An organotypic model of high-grade serous ovarian cancer to test the anti-metastatic potential of ROR2 targeted Polyion complex nanoparticles.

Nidhi Joshi[1‡], Dongli Liu[2‡], Kristie-Ann Dickson[3], Deborah J. Marsh[3,4], Caroline E. Ford [2 *], Martina Stenzel[1 *]

1. School of Chemistry, University of New South Wales, Sydney, 2052, Australia;
2. School of Women's and Children's Health, Faculty of Medicine and Health, University of New South Wales, Australia;
3. Translational Oncology Group, School of Life Sciences, Faculty of Science, University of Technology Sydney, Ultimo, NSW 2007, Australia
4. Northern Clinical School, Faculty of Medicine and Health, University of Sydney, Camperdown, NSW 2006, Australia

‡Joint First Author

*E-mail: m.stenzel@unsw.edu.au, Caroline.Ford@unsw.edu.au

Analytical instruments

Nuclear Magnetic Resonance (NMR).

^{1}H NMR spectroscopy was performed using a Bruker Avance 300 MHz, and 400 MHz, processed using Bruker Topspin software package. $CDCl_3$ was used as solvent with 32 number of scans.

Size Exclusion Chromatography (SEC).

Size exclusion chromatography (SEC) was used to determine the molecular weight and poly dispersity (*Đ*) of furan protected and deprotected PDMAEMA. DMF-SEC measurements were performed using a Shimadzu modular system containing a DGU-12A degasser, an LC-10AT

pump, a SIL-10AD automatic injector, a CTO-10A column oven and a RID-10A refractive index detector. HPLC grade DMF (0.03% w/v LiBr) was used as a mobile phase with the flow rate of 1 $mLmin^{-1}$. The polymer samples for SEC measurements were prepared in DMF ($1mgmL^{-1}$ of polymer sample) followed by filtering with 0.45µm filter. The injection volume of 50µL was maintained in each measurement using the small insert. Chromatograms were processed using Cirrus 2.0 software (Polymer Laboratories).

Dynamic Light Scattering (DLS).

The hydrodynamic size and zeta potential of the nanoparticles were measured by Dynamic Light Scattering (DLS) using Malvern Zetasizer Nano ZS instrument equipped with a 4 mV He-Ne laser operating at $\lambda = 632nm$ as incident beam and backscatter detection angle at 173°. The nanoparticle solutions were filtered through a 0.45µm syringe filters before measurement 25°C. Each sample was measured at least 10 scans, in triplicates using disposable cells (ZEN).

Transmission Electron Microscopy (TEM).

TEM analysis was performed using a JEOL1400 TEM and a FEI Tecnai-G2 at 80-100 kV beam voltage. BSA decorated PICs nanoparticle samples were prepared and deposited on the formvar-coated copper grids and draining the excess sample solution using filter paper. Samples were exposed to uranyl acetate (3% aqueous solution) for 2 min and then completely air-dried before measurements

Fluorescence Spectrophotometer.

Fluorescence measurements of FITC labelled BSA-PDMAEMA conjugates and their respective PICs were performed on Agilent Cary Eclipse fluorescence spectroscopy. All the fluorescence spectra were recorded between 450 and 750 nm at $\lambda ex = 490$ nm, $\lambda em = 530$ nm with slit width of 10 mm.

