SUPPLEMENTARY INFORMATION

The HIF pathway regulates oxygen sensing in the simplest animal, *Trichoplax adhaerens*

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1. Supplemental Experimental Procedures

RNA isolation and RT-q-PCR analysis (continued)

Melting curve analyses were performed after PCR amplifications; primer pairs giving non-uniform amplification products or C_t values very different to those of control genes were not investigated further. Primers were designed to bridge exon-intron boundaries in highly conserved gene regions, wherever possible.

Table. Oligonucleotide primers used in RT-q-PCR experiments.

Entry	Target gene	Sequence	Direction
1	*taACTB*	GGGATGATATGGAAAAGATCTGG	Forward
2	*taACTB*	GCCGGAATCAAGAACGATAC	Reverse
3	*taCDO*	AAGGCCAAGGGAGTGGTATT	Forward
4	*taCDO*	TTCTCCCTCAGGCCATTCTA	Reverse
5	*taPGK*	GGAATGGACCTCCTGGTGTA	Forward
6	*taPGK*	GGCAACTCCCGGTAGATTTT	Reverse
7	*taPHD*	GAACAGCCAGGACTGTGGAG	Forward
8	*taPHD*	TCGTTCCTTCTCGTCGAAAT	Reverse
9	*taPLOD*	GATCATGAGGGTGGAGGTTG	Forward
10	*taPLOD*	CAAGGATCAATAAACGAGACCA	Reverse
11	*taIDE*	TCAATGAAGGAACACTTGGAA	Forward
12	*taIDE*	CGCTTAGGAGCGTCTTTTTC	Reverse
13	*taALDO*	GGCGATGTGTCCTCAAGATT	Forward
14	*taALDO*	TAAGTCGCCGCCAGTACTCT	Reverse
15	*taPDK*	TGAGAGCAACGGTTGAAAAA	Forward
16	*taPDK*	CCTCTGTCAACAACTCTAATCACTAT	Reverse
17	*taHIFα*	CGCTTCGGTTGTCTGTGATA	Forward
18	*taHIFα*	CGCGTTATAATCGCATAGCA	Reverse
19	*taHIFα*	GCTGTGTTATAGGGACATCAAGTG	Forward
20	*taHIFα*	CCCTTCATACGGGATTCTGA	Reverse
21	*taHIFα*	TTGAGCAGGACATCAAGTGG	Forward
22	*taHIFα*	ATTCCACGTCGGATTCTGAT	Reverse

Human cell culture, transfections, and immunoblots (full method)

Cultures of HEK 293T cells were maintained in Dulbecco's modified Eagle's Medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 50 IU/ml penicillin G, and 50 μg/ml streptomycin. Cells were either grown in normoxia (21% O_2 / 5% CO_2) or moderate hypoxia (6% O_2 / 5% CO_2 for 4 hours) using an Invivo$_2$ hypoxic workstation (Ruskin Technologies, UK). Plasmid transfections were performed in HEK 293T cells with FuGENE 6 (Roche) according to the manufacturer's instructions. HEK 293T cells were transfected with pEF6 HA-taPHD plasmid plus 50 nM control (*Drosophila* HIFα, dHIF) or PHD2 siRNA oligonucleotide using DharmaFECT Duo (Fisher Scientific) according to the manufacturer's instructions. Control and PHD2 siRNA oligonucleotide sequences were as reported (Appelhoff *et al*, 2004). 48 h post-treatment, cells were lyzed (20 mM Tris-HCl (pH 7.4), 100 mM NaCl, 5 mM $MgCl_2$, 0.5% (v/v) Igepal CA-630, 1x Complete protease inhibitor mixture (Roche)); proteins were analyzed by immunoblotting following SDS-PAGE separation. HRP-conjugated anti-HA antibody (Roche) was used at 1:2,000. HRP-conjugated anti-β-actin antibody (Sigma) was used at 1:25,000. Mouse anti-HIF1α antibody (Transduction Labs) was used at 1:1,000, mouse anti-PHD2 antibody (Appelhoff *et al*, 2004) was used at 1:50, and goat anti-mouse HRP conjugated secondary antibody (Dako) at 1:2,000.

Enzymatic assay conditions

Assays with recombinant enzymes / substrates utilized similar conditions to those described (Hewitson *et al*, 2007), except without the 1-[^{14}C]-2OG; reactions were quenched with an equal volume of MeCN (4°C). Peptide modifications were analyzed using a MALDI-TOF microMX machine in negative or positive ion modes using an α-cyano-4-hydroxy-cinnamic acid (CHCA) matrix (1:1). MS/MS analyses used a

Bruker Daltonics Ultraflex MALDI TOF/TOF machine. Proteins were analyzed by 'non-denaturing' MS using a Waters Q-Tof spectrometer (Loenarz *et al*, 2009); typically, experiments were performed at least twice. To analyze peptide binding to the VHL complex, a modification of a fluorescence resonance energy transfer assay (Dao *et al*, 2009) was used (Loenarz *et al*, 2009) with *N*-terminally biotinylated peptides (50 nM). The data output ('FRET signal') from the EnVision Multilabel plate reader (PerkinElmer) is the ratio of the 665 nm and 615 nm emission signals, multiplied by 10,000. Assays were performed in duplicate. Peptides were prepared by solid phase peptide synthesis (CS Bio CS336), as described (Loenarz *et al*, 2009).

