Supporting Information

Center of Mass Calculation in Combination with MS/MS allows Robust Identification of Single Amino Acid Polymorphisms in Clinical Measurements of Insulin-Like Growth Factor 1

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Table of Contents.

Figure S8. ETD spectrum from the A38V variant following reduction/alkylation. Relatively few fragments are produced and much of the precursor remains intact. However, several unique internal fragments are detected as well. Note: the y-axis has been zoomed for visual enhancement.

Figure S9. EThcD spectrum from the A38V variant following reduction/alkylation. Relatively few fragments are produced and none of the fragments detected are unique to this technique.

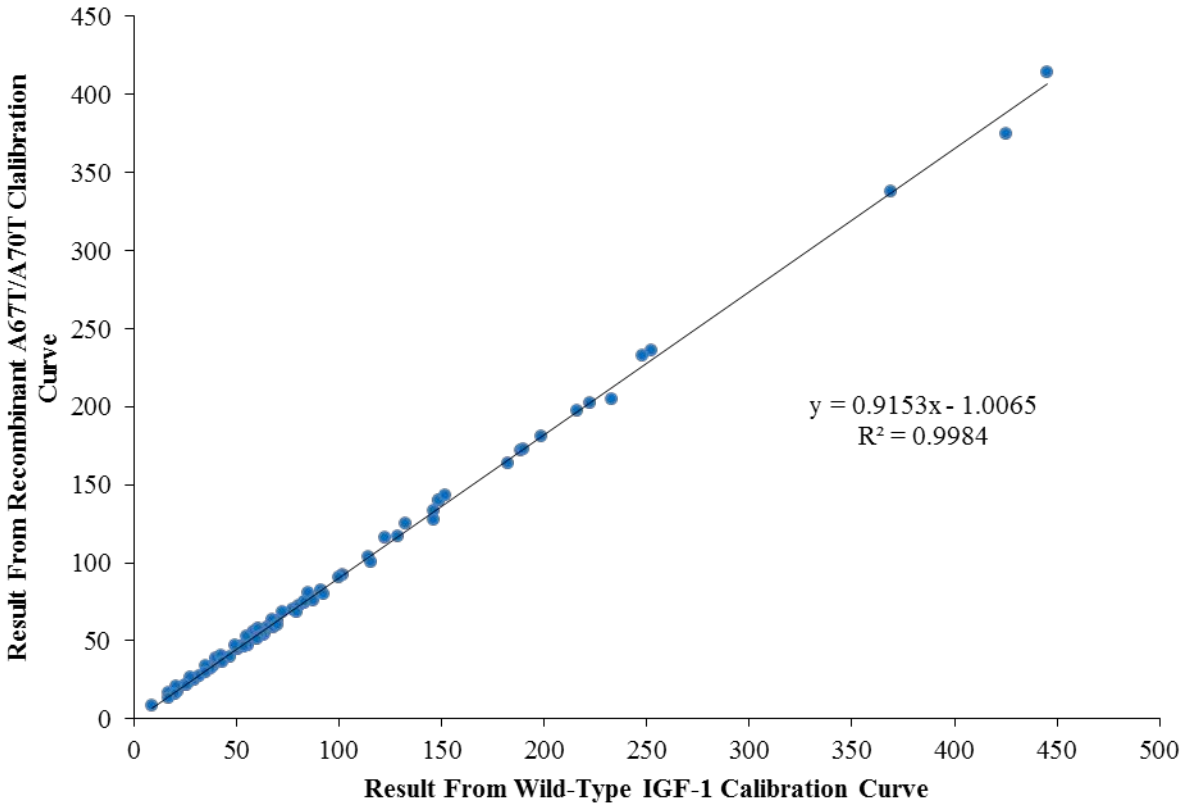


Figure S1. Comparison of A67T and A70T results from a recombinant A67T/A70T (mixed in equal portions) calibration curve and our routine IGF-1 wild-type calibration curve. There was no significant bias between the results, and the bias that was observed was attributed to the different sources of the material. Therefore, we determined that it is appropriate to quantitate A67T or A70T from the wild-type IGF-1 curve.

