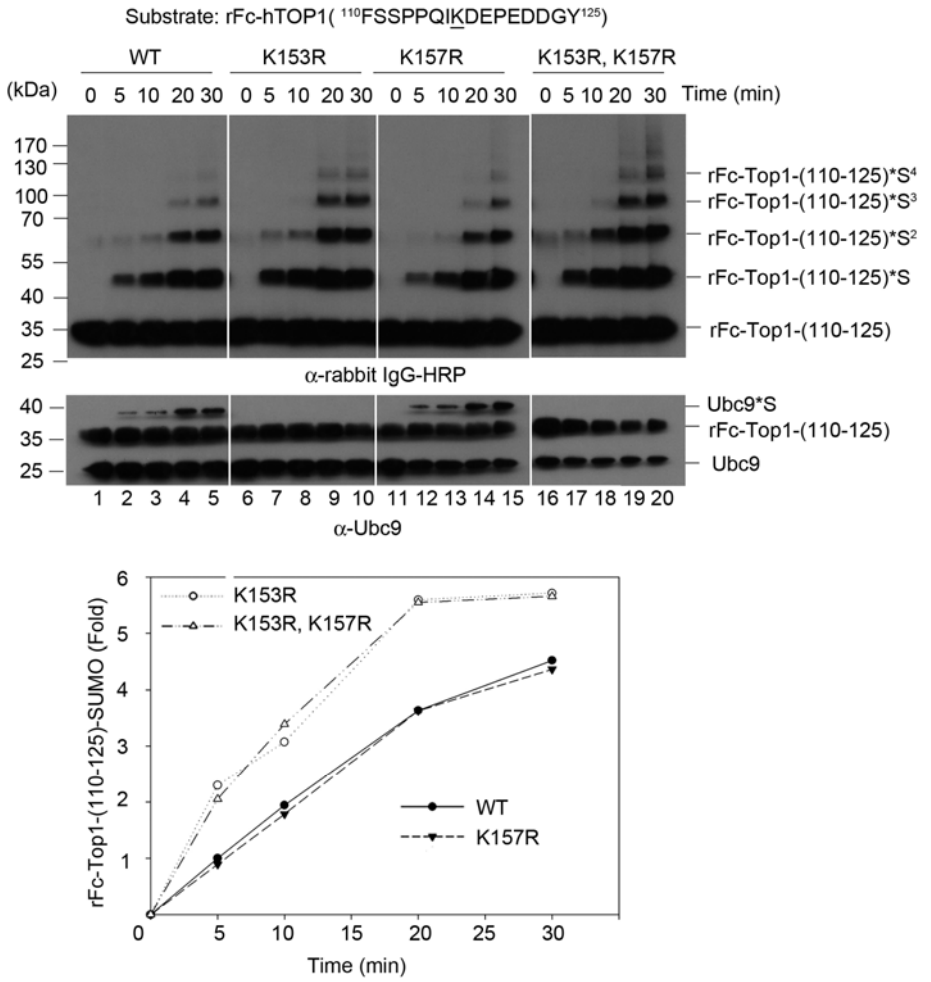

Figure S4. Autosumoylation of Ubc9 negatively regulates sumoylation of substrates. Substrate sumoylation by Ubc9 variants. *In vitro* sumoylation assays were performed using 0.5 μg rFc-Top1 (110-125) as the SUMO substrate in the presence of 400 ng Aos1-Uba2, 0.1 μg wild type (WT) or indicated Ubc9 variants, 0.5 μg Smt3 and 5 mM ATP at 37°C for the indicated times. Reaction mixtures were then subjected to SDS-PAGE, followed by immunoblotting with anti-rabbit immunoglobulin antibody (α-rabbit IgG HRP; top panel) to reveal SUMO-conjugation of the Fc-containing substrate and anti-Ubc9 antibody (α-Ubc9; middle panel) to detect Ubc9 autosumoylation. Bands corresponding to SUMO-conjugated substrate and Ubc9 are indicated. Bottom plot: fold change of sumoylation level as a function of time. Levels of substrate sumoylation were obtained by calculating the sum of the intensities of all bands corresponding to SUMO conjugates in each reaction. The fold change is derived from normalization relative to the intensity level in the reaction using wild type (WT) Ubc9 at 5 min. rFc-Top1 (110-125)*S, rFc-Top1 (110-125)*S^2 and rFc-Top1 (110-125)*S^n correspond to rFc-Top1 (110-125) conjugated to monomeric, dimeric and higher oligomeric forms of Smt3.