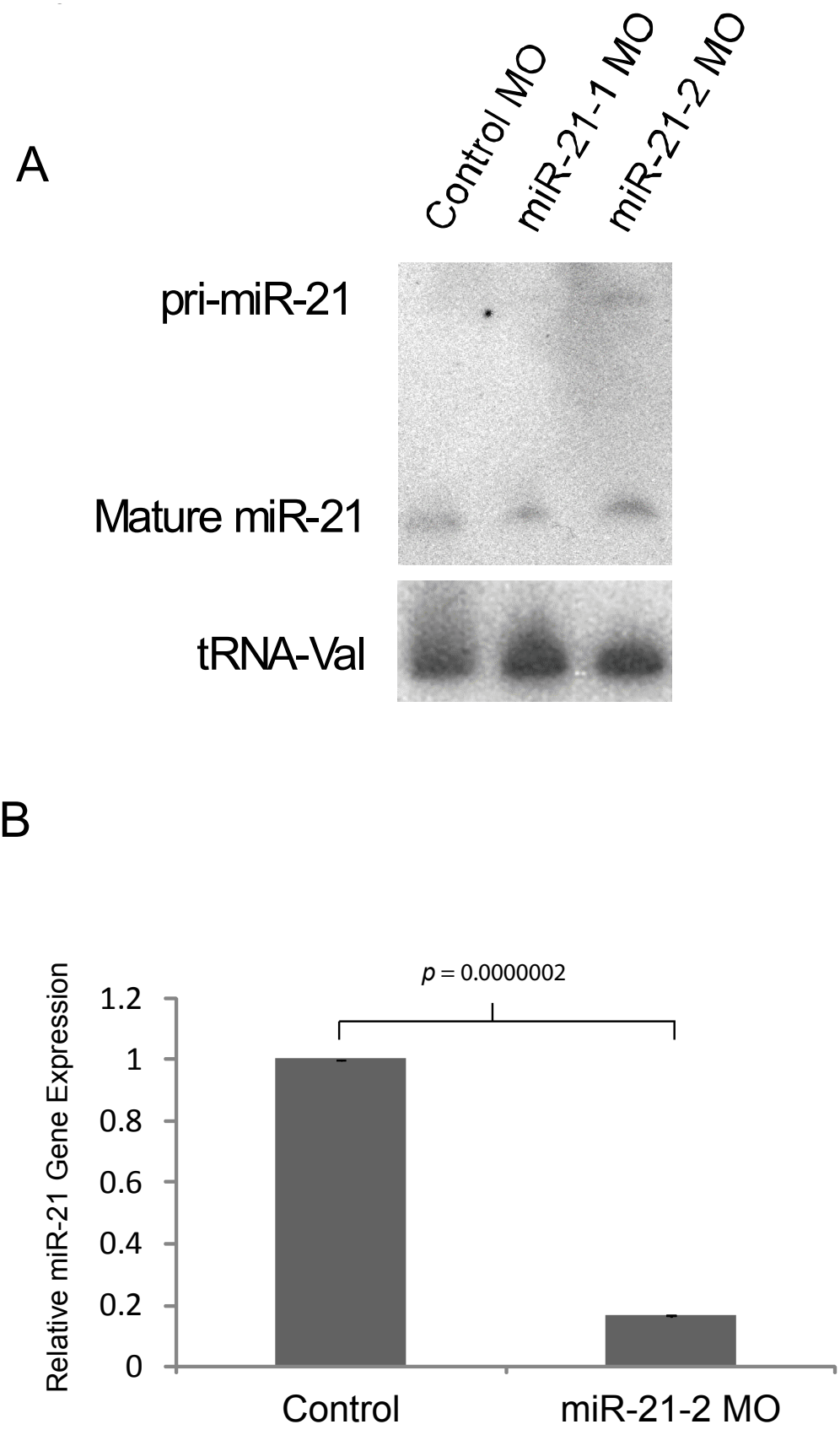



**Fig. S1. miR-21 knockdown results in accumulation of miR-21 precursor and loss of mature miR-21.** (**A**) Northern blot for short RNA molecules from control, miR-21-1 MO-injected, and miR-21-2 MO-injected embryos. Injection of miR-21-2 MO results in accumulation of the pre-miR-21 precursor (top band), which may be detected faintly in the miR-21-1 lane as well, but is absent from the control lane. (**B**) Injection of miR-21-2 MO causes a 90% reduction in the amount of mature miR-21 detectable by qPCR.

