SUPPLEMENTAL MATERIAL

Supplemental Table 1. Bovine primers for real time RT-PCR

	Forward	Reverse
P21	AGAGCGGTGGAACTTCGACTTTGT	ACAGGTCCAAGTGGTCCTCCTGA
FOXO3	GAATTCCGTCAGCAACATGGGCTT	TGGAAGGTTCGCACTGGTTGAGTA
Bcl2	ATGACCGAGTACCTGAACCG	CCTTCAGAGACAGCCAGGAG
survivin	TTCATCCACTGTCCCACTGA	TAGGGTCGTCATCTGGTTCC
HDAC-1	GCCTCACCGAATCCGCATGA	GGGCGGATGGAGCGTAAGAA
cKIT	TCCTGATTGACCTTCCCTTG	TGTCAAATCCTTGGGGAGAG
MCP-1	CGCCTGCTGCTATACATTCA	ACACTTGCTGCTGGTGACTC
SDF-1	CTTGCCGATTCTTTGAGAGC	CTGAAGGGAGCAGTTTGGAG
IL-6	GTGAAAGCAGCAAGGAGACA	ATCCGTCCTTTTCCTCCATT
PDGFb	AGTGACCACTCCATCCGTTC	TCAGCCCCGTCTTCATCTAC
S100A4	GCAAGGAGGGTGACAAGTTC	TCGTTGTCCTTGTTGCAGTC
HPRT	CTGGCTCGAGATGTGATGAA	CAACAGGTCGGCAAAGAACT

Supplemental Figures

Figure I. HDAC protein expression in lung extracts from rats. (A) HDAC1, (B) HDAC2, (C) HDAC3, (D) HDAC4, (E) HDAC5, (F) HDAC7 (G) Bcl-2, (H) representative bands and (I) kidney. Rats were exposed to normal air (NC) or hypoxia for 2 days (2D), 1 week (1W) and 2 weeks (2W). The data are generated from optical density measurements of individual bands from Western blots and normalised to β-actin. The ratios are presented as mean ± SEM of fold change relative to NC. n=3 in each group. Welch's ANOVA was used for (C) HDAC3 and (E) HDAC5 and standard ANOVA for the others, comparing each group with NC. * $p<0.05$, ** $p<0.001$compared with NC group.

