SUPPLEMENTARY DATA

ASPARAGINE β-HYDROXYLATION STABILIZES THE ANKYRIN REPEAT DOMAIN FOLD

Figure S1. Stimulation of FIH mediated 2OG decarboxylation, as measured by $^{14}CO_2$ formation normalized against the turnover stimulated by HIF-1α (788-806). Assays were performed in triplicate and the results averaged. Background counts observed in the absence of FIH were subtracted. Error bars indicate one standard deviation.

Figure S2. MS/MS and LC-MS (inset) analysis of 1CA incubated in the absence (top) and presence (bottom) of FIH; peaks with m/z = 721.45 and 726.77 correspond to the $[M+3H]^{3+}$ of unhydroxylated and hydroxylated 1CA. A +16 Da shift is observed in the y ion series appearing at y_5, corresponding to fragments containing hydroxylated Asn. A slight decrease in retention time was observed for the hydroxylated peptide, consistent with previous reports (1,2).

Figure S3. Stimulation of FIH mediated 2OG decarboxylation, as measured by $^{14}CO_2$ formation normalized against the turnover stimulated by the IκBα ARD. Assays were performed in triplicate and averaged. Error bars indicate one standard deviation.

Figure S4. Extent of hydroxylation quantified by tryptic digestion followed by LC-MS analysis for $3CA_{1A2N}$ hydroxylation compared to that of recombinant IκBα and mouse Notch 1 (N1) ARD.

Figure S5. Stereoview close up of the $3CA_{1A2N\text{ -OH}}$ structure illustrating the $|F_o\text{-}F_c|$ difference electron density contoured to 3σ (green mesh) of the Asn-84 β hydroxyl group positioned to form a hydrogen bond (2.9Å) with the Asp-82 side-chain carboxylate that may stabilize the type I β-turn within the β-hairpin loop.

Figure S6. Figure S1. Stereo view of the "DVNA" motif of $3CA_{1A2N\text{-OH}}$ in stick representation (white) with experimental |2Fo-Fc| electron density (blue mesh) contoured to 1σ.

Table S1
Diffraction pattern analyses indicated that both 3CA1A2N and His-tagged 3CA1A2N-OH crystallized in the same space group, hexagonal P3121 with each having one molecule in the asymmetric unit. The 3CA1A2N crystal had a 12 Å longer unit cell edge (C) than that of the His-3CA1A2N-OH crystal but both had similar A and B cell edge lengths (~49 Å). This discrepancy in the C cell edge length indicates that the 3CA1A2N crystals have less efficient packing than that of the 3CA1A2N-OH crystal. In fact, the N-terminal His-tag of 3CA1A2N-OH may be partly responsible for the increased packing efficiency as it is positioned along the C-terminal end of helix 1 of each repeat (close to the archetypical binding groove of AR proteins) and also is observed to interact with the C-terminal using two histidines from the His-tag.

1. Cockman, M. E., Lancaster, D. E., Stolze, I. P., Hewitson, K. S., McDonough, M. A., Coleman, M. L., Coles, C. H., Yu, X., Hay, R. T., Ley, S. C., Pugh, C. W., Oldham, N. J., Masson, N., Schofield, C. J., and Ratcliffe, P. J. *Proc Natl Acad Sci USA 2006,* 103(40), 14767-14772
2. Coleman, M. L., McDonough, M. A., Hewitson, K. S., Coles, C., Mecinovic, J., Edelmann, M., Cook, K. M., Cockman, M. E., Lancaster, D. E., Kessler, B. M., Oldham, N. J., Ratcliffe, P. J., and Schofield, C. J. *J Biol Chem* 2007, 282(33), 24027-24038

	$3CA_{1A2N-OH}$	**$3CA_{1A2N}$**
Data collection	SRS Daresbury beamline 14.2	SRS Daresbury beamline 14.2
Wavelength (Å)	0.9790	0.9790
Resolution (Å)	50-1.9 (1.97-1.90)	50-2.0 (2.07-2.00)
Space-group	$P3_121$	$P3_121$
Unit cell (Å)	a=48.9, b= 48.9, c= 67.1	a=49.0, b= 49.0, c= 79.0
Reflections observed/unique	35424 / 7197	58446/ 7815
Mean I/σ (I)	10.7 (3.8)*	22.4 (4.0)*
R_{merge}	0.131 (0.363)*	0.083 (0.444)*
Completeness (%)	93.3 (95.8)*	99.9 (99.7)*
Redundancy	4.9 (4.8)*	7.5 (6.8)*
Refinement		
Resolution (Å)	26.3 – 1.9	28.9 – 2.0
Completeness (%)	89.4	95.6
R_{cryst} / R_{free}	0.173/0.224	0.213/0.238
Rmsd bond length (Å)	0.010	0.011
Rmsd bond angle (°)	1.30	1.20
Numbers of atoms		
Protein [Avg. B_{factor} Å^2]	698 [18.2]	645 [27.7]
Ions [Avg. B_{factor} Å^2]	7 [33.5]	3 [28.9]
Waters [Avg. B_{factor} Å^2]	77 [30.9]	125 [41.5]
Ramachandran Statistics	Core 88.1% Allowed 11.9% Generous 0.0% Disallowed 0.0%	Core 93.5% Allowed 6.5% Generous 0.0% Disallowed 0.0%

*Values in parentheses are for data in the highest resolution shell.

