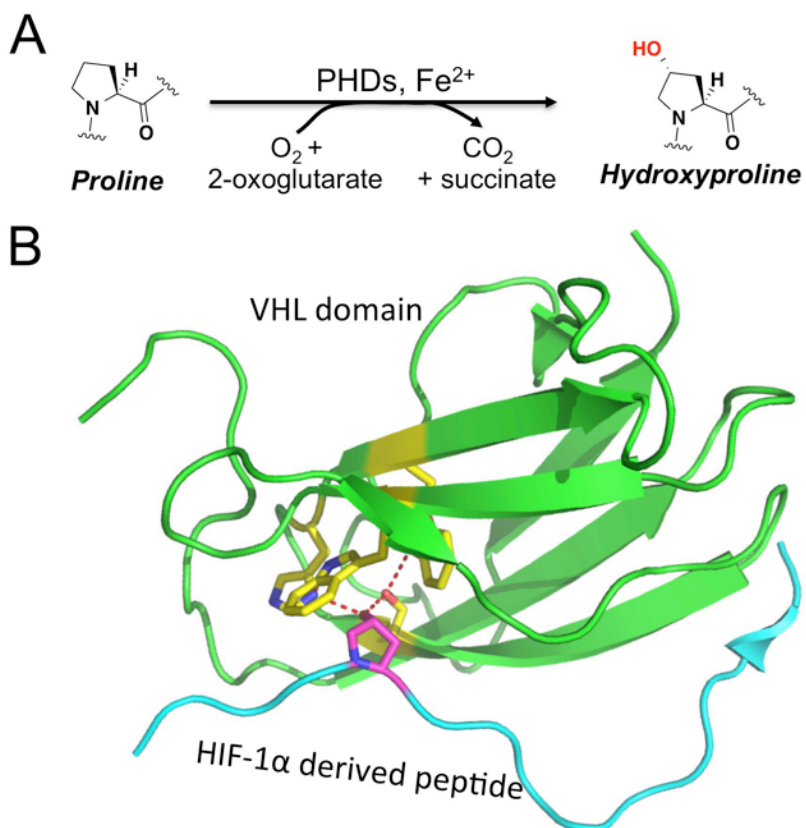


## **Supporting Information**

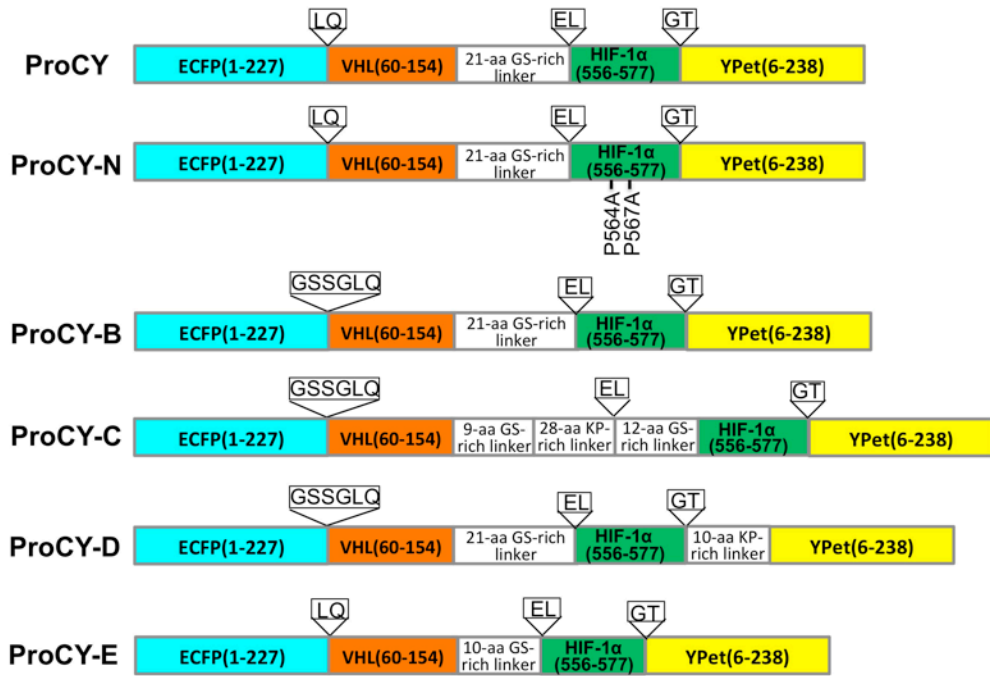
### **A Genetically Encoded FRET Sensor for Hypoxia and Prolyl Hydroxylases**

