Figure 1S

A

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SENP1
SENP1m
SUMO-1
R5020
PR-B-SUMO-1
PR-B
β-actin

B

T47D Y-B cells/
PRE₂-Luc

RLU

R5020
SENP1

- | + | - | + | - | + | - | + | - | + | - | + |

0 | 1000 | 2000 | 3000 | 4000 |

C

PR-B/ PRE₂-Luc

RLU

R50
RU
ZK98

SENP1
SENP1m
Figure 2S

The image shows a bar graph with the Y-axis labeled as RLU (Relative Light Units) ranging from 0 to 600. The X-axis represents different conditions: R5020, SENP1, PR-B, PR-B K388R, and PR-B DX. The conditions are indicated with '−' for negative and '+' for positive. The graph displays the relative light units for each condition, with PR-B DX showing the highest RLU value.
**Figure 3S**

A. Bar graph showing NT-B/ PRE$_2$-Luc RLU at different MEKK1 concentrations (5, 20, 50, 100, 200 ng).

B. Western blot analysis of SUMO-1, PR-SUMO-1, PR-B, and β-actin expression under different conditions with R5020 and MEKK1.

- **Bar Graph:**
  - x-axis: MEKK1 concentration (5, 20, 50, 100, 200 ng)
  - y-axis: RLU

- **Western Blot:**
  - Lanes: 1-8
  - Stained proteins: SUMO-1, PR-SUMO-1, PR-B, β-actin

**Legend:**
- + indicates presence
- - indicates absence
- SUMO-1
- R5020
- MEKK1
- PR-SUMO-1
- PR-B
- β-actin
Supplemental Figure Legends

Supplemental Figure 1S. A) DeSUMOylation of PR by SENP1 depends on its catalytic activity. HeLa cells were transiently transfected with expression vectors encoding wild type PR-B together with a GFP-SUMO-1 expression vector (+), and wild type or mutant (m) SENP1. Cells were treated 24 hrs without (-) or with (+) 10 nM R5020. Western blot analysis was performed on cell extracts probed with the anti-PR1294 monoclonal antibody or anti β-actin control. B) SENP1 enhances PR-B activity in T47D breast cancer cells. PR-negative T47D breast cancer cells stably expressing PR-B were transfected with the PRE2-Luc reporter plasmid in the presence of pSV40-Renilla as internal control along with increasing amount (20-1000 ng) of SENP1 expression vector, or an empty vector control (-). Cells were treated without (-) or with (+) 10 nM R5020 for 24 hrs before being assayed for luciferase activity. C) SENP1 enhances transcription by the partial agonist RU486. HeLa cells were transfected with 2 μg of PRE2-luciferase reporters together with 50 ng of a PR-B expression vector and Renilla-Luc as an internal control in the presence or absence of 100ng SENP1 or SENP1m expression vectors. The cells were treated for 24 hrs with the agonist R5020 (10 nM), partial agonist RU486 (100 nM), or the pure antagonist ZK98299 (100 nM) then harvested and lysed. The extracts were assayed for luciferase activities as in Figure 1.

Supplemental Figure 2S. The PR DBD dimerization interface is necessary for effective synergy control. HeLa cells were transfected with 2 μg of PRE2-luciferase reporters together with 50 ng of a wild type PR-B, the PR-B K388R SUMOylation deficient, or a PR-B DBD dimerization mutant (PR-B DX) expression vector and Renilla-Luc as an internal control in the presence or absence of 100 ng SENP1 expression vectors. The cells were treated for 24 hrs with the agonist R5020 (10 nM), then harvested and lysed. The extracts were assayed for luciferase activities as in Figure 1.

Supplemental Figure 3S. A) The stimulatory effect of MEKK1 on PR-B transcriptional activity is LBD and hormone independent. HeLa cells were transfected with 2 μg of PRE2-luciferase reporters together with 500 ng of NTB-DBD, a constitutively active PR N-terminal expression vector in the presence of pSV40-Renilla as internal control along with increasing amount (5-200 ng) of constitutively active MEKK1 expression vector, or an empty vector control (-). The extracts were assayed for luciferase activities as in figure 1. B) Concentration dependent effect of MEKK1 on PR SUMOylation. HeLa cells were transiently transfected with expression vectors encoding wild type PR-B together with a GFP-SUMO-1 expression vector (+) in the absence (-) or presence of increasing amount of MEKK1 expression vector. Cells were treated 24 hrs without (-) or with (+) 10 nM R5020. Western blot analysis was performed on cell extracts probed with the anti-PR1294 monoclonal antibody or anti β-actin control.