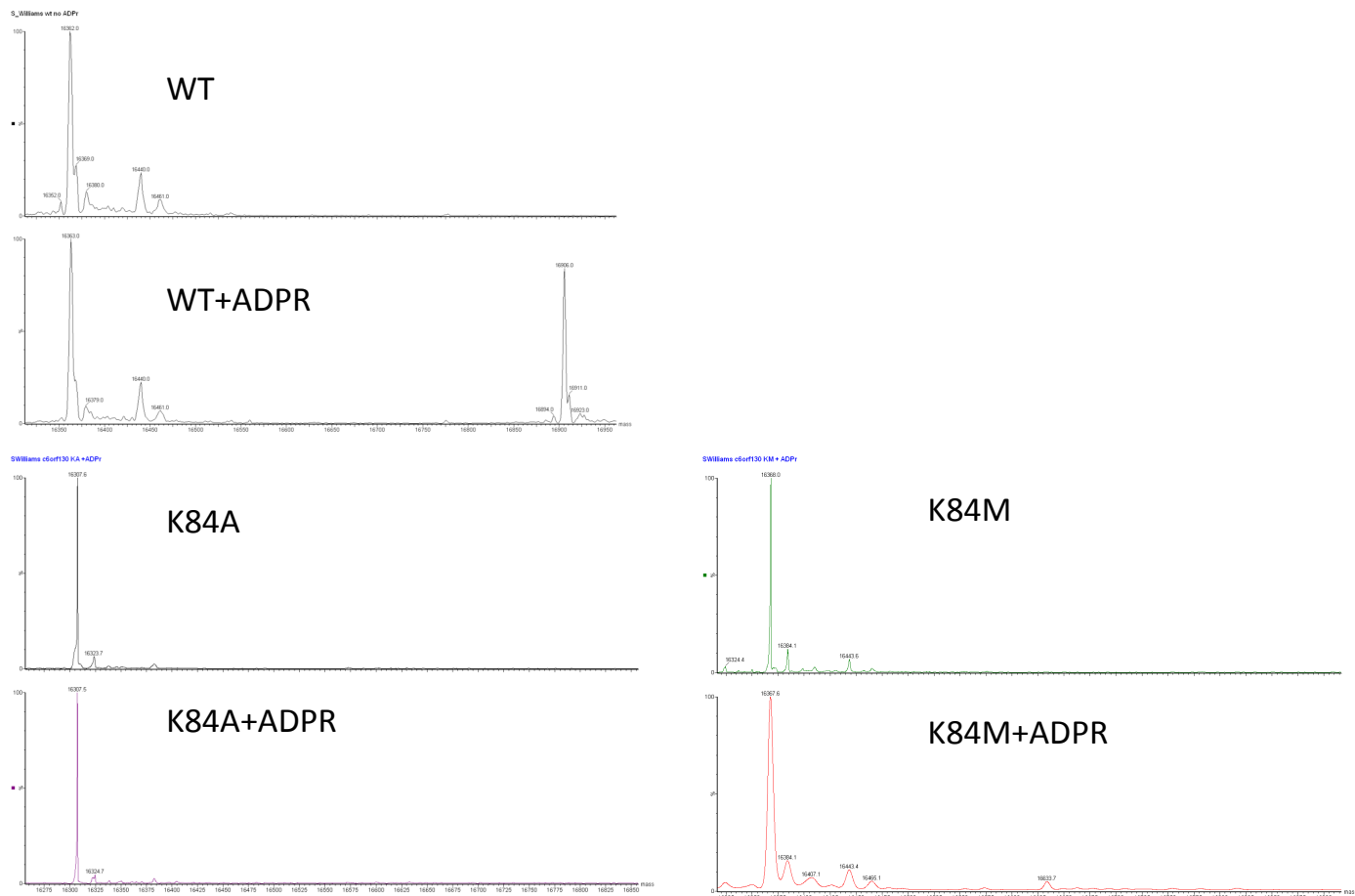
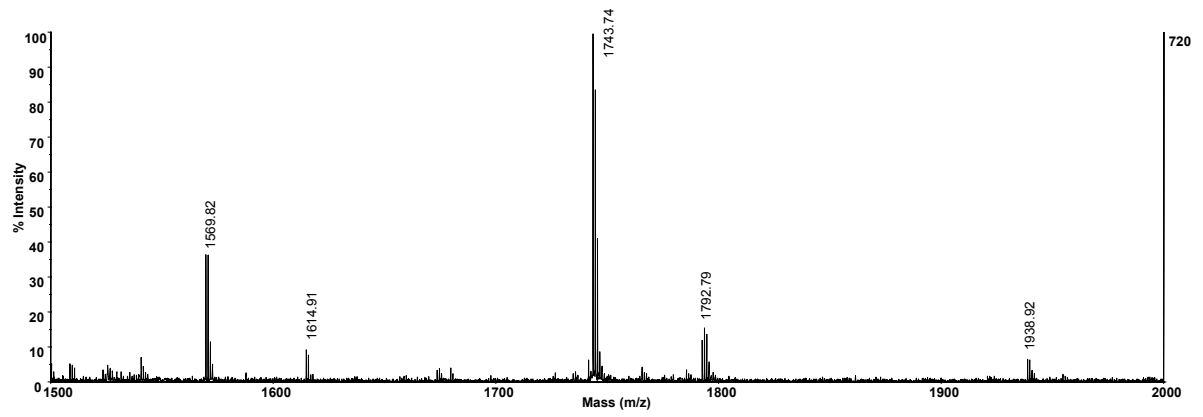
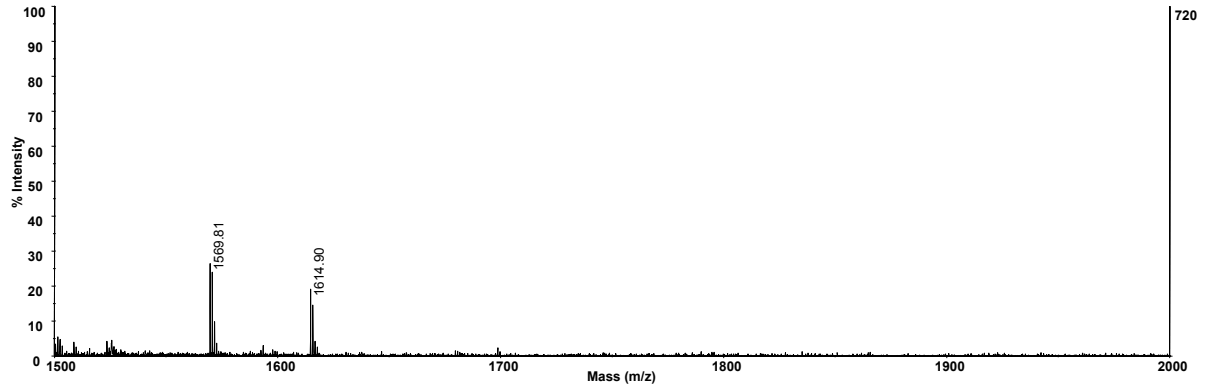