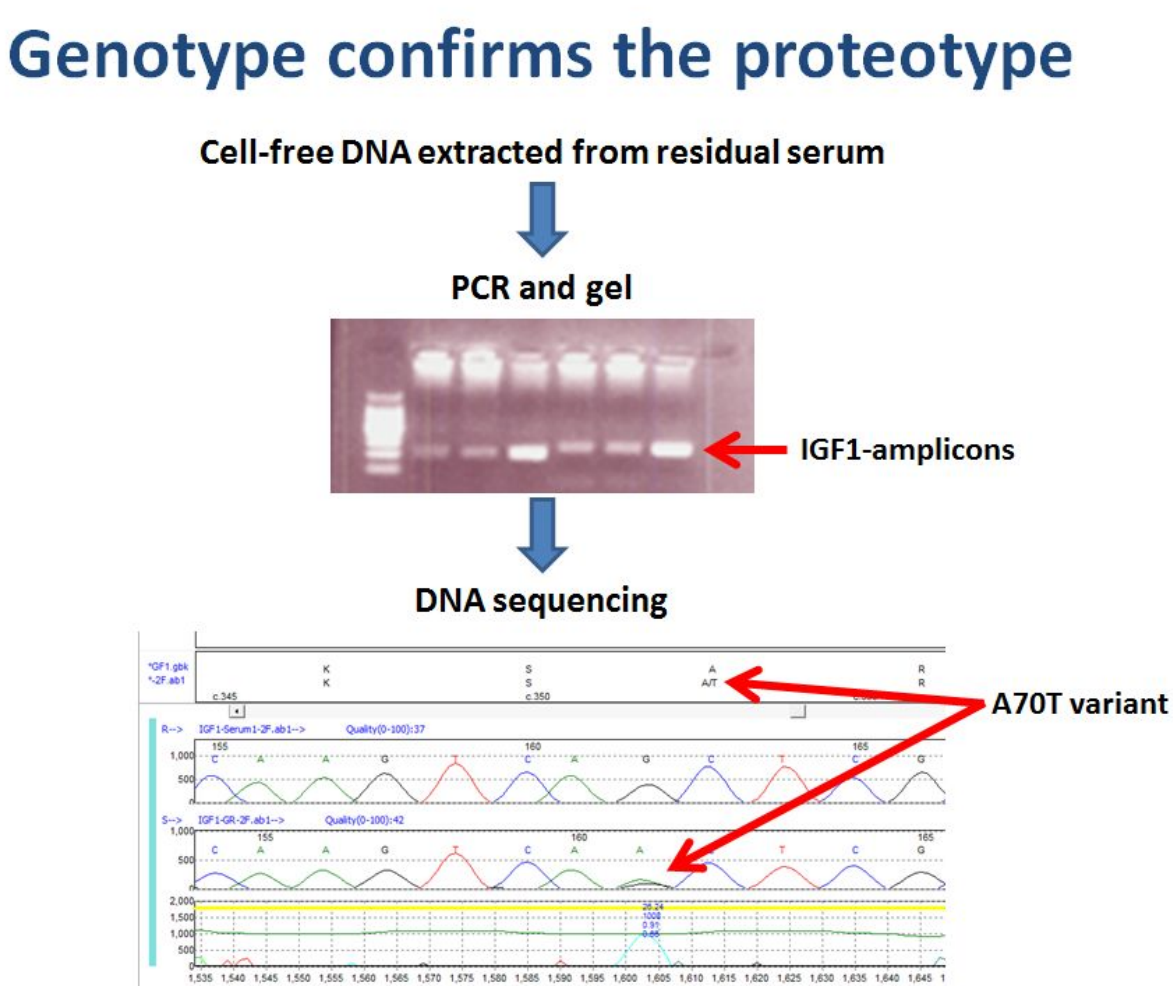


Figure S2. DNA Sequencing workflow and example trace.

