Supplementary Materials

Titles and Legends for Supplementary Figures

Supplementary Figure 1 (A) HCT-116 cells were continuously treated with 500 nM calcitriol. Untreated and calcitriol-treated cell samples were collected at 0h, 24h, 48h, and 72h. Cell number was determined using a hemacytometer and counting under a microscope. The data was used to calculate cell doubling time using an online doubling time calculator (http://www.doubling-time.com/compute.php). The experiment was repeated three times, data shown are mean values + S.D. (B and C) HCT-116 cells were treated with 500 nM calcitriol for 72 hours. Untreated and calcitriol-treated cells were labeled with BrdU for 30 min, washed and further cultured in normal growth media. At 0h, 4h, 8h, 12h, and 16h post labeling, the cells were stained with FITC-conjugated anti-BrdU antibody and propidium iodide as described in Materials and Methods, followed by flow cytometry analysis. The experiment was repeated three times; representative results of three independent experiments were shown. The percentages of BrdU-labeled cells in each phase of cell cycle were summarized in B, data shown are mean values + S.D. (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

Supplementary Figure 2 (A) HCT-116 cells were treated with 500 nM calcitriol for 48 hours. ChIP assay was performed as described in Materials and Methods, using primers specific for the GDF15 promoter and the indicated antibodies. The experiment was repeated three times. Representative images of three independent experiments were shown. (B) HCT-116 cells were treated with 500 nM calcitriol for 48 hours. Total RNA was isolated and analyzed for *HOXA1 and CCND1* expression by real-time PCR.

The experiments have been repeated three times, data shown are mean values + S.D. (***p<0.001).

Supplementary Figure 3 (A) HCT-116 cells were transfected with 100 nM negative control or JMJD1A specific siRNAs. Knockdown of JMJD1A was confirmed by western blot. Cell growth was determined at various time points after transfection by WST-1 assay. (B) HCT-116 cells were transfected with empty vector or pCEP4-JMJD1A plasmid to express Flag-tagged JMJD1A. This construct does not contain the JMJD1A 3'UTR, thus is not a target of miR-627. At 24h after plasmid transfection, cells were transfected with 100 nM negative control miRNA or miR-627 mimic. JMJD1A overexpression was confirmed by western blot. Cell growth was determined at various time points after miRNA transfection by WST-1 assay. The experiment was repeated three times, data shown are mean values + S.D. (**p<0.01, ***p<0.001). The mean protein levels ± S.D. are: siR-NC (1.0 ± 0), siR-JMJD1A (0.36 ± 0.15).

Supplementary Figure 4 HCT-116 cells stably transfected with pRNAT-CMV3.2/Puro-3'UTR (to overexpress *JMJD1A* 3'UTR sponge and block miR-627) or pRNAT-CMV3.2/Puro-3'UTR-mut (to overexpress JMJD1A 3'UTR sponge with miR-627 binding site mutation) were treated with 500 nM calcitriol for 48h, and western blots were done using the indicated antibodies. Relative protein levels were quantified and shown under the gel. The experiment was repeated three times. The mean protein levels ± S.D. are: left panel, Ctr (1.0 ± 0), Calcitriol (1.01 ± 0.09); right panel, Ctr (1.0 ± 0), Calcitriol (0.14 ± 0.03).

Supplementary Figure 5 A diagram illustrates the mechanism of vitamin D regulation of miR-627 and JMJD1A.

Supplementary Materials and Methods

Drugs and Chemicals. Calcitriol was purchased from Cayman Chemical. Bromodeoxyuridine was purchased from Sigma Aldrich.

Creation of Stable Expression Cell Lines. Stable cell lines expressing miR-627 or negative control miRNA were established by transfection of HCT-116 cells with Block-iT^{TM} Pol II miR expression vectors: pcDNA6.2-GW/EmGFP-miR-627 or pcDNA6.2-GW/EmGFP-nega-control. Colonies were selected in Blasticidin-containing media and cloned with cloning cylinders. Overexpression of miR-627 was determined by real-time PCR analysis. The pRNAT-CMV3.2/Puro-3'UTR or pRNAT-CMV3.2/Puro-3'UTR-mut plasmids were transfected into HCT-116 cells, and cell lines stably expressing the JMJD1A 3'UTR sponge or miR-627 binding site mutated 3'UTR sponge were selected using Puromycin.

