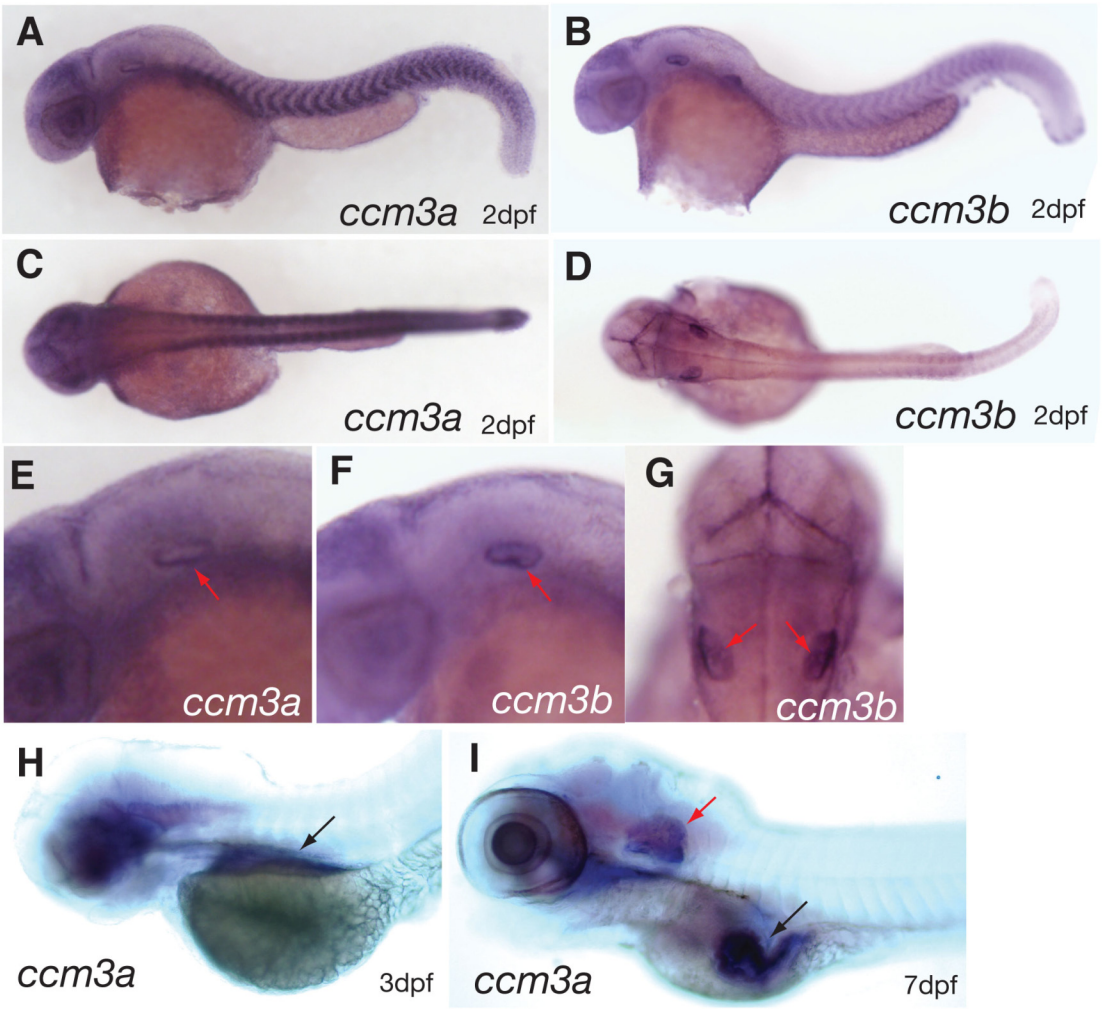
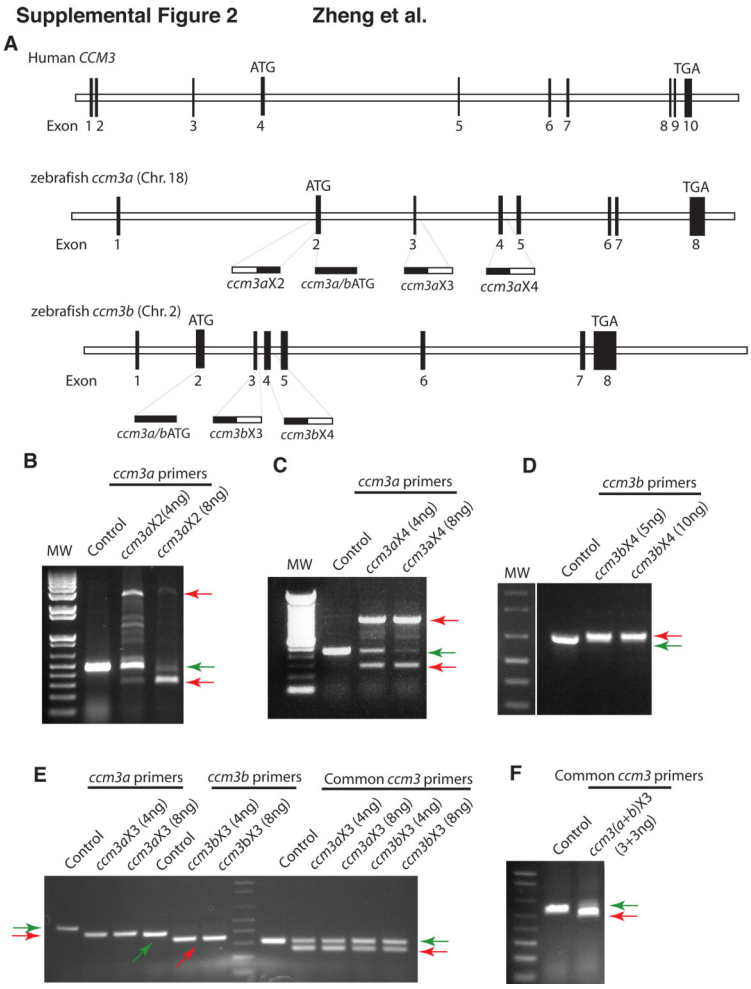
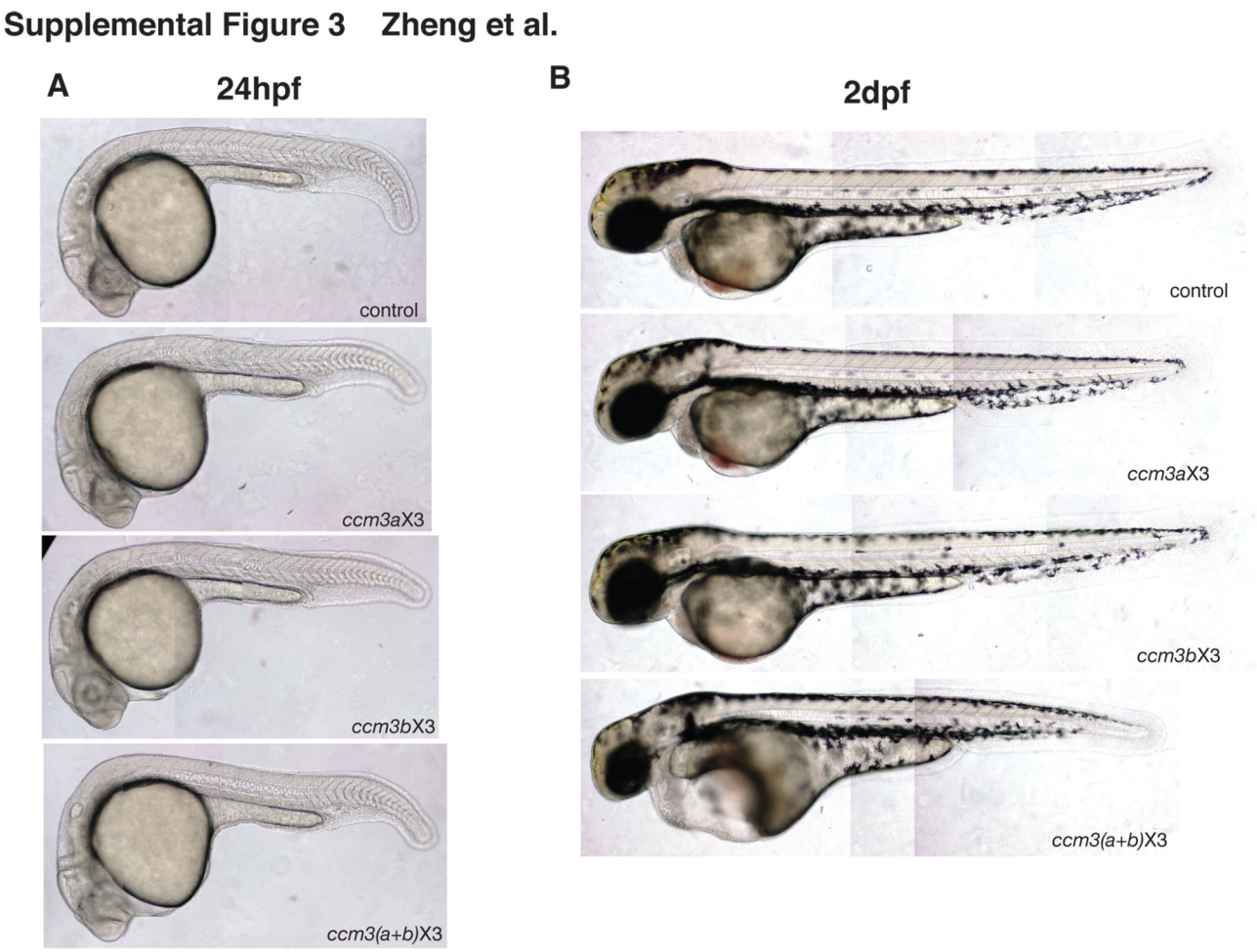
Supplemental Figure 1 Zheng et al.



Supplemental Figure 1. Expression of *ccm3* genes in zebrafish embryos. Shown are in situ hybridization studies for *ccm3a* and *ccm3b*. Both *ccm3a* (A, lateral view and C, dorsal view) and *ccm3b* (B, lateral view and D, dorsal view) are expressed in the somites and head of 2dpf embryos. *ccm3a* (E and I) and *ccm3b* (B, D, F and G) are also strongly expressed in the developing ear. In 3dpf (H) and 7dpf (I) embryos, *ccm3a* expression is detected in the developing intestine (arrow).

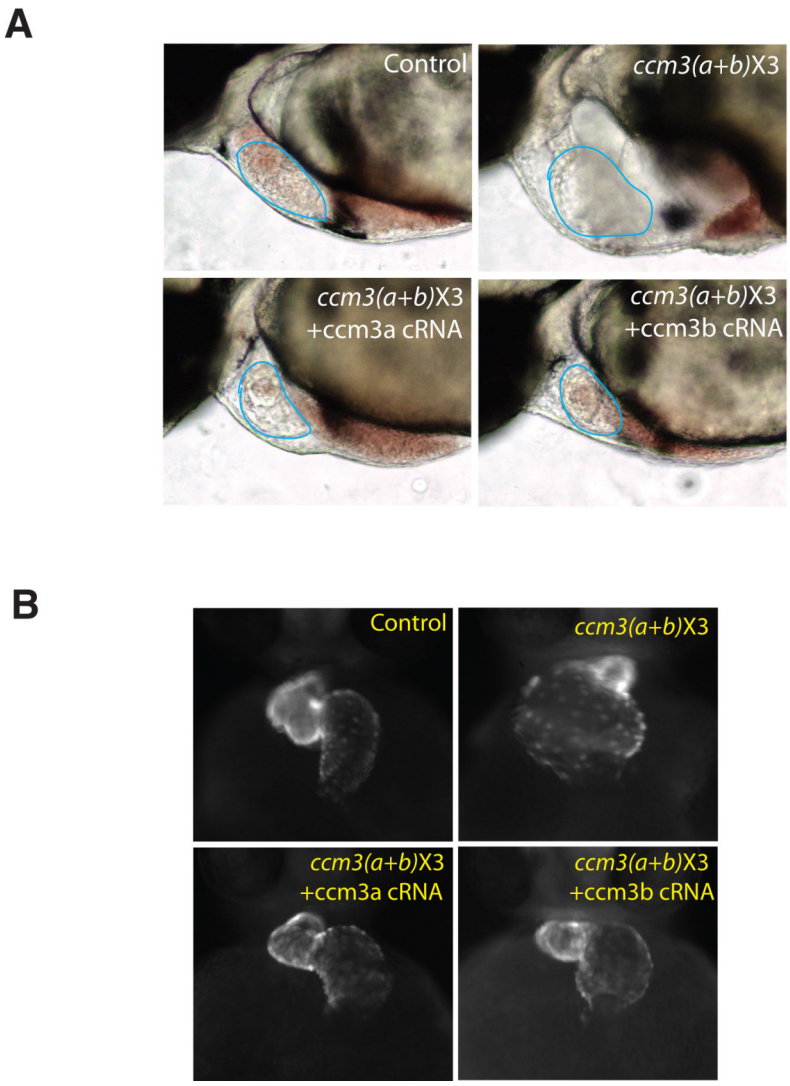