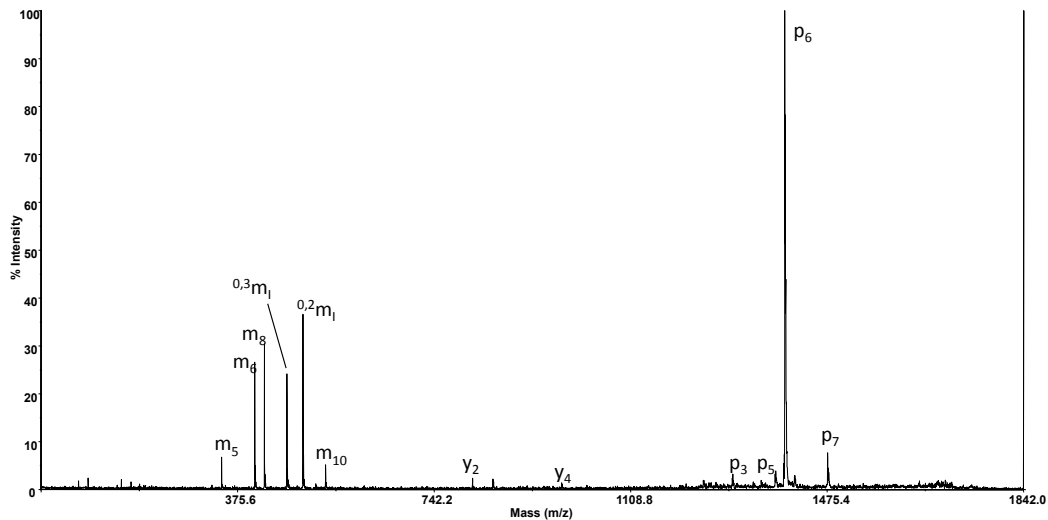
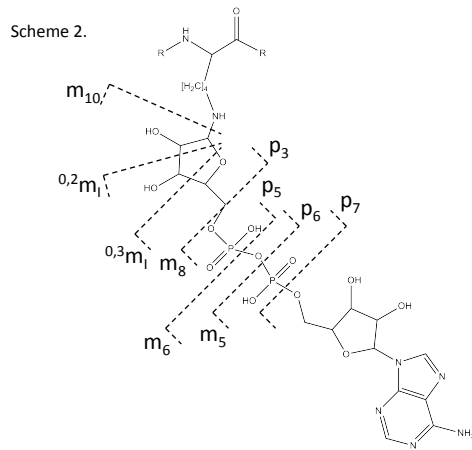
## Supplementary Mass spectrometry Data 2