A67V Variant Sequence:

GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPVKSA

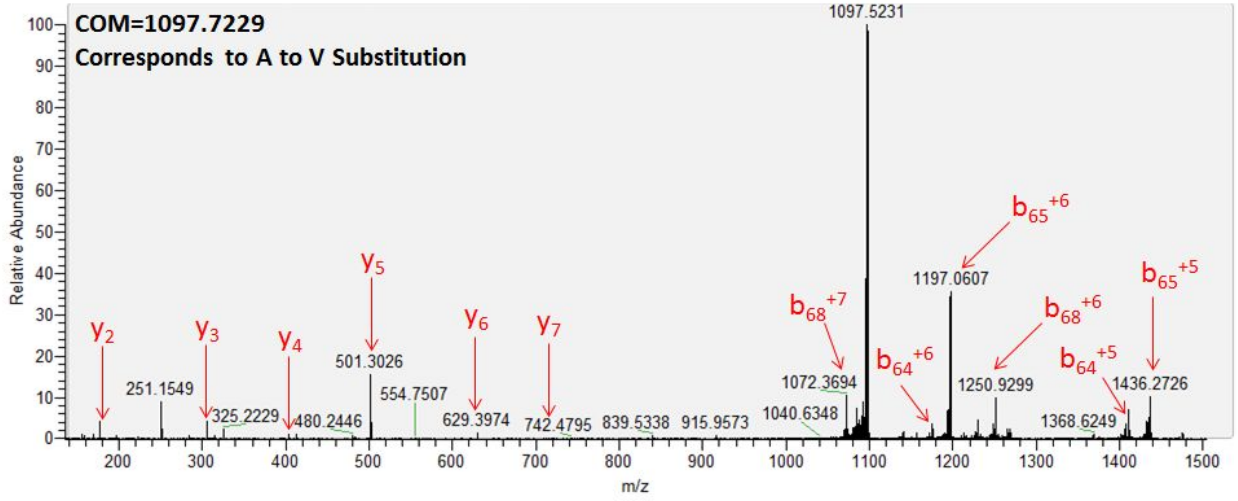


Figure S3. HCD spectrum of the A67V variant without reduction/alkylation. Under these conditions, only C-terminal fragments are produced. However, due to the location of the amino acid substitution the position can be conclusively determined.

A38V Variant Sequence:

GPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRVPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSA

