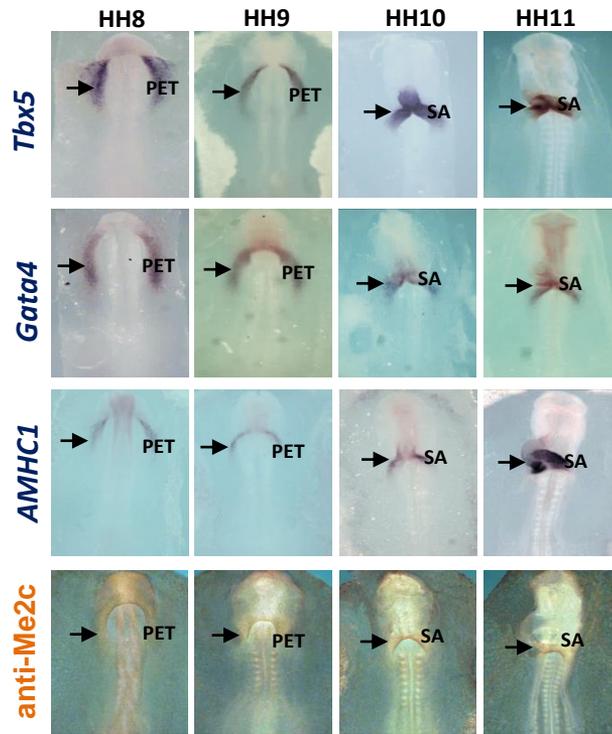
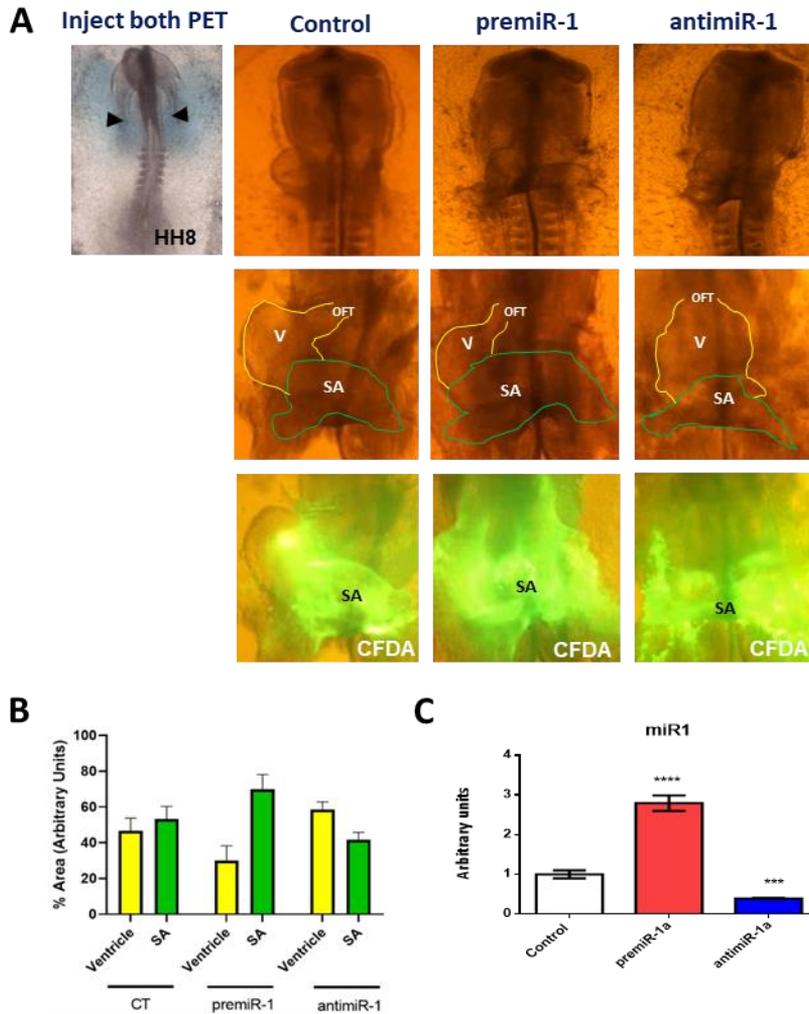


