Electronic Supplementary Material (ESI) for Chemical Science.

Supporting Information

Non-competitive cyclic peptides for targeting enzyme-substrate complexes

T. E. McAllister[a], T.-L Yeh[a], M. I. Abboud[a], I. K. H. Leung[a,b], E. S. Hookway[c], O. N. F. King[a], B. Bhushan[a,d], S. T. Williams[a], R. J. Hopkinson[a], M. Münzel[a], N. D. Loik[e], R. Chowdhury[a], U. Oppermann[c], T. D. W. Claridge[a], Y. Goto[e], H. Suga[e,f], C. J. Schofield[a], & A. Kawamura[a,d]

Affiliations:
[a]*Department of Chemistry, University of Oxford, Chemistry Research Laboratory, 12 Mansfield Road, Oxford OX1 3TA, United Kingdom*
[b]*Current address: School of Chemical Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*
[c]*Botnar Research Centre, NIHR Oxford Biomedical Research Unit, University of Oxford, Windmill Road, Oxford, OX3 7LD, United Kingdom.*
[d]*Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford OX3 7BN*
[e]*Department of Chemistry, Graduate School of Science, The University of Tokyo, Tokyo 113-0033*
[f]*JST, CREST, The University of Tokyo, Tokyo 113-0033, Japan*

Correspondence:
Akane Kawamura, akane.kawamura@chem.ox.ac.uk

Supplementary Methods.

Production of recombinant proteins

Production of truncated PHD2. Truncated PHD2 (tPHD2, residues 181-426) used for activity assays was prepared as described[1, 2]. For tPHD2$_{\mathrm{HisBio}}$, DNA encoding for tPHD2 (181-426) was cloned into pNIC-Bio3 plasmid which produces protein with an *N*-terminal His$_6$-tag and *C*-terminal Avi-tag. The resultant construct was then transformed into BL21 (DE3) Rosetta with pCDF-BirA[3]. Cells were grown in 2TY media supplemented with kanamycin (50 μg/mL) and spectinomycin (50 μg/mL) at 37 °C with shaking (180 rpm), and induced at OD$_{600}$ at approximately 0.8 with isopropyl-β-D-1-thiogalactopyranoside (IPTG, 0.5 mM) and supplemented with biotin (0.1 mM) simultaneously. The cells were grown for 16 hrs at 18 °C, and then harvested. After cell lysis using sonication, tPHD2$_{\mathrm{HisBio}}$ was purified from cell lysates using nickel affinity chromatography and imidazole gradient as described[3]. The protein was then exchanged into 50 mM Tris-HCl, pH 7.5, 100 mM NaCl using a PD10 desalting column (GE Healthcare). Biotinylation was confirmed using ESI-MS (Waters Micromass LCT Premier). Enzyme activity was confirmed in an assay utilizing MALDI TOF MS to monitor hydroxylation of a HIF-1α substrate peptide[4].

For NMR experiments, isotopically labelled ^{15}N-tPHD2$_{181\text{–}402}$ was produced in *E. coli* BL21(DE3)[5]. Cells were grown at 37 °C (to an OD$_{600}$ of 0.8) in M9 minimal media supplemented with 1 g/L ^{15}N-labelled NH_4Cl and 10 g/L D-glucose. Protein production was induced with 0.2 mM isopropyl β-D-1-thiogalactopyranoside (16 h, 28 °C). *Apo*-PHD2$_{181\text{–}402}$ was produced by incubation (1 mg/mL protein) with EDTA (0.2 M) in 15 mM ammonium acetate (pH 7.5) for 16 h at 4 °C. Prior to the NMR experiments, protein samples were buffer exchanged in 50 mM Tris-D$_{11}$, pH 6.6, containing 0.02 % NaN_3, using PD-10 columns.

Production of full-length PHD1-3. Full length PHD1/2 genes were cloned into pFastbac vector (Life technologies) for the production of proteins with tobacco etch virus (TEV) cleavable *N*-terminal histidine tags in *Spodoptera frugiperda* (Sf9). Recombinant baculoviruses were produced following manufacturer´s instructions (Life technologies). Sf9 insect cells were grown in suspension in Insect Xpress (Lonza) supplemented with penicillin/ streptomycin. Cells were infected with recombinant baculoviruses at low multiplicity of infection (MOI, 1ml of virus stock/1L of culture). 72 hpi (hours post insemination) cultures were collected and cells were pelleted by centrifugation (2000 rpm, 30 min, 4 °C) and washed with cold PBS. A construct encoding for full-length PHD3 was cloned into the pET-28a(+) vector for the production of PHD3 with an *N*-terminal His$_6$-tag in *E. coli* BL21(DE3)[6]. Cells were induced with 0.5 mM IPTG for 6 hours at 37 °C before being pelleted by centrifugation. All cells were freeze-thawed and resuspended in binding buffer (20 mM Tris·HCl pH 7.0-7.5, 0.5 M NaCl, 5% glycerol (*v/v*) and 0.5 mM Tris(2-carboxyethyl)phosphine (TCEP)), supplemented with protease inhibitors. Cells were then lyzed by sonication. The proteins were purified by Ni^{2+} affinity followed by size exclusion chromatography (50 mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol and 0.5 mM TCEP). Protein purity was assessed by SDS-PAGE; proteins were characterized by MS analyses under denaturing conditions.

Recombinant FIH[7] and KDM4A[8] were produced as described.

Screening of tPHD2 using RaPID system

In brief, translation of the first round selection was performed using mRNA-puromycin (156 pmol) and 150 μL of translation mixture at 37 °C for 30 min followed by room temperature incubation at 12 min. EDTA (200mM, pH 7.5, 15 μL) was added and incubated at 37 °C for further 30 min. The library was buffer exchanged with the Selection Buffer [50 mM Tris-HCl (pH 7.6), 150 mM NaCl, 0.05% (*v/v*) tween 20] by gel filtration. The resultant peptide library was applied to tPHD2-immobilized magnetic beads (Dynabeads® M-280 Streptavidin (Life-technologies), loaded at 4 pmol / μL loading), and incubated at 4 °C for 30 min. After the supernatant was removed, the bead was washed 3 times with 300 μL of cold selection buffer. RT reaction buffer I [50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl2, 10 mM DTT, 0.5 mM dNTPs, 2 μM CGS3an13.R39] (40 μL) was added to the beads containing

200 units of M-MLV reverse transcriptase (Promega) and 8 units of RNase inhibitor (Promega), and reverse-transcribed at 42 °C for 60 min. The collected cDNA was eluted with 800 µL of PCR buffer [10 mM Tris-HCl (pH 7.5), 50 mM KCl, 0.1% Triton X-100, 2.5 mM MgCl2, 0.25 mM dNTPs, 0.25 µM T7g10M.F48, 0.25 µM CGS3an13.R39 (see **Supplementary Table 4** for primer sequences)] at 95 °C for 5 min. After addition of Taq DNA polymerase to the eluate, the mixture was used for PCR amplification. The amplified DNA was purified by phenol/chloroform extraction followed by ethanol purification. The resultant DNA library was transcribed in vitro, ligated with the puromycin linker, and used for the next round of selection. For the 2nd-5th rounds of selection, small-scale (5 µL in 2nd, 2.5 µL in 3rd-5th) translation reactions were carried out. Reverse transcription of the mRNAs fused on peptides was buffer exchanged with selection buffer by gel-filteration. The resultant peptide library was subjected to biotin-bound dynabeads magnetic beads at 4 °C for 30 min, and then the supernatant was recovered (negative selection). After repeating the negative selection step 3 times, the recovered peptide solution was mixed with tPHD2-immobilized magnetic beads (4 pmol tPHD2 / µL loading), and incubated at 4 °C for 30 min. After the supernatant was removed, the beads were washed 3 times with 60 µL of cooled selection buffer. The collected cDNA-mRNA-peptide fusion was recovered with 100 µL of PCR buffer by incubation at 95 °C for 5 min and then amplified by Taq DNA polymerase. The amplified DNA was purified by phenol/chloroform extraction followed by ethanol purification. The resulting DNA library was transcribed in vitro, ligated with the puromycin linker, and used for the next round of selection. After the 5th round of the selection, the resulting cDNA library was cloned into the pGEM-T Easy Vector (Promega) and the individual clones were sequenced.

Illumina Sequencing

After sequencing, the variable region of each amplicon was extracted by removing the constant region and sequencing adaptors from reads using Cutadapt (version 1.8.1)[9]. The forward and reverse reads for each variable region were compared and non-identical reads were discarded in order to reduce downstream error due to sequencing errors. The resulting validated variable region sequences were tabulated using R and the resultant peptide determined using the Biostrings package[10].

Chemical synthesis of selected macrocyclic peptides

CP linear precursors were prepared with an amidated '*C*-terminus' by standard solid phase synthesis on a CS Bio CS336X peptide synthesizer on Rink Amide MBHA resin using N,N′-diisopropylcarbodiimide (DIC) as coupling reagent. After cleavage of the *N*-terminal Fmoc-protecting group a solution of 150 mg of chloroacetic acid succinimidyl ester in 4 mL DMF was added to the resin and the mixture shaken for 3 h. The resin was filtered off, washed and then subsequently treated with 4 mL of deprotection solution (95 % (v/v) CF_3CO_2H (TFA), 2.5 % (v/v) triisopropylsilane, 2.5 % (v/v) water). After 3 h the volume was reduced to 1 mL under a nitrogen stream and the peptides were precipitated with cold Et_2O. The mixture was centrifuged and the supernatant discarded. The solid was taken up in 1.5 mL of triethylammoniumacetate buffer (1 M, pH 8.5) and the pH readjusted to > 8 if necessary. In a microwave (Biotage Initiator) the mixture was heated to 80 °C for 10 min and subsequently purified by HPLC (0-45 % MeCN in 45 min, 0.1 % TFA, Dionex Ultimate 3000 series, Grace Vydac 218TP101522 column).[8]

Alternatively, peptides were prepared using a CEM Liberty Blue peptide synthesizer using Rink Amide MBHA resin with DIC as coupling reagent. After cleavage of the *N*-terminal Fmoc-protecting group, a solution of 150 mg chloroacetic anhydride in 5 mL dimethylformamide ($(CH_3)_2NCH$, DMF) was added and incubated for 40 min at room temperature. The resin was filtered, washed with DMF (3 × 10 mL) and dichloromethane (CH_2Cl_2, DCM) (3 × 10 mL), dried and cleaved from the resin with 5 mL of deprotection solution (92.5 % TFA, 2.5 % triisopropylsilane, 2.5 % water, 2.5% 1,3-dimethoxybenzene v/v) for 3 h. The cleaved peptide was precipitated with cold Et_2O, dissolved in 10 mL of water/acetonitrile (1:1), and lyophilised. The dried linear peptides were dissolved in 6 mL of a 1:1 solution of acetonitrile/1M Triethylammonium acetate (pH 8.5), and transferred to microwave vials (0.5 - 2 mL, glass vials). The contents were incubated at 80 °C for 10 minutes in a microwave synthesiser (Biotage Initiator) with stirring to induce cyclisation. The peptide solution was acidified using TFA, then purified by reverse-phase HPLC as described above. Peptide concentrations were determined using

^{1}H NMR and comparing the integrals of characteristic peaks in comparison with an internal standard of 3-(trimethylsilyl)propionic-2,2,3,3-d4 acid.

