The cellular response to hypoxia is critical for cell survival and is fine-tuned to allow cells to recover from hypoxic stress and adapt to heterogeneous or fluctuating oxygen levels\(^1,2\). The hypoxic response is mediated by the α-subunit of the transcription factor HIF-1 (HIF-1α)\(^3\), which interacts through its intrinsically disordered C-terminal transactivation domain with the TAZ1 (also known as CH1) domain of the general transcriptional coactivators CBP and p300 to control the transcription of critical adaptive genes\(^4\,6\). One such gene encodes CITED2, a negative feedback regulator that attenuates HIF-1 transcriptional activity by competing for TAZ1 binding through its own disordered transactivation domain\(^7\,9\). Little is known about the molecular mechanism by which CITED2 displaces the tightly bound HIF-1α from their common cellular target. The HIF-1α and CITED2 transactivation domains bind to TAZ1 through helical motifs that flank a conserved LP(Q/E)L sequence that is essential for negative feedback regulation\(^6\,8\,9\). Here we show that human CITED2 displaces HIF-1α by forming a transient ternary complex with TAZ1 and HIF-1α and competing for a shared binding site through its LPEL motif, thus promoting a conformational change in TAZ1 that increases the rate of HIF-1α dissociation. Through allosteric enhancement of HIF-1α release, CITED2 activates a highly responsive negative feedback circuit that rapidly and efficiently attenuates the hypoxic response, even at modest CITED2 concentrations. This hypersensitive regulatory switch is entirely dependent on the unique flexibility and binding properties of these intrinsically disordered proteins and probably exemplifies a common strategy used by the cell to respond rapidly to environmental signals.

The diverse functionality of intrinsically disordered proteins arises from their inherent flexibility and their ability to adopt an ensemble of conformations of similar energy, permitting rapid but specific interactions with numerous cellular partners through short peptide motifs\(^10\). Individual motifs in intrinsically disordered proteins can function synergistically to enhance binding affinity or modulate the biological response\(^11\,12\), but little is known about how these motifs compete for occupancy of common target molecules during cellular signalling.

Under normoxic conditions, the proteins that mediate the hypoxic response are tightly regulated. Accumulation of HIF-1α is suppressed by hydroxylation events that target it for degradation\(^13\) and inhibit binding to the TAZ1 domain of CBP/p300 (ref. 14). In hypoxia, HIF-1α is no longer hydroxylated and binds tightly to TAZ1 to promote rapid activation of adaptive genes\(^5\,6\,14\). The hypoxic response is remarkably efficient; HIF-1α stabilization and transcriptional activity exhibits a switch-like dependence on oxygen concentration\(^15\,16\). Like HIF-1α, CITED2 is unstable in normoxia\(^7\), subject to proteasomal degradation\(^17\), and forms a high-affinity complex with TAZ1 (ref. 8). CITED2 is stabilized in hypoxia and nearly all detectable CITED2 is found in complex with CBP/p300 (ref. 7), suggesting that CITED2 competes with HIF-1α in an exceptionally efficient manner.

The activation domains of HIF-1α (residues 776–826) and CITED2 (residues 216–269) utilize partially overlapping binding sites to form high-affinity complexes with TAZ1 (Fig. 1)\(^5\,6\,8\,9\). The α\(_\text{A}\) helices of HIF-1α and CITED2 and their conserved LP(Q/E)L motifs bind to the same surfaces of TAZ1. The region of CITED2 that is C-terminal to the LPEL motif binds in an extended conformation in the same site as the α\(_\text{B}\) helix of HIF-1α. Only the α\(_\text{C}\) helix of HIF-1α binds to a fully non-overlapping site on TAZ1.

Competition between HIF-1α and CITED2 was characterized by NMR spectroscopy. The \(^1\)H–\(^15\)N heteronuclear single quantum coherence (HSQC) spectrum of \(^15\)N-labelled TAZ1 bound to HIF-1α differs from the spectrum of \(^15\)N-TAZ1 bound to CITED2 (Extended Data Fig. 1), which allows us to discriminate between HIF-1α- and CITED2-bound TAZ1 resonances and obtain site-specific information on the competition mechanism. Consistent with literature reports\(^5\,8\), the HIF-1α and CITED2 transactivation domains bind TAZ1 with the same affinity under the conditions of our NMR experiments (dissociation constant (\(K_d\)) = 10 ± 2 nM, Extended Data Fig. 2). As their binding affinities are the same, we expected that a sample of \(^15\)N-TAZ1 mixed with both the HIF-1α and CITED2 peptides in a 1:1:1 molar ratio would yield an HSQC spectrum with two sets of resonances of approximately equal intensity, corresponding to an equally populated mixture of the \(^15\)N-TAZ1–HIF-1α and \(^15\)N-TAZ1–CITED2 complexes. However, this experiment yielded an unexpected result. Regardless of the order of addition, the only cross peaks observed in spectra of \(^15\)N-TAZ1 samples containing equimolar HIF-1α and CITED2 are those of the binary CITED2 complex (Fig. 2a, Extended Data Fig. 3). Observation of solely the TAZ1–CITED2 complex, despite the equal affinities of HIF-1α and CITED2 for binding to TAZ1, suggests that CITED2 binds to the TAZ1–HIF-1α complex in a strongly cooperative process to fully and efficiently displace HIF-1α at equimolar concentration.

