Supplementary Information for

JMJD5 couples with CDK9 to release the paused RNA polymerase II

Haolin Liu[a,b], Srinivas Ramachandran[c], Nova Fong[c], Tzu Phang[d], Schuyler Lee[a,b], Pirooz Parsa[e], Xinjian Liu[f], Laura Harmacek[a], Thomas Danhorn[a], Tengyao Song[a], Sangphil Oh[g], Qianqian Zhang[h], Zhongzhou Chen[h], Qian Zhang[i], Ting-Hui Tu[a], Carrie Happoldt[a], Brian O'Conner[a], Ralf Janknecht[g], Chuan-Yuan Li[f], Philippa Marrack[a,b], John Kappler[a,b,1], Sonia Leach[a], Gongyi Zhang[a,b,1]

[1]Gongyi Zhang, zhangg@njhealth.org; John Kappler, kapplerj@njhealth.org

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Figures S1 to S6

Tables S1

Other supplementary materials for this manuscript include the following:

Dataset S1

www.pnas.org/cgi/doi/10.1073/pnas.2005745117

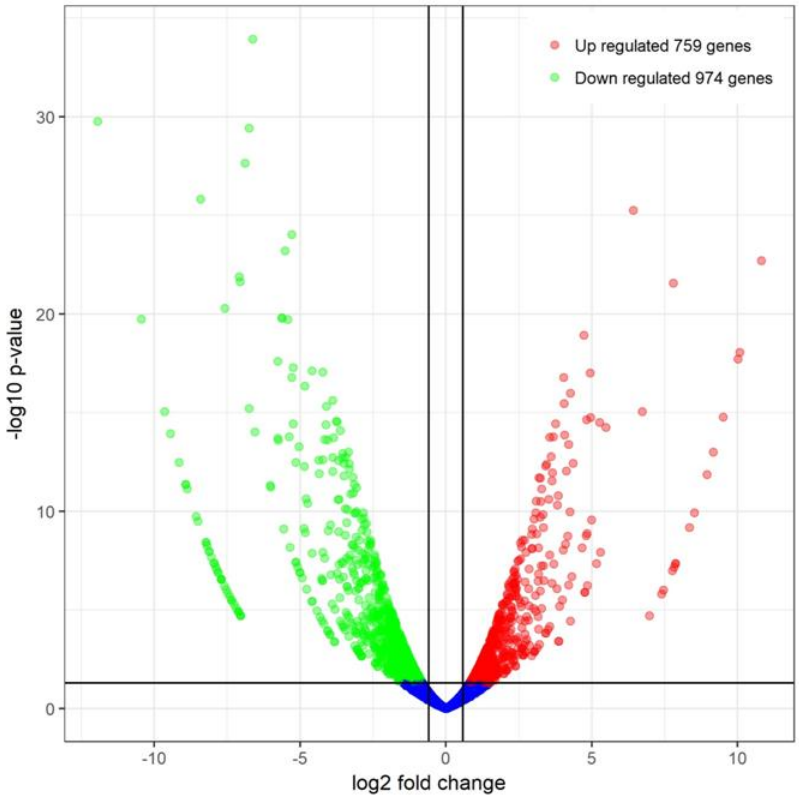

Fig. S1. RNA-seq of wildtype and JMJD5 knockout cells. A volcano diagram of up-regulated and down-regulated RNA transcripts with JMJD5 knockout compered to the wildtype MEF cell was shown. The full list of up-regulated and down-regulated RNA transcripts can be found in the supplement Dataset S1 (PNAS_202005745_s2.xlsx).

