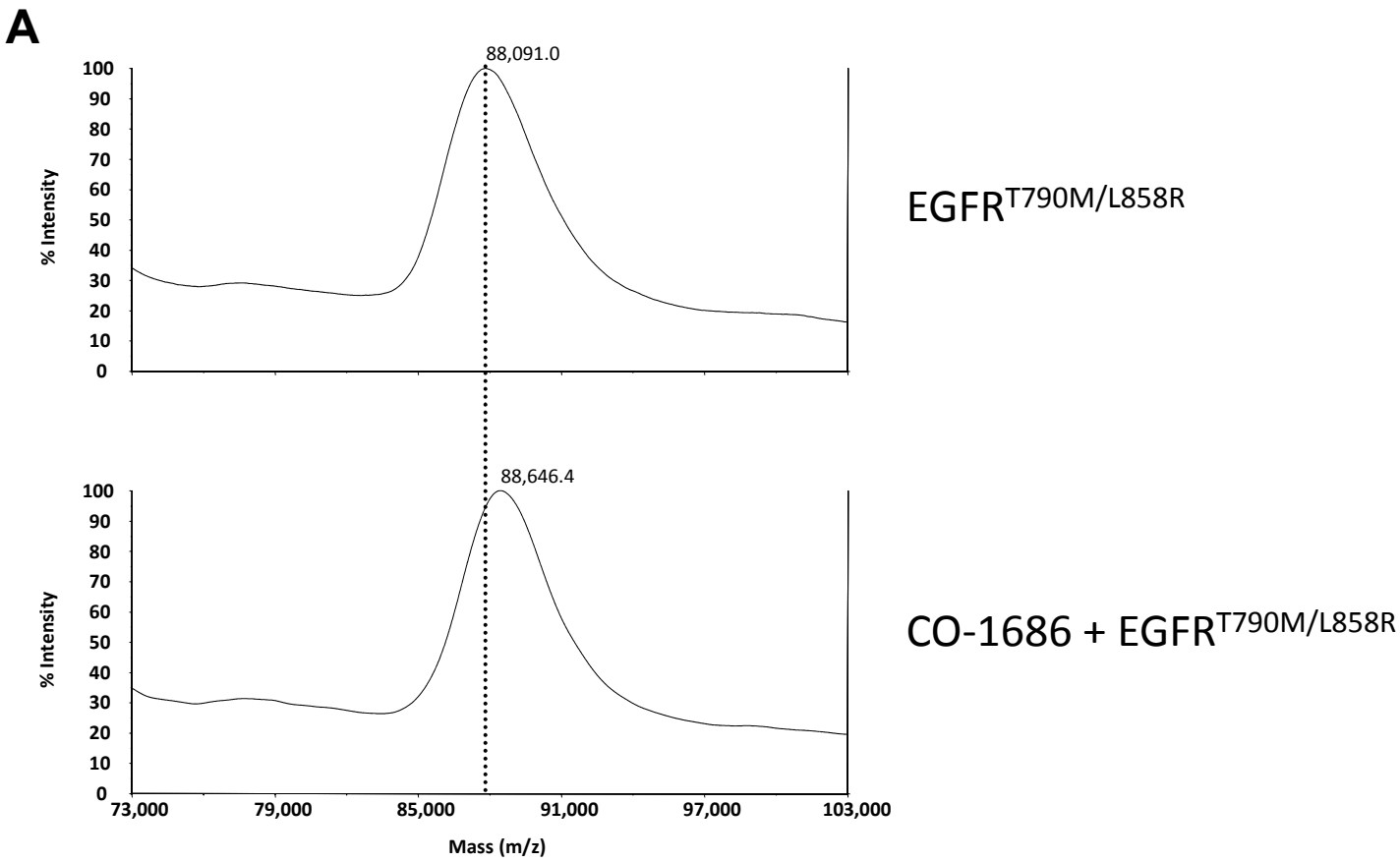
