## Supplemental Materials and Methods

### Antibodies and Reagents

The sources of antibodies against the following proteins were: JMJD2B (sc-67192), ER (sc-7207), WDR5 (sc-100895) from Santa Cruz Biotechnology; HDAC1 (AV38530), β-actin (A1978) and FLAG (F3165) from Sigma; MLL2 (ab15962), MLL3 (ab71200), JARID1B (ab56759), JMJD2A (ab24545), JMJD2C (ab85454), JMJD2D (ab93694), H3K9me3 (ab8898), H3K9me2 (ab1220), H3K9me1 (ab9045), H3K4me3 (ab8580), H3K4me2 (ab32356), H3K4me1 (ab8895), and H3 (ab1791) from Abcam; and MLL1 (A300-374A), MLL4 (A300-113A), RbBP5 (A300-109A), and ASH2L (A310-028A) from Bethyl Laboratories. 17β-estradiol (E2) was purchased from Sigma. Control siRNA and specific siRNAs were synthesized by Shanghai GeneChem Inc (Shanghai, China). The sequence for control siRNA was: 5'-TTCTCCGAACGTGTCACGT-3'. The sequence for JMJD2B siRNA: #1, 5'-GACCTGTACAGCATCAACT-3'; #2, 5'-GCGGCTCTTTGTATGATGA-3'; for JMJD2C: 5'- GGACCTCTATAGCATTAAT-3'; for MLL2: 5'-GCAGTTTGTGCACTCCAAG-3'; for MLL3: 5'-GCAATGGTCTTTCTGGATA-3'. FLAG-tagged mJmjd2b and FLAG-tagged was kindly provided by Dr. Roland Schüle.

### Immunopurification and Mass Spectrometry

MCF-7 cells stably expressing FLAG-JMJD2B were generated via transfection of the cells with FLAG-tagged JMJD2B and selection in medium containing 1 mg/ml of G418.

Anti-FLAG immunoaffinity resin (Sigma) was prepared according to the manufacturer's protocol. Cell lysates were obtained from about $10^9$ cells and applied to anti-FLAG immunoaffinity resin to allow for adsorption of the protein complex to the resin. After binding, the resin was washed with cold PBS plus 0.1% Nonidet P-40. FLAG peptide (Sigma) was applied to the resin to elute the FLAG protein complex as described by the vendor. Eluates were collected and resolved on SDS-PAGE, silver stained, and subjected to LC-MS/MS sequencing and data analysis.

**FPLC Chromatography**

MCF-7 nuclear extracts were prepared and dialyzed against buffer D (20 mM Tris-HCl, 0.2 mM EDTA, 5 mM $MgCl_2$, 0.1 M KCl, 10% (v/v) glycerol, 0.5 mM DTT, 1 mM benzamidine, 0.2 m MPMSF, pH7.9). Approximately 6 mg of nuclear protein was concentrated to 300 μl using a Millipore Ultrafree centrifugal filter apparatus (10 kDa nominal molecular mass limit) and then applied to a Superose 6 size exclusion column (10/300 GL GE Healthcare) that had been equilibrated with buffer D and calibrated with protein standards. The column was eluted at a flow rate of 0.5 ml/min and fractions were collected.

**Histone Methylation (HMT) and Histone Demethylation (HDM) Assays**

FLAG-JMJD2B or FLAG-MLL2/SET immunoprecipitates were incubated with 2 μg of substrate in a 50 μl reaction volume with HMT buffer (20 mM Tris-HCl, 4 mM EDTA, 1 mM PMSF, 0.5 mM DTT, pH 7.9) and 0.3 μM S-adenosyl-L-(methyl-$^3$H) methionine (1

μCi, GE Healthcare) for 1 h at 30°C. The reaction was spotted on Whatman P81 paper, dried and washed with carbonate buffer (pH 9.2). The spotted P81 paper was then soaked with Atomlight fluid (Packard Biosciences) and subjected to liquid scintillation spectrometry (Beckman). In assays without isotope, the HMT reaction was stopped by adding SDS buffer. The samples were subjected to Western blotting with specific antibodies. For HDM assay, free histones or native nucleosomes were incubated with purified FLAG-JMJD2B complex in demethylation buffer (50 mM Tris-HCl, pH 8.0, 50 mM KCl, 10 mM $MgCl_2$, 1 mM α-ketoglutarate, 40 mM $FeSO_4$, 2 mM ascorbic acid) at 37°C. $MgCl_2$ was omitted when nucleosomes were used as the substrate. Reaction mixtures were analyzed by Western blotting using specific antibodies.

**Immunoprecipitation**

MCF-7 cell lysates were prepared by incubating the cells in lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% Nonidet P-40) for 20 min at 4 ℃. This was followed by centrifugation at 14,000 g for 15 min at 4 ℃. For immunoprecipitation, 500 μg of protein was incubated with specific antibodies (1-2 μg) for 12 h at 4 ℃ with constant rotation; 50 μl of 50% protein A or G agarose beads was then added and the incubation was continued for an additional 2 h. Beads were then washed five times using the lysis buffer. Between washes, the beads were collected by centrifugation at 3,000 g for 30 s at 4 ℃. The precipitated proteins were eluted from the beads by resuspending the beads in 2 x SDS-PAGE loading buffer and boiling for 5 min. The boiled immune complexes were subjected to SDS-PAGE followed by immunoblotting with appropriate antibodies.

Immunodetection was performed using enhanced chemiluminescence (ECL System, Amersham Biosciences) according to the manufacturer's instructions.

**Cell Culture and Reporter Assay**

MCF-7 and 293T cells were from the American Type Culture Collection (Manassas, VA). Cells were maintained in DMEM supplemented with 10% FBS. Transfections were carried out using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Luciferase activity was measured using a dual luciferase kit (Promega, Madison, WI) according to the manufacturer's protocol. Each experiment was performed in triplicate and repeated at least three times.