Strain promoted Azide-Alkyne cycloaddition

One equivalent of the azide-containing peptide (3CAz) was mixed with 1.5 eq of N-[(1R,8S,9S)-Bicyclo[6.1.0]non-4-yn-9-ylmethyloxycarbonyl]-N′-biotinyl-1,8-diamino-3,6-dioxaoctane (biotin-BCN) in 1:1 H2O/MeCN and shaken at 37 °C for 5h. The desired product was obtained in high yield (>90%) and the purified by HPLC purification as above.

Biolayer interferometry

Experiments were performed using an OctetRed 384 (ForteBio) in BLI buffer (50 mM Tris-HCl (pH 7.5 at 22 °C), 200 mM NaCl, 0.05% (*v*/*v*) Tween-20 in some instances supplemented with 0.1% (*w*/*v*) BSA), at 25 °C. Protein was diluted into BLI buffer supplemented with 5-fold molar excess of $ZnSO_4$ and 10-fold excess of 2-OG was loaded onto biosensors using a 1μM solution for Ni-NTA (Ni-nitrilotriacetic acid functionalised) biosensors and 50-100 nM for SA (streptavidin-functionalised) biosensors. The sensors were then subjected to a cycle of association/dissociation with different concentrations of peptides in BLI buffer + 1% DMSO. SA biosensors were additionally subjected to a 60 second blocking step with 1% (*w*/*v*) BSA in BLI buffer. Ni-NTA biosensors were regenerated as described in ForteBio Technical Note #31 and reused up to 10 times. Experiments with unfunctionalized SA biosensors to establish background binding to the sensors themselves showed negligible binding of peptides across the concentration ranges used. Higher peptide concentrations displaying complex binding curves and lower concentrations with insufficient signal magnitudes were excluded from fitting. Unless otherwise stated, all values are derived from fitting the kinetic data for the experiment – in some instances (for low affinity interactions) a steady-state approximation was used instead. Supplementary Figs. 2, 3 and 4 show all the traces used for the fitting and concentrations used in each experiment are indicated. 3-fold dilution series of 3C and 4C with highest concentrations indicated were used in the binding experiment with KDM4A in figure 3A/3B.

PHD2 enzyme activity assays

MALDI-TOF MS inhibition assays: Compounds (cyclic peptides/NOG) or DMSO-only controls were pre-incubated with enzyme (2 μM) for 20 min in 2% (*v/v*) DMSO at room temperature. The reaction was initiated by addition of an equal volume of a solution containing all other assay components to give final concentrations of: tPHD2 (1 μM), CODD^{mut} (H-DLDLEALAPYIPADDDFQL-OH; 50 μM), 2-oxoglutarate (10 μM), Na-ascorbate (100 μM), $(NH_4)_2Fe(SO_4)_2$ (5 μM), HEPES pH 7.5 (50 mM) + 1% (*v/v*) DMSO. The reactions was allowed to proceed for 15 min (ascertained to be within the linear range of the reaction; data not shown) before being quenched with 1 volume of 2% (*v*/*v*) formic acid. Comparisons showed activity equivalent to the 'natural' substrate peptide. Concentrations used were as follows: 1C - 8.3 μM, 3C – 6.1 μM, 4C – 4.5 μM, 5C – 7.9 μM, 6C – 6.4 μM and NOG – 10 μM.

Cyclic peptides as substrates: Reaction components except enzyme were combined and incubated at 37 °C for 5 min before initiating the reaction by addition of enzyme from a stock of 5 × final concentration (also pre-incubated at 37 °C). Final concentrations of assay components were: tPHD2 (2 μM)*, 2-oxoglutarate (300 μM), Na-ascorbate (4 mM), $(NH_4)_2Fe(SO_4)_2$ (50 μM)*, Tris pH 7.5 (50 mM), cyclic peptides*, CODD^{mut} (100 μM)* + 1% (*v/v*) DMSO. Reactions were left for the times outlined in Fig S3, then quenched with 1 volume of 2% (*v*/*v*) formic acid. Concentrations of cyclic peptides used were: 4C – 225 μM, 5C – 395 μM, 6C – 320 μM. *In some reactions these components were omitted – see **Supplementary Fig 5A**

After quenching the resultant solutions were mixed 1:1 (*v*/*v*) with saturated α-cyano-4-hydroxycinammic acid solution (1:1 H_2O/MeCN + 0.1% (*v/v*) TFA), dried and analysed by Matrix Assisted Laser Desorption/Ionization –Time of Flight mass-spectrometry.

NMR experiments

All spectra were acquired at 310 K. For ^1H-^{15}N HSQC experiments, the Bruker sequence *hsqcetfpf3gpsi* was used. The size of the data matrix was 2048 and 512 points in the ^1H and the ^{15}N dimension, respectively. The spectral width was set to 16 ppm (^1H) and 40 ppm (^{15}N), and the centre of the spectrum was set to 4.7 ppm (^1H) and 119 ppm (^{15}N), respectively. $^1J_{NH}$ was set to 90 Hz. ^{15}N decoupling was achieved using the GARP. The relaxation delay was 1 s.[2] Data were processed with Bruker Topspin 3.1 software and analysed with CCPNmr Analysis[11]. Unless otherwise stated, assay mixtures contained 40 µM *apo*-^{15}N-PHD2$_{181\text{-}402}$, 100 µM Zn(II), 100 µM 2-oxoglutarate, 200 µM cyclic peptide (3C or 4C, where necessary), and 200 µM CODD (H-DLDLEMLAPYIPMDDDFQL-OH , where necessary) buffered with 50 mM Tris-D_{11} (Cortectnet), pH 6.6, in 10 % D_2O and 90 % H_2O containing 0.02 % NaN_3.

Cell culture and cell-based assays

U2OS, Hep3B and RCC4 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Sigma Aldrich D6546) supplemented with 10% fetal bovine serum (FBS)(Sigma Aldrich F7524), 2 mM L-glutamine (Sigma Aldrich G7513), 100 U/mL penicillin and 100 µg/mL streptomycin (Sigma Aldrich P0781). Cells were seeded to reach 60-70% confluency prior to compound and hypoxia treatment.

For investigation of the effect of CP treatments on PHD activity in cells, culture media was replaced with media pre-incubated in the targeted oxygen level hypoxia workstation overnight immediately after cells were moved into the hypoxic conditions. For testing CPs as inhibitors of HIF hydroxylases, the cells exposed to compounds at final 1% DMSO and in 20%, 5% or 1% oxygen level for designated times. Cells were harvested in urea/SDS buffer (6.7 M urea, 10 mM Tris-HCl pH 6.8, 10% glycerol, and1% SDS) supplemented with 1 mM dithiothreitol following a phosphate-buffered saline (PBS) rinse. Cell extracts were analyzed with SDS-PAGE and immunoblotted as described below.[12]

Capture assays

Cell pellets were lysed in lysis buffer (20 mM Tris pH 7.4 at 4 °C, 137 mM NaCl, 2 mM EDTA, 10% (*v*/*v*) glycerol, 1% (*v*/*v*) Triton™ X-100) or cell extraction buffer (CEB; Thermo #FNN0011) with the addition of protease inhibitor cocktail (Sigma, #P8340) and 1 mM phenylmethylsulfonyl fluoride) for 30 min on ice with gentle mixing to resuspend every 10 min. The insoluble fraction was then precipitated by centrifugation (15 min, 13,000 × *g*) and the protein supernatant concentration determined by bicinchoninic acid BCA assay (Thermo #23227).

Western Blotting

Proteins were transferred at 320 mA for 25 min at room temperature with an ice-pack, in transfer buffer (10 mM Tris, 100 mM glycine, 10% (*v*/*v*) MeOH, 0.01% (*w*/*v*) SDS). The membrane was blocked using 5% (*w*/*v*) skimmed milk powder in PBST buffer (10 mM Na_2HPO_4, 1.8 mM K_2HPO_4 pH 7.4, 137 mM NaCl, 2.7 mM KCl, 0.1% (*v*/*v*) Tween-20) for 10 min and probed for 1 hr at room temperature or 4 °C overnight with primary antibodies. The membrane was washed PBST (3 × 10 min) before the membrane was incubated for 1 hr with HRP-conjugated secondary antibodies (Dako, #P0447). All antibody solutions contained 5% (*w*/*v*) skimmed milk powder in PBST buffer. The membrane was further washed with PBST (3 × 10 min) and bands visualised using super signal west dura ECL (Thermo #34075) and detected on a ChemiDoc™ (Bio-Rad #1708280).

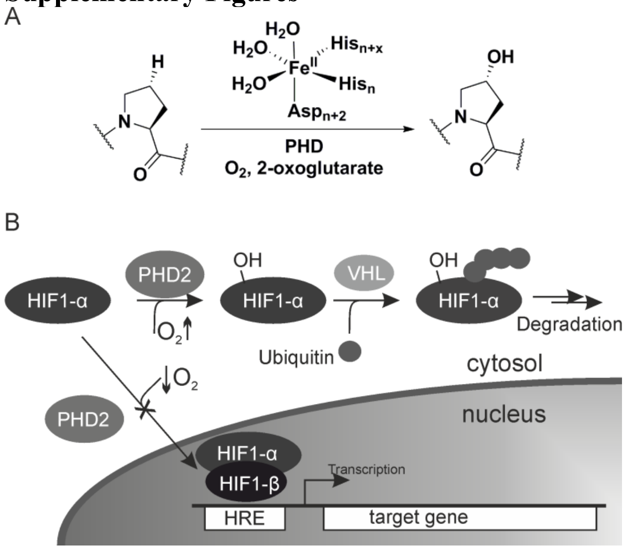
Size-exclusion chromatography/multi-angle laser scattering

Samples were prepared with a tPHD2 concentration of 1 mg/mL, 36 µM in TBS (50 mM Tris pH 7.4 at 4 °C, 200 mM NaCl). To portions of this solution a series of 3C solutions in DMSO were added to give a final DMSO concentration of 1% *v*/*v* and final 3C concentrations of 0 µM, 11 µM, 22 µM and 44 µM. 100 µL of protein solution was injected onto a Superdex 200 HR 10/30 column (GE healthcare) pre-equilibrated with TBS and eluted species monitored using UV, multi-angle light scattering (Wyatt Dawn HELEOS-II 8-angle light scattering detector) and refractive index (Wyatt Optilab rEX refractive index monitor).