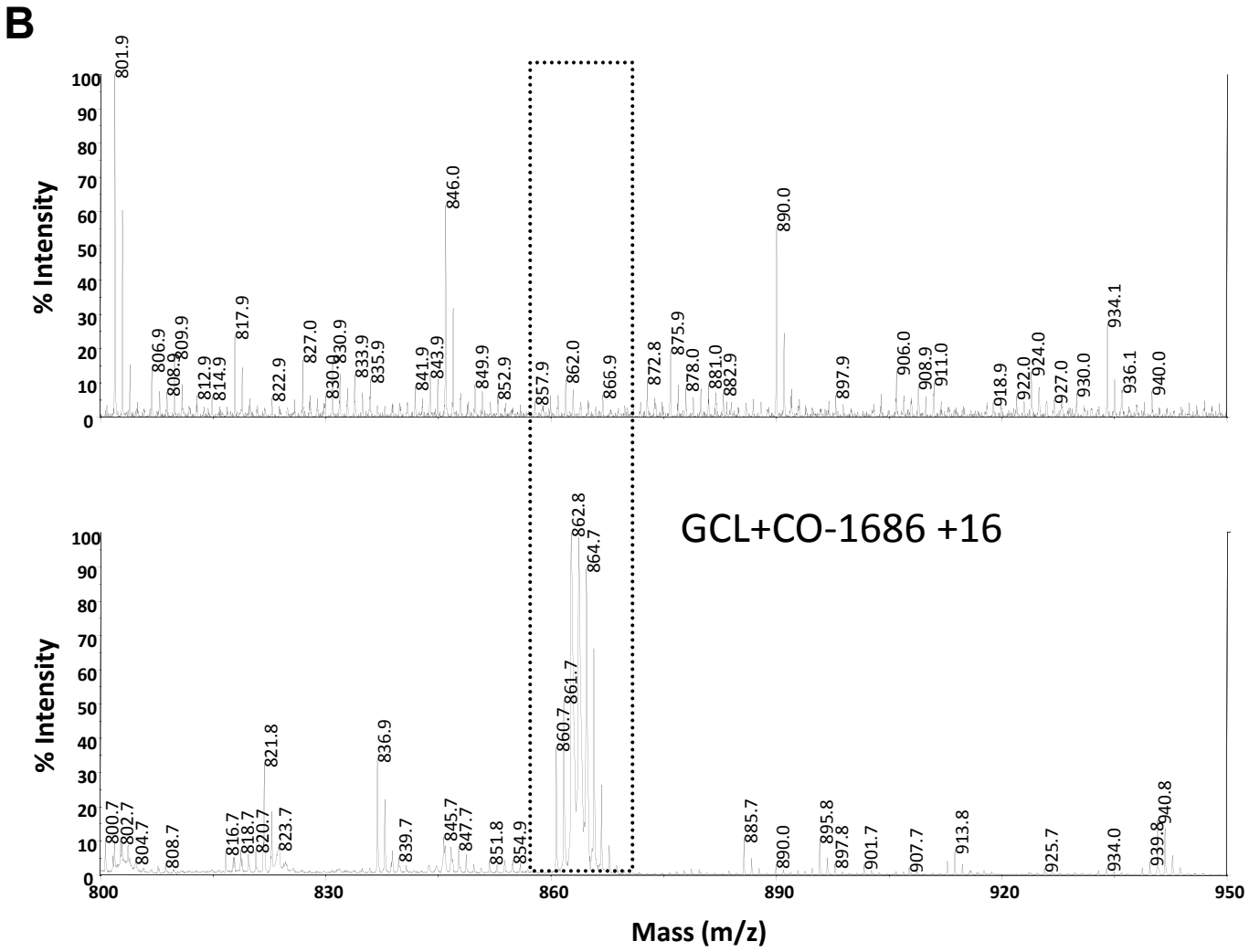
Supplemental Figure 1



