Supplementary figure S1-12

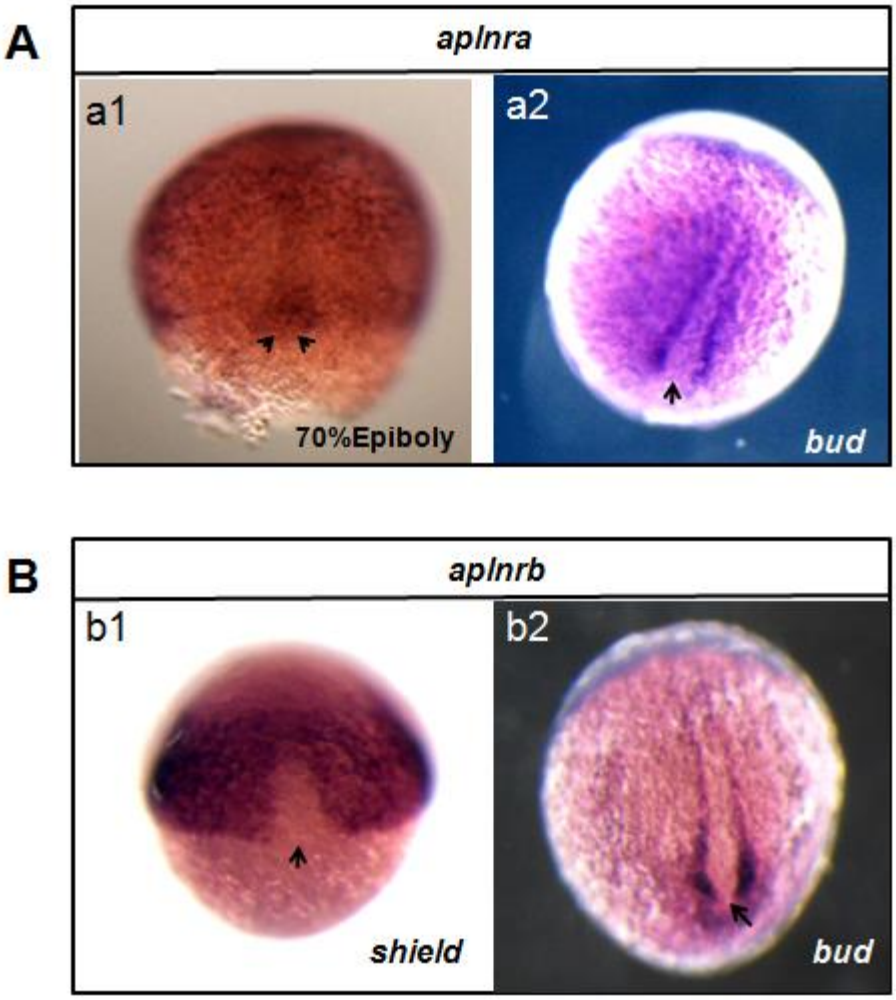

Fig.S1 The expression pattern of *aplnra/aplnrb* at gastrulation stage and bud stage (A) The expression pattern of *aplnra*. *aplnra* is expressed ubiqiuously at 70% epiboly, it is also expressed in DFCs(a1, arrow head). At bud stage, it is expressed in the cells near midline while not in KV progenitors(a2, arrow). (B) The expression pattern of *aplnrb*. *aplnrb* is not expressed in the DFCs at shield stage (b1, arrow), the expression pattern is similar to that of *aplnra* at bud stage (b2).

