SUPPLEMENTARY INFORMATION

Multiple *pkd* and *piezo* gene family members are required for atrioventricular valve formation

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Fig. S1 Wild-type-like cardiac chamber size in 78 hpf *pkd1a* mutants. a Schematics of Pkd1a (wild-type and predicted mutants) showing the disruption of the conserved PCCD. b-e' Diameter quantification method along the heart tube during atrial diastole (b-e) and systole (b'-e'); brightfield images of hearts (b,b') are segmented along the heart midline (c-d') and displayed using a thermal lookup table (e,e'). f Diameter along the heart tube of 78 hpf wild-type larvae during atrial diastole and systole. g-h Diameter along the heart tube of 78 hpf wild-type, *tnnt2a* mutant, and *pkd1a* mutant larvae during atrial diastole (g) and systole (h)); arrow points to the decreased heart diameter during atrial systole. i Heart rate of 78 hpf wild-type, *pkd1a* mutant, and *klf2a; klf2b* double mutant larvae. The center of the error bar represents the mean in i. Error bars indicate S.E.M. in f, g, h; s.d. in i. P-values were calculated using a two-sided Student's t-test in i and are relative to wild type. Scale bar: 50 µm. Source data are provided as a Source Data file.







Fig. S2 *pkd2 and pkd111* mutants display heart looping defects. a-a' Schematics of Pkd2 (a) and Pkd111 (a') (wild-type and predicted mutants) showing the disruption of the conserved PCCD. b Relative mRNA levels of *pkd1a*, *pkd111*, and *pkd2* in 78 hpf wild types and *pkd1a* mutants; n= 3 biologically independent samples; Ct values are listed in Supplementary Data 1. c-f Brightfield images of 78 hpf wild-type (c) and *pkd2* mutant hearts which display normal (d) or defective looping (e-f); one time point of a spinning disc movie at atrial diastole; green and red lines outline the heart. g Heart looping defects of 78 hpf wild-types and *pkd* mutants. h-i Diameter along the heart tube of 78 hpf wild-type, *tnnt2a* mutant, and *pkd* triple mutant larvae during atrial diastole (h) and systole (i); arrow points to the decreased heart diameter during atrial systole. j Heart rate of 78 hpf wild-type and *pkd* triple mutant larvae. Hearts imaged in ventral view, anterior to the top, ventricle (V) on the left and atrium (A) on the right. The center of the error bar represents the mean in b, j. Error bars indicate S.E.M. in h, i; s.d. in b, j. P-values were calculated using a two-sided Student's t-test in b, j and are relative to wild type. Scale bar: 50 µm. Source data are provided as a Source Data file.

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a'	Piezo2a ^{wt} - 2622aa	CG0 R	STTC L	GGT V	GCC P	GTT(F	CCT(L	GACG T	PFI GAG E	EW CTG L	dom CGG	ain GCC A	GTG V	AT(M	GGA D	CTC.	GGG:	TCT V	gga W	CG T	GAC D	ACG T	ACG T	CTC L	AGT S	TTA L	TCC. S	AGC' S	TGG W
a'	Piezo2a ^{wt} - 2622aa Piezo2a ^{bns367} - 2620aa -2aa	CGG R	STTC L	GGT V	GCC P	GTTC F	CCT(L	GACG T	PFI GAG E	EW CTG L	dom CGG R	ain GCC A	GTG V	GATO M	GGA D	CTC	GGG: 1 T	TCT V	GGA W	ICG T	GAC D	ACG T	ACG T	CTC L	AGT S	TTA L	TCC S	AGC' S	TGG W
a'	Piezo2a ^{wt} - 2622aa Piezo2a ^{bns367} - 2620aa -2aa	CGO R CGO	GTT(L GTT(GGT V	GCC P III GCC	GTTC F I G	CCT(L	FACG T -ACG	PFI GAG E I I I I	EW CTG L	dom CGG R III CGG	ain GCC A IIII GCC	GTG V	SAT(M SAT(GGA D III GGA	CTC	GGG: 7 7 1 GGG:	TCT V TCT	GGA W GGA	ICG T 	GAC D III GAC	ACG T ACG	ACG T ACG	CTC L CTC	AGT S AGT	TTA L TTA	TCC S IIII TCC	AGC' S IIII AGC	TGG W III TGG





