Germline-specific dgcr8 knockout in zebrafish using a BACK approach

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Supplementary figures

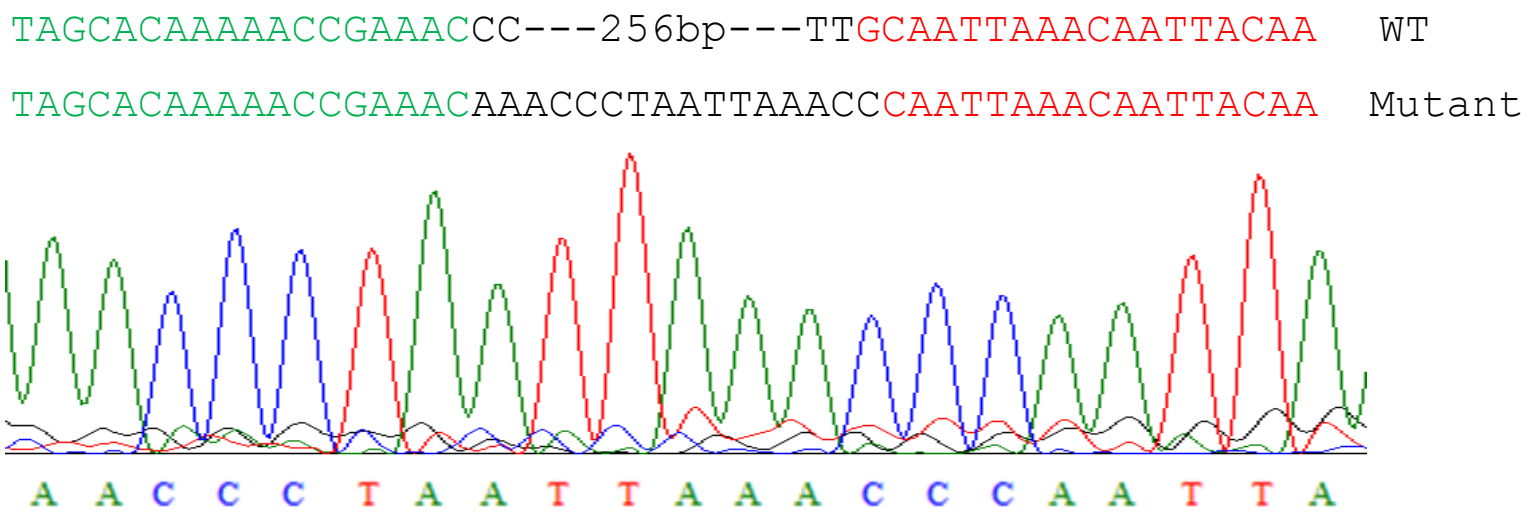

Figure S1. Germline transmitted *dgcr8* deletion. Sequencing results of the *dgcr8* deletion detected in F2 generation.