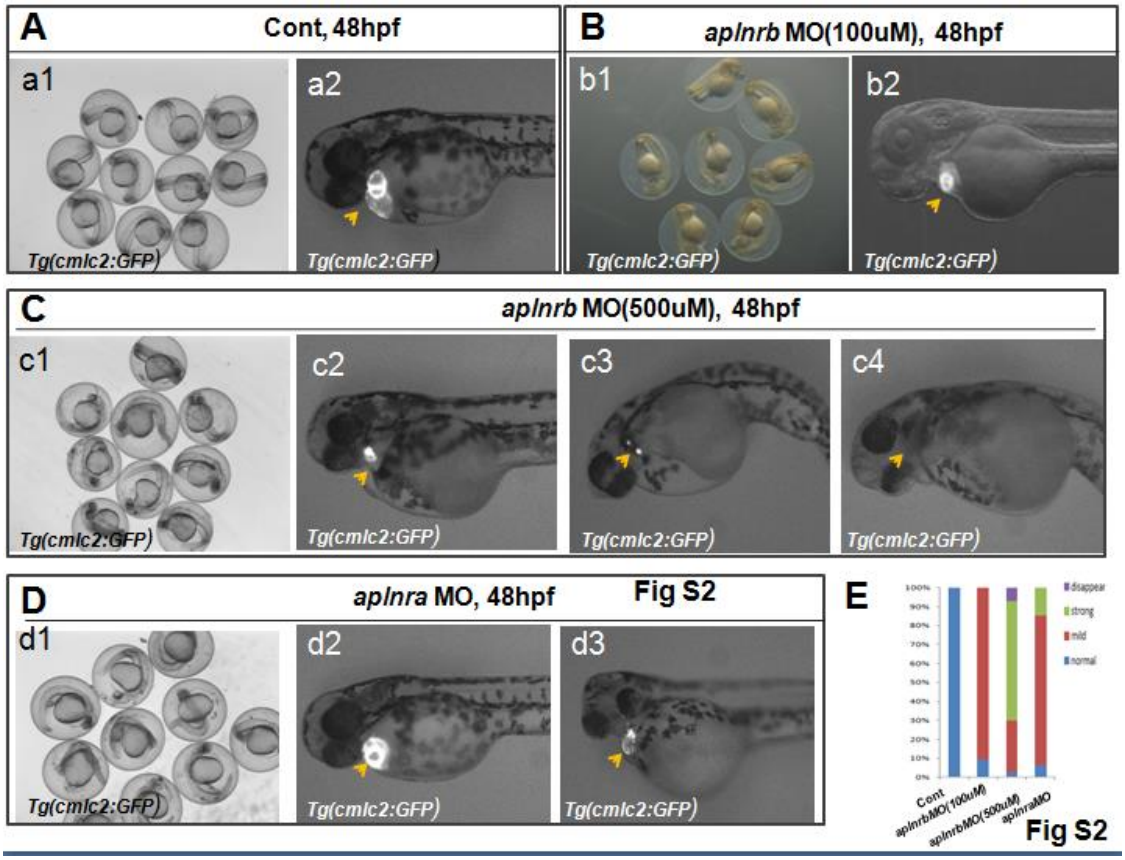

Figure S2: The heart phenotype for *aplnrb* or *aplnra* loss of function

(A-E) The heart development in control and *aplnra/b* morphants. Compared with the control morphants (A. a2, E, 100%, n=57), the heart in most of embryos injected with *aplnrb* MO(100uM) displayed mild smaller (B. b2, E, 89.3%, n=103), while in most of *aplnrb* morphants(500uM), the heart became very small (C. c3, E, 63.6%, n=99) or the heart disappeared (C. c4, E, 27.3%, n=99), only small part of embryos had the mild smaller heart (C. c2, E, 7.1%, n=99). For *aplnra* morphants, the phenotype was not strong, majority of embryos displayed mild smaller heart (D.d2, E, 78.7%, n=108), only smaller number of embryos displayed very small heart (D. d3, E, 14.8%, n=108).

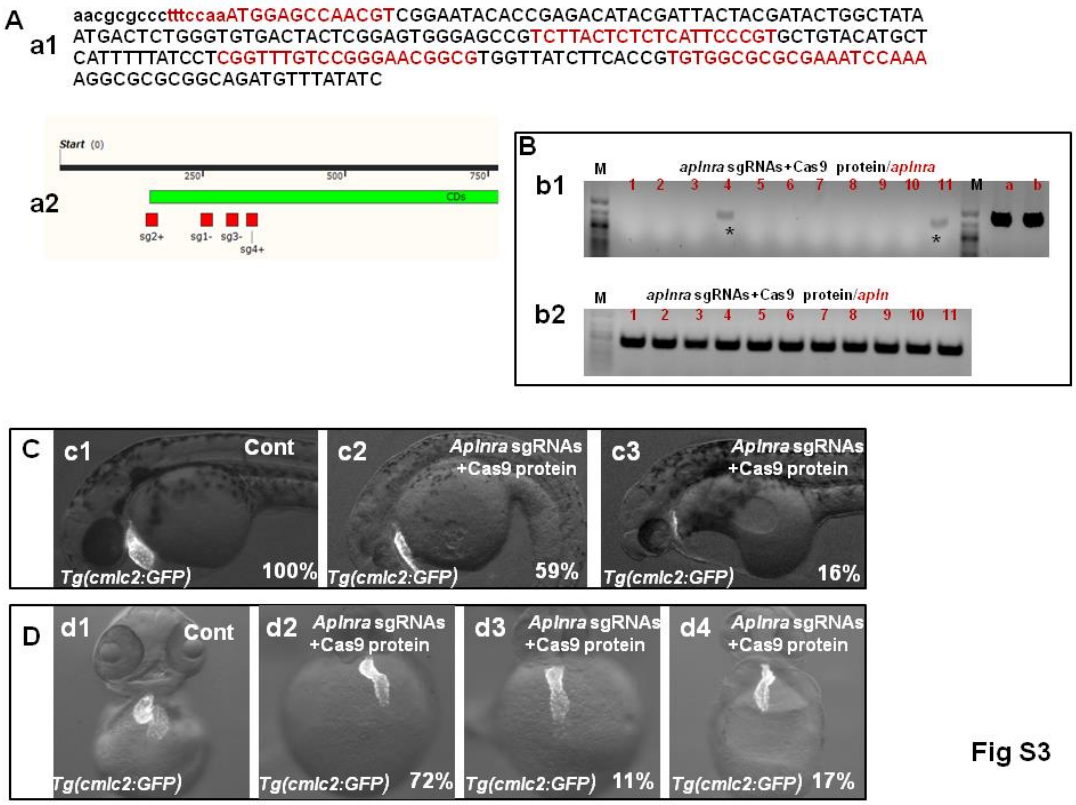

Fig S3

Figure S3 The heart phenotype of embryos injected with *aplnra* sgRNAs and Cas9 protein

(A. a1-a2) The four target points for *aplnra* gene. a1 showed the detailed sequence for each targeting point in the genomic DNA. a2 showed the location of the target points in the genome map. (B. b1-b2) The efficiency of genome editing using CRISPR/Cas9 system. The sorted embryos injected with *aplnra* sgRNAs and Cas9 protein were used to prepare the genomic DNA, PCR experiment showed that the genomic DNA in control embryos was intact (B. b1, line a and b), while the genomic DNA was edited successfully in nearly all the embryos (88.9%, n=54), 11.1% of embryos' genomic DNA was not completely edited (cutting off part of target genome) (B. b1, line 1-11, stars showed, n=27, amplified with *aplnra* primers). Using the genomic DNA from the embryos injected with sgRNAs and Cas9 protein as templates, the *apln* can be amplified (B. b2, line1-11), meaning all the templates worked well. (C) The heart progenitor number decreased in most of embryos injected with *aplnra* sgRNAs and Cas9 protein (c2 showed the embryos with mild decreased heart number, c3 showed the embryos with heart number decreased greatly.) (D) The heart left right asymmetry defect in embryos with mild heart number decreased (D. d2-d4, n=89).

