Small molecule targeting of PCNA chromatin association inhibits tumor cell growth

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Molecular Pharmacology

Supplemental data

1. The Surface plasmon resonance (SPR) analysis of the binding of PCNA-I1 and PCNA-I3 to PCNA

A. Materials and Methods

The interaction of PCNA-I1 and PCNA-I3 with PCNA protein was determined using a Biacore 100T surface plasmon resonance biosensor instrument. Recombinant PCNA protein (Surmodics; Eden Prarie, MN) was immobilized on a Biacore CM5 Chip (GE Healthcare; Piscataway, NJ) using the EDC/NHS amine coupling chemistry specified by the manufacturer. Approximately 10,000 RU of PCNA protein was immobilized on the activated dextran surface, which is approximately equivalent to 10 ng/mm^2 surface coverage.

For the kinetic binding analysis, PCNA-I1 and PCNA-I3 were dissolved into 100% DMSO to create 10 mM stock solutions. The inhibitors were serially diluted into nM concentrations in a phosphate buffer saline solution with 5% DMSO (PBS + 5% DMSO). Prior to running SPR analysis, the inhibitor solutions were filtered using a Millex-GX 0.22 μM PVDF syringe-driven filter unit (Millipore; Billerica, MA). The SPR binding experiments were conducted using PBS + 5% DMSO running buffer.

B. Results

PCNA-I1 and PCNA-I3 were serially diluted to concentrations ranging from 0 to 5000 nM in PBS + 5% DMSO running buffer. Based on the size of the PCNA inhibitors (≈300 Da) and the PCNA (≈30,000 Da), the maximum response (RU) resulting from the interaction would be approximately 100 RU, assuming a 1:1 stoichiometric ratio of interaction. This predicted maximum RU was calculated by: $\mathrm{R_{max}} = \frac{\mathrm{MW_{analyte}}}{\mathrm{MW_{ligand}}} \times \mathrm{RU(immobilized)} \times \mathrm{Stoichiometric\ Ratio.}$

Both PCNA inhibitors exhibit dose-dependent response curves. The response was within range of the theoretical R_{mzx}. A change in refractive index due to buffer mismatch with 5% DMSO is observed, as shown by the sudden jump in RU when the samples are injected at the association phase(time = 60 seconds) and dissociation phase (time = 120 seconds). When the buffer mismatch is further adjusted for in the sensorgram, the dose-dependent response is more clearly illustrated. The kinetic binding constants were determined for the PCNA inhibitors using the BIAevaluation software. This iterative model used to determine the best fit for the interaction parameters in a Langmuir 1:1 binding resulted in Kd values of 0.14 μM for PCNA-I1 and 0.17 μM for PCNA-I3, respectively.

2. Cell cycle distribution of PC-3 cells treated with PCNA-Is

The cell cycle distribution of control and treated cells were shown Fig. 2. PC-3 cells starved for 24 hours and treated in fresh medium with PCNA-Is at concentrations, approximately 2-3 times of their IC50 values or at the highest concentration of 10 μM, shown in the supplemental Fig. 2. The control and treated cells were sampled 24, 48, or 72 hours after the treatment for flow cytometry analysis. Data shown were one representative of two experiments.

3. Figure legends

Supplemental Figure 1. SPR analysis of the binding kinetics of PCNA-I1 and PCNA-I3 to PCNA.

