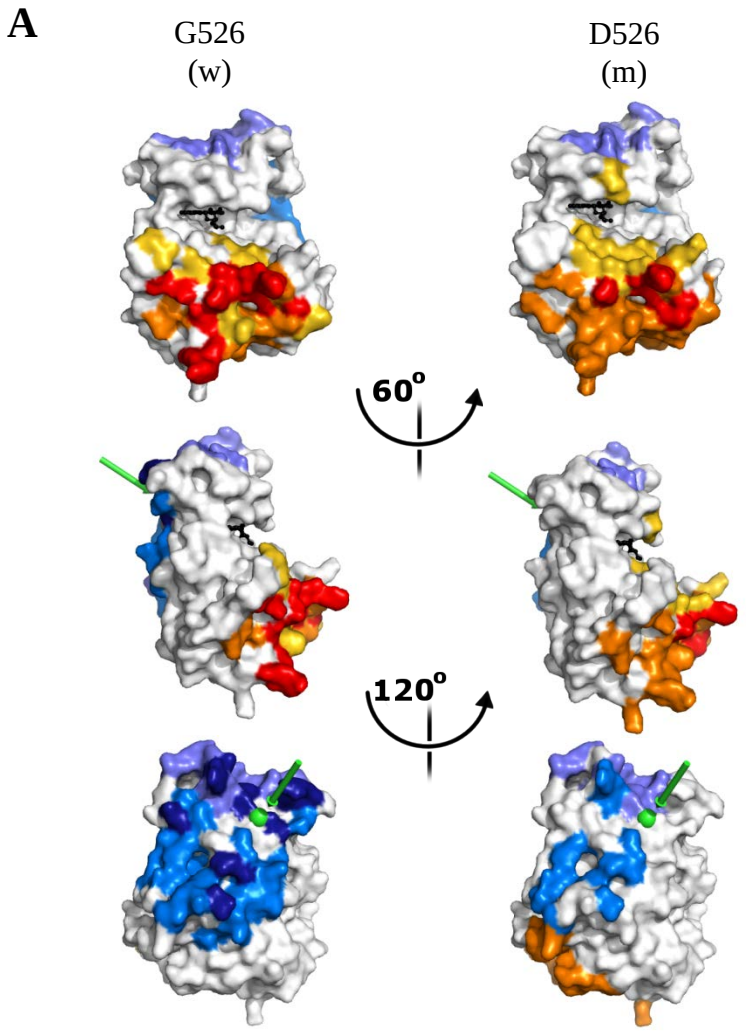
SUPPLEMENTAL MATERIAL

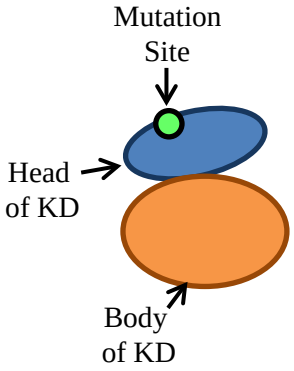


B

Docking Poses			
Cluster	w-w	w-m	m-m
1	95	75	133
2	84	72	108
3	43	65	47
4	35	45	47
5	35	39	47
6	35	36	41
7	32	34	40
8	32	32	35
9	26	30	30
10	26	30	26

-	+
+	--
+	++
-	---

C



Orientation of Top 10 Clusters				
Orientation		w-w	w-m	m-m
	head to head	5	4	0
	body to head	3	4	4
	body to body	2	2	6

Figure S1. Molecular docking of mutant TNNI3K shows alterations in predicted binding interfaces as compared to wild type. A, To examine the predicted impact of the mutation on TNNI3K dimerization, ClusPro docking poses were manually examined for the kinase domains (KDs) of wild type (w) and mutant (m) TNNI3K to determine the binding interfaces. The top 10 poses were mapped into four groups for w-w, w-m and m-m interactions. Two of the four groups utilize the body of the KD as the binding interface (orange and yellow with overlapping residues in red) and two utilize the opposite side of the molecule at the head of the KD (periwinkle and blue with overlapping residues in dark purple). The alpha carbon of residue 526 is shown as a green sphere and an inhibitor bound in the ATP binding site as black sticks. **B**, The presence of each of the 4 binding classes (indicated by color) is summarized for the heterozygous and homozygous mutant docking conditions (w-m and m-m, respectively), relative to w-w. An amplified (+) or diminished (-) presence of the pose upon mutation is shown for each class. **C**, Consolidating the top 10 clusters into the precise orientation of the KDs upon dimerization highlights the direct impact that the mutation has on each pose and the loss of head-to-head interactions in the presence of the D526 mutant.

