

Supplementary Figures

SUMO2/3 modification of transcription-associated proteins controls cell viability in response to oxygen and glucose deprivation-mediated stress

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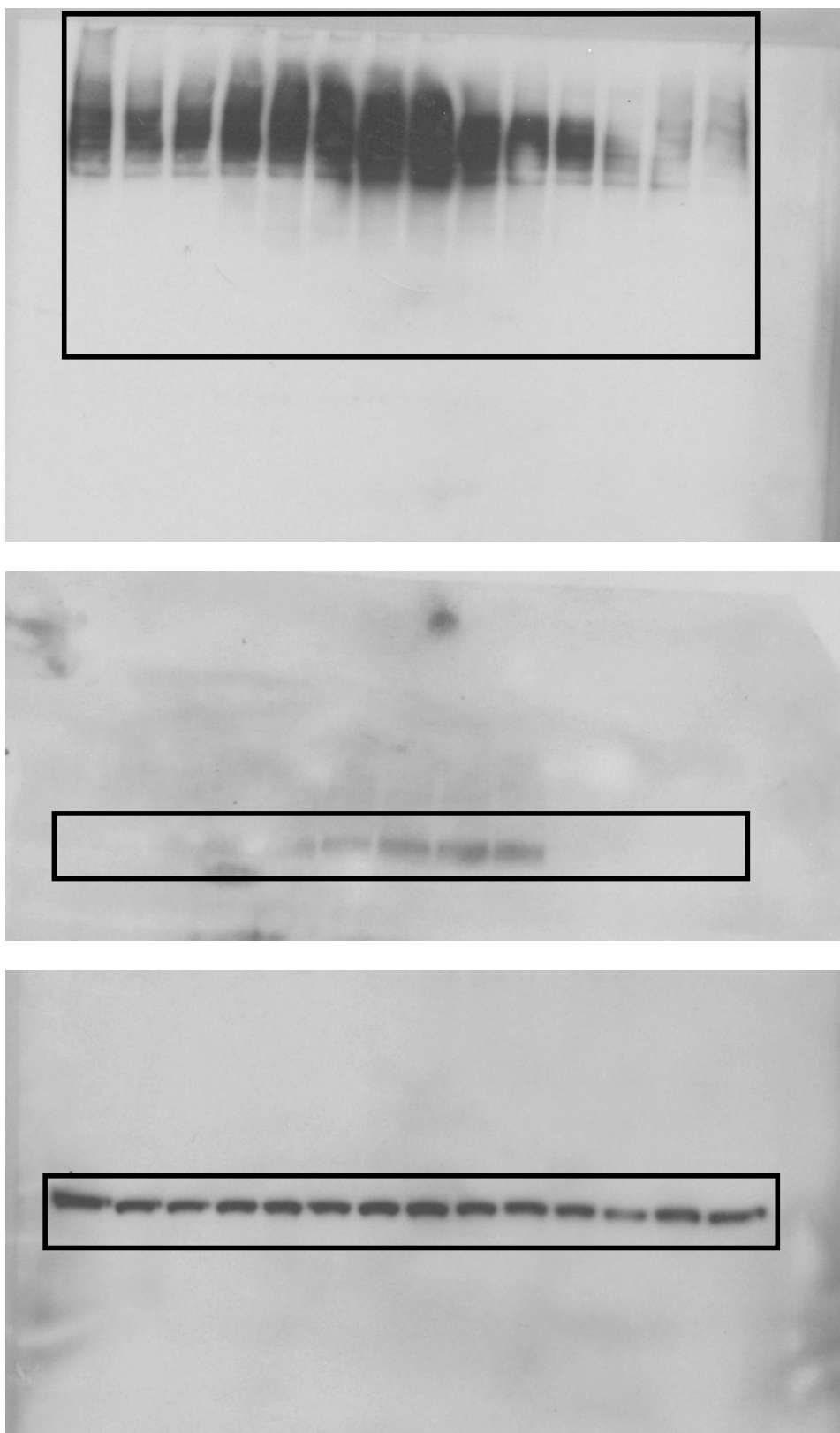


