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Supplemental Material to:

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Expression and epigenetic regulation of angiogenesis-related factors during dormancy and recurrent growth of ovarian carcinoma

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Supplemental table 1.Primers for real-time PCR

Gene	Primer sequences	Product length(bp)
ANG1	S:AGCTACCACCAACAACAGTG A:CAAAGATTGACAAGGTTGTGG	79
ANG2	S:TGCCACGGTGAATAATTCAG A:TTCTTCTTTAGCAACAGTGGG	124
ANG4	S:AACAGCGCGCTCGAGAAG A:GCTTCGCCTTCTTGCTGA	85
TSP1	S:CCTCAGGAACAAAGGCTGCTC A:GCCAATGTAGTTAGTGCGGATG	103
IGFBP3	S:AACTGTGGCCATGACTGAGGA A:CTCCCTGAGCCTGACTTTGC	100
ANGPTL2	S:CCACCCTGGACAGAGATCAT A:CTCGGAACTCAGCCCAGTAG	168
RECK	S:CGCGTGGCAGTCGATTACTAT A:GCTGCCAAGAGCGAAGGA	107
SPARC	S:GCGAGCTGGATGAGAACAACAC A:GTGGCAAAGAAGTGGCAGGAAG	136
TIE1	S:GCCCAGATTGCGCTACAG A:ATCAATGCCCGCGTAAGT	96
TIE2	S:TTGAAGTGGAGAGAAGGTCTG A:GTTGACTCTAGCTCGGACCAC	128
TIMP3	S:TTCTCAGCGAGGATGGCACTT A:AAACACGGTTCAGGATGCTGG	200
EFEMP1	S:CAGCTGACCCTCAGCGCATT A:CAGTTGTGCGTCCCTGCAGT	124
SULF1	S:GGCATTTTGAATCAGCTACACGTA A:TCCCATCCATCCCATAACTGTC	152
CDH13	S:TTCAGCAGAAAGTGTTCCATAT A:GTGCATGGACGAACAGAGT	204

