Supporting Information

Protein painting for structural and binding sites analysis via intracellular lysine reactivity profiling with o-phthalaldehyde

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Supplementary Method

Supplementary Figures

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Cell Viability Assay. Cell Viability was determined by CellTiter-Glo Luminescent Cell Viability Assay (Promega, USA). HEK293T cells incubated at different times (5 min, 15 min, and 30 min) of 800 μM OPA were seeded in a 96-well plate at 4 thousand cells per well. Cells were lysed using Cell Titer-Glo® reagent and mixed for 2 min on an orbital shaker. The plate was incubated for 10 min and analyzed by microplate reader (EnSpire). All Cell Viability was performed with four independent experiments. Data was analyzed by GraphPad Prism (version 8.0.2).

Supplementary Figure 1-9

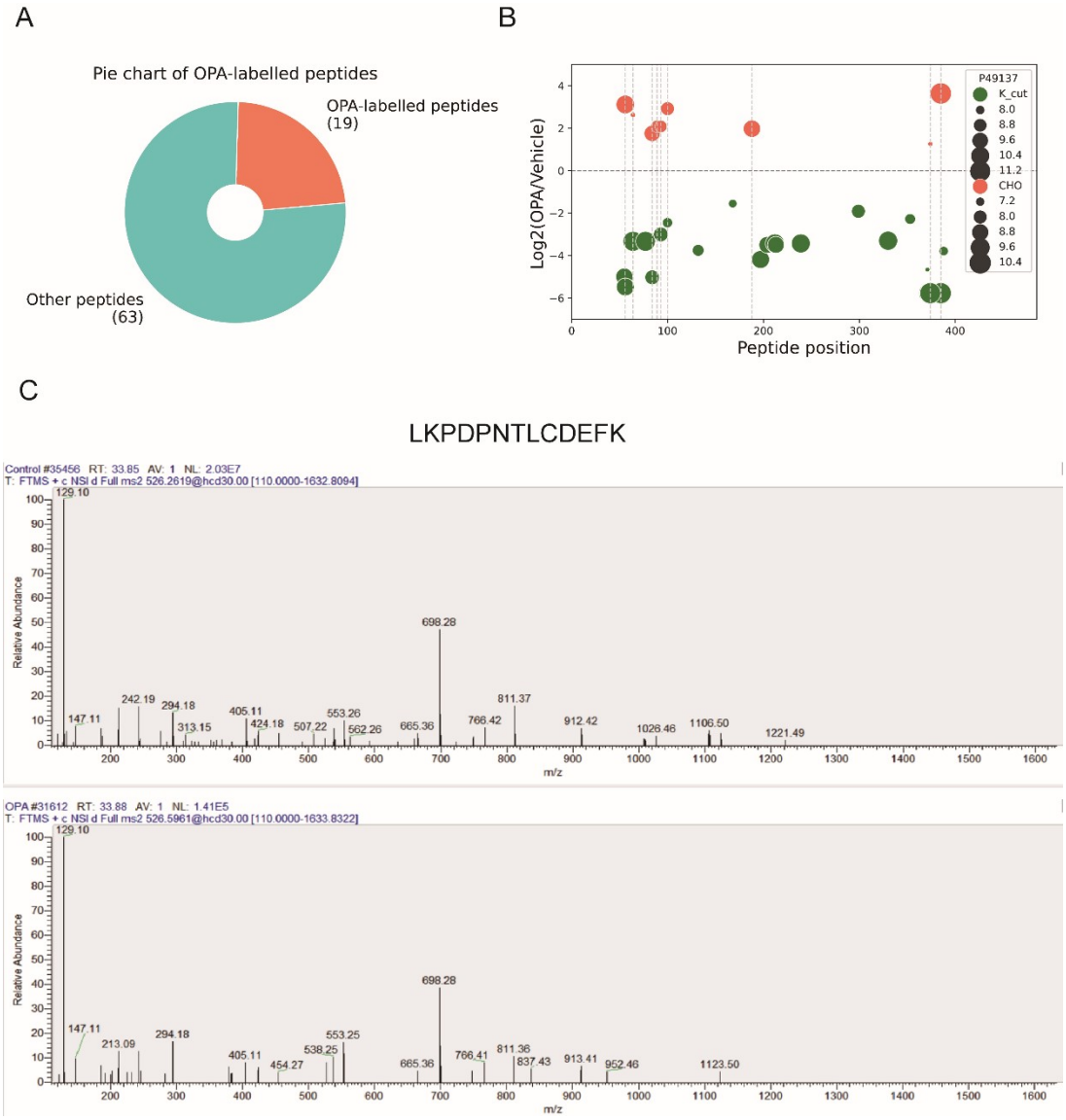

Fig. S1. Feasibility of RAPID method. (A) Fraction of total quantified peptides that were liganded by OPA in MAPKAPK2 protein. (B) Bubble diagram shows the distributions and labeling reactivity shifts of identified lysine residues in MAPKAPK2 protein. (C) Compared intensity of MS/MS spectra for peptide LKPDPNTLCDEFK in the control group (upper, 2.03E7) and OPA-treated group (below, 1.41E5).

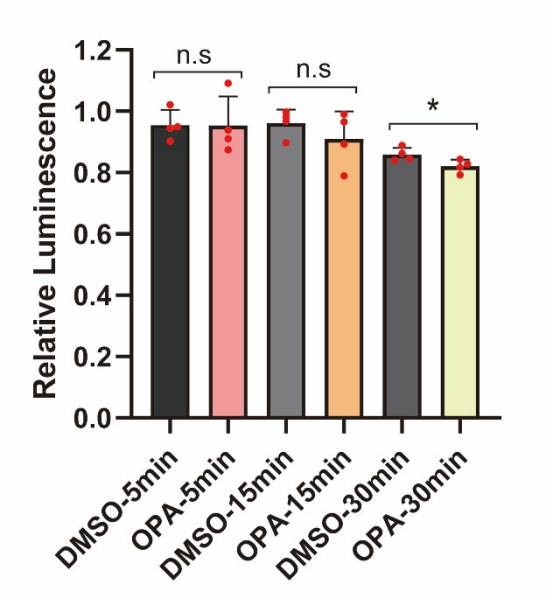

Fig. S2. Cell Viability at different incubation times of OPA. HEK293T cells were incubated at different times (5 min, 15 min, and 30 min) of 800 μM OPA followed by quantification of ATP based on luminescence intensity (n = 4 independent biological experiments). *$p < 0.05$, n.s $p > 0.05$ compared with DMSO group.

