

Supplementary materials for

Manipulating Histone Acetylation Leads to Adverse Effects in Hemangiosarcoma Cells

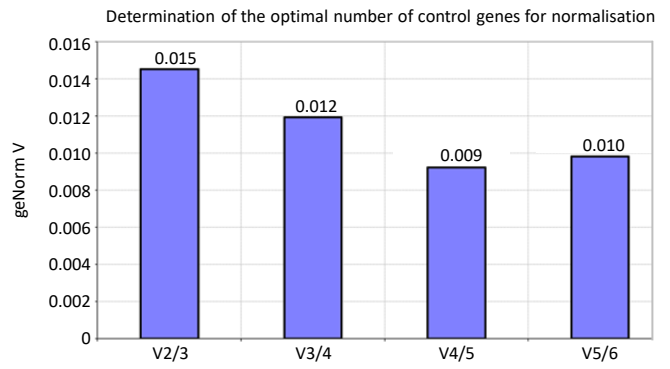
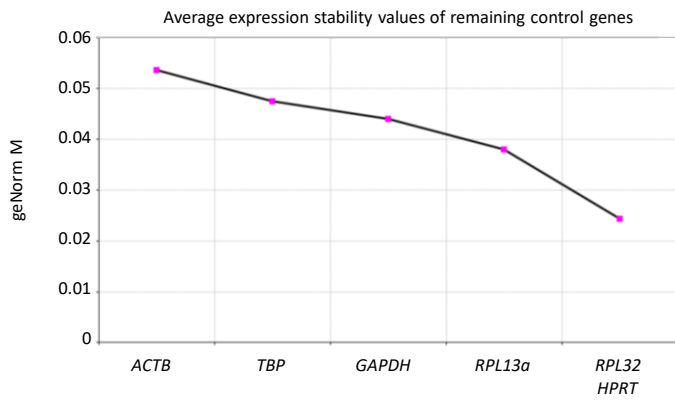
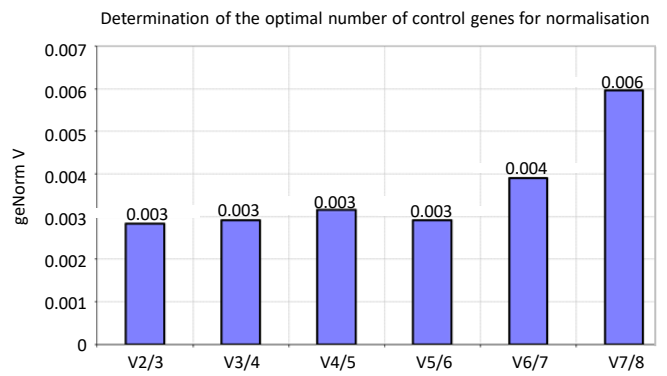
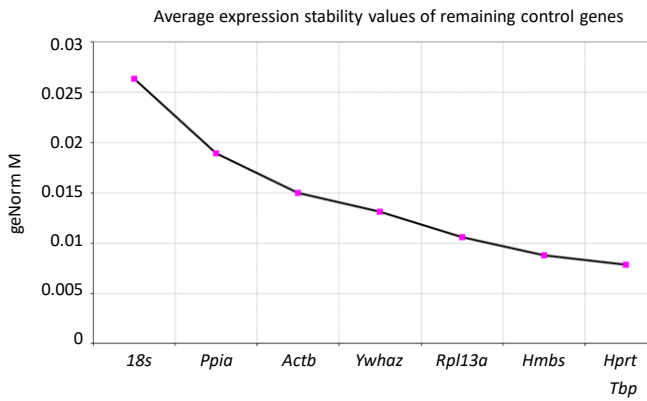