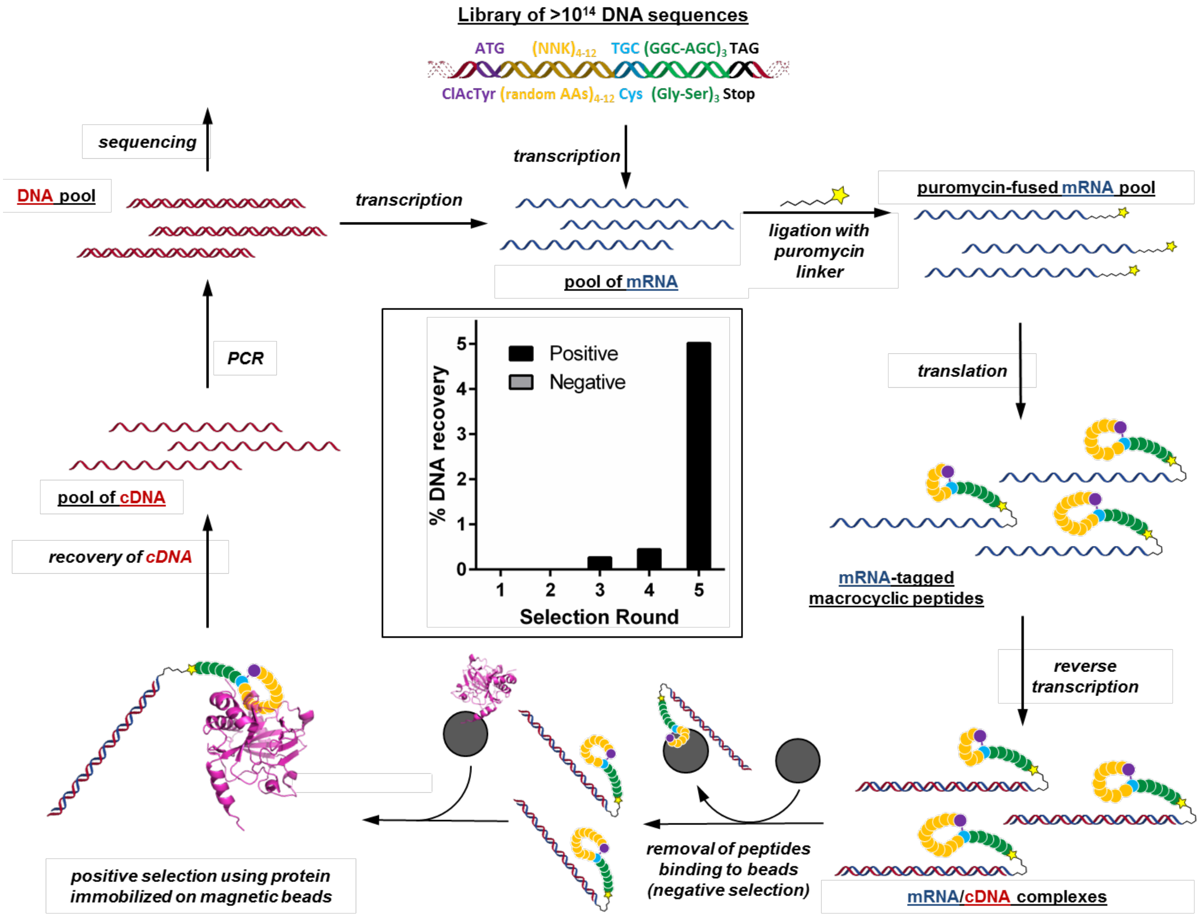
Non-denaturing ESI-MS Experiments

tPHD2 was desalted using a BioSpin 6 column (Bio-Rad, Hemel Hempstead, UK) into 15 mM ammonium acetate (pH 7.5). The stock of protein was then diluted with the same buffer to a final concentration of 100 μM. CODD peptide, 2OG, PHD2_004C and the inhibitor IOX3 (1-chloro-4-hydroxyisoquinoline-3-carbonyl)glycine [13] were also dissolved in 15 mM ammonium acetate (pH 7.5) to a final concentration of 100 μM. Combinations of these components were mixed and data were acquired as previously described [14].

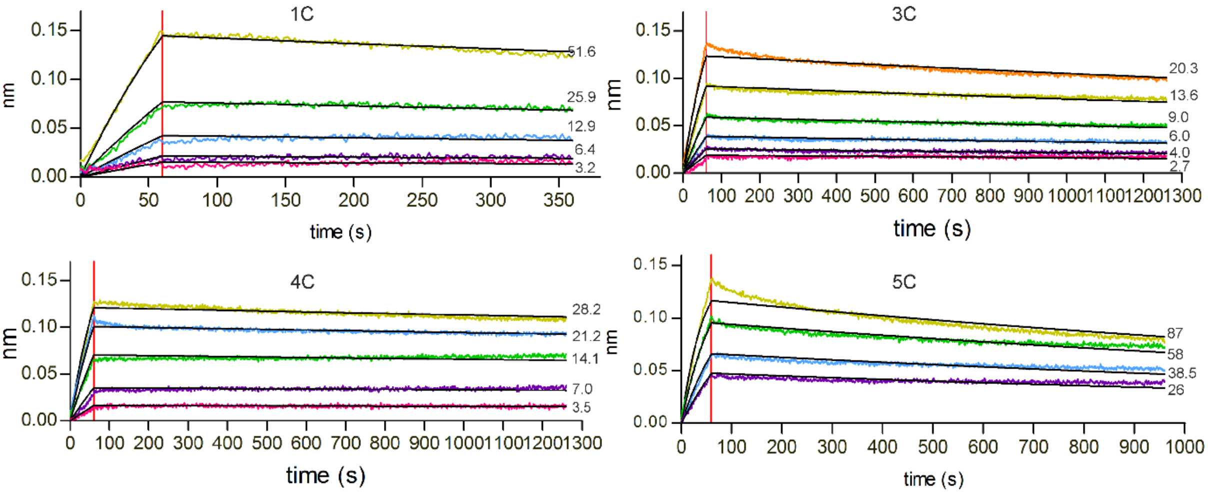
Supplementary Figures

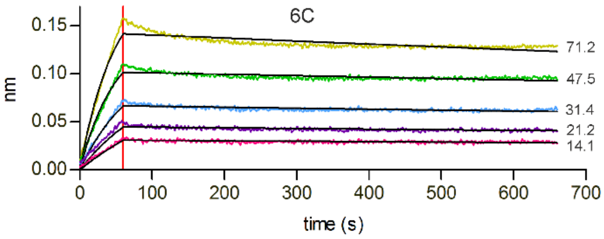


Supplementary Figure 1. The PHD2 reaction. *(A) PHD2 catalyzes hydroxylation of proline residues in HIF1-α using O2, 2OG, and a Fe(II). (B) O2 availability regulates activity of PHD2. In normoxia PHD2 hydroxylates HIF1-α which increases its affinity towards the von Hippel-Lindau protein, an E3 ubiquitin ligase, which marks HIF1-α for degradation by the proteasome. In hypoxia HIF1-α hydroxylation is reduced, leading to HIF1-α accumulation and translocation into the nucleus where it forms an active heterodimer with HIF1-β. This complex can bind to hypoxia response elements and increase transcription of HIF target genes, e.g.erythropoietin.*

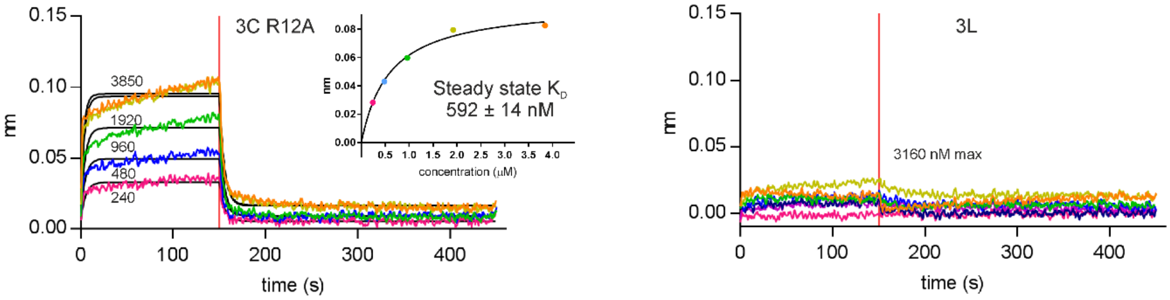


Supplementary Figure 2. Overview of the RaPID selection procedure.
The starting DNA template library (from 5'- to 3'-) consists of a T7 promoter, a GGG triplet, an epsilon sequence, a Shine-Dalgano sequence and a start codon (ATG), followed by 4-12 repeats of NNK codons encoding for all 20 proteinogenic amino acids, a cysteine codon, three glycine-serine repeats then a stop codon.[15] *This library is transcribed into mRNA and ligated to a puromycin-derivatised oligonucleotide, and then used as the template for an in vitro translation reaction. This translation reaction mixture contains 19 proteinogenic amino acids (methionine omitted) and is supplemented with an initiator tRNA acylated with chloroacetyl* D-*tyrosine, allowing reprogramming of the start codon. After the translation step, the peptide is cyclised by an intramolecular reaction between the chloroacetyl group and a C-terminal cysteine. Crucially, the puromycin covalently links the coding mRNA strand to the corresponding translated CPs. Reverse transcription generates mRNA/cDNA-linked CPs and the mixture is incubated with magnetic beads to select for CPs that bind to the immobilized target. After series of washes, the cDNA associated with bound CPs are PCR amplified and sequenced. qPCR is used to measure the proportion of the input DNA recovered during each round. tPHD2$_{HisBio}$ selection recovery is shown (centre).*



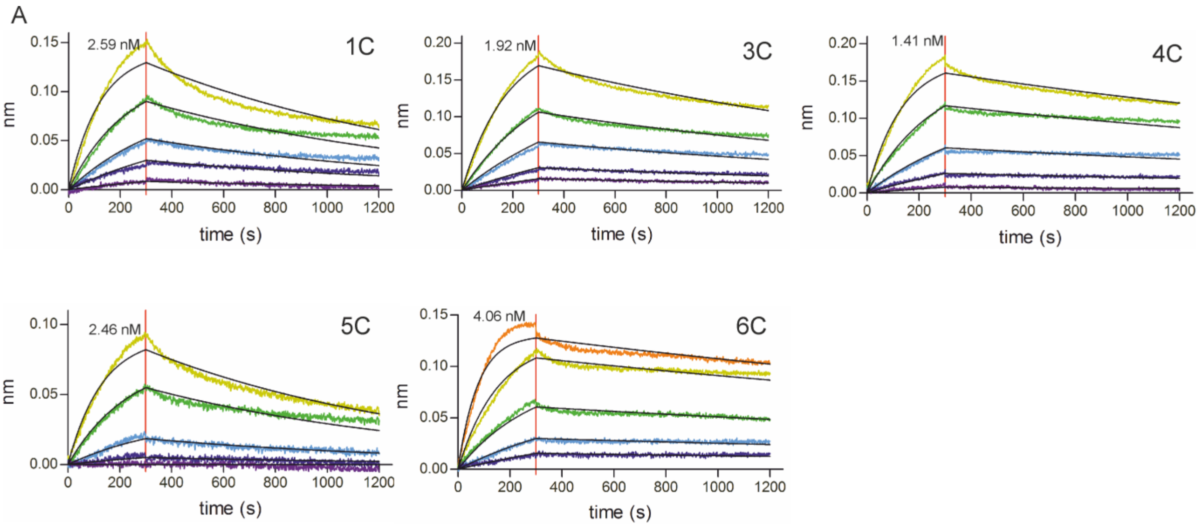


	k_{on} (10^5 M^{-1} s^{-1})	k_{off} (10^{-4} s^{-1})	K_D (nM)	R^2	t½ (h)
1C	1.62 ± 0.05	9.01 ± 0.12	5.51 ± 0.19	0.995	0.21
3C	6.15 ± 0.05	1.70 ± 0.00	0.27 ± 0.01	0.997	1.13
4C	4.23 ± 0.08	0.86 ± 0.01	0.17 ± 0.03	0.996	2.24
5C	2.66 ± 0.08	3.87 ± 0.03	1.46 ± 0.04	0.979	0.50
6C	2.68 ± 0.06	1.95 ± 0.03	0.75 ± 0.02	0.995	0.99
3C R12A	0.94 ± 0.14	737 ± 25	783 ± 90	0.888	0.003
3L	-	-	-	-	-