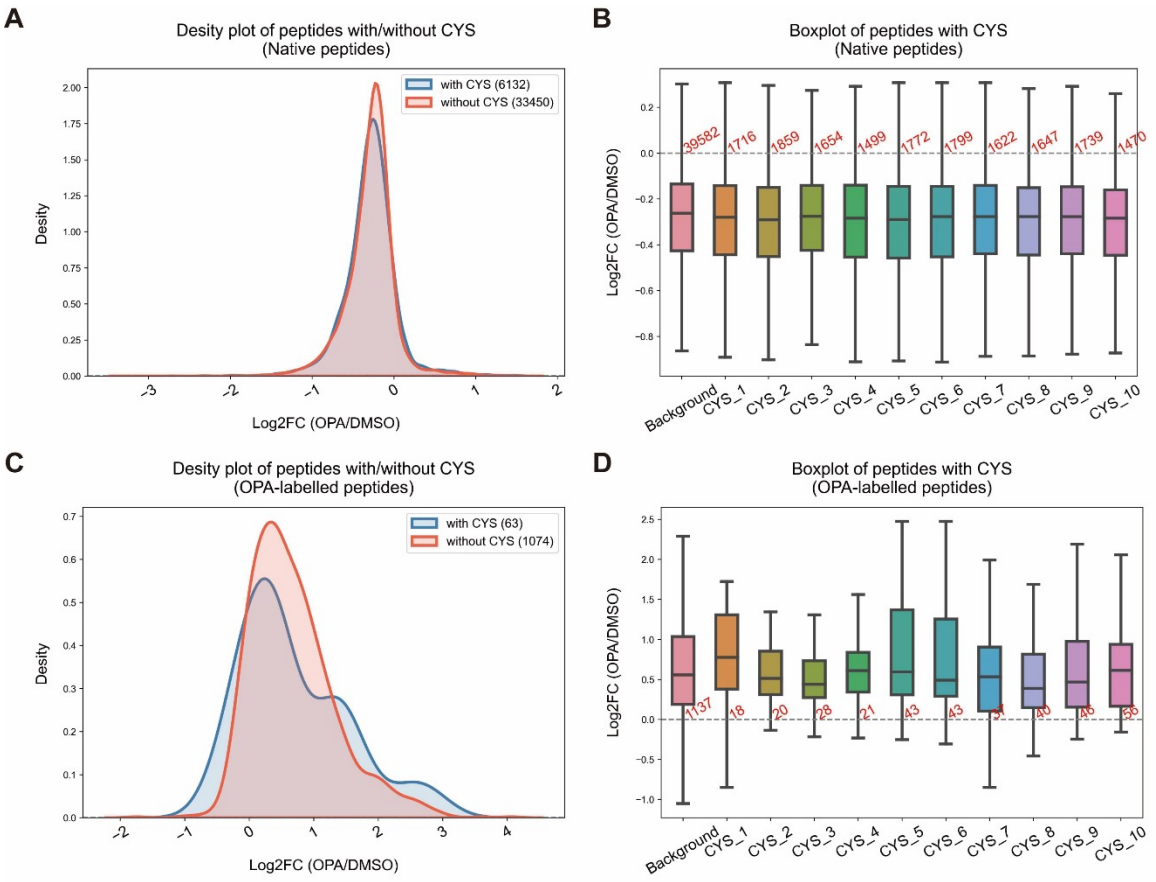

Fig. S3. Potential effects of cysteine on lysine reactivity. (A) Overall density plot of native peptide abundance changes with or without cysteine. (B) Bloxplot of native peptide FC with the amount of cysteine near lysine. (C) Overall density plot of OPA-labelled peptide abundance changes with or without cysteine. (D) Bloxplot of OPA-labelled peptide FC with the amount of cysteine near lysine.

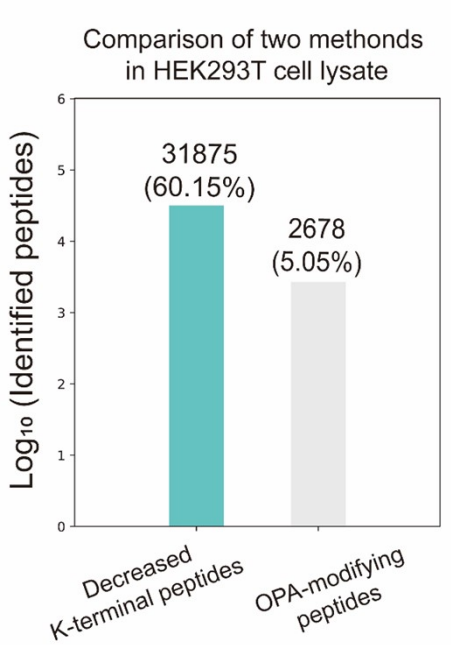

Fig. S4. RAPID-OPA analysis in HEK293T cell lysate. The number of peptides with differentiated abundance identified in HEK293T cells lysate.

