SUPPORTING INFORMATION

A Photo-Crosslinking Approach to Identify Class II SUMO-1 Binders

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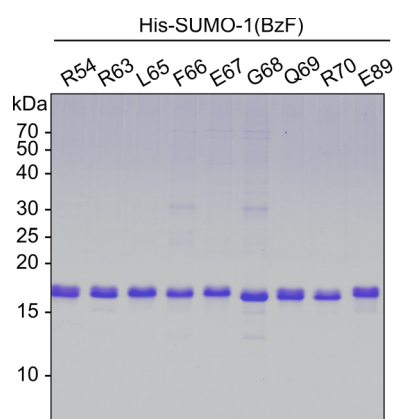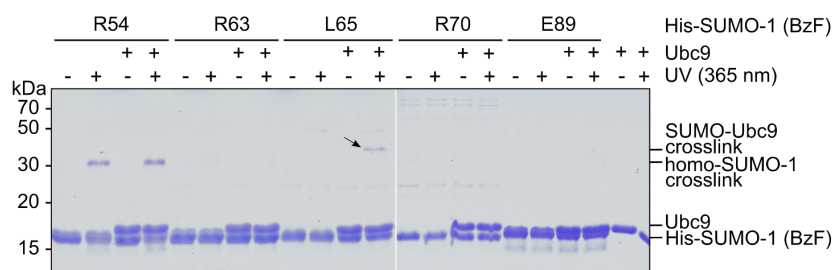
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Figure S1. SUMO-1 mutants. SUMO-1 mutants with BzF at different positions were recombinantly expressed in *E.coli* and purified by N-terminal His$_6$-tag using Ni-NTA and size exclusion chromatography.
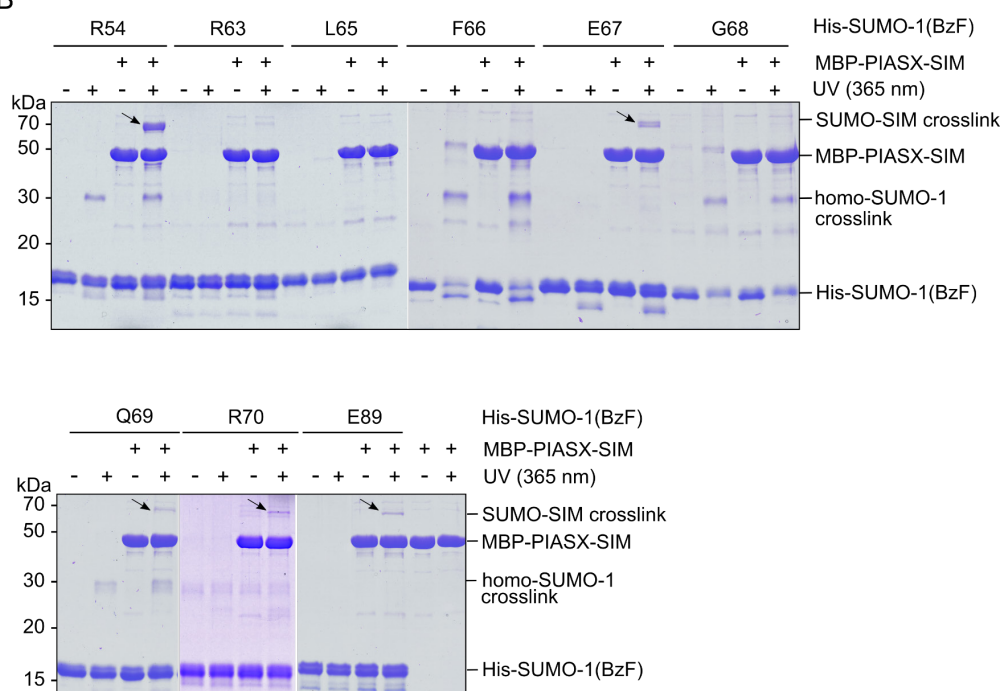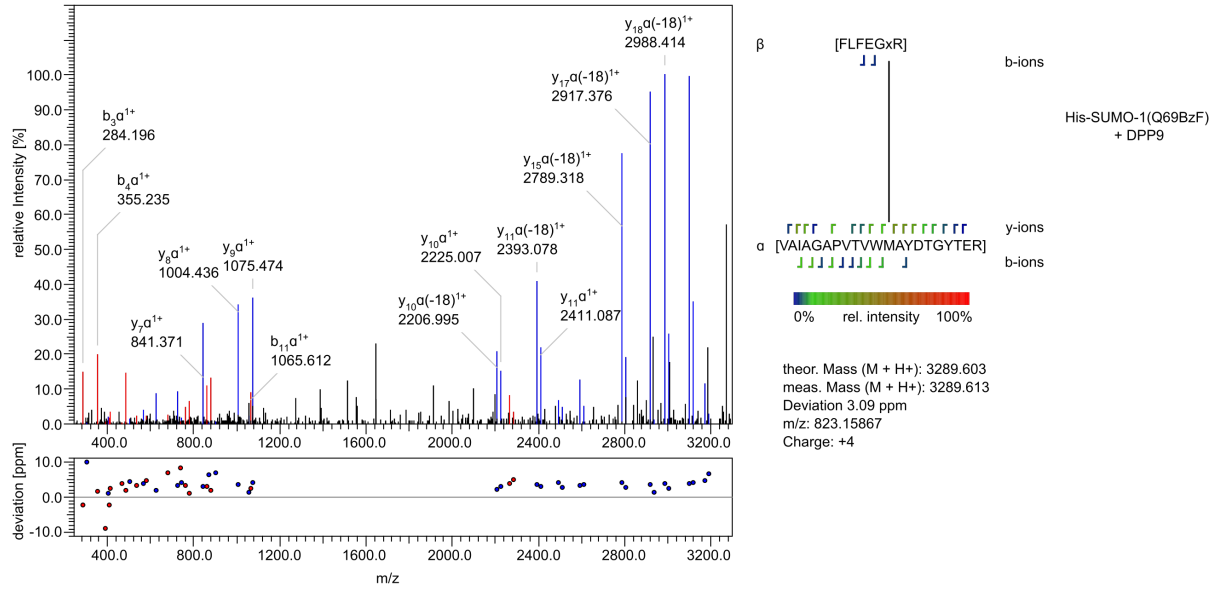

