

## **Supplementary Information**

### **Analysis of SUMOylated proteins using SUMO-traps.**

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## SUPPLEMENTARY INFORMATION

Supplementary Fig 1. Effect of cysteine protease inhibitors on the protection of SUMOylated proteins. Cell extracts were prepared with heat shock stimulated HeLa cells. Lysates were treated with the indicated cysteine protease inhibitors and samples collected at the indicated times. IAA, NEM and PR-619 were used at standard dosis [19]. Western blot analysis of cell lysates using anti-SUMO-2/-3 antibody.

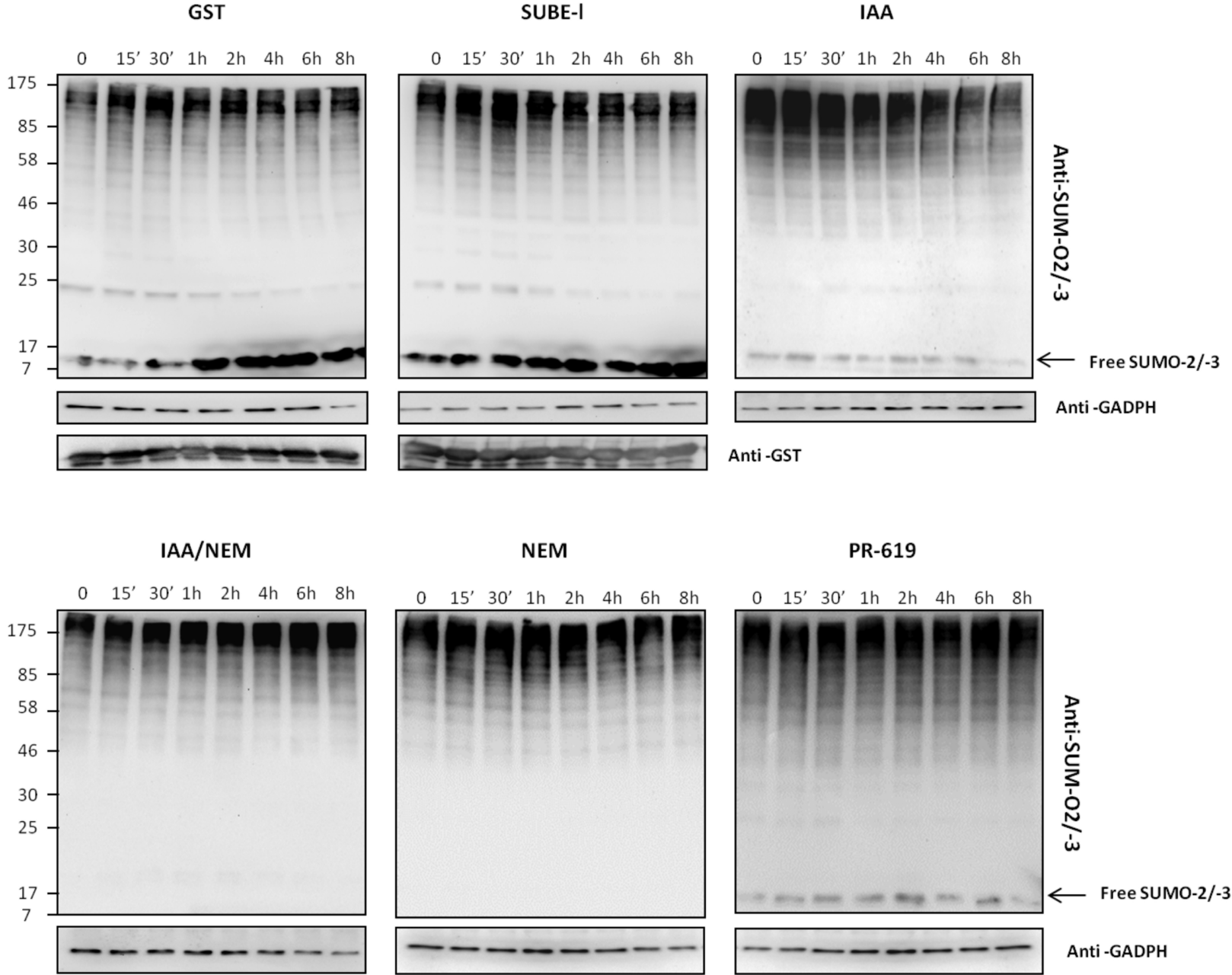
Supplementary Fig 2. SUMO-2/-3 chains generated *in vitro* can be captured by SUBE-1. *In vitro* SUMOylation assays (input) were used to SUMO-2/-3 (A) or SUMO-1 (B) chains. SUMOylated SUMO-2/-3 but not SUMO-1 was mainly captured by SUBE-1 (bound). GST control captured material shows background levels. SUMOylated proteins were monitored by Western blot analysis with anti-SUMO-2/-3 or SUMO-1 antibodies.

