Table 1 qRT-PCR primer sequences

Name	Forward primer (5′-3′)	Reverse primer (5′-3′)
RUNx	ACCACGAGCCACTTCAGCAG	CGATGGTGTGGCGCTGTA
HLTF	GCTCCTCTTGTCATCCCACTCA	CGTCTTTGCTTAGTCCATCTGCCTT
CDKN2A	CATAGATGCCGCGGAAGGT	ATCTAAGTTTCCCGAGGTTTCTCA
HPP1	TGCTTTCCCTACCTCCTTAAGTGA	CTGTCATCATAACCAGAGCAATTCC
PTEN	CAAGATGATGTTTGAAACTATTCCAATG	CCTTTAGCTGGCAGACCACAA
APC	GGA AGC AGA GAA AGT ACT GGA	CTG AAG TTG AGC GTA ATA CCA G
MLH	GAGTGGCTGGACAGAGGAAG	TTTCTTCGTCCCAATTCACC
RASSF1A	GGCGTCGTGCGCAAAGGCC	GGGTGGCTTCTTGCTGGAGGG
MST1	GATGGGCACTGTCCGAGTAG	GCAACGTGTCATCGTGCTC
LATS	TAGAGCAGAGGGCGCGGAAG	CCAACACTCCACCAGTCACAGA
$VEGF_{165}$	ATCTTCAAGCCATCCTGTGTGC	CAAGGCCCACAGGGATTTTC
$VEGF_{189}$	ATCTTCAAGCCATCCTGTGTGC	CACAGGGAACGCTCCAGGAC
TSP2	TCGTGCGCTTTGACTACATC	GTGCCGTCAATCCAGAGGT
TSP1	TTGTCTTTGGAACCACACCA	CTGGACAGCTCATCACAGGA
Foxo-3a	ACAATAGCAACAAGTATACCAAGAGC	GACTGTCGTCAGCTGATTCG
FOXM1	GGAGGAAATGCCACACTTAGCG	TAGGACTTCTTGGGTCTTGGGGTG
PTTG1	TGATCCTTGACGAGGAGAGAG	GGTGGCAATTCAACATCCAGG
GAPDH	CGACCACTTTGTCAAGCTCA	AGGGGAGATTCAGTGTGGTG

Figure S1.

