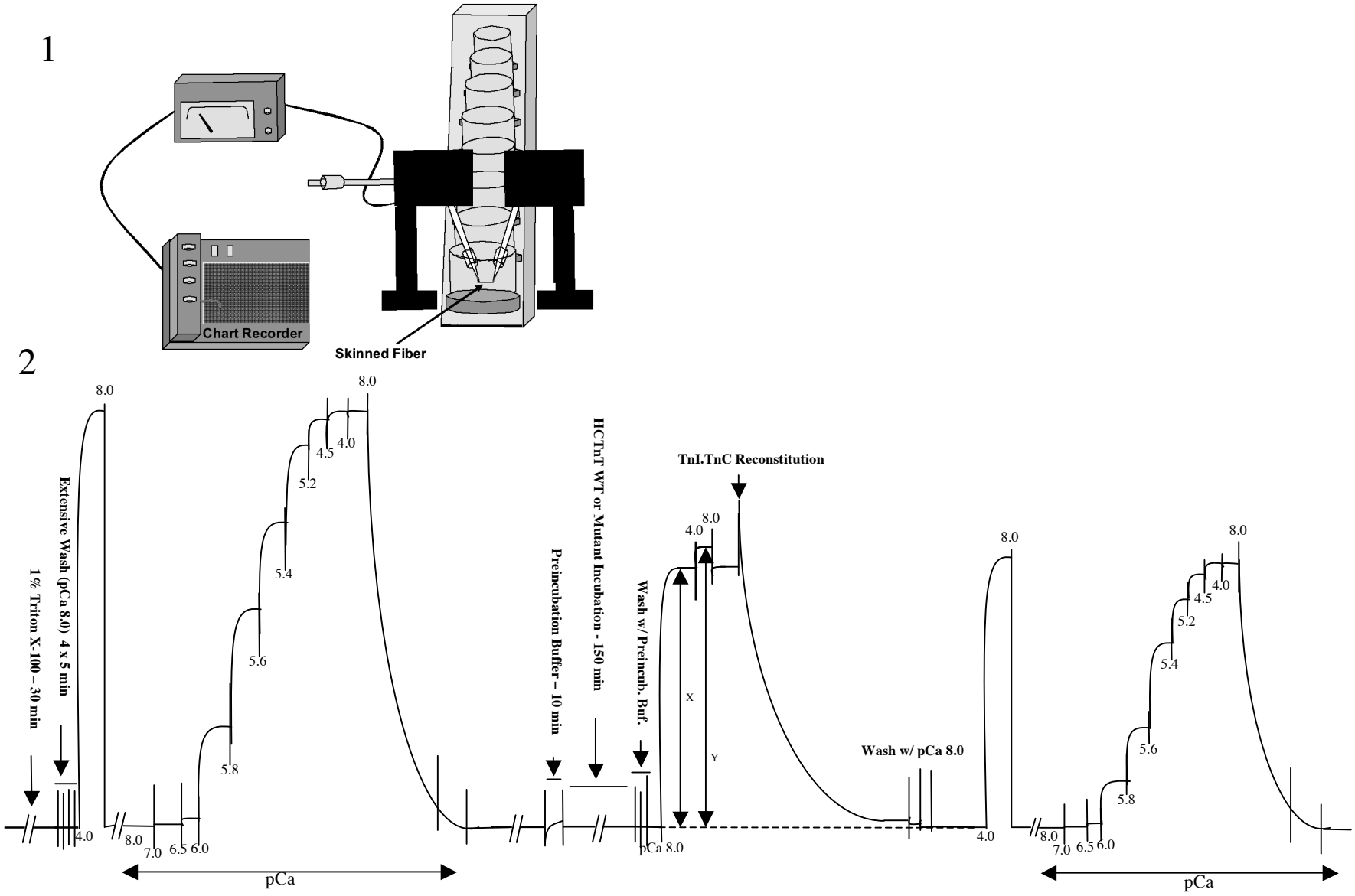
Supplemental Material

Supplemental Figure A



Supplemental Figure A. Skinned fiber setup and experimental design. 1) Schematic of the Güth system used for skinned fiber measurements. The skinned fiber with a diameter of 75 - 120 μm mm and length of 1.0 -1.2 mm is mounted between two clips, one side fixed and the other movable, to allow for changes in the fiber sarcomere length. The information such as increased or decreased tension in the fiber is captured by the force transducer. The signal is amplified and recorded by a chart recorder that allows evaluation of the changes in tension of the fiber as shown in the tracing in B. 2) Experimental design of skinned fibers used to assess contractile effects of wild type and DCM-troponin T mutants. First, the fiber is treated with Triton X-100 to remove the sarcolemma, thus disrupting the fibers internal Ca^{2+} storage. The Triton X-100 is washed away by subsequent washes with pCa 8 (10^{-8}) Ca^{2+} concentration, essentially a minus Ca^{2+} solution. Before the experiment is conducted, the ability of the fiber to contract is assessed by placing it in a high Ca^{2+} solution pCa 4 (10^{-4}) Ca^{2+} and then relaxed with pCa 8 solution. The endogenous pCa curve is performed by incubating the fiber in solutions of subsequently higher Ca^{2+} concentrations and calculating the pCa_{50} and Hill coefficient using a Hill equation (see methods) which are the $[Ca^{2+}]$ value at half maximal contraction and the cooperativity index of the thin filament activation, respectively. This establishes the pCa_{50} value of the fiber with all native proteins intact. Next, the fiber is incubated for 2.5h with the experimental exogenous human cardiac troponin T (wild-type or mutant) and will displace native cardiac troponin complex. To check the efficiency of the endogenous cardiac troponin complex displacement the ratio between Ca^{2+} unregulated tension measured at pCa8 (X) and sum of Ca^{2+} unregulated force at low $[Ca^{2+}]$ plus Ca^{2+} regulated force at high $[Ca^{2+}]$ (Y) is evaluated (the percentage of displacement is reported in Table 3 for each protein). Because a certain amount of native cardiac troponin I and troponin C is lost during this procedure, the fiber is incubated with binary

