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Proteomics and Functional Investigation of SUMO and Ubiquitin E3 ligases

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ABBREVIATIONS

| | |
|-------|---|
| ABC | Ammonium Bicarbonate |
| AML | Acute Myeloid Leukemia |
| BARD1 | BRCA1-Associated RING Domain 1 |
| BCA | Bicinchoninic Acid |
| BioID | Proximity-dependent Biotin Identification |
| BrdU | 5'-bromo-2'-deoxyuridine |
| BRCA1 | Breast cancer susceptibility type 1 |
| ChIP | Chromatin Immunoprecipitation |
| CldU | 5-chloro-2'-deoxyuridine |
| CUL | Cullin |
| DDA | Data Dependent Acquisition |
| DDR | DNA Damage Response |
| DIA | Data Independent Acquisition |
| DiGly | Di Glycine (Ubiquitin tryptic remnant) |
| DMSO | Dimethyl Sulfoxide |
| DNA | Deoxyribonucleic acid |
| DTT | Dithiothreitol |
| DUB | Deubiquitinating Enzyme |
| Dox | Doxycycline |
| DSB | Double Strand Break |
| HR | Homologous Recombination |
| HU | Hydroxyurea |
| IdU | 5-Iodo-2'-deoxyuridine |

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|------------|--|
| iPOND | isolation of Proteins On Nascent DNA |
| IR | Ionizing Radiation |
| K | Lysine |
| LFQ | Label Free Quantification |
| LUBAC | Linear Ubiquitin Chain Assembly Complex |
| MoaD | Bacteria protein molybdopterin converting factor subunit 1 |
| MS | Mass Spectrometry |
| NHEJ | Non-Homologous End Joining |
| PARylation | ADP-ribosylation |
| PARPi | PARP inhibitor |
| PBS | Phosphate-Buffered Saline |
| PBST | PBS supplemented with 0.05% Tween 20 |
| PCNA | Proliferating Cell Nuclear Antigen |
| PIAS | Protein Inhibitor of Activated STAT |
| PINK1 | PTEN-induce putative kinase 1 |
| PRC1 | Polycomb Repressive Complex 1 |
| PTMs | Post Translational Modifications |
| RING | Really Interesting New Gene |
| RT | Room Temperature |
| SILAC | Stable Isotope Labeling by Amino acids in Cell culture |
| SIMs | SUMO Interacting Motifs |
| SSA | Single Strand Annealing |
| SSB | Single Strand Break |
| ssDNA | Single-Stranded DNA |
| SR | Substrate Receptor |

| | |
|--------|--|
| STUbLs | SUMO-targeted Ubiquitin Ligases |
| SUMO | Small Ubiquitin-Like Modifier |
| ThiS | Thiamine biosynthesis protein S |
| TLS | Trans-Lesion Synthesis |
| TS | Temple Switching |
| TULIP | Targets for Ubiquitin Ligases Identified by Proteomics |
| Ub | Ubiquitin |
| UBDs | Ubiquitin Binding Domains |
| Ubls | Ubiquitin-Like proteins |
| USP | Ubiquitin Proteasome System |
| UV | Ultraviolet Light |
| WB | Western Blot |
| WT | Wild Type |
| ZNF451 | Zinc finger 451 |



