SUPPLEMENTAL INFORMATION

Endogenous Cardiac Troponin T Modulates Ca²⁺-Mediated Smooth Muscle Contraction

By

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a cTnT sequence (238-497 bp)

	TTCATGCCCA ACTTGGTGCC	TCCCAAGAT	CCCGATGGAG	AGAGAGTGGI	CTTTGATGAC	human cTNT
	TTCATGCCCA ACTTGGTGCC	TCCCAAGAT	CCCGATGGAG	AGAGAGTGG	CTTTGATGAC	sample
	ATCCACCGGA AGCGCATGGA	GAAGGACCTO	G AATGAGTTGC	AGGCGCTGAT	CGAGGCTCAC	human cTNT
	ATCCACCGGA AGCGCATGGA	GAAGGACCTO	G AATGAGTTGC	AGGCGCTGAT	CGAGGCTCAC	sample
	TTTGAGAACA GGAAGAAAGA	GGAGGAGGAG	G CTCGTTTCTC	TCANAGACAC	GATCGAGAGA	human cTNT
	TTTGAGAACA GGAAGAAAGA	GGAGGAGGAG	G CTCGTTTCTC	TCAAAGACAG	GATCGAGAGA	sample
	COTCOSCIO ISCOSCCOI	CONCENSES	ATCCCCANTG	ACCCCC ACH	GENERGERIG	human cTNT
	CGTCGGGCAG AGCGGGCCGA	GCAGCAGCOC	ATCCGGARIG	AGCGGGAGAG	GGAGCGGCAG	comple
	NGGGGGCAG AGCGGGGCCGA	- GCRGCRGCOG	AICCOORATO	AGCOGOROAN	GONOCOOCNO	admpie
	ARCCOCCIOG CIGARGAGAG					
	ARCCGCCIGG CIGRAGAGAG	- Sal	lihie			
	primer-R					
h	cTnl sequence (117	-538 hn)			
N	erni sequence (rn	-990 pb	,			
	primer-F					
	TAAGATCTCC GCCTCGAGAA	AATTGCAGCT	GAAGACTCTG	CTGCTGCAGA	TTGCAAAGCA	human cTNI
	TARGATCTCC GCCTCGAGAA	AATTGCAGCT	GAAGACTCTG	CTGCTGCAGA	TTGCAAAGCA	sample
	AGAGCTGGAG CGAGAGGCGG	AGGAGCGGCG	CGGAGAGAAG	GGGCGCGCTC	TGAGCACCCG	human cTNI
	AGAGCTGGAG CGAGAGGCGG	AGGAGCGGCG	CGGAGAGAAG	GGGCGCGCTC	TGAGCACCCG	sample
	CTGCCAGCCG CTGGAGTTGG	CCGGGCTGGG	CTTCGCGGAG	CTGCAGGACT	TGTGCCGACA	human cTNI
	CTGCCAGCCG CTGGAGTTGG	CCGGGCTGGG	CTTCGCGGAG	CTGCAGGACT	TGTGCCGACA	sample
	GCTCCACGCC CGTGTGGACA	AGGTGGATGA	AGAGAGATAC	GACATAGAGG	CAAAAGTCAC	human cTnl
	GCTCCACGCC CGTGTGGACA	AGGTGGATGA	AGAGAGATAC	GACATAGAGG	CAAAAGTCAC	sample
	CAAGAACATC ACGGAGATTG	CAGATCTGAC	TCAGAAGATC	TTTGACCTTC	GAGGCAAGTT	human cTnl
	CAAGAACATC ACGGAGATTG	CAGATCTGAC	TCAGAAGATC	TTTGACCTTC	GAGGCAAGTT	sample
	TAAGCGGCCC ACCCTGCGGA	GAGTGAGGAT	CTCTGCAGAT	GCCATGATGC	AGGCGCTGCT	human cTnl
	TAAGCGGCCC ACCCTGCGGA	GAGTGAGGAT	CTCTGCAGAT	GCCATGATGC	AGGCGCTGCT	sample
	GGGGGCCCGG GCTAAGGAGT	CCCTGGACCT	GCGGGGCCCAC	CTCAAGCAGG	TGAAGAAGGA	human cTnl
	GGGGGCCCGG GCTAAGGAGT	CCCTGGACCT	GCGGGGCCCAC	CTCAAGCAGG	TGAAGAAGGA	sample
	GG human cTnl			•		
	GG sample primer-R					
C	cTnC sequence (12	-341bp)				
•						
	primer-r					
	CTACAAGGCT GCGGTAGAGC A	GCTGACAGA .	AGAGCAGAAA A	ATGAGTTCA .	AGGCAGCCTT	human cTnC
	CTACAAGGCT GCGGTAGAGC A	GCTGACAGA .	AGAGCAGAAA A	ATGAGTTCA .	AGGCAGCCTT	sample
	CGACATCTTC GTGCTGGGCG C	TGAGGATGG	CTGCATCAGC A	CCARGGAGC '	FGGGCAAGGT	human cTnC
	CGACATCTTC GTGCTGGGCG C	TGAGGATGG	CTGCATCAGC A	CCARGGAGC '	FGGGCAAGGT	sample
	GATGAGGATG CTGGGCCAGA A	CCCCACCCC .	TGAGGAGCTG C	AGGAGATGA '	FCGATGAGGT	human cTnC
	GATGAGGATG CTGGGCCAGA A	. ccccacccc	TGAGGAGCTG C	AGGAGATGA '	FCGATGAGGT	sample
	GGACGAGGAC GGCAGCGGCA C	GGTGGACTT	TGATGAGTTC C	TGGTCATGA	IGGTTCGGTG	human cTnC
	GGICGIGGIC GGCIGCGGCI C	GGTGGACTT	TGATGAGTTC C	TGGTCATGA	IGGTTCGGTG	samnle
	CITGINGEL GICIGCING G	GARATCIGA	GAGGAGCTG T	CTGACCTCT '	CCGCATGTT	human cTnC
	CITGINGEL GICIGCING G	GARATCTON	SGAGGAGCTG T	CTGACCTCT '	CCGCATGTT	sample
	TGICININT SCTGITCCCT A	CATCGACCT	human cTrC	010100101		a surface
	TGICININT SCTGITGSCT N	CATCGACCT	comple			
	ICACARARAT GLIGAIGGCI A	-	Sample			
	prime	r-R				

