Supporting information for

DNA methylation profiling and growth inhibitory effects of decitabine in canine hemangiosarcoma cell lines

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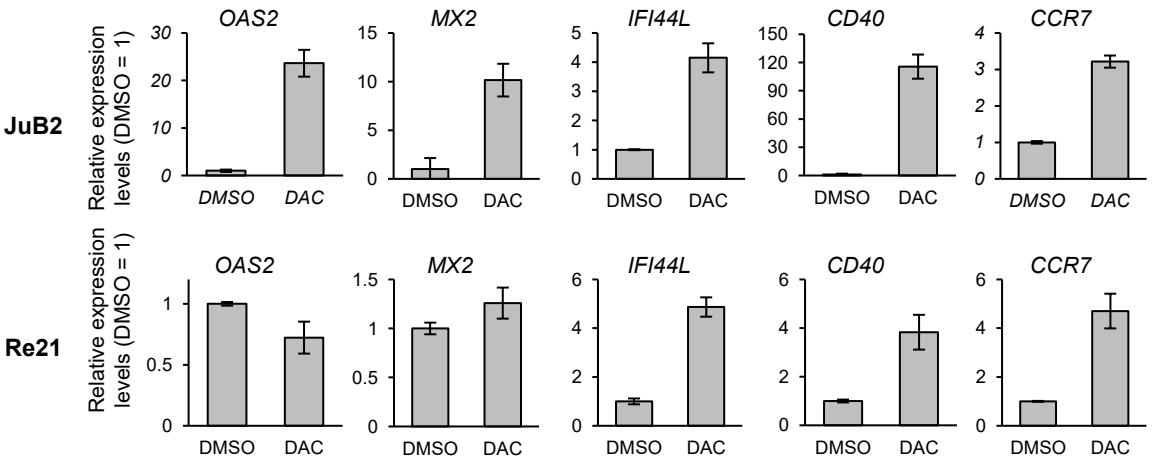

Fig. S1. Expression of inflammation-related genes.
Expression levels of inflammation-related genes in HSA cell lines treated with DMSO or DAC for 72 h followed by culture without either treatment for 96 h. Samples were analyzed in triplicate, and values are presented as mean ± SD relative to the DMSO control.