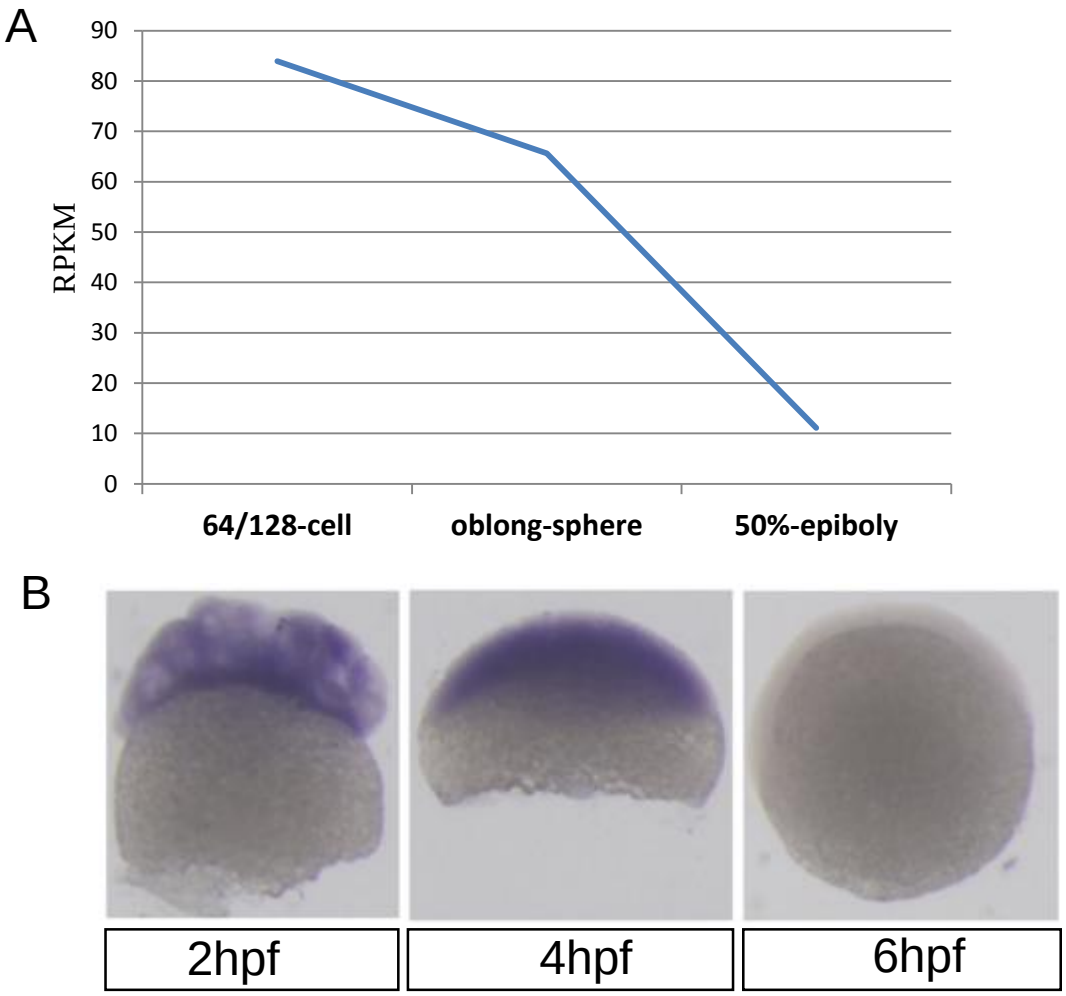

Figure S2. *Dgcr8* transcripts are maternally provided. **a** Transcript level of zebrafish *dgcr8* at different early developmental stages by transcriptome analysis. **b** *Dgcr8* transcripts were detected in wild-type embryos collected at 2 hpf and 4 hpf but not at 6 hpf using whole mount *in situ* hybridization.

