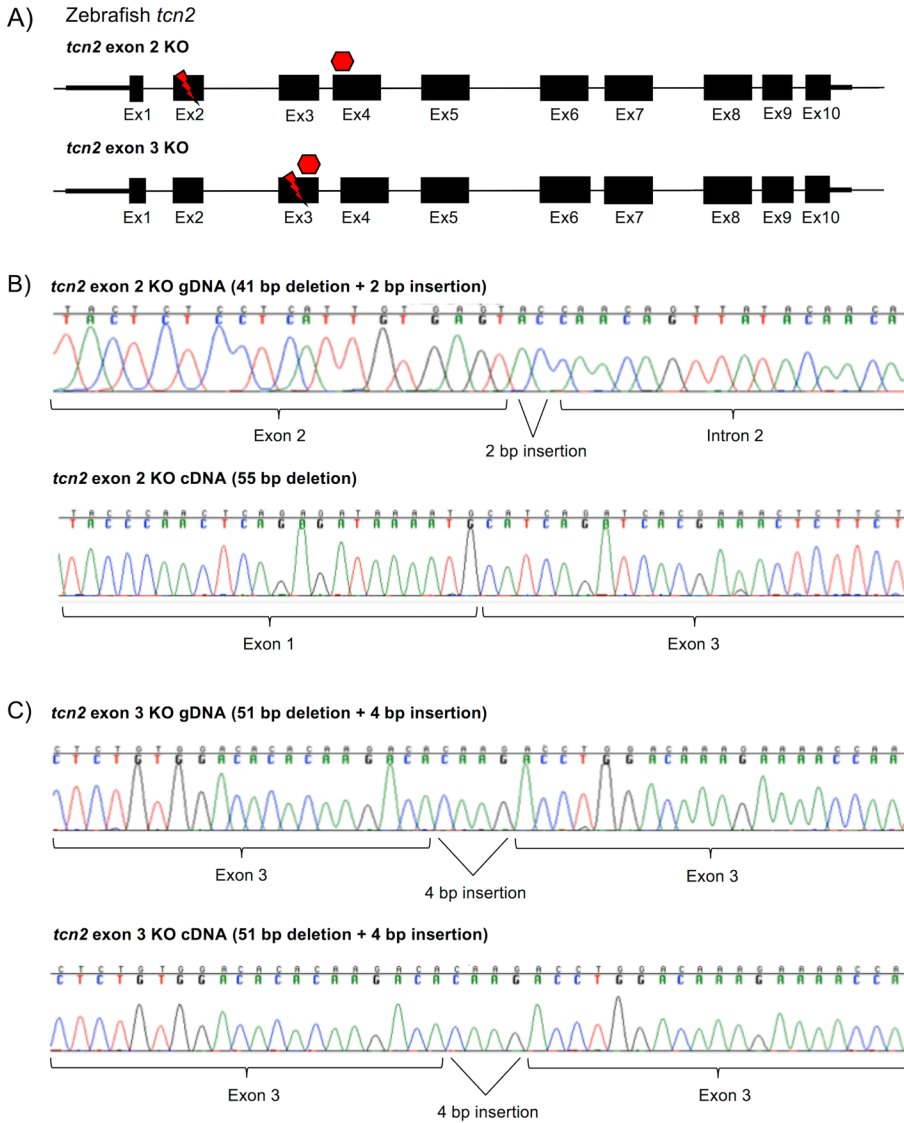


## SUPPORTING INFORMATION



**Figure S1. Design and characterization of CRISPR/Cas9 mutagenized *tcn2*<sup>-/-</sup> zebrafish lines.** (A) Two sgRNAs were designed to target either the second or the third exon out of ten in zebrafish *tcn2*. Exons and introns not to scale. Red hexagon demonstrates approximate location of early stop codon in the resulting transcript. (B) Chromatograms of gDNA and cDNA sequencing results for *tcn2* exon 2 KO line. gDNA sequencing demonstrates a 41-bp deletion/2-bp insertion overlapping the exon 2/intron 2 boundary, while cDNA sequencing demonstrates the alternative splicing removal of exon 2 in the cDNA transcript, causing the resulting transcript to be out of frame. (C) Chromatograms of gDNA and cDNA sequencing results for *tcn2* exon 3 KO line. gDNA and cDNA sequencing confirm a 51-bp deletion/4-bp insertion in exon 3, causing the resulting transcript to be out of frame.

