Supplemental Experimental Procedures

Protein purification and Crystal structures

Proteins were expressed from BL21 DE3 pLysS codon (+) at 16 °C overnight after induction with 0.1 mM IPTG in mid-log phase of bacterial growth. Cells were harvested by centrifugation and protein was purified by the His-tag on Ni-NTA resin (Qiagen). The SUMO tag was removed from RbBP5, ASH2L and MLL1 by incubation with the ULP1 protease at 4 °C overnight. Protease and cleaved SUMO-His tag were collected by batch binding with the Ni-NTA resin for 1h. For GST pull down, MLL1$^{3762\text{-}C'}$and MLL3$^{4707\text{-}C'}$ were cloned into pGEX6P1 and expressed and lysed as described above. Proteins were lysed and purified in 50mM NaPi, pH 7.8, 400mM KCl and 10% glycerol, with 0.1mM PMSF, 0.1mM benzamidine for protease inhibition. GST fusion proteins were collected by batch binding with 1mL glutathione 4B sepharose (GE Healthcare) and eluted according to the manufacturer's directions.

The crystallization solution is 30%PEG4000, 0.2M magnesium chloride, and 0.1 M Tris-HCl, pH8.5. All the crystals are gradually transferred to harvesting solution (30% PEG 4000, 20% Glycerol, 0.2M magnesium chloride, and 0.1 M Tris-HCl, pH8.5), followed by flash-frozen in liquid nitrogen. The 2.1Å dataset was collected at Advanced Photon Source beamline 21ID-G and was processed by HKL2000. The crystal belongs to *P1* space group. The structure was solved by molecular replacement by Phaser using the previously published WDR5 structure (2H14). There are two WDR5 molecules in one asymmetric unit. Iterative cycles of refinement and modeling were carried out using Phenix and Coot.

Histone methylation assay and Mass spectrometry

Methylation reaction is carried out at romm temperature for 2 hours using 0.5 μM (final concentration) MLL1 complex or other HMTs is mixed with 50 μM H3 10-mer peptides (substrate) and 1.0 μCi of cofactor ^{3}H-*S*-adenosyl methionine (Perkin Elmer). The Reaction mix was spotted onto P81 phosphocellulose paper (Whatman), washed 30min with bicarbonate, pH 9.0 and counted by scintillation counter.

For mass spec analyses, methylation reactions were carried out at room temperature for 8 hours, using 5μM MLL3 complexes reconstituted with and without WDR5, 50μM biotin-H3 (1-21), and 2.5mM SAM. 1μL of the reaction mixtures was diluted 10X in water and desalted with EMD Millipore C18 ZipTip; peptides were eluted into solvent containing

70% acetonitrile and 0.1% trifluoroacetic acid. Samples were mixed with MALDI matrix in 1:1 ratio before being spotted onto the plate for data acquisition.

Cell cycle, apoptosis and differentiation assays by flow cytometer

For cell cycle analyses, cells were treated with 10, 20 and 40μM MM-401, or 40μM MM-NC-401 or DMSO vehicle for 2 days. Cells were harvested and fixed in ice-cold ethanol overnight. After fixation, cells were stained with propidium iodide for 30 minutes.

For apoptosis assays, cells were treated with MM-401, MM-NC-401 or DMSO for 2 days and harvested, washed and resuspended in 1X AnnexinV binding buffer (BD Pharmingen) staining buffer with APC-conjugated AnnexinV (BD Pharmingen) and propidium iodide for 15 minutes.

For surface marker detection, cells were treated with MM-401, MM-NC-401 or DMSO for 4 days and harvested by centrifugation at 500xg for 5 minutes, washed and resuspended in staining buffer (1xPBS + 2% FCS) with Pacific-Blue-conjugated anti-CD11b (BioLegend) and APC-conjugated anti-c-Kit (eBio Sciences) antibodies for 30 minutes. All experiments were analyzed on FACSDiva (BD Pharmingen). Data was analyzed using FloJo software.

BLI/OctetRED assay

Purified recombinant WDR5 protein was biotinylated using the Thermo EZ-Link long-chain biotinylation reagent. Relatively low biotinylation reagent concentration was applied to avoid protein over-biotinylation. Briefly, protein and biotinylation reagent were mixed with 1:1 molar ratio in PBS at 4 °C. This reaction mixture was incubated at 4°C for 2 hours to allow reaction being finished. Reaction mixture was then dialyzed using Fishersci 10K MWCO dialysis cassettes to remove unreacted biotinylation reagent.

