Supporting information

Spectomycin B1 as a novel SUMOylation inhibitor that directly binds to SUMO E2

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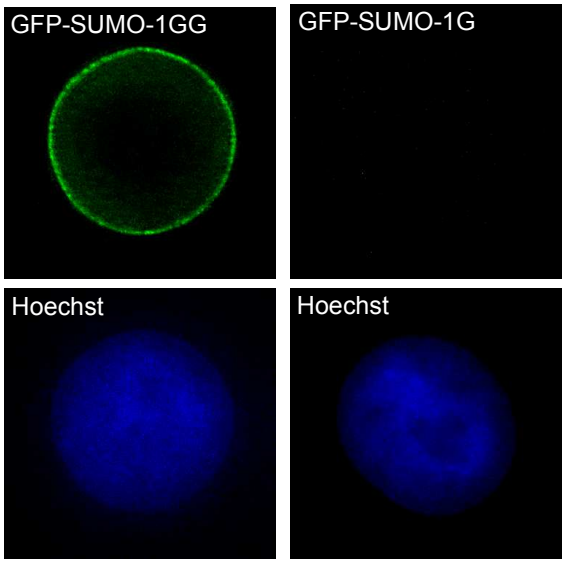
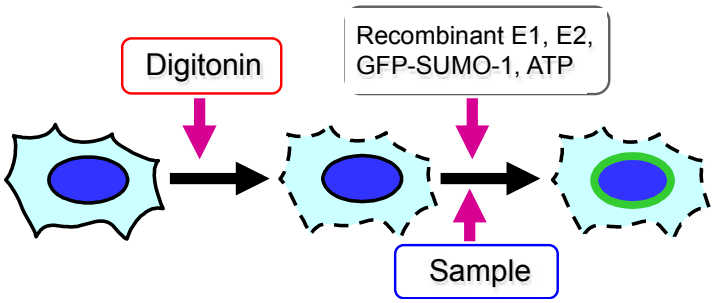
Supplemental Table S1. Oligonucleotides sequences used for RT-PCR

Oligonucleotides	Sequence
pS2 sense	5'-CCCCTGGTGCTTCTATCCTAA-3'
pS2 antisense	5'-GATCCCTGCAGAAGTGTCTAAAA-3'
Ubc9 sense	5'-GGCACGATGAACCTCATGAA-3'
Ubc9 antisense	5'-TCCCACGGAGTCCCTTTCT-3'
Uba2 sense	5'-TCAAGAAGTATCTCCTGACAGAGC-3'
Uba2 antisense	5'-TGCTCTAGCTCTGGCTTCG-3'
β-actin sense	5'-ATGAAGATCAAGATCATTGCTCCTC-3'
β-actin antisense	5'-ACATCTGCTGGAAGGTGGACA-3'

