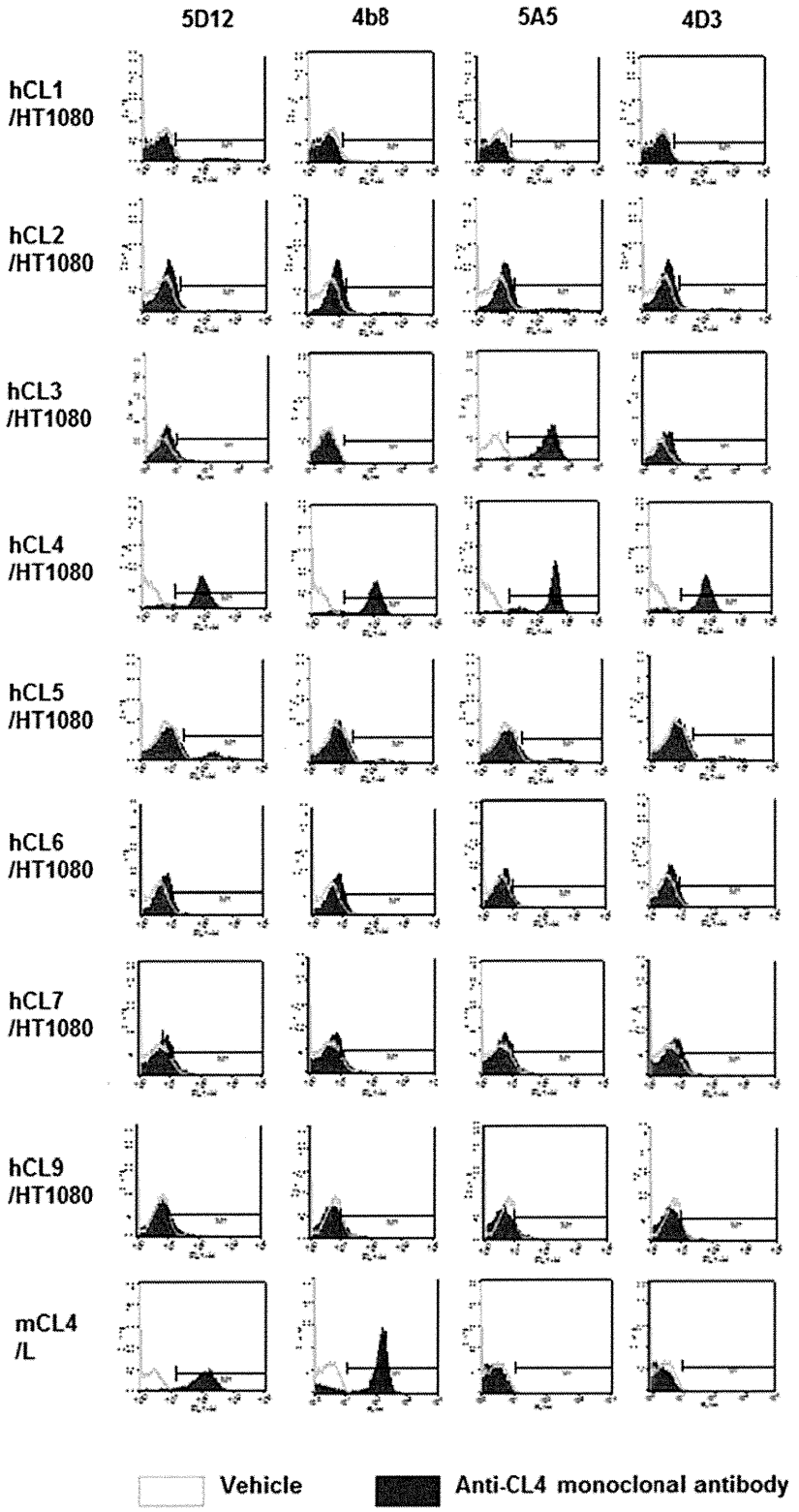



**Figure 19**
**To be continued**

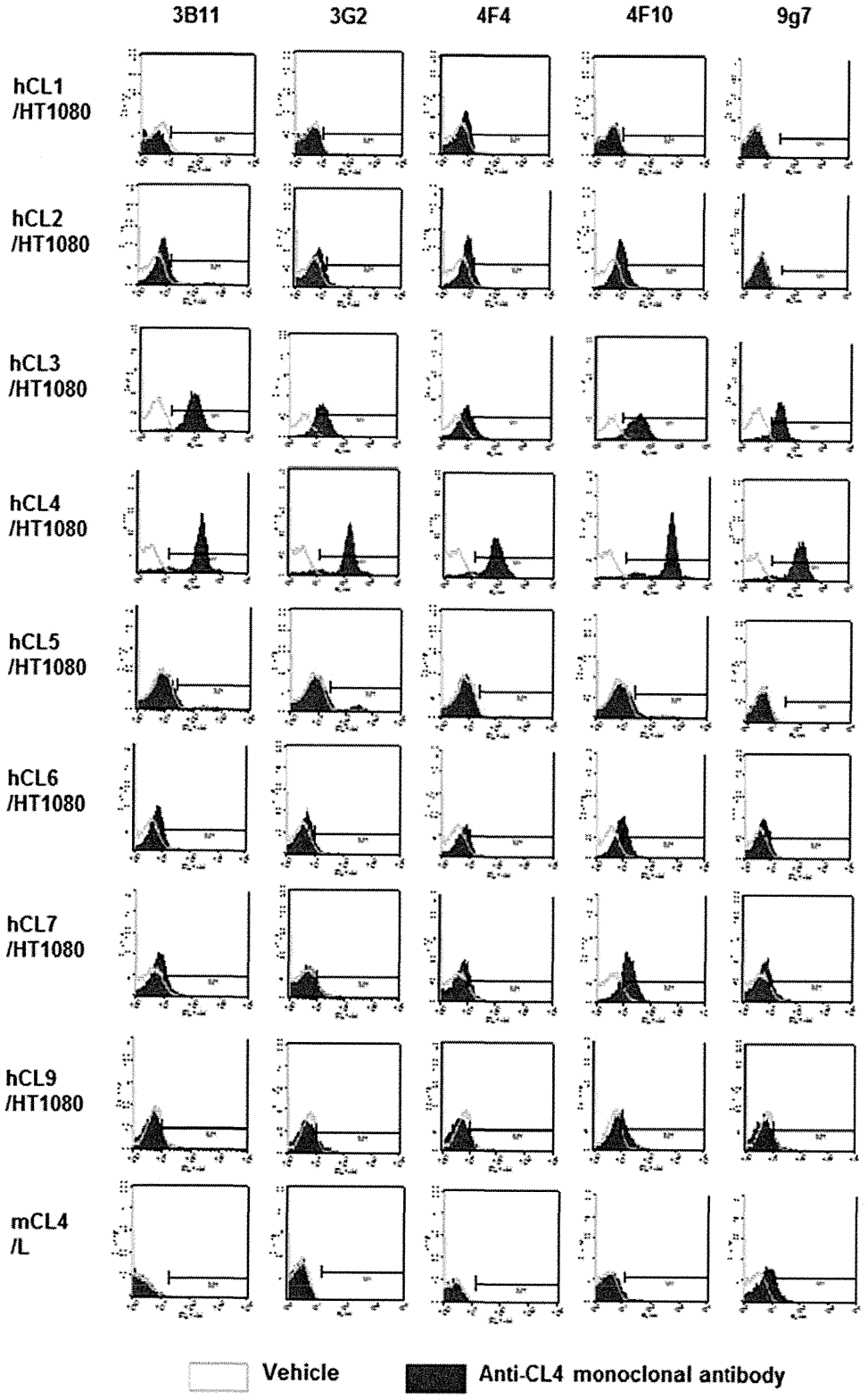



**Figure 19 Flow cytometry analysis of the interaction between rat anti-hCL-4 antibodies and CLs-expressing cells.**

CLs-expressing cells were incubated with rat anti-hCL4 antibodies and FITC-conjugated goat anti-rat IgG (H+L). The antibodies-bound cells were detected using a flow cytometer. As a control, cells were incubated with PBS.

