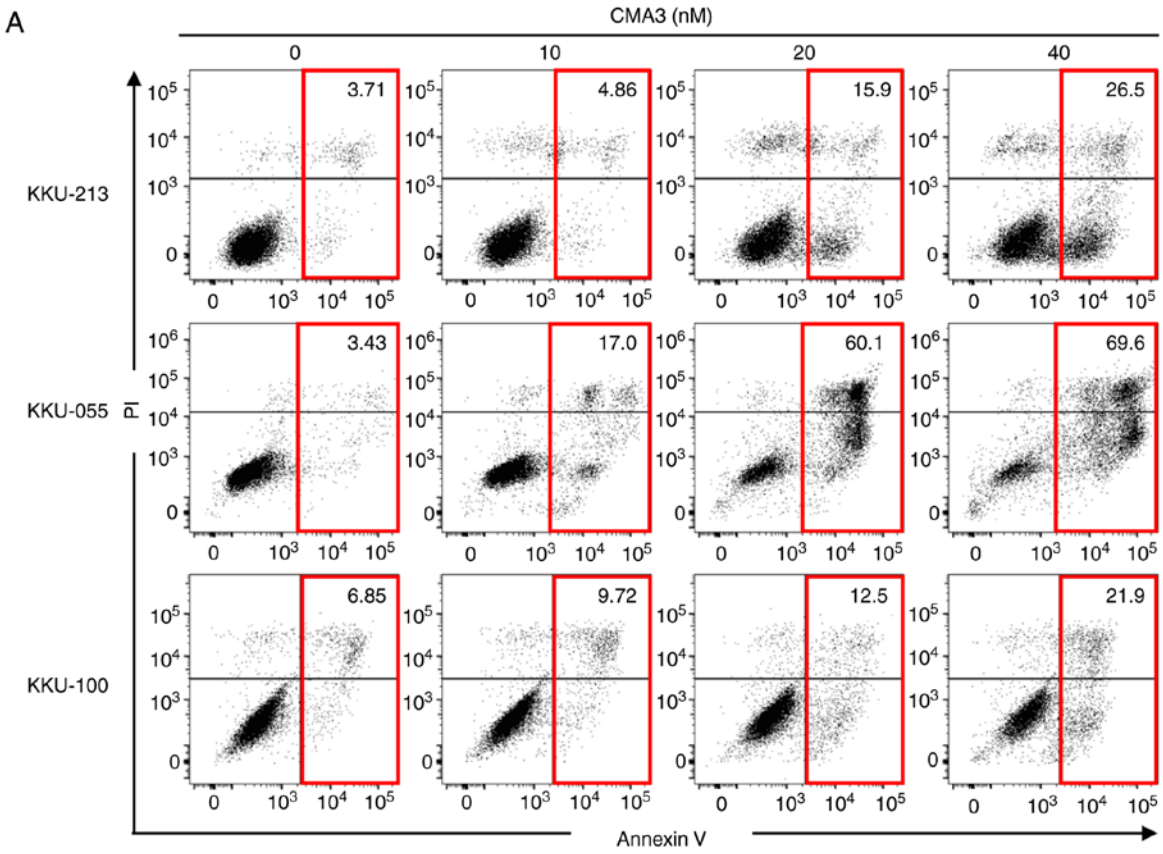
Figure S1. CMA3 induces apoptosis in CCA cell lines in a dose-dependent manner. CCA cells were treated with increasing concentrations of CMA3 (0-40 nM) for 24 h. After that cells were stained with Annexin V/PI and were analyzed by flow cytometry. (A) The representative dot plots of three CCA cell lines shows Annexin V-positive cells (in red boxes) after treatment with various concentrations of CMA3 for 24 h. Numbers indicate Annexin V-positive cells (B) The bar graph represents the mean ± standard deviation of % Annexin V-positive cells from three independent experiments. *P<0.05, **P<0.01 and ***P<0.001 vs. 0 nM. CMA3, Chromomycin A3; CCA, cholangiocarcinoma; PI, propidium iodide.



