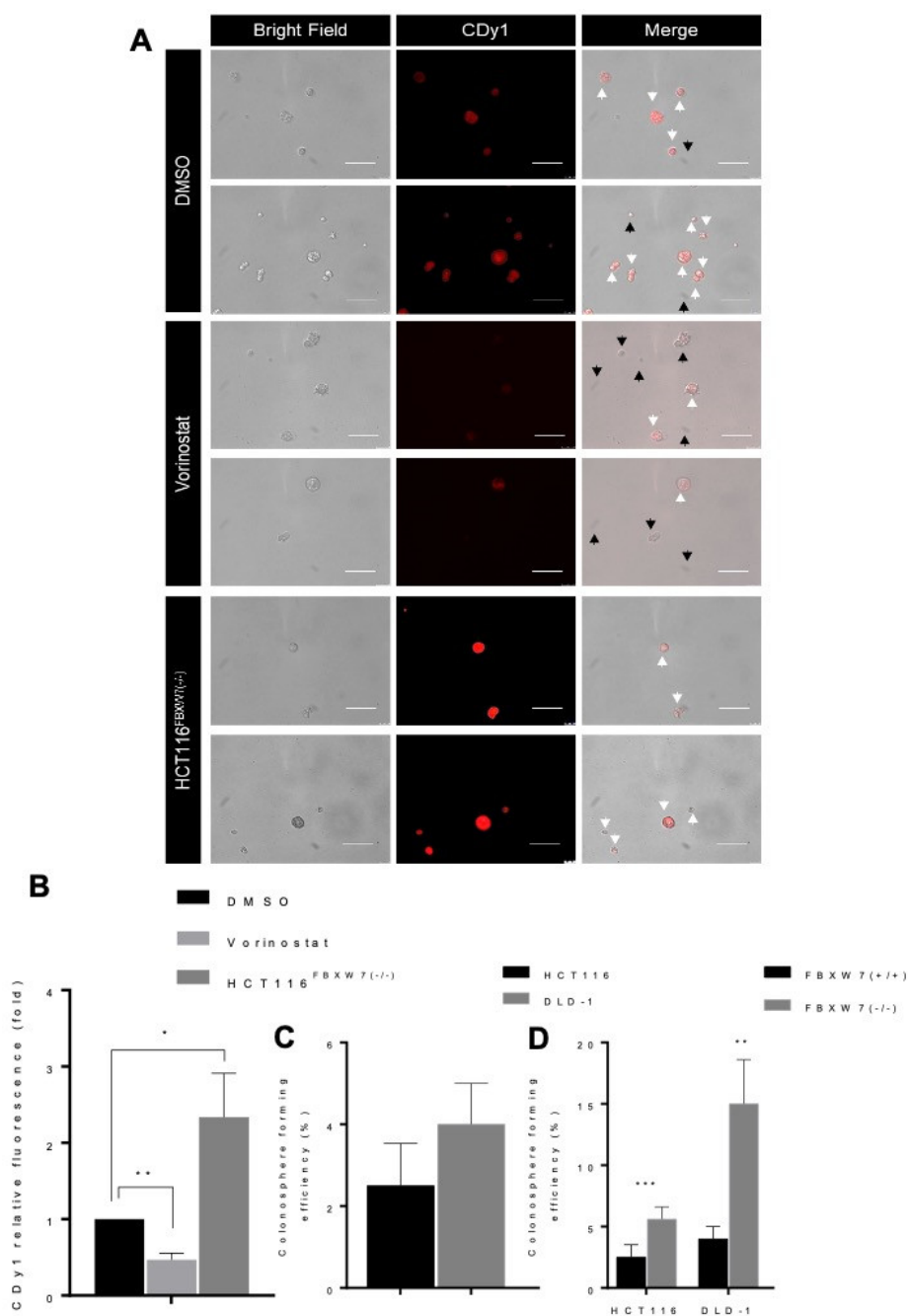


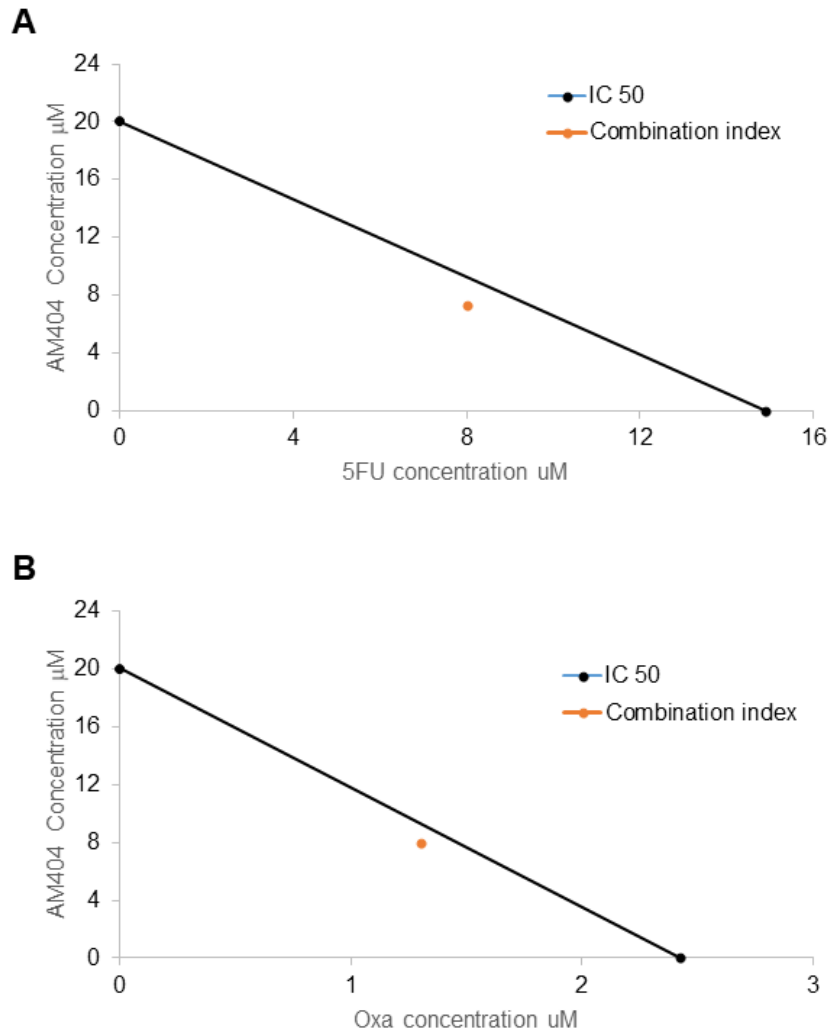
# Supplementary material: Repurposing Antibacterial AM404 As a Potential Anticancer Drug for Targeting Colorectal Cancer Stem-Like Cells

Mehreen Ahmed, Nicholas Jinks, Roya Babaei-Jadidi, Hossein Kashfi, Marcos Castellanos-Urbe, Sean T. May, Abhik Mukherjee and Abdolrahman S. Nateri

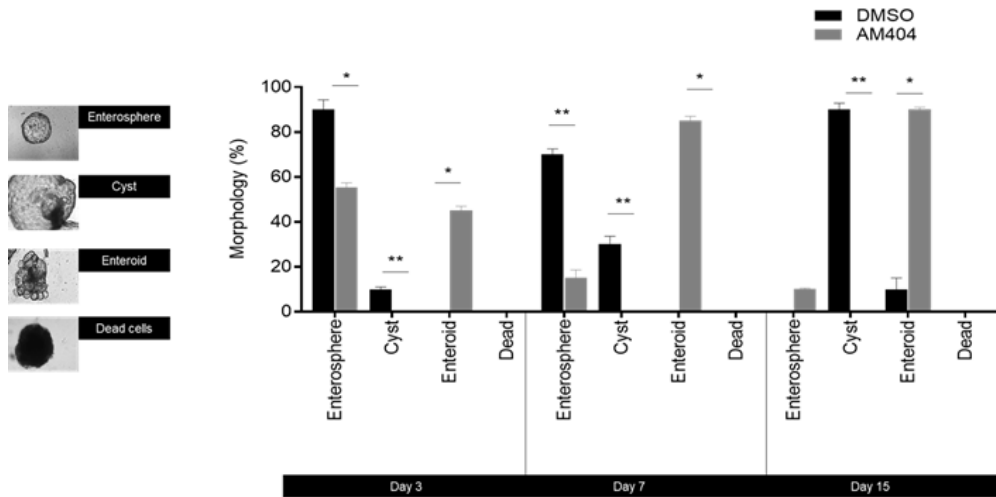


**Figure 1.** A) Vorinostat (SAHA) induces differentiation and FBXW7 deletion indicates attainment of more stemness activity in HCT116 derived colonospheres. CDy1 dye marks the stem-like activity of formed colonospheres. Black arrows show the colonospheres that were not stained with CDy1, indicating loss of their stem-like activity. Whereas, white arrows show the colonospheres with stem-like characteristics. Scale bars: 25  $\mu$ m. B) CDy1 confers reduced fluorescence intensity in Vorinostat

