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Supplemental Information

Characterization of Zebrafish Cardiac and Slow Skeletal Troponin C Paralogs by MD Simulation and ITC

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Table S1. Dimensions, Ions, and water molecules for each simulation system

		100 ns Ca^{2+}-bound	PMF	200 ns TnC+Ca^{2+}+TnI_{SW}	1 µs Ca^{2+}-free	1 µs Ca^{2+}-bound
cTnC 18ºC	Box Dimensions (nm)	596 x 5.96 x 5.96	14.94 x 5.98 x 5.98	7.68 x 7.68 x 7.68	596 x 5.96 x 5.96	5.96 x 5.96 x 5.96
	K^+ Ions	14	14	11	15	14
	Cl^- Ions	1	1	1	0	1
	Ca^{2+} Ions	1	1	1	0	1
	Water Molecules	6612	17349	14555	6655	6612
cTnC 28ºC	Box Dimensions (nm)	5.97 x 5.97 x 5.97	14.99 x 5.99 x 5.99	7.72 x 7.72 x 7.72	5.98 x 5.98 x 5.98	5.97 x 5.97 x 5.97
	K^+ Ions	14	13	11	15	14
	Cl^- Ions	1	0	1	0	1
	Ca^{2+} Ions	1	1	1	0	1
	Water Molecules	6612	17352	14555	6655	6612
ssTnC 18ºC	Box Dimensions (nm)	5.95 x 5.95 x 5.95	14.95 x 5.98 x 5.98	7.68 x 7.68 x 7.68	5.97 x 5.97 x 5.97	5.96 x 5.96 x 5.96
	K^+ Ions	15	14	12	16	15
	Cl^- Ions	1	0	1	0	1
	Ca^{2+} Ions	1	1	1	0	1
	Water Molecules	6612	17343	14555	6657	6612
ssTnC 28ºC	Box Dimensions (nm)	5.97 x 5.97 x 5.97	15.00 x 6.00 x 6.00	7.72 x 7.72 x 7.72	5.98 x 5.98 x 5.98	5.97 x 5.97 x 5.97
	K^+ Ions	15	14	12	16	15
	Cl^- Ions	1	0	1	0	1
	Ca^{2+} Ions	1	1	1	0	1
	Water Molecules	6612	17340	14555	6657	6612

Table S2. Homology model quality indicators for representative structures from 100 ns TnC+Ca^{2+} simulations.

	cTnC (18ºC)	cTnC (28ºC)	ssTnC (18ºC)	ssTnC (28ºC)
RAMPAGE				
Favored	85 (98.84%)	83 (96.51%)	84 (97.67%)	86 (100.00%)
Allowed	1 (1.16%)	3 (3.49%)	2 (2.33%)	0 (0.00%)
Outlier	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
PROCHECK				
Bad Backbone Bonds	0.00%	0.00%	0.00%	0.00%
Bad Backbone Angles	4.60%	5.40%	4.10%	4.60%
Bad Contacts	0	0	0	0
Molprobity Score	1.62	1.69	1.54	1.70
QMEAN Score	0.627	0.669	0.767	0.69
Whatcheck Structure Z-Score				
1st Generation Packing	-1.836	-1.079	-1.237	-1.322
2n Generation Packing	-0.89	-1.245	-1.349	-1.079
χ_1/χ_2 Rotamer Normality	-1.45	-1.685	-2.472	-1.836
Backbone Conformation	0.672	0.93	1.177	0.662
Inside/Outside	0.955	0.966	0.926	0.999
Whatcheck RMS Z-Score				
Bond Lengths	0.644	0.635	0.642	0.662
Bond Angles	1.173	1.175	1.109	1.177
Omega Angle Restraints	1.471	1.235	1.422	1.369
Side Chain Planarity	1.886	1.732	1.488	1.789
Improper Dihedral Distribution	1.175	1.163	1.211	1.255

Table S3. Homology model quality indicators for representative structures of TnC+Ca^{2+} in complex with TnI_{SW}.

