**In vivo** characterization of candidate genes for heart rate variability identifies culprits for sinoatrial pauses and arrests

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ABSTRACT

A meta-analysis of genome-wide association studies (GWAS) recently identified eight loci that are associated with heart rate variability (HRV) in data from 53,174 individuals. However, functional follow-up experiments - aiming to identify and characterize causal genes in these loci - have not yet been performed. We developed an image- and CRISPR-Cas9-based pipeline to systematically characterize candidate genes for HRV in live zebrafish embryos and larvae. Nine zebrafish orthologues of six human candidate genes were targeted simultaneously in fertilized eggs from fish that transgenically express GFP on smooth muscle cells (Tg(acta2:GFP)), to visualize the beating heart. An automated analysis of 30s recordings of 384 live zebrafish atria at 2 and 5 days post-fertilization helped identify genes that influence HRV (kiaa1755 and gngt1); heart rate (kiaa1755); sinoatrial pauses and arrests (syt10, hcn4 and kiaa1755); and early cardiac development (gngt1, neo1a). Hence, comprehensively characterizing candidate genes in GWAS-identified loci for HRV in vivo helped us identify previously unanticipated culprits for life-threatening cardiac arrhythmias.
INTRODUCTION

Heart rate variability (HRV) reflects the inter-beat variation of heart rate. HRV is controlled by the sinoatrial node, which receives input from the autonomic nervous system. Autonomic imbalance, which has been associated with work stress and other modifiable or non-modifiable risk factors (Thayer et al. 2010), is reflected in lower HRV. Lower HRV has been associated with higher cardiac morbidity and mortality (de Bruyne et al. 1999), and with a higher risk of all-cause mortality (Dekker et al. 1997). HRV can be quantified non-invasively using the RR interval of a 12-lead ECG, making HRV a useful clinical marker for perturbations of the autonomic nervous system. However, the genetic basis of HRV remains largely elusive.

Recently, we and others identified the first loci that are robustly associated with HRV, using a meta-analysis of genome-wide association studies (GWAS) with data from 53,174 participants (Nolte et al. 2017). Eleven of the 17 associated single nucleotide polymorphisms (SNPs) overlapped with loci that we previously identified as being associated with resting heart rate (den Hoed et al. 2013). The heart rate associated loci in turn had on aggregate been associated with altered cardiac conduction and risk of sick sinus syndrome (den Hoed et al. 2013). In silico functional annotation of the five loci that are associated with both HRV (Nolte et al. 2017) and heart rate (den Hoed et al. 2013) previously resulted in the prioritization of six candidate genes that are anticipated to be causal (Nolte et al. 2017). Functional follow-up experiments - ideally in vivo - are required to conclude if these genes are indeed causal, and examine if they influence HRV, heart rate, and/or cardiac conduction.

Mouse models are commonly used in cardiac research, but mice show substantial differences in cardiac rate and electrophysiology compared with humans (Poon and
Brand 2013). Inherently, these differences complicate extrapolation of results from mouse models to humans (Poon and Brand 2013). Additionally, rodents are not suitable for high-throughput, in vivo screens of cardiac rhythm and rate. Such screens are essential to systematically characterize positional candidate genes in the large number of loci that have now been identified by GWAS for cardiac rhythm, rate (Eppinga et al. 2016) and conduction (Arking et al. 2014). Hence, novel model systems that facilitate systematic, in vivo characterization of a large number of candidate genes are desirable.

In recent years, the zebrafish has become an important model system for genetic and drug screens for human disease (Lieschke and Currie 2007; MacRae and Peterson 2015). Amongst the many advantages of zebrafish larvae as a model system are their short generation time, large number of offspring produced regularly, relatively low maintenance costs, rapid early development, and optical transparency. Zebrafish have a fully functional, beating heart at ~24 hours post-fertilization, and the ECG of the two-chambered zebrafish heart is comparable to that of humans (Liu et al. 2016). Fluorescently labeled transgenes facilitate visualization of cell types and tissues of interest, which can now be accomplished in high-throughput thanks to advances in automated positioning of non-embedded, live zebrafish embryos (Pardo-Martin et al. 2013). In addition, the zebrafish has a well-annotated genome, with orthologues of at least 71.4% of human genes (Howe et al. 2013). These genes can be targeted efficiently and in a multiplexed manner (i.e. in multiple genes simultaneously) thanks to recent advances in Clustered, Regulatory Interspaced, Short Palindromic Repeats (CRISPR) and CRISPR-associated systems (Cas) (Varshney et al. 2016). All characteristics combined make zebrafish embryos an attractive model system to systematically characterize candidate genes for cardiac rhythm, rate and conduction.
The aim of this study was to objectively characterize the most promising candidate genes in GWAS-identified loci for HRV and heart rate for a role in cardiac rhythm, rate and function using a large-scale, image-based screen in zebrafish embryos and larvae.
RESULTS

Experimental pipeline

Our experimental pipeline is outlined in Figure 1. Founder mutants ($F_0$ generation) were generated in which all nine zebrafish orthologues of six human candidate genes were targeted using a multiplexed CRISPR-Cas9 approach (Table 1, Supplemental Table S1) (Varshney et al. 2016). Generating mutants in a background with a transgenically expressed fluorescent label on smooth muscle cells (Tg($acta2$:gfp)) allowed us to visualize the beating atrium with minimal background noise. $F_0$ mutants were in-crossed, and the atria of 384 $F_1$ embryos were recorded for 30s at 2 days post fertilization (dpf). In addition, we captured bright field images of the embryos to quantify body length, surface area and volume. Of the 384 imaged embryos, 326 were imaged again at 5dpf (Supplemental Fig. S1), to capture genetic effects on cardiac outcomes and body size at two key stages of early cardiac development (Hou et al. 2014).
Descriptive information

At 2dpf, some embryos showed sinoatrial pauses (n=39, 10.3%, Supplemental Movie S1) and arrests (n=36, 9.5%, Supplemental Movie S2); cardiac edema (n=15, 4.0%); or uncontrolled atrial contractions (n=1, 0.2%, Supplemental Movie S3). At 5dpf, a subset of larvae displayed sinoatrial pauses (n=9, 2.7%); sinoatrial arrests (n=3,
0.9%); cardiac edema (n=14, 4.2%); uncontrolled atrial contractions (n=9, 2.7%); or an abnormal cardiac morphology and impaired cardiac contractility (n=6, 1.8%, Supplemental Movie S4). In the 279 embryos without cardiac abnormalities that survived quality control at 2dpf (Supplemental Fig. S1), HRV was 15.5±10 ms (mean±SD) and heart rate 208±24 bpm. In the 291 larvae included in the analysis at 5dpf, HRV was 13.18±17.06 ms and heart rate 265±35 bpm (Table 2).

| Age | Trait | Mean | SD | 1st | Median | 3rd |
|-----|-------|------|----|-----|--------|-----|
| 2   | SDNN  | 19.52| 12.60| 9.84| 17.39  | 26.55|
|     | RMSSD | 11.60| 8.93 | 6.43| 8.73   | 13.12|
|     | HR    | 15.56| 10.04| 8.74| 12.96  | 19.65|
|     | SDNN  | 13.30| 17.18| 5.18| 7.60   | 11.72|
|     | RMSSD | 13.05| 18.26| 5.93| 7.48   | 12.01|
| 5   | HR    | 13.18| 17.06| 5.65| 7.72   | 12.06|
|     | HR    | 265  | 35  | 244 | 261    | 286  |

Information based on data from 279 embryos at 2 days post-fertilization (dpf) and 291 larvae at 5 dpf. HRV was calculated by averaging SDNN and RMSSD. HRV, SDNN and RMSSD are shown in ms, whereas HR is shown in beats per min. 1st and 3rd refers to the respective quartile. HRV: heart rate variability; SDNN: standard deviation of the normal-to-normal interval; RMSSD: root mean square of successive heart beat interval differences; HR: heart rate.

After imaging at 5dpf, all larvae were sequenced at the nine CRISPR-Cas9 targeted and three putative off-target sites (2x250 bp paired end), and transcript-specific dosages were calculated by weighting the number of mutated alleles by their predicted impact on protein function based on Ensembl’s variant effect predictor (VEP). At the three putative off-target sites, no CRISPR-Cas9 induced mutations were identified (Supplemental Table S2). A total of 164 unique mutations were identified across the nine CRISPR-targeted sites, ranging from three unique mutations in hcn4l to 37 in kiaa1755_2 (Supplemental Table S3). Frameshift mutations were most common (48.5%), followed by missense variants (24.5%), in-frame deletions (14.7%), and synonymous variants (5.5%). Eighty-five, sixty-nine and nine variants were predicted to have a high, moderate, and low impact on protein function,
respectively (Supplemental Table S3). Mutant allele frequencies ranged from 4.1% for *hcn4l* to 92.0% for *neo1b* (Supplemental Table S4).

**Genetic effects on cardiac rhythm, rate and function**

We examined the effect of the induced mutations on HRV and heart rate after excluding cases with cardiac abnormalities that would drive effects on HRV and heart rate, i.e. sinoatrial pauses or arrests, uncontrolled atrial contractions, cardiac edema, and abnormal cardiac morphology or contractility (primary analyses, Supplemental Fig. S1). We also examined the impact of mutations on cardiac abnormalities for events that affected at least five embryos (2dpf) or larvae (5dpf), and we examined the effect of mutations on body size (secondary analyses, Supplemental Fig. S1).

Each additional mutated allele in *gngt1* was associated with higher odds of abnormal cardiac morphology and impaired cardiac contractility at 2dpf (Supplemental Table S5), as well as with higher odds of cardiac edema and a higher HRV at 5dpf (Figure 2, Supplemental Table S6-S7). *Gngt1* mutants also had a smaller dorsal body surface area at 5dpf.

Each additional mutated allele in the main transcript of *kiaa1755* was associated with a higher HRV and a trend for a higher heart rate at 2dpf (Figure 2, Supplemental Tables S6-S7). Mutations in *kiaa1755* were also associated with a larger body volume at 5dpf (Supplemental Table S8). Embryos with mutations in *kiaa1755* tended to be shorter and smaller at 2dpf (Supplemental Table S8), and had higher odds of sinoatrial pauses and a higher heart rate at 5dpf (Figure 2, Supplemental Tables S5-S7).
Each additional mutated allele in *hcn4* was associated with higher odds of sinoatrial pauses and arrests at 2dpf (Supplemental Table S5). In larvae free from severe cardiac abnormalities, each additional mutated allele in *hcn4* was associated with a trend for higher heart rate at 5dpf (Figure 2, Supplemental Tables S6-S7), without affecting body size (Supplemental Table S8). Mutations in *hcn4l* were positively associated with lateral body surface area at 5dpf (Supplemental Table S8), but not with cardiac rhythm, rate, or abnormalities at 2dpf or 5dpf.

Mutations in *neo1a* and *neo1b* did not affect cardiac rhythm, rate, or abnormalities at 2dpf. However, embryos with mutations in *neo1a* tended to be shorter and had a larger dorsal body surface area normalized for length. Embryos with mutations in *neo1b* had a larger lateral body surface area at 2dpf. At 5dpf, mutations in *neo1a* were associated with lower odds of abnormal cardiac morphology and impaired cardiac contractility, uncontrolled atrial contractions (trend), and edema (Supplemental Table S5), but not with body size.

Each additional mutated allele in *syt10* was associated with higher odds of a sinoatrial pause at 2dpf (Supplemental Table S5), but did not affect HRV, heart rate or body size (Supplemental Tables S6-S8).
Five of the six human candidate genes taken forward for experimental follow-up showed at least one relevant cardiac phenotype in zebrafish embryos or larvae (i.e. GNGT1, SYT10, HCN4, NEO1, KIAA1755). Only a trend towards an effect - on sinoatrial pauses at 2dpf - was observed for the RGS6 orthologue, for which the mutant allele frequency was only 4%. We next examined the druggability of the five genes for which we showed most evidence of a causal role using the drug-gene interaction (DGI) database. This only highlighted HCN4 as being druggable (Cotto et
Indeed, ivabradine is already being used as an open channel blocker of HCN4 (Böhm et al. 2010). We next identified interaction partners of the proteins encoded by the five genes using GeneMania (Warde-Farley et al. 2010) and STRING (Szklarczyk et al. 2017). A total of 49 interacting partners were identified, 31 of which were considered to be druggable or belong to a “favorable” protein family. Seven of these are targeted by FDA-approved medication (Supplemental Table S9), amongst which are several anti-hypertensive agents (e.g. Telmisartan, Iloprost and Diazoxide), as well as a neuromuscular blocking agent (i.e. Botulinum toxin type A). It would be worthwhile to examine if these agents can be repurposed to prevent cardiac arrhythmias in individuals at risk, or whether cardiac arrhythmias represent on-target, adverse side effects of these drugs.
DISCUSSION

Large-scale, *in vivo* follow-up studies of candidate genes in GWAS-identified loci remain sparse. Here we present an objective, image-based pipeline to systematically characterize candidate genes for cardiac rhythm, rate, and conduction-related disorders. Using a zebrafish model system, we identified orthologues of genes in HRV and heart rate associated loci that influence not only the complex traits for which they were identified (i.e. *kiaa1755, gngt1*), but also body size (*kiaa1755, hcn4, gngt1, neo1a, neo1b*), early cardiac development (*gngt1, neo1a, neo1b*), and/or the odds of sinoatrial pauses and arrests (*hc4n, syt10, kiaa1755*). These results add weight to the notion that GWAS-identified common variants for complex traits can flag genes for which rare, detrimental mutations cause severe, early-onset disorders (Arking et al. 2014; Flannick et al. 2016; Ganna et al. 2018). We show here that systematic characterization of candidate genes in GWAS-identified loci for complex traits using an image- and CRISPR-Cas9-based zebrafish model system can help identify clinically relevant causal genes, and aid confirmation of the role of such genes in whole-exome sequencing efforts in humans by reducing the multiple testing burden (Flannick et al. 2018).

The HRV increasing T-allele of rs4262 in the 5′ untranslated region of *GNG11* is associated with a lower expression of *GNG11* in the tibial artery, atrial appendage and aorta (GTEx (Battle et al. 2017)). This is directionally consistent with 5dpf *gngt1* mutant zebrafish larvae - i.e. mutants for the zebrafish orthologue of the human paralogues *GNGT1* and *GNG11* (Lagman et al. 2012) - having a higher HRV. *GNG11* encodes the γ-subunit of a G-protein complex, which interacts with G-protein-coupled inwardly rectifying potassium channels (GIRKs) that transduce signals rapidly enough to induce changes in HRV (Mark and Herlitze 2000). The zebrafish heart
normally has a tube-like appearance at 28 hours post fertilization (hpf) (Bakkers 2011). Higher odds of abnormal cardiac morphology, contractility and edema with each additional mutated gngt1 allele at 5 but not 2dpf suggests that this gene affects cardiac development between 2 and 5dpf.

Non-synonymous SNPs in KIAA1755 have been identified in GWAS for HRV (Nolte et al. 2017) and heart rate (den Hoed et al. 2013). In humans, the C-allele of the HRV-associated rs6123471 in the 3’ UTR of KIAA1755 is associated with a lower expression of KIAA1755 in brain and aorta (GTEx (Battle et al. 2017)), a higher HRV (Nolte et al. 2017), and a lower heart rate (den Hoed et al. 2013). Our results show that mutations in kiaa1755_1 predispose to a higher HRV and, in a sensitivity analysis, a higher heart rate at 2dpf. Mutations in kiaa1755_2 on the other hand result in a higher heart rate and higher odds of sinoatrial pauses at 5dpf. Hence, a higher HRV at 2dpf for each additional mutated allele in kiaa1755_1 is directionally consistent with results in humans, while the effects of mutations in kiaa1755_1 and kiaa1755_2 on heart rate are not. Genetic effects on HRV and heart rate were mutually adjusted in our study, which may have revealed the unbiased direction of effect for mutations in KIAA1755 on heart rate. In other words: the GWAS-identified association of the KIAA1755 locus - and possibly other loci - with heart rate may have been driven by HRV. This would explain why the eleven HRV-associated loci that showed evidence of an association with heart rate all did so in the expected (i.e. opposite) direction from a phenotypic point of view (Nolte et al. 2017). KIAA1755 is a previously uncharacterized gene that shows a broad expression pattern, including different brain regions and the left atrial appendage (GTEx (Battle et al. 2017)). Future mechanistic studies are required to distill how KIAA1755 influences heart rhythm and rate.
The locus harboring *HCN4* has been flagged in GWAS for HRV (Nolte et al. 2017), heart rate (den Hoed et al. 2013) and atrial fibrillation (Ellinor et al. 2012). 

