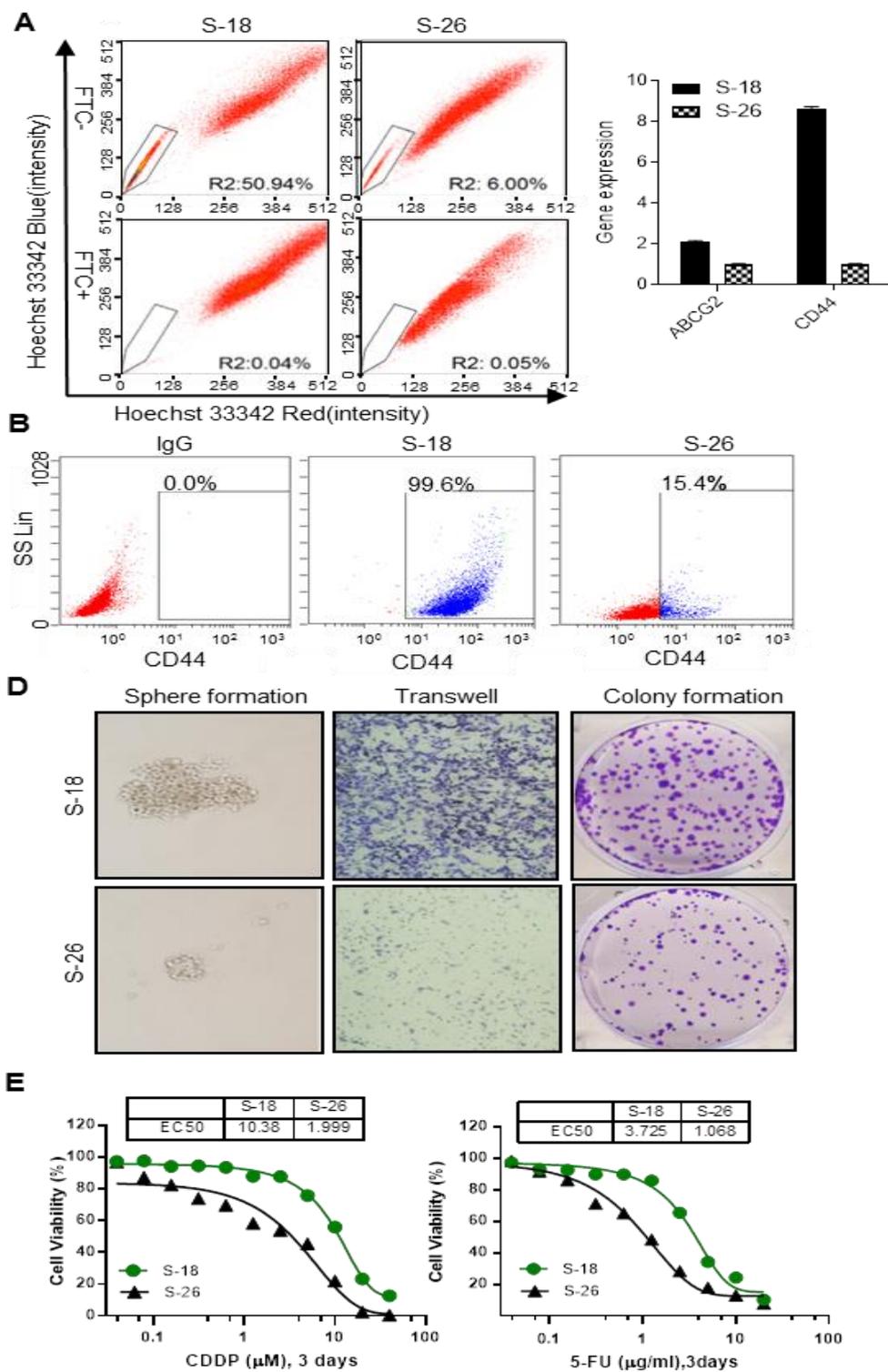


Supplementary Materials



Supplementary Figure 1. NPC S-18 cells have cancer stem cell characteristics.

