**SUPPLEMENTARY FIGURE LEGENDS**

**Figure S1** Distribution of SILAC ratios and calibration curves determine proper amount of SILAC standard for LCM samples. (A) Histograms of $Log_2$ (peptide/protein SILAC ratio) from LCM samples spiked with 50, 100 and 400 ng of SILAC standard. Distance from the center represents a skewed ratio between sample and SILAC standard; (B) Calibration curves were constructed from median ratio of quantified peptides and proteins from triplicate LCM samples spiked with 50, 100, 200 and 400 ng of SILAC standard (blue dots with black error bars). LCM samples with 200 ng of SILAC standard resulted in $Log_2$ (SILAC ratio) close to 0 (calculated 1:1 ratio, green dots) at both peptide and protein level, indicating approximate 1:1 ratio between tumor tissues and SILAC standard.

**Figure S2** Evaluation of LFQ and SILAC based quantification in WTL samples. (A) CVs of peptide and protein ratios quantified from quadruplicate WTL samples (only peptides quantified in ≥3 measurements were considered); (B) Number of quantified peptides and proteins from quadruplicate WTL samples. (**: $P<0.01$, ****: $P<0.0001$).

**Figure S3** Reproducibility of peptides (A) and proteins (B) quantified by shotgun global proteome profiling in replicate LCM samples (n=4), replicate WTL samples (n=4) and experimental LCM samples (n=8) (obs/observation: number of times which a given peptide was quantified by LC-MS/MS).

**Figure S4** Enriched KEGG pathways between ER+ and ER- breast tumor tissues were identified from LFQ quantitative data of 8 experimental LCM samples using GSEA software. (A) Some enriched pathways share the same set of differentially expressed proteins and therefore cluster together. Two clusters were selected for further investigation, including an ER+ region (red rectangle) and an ER- (blue rectangle); (B) Enrichment plots provide detailed enrichment information of two representative KEGG pathways between ER+ and ER- breast tumor tissues from the two clusters.

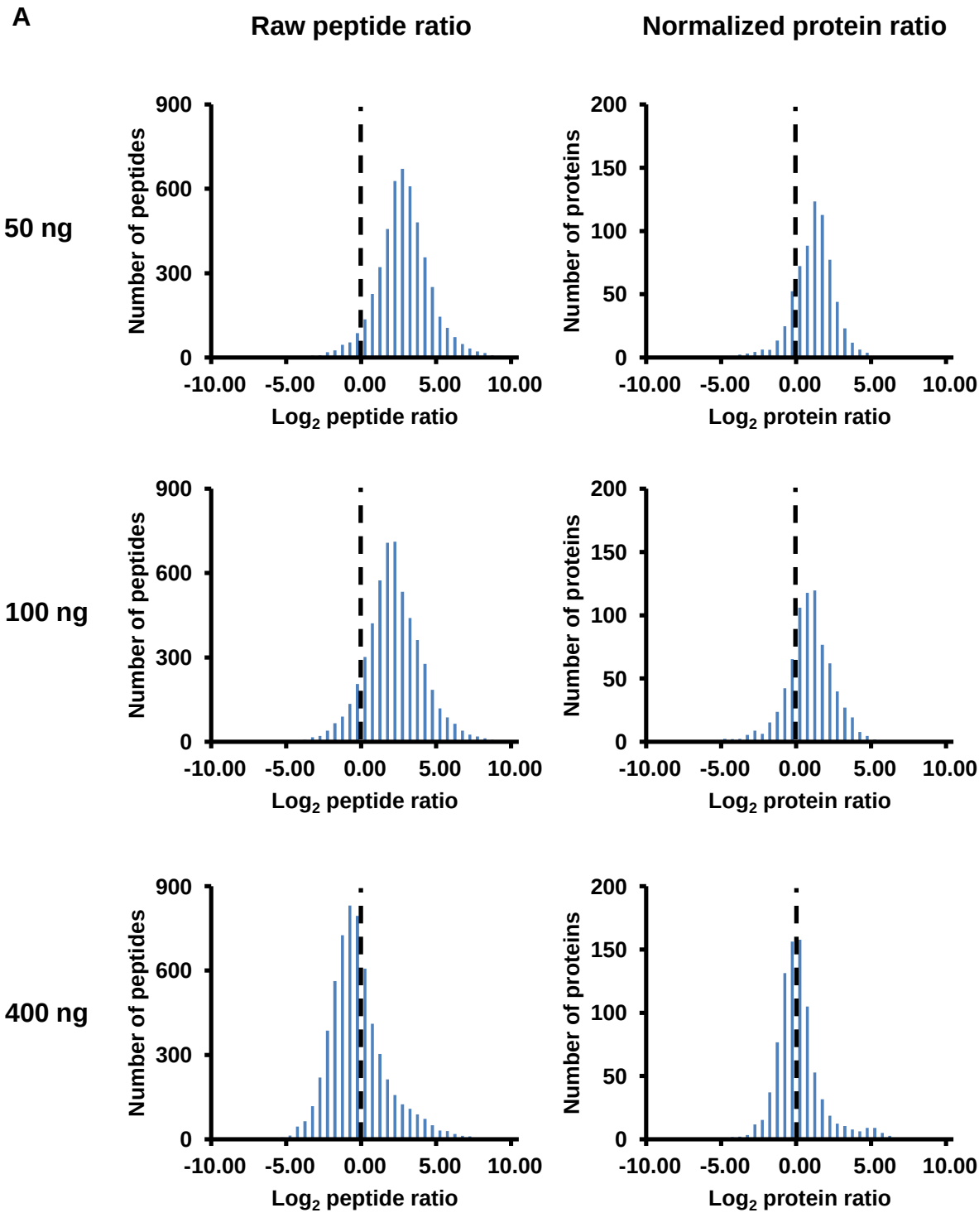
**Figure S5** Comparison of different methods in quantifying proteins involved in focal adhesion pathway in quadruplicate WTL samples. (A) CVs of peptide and protein ratios quantified from replicate WTL samples (n=4) with 3 different quantitative methods (only peptides quantified in ≥3 measurements were considered); (B) Number