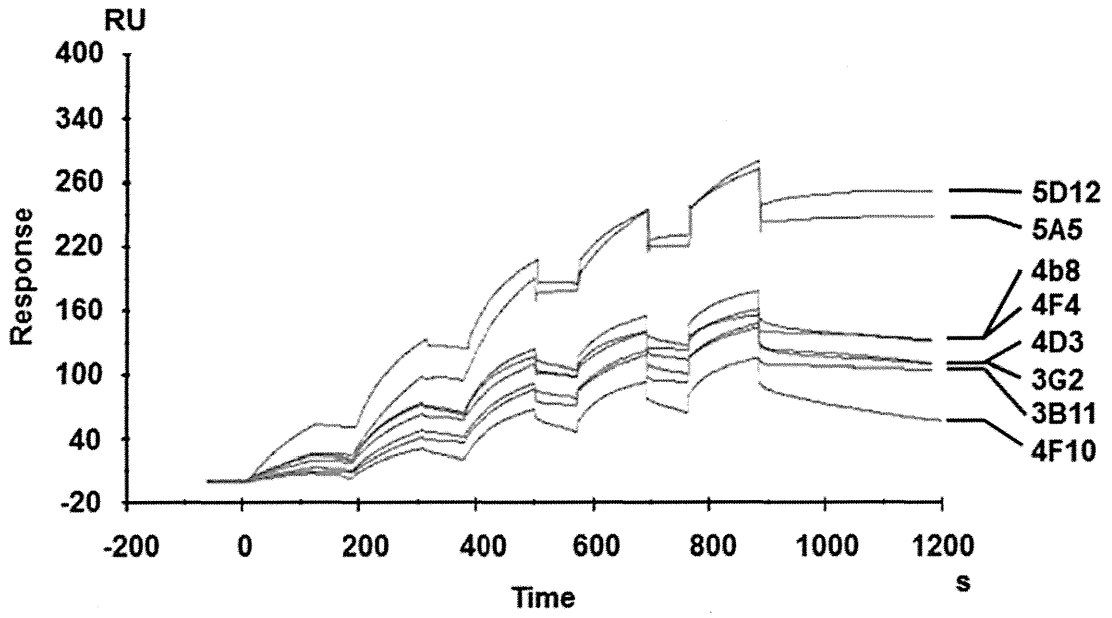



**Figure 20 Interaction of rat Abs with hCL-4 using Surface plasma resonance(SPR) analysis.**

Anti-rat IgG and each anti-CL-4 antibodies was immobilized onto the CM5 chip and binding analysis was carried out with different dilutions (10、100、200、300、500 nM ) of hCL-4 protein passed at a flow rate of 30 μL/mL. Sensogram showed the binding kinetics of anti-CL-4 antibodies with hCL-4 protein at different dilutions.

**Table 4 Binding kinetics of rat Abs to hCL-4.**

| Clone | 5D12 | 5A5 | 4b8 | 4F4 | 4D3 | 3G2 | 3B11 | 4F10 |
|---|---|---|---|---|---|---|---|---|
| Ka (1/Ms, $\times 10^4$) | 2.38 | 1.33 | 1.70 | 2.50 | 1.99 | 1.33 | 1.27 | 18.60 |
| Kd (1/s, $\times 10^{-4}$) | 0.34 | 0.58 | 4.03 | 9.65 | 8.27 | 7.99 | 8.68 | 141.0 |
| KD (nM) | 1.41 | 4.35 | 23.72 | 38.62 | 41.50 | 59.88 | 68.51 | 75.73 |

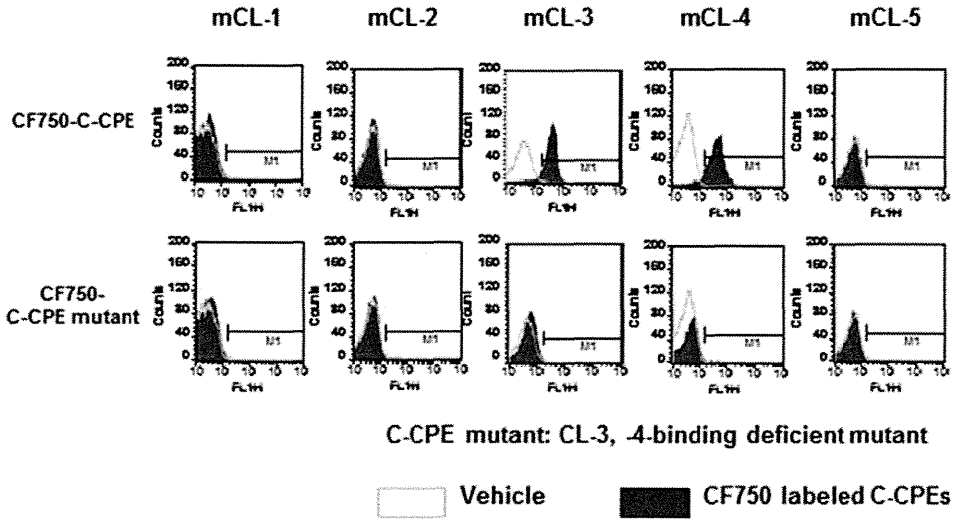



**Figure 21 Flow cytometry analysis of the interaction between CF750-C-CPE and CLs-expressing cells.**

CLs-expressing cells were incubated with CF750-C-CPEs and FITC-conjugated goat anti-mouse IgG (H+L). The C-CPE-bound cells were detected using a flow cytometer. As a control, cells were incubated with PBS.

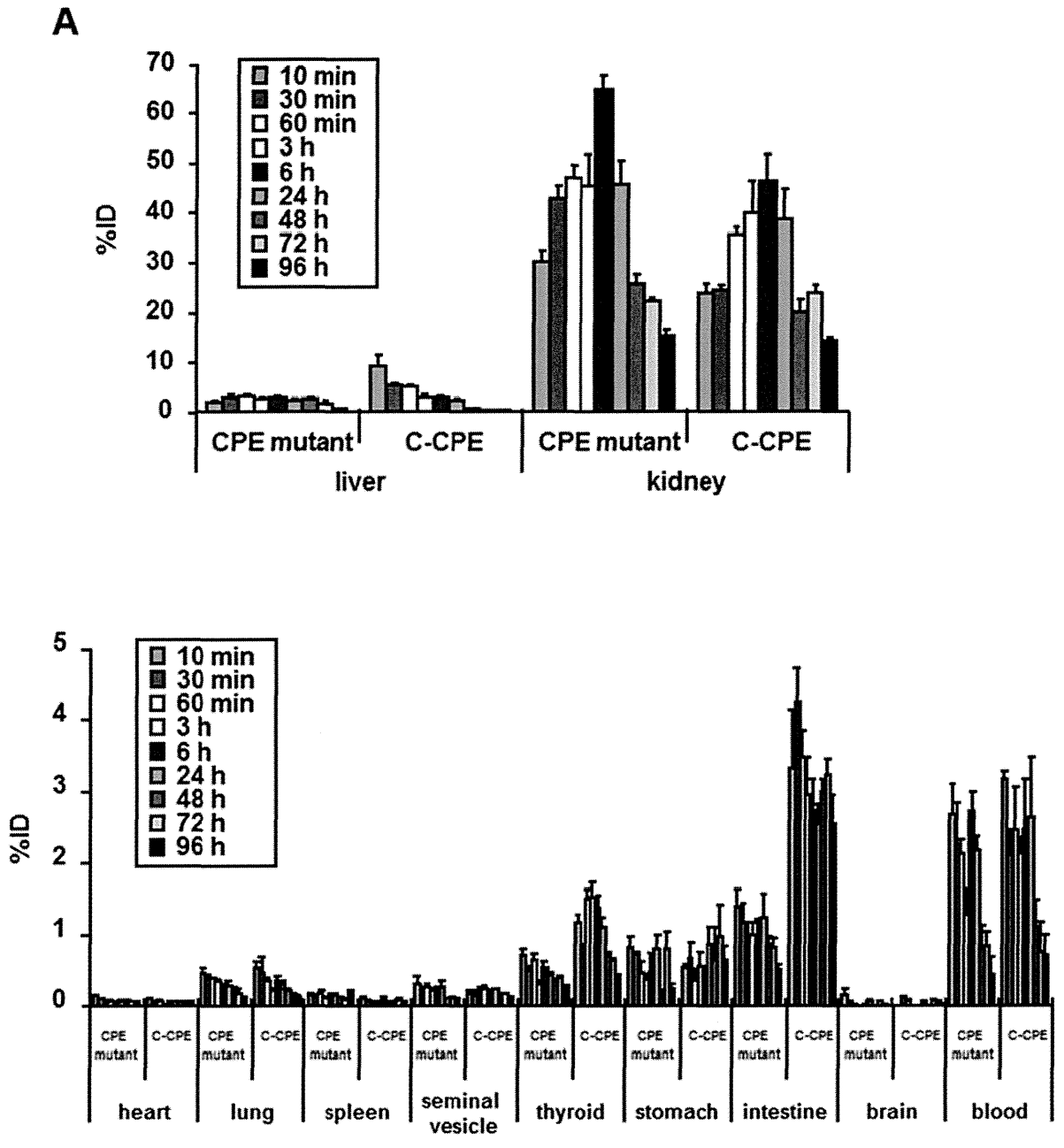



**Figure 22 The distribution of the CF750-C-CPE in mice.**
BALB/c mice were intravenously injected with 2 μg/mouse CF750-C-CPE or CF750-C-CPE mutant. Tissues were removed at the indicated times after injection and the intensity of fluorescence of each tissue was measured. A showed the fluorescence intensity of liver and kidney, and B showed the fluorescence intensity of other tissues. Tissue labeling C-CPEs levels were calculated as percentages of injected doses. Data are means ± SEM (n = 5). ID: injected dose.

