Supporting Information (SI Appendix)

Inhibition of Enhancer of zeste homolog 2 (EZH2) induces natural killer cell-mediated eradication of hepatocellular carcinoma cells

Suresh Bugide[a], Michael R. Green[b,c,1], and Narendra Wajapeyee[a,1]

[a]Department of Pathology, Yale University School of Medicine, New Haven, CT, 06510
[b]Department of Molecular, Cell and Cancer Biology, University of Massachusetts Medical School, Worcester, MA, 01605
[c]Howard Hughes Medical Institute, University of Massachusetts Medical School, Worcester, MA, 01605

[1]To whom correspondence should be addressed:

Narendra Wajapeyee
Department of Pathology, 310 Cedar Street, Room 315A, Yale University School of Medicine, New Haven, CT 06510, USA. Tel: (203)-737-5070
Email: Narendra.Wajapeyee@yale.edu

Michael R. Green
Department of Molecular, Cell and Cancer Biology, University of Massachusetts Medical School, 364 Plantation Street, Room 628, Worcester, MA 01605, USA. Tel: (508)-856-5331
Email: Michael.Green@umassmed.edu

SI Materials and Methods

LDH cytotoxic assay. The LDH cytotoxicity assay was performed using the LDH cytotoxicity assay kit from Thermo Fisher Scientific (cat. no. 88953), as previously described (1). NK92MI cells (2×10^6 cells/ml; 100 μL) served as effector cells and were incubated with different HCC cell lines (10×10^3) cells in an effector to target ratio of 20:1 in 96-well tissue culture plates. The plates were incubated at 37°C in a CO_2 incubator for 2 hrs. After incubation, the 96-well tissue culture plates were centrifuged at 1000 rpm for 3 min. The supernatants were then collected from each well into a fresh 96-well plate and 50 μL of LDH substrate mixture was added to each well. The plate was incubated for 10–20 min at room temperature in the dark and absorbance at 490 nm and 680 nm was measured using an ELISA plate reader. The absorbance at 680 nm was subtracted from the absorbance at 490 nm to calculate the percent (%) cytotoxicity using the formula below:

$$\frac{\text{LDH experimental} - \text{LDH effector cells} - \text{LDH spontaneous}}{\text{LDH maximal - LDH spontaneous}} \times 100$$

Calcein AM cytotoxicity assay. A Calcein AM cytotoxicity assay was performed as previously described (2). To stain the HCC cells, 1 μM of Calcein AM (cat. no. C1359, Sigma-Aldrich) was added to cells in DMEM (1×10^5 cells/ml) and the cells were incubated for 30 min at room temperature. The HCC cells were washed twice with 1× PBS and re-suspended in complete DMEM medium at a concentration of 1×10^5 cells/ml. The NK92MI cells were plated in triplicate in 96-microwell plates at a concentration of 1×10^6 cells/ml to achieve a 1:10 ratio. The plates were incubated at 37°C in CO_2 for 4 hrs. After incubation, fluorescent images of the cells

were captured using a microscope (Olympus). For complete cell lysis, the HCC cells were incubated with 20 µl of 10× lysis buffer for 1 hr at 37°C.

Chemical genetics screen using small molecule inhibitors targeting specific epigenetic regulators. To identify the modulators of NK cell ligand expression on HCC cells, 32 different small molecule inhibitors obtained from Structural Genomics Consortium (SGC) were tested. All of the inhibitors were dissolved in DMSO to prepare 10 mM stocks. The inhibitors and their targets are listed in the SI Appendix, Table S2. Additionally, the EZH2 inhibitor GSK126 was purchased from MedChem Express (cat.no. HY-13470), which was also dissolved in DMSO and prepared as a 10 mM stock solution. SK-HEP-1 cells (3×10^6) were seeded in 6-well plates and treated with different doses of small molecule inhibitors (listed in **SI Appendix, Table S2)** or DMSO as a control. After 48 hrs of treatment with the inhibitors, the cells were either harvested in TRIzol® reagent for analyzing the expression of NK cell ligands or used for NK cell cytotoxicity assays.

Based on their effect on the re-expression of at least seven or more NK cell ligands in the primary screen using SK-HEP-1 cells and the HCC patient data samples indicating the alteration of these epigenetic regulators in HCC, we selected six inhibitors for follow-up studies. The six inhibitors, which were against the epigenetic regulators EZH2, SMYD2, BRPF1/2/3, and BAZ2A/B proteins, were further tested in another HCC cell line PLC-PRF-5. PLC/PRF/5 cells (3×10^6) were seeded in 6-well plates and treated with EZH2, SMYD2, BRPF1/2/3, or BAZ2A/B inhibitors or DMSO vehicle control. After 48 hrs of treatment with the inhibitors, the cells were harvested in TRIzol® reagent to analyze the expression of the NK cell ligands.

NK cell cytotoxicity assay using EZH2 shRNAs or EZH2 inhibitor. SK-HEP-1 or PLC/PRF/5 cells expressing *EZH2* shRNAs or a non-specific shRNA as a negative control were analyzed for NK cell-mediated cytotoxicity using LDH or Calcein AM, as described. For similar experiments with the EZH2 inhibitor, SK-HEP-1 and PLC/PRF/5 cells were treated with the EZH2 inhibitors GSK343 and GSK126 at the concentrations of 3 μM or 2 μM, respectively, for 48 hrs. After treatment, the cells were analyzed for NK cell-mediated cytotoxicity using LDH or Calcein AM, as described.

Similarly, SK-HEP-1 or PLC/PRF/5 cells expressing *ULBP1*, *MICA*, or *MICB* shRNAs or a non-specific shRNA as a negative control were treated with either DMSO or 3 μM GSK343 and analyzed for NK cell-mediated cytotoxicity using an LDH-based method.

Chromatin Immunoprecipitation. Chromatin immunoprecipitation (ChIP) was performed as previously described (3). Briefly, a ChIP assay was performed in SK-HEP-1 and PLC/PRF/5 cells using the Simple ChIP Enzymatic Chromatin IP kit (cat.no. 9002S, Cell Signaling) according to the manufacturer's protocol. The lysates were diluted in the ChIP buffer containing protease inhibitor cocktail (Roche), and the samples were incubated with antibodies against EZH2, trimethyl H3K27, DNMT1, DNMT3a (Active Motif, CA, USA), or control IgG (Cell Signaling), followed by immobilization on protein A/G agarose beads (Life Technologies). The chromatin was eluted, and DNA was extracted using DNA purification columns. Quantitative PCR (qPCR) was performed using *ULBP1* and *MICA* promoter-specific primers. Relative fold-change was calculated as the ratio of immunoprecipitated DNA to IgG. The primer sequences and antibodies used for the ChIP assays are listed in SI Appendix, Table S5.

5Aza2dC and TSA treatment. SK-HEP-1 and PLC/PRF/5 cells were treated with 5 μM 5-Aza-2-deoxyCytidine (5Aza2dC) demethylating agent for 3 days followed by 1 μM Trichostatin A (TSA) for 12 hrs. Post 5Aza2dC /TSA treatment, the cells were either harvested in TRIzol® for total RNA isolation or lysed in IP lysis buffer to evaluate the expression of the ULBP1 and MICA ligands by western blotting.

Methylated DNA Immunoprecipitation (MeDIP). SK-HEP-1 and PLC/PRF/5 cells were treated with azacytidine (5Aza2dC) and TSA or the EZH2 inhibitor GSK343 or DMSO. Post treatment, DNA was extracted from the cells using a DNeasy Blood & Tissue kit (Qiagen). The extracted DNA was sonicated (Qsonica) for 15 30-second pulses (60% amplitude) with a 45-second pause between each pulse. The sonicated DNA (1 μg) from each sample was used for MeDIP analysis with the MeDIP kit (cat.no. 55009, Active Motif) according to the manufacturer's protocol. The samples were incubated with antibodies against 5-methylcytosine or control IgG, followed by immobilization on Protein G magnetic beads. The chromatin was eluted, and the DNA was extracted using DNA purification columns (Qiagen). Quantitative PCR was performed using *ULBP1* promoter-specific primers. The relative fold-enrichment was calculated as the ratio of immunoprecipitated DNA to IgG. The primer sequences used for the MeDIP assay are listed in SI Appendix, Table S5.

