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Supplementary Data for

Kdm2b promotes cell viability by enhancing DNA damage response in canine hemangiosarcoma

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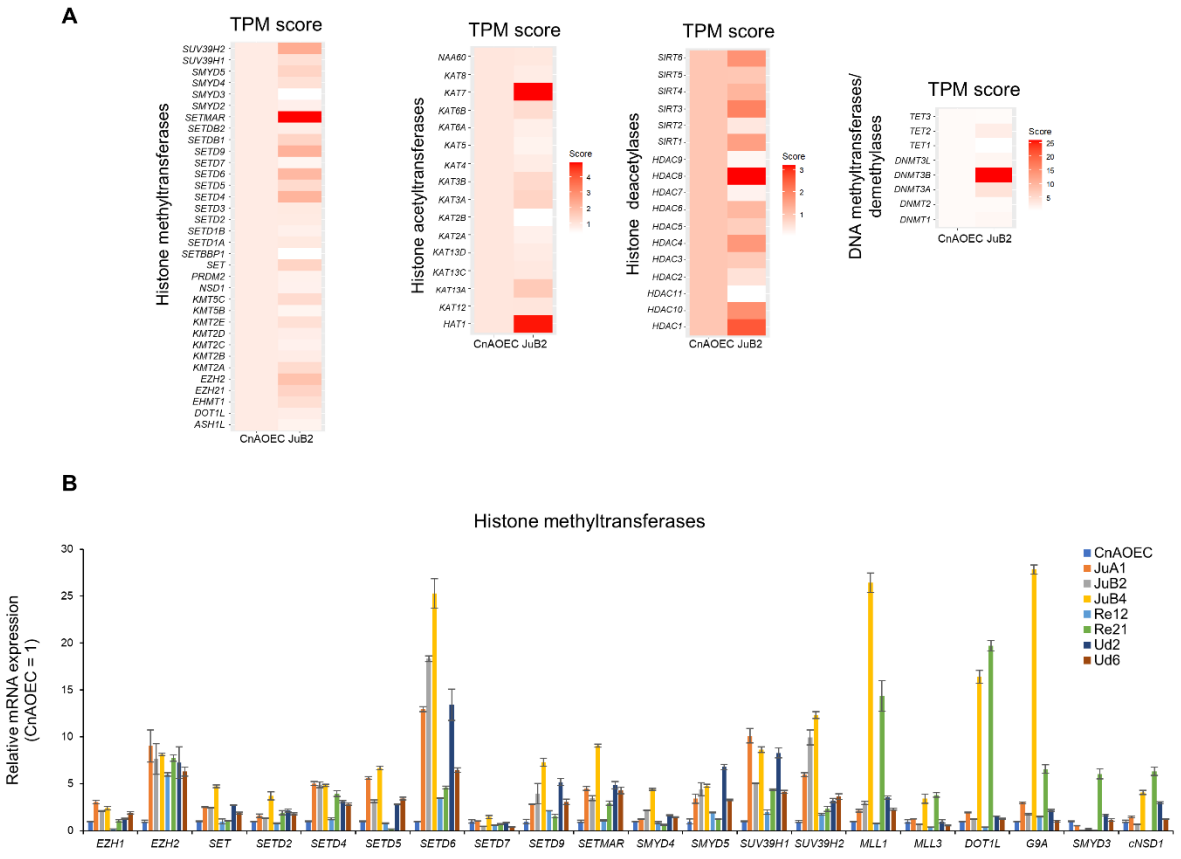

Fig. S1. Histone methyltransferases are differentially expressed in HSA.

(A) TPM scores of histone methyltransferases, histone acetyltransferases, histone deacetylases, DNA methyltransferases, and DNA demethylases in CnAOEC and JuB2 HSA cell line. **(B)** qRT-PCR verification of select histone methyltransferases in CnAOEC and HSA cell lines. Data are presented as mean values ± SD, standard deviation.

