SUPPORTING INFORMATION

Materials. N,N'-cystaminebisacrylamide (CBA) was obtained from Polysciences, Inc. Heparin (sodium salt) and AMD3100 octahydrochloride hydrate was purchased from Sigma-Aldrich (St. Louis, MO). The base form of AMD3100 used in synthesis was obtained from Ontario Chemicals, Inc. (Guelph, ON, Canada). Plasmid DNA, gWiz high-expression luciferase (gWiz-Luc) containing luciferase reporter gene was from Aldevron (Fargo, ND). Cell culture inserts (for 24-well plates, 8.0 μm pores, Translucent PET Membrane, cat# 353097) and BD Matrigel™ Basement Membrane Matrix (cat# 356237) were purchased from BD Biosciences (Franklin Lakes, NJ). Human CXCL-12 (SDF-1α) was from Shenandoah Biotechnology, Inc. (Warwick, PA). Dulbecco's Modified Eagle Medium (DMEM), Dulbecco's Phosphate Buffered Saline (PBS), Fetal Bovine Serum (FBS), L-Glutamine, and Penicillin-Streptomycin (Pen-Strep) solution were from Thermo Scientific (Waltham, MA). G418 sulfate and Minimum Essential Medium (MEM) were from Mediatech, Inc. (Manassas, VA). Diff-Quick staining kit was from IMEB Inc. (San Marcos, CA). All other reagents and chemicals were obtained from Fisher Scientific or VWR International unless otherwise noted.

Synthesis and Characterization of RPA. RPA was synthesized by Michael addition of equal molar ratio of AMD3100 and CBA. Typically, CBA (104 mg, 0.4 mmol) and AMD3100 (200.8 mg, 0.4 mmol) were added into a glass vial containing methanol/water mixture (4 mL, 7/3 v/v). Polymerization was carried out under nitrogen protection in dark at 37 °C for 72 h. Then, additional 20 mg of AMD3100 was added to the reaction mixture

to consume any residual acrylamide groups, and stirred was continued for another 6 h. The reaction mixture was then added dropwise to excess of 1.25 M HCl in ethanol so that pH of the mixture was kept around 3. The resulting precipitated RPA.HCl was then isolated by centrifugation, washed twice with ethanol and dried in vacuum. The polymer was then dissolved in water and dialyzed against water for 2 days (MWCO 3.5kDa) before final freeze-drying.

The polymer was analyzed by 1H NMR to confirm completion of the reaction from disappearance of the acrylamide signal of CBA (Fig. S1). The composition of the polymer was determined by elemental analysis from N, S, and Cl content (Atlantic Microlab). Removal of any potentially unreacted AMD3100 was confirmed by analyzing its content in RPA using LC-MS/MS (AQUITY UPLC® TQD system, Waters, MA) equipped with AQUITY UPLC® BEH Shield RP18 column (2.1mm×100mm, 1.7 μm). A gradient of aqueous solution of (2 mM ammonium formate + 0.1% formic acid) and acetonitrile was used. AMD3100 was monitored at the parent/daughter ions of (m/z) 503.61 → (m/z) 105.00. The purity of RPA was >99.8%.

Weight- and number-average molecular weights and polydispersity index (PDI) were determined by Size Exclusion Chromatography (SEC) using Viscotek GPCmax chromatography system consisting of an autosampler, a pump, a CTO-10ASVP Shimadzu column oven, a refractive index detector, a low- and right-angle light scattering detector, and OmniSEC software for chromatographic data analysis/storage (Malvern Instruments, UK). The columns used were single pore AquaGel^{TM} columns (cat# PAA-202 and PAA-203) by PolyAnalytik (London, ON, Canada). Sodium acetate buffer (0.3 M, pH 5) was used as an eluent at flow rate of 0.3 mL/min.