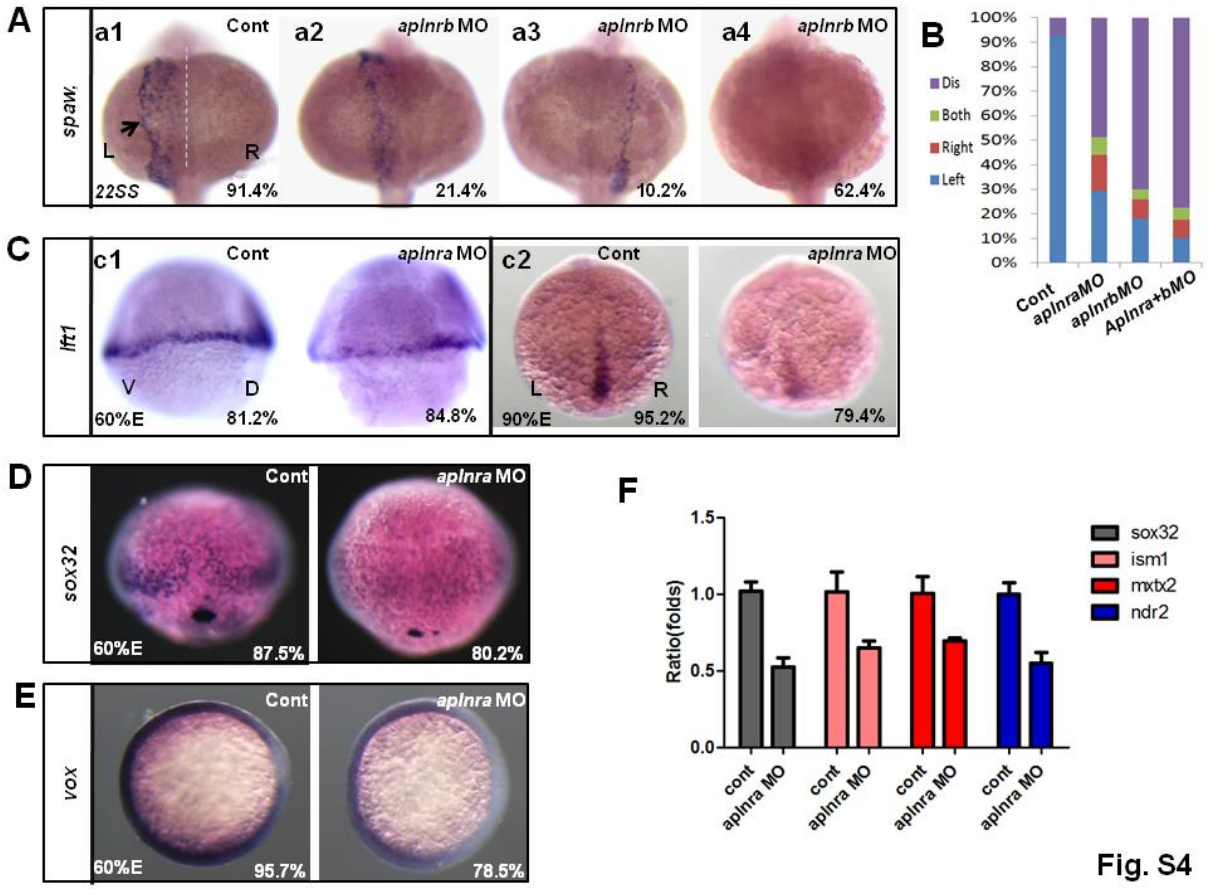

Fig.S4 The expression of nodal related genes in *aplnra* morphants

(A-B) The expression of *spaw* in control embryos and *aplnra* morphants. In control, 91.4% of embryos displayed left-sided *spaw*(A. a1 and B, n=93), 21.4%, 10.2% and 62.4% of embryos injected with *aplnrb* MO(500uM) displayed left-sided *spaw*, right-sided *spaw* and *spaw* disappeared (A. a2-a4 and B, n=98). (C) Compared with control embryos, at 60% epiboly, 84.8% of embryos injected with *aplnra* MO displayed mild decreased *lft1* expression (Cc1, n=33), at 95% epiboly, 79.4% of embryos injected with *aplnra* MO displayed greatly decreased *lft1* expression (Cc2, n=33). (D) The expression of *sox17* indicated that the transcription level of *sox17* was down-regulated in *aplnra* morphants (80.2%, n= 26), most of control embryos displayed high level of *sox17* (87.5%, n=24). (E) In control, 95.7% of embryos displayed high level of *vox* expression (n=23), while *vox* was down-regulated in 78.5% of embryos injected with *aplnra* MO (n=28). (F) Q-PCR analysis for the nodal related genes *sox32*, *ism1*, *ndnr2* and *mxtx2* in control and *aplnra* morphants. The result indicated

that all the four genes were down-regulated in *aplnra* morphants.

Note: L, Left; R, Right; D, Dorsal; V, Ventral; Three repeated experiments were done for Q-PCR. *, P<0.05; **, P<0.01; NS, not significant difference.