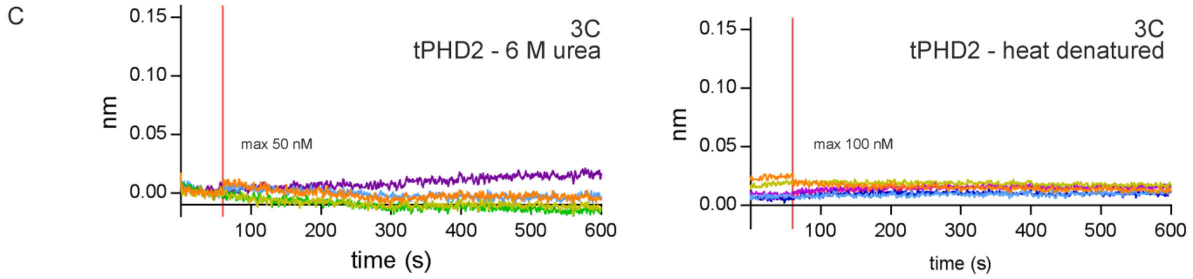
Supplementary Figure 3. Biolayer interferometry data for CP binding to tPHD2$_{HisBio}$ immobilised via C-terminal biotin tag.

*Full traces of data presented in **Figure 3** for different concentrations of CPs (colours) binding to tPHD2 are shown. The data was fitted with one-site binding model shown as black lines. All peptide concentrations are in nM. As 3C R12A binding reached equilibrium, a steady state approximation was also used (inset) which gave a K$_D$ in good agreement with the kinetic fit. Dissociation half-life ($t_{1/2} = 0.693 / k_{off}$)*[16]



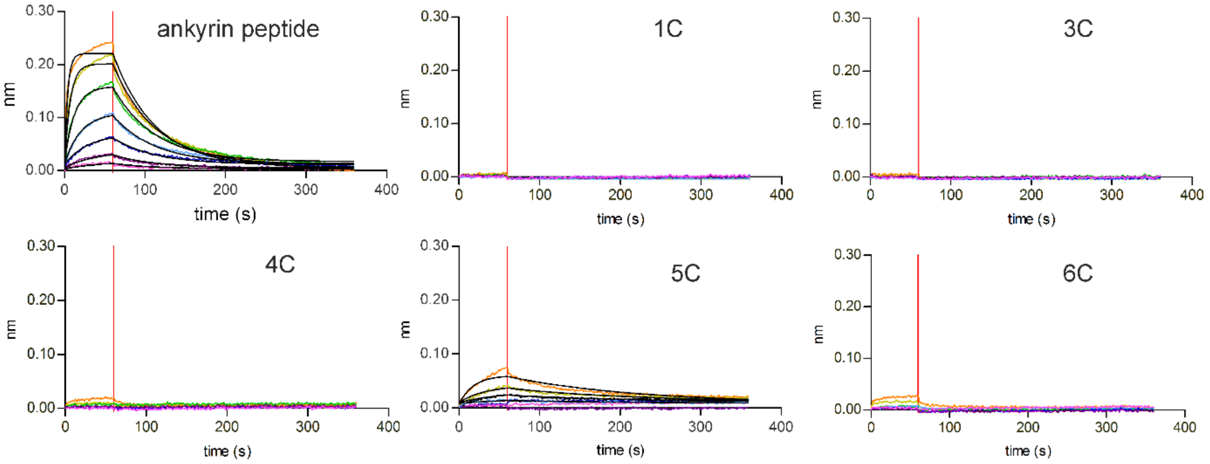
B

	k_{on} (10^6 M^{-1} s^{-1})	k_{off} (10^{-4} s^{-1})	K_D (pM)	R^2
1C	2.88 ± 0.04	8.32 ± 0.05	288 ± 6	0.980
3C	2.77 ± 0.02	4.97 ± 0.02	180 ± 3	0.995
4C	5.19 ± 0.01	3.21 ± 0.03	62 ± 2	0.994
5C	2.75 ± 0.03	9.10 ± 0.05	331 ± 6	0.988
6C	3.21 ± 0.02	2.46 ± 0.03	78 ± 2	0.992



Supplementary Figure 4. Biolayer interferometry data for CP binding to tPHD2$_{HisBio}$ immobilised via an N-terminal His-tag.

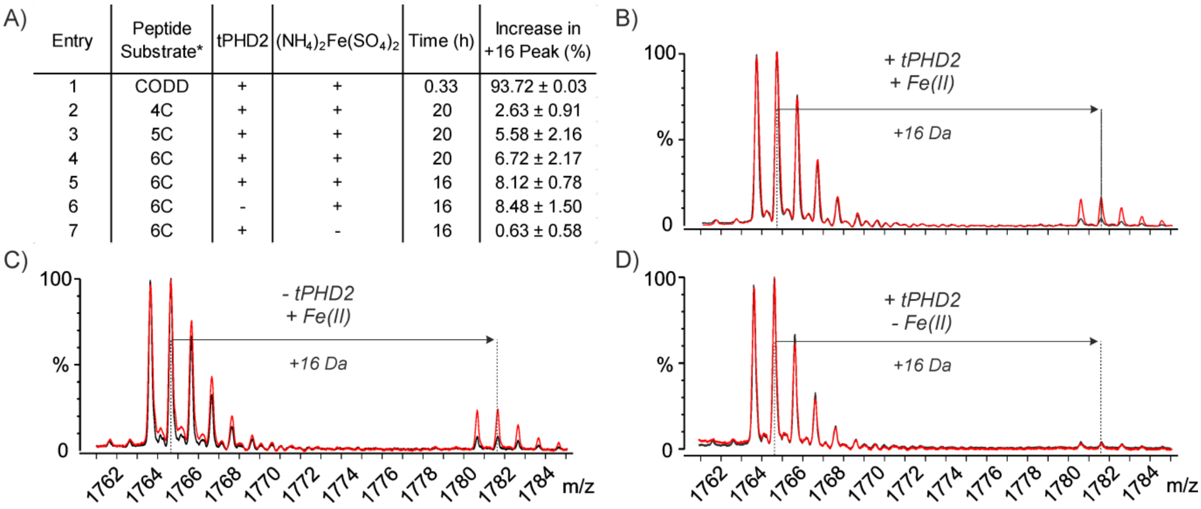
(A) *BLI traces and kinetic parameters as determined by global fitting of the data for CPs; the top concentration for a 2-fold dilution series is indicated, with fitted data from a one-site binding model shown as black lines.* ***(B)*** *Global fitting kinetic data of all CPs from the selection. ± represents the standard error.* ***(C)*** *3C showed no detectable binding response with tPHD2 denatured chemically or thermally.*



	k_{on} (10^4 M^{-1} s^{-1})	k_{off} (10^{-4} s^{-1})	K_D (nM)	R^2
Ankyrin	2.42 ± 0.02	191 ± 0.5	788 ± 6	0.992
1C	-	-	-	-
3C	-	-	-	-
4C	-	-	-	-
5C	51.4 ± 1.5	60.5 ± 1.0	11.8 ± 0.5	0.894
6C	-	-	-	-

Supplementary Figure 5. Biolayer interferometry traces of consensus substrate ankyrin peptide and cyclic peptide binding to FIH immobilized via N-terminal His-tag.

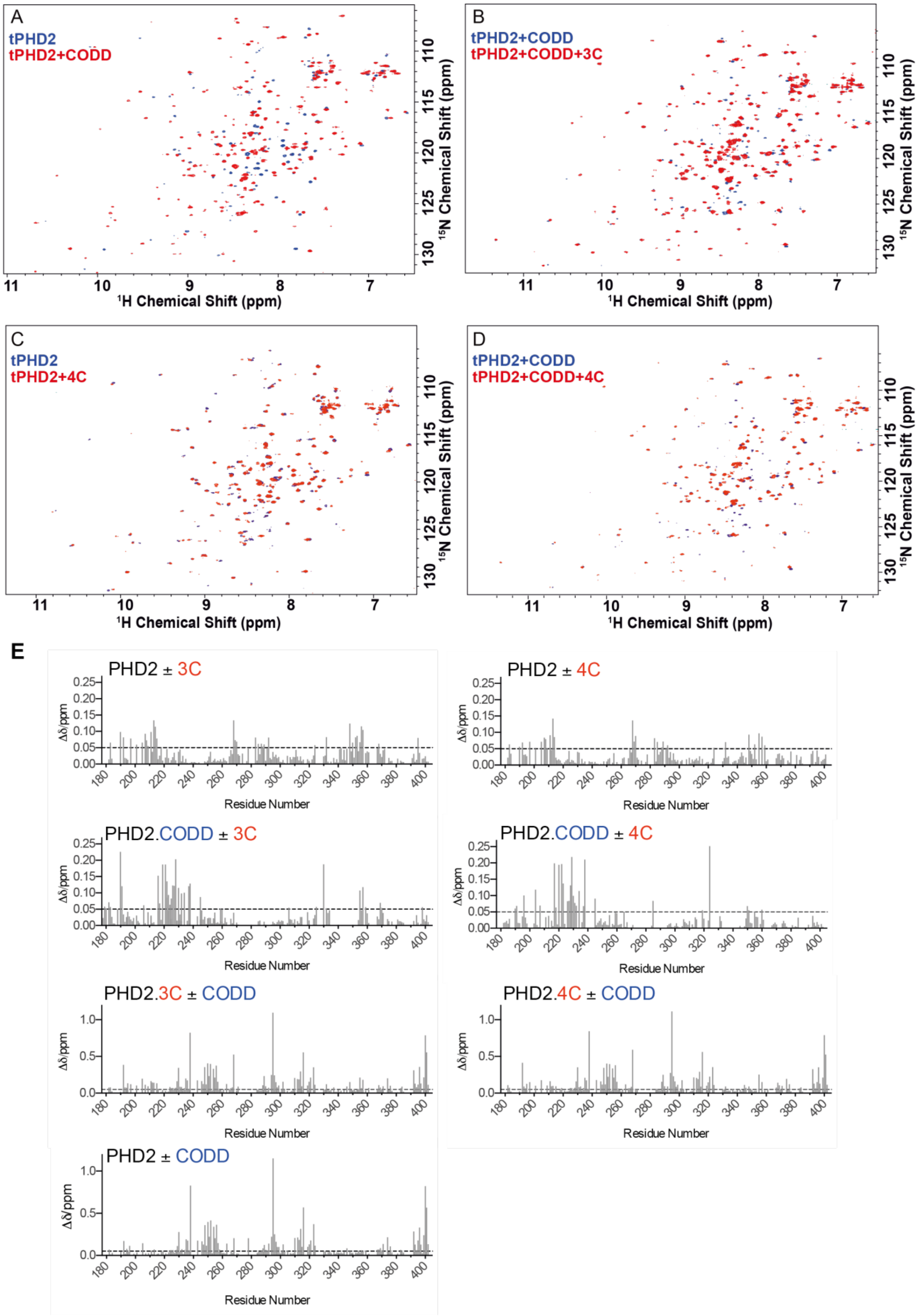
Results of fitting the data to a one-site binding model are shown. Only 5C gave a curve fitting result with $R^2 > 0.85$*, 1C and 3C showed no detectable response at any concentration while 4C and 6C showed some interaction at higher concentrations; this was attributed to non-specific binding. Standard errors from the curve fitting are shown. Coloured curves (highest to lowest concentration in the order orange, yellow, green, blue, indigo, violet, pink) represent processed data while black lines show fits. The ankyrin fragment (sequence H-HLEVVKLLLEHGADVNADQK-OH) was used in a 2-fold dilution series with 10 µM highest concentration. 1C, 3C,4C, 5C and 6C were used in 2-fold dilution series from 83, 61, 45, 55 and 64 nM respectively.*