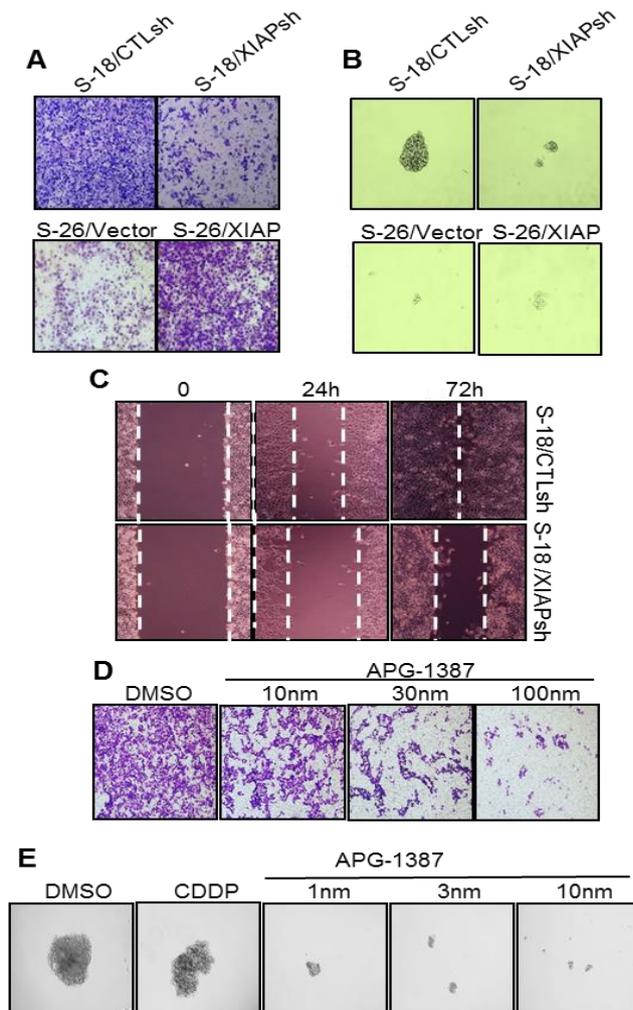
(A) Human NPC S-18 and S-26 cell lines were stained with Hoechst 33342. The percentage of SP cells is indicated by the dye and was analysed by flow cytometry with or without pre-treatment with Fumitremorgin C (FTC, 5ug/ml for 5min).

(B) Human NPC S-18 and S-26 cell lines were labelled with the CD44-PE or IgG-PE antibody, and the percentage of CD44-positive cells is indicated by the blue dye.

(C) mRNA expression level of CD44 and ABCG2 quantified using real-time PCR in S18 and S26 cells.

(D) Sphere formation assay, uncoated transwell assay, and colony formation assay using S18 and S26 cells.

(E) IC50 values for cisplatin and 5-fluorouracil in S18 and S26 cells. Cells were treated with a range of concentrations of each compound for 72 h, and cell survival was measured by using the Cell Counting Kit-8.



Supplementary Figure 2. XIAP is important in maintaining the migratory ability and sphere-forming ability of NPC.