	cTnC (18ºC)	cTnC (28ºC)	ssTnC (18ºC)	ssTnC (28ºC)
RAMPAGE				
Favored	99 (99.00%)	96 (96.00%)	94 (94.00%)	98 (98.00%)
Allowed	1 (1.00%)	4 (4.00%)	6 (6.00%)	2 (2.00%)
Outlier	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
PROCHECK				
Bad Backbone Bonds	0.00%	0.20%	0.00 %	0.00%
Bad Backbone Angles	4.30%	4.30%	5.00%	6.40%
Bad Contacts	0.00%	0.00%	0.00%	0.00%
Molprobity Score	1.22	1.59	1.93	2.25
QMEAN Score	0.563	0.512	0.515	0.463
Whatcheck Structure Z-Score				
1st Generation Packing	-1.027	-0.607	-1.175	-1.329
2n Generation Packing	0.614	0.548	-1.175	-0.09
χ_1/χ_2 Rotamer Normality	-1.924	1.122	-0.118	-2.428
Backbone Conformation	0.856	0.959	-1.724	1.234
Inside/Outside	0.988	0.959	0.928	1.077
Whatcheck RMS Z-Score				
Bond Lengths	0.505	0.600	0.485	0.547
Bond Angles	-1.027	1.157	1.216	1.223
Omega Angle Restraints	1.297	1.419	1.608	1.638
Side Chain Planarity	2.012	1.767	1.972	2.083
Improper Dihedral Distribution	1.305	1.19	1.447	1.281

Table S4. Homology model quality indicators for representative structures of Ca^{2+}-free TnC from 1 μs simulations.

	cTnC (18ºC)	cTnC (28ºC)	ssTnC (18ºC)	ssTnC (28ºC)
RAMPAGE				
Favored	85 (98.80%)	83 (96.50%)	84 (97.70%)	84 (97.70%)
Allowed	1 (1.20%)	3 (3.50%)	2 (23.00%)	2 (23.00%)
Outlier	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
PROCHECK				
Bad Backbone Bonds	0.00%	0.00%	0.00%	0.00%
Bad Backbone Angles	5.60%	4.60%	4.30%	4.60%
Bad Contacts	0.00%	0.00%	0.00%	0.00%
Molprobity Score	0.83	0.82	0.57	1.37
QMEAN Score	0.842	0.605	0.794	0.72
Whatcheck Structure Z-Score				
1st Generation Packing	-0.916	-0.814	-0.855	-1.237
2n Generation Packing	-0.658	-0.518	-0.365	-1.074
χ_1/χ_2 Rotamer Normality	-2.497	-2.265	-1.093	-0.818
Backbone Conformation	1.771	1.23	1.23	0.934
Inside/Outside	0.961	0.984	0.982	0.955
Whatcheck RMS Z-Score				
Bond Lengths	0.513	0.553	0.551	0.501
Bond Angles	1.156	1.241	1.17	1.232
Omega Angle Restraints	1.428	1.377	1.512	1.38
Side Chain Planarity	1.524	1.69	1.774	1.496
Improper Dihedral Distribution	1.177	1.206	1.16	1.224

Table S5. Homology model quality indicators for representative structures of TnC+Ca^{2+} from 1 μs simulations.

	cTnC (18ºC)	cTnC (28ºC)	ssTnC (18ºC)	ssTnC (28ºC)
RAMPAGE				
Favored	84 (97.70%)	85 (98.80%)	81 (94.20%)	85 (98.80%)
Allowed	2 (23.00%)	1 (1.20%)	5 (5.80%)	1 (1.20%)
Outlier	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
PROCHECK				
Bad Backbone Bonds	0.00%	0.00%	0.00%	0.00%
Bad Backbone Angles	7.10%	7.60%	6.10%	4.40%
Bad Contacts	0.00%	0.00%	0.00%	0.00%
Molprobity Score	1.2	0.57	1.08	0.81
QMEAN Score	0.664	0.584	0.718	0.684
Whatcheck Structure Z-Score				
1st Generation Packing	-0.875	-1.506	-2.138	-0.616
2n Generation Packing	-0.398	-1.155	-2.061	-0.606
χ_1/χ_2 Rotamer Normality	-3.038	-2.781	-3.188	-2.315
Backbone Conformation	1.06	1.013	0.356	0.948
Inside/Outside	1.019	0.985	0.993	0.95
Whatcheck RMS Z-Score				
Bond Lengths	0.488	0.525	0.542	0.495
Bond Angles	1.265	1.273	1.209	1.122
Omega Angle Restraints	1.73	1.554	1.57	1.786
Side Chain Planarity	2.332	1.775	1.686	1.797
Improper Dihedral Distribution	1.441	1.31	1.224	1.258

Table S6. Cumulative interhelical angle frequencies combined over replicated long timescale simulations.