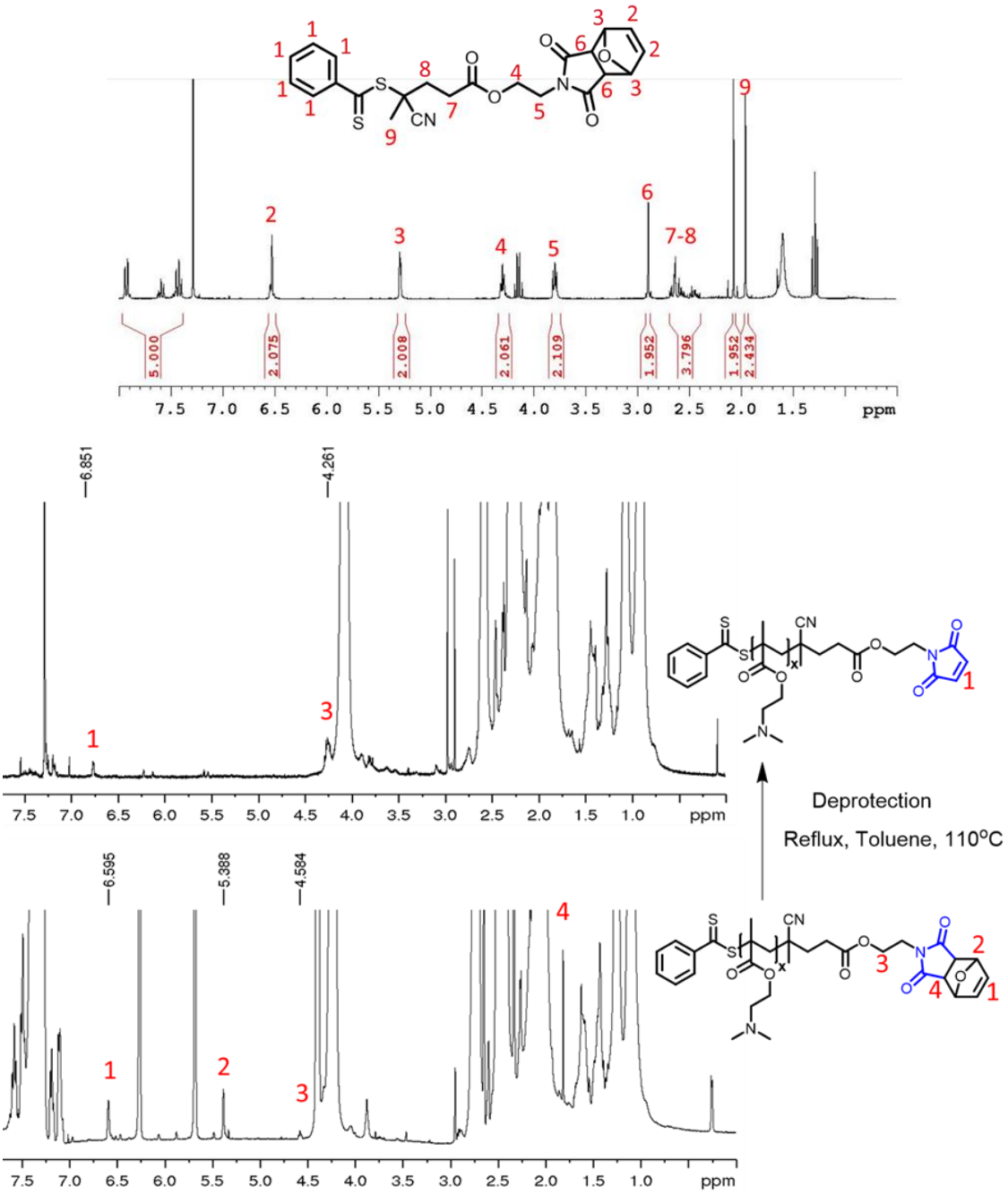

Figure S1. ^{1}H NMR spectra of the furan protected RAFT agent MCPADB in $CDCl_3$ and**,** furan protected PDMAEMA before deprotection and PDMAEMA containing maleimide end group after deprotection in $CDCl_3$ (400MHz)