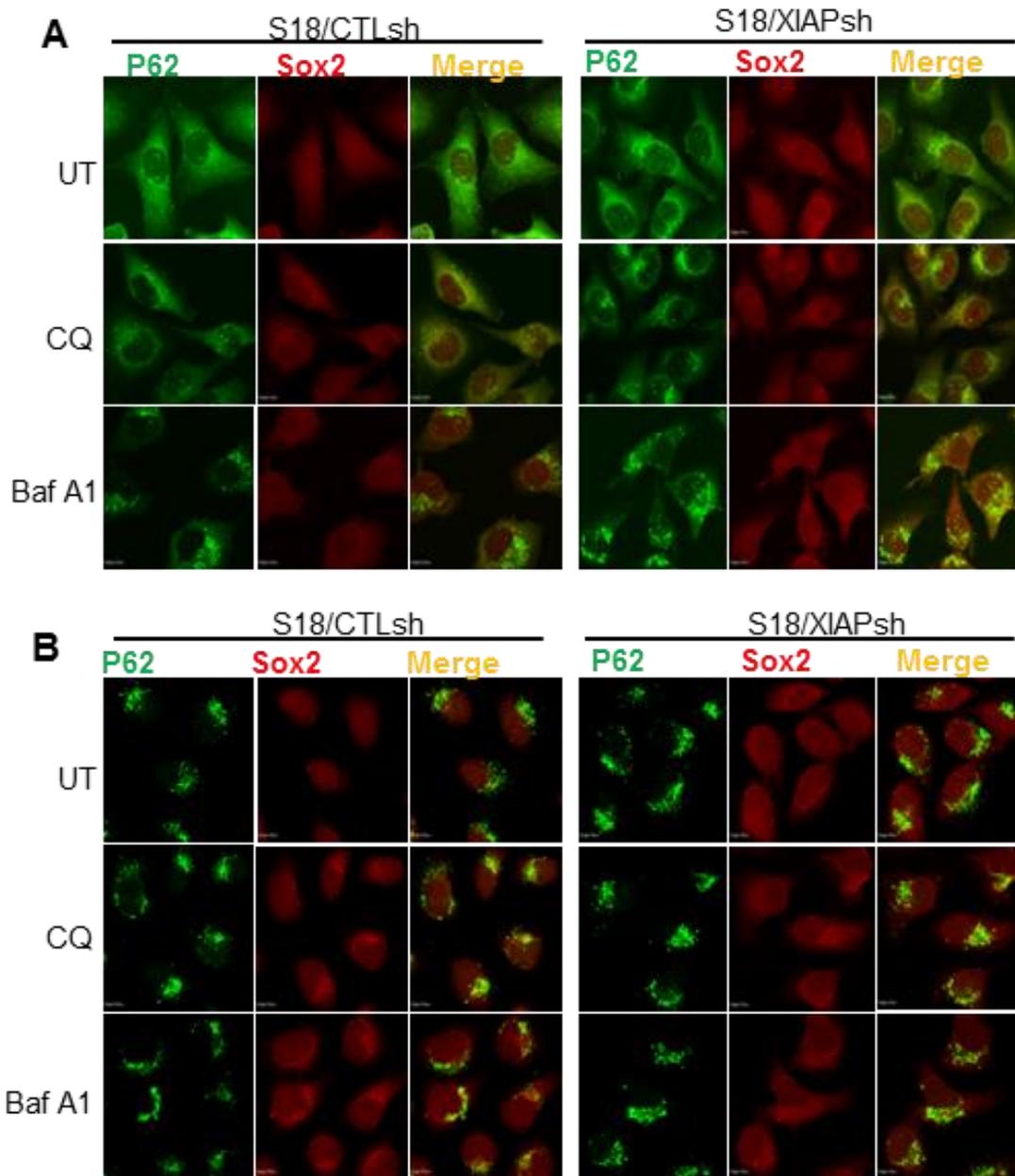
(A) The migratory ability of S-18/CTLsh, S-18/XIAPsh, S-26/vector and S-26/XIAP cells was assayed using an uncoated transwell assay.

(B) The sphere-forming ability of S-18/CTLsh, S-18/XIAPsh, S-26/vector, and S-26/XIAP cells was assayed using a sphere formation assay.

(C) The cell scratch was monitored after 24h and 72h in S-18/CTLsh and S-18/XIAPsh cells.

