Supplementary Data

Regulation of the Human Plasminogen Activator Inhibitor Type 2 (PAI-2) Gene: Cooperation of an Upstream Silencer and Transactivator

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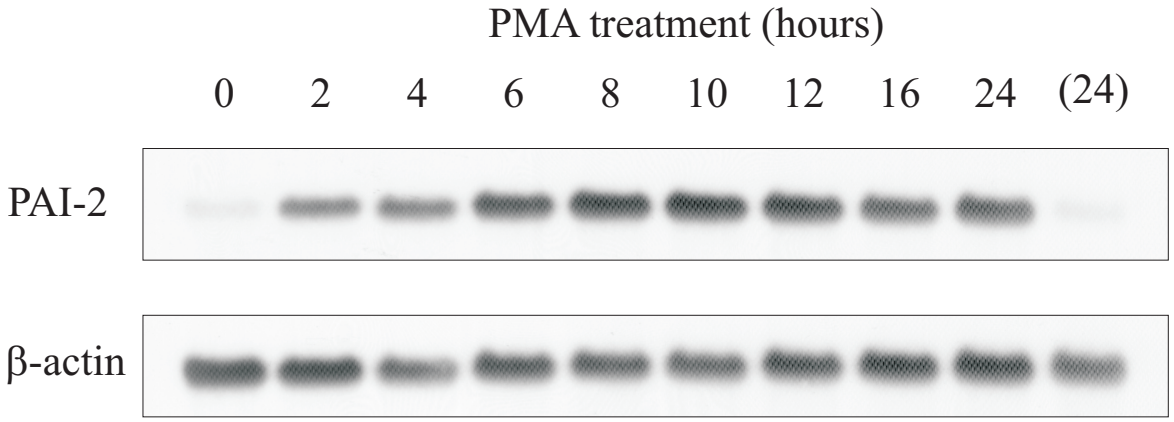
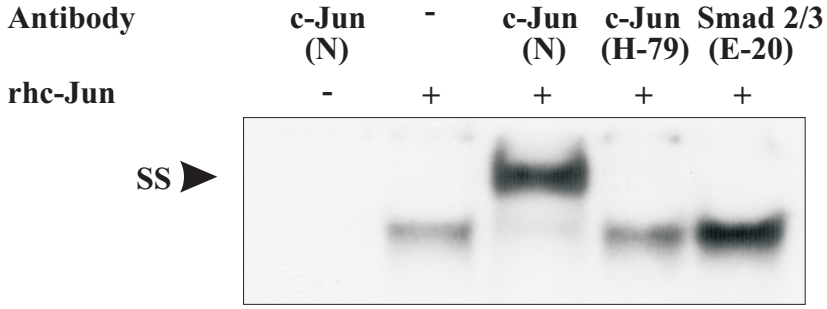


FIGURE S1. **PAI-2 mRNA expression is up-regulated in U937 cells in response to PMA.** Total RNA was isolated from $1x10^7$ U937 cells treated for the indicated times with 40 ng/ml PMA. Twenty micrograms of total RNA per lane was analyzed by Northern blot using ^{32}P-labeled human PAI-2 cDNA and ^{32}P-labeled human β-actin cDNA.

A.



B.

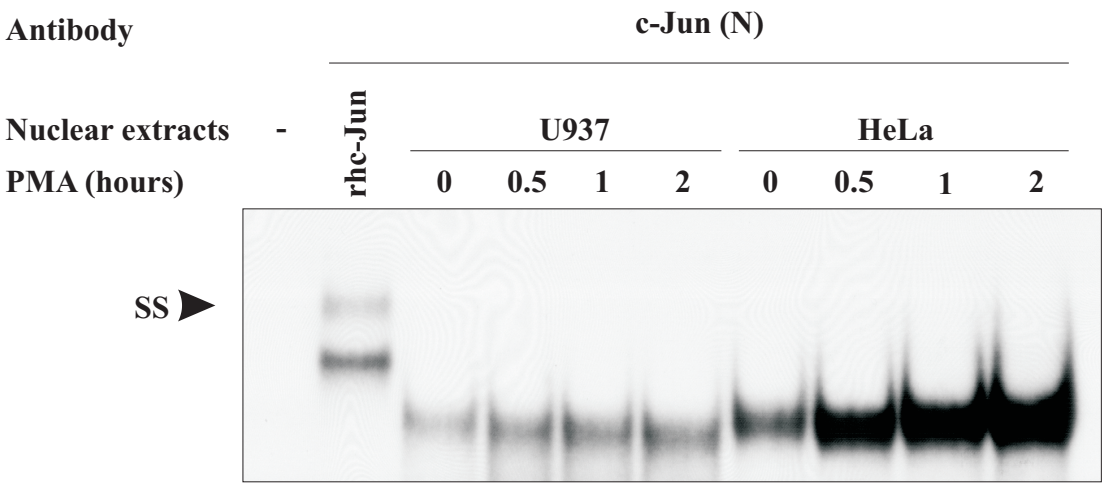


FIGURE S2. **c-Jun does not bind to the transactivator AP-1 site in nuclear extracts prepared from untreated or PMA-treated U937 or HeLa cells.** *(A)* Supershift assays were performed with recombinant human c-Jun (rhc-Jun) (Promega) and a ^{32}P-labeled double stranded oligonucleotide probe representing the human PAI-2 promoter transactivator region -4952/-4932. Labeled probe was incubated with 0.64 μg of rhc-Jun and 0.1 μg of poly(dI-dC) for 20 minutes at room temperature. rhc-Jun was pre-incubated with 4 μg of antibody for 2 hours on ice. The antibodies used for supershift assays with rhc-Jun were anti-c-Jun (N), anti-c-Jun (H-79) or anti-Smad 2/3 (E-20). *(B)* Supershift assays with nuclear extracts prepared from U937 cells or HeLa cells cultured in the presence of 40 ng/ml PMA for the indicated times were performed similarly, except 5 μg of nuclear extracts was incubated with 2 μg of poly (dI-dC). Supershifted (SS) complexes are indicated with arrows.