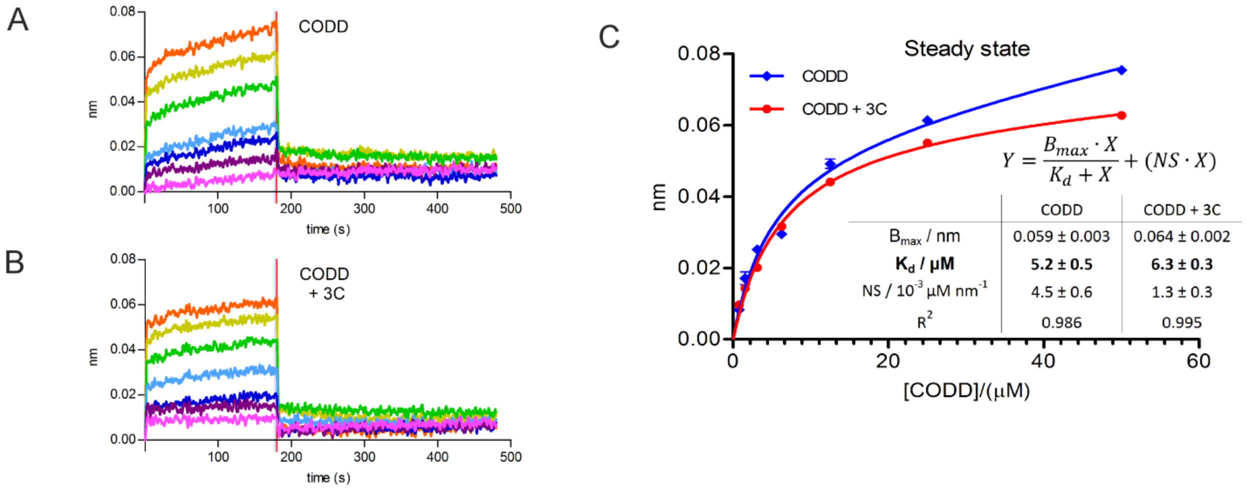
A)

Entry	Peptide Substrate*	tPHD2	$(NH_4)_2Fe(SO_4)_2$	Time (h)	Increase in +16 Peak (%)
1	CODD	+	+	0.33	93.72 ± 0.03
2	4C	+	+	20	2.63 ± 0.91
3	5C	+	+	20	5.58 ± 2.16
4	6C	+	+	20	6.72 ± 2.17
5	6C	+	+	16	8.12 ± 0.78
6	6C	-	+	16	8.48 ± 1.50
7	6C	+	-	16	0.63 ± 0.58

Supplementary Figure 6. The CPs are likely not substrates for tPHD2 within limits of detection.
(A) *PHD2 activity assays using MALDI-TOF MS were carried out with proline-containing cyclic peptides (4C, 5C, 6C) as substrates under standard assay conditions.* ***(B-D)*** *Representative MALDI-TOF MS spectra assessing 6C peptide as a substrate under different assay conditions as shown (entries 5-7 respectively), with t = 0 (black) and t = 16 h (red) overlaid.*



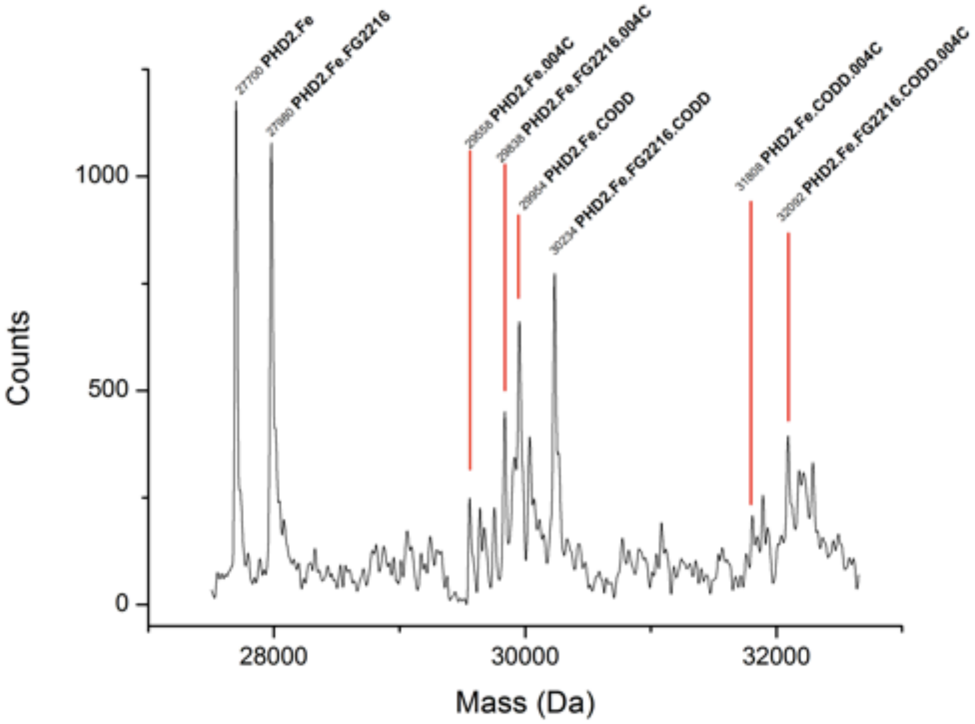
Supplementary Figure 7. *^{1}H-^{15}N HSQC spectra for tPHD2 with different combinations of CODD (200 μM) and CPs (200 μM) (A-D); (E) Weighted changes in chemical shift of residues in tPHD2 upon binding of CPs or CODD or both to tPHD2. Dashed line (Δδ = 0.05) denotes the threshold for significant changes as previously (Fig 3). PHD2 ± CODD from previous data.*[5]



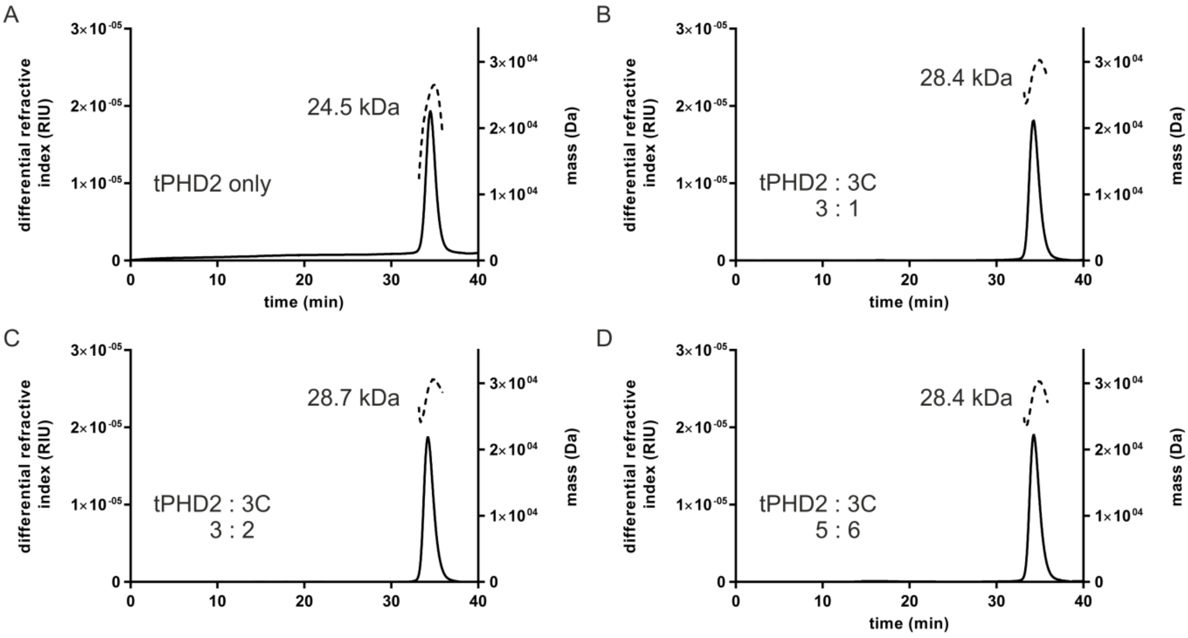
$$Y = \frac{B_{max} \cdot X}{K_d + X} + (NS \cdot X)$$

	CODD	CODD + 3C
B_{max} / nm	0.059 ± 0.003	0.064 ± 0.002
K_d / μM	**5.2 ± 0.5**	**6.3 ± 0.3**
NS / 10^{-3} μM nm^{-1}	4.5 ± 0.6	1.3 ± 0.3
R^2	0.986	0.995

