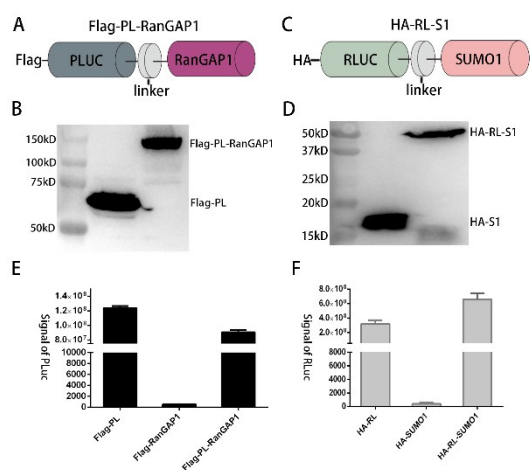


## Fast and sensitive quantification of protein SUMOylation using dual luciferase biosensor

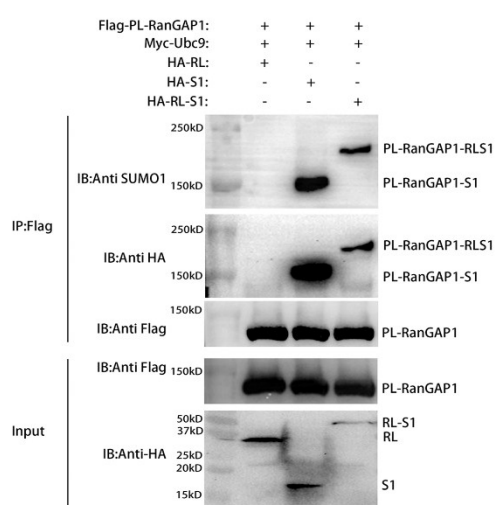
Yuan Zhang, Xianliang Ke, Yan Liu, Zhenhua Zheng, Jin Meng and Hanzhong Wang \*

Key Laboratory of Special Pathogens and Biosafety, Center for Emerging Infectious Diseases, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China

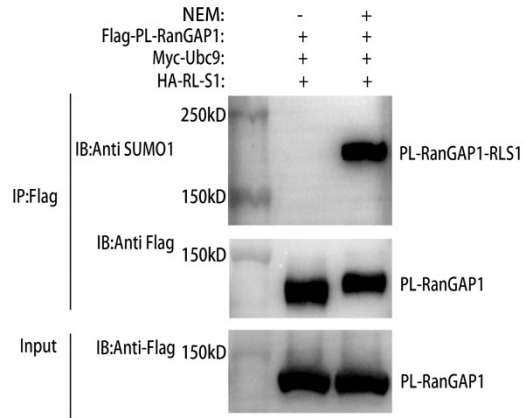
\*E-mail: wanghz@wh.iov.cn. Phone: +86-027-8719-7295



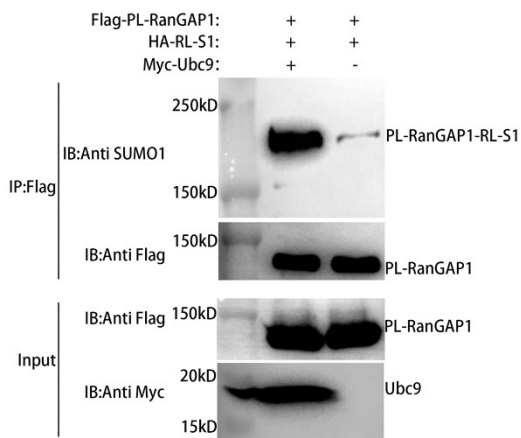
**Fig. S1.** Characterization of luciferase-tagged RanGAP1 and SUMO1. (A) Construct of pFlag-PL-RanGAP1. (B) Eukaryotic expression of Flag-RanGAP1 and Flag-PL-RanGAP1. (C) Construct of pHA-RL-S1. (D) Eukaryotic expression of HA-SUMO1 and HA-RL-S1. (E) Measurement of luminance catalyzed by Flag-PL, Flag-RanGAP1, and Flag-PL-RanGAP1 with the substrate of *Photinus pyralis* luciferase. (F) Measurement of luminance catalyzed by HA-RL, HA-SUMO1, and HA-RL-S1, with the substrate of *Renilla* luciferase. Error bars show the standard deviation of three experiments.



