Legends to Supplementary Videos

Video S1. Asymmetric cell movements during heart looping in zebrafish

The Tg(tbx5a:eGFP) line shows both the heart tube and late cardiac progenitors contributing to the posterior pole. Embryos were microinjected with Control MO at 1 cell stage and the time-lapse 4D videos were recorded from 28 hpf to 44 hpf taking one picture every 10 minutes. The video shows a ventral view (right side on the left) of surface rendering of a maximum intensity projection. Note the bilaterally asymmetric cell contribution, with more cells from the right side.

Video S2. *prrx1a* knockdown prevents heart looping in zebrafish

Tg(tbx5a:eGFP) embryos were microinjected with *prrx1a*^{MO1} at 1 cell stage and the time lapse 4D videos were recorded from 28 hpf to 44 hpf stage taking one picture every 10 minutes. The video shows images similar to those described in legend to Video S1. Note the decrease in the Tbx5a+ progenitor cells and the absence of heart looping.

Video S3. Down regulation of *prrx1a* produces randomized heart jogging

Tg(tbx5a:eGFP) embryos were microinjected with *prrx1a*^{MO1} at 1 cell stage and the time lapse 4D videos were recorded from 27hpf to 48hpf taking one picture every 10 minutes. The video shows a maximum intensity projection of confocal images. While the jogging is to the left side as in control embryos the heart fails to loop and remains as a straight tube in the midline.

Video S4. Down regulation of *prrx1a* produces randomized heart jogging

Tg(tbx5a:eGFP) embryos were microinjected with *prrx1a*^{MO1} at 1 cell stage and the time lapse 4D videos were recorded from 27hpf to 48hpf taking one picture every 10 minutes. The video shows a maximum intensity projection of confocal images. While the jogging is to the right side the heart fails to loop and remains as an straight tube in the midline.

Video S5. Asymmetric actomyosin forces during heart looping in zebrafish

Tg(tbx5a:eGFP) embryos were microinjected with Control MO plus LifeAct:*RFP* mRNA at 1 cell-stage and the time-lapse 4D videos were recorded from 28hpf to 38hpf stage taking one picture every 10 minutes. The video shows ventral views (right side on the left) of a maximum intensity projection and was generated with a colocalization channel. Note how actomyosin fibres make contact at the posterior pole of the heart on the right side.

Video S6. Prrx1a drives the asymmetric actomyosin forces necessary for the heart looping in zebrafish

Tg(tbx5a:eGFP) embryos were microinjected with *prrx1a*^{MO1} plus LifeAct:*RFP* mRNA at 1 cell-stage and the time-lapse 4D videos were recorded and presented as described in Video S5. Note the disruption of actomyosin fibres and the absence of the cable-like structure upon *prrx1a* downregulation.

Video S7. *prrx1a* regulates cell shape and actomyosin cytoskeleton-Control.

3D reconstruction at 30-32 hpf in a *Tg*(*actb2:myl12.1-mCherry*) zebrafish embryo injected with LifeAct*:GFP* mRNA and control morpholino (Ctrol MO) at 1 cell-stage embryo to visualize F-actin (lifeAct:GFP, green), myosin II (*myl12.1 mCherry*, red) and Prrx1 expression (anti-Prrx1, white).

Video S8. *prrx1a* regulates cell shape and actomyosin cytoskeleton-*prrx1a*^{MO1}.

3D reconstruction at 30-32 hpf in a *Tg*(*actb2:myl12.1-mCherry*) zebrafish embryo injected with LifeAct*:GFP* mRNA and *prrx1a* morpholino (*prrx1a*^{MO1}) at 1 cell-stage embryo to visualize, F-actin (lifeAct:GFP, green), myosin II (*myl12.1-mCherry*, red) and Prrx1 expression (anti-Prrx1, white).

Video S9. Heart looping in zebrafish-control

Tg(tbx5a:eGFP) embryos were treated with vehicle from 26hpf and the time lapse 4D videos were recorded from 26hpf to 44hpf stage taking one picture every 10 minutes. The video shows a maximum intensity projection. Note the proper dextral looping of the heart.

Video S10. Actomyosin inhibition prevents heart looping in zebrafish

Tg(tbx5a:eGFP) embryos were treated with blebbistatin from 26hpf and the time lapse 4D videos were recorded and presented as described for Video S9. Note how upon blebbistatin treatment the dextral looping of the heart is impeded and the heart remains as a straight tube.