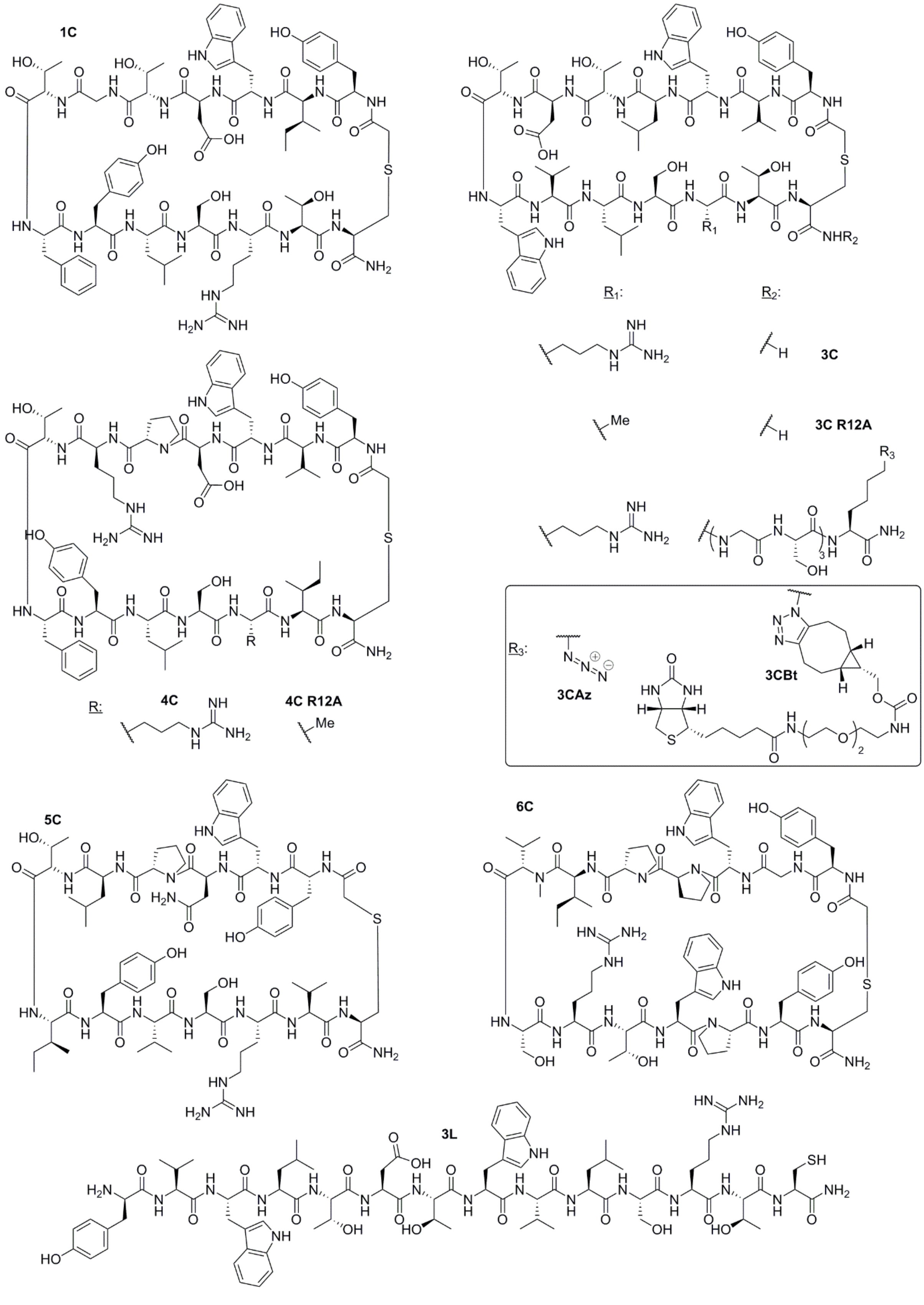
Supplementary Figure 8. CODD binding to PHD2 is not substantially perturbed by 3C binding.
Biolayer interferometry traces of CODD substrate peptide binding to tPHD immobilized on Ni-NTA sensors in the presence ***(A)*** *and absence* ***(B)*** *of 3C (10 nM).* ***(C)*** *A steady state model using the final 2 seconds of the association step was used to determine the binding affinity of CODD for tPHD2 under each condition. CODD was used in a 2-fold dilution series with highest concentration of 50 μM (coloured orange, yellow, green, blue, indigo, violet and magenta).*



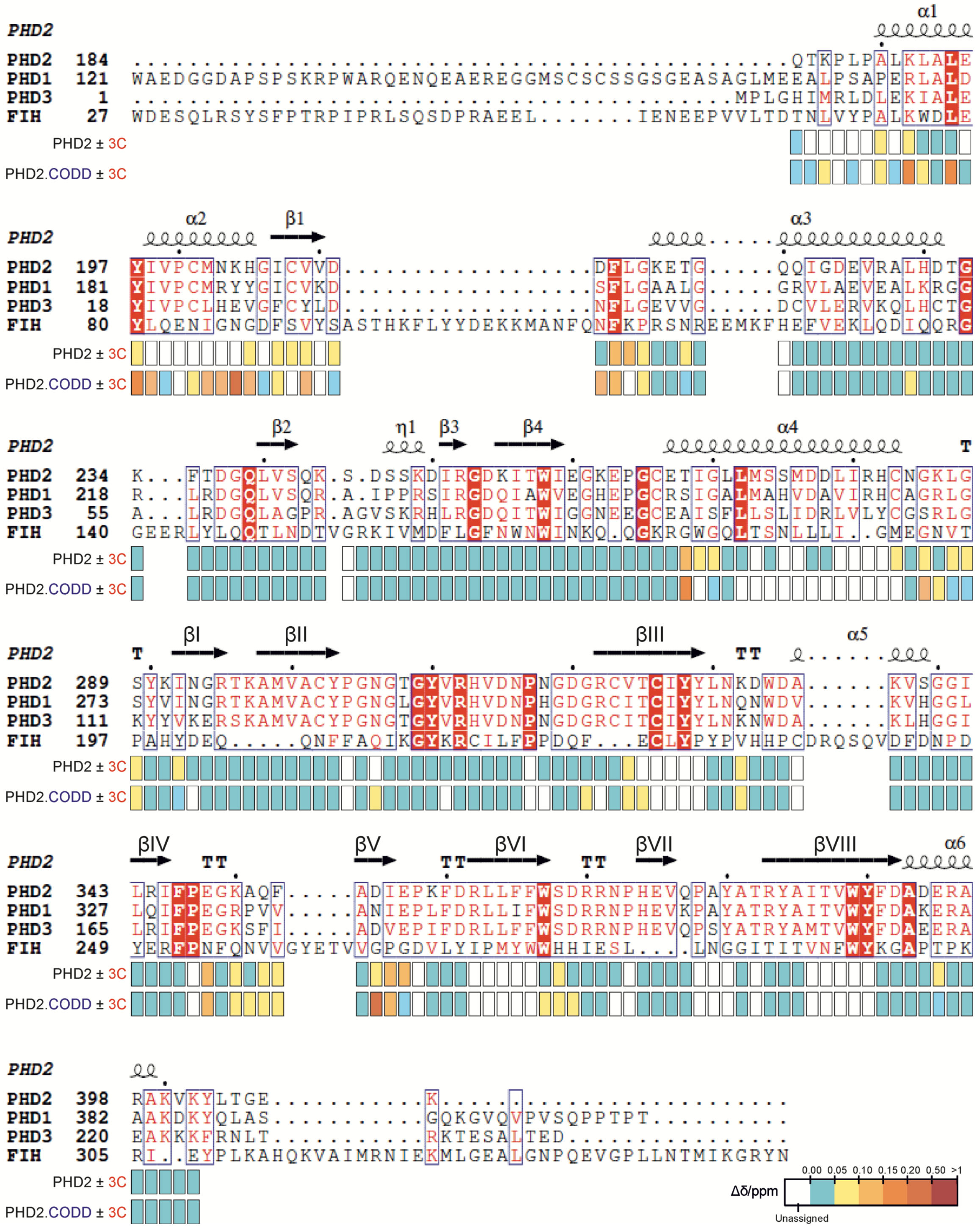
Supplementary Figure 9. Non-denaturing ESI-MS analysis of PHD2 with co-factors, CODD and CPs.
The assay mixture contained: PHD2 15uM and Fe(II) 15uM, 15uM FG2216, 15uM CODD and 48 uM PHD2_4C. The peak at 32092 corresponds to a PHD2.Fe(II).FG2216.CODD.4C complex (mass difference to PHD2.Fe.FG2216.CODD =1858. (4C mass= 1861)). The small peak at 31808, corresponds to the PHD2.Fe.CODD.4C complex (Mass difference to PHD2.Fe.CODD = 1854). The peak at 29558 corresponds to a PHD2.Fe(II).4C complex (Mass difference to PHD2.Fe(II).CODD.4C complex = 2250, (mass of CODD = 2253)). The peak at 29838 corresponds to the PHD2.Fe.FG2216.4C complex (Mass difference to PHD2.Fe.FG2216.CODD.4C = 2254). When all components are combined peaks can be resolved for complexes including the PHD2.Fe.FG2216.CODD.4C complex. The ability of the 4C peptide to bind at the same time as CODD and FG2216 suggests a binding site for 4C away from the active site of PHD2.



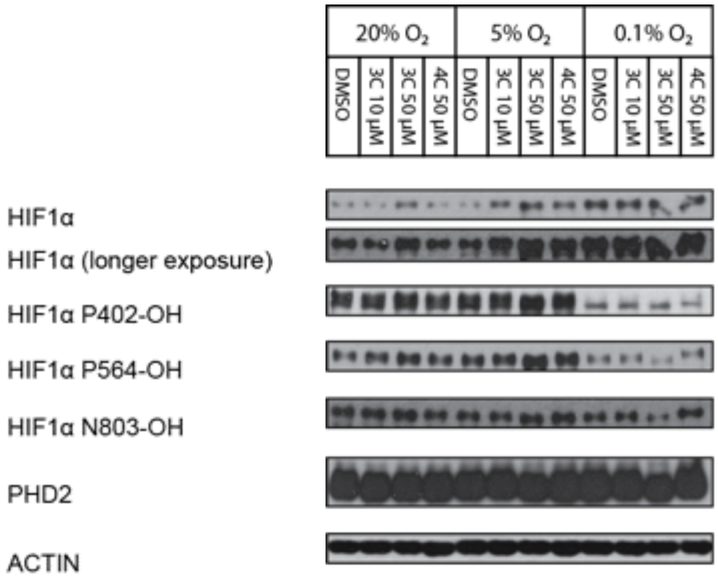
Supplementary Figure 10. SEC-MALS of tPHD2 with and without 3C.
Size exclusion chromatography with multi-angle light scattering analysis of PHD2 in the absence and presence of 3C (stoichiometric concentration ratios of 0.3, 0.6, 1.2 relative to tPHD2) were carried out.



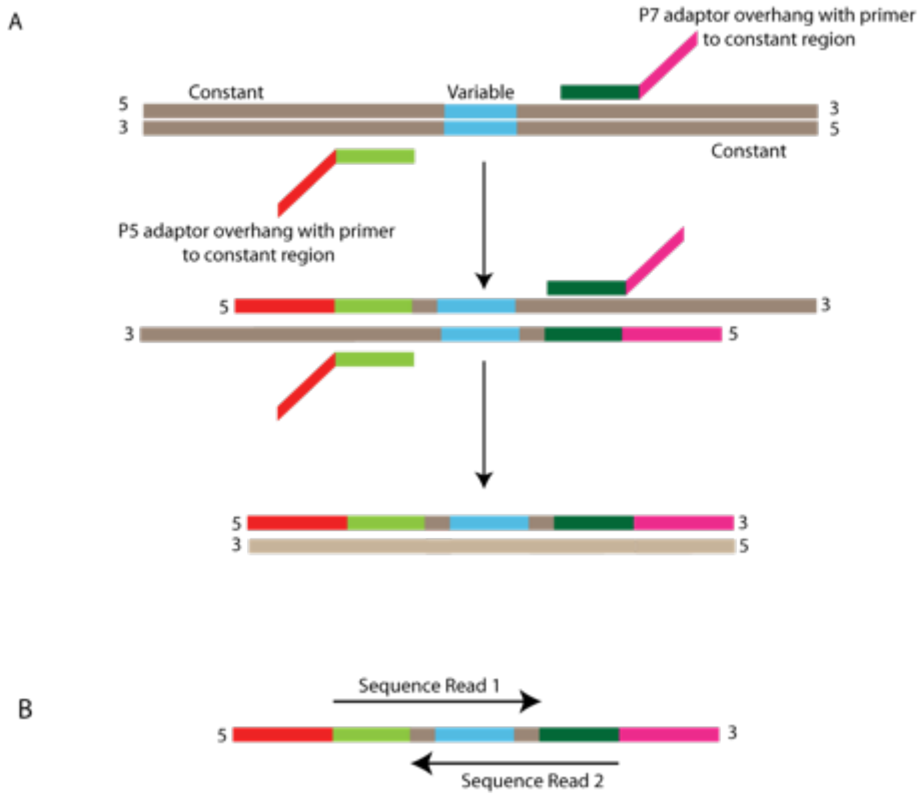
Supplementary Figure 11. Chemical structures of synthesized peptides.