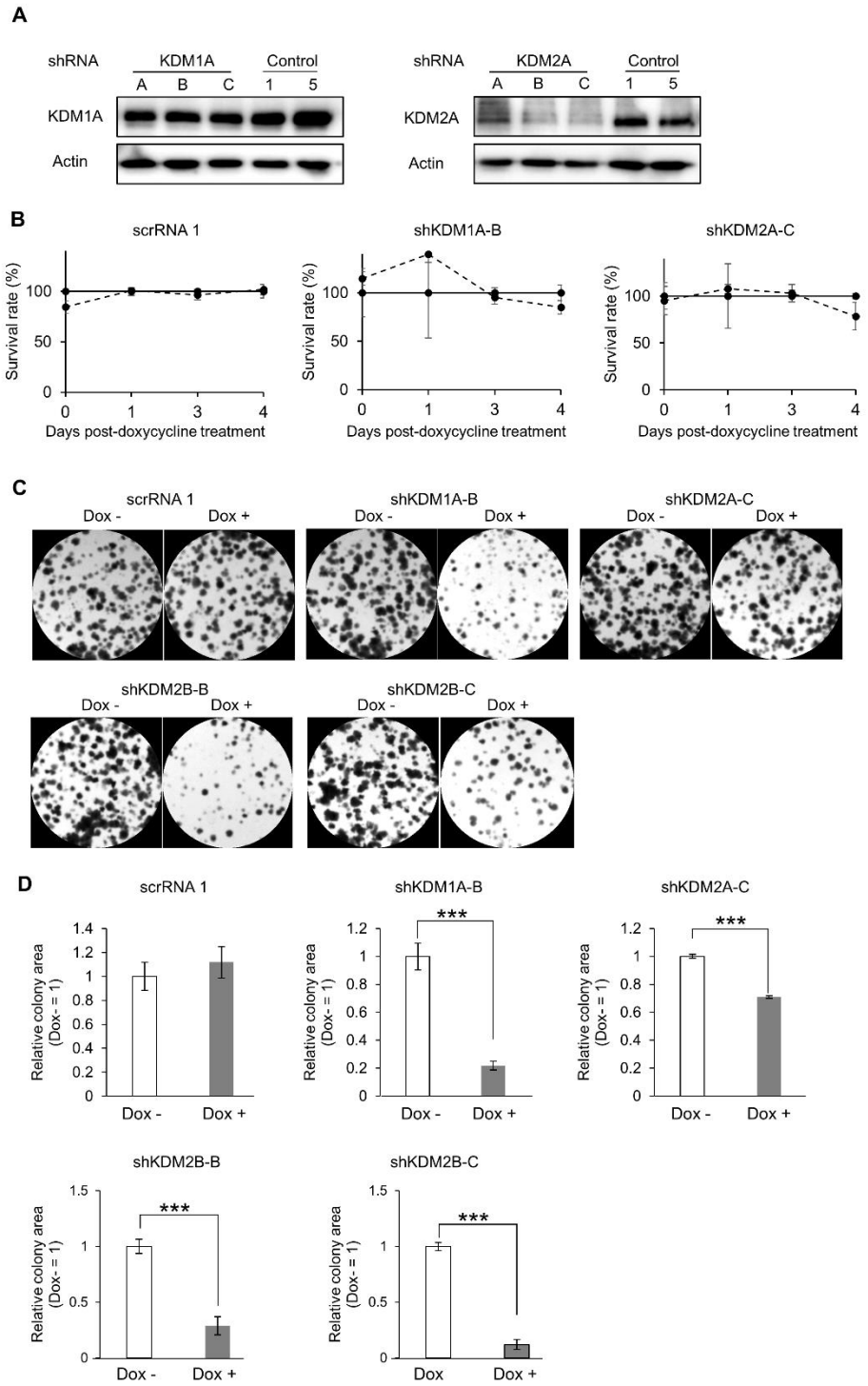

Fig. S2. KDM2B is important for HSA cell survival *in vitro*.

(A) Western blotting of KDM1A and KDM2A to verify the silencing efficiency of shRNA vectors developed for KDM1A and KDM2A. (**B)** Cell viability analysis after the induction of the inducible shRNA vector using doxycycline. (**C)** Colony formation assay of JuB2 cells after KDM1A, KDM2A, or KDM2B silencing. (**D)** Quantitative analysis of (C). N.D. means not detected. ***, $P < 0.001$, Student-*t* test.

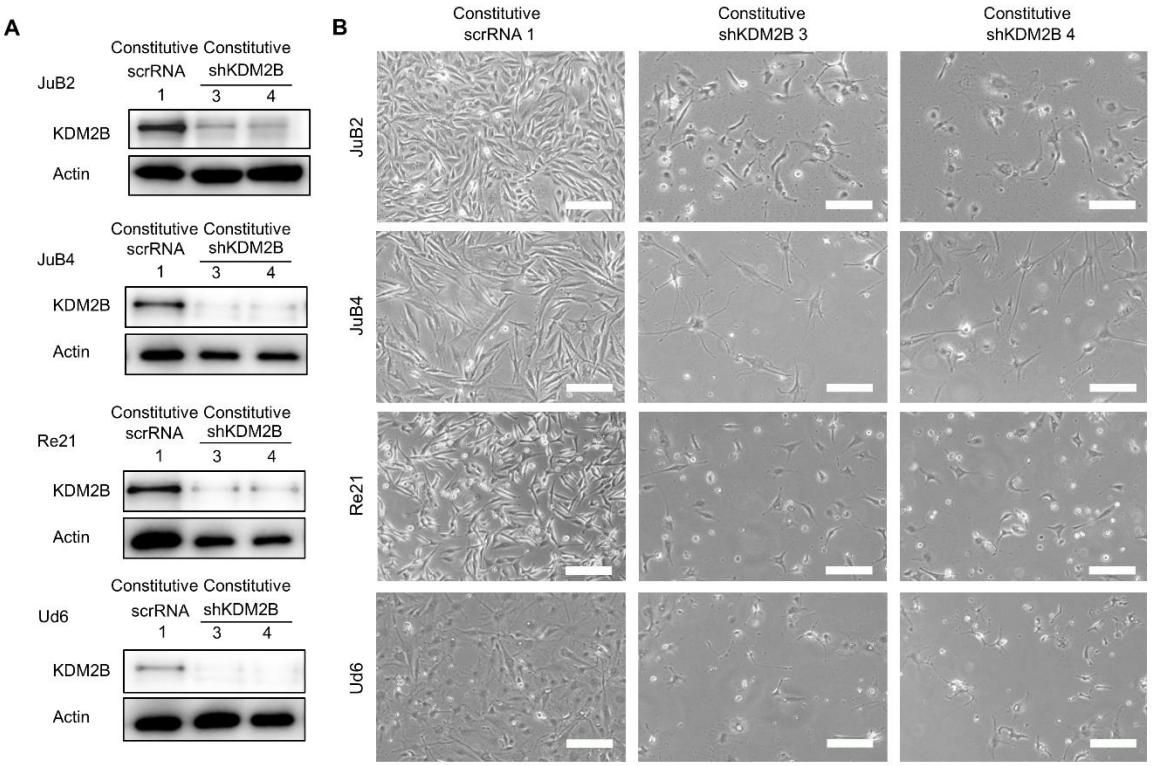

Fig. S3. KDM2B is also essential in different HSA cell lines.

(A) Western blotting for KDM2B following KDM2B knockdown with constitutively expressed silencing vectors. (**B)** Viability and morphology of JuB2, JuB4, Re21, and Ud6 HSA cell lines four days after silencing of KDM2B. Scale bars, 100 μm. Data are presented as mean values ± SD. ***, $P < 0.001$, Student's *t*-test.

