

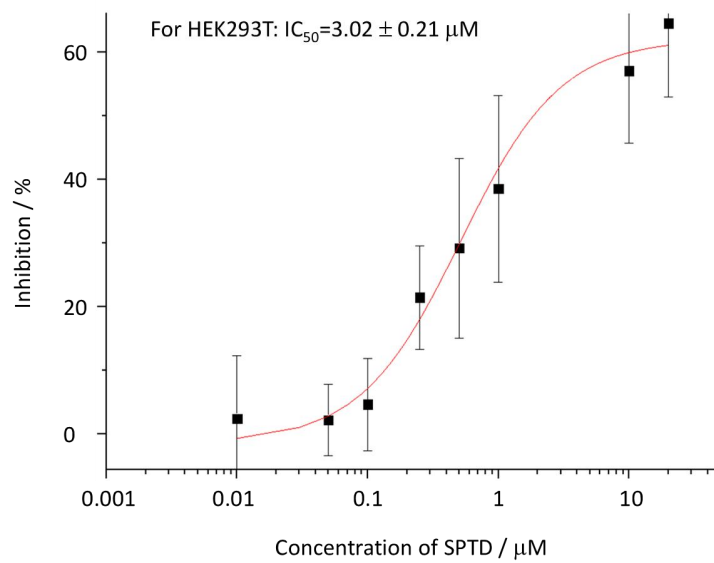
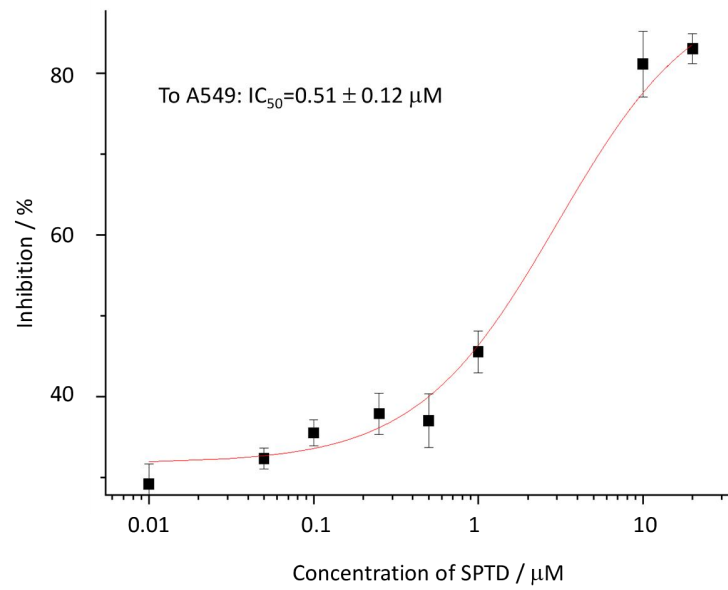
# **Strophanthidin induces apoptosis of human lung adenocarcinoma cells by promoting TRAIL-DR5 signaling**

Xiao Tian <sup>1,2,+</sup>, Liangzhen Gu <sup>2,3,+</sup>, Fangang Zeng<sup>4</sup>, Xingkai Liu<sup>2</sup>, Yang Zhou <sup>2,3</sup>,  
Yang Dou<sup>2,3</sup>, Juanjuan Han<sup>2</sup>, Yao Zhao<sup>2,3</sup>, Yanyan Zhang<sup>2</sup>, Qun Luo <sup>\*2,3</sup> and Fuyi  
Wang<sup>\*1,2,3</sup>