**Table 5 The amino acid sequences of L chain and H chain of HKH189.J9.**

| | Signal peptide | FR1 | CDR1 | FR2 | CDR2 | FR3 | CDR3 | FR4 |
|---|---|---|---|---|---|---|---|---|
| VH | MDIRLSL AFLVLFI KGVQC | EVQLVESGG GLVQPGRS MKLSCAAS GFTFS | | | | | | WGQGT LVTVSS |
| VL | MRVQIQF LGLLLL WTSGAQ C | VVQMTQSPS YLAASPGES VSISC | | | | | | FGAGT KLELK R |

未公表データにつき、一部データを削除

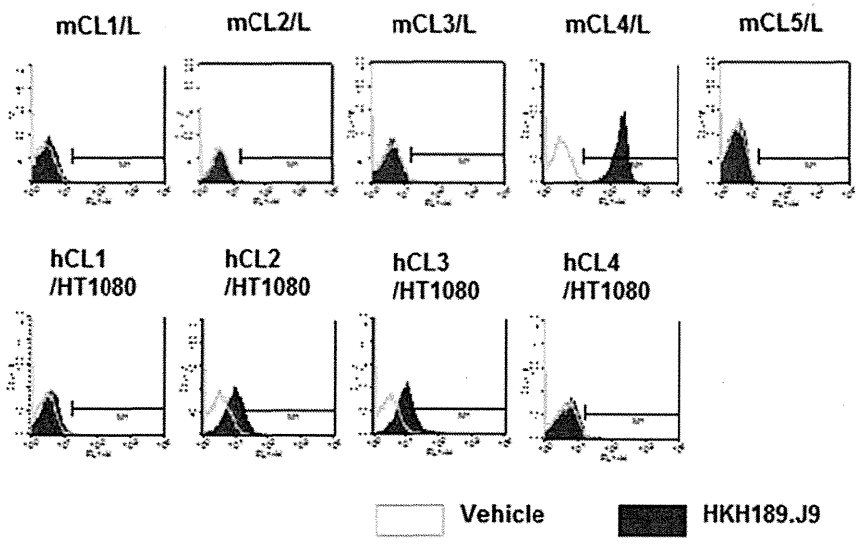



**Figure 23 Flow cytometry analysis of the interaction between HKH189.J9 and CLs-expressing cells.**

CLs-expressing cells were incubated with HKH189.J9 and FITC-conjugated goat anti-rat IgG (H+L). The antibodies-bound cells were detected using a flow cytometer. As a control, cells were incubated with PBS.

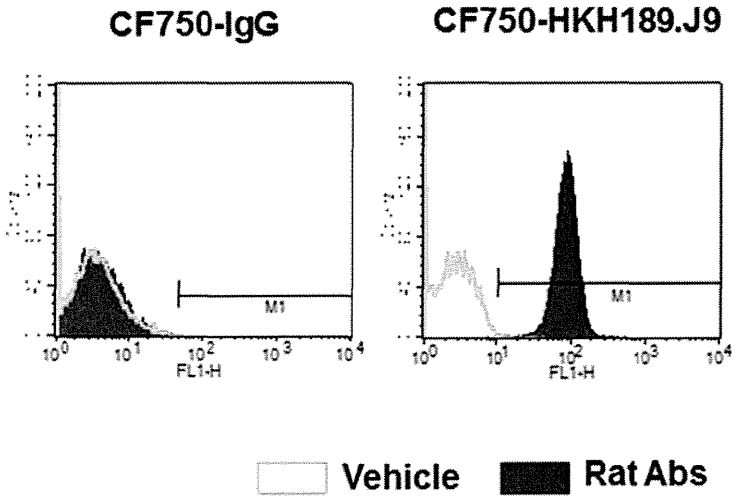



**Figure 24 Flow cytometry analysis of the interaction between CF750 labeling antibodies and mCL-4-expressing cells.**
CLs-expressing cells were incubated with CF750 labeling antibodies and FITC-conjugated goat anti-rat IgG (H+L). The antibodies-bound cells were detected using a flow cytometer. As a control, cells were incubated with PBS.

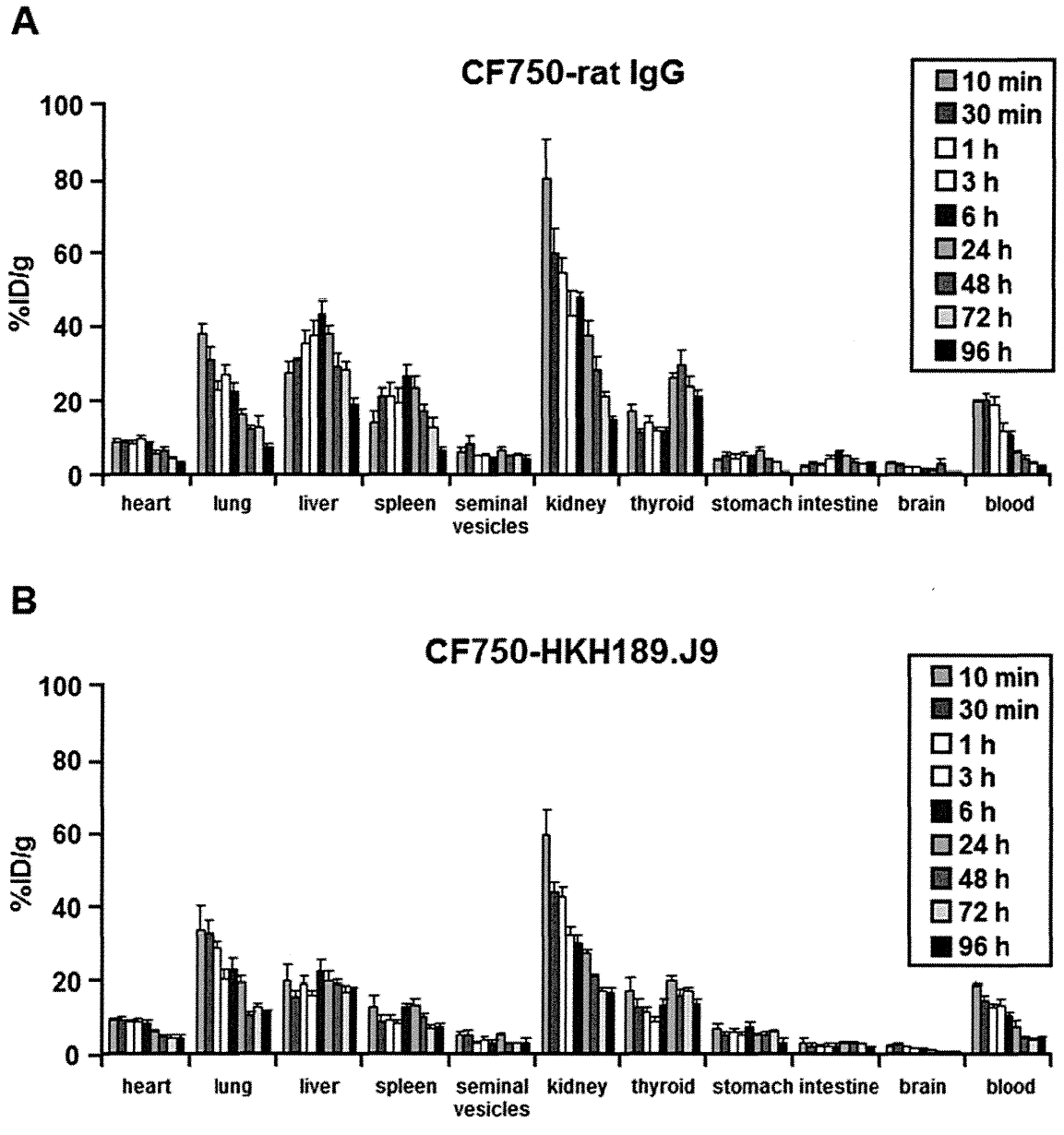
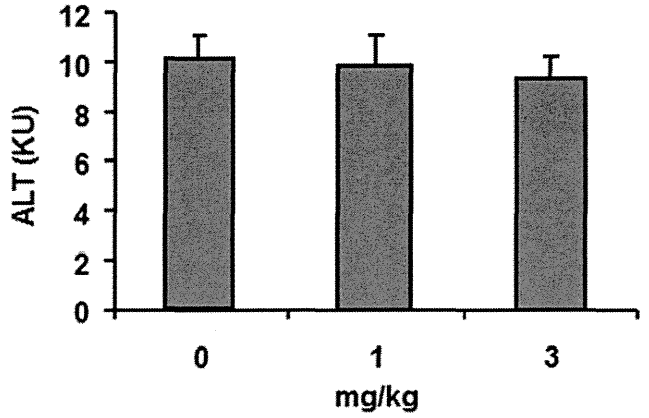
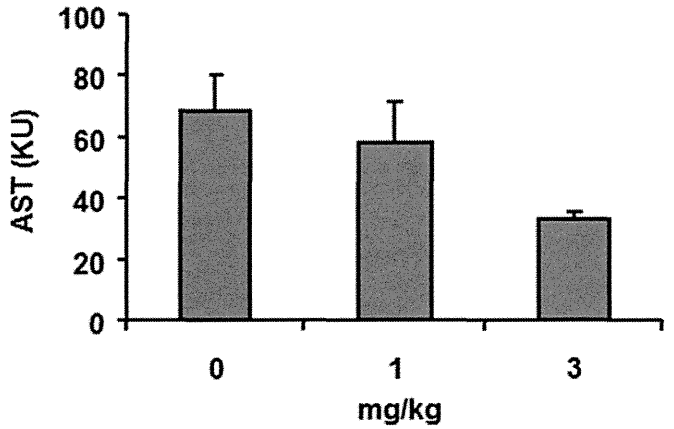



