# Supplementary Information

## Natural (dihydro)phenanthrene plant compounds are direct activators of AMPK through its allosteric drug and metabolite-binding site

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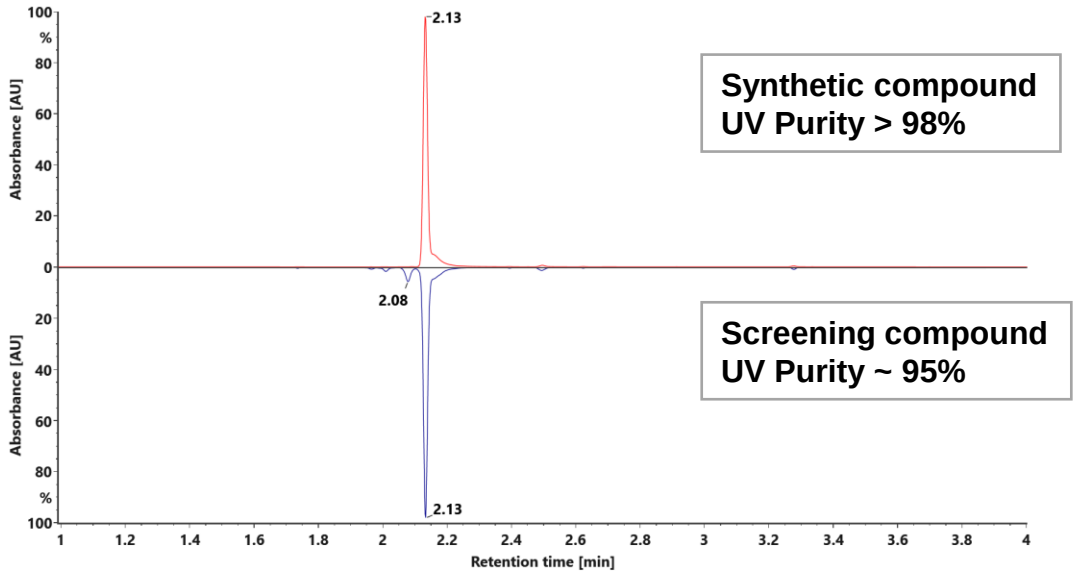
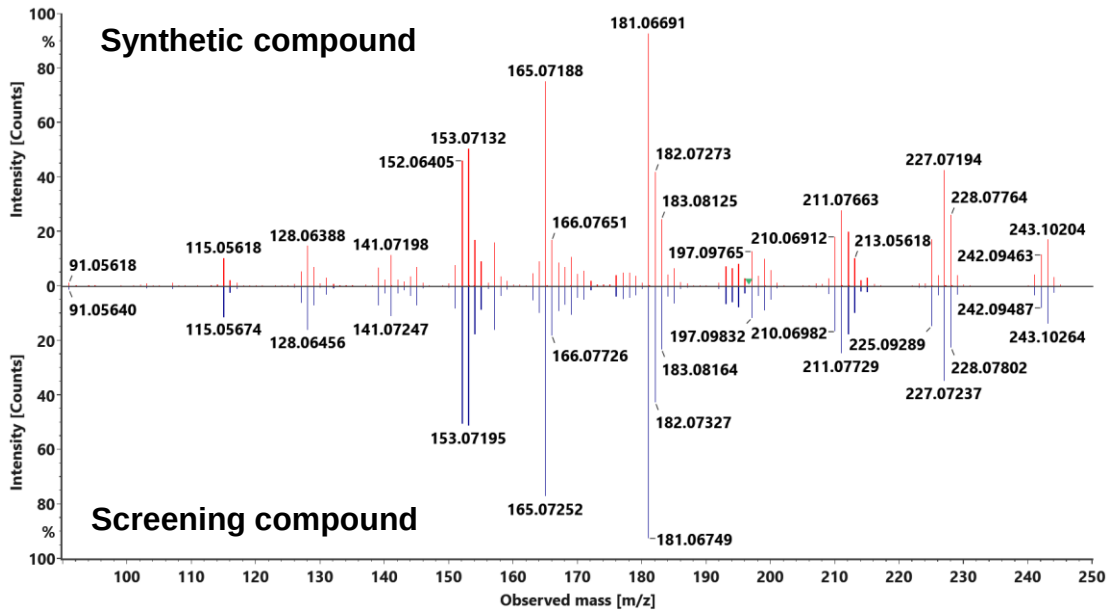
# Supplementary Figure 1