Following the surprising results from the NMR experiments, the interactions of the HIF-1α and CITED2 activation domains with TAZ1 were characterized by fluorescence anisotropy competition experiments\(^18\,19\). Unlabelled HIF-1α or CITED2 peptide was titrated into a pre-formed complex of AlexaFluor-594-labelled HIF-1α peptide bound to unlabelled TAZ1 (Fig. 2b, c). As the HIF-1α and CITED2 peptides have equal affinities for TAZ1, the two proteins should behave similarly in displacing fluorescently labelled HIF-1α from the TAZ1 complex. Unlabelled HIF-1α peptide displaces fluorescently labelled HIF-1α from the TAZ1 complex with an apparent dissociation constant (\(K_d\)) of 10 ± 2 nM versus \(K_d,\text{binary} = 10 ± 2 \text{ nM}\) (Fig. 2b). In contrast, CITED2 is a much more efficient competitor than HIF-1α (Fig. 2c), displacing fluorescently labelled HIF-1α from its complex with TAZ1 with an apparent dissociation constant (\(K_d\)) of 0.2 ± 0.1 nM, 50-fold smaller than the \(K_d\) for the binary TAZ1–CITED2 complex (\(K_d,\text{binary} = 10 ± 1 \text{ nM}\)). Thus, both the fluorescence and NMR results indicate that CITED2 is extremely effective in displacing HIF-1α from the TAZ1–HIF-1α complex. Remarkably, the reverse process is
inefficient: very high concentrations of HIF-1α peptide are required to displace AlexaFluor-594-labelled CITED2 peptide from the pre-formed TAZ1–CITED2 complex (apparent $K_d = 0.9 \pm 0.1 \mu M$, almost 100-fold weaker than the $K_a$ of the binary TAZ1–HIF-1α complex) (Fig. 2d).

To obtain further insights into the molecular mechanism of HIF-1α displacement by CITED2, stopped-flow fluorescence methods were used to measure the rates at which unlabelled HIF-1α or CITED2 peptides displace AlexaFluor-488-labelled HIF-1α peptide from its complex with unlabelled TAZ1. The difference in the kinetics of HIF-1α and CITED2 competition is notable (Fig. 2e, f, Extended Data Figs 4, 5). CITED2 fully displaces labelled HIF-1α from TAZ1 within 10 s (observed rate ($k_{obs} = 2.2 \pm 0.2 s^{-1}$ at 100μM competing CITED2) (Fig. 2e, g, Extended Data Fig. 4), whereas unlabelled HIF-1α peptide fails to displace the fluorescently labelled HIF-1α in this time frame (Fig. 2e, black curve). Instead, displacement by HIF-1α is slow ($k_{obs} = 0.04 \pm 0.001 s^{-1}$ at 100μM competing HIF-1α) and must be monitored by manual mixing experiments (Fig. 2f, g, Extended Data Fig. 5). Competition by unlabelled HIF-1α peptide is only complete after 200 s, whereas competition by CITED2 is complete within the 10 s interval before data can be acquired (dead time) in the manual mixing experiment (Fig. 2f, grey curve). The observed rate of dissociation of the fluorescently labelled HIF-1α peptide is directly proportional to the concentration of the competing CITED2 (Fig. 2g, Extended Data Fig. 4b), strongly suggesting that CITED2 forms a transient ternary complex with TAZ1 and HIF-1α, from which HIF-1α is displaced very rapidly. In the absence of ternary complex formation, dissociation of fluorescently labelled HIF-1α would be absolutely required before either unlabelled HIF-1α or CITED2 could bind, resulting in identical kinetic profiles for competition by both peptides. Thus, the efficient displacement of HIF-1α from its complex with TAZ1 by CITED2 is kinetically driven and proceeds through a transient ternary intermediate.

Conformational fluctuations in the TAZ1–HIF-1α complex might play a critical role in allowing formation of a ternary complex and modulating competition between HIF-1α and CITED2. The [1H]–15N heteronuclear nuclear Overhauser effect (NOE) reports on dynamics.
of the protein backbone, with higher NOE values corresponding to ordered regions and lower values indicative of dynamic disorder due to fluctuations on the picosecond-nanosecond timescale. Measurements of the $[{\Lambda}H^{-15}N]$ NOE for the $^{15}N$-HIF-$\alpha$ and $^{15}N$-CITED2 peptides in complex with TAZ1. The amino acid sequences of the HIF-$\alpha$ and CITED2 transactivation domains and the positions of the helical motifs formed upon TAZ1 (Fig. 3) show major differences in flexibility. HIF-$\alpha$ displays a wide range of dynamics throughout its sequence (Fig. 3a). The $\alpha_A$ and $\alpha_C$ helices adopt well-ordered, structured states with elevated heteronuclear NOE values, whereas the linker between $\alpha_A$ and $\alpha_C$ and residues in the N-terminal region, encompassing the $\alpha_A$ helix and the LPQL motif, remain highly flexible. In contrast, the distribution of $[{\Lambda}H^{-15}N]$ NOE values is more uniform for residues 222–250 of the CITED2 peptide, with elevated values observed throughout the $\alpha_A$ helix, the LPQL motif, and several neighbouring residues that form stabilizing interactions in the complex with TAZ1 (Fig. 3b).