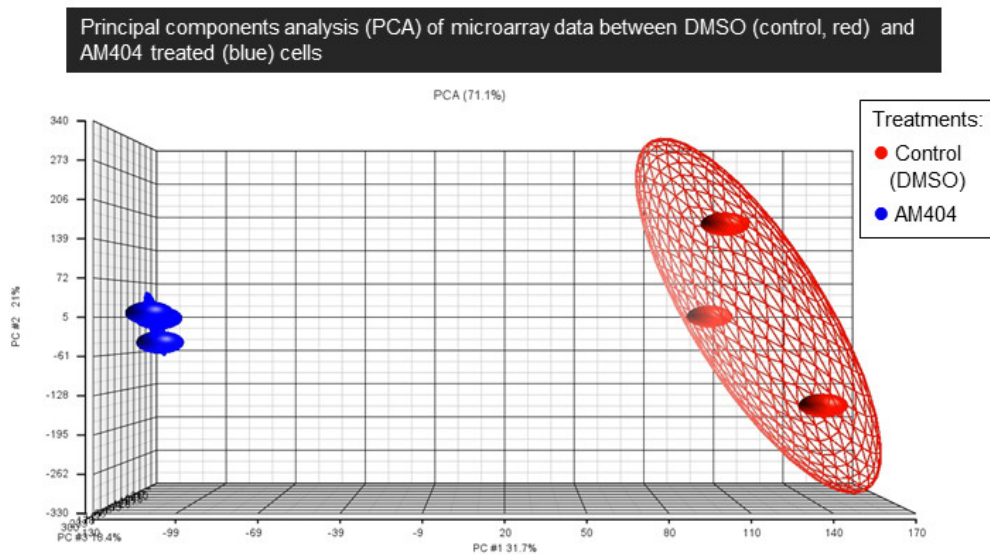
treated colonospheres, possibly due to the drug induced differentiation and greater intensity in HCT116-FBXW7(-/-) cell derived colonospheres indicating higher tumorigenic properties as observed by CDy1 staining. Bars represent mean  $\pm$  SD,  $n = 15$ ; \*  $p < 0.05$ , \*\*  $p < 0.01$ , using Student's  $t$  test. **C)** Quantification of the colonosphere-forming ability of the HCT116 and DLD-1 cell lines. **D)** Quantification of the colonosphere-forming ability of the HCT116 cells  $\pm$  FBXW7 (left) and DLD1 cells  $\pm$  FBXW7 (right). Bars represent the mean of three different experiments  $\pm$  SEM; \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , using Student's  $t$  test.



**Figure S2.** Isobologram analysis at the combination effect. **(A)** Combination therapy of AM404 and 5FU with combination index of 0.89. **(B)** Combination therapy of AM404 and Oxa with combination index of 0.94.



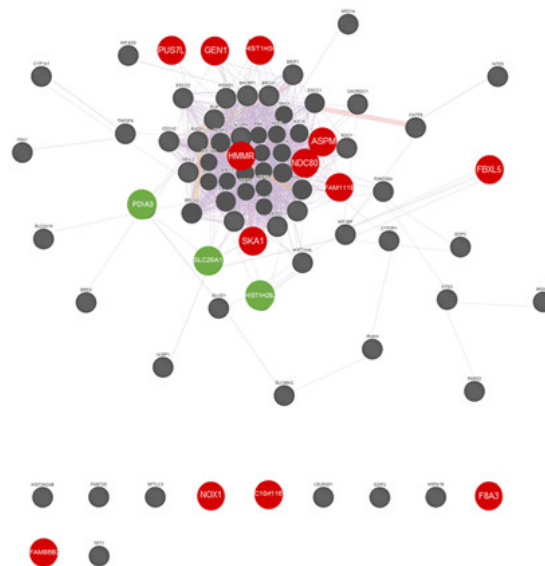
**Figure S3.** AM404 treatment presents enteroid-like structures and induces differentiation in organoids. Percentage of enterosphere, cyst, enteroids and dead cells at day 3, 7 and 15 days of treatment. Bars represent the mean of three different experiments  $\pm$  SEM; \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , using Student's t test.