of quantified peptides and proteins involved in focal adhesion pathway from quadruplicate WTL samples using 3 different quantitative methods. (*: P<0.05, **: P<0.01, ****: P<0.0001, NS: P>0.05).

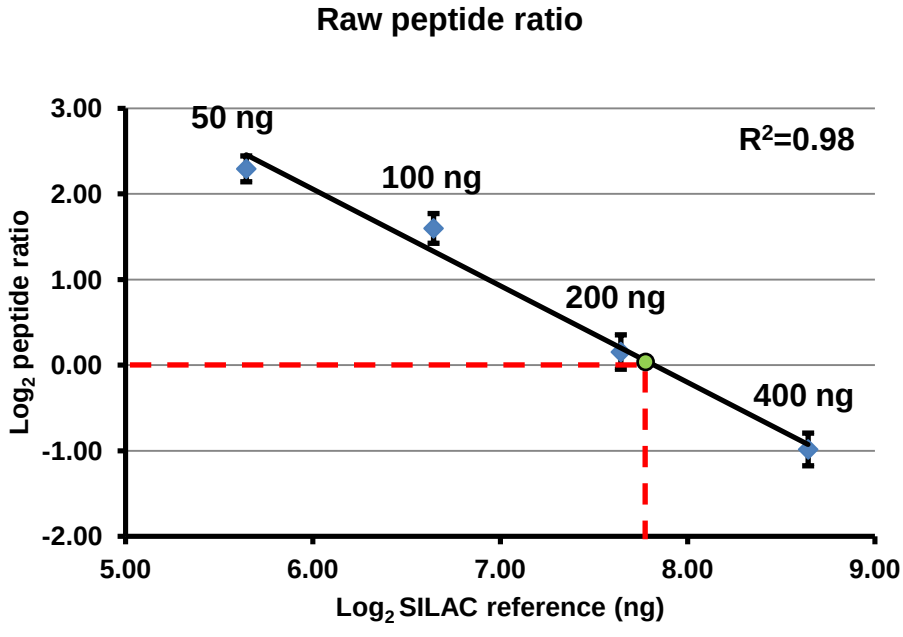
**Figure S6** Reproducibility of "focal adhesion" peptides (A) and proteins (B) quantified by shotgun and directed AIMS method in replicate LCM samples (n=4) and replicate WTL samples (n=4) (obs/observation: number of times which a given peptide was quantified by LC-MS/MS).

**Figure S7** An example of SILAC based SRM assay. (A) A linear calibration curve show a good correlation between observed retention time and predicted normalized retention time. This information and masses of parent and daughter ions ensure correct assignment of observed LC-MS features to their peptide sequences. The red dot is peptide "K.SPFEVYVDK.S"; (B) LC-MS feature of "light" and "heavy" versions of the peptide "K.SPFEVYVDK.S". Baseline of this feature is not noisy, which is important to precise quantification.

