Supplementary Information

Studies on the Interaction of the Histone Demethylase KDM5B with Tricarboxylic Acid Cycle Intermediates

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Figure S1. Determination of kinetic parameters for KDM5B with H3K4me2(1-21). A. Conditions: 1 µM KDM5B was incubated with different concentrations of 2OG, 5 µM H3K4me2(1-21), 10 µM Fe(II), 500 µM L-ascorbate in 50 mM HEPES 150 mM NaCl (pH 7.5). B. Conditions: 0.6 µM KDM5B was incubated with 10 µM 2OG, various concentrations of H3K4me2(1-21), 10 µM Fe(II), 500 µM L-ascorbate in 50 mM HEPES 150 mM NaCl pH 7.5. Error bars represent standard deviations for triplicate assays. The levels of methylated and demethylated peptides were analysed by MALDI-TOF-MS.
Figure S2. Inhibition of KDM5B by TCA cycle intermediates. A. pyruvate, B. succinate, C. fumarate, D. malate, E. D-2HG, F. L-2HG, G. oxaloacetate, H. citrate. Assay conditions: 0.6 µM KDM5B was incubated with 3 µM 2OG, 5 µM H3K4me2(1-21), 10 µM Fe(II), 500 µM L-ascorbate in 50 mM HEPES 50 mM NaCl (pH 7.5). Error bars represent standard deviations for triplicate assays. The levels of methylated and demethylated peptides were analysed by MALDI-TOF-MS.
Figure S3: Views from the crystal structures of KDM5B in complex with inhibitors occupying the 2OG binding pocket. Active site of KDM5B (green) in complex with 2OG (yellow) (PDB ID 5FUP) overlaid with (A) KDM5B:KDOAM25A complex (PDB ID 5A3N, silver and magenta respectively) and (B) KDM5A:CPI-455 complex (PDB ID 5A3N, silver and cyan respectively). Metal ions are shown as purple and orange spheres, and water molecules in the KDM5B:KDOAM25 complex as grey spheres. Amino acid residues are labelled as in PDB ID 5FUP. Note the overlap of the inhibitor and 2OG binding sites.
Table S1. Results of $K_i$ determination for succinate using GraphPad Prism 5.0.

The data were globally fitted using a competitive inhibition model.

| Competitive inhibition | Best-fit values | Std. Error | 95% Confidence Intervals | Goodness of Fit |
|-------------------------|-----------------|------------|--------------------------|-----------------|
| $K_m$                   | 3.006 3.006 3.006 3.006 3.006 | 0.5003 0.5003 0.5003 0.5003 0.5003 | 1.989 to 4.023 1.989 to 4.023 1.989 to 4.023 1.989 to 4.023 1.989 to 4.023 | 0.9523 |
| $I$                     | 30.00 30.00 30.00 30.00 30.00 | 5.583 5.583 5.583 5.583 5.583 | 15.42 to 38.13 15.42 to 38.13 15.42 to 38.13 15.42 to 38.13 15.42 to 38.13 | 0.9085 |
| $K_i$                   | 26.77 26.77 26.77 26.77 26.77 | 0.0001377 0.0001377 0.0001377 0.0001377 0.0001377 | 0.004170 to 0.004170 to 0.004170 to 0.004170 to 0.004170 to | 0.9725 |
| $V_{max}$               | 0.004450 0.004450 0.004450 0.004450 0.004450 | 0.0001377 0.0001377 0.0001377 0.0001377 0.0001377 | 0.004730 0.004730 0.004730 0.004730 0.004730 | 0.9713 |

Number of points Analyzed: 9 9 9 10
Table S2. Results of $K_i$ determination for oxaloacetate using GraphPad Prism 5.0. The data were globally fitted using a competitive inhibition model.

