Supplemental Material to:

Wen-Jie Guo, Yang-Miao Zhang, Li Zhang, Bin Huang, Fei-Fei Tao, Wei Chen, Zi-Jian Guo, Qiang Xu and Yang Sun

Novel monofunctional platinum (II) complex Mono-Pt induces apoptosis-independent autophagic cell death in human ovarian carcinoma cells, distinct from cisplatin

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Supplementary Fig. S1

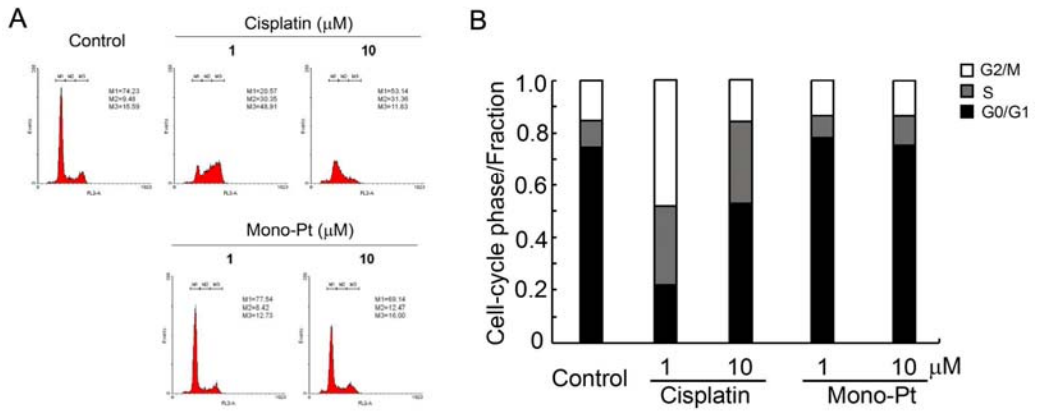

Figure S1. Cisplatin but not Mono-Pt induces cell cycle arrest in human ovarian carcinoma Skov-3 cells. About 5×10^5 synchronized cells were grown/well in 6-well plates and treated with 1 μM or 10 μM of cisplatin or Mono-Pt for 24 h. M1, M2 and M3 indicated G_0/G_1 phase, S phase and G_2/M phase. The results shown are representative of three different experiments.

