Supporting Information

Dissecting Human Skeletal Muscle Troponin Proteoforms by Top-down Mass Spectrometry

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SP|P45379|TNNT2 HUMAN|
                      MSDI --EEVVEEYEEEEOEEAAVEEEEDWREDEDEOEEAAEEDAEAEAETEETRAEEDEE 58
SP|P45378|TNNT3 HUMAN|
                      MSDEEVEQVEEQYEEEE-----HEP 33
                      MSDEEVEOVEEOYEEEE-----EAOE-----E 22
SP|P45378-2|TNNT3 HUMAN|
                      SPIP45378-3ITNNT3 HUMANI
                      MSDEEVEQVEEQYEEEE-----EAQEEE------
SPIP45378-4|TNNT3 HUMAN|
                      MSDEEVEQVEEQYEEEE------EAQE------
SPIP45378-5|TNNT3 HUMANI
SP|P45378-6|TNNT3_HUMAN|
                      MSDEEVEQVEEQYEEEE-----HEP 33
SP|P45378-7|TNNT3 HUMAN|
                      MSDEEVEQVEEQYEEEE------EAQEEE------
                      MSDTEEQ----EYEEEQ------EP 29
SPIP13805|TNNT1 HUMAN|
SP|P13805-2|TNNT1 HUMAN|
                      MSDTEEQ---EYEEEQ------PEEEAAEEEE-----
SP|P13805-3|TNNT1 HUMAN|
                      MSDTEEQ----EYEEEQ-----EPEEEAAEEEEEAP-----EEP 29
                      EEEAKEAEDGPMEESKPKPRSFMPNLVPPKIPDGERVDFDDIHRKRMEKDLNELQALIEA 118
SP|P45379|TNNT2 HUMAN|
SPIP45378 ITNNT3 HUMAN I
                       EEVOEDT----AEEDAEEEKPRPKLTAPKIPEGEKVDFDDIOKKRONKDLMELOALIDS 88
                      EEVOEDT----AEEDAEEEKPRPKLTAPKIPEGEKVDFDDIOKKRONKDLMELOALIDS 77
SPIP45378-2|TNNT3 HUMANI
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SP|P45378-3|TNNT3 HUMAN|
SP|P45378-4|TNNT3 HUMAN|
                       -----EVQEEEKPRPKLTAPKIPEGEKVDFDDIQKKRQNKDLMELQALIDS
SP | P45378-5 | TNNT3_HUMAN |
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                      E-----EKPRPKLTAPKIPEGEKVDFDDIQKKRQNKDLMELQALIDS 75
SPIP45378-6|TNNT3 HUMANI
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SP|P45378-7|TNNT3 HUMAN|
SP|P13805|TNNT1 HUMAN|
                       EPVAEPE----EERPKPSRPVVPPLIPPKIPEGERVDFDDIHRKRMEKDLLELQTLIDV 84
SP|P13805-2|TNNT1_HUMAN|
                      ----EE----EERPKPSRPVVPPLIPPKIPEGERVDFDDIHRKRMEKDLLELQTLIDV 73
SP|P13805-3|TNNT1_HUMAN| EPVAEPE-----EERPKPSRPVVPPLIPPKIPEGERVDFDDIHRKRMEKDLLELQTLIDV 84
                                                ****:**:******::** :*** ***:**:
                       HFENRKKEEEELVSLKDRIERRRAERAEQQRIRNEREKERQNRLAEERARREEEENRRKA
SPIP45379ITNNT2 HUMANI
SP|P45378|TNNT3 HUMAN|
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SPIP45378-2|TNNT3 HUMANI
                       HFEARKKEEEELVALKERIEKRRAERAEOORIRAEKERERONRLAEEKARREEEDAKRRA
SP|P45378-3|TNNT3_HUMAN|
                      HFEARKKEEEELVALKERIEKRRAERAEQQRIRAEKERERQNRLAEEKARREEEDAKRRA
SP|P45378-4|TNNT3 HUMAN|
                       HFEARKKEEEELVALKERIEKRRAERAEQQRIRAEKERERQNRLAEEKARREEEDAKRRA
SP|P45378-5|TNNT3 HUMAN|
                       HFEARKKEEEELVALKERIEKRRAERAEQQRIRAEKERERQNRLAEEKARREEEDAKRRA
SP|P45378-6|TNNT3 HUMAN|
                       HFEARKKEEEELVALKERIEKRRAERAEQQRIRAEKERERQNRLAEEKARREEEDAKRRA
SP|P45378-7|TNNT3_HUMAN|
                      HFEARKKEEEELVALKERIEKRRAERAEQQRIRAEKERERQNRLAEEKARREEEDAKRRA 129