Isolation of mRNA and RT-qPCR analysis. Total RNA was extracted with TRIzol® (Invitrogen) and purified with RNeasy mini columns (Qiagen) for the mRNA expression analyses. The cDNA was generated using the M-MuLV first-strand cDNA synthesis kit (New England Biolabs) according to the manufacturer's instructions. Quantitative reverse transcription

polymerase chain reaction (RT-qPCR) was performed using the Power SYBR® Green kit (Applied Biosystems) according to the manufacturer's instructions. Actin was used as an internal control. Primer sequences used in the study are provided in SI Appendix, Table 5.

Preparation of the shRNA lentivirus and generation of stable cell lines. Gene specific lentiviral shRNAs were obtained from the Open Biosystems. The catalogue numbers for the shRNAs are provided in SI Appendix, **Table S5**. For the lentivirus production, shRNAs were transfected into 293T cells along with the PDM2.G and psPAX2 packaging plasmids using Effectene Transfection Reagent (Qiagen) per the manufacturer's instructions. After 48 hrs, the lentivirus-containing supernatants were harvested, filtered, and used for infections. Lentiviral shRNA-infected HepG2/C3A cells were selected using 1.5 μg/ml of puromycin and the PLC/PRF/5 and SK-HEP-1 cells were selected using 0.75 μg/ml of puromycin.

Cloning and expression of *ULBP1*, *ULPB2*, *ULBP5*, and *ULBP6*

ULBP1, *2*, *5*, and *6* were PCR amplified using HepG2/C3A cDNA as a template and the primers listed in SI Appendix, Table S5. The amplified fragments were digested with the Xba1 and Xho1 restriction enzymes and ligated into the FG12 vector. Lentiviral particles were generated from the FG12 vector and the *ULBP1*, *2*, *5*, and *6* plasmids as described in the previous section. The SK-HEP-1 cells were infected with FG12 lentiviral particles or lentiviral particles generated from vectors expressing *ULBP1*, *2*, *5*, and *6*. The infected SK-HEP-1 cells were selected using 0.75 μg/ml of puromycin.

SDS-PAGE and Immunoblotting analysis

The cells were lysed using IP lysis buffer (Pierce) containing protease inhibitor cocktail (Roche, Basel, Switzerland). The cell lysates were centrifuged at 10,000 rpm for 10 min. The total protein concentration was estimated using the Bradford assay (Bio-Rad) and an equal amount of protein was subjected to SDS-PAGE. After transferring the proteins to polyvinylidene fluoride (PVDF) membranes, the membranes were blocked with 5% non-fat milk prior to treatment with antibodies at 4°C overnight, followed by three 15 min washes with 1× TBST. After incubation with a secondary antibody, the membrane signals were detected using the SuperSignal West Pico Chemiluminescent Substrate kit (Thermo Fisher Scientific) or the SuperSignal West Femto Chemiluminescent Substrate kit (Thermo Fisher Scientific). The antibodies used for this study are listed in SI Appendix, Table S5.

SI References

1. Jurisic V, Spuzic I, & Konjevic G (1999) A comparison of the NK cell cytotoxicity with effects of TNF-alpha against K-562 cells, determined by LDH release assay. *Cancer Lett* 138(1-2):67-72.
2. Somanchi SS, McCulley KJ, Somanchi A, Chan LL, & Lee DA (2015) A Novel Method for Assessment of Natural Killer Cell Cytotoxicity Using Image Cytometry. *PLoS One* 10(10):e0141074.
3. Gazin C, Wajapeyee N, Gobeil S, Virbasius CM, & Green MR (2007) An elaborate pathway required for Ras-mediated epigenetic silencing. *Nature* 449(7165):1073-1077.

SI Figure Legends and Figures

Fig. S1. Role of NK cell ligands in NK cell-mediated cytotoxicity against HCC cells. (A) HepG2/C3A cells expressing a non-silencing (NS) shRNA or shRNAs against *ULBP1*, *2*, *5* or *6* were analyzed for the mRNA expression of the indicated NK cell ligands by RT-qPCR. Relative mRNA expression compared to NS shRNA expressing cells is shown. (B) HepG2/C3A cells expressing a NS shRNA or shRNAs against *ULBP1*, *2*, *5* or *6* were analyzed for the indicated proteins by immunoblotting. (C) SK-HEP-1 cells expressing an empty vector or cDNAs for *ULBP1*, *2*, *5* or *6* were analyzed for the mRNA expression of the indicated ULBP ligands by RT-qPCR. Relative mRNA expression compared to SK-HEP-1 cells expressing an empty vector is shown. (D) SK-HEP-1 cells expressing an empty vector or cDNAs for *ULBP1*, *2*, *5* or *6* were analyzed for the indicated proteins by immunoblotting. Data are presented as mean ± SEM; *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001.

Fig. S2. Chemical genetic screen to identify the epigenetic factors that regulate NK cell ligand expression. SK-HEP-1 cells were treated with two different concentrations of the indicated inhibitors as listed in Supplementary Table 2 for 48 hrs and the expression of the indicated NK cell ligands were analyzed by RT-qPCR. The relative mRNA expression compared to DMSO-treated cells is shown. Data are presented as mean ± SEM; ns=not significant, *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001.

Fig. S3. Chemical genetic screen to identify the epigenetic factors that regulate NK cell ligand expression. SK-HEP-1 cells were treated with two different concentrations of the indicated inhibitors as listed in Supplementary Table 2 for 48 hrs and the expression of the indicated NK cell ligands were analyzed by RT-qPCR. The relative mRNA expression compared to DMSO-treated cells is shown. Data are presented as mean ± SEM; ns=not significant, *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001.

Fig. S4. Testing the deregulation of epigenetic regulators in HCC patient samples and role of epigenetic factors that regulate NK cell ligand expression. (A) TCGA data for HCC were analyzed using the cBIO portal. All 360 patient samples containing information on mutations, copy number alterations, and mRNA expression were analyzed. The alterations in EZH2, SMYD2, BAZ2A/2B, and BRPF1, BRPF2 (BRD1), and BRPF3 is shown. (B) The indicated oncomine dataset was analyzed for the expression of the indicated epigenetic regulators. (C) PLC/PRF/5 cells were treated with two different concentrations of the indicated inhibitors as listed in Supplementary Table 2 for 48 hrs and the expression of the indicated NK cell ligands were analyzed by RT-qPCR. The relative mRNA expression for indicated NK cell ligands compared to DMSO-treated cells is shown. Data are presented as mean ± SEM; ns=not significant, *p<0.05, **p<0.01, and ***p<0.001.

Fig. S5. EZH2 inhibition results in the re-expression of NK cell ligands and role of NK cell ligands in NK cell-mediated HCC cell eradication. (A) SK-HEP-1 or PLC/PRF/5 cells were treated with either DMSO or the EZH2 inhibitor GSK126 (2 μM) for 48 hrs. Immunoblotting for the H3K27TriMe histone mark and histone H3 was performed for DMSO- or GSK126-treated