| Competitive inhibition | Best-fit values | Std. Error | 95% Confidence Intervals | Goodness of Fit | Constraints |
|------------------------|-----------------|------------|--------------------------|----------------|-------------|
|                        | $K_m$           | $I$        | $K_i$                    | $V_{max}$      |              |
|                        | 5.905           | $= 0.0$    | 54.00                    | 0.006615       | $K_m > 0.0$ and shared | $K_m > 0.0$ and shared |
|                        | 5.905           | $= 15.00$  | 54.00                    | 0.006615       | $K_m > 0.0$ and shared |
|                        | 5.905           | $= 50.00$  | 54.00                    | 0.006615       | $K_m > 0.0$ and shared |
|                        | 5.905           | $= 100.0$  | 54.00                    | 0.006615       | $K_m > 0.0$ and shared |
|                        | $I = 0.0$       | 6.109      | 0.0002073                | 4.860 to 6.950 | $K_m > 0.0$ and shared |
|                        | $I = 15.00$     | 6.109      | 0.0002073                | 4.860 to 6.950 | $K_m > 0.0$ and shared |
|                        | $I = 50.00$     | 6.109      | 0.0002073                | 4.860 to 6.950 | $K_m > 0.0$ and shared |
|                        | $I = 100.0$     | 6.109      | 0.0002073                | 4.860 to 6.950 | $K_m > 0.0$ and shared |
|                        | $V_{max} > 0.0$ | 0.007042   | 0.007042                 | 2.669e-007     | $V_{max} > 0.0$ and shared |
|                        | $V_{max} > 0.0$ | 0.007042   | 0.007042                 | 3.110e-007     | $V_{max} > 0.0$ and shared |
|                        | $V_{max} > 0.0$ | 0.007042   | 0.007042                 | 1.220e-007     | $V_{max} > 0.0$ and shared |
|                        | $V_{max} > 0.0$ | 0.007042   | 0.007042                 | 8.780e-007     | $V_{max} > 0.0$ and shared |
|                        | $V_{max} > 0.0$ | 0.007042   | 0.007042                 | 8.780e-007     | $V_{max} > 0.0$ and shared |
|                        | $V_{max} > 0.0$ | 0.007042   | 0.007042                 | 8.780e-007     | $V_{max} > 0.0$ and shared |
|                        | $V_{max} > 0.0$ | 0.007042   | 0.007042                 | 8.780e-007     | $V_{max} > 0.0$ and shared |

| Degrees of Freedom | 26 |
| R square           | 0.9869 |
| Absolute Sum of Squares | 2.669e-007, 1.782e-007, 3.110e-007, 1.220e-007, 8.780e-007 |
| Sy.x               | 0.0001838 |

Number of points Analyzed: 7, 7, 7, 8
**Table S3. Results of determination of 2OG $K_m^{\text{app}}$ in the presence of different concentrations of succinate and oxaloacetate.** The data were analysed by non-linear regression using GraphPad Prism 5.0.

