



**Figure S1. SUMOylation Deficient PML Binds SUMO1**. *Pml<sup>+/+</sup>*, *Pml<sup>+/+</sup>* immortalized MEFs or 293T cells were transfected as indicated. Cell lysates were immunoprecipitated using anti-FLAG, or anti-GFP antibodies. The immunoprecipitates and 10% of the inputs used for immunoprecipitation were analyzed by Western blotting with anti-GFP or anti-FLAG antibodies. Asterisk (\*) indicates a cross-reacting band. HC: immunoglobulin heavy chains. Molecular weight markers (kDa) are indicated.





*Pml*<sup>/-</sup> MEFs

Figure S2. SUMOylation Deficient PML Has Decreased SUMO Binding Capacity When Its SUMO Binding Motif Is Mutated or Deleted. (A) 293T cells were transfected as indicated. The immunoprecipitation and Western blot analysis were performed using anti-GFP and anti-FLAG antibodies, respectively (top panel). Ten percent of the inputs used for immunoprecipitation was also analyzed by Western blot (bottom panel). (B)  $Pmt^{-/-}$  immortalized MEFs were transfected as indicated. Cell lysates were immunoprecipitated with anti-FLAG antibodies. The immunoprecipitates and 4% of inputs used for immunoprecipitation were analyzed by Western blotting with antibodies against GFP (left panel) or FLAG (right panel). Asterisk (\*) indicates a cross-reacting band. Molecular weight markers (kDa) are indicated. HC: immunoglobulin heavy chains. LC: immunoglobulin light chains.



**Figure S3. A PML RING Domain Mutant Is Partially Impaired in Its Ability to Bind High Molecular Weight SUMOylated Proteins in vivo.** *Pml<sup>-/-</sup>* immortalized MEFs were transfected as indicated. Cell lysates were immunoprecipitated with anti-FLAG antibodies. The immunoprecipitates and 10% of inputs were analyzed by Western blot with antibodies against GFP (upper panel) or FLAG (lower panel). Molecular weight markers (kDa) are indicated. HC: immunoglobulin heavy chains.



Figure S4. PML RING Domain and SUMO Binding Motif Are Essential for PML-NB Formation as Revealed by Staining of Endogenous SUMO1.  $Pml^{-1}$  immortalized MEFs were transfected with the indicated plasmids and analyzed by immunofluorescence. Representative confocal microscopy images are presented. Scale bar, 10 µm.



Figure S5. PML RING Domain and SUMO Binding Motif Are Essential for PML-NB Formation as Revealed by Staining of Endogenous Daxx.  $Pml^{-/-}$  immortalized MEFs were transfected with the indicated plasmids and analyzed by immunofluorescence. Representative confocal microscopy images are presented. Scale bar, 10 µm



**Figure S6. The SUMO Binding Motif and RING Domain Are Required for PML Proapoptotic activity.** *Pml*<sup>-/-</sup> immortalized MEFs were infected with retroviruses expressing the indicated PML or its mutants. After drug selection, cells were subjected to PI staining and FACS analysis to quantify the sub-G1 population of cells.