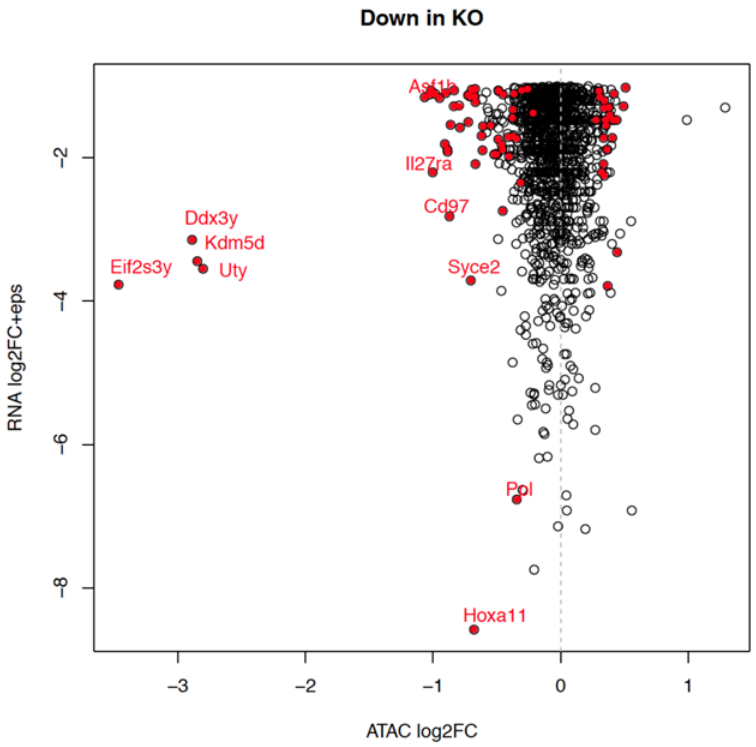

Fig. S2. Down-regulated genes in both RNA-seq and ATAC-seq methods in JMJD5 knock out cell line. The RNA-seq log2-fold expression change of genes significantly down-regulated in KO relative to WT (y-axis) is compared to ATAC-seq read coverage in open chromatin regions spanning each gene (-6000 nt to +3500 nt) (x-axis). Genes with significantly (fdr=0.1) differential chromatin accessibility between KO and WT are highlighted in red, with select gene names illustrated.

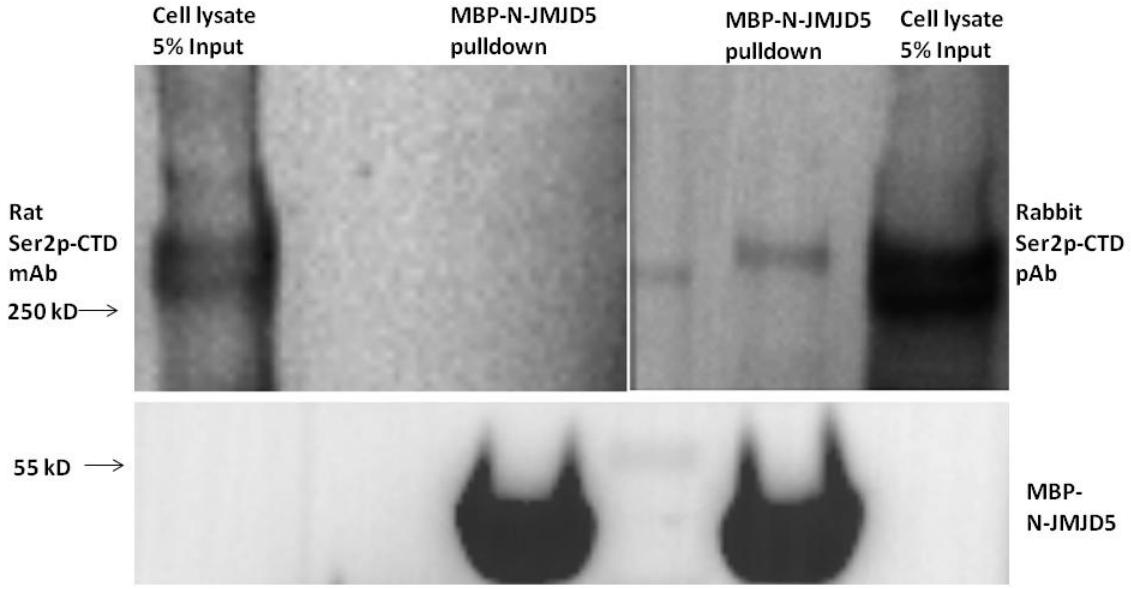

Fig. S3. Comparation of Rat 3E10 and Rabbit S2p-CTD pAb in recognization of MBP-N-JMJD5 pulldown. Equal amount of cell lysate input and MBP-N-JMJD5 pulldown product is ran on the SDS gel and transferred to the membrane and blotted by rat and rabbit serine2-CTD antibodies side by side. MBP-N-JMJD5 pulldown can be recognized by Rabbit S2p-CTD pAb (right) but not by Rat monoclonal 3E10 (left). MBP-N-JMJD5 was also blotted by MBP antibody as loading control.