Supplemental Figure 2. Morpholino knockdown of *ccm3* genes in zebrafish embryos. (A) The zebrafish *ccm3a* and *ccm3b* gene structures are shown in comparison to that of the human *CCM3* gene. The binding sites for morpholinos targeting exon splice sites and the ATG of the zebrafish *ccm3* genes are shown. *ccm3a*X2, *ccm3a*X3, and *ccm3a*X4 target the acceptor site of exon 2 and the donor sites of exons 3 and 4 of the *ccm3a* gene respectively. *ccm3b*X3 and *ccm3b*X4 target the donor sites of exons 3 and 4 of the *ccm3b* gene. *ccm3(a+b)*ATG targets a conserved sequence in the 5'-UTR of the *ccm3a* and *ccm3b* genes to block the translation of both genes simultaneously. Note that zebrafish *ccm3* exon 3 is equivalent to human *CCM3* exon 5. (B) *ccm3a*X2 causes the retention of intron 1 (top red arrow) or deletion of exon 2 (bottom red arrow) of the *ccm3a* gene with introduction of a premature stop codon (not shown). The green arrow indicates the small amount of residual wild-type transcript detected following 4ng and 8ng morpholino injection. (C) *ccm3a*X4 causes the retention of intron 4 (top red arrow) or deletion of exons 3 and 4 (bottom red arrow) of the *ccm3a* gene with introduction of a premature stop codon (not shown). The green arrow indicates the residual wild-type transcript detected following 4ng and 8ng injection. (D) *ccm3b*X4 causes partial retention of intron 4 (top arrow) in the *ccm3b* gene and introduces a premature stop codon (not shown). The green arrow indicates the small amount of residual wild-type transcript detected following 4ng and 8ng injection. (E) *ccm3a*X3 and *ccm3b*X3 morpholinos target the donor sites of exon 3 in *ccm3a* and *ccm3b* respectively, and result in *ccm3a* or *ccm3b* transcripts that are in frame but lack 54 bp that encode 18 amino acids (red arrows). The amplification of total ccm3 transcripts following 4 and 8 ng injections of *ccm3a*X3 and *ccm3b*X3 morpholinos is shown on the right. (F) Simultaneous targeting of exon 3 in *ccm3a* and *ccm3b* is shown. The red arrow indicates amplification of truncated transcripts lacking exon 3 and the green arrow indicates the residual amount of wild-type ccm3 transcripts.