**Histone Methylation (HMT) and Histone Demethylation (HDM) Assays**

FLAG-JMJD2B or FLAG-MLL2/SET immunoprecipitates were incubated with 2 μg of substrate in a 50 μl reaction volume with HMT buffer (20 mM Tris-HCl, 4 mM EDTA, 1 mM PMSF, 0.5 mM DTT, pH 7.9) and 0.3 μM S-adenosyl-L-(methyl-$^3$H) methionine (1 μCi, GE Healthcare) for 1 h at 30$^o$C. The reaction was spotted on Whatman P81 paper, dried and washed with carbonate buffer (pH 9.2). The spotted P81 paper was then soaked with Atomlight fluid (Packard Biosciences) and subjected to liquid scintillation spectrometry (Beckman). In assays without isotope, the HMT reaction was stopped by adding SDS buffer. The samples were subjected to Western blotting with specific antibodies. For HDM assay, free histones or native nucleosomes were incubated with purified FLAG-JMJD2B complex in demethylation buffer (50 mM Tris-HCl, pH 8.0, 50 mM KCl, 10 mM $MgCl_2$, 1 mM α-ketoglutarate, 40 mM $FeSO_4$, 2 mM ascorbic acid) at

37$^{o}$C. $MgCl_2$ was omitted when nucleosomes were used as the substrate. Reaction mixtures were analyzed by Western blotting using specific antibodies.

**Real-time RT-PCR (qPCR)**

Total cellular RNAs were isolated with TRIzol reagent (Invitrogen) and used for first strand cDNA synthesis with the Reverse Transcription System (Promega, A3500). Quantitation of all gene transcripts was done by qPCR using a Power SYBR Green PCR Master Mix and an ABI PRISM 7300 sequence detection system (Applied Biosystems, Foster City, CA) with the expression of GAPDH as the internal control. The primers used were: *TFF1* forward primer: TTGTGGTTTTCCTGGTGTCA, reverse primer: CCGAGCTCTGGGACTAATCA; *EBAG9* forward primer: GATGCACCCACCAGTGTAAAGA, reverse primer: AGTCAGGTTCCAGTTGTTCCAAAG; *Cathepsin D* forward primer: GTACATGATCCCCTGTGAGAAGGT, reverse primer: GGGACAGCTTGTAGCCTTTGC;
*GREB1* forward primer: GGCAGGACCAGCTTCTGA, reverse primer: CTGTTCCCACCACCTTGG; *JMJD2B* forward primer: GGCCGGAGCTGCACACT, reverse primer: CGCGTCTTTGCACAGAGTAAGA.

**ChIP/qChIP and Re-ChIP**

ChIP and Re-ChIP experiments were performed according to the procedure described previously (1-3). qChIP was performed using SYBR Green PCR Master Mix on the ABI PRIZM 7300 Sequence Detector. The amounts of immunoprecipitated DNA were

normalized to the input. The primers used were: *TFF1* forward primer: GGCCATCTCTCACTATGAATCACTTCTGC, reverse primer: GGCAGGCTCTGTTTGCTTAAAGAGCG; *EBAG9* forward primer: TCCCACCTTCCTTTCGC, reverse primer: CCAGTCGCAGTTTCCTCAC; *JMJD2B*_ ER3291 forward primer: AGGCTGTTTTGGTGGA, reverse primer: AGGCTGAGGAAGGGTC; *JMJD2B*_ER3292 forward primer: TGACATCCACCCAGACA, reverse primer: TGAATCCCAGAGCAAGA;

**Colony Formation Assay**

MCF-7 cells were stably transfected with JMJD2B shRNA plasmid. The cells were maintained in culture media supplemented with 1 mg/ml G418 for 14 days in the presence or absence of E2 and were then stained with crystal violet.

**Cell Flow Cytometry**

MCF-7 cells stably transfected with JMJD2B shRNA plasmid were maintained in charcoal-stripped serum medium for 3 days and then 100 nM E2 was added for an appropriate period of time; cells were then trypsinized, washed with PBS, and fixed in 70% ethanol at 4 ℃ overnight. After being washed with PBS, cells were incubated with RNAase A (Sigma) in PBS for 30 min at 37 ℃ and then stained with 50 mg/ml propidium iodide (PI). Cell cycle data were collected with FACS Calibur (Becton Dickinson) and analyzed with ModFit LT 3.0 (Verity Software House Inc., Topsham, ME).

**Supplemental References**


1. Li R, *et al.* (2009) ZIP: a novel transcription repressor, represses EGFR oncogene and suppresses breast carcinogenesis. *Embo J.* 28:2763-2776.
2. Wang Y, *et al.* (2009) LSD1 is a subunit of the NuRD complex and targets the metastasis programs in breast cancer. *Cell* 138:660-672.
3. Zhang H, *et al.* (2004) Differential gene regulation by the SRC family of coactivators. *Genes Dev.* 18:1753-1765.

**Supplemental Figure Legends**

**Supplemental Figure 1. The recruitment of JMJD2B and the level of H3K9me3 on ERα target genes.** Cells were deprived of estrogen for 3 days and then treated with E2 for 1 h. Soluble chromatin from ZR-75-1 (A) and T47D (B) cells was prepared and qChIP was performed with antibodies against the indicated proteins. Each bar represents the mean ± S.D. for triplicate experiments. P values were determined by using Student's t-test. The star (*) indicates that p value is below 0.001.

**Supplemental Figure 2. Coordinated H3K9 Demethylation and H3K4 Methylation by the JMJD2B/MLL2 Complex.** (A) MCF-7 cells were treated with control siRNA or JMJD2B siRNA. Cells were deprived of estrogen for 3 days and then treated with E2 for 1 h. Soluble chromatin was then prepared for ChIP or qChIP assays with antibodies against the indicated proteins or with anti-H3K9me3 or anti-H3K4me1/2/3. Each bar represents the mean ± S.D. for triplicate experiments. (B) MCF-7 cells were treated with control siRNA or MLL2 siRNA. Cells were deprived of estrogen for 3 days and then treated with E2 for 1 h. Soluble chromatin was then prepared for ChIP or qChIP assays with antibodies against the indicated proteins or with anti-H3K9me3 or anti-H3K4me2/3. Each bar represents the mean ± S.D. for triplicate experiments. (C) JMJD2B-depleted MCF-7 cells were transfected with W-mJmjd2b or M-mJmjd2b. Cells were deprived of estrogen for 3 days and then treated with E2 for 1 h. Soluble chromatin was then prepared for ChIP or qChIP assays with antibodies against the indicated proteins or with anti-H3K9me3 or anti-H3K4me2/3. Each

bar represents the mean ± S.D. for triplicate experiments. (D) Mammalian cells-purified FLAG-JMJD2B or/and FLAG-MLL2/SET was incubated with human H3 peptides corresponding to N-terminal 21 amino acids, naked or K9 trimethylated, in methylation/demethylation buffer. The reactions were stopped and the mixtures were resolved on SDS-PAGE for Western blotting analysis with anti-H3K4me1/2 or anti-H3K9me3 (left). Scintillation counting was also performed by incubating the corresponding enzymes and substrates in the presence of S-adenosyl-L-[methyl-$^{3}$H] methionine (right). Each bar represents the mean ± S.D. for triplicate measurements. cpm: counts per minute. P values were determined by using Student's t-test. The * indicates that p value is below 0.001, whereas # indicates that p value is below 0.01.