Fig. S3 piezo2a mutants display heart looping defects. a-a' Schematics of Piezo1 (a) and Piezo2 (a') (wild-type and predicted mutants) showing the disruption of the conserved PFEW domain. b Relative mRNA levels of piezo1 and piezo2a in 78 hpf wild types and pkd1a mutants; n= 3 biologically independent samples; Ct values are listed in Supplementary Data 1. c-f Brightfield images of a 78 hpf wild type (c) and 78 hpf piezo2a mutants injected at the one-cell stage with a pkd1a morpholino which display normal (d) or defective (e-f) cardiac looping; one time point of a spinning disc movie at atrial diastole; green and red lines outline the heart. g Heart looping defects of 78 hpf wild-type, piezo1 mutant, and piezo2a mutant larvae, non-injected or injected at the one-cell stage with a pkd1a morpholino. h-i Diameter along the heart tube of 78 hpf wild-type, tnnt2a mutant, piezo1 mutant, and piezo2a mutant larvae during atrial diastole (h) and systole (i); arrow point to the decreased heart diameter during atrial systole state. j Heart rate of 78 hpf wild-type, piezo1 mutant, piezo2a mutant, and piezo2a; pkd1a double mutant larvae. k-l In situ hybridization at 78 hpf for piezo1 (k) and piezo2a (l) expression. Hearts imaged in ventral view, anterior to the top, ventricle (V) on the left and atrium (A) on the right. The center of the error bar represents the mean in b, j. Error bars indicate S.E.M. in h, i; s.d. in b, j. P-values were calculated using a two-sided Student's t-test in b, j and are relative to wild type. Scale bars: 100 µm in k,l; 50 µm in c-f. Source data are provided as a Source Data file.



Fig. S4 Elevated calcium levels in the atrioventricular canal at an early larval stage. a-c' Confocal imaging and quantification method of 78hpf *fli1a:Gal4FF; UAS:GCamP6s* hearts during atrial diastole (a) and systole (a'), displayed using a thermal lookup table (b,b') and segmented along the cardiac midline using the GCamP6s fluorescence signal (c,c'); maximum projection of 4D-assembled spinning disc movies. d Diameter and endocardial calcium levels along the contracting heart tube in 78 hpf wild-type larvae during atrial diastole and systole. Hearts imaged in ventral views, anterior to the top, ventricle (V) on the left and atrium (A) on the right. Error bars indicate S.E.M. Scale bar: 50 µm. Source data are provided as a Source Data file.





Fig. S5 Transcriptional modulation in the atrioventricular canal by Pkd and Piezo members. a-b Confocal projection and quantification method of a 78 hpf wild-type heart stained for MHC and *klf2a* (a), segmented along the cardiac midline using MHC staining to analyze *klf2a* mean fluorescence intensity (b). c *klf2a* mean fluorescence intensity along the heart tube in 78 hpf wild-type larvae. d-f, h-j, l-n, p-r Confocal projections of 78 hpf wild-type (d,h,l,p), *pkd2* mutant (e,m), *pkd1a* mutant (i,q), *pkd1a; pkd2* double mutant (f,n), and *piezo2a; pkd1a* double mutant (j,r) hearts stained for MHC, and *klf2a* (d-f, h-j), or *klf2b* (l-n, p-r) expression. g, k, o, s *klf2a* (g,k) and *klf2b* (o,s) mean fluorescence intensity along the heart tube in 78 hpf wild-type, *pkd2* mutant, *pkd1a* mutant, *pkd1a; pkd2* double mutant, and *piezo2a; pkd1a* double mutant, *pkd1a; pkd2* double mutant, and *piezo2a; pkd1a* double mutant, *pkd1a; pkd2* double mutant, and *piezo2a; pkd1a* double mutant, *pkd1a; pkd2* double mutant, and *piezo2a; pkd1a* double mutant, *pkd1a; pkd2* double mutant, and *piezo2a; pkd1a* double mutant (j, on the right. Error bars indicate S.E.M. in c, g, k, o, s. Scale bars: 50 µm. Source data are provided as a Source Data file.

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Fig. S6 Wild-type-like cardiac chamber size in 78 hpf *camk2g* mutants. a-a' Schematics of Camk2g1 (a) and Camk2g2 (a') (wild-type and predicted mutants) showing the disruption of the conserved CamKII-AD. b-c Diameter along the heart tube in 78 hpf wild-type, *tnnt2a* mutant, and *camk2g1; camk2g2* double mutant larvae during atrial diastole (b) and systole (c); arrow points to the decreased heart diameter during atrial systole. d Heart rate of 78 hpf wild-type, *camk2g1* mutant, *camk2g2* mutant, and *camk2g1; camk2g2* double mutant larvae. The center of the error bar represents the mean in d. Error bars indicate S.E.M. in b,c; s.d. in d. P-values are calculated using a two-sided Student's t-test in d and are relative to wild type. Source data are provided as a Source Data file.