Supporting Information

Phosphorylation but not Alternative Splicing nor Proteolytic Degradation Is Conserved in Human and Mouse Cardiac Troponin T

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Running title: Top-Down Mass Spectrometry of Human and Mouse Cardiac Troponin T

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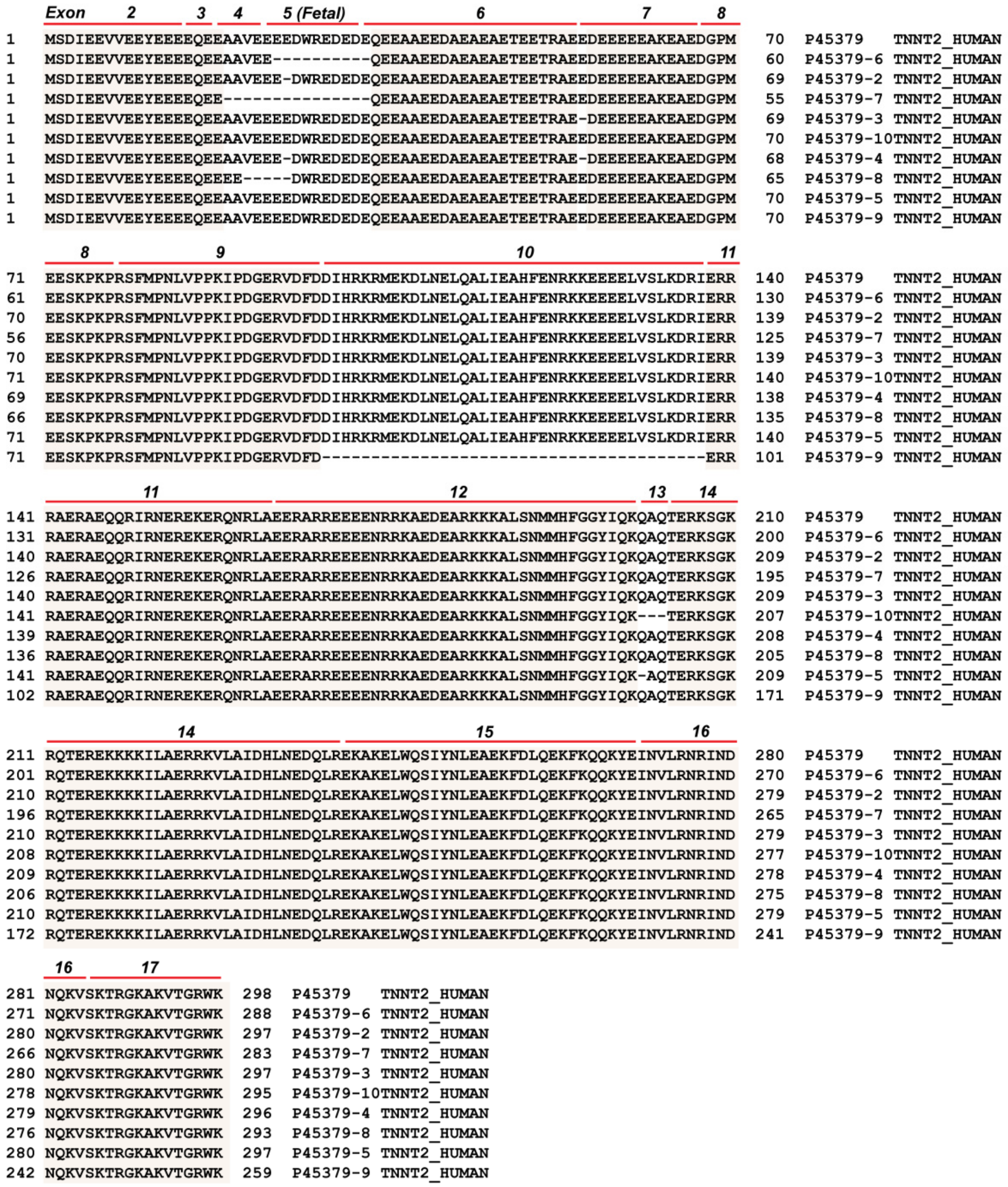
Supplemental Figures



