Supplemental figures to

Specificity of protein covalent modification by the electrophilic proteasome inhibitor carfilzomib in human cells

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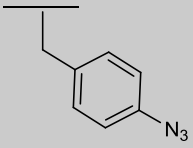
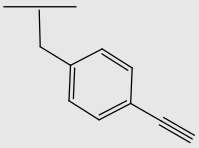
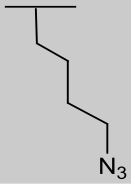
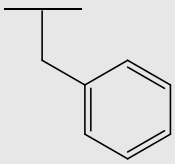
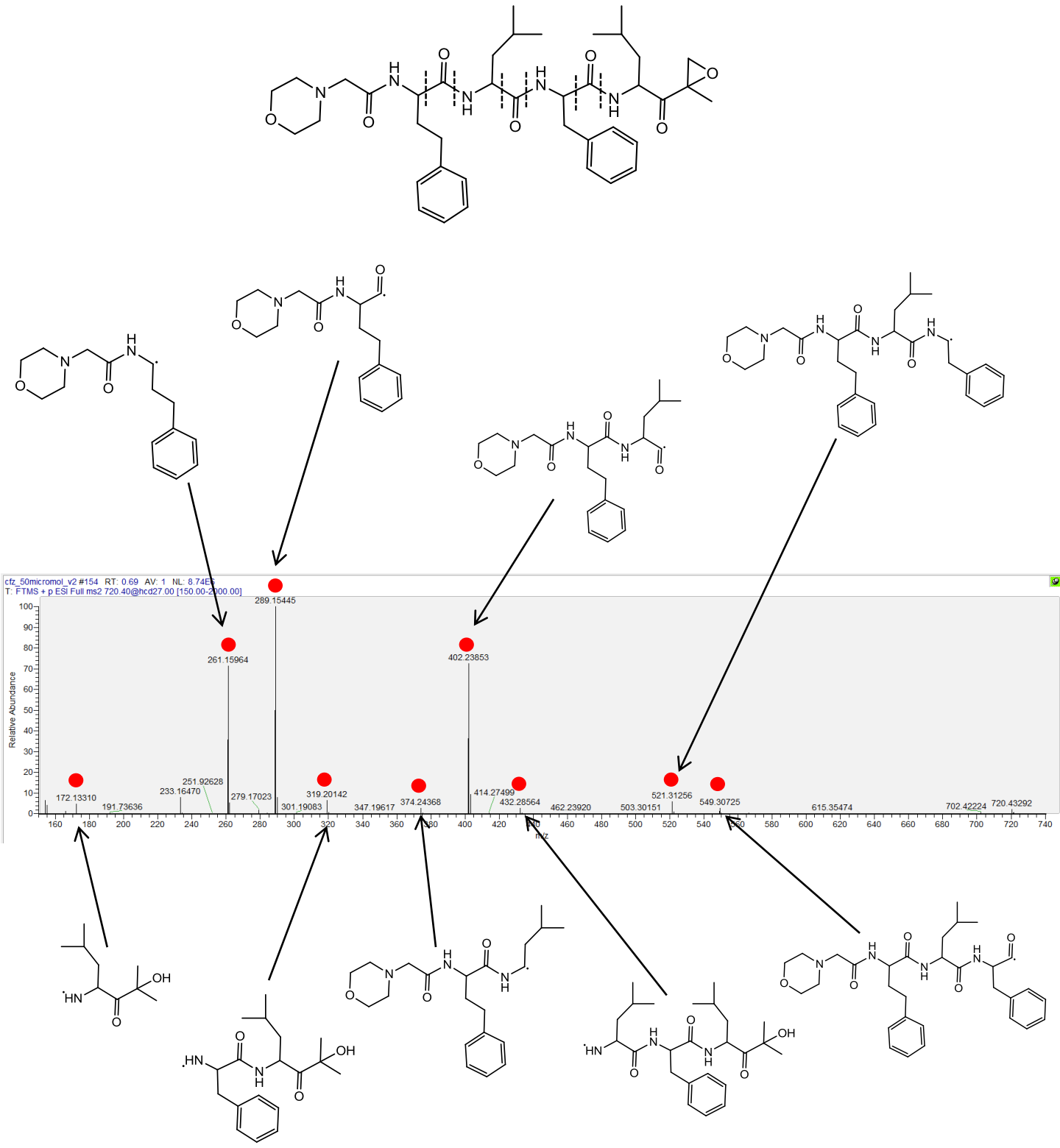
A

Metabolic stability of CFZ and analogue compounds

Compound (1 uM)	P2 moiety	% Remaining at 120 min	$T_{½}$ (min)	CL_{int} (mL/min/kg)	Cl_{hep}, predicted (mL/min/kg)
OP-828		0.105	7.34	437	19.8
OP-829		0.740	13.1	245	19.1
OP-830		0.391	11.4	280	19.3
CFZ		0.422	8.51	377	19.6