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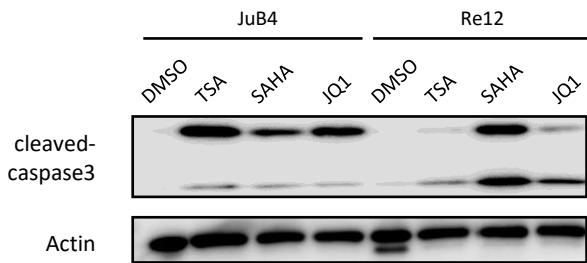
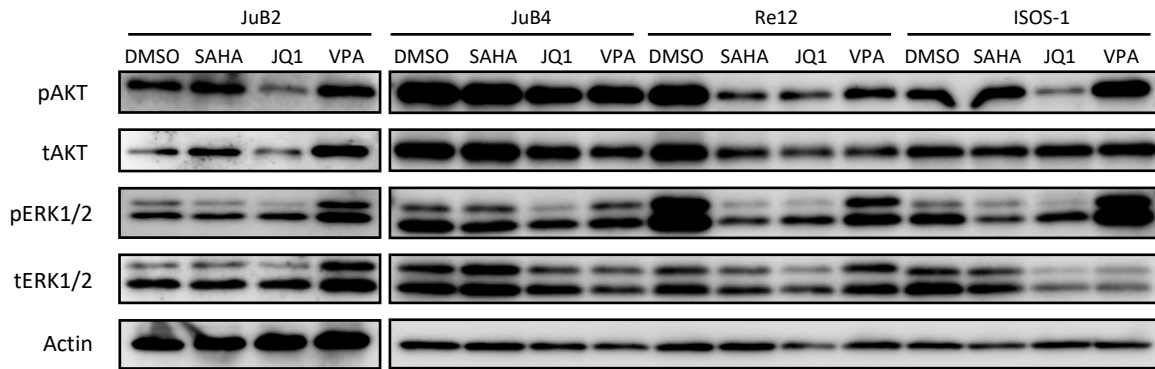
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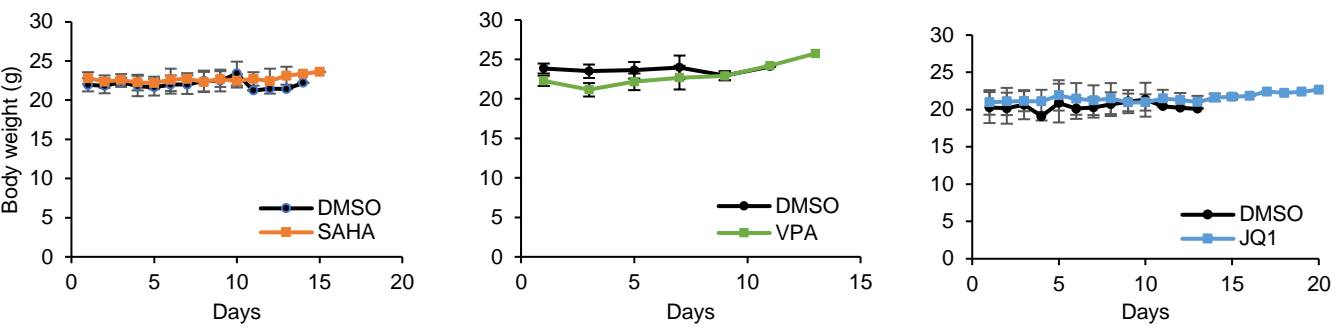
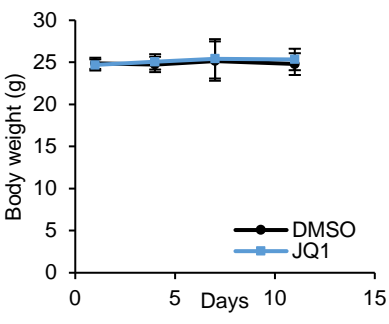
A**B****Supplementary figure 1.****geNorm analysis to determine appropriate reference gene sets.**