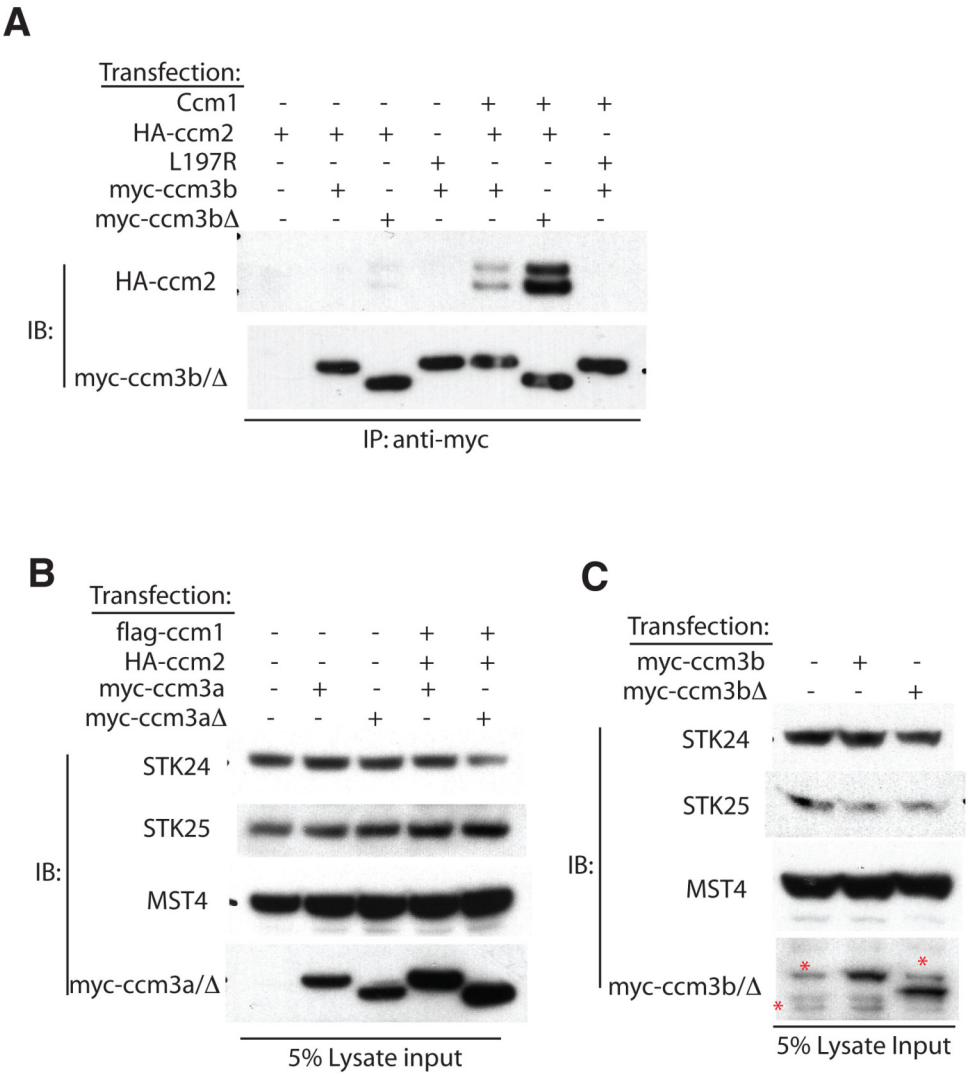
Supplemental Figure 3. Morphology of zebrafish embryos expressing ccm3 proteins lacking the 18 amino acids coded by exon 3. Bright field of images of 24hpf (A) and 2dpf (B) zebrafish embryos injected with control, *ccm3aX3* (3ng/embryo), *ccm3bx3* (3ng/embryo) or the combination of *ccm3aX3* and *ccm3bX3* morpholinos are shown.

Supplemental Figure 4 Zheng et al.

