Supporting info

The activity of JmjC histone lysine demethylase KDM4A is highly sensitive to oxygen concentrations

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Contents:

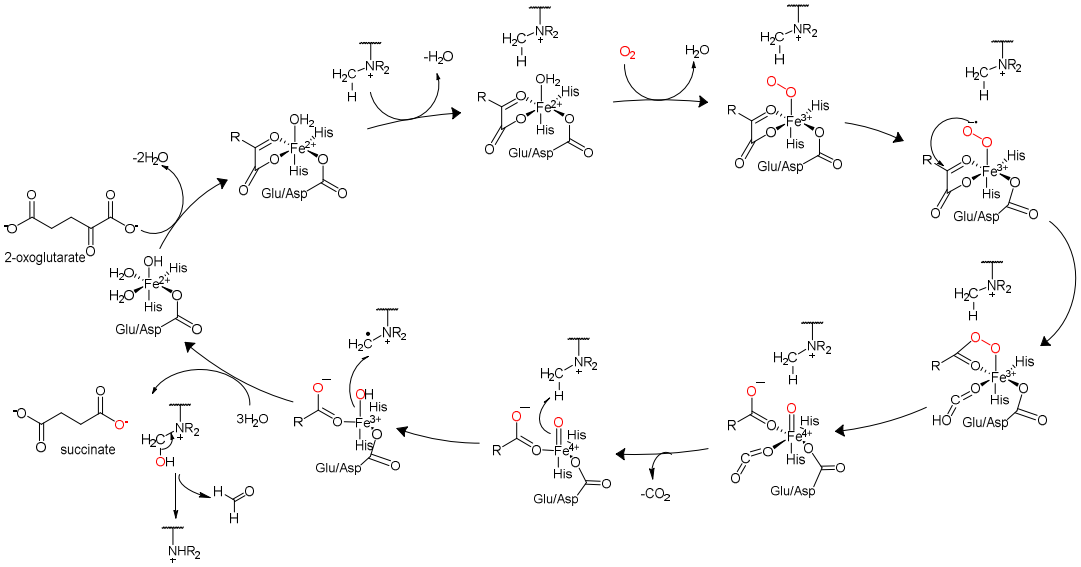


Figure S1. Consensus mechanism for the 2OG oxygenases. 2OG coordinates the Fe^{2+} centre in the active site, prior to binding of substrate and oxygen. 2OG then undergoes a 2-electron oxidation, potentially via a cyclic peroxide, to produce succinate, carbon dioxide and an Fe^{4+} oxo species, and the substrate is subsequently oxidised in a radical rebound mechanism, and released along with succinate to regenerate the catalytic centre. Oxygen atoms deriving from molecular oxygen are highlighted in red. Demethylation of methylated lysines by the JmjC-KDMs produces formaldehyde as a side product upon collapse of the hydroxylated product.

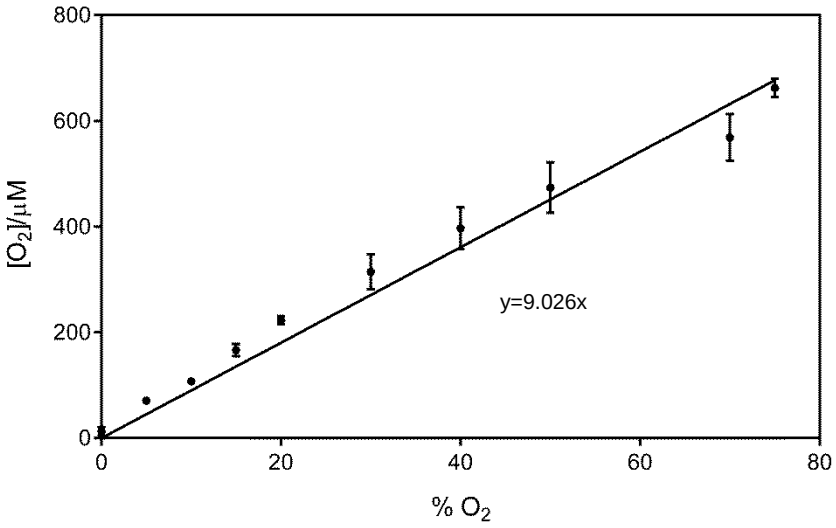


Figure S2. Calibration curve used to calculate dissolved oxygen concentration in the *in vitro* oxygen dependence assay. HEPES buffer in sealed vials was incubated at the desired percentage O_2 using a mass flow controller (Brooks Instruments) for 10 min at 37°C. A FOXY electrode (Ocean Optics) was used to determine dissolved oxygen concentration in ppm, before subsequent conversion to μM. GraphPad Prism was used to fit the curve to a linear regression model. N=3, error bars denote standard deviation.