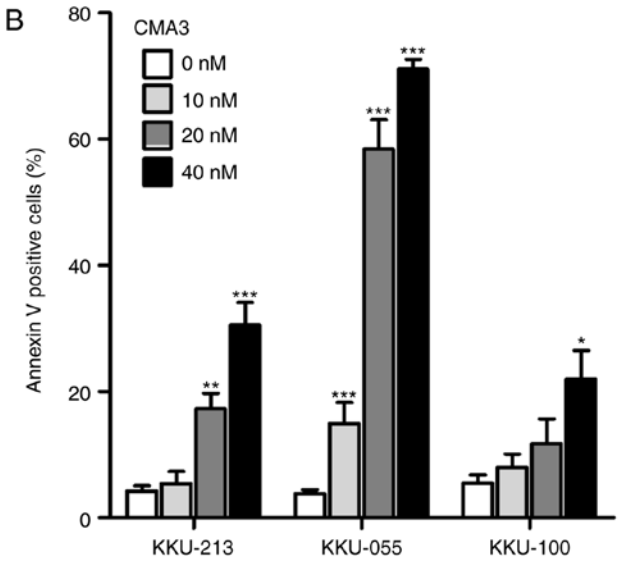


Figure S2. CMA3 suppresses Sp1-related apoptotic proteins and induces caspase-dependent apoptosis in KKU-055. (A) Expression of activated caspase-8, -9 and -3 in CMA3-treated KKU-055 (0-40 nM) for 24 h are demonstrated by western blotting. (B) KKU-055 cells were treated with 20 nM CMA3 at 0-24 h and activated caspase-8, -9, and -3 were determined. (C) Expression of Sp1-related anti-apoptotic proteins are shown in 20 nM CMA3-treated KKU-055 at various times. Intensities of protein bands were normalized with Hsc70 and compared with those without CMA3 (0 nM or 0 h, intensity=1), as indicated by the number shown on the top of each band. For $FLIP_{S/L}$, only $FLIP_S$ isoform was quantitated. CMA3, Chromomycin A3; CCA, cholangiocarcinoma; FLIP, FADD-like IL-1β-converting enzyme-inhibitory protein; SP1, specificity protein 1; cIAP2, cellular inhibitor of apoptosis; XIAP, X-linked inhibitor of apoptosis protein; Mcl-1, myeloid cell leukemia-1.