**Figure 25 The distribution of the CF750-HKH189.J9 in mice.**
BALB/c mice were intravenously injected with 20 μg/mouse CF750-rat IgG (A) or CF750-HKH189.J9 (B). Tissues were removed at the indicated times after injection and the intensity of fluorescence of each tissue was measured. Tissue labeling antibody levels were calculated as percentages of injected doses per gram of tissue. Data are means ± SEM (n = 5). ID/g: injected dose per gram of tissue.

A




B




C

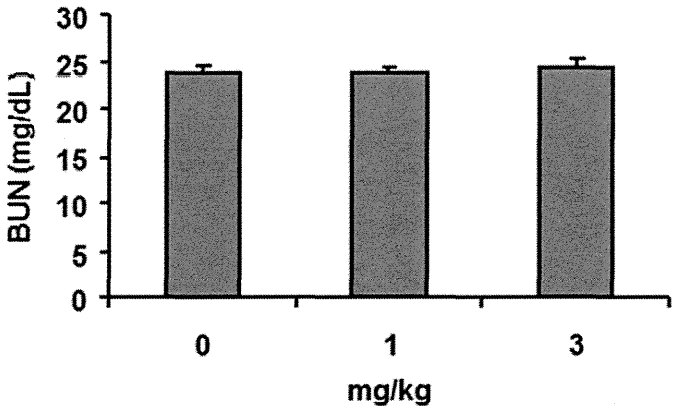



**Figure 26 Toxicity of 5D12 in the liver and kidney.**
BALB/c mice were intraperitoneally injected with 5D12 at the indicated dose once. After 48 hours, the serum ALT (A), AST (B) and BUN (C) levels were measured. Data are means ± SEM (n = 5).

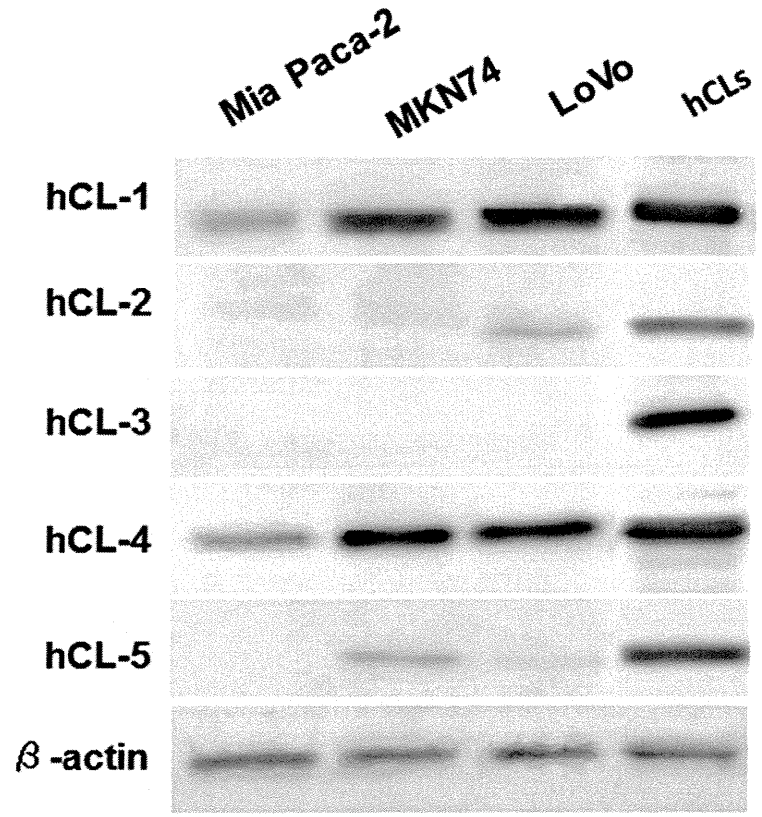



**Figure 27 Expression of CLs in cancer cells.**
Mia Paca-2, MKN74, LoVo cell lysates were subjected to SDS-PAGE, followed by Western blotting. hCL-1, -2, -3, -4, -5/HT1080 cells are as positive controls. β-actin is an endogenous control.

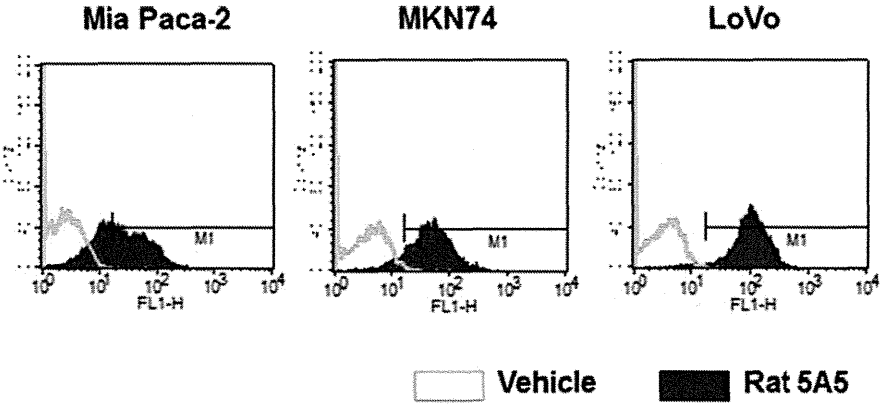



**Figure 28 Flow cytometry analysis of the interaction between 5A5 and Mia Paca-2、MKN74、LoVo cells.**
Mia Paca-2、MKN74、LoVo cells were incubated with 5A5 and FITC-conjugated goat anti-rat IgG (H+L). The antibodies-bound cells were detected using a flow cytometer. As a control, cells were incubated with PBS.

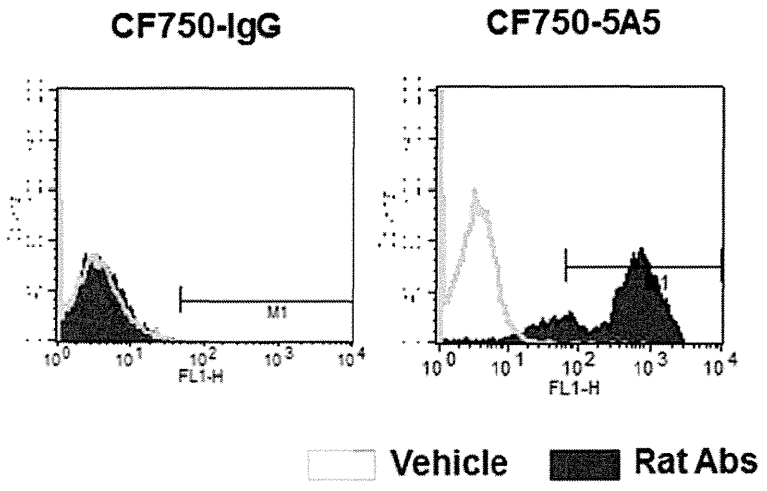



**Figure 29 Flow cytometry analysis of the interaction between CF750 labeling antibodies and hCL-4-expressing HT1080.**
hCL-4-expressing HT1080 was incubated with CF750 labeling antibodies and FITC-conjugated goat anti-rat IgG (H+L). The antibodies-bound cells were detected using a flow cytometer. As a control, cells were incubated with PBS.