A) LC-UV trace @ 280 nm



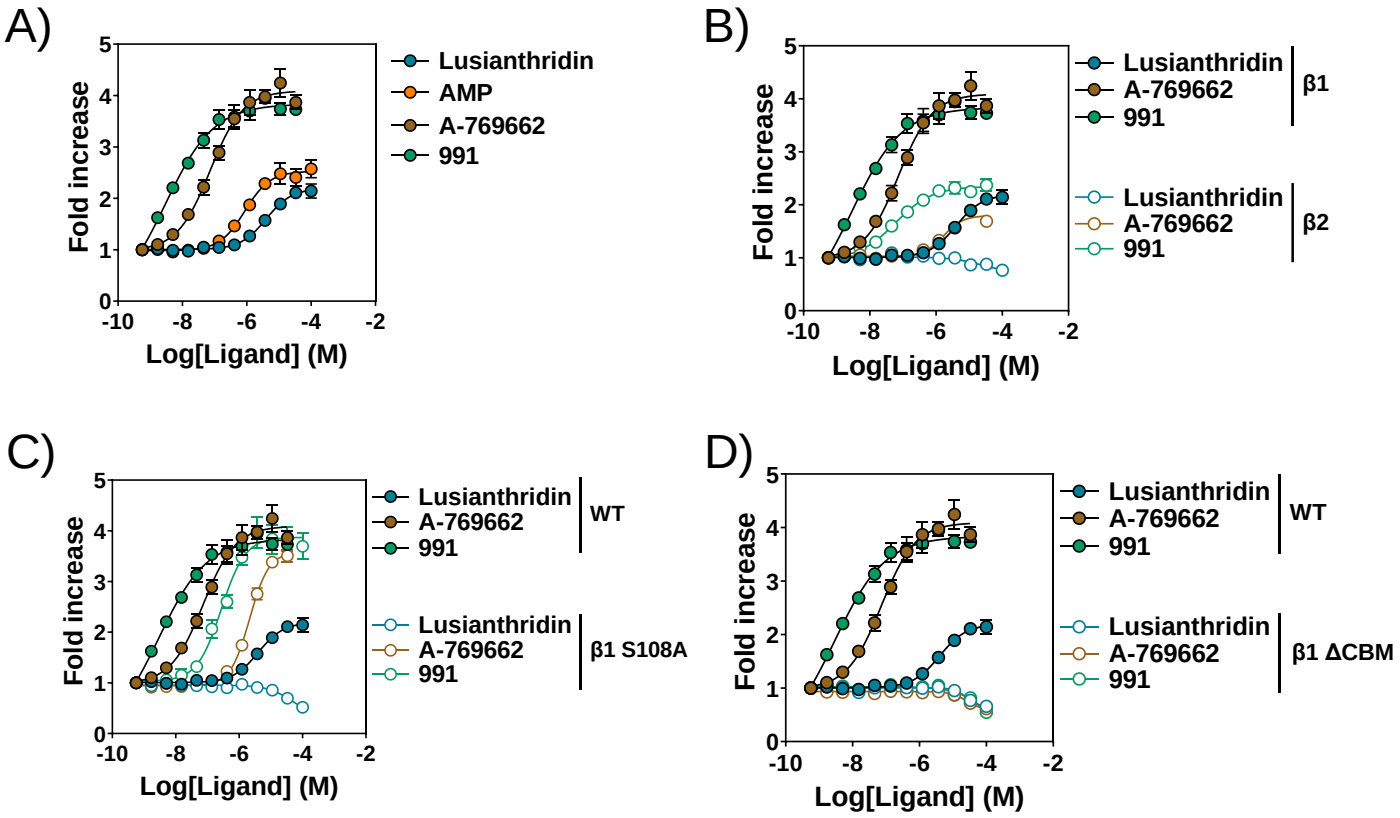

B) Tandem mass spectrometry spectra




**Supplementary Figure 1. Quality control of Lusianthridin samples from the initial screening library compared to the one obtained by synthesis**

A) Determination of the Lusianthridin purity by RPLC-UV at 280 nm: HSS C18 T3 column (2.1 i.d. x 50 mm) using linear gradient of ACN and $H_2O$ + 0.1% formic acid from 2% to 98% in 5 min. B) Tandem mass spectrometry spectra of Lusianthridin obtained by synthesis (upper) compared to the sample from the initial screening library (lower); spectra were acquired on a QqToF system with a ramp of energy from 20 to 60 eV.

