Figure S1

Sequence Alignments. Alignment and conservation of the Human and Zebrafish cDNA and protein sequences of the genes targeted by CRISPR/Cas9 pertaining to this study.

ABCC9_p.G989E

```
Human      ACCTGCTGGCGCTACCTGACATCTggaGGATTCTTCCTGCTCATCCTGATG   2991
            T  C  W  R  Y  L  T  S  G  G  F  F  L  L  I  L  M    997
Zebrafish  ATGTGCTGCTGCTATCTCTCCTCAggcGGCTTCCTCATGGTCTTCCTAATG   2972
            M  C  C  C  Y  L  S  S  G  G  F  L  M  V  F  L  M    991
```

ABCC9 p.C1043Y

```
Human      TATGTGGCTGGCTTTAGCATACTCtgtGGAGCAGGCATTTTCCTTTGCCTT   3052
            Y  V  A  G  F  S  I  L  C  G  A  G  I  F  L  C  L   1051
Zebrafish  TATGTGCCGGTTTTTATTATCCTGtgcGCAGCGGCAATAGCTCTTTGCCTC   3079
            Y  V  P  V  F  I  I  L  C  A  A  A  I  A  L  C  L   1060
```

KCNJ8 p.V65M

```
Human      CTACAGGACATCTTCACCACCTTGgtgGACCTGAAATGGCGCCACACGCTG    219
            L  Q  D  I  F  T  T  L  V  D  L  K  W  R  H  T  L     73
Zebrafish  CTGCAGGACGTCTTCACCACTCTGgtgGATCTGAAGTGGCGCTTCACGCTG    219
            L  Q  D  V  F  T  T  L  V  D  L  K  W  R  F  T  L     73
```

PLN p. R14Del

```
Human      TACCTCACTCGCTCAGCTATAAGAagaGCCTCAACCATTGAAATGCCTCAA     66
            Y  L  T  R  S  A  I  R  R  A  S  T  I  E  M  P  Q     22
Zebrafish  CACATGACACGGGCGGCCATTCGGcggGCGTCCACCATGGAGGTTCCCCAA     66
            H  M  T  R  A  A  I  R  R  A  S  T  M  E  V  P  Q     22
```

Figure S2

Reference sequences for primers and oligonucleotides used in this study and targeting strategy. (A) Sequences of PCR primers used for sequencing. **(B)** Sequences of constant oligonucleotide and sgRNAs used for targeting of the Cas9 nuclease to the specific site(s) of mutation(s). **(C)** Step-by-step explanatory design of the template oligonucleotides used to introduce patient-specific mutations in the *abcc9*, *kcnj8* and *pln* genes.

A

Sequencing Primers

Primer name	Sequence	Size
Abcc9_ZF.G989E_F	CACTGAAAAATGGTCTTGGACA	222
Abcc9_ZF.G989E_R	AATAGTCGATGGCCACCATAAC	222
Abcc9_ZF.C1043Y_F	TGCATCAGCTCTTTTTCTTTCA	168
Abcc9_ZF.C1043Y_R	AGCCACTCCAAGAAACTCTACG	168
Kcnj8_ZF.V65M_F	GACACGCTCAGGAGAAGTTTTT	278
Kcnj8_ZF.V65M_R	GCACAGAAATGTCATGGTGAAG	278
Pln_ZF.R14Del_F	ACCTCTTCCATCACCACAAC	320
Pln_ZF.R14Del_R	ACAGTCCTTCGCTCTCATTG	320

B

Constant Oligo (80bp)

AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC

sgRNAs (60bp)

Name of sgRNA	Promoter region(17nt)	Gene specific region (20nt)	Overlap region (23nt)
Abcc9_p.G989E	TAATACGACTCACTATA	GACCATGAGGAAGCCGCCTG	GTTTTAGAGCTAGAAATAGCAAG
Abcc9_p.C1043Y	TAATACGACTCACTATA	GAGCTATTGCCGCTGCGCAC	GTTTTAGAGCTAGAAATAGCAAG
Kcnj8_p.V65M	TAATACGACTCACTATA	GCAGGACGTCTTCACCACTC	GTTTTAGAGCTAGAAATAGCAAG
Pln_p.R14Del	TAATACGACTCACTATA	GGCACGGGCGGCCATTCGGC	GTTTTAGAGCTAGAAATAGCAAG

C

Template Oligonucleotides (injected in **bold**)

Abcc9_p.Gly989Glu

Genomic:
ATGTGCTGCTGCTATCTCTCCTCAGGCGGCTTCCTCATGGTCTTCCTAATG

SNP change:
ATGTGCTGCTGCTATCTCTCCTCAGAAGGCTTCCTCATGGTCTTCCTAATG

PAM change:
ATGTGCTGCTGCTATCTCTCATCAGAAGGCTTCCTCATGGTCTTCCTAATG

Abcc9_p.Cys1043Tyr

Genomic:
TATGTGCCGGTTTTTATTATCCTGTGCGCAGCGGCAATAGCTCTTTGCCTC

SNP change:
TATGTGCCGGTTTTTATTATCCTGTACGCAGCGGCAATAGCTCTTTGCCTC

PAM change:
TATGTGCCGGTTTTTATTATACTGTACGCAGCGGCAATAGCTCTTTGCCTC

Kcnj8_p.V65M

Genomic:
CCTGCAGGACGTCTTCACCACTCTGGTGGATCTGAAGTGGCGCTTCACGCT

SNP change:
CCTGCAGGACGTCTTCACCACTCTGATGGATCTGAAGTGGCGCTTCACGCT

PAM change:
CCTGCAGGACGTCTTCACCACTCTGATGGATCTGAAGTGGCGCTTCACGCT

Pln_p.R14Del

Genomic:
TGACACGGGCGGCCATTCGGCGGGCGTCCACCATGGAGGTTCCCCAACAGGCCA

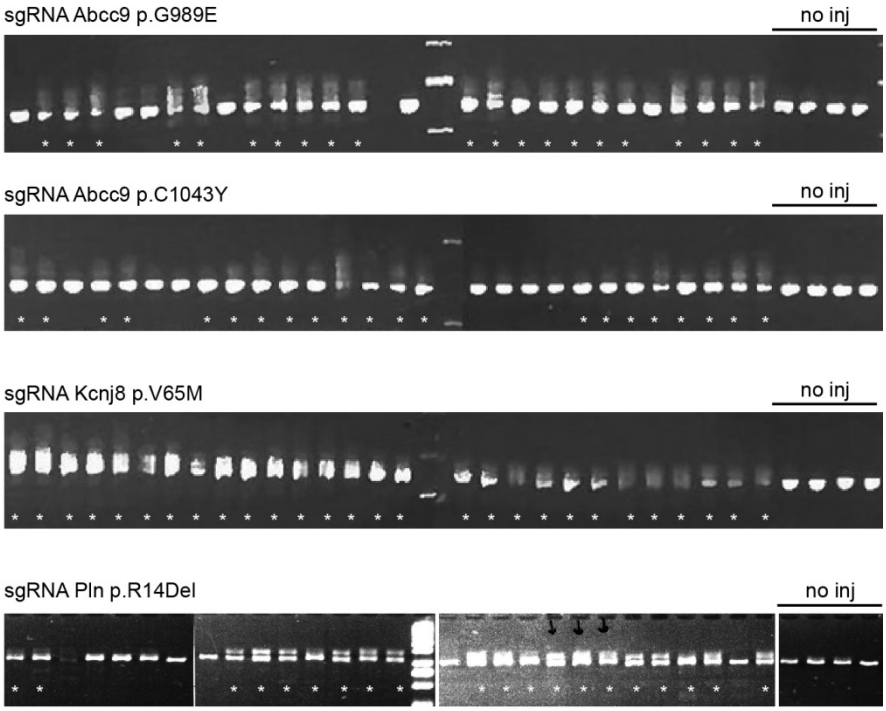
SNP (deletion):
TGACACGGGCGGCCATTCGGGCGTCCACCATGGAGGTTCCCCAACAGGCCA

PAM change (deleted):
TGACACGGGCGGCCATTC*GGGCGTC*CACCATGGAGGTTCCCCAACAGGCCA
/\
BglI (GCCNNNNNGGC)

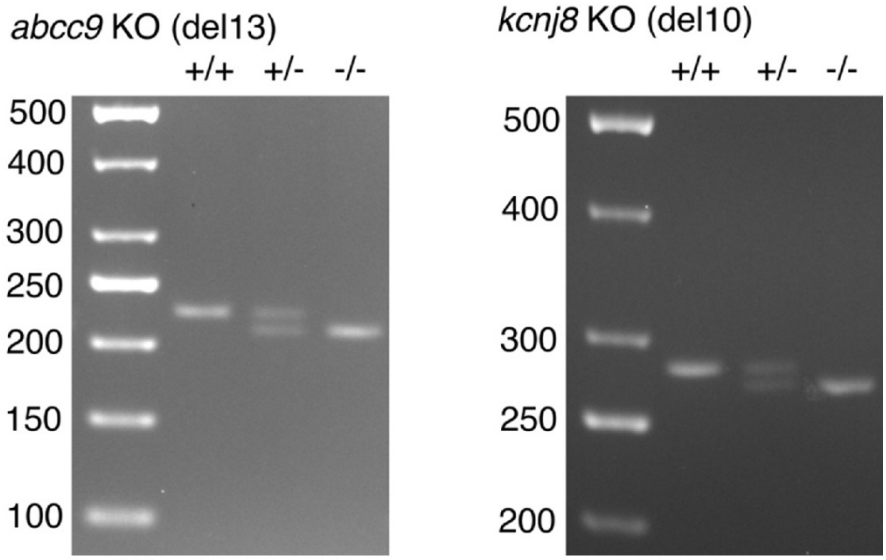
Figure S3

Electrophoresis-based genotyping strategy. (a) Efficiency of sgRNAs is initially tested by observing PCR products encompassing the target sites on a 4% agarose gel. While non-injected controls yield sharp PCR bands, sgRNAs induce the appearance of extra PCR bands or smears (denoted by asterisks), denoting the occurrence of DNA indels at the target site. **(b)** CRISPR/Cas9-induced deletions or insertions of 5 nucleotides or more can be visualized by running PCR products of approximately 200bp encompassing the site of mutations on a 4% agarose gel. The example given here is of a *abcc9* KO line harboring a CRISPR/Cas9-induced deletion of 13 bp and a *kcnj8* KO line presenting with a deletion of 10 bp. **(c)** In case of introduction of a restriction site, carriers of the mutation can be identified by amplifying the genomic sequence encompassing the mutation by PCR, enzymatic restriction and electrophoresis on a 2% agarose gel. Identification of $pln^{+/R14del}$ after *BglI* restriction is shown as example.

