

Supplementary Figure Legends:

Figure 1. Sumoylation of PRB and effects of SUMO-1 on PRB-mediated gene transcription activation.

(A) 293T cells were transiently transfected with pMMTV luciferase reporter construct, Myc-PRB vectors and increasing amounts of HA-SUMO-1 vector (0.05-0.5 μ g). Cells were either untreated (P-) or treated with progesterone (10 nM, P+) for 24 h, and cell extracts were measured for luciferase activity. (B) 293T cells were transfected with pMMTV luciferase reporter construct with Myc-PRB or Myc-PRB K388R vectors as indicated. The cells were then treated with progesterone (10 nM, 24 h) before harvesting; Mean \pm S.D. (n = 3)

Figure 2. Ligand-induced dimerization of PRB and its mutants.

293T cells were co-transfected with expression vectors for Myc-PRB, HA-PRB, Myc-PRB K7/531R and HA-PRB K7/531R as indicated. Lysates from transfected cells were immunoprecipitated using an anti-Myc antibody and immunoprecipitates were probed with anti-HA and anti-Myc antibodies.

Figure 3. PIAS3 and PIASy induced PRB sumoylation.

293T cells were transfected with HA-SUMO-1, Myc-PRB, Flag-PIAS3 or PIASy as indicated. Cells were treated, or not, with progesterone (100 nM, 2 h) prior to harvesting. Cell extracts were lysed with RIPA buffer and immunoblotted with an anti-Myc antibody.

Figure 4. PIAS3 interacted with PRB and PRB mutants *in vivo*.

293T cells were co-transfected with expression vectors for Myc-tagged PRB, its mutants (K388R, K7/531R or K7/388/531R) and HA-tagged PIAS3 as indicated. Lysates from transfected cells were immunoprecipitated using an anti-HA antibody and immunoprecipitates were probed with an anti-Myc antibody.

Figure 5. PIAS3 C334S destabilized PRB retention in the nucleus.

HeLa cells were cultured in medium containing charcoal-stripped serum then co-transfected with Myc-PRB and GFP-PIAS3 C334S, treated with progesterone (100 nM) for 0 or 2 h, and subjected to immunofluorescence staining with Myc-specific monoclonal antibody. *Scale bar*, 20 μ m. At least 300 transfected cells were scored for PRB subcellular distribution.