HIF-$\alpha$ and CITED2 form both well-ordered and dynamic contacts with TAZ1 (Fig. 3), suggesting a critical role for disorder in modulating competition for TAZ1 binding. The $\alpha_A$ helices of HIF-$\alpha$s and CITED2 occupy the same surface of TAZ1 (Fig. 1a), but they exhibit different dynamic characteristics, with HIF-$\alpha$ retaining considerable flexibility, whereas CITED2 adopts a stable, well-ordered helical structure (Fig. 3). Complete dissociation of HIF-$\alpha$ from the surface of TAZ1 is not a prerequisite for CITED2 binding: HIF-$\alpha$ could remain anchored to TAZ1 through its $\alpha_B$ and/or $\alpha_C$ helices while allowing CITED2 to bind to the opposite face of TAZ1 through its $\alpha_A$ helix, displacing the dynamically disordered $\alpha_A$ helix of HIF-$\alpha$.

Simultaneous binding of HIF-$\alpha$s and CITED2 to TAZ1 would facilitate competition for binding at the common LP(Q/E)L site, providing a rational mechanism for the enhanced dissociation of HIF-$\alpha$s in the presence of CITED2.

To determine the importance of the LP(Q/E)L motif in modulating competition between the HIF-$\alpha$s and CITED2 transactivation domains, we designed a truncated CITED2 peptide (CITED2 (216–242)) encompassing the well-ordered $\alpha_A$ helix but lacking the conserved LPEL motif (Fig. 4a). Removal of the LPEL motif and the C-terminal residues results in a marked reduction in the TAZ1 binding affinity ($K_{d,\text{binary}} = 36 \pm 10 \mu M$) (Extended Data Fig. 2c, g). Similarly, a truncated HIF-$\alpha$ peptide (HIF-$\alpha$ (796–826)) lacking the $\alpha_A$ region and LPQL motif (Fig. 4a) binds weakly to TAZ1 ($K_{d,\text{binary}} = 1.9 \pm 0.1 \mu M$) (Extended Data Fig. 2d, g). Despite the observed decrease in TAZ1 binding affinity for these truncated peptides, NMR spectra show that CITED2 (216–242) and HIF-$\alpha$ (796–826) bind to $^{15}N$-TAZ1 in the same manner as the corresponding regions of the full-length CITED2 and HIF-$\alpha$s peptides (Extended Data Fig. 6).

Direct evidence for ternary complex formation was obtained from NMR titrations (Fig. 4, Extended Data Figs 7, 8). When unlabelled CITED2 (216–242) peptide was added to a 1:1 complex of $^{15}N$-TAZ1 with the full-length HIF-$\alpha$s activation domain (residues 776–826), chemical shift changes and/or broadening were observed for HSQC cross peaks of TAZ1 residues located in the binding site of the CITED2 $\alpha_A$ helix (Fig. 4b, Extended Data Figs 7, 9), showing clearly that in the absence of the LPEL motif, the N-terminal region of the CITED2 peptide can bind to the TAZ1–HIF-$\alpha$s complex without displacing the bound HIF-$\alpha$s. The cross peaks of several residues in the C-terminal region of helix $\alpha_B$ and helix $\alpha_C$ of TAZ1 are also perturbed by binding of the CITED2 peptide, shifting towards their positions in the spectrum of the TAZ1–CITED2 binary complex as excess peptide is added. These residues correspond to regions where there are differences in structure and relative helix orientation between the HIF-$\alpha$s and CITED2 complexes (Extended Data Fig. 10). Binding of the truncated CITED2 (216–242) peptide to the pre-formed TAZ1–HIF-$\alpha$s complex is relatively weak, as TAZ1 is in the HIF-$\alpha$s-bound conformation (orange structure in Extended Data Fig. 10). Importantly, binding of the truncated HIF-$\alpha$s peptide lacking the LPQL motif (HIF-$\alpha$ (796–826)) to the TAZ1–CITED2 complex appears to be even weaker, with negligibly small changes observed in the HSQC spectrum of the $^{15}N$-TAZ1–CITED2 complex on addition of a fivefold excess of HIF-$\alpha$s peptide (Fig. 4c, Extended Data Fig. 8), suggesting that the conformation of TAZ1 in complex with CITED2 (blue structure in Extended Data Fig. 10) does not favour binding of HIF-$\alpha$s (796–826). This contrasts markedly with binding of HIF-$\alpha$s (796–826) to free TAZ1, which occurs with a $K_d$ of $1.9 \pm 0.1 \mu M$ (Extended Data Fig. 2) and leads to large chemical shift changes in the TAZ1 HSQC spectrum (Extended Data Fig. 6). Thus, when TAZ1 is in the CITED2-bound conformation, the affinity for binding the $\alpha_B$–$\alpha_C$ region of HIF-$\alpha$s is greatly decreased.

