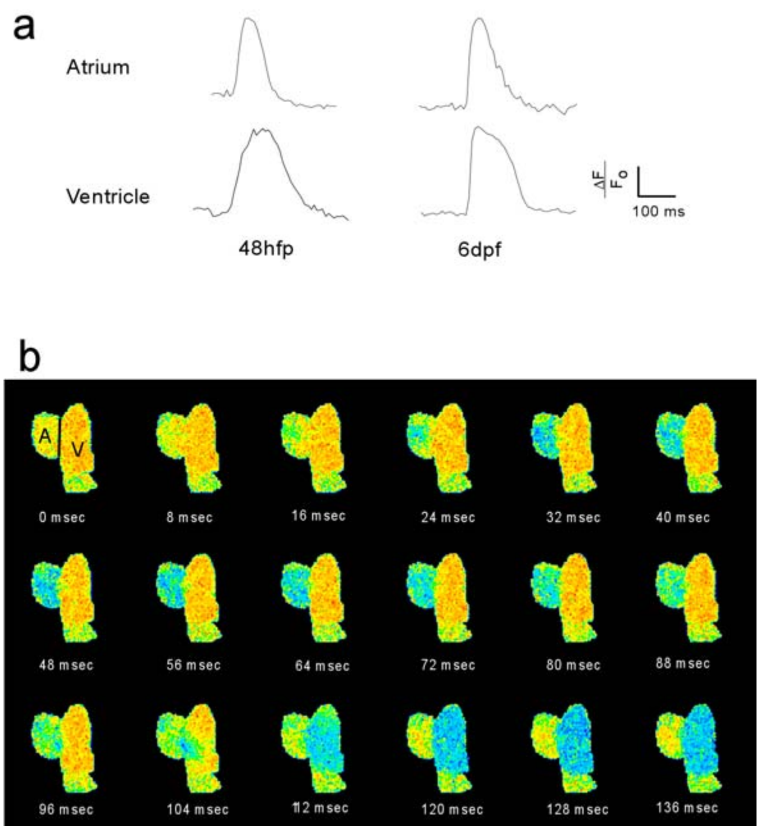
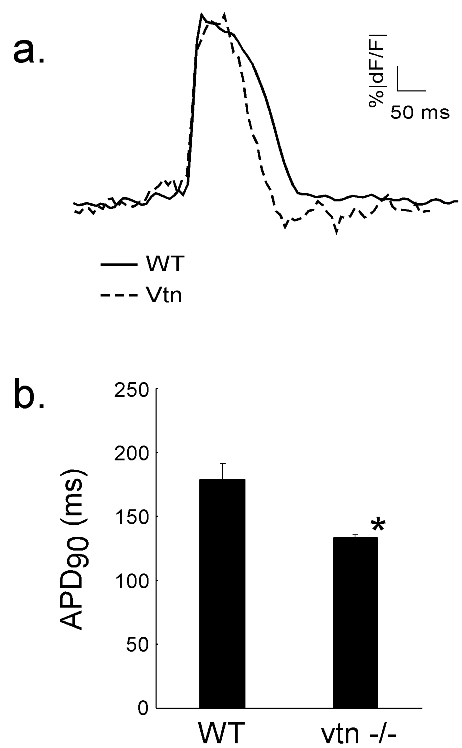
Supplemental Figure 1: Optical voltage recordings from zebrafish embryos