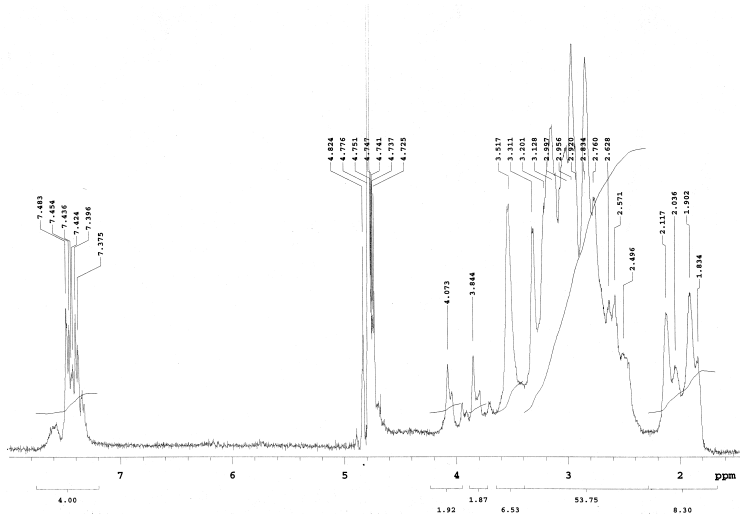

Figure S1. 1H NMR of RPA in D_2O. From the NMR spectrum, there are no unreacted acrylamide residues. Furthermore, the peaks for the center methylenes of the $(CH_2)_3$ sections of the cyclam rings (~2 ppm) show a double peak with integral intensity ratios of 2:1. From that, we can infer no selectivity in the reaction of CBA with the three available amines in each cyclam ring.

Synthesis and Characterization of RHB. RHB was synthesized by Michael addition of equal molar ratio of DMDPTA and CBA (Fig. S2). Typically, CBA (0.54 g, 2.0 mmol) and DMDPTA (0.318 g, 2.0 mmol) were dissolved in 3.5 mL methanol/water (8/2 v/v) and the polymerization was carried out at 50 °C for 72 h. Then, additional 0.1 mmol of DMDPTA was added to the reaction mixture to consume any residual acrylamide groups, and stirring was continued for 12 h at 50 °C. The RHB polymer was isolated by freeze-drying after extensive dialysis against distilled water acidified with HCl to pH 3 and a final dialysis against DI water. Typical yield after dialysis was 30-40%. Molecular weights and PDI were determined by GPC.

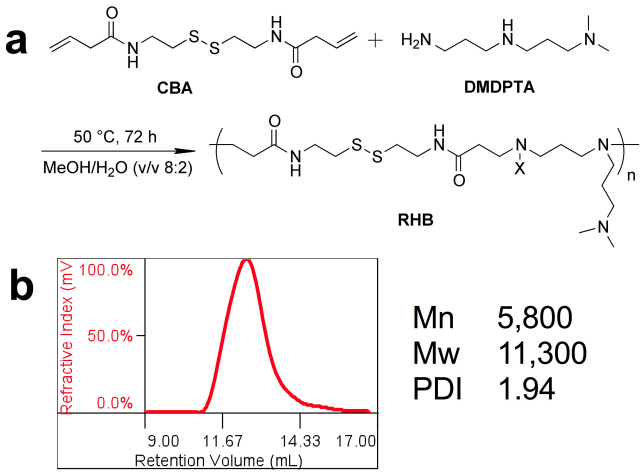

Figure S2. a) Synthetic scheme of RHB, X=H or polymer chain; b) GPC chromatogram, molecular weights and PDI of RHB.

Ethidium Bromide (EtBr) Exclusion Assay. The ability of the studied polycations to condense gWiz-Luc DNA was determined by EtBr exclusion assay by measuring the changes in EtBr/DNA fluorescence. DNA solution at a concentration of 20 μg/mL in 10 mM HEPES buffer (pH 7.4) was mixed with EtBr (1 μg/mL) and fluorescence was measured and set to 100% using an excitation wavelength of 540 nm and an emission wavelength of 590 nm. Fluorescence readings were taken following a stepwise addition of a polycation solution, and the condensation curve for each polycation was constructed (Fig. S3).

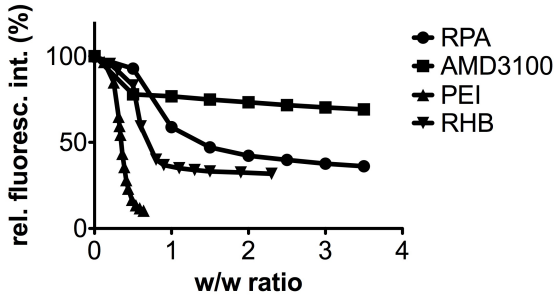

Figure S3. DNA condensation by EtBr exclusion assay.

