Supplementary Information

Site-Specific Quantification of Protein Ubiquitination on MS2 Fragment Ion Level via Isobaric Peptide Labeling

Ting Cao¹, Lei Zhang², Ying Zhang², Guoquan Yan^{1,2}, Caiyun Fang¹, Huimin Bao¹, Haojie Lu^{1,2*}

¹ Shanghai Cancer Center and Department of Chemistry, Fudan University, Shanghai 200032, P. R. China

² Institutes of Biomedical Sciences and Key Laboratory of Glycoconjugates Research Ministry of Public Health, Fudan University, Shanghai 200032, P. R. China

E-mail: luhaojie@fudan.edu.cn.

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Table S-1. SILAC labeling efficiency of MCF-7 cells.

Table S-2. Labeling efficiency of peptide N-terminus dimethylation.

Excel S-1. K-ε-GG peptides identified in ubiquitinated proteome.

Excel S-2. Peptides identified in supernatant proteome.

Excel S-3. K-ɛ-GG peptides quantified in ubiquitinated proteome.

Excel S-4. Peptides quantified in supernatant proteome.

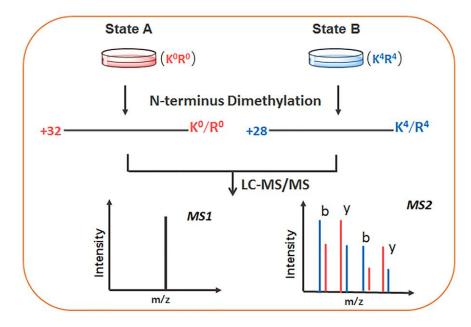


Figure S-1. Schematic diagram of quantification of the supernatant proteome after K-E-GG enrichment.

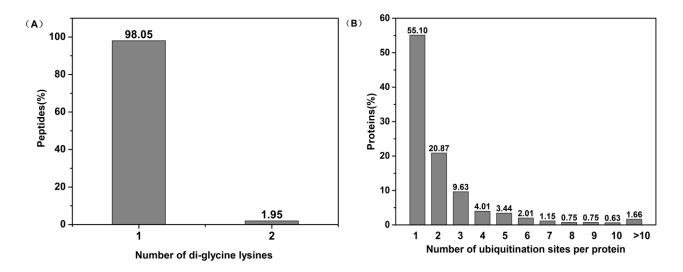


Figure S-2. (A) Fraction of peptides with one or two di-glycine-lysines in the enriched dataset. (B) The distribution of proteins with different numbers of ubiquitination sites.

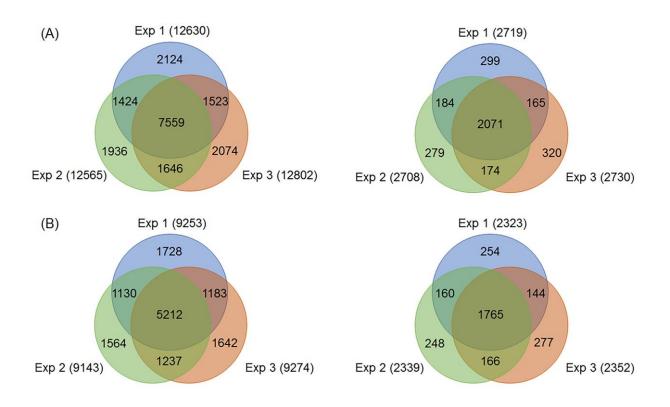


Figure S-3 Identification and quantification of supernatant proteome. Venn diagram showing the overlap of identified peptides (A, left) and proteins (A, right), quantified peptides (B, left) and proteins (B, right).

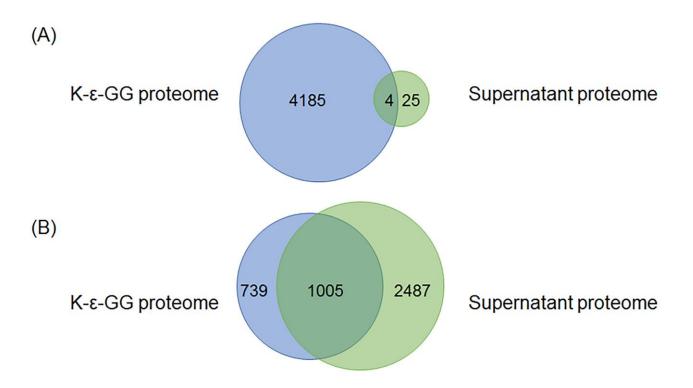


Figure S-4. Overlap of K- ϵ -GG peptides (A) and proteins (B) identified in K- ϵ -GG modified proteome and supernatant proteome.