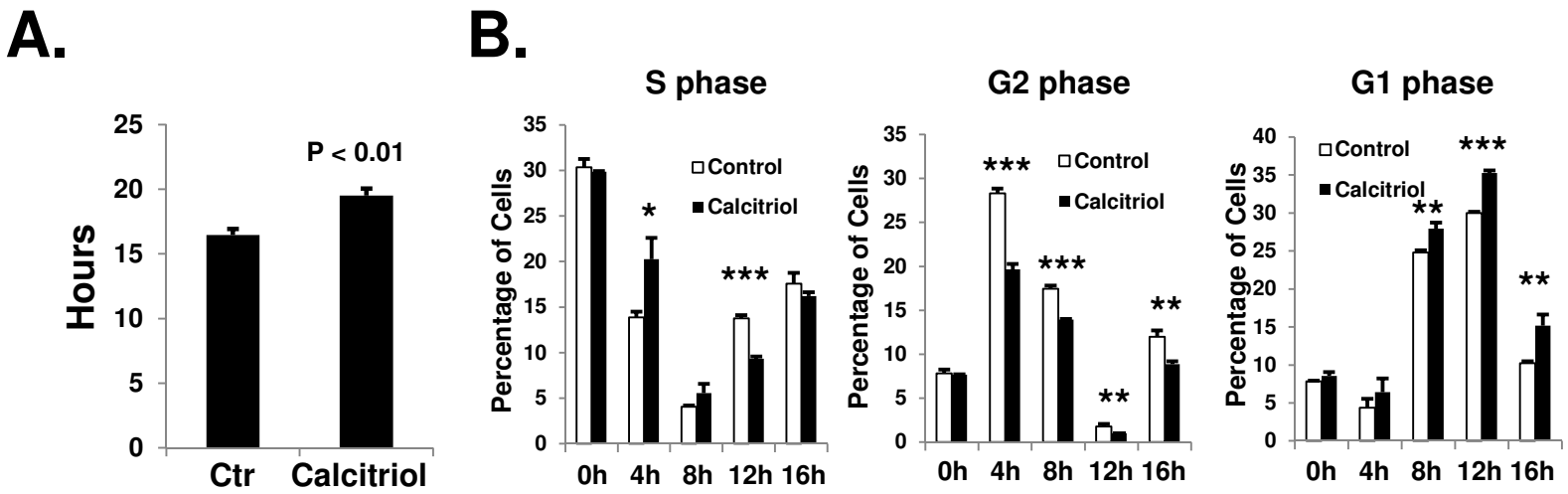
WST-1 Assay. *In vitro* cell proliferation was assessed with the WST-1 reagent (Roche). Briefly, WST-1 reagent was added to cell culture and incubated for 4h at 37℃. The absorbance of the formazan product was measured at 440 nm.

Cell cycle analysis. Cell cycle distribution was analyzed using an Accuri C6 Flow Cytometer. Cells were fixed and stained with propidium iodide using the Cell Cycle Phase Determination Kit (Accuri Cytometers) and analyzed following the manufacturer's

protocol. For BrdU labeling, BrdU was dissolved in PBS and added to culture medium at a final concentration of 10 μM. After incubation for 30 min at 37℃, the cells were rinsed twice with culture media and further cultured in normal growth media for various time. The cells were then washed with PBS and fixed in 70% ice-cold ethanol. The cells were washed and incubated in 2 N HCl for 30 min at room temperature for DNA denaturation. The cells were washed twice in PBS and then stained with FITC-conjugated anti-BrdU antibody (Enzo Life Sciences) and propidium iodide, followed by flow cytometry analysis.

Cell doubling time. The cell numbers were determined by using a hemacytometer and counting under a microscope, at 0h, 24h, 48h, and 72h. The data was used to calculate cell doubling time using an online doubling time calculator (http://www.doubling-time.com/compute.php).