Figure S2. Photo-crosslinking of SUMO-1 mutants with Ubc9 (A) and MBP-PIASX-SIM (B). Proteins were incubated followed by UV-irradiated for 60 min. Samples before and after irradiation were analyzed by SDS-PAGE analysis and Coomassie staining.
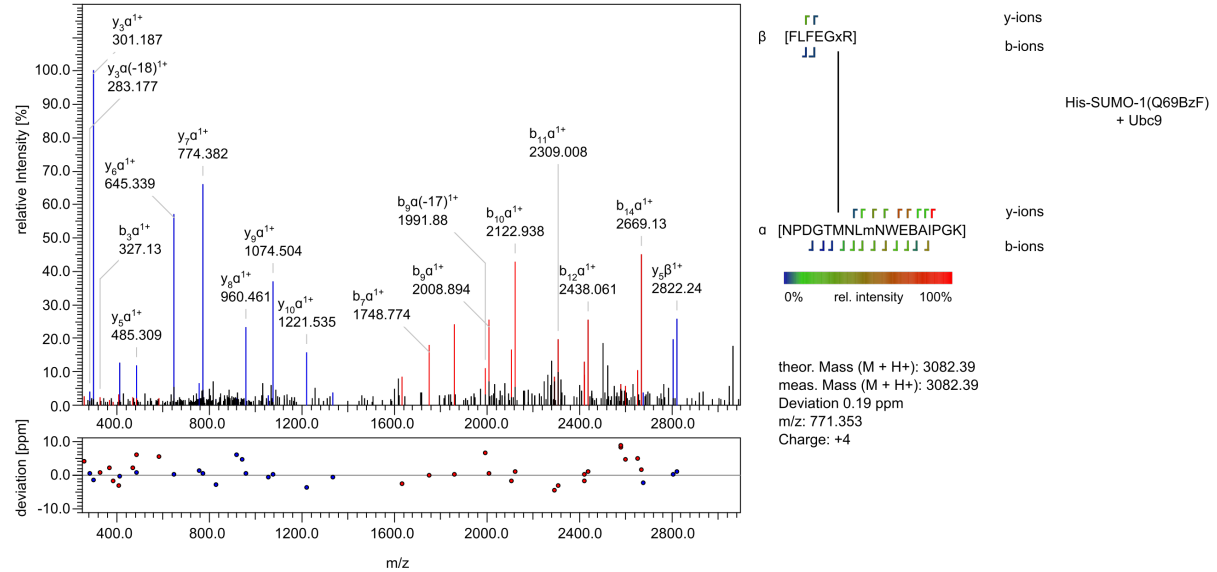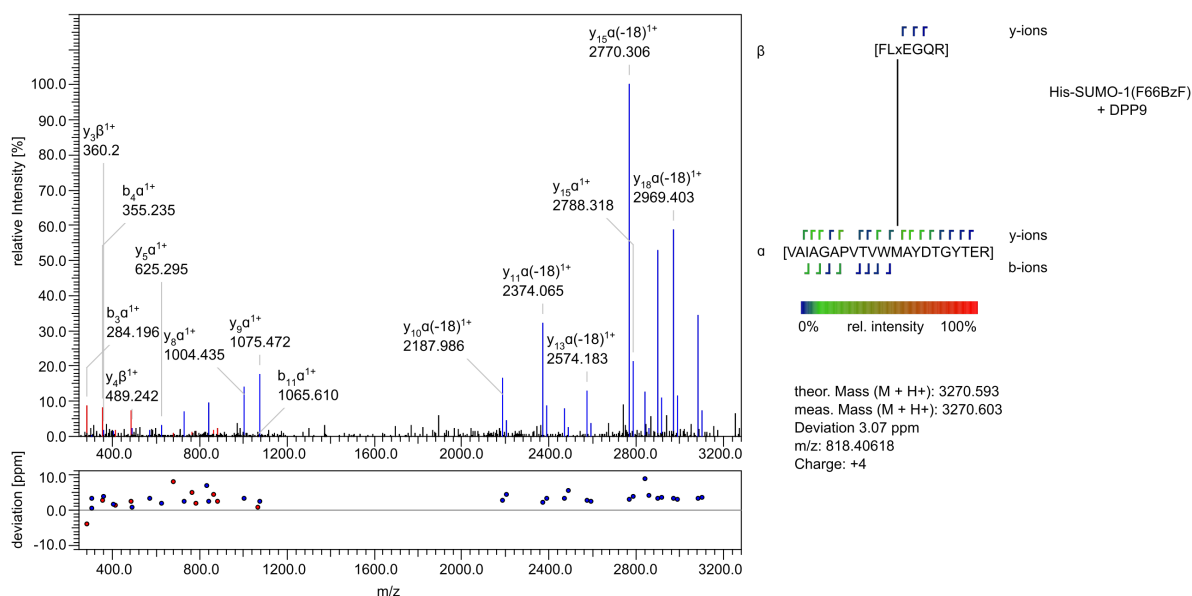
Figure S3. Analyses of photo-crosslinking products of His-SUMO-1(Q69BzF) with class II SUMO-1 binding partners DPP9 and Ubc9. (A) MS/MS spectra and fragmentation representation of the α-β crosslinked product of His-SUMO-1(Q69BzF) (β) and DPP9 (α) peptides. The precursor ion with a charge of +4 was measured at m/z 823.15867. The deviation of the singly charged precursor mass ([M+H$^+$]: 3289.613) from the theoretical singly charged crosslinked product ([M+H$^+$]: 3289.603) is 3.09 ppm. (B) MS/MS spectra and fragmentation representation of the α-β crosslinked product of His-SUMO-1(Q69BzF) (β) and Ubc9 (α) peptides. The precursor ion with a charge of +4 was measured at m/z 771.353. The deviation of the singly