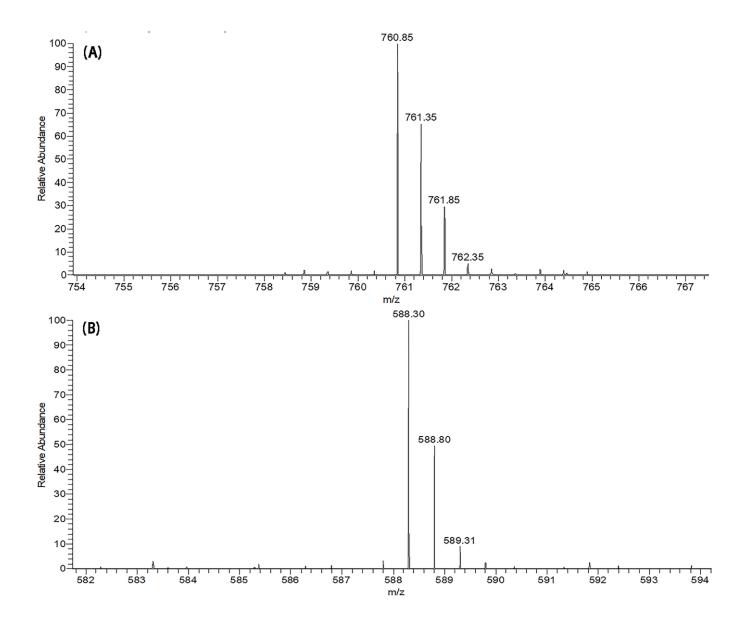


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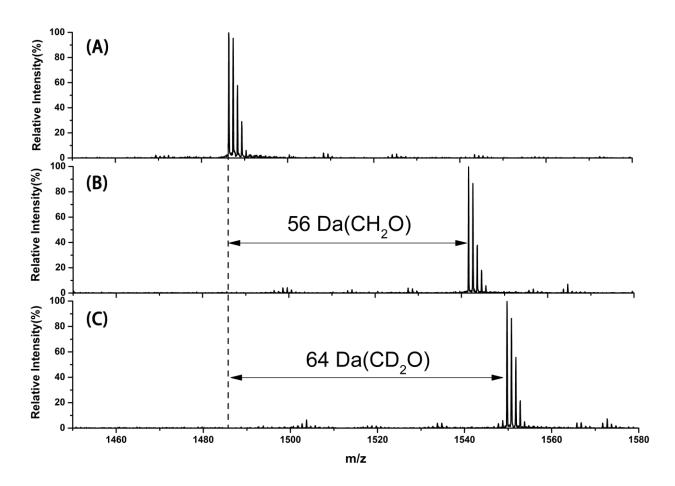


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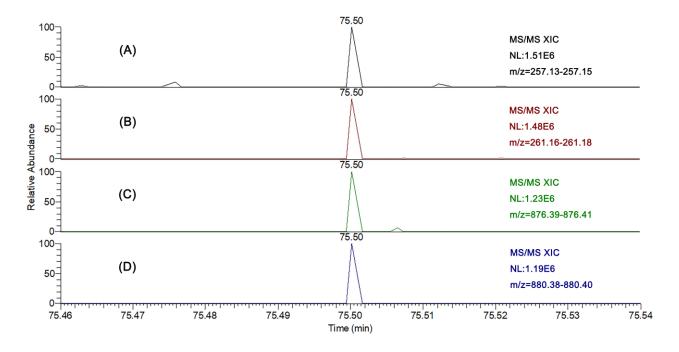


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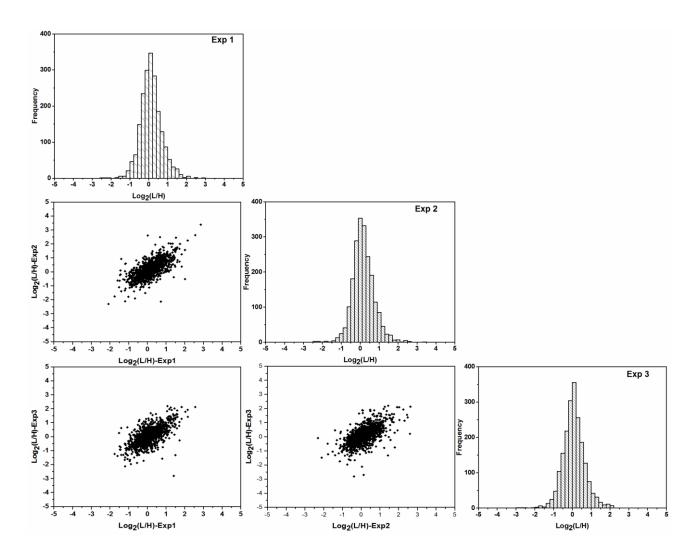


Figure S-8. Scatterplots and histograms for quantified K-ε-GG peptide ratios from experiment 1 versus 2 versus 3.