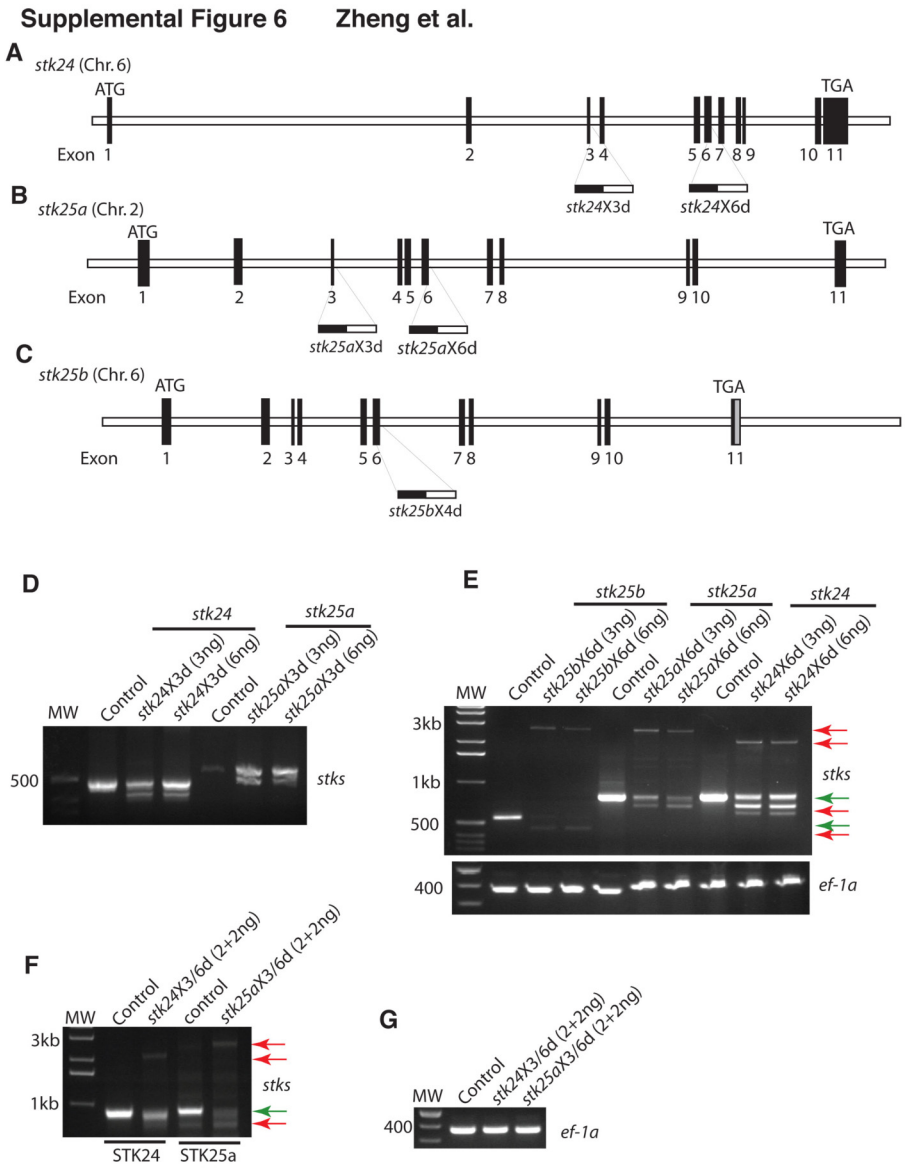


Supplemental Figure 4. Rescue of the big heart phenotype conferred by loss of *ccm3* exon 3 with cRNAs encoding full length ccm3a or ccm3b. Bright field (A) and fluorescence (B) images of the hearts of 48 hpf Tg (*i-fabp*:GFP) embryos following injection of control morpholino, *ccm3(a+b)*X3 morpholinos and *ccm3(a+b)*X3 morpholinos + cRNAs encoding full length ccm3a or ccm3b are shown.