*HCN4* is arguably the most well-characterized gene in the context of cardiac rhythm. It belongs to the family of “funny” channels, aptly named for being activated upon hyperpolarization. It is expressed in the sinoatrial node (Ludwig et al. 1999; Shi et al. 1999) and plays an important role in cardiac pace making (Stieber et al. 2003). The heart-rate lowering agent ivabradine (Bucchi et al. 2013) is an open channel blocker of *HCN4*, and reduces cardiovascular and all-cause mortality in heart failure patients (Swedberg et al. 2010). Hcn4+/− mice die prenatally, and analyses of their hearts show no arrhythmias and a lower heart rate compared with wildtype and heterozygotes (Stieber et al. 2003). In line with this, humans that are heterozygous for an *HCN4* loss of function mutation are typically characterized by bradycardia. We identified nine zebrafish embryos that were compound heterozygous for mutations in *hcn4*, and one embryo that was compound heterozygous for *hcn4l*, without a deviation from HWE for either gene (Supplemental Table S4). Four of the nine compound heterozygous larvae for *hcn4*, and six of the eight larvae that were heterozygous for mutations in *hcn4* as well as *hcn4l* showed a sinoatrial arrest before or during the atrial recording at 2dpf. Zebrafish embryos can survive without a functional heart at this stage, thanks to tissue oxygenation by diffusion (Burggren and Pinder 1991). This allowed us to witness genetically driven cardiac arrests that would have been lethal in embryos of other species. At 5dpf, larvae carrying mutations in *hcn4* that were free from severe cardiac abnormalities tended to have a higher heart rate than larvae without CRISPR-induced mutations. This unexpected result may reflect overcompensation by *hcn4l* or cardiac compensation in response to unobserved sinoatrial arrests outside the 30s recording.
In addition to the locus in \( HCN4 \), the GWAS for HRV identified an independent locus in the adjacent \( NEO1 \) (Nolte et al. 2017). Neogenin-1 is a transmembrane receptor that is involved in cell migration, angiogenesis and neuronal survival (reviewed by Wilson et al. (Wilson and Key 2007)). In line with our results, morpholino-mediated knockdown of \textit{neola} expression in zebrafish larvae was previously shown to result in shorter, thicker larvae due to defects in somitogenesis, with loss of neuronal differentiation due to malformation of the neural tube (Mawdsley et al. 2004). \( NEO1 \) has a pro-apoptotic function in humans (Wilson and Key 2007), so the cardioprotective effect we observed in 5dpf mutants may result from inefficient propagation of apoptotic signals by specific effectors. Inhibiting \( NEO1 \) in early development may thus help protect cardiac integrity - perhaps temporarily - but will likely have adverse effects due to inadequate apoptosis at later stages of development.

\( SYT10 \) belongs to the family of synaptogamins: transmembrane proteins that are involved in regulation of membrane trafficking in neurons (Sudhof 2002), which is important for neurotransmitter release (Chapman 2008). Variants in/near \( SYT10 \) have previously been associated with HRV (Nolte et al. 2017) as well as with heart rate (den Hoed et al. 2013) and heart rate recovery (Verweij et al. 2018). We observed an effect of mutations in \( syt10 \) on the odds of sinoatrial pauses at 2dpf. Since \( SYT10 \) is mainly expressed in brain, tibial nerve and pituitary (GTEx (Battle et al. 2017)), mutations in \( syt10 \) may affect the odds of sinoatrial pauses through altered neurotransmitter release.

Of the five genes for which we show causal effects, only \( HCN4 \) is highlighted as being druggable by DGIdb. The protein encoded by \( HCN4 \) has already been targeted using several compounds (Stieber et al. 2006). Although four of the five causal genes
are not highlighted as being druggable by current pharmaceutical standards, targeting via antisense oligonucleotides or antibodies can still be explored. Expanding our search revealed several druggable interaction partners of causal genes that are already targeted by FDA-approved anti-hypertensive and neuromuscular blocking agents, amongst others. For existing drugs that target interaction partners of the five causal genes, it is worthwhile examining their effect on cardiac rhythm, rate and development, since repurposing FDA-approved drugs would imply the quickest and safest route to the clinic, while quantifying possibly unknown adverse side effects would also be informative.

A few limitations of our study should be discussed. Firstly, acquiring 30s recordings implies that false negatives for sinoatrial pauses or arrests are inevitable. In fact, we observed 54 pauses or arrests at 2dpf (14.3%) and eight at 5dpf (2.5%) while positioning the larva for imaging, without observing abnormalities during the subsequent 30s recording. This suggests that at least 15.9% of all embryos without a sinoatrial pause at 2dpf are false negatives, which negatively affects the statistical power to find genetic effects. This limitation will have resulted in conservative effect estimates. Secondly, CRISPR guide-RNAs with predicted off-targets free from mismatches were avoided. However, two of the selected targets - i.e. for hcn4 and kiaa1755_2 - have exonic off-targets with three mismatches (Supplemental Table S1). Human orthologues of potential off-targets dclk1 and galnt10 have previously been associated with heart-rate variability-related traits (Newton-Cheh et al. 2007) (dclk1), as well as with carotid intima-media thickness (Dong et al. 2015) and body mass index (Locke et al. 2015; Justice et al. 2017; Ng et al. 2017) (galnt10). Sequencing larvae at the three predicted off-target regions did not show any CRISPR-Cas9 induced mutations (Supplemental Table S2). In line with predictions by Varshney et
off-target effects are thus unlikely to have influenced our results (Varshney et al. 2015). Thirdly, we in-crossed mosaic founder mutants (F₀) and phenotypically screened and sequenced the F₁ generation. For some genes, this yielded a small number of larvae with 0 or 2 mutated alleles (i.e. rgs6, hcn4, and hcn4l), resulting in a lower power to find true genetic effects for these genes. In spite of this limitation, we were able to detect significant effects of mutations in all genes except rgs6, for which we observed a trend for an effect on the odds of sinoatrial pauses at 2dpf. Furthermore, screening the F₀ or F₂ generation instead has its own advantages and disadvantages, and we argue that non-conclusive results for a small subset of candidate genes in the primary screen is not problematic when aiming to systematically characterize a large number of candidate genes. Finally, we recorded the atrium only, to enable a higher frame rate, a higher resolution in time for HRV, and a higher statistical power to detect small genetic effects on HRV. As a result, any ventricular abnormalities were not registered, and uncontrolled atrial contractions may thus reflect atrial fibrillation, premature atrial contractions, high atrial rate, or atrial tachycardia. Additional studies with a larger frame and longer acquisition time are required to replicate and clarify the effect of mutations in neo1a on uncontrolled atrial contractions.

Our study benefits from a repeated measures design, which enabled us to capture genetic effects at different stages of early development in zebrafish. Furthermore, the throughput of the setup allowed us to examine the effect of multiple genes simultaneously, in an unprecedented sample size for in vivo genetic screens. Our results demonstrate that a large sample size is paramount to robustly detect genetic effects on complex traits in zebrafish embryos and larvae, even for functional knockout mutations. Identifying CRISPR-Cas9-induced mutations allele-specifically
helped us classify larvae as heterozygous and compound heterozygous, which in turn helped pinpoint the true effect of mutations in these genes.

Our study is based on an objective, high-throughput imaging approach and aimed to identify and characterize causal genes in GWAS-identified loci for HRV. In all genes that show an effect on HRV (*kiaa1755* and *gngt1*), directions of effect were directionally consistent with eQTL associations in humans (based on GTEx (Battle et al. 2017)). This emphasizes the merit of our model system and the robustness of our findings. Furthermore, our results extend beyond the complex outcomes for which these genes were prioritized, and helped identify *KIAA1755*, *HCN4* and *SYT10* as likely effectors of detrimental mutations that predispose to sinoatrial pauses and arrests.
METHODS

Candidate gene selection

Candidate genes in GWAS-identified loci for HRV were identified as described in detail in Nolte et al. (2017). Of the 18 identified candidate genes, six were selected for experimental follow-up. This selection was based on overlap with findings from GWAS for heart rate (KIAA1755, SYT10, HCN4, GNG11) (den Hoed et al. 2013), as well as with results from eQTL analyses in sinoatrial node and brain (RGS6). Additional candidate genes from the same or nearby loci were also selected for experimental follow-up, i.e. NEO1, which resides next to HCN4. Zebrafish orthologues of the human genes were identified using Ensembl, as well as using a comprehensive synteny search using Genomicus (Louis et al. 2015) (Supplemental Table S1). Of the selected genes, GNG11, SYT10 and RGS6 have one orthologue in zebrafish, and KIAA1755, HCN4 and NEO1 each have two orthologues, resulting in a total of nine zebrafish orthologues for six human candidate genes (Table 1). For simplicity, the KIAA1755 orthologues quo and si:dkey-65j6.2 have been referred to as kiaa1755_1 and kiaa1755_2.

Mutagenesis

All nine zebrafish genes were targeted together using a multiplexed CRISPR-Cas9 approach described recently (Varshney et al. 2016). Briefly, guide-RNAs (gRNAs) were selected using ChopChop (Labun et al. 2016) and CRISPRscan (Moreno-Mateos et al. 2015) (Supplemental Table S1), based on their predicted efficiency, a moderate to high GC-content, location in an early exon, and absence of predicted off-target effects without mismatches. Oligonucleotides were designed as described (Varshney...
et al. 2016), consisting of a T7 or SP6 promoter sequence (for gRNAs starting with
‘GG’ or ‘GA’, respectively), a gene-specific gRNA-target sequence, and an overlap
sequence to a generic gRNA. The gene-specific oligonucleotides were annealed to a
generic 80 bp long oligonucleotide at 98°C for 2 mins, 50°C for 10 mins, and 72°C
for 10 mins. The products were checked for correct length on a 2% agarose gel. The
oligonucleotides were subsequently transcribed in vitro using the manufacturer’s
instructions (TranscriptAid T7 high yield transcription kit / MEGAscript SP6
transcription kit, both ThermoFisher Scientific, Waltham, USA). The gRNAs were
purified, after which the integrity of the purified gRNAs was examined on a 2%
agarose gel. The zebrafish codon-optimized plasmid pT3TS-nls-zCas9-nls was used
as a template to produce Cas9 mRNA (Jao et al. 2013). The plasmid was linearized
with Xba1, and then purified using the Qiaprep Spin Miniprep kit (Qiagen, Hilden,
Germany). The DNA was transcribed using the mMESSAGE mMACHINE T3
Transcription Kit (ThermoFisher Scientific, Waltham, USA), followed by LiCl
precipitation. The quality of the RNA was confirmed on a 1% agarose gel.

**Husbandry & microinjections**

A zebrafish line with GFP-labelled α-smooth muscle cells Tg(acta2:GFP) (Whitesell
et al. 2014) was used to visualize the beating heart. To this end, eggs from an in-cross
of Tg(acta2:GFP) fish were co-injected with a mix of Cas9 mRNA (final
concentration 150 ng/µl) and all nine gRNAs (final concentration 25 ng/µl each) in a
total volume of 2nL at the single-cell stage. CRISPR-Cas9 injected embryos were
optically screened for the presence of Tg(acta2:GFP) at 2 days post fertilization (dpf),
using an automated fluorescence microscope (EVOS FL Cell imaging system,
ThermoFisher Scientific, Waltham, USA). Tg(acta2:GFP) carriers were retained and
raised to adulthood in systems with circulating, filtered and temperature controlled water (Aquaneering, Inc, San Diego, CA). All procedures and husbandry were conducted in accordance with Swedish and European regulations, and have been approved by the Uppsala University Ethical Committee for Animal Research (C142/13 and C14/16).

Experimental procedure imaging

The founder mutants (F₀ generation) were only used for reproduction. After crossing the founder mutants, F₁ embryos were used for experiments. Eggs were collected after F₀ fish were allowed to reproduce for 45 mins to minimize variation in developmental stage. Fertilized eggs were placed in an incubator at 28.5°C. At 1dpf, embryos were dechorionated using pronase (Roche Diagnostics, Mannheim, Germany).

At 2dpf, embryos were removed from the incubator and allowed to adapt to controlled room temperature (21.5 °C) for 20 mins. Individual embryos were exposed to 100 µg/ml Tricaine (MS-222, Sigma-Aldrich, Darmstadt, Germany) for 1 min before being aspirated, positioned in the field of view of a fluorescence microscope, and oriented dorsally using a Vertebrate Automated Screening Technology (VAST) BioImager (Union Biometrica Inc., Geel, Belgium). We subsequently acquired twelve whole-body images, one image every 30 degrees of rotation, using the camera of the VAST BioImager to quantify body length, dorsal and lateral surface area and volume, as well as the presence or absence of cardiac edema. The VAST BioImager then positioned and oriented the larva to visualize the beating atrium and triggered the upright Leica DM6000B fluorescence microscope to start imaging using a HCX APO L 40X/0.80 W objective and L5 ET, k filter system (Micromedic AB, Stockholm,
Images of the beating atrium were acquired for 30s at a frame rate of 152 frames/s using a DFC365 FX high-speed CCD camera (Micromedic AB, Stockholm, Sweden). After acquisition, the larvae were dispensed into a 96-well plate, rinsed from tricaine, and placed back into the incubator. The procedure was repeated at 5dpf (i.e. the larval stage), to allow genetic effects that influence HRV and heart rate differently at different stages of development to be captured (Hou et al. 2014). After imaging at 5dpf, the larvae were once again dispensed into 96-well plates, sacrificed, and stored at -80°C for further processing.

**Quantification of cardiac traits and body size**

A custom-written MATLAB script was used to convert the images acquired by the CCD camera into quantitative traits. To acquire the heart rate, each frame of the sequence was correlated with a template frame. The repeating pattern yields a periodic graph from the correlation values and by detecting the peaks in the graph we can assess the heart rate. The template frame should represent one of the extreme states in the cardiac cycle, i.e. end-systole or end-diastole. To detect these frames, we examined the correlation between the first 100 frames. The combination of frames that showed the lowest correlation corresponded to the heart being in opposite states. One of these frames was chosen as the template (Niazi et al. 2009).

This numeric information was subsequently used to quantify: 1) heart rate as the inverse of RR-interval; 2) the standard deviation of the normal-to-normal RR interval (SDNN); and 3) the root mean square of successive heart beat interval differences (RMSSD). Finally, a graph of pixel changes over time was generated across the 30s recording to help annotate the script’s performance. The files containing the inter-
beat-intervals were used to objectively quantify sinoatrial pauses (i.e. the atrium stops beating for longer than 3x the median inter-beat-interval of the larva, Supplemental Movie S1) and sinoatrial arrests (i.e. the atrium stops beating for longer than 2s, Supplemental Movie S2) using a custom-written Stata script. The graphs of pixel changes over time were also used to identify larvae with other abnormalities in cardiac rhythm. Such abnormalities were annotated as: uncontrolled atrial contractions (Supplemental Movie S3); abnormal cardiac morphology (i.e. a tube-like atrium, Supplemental Movie S4); or impaired cardiac contractility (i.e. a vibrating rather than a contracting atrium, Supplemental Movie S4). These phenotypes were annotated independently by two investigators (B.v.d.H. and M.d.H.), resulting in an initial concordance rate >90%. Discrepancies in annotation were discussed and re-evaluated to reach consensus.

