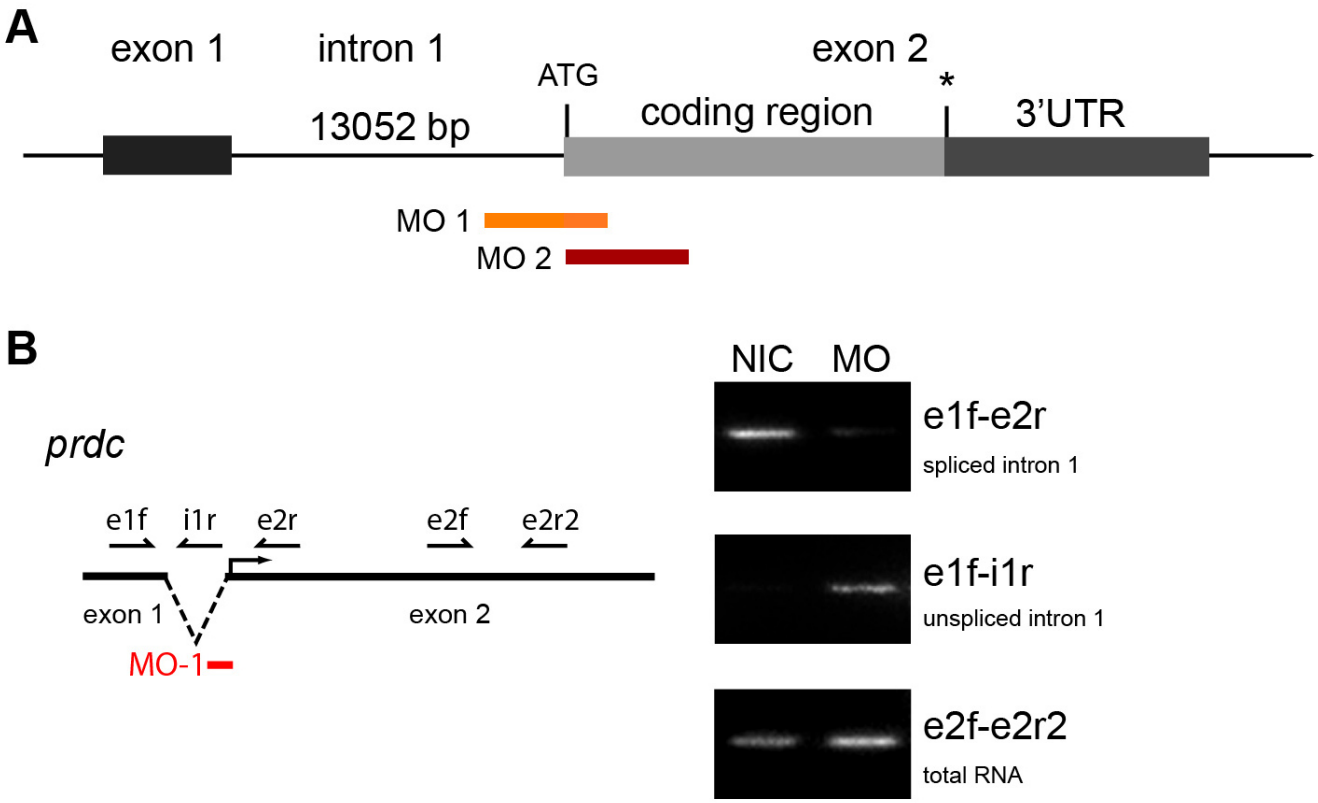
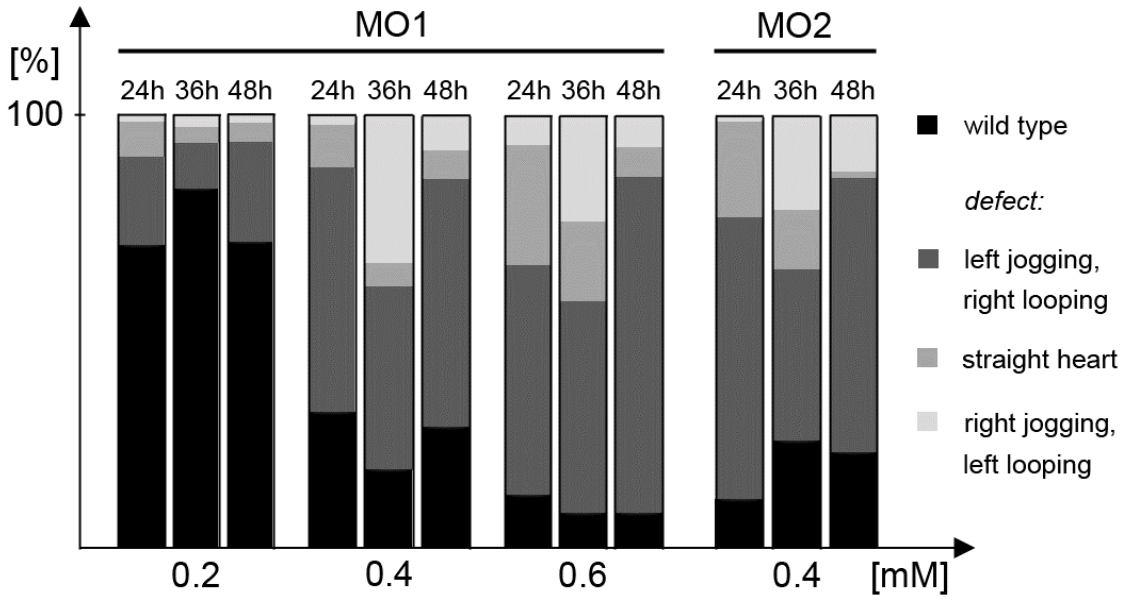