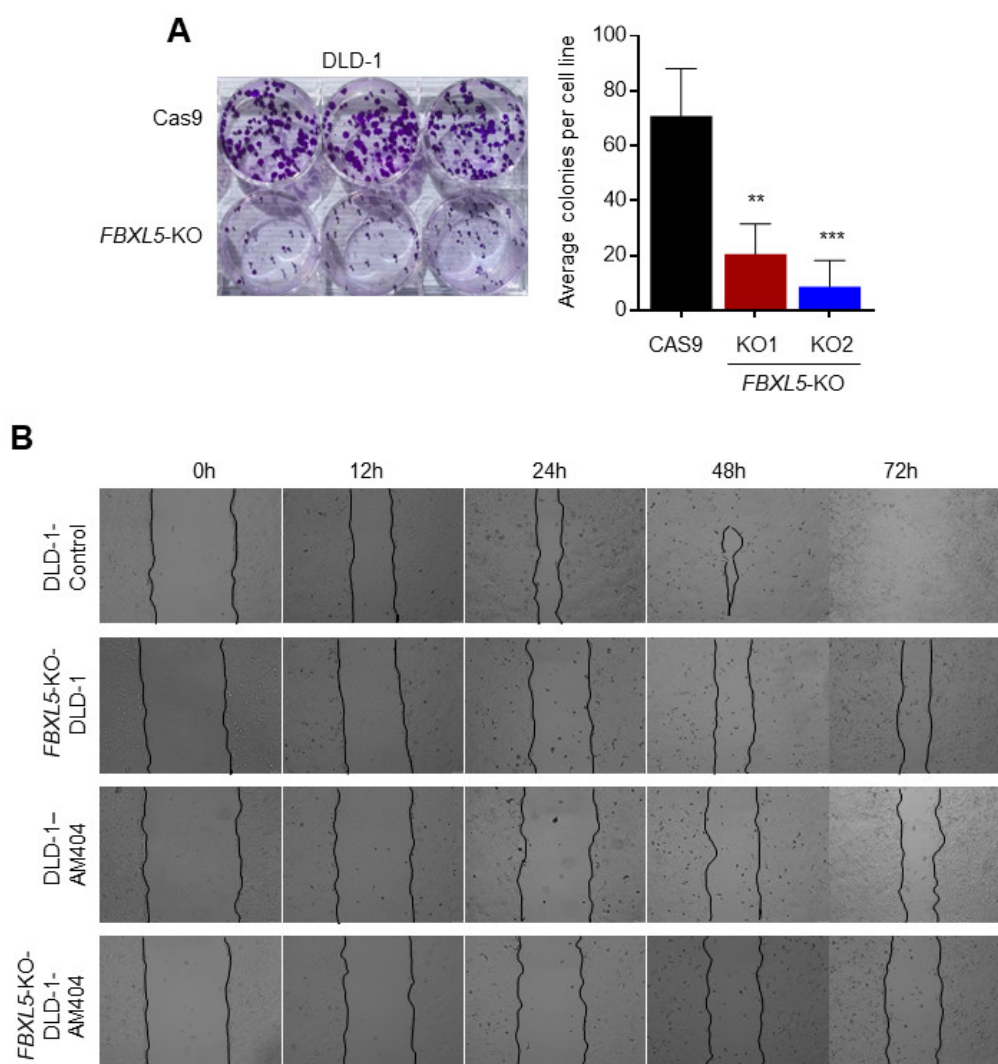
**Figure S4.** Principal components analysis (PCA) of microarray data between DMSO (control, red) and AM404 treated (blue) cells transcriptomes.

