

Supplementary Materials

Tris-(Nitrilotriacetic Acid)-Decorated Polymer Conjugates as Tools for Immobilization and Visualization of His-Tagged Proteins

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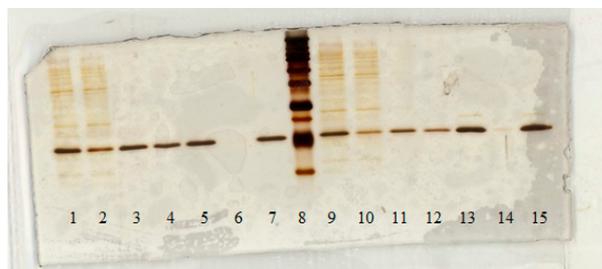
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Table S1. Overview of samples collected during purification of the 6xHis- and 10xHis-SUMO1 proteins and amounts loaded onto 16% polyacrylamide gels used for subsequent silver staining and WB analyses.

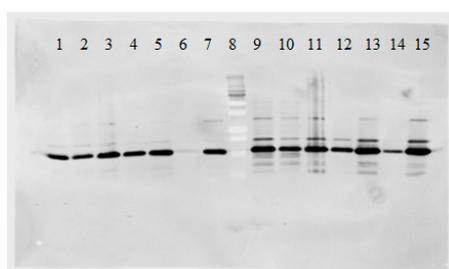
#	Sample name	* Concentration	Sample load [μL/lane]	Sample load [ng/lane]
1	Supernatant after homogenization	50xdil ~ 10 ng/μL	7.5	~ 75
2	Flow though 1 (FT1)	50xdil ~ 10 ng/μL	7.5	~ 75
3	FT2	50xdil ~ 10 ng/μL	7.5	~ 75
4	FT3	50xdil ~ 10 ng/μL	7.5	~ 75
5	Elution (EL) 1+2 before dialysis	50xdil ~ 10 ng/μL	7.5	~ 75
6	EL3	50xdil ~ 10 ng/μL	7.5	~ 75
7	Elution (EL) 1+2 after dialysis	50xdil ~ 10 ng/μL	7.5	~ 75
8	All Blue marker	-	2.0	-
9	Supernatant after homogenization	50xdil ~ 10 ng/μL	7.5	~ 75
10	Flow though 1 (FT1)	50xdil ~ 10 ng/μL	7.5	~ 75
11	FT2	50xdil ~ 10 ng/μL	7.5	~ 75
12	FT3	50xdil ~ 10 ng/μL	7.5	~ 75
13	Elution (EL) 1+2 before dialysis	50xdil ~ 10 ng/μL	7.5	~ 75
14	EL3	50xdil ~ 10 ng/μL	7.5	~ 75
15	Elution (EL) 1+2 after dialysis	50xdil ~ 10 ng/μL	7.5	~ 75

* Concentrations of fractions collected during the 6xHis- and 10xHis-SUMO1 purification are estimated according to the measured concentrations of the joint elutions 1+2 which was 500 ng/ μ L according to Nanodrop spectrophotometer).

Figure S1 Analysis of His-SUMO1 protein purification process: results of silver staining



(a) Analysis of His-SUMO1 protein purification process: results of Western blotting (4 nM **iBody 2** used for detection of proteins)



(b) Analysis of His-SUMO1 protein purification process: results of Western blotting