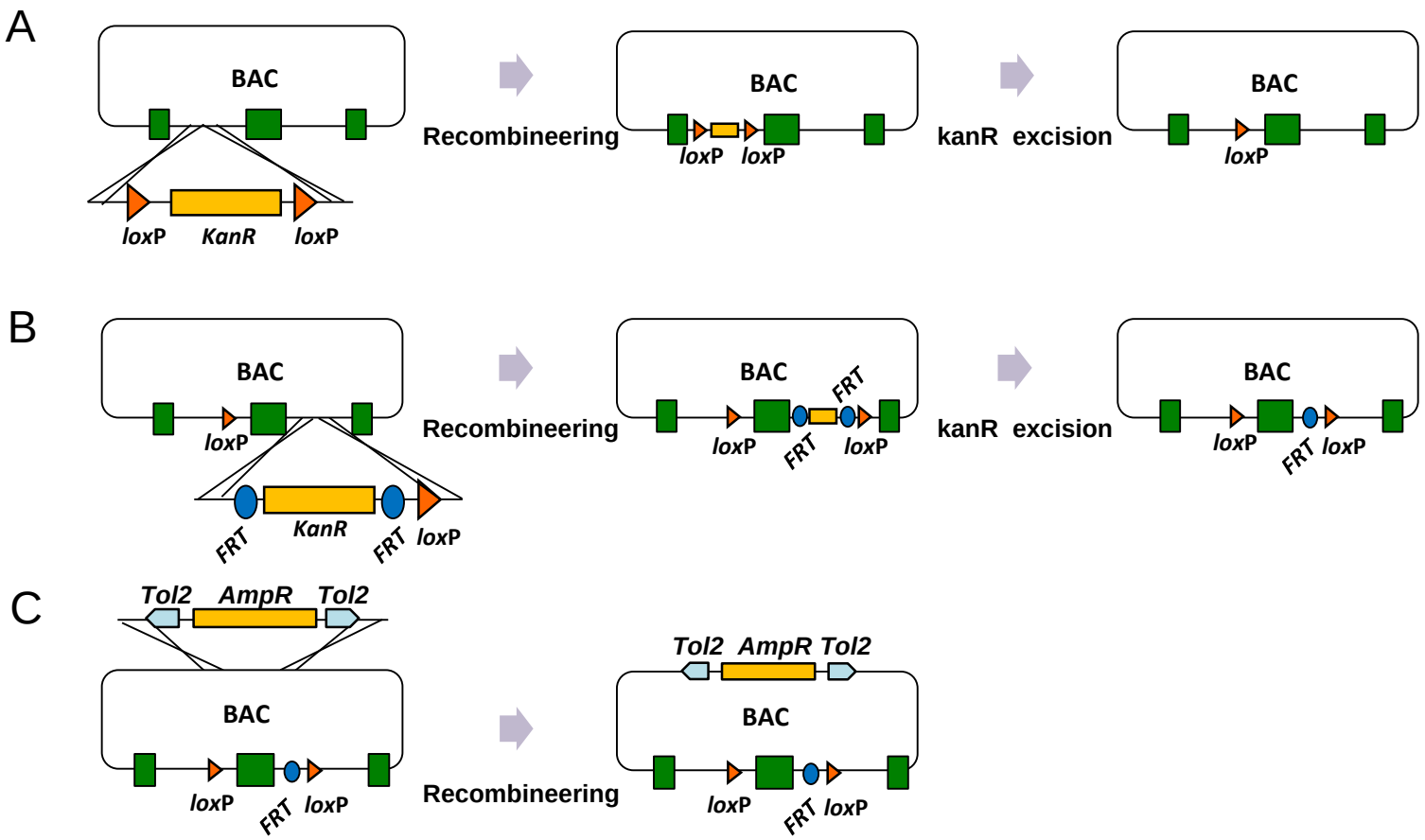

Figure S3. Recombineering of *loxP* cassettes and *iTol2* cassette into the *dgcr8* BAC clone. **a** Targeting the first *loxP* cassette into BAC and excision of the *Neomycin* cassette. **b** Targeting the second *loxP* cassette into BAC and excision of the *Neomycin* cassette. **c** Introducing the *iTol2* cassette into BAC.