Formation and Characterization of polycation/DNA complexes (polyplexes). gWiz-Luc DNA solution in 10 mM HEPES (pH 7.4) was prepared to give a DNA concentration in the final polyplexes = 20 μg/mL. Polyplexes were formed by adding predetermined volume of polymer to achieve the desired polycation/DNA weight/weight (w/w) ratio and mixed by vigorous vortexing for 10 s. Polyplexes were further allowed to stand for 30 min prior to use. The determination of hydrodynamic diameters and zeta potentials of polyplexes was performed by Dynamic Light Scattering following previously published method. Results were expressed as mean ± standard deviation (S.D.) of 3-10 experimental runs (Table S1).

Table S1. Sizes and zeta-potentials for different w/w ratios of RPA, RHB and PEI.

	w/w	**Size (nm)**	**Zeta-potential (mV)**
RPA	5	56 ± 0	25 ± 4
	10	70 ± 6	28 ± 4
	15	57 ± 1	31± 4
	20	62 ± 4	33 ± 4
	25	57 ± 0	26 ± 4
RHB	5	157 ± 2	31 ± 5
PEI	1.2 (N/P 10)	84 ± 1	33 ± 4

Reduction-triggered release of DNA from polyplexes. Redox-triggered disassembly of the polyplexes was examined by agarose gel electrophoresis (Fig. S4). Briefly, gWiz-Luc DNA polyplexes were incubated with or without 20 mM GSH either in the presence or

absence of 0.15 M NaCl at 37 °C for 1 h. Samples were then loaded onto a 0.8% agarose gel containing 0.5 μg/mL EtBr and run for 75 min at 120 V in 0.5x Tris/Borate/EDTA (TBE) running buffer. The gel was visualized under UV.

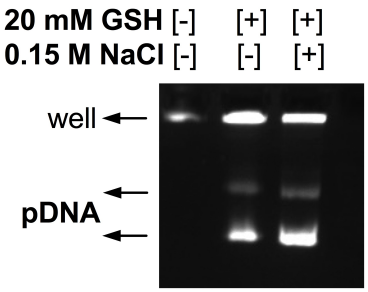

Figure S4. Reduction-triggered DNA release from RPA/DNA polyplexes.

Cell Culture. Murine melanoma cell line B16F10 and human hepatocellular carcinoma cell line HepG2 were purchased from ATCC (Manassas, VA). B16F10 cells were maintained in DMEM supplemented with 10% FBS. Hep G2 cells were maintained in MEM supplemented with 10% FBS. Human epithelial osteosarcoma U2OS cells stably expressing human CXCR4 receptor fused to the N-terminus of enhanced green fluorescent protein were purchased form Fisher Scientific. The cells were cultured in DMEM supplemented with 2 mM L-Glutamine, 10% FBS, 1% Pen-Strep and 0.5 mg/ml G418.

Polycation toxicity. Toxicity of polycations was evaluated by MTS assay in Hep G2 cells. The cells were plated into 96-well microtiter plates at a density of 20,000 cells/well. After 24 h, culture medium was replaced by 150 μl of serial dilutions of a polymer in serum-supplemented medium and the cells were incubated for 24 h. Polymer solutions were aspirated and replaced by a mixture of 100 μl serum-free media and 20 μl of MTS

reagent (CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay, Promega). After 2 h incubation, the absorbance was measured spectrophotometrically in Synergy 2 Microplate Reader (BioTek, VT) at a wavelength of 490 nm. The relative cell viability (%) was calculated as [A]sample/[A]untreated × 100%. The IC50 were calculated as polymer concentration, which inhibits growth of 50% of cells relative to untreated cells. The IC50 values were calculated based on "log(inhibitor) vs. response - absolute IC50" curve fitting procedure in GraphPad Prism, with constrains of Fifty=50, Top=100 and a formula Y = Bottom + (Top-Bottom)/(1+10^((LogIC50-X)*HillSlope + log((Top-Bottom)/(Fifty-Bottom)-1))).

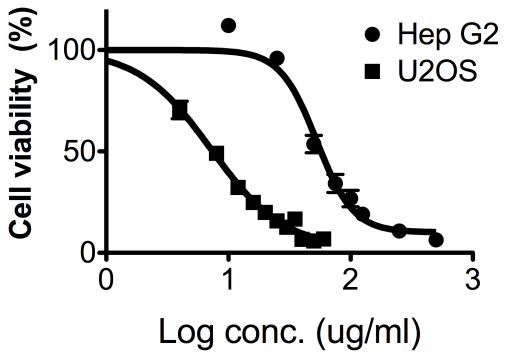

Figure S5. Cytotoxicity of RHB in Hep G2 and U2OS cells by MTS assay. IC50 values for RHB are 57±6 μg/ml in Hep G2 cells and 7±1 μg/ml in U2OS cells.

