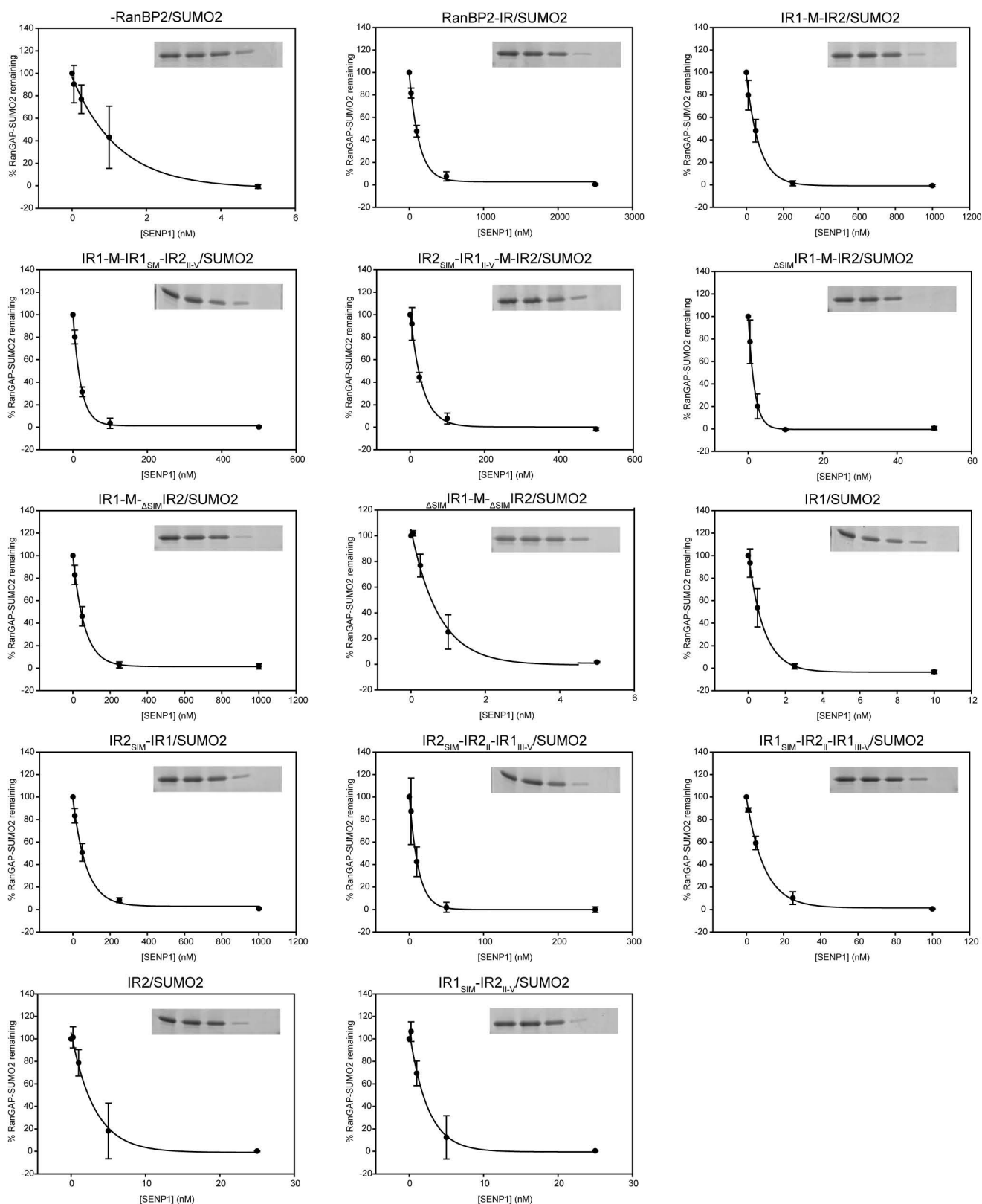
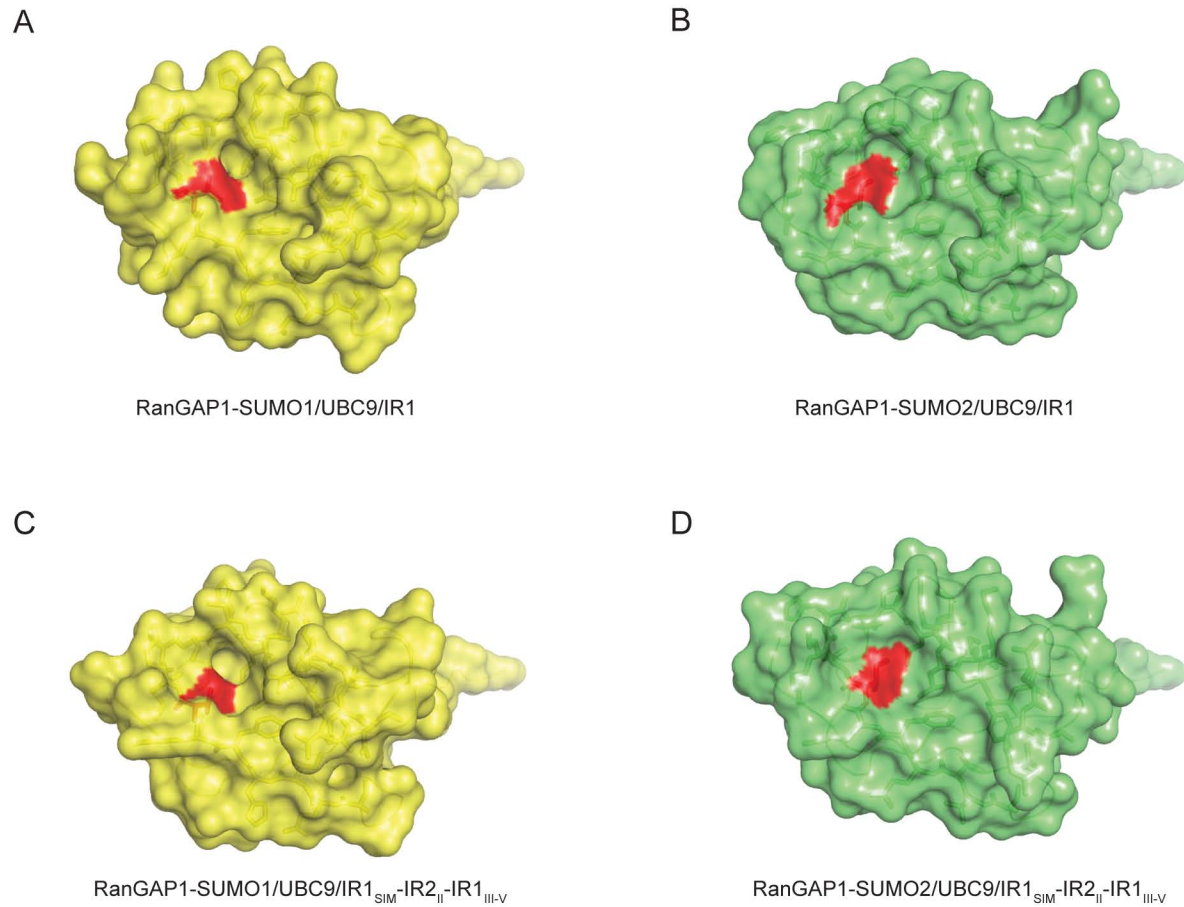