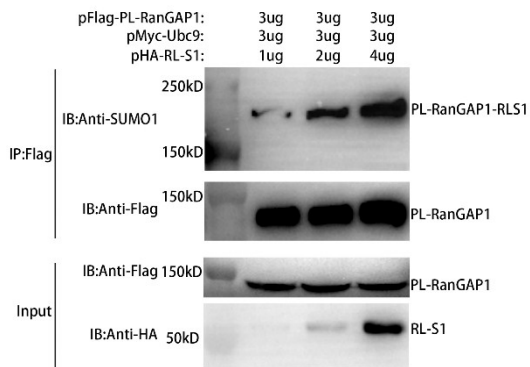
**Fig. S2** Detection of the SUMOylation of Flag-PL-RanGAP1 with HA-RL, HA-S1 and HA-RL-S1, respectively, by Western blot assay.



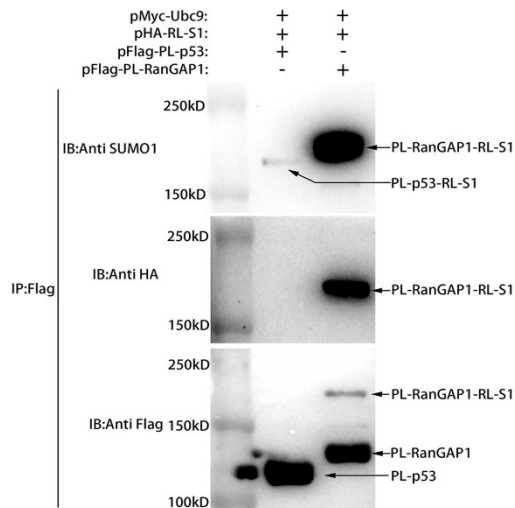
**Fig. S3** Detection of the SUMOylation of Flag-PL-RanGAP1 by HA-RL-S1 treated with or without NEM via Western blot analysis.



**Fig. S4** Detection of the SUMOylation of Flag-PL-RanGAP1 by HA-RL-S1 co-transfected with or without pMyc-Ubc9 via Western blot analysis.



**Fig. S5** Detection of the SUMOylation of Flag-PL-RanGAP1 co-transfected with 1, 2 and 4  $\mu$ g of pHA-RL-S1 by Western blot assay.



**Fig. S6** Detection of the SUMOylation of Flag-PL-p53 and Flag-PL-RanGAP1 by HA-RL-S1 via Western blot analysis.

## Methods

### Antibodies, Plasmids, and Reagents

Mouse anti-Flag and mouse anti-HA antibodies were purchased from Sigma-Aldrich (St Louis, MO). The rabbit anti-SUMO1 antibody was acquired from Cell Signaling Technology (USA). The Dual-Luciferase® Reporter Assay System was purchased from Promega. Plasmid pNF- $\kappa$ B-luc was purchased from Stratagene, and plasmid pRL-TK was obtained from Promega. pCDNA3.1-HA-SUMO1 (pHA-SUMO1, pHA-S1) was provided as a gift by Junying Yuan (Addgene plasmid 21154). pCDEF-Myc-Ubc9 (pMyc-Ubc9) was generously provided by Hong Tang (Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China). N-Ethylmaleimide (NEM) and Flag peptide were purchased from Sigma-Aldrich.