Figure S1


A
Raw peptide ratio
Normalized protein ratio
50 ng
100 ng
400 ng
Number of peptides
Number of proteins
900
600
300
0
200
150
100
50
-10.00
-5.00
0.00
5.00
10.00
Log2 peptide ratio
Log2 protein ratio

B


Raw peptide ratio
50 ng
100 ng
200 ng
400 ng
R²=0.98
Log2 peptide ratio
3.00
2.00
1.00
0.00
-1.00
-2.00
5.00
6.00
7.00
8.00
9.00
Log2 SILAC reference (ng)

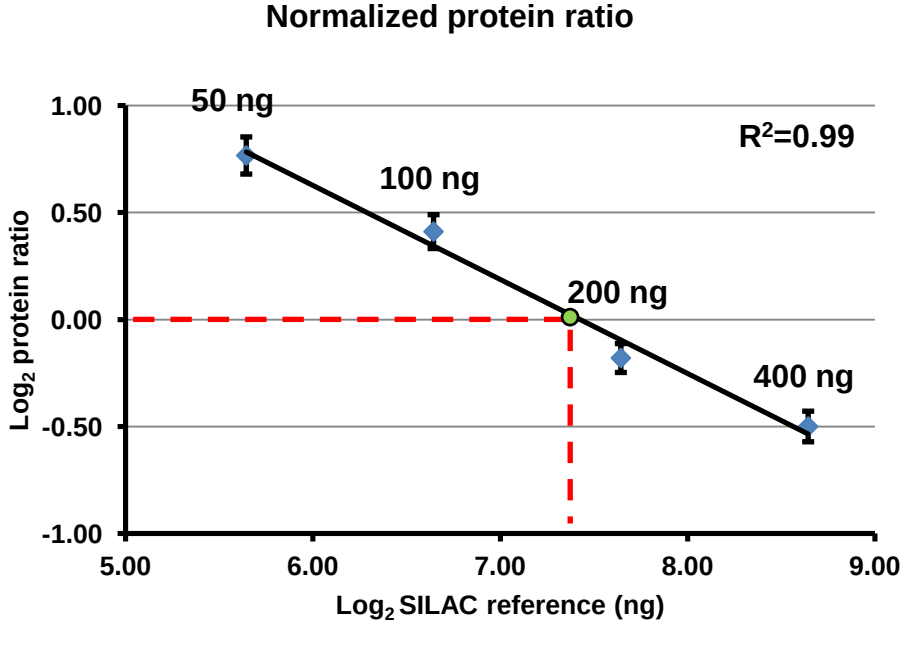

Normalized protein ratio
50 ng
100 ng
200 ng
400 ng
R²=0.99
Log2 protein ratio
1.00
0.50
0.00
-0.50
-1.00
5.00
6.00
7.00
8.00
9.00
Log2 SILAC reference (ng)