Fig. 1

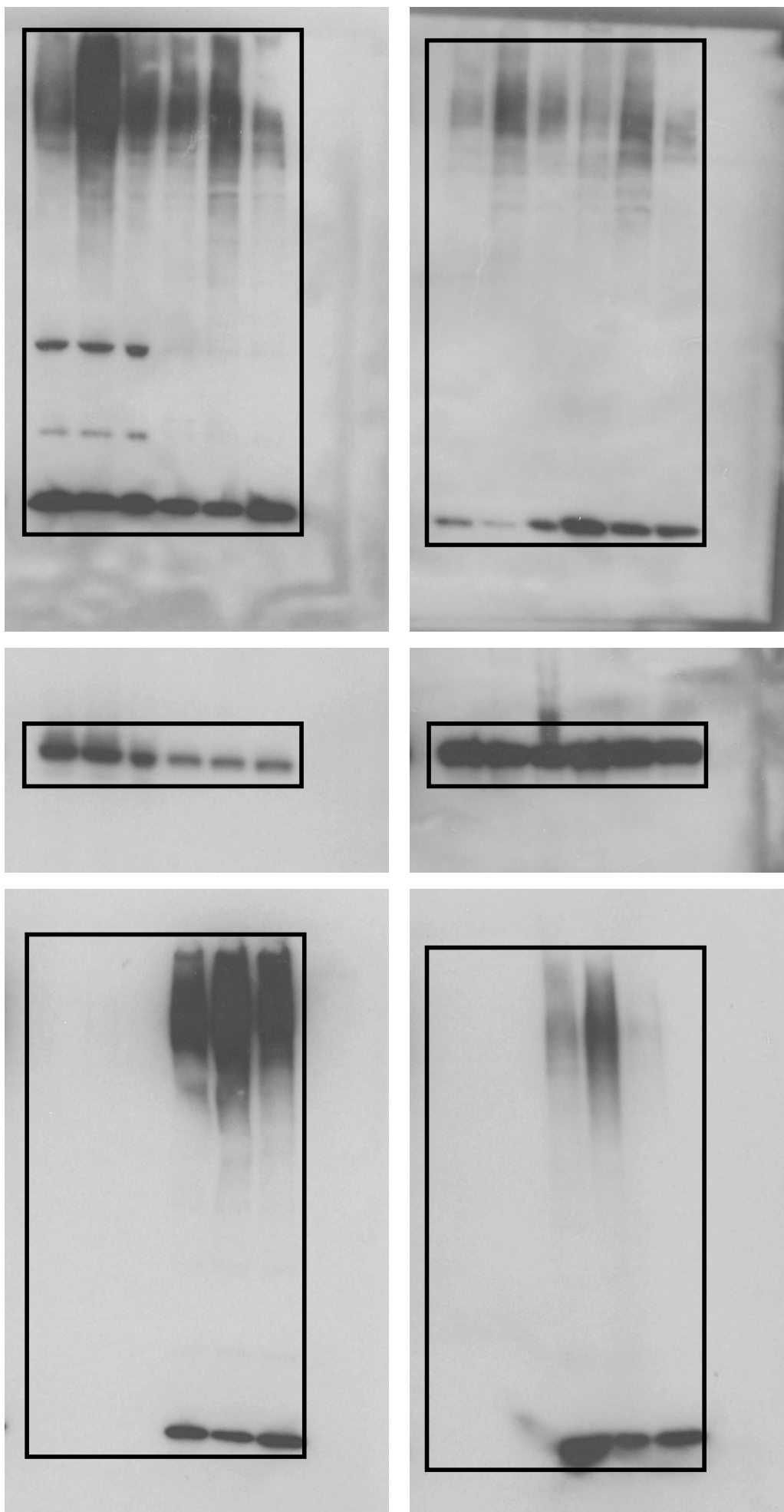


Fig. 2

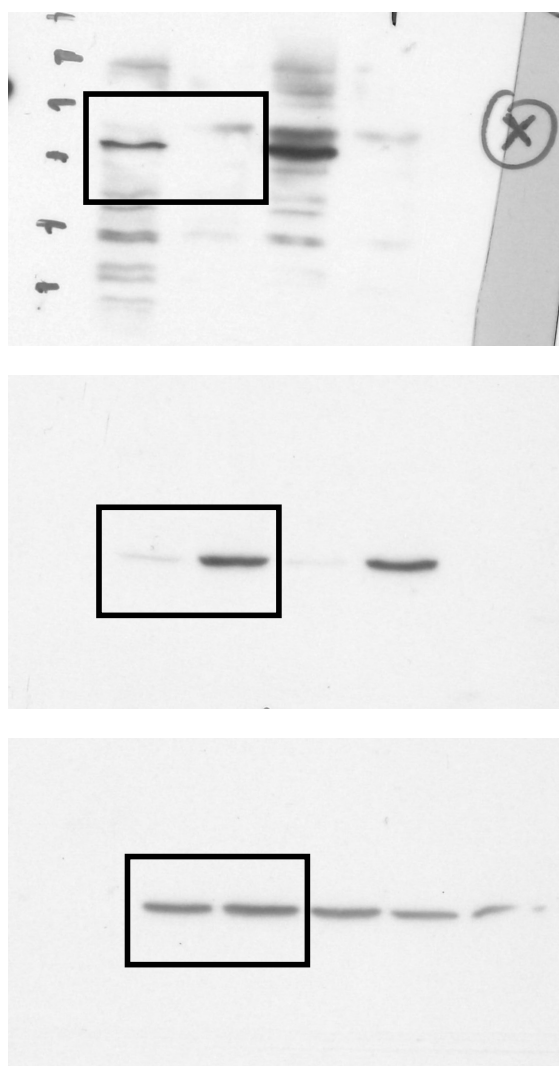


Fig. 3