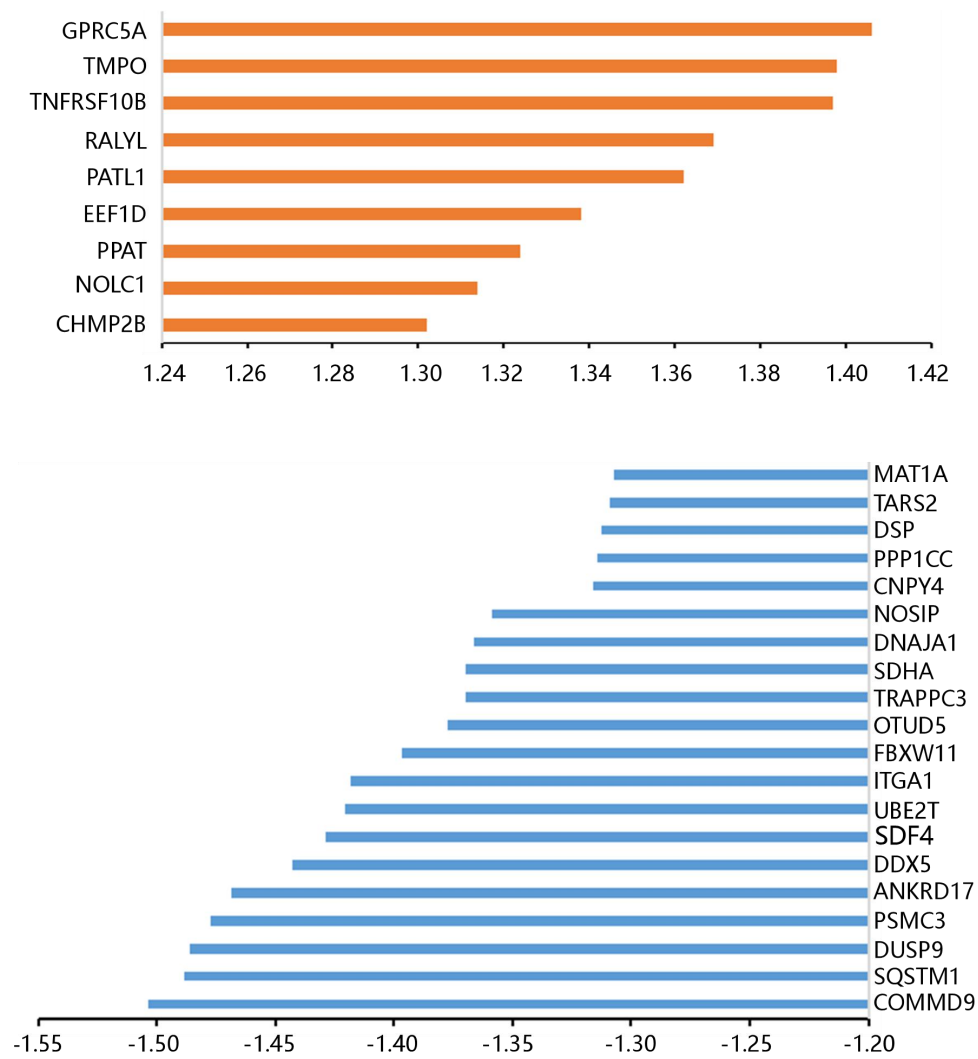
## **Supplementary Materials**

Figure S1 – S12

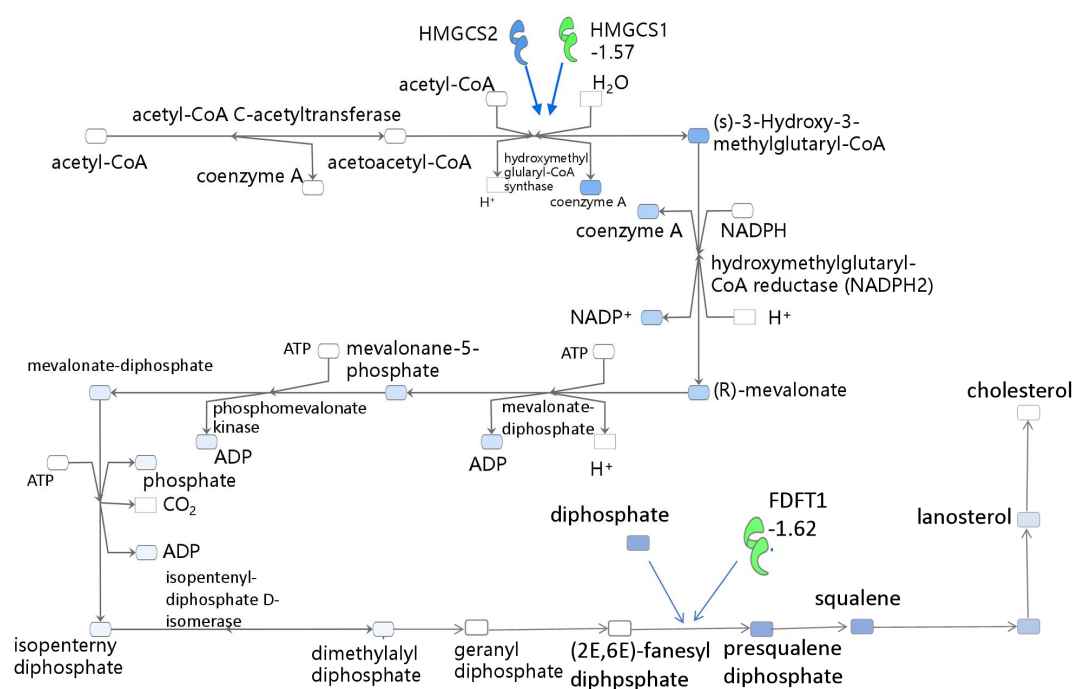
Table S1 – S2



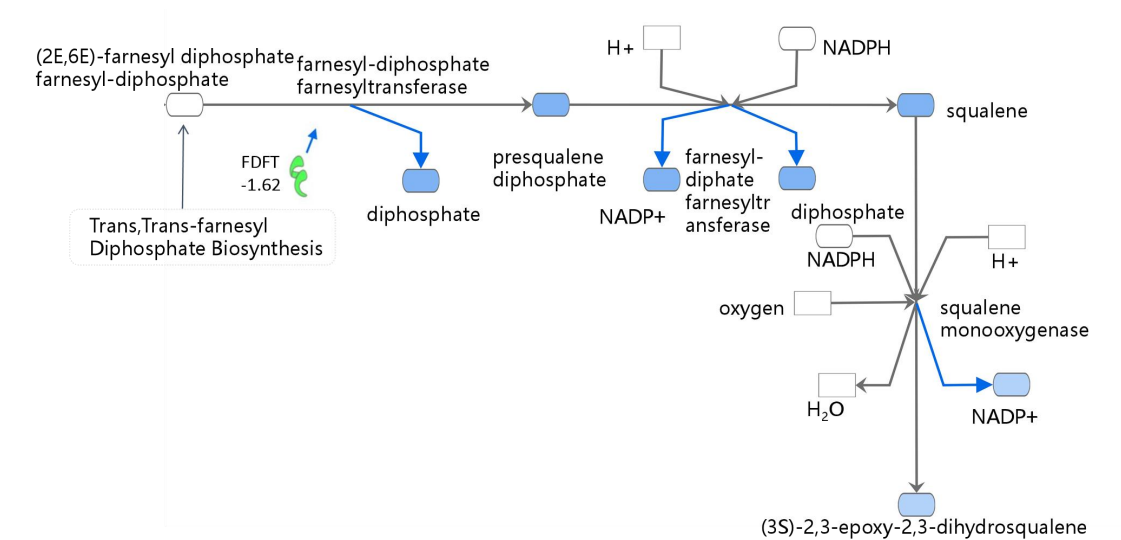
**Figure S1.** The inhibition of Strophanthidin (SPTD) on the growth of (top) A549 cells and (bottom) HEK293T cells incubated with various concentrations of SPTD at 37 °C