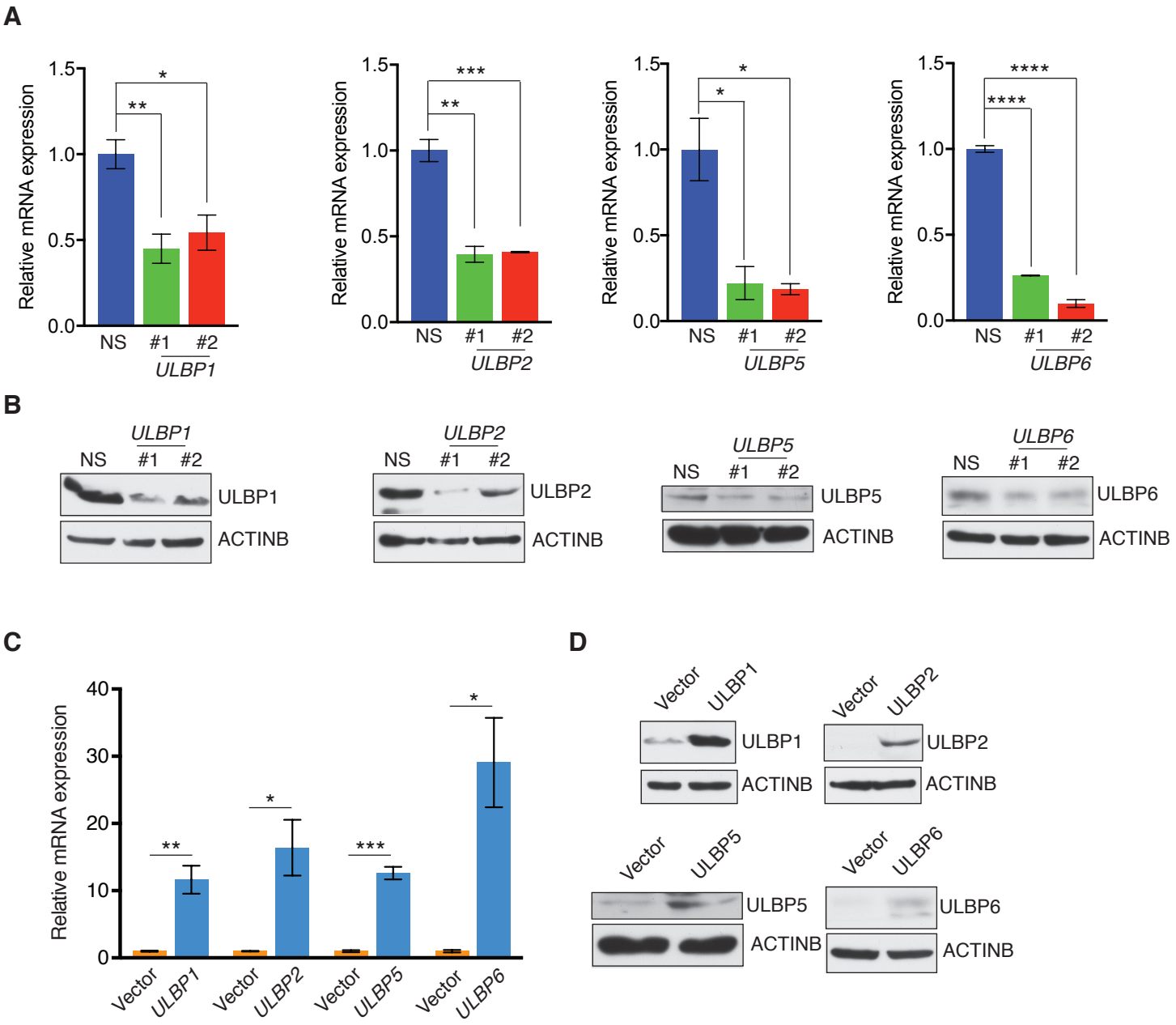
cells. (B) SK-HEP-1 or PLC/PRF/5 cells were treated with either DMSO or the EZH2 inhibitor GSK126 (2 μM) for 48 hrs. The mRNA expression of the indicated NK cell ligands was measured by RT-qPCR and compared to DMSO-treated cells. (C) SK-HEP-1 or PLC/PRF/5 cells expressing a non-silencing (NS) shRNA or *ULBP1, MICA or MICB* shRNAs were analyzed for *ULBP1, MICA or MICB* mRNA expression by RT-qPCR. The relative *ULBP1, MICA or MICB* mRNA expression compared to NS shRNA-expressing cells is shown. (D) SK-HEP-1 (left) or PLC/PRF/5 (right) cells expressing a non-silencing (NS) shRNA or *ULBP1, MICA or MICB* shRNAs were analyzed for the indicated proteins by immunoblotting. Data are presented as mean ± SEM; *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001.

Fig. S6. MICB in NK cell mediated HCC cell eradication and the role of DNA methylation in the regulation of ULBP1 and MICA. (A) SK-HEP-1 or PLC/PRF/5 cells expressing either non-silencing (NS) or *MICB* shRNAs were treated with DMSO or the EZH2 inhibitor GSK343 (3 μM) for 48 hrs and incubated with NK cells at a ratio of 20:1. (B) The 2-kb upstream promoter DNA sequences and the 5'UTR/CDS for *ULBP1* and *MICA* were downloaded from the UCSC genome browser and analyzed using EBI CpG plot to predict putative CpG islands. (C) Indicated HCC cell lines were analyzed by immunoblotting for DNA methyltransferase expression. Data are presented as mean ± SEM; ns=not significant, *p<0.05, and **p<0.01.

Fig. S7. Role of DNA methyltansferase 3A in EZH2-induced *ULBP1* silencing. (A) SK-HEP-1 or PLC/PRF/5 cells expressing non-silencing (NS) shRNA, DNMT1, or DNMT3A shRNAs were analyzed for DNMT1 or DNMT3A expression using RT-qPCR. DNMT1 or DNMT3A mRNA expression relative to NS shRNA is shown. (B) SK-HEP-1 or PLC/PRF/5 cells

expressing non-silencing (NS) shRNA or DNMT1 or DNMT3A shRNAs were analyzed for DNMT1 or DNMT3A expression by immunoblotting. (C) SK-HEP-1 or PLC/PRF/5 cells were treated with DMSO or GSK343 (3 μM) for 48 hrs and analyzed for DNMT1 recruitment on the *ULBP1* promoter or on *ACTINB* (control) using a chromatin immunoprecipitation (ChIP) assay. (D) SK-HEP-1 or PLC/PRF/5 cells expressing non-silencing (NS) shRNA or *DNMT1* shRNAs were analyzed for *ULBP1* mRNA expression by RT-qPCR. Relative *ULBP1* mRNA expression compared to NS shRNA-expressing cells are shown. Data are presented as mean ± SEM; ns=not significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$, and ****$p<0.0001$.