charged precursor mass ([M+H$^+$]: 3082.39) from the theoretical singly charged crosslinked product ([M+H$^+$]: 3082.39) is 0.19 ppm. The b- and y-type fragment ions of the peptide backbone are shown as red and blue peaks in the respective MS/MS spectra. Each of the depicted crosslink positions is one of the most likely positions hit by the photo-crosslinker, as can be determined from the accuracy of the data. 'x' represents photo-crosslinker BzF. 'm' indicates oxidized methionine.
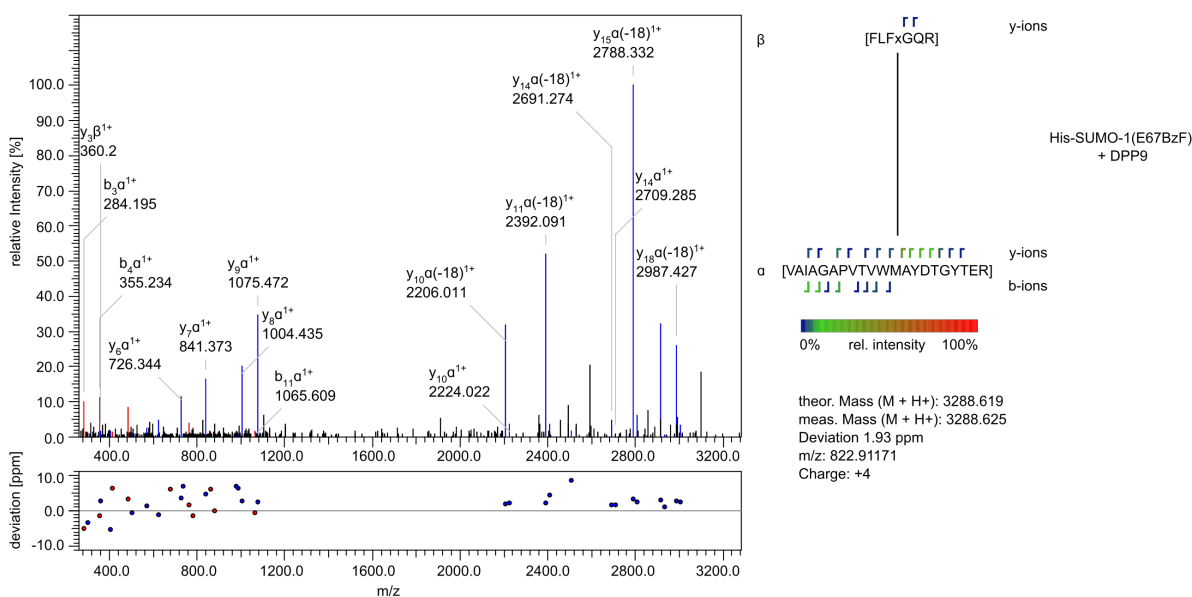
Figure S4. Analyses of photo-crosslinking products of His-SUMO-1(F66BzF) and His-SUMO-1(E67BzF) with DPP9. (A) MS/MS spectra and fragmentation representation of the α-β crosslinked product of His-SUMO-1(F66BzF) (β) and DPP9 (α) peptides. The precursor ion with a charge of +4 was measured at m/z 818.40618. The deviation of the singly charged precursor mass ([M+H$^+$]: 3270.603) from the theoretical singly charged crosslinked product ([M+H$^+$]: 3270.593) is 3.07 ppm. (B) MS/MS spectra and fragmentation