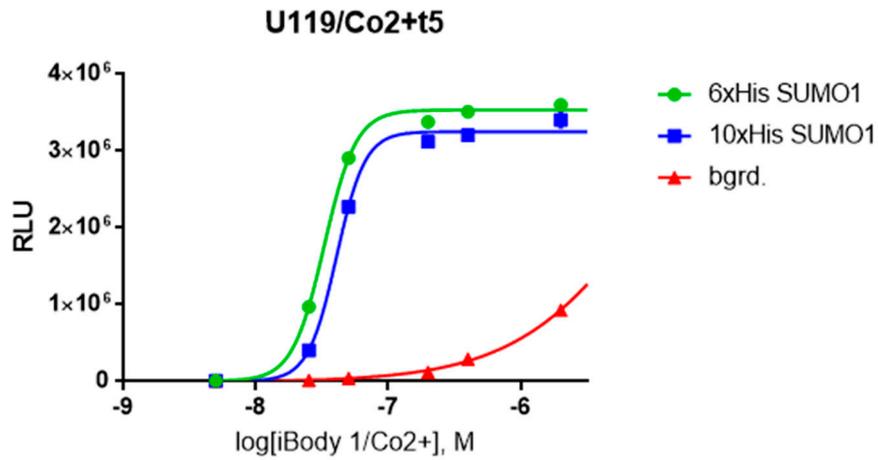
Supplementary Table S2

Concentration series of iBodies used for K_D determination with ELISA

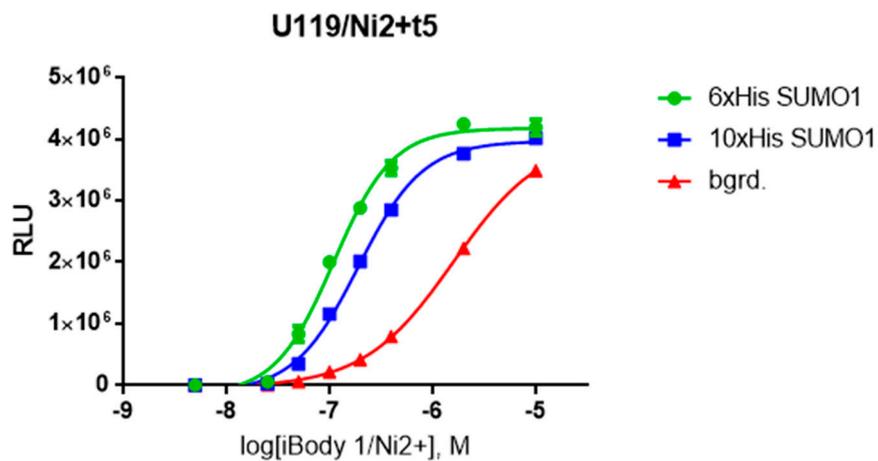
	iBody 1/Co²⁺	iBody 1/Ni²⁺	iBody 2/Co²⁺	iBody 2/Ni²⁺
Concentration series of iBodies [M]	1.00E-05	1.00E-05	1.70E-06	3.00E-06
	2.00E-06	2.00E-06	4.25E-07	7.50E-07
	4.00E-07	4.00E-07	1.06E-07	1.88E-07
	2.00E-07	2.00E-07	5.31E-08	9.38E-08
	1.00E-07	1.00E-07	2.66E-08	4.69E-08
	5.00E-08	5.00E-08	1.33E-08	2.34E-08
	2.50E-08	2.50E-08	6.64E-09	1.17E-08
	5.00E-09	5.00E-09	1.66E-09	2.93E-09

Supplementary Figure S2

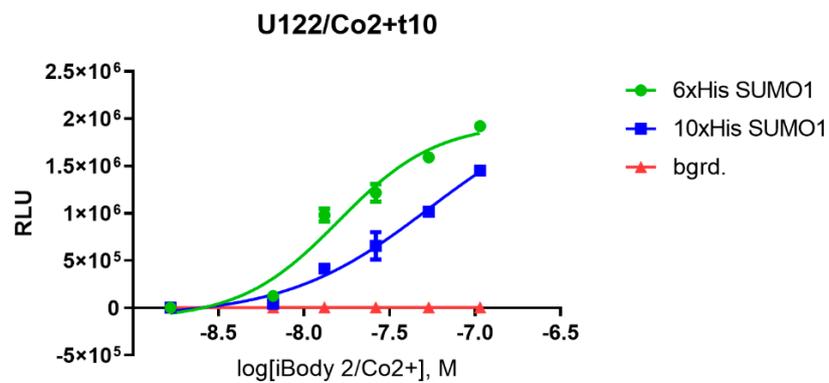
(a) Graphical representation of binding of **iBody 1/Co²⁺** to His-tagged SUMO1 proteins measured with ELISA



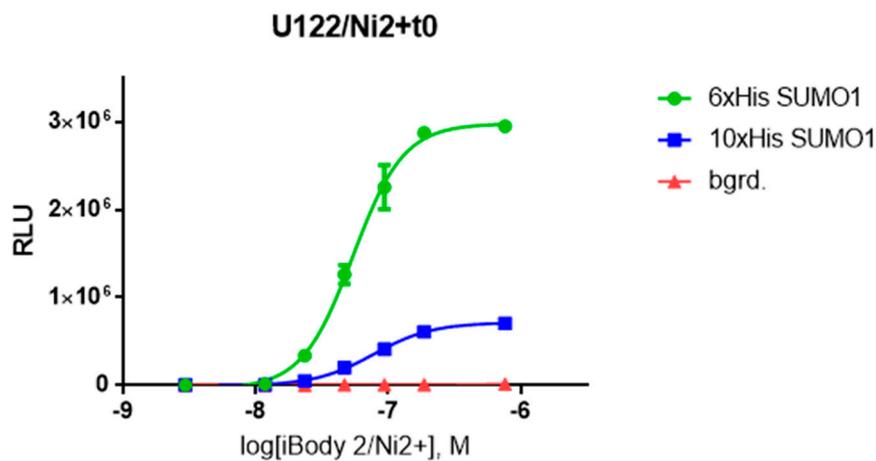
(b) Graphical representation of binding of **iBody 1/Ni²⁺** to His-tagged SUMO1 proteins measured with ELISA