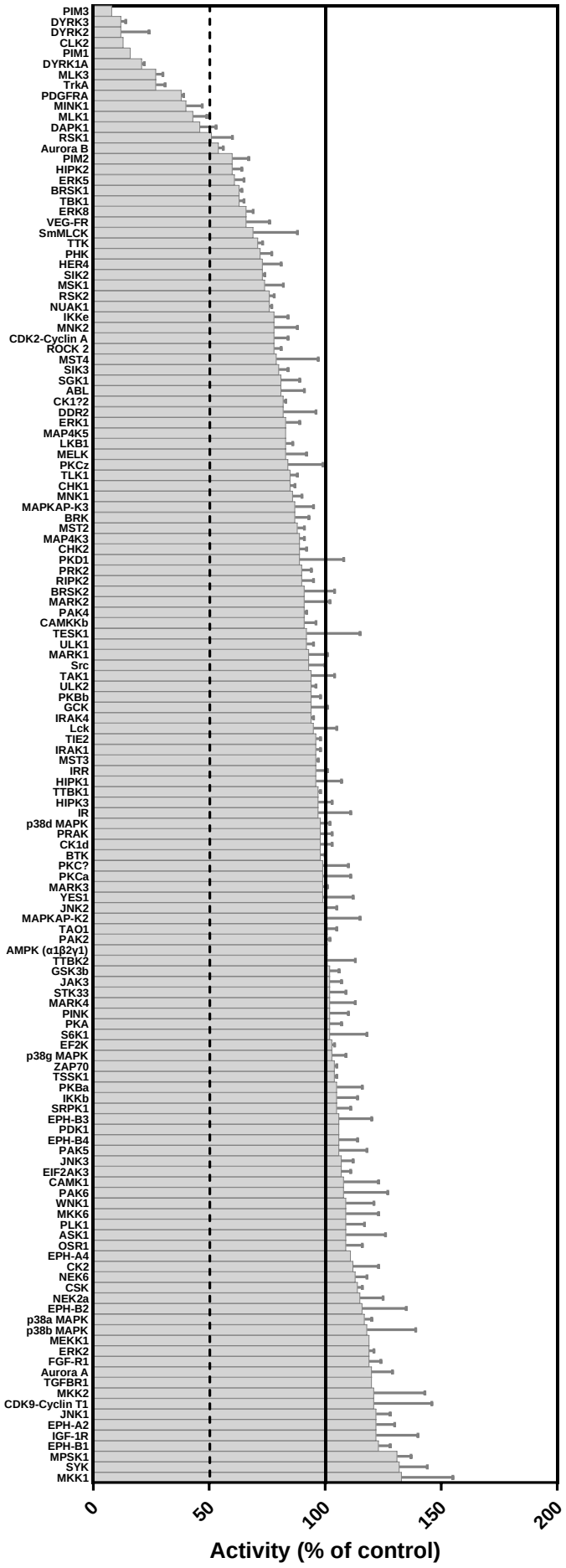
# Supplementary Figure 2




**Supplementary Figure 2. Regulation of AMPK complexes by activators**

A) AMPK activity of bacterially-expressed recombinant α2β1γ1 in the presence of increasing concentrations of AMP, 991, A-769662 or Lusianthridin. 991, A-769662 or Lusianthridin activation of α2β1γ1 compared to α2β2γ1 (B), α2β1γ1 S108A mutant (C) or α2β1γ1 lacking the carbohydrate binding module (CBM) of the β1 subunit (ΔCBM) (D). AMPK activity was determined using the HTRF KinEASE assay kit and results are presented as fold increase in AMPK activity (± SEM n=3) relative to the activity in the absence of activator.

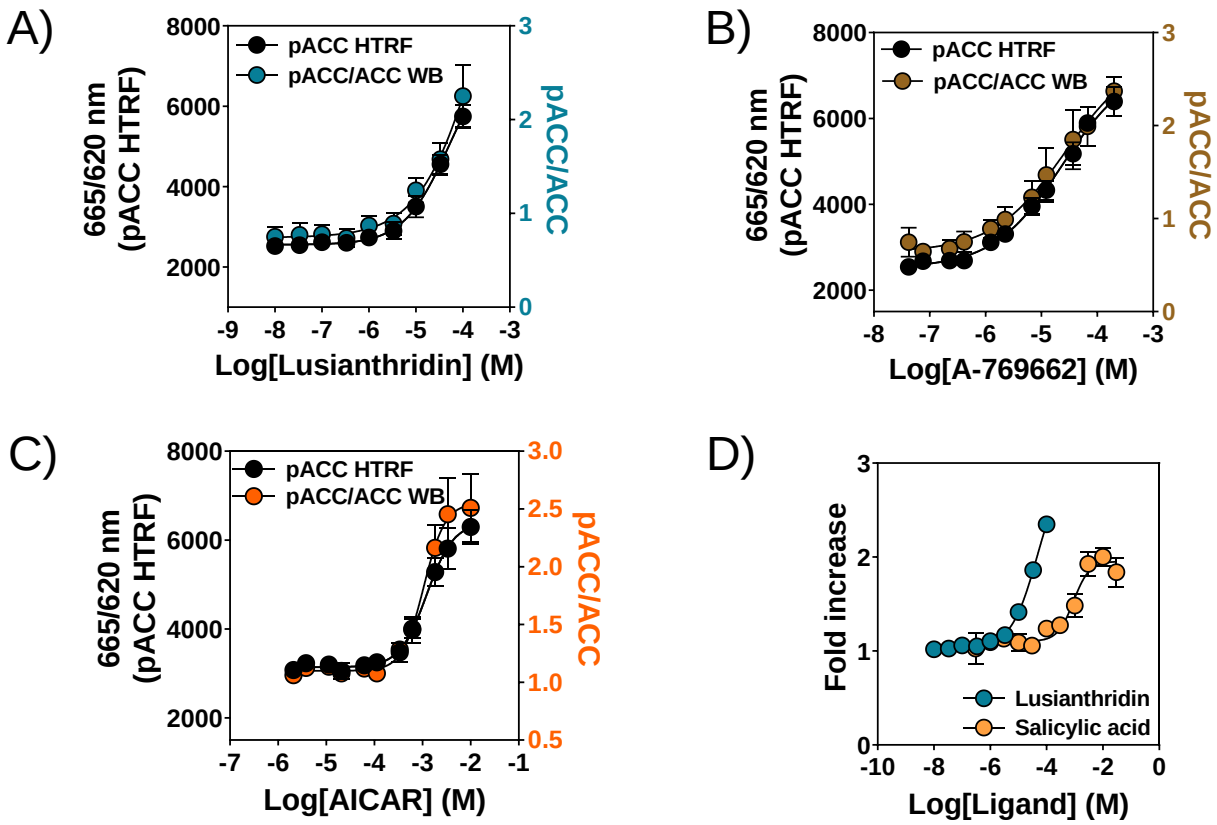
# Supplementary Figure 3




**Supplementary Figure 3. Lusianthridin effect on a panel of protein kinases**

Lusianthridin (10 μM) was used in a protein kinase panel screen (140 kinases) by the International Centre for Kinase Profiling (University of Dundee). Results are displayed as the mean percentage activity compared with the activity of each kinase in the absence of Lusianthridin. The kinases are ranked in order of least to most activity (top to bottom).