Supplemental Figure 5 Zheng et al.

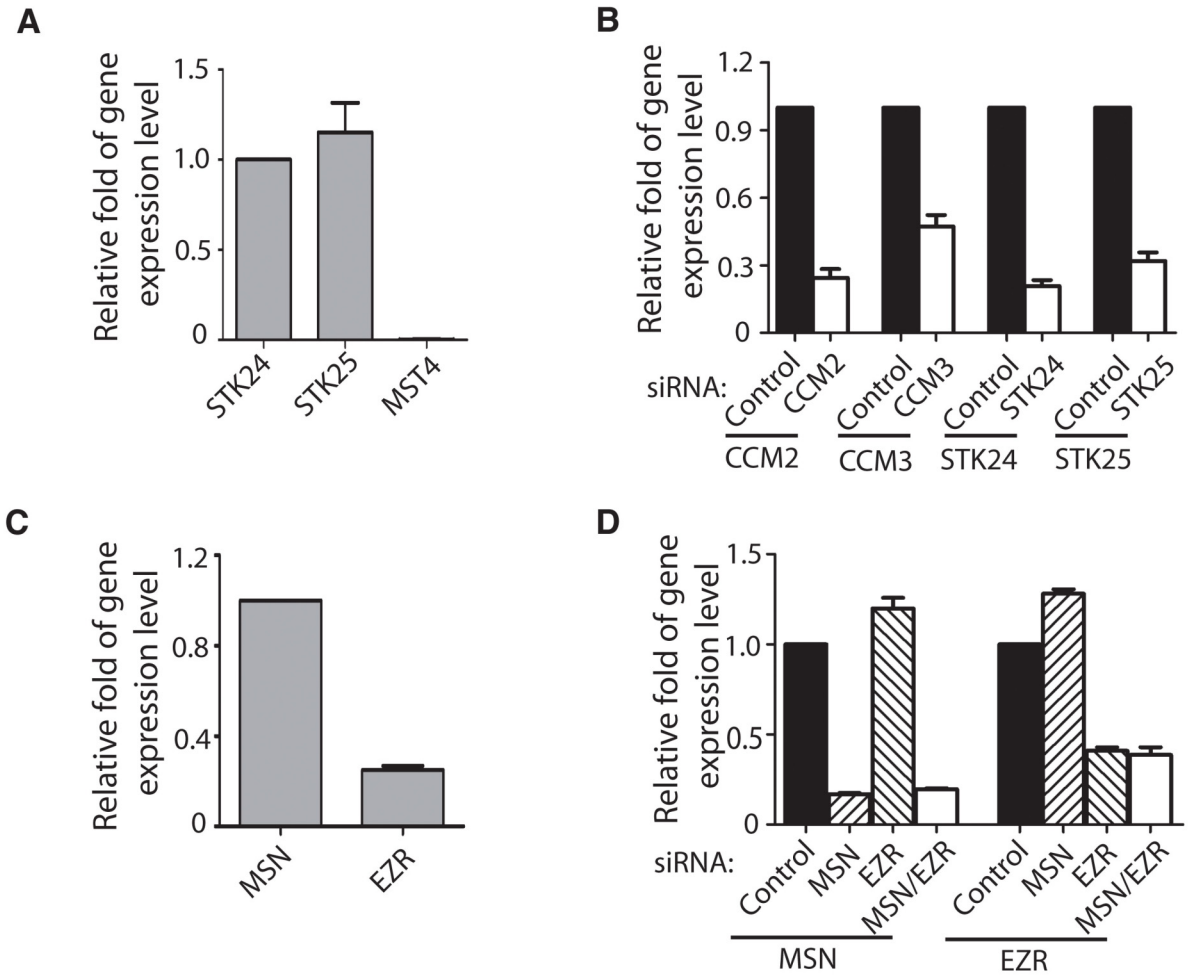


Supplemental Figure 5. ccm3bD interacts with ccm1 and ccm2 but not with STKs. (A) Co-immunoprecipitation of ccm1, ccm2 and ccm3b requires the ccm2 phosphotyrosine binding (PTB) domain but not exon 3 of ccm3. FLAG-ccm1, HA-ccm2 and myc-ccm3b were co-expressed in HEK293T cells and immunoprecipitations performed using anti-myc antibodies. ccm2L197R contains a point mutation in the PTB domain that blocks interaction with ccm1 {Kleaveland, 2009 #1121}. ccm3bD indicates ccm3b proteins lacking exon 3. (B) The expression levels of endogenous STK24, STK25, MST4, and heterologous ccm3a, ccm3aΔ in 5% of the lysate used for the co-immunoprecipitation experiments in Figure 3D are shown. (C) The expression levels of endogenous STK24, STK25, MST4, and heterologous ccm3b, ccm3bΔ in 5% of the lysate used for the co-immunoprecipitation experiments in Figure 3E are shown. Red asterisks indicate background bands present in all lanes.