CDH1	S:CAAGCAGCAGTACATTCTA A:CACTTCCACTCTCTTTTC	148
β-actin	S:GAAGCTGTGCTATGTTGCTCTA A:GGAGGAAGAGGATGCGGCA	64

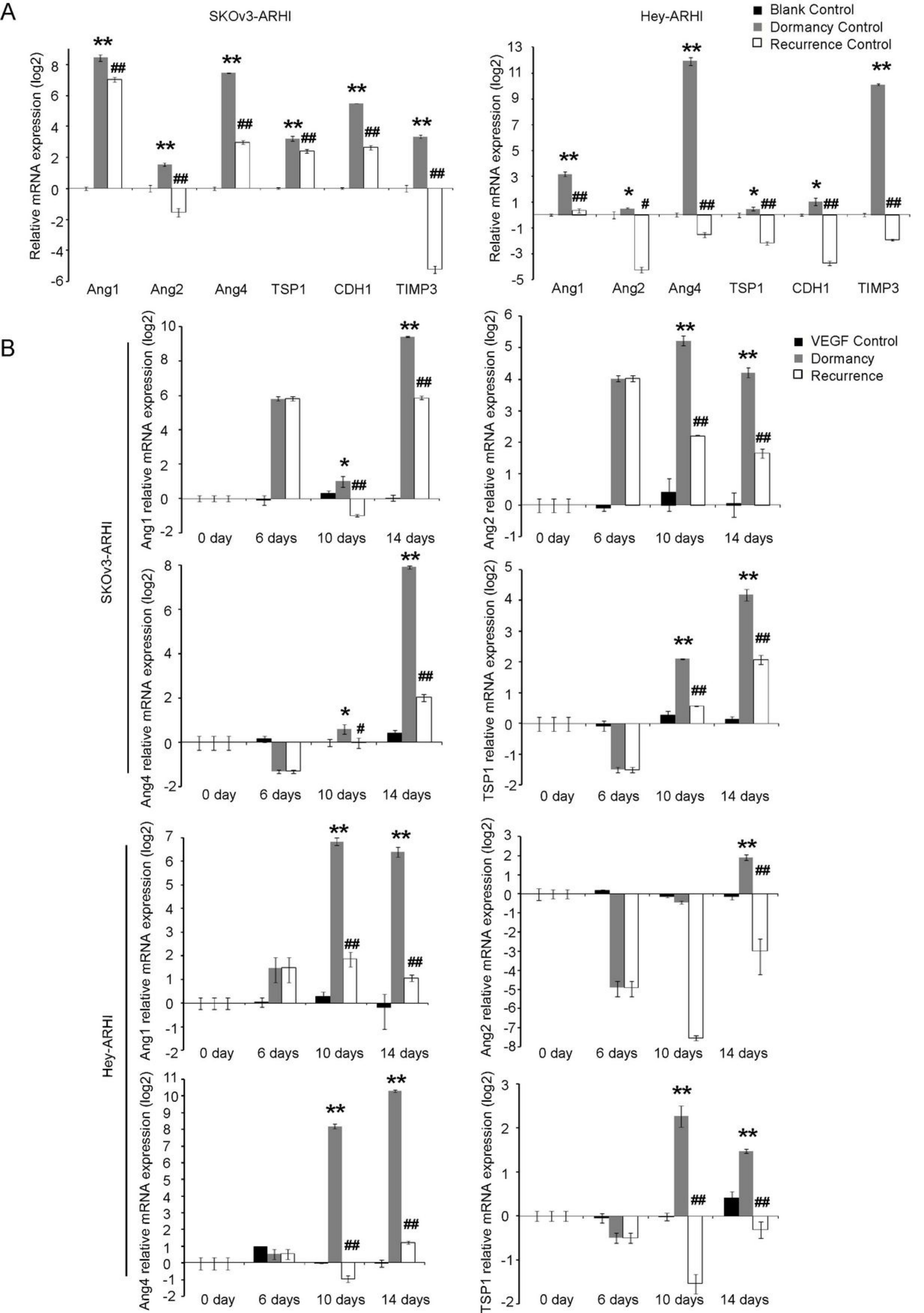
Supplemental table 2. Primers for bisulfite sequencing PCR

