

## **Supporting Information for:**

### **Large-Scale Filter-Aided Sample Preparation Method (LFASP) for the Analysis of the Ubiquitinome**

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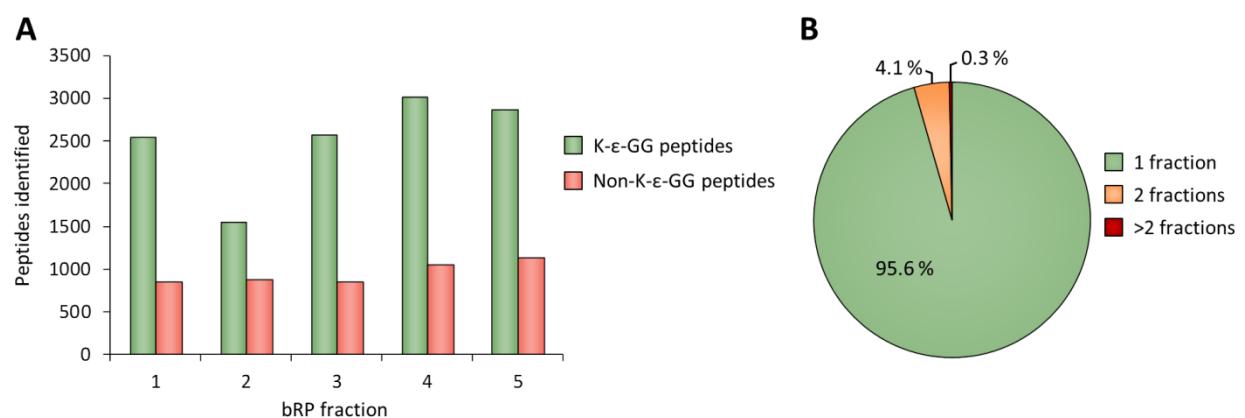
**Figure S1. Analysis of K-ε-GG peptide data.**

**Figure S2. Digestion efficiency of LFASP at different trypsin-to-protein ratios**

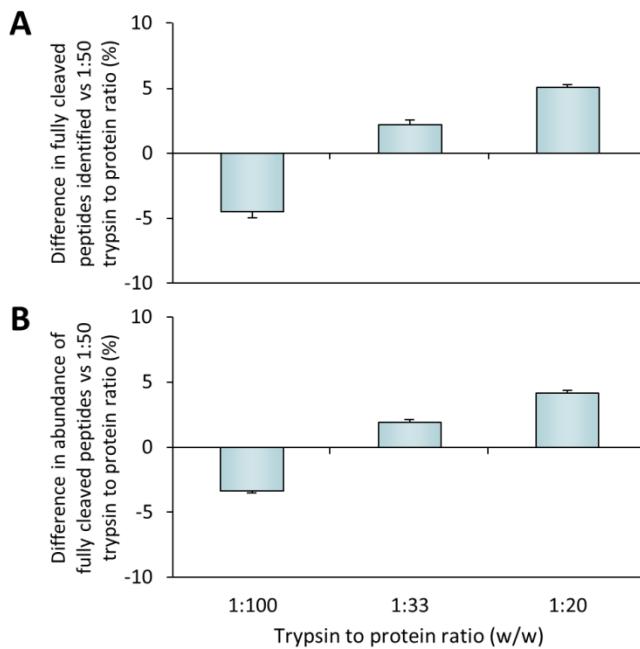
**Figure S3. Comparison of dynamic ranges of the comprehensive proteomic analysis in this study and the data set by Udeshi et al. 2013.**

**Table S1. Raw files from state-of-the-art ubiquitinome studies used for comparison with LFASP (this study).**

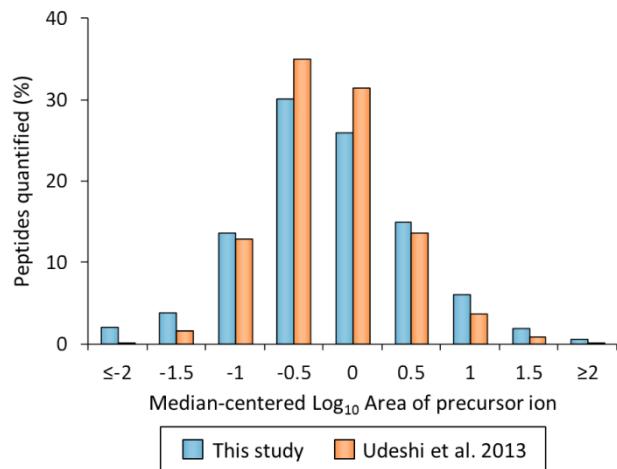
**Table S2. Peptides identified in raw files from this and state-of-the-art ubiquitinome studies.**



**Figure S1. Analysis of K-ε-GG peptide data.** (A) Number of K-ε-GG peptides and non-K-ε-GG peptides in five fractions derived from non-contiguous combination of 25 bRP fractions (see Figure 1). (B) Percentage of K-ε-GG peptides identified in 1, 2 or >2 bRP fractions.



**Figure S2. Digestion efficiency of LFASP at different trypsin-to-protein ratios.** Jurkat cells were lysed and digested by LFASP using different trypsin-to-protein ratios (1:100, 1:50, 1:33 and 1:20, w/w). The difference in the percentage of identifications (A) or abundance (B) of fully cleaved peptides is compared to the 1:50 (w/w) trypsin-to-protein ratio employed in the ubiquitinome studies used for comparison with LFASP (Udeshi et al. 2013, Quadroni et al. 2015). The results are expressed as the mean  $\pm$  SD of three replicate digestions per trypsin-to-protein ratio.