**Supplemental Figure 3.** Bioinformatics analysis of JMJD2B expression in human breast carcinoma samples that differ in ERα status using database at Oncomine.

**Supplemental Figure 4. *JMJD2B* is an ERα-inducible Gene.** (A) Estrogen induces the mRNA expression of JMJD2B. MCF-7 cells were deprived of estrogen for 3 days and then treated with different doses of E2 for 3 h (left) or treated with 100 nM E2 for different periods of time (right). Total RNAs were extracted from the cells and analyzed for the mRNA expression of JMJD2B and other members of the JMJD2 family histone demethylases by real time RT PCR. Each point represents the mean ± S.D. for triplicate measurements. (B) The induction of JMJD2B mRNA expression by E2 is dependent on ERα. MCF-7 cells were transfected with ERα siRNA. Cells were deprived of estrogen for

3 days and then treated with E2. Total RNAs were extracted from the cells and analyzed for the mRNA expression of JMJD2B. The ERα knockdown was confirmed by Western blotting. Each point represents the mean ± S.D. for triplicate measurements. (C) Estrogen induces JMJD2B protein expression. MCF-7 cells were deprived of estrogen for 3 days and then treated with E2 for indicated periods of time. Cellular lysates were immunoblotted with antibodies against the indicated proteins. (D) Transcriptional targeting of JMJD2B by ERα. The identification of two half-ERE sites in intron 1 of *JMJD2B*. The number represents the nucleotide position relative to the transcription start site (+1). Lower left panel: ChIP assays of the occupancy of ERα on the indicated region of *JMJD2B* intron 1, ER_3291 and ER_3292, in the presence or absence of E2 treatment in MCF-7 cells. The occupancy of ERα in +11,944 to +12,121 region of *JMJD2B* was also examined. Lower right panel: Luciferase reporter assays in MCF-7 cells transfected with ER_3291-LUC, ER_3292-LUC, or their mutant counterparts in the presence of increased concentrations of E2. Each bar represents the mean ± S.D. for triplicate experiments.

**Supplemental Figure 5. The recruitment of JMJD2B and MLL2 on MLL2 target genes.** (A) The occupancy of JMJD2B and MLL2 on ERα binding sites of *TFF1*, *XPB1*, *NRIP1*, *GREB1*, *CCND1*, *EBAG9*, *CYP1b1*, *ESR1*, *CASP7*, *MYC*, *CTSD*, and *PR* genes. MCF-7 cells were deprived of estrogen for 3 days and then treated with E2 for 1 h. Soluble chromatin was prepared and qChIP was performed with antibodies against the indicated proteins. Each bar represents the mean ± S.D. for triplicate experiments. promoter (p), enhancer (e). (B) The occupancy of JMJD2B and MLL2 on the p53 binding sites of *p21*

and *MDM2* genes. MCF-7 cells were treated with vehicle (DMSO) or Etoposide for 8 h. Soluble chromatin was prepared and qChIP was performed with antibodies against the indicated proteins. Each bar represents the mean ± S.D. for triplicate experiments. (C) The occupancy of JMJD2B and MLL2 on the RARa binding sites of *RARb-2, HoxA1* and *HoxB1* genes. MCF-7 cells were deprived of hormones for 3 days and then treated with all *trans*-retinoic acid (RA) for 1 h. Soluble chromatin was prepared and qChIP was performed with antibodies against the indicated proteins. Each bar represents the mean ± S.D. for triplicate experiments. P values were determined by using Student's t-test. The star (*) indicates that p value is below 0.001 while the sign(#) indicates that p value is below 0.05 and the excalmatory mark (!) indicates that p value is above 0.05.

