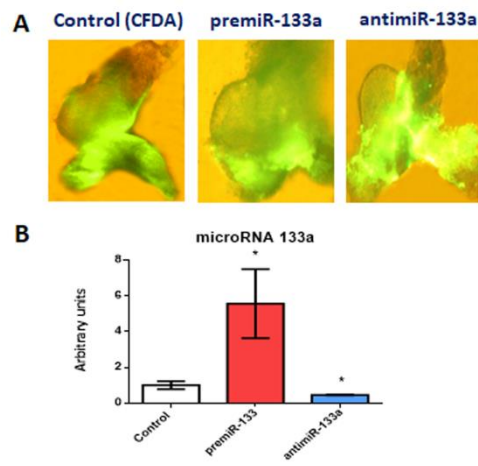


**Supplementary Figure S1.** TargetScan analysis of putative microRNA binding sites in Human RhoA 3' UTR and Cdc42 3' UTR. As can be observed, miR-133a is a putative microRNA targeting Rho A (panel A), and Cdc42 (panel B). Note their binding site are highly conserved along the RhoA and Cdc42 3' UTR in several species, including chicken.



**Supplementary Figure S2.** Panel **A** shows the visualization of CFDA in dissected cardiac *asa* (HH11) from embryos microinjected, either with CFDA, premiR-133a or antimiR-133a, at the level of the posterior cardiac precursors into both primitive endocardial tubes. Panel **B** shows qRT-PCR results validating the miR-133a gain- and loss-of-function respect to control on cardiac *asa*. Student's t-test: \* $p < 0.05$  with respect to control (CFDA) embryos.

qPCR primers		
Gene	Specie	Sequence
Tbx5_Foward	Gallus Gallus	AACCCCTACCCGATCTCCCC
Tbx5_Reverse	Gallus Gallus	CCATGTAGGGCTTCTTGTAGG G
AMHC1_Foward	Gallus Gallus	AAGACCGGAAGAACATGGTG
AMHC1_Reverse	Gallus Gallus	GAGACTCAGCCATGTCAGCA
RhoA_Foward	Gallus Gallus	ATTTCTGTCCCAACGTGCCT
RhoA_Reverse	Gallus Gallus	CAAAAACCTCCCTCACACCG
Cdc42_Foward	Gallus Gallus	GCTGTAACAGTGATGATTGGAGG
Cdc42_Reverse	Gallus Gallus	TCTGTGGATAGCTGAGGGGT
Raldh2_Foward	Gallus Gallus	ACTGAGATTTAAACGGTGGATGA
Raldh2_Reverse	Gallus Gallus	TCTCTTACCATTGCCAGACA
p21_Foward	Gallus Gallus	AGCCAACACAACAGAAGAGGA
p21_Reverse	Gallus Gallus	TCCCATGGAGACCGACGATA
Cyclin A_Foward	Gallus Gallus	TAAGCCTTGCCTCATGGACC
Cyclin A_Reverse	Gallus Gallus	TCTGGTGCGTCAATAAGCGA
Gapdh_Foward	Gallus Gallus	TGTCCTCTCTGGCAAAGTCC
Gapdh_Reverse	Gallus Gallus	TGCCCATTGATCACAAGTTT

**Supplementary Table S1.** Primer sequences and corresponding genes for qRT-PCR (Gallus gallus).

3'UTRs cloning primers		
RhoA_3'UTR_F	Gallus Gallus	GGACTAGTGAGCCTGTCAAACCTGAGG
RhoA_3'UTR_R	Gallus Gallus	GGAAGCTTTGCTTGGCCATCTTCCTCT
Cdc42_3'UTR_F	Gallus Gallus	GGGAAGCTTATTGGAATGAGAGCTGGCCC
Cdc42_3'UTR_R	Gallus Gallus	GGGACTAGTATCCTTGTTGGGCTGGAACC

**Supplementary Table S2.**  
Primer sequences 3'UTR corresponding RhoA and Cdc42 genes for luciferase assays (Gallus gallus).