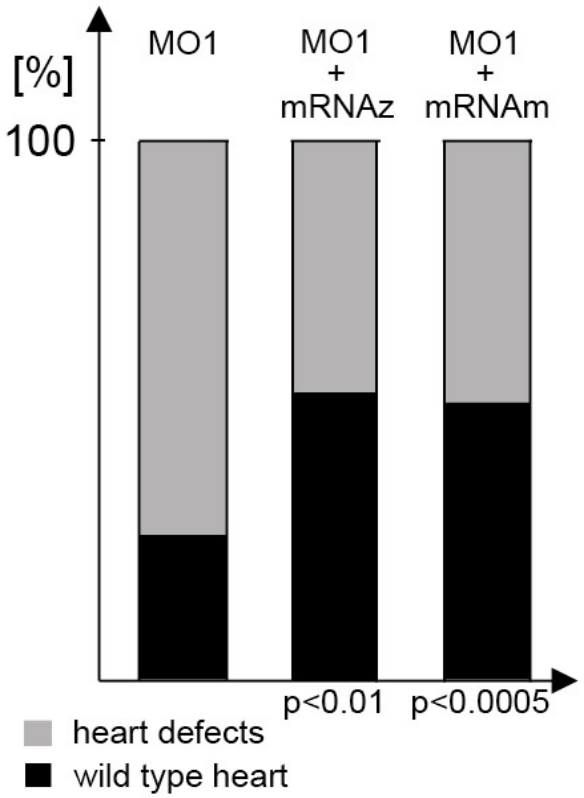
Fig. S1. Design and evaluation of *grem2*-targeting morpholinos. (**A**) Schematic diagram of the positioning of the two anti-*grem2* morpholinos (MO1 and MO2) relative to *grem2* gene structure. MO1 straddles the exon/intron boundary at the 3′ splice site, blocking splicing of the *grem2* transcript. MO2 starts at the beginning of exon 2, the first bases of which contain the initiation of translation ATG codon, blocking synthesis of Grem2 protein. The location of the coding region, stop codon (*) and 3′UTR are indicated. (**B**) MO-1 morpholino effectively blocks splicing of *grem2* as compared to non-injected control embryos (NIC). A set of primers specific for the unspliced (e1f/i1r) and spliced (e1f/e2r) forms of the *grem2*/*prdc* transcripts were used to show that MO-1 blocks splicing of the *grem2* transcript. The primer pair e2f/e2r2 from the 3′UTR area of the *grem2* exon 2 that detects both unspliced and spliced transcripts shows similar levels of *grem2* in both samples. The locations of the different primers and MO-1 within the *grem2* gene locus are indicated in the left diagram. RNA was extracted from ~30 embryos at 24 hour using the TRIzol reagent and 2 µg of total RNA were reverse transcribed to cDNA for PCR. Primer sequences: e1f: 5′ACTGAAGACTCTCAGCGGCT3′; e2r: 5′TGGCTGACCGTCTG-CCGCAA3′; i1r: 5′GATCCTCTGCTTCATCAGAC3′; e2f: 5′AA CACCTCTGACCGCC-GCAC3′; e2r2: TGCGGCCTGCAGAATGCACA.