Table S1. Cardiac Phenotype of Family Members with TNNI3K-G526D Mutation

ID	Age at diagnosis of arrhythmia, DCM	Rhythm	Conduction	LV ejection fraction % (age)	Other findings on initial echo	Coronary artery disease	Medical therapy	Status	Cardiac phenotype
II.2	66,71	CAF→ AVN ablation, RV-pacing	LAFB, RBBB	10(71)→ 25(80)[1]	Mod. LVE, Sev. RVE, Sev. BAE, Mod.-Sev. MR	Mild LAD	Class II, V; ACEI, AA, D, AC	Died at 81 in CHF	AF, CSD, chronic DCM
III.1	29,60	PAF	1° AVB, LAFB, RBBB, EPS: normal SAN & AVN	40(60)→ 50(65)[2]	Mild LVE, Mild LAE; Mild MR	Normal ECG stress test	ACEI, D, Class IC, V	Alive at 66	AF, CSD, temporary DCM
III.3	36,-	PAF	1° AVB, LAE, LPFB, RBBB, PAC's, PVC's	55(53)	RVE	No testing	AC	CVA at 53; Alive at 56	AF, CSD
III.4	20,39	MAT	1° AVB, LAFB, RBBB, PAC's, PVC's	45(39)→ 55(50)[3]	Mild LVE	Normal coronary angiogram	ACI, Class II	Alive at 52	MAT, CSD, temporary DCM (abnl RV biopsy)
IV.1	13,-	MAT→ RFA[4] at age 23	1° AVB, LAFB, EPS: normal SAN & AVN	"mildly reduced" (19)→60(24)[5]	-	No testing	Class II	Alive at 29	MAT, CSD, temporary DCM
IV.2	14,11	EAT; RFA[4] at age 19	LAFB	56(11)	Mod. LVE	No testing	Class IA,V	Alive at 26	EAT, CSD, LVE
IV.4	11,-	AFL; RFA[4] at age 11	RAD, RVH	63(17)	Mild LAE	No testing	-	Alive at 17	AFL, CSD

AA, aldosterone antagonist; AC, anticoagulant; ACEI, angiotensin-converting enzyme inhibitor; AFL, atrial flutter; AVB, atrioventricular block; AVN, atrioventricular node; BAE, biatrial enlargement; BB, beta-receptor blocker; CAF, chronic atrial fibrillation; CHF, congestive heart failure; Class, Vaughan Williams antiarrhythmic drug classification; CSD, cardiac conduction system disease; CVA, cerebrovascular accident; DCM, dilated cardiomyopathy; D, diuretic; EAT, ectopic atrial tachycardia; EF, left ventricular ejection fraction; EPS, invasive electrophysiologic study; LAFB, left anterior fascicular block; LAE, left atrial enlargement; LVE, left ventricular enlargement; MAT, multifocal atrial tachycardia; Mod., moderate; MR, mitral valve regurgitation; PAF, paroxysmal atrial fibrillation; RAD, right axis deviation; RBBB, right bundle branch block; RFA, radiofrequency ablation; RVE, right ventricular enlargement; RVH, right ventricular hypertrophy; SAN, sinoatrial node; Sev., severe.