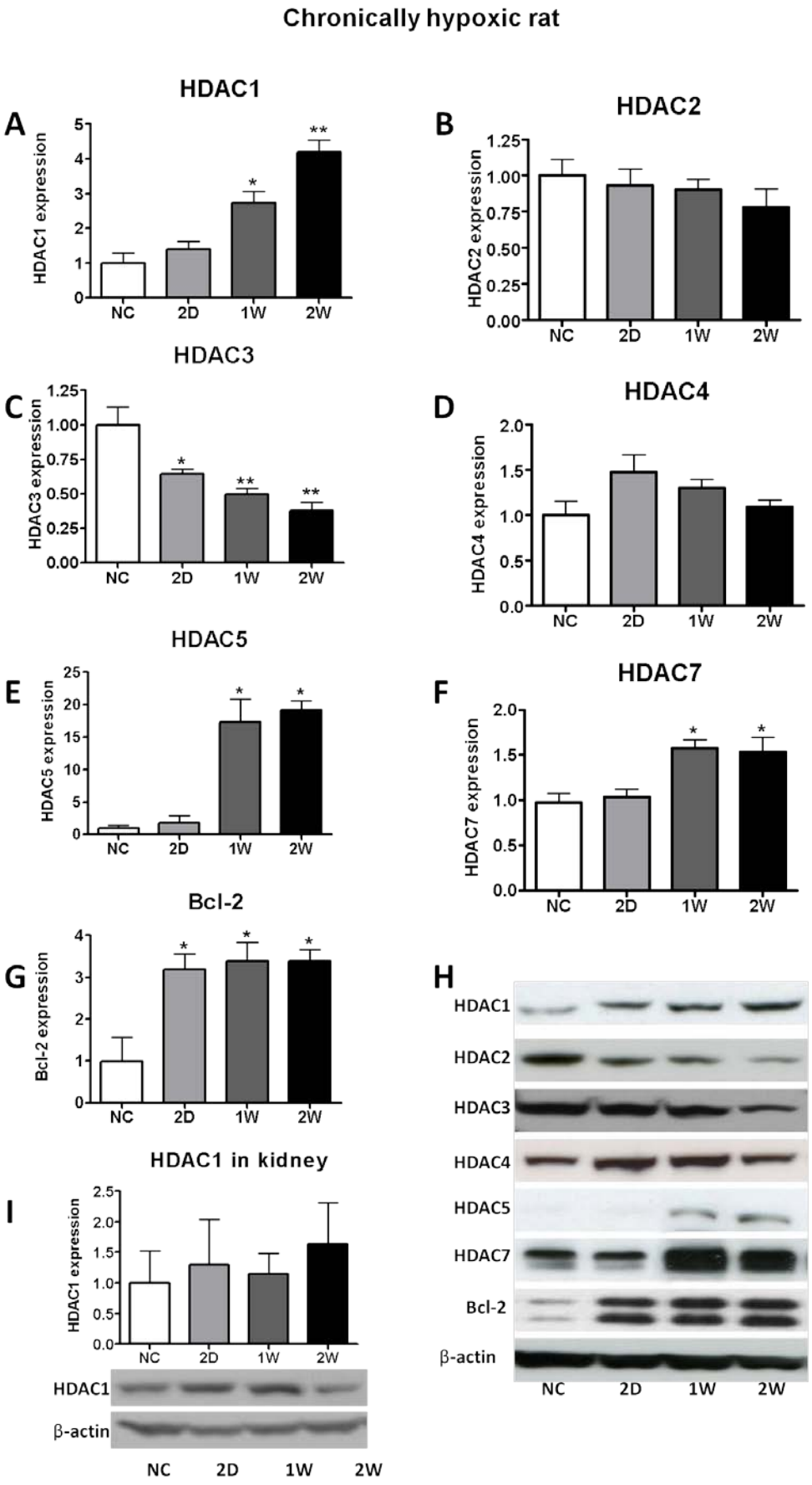

Chronically hypoxic rat
HDAC1
A
HDAC1 expression
NC
2D
1W
2W
HDAC2
B
HDAC2 expression
HDAC3
C
HDAC3 expression
HDAC4
D
HDAC4 expression
HDAC5
E
HDAC5 expression
HDAC7
F
HDAC7 expression
Bcl-2
G
Bcl-2 expression
H
HDAC1
HDAC2
HDAC3
HDAC4
HDAC5
HDAC7
Bcl-2
β-actin
HDAC1 in kidney
I
HDAC1 expression
HDAC1
β-actin

Figure II. Distribution of HDAC1 and HDAC5 expression in IPAH lung sections, low power view. Bar = 25 µm.