Figure S1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1.0
0.5
0.0
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#2
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ULBP2
ULBP5
ULBP6
B
ULBP1
ULBP2
ULBP5
ULBP6
ACTINB
C
Relative mRNA expression
40
30
20
10
0
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ULBP2
ULBP5
ULBP6
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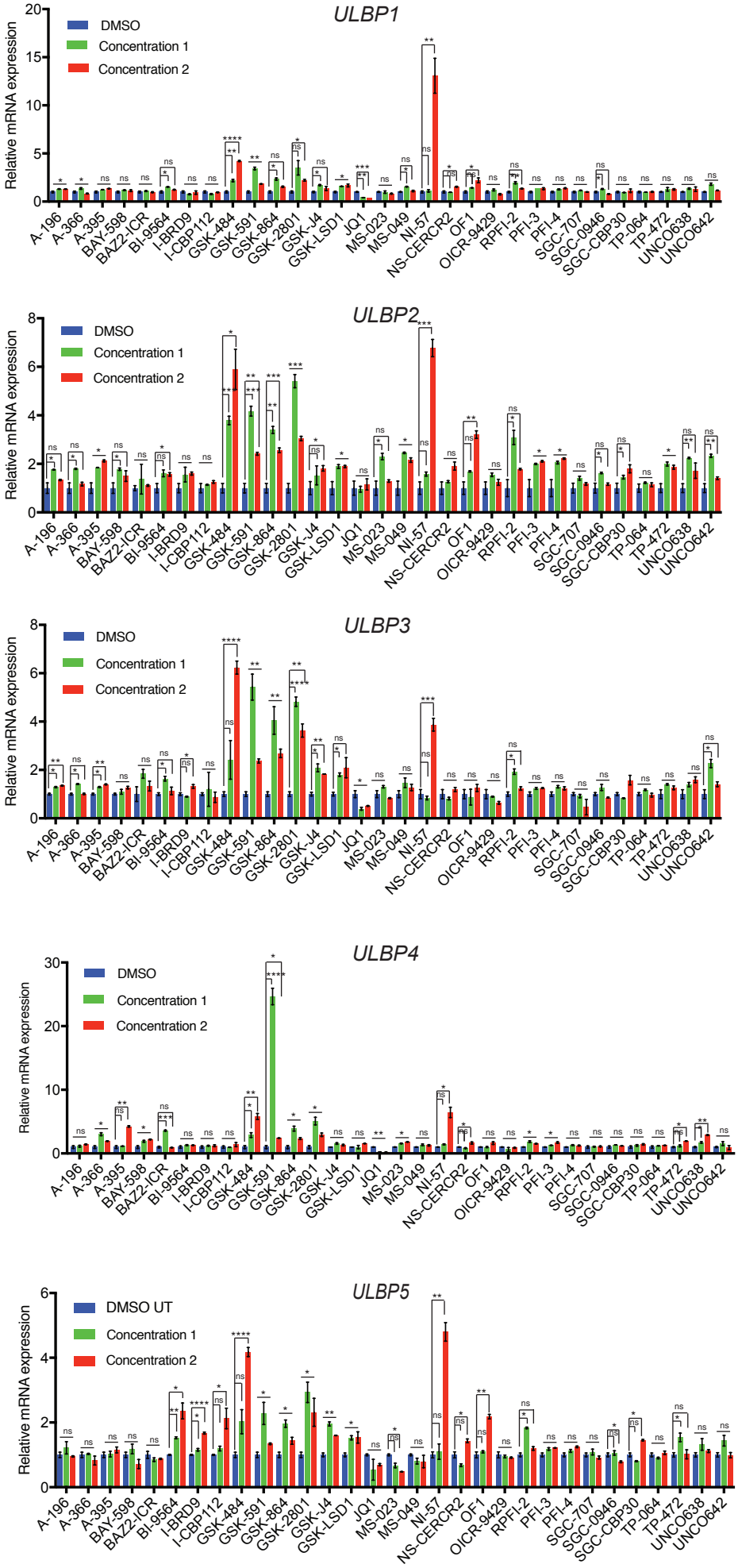
Vector
ULBP1
ULBP2
ULBP5
ULBP6
ACTINB

Figure S2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ULBP2
ULBP3
ULBP4
ULBP5
DMSO
DMSO UT
Concentration 1
Concentration 2
Relative mRNA expression
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BAY-598
BAZ2-ICR
BI-9564
I-BRD9
I-CBP112
GSK-484
GSK-591
GSK-864
GSK-2801
GSK-J4
GSK-LSD1
JQ1
MS-023
MS-049
NI-57
NS-CERCR2
OF1
OICR-9429
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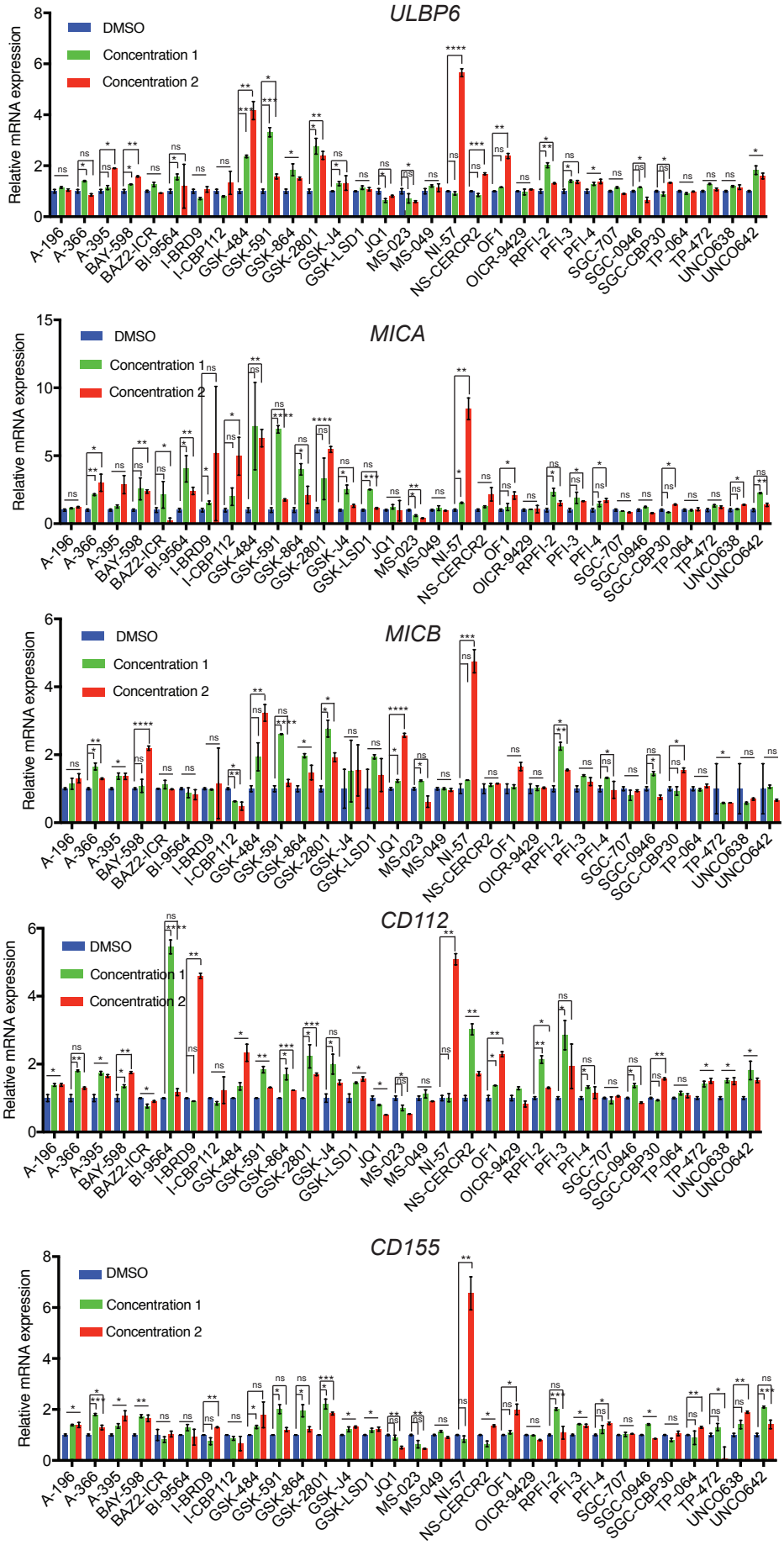
Figure S3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MICB
CD112
CD155
Relative mRNA expression
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OF1
OICR-9429
RPFI-2
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Figure S4

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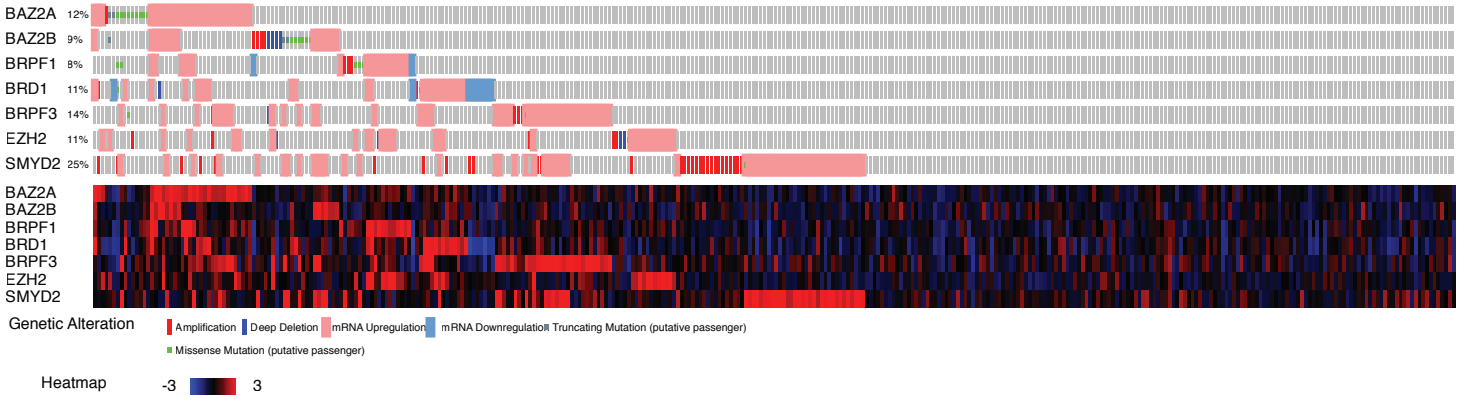