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1    MSDIEEVVEEYEEEEQEEAAVEE----------QEEAAEEDAEAEAETEETRAEEDEEEEEAKEAEDGPM   60  P45379-6 TNNT2_HUMAN
1    MSDIEEVVEEYEEEEQEEAAVEEE-DWREDEDEQEEAAEEDAEAEAETEETRAEEDEEEEEAKEAEDGPM   69  P45379-2 TNNT2_HUMAN
1    MSDIEEVVEEYEEEEQEE---------------QEEAAEEDAEAEAETEETRAEEDEEEEEAKEAEDGPM   55  P45379-7 TNNT2_HUMAN
1    MSDIEEVVEEYEEEEQEEAAVEEEEDWREDEDEQEEAAEEDAEAEAETEETRAE-DEEEEEAKEAEDGPM   69  P45379-3 TNNT2_HUMAN
1    MSDIEEVVEEYEEEEQEEAAVEEEEDWREDEDEQEEAAEEDAEAEAETEETRAEEDEEEEEAKEAEDGPM   70  P45379-10TNNT2_HUMAN
1    MSDIEEVVEEYEEEEQEEAAVEEE-DWREDEDEQEEAAEEDAEAEAETEETRAE-DEEEEEAKEAEDGPM   68  P45379-4 TNNT2_HUMAN
1    MSDIEEVVEEYEEEEQEEEE-----DWREDEDEQEEAAEEDAEAEAETEETRAEEDEEEEEAKEAEDGPM   65  P45379-8 TNNT2_HUMAN
1    MSDIEEVVEEYEEEEQEEAAVEEEEDWREDEDEQEEAAEEDAEAEAETEETRAEEDEEEEEAKEAEDGPM   70  P45379-5 TNNT2_HUMAN
1    MSDIEEVVEEYEEEEQEEAAVEEEEDWREDEDEQEEAAEEDAEAEAETEETRAEEDEEEEEAKEAEDGPM   70  P45379-9 TNNT2_HUMAN

        8           9                         10                          11
71   EESKPKPRSFMPNLVPPKIPDGERVDFDDIHRKRMEKDLNELQALIEAHFENRKKEEEELVSLKDRIERR  140  P45379    TNNT2_HUMAN
61   EESKPKPRSFMPNLVPPKIPDGERVDFDDIHRKRMEKDLNELQALIEAHFENRKKEEEELVSLKDRIERR  130  P45379-6 TNNT2_HUMAN
70   EESKPKPRSFMPNLVPPKIPDGERVDFDDIHRKRMEKDLNELQALIEAHFENRKKEEEELVSLKDRIERR  139  P45379-2 TNNT2_HUMAN
56   EESKPKPRSFMPNLVPPKIPDGERVDFDDIHRKRMEKDLNELQALIEAHFENRKKEEEELVSLKDRIERR  125  P45379-7 TNNT2_HUMAN
70   EESKPKPRSFMPNLVPPKIPDGERVDFDDIHRKRMEKDLNELQALIEAHFENRKKEEEELVSLKDRIERR  139  P45379-3 TNNT2_HUMAN
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66   EESKPKPRSFMPNLVPPKIPDGERVDFDDIHRKRMEKDLNELQALIEAHFENRKKEEEELVSLKDRIERR  135  P45379-8 TNNT2_HUMAN
71   EESKPKPRSFMPNLVPPKIPDGERVDFDDIHRKRMEKDLNELQALIEAHFENRKKEEEELVSLKDRIERR  140  P45379-5 TNNT2_HUMAN
71   EESKPKPRSFMPNLVPPKIPDGERVDFD---------------------------------------ERR  101  P45379-9 TNNT2_HUMAN