Supplementary Table I. Antibodies used in this work. Source and dilution

Primary Antibodies	Source	Catalog Number	Dilution
Anti-GPF-chicken	Aveslab	GFP-1020	1/500
Anti-RFP-mouse	Life technologies	MA515257	1/500
Phalloidin-TRITC	Sigma	P1951	1/50
Phalloidin-FITC	Sigma	P5282	1/50
Anti-Prrx1-rabbit	Tanaka lab	-	1/200
Anti-S46-mouse	Hybridoma bank	-	1/250
Anti-MF-20-mouse	Hybridoma bank	-	1/250
Anti-Islet1-mouse	Hybridoma bank	40.2D6	1/500
Anti-Palladin-mouse	Thermo Fisher Scientific	MA5-16141	1/50
Anti-Fluorescein-POD	Roche	11 426 320 001	1/500
Anti-Histone3	Abcam	AB1791	1/250
Anti-Smad 5-rabbit	Cell Signaling	12534	1/100
Anti-IgG- rabbit	Diagenode	C15410206	1/500
Secondary Antibodies	**Source**	**Catalog Number**	**Dilution**
Alexa Fluor 488 goat anti-chicken	Life technologies	A11039	1/500
Alexa Fluor 488 goat anti-mouse	Invitrogen	A11001	1/500
Alexa Fluor 488 goat anti-rabbit	Invitrogen	A11008	1/500
Alexa Fluor 568 goat anti-mouse	Invitrogen	A11004	1/500
Alexa Fluor 568 goat anti-rabbit	Invitrogen	A11011	1/500
Alexa Fluor 647 goat anti-rabbit	Life technologies	A27040	1/500
Alexa Fluor 647 goat anti-mouse	Life technologies	A31571	1/500

Supplementary Table II. Oligonucleotides used in this work

Name	Sequence 5´-3´	Type
***prrx1a*^{MO1}**	TTTTGTTCTCCAGCACTTACTCTCC	Morpholino
***prrx1a*^{MO2}**	ATATCCTCTACCTACAACCAACATG	Morpholino
***spaw*^{MO}**	TGGTAGAGCTTCAACAGACTCTGCA	Morpholino
***snail1b*^{MO}**	CTCTTAATTTCACTCTCACCAGTCT	Morpholino
Snail1bF	ATGCCACGCTCATTTCTTGT	RT-PCR
Snail1bR	GCGGGACGACTGCATATC	RT-PCR
***Snail1*^{MO1}**	CCAGCACTGTAGGCAACAGGCAGCA	Morpholino
***Snail1*^{MO2}**	GCCAAGTAGCGTCATTACCAGTGTG	Morpholino
gSnai1PF1	GCAAGAAGCCCAACTACAGC	RT-PCR
gSnail1PR1	TGCCCTCATCCTCCTCACTA	RT-PCR
gSnail1PF2	CCCTGTGTCTGCAAGATGTG	RT-PCR
gSnail1PR2	ATGTATCTGCACGGGAAAGG	RT-PCR
zfgapdh-F	GTACGACTCCACCCATGGAA	RT-PCR
zfgapdh-R	CCTGCATCACCCCACTTAAT	RT-PCR
siRNA1-Prrx1	UUGGCUUGUAAAUUGUCCAGGCCGG	siRNA
siRNA2-Prrx1	GGACAAUGAUCAGCUGAAUUCAGAA	siRNA
gPcA13F	TAGGAGCCACACTACCAAACCGAC	sgRNA cloning
gPcA13R	AAACGTCGGTTTGGTAGTGTGGCT	sgRNA cloning
gPcT21F	TAGGACTGCGATCACCGCGAATC	sgRNA cloning
gPcT21R	AAACGATTCGCGTTGATCGCAGT	sgRNA cloning
zfmyl7	AGGGGGAAAACTGCTCAAAG	Probe cloning
zfmyl7	GGCCTTAAACCAAATGTCCA	Probe cloning
zfamhc F	TCAACTGGATGGTGGTGAGA	Probe cloning
zfamhc R	CTCTTGAAGCAACGACACCA	Probe cloning
zfvmhc F	GCTCCTCCTTCCAGACTGTG	Probe cloning
zfvmhc R	CTGCTGCTTGTCATTTTCCA	Probe cloning
caMHC F	ACTGGATGCTGAGACACGAA	Probe cloning
caMHC R	TCAGAGATTGGGGCTGGAAG	Probe cloning
cvMHC F	TGCAGTCCACCCTTGATTCT	Probe cloning
cvMHC R	TCCCTTGACTTGCTCCTCAG	Probe cloning
cPalld F	TGATGGAACTTGCTCACTGC	Probe cloning
cPalld R	GCTTGGTTGTTTGAGGTGGT	Probe cloning
cPrrx2 F	GGAAAAACTTCAGCGTGAGC	Probe cloning
cPrrx2 R	ACCCAAATTGGTCAAATCCA	Probe cloning
cTbx18 F	AGGGGATGAGTGTAGGGTCT	Probe cloning
cTbx18 R	CTTCTTGGAGCGTTCGGATG	Probe cloning
cTbx5 F	GTGGGGACGGAGATGATCAT	Probe cloning
cTbx5 R	GAGCTGGCGTACATACATGC	Probe cloning
cNkx2.5 F	CATGTTGGCCACCTTCAAGC	Probe cloning
cNkx2.5 R	ATGAGTTGTAGGGCTTGGCT	Probe cloning
MmPrrx1 F	GACCATGACCTCCAGCTACGG	Probe cloning
MmPrrx1 R	TCAGTTGACTGTTGGCACCT	Probe cloning
MmPrrx2 F	GCAAGAACTTCTCGGTGAGC	Probe cloning
MmPrrx2 R	GCCTTAGGCACAATCTCAGC	Probe cloning
***GgRS*17F**	ACACCCGTCTGGGCAACGACT	qRT-PCR
***GgRS*17R**	CCCGCTGGATGCGCTTCATCA	qRT-PCR
GgPrrx1F	CACTACCCCGATGCCTTTGTA	qRT-PCR
***GgPrrx1* R**	GAAACTTGGCTCTCCGGTTCT	qRT-PCR
GgPalladin F	GCAGCGCACAACTAACTGTC	qRT-PCR
GgPalladin R	TGGGAAATGGTGGAAATCAT	qRT-PCR
MmEef2 F	AGCGAGGACAAAGACAAGGA	qRT-PCR

