Supporting Information

Chi et al. 10.1073/pnas.0909432107

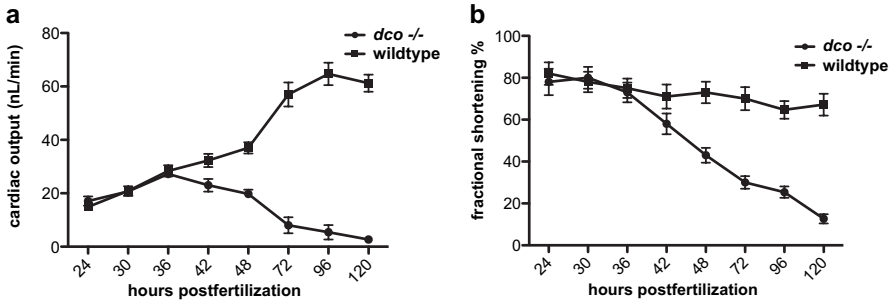


Fig. S1. *dco* Mutants exhibit severe heart failure. (*A*) *dco* Mutants exhibit severely reduced cardiac output by 42 hpf, (*B*) which is due to severely impaired ventricular function as assessed by fractional shortening measurements. Both cardiac output and ventricular function deteriorate as *dco* mutants mature. Cardiac output and fractional shortening were measured in 12 WT and $dco^{-/-}$ hearts at 24, 30, 36, 42, 48, 72, 96, and 120 hpf. Data are mean for each data set; error bars indicate SEM.