a



b



c

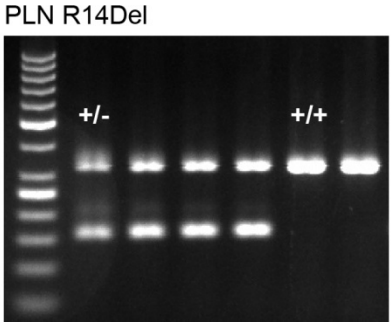
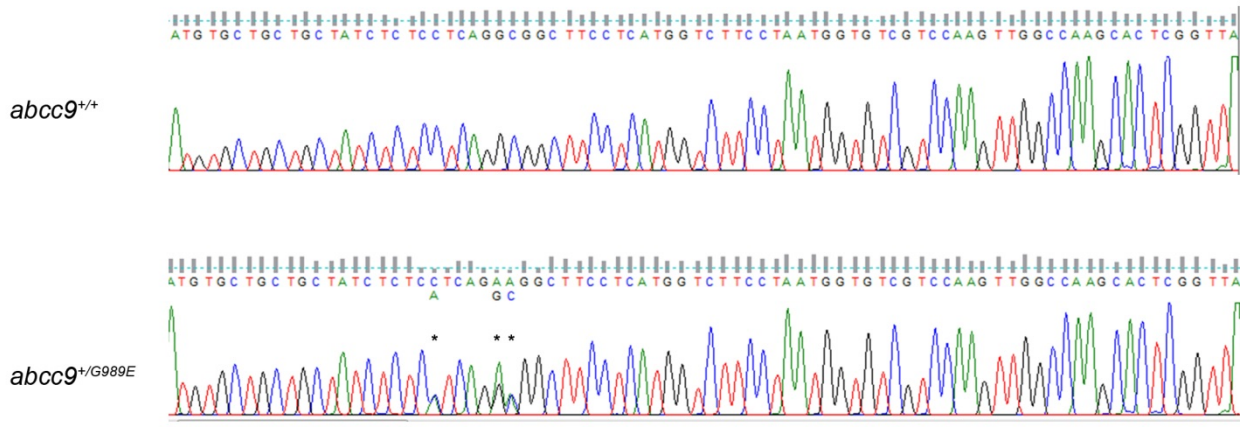


Figure S4

Sequencing traces for (A) wildtype and heterozygous *abcc*$^{9+/G989E}$ and (B) wildtype and *abcc9*$^{+/C1043Y}$ zebrafish. Single nucleotide changes are denoted by an asterisk

A



B

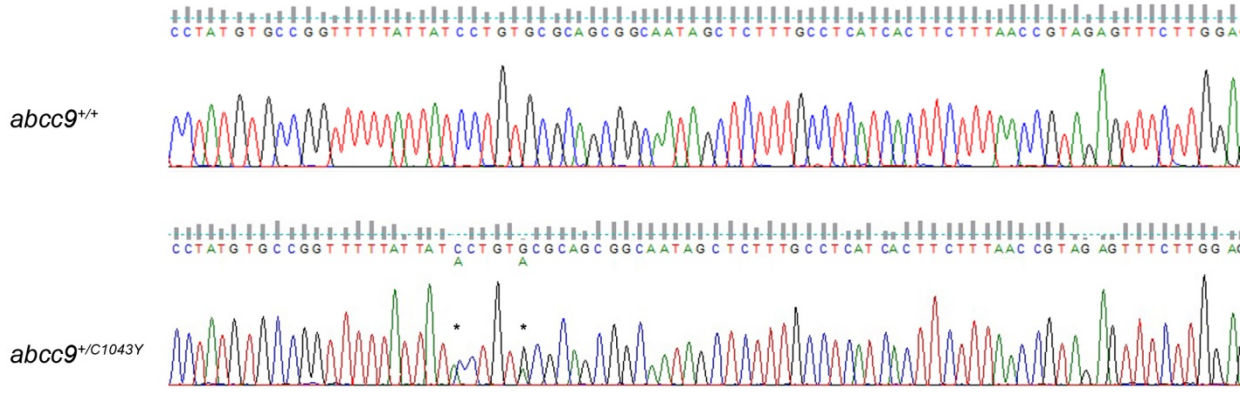


Figure S5

Testing of sgRNA for off-target effects. For each genomic location tested, the partial homologous sequence to the sgRNA is highlighted in magenta.

ABCC9 G989E: Gene-specific region sgRNA: GACCATGAGGAAGCCGCCTG

Locus tested:

- Danio rerio strain Tuebingen chromosome 13, GRCz11 Primary Assembly

Features:
attractin-like protein 1 isoform X1

```
Query  3         CCATGAGGAAGCCGC  17
                 |||||||||||||||
Sbjct  20091645  CCATGAGGAAGCCGC  20091659
```

Sequencing:

```
GGGAGAGCAAAAATAGCAAAACAGACTCTGCAATACTGAATCTGAAAGTTATTAAAAACGTATATGTTTGTATTGAATTGTTGTGT
GAATTTGCAGTAGTGTTAATATGATCTTTGTGAATGGTTGCATCAGTTTGGTACTTGAATATCATATATATTGAAACAAAGTACAA
GTATAAGACATGGGAGAAATTCAAACCGTAAACCATCAATCTTAACTATTACTTTTGCATTAAATCATTCAGCAAACTTTTTTTTT
TAAAGTTAATTAAAAATGAGATGAGGTAATTAATTACTTTATATGACTTATTTTGGTTGCCCTAGCATTCTGTACACATACTAATA
ATTCATATCAACCCTACCATTTAAATTTACCTGGTTATTTCAACATTTGTCAAGCCAACATCAAAGTTTGTTTCAAGCTACTTATT
ATTACTTTAATAAAATATAATTTTTGGCTGTTATGATTTTTAATTTCATTTCAATGTAATATCCCTTTTTGTAATGTTGAGAGTTG
TGTGTGTGTGTGTTTGATCCATGAGGAAGCCGCTATTTAATTAGACAGGCCCTCCTGAGGAGCACTCAAGCGAGAACGCTTAAACC
CCGCAAGCAACAATCGCGTGTGTTATTGTGAGACTCTCACACACACACACGTAGCTGTCTCAGTGTGAAATGTGGCACTCGGAGGA
TTTATCTGCTAATTCGGTCTCCTGCAAGGAGACACCACACACACACACTGTTTATTTAGTCAGGTTTGAGGGTCCGTCGCTGTGTGGC
TGGAAGGATCAACAGTGGGCAGTTAGAGAAACACTCTTTTCCTCTCTCACCTGTTTCTCATCTTTCTTTCCTTTCAAACTAAGAGA
GCAGTAGGACTAGACAGATGACTAGAGGGCATGCCCATTGTCTGACACCTGCTGTTTTCATTTTCCATCCCTTTATTTGTTCTCTC
AGTCCTTGTCTTGTTATGTCTTTCTCTTGCACTTCTTTTTTTTTTTTGGACCTCATGTTGTTCTGAACTGGGTTCACTTGCTTTAA
TTTGCTGTCGTTCAATAGCAAAGAAGTCAGTTAAACCTGGAAAAAATACCTGGCATTTGCATTCATCTCTGGCAGTTATGTTGAAA
TACGTGCTTTAAAAAAAAGAAAAATAAATCAACAAAATACACAATTTAATGA
ABCC9_989_ctrl2_F       TTTTGGTTGCCCTAGCATTC
ABCC9_989_Ctrl2_R       TAACACACGCGATTGTTGCT
```

Result: Wild type

ABCC9 C1043Y: Gene-specific region sgRNA: GAGCTATTGCCGCTGCGCAC

Loci tested:

- Danio rerio strain Tuebingen chromosome 7, GRCz11 Primary Assembly

57861 bp at 5' side: synaptic vesicle membrane protein VAT-1 homolog-like15359 bp at 3' side: C-type lectin domain family 3 member A

```
Query  2         AGCTATTGCCGCTGC  16
                 ||||||||||||||
Sbjct  65372001  AGCTATTGCCGCTGC  65372015
```

Sequencing:

ATTCCGTCTCGCCCTGCCTATAAGGCCTATAGTAGCACACACACAAAACCTCACACAAATCTTTAAAAGAAATACATATAGCTGCA
GAACATCTTTTAACACAATGGTGTTCATCAATTTTAAGAGCATATTGTCTCCTTCAGAGTAGTACAGATAATAAAAACTCATTCAT
ATATATATATATATATATATATATATATATATATAAATGGAATGAATTCACTACTTTATTTGTTTATTTATTTGGTGAACGTGAAGTA
GGGTACTTCACACTGTAATCTGTTTGAGACTCTGCTGCCCCCTGTAGGCTTGTCTATGGCGCTACACACTTGACATCAGACAACAT
TGAACTTAATGGAATCAGAAGCACATATACTAGATATACTGAAGTGTTTTGTGTGAATGTTTGTATGTAATTTCTTACTTTTTTGT
GTGTTAACCTGTAGGTGAAGGAAGCAATGCAGAGAATCCACGACAGAGGAAACATAGGGAAGCTCATTCTGGACGTGGAAAAGTCT
CCCACGCCTTTGGTGAGTGATTCATCCACAAACCTGCTTTAGAATTAAACCACAGTAATAAGATGAGCAAAACATCATGTGCAAAA
CATAGTAATTATTGTGTCAGCTATTGCCGCTGCCCTTTCATCATTTTGTTTACGGAATGTCTTTCATTGTTGGGTTTTCGAACCAT
GTTATTTTTTTTATGTTCAATCTACGCGCTCCAAAGGGGTTCGAGAGAGACTCATTTTATGAAGCATGCTTAAACAATACACCTGT
TTACTTCTAGTAGGAACACCAAATGGTCCTGTGAGAGTGATTGAAGAGTCAGGCGACATCACCTCAGCAGTTTCCATGGTGACAGT
GCCTTATGACGCTGTTTTCAGAAGAGACAATCTGTTTTAACAGTGGCCTTCAACGGCACACTTTCATCACAACTACTCATAGCGGC
TTCACTTTAACCTGGGGGATTCTTAAAGGAGCCACTCAAGAGCAGCAGATGAACAATTGATTTCTCCTTTCTTTTACAGAAGATTT
TTGAACGTACTGAAGAAAAAAAAACATACAAAATTAAAAAATATCTAATAAAAATGACAAGTATATAAATAAATAAATGCATGTAC
ATACACACTCAGGGCCACACGGTTGCGCAGTGGGTAGCATGATCACCTCACAGCAAGAAGGTCACTGGTTCGAGCCTCGGTTGGGC
CAGTTGGCATTTCTGTGTGGAGTTTGCATGTTCTCCCCTTGTTGGTGTGGGTTTTC

```
ABCC9_1043_Ctrl1_F      ACGCCTTTGGTGAGTGATTC
ABCC9_1043_Ctrl1_R      AGGTGATGTCGCCTGACTCT
```

