## Germline-specific dgcr8 knockout in zebrafish using a BACK approach

## **Cellular and Molecular Life Sciences**

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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 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  $\pm$  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  $\pm$  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

dgcr8_F0       CTTGAGTCAGACTCGAGAGGAG       Genotyping of global dgcr8 knockout         dgcr8_R0       CTCACTGGAGCGAGCCGATGAG       Genotyping of global dgcr8 knockout         dgcr8-lox-F       TACCAGAAGAAACTCAAAGGTTTGAAATAAACGTAAATGA         dgcr8-lox-R       TACCAGAACTAATGGATCC       Amplification of 1st loxP cassette for BAC         dgcr8-lox-R       GCTCTAGAACTAGTGGATCC       Amplification of positive clones of         dgcr8-F1       ACTCAAAGGTTTGAAATAAACG       PCR identification of positive clones of         dgcr8-F1       CAACTACGTTGGAAGTCTGTG       PCR identification of 2nd loxP cassette         dgcr8-FRT-F       GGAAGGGCTTCAAAATTTTCTCCTAAGAATTGAGACTGCA       Amplification of 2nd loxP cassette for BAC         dgcr8-FRT-F       GGAAGGACTATGACCAGCGCCAGTGA       Amplification of 2nd loxP cassette for BAC         dgcr8-FRT-R       GTGAGGAAACAGCAAGCAGCAGTGA       Amplification of 2nd loxP cassette for BAC         dgcr8-F2       CTCCAATGAAGCAGCAGCAGTGA       Amplification of 2nd loxP cassette for BAC         dgcr8-F2       CTCCAAGAACAGCAAGGGCCAC       PCR identification of positive clones of targeting 2nd loxP cassette         dgcr8-F2       CTGCCAAAGAGAATGATCGGGACCCAC       PCR identification of positive clones of targeting 2nd loxP cassette         dgcr8-FRT-R1       CTGGAAAAAATAAGTACAGCAGCGGAACCCTTAATAAGT       Amplification of iTol2 cassette         fol2-hom-F1       GCC
dgcr8_R0     CTCACTGGAGCGAGCCGATGAG     Genotyping of global ogc/s kitckout       dgcr8-lox-F     TACCAGAAGAAAACTCAAAGGTTTGAAATAAACGTAAATGA TCTCCGATCATATTCAATAACC     Amplification of 1st /oxP cassette for BAC       dgcr8-lox-R     GCTCTAGAACTAGTGGATCC     Amplification of 1st /oxP cassette for BAC       dgcr8-F1     ACTCAAAGGTTTGAAATAAACG     PCR identification of positive clones of targeting 1st /oxP cassette       dgcr8-F1     CAACTACGTTGGAAGTCGTG     PCR identification of 2nd /oxP cassette       dgcr8-FRT-F     GGAAGGGCTTCAAAATTTCCCTAAGAATTGAGACTGCA CTGTGTAAAACGACGGCCAGTGA     Amplification of 2nd /oxP cassette for BAC       dgcr8-FRT-F     GGAAGGGCTTCAAAATTTCTCCTAAGAATTGAGACTGCA CTGCACATGAAGCAAGAGATCATGATGGCACATGATGACGT GGCAGGAAACAGCTAGAGCAGTGC     PCR identification of positive clones of targeting 2nd /oxP cassette for BAC       dgcr8-F2     CTCCTAAGAATTGAGCACAGCAGCAC     PCR identification of positive clones of targeting 2nd /oxP cassette       dgcr8-F2     CTCCTAAGAATGAGCAGCACC     PCR identification of positive clones of targeting 2nd /oxP cassette       dgcr8-F2     GCTGTCAAAACATGAGAATTGATCCGGAACCCTTAATAAGT GATCTCCAAAAATAACTACAGCAGTCAC     PCR identification of iTo/2 cassette       tol2-hom-F1     GCTGTCGAACAGTGAGCAGCCAATGCCGAGGGACCTAAA TACTCAAGTACAACATGAGGAATTGATCCAGG     Amplification of iTo/2 cassette for BAC       tol2-hom-R1     GTCGCTGTCGACGGGGACCCTATAGTCGAGGGGACCTAAA TACTCAAGTACAGCATGCTGCAGGGGACCTAAAC     Amplification of positive clones of iTol2-L200-F1       AGCCTATGCCTACAGCAGTGACCTATGAGTGA     P
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Tol2-hom-R1       GTCGCTGTCGACGGTGACCCTATAGTCGAGGGACCTAAA TACTCAAGTACAATTTTAATGG       recombineering         Tol2-L200-F1       AGCCTATGCCTACAGCATCCAGG       PCR identification of positive clones of targeting <i>iTol2</i> cassette         cre-F1       GAACCTGATGGACATGTTCAGG       Output targeting <i>iTol2</i> cassette
TACTCAAGTACAATTTTAATGG         Tol2-L200-F1       AGCCTATGCCTACAGCATCCAGG       PCR identification of positive clones of targeting <i>iTol2</i> cassette         Cre-F1       GAACCTGATGGACATGTTCAGG       Output for the second
Tol2-L200-F1     AGCCTATGCCTACAGCATCCAGG     PCR identification of positive clones of targeting <i>iTol2</i> cassette       Tol2-L200-R1     ACTTGATTACTGTACTTAAGTA     targeting <i>iTol2</i> cassette       cre-F1     GAACCTGATGGACATGTTCAGG     Output targeting it target it t
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cre-F1 GAACCTGATGGACATGTTCAGG
Genotyping positive (Cre transgenic tish
cre-R1 AGTGCGTTCGAACGCTAGAGCCTGT
dgcr8_T7F ACAGTACGTTAATACGACTCACTATAG
GGCCCAATTGTGTTCTTGTATCAG
dgcr8_T7R GTGCACTCTCAGGTGCAGAAGTG
Pri-miR-21F TTTCAGCCCCACCCTCTCCTCT O-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 TCTTCTTGGTGAATGGAGGGG
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 ef1q
et1a- qPCR-R ATCAAGAAGAGTAGTACCGCTAGCATTAC
chd-qPCR-F GGGAGGAGGAGGAGGICGAGIC Q-PCR amplification of zebrafish <i>chd</i>
chd-qPCR-R ACAAAAICGGIGGIGGIGGIGCI
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 IAGACACAGICCAGICCGCC Q-PCR amplification of zebrafish gata1
RKX2.5-qPCR-F CTTCAGTGCTTCAGGCTTTTACGCG Q-PCR amplification of zebrafish nkx2.5
PULI-QPOR-F GGGCAGITTIAACCAAAGATCA Q-PCR amplification of zebrafish pu.1
Pax2a-qPCR-F CCGCGTTATTAAGTTCCCCT Q-PCR amplification of zebrafish pax2a
cd82b-qPCR-P TCCACACCACCACCACCACCACCACCACCACCACCACCAC
gstm-qPCR-R GAGCTGGACAAATCCATTGCG