representation of the α-β crosslinked product of His-SUMO-1(E67BzF) (β) and DPP9 (α) peptides. The precursor ion with a charge of +4 was measured at m/z 822.91171. The deviation of the singly charged precursor mass ([M+H$^+$]: 3288.625) from the theoretical singly charged crosslinked product ([M+H$^+$]: 3288.619) is 1.93 ppm. Each of the depicted crosslink positions is one of the most likely positions hit by the photo-crosslinker, as can be determined from the accuracy of the data. 'x' represents photo-crosslinker BzF.
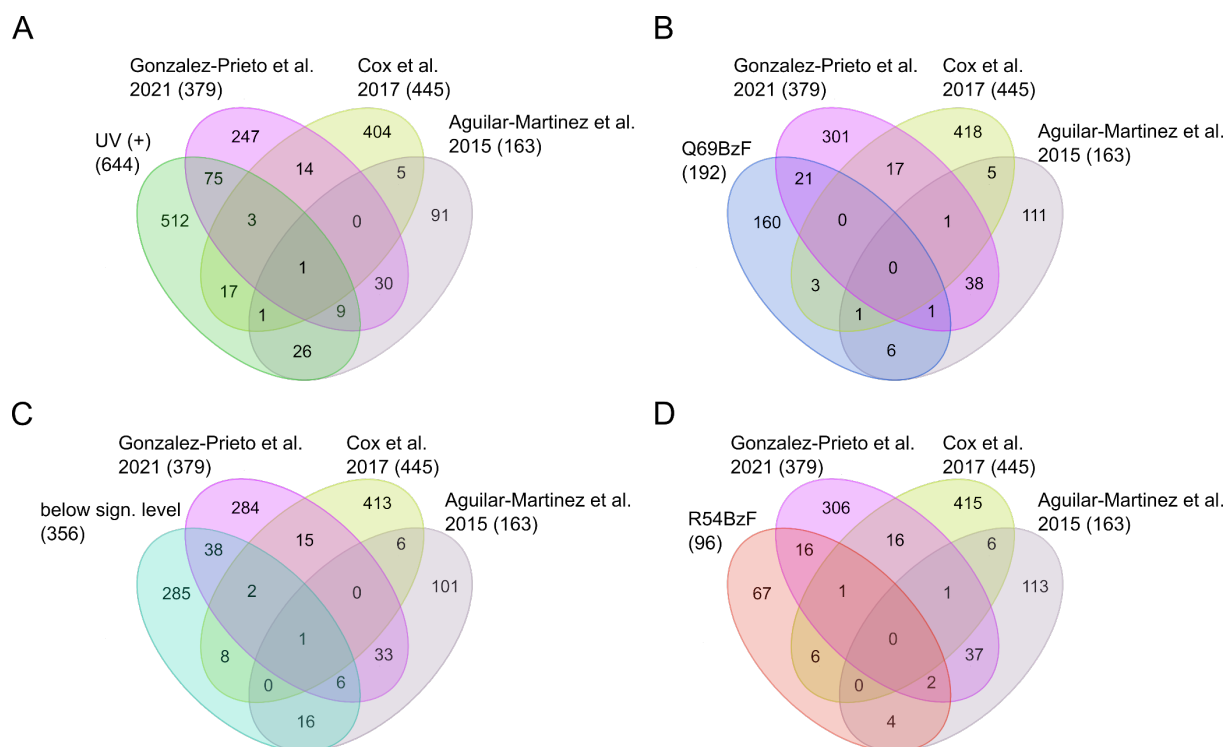
Figure S5. Comparison of all SUMO-binders identified in this study (A), proteins enriched by SUMO-1(Q69BzF) (B), proteins below the significance level (C) and proteins enriched by SUMO-1(R54BzF) (D) with SUMO interactors identified in the recent three major proteomic studies of (González-Prieto et al., 2021), (Cox et al., 2017) and (Aguilar-Martinez et al., 2015). For the comparison analyses, only proteins that could be unambiguously identified with Uniprot were considered.