Figure S1 | The partial DNA sequence of human cTnT, cTnI and cTnC. PCR primers used are indicated by arrows. The sequences were exactly the same as known sequences of cDNAs of cTnT (238-497 bp), cTnI (117-538 bp) and cTnC (12-341bp) in human detrusor, respectively.



Figure S2 | **Real-time PCR quantification of cTnI (a) and cTnC (b) in tRNA of various human smooth muscle tissues.** All tRNA were purchased commercially. The mRNA expression of cTn in each smooth muscle tissue was compensated by that of GAPDH, and is plotted relative to that of trachea.



Figure S3 | Time dependency of cTnI and cTnC effects on pig detrusor strip. Pig detrusor strips were permebilised with 20 μ M β -escin for 30 min. a) Each trace indicates an experimental protocol performed to examine the effect of exposure time to cTnI and/or cTnC. b-d) Changes in force development are summarized (n=8-12). Each column represents the mean \pm S.E. measured at each condition indicated by arrows when the amplitude of the contraction obtained at 1 μ M Ca²⁺ was normalized as 1.0. The *P* value of each column was expressed when there was a significant difference (*P* < 0.05) in comparison with that induced by 1 μ M Ca²⁺.



Figure S4 | **RT-PCR detection of cTnT in murine smooth muscle.** tRNA were extracted from the several tissues of mice. In urinary bladder, colon, ileum and stomach, the mucous was removed carefully.



Figure S5 | **Negative controls.** Neither cTnT nor tropomyosin was detected in UMUCS3 and TCCSuP human urinary bladder carcinoma cells. Micrographs of cellular nuclei (blue: DAPI), and transmission are also shown.



Figure S6 | Lack of effects of cTnI and cTnC in intact detrusor strips from human. Either cTnI or cTnC were applied between CCh-induced contractions (**a**, **c**). The peak amplitude of contraction did not change after applications of cTnI and cTnC (P > 0.05, n=6) (**b**, **d**).