Paralog	cTnC	cTnC	cTnC	cTnC	ssTnC	ssTnC	ssTnC	ssTnC
Temperature	18ºC	28ºC	18ºC	28ºC	18ºC	28ºC	18ºC	28ºC
Ca^{2+}			+	+			+	+
<90	0	0	0	0	0	0	0	0
<95	0	0	0	10	0	0	0	0
<100	0	0	0	66	0	0	0	0
<105	0	7	0	283	0	0	0	1
<110	0	36	0	876	0	0	0	4
<115	2	121	0	1743	2	4	10	56
<120	25	447	1	2920	13	24	114	210
<125	205	2147	19	7330	109	540	1056	1189
<130	3123	9532	216	12210	2426	5932	5761	5745
>130	21666	15420	24744	5412	22456	18497	18142	18059

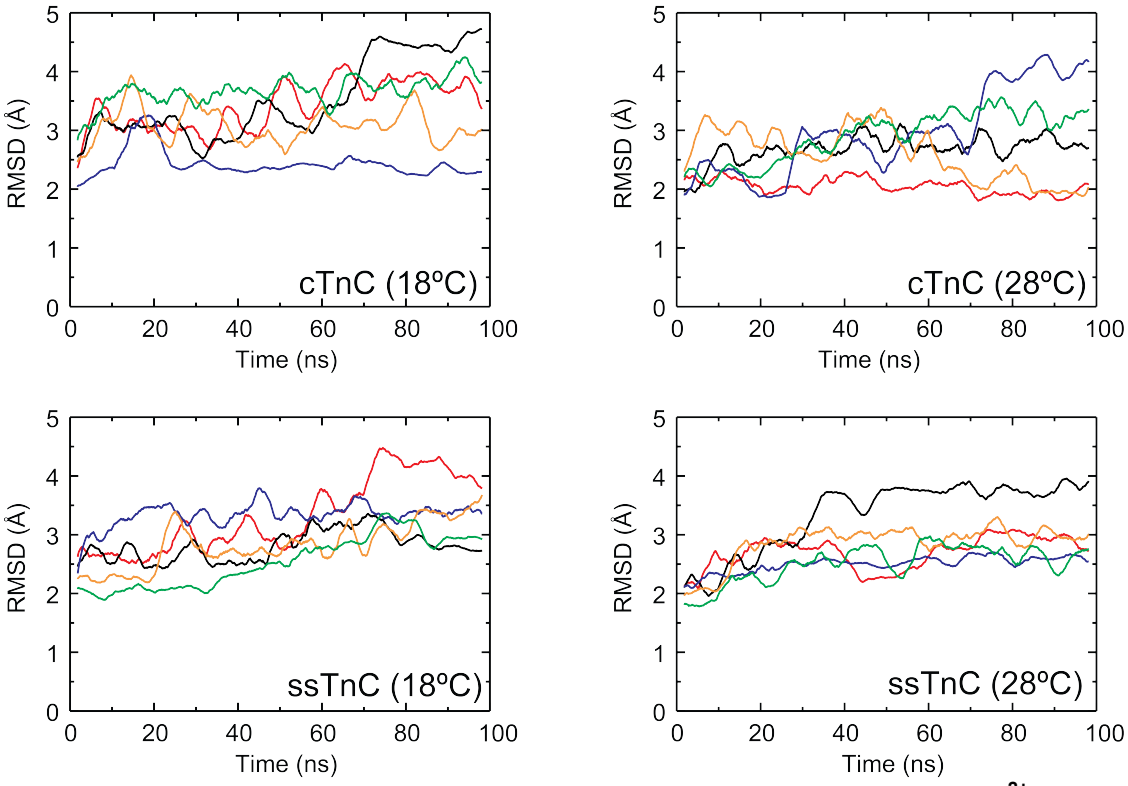

Figure S1. RMSD as a function of time for 100 ns simulations of TnC+Ca^{2+} that preceded PMF calculations for each temperature paralog combination. These indicate that each of the simulations has diverged substantially from the starting coordinates. Plots are a running average over 0.5% of the total number of data points.