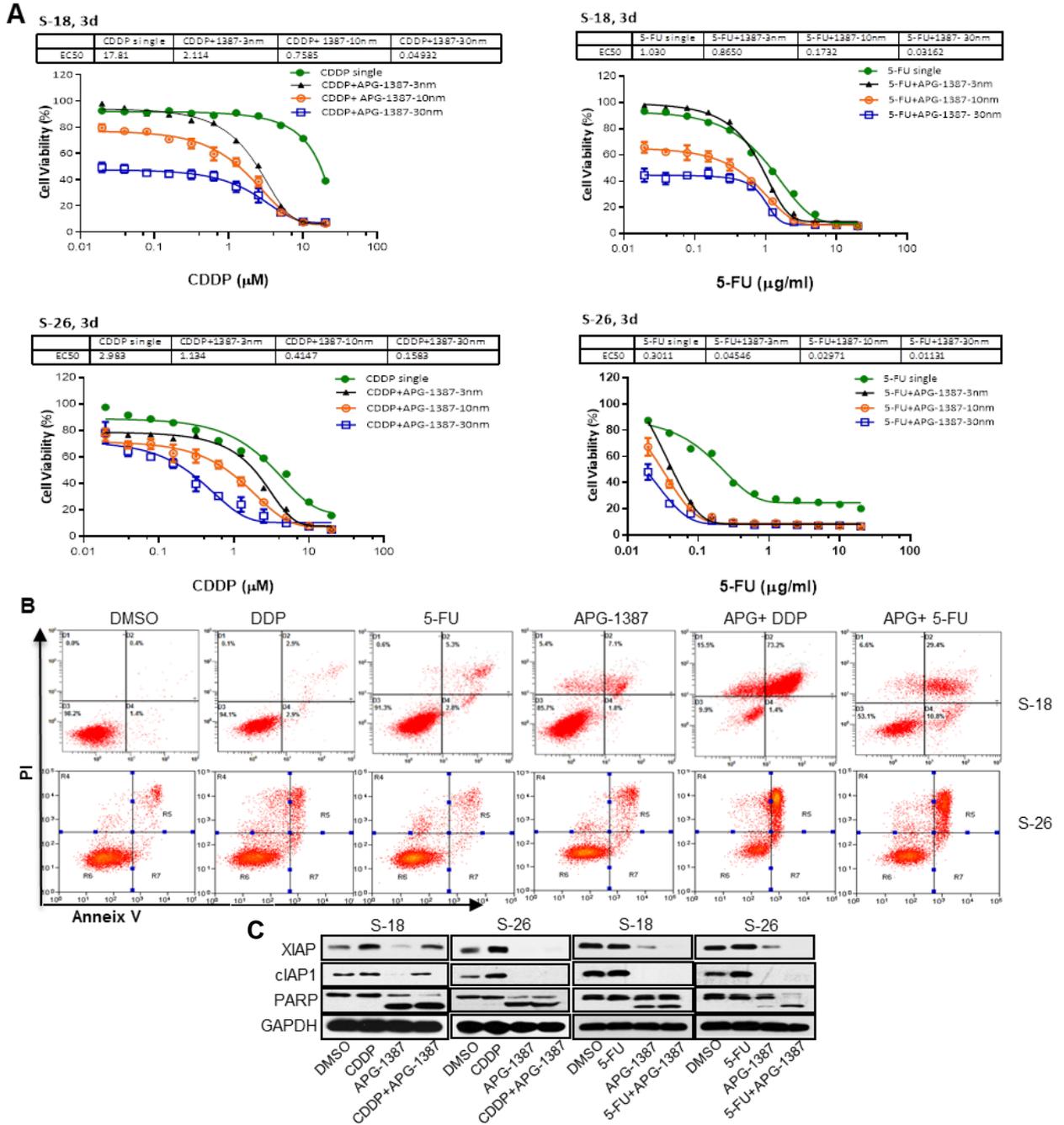
(D) S-18 cells were incubated with APG-1387 (10-100 nmol/L) or DMSO for 24 h and then seeded in an un-coated matrigel transwell chamber for 24 h. The cells that migrated through the membrane were stained by crystal violet and photographed with an optical microscope.