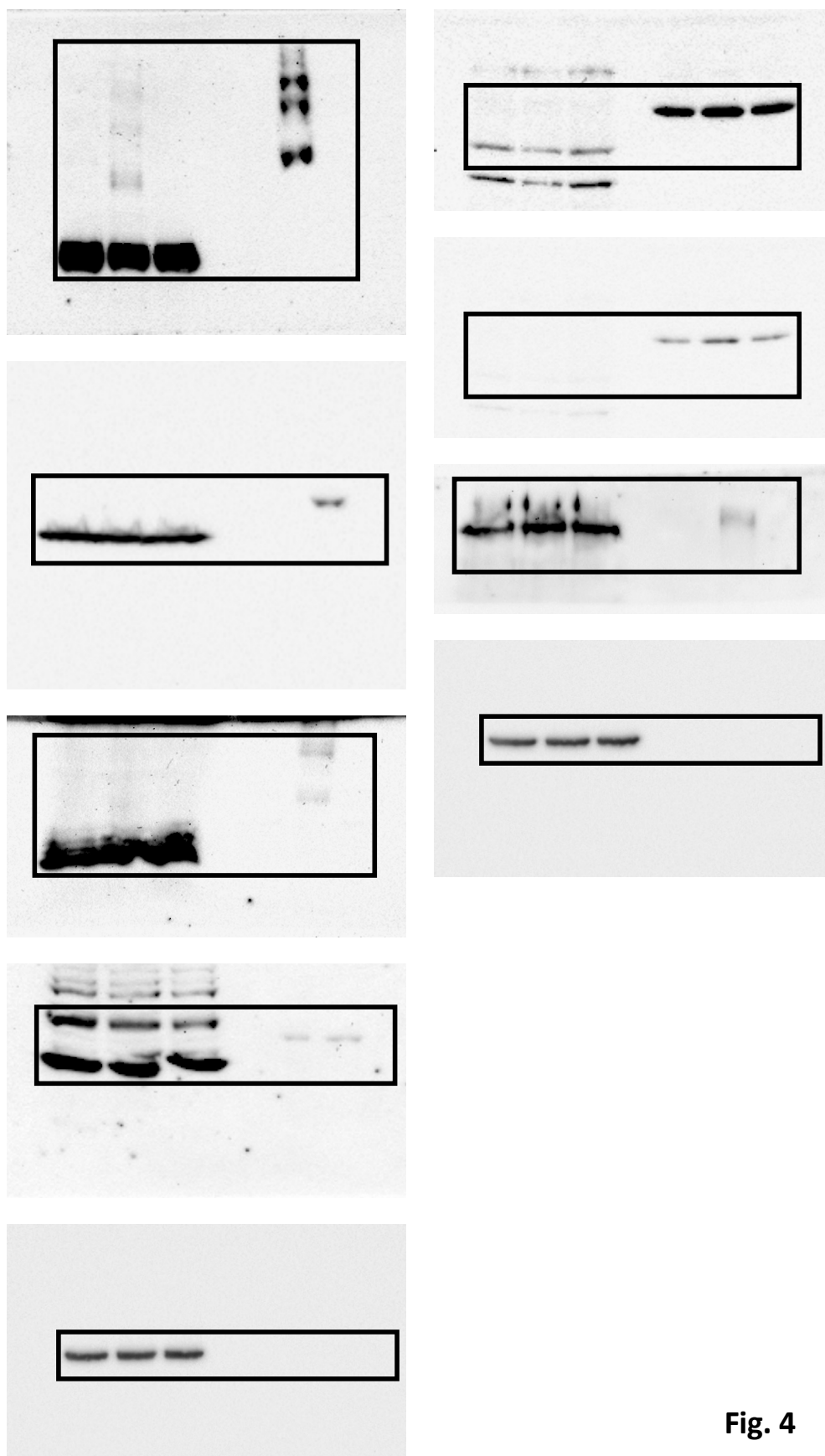


Fig. 4

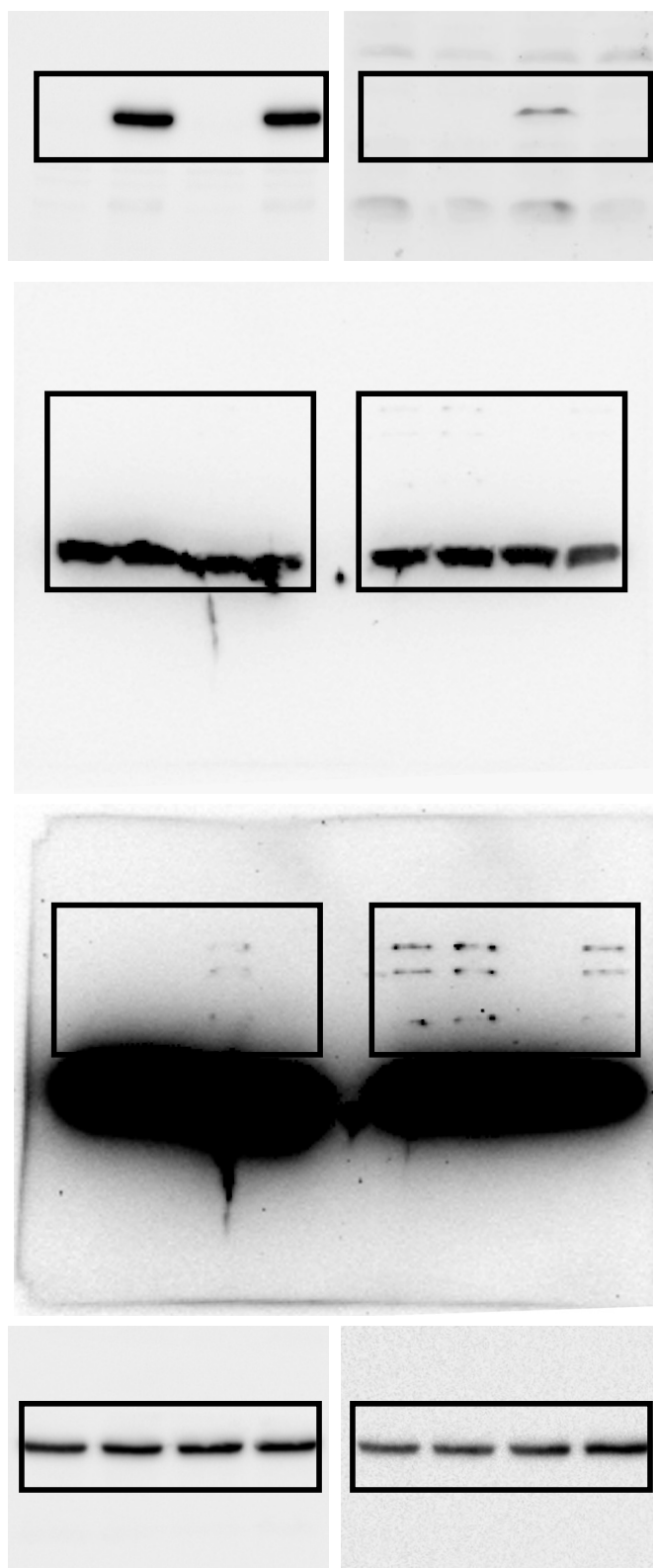
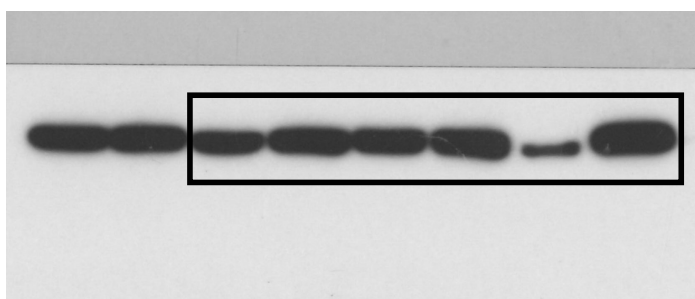