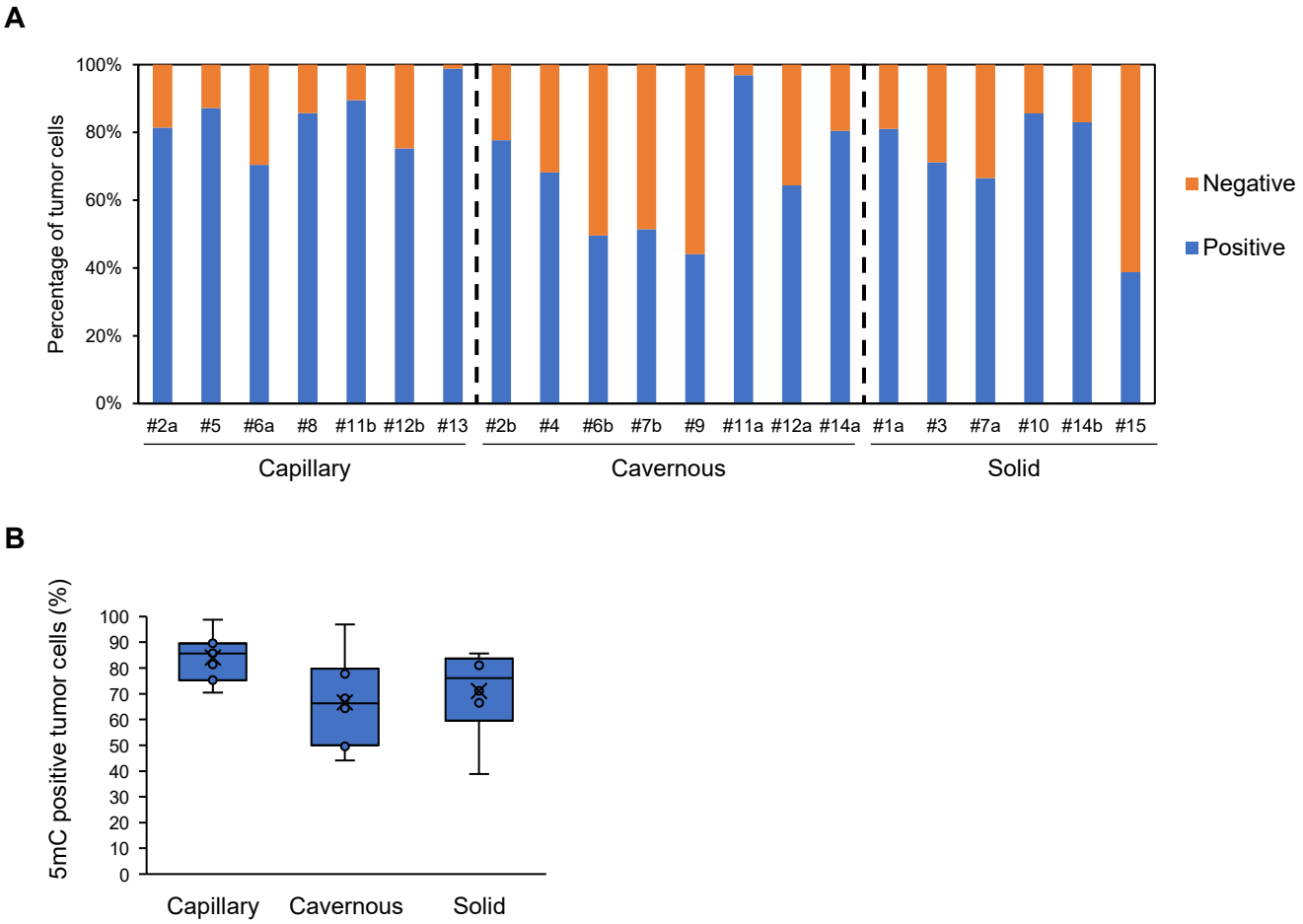

Fig. S2. 5mC positivity across histopathological patterns in clinical canine HSA.
(A) Stacked bar graphs showing the proportions of 5mC-positive and -negative tumor cells in 21 regions from 15 dogs. Some dogs contributed two regions with distinct morphological characteristics. (B) Boxplots showing the percentages of 5mC-positive tumor cells in capillary, cavernous, and solid regions.

