Supporting Information

An Effective Top-down LC/MS+ Method for Assessing Actin Isoforms as a Potential Cardiac Disease Marker

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Supplementary Tables

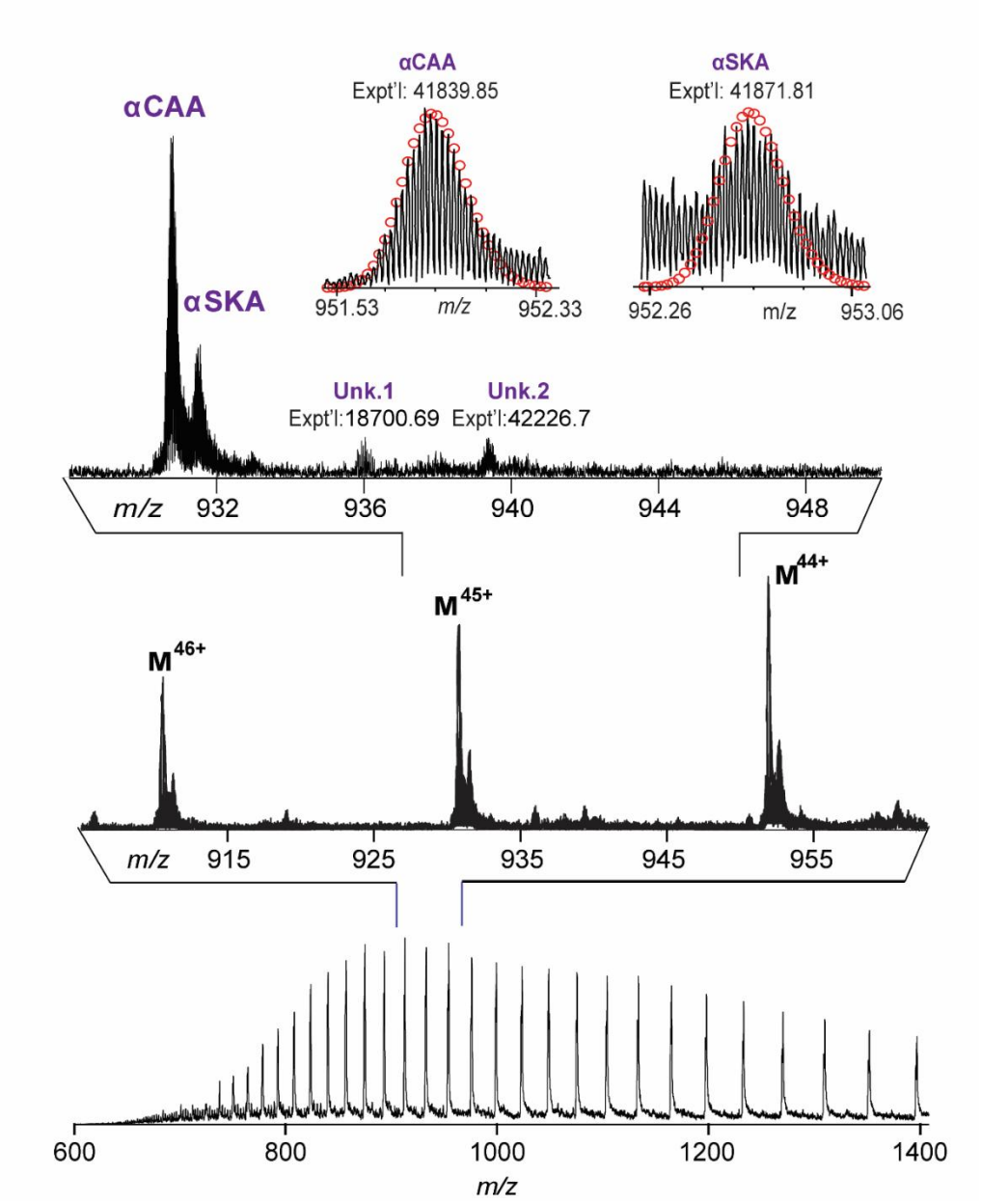
Supplementary Table 1. Investigation of the impact of the number of isotopomers on the quantification of relative abundances. The calculation is based on three MS spectra of α-actin isolated from swine hearts (Figure 5).

# of isotopomer	αCAA%	αSKA%
3	37.1	62.9
5	37.0	63.0
7	37.2	62.8
11	37.2	62.8

Supplementary Table 2. Individual values of relative abundances of αCAA and αSKA from each human heart. The calculated values are based on the data presented in Figure 6. Three biological replicates in control (Ctrl), Ctrl-1, Ctrl-2, Ctrl-3, and dilated cardiomyopathy (DCM), DCM-1, DCM-2, DCM-3, respectively.