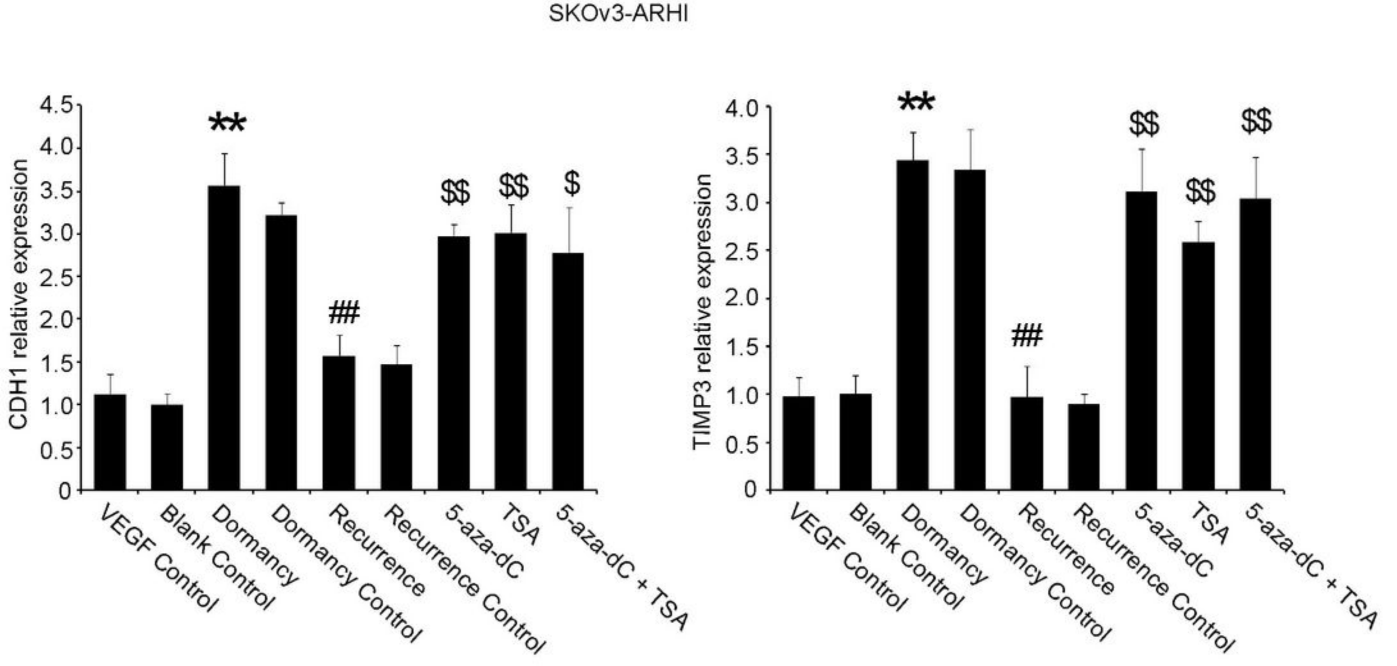
Gene	Primer sequences	Product length(bp)	Annealing temperature(℃)
ANG1	S:TTAGAAAATAGTGGGAGAAGATATAAT A:CCAAATATTAAAATTTCTAAAAAAA	184	56
ANG2	S:TATTTTGAGTTGTGATTTTGTTTTG A:TCTCCAACACTTACAACCTCTACAC	233	64
ANG4	S:TGTTATTAAGATTGAGGAAGGAGGA A:ACAACCAACAATAACCAAACTTACC	205	61
TSP1	S:GTGGGGTTAGTTTAGGATAGG A:CAAAAAACACCAAAAAAACCATT	124	54
IGFBP3	S:GTGGGTTTTTGGGGATATAAATAGT A:AATCACTCCTAACCAACTCAACAC	278	65
ANGPTL2	S:TTTAGAGATGAGAATGAGGTTTTTA A:AACCTAACCAACTCTACTCCCTAAC	280	64
RECK	S:TTTAGTGATGAATTTTTGTTAGGGG A:AACTTCTCTCCTTCATATACCCTCAA	142	62
SPARC[22]	S:ATTTAGTTTAGAGTTTTGAGTGG A:ACAAAACTTCCCTCCCTTAC	221	56.5
TIE1	S:TGTTGTTTATAAATGGTTAAGATGGTAAA A:CTTACCTCAACCTCCCAAATAACTA	220	57.2
TIE2	S:TGGTTTTTTGGGGTTATATTGAGTA A:ACTAAAAATAACAAACCCTCCACCTATA	270	51.1
TIMP3[23]	S:GCGGCCGCGTTAGAGATATTTAGTGGTTTAG A:CCCTCAAACCAATAACAAAAC	263	50.3