CXCR4 Redistribution Assay. CXCR4+ U2OS cells were plated in 96-well plate 18-24 h before the experiment at a seeding density of 8,000 cells per well. The cells were first washed with 100 μL assay buffer (DMEM supplemented with 2 mM L-Glutamine, 1% FBS, 1% Pen-Strep and 10 mM HEPES) twice and then incubated with different concentrations of the polycations or AMD3100 (Fig. S6) in assay buffer containing 0.25% DMSO at 37 °C for 30 min. In experiments with RPA/DNA and RHB/DNA

polyplexes, DNA concentration was 0.5 μg/mL and polyplexes were prepared at w/w 1 and 5 (i.e., polycation concentrations were 0.5 and 2.5 μg/mL). Human SDF-1α (CXCL-12) was then added to each well to make final concentration 10 nM. DMSO alone was used as the negative control, and hSDF-1α alone was used as the positive control. After 1 h incubation at 37 °C, the cells were fixed with 4% formaldehyde at room temperature for 20 min followed by 4-time washing with PBS. All the images were taken by EVOS fl microscope at 20X (Fig. S6).

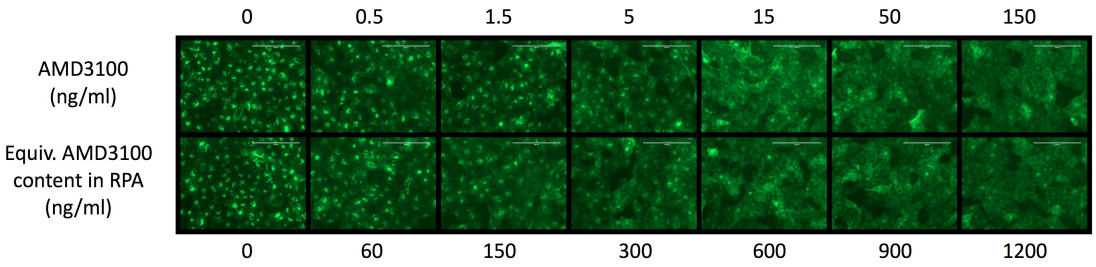

Figure S6. Dose-dependent CXCR4 antagonistic ability of AMD3100 and RPA. Equivalent AMD3100 content in RPA was calculated based on elemental analysis of the polymer.

RPA does not inhibit phorbol 12-myristate induced internalization of CXCR4 receptor. The CXCR4+ U2OS cells were treated with RPA, RPA/DNA or AMD3100 before incubation with 100 ng/mL of phorbol 12-myristate 13-acetate and the cells were imaged by fluorescence microscope (Fig. S7). The results show that internalization of CXCR4 receptor is not inhibited by RPA or AMD3100 when the cells are stimulated with phorbol myristate, unlike when they are stimulated with CXCL12.

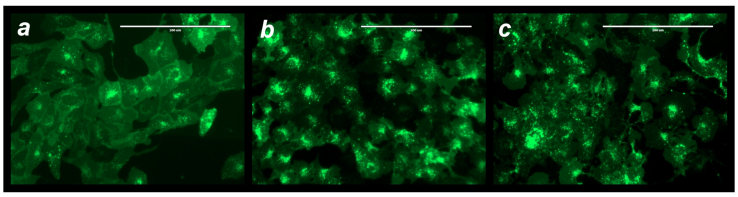

Figure S7. AMD3100 and RPA do not inhibit phorbol-stimulated CXCR4 internalization. CXCR4+ U2OS cells were treated with AMD3100.8HCl (0.24 μg/mL) and then stimulated with a) 10 nM CXCL12 or b) 100 ng/ml of phorbol myristate acetate. c) CXCR4+ U2OS cells were treated with RPA/DNA (w/w 5) polyplexes (i.e., 0.5 μg/mL RPA, 0.1 μg/mL DNA) and then stimulated with 100 ng/ml of phorbol myristate acetate.