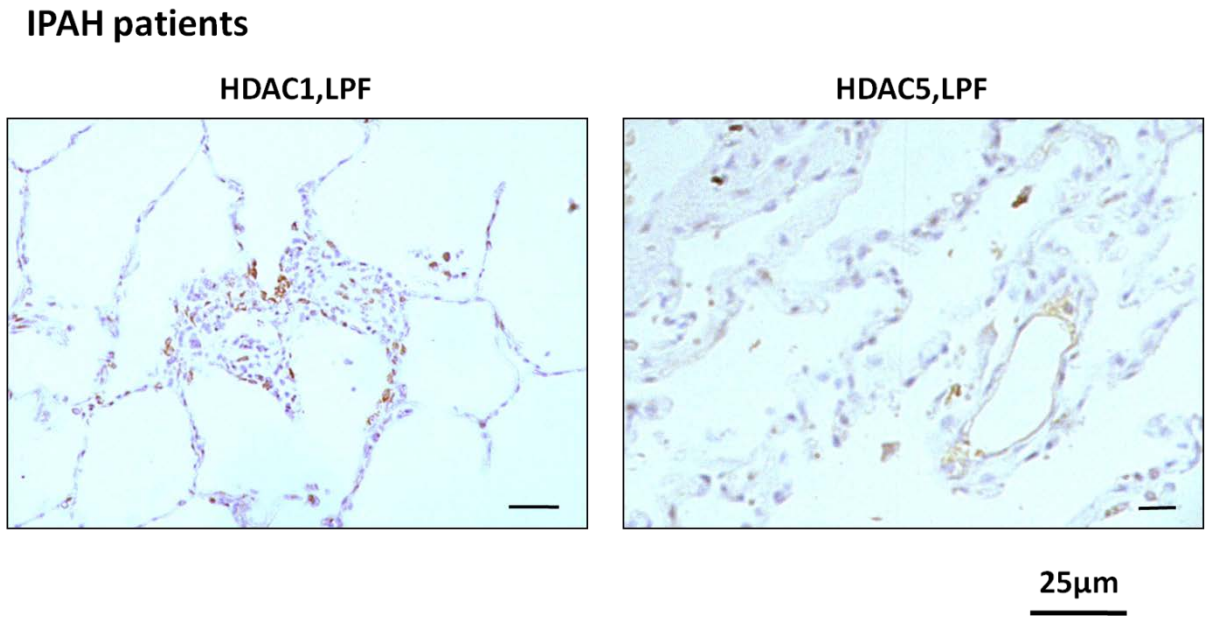


Figure III. Distribution of HDAC1 and HDAC5 in normoxic rat lung sections, high power view. Bar = 25 µm.

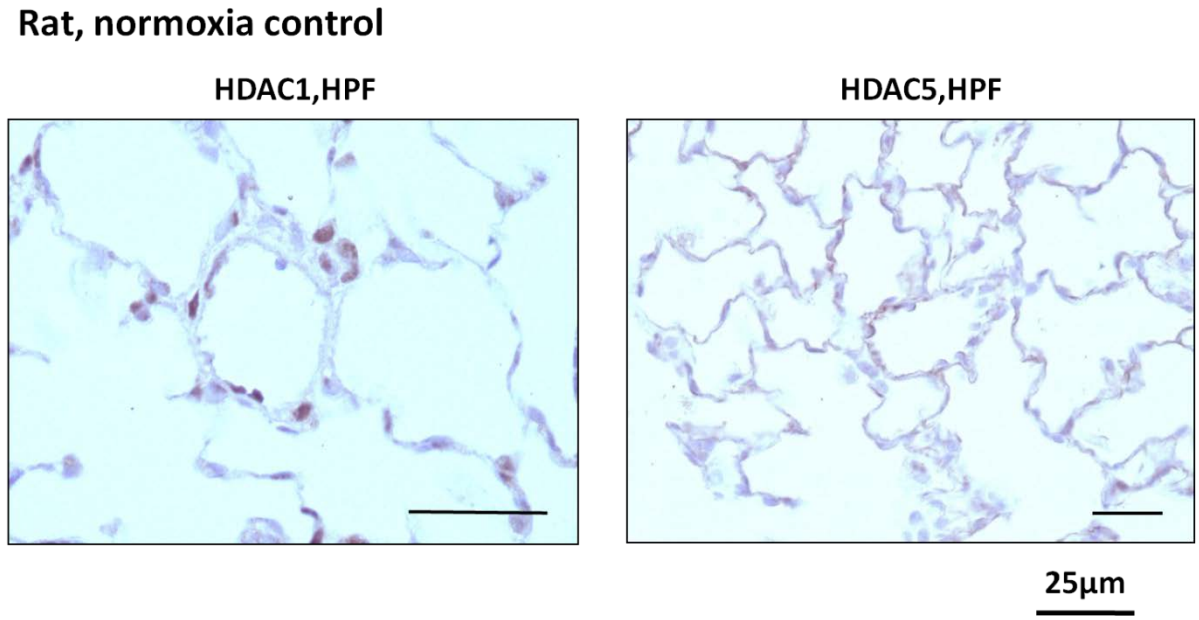


Figure IV. Prevention study: Valproic acid (VPA) was administered during 2 weeks hypoxia. (A) PAP, mean pulmonary artery pressure (B) RV/LV+sep, right ventricular hypertrophy, (C) SBP, systolic blood pressure, (D) percentage of muscularised vessels, (E) histology figures with van Gieson's elastic stain. NC: normoxia; HC: hypoxia for 2 weeks; low VPA: hypoxia with VPA 100 mg/kg/day; and high VPA: hypoxia with VPA 300 mg/kg/day. The data are presented as mean± SEM. n=6 in each group. * $p<0.05$, ** $p<0.001$ compared with HC, ## $p<0.001$ compared with NC. One way standard ANOVA was used, compairing all groups pairwise. Bar = 25 µm.

