Supplementary Data

Supplementary Figure Legends

Figure S1. Mammalian and zebrafish KLF6/Copeb protein sequences are highly homologous. (a). Alignment of human, mouse and zebrafish KLF6/Copeb protein sequences compared to the *similar to kruppel like factor 6* (official gene name *si:dkeyp-12a9.2).* There is 75% identity between the human and zebrafish KLF6/Copeb proteins. Only 54% identity is seen between zebrafish Copeb and the related protein encoded by ***si:dkeyp-12a9.2 (*called** "similar to klf6" in figure). * indicates identical amino acids and : indicates isoelectric amino acids. Blue boxes delineate the C2H2 zinc finger DNA binding domains. (b) Phylogram demonstrates that Copeb is closely related to mammalian KLF6.

Figure S2. *copeb* ATG morpholino injection induces early embryonic lethality in few embryos. The percent survival of embryos injected with either 2 or 4 µM *copeb* ATG morpholino was normalized to that observed in embryos from the same clutch that were either injected with a control morpholino or were uninjected. No death was observed in any clutches after 1 dpf. Values are averaged from over 300 embryos at each time point from at least 4 clutches. The error bars indicate the standard deviation. Similar results were obtained with the exon 2 splice donor blocking morpholino.

Figure S3. *copeb*-splice blocking morpholino creates a truncated protein due to mis-splicing of zygotic Copeb. (a) A *copeb*-splicing morpholino was designed to target the splicing donor site of *copeb* exon 2. (b) RT-PCR was performed on cDNA samples prepared from uninjected control and morphants on 1-4 dpf embryos with a pair of primers on exons 2 and 3, (copeb-e2-f and copeb-e3-r, respectively) as indicated by the arrows in (a). (c) The bands generated by the PCR from control and morphant cDNA shown in (c) were sequenced, revealing a 49 bp deletion in the morphant mRNA due to splicing to a cryptic splice donor site in exon 2 (black box). The corresponding base pair of

the *copeb* coding sequence is labeled below the sequencing traces. (d) The product of the mis-spliced mRNA is predicted to be prematurely truncated due to a frame shift, eliminating the C2H2 zinc finger that is essential for Copeb function.

Figure S4. Restoration of *copeb* rescues the liver and pancreas phenotypes in *copeb* morphants. Embryos from *Tg(fabp10:RFP;ela3l:EGFP)* carriers were injected with 2 µM *copeb* ATG morpholino, *copeb* mRNA, a combination of the two or uninjected as controls and were scored for viability (a) and any developmental abnormality, termed "affected" (b); n is the total number of embryos scored in all experiments, which were carried out on at least 3 clutches. (c) Live images of representative 4 dpf larvae shows normal size pancreas in control larvae, marked by GFP expression, whereas the liver and pancreas is reduced in larvae injected with either the *copeb* splice donor morpholino or ATG morpholino. The liver and pancreas size is rescued by co-injecting the ATG morpholino with *copeb* mRNA. (d) 4 dpf larvae from *Tg(fabp10:RFP;ela3l:EGFP)* fish were injected with the *copeb* ATG morpholino, *copeb* RNA or both were scored for liver size based on RFP. Total n from 4 clutches is indicated below graph.

Figure S5. *copeb* morphants have decreased expression of blood cell genes but normal hepatic vasculature . (a) mRNA levels of marker genes for mesoderm-derived organ system such as blood and kidney were analyzed using QPCR in control embryos and *copeb* morphants at 4 dpf , and their levels in control embryos were set to be 1. Compared to control embryos, *copeb* morphants exhibited significantly lower expression of genes involved in vasculogenesis and hematopoiesis. However, expression of the kidney marker (*npsh1l*) was not significantly different between control and morphants. Errors bars show standard deviation from three independent experiments; * indicates $p<0.05$. (b) The *copeb* morpholino was injected into embryos expressing GFP in endothelial cells *Tg(fli1a:EGFP)* were imaged live on 4 dpf. The vascular network is similar in the liver in control (b-c) and *copeb* morphants (d-e). Scale bars = 500µm (b,d) and 100µm (c,e)

Figure S6. *Klf6*+/+ ES cells are able to differentiated in to the hepatic lineage using the serum-free hepatic differentiation protocol. (a) A schematic drawing showing the serum-free hepatic differentiation protocol. *Klf6*+/+ ES cells were pushed into the hepatic lineage using the serum-free protocol. Expression of marker genes for hepatic differentiation was analyzed in these cells using QPCR from day 0-12. mRNA levels at day 0 were set to be 1. *Klf6* expression exhibited a biphasic increase at day 2 and day 8 (b). Expression of early liver marker *Afp* (c) and *Ttr* (d) could be detected at day 8, while expression of *Albumin*, a later hepatic marker, peaked at day 12 (e).

