Supplementary information

Detecting endogenous SUMO targets in mammalian cells and tissues

Janina Becker, Sina V. Barysch, Samir Karaca, Claudia Dittner, He-Hsuan Hsiao, Mauricio Berriel Diaz, Stephan Herzig, Henning Urlaub, Frauke Melchior

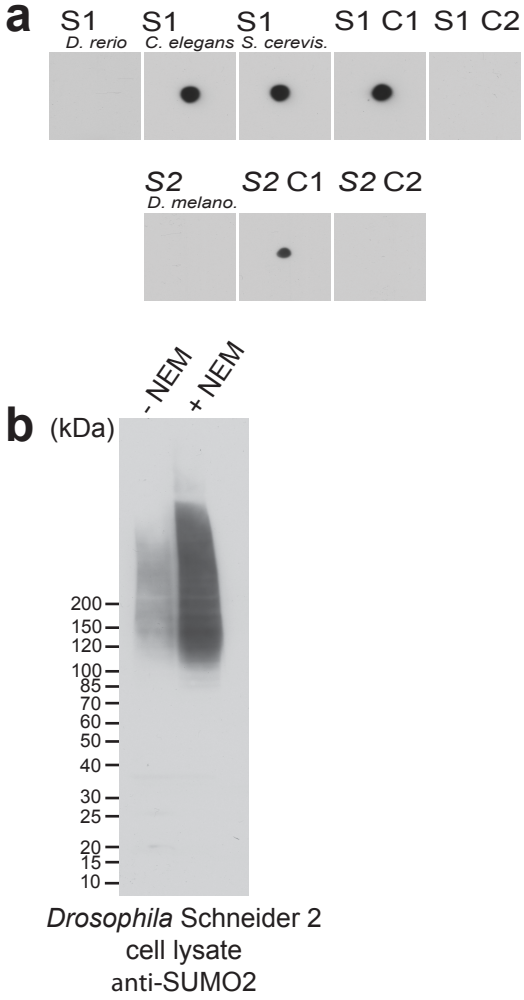
Legends belonging to Supplementary Table S1 and S2 (excel file)

Supplementary Table S1: List of unambiguously identified SUMO1 and SUMO2/3 candidates (for details see Materials and Methods). 0–10%: proteins for which less than 10% of the total signal intensity was found in the control immunoprecipitation (IP); 10–25%: proteins for which 10–25% of the total signal intensity was found in the control IP.

Supplementary Table S2: List of 296 SUMO candidates that were identified both in our study (immunoprecipitation with SUMO1 and/or SUMO2 monoclonal antibodies; HeLa suspension cells, no stress) and by Bruderer, R. et al. (2011) *EMBO Rep* **12**, 142-8 (enrichment of polysumoylated proteins by pulldown; heat stressed HeLa cells). See Supplemental Figure 2.

