SUPPLEMENTAL DATA

LEGENDS TO SUPPLEMENTAL FIGURES

SUPPLEMENTAL FIGURE S1. **Comparison of the amino acid sequences of P4H-TM homologues from zebrafish (z), mouse (m) and human (h) sources.** The black boxes represent identical amino acids. Gaps were introduced for optimal alignment. A dashed line indicates the transmembrane domain and asterisks the conserved catalytically critical amino acids.

SUPPLEMENTAL FIGURE S2. **Comparison of the cerebral structures of P4H-TM deficient and control embryos.** Hematoxylin and eosin stained sections of 4 dpf MM and TM1+p53 larvae are shown. Dc indicates diecephalon and Tc indicates telencephalon.

SUPPLEMENTAL FIGURE S3. **P4H-TM deficient larvae develop no signs of polycythemia or anemia.** *O*-dianisidine stainings of 2 dpf RC, MM, TM1 and TM3 embryos. Intensity of staining was scored 1-3.
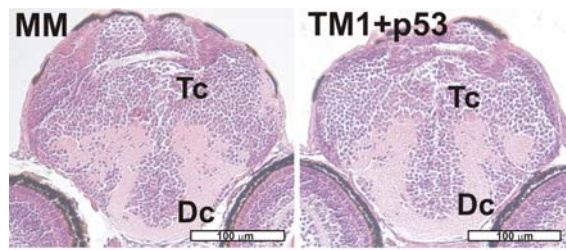
Supplemental Figure S1.

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zP4H-TM    1 MLSGKMMEPQ    DDIEEDDSPSASAPPSSSPSPSSSPPPRALPRQRTSLQKS SVCSRSYFMVVMVFFHVYIINVIALL
mP4H-TM    1 MAAAVATVQRPEAETVEEASNLQWPLPPEHRPSGAATRPGDSEDAPVRPLCKPRGICSRAYFLVLMVFVHLYLGNVLALL
hP4H-TM    1 MAAAAVTGQRPETAAAEEASRPQW-APPDHCQAQAAAGLGDGEDAPVRPLCKPRGICSRAYFLVLMVFVHLYLGNVLALL


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zP4H-TM   77 LYVHYNNGSNAISANRDFTSSDSSSHKVTGASDAQATADSGFPRNMFLPRIEGIRVGHVQKVSLVSGKVHEMKTLSLKPL
mP4H-TM   81 LFVHYSNGD       ESTDPGPQRREQSPQPVPTLGP      LTRLEGIKVGYERKVQVVAGRDHFIRTLSLKPL
hP4H-TM   80 LFVHYSNGD       ESSDPGPQHRVQGPGPEPTLGP      LTRLEGIKVGHERKVQLVTDRDHFIRTLSLKPL


zP4H-TM  157 LFEIPDFLSEEECAVVVRLAQLKGLMESQVMVPEGQEELDQQLNLSPEEIFNFLDLNQDGQLQPHEILTHSRVRDGIWLT
mP4H-TM  145 LFEIPGFLSDEECRLIIHLAQMKGLQRSQILPTEEYEEAMSAMQVSQLDLFQLLDQNEDGRLQLREVLAQTRLGNGRWMT
hP4H-TM  144 LFEIPGFLTDEECRLIIHLAQMKGLQRSQILPTEEYEEAMSTMQVSQLDLFRLLDQNRDGHLQLREVLAQTRLGNGWMT


zP4H-TM  237 SENLKEIYDGLKADLDGNGLLSLEEFGRLRSDAFQRFLLQRGVERSQLVRNSRHTWLYQGQGAHQVLQDLRKSLSRVTLL
mP4H-TM  225 PENIQEMYSAIKADPDGDGVLSLQEFSNMDLRDFHKYMRSHKAESNELVRNSHHTWLHQGEGAHHVMRAIRQ    RVLRL
hP4H-TM  224 PESIQEMYAAIKADPDGDGVLSLQEFSNMDLRDFHKYMRSHKAESSELVRNSHHTWLYQGEGAHHIMRAIRQ    RVLRL


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zP4H-TM  317 TRLPSSLVELSEPLQVVRYEQGGHYHAHHDSGPVYPETACTHTRLAANTTSPFQTSCRYITVLFYLNNVQEGGETTFPVA
mP4H-TM  302 TRLSPEIVEFSEPLQVVRYGEGGHYHAHVDSGPVYPETICSHTKLVANESVPFETSCRYMTVLFYLNNVTGGGETVFPVA
hP4H-TM  301 TRLSPEIVELSEPLQVVRYGEGGHYHAHVDSGPVYPETICSHTKLVANESVPFETSCRYMTVLFYLNNVTGGGETVFPVA