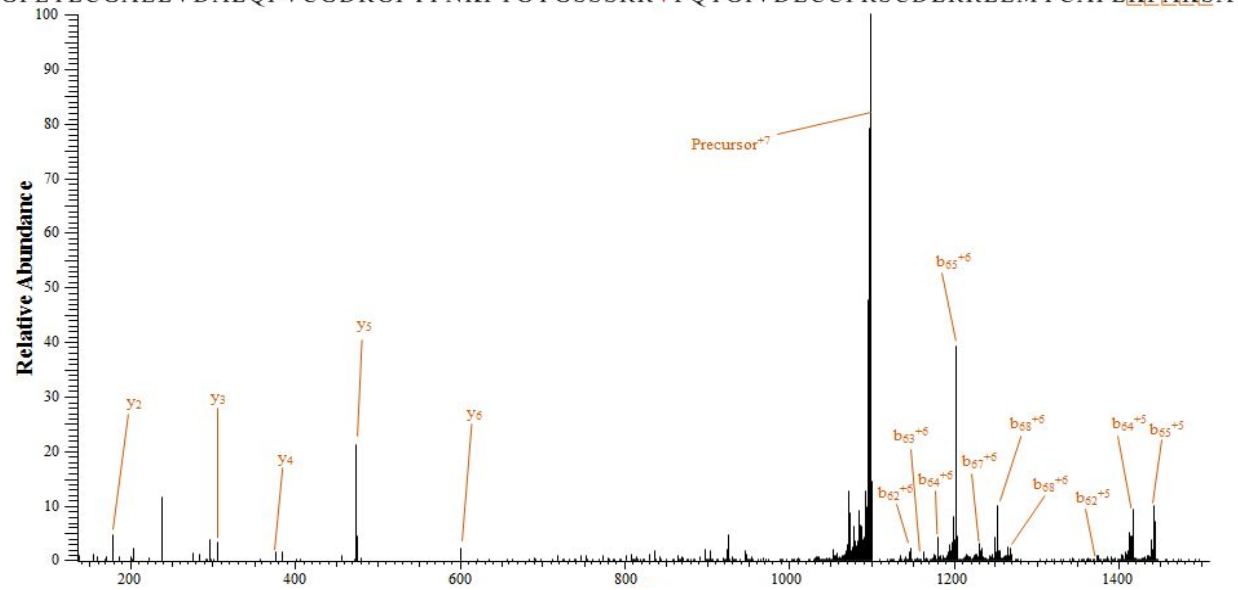


Figure S4. HCD spectrum from the A38V variant without reduction/alkylation. Under these conditions, only C-terminal fragments are produced. Due to the location of the substitution, this technique cannot be used to determine the location of the substitution.

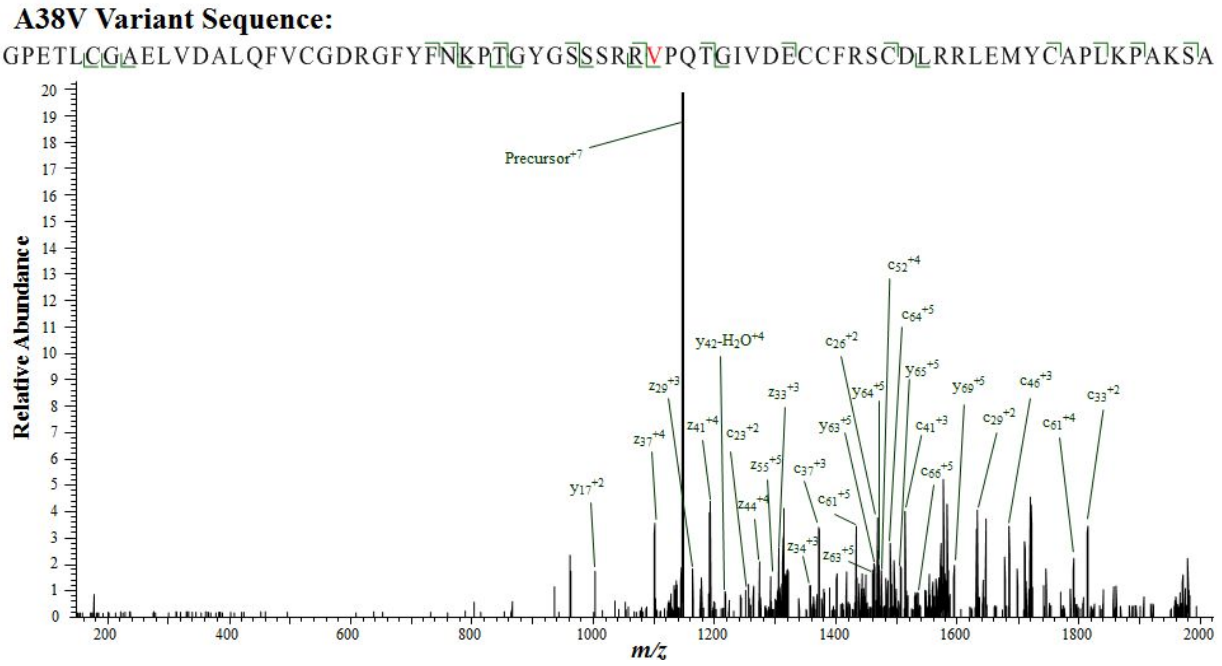


Figure S5. ETciD spectrum from the A38V variant following reduction/alkylation. This technique produced the most identifiable fragments from this polypeptide, including many unique internal fragments. Note: the y-axis has been zoomed for visual enhancement.