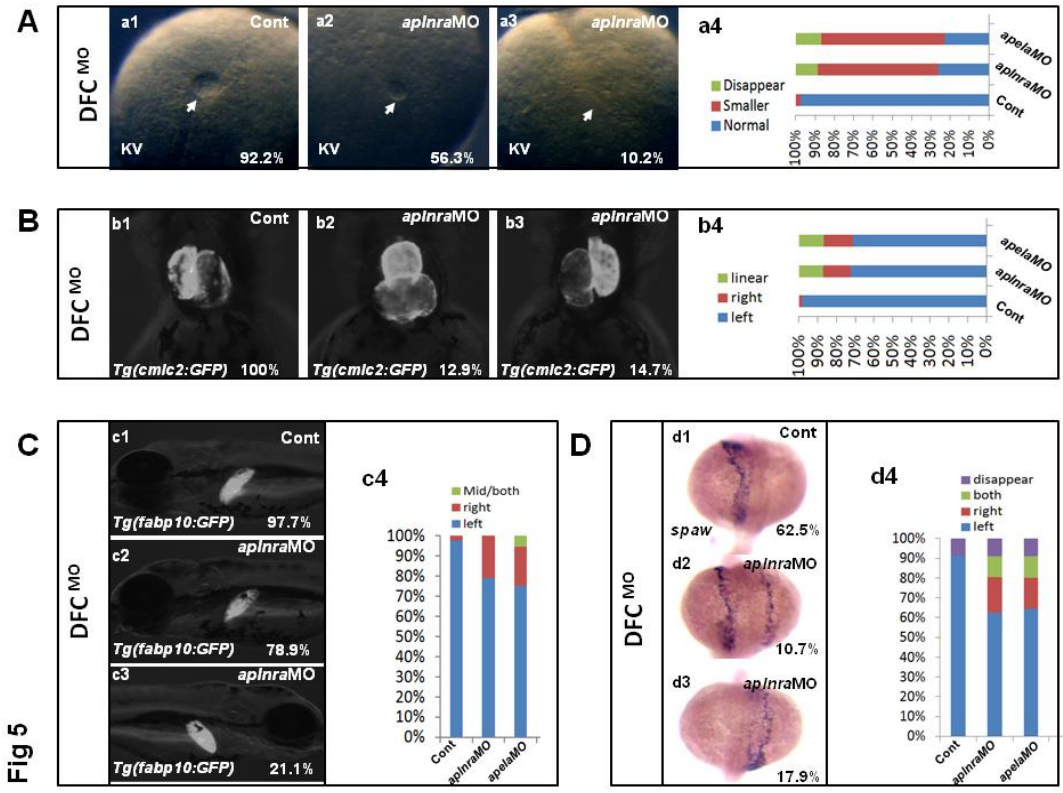

Fig.S5 *aplnra* or *apela* loss of function in DFCs resulted in LR patterning defect.

(A. a1-a4) The KV phenotype in control and *aplnra or apela* loss of function in DFCs. At 10 somite stage, 56.3% and 10.2% of embryos injected with *aplnra* MO in DFCs displayed smaller KV and disappeared KV(A. a2-a4, n=119), 57.6% and 12.0% of embryos injected with *apela* MO in DFCs displayed smaller KV and disappeared KV (A. a4, n=90), while 92.2% of control embryos displayed normal KV (A. a1 and a4, n=103). (B. b1-b4) At 60hpf, part of embryos injected with *aplnra* MO displayed linear heart (B. b2 and b4, 14.7%, n=116) and reversed heart (B. b3 and b4, 12.9%, n=116), 15.5% and 13.1% of embryos injected with *apela* MO^{DFCs} displayed reversed heart and linear heart (B. b4, n=90). (C. c1-c3) Liver LR defect was also observed in *aplnra* MO (total 21.1%, n=95) or *apela* MO (total 24.8. %, n=90) injected embryos in DFCs (C. c3 and c4). (D. d1-d4) *spaw* expression was examined in embryos, right-sided and both-sided *spaw* was discovered in 17.9% and 10.7% of embryos

with *aplnra* loss of function in DFCs (D. d2-d4, n=56) or 15.5%, 11.1% and 8.9% of embryos displayed right-sided, both-sided and disappeared *spaw* with *apela* loss of function in DFCs(Dd4, n=56)

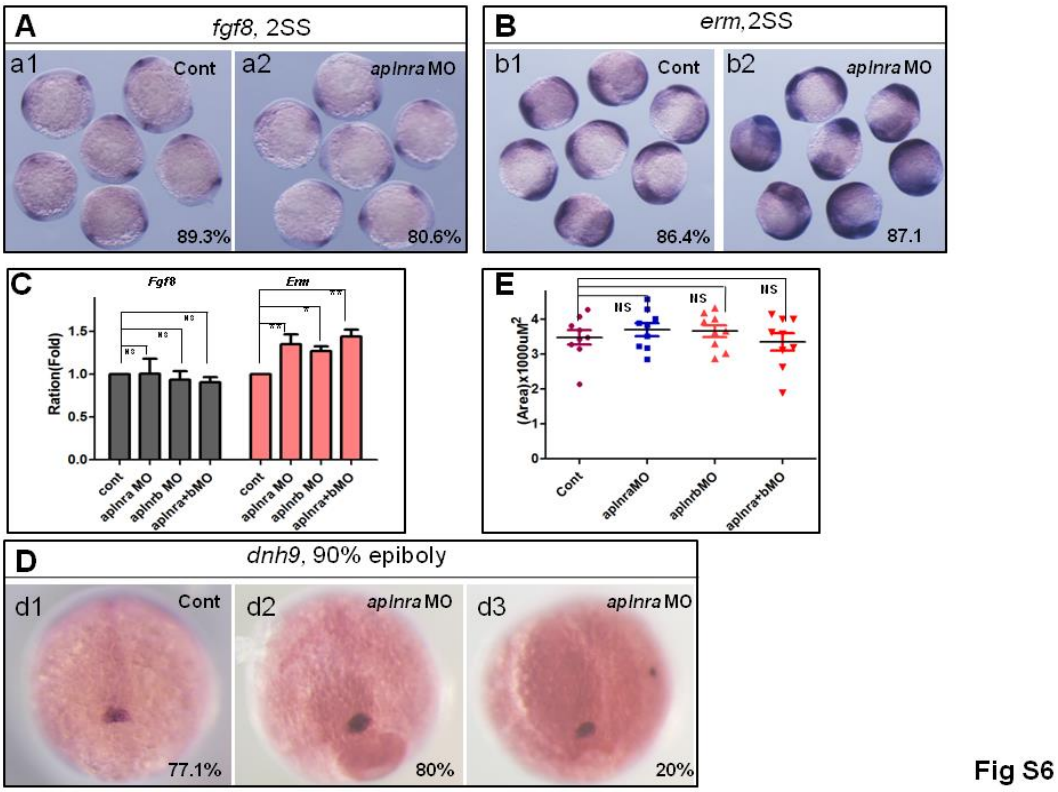

Fig S6

Figure S6: The expression of some critical regulators being related to LR patterning.