Result: Wild type

- Danio rerio strain Tuebingen chromosome 1, GRCz11 Primary Assembly

70372 bp at 5' side: VPS10 domain-containing receptor SorCS327098 bp at 3' side: VPS10 domain-containing receptor SorCS1

```
Query  5         TATTGCCGCTGCGC  18
                 ||||||||||||||
Sbjct  48556680  TATTGCCGCTGCGC  48556693
```

Sequencing:

AGTAGGTAGTGCTGTAGCATCACAGCAAGAAGGTCTCTGGTTCGAGCCTCGGCTAGGACAGTTGACATTTCTGTGTGGAGTTTGCA
TGTTCTCTCCACGTTCATGTGGGTTTCATCCGGGTGCTCCGGTTTCCCCTACAGTCCAAAGACATGTGAATTGGGTAGGCTATTGT
CCATAATGTATGTGTGTGAATGAGTGTGTATGGATGTTTCCCATTATGGATGGGCATCCGCTGCGTTAACATGTGCTGGATAAGTT
GGCCGTTCATTAAGCTGTGGCGACCCTGTATAAATAAAAGGACTAAGCCGAAAAGAAAACGAATGAATGGGTTTTGTCTGTCTTAT
TTTTATTTAATGCTTTTTCTCTGTTCTTATGTTGTTCCAACCTACATTTCAACTTATTTTCATCATATCTTCTCACCTCGTTTAAC
CCCGACTAAGAACATTTTACAAGAGAGTAGCTTTATTTTTTATAATTTACTGTGTGTTATTTTTATTTTGCTGTTGTTGTTCTGTT
TAAATTTAATAAGAGCCAGGTCCCATCTGCCATGTTCAGTGTGCCGCTGCTTAAAAGCGCCATGCTAACGCTACTCAAAGACCGAC
TCTTTGCTAAGCTTCAAACCAAAGAACAGTCACTGAAAATAAAAACCCACCACCGCCGCTCGTCCACGTACAGCTCATTTTTTCAT
CATCATCGTTTGTAGTTTTCATATTGCCGCTGCGCCTTTGGAGGCCTTCCATGCCATCGGATTGATTTCTCTTTGAGACAGATAAT
TACTCTCTCCGTTTGGCAAAGGAAATGTGTAAATAGCTTTGCGAGTCCCACCCCGGAGACGATGTGTCTTTGCGTGCCTTCCATCT
GCCAGAGCTCAGGGGTAACTGAGAACAACATTTAAAACATTACAAGCTATTATACAAGCCCTGCTCCCCTAGAATTACAGCTATCG
TCTTTATGGAAATAAATTAATTAATAAATAAATAACCTGGAGGTGCAGCGTGGAGTTGAGCTGCACGGCAGGTGTGGGATTACAGT
TATGGCATAAATAACAAACACTTCATCATTGGCTGGAATGAGAACATGGCTCTTAATGAG

```
ABCC9_1043_Ctrl2_F      ATCTGCCATGTTCAGTGTGC
ABCC9_1043_Ctrl2_R      TAGGGGAGCAGGGCTTGTAT
```

Result: Wild type

- Danio rerio strain Tuebingen chromosome 9, GRCz11 Primary Assembly

Features:

FERM and PDZ domain-containing protein 4 isoform X2FERM and PDZ domain-containing protein 4 isoform X1

```
Query  1         GAGCTATTGCCGCT  14
                 ||||||||||||||
Sbjct  54082218  GAGCTATTGCCGCT  54082205
```

Sequencing:

```
TCAATAGCAGTCAAATGTTGTTTTGGACGGTTTAAAACTTGAGTATCGATCGGTACCGAATTCCAGTATCGTGACAACACCTAATA
ATATGTATTTGGGTTGTCACGATACTGGAATTTAGTACCATTCATTCATTCATTCATTAATTTATTTTTTGTCAGCTTAGTCTCTT
TATTAATTCGAGGTCGCCATAGCGGAATGAACCGCCAGCTTATCCAGTAGGGATGTCAAAATTAATTGTTTTTTCGGTGCACTGCG
ATGCAGACGCGGATAATTCGGTATCGGTTCAGTAAAAATCATGTACCGACGTCATTTATCTCCTATGCGCTCTGTCGCGAGGGAGG
TGTATTTACAACACTCAGGAGCCAACTCAGGGCTGGCTCGTGGCATAGGCAGTATAGGCAAATGCCAAGGGTGCTGTAAATCCAGG
GGGGCACCAGAAATAGCGGCAATAGCTCAAAAACCTCTTACCCTAAAAACATTTAAAGTGTGTTTCCTACAAAAAGACAAAGCTGG
TTATGTTTCACAGCTGTCCTTTTCCTTTTTTTTTTTTGCTCGACATTACGACCACTGCTCCCTTTAAGCTCTCACAATATGCTTTT
AGCCGGGATAGCTCGCACGGAGCCGAACTCTCAGTAAGGGATCCTCGGAAAAGTGCGTCTTTATTTTCTTTTCTTTTTCTTGCTCC
GCTCAGCGCGAGCTCTCCCGGTTAAACGCATATTGAGAGGGCTGTGTGTGGAAAGTGTGGAGGGCTGTGTGTGGAGAGGAAGTGTG
TGTGTGGGTTTGTTTGTTTGGTGCTGTCTATGTTTGTGTATGTGTGTGAATGAGAGACTGTGTGTGTGTGTGTGTGTGTGTGTG
TGTGTGTGTTTGTTTATATAGCTTGTTATAGCCTTCCCCCAAAATATAACTGTGTATGGAAAGCATCGTCAATGCACCAAGATATC
GAATTGAACCGAATCGATGGCATGATAATCGTAACCGAACCGTG
ABCC9_1043_Ctrl3_F     AACCGCCAGCTTATCCAGTA
ABCC9_1043_Ctrl3_R     GGGAGCAGTGGTCGTAATGT
```

Result: Wild type

KCNJ8 V65M: Gene-specific region sgRNA: GCAGGACGTCTTCACCACTC

Loci tested:

- Danio rerio strain Tuebingen chromosome 5, GRCz11 Primary Assembly

Features:
10462 bp at 5' side: GTPase Era, mitochondrial290 bp at 3' side: T-box transcription factor TBX6L isoform X2

```
Query  1         GCAGGACGTCTTCACCACTC  20
                 |||||| ||||||||||||
Sbjct  42280311  GCAGGAAGTCTTCACCACTC  42280292
```

Sequencing:

```
ATGCTTTGAAATGCATATCTTTGTTGTTATTCTTTATTATTATTATTATTATTATGTTTGCCACACTGTTAGAAAGTCTAAACACA
TTCAAAACCAATATAGAAATGCACTCAAGTTGTCATAAATGTGCATCCAGGATGACTCTGCTCAGCTTTCAGTAAACACCCTCAGC
AAATTACCTGTAAGCAGATTTGAATGTTGAGAATTGGAAAATATCCACCAAACATGGAACTTTGACAGTTCTGAGAGTGTGGCATT
CAAGAGTGGTGAAGACTTCCTGCAGTAATGAGGGGACAAACTGCTGGGGTGATTAACACCTATATGTGCGTCTCTATGGGTTTGGA
GGAAGTGTCAGGGAAGCCTTGGCCTCTAAAGAAAGACGGACCCCGTTTCCCCCTCAATTCCTCTCAATGTTGTTTCTACACACCTT
TGTGGGCTGGCTGGAGACAAAAGACAGATCAAAGAGGAGACTGATGTTTTGACAGAAGTGCACATACACACCTCTGGCCCCCTTTG
AAGTGATGGAAGAAGGTAGAAGCCTAGTCAGTTTGTAGCTTCAGTCTGGTGGACATGTATCTCCCAGAGGAACGGCCTGTGCTGGA
CTTACCCTACCAGATGAATCATTTGGCTAATAACTATGGATACTATCCACAAGGTAGGAATTTCACGTTCCTTATATTAAAGAATG
TCAAATTTCAGGTTTATTTTAAATATACATTT
```

```
KCNJ8_65_Ctrl1_F       CCACCAAACATGGAACTTTGA
KCNJ8_65_Ctrl1_R       CCGTTCCTCTGGGAGATACA
```

Result: Wild type

- Danio rerio strain Tuebingen chromosome 3, GRCz11 Primary Assembly

Features:
nucleoside diphosphate kinase, mitochondrial isoform X1nucleoside diphosphate kinase, mitochondrial

```
Query  4         GGACGTCTTCACCAC  18
                 ||||||||||||||
Sbjct  15071796  GGACGTCTTCACCAC  15071810
```

Sequencing:

```
ACACTCAAACAGGTTTAAACGGCGAGTAATGATTGAATGTTTATGTGAACTATCCCTTTAATACTTGTCAATGCTAATTTATTATT
ATTTTTTAAATACAGCTTTGCATTTTTAGTTCATGTTAGCTCATGTTTAGTAAATGTTAAGCTTTAATTATTCGTGTTAAATGAGT
TATACGTTTATTTGCATTGTTAGTTTATGTTAATGGCTTTAGTATATTGACTAACCTGAAATGTTAGTGTGGTTTAGAGCTAGTCT
GAGGTCAGTGTGAGTGTAATAATACCTGCTGATGTGCACACTGAAGTCTCCTCTGATAGTCCCCGGTGCGGCGGCTGCAGGGTCTG
TGTCTCCCACCATCATGCGGGACGTCTTCACCACATTATGACCCTCCCACACCTAAACACAAGTTAAAGCAGGACAGTCTGTGATG
GTCTGTGATCGGCAACCTTTTTCTGTTGCATTGCATCATTAATTAACAGACTAAACCACTTTAAGAAGTAAAAATGTAACTAGATA
AATACAGTTGCATTATTGGTATGCGTTTTAATATCTTAGACTGAACCTAATACAGTTGGGGCGGCACGATGACTCAGTGGTTGGCA
CTGTGGACTCACAGCAAGAAGGTTGCTGGTTTGAGTCCCGGCTGGGTCAGTTGGCGATTCTGTGTGGAGTTTGCATGTTCTCCCTG
TGTTCGCATGGGTTTGCTCCTGGTGCTCCGGTTTCCCCCACAGTCAAAACATATTCGCTATAGGTGAATTGGATGAACTAAATTAT
CTGTAGTGTGTGAATGAGTGTGTATGTGTGTTTTCCAGTAGTGAA
```

```
KCNJ8_65_Ctrl2_F       CGGCGAGTAATGATTGAATG
KCNJ8_65_Ctrl2_R       GTTTAGGTGTGGGAGGGTCA
```