(A, B) (Left) Average expression stability values (geNorm M) of candidate genes. The gene with the lowest M value has the most stable expression in dogs (A) and mice (B). (Right) Determination of optimal number of control genes for geometric normalisation for dogs (A) and mice (B). To determine the optimal number of reference genes, 0.15 V value was used as the cut-off value as Vandesompele et al. (2002) recommended.

A**B**

Supplementary figure 2.

(A) Uncropped images of western blot analysis for cleaved caspase 3 and Actin in JuB4 and Re12 treated with DMSO, TSA, SAHA and JQ1. TSA: Trichostatin A. (B) Western blot analysis for phosphorylated and total AKT and ERK expression in canine and murine HSA cell lines treated with DMSO, SAHA, JQ1 or VPA.

A**B**

Supplementary Figure 3.

Average body weight of mice in *in vivo* study

(A) Average body weight of Balb/c mice treated with DMSO, SAHA, VPA or JQ1 after starting treatments. (B) Average body weight of KSN/Slc mice treated with DMSO or JQ1 after starting treatments.

Case Number	Breed	Age	Sex	Location
1	Miniature Dachshund	10y	F Spay	Spleen
2	Jack Russell Terrier	9y	M Cast	Spleen
3	Jack Russell Terrier	7y	F Spay	Spleen
4	Miniature Dachshund	14y	M Cast	Spleen
5	French Bulldog	7y	M Cast	Spleen
6	Miniature Dachshund	9y	F	Spleen
7	Miniature Schnauzer	11y	M	Spleen
8	Miniature Dachshund	10y	F	Spleen
9	Flat-coated retriever	11y	F	Spleen
10	Golden Retriever	9y	M	Liver

*F: female, M: male, Spay: spayed, Cast: castrated

Supplementary Table 1. Case information

Protein Name	Maker	Host	Dilution	Catalog Number
Acetylated Histone H2B	Santa Cruz Biotechnology, Inc.	Mouse	1:500	sc-515937
Acetylated Histone H3	Active Motif	Rabbit	1:5000	39040
Acetylated Histone H4	Santa Cruz Biotechnology, Inc.	Mouse	1:500	sc-377520
Cleaved-caspase 3	Cell Signaling Technology	Rabbit	1:1000	9661S
pAKT	Cell Signaling Technology	Rabbit	1:1000	4060
tAKT	Cell Signaling Technology	Rabbit	1:1000	4691
pERK1/2	Cell Signaling Technology	Rabbit	1:1000	4370S
tERK1/2	Cell Signaling Technology	Rabbit	1:1000	4695S
LC3	MBL	Rabbit	1:1000	PM036
Actin	Sigma-Aldrich	Mouse	1:10000	MAB1501
H3	MAB Institute	Mouse	1:10000	MABI0001-20
Goat anti-Mouse IgG (H+L)	Thermo Fisher Scientific	Goat	1:10000	G21040
Goat anti-Rabbit IgG (H+L)	Thermo Fisher Scientific	Goat	1:10000	G21234