SP|P13805|TNNT1_HUMAN|
                       HFEQRKKEEEELVALKERIERRRSERAEQQRFRTEKERERQAKLAEEKMRKEEEEAKKRA 144
SP|P13805-2|TNNT1 HUMAN|
                       HFEORKKEEEELVALKERIERRRSERAEOORFRIEKEREROAKLAEEKMRKEEEEAKKRA
SP|P13805-3|TNNT1 HUMAN|
                      HFEORKKEEEELVALKERIERRRSERAEOORFRTEKEREROAKLAEEKMRKEEEEAKKRA
                       SPIP45379|TNNT2 HUMAN|
                       EDEARKKKALSNMM-HFGGYIQKQAQTERKSGKRQTEREKKKKILAERRKVLAIDHLNED 237
SP|P45378|TNNT3 HUMAN|
                       EDDLKKKKALSSMGANYSSYLAKA---DQKRGKKQTAREMKKKILAERRKPLNIDHLGED 205
                       EDDLKKKKALSSMGANYSSYLAKA---DQKRGKKQTAREMKKKILAERRKPLNIDHLGED 194
SP|P45378-2|TNNT3 HUMAN|
                       EDDLKKKKALSSMGANYSSYLAKA---DQKRGKKQTAREMKKKILAERRKPLNIDHLGED
SPIP45378-3|TNNT3 HUMAN|
                       EDDLKKKKALSSMGANYSSYLAKA---DQKRGKKQTAREMKKKILAERRKPLNIDHLGED 186
SP|P45378-4|TNNT3_HUMAN|
SP | P45378-5 | TNNT3_HUMAN |
                       EDDLKKKKALSSMGANYSSYLAKA---DQKRGKKQTAREMKKKILAERRKPLNIDHLGED 181
SP | P45378-6 | TNNT3_HUMAN |
                       EDDLKKKKALSSMGANYSSYLAKA---DQKRGKKQTAREMKKKILAERRKPLNIDHLGED 192
SP|P45378-7|TNNT3 HUMAN|
                       EDDLKKKKALSSMGANYSSYLAKA---DOKRGKKOTAREMKKKILAERRKPLNIDHLGED 186
                       EDDAKKKKVLSNMGAHFGGYLVKA---EQKRGKRQTGREMKVRILSERKKPLDIDYMGEE 201
SPIP13805|TNNT1 HUMAN|
                      EDDAKKKKVLSNMGAHFGGYLVKA---EOKRGKROTGREMKVRILSERKKPLDIDYMGEE 190
SPIP13805-2|TNNT1 HUMAN|
                      EDDAKKKKVLSNMGAHFGGYLVKA---EQKRGKRQTGREMKVRILSERKKPLDIDYMGEE 201
SPIP13805-3|TNNT1 HUMAN|
                                               ::* **:** ** * :**:**: * **::
                       SP|P45379|TNNT2 HUMAN|
                       KLR-----DKAKELWETLHQLEIDKFEFGEKLKRQKYDITTLRSRIDQA 249
SPIP45378|TNNT3 HUMAN|
                           -----d<mark>kakel</mark>wetlhq<mark>leidkf</mark>efgeklkrqkydittlrs<mark>r</mark>idqa
SP|P45378-2|TNNT3 HUMAN|
SP|P45378-3|TNNT3 HUMAN|
                      KLR-----DKAKELWETLHQLEIDKFEFGEKLKRQKYDITTLRSRIDQA
SP|P45378-4|TNNT3_HUMAN|
                      KLR-----DKAKELWETLHQLEIDKFEFGEKLKRQKYDIMNVRARVQML 230
SP|P45378-5|TNNT3 HUMAN|
                      KLR-----DKAKELWETLHQLEIDKFEFGEKLKRQKYDITTLRSRIDQA 225
SPIP45378-6ITNNT3 HUMANI
                      KLR-----DKAKELWETLHQLEIDKFEFGEKLKRQKYDITTLRSRIDQA 236
                      KLR-----DKAKELWETLHQLEIDKFEFGEKLKRQKYDITTLRSRIDQA 230
SPIP45378-7|TNNT3 HUMAN|
SPIP13805|TNNT1 HUMAN|
                       QLRARSAWLPPSQPSCPAREKAQELSDWIHQLESEKFDLMAKLKQQKYEINVLYNRISHA 261
                      OLR-----EKAOELSDWIHOLESEKFDLMAKLKOOKYEINVLYNRISHA 234
SP|P13805-2|TNNT1 HUMAN|
SP|P13805-3|TNNT1 HUMAN|
                              -----EKAQELSDWIHQLESEKFDLMAKLKQQKYEINVLYNRISHA 245
                      QLR-
                                        :**:** : :::** :**::
                       QKVSKTRG---KAKVTGRWK 298
SP|P45379|TNNT2 HUMAN|
SP|P45378|TNNT3 HUMAN|
                       QKHSKKAGTPAKGKVGGRWK 269
SP|P45378-2|TNNT3_HUMAN|
                      QKHSKKAGTPAKGKVGGRWK 258