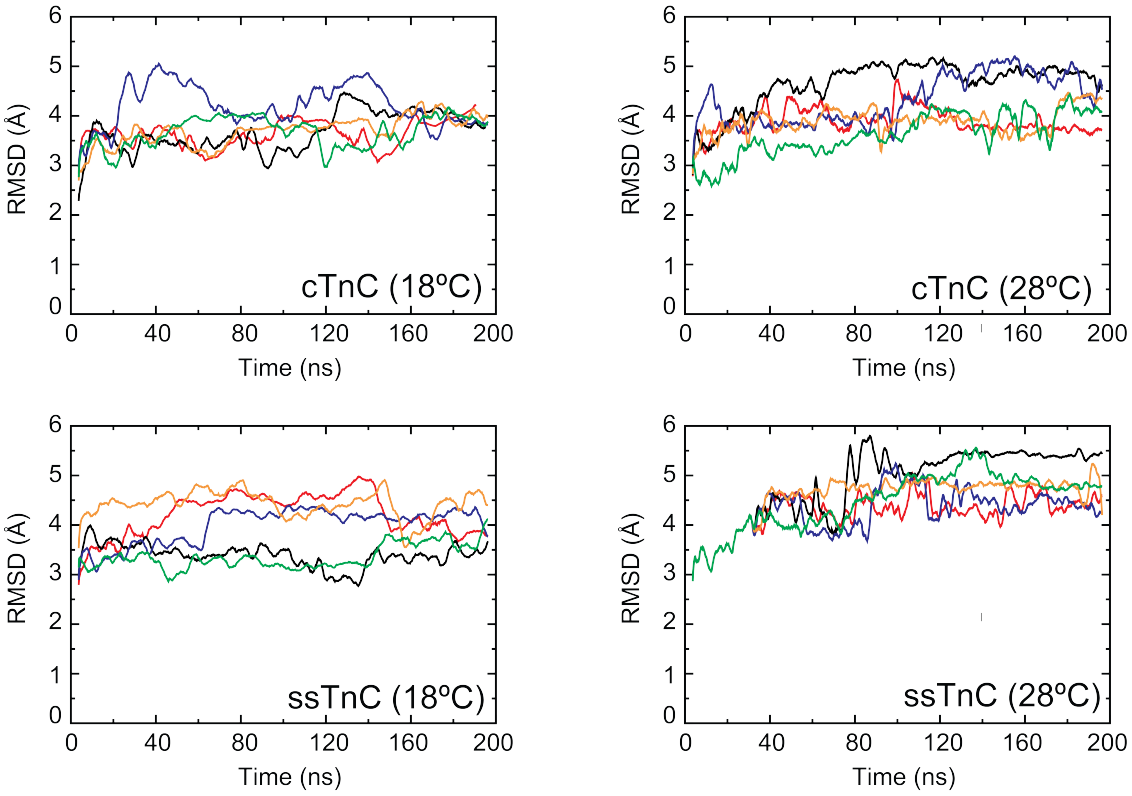

Figure S2. RMSD as a function of time for 200 ns simulations of TnI_{sw}+TnC+Ca^{2+} for each temperature paralog combination. These indicate that each of the simulations has diverged substantially from the starting coordinates. Plots are a running average over 0.5% of the total number of data points.