(E) S-18 cells were cultured in sphere-forming conditions and incubated with APG-1387 (1-10 nmol/L), DMSO, or CDDP (5 μ mol/L) for 7 d. The image of the sphere is presented.



Supplementary Figure 3. The co-localization of Sox2 with p62 and LAMP2.

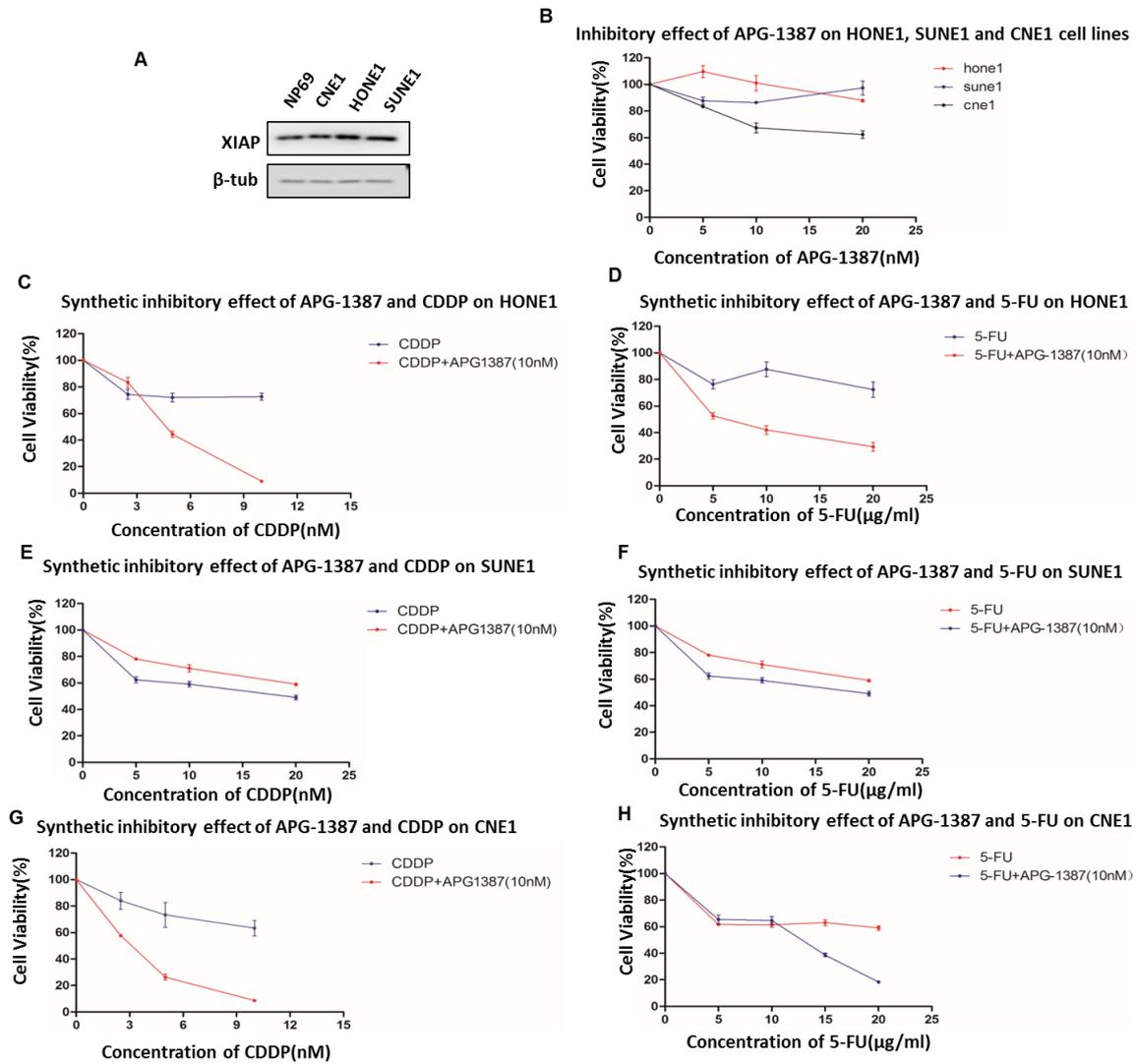
(A, B) S-18/CTLsh and S-18/XIAPsh cells were treated with Bafilomycin A1 (100nM for 6 h) and CQ (20 μ M for 6h). The cells were fixed, immunofluorescence-stained with Sox2 and p62 or LAMP2a antibodies, and photographed using confocal fluorescence microscopy.