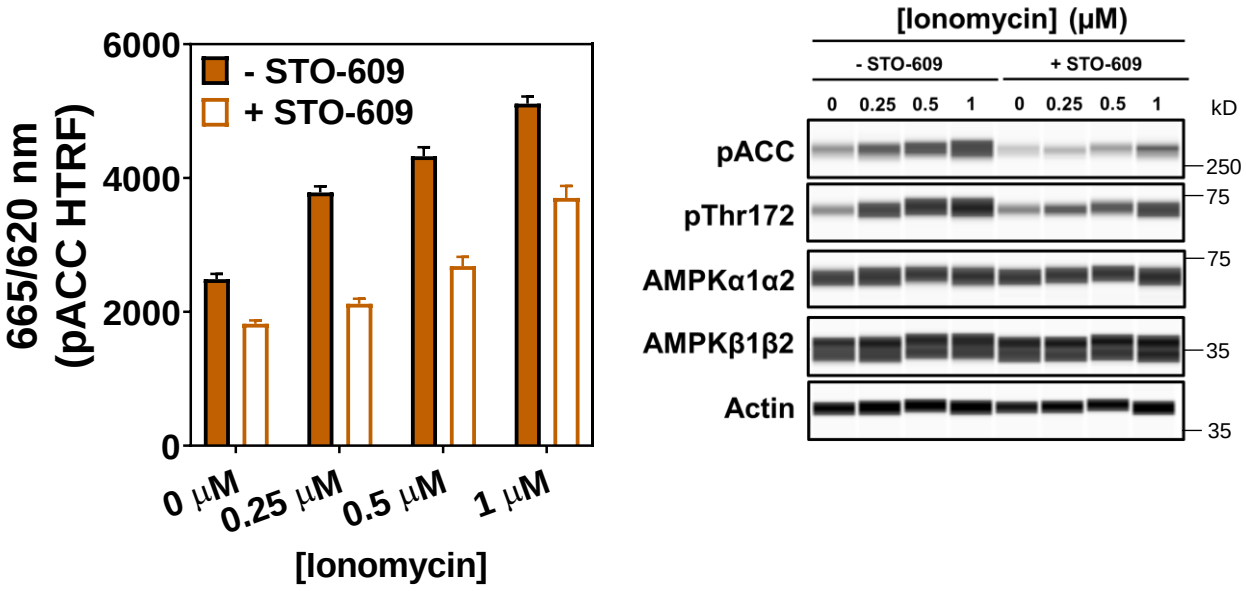
# Supplementary Figure 4




**Supplementary Figure 4. Lusianthridin activation of AMPK in intact cells**

U2OS cells were treated with varying concentrations of Lusianthridin (A), A-769662 (B) or AICAR (C) for 30 min at 37 ºC. Phosphorylation of ACC was determined by western blot (WB) analysis, and a graph is shown comparing the results (mean ± SEM of at least 3 independent experiments) from the western blot analysis with the data obtained from the pACC HTRF assay. D) U2OS cells were treated with varying concentrations of Lusianthridin or salicylic acid for 30 min at 37 ºC. Phosphorylation of ACC was determined using the pACC HTRF assay (mean ± SEM of at least 3 independent experiments) .