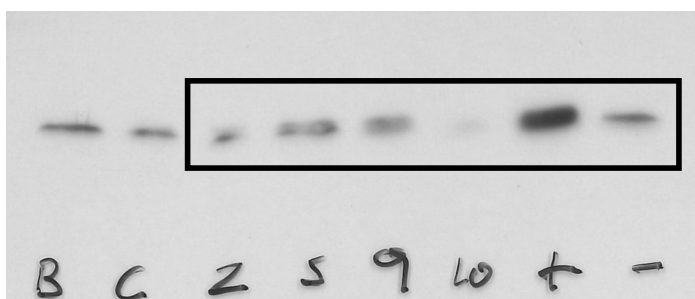
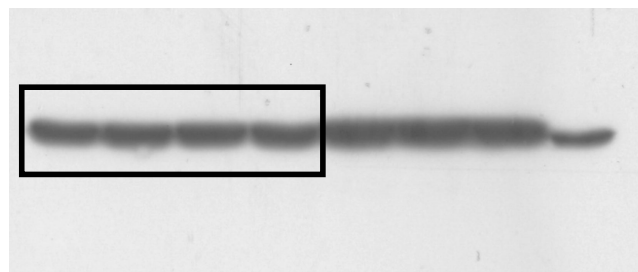
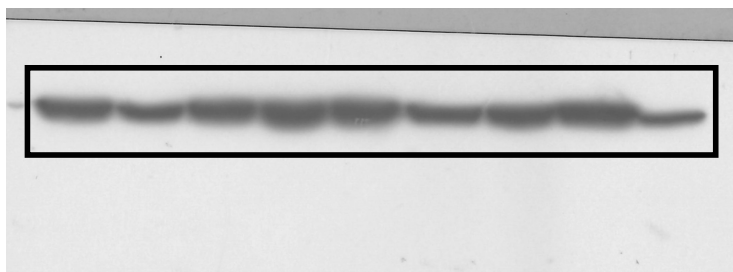