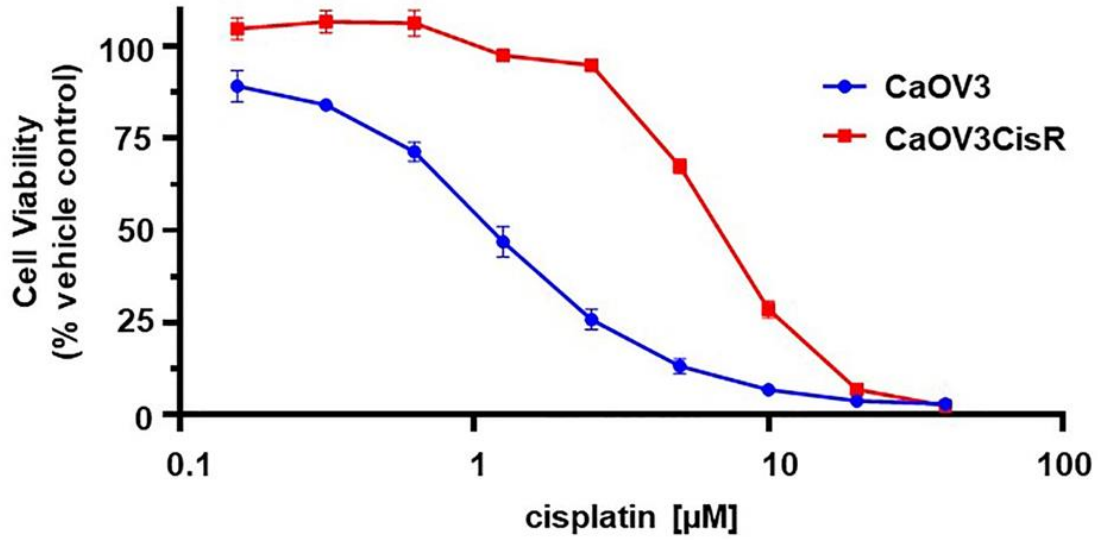

Figure S2. Cisplatin dose curves in the CaOV3 and CaOV3CisR cell line pair measuring cell viability (MTS assay) for the purpose of calculating IC50 doses expressed as a % of saline vehicle control. Data points represent the experimental mean ± SEM (N = 3).

Table S1. Summary of experimental data of BSA conjugation to PDMAEMA with different chain length. The molar ratio of polymer to BSA was kept 1:1.

Sample name	PDMAEMA	BSA
BSA-PDMAEMA$_{72}$	12 mg 1 µmol	66.4 mg 1 µmol
BSA-PDMAEMA$_{87}$	14 mg 1 µmol	66.4 mg 1 µmol
BSA-PDMAEMA$_{150}$	25 mg 1 µmol	66.4 mg 1 µmol
BSA-PDMAEMA$_{220}$	36 mg 1 µmol	66.4 mg 1 µmol

Table S2. PICs nanoparticles formed between BSA-PDMAEMA conjugates differ in the cationic block length (Molecular weight (12kDa to 36kDa) and siRNA at a N/P ratio of 10

Sample	N /P ratio	Polymer Volume (µL)	siRNA (20µM) Volume (µL)	HEPES (10mM) Volume (µL)
PICs1	10	29	20	31
PICs2	10	27	20	33
PICs3	10	28	20	32
PICs4	10	27.8	20	32.2

Table S3. Summary of molecular weights, molecular weight distribution of polymer and BSA-polymer conjugates[a], N/P ratio and PICs concentration.

Polymer sample	M_n[a] (kDa)	M_n BSA-PDMAEMA conjugates (kDa)	($Đ$)[b]	N/P ratio PICs	PICs ROR2 siRNA (μM)
BSA	66	…		…	…..
P1	12	78	1.1	10	5
P2	14	80	1.1	10	5
P3	25	90	1.1	10	5
P4	36	101	1.23	10	5

[a]Polymer molecular weight and [b]Molecular weight distribution ($Đ$) was measured by the ^{1}HNMR and Size Exclusion Chromatography (SEC) in DMF, respectively.

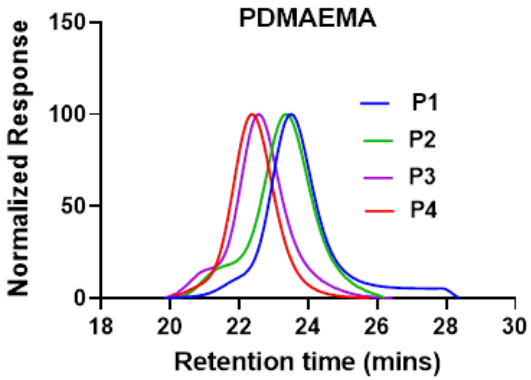

Figure S3. SEC traces of PDMAEMA containing maleimide end group, differ in their molecular weights P1 ($PDMAEMA_{12KDa}$), P2 ($PDMAEMA_{14KDa}$), P3 ($PDMAEMA_{25KDa}$) and P4 ($PDMAEMA_{36KDa}$) in DMF as an eluent.