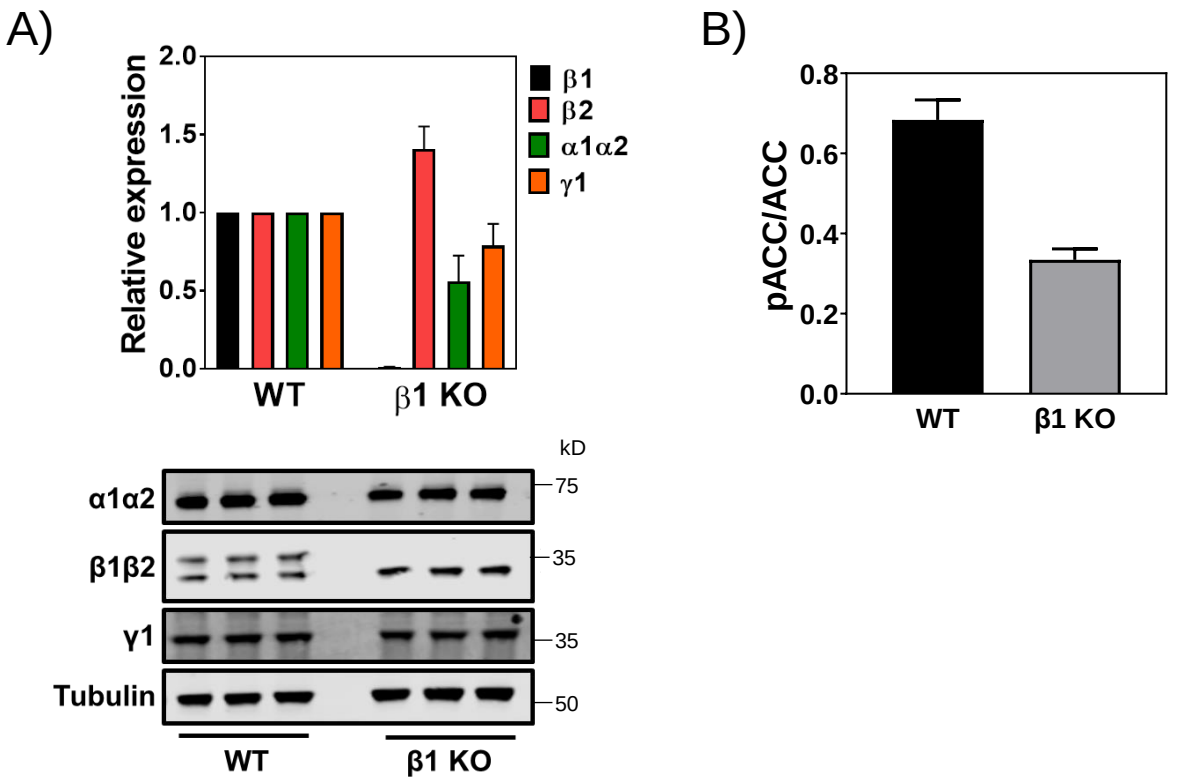
# Supplementary Figure 5




**Supplementary Figure 5. Effect of STO-609 on activation of AMPK by ionomycin**

Left, a graph showing the results from U2OS cells treated with varying concentrations of ionomycin for 5 min with or without prior treatment with STO-609 (10 µg/ml). pACC was determined by performing a HTRF assay with the pACC assay kit and results are presented as the ratio of 665/620 nm (pACC HTRF). Right, western blot analysis of the results using capillary electrophoresis (Sally Sue, ProteinSimple).