**Figure S2**

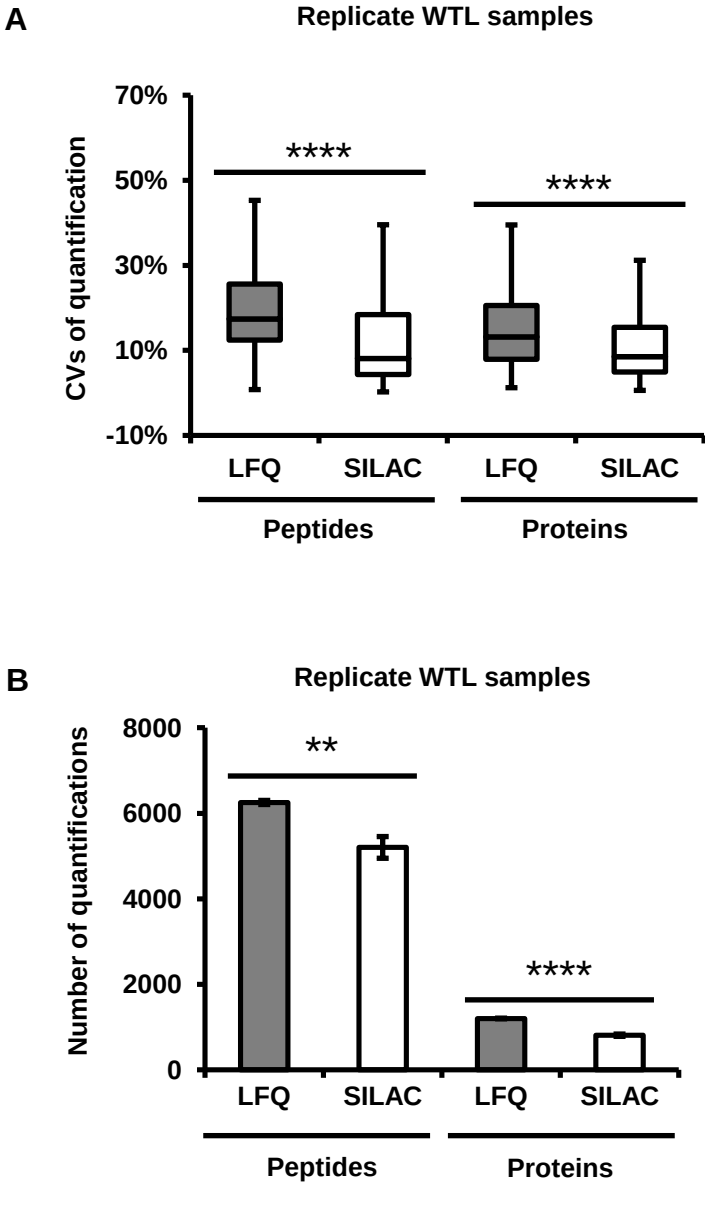
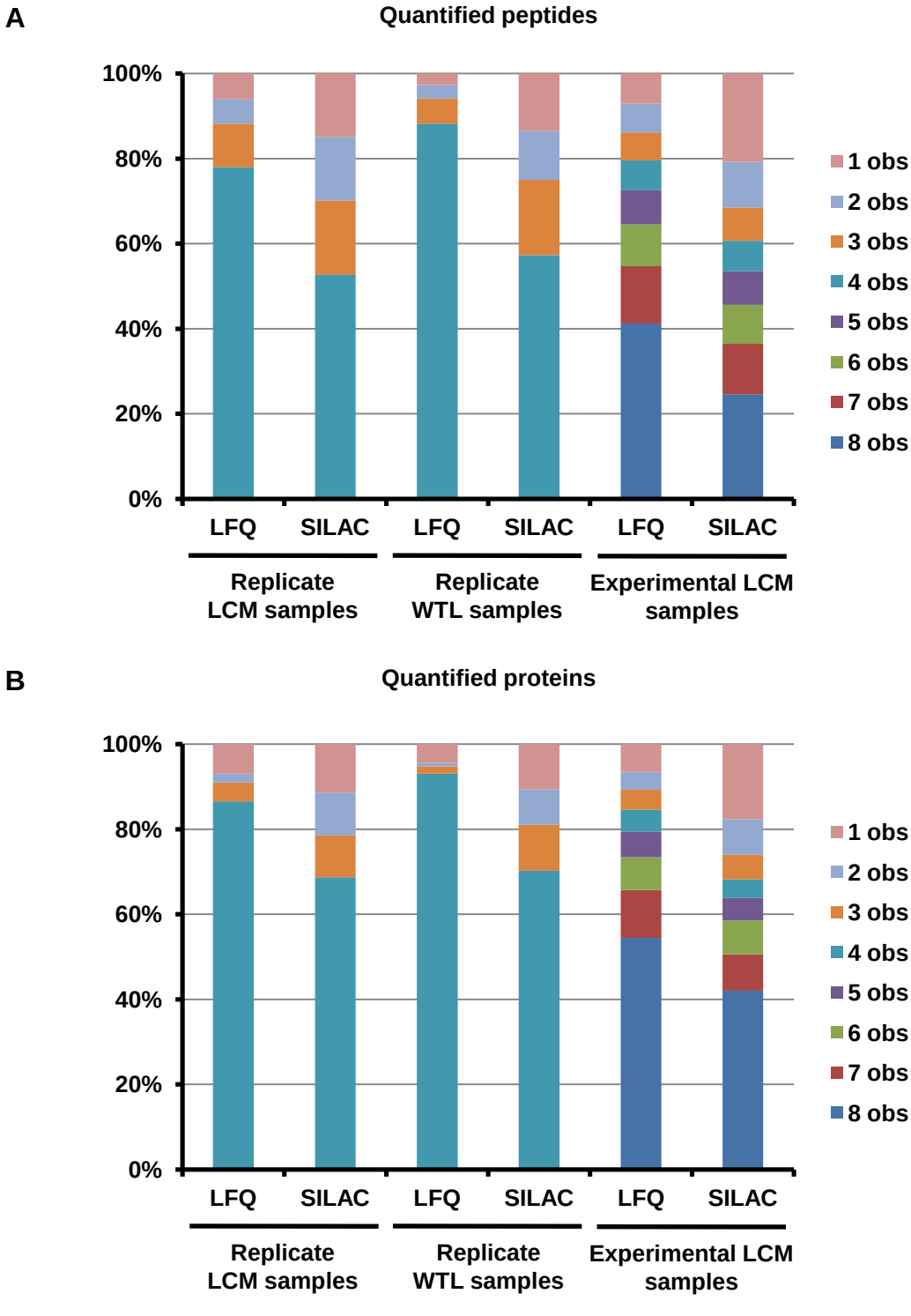
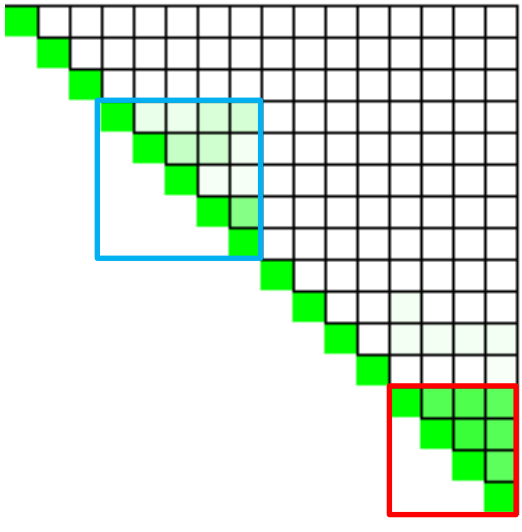