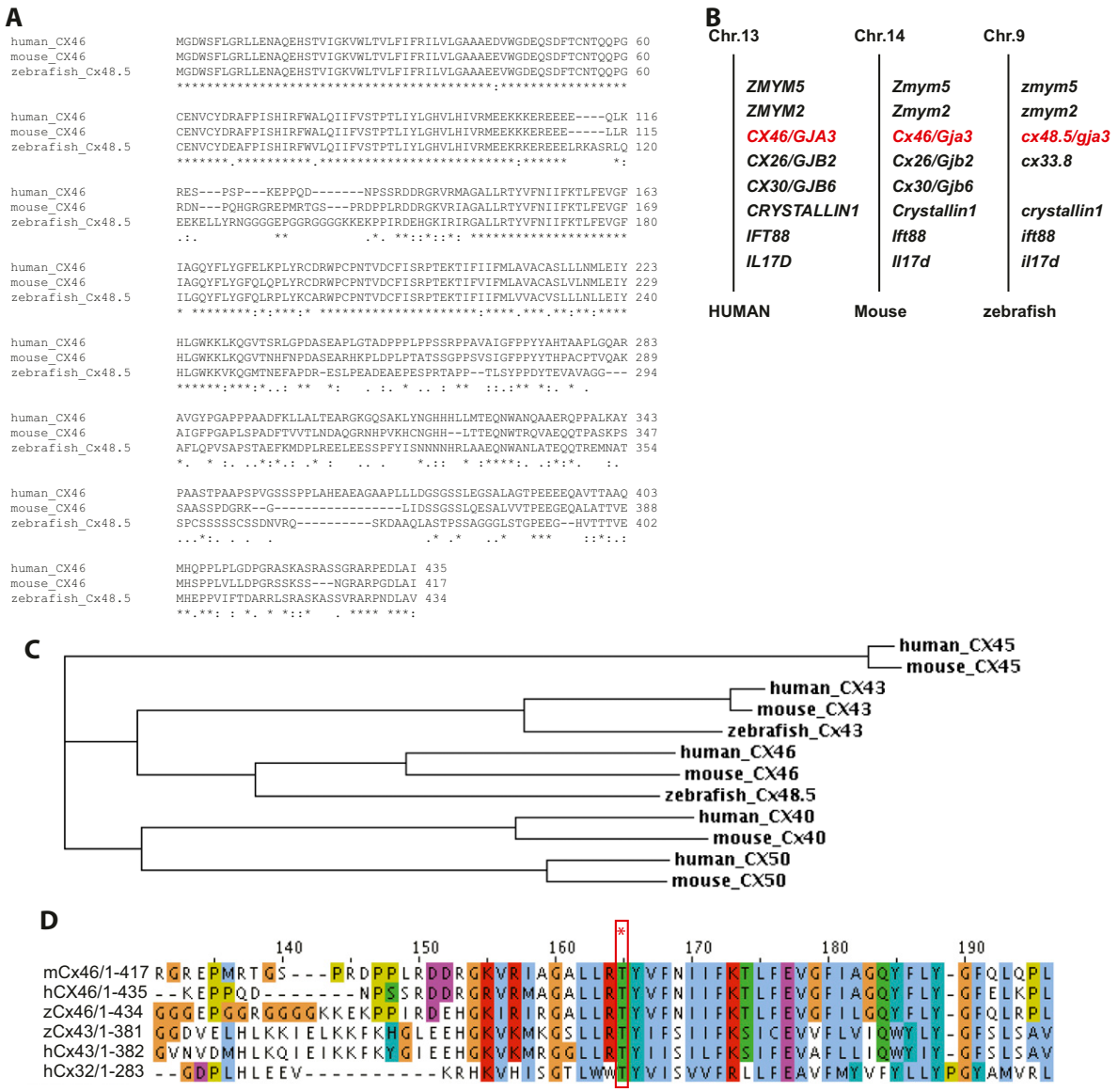


Fig. S2. Cx48.5 is a functional ortholog of the mammalian CX46/GJA3 subfamily members. (*A*) ClustalW alignment of human, mouse, and zebrafish GJA3 sequences. Below protein sequences, identical and similar amino acids are labeled with an asterisk and colon/period, respectively. Zebrafish Cx48.5 and human CX46 proteins share 64% homology. National Center for Biotechnology Information blast search analysis of the human genome as well as the zebrafish genome reveals that zebrafish *cx48.5* is most closely homologous to human *CX46*. GenBank accession numbers used for the analysis are as follows: human CX46 (NP_068773), mouse CX46 (NP_058671), and zebrafish Cx48.5 (NP_997525). Human and mouse CX46 proteins are highly similar to zebrafish Cx48.5 protein. hCX46, human CX46 protein; mCX46, mouse CX46 protein; zCx48.5, zebrafish Cx48.5 protein. (*B*) Chromosomal location of zebrafish *cx48.5,* and human and mouse *CX46*. *CX46*/*cx48.5* genes, highlighted in red are syntenic. (*C*) Phylogenetic analysis of zebrafish Cx48.5 and its closest human, mouse, and fish homologs. Phylogenetic tree is built using the clustalW program. Length of horizontal branches is proportional to the evolutionary distance between the proteins. GenBank accession numbers used for the analysis are as follows: human CX46 (NP_068773), mouse CX46 (NP_058671), zebrafish Cx48.5 (NP_997525), mouse CX43 (NP_034418), human CX43 (NP_000156), zebrafish Cx43 (NP_571113), human CX50 (NP_005258), mouse CX50 (AAG42241), human CX40 (NP_005257), mouse CX40 (NP_032147), human CX45 (NP_00548), and mouse CX45 (CAC21557). (*D*) Alignment of mutant zebrafish Cx46 protein to various connexins from zebrafish, mouse, and human reveals that the *s226* mutation results in a change in a highly conserved amino acid residue (red asterisk).