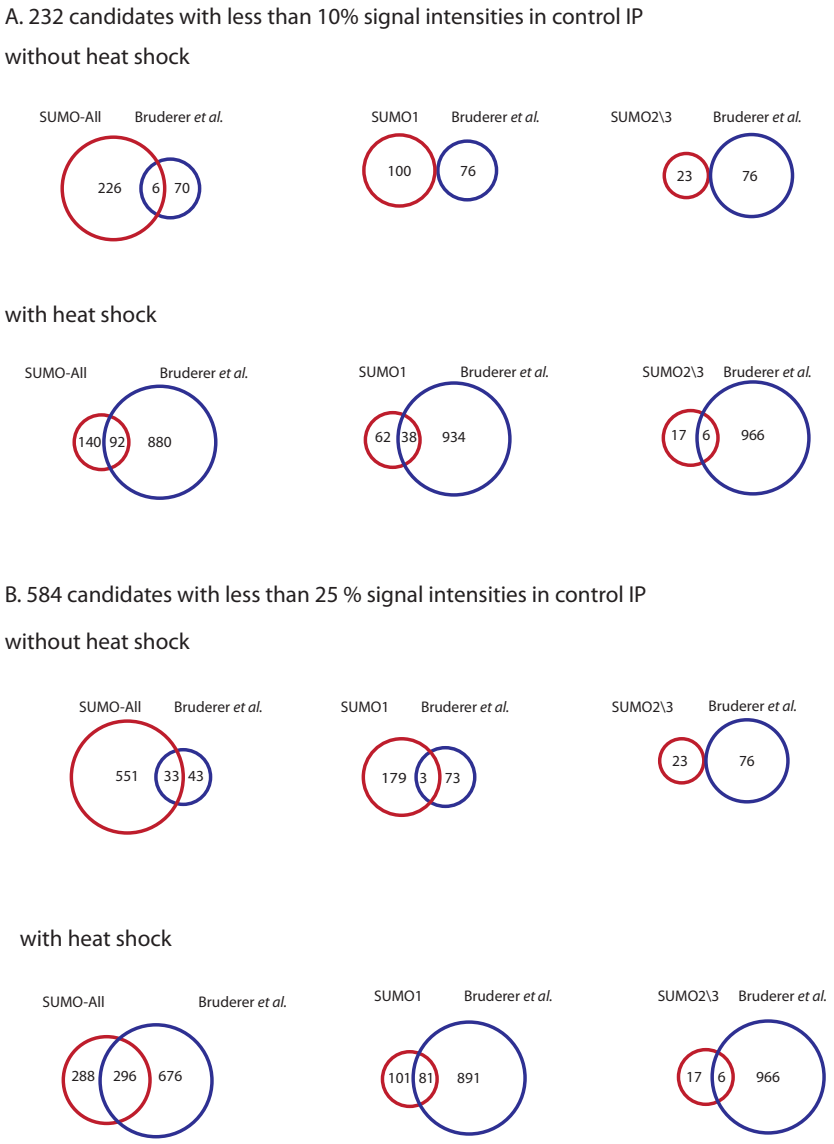
Nature Structural & Molecular Biology: doi:10.1038/nsmb.2526

Supplementary Figure 1



Supplementary Fig. 1: Monoclonal antibody SUMO1 21C7 recognizes zebrafish SUMO1, monoclonal antibody SUMO2 8A2 recognizes drosophila SUMO. **(a)** Peptide competition assays with species-specific peptides that correspond to the human epitope-spanning peptides described in Fig. 2. Peptides were tested for their ability to compete with immobilized SUMO1 or SUMO2 in immunoblotting. Shown are dot blots for peptides from *D. rerio, C. elegans, S. cerevisiae* and *D. melanogaster* SUMO proteins. Top: human SUMO1 was spotted, monoclonal SUMO1 21C7 was used; Bottom: human SUMO2 was spotted, monoclonal SUMO2 8A2 was used. C1=control without peptide, C2= control with human epitope-spanning peptides. **(b)** *Drosophila* Schneider 2 cells were lysed in RIPA buffer with or without 10 mM NEM, and analyzed by immunoblotting with anti SUMO2 8A2.

Supplementary Figure 2



Supplementary Fig. 2: Comparison of endogenous SUMO candidates identified in this study with those identified as candidates for endogenous polySUMO conjugation with or without heat shock by Bruderer, R. et al. *EMBO Rep* **12**, 142-8 (2011). **A**. Comparison of 232 SUMO candidates that have less than 10% total signal intensities in the control IP (SUMO All) with two candidate lists provided by Bruderer *et al.*; Upper panel: polySUMO conjugates identified by Bruderer et al. without heat stress; Lower panel: candidates identified by Bruderer et al. upon heat stress. Left: all candidates; Middle: proteins showing preference for SUMO1 (proteins with >80% of the total SUMO intensity found in SUMO1 IP); Right; proteins showing preference for SUMO2/3 (proteins with >80% of the total SUMO intensity found in SUMO2 IP). **B.** Same comparison as in **A**, but using our extended candidate list (584 candidates having less than 25 % total signal intensities in control IP). See **Supplementary Table S2** for the list of 296 overlapping proteins.