Our data suggest that HIF-$\alpha$s and CITED2 function synergistically, forming a hypersensitive unidirectional switch that stimulates release of HIF-$\alpha$s from its complex with TAZ1 to maintain tight control of the transcriptional response to hypoxia. In the absence of such synergy, a large excess of CITED2 would be required to fully displace HIF-$\alpha$s (hatched line in Fig. 2c); however, CITED2 fully displaces HIF-$\alpha$s at close to equimolar concentration, creating a highly responsive feedback circuit. These data suggest a mechanistic model, illustrated schematically in Fig. 4d, that involves elements of direct competition, allostery and differential thermodynamic coupling between distinct binding motifs within the intrinsically disordered CITED2 and HIF-$\alpha$s peptides. The apparent equilibrium constant for displacement of HIF-$\alpha$s by CITED2 ($K_{d,\text{app}} = 0.2 \mu M$) does not represent the affinity with which CITED2 binds to the TAZ1–HIF-$\alpha$s complex, but is the product of the equilibrium constants for the individual steps. In this model, the CITED2 transactivation domain binds transiently to the TAZ1–HIF-$\alpha$s complex through its N-terminal region, displacing the dynamic and weakly interacting $\alpha_B$ helix of HIF-$\alpha$s, then competing through an intramolecular process for binding to the LP(Q/E)L site. Plasticity in TAZ1 (denoted by changes in helix length and angle in Fig. 4d) is vital to the operation of the allosteric switch. The CITED2 $\alpha_A$ and LPQL motifs act cooperatively to strengthen the interactions between CITED2 and the TAZ1–HIF-$\alpha$s complex, displace the HIF-$\alpha$s LPQL motif, and shift the TAZ1 conformational ensemble towards the structure in the CITED2-bound state (Extended Data Fig. 10), thereby
The conserved LP(Q/E)L motif mediates competition between HIF–1α and CITED2. a, Amino acid sequences of HIF–1α (top) and CITED2 (bottom) transactivation domain peptides and locations of helical motifs. The LP(Q/E)L motif is shown in red. Truncated constructs are indicated by coloured bars. b, Superimposed 1H–15N HSQC spectra of 100 μM 15N-TAZ1 in the presence of one molar equivalent of HIF–1α peptide (black), five molar equivalents of CITED2(216–242) (green), and one molar equivalent of HIF–1α peptide plus one (gold), three (orange), or five (magenta) molar equivalents of CITED2(216–242). c, Superimposed 1H–15N HSQC spectra of 100 μM 15N-TAZ1 in the presence of one molar equivalent of CITED2 peptide (cyan), five molar equivalents of HIF–1α (796–826) (purple), and one molar equivalent of CITED2 peptide plus one (gold), three (orange), or five (magenta) molar equivalents of HIF–1α (796–826). The cyan, gold, orange and magenta spectra cross peaks are almost exactly superimposed. d, Schematic mechanism for displacement of HIF–1α from its complex with TAZ1 by CITED2. The αA, α3 and αC helices of TAZ1 are represented as grey cylinders, HIF–1α as cyan, LPEL motifs of CITED2 as magenta, and CBP/p300 as purple. TAZ1–LPEL–HIF–1α complexes (Fig. 3a), resulting in weak coupling and limited binding cooperativity. The HIF–1α αA and αC regions bind extremely weakly to the TAZ1–CITED2 complex (Fig. 4c) and, as coupling between them is weak, HIF–1α is ineffective in displacing CITED2 from the TAZ1–CITED2 complex.

Fine-tuning of transcriptional output is especially critical in the case of hypoxia as cells must respond rapidly to changes in oxygen levels and uncontrolled expression of oxygen stress genes would lead to unacceptable levels of tissue damage. Here we show that HIF–1α and CITED2 function as a fine-tuned molecular on/off switch for the hypoxic response. This function is entirely dependent on their intrinsic disorder, which allows them to undergo energetically and dynamically heterogeneous interactions with their common target, the TAZ1 domain of CBP/p300. The unique characteristics of intrinsically disordered proteins make them well-suited for allosteric regulation of cellular signalling. Given the prevalence of disorder in the proteome and the pleiotropic nature of cellular hub proteins such as CBP/p300, we anticipate that the mechanism described here may represent a general regulatory strategy used by intrinsically disordered proteins to compete for binding of common molecular targets in response to specific stimuli throughout the cell cycle. The existence of such allosteric regulatory mechanisms has important implications for systems biology, since computational modelling of cellular networks on the basis of widely available binary dissociation constants would be invalid when allosterism is involved.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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1. Semenza, G. L. Oxygen sensing, hypoxia-inducible factors, and disease pathophysiology. Annu. Rev. Pathol. 9, 47–71 (2014).
2. Henze, A.-T. & Acker, T. Feedback regulators of hypoxia-inducible factors and their role in cancer biology. Cell Cycle 9, 2821–2835 (2010).
3. Wang, G. L., Jiang, B. H., Rue, E. A. & Semenza, G. L. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc. Natl Acad. Sci. USA 92, 5510–5514 (1995).
4. Arany, Z. et al. An essential role for p300/CBP in the cellular response to hypoxia. Proc. Natl Acad. Sci. USA 93, 12969–12973 (1996).
5. Dames, S. A., Martinez-Yamout, M., De Guzman, R. N., Dyson, H. J. & Wright, P. E. Structural basis for Hif-1α/CBP recognition in the cellular hypoxic response. Proc. Natl Acad. Sci. USA 99, 5271–5276 (2002).
6. Freedman, S. J. et al. Structural basis for recruitment of CBP/p300 by hypoxia-inducible factor-1 α. Proc. Natl Acad. Sci. USA 99, 5367–5372 (2002).
7. Bhattacharya, S. et al. Functional role of p35Srf, a novel p300/CBP binding protein, during transactivation by HIF-1. Genes Dev. 13, 64–75 (1999).
8. De Guzman, R. N., Martinez-Yamout, M. A., Dyson, H. J. & Wright, P. E. Interaction of the TAZ1 domain of the CREB-binding protein with the activation domain of CITED2: regulation by competition between intrinsically unstructured ligands for non-identical binding sites. J. Biol. Chem. 279, 3049–3056 (2004).

