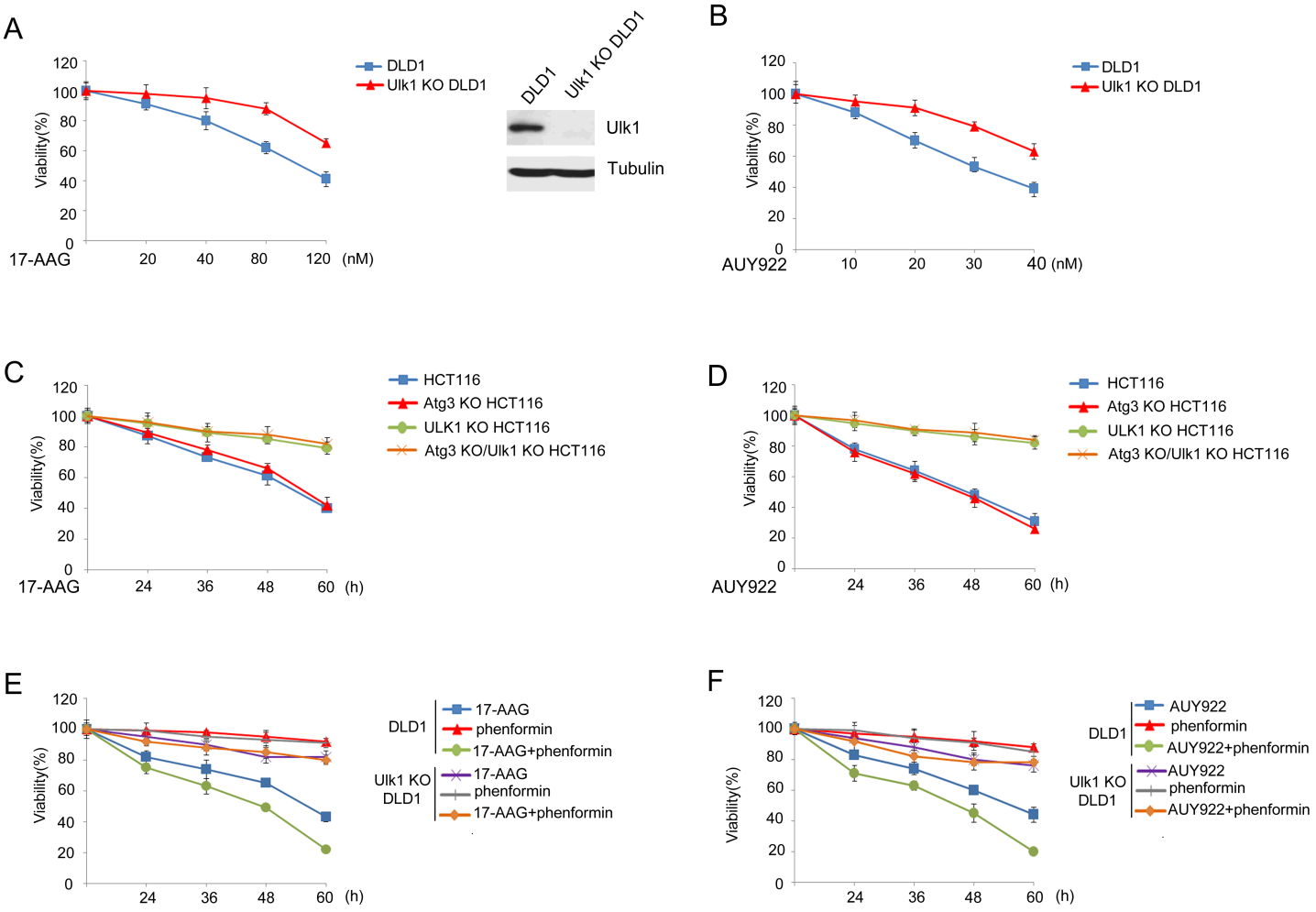
Supplemental Figure 1



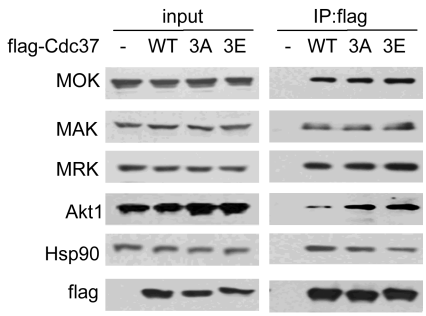
Supplemental Figure 2