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Figure S1

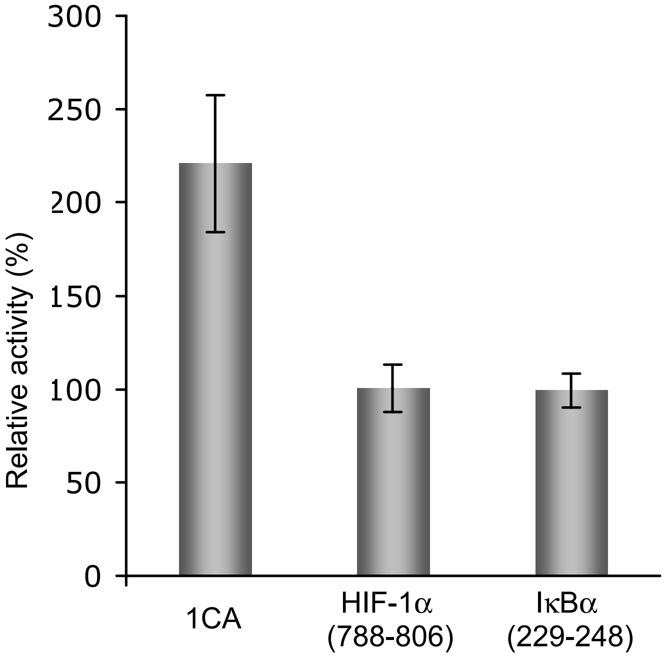
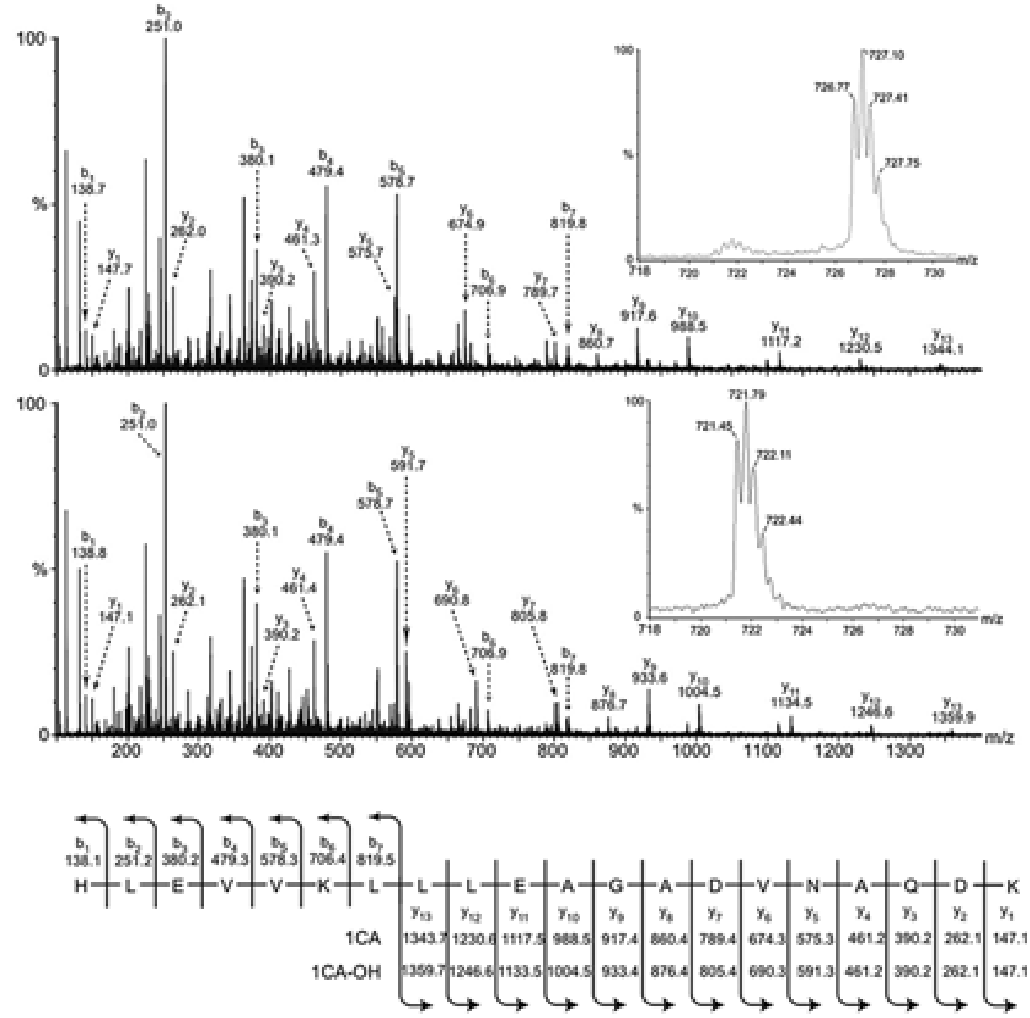


