

SUPPLEMENTAL FIGURE LEGEND

Supplemental Figure 1. Transition of signal molecules protein expression and phosphorylation in CAR activated cells during adriamycin treatment. Huh7 cells were infected with Adenovirus- β -gal or Adenovirus-mCAR at 10 MOI. NI means No-Infection. After following treatment with TCPOBOP, treated cells were harvested after indicated time course of adriamycin treatment. p38/p-p38, JNK/p-JNK, Cyclin B1, Cdc2/p-Cdc2 (Y15) and β -actin protein levels were determined by Western blotting.

SUPPLEMENTAL MATERIALS AND METHODS

Western blot

Treated cells were washed three times with ice-cold phosphate-buffered saline (PBS) and collected by cell scraper. After centrifugation, cells were suspended into RIPA lysis buffer (150 mM NaCl, 1% NP-40, 0.5% DOC (deoxycholate-Na), 0.1% SDS and 50 mM Tris-HCL) containing complete protease inhibitor cocktail (Roche Diagnostics, IN, USA). Whole cell lysates were separated on a 10% SDS-polyacrylamide gel and were transferred onto an Immobilon-P membrane (Millipore, MA, USA). The membrane was then incubated overnight at 4°C in Tris-buffered saline, 0.05% (v/v) Tween20 (TBS-T) with primary antibodies including anti-JNK (#9252), anti-phospho-JNK (#9251), anti-p38 MAP kinase (#9212), anti-phospho-p38

MAP kinase (#9211), anti-Cyclin B1 (#4138), anti-Cdc2 (#9112), anti-phospho-Cdc2 (Tyr15) (#9111), anti-phospho-Cdc2 (Thr14) (#2543), anti-phospho-Cdc2 (Thr161) (#9114) from Cell Signaling technology (MA, USA) or anti- β -actin (sc-47778) from Santa Cruz Biotechnology (CA, USA). After incubation with HRP-conjugated anti-rabbit IgG (Cell Signaling technology), protein bands on the membrane were visualized using ECL Plus Western Blotting Detection Reagent (GE Healthcare Bio-Science Corp. WI, USA).