Supplemental Figure 1. Deconvoluted mass spectra of WT c6orf130, K84A and K84M mutants with or without pre-incubation with ADP-ribose. A mass gain of approximately 541 Da corresponds to the ADP-ribosylated form of the protein that is only observed in the wild-type protein



Supplementary Figure 2. Negative ion MALDI-MS of tryptic digest of C6orf130 in the absence (Top) or the presence (bottom) of C6orf130 pre-incubation with ADP-ribose.



Supplementary Figure 3. Top: Fragmentation patterns of the ADP-ribosylated peptide **YYIYLITKK** where the ADP-ribose moiety is located at the penultimate lysine. Scheme 1 is the standard peptide fragmentation described by Roepstorff with the y-ions observed for this peptide labeled. Scheme 2 shows the fragmentation of the ADP-ribose moiety as described by Hengel, et al. with the fragments observed labeled. Bottom: Negative ion MALDI-MS/MS spectrum of ion m/z 1743.7 corresponding to the ADP-ribosylated form of residues 71-79 of C6orf130. It is concluded that the ADP-ribose resides on K84 due to its expected reactivity, that it K84 is no longer cleaved by trypsin, and mutation of K84 to Ala or Met blocks reactivity of C6orf130 with ADP-Ribose. Fragment ions are labeled as described in the literature and outlined in Supplementary Figure X

References:

Roepstorff P, Fohlman J. "Proposal for a common nomenclature for sequence ions in mass spectra of peptides." *Biomed Mass Spectrom.* 1984 Nov;11(11):601.

Hengel SM, Shaffer SA, Nunn BL, Goodlett DR. Tandem mass spectrometry investigation of ADP-ribosylated kemptide. *J Am Soc Mass Spectrom.* 2009 Mar;20(3):477-83.

Hengel, SM, Goodlett DR. "A review of tandem mass spectrometry characterization of adenosine diphosphate-ribosylated peptides. Hengel, Shawna M.; Goodlett, David R. *Int J Mass Spectrom* 2012 Feb;312(SI):114-121)