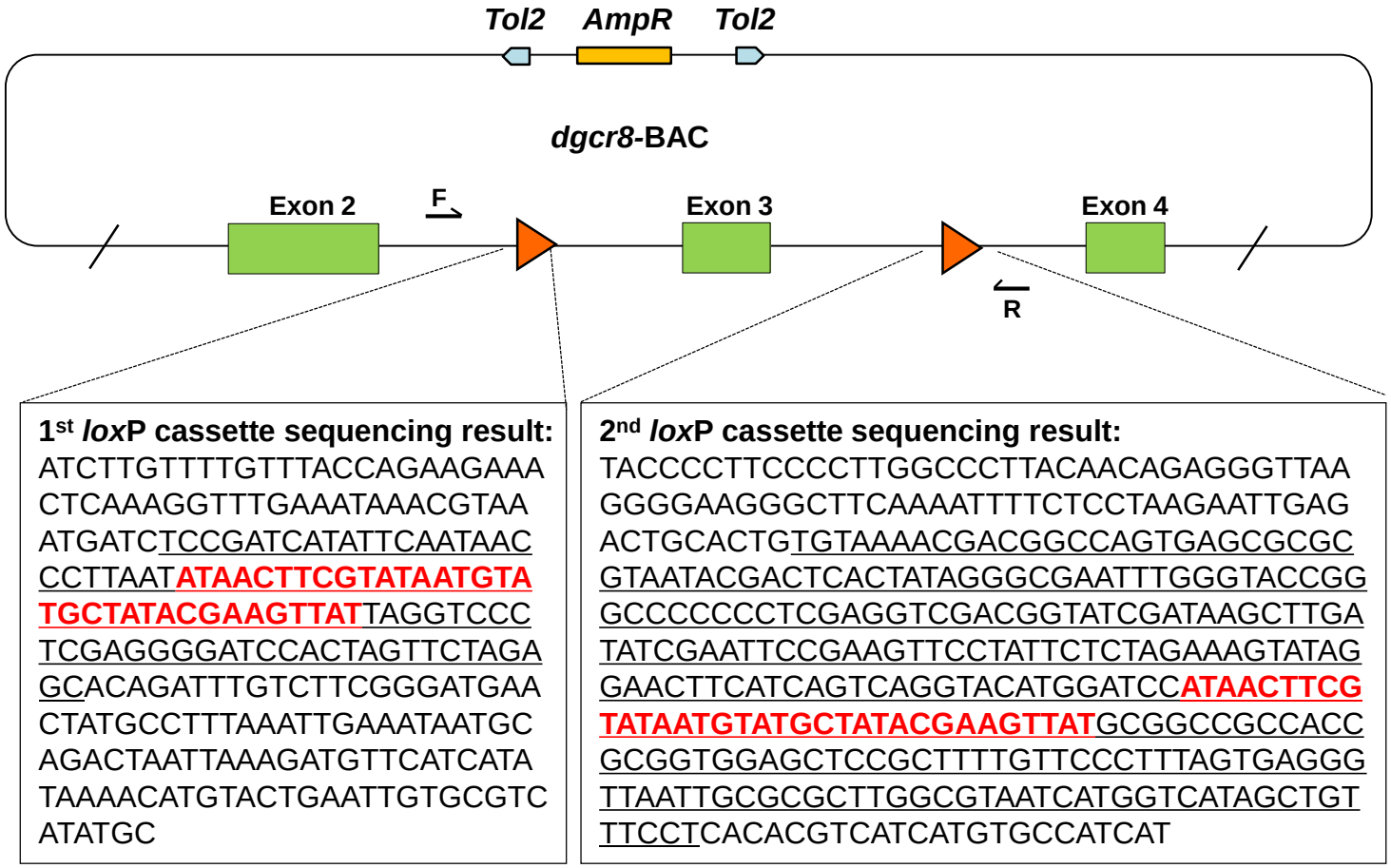

Figure S4. Squencing results of BAC *loxP* cassettes. The first *loxP* cassette and second *loxP* cassette were underlined and the *loxP* sites were shown in red.

