

Supplemental Figure 1. Creation and characterization of *hnf4g* mutants. A) CRISPR guides were designed to target the second exon of the zebrafish *hnf4g* locus, an exon which is present in all isoforms (Figure 1). Isoform depicted is ENSDART00000164750.3-hnf4g203. Two lines with nucleotide changes that cause frameshifts resulting in premature stop codons were identified and designated *hnf4g*^{rdu58} and *hnf4g*^{rdu59}. Their nucleotide sequence is shown in the box below the gene model. B) Alignment of the predicted peptide sequences for *hnf4g*^{rdu58} and *hnf4g*^{rdu59} with the first 180 amino acids of the wild type *hnf4g* coding sequence. Stop codon position is indicated in the protein model by vertical red bars. In the alignment, the consensus sequence is shown below the amino acid sequences and an asterisk indicates stop codons. C) Brightfield images of 6 dpf larvae resulting from an in-cross of *hnf4g*^{-/+}adults. No obvious

morphological defects were observed in these animals at this developmental stage. D and E) Animals resulting from an in-cross of $hnf4g^{-/+}$ adults were genotyped at 6 dpf and as adults. No significant difference in survival from the expected allelic ratios was observed (Chi-square test, larvae p=0.4724; adults p=0.4826). F) Levels of mRNA transcript for each of the *hnf4* genes were measured by qRT-PCR and compared in *hnf4g*^{+/+} and *hnf4g*^{-/-} larvae at 6 dpf. *hnf4g* transcript was significantly reduced in *hnf4g*^{-/-} compared to wild-type larvae, but no significant change in transcript level was observed for *hnf4a* or *hnf4b* (n=3-5 groups of 10 pooled larvae for each condition; error bars represent the standard error of the mean (SEM); unpaired t-test Bonferroni-Dunn Method *hnf4g* p>0.0001; *hnf4a* p=0.0803; *hnf4b* p=0.0933).