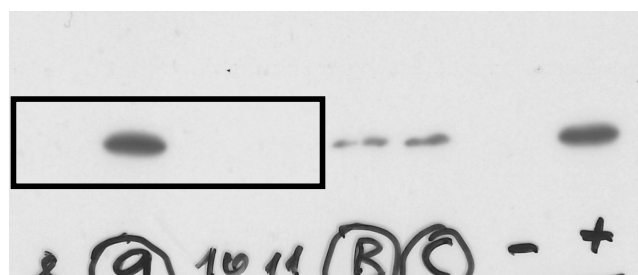
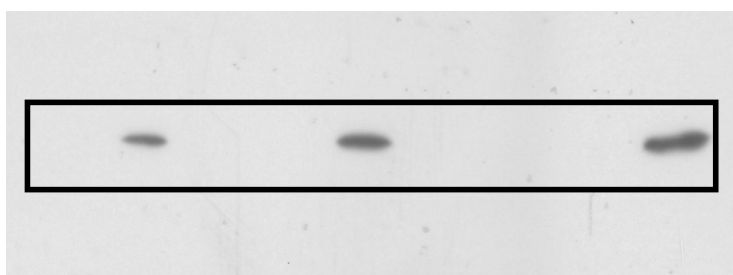
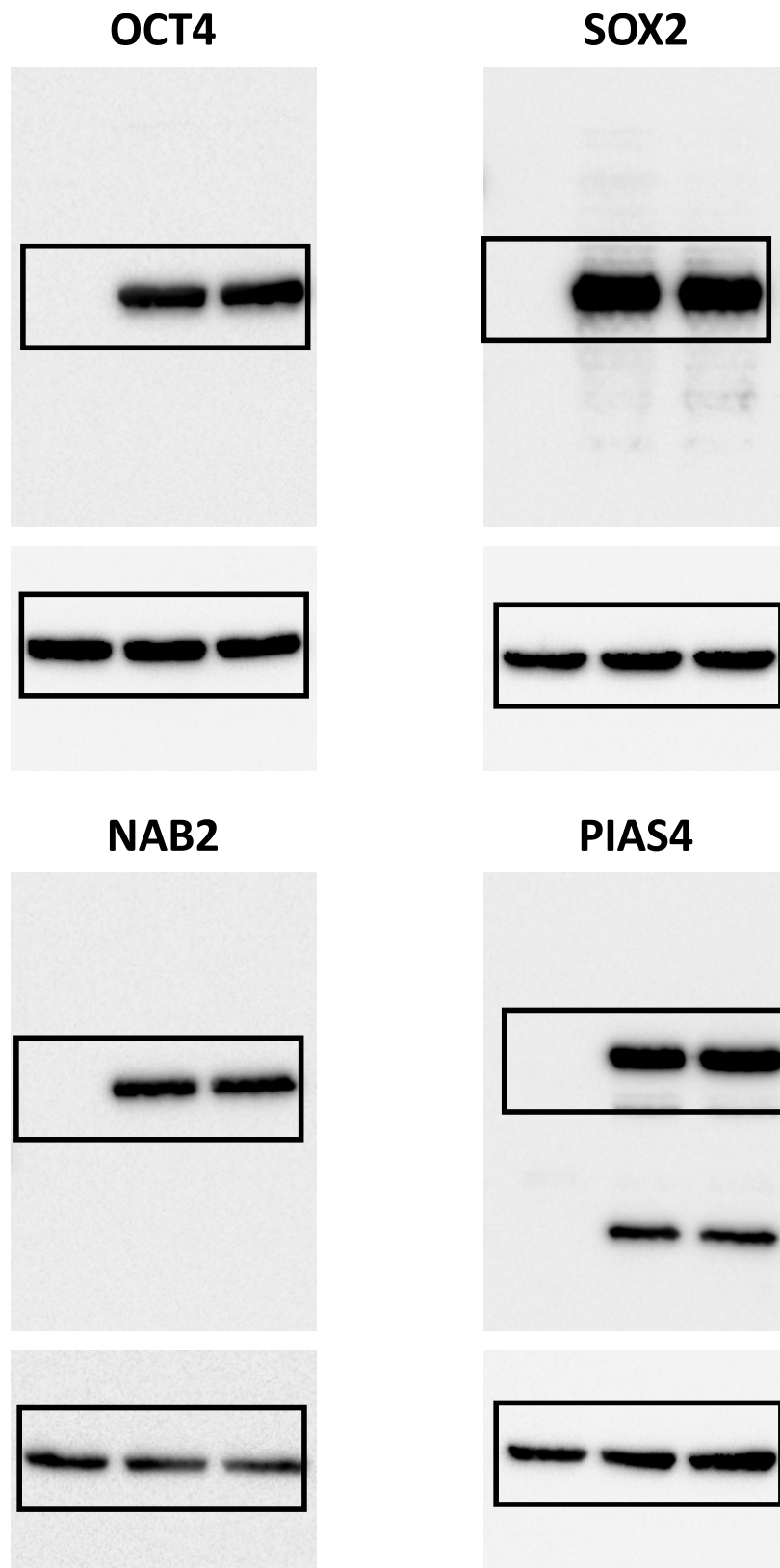