BLI experiments were performed using an OctetRED96 instrument from PALL/ForteBio. All assays were run at 30 °C using PBS (pH 7.4) as the assay buffer, in which 0.01% BSA, 0.01% Tween-20 and 5% DMSO were included, with continuous 1000 RPM shaking. Biotinylated WDR5 was immobilized on Super Streptavidin (SSA) biosensors (ForteBio) by dipping sensors into 15 μg/mL protein solutions. Average saturation response level of 11-12 nm was achieved in 15 minutes. Biotinylated blocked Streptavidin (SAB4) sensors were prepared as well by following the protocol provided by the manufacturer as the inactive reference controls. Loaded sensors were washed in the

buffer for 10 minutes to eliminated loose nonspecific bound protein and establish stable base line before starting association-dissociation cycles of compound from the lowest concentration. For very potent compound with K_d value lower than 10 nM, column format was utilized to perform the assay in which an individual sensor was prepared for each concentration to avoid significant carry-over due to slow dissociation rates. Compound was allowed to associate with the sensors for 10 minutes followed by 10 minute of dissociation. Buffer only reference was included in all assays. Raw kinetic data collected were processed in the Data Analysis software provided by the manufacturer using double reference subtraction in which both buffer only reference and inactive protein reference were subtracted. Resulting data were analyzed based on 1:1 binding model from which k_{on} and k_{off} values were obtained and then K_d values were calculated.

RNA-seq data analyses

TopHat2 [5] was used to map reads to mouse reference genome assembly mm9. Mapped reads from two conditions were then used as input in cuffdiff software [6] to calculate FPKM for each gene under each condition. Genes are considered significantly differentially expressed if both below criteria are met: 1) P value given by cuffdiff is less than 0.05; and 2) $\log_2$ (fold changes) greater than 0.7 or less than -0.7. Scatter plot of correlation studies for MLL1 deletion and MM-401 data set was obtained by R software using the following procedures: transcripts with enough aligned reads and corresponding to the same gene are combined. Transcripts with not enough aligned reads or show opposite trends for the same gene are discarded. Total of 8100 genes with valid expression level information in both compound and deletion data sets were used for the analyses. Gene set enrichment analysis (GSEA) was done using downloaded version of GSEA softward. Differential expressed genes were used as ranked gene list and GSEA was run against a group of genes. FDR was calculated based on 1,000 permutations. R package GOstats was used for GO term association. For each gene list, GO term enrichment was tested with conditional single-sided hypergeometric distribution and P value was calculated.

Supplemental Figure Legends

Supplemental Figure 1. (A-D), Affinities and kinetic parameters of the interactions between WDR5 and MM-401 and MM-NC-401 determined by biophysical assays. **A.** Kinetic binding **s**ensorgrams of MM-401 with concentrations ranging from 0.4 to 25 nM. Interferometric responses increased upon the binding of MM-401 to the WDR5 protein immobilized on the Super Streptavidin (SSA) bio-sensor surfaces. Signals were monitored and recorded every second while sample plates being continuously shaken at 1000 RPM to eliminate mass transport effect. MM-401 does-dependently binds to WDR5 and saturation was achieved for sensorgrams with high compound concentration, which are two main indications of the specificity of the interaction. The sensorgram of each concentration was obtained using a fresh biosensor to avoid the carry-over due to low dissociation rate. **B.** Steady state fitting of the equilibrium responses *vs* compound concentrations based on 1:1 binding model. **C.** Kinetic binding **s**ensorgrams of MM-NC-401 with concentrations ranging from 0.3 to 10 μM. There was no binding signal detected at the highest concentration tested, 10 μM. **D.** Summary of K_d, k_{on} and k_{off} values of MM-401 and MM-NC-401 to WDR5. **E.** Competitive fluorescence polarization experiments for WDR5 in the presence of increasing concentrations of MM-401 or MM-NC-401 as well as fluorescence-labeled Ac-ARA peptide as tracer. **F.** Summary of IC50 of MM-101, MM-401 as well as MM-NC-401 in binding, HMT and cell growth assays. This figure is mostly related to Figure 1.

Supplemental Figure 2. A. Steady-state kinetic analysis of the MLL1 complex at standard reaction conditions to evaluate MLL1 complex function and establish peptide concentration for inhibitor testing. Error bars represent standard error from two replicates. The kinetic parameters were determined and summarized in the Table. **B.** Lineweaver-Burk plot in the presence of increasing concentrations of MM-401, ranging from 0 to 12.8 μM. The enzyme kinetics for MLL1 complex in the presence of inhibitor shows that MM-401 is likely to be a non-competitive inhibitor for the MLL1 complex. **C.** *In vitro* HMT assays for evaluation of specificity of MM-401 for other MLL subfamily histone methyltransferases (G9a, Suv39h1, MMSET and DOT1L). 250 μM MM-401 is used in each reaction. **D.** *In vitro* HMT assays for the recombinant MLL family HMT complexes with (black) or without WDR5 (white). Y-axis, count per minute for activities detected by scintillation counter. Data represent mean values for triplicates ± s.d. Experiment was performed at least two times. **E.** MALDI mass spec to detect MLL3 methylation product

with no enzyme (top), 5 μM MLL3 complex (middle) and 5 μM MLL3 complex without WDR5 (bottom). Expected molecular weights of the products are highlighted. This figure is related to Figure 2.