9. Freedman, S. J. et al. Structural basis for negative regulation of hypoxia-inducible factor-1α by CITED2. Nat. Struct. Biol. 10, 504–512 (2003).

10. Wright, P. E. & Dyson, H. J. Intrinsically disordered proteins in cellular signalling and regulation. Nat. Rev. Mol. Cell Biol. 16, 18–29 (2015).

11. Berlow, R. B., Dyson, H. J. & Wright, P. E. Functional advantages of dynamic protein disorder. FEBS J. 285, 2433–2440 (2015).

12. Van Roey, K. et al. Short linear motifs: ubiquitous and functionally diverse protein interaction modules directing cell regulation. Chem. Rev. 114, 6733–6778 (2014).

13. Jaakkola, P. et al. Targeting of HIF-1α to the von Hippel–Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. Science 292, 468–472 (2001).

14. Lando, D., Peet, D. J., Whelan, D. A., Gorman, J. J. & Whitelaw, M. L. Asparagine hydroxylation of the HIF transactivation domain: a hypoxic switch. Science 295, 858–861 (2002).

15. Jewell, U. R. et al. Induction of HIF-1α in response to hypoxia is instantaneous. FASEB J. 15, 1312–1314 (2001).

16. Kohn, K. W. et al. Properties of switch-like bioregulatory networks studied by simulation of the hypoxia response control system. Mol. Biol. Cell 15, 3042–3052 (2004).

17. Shin, D. H. et al. CITED2 mediates the paradoxical responses of HIF-1α to proteasome inhibition. Oncogene 27, 1939–1944 (2008).

18. Lee, C. W., Ferreon, J. C., Ferreon, A. C., Arai, M. & Wright, P. E. Graded enhancement of p53 binding to CREB-binding protein (CBP) by multisite phosphorylation. Proc. Natl Acad. Sci. USA 107, 19290–19295 (2010).

19. Roehrl, M. H. A., Wang, J. Y. & Wagner, G. A general framework for development and data analysis of competitive high-throughput screens for small-molecule inhibitors of protein-protein interactions by fluorescence polarization. Biochemistry 43, 16066–16066 (2004).

20. Motlagh, H. N., Li, J., Thompson, E. B. & Hilser, V. J. Interplay between allosterism and intrinsic disorder in an ensemble. Biochem. Soc. Trans. 40, 975–980 (2012).

21. Ferreon, A. C., Ferreon, J. C., Wright, P. E. & Deniz, A. A. Modulation of allosteric disorder by protein intrinsic disorder. Nat. Rev. Mol. Cell Biol. 14, 390–394 (2013).

22. Garcia-Pino, A. et al. Allostery and intrinsic disorder mediate transcription regulation by conditional cooperativity. Cell 142, 101–111 (2010).

23. Motlagh, H. N., Wrabl, J. O., Li, J. & Hilser, V. J. The ensemble nature of allostery. Nature 508, 331–339 (2014).

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Methods preparation. All HIF-1α and CITED2 constructs (HIF-1α residues 776–826, HIF-1α residues 796–826; CITED2 residues 216–269; CITED2 residues 216–242) were expressed in E. coli BL21 (DE3) (DNAY) as His-tagged GB1 fusion proteins in a coexpression vector with TAZ1 (residues 340–439 of mouse CBP)34. Cell pellets were resuspended in 40 ml of buffer containing 25 mM Tris (pH 8.0), 200 mM NaCl, 8 M urea, and 20 mM imidazole per litre of culture and lysed by sonication. The soluble fraction was isolated by centrifugation. The supernatant was purified by nickel affinity chromatography using NiNTA resin and the HIF-1α and CITED2 peptides were separated from the His-, GB1 tag by thrombin cleavage on the resin. The cleaved HIF-1α and CITED2 peptides were further purified by reversed phase HPLC, using a C4 cartridge (Waters) in standard acetonitrile/TFA mobile phase. Pure HIF-1α and CITED2 peptides were lyophilized and stored at −80 °C. Lyophilized peptides were dissolved in 50–100 mM Tris buffer (pH 8.2) and dialysed overnight in buffer containing 20 mM Tris (pH 6.8), 50 mM NaCl, and 2 mM diithiothreitol (DTT) before use. TAZ1 was expressed and purified under native conditions as described previously35, with additional purity achieved by size-exclusion chromatography on a Superdex 75 column (GE Healthcare) in buffer containing 20 mM Tris (pH 8.0), 200 mM NaCl, and 2 mM DTT. Uniform isotopic labelling of [15N-HIF-1α], [15N-CITED2], and [15N]-TAZ1 for NMR experiments was performed by expression in minimal media containing [15N] ammonium chloride (0.5 g l−1) and [15N] ammonium sulfate (0.5 g l−1) as the sole nitrogen sources. HIF-1α constructs for fluorescence experiments required additional mutations to allow single-site labelling. The full-length HIF-1α transactivation domain construct (residues 776–826) contains two cysteine residues at positions 780 and 800. A non-perturbing C808V point mutation36 was introduced to allow for fluorescent labelling of HIF-1α at position 780. The native sequence of the truncated HIF-1α construct (residues 796–826) does not contain any cysteine residues. The HIF-1α C terminus was extended by three residues (Gly-Ser-Cys) to introduce an exposed cysteine residue for labelling. In this extended HIF-1α truncation construct, the C-terminal residue of HIF-1α (Asn826) was mutated to Asp to preserve the native negative charge in this region. All single-Cys HIF-1α mutants were labelled with a 3–5-fold molar excess of AlexaFluor 488 or AlexaFluor 594 maleimide dye (Molecular Probes) in 50 mM Tris (pH 7.2), 2 mM TCEP. The labelling reaction was carried out overnight at 4 °C. Dye-labelled HIF-1α peptides were separated from free dye and unlabelled peptide on an analytical C18 reverse-phase HPLC column. Full-length CITED2 was labelled with AlexaFluor 488 or 594 at Cys 261 in the same manner.

