**Supplemental Material**

**Supplemental Figure 1: *MZbcl9l∆4* mutants are viable and fertile.**

(**A,B**) Schematics showing the mutation induced in *bcl9l∆4* mutants. Analogous to *bcl9* mutants (see Figure 1B,C), we designed a sgRNA that induces mutagenesis between the HD1 and HD2 domain. Gene locus represented as per genome annotation *Zv10* (**A**) with two isoforms that differ in the first coding exon and the untranslated regions (UTRs). The green dotted box represents a zoomed region of the gene locus, with the red line representing the location of the sgRNA used for mutagenesis; black boxes mark coding exons (CDS), white boxes mark UTRs, the blue boxes represent the part of the CDS that will contribute to HD1 and purple boxes to HD2. A 4 bp deletion at the Cas9 cutting side (**B**) leads to a frame-shift allele with a premature *STOP* codon before HD2. (**C-G**) Brightfield images of homozygous *bcl9l∆4* mutants at different stages from gastrulation to adulthood, lateral views, anterior to the left. Maternal zygotic *bcl9l* mutants (*MZbcl9l∆4)* mutants did not show any gastrulation defects (**C,D**). At 5 dpf, we could not detect any cardiac and craniofacial phenotypes (**E,F**) as observed in zygotic *bcl9∆29* mutants (see Figure 1). (**G**) Representative image of a five-month old (5 mo) *MZbcl9l∆4* F4 mutant obtained from a cross of two adult homozygous *bcl9l∆4* mutants. Scale bars, 500 µm.

**Supplemental Figure 2: *bcl9* mutants feature normal early cardiac patterning, but variable cardiac phenotypes in *bcl9Δ29* mutants between 2-3 dpf.**

(**A-L**)*bcl9* mutants displayedunchanged *nkx2.5* (**A-D**),*myh6/amhc* (**E-H**), and *gata4* (**I-L**) expression at 24 hpfcompared to wildtype siblings: lateral and dorsal views,anterior to the left. Scale bar, 250 µm. (**M-O**) Fluorescent and brightfield (inlets) images of *drl*:EGFP transgenic wildtype and *bcl9Δ29* mutant embryos at 72 hpf (**M,N**), lateral views, anterior to the left.The hearts of *bcl9Δ29* are patterned into atria and ventricle. The two chambers are slightly misaligned and the embryos develop cardiac edema (**O**). (**O,P**) Whole-mount ISH for *vcana* reveals expanded expression around the atrio-ventricular canal and in the atrium (asterisks) suggesting a defect in valve formation, ventral views, anterior to the left. Scale bars, 200 µm (**M,N**), 500 µm (**P,Q**). Diagram in **O** depicts mean with s.d. from representative clutch of embryos, genotype confirmed by PCR.

**Supplemental Figure 3: Cardiac phenotypes *of* zebrafish *bcl9Δ29* mutants.**

(**A-O**) SPIM images of *hand2:EGFP;drl:mCherry-*expressing wildtype siblings and homozygous *bcl9Δ29* embryos at 5 dpf; ventral views, anterior to the top, imaged after viable heart-stopping BDM treatment, two individual *bcl9Δ29*-mutant embryos are shown. **A,F,K**, bright field views to illustrate the absent swimbladder in mutants (sb, asterisks in **F,K**) and different expressivity of craniofacial defects (arrowhead, **K**); **B-O** maximum-intensity projection fluorescence close-ups of the heart (red dotted outlines in **B**,**G**,**L**); **D,E,I,J,N,O** depict optical sections at AV canal level. (**P,Q**) Confocal sections of sibling control (**P**) and homozygous *bcl9Δ29* (**Q**) hearts, dissected and stained at 5 dpf to reveal the details of cardiac architecture. Also compare to Figure 2. ba, bulbus arteriosus; a, atrium; v ventricle; av, atrioventricular canal. Scale bars **B-E,G-J,L-O** 100 m, **S**,**T** 20 m.