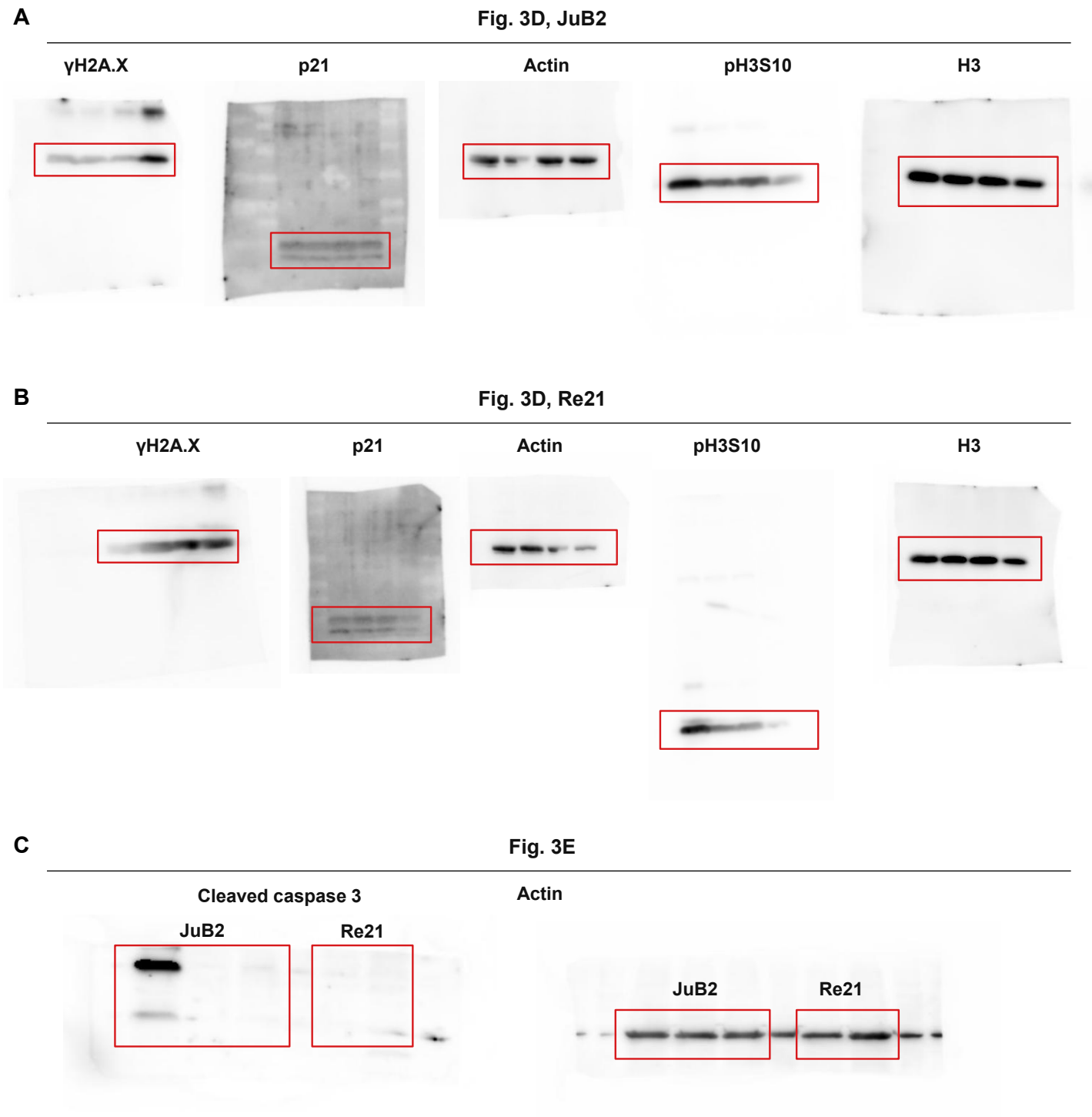

Fig. S3. Full-membrane images corresponding to Fig. 3D and E.
(A, B) Full-length γH2A.X, p21, pH3S10, actin, and H3 blot images for JuB2 (A) and Re21 (B). (C) Full-length cleaved caspase 3 and actin blot images corresponding to Fig. 3E. Red boxes indicate the regions shown in Fig. 3D and E; the two boxes in (C) indicate the non-adjacent JuB2 and Re21 lane groups. Treatment conditions and controls are labelled as in Fig. 3.