### Plasmid Construction.

The PLuc fragment was amplified from pNF- $\kappa$ B-luc and inserted into the pCAGGS-Flag vector to generate the pFlag-PL plasmid. The RanGAP1 and human p53 gene was amplified from a whole human cDNA library and cloned into the pCAGGS-Flag to generate the pFlag-RanGAP1 and pFlag-p53 construct. The pFlag-PL-RanGAP1 and pFlag-PL-p53, which could express Flag-tagged PLuc-RanGAP1 and PLuc-p53 fusion proteins, was generated by overlap PCR and a traditional cloning step. pHA-RL was generated by inserting the RLuc gene into a pCAGGS-HA vector. pHA-RL-S1, which expresses HA-tagged RLuc-SUMO1 fusion proteins, was generated by fusing the RLuc gene and SUMO1 fragment by overlap extension PCR and then inserted into the pCAGGS-HA vector.

### Cell Culture, Transfection, and IP

Human embryonic kidney cells (HEK 293T) were cultured in Dulbecco's modified Eagle's medium (DMEM, Life Technologies) supplemented with 10% fetal bovine serum (FBS, Gibco) at 37 °C with 5% CO<sub>2</sub>. For SUMOylation detection by Western blot analysis,  $3 \times 10^6$  cells were seeded in a 60mm dish to allow growth for 18h. These cells were transfected with 3  $\mu$ g of the target protein expression plasmid, 3  $\mu$ g of pMyc-Ubc9 and 3  $\mu$ g of pHA-S1 with calcium phosphate reagents (Promega). About 30 h after transfection, the cells were lysed with Western and IP lysis buffer (20mM Tris, PH=7.5/150mM NaCl/1% Triton X-100, Beyotime Institute of Biotechnology, China) containing a complete protease inhibitor cocktail (Roche) and 20 mM NEM. The lysates were centrifuged at 13,000 rpm for 15 min. Supernatants were incubated with 4  $\mu$ g of

mouse anti-Flag antibody and 50  $\mu$ L of Protein G Agarose (MILLIPORE). The agarose was washed and boiled in protein loading buffer. For SUMOylation detection based on luciferase reporter system,  $2 \times 10^5$  cells were seeded in a 24-well plate to allow growth for 18h. These cells were transfected with 0.3  $\mu$ g of the target protein expression plasmid, 0.3  $\mu$ g of pMyc-Ubc9, and 0.3  $\mu$ g of pHA-RL or pHA-RL-S1. About 30 h after transfection, cells were lysed with Passive Lysis Buffer (Promega) containing the protease inhibitor cocktail and 20 mM NEM. The lysates were centrifuged at 13,000 rpm for 15 min. The supernatants were incubated with 0.5  $\mu$ g of mouse anti-Flag antibody and 5.5  $\mu$ L of Protein G Agarose. The agarose was fully washed, eluted with the Flag peptide, and centrifuged at  $14,000 \times g$  for 30 s. Elution by boiling was not used in this step to keep activity of luciferases.

#### **Western Blot, Imaging Analysis, and Luciferase Assay.**

To detect SUMOylation by Western blot analysis, samples were subjected to SDS-PAGE and transferred to PVDF membranes (Millipore) at 4  $^{\circ}$ C for 3 h with a 250 mA current. The membranes were blocked with TBST (50 mM Tris-HCl, 500 mM NaCl, pH 7.4, and 0.1% Tween20) containing 5% bovine serum albumin (BSA) and incubated with the respective primary antibody at 4  $^{\circ}$ C overnight. The membranes were washed with TBST and incubated with the horseradish peroxidase (HRP)-conjugated secondary antibody (Pierce) for 2 h at room temperature. After washing with TBST, the membranes were incubated with the Immobilon Western chemiluminescent HRP substrate (Millipore) and subjected to the Bio-rad Imaging System for imaging analysis. For SUMOylation detection based on luciferase assay, the luciferase activity of the immunoprecipitated proteins were measured using the Dual-Luciferase<sup>®</sup> Reporter Assay System in accordance with the manufacturer's instruction on GloMax<sup>®</sup>20/20 Luminometer (Promega).