**Supplemental Figure 4:** **Craniofacial defects in *bcl9* and *pygo1/2* mutants.**

(**A-F**) Alcian blue staining of the pharyngeal cartilage of 5 dpf wildtype, *bcl9Δ29*, and double homozygous *pygo1Δ5*;*pygo2Δ1* embryos shown in ventral (**A,C,E**) and lateral (**B,D,F**) views, anterior to the left. *bcl9*and *pygo1/2* mutants have severe malformations of the pharyngeal apparatus with fusions defect of the ceratohyal (ch) and ceratobranchial 1 (cb1) arches and miss-shaped Meckel’s (m) and palatoquadrate (pq) cartilage. Scale bars, 100 µm. (**G-L**) SPIM-based brightfield imaging of wildtype-appearing siblings and homozygous *pygo1Δ5*;*pygo2Δ1* embryos. Note absence of swim bladder (sb) and craniofacial defects in double-mutants (**G,J**). Ventral close-up of cardiac region (square in **H**,**K**, enlarged in **I**,**L** with red outline depicting the heart), showing abnormal heart looping and smaller bulbus arteriosus region in homozygous mutants. (**M**) Quantification of phenotypes in four individual *pygo1Δ5/wt* x *pygo2Δ1/wt* crosses reveal defects in 7-18% of all embryos. Genotyping of phenotypic embryos revealed phenotype occurrence in homozygous *pygo2Δ1* mutants in combination with homozygous or heterozygous *pygo1Δ5* mutation. (**N-P**) *MZpygo1Δ5* (**N**) and homozygous *pygo2Δ1* (**O**) mutants, as well as mutants carrying a homozygous *pygo1Δ5* combined with heterozygous *pygo2Δ1* alleles (**P**) are viable and fertile; lateral views, anterior to the left.

**Supplemental Figure 5: *Pygo1/2*-KO mouse embryos display severe valve and septal defects.**

(**A**) Volume renderings generated from SPIM images of control (left panels) and *Pygo1/2*-KO (right panels) 13.5 dpc mouse heart embryos. The images show hearts from different perspectives. Additionally, an internal view is shown on the bottom of the panel. Dashed blues lines indicate the atrio-ventricular septum. Dashed white lines indicate the opening, found only in mutant hearts due to septum malformations, between the cardiac chambers. (**B**) Virtual sections generated by SPIM imaging of 13.5 dpc mouse control (left panels) or *Pygo1/2*-KO (right panels) embryonic hearts. Dashed yellow lines mark atrio-ventricular valves. Reduction in valve leaflet thickness, resulting in aberrantly communicating chambers, is evident in the mutant.

**Supplemental Figure 6: *Bcl9/9l-HD1 and* Bcl9/9l-ΔHD1/ΔHD2 mouse embryos display heart defects recapitulating the *Pygo1/2*-KO phenotype.**

(**A-L**) Haematoxylin/eosin stained transverse sections of the murine heart at 13.5 dpc. Compare control (**A**,**B**) with *Bcl9/9l-HD1* (**C-F**), and control (**G,L**) with *Bcl9/9l-HD1/-HD2* mutant littermates (**I-L**). The reduction of the myocardium is indicated by arrowheads, while defective formation of the atrial septum by asterisks.

**Supplementary Figure 7: Loss of the tripartite PYGO-BCL9--catenin complex formation leads to cartilage defects in the mouse.**

(**A-F**) Alcian blue cartilage staining reveals several cartilage defects in *Bcl9/9l-Δ1/Δ2* embryos, such as loss of digit formation (compare **A,B** with **D,E**) and rib bifurcations (**C,D**).

**Supplemental Figure 8: Functional inhibition of Bcl9--catenin-interaction in *bcl9∆29* mutants and wildtype leads to craniofacial, cardiac, and fin defects.**

(**A-E**) Embryos obtained from a heterozygous *bcl9∆29 (bcl9∆29/wt)* incross were treated with 10 µM LH-2-40 from 4-cell stage on, lateral views, anterior to the left. Phenotype classes as described in Figure 4 were observed independent of the genotype of the embryo.(**F**) General phenotype penetrance is comparable to wildtype embryos treated with 10 µM LH-2-40 from 4-cell stage on (see Figure **4F**). Nevertheless, the penetrance of strong phenotypes is increased in treated *bcl9∆29* crosses.(**G,H**)In addition to cardiac and craniofacial defects, Bcl9-inhibited embryos (wildtype or *bcl9∆29* incrosses) are characterized by readily observable fin defects not observed in untreated *bcl9∆29* mutants (arrow heads **H**, compare to control in **G**).

**Supplemental Figure 9: Total set of differentially expressed genes in *bcl9∆29* mutants compared to wildtype.**

Volcano plot depicting the set of upregulated (74) and downregulated (83) genes in *bcl9∆29*mutant zebrafish embryos with an absolute value of the logFC above 1, p<0.12.

**Supplementary Table S1: De-regulated genes in *bcl9∆29* zebrafish mutants with expression in cardiac, pharyngeal, and craniofacial precursors/derivatives as per ZFIN annotations and selected publications.**

**Supplementary Table S2: Examples of candidate genes deregulated in *bcl9∆29* zebrafish mutants and with known functions in heart and craniofacial development**

**Supplementary Table S3: *Pygo1/2* loss-of-function leads to broad secondary gene expression changes.**