

Supporting Information

Visualizing and Quantifying Protein PolySUMOylation at the Single-Molecule Level

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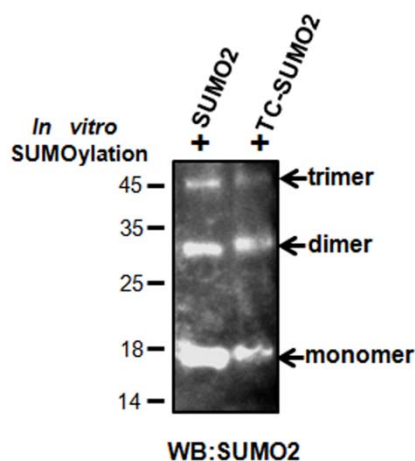


Figure S1. *In vitro* SUMOylation reaction of SUMO-2 and TC-SUMO2. The SUMOylation reaction was performed in the presence of either 50 ng of SUMO-2 or 40 ng of TC-SUMO2 at 37°C for 2 h. The reaction products were harvested for SDS-PAGE and subsequent western blotting analysis with SUMO2-specific antibodies. The positions of the SUMO monomers and multimers are indicated.

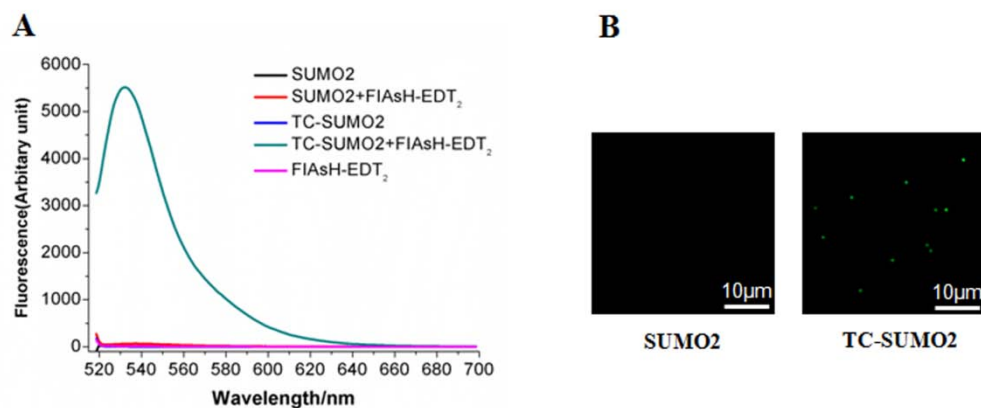


Figure S2. (A) Fluorescence emission spectra of SUMO-2 and TC-SUMO2 protein complex with and without FIAsH-EDT₂ labeling, respectively. The FIAsH-EDT₂ dye was used as the negative control ($\lambda_{ex} = 510$ nm). (B) Representative images of SUMO-2 (left panel) and TC-SUMO2 (right panel) protein complexes with FIAsH-EDT₂ labeling at the single-molecule level.

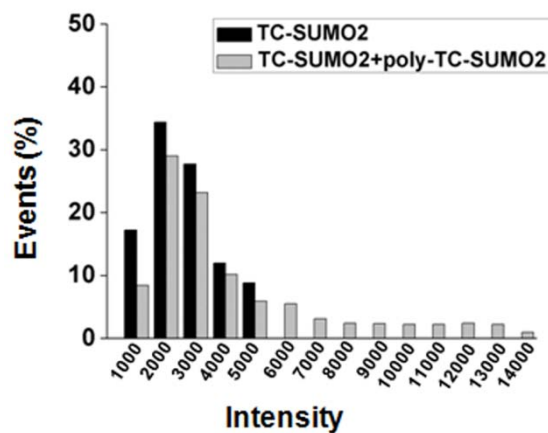


Figure S3. Intensity distribution histogram of TC-SUMO2 proteins without (black) and with (grey) *in vitro* SUMOylation reaction.

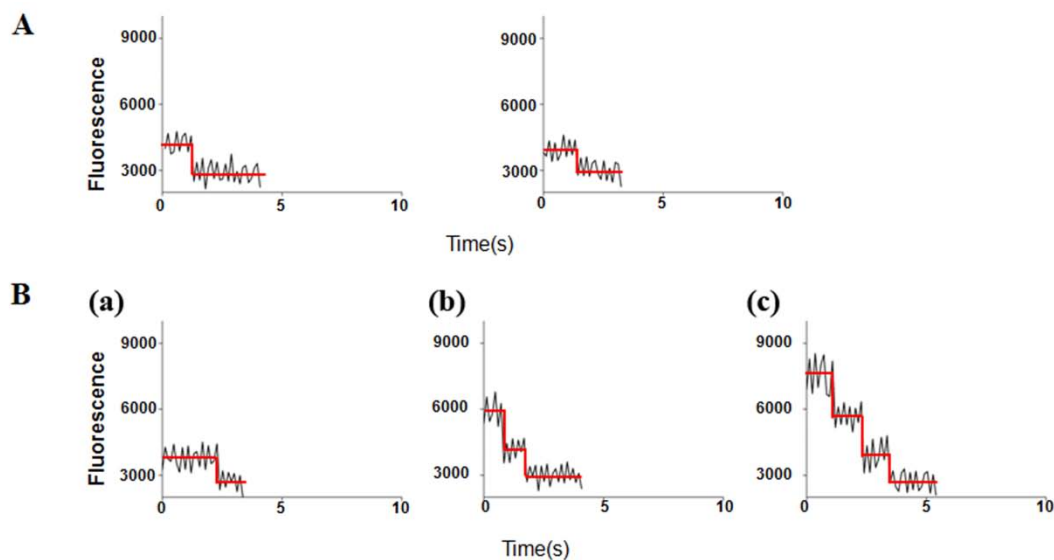


Figure S4. Representative intensity profiles (black) and the results of the step fitting algorithm (red) obtained from TC-SUMO2 without (A) and with (B) *in vitro* SUMOylation reaction. Note: the panel B contain a mixture of monomeric (a), dimeric(b) and trimeric(c) SUMO.

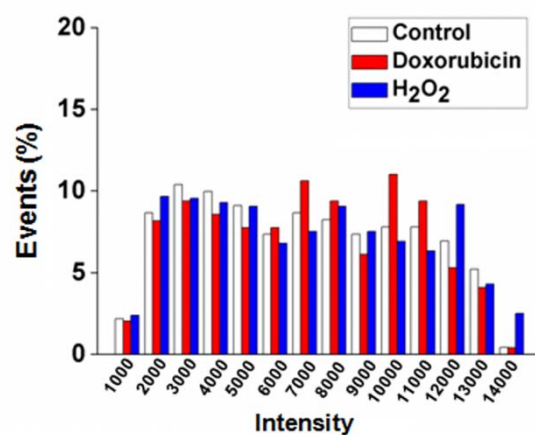


Figure S5. Intensity distribution histogram of SUMOylated Sp100 under the basal condition (white) and the stimulation by doxorubicin (red) and H₂O₂ (blue).