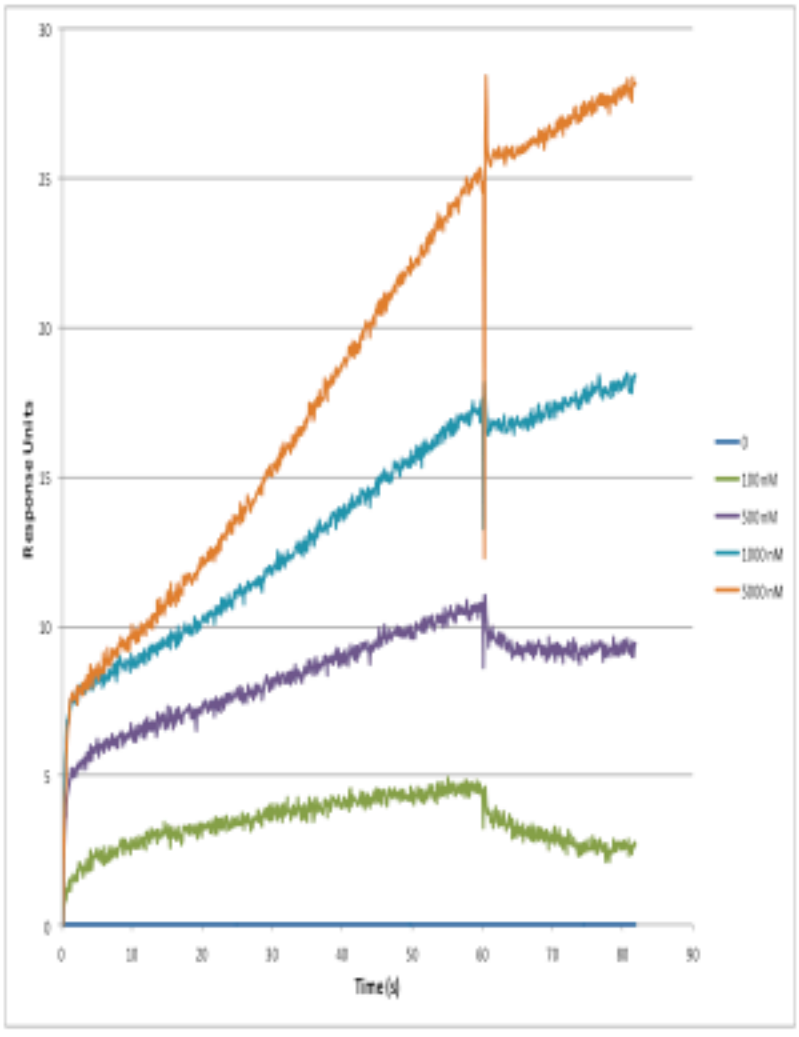
The binding responses for PCNA-I1 (Fig. 1A) and PCNA-I3 (Fig. 1B) in the SPR analysis were shown in Supplemental Figure 1.

Supplemental Figure 2. Effects of PCNA-Is on cell cycle progression

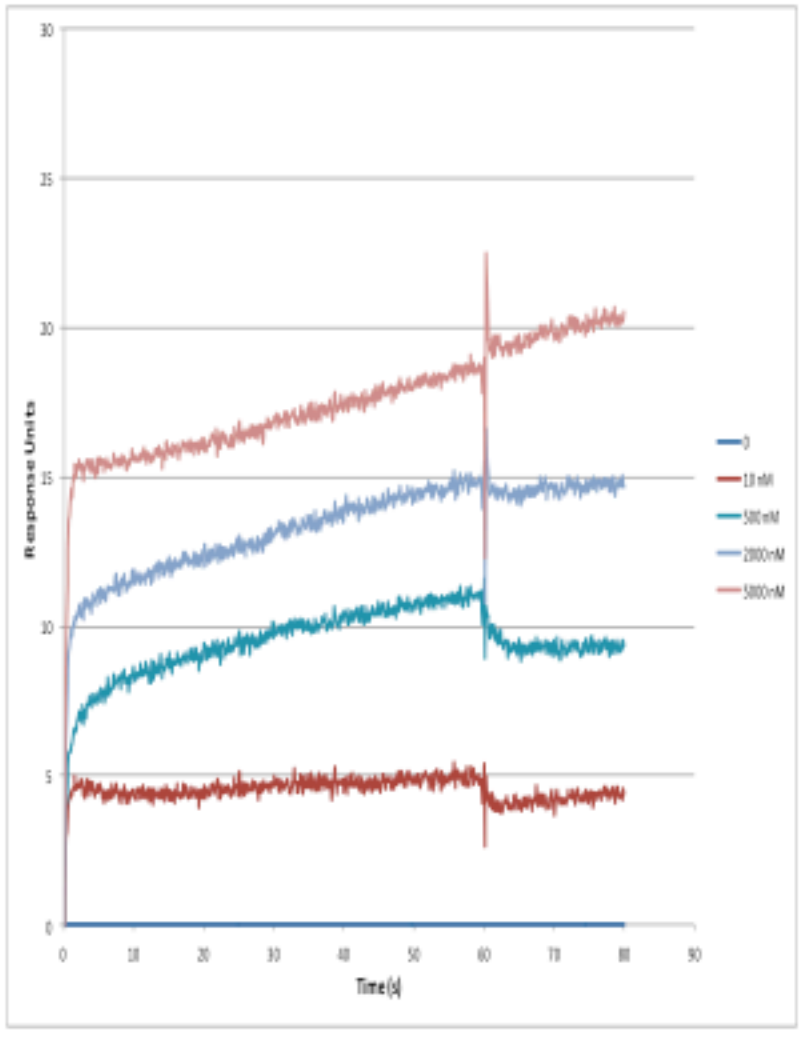
PC-3 cells were plated onto 60-mm plates at 2 x 10^5/plate. After an overnight incubation, the cells were starved for 24 hours in serum-free medium (SFM). The starved cells were then cultured in fresh SFM or stimulated in the medium supplemented with 5% FBS and sampled 24, 48, or 72 hours later for flow cytometry analysis. Cells treated with PCNA-I2, 3, and 4 were shown in Supplemental Figure 2-1 and those treated with PCNA-I4, 6, 7, 8, 9, and 10 were shown in Supplemental Figure 2-2.