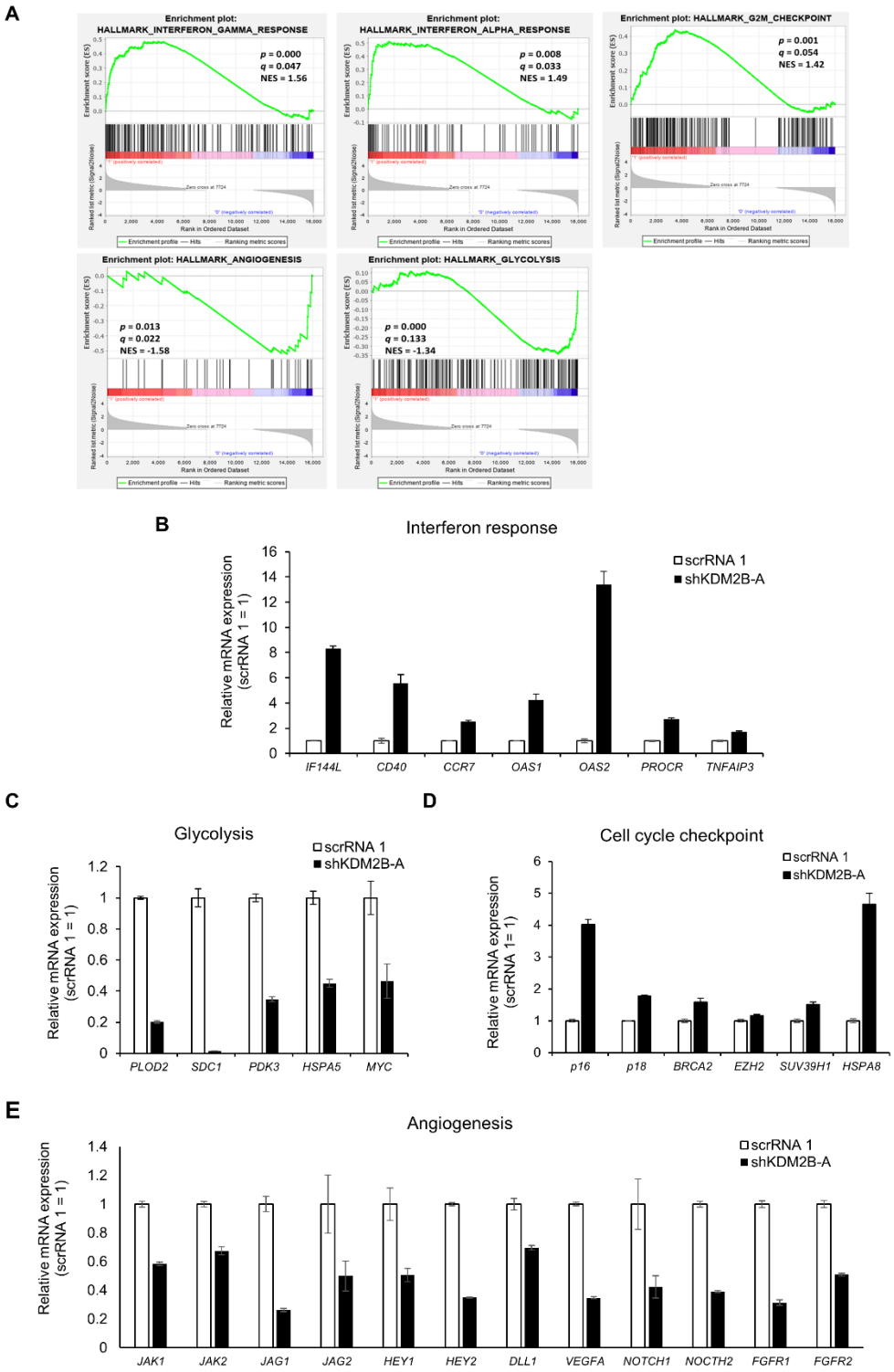

Fig. S4. KDM2B regulates the interferon responses, cell cycle checkpoints, angiogenesis, and glycolysis pathways.

(A) GSEA enrichment plots in shKDM2B-A JuB2 cells versus scrRNA-1 JuB2 cells. (**B–E)** qRT-PCR results of genes related to the enriched pathways in A. Data are presented as mean values ± SD.