Figure S6. Uncropped images of SDS-gels and blots in Fig. 2, Fig. 3, Fig. S1, Fig. S2.

SUPPORTING TABLES

Tables S1-S5 (Excel sheets) are provided as separate Supporting Information file.

Table S6: List of proteins recombinantly expressed in *E.coli* and their expression plasmids.

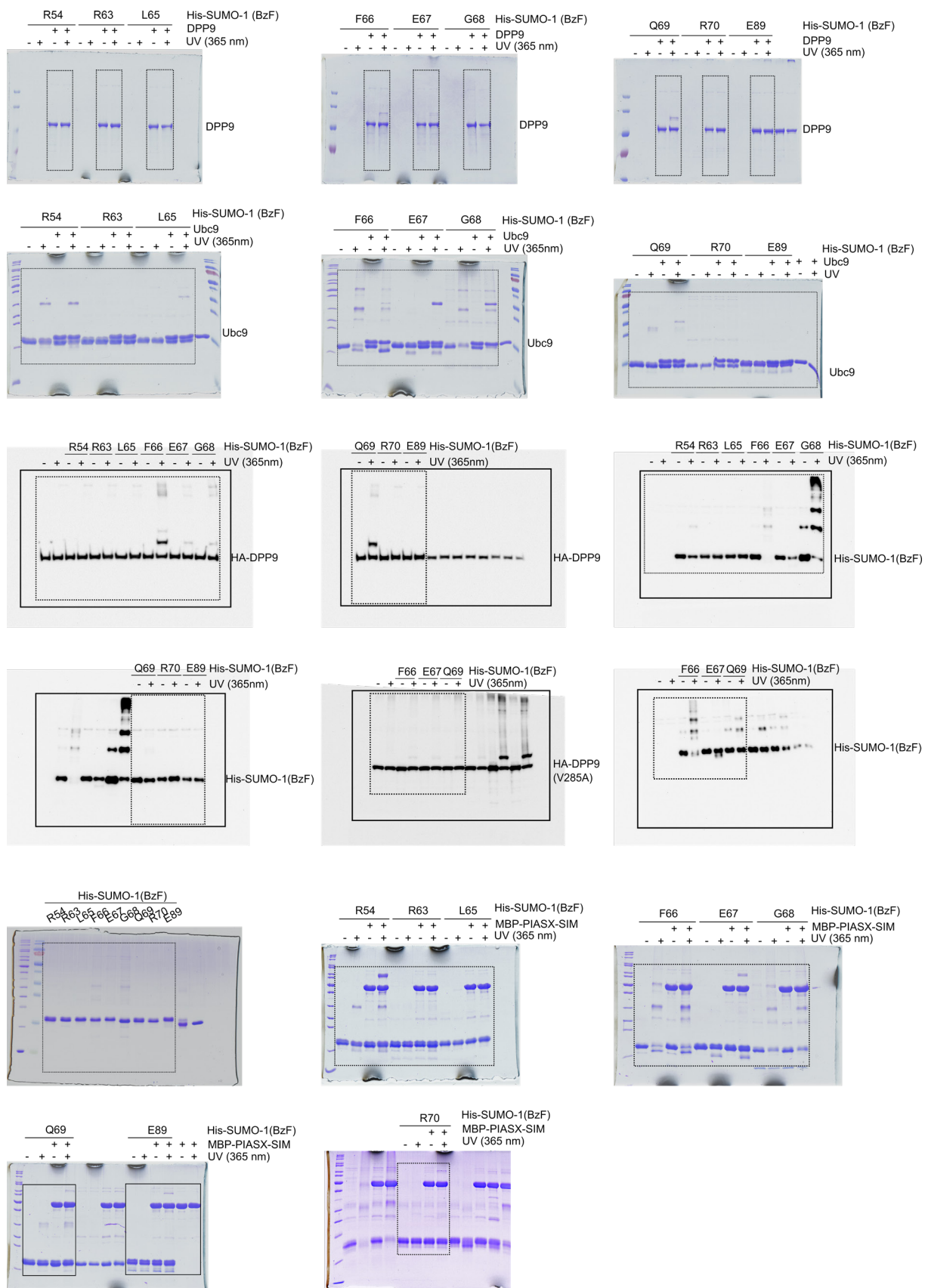| Name of construct	Plasmid name	Vector backbone
Met-Gly-Cys-Ser-His$_6$-SUMO-1(C52A, R54BzF)	pKBR42 + pEVOL(BzF)	pET28a
Met-Gly-Cys-Ser-His$_6$-SUMO-1(C52A, R63BzF)	pKBR105 + pEVOL(BzF)	pET28a
Met-Gly-Cys-Ser-His$_6$-SUMO-1(C52A, L65BzF)	pKBR106 + pEVOL(BzF)	pET28a
Met-Gly-Cys-Ser-His$_6$-SUMO-1(C52A, F66BzF)	pKBR111 + pEVOL(BzF)	pET28a
Met-Gly-Cys-Ser-His$_6$-SUMO-1(C52A, E67BzF)	pKBR112 + pEVOL(BzF)	pET28a
Met-Gly-Cys-Ser-His$_6$-SUMO-1(C52A, G68BzF)	pKBR113 + pEVOL(BzF)	pET28a
Met-Gly-Cys-Ser-His$_6$-SUMO-1(C52A, Q69BzF)	pKBR110 + pEVOL(BzF)	pET28a
Met-Gly-Cys-Ser-His$_6$-SUMO-1(C52A, R70BzF)	pKBR107 + pEVOL(BzF)	pET28a
Met-Gly-Cys-Ser-His$_6$-SUMO-1(C52A, E89BzF)	pKBR108 + pEVOL(BzF)	pET28a
Ubc9	pUbc9	pET23a (provided by (Flotho et al., 2012))
MBP-PIASX-SIM-His$_6$	pKT57	pMal-c2X