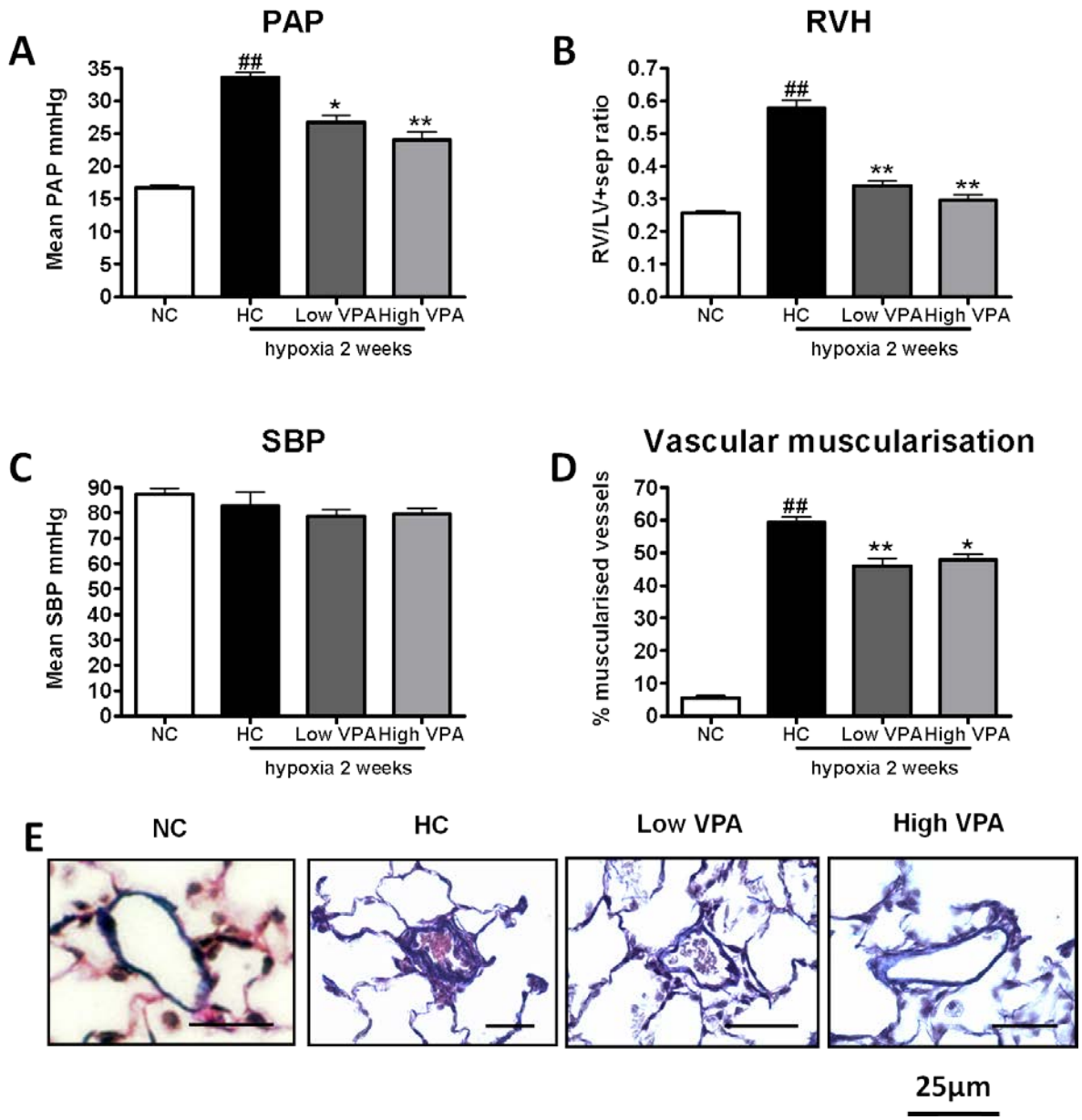

A
PAP
Mean PAP mmHg
35
30
25
20
15
10
5
0
##
*
**
NC
HC
Low VPA
High VPA
hypoxia 2 weeks
B
RVH
RV/LV+sep ratio
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
##
**
**
NC
HC
Low VPA
High VPA
hypoxia 2 weeks
C
SBP
Mean SBP mmHg
90
80
70
60
50
40
30
20
10
0
NC
HC
Low VPA
High VPA
hypoxia 2 weeks
D
Vascular muscularisation
% muscularised vessels
70
60
50
40
30
20
10
0
##
**
*
NC
HC
Low VPA
High VPA
hypoxia 2 weeks
E
NC
HC
Low VPA
High VPA
25µm

Figure V. HDAC1 protein levels in chronically hypoxic rat lungs. The data are generated from optical density