Table. Peptide sequences used in analyses.

Entry	Species	Name	Database	Sequence*
1	HUMAN	HIF-1$\alpha_{556-574}$ (CODD)	gi: 4504385	DLDLEMLA**P**YIPMDDDFQL
2	HUMAN	HIF-1$\alpha_{395-413}$ (NODD)	gi: 4504385	DALTLLA**P**AAGDTIISLDF
3	HUMAN	HIF-1$\alpha_{788-806}$ (CAD)	gi: 4504385	DESGLPQLTSYDCEV**N**API
4	HUMAN	*trans*4Hyp-564 HIF-1$\alpha_{556-574}$	gi: 4504385	DLDLEMLA-**Hyp**-YIPMDDDFQL
5	HUMAN	*trans*4Hyp-402 HIF-1$\alpha_{395-413}$	gi: 4504385	DALTLLA-**Hyp**-AAGDTIISLDF
6	TRIAD	HIF$\alpha_{477-497}$ (ODD)	JGI: scaffold_5	EKEDYDDLA**P**FVPPPSFDNRL
7	TRIAD	*trans*4Hyp-486 HIF$\alpha_{477-497}$	JGI: scaffold_5	EKEDYDDLA-**Hyp**-FVPPPSFDNRL
8	DICDI	Skp1$_{135-153}$ (ddSkp1)	gi: 66822139	FNIKNDFT**P**EEEEQIRKEN
9	DICDI	*trans*4Hyp-143 Skp1$_{135-153}$	gi: 66822139	FNIKNDFT-**Hyp**-EEEEQIRKEN
10	NEMVE	HIFα (CAD)	JGI: scaffold_26	VKALFPYVTQSDAEV**N**APV
11	NEMVE	HIFα (ODD)	JGI: scaffold_26	ESNELQNRA**P**YIPPPTGDAAL
12	ANOGA	HIF$\alpha_{397-415}$ (CODD)	gi: 158290352	ELDLSMRA**P**YISMSEVDDL
13	TRICA	HIF$\alpha_{543-561}$ (CODD)	gi: 189237669	ESDLVAKA**P**YITMNMGDDL
14	CANMG	HIF$\alpha_{468-486}$ (NODD)	gi: 107051811	PEDLTHLA**P**SGGDTCVPLP
15	CANMG	HIF$\alpha_{657-675}$ (CODD)	gi: 107051811	SDEFEMRA**P**YIPPSNELLL
16	PALPU	HIF$\alpha_{629-647}$ (CODD)	gi: 50261639	LDEFDMRA**P**FIPLSNELLM
17	STRPU	HIF$\alpha_{404-423}$ (NODD)	gi: 115653070	VEEKLAYLA**P**TAGDVMIELD
18	STRPU	HIF$\alpha_{519-536}$ (CODD)	gi: 115653070	DELAMRA**P**YIPMGEDFDL
19	BRAFL	HIFα (NODD)	JGI: Bf_V2_6	PEDLTRVA**P**AAGDAMIPLG
20	BRAFL	HIFα (CODD)	JGI: Bf_V2_6	AEELSYRA**P**YIPAYQMPLN
21	DANRE	HIF1αl2$_{435-454}$ (CODD)	gi: 59933250	ELDLDSLA**P**YIPMHGEDFLL
22	CAEEL	HIF$\alpha_{607-634}$ (ODD)	gi: 193207991	DDLQWEEPDLSCLA**P**FVDTYDMMQMDEG
23	DROME	HIF$\alpha_{843-861}$ (ODD)	gi: 195574995	FEAFAMRA**P**YIPIDDDMPL

If available, the GenBank protein entry (gi) is given (www.ncbi.nlm.nih.gov/Genbank); otherwise the JGI entry specifies the gene scaffold

(www.jgi.doe.gov). *Abbreviations: Hyp, *trans*-4-hydroxyprolyl; see supplementary Table S3 for organism abbreviations.

Trichoplax RNA interference experiments

RNAi analyses employed a modification of a reported procedure (Jakob *et al*, 2004). Probes for genes were prepared as pGEM-T easy vector (Promega) inserts: Following linearization by PCR, they were transcribed using T7 or SP6 RNA polymerase (Roche), and purified by Li^+/EtOH precipitation after treatment with DNase I. Single-stranded RNA from T7 and SP6 transcriptions were annealed overnight (100 mM NaCl, 10 mM $MgCl_2$, 1 mM dithiothreitol in 50 mM Tris, pH 7.9, 95 °C) and cooled over 16 hours; a transfection complex consisting of the annealing product (~2 μg) and 5 μl FuGENE HD (Roche) in seawater (1 ml) was incubated (16 hours) with ~10 *Trichoplax*. Primers for the *taPHD* probe were 5'-GGAGCATCAACAATCCCATT-3' and 5'-CTACAACTGCTCCCCAGGAA-3'. Double-stranded RNA corresponding to the *C. elegans egl-9* gene (strain VC575) was used as a control (primers: 5'-CAAGGGAACACCTTCTACCG-3', 5'-TGCAGGATCATCAAACGAAA-3').