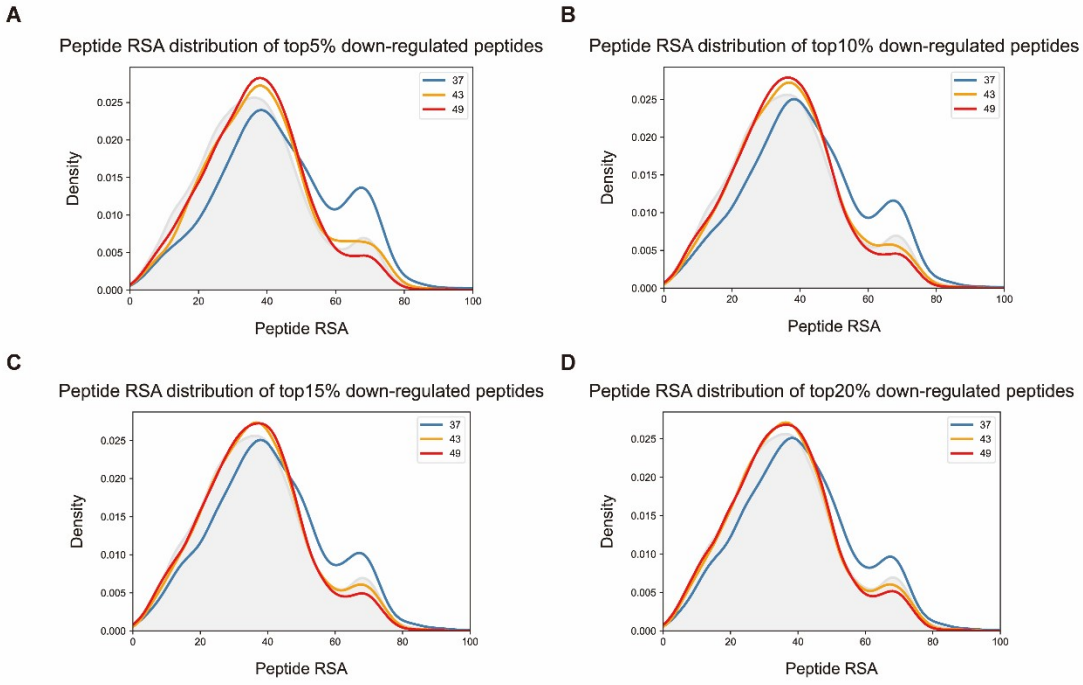

Fig. S5. RSA distribution of top 5%/10%/15%/20% down-regulated peptides at 37°C, 43°C, and 49°C. (A-D) Peptide RSA.

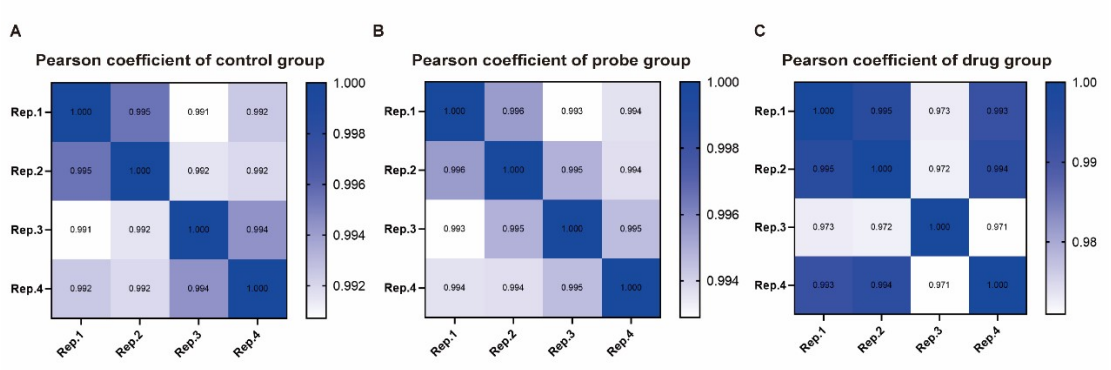

Fig. S6. Pearson coefficient among four technical replicates. (A) Pearson coefficient for four replications of the control group (treated with DMSO). (B) Pearson coefficient for four replications of the probe group (treated with OPA). (C) Pearson coefficient for four replications of the drug group (treated prior with drug before OPA incubation).

