

Supporting Information

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

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SP|P45379|TNNT2_HUMAN|MSDI--EEVVEEYEEEEQEAAVEEEEDWREDEQEAAAEADAEEAEATEETRAEED 58
SP|P45378|TNNT3_HUMAN|MSDEEVEQVEEQYEEEE-----EAQEEAAEVHEEV-----HEP 33
SP|P45378-2|TNNT3_HUMAN|MSDEEVEQVEEQYEEEE-----EAQE-----E 22
SP|P45378-3|TNNT3_HUMAN|MSDEEVEQVEEQYEEEE-----EAQEEAAEVHEEV-----HEP 33
SP|P45378-4|TNNT3_HUMAN|MSDEEVEQVEEQYEEEE-----EAQEEE-----23
SP|P45378-5|TNNT3_HUMAN|MSDEEVEQVEEQYEEEE-----EAQE-----21
SP|P45378-6|TNNT3_HUMAN|MSDEEVEQVEEQYEEEE-----EAQEEAAEVHEEV-----HEP 33
SP|P45378-7|TNNT3_HUMAN|MSDEEVEQVEEQYEEEE-----EAQEEE-----23
SP|P13805|TNNT1_HUMAN|MSDTEEQ---EYEEEQ-----PEEEAAEEEEAP-----EEP 29
SP|P13805-2|TNNT1_HUMAN|MSDTEEQ---EYEEEQ-----PEEEAAEEEE-----23
SP|P13805-3|TNNT1_HUMAN|MSDTEEQ---EYEEEQ-----PEEEAAEEEEAP-----EEP 29
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SP|P45379|TNNT2_HUMAN|EEEAKEAEDGPMEESEKPKFRSFMNVLVPPKIPDGERVDFDDIHRKRMEKDLNELQALIEA 118
SP|P45378|TNNT3_HUMAN|EEVQEDT----AEEDAEEKPRPKLTAPKIPERGEKVDFFDIQKKRQNKDLMELQALIDS 88
SP|P45378-2|TNNT3_HUMAN|EEVQEDT----AEEDAEEKPRPKLTAPKIPERGEKVDFFDIQKKRQNKDLMELQALIDS 77
SP|P45378-3|TNNT3_HUMAN|EEVQ-----EEEKPRPKLTAPKIPERGEKVDFFDIQKKRQNKDLMELQALIDS 80
SP|P45378-4|TNNT3_HUMAN|-----EVQEEKPRPKLTAPKIPERGEKVDFFDIQKKRQNKDLMELQALIDS 69
SP|P45378-5|TNNT3_HUMAN|-----EEEKPRPKLTAPKIPERGEKVDFFDIQKKRQNKDLMELQALIDS 64
SP|P45378-6|TNNT3_HUMAN|E-----EKPRPKLTAPKIPERGEKVDFFDIQKKRQNKDLMELQALIDS 75
SP|P45378-7|TNNT3_HUMAN|-----EVQEEKPRPKLTAPKIPERGEKVDFFDIQKKRQNKDLMELQALIDS 69
SP|P13805|TNNT1_HUMAN|EPVAEPE----EERPSPSRPVVPLIPPKIPERGEVDFDDIHRKRMEKDLLELQTLIDV 84
SP|P13805-2|TNNT1_HUMAN|----EE----EERPSPSRPVVPLIPPKIPERGEVDFDDIHRKRMEKDLLELQTLIDV 73