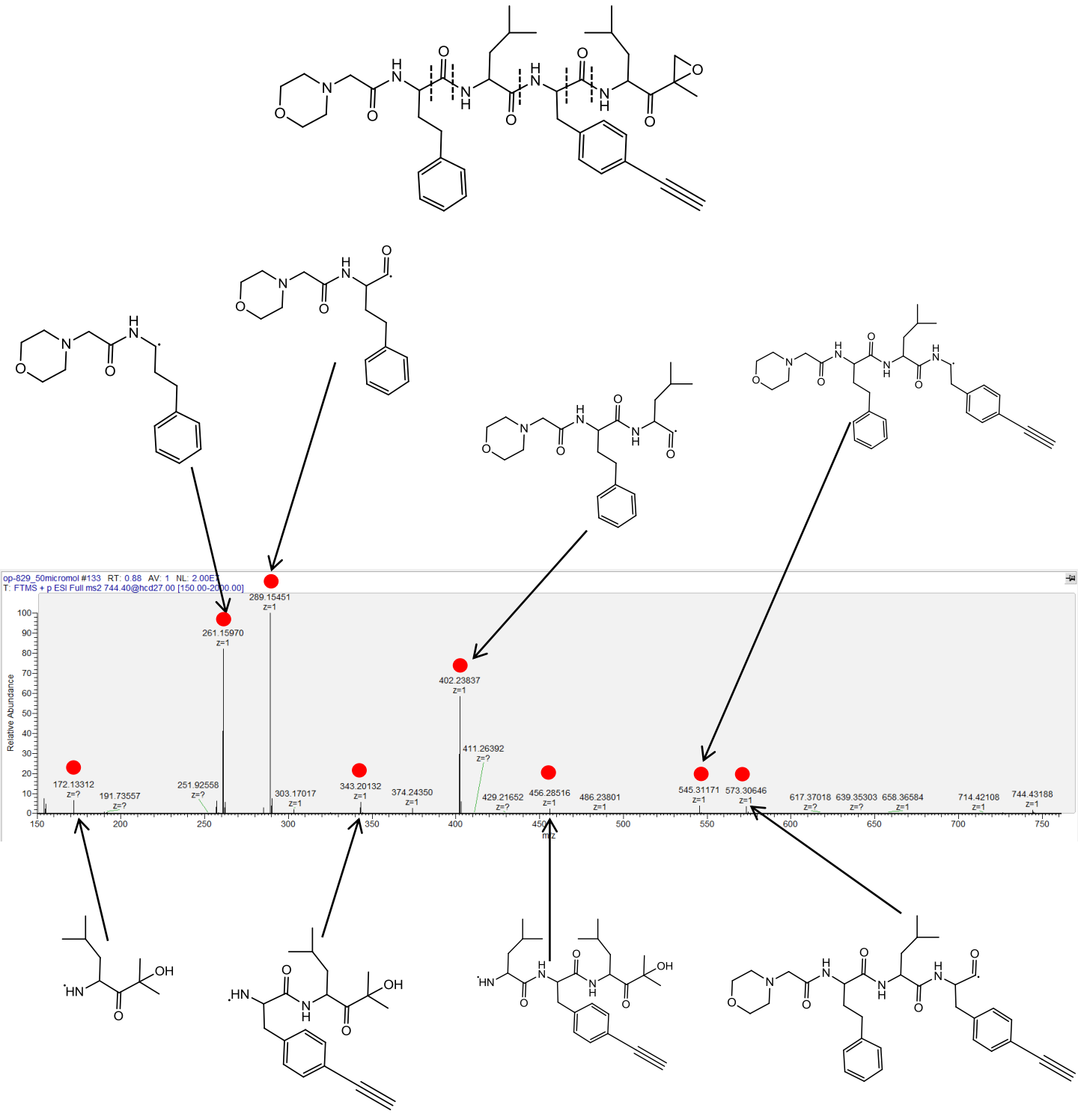
Supplemental Figure 1. (A) Table of metabolic stability values for the parent carfilzomib compound and the three analogues. (B) MS/MS fragmentation of carfilzomib. (C) MS/MS fragmentation of OP-829.

B



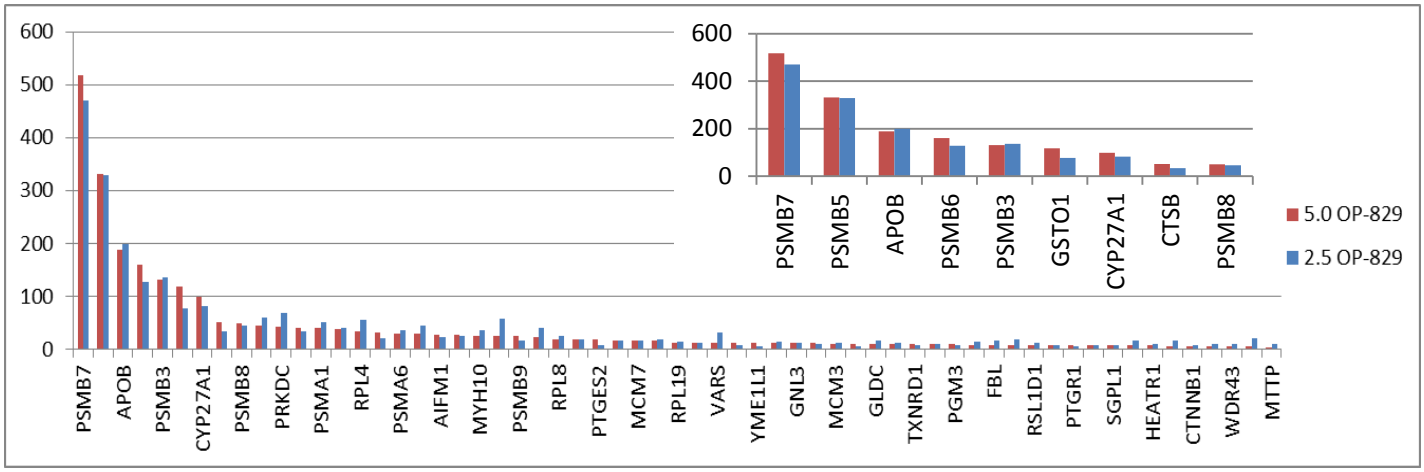
Supplemental Figure 1.