for 24 h. The inhibition rate (%) are present as mean  $\pm$  SD, n=6.



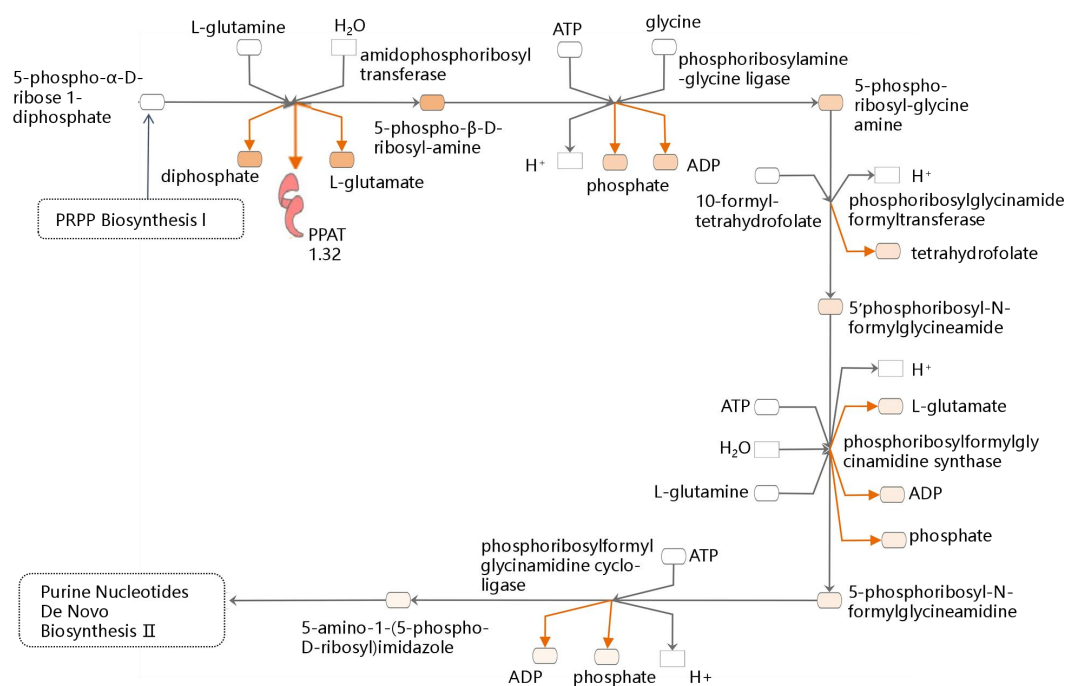
**Figure S2.** Differentially expressed proteins (DEPs) with a fold change  $|FC| \geq 1.3$  identified in A549 cells incubated with 1.0  $\mu\text{M}$  Strophanthidin (SPTD) at 37 °C for 24 h, compared to those in A549 cells without treatment.