Figure S7. *p21* expression does not require *Klf6* in ES cell differentiation cultures. *Klf6*$^{-/-}$ ES cells on days 2-6 of differentiation were analyzed for *p21* expression by qPCR. Expression was normalized to WT cells, averaged over 3 experiments and the error bars display the standard error.

Table S1. Primer sequences for qPCR

Mouse primers (3'-5')	
Afp F	GCCACCGAGGAGGAAGTG
Afp 2R	AGTCTTCTTGCGTGCCAGC
Albumin F	GGTGTGTTTCGCCGAGAACGAC
Albumin R	GGCGGCAGACTCATCGGC
Cxcr4 F	CGGGATGAAAACGTCCATTT
Cxcr4 R	ATGACCAGGATCACCAATCCA
Gata4 F	GCGGACTCACGGAGATCG
Gata4 R	CTGCTACACACCCAGGCG
Gapdh F	GCCAAAAGGGTCATCATCTC
Gapdh R	ATGGCATGGACTGTGGTCAT
Hnf3β F	ACTGTAACGGGGGAGGGGC
Hnf3β R	CAGTCGGATGGCTCGTGC
Klf6 F	ACCCGACATGGATGTGCTCCCAAT
Klf6 R	GCAGGGCTCACTCTGAAGATA
Sox17 F	CAGGAAAACCTCAGCATGTCACC
Sox17 R	CTTGGGGAAAACTGGCTGGAG
Ttr F	CTCACCCACAGATGAGAAG
Ttr R	GGCTGAGTCTCTCAATTC
Zebrafish primers (3'-5')	
amy F	CATCAACCCTGATTCCACCT
amy R	GTCCCACCAATTGGAAAATG
ces F	AGCGCTCCTTTCACAGAAGA
ces R	TTTTCCCAGGTTTCTGTTGC
copeb F	CGTCCACGACACTGGC
copeb R	GCTCCAAAGATCCTCCTGGC
zcopeb-E2-F	TCTGACGGAGTCAGCACATCA
zcopeb-E3-R	GGTTAGCTCATCGCTCCTTG
cyp2E1 F	TATTCCCATGCTGCACTCTG
cyp2E1 R	AGGAGCGTTTACCTGCAGAA
cyp3c1 F	AAACCCTGATGAGCATGGAC
cyp3c1 R	CAAGTCTTTGGGGATGAGGA
fabp10 F	CATTGTGGCTGCAGGTTATG
fabp10 R	TAATCCTCACATCCCGATCC
hgae2 F	CTCTGCCCCAGTGAGGAAA

hgae2 R	CGAGGATGTTGTGGGACAGA
hpx F	TAGATGCTGCCTTTGTGTGC
hpx R	TGGTTCCTTCCTTCACAAGC
ifapb F	CACCTCCAAAACTCCTGGAA
ifabp R	TTCTGCAGACCAGCTTTCCT
insulin F	GCTCTGTTGGTCCTGTTGGT
insulin R	GGGCAGATTTAGGAGGAAGG
kdr F	GGTGGGACACTCACACTCATTT
kdr R	TCGCTGGACTTCTTGTGACTG
lcp1 F	GATGGCAGCAGCACAGATC
lcp1 R	CGGCAGAGGGAGATTGGCGGC
mpx F	TGTTGTGCTCTTTCAGTGGGGG
mpx R	ACTTGTAAGCAGCGTCTACTATT
mtp F	CTCAGCTGGTGGATGCAGTA
mtp R	ATCTCTGTGCTGCCGATCTT
npsh1l F	CAGCATGACAGGAGACCAGA
npsh1l R	GCTGTGAAGAAGGGATCTGC
rppo F	CTGAACATCTCGCCCTTCTC
rppo R	TAGCCGATCTGCAGACACAC
tfa F	TGCAGAAAAAGCTGGTGATG
tfa R	ACAGCATGAACTGGCACTTG
try F	CCCTCACCCGAAATACAATG
try R	GAAACCAAGCACTGCTCTCC