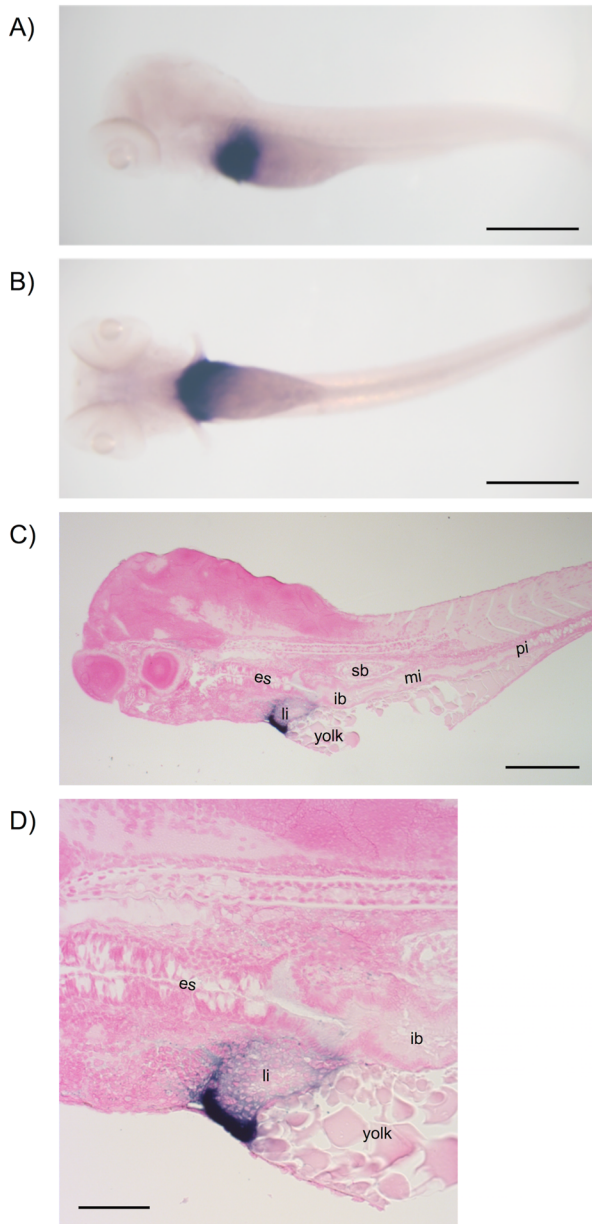
## Non-canonical vitamin B<sub>12</sub>-binding proteins in zebrafish

