Structural Mechanism of the Oxygenase JMJD6 Recognition by the Extraterminal (ET) Domain of BRD4

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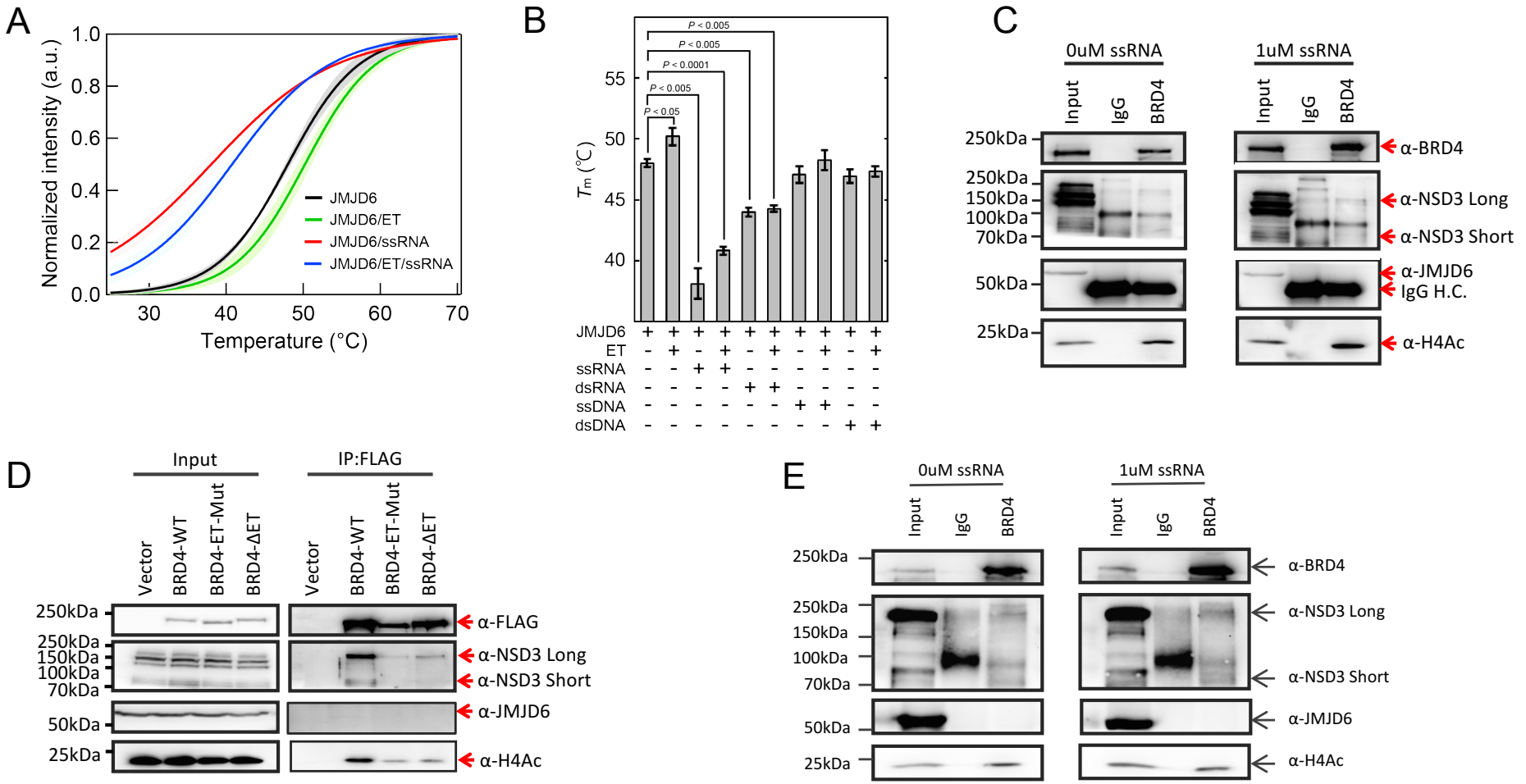
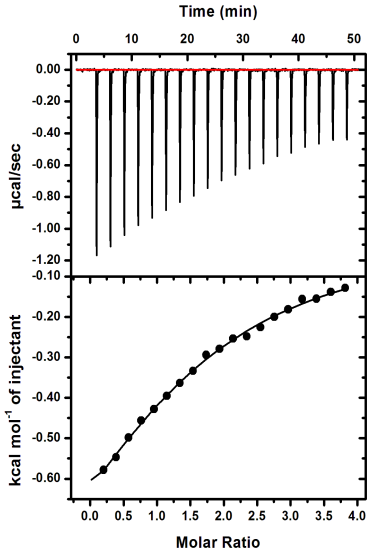