Supplementary Figure 4. APG-1387 sensitized NPC cells to chemotherapies (CDDP and 5-FU) in vitro.

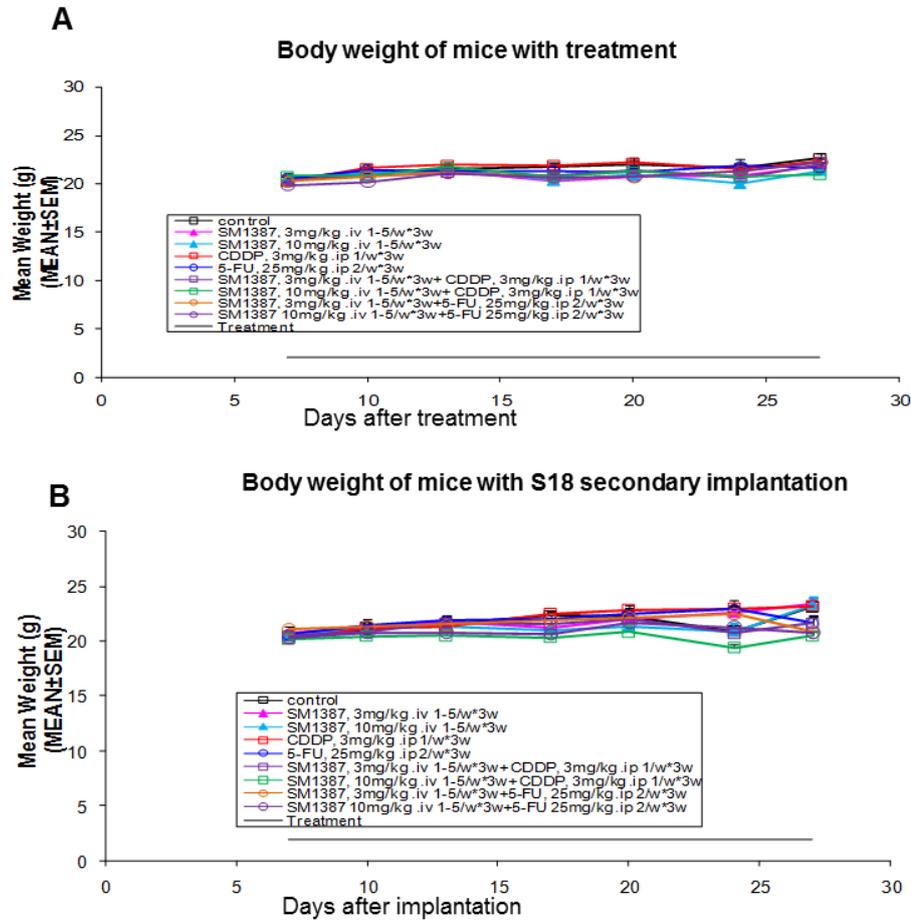
(A) S-18 and S-26 cells were treated with increasing concentrations of CDDP or 5-FU and APG-1387 (3, 10, 30 nmol/L) for 3 days. The anti-proliferative effect was measured by using Cell Counting Kit-8.