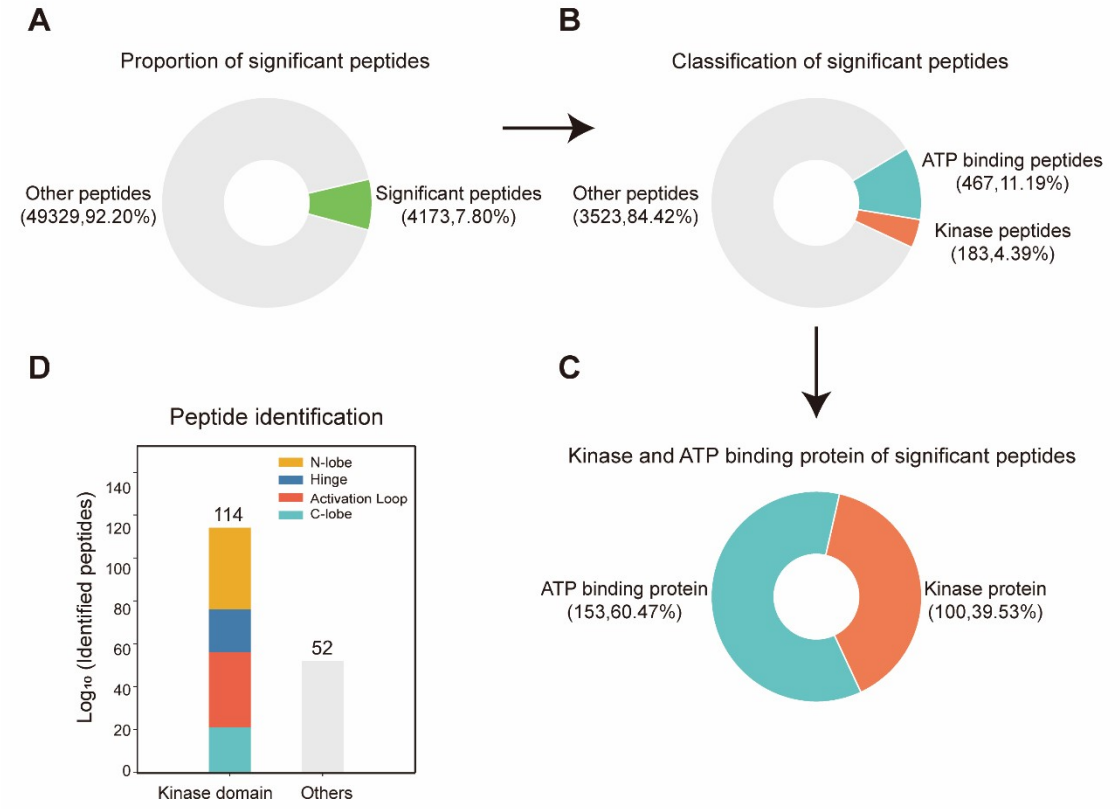

Fig. S7. The effect of identifying kinase for staurosporine by RAPID-OPA. (A) Fraction of total quantified significant peptides. (B) Fraction of total quantified kinase peptides, ATP binding peptides, and other peptides. (C) Fraction of significant peptides corresponding to kinase and ATP binding protein. (D) Bar plot visualization of the locations of the identified peptides with significantly changed labeling reactivity of kinase (adj p value < 0.05).