Suzan Youssef, Wei Ren, and Hui-wang Ai\*

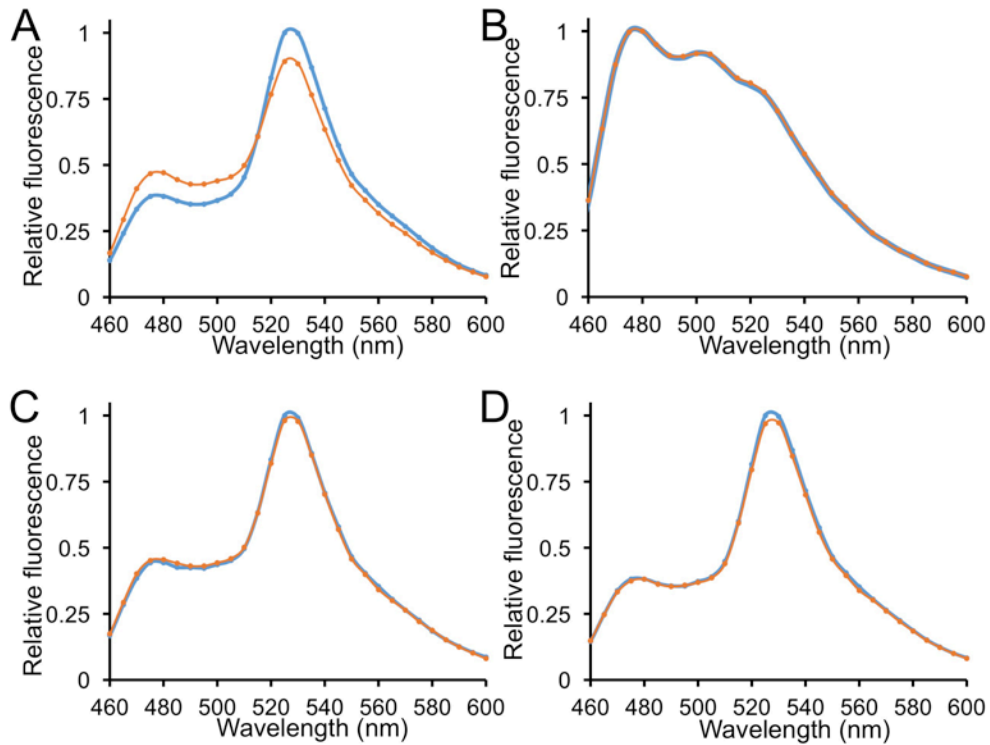
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**Figure S1.** (A) Chemical reaction for conversion of proline into 4-hydroxyproline by prolyl hydroxylase domain enzymes (PHDs). (B) Interactions between a VHL domain (residues 60-154) and a HIF-1 $\alpha$  derived peptide, showing several interfacial H-bonds through the hydroxyl group of 4-hydroxyproline (residue 564). The structure is redrawn based on Protein Data Bank (PDB) entry 4AJY.



**Figure S2.** Domain arrangements of biosensors constructed in this study. Residues are numbered by following PDB entries 2WSN and 4AJY.



**Figure S3.** Fluorescent emission spectra for (A) ProCY-B, (B) ProCY-C, (C) ProCY-D, and (D) ProCY-E before (blue) and after (orange) treatment with a catalytically active PHD2 fragment.