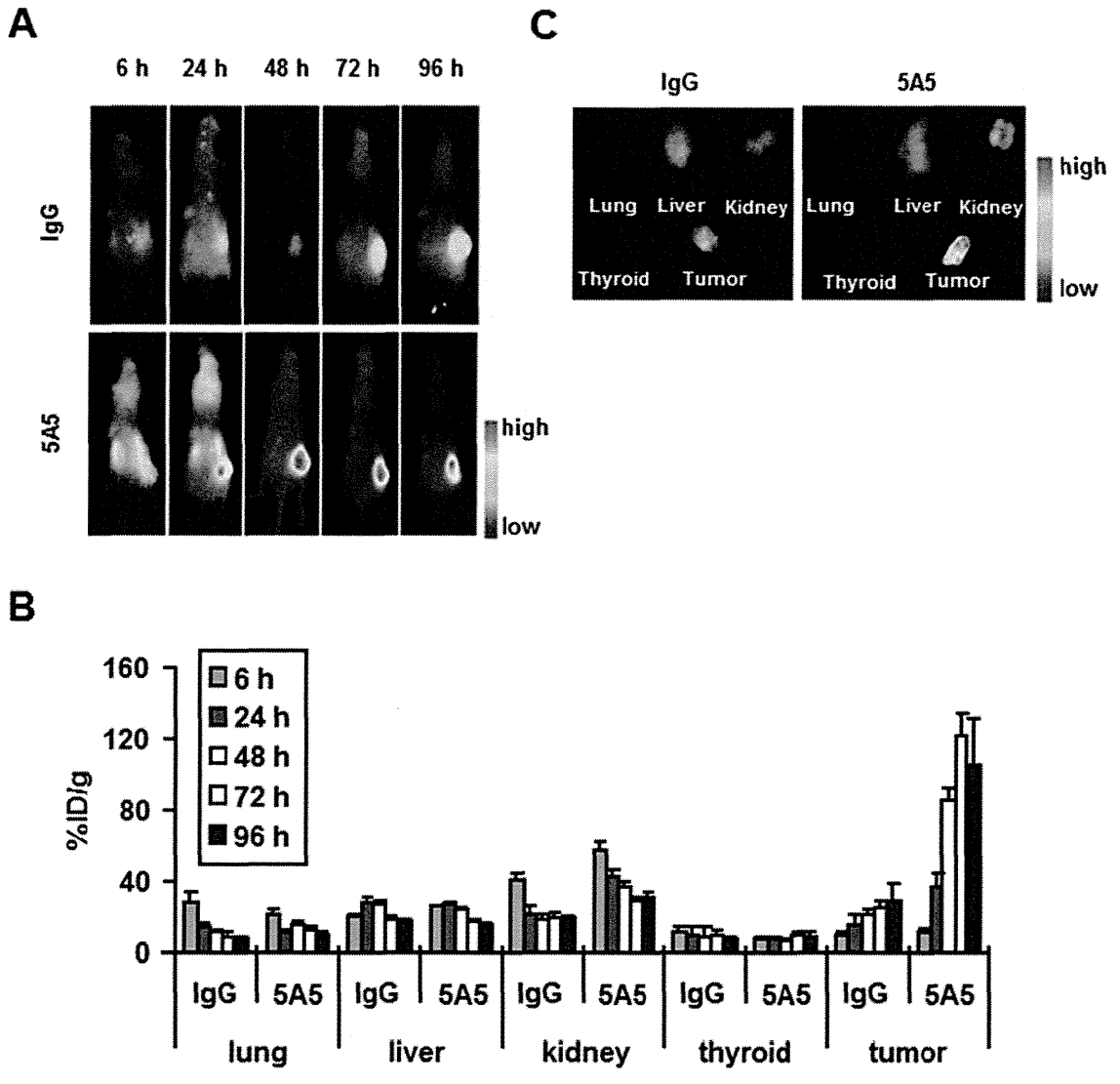



**Figure 30 Distribution of the CF750-5A5 in mice bearing tumor MKN74.**
MKN74 cells were transplanted s.c. into BALB/c Slc-nu/nu mice. CF750-5A5 or CF750-rat IgG were intraperitoneally injected at 20 μg/mouse after 5 weeks. The intensity of fluorescence of mice at the indicated times after injection were observed (A). The tissues were removed at the indicated times and the intensity of fluorescence of each tissue was measured (B). The intensity of fluorescence of tissues at 72 hour point was shown (C). Tissue labeling antibody levels were calculated as percentages of injected doses per gram. Data are means ± SEM (n = 3). ID/g: injected dose per gram of tissue.

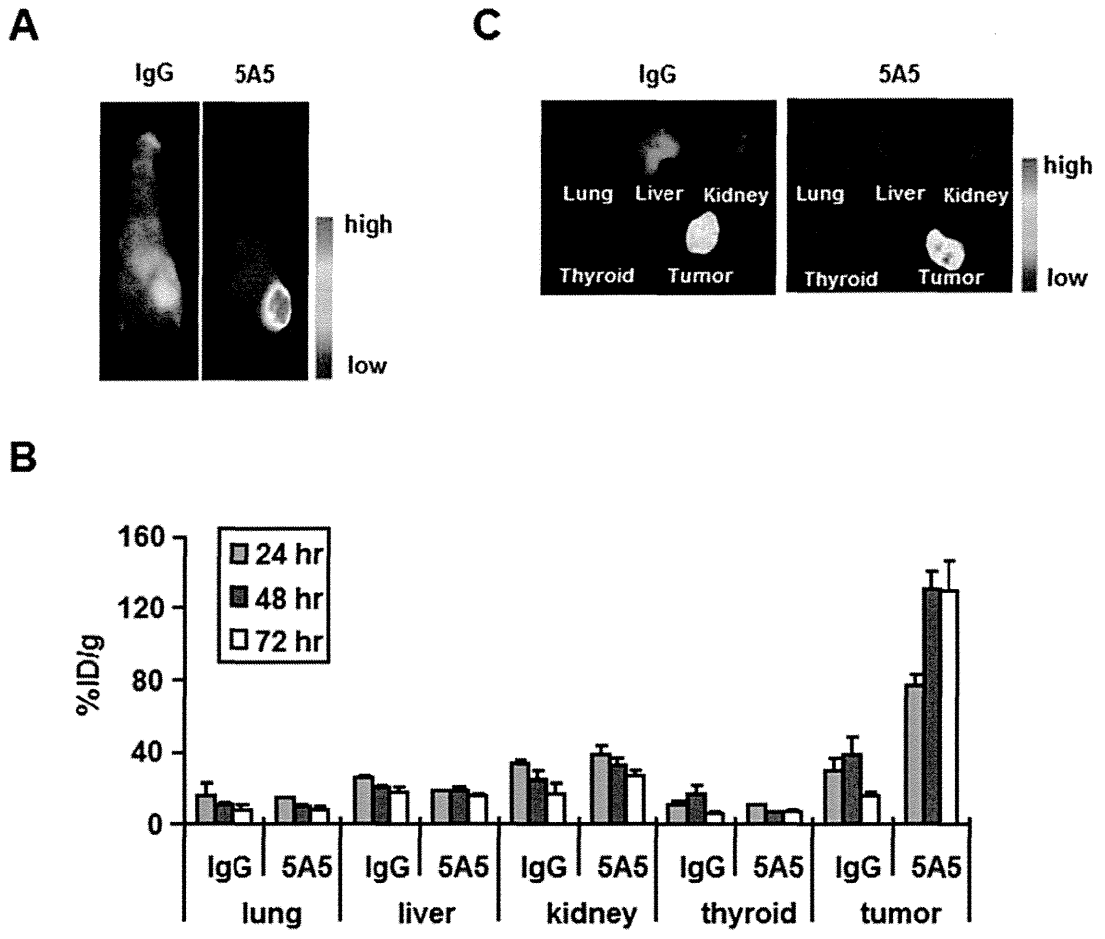



**Figure 31 Distribution of the CF750-5A5 in mice bearing tumor Mia Paca-2.**
Mia Paca-2 cells were transplanted s.c. into BALB/c Slc-nu/nu mice. CF750-5A5 or CF750-rat IgG were intraperitoneally injected at 20 μg/mouse after 5 weeks. The intensity of fluorescence of mice at the indicated times after injection were observed (A). The tissues were removed at the indicated times and the intensity of fluorescence of each tissue was measured (B). The intensity of fluorescence of tissues at 72 hour point was shown (C). Tissue labeling antibody levels were calculated as percentages of injected doses per gram. Data are means ± SEM (n = 3). ID/g: injected dose per gram of tissue.