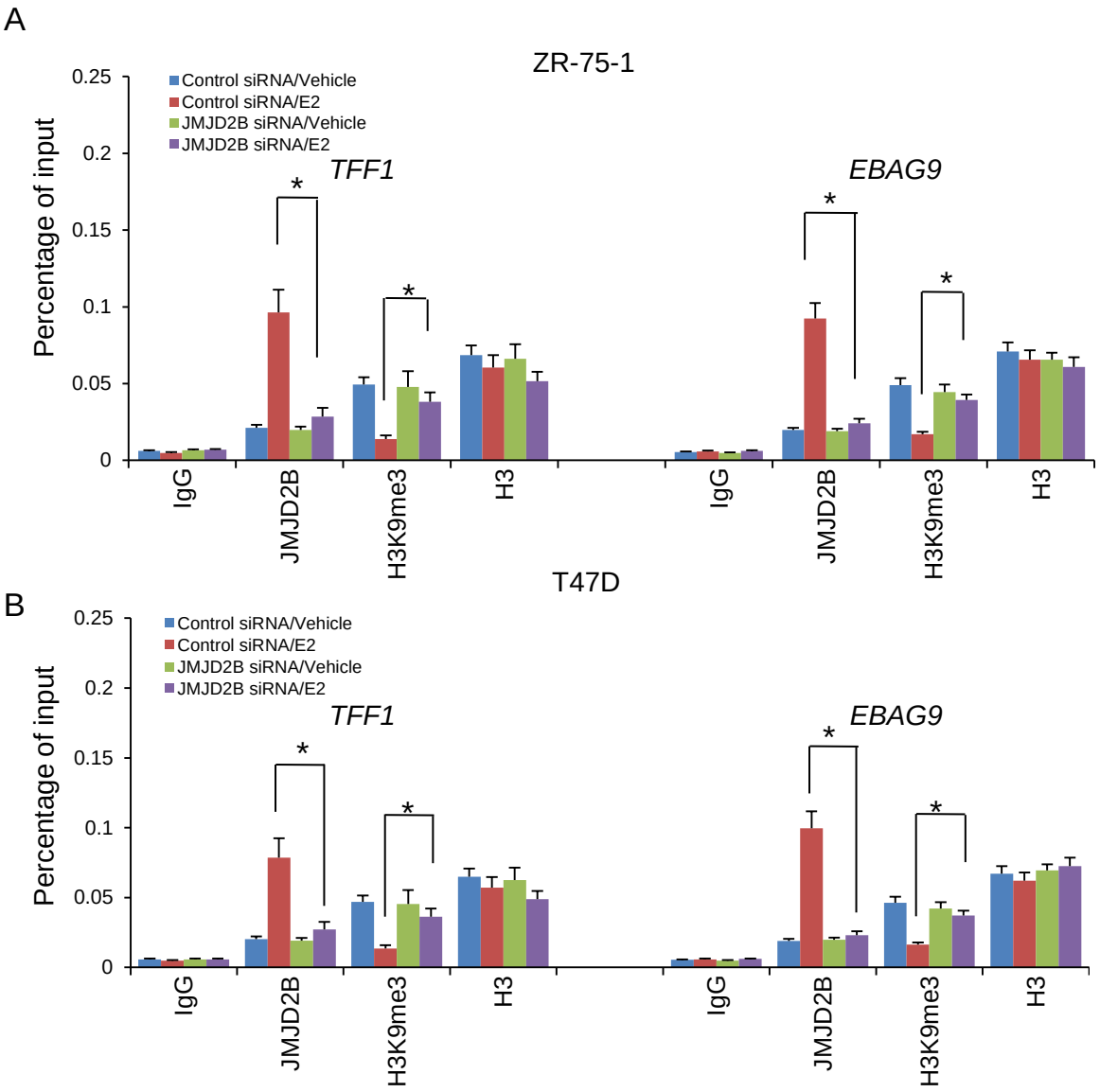
**Supplemental Figure 6. JMJD2B binds to the promoters (Pr) but not the transcribed regions (Tr) of *TFF1* and *EBAG9.*** MCF-7 cells were deprived of estrogen for 3 days and then treated with E2 for 1 h. Soluble chromatin was prepared for qChIP assays with antibodies against the indicated proteins. Each bar represents the mean ± S.D. for triplicate experiments. P values were determined by using Student's t-test. The star (*) indicates that p value is below 0.001 while the excalmatory mark (!) indicates that p value is above 0.05.

**Supplemental Figure 7. The integrity of JMJD2B/MLL2 complex.** MCF-7 cells were transfected with control siRNA, MLL2 siRNA, WDR5 siRNA or JMJD2B siRNA. Whole cell lysates were immunoprecipitated with antibodies against the indicated proteins. Immunocomplexes were then immunoblotted using antibodies against the indicated

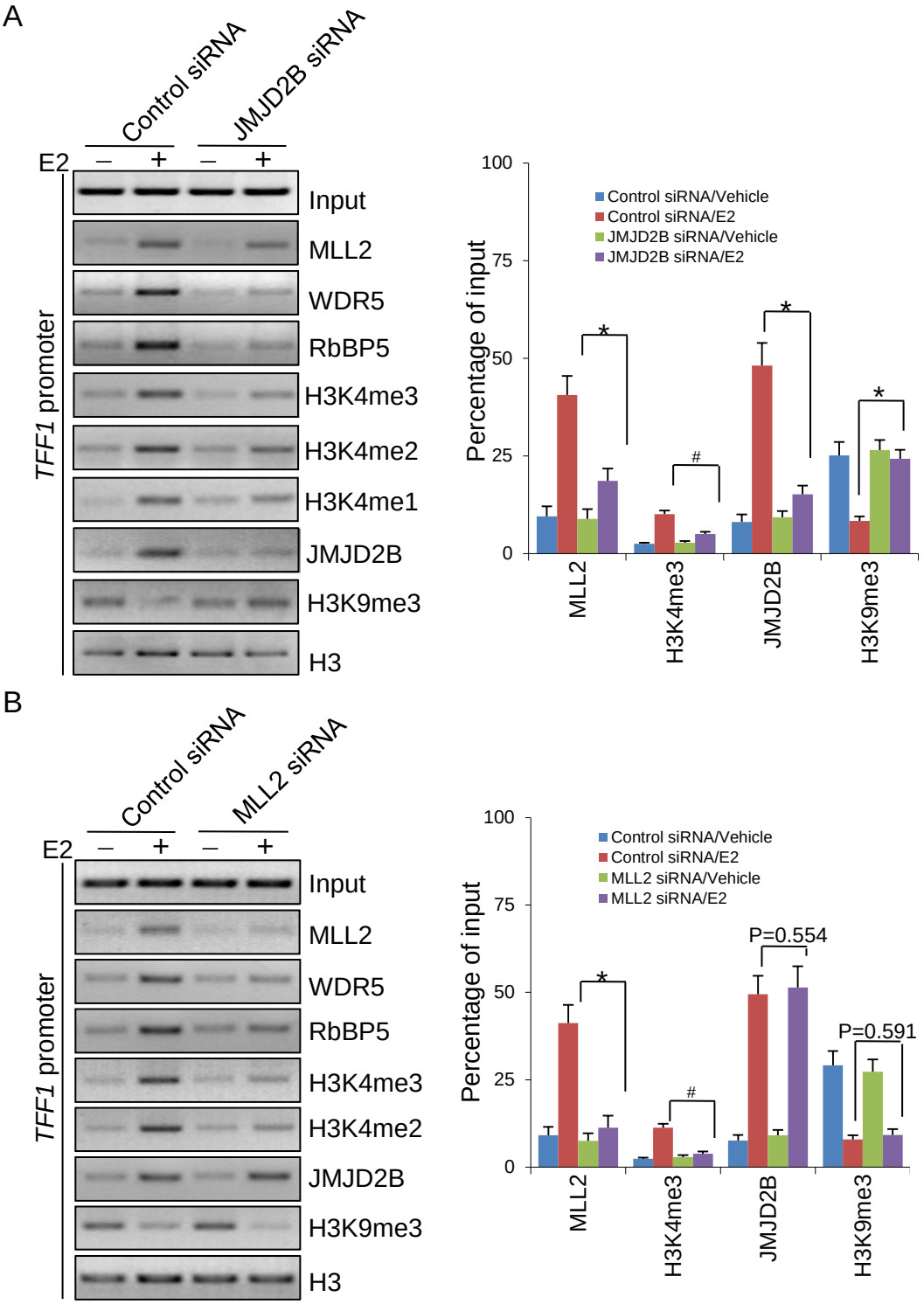
proteins. The knock down effect for MLL2, WDR5 and JMJD2B was validated by Western blotting.

