

## **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

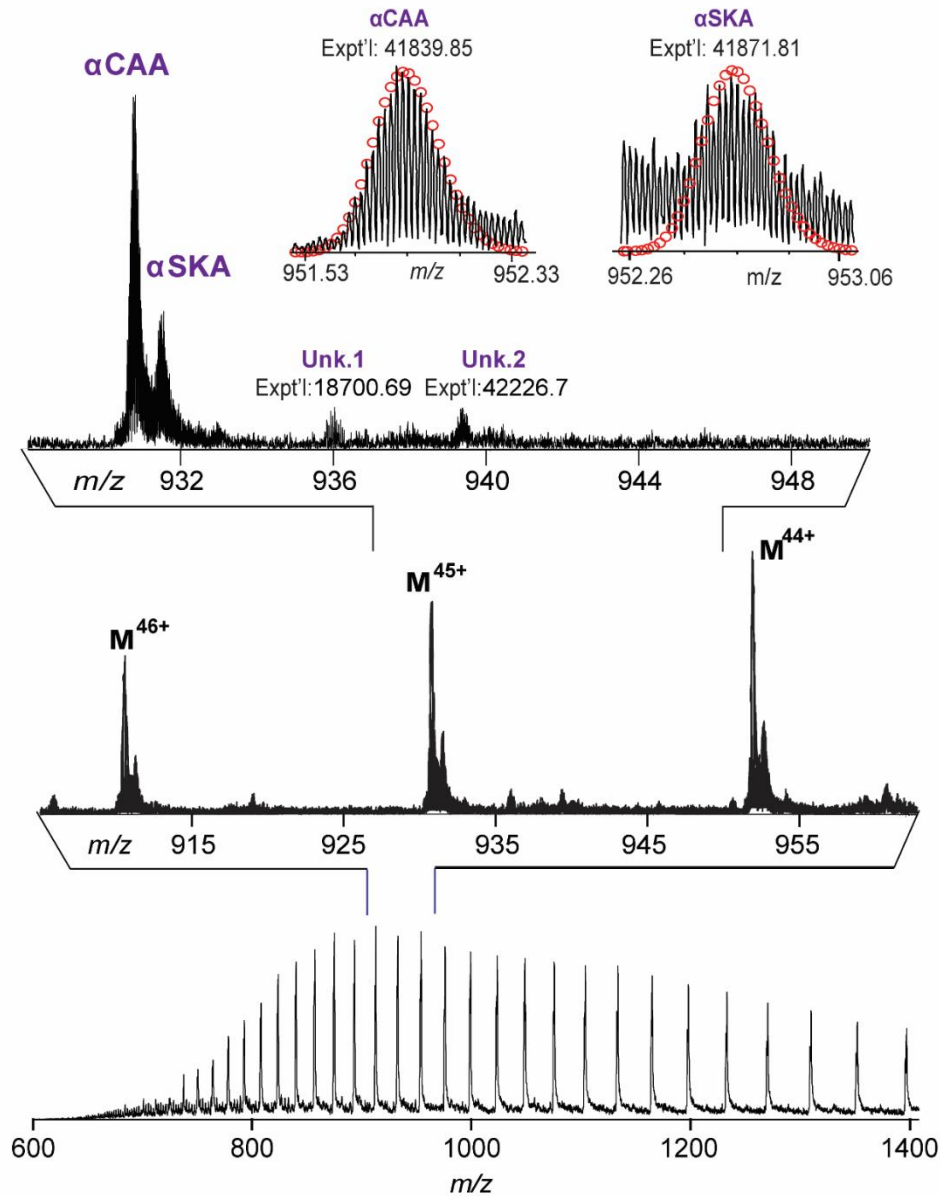
**Supplementary Table 1. Investigation of the impact of the number of isotopomers on the quantification of relative abundances.** The calculation is based on three MS spectra of  $\alpha$ -actin isolated from swine hearts (Figure 5).