## Supplementary Figure S1



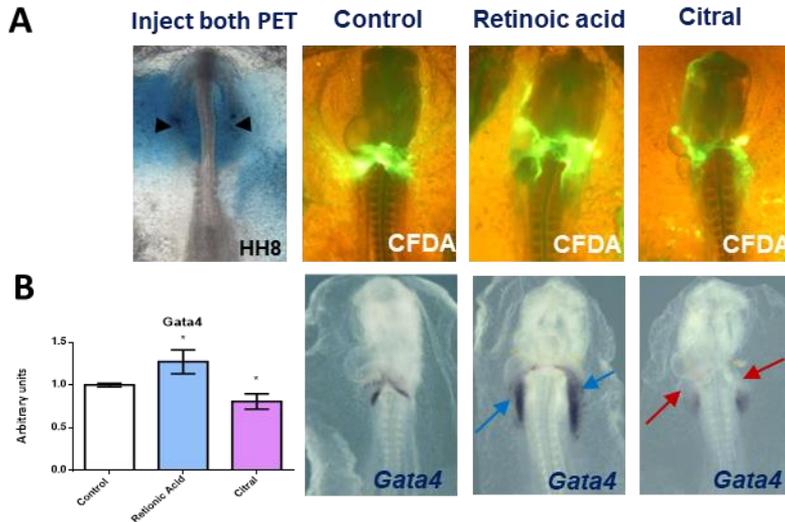
**Supplementary Figure S1. A:** Whole-mount ISH for *Tbx5*, *Gata4* and *AMHC1* and whole-mount IMH for *Mef2c* during early chick cardiac development, from HH8 through HH11, in control embryos. Note *Tbx5*, *Gata4* and *AMHC1* expression pattern in both primitive endocardial tubes (PET), being observable in sinoatrial (SA) region at later stages. Note the location of *Mef2c* in the PET and, subsequently, in the SA region. Arrows: positive stains.

## Supplementary Figure S2



**Supplementary Figure S2. A:** Embryo microinjected with CFDA (control), premiR-1 or antimiR-1, at the level of the prospective atrial cells of both primitive endocardial tubes (arrowheads) and light microscopy of control and experimental embryos (HH11) subjected to miR-1 gain- and loss-of-function. Note in the experimental embryos and dissected cardiac *asa* (HH11) the morphological alterations based on an expansion of the posterior domain of the heart after miR-1 administration, concomitantly with a reduced ventricle region. The opposite effects are observed after antimiR-1 administration. **B:** Area percentage of structural SA and V after miR-1 gain- and loss-of-function experiments. **C:** RT-qPCR validating the miR-1 gain- and loss-of-function results respect to control on cardiac *asa* and fluorescence visualization of dissected cardiac *asa*. OFT: outflow tract; V: ventricle; SA: sinoatrial region. Student's *t*-test: \*\*\* $p < 0.005$ , \*\*\*\* $p < 0.0001$  with respect to control (CFDA) embryos.

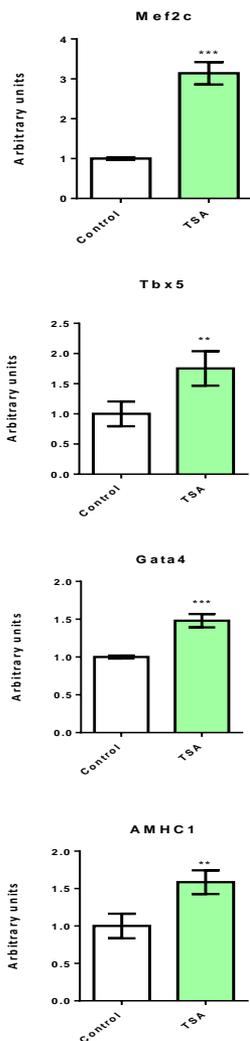
## Supplementary Figure S3



**Supplementary Figure S3. A:** Embryos microinjected with CFDA (control), Retinoic acid (RA) or Citral, at the level of the posterior cardiac precursors into both primitive endocardial tubes (arrowheads), and visualization of CFDA. **B:** The gain-of-function of RA leads to expanded expression of *Gata4* in the heart tube and in the inflow tract (blue arrows). Note atrophic sinoatrial region with diminished *Gata4* expression (red arrows). RT-qPCR of RNA from dissected cardiac area (left side) in embryos microinjected either with CFDA, RA or Citral. Standard deviations are from three independent experiments. Student's t-test: \* $p < 0.05$  with respect to control (CFDA) embryos.



## Supplementary Figure S5



**Supplementary Figure S5.** Effect of Trichostatin A (TSA) on the expression of Mef2c, Tbx5, Gata-4 and AMHC-1. RT-qPCR of RNA from dissected cardiac *asa* (right side) in embryos microinjected either with CFDA, or TSA (histone deacetylase –HDAC- inhibitors) at the level of the posterior cardiac precursors into both primitive endocardial tubes. Treatment with TSA leads to increased Mef2c, Tbx5, Gata-4 and AMHC1 transcripts. Student's t-test: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.005$ , with respect to control (CFDA) embryos.