(B,C) S-18 and S-26 cells treated with CDDP (2.5 $\mu\text{mol/L}$), 5-FU (2.5 $\mu\text{g/mL}$), APG-1387 (30 nmol/L), or a combination of APG-1387 and CDDP or 5-FU for 2 days. Scatterplot of Annexin V / propidium iodide staining measured by flow cytometry. Western blot analysis was conducted and probed with indicated antibodies. Blots shown are representative of three independent experiments.



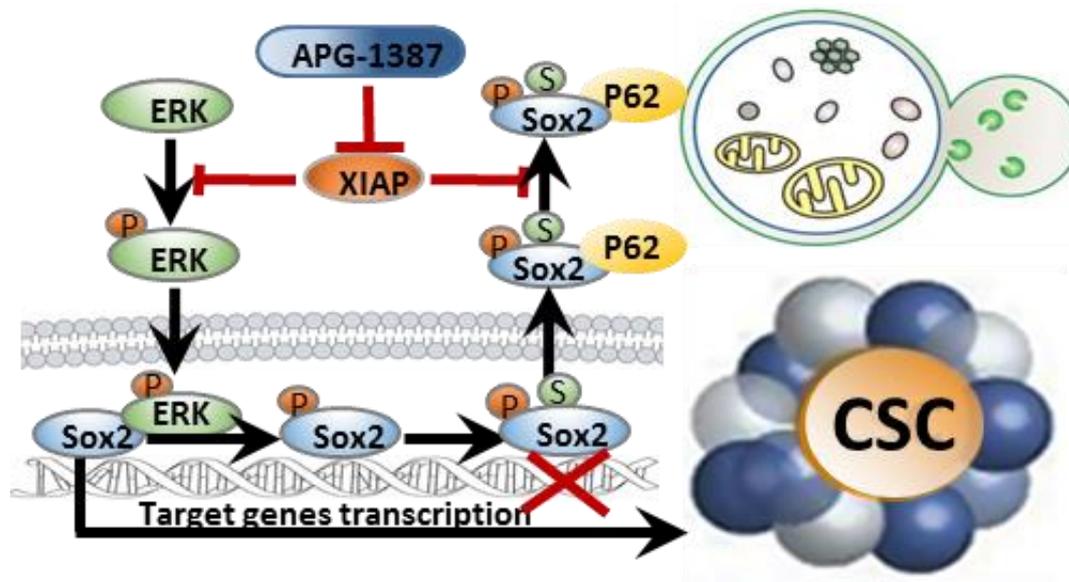
Supplementary Figure 5. APG-1387 sensitized other NPC cells to chemotherapies (CDDP and 5-FU) in vitro.

Western blot analysis was conducted and probed with XIAP antibody in CNE1, HONE1, SUNE1 and NP69 cells. Blots shown are representative of three independent experiments. CNE1, HONE1 and SUNE1 cells were treated with increasing concentrations of CDDP or 5-FU and APG-1387 (10 nmol/L) for 3 days. The anti-proliferative effect was measured by using Cell Counting Kit-8.



Supplementary Figure 6. The body weights of mice were unaffected upon tumor xenografts implantation or drug treatment.

(A, B) Average body weights for mice treated with single drug APG-1387, CDDP or 5-FU, or combined therapeutic of APG-1387 with CDDP or 5-FU. The body weights were monitored twice per week. *, $P < 0.05$; **, $P < 0.01$. Error bars represent the SEM from three independent experiments. (B) Mice were implanted with cells derived from each group of primary tumor xenografts, and then body weights were measured twice per week. Error bars represent the SEM from three independent experiments. *, $P < 0.05$.



Supplementary Figure 7. Schematic depicting the effect of XIAP or APG-1387 on the cancer stem cells and the underlying mechanism.