Supplementary Fig 3. SUMOylated proteins are specifically captured by SUBE-1. (A) SUMOylated p53 and (B) I $\kappa$ B $\alpha$  were generated by *in vitro* SUMOylation assays (input). SUMOylated p53 and I $\kappa$ B $\alpha$  were captured using SUBE-1 or GST control. Western-blot detection with specific antibodies.

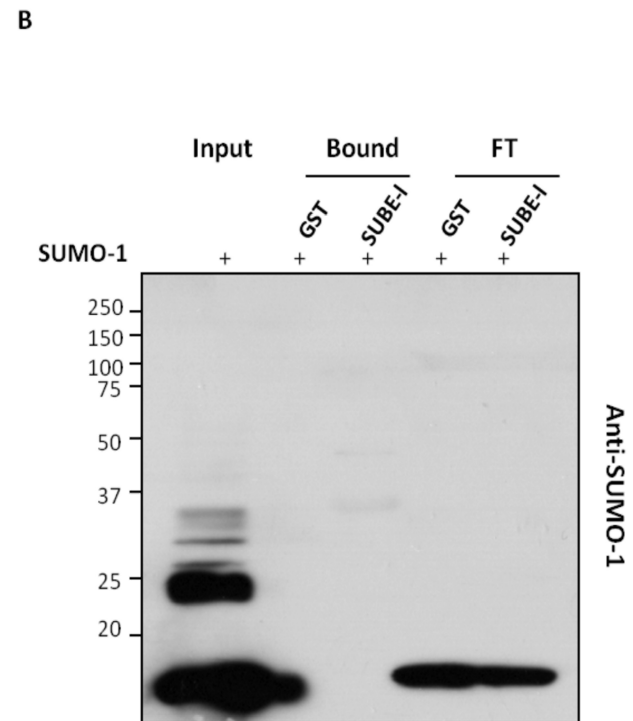
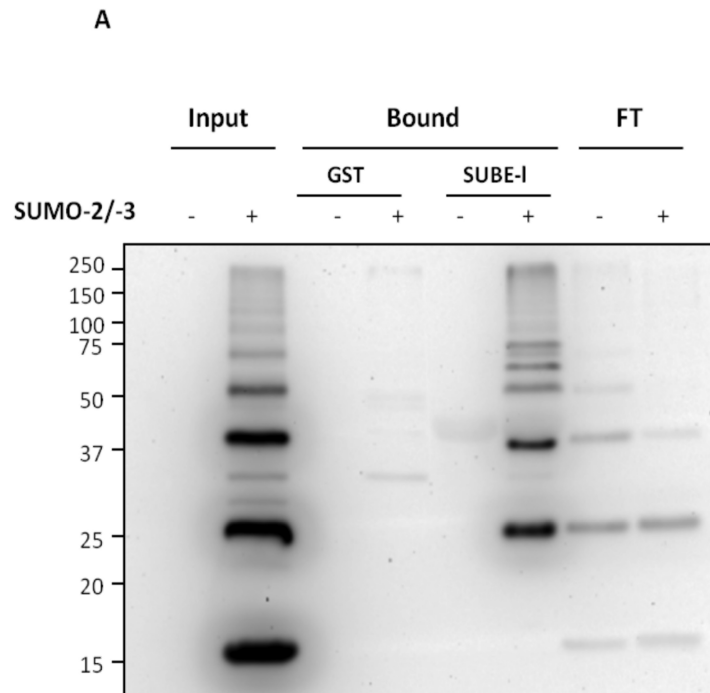
Supplementary Fig 4. SUMO-binding entities (SUBEs) present a better capacity to bind SUMOylated proteins and polySUMO chains than the natural SIMs of RNF4. (A) Capture of SUMOylated proteins from heat-shock induced HeLa cells using SUBE-1 or commercially available SUMO captured system based in the natural SIM domains of RNF4 (Kit). (A) SUBE-1 capture polySUMO chains better than the SUMO-purification kit. Western-blot detection with anti-SUMO-2/-3 or Sam68 antibodies.