Construct design, heterologous expression, and purification of proteins

DNA for *Trichoplax* taPHD was synthesized with codon-optimization for expression in *E. coli* (Geneart AG, Germany), and inserted into the pET-28b (NheI/SacI for $taPHD_{64-300}$) and pEF6-HA (BamHI/SalI) vectors for expression with an *N*-terminal His_6-tag (*E. coli*) or an *N*-terminal HA-tag (HEK293T cells). Expression vectors for *N*-terminally HA-tagged human $PHD2_{2-426}$ and *C. elegans* $EGL\text{-}9_{2-723}$ were constructed using the pEF6-HA vector (BamHI/EcoRI, a gift from R. Marais, London, UK). The vector for expression of $PHD2_{181-426}$ (pET-28a) has been described (McNeill *et al*, 2005). All constructs were verified by sequencing. Recombinant proteins were produced with an *N*-terminal His_6-tag using *E. coli* Rosetta 2 cells;

Expression was induced by isopropyl-β-D-thiogalactosidase (typically 0.5 mM, ~14 hours at 18 °C). Cells were harvested and lyzed by sonication in 20 mM Tris-HCl (pH 7.9), 0.5 M NaCl and 10 mM $MgCl_2$; soluble protein was purified by immobilized Ni(II) affinity chromatography then by gel filtration chromatography. Protein of >95% purity (by SDS/PAGE analysis) was exchanged into 50 mM Tris-HCl (pH 7.5) buffer with 500 mM NaCl and concentrated.

Statistical analyses

Intergroup comparisons were statistically analyzed using unpaired *t* tests.

Bioinformatic analyses

Bioinformatic analyses used BioPerl (www.bioperl.org), BLAST 2.2.17 (NCBI), HMMER v2.3.2 (http://hmmer.wustl.edu), and Pfam (Sanger). Analyzed genomes (JGI, www.jgi.doe.gov; Ensembl, www.ensembl.org; NCBI, www.ncbi.nlm.nih.gov) included datasets of masked assemblies, gene, and protein models (see supplementary Table S1). Contour plots used functions within the 'gplots' library of the R-statistical programming language (www.r-project.org). For detailed analyses of protein domains, models from databases were checked using Genscan (http://genes.mit.edu/GENSCAN.html) and curated as necessary. Protein homology models used Modeller v8.3 (UCSF) and PDBs 3HQR, 1AN4, and 1LQB. Multiple alignments of protein sequences used ClustalW2 (www.ebi.ac.uk/Tools/clustalw2), refined using GeneDoc (www.nrbsc.org/gfx/genedoc).