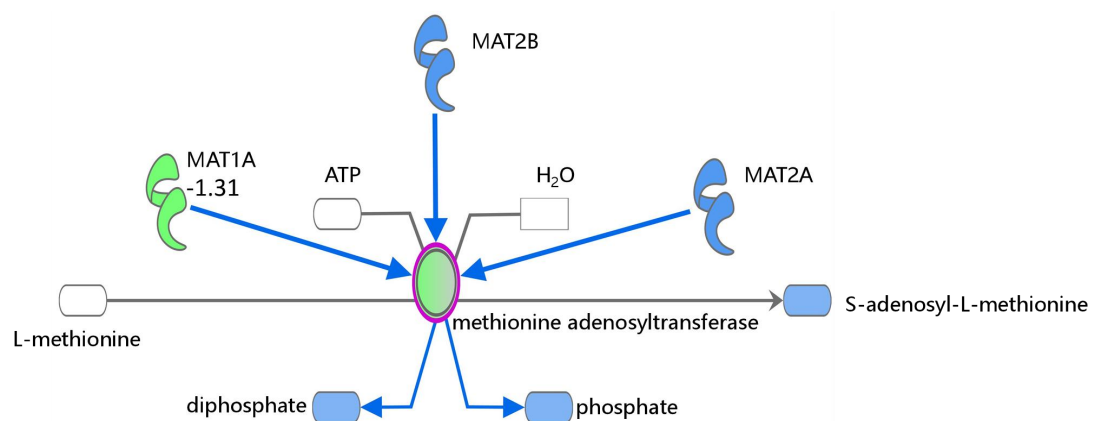
**Figure S3.** Schematic diagram super-pathway of cholesterol biosynthesis signaling pathway with which the DEPs with  $|FC| \geq 1.3$  identified in A549 cells incubated with 1.0  $\mu\text{M}$  strophanthidin (SPTD) at 37  $^{\circ}\text{C}$  for 24 h, compared to those in A549 cells without treatment. The red color represents the protein (or complex) upregulated, green downregulated; the orange color represents the protein (or complex) predicted to be upregulated, blue to be downregulated.



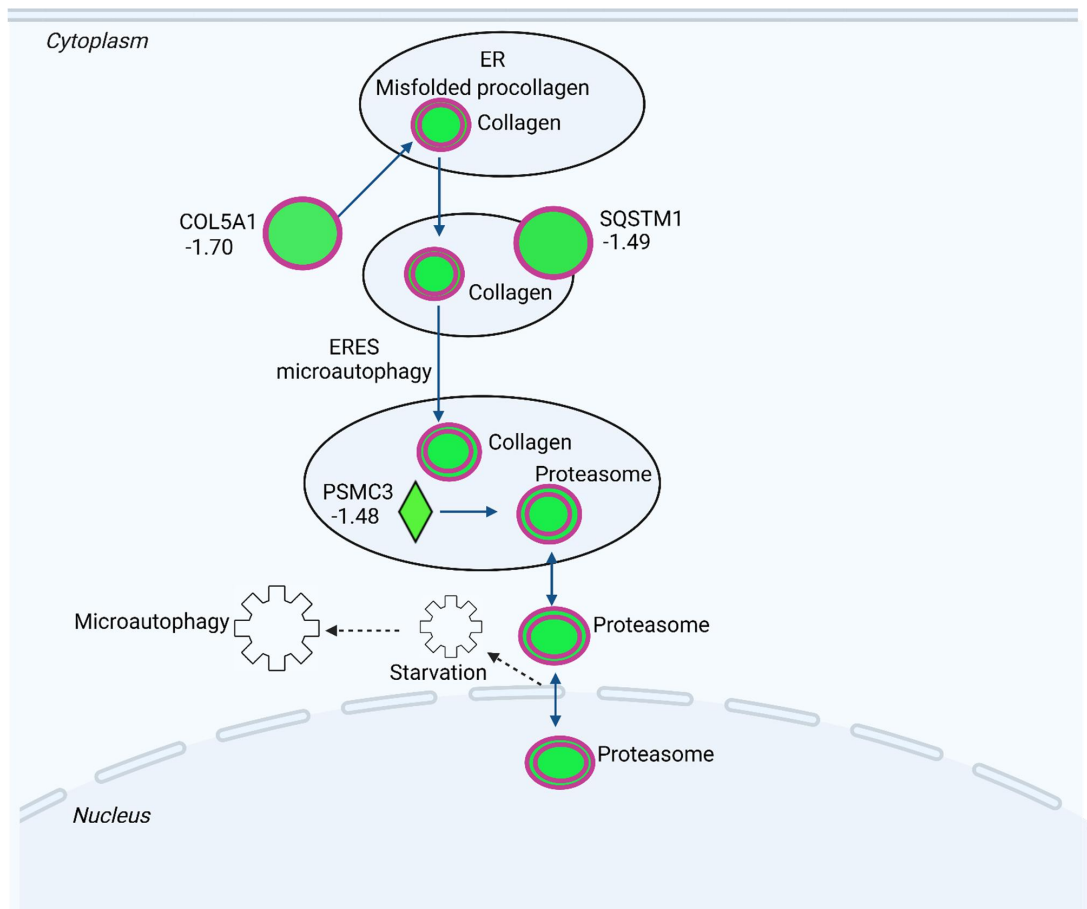
**Figure S4.** Schematic diagram super-pathway of epoxy-squalene biosynthesis signaling pathway with which the DEPs with  $|FC| \geq 1.3$  identified in A549 cells incubated with 1.0  $\mu\text{M}$  SPTD at 37 °C for 24 h, compared to those in A549 cells without treatment. The red color represents the protein (or complex) upregulated, green downregulated; the orange color represents the protein (or complex) predicted to be upregulated, blue to be downregulated.