Supplementary Fig. S2

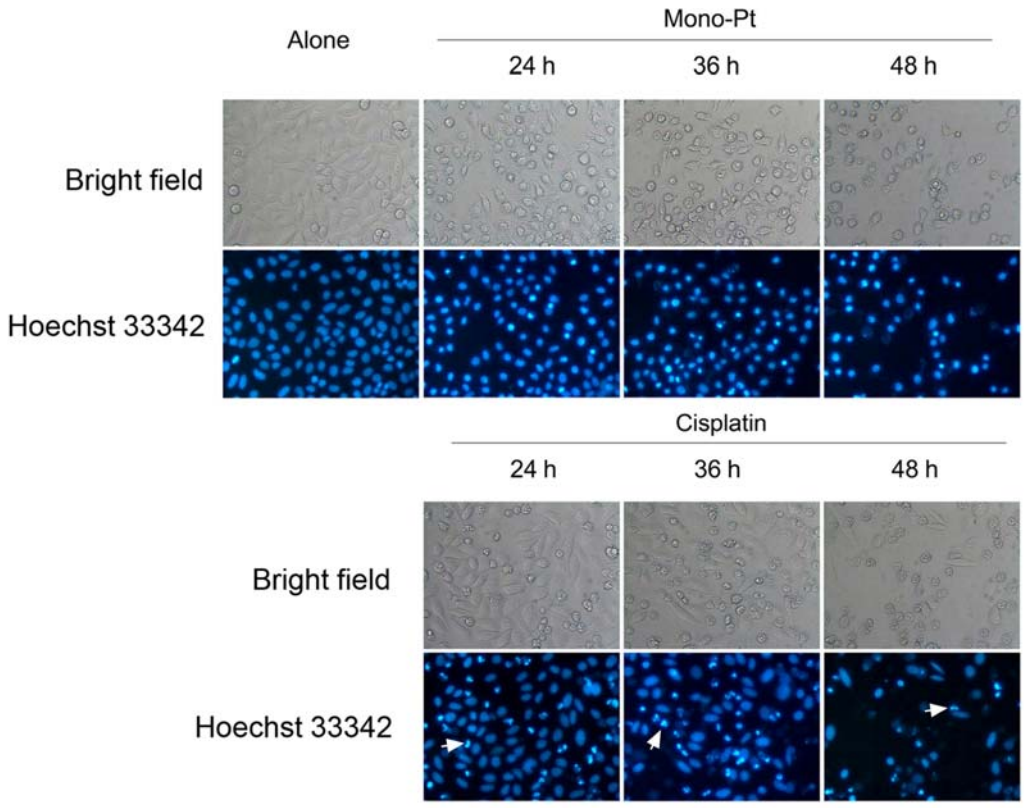

Figure S2. Cisplatin but not Mono-Pt induces apoptotic bodies in human ovarian carcinoma Caov-3 cells. The cells treated with 10 μM Mono-Pt or 50 μM cisplatin were stained and incubated with 1 μg/ml Hoechst 33342 (DNA dye, Sigma) in dark for 30 min and observed by converted fluorescence microscopy. The microscopy imaging of cells showed fragmented nuclei (white arrows) in 50 μM cisplatin-treated cells. The results shown are representative of three different experiments.

Supplementary Fig. S3

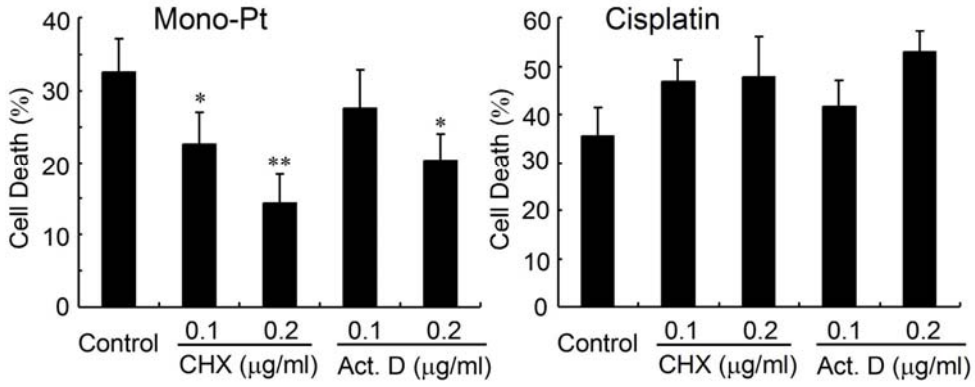

Figure S3. Mono-Pt-induced cell death required for *de novo* protein synthesis. Cycloheximide (CHX, Sigma) and actinomycin D (Act. D, Sigma) were incubated Caov-3 cells for 12 h, then cells were treated with 10 μM Mono-Pt or 50 μM cisplatin for 24 h. Cell death was assessed by trypan blue dye exclusion assay. Data represent mean ± SEM of three different experiments. **P*<0.05, ***P*<0.01 vs Control group.