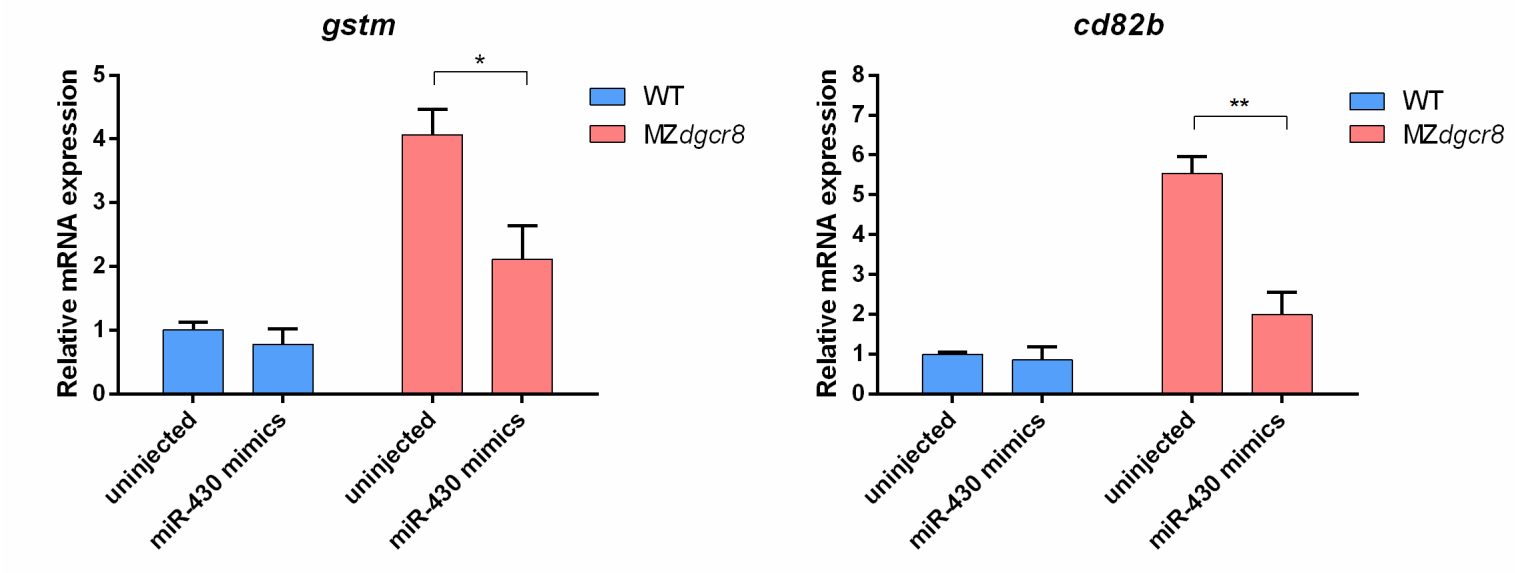

Figure S5. Q-PCR analysis of mRNA expression of miR-430 targets. The WT, MZ*dgcr8* and miR-430 rescued embryos were collected at shield stage. The mRNA level of *gstm* and *cd82b* was analyzed by real-time PCR and normalized to the expression levels in the WT control. Data are expressed as mean values ± S.E.M (n = 3). **P*<0.05 and ***P*<0.01 versus corresponding control.