**Figure S5.** Schematic diagram super-pathway of 5-aminoimidazole ribonucleotide biosynthesis I signaling pathway with which the DEPs with  $|FC| \geq 1.3$  identified in A549 cells incubated with 1.0  $\mu\text{M}$  SPTD at 37 °C for 24 h, compared to those in A549 cells without treatment. The red color represents the protein (or complex) upregulated, green downregulated; the orange color represents the protein (or complex) predicted to be upregulated, blue to be downregulated.

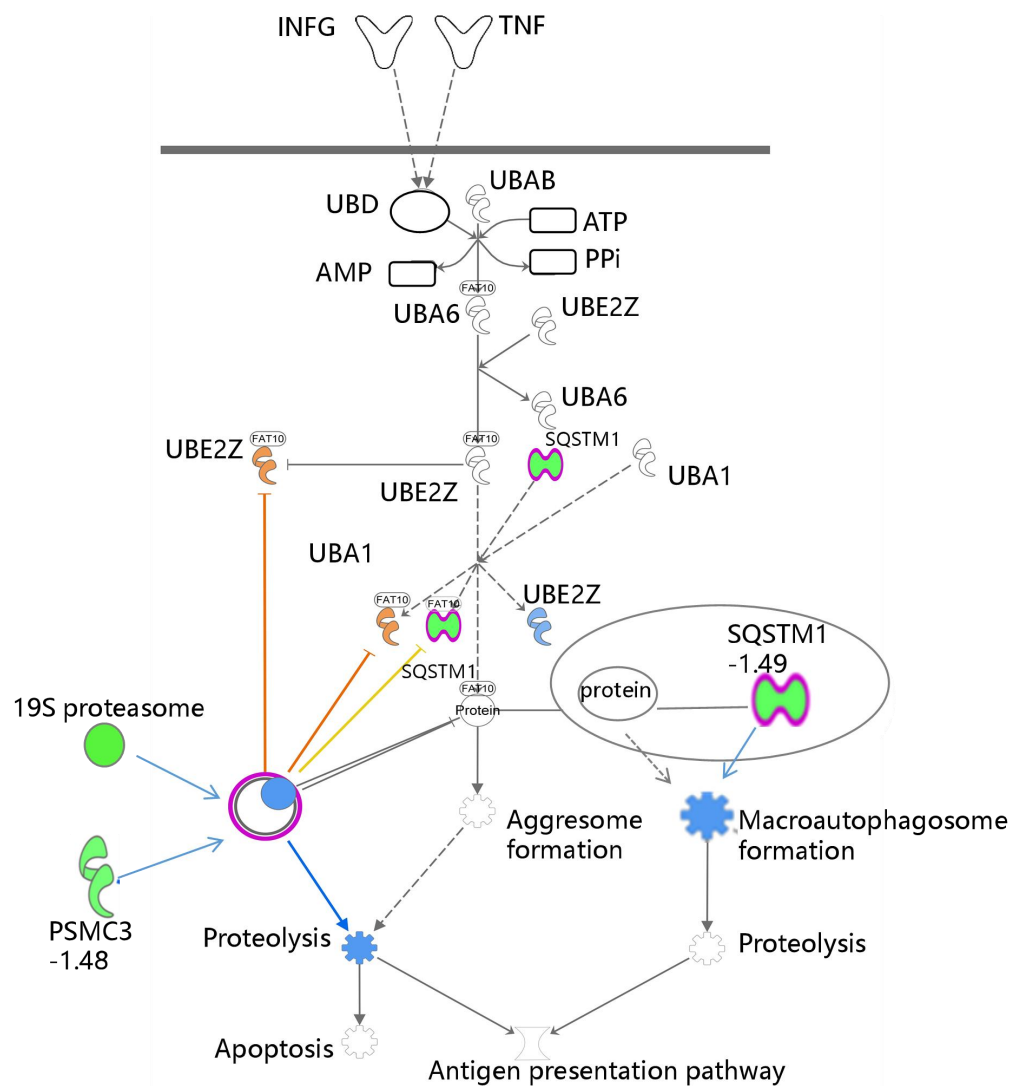


**Figure S6.** Schematic diagram super-pathway of S-adenosyl-L-methionine biosynthesis signaling pathway with which the DEPs with  $|FC| \geq 1.3$  identified in A549 cells incubated with 1.0  $\mu\text{M}$  SPTD at 37 °C for 24 h, compared to those in A549 cells without treatment. The red color represents the protein (or complex) upregulated, green downregulated; the orange color represents the protein (or complex) predicted to be upregulated, blue to be downregulated.