A
Replicate WTL samples
CVs of quantification
70%
50%
30%
10%
-10%
****
****
LFQ
SILAC
LFQ
SILAC
Peptides
Proteins
B
Replicate WTL samples
Number of quantifications
8000
6000
4000
2000
0
**
****
LFQ
SILAC
LFQ
SILAC
Peptides
Proteins

**Figure S3**


A
Quantified peptides
100%
80%
60%
40%
20%
0%
LFQ
SILAC
LFQ
SILAC
LFQ
SILAC
Replicate LCM samples
Replicate WTL samples
Experimental LCM samples
1 obs
2 obs
3 obs
4 obs
5 obs
6 obs
7 obs
8 obs
B
Quantified proteins
100%
80%
60%
40%
20%
0%
LFQ
SILAC
LFQ
SILAC
LFQ
SILAC
Replicate LCM samples
Replicate WTL samples
Experimental LCM samples
1 obs
2 obs
3 obs
4 obs
5 obs
6 obs
7 obs
8 obs

## Figure S4

### A



| KEGG pathway |
|---|
| Ribosome |
| Peroxisome |
| Glutathione metabolism |
| Pathways in cancer |
| Leukocyte transendothelial migration |
| Cell adhesion molecules |
| Focal adhesion |
| ECM receptor interaction |
| Cell cycle |
| Lysosome |
| Citrate cycle |
| Valine, leucine and isoleusine degradation |
| Oxidative phosphorylation |
| Parkinsons disease |
| Huntingtons disease |
| Alzheimers disease |