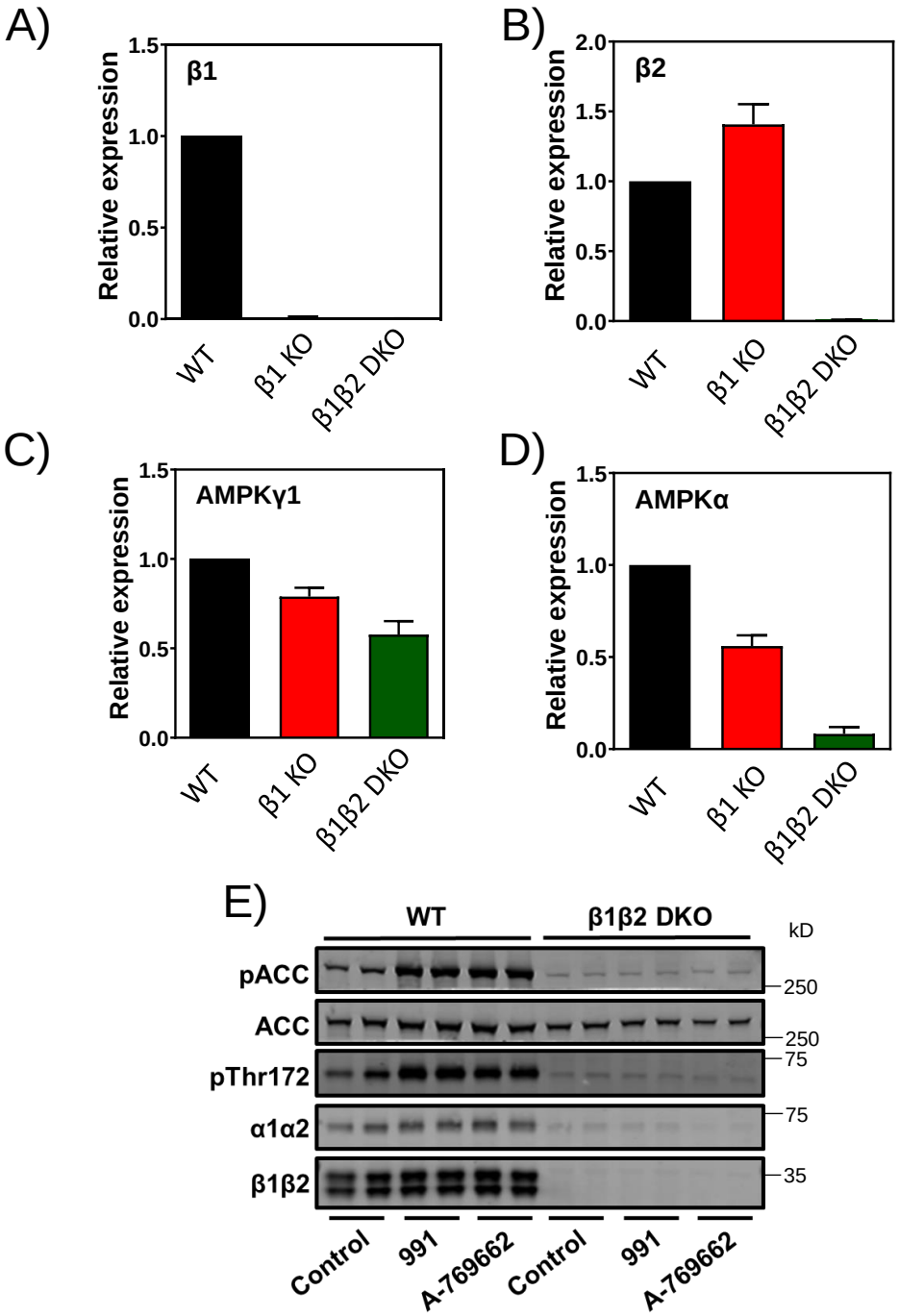
# Supplementary Figure 6




**Supplementary Figure 6. AMPK subunit expression and AMPK activity in β1 KO U2OS cells**

A) AMPK subunit analysis of U2OS wild-type (WT) or β1 knockout (β1 KO) cells using the indicated subunit antibodies. Quantification of 3 independent experiments is displayed along with a representative blot. Results are shown as relative expression to the U2OS WT cells. B) Comparison of the pACC/ACC levels in U2OS WT versus β1 KO cells.

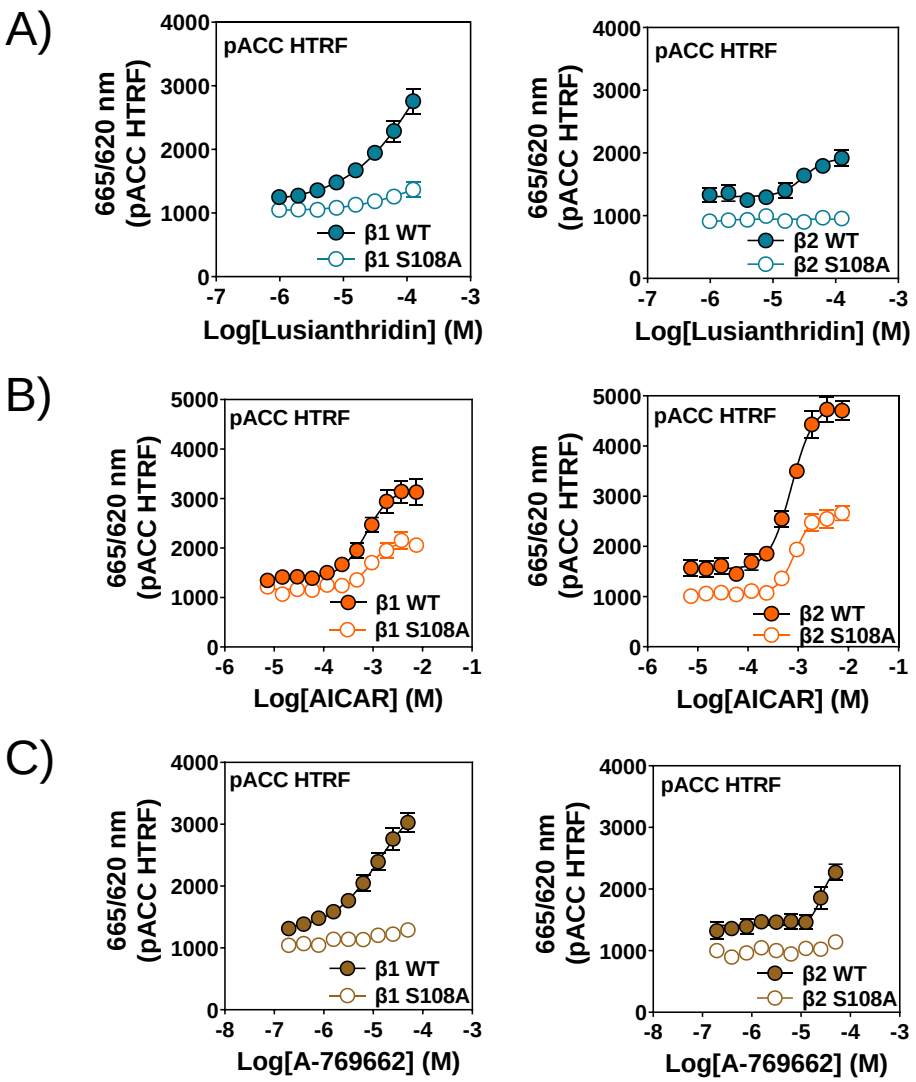
# Supplementary Figure 7




**Supplementary Figure 7. Subunit expression of WT, β1 KO and β1β2 double knockout (DKO) U2OS cells**

Comparison of the expression of β1 (A), β2 (B), γ1 (C) and total AMPKα (D) in U2OS WT, β1 KO or β1β2 DKO cells. The bands were quantified and the relative subunit expression compared U2OS WT cells was determined and shown in the graph (mean ± SEM of at least 3 independent experiments). E) U2OS WT and β1β2 DKO cells were treated with control (DMSO), 991 (30 μM) or A-769662 (100 μM) for 30 min at 37 ºC and subjected to western blot analysis with the indicated antibodies.