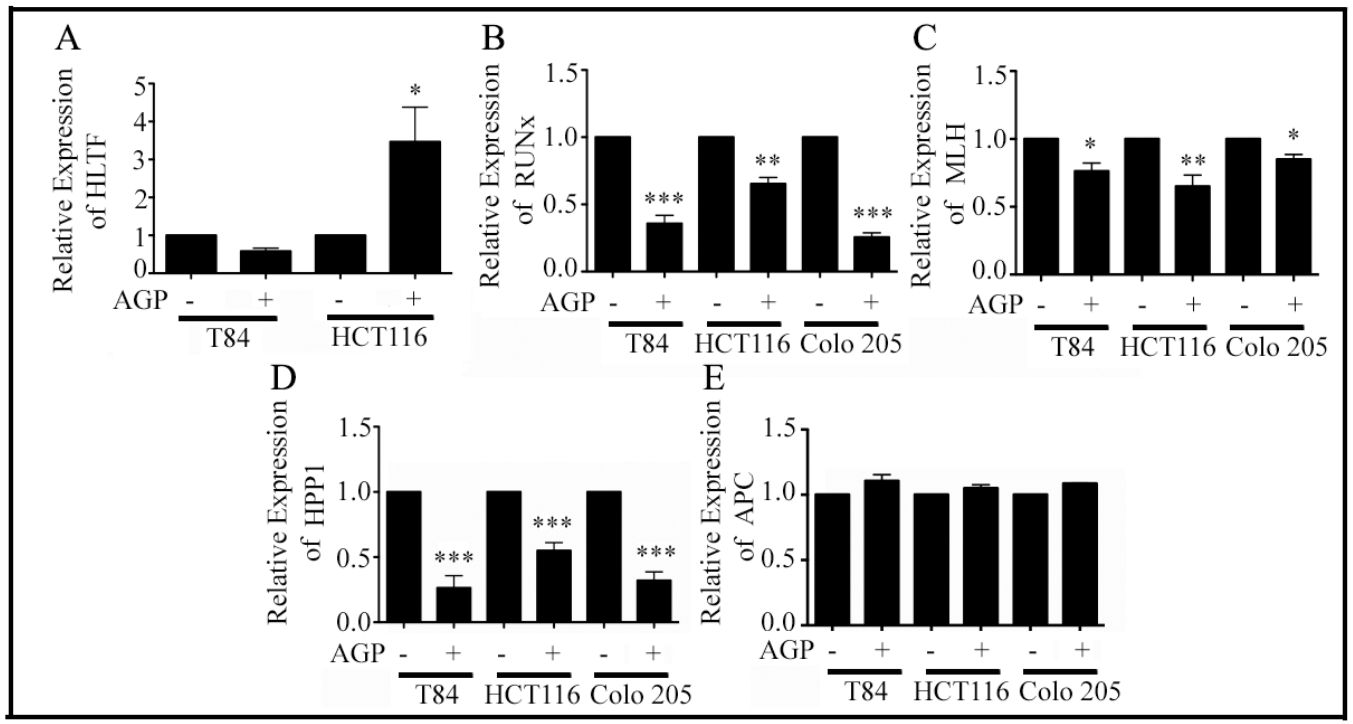

A
Relative Expression of HLTF
5
4
3
2
1
0
*
AGP - + - +
T84
HCT116
B
Relative Expression of RUNx
1.5
1.0
0.5
0.0
***
**
***
AGP - + - + - +
T84
HCT116
Colo 205
C
Relative Expression of MLH
1.5
1.0
0.5
0.0
*
**
*
AGP - + - + - +
T84
HCT116
Colo 205
D
Relative Expression of HPP1
1.5
1.0
0.5
0.0
***
***
***
AGP - + - + - +
T84
HCT116
Colo 205
E
Relative Expression of APC
1.5
1.0
0.5
0.0
AGP - + - + - +
T84
HCT116
Colo 205

Figure S2.