Figure S2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%
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y1 147.7
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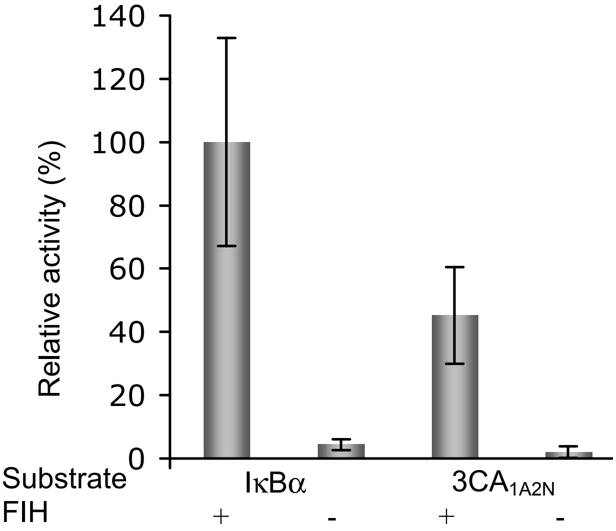
b4 479.4
y5 575.7
b5 578.7
y6 674.9
b6 706.9
y7 789.7
b7 819.8
y8 860.7
y9 917.6
y10 988.5
y11 1117.2
y12 1230.5
y13 1344.1
726.77
727.10
727.41
727.75
718 720 722 724 726 728 730
m/z
b2 251.0
b1 138.8
y1 147.1
y2 262.1
b3 380.1
y3 390.2
y4 461.4
b4 479.4
b5 578.7
y5 591.7
y6 690.8
b6 706.9
y7 805.8
b7 819.8
y8 876.7
y9 933.6
y10 1004.5
y11 1134.5
y12 1246.6
y13 1359.9
721.45
721.79
722.11
722.44
200 300 400 500 600 700 800 900 1000 1100 1200 1300
m/z
b1 138.1
b2 251.2
b3 380.2
b4 479.3
b5 578.3
b6 706.4
b7 819.5
H L E V V K L L L E A G A D V N A Q D K
y13 y12 y11 y10 y9 y8 y7 y6 y5 y4 y3 y2 y1
1CA 1343.7 1230.6 1117.5 988.5 917.4 860.4 789.4 674.3 575.3 461.2 390.2 262.1 147.1
1CA-OH 1359.7 1246.6 1133.5 1004.5 933.4 876.4 805.4 690.3 591.3 461.2 390.2 262.1 147.1

Figure S3



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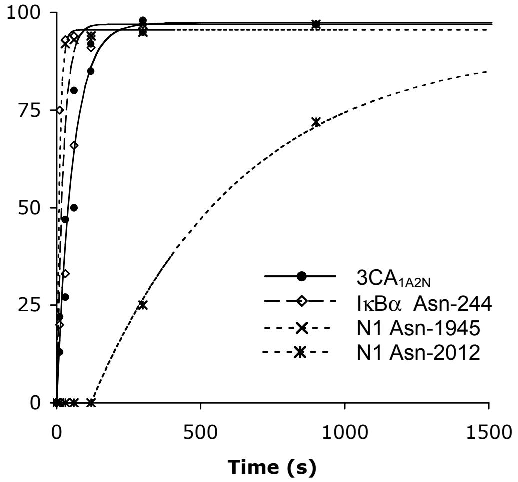

Figure S5

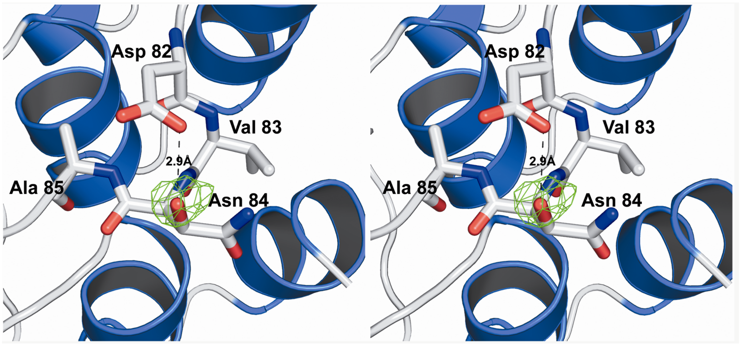

Figure S6