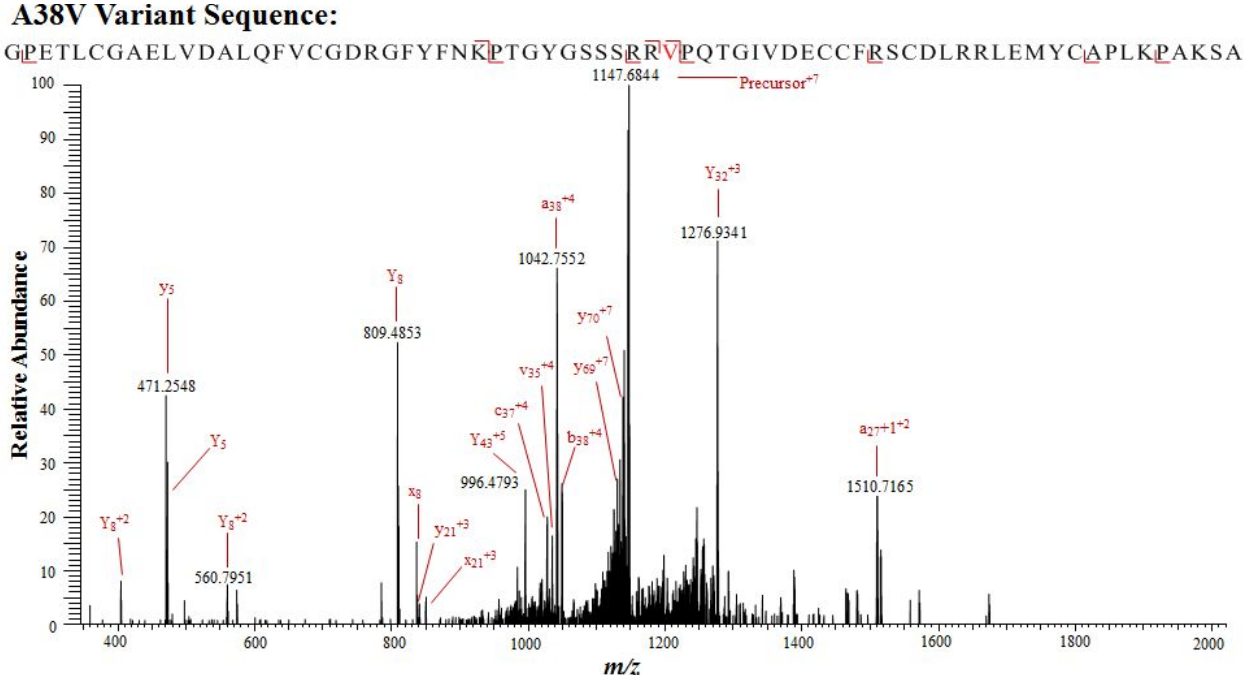


Figure S6.UVPD spectrum from the A38V variant following reduction/alkylation. This technique produced several unique internal fragments, including fragments bracketing the location of the amino acid substitution. Thus, the location of the substitution could be conclusively determined from this technique alone. "Y" corresponds to y-2 ion; we have adopted this convention from Protein Prospector.