Specie	Target		Sequence	Gene ID
Canine	*TBP*	F	ATAAGAGAGCCCCGAACCAC	ENSCAFG00000004119
		R	TTCACATCACAGCTCCCCAC	
	ROBO1	F	AAGAGCGAGCCACAGTTCAT	ENSCAFG00845020071
		R	GGCTGCTTCACAACATCTGA	
	ROBO2	F	ATGGCTTGCTGATTCATGGC	ENSCAFG00845020871
		R	TGCTGTTTTATCCCCTTGGC	
	ROBO3	F	CTTTCCAATGCGAGACCAAAGG	ENSCAFG00845011736
		R	GCACATCCGTAATGTTGAGCTG	
	ROBO4	F	GTGCACATTCAGCTGGAAAA	ENSCAFG00845011649
		R	CCCTGAAGAGGGCTGTGTAG	
	SLIT2	F	ATCCACGCATGCATCAGTAA	ENSCAFG00845010097
		R	CATCGGCACAAATACACCAG	
	SLIT3	F	AAGACTGCACGGTACCCATC	ENSCAFG00845002910
		R	CTCACACTGCTGTCCCTCAA	
	OAS2	F	TGACCCAGATCCAGAAAACC	ENSCAFG00845022313
		R	CCATTCGGTAGCGTCTTTTG	
	MX2	F	TGGCGGAAAGACTTACCAAG	ENSCAFG00000010167
		R	TGGTGGCTCTCCCTTATTTG	
	IFI44L	F	TCTATTTTCCGAGGCCAGAG	ENSCAFG00845003006
		R	TCATGTATCCCCATGGAGTC	
	CD40	F	TATTCACCTCGCCATGGTTC	ENSCAFG00845018444
		R	GAGTGCATTCCGTGTCAATG	
	CCR7	F	GGCTCTCCTTGTCATTTTCC	ENSCAFG00845007930
		R	TCCACCGTGGTATTTTCTCC	

Table S1. Primer sequences for qPCR

Protein name	Manufacturer	Host	Dilution	Clone #	Catalog #
5-methylated cytosine	Abcam, Cambridge, UK	Mouse	IHC 1:150	33D3	ab10805
5-hydroxymethylated cytosine	Active Motif, Carlsbad, CA, USA	Rabbit	IHC 1:500	N/A	39770
pH3S10	Cell Signaling Technology, Danvers, MA, USA	Rabbit	WB 1:1000 IHC 1:1000	D2C8	3377
γH2A.X (Ser139)	Santa Cruz Biotechnology, Dallas, TX, USA	Mouse	WB 1:1000 IHC 1:200	N/A	sc-517348
p21	Cell Signaling Technology, Danvers, MA, USA	Rabbit	WB 1:1000	12D1	2947
Histone H3	Monoclonal Antibody Institute Co., Ltd., Iida, Nagano, Japan.	Mouse	WB 1:2500	CMA301	MABI0001-20
Cleaved-caspase 3	Cell Signaling Technology, Danvers, MA, USA	Rabbit	WB 1:1000	N/A	9661S
Actin	MilliporeSigma (Merck), Burlington, MA, USA	Mouse	WB 1:10000	C4	MAB1501

Table S2. Antibody list

Case No.	Breed	Age	Sex	Location
1	Great Pyrenees	9y	Castrated	Spleen
2	Golden Retriever	9y	Castrated	Spleen
3	Labrador Retriever	10y	Male	Spleen
4	Jack Russell Terrier	7y	Male	Spleen
5	Bichon Frise	8y	Spayed female	Kidney
6	Golden Retriever	9y	Male	Liver
7	Miniature dachshund	9y	Female	Spleen
8	Miniature Schnauzer	11y	Male	Spleen
9	Miniature Schnauzer	9y	Male	Spleen
10	Miniature dachshund	10y	Female	Spleen
11	Scottish terrier	11y	Spayed female	Intrathoracic mass
12	Golden Retriever	11y	Male	Spleen
13	Maltese	10y	Unknown	Spleen
14	Border Collie	13y	Male	Spleen
15	Labrador Retriever	10y	Spayed female	Spleen