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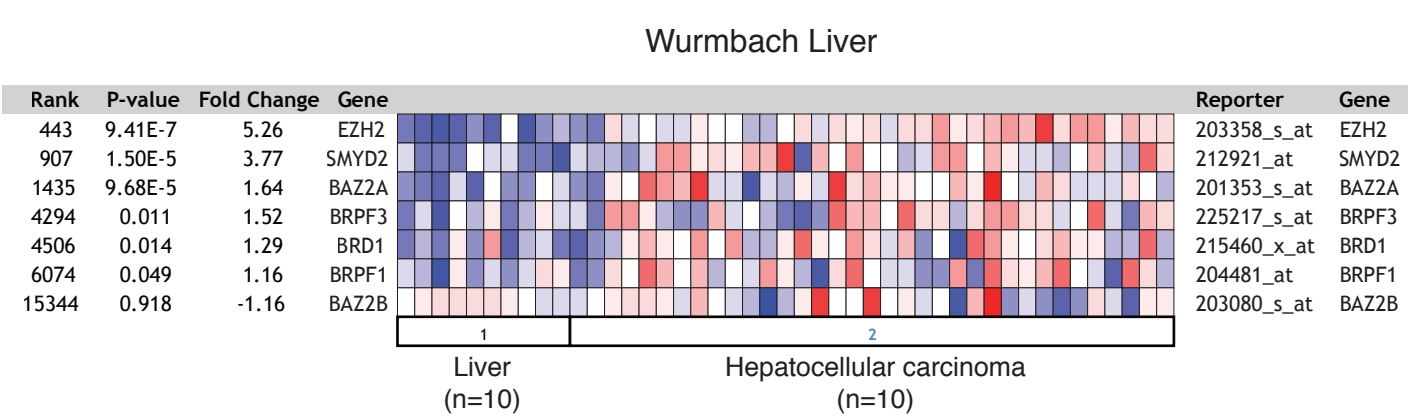

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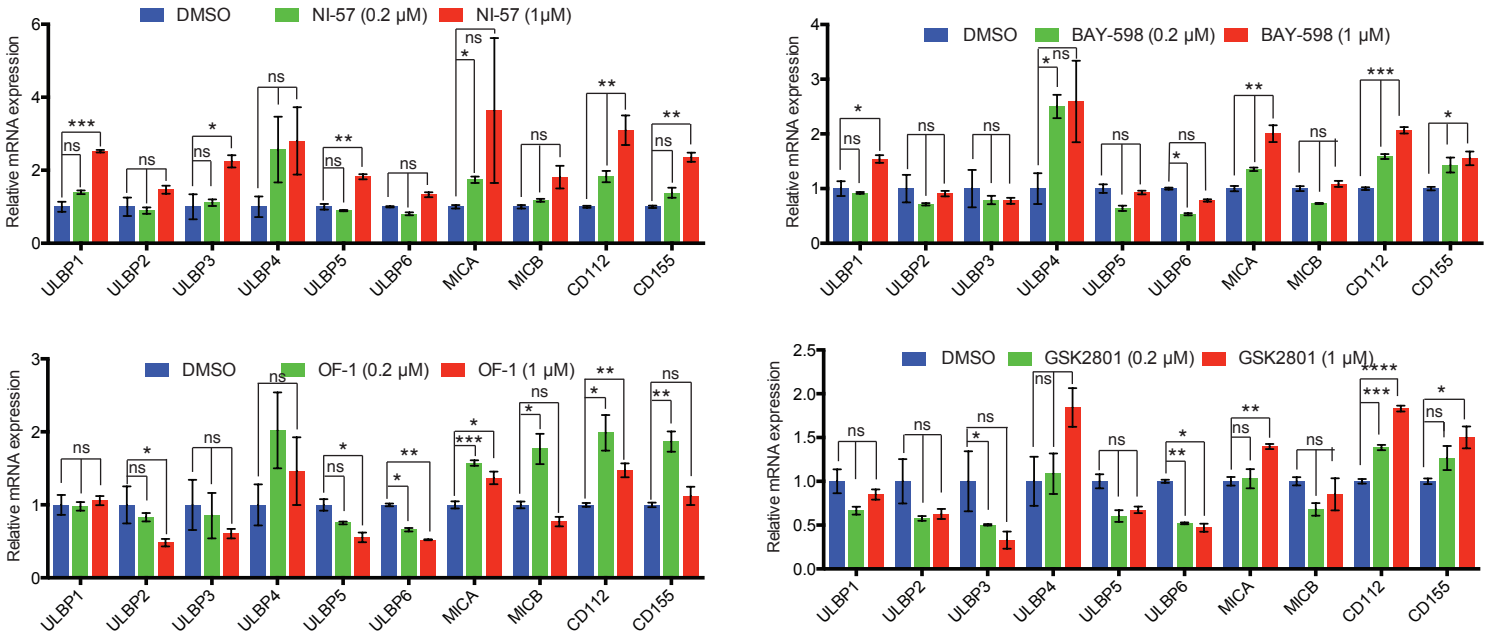
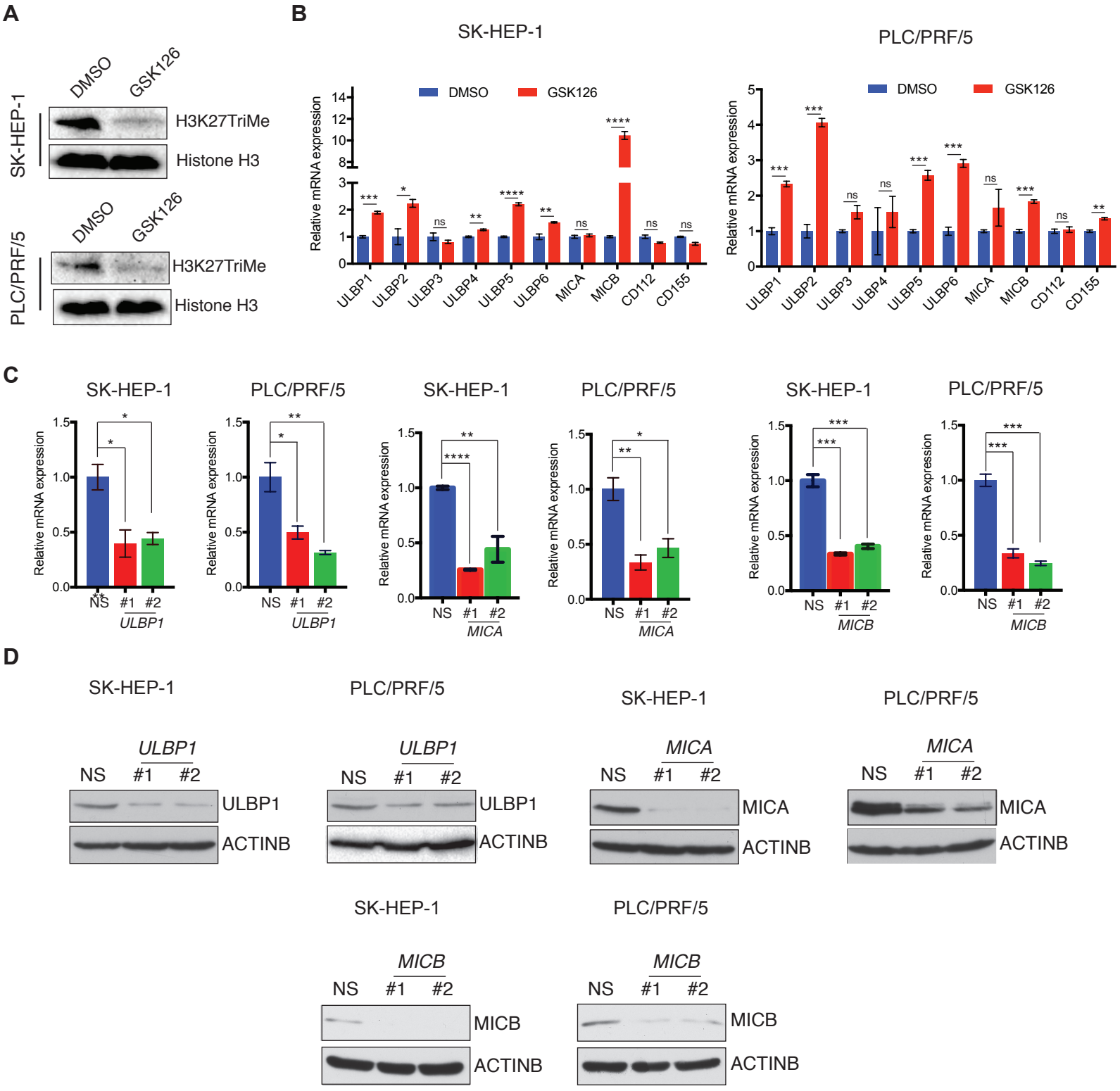