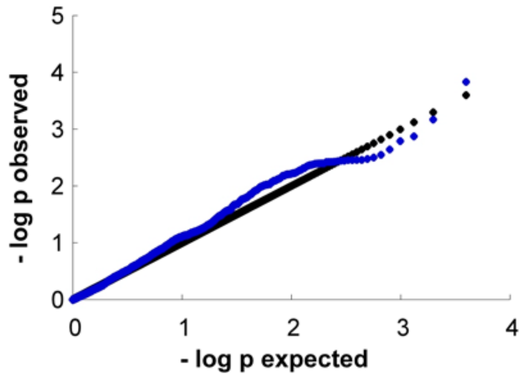
Supplemental Fig 1: Optical voltage recordings in the zebrafish embryo. **(1a)** We obtained high-resolution action potentials from embryonic atrium and ventricle as early as 48 hours post fertilization (hpf). Normal embryos display subtle differences in atrial and ventricular action potential profiles at this stage, which resemble the adult human transients in duration and morphology. **(1b)** Sequential optical maps obtained during a spontaneous cardiac depolarization. The impulse arises in the atrium and pauses at the atrioventricular boundary (t=32ms), before emerging into the ventricle (t=104ms). Activation maps demonstrate differential conduction velocities in atrium and ventricle (2.6 ± 0.5mm/s and 8.2 ± 2.1mm/s, respectively), and confirm the emergence of physiologic AV conduction delay (88 ± 38msec). A = atrium, V=ventricle, and the line at 0 msec demarcates the atrioventricular boundary.

Supplemental Figure 2: Valentine mutants show shortened action potential duration.



Supplemental Fig 2: Valentine mutants display shortened action potential duration.
2a) Representative action potential recordings from wildtype and *valentine* homozygotes are shown. 2b) Action potentials are shortened in *valentine* homozygous mutants, * denotes P<0.05. Values expressed as mean +/- S.D.

Supplemental Figure 3: Quantile-Quantile Plot Excluding GINS3 Locus



Supplemental Fig3: Quantile-quantile plot of observed versus expected p values from SNPs within 150kb of each human ortholog of the zebrafish repolarization genes, excluding GINS3. Association data are from the QTSCD GWAS. Blue data points are observed p values, black points indicate results that would be anticipated by chance alone. In contrast to Figure 3b, when GINS3 is excluded, the observed values do not deviate from the null hypothesis.

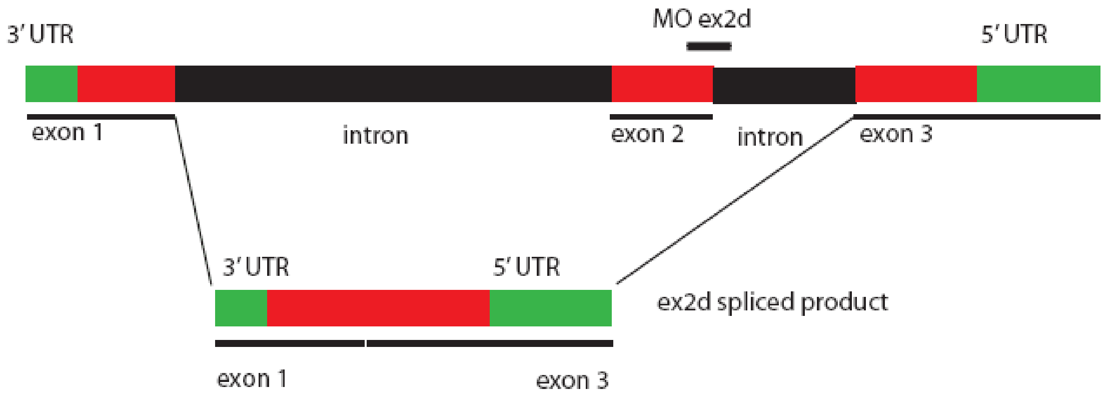
Supplemental Figure 4: GINS3 Knockdown in Zebrafish