2. Supplementary Data

2.1. Supplementary Figures

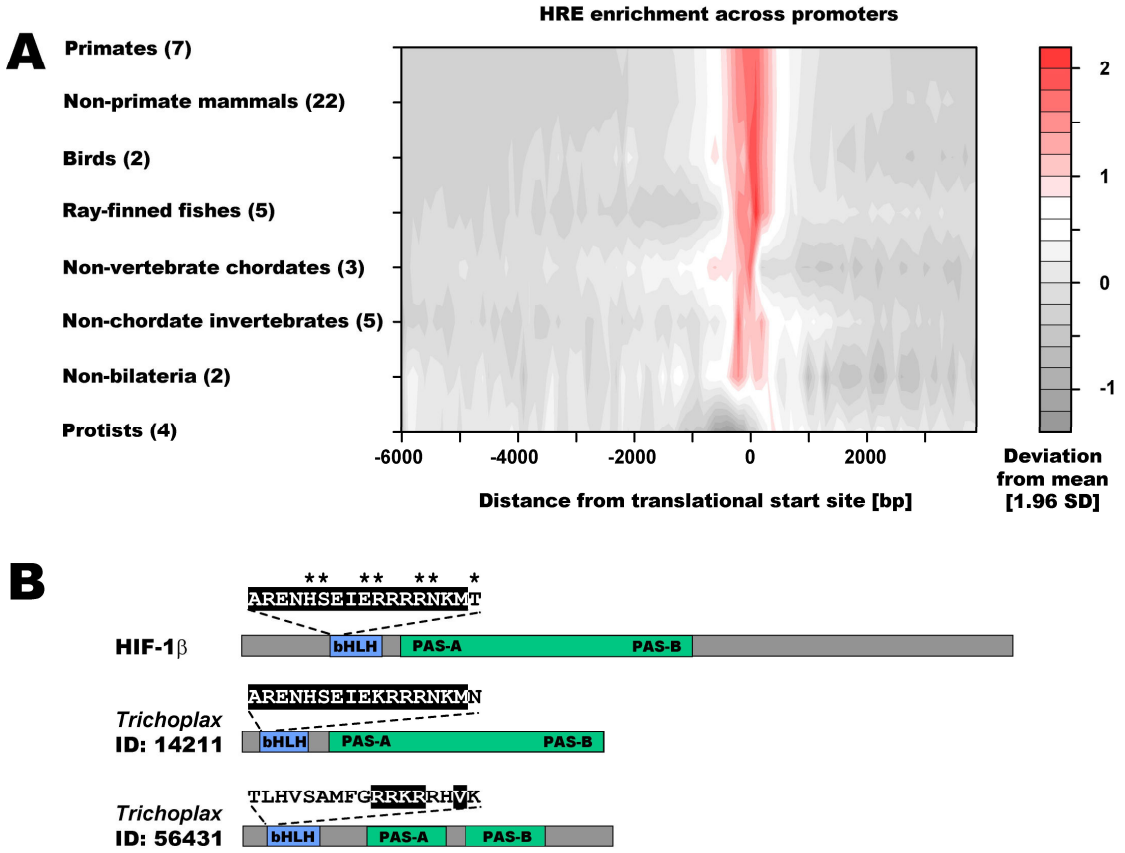
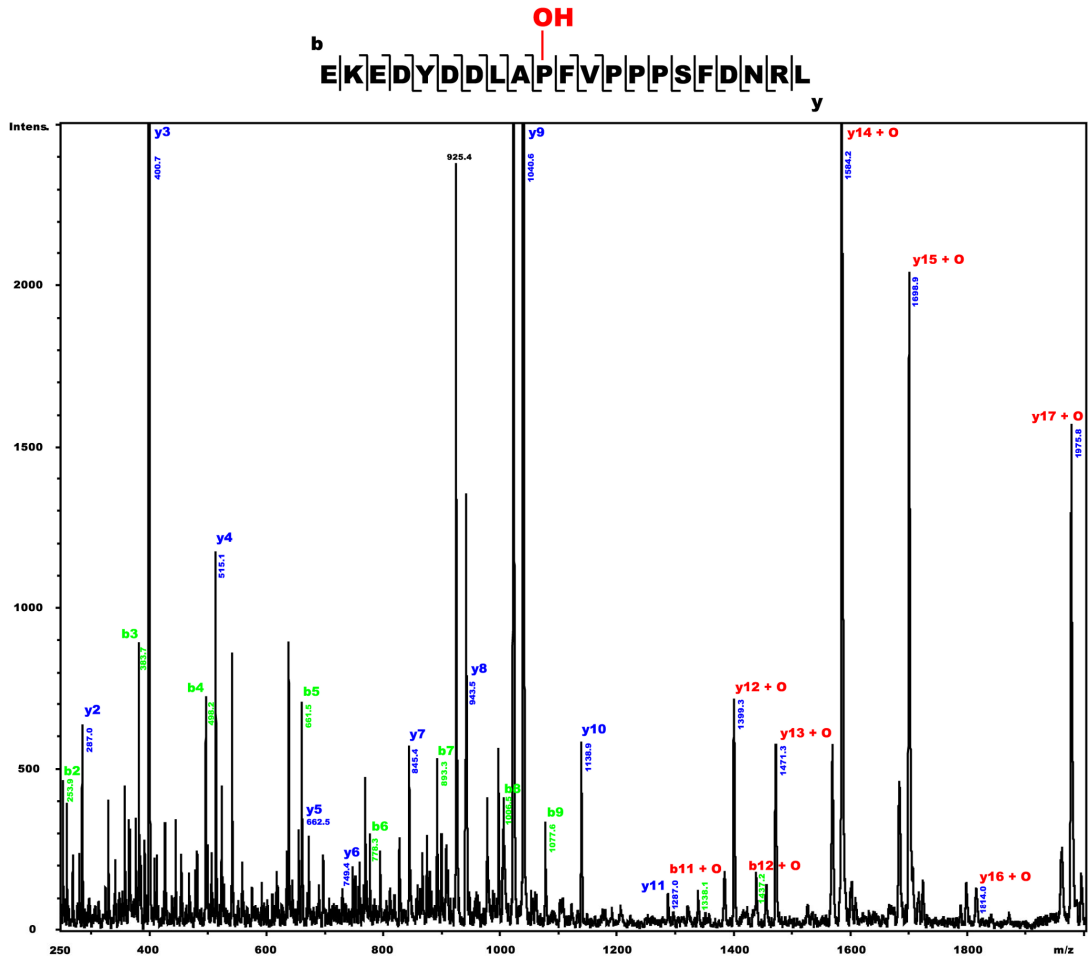