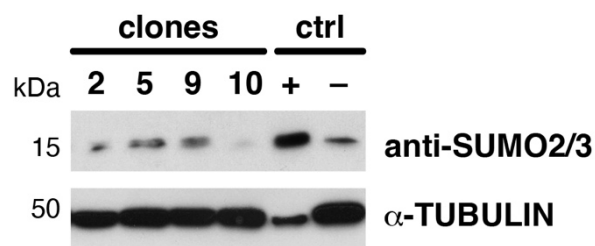
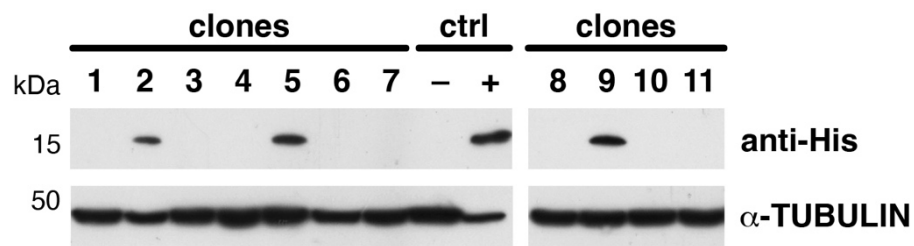
Fig. 5



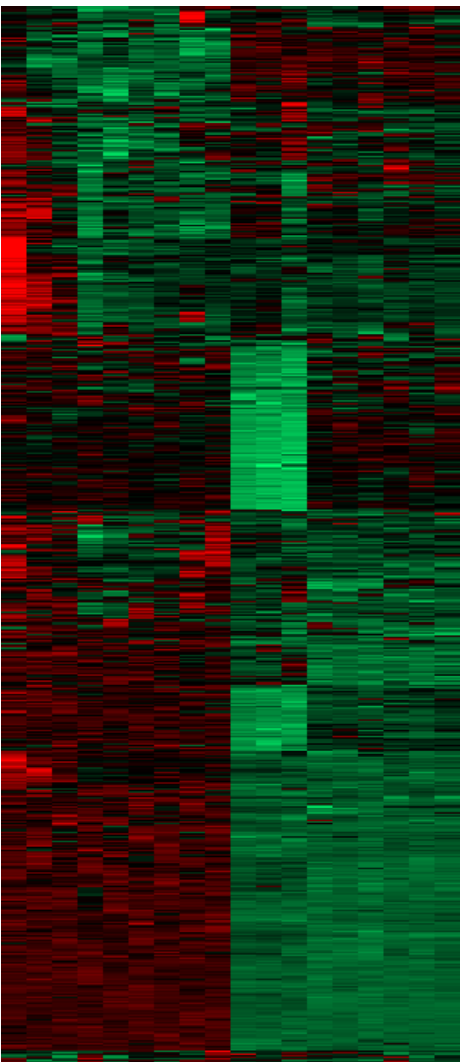
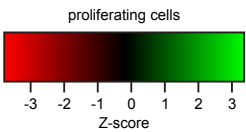
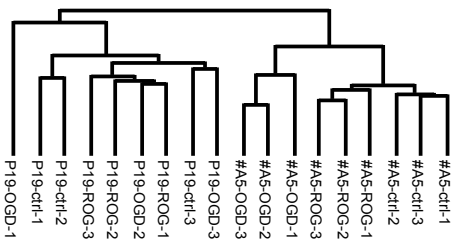
Supplementary Fig. S2