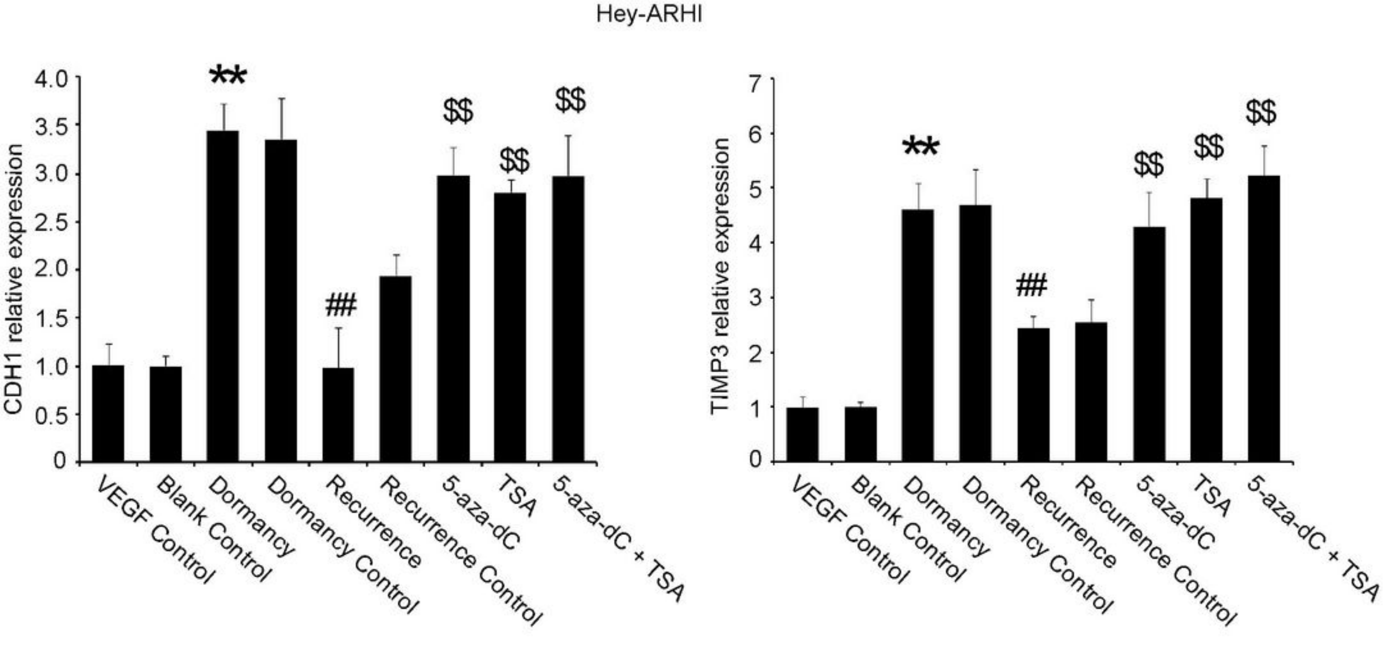
EFEMP1	S:TATTTGGATTTTATAGGAGTTGGTTAGA A:CTCTTTTTTCTTATCAATCTAAATCCC	223	59
SULF1	S:GGTTATTTGATTGGGAGTTTTTAGAT A:AACAAAACAACCTTCCTTCTCTTAAT	212	51.7
CDH13	F:TTGGAAAAGTGGAATTAGTTGGTAT R:ACCAAAACCAATAACTTTACAAAAC	187	55
CDH1	S:GTTGTTGTTGTTGTTGTAGGTATTT A:CCACTCCCATCACTAAAAAATC	197	54.9