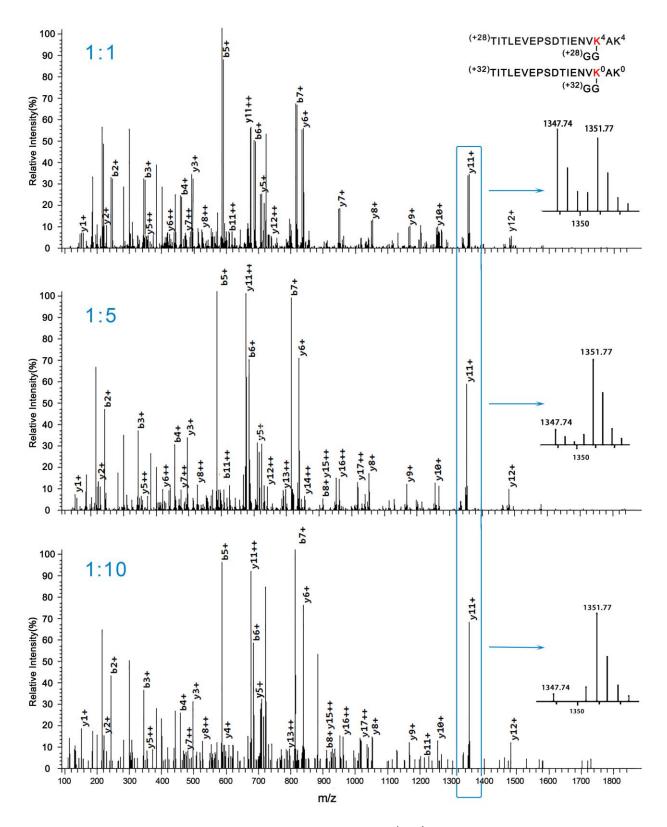


Figure S-9. MS/MS spectra of the peptide TITLEVEPSDTIENVK(GG)AK with the ratio of 1:1, 1:5 and 1:10.

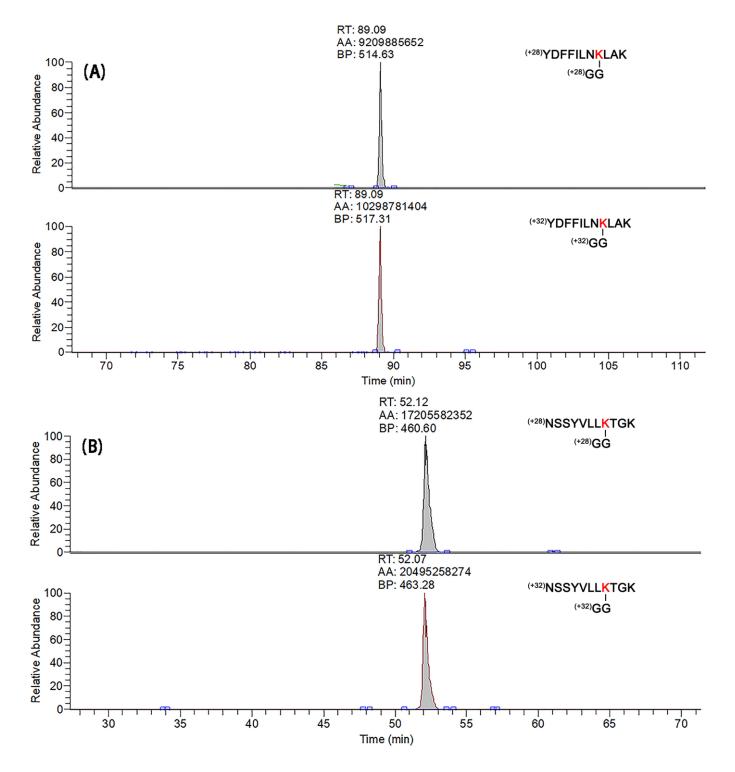


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Table S-1. SILAC labeling efficiency of MCF-7 cells.

ſ	Identified peptides	Identified peptides with R ⁴ or K ⁴	Labeled ratio (%)
	12147	12088	99.5

Table S-2. Labeling efficiency of peptide N-terminus dimethylation.

	Labeled N-terminus	Non-labeled N-terminus	Labeled ratio (%)
Supernatant 1	11857	80	99.3
Supernatant 2	11797	71	99.4
Supernatant 3	12106	94	99.2
Elute 1	2381	22	99.1
Elute 2	2511	18	99.3
Elute 3	2218	21	99.1