Bright-field images of the larvae were used to assess body length, dorsal and lateral surface area, and volume. Images were automatically segmented and quantified using a custom-written CellProfiler (Lamprecht et al. 2007) pipeline, followed by manual annotation for segmentation quality. Larvae with suboptimal segmentation quality due to the presence of an air-bubble, a bent body, an incomplete rotation within the capillary, partial capturing of the larva, or an over-estimation of size were replaced by images with a 180° difference in rotation, or excluded if that image was also sub-optimally segmented. The larva was excluded from the analysis for body volume if more than four of the 12 images had a bad segmentation. Imaging, image quantification and image quality control were all performed blinded to the sequencing results.
Quality control of phenotype data

A series of quality control steps was performed to ensure only high-quality data was included in the genetic association analysis (Supplemental Fig. S1). First, graphs indicating that one or more true beats were missed by the script were removed from the analysis a priori (Supplemental Fig. S1). Second, larvae with any secondary phenotype (i.e. uncontrolled atrial contractions, sinoatrial pause or arrest, edema, abnormal morphology and/or reduced contractility) were excluded from the analyses for HRV and heart rate (primary analysis) (Supplemental Fig. S1). Third, genetic effects on these secondary traits were examined at 2dpf and 5dpf for traits with at least five cases (secondary analysis). Furthermore, embryos and larvae that showed a sinoatrial pause or arrest in between positioning and video acquisition, but not during the recording, were excluded from the analysis for sinoatrial pauses and arrests, since we cannot ascertain case status in the same rigorous manner for such samples, but they are not appropriate controls either.

Sample preparation for sequencing

After imaging at 5dpf, larvae were sacrificed by prolonged exposure to tricaine, and DNA was extracted by exposure to lysis buffer (10mM Tris-HCl pH8, 50mM KCl, 1mM EDTA, 0.3% Tween 20, 0.3% Igepal) and proteinase K (Roche Diagnostics, Mannheim, Germany) for 2 h at 55°C, followed by 10 min at 95°C to deactivate the proteinase K. Gene-specific primers (150bp-300bp) amplifying gRNA-targeted and putative off-target regions in dclk1b and both galnt10 orthologues (Supplemental Table S1) were distilled from ChopChop (Labun et al. 2016) and Primer3 (Koressaar and Remm 2007), and Illumina adaptor-sequences were added. The first PCR was
conducted by denaturation at 98°C for 30s; amplification for 35 cycles at 98°C for 10s, 62°C for 30s and 72°C for 30s; followed by a final extension at 72°C for 2 mins. Amplified PCR products were cleaned using magnetic beads (Mag-Bind PCR Clean-up Kit, Omega Bio-tek Inc. Norcross, GA). The purified products were used as a template for the second PCR, in which Illumina Nextera DNA library sequences were attached to allow multiplexed sequencing of all CRISPR-targeted sites across 384 larvae in a single lane. The second PCR amplification was performed by denaturation at 98°C for 30s; amplification for 25 cycles at 98°C for 10s, 66°C for 30s and 72°C for 30s; followed by a final extension at 72°C for 2 mins. Products were then purified using magnetic beads. All liquid handling was performed using a Hamilton Nimbus robot equipped with a 96-head (Hamilton robotics, Kista, Sweden). Samples were pooled and sequenced in a single lane on a MiSeq (300 bp paired-end, Illumina Inc., San Diego, CA) at the National Genomics Infrastructure, Sweden.

Processing of sequencing data

A custom-written bioinformatics pipeline was developed in collaboration with the National Bioinformatics Infrastructure Sweden to prepare .fastq files for analysis. First, a custom-written script was used to de-multiplex the .fastq files by gene and well. PEAR (Zhang et al. 2014) was then used to merge paired-end reads, followed by removal of low-quality reads using FastX (Pearson et al. 1997). The reads were then mapped to the wildtype zebrafish genome (Zv11) using STAR (Dobin et al. 2013). Next, we converted files from .sam to .bam format using samtools (Li et al. 2009), after which variants - mostly indels and SNVs - were called allele specifically using a custom-written variant calling algorithm in R (Danio rerio Identification of Variants by Haplotype - DIVaH). A summary of all unique sequences identified for the
orthologues and their respective alignment report string (Concise Idiosyncratic Gapped Alignment Report (CIGAR)) is shown in Supplemental Table S2. All unique variants (Supplemental Table S3) located within ±30 bps of the CRISPR-targeted sites that were identified across the two alleles were subsequently pooled, and used for functional annotation using Ensembl’s VEP (McLaren et al. 2016). Transcript-specific dosage scores were then calculated by retaining the variant with the highest predicted impact on protein function, and assigning it a score of 0 (no effect), 0.2 (modifier variant), 0.33 (low), 0.66 (moderate) or 1 (high). Transcript-specific dosage scores were subsequently calculated by summing the allele-specific scores at each embryo and target site. Since all transcripts within a target site were affected virtually identically, we only used the main transcript of each target site for the genetic association analysis.

Most larvae had successfully called sequences in all nine CRISPR-targeted sites (n=288, 75%). Of the 96 remaining embryos, 83 larvae (22%) had a missing sequence at one target site, and 11 larvae (3%) had two missing sequences. The remaining two larvae (0.5%) had three and four missing sequences, respectively. These two larvae have been excluded from the genetic association analysis. For the remaining larvae, the mean dosage of the transcript was imputed for missing calls. At a gene-level, a median of 380 larvae were successfully sequenced and called across all nine main transcripts, and all but one gene had successfully called mutations in ≥375 larvae. For neo1b, calling failed in 77 larvae (Supplemental Table S4). The imputed mean dosage for the main transcript of neo1b was still included in the genetic association analysis, since the mutant allele frequency in successfully called larvae was high (i.e. 0.798), and using an imputed dosage was anticipated to influence the results less than to discard the gene while it had been targeted. However, the results for neo1b should be
interpreted in light of its call rate. The mutant allele frequency was low for \textit{hc}n4, \textit{hc}n4\textit{l} and \textit{rg}6. For the remaining orthologues we had at least 10 wildtype and 10 compound heterozygous mutants. All three putative off-targets regions (\textit{dcl}k\textit{1b}, both \textit{gal}n10 orthologues, Supplemental Table S1) were classified as wildtype across all larvae and had no CRISPR-Cas9 induced mutations within ±30bps of the cut-site (Supplemental Table S2).

\textit{Interrogating the druggability of the candidate genes}

All human orthologues of the zebrafish genes that we found an effect for were interrogated in the drug-gene interaction database (DGIdb) v3.0.2 (Cotto et al. 2018). The human interaction partners of the candidates (Supplemental Table S9) were distilled from STRING v10.5 (Szklarczyk et al. 2017) and GeneMania (Warde-Farley et al. 2010). The GeneMania search was limited to physical interactions and pathway data.

\textit{Statistical analysis}

The standard deviation of NN-intervals (SDNN) and the root mean square of successive differences (RMSSD) were strongly correlated at 2dpf ($r^2=0.77$) and 5dpf ($r^2=0.85$), so a composite endpoint ‘HRV’ was calculated as the average of SDNN and RMSSD. In larvae free from sinoatrial pauses and arrests, abnormal cardiac morphology, impaired cardiac contractility, and edema HRV and heart rate at 2dpf (n=279) or 5dpf (n=291) were inverse-normally transformed to ensure a normal distribution, and to allow comparison of effect sizes across traits. Please see the Supplemental methods for a detailed description of the cardiac phenotypes that were
used as exclusion criteria for the analysis for HRV and heart rate. Genetic effects on HRV and heart rate were subsequently examined using hierarchical linear models at 2dpf and 5dpf separately (Stata’s xtmixed), mutually adjusted for the other outcome (i.e. heart rate and HRV) and time of imaging (fixed factors), and with larvae nested in six batches (random factor with fixed slope). Embryos and larvae in which a sinoatrial pause and/or arrest was observed in between positioning and recording were excluded from the analysis (see Supplemental Table S7 for a sensitivity analysis). In secondary analyses, genetic effects were examined for cardiac abnormalities that were observed in ≥5 larvae at 2dpf and/or 5dpf, using a logistic regression analysis. Genetic effects on body size were also examined, using hierarchical linear models on inverse-normally transformed outcomes.

For dichotomous as well as continuous outcomes, genetic effects were examined using an additive model, with dosage scores for all target sites as independent exposures in the same model, i.e. mutually adjusted for effects of mutations in the other orthologues. Missing genotypes were imputed at the mean in this analysis. For each outcome, association analyses were examined for the main transcript of the orthologue. We performed two independent primary and six independent secondary tests at 2 and 5dpf – on different data – and considered $P$-values $<$0.05 to reflect statistically significant effects. All statistical analyses were performed using Stata MP 14.2.
DATA ACCESS

Raw data and scripts will be made publicly available after publication if desirable.

Supplemental videos have been deposited at https://www.dropbox.com/s/3dboz7nq3emkz6z/Supp_videos.zip?dl=0.

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AUTHOR CONTRIBUTIONS: B.v.d.H. and M.d.H. conceived the study design. B.v.d.H., A.E., T.K., S.V. and S.J. performed the experiments. B.v.d.H., A.E., E.M., O.D. and M.d.H. conceived the sequencing quality control and variant calling pipeline. B.v.d.H., A.A., H.L.B. and M.d.H. performed the image quantification and statistical analysis. B.v.d.H. and M.d.H. wrote the manuscript. All authors provided critical feedback to the manuscript.
DISCLOSURE DECLARATION

There are no competing interests.
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384 embryos imaged at 2dpf

Secondary trait analysis:
Cardiac traits (left), body size analysis (right)

Sinoatrial pauses (n=39) & arrests (n=36),
uncontrolled atrial contraction (n=1),
edema (n=15), fish moved during
recording/missed beats (n=38)

Embryos excluded due to
suboptimal segmentation
and/or presence of edema:
length (n=46), dorsal (n=56),
lateral (n=78), volume (n=79)

Primary analysis:
279 embryos included in HRV and
heart rate analysis at 2dpf

384 embryos imaged at 2dpf

Larvae excluded due to
technical issues* (n=41), death (n=3)
or larva not detected by VAST (n=14)

326 larvae imaged at 5dpf

Secondary trait analysis:
Cardiac traits (left), body size analysis (right)

Sinoatrial pauses (n=9) & arrests (n=3),
uncontrolled atrial contraction (n=9),
abnormal morphology/reduced contractility (n=6)
edema (n=14), missed beats (n=1)

Larvae excluded due to
suboptimal segmentation
and/or presence of edema:
length (n=84), dorsal (n=93),
lateral (n=70), volume (n=128)

Primary analysis:
291 larvae included in HRV and
heart rate analysis at 5dpf

Supplementary Figure S1: Flow chart showing the number of included larvae in the analysis and reasons for exclusions. Embryos/larvae can show more than one phenotype. *Technical issues refers to not being able to image due to microscope complications. VAST: Vertebrate automated screening technology.
| Gene   | Orthologue  | Target Sequence gRNA | Exon Target (Transcript Name) | CRISPRscan Rank | ChopChop Rank | Activity Test Rank | Predicted Off-targets Nr of Mismatches | Genomic Location of Potential Off-target 1, Feature ENSG (Gene-ID) of Potential Off-target 1 | Genomic Location of Potential Off-target 2, Feature ENSG (Gene-ID) of Potential Off-target 2 |
|--------|-------------|----------------------|-------------------------------|-----------------|---------------|-------------------|--------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| gngt1  | ENSDARG00000035798 | gGACCTGGATAAGGCGAAGA | 2/3 (gngt1-001); 1/2 (gngt1-002) | 62              | 4/13 (yellow) | Moderate          | 1                                   | chr25:17488676                                                                  | ENSDARG00000051950 (zgc:66449), Intron (3/6), Intron (4/4)                 |
| syt10  | ENSDARG00000045750 | GGCTGATGCCGTCCTCTGTG | 1/7 (syt10-001); 1/7 (syt10-201) | 36              | 6/129 (green) | Low               | 0                                   |                                                                                   |                                                                                  |
| rgs6   | ENSDARG00000015627 | GGCCTCTCAGGACCAAGG | 1/17 (rgs6-201); 2/18 (rgs6-001) | 62              | 1/107 (green) | Low               | 0                                   |                                                                                   |                                                                                  |
| hcn4   | ENSDARG00000061685 | GGAGAGCCCTCCTGGAGCCG | 1/8 (hcn4-001) | 59              | 11/280 (green) | High              | 2                                   | chr21:45844184                                                                  | ENSDARG00000100453 (galnt10), Exon (1/3)                                     |
| hcn4l  | ENSDARG00000074419 | gGCCTGGATAGTGTTTAACG | 2/8 (hcn4l-201); 1/7 (hcn4l-001) | 2/191 (green)   | Low            | 0                  | 0                                   | chr15:32969618                                                                  | ENSDARG00000104664 (dckl1b), Exon (3/16), Exon (4/17)                       |
| neo1a  | ENSDARG00000102855 | GGAGCCGTCGGATACACTAG | 1/4 (neo1a-001); 2/30 (neo1a-202); 2/29 (neo1a-201) | 48              | 2/55 (green)   | Very High          | 0                                   |                                                                                   |                                                                                  |
| neo1b  | ENSDARG00000075100 | GGACAGAGATGCTCGGCCTG | 3/29 (neo1b-201) | 58              | 18/354 (green) | Very High          | 0                                   |                                                                                   |                                                                                  |
| quo    | ENSDARG00000073684 | GGAGATCCGGTTGCCCTGTG | 2/22 (quo-001); 3/23 (quo-201) | 83              | 12/331 (green) | Very High          | 0                                   |                                                                                   |                                                                                  |
| si:dkey-65j6.2 | ENSDARG00000103586 | GGACGGTTTGGCTCCAGCAG | 1/23 (si:dkey-65j6.2-201); 3/25 (si:dkey-65j6.2-001) | 59              | 14/303 (green) | Very High          | 2                                   | chr15:32969618                                                                  | ENSDARG00000104664 (dckl1b), Exon (3/16), Exon (4/17)                       |

Supplemental Table 1: Overview of zebrafish orthologues and designed gRNAs. Table showing zebrafish genes, main transcript used for the analysis and gRNAs; rsID refers to the lead S-S-identified loci (Nolte et al., 2017), multiple independent SNPs per locus can be present; for simplicity, quo is referred to as kiaa1755_1, and si:dkey-65j6.2 as kiaa1755_2; Conservation refers to the number of genes that are preserved in the same locus across species according to Zebrafish-spotted gar and human conservation scores (Nolte et al., 2017); Predicted off-targets indicate a manually introduced nucleotide change to guanine; Exon target lists the targeted transcript name according to gRNA target sequence. Intron conservation indicates that the sequence target indicates an introduced nucleotide change to guanine; Exon target lists the targeted transcript name according to gRNA target sequence.
### Supplemental Table 2: Unique Cigars