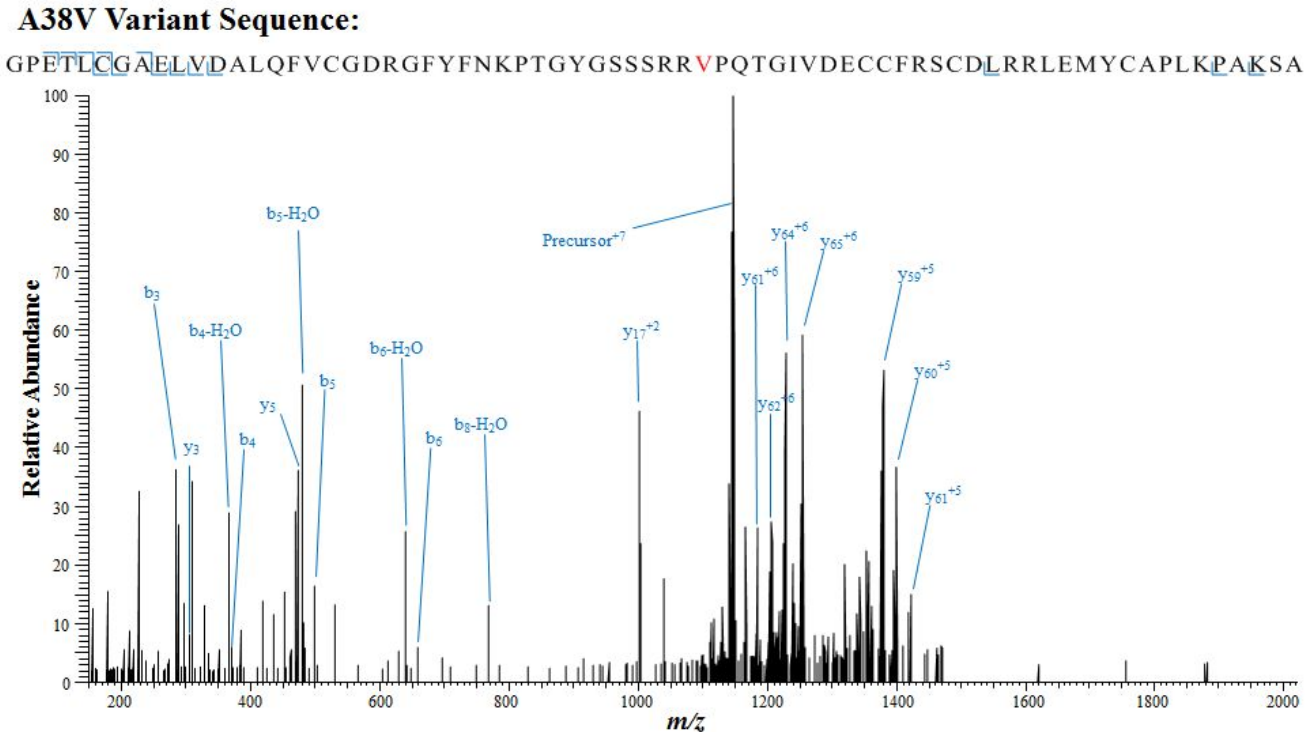
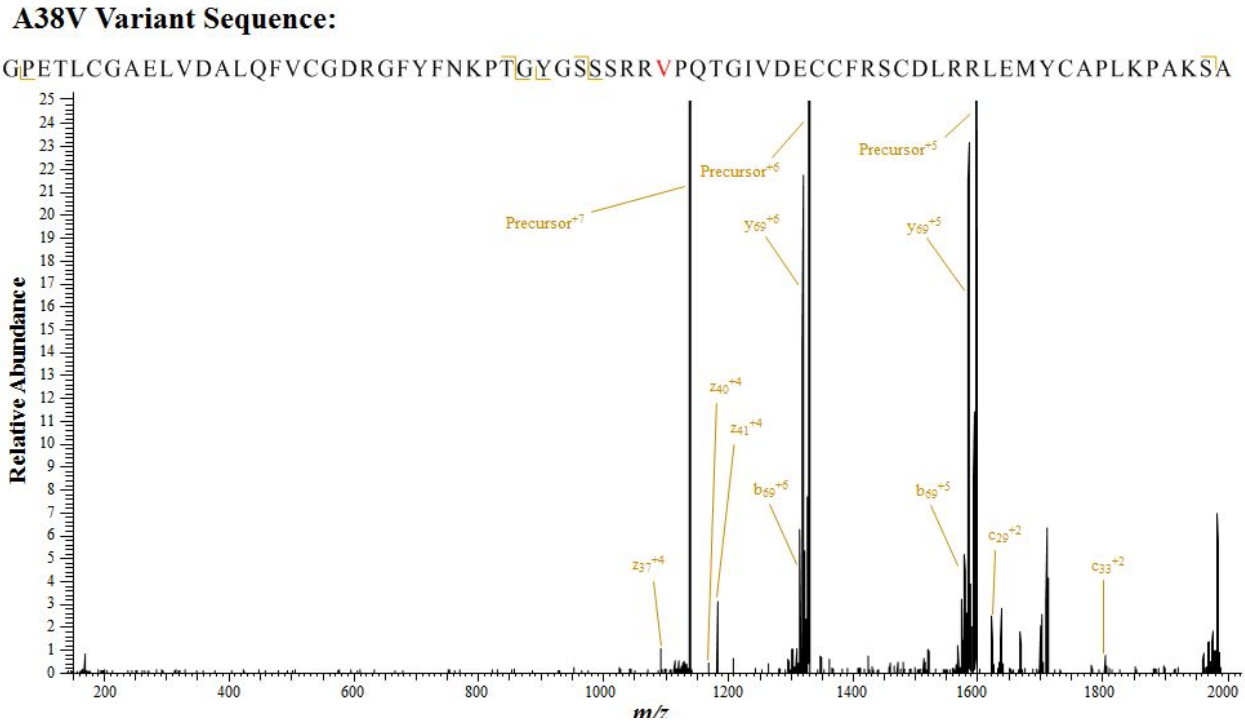