# of isotopomer	$\alpha$ CAA%	$\alpha$ SKA%
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  $\alpha$ CAA and  $\alpha$ SKA 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	$\alpha$ CAA %	$\alpha$ SKA %
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



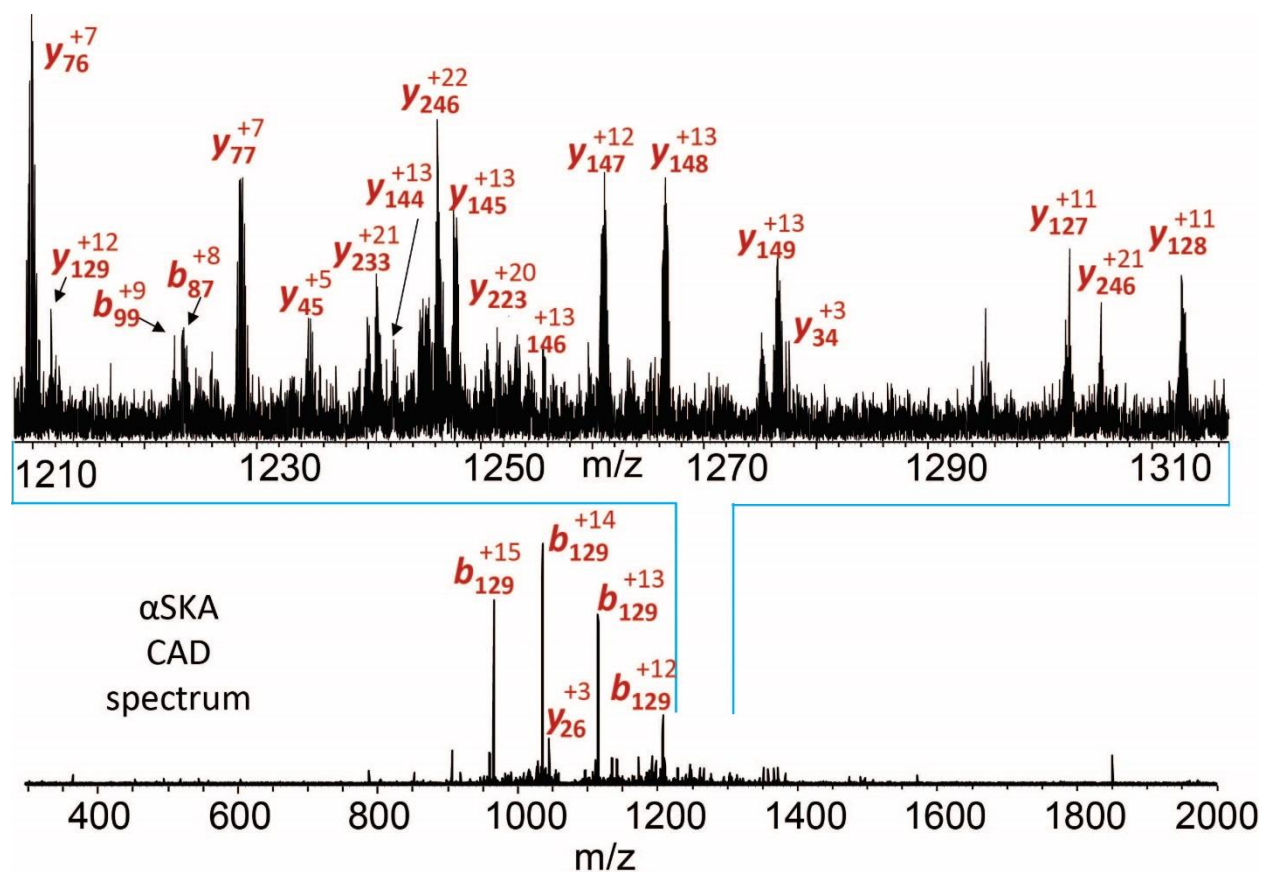
**Supplementary Figure 1. LC/MS<sup>+</sup> analysis of human  $\alpha$ -actin.** Bottom; MS detection of human  $\alpha$ -actin in on-line LC/MS using low-resolution MS (shown in multiply charged ions); middle, off-line FTMS analysis of intact  $\alpha$ -actin ( $M^{46+}$ ,  $M^{45+}$ ,  $M^{44+}$ ); and top, isotopically resolved molecular ions  $\alpha$ CAA and  $\alpha$ SKA with two unknown proteins. Circles represent the theoretical isotopic abundance distribution of the isotopomer peaks corresponding to the assigned mass.