measurements of individual bands from Western blots and normalised to β-actin. Data are presented as mean ± SEM of fold change relative to 4WH. n=6. 4WH: hypoxia for 4 weeks; VPA: hypoxia with VPA 300 mg/kg/day; SAHA: hypoxia with SAHA 50 mg/kg/day. * $p<0.05$ compared with 4WH. Welch's ANOVA was used, comparing each group with 4WH.

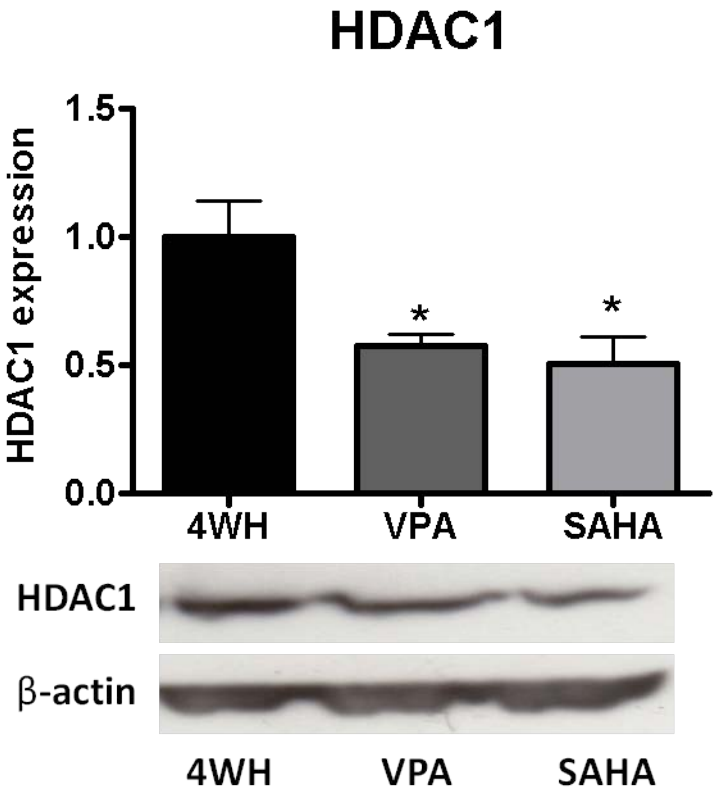


Figure VI. HDAC-1 mRNA levels are significantly increased in R-cells compared to CO-SMC. Data is analyzed as relative expression to HPRT and presented as mean ± SEM. *, $p<0.05$; compared with CO-SMC using Student's t test.

