

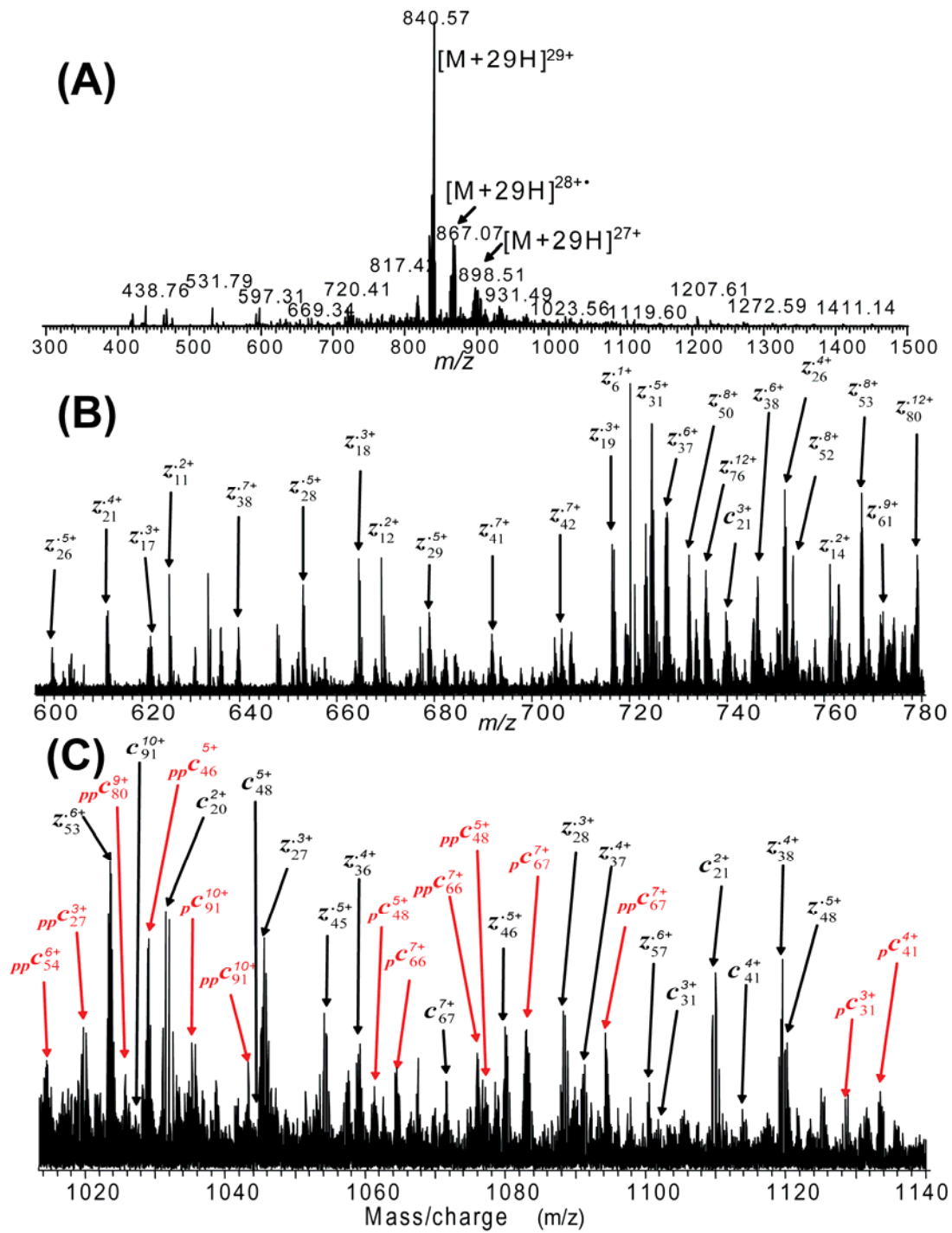
## Figure Legends

**Supplemental Fig. 1. A representative ECD spectrum of cTnI.** (A) ECD of a single charge state ( $M^{29+}$ ) of a mixture of un-, mono-, and doubly-phosphorylated cTnI at  $m/z$  840; (B) expanded region of (A) from  $m/z$  600-780 with fragment assignments of predominantly  $z'$  ions; (C) expanded region of (A) from  $m/z$  1014-1040 with fragment assignments of both  $c$  and  $z'$  ions. Phosphorylated  $c$  ions are labeled with a "p".

**Supplemental Fig. 2. MS/MS Product map from the ECD spectra for assignments to cTnI-*Ala2*.** Fragment assignments were made to the DNA-predicted sequence of transgenic mouse *cTnI-Ala2* where Ser22/23 are replaced with Ala22/23 (highlighted in circles), with the removal of N-terminal Met and acetylation at the new N-terminus.

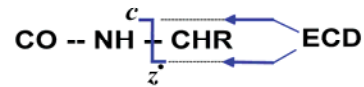
**Supplemental Fig. 3. High resolution FTMS analysis of cTn affinity purified *cTnI-Ala2* transgenic mouse hearts.** (A) Broadband full spectrum of cTn mixture revealing 12 major and 63 minor protein forms. Inset, SDS-Gel image of cTn mixture. (B) Expanded spectrum of cTnI showing cTnI is not phosphorylated confirming Ser22/23 are the only basally phosphorylated sites in wild-type mouse cTnI. Inset, isotopically resolved molecular ions of unphosphorylated cTnI ( $M^{27+}$ ) with highly accurate molecular weight measured. Dashed arrow indicates the expected position of monophosphorylated cTnI from cTnI-*Ala2* transgenic mice ( $_p$ cTnI-*Ala2*) which is not observed here. (C) Expanded spectrum of cTnT revealing cTnT is present as un- and mono-phosphorylated forms. Inset, isotopically resolved molecular ions of unphosphorylated cTnT ( $M^{38+}$ ) with highly accurate molecular weight measured. Calc'd, calculated most abundant molecular weight; Expt'l: experimental most abundant molecular weight. Phosphorylated protein ions (+80 Da,  $HPO_3$ ) are labeled with "p". Non-covalent adducts of phosphoric acids (+98 Da,  $H_3PO_4$ ) were also observed.

Supplemental Fig. 1.



Supplemental Fig. 2.

1 A D E S S D A A G E P Q P A P A P V R R R A A A N Y R A Y A  
 31 T E P L H A K K K S K I S A S R K L Q L K T L M L Q I A K Q E  
 61 M E R E A E E R R G E K G R V L R T R C Q P L L E L D G L G F  
 91 E E L Q D L C R Q L H A R V D K V D E E R Y D V E A K V T K  
 121 N I T E I A D L T Q K I Y D L R G K F L K R P T L R R V R I S  
 151 A L D A M M L Q A L L L G L T R A K E S L D L R A H L L K Q V K K E L D  
 181 I L E L K E N L R L E V L G L D W L R L K N I D A L L S G M L E L G L R L K K K F E G



Supplemental Fig. 3.