Supplemental Figure 6. Morpholino knockdown of the *stk24* and *stk25* genes in zebrafish embryos. (A-C) The zebrafish *stk24, stk25a* and *stk25b* genes are shown with the binding sites for morpholinos targeting the splice sites of the genes indicated. *stk24*X3 and *stk24*X6 target the donor sites of exon3 and exon 6 of *stk24* gene. *stk25a*X3 and *stk25a*X6 target the donor sites of exon3 and exon 6 of *stk25a* gene. *stk25b*X6 targets the donor site of exon 6 of *stk25b* gene. The effect of these morpholinos on *stk24* and *stk25* gene expression was characterized using RT-PCR (D-G, below) and sequencing of the PCR products (not shown). (D) *stk24*X3 and *stk25a*X3 morpholinos drive partial deletion of exon 3 in *stk24* and *stk25*b. The mutant mRNAs are detected as the smaller bands relative to the control. (E) *stk24*X6, *stk25a*X6 and *stk25b*X6 morpholinos drive partial deletion of exon 6 and retention of intron 6 in *stk24*, *stk25a* and *stk25b*. Green arrows indicate bands amplified from wild-type transcripts and red arrows indicate those with either truncated (smaller) or intron-containing (larger) transcripts. (F) The morpholino combinations of *stk24*X3 + *stk24*X6 and *stk25a*X3 + *stk25a*X6 drive more efficient knock-down of *stk24* or *stk25* expression. (G) Expression of the control gene *ef1a* is unchanged in embryos injected with *stk24* and *stk25* morpholinos. The dosages of for each morpholino are indicated.

Supplemental Figure 7 Zheng et al.



Supplemental Figure 7. Expression and siRNA knockdown of GCK-III family STKs, Moesin and Ezrin in HMVECs. (A) Relative expression level of *STK24, STK25* and *MST4* in HMVECS as detected with real-time RT-PCR. The expression levels were normalized to the level of *STK24*. Note that *STK24* and *STK25* are expressed at comparable level in HMVECs whereas *MST4* expression is not detectable. (B) The knockdown efficiency of siRNAs directed against *CCM2, CCM3, STK24* and *STK25*. HMVECs were transfected with 8nM of the indicated siRNAs using siPORT ™ *Amine* Transfection Agent (Ambion) and total RNA harvested 2 days after transfection. Real-time RT-PCR analysis was performed to measure the gene expression level. The results represented the mean and SEM of two independent experiments. (C) Relative expression level of *MSN* and *EZR* in HMVECS as detected with real-time RT-PCR. The expression levels were normalized to the level of *MSN*. The expression level of *MSN* is 4 fold higher that of *EZR* in HMVECs. (D) The knockdown efficiency of siRNAs directed against *MSN* and *EZR*. HMVECs were transfected with 8nM of the indicated siRNAs and the expression levels of *MSN* and *EZN* measured as described in (B). Data shown in this figure are mean and SEM of 3 replicated experiments.