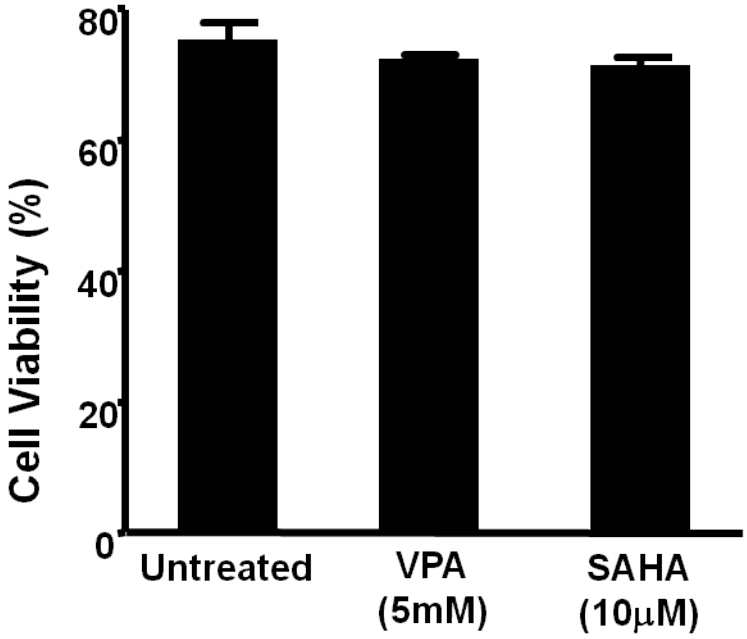


Figure VII. HDAC inhibitors, VPA and SAHA, do not induce significant cell death at the concentrations tested. Fibroblast cells isolated from chronically hypoxic pulmonary hypertensive calves (PH-Fibs) were treated with VPA (5mM), SAHA (10μM), or left untreated. Cell viability was measured by cell count using automatic cell counter. Data are presented as percentage of live cells to total cells.

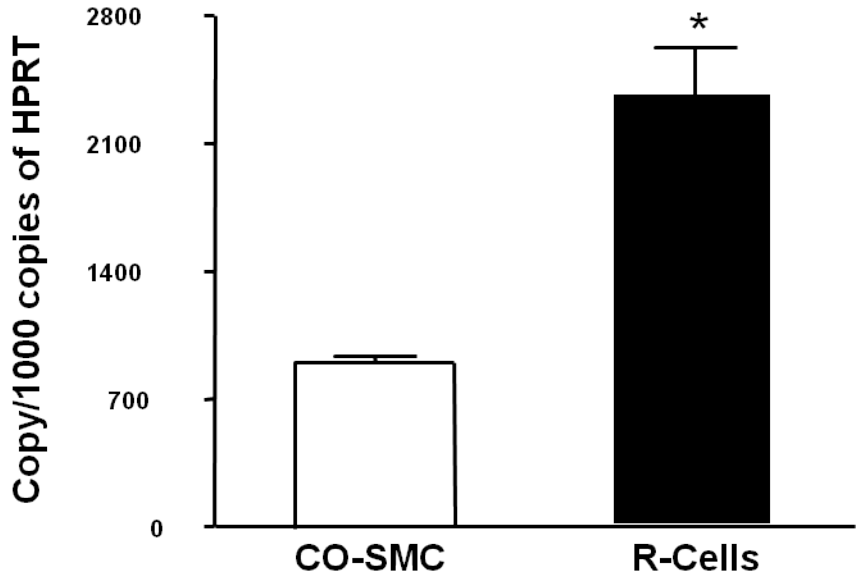
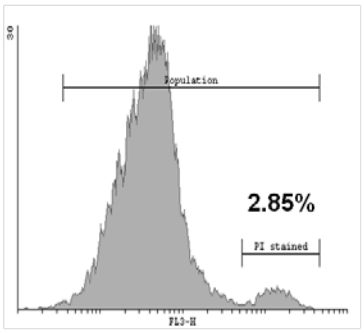
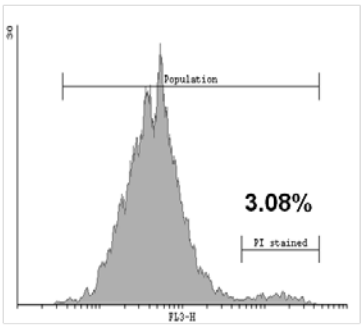


Figure VIII. FACS analysis of (A) viability staining with PI and (B) cell cycle distribution in PSMC stimulated with PDGF 50μg for 72 hours, with or without VPA or SAHA treatment. The calculations of defined areas are performed by software Cyflogic v1.2.1. Each experiment was repeated at least 3 times with separate cell preperations.