SP|P13805-3|TNNT1_HUMAN|EPVAEPE----EERPSPSRPVVPLIPPKIPERGEVDFDDIHRKRMEKDLLELQTLIDV 84
* * ****:*:*****:*:*** **:*:
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SP|P45378|TNNT3_HUMAN|HFEARKEEEEELVALKERIEKRAERAQQIRAEKERERQNLAEERARREEENRRA 148
SP|P45378-2|TNNT3_HUMAN|HFEARKEEEEELVALKERIEKRAERAQQIRAEKERERQNLAEERARREEENRRA 137
SP|P45378-3|TNNT3_HUMAN|HFEARKEEEEELVALKERIEKRAERAQQIRAEKERERQNLAEERARREEENRRA 140
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SP|P45378-7|TNNT3_HUMAN|HFEARKEEEEELVALKERIEKRAERAQQIRAEKERERQNLAEERARREEENRRA 129
SP|P13805|TNNT1_HUMAN|HFEQRKKEEEEELVALKERIERRSERAEQQFRTEKERERQAKLAEKMRKEEEEAKKRA 144
SP|P13805-2|TNNT1_HUMAN|HFEQRKKEEEEELVALKERIERRSERAEQQFRTEKERERQAKLAEKMRKEEEEAKKRA 133
SP|P13805-3|TNNT1_HUMAN|HFEQRKKEEEEELVALKERIERRSERAEQQFRTEKERERQAKLAEKMRKEEEEAKKRA 144
*** *****:*:***:*:*****:*:*** **:*:*** :**** : * : :
SP|P45379|TNNT2_HUMAN|EDEARKKKALSMM-HFGGYIQKQAQTERKSGKRQTEREKKKKILAERRKVLADHLDNED 237
SP|P45378|TNNT3_HUMAN|EDDLKKKKALS SMGANYSYLAKA---DQKRGGKQTAREMKKKILAERRKPLNIDHLDG 205
SP|P45378-2|TNNT3_HUMAN|EDDLKKKKALS SMGANYSYLAKA---DQKRGGKQTAREMKKKILAERRKPLNIDHLDG 194
SP|P45378-3|TNNT3_HUMAN|EDDLKKKKALS SMGANYSYLAKA---DQKRGGKQTAREMKKKILAERRKPLNIDHLDG 197
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SP|P45378-7|TNNT3_HUMAN|EDDLKKKKALS SMGANYSYLAKA---DQKRGGKQTAREMKKKILAERRKPLNIDHLDG 186
SP|P13805|TNNT1_HUMAN|EDDAKKKVLSNMGAHFGGYLVKA---EQKRGRQGTGREMKVRIISERKKPLIDYMGEE 201
SP|P13805-2|TNNT1_HUMAN|EDDAKKKVLSNMGAHFGGYLVKA---EQKRGRQGTGREMKVRIISERKKPLIDYMGEE 190
SP|P13805-3|TNNT1_HUMAN|EDDAKKKVLSNMGAHFGGYLVKA---EQKRGRQGTGREMKVRIISERKKPLIDYMGEE 201