GST–TAZ1 for bio-layer interferometry assays was obtained by cloning the TAZ1 sequence (mouse CBP residues 340–439) into the PGEX-4T2 vector. Soluble GST–TAZ1 was purified by affinity chromatography on a glutathione sepharose 4B column followed by size-exclusion chromatography on a Superdex 75 column (GE Healthcare).

Concentration measurements. Protein concentrations were determined by absorbance at 280 nm. For CITED2 (216–242), which does not contain tyrosine or tryptophan residues, concentrations were determined by absorbance at 205 nm21. To ensure that concentrations determined from absorbance measurements at 280 nm and 205 nm are in agreement, we measured the absorbance at both wavelengths for proteins containing tryptophan and tyrosine residues. Protein concentrations determined by both methods were in excellent agreement, with less than 5% variation between concentrations determined by absorbance at 280 nm and 205 nm.

NMR spectroscopy. All NMR samples were prepared in buffer containing 20 mM Tris (pH 6.8), 50 mM NaCl, 2 mM DTT, and 5% D2O. Spectra were recorded at 25 °C on Bruker 500, 600 and 900 MHz spectrometers. NMR data were processed using NMRPipe37 and analysed using SPARKY29. Data collected in the first 2 ms were removed before fitting to account for the Protein Data Bank under accession number 1L8C and the corresponding NOE measurements in the presence of cross-correlated relaxation.

Fluorescence anisotropy. Fluorescence anisotropy binding and competition assays were carried out in buffer containing 20 mM Tris (pH 6.8), 50 mM NaCl, and 2 mM DTT at 25 °C on an ISS-PC1 photon-counting steady-state fluorimeter. Dissociation constants (Kd) for HIF-1α(776–826), CITED2(216–269), and CITED2(216–242) binding to TAZ1 were determined by a competition method18,19 to enable detection of high-affinity binding and account for any effects of labelling. In this method, 20 nM labelled HIF-1α(776–826) or CITED2(216–269) was initially bound to 250 nM TAZ1. Unlabelled HIF-1α and CITED2 peptides were titrated into the pre-formed complex of labelled peptide with TAZ1 and anisotropy of the fluorescent HIF-1α or CITED2 peptide was recorded at each concentration of competing unlabelled peptide. Data were fit as previously described18 using Prism 7.0 (GraphPad Software, Inc.). HIF-1α(796–826) was labelled as described above and unlabelled TAZ1 was titrated directly into a cuvette containing 20 nM fluorescent-labelled HIF-1α(796–826). Anisotropy measurements were carried out after each addition of TAZ1 and the data were fit to a standard one-site binding model using Prism 7.0 (GraphPad Software, Inc.). Anisotropy measurements were carried out three times using different protein preparations. Each competition experiment was performed under carefully controlled conditions, using the same stock solutions of CITED2 and HIF-1α peptides to displace bound, fluorescently labelled CITED2 and HIF-1α peptides from TAZ1. In this way potential errors arising from slight variations in peptide concentration are eliminated. Reported anisotropy values are the average of the three independent measurements, with error bars depicting the standard deviation.

Time-resolved fluorescence measurements. Kinetic curves describing the competition of unlabelled HIF-1α and CITED2 peptides (12.5–100 μM, after mixing) with a pre-formed complex of AlexaFluor 488-labelled HIF-1α peptide and unlabelled TAZ1 (0.25 μM after mixing) were obtained by measuring the fluorescence intensity of the labelled HIF-1α peptide as a function of time after mixing with the competing unlabelled peptide. All samples were prepared in buffer containing 20 mM Tris (pH 6.8), 50 mM NaCl, and 2 mM DTT. For manual mixing fluorescence experiments, samples were mixed by hand in a quartz cuvette and kinetic traces were obtained by monitoring the fluorescence intensity at 517 nm with an excitation wavelength of 494 nm for 200 s on an ISS-PC1 photon-counting steady-state fluorimeter. The delay between mixing and fluorescence detection was 10 s. Observed rates (kobs) were obtained by fitting the kinetic traces to a single exponential decay function using Prism 7.0 (GraphPad Software, Inc.). Each curve was measured three times and reported rates are the average and standard deviation of the three independent measurements.

Stopped-flow kinetic traces were obtained using an Applied Photophysics DX-17 stopped flow spectrophotometer operating at 25 °C. Fluorescence intensity was measured for 1–10 s after rapid mixing using an excitation wavelength of 488 nm and a 515 nm long-pass cutoff filter for AlexaFluor 488-labelled samples. Data collected in the first 2 ms were removed before fitting to account for the instrumental dead time. 5–10 kinetic traces were averaged and the data were fit to a single exponential decay function using Prism 7.0 (GraphPad Software, Inc.). Data at each concentration of peptide were measured three times and the reported rates are the average and standard deviation of the three independent measurements.