Result: Wild type

Pln R14Del: Gene-specific region sgRNA: GGCACGGGCGGCCATTCGGC

Locus tested:

- Danio rerio strain Tuebingen chromosome 3, GRCz11 Primary Assembly

Features:
serine/threonine-protein kinase TAO2 isoform X2serine/threonine-protein kinase TAO2 isoform X1

```
Query  1         GGCACGGGCGGCCAT  15
                 ||||||||||||||
Sbjct  21142969  GGCACGGGCGGCCAT  21142983
```

Sequencing:

TTTGCTCGCCGGCTGACGGCTCACCTCTGTGTGAAGGGCTTTCCCACTTCAACCAGTTTGTCCGGTTAGGCCATCGTGTTGCGTCG
GCGAAGCGGAGGCCCGGAGGAGGAGGAGCAGTCTGCGGCAACGACCGAGTTCGAGTCCAGGGAAAAGCGGTTCCAGAAATCAGGTA
AGAATAAAAAACAGATTCCATAAATAAAGTGAATGAGTTCGTAACGGGGCGAGAATGCGATGAAATCCGAAAAAGAGGTCAAAATC
GGACTAGGCCTTATCTTTTTCTATACGGCTTTTATAAATCATCGCTTGGGTTTAGGGAAGGAGGAGGAGGAGGGTGGCTGGCTGGG
CCGAATGGCCGCCCGCCCAGTCAATCGTTCAGTCATTCAGTCAGTCAGTCAGACAGAAGGTCACTCGACAGCAGCCTCCAGCGCCT
TCCACTGGGCTTCGTTTCCAGAATGAGCCTGGGTTGACTAAAAACTCACCAGTGCACTTGAAAAAATATAAAAAAATACTCCCCCT
CCATACACTAGCTCTTTATTTTCTGAACACTAACAGCTACATTTTAAGAGGTCCAGTTTGAGAGACTTTTTATTAAGCTCGAGCTC
CTAACATGCACTATTTTTTTTTATTTTTTATTTGAGCTTAGGTTGGTTTTCTTTATATTTAGTTTGGATGTTAGGTGTAGAAGGTT
AGACTTCTTATATTGTAAGGACAATTAGAAATCAATGTTTCATCTGCAAGTTTGTGTGGCTAACCGACTGATTCAATGTATTTGAG
CACACCTTCTACAGGACAATTTTTTTCTGAATCAATTGTTTACTAAGGAATGAAGACAATCCCAGATGAGATAATTTTTTTCCGTT
TCTTTTTTCAAGTGTAAATAGTATGCTTAAAAGTGGAAAAAATATGGGGAATAATGATTTTTAAGTATAGAAGTTTTTATTTCACT
TGATTTGCTCTTCATTAAGAAAAGCCTGAACA

Pln_14_Ctrl2_F CTCTGTGTGAAGGGCTTTCC
Pln_14_Ctrl2_R TGCTGTCGAGTGACCTTCTG

Result: Wild type

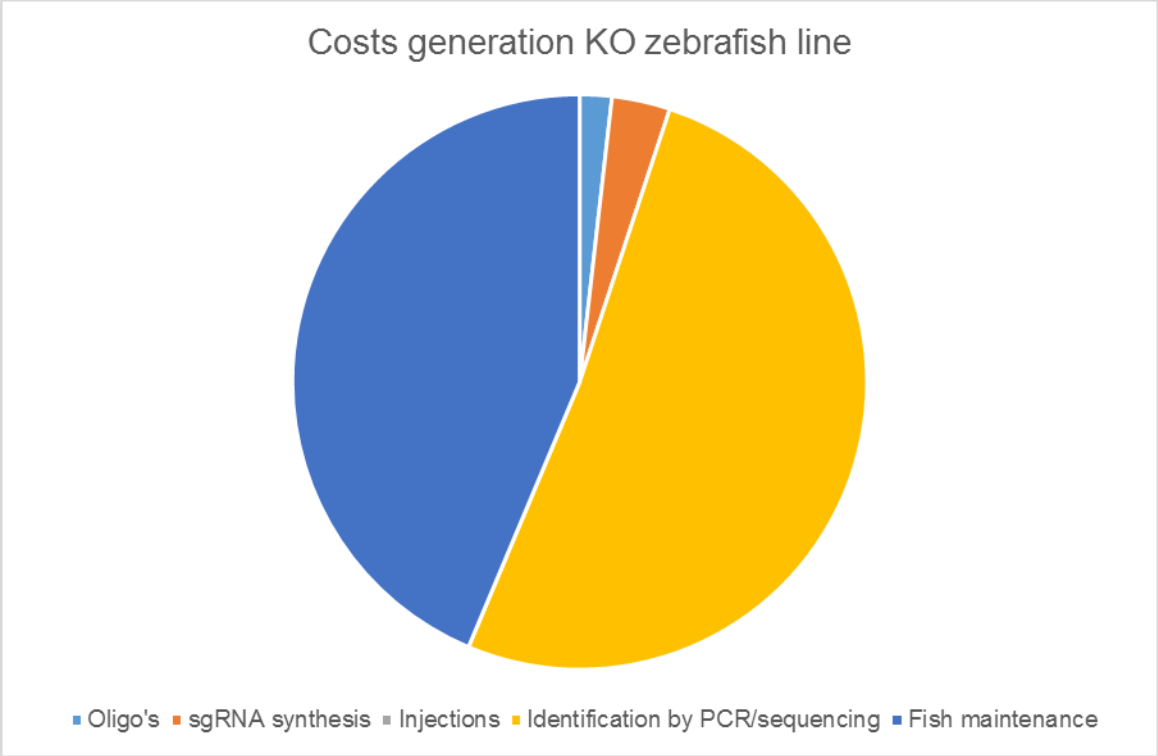
Figure S6

Costs estimate for the generation of KO zebrafish lines and patient-specific KI zebrafish lines. (**a**) Overview of the workflow for generating KO and KI zebrafish lines with estimated hands-on time shown in days. Costs are shown in euro, per procedure and are calculated for the generation of a zebrafish line for one gene, starting with two sgRNAs. A distinction has been made between the costs for a KO or a KI zebrafish line. Labor costs are not taken into account. (**b**) Contribution of distinct procedures to the costs for the generation of a KO zebrafish line. Note that the identification of the mutated alleles by PCR and sequencing and fish maintenance are relatively the highest expenditures.

a

Workflow		Hands-on time (days)	Material costs (€)	
			KO	KI
Design sgRNA's	Determine target sites, Template Oligo's	0,5	20	32
Test primers and determine SNP's	Sequence CRISPR target sites	2,0	20	20
Make sgRNA's	Synthesis of 2 sgRNA's	1,5	35	35
Test efficiency sgRNA's	- Inject embryos - Check PCR on gel	0,5 1,5	20	20
Inject founders (F0)	- Inject 2 sgRNA's separately (2 tanks)	0,5		15
Wait 3 months			110	110
Identify founders	- F0 outcross with WT	0,5		
	- Test embryos (24 per founder)			
	KO: test ±8 founders (2 plates)	5,0	275	
	KI: test ±12 founders (3 plates)	10,0		705
Grow interesting F1 (1 allele)				
Wait 3 months			160	160
Genotype F1 (HETs)	Fin clip 1 tank – PCR + sequence (1 plate)	4,0	225	225
Incross – Grow F2 (1 allele)				
Wait 3 months			215	215
Genotype F2 (HOMs)	Fin clip , PCR + gel electrophoresis (1 plate)	4,0	30	225
Total costs			1.110	1.762

b



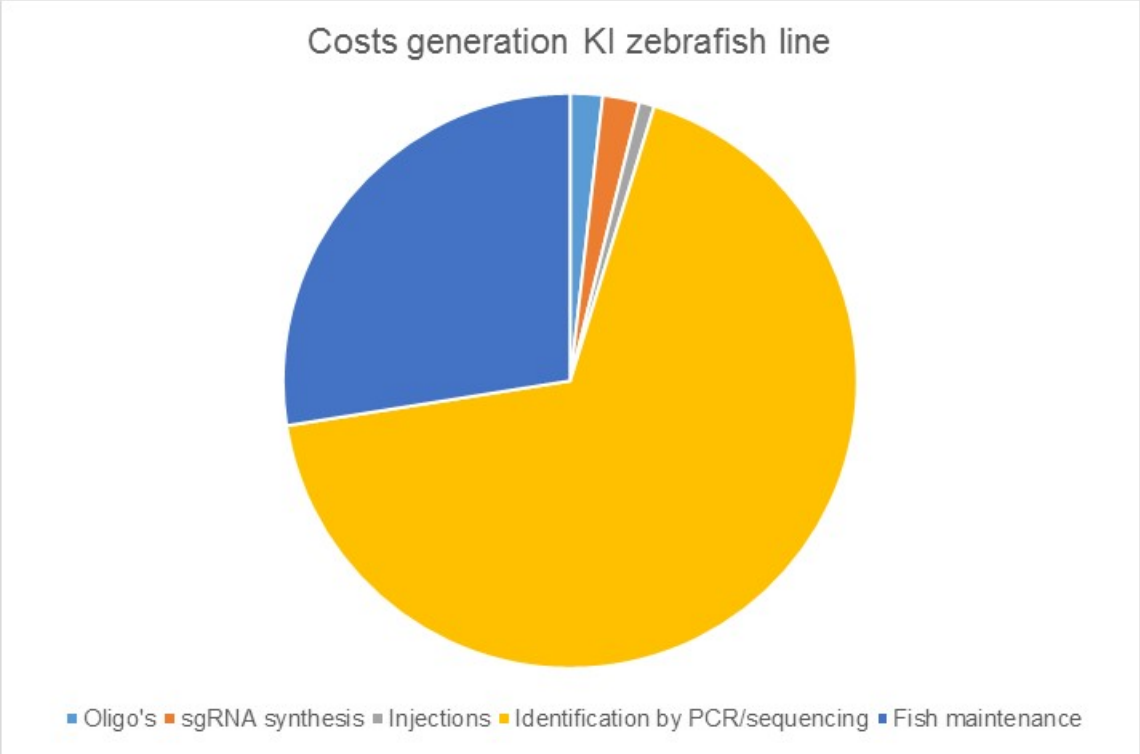


Figure S7

Homozygous *kcnj8*$^{V65M/V65M}$ mutation induces CS-related cardiac anomalies and cerebral vasodilation in zebrafish embryos. (**A**) Representative images illustrating the morphology of 5 dpf wild-type and *kcnj8*$^{V65M/V65M}$ mutants as seen from a left lateral (top) and dorsal view (bottom). (**B**) Quantification of cardiac function using individual characteristic confocal sections from a time series of the embryonic cardiac cycle at 5 dpf. Pericardial edema was quantified by measuring pericardial area using striking morphological landmarks indicated by white boxes. (**C**) Tracking of individual red blood cells (RBCs) measuring blood flow velocity in the cardinal vein. RBCs were tracked for 10 frames using ImageJ (NIH) and the plugin MTrackJ(35). (**D**) Quantification of vascular dilations in a Tg(*kdrl:GFP*) background. 3D reconstruction of vascular structure in Imaris was used to calculate vessel volume. *kcnj8*$^{+/+}$ controls are the same as in Fig.2B-D. For all graphs, significance was determined by two-tailed unpaired Student's t test or Mann–Whitney two-tailed U test: * p≤0.05; ** p≤0.01; *** p≤0.001; **** p≤0.0001. The black horizontal bar indicates the mean value for each condition. Sample size, *kcnj8*$^{+/+}$, n=21; *kcnj8*$^{V65M/V65M}$, n=19 in **B**, *kcnj8*$^{+/+}$, n=10; *kcnj8*$^{V65M/V65M}$, n=10 in **C**, *kcnj8*$^{+/+}$, n=20; *kcnj8*$^{V65M/V65M}$, n=11 in **D**. Scale bars, 1 mm **A**. All embryos analyzed originated from group matings of adult zebrafish.