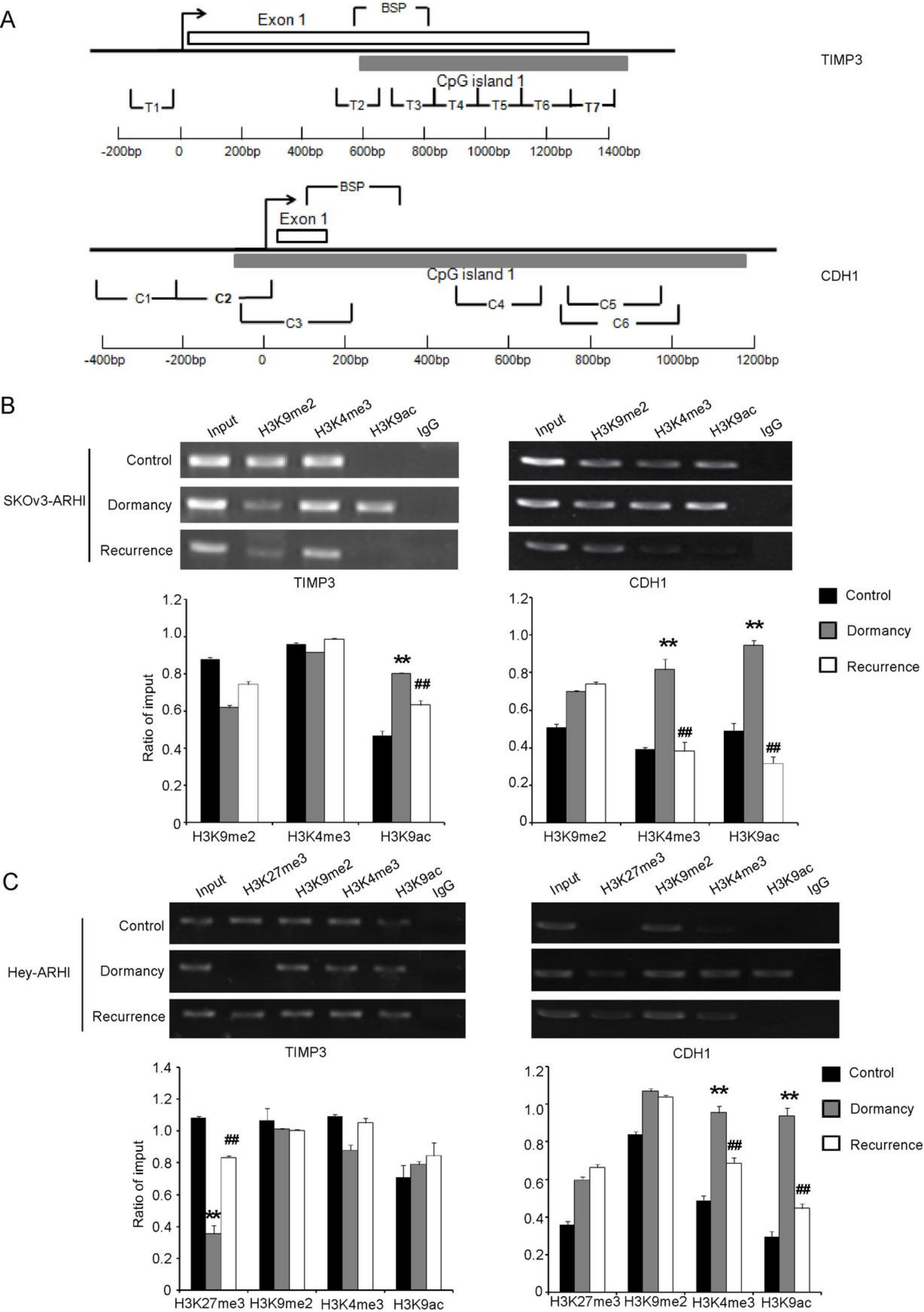
Supplemental table 3. Primers for ChIP-PCR

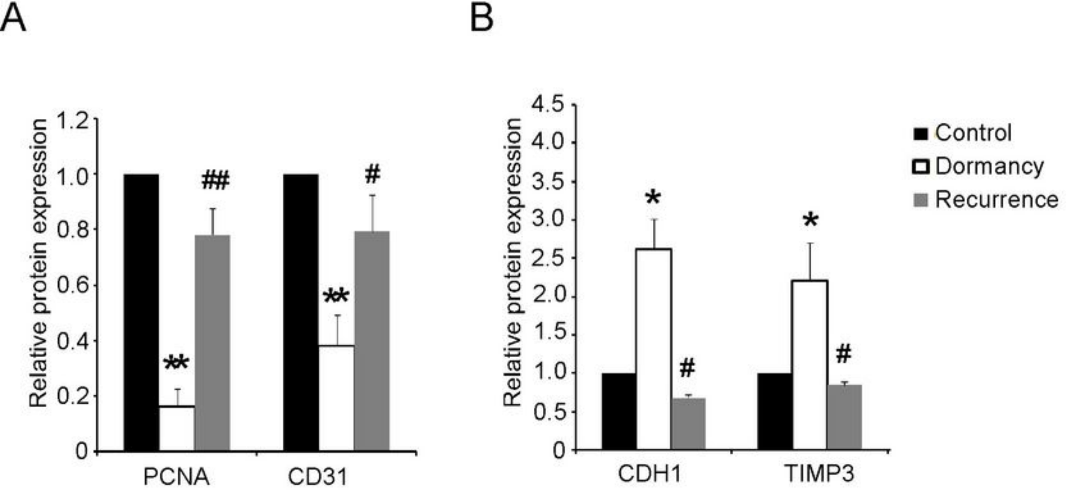
Gene	Primer sequences	Product length(bp)
CDH1	S: ACTCCAGGCTAGAGGGTCACC A: CCGCAAGCTCACAGGTGCTTTGCAGTTCC	219
TIMP3	S:GCAACTCCGACATCGGTAAG A:GCCCAAGGACATCGAGTTT	167