**A****Gene ontology (GO) biological pathways**

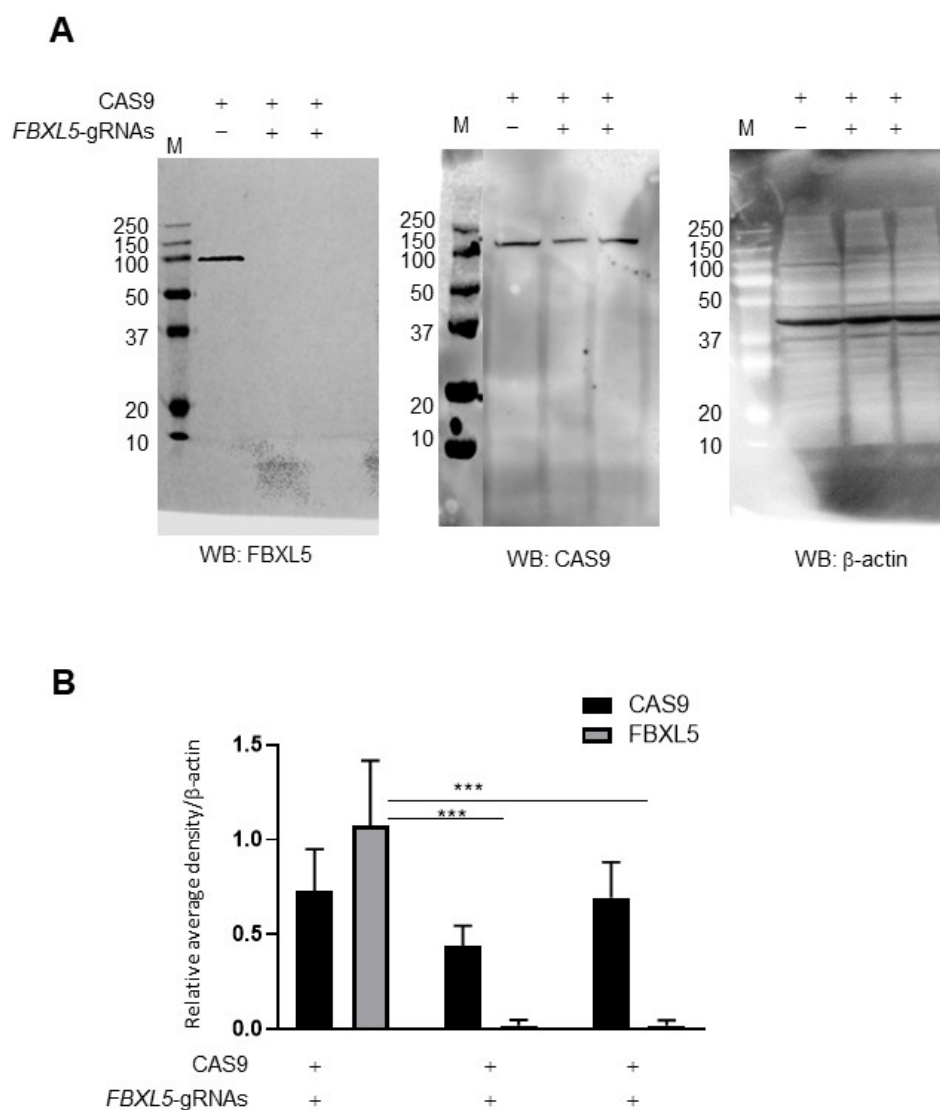
Negative regulation of transcription from RNA polymerase II promoter (GO: 0000122)
DNA damage induced protein phosphorylation (GO: 0006975)
DNA repair (GO: 0006281)
Protein ubiquitination involved in ubiquitin-dependent protein catabolic process (GO: 0042787)
Reduction of transcription involved in G2/M transition of mitotic cell cycle (GO: 0000117)
Negative regulation of G2/S transition of mitotic cell cycle by negative regulation of transcription from RNA polymerase II
Ciliary basal body plasma membrane docking (GO: 0097711)
Histone H4 acetylation involved in response to DNA damage stimulus (GO: 20000776)
Regulation of transcription involved in G1/S transition of mitotic cell cycle (GO: 0000083)
Telomere maintenance in response to DNA damage (GO: 0043247)

**B****Genetic and physical interaction network of differentially expressed genes by AM404**

**Figure S5.** (A) Gene Ontology (GO) biological pathways for the 323 genes (mean  $\geq 2$  fold change) that had significantly altered their expression by AM404 treatment. Results were obtained using DAVID Bioinformatics Resources 6.8. (B) Interaction network of 75 differentially expressed genes (mean  $\geq 2.5$  fold change) by AM404 treatment. The GeneMANIA plug-in for Cytoscape was used to generate an interaction network based on previously documented interactions. Red and green nodes mark down- and upregulated 16 genes amongst other genes. .



**Figure S6.** (A) Colony forming efficiency assays of DLD-Cas9 (control) and DLD-Cas9:FBXL5-gRNAs (CRISPR-knockout) cell lines (KO1 and KO2). Bars are expressed as mean  $\pm$  S.E.M. ( $n = 3$ ). \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ . (B) Representative images of wound healing assay of DLD-Cas9 (control) and DLD-Cas9:FBXL5-gRNAs (CRISPR-knockout) cell lines treated with DMSO (control) and AM404 were taken at 0, 12, 24, 48 and 72 hours with phase-contrast microscope for quantification in triplicate samples as shown in Figure. 6E.



**Figure S7. (A)** Whole western blot images of Figure 6B. **(B)** FBXL5 and Cas9 intensity ratio versus  $\beta$ -actin of each band showed in Figure 6B. Bars represent the mean of three different experiments  $\pm$  SEM; \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , using Student's  $t$  test.