              11                          12                        13   14
141  RAERAEQQRIRNEREKERQNRLAEERARREEEENRRKAEDEARKKKALSNMMHFGGYIQKQAQTERKSGK  210  P45379    TNNT2_HUMAN
131  RAERAEQQRIRNEREKERQNRLAEERARREEEENRRKAEDEARKKKALSNMMHFGGYIQKQAQTERKSGK  200  P45379-6 TNNT2_HUMAN
140  RAERAEQQRIRNEREKERQNRLAEERARREEEENRRKAEDEARKKKALSNMMHFGGYIQKQAQTERKSGK  209  P45379-2 TNNT2_HUMAN
126  RAERAEQQRIRNEREKERQNRLAEERARREEEENRRKAEDEARKKKALSNMMHFGGYIQKQAQTERKSGK  195  P45379-7 TNNT2_HUMAN
140  RAERAEQQRIRNEREKERQNRLAEERARREEEENRRKAEDEARKKKALSNMMHFGGYIQKQAQTERKSGK  209  P45379-3 TNNT2_HUMAN
141  RAERAEQQRIRNEREKERQNRLAEERARREEEENRRKAEDEARKKKALSNMMHFGGYIQK---TERKSGK  207  P45379-10TNNT2_HUMAN
139  RAERAEQQRIRNEREKERQNRLAEERARREEEENRRKAEDEARKKKALSNMMHFGGYIQKQAQTERKSGK  208  P45379-4 TNNT2_HUMAN
136  RAERAEQQRIRNEREKERQNRLAEERARREEEENRRKAEDEARKKKALSNMMHFGGYIQKQAQTERKSGK  205  P45379-8 TNNT2_HUMAN
141  RAERAEQQRIRNEREKERQNRLAEERARREEEENRRKAEDEARKKKALSNMMHFGGYIQK-AQTERKSGK  209  P45379-5 TNNT2_HUMAN
102  RAERAEQQRIRNEREKERQNRLAEERARREEEENRRKAEDEARKKKALSNMMHFGGYIQKQAQTERKSGK  171  P45379-9 TNNT2_HUMAN

                  14                           15                      16
211  RQTEREKKKKILAERRKVLAIDHLNEDQLREKAKELWQSIYNLEAEKFDLQEKFKQQKYEINVLRNRIND  280  P45379    TNNT2_HUMAN
201  RQTEREKKKKILAERRKVLAIDHLNEDQLREKAKELWQSIYNLEAEKFDLQEKFKQQKYEINVLRNRIND  270  P45379-6 TNNT2_HUMAN
210  RQTEREKKKKILAERRKVLAIDHLNEDQLREKAKELWQSIYNLEAEKFDLQEKFKQQKYEINVLRNRIND  279  P45379-2 TNNT2_HUMAN
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208  RQTEREKKKKILAERRKVLAIDHLNEDQLREKAKELWQSIYNLEAEKFDLQEKFKQQKYEINVLRNRIND  277  P45379-10TNNT2_HUMAN
209  RQTEREKKKKILAERRKVLAIDHLNEDQLREKAKELWQSIYNLEAEKFDLQEKFKQQKYEINVLRNRIND  278  P45379-4 TNNT2_HUMAN
206  RQTEREKKKKILAERRKVLAIDHLNEDQLREKAKELWQSIYNLEAEKFDLQEKFKQQKYEINVLRNRIND  275  P45379-8 TNNT2_HUMAN
210  RQTEREKKKKILAERRKVLAIDHLNEDQLREKAKELWQSIYNLEAEKFDLQEKFKQQKYEINVLRNRIND  279  P45379-5 TNNT2_HUMAN
172  RQTEREKKKKILAERRKVLAIDHLNEDQLREKAKELWQSIYNLEAEKFDLQEKFKQQKYEINVLRNRIND  241  P45379-9 TNNT2_HUMAN