[1]reverse ventricular remodeling therapy optimized; ventricular rate control with AV node ablation and RV pacing at 70 beats per minute.
[2]blood pressure control optimized; in sinus rhythm on serial evaluation.
[3]reverse ventricular remodeling therapy optimized
[4]right atrial foci
[5]decreased burden of atrial tachycardia

Table S2. Primer Sequences and Annealing Temperatures for PCR of *TNNI3K* Exons

Primer	Sequence (5'→3')	PCR Annealing Temperature (°C)
TNNI3K Exon 1F	TGATTAGTTCCCTTTATGAT	52
TNNI3K Exon 1R	TCCGTATACAAATTAACACA	
TNNI3K Exon 2F	TGGGTTCTTCATTACTACAA	54
TNNI3K Exon 2R	ATGTCTGAGGTTCTACGG	
TNNI3K Exon 3F	ATTCTTCATATAGAGGGTTC	54
TNNI3K Exon 3R	CTATTAAGCAGCTCAAGTC	
TNNI3K Exon 4F	CAGAGTCAAAATACACAGCA	54
TNNI3K Exon 4R	AAAAAGCCTAAAGATACACA	
TNNI3K Exon 5F	AGGCAGAAAATCAATACATC	54
TNNI3K Exon 5R	CCCCAATGTGTTCAGTAG	
TNNI3K Exon 6F	AAACTGAATCTTAATAGGCA	52
TNNI3K Exon 6R	AGTAACATGACAACCGTAA	
TNNI3K Exon 7F	TTGTATTAATCAACCCTAGA	50
TNNI3K Exon 7R	AAGGATTCGCATGTAGG	
TNNI3K Exon 8F	TGGTAGTAGGCAATAGA	48
TNNI3K Exon 8R	GCATGCCCTAGATTTGA	
TNNI3K Exon 9F	GGAGATACCCCCTTACACCT	62
TNNI3K Exon 9R	GCCCTACCTCTGCTATTGCT	
TNNI3K Exon 10F	AGAGGGCACAATTTAGTTCA	56
TNNI3K Exon 10R	CCTAGTGCTGGCCATAAG	
TNNI3K Exon 11F	ATCCTTACTTCATACCTTTG	54
TNNI3K Exon 11R	AGTTGCCTTCTCTGATTAC	
TNNI3K Exon 12F	GAGTGCCTGACATAGTGC	56
TNNI3K Exon 12R	CCCCTGAAAGAATGATAGTA	
TNNI3K Exon 13F	GCTTGTGCATGACTAGG	52
TNNI3K Exon 13R	AAGGGTAAGGTTAATTATGA	
TNNI3K Exon 14F	GATCATTTGGGTTAAGGT	50
TNNI3K Exon 14R	ATTTTATTTCTGCATCGTC	
TNNI3K Exon 15F	TATTGGCTCAGGTAACCTA	54
TNNI3K Exon 15R	AGGTATTGGCTCGATAACT	
TNNI3K Exon 16F	GAGAAGCATGTGCATGG	52
TNNI3K Exon 16R	TGAAACACAGTTATTAGGCA	
TNNI3K Exon 17F	AATTTCTAGCTTAACCTTCA	52
TNNI3K Exon 17R	GTTAGCTGGTGCATCTTA	
TNNI3K Exon 18F	GAGGGTTTGTCTAAAGTAGG	58
TNNI3K Exon 18R	AGAGTTTCAAAATGTAGGGTC	
TNNI3K Exon 19F	ACAGAAGTCTCTTGAGGTT	50
TNNI3K Exon 19R	ACATAAGCCATTATCCC	
TNNI3K Exon 20F	TGAGGGCAATATGTATGG	52
TNNI3K Exon 20R	AGATTGTTTGCTTACCTCC	
TNNI3K Exon 21F	ATGTCTGCTGATGTTGCTC	50
TNNI3K Exon 21R	CCCGCCTTGACAGTC	
TNNI3K Exon 22F	AGAGGGAGACTCAACA	50
TNNI3K Exon 22R	GTAACTTTTCATAAGCACC	
TNNI3K Exon 23F	TAAAATATGGGAAACCTTGG	54
TNNI3K Exon 23R	AAGTTTTTCAGGGGGTAATC	
TNNI3K Exon 24F	CAAGGTTATACCGCTAGAA	54
TNNI3K Exon 24R	TCTCCCTTTTTGTCAAATAG	
TNNI3K Exon 25F	CTACTCACCAGCAACCGT	56
TNNI3K Exon 25R	GTGGGGAATTAAATAGGACA	