**Supplemental Figure 8. Association of JMJD2B with the MLL2 complex.** Whole cell lysates from MDA-MB-231 cells were immunoprecipitated with antibodies against the indicated proteins. Immunocomplexes were then immunoblotted using antibodies against the indicated proteins.

# Supplemental Figure 1



# Supplemental Figure 2



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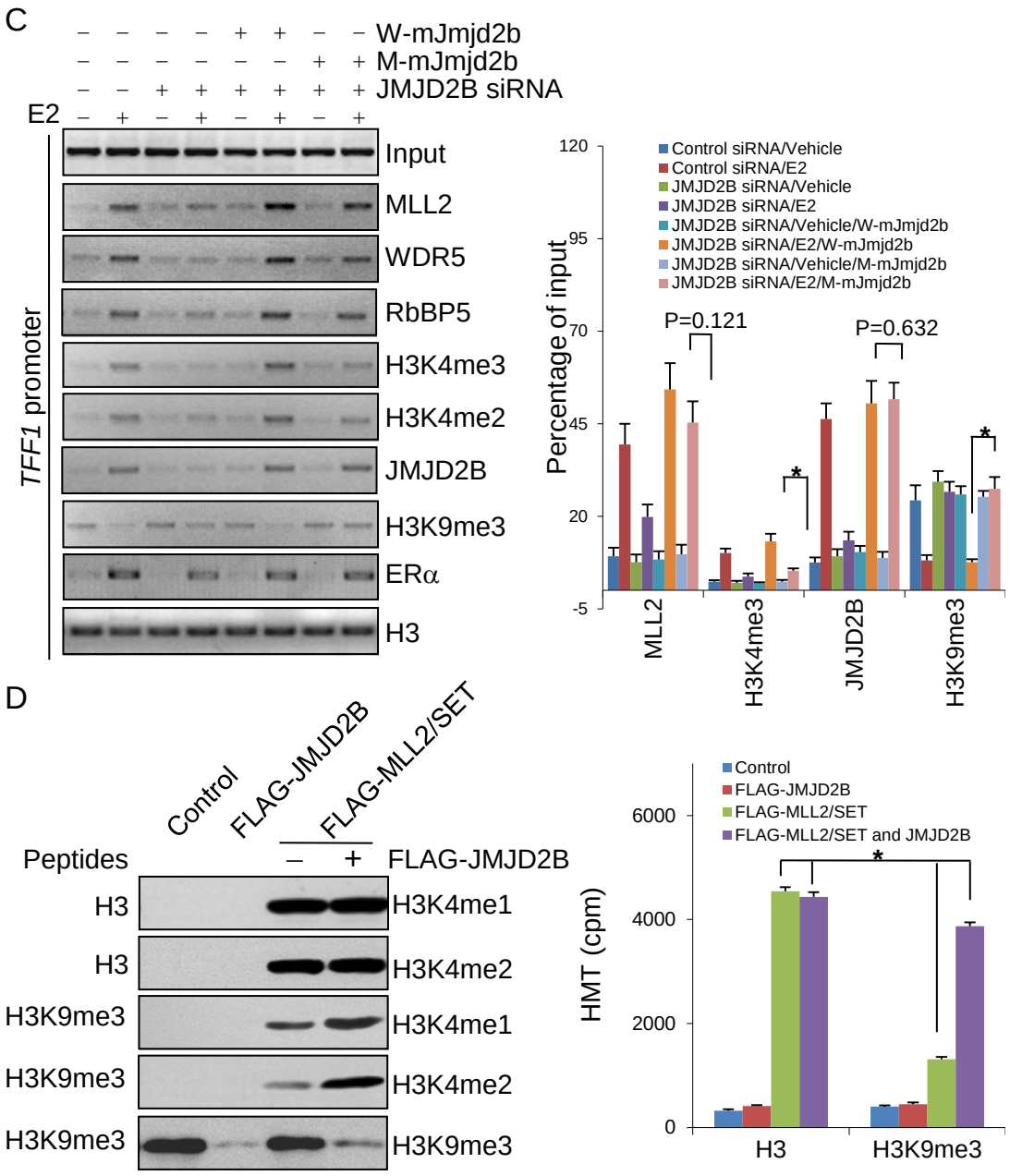

