**Supplementary Data**

**Materials and Methods**

***Gene-expression microarray analysis***

Total RNA was extracted from fresh cell pellets collected from human CRC cell lines using the RNeasy Mini Kit (Qiagen, Valencia, CA) and amplified and labeled using the Illumina’s Total Prep RNA Amplification kit (Illumina, San Diego, CA). RNA integrity values were assessed using Labchip GX (Caliper LifeSciences, Hokinton, MA). Labeled cRNA was hybridized overnight, followed by washing and scanning the Human HT12-V4 arrays (IIlumina) following the manufacturer's protocols. Illumina's GenomeStudio v2011.1 software was used to generate and normalize signal intensity values from the scans. The signal intensity values were normalized and rescaled across multiple arrays and chips. The array data were further normalized using GeneSpring GX7.3 software (Agilent Technologies, Santa Clara, CA). In brief, unsupervised analysis was conducted with following criteria: transcripts must be detected in at least one sample (p<0.01)-PALO. The hierarchical clustering was then performed on the expressed genes (i.e. the transcripts) with a detection p-value less than 0.01 in at least one sample, yielding 21997 transcripts, while the similarity or dissimilarity of gene expression profiles was measured using Pearson’s correlation. By using this algorithm, samples were segregated into distinct groups based on similarity in expression patterns. Differential gene expression analysis was performed using T-test analysis at FDR 0.05 with Benjamini-Hochberg correction. Supervised analysis was then conducted to identify probes that are differentially expressed by a fold change of 1.5 and difference of 100 in the gene signal intensity compared to the control groups. Ingenuity Pathway Analysis (IPA) (Ingenuity systems Inc., Redwood City, CA) was used to categorize differentially expressed genes into various functional pathways.

**Supplementary Tables:**

**Supplementary Table 1:**

|  |  |
| --- | --- |
| **Primers** | **Sequence** |
| cMyc (foward) | 5’- CGT CTC CAC ACA TCA GCA CAA |
|  (reverse) | 5’- TCT TGG CAG CAG GAT AGT CCT T |
| FBXW7 (forward) | 5’- AAA GAG TTG TTA GCG GTT CTC G  |
|  (reverse) | 5’- CCA CAT GGA TAC CAT CAA ACT G |
| CDK4 (forward) | 5’- ATG TTG TCC GGC TGA TGG A |
|  (reverse) | 5’- CAC CAG GGT TAC CTT GAT CTC C |
| CDK6 (forward) | 5’- GCC TAT GGG AAG GTG TTC AA |
|  (reverse) | 5’- CAC TCC AGG CTC TGG AAC TT |
| Cyclin D1 (forward) | 5’- AAT GAC CCC GCA CGA TTT C |
|  (reverse) | 5’- TCA GGT TCA GGC CTT GCA C  |
| Cyclin E1 (forward) | 5’- ATC CTC CAA AGT TGC ACC AG |
|  (reverse) | 5’- AGG GGA CTT AAA CGC CAC TT |
| GAPDH (forward) | 5’- ACC CAG AAG ACT GTG GAT GG |
|  (reverse) | 5’- CAG TGA GCT TCC CGT TCA G |

**Supplementary Table 2:**

|  |  |
| --- | --- |
| **Antibody** | **Manufacturer** |
| p53 | Santa Cruz Biotechnology |
| c-Myc | Chemicon |
| CDK4 | BD Biosciences |
| CDK6 | Cell Signaling |
| Cyclin D1 | Santa Cruz Biotechnology |
| Cyclin E1 | Cell Signaling |
| Bax | Cell Signaling |
| Bcl-2 | Santa Cruz Biotechnology |
| Bcl-xl | Santa Cruz Biotechnology |
| PARP1 | Santa Cruz Biotechnology |
| FBXW7 | Abcam |
| Beta-actin | Sigma |

**Supplementary Figure Legends:**

**Suppl. Figure 1: Inhibitory concentration 50 of curcumin and AKBA** (A) Four CRC cell lines (HCT116, RKO, SW480 and SW620) were treated with various concentrations of curcumin and AKBA, and cell viability was determined using the MTT assay. (B) Inhibitory concentration 50 (IC50) of curcumin and AKBA were determined across four cell lines and the ratio between AKBA and curcumin was calculated.

**Suppl. Figure 2: Curcumin and AKBA exert cytotoxicity and suppress colony formation on CRC cell lines** (A) Cytotoxicity of different doses of curcumin and/or AKBA on RKO, Caco2, and HT29 cell lines. Insert: Synergy between curcumin and AKBA was calculated by use of the Combined Index (CI). (B) Colony formation assay of CRC cell lines treated with various concentrations of curcumin and/or AKBA

**Suppl. Figure 3: Curcumin and AKBA inhibit cellular proliferation in 3D cultures.** 3D cultures were generated using HCT116 cells followed by curcumin (10µM) and/or AKBA (30µM) treatment. Diameter of 3D cultures was evaluated on days 0, 3, 7, 10.

**Suppl. Figure 4: The effects of curcumin and AKBA on miR-34a, miR-27a, miR-16 and miR-145 expression in CRC cell lines.** (A) Relative expression of miR-34a and miR-27a in RKO, HT29 and Caco2 cell lines treated with curcumin and/or AKBA (B) Relative expression of miR-16 and miR-145 in HCT116, HCT116p53-/- and SW480 cell lines treated with curcumin and/or AKBA. All data normalized to RNU6B.

**Suppl. Figure 5: Curcumin and AKBA modulate genes involved in cell cycle regulation** Comparative gene-expression analysis of FBXW7, cMyc, CDK4, CDK6, Cyclin E1 and Cyclin D1 assessed by qRT-PCR. All expression was normalized to GAPDH.

**Suppl. Figure 6: The effects of miR-34a over-expression on cell function colorectal cancer cells** (A) qPCR analysis of miR-34a expression after pre-miR-34a transfection in HCT116, HCT116p53-/-, and SW480 cell lines. (B) The effects of miR-34a over-expression on cell apoptosis (B), the cell cycle (C), cell colony formation (D), and cell proliferation (E).

**Suppl. Figure 7: The effects of miR-27a knockdown on cell function in colorectal cancer cells** (A) qPCR analysis of miR-27a expression after anti-miR-27a transfection in HCT116, HCT116p53-/- and SW480 cell lines. The effects of miR-27a knockdown on cell apoptosis (B), the cell cycle (C), cell colony formation (D), and cell proliferation (E).

**Suppl. Figure 8:** Comparative gene-expression analysis of FBXW7, cMyc, CDK4, CDK6, Cyclin E1 and Cyclin D1 from xenograft tumors assessed by qRT-PCR. All expression was normalized to GAPDH.

**Suppl. Table 1:** List of various primers used for various assays in this study

**Suppl. Table 2:** List of various antibodies used for various assays in this study