### B

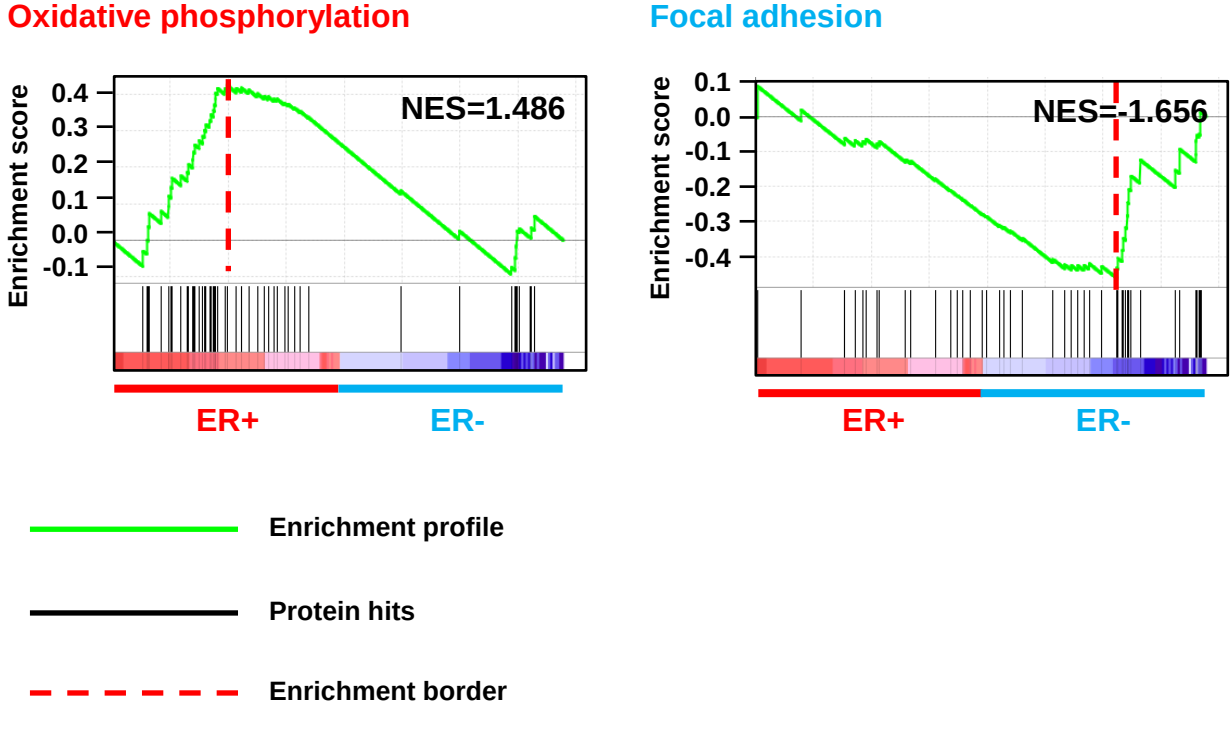


**Figure S5**

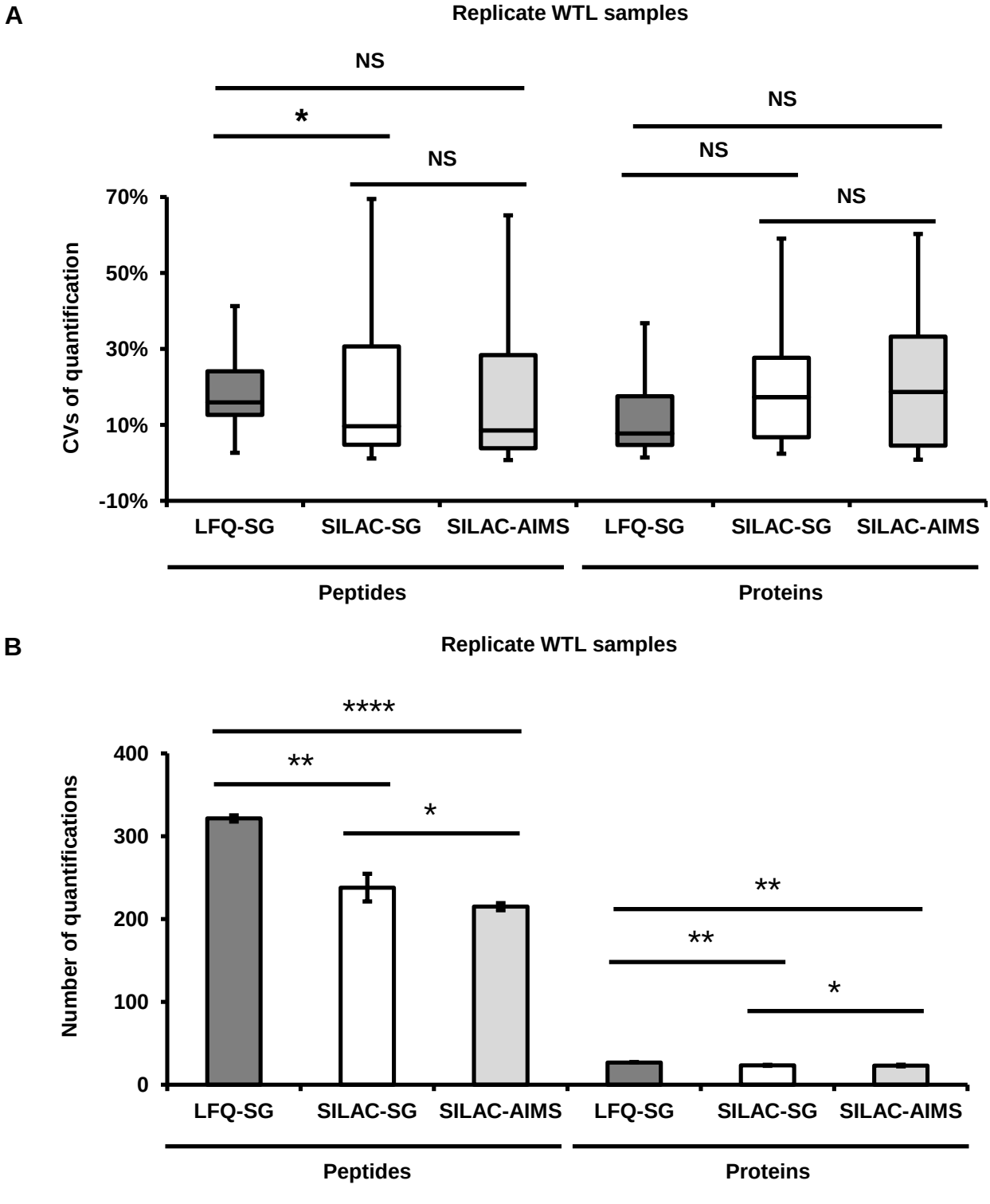

A
Replicate WTL samples
NS
*
NS
NS
NS
NS
70%
50%
30%
10%
-10%
CVs of quantification
LFQ-SG
SILAC-SG
SILAC-AIMS
LFQ-SG
SILAC-SG
SILAC-AIMS
Peptides
Proteins
B
Replicate WTL samples
****
**
*
**
**
*
400
300
200
100
0
Number of quantifications
LFQ-SG
SILAC-SG
SILAC-AIMS
LFQ-SG
SILAC-SG
SILAC-AIMS
Peptides
Proteins