Supplementary Table 2. Antibody list

Species	Target	Sequence (Forward)	Sequence (Forward)	Gene ID
Canine	<i>OAS1</i>	TGTGCGGGTGTCTAAAGTTG	TGAACTGTCTCGTTTCTCG	ENSCAFG 00000023556
	<i>OAS2</i>	TGACCCAGATCCAGAAAACC	CCATTCCGGTAGCGTCTTTTG	ENSCAFG 00000023107
	<i>CXCL8</i>	GGCAGCTTTTGTCTTTCTG	ACACTGGCATCGAAGTTCTG	ENSCAFG 00000003029
	<i>CXCL12</i>	AGCCAACGTCAAGCATCTCA	TCAATGCACACCTGTCTGCT	ENSCAFG 00000007026
	<i>IL6</i>	TCGGCAAAATCTCTGCACTG	TTTCTGCCAGTGCCTCTTTG	ENSCAFG 00000002733
	<i>RPL32</i>	TGGTTACAGGAGCAACAAGAAA	GCACATCAGCAGCACTTCA	ENSCAFG 00000004871
	<i>HPRT1</i>	CACTGGGAAAACAATGCAGA	ACAAAGTCSGGTTTATAGCCAACA	ENSCAFG 00000018870
	<i>GAPDH</i>	ATTCCACGGCAGTCAAG	TACTCAGCACCAGCATCACC	ENSCAFG 00000015077
	<i>TBP</i>	ATAAGAGAGCCCCGAACCAC	TTCACATCACAGTCCCCAC	ENSCAFG 00000004119
<i>RPL13A</i>	GCCGGAAGGTTGTAGTCGT	GGAGGAAGGCCAGTAATTC	ENSCAFG 00000029892	
Mouse	<i>Atg9a</i>	ATCACCTTGGCACCACATTG	TGGTGAAGGCAACCACAAAAG	ENSMUSG 00000033124
	<i>Atg13</i>	ATTTGCACCCGCTCATCATC	AGGGCCTTCTTTGCTTCATG	ENSMUSG 00000027244
	<i>Lamp1</i>	AGTCTTGTTGGCGTTCAG	AGGCAATGCATTACGTGAGC	ENSMUSG 00000031447
	<i>Ulk1</i>	AAACATCGTGGCGCTGTATG	TTCACTCAGTGTGCGCATAG	ENSMUSG 00000029512
	<i>Ccne1</i>	AAGCCCAAGCAAAGAAAGCC	TGGCAGGTTTGGTCATTCTG	ENSMUSG 00000002068
	<i>Cdk6</i>	AAGCTGCTGACCAATTGTGC	ATACGCATGCACACACTC	ENSMUSG 00000040274
	<i>Gmn</i>	AGGAGAACGCTGAAGATGATCC	TGCTAGCTGGTCATCCCAAAG	ENSMUSG 00000006715
	<i>Mcm3</i>	CGTTCCAAGGATGTCTTTGAGC	ATGTGGCTGCCGTTTCAAG	ENSMUSG 00000041859
	<i>Il6</i>	TACCACTTACAAGTCGGAGGC	CTGCAAGTGCATCATCGTTGTTT	ENSMUSG 00000025746
	<i>Cxcl1</i>	CAAACCGAAGTCATAGCCACAC	TTTCTCCGTTACTTGGGGACAC	ENSMUSG 00000029380
	<i>Ccl2</i>	TTTCCACAACCACCTCAAGC	TAAAGGCATCACAGTCCGAGTC	ENSMUSG 00000035385
	<i>Ccl7</i>	AAAACCCCAACTCAAAGCC	ACAGCGGTGAGGAATTTTGC	ENSMUSG 00000035373
	<i>Oas1a</i>	AAGCACTGGTACCAACTGTG	AGGCAAAGACAGTGAGCAAC	ENSMUSG 00000052776
	<i>Oas2</i>	TAGACCAGGCCGTGGATG	GTTTCCCGGCCATAGGAG	ENSMUSG 00000032690
	<i>Hprt</i>	GCTTGCTGGTGAAAAGGACCTCTCGAAG	CCCTGAAGTACTCATTATAGTCAAGGGCAT	ENSMUSG 00000025630
	<i>Tbp</i>	CCTTGTAACCTTCACCAATGAC	ACAGCCAAGATTCACGGTAGA	ENSMUSG 00000014767
	<i>Ywhaz</i>	GAAAAGTTCTTGATCCCCAATGC	TGTGACTGGTCCACAATTCCTT	ENSMUSG 00000022285
	<i>Actb</i>	AGGCCAACCGTGAAAAGATG	TGGATGGCTACGTACATGGC	ENSMUSG 00000029580
	<i>Hmbs</i>	ATGAGGGTGATTGAGTGGG	TTGTCTCCCGTGGTGGACATA	ENSMUSG 00000032126
	<i>Rpl13a</i>	AGGGGCAGGTTCTGGTATTG	TGTTGATGCCTTCACAGCGT	ENSMUSG 00000074129
<i>Ppia</i>	CGCGTCTCCTTCGAGCTGTTTG	TGTAAAGTCACCACCCTGGCACAT	ENSMUSG 00000071866	
<i>18s</i>	CGGCTACCACATCCAAGGAA	AGCTGGAATTACCGCGGC	ENSMUSG 00000119584	

Supplementary Table 3. Primer list