Supplemental Figure 1, (A) Thermal stability of the full-length JMJD6 determined by differential scanning fluorimetry with concentrations of JMJD6 at 1μM and ET at 1μM. The averaged fluorescent intensities were normalized using T_m and *a* obtained by fitting thermal shift data to a theoretical equation (see Experimental procedures). The profiles in black, green, red and blue were obtained from free JMJD6, JMJD6/ET, JMJD6/ssRNA and JMJD6/ET/ssRNA, respectively. The standard deviations of the profiles were calculated from three independent experiments. In control experiments, samples of only ET, ssRNA, dsRNA, ssDNA or dsDNA without JMJD6 had no fluorescent intensity; (B) Thermal stability chart of JMJD6 with/without ET, ssRNA, dsRNA, ssDNA and dsDNA. T_m is presented as mean ± SEM (n = 3). P-values calculated by the t-test indicate a statistically significant difference; (C) Endogenous IP experiments. Nuclear extracts added with or without ssRNA purified from HEK293T cells were subjected to immunoprecipitation (IP) and followed by western blotting; (D) HEK293T cells were transfected with Flag-BRD4WT, Flag-BRD4-Mut and Flag-BRD4-ΔET and cell lysates were treated with ssRNA and subjected to IB analysis; (E) HEK293T cells were transfected with His-JMJD6 and subjected ssRNA treatment and IB analysis.

Supplemental Figure 2, ITC binding affinity assay of JMJD6 peptides



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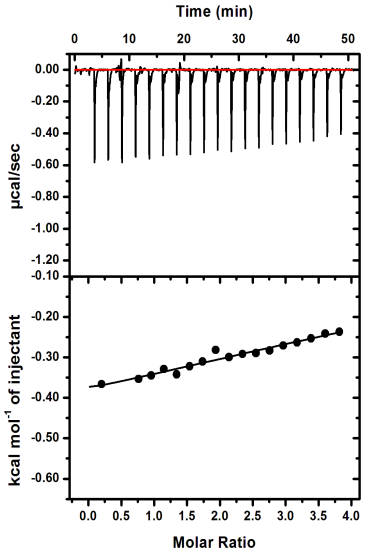

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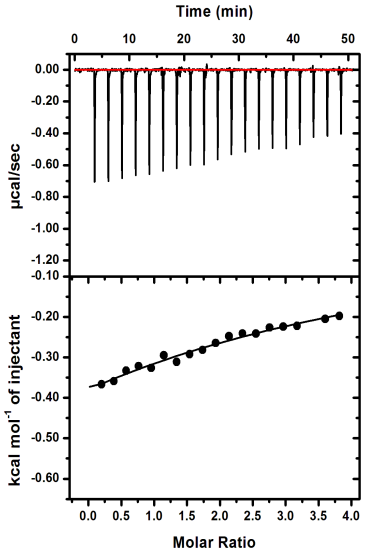

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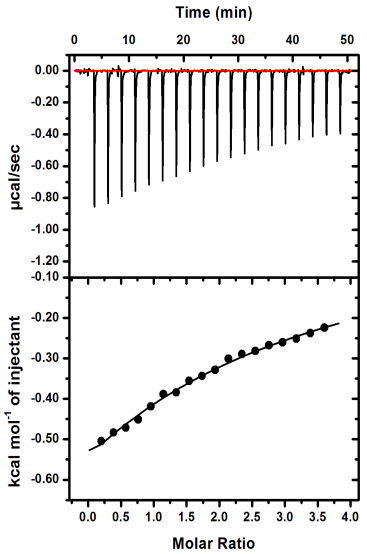
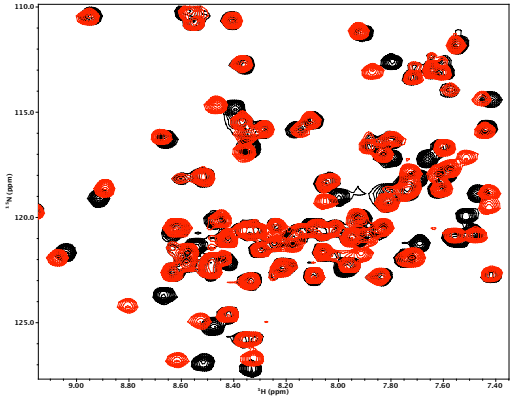