C

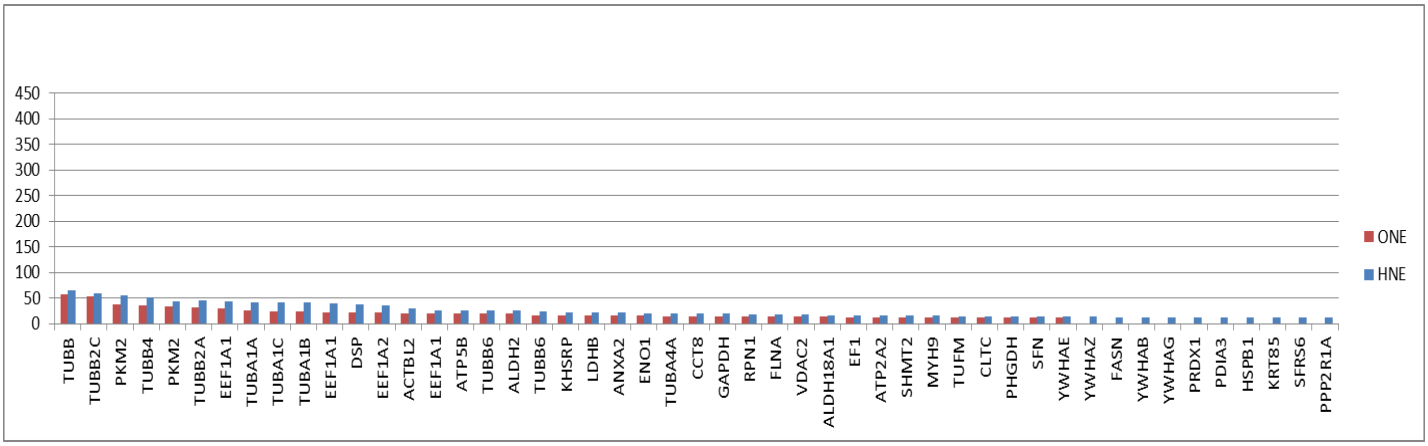


Supplemental Figure 1.

A



B

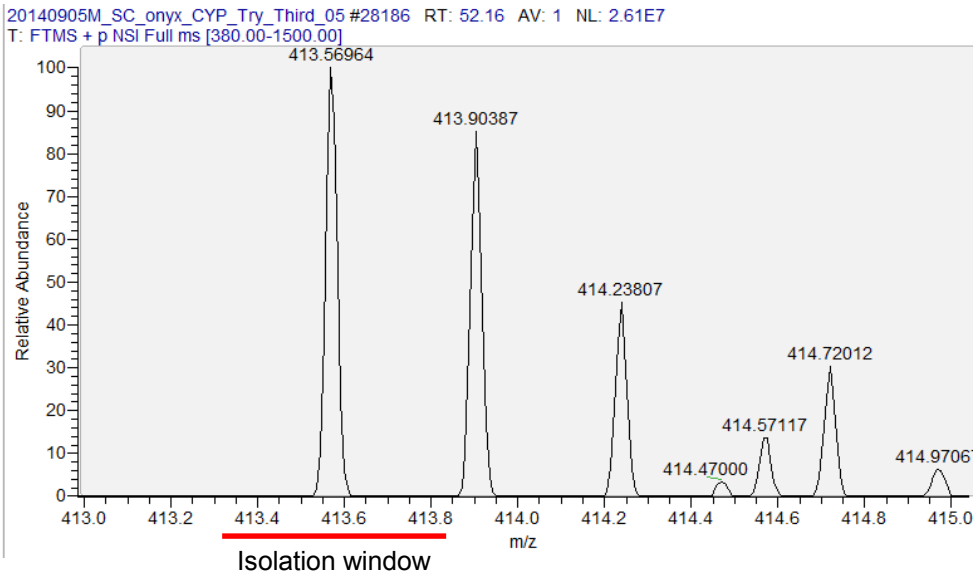


Supplemental Figure 2. Comparison of (A) OP-829 specificity to the relatively non-specific electrophiles (B) HNE and ONE at a 10μM concentration. Protein names are on the x-axis and spectral counts are on the y-axis. Proteins shown in (A) were enriched at least 4-fold in the OP-829 treatment over DMSO controls.