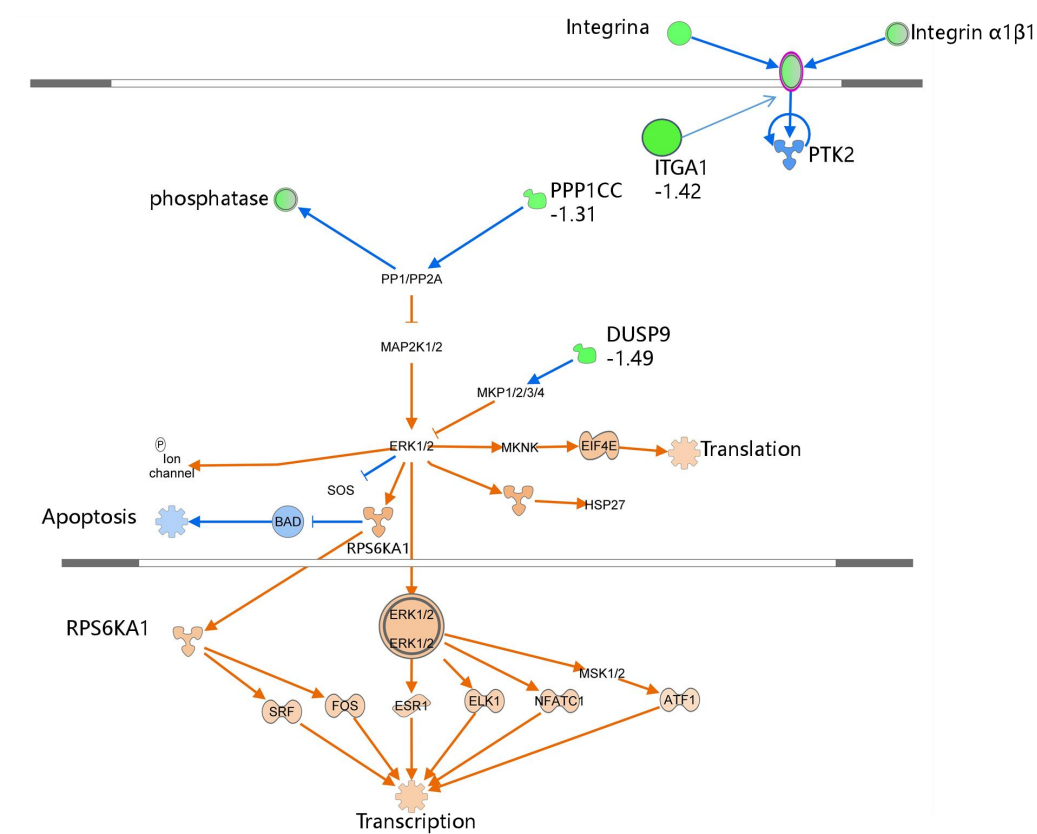


**Figure S7.** Schematic diagram super-pathway of micro-autophagy signaling pathway with which the DEPs with  $|FC| \geq 1.3$  identified in A549 cells incubated with 1.0  $\mu\text{M}$  SPTD at 37 °C for 24 h, compared to those in A549 cells without treatment. The red color represents the protein (or complex) upregulated, green downregulated; the orange color represents the protein (or complex) predicted to be upregulated, blue to be downregulated.