Table S3. Case information

Case #	Histological pattern	Cellular atypia	Mitotic activity	Pleomorphism	Total score	Morphological severity	5mC positive (%)	5mC negative (%)
#1	Solid	3	2	2	7	Severe	81.04	18.96
#2a	Cavernous	3	2	3	8	Severe	77.7	22.3
#2b	Capillary	3	2	3	8	Severe	81.45	18.55
#3	Solid	2	1	2	5	Mild	71.18	28.82
#4	Cavernous	2	1	1	4	Mild	68.19	31.81
#5	Capillary	3	2	2	7	Severe	87.18	12.82
#6a	Cavernous	3	1	2	6	Mild	49.59	50.41
#6b	Capillary	3	1	2	6	Mild	70.42	29.58
#7a	Cavernous	2	2	2	6	Mild	51.43	48.57
#7b	Solid	2	2	3	7	Severe	66.52	33.48
#8	Cavernous	3	2	2	7	Severe	85.71	14.29
#9	Cavernous	3	2	2	7	Severe	44.08	55.92
#10	Solid	3	1	3	7	Severe	85.61	14.39
#11a	Capillary	3	3	3	9	Severe	89.57	10.43
#11b	Cavernous	3	3	3	9	Severe	96.93	3.07
#12a	Cavernous	2	2	2	6	Mild	64.4	35.6
#12b	Capillary	1	2	2	5	Mild	75.28	24.72
#13	Capillary	3	3	3	9	Severe	98.81	1.19
#14a	Cavernous	3	2	1	6	Mild	80.47	19.53
#14b	Solid	3	2	3	8	Severe	83.04	16.96
#15	Solid	1	2	2	5	Mild	38.8	61.2

Table S4. Clinical case analysis scores

GO term	Gene
GO:0001944 Vasculature development	*ACTA2, APOE, AQP1, CEACAM1, CDH2, COL1A1, COL1A2, COL15A1, COMP, COMT, CCN2, CYP1B1, EDNRA, EGR1, ENPEP, EPAS1, FLNA, GAA, NR4A1, NDST1, ITGAV, LEPR, LIF, LOX, LOXL2, MEIS1, MMP2, MMP14, MYH9, MYLK, NOTCH3, NPR3, PDGFA, PDGFRB, PTPRB, ROBO1, SHC1, TCF7L2, TGFBR2, THY1, NRP1, SEMA5A, ADGRA2, EFEMP2, NSDHL, AMOTL2, HDAC7, PARVA, ZMIZ1, ARHGAP22, FKBP10, ADM2, SVEP1, ZC3H12A, COL27A1, CSF1, HOXA5, PBX1, PML, TGM2, TSC2, PAX8, LMO4, DACT1, CC2D2A, IRX3, STARD13*

Table S5. A gene list enriched in the Vasculature development gene set.

Gene	Canonical pathway	Promoter meth.diff (%)	Relative TPM (JuB2/CnAOEC)
PDGFRB	PDGF	100	0.013
NPR3	Natriuretic peptide	100	0.037
ROBO1	SLIT–ROBO	100	0.044
PTPRB	ANGPT–TIE2 / VEGFR2	100	0.045
SEMA5A	Semaphorin	100	0.054
ADGRA2	WNT7/β-catenin	100	0.114
DACT1	Wnt/PCP	100	0.144
EDNRA	Endothelin	100	0.218
ADM2	CALCRL–RAMP (Adrenomedullin)	100	0.256
ENPEP	RAAS modulator	100	0.335
NRP1	VEGF/Semaphorin	100	0.375
STARD13	Rho/Actin	90.839	0.498
LIF	LIF/IL-6 family	88.462	0.019
PDGFA	PDGF	87.5	0.085
ARHGAP22	Rho/Actin	78.571	0.217
THY1	Integrin/FAK	77.717	0.165
TGFBR2	TGF-β	75	0.415
CSF1	CSF1R/M-CSF	64.192	0.418
ITGAV	Integrin	60.274	0.047
CCN2	TGF-β/Integrin	60	0.012
LEPR	Leptin/JAK–STAT	40.5	0.014
TSC2	PI3K–AKT–mTOR	39.938	0.478
NOTCH3	Notch	30	0.002
SHC1	RTK–RAS–MAPK	26.596	0.406

Table S6. Signaling pathway-related genes