Fig S1. Analysis of potential HRE sequences in transcription factor binding sites, and candidate HIFβ proteins. (**A**) Contour plot showing the frequency of potential HRE sequences per 100 base pairs (bp) across promoter regions of 50 metazoan and protist genomes (plotted as ± 1.96 SD from the mean; number of analyzed organisms in brackets; for analyzed organisms see supplementary Table S1). Average deviations from the mean in the 'enriched' region from -300 to +299 bp are: Protists (-1.2 SD), non-bilateria (+2.2 SD), non-chordate invertebrates (+2.2 SD), non-vertebrate chordates (+1.8 SD), ray-finned fishes (+2.9 SD), birds (+2.8 SD), non-primate mammals (+3.0 SD), and primates (+3.1 SD). Control analyses of predicted RNA polymerase II binding site sequences (eukaryotic TATA box, i.e. TATAWAA, where W is A

or T) across the same organisms were qualitatively as anticipated ('enriched' from -300 to -1 bp in protists (+1.8 SD) and animals (+0.67 SD); 'lowered' from 0 bp to +299 bp in protists (-0.6 SD) and animals (-1.3 SD)). (**B**) Predicted domain structures of *Trichoplax* bHLH-PAS proteins; sequence 14211 (Srivastava *et al*, 2008) corresponds to the likely taHIFβ homolog. Asterisks indicate (predicted) DNA interacting residues based on homology modeling (PDB 1AN4).



fragment	m/z (exp.)	m/z (obs.)
b2	258.1	253.9
b3	387.2	383.7
b4	502.2	498.2
b5	665.3	661.5
b6	780.3	778.3
b7	895.3	893.3
b8	1008.4	1006.5
b9	1079.5	1077.6
b10 + O	1192.5	-
b11 + O	1339.6	1338.1
b12 + O	1438.6	1437.2

fragment	m/z (exp.)	m/z (obs.)
y1		-
y2	287.2	287.0
y3	401.2	400.7
y4	516.2	515.1
y5	663.3	662.5
y6	750.3	749.4
y7	847.4	845.4
y8	944.4	943.5
y9	1041.5	1040.6
y10	1140.6	1138.9
y11	1287.7	1287.0
y12 + O	1400.7	1399.3
y13 + O	1471.8	1471.3
y14 + O	1584.9	1584.2
y15 + O	1699.9	1698.9
y16 + O	1814.9	1814.0
y17 + O	1978.0	1975.8

Fig S2. MS/MS analysis of taHIFα ODD hydroxylation by taPHD. MALDI TOF MS/MS analysis of a hydroxylated taHIF$\alpha_{477\text{-}497}$ peptide (2479.5 Da peak). The b-ion (b2-b9, b11-b12) and y-ion series (y2-y17) are in green and blue, respectively (not all peaks, including those for internal fragments, are assigned). The data reveals hydroxylation of taHIFα at Pro-486 (note +16 Da mass increase for peaks from y12/b11 onwards).