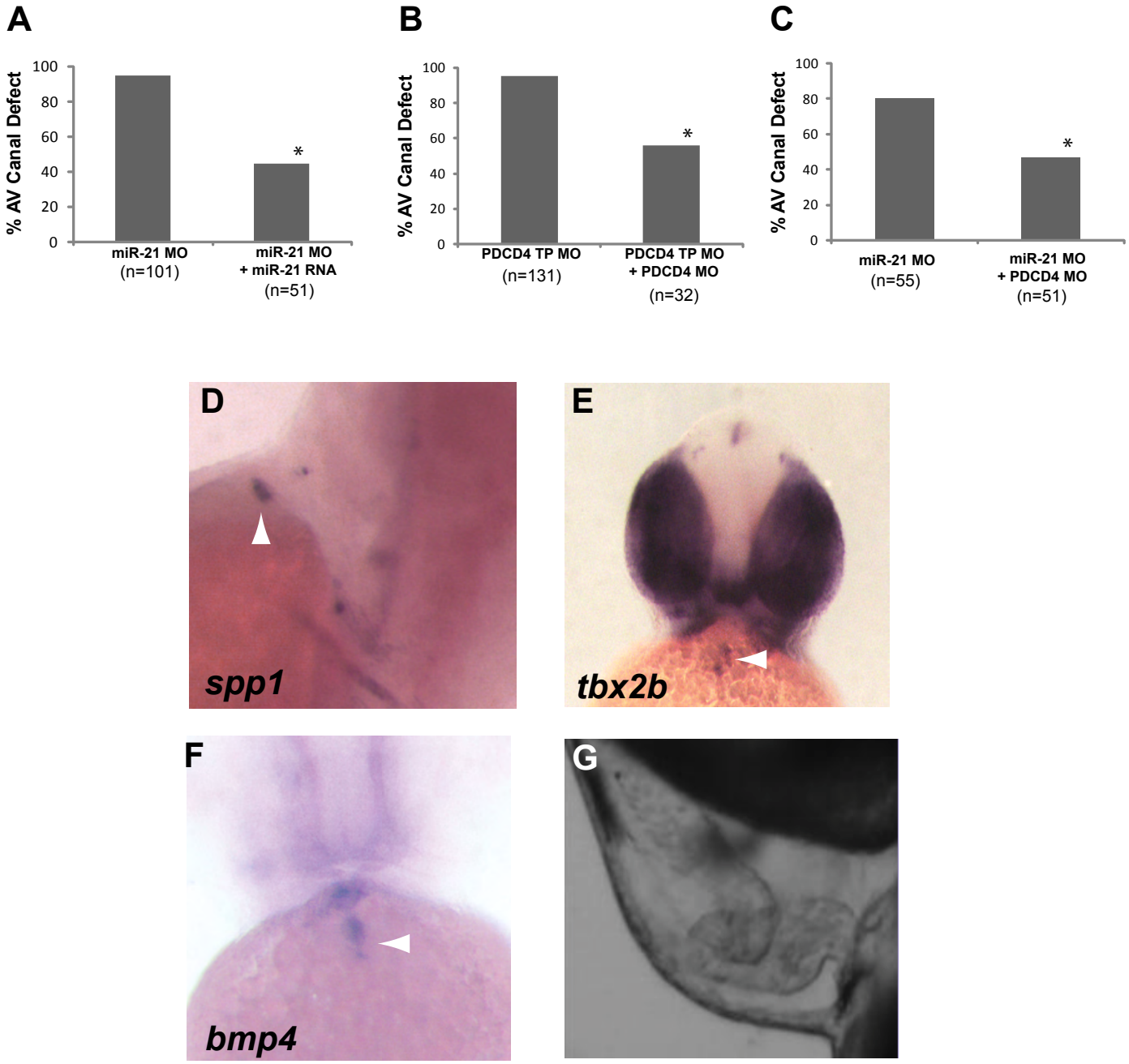



**Fig. S2. Rescue of miR-21 phenotypes.** (**A**) The miR-21 MO AVC defect is rescued by co-injecting mature miR-21 RNA into embryos. *$P$=6×10$^{-12}$. (**B**) The PDCD4 target protector (TP) MO AVC defect is rescued by co-injecting a splice-acceptor PDCD4 MO. *$P$=5×10$^{-8}$. (**C**) The miR-21 MO AVC defect is rescued by co-injecting a splice-acceptor PDCD4 MO into embryos. *$P$=0.0005. (**D-F**) Representative embryos demonstrating recovery of *in situ* expression patterns of the indicated genes after the rescue experiments performed above. The AVC is indicated by white arrowheads. (**G**) Representative embryonic heart demonstrating recovery of AV constriction and looping after the rescue experiments performed above.