A

CYP27A1: ^{474}AC[719]LGR^{478}

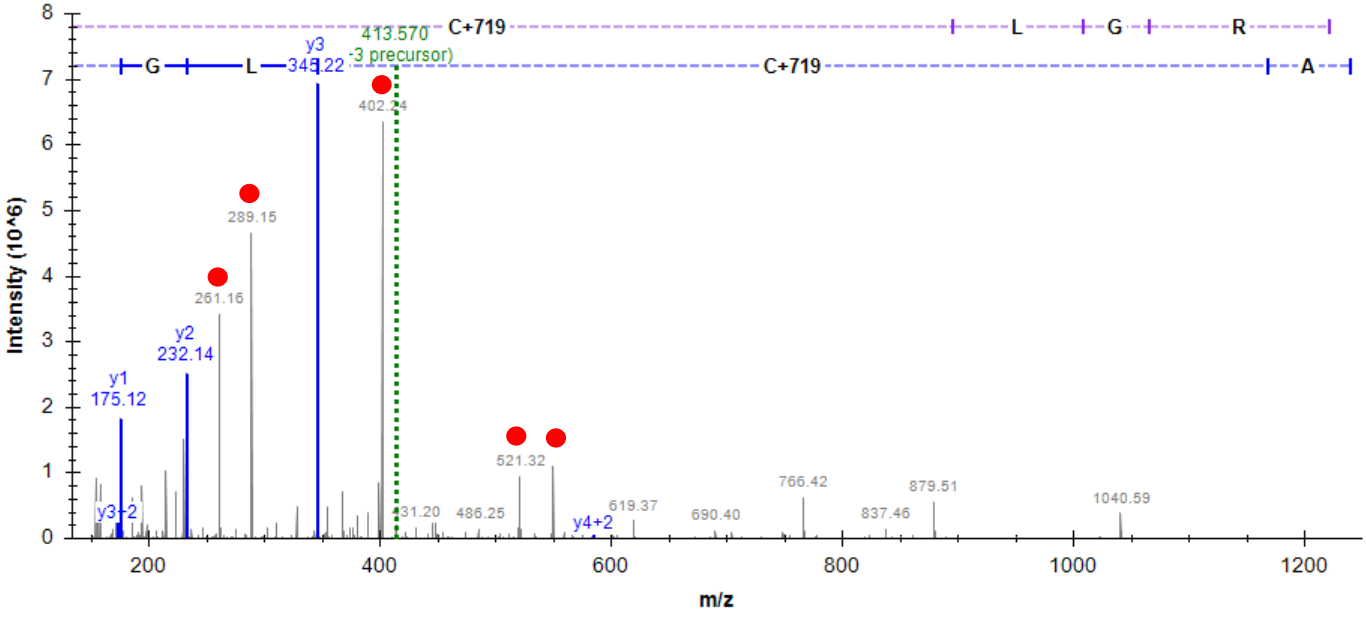
MS1



Precursor mass error: -0.91ppm

Evidence of adduct: CFZ fragment ions (red circles) and y4^{+2} ion showing mass shifted Cys residue

MS2

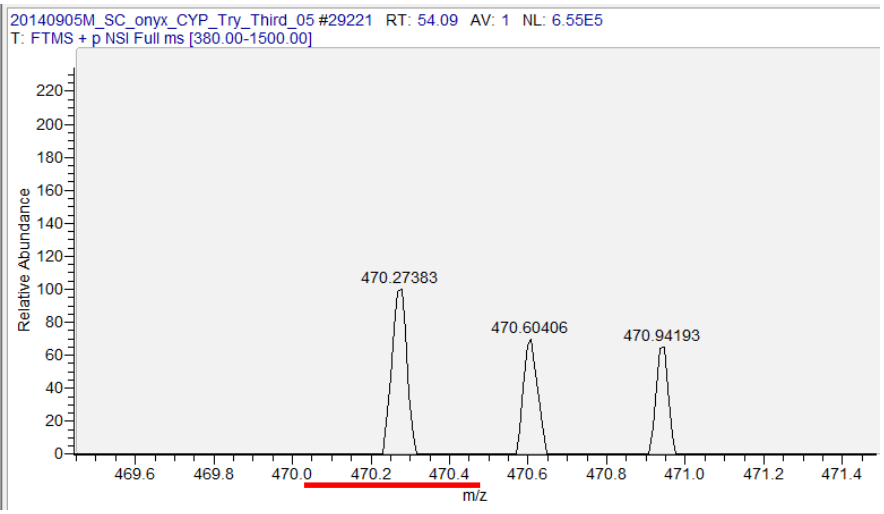


Supplemental Figure 3. MS/MS spectra of carfilzomib adducted peptides from (A) CYP27A1 and (B) GSTO1. Red circles denote fragment ions originating from the CFZ compound as in Fig S1B.