（A, B）The expression of *fgf8* and *erm*. No difference was observed for *fgf8* expression in control embryos and *aplnra* morphants at 2 somite stage (A. a1-a2, n=36). But *erm*, the downstream gene of *fgf8*, was up regulated in *aplnra* morphants (B. b1- b2, n=31). (C) The expression of *fgf8* and *erm* in the control embryos, *aplnra* morphants, *aplnrb* morphants and *aplnra+b* morphants were checked by Q-PCR. The result demonstrated that, among them, no difference was observed for *fgf8* expression, but all the morphants displayed increased expression of *erm*. (D, E) The expression of *dnah9* in embryos treated differently . Compared with that in control (D. d1, n=35), the expression of *dnh9* was not changed greatly in *aplnra* morphants (D. d2, d3, n=30), this result was confirmed by measuring the area in control embryos and *aplnra* morphants (E). The expression of *dnh9* was also checked in *aplnrb* and *aplnra+b* morphant, no distinct difference was observed among these embryos (E). Note: *, $P<0.05$; **, $P<0.01$; NS, not significant difference.

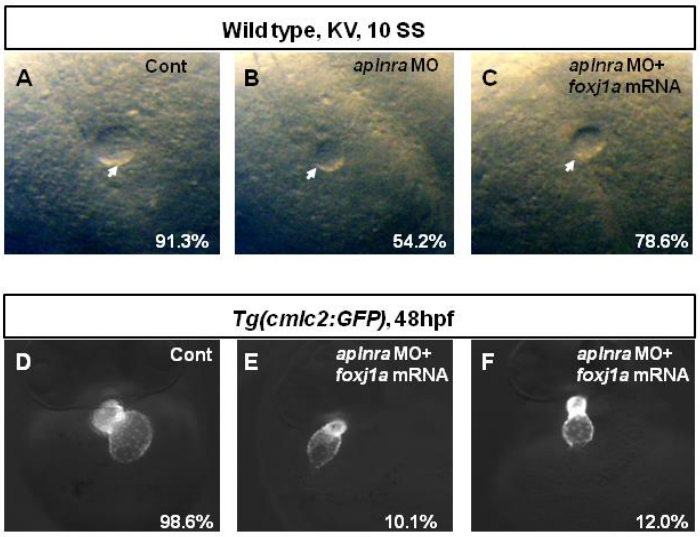

Fig.S7

Fig.S7 *foxj1a* mRNA partially rescued the LR patterning defect in *aplnra* morphants.

(A-C) The KV phenotype in different cluster of embryos. The KV in control embryos were normal (A, arrow, n=181), the KV in *aplnra* morphants were smaller than those in controls (B, arrow, n=213). But in embryos injected with *aplnra*MO and *foxj1a* mRNA, most of KVs were bigger than those in *aplnra* morphants (C, arrow n=168). (D-F) The heart LR patterning in control embryos and rescued embryos. Compared with that in control (D, n=72), very low ratio of embryos injected with *aplnra*MO and *foxj1a* mRNA displayed reversed heart (E, n=158) and linear heart (F, 5 n=158).