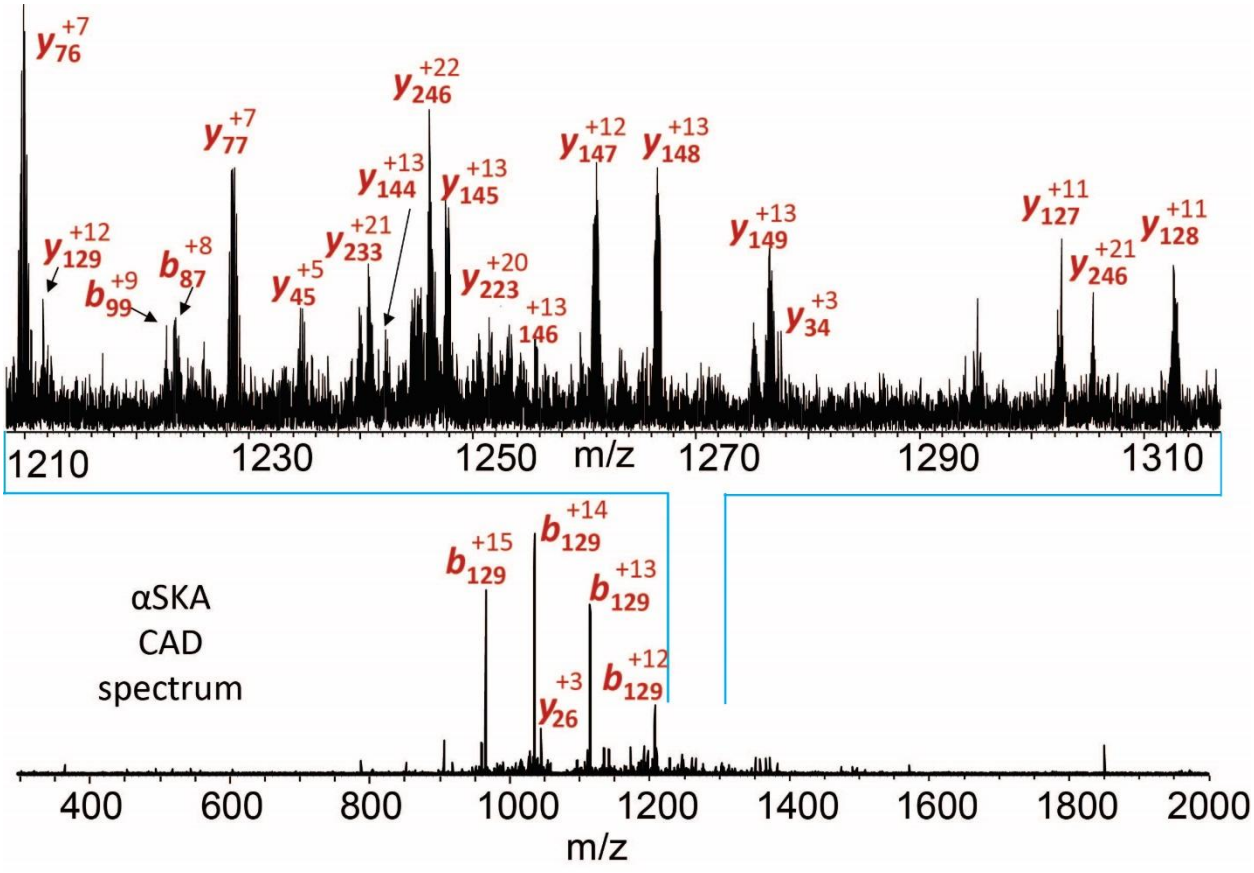
Samples	αCAA %	αSKA %
Ctrl1	73.3	26.7
Ctrl2	75.6	24.4
Ctrl 3	87.4	12.6
DCM 1	57.0	43.0
DCM 2	58.2	41.8
DCM 3	53.5	46.5