A

CYP27A1: ^{225}IGC[719]LQR^{230}

MS1

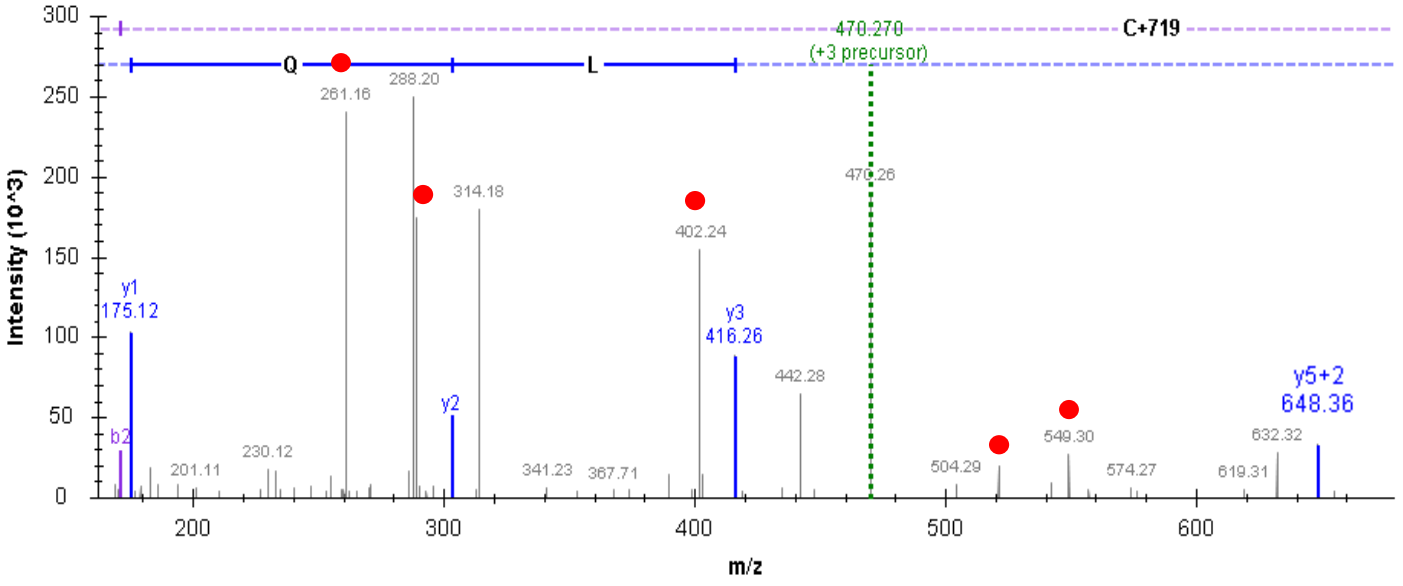


Isolation window

Precursor mass error: -4.71ppm

Evidence of adduct: CFZ fragment ions (red circles) and y5 ion showing mass shifted residues

MS2

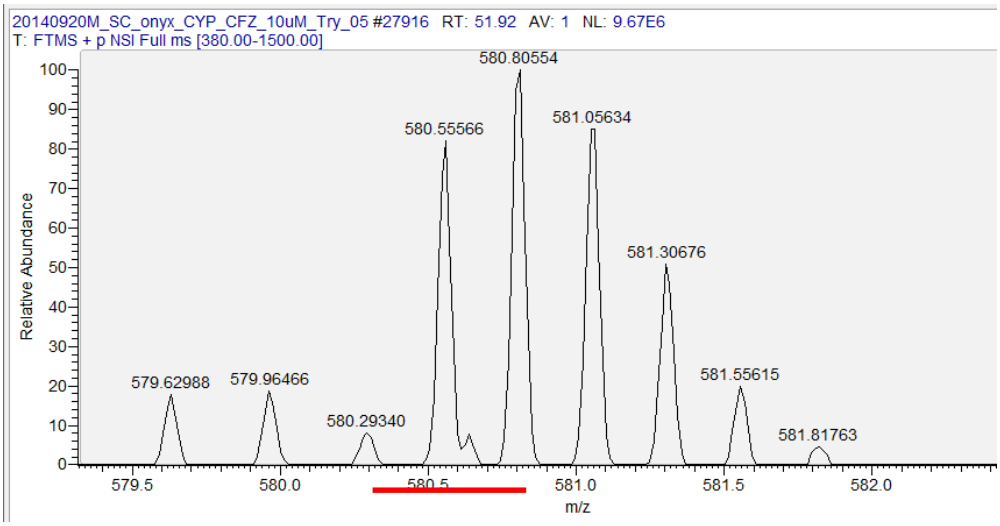


Supplemental Figure 3.