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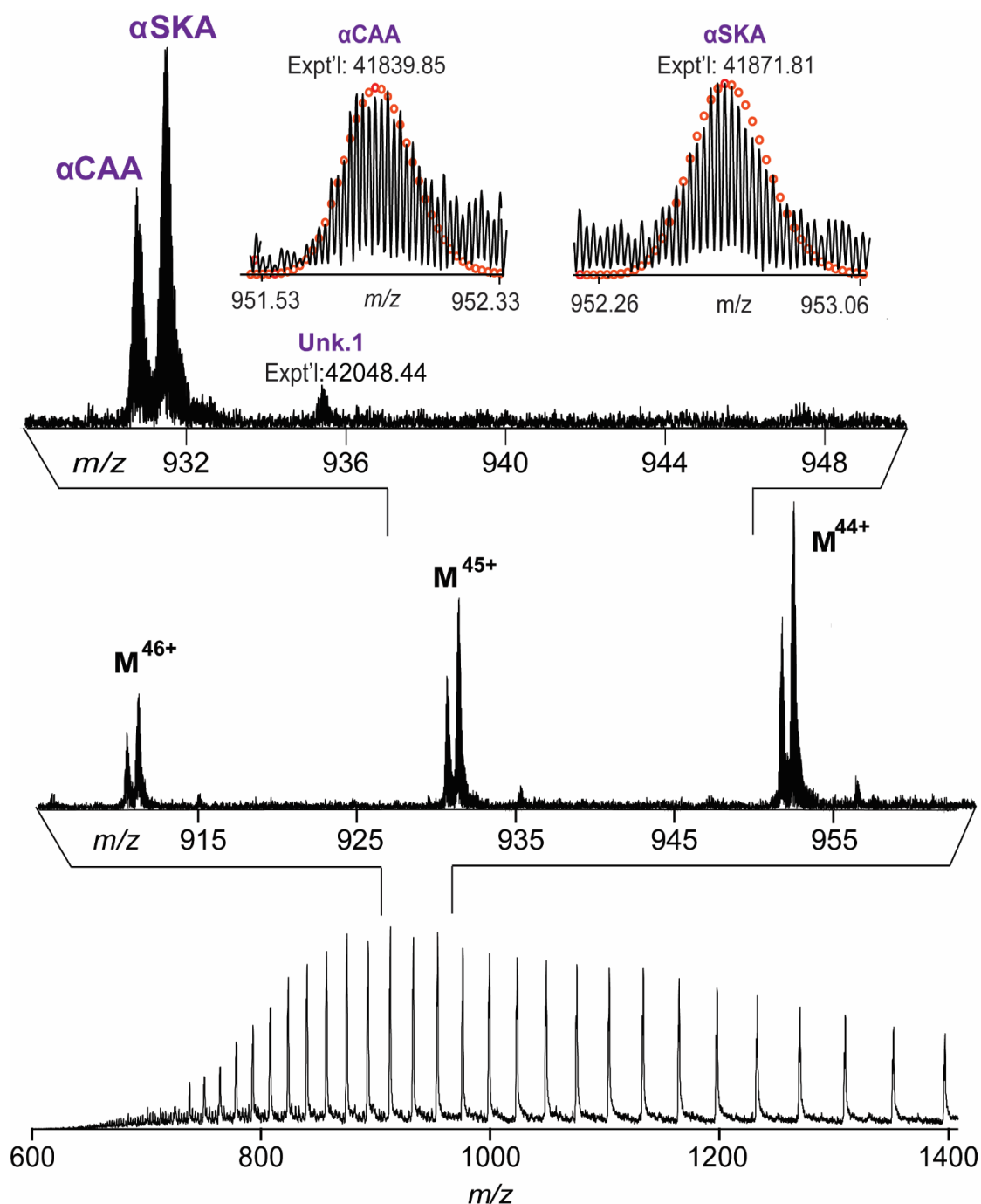
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P68133|ACTS MCDDEETALVCDNGSGLVKAGFAGDDAPRAVFPSIVGRPRHQGMVGMGQKDSYVGDEA 60
***:*****
P68032|ACTC QSKRGILTLKYPIEHGIITNWDDMEKIWHHTFYNELRVAPEEHPTLLTEAPLNPKANREK 120
P68133|ACTS QSKRGILTLKYPIEHGIITNWDDMEKIWHHTFYNELRVAPEEHPTLLTEAPLNPKANREK 120
*****
P68032|ACTC MTQIMFETFNPAMYVAIQAVLSLYASGRTTGIVLDSGDGVTHNVPIYEGYALPHAIMRL 180
P68133|ACTS MTQIMFETFNPAMYVAIQAVLSLYASGRTTGIVLDSGDGVTHNVPIYEGYALPHAIMRL 180
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P68032|ACTC DLAGRDLTDYLMKILTERGYSFVTTAEREIVRDIKEKLCYVALDFENEMATAASSSSLEK 240
P68133|ACTS DLAGRDLTDYLMKILTERGYSFVTTAEREIVRDIKEKLCYVALDFENEMATAASSSSLEK 240
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P68133|ACTS SYELPDGQVITIGNERFRCPETLFQPSFIGMESAGIHETTYSIMKCDIDIRKDLYANNV 300
*****
P68032|ACTC LSGGTTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGSILASLSTFQQMWIS 360
P68133|ACTS MSGGTTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGSILASLSTFQQMWIT 360
:*****:
P68032|ACTC KQEYDEAGPSIVHRKCF 377
P68133|ACTS KQEYDEAGPSIVHRKCF 377