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ProCY      MRGSHHHHHHGMASMTGGQQMGRDLYENLYFQGSMSVSKGEELFTGVVPILEVELDGDVNG
ProCY-N    MRGSHHHHHHGMASMTGGQQMGRDLYENLYFQGSMSVSKGEELFTGVVPILEVELDGDVNG
ProCY-B    MRGSHHHHHHGMASMTGGQQMGRDLYENLYFQGSMSVSKGEELFTGVVPILEVELDGDVNG
ProCY-C    MRGSHHHHHHGMASMTGGQQMGRDLYENLYFQGSMSVSKGEELFTGVVPILEVELDGDVNG
ProCY-D    MRGSHHHHHHGMASMTGGQQMGRDLYENLYFQGSMSVSKGEELFTGVVPILEVELDGDVNG
ProCY-E    MRGSHHHHHHGMASMTGGQQMGRDLYENLYFQGSMSVSKGEELFTGVVPILEVELDGDVNG

ProCY      HRFSVSGEGEGDATYGKLTLLKFICTTGKLPVPWPTLVTTLTWGVQCFSRYPDHMKQHDFD
ProCY-N    HRFSVSGEGEGDATYGKLTLLKFICTTGKLPVPWPTLVTTLTWGVQCFSRYPDHMKQHDFD
ProCY-B    HRFSVSGEGEGDATYGKLTLLKFICTTGKLPVPWPTLVTTLTWGVQCFSRYPDHMKQHDFD
ProCY-C    HRFSVSGEGEGDATYGKLTLLKFICTTGKLPVPWPTLVTTLTWGVQCFSRYPDHMKQHDFD
ProCY-D    HRFSVSGEGEGDATYGKLTLLKFICTTGKLPVPWPTLVTTLTWGVQCFSRYPDHMKQHDFD
ProCY-E    HRFSVSGEGEGDATYGKLTLLKFICTTGKLPVPWPTLVTTLTWGVQCFSRYPDHMKQHDFD

ProCY      KSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGI DFKEDGNILGHKLEYN
ProCY-N    KSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGI DFKEDGNILGHKLEYN
ProCY-B    KSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGI DFKEDGNILGHKLEYN
ProCY-C    KSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGI DFKEDGNILGHKLEYN
ProCY-D    KSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGI DFKEDGNILGHKLEYN
ProCY-E    KSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGI DFKEDGNILGHKLEYN

ProCY      YISHNVYITADKQKNGIKAHFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSLSTQ
ProCY-N    YISHNVYITADKQKNGIKAHFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSLSTQ
ProCY-B    YISHNVYITADKQKNGIKAHFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSLSTQ
ProCY-C    YISHNVYITADKQKNGIKAHFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSLSTQ
ProCY-D    YISHNVYITADKQKNGIKAHFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSLSTQ
ProCY-E    YISHNVYITADKQKNGIKAHFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSLSTQ

ProCY      SALS KDPNEKRDMVLEFVTA----LQRPVLRVNSREPSQVIFCNRSRPRVLPVWLN
ProCY-N    SALS KDPNEKRDMVLEFVTA----LQRPVLRVNSREPSQVIFCNRSRPRVLPVWLN
ProCY-B    SALS KDPNEKRDMVLEFVTAAGGSGLQRPVLRVNSREPSQVIFCNRSRPRVLPVWLN
ProCY-C    SALS KDPNEKRDMVLEFVTAAGGSGLQRPVLRVNSREPSQVIFCNRSRPRVLPVWLN
ProCY-D    SALS KDPNEKRDMVLEFVTAAGGSGLQRPVLRVNSREPSQVIFCNRSRPRVLPVWLN
ProCY-E    SALS KDPNEKRDMVLEFVTA----LQRPVLRVNSREPSQVIFCNRSRPRVLPVWLN