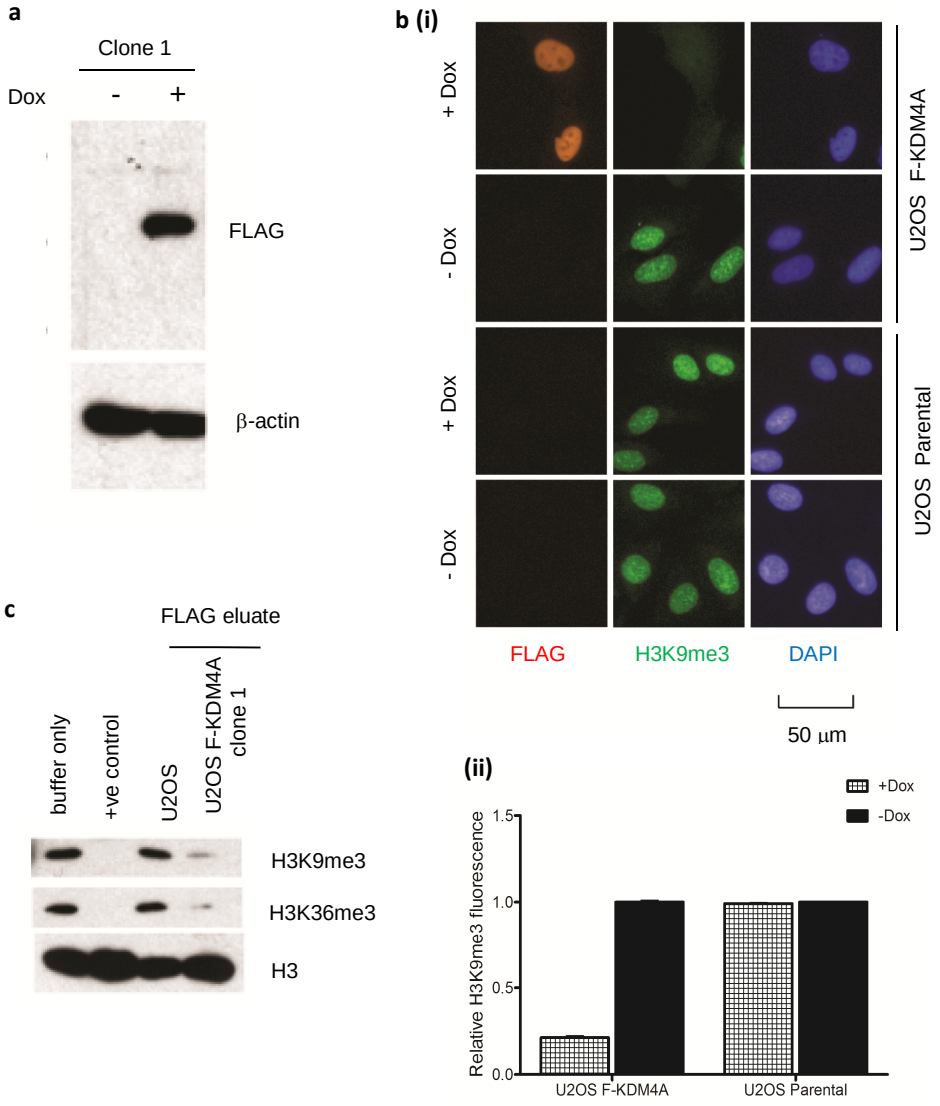
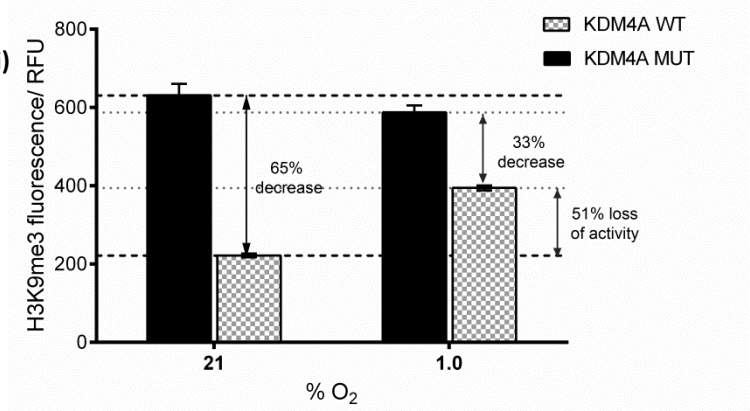


Figure S3. Validation of the U2OS F-KDM4A cell line. (a) Expression of F-KDM4A is dox-inducible. Cells treated - /+ doxycycline (Dox) for 24 h were harvested in SDS-Urea buffer, run on a 9% gel and immunoblotted with the indicated antibodies. Western blots were visualised using ECL substrate. **(b)**(i) Selected images from immunofluorescence analysis of U2OS F-KDM4A and U2OS parental cells treated with +/- dox for 24 h. Cells were fixed and stained for FLAG and H3K9me3, and nuclear co-localisation of KDM4A expression was verified using DAPI. FLAG signal in this clone was heterogenous, hence in experiments, only strongly overexpressing cells (Figure 2, Figure 3, marked with arrows) were considered for analysis. (ii) Quantification of H3K9me3 fluorescence in U2OS F-KDM4A and parental cells treated ± dox. H3K9me3 levels in dox treated cells were calculated relative to that in untreated cells of the same type. **(c)** F-KDM4A purified from cells exhibits histone demethylase activity in vitro. Purified F-KDM4A was prepared as follows. Parental U2OS cells (as control) or U2OS F-KDM4A cells were treated with dox for 24 h and then harvested in Igepal lysis buffer (100 mM NaCl, 0.5% *Igepal* CA630, 20 mM Tris, pH 7.5, 5 mM $MgCl_2$). F-KDM4A was then purified from extracts using anti-FLAG M2-Agarose beads (Sigma), followed by FLAG peptide elution (250 μg mL^{-1} FLAG peptide (Sigma) in 20 mM Tris, pH 7.5, 5 mM KCl, 1 mM $MgCl_2$). FLAG eluate was then tested for histone demethylase activity using reactions containing purified histones (25 μg), ascorbate (2 mM), 2OG (1 mM) and $FeSO_4$ (50 μM). Purified recombinant His_6-tagged $KDM4A_{1-359}$ (10 μg) was used as a positive control. Reactions were incubated at 37 °C for 16 h and then stopped by the addition of Laemmli sample buffer. Reaction products were run on 15% gels and immunoblotted.