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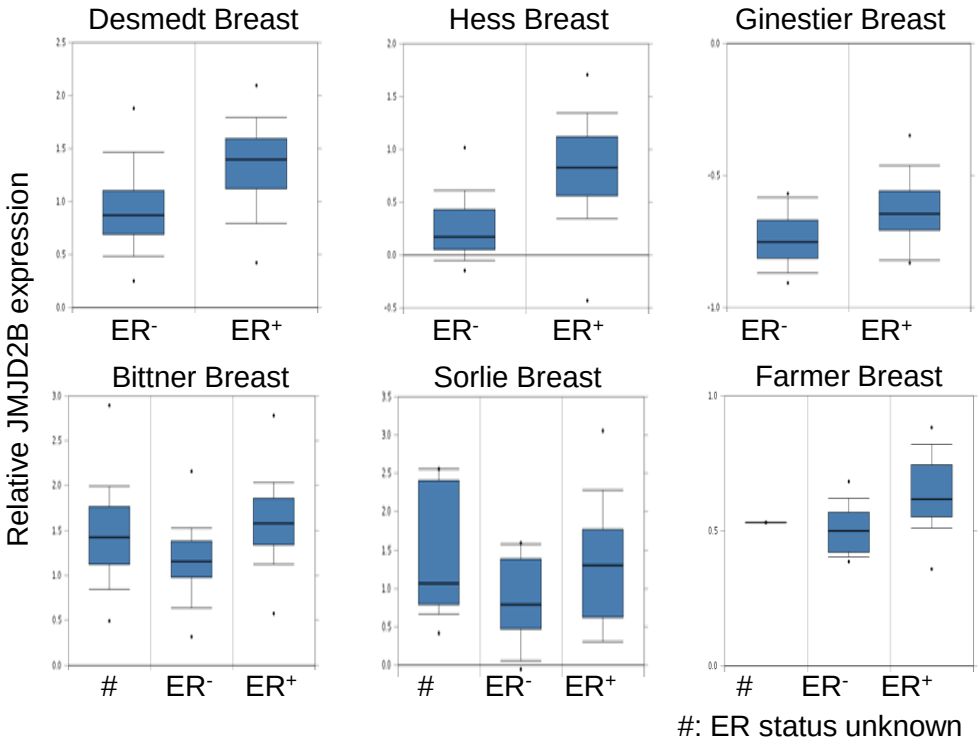
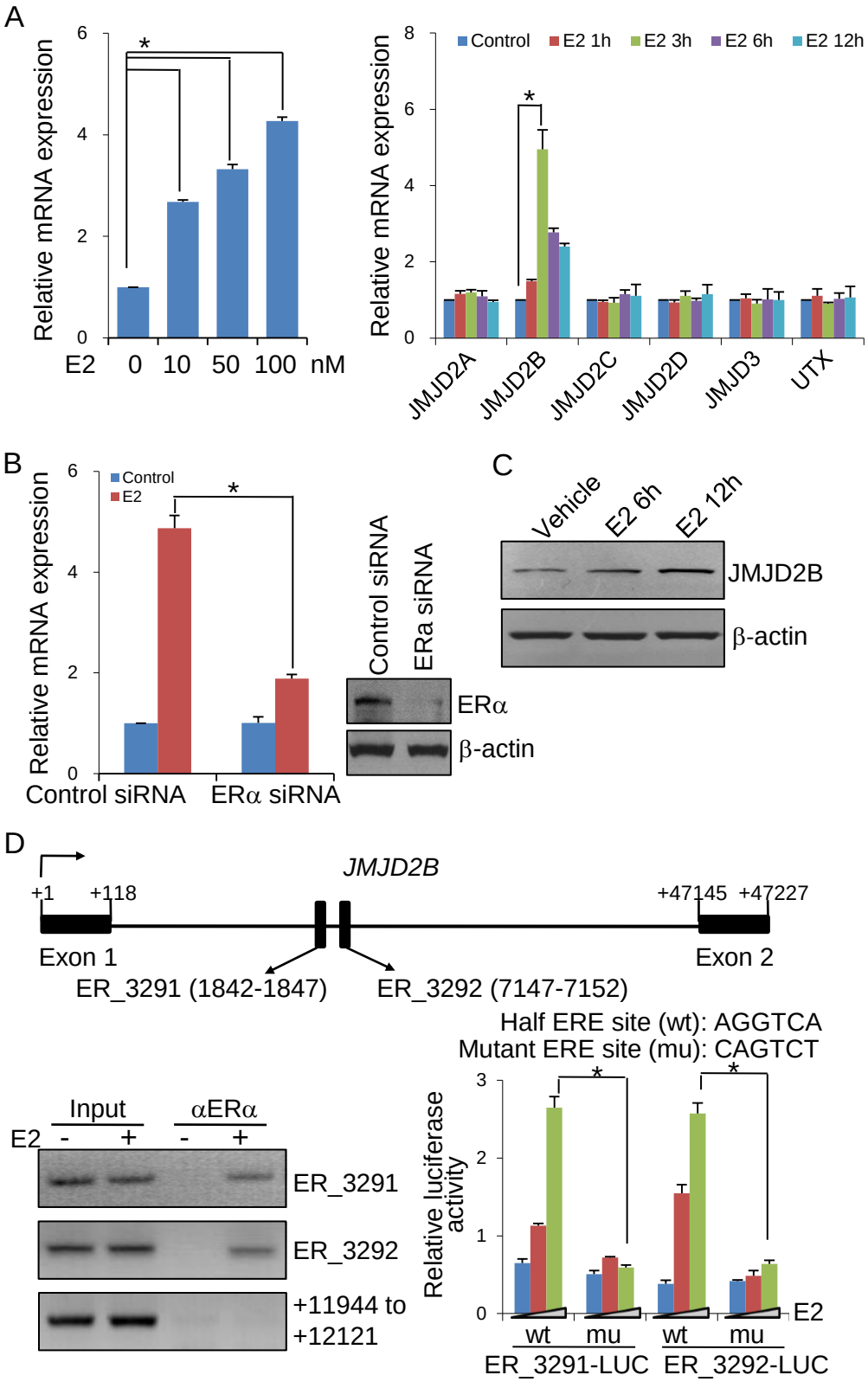