ProCY      FDGEPQPYPTLPPGTGRRISHSYRGLWLF RDAGTHDGLLVNQTELFVPSLNVDGQPIFAN
ProCY-N    FDGEPQPYPTLPPGTGRRISHSYRGLWLF RDAGTHDGLLVNQTELFVPSLNVDGQPIFAN
ProCY-B    FDGEPQPYPTLPPGTGRRISHSYRGLWLF RDAGTHDGLLVNQTELFVPSLNVDGQPIFAN
ProCY-C    FDGEPQPYPTLPPGTGRRISHSYRGLWLF RDAGTHDGLLVNQTELFVPSLNVDGQPIFAN
ProCY-D    FDGEPQPYPTLPPGTGRRISHSYRGLWLF RDAGTHDGLLVNQTELFVPSLNVDGQPIFAN
ProCY-E    FDGEPQPYPTLPPGTGRRISHSYRGLWLF RDAGTHDGLLVNQTELFVPSLNVDGQPIFAN

ProCY      ITLPGSAGSSAGG-----SAGGSAGSAGGELDL DLEM
ProCY-N    ITLPGSAGSSAGG-----SAGGSAGSAGGELDL DLEM
ProCY-B    ITLPGSAGSSAGG-----SAGGSAGSAGGELDL DLEM
ProCY-C    ITLPGSAGSSAGGKPKPKPKPKPKELPKPKPKPKPKPKPAGGSAGSAGGKLDLDLEM
ProCY-D    ITLPGSAGSSAGG-----SAGGSAGSAGGELDL DLEM
ProCY-E    ITLPGSAGSSAG-----GSELDLDLEM

ProCY      LAPIYIMDDDFQLRSFGT-----ELFTGVVPILEVELDGDVNGHKFVSVEGEGDA
ProCY-N    LAAYIAMDDDFQLRSFGT-----ELFTGVVPILEVELDGDVNGHKFVSVEGEGDA
ProCY-B    LAPIYIMDDDFQLRSFGT-----ELFTGVVPILEVELDGDVNGHKFVSVEGEGDA
ProCY-C    LAPIYIMDDDFQLRSFGT-----ELFTGVVPILEVELDGDVNGHKFVSVEGEGDA
ProCY-D    LAPIYIMDDDFQLRSFGTKPKPKPKPKPELFTGVVPILEVELDGDVNGHKFVSVEGEGDA
ProCY-E    LAPIYIMDDDFQLRSFGT-----ELFTGVVPILEVELDGDVNGHKFVSVEGEGDA

ProCY      TYGKLTLLKLLCTTGKLPVPWPTLVTTTLGYGVQCFARYPDHMKQHDFFKSAMPEGYVQERT
ProCY-N    TYGKLTLLKLLCTTGKLPVPWPTLVTTTLGYGVQCFARYPDHMKQHDFFKSAMPEGYVQERT
ProCY-B    TYGKLTLLKLLCTTGKLPVPWPTLVTTTLGYGVQCFARYPDHMKQHDFFKSAMPEGYVQERT
ProCY-C    TYGKLTLLKLLCTTGKLPVPWPTLVTTTLGYGVQCFARYPDHMKQHDFFKSAMPEGYVQERT
ProCY-D    TYGKLTLLKLLCTTGKLPVPWPTLVTTTLGYGVQCFARYPDHMKQHDFFKSAMPEGYVQERT
ProCY-E    TYGKLTLLKLLCTTGKLPVPWPTLVTTTLGYGVQCFARYPDHMKQHDFFKSAMPEGYVQERT