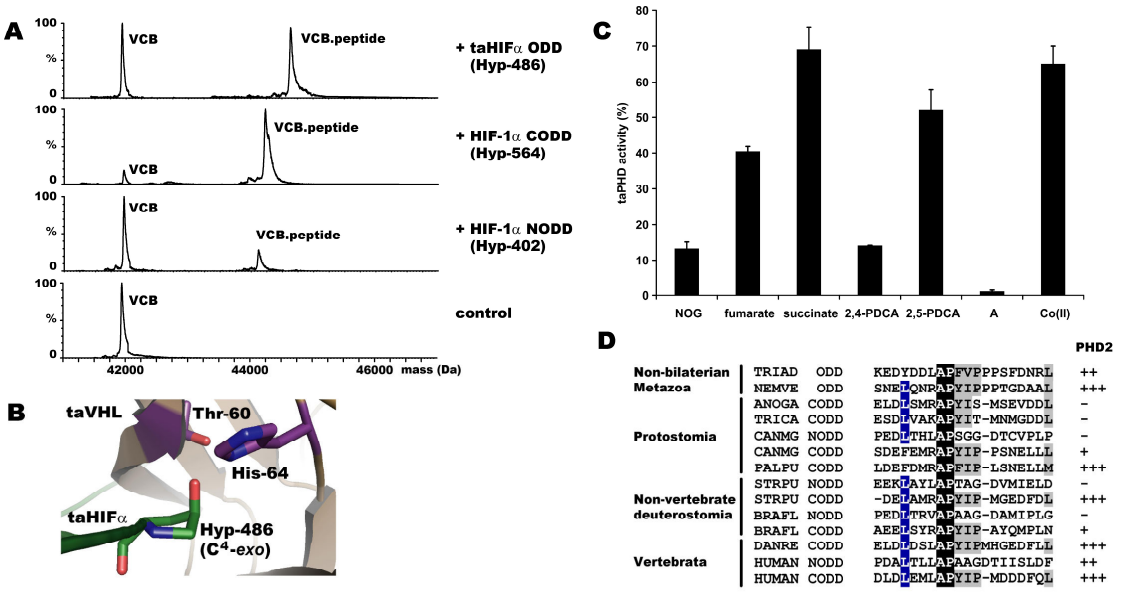


Fig S3. (**A**) **taHIFα binds the VHL complex in a prolyl-hydroxylation dependent manner; taPHD inhibition studies; Many HIFα ODDs from across metazoans are substrates of human PHD2.** Non-denaturing electrospray ionization MS analyses showing that binding of human and *Trichoplax* HIFα peptides to the VHL complex is increased by prolyl *trans*-4-hydroxylation. Non-hydroxylated peptides did not bind substantially (<5%) to the VHL complex. (**B**) Structure homology model showing recognition of taHIFα *trans*-4-hydroxyprolyl-486 in the C^4-*exo* conformation at the taVHL binding pocket (His-64 and Thr-60 are equivalent to His-115 and Ser-111 in human VHL, PDB 1LQB). (**C**) Inhibition of taPHD-catalyzed taHIFα hydroxylation by small molecules (1 mM; n = 3; ± SEM). NOG, *N*-oxalylglycine; PDCA, pyridine dicarboxylate; A, *N*-[(1-chloro-4-hydroxy-3-isoquinolinyl)carbonyl]glycine. (**D**) Despite their low sequence conservation, HIFα substrates from most other tested species are hydroxylated by PHD2 (results were similar with taPHD). The extent of hydroxylation of 100 μM peptide after 30 min incubation with 4 μM PHD2 is indicated (+++, >80%; ++, >50%; +, >15%; -, no hydroxylation); see supplementary Table S3 for organism abbreviations.

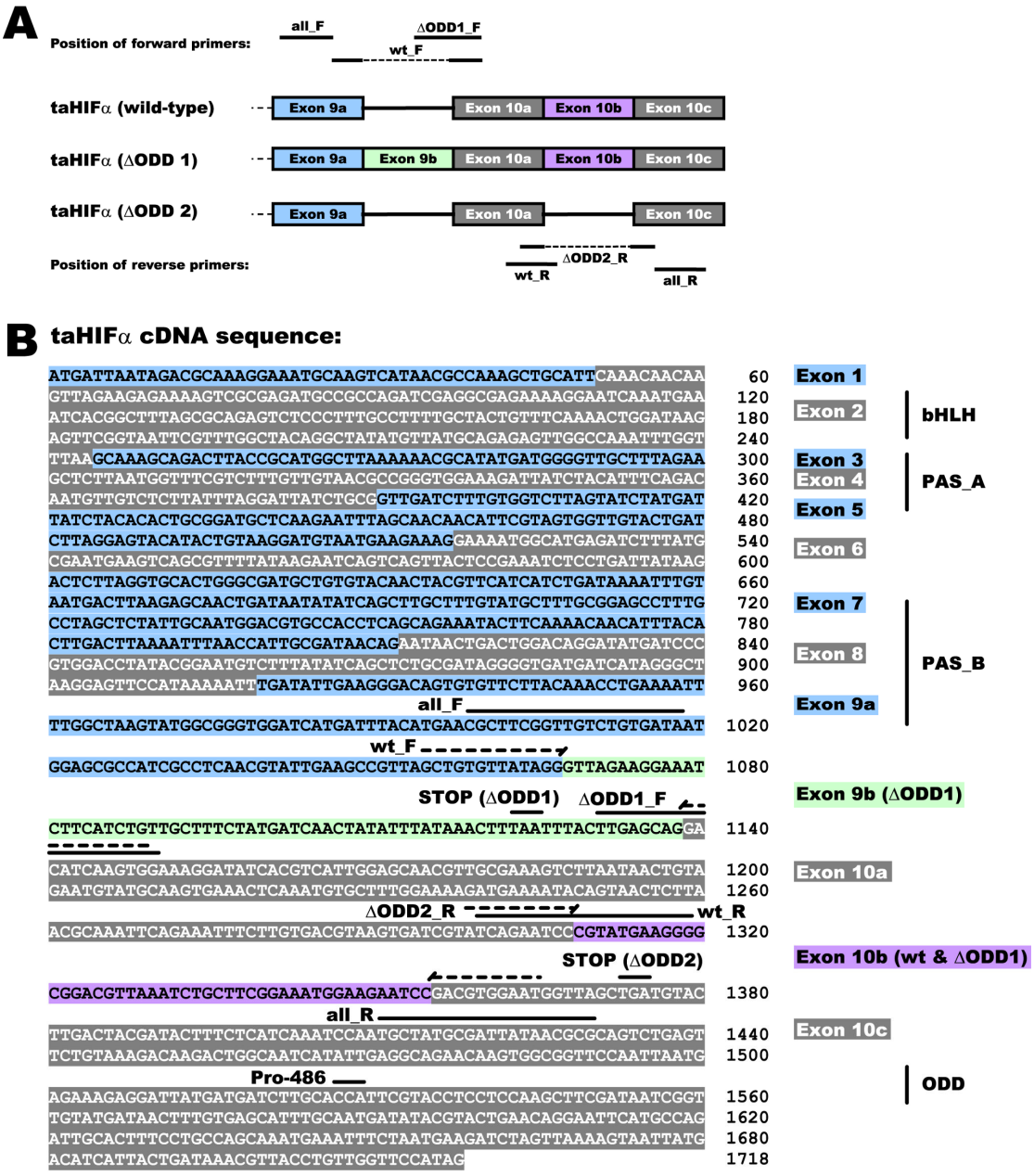


Fig S4. Hypoxia causes variation in *taHIFα* splicing of *Trichoplax*. (**A** and **B**) Alternative splicing of *taHIFα*, and location of primers for RT-q-PCR analyses.