Figure S5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Histone H3
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GSK126
Relative mRNA expression
ULBP1
ULBP2
ULBP3
ULBP4
ULBP5
ULBP6
MICA
MICB
CD112
CD155
C
SK-HEP-1
PLC/PRF/5
Relative mRNA expression
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#2
ULBP1
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D
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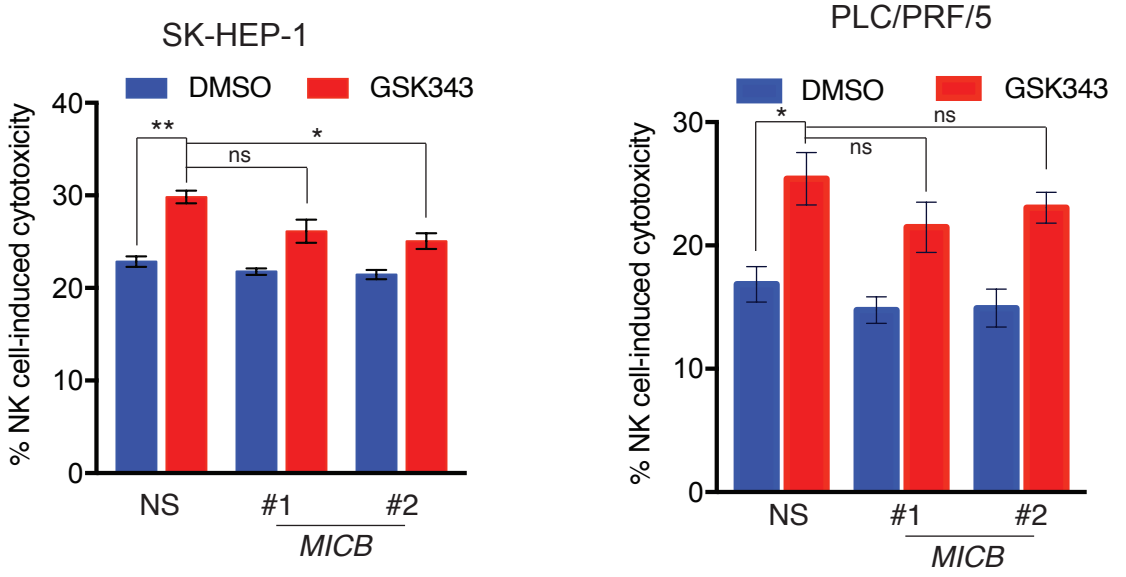
MICB
ACTINB

Figure S6

A



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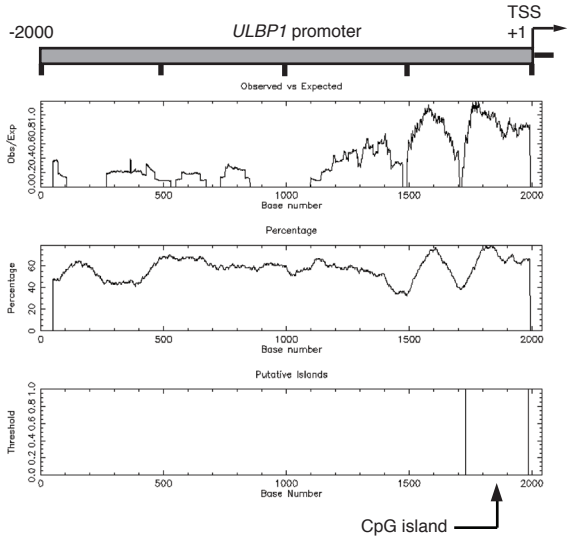