**Table S1.** List of primers and their sequences used for qRT-PCR assay.

CD44	Forward	AGAAGGTGTGGGCAGAAGAA
	Reverse	AAATGCACCATTTCTGAGA
NANOG	Forward	GCAGGCAACTCACTTTATCCCA
	Reverse	GGCAGCTTTAAGACTTTTCTGG
LGR5	Forward	GACAACAGCAGTATGGACG
	Reverse	GCATTACAAGTAAGTGCCAG
OCT4	Forward	AAGCTGCGGCCCTTGCTGCAG
	Reverse	AGCCCAAGCTGCTGGGCGATG
Krt20	Forward	TGAAGAGCTGCGAAGTCAGAT
	Reverse	TCCCTCTCAGTCTCATACTTCAGTC
CDX2	Forward	AGCCAAGTGAAAACCAGGAC
	Reverse	CCAGATTTTAACCTGCCTCTCA
C-Jun	Forward	ACCCCAAGATCCTGAAACAG
	Reverse	ATCAGGCGCTCCAGCTCG
C-Myc	Forward	GGAGACACCGCCACCA
	Reverse	GCGCTGCGTAGTTGTGCTG

CCND1	Forward	GCTGGAGGTCTGCGAGGA
	Reverse	CATCTTAGAGGCCACGAACA
CTNNB1	Forward	TATTACGACAGACTGCC
	Reverse	TGAAGTCCTAAAGCTTG
CXCR4	Forward	TTCTACCCCAATGACTTGTG
	Reverse	ATGTAGTAAGGCAGCCAACA
BMI-1	Forward	CCAGGGCTTTTCAAAAATGA
	Reverse	CCGATCCAATCTGTTCTGGT
N-Cadherin	Forward	GACAATGCCCCTCAAGTGT
	Reverse	CCATTAAGCCGAGTGATGGT
Vimentin	Forward	GAGAACTTTGCCGTTGAAGC
	Reverse	TCCAGCAGCTTCCTGTAGGT
FBXW7	Forward	GAGACTTCATCTCCTTGCTTCCTAAA
	Reverse	CGCTTGACAGCAGGTCTTTG
B-actin	Forward	GCGCGGCTACAGCTTCA
	Reverse	CTTAATGTCACGCACGATTCC

Table S2. List of drugs selected during different stages of the screening.

Primary Screening (50 drugs)	Re-screening			Common Compounds (11 Drugs)
	HCT116 (16 Drugs)	DLD-1 (11 Drugs)	SW480 (11 Drugs)	
3,5,3'-Triiodothyronine				
5-Nonyloxytryptamine HCl				
Acitretin				
AM 404				
Benidipine HCl				
Bifemelane HCl				
Calcitriol				
CGS 15943				
Chloroxine				
(+)-cis-Diltiazem HCl	5-Nonyloxytryptamine HCl	AM 404		
Clofazimine	AM 404	Daunorubicin		
Dactinomycin	Epirubicin HCl	HCl	AM 404	AM 404
Daunorubicin HCl	Dactinomycin	Doxorubicin	Daunorubicin HCl	Daunorubicin HCl
Doxorubicin HCl	Daunorubicin HCl	HCl	Doxorubicin HCl	Epirubicin HCl
Ebselen	Doxorubicin HCl	Epirubicin HCl	Epirubicin HCl	Hexachlorophene
Efavirenz	Hexachlorophene	Hexachlorophene	Hexachlorophene	Honokiol
Epigallocatechin gallate	Honokiol	ne	Honokiol	Honokiol
Epirubicin HCl	Idarubicin HCl	Honokiol	Idarubicin HCl	Idarubicin HCl
Fenoldopam mesylate	MK 886	Honokiol	Itraconazole	Itraconazole
Galanthamine HBr	Mitoxantrone HCl	Idarubicin HCl	MK 886	MK 886
Hexachlorophene	Oligomycin A	Itraconazole	Rifabutin	Rifabutin
Honokiol	Rifabutin	MK 886	Rifabutin	Rifapentine
Icariin	Rifampicin	Rifabutin	Rifapentine	
Idarubicin HCl	Rifapentine	Rifapentine		
Idebenone	Zafirlukast			
Lacidipine				
Methyldopa				
MK 886				
Mitoxantrone HCl				
Nicotinamide				
Nisoldipine				
Oligomycin A				
Oxytetracycline HCl				
PD 81723				
Pefloxacin mesylate				

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Phylloquinone  
 Pyrazinamide  
 Raloxifene HCl  
 Rifabutin  
 Rifampicin  
 Rifapentine  
 Rolitetracycline  
 Symmetrel  
 Taxifolin-(+/-)  
 Tolterodine tartrate  
 Trans-Retinoic acid  
 Tremulacin  
 Trimebutine maleate  
 Vinorelbine tartrate  
 Zafirlukast

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**Table S3.** List of differentially expressed genes (mean  $\geq 2$  fold change) in AM404 treated colonospheres.