      16     17
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271  NQKVSKTRGKAKVTGRWK  288  P45379-6 TNNT2_HUMAN
280  NQKVSKTRGKAKVTGRWK  297  P45379-2 TNNT2_HUMAN
266  NQKVSKTRGKAKVTGRWK  283  P45379-7 TNNT2_HUMAN
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278  NQKVSKTRGKAKVTGRWK  295  P45379-10TNNT2_HUMAN
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276  NQKVSKTRGKAKVTGRWK  293  P45379-8 TNNT2_HUMAN
280  NQKVSKTRGKAKVTGRWK  297  P45379-5 TNNT2_HUMAN
242  NQKVSKTRGKAKVTGRWK  259  P45379-9 TNNT2_HUMAN
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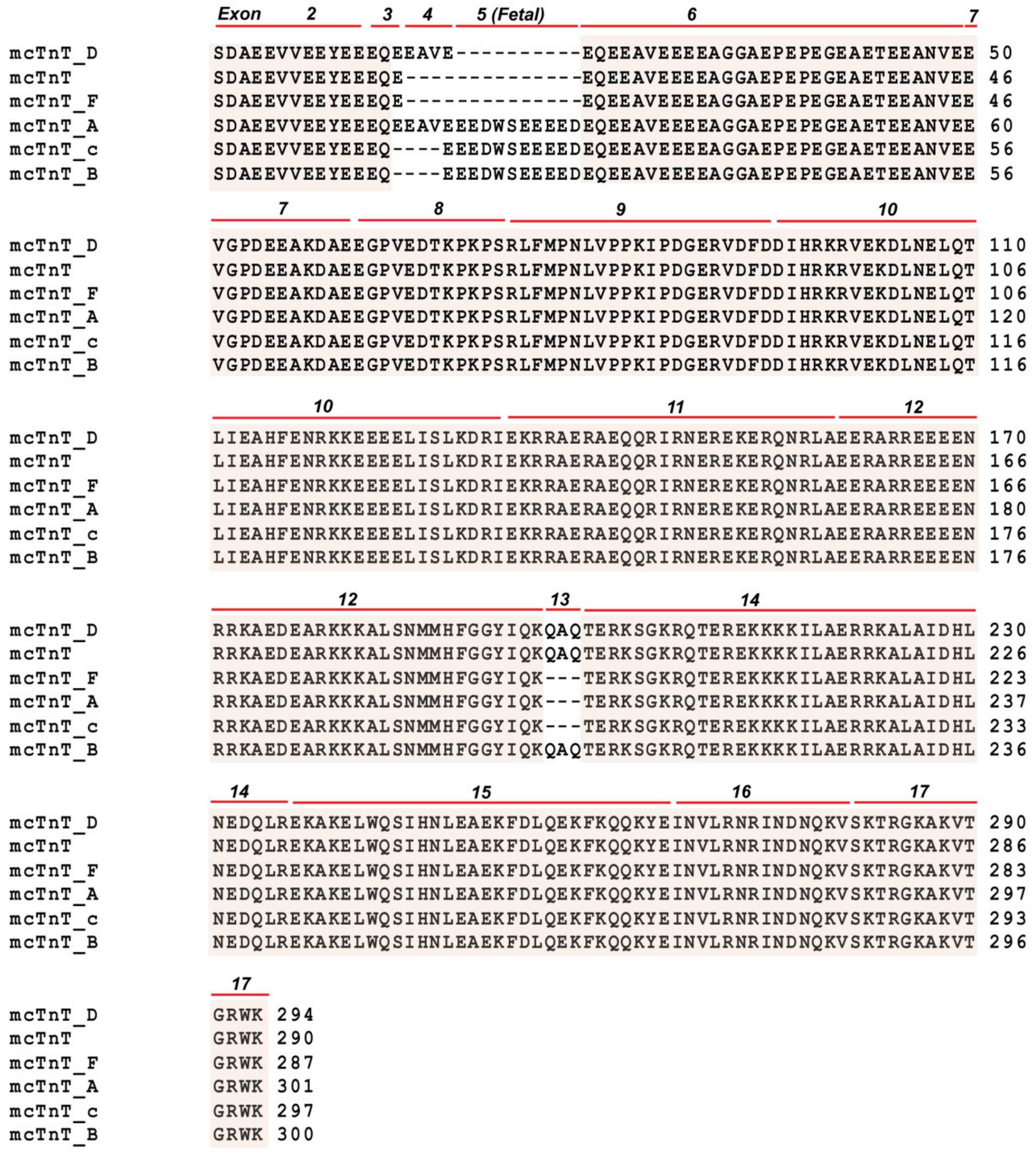
Figure S1. Amino acid sequence alignment of multiple hcTnT isoforms. Sequences were retrieved from UniProtKB/Swiss-Prot database. Isoform 1, accession number P45379 or P45379-1, also known as TNT, which has been chosen as the 'canonical' sequence; Isoform 2, P45379-2; Isoform 3, P45379-3; Isoform 4, P45379-4; Isoform 5, P45379-5; Isoform 6, P45379-6 also known as TNT3; Isoform 7, P45379-7, also known as TNT4; Isoform 8, P45379-8, also known as TNT2; Isoform 9, P45379-9; Isoform 10, P45379-10. Exon numbers and boundaries are indicated. Shaded areas indicate the conserved sequence regions.