Supplementary Figure 12. Sequence alignment of the catalytic domains of HIF hydroxylases.
The sequences of PHD1-3 and FIH catalytic domains were aligned using ClustalW, and overlaid with the crystallographically observed secondary assignment of PHD2 (PDB 2G19) using ESPRIPT(ESPript - http://espript.ibcp.fr).[17] *The NMR shifts for PHD2.3C relative to PHD2 and PHD2.CODD.3C relative to PHD2.CODD are annotated below the alignments, with colours corresponding to NMR shifts as in* ***Figure 4B-D****.*



Supplementary Figure 13. Graded hypoxia treatment with cyclic peptides.
Western blot analysis of RCC4_VA cells, following 16 hours treatment with DMSO or cyclic peptides (3C/4C) under different oxygen concentrations (20%, 5%, 0.1%). Hydroxylation of HIF1α by PHD (at Pro402, Pro564) and FIH (Asp803) were probed using hydroxylation specific HIF1α antibodies.



Supplementary Figure 14. Generation of Illumina-compatible libraries.

(A) *Specific forward (P5) and reverse (P7) primers (Supplementary Table 1) were designed consisting of priming sequences complimentary to the constant region of the recovered DNA at the 3' end of the primer and incorporating the adaptor sequences necessary for sequencing at the 5' end. During the first cycle of PCR, extension occurs such that the product has the required adaptor at one end with addition of the adaptor at the second end during the second cycle of PCR. Subsequent cycles amplify the library containing the sequence of interest with adaptors at both ends* ***(B)*** *During sequencing only strands with P5 adaptor at the 5' end will initially bind the flow cell ensuring all reads are occurring in the same orientation. Libraries are sequenced in a paired-ended fashion with each read incorporating the region of interest.*

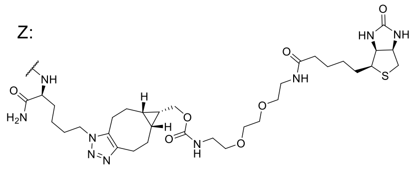
Supplementary Table 1.

Amino acid sequences of the variable region of the 200 most abundant peptides from round 5 of the selection procedure – values shown are percentage total sequencing reads. All sequences preceded by a D-Tyrosine residue and are followed by Cys-(Gly-Ser)$_3$ as depicted in ***Fig 2****. Sequences with an asterisk contain frame-shift mutations which result in read through of the conserved stop codon and the C-terminus is altered to AAAAAAARTGGG.*

	Sequence	R4	R5		Sequence	R4	R5		Sequence	R4	R5		Sequence	R4	R5
1	VWDPRTFYLSRI	8.222	51.390	**51**	VWDPSTFYLSRI	0.021	0.125	**101**	HSPTWECFVSL	0.072	0.060	**151**	LVSWWPPIIKRV	0.060	0.035
2	IWDTGTFYLSRT	3.025	7.549	**52**	VWDPRTFYLPRI	0.051	0.125	**102**	VWDPRAFYLSRI	0.024	0.058	**152**	ESPTWTCYQPLY	0.046	0.035
3	VWLTDTWVLSRT	1.321	5.750	**53**	VWDPRTFYSSRI	0.060	0.124	**103**	IWFYATWTLSRS	0.059	0.058	**153**	VWDPRTFYFSRI	0.008	0.035
4	WNPETIYIRRD	2.260	2.276	**54**	WDQRTFYISRV	0.079	0.123	**104**	WDSFYVSRLYG	0.069	0.057	**154**	VWDPRTFYLSKI	0.014	0.035
5	VWDPRTFYLSRT	0.603	1.619	**55**	YWDTNTFYLSRV	0.035	0.122	**105**	WPDTDSWYIRRT	0.105	0.057	**155**	IWDTRSWYLSRN	0.009	0.034
6	GWPPIVSRTWPY	0.426	1.405	**56**	LIQYWPPIVSRR	0.109	0.119	**106**	QSPTWQCWTSLY	0.036	0.055	**156**	IWYMNTWILSRN	0.045	0.034
7	WIDRRSFYIQRN	0.603	0.858	**57**	VWDPKTFYLSRI	0.023	0.117	**107**	VWDQRTFYLSRI	0.010	0.055	**157**	FTWPFYVQRL	0.033	0.034
8	PNSFYFSRLYG	0.271	0.806	**58**	IWDTWSWYLSRN	0.144	0.117	**108**	WPNRDTYYMRRA	0.080	0.054	**158**	LVKHWPPIVLRS	0.038	0.034
9	MWYTDTWQLSRT	0.229	0.666	**59**	WDPSSYYIQRV	0.152	0.115	**109**	WKDYTSYAIFSR	0.059	0.053	**159**	NWNRETWYLSRI	0.069	0.033
10	IWDTGTFYLSRI	0.301	0.642	**60**	VWDTRTFYLSRI	0.018	0.113	**110**	VWDPRTYYLSRI	0.017	0.053	**160**	WDRVSFYITRT	0.088	0.033
11	IWDPRTFYLSRI	0.087	0.545	**61**	VWDSRTFYLSRI	0.020	0.113	**111**	VWMTSQWMLSRT	0.038	0.053	**161**	TWLYETWILSRI	0.020	0.033
12	IWDTDTFYLSRT	0.064	0.507	**62**	VVWHTVYAIWRN	0.077	0.112	**112**	VWDTRSYYLSRL	0.059	0.052	**162**	LWHYDTYILSRV	0.049	0.033
13	VNSWILSRLTGI	0.281	0.433	**63**	VWDPRTSYLSRI	0.051	0.111	**113**	FTWPFFVKRS	0.110	0.052	**163**	VEELAVYSIWRN	0.033	0.033
14	IASFYLNRATGI	0.378	0.428	**64**	WIDPNTFYIRRS	0.117	0.111	**114**	WDPDTFIIKRI	0.172	0.050	**164**	WDPRTFYLSRI	0.007	0.033
15	WDPNTIFIKRI	0.212	0.383	**65**	HFSFYINRRTGT	0.154	0.110	**115**	IWYTGTFYLSRT	0.016	0.050	**165**	FKPPFYIIRR	0.050	0.032
16	TWLYETWMLSRI	0.375	0.376	**66**	WNRNTFYISRV	0.126	0.110	**116**	VQWHATYAIFKF	0.032	0.049	**166**	VWDPRTFYLTRI	0.018	0.032
17	WNPLTIYVSRV	1.075	0.357	**67**	IWDTRTFYLSRT	0.018	0.110	**117**	VWDPRTFYLSWI	0.014	0.048	**167**	SSPTWDCFTPLG	0.038	0.032
18	IWDTSTFYLSRT	0.044	0.310	**68**	VWDPGTFYLSRI	0.034	0.109	**118**	VIWYVTHAIFKR	0.020	0.047	**168**	WDPSTYYVSRI	0.080	0.031
19	WDPSTIFIKRN	0.755	0.304	**69**	VWDPRSFYLSRI	0.023	0.106	**119**	WVHTYYIKRI	0.081	0.046	**169**	WPDKSSFYVSRM	0.040	0.030
20	LWYINTFYLSRT	0.339	0.294	**70**	IWYTDTWQLSRT	0.013	0.104	**120**	STPVWSCWTPLY	0.059	0.046	**170**	WIDRRSFYIQRI	0.021	0.030
21	NWTNWFTISRV	0.364	0.291	**71**	VWDPWTFYLSRI	0.027	0.099	**121**	DWDTTSFYLSRI	0.080	0.045	**171**	FWVTDQWMLSRT	0.098	0.030
22	FWDPRTFYLSRI	0.087	0.276	**72**	VWHFDTYMLSRS	0.106	0.094	**122**	VWVPRTFYLSRI	0.013	0.045	**172**	LWFPETWILSRT	0.057	0.030
23	VISHAVWQQYVR	0.181	0.270	**73**	IWDTATFYLSRT	0.013	0.091	**123**	WNPETIYIRRV	0.045	0.045	**173**	DWDTNTFYLSRV	0.062	0.030
24	VWDTGTFYLSRT	0.058	0.269	**74**	VWYTDTWQLSRT	0.015	0.089	**124**	WVPTTIYIKRS	0.077	0.045	**174**	WVHTFYMKRN	0.044	0.030
25	FWVTSSWWISRT	0.295	0.240	**75**	WPNKDTFYIHRS	0.076	0.086	**125**	WDPETVYIKRV	0.123	0.045	**175**	VWDPRTFICLGF*	0.017	0.029
26	WDPNTIFIKRM	0.243	0.225	**76**	WNPDTVFIRRT	0.249	0.085	**126**	VWDPRTFCLSRI	0.021	0.045	**176**	VWEPRTFYLSRI	0.015	0.029
27	VWDPRTFYLSRV	0.076	0.223	**77**	TWNTNTWYLSRM	0.050	0.084	**127**	WLGPFHLSRNP	0.068	0.044	**177**	FVRSWPPTIRRR	0.033	0.029
28	WIDRTSWYIRRS	0.307	0.216	**78**	VZDPRTFYLSRI	0.035	0.083	**128**	PKSFYLSRQTGL	0.042	0.044	**178**	WNPKTIYILRS	0.067	0.029
29	VWYTSTFRISRT	0.169	0.214	**79**	IWYTNTWILSRN	0.034	0.083	**129**	TWLYETWMLSRT	0.029	0.044	**179**	VICDGGFYYC	0.022	0.029
30	WNPLTIYVSRR	0.225	0.208	**80**	FFEPFYIRRN	0.111	0.081	**130**	VWDPRTFYZSRI	0.031	0.044	**180**	TKSFYLSRATGM	0.028	0.028
31	VTWYETWALFRA	0.056	0.197	**81**	VWDPRTFFLSRI	0.020	0.080	**131**	IVDLGTHWLFRR	0.026	0.044	**181**	VWDPRTFYLFRI	0.011	0.028
32	VWDPRTFYLSRN	0.152	0.194	**82**	WPNKQTFYIKRS	0.075	0.080	**132**	GWDPRTFYLSRI	0.008	0.042	**182**	VWDTNTFYLSRV	0.004	0.027
33	VWDPRTFYLSRM	0.127	0.187	**83**	WIDHGSWALWKN	0.039	0.080	**133**	GNPLYTFYVRRI	0.118	0.042	**183**	IWDTGSFYLSRT	0.013	0.027
34	VTWYVTHAIFKR	0.054	0.182	**84**	SWDTRTFYLSRR	0.110	0.076	**134**	GWPPLLNRRTRA	0.040	0.041	**184**	IWDTGTFYLSRS	0.038	0.027
35	VWLTDTWVLSRI	0.062	0.174	**85**	TWDTRTWYISRT	0.115	0.071	**135**	VWDPRTFYMSRI	0.016	0.041	**185**	VTWYDTHAMFKK	0.014	0.027
36	TWNTNTWYLSRI	0.059	0.156	**86**	VTDHQVWASFRY	0.035	0.071	**136**	IWLTDTWVLSRT	0.011	0.040	**186**	WNPETIYIRRI	0.027	0.026
37	VWYTQGWIISRT	0.172	0.155	**87**	VWNPRTFYLSRI	0.020	0.071	**137**	VLDPRTFYLSRI	0.010	0.040	**187**	IWYSDTFYLSRR	0.018	0.026
38	VWDPRTLYLSRI	0.072	0.153	**88**	WNEATFYVKRT	0.115	0.070	**138**	VWITDTWTISRM	0.063	0.040	**188**	VWDPTTFYLSRI	0.004	0.025
39	LASFYLSRLTGI	0.119	0.152	**89**	VCDPRTFYLSRI	0.037	0.070	**139**	VWITDTWTISRI	0.038	0.039	**189**	WNPTTIFVSRR	0.103	0.025
40	WTHRWPPTLSRI	0.239	0.151	**90**	VTHFSVFAVFRR	0.034	0.069	**140**	IASFYLSRATGI	0.007	0.039	**190**	IWDTGTFYLSRN	0.048	0.025
41	FTWPYWISRN	0.152	0.147	**91**	VWDPRMFYLSRI	0.025	0.068	**141**	WDQNTFYIRRT	0.039	0.038	**191**	VWDTGTFYLSRI	0.007	0.025
42	FWDTGTFYLSRT	0.095	0.147	**92**	VTEYHVWSMYKN	0.073	0.067	**142**	TWNTNTWYLSRT	0.015	0.038	**192**	LISNWPPIIKRA	0.050	0.025
43	VWDPRTFYLSRS	0.154	0.142	**93**	WDDRSFYIHRV	0.131	0.067	**143**	FWLTDTWVLSRT	0.012	0.038	**193**	FGIRGRFICLGF*	0.068	0.025
44	VTWYSSYAVYRP	0.050	0.142	**94**	HESFYVSRIYG	0.131	0.066	**144**	VWYPRTFYLSRI	0.010	0.037	**194**	FTWPYYINRR	0.036	0.025
45	VRDPRTFYLSRI	0.067	0.138	**95**	VWDPRTFHLSRI	0.034	0.066	**145**	IWDTDTFYLSRI	0.006	0.037	**195**	GDPQQTFYVRRI	0.028	0.025
46	WNPRTIYIKRI	0.117	0.136	**96**	GSLDRWRIWSPL	0.067	0.065	**146**	IWHYDSWYISRI	0.033	0.037	**196**	DWDPRTFYLSRI	0.011	0.025
47	IHRFFDFTYVW	0.111	0.135	**97**	VWDPRTFYLSRF	0.022	0.063	**147**	VWDPMTFYLSRI	0.009	0.037	**197**	VWIRGRFICLGF*	0.017	0.025
48	AWDPRTFYLSRI	0.058	0.135	**98**	VWGPRTFYLSRI	0.027	0.063	**148**	IWHYDSWYISRM	0.047	0.037	**198**	PDSWYISRATGL	0.042	0.025
49	EHSFYLSRATGV	0.119	0.134	**99**	VWDPRTFYLSRI*	0.051	0.061	**149**	WIDNNVFAILKR	0.026	0.036	**199**	VWDPRTFYLSMI	0.010	0.025
50	VWDLRTFYLSRI	0.044	0.128	**100**	VWDPRTFYLSGI	0.032	0.060	**150**	WDQRTFYVQRIC	0.041	0.035	**200**	IWDTSTFYLSRI	0.004	0.025