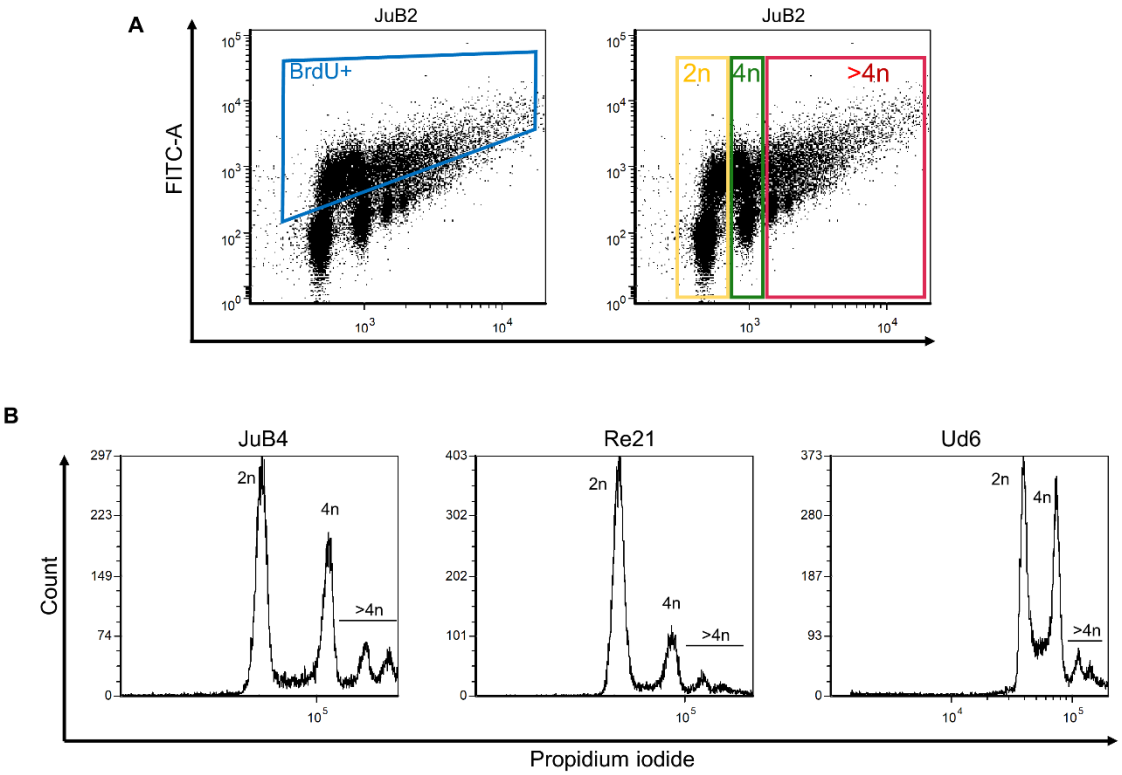

Fig. S5. Parental HSA cells are aneuploid.

(A) Dot plots illustrating the gating methods for 2n, 4n, >4n, and dividing cell populations.

(B) Histograms of propidium iodide intensities in JuB4, Re21, and Ud6 cell lines.

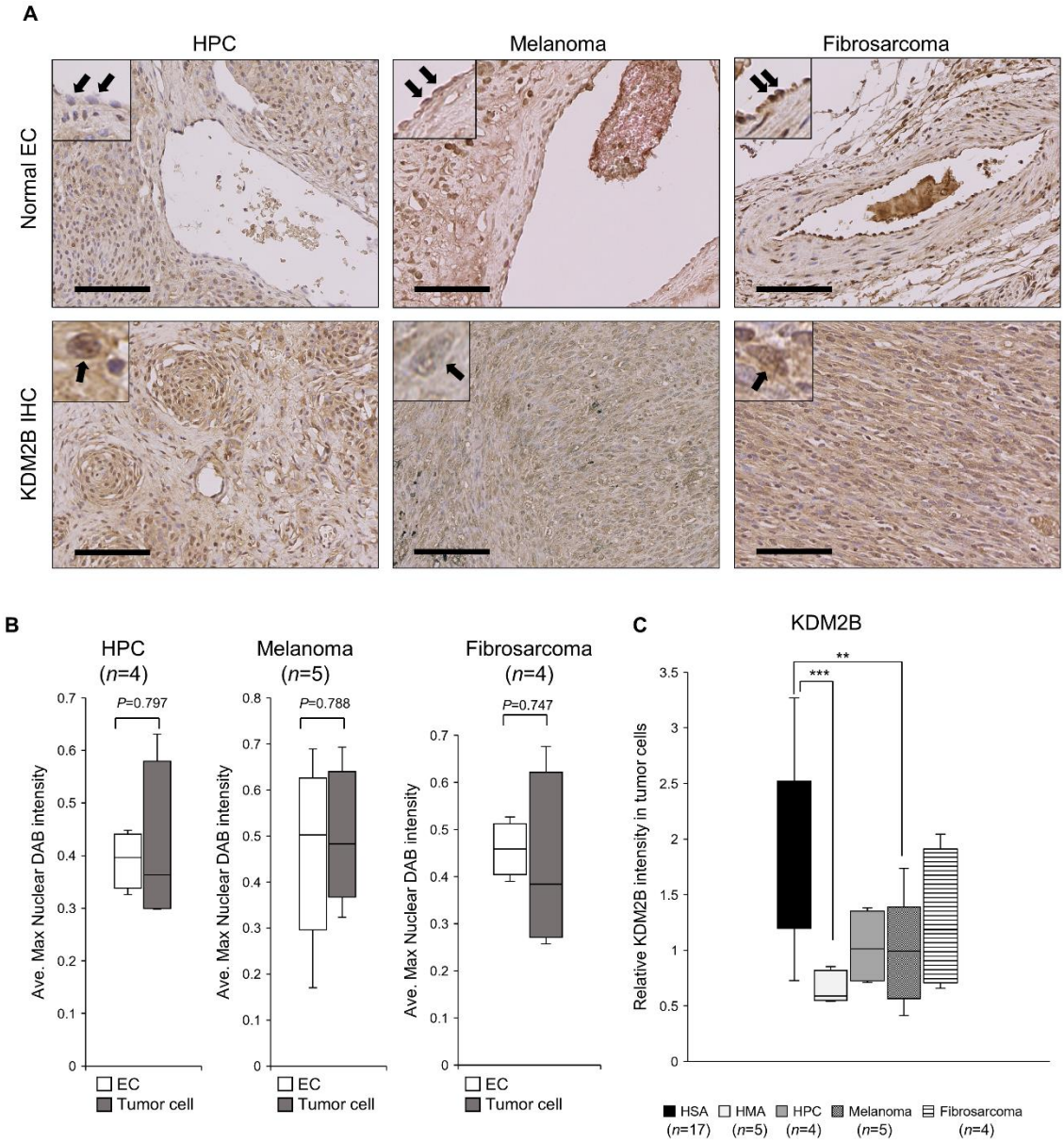

Fig. S6. KDM2B can be used as a potential marker for hemangiosarcoma.

(A) Representative images of IHC analysis of clinical cases of HPC (*n*=4), melanoma (*n*=5), and fibrosarcoma (*n*=4). Scale bars, 100 μm. **(B)** Average maximum nuclear DAB intensity in HPC, melanoma, and fibrosarcoma cases. **(C)** Comparison of the relative KDM2B intensities in tumor cells normalized by the average KDM2B intensities in EC on the same slides between HSA, HMA, HPC, melanoma, and fibrosarcoma. Data are presented as mean values ± SD. **, $P < 0.01$, Bonferroni test.