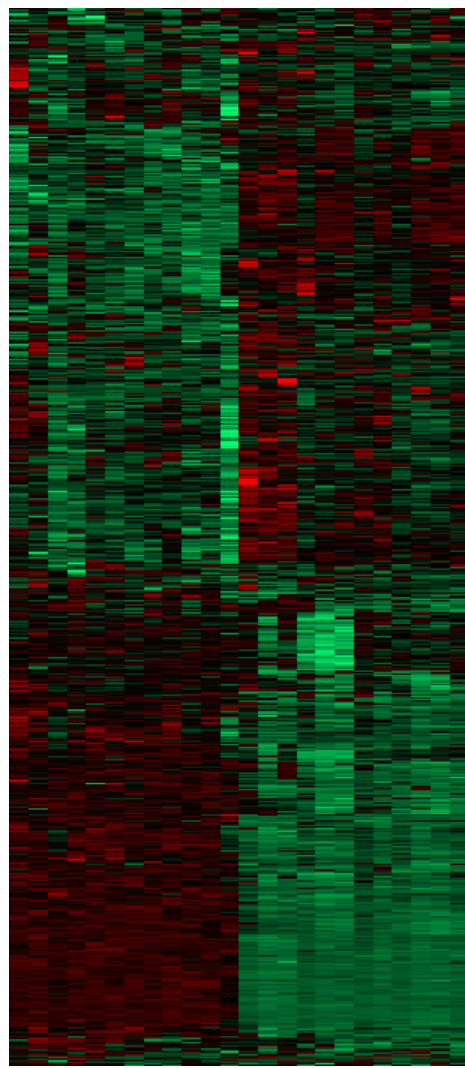
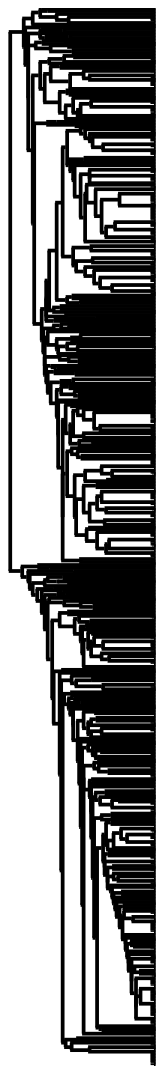
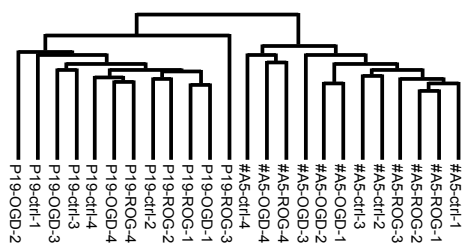
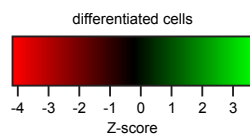


Supplementary Fig. S5



Supplementary Fig. S2 Generation of stable cell lines from P19 cells expressing His10-SUMO2. Clones resistant to puromycin after introducing an expression construct for His10-SUMO2 were tested by western blot with anti-His antibodies (upper panel) for stable integration of the construct. Positive clones were subsequently tested with anti-SUMO2/3 antibodies (lower panel). Positive (+) and negative (-) control (ctrl) samples, corresponding to cells transiently transfected with the expression construct or not transfected, respectively, were also analyzed. 20 μ g of total protein were loaded per lane. α -TUBULIN was analyzed as a loading marker.

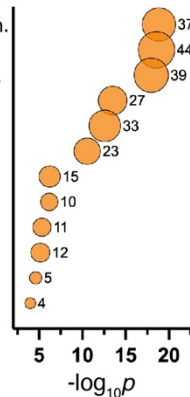




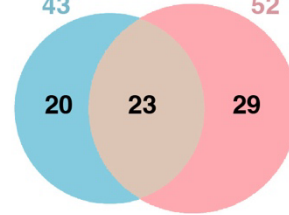
Supplementary Fig. S3 Heatmaps of His10-SUMO2 modified proteins under OGD and ROG in proliferating and differentiated cells. Heatmaps of sample clustering after mass spectrometry data analysis of proliferating and differentiated cells are shown. Color code for Z-score values of each heatmap is also indicated.

A**proliferating (only sum.-desum.)**

negative regulation of transcription from RNA pol. II prom.
 regulation of transcription from RNA pol. II prom.
 positive regulation of transcription from RNA pol. II prom.
 positive regulation of transcription, DNA-templated
 regulation of transcription, DNA-templated
 negative regulation of transcription, DNA-templated
 regulation of gene expression
 mRNA splicing, via spliceosome
 RNA splicing
 chromatin organization
 protein sumoylation
 3'-UTR-mediated mRNA stabilization

**B**

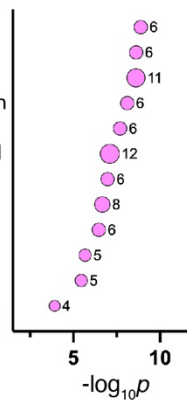
OGD vs. Ctrl 43 OGD vs. ROG 52



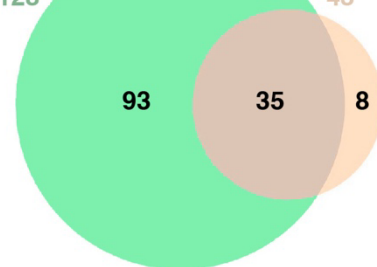
enrich.: 54.81 fold
 $p\text{-val: } 1.61 \cdot 10^{-37}$

C**proliferating (desum. under OGD)**

regulation of G0 to G1 transition
 regulation of nucleotide-excision repair
 chromatin organization
 regulation of mitotic metaphase/anaphase transition
 positive regulation of double-strand break repair
 negative regulation of transcription, DNA-templated
 regulation of G1/S transition of mitotic cell cycle
 chromatin remodeling
 positive regulation of cell differentiation
 positive regulation of T cell differentiation
 positive regulation of myoblast differentiation
 positive regulation of stem cell population mainten.