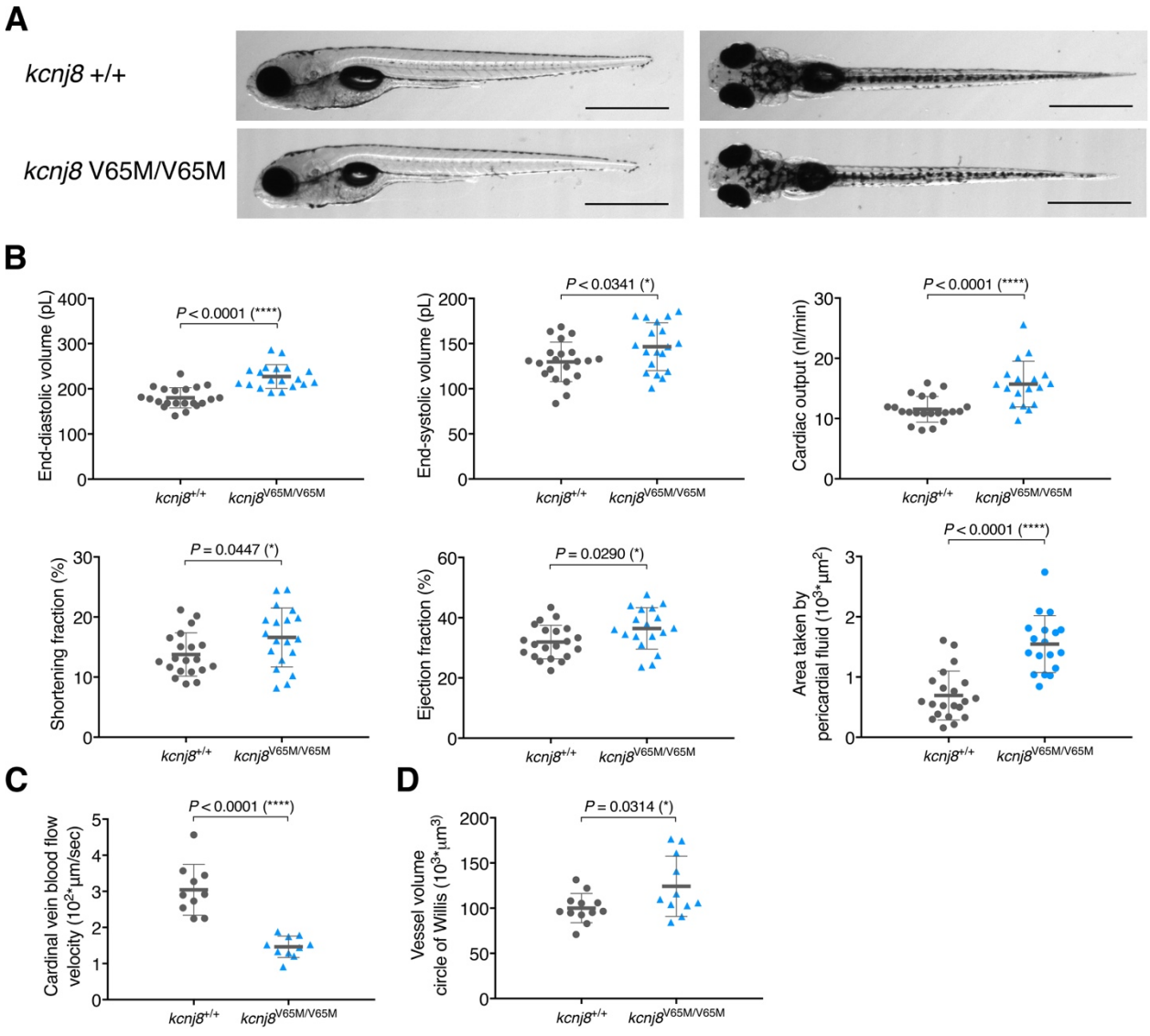


Figure S8

***kcnj8*[V65M] mutants present with enhanced stroke volume and contractile activity as well as reduced dorsal aorta blood flow velocity at 5 days post fertilization (dpf).** (**A**) Stroke volume in *kcnj8*$^{+/V65M}$ and *kcnj8*$^{V65M/V65M}$ mutants. (**B**) Fractional area change in *kcnj8*$^{+/V65M}$ and *kcnj8*$^{V65M/V65M}$ mutants to assess ventricular contractility. (**C**) Tracking of individual red blood cells (RBCs) measuring blood flow velocity in the dorsal aorta. RBCs were tracked over complete imaged area using ImageJ (NIH) and the plugin MTrackJ (35). *kcnj8*$^{+/+}$ controls are the same as in Fig.2B-C and S6. For all graphs, significance was determined by two-tailed unpaired Student's t test: * p≤0.05; ** p≤0.01; *** p≤0.001; **** p≤0.0001. The black horizontal bar indicates the mean value for each condition. Sample size, *kcnj8*$^{+/+}$, n=21; *kcnj8*$^{+/V65M}$, n=13; *kcnj8*$^{V65M/V65M}$, n=19 in **A** and **B**; *kcnj8*$^{+/+}$,n=10; *kcnj8*$^{+/V65M}$, n=7; *kcnj8*$^{V65M/V65M}$, n=7 in **C.** All embryos analyzed originated from group matings of adult zebrafish.

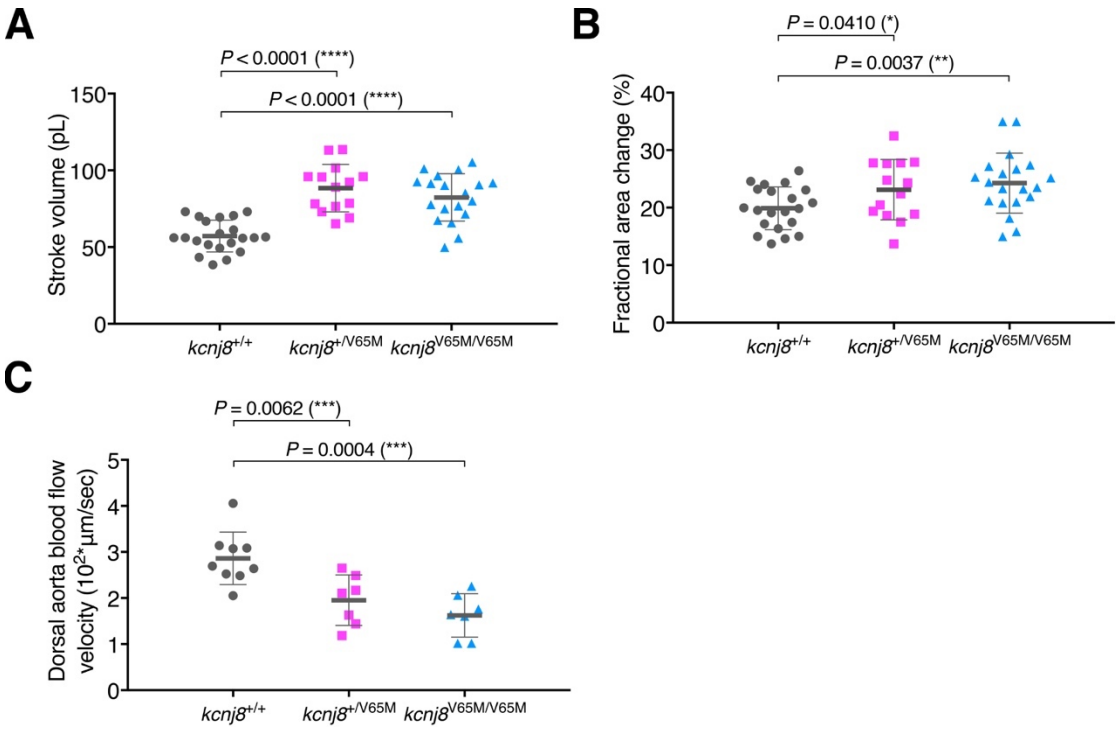


Figure S9

Adult *kcnj8*[V65M] fish show enlarged ventricular area. (**A**) Ventricular area in homozygous *kcnj8*[V65M] mutants. Representative heart histology of adult *kcnj8*$^{V65M/V65M}$ mutants and wildtype siblings after H&E staining. Exemplary depiction of 1 WT and 1 *kcnj8*[V65M] heart. For assessment of ventricular chamber size, tissue sections showing the largest ventricular area were selected and area was quantified using ImageJ (NIH). (**B**) Atrial area in heterozygous and homozygous *kcnj8*[V65M] mutants. For assessment of atrial chamber size, tissue sections showing the largest atrial area were selected and area was quantified using ImageJ (NIH). (**C**) Total body length in *kcnj8*[V65M] mutants. To account for variations in heart size overall body length was measured from the tip of the head to the end of the trunk (before the caudal fin). *kcnj8*$^{+/+}$ controls are the same as in Fig.2E. For all graphs, significance was determined by two-tailed unpaired Student's t test or Mann–Whitney two-tailed U test: * p≤0.05; ** p≤0.01; *** p≤0.001; **** p≤0.0001. The black horizontal bar indicates the mean value for each condition. Sample size, *kcnj8*$^{+/+}$, n=6; *kcnj8*$^{+/V65M}$, n=6; *kcnj8*$^{V65M/V65M}$, n=6 in **A**-**C**. Scale bars, 500 μm in **A**. All embryos analyzed originated from group matings of adult zebrafish.