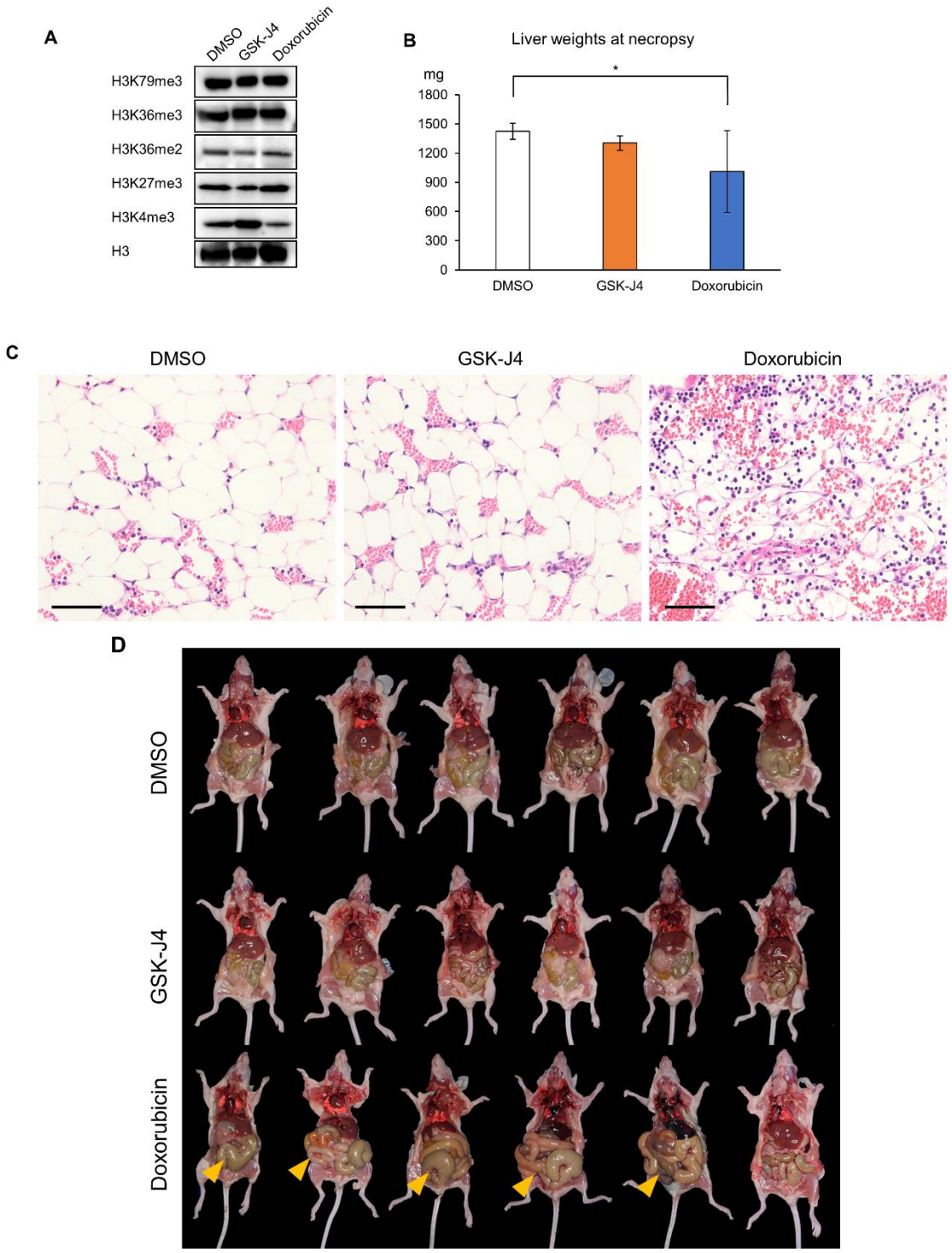

Fig. S7. GSK-J4 can be used as a therapeutic alternative to doxorubicin treatment in HSA. **(A)** Western blotting of histone modifications in JuB2 cells treated with DMSO, GSK-J4, or doxorubicin. **(B)** Liver weights of mice treated with DMSO, GSK-J4, or doxorubicin. Data are presented as mean values ± SD. *, $P < 0.05$, Tukey's test. **(C)** Representative images of bone marrow sections from mice treated with DMSO, GSK-J4, or doxorubicin. Scale bars, 100 μm. **(D)** Gross images of nude mice treated with DMSO, GSK-J4, or doxorubicin. Arrowheads indicate enlargement of intestinal segments.

Table S1. List of antibodies used for western blotting in this study.

Protein Name	Maker	Host	Dilution	Catalog Number
KDM1A	Cell Signaling Technology	Rabbit	1:1000	2139S
KDM2A	Abcam	Rabbit	1:1000	ab191387
KDM2B	Santa Cruz Biotechnology, Inc.	Mouse	1:1000	sc-293279
Actin	Sigma-Aldrich	Mouse	1:1000	MAB1501
p-ATM	Santa Cruz Biotechnology, Inc.	Mouse	1:1000	sc-47739
t-ATM	Santa Cruz Biotechnology, Inc.	Mouse	1:1000	sc-377293
c-FOS	Santa Cruz Biotechnology, Inc.	Mouse	1:1000	sc-166940
γH2A.X	Bethyl Laboratories, Inc.	Rabbit	1:1000	A300-081A-T
Cleaved-caspase 3	Cell Signaling Technology	Rabbit	1:1000	9661S
p-ERK1/2	Cell Signaling Technology	Rabbit	1:1000	4370S
t-ERK1/2	Cell Signaling Technology	Rabbit	1:1000	4695S
H3K79me3	Cell Signaling Technology	Rabbit	1:1000	74073S
H3K36me3	Abcam	Rabbit	1:1000	ab9050
H3K36me2	Active Motif	Rabbit	1:4000	39255
H3K27me3	Abcam	Mouse	1:1000	ab6002
H2AK119ub1	Sigma-Aldrich	Mouse	1:1000	05-678
H3	Active Motif	Rabbit	1:5000	39140
Antirabbit	Sigma-Aldrich	Donkey	1:10000	NA934VS
Antimouse	Thermo Fisher Scientific	Goat	1:10000	G21040

Table S2. List of qRT-PCR primers used in this study.