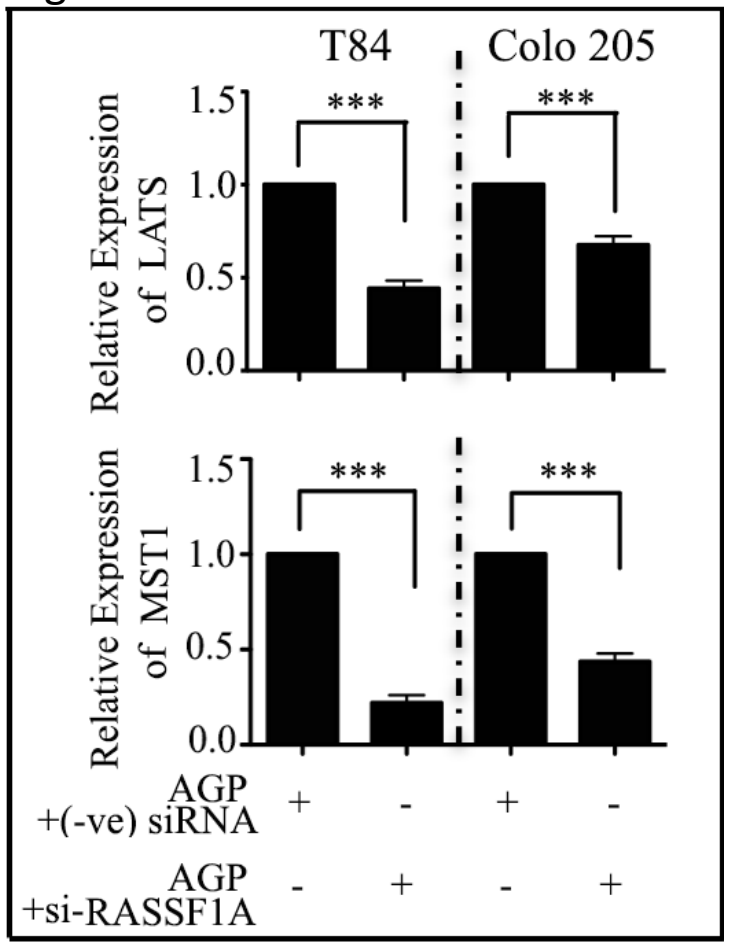

T84
Colo 205
Relative Expression of LATS
Relative Expression of MST1
1.5
1.0
0.5
0.0
***
***
***
***
AGP
+(-ve) siRNA
+ - + -
AGP
+si-RASSF1A
- + - +

Figure S3.