Supplemental Figure 3. A. Competitive fluorescence polarization experiments for WDR5 and WIN motifs for MLL1-4. Fluorescence-labeled 12mer MLL1 WIN peptide is used as the tracer. **B.** *In vitro* Ni-NTA pull down experiments for the MLL4 (WBP7) complex with or without WDR5 as indicated on top. MLL4, RbBP5 and WDR5 in the bound fraction were detected in immunoblots using antibodies as indicated on left. **C.** *In vitro* GST pull down experiments for the MLL4 complex and HeLa nuclear extract in the presence of increasing concentration of MM-401 or 25 μM MM-NC-401 as indicated on top. MLL4, RbBP5 and WDR5 in the bound fraction were detected in immunoblots using antibodies as indicated on left. **D.** *In vitro* HMT assays for the recombinant MLL1 or MLL3 complexes with (black) or without RbBP5 (dark blue) or ASH2L (light blue). Y-axis, relative activities to the full complex in each case, which is arbitrarily set as 1. Data represent mean values for triplicates ± s.d. Experiment was performed at least two times. **E.** Genotyping PCR to show Mll1 allele before and after excision. Mll $^{f/f}$ and Mll knockout allele are indicated on right. **F.** Immunoblot for MLL, RbBP5 and WDR5 proteins after MM-401 treatment for MLL1 deletion. **G.** ChIP experiments for WDR5 and RbBP5 at Hoxa9 loci with MM-401 treatment or MLL1 gene deletion. Y-axis represents relative value to 5% input. Data are presented as mean ± s.d. from three experiments. **H.** Immunofluorescence for MLL1 with or without MM-401 treatment as indicated on left. This figure is related to Figure 2 and 3.

Supplemental Figure 4 A. ChIP analyses for MLL1 at MLL1 dependent or independent gene targets as indicated on bottom using murine *MLL-AF9* leukemia cells. **B**. Real-time PCR for 10 MLL1 independent genes as indicated on bottom in MLL-AF9 transduced BM cells treated with Mock or 4-OHT. Gene expression was normalized against total RNA and presented as fold change against Mock treated cells, which was arbitrarily set at 1. **C.** ChIP analyses for H3K4me2 (top) and H3K4me3 (bottom) at promoters of genes described in B. **D.** Real-time PCR for all Hox a genes (except Hoxa1) as indicated on bottom. Gene expression was normalized against total RNA and presented as fold change against Hoxa9 in mock treated cells, which was arbitrarily set at 1. **E.** ChIP analyses for H3K4me2 at Hox a1 to a6 as indicated. Primer pairs used for real time PCR

are indicated as black lines in Hox a gene schematics above. For **A-E**, data are presented as mean ± s.d. from three experiments. For CHIP assays, Y-axis represents relative value to 5% input. This figure is related to Figure 3.

Supplemental Figure 5 GO term enrichment for MLL1 targets that change expression upon MM-401 treatment. Selected developmental pathways were listed for both down-regulated (**A**) and up-regulated pathways (**B**). Full list see Supplemental Table 6. This figure is related to Figure 4.

Supplemental Figure 6 MLL1 methyltransferase activity is required for MLL leukemia but dispensable for normal hematopoiesis. **A.** Schematics for the bone marrow transformation assay using retroviruses expressing MLL-AF9 and shRNAs against MLL1 or WDR5. **B.** Clonogenic assays for MLL-AF9 cells treated with control, MLL1 and WDR5 shRNAs as indicated on top. **C.** Quantitation of the colonies in experiment shown in B. Average of three experiments is presented and error bars represent standard deviation. **D.** Growth curve for freshly isolated wild type murine bone marrow cells in the presence of MM-401 (left) or MM-NC-401 (right). Y-axis, equivalent cell numbers in culture; X-axis, days in culture. Data represent mean values for triplicates ± s.d. **E.** Colony assay for MM-401 and MM-NC-401 treated wild type murine bone marrow cells. For **D** and **E**, NT, no treatment control; DMSO, 0.025% DMSO in culture media; 20-160μM, final compound concentration in media. This figure is related to Figure 5.

Supplemental Figure 7 MM-401 shows specific inhibition of MV4;11 (MLL-AF4) but not K562 (non-MLL) cells. **A.** Dose-dependent effects of MM-401 on apoptosis for human *MV4:11* or *K562* cells. FACS analyses for Annexin V and PI double stained cells were quantified and presented as percent total. **B.** Dose-dependent effects of MM-401 on cell cycle progression for human *MV4:11* or *K562* cells as indicated on top. Cell cycle index was analyzed by FACS after PI staining and cell cycle phases were indicated on top. Y-axis is % cells in each cell cycle phase. **C.** Dose-dependent effects of MM-401 on myeloid differentiation for human *MV4:11* or *K562* cells. FACS analyses for myeloid surface marker CD11b. Y-axis is normalized % events defined by Flow-Jo on overlay histogram and X-axis is signal intensity in arbitrary unit. This figure is related to Figure 5 and Figure 6.

Supplemental Tables

Supplemental Table 1 Genes that show differential expression in MLL1 deletion vs. Mock treated cells. Selection Criteria is *p* value<0.05, abs(log2(fold changes))>1 ("abs" means absolute value)

Supplemental Table 2 Genes that show differential expression in MM-NC-401 vs. MM-401 treated cells. Selection Criteria is *p* value<0.05, abs(log2(fold changes))>1 ("abs" means absolute value).