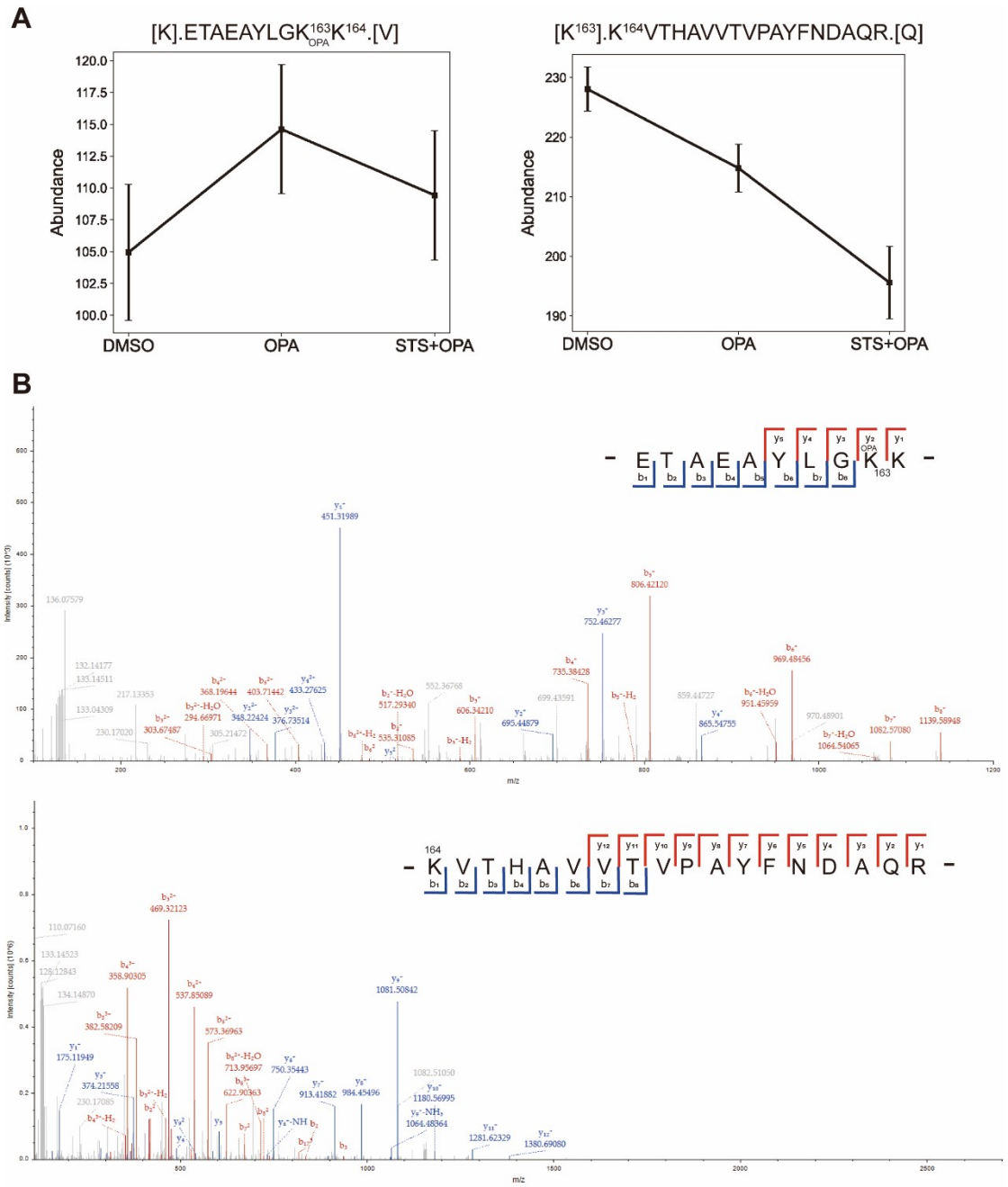

Fig. S8. Covalent modification of lysine 163 in HSPA5 by OPA. (A) Abundance of peptides [K].ETAEAYLGK163 OPAK.[V] and [K163].K164VTHAVVTVPAYFNDAQR.[Q] in HSPA5. Data are presented as mean values ± s.d. (error bars). (B) MS/MS result of peptides ETAEAYLGK163 OPAK (upper) and K164VTHAVVTVPAYFNDAQR (below).

