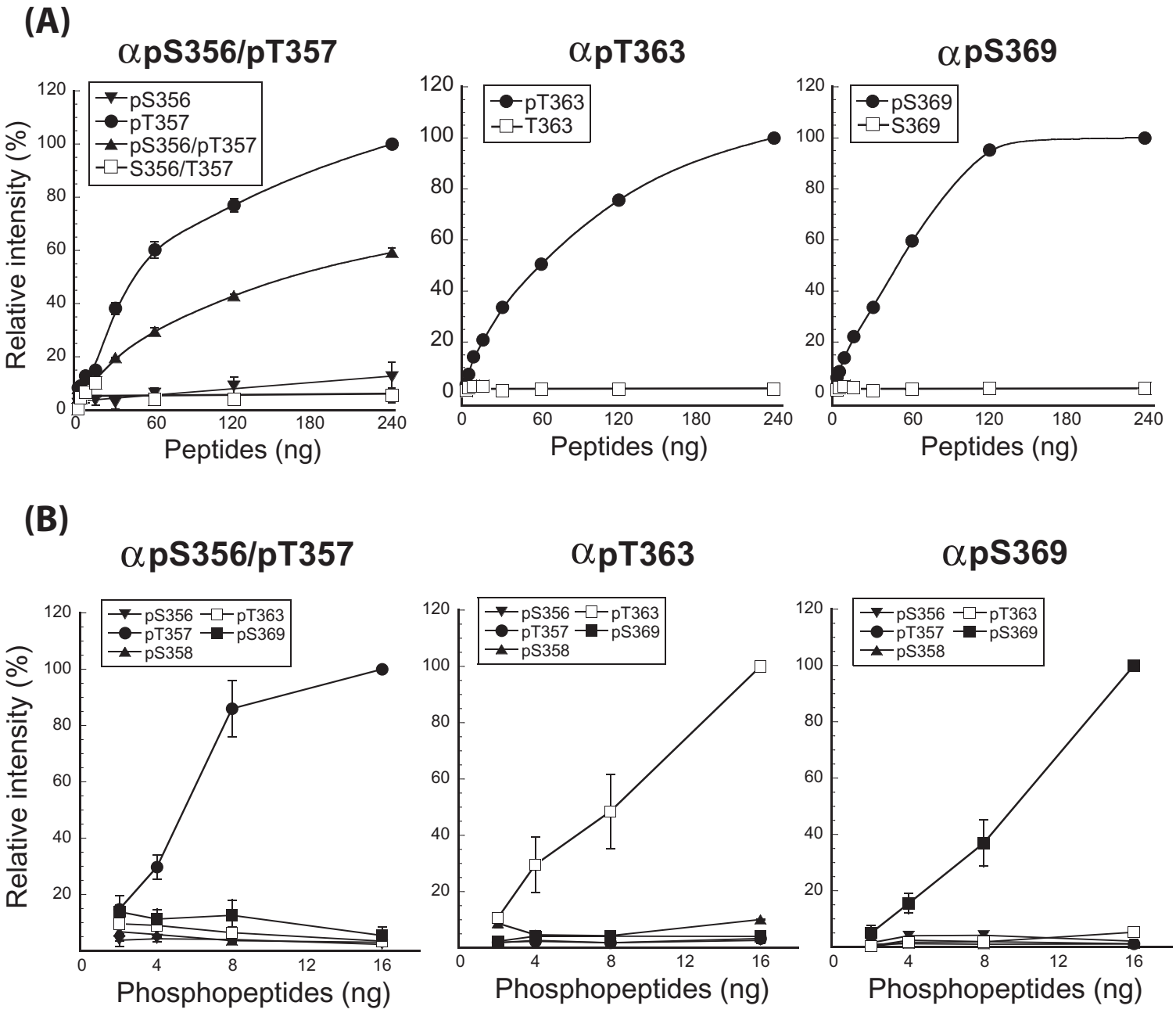
Supplemental Materials

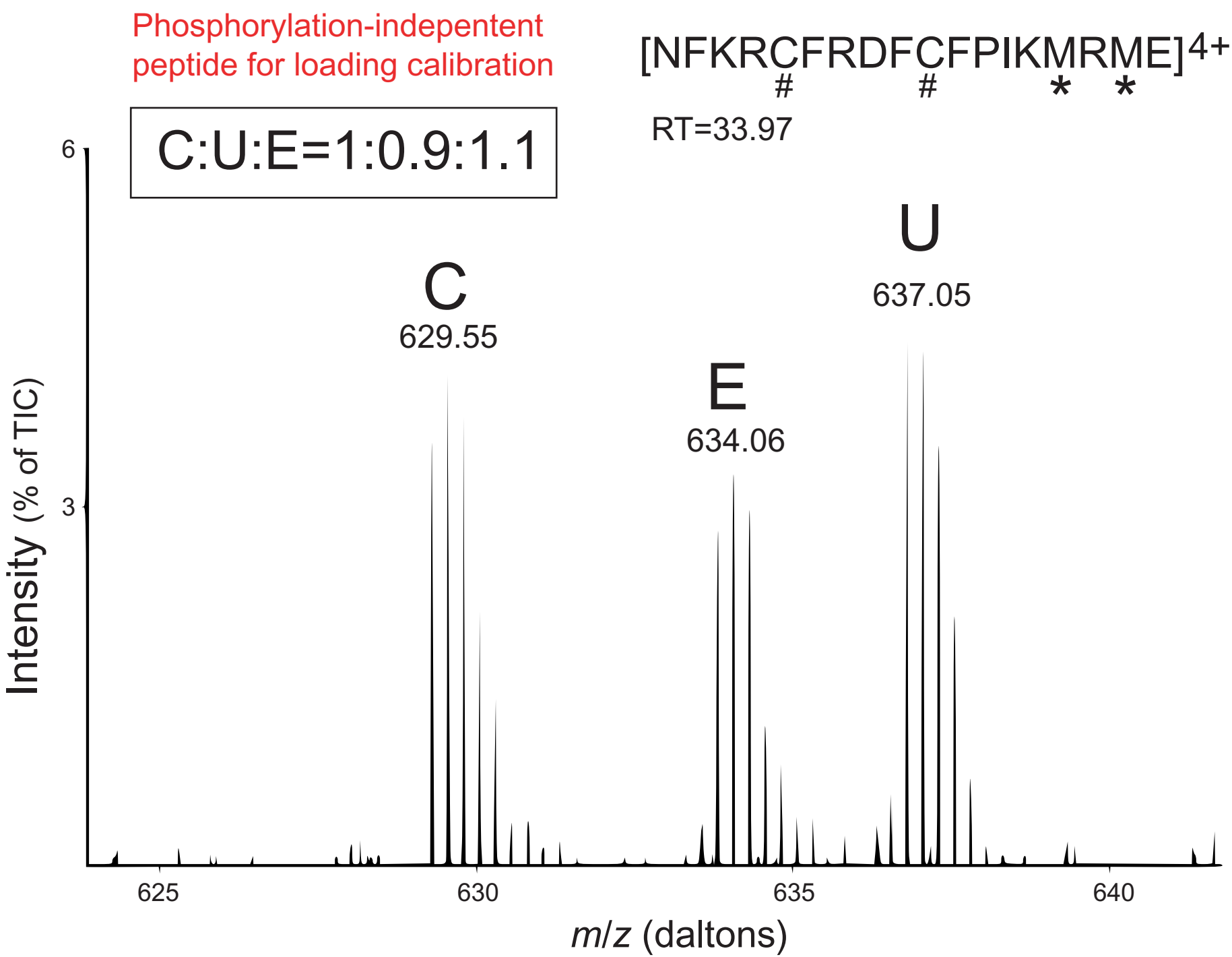
S.Figure 1. (A) Phospho-peptide antibodies have high phospho-specificity for peptides. Reactivity of antibodies with phospho-peptide antigens vs. corresponding unphosphorylated peptides was determined. **(A-1) anti-pS356/pT357** with pS356 vs. S356, pT357 vs. T357; pS356/pT357 vs. S356/T357**; (A-2) anti-pT363** with pT363 vs. T363; **(A-3) anti-pS369** with pS369 vs. S369**.**
(B) Each Phospho-peptide antibody has high specificity for its cognate antigen phospho-peptide. Cross-reactivity of antibodies with other phospho-peptide antigens was examined. **(B-1) anti-pS356/pT357, (B-2) anti-pT363 and (B-3) anti-pS369** with pS356, pT357, pT363 and pS369 peptides**.**
Dot blot analysis was performed as described in Materials and Methods to determine relative reactivity of partially purified antibodies with the phosphopeptide antigens and corresponding unphosphorylated peptides. In each panel, the highest blotting signal is set as 100% and those of all the other dots were normalized against this peak signal and expressed as relative intensity (%). Each value represents the mean ± s.e.m. of three experiments in duplicate.

S.Figure 2. Relative abundance of the phosphorylation-independent KOPR peptide (N^{336}FKRCFRDFCFPIKMRME^{353}**), in control (C), etorphine (E) and U50,488H (U) samples**. Experiments were performed as described in Fig. 7 legend. A phosphorylation-independent peptide, KOPR(336-353), was identified in LC-MS/MS analysis. The relative abundance of this peptide was determined to be C:U:E=1:0.9:1.1, which represented the ratio of total receptor input and was used to normalize all the phosphophopeptide ratios. Results shown are the averages of the two experiments performed.

Suppl Fig. 1


(A)
αpS356/pT357
αpT363
αpS369
Relative intensity (%)
pS356
pT357
pS356/pT357
S356/T357
pT363
T363
pS369
S369
Peptides (ng)
(B)
αpS356/pT357
αpT363
αpS369
pS358
Phosphopeptides (ng)

Suppl Fig. 2


Phosphorylation-indepentent
peptide for loading calibration
[NFKRCFRDFCFPIKMRME]4+
# # * *
RT=33.97
C:U:E=1:0.9:1.1
C
629.55
E
634.06
U
637.05
Intensity (% of TIC)
6
3
625
630
635
640
m/z (daltons)