Transfection of DNA polyplexes. All transfection experiments were conducted in 48-well plates with cells at logarithmic growth phase. Cells were seeded at a density of 40,000 cells/well 24 h prior to transfection. On a day of transfection, the cells were incubated with the polyplexes (DNA conc. 2.35 μg/ml) in 170 μL of serum-free or 10% FBS-containing media. After 4 h incubation, polyplexes were completely removed and the cells were cultured in complete culture medium for 24 h prior to measuring luciferase expression. The medium was discarded and the cells were lysed in 100 μL of 0.5x cell culture lysis reagent buffer (Promega, Madison, WI) for 30 min. To measure the luciferase content, 100 μL of 0.5 mM luciferin solution was automatically injected into each well of 20 μL of cell lysate and the luminescence was integrated over 10 s using Synergy 2 Microplate Reader (BioTek, VT). Total cellular protein in the cell lysate was determined by the Bicinchoninic acid protein assay using calibration curve constructed with standard bovine serum albumin solutions (Pierce, Rockford, IL). Transfection activity was expressed as RLU/mg cellular protein ± SD of quadruplicate samples.

Effect of phorbol myristate on transfection activity of RPA/DNA polyplexes. The effect of treating the CXCR4+ U2OS cells with phorbol myristate (PMA) on transfection activity of RPA/DNA polyplexes (Fig. S8) was evaluated using the same conditions described in the section "Transfection of DNA polyplexes" except that the cells were co-incubated with polyplexes and 100 ng/mL of phorbol myristate acetate in serum-free medium for 4 h. Luciferase expression was measured 24 h post-incubation. No toxicity for phorbol myristate was observed.

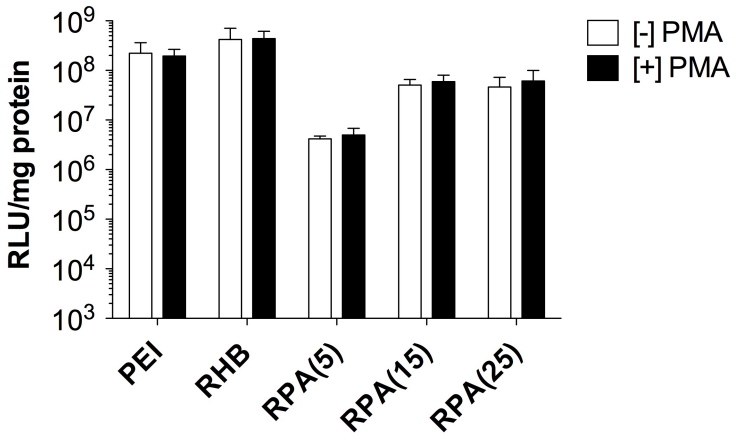

Figure S8. Effect of phorbol myristate (+/- PMA) treatment on transfection activity of RPA/DNA prepared at w/w 5, 15 and 25.

Intracellular distribution of RPA/DNA polyplexes. Luciferase DNA was labeled with Label IT®-Tracker™ CX-Rhodamine Kit (Mirus, Madison, WI) according to manufacturer's protocol. 120,000 CXCR4+ U2OS cells were plated in glass-bottom dish (MatTek P35GC-0-14-C) 24 h before the experiment. The cells were incubated with RPA/DNA polyplexes prepared at w/w 5 (2.35 μg/mL DNA) for 3 h before adding 10

nM hCXCL12. The cells were incubated for another 1 h before a PBS wash, fixation and imaging by Perkin Elmer Spinning Disk confocal microscope (Fig. S9).

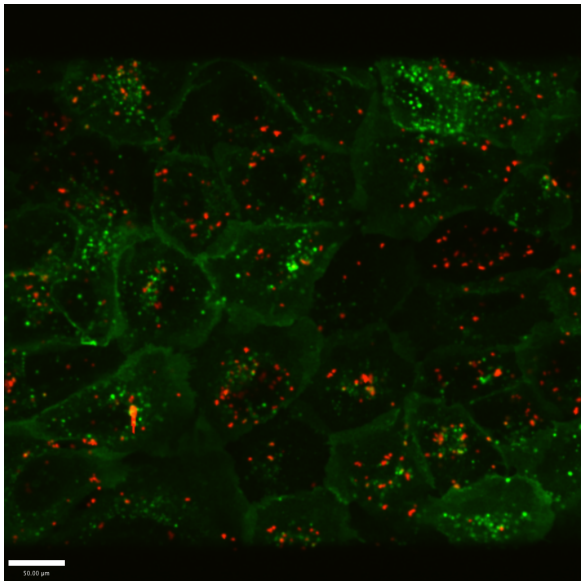

Figure S9. Intracellular distribution of RPA/DNA polyplexes in CXCR4+ U2OS cells. (red fluorescence: CX-Rhodamine labeled plasmid DNA; green fluorescence: GFP-CXCR4 receptor)