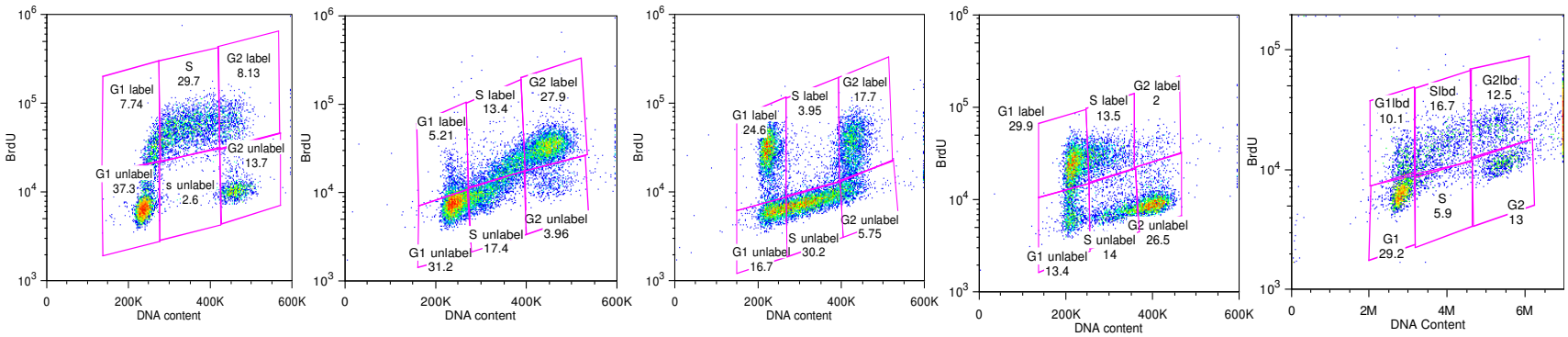
Detection of Apoptosis. For annexin V staining, cells were washed with cold phosphate-buffered saline (PBS) and diluted in annexin-binding buffer at $1x10^6$ cells/ml. 5 μl annexin V fluorescein conjugate (Invitrogen) was added to each 100 μl of cell suspension and cells were incubated at room temperature for 15 minutes. After incubation, cells were analyzed by flow cytometry.



Supplementary Figure 1AB

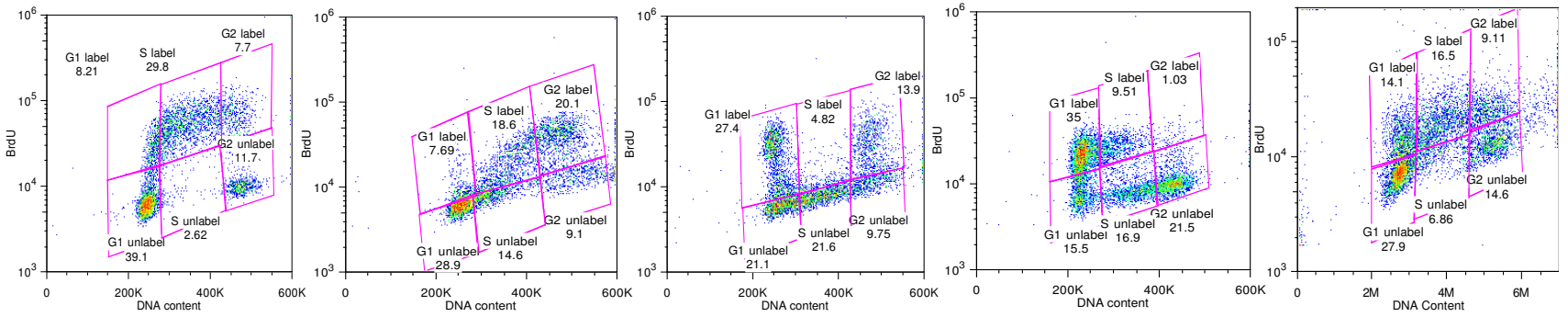
C.