Gene Symbol	Fold-Change
SLC20A1	3.40922
SNORA31	3.23582
SNORA28	3.13565
MIR3115	3.11244
HIST1H2BJ	3.09949
MIR637	2.80158
SPDYA	2.77065
TNFSF9	2.74608
CHORDC1	2.71431
SNORD59B	2.68054
TPT1	2.67106
OTTHUMG00000165727	2.57599
BRD2	2.5751
GLUD1	2.54969
MIR3662	2.54056
HSPA1B	2.53474
HIST2H2BE	2.40498
BHLHE40	2.40393
FDPS	2.38535
HSPA1B	2.36471
SNORA10	2.35812
HSPA1B	2.35085
MPZL3	2.3379
IL8	2.32806
HSPA1A	2.32556
SNORD51	2.32159
CTGF	2.30568
HSPA1B	2.29133
KLF6	2.27076
HMGCS1	2.26364
LOC100129785	2.26142
TSC22D3	2.26104
LOC100506766	2.25716
OTTHUMG00000171211	2.24005
PPP1R2	2.22361
HSPA1A	2.19472
LOC554174	2.1828



ZBTB40-IT1	2.16383
TMEM55B	2.14921
MAFG	2.14053
PRPF39	2.12726
PER1	2.11071
INSIG1	2.09923
ZBTB34	2.08724
ECM1	2.067
MIR3143	2.05838
ADM	2.05594
GRHL1	2.05407
JUN	2.05354
MVD	2.05347
CLK1	2.05199
HIST1H4B	2.04491
SNORD58A	2.03921
RNF39	2.03291
YOD1	2.02659
ARL14	2.02412
NR4A2	2.02206
CDKN1C	2.01733
LRCH4	2.0168
RPLP1	2.01204
OTTHUMG00000154962	2.00453
FANCD2	-2.00047
ACADM	-2.00231
OMA1	-2.00622
IRAK1BP1	-2.00897
FASTKD3	-2.01007
CCP110	-2.01069
NCAPG	-2.01211
LAMP3	-2.01324
SEPHS2	-2.01373
MLF1IP	-2.01385
CCDC71	-2.01528
KIF20A	-2.01628
HENMT1	-2.01765
SNORD72	-2.01799
H2AFY2	-2.01904
ADAT1	-2.01967
SULT1C2	-2.02463
TIRAP	-2.02682
C12orf66	-2.0271
MINA	-2.02816
UBLCP1	-2.0351
ZNF594	-2.03544
RFC5	-2.03811
DSCC1	-2.03918
UEVLD	-2.03929
KLK10	-2.03937
CEP19	-2.04253
ID3	-2.04304
DHODH	-2.04324
FECH	-2.04569
SELRC1	-2.0464
YEATS4	-2.04749

TTC26	-2.04789
SFXN2	-2.05314
TRIM32	-2.0533
DLAT	-2.05431
FAM114A2	-2.05534
DSTNP2	-2.05573
GPR1	-2.0561
FJX1	-2.05917
BUB1	-2.06027
LOC100129502	-2.06418
SKAP1	-2.06454
GSG2	-2.06499
KIAA0586	-2.06713
USP13	-2.06889
POLI	-2.06894
KIAA1107	-2.07024
DCLRE1A	-2.07025
HCG15	-2.07153
ERAL1	-2.07185
PLK4	-2.07297
CABYR	-2.07395
AASDHPPT	-2.07559
LOC100507449	-2.07571
KLHL4	-2.07778
CCDC113	-2.07969
RBL1	-2.08169
ASF1B	-2.0852
SKA3	-2.10319
TICRR	-2.10426
WDR92	-2.1047
FXYD3	-2.10478
SYK	-2.10558
TBCK	-2.10995
RPGRIPL	-2.11011
HAUS1	-2.11344
TRMT61B	-2.11432
CENPK	-2.11466
DNAJC11	-2.12161
TRMT1L	-2.1234
CASP6	-2.12733
SNORD111	-2.13249
OSGEPL1	-2.13254
TMEM9B-AS1	-2.13855
SNORD100	-2.1433
CDK1	-2.14947
FAM173B	-2.15377
THEM4	-2.15445
ASCL2	-2.15581
SIRT5	-2.15946
ZMYND11	-2.16142
MBLAC2	-2.16328
IGFL4	-2.16683
MRPL15	-2.16767
KLHDC1	-2.16798
FARSB	-2.16817
NMRK1	-2.17103