CURRICULUM VITAE

Daniel Salas Lloret was born on the 29th of June 1994 in Alicante (Spain), although he was raised in Alcázar de San Juan, Ciudad Real (Spain). It was there where he completed the International Bachillerato (High School). In 2012, he started his undergraduate studies in the field of Biochemistry with mention in Biotechnology at University of Castilla La-Mancha (UCLM) in Toledo, Spain. At the end of his undergraduate studies, he performed his bachelor thesis in the laboratory of Professor Christoph Wülfing in the School of Cellular and Molecular Medicine at Bristol University, UK. There, he studied the role of actin regulators at the immune synapse of CD4 and CD8 T-cells. Subsequently, he enrolled in a post-graduate Biotechnology MSc qualification at Autonomous University of Madrid (UAM) in Spain. His master thesis was completed in Dr. Mark J. van Raaij laboratory at National Center of Biotechnology (CNB-CSIC), where Daniel gained expertise in the field of protein origami working on the design of putative self-assembling viral proteins-based building blocks. After completing his MSc education, Daniel was awarded with an European funded grant to work in the laboratory of Professor Andrés Aguilera López in the department of Genetic Instability and Cancer at the Andalusian Molecular Biology and Regenerative Medicine Center (CABIMER). There, he learned how to work with yeast and developed a project focused on topoisomerases and genetic instability. In June 2018, Daniel joined Dr. Román González Prieto as PhD student funded by the Dutch Cancer Society (KWF) in the department of Cell and Chemical Biology at Leiden University Medical Center (LUMC) in The Netherlands. Here, he developed new mass-spectrometry technologies for the identification of E3 ligases substrates for both ubiquitin and small ubiquitin-like modifiers (SUMO). He employed this technology for the development of a comprehensive and interactive E3-specific SUMO proteome and studying the BRCA1-BARD1 E3 ligase for breast cancer vulnerabilities. During his PhD, Daniel attended several conferences and workshops around Europe where he presented his work through posters and oral presentations. In 2023, Daniel joined Professor Dr. Alfred Vertegaal laboratory as post-doctoral fellow.

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As every journey, the PhD also has an end, even if you do not believe it yet. You would agree with me that it is not a smooth journey and that it requires a lot of effort and dedication. Here, I want to make clear that not only in the PhD but in science in general, when there is success, it is never a one person achievement. Do not ever think that only one person can achieve something big in science. It is just not possible. Therefore, it would not be different in my case. I would like to thank everybody who has been involved in this journey and manifest that I could have never finished my PhD without them. There are that many people that I could not fit everyone in this section, thus I apologize to anyone left out.

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No tiene sentido escribir esta parte en inglés, por lo que iré directo al grano. A mi familia, muchas gracias por apoyarme desde el primer momento en hacer una tesis en el extranjero.

Sabíais que el doctorado iba a suponer verme menos, faltar en cumpleaños, viajes, rutas, actividades, comidas y una larga lista que, espero cambie gracias a la consecución de este título. Aun así, no dudasteis en ningún momento. Cada minuto que nos veíamos, ya fuera aquí en Los Países Bajos o en España, contaba el doble.

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LIST OF PUBLICATIONS

1. **D. Salas-Lloret**, G. Agabiti, R. Gonzalez-Prieto, TULIP2: An Improved Method for the Identification of Ubiquitin E3-Specific Targets. *Front Chem* 7, 802 (2019).
2. **D. Salas-Lloret**, R. Gonzalez-Prieto, Insights in Post-Translational Modifications: Ubiquitin and SUMO. *Int J Mol Sci* 23 (2022).
3. **D. Salas-Lloret** et al., SUMO-activated target traps (SATTs) enable the identification of a comprehensive E3-specific SUMO proteome. *Sci Adv* 9, eadh2073 (2023).
4. **D. Salas-Lloret** et al., BRCA1/BARD1 ubiquitinates PCNA in unperturbed conditions to promote replication fork stability and continuous DNA synthesis. *bioRxiv* 10.1101/2023.01.12.523782, 2023.2001.2012.523782 (2023). Under revision in *Mol. Cell* (2023)
5. Z. Yalcin, **D. Salas-Lloret** et al., Ubiquitinome Profiling Reveals in Vivo UBE2D3 Targets and Implicates UBE2D3 in Protein Quality Control. *Mol Cell Proteomics* 22, 100548 (2023).
6. **D. Salas-Lloret** and R. Gonzalez-Prieto, Unveiling BRCA1-BARD1 ubiquitin ligase heterodimer. *DNA repair, Ubiquitin and Cancer*. Revised in *DNA repair*, 2022.
7. Zeliha Yalcin, Shiu Yeung Lam, Marieke Peuscher, Jaco Torr, Prasanna Iyengar, **Daniel Salas-Lloret**, Inge de Krijger, Nathalie Moatti, Aurora Cerutti, Roman Gonzalez-Prieto, Jacqueline Jacobs, UBE2D3 facilitates NHEJ by orchestrating ATM signalling through multi-level control of RNF168. *Nat. Struct. Mol. Biol.* (2023). Under revision.

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