| Orthologue | Sequence | Cigar | Mean Nr reads | Nr alleles | SD reads |
|------------|----------|-------|---------------|------------|----------|
| gngt1      | CTACATTAGCGAGGCGCTATATTCCCTTCCAGTATATTGGAACATCACATAATAATCCATTCCTACACCAACACAT | 45M1S41M1S119M | 498 | 336 | 381 |
|            | GCGAGTTAGAGAAGTCAGTGGTGGGCTCACAGAAAAACATGTGCGAGGCGATGGTGAAGTCGGAAGTGGGAAGGGCGGAAAATGCGCGGATATATTTCCAC | | | | |
| gngt1      | GTAAATGTGATGTGATGTGTCCTGTGCACTTGAGCAGATGGAATGAAAAATGACTAGCTGGATAAG | 207M | 517 | 267 | 372 |
|            | GCGAGTTAGAGAAGTCAGTGGTGGGCTCACAGAAAAACATGTGCGAGGCGATGGTGAAGTCGGAAGTGGGAAGGGCGGAAAATGCGCGGATATATTTCCAC | | | | |
| gngt1      | GTAAATGTGATGTGATGTGTCCTGTGCACTTGAGCAGATGGAATGAAAAATGACTAGCTGGATAAG | 45M1S41M1S119M | 544 | 69 | 405 |
|            | GCGAGTTAGAGAAGTCAGTGGTGGGCTCACAGAAAAACATGTGCGAGGCGATGGTGAAGTCGGAAGTGGGAAGGGCGGAAAATGCGCGGATATATTTCCAC | | | | |
| gngt1      | GTAAATGTGATGTGATGTGTCCTGTGCACTTGAGCAGATGGAATGAAAAATGACTAGCTGGATAAG | 151M6D50M | 500 | 63 | 364 |
|            | GCGAGTTAGAGAAGTCAGTGGTGGGCTCACAGAAAAACATGTGCGAGGCGATGGTGAAGTCGGAAGTGGGAAGGGCGGAAAATGCGCGGATATATTTCCAC | | | | |
| gngt1      | GTAAATGTGATGTGATGTGTCCTGTGCACTTGAGCAGATGGAATGAAAAATGACTAGCTGGATAAG | 158M1S48M | 504 | 4 | 221 |
|            | GCGAGTTAGAGAAGTCAGTGGTGGGCTCACAGAAAAACATGTGCGAGGCGATGGTGAAGTCGGAAGTGGGAAGGGCGGAAAATGCGCGGATATATTTCCAC | | | | |
| gngt1      | GTAAATGTGATGTGATGTGTCCTGTGCACTTGAGCAGATGGAATGAAAAATGACTAGCTGGATAAG | 1S44M1S41M1S69M1D3S46M | 584 | 13 | 479 |
|            | GCGAGTTAGAGAAGTCAGTGGTGGGCTCACAGAAAAACATGTGCGAGGCGATGGTGAAGTCGGAAGTGGGAAGGGCGGAAAATGCGCGGATATATTTCCAC | | | | |
| gngt1      | GTAAATGTGATGTGATGTGTCCTGTGCACTTGAGCAGATGGAATGAAAAATGACTAGCTGGATAAG | 45M1S41M1S119M | 504 | 4 | 221 |
|            | GCGAGTTAGAGAAGTCAGTGGTGGGCTCACAGAAAAACATGTGCGAGGCGATGGTGAAGTCGGAAGTGGGAAGGGCGGAAAATGCGCGGATATATTTCCAC | | | | |
| gngt1      | GTAAATGTGATGTGATGTGTCCTGTGCACTTGAGCAGATGGAATGAAAAATGACTAGCTGGATAAG | 87M1S119M | 3 | 1 | - |
|            | GCGAGTTAGAGAAGTCAGTGGTGGGCTCACAGAAAAACATGTGCGAGGCGATGGTGAAGTCGGAAGTGGGAAGGGCGGAAAATGCGCGGATATATTTCCAC | | | | |
| syt10      | CGGTGTCATCTATCCTACCCCTTGCACGTCAGCTAGCCTACACATGCTGGCTCCCAATGAATCCTGCGCTCCACG | 227M | 1024 | 518 | 644 |
|            | GGGCTTCCCATACAAATGAGCTAGCTATGCTGCGCTCCCAAGAAGGGACGAAGGCTCTAGGGCGCCGGAAGGGAGAGGCTCTAGGATATATTTCCAC | | | | |
| syt10      | CGGTGTCATCTATCCTACCCCTTGCACGTCAGCTAGCCTACACATGCTGGCTCCCAATGAATCCTGCGCTCCACG | 126M1I101M | 735 | 46 | 462 |
|            | GGGCTTCCCATACAAATGAGCTAGCTATGCTGCGCTCCCAAGAAGGGACGAAGGCTCTAGGGCGCCGGAAGGGAGAGGCTCTAGGATATATTTCCAC | | | | |
| syt10      | CATGTCGCCACATACATGATATGGGTTTATCCACATGCTGGCTCCCAATGAATCCTGCGCTCCACG | 122M8BD97M | 606 | 45 | 397 |
|            | GGGCTTCCCATACAAATGAGCTAGCTATGCTGCGCTCCCAAGAAGGGACGAAGGCTCTAGGGCGCCGGAAGGGAGAGGCTCTAGGATATATTTCCAC | | | | |
| Orthologue | Sequence |
|------------|----------|
| syt10      | CGGTCTCTTCCATTCCGTGCACTCAGCAGTATGCTGCGCCGAGGACGGCGGCAGTCGCGCCGAGGAAATGCGCGGATATATTTCAC |
|            | Cigar     | Mean Nr reads | Nr alleles | SD reads |
| syt10      | 120M1S9I2M1S2M8I101M | 793 | 43 | 405 |
| syt10      | 109M21D98M | 667 | 19 | 267 |
| syt10      | 124M7D96M | 502 | 11 | 300 |
| syt10      | 124M100D95M | 1096 | 16 | 431 |
| rgs6       | GTGACAGTACCTTCCTGAGCACTCACTAGTGCCAGTGTCCAGAGAGGCTGAGCCATGCTTGATTGCTGGGTCTCTCTTTCTGTGTCTGTGGGGAACGGGCACAACAGAAAAGGAATGAGTCCCAGAACAGG |
|            | Cigar     | Mean Nr reads | Nr alleles | SD reads |
| rgs6       | 169M | 1431 | 728 | 813 |
| rgs6       | 68M1D100M | 784 | 24 | 453 |
| rgs6       | 68M2D99M | 540 | 7 | 195 |
| hcn4       | GTCCGGCAGTATCTGCTCGATGCCTTCCAGCTTAATAAAGCCAGCCTGCTGATCCGGGATGCCCTGATGCTGGCTCGAAACCGGCGGCTTCCTCTCGGCCTCCCCCGCCGCTCTGCGCACCGGGCTCCGCGCCGCCACCCGCGGCTCCAGGAGGGCTCTCCTCGCTAGGAGTGACCTCTCCGTCCGTGATCAGACGCCTTTCCTCGGCTACATCCGAGTCGTGGACGTGCC |
|            | Cigar     | Mean Nr reads | Nr alleles | SD reads |
| hcn4       | 68M1D100M | 1424 | 1 | 30 |
| hcn4       | 59M120D89M | 1380 | 638 | 789 |
| Orthologue | Sequence | Cigar | Mean Nr reads | Nr alleles | SD reads |
|------------|----------|-------|---------------|------------|----------|
| hcn4       | GTCCGGCAGTATCTGCTGATGCCCTCCAGCTTTAAAGGCAGCCGTCTGATCCCCGGGTAGCTGCTGATGCCCTGATGCTGGCTCGAACCGGCGGCTTCCTCTCCGCCTCCCCCGCCGCTCTGCGCACCGGGCTCCGCGCCGCCACCCGCGCCGCCACCCAGGGAGGGCTCTCCTCGCTAGGAGTGACCTCTCCGTCCGTGATCAGACGCCTTTCCTCGGCTACATCCGAGTCGTGGACGTGCC | 142M1S1M4I1S85M | 725 | 48         | 343       |
| hcn4       | GTCCGGCAGTATCTGCTGATGCCCTCCAGCTTTAAAGGCAGCCGTCTGATCCCCGGGTAGCTGCTGATGCCCTGATGCTGGCTCGAACCGGCGGCTTCCTCTCCGCCTCCCCCGCCGCTCTGCGCACCGGGCTCCGCGCCGCCACCCGCGCCGCCACCCAGGGAGGGCTCTCCTCGCTAGGAGTGACCTCTCCGTCCGTGATCAGACGCCTTTCCTCGGCTACATCCGAGTCGTGGACGTGCC | 136M1SS5M11S78M | 1314 | 16         | 225       |
| hcn4       | GTCCGGCAGTATCTGCTGATGCCCTCCAGCTTTAAAGGCAGCCGTCTGATCCCCGGGTAGCTGCTGATGCCCTGATGCTGGCTCGAACCGGCGGCTTCCTCTCCGCCTCCCCCGCCGCTCTGCGCACCGGGCTCCGCGCCGCCACCCGCGCCGCCACCCAGGGAGGGCTCTCCTCGCTAGGAGTGACCTCTCCGTCCGTGATCAGACGCCTTTCCTCGGCTACATCCGAGTCGTGGACGTGCC | 143M5I7M6I80M | 799 | 15         | 383       |
| hcn4       | GTCCGGCAGTATCTGCTGATGCCCTCCAGCTTTAAAGGCAGCCGTCTGATCCCCGGGTAGCTGCTGATGCCCTGATGCTGGCTCGAACCGGCGGCTTCCTCTCCGCCTCCCCCGCCGCTCTGCGCACCGGGCTCCGCGCCGCCACCCGCGGCTCTCCTCCAGGGAGCTCCAGGGCTCTCCTCGCTAGGAGTGACCTCTCCGTCCGTGATCAGACGCCTTTCCTCGGCTACATCCGAGTCGTGGACGTGCC | 141M1S1M4I1S87M | 987 | 11         | 377       |
| hcn4       | GTCCGGCAGTATCTGCTGATGCCCTCCAGCTTTAAAGGCAGCCGTCTGATCCCCGGGTAGCTGCTGATGCCCTGATGCTGGCTCGAACCGGCGGCTTCCTCTCCGCCTCCCCCGCCGCTCTGCGCACCGGGCTCCGCGCCGCCACCCGCGGGCCCTCCAGGGAGGGCTCTCCTCGCTAGGAGTGACCTCTCCGTCCGTGATCAGACGCCTTTCCTCGGCTACATCCGAGTCGTGGACGTGCC | 143M3I87M | 694 | 2          | 49        |
| hcn4       | GTCCGGCAGTATCTGCTGATGCCCTCCAGCTTTAAAGGCAGCCGTCTGATCCCCGGGTAGCTGCTGATGCCCTGATGCTGGCTCGAACCGGCGGCTTCCTCTCCGCCTCCCCCGCCGCTCTGCGCACCGGGCTCCGCGCCGCCACCCGCGCCCTCCAGGGAGGGCTCTCCTCGCTAGGAGTGACCTCTCCGTCCGTGATCAGACGCCTTTCCTCGGCTACATCCGAGTCGTGGACGTGCC | 98M11J4M2S1D86M | 346 | 2          | 36        |
| hcn4l      | AGGAGAGGAAATCCACGGCAAACCAGCTGCGCAAGTACCTGACCTTGATCTGCTGAGGGTCCAGGATGATTTCTGTACTGTCCTCTTTCACAATCCCCGTGCGGAAGTTCAGCACGAGGTCGACCAGGAAGAAGGTGTCGGAGACCACGTAAACACTATCCAGGCGGGTGTGTGTTCGTCTTTGAAGAAGGTGATGCCCACGGGGATGATGATCAGGTTGCCCACCATGTTAGAAG | 115M1S34M2D53M1S29M | 585 | 18         | 295       |
| Orthologue | Sequence | Cigar | Mean Nr reads | Nr alleles | SD reads |
|------------|----------|-------|---------------|------------|----------|
| hcn4l      | AGGAGAGAAATCCAGGAGAACGTCGAGACTGACATTCTGCTGAGGTTCAGGATTCTCTG | 115M1S28M16D45M1S29M | 523         | 9          | 176      |
| hcn4l      | AGGAGAGAAATCCAGGAGAACGTCGAGACTGACATTCTGCTGAGGTTCAGGATTCTCTG | 115M1S33M15S6M1S29M | 1218        | 4          | 915      |
| neo1a      | CAGTGAGGAACTTCAGCCCATTCTGGTTCTCCACGGACGAGAGCGCCAGTTC | 50M16D10M1I91M1S57M | 957         | 95         | 545      |
| neo1a      | CAGTGAGGAACTTCAGCCCATTCTGGTTCTCCACGGACGAGAGCGCCAGTTC | 40M16D10M1I91M1S57M | 696         | 72         | 413      |
| neo1a      | CAGTGAGGAACTTCAGCCCATTCTGGTTCTCCACGGACGAGAGCGCCAGTTC | 40M16D10M1I91M1S57M | 899         | 82         | 475      |
| neo1a      | CAGTGAGGAACTTCAGCCCATTCTGGTTCTCCACGGACGAGAGCGCCAGTTC | 30M4D30M1I91M1S57M | 703         | 66         | 359      |
| neo1a      | CAGTGAGGAACTTCAGCCCATTCTGGTTCTCCACGGACGAGAGCGCCAGTTC | 42M12D10M3S57M | 589         | 72         | 305      |
| neo1a      | CAGTGAGGAACTTCAGCCCATTCTGGTTCTCCACGGACGAGAGCGCCAGTTC | 42M12D10M3S57M | 696         | 72         | 413      |
| neo1a      | CAGTGAGGAACTTCAGCCCATTCTGGTTCTCCACGGACGAGAGCGCCAGTTC | 30M4D30M1I91M1S57M | 985         | 57         | 521      |
| neo1a      | CAGTGAGGAACTTCAGCCCATTCTGGTTCTCCACGGACGAGAGCGCCAGTTC | 30M4D30M1I91M1S57M | 899         | 82         | 475      |
| neo1a      | CAGTGAGGAACTTCAGCCCATTCTGGTTCTCCACGGACGAGAGCGCCAGTTC | 30M4D30M1I91M1S57M | 703         | 66         | 359      |
| neo1a      | CAGTGAGGAACTTCAGCCCATTCTGGTTCTCCACGGACGAGAGCGCCAGTTC | 30M4D30M1I91M1S57M | 589         | 72         | 305      |
| neo1a      | CAGTGAGGAACTTCAGCCCATTCTGGTTCTCCACGGACGAGAGCGCCAGTTC | 42M12D10M3S57M | 696         | 72         | 413      |
| Orthologue | Sequence | Cigar | Mean Nr reads | Nr alleles | SD reads |
|------------|----------|-------|---------------|------------|----------|
| neo1a      | CAGTGAGGACATTCCGCCATTCGTTCCACCGAGAGGGATATACAAAGGAGGACGGCCAGTCCGTCTCA | 50M6D2S99M1S57M | 569 | 10 | 314 |
| neo1a      | ACTGTCAGCTCCAGGAGAGTTCTCAGGCAAGATGGTTGAAAAAGATGGCTCTTCTCCAGGGTCTCAAGA | 597M1S105M1S57M | 1569 | 6 | 1406 |
| neo1a      | CAGTGAGGACATTCCGCCATTCGTTCCACCGAGAGGGATATACAAAGGAGGACGGCCAGTCCGTCTCA | 48M2S1M14I1S33M1S72M1S57M | 1242 | 1 | - |
| neo1b      | TGGCAGGTCTTCCGCGGTTCCTCAGTCAGCCGGAGGGTGTTTCTGTTCGAGGCTGGGACTGAGATGCC | 123M6D64M1S12M | 734 | 4 | 441 |
| neo1b      | TGGCAGGTCTTCCGCGGTTCCTCAGTCAGCCGGAGGGTGTTTCTGTTCGAGGCTGGGACTGAGATGCC | 128M4I77M | 991 | 77 | 818 |
| neo1b      | TGGCAGGTCTTCCGCGGTTCCTCAGTCAGCCGGAGGGTGTTTCTGTTCGAGGCTGGGACTGAGATGCC | 129M6D71M | 700 | 75 | 505 |