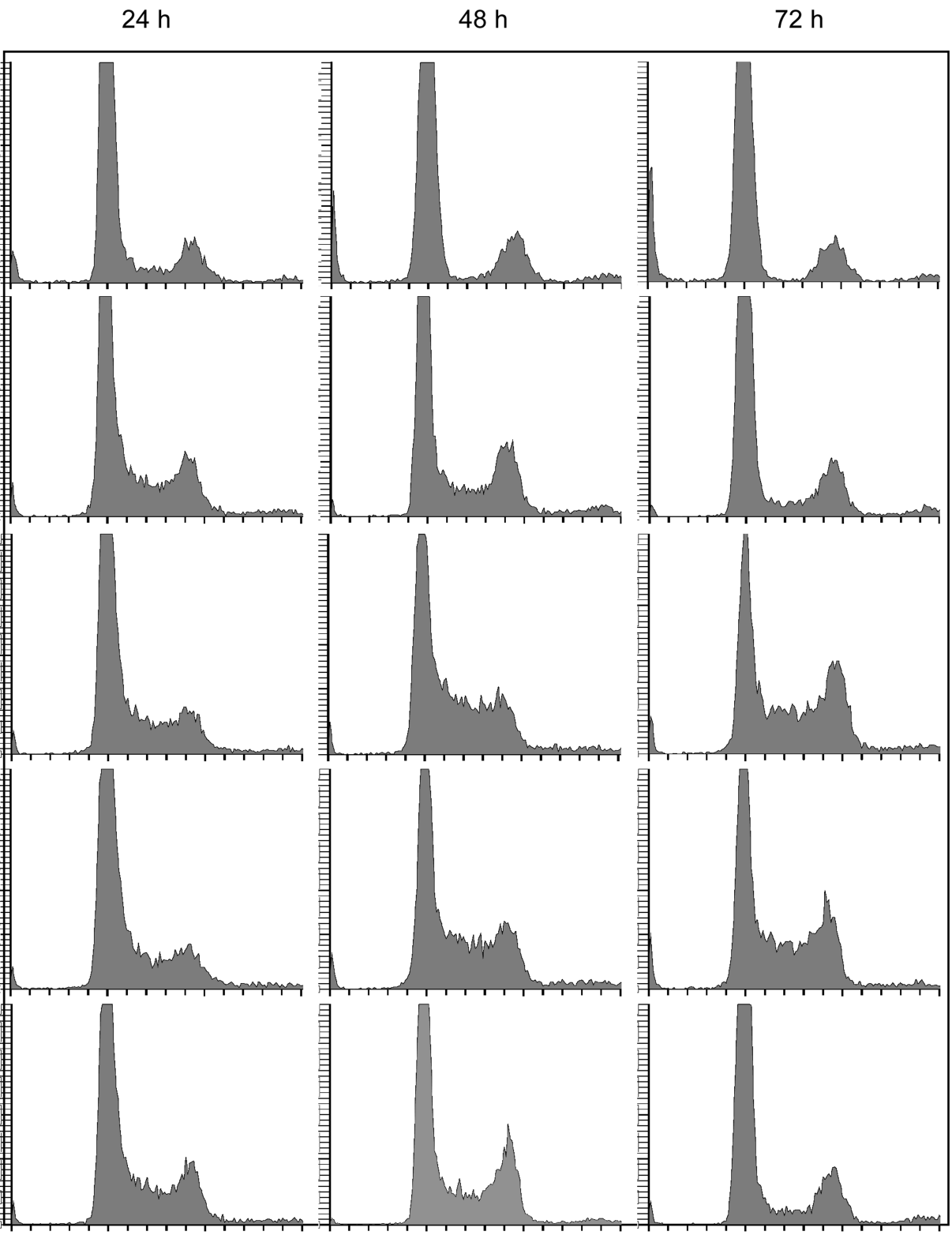
Supplemental Fig. 1

A. PCNA-I1