                                                         *             *
zP4H-TM  397 DNRTYEEASLIQNDVDLLDTRKHCDKGNLRVKPVKGTAVFWYNYLSDGRGWVGEQDEYSLHGGCVVTQGTKWVANNWINV
mP4H-TM  382 DNRTYDMSLIQDDVDLRDTRRHCDKGNLRVKPQQGTAVFWYNYLPDGQGWVGEVDDYSLHGGCLVTRGTKWIANNWINV
hP4H-TM  381 DNRTYDMSLIQDDVDLRDTRRHCDKGNLRVKPQQGTAVFWYNYLPDGQGWVGDVDDYSLHGGCLVTRGTKWIANNWINV


zP4H-TM  477 DPDYQRQARYQ 487
mP4H-TM  462 DPSRARQALFQQEMARLAREGGMDSQPEWALDRAYSDARVEL 503
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Supplemental Fig. S2

Supplemental Fig. S3
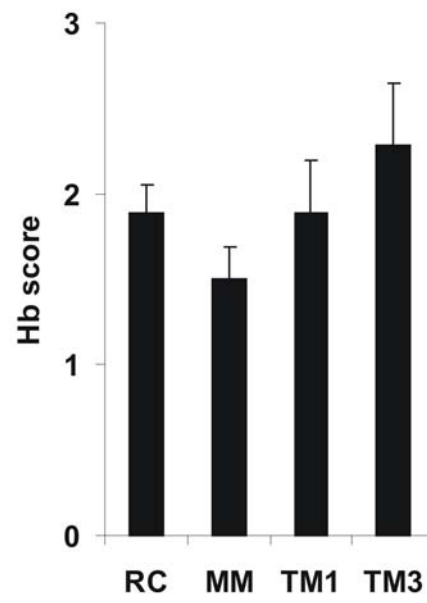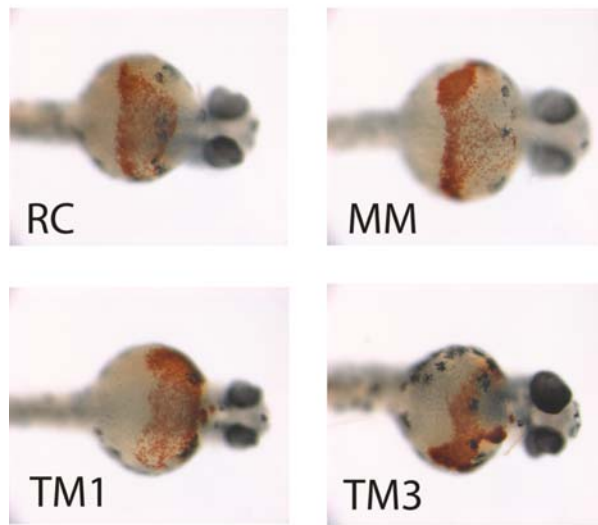
SUPPLEMENTAL MOVIES
Movie S1. Time-lapse movie of the heart of a 2 dpf RC embryo.
Movie S2. Time-lapse movie of the heart of a 2 dpf MM embryo.
Movie S3. Time-lapse movie of the heart of a 2 dpf TM1 embryo.
Movie S4. Time-lapse movie of the heart of a 2 dpf TM3 embryo.

SUPPLEMENTAL TABLE S1. **Primers used in the study.**

Sequences of Primers Used in Q-PCR

Gene	Forward Primer	Reverse Primer
P4H-TM	TACGGGTGGGCCATG	CAGCAGAGGCTTTAGACTGAG
Hif-1α	GCGTAAGAGGAAGCTGAACG	TCCACACTGTGAAGCAAAGC
Hif-2α	TCAGTTGCACCGACTGTCTC	TGCACTACTTGGAGGTGCAG
Hif-p4h-1	GGGGATTGTGTCATCAATG	GGGGATTGTGTCATCAATG
Hif-p4h-2	GGAGATGGGAGATGTGTCA	TGAGCTGTTCCCTCTGGA
Hif-p4h-3	AGTTCAGCCGTCGTATGC	CTGTGAGATGGCTGTGAGA
Epo	CTCTTTGCCTTACTGCTGATG	CATAGCAGCCTCTGCATC
Vegf-a	TCCTGTGTGGTTCTCATGC	TGCATTCACACTTGGTGTGTTC
Glut-1	GTGGGTTTGGAAACGGTA	CTCAGGGCAAAAAGGAAGA
Ldha	GGCTATGGACTTGCAGCA	CTTTTGAGTTTGCGGTCAC
C-P4H-α(I)	GGCGAATTATGGTGTTGGAG	GTTGCTATGCGATTCCCTGT
Lox	GGAGGACACGTCCTGTGACT	TTGGCAGTCAATGTCAGCAT
Col(IV)α3	CAATTGTTTGCCGATGTTCA	GAGGCGTAGCGACAAGTTTC
Col(XVIII)α1	CGGGACGGTGTTAGACAAG	GGTGGAGAAATCTGCTCTG