| Succinate, μM | $V_{m^{\text{app}}}$, μM/s | $K_{m^{\text{app}}}$, μM | Oxaloacetate, μM | $V_{m^{\text{app}}}$, μM/s | $K_{m^{\text{app}}}$, μM |
|--------------|-----------------|----------------|-----------------|-----------------|----------------|
| 0            | 0.0043          | 3.4            | 0               | 0.0062          | 5.0            |
| 30           | 0.0044          | 4.0            | 15              | 0.0066          | 7.2            |
| 100          | 0.0046          | 19.5           | 50              | 0.0070          | 15.0           |
| 300          | 0.0046          | 44             | 100             | 0.0069          | 18.5           |
| 2OG     | ZUP | Succinate | Fumarate | Oxaloacetate | Pyruvate | Malate | D-2HG | L-2HG |
|---------|-----|-----------|----------|--------------|----------|--------|-------|-------|
| Wavelength (Å) | 0.9173 | 0.9173 | 0.9173 | 0.9795 | 0.9795 | 0.9795 | 0.9795 | 0.9173 |
| Resolution range (Å) | 71.17 | 71.07-2.1 | 64.28-2.47 | 57.07-1.87 | 57.13-2.03 | 61.57-1.86 | 61.57-1.86 | 47.88-1.89 |
| | -2.15 | (2.175-2.558) | -2.47 | (1.937-1.87) | (2.103-2.03) | (1.926-1.86) | (1.926-1.86) | (1.958-1.89) |
| | -2.27 | (2.1) | -2.15 | -1.87 | -1.87 | -1.87 | -1.87 | -2.05 |
| | -2.15 | (2.15) | -2.47 | -1.87 | -1.87 | -1.87 | -1.87 | -2.05 |
| Space group | P 6 2 2 | P 6 2 2 | P 6 2 2 | P 6 2 2 | P 6 2 2 | P 6 2 2 | P 6 2 2 | P 6 2 2 |
| Unit cell | α 142.34 | 142.14 | 141.68 | 141.68 | 141.68 | 141.68 | 142.29 | 142.08 |
| | β 142.34 | 142.14 | 141.68 | 141.68 | 141.68 | 141.68 | 142.29 | 142.08 |
| | γ 152.26 | 90.90 | 90.90 | 90.90 | 90.90 | 90.90 | 152.47 | 151.41 |
| | 90.120 | 120 | 120 | 120 | 120 | 120 | 90.120 | 90.120 |
| Completeness (%) | 623357 | 1057061 | 1057061 | 1488447 | 1173428 | 1516065 | 1438569 | 1108750 |
| Unique reflections | 623357 | 1057061 | 1057061 | 1488447 | 1173428 | 1516065 | 1438569 | 1108750 |
| Multiplicity | 193 | 193 | 193 | 193 | 193 | 193 | 193 | 193 |
| Compleness (%) | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Mean l/σ(l) | 29.51 | 19.90 | 10.31 | 15.89 | 15.64 | 15.85 | 15.78 | 15.78 |
| Wilson B-factor | 38.12 | 45.47 | 57.76 | 40.40 | 40.40 | 35.77 | 41.76 | 41.76 |
| R-merge (%) | 0.06963 | 0.1085 | 0.2032 | 0.1285 | 0.1285 | 0.1153 | 0.1097 | 0.1344 |
| R-work (%) | 0.07154 | 0.1114 | 0.2102 | 0.1636 | 0.1184 | 0.1126 | 0.1472 | 0.1472 |
| CC1/2 | 1 (0.954) | 0.999 | 0.994 | 0.999 | 0.999 | 0.999 | 0.999 | 0.999 |
| CC* | 1 (0.988) | 1 | 0.999 | 0.999 | 0.999 | 0.999 | 0.999 | 0.999 |
| R-work‡ | 0.1830 | 0.1977 | 0.2097 | 0.2432 | 0.2334 | 0.2566 | 0.2566 | 0.2566 |
| R-free§ | 0.2259 | 0.2337 | 0.2333 | 0.2333 | 0.2333 | 0.2333 | 0.2333 | 0.2333 |
| Number of non-hydrogen atoms | 4263 | 3935 | 3855 | 4242 | 3904 | 4161 | 4266 | 4256 |
| macromolecules | 3708 | 3667 | 3727 | 3750 | 3762 | 3770 | 3768 | 3762 |
| ligands | 66 | 50 | 60 | 72 | 69 | 68 | 73 | 73 |
| water | 489 | 218 | 68 | 420 | 73 | 323 | 425 | 421 |
| Protein residues | 454 | 465 | 452 | 453 | 453 | 453 | 453 | 453 |
| RMS (bonds) †† | 0.008 | 0.009 | 0.009 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 |
| RMS (angles) †† | 1.09 | 1.10 | 1.20 | 1.06 | 1.18 | 1.10 | 1.10 | 1.10 |
| Ramachandran favored (%) | 98 | 97 | 96 | 98 | 98 | 98 | 97 | 98 |
| Ramachandran outliers (%) | 0 | 0.22 | 0.44 | 0 | 0 | 0 | 0.44 | 0.22 |
| Clashscore | 5.14 | 4.12 | 5.93 | 3.74 | 4.12 | 5.98 | 4.25 | 7.58 |
| Average B-factor (‡) | 44.80 | 50.10 | 65.30 | 44.40 | 48.10 | 43.40 | 43.50 | 48.30 |
| macromolecules | 43.20 | 50.00 | 65.20 | 43.30 | 47.80 | 42.80 | 42.80 | 47.10 |
| ligands | 47.90 | 53.80 | 77.10 | 49.70 | 54.40 | 49.40 | 47.70 | 54.50 |
| solvent | 56.00 | 50.90 | 56.20 | 53.50 | 56.70 | 48.80 | 53.70 | 57.80 |

Statistics for the highest-resolution shell are shown in parentheses.

† $R_{merge} = \frac{\sum_{kkl} \sum_{i} |I_i(hkl) - \langle I(hkl)\rangle|}{\sum_{kkl} \sum_{i} I_i(hkl)}$, where $I_i(hkl)$ is the intensity of the $i$th measurement of reflection $hkl$ and $\langle I(hkl)\rangle$ is the mean value of $I(hkl)$ for all $i$ measurements.

‡ $R_{work} = \frac{\sum_{kkl} |F_{obs} - F_{calc}|}{\sum_{kkl} |F_{calc}|}$, where $F_{obs}$ is the observed structure factor and $F_{calc}$ is the calculated structure factor.

§ $R_{free}$ is the same as $R_{free}$ except calculated with a subset (5%) of data that were excluded from the refinement calculations.

†† Engh Huber (1991).