2.2. Supplementary Tables

Table S1. Organism groups and genome annotation files employed in protist and animal promoter analyses.

Note: This table is provided as a separate XLS format file.

The table lists analyzed datasets of masked assemblies from either JGI (www.jgi.doe.gov) or Ensembl (www.ensembl.org). The associated gene and protein models were also used.

Table S2. Conservation of HIFα transcription factor domains that are hydroxylation targets of the 2OG oxygenases PHD and FIH.

Clade	Species	Database ID	HIFα NODD	HIFα CODD	HIFα CAD
Placozoa	TRIAD	JGI:56360		**KEDYDDLAPFVPPPSFDNRL**	
Cnidaria	NEMVE	JGI:scaffold_26 GenScan		**SNELQNRAPYIPPPTGDAAL**	**GVKALFPYVTQSDAEVNAPV**
Ecdysozoa	CAEEL	gi:3876881		**EPDLSCLAPFVDTYDMMQM**	
	DROME	gi:24651293		**FEAFAMRAPYIPIDDDMPLL**	
	CULQU	gi:170035200	EPDDLTHLAPTAGDACIPLEE	DLDLSMRAPYISMSEVDDL	
	APIME	gi:110756935	EPEDLTHLAPTPGDVCVPLED	DDELELRAPYIPMSDQDEAL	
	ANOGA	gi:158290352	EPDDLTHLAPTAGDACIPLEE	ELDLSMRAPYISMSEVDDL	
	AEDAE	gi:157114231	EPDDLTHLAPTAGDACIPLEE	DLTMSMRAPYISMSEVDDL	
	NASVI	gi:156551204	EPEDLTHLAPTAGDVCVPLEE	DDELALRAPYIPMSDQDEAL	
	TRICA	gi:189237669	EPDDLTHLAPVAGDVCVPLDD	ESDLVAKAPYITMNMGDDL	ATIPSLLDLTQQDFEVNAPV
	PALPU	gi:50261639	EPDDLTHLAPSGGDTCVPLEV	**LDEFDMRAPFIPLSNELLML**	LTIPSLSELSQLDFEVNAPA
	CANMG	gi:107051811	EPEDLTHLAPSGGDTCVPLPT	**SDEFEMRAPYIPPSNELLL**	DTIPTLLELTQQDYEVNAPA
Lophotrochozoa	LOTGI	JGI:169204	EPEDLTHLAPMPSGACTLGSH	LIDMNERSPFIPMSRQSDHSL	SLSTILPCLTQQDYEVNAPT
Echinodermata	STRPU	gi:115929387	VEEKLAYLAPTAGDVMIELDP	**CDELAMRAPYIPMGEDFDL**	PLGAVLPLITNLDAEVNAPL
Cephalochordata	BRAFL	JGI:scaffold_35 GenScan	SPEDLTRVAPAAGDAMIPLGF	**AEELSYRAPYIPAYQMPLN**	LPVDFLPPLTRADVEVNAPI
Fish	DANRE	ENSDARG00000034293	EPEALTVLAPAAGDAIISLDF	**DLDLEMLAPYIPMDDDFQL**	EGSGGLPQLTRYDCEVNAPV
		ENSDARG00000008697	EPEELAQLAPMPGDAIIALDF	DLDLETLAPYIPMDGEDFQL	FDSYCLPELTRYDCEVNMPL
		ENSDARG00000041169	NPEELLQLAPHSGDAIISLTE	ELDLDMLAPYISMDDDFQL	
		ENSDARG00000006181		GLDLEMLAPYIPMDDDFQL	AIAMPLPQITHHDCEVNAPV
		ENSDARG00000057671	EPEDLTQLAPTPGDTIISLDF	DLDLETLAPYIPMDGEDFQL	FETYSLPELTRYDCEVNVPL
		ENSDARG00000044550		**ELDLDSLAPYIPMHGEDFLL**	AALLTLPVLSGWECEVNAPL
	TETNG	ENSTNIG00000017339	RPGALTMLAPAAGDTVVPLDF	DLDLEMLAPYIPMDYDFQL	HSLFSLPQLTRDDCEVNAPL
		ENSTNIG00000017338	RPGALTMLAPAAGDTVVPLDF	DLDLEMLAPYIPMDYDFQL	HSLFSLPQLTRDDCEVNAPL
		ENSTNIG00000019821	n/a	n/a	n/a
		ENSTNIG00000009866	EPEDLTQLAPTPGDTIITLDF	DLDLETLAPYIPMDGEDFQL	FESTCLPELTRYDCEVNVPL
		ENSTNIG00000006798	KPEQLLQLAPEAGDVVPLTE	EMDLEMLAPYISMDDDFQL	
		ENSTNIG00000005330	n/a	n/a	n/a
Amphibia	XENTR	ENSXETG00000014449	EPESLTVLAPDAGDEIISLDF	**DLDLEMLAPYIPMDDDFQL**	LDGTVLPQLTGYDCEVNAPV
		ENSXETG00000026167	EPEDLAQLAPTPGDEIVSLDF	DLDLETLAPYIPMDGEDFQL	FEPYLLPELTRYDCEVNVPV
	XENLA	gi:148229705	EPESLTVLAPDAGDEIIPLDF	**DLDLEMLAPYIPMDDDFQL**	FDGTVLPQLTGYDCEVNAPV
		gi:147900690	EPESLTALAPDAGDDIIPLDF	**DLDLEMLAPYIPMDDDFQL**	LDGTGLPQLTGYDCEVNAPV
		gi:148227427	EPEELAQLAPTPGDEIVSLDF	DLDLETLAPYIPMDGEDFQL	**FESYLLPELTRYDCEVNVPV**
		gi:147904360	EPEDLAQLAPTPGDEIVSLDF	DLDLETLAPYIPMDGEDFQL	**FESYLLPELTRYDCEVNVPV**
Aves	CHICK	gi:45383550	EPDALTVLAPAAGDTIISLDF	**DLDLEMLAPYIPMDDDFQL**	**DESGLPQLTSYDCEVNAPI**
		gi:46048879	**EPEELAQLAPTPGDAIISLDF**	**ELDLETLAPYIPMDGEDFQL**	FEPYLLPELTRYDCEVNVPV
	TAEGU	gi:224051853	EPDALTVLAPAAGDTIISLDF	**DLDLEMLAPYIPMDDDFQL**	**DESGLPQLTSYDCEVNAPI**
		gi:224047239	**EPEELAQLAPTPGDAIISLDF**	**ELDLETLAPYIPMDGEDFQL**	FEPYLLPELTRYDCEVNVPV
Mammalia	ORNAN	gi:149554358	EPDALTLLAPAAGDTIIYLDF	**DLDLEMLAPYIPMDDDFQL**	**DESGLPQLTSYDCEVNAPI**
		gi:149429726	**EPEELAQLAPTPGDAIISLDF**	**ELDLETLAPYIPMDGEDFQL**	LEPYLLPELTRYDCEVNVPV
		gi:149517086		ALDLEMLAPYISMDDDFQL	
	MOUSE	gi:226061948	**EPDALTLLAPAAGDTIISLDF**	**DLDLEMLAPYIPMDDDFQL**	**DESGLPQLTSYDCEVNAPI**
		gi:149269519	**EPEELAQLAPTPGDAIISLDF**	**ELDLETLAPYIPMDGEDFQL**	FEPYLLPELTRYDCEVNVPV
		gi:251823727		TLDLEMLAPYISMDDDFQL	
	HUMAN	gi:4504385	**EPDALTLLAPAAGDTIISLDF**	**DLDLEMLAPYIPMDDDFQL**	**DESGLPQLTSYDCEVNAPI**
		gi:40254439	**EPEELAQLAPTPGDAIISLDF**	**ELDLETLAPYIPMDGEDFQL**	**FESYLLPELTRYDCEVNVPV**
		gi:23065535		**DALDLEMLAPYISMDDDFQL**	

