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

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 is 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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LIST OF PUBLICATIONS

1. **D. Salas-Lloret**, G. Agabitini, 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.