exogenous human cardiac troponin I.C binary complex to replenish the regulatory units in the fiber and restore Ca^{2+} regulation of force in the fiber. The fiber is then rinsed with pCa8 to remove the excess exogenous human cardiac troponin I.C binary complex. Finally, a second pCa curve is performed by incubating the fiber in solutions of subsequently higher Ca^{2+} concentrations and from this data the pCa_{50} and Hill coefficient are calculated. In addition, after the human cardiac troponin T displacement and reconstitution with binary complex the maximal force recovery was checked by briefly exposing the fibers to pCa 4.0. The percentage of maximal force recovery calculated in relation to the initial maximal force of the fiber is shown in Table 3.

Supplemental Table A.

Dilated Cardiomyopathy Mutations

Mutation Number	Mutation	Exon	References
A	Arg134Gly	11	Novel, this report
B	Arg151Cys	11	Novel, this report
C	Arg159Gln	12	Novel, this report
D	Arg205Trp	14	[1], this report
E, F, G, H	Lys210del	14	[1-4], this report
I	Glu244Asp	15	[5-7], this report
J	Arg131Trp	11	[1]
K	Arg141Trp	11	[4, 8, 9]
L	Ala172Ser	12	[10]
M	Arg205Leu	14	[1]
N	Asp270Asn	16	[1]

Hypertrophic Cardiomyopathy Mutations

Mutation Number	Mutation	Exon	References
1	Ser69Phe	8	[11]
2	Phe70Leu	9	[12]
3	Pro77Leu	9	[11]
4	Ile79Asn	9	[5, 6, 11, 13, 14]
5	Glu83Lys	9	[15]
6	Val85Leu	9	[16]
7	Asp86Ala	9	[17]
8	Arg92Trp	10	[11, 14, 16-23]
9	Arg92Gln	10	[5, 6, 13, 14, 24-30]
10	Arg92Leu	10	[11, 12, 14, 31]
11	Arg94Leu	10	[11, 14, 32]
12	Arg94Cys	10	[15]
13	Lys97Asn	10	Genomics of Cardiovascular Development, Adaptation, and Remodeling. NHLBI Program for Genomic Applications, Harvard Medical School. URL: http://www.cardiogenomics.org [*Oct, 2008*].
14	Ala104Val	10	[14, 26, 33, 34]
15	Phe110Ile	10	[5, 6, 14, 16, 26, 35-37]
16	Phe110Leu	10	[28]
17	Phe110Val	10	[12]
18	Lys124Asn	10	[38]

19	Arg130Cys	11	[28, 39]
20	Glu160del	12	[5, 12, 14, 15, 28, 40]
21	Glu163Lys	12	[5, 14]
22	Ser179Phe	12	[41]
23	Gln191del	13	[30]
24	Glu244Asp	15	[5-7]
25	Lys247Arg	15	[42]
26	Asn271Ile	16	[12]
27	Lys273Glu	16	[4, 16, 43]
28	Arg278Cys	17	[5, 6, 17, 28, 37, 42, 44-48]; Genomics of Cardiovascular Development, Adaptation, and Remodeling. NHLBI Program for Genomic Applications, Harvard Medical School. URL: http://www.cardiogenomics.org [*Oct, 2008*].
29	Arg278Pro	17	[17, 47, 49, 50]
30	Arg286Cys	17	[12, 47]
31	Arg286His	17	[17, 30]
32	Trp287ter	17	[12]

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