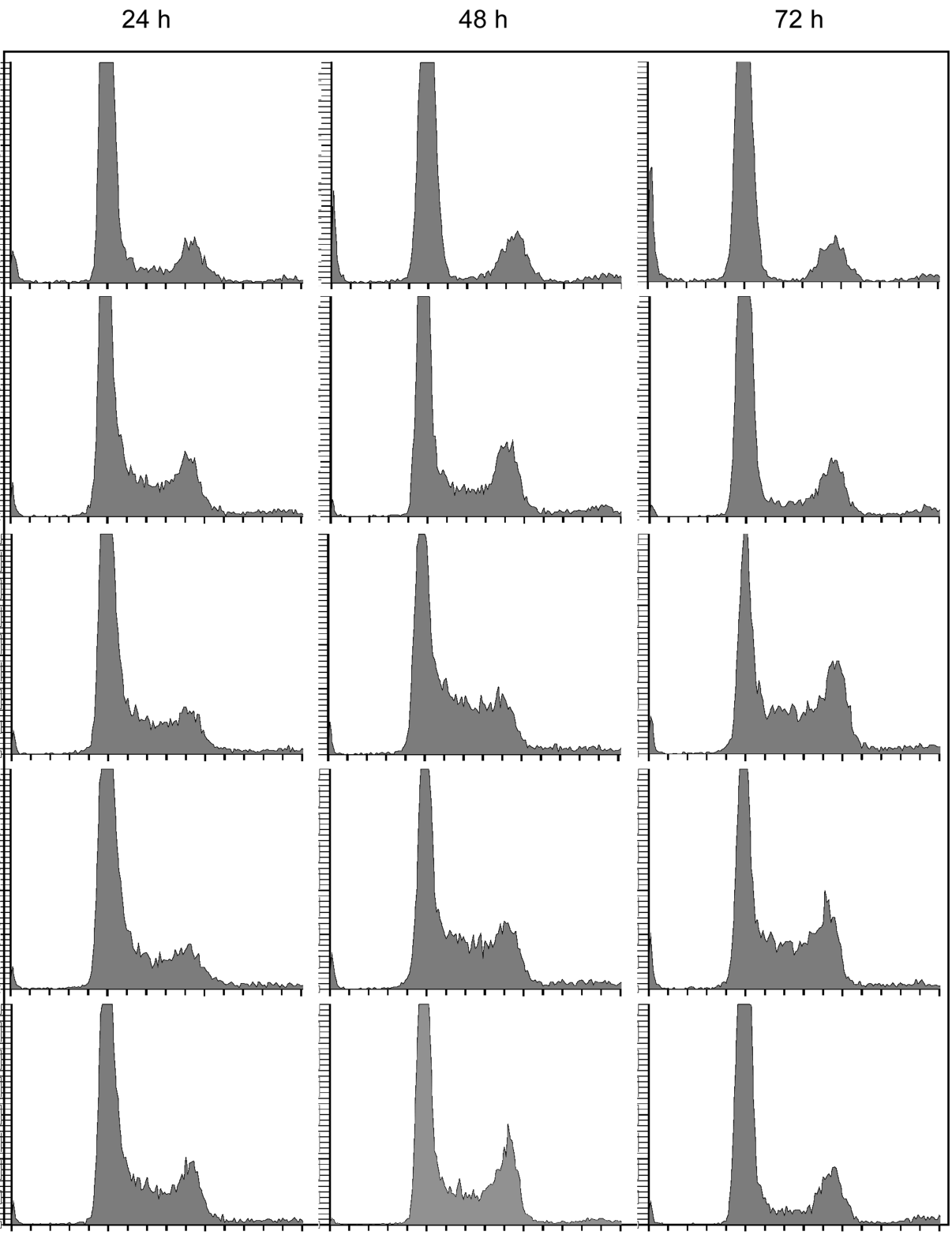


B. PCNA-I3

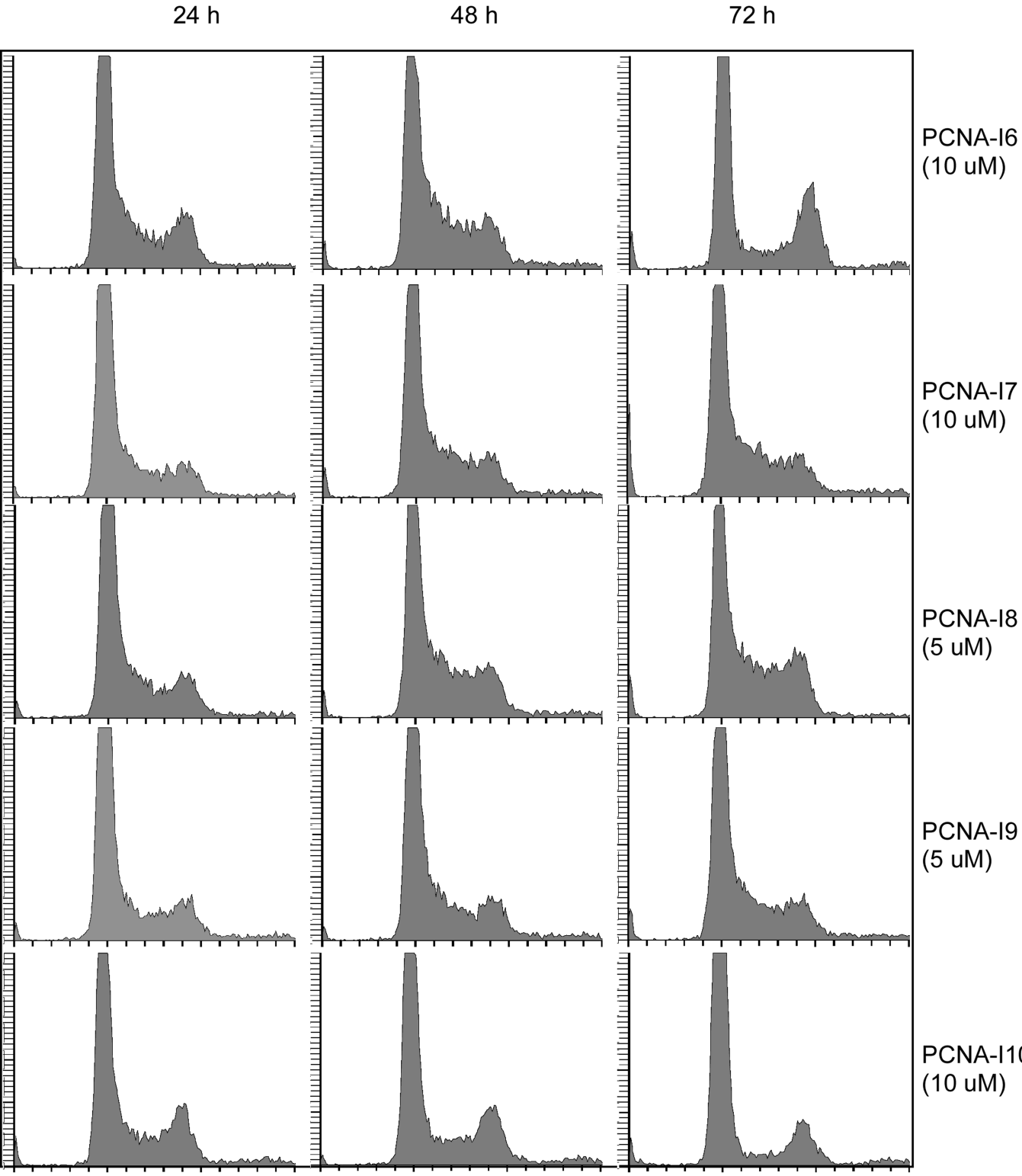




Supplemental Figure 2-1



Supplemental Figure 2-1



Supplemental Figure 2-2