Supplement

System-wide analysis of SUMOylation dynamics in response to replication stress reveals novel SUMO target proteins and acceptor lysines relevant for genome stability

Authors

Zhenyu Xiao[1], Jer-Gung Chang[1], Ivo A. Hendriks[1], Jón Otti Sigurðsson[2], Jesper V. Olsen[2] and Alfred C.O. Vertegaal[1]*

Affiliation

[1]Department of Molecular Cell Biology, Leiden University Medical Center, 2300 RC Leiden, the Netherlands

[2]Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3B, 2200 Copenhagen, Denmark

* To whom correspondence should be addressed: E-mail: vertegaal@lumc.nl

Running title

SUMOylation and replication stress

Legends Supplemental Figures

Figure S1. A strategy for identifying SUMO-2 acceptor lysines in endogenous proteins during replication stress.

A, Schematic representation of the His10-SUMO-2-K0-Q87R-IRES-GFP construct used in this project.

B, Cartoon depicting our strategy to identify SUMO-2 acceptor lysines and their dynamics during replication stress. U2OS cells expressing His10-SUMO-2-K0-Q87R were treated with 2 mM Hydroxyurea (HU) for 2 hours or 24 hours to induce DNA replication fork stalling and double strand breaks, respectively. U2OS cells expressing His10-SUMO-2-K0-Q87R cells were mock treated as negative controls. SUMO-2 target proteins were enriched on Ni-NTA beads. SUMOylated peptides were obtained by Lys-C digestion and Ni-NTA re-purification and subsequently analyzed by mass spectrometry.

Figure S2. A U2OS cell line stably expressing His10-SUMO-2-K0-Q87R.

Immunoblotting analysis was used to verify the expression levels of SUMO-2 in U2OS cells stably expressing His10-SUMO-2-K0-Q87R (His10-K0-S2-Q87R).

Legends Supplemental Tables

Supplemental Table 1. SUMO-2 target proteins. A complete list of 566 His10-SUMO-2 target proteins including gene names, intensities, PEP, ANOVA log(*P* value) and other relevant information.

Supplemental Table 2. Bioinformatics analysis of SUMOylated proteins. Complete term enrichment analysis of all SUMOylated proteins identified in our site-independent approach. Annotated terms include GOBP, GOMF, GOCC, CORUM, Keywords, KEGG, Pfam and GSEA.

Supplemental Table 3. Fully annotated list of all SUMOylated proteins.

Supplemental Table 4. HU-regulated SUMO-2 target proteins. Label-free quantification of SUMOylated proteins with significantly regulated proteins after 2 hours or 24 hours of HU treatment. The proteins are sorted by their average $\log_2$ LFQ ratio for 2 hours HU treatment compared to control, and for 24 hours HU treatment compared to control.

Supplemental Table 5. SUMOylation sites. A list of SUMO-2 acceptor lysines obtained by our site-specific purification approach, including gene names, intensities, sequence windows, overlap with Hendriks *et al.*(39) and other relevant information.

Supplemental Table 6. SUMO-2 targets identified in a site-specific manner. A complete list of all proteins identified by SUMOylation site, with number of sites, gene names, site positions and other relevant information.

Supplemental Table 7. HU-regulated SUMOylation sites. Sites quantification with significantly regulated sites after 2 hours or 24 hours of HU treatment compared to the control. The SUMOylation sites are sorted by average $\log_2$ ratio for 2h HU treatment compared to control, and for 24h HU treatment compared to control.

Supplemental Table 8. Overlap of dynamic SUMO-2 target proteins identified in our site-independent and our site-specific approach. A comparison of dynamically regulated SUMO-2 target proteins identified, using the SUMOylation site-independent approach or the SUMOylation site-specific approach. Overlap, and other relevant information are provided.

Supplemental Table 9. Peptides co-modified by SUMOylation and phosphorylation. List of peptides simultaneously modified by both SUMO-2 and phosphorylation.