Data availability. All structures referred to in this work have been previously published24,25,26,27,28,29,30,31. Coordinates for the TAZ1–HIF-1α complex are available from the Protein Data Bank under accession number 1L8C and the corresponding NMR data are available under BMRB accession number 5327. Coordinates for the TAZ1–CITED2 (ref. 8) complex are available from the Protein Data Bank under accession number 1R8U and the corresponding NMR data are available under BMRB accession number 5987. Resistance assignments for the isolated TAZ1 domain of CBP/p300 are available under BMRB accession number 6268 (ref. 25).

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Extended Data Figure 1 | Representative $^1$H–$^{15}$N HSQC spectra of $^{15}$N-TAZ1, $^{15}$N-TAZ1 bound to HIF-1α, and $^{15}$N-TAZ1 bound to CITED2. Superimposed $^1$H–$^{15}$N HSQC spectra are shown for $^{15}$N-TAZ1 in black, $^{15}$N-TAZ1 bound to HIF-1α (residues 776–826) in orange, and $^{15}$N-TAZ1 bound to CITED2 (residues 216–269) in blue. Selected cross peaks are labelled with residue assignments. The tryptophan indole resonances are shown as an inset in the lower left corner.
Extended Data Figure 2 | Determination of binding affinities for HIF-1α and CITED2 peptides by fluorescence anisotropy and bio-layer interferometry (Octet). a, Fluorescence anisotropy data for titration of unlabelled HIF-1α peptide into a pre-formed complex of AlexaFluor-594-labelled HIF-1α peptide and unlabelled TAZ1. b, Fluorescence anisotropy data for titration of unlabelled CITED2 peptide into a pre-formed complex of AlexaFluor-594-labelled CITED2 peptide and unlabelled TAZ1. c, Fluorescence anisotropy data for titration of unlabelled CITED2 (216–242) into a pre-formed complex of AlexaFluor-594-labelled CITED2 peptide and unlabelled TAZ1. d, Fluorescence anisotropy data for titration of unlabelled TAZ1 into AlexaFluor-488-labelled HIF-1α (796–826). Data shown represent the average (circles) and standard deviation (error bars) of three independent measurements (a–d). e, Representative bio-layer interferometry (Octet) data for HIF-1α(776–826) binding to GST–TAZ1. Data are shown in blue for three concentrations of HIF-1α as marked. The red lines are the result of fitting the data globally to obtain a shared $K_d$ value for the three concentrations shown. f, Representative bio-layer interferometry (Octet) data for CITED2(216–269) binding to GST–TAZ1. Data are shown in blue for three concentrations of CITED2 as marked. The red lines are the result of fitting the data globally to obtain a shared $K_d$ value for the three concentrations shown. g, Tabulated $K_d$ values for the HIF-1α and CITED2 peptides included in this study. ND, not determined. The reported $K_d$ values are the average and standard deviation of the $K_d$ values obtained from nonlinear least squares fitting of at least three independent sets of experimental data.
Extended Data Figure 3 | Monitoring HIF-1α and CITED2 competition for 15N-TAZ1 binding by NMR spectroscopy. a, Full 1H-15N HSQC spectra from NMR competition experiments with HIF-1α and CITED2 transactivation domain peptides. Superimposed spectra are shown for 15N-TAZ1 in the presence of one molar equivalent of HIF-1α (black), one molar equivalent of CITED2 (cyan), and one molar equivalent of both HIF-1α and CITED2 peptides (red, with fewer contours displayed for visibility). The tryptophan indole amide resonances are shown as an inset in the lower left corner. b, Detailed view of selected 15N-TAZ1 resonances. The spectral region highlighted in b is marked by the dotted lines on the full spectra in a. The spectra are displayed as described for a.
Extended Data Figure 4 | Monitoring CITED2 competition for TAZ1 binding by stopped-flow fluorescence. a, Representative time-resolved fluorescence data for rapid mixing of unlabelled CITED2 peptide with AlexaFluor-488-labelled HIF-1α peptide in a pre-formed complex with unlabelled TAZ1 (complex concentration = 0.25 μM). The data shown are the average of 10 shots. The concentrations of CITED2 used in each experiment are indicated in the upper left corner of each graph. The red lines are fits to a single exponential function, and the residuals from fitting are shown below the graph of the data obtained at each concentration of CITED2. b, Concentration dependence of observed rates ($k_{obs}$) from time-resolved fluorescence experiments monitoring CITED2 competition for TAZ1 binding, determined from stopped-flow fluorescence measurements. The data shown represent the average (circles) and standard deviation (error bars) of three independent measurements. The solid red line is the result of fitting to a linear function.
Extended Data Figure 5 | Monitoring HIF-1α competition for TAZ1 binding by fluorescence. a, Representative time-resolved fluorescence data for mixing of unlabelled HIF-1α peptide with AlexaFluor-488-labelled HIF-1α peptide in a pre-formed complex with unlabelled TAZ1 (complex concentration = 0.25 μM). The data shown are the average of 3 independent measurements. The concentrations of HIF-1α peptide used in each experiment are indicated in the upper left corner of each graph. The red lines are fits to a single exponential function, and the residuals from fitting are shown below the graph of the data obtained at each concentration of HIF-1α. b, Concentration dependence of observed rates ($k_{obs}$) from time-resolved fluorescence experiments monitoring HIF-1α competition for TAZ1 binding, determined by standard fluorescence intensity measurements. The data shown represent the average (circles) and standard deviation (error bars) of three independent measurements. The solid black line is the result of fitting to a linear function.
Extended Data Figure 6 | Binding of full-length and truncated HIF-1α and CITED2 transactivation domain peptides to $^{15}$N-TAZ1.