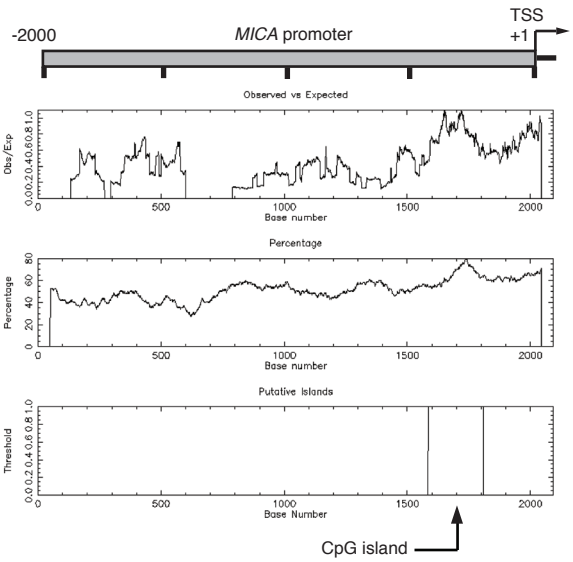

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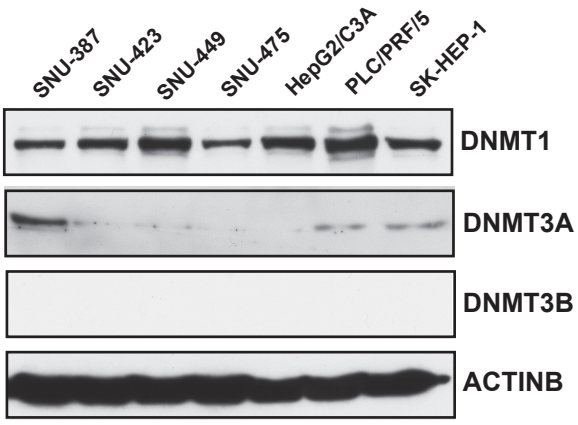
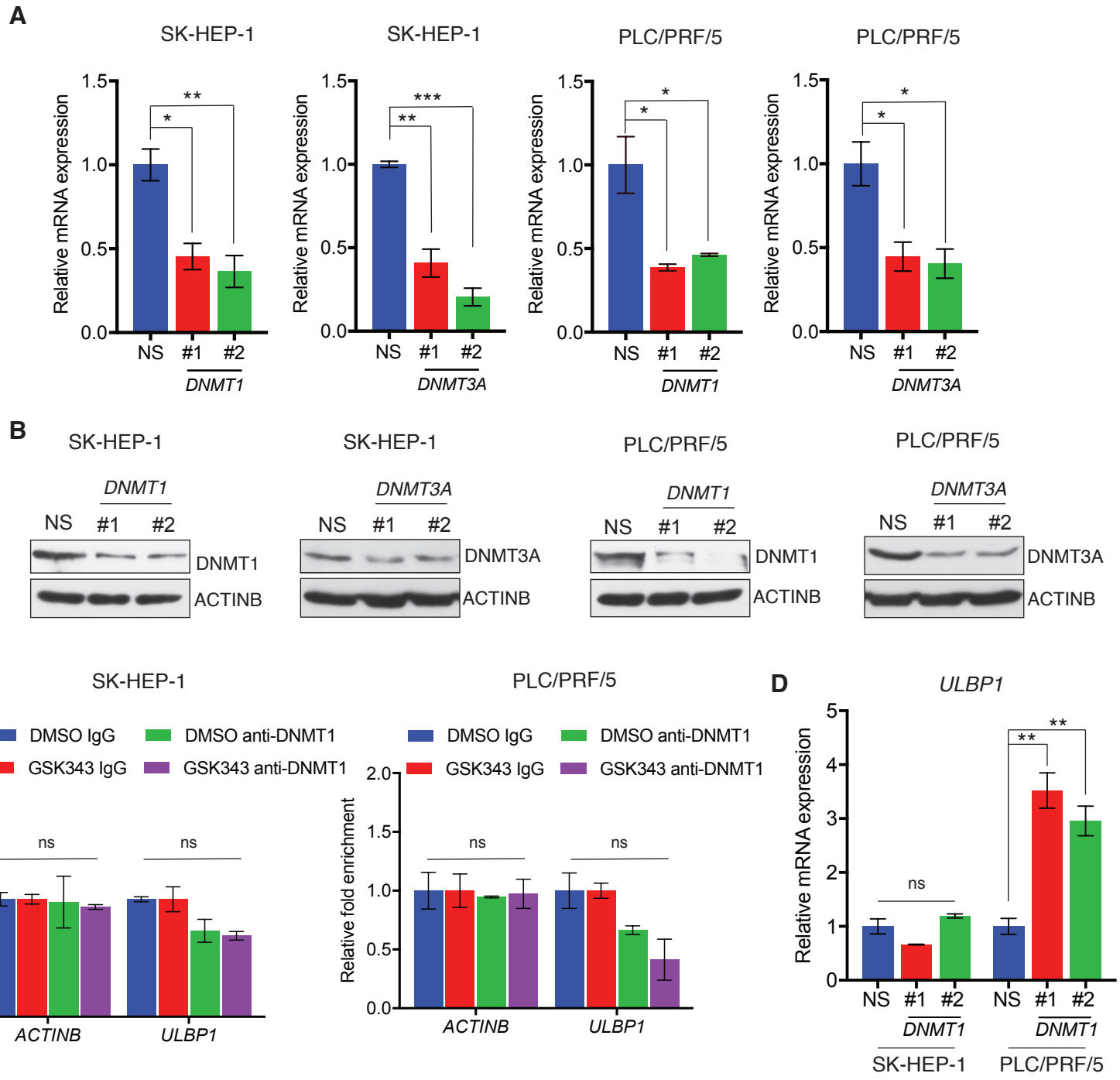


Figure S7


A
SK-HEP-1
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PLC/PRF/5
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Relative mRNA expression
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#2
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PLC/PRF/5
PLC/PRF/5
DNMT1
DNMT3A
ACTINB
C
SK-HEP-1
PLC/PRF/5
DMSO IgG
DMSO anti-DNMT1
GSK343 IgG
GSK343 anti-DNMT1
Relative fold enrichment
ns
ACTINB
ULBP1
D
ULBP1
Relative mRNA expression
SK-HEP-1
PLC/PRF/5

SI Tables

Table S1. List of HCC panel cell lines used in this paper. Cell lines genotype and their tumor status.

S. No.	Cell lines	Tumor Progression status	Tumor Grade	Key genetic mutations (as provided by ATCC)
1	HepG2/C3A	Primary	-	*NRAS, CTNNB1*
2	PLC/PRF/5	Primary	-	*TP53, CDKN2A, STK11*
3	SK-HEP-1	Metastasis, ascites	-	*CDKN2A, CDKN2a (p14), BRAF*
4	SNU-387	Primary	Grade IV/V	*TP53, CDKN2A, CDKN2a (p14), NRAS*
5	SNU-423	Primary	Grade III/IV	Not Determined
6	SNU-449	Primary	Grade II-III/IV	*TP53, CDKN2A, CDKN2a (p14)*
7	SNU-475	Primary	Grade II-IV/V	*TP53*

Table S2: List of inhibitors targeting indicated chromatin modifiers and the concentrations at which they were used in the chemical genetic screen.

S.No.	Target protein	Inhibitors	Inhibitor concentrations (μm)
1	BAZ2A/2B	BAZ2-ICR	0.2 or 1
2	BAZ2A/2B	GSK2801	0.2 or 1
3	BET family	JQ1	0.2 or 1
4	BRD9/7	BI-9564	0.2 or 1
5	BRD9/7	TP-472	0.2 or 1
6	BRD9	I-BRD9	0.2 or 1
7	BRPF1/2/3; BRPF1B	NI-57	0.2 or 1
8	BRPF1/2/3; BRPF1B	OF1	0.2 or 1
9	BRPF1/2/3; BRPF1B	PFI-4	0.2 or 1
10	CECR2	NVS-CECR2-1	0.2 or 1
11	CREBBP, EP300	I-CBP112	0.2 or 1
12	CREBBP, EP300	SGC-CBP30	0.2 or 1
13	DOT1L	SGC0946	0.2 or 1
14	EED	A-395	0.2 or 1
15	EZH2/H1	GSK343	0.6 or 3
16	G9a (EHMT2)/GLP	A-366	0.2 or 1
17	G9a (EHMT2)/GLP	UNC0638	0.2 or 1
18	G9a (EHMT2)/GLP	UNCO642	0.2 or 1
19	IDH1 mutant	GSK864	0.2 or 1
20	JMJD3/UTX (KDM6A/B)	GSK-J4	1 or 5
21	LSD1 (KDM1A)	GSK-LSD1	0.2 or 1
22	PAD4 (PADI4)	GSK484	2 or 10
23	PRMT Type I	MS023	0.2 or 1
24	PRMT3	SGC707	0.2 or 1
25	PRMT4	TP-064	0.2 or 1
26	PRMT4/6	MS049	1 or 5
27	PRMT5	GSK591	0.2 or 1
28	SETD7	(R)-PFI-2	0.2 or 1
29	SMARCA2/4, PB1	PFI-3	0.2 or 1
30	SMYD2	BAY-598	0.2 or 1
31	SUV420H1/H2 (KMT5B/C)	A-196	0.2 or 1
32	WDR5	OICR-9429	0.6 or 3

Table S3: Table showing list of chromatin modifier inhibitors used in this study and their effect on NK cell ligands expression in SK-HEP-1 cells. (+) mRNA expression increased more than 1.5 fold; (-) mRNA expression less than 1.5 fold; low, low dose of inhibitor used; High, High dose of inhibitor used. The concentrations of each drug used for the experiment listed in Table S2.

			ULBP1		ULBP2		ULBP3		ULBP4		ULBP5		ULBP6		MICA		MICB		CD112		CD155	
S. NO	**Target**	**Inhibitor**	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
1	BAZ2A/2B	BAZ2-ICR	-	-	-	-	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-
2	BAZ2A/2B	GSK2801	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	BET family	JQ1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
4	BRD9/7	BI-9564	-	-	+	+	+	-	-	-	+	+	+	-	+	+	-	-	+	-	+	-
5	BRD9/7	TP-472	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+	-	-
6	BRD9	I-BRD9	-	-	-	+	-	-	-	-	-	+	-	-	+	+	-	-	-	+	-	-
7	BRPF1/2/3; BRPF1B	NI-57	-	+	+	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
8	BRPF1/2/3; BRPF1B	OF1	-	+	+	+	-	-	-	+	-	+	-	+	-	+	-	+	-	+	-	+
9	BRPF1/2/3; BRPF1B	PFI-4	-	-	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
10	CECR2	NVS-CECR2-1	-	+	-	+	-	-	-	+	-	-	-	+	-	+	-	-	+	+	-	+
11	CREBBP, EP300	I-CBP112	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-
12	CREBBP, EP300	SGC-CBP30	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-
13	DOT1L	SGC0946	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
14	EED	A-395	-	-	+	+	-	-	-	+	-	-	-	+	-	+	-	-	+	+	-	-
15	EZH2/H1	GSK126	-	+	+	+	-	-	+	-	-	+	-	+	-	-	+	+	-	-	-	+
16	EZH2/H1	GSK343	-	+	-	+	-	+	-	+	-	+	-	+	+	+	-	+	-	+	-	+
17	G9a (EHMT2)/GLP	A-366	-	-	+	-	-	-	+	+	-	-	-	-	+	+	+	-	-	-	-	-
18	G9a (EHMT2)//GLP	UNC0638	-	-	+	+	-	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-