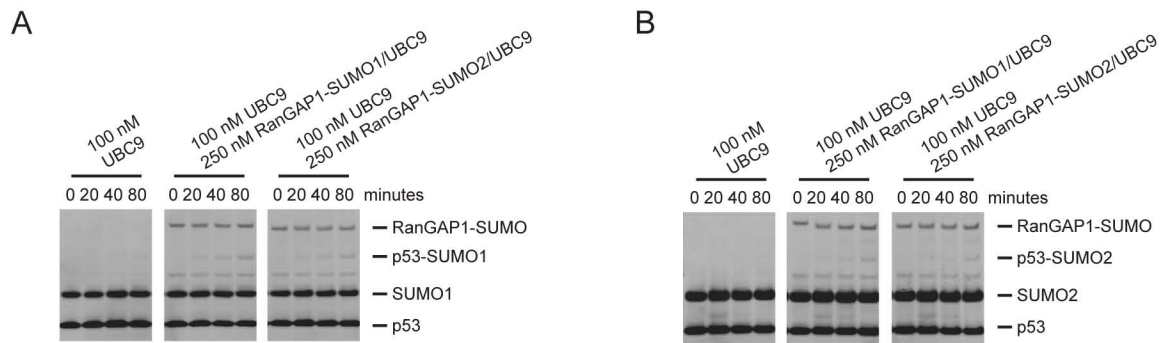
**SUPPLEMENTARY FIGURE 1.** Protease protection assays with RanGAP1-SUMO1. The quantitated data for RanGAP1-SUMO1 cleavage by SENP1 in the presence of UBC9 and the noted RanBP2 constructs were fit to exponential decay curves ( $y = y_0 + ae^{-bx}$ ). Error bars represent one standard deviation and were calculated from three independent experiments. Equations for the fit curves were used to interpolate the SENP1 concentration required to deconjugate 50% of RanGAP1-SUMO1 after 20 minutes. Gel insets show bands corresponding to RanGAP-SUMO1 from one set of data used for quantitation.



**SUPPLEMENTARY FIGURE 2.** Protease protection assays with RanGAP1-SUMO2. The quantitated data for RanGAP1-SUMO2 cleavage by SENP1 in the presence of UBC9 and the noted RanBP2 constructs were fit to exponential decay curves ( $y = y_0 + ae^{-bx}$ ). Error bars represent one standard deviation and were calculated from three independent experiments. Equations for the fit curves were used to interpolate the SENP1 concentration required to deconjugate 50% of RanGAP1-SUMO2 after 20 minutes. Gel insets show bands corresponding to RanGAP-SUMO2 from one set of data used for quantitation.



**SUPPLEMENTARY FIGURE 3.** A deeper hydrophobic pocket is apparent on the SUMO1 surface compared to SUMO2. Surface representation of SUMO from *A*) SUMO1-RanGAP1-UBC9-IR1, *B*) SUMO2-RanGAP1-UBC9-IR1, *C*) SUMO1-RanGAP1-UBC9-IR1<sub>SIM</sub>-IR2<sub>II</sub>-IR1<sub>III-V</sub> and *D*) SUMO2-RanGAP1-UBC9-IR1<sub>SIM</sub>-IR2<sub>II</sub>-IR1<sub>III-V</sub>. SUMO1 is shown in yellow and SUMO2 is shown in green. The positions for Val38 in SUMO1 and Ile34 in SUMO2 are colored red. The deeper hydrophobic pocket on the SUMO1 surface is located above and to the right of Val38.



**SUPPLEMENTARY FIGURE 4.** Conjugation assays in the absence of RanBP2. *A*, Time course of SUMO1 conjugation to p53. Reactions were performed in the presence of the specified concentrations of UBC9 and RanGAP-SUMO/UBC9 and p53 conjugation was detected by SYPRO staining. *B*, same as *A*, except that conjugation assays were performed with SUMO2.