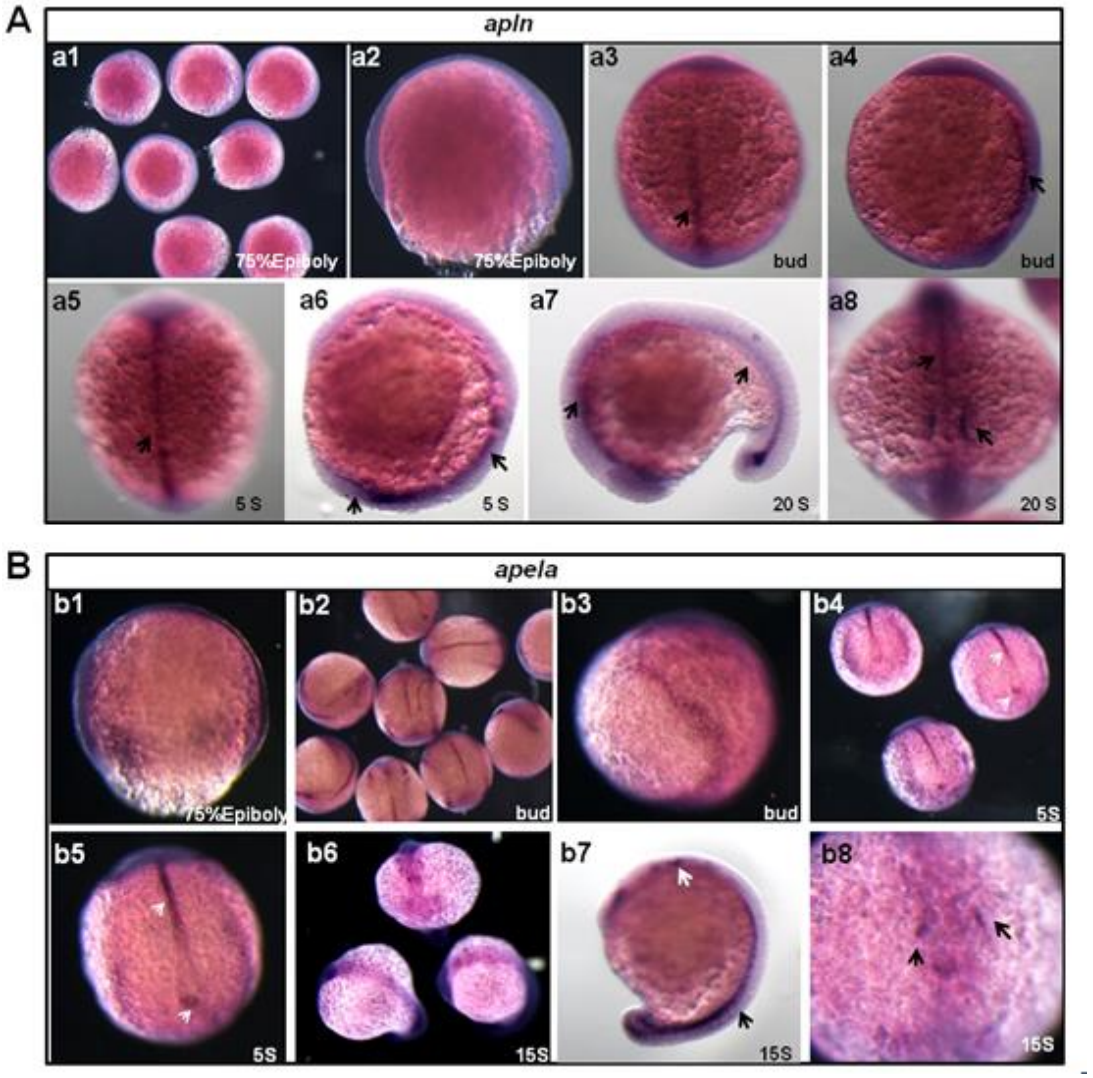

Fig.S8 The expression of *apln* and *apela* at different stages.

(A. a1-a8) The expression of *apln* from 75% E to 20 SS. At 75% E, *apln* was not expressed in the embryos (A. a1-a2). At bud to 5 SS, *apln* was expressed in the midline (A. a3-a6, arrow showed). At 20SS, *apln* was expressed in midline (A. a7, right arrow; a8, up arrow) and heart progenitor field (A. a7, left arrow; a8, down arrow). (B. b1-b8) The expression of *apela* from 75% E to 15 SS. At 75% E, *apela* was expressed ubiquitously (B. b1). At bud stage, *apela* was expressed in the midline and presomite mesoderm (PSM) (B. b2-b3). At 5SS, *apela* was expressed in the midline and KV epithelium (B. b4-b5, arrow head). At 15SS, *apela* was expressed in midline and heart progenitor field (B. b6-b8, arrow).

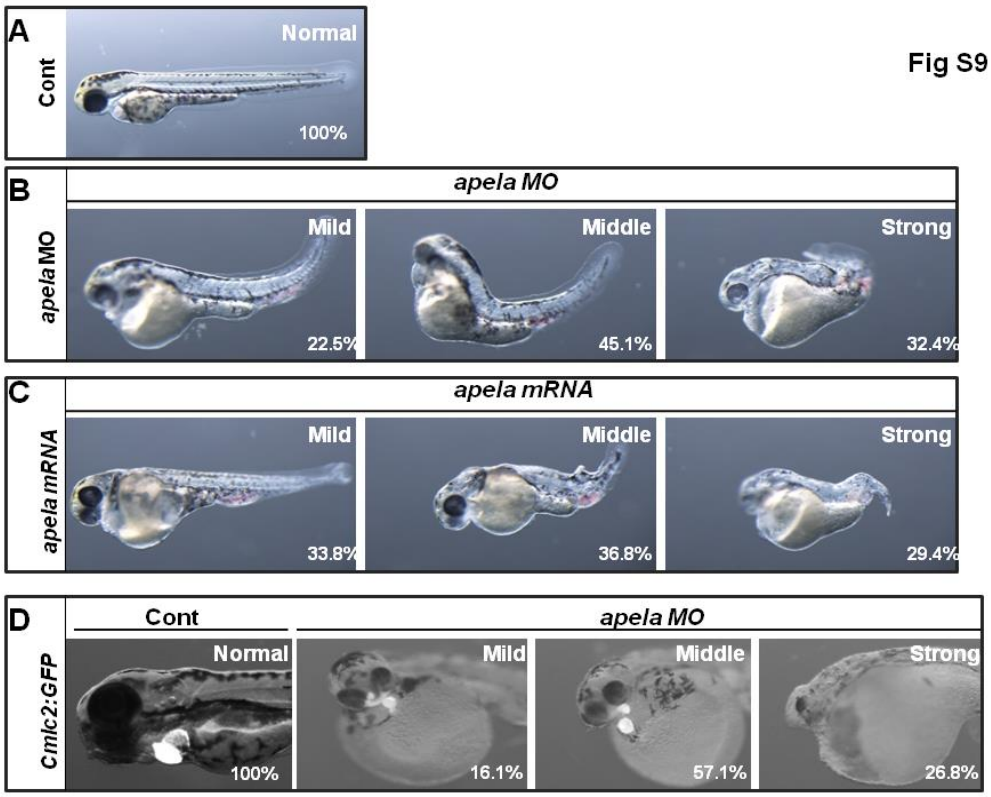

Fig S9

Figure S9: The similar phenotypes between embryos injected with *apela* mRNA or *apela* MO (A-C) The living embryos injected with control MO, *apela* MO or *apela* mRNA. At 2 days post fertilization, embryos injected with control MO developed normally (A, 100%, n=45). The embryos injected with *apela* MO or *apela* mRNA had the similar phenotype, displayed mild (22.5% in *apela* MO, n=71; 33.8% in *apela* mRNA, n=68), middle (45.1% in *apela* MO, n=71; 36.8% in *apela* mRNA, n=68) and strong phenotype (32.4% in *apela* MO, n=71; 29.4% in *apela* mRNA, n=68) (B, C). Meanwhile, the heart cell number was also decreased greatly in *apela* MO injected embryos (D, n=56).