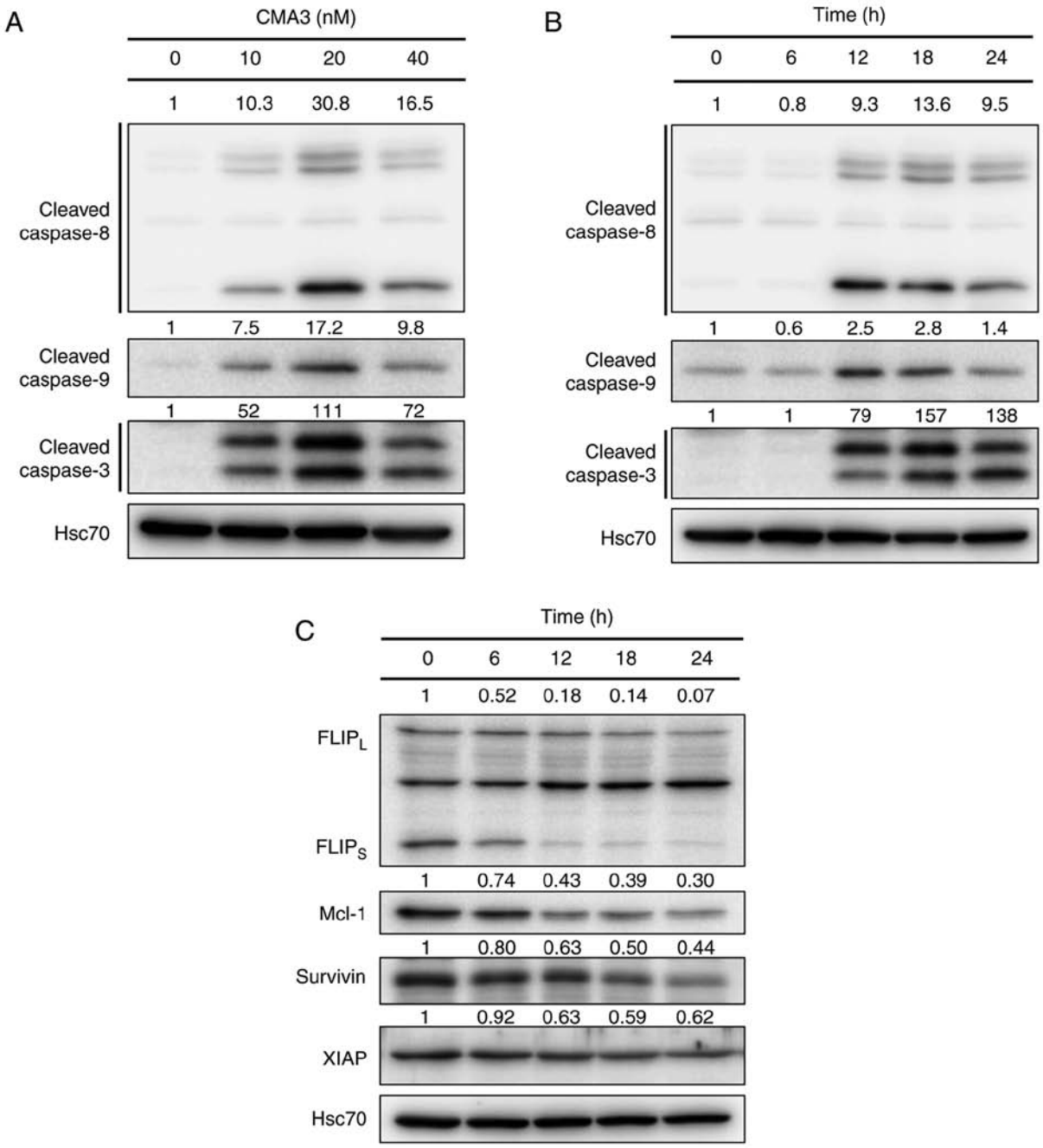


Figure S3. mRNA expression of *Mcl-1* and *XIAP* in KKU-213 after treatment with 40 nM CMA3 (CMA3-*Mcl-1* and CMA3-*XIAP*) or 200 nM MTA (MTA-*Mcl-1* and MTA-*XIAP*) for 0-24 h. The data are presented as the mean ± standard error from the representative experiment. CMA3, Chromomycin A3; CCA, cholangiocarcinoma; XIAP, X-linked inhibitor of apoptosis protein; Mcl-1, myeloid cell leukemia-1; MTA, mithramycin A.

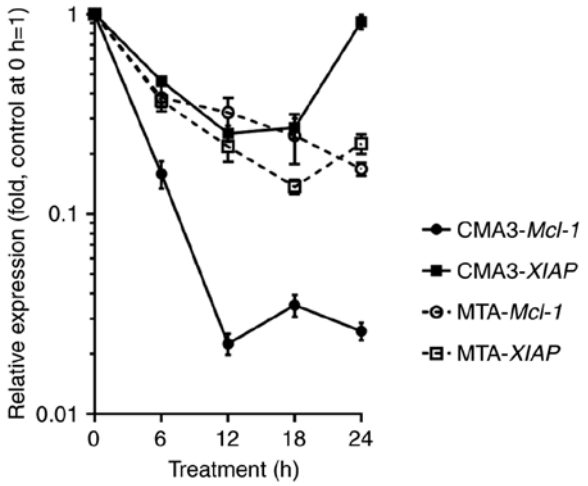


Figure S4. CMA3 reduces tumor growth *in vivo* without any serious side effects. (A) Tumor tissues from control and CMA3-treated group are shown (n=12/group). (B) Tumor weights of control and treatment groups are compared. Each tumor weight is presented and the bar represents the mean ± SD of tumor weights. The smallest and biggest tumors of each group were omitted to avoid technical error. (C) Body weights of mice in control and CMA3-treated group are presented as the mean ± SD of percentage of body weight (at day 0=100%, n=7/group). *P<0.05 and ***P<0.001. SD, standard deviation; CMA3, Chromomycin A3.