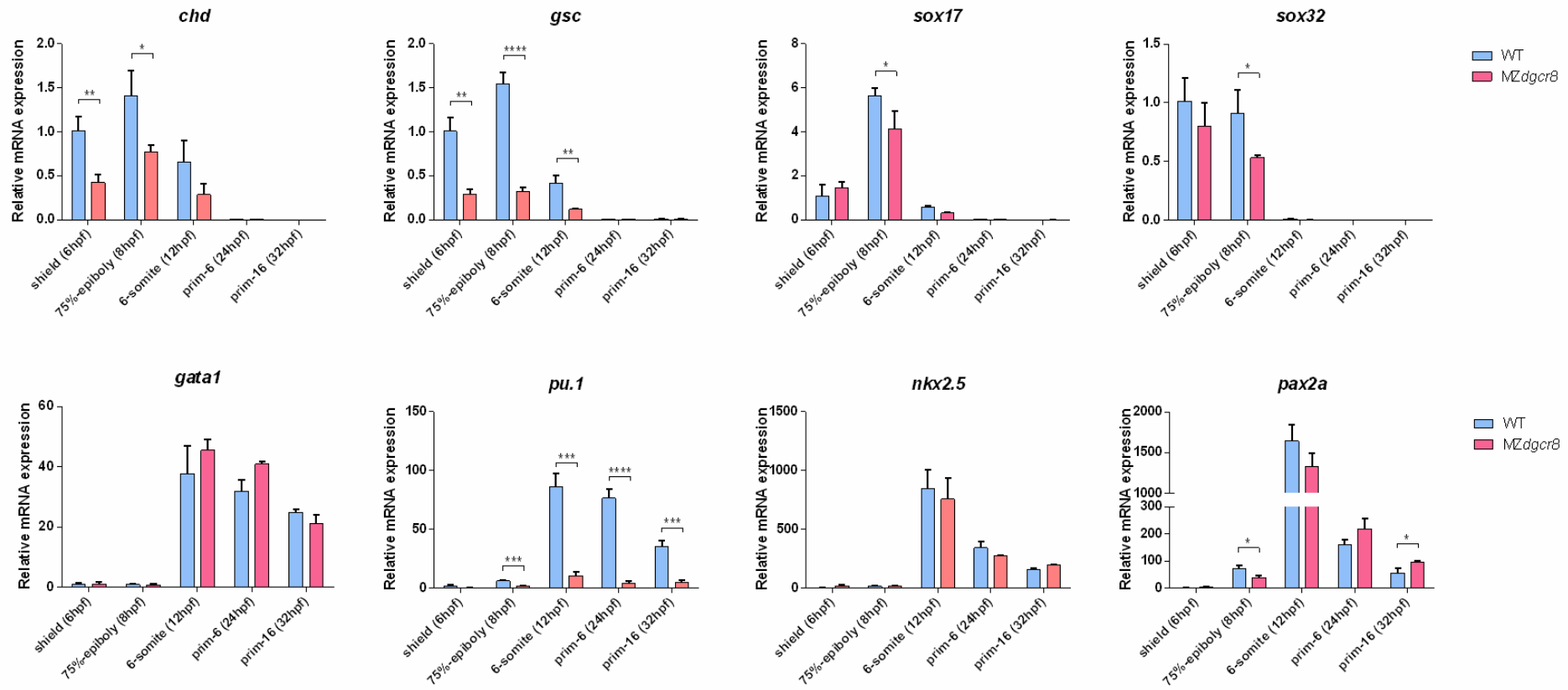

Figure S6. Q-PCR analysis of marker gene expression in the MZ*dgcr8* mutant. The WT and MZ*dgcr8* embryos were collected at various developmental stages (shield, 75%-epiboly, prim-6, prim-16). The mRNA level of the indicated maker genes was analyzed by real-time PCR and normalized to the expression levels at shield stage of the WT control. Data are expressed as mean values ± S.E.M (n = 3). **P*<0.05 , ***P*<0.01 and ****P*<0.001versus corresponding control.

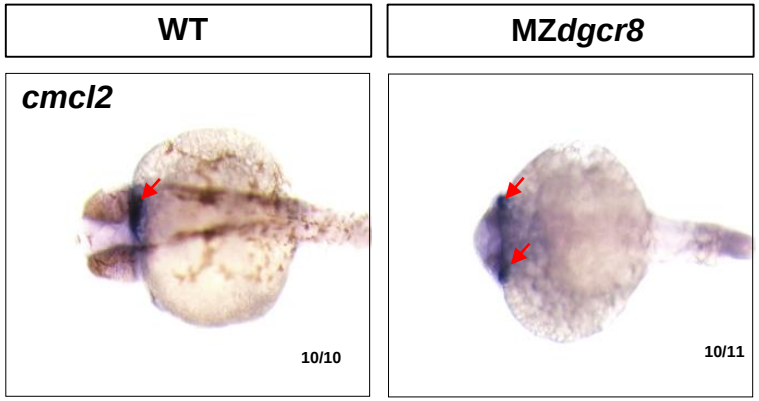

Figure S7. *In situ* analysis of *cmlc2* expression in the MZ*dgcr8* mutant. The WT and MZ*dgcr8* embryos were collected at 32 hpf. The *cmlc2* expressing cardiocytes (indicate by arrows) failed to migrate to the middleline in the MZ*dgcr8* embryos.