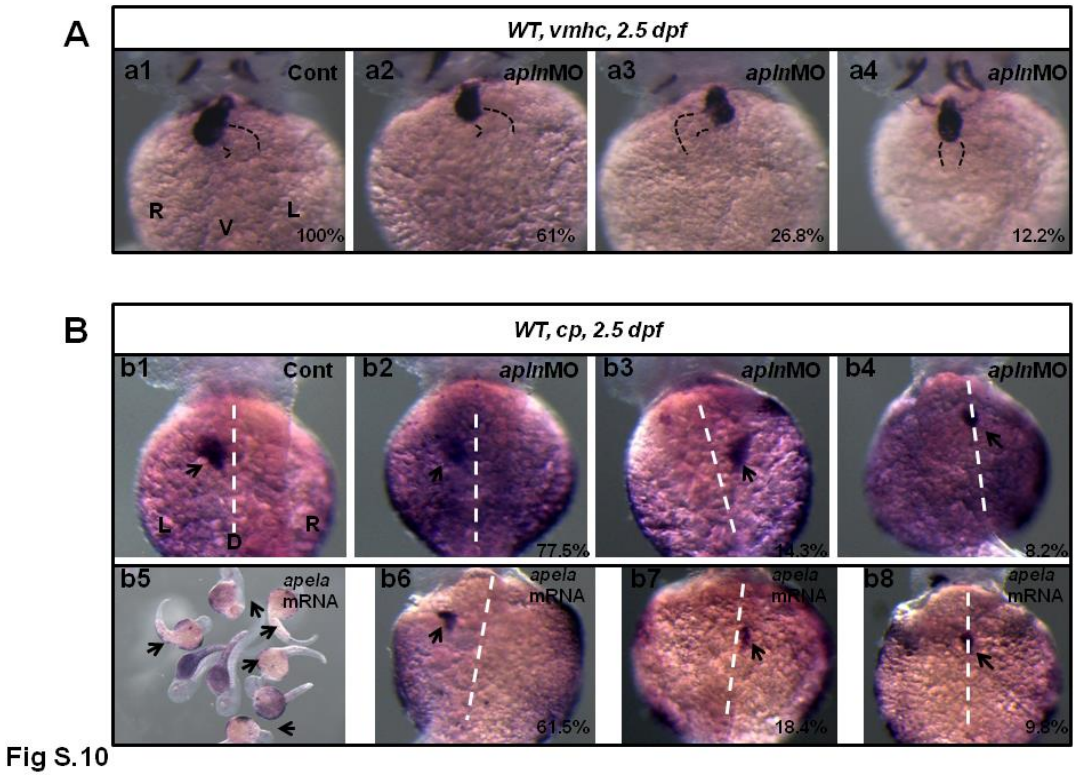

Fig S.10

Fig.S10 The organ LR patterning defect in *apln* morphants and *apela* mRNA injected embryos.

(A. a1-a4) The heart LR patterning in control and *apln* MO injected embryos. In control embryos, 100% of embryos displayed normal heart loop (n=112). In *apln* morphants, 61%, 26.8% and 12.2% of embryos displayed normal, reversed and linear heart (A. a2-a4, n=41). For liver LR patterning, 97.3% of control embryos displayed left liver (B. b1, n=112). In *apln* morphants, 77.5%, 14.3% and 8.2% of embryos displayed left-sided, right-sided and middle/both sided liver (B. b2-b4, n=49). In embryos injected with *apela* mRNA, most of them displayed abnormal development (B. b5, arrow showed), 61.5%, 18.4% and 9.8% of embryos displayed left-sided, right-sided and middle/both sided liver (B. b6-b8, n=103).