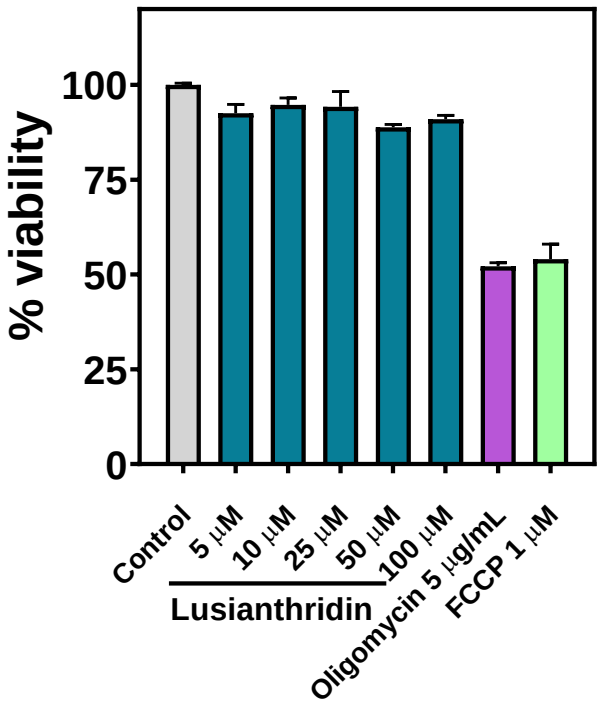
# Supplementary Figure 8




**Supplementary Figure 8. Treatment of β1 and β2 WT, and β1 and β2 S108A mutant stable cell lines with activators**

β1 and β2 WT, and β1 and β2 S108A stable cell lines were treated with varying concentrations of Lusianthridin (A), AICAR (B), A-769662 (C). Phosphorylation of ACC was determined using the pACC HTRF assay and the ratio of 665/620 nm is displayed. Results are the mean ± SEM of at least 3 independent experiments.

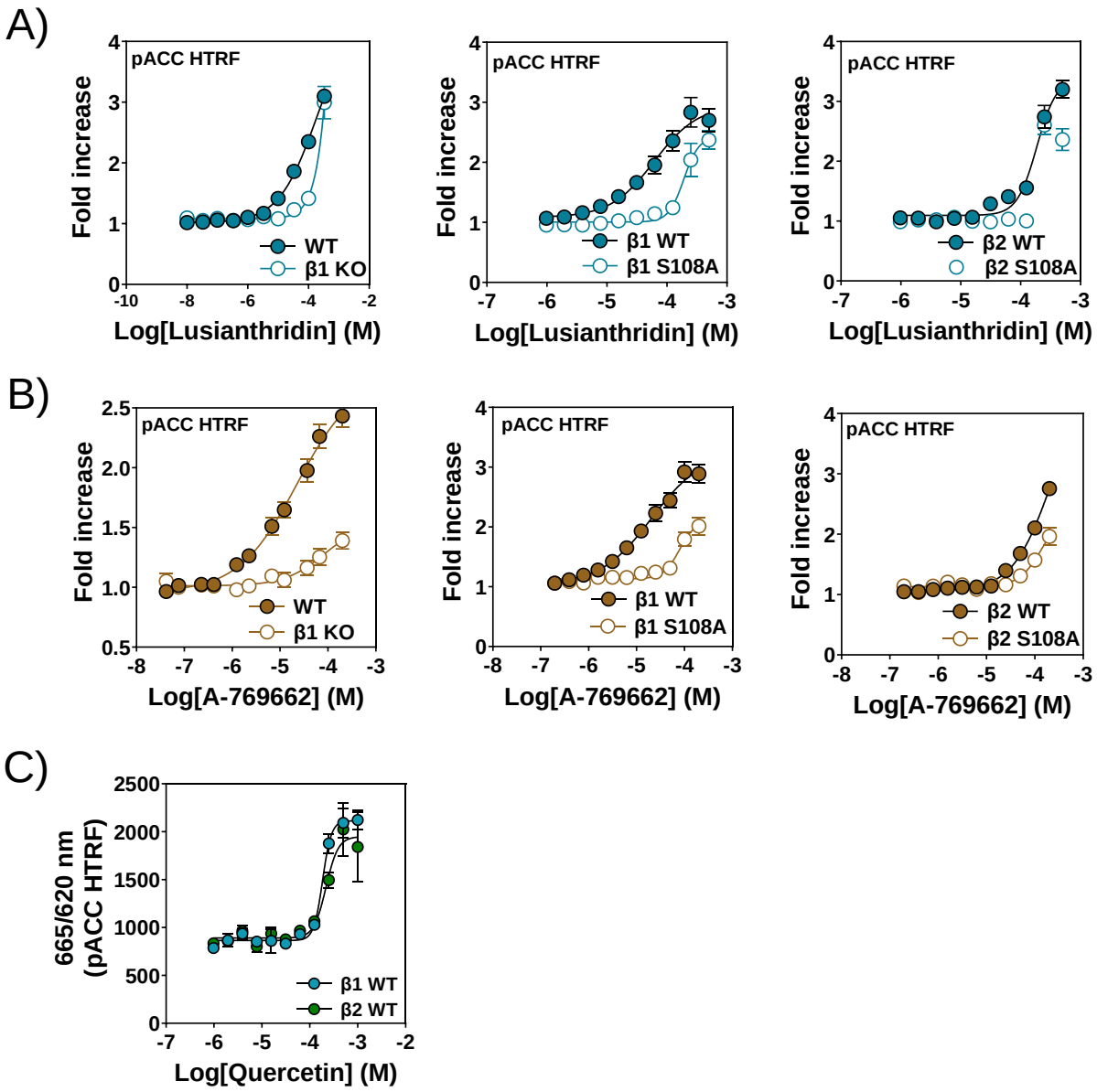
# Supplementary Figure 9




**Supplementary Figure 9. Lusianthridin does not decrease the cell viability of mouse primary hepatocytes**

Mouse primary hepatocytes were isolated and seeded at 10k cells per well in a 96 well plate. The following day, cells were treated for 1 h or 4 h (Lusianthridin) in media without phenol red. The cells were then labelled with the MTT compound (Vybrant® MTT Cell Proliferation Assay Kit - Thermo - V13154) to measure cell viability according to the manufacturer`s instructions.