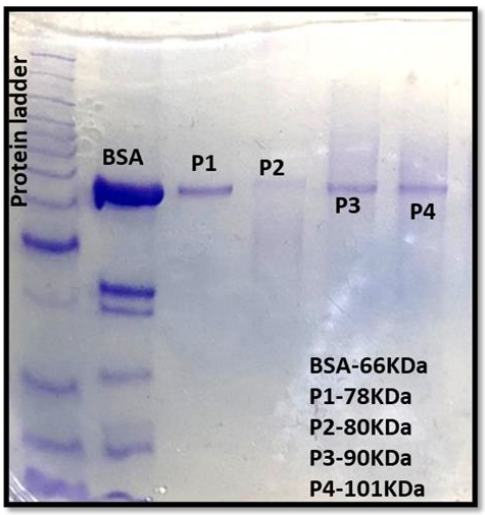

Figure S4. SDS PAGE analysis of 4 different BSA conjugated PDMAEMA, differ in their molecular weights (BSA conjugates P1 to P4 = 78 kDa, 80 kDa, 90 kDa and 101 kDa**)**

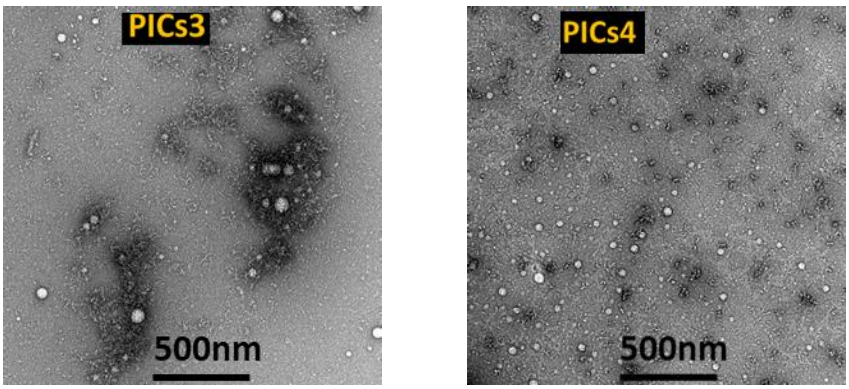

Figure S5. TEM images of inactive siRNA loaded PICs3 and PICs4 nanoparticles (scale bar 500 nm).

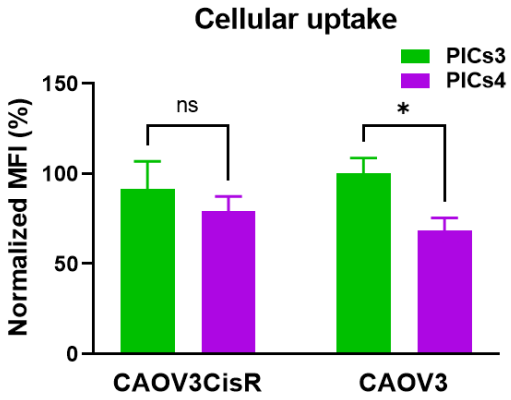

Figure S6. *In vitro* cellular uptake of PICs3 and PICs4. Quantitative analysis of FITC labelled PICs3 and PICs4 uptake after 4 h incubation with CaOV3CisR and CaOV3 cells by flow cytometry.