** : ***.*.* . : . : * : : * **:*:*** ** * : **:*:*** ** * : : * :
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SP|P45378|TNNT3_HUMAN|KLR-----DKAKELWETLHQLEIDKFEFGEKLRQKYDITTLRSRIDQA 249
SP|P45378-2|TNNT3_HUMAN|KLR-----DKAKELWETLHQLEIDKFEFGEKLRQKYDITTLRSRIDQA 238
SP|P45378-3|TNNT3_HUMAN|KLR-----DKAKELWETLHQLEIDKFEFGEKLRQKYDITTLRSRIDQA 241
SP|P45378-4|TNNT3_HUMAN|KLR-----DKAKELWETLHQLEIDKFEFGEKLRQKYDINVRVRVQML 230
SP|P45378-5|TNNT3_HUMAN|KLR-----DKAKELWETLHQLEIDKFEFGEKLRQKYDITTLRSRIDQA 225
SP|P45378-6|TNNT3_HUMAN|KLR-----DKAKELWETLHQLEIDKFEFGEKLRQKYDITTLRSRIDQA 236
SP|P45378-7|TNNT3_HUMAN|KLR-----DKAKELWETLHQLEIDKFEFGEKLRQKYDITTLRSRIDQA 230
SP|P13805|TNNT1_HUMAN|QLRARSAWLPPSPQSPAREKAQELSDWIHQLESEKFDLMAKLRQKYEINVLYNRISHA 261
SP|P13805-2|TNNT1_HUMAN|QLR-----EKAQELSDWIHQLESEKFDLMAKLRQKYEINVLYNRISHA 234
SP|P13805-3|TNNT1_HUMAN|QLR-----EKAQELSDWIHQLESEKFDLMAKLRQKYEINVLYNRISHA 245
** : ***.*.* : : : * : : * **:*:*** ** * : **:*:*** ** * : : * :
SP|P45379|TNNT2_HUMAN|QKVSKEGTRG---KAKVTGRWK 298
SP|P45378|TNNT3_HUMAN|QKHSKKAQTPAKGKVGGRWK 269
SP|P45378-2|TNNT3_HUMAN|QKHSKKAQTPAKGKVGGRWK 258
SP|P45378-3|TNNT3_HUMAN|QKHSKKAQTPAKGKVGGRWK 261
SP|P45378-4|TNNT3_HUMAN|AKFSKKAQTPAKGKVGGRWK 250
SP|P45378-5|TNNT3_HUMAN|QKHSKKAQTPAKGKVGGRWK 245
SP|P45378-6|TNNT3_HUMAN|QKHSKKAQTPAKGKVGGRWK 256
SP|P45378-7|TNNT3_HUMAN|QKHSKKAQTPAKGKVGGRWK 250
SP|P13805|TNNT1_HUMAN|QKFRKGAG---KGRVGGGRWK 278
SP|P13805-2|TNNT1_HUMAN|QKFRKGAG---KGRVGGGRWK 251
SP|P13805-3|TNNT1_HUMAN|QKFRKGAG---KGRVGGGRWK 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”

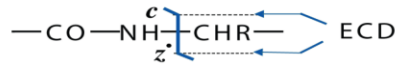
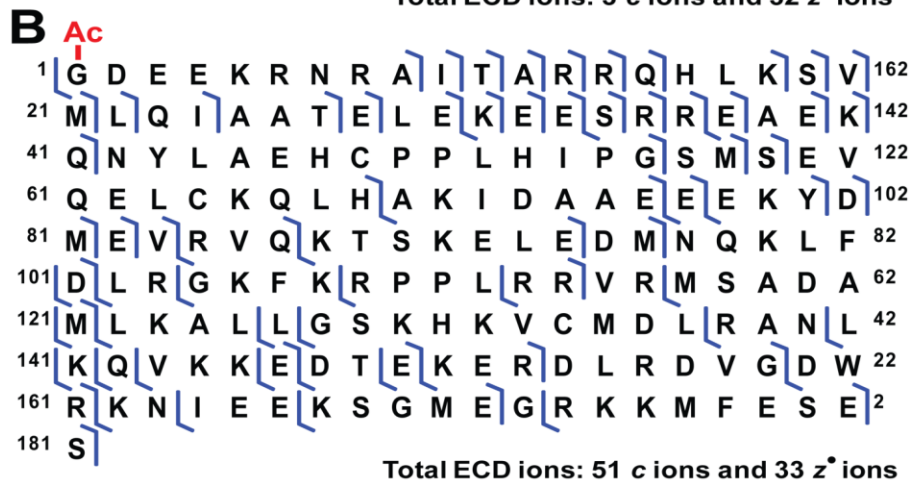
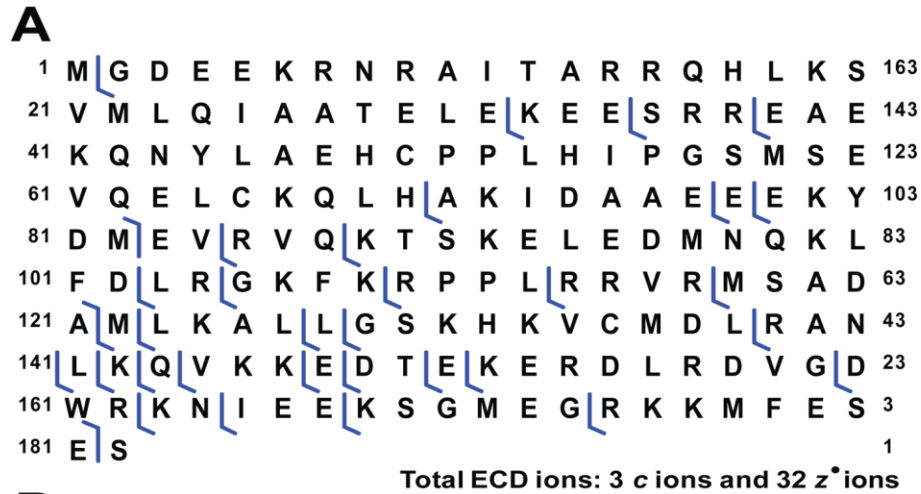