a, c, Representative regions from superimposed $^1$H–$^{15}$N HSQC spectra of $^{15}$N-TAZ1 bound to HIF-1α and CITED2 peptides. In a, free $^{15}$N-TAZ1 is shown in grey, $^{15}$N-TAZ1 bound to HIF-1α(796–826) is shown in red, and $^{15}$N-TAZ1 bound to HIF-1α(776–826) is shown in black. In c, $^{15}$N-TAZ1 is free (grey), bound to CITED2(216–242) (red), and bound to CITED2 (216–269) (black).

b, d, Weighted average $^1$H–$^{15}$N chemical shift changes ($\Delta\delta_{av}$) for each $^{15}$N-TAZ1 residue upon addition of HIF-1α(776–826) (black) and HIF-1α(796–826) (red). d, Weighted average $^1$H–$^{15}$N chemical shift changes ($\Delta\delta_{av}$) for each $^{15}$N-TAZ1 residue upon addition of CITED2(216–269) (black) and CITED2(216–242) (red). Weighted average $^1$H–$^{15}$N chemical shift changes were calculated as $\Delta\delta_{av} = ((\Delta\delta_H)^2 + (\Delta\delta_N/5)^2)^{1/2}$.
Extended Data Figure 7 | Monitoring HIF-1α and CITED2(216–242) competition for 15N-TAZ1 binding by NMR spectroscopy. a, Full 1H–15N HSQC spectra from NMR competition experiments with HIF-1α peptide and CITED2(216–242). Superimposed spectra are shown for 15N-TAZ1 in the presence of one molar equivalent of HIF-1α peptide (black), five molar equivalents of CITED2(216–242) (green), and one molar equivalent of HIF-1α peptide plus one (gold), three (orange), and five (magenta) molar equivalents of CITED2(216–242). The tryptophan indole amide resonances are shown as an inset in the lower left corner. 
b, Detailed view of selected 15N-TAZ1 resonances. The spectral region highlighted in b is marked by the dotted lines on the full spectra in a. The spectra are displayed as described for a. 
c, Weighted average 1H–15N chemical shift changes (Δδav) for each 15N-TAZ1–HIF-1α residue upon addition of one (gold), three (orange), or five (magenta) molar equivalents of CITED2(216–242). Weighted average 1H–15N chemical shift changes were calculated as Δδav = ((ΔδH)² + (ΔδN/5)²)½.
Extended Data Figure 8 | Monitoring CITED2 and HIF-1α(796–826) competition for \(^{15}\text{N}-\text{TAZ1}\) binding by NMR spectroscopy. a, Full \(^1\text{H}–^{15}\text{N}\) HSQC spectra from NMR competition experiments with CITED2 peptide and HIF-1α(796–826). Superimposed spectra are shown for \(^{15}\text{N}-\text{TAZ1}\) in the presence of one molar equivalent of CITED2 peptide (cyan), five molar equivalents of HIF-1α(796–826) (purple), and one molar equivalent of CITED2 peptide plus one (gold), three (orange), and five (magenta) molar equivalents of HIF-1α(796–826). The tryptophan indole amide resonances are shown as an inset in the lower left corner. b, Detailed view of selected \(^{15}\text{N}-\text{TAZ1}\) resonances. The spectral region highlighted in b is marked by the dotted lines on the full spectra in a. The spectra are displayed as described for a. c, Weighted average \(^1\text{H}–^{15}\text{N}\) chemical shift changes (\(\Delta \delta_{\text{av}}\)) for each \(^{15}\text{N}-\text{TAZ1–CITED2}\) residue upon addition of one (gold), three (orange), or five (magenta) molar equivalents of HIF-1α(796–826). Weighted average \(^1\text{H}–^{15}\text{N}\) chemical shift changes were calculated as \(\Delta \delta_{\text{av}} = ((\Delta \delta_H)^2 + (\Delta \delta_N/5)^2)^{1/2}\).
Extended Data Figure 9 | Location of spectral changes for 15N-TAZ1–HIF-1α upon titration with the CITED2(216–242) peptide. 15N-TAZ1–HIF-1α resonances that shift and/or broaden upon addition of an equimolar amount of CITED2(216–242) are mapped onto the structure of the TAZ1–HIF-1α complex as red spheres. TAZ1 (residues 340–439) is shown in grey and the HIF-1α transactivation domain is shown in orange (residues 776–826). The expected structure of the CITED2(216–242) peptide in complex with TAZ1 is shown in blue. Structural motifs of HIF-1α and CITED2 are labelled for reference.
Extended Data Figure 10 | Structural differences in the TAZ1 domain of CBP upon binding HIF-1α and CITED2. Superposition of the TAZ1 structures in complex with HIF-1α (orange) and CITED2 (blue). The structures of the bound HIF-1α and CITED2 peptides are omitted for clarity, and the TAZ1 helices are labelled for reference.