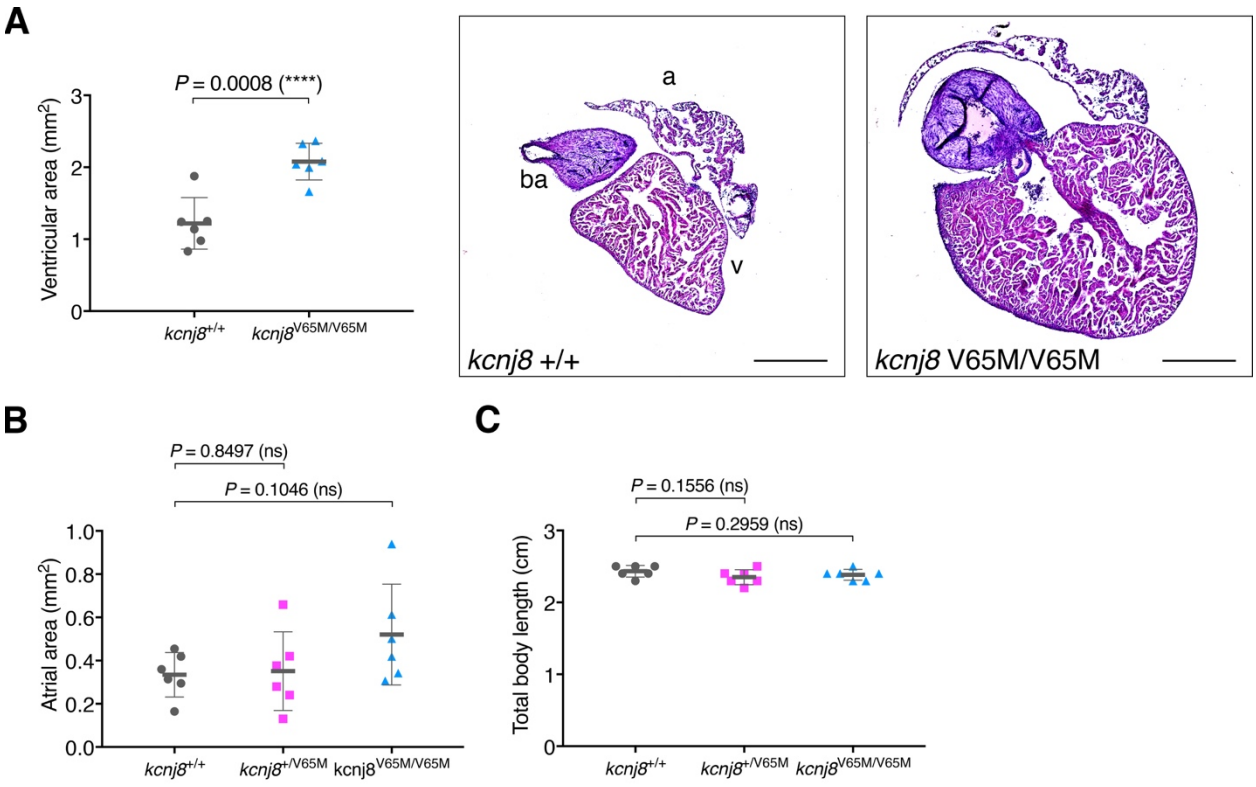


Figure S10

***abcc9*[G989E] embryos show CS-related anomalous heart function and pericardial edema at 5 dpf**. (**A**) Comparison of wild-type and *abcc9*[G989E] mutants at 5 dpf seen from a left lateral (left) and dorsal view (right). (**B**-**D**) Quantification of cardiac function using sequential still frames from high-speed imaging of the embryonic cardiac cycle at 5 dpf. Ventricular volume (B), cardiac output (**C**) and contractile function (**D**) were calculated. (**E**) Quantification of pericardial edema by measuring pericardial area using striking morphological landmarks. In order to correct for possible enlarged ventricular size in mutants, ventricular area was subtracted. (**F**) Quantification of vascular dilations in a Tg(*kdrl:GFP*) background. 3D reconstruction of vascular structure in Imaris was used to calculate vessel volume. For all graphs, significance was determined by two-tailed unpaired Student's t test or Mann–Whitney two-tailed U test: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$. The black horizontal bar indicates the mean value for each condition. Sample size, *abcc9*$^{+/+}$, n=20; *abcc9*$^{+/G989E}$, n=17; *abcc9*$^{G989E/G989E}$, n=19 in **B**-**E**; *abcc9*$^{+/+}$, n=5; *abcc9*$^{+/G989E}$, n=10; *abcc9*$^{G989E/G989E}$, n=5 in **F**. Scale bar, 1 mm in **A**. All embryos analyzed originated from group matings of adult zebrafish.

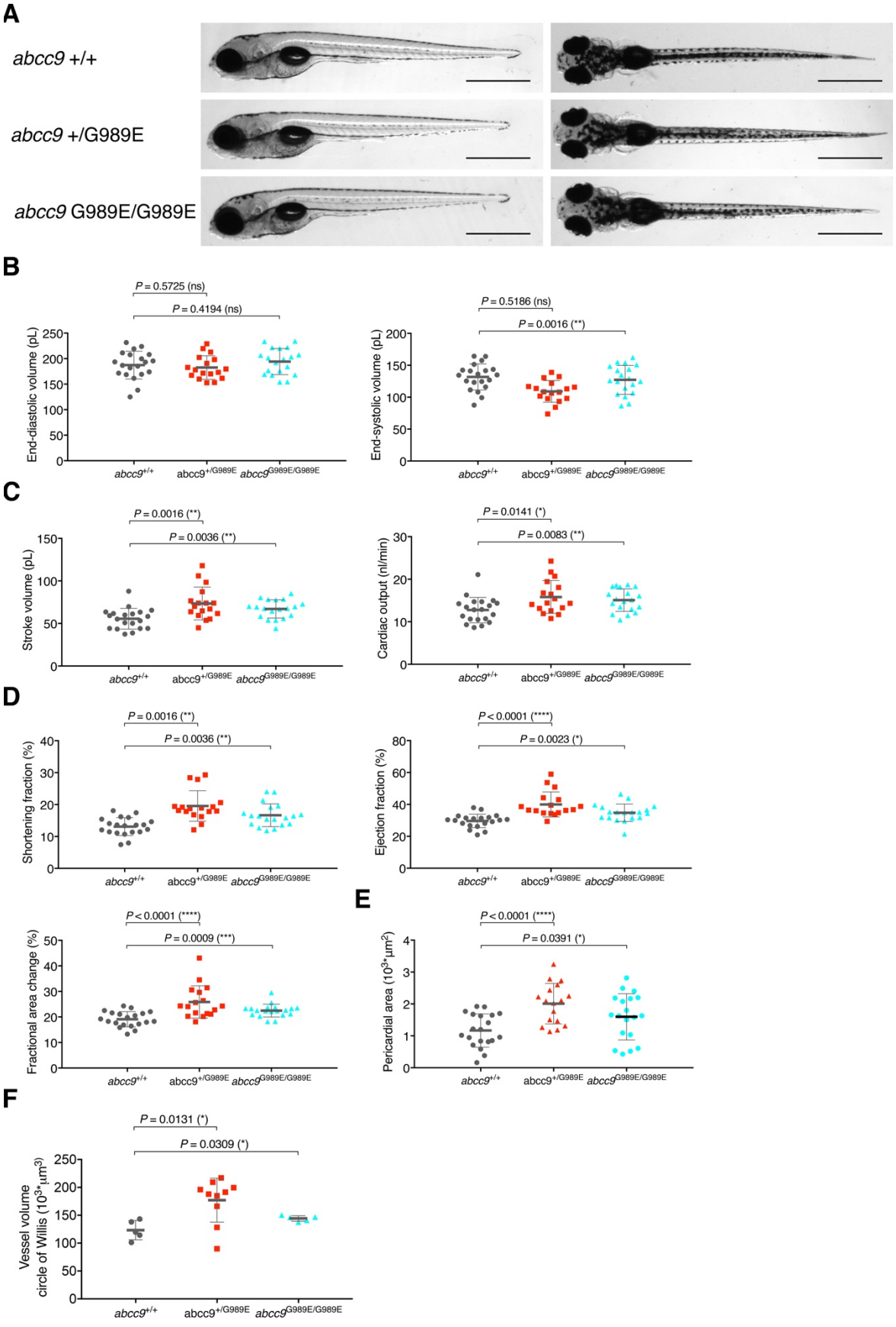

A
abcc9 +/+
abcc9 +/G989E
abcc9 G989E/G989E
B
P = 0.5725 (ns)
P = 0.4194 (ns)
End-diastolic volume (pL)
P = 0.5186 (ns)
P = 0.0016 (**)
End-systolic volume (pL)
C
P = 0.0016 (**)
P = 0.0036 (**)
Stroke volume (pL)
P = 0.0141 (*)
P = 0.0083 (**)
Cardiac output (nl/min)
D
P = 0.0016 (**)
P = 0.0036 (**)
Shortening fraction (%)
P < 0.0001 (****)
P = 0.0023 (*)
Ejection fraction (%)
P < 0.0001 (****)
P = 0.0009 (***)
Fractional area change (%)
E
P < 0.0001 (****)
P = 0.0391 (*)
Pericardial area (10³*µm²)
F
P = 0.0131 (*)
P = 0.0309 (*)
Vessel volume circle of Willis (10³*µm³)
abcc9+/+
abcc9+/G989E
abcc9G989E/G989E

Figure S11

***abcc9*[C1043Y] embryos show torturous cerebral vessels at 5 dpf**. (**A**) Comparison of wild-type and *abcc9*[C1043Y] mutants at 5 dpf seen from a left lateral (left) and dorsal view (right). (**B**-**D**) Quantification of cardiac function using sequential still frames from high-speed of the embryonic cardiac cycle at 5 dpf. Ventricular volume (B), cardiac output (C) and contractile function (D) were calculated. (**E**) Quantification of pericardial edema by measuring pericardial area using striking morphological landmarks. In order to correct for possible enlarged ventricular size in mutants, ventricular area was subtracted. (**F**) Representative confocal images of circular structure comprised of BCA and PCS in wild type and mutant 5 dpf fish are highlighted in red. (**G**) Quantification of phenotype shown in (F) in heterozygous, homozygous and wildtype siblings, (**H**) Quantification of vascular dilations in a Tg(*kdrl:GFP*) background. 3D reconstruction of vascular structure in Imaris was used to calculate vessel volume. For all graphs, significance was determined by two-tailed unpaired Student's t test: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$. The black horizontal bar indicates the mean value for each condition. Sample size, *abcc9*$^{+/+}$, n=20; *abcc9*$^{+/C1043Y}$, n=20; *abcc9*$^{C1043Y/C1043Y}$, n=11 in **B**-**E**; *abcc9*$^{+/+}$, n=6; *abcc9*$^{+/C1043Y}$, n=12; *abcc9*$^{C1043Y/C1043Y}$, n=5 in **G-H**. Scale bar, 1 mm in **A**, 50 μm in **F**. All embryos analyzed originated from group matings of adult zebrafish.