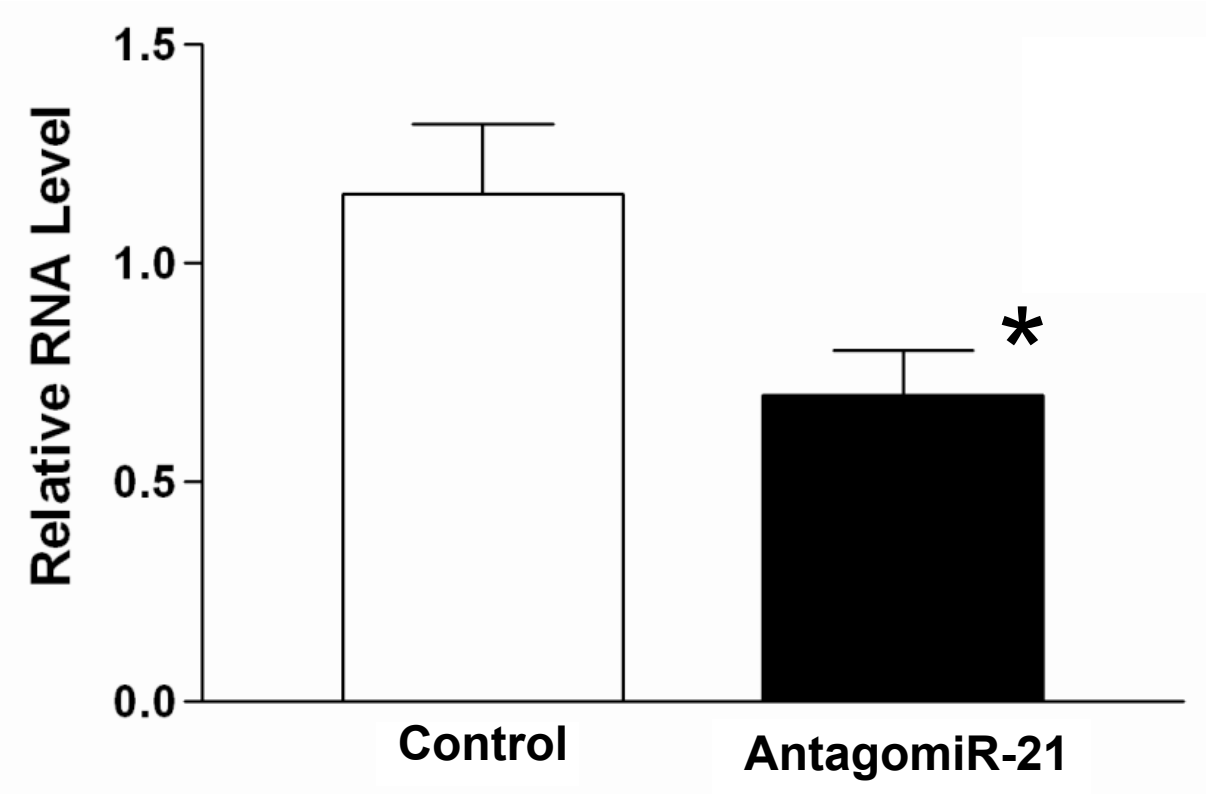



**Fig. S3. miR-21 is expressed in mouse cardiac cushion endothelial cells.** qPCR from murine cardiac cushion cells demonstrates miR-21 expression, which is reduced upon transfection of a miR-21 antagomir ($P<0.05$).

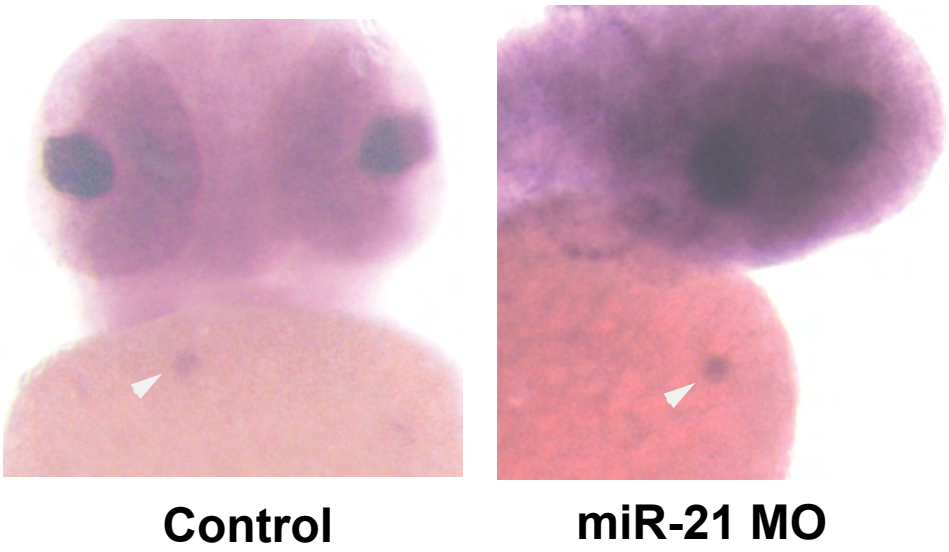
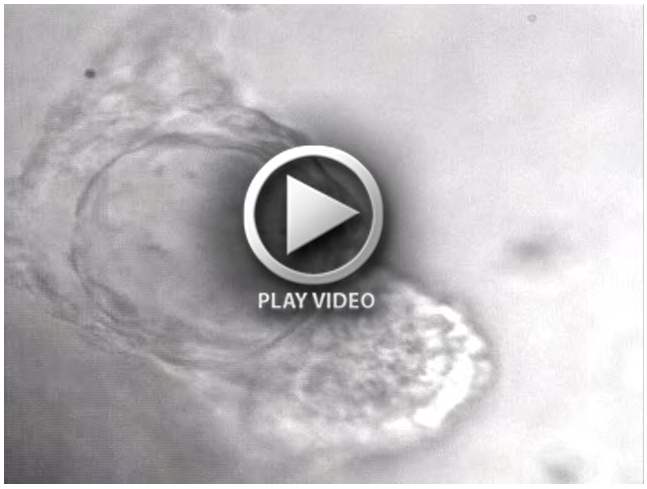



**Fig. S4. *In situ* expression pattern of *pdcd4b*.** Shown are the expression patterns of *pdcd4b* under control and miR-21 knockdown conditions.


**Movie 1. Control zebrafish embryo heart at 5 days post-fertilization.** Atrium is on the left, ventricle on the right.




**Movie 2. mirR-21 morphant heart at 5 days post-fertilization.** Atrium is on the left, ventricle on the right.


**Movie 3. PDCD4-TP$^{miR-21}$ morphant embryo at 48 hours post-fertilization.** Atrium is on the right, ventricle on the left.