Supplementary Fig. S4

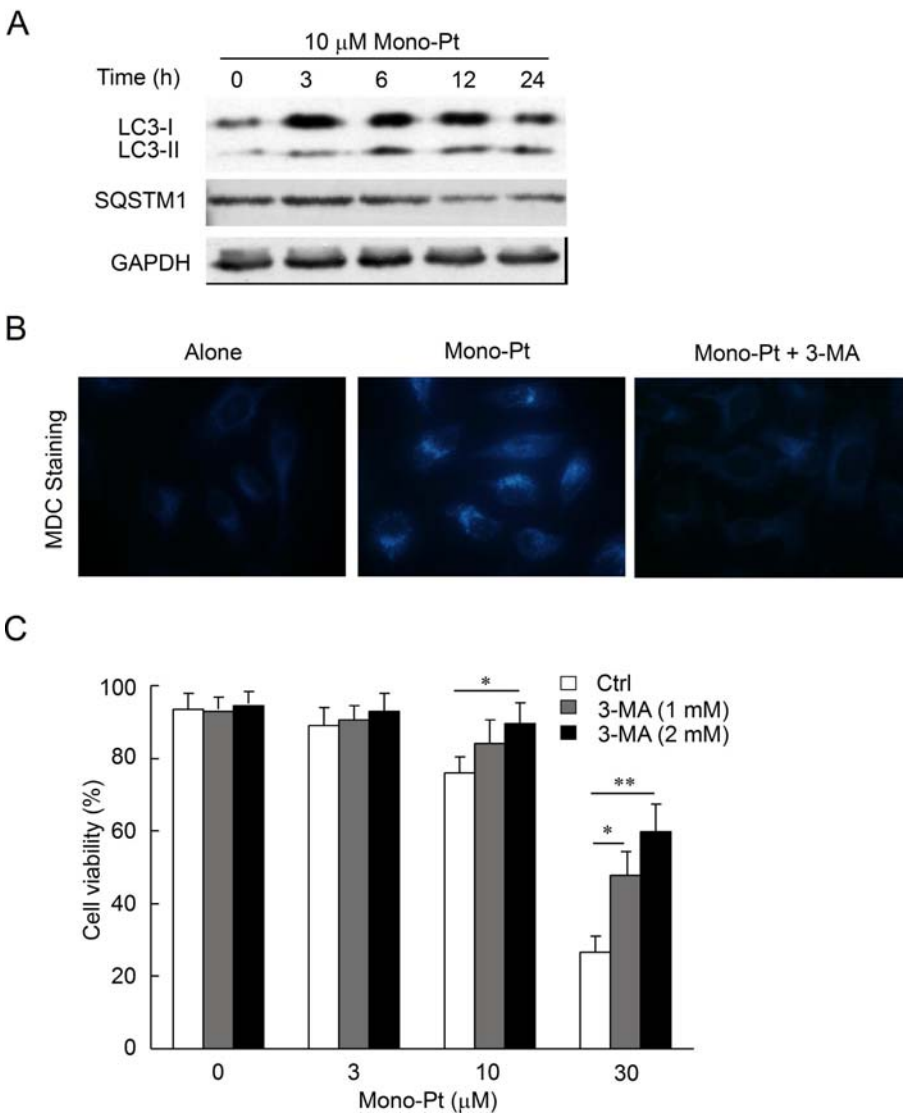

Figure S4. Mono-Pt induces autophagy in human ovarian carcinoma Skov-3 cells. (**A**) Skov-3 cells were cultured with 10 μM Mono-Pt for 3 h, 6 h, 12 h and 24 h. Then cells were subjected to immunoblotting for LC3 and SQSTM1. (**B**) Skov-3 cells were treated with 10 μM Mono-Pt for 12 h in the absence or presence of 2 mM 3-MA and incubated with 0.05 mM monodansylcadaverine (MDC) for 10 min. Cells were then analysed by fluorescence microscopy. The results shown are representative of three experiments. (**C**) After Skov-3 cells were treated with 0, 3, 10 or 30 μM Mono-Pt with or without 1 or 2 mM 3-MA for 24 h, cell viability was measured by trypan blue dye exclusion assay. **P*<0.05, ***P*<0.01.

Supplementary Fig. S5

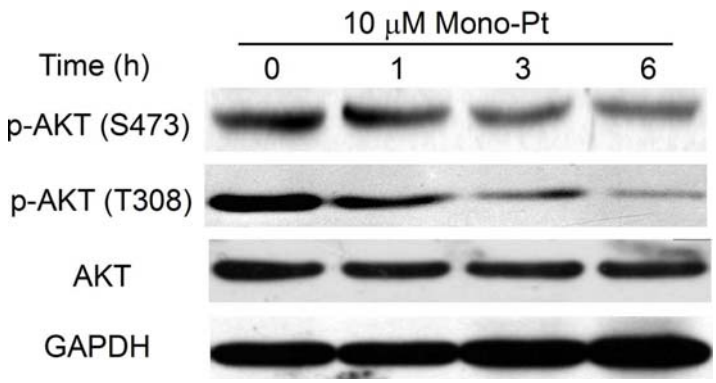

Figure S5. Mono-Pt inhibits AKT signaling in human ovarian carcinoma Skov-3 cells. Skov-3 cells treated with 10 μM Mono-Pt for indicated time were analyzed by immunoblotting for levels of phospho- and total AKT. The results shown are representative of three different experiments.