**D**

prol. 128 diff. 43



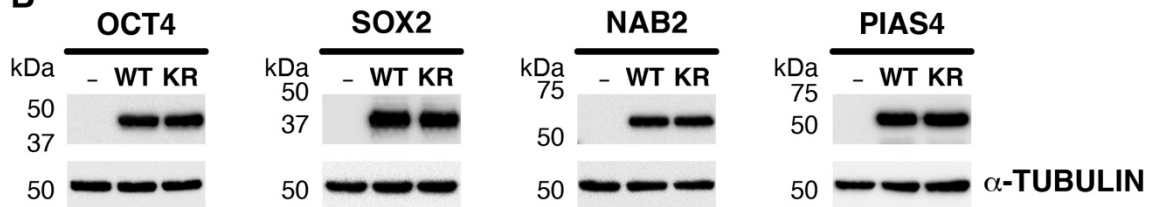
enrich.: 33.88 fold
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Supplementary Fig. S4 GO analysis of proteins experiencing sumoylation changes in response to OGD and ROG. **A** Gene ontology (GO) analysis of proteins increasing sumoylation in response to OGD in proliferating cells and subsequently decreasing it after ROG has been represented by bubble graphics. **B** Overlapping between proteins undergoing desumoylation in response to OGD (OGD vs. control, Ctrl) and those undergoing increasing sumoylation after ROG (OGD vs. ROG) in proliferating cells, has been represented by Venn diagrams. **C** Gene ontology (GO) analysis of proteins decreasing sumoylation in response to OGD in proliferating cells has been represented by bubble graphics. **A, C** Bubble size represents the number of proteins in each category, also indicated next to each bubble. p -value cutoff $2 \cdot 10^{-4}$. **D** Overlapping between proteins undergoing decreased sumoylation under ROG after OGD in proliferating (prol.) and differentiated (diff.) cells, has been represented by Venn diagrams. **B, D** Numbers on the top indicate the total number of proteins in each condition. Enrichment (enrich.) of the overlapping, together with the associated p -value, as determined by the hypergeometric test, is indicated below.

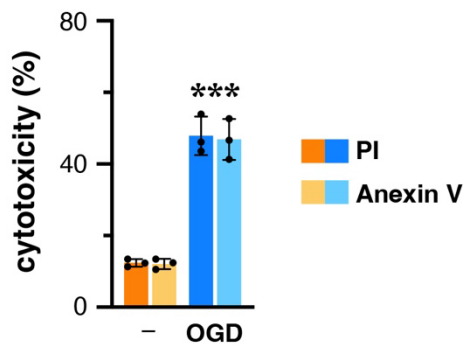
A

Protein	mutation	sequence	references
OCT4	K123R	DGASPEPCTVTPGAV K LEKEKLEQNPEESQD	[30, 31]
SOX2	K247R	QGTPGMALGSMGSSV K SEASSSPVVTSSSH	[29]
NAB2	K379R	RLHSEELGGPPLKKL K QEVGEQSHNEIQQPP	[22]
	K517R	PGPHPALVEGRRSSV K VEAEASRQ*	
PIAS4	K35R	LQMLLGFVGRSKSGL K HELVTRALQLVQFDC	[34]

B



C



Supplementary Fig. S5 Sumoylation mutants of selected factors for cytotoxicity analysis in response to OGD. **A** Schematic representation of the mutated Lys residue (K, red) and the surrounding amino acid sequence of the indicated proteins. References previously describing these mutations are also indicated. **B** Comparison of expression levels between wild-type (WT) and sumoylation mutant (KR) versions of the indicated proteins tagged with a Flag epitope at the N-terminus, determined in P19 cells by western blot with anti-Flag antibodies. As a negative control, extracts from cells transfected with empty vector (-) have been also analyzed. 20 µg of total protein were loaded per lane. -TUBULIN was registered as a loading marker. **C** The percentage of cytotoxicity was determined in proliferating cells transfected with the empty vector used for protein expression constructs (pAdRSV-Sp) subjected or not (control, -) to harmful OGD, by propidium iodide incorporation and Annexin V labeling. Values are means ± s.d. of three independent determinations. Statistical significance of the differences in cytotoxicity was analyzed by one-way ANOVA ($p < 0.05$) followed by Tukey's post-test. Differences with the corresponding controls are indicated on top of the bars. *** $p < 0.001$.