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mcTnT      SDAEEVVEEYEEEQE--------------EQEEAVEEEEAGGAEPEPEGEAETEEANVEE 46
mcTnT_F    SDAEEVVEEYEEEQE--------------EQEEAVEEEEAGGAEPEPEGEAETEEANVEE 46
mcTnT_A    SDAEEVVEEYEEEQEEAVEEEDWSEEEEDEQEEAVEEEEAGGAEPEPEGEAETEEANVEE 60
mcTnT_c    SDAEEVVEEYEEEQ----EEEDWSEEEEDEQEEAVEEEEAGGAEPEPEGEAETEEANVEE 56
mcTnT_B    SDAEEVVEEYEEEQ----EEEDWSEEEEDEQEEAVEEEEAGGAEPEPEGEAETEEANVEE 56

mcTnT_D    VGPDEEAKDAEEGPVEDTKPKPSRLFMPNLVPPKIPDGERVDFDDIHRKRVEKDLNELQT 110
mcTnT      VGPDEEAKDAEEGPVEDTKPKPSRLFMPNLVPPKIPDGERVDFDDIHRKRVEKDLNELQT 106
mcTnT_F    VGPDEEAKDAEEGPVEDTKPKPSRLFMPNLVPPKIPDGERVDFDDIHRKRVEKDLNELQT 106
mcTnT_A    VGPDEEAKDAEEGPVEDTKPKPSRLFMPNLVPPKIPDGERVDFDDIHRKRVEKDLNELQT 120
mcTnT_c    VGPDEEAKDAEEGPVEDTKPKPSRLFMPNLVPPKIPDGERVDFDDIHRKRVEKDLNELQT 116
mcTnT_B    VGPDEEAKDAEEGPVEDTKPKPSRLFMPNLVPPKIPDGERVDFDDIHRKRVEKDLNELQT 116

mcTnT_D    LIEAHFENRKKEEEELISLKDRIEKRRAERAEQQRIRNEREKERQNRLAEERARREEEEN 170
mcTnT      LIEAHFENRKKEEEELISLKDRIEKRRAERAEQQRIRNEREKERQNRLAEERARREEEEN 166
mcTnT_F    LIEAHFENRKKEEEELISLKDRIEKRRAERAEQQRIRNEREKERQNRLAEERARREEEEN 166
mcTnT_A    LIEAHFENRKKEEEELISLKDRIEKRRAERAEQQRIRNEREKERQNRLAEERARREEEEN 180
mcTnT_c    LIEAHFENRKKEEEELISLKDRIEKRRAERAEQQRIRNEREKERQNRLAEERARREEEEN 176
mcTnT_B    LIEAHFENRKKEEEELISLKDRIEKRRAERAEQQRIRNEREKERQNRLAEERARREEEEN 176

mcTnT_D    RRKAEDEARKKKALSNMMHFGGYIQKQAQTERKSGKRQTEREKKKKILAERRKALAIDHL 230
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mcTnT_F    RRKAEDEARKKKALSNMMHFGGYIQK---TERKSGKRQTEREKKKKILAERRKALAIDHL 223
mcTnT_A    RRKAEDEARKKKALSNMMHFGGYIQK---TERKSGKRQTEREKKKKILAERRKALAIDHL 237
mcTnT_c    RRKAEDEARKKKALSNMMHFGGYIQK---TERKSGKRQTEREKKKKILAERRKALAIDHL 233
mcTnT_B    RRKAEDEARKKKALSNMMHFGGYIQKQAQTERKSGKRQTEREKKKKILAERRKALAIDHL 236

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mcTnT      NEDQLREKAKELWQSIHNLEAEKFDLQEKFKQQKYEINVLRNRINDNQKVSKTRGKAKVT 286
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mcTnT_A    NEDQLREKAKELWQSIHNLEAEKFDLQEKFKQQKYEINVLRNRINDNQKVSKTRGKAKVT 297
mcTnT_c    NEDQLREKAKELWQSIHNLEAEKFDLQEKFKQQKYEINVLRNRINDNQKVSKTRGKAKVT 293
mcTnT_B    NEDQLREKAKELWQSIHNLEAEKFDLQEKFKQQKYEINVLRNRINDNQKVSKTRGKAKVT 296

mcTnT_D    GRWK 294
mcTnT      GRWK 290
mcTnT_F    GRWK 287
mcTnT_A    GRWK 301
mcTnT_c    GRWK 297
mcTnT_B    GRWK 300
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Figure S2. Sequence alignment of multiple mouse cTnT isoforms. Sequences were retrieved from NCBI database. The mcTnT isoforms D, E, F, A, C, and B were denoted as mcTnT_D, mcTnT, mcTnT_F, mcTnT_A, mcTnT_C and mcTnT_B respectively. Isoforms D, E, and F also correspond to P50752-4 (isoform A1), P50752-3 (isoform A2), and P50752-2 (isoform A3B) of mouse cTnT in UniProtKB/Swiss-Prot database. Exon numbers and boundaries are indicated. Shaded areas indicate the conserved sequence regions.