# Supplementary Figure 10

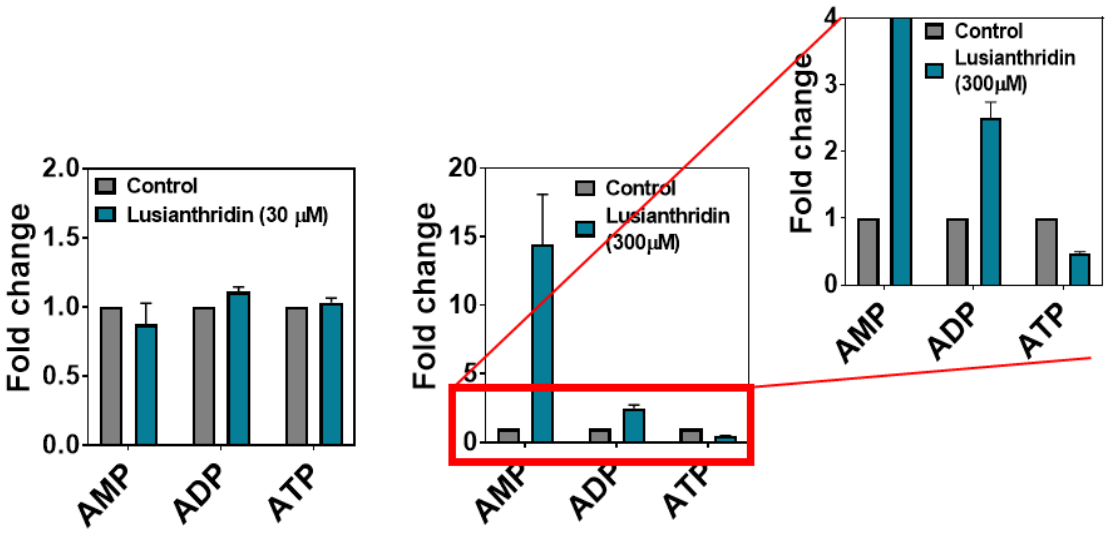



**Supplementary Figure 10. Treatment of WT or β1 KO U2OS cells, β1 and β2 WT, and β1 and β2 S108A mutant stable cell lines with activators**

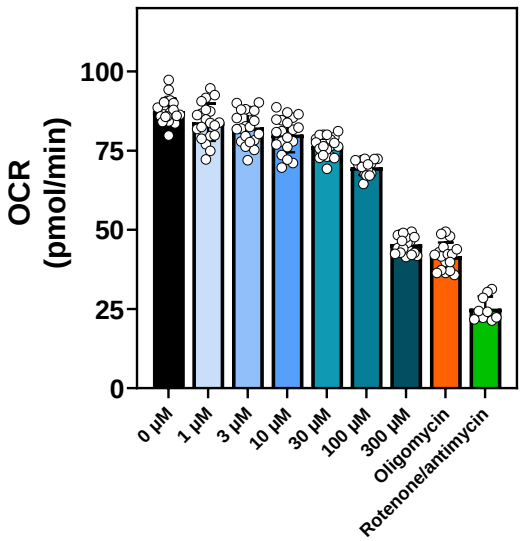
WT or β1 KO U2OS cells, β1 and β2 WT, and β1 and β2 S108A stable cell lines (as indicated in the figure legends) were treated with varying concentrations of Lusianthridin (A) or A-769662 (B). Phosphorylation of ACC was determined using the pACC HTRF assay and fold increase is displayed. Results are the mean ± SEM of at least 3 independent experiments. C) β1 WT and β2 WT stable cell lines were treated with varying concentrations of quercetin for 30 min at 37 °C. Phosphorylation of ACC was determined using the pACC HTRF assay displayed as the ratio of the 665/620 nm.

# Supplementary Figure 11

A)




B)




**Supplementary Figure 11. Effect of Lusianthridin on adenine nucleotides and oxygen consumption rate.** A) Adenine nucleotides were measured in U2OS cells treated for 30 mins with 30 or 300 µM of Lusianthridin. Methods can be found in Elia, I., Broekaert, D., Christen, S. et al. Proline metabolism supports metastasis formation and could be inhibited to selectively target metastasizing cancer cells. Nat Commun 8, 15267 (2017). Results are shown as fold change versus control treated cells. B) HepG2 cells were incubated with varying concentrations of Lusianthridin (as indicated) for 30 mins and oxygen consumption rate was determined using a XF96 instrument (Seahorse Biosciences, MA, USA) at 37°C. The effects of Oligomycin and Rotenone/antimycin A are shown as a comparison.