sequence for cTnT. Isoform I of human fsTnT, P45378, also known as Tnt1, has been chosen as the “canonical” form for fsTnT; isoform II, P45378-2, also known as Tnt3; isoform III, P45378-3, also known as Tnt1f; isoform IV, P45378-4, also known as Tnt3f; isoform V, P45378-5, also known as Tnt3f*; isoform 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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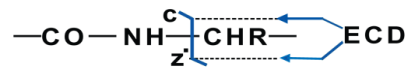
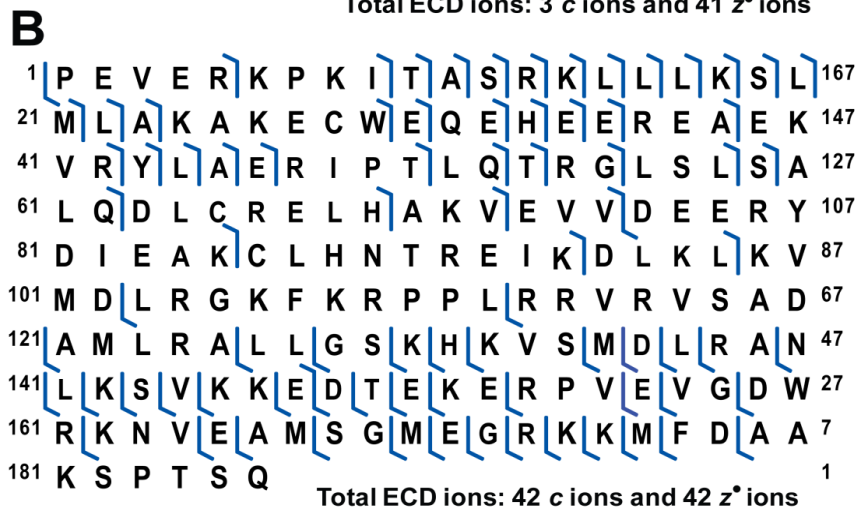
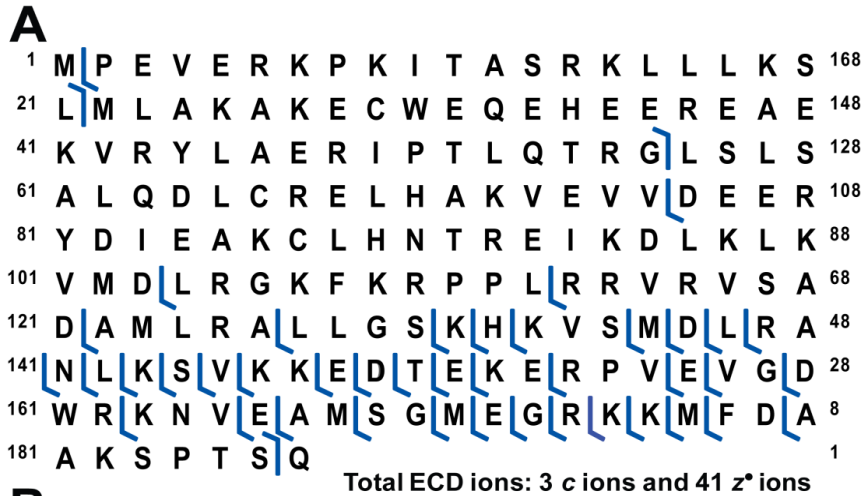
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SP|P48788|TNNI2_HUMAN| -----MGDEEKRNR AITARRQH LKSVMLQIAATE 29
SP|P19237|TNNI1_HUMAN| -----MPEVERKPKI TASRKL LKSLMLAKAKEC 29
                                     . : : : : : * : * : * : *
SP|P19429|TNNI3_HUMAN| LEREA EERRG EKGRALSTR CQPLEL AGLGFAELQDLCRQLHARVDKVD EERYDIEAKVTK 120
SP|P48788|TNNI2_HUMAN| LEKEESRREAEKQNYLA EHC PPLHIPG-SMSEVQELCKQLHAKIDAA EEEKYDMEV RVQK 88
SP|P19237|TNNI1_HUMAN| WEQEHEEREAEKVRYLAERI PTLQTRGLSLSALQDLCRELHAKVEVVD EERYDIEAKCLH 89
      * : * . * . * . * : : * . * . : : * : * : * : * : * : * : * : * : * : * :
SP|P19429|TNNI3_HUMAN| NITEIADLTQKIFDLRGKFKRPTLRRVRI SADAMM QALLGARAKESLDLRAHLKQVKKED 180
SP|P48788|TNNI2_HUMAN| TSKELEDMNQKLFDLRGKFKR PPLRRVRMSADAMLKALLGSKHKVCM DLRANL KQVKKED 148
SP|P19237|TNNI1_HUMAN| NTREIKDLKLVMDLRGKFKR PPLRRVRVSADAMLRALGSKHKVSM DLRANL KSVK KED 149
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SP|P19429|TNNI3_HUMAN| TEKEN--REVDWRKNIDALSGMEGRKKKFES----- 210
SP|P48788|TNNI2_HUMAN| TEKERDLRDVGDWRKNIEEKSGMEGRKKMFESES----- 182
SP|P19237|TNNI1_HUMAN| TEKERP-VEVDWRKNVEAMSGMEGRKKMFDAAKSPTSQ 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.



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.



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.