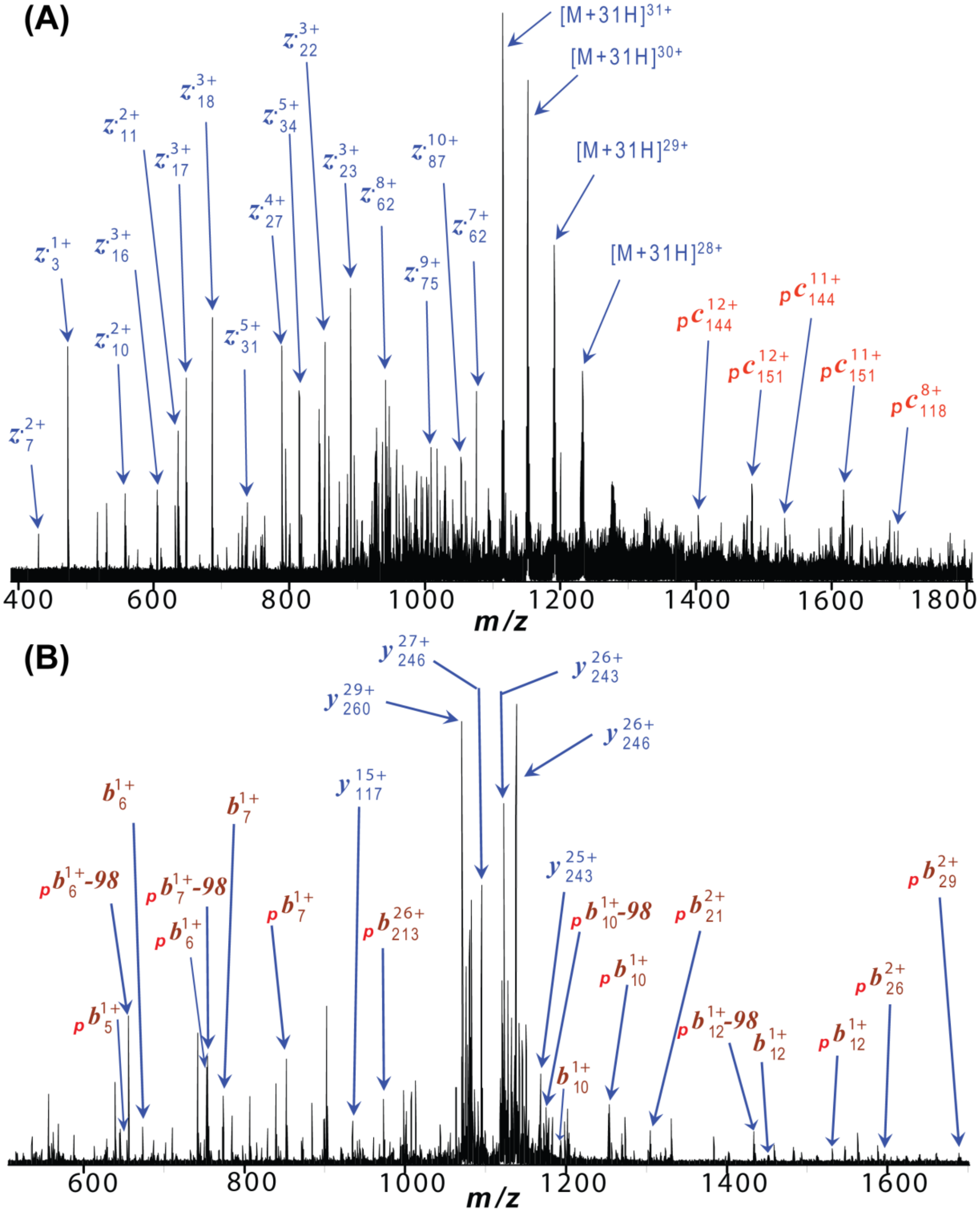

Figure S3. Representative MS/MS spectra of phosphorylated mcTnT for determination of phosphorylation site in mouse heart samples. (A) ECD spectrum of mouse ${}_p$cTnT; No neutral loss of phosphate moiety is detected. (B) CAD spectrum of mouse ${}_p$cTnT. Subscript *p* stands for product ions carrying phosphorylation. Neutral loss of phosphate moiety is evident. -98 indicates the loss of H_3PO_4 (-98 Da). Note that the masses of ***b*** ions (e.g. $\boldsymbol{b}_6$) equal to those with the loss of HPO_3 (-80 Da) from their phosphorylated counterparts (e.g. ${}_p\boldsymbol{b}_6$).