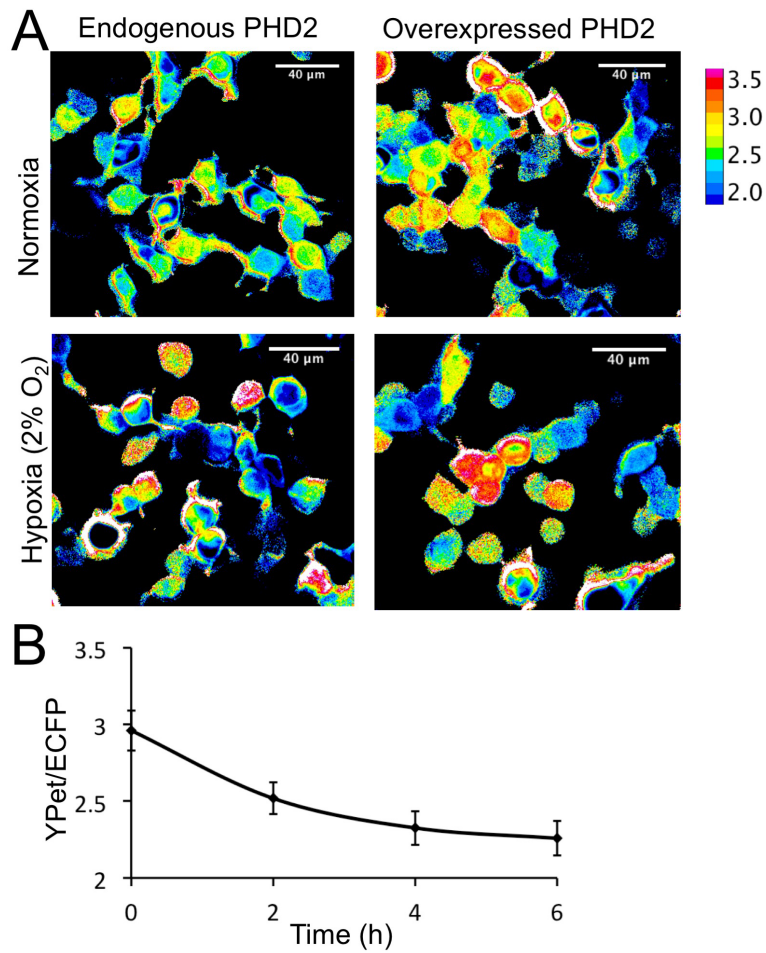
ProCY      IFFKDDGNYKTRAEVKFEGDTLVNRIELKGI DFKEDGNILGHKLEYNYNHNVYITADKQ
ProCY-N    IFFKDDGNYKTRAEVKFEGDTLVNRIELKGI DFKEDGNILGHKLEYNYNHNVYITADKQ
ProCY-B    IFFKDDGNYKTRAEVKFEGDTLVNRIELKGI DFKEDGNILGHKLEYNYNHNVYITADKQ
ProCY-C    IFFKDDGNYKTRAEVKFEGDTLVNRIELKGI DFKEDGNILGHKLEYNYNHNVYITADKQ
ProCY-D    IFFKDDGNYKTRAEVKFEGDTLVNRIELKGI DFKEDGNILGHKLEYNYNHNVYITADKQ
ProCY-E    IFFKDDGNYKTRAEVKFEGDTLVNRIELKGI DFKEDGNILGHKLEYNYNHNVYITADKQ

ProCY      KNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALFKDPNEKRKH
ProCY-N    KNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALFKDPNEKRKH
ProCY-B    KNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALFKDPNEKRKH
ProCY-C    KNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALFKDPNEKRKH
ProCY-D    KNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALFKDPNEKRKH
ProCY-E    KNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALFKDPNEKRKH

ProCY      MVLLEFLTAAGITEGMNELYKEF-
ProCY-N    MVLLEFLTAAGITEGMNELYKEF-
ProCY-B    MVLLEFLTAAGITEGMNELYKEF-
ProCY-C    MVLLEFLTAAGITEGMNELYKEF-
ProCY-D    MVLLEFLTAAGITEGMNELYKEF-
ProCY-E    MVLLEFLTAAGITEGMNELYKEF-

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**Figure S4.** Alignment of protein sequences of biosensors constructed in this study.



