Supplementary Information

Screening method for SUMOylated isopeptides by ion mobility mass spectrometry

Quentin Dumont, David L. Donaldson and Wendell P. Griffith✉

Department of Chemistry, University of Toledo, Toledo, Ohio 43606

Supplementary Table 1. List of the major linear peptides detected. These peptides are indicated on the mass spectrum in Figure 1 of the main text. m/z: experimentally measured *m/z*: value of peptide ion; *z*: charge of peptide ion; $M_{calculated}$: neutral mass of peptide calculated from the monoisotopic experimentally measured *m/z* value; $M_{theoretical}$: neutral mass of peptide calculated from sequence; ΔM: monoisotopic mass error of measurement.

#	Sequence position	*m/z*	*z*	$M_{calculated}$	$M_{theoretical}$	ΔM	Sequence
1	12-20	535.2824	+2	1068.5648	1068.4909	0.0739	TENNDHINL
2	22-32	553.8039	+2	1105.6078	1105.5477	0.0601	VAGQDGSVVQF
3	12-21	599.3212	+2	1196.6424	1196.5858	0.0566	TENNDHINLK
4	21-32 22-33	617.8597	+2	1233.7194	1233.6426	0.0768	KVAGQDGSVVQF VAGQDGSVVQFK
5	77-87	671.8137	+2	1341.6274	1341.5355	0.0919	EMEDEDTIDVF
6	63-76	749.8804	+2	1497.7608	1497.7020	0.0588	DGQPINETDTPAQL
7	62-76	823.4124	+2	1644.8248	1644.7704	0.0544	FDGQPINETDTPAQL
8	61-76	901.4847	+2	1800.9694	1800.8715	0.0979	RFDGQPINETDTPAQL
9	22-32	1106.6132	+1	1105.6132	1105.5477	0.0655	VAGQDGSVVQF
10	44-53	1198.6930	+1	1197.6930	1197.5707	0.1223	MKAYCERQGL
11	77-87	1342.6472	+1	1341.6472	1341.5355	0.1117	EMEDEDTIDVF
12	63-87	1411.6888	+2	2821.3776	2821.2197	0.1579	DGQPINETDTPAQLEMEDEDTIDVF
13	62-87	1485.2484	+2	2968.4968	2968.2881	0.2087	FDGQPINETDTPAQLEMEDEDTIDVF
14	61-87	1563.3177	+2	3124.6354	3124.3892	0.2462	RFDGQPINETDTPAQLEMEDEDTIDVF

Supplementary Figure 1. Amino acid sequence of human SUMO2 (SWISSPROT ID: P61956). Sequence covered by MS is indicated as underlined.

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 1 MADEKPKEGV KTENNDHINL KVAGQDGSVV QFKIKRHTPL SKLMKAYCER

51 QGLSVRQIRF RFDGQPINET DTPAQLEMED EDTIDVFQQQ TGG
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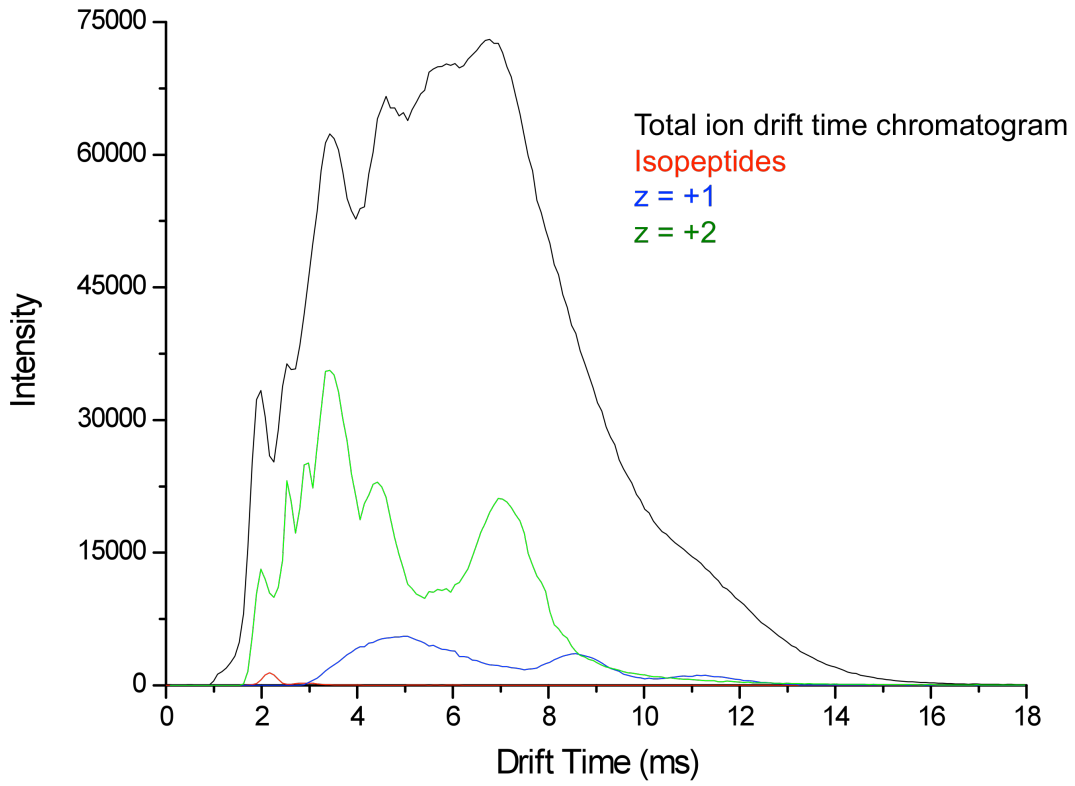
Supplementary Table 2. List of the SUMO isopeptides peptides detected using ion mobility mass spectrometry. These peptides are indicated on the mass spectrum in Figure 4 of the main text.

#	*m/z*	*z*	$M_{calculated}$	$M_{theoretical}$	ΔM
1	689.0397	+3	2064.0957	2063.9946	0.1011
2	694.6915	+3	2081.0511	2080.9773	0.0738
3	731.7465	+3	2192.2240	2192.0696	0.1544
4	737.4106	+3	2209.2084	2209.0723	0.1361

Supplementary Figure 2. Drift time total ion chromatogram (black trace) of peptides detected from the trypsin/chymotrypsin proteolysis of poly-SUMO-2. Extracted drift time ion chromatograms of only larger more highly charged isopeptides with z ≥ +3 (red trace), peptides with z = +1 (blue trace) and z = +2 (green trace).