A

CYP27A1: ^{420}NTQFVFC[719]HYVVSR^{432}

MS1

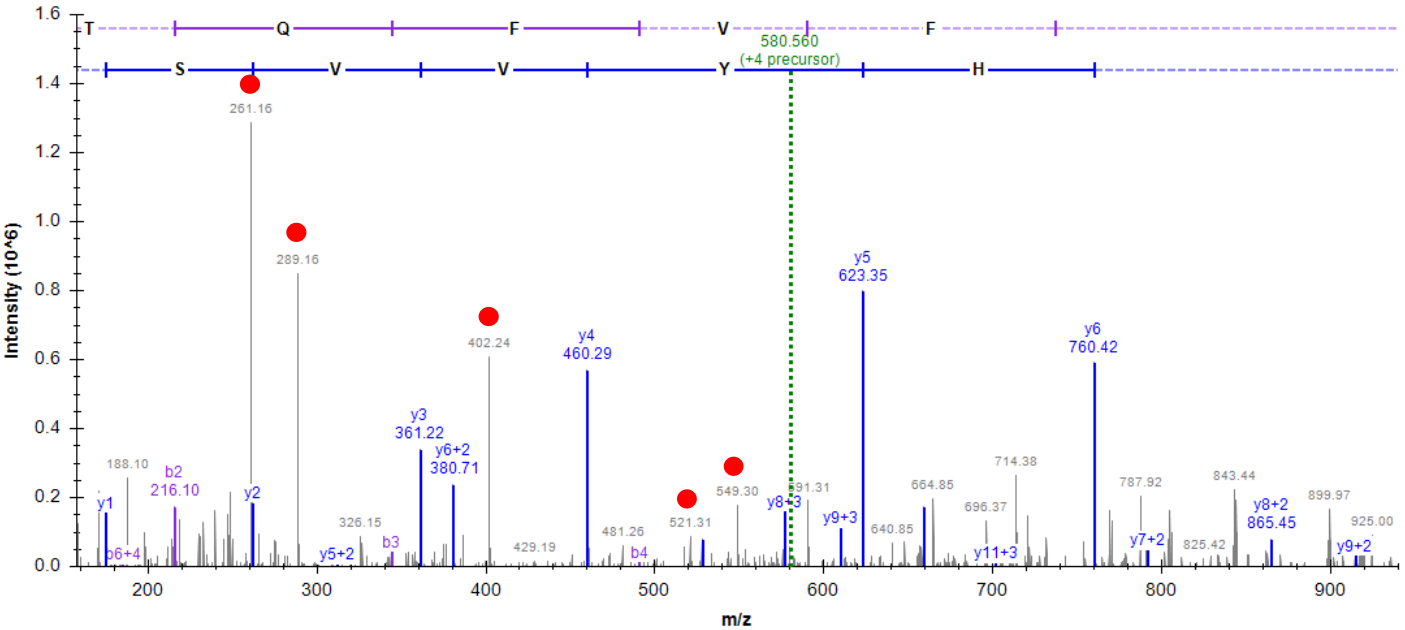


Isolation window

Precursor mass error: 8.06ppm

Evidence of adduct: CFZ fragment ions (red circles) and y7 ion showing mass shifted Cys residue

MS2

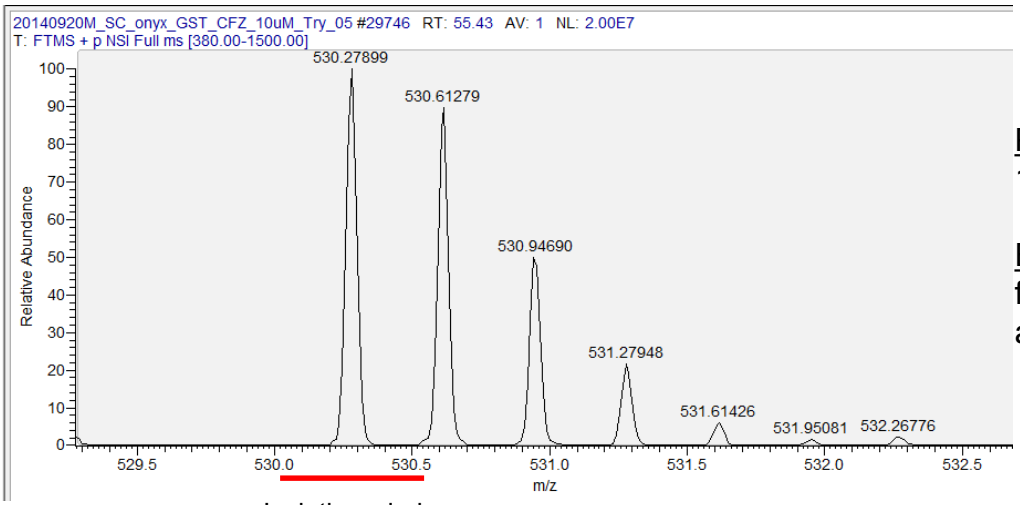


Supplemental Figure 3.