CLUSTAL O(1.2.4) multiple sequence alignment

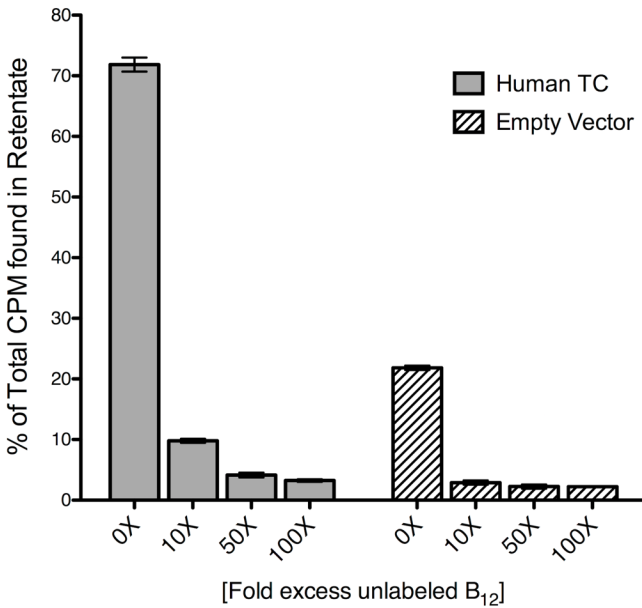
human_TC	-----EMCEIPEMDSHLVEKLGQHLLPWM--DRLSLEHLN----PSIYVGLRLSSLQAG	48
human_IF	TSQTQSSCSVPSAQ---EPLVNGIQVLMENSVTSSAYPN---PSILIAMNLGAYNL	52
human_HC	-----EICEVSEENYIRLKPLLNTM-----IQSNYNRGTSAVNVVSLKLVGIQIQ	46
zebrafish_Tcn2	-----KPCAS--DHETLLQSLNKQLLRV--DT-QDMLPN---PSVHIALRLSTQHNL	45
zebrafish_Tcnba	-----	0
zebrafish_Tcnbb	-----	0
human_TC	TKEDLYLHSLKLGYYQQCLLGSFSEDDGDCQCGKPSMGQLALYLALRANCEFVRGH---	104
human_IF	---KAQKLLTYQ-----LMSDDNDLTIQGLGLTSMALTSSCRDPGDKVSI-	95
human_HC	TLMQKMIQQIKYN-----VKSRSDVSSGELALILAL-GVCRNAENLIY-	91
zebrafish_Tcn2	DKEHQYLNRLKKEFHEDIE-----KSLRNGELVVGRALALYLALRSCDLSLHLNHN	98
zebrafish_Tcnba	-----	0
zebrafish_Tcnbb	-----	0
human_TC	-----KGDRVLSQKWFLEDEKRAIGHDHKGHPHTSYQYGLGILALCLHQKRVHDSVVDKLLY	163
human_IF	-----LQRQMENWAPSSPNAEASAFYGFSLALALCQKNSATLPIAVR-F-	140
human_HC	--DYHLIDKLENKFAELENME-AHNGTPLTNYQLSLDLVLCCLFNGNYSTAEVNHNF-	147
zebrafish_Tcn2	EKNEFLTLHKEMEEEKQNIAF--SHRPKTYQYSLGILALCVSGVRVSTHVSHKLIH	156
zebrafish_Tcnba	-----	0
zebrafish_Tcnbb	-----	0
human_TC	-----AVEPFH---QGHHSVDTAAMAGLAFTCLKRSNFPN-----GRRQRITMAIRTVREILK	214
human_IF	--AKTLANSSPNVDTGAMATLALTCMYNKPVGS---EEGYRSLFGQVLDIIVEKISM	195
human_HC	TPENKNYYFGSQFSDVTGAMAVLALTCVKKSLINGQIKADEGSLKNISYTKSLVEKILS	207
zebrafish_Tcn2	AVEHGQIKHGESLDCIDSHAMAGMALQCLKNEGISV-----KDEAELDKALATIKQKLV	210
zebrafish_Tcnba	-----	0
zebrafish_Tcnbb	-----	0
human_TC	-----AQTPEGHFGNVYSTPLALQFLMTSPMRGAELGTACLKARVALLASLDGAFQNALMISQL	274
human_IF	KIKDNGIIGDIYSTGLAMQALSVTPE--PSKKEWNCKTTDMILNEIKQKGFHPMSIAQI	254
human_HC	EKKENGLIGNTFSTGEAMQALFVSSDYNNENDWNCQQLTNTVLTEISQGFASNPNAAAQV	267
zebrafish_Tcn2	SKRADGHMGNEFSTGLAVQALLAMGVEMECCG---TAIEALRGDIRKGYHNPMAASQV	266
zebrafish_Tcnba	-----	0
zebrafish_Tcnbb	-----	0
human_TC	LPVLNHKTYIDLIFPDCLAPR---VMLEPAAE---TIPQOEIISVTLQVLSL---LPP	324
human_IF	LPSLKGKTYLDVPQVTCSPD---HEVQPTLPSNPGGPTASNITVIYTIINNQLRGVEL	310
human_HC	LPALMGKTFLDINKDSSCVSAGNFNISADEPITV--TPPDSQSYISVNYSV---RINET	322
zebrafish_Tcn2	LPALYQQSYLHLKSKCEKRESD---DTLTADVESASEVLPSLGQV-AVQVEVKS--NGEA	320
zebrafish_Tcnba	-----LPADSGKL---EELPKVVTIVNDFTN-EQ	25
zebrafish_Tcnbb	-----GHSGEQHGQV---GQVSVINVVTKNFAN-EL	28
human_TC	Y---RQISISVLAGSTVE-DVLKKAHEL-GGFTYET-QASLSGPI <sup>d</sup> LTSVMGKA--AGEREF	376
human_IF	LFNETINVSVKSGSVLLVLEEAQRK-NPMFKPET-TMTSWGIV <sup>s</sup> VSSINNIENVNHRKY	368
human_HC	Y---FTNVTVLNKGSVFLSVMEKAKMNDTIFGFTM-EERSWGPY <sup>t</sup> ITCIQGLCANNDRTY	378
zebrafish_Tcn2	S---VFPINVPKGSFLFEALNLLQDKQ-TGFTFKT-EDSLWGAF <sup>s</sup> LSVLNDEQARQTDRTY	375
zebrafish_Tcnba	L---SYSTTVIQEGLMFGVNLQMESN-ADFKFSYTIHHTFGIY <sup>s</sup> LESVNLGASDEDDQTY	81
zebrafish_Tcnbb	N---TYPVTAPKMPIFGVNLQDQSN--QLNFTYSISKSYGIF <sup>s</sup> LESVNLGASSTENKTY	83
human_TC	WQLLRDP---NTPLLQ <sup>cb</sup> GIADYRPKDGETIELRLVSW--- 409	
human_IF	WQFLSGV---TPLNEGVADYIPFNHEHITANFTQY--- 400	
human_HC	WELLSGG---EPLSQAGSYVVRNGENLEVRWSKY--- 410	
zebrafish_Tcn2	WHVSSD---GTSLTQGIKDYKIDSAQRITIKNTGY--- 407	
zebrafish_Tcnba	WELLSKSGVVTRLEVIGICYQVQRDENLILRFTTWATKK 121	
zebrafish_Tcnbb	WELLSKREKTRRLNVI <sup>g</sup> ICYQPERNENFIMNFTTWA--- 120	
* * * *		