SP|P45378-3|TNNT3 HUMAN|
                      QKHSKKAGTPAKGKVGGRWK 261
SP|P45378-4|TNNT3 HUMAN|
                      AKESKKAGTPAKGKVGGRWK 250
SPIP45378-5|TNNT3 HUMAN|
                      OKHSKKAGTPAKGKVGGRWK 245
SP|P45378-6|TNNT3 HUMAN|
                      OKHSKKAGTPAKGKVGGRWK 256
SP|P45378-7|TNNT3 HUMAN|
                      QKHSKKAGTPAKGKVGGRWK 250
                       OKFRKGAG---KGRVGGRWK 278
SP|P13805|TNNT1 HUMAN|
SP|P13805-2|TNNT1 HUMAN|
                      OKFRKGAG---KGRVGGRWK 251
SP|P13805-3|TNNT1_HUMAN| QKFRKGAG---KGRVGGRWK 262
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Supplementary Figure 1. Sequence alignment of human cTnT and all isoforms of human sTnT. All sequences listed above were retrieved from UniProtKB/Swiss-Prot database. The sequences are human cTnT, seven human fsTnT isoforms, and three ssTnT isoforms, respectively. Human cTnT, accession number P45379, also known as TNT, has been chosen as the "canonical"

sequence for cTnT. Isoform I of human fsTnT, P45378, also known as Tnt1, has been chosen as the "canonical" form for fsTnT; isoform II, P45378-2, also known as Tnt3; isoform III, P45378-3, also known as Tnt1f; isoform IV, P45378-4, also known as Tnt3f; isoform V, P45378-5, also known as Tnt3f*; isoform VI, P45378-6; isoform VII, P45378-7. Isoform I of human ssTnT, P13805, has been chosen as the "canonical" form for ssTnT; isoform II, P13805-2; isoform III, P13805-3. Shaded areas represent the conserved region between these human TnT isoforms.

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SP|P19429|TNN13 HUMAN| MADGSSDAAREPRPAPAPIRRRSSNYRAYATEPHAKKKSKISASRKLQLKTLLLQIAKQE 60
. ::: : ::*: **:::* *
SP|P19429|TNNI3 HUMAN| LEREAEERRGEKGRALSTRCQPLELAGLGFAELQDLCRQLHARVDKVDEERYDIEAKVTK 120
SP|P48788|TNN12 HUMAN| LEKEESRREAEKONYLAEHCPPLHIPG-SMSEVOELCKOLHAKIDAAEEEKYDMEVRVOK 88
SP|P19237|TNNI1 HUMAN| WEQEHEEREAEKVRYLAERIPTLQTRGLSLSALQDLCRELHAKVEVVDEERYDIEAKCLH 89
                   *:* ..*..** . *: : *. * .:: :*:**::**::: .:**:**:: :
SP|P19429|TNN13 HUMAN| NITEIADLTQKIFDLRGKFKRPTLRRVRISADAMMQALLGARAKESLDLRAHLKQVKKED 180
SP|P48788|TNN12 HUMAN| TSKELEDMNQKLFDLRGKFKRPPLRRVRMSADAMLKALLGSKHKVCMDLRANLKQVKKED 148
SP|P19237|TNNI1 HUMAN| NTREIKDLKLKVMDLRGKFKRPPLRRVRVSADAMLRALLGSKHKVSMDLRANLKSVKKED 149
                   . *: *:. *::******* *****::***:: * .:***.**
SP|P19429|TNN13 HUMAN| TEKEN--REVGDWRKNIDALSGMEGRKKKFES----- 210
SP|P48788|TNN12 HUMAN| TEKERDLRDVGDWRKNIEEKSGMEGRKKMFESES---- 182
SP|P19237|TNN11 HUMAN| TEKERP-VEVGDWRKNVEAMSGMEGRKKMFDAAKSPTSQ 187
                   **** : ******: ****** *::
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Supplementary Figure 2. Sequence alignment of all human TnI isoforms including cTnI, fsTnI and ssTnI. All sequences listed above were retrieved from UniProtKB/Swiss-Prot database. The sequences are human cTnI, fsTnI, and ssTnI, respectively. Human cTnI, accession number P19429; human fsTnI, P48788; human ssTnI, P19237. Shaded areas represent the conserved region between these human TnI isoforms.