B

GSTO1: ^{30}F**C[719]**PFAER^{36}

MS1

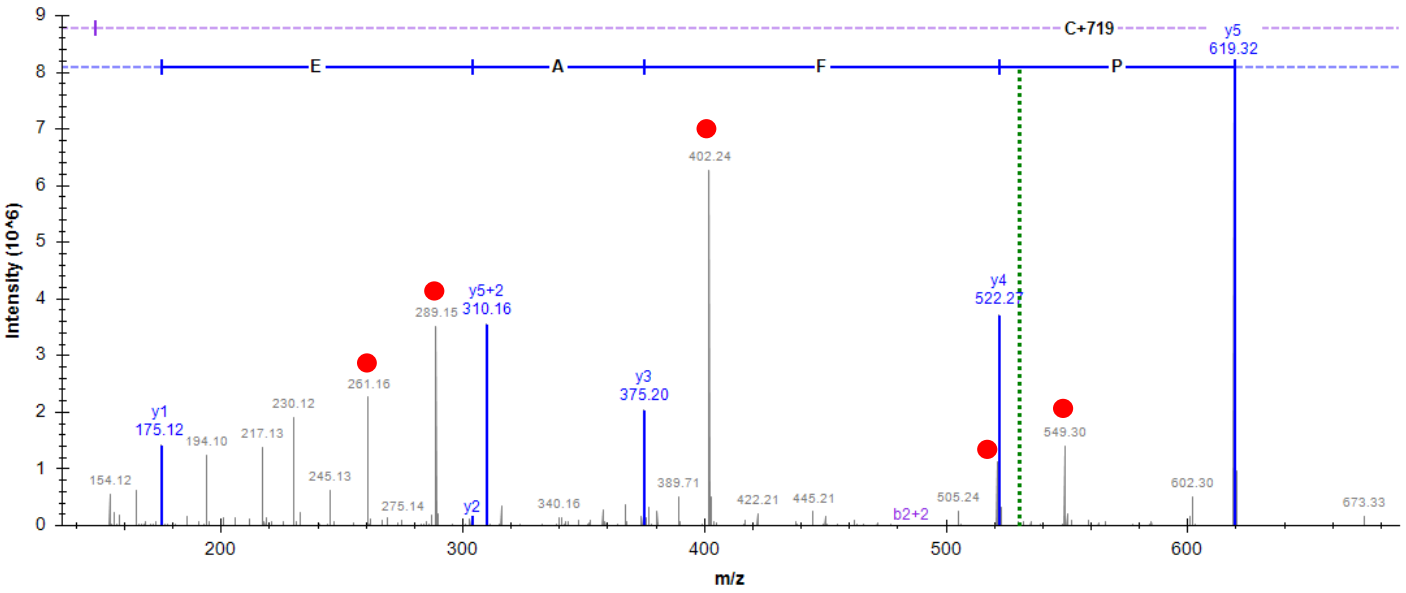


Isolation window

Precursor mass error: 1.39ppm

Evidence of adduct: CFZ fragment ions (red circles) and mass shifted b2 ion

MS2

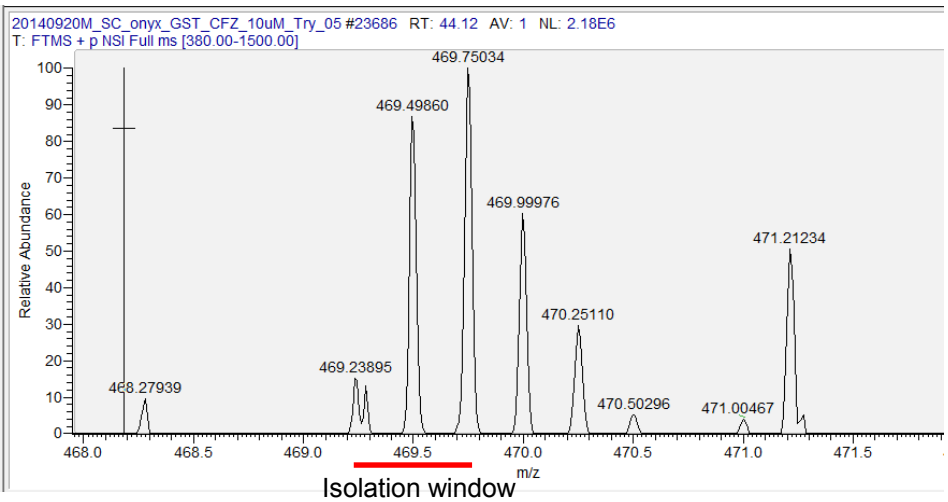


Supplemental Figure 3.