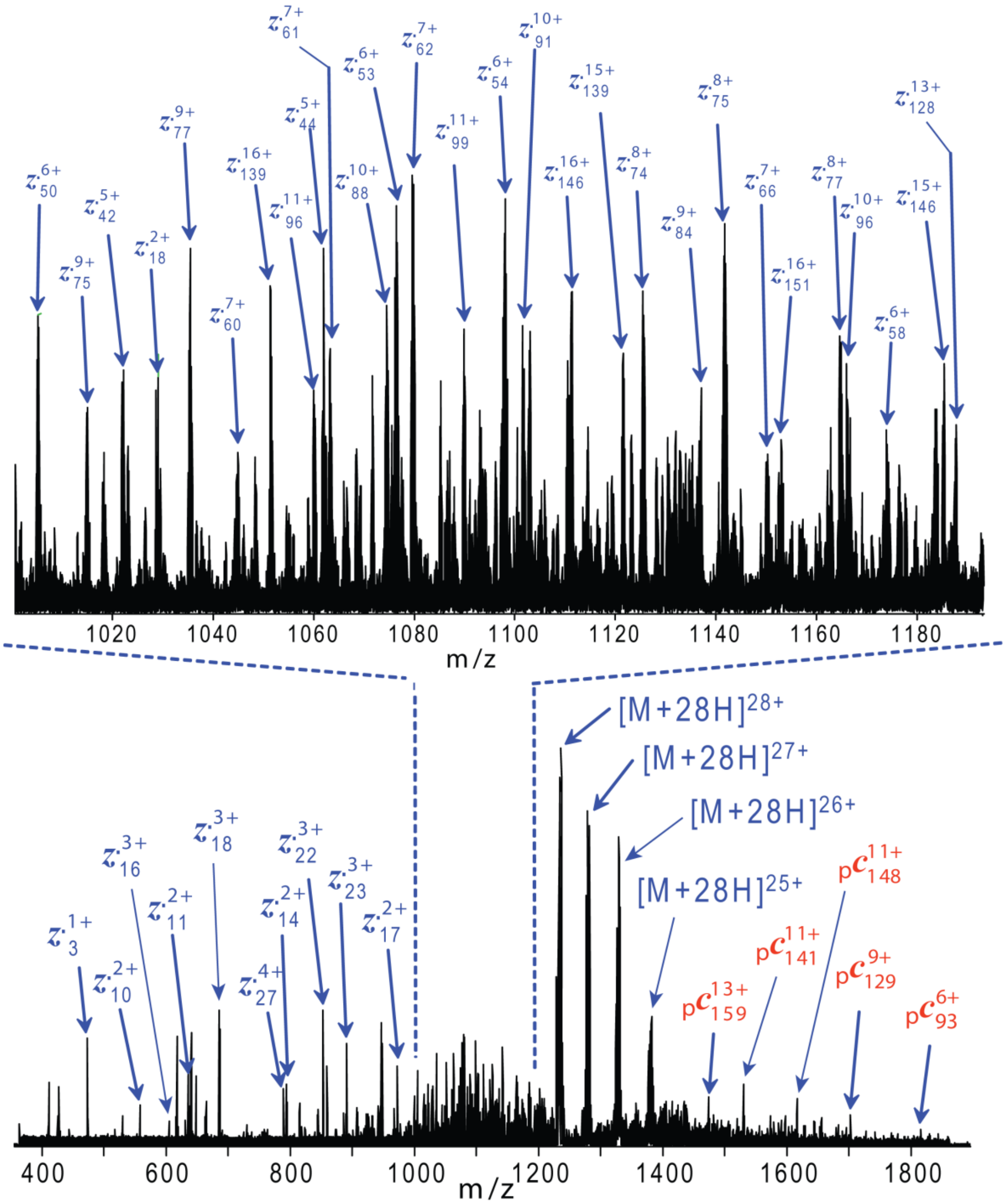

Figure S4. Representative ECD spectra of $_p$hcTnT for determination of phosphorylation site in cTnT purified from normal human heart samples. No neutral loss of phosphate moiety is detected.