Supplementary Table 2. Structures and high resolution MS analyses of the synthesised cyclic peptides.

HRMS of synthesized peptides used in this study. All peptides are C-terminal amides. Peptides with underlined amino acids are cyclic with a thioether bond between an N-terminal acetyl group and the cysteine sidechain. Abbreviations: ^{D}*Y – D-Tyrosine; Z – biotin-tag with a linker, structure as shown. Full chemical structures for all peptides are shown in* ***Supplementary Fig. 10.***

Peptide	Sequence/Formula	HRMS:	Calculated	Observed
1C	^{D}YIWDTGTFYLSRTC $C_{82}H_{113}O_{23}N_{19}S$	$[M+H]^+$	1764.80502	1764.80664
3C	^{D}YVWLTDTWVLSRTC $C_{82}H_{113}O_{23}N_{19}S$	$[M+H]^+$	1781.86795	1781.86865
4C	^{D}YVWDPRTFYLSRIC $C_{88}H_{124}O_{21}N_{22}S$	$[M+H]^+$	1857.91049	1857.90735
5C	^{D}YWNPLTIYVSRVC $C_{78}H_{113}O_{19}N_{19}S$	$[M+H]^+$	1652.82536	1652.82629
6C	^{D}YGWPPIVSRTWPYC $C_{86}H_{114}O_{19}N_{20}S$	$[M+H]^+$	1763.83626	1763.83752
3C (R12A)	^{D}YVWLTDTWVLSATC $C_{80}H_{114}O_{22}N_{17}S$	$[M+H]^+$	1696.80395	1696.80261
3L	^{D}YVWLTDTWVLSRTC $C_{81}H_{121}O_{21}N_{20}S$	$[M+H]^+$	1741.87304	1741.86951
3CBt	^{D}YVWLTDTWVLSRTCGSGSGSZ Z:  $C_{131}H_{196}O_{38}N_{34}S_2$	$[M+2H]^{2+}$	1459.70184	1459.70337

Supplementary Table 3. Conditions used in 3CBt pull down assays.

The conditions used for each experiment in ***Figure 5****. Inputs samples were typically diluted ten-fold relative to the recovered supernatants from the beads for loading. For example: if 50 µL of 10 mg/mL cell lysate was incubated with the beads and recovered by heating in 20 µL of 1 × loading dye, the sample has been concentrated 2.5-fold (if all protein bound to and was recovered from the beads this solution would be at 25 mg/mL protein). Hence the input sample would be diluted 1 in 4 to 2.5 mg/mL as this corresponds to one tenth of the effective concentration of the lysate after elution.*

Experiment	Cells	Lysate	Added protein	3CBt/biotin	Beads pmol/µL
A	-	-	100 nM	520 nM	8.66
B	-	-	10 µM	26 µM	10.4
C	U2OS	8.7 mg/mL	5 µM	26 µM	8.66
D	Hep3B	5 mg/mL	-	13 µM	4.32
E	Hep3B	Hyp: 6.5 mg/mL Norm: 10 mg/mL	-	26 µM	8.22
F	RCC4	7.5 mg/mL	0 µM 1 µM 5 µM	26 µM	8.66
G	RCC4	8.4 mg/mL	-	52 µM	7.8

Supplementary Table 4. List of primers used in this study.

Primer	Sequence
CGS3an13.R39	TTTCCGCCCCCCGTCCTAGCTGCCGCTGCCGCTGCCGCA
T7g10M.F48	TAATACGACTCACTATAGGGTTAACTTTAAGAAGGAGATATACATATG
P5 adaptor	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTAGGGTTAACTTTAAGAAGGAGATATAC
P7 adaptor	CAAGCAGAAGACGGCATACGAGATXXXXXXGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCCTAGCTGCCGCTG*

**XXXXXX represents the index, which was one of: GCGGAC, TTTCAC, GGCCAC or CGAAAC.*

Supplementary References

1. E. Flashman, L. M. Hoffart, R. B. Hamed, J. M. Bollinger Jr, C. Krebs and C. J. Schofield, *FEBS Journal*, 2010, **277**, 4089-4099.
2. R. Chowdhury, M. A. McDonough, J. Mecinovic, C. Loenarz, E. Flashman, K. S. Hewitson, C. Domene and C. J. Schofield, *Structure*, 2009, **17**, 981-989.
3. T. Keates, C. D. Cooper, P. Savitsky, C. K. Allerston, C. Phillips, M. Hammarstrom, N. Daga, G. Berridge, P. Mahajan, N. A. Burgess-Brown, S. Muller, S. Graslund and O. Gileadi, *New biotechnology*, 2012, **29**, 515-525.
4. E. Flashman, E. A. L. Bagg, R. Chowdhury, J. Mecinović, C. Loenarz, M. A. McDonough, K. S. Hewitson and C. J. Schofield, *Journal of Biological Chemistry*, 2008, **283**, 3808-3815.
5. R. Chowdhury, I. K. H. Leung, Y.-M. Tian, M. I. Abboud, W. Ge, C. Domene, F.-X. Cantrelle, I. Landrieu, A. P. Hardy, C. W. Pugh, P. J. Ratcliffe, T. D. W. Claridge and C. J. Schofield, *Nature Communications*, 2016, **7**, 12673.
6. R. Chowdhury, I. K. Leung, Y. M. Tian, M. I. Abboud, W. Ge, C. Domene, F. X. Cantrelle, I. Landrieu, A. P. Hardy, C. W. Pugh, P. J. Ratcliffe, T. D. Claridge and C. J. Schofield, *Nat Commun*, 2016, **7**, 12673.
7. K. S. Hewitson, L. A. McNeill, M. V. Riordan, Y.-M. Tian, A. N. Bullock, R. W. Welford, J. M. Elkins, N. J. Oldham, S. Bhattacharya, J. M. Gleadle, P. J. Ratcliffe, C. W. Pugh and C. J. Schofield, *Journal of Biological Chemistry*, 2002, **277**, 26351-26355.
8. A. Kawamura, M. Münzel, T. Kojima, C. Yapp, B. Bhushan, Y. Goto, A. Tumber, T. Katoh, O. N. F. King, T. Passioura, L. J. Walport, S. B. Hatch, S. Madden, S. Müller, P. E. Brennan, R. Chowdhury, R. J. Hopkinson, H. Suga and C. J. Schofield, *Nature Communications*, 2017, **8**, 14773.
9. M. Martin, *EMBnet.journal*, 2011, **17**, 10-21.
10. H. Pagès, P. Aboyoun, R. Gentleman and S. DebRoy, 2016.
11. W. F. Vranken, W. Boucher, T. J. Stevens, R. H. Fogh, A. Pajon, M. Llinas, E. L. Ulrich, J. L. Markley, J. Ionides and E. D. Laue, *Proteins*, 2005, **59**, 687-696.
12. W. Aik, M. A. McDonough, A. Thalhammer, R. Chowdhury and C. J. Schofield, *Curr Opin Struct Biol*, 2012, **22**, 691-700.
13. L. Yan, V. J. Colandrea and J. J. Hale, *Expert Opinion on Therapeutic Patents*, 2010, **20**, 1219-1245.
14. J. Mecinovic, R. Chowdhury, E. Flashman and C. J. Schofield, *Analytical biochemistry*, 2009, **393**, 215-221.
15. Y. Yamagishi, I. Shoji, S. Miyagawa, T. Kawakami, T. Katoh, Y. Goto and H. Suga, *Chemistry & Biology*, 2011, **18**, 1562-1570.
16. R. A. Copeland, *Methods Biochem Anal*, 2005, **46**, 1-265.
17. X. Robert and P. Gouet, *Nucleic Acids Research*, 2014, **42**, W320-W324.