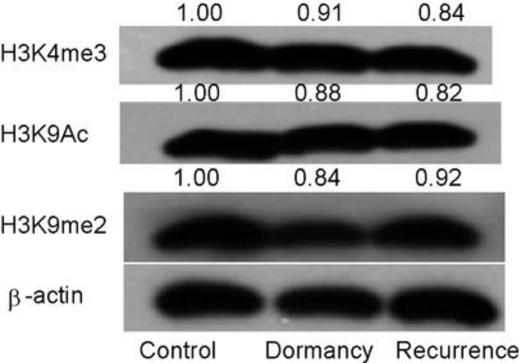

A
SKOv3-ARHI
Relative mRNA expression (log2)
8
6
4
2
0
-2
-4
-6
Ang1
Ang2
Ang4
TSP1
CDH1
TIMP3
**
##
Hey-ARHI
Blank Control
Dormancy Control
Recurrence Control
13
11
9
7
5
3
1
-1
-3
-5
*
#
B
SKOv3-ARHI
Hey-ARHI
Ang1 relative mRNA expression (log2)
Ang2 relative mRNA expression (log2)
Ang4 relative mRNA expression (log2)
TSP1 relative mRNA expression (log2)
VEGF Control
Dormancy
Recurrence
0 day
6 days
10 days
14 days


SKOv3-ARHI
CDH1 relative expression
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
**
##
$$
$$
$
TIMP3 relative expression
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
**
##
$$
$$
$$
VEGF Control
Blank Control
Dormancy
Dormancy Control
Recurrence
Recurrence Control
5-aza-dC
TSA
5-aza-dC + TSA


Hey-ARHI
CDH1 relative expression
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
**
##
$$
$$
$$
TIMP3 relative expression
0
1
2
3
4
5
6
7
**
##
$$
$$
$$
VEGF Control
Blank Control
Dormancy
Dormancy Control
Recurrence
Recurrence Control
5-aza-dC
TSA
5-aza-dC + TSA


A
BSP
Exon 1
TIMP3
CpG island 1
T1
T2
T3
T4
T5
T6
T7
-200bp
0
200bp
400bp
600bp
800bp
1000bp
1200bp
1400bp
BSP
Exon 1
CDH1
CpG island 1
C1
C2
C3
C4
C5
C6
-400bp
-200bp
0
200bp
400bp
600bp
800bp
1000bp
1200bp
B
Input
H3K9me2
H3K4me3
H3K9ac
IgG
SKOv3-ARHI
Control
Dormancy
Recurrence
TIMP3
CDH1
Ratio of imput
1.2
1.0
0.8
0.6
0.4
0.2
0
H3K9me2
H3K4me3
H3K9ac
**
##
**
##
**
##
Control
Dormancy
Recurrence
C
Input
H3K27me3
H3K9me2
H3K4me3
H3K9ac
IgG
Hey-ARHI
Control
Dormancy
Recurrence
TIMP3
CDH1
Ratio of imput
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
H3K27me3
H3K9me2
H3K4me3
H3K9ac
**
##
**
##
**
##
Control
Dormancy
Recurrence