MmEef2 R	TACTTCTGTGCAGTGACGGG	qRT-PCR
MmSnail1 F	CAGCTGCTTCGAGCCATAGA	qRT-PCR
MmSnail1 R	TAGGGGAGGTAGGGAAGTGG	qRT-PCR
MmPalladin F	GTCGCGTCAGTTGTACAGGA	qRT-PCR
MmPalladin R	CACTGTCCCGTGATCGAGAG	qRT-PCR
***DrEF1α* F**	GTCAAGGACATCCGTCGTG	qRT-PCR
***DrEF1α* R**	ACCAGGGTGGTTCAGGATG	qRT-PCR
***Drprrx1a* F**	AGTCTCCGGGACTCACCAG	qRT-PCR
***Drprrx1*a R**	TCTCCTCTGCGTTGAGCTG	qRT-PCR
QPCR-DF	TTAAATGCATGGAGAGGCTCG	QPCR-ChIP
QPCR-DR	ACCGGGCCCCCTAAGAC	QPCR-ChIP
QPCR-PF	CCCTAGCAGCTCTCCGTTGG	QPCR-ChIP
QPCR-PR	CTCTCCATGGCGTGGGCATA	QPCR-ChIP
QPCR-CRF	GCATCATCAGGCCATTCGGT	QPCR-ChIP
QPCR-CRR	CCCAAGAGTTTGGTGACCCTA	QPCR-ChIP
GgDF	TGCTCCCTTATATTAATACCCATC	Gg Distal Cloning
GgDR	GCAAAGGAGACTGAAAGACAGG	Gg Distal Cloning
GgPF	CCTCTCTCTCCCCTCCGG	Gg Prox. Cloning
GgPR	GCAGGTGACTCACGGAAAAG	Gg Prox. Cloning
Distal Mut F	gctcggggaagcacatctgttttcaatcGAATTCActttgatggcatcaaat gttctttcccc	Mutagenesis
Distal Mut R	ggggaaagaacatttgatgccatcaaagTGAATTCgattgaaaacaga gtgcttccccgagc	Mutagenesis
Prox Mut F	gagcgggggcggtggAATTCAgggggggcgagggca	Mutagenesis
Prox Mut R	tgccctcgccccccTGAATTccaccgcccccgctc	Mutagenesis

Supplementary Table III. Gene probes and reporters used in this work.