Continued
| Orthologue | Sequence                                                                 | Cigar                  | Mean Nr reads | Nr alleles | SD reads |
|------------|--------------------------------------------------------------------------|------------------------|---------------|------------|----------|
| neo1b      | TGGCAGGTCTTCGGCCTGTTCTCAGTCAGTCAGTCAGCCGGAGGGTGTTTCTGTTCGCAGCGGTGGGACTGAGATGCTGAGCTGTTTGAGGTGACGGAGGAGCTGGTCCCGTTCACCCGCTGGGACAGAGATGTGGAGCTGGACAGCAGGGTCATTCAGCTGCCCAGCGGAGCTCTGGTCATCAGCAACGCCTCACAGGCTGACGCC | 121M9D63M12M           | 739           | 33         | 686      |
| neo1b      | TGGCAGGTACGGAGGAAGAGCTGTCGCCGTTCTACCCGCGTGGACAGAATGTGCGAGCTGAAGCAGGACTTATTAGCAGGGTTCGAGGTGGGACTGAGATGCTGAGCTGTTTGAGGTGACGGAGGAGCTGGTCCCGTTCACCCGCTGGGACAGAGATGTGGAGCTGGACAGCAGGGTCATTCAGCTGCCCAGCGGAGCTCTGGTCATCAGCAACGCCTCACAGGCTGACGCC | 406x506                | 739           | 33         | 686      |
| neo1b      | TGGCAGGTACGGAGGAAGAGCTGTCGCCGTTCTACCCGCGTGGACAGAATGTGCGAGCTGAAGCAGGACTTATTAGCAGGGTTCGAGGTGGGACTGAGATGCTGAGCTGTTTGAGGTGACGGAGGAGCTGGTCCCGTTCACCCGCTGGGACAGAGATGTGGAGCTGGACAGCAGGGTCATTCAGCTGCCCAGCGGAGCTCTGGTCATCAGCAACGCCTCACAGGCTGACGCC | 193M1512M              | 970           | 27         | 739      |
| neo1b      | TGGCAGGTACGGAGGAAGAGCTGTCGCCGTTCTACCCGCGTGGACAGAATGTGCGAGCTGAAGCAGGACTTATTAGCAGGGTTCGAGGTGGGACTGAGATGCTGAGCTGTTTGAGGTGACGGAGGAGCTGGTCCCGTTCACCCGCTGGGACAGAGATGTGGAGCTGGACAGCAGGGTCATTCAGCTGCCCAGCGGAGCTCTGGTCATCAGCAACGCCTCACAGGCTGACGCC | 123M6D77M              | 915           | 24         | 914      |
| neo1b      | TGGCAGGTACGGAGGAAGAGCTGTCGCCGTTCTACCCGCGTGGACAGAATGTGCGAGCTGAAGCAGGACTTATTAGCAGGGTTCGAGGTGGGACTGAGATGCTGAGCTGTTTGAGGTGACGGAGGAGCTGGTCCCGTTCACCCGCTGGGACAGAGATGTGGAGCTGGACAGCAGGGTCATTCAGCTGCCCAGCGGAGCTCTGGTCATCAGCAACGCCTCACAGGCTGACGCC | 129M8I3M4I74M           | 564           | 21         | 525      |
| neo1b      | TGGCAGGTACGGAGGAAGAGCTGTCGCCGTTCTACCCGCGTGGACAGAATGTGCGAGCTGAAGCAGGACTTATTAGCAGGGTTCGAGGTGGGACTGAGATGCTGAGCTGTTTGAGGTGACGGAGGAGCTGGTCCCGTTCACCCGCTGGGACAGAGATGTGGAGCTGGACAGCAGGGTCATTCAGCTGCCCAGCGGAGCTCTGGTCATCAGCAACGCCTCACAGGCTGACGCC | 206M                   | 564           | 21         | 525      |
| neo1b      | TGGCAGGTACGGAGGAAGAGCTGTCGCCGTTCTACCCGCGTGGACAGAATGTGCGAGCTGAAGCAGGACTTATTAGCAGGGTTCGAGGTGGGACTGAGATGCTGAGCTGTTTGAGGTGACGGAGGAGCTGGTCCCGTTCACCCGCTGGGACAGAGATGTGGAGCTGGACAGCAGGGTCATTCAGCTGCCCAGCGGAGCTCTGGTCATCAGCAACGCCTCACAGGCTGACGCC | 125M4077M              | 801           | 10         | 567      |
| neo1b      | TGGCAGGTACGGAGGAAGAGCTGTCGCCGTTCTACCCGCGTGGACAGAATGTGCGAGCTGAAGCAGGACTTATTAGCAGGGTTCGAGGTGGGACTGAGATGCTGAGCTGTTTGAGGTGACGGAGGAGCTGGTCCCGTTCACCCGCTGGGACAGAGATGTGGAGCTGGACAGCAGGGTCATTCAGCTGCCCAGCGGAGCTCTGGTCATCAGCAACGCCTCACAGGCTGACGCC | 117M22D54M1512M        | 1170          | 3          | 685      |
| neo1b      | TGGCAGGTACGGAGGAAGAGCTGTCGCCGTTCTACCCGCGTGGACAGAATGTGCGAGCTGAAGCAGGACTTATTAGCAGGGTTCGAGGTGGGACTGAGATGCTGAGCTGTTTGAGGTGACGGAGGAGCTGGTCCCGTTCACCCGCTGGGACAGAGATGTGGAGCTGGACAGCAGGGTCATTCAGCTGCCCAGCGGAGCTCTGGTCATCAGCAACGCCTCACAGGCTGACGCC | 117M22D54M1512M        | 1170          | 3          | 685      |
| neo1b      | TGGCAGGTACGGAGGAAGAGCTGTCGCCGTTCTACCCGCGTGGACAGAATGTGCGAGCTGAAGCAGGACTTATTAGCAGGGTTCGAGGTGGGACTGAGATGCTGAGCTGTTTGAGGTGACGGAGGAGCTGGTCCCGTTCACCCGCTGGGACAGAGATGTGGAGCTGGACAGCAGGGTCATTCAGCTGCCCAGCGGAGCTCTGGTCATCAGCAACGCCTCACAGGCTGACGCC | 120M19D54M1512M        | 801           | 10         | 567      |
| neo1b      | TGGCAGGTACGGAGGAAGAGCTGTCGCCGTTCTACCCGCGTGGACAGAATGTGCGAGCTGAAGCAGGACTTATTAGCAGGGTTCGAGGTGGGACTGAGATGCTGAGCTGTTTGAGGTGACGGAGGAGCTGGTCCCGTTCACCCGCTGGGACAGAGATGTGGAGCTGGACAGCAGGGTCATTCAGCTGCCCAGCGGAGCTCTGGTCATCAGCAACGCCTCACAGGCTGACGCC | 125M17D51M1512M        | 960           | 10         | 710      |
| neo1b      | TGGCAGGTACGGAGGAAGAGCTGTCGCCGTTCTACCCGCGTGGACAGAATGTGCGAGCTGAAGCAGGACTTATTAGCAGGGTTCGAGGTGGGACTGAGATGCTGAGCTGTTTGAGGTGACGGAGGAGCTGGTCCCGTTCACCCGCTGGGACAGAGATGTGGAGCTGGACAGCAGGGTCATTCAGCTGCCCAGCGGAGCTCTGGTCATCAGCAACGCCTCACAGGCTGACGCC | 129M1S84M1512M         | 503           | 11         | 266      |
| neo1b      | TGGCAGGTACGGAGGAAGAGCTGTCGCCGTTCTACCCGCGTGGACAGAATGTGCGAGCTGAAGCAGGACTTATTAGCAGGGTTCGAGGTGGGACTGAGATGCTGAGCTGTTTGAGGTGACGGAGGAGCTGGTCCCGTTCACCCGCTGGGACAGAGATGTGGAGCTGGACAGCAGGGTCATTCAGCTGCCCAGCGGAGCTCTGGTCATCAGCAACGCCTCACAGGCTGACGCC | 126M7D60M1512M         | 503           | 11         | 266      |
| kiaa1755_1 | CGCAGAGTCTCCTGACCAACATAGCACCACCCCCAATCTACCCGCGTGACAGAATGTGCGAGCTGAAGCAGGACTTATTAGCAGGGTTCGAGGTGGGACTGAGATGCTGAGCTGTTTGAGGTGACGGAGGAGCTGGTCCCGTTCACCCGCTGGGACAGAGATGTGGAGCTGGACAGCAGGGTCATTCAGCTGCCCAGCGGAGCTCTGGTCATCAGCAACGCCTCACAGGCTGACGCC | 6M132321MM1512M        | 997           | 119        | 507      |
| kiaa1755_2 | CGCAGAGTCTCCTGACCAACATAGCACCACCCCCAATCTACCCGCGTGACAGAATGTGCGAGCTGAAGCAGGACTTATTAGCAGGGTTCGAGGTGGGACTGAGATGCTGAGCTGTTTGAGGTGACGGAGGAGCTGGTCCCGTTCACCCGCTGGGACAGAGATGTGGAGCTGGACAGCAGGGTCATTCAGCTGCCCAGCGGAGCTCTGGTCATCAGCAACGCCTCACAGGCTGACGCC | 6M132321MM1512M        | 997           | 119        | 507      |
| kiaa1755_3 | CGCAGAGTCTCCTGACCAACATAGCACCACCCCCAATCTACCCGCGTGACAGAATGTGCGAGCTGAAGCAGGACTTATTAGCAGGGTTCGAGGTGGGACTGAGATGCTGAGCTGTTTGAGGTGACGGAGGAGCTGGTCCCGTTCACCCGCTGGGACAGAGATGTGGAGCTGGACAGCAGGGTCATTCAGCTGCCCAGCGGAGCTCTGGTCATCAGCAACGCCTCACAGGCTGACGCC | 6M132321MM1512M        | 997           | 119        | 507      |
| kiaa1755_4 | CGCAGAGTCTCCTGACCAACATAGCACCACCCCCAATCTACCCGCGTGACAGAATGTGCGAGCTGAAGCAGGACTTATTAGCAGGGTTCGAGGTGGGACTGAGATGCTGAGCTGTTTGAGGTGACGGAGGAGCTGGTCCCGTTCACCCGCTGGGACAGAGATGTGGAGCTGGACAGCAGGGTCATTCAGCTGCCCAGCGGAGCTCTGGTCATCAGCAACGCCTCACAGGCTGACGCC | 6M132321MM1512M        | 997           | 119        | 507      |
| Orthologue  | Sequence                                                                 | Cigar                        | Mean Nr reads | Nr alleles | SD reads |
|------------|--------------------------------------------------------------------------|------------------------------|---------------|------------|----------|
| kiaa1755_1 | CGCAGAGTCTCTGGACCAAATATGACCACTACCACTATCACCAACATGTTCAACAGGAACACGGATCCT    | 6M1S20M11I2M1541M312556M1513M | 882           | 68         | 903      |
|            | ATACCTTTCTGTGAA                                                          | 6M1S32M1538M100D51M1513M     | 541           | 57         | 417      |
|            | kiaa1755_1                                                              | 6M1S32M1542M3157M1513M       | 780           | 55         | 596      |
|            | kiaa1755_1                                                              | 6M1S32M1521M17D56M           | 1077          | 38         | 490      |
|            | kiaa1755_1                                                              | 6M1S32M1521M1518M151D70M     | 876           | 30         | 459      |
|            | kiaa1755_1                                                              | 6M1S32M15199M1513M           | 775           | 30         | 588      |
|            | kiaa1755_1                                                              | 6M1S32M151542M1511S55M1513M | 822           | 22         | 547      |
|            | kiaa1755_1                                                              | 6M1S32M1522M1511M3515M1512S51M1560M | 1190          | 21         | 408      |
|            | kiaa1755_1                                                              | 6M1S32M1522M1511M1511M1517M | 1469          | 20         | 683      |
|            | kiaa1755_1                                                              | 6M1S32M1522M1517M5D68M       | 1365          | 18         | 561      |
|            | kiaa1755_1                                                              | 6M1S32M1522M1518M15172M      | 1602          | 14         | 834      |
|            | kiaa1755_1                                                              | 6M1S32M1522M1518M15172M      | 1602          | 14         | 834      |
|            | kiaa1755_1                                                              | 6M1S32M151542M1511S55M1513M | 822           | 22         | 547      |
|            | kiaa1755_1                                                              | 6M1S32M1522M1511M3515M1512S51M1560M | 1190          | 21         | 408      |
|            | kiaa1755_1                                                              | 6M1S32M1522M1511M1511M1517M | 1469          | 20         | 683      |
|            | kiaa1755_1                                                              | 6M1S32M1522M1517M5D68M       | 1365          | 18         | 561      |
|            | kiaa1755_1                                                              | 6M1S32M1522M1518M15172M      | 1602          | 14         | 834      |
| Orthologue       | Sequence                                                                 | Cigar                  | Mean Nr reads | Nr alleles | SD reads |
|------------------|---------------------------------------------------------------------------|------------------------|---------------|-----------|----------|
| kiaa1755_1       | CGCAGAGTCTCTGGACCAAGGACATGAATGACCCAGGATCTCTGGAGCAATGATGAGGGTACAGGAGACACGAGATC | 6M1S20M11I2M154M312570M | 136           | 6         | 89       |
|                  | ACATGCCATCTCTTGGAGCAGGAGAAATGATGACCCAGGATCTCTGGAGCAATGATGAGGGTACAGGAGACACGAGATC |                        |               |           |          |
|                  | kiaa1755_1       | CGCAGAGTCTCTGGACCAAGGACATGAATGACCCAGGATCTCTGGAGCAATGATGAGGGTACAGGAGACACGAGATC | 6M1S20M11I2M154M312570M | 1101       | 5         | 323      |
|                  | ACATGCCATCTCTTGGAGCAGGAGAAATGATGACCCAGGATCTCTGGAGCAATGATGAGGGTACAGGAGACACGAGATC |                        |               |           |          |
|                  | kiaa1755_1       | CGCAGAGTCTCTGGACCAAGGACATGAATGACCCAGGATCTCTGGAGCAATGATGAGGGTACAGGAGACACGAGATC | 6M1S20M11I2M154M312570M | 120        | 4         | 48       |
|                  | ACATGCCATCTCTTGGAGCAGGAGAAATGATGACCCAGGATCTCTGGAGCAATGATGAGGGTACAGGAGACACGAGATC |                        |               |           |          |
|                  | kiaa1755_1       | CGCAGAGTCTCTGGACCAAGGACATGAATGACCCAGGATCTCTGGAGCAATGATGAGGGTACAGGAGACACGAGATC | 6M1S20M11I2M154M312570M | 107        | 2         | 65       |
|                  | ACATGCCATCTCTTGGAGCAGGAGAAATGATGACCCAGGATCTCTGGAGCAATGATGAGGGTACAGGAGACACGAGATC |                        |               |           |          |
|                  | kiaa1755_1       | CGCAGAGTCTCTGGACCAAGGACATGAATGACCCAGGATCTCTGGAGCAATGATGAGGGTACAGGAGACACGAGATC | 6M1S20M11I2M154M312570M | 1184       | 1         | -        |
|                  | ACATGCCATCTCTTGGAGCAGGAGAAATGATGACCCAGGATCTCTGGAGCAATGATGAGGGTACAGGAGACACGAGATC |                        |               |           |          |
|                  | kiaa1755_1       | CGCAGAGTCTCTGGACCAAGGACATGAATGACCCAGGATCTCTGGAGCAATGATGAGGGTACAGGAGACACGAGATC | 6M1S20M11I2M154M312570M | 233        | 1         | -        |
|                  | ACATGCCATCTCTTGGAGCAGGAGAAATGATGACCCAGGATCTCTGGAGCAATGATGAGGGTACAGGAGACACGAGATC |                        |               |           |          |
|                  | kiaa1755_1       | CGCAGAGTCTCTGGACCAAGGACATGAATGACCCAGGATCTCTGGAGCAATGATGAGGGTACAGGAGACACGAGATC | 6M1S20M11I2M154M312570M | 25         | 1         | -        |
|                  | ACATGCCATCTCTTGGAGCAGGAGAAATGATGACCCAGGATCTCTGGAGCAATGATGAGGGTACAGGAGACACGAGATC |                        |               |           |          |
|                  | kiaa1755_2       | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC | 48M1522M21D45M152151M | 985        | 120       | 638      |
|                  | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC |                        |               |           |          |
|                  | kiaa1755_2       | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC | 48M1522M21D45M152151M | 891        | 78        | 613      |
|                  | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC |                        |               |           |          |
|                  | kiaa1755_2       | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC | 48M1522M21D45M152151M | 1070       | 62        | 464      |
|                  | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC |                        |               |           |          |
|                  | kiaa1755_2       | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC | 48M1522M21D45M152151M | 772        | 62        | 333      |
|                  | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC |                        |               |           |          |
|                  | kiaa1755_2       | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC | 78M1520D59M | 856        | 59        | 414      |
|                  | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC |                        |               |           |          |
|                  | kiaa1755_2       | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC | 81M1520D59M | 829        | 55        | 457      |
|                  | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC |                        |               |           |          |
|                  | kiaa1755_2       | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC | 81M1520D59M | 935        | 35        | 587      |
|                  | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC |                        |               |           |          |
|                  | kiaa1755_2       | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC | 76M120D64M | 837        | 33        | 470      |
|                  | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC |                        |               |           |          |
|                  | kiaa1755_2       | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC | 564        | 32        | 351      |
|                  | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC |                        |               |           |          |
|                  | kiaa1755_2       | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC | 48M1522M14D52M152151M | 964        | 26        | 370      |
|                  | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC |                        |               |           |          |
|                  | kiaa1755_2       | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC | 48M1522M14D52M152151M | 793        | 20        | 426      |
|                  | TCTGATCATCCTAATGGAAGAACAAAATGCTGGACACTCTGGAGGACTGAGAACAAATCAGGAGGATCCGAGGACGCTAGC |                        |               |           |          |
| Orthologue      | Sequence                                                                 | Cigar                  | Mean Nr reads | Nr alleles | SD reads |
|----------------|--------------------------------------------------------------------------|------------------------|---------------|------------|----------|
| kiaa1755_2     | TCTGATCATCTAATGAAAGCAGAAACTGCTCAGCAGGACGAGAGAGGAAATCCAGAGGCGCATACCGGGACGGT | 48M1S25M1SBD1M1S2M1S2M1S11M | 787           | 19         | 595      |
| kiaa1755_2     | TCTGATCATCTAATGAAAGCAGAAACTGCTCAGCAGGACGAGAGAGGAAATCCAGAGGCGCATACCGGGACGGT | 48M1S262M1D41M1S2M1S11M | 1035          | 18         | 370      |
| kiaa1755_2     | TCTGATCATCTAATGAAAGCAGAAACTGCTCAGCAGGACGAGAGAGGAAATCCAGAGGCGCATACCGGGACGGT | 48M1S29M1D48M1S2M1S11M | 657           | 17         | 443      |
| kiaa1755_2     | TCTGATCATCTAATGAAAGCAGAAACTGCTCAGCAGGACGAGAGAGGAAATCCAGAGGCGCATACCGGGACGGT | 48M1S21M1D74M1S2M1S11M | 1152          | 15         | 331      |
| kiaa1755_2     | TCTGATCATCTAATGAAAGCAGAAACTGCTCAGCAGGACGAGAGAGGAAATCCAGAGGCGCATACCGGGACGGT | 48M1S21M1D74M1S2M1S11M | 1152          | 15         | 331      |
| kiaa1755_2     | TCTGATCATCTAATGAAAGCAGAAACTGCTCAGCAGGACGAGAGAGGAAATCCAGAGGCGCATACCGGGACGGT | 48M1S21M1D74M1S2M1S11M | 1152          | 15         | 331      |
| kiaa1755_2     | TCTGATCATCTAATGAAAGCAGAAACTGCTCAGCAGGACGAGAGAGGAAATCCAGAGGCGCATACCGGGACGGT | 48M1S21M1D74M1S2M1S11M | 1152          | 15         | 331      |
## Continued Supplemental Table 2