## Supplementary Table STI

qPCR primers		
Gene	Specie	Sequence
Tbx5_Foward	Gallus Gallus	AACCCCTACCCGATCTCCCC
Tbx5_Reverse	Gallus Gallus	CCATGTAGGGCTTCTTGATAGGG
AMHC1_Foward	Gallus Gallus	AAGACCGGAAGAACATGGTG
AMHC1_Reverse	Gallus Gallus	GAGACTCAGCCATGTCAGCA
Gata4_Foward	Gallus Gallus	TCAACTGTGGGGCCATGTC
Gata4_Reverse	Gallus Gallus	GCATTACAGACGGGCTCTCC
Mef2c_Foward	Gallus Gallus	CCCCAGGAATGCTGTCTTTA
Mef2c_Reverse	Gallus Gallus	AGTGGAATTCATCCGGTGA
HDAC4_Foward	Gallus Gallus	ATGGCATCTGTGCGGTAGG
HDAC4_Reverse	Gallus Gallus	CATGACAGGCAGGAGTA
Calmodulin_Foward	Gallus Gallus	TATCAGTGCAGCAGAGCTACG
Calmodulin_Reverse	Gallus Gallus	GGAGGTCTCTTCACTTTGACG
Erk2/MAPK1_Foward	Gallus Gallus	GAGCATCAGACGTACTGCCA
Erk2/MAPK_Reverse	Gallus Gallus	AATGTGGTCTGTGCTGAGGT
Raldh2_Foward	Gallus Gallus	ACTGAGATTTAAAACGGTGGATGA
Raldh2_Reverse	Gallus Gallus	TCTCTACCATTGCCAGACA
RAR $\alpha$ _Foward	Gallus Gallus	AGGTGGACAAACTGCAGGAG
RAR $\alpha$ _Reverse	Gallus Gallus	CGGGATCTCCATCTTCAGTG
RAR $\beta$ _Foward	Gallus Gallus	CTGTAAAAGTTCGATGAAGTCT
RAR $\beta$ _Reverse	Gallus Gallus	TTTTATCTTTTACGTTTGAAAACITTT
RAR $\gamma$ _Foward	Gallus Gallus	ATGTTCCCCAGAATGCTGAT
RAR $\gamma$ _Reverse	Gallus Gallus	GGCTCCTCCTCGAACATCTC
RXR $\alpha$ _Foward	Gallus Gallus	GGCTGAAGTGGAGGCATTAC
RXR $\alpha$ _Reverse	Gallus Gallus	AAGAGGTGCTCCAGGCATTT
RXR $\gamma$ _Foward	Gallus Gallus	GGAAGCCTACACGAAGCAGA
RXR $\gamma$ _Reverse	Gallus Gallus	GGTGTCTCCAGCATCTCCAT
CRABPI_Foward	Gallus Gallus	CCACCTGGGAGAATGAAAAC
CRABPI_Reverse	Gallus Gallus	CATTCTACTGGGGCTTTCCA
CRABPII_Foward	Gallus Gallus	TCAAGACCTCAACCACCGTG
CRABPII_Reverse	Gallus Gallus	GATGAGCTCCCCATCGTTGG
Gapdh_Foward	Gallus Gallus	TGTCTCTCTGGCAAAGTCC
Gapdh_Reverse	Gallus Gallus	TGCCATTGATCACAAAGTTT

Supplementary Table STI. Primer sequences and corresponding genes for RT-qPCR (Gallus gallus).

## Supplementary Table STII

3'UTRs cloning primers		
HDAC4_3'UTR_F position 1	Gallus Gallus	GGAAGCTTCAGCCACCTCACCCTTACA
HDAC4_3'UTR_R position 1	Gallus Gallus	GGACTAGTTAGAGGGAGAACCAGCTCCA
HDAC4_3'UTR_F position 2	Gallus Gallus	GGAAGCTTCCCTCCTGCAGTTCTCTAA
HDAC4_3'UTR_R position 2	Gallus Gallus	GGACTAGTGGGTCACATGGGTTGATAC
Calmodulin_3'UTR_F	Gallus Gallus	GGACTAGTCACTTGGTGCATGGCTTAGA
Calmodulin_3'UTR_R	Gallus Gallus	GGAAGCTTTGCATCAGTTGAGAGCAACA
MAPK1/Erk2_3'UTR_F	Gallus Gallus	GGACTAGTAAACCATTGGCCAGAAGAA
MAPK1/Erk2_3'UTR_R	Gallus Gallus	GGAAGCTTGAGCATTGAAGCCATGTGA

**Supplementary Table STII.** Primer sequences 3'UTR corresponding HDAC4 (position 1 and position 2); Calmodulin and MAPK1/Erk2 genes for luciferase assays (Gallus gallus).