# Supplemental Figure 4



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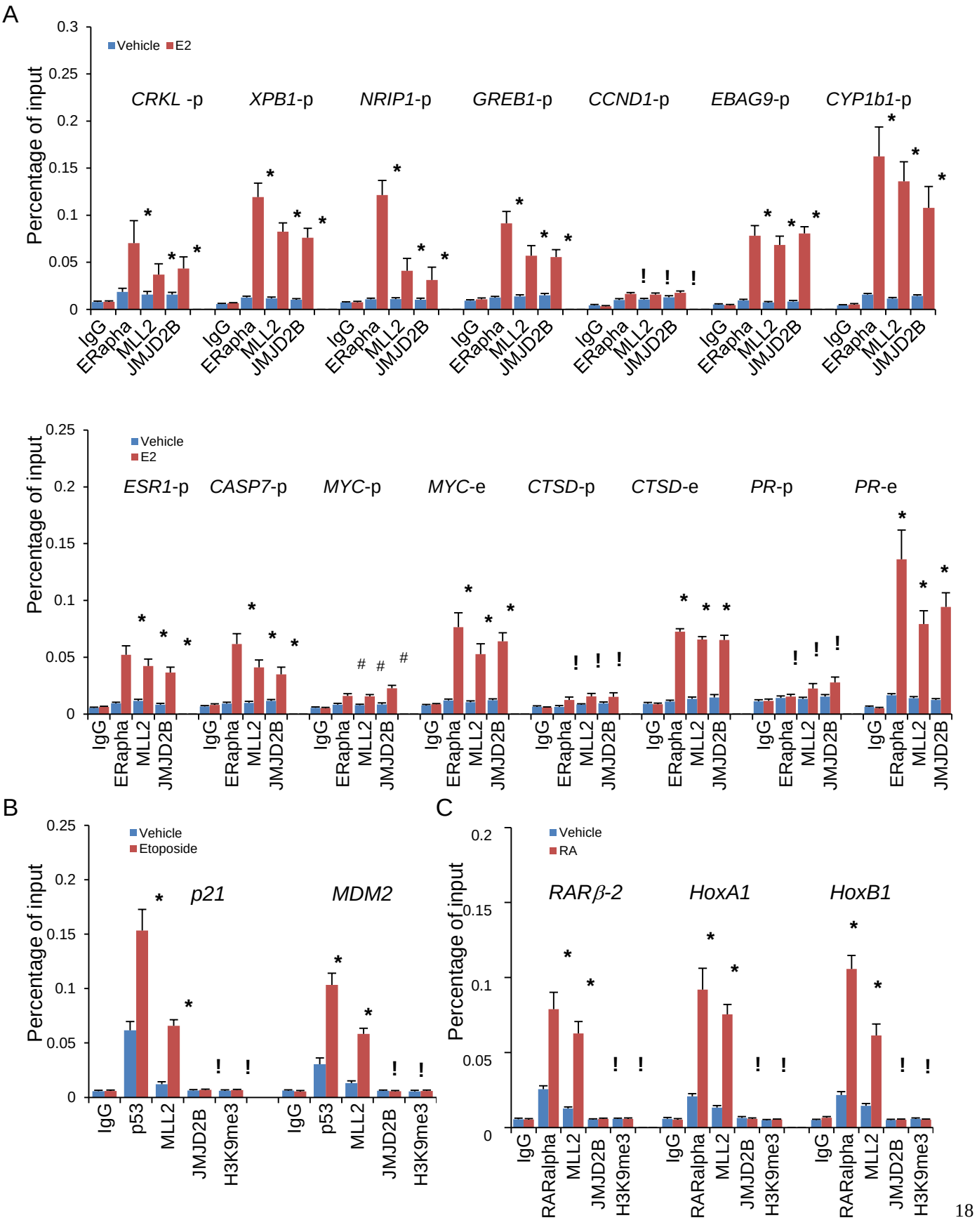

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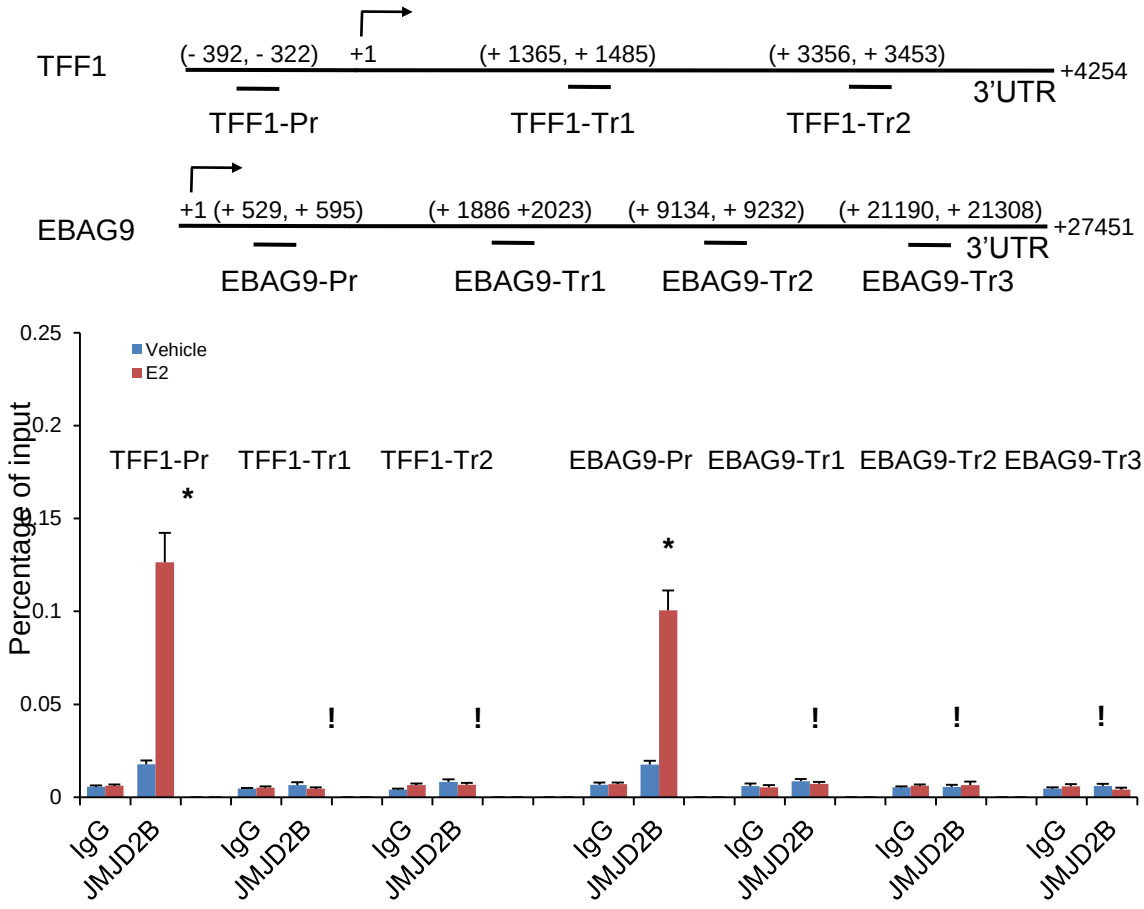