a **(i)**



(ii)

(1) $$\%\ \text{demethylation H3K9me3} = 100\left(1-\left(\frac{\text{H3K9me3 fluorescence}_{WT}}{\text{H3K9me3 fluorescence}_{MUT}}\right)\right)$$

(2) $$\text{Relative loss of H3K9me3} = \left(\frac{\%\ \text{demethylation H3K9me3}_{1.0\%\ O_2}}{\%\ \text{demethylation H3K9me3}_{21\%\ O_2}}\right)$$

b

(3) $$\%\ \text{demethylation H3K9me3} = 100\left(1-\left(\frac{\text{H3K9me3 fluorescence}_{+Dox}}{\text{H3K9me3 fluorescence}_{-Dox}}\right)\right)$$

(4) $$\text{Relative loss of H3K9me3} = \left(\frac{\%\ \text{demethylation H3K9me3}_{1.0\%\ O_2}}{\%\ \text{demethylation H3K9me3}_{21\%\ O_2}}\right)$$

Figure S4 Calculation of loss of H3K9me3 and relative KDM4A activity in immunofluorescence experiments. (a) Scheme detailing calculation of percentage demethylation and relative loss of H3K9me3 in transiently transfected HeLa. (i) Graph depicts raw mean fluorescence intensities of H3K9me3 in WT & MUT-transfected cells in both 1.0 & 21 % O_2 for a single biological repeat. FLAG fluorescence signal was used to identify transfected cells (indicated by arrows) and >50 cells were selected for analysis. Error bars denote standard error of the mean. (i) Equations detailing the calculation procedure. (b) Equations used to calculate relative KDM4A activity in U2OS F-KDM4A cells in different oxygen concentrations.

	Experiment	Epitope	Conjugate	Host species	Source & cat no	Dilution
Primary antibodies	IF, Western blot	H3K9me3	--	Rabbit	Abcam, ab8898	1:1000
	IF, Western blot	H3K36me3	--	Rabbit	Abcam, ab9050	1:500
	IF, Western blot	FLAG	--	Mouse M2	Sigma, F1804	1:1000
	Western blot	KDM4A	--	Rabbit	Bethyl Laboratories, A300-861A	1:1000
	Western blot	H3K4me3	--	Rabbit	Diagenode, C15410003-50	1:500
	Western blot	H3K27me3	--	Rabbit	Millipore, 07-449	1:500
	Western blot	H3	--	Mouse	Abcam, ab10799	1:1000
	Western blot	HIF-1α (human)	--	Mouse	BD Transduction Laboratories,	1:1000[a]
	Western blot	β-actin	--	Chicken	Abcam, ab13822	1:500[a]
Secondary antibodies	IF	Rabbit IgG (H+L)	Alexa Fluor® 488	Goat	Life Technologies, A11034	1:500
	IF	Mouse IgG (H+L)	Alexa Fluor® 594	Goat	Life Technologies, A11032	1:500
	Western blot	Rabbit IgG (H+L)	Dylight 680	Goat	Life Technologies, 35568	1:10,000
	Western blot	Mouse IgG (H+L)	Dylight 800	Goat	Life Technologies, 35521	1:10,000
	Western blot	Chicken IgY (H+L)	Dylight 680	Goat	Thermo Fisher Scientific, SA5-10074	1:10,000

Table S1: Antibodies used in immunofluorescence (IF) and western blot experiments. Antibodies were diluted in 3% SEABLOCK blocking reagent (Thermo Fisher Scientific) in PBS with 0.1% Tween-20. *a* antibodies were diluted in 5% milk in PBS with 0.1% Tween-20.

	Forward sequence	Reverse sequence	Reference
KDM4A	GCCGCTAGAAGTTTCAGTGAG	GCGTCCCTTGGACTTCTTATT	[1]
β-actin	GCTGTGCTACGTCGCCCTG	GGAGGAGCTGGAAGCAGCC	

Table S2: Sequences of primers used in RT-qPCR analysis of KDM4A levels.

Supplementary references:

(1) Mallette, F. A.; Richard, S. *Cell Rep.* **2012**, *2* (5), 1233–1243.