Quantification of anti-*grem2* morpholino MO1 and MO2 effects on cardiac jogging and looping

	Concen-tration	hpf	WT (%)	Jogging left, looping right (%)	Straight heart (%)	Jogging right, looping left (%)	N: number of experiments n: number of embryos
MO 1	0.2 mM	24 h	67	21	10	2	N = 3, n = 152
		36 h	83	10	2	5	N = 3, n = 183
		48 h	68	27	2	3	N = 4, n = 164
	0.4 mM	24 h	28	55	13	4	N = 4, n = 196
		36 h	16	42	5	37	N = 4, n = 129
		48 h	25	57	7	9	N = 4, n = 169
	0.6 mM	24 h	12	50	31	7	N = 4, n = 198
		36 h	9	45	20	26	N = 4, n = 193
		48 h	9	75	8	8	N = 4, n = 165
MO 2	0.4 mM	24 h	12	60	26	2	N = 4, n = 182
		36 h	27	35	14	23	N = 3, n = 127
		48 h	24	60	4	13	N = 3, n = 178

Fig. S2. Quantification of looping and jogging defects in *grem2* morphants. Phenotypic analysis of *grem2* morphants generated using various concentrations of MO1 and MO2 as indicated at 24, 36 and 48 hour. The two independently designed morpholinos gave comparable results, confirming that the phenotype is specific to loss of Grem2 function. The data for the graph are included in the Table below.



Quantification of the rescue of the *grem2* morpholino MO1 cardiac defects by co-injection of zebrafish or mouse *grem2* mRNA

24 hpf	Concentration	WT (%)	Cardiac defects (%)	*N*, number of experiments *n*, number of embryos
MO1	0.4 mM MO	28	72	N = 4, n = 196
MO1 + zebrafish mRNA	0.4 mM MO + 15 ng/μl mRNAz	50	50	N = 1, n = 42
MO1 + mouse mRNA	0.4 mM MO + 10 ng/μl mRNAm	48	52	N = 3, n = 138

Fig. S3. Confirmation of *grem2* morpholino specificity by mRNA injection rescue experiments. To further test the specificity of the morpholino effects, we tested whether zebrafish *grem2* or mouse *Grem2* mRNA rescues the morphant phenotype. The results showed that both zebrafish *grem2* (mRNAz) and mouse *Grem2* (mRNAm) partially reversed the MO1 morpholino-incurred defects, supporting the notion that the cardiac jogging and looping phenotypes are caused by loss of Grem2 function. The data for the graph are included in the Table below.

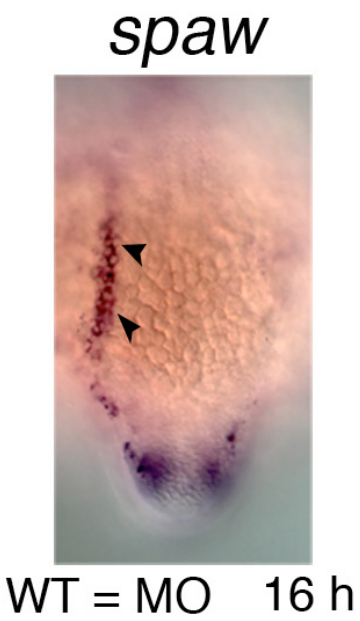


Fig. S4. Loss of Grem2 does not affect early asymmetric gene expression of the nodal gene *spaw* in posterior mesoderm. *Spaw* expression at 16 hours post-fertilization (h) in *grem2* morphants is indistinguishable from wild-types.

Table S1. Phenotypic characteristics of probands and families with GREM2 variants.

Pedigree No.	AF	GREM-2	Age at diagnosis/ enrollment	AF Triggers	AF risk factors
B-II-1	Affected	Heterozygous variant	37/52	Vagal-OSA	OSA Obesity (BMI 43)
A-I-2	Unaffected	Heterozygous variant	**/90	N/A	Unknown
A-II-1	Affected	Heterozygous variant	62/73	Vagal-post-prandial and nocturnal	None
A-II-3	Unaffected	Heterozygous variant	**/72	N/A	HTN
A-II-5	Unaffected	Wild type	**/68	N/A	Unknown
A-II-6	Unaffected	Heterozygous variant	**/67	N/A	Unknown

BMI=body mass index expressed in kg/m^2, N/A= not applicable, HTN=hypertension, OSA= obstructive sleep apnea

Table S2. PCR primer sequences.

Gene	Forward Primer	Reverse Primer
β−Actin	CTACGAGGGCTATGCTCTCCC	CCGGACTCATCGTACTCCTGC
GAPDH	AAGGTGAAGGTCGGAGTCAAC	GGGGTCATTGATGGCAACAATA
Cacna1c	CAGGAGATATTTCCAGATGAGACC	GATCCTTTTGTCGCTTTAGACATT
Gja5	ATAACAGTGGGCAGTTGAACAGCAG	TACCCAATAACGAATGTGGGAGATG
Kcne2	ACGTCATCCTGTACCTCATGGTGAT	GCTTCATGTTTGCCTCTGTTCTCAT
Nppa	GGAGCCTACGAAGATCCAGC	TCCAATCCTGTCAATCCTACCC
Sln	TTGGTAGCCTGAGTGTGCCCCTGCT	TCACGAGGAGCCACATAAGG
ID2	CGACCCGATGAGTCTGCTCTACAAC	GTGTTCTCCTGGTGAAATGGCTGATAAC