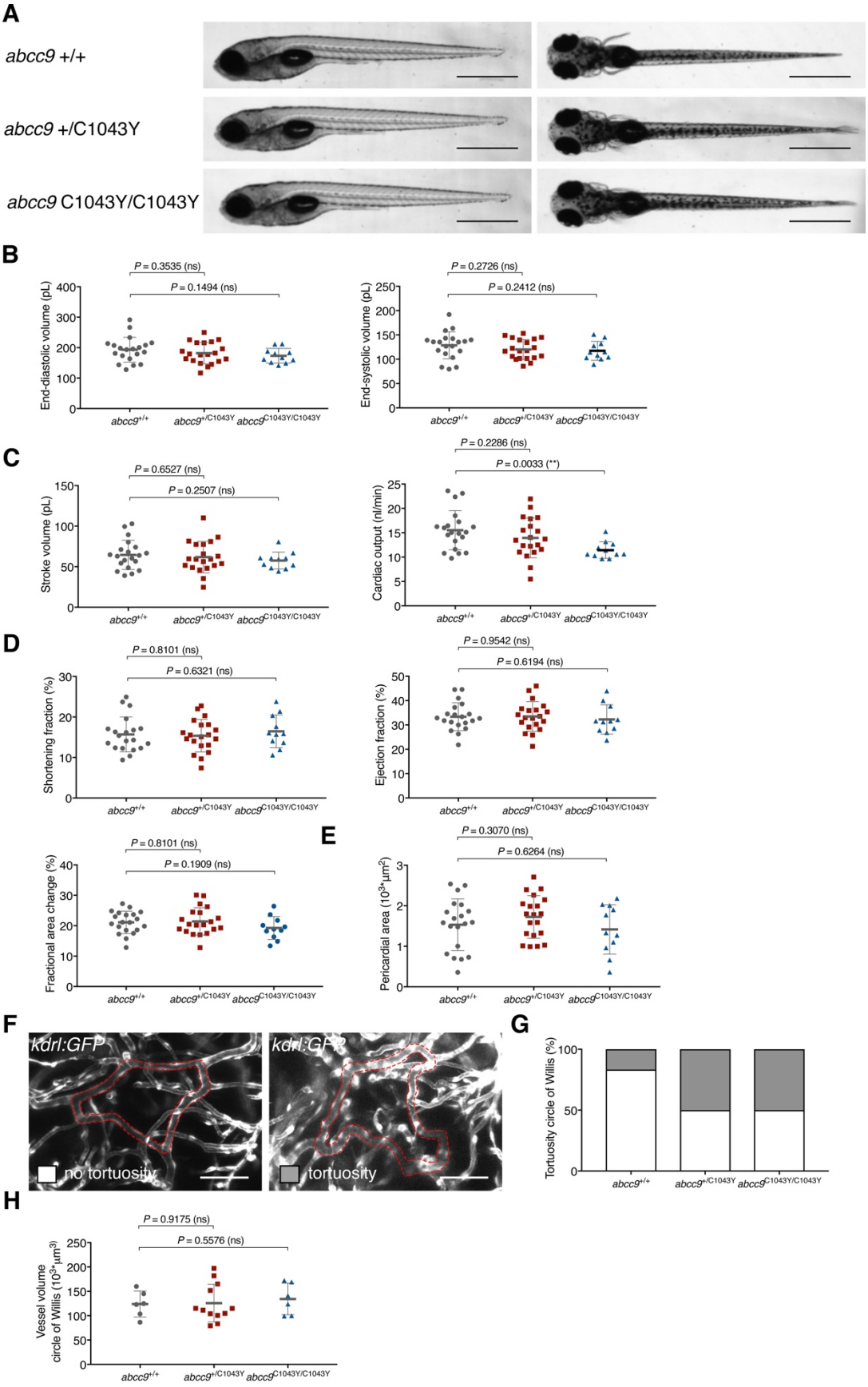


Figure S12

Alcian blue staining in *kcnj8*[V65M] mutants. (A) Comparison of wild-type and *kcnj8*[V65M] mutants stained with Alcian blue at 5 dpf seen from a dorsal (left) and left lateral view (right). Sample size, $kcnj8^{+/+}$, n=5; $kcnj8^{+/V65M}$, n=12; $kcnj8^{V65M/V65M}$, n=7 in **A.** Scale bar, 500 μm in **A**. All embryos analyzed originated from group matings of adult zebrafish.

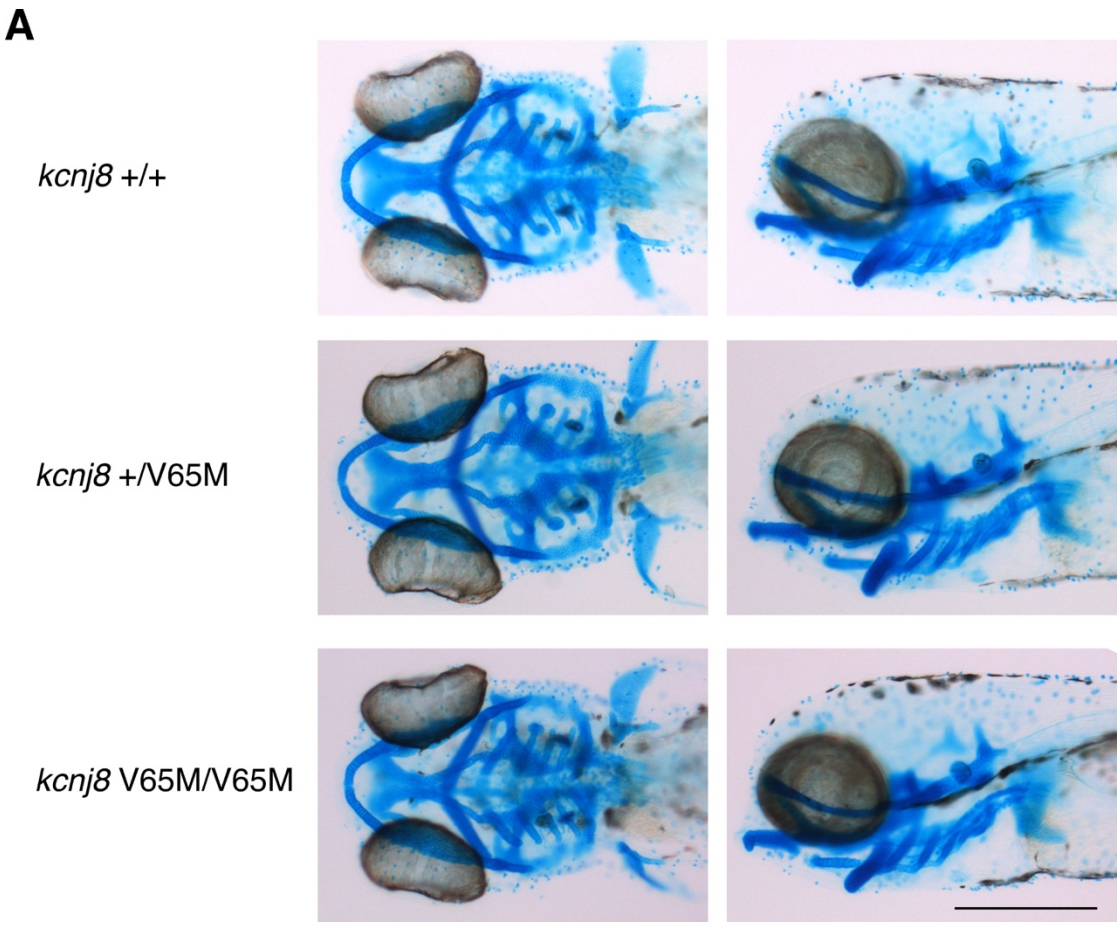


Table S1

Major clinical features of Cantú Syndrome.

Neonatal features
Macrosomia
Macrocephaly
Maternal polyhydramnios
Craniofacial dysmorphology
Epicanthal folds
Broad nasal bridge
Small nose/anteverted nostrils
Long philtrum
Prominent mouth/full lips
Macroglossia
Gingival hyperplasia
Hair
Thick scalp hair
Excessive body hair on forehead, face, back and limbs
Skin and joints
Loose and/or wrinkled skin
Deep palmar and plantar creases
Hyperflexibility of joints
Cardiovascular
Cardiomegaly
Pericardial effusions
Patent ductus arteriosus
Pulmonary hypertension
Increased cardiac output
Ventricular hypercontractility
Reduced vascular tone
Tortuosity and dilation of cranial blood vessels
Brain
Headaches
Seizures
Skeletal abnormalities
Narrow thorax
Pectus carinatum
Broad ribs
Delayed bone age
Other reported features
Lymphedema
Immune dysfunction or recurrent infections
Umbilical hernia
Developmental delay