Species	*Target*		Sequence	Gene ID	Reference
Canine	*TBP*	F	ATAAGAGAGCCCCGAACCAC	ENSCAFG00000004119	Peters *et al.*, 2007
		R	TTCACATCACAGCTCCCCAC		
	KDM1A	F	CCCACCTGAGGAAGAAAATG	ENSCAFG00000013361	This paper
		R	GGAAAACAGGCTGCTTCTTG		
	KDM2A	F	AAGAAATGTGTCCCCACAGG	ENSCAFG00000011792	This paper
		R	CAAGCTCCTCCAGCAAAATC		
	KDM2B	F	AGAAGCCACGAATGCCATTG	ENSCAFG00000008259	This paper
		R	AACAGCCTTTCACGCTCATC		
	KDM3A	F	ACTTTAGGATGCCGGTCACAG	ENSCAFG00000007522	This paper
		R	TGCAGCTTCTTGAGTTTGGC		
	KDM4A	F	GATTTCCCCTTTGATGCTGA	ENSCAFG00000004903	This paper
		R	TGGTAGACTCCGCACAGTTG		
	KDM4B	F	CAGTCAGGCCTCTTCACACA	ENSCAFG00000018925	This paper
		R	CCAATACTTGCGTTCCAGGT		
	KDM4C	F	CCACTGACTCTGGTGAAGCA	ENSCAFG00000001434	This paper
		R	TCGCCCAAGACTCTGTTTCT		
	KDM5A	F	GGACCTTGAGCCTCTGAGTG	ENSCAFG00000015781	This paper
		R	GGAATGCATGGCTTCAATCT		
	KDM5B	F	GCTGTCCAACTCCCAAATGT	ENSCAFG00000010452	This paper
		R	GGCTCTTGGGTTTTTCCTTC		
	KDM6A	F	ATAACCGCACAAACCTGACC	ENSCAFG00000014589	This paper
		R	AGGACCTGCCAAATGTGAAC		
	KDM6B	F	TCTTCGATTTTCCCCCTACC	ENSCAFG00000016817	This paper
		R	GAATGGATTCGTCCAGCATC		
	KDM7A	F	AGCAACCAGGCAACAAAAGG	ENSCAFG00000003990	This paper
		R	TCTTCCCAAGACGCTGTTTG		
	COL1A2	F	TCTCCCTGGTGAATTTGGTC	ENSCAFG00000002069	This paper
		R	GTTCACCCTTGTTTCCATCG		
	INSIG1	F	TGGGATCACTATTGCCTTCC	ENSCAFG00000005081	This paper
		R	AGCGGATGTAGAGAAAGTCTGG		
	ITGB3	F	GGATTCCAGCAATGTCCTTC	ENSCAFG00000013735	This paper
		R	TGGCGTTGAACGATAGAGAC		
	PPARG	F	TTCTCCAGCATTTCCACTCC	ENSCAFG00000004991	This paper
		R	AGGCTCCACTTTGATTGCAC		

	TGFBR3	F	CACATTGTGCACCAAGAAGG	ENSCAFG00000020179	This paper
		R	TCATTGAGGCATCCAGTGAG		
	VLDLR	F	GACGAACCCCTGAAAGAATG	ENSCAFG00000002028	This paper
		R	TGCGCAGTCACATTCATAGC		
	EZH1	F	TGAGGAGTCCCTTTTTCGAG	ENSCAFG00000014827	This paper
		R	TCATCTGTTGGCAGCTTCAG		
	EZH2	F	CAGACCGGTGAAGAGCTGTT	ENSCAFG00000003411	This paper
		R	GGGGAGGAAGAGGTAGCAGA		
	SET	F	TCCATCGTCAAAGTCCACTG	ENSCAFG00000032728	This paper
		R	TCCTGCTGGCTTTATTCTGC		
	SETD2	F	ACAGCAGAAGCAGACACCTC	ENSCAFG00000013392	This paper
		R	AGGCACTGGACGATGAACTG		
	SETD4	F	TGAATCATAGCCCCGAAGTC	ENSCAFG00000009616	This paper
		R	AGCCGTTGGTTATCATGAGG		
	SETD5	F	ACCCCCAAACACTACATTCG	ENSCAFG00000005486	This paper
		R	CCAAGGCTTGCTTTATCCAG		
	SETD6	F	CAAACTCCCCTTTGATGGTG	ENSCAFG00000008461	This paper
		R	TTTAGGAATGGGCTGAGTGG		
	SETD7	F	AGGTAGCGGTGGGACCTAAT	ENSCAFG00000003703	This paper
		R	GTTGTAGGGCTCAGGCACAT		
	SETD9	F	AAGGCGCGGTTGTATCTATG	ENSCAFG00000006922	This paper
		R	TCAATGAGTACCCCATCCAG		
	SETMAR	F	CTTGGAGAACGTGCCTGTGA	ENSCAFG00000005958	This paper
		R	CAAATGCATCCGGGAAAGGT		
	SMYD3	F	CGGAGATGCAGGAAGTTGGT	ENSCAFG00000029171	This paper
		R	CTCGATGTCTCGGACTGCTC		
	SMYD4	F	TGTGGGAAAGGACCCTAATG	ENSCAFG00000019244	This paper
		R	AGTGTGAGGCCTTGAATGTG		
	SMYD5	F	TGAAGGATCTGGCCTGTATG	ENSCAFG00000008932	This paper
		R	TGGCTTGATATCCTCCAAGG		
	SUV39H1	F	TGGAGAAGATCCGCAAGAAC	ENSCAFG00000015545	This paper
		R	TGTACACGTCCTCCACGTAGTC		
	SUV39H2	F	CGATTGGAATCACCAAAAGG	ENSCAFG00000004696	This paper
		R	AGAATCTGGCCATCCTTTCC		
	MLL1	F	GCTTTGGCTCCAGCAAGAAC	ENSCAFG00000012691	This paper
		R	CGTCAGTGACTTCCAGGCAT		