**Figure S2. Multiple sequence alignment of known and potential cobalamin carrier proteins in humans and zebrafish.** A multiple sequence alignment for human TC, IF, and HC and zebrafish Tcn2, Tcnba and Tcnbb was performed using Clustal-Omega software. Specific annotations were added according to findings in (1). Blue lettering indicates residues involved in hydrophobic interactions with cobalamin, while letters above the alignment indicate residues involved in hydrogen bonds to a specific side chain of cobalamin (side chain indicated by the letter shown). Dots below alignment indicate levels of local residue conservation, while asterisks signify complete residue conservation within this set of sequences. Red bars below alignment indicate regions targeted for site-directed mutagenesis.

**Table S1. Cobalamin carrier protein homologs in vertebrate species.** Species name, prepended organism abbreviation, common name, lineage, and accession code are notated for each sequence included in Fig 2. Further divisions based on homology classification are shown in alternate sheets.



**Figure S3. Whole-mount *in situ* hybridization and sectioning of liver-specific probe *fabp10a*.** Whole-mount *in situ* hybridizations were performed on zebrafish embryos at 5 dpf with a DIG-labelled RNA probe and subsequent treatment with an anti-DIG antibody conjugated to alkaline phosphatase (AP) and BM-purple staining for spatial comparison of the known liver-specific *fabp10a* with our genes of interest (A-B). Embryos were then sectioned and stained with Nuclear Fast Red (NFR) to visualize at higher resolution (C-D). (A) Lateral view of *fabp10a* expression. Scale bar: 400  $\mu$ m. (B) Ventral view of *fabp10a* expression. Scale bar: 400  $\mu$ m. (C) Sagittal section of *fabp10a*. Scale bar: 200  $\mu$ m. (D) Zoom-in of the sagittal section of *fabp10a*. Scale bar: 50  $\mu$ m. Abbreviations: es = esophagus, li = liver, ib = intestinal bulb, sb = swim bladder, mi = mid-intestine, pi = posterior intestine.



**Figure S4. Competition assay demonstrates binding specificity of radiolabeled cobalamin.** Specific activity (191  $\mu\text{Ci}/\mu\text{g}$ ) of  $^{57}\text{Co}$ -labelled vitamin B<sub>12</sub> solution was used to calculate input concentration of radiolabeled cobalamin used in binding assay experiments (145 pM). Media samples (Human TC and empty vector) were incubated with  $^{57}\text{Co}$ -containing cobalamin and varying concentrations of excess unlabeled cobalamin competitor (0X, 10X, 50X, 100X). Samples were spun through 10 kDa filters to separate protein-bound and unbound radioactive cobalamin. Percent of total counts per minute (CPM) in the retentate (protein-bound fraction) are shown,  $\pm$  SD (n=3).

## REFERENCES

1. Wuerges, J., Geremia, S., and Randaccio, L. (2007) Structural study on ligand specificity of human vitamin B<sub>12</sub> transporters. *Biochem J* **403**, 431-440