a

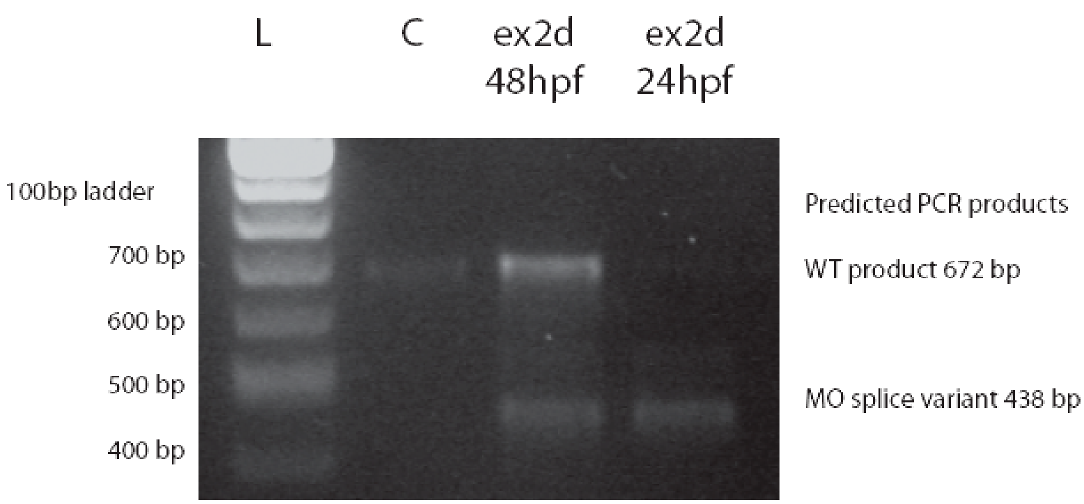
	Wildtype	GINS3 Morpholino
1:1 AV rhythm	0	33
2:1 AV block	38	10

P< 0.0001, Fisher's exact test

b



c



Supplemental Figure 4: GINS3 Knockdown in Zebrafish. (4a) Results of exposure of GINS3 morpholino injected embryos and wild type controls to 12uM dofetilide. Embryos are scored for cardiac rhythm, with 100% of wild type embryos showing 2:1 AV block, while the majority of GINS3 morpholino injected embryos did not develop 2:1 AV block, phenocopying GINS3 mutant embryos. 4b) Schematic showing the predicted effect of GINS3 morpholino injection on GINS3 mRNA processing and 4c) the observed exclusion of exon 2 in GINS3 MO injected embryos at 24 and 48 hpf, L = 100bp ladder, C = control, wild type embryo sample.

Supplemental Table 1. Dofetilide responses for heg-san-vtn mutants.

Gene	Allele	Proportion of mutants with 2:1 AV block
Valentine (CCM2)	Insertional	0/40
Valentine (CCM2)	ENU	0/40
Heg (Heart of Glass)	ENU	0/40
Santa (CCM1)	ENU	0/40
Wild-type	NA	40/40

Abbreviations: ENU: ethyl-nitrosourea. All fish were exposed to a dose of dofetilide (12uM) which results in uniform AV block in wild-type embryos. All findings were significant at $p<0.001$ when compared with wild type siblings.

Supplemental Table 2. Evidence for Novel Cardiac Repolarization Pathway

	Gene	Allele(s)	2:1 AV block with drug	Zebrafish genome ID	C. elegans ortholog	Probability of genetic interaction	Other evidence of interaction	References for additional data
a	valentine (CCM2)	hi296a	0/39	AY648715	None		Independent zebrafish genetic interaction with other CCM genes	Mably JD et al., Development 2006;133:3139, Mably JD et al. Current Biol 2003;13:2138, Gotthardt M et al. JBC 2000;275:25616
b	snriboD1	hi601	0/40	AF506225	snr-3	g=0.81, k=0.79, n=0.80		
c	casein kinase I	hi1002, hi2069	0/40	AY099516	kin-19	g=0.80, k=0.83, m=0.83, n=0.80, p=0.77		
d	gins3	hi1241	0/20	AY648732	Y65B4B2.8	p=0.76		
e	survivin	hi1326, hi2111, hi3018	20/60	AY057057	bir-1		Genetic interaction with j and l, possible physical interaction with j, regulates Kv channels through unknown mechanism	Chang JL et al., Exp Cell Res 2006;312:962 McMurtry MS et al., J Clin Invest. 2005 Jun;115(6):1479-91.
f	WRB	hi1482	NA	AY648739	Y50D4A.2			
g	Suppressor of Ty6 homolog	hi1621, hi2505b	0/60	NM145118	emb-5	b=0.81, h=0.83, k=0.83, n=0.87, p=0.78		
h	surfeit 6	hi1769	NA	AY648750	ZK546.14B	g=0.83, n=0.92		
j	nuf2	hi1780a	0/40	NM1007456	None		Genetic interaction with e and l	Chang JL et al., Exp Cell Res 2006;312:962
k	rpa1	hi2618	0/40	AY648787	F18A1.5	b=0.82, m=0.79, n=0.91		
l	cdca11	hi2769	0/40	BC050181	None		Genetic interaction with e and j	Tulu US et al. Current Biol 2006;16:536
m	esco1	hi2865	0/40	AY648804	FO8F8.4	d=0.83, k=0.79		
n	nop56	hi3101	0/40	AY648814	KO7C5.4	b=0.80, c=0.80, g=0.87, h=0.92, k=0.91		
o	polyA BP	hi3202b	0/40	BC047811	pab-1			
p	pkc λ	hi3208	0/60	AF390109	pkc-3	c=0.77, d=0.76, g=0.78		