Simultaneous transfection and CXCR4 inhibition of RPA/DNA polyplexes. CXCR4+ U2OS cells were plated in black 96-well plate with optical bottom 24 h before the experiment at a seeding density of 8,000 cells per well. The cells were incubated with RPA/DNA polyplexes prepared at w/w 5, 10 and 15 (2.35 μg/mL DNA) or RHB/DNA polyplexes (negative control) prepared at w/w 5 in serum-free media. The polyplexes were removed after 4 h incubation and the cells were continued to grow in complete culture media. The luciferase transfection was measured after 24 h as described above in the "Transfection of DNA polyplexes" section. The CXCR4 antagonism was evaluated in the same cells at 0 h and 24 h after polyplex incubation by stimulating the cells with 10 nM hCXCL12 (Fig. S10).

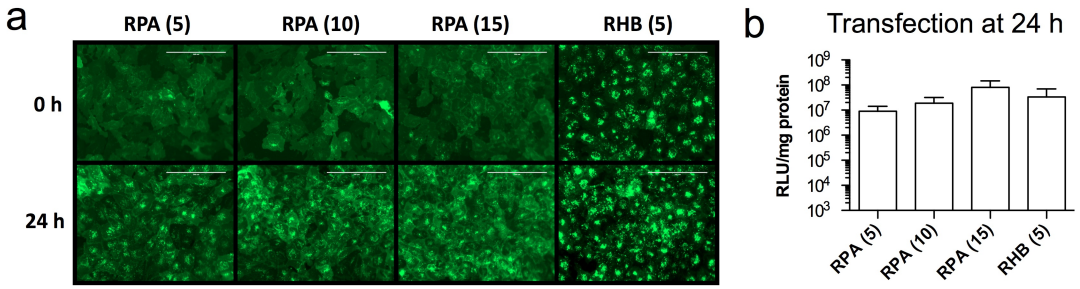

Figure S10. Simultaneous transfection and CXCR4 inhibition by RPA/DNA polyplexes in CXCR4+ U2OS cells. a) Cells treated with RPA/DNA polyplexes (RPA/DNA w/w = 5, 10 and 15) showed CXCR4 inhibition both at 0 h and, a weaker one, at 24 h after polyplex incubation. In contrast, RHB/DNA polyplexes (RHB(5)) didn't show any CXCR4 antagonism at any time. b) Simultaneously, RPA/DNA polyplexes exhibit similar transfection (luciferase expression) as control RHB polyplexes at 24 h after polyplex incubation.

***Cell invasion assay*.** The upper sides of the transwell inserts were coated with 40 μl Matrigel diluted in serum-free medium (v/v 1:3) per insert. The 24-well plates with coated inserts were then placed in 37 °C incubator for 2 h. CXCR4+ U2OS cells were trypsinized and resuspended in different concentrations of drugs in serum-free medium for 30 min before adding to the inserts at a final concentration of 10,000 cells in 300 μl medium per insert. 20 nM CXCL12 in serum-free medium as the chemo-attractant was then added to corresponding wells in the companion plate. After 16 h, the non-invaded cells on the upper surface of the inserts were removed with a cotton swab. The invaded cells were then fixed and stained by dipping the inserts into Diff-Quick solution. The images were taken by EVOS xl microscope. Five 20X imaging areas were randomly selected for each insert and each sample was conducted in triplicate.

Statistical significance of the observed differences in cell invasion was analyzed using non-parametric ANOVA with Dunn's multiple comparison test using GraphPad InStat (v. 3.10). P values for selected comparisons are shown below ($P<0.05$ was considered significant).

Comparison	P value
Non-treated vs. AMD3100	P<0.001
Non-treated vs. RHB (2 mg/ml)	P>0.05
Non-treated vs. RPA (2 mg/ml)	P<0.001
Non-treated vs. RPA (5 ug/ml)	P<0.001
Non-treated vs. RPA/DNA	P<0.001
AMD3100 vs. RHB (2 mg/ml)	P<0.001
AMD3100 vs. RPA (2 mg/ml)	P>0.05
AMD3100 vs. RPA (5 ug/ml)	P>0.05
AMD3100 vs. RPA/DNA	P>0.05