| Orthologue | Sequence                                                                 | Cigar                  | Mean Nr reads | Nr alleles | SD reads |
|------------|--------------------------------------------------------------------------|------------------------|---------------|------------|----------|
| galnt10scaff | GTGGGCACTGGGCTGTGAGGAGAGCAACACTTCTCCGGGTCCCCGATCCGGGCTGAGG              | 216M                   | 148           | 137        | 106      |
| galnt10scaff | AGCCCGGAGACCCAGGAGCCAGGGCTGAGAGACCAGGGCACTGGACAGCCAGACGCTGAGAGACACA   | 41M1S135M14D25M        | 157           | 143        | 94       |
| galnt10scaff | AGCCCGGAGACCCAGGAGCCAGGGCTGAGAGACCAGGGCACTGGACAGCCAGACGCTGAGAGACACA   | 147                    | 82            | 135        | 107      |
| galnt10scaff | AGCCCGGAGACCCAGGAGCCAGGGCTGAGAGACCAGGGCACTGGACAGCCAGACGCTGAGAGACACA   | 136                    | 13            | 136        | 127      |
| galnt10scaff | AGCCCGGAGACCCAGGAGCCAGGGCTGAGAGACCAGGGCACTGGACAGCCAGACGCTGAGAGACACA   | 4                       | 2                          | 2          | 1        |

All unique sequences identified for targeted orthologues and their respective alignment report string (Cigar). Mean number of reads, the number of alleles the cigar was found in and the standard deviation (SD) are reported. Cigar letters: M=match, D=deletion, I=insertion, S=substitution.
**Supplemental Table 3: Unique variants and affected transcripts**

| Orthologue | Chr | ENSDARG | Affected transcripts | Start (bp) | End (bp) | Mutation | Annotation | Base pair change | VEP impact | Nr affected alleles |
|------------|-----|---------|----------------------|------------|----------|----------|------------|------------------|------------|---------------------|
| gngt1      | 19  | ENSDARG00000035798 | 40856888 | 40856893 | AAGGGC/- | inframe_deletion | -6          | MODERATE  | 75               |
|            |     | ENSDARG000001950 | 40856893 | 40856895 | GAAG/-    | frameshift_variant | -3          | MODERATE  | 3                |
|            |     | ENSDARG000013983 | 40856894 | 40856894 | A/-       | frameshift_variant | -1          | HIGH      | 69               |
|            |     |                  | 40856895 | 40856895 | A/T       | missense_variant | 0           | MODERATE  | 3                |
|            |     |                  | 40856895 | 40856897 | AGA/TTT   | missense_variant | 0           | MODERATE  | 69               |
|            |     |                  | 40856897 | 40856896 | /TAAC     | stop_gained,frameshift_variant | 4           | HIGH      | 2                |
|            |     |                  | 40856897 | 40856897 | ATG/CCA   | missense_variant | 0           | MODERATE  | 2                |
|            |     |                  | 10889933 | 10889933 | GACATGATGGTCCGCAGACAG/- | inframe_deletion | -21         | MODERATE  | 19               |
|            |     |                  | 10889944 | 10889944 | C/A       | synonymous_variant | 0           | LOW       | 42               |
|            |     |                  | 10889945 | 10889945 | /CAGTGGATG | inframe_insertion | 9           | MODERATE  | 42               |
|            |     |                  | 10889946 | 10889946 | GCACA/-   | frameshift_variant | -5          | HIGH      | 3                |
|            |     |                  | 10889946 | 10889946 | GCACAGA/- | frameshift_variant | -7          | HIGH      | 15               |
|            |     |                  | 10889947 | 10889947 | GCACAGAG/- | frameshift_variant | -8          | HIGH      | 40               |
|            |     |                  | 10889948 | 10889948 | A/C       | synonymous_variant | 0           | LOW       | 42               |
| sop10      | 4   | ENSDARG0000045750 | 10889948 | 10889948 | ACAGAG/- | frameshift_variant | -7          | HIGH      | 11               |
|            |     | ENSDARG0000136049 | 10889949 | 10889950 | 10889950 | frameshift_variant | -10         | HIGH      | 4                |
|            |     |                  | 10889950 | 10889950 | /GGAATGG  | frameshift_variant | 8           | HIGH      | 42               |
|            |     |                  | 10889950 | 10889950 | /A        | frameshift_variant | 1           | HIGH      | 44               |
|            |     |                  | 10889950 | 10889950 | A/G       | synonymous_variant | 0           | LOW       | 11               |
|            |     |                  | 10889951 | 10889951 | G/T       | stop_gained   | 0           | HIGH      | 42               |
|            |     |                  | 10889952 | 10889952 | /CAT      | stop_gained,protein_altering_variant | 3           | HIGH      | 11               |
|            |     |                  | 10889952 | 10889952 | A/-       | frameshift_variant | -1          | HIGH      | 42               |
|            |     |                  | 10889952 | 10889952 | AG/CA     | missense_variant | 0           | MODERATE  | 11               |
|            |     |                  | 10889959 | 10889959 | 0/0       | missense_variant | 0           | MODERATE  | 4                |
| rs6        | 20  | ENSDARG00000015627 | 28638811 | 28638820 | CCCAGCCGCCCT/- | inframe_deletion | -12         | MODERATE  | 1                |
|            |     | ENSDARG0000135513 | 28638820 | 28638820 | C/-       | frameshift_variant | -1          | HIGH      | 24               |
|            |     | ENSDARG0000184779 | 28638820 | 28638820 | CCT/-     | frameshift_variant | -2          | HIGH      | 7                |
|            |     |                  | 28638820 | 28638820 | CCT/-     | inframe_deletion | -3          | MODERATE  | 4                |
|            |     |                  | 28638824 | 28638824 | G/A       | stop_gained | 0           | HIGH      | 4                |
|            |     |                  | 28638837 | 28638836 | /CTGAGCCATG | frameshift_variant | 10          | HIGH      | 4                |
|            |     |                  | 1143637 | 1143644 | ACCGCCGG/- | frameshift_variant | -8          | HIGH      | 13               |
|            |     |                  | 1143638 | 1143638 | G/T       | missense_variant | 0           | MODERATE  | 16               |
|            |     |                  | 1143641 | 1143641 | G/A       | missense_variant | 0           | MODERATE  | 9                |
|            |     |                  | 1143643 | 1143643 | G/-       | frameshift_variant | -1          | HIGH      | 11               |
|            |     |                  | 1143643 | 1143643 | G/G/C/A   | missense_variant | 0           | MODERATE  | 2                |
|            |     |                  | 1143643 | 1143643 | GGCT/-    | frameshift_variant | -4          | HIGH      | 9                |
|            |     |                  | 1143644 | 1143644 | /CCGCCC   | frameshift_variant | 7           | HIGH      | 16               |
|            |     |                  | 1143644 | 1143644 | G/C       | missense_variant | 0           | MODERATE  | 75               |
| hcn4       | 18  | ENSDARG00000061685 | 1143644 | 1143644 | 1143644 | inframe_deletion | -3          | MODERATE  | 1                |
|            |     | ENSDARG0000136140 | 1143644 | 1143644 | 1143644 | frameshift_variant | 5           | HIGH      | 15               |
|            |     | ENSDARG0000189186 | 1143645 | 1143645 | 1143645 | frameshift_variant | 3           | MODERATE  | 4                |
|            |     |                  | 1143645 | 1143645 | /GTGTCCTCC | protein_altering_variant | 1           | HIGH      | 2                |
|            |     |                  | 1143646 | 1143646 | /CACA     | frameshift_variant | 4           | HIGH      | 48               |
|            |     |                  | 1143646 | 1143646 | T/C       | synonymous_variant | 0           | LOW       | 48               |
|            |     |                  | 1143652 | 1143652 | /AGCTCC   | inframe_insertion | 6           | HIGH      | 15               |
| Orthologue | Chr | Ensembl Transcript ID | Start (bp) | End (bp) | Mutation | VEP impact | Base pair change | Annotation | Nr affected alleles |
|------------|-----|-----------------------|------------|----------|----------|------------|-----------------|------------|---------------------|
| hcn4       | 25  | ENSDARG00000074419    | 29273086   | 29273101 | CGACGTAAAACACTAT/-| frameshift_variant| -16            | HIGH       | 9                   |
|            |     | ENSDART00000088249    | 29273081   | 29273090 | CA/-      | frameshift_variant| -1            | HIGH       | 4                   |
|            |     | ENSDART0000148940    | 29273092   | 29273099 | TA/-      | frameshift_variant| -2            | HIGH       | 18                  |
|            |     |                       | 53879228   | 53879244 | CAGCGAGCCGTGGATA/-| frameshift_variant| -17           | HIGH       | 1                   |
|            |     |                       | 53879231   | 53879258 | GGAGCGCCTGGATACACACTAGCGTGAGG/-| frameshift_variant| -28           | HIGH       | 11                  |
|            |     |                       | 53879235   | 53879255 | CTGCCGATACACTAGCGTGAGGAGGAGG/-| frameshift_variant| -31           | HIGH       | 57                  |
|            |     |                       | 53879237   | 53879252 | CTGCCGATACACTAGCG/-| frameshift_variant| -16           | HIGH       | 157                 |
|            |     |                       | 53879227   | 53879267 | CTGCCGATACACTACGGTGAGCAGAGGCGC/ CA/-| frameshift_variant| -31           | HIGH       | 9                   |
|            |     |                       | 53879239   | 53879250 | CGATACACTAG/-   | inframe_deletion  | -12           | MODERATE  | 66                  |
|            |     |                       | 53879240   | 53879246 | GAGATCA/-     | frameshift_variant| -7            | HIGH       | 2                   |
|            |     |                       | 53879242   | 53879248 | ATACCT/-     | frameshift_variant| -7            | HIGH       | 2                   |
|            |     |                       | 53879243   | 53879247 | TACAC/-      | frameshift_variant| -5            | HIGH       | 17                  |
|            |     |                       | 53879243   | 53879268 | TACACTACCGTGTCGAGAGGAGCGCAG/-| frameshift_variant| -26           | HIGH       | 16                  |
|            |     |                       | 53879244   | 53879245 | AC/G        | missense_variant | 0             | MODERATE  | 1                   |
|            |     |                       | 53879245   | 53879252 | CA/GT       | missense_variant | 0             | MODERATE  | 5                   |
|            |     |                       | 53879245   | 53879255 | CACTACGGTG/-  | frameshift_variant| -11           | HIGH       | 20                  |
|            |     |                       | 53879246   | 53879248 | ACT/-       | inframe_deletion | -3           | MODERATE  | 20                  |
|            |     |                       | 53879247   | 53879248 | -/CGGGTCCGAGGGAGG/ CT/GC/| frameshift_variant| 14           | HIGH       | 1                   |
|            |     |                       | 53879247   | 53879248 | -/TCCAT/     | frameshift_variant| 5             | HIGH       | 14                  |
|            |     |                       | 53879247   | 53879247 | C/G         | missense_variant | 0             | MODERATE  | 1                   |
|            |     |                       | 53879247   | 53879247 | CT/TC       | missense_variant | 0             | MODERATE  | 14                  |
|            |     |                       | 53879247   | 53879250 | CTAG/-      | frameshift_variant| -4            | HIGH       | 82                  |
|            |     |                       | 53879247   | 53879252 | CTAGCG/-    | inframe_deletion  | -6           | MODERATE  | 10                  |
|            |     |                       | 53879247   | 53879253 | CTAGCGTG/-  | inframe_deletion  | -9           | MODERATE  | 69                  |
|            |     |                       | 53879248   | 53879247 | GCGGTT/ GCGG/ | protein_altering_variant | 6           | MODERATE  | 6                   |
|            |     |                       | 53879248   | 53879248 | T/G         | missense_variant | 0             | MODERATE  | 6                   |
|            |     |                       | 53879248   | 53879248 | T/C         | missense_variant | 0             | MODERATE  | 6                   |
|            |     |                       | 53879248   | 53879248 | T/C         | missense_variant | 0             | MODERATE  | 10                  |
|            |     |                       | 53879249   | 53879249 | A/G         | synonymous_variant | 0             | LOW       | 2                   |
|            |     |                       | 53879249   | 53879260 | AGGGTCCGGAGG/ | inframe_deletion  | -12          | MODERATE  | 5                   |
|            |     |                       | 53879250   | 53879254 | GCGGT/-     | frameshift_variant| -5            | HIGH       | 2                   |
|            |     |                       | 53879250   | 53879254 | GT/AA       | missense_variant | 0             | MODERATE  | 10                  |
|            |     |                       | 53879262   | 53879262 | GCGG/-      | frameshift_variant| -4            | HIGH       | 5                   |
|            |     |                       | 53879262   | 53879262 | -/CGCGCTTCT  | frameshift_variant| 10           | HIGH       | 89                  |
|            |     |                       | 53879266   | 53879266 | C/T         | missense_variant | 0             | MODERATE  | 5                   |
|            |     |                       | 2899698    | 2899698  | G/A         | missense_variant | 0             | MODERATE  | 2                   |
|            |     |                       | 2899707    | 2899708  | GAGGATCGTCGCGCGCTGGAGGACT/-| frameshift_variant| -22           | HIGH       | 2                   |
|            |     |                       | 2899710    | 2899710  | ATGCTGGGCGGCTGGGAGGCT/-| frameshift_variant| -19           | HIGH       | 7                   |
|            |     |                       | 2899711    | 2899710  | TGCTGCGG/   | inframe_deletion  | -9           | MODERATE  | 19                  |
|            |     |                       | 2899713    | 2899713  | CTGGGC/-    | inframe_deletion  | -6           | MODERATE  | 120                 |
|            |     |                       | 2899715    | 2899715  | CGCG/-      | frameshift_variant| -4            | HIGH       | 13                  |
|            |     |                       | 2899715    | 2899715  | CGCGGCGGCTGGAGGCGG/-| frameshift_variant| -17           | HIGH       | 6                   |
|            |     |                       | 2899716    | 2899716  | GCGGCGGCTGTCGAGGCGG/-| frameshift_variant| -7            | HIGH       | 7                   |
|            |     |                       | 2899718    | 2899718  | C/-         | frameshift_variant| -1            | HIGH       | 58                  |
|            |     |                       | 2899719    | 2899719  | -/AGGAAGGT  | frameshift_variant| 8             | HIGH       | 15                  |
|            |     |                       | 2899719    | 2899719  | -/TGGG      | frameshift_variant| 4             | HIGH       | 50                  |
|            |     |                       | 2899719    | 2899719  | -/AG        | frameshift_variant| 2             | HIGH       | 1                   |
|            |     |                       | 2899719    | 2899719  | CTGGG/-     | frameshift_variant| -6           | MODERATE  | 50                  |
|            |     |                       | 2899722    | 2899722  | -/GTTC      | frameshift_variant| 4             | HIGH       | 15                  |
|            |     |                       | 2899734    | 2899737  | GCATGTTCA   | missense_variant | 0             | MODERATE  | 7                   |
|            |     |                       | 2899740    | 2899740  | T/A         | missense_variant | 0             | MODERATE  | 7                   |
| Orthologue | Chr | ENSDARG | Affected transcripts | Start (bp) | End (bp) | Mutation | Annotation | Base pair change | VEP impact | Nr affected alleles |
|------------|-----|---------|---------------------|------------|---------|----------|------------|------------------|------------|---------------------|
| 2051654    | 2051734 | ATGACCCAATCTATCAGAGCATGCTCAGAAGACAGCAGCAGCAC | AAGTCATTCCCTCACAGGCAACGAGGCTCCCTCAAGC/ | GGT | AAGTCATTCCCTCACAGGCAACGAGGCTCCCTCAAGC | inframe_deletion | -81 | MODERATE | 13 |
| 2051699    | 2051715 | GTCATTCCCTCACAGG/ | AAGTCATTCCCTCACAGGCAACGAGGCTCCCTCAAGC | GGT | AAGTCATTCCCTCACAGGCAACGAGGCTCCCTCAAGC | inframe_deletion | -15 | MODERATE | 10 |
| 2051703    | 2051716 | CATTCTCACAGGG/ | AAGTCATTCCCTCACAGGCAACGAGGCTCCCTCAAGC | GGT | AAGTCATTCCCTCACAGGCAACGAGGCTCCCTCAAGC | inframe_deletion | -14 | HIGH | 61 |
| 2051706    | 2051708 | TCC/ | inframe_deletion | -13 | MODERATE | 17 |
| 2051709    | 2051715 | TCACAGGG/ | frameshift_variant | -7 | HIGH | 9 |
| 2051710    | 2051716 | CACAGGG/ | frameshift_variant | -7 | HIGH | 53 |
| 2051710    | 2051719 | CACAGGGCAA/ | frameshift_variant | -10 | HIGH | 51 |
| 2051712    | 2051711 | /CCGG | frameshift_variant | 4 | HIGH | 1 |
| 2051712    | 2051711 | /ACCGGACT | frameshift_variant | 8 | HIGH | 7 |
| 2051712    | 2051712 | C/T | stop_gained | 0 | MODERATE | 15 |
| 2051712    | 2051714 | CAG/ACC | frameshift_variant | -5 | HIGH | 14 |
| 2051713    | 2051728 | CAGGG/ | frameshift_variant | -17 | HIGH | 23 |
| 2051713    | 2051713 | /G | frameshift_variant | 1 | HIGH | 15 |
| 2051713    | 2051712 | /A | frameshift_variant | 1 | HIGH | 19 |
| 2051713    | 2051712 | /TTG | stop_gained,inframe_insertion | 3 | HIGH | 70 |
| 2051713    | 2051714 | AG/TA | frameshift_variant | -1 | HIGH | 22 |
| 2051713    | 2051713 | AGGG/ | frameshift_variant | 4 | MODERATE | 4 |
| 2051713    | 2051713 | /ACC | frameshift_variant | 3 | MODERATE | 51 |
| 2051714    | 2051714 | G/ | frameshift_variant | -1 | HIGH | 22 |
| 2051714    | 2051714 | G/A | frameshift_variant | 0 | MODERATE | 40 |
| 2051714    | 2051714 | G/C | frameshift_variant | 0 | MODERATE | 15 |
| 2051715    | 2051715 | G/C | frameshift_variant | 1 | HIGH | 22 |
| 2051715    | 2051715 | G/C | frameshift_variant | 0 | MODERATE | 22 |
| 2051716    | 2051716 | G/T | synonymous_variant | 0 | LOW | 18 |
| 2051716    | 2051716 | G/A | synonymous_variant | 0 | LOW | 10 |
| 2051719    | 2051720 | AC/TT | frameshift_variant | 0 | MODERATE | 1 |
| 2051719    | 2051723 | ACCGGG/ | frameshift_variant | -5 | HIGH | 17 |
| 2051724    | 2051724 | A/T | frameshift_variant | 0 | MODERATE | 17 |
| 2051736    | 2051736 | C/A | frameshift_variant | 0 | MODERATE | 13 |
| Orthologue | Chr | ENSDARG | Affected transcripts | Start (bp) | End (bp) | Mutation | Annotation | Base pair change | VEP impact | Nr affected alleles |
|------------|-----|---------|----------------------|------------|----------|----------|------------|-----------------|------------|---------------------|
| 43213133   | 43213135 | 43213149 | 43213156 | 43213159 | 43213190 | 43213190 | 43213190 | 43213190 | | | |