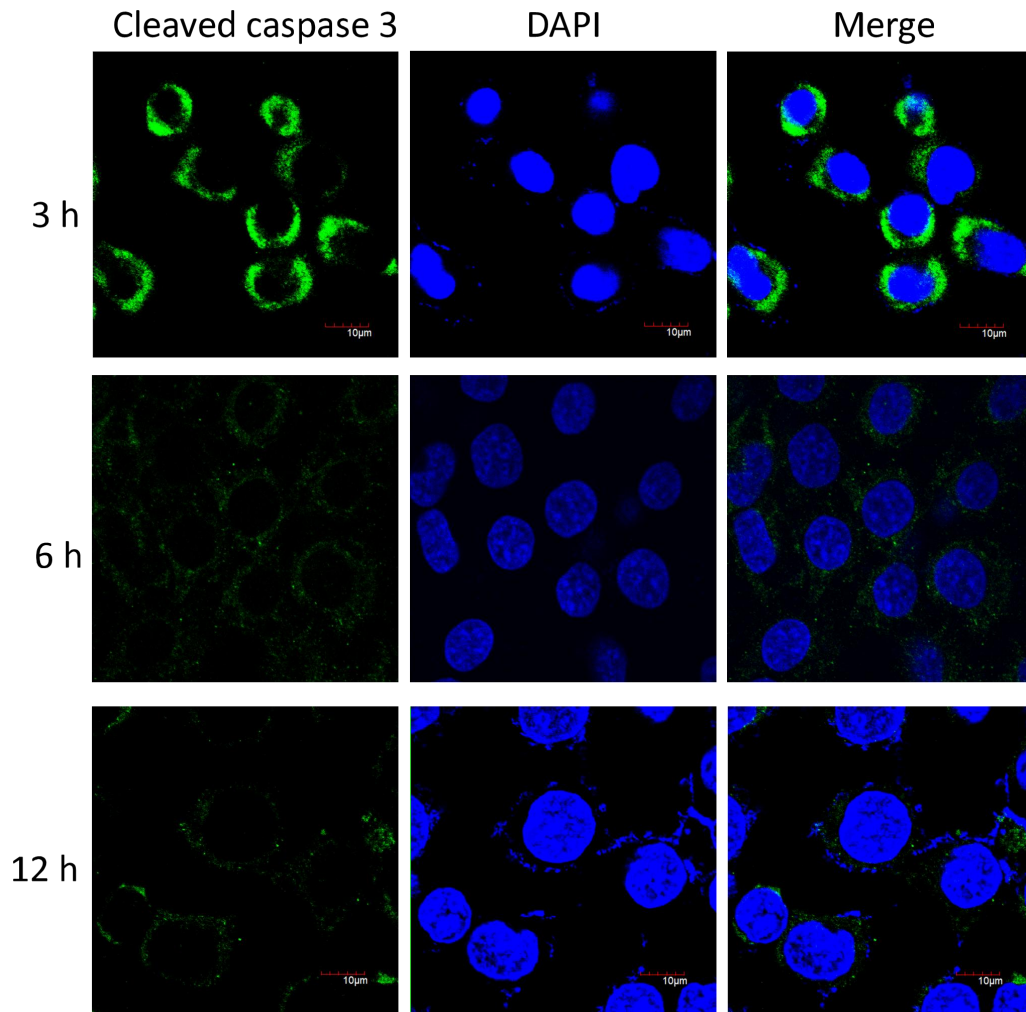




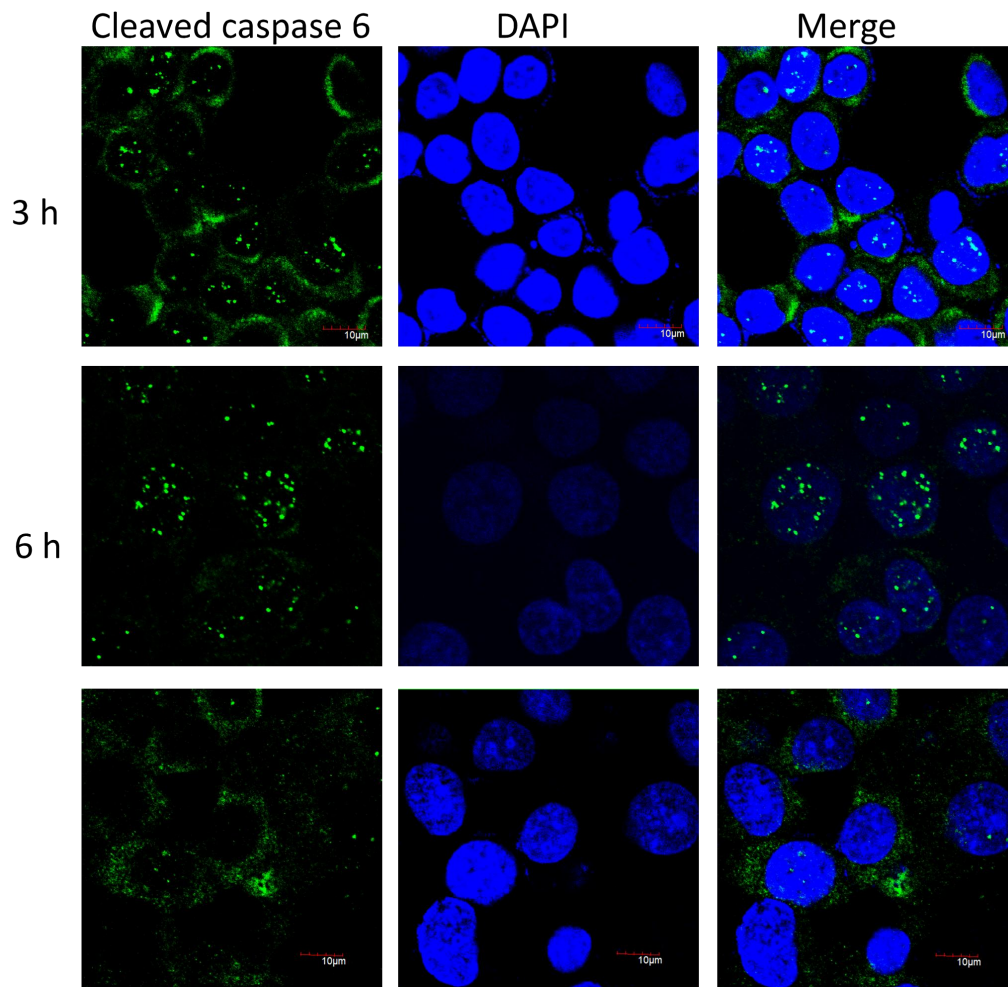
**Figure S8.** Schematic diagram super-pathway of FAT10 signaling pathway with which the DEPs with  $|FC| \geq 1.3$  identified in A549 cells incubated with 1.0  $\mu\text{M}$  SPTD at 37 °C for 24 h, compared to those in A549 cells without treatment. The red color represents the protein (or complex) upregulated, green downregulated; the orange color represents the protein (or complex) predicted to be upregulated, blue to be downregulated.



