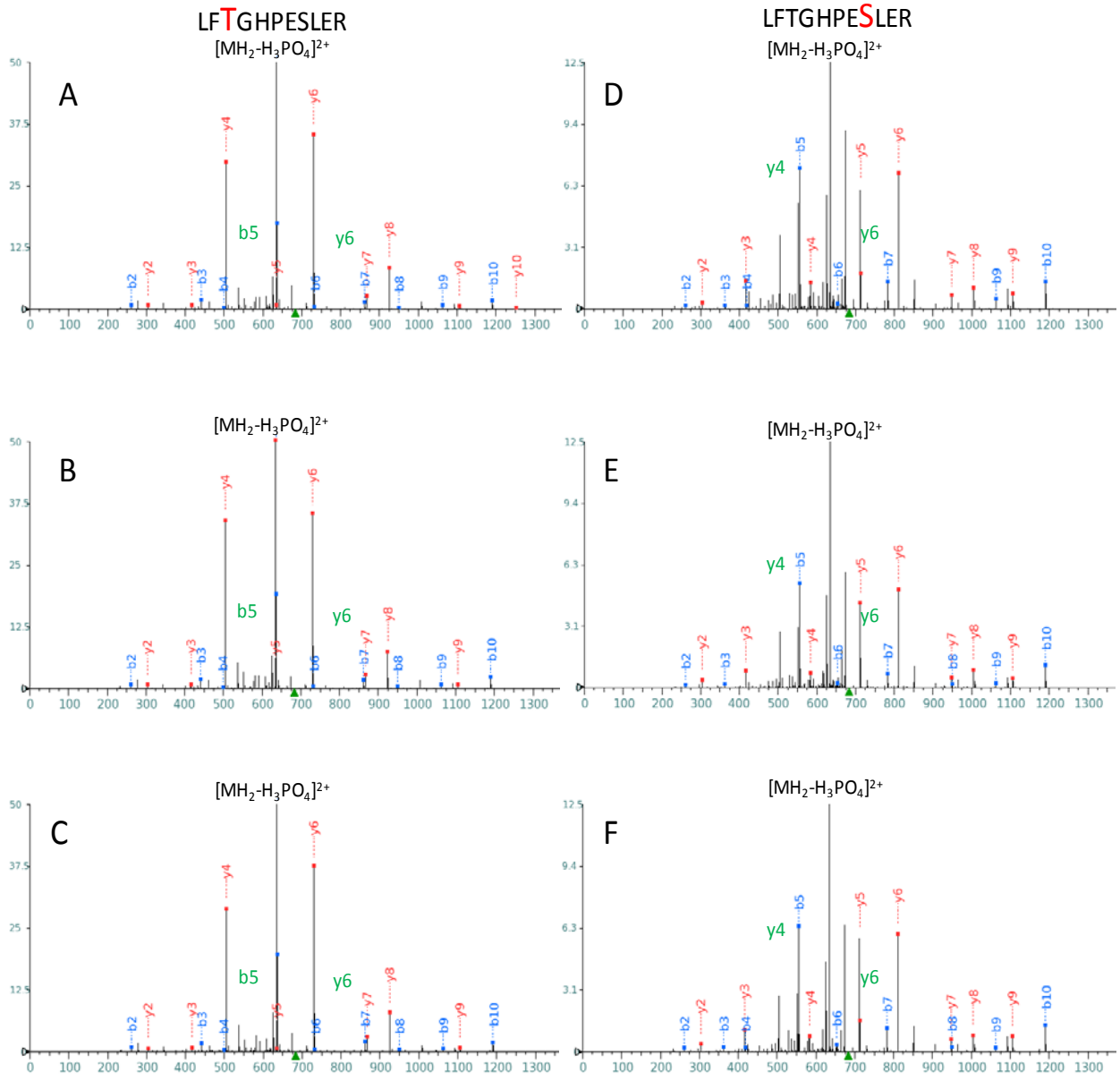

Figure S1. Example spectra from each library shows the presence of candidate peaks supporting gas phase rearrangement of the phosphorylation group. Two libraries were synthesized as described in the Methods. Each library contained 960 phosphopeptides and was analyzed by shotgun sequencing with MS/MS collected in an ion trap mass spectrometer (LTQ). The peptide LFTGHPESLER was highlighted in the paper by Palumbo and Reid[1]. The doubly-charged precursor ion for this peptide was identified in all three replicates and is shown in **A-C**, respectively. The doubly-charged precursor ion for the corresponding peptide from each of three replicates from the other library (LFTGHPESLER) is shown in **D-F**, respectively. Correct b/y ions are indicated in blue/red, respectively. Site-determining ions for the alternative (incorrect) assignment are indicated in green. Ions are only labeled if they are within 0.6 m/z Da of the predicted fragment ion mass and if they were among the ten most abundant peaks/ 100 m/z window.