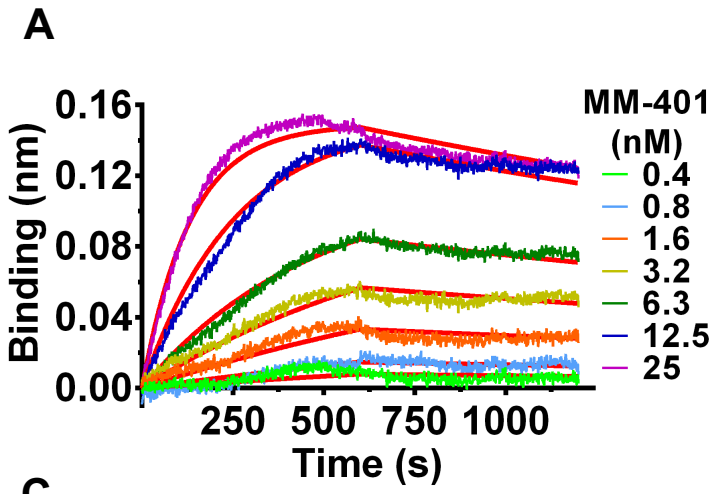
Supplemental Table 3 Common down regulated genes for MLL1 deletion and MM-401 treatment. Selection Criteria is *p* value<0.05, abs(log2(fold changes))>0.7 ("abs" means absolute value)

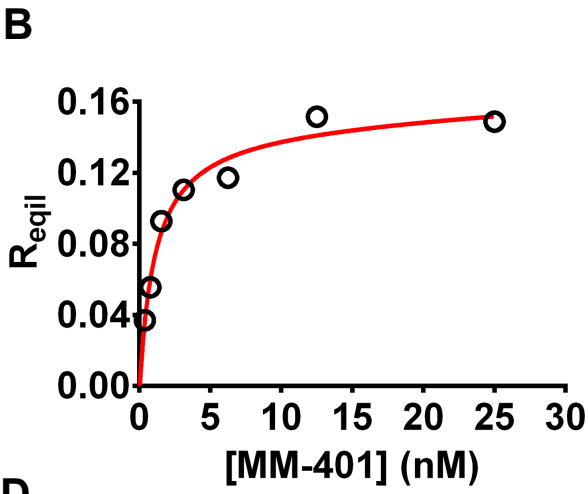
Supplemental Table 4 Common up regulated genes for MLL1 deletion and MM-401 treatment. Selection Criteria is P value<0.05, abs(log2(fold changes))>0.7 ("abs" means absolute value)

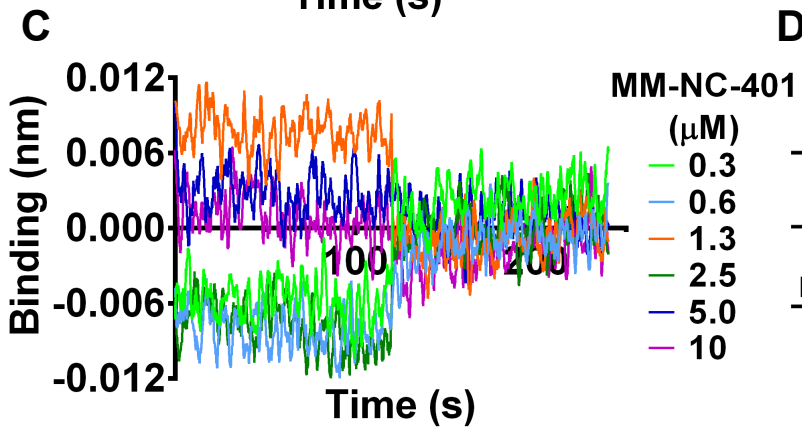
Supplemental Table 5 GO-term analysis for genes regulated by both MLL1 deletion and MM-401 treatment

Supplemental Table 6 Cytogenetic information for patients whose blood samples are used in Figure 7B

Supplementary Figure 1





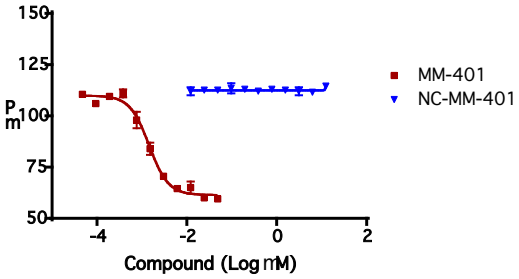


D

	K_d (nM)	k_{on} (10^5 1/Ms)	k_{off} (10^{-4} 1/s)
MM-401	0.78±0.20	3.3±0.4	2.5±0.4
MM-NC-401	> 10 μM	N/A	N/A