A
B
Relative protein expression
1.2
1.0
0.8
0.6
0.4
0.2
0
**
##
**
#
PCNA
CD31
Relative protein expression
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
*
#
*
#
CDH1
TIMP3
■ Control
□ Dormancy
■ Recurrence


1.00
0.91
0.84
H3K4me3
1.00
0.88
0.82
H3K9Ac
1.00
0.84
0.92
H3K9me2
β-actin
Control
Dormancy
Recurrence

Supplemental Figure 1. Angiogenesis-related genes were differentially expressed in ovrain cancer cells treated with DOX or DOX withdrawal. Ang1, Ang2 Ang4 and TSP1 dynamically express during the dormancy-to-recurrence transition. (**A**) Expression of angiogenesis-related factors in three non-VEGF control groups of SKOv3-ARHI and Hey-ARHI cells. (**B**) Dynamic expression of Ang1, Ang2 Ang4 and TSP1 during the dormancy-to-recurrence transition during a time line of 0, 6, 10 and 14 days, as measured by real-time PCR in SKOv3-ARHI and Hey-ARHI cells. *Compared with the VEGF control group, $P < 0.05$, ** Compared with the VEGF control group, $P < 0.01$, $^{\#}$Compared with the dormancy group, $P < 0.01$. $^{\#\#}$Compared with the dormancy group, $P < 0.01$.

Supplemental Figure 2. The TIMP3 and CDH1 protein levels were semi-quantified according to immunocytochemistry experiments. **Compared with the control group, $P < 0.05$. $^{\#\#}$Compared with the dormancy group, $P < 0.05$. $^{\$}$Compared with the recurrence group, $P < 0.05$; $^{\$\$}$Compared with the recurrence group, $P < 0.05$

Supplemental Figure 3. H3K9Ac, H3K4me3, and H3K27me3 may regulate the expression of *TIMP3* and *CDH1*. (**A**) Design of primers for BSP and ChIP analysis across the promoter and exon 1 of *TIMP3* and *CDH1*. Seven pairs of ChIP primers for *TIMP3* and 6 pairs of ChIP primers for *CDH1* were tested. T1~T7 and C1~C6 mean the respective location of the PCR products. The results showed that the histone markers were modulated in the T7 (*TIMP3*) and C2 (*CDH1*) regions. The T7 and C2 primers were used in the subsequent ChIP experiments. (**B**) H3K4me3, H3K9me2, and H3K9Ac at the

TIMP3 and *CDH1* promoter were analyzed by ChIP analysis in the SKOv3-ARHI cells. (**C**) H3K27me3, H3K4me3, H3K9me2, and H3K9Ac at the *TIMP3* and *CDH1* promoter were analyzed by ChIP analysis in the Hey-ARHI cells. The acetylation and methylation levels were expressed as the ratio of the signal intensity of the immunoprecipitation product (IP) to the input (see Methods). **Compared with the control group, P <0.01. $^{\#\#}$Compared with the recurrence group, P <0.01. Independent ChIP experiments were repeated at least twice to confirm the reproducibility of the results.

Supplemental Figure 4. PCNA, CD31, TIMP3 and CDH1 protein levels were semi-quantified in the in-vivo dormancy-to-recurrence transition model. (**A**) Semi-quantitative PCNA and CD31 protein levels in the in-vivo dormancy-to-recurrence transition model. The scores were made according to immunohistochemistry experiments (**Fig. 7**) (**B**) Semi-quantitative TIMP3 and CDH1 protein levels in the in-vivo dormancy-to-recurrence transition model. The sores were made according to immunohistochemistry experiments (**Fig. 8**). * Compared with the control group, P <0.05.** Compared with the control group, P <0.01. $^{\#}$Compared with the dormancy group, P <0.05. $^{\#\#}$Compared with the dormancy group, P <0.01.

Supplemental Figure 5. No significant changes in the general histone modification were found during the transition from dormancy to recurrent growth in vivo.

Supplemental Table 1. Primers for real-time PCR.

Supplemental Table 2. Primers for bisulfite sequencing PCR.

Supplemental Table 3. Primers for ChIP-PCR.