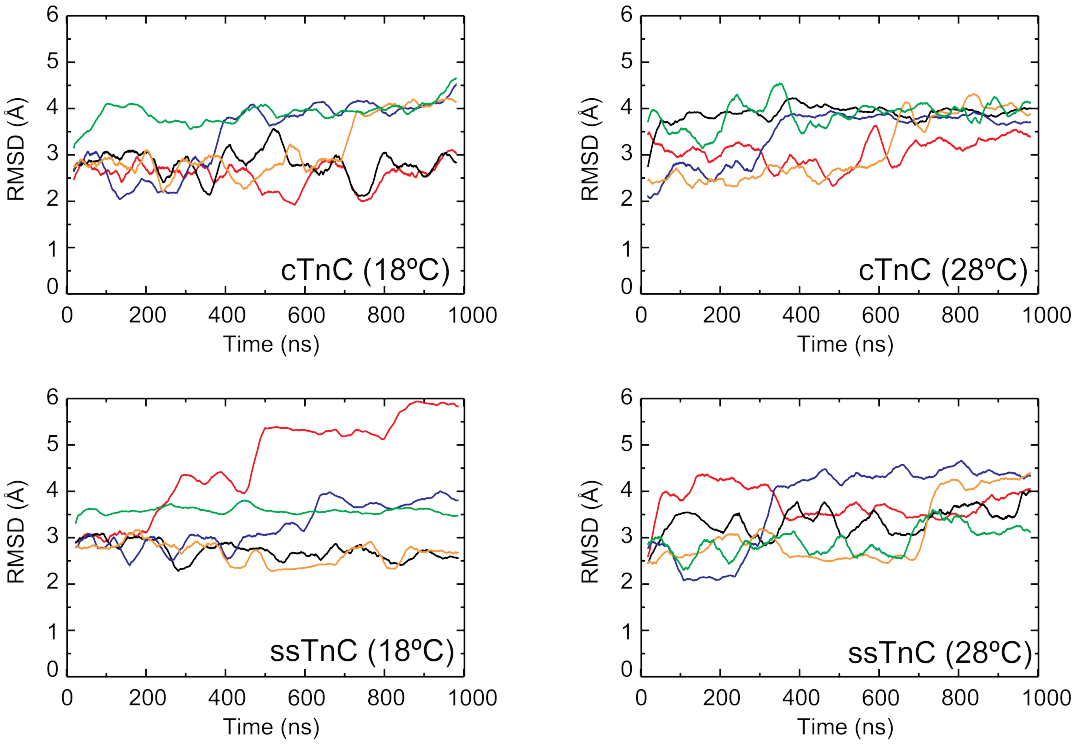

Figure S3. RMSD as a function of time for 1 μs simulations of TnC+Ca^{2+} for each temperature paralog combination. These indicate that each of the simulations has diverged substantially from the starting coordinates. Plots are a running average over 0.5% of the total number of data points.