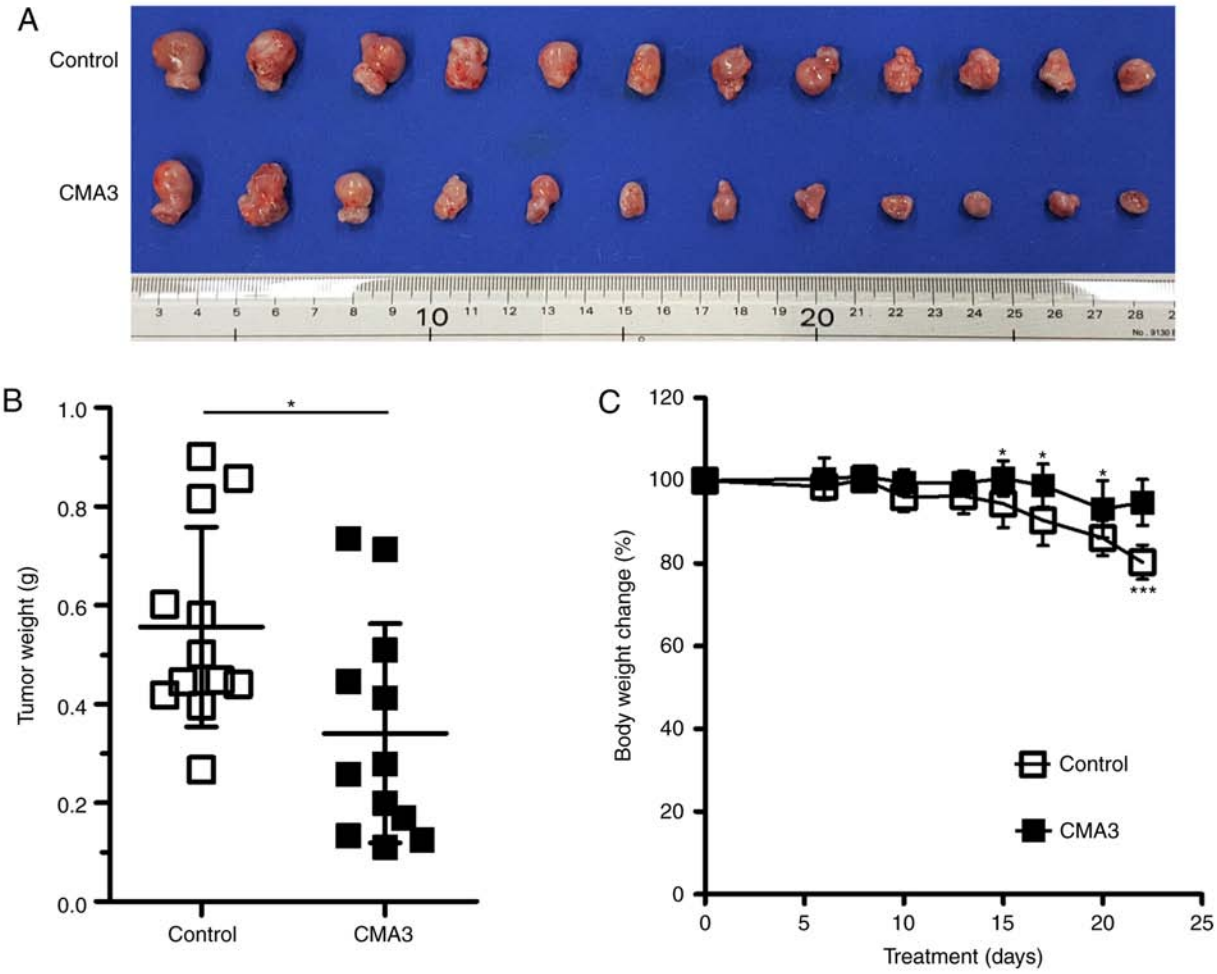


Figure S5. Chromomycin A3 toxicity test in mouse model. A total of eight mice were randomly divided into 4 groups (n=2/group) and different treatments were given intravenously for 3 weeks. The body weights were measured 3 times a week. The data are shown as mean body weight changes (%). Mean body weight on day 0 of each group was set as 100%.

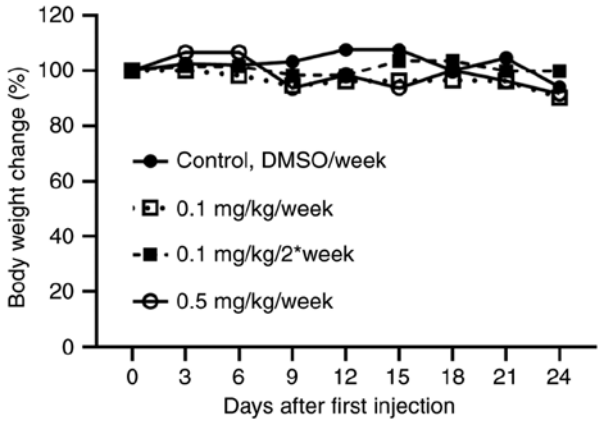


Figure S6. CMA3 suppressed tumor growth by induction of apoptosis. The areas of caspase-9- and Bax-positive were determined and are presented as AU/cells/field (n=5/group, 5 images/tumor, total =25 images/group). The data are shown as mean ± standard deviation. *P<0.05 and ***P<0.001. AU, arbitrary units; CMA3, Chromomycin A3; CK19, cytokeratin 19.