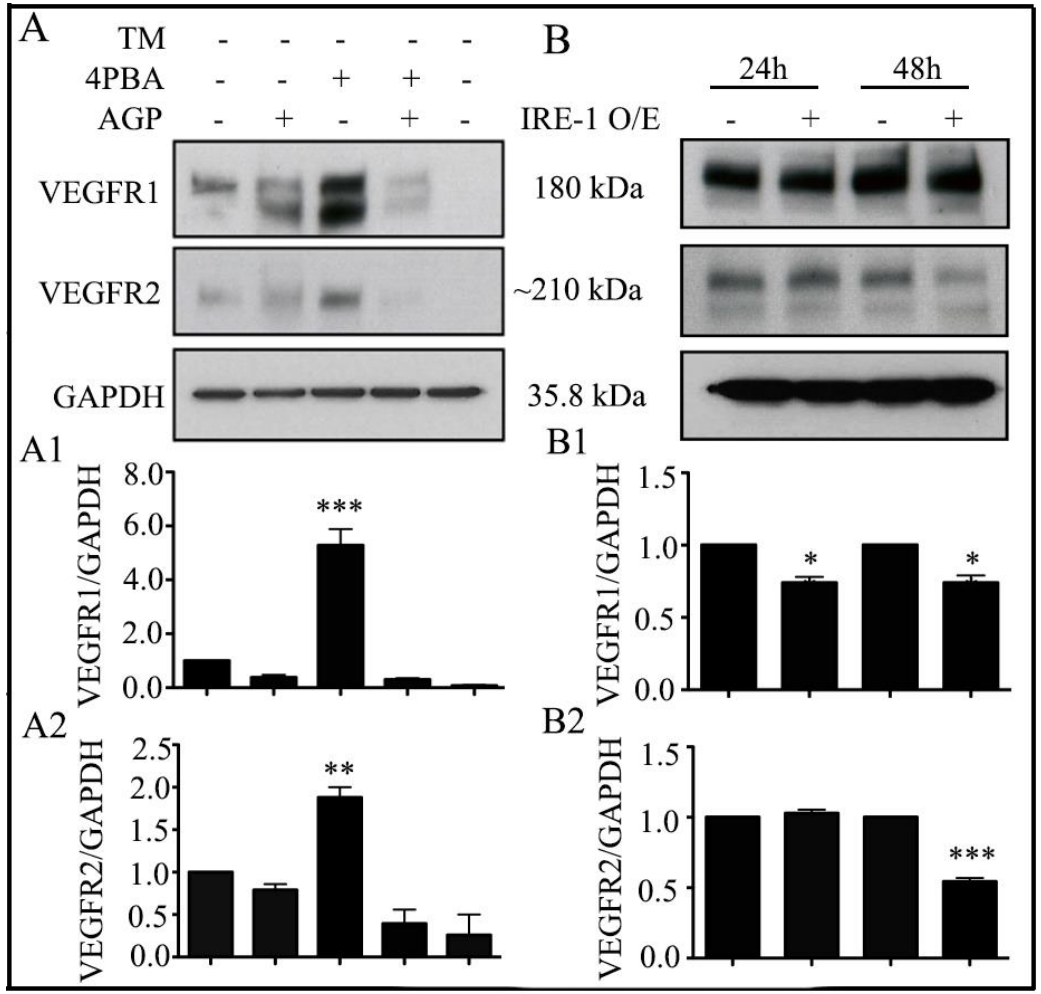

A
TM - - - - -
4PBA - - + + -
AGP - + - + -
VEGFR1
VEGFR2
GAPDH
B
24h
48h
IRE-1 O/E - + - +
180 kDa
~210 kDa
35.8 kDa
A1
VEGFR1/GAPDH
8.0
6.0
4.0
2.0
0.0
***
A2
VEGFR2/GAPDH
2.5
2.0
1.5
1.0
0.5
0.0
**
B1
VEGFR1/GAPDH
1.5
1.0
0.5
0.0
*
*
B2
VEGFR2/GAPDH
1.5
1.0
0.5
0.0
***

Figure S4.

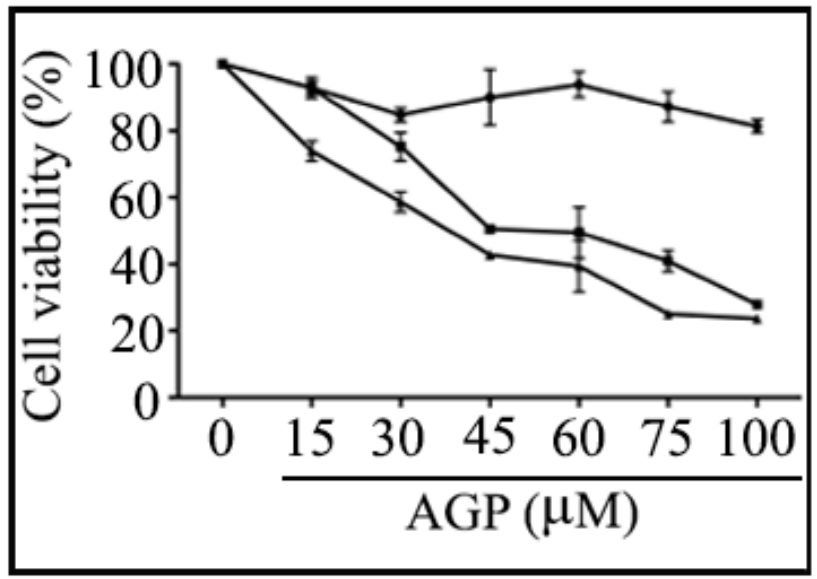
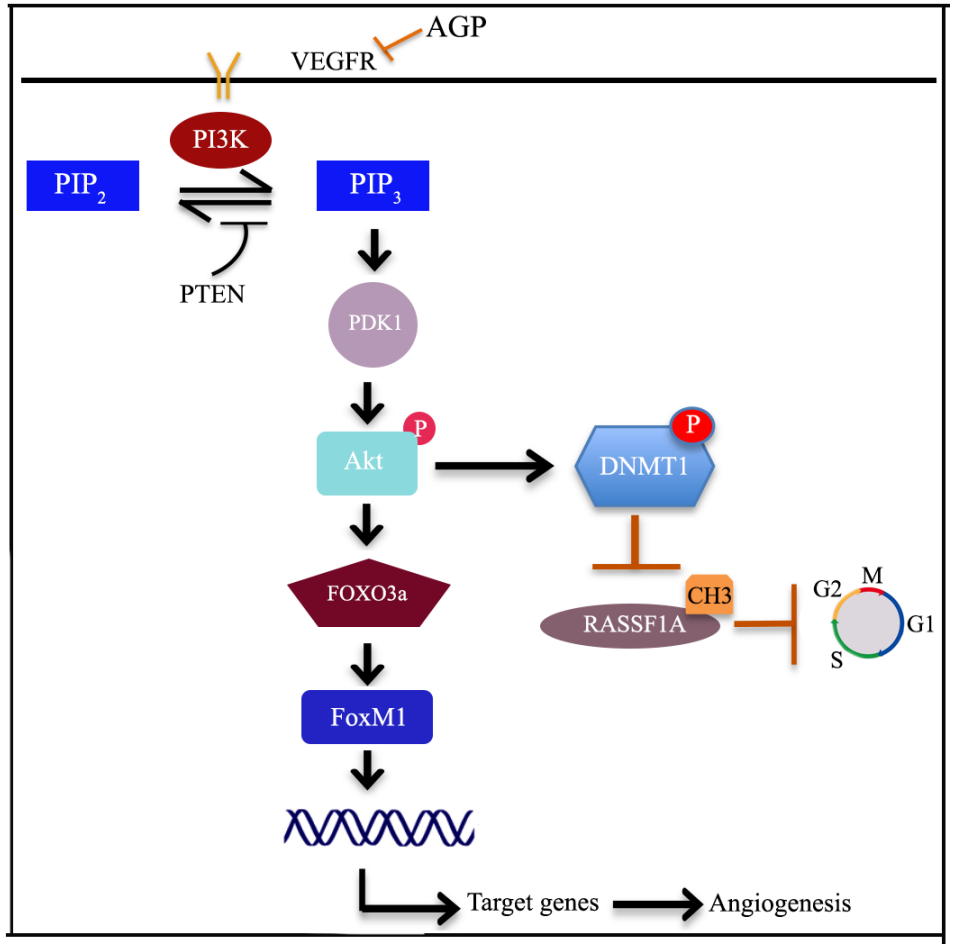