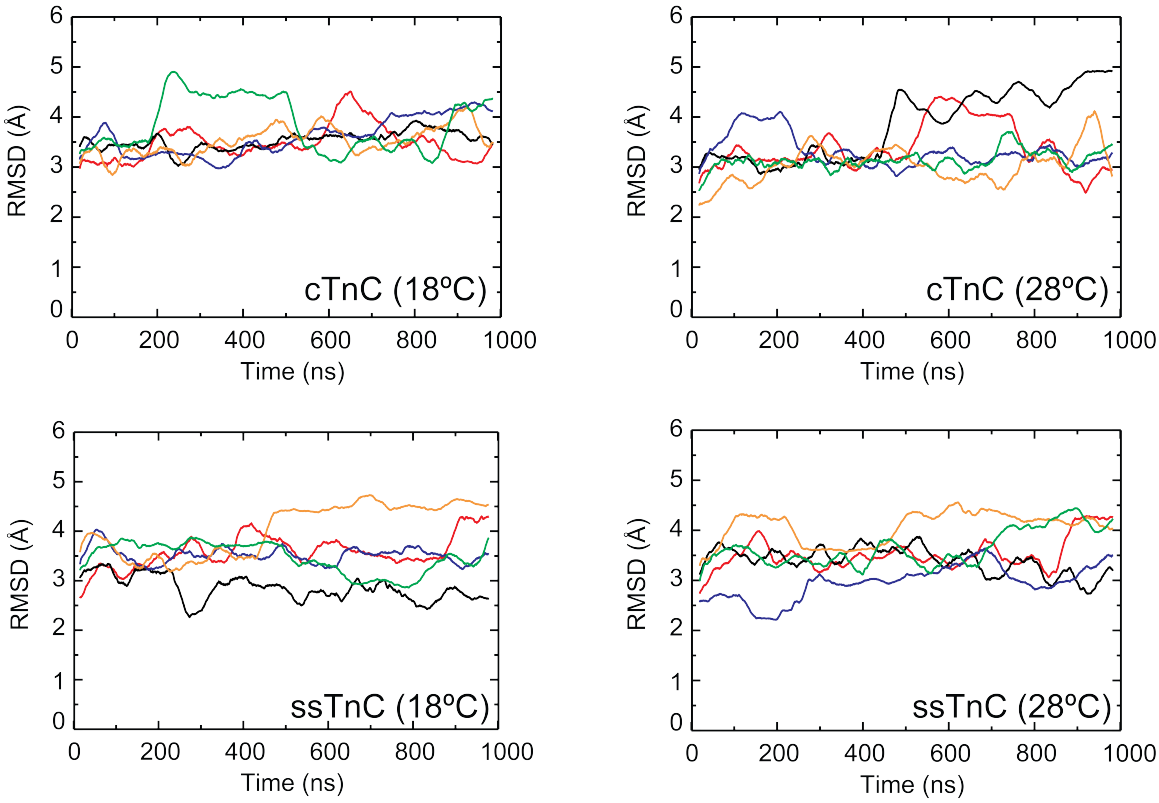

Figure S4. RMSD as a function of time for 1 μs simulations of TnC in the absence of Ca^{2+} for each temperature paralog combination. These indicate that each of the simulations has diverged substantially from the starting coordinates. Plots are a running average over 0.5% of the total number of data points.

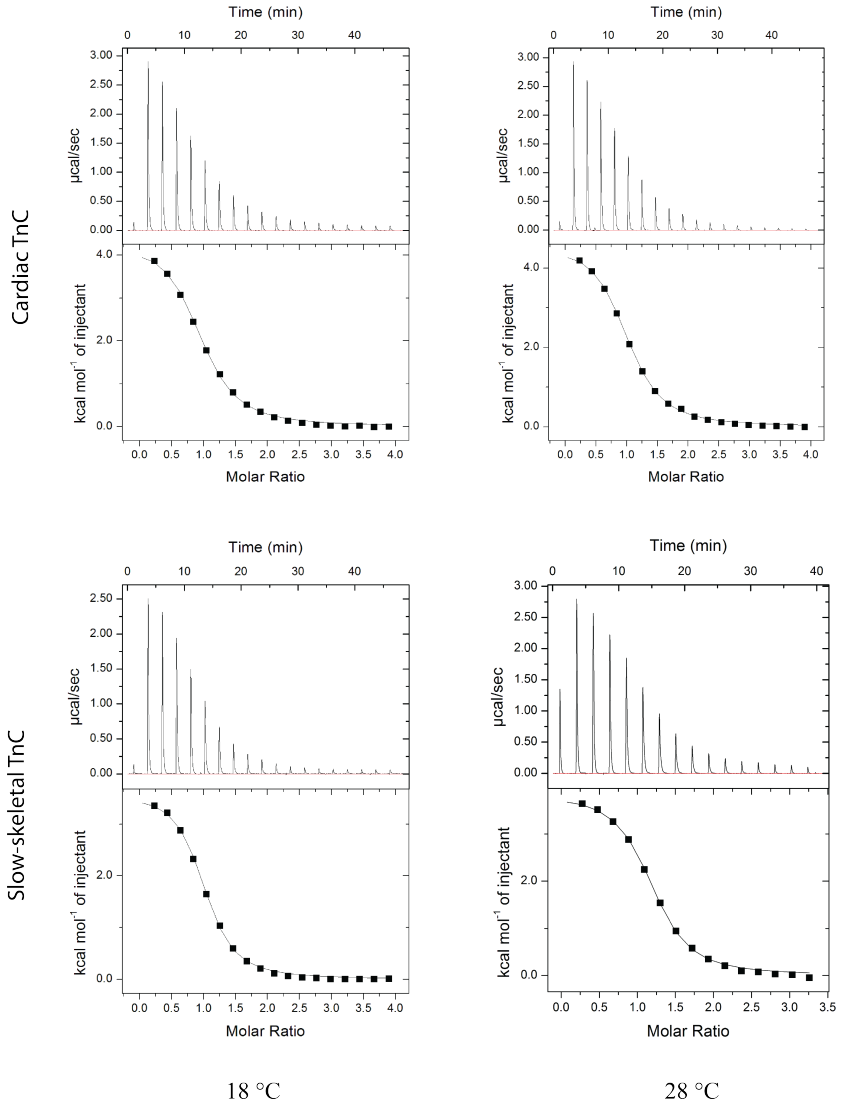

Figure S5. Representative ITC binding isotherms show the interaction between N-TnC and Ca^{2+} for each TnC paralog at each temperature. Thermodynamic parameters and K_d values are listed in Table 4.