Supplementary material for

Auto-hydroxylation of FIH-1, an Fe(II), α-ketoglutarate dependent human hypoxia sensor

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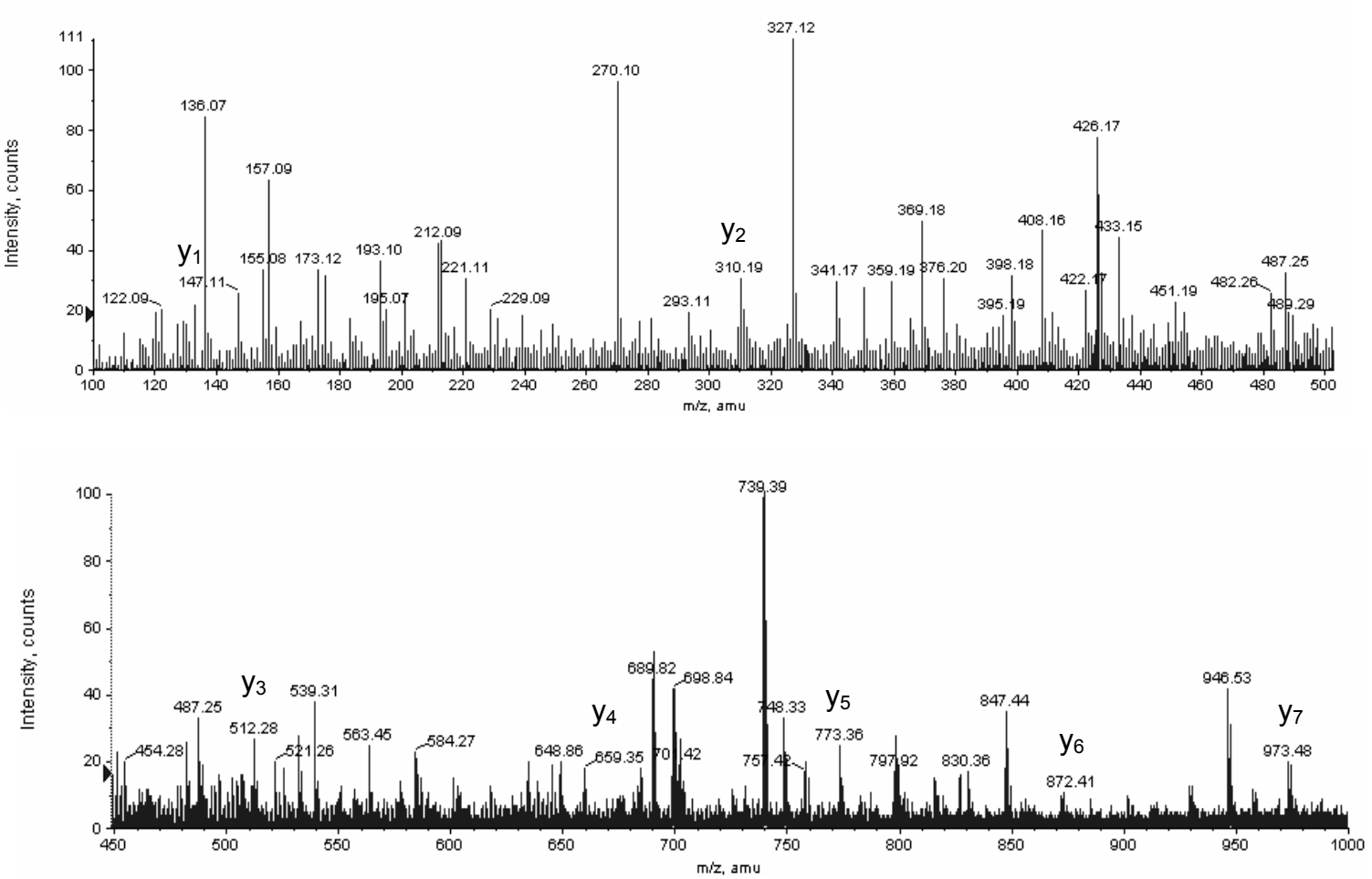

Figure S1. ESI-MS/MS spectra of trypsin-digested purple FIH-1, showing selected *y*-ions from the OMH^+ ion for peptide 252-298 (*m* = 5533 amu). Purple FIH-1 was prepared by anaerobically mixing apo FIH-1 (100 μM), $FeSO_4$ (500 μM), and αKG (500 μM) in 50 mM HEPES, pH 7.50, then opening the cuvette to air. The purple FIH-1 was then concentrated and buffer exchanged to remove excess metal. Purple FIH-1 (10 mM) was digested by trypsin overnight (37°C, 50 mM Tris, pH 8.00, 1 mM $CaCl_2$), then separated by RP-HPLC over a 150 x 2.1 mm PLRP-S column (Polymer Laboratories, Amherst, MA) using an H_2O/CH_3CN (0.1% formic acid) gradient. Peptide 252-298 was collected, dried, and then injected into a Qstar XL hybrid ESI-TOF for analysis.