The predicted impact of unique CRISPR-Cas9 induced mutations on protein function was assessed using Ensembl’s Variant Effect Predictor (VEP). An allele- and target-specific dosage score was subsequently calculated for each larva by weighting

the mutation with the highest predicted impact by a factor 0.33, 0.66, or 1 for mutations with a low, moderate or high predicted impact, respectively. Modification of expression was only observed for transcripts of genes located near the targeted gene. Start and end coordinates are based on GRCh11. For simplicity, kia1755_1 refers to quo, and kia1755_2 to sicley-65/j6.2.
Supplemental Table 4: Mutant allele frequencies

| Gene   | Nr mutated alleles | Expected nr mutated alleles | P_HWE |
|--------|--------------------|----------------------------|-------|
|        | 0      | 1    | 2    | Missing | Sample call rate | Mutant allele freq | 0 | 1 | 2 |       |
| gngt1  | 234    | 132  | 12   | 4       | 0.990            | 0.206               | 238 | 124 | 16 | 2.70E-01 |
| syt10  | 193    | 131  | 54   | 4       | 0.990            | 0.316               | 177 | 163 | 38 | 2.10E-04 |
| rgs6   | 346    | 34   | 1    | 1       | 0.997            | 0.047               | 346 | 34  | 1  | 5.79E-01 |
| hcn4   | 268    | 100  | 9    | 5       | 0.987            | 0.156               | 268 | 100 | 9  | 4.69E-01 |
| hcn4l  | 351    | 29   | 1    | 1       | 0.997            | 0.041               | 351 | 30  | 1  | -      |
| neo1a  | 20     | 113  | 241  | 8       | 0.979            | 0.795               | 16  | 122 | 237| 1.16E-01 |
| neo1b  | 15     | 19   | 271  | 77      | 0.798            | 0.920               | 2   | 45  | 258| 2.12E-13 |
| kiaa1755_1 | 55    | 42   | 283  | 2       | 0.995            | 0.800               | 15  | 122 | 243| 3.33E-32 |
| kiaa1755_2 | 24    | 60   | 295  | 3       | 0.992            | 0.858               | 8   | 93  | 279| 5.65E-10 |

Number of larvae with 0, 1 and 2 mutated alleles, as well as the number of larvae with a missing call. P-value for a Hardy-Weinberg equilibrium (HWE) exact test, considering a ±30 bp window around the CRISPR targeted site as a single locus (P<2.9E-3 is significant after Bonferroni correction). Larvae with more than two missing genotypes were excluded from the statistical analysis (i.e. n_total=382). For simplicity, kiaa1755_1 refers to quo, and kiaa1755_2 to si:dkey-65j6.2.
### Supplemental Table 5: Transcript-specific association analysis for dichotomous cardiac outcomes

#### Sinoatrial pause

| Gene      | 2dpf (n=323, 39 cases) |   |   | P     | 5dpf (n=318, 9 cases) |   |   | P     |
|-----------|-------------------------|---|---|------|------------------------|---|---|------|
|           | OR     | LCI    | UCI    |      | OR     | LCI    | UCI    |      |
| gngt1     | 1.581  | 0.711  | 3.517  | 2.61E-01  | 0.123  | 0.009  | 1.649  | 1.13E-01  |
| syt10     | 1.734  | 1.018  | 2.953  | 4.27E-02  | 0.732  | 0.241  | 2.221  | 5.81E-01  |
| rgs6      | 2.209  | 0.862  | 5.666  | 9.90E-02  | 3.862  | 0.637  | 23.411 | 1.42E-01  |
| hcn4      | 3.041  | 1.656  | 5.584  | 3.34E-04  | 1.209  | 0.359  | 4.072  | 7.59E-01  |
| hcn4l     | 1.269  | 0.327  | 4.930  | 7.31E-01  | 3.112  | 0.328  | 29.534 | 3.23E-01  |
| neo1a     | 0.936  | 0.465  | 1.881  | 8.52E-01  | 0.747  | 0.218  | 2.558  | 6.42E-01  |
| neo1b     | 2.014  | 0.705  | 5.749  | 1.91E-01  | 0.379  | 0.081  | 1.785  | 2.20E-01  |
| kiaa1755_1| 0.749  | 0.426  | 1.318  | 3.16E-01  | 0.582  | 0.219  | 1.547  | 2.78E-01  |
| kiaa1755_2| 1.045  | 0.479  | 2.278  | 9.12E-01  | 8.170  | 1.077  | 61.988 | 4.22E-02  |
| time of day| 0.932  | 0.704  | 1.234  | 6.25E-01  | 1.079  | 0.583  | 1.998  | 8.09E-01  |
| intercept | 0.030  | 0.003  | 0.298  | 2.81E-03  | 0.017  | 0.000  | 0.830  | 4.00E-02  |

#### Sinoatrial arrest

| Gene      | 2dpf (n=321, 36 cases) |   |   | P     |
|-----------|-------------------------|---|---|------|
|           | OR     | LCI    | UCI    |      |
| gngt1     | 1.586  | 0.707  | 3.556  | 2.63E-01  |
| syt10     | 1.639  | 0.952  | 2.821  | 7.47E-02  |
| rgs6      | 1.988  | 0.737  | 5.357  | 1.75E-01  |
| hcn4      | 2.906  | 1.556  | 5.428  | 8.19E-04  |
| hcn4l     | 1.267  | 0.331  | 4.852  | 7.30E-01  |
| neo1a     | 1.016  | 0.491  | 2.101  | 9.67E-01  |
| neo1b     | 1.590  | 0.549  | 4.605  | 3.93E-01  |
| kiaa1755_1| 0.823  | 0.458  | 1.480  | 5.16E-01  |
| kiaa1755_2| 1.143  | 0.507  | 2.578  | 7.47E-01  |
| time of day| 0.928  | 0.697  | 1.236  | 6.10E-01  |
| intercept | 0.030  | 0.003  | 0.303  | 3.02E-03  |

### Cardiac edema

| Gene      | 2dpf (n=369, 15 cases) |   |   | P     | 5dpf (n=322, 14 cases) |   |   | P     |
|-----------|-------------------------|---|---|------|------------------------|---|---|------|
|           | OR     | LCI    | UCI    |      | OR     | LCI    | UCI    |      |
| gngt1     | 1.518  | 0.489  | 4.706  | 4.70E-01  | 7.780  | 1.755  | 34.480 | 6.92E-03  |
| syt10     | 0.584  | 0.226  | 1.509  | 2.67E-01  | 0.901  | 0.291  | 2.787  | 8.57E-01  |
| rgs6      | 0.702  | 0.146  | 2.043  | 2.68E-01  | 2.730  | 0.456  | 16.336 | 2.71E-01  |
| hcn4      | 0.546  | 0.146  | 2.043  | 3.68E-01  | 0.380  | 0.065  | 2.213  | 2.82E-01  |
| hcn4l     | 0.800  | 0.111  | 2.107  | 8.25E-01  | 0.039  | 0.000  | 63.816 | 3.90E-01  |
| neo1a     | 0.844  | 0.309  | 2.307  | 7.41E-01  | 0.253  | 0.080  | 0.796  | 1.88E-02  |
| neo1b     | 1.154  | 0.328  | 2.043  | 8.23E-01  | 0.217  | 0.046  | 1.028  | 5.42E-02  |
| kiaa1755_1| 1.137  | 0.505  | 2.563  | 7.56E-01  | 2.126  | 0.604  | 7.486  | 2.40E-01  |
| kiaa1755_2| 1.317  | 0.430  | 4.032  | 6.29E-01  | 2.011  | 0.414  | 9.761  | 3.86E-01  |
| time of day| 0.963  | 0.633  | 1.465  | 8.59E-01  | 1.675  | 0.930  | 3.016  | 8.57E-02  |
| intercept | 0.036  | 0.002  | 0.697  | 2.78E-02  | 0.017  | 0.001  | 0.513  | 1.90E-02  |
Continued Supplemental Table 5

| Gene    | OR     | LCI    | UCI     | P       |
|---------|--------|--------|---------|---------|
| gngt1   | 2.578  | 0.502  | 13.247  | 2.57E-01|
| syt10   | 0.343  | 0.065  | 1.813   | 2.08E-01|
| rgs6    | 1.313  | 0.138  | 12.517  | 8.13E-01|
| hcn4    | 1.310  | 0.339  | 5.064   | 6.96E-01|
| hcn4l   |        |        |         |         |
| neo1a   | 0.294  | 0.085  | 1.012   | 5.23E-02|
| neo1b   | 0.988  | 0.203  | 4.810   | 9.88E-01|
| kiaa1755_1 | 0.644 | 0.230  | 1.802   | 4.02E-01|
| kiaa1755_2 | 2.107 | 0.352  | 12.615  | 4.14E-01|
| time of day | 0.648 | 0.335  | 1.251   | 1.96E-01|
| intercept | 0.245 | 0.004  | 14.946  | 5.03E-01|

Uncontrolled atrial contractions 5dpf (n=326, 9 cases)

| Gene    | OR     | LCI    | UCI     | P       |
|---------|--------|--------|---------|---------|
| gngt1   | 41.908 | 3.402  | 516.185 | 3.55E-03|
| syt10   | 0.928  | 0.206  | 4.187   | 9.23E-01|
| rgs6    | 0.348  | 0.021  | 5.885   | 4.64E-01|
| hcn4    | 0.348  | 0.021  | 5.885   | 4.64E-01|
| hcn4l   |        |        |         |         |
| neo1a   | 0.15   | 0.026  | 0.851   | 3.21E-02|
| neo1b   | 0.145  | 0.015  | 1.379   | 9.29E-02|
| kiaa1755_1 | 1.915 | 0.371  | 9.882   | 4.38E-01|
| kiaa1755_2 | 0.628 | 0.099  | 3.98    | 6.21E-01|
| time of day | 1.316 | 0.582  | 2.978   | 5.09E-01|
| intercept | 0.076 | 0.001  | 5.739   | 2.43E-01|

Abnormal morphology and impaired contractility 5dpf (n=326, 6 cases)