B

GSTO1: ^{188}LNE**C[719]**VDHTPK^{197}

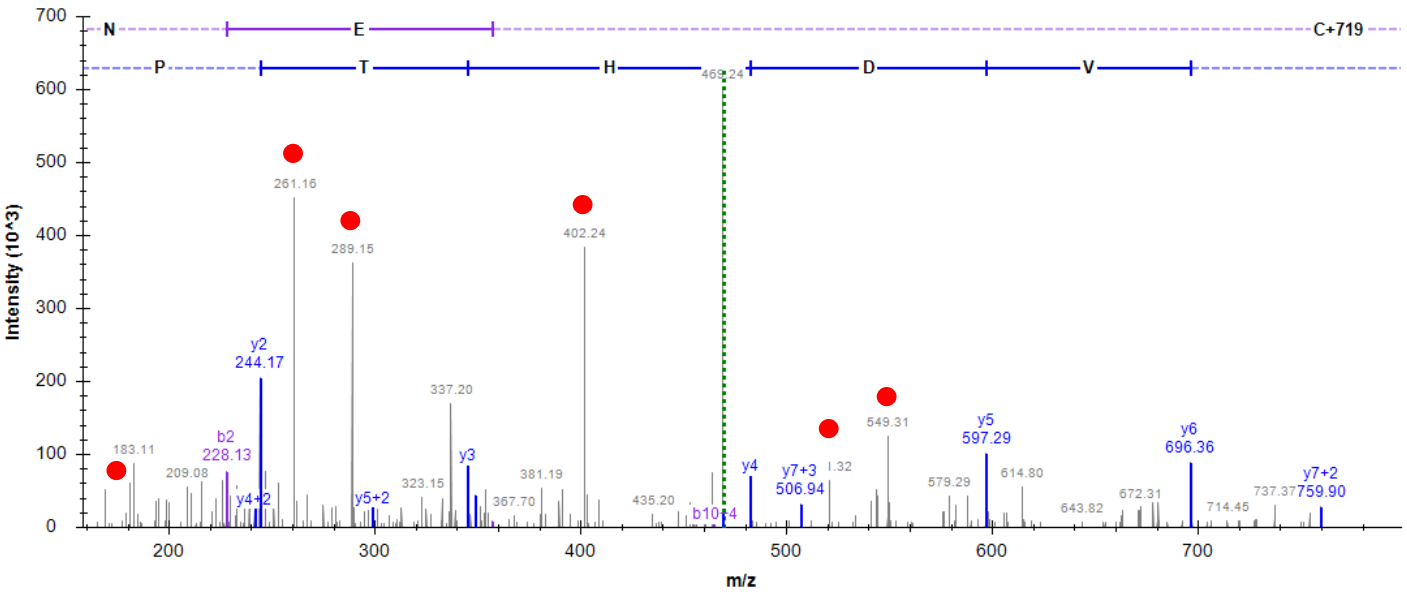
MS1



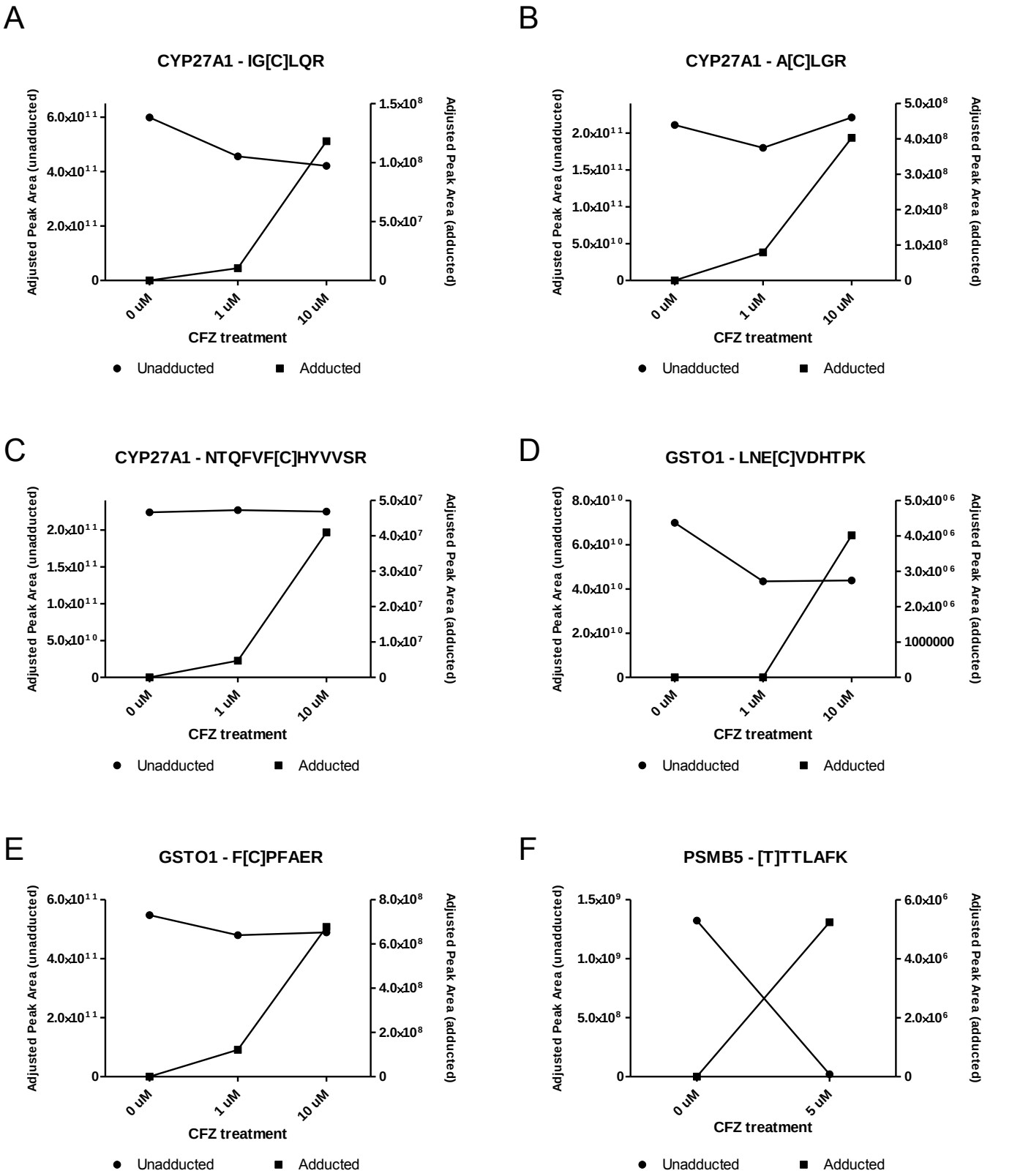
Precursor mass error: 3.24ppm

Evidence of adduct: CFZ fragment ions (red circles) and mass shifted y7 ions

MS2



Supplemental Figure 3.



Supplemental Figure 4. Comparison of adducted and unadducted peptide forms. (A-C) CYP27A1 peptides and (D-E) GSTO1 peptides show minimal decreases in the amount of unadducted peptide compared to the target CFZ protein (F) PSMB5.