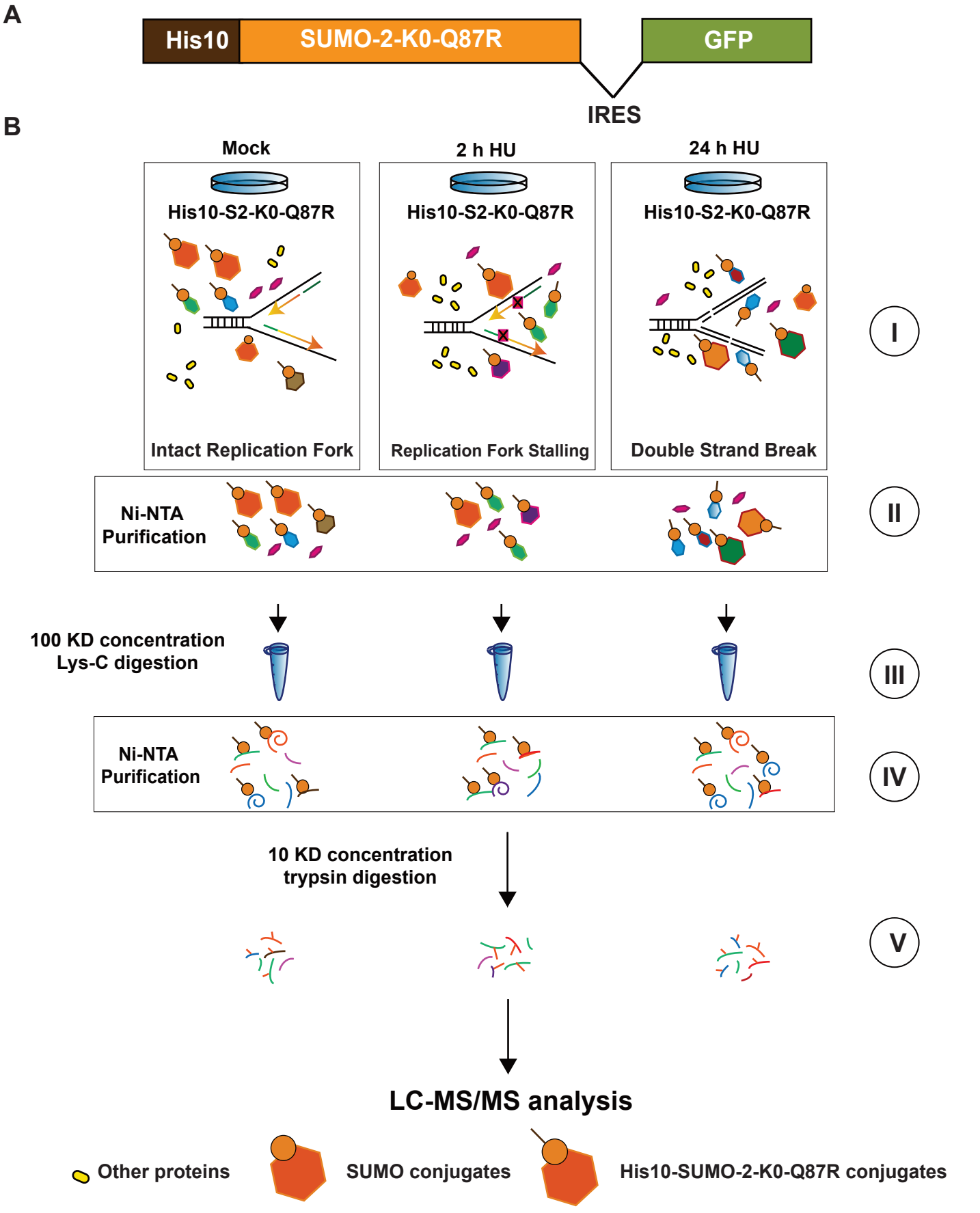
A
His10
SUMO-2-K0-Q87R
GFP
IRES
B
Mock
2 h HU
24 h HU
His10-S2-K0-Q87R
His10-S2-K0-Q87R
His10-S2-K0-Q87R
Intact Replication Fork
Replication Fork Stalling
Double Strand Break
I
Ni-NTA Purification
II
100 KD concentration
Lys-C digestion
III
Ni-NTA Purification
IV
10 KD concentration
trypsin digestion
V
LC-MS/MS analysis
Other proteins
SUMO conjugates
His10-SUMO-2-K0-Q87R conjugates
His10-SUMO-2-K0-Q87R peptids
QQTGG-remnants
Ni-NTA Beads Aspecific Binding Proteins

Figure S1

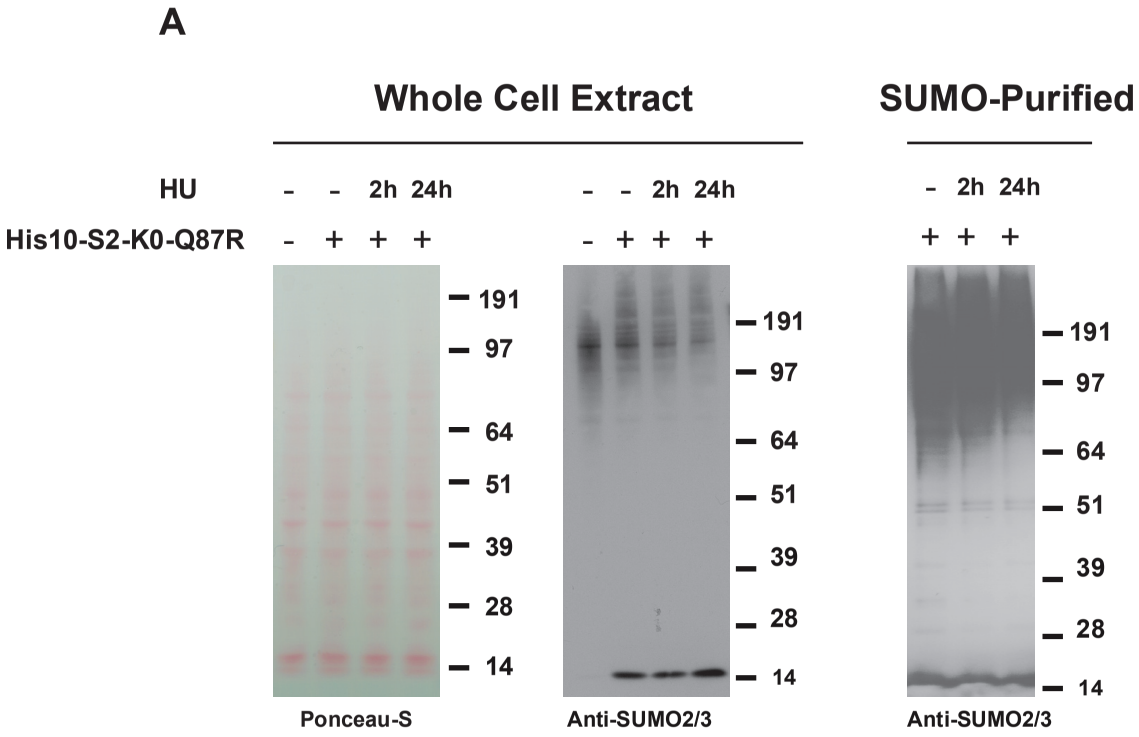
A
Whole Cell Extract
SUMO-Purified
HU
His10-S2-K0-Q87R
- - 2h 24h
- + + +
191
97
64
51
39
28
14
Ponceau-S
Anti-SUMO2/3
Anti-SUMO2/3

Figure S2