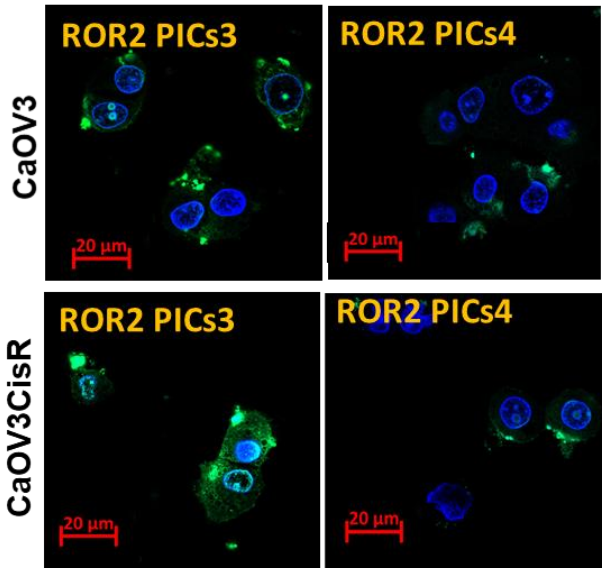

Figure S7. Confocal laser scanning microscope visualization of PICs nanoparticles (PICs3 and PICs4) localization in CaOV3 and CaOV3CisR cells after 4 h of incubation. (Scale bar, 20 µm; Hoerst stained nuclei, blue; nanoparticles, PICs3 & PICs4; FITC-BSA conjugates, green.

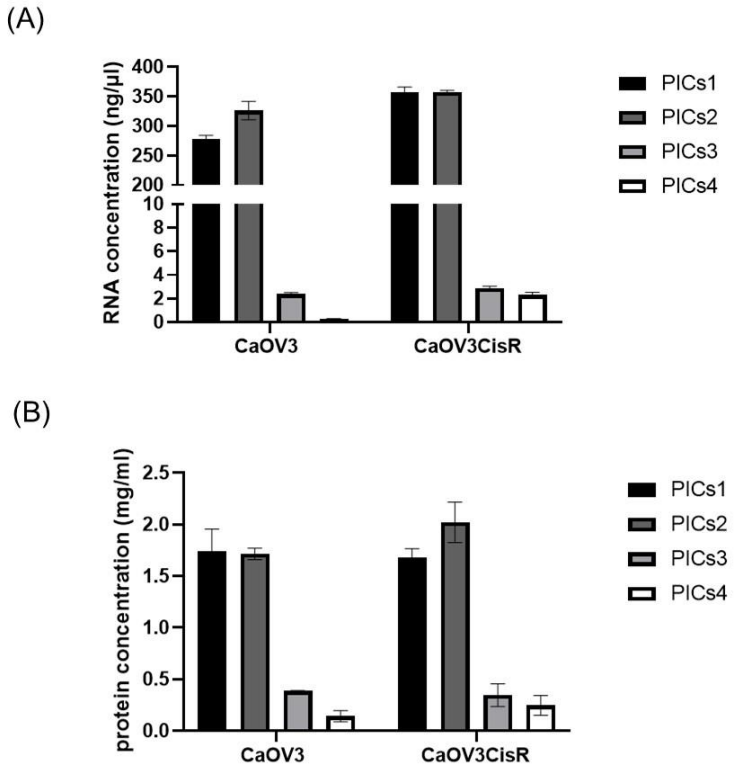

Figure S8. The total RNA and protein concentrations in CaOV3 and CaOV3CisR cancer cell lines treated with PICs (1-4) conjugated non-targeting siRNA. **(A)** RNA concentrations of CaOV3 and CaOV3CisR cells treated with PICs conjugated siRNA detected via Nanodrop. **(B)** Protein concentrations of the CaOV3 and CaOV3CisR cell lysates after treatment with PICs conjugated siRNA estimated by the BCA protein assay. For both panels, n=2, error bar represents standard deviation.

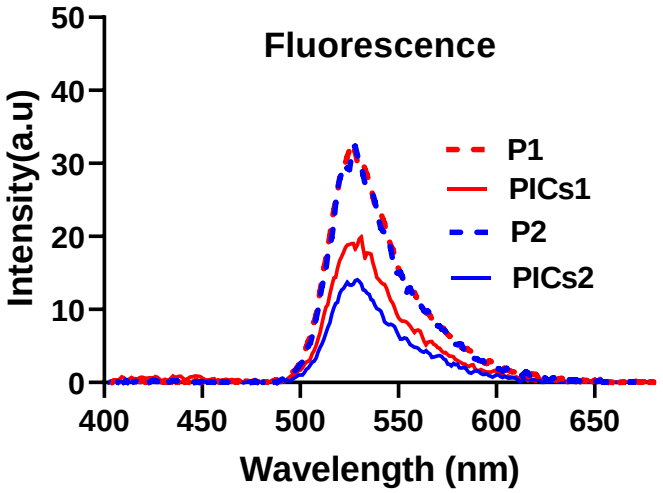

Figure S9. Fluorescence emission spectra of FITC labelled BSA-PDMAEMA conjugates P1 and P2 and their respective PICs nanoparticles (PICs1 and PICs2) ($\lambda_{ex}/\lambda_{em}$ = 490 nm/530 nm) in HEPES buffer.