Selected entries were prepared as peptides (see Experimental Procedures for details) and tested as substrates of human PHD2 or *Trichoplax* taPHD (for HIFα NODD and CODD domains) or FIH (for HIFα CAD domains) enzymes; entries in bold were

found to be hydroxylated *in vitro* (see supplementary Fig S3D). See supplementary Table S3 for organism abbreviations.

Table S3. Abbreviations used for organisms.

ExPASy code	Taxonomical name	Common name
AEDAE	*Aedes aegypti*	Yellowfever mosquito
ANOGA	*Anopheles gambiae*	African malaria mosquito
APIME	*Apis mellifera*	Honeybee
BRAFL	*Branchiostoma floridae*	Florida lancelet
CAEEL	*Caenorhabditis elegans*	-
CANMG	*Cancer magíster*	Dungeness crab
CHICK	*Gallus gallus*	Chicken
CULQU	*Culex quinquefasciatus*	Southern house mosquito
DANRE	*Danio rerio*	Zebrafish
DAPPU	*Daphnia pulex*	Water flea
DICDI	*Dictyostelium discoidum*	Slime mold
DROME	*Drosophila melanogaster*	Fruit fly
HUMAN	*Homo sapiens*	Human
LOTGI	*Lottia gigantea*	Owl limpet
MOUSE	*Mus musculus*	Mouse
NASVI	*Nasonia vitripennis*	Parasitic wasp
NEMVE	*Nematostella vectensis*	Starlet sea anemone
ORNAN	*Ornithorhynchus anatinus*	Duckbill platypus
PALPU	*Palaemonetes pugio*	Daggerblade grass shrimp
STRPU	*Strongylocentrotus purpuratus*	Purple sea urchin
TAEGU	*Taeniopygia guttata*	Zebra finch
TETNG	*Tetraodon nigroviridis*	Green puffer
TRIAD	*Trichoplax adhaerens*	-
TRICA	*Tribolium castaneum*	Red flour beetle
XENLA	*Xenopus laevis*	African clawed frog
XENTR	*Xenpus tropicalis*	Western clawed frog

ExPASy codes were from www.expasy.ch/cgi-bin/speclist.

3. Supplementary References

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