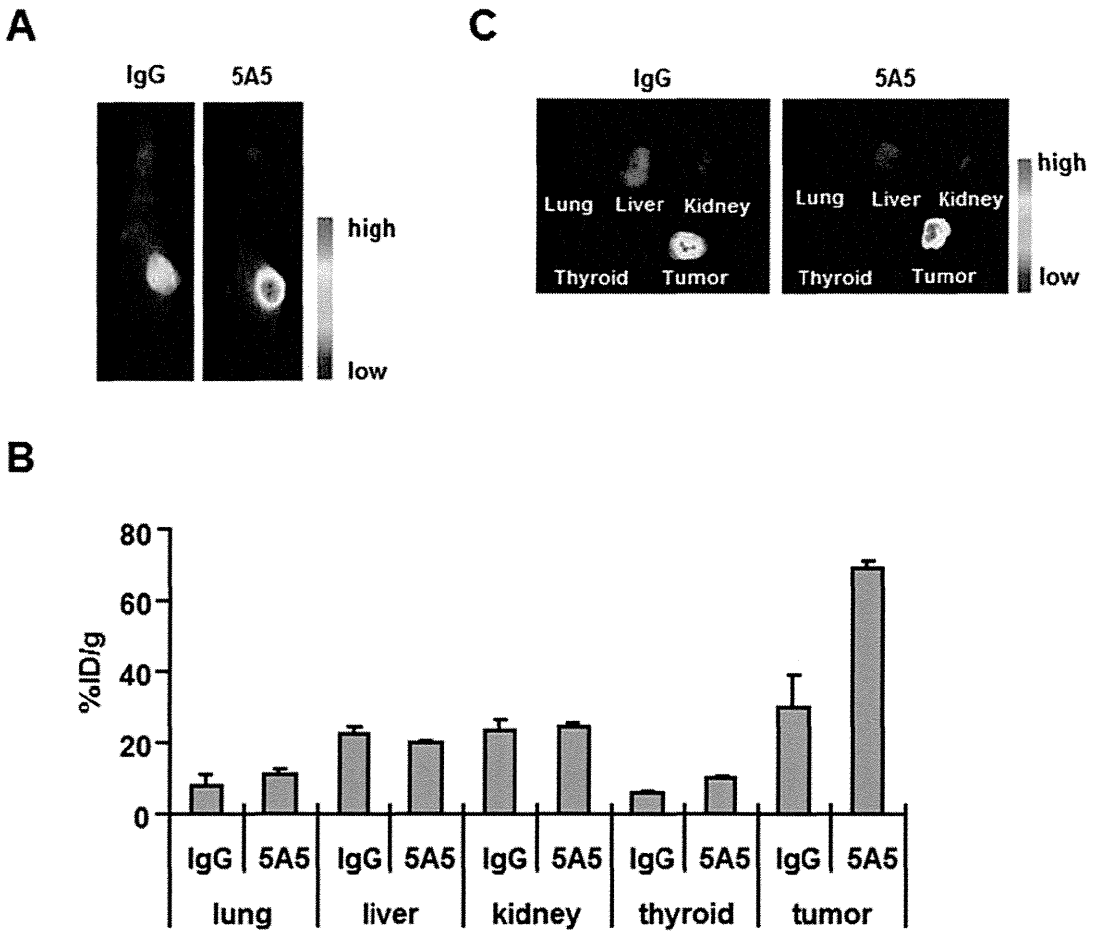



**Figure 32 Distribution of the CF750-5A5 in mice bearing tumor LoVo.**
LoVo cells were transplanted s.c. into BALB/c Slc-nu/nu mice. CF750-5A5 or CF750-rat IgG were intraperitoneally injected at 20 μg/mouse after 5 weeks. The intensity of fluorescence of mice at 72 hour point after injection were observed (A). The tissues were removed at 72 hour point and the intensity of fluorescence of each tissue was measured (B). The intensity of fluorescence of tissues at 72 hour point was shown (C). Tissue labeling antibody levels were calculated as percentages of injected doses per gram. Data are means ± SEM (n = 3). ID/g: injected dose per gram of tissue.

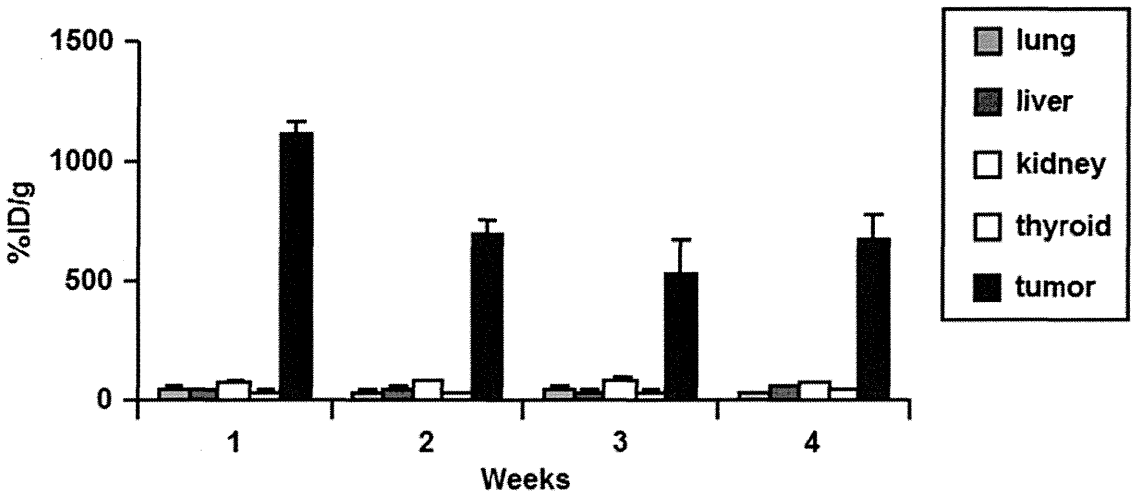



**Figure 33 Tumor implantation time-dependent accumulative of CF750-5A5 in mice bearing tumor Mia Paca-2.**

Mia Paca-2 cells were transplanted s.c. into BALB/c Slc-nu/nu mice. CF750-5A5 was intraperitoneally injected at 20 μg/mouse each week respectively until 4th week. The tissues were removed at each week and the intensity of fluorescence of each tissue was measured. Tissue labeling antibody levels were calculated as percentages of injected doses per gram. Data are means ± SEM (n = 3). ID/g: injected dose per gram of tissue.