(c) Graphical representation of binding of **iBody 2/Co²⁺** to His-tagged SUMO1 proteins measured with ELISA



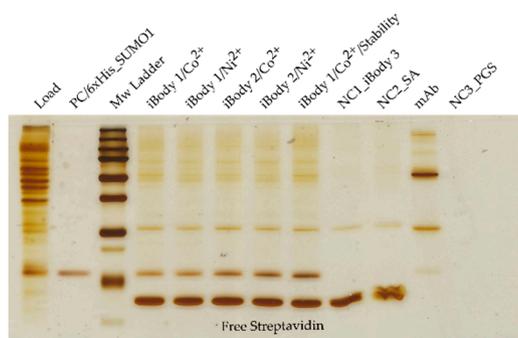
(d) Graphical representation of binding of **iBody 2/Ni²⁺** to His-tagged SUMO1 proteins measured with ELISA



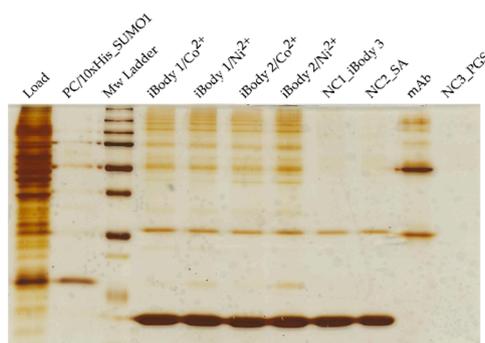
Supplementary Figure S3

Repetition of the immunoprecipitation experiments showing the reproducibility of the results.

(a) Pull down of 6xHis-SUMO1 protein from cell lysate: results of silver staining

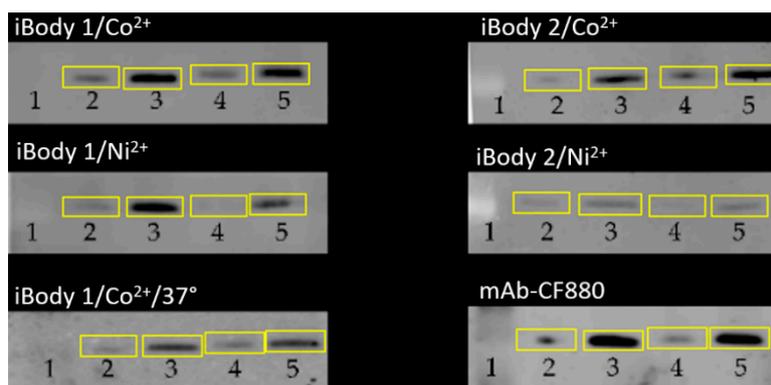


(b) Pull down of 10xHis-SUMO1 protein from cell lysate: results of silver staining



Supplementary Figure S4

Relative quantification of the western blotting results. The signals were analysed with Image Lite Studio from LI-COR.



Supplementary Table S3

Relative signal intensities on western blots

Detection molecule	Sample	Lane	Relative Signal Intensities
iBody 1/Co ²⁺	50 ng 6xHis SUMO	2	775276
	100 ng 6xHis SUMO	3	3478196
	50 ng 10xHis SUMO	4	948963
	100 ng 10xHis SUMO	5	3031705
iBody 1/Ni ²⁺	50 ng 6xHis SUMO	2	476059
	100 ng 6xHis SUMO	3	3470561
	50 ng 10xHis SUMO	4	148707
	100 ng 10xHis SUMO	5	1689053
iBody 1/Co ²⁺ /Stab. 37°C	50 ng 6xHis SUMO	2	283668
	100 ng 6xHis SUMO	3	2026090
	50 ng 10xHis SUMO	4	748055
	100 ng 10xHis SUMO	5	2108265
iBody 2/Co ²⁺	50 ng 6xHis SUMO	2	199759
	100 ng 6xHis SUMO	3	2293775
	50 ng 10xHis SUMO	4	940122
	100 ng 10xHis SUMO	5	3361312
iBody 2/Ni ²⁺	50 ng 6xHis SUMO	2	190389
	100 ng 6xHis SUMO	3	746595
	50 ng 10xHis SUMO	4	66201
	100 ng 10xHis SUMO	5	446170
mAb-CF880	50 ng 6xHis SUMO	2	888298
	100 ng 6xHis SUMO	3	5398132
	50 ng 10xHis SUMO	4	807643
	100 ng 10xHis SUMO	5	4590556