Figure S7. HCD spectrum from the A38V variant following reduction/alkylation. Under these conditions, predominantly N-terminal fragments are produced.



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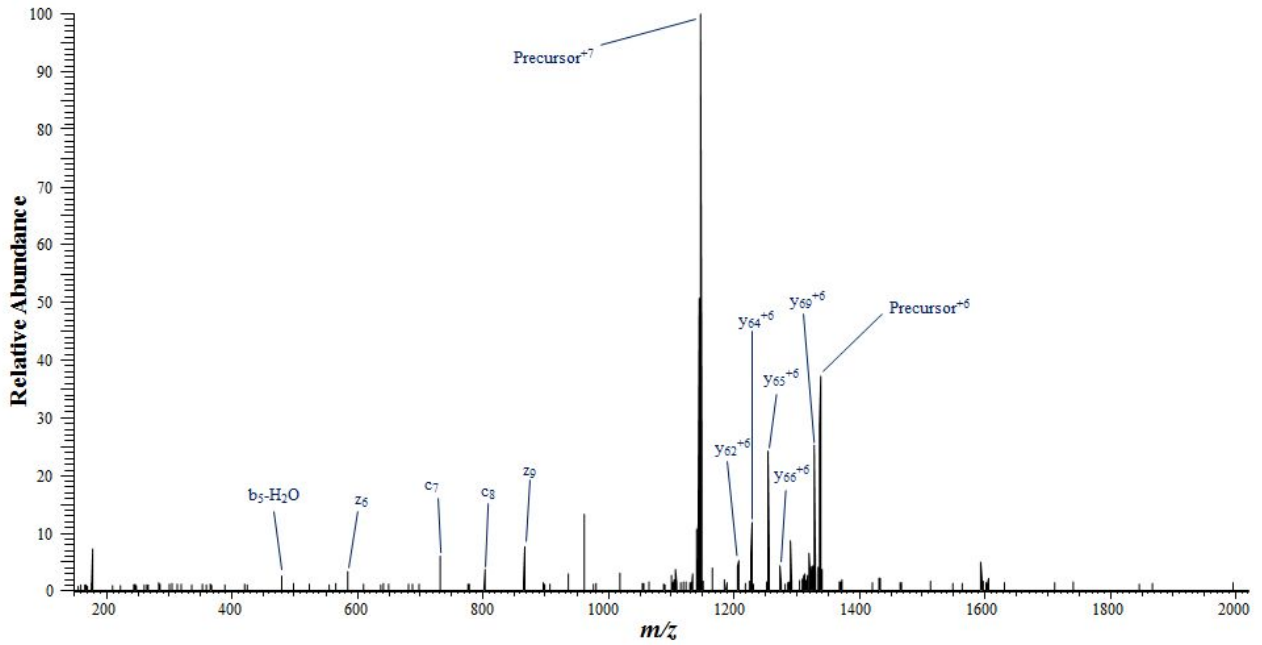

Figure S9. EThcD spectrum from the A38V variant following reduction/alkylation. Relatively few fragments are produced and none of the fragments detected are unique to this technique.