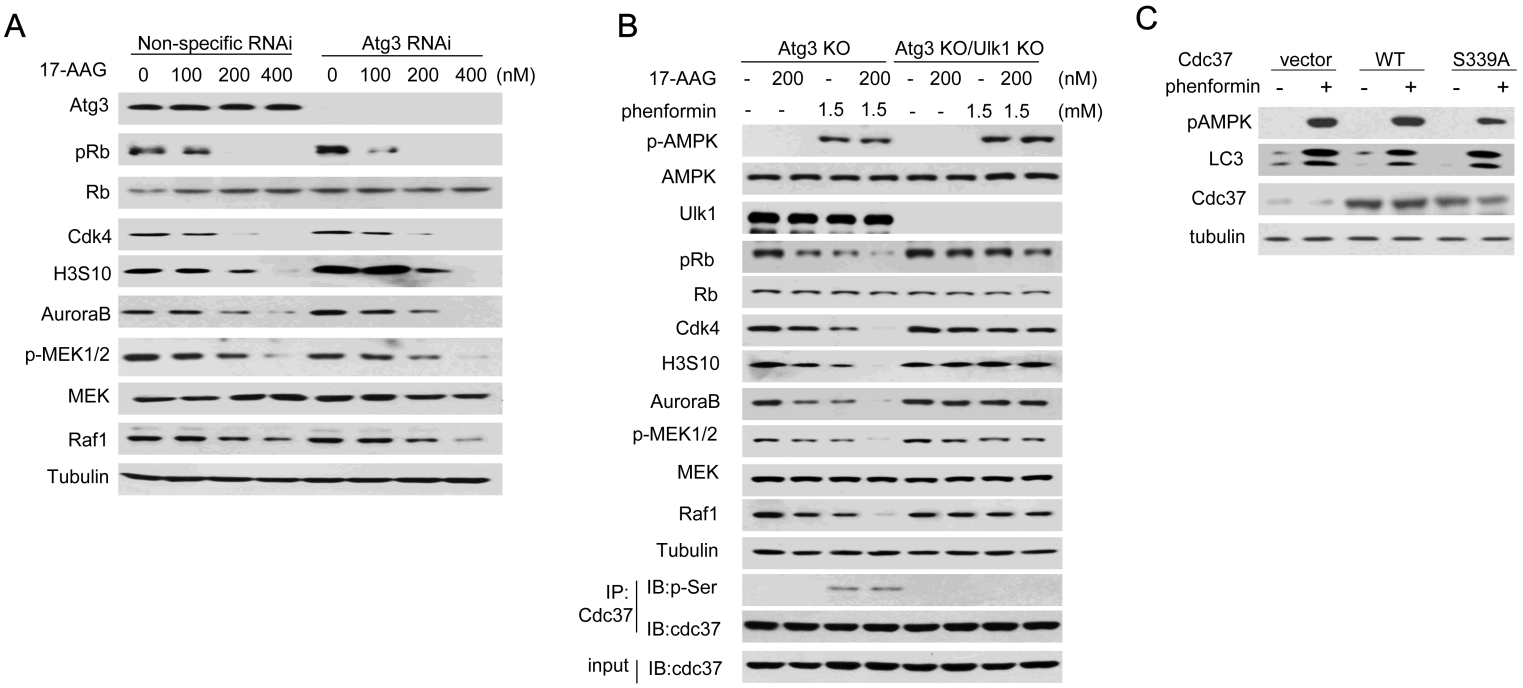
Supplemental Figure 3



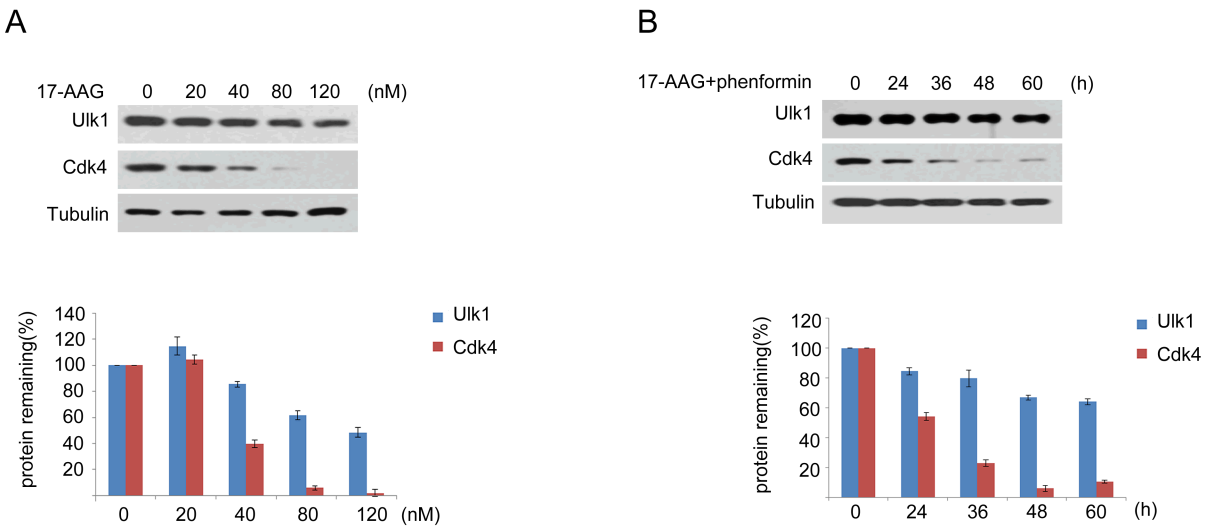
Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7