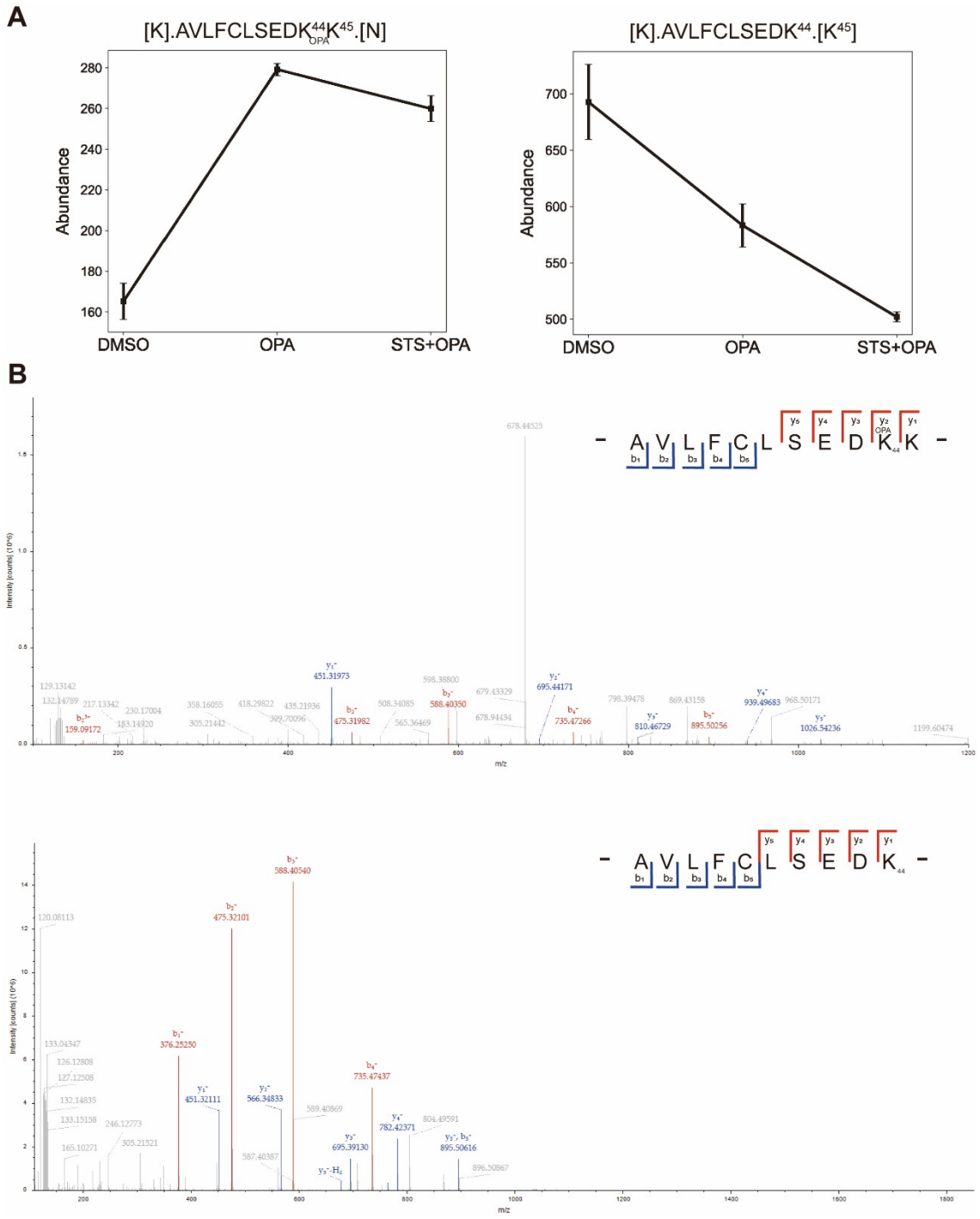

Fig. S9. Covalent modification of lysine 44 in CFL1 by OPA. (A) Abundance of peptides [K].AVLFCLSEDK44 OPAK.[N] and [K].AVLFCLSEDK^{44}.[K] in CFL1. Data are presented as mean values ± s.d. (error bars). (B) MS/MS result of peptides AVLFCLSEDK44 OPAK (upper) and AVLFCLSEDK^{44} (below).