**Figure S5.** (A) Pseudocolored ratio images of representative ProCY-N expressing HEK 293T cells under normoxia or hypoxia. The color bar represents the ratio of sensitized YPet fluorescence emission to direct ECFP donor emission (YPet/ECFP). (B) The time-course of FRET ratios of ProCY expressed in HEK 293 under hypoxia (2% O<sub>2</sub>) in response to re-oxygenation under normoxic conditions.

**Table S1: Oligonucleotides used in this study.**

Oligo name	Nucleotide sequence
YPet-For	GATTGGTACCGAGTTATTCAGTGGTGT
YPet-Rev	TTCTAAGCTTAGAATTCTTTGTACAATTCATT
ECFP-For	AGGGCTCGAGCATGGTGAGCAAGGGCGA
PVHL-Rev	CAGCACTGGGCGCTGCAGGGCGGCGGTACGAACTC
Pst1-For	CCGCCCTGCAGCGCCAGTGCTGCGCAGC
Kpn1-Rev1	GGGGATGTATGGAGCCAACATCTCTAAATCAAGGTCGAGCTCACCACCTG CTGAACCTGC
Kpn-Rev2	ACTCGGTACCAAACTGCGAAGCTGGAAGTCATCATCCATGGGGATGTAT GGAGCCAA
Kpn-Rev3	ACCACCTGCTGAACCTGCTCCGCTACCACCGGCAGAGCCACC
GGSG-For	GGAGGCAGCGGACGCCAGTGCTGCGCAGC
GGSG-Rev	GCGTCCGCTGCCTCCGGCGGCGGTACGAACTC
MidFloppy-R	GAATGAGCTCTTTAGGCTTAGGTTGGGCTTCGGCTTTGGTTGCCACCTG CGCTTGA
MidFloppy-F1	CTTAGAGCTCCCCAAGCCAAAACCAAGCCCAAGCCGAAGCCAAAGCCG AAGCCC
MidFloppy-F2	CCAAAGCCGAAGCCCGCCGGTGGTAGCGGAGCAGGTTACAGCAGGTGGTA AGCTC
pBAD-Rev	GATTTAATCTGTATCAGG
HIF-Rigid-F	ATTAGGTACCAAGCCCAAGCCGAAGCCAAAGCCGAAGCCCGAGTTATTC ACTGGTGT
SacI-Rev	GGTCGAGCTCAGAGCCACCTGCGCT
PAPA-Rev	ACTCGGTACCAAACTGCGAAGCTGGAAGTCATCATCCATTGCGATGTAA GCAGCCAA
PHD2-Kpn1-F	CGTGGGTACCATGCCCAACGGGCAGACGAAG
PHD2-HindIII-R	AAGCGAAGCTTAGAAGACGTCTTTACCGAC
HydpcDNA3-F	CGCCTAAGCTTGCCGCCACCATGGTGAGCAAGGGCGAG
HydpcDNA3-R	ACCTTCTAGATTAGAATTCTTTGTACAATTCATT
PHD2-pcDNA3-F	TCGCCTAAGCTTGCCGCCACCATGGGTCCCAACGGGCAGACGAAG
PHD2-pcDNA3-R	GATCTCTAGATTAGAAGACGTCTTTACCGACCGA
mycPHD2-R	CCCTCTAGATTACAGATCCTCTTCTGAGATGAGTTTTTGTTCGAAGACGTC TTTACCGAC
NES-HP-F1	CCCAAGCTTGCCGCCACCATGGCACTTCAACTCCTCCTTGAACG
NES-HP-F2	CAACTTCCTCCTTTGAACGTCTTACTCTTGTGAGCAAGGGCGAGGAG
NES-HP-XbaI-R	GCATTCTAGAGAATTCTTTGTACAATTCATT
PLJMI-NheI-F	TCCGCTAGCGCCGCCACCATGGGAGCAAGGGCGAGG
pHP-Nuc-R	TCAGCTCGAGAGAACTCTTTGTACAATTCATT