E

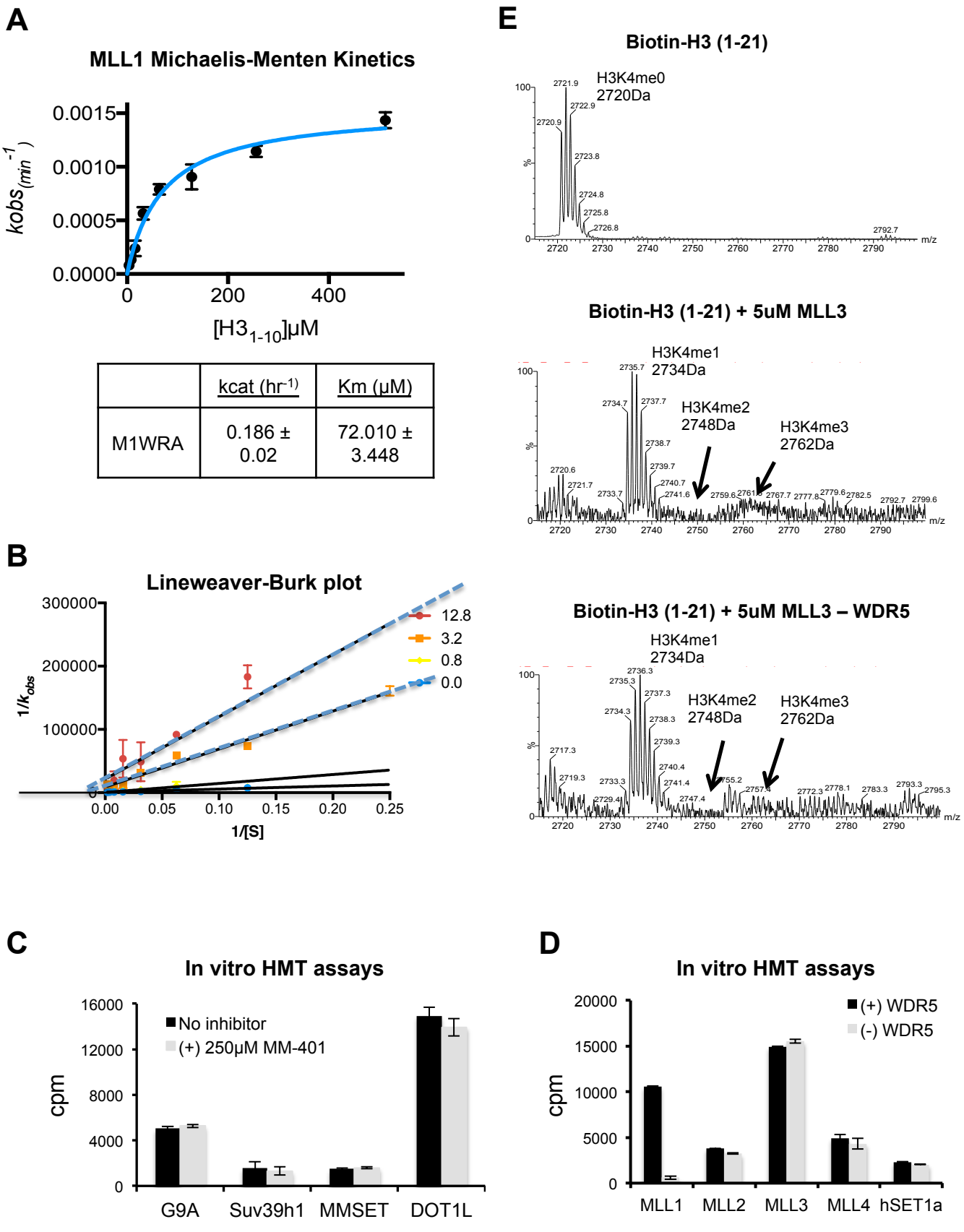
	MM-401	NC-MM-401
Sigmoidal dose-response (variable slope)		Ambiguous
Best-fit values		
Bottom	61.41	~ 290.1
Top	109.9	112.5
LogEC50	-2.839	~ 1.167
HillSlope	-2.028	~ -27.41
EC50	0.001448	~ 14.70



F

Compound	IC_{50} ± SD		
	Binding affinity to WDR5 (nM)	HMT inhibition (μM)	Cell growth inhibition (μM)
MM-101	2.9 ± 1.4	5.4 ± 0.28	134
MM-401	0.9 ± 0.2	0.32 ± 0.10	9.76 ± 0.04
MM-NC-401	> 100 μM	> 100	> 100