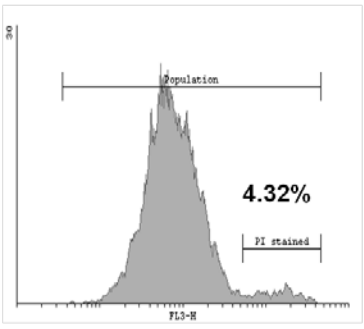
A



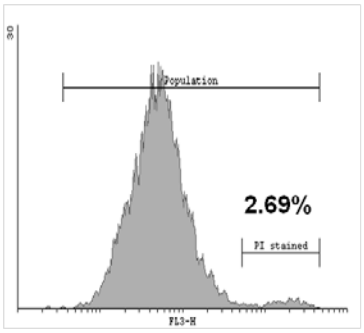
Control



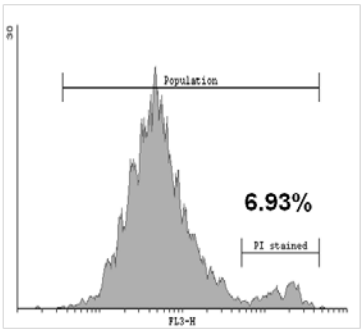
PDGF



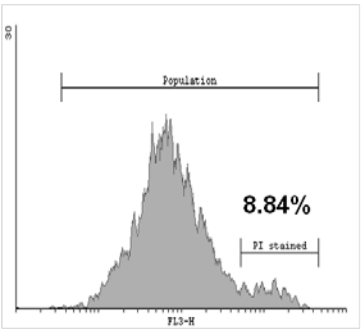
PDGF + VPA 1mM



PDGF + VPA 2mM

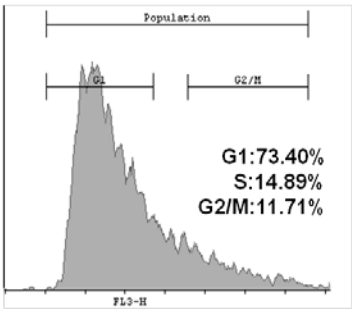


PDGF + SAHA 2.5μM



PDGF + SAHA 10μM

B



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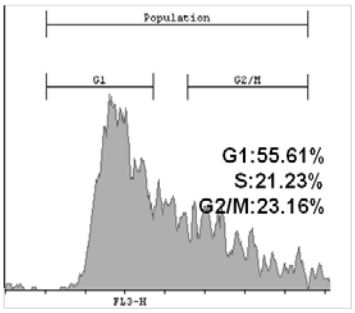
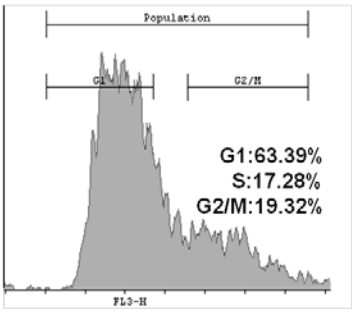
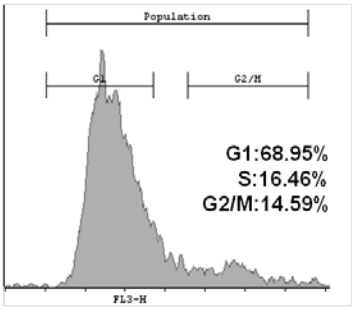


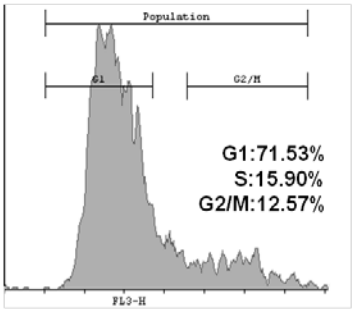
PDGF



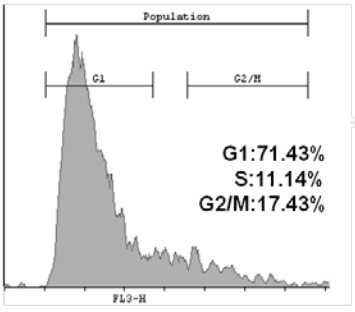
PDGF + VPA 1mM



PDGF + VPA 2mM



PDGF + SAHA 2.5μM



PDGF + SAHA 10μM