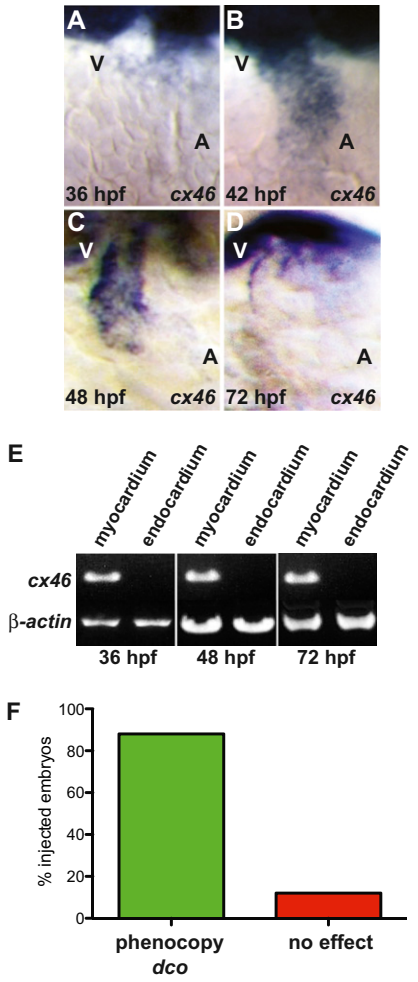


Fig. S3. *cx46* Expression and morpholino knockdown confirms that *cx46* is the affected gene in *dco* mutants. (*A–D*) Whole-mount RNA in situ hybridization reveals *cx46* expression in the heart and lens. Expression of *cx46* at (*A*) 36, (*B*) 42, (*C*) 48, and (*D*) 72 hpf. A, atrium; V, ventricle. (*E*) *cx46* and β-Actin control RT-PCR from FACS-sorted myocardial and endocardial cells at 36, 48, and 72 hpf. (*F*) Morpholino knockdown of *cx46* recapitulates *dco* cardiac phenotype. Of WT embryos injected with an ATG *cx46* morpholino, 88% are indistinguishable from *dco* mutant embryos and display severe impairment of cardiac conduction (*n* = 125/142).

Table S1. *Cx46/Gja3* mRNA orthologs can rescue *dco* cardiac phenotype

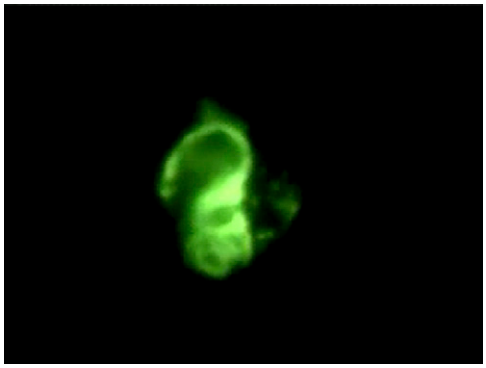
	Analyzed embryos	Heart defects	Genotyped mutants	Rescued hearts	% Rescued	%WT embryos with cardiac defect
capped mRNA WT *cx46*	239	28	62	35	56	0
capped mRNA MT *cx46*	228	84	60	0	0	14
capped mRNA WT *mCx46*	233	47	59	15	25	1
Capped mRNA WT *cx43*	213	55	55	0	0	0
Tg(cmlc2:cx46 WT-GFP)	288	0	74	74	100	0
Tg(cmlc2:cx46 mut-GFP)	271	83	67	0	0	0

Mutant zebrafish *cx46* can cause a cardiac conduction phenotype in WT hearts (% WT embryos with cardiac defects). To determine the number of WT embryos with cardiac defects, we subtracted the number of genotyped mutant embryos from the total number of embryos exhibiting heart defects. The number of WT embryos was calculated by subtracting the number of genotyped mutant embryos from the total number of analyzed embryos. Thus, the % WT embryos with cardiac defects was calculated by dividing the number of WT embryos exhibiting cardiac defects by the total number of WT embryos.

Table S2. Conduction parameters determined by ECG analysis of *Cx46*$^{+/+}$, *Cx46*$^{lacZ/+}$, *and Cx46*$^{lacZ/lacZ}$ mice reveal conduction defects in absence of *Cx46* function