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**Supplementary Figure 2. The sequence alignment of human  $\alpha$ -cardiac actin ( $\alpha$ CAA) and  $\alpha$ -skeletal actin ( $\alpha$ SKA).** The sequence alignment of human  $\alpha$ CAA (P68032), and  $\alpha$ SKA (P68133) from UnitProtKB/Swiss-Prot database. Human  $\alpha$ CAA and  $\alpha$ SKA vary by two juxtaposed amino acids (Asp2Glu3 for  $\alpha$ CAA, and Glu2Asp3 for  $\alpha$ SKA) and two amino acids substitution (Met299 and Thr358 in  $\alpha$ SKA, versus Leu299 and Ser358 in  $\alpha$ CAA), 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  $\alpha$ SKA. Representative MS/MS spectra of  $b$  and  $y$  ions from CAD spectra of  $\alpha$ SKA.



**Supplementary Figure 4. LC/MS+ analysis of swine  $\alpha$ -actin.** Bottom; MS detection of swine  $\alpha$ -actin proteins in on-line LC/MS using low-resolution MS (shown in multiply charged ions); middle, off-line FT analysis of intact  $\alpha$ -actin ( $M^{46+}$ ,  $M^{45+}$ ,  $M^{44+}$ ); and top, isotopically resolved molecular ions  $\alpha$ CAA and  $\alpha$ SKA with one unknown protein. Circles represent the theoretical isotopic abundance distribution of the isotopomer peaks corresponding to the assigned mass.