	MLL3	F	TCGCTCCAAGAAAAGGAAGA	ENSCAFG00000004955	This paper
		R	CAAGCCATAGGAGGTGGTGT		
	DOT1L	F	ACCACGATGCTGCTCATGAA	ENSCAFG00000019420	This paper
		R	GCCTCTGCATGCTTTCGAAG		
	G9A	F	GAAGAAGTGGCGGAAGGACA	ENSCAFG00000000669	This paper
		R	ACTCACTAGGGCCTGAGGAG		
	NSD1	F	TATGGAGGGGGATGTGAGCA	ENSCAFG00000016473	This paper
		R	GGTCTTCCTGCAGCTGTCTT		
	P15INK4B	F	GTGCGGCAGCTCCTGGAAGC	ENSCAFG00000001675	This paper
		R	GCCCATCATCATGACCTGGATCG		
	P16INK4A	F	GTGGACCTGGCTGAGGAGCG	ENSCAFG00040004457	This paper
		R	TTCTTGAAGTCCGGGCTGTCTG		
	ATR	F	CAGCGCTTCCTAGTACTCCG	ENSCAFG00000007863	This paper
		R	TTGGCAGCAAGGTCAGGTAG		
	IF144L	F	TCTATTTTCCGAGGCCAGAG	ENSCAFG00000020343	This paper
		R	TCATGTATCCCCATGGAGTC		
	CD40	F	TATTCACCTCGCCATGGTTC	ENSCAFG00000009994	This paper
		R	GAGTGCATTCCGTGTCAATG		
	CCR7	F	GGCTCTCCTTGTCATTTTCC	ENSCAFG00000030300	This paper
		R	TCCACCGTGGTATTTTCTCC		
	OAS1	F	TGTGCGGGTGTCTAAAGTTG	ENSCAFG00000023556	This paper
		R	TGAACTGTCCTCGTTTCTCG		
	OAS2	F	TGACCCAGATCCAGAAAACC	ENSCAFG00000023107	This paper
		R	CCATTCGGTAGCGTCTTTTG		
	PROCR	F	GCAGGAACACAATGCTTCAA	ENSCAFG00000007945	Aoshima *et al.*, 2018
		R	AAGATGCCTACAGCCACACC		
	TNFAIP3	F	GGTGATCGAAATTCCTGTCC	ENSCAFG00000000267	This paper
		R	TGGGTAAGTTGGCTTCATCC		
	BAX	F	ACATGGAGTTGCAGAGGATG	ENSCAFG00000003867	This paper
		R	CCAGTTGAAGTTGCCATCAG		
	RB1	F	AAGCAGAAGCCAACTTGACCAG	ENSCAFG00000004436	This paper
		R	GTCCTTCTCGGTCTTTTGCTTG		
	MCL1	F	AGCTGCATCGAACCATTAGC	ENSCAFG00000012050	This paper
		R	AGAACTCCACAAACCCATCC		
	E2F1	F	GATGGTCATGGTGATCAAGG	ENSCAFG00000007429	This paper
		R	GCACAGGAAAACGTCAATGG		

	CDC25A	F	CCTGAAAAGGAGCCATTCTG	ENSCAFG00000012919	This paper
		R	ACGGGGTCTCTTCATCATTG		
	p18	F	GCTGCAGGTTATGAAACTTGG	ENSCAFG00000028905	This paper
		R	CGAAACCAGTTCGGTCTTTC		
	BRCA2	F	CGGGAGATTGACTGTGGGTC	ENSCAFG00000006383	This paper
		R	GCAAGCAGGACGAGTACTGT		
	HSPA8	F	AACCACCCCAAGTTATGTCG	ENSCAFG00000011666	This paper
		R	TCAGATTGGACGACAGCATC		
	JAK1	F	TACGGACAACATCAGCTTCG	ENSCAFG00000018615	This paper
		R	TGAGGATCCGGTCAAAACTC		
	JAK2	F	TCAGATGTCTGGAGCTTTGG	ENSCAFG00000002102	This paper
		R	TCATCTGTCCTTGCTTGTCG		
	JAG1	F	GAAGCGTGGGATTCCAGTAA	ENSCAFG00000005627	This paper
		R	CAGAACTTGTTGCAGCCAAA		
	JAG2	F	AGGGCAGTACCTGCAACATC	ENSCAFG00000018401	This paper
		R	TGCAGGAGAACGAGTCAATG		
	HEY1	F	GCGCGGATGAGAATGGAAAC	ENSCAFG00000008391	Aoshima *et al.*, 2018
		R	GTCGGCGCTTCTCAATGATG		
	HEY2	F	CGGCGAGATCGGATAAATAA	ENSCAFG00000032212	Aoshima *et al.*, 2018
		R	CGCGTCGAAGTAGCCTTTAC		
	DLL1	F	CCGATGACCTCACAACAGAA	ENSCAFG00000004094	This paper
		R	GCAGACCTTCTCCCCTCTCT		
	VEGFA	F	TATGGCAGGAGGAGAGCACAAACC	ENSCAFG00000001938	This paper
		R	CAGCCCCCACACCGCATCAG		
	NOTCH1	F	TACCGGCCAGAACTGTGAGGAGAA	ENSCAFG00000019633	Aoshima *et al.*, 2018
		R	GGAGGGCAGCGGCAGTTGTAAGTA		
	NOCTH2	F	TCGGGATAGCTATGAGCCCT	ENSCAFG00000010476	Aoshima *et al.*, 2018
		R	GGCATGTTGCTTTCCCCAAC		
	FGFR1	F	TCCGTCAATGTCTCAGATGC	ENSCAFG00000005970	This paper
		R	CCATCTTTTCTGGGGATGTC		
	FGFR2	F	TCCGTCAATGTCTCAGATGC	ENSCAFG00000012374	This paper
		R	CCATCTTTTCTGGGGATGTC		
	PLOD2	F	TATGGCTCTCTGCCGAAATG	ENSCAFG00000008101	This paper
		R	TGGAATGTTTCCGGAGTAGG		
	SDC1	F	TCTGGGGATGACTCTGACAAC	ENSCAFG00000003833	This paper
		R	TCTGCTGTGACAAGGTGATG		