Table S7: Sequences of proteins used in this study.

Protein	Sequence
Met-Gly-Cys-Ser-His$_6$-SUMO-1(C52A, R54BzF)	MGCSHHHHHHGSDQEAKPSTEDLGDKKEGEYIKLK VIGQDSSEIHFKVKMTTHLKKLKESYAQ[BzF]QGVP MNSLRFLFEGQRIADNHTPKELGMEEEDVIEVYQEQ TGG
Met-Gly-Cys-Ser-His$_6$-SUMO-1(C52A, R63BzF)	MGCSHHHHHHGSDQEAKPSTEDLGDKKEGEYIKLK VIGQDSSEIHFKVKMTTHLKKLKESYAQRQGVPMNS L[BzF]FLFEGQRIADNHTPKELGMEEEDVIEVYQEQT GG
Met-Gly-Cys-Ser-His$_6$-SUMO-1(C52A, L65BzF)	MGCSHHHHHHGSDQEAKPSTEDLGDKKEGEYIKLK VIGQDSSEIHFKVKMTTHLKKLKESYAQRQGVPMNS LRF[BzF]FEGQRIADNHTPKELGMEEEDVIEVYQEQT GG
Met-Gly-Cys-Ser-His$_6$-SUMO-1(C52A, F66BzF)	MGCSHHHHHHGSDQEAKPSTEDLGDKKEGEYIKLK VIGQDSSEIHFKVKMTTHLKKLKESYAQRQGVPMNS LRFL[BzF]EGQRIADNHTPKELGMEEEDVIEVYQEQT GG
Met-Gly-Cys-Ser-His$_6$-SUMO-1(C52A, E67BzF)	MGCSHHHHHHGSDQEAKPSTEDLGDKKEGEYIKLK VIGQDSSEIHFKVKMTTHLKKLKESYAQRQGVPMNS LRFLF[BzF]GQRIADNHTPKELGMEEEDVIEVYQEQT GG
Met-Gly-Cys-Ser-His$_6$-SUMO-1(C52A, G68BzF)	MGCSHHHHHHGSDQEAKPSTEDLGDKKEGEYIKLK VIGQDSSEIHFKVKMTTHLKKLKESYAQRQGVPMNS LRFLFE[BzF]QRIADNHTPKELGMEEEDVIEVYQEQT GG
Met-Gly-Cys-Ser-His$_6$-SUMO-1(C52A, Q69BzF)	MGCSHHHHHHGSDQEAKPSTEDLGDKKEGEYIKLK VIGQDSSEIHFKVKMTTHLKKLKESYAQRQGVPMNS LRFLFEG[BzF]RIADNHTPKELGMEEEDVIEVYQEQT GG
Met-Gly-Cys-Ser-His$_6$-SUMO-1(C52A, R70BzF)	MGCSHHHHHHGSDQEAKPSTEDLGDKKEGEYIKLK VIGQDSSEIHFKVKMTTHLKKLKESYAQRQGVPMNS LRFLFEGQ[BzF]IADNHTPKELGMEEEDVIEVYQEQT GG
Met-Gly-Cys-Ser-His$_6$-SUMO-1(C52A, E89BzF)	MGCSHHHHHHGSDQEAKPSTEDLGDKKEGEYIKLK VIGQDSSEIHFKVKMTTHLKKLKESYAQRQGVPMNS LRFLFEGQRIADNHTPKELGMEEEDVI[BzF]VYQEQT GG
Ubc9	MSGIALSRLAQERKAWRKDHPFGFVAVPTKNPDGT MNLMNWECAIPGKKGTPWEGGLFKLRMLFKDDYP SSPPKCKFEPPLFHPNVYPSGTVCLSILEEDKDWRPAI TIKQILLGIQELLNEPNIQDPAQAEAYTIYCQNRVEYE KRVRAQAKKFAPS
MBP-PIASX-SIM-His$_6$	MKTEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIK VTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRFGGY AQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAY PIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKG KSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDI KDVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIA

	EAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLP TFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLL TDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAAT MENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQ TVDEALKDAQTNSSSNNNNNNNNNNLGIEGRISEFL VPRGSKKVDVIDLTIESSSDEKVDHHHHHH
His6-TEV-DPP9-S	MSYYHHHHHHDYDIPTTENLYFQGAMGSATTGTPT ADRGDAAATDDPAARFQVQKHSWDGLRSIIHGSRK YSGLIVNKAPHDFQFVQKTDESGPHSHRLYYLGMPY GSRENSLLYSEIPKKVRKEALLLLSWKQMLDHFQAT PHHGVYSREEELLRERKRLGVFGITSYDFHSESGLFL FQASNSLFHCRDGGKNGFMVSPMKPLEIKTQCSGPR MDPKICPADPAFFSFINNSDLWVANIETGEERRLTFC HQGLSNVLDDPKSAGVATFVIQEEFDRFTGYWWCP TASWEGSEGLKTLRILYEEVDESEVEVIHVPSPALEE RKTDSYRYPRTGSKNPKIALKLAEFQTDSQGKIVSTQ EKELVQPFSSLFPKVEYIARAGWTRDGKYAWAMFL DRPQQWLQLVLLPPALFIPSTENEEQRLASARAVPR NVQPYVVYEEVTNVWINVHDIFYPFPQSEGEDELCF LRANECKTGFCHLYKVTAVLKSQGYDWSEPFSPGE DEFKCPIKEEIALTSGEWEVLARHGSKIWVNEETKL VYFQGTKDTPLEHHLYVVSYEAAGEIVRLTTPGFSH SCSMSQNFDMFVSHYSSVSTPPCVHVYKLSGPDDDP LHKQPRFWASMMEAASCPPDYVPPEIFHFHTRSDVR LYGMIYKPHALQPGKKHPTVLFVYGGPQVQLVNNS FKGIKYLRLNTLASLGYAVVVIDGRGSCQRGLRFEG ALKNQMGQVEIEDQVEGLQFVAEKYGFIDLSRVAIH GWAYGGFLSLMGLIHKPQVFKVAIAGAPVTVWMA YDTGYTERYMDVPENNQHGYEAGSVALHVEKLPN EPNRLLILHGFLDENVHFFHTNFLVSQLIRAGKPYQL QIYPNERHSIRCPESGEHYEVTLLHFLQEYL
HA-DPP9	MASYPYDVPDYASLGSEFATTGTPTADRGDAAATD DPAARFQVQKHSWDGLRSIIHGSRKYSGLIVNKAPH DFQFVQKTDESGPHSHRLYYLGMPYGSRENSLLYSE IPKKVRKEALLLLSWKQMLDHFQATPHHGVYSREE ELLRERKRLGVFGITSYDFHSESGLFLFQASNSLFHC RDGGKNGFMVSPMKPLEIKTQCSGPRMDPKICPADP AFFSFINNSDLWVANIETGEERRLTFCHQGLSNVLDD PKSAGVATFVIQEEFDRFTGYWWCPTASWEGSEGL KTLRILYEEVDESEVEVIHVPSPALEERKTDSYRYPR TGSKNPKIALKLAEFQTDSQGKIVSTQEKELVQPFSS LFPKVEYIARAGWTRDGKYAWAMFLDRPQQWLQL VLLPPALFIPSTENEEQRLASARAVPRNVQPYVVYEE VTNVWINVHDIFYPFPQSEGEDELCFLRANECKTGF CHLYKVTAVLKSQGYDWSEPFSPGEDEFKCPIKEEIA LTSGEWEVLARHGSKIWVNEETKLVYFQGTKDTPL EHHLYVVSYEAAGEIVRLTTPGFSHSCSMSQNFDMF VSHYSSVSTPPCVHVYKLSGPDDDPLHKQPRFWAS MMEAASCPPDYVPPEIFHFHTRSDVRLYGMIYKPHA LQPGKKHPTVLFVYGGPQVQLVNNSFKGIKYLRLNT LASLGYAVVVIDGRGSCQRGLRFEGALKNQMGQVE