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Α
 <sup>1</sup> M G D E E K R N R A I T A R R Q H L K S <sup>163</sup>
<sup>21</sup> V M L Q I A A T E L E K E E S R R E A
41 K Q N Y L A E H C P P L H I P G S M S E 123
61 V Q E L C K Q L H A K I D A A E E E K Y 103
81 D M E V R V Q K T S K E L E D M N Q K L 83
101 F D L R G K F K R P P L R R V R M S A D 63
121 A M L K A L L G S K H K V C M D L R A N 43
141 L K Q V K K E D T E K E R D L R D V G D 23
161 W R K N I E E K S G M E G R K K M F E S 3
181 E S
                    Total ECD ions: 3 c ions and 32 z ions
B Ac
 1 G D E E K R N R A I T A R R Q H L K S V 162
21 M L Q I A A T E L E K E E S R R E A E K 142
41 QNYLAEHCPPLHIPGSMSEV 122
61 Q E L C K Q L H A K I D A A E E E K Y D 102
81 MEVRVQKTSKELEDMNQKLF82
101 D L R G K F K R P P L R R V R M S A D A 62
121 M L K A L L G S K H K V C M D L R A N L 42
141 K Q V K K E D T E K E R D L R D V G D W 22
161 RKN I E E K S G M E G R K K M F E S E 2
<sup>181</sup> S
                    Total ECD ions: 51 c ions and 33 z ions
                           -co-NH
-chR
-chR
-chR
-chR
```

Supplementary Figure 3. The product map of fsTnI from one ECD spectrum. The assignments were made based on DNA-predicted sequence of human fsTnI (UnitprotKB/Swiss-Prot **P48788** (TNNI2_HUMAN)) before (A) and after (B) consideration the removal of methionine and acetylation (+42 Da) of the first amino acid at the N-terminus.

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<sup>1</sup> M P E V E R K P K I T A S R K L L L K S <sup>168</sup>
<sup>21</sup> LMLAKAKECWEQEHEEREAE <sup>148</sup>
41 K V R Y L A E R I P T L Q T R G L S L S 128
61 A L Q D L C R E L H A K V E V V D E E R 108
81 Y D I E A K C L H N T R E I K D L K L K
101 V M D L R G K F K R P P L R R V R V S A 68
121 D A M L R A L L G S K H K V S M D L R A 48
141 N L K S V K K E D T E K E R P V E V G D 28
161 W R K N V E A M S G M E G R K K M F D A 8
181 AKSPTSQ
                  Total ECD ions: 3 c ions and 41 z ions
B
 1 P E V E R K P K I T A S R K L L L K S L 167
21 M L A K A K E C W E Q E H E E R E A E K 147
41 V RIYLAER I P TL QTR GL S LISIA 127
61 L Q D L C R E L H A K V E V V D E E R Y 107
81 DIEAKCLHNTREIKDLKLKV87
101 M D L R G K F K R P P L R R V R V S A D 67
121 A M L R A L L G S K H K V S M D L R A N 47
141 L K S V K K E D T E K E R P V E V G D W 27
161 RKN VELAMIS GMELGRKKMF DAA7
<sup>181</sup> K S P T S Q
                 Total ECD ions: 42 c ions and 42 z ions
                      -CO-NH-CHR-ECD
```

Supplementary Figure 4. The product map of ssTnI from one ECD spectrum. The assignments were made based on DNA-predicted sequence of human ssTnI (UnitprotKB/Swiss-Prot **P19237** (TNNI1_HUMAN)) before (A) and after (B) consideration of removal of methionine at the N-terminus.