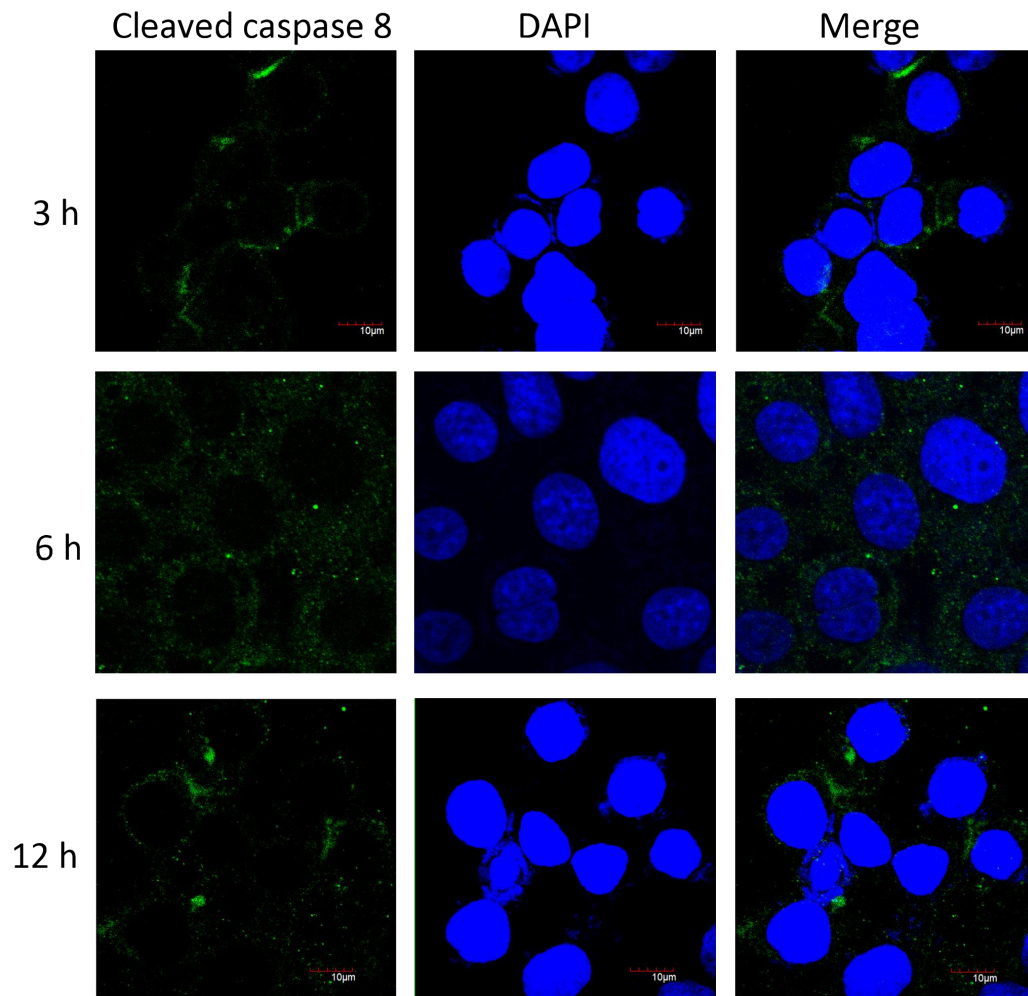
**Figure S9.** Schematic diagram super-pathway of ERK/MAPK signaling pathway with which the DEPs with  $|FC| \geq 1.3$  identified in A549 cells incubated with  $1.0 \mu\text{M}$  SPTD at  $37^\circ\text{C}$  for 24 h, compared to those in A549 cells without treatment. The red color represents the protein (or complex) upregulated, green downregulated; the orange color represents the protein (or complex) predicted to be upregulated, blue to be downregulated.



**Figure S10.** Immunofluorescence images of cleaved/activated caspase-3 in A549 cells treated with 1.0  $\mu\text{M}$  SPTD for different times at 37 °C. Green fluorescence represents the target protein and blue fluorescence represents the nucleus stained by DAPI.



**Figure S11.** Immunofluorescence images of cleaved/activated caspase-6 in A549 cells treated with 1.0  $\mu$ M SPTD for different times at 37 °C. Green fluorescence represents the target protein and blue fluorescence represents the nucleus stained by DAPI.



**Figure S12.** Immunofluorescence images of cleaved/activated caspase-8 in A549 cells treated with 1.0  $\mu\text{M}$  SPTD for different times at 37 °C. Green fluorescence represents the target protein and blue fluorescence represents the nucleus stained by DAPI.