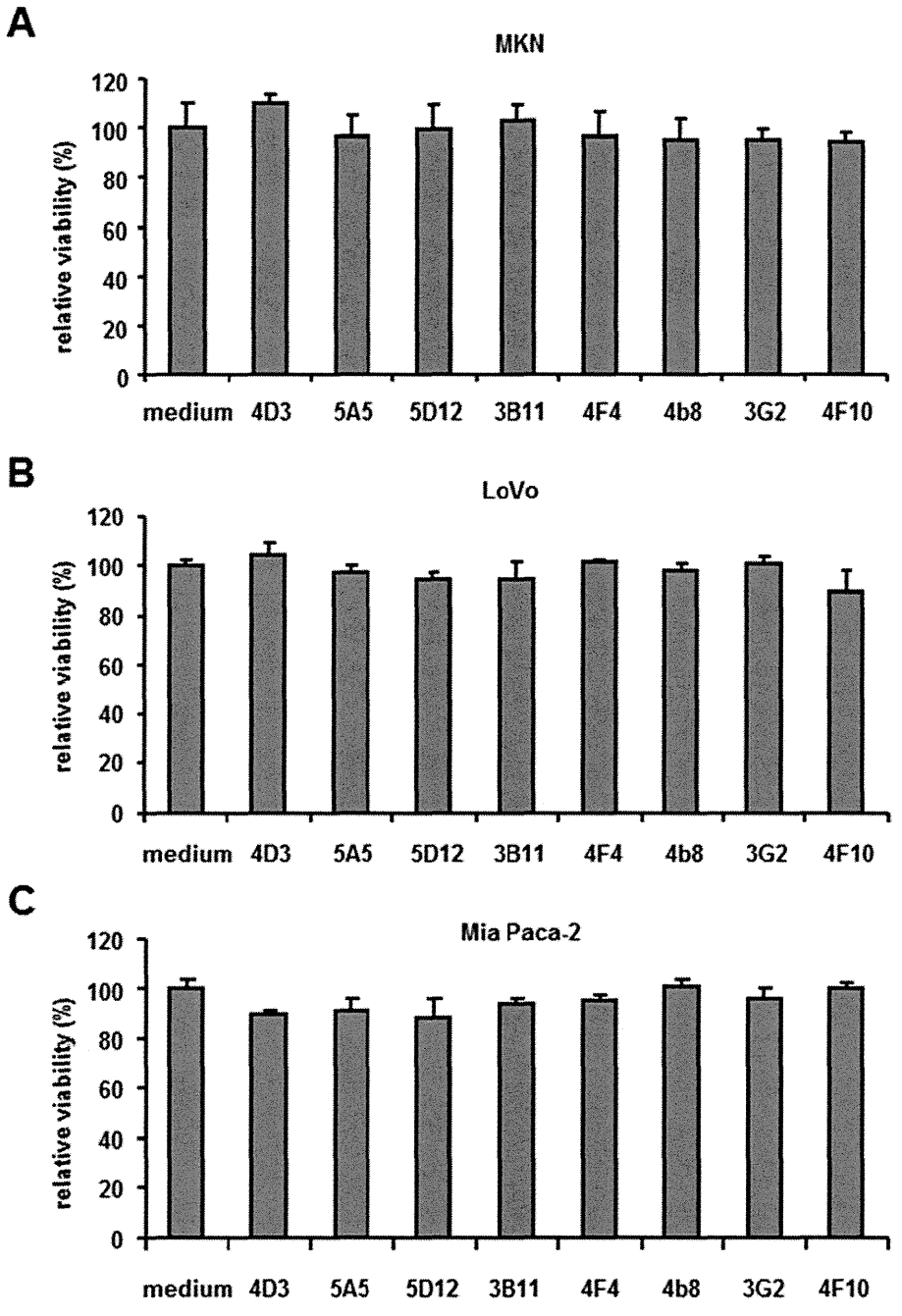



**Figure 34 The CDC activity of rat antibodies.**

MKN74 (A) or LoVo (B) or Mia Paca-2 (C) were seeded in 96-well plate. Human serum were added with 10 μg/mL rat antibodies and incubated for 3 hours, followed by measurement of the relative viability of cells. The data represented the means ± SD (n=3).

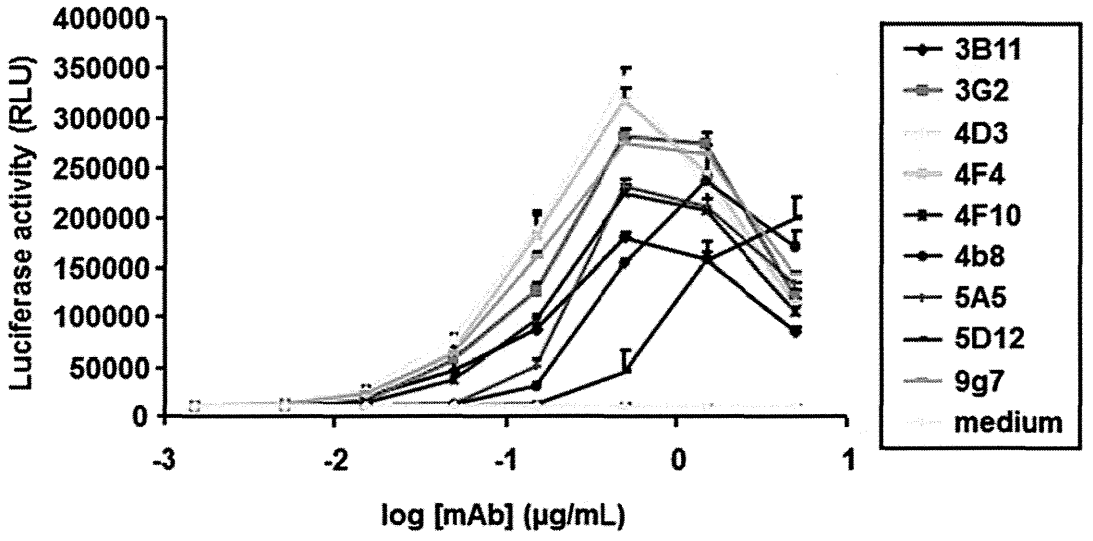



**Figure 35 The FcγRIIIa reporter activity of rat antibodies.**
hCL-4/HT1080 were seeded in 96-well plate and cultured for 24 hours. After removing the medium, Jurkat/FcγRIIIa/NFAT-Luc suspended in Opti-MEM I Reduced Serum Media were added with serially diluted rat antibodies and incubated for 5 hours, followed by measurement of the luciferase activity. The data represented the means ± SD (n=3).

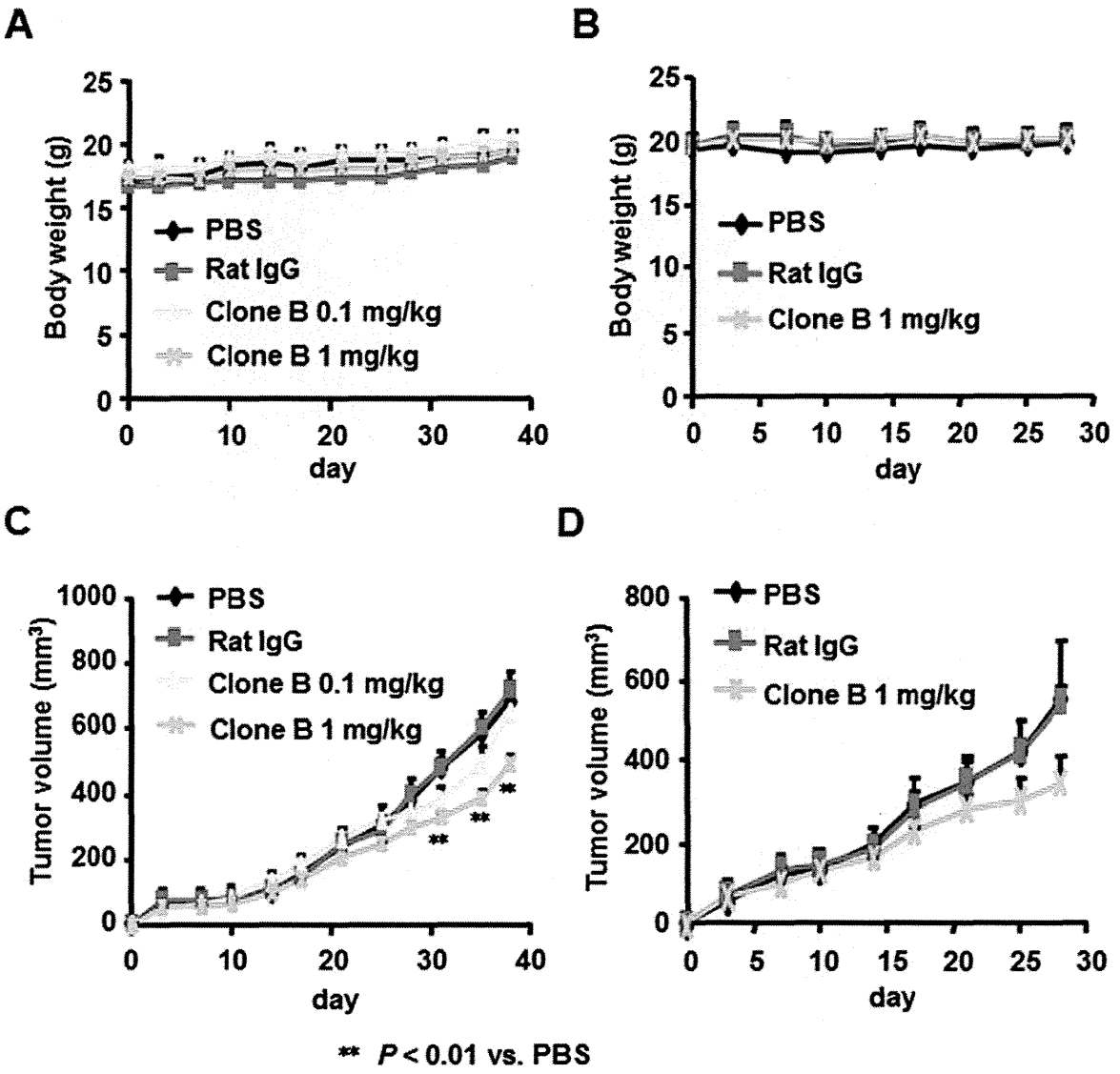



**Figure 36 The body weight and anti-tumor effect of 5A5 in MKN74 or LoVo cells bearing mice.**

MKN74 or LoVo cells were transplanted s.c. into BALB/c Slc-nu/nu mice. Rat IgG or 5A5 were intraperitoneally injected at 1 mg/kg body weight twice a week for 4~5 weeks. The body weight (A for MKN74, B for LoVo) and tumor size (C for MKN74, D for LoVo) were measured before each administration. Data are means ± SEM (n = 5). ＊＊: $p < 0.01$ vs. PBS.