Table S2

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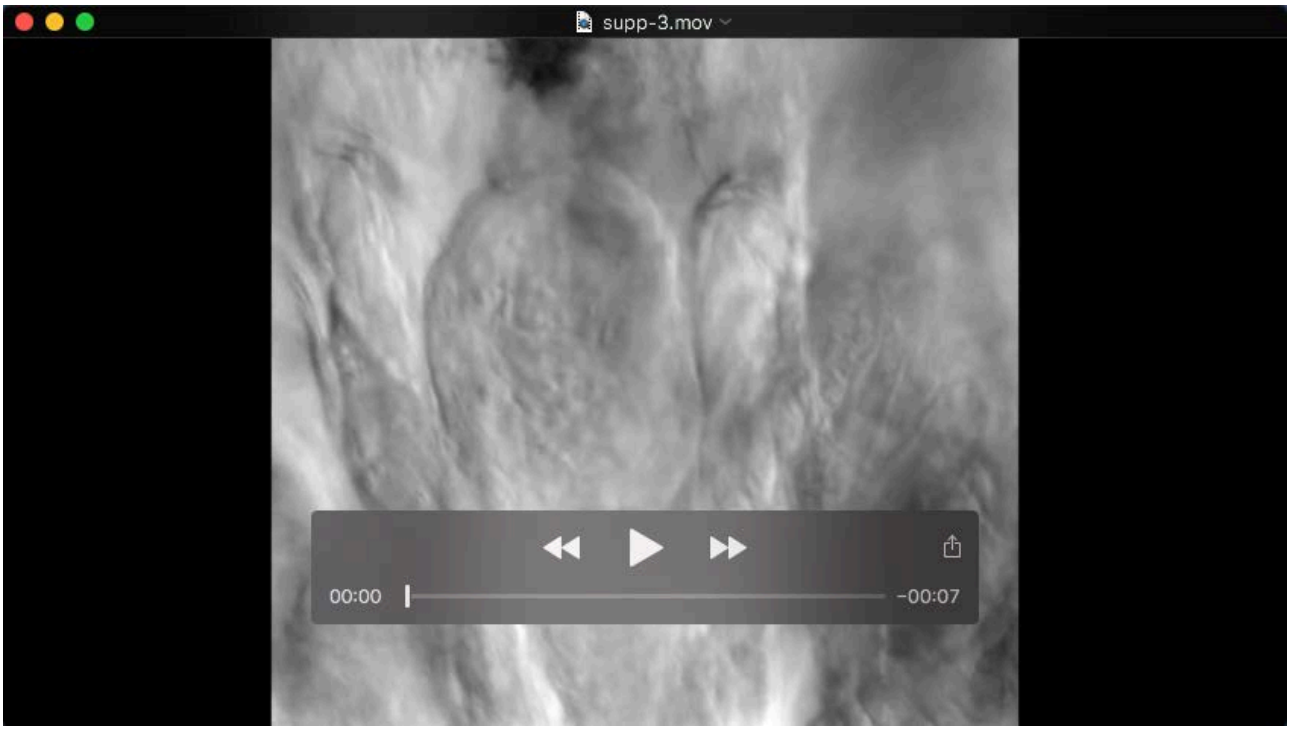
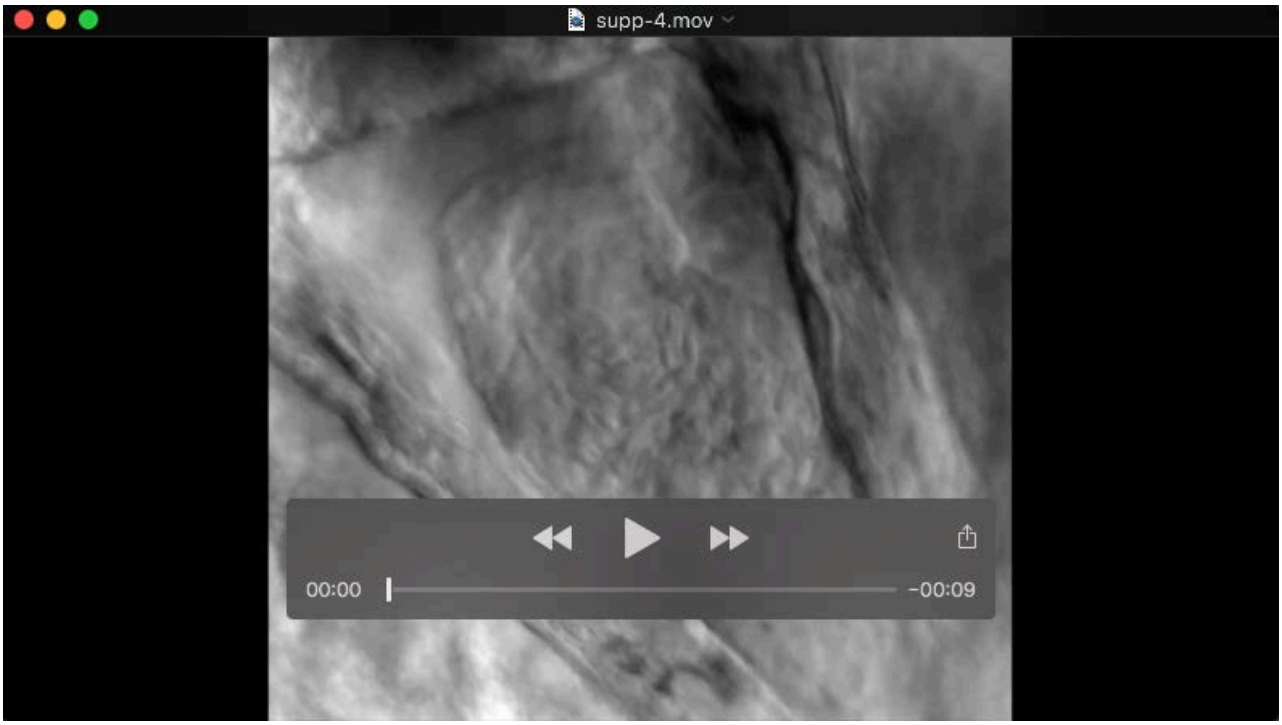
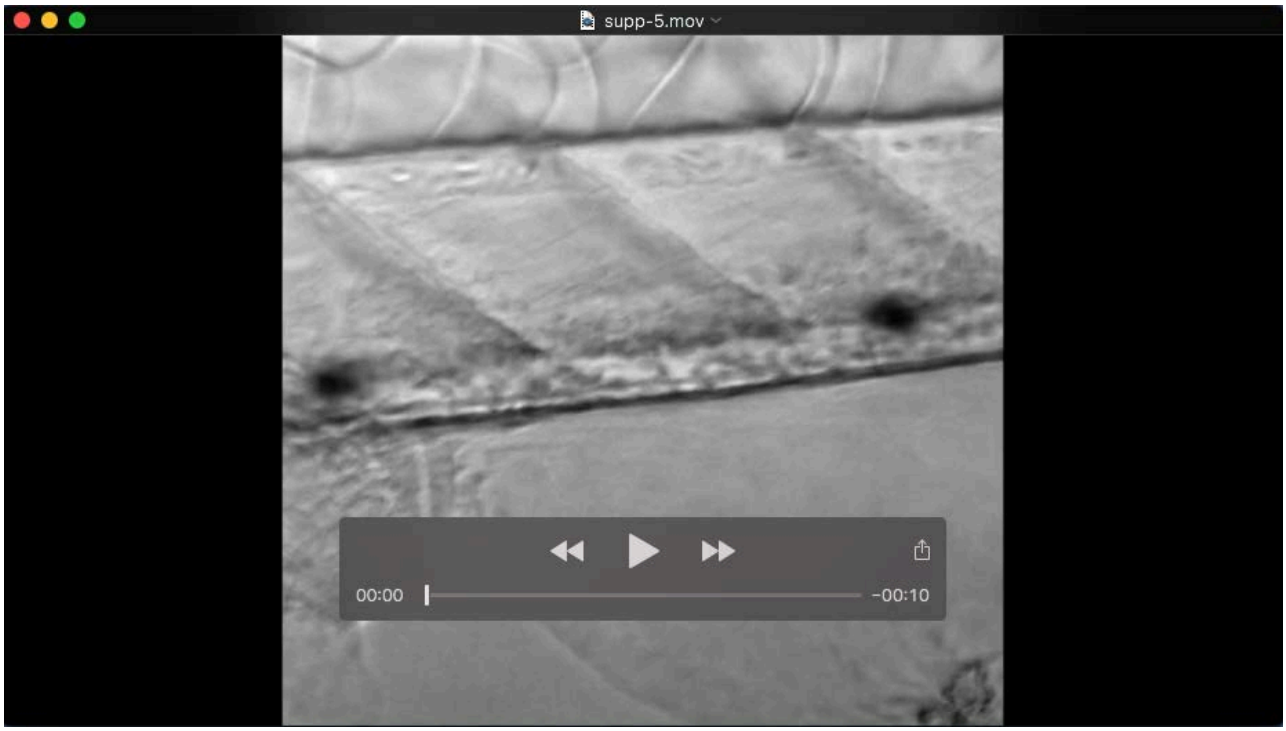


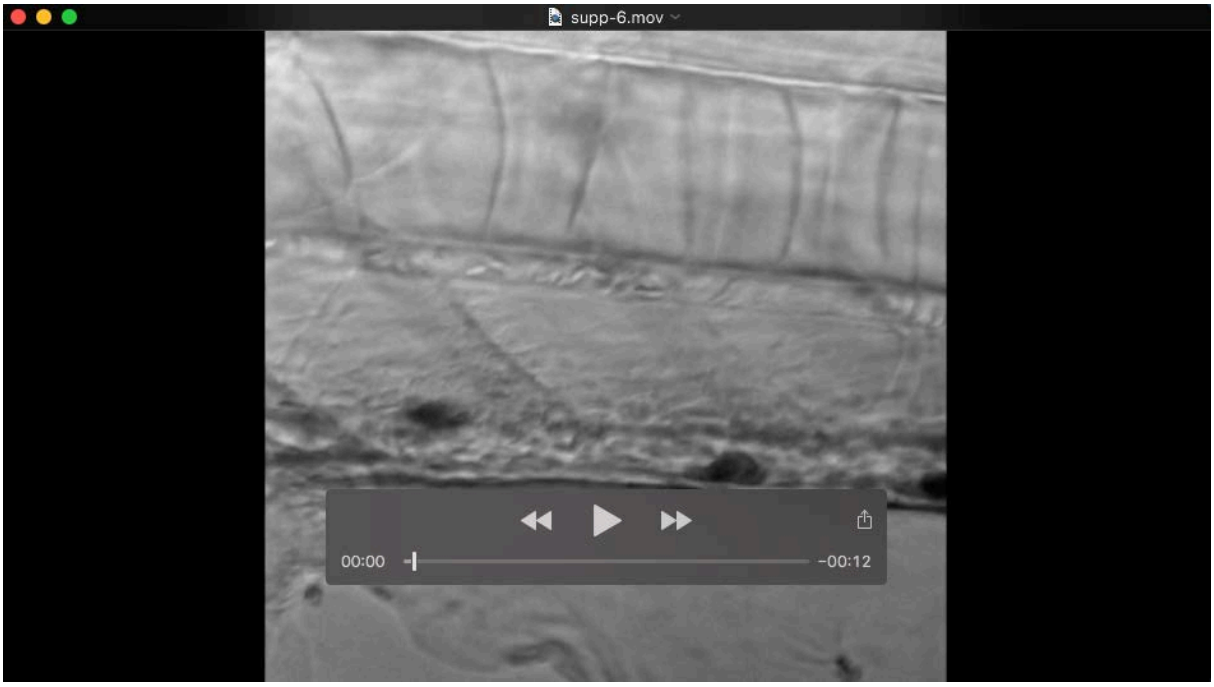
Movie 1. High-speed video imaging of ventricular area of *kcnj8*$^{+/+}$ KI zebrafish. Three entire cardiac cycles are shown which are slowed down by a factor of 10.



Movie 2. High-speed video imaging of ventricular area of *kcnj8*$^{+/-}$ KI zebrafish. Three entire cardiac cycles are shown which are slowed down by a factor of 10.



Movie 3. High-speed video imaging of caudal vein of *kcnj8*$^{+/+}$ KI zebrafish. Three entire cardiac cycles are shown which are slowed down by a factor of 10.



Movie 4. High-speed video imaging of caudal vein of *kcnj8*$^{+/-}$ KI zebrafish. Three entire cardiac cycles are shown which are slowed down by a factor of 10.