Name	Description	Structures Detected	Type of probe
caMHC	myosin, heavy chain 7, cardiac muscle	atrial marker	in situ probe
cNkx2.5	NK2 homeobox 5	Probe for endogenous expression of Nkx2.5	in situ probe
cPALLD	palladin, cytoskeletal associated protein	Probe for endogenous expression of Palladin	in situ probe
cPrrx1	paired related homeobox 1	Probe for endogenous expression of Prrx1	in situ probe
cPrrx2	paired related homeobox 2	Probe for endogenous expression of Prrx2	in situ probe
crestin	crestin	pan neural crest marker	in situ probe
cTbx18	T-box 18	cardiac posterior pole marker	in situ probe
cTbx5	T-box 5	Probe for endogenous expression of Tbx5	in situ probe
cvMHC	myosin, heavy chain 15	ventricular marker	in situ probe
Islet1	ISL LIM homeobox 1	cardiac posterior pole marker	Antibody
MF20	myosin, heavy chain 1E, skeletal muscle	cardiomyocyte marker	Antibody
mPrrx1	paired related homeobox 1	Probe for endogenous expression of Prrx1	Antibody
mPrrx2	paired related homeobox 2	Probe for endogenous expression of Prrx2	in situ probe
Palld	palladin, cytoskeletal associated protein	Probe for endogenous expression of Palladin	Antibody
Prrx1	paired related homeobox 1	Probe for endogenous expression of Prrx1	Antibody
S46	myosin, heavy chain 15	atrial marker	Antibody
snail1a	snail family zinc finger 1a	Probe for endogenous expression of snail1a	in situ probe
snail1b	snail family zinc finger 1b	Probe for endogenous expression of snail1b	in situ probe
zfamhc	myosin, heavy chain 6, cardiac muscle, alpha	atrial marker	in situ probe
zfltbp3	latent TGF-β binding protein-3	anterior SHF marker	in situ probe
zfmyl7	myosin light-chain 7	cardiac myosin marker	in situ probe
zfpitx2c	paired-like homeodomain 2	Probe for endogenous expression of pitxc2	in situ probe
zfprrx1a	paired related homeobox 1a	Probe for endogenous expression of prrx1a	in situ probe
zfvmhc	ventricular myosin heavy chain	ventricular marker	in situ probe
Kaede	photoactivable fluorescent protein	Photoactivable reporter for cell tracing	Fluorescent Protein
LifeAct	17-amino-acid peptide fusion to GFP or RFP	Reporter for filamentous actin, stress fibers	Fluorescent Probe
Phalloidin	phallotoxin	Reporter for polymeric F actin, stress fibers	Fluorescent Probe
tdTomato	tandem dimer Tomato	Reporter for CRE driven recombination in mouse	Fluorescent Reporter
tbx5a:eGFP	T-box 5a	Reporter for endogenous expression of Tbx5a	Fluorescent Reporter
Myl12.1-mCherry	myosin, light chain 12, genome duplicate 1	Reporter for non-cardiac myosin II	Fluorescent Reporter

Supplementary Table IV. Abbreviations used in this work

Abbreviation	Full Name
A	atrium
AIP	anterior intestinal portal
CF	cardiac folds
C	coelom
DSpLM	dorsal splanchnic lateral mesoderm
E	eyes
EC	ectoderm
FG	foregut
HT	heart tube
IFT	inflow tract
LPM	lateral plate mesoderm
LSH	left sinus venosus horn
LVV	left vitelline vein
NC	neural crest
N	notochord
NP	neural plate
NT	neural tube
OFT	outflow tract
P	pericardium
PHT	primary heart tube
PLPM	posterior lateral plate mesoderm
PP	posterior pole
PV	pericardial vesicle
RSH	right sinus venosus horn
RVV	right vitelline vein
S	somite
SHF	secondary heart field
SH	sinus venosus horns
SoLM	somatic lateral mesoderm
SpLM	splanchnic lateral mesoderm
SV	sinus venosus
V	ventricle
VP	venous pole
VSpLM	ventral splanchnic lateral mesoderm
VV	vitelline veins
YS	yolk sac