Supplementary Figure 2

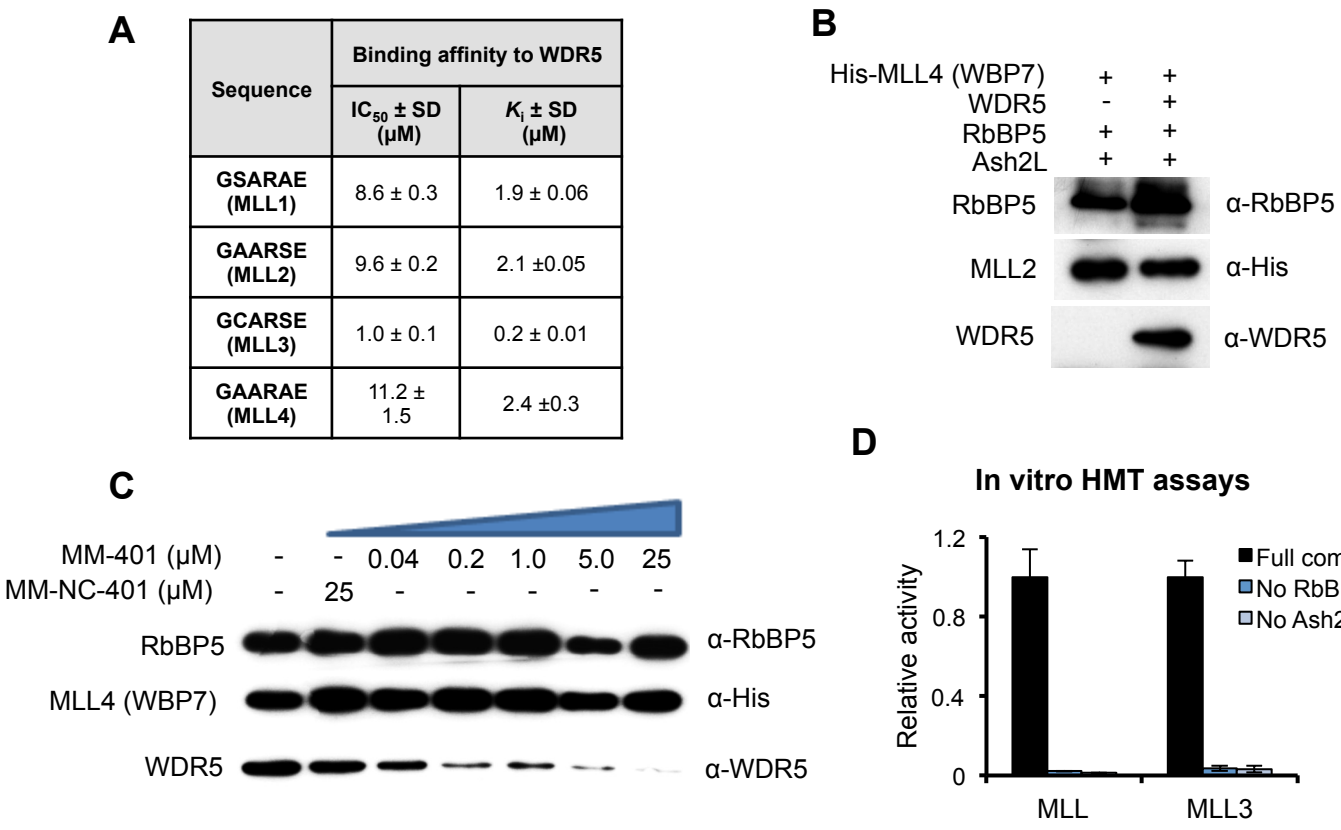


	kcat (hr^{-1})	Km (μM)
M1WRA	0.186 ± 0.02	72.010 ± 3.448

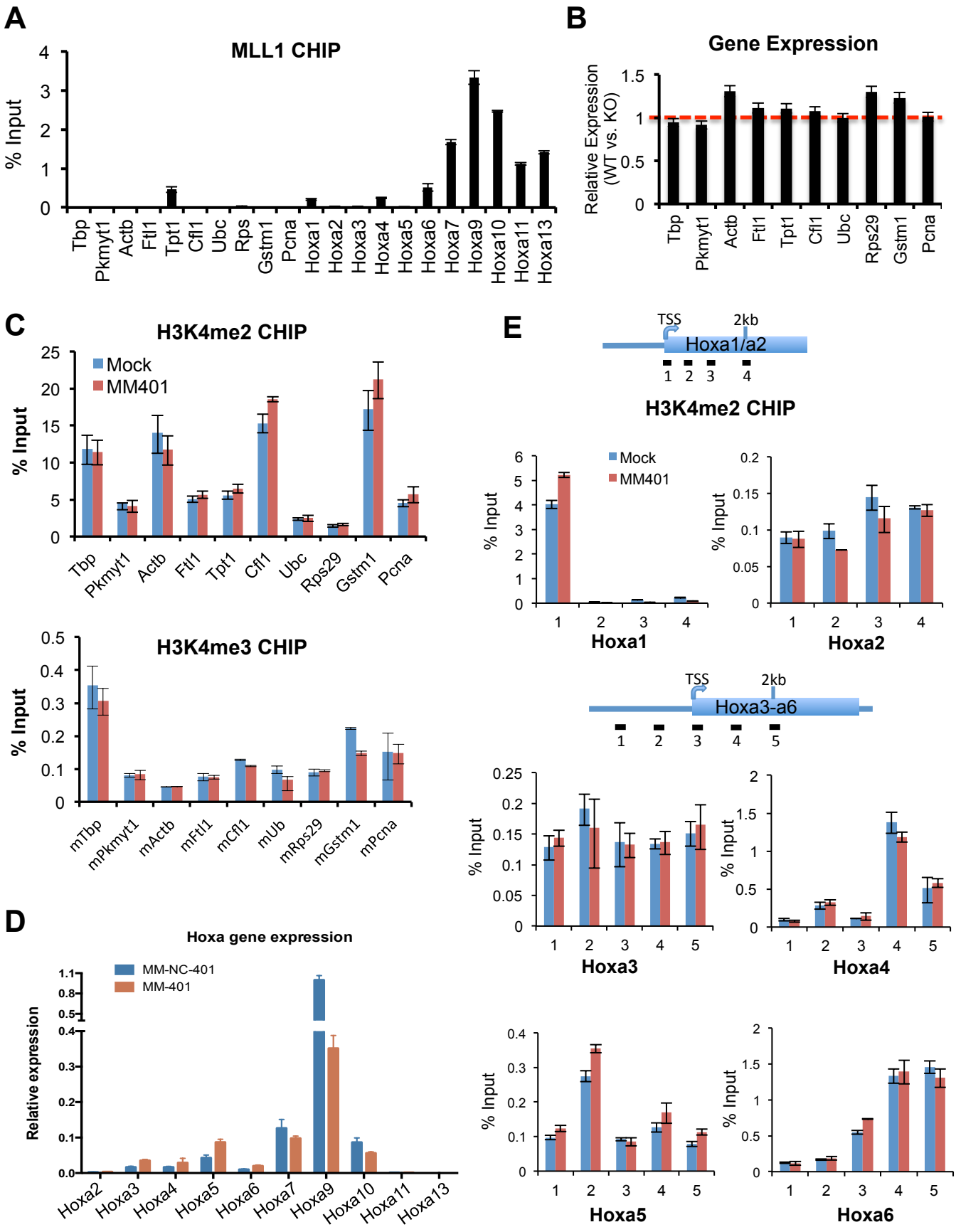
Supplementary Figure 3

A

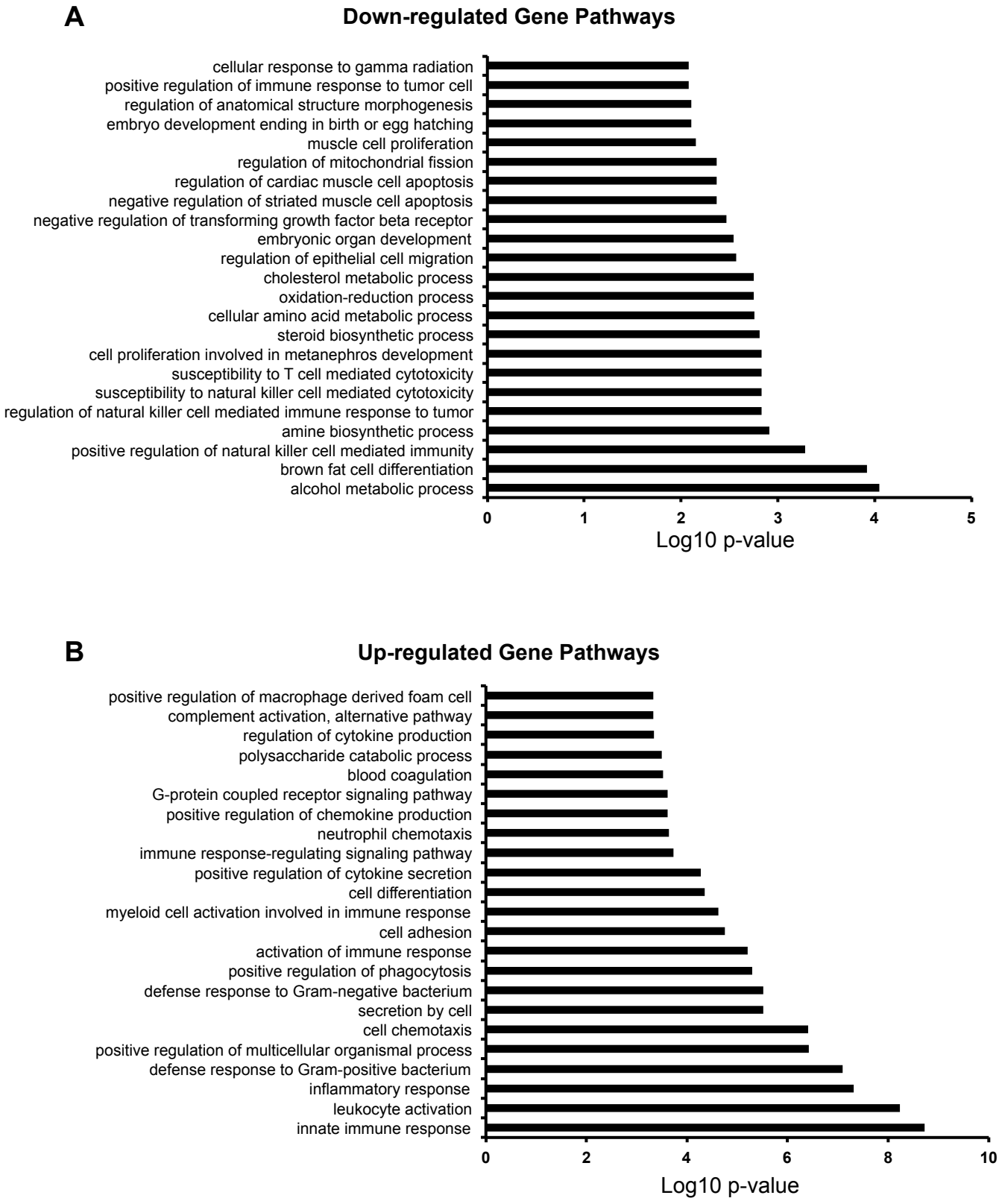
Sequence	Binding affinity to WDR5	
	IC_{50} ± SD (μM)	K_i ± SD (μM)
GSARAE (MLL1)	8.6 ± 0.3	1.9 ± 0.06
GAARSE (MLL2)	9.6 ± 0.2	2.1 ±0.05
GCARSE (MLL3)	1.0 ± 0.1	0.2 ± 0.01
GAARAE (MLL4)	11.2 ± 1.5	2.4 ±0.3