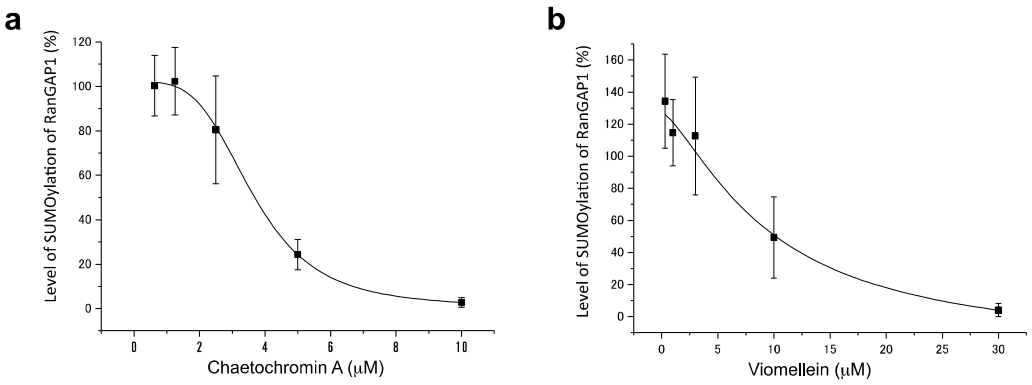
a



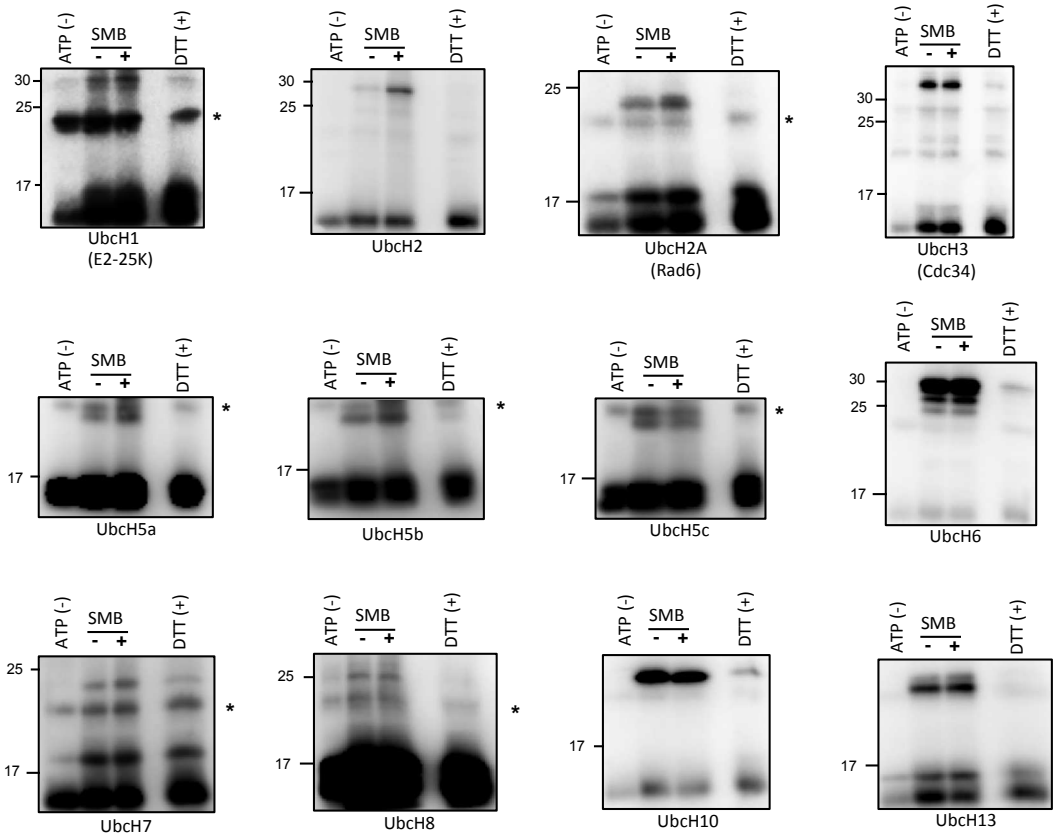
b



Supplemental Figure S1. System of screening for SUMOylation inhibitors. (**a**) *In situ* SUMOylation assay. *In situ* SUMOylation assay was performed as described in the Supplemental Method. GFP-SUMO-1GG but not GFP-SUMO-1G, defective mutant for SUMO conjugation activity, accumulated around the nuclear rim, indicating that the GFP signal at nuclear rim is attributed to SUMO conjugation activity in semi-intact cells. (**b**) Schematic model of the *in situ* cell-based screening system.



Supplemental Figure S2. Dose dependent inhibition of *in vitro* SUMOylation by chaetochromin A (a) and viomellein (b). The error bars show the standard deviations from three independent assays.



Supplemental Figure S3. Effects of spectomycin B1 and DTT on thioester-bond formation between various ubiquitin E2s and biotinylated ubiquitin. The asterisk represents a non-specific band.

Supplemental Method

***In situ* SUMOylation Assay**

HeLa cells were grown on a 96-well plate, briefly rinsed with cold TRB buffer (20 mM HEPES [pH 7.3], 110 mM KOAc, 2 mM $MgCl_2$, 1 mM EGTA, 2 mM DTT) and permeabilized with TRB buffer containing 50 μg/ml digitonin (Calbiochem, Darmstadt, Germany) for 5 min on ice. After the cells were rinsed twice with ice-cold TRB buffer, 100 μl of assay solution containing 0.2 μg of GST-Aos1/Uba2 (E1), 0.2 μg of His-tagged Ubc9 (E2), 2 μg of GFP-SUMO, and ATP was added and incubated for 15 min at 30°C. The cells were washed with pre-warmed TRB buffer for 5 min at 30°C, followed by fixation with 3.7% formaldehyde in PBS for 15 min at room temperature. The cells were rinsed three times with PBS for 5 min, and then treated with 1 mg/ml Hoechst 33342 (Invitrogen) for 5 min. GFP signal around the nuclear rim was observed using a DeltaVision fluorescent microscope (GE Healthcare).