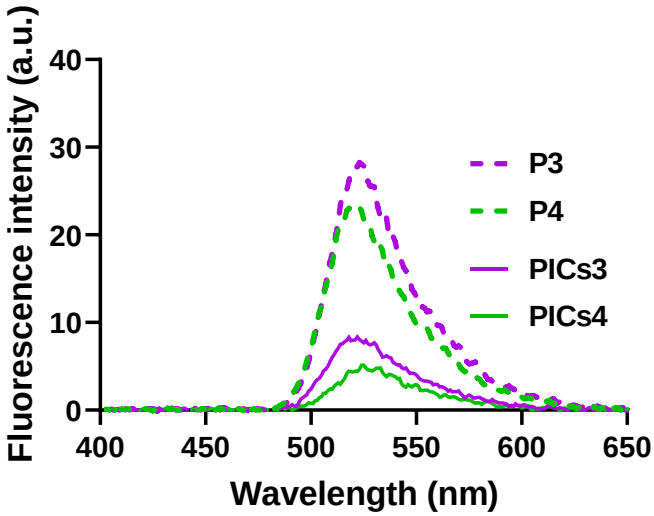

Figure S10. Fluorescence emission spectra of FITC labelled BSA-PDMAEMA conjugates (P3 and P4) and their respective PICs nanoparticles (PICs3 and PICs4) ($\lambda_{ex}/\lambda_{em}$ = 490 nm/530 nm) in HEPES buffer

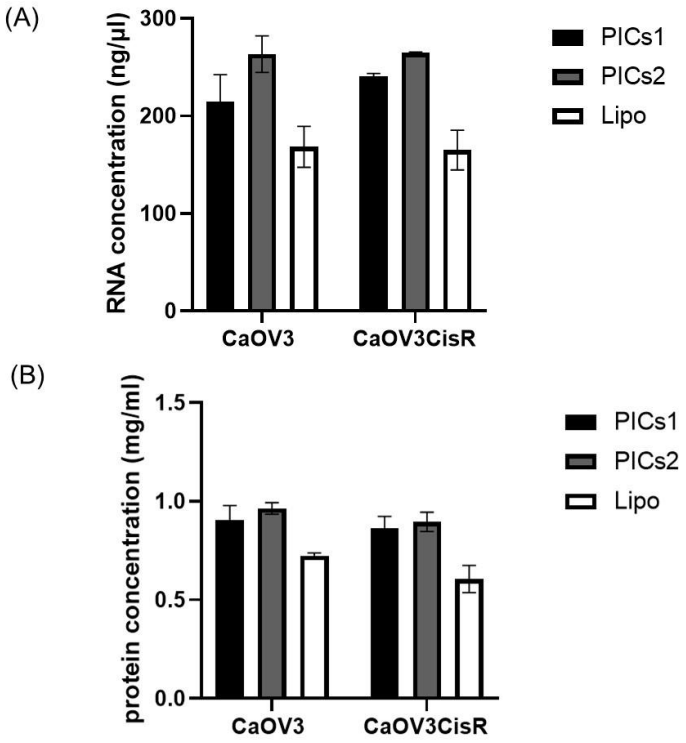

Figure S11. The mRNA and protein expression level of total RNA and protein concentrations in CaOV3 and CaOV3CisR cancer cell lines treated with non-targeting siRNA delivered via PICs1 and PICs2 nanoparticles and lipofectamine 2000 system. **(A)** RNA concentrations of CaOV3 and CaOV3CisR cells following transfections detected via Nanodrop. **(B)** Protein concentrations of the CaOV3 and CaOV3CisR cell lysates after treatments estimated by the BCA protein assay. For both panels, n=2, error bar represents standard deviation.