	IEDQVEGLQFVAEKYGFIDLSRVAIHGWSYGGFLSL MGLIHKPQVFKVAIAGAPVTVWMAYDTGYTERYM DVPENNQHGYEAGSVALHVEKLPNEPNRLLILHGFL DENVHFFHTNFLVSQLIRAGKPYQLQIYPNERHSIRC PESGEHYEVTLLHFLQEYL
HA-DPP9(V285A)	MASYPYDVPDYASLGSEFATTGTPTADRGDAAATD DPAARFQVQKHSWDGLRSIIHGSRKYSGLIVNKAPH DFQFVQKTDESGPHSHRLYYLGMPYGSRENSLLYSE IPKKVRKEALLLLSWKQMLDHFQATPHHGVYSREE ELLRERKRLGVFGITSYDFHSESGLFLFQASNSLFHC RDGGKNGFMVSPMKPLEIKTQCSGPRMDPKICPADP AFFSFINNSDLWVANIETGEERRLTFCHQGLSNVLDD PKSAGVATFVIQEEFDRFTGYWWCPTASWEGSEGL KTLRILYEEVDESEAEVIHVPSPALEERKTDSYRYPR TGSKNPKIALKLAEFQTDSQGKIVSTQEKELVQPFSS LFPKVEYIARAGWTRDGKYAWAMFLDRPQQWLQL VLLPPALFIPSTENEEQRLASARAVPRNVQPYVVYEE VTNVWINVHDIFYPFPQSEGEDELCFLRANECKTGF CHLYKVTAVLKSQGYDWSEPFSPGEDEFKCPIKEEIA LTSGEWEVLARHGSKIWVNEETKLVYFQGTKDTPL EHHLYVVSYEAAGEIVRLTTPGFSHSCSMSQNFDMF VSHYSSVSTPPCVHVYKLSGPDDDPLHKQPRFWAS MMEAASCPPDYVPPEIFHFHTRSDVRLYGMIYKPHA LQPGKKHPTVLFVYGGPQVQLVNNSFKGIKYLRLNT LASLGYAVVVIDGRGSCQRGLRFEGALKNQMGQVE IEDQVEGLQFVAEKYGFIDLSRVAIHGWSYGGFLSL MGLIHKPQVFKVAIAGAPVTVWMAYDTGYTERYM DVPENNQHGYEAGSVALHVEKLPNEPNRLLILHGFL DENVHFFHTNFLVSQLIRAGKPYQLQIYPNERHSIRC PESGEHYEVTLLHFLQEYL

Table S8: DPP9 plasmid information for expression in SF9 insect cells.

Name of construct	Plasmid name	Vector backbone
His$_6$-TEV-DPP9-short	Human DPP9-short	pFASTBacHT

Table S9: List of plasmids used for transfection.

Name of construct	Plasmid name	Vector backbone
HA-DPP9	pDPP9short	pcDNA3.1 (provided by (Pilla et al., 2012))
HA-DPP9(V285A)	pKBR119	pcDNA3.1

Supporting References

Aguilar-Martinez, E., Chen, X., Webber, A., Mould, A. P., Seifert, A., Hay, R. T., et al. (2015). Screen for multi-SUMO-binding proteins reveals a multi-SIM-binding mechanism for recruitment of the transcriptional regulator ZMYM2 to chromatin. *Prot. Natl. Acad. Sci. U. S. A.* 112, E4854-63. doi: 10.1073/pnas.1509716112

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Flotho, A., Werner, A., Winter, T., Frank, A. S., Ehret, H., and Melchior, F. (2012). Recombinant reconstitution of sumoylation reactions in vitro. *Methods Mol. Biol.* 832, 93–110. doi: 10.1007/978-1-61779-474-2_5

González-Prieto, R., Eifler-Olivi, K., Claessens, L. A., Willemstein, E., Xiao, Z., Talavera Ormeno, C. M. P., et al. (2021). Global non-covalent SUMO interaction networks reveal SUMO-dependent stabilization of the non-homologous end joining complex. *Cell Rep.* 34, 108691. doi: 10.1016/j.celrep.2021.108691

Pilla, E., Möller, U., Sauer, G., Mattiroli, F., Melchior, F., and Geiss-Friedlander, R. (2012). A novel SUMO1-specific interacting motif in dipeptidyl peptidase 9 (DPP9) that is important for enzymatic regulation. *J. Biol. Chem.* 287, 44320–44329. doi: 10.1074/jbc.M112.397224