Supplemental Figure 1



Supplemental Figure 1

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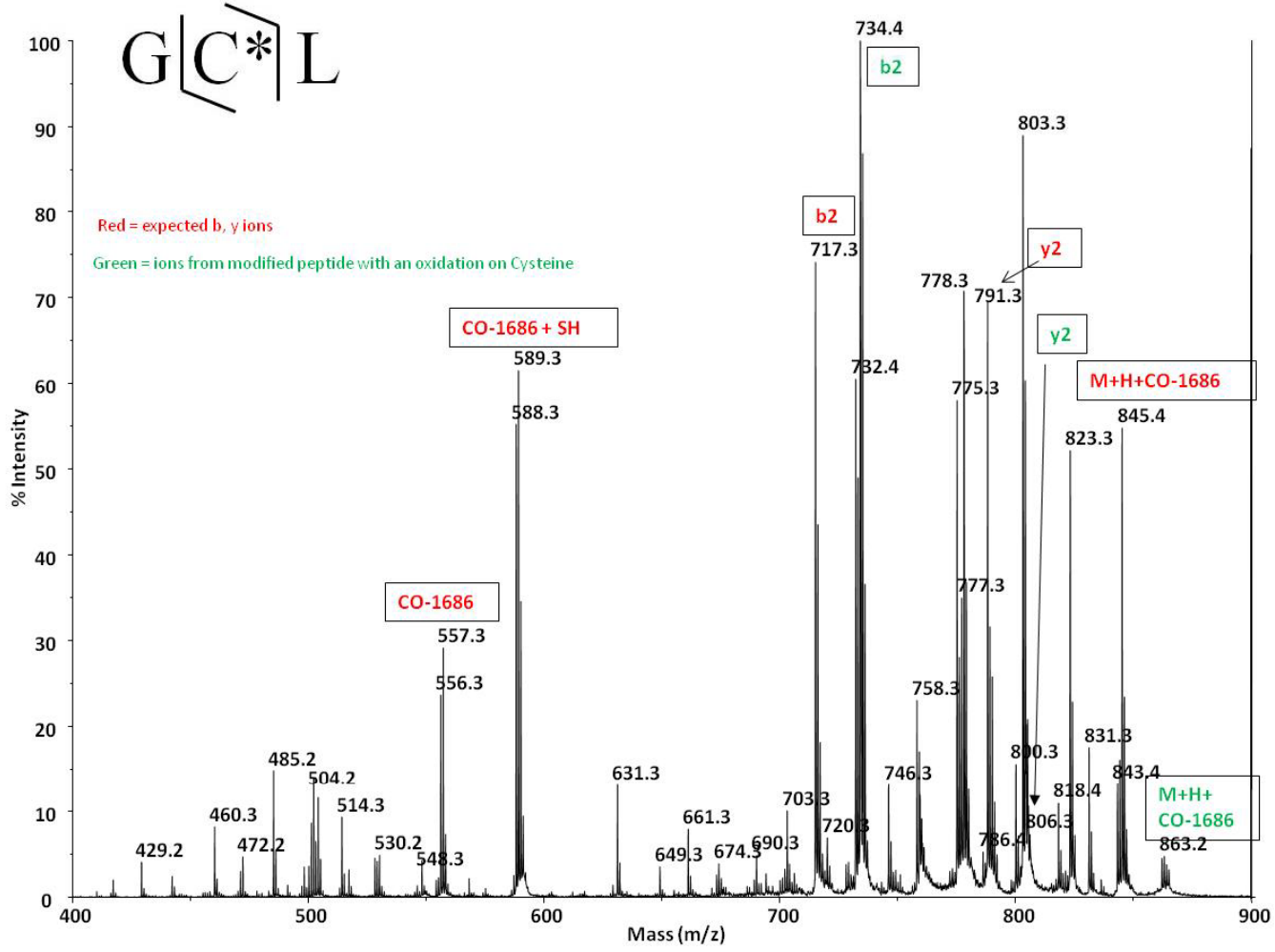

Supplemental Figure 1

SI figure 1. Covalent bonding of the EGFR $^{L858R/T790M}$ protein by CO-1686. (A) Comparison of the mass spectrum of the intact EGFR$^{T790M/L858R}$ protein (m/z 87,547 Da) without (top panel) and with (lower panel) CO-1686 incubation. The EGFR $^{L858R/T790M}$ protein was incubated for 60 minutes with CO-1686 (MW=555.56). The centroid mass (m/z= 88,091 Da) showed a positive shift of 555.4 Da indicating complete stoichiometric modification of EGFR $^{L858R/T790M}$ protein by CO-1686. (B) Peptide fingerprint showing C797 is covalently modified by CO-1686. Pepsin digest analysis of EGFR$^{L858R/T790M}$ incubated with CO-1686 confirms that Cys797 is being modified. The EGFR T790M/L858R mutant was reacted with 10-equivalence of CO-1686 for 30 min at 37^{o}C and then subjected to a pepsin digest in-solution. After digestion, the sample was purified, spotted on a MALDI target plate and then analyzed on an ABSciex 4800 MALDI TOF/TOF. The top panel shows the peptide fingerprint from the control protein, while the bottom panel shows the peptide fingerprint from the CO-1686 treated protein. There is a unique peptide observed in the treated digest with a mass of 864.7 that corresponds with the mass of the peptide (GCL) containing C797, the mass of CO-1686 and the mass of a single oxygen (MW = 16) occurring on the peptide. (C) MS/MS confirms the identity of the ^{796}GCL^{798} peptide with a single CO-1686 modification on cysteine 797. The expected b and y ions are indicated. Other unique detected ions are not labeled.