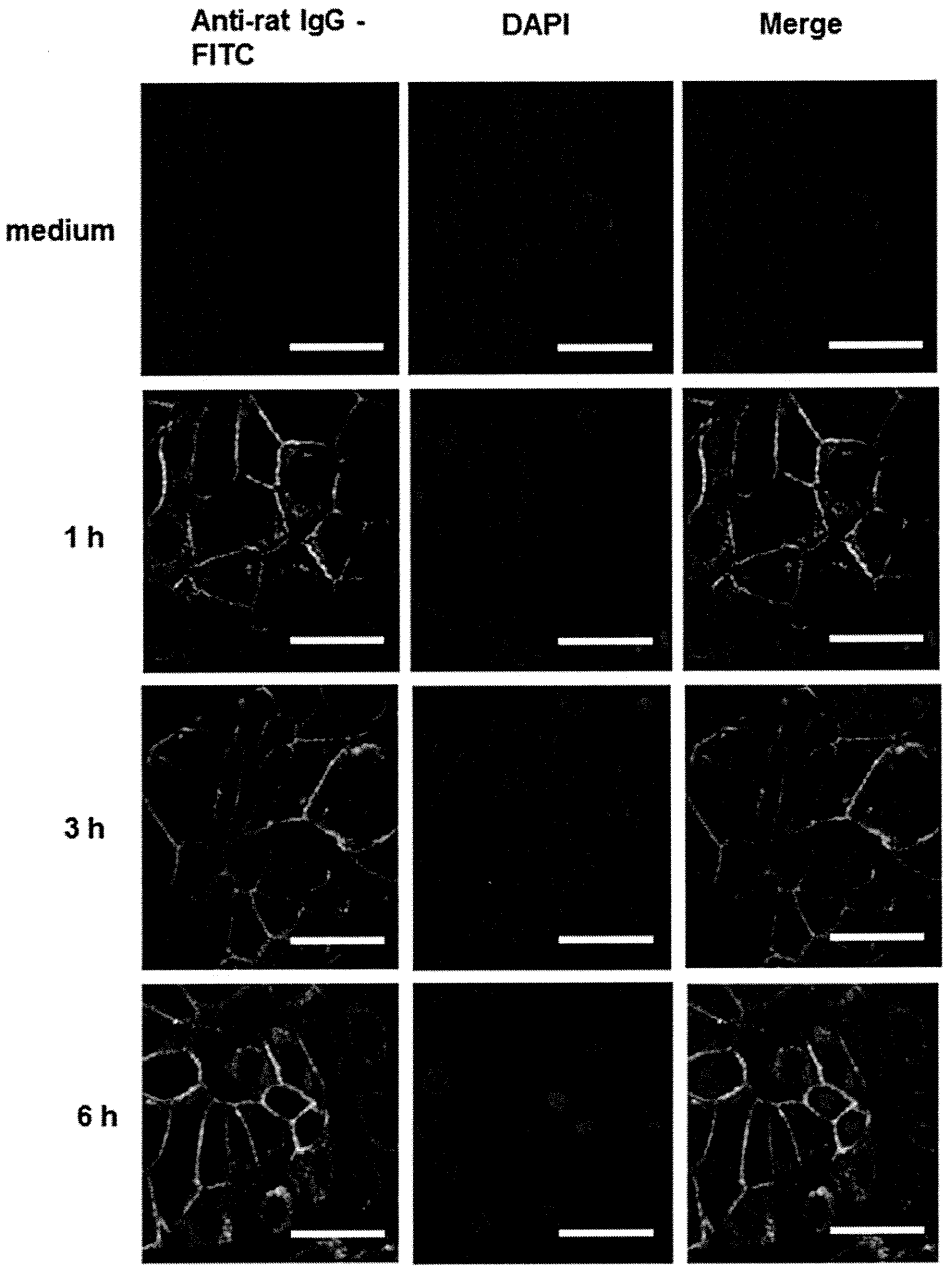



**Figure 37 Immunofluorescence staining of the tendency of 5A5 in hCL-4 expressing MKN74.**
MKN74 cells were treated with 5A5 at 5 μg/mL or medium only, and was fixed with 4% PFA after 1 h, 3 h or 6 h. 5A5 was then stained by anti-rat IgG –FITC, and cell nucleus was stained by DAPI. Bar, 50 μm.

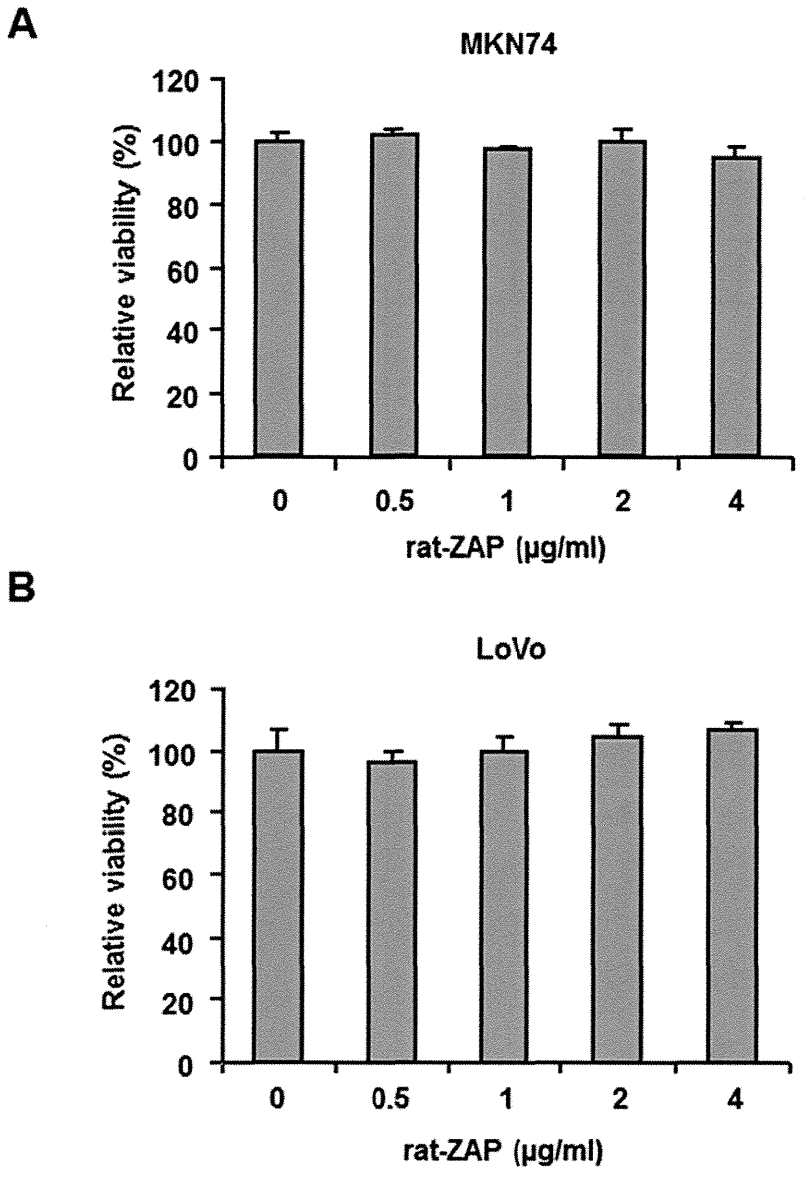



**Figure 38 Cytotoxicity of rat-ZAP alone in hCL-4 expressing cancer cells.** Cytotoxicity of rat-ZAP alone in MKN74 (A) and LoVo (B) cells. Cells were treated with rat-ZAP for 72 h at the indicated concentration. The cell viability was measured by WST-8 assay. The data represented the means ± SD (n=3).