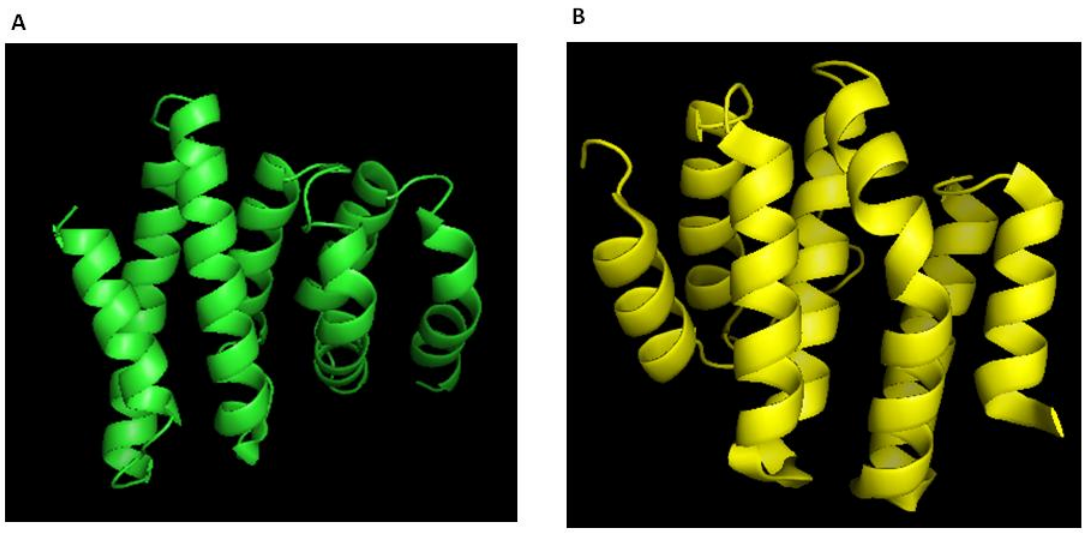

Fig. S4. The similarity of structures between N-JMJD5 and RPRD1A. A. The predicted structure of N-JMJD5 is based on Jpred software for secondary structure prediction and 3D prediction using Raptor X software. B. Structure of CTD binding domain of RPRD1A, 4JXT, from PDB bank.

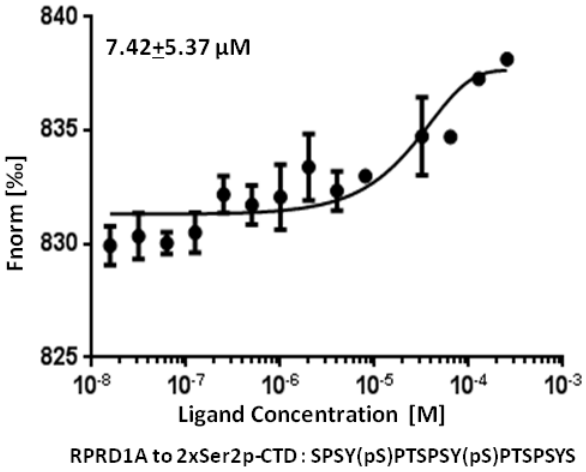

Fig. S5. MST measurement of the binding between RPRD1A and Pol II CTD peptide with Ser2 phosphorylation at two heptad repeats.

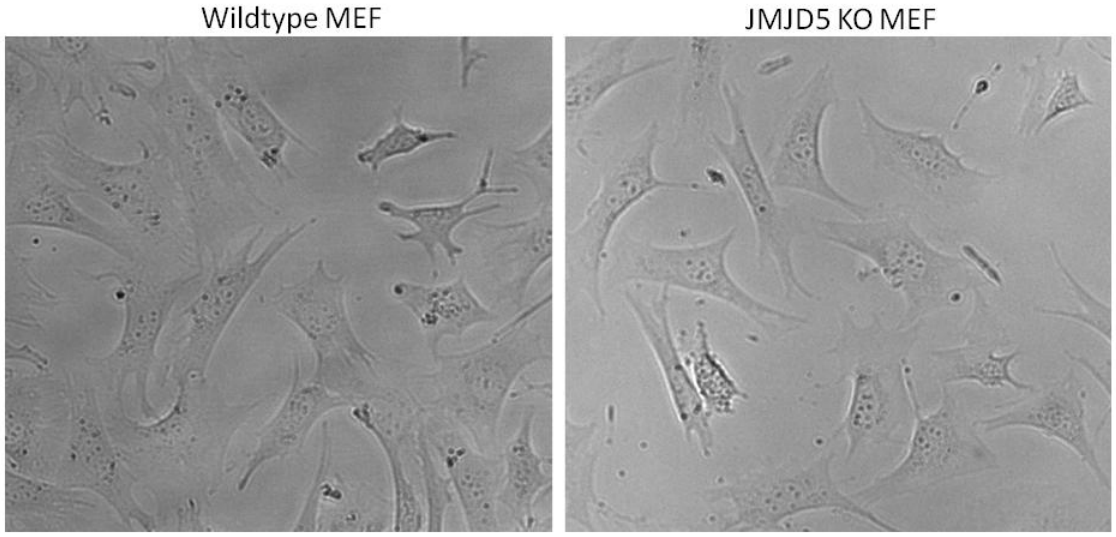

Fig. S6. Bright field image of wildtype and JMJD5 KO MEF cells. Both cells were culture at regular DMEM medium with 10% of FBS. The images were captured by the 10 fold magnification camera.

Table S1. Antibodies used in this research

Antibody name	Species	Vendor	Catalog number or Reference
Phospho-Serine2-CTD Pol II for blotting	Rabbit	Dr. David Bentley Lab	Nat Struct Mol Biol 15, 71-78 (2008)
Phospho-serine2-CTD Pol II 3E10 for ChIP-seq	Rat	Active Motif	# 61083
Phospho-serine5-CTD Pol II for blotting	Rabbit	Abcam	# 5131
CTD Pol II antibody for ChIP-seq and blotting	Rabbit	Dr. David Bentley Lab	Nat Struct Mol Biol 15, 71-78 (2008)
CDK9	Mouse	SantaCruz Biotechnology	sc-13130
β-Actin	Mouse	SantaCruz Biotechnology	Sc-69879
MBP antibody	Mouse	Made in our lab	H71-22, PLoS One 10, e0136613 (2015)