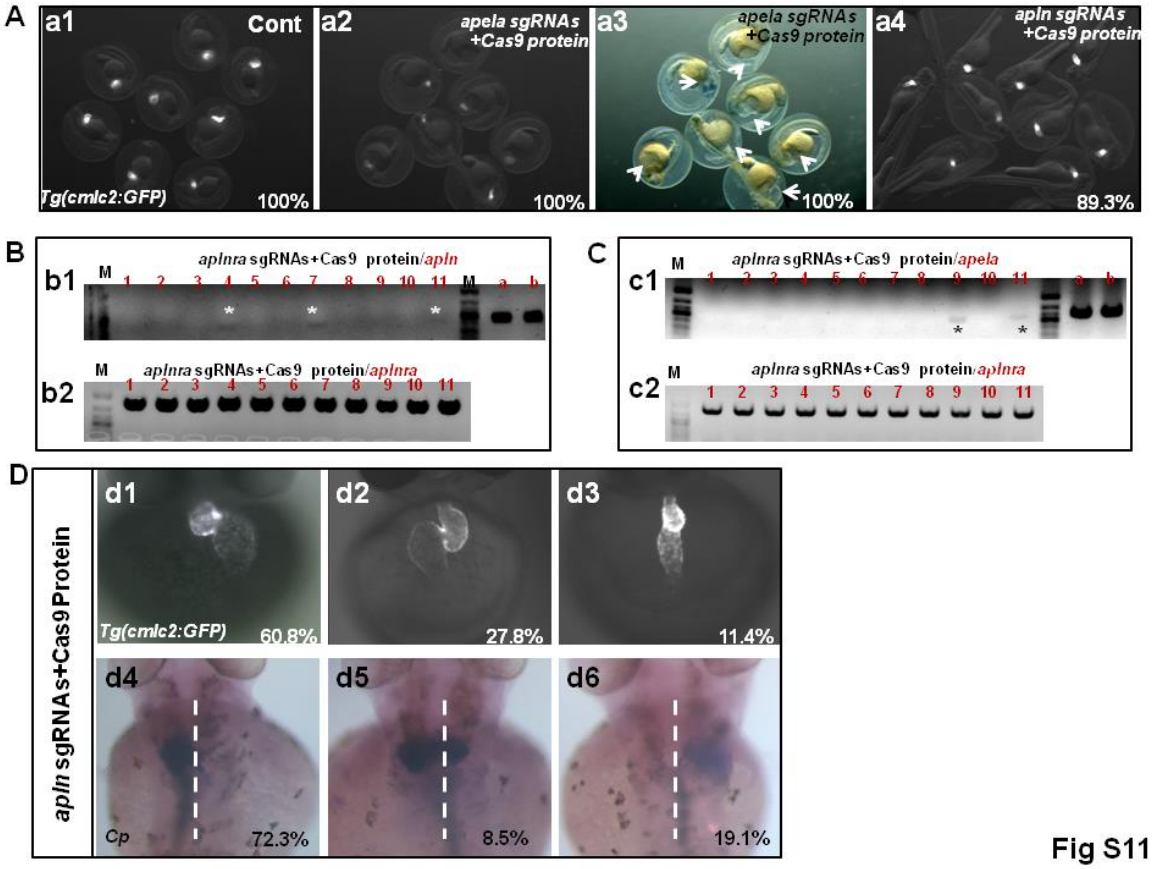

Fig S11

Fig.S11 The phenotype in embryos co-injected with *apela/apln* sgRNAs and Cas9 protein.

(A. a1-a4) The heart phenotype in living embryos co-injected with *apela/apln* sgRNAs and Cas9 protein. In control embryos, 100% of them had normal heart (A. a1, n=107). In embryos co-injected with *apela* sgRNAs and Cas9 protein, all the embryos displayed very smaller heart or heart disappear (A. a2, 100%, n=136), meanwhile all the embryos displayed intumescent cardiocoelom (A. a3, arrow head, 100%, n=136. Note that a2 and a3 are the same sample). In embryos co-injected with *apln* sgRNAs and Cas9 protein, no difference about heart size was observed (89.3%, n=141). (B, C) *apela* and *apln* genomic DNA was edited by co-injected Cas9 protein and sgRNAs. (D. d1-d6) The heart and liver LR patterning defect in embryos injected with Cas9 protein and *apln* sgRNAs. There were 60.8%, 27.8% and 11.4% of the embryos showed normal looped heart, reverse looped heart and linear heart (D. d1-d3, n=79), 72.3%, 8.5% and 19.1% of them displayed left-sided, middle and right-sided liver (D.

d4-d6, n=49).

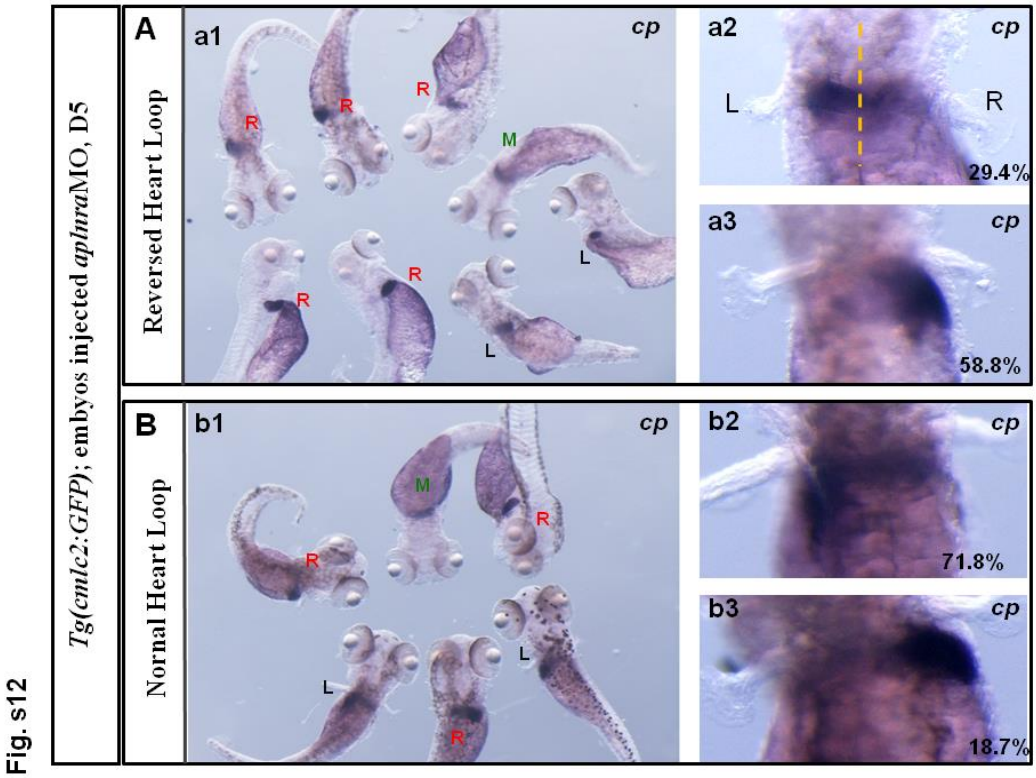

Fig.S12 The heart and liver LR patterning defect in *aplnra* morphants did not occur at the same time.

(A. a1-a3) The liver LR patterning for the embryos displayed reversed loop heart in *aplnra* morphants. Of them, 29.4% of embryos displayed left-sided liver (A. a2, n=17); 58.8% of the embryos displayed right-sided liver (A. a3, n=17). (B. b1-b3) The liver LR patterning for the embryos displayed normal loop heart in *aplnra* morphants. Of the embryos, 71.8% of the embryos displayed left-sided liver (B. b2, n=32); 18.7% of embryos displayed right-sided liver (B. b3, n=32).