	HSPA5	F	CGGAGGCTTATTTGGGAAAG	ENSCAFG00000020196	This paper
		R	TGGGCATCATTGAAGTAGGC		
	PDK3	F	TGTCCATCAAGCAGTTCCTG	ENSCAFG00000013476	This paper
		R	AGCAAGTTATCCGGCAGAAG		
	MYC	F	CGCTGGTCCTTAAGAGATGC	ENSCAFG00000001086	This paper
		R	CGCCTCTTGTCATTCTCCTC		
Human	*KDM1A*	F	CCCATGGAAACTGGAATAGC	ENSG00000004487	This paper
		R	GCCAAGCTTTCATCCATCTC		
	KDM2A	F	AAGAAATGTGTCCCCACAGG	ENSG00000173120	This paper
		R	CAAGCTCCTCCAGCAAAATC		
	KDM2B	F	ATGCCTGACCCTGATTTCAC	ENSG00000089094	This paper
		R	TGGGTGTTCACATCCATCAC		
	EZH2	F	TTTCCAGATAAGGGCACAGC	ENSG00000106462	This paper
		R	ATGTTGGGGGTACATTCAGG		
	G9A	F	GGTTTGCGCTTCAACTCAAC	ENSG00000204371	This paper
		R	AATGGGCACGTTCTCATAGC		
	MLL1	F	TGCCTGGAAGTCATTGACAG	ENSG00000118058	This paper
		R	GGAACACAACTGCATCATGG		
	SETD6	F	AGGATGAAAAGGAGCCCAAC	ENSG00000103037	This paper
		R	TTTAGGAATGGGCTGAGTGG		
	SMYD5	F	TGAAGGATCTGGCCTCTTTG	ENSG00000135632	This paper
		R	AAAGGAGGTCTCTGCATTGG		
	SUV39H2	F	TCGATACGGCAATGTGTCTC	ENSG00000152455	This paper
		R	CAATGCTATTCGGGGAAGAC		

Table S3. List of oligos used for shRNA expression vector construction in this study.

Species	Target	Sequence	Reference
Canine	KDM1A-A	AAGUGAUACUGUGCUAGUCCAC	This paper
	KDM1A-B	ACUUCAGGAUGUGAAGUGAUAG	This paper
	KDM1A-C	GUAUAGAGCAAGAGAAGCAGAU	This paper
	KDM2A-A	AGCUCUCAGUGGCAUCAUCAAG	This paper
	KDM2A-B	CAUGCUGUAUCUGCAAUGAGAU	This paper
	KDM2A-C	CGCCCACAACCUGGAGCUGUAC	This paper
	KDM2B-A	ACCACAGCAUCUGAAGGAGAAG	This paper
	KDM2B-B	CCCAGUGCCUGUCCUUCUUCAA	This paper
	KDM2B-C	CCCAGAGAGAAUCCAUGCUUAU	This paper
	KDM2B-3	UGGAAAUAUCUGUCAUAUUGA	This paper
	KDM2B-4	CAGUUCAUAGCUGAAAUGUCU	This paper
	scramble 1	AUAAGAGACGAACGUAACAUA	This paper
	scramble 5	AGAAGAUUAACGUAGAAGGUG	This paper

Table S4. List of oligos used for canine *KDM2B* cloning and mutagenesis in this study.

Species	Target		Sequence	Reference
Canine	WT KDM2B	F	CGCTACCGGTCTCGAGACCATGCATCGGGCAGTGGACCCTC	This paper
		R	CGACGGTACCGAATTCCAGAGGCGGGACCTAGGTCCAGC	
	Silent mutation for shKDM2B C	F	CGCGAGTCTATGCTGATTGATGCCCCAAGAAAGCC	This paper
		R	CAGCATAGACTCGCGTTGGTATTCCTGAGTGAGGT	
	$KDM2B^{H283Y}$	F	TGGATTTACGCAGTCTACACCCCCGTA	This paper
		R	GACTGCGTAAATCCAACCGGAAGG	
	$KDM2B^{C586A}$	F	ACGCGAGCCCGCAAGTGCGAGGCC	This paper
		R	CGCCGGACGCGAGCCCGCAAG	
	$KDM2B^{\triangle PHD}$	F	GCGCCAGTGCTGCCCCATGGCAAGACCGGGAAACAAAA	This paper
		R	TTTCCCGGTCTTGCCATGGGGCAGCACTGGCGCGA	

Table S5. List of oligos used for CUT&RUN assay.

Species	Target		Sequence	Reference
Canine	*GAPDH* promoter	F	AACATTCTCTGGGGGATTAGG	This paper
		R	TGCGCTACCACACCTTTTTC	
	KDM2B promoter	F	TTGGTGCCAACACTGTTCTG	This paper
		R	ATCCTCCCTGGTGTTTATGC	
	INSIG1 promoter	F	GCCTGTTCACTCCTTACAACAG	This paper
		R	GGGATGCTTAAAGTCTCTGCAC	
	OAS2 promoter	F	GCGGTTCTTCACCGTTATTG	This paper
		R	AATCACAGAGCGACCTTTGG	
	GAPDH gene body	F	ACTCTTGCCCTTTGAGCTTG	This paper
		R	TAGCTGTGCCCTGAGAAATG	
	KDM2B gene body	F	GCCTTCCTTGAAAGCACAAG	This paper
		R	AGGCCACTGACCACAAAATC	
	INSIG1 gene body	F	CCAAAATCCATACCCACCTG	This paper
		R	TCCATAGGGCTGTGGAAAAC	
	OAS2 gene body	F	TTGGAGAAGGACGATCCAAC	This paper
		R	ATGTGGCCTCAAAAGACACC	