**Figure S6**

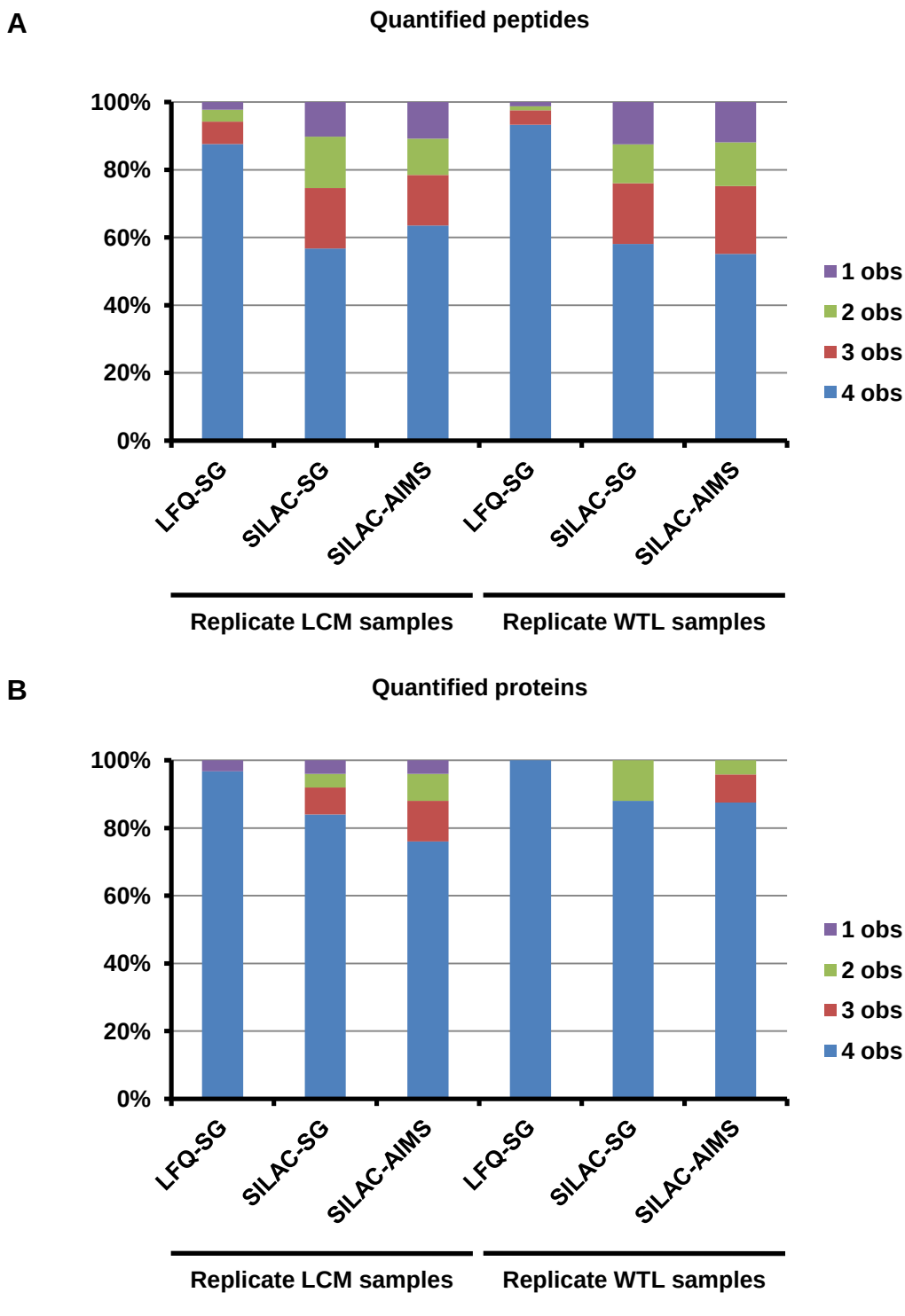
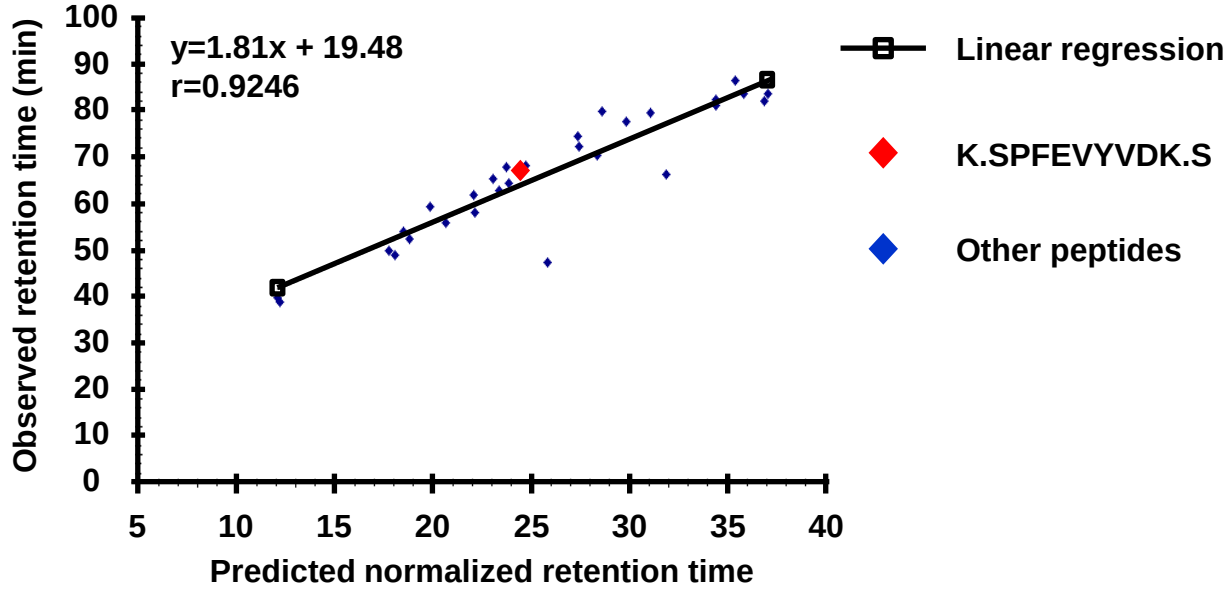
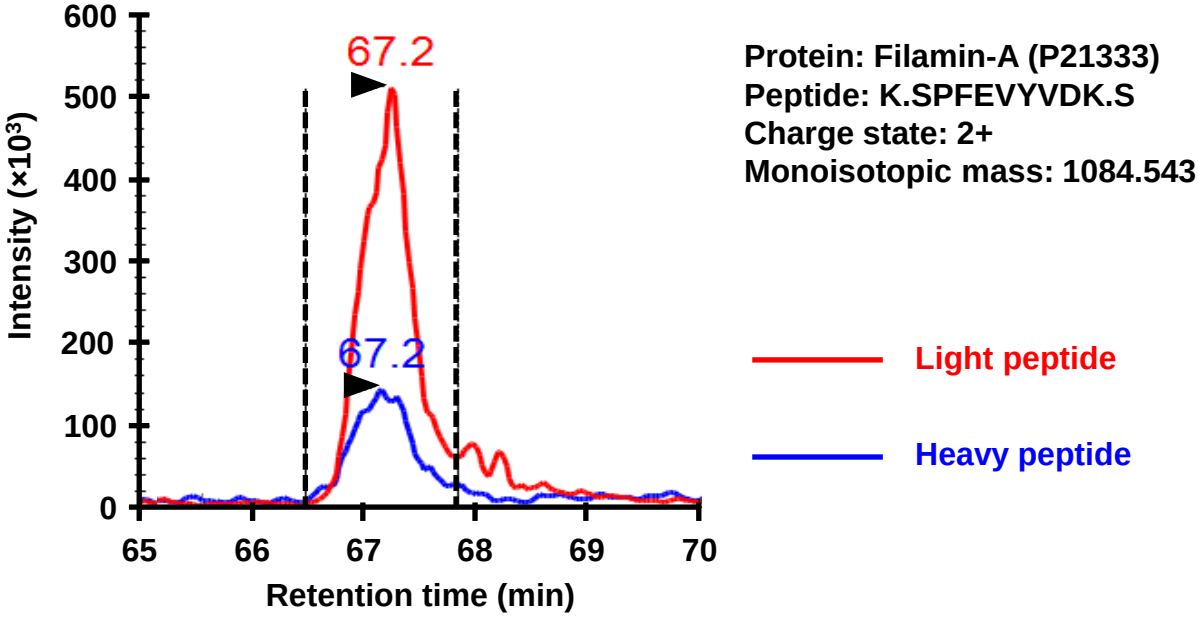

A
Quantified peptides
100%
80%
60%
40%
20%
0%
LFQ-SG
SILAC-SG
SILAC-AIMS
LFQ-SG
SILAC-SG
SILAC-AIMS
1 obs
2 obs
3 obs
4 obs
Replicate LCM samples
Replicate WTL samples
B
Quantified proteins
100%
80%
60%
40%
20%
0%
LFQ-SG
SILAC-SG
SILAC-AIMS
LFQ-SG
SILAC-SG
SILAC-AIMS
1 obs
2 obs
3 obs
4 obs
Replicate LCM samples
Replicate WTL samples

Figure S7

A


y=1.81x + 19.48
r=0.9246
Observed retention time (min)
Predicted normalized retention time
Linear regression
K.SPFEVYVDK.S
Other peptides

B


67.2
67.2
Intensity (×10³)
Retention time (min)
Protein: Filamin-A (P21333)
Peptide: K.SPFEVYVDK.S
Charge state: 2+
Monoisotopic mass: 1084.543
Light peptide
Heavy peptide