Associations of cardiac outcomes with the number of mutated alleles across the nine orthologues, weighted by their predicted effect on protein function. At 2 and 5 days post fertilization (dpf), associations were analyzed using logistic regression for outcomes that were observed in at least five larvae. Associations were adjusted for time of day and for the weighted number of mutated alleles in the other genes as fixed factors. '-' indicates that parameters could not be estimated. For simplicity, *kiaa1755_1* refers to *quo*, and *kiaa1755_2* to *si:dkey-65j6.2*. 
### Supplemental Table 6: Transcript-specific association analysis for HRV and heart rate

| Age n | Factor               | Gene   | Effect size | Heart rate variability | SE  | LCI       | UCI       | P     |
|-------|----------------------|--------|-------------|------------------------|-----|----------|----------|-------|
|       | fixed effects        |        |             |                         |     |          |          |       |
|       | kiaa1755_1           | gnt1   | -0.052      | -0.107                 | 0.157 | 6.25E-01 |          |       |
|       | syt10                |        | -0.070      | -0.077                 | 0.081 | 3.62E-01 |          |       |
|       | rg56                 |        | 0.000       | 0.175                  | 0.344 | 9.98E-01 |          |       |
|       | hcn4                 |        | -0.004      | 0.101                  | 0.194 | 9.70E-01 |          |       |
|       | hcn4l                |        | 0.165       | 0.178                  | 0.514 | 3.55E-01 |          |       |
|       | neo1a                |        | -0.028      | 0.095                  | 0.158 | 7.69E-01 |          |       |
|       | neo1b                |        | -0.065      | 0.113                  | 0.158 | 5.68E-01 |          |       |
|       | time of day          |        | 0.074       | 0.097                  | 0.264 | 4.42E-01 |          |       |
|       | Heart rate (in SD)   |        | 0.503       | 0.045                  | 0.592 | 1.77E-28 |          |       |
|       | intercept            |        | -0.459      | 0.056                  | -0.348 | 4.59E-16 |          |       |
|       |                     |        | -1.614      | 0.300                  | -1.026 | 7.54E-08 |          |       |
|       | random effects       |        | variation by batch | 0.340  | 0.113  | 0.177 | 0.652 | - |
|       |                      |        | residual    | 0.765  | 0.033  | 0.704 | 0.832 | - |
|       | fixed effects        |        |             |                         |     |          |          |       |
|       | hcn4                 |        | -0.009      | 0.157                  | 0.299 | 9.56E-01 |          |       |
|       | hcn4l                |        | -0.032      | 0.095                  | 0.154 | 7.35E-01 |          |       |
|       | hcn4b                |        | 0.142       | 0.177                  | 0.489 | 4.22E-01 |          |       |
|       | neo1a                |        | 0.017       | 0.096                  | 0.204 | 8.02E-01 |          |       |
|       | neo1b                |        | 0.077       | 0.121                  | 0.313 | 5.25E-01 |          |       |
|       | kiaa1755_1           |        | 0.000       | 0.077                  | 0.150 | 1.00E+00 |          |       |
|       | kiaa1755_2           |        | 0.008       | 0.100                  | 0.204 | 9.40E-01 |          |       |
|       | time of day          |        | 0.524       | 0.052                  | 0.626 | 7.54E-24 |          |       |
|       | Heart rate (in SD)   |        | -0.645      | 0.054                  | -0.540 | 2.86E-33 |          |       |
|       | intercept            |        | -1.893      | 0.383                  | -1.142 | 7.67E-07 |          |       |
|       | random effects       |        | variation by batch | 0.540  | 0.185  | 0.276 | 1.056 | - |
|       |                      |        | residual    | 0.769  | 0.032  | 0.708 | 0.834 | - |

| Age n | Factor               | Gene   | Effect size | Heart rate variability | SE  | LCI       | UCI       | P     |
|-------|----------------------|--------|-------------|------------------------|-----|----------|----------|-------|
|       | fixed effects        |        |             |                         |     |          |          |       |
|       | kiaa1755_1           | gnt1   | -0.061      | 0.102                 | 0.139 | 5.48E-01 |          |       |
|       | syt10                |        | -0.026      | 0.074                 | -0.118 | 7.22E-01 |          |       |
|       | rg56                 |        | -0.045      | 0.168                 | 0.284 | 7.88E-01 |          |       |
|       | hcn4                 |        | -0.047      | 0.096                 | 0.236 | 6.27E-01 |          |       |
|       | hcn4l                |        | 0.105       | 0.169                 | 0.436 | 5.35E-01 |          |       |
|       | neo1a                |        | 0.055       | 0.090                 | 0.233 | 5.39E-01 |          |       |
|       | neo1b                |        | -0.121      | 0.108                | -0.333 | 2.62E-01 |          |       |
|       | kiaa1755_1           |        | 0.120       | 0.073                 | 0.263 | 9.83E-02 |          |       |
|       | kiaa1755_2           |        | 0.078       | 0.092                 | 0.259 | 4.01E-01 |          |       |
|       | time of day          |        | 0.550       | 0.040                 | 0.627 | 9.14E-44 |          |       |
|       | HRV (in SD)          |        | -0.408      | 0.051                 | -0.308 | 1.23E-15 |          |       |
|       | intercept            |        | -1.770      | 0.258                | -2.276 | 7.14E-12 |          |       |
|       | random effects       |        | variation by batch | 0.185  | 0.077  | 0.082 | 0.420 | - |
|       |                      |        | residual    | 0.734  | 0.031  | 0.675 | 0.798 | - |

| Age n | Factor               | Gene   | Effect size | Heart rate variability | SE  | LCI       | UCI       | P     |
|-------|----------------------|--------|-------------|------------------------|-----|----------|----------|-------|
|       | fixed effects        |        |             |                         |     |          |          |       |
|       | hcn4                 |        | -0.087      | -0.139                 | 0.186 | 5.32E-01 |          |       |
|       | hcn4l                |        | 0.150       | -0.084                 | 0.315 | 7.37E-02 |          |       |
|       | neo1a                |        | 0.204       | -0.157                | 0.512 | 1.94E-01 |          |       |
|       | neo1b                |        | 0.002       | 0.085                 | 0.168 | 9.82E-01 |          |       |
|       | kiaa1755_1           |        | 0.006       | 0.107                 | 0.216 | 9.57E-01 |          |       |
|       | kiaa1755_2           |        | 0.031       | 0.068                 | 0.164 | 6.50E-01 |          |       |
|       | time of day          |        | 0.564       | 0.043                 | 0.648 | 1.97E-39 |          |       |
|       | HRV (in SD)          |        | -0.516      | 0.043                 | -0.599 | 7.79E-34 |          |       |
|       | intercept            |        | -2.137      | 0.375                | -2.872 | 1.20E-08 |          |       |
|       | random effects       |        | variation by batch | 0.619  | 0.204  | 0.324 | 1.181 | - |

Associations of heart rate variability (HRV) and heart rate (mutually adjusted) with the number of mutated alleles across the nine orthologues, weighted by their predicted effect on protein function. Associations were examined for the main transcript of each targeted zebrafish orthologue using hierarchical linear models (xtmixed in Stata), using inverse-normally transformed outcomes, so effect sizes and SEs can be interpreted as z-score units.

Associations were adjusted for time of day and the weighted number of mutated alleles in the other genes as fixed factors, with larvae nested in batches (random factor). For simplicity, kiaa1755_1 refers to kiaa1755_1 and kiaa1755_2 to si:kiaa1755_2.
Supplemental Table 7: Transcript-specific association analysis for heart rate variability and heart rate in larvae without a sinoatrial pause before or after the recording

| Age | n | Factor | Gene      | Effect size | Heart rate variability | SE  | LCI   | UCI   | P        |
|-----|---|--------|-----------|-------------|------------------------|-----|-------|-------|----------|
| 2   | 234 | fixed effects | gnt1      | -0.066      | 0.122 -0.306 0.173 5.88E-01 |     |       |       |          |
|     |     |         | syst10    | -0.058      | 0.084 -0.223 0.107 4.91E-01 |     |       |       |          |
|     |     |         | rgs6      | 0.036       | 0.201 -0.358 0.429 8.60E-01 |     |       |       |          |
|     |     |         | hcn4      | 0.029       | 0.111 -0.189 0.247 7.94E-01 |     |       |       |          |
|     |     |         | hcn4d     | 0.171       | 0.206 -0.232 0.575 4.05E-01 |     |       |       |          |
|     |     |         | neo1a     | -0.056      | 0.105 -0.263 0.151 5.95E-01 |     |       |       |          |
|     |     |         | neo1b     | -0.094      | 0.125 -0.338 0.150 4.50E-01 |     |       |       |          |
|     |     |         | kiaa1755_1| 0.180       | 0.082 0.019 0.340 2.81E-02 |     |       |       |          |
|     |     |         | kiaa1755_2| 0.093       | 0.102 -0.107 0.292 3.64E-01 |     |       |       |          |
|     |     |         | time of day| 0.555       | 0.050 0.456 0.654 4.10E-28 |     |       |       |          |
|     |     |         | Heart rate (in SD) | -0.534 | 0.063 -0.658 -0.411 1.81E-17 |     |       |       |          |
|     |     |         | intercept  | -1.770      | 0.316 -2.388 -1.151 2.05E-08 |     |       |       |          |
|     |     | random effects | variation by batch | 0.344 | 0.118 0.176 0.674 - |     |       |       |          |
|     |     |         | residual   | 0.769       | 0.036 0.702 0.843 - |     |       |       |          |
| 5   | 283 | fixed effects | gnt1      | 0.264       | 0.106 0.056 0.472 1.27E-02 |     |       |       |          |
|     |     |         | syst10    | -0.005      | 0.071 -0.144 0.134 9.42E-01 |     |       |       |          |
|     |     |         | rgs6      | -0.025      | 0.157 -0.333 0.282 8.72E-01 |     |       |       |          |
|     |     |         | hcn4      | -0.042      | 0.094 -0.226 0.142 6.56E-01 |     |       |       |          |
|     |     |         | hcn4d     | 0.135       | 0.173 -0.203 0.474 4.33E-01 |     |       |       |          |
|     |     |         | neo1a     | -0.002      | 0.094 -0.185 0.182 9.86E-01 |     |       |       |          |
|     |     |         | neo1b     | 0.055       | 0.118 0.176 0.286 6.41E-01 |     |       |       |          |
|     |     |         | kiaa1755_1| 0.020       | 0.077 -0.131 0.170 7.99E-01 |     |       |       |          |
|     |     |         | kiaa1755_2| 0.081       | 0.102 -0.119 0.281 4.28E-01 |     |       |       |          |
|     |     |         | time of day| 0.534       | 0.051 0.434 0.633 8.87E-26 |     |       |       |          |
|     |     |         | Heart rate (in SD) | -0.623 | 0.053 -0.727 -0.519 5.00E-32 |     |       |       |          |
|     |     |         | intercept  | -1.978      | 0.372 -2.707 -1.250 1.01E-07 |     |       |       |          |
|     |     | random effects | variation by batch | 0.516 | 0.178 0.263 1.013 - |     |       |       |          |
|     |     |         | residual   | 0.747       | 0.032 0.687 0.812 - |     |       |       |          |

**Notes:**
- Associations of heart rate variability (HRV) and heart rate (mutually adjusted) with the number of mutated alleles across the nine orthologues, weighted by their predicted effect on protein function. Alleles were examined for the main transcript of each targeted zebrafish orthologue using hierarchical linear models (xtmixed in Stata), using inverse-normally transformed outcomes, so effect sizes and SEs can be interpreted as z-score units. Larvae with a sinoatrial pause before or after the recording were excluded from the analysis in this sensitivity analysis. Analyses were adjusted for time of day and the weighted number of mutated alleles in the other genes as fixed factors, with larvae nested in batches (random factor). For simplicity, kiaa1755_1 refers to quo, and kiaa1755_2 to ak:deley-65j62.
Supplemental Table 8: Transcript-specific association analyses for body size

### Dorsal body surface area

| Factor     | Gene   | Effect size | SE   | LCI  | UCI  | P     |
|------------|--------|-------------|------|------|------|-------|
| fixed effects | gnt1   | 0.112       | 0.109 | -0.101 | 0.324 | 3.041E-01 |
|            | syt1D  | -0.057      | 0.076 | -0.206 | 0.091 | 4.84E-01 |
|            | rgs6   | 0.038       | 0.163 | -0.282 | 0.358 | 8.16E-01 |
|            | hcn4   | -0.106      | 0.098 | -0.298 | 0.086 | 2.80E-01 |
|            | hcn4f  | -0.151      | 0.196 | 0.535  | 0.223 | 4.41E-01 |
| fixed effects | neo1a  | 0.218       | 0.099 | 0.025  | 0.412 | 2.66E-02 |
|            | neo1b  | -0.100      | 0.115 | -0.325 | 0.126 | 3.86E-01 |
|            | kiao175s_1 | 0.016   | 0.075 | -0.131 | 0.163 | 8.32E-01 |
|            | kiao175s_2 | 0.060  | 0.102 | 0.129  | 0.259 | 5.58E-01 |
| Time of day | -0.046  | 0.040       | -0.125 | 0.032  | 2.49E-01 |
| Intercept  | -0.152  | 0.338       | 0.815  | 0.510  | 6.32E-01 |
| random effects | Variation by batch | 0.431 | 0.254 | 0.267 | 0.910 | 5.54E-02 |
| Residual   | 0.842   | 0.034       | 0.779  | 0.911  | -   | 0.852 |

### Lateral body surface area

| Factor     | Gene   | Effect size | SE   | LCI  | UCI  | P     |
|------------|--------|-------------|------|------|------|-------|
| fixed effects | gnt1   | -0.056      | 0.121 | -0.293 | 0.181 | 6.41E-01 |
|            | syt1D  | 0.042       | 0.085 | -0.124 | 0.208 | 6.21E-01 |
|            | rgs6   | -0.076      | 0.183 | -0.398 | 0.135 | 9.01E-01 |
|            | hcn4   | 0.151       | 0.108 | 0.061  | 0.363 | 1.64E-01 |
|            | hcn4f  | -0.047      | 0.221 | -0.479 | 0.385 | 8.32E-01 |
| fixed effects | neo1a  | -0.036      | 0.122 | -0.252 | 0.180 | 7.42E-01 |
|            | neo1b  | 0.257       | 0.130 | 0.002  | 0.512 | 4.80E-02 |
|            | kiao175s_1 | 0.027 | 0.085 | -0.140 | 0.194 | 7.52E-01 |
|            | kiao175s_2 | -0.224  | 0.117 | -0.454 | 0.005 | 5.33E-01 |
| Time of day | -0.049  | 0.045       | -0.138 | 0.092  | 2.72E-01 |
| Intercept  | 0.061   | 0.335       | -0.595 | 0.718  | 8.55E-01 |
| random effects | Variation by batch | 0.340 | 0.117 | 0.173 | 0.669 | -1.16E-01 |
| Residual   | 0.914   | 0.038       | 0.843  | 0.991  | -   | 0.829 |

### Body volume

| Factor     | Gene   | Effect size | SE   | LCI  | UCI  | P     |
|------------|--------|-------------|------|------|------|-------|
| fixed effects | gnt1   | -0.051      | 0.111 | -0.270 | 0.167 | 6.45E-01 |
|            | syt1D  | 0.008       | 0.079 | -0.147 | 0.163 | 9.20E-01 |
|            | rgs6   | -0.250      | 0.181 | -0.606 | 0.106 | 1.68E-01 |
|            | hcn4   | 0.123       | 0.099 | -0.070 | 0.317 | 2.12E-01 |
|            | hcn4f  | -0.066      | 0.194 | -0.446 | 0.314 | 7.35E-01 |
| fixed effects | neo1a  | 0.105       | 0.103 | -0.097 | 0.307 | 3.07E-01 |
|            | neo1b  | 0.175       | 0.121 | -0.063 | 0.413 | 1.49E-01 |
|            | kiao175s_1 | -0.035 | 0.079 | -0.190 | 0.120 | 6.57E-01 |
|            | kiao175s_2 | -0.019  | 0.106 | -0.226 | 0.188 | 8.58E-01 |
| Time of day | -0.056  | 0.042       | -0.139 | 0.027  | 1.85E-01 |
| Intercept  | -0.184  | 0.345       | -0.860 | 0.402  | 5.94E-01 |
| random effects | Variation by batch | 0.496 | 0.155 | 0.268 | 0.916 | -1.16E-01 |
| Residual   | 0.841   | 0.035       | 0.776  | 0.912  | -   | 0.733 |

Associations were analyzed for the main transcript of each targeted zebrafish orthologue using hierarchical linear models (mixed in Stata) using inversenormally-transformed outcomes, so effect sizes and SEs can be interpreted as z-score units. Associations were adjusted for time of day and for the weighted number of mutated alleles in the other genes as fixed factors, with larvae nested in batches (random factor). For simplicity, kiao175s_1 refers to quo, and kiao175s_2 to sidkay 65.6.2.
Supplemental Table 9: Druggability of interacting partners

| Candidate | Interaction partner | DGIdb categories | Drug available? (y/n) | Drug name (FDA approved) | Drug category |
|-----------|---------------------|------------------|----------------------|--------------------------|---------------|
| AGTR1     | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
| ATR1      | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
| AGTR1     | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
| ATR1      | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
| AGTR1     | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
| ATR1      | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
| AGTR1     | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
| ATR1      | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
| AGTR1     | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
| ATR1      | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
| AGTR1     | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
| ATR1      | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
| AGTR1     | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
| ATR1      | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
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| ATR1      | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
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| AGTR1     | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
| ATR1      | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
| AGTR1     | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
| ATR1      | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
| AGTR1     | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
| ATR1      | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
| AGTR1     | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
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| ATR1      | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
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| ATR1      | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
| AGTR1     | G-protein coupled receptor | Druggable genome, Drug resistance, Clinically actionable | yes | Fluorouracil | Anti-neoplastic agents |
| ATR1      | G-prote...