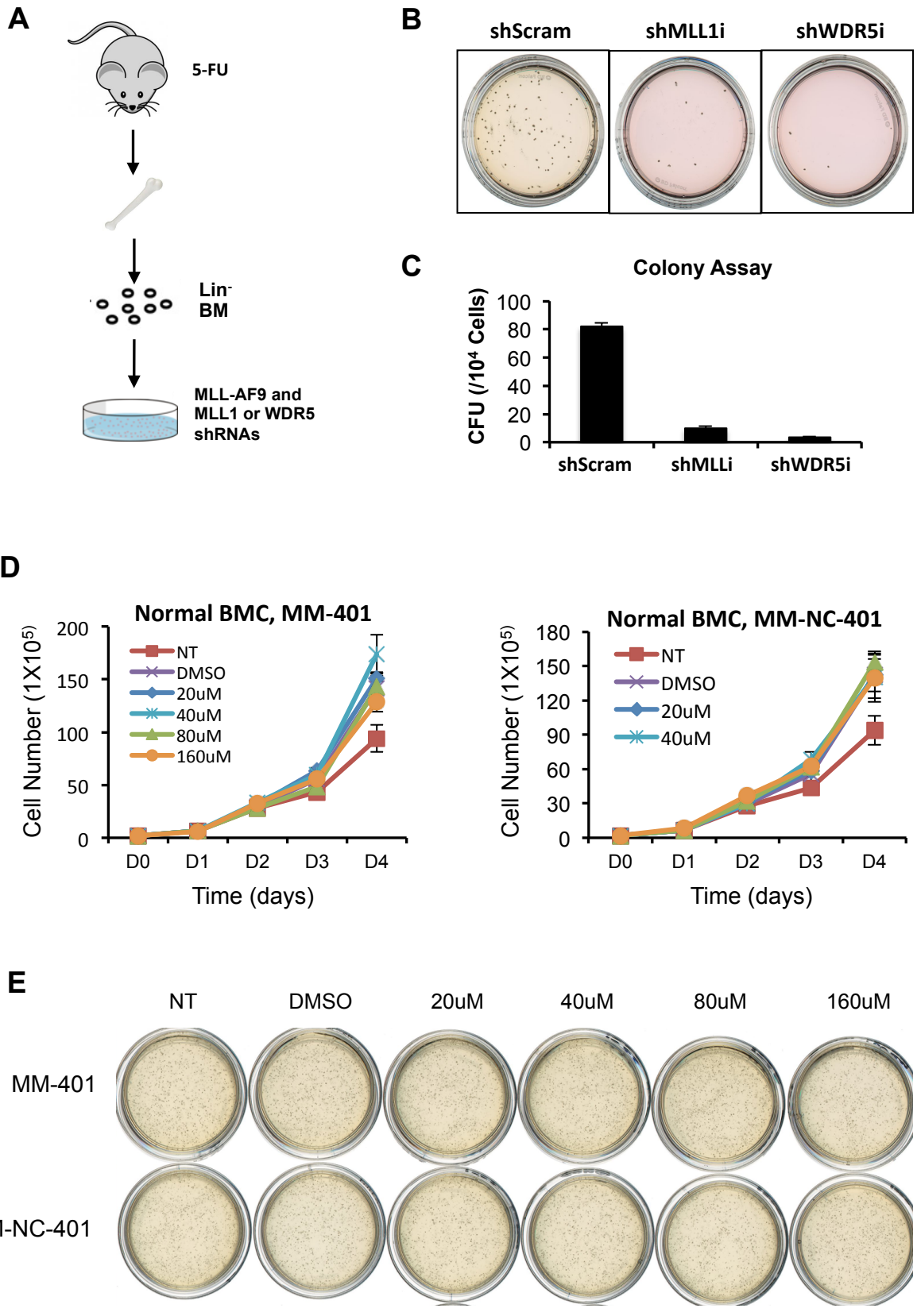
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7