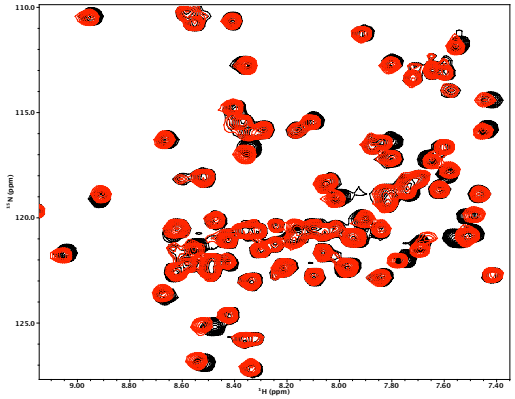
R95A

Peptide Name	Peptide Sequence	ITC Binding (Kd)
JMJD6 WT	KWTLERLKRKYRN	158 ± 14 µM
JMJD6 W85A	K**A**TLERLKRKYRN	675 ± 66 µM
JMJD6 L90A	KWTLER**A**KRKYRN	No Binding
JMJD6 K91A	KWTLERL**A**RKYRN	No Binding
JMJD6 Y94A	KWTLERLKRK**A**RN	N/A
JMJD6 R95A	KWTLERLKRKY**A**N	No Binding

Supplemental Figure 3, ^{15}N-HSQC titration of JMJD6 peptide with ET domain in the PBS buffer of pH7.5 at 298K, black color spectrum is free protein, red is JMJD6/ET complex. The protein concentration is 0.1mM and the molar ratio of the protein to peptide is 1:5.



Wild type



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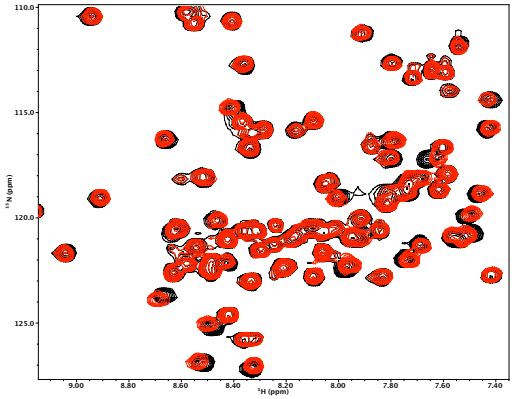

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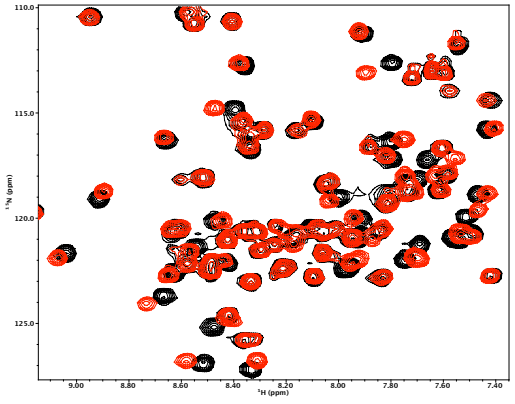

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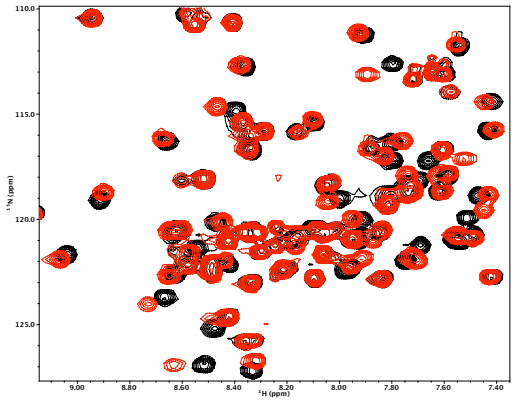

R95A

Peptide Name	**HSQC titration**
JMJD6 WT	Good binding
JMJD6 W85A	Very weak
JMJD6 L90A	No Binding
JMJD6 K91A	weak
JMJD6 Y94A	N/A
JMJD6 R95A	weak