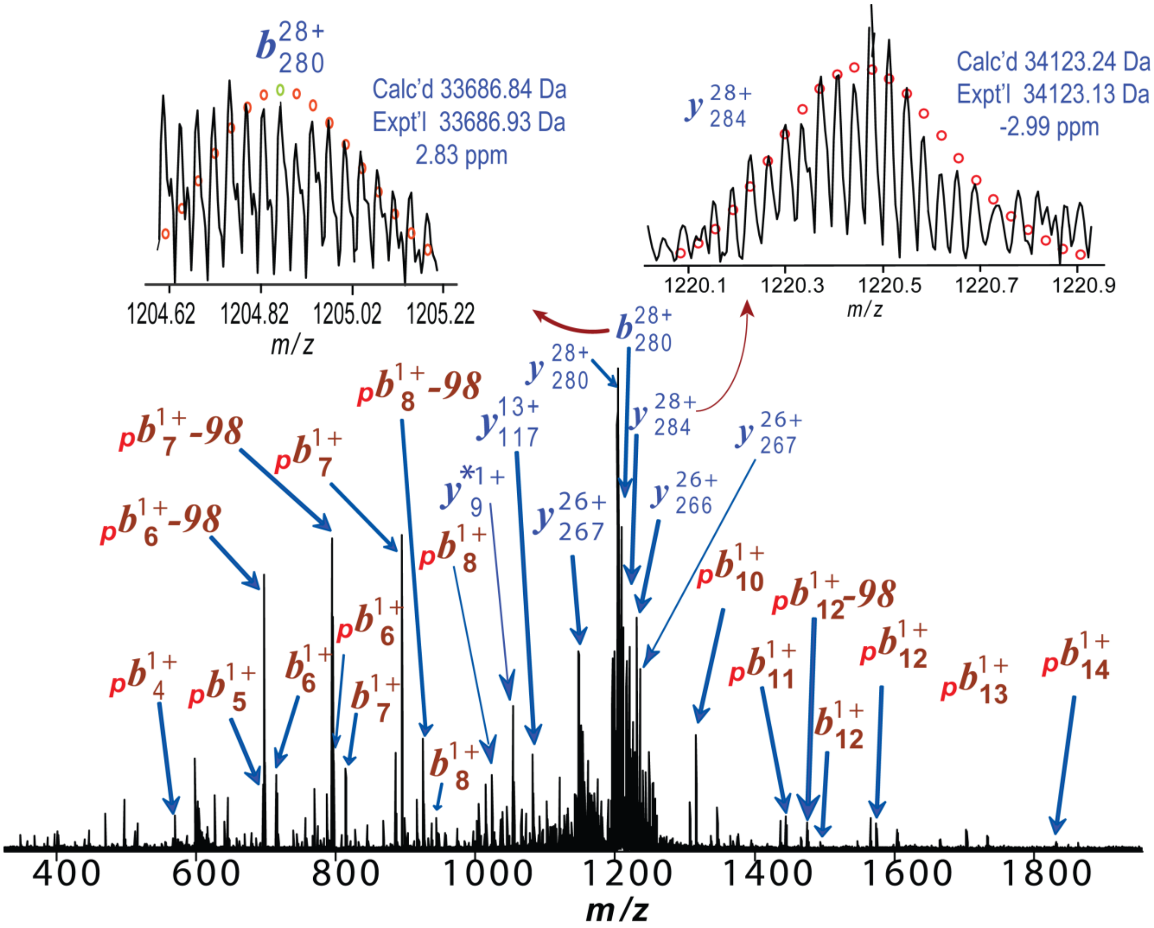

Figure S5. Representative CAD spectra of $_{p}$hcTnT for determination of phosphorylation site in cTnT purified from normal human heart samples. Neutral loss of phosphate moiety is evident. -98 indicates the loss of H$_3$PO$_4$ (-98 Da). Note that the masses of ***b*** ions (e.g. $\boldsymbol{b}_6$) equal to those with the loss of HPO$_3$ (-80 Da) from their phosphorylated counterparts (e.g. $_{p}\boldsymbol{b}_6$).