Competing financial interests: The authors declare no competing financial interests.

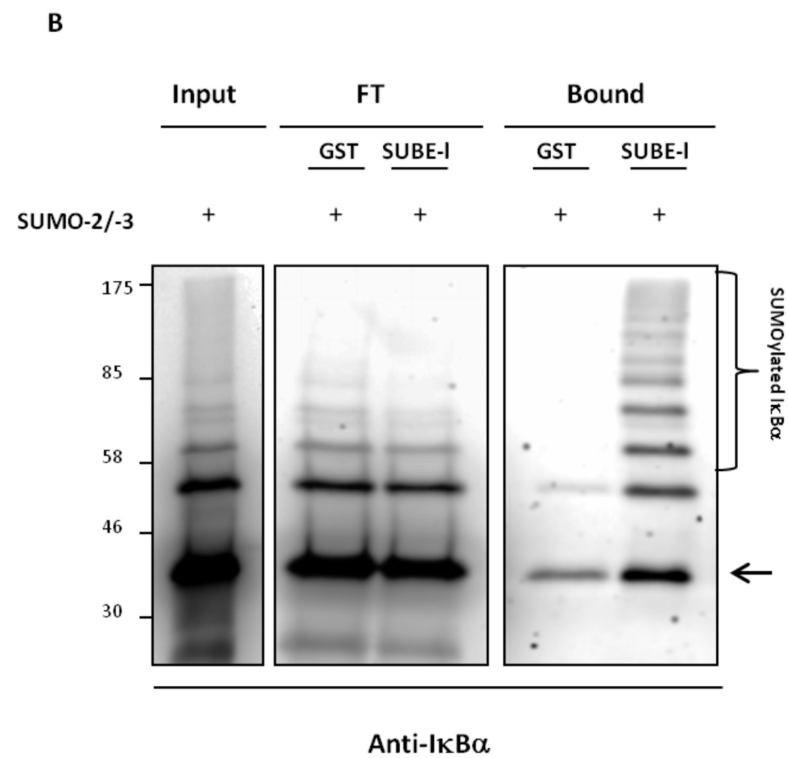
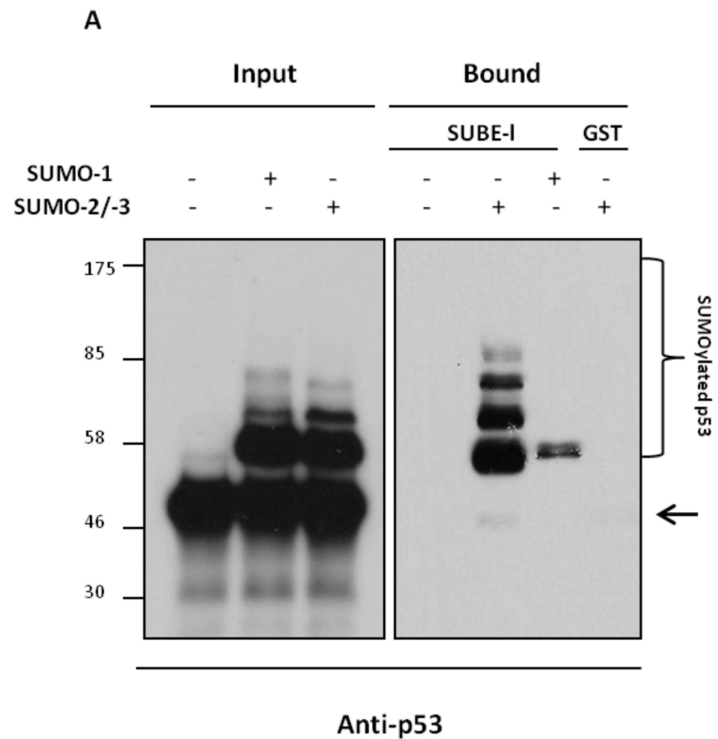
**Supplementary 1**



## Supplementary 2



### Supplementary 3



Supplementary 4