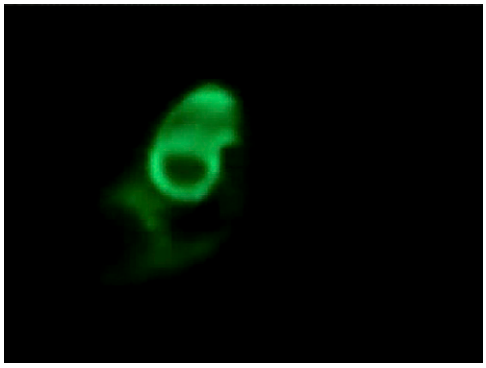
Parameter	*Cx46*$^{+/+}$ (n = 15)	*Cx46*$^{lacZ/+}$ (n = 15)	*Cx46*$^{lacZ/lacZ}$ (n = 15)	P value
PR interval (ms)	40.5 ± 1.0	40.2 ± 1.7	41.2 ± 1.3	0.88
QRS interval (ms)	9.2 ± 0.8	10.2 ± 1.0	14.2 ± 1.2*	0.005
QT interval (ms)	18.6 ± 0.9	19.8 ± 0.9	23.0 ± 1.2*	0.01
Heart rate (beats/min)	504 ± 10	498 ± 9	473 ± 10*	0.05

Mean (±SEM) heart rates and durations of the intervals defined in Fig. 5*D* are shown for the different mice.
*Statistically significant difference between normal (*Cx46*$^{+/+}$ *and Cx46*$^{lacZ/+}$) and mutant (*Cx46*$^{lacZ/lacZ}$) hearts ($P < 0.05$).



Movie S1. A 72-hpf *Tg(cmlc2:GFP)* WT heart. Epifluorescence movie of 72 hpf WT heart reveals that ventricular wall contraction is coordinated. Ventricle is in the foreground and to the left, and atrium is in the background and to the right.

Movie S1



Movie S2. A 72-hpf *dco*^{s226}*; Tg(cmlc2:GFP)* mutant heart. Epifluorescence movie of 72 hpf *dco*^{s226} mutant heart reveals that *dco* ventricular wall contraction is incoordinate. Ventricle is in the foreground and to the right, and atrium is in the background and to the left.

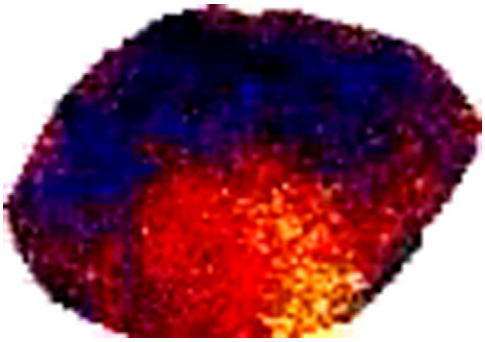
Movie S2

Movie S3. Morpholino *cx46* mutant heart. Brightfield movie of morpholino *cx46* mutant heart. The 48-hpf morpholino *cx46* mutant hearts exhibit uncoordinated ventricular contractions.

Movie S3

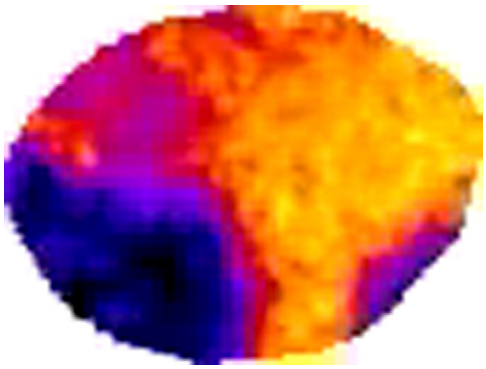
Movie S4. Optical mapping of 60 hpf *Tg(cmlc2:gCaMP)*^{s878} WT hearts reveals linear conduction from the atrioventricular canal to the outflow tract. Calcium activation initiates at the atrioventricular canal (left) and travels across the ventricle to the outflow tract (right).

Movie S4



Movie S5. Optical mapping of 60 hpf *Tg(cmlc2:gCaMP)*^{s878} *dco* mutant hearts reveals aberrant conduction throughout the ventricle. Calcium activation initiates at the atrioventricular canal (*Left*) but fails to conduct uniformly across the ventricle.

Movie S5



Movie S6. Optical mapping of *dco*$^{-/-}$; *Tg(cmlc2:gCaMP)*^{s878} transplanted donor cardiomyocytes in 60 hpf *Tg(cmlc2:gCaMP)*^{s878} WT ventricle reveals disrupted conduction within the ventricle. Calcium activation initiates at the atrioventricular canal (left) but fails to uniformly travel to the outflow tract.

Movie S6