Supplementary table

Table S1 Primers used in the present study

Primer name	Sequence (5′-3′)	Purpose
dgcr8_F0	CTTGAGTCAGACTCGAGAGGAG	Genotyping of global *dgcr8* knockout
dgcr8_R0	CTCACTGGAGCGAGCCGATGAG	
dgcr8-lox-F	TACCAGAAGAAACTCAAAGGTTTGAAATAAACGTAAATGA TCTCCGATCATATTCAATAACC	Amplification of 1st *lox*P cassette for BAC recombineering
dgcr8-lox-R	TTTCAATTTAAAGGCATAGTTCATCCCGAAGACAAATCTGT GCTCTAGAACTAGTGGATCC	
dgcr8-F1	ACTCAAAGGTTTGAAATAAACG	PCR identification of positive clones of targeting 1st *lox*P cassette
dgcr8-F1-1	CAACTACGTTGGAAGTCTGTG	
dgcr8-Lox-R1	GCATATGACGCACAATTCAGTAC	
dgcr8-FRT-F	GGAAGGGCTTCAAAATTTTCTCCTAAGAATTGAGACTGCA CTGTGTAAAACGACGGCCAGTGA	Amplification of 2nd *lox*P cassette for BAC recombineering
dgcr8-FRT-R	CTCACATGAAGCAAGAGATCATGATGGCACATGATGACGT GTGAGGAAACAGCTATGACCATG	
dgcr8-F2	CTCCTAAGAATTGAGACTGC	PCR identification of positive clones of targeting 2nd *lox*P cassette
dgcr8-F2-1	AGCATGTGGCCACAAGGCCAC	
dgcr8-FRT-R1	CTGGAATAACTACAGCAGTCAC	
Tol2-hom-F1	GCTGTCAAACATGAGAATTGATCCGGAACCCTTAATAAGT GATCTCCAAAAAATAAGTAC	Amplification of *iTol2* cassette for BAC recombineering
Tol2-hom-R1	GTCGCTGTCGACGGTGACCCTATAGTCGAGGGACCTAAA TACTCAAGTACAATTTTAATGG	
Tol2-L200-F1	AGCCTATGCCTACAGCATCCAGG	PCR identification of positive clones of targeting *iTol2* cassette
Tol2-L200-R1	ACTTGATTACTGTACTTAAGTA	
cre-F1	GAACCTGATGGACATGTTCAGG	Genotyping positive *Cre* transgenic fish
cre-R1	AGTGCGTTCGAACGCTAGAGCCTGT	
dgcr8_T7F	ACAGTACGTTAATACGACTCACTATAG GGCCCAATTGTGTTCTTGTATCAG	Amplification of zebrafish *dgcr8* cDNA containing open reading frame
dgcr8_T7R	GTGCACTCTCAGGTGCAGAAGTG	
Pri-miR-21F	TTTCAGCCCCACCCTCTCCTCT	Q-PCR amplification of zebrafish Pri-miR-21
Pri-miR-21R	GCTATCTGACACACTGGGAAAG	
Pri-miR-25F	GCTACGCTACACTGATGCTACGC	Q-PCR amplification of zebrafish Pri-miR-25
Pri-miR-25R	TCTTCTTCTGGTGAATGGAGGGG	
Pri-miR-430F	AGTAACATGGGGACACTCCTTT	Q-PCR amplification of zebrafish Pri-miR-430
Pri-miR-430R	CCCCAACTTGATAGCACTTTCT	
ef1α-qPCR-F	TGGAGGCCAGCTCAAACAT	Q-PCR amplification of zebrafish *ef1α*
ef1α- qPCR-R	ATCAAGAAGAGTAGTACCGCTAGCATTAC	
chd-qPCR-F	GGGAGGAAGGAGGTCGAGTC	Q-PCR amplification of zebrafish *chd*
chd-qPCR-R	ACAAAATCGGTGGTGGTGCT	
sox17-qPCR-F	CATCCGAAGGCCAATGAACG	Q-PCR amplification of zebrafish *sox17*
sox17-qPCR-R	CGAATGGACGTTTGTCCACC	
gsc-qPCR-F	ACGTCGGCACAAGAGAACAA	Q-PCR amplification of zebrafish *gsc*
gsc-qPCR-R	TCCTCTGACGACGACCTTTTC	
gata1-qPCR-F	TAGACACAGTCCAGTTCGCC	Q-PCR amplification of zebrafish *gata1*
gata1-qPCR-R	TGTGGGGTTGTAGGGAGAGT	
nkx2.5-qPCR-F	CTTCAGTGCTTCAGGCTTTTACGCG	Q-PCR amplification of zebrafish *nkx2.5*
nkx2.5-qPCR-R	GCTCCGCATCATCCAGCTTCAGATC	
pu.1-qPCR-F	GGGCAGTTTTAACCAAAGATCA	Q-PCR amplification of zebrafish *pu.1*
pu.1-qPCR-R	CCCAAGAGTGATCGTTCTGAC	
pax2a-qPCR-F	CCGCGTTATTAAGTTCCCCT	Q-PCR amplification of zebrafish *pax2a*
pax2a-qPCR-R	TGGCGTATCCATCTTCAATCC	
cd82b-qPCR-F	GTTTGTCCCCTCAATGGCCT	Q-PCR amplification of zebrafish *cd82b*
cd82b-qPCR-R	TGGACAGGGTGATGACTGGA	
gstm-qPCR-F	CACCCTCTTGTTGCAAAATGGC	Q-PCR amplification of zebrafish *gstm*
gstm-qPCR-R	GAGCTGGACAAATCCATTGCG	