Supplementary Figures

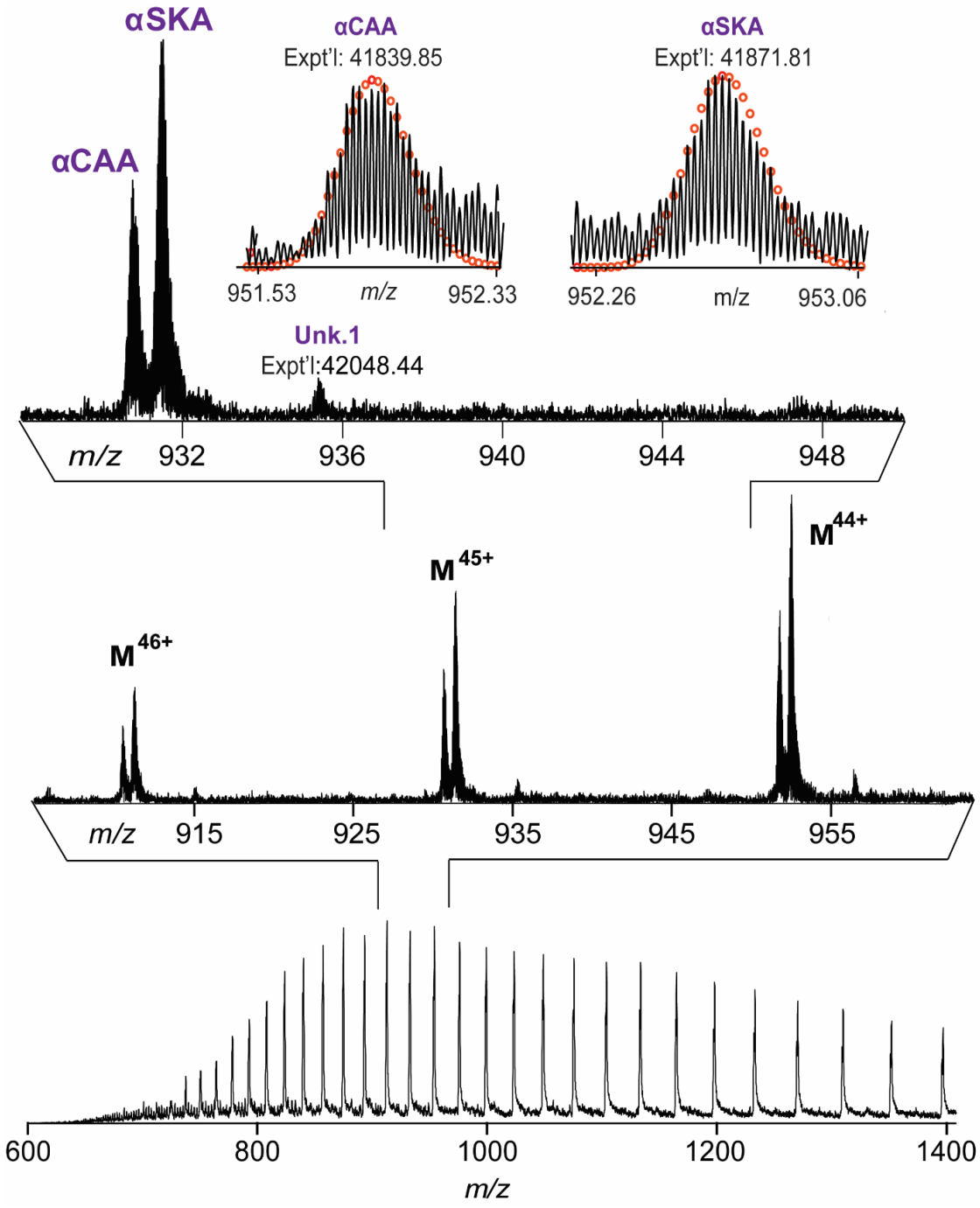


Supplementary Figure 1. LC/MS+ analysis of human α-actin. Bottom; MS detection of human α-actin in on-line LC/MS using low-resolution MS (shown in multiply charged ions); middle, off-line FTMS analysis of intact α-actin (M^{46+}, M^{45+}, M^{46+}); and top, isotopically resolved molecular ions αCAA and αSKA with two unknown proteins. Circles represent the theoretical isotopic abundance distribution of the isotopomer peaks corresponding to the assigned mass.

Supplementary Figure 2. The sequence alignment of human α-cardiac actin (αCAA) and α-skeletal actin (αSKA). The sequence alignment of human αCAA (P68032), and αSKA (P68133) from UnitProtKB/Swiss-Prot database. Human αCAA and αSKA vary by two juxtaposed amino acids (Asp2Glu3 for αCAA, and Glu2Asp3 for αSKA) and two amino acids substitution (Met299 and Thr358 in αSKA, versus Leu299 and Ser358 in αCAA), resulting in 32 Da difference. The two juxtaposed amino acids and the two amino acids substitution are both indicated in red.



Supplementary Figure 3. MS/MS mapping of αSKA. Representative MS/MS spectra of ***b*** and ***y*** ions from CAD spectra of αSKA.



Supplementary Figure 4. LC/MS+ analysis of swine α-actin. Bottom; MS detection of swine α-actin proteins in on-line LC/MS using low-resolution MS (shown in multiply charged ions); middle, off-line FT analysis of intact α-actin (M^{46+}, M^{45+}, M^{46+}); and top, isotopically resolved molecular ions αCAA and αSKA with one unknown protein. Circles represent the theoretical isotopic abundance distribution of the isotopomer peaks corresponding to the assigned mass.