19	G9a (EHMT2)//GLP	UNCO642	+	-	+	-	+	-	+	-	-	-	+	+	+	-	-	-	+	+	+	-
20	IDH1 mutant	GSK864	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	-	+	+
21	JMJD3/UTX (KDM6A/B)	GSK-J4	+	-	+	+	+	+	+	-	+	+	-	-	+	-	+	+	+	-	+	-
22	LSD1 (KDM1A)	GSK-LSD1	+	+	+	+	+	+	-	+	+	+	-	-	+	-	+	-	-	+	+	+
23	PAD4 (PADI4)	GSK484	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
24	PRMT Type I	MS023	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
25	PRMT3	SGC707	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	PRMT4	TP-064	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	PRMT4/6	MS049	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	PRMT5	GSK591	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	-	+	+
29	SETD7	®-PFI-2	+	-	+	+	+	-	+	+	+	-	+	-	+	+	+	-	+	-	+	-
30	SMARCA2/4, PB1	PFI-3	-	-	+	+	-	-	+	-	+	-	-	-	+	+	-	-	+	+	-	-
31	SMYD2	BAY-598	-	-	+	-	-	-	+	+	-	-	-	+	+	+	-	+	-	+	-	-
32	SUV420H1/H2 (KMT5B/C)	A-196	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	WDR5	OICR-9429	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table S4: Table showing list of chromatin modifier inhibitors used in this study and their effect on NK cell ligands expression in PLC/PRF/5 cells. (+) mRNA expression increased more than 1.5 fold; (-) mRNA expression less than 1.5 fold; low, low dose of inhibitor used; High, High dose of inhibitor used. The concentrations of each drug used for the experiment listed in Supplementary Table 2.

			ULBP1		ULBP2		ULBP3		ULBP4		ULBP5		ULBP6		MICA		MICB		CD112		CD155	
S. No.	Target	Inhibitor	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
1	BAZ2A/2B	GSK2801	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+
2	BRPF1/2/3; BRPF1B	NI-57	-	+	-	-	-	+	+	+	-	+	-	-	+	+	-	+	+	+	-	+
3	BRPF1/2/3; BRPF1B	OF1	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-	+	-	+	-
4	EZH2/H1	GSK126	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-
5	EZH2/H1	GSK343	-	+	+	+	-	-	-	+	+	+	-	+	-	+	-	+	-	-	-	-
6	SMYD2	BAY-598	-	-	+	-	-	-	+	+	-	-	-	+	+	+	-	+	-	+	-	-

Table S5: Primer sequences, clone IDs, catalog numbers, antibodies, and chemical inhibitors used in this study. Primers were used for qRT-PCR analysis, ChIP experiments, and cloning. The shRNAs used herein were obtained from Open Biosystems; clone IDs and catalog numbers are listed. The antibodies were used for immunoblot analyses. The source and concentrations of chemical inhibitors used for drug treatment experiments are summarized.

Application	**Gene symbol**	**Forward primer (5′-3′)**	**Reverse primer (5′-3′)**
RT-qPCR	*CD112*	ACGGTCACCTGCAAAGTGGA	ACGGCCGAGGTACCAGTTGT
	CD155	TGTCCCGTAACGCCATCATC	CCAAAGGACCTCACGGGAAC
	DNMT1	CAGCAACGGGCAGATGTTTC	CGGAGGGGGCTTTGTAGATG
	DNMT3a	CTACGCACCACCTCCACCAG	CAATGTTCCGGCACTTCTGC
	EZH2	TCCCGCTGAGGATGTGGATA	GGGCACGAACTGTCACAAGG
	MICA	CCTGCAATCCCAGCACTTTG	ATTCACCACCAAGCCCGTCT
	MICB	CACGTTCGCCCTTTGTTCAG	GGAGGCAGAGGTTGCAGTGA
	ULBP1	CCACCAGGACTGGCAAACTG	ATTGGGAGGCCAAGGTGGTA
	ULBP2	CAGGCACAACCCAACTCAGG	GCCAGACAGAAGGGCGAGTT
	ULBP3	CCTCGCGATTCTTCCGTACC	GCCCCACCTCTCTCAGCAT
	ULBP4	TCGCCACCAATGGAGAGAAA	ATTGCCTCCCAGTGCCCTAA
	ULBP5	GCTTCTGCTCCTGCTGTCCA	GGGACTGACGGGTGTGACTG
	ULBP6	GCCATGTCCTCAGGCACAAC	TCAGATGCCAGGGAGGATGA
Cloning	*ULBP1*	GCAGTCTAGAATGGCAGCGGCCGCCAGC	GCATCTCGAGTCATCTGCCAGCTAGAATGAA
	ULBP2	GCAGTCTAGAATGGCAGCAGCCGCCGCT	GCATCTCGAGTCAGATGCCAGGGAGGATGA
	ULBP5	GCAGTCTAGAATGGCAGCGGCCGCCAGCC	GCATCTCGAGTCAAGATATGGAGACCTGAGTG
	ULBP6	GCAGTCTAGAATGGCAGCAGCCGCCATCC	GCATCTCGAGTCAGATGCCAGGGAGGATGAA
ChIP primers	*MICA*	GTGGCATGATCTCGGCTCAC	TGGTGGTGGGTGCCTGTAGT
	ULBP1	CTGGGCCCACCAAGCTATTC	CCTCTGTCCAGGAGGGGCTA
MeDIP primers	*ULBP1*	GAGTTGCGTCAGCCAGGCC	TATAAAGCTGCCCAGCCCGG
shRNAs	**Gene symbol**	**Clone ID**	**Catalog number**
	DNMT1	TRCN0000021892	RHS3979-9589300
		TRCN0000021893	RHS3979-9589301
	DNMT3a	TRCN0000035755	RHS3979-9603163
		TRCN0000035757	RHS3979-9603165
	MICA	V3LHS_354146	RHS4430-101105008
		V3LHS_354144	RHS4430-101106880
	MICB	TRCN0000061333	RHS3979-9628517
		TRCN0000061336	RHS3979-9628520
	EZH2	TRCN0000040073	RHS3979-9607462
		TRCN0000010475	RHS3979-9630938
	ULBP1	V3LHS_412149	RHS4430-101103656
		V3LHS_384642	RHS4430-101106707
	ULBP2	V3LHS_408382	RHS4430-101063492
		V3LHS_408380	RHS4430-101064935
	ULBP5	V3LHS_362960	RHS4430-101162352
		V3LHS_362957	RHS4430-101166818
	ULBP6	V3LHS_311159	RHS4430-101128069

		V3LHS_311158	RHS4430-101131137
		V3LHS_311161	RHS4430-101132422
Immunoblotting	**Protein symbol**	**Antibody source**	**Dilution**
	Actin	Cell signaling	1:5000
	DNMT1	Active Motif	1:1000
	DNMT3A	Active Motif	1:1000
	DNMT3A	Santa Cruz Biotechnology	1:200
	DNMT3B	Santa Cruz Biotechnology	1:200
	MICA/B	Santa Cruz Biotechnology	1:200
	MICB	Santa Cruz Biotechnology	1:200
	EZH2	Cell signaling	1:2000
	Histone H3	Cell signaling	1:2000
	H3K27TriMe	Cell signaling	1:2000
	ULBP1	Santa Cruz Biotechnology	1:250
	ULBP2	VWR International	1:1000
	ULBP5	Novus Biologicals	1:1000
	ULBP2/5/6	Novus Biologicals	1:250