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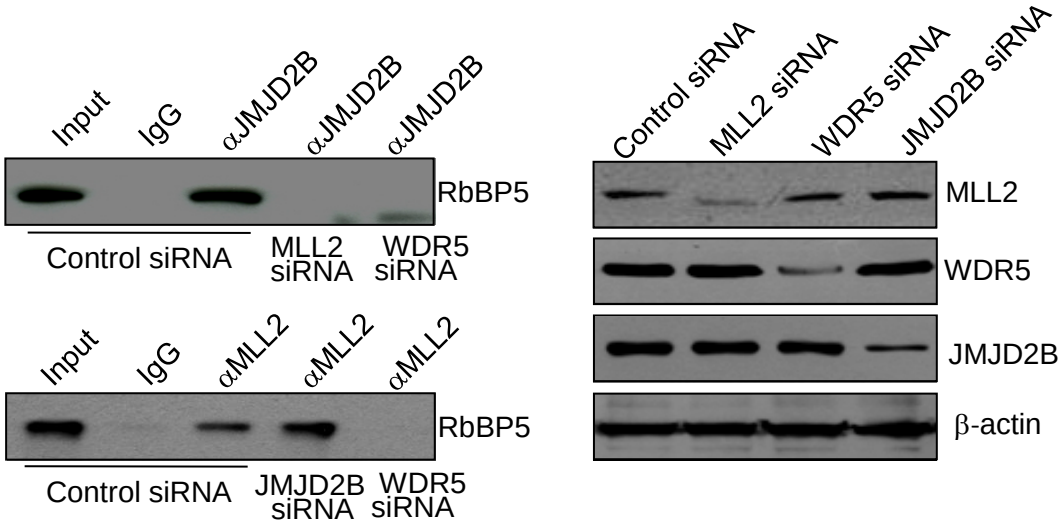
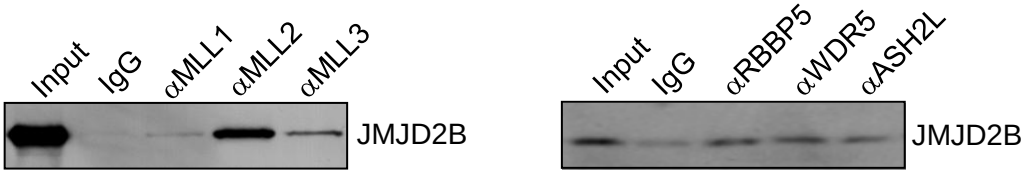


## Supplemental Figure 8



Supplemental File 1

**Mass Spectrometry Analysis of JMJD2B-containing Protein Complex**

| Band | Identified proteins | Peptides |
|---|---|---|
| 500 kDa | MLL2 | YHFPCATASGSFLSMK |
| | | VCQACRKPGNDSK |
| | | LSPLPVVSR |
| | | LLLCDDCDISYHTYCLDPPLLTVPKGGWK |
| | | PVAPVAPPELVPMKVKEPEPQYFR |
| | | FEGVWLTETGMALLR |
| | | PFLQGGLPLGNLPSSSPMDSYPGLCQSPFLDSR |
| | | QDYPDWSSR |
| | | PQPPPPESCCALPPR |
| | | FTFPQAVGEPSLK |
| | | QRLRELLIR |
| | | HNVQK |
| | | DIFNEHLRLVESANEKAER |
| | | LVESANEKAEREALLR |
| | | PGQSMMGSR |
| | | VMAQGSIGVAPGMNR |
| | | REANGEPIGAPGTSNHLLLAGPR |
| | | KEDGVR |
| | | GSEVSVMLTVSAAAAK |
| | | CSLCQRTGATSSCNRMR |
| | | TGATSSCNRMRCPNVYHFACAIR |
| | | EADMLRLFPEYLK |
| | | YGRHPLMELPLMINPTGCAR |
| | | YINHSCAPNCVAEVVTFDKEDK |
| 135 kDa | JMJD2B | YVAYIESQGAHR |
| | | AMTVGEYRRLANSEK |
| | | HQDFDDLER |
| | | TILDMVER |
| | | SWYAIPPEHGK |
| | | LAIGFFPGSSQGCDAFLR |
| | | MTLISPIILK |
| | | YGIPFSR |
| | | KPKPEDPK |
| | | TEPYCAICTLFYPYCQALQTEK |
| | | FLNVIER |

| | | |
|---|---|---|
| | | HKSGGHAVQLLRAVSLGQVVITK |
| | | VGTPLATEDSGR |
| | | |
| 110 kDa | hnRNP U | VSELKEELK |
| | | GVKRPR |
| | | DIDIHEVR |
| | | YNILGTNTIMDKMMVAGFK |
| | | DLPEHAVLK |
| | | LLEQYKEESK |
| | | KALPPEK |
| | | GRGGFNMRGGNFR |
| | | |
| 75 kDa | ASH2L | DVFDKEEFYLYSK |
| | | YGDKNFPHELPYGHPPLR |
| | | QNDYAPGINSLDQDLLGFKPYDEEPDK |
| | | SLPDTYK |
| | | |
| 70 kDa | heat shock 70kDa protein 8 | RFDDAVVQSDMK |
| | | VEIIANDQGNR |
| | | STAGDTHLGGEDFDNR |
| | | MKEIAEAYLGK |
| | | NQVAMNPTNTVFDAK |
| | | SQIHDIVLVGGSTR |
| | | NSLESYAFNMK |
| | | TTPSYVAFTDTER |
| | | DAGTIAGLNVLR |
| | | |
| 70 kDa | heat shock 70kDa protein 1B | KFGDPVVQSDMK |
| | | AQIHDLVLVGGSTR |
| | | NALESYAFNMK |
| | | LVNHFVEEFK |
| | | NQVALNPQNTVFDAK |
| | | IINEPTAAAIAYGLDR |
| | | DAGVIAGLNVLR |
| | | |
| 62 kDa | RbBP5 | SAPVMLTLSDSK |
| | | KGSCFLINTADRIIR |
| | | EILTCGRDGEPEPMQK |
| | | SIGNLVK |
| | | |
| 62 kDa | ERα | LRKCYEVGMMK |

| | | |
|---|---|---|
| | | KCYEVGMMKGGIR |
| | | LAQLLLILSHIRHMSNK |
| | | HMSNKGMEHLYSMKCK |
| | | |
| 40 kDa | WDR5 | LGISDVAWSSDSNLLVSASDDK |
| | | IWDVSSGKCLK |
| | | |
| 20 kDa | Ribosomal protein S9 | SRLDQELK |
| | | IGVLDEGK |
| | | RLQTQVFK |
| | | HIDFSLR |
| | | SPYGGGRPGR |