Figure S5.


AGP
VEGFR
PI3K
PIP2
PIP3
PTEN
PDK1
P
Akt
P
DNMT1
FOXO3a
CH3
RASSF1A
G2
M
G1
S
FoxM1
Target genes
Angiogenesis

Supplementary Figure 1. Andrographolide induced tumor suppressor gene expression. Colon cancer cells were treated with AGP IC_{50} for 48h and relative gene expression for (A) HLTF, (B) RUNx, (C) MLH, (D) HPP1 and (E) APC was determined by qRT-PCR.

Supplementary Figure 2. Andrographolide induced RASSF1A influences hippo pathway signaling. T84 and Colo 205 cells were transfected with RASSF1A siRNA and then treated with AGP for 48h. Cells were evaluated for mRNA expression by qRT-PCR for LATS (*upper histogram*) and for MST1 (*lower histogram*) (***P<0.001).

Supplementary Figure 3. ER stress regulates VEGFR expression in andrographalide treated cells. **A.** T84 were treated with AGP in the presence or absence of the ER stress chemical inhibitor 4PBA for 48h and cell lysates were evaluated for protein expression of VEGFR1 and VEGFR2 by western blot. Histogram representing quantification of VEGFR1 and VEGFR2 by scanning densitometry are presented. B. T84 cells were transfected with plasmid for overexpression of IRE-1 and then treated with AGP. Cell lysates were analyzed by western blot for VEGFR1 and VEGFR2 and quantified by densitometry. Expression is normalized against GAPDH expression. (*P<0.05, **P<0.01, ***P<0.001).

Supplementary Figure 4. Andrographolide suppresses cell proliferation in endothelial cells. Endothelial cells were treated with AGP for 24, 48, and 72 h and cell viability was quantified by MTT assay.

Supplementary Figure 5. Schematic representation for RASSF1A regulation in cells treated with andrographolide. AGP treatment results in reduced levels of angiogenesis signaling events including phospho-Akt. Lower levels of activated Akt results in less stable DNMT which then results in reduced methylation of the RASSF1A gene allowing for increased RASSF1A expression. AGP also significantly increases PTEN expression which blocks the conversion of PIP_2 to PIP_3 and consequently results in reduced activation of Akt downstream.