DYNC2LI1	-2.1717
PIGV	-2.17174
ZNF280B	-2.18593
SLC25A10	-2.19245
C2orf43	-2.19248
PRSS35	-2.20022
ERCC4	-2.20079
MOCOS	-2.20219
CPT2	-2.204
PNPO	-2.20535
SLC16A13	-2.21061
ANKEF1	-2.21115
GTF2H3	-2.21185
TRIP11	-2.21495
KIF23	-2.2182
TST	-2.22501
ZFP69	-2.22759
COX10-AS1	-2.22822
PLK1	-2.22996
C6orf70	-2.23309
C5orf63	-2.23424
ZNF266	-2.23441
RRS1	-2.2374
C4orf32	-2.2399
KIAA1524	-2.24724
DET1	-2.24786
RMI1	-2.25043
CDX2	-2.25241
SCRN3	-2.25434
PLA2G7	-2.25701
PRMT3	-2.25733
LOC100506963	-2.25794
SBSPON	-2.26796
SNORD9	-2.26939
C6orf203	-2.26952
POLQ	-2.27153
ZNF554	-2.27756
PPIL1	-2.28812
KRCC1	-2.28819
MRM1	-2.28824
NARG2	-2.29049
LCN2	-2.29312
THAP10	-2.29872
CETN3	-2.29988
MCM10	-2.30303
HJURP	-2.30314
OBSCN	-2.30829
HSD17B1	-2.30857
LOC202181	-2.30882
PMS2	-2.31583
SRBD1	-2.31753
KIF11	-2.31855
XRCC2	-2.31985
RBM45	-2.32138
ZBED3	-2.32373
CCDC125	-2.32511

SLC9A8	-2.32792
TMEM52B	-2.3406
CKAP2L	-2.34853
GTF2IRD2	-2.35277
LOC100507516	-2.35412
KIAA1377	-2.35468
FAM83D	-2.35958
MARS2	-2.36325
NCOA5	-2.36498
TSEN2	-2.36718
KIF20B	-2.37419
FKTN	-2.3758
SDAD1	-2.3768
SNORD126	-2.37717
STRADA	-2.38501
IFT80	-2.38685
FAM86DP	-2.38812
LYRM7	-2.38851
CHAF1B	-2.39505
OTTHUMG00000059903	-2.39824
PRIM1	-2.39825
PSMD5-AS1	-2.40089
SNORD69	-2.40203
CSRP2BP	-2.40465
BRCA2	-2.40876
CFTR	-2.41427
GEMIN4	-2.41471
CENPF	-2.42261
ZFP90	-2.42381
RWDD2B	-2.4249
MGME1	-2.42706
SYCE2	-2.44092
C12orf4	-2.44353
OTTHUMG00000037424	-2.44592
FLJ14186	-2.44863
ZNF252P	-2.4538
ACER2	-2.45416
CDC25C	-2.45885
PTCD2	-2.46617
ABO	-2.46676
GINS1	-2.46788
ZNF23	-2.47319
CES2	-2.48362
CLUAP1	-2.48464
USP40	-2.49191
RNF170	-2.49736
WDR76	-2.49784
CYP2R1	-2.5001
LDLRAD1	-2.51564
SLC2A10	-2.52026
SHCBP1	-2.5219
MGAT2	-2.53704
CDC7	-2.57597
NEIL3	-2.57773
BRCA1	-2.58799
KIAA1279	-2.58836

FAM72D	-2.60089
FAM86HP	-2.60142
NCAPH	-2.61352
MIF4GD	-2.61989
WDHD1	-2.6237
SGOL1	-2.63519
GOPC	-2.65037
PARS2	-2.65689
BLM	-2.67003
FAM208A	-2.67272
F8A1	-2.68143
SNORD121A	-2.69081
CYP1A1	-2.69622
RAD51	-2.73685
SLC46A3	-2.7375
CHTF8	-2.74581
ESCO2	-2.74641
DZIP3	-2.74818
SPTLC3	-2.76889
IRAK4	-2.77648
DTD2	-2.78523
SNORD90	-2.79634
OTTHUMG00000163993	-2.80351
SNORD63	-2.81223
DTL	-2.81506
CDCA2	-2.85031
HIST1H4L	-2.85568
LINC00858	-2.87467
HIST2H2AB	-2.88008
DEPDC1	-2.94581
INTS5	-2.94694
ULBP1	-2.99393
F8A3	-2.99583
BRIP1	-2.99929
FBXL5	-3.0001
FAM111B	-3.06187
HMMR	-3.06684
NOX1	-3.09866
PDIA3	-3.20377
FAM86B2	-3.22522
F8A3	-3.26485
MIR1184-1	-3.26485
C1orf116	-3.41795
ASPM	-3.44786
SKA1	-3.45407
NDC80	-3.46322
PUS7L	-3.50814
SNORA2A	-3.6422
GEN1	-3.64797
HIST1H3I	-3.89911