**Figure S3. Comparison of dynamic ranges of the comprehensive proteomic analysis in this study and the data set by Udeshi et al. 2013.** Peptide abundance data were median-centered for comparison of dynamic ranges between data sets.

**Table S1. Raw files from state-of-the-art ubiquitinome studies used for comparison with LFASP (this study).**

Raw file name	Study
JurkatRep01_KGGIP_L-noM-PR619H-MG132_2i_bRPFxn01	Udeshi et al. 2013
JurkatRep01_KGGIP_L-noM-PR619H-MG132_2i_bRPFxn02	Udeshi et al. 2013
JurkatRep01_KGGIP_L-noM-PR619H-MG132_2i_bRPFxn03	Udeshi et al. 2013
JurkatRep01_KGGIP_L-noM-PR619H-MG132_2i_bRPFxn04	Udeshi et al. 2013
JurkatRep01_KGGIP_L-noM-PR619H-MG132_2i_bRPFxn05	Udeshi et al. 2013
JurkatRep01_KGGIP_L-noM-PR619H-MG132_2i_bRPFxn06	Udeshi et al. 2013
JurkatRep01_KGGIP_L-noM-PR619H-MG132_2i_bRPFxn07	Udeshi et al. 2013
JurkatRep01_KGGIP_L-noM-PR619H-MG132_2i_bRPFxn08	Udeshi et al. 2013
JurkatRep02_KGGIP_L-MG132M-noH-PR619_bRPFxn01	Udeshi et al. 2013
JurkatRep02_KGGIP_L-MG132M-noH-PR619_bRPFxn02	Udeshi et al. 2013
JurkatRep02_KGGIP_L-MG132M-noH-PR619_bRPFxn03	Udeshi et al. 2013
JurkatRep02_KGGIP_L-MG132M-noH-PR619_bRPFxn04	Udeshi et al. 2013
JurkatRep02_KGGIP_L-MG132M-noH-PR619_bRPFxn05	Udeshi et al. 2013
JurkatRep02_KGGIP_L-MG132M-noH-PR619_bRPFxn06	Udeshi et al. 2013
JurkatRep02_KGGIP_L-MG132M-noH-PR619_bRPFxn07	Udeshi et al. 2013
JurkatRep02_KGGIP_L-MG132M-noH-PR619_bRPFxn08	Udeshi et al. 2013
JurkatRep02_KGGIP_L-MG132M-noH-PR619_bRPFxn09	Udeshi et al. 2013
JurkatRep03_KGGIP_L-MG132_M-No_H-PR619_bRPFxn01	Udeshi et al. 2013
JurkatRep03_KGGIP_L-MG132_M-No_H-PR619_bRPFxn02	Udeshi et al. 2013
JurkatRep03_KGGIP_L-MG132_M-No_H-PR619_bRPFxn03	Udeshi et al. 2013
JurkatRep03_KGGIP_L-MG132_M-No_H-PR619_bRPFxn04	Udeshi et al. 2013
JurkatRep03_KGGIP_L-MG132_M-No_H-PR619_bRPFxn05	Udeshi et al. 2013
JurkatRep03_KGGIP_L-MG132_M-No_H-PR619_bRPFxn06	Udeshi et al. 2013
JurkatRep03_KGGIP_L-MG132_M-No_H-PR619_bRPFxn07	Udeshi et al. 2013
JurkatRep03_KGGIP_L-MG132_M-No_H-PR619_bRPFxn08	Udeshi et al. 2013
151013_6225-1	Quadroni et al. 2015
151013_6225-2	Quadroni et al. 2015
151013_6226-1	Quadroni et al. 2015
151013_6226-2	Quadroni et al. 2015
151013_6227-1	Quadroni et al. 2015
151013_6227-2	Quadroni et al. 2015

**Table S2. Peptides identified in raw files from this and state-of-the-art ubiquitinome studies.**

# K-ε-GG sites	# missed cleavages	Study								
		This study			Udeshi et al. 2013			Quadroni et al. 2015		
		Peptides identified	% peptides identified <sup>1</sup>	% peptide abundance <sup>1</sup>	Peptides identified	% peptides identified <sup>1</sup>	% peptide abundance <sup>1</sup>	Peptides identified	% peptides identified <sup>1</sup>	% peptide abundance <sup>1</sup>
0	0	3885	91.0	93.6	15955	71.0	72.3	2650	60.5	56.5
	1	353	8.3	6.2	5365	23.9	22.7	1343	30.7	31.6
	2	24	0.6	0.1	971	4.3	4.3	317	7.2	10.5
	3	6	0.1	0.1	167	0.7	0.7	67	1.5	1.4
1	0	465	4.0	3.8	2323	4.4	4.6	145	4.1	3.5
	1	10448	89.9	91.2	40975	78.2	81.3	2559	71.9	64.2
	2	673	5.8	4.7	8180	15.6	12.8	733	20.6	27.1
	3	32	0.3	0.2	908	1.7	1.4	123	3.5	5.2
2	0	-	-	-	1	0.2	0.1	-	-	-
	1	33	9.2	7.6	61	10.5	11.1	3	7.5	2.4
	2	312	87.4	90.7	470	80.8	80.3	30	75.0	59.1
	3	12	3.4	1.7	50	8.6	8.5	7	17.5	38.5
3	2	2	15.4	9.9	3	50.0	44.9	-	-	-
	3	11	84.6	90.1	3	50.0	55.1	1	100.0	100.0

<sup>1</sup>Percentage calculated for peptides with the same number of K-ε-GG sites.