	Additional known interactions							
q	santa (krit-1/CCM1)						Genetic interaction with a, r and s. Physical interaction with a and r	Mably JD et al., Development 2006;133:3139, Mably JD et al. Current Biol 2003;13:2138, Hilder TL et al. J Proteome Res 2007:6:4343
r	pcd10 (CCM3)						Physical interaction with a and q	Hilder TL et al. J Proteome Res 2007:6:4343 Mably JD et al., Development 2006;133:3139, Mably JD et al. Current Biol 2003;13:2138
t	integrin cytoplasmic domain associated protein						Physical interaction with q and u	Zhang J et al. Hum Mol Gen 2001;10:2953
u	integrin beta 1						Physical interaction with t and v	Cherubini A. at el. Mol Cell Biol 2005;16:2972
v	KCNH2						Physical interaction with u	Cherubini A. at el. Mol Cell Biol 2005;16:2972
w	snx17						Physical interaction with q and x	Czubayko M. et al. Biochem Biophys Res Commun 2006;345:1264
x	lrp5						Physical interaction with w, y and z	Gotthardt M et al. JBC 2000;275:25616
y	psd95						Genetic interaction with c, Physical interaction with multiple channels including Kv family and sodium channel	
z	nos1ap						Physical interaction with x	Gotthardt M et al. JBC 2000;275:25616

Supplemental Table 3: Position of the Genomic Blocks encompassing 15 zebrafish repolarization genes

Locus	*GENE*	CHROM	START_build35	STOP_build35	START-150kb	STOP+150kb
ZF#1	*CDCA8*	chr1	37,827,251	37,844,482	**37677251**	**37994482**
ZF#2	*CDCA1*	chr1	160,023,382	160,057,205	**159873382**	**160207205**
ZF#3	*PRKCI*	chr3	171,422,926	171,506,466	**171272926**	**171656466**
ZF#4	*CSNK1A1*	chr5	148,855,037	148,911,200	**148705037**	**149061200**
ZF#5	*CCM2*	chr7	44,813,026	44,889,308	**44663026**	**45039308**
ZF#6	*ESCO2*	chr8	27,687,976	27,718,341	**27537976**	**27868341**
ZF#7	*PABPC1*	chr8	101,784,319	101,803,491	**101634319**	**101953491**
ZF#8	*SURF6*	chr9	133,227,106	133,232,598	**133077106**	**133382598**
ZF#9	*GINS3*	chr16	56,983,923	56,997,536	**56833923**	**57147536**
ZF#10	*RPA1*	chr17	1,680,094	1,748,119	**1530094**	**1898119**
ZF#11	*SUPT6H*	chr17	24,013,428	24,053,375	**23863428**	**24203375**
ZF#12	*BIRC5*	chr17	73,721,871	73,733,310	**73571871**	**73883310**
ZF#13	*SNRPD1*	chr18	17,446,257	17,464,206	**17296257**	**17614206**
ZF#14	*NOL5A*	chr20	2,581,253	2,587,038	**2431253**	**2737038**
ZF#15	*WRB*	chr21	39,674,139	39,691,486	**39524139**	**39841486**