Control



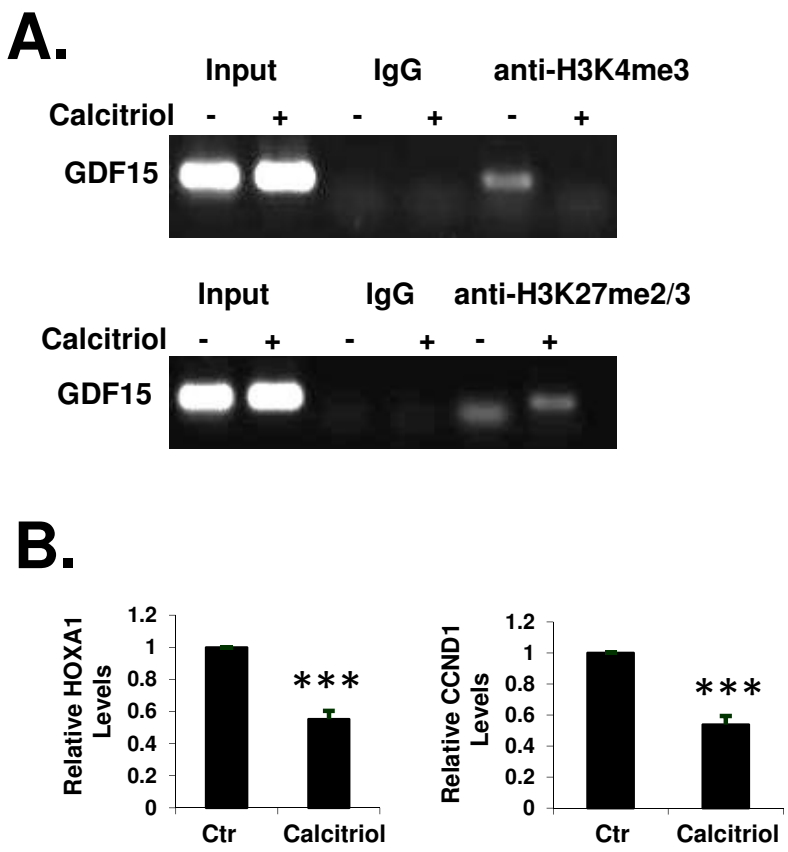
0h 4h 8h 12h 16h

Calcitriol

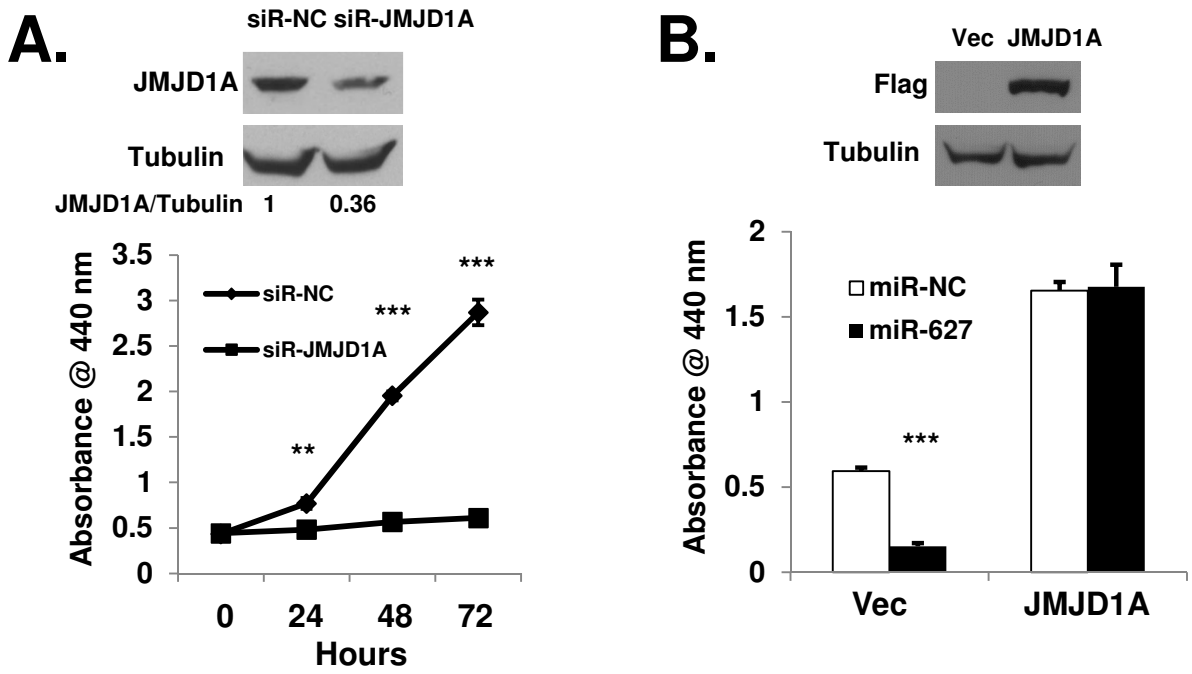


0h 4h 8h 12h 16h

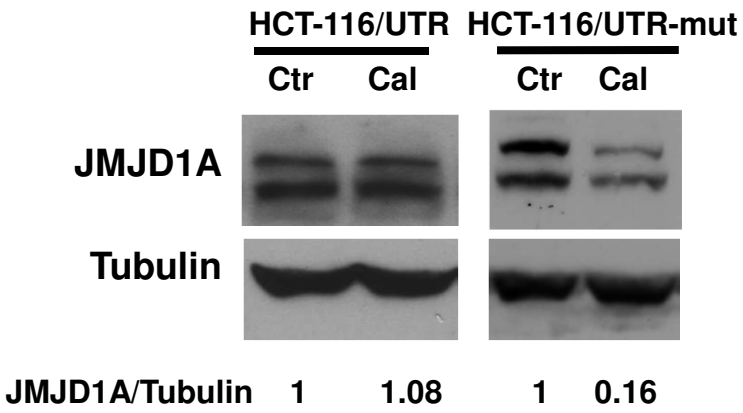
Supplementary Figure 1C



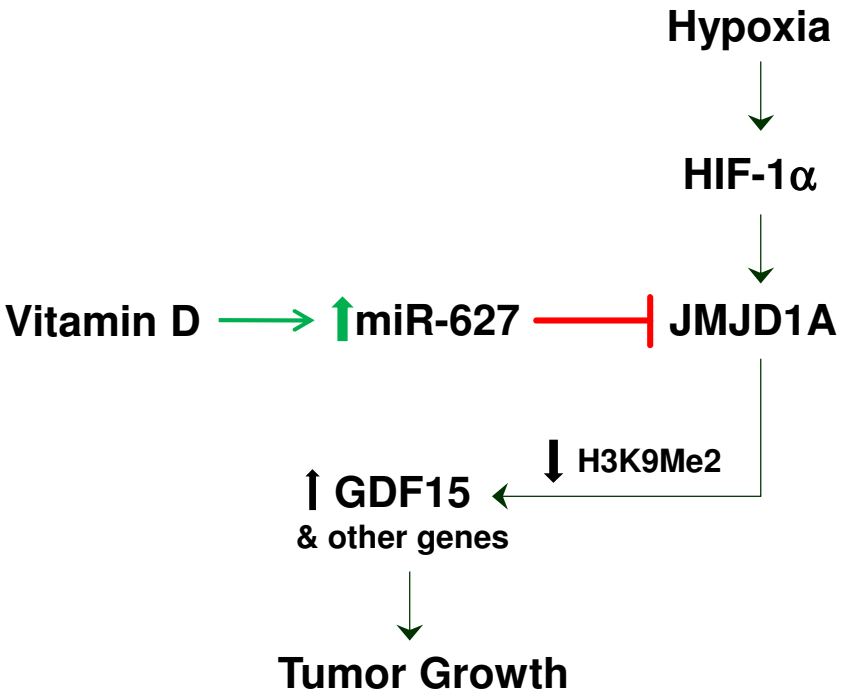
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5

Supplementary Table 1

List of miRNAs that are Increased by Calcitriol

Name of miRNA	Average Fluorescence Ratio of Calcitriol/Control	Standard Deviation
let-7g	1.279	0.073
miR-28	1.342	0.091
miR-346	1.345	0.256
miR-627	**6.458**	**1.239**

The fold of changes in miRNA levels in calcitriol-treated cells comparing to control cells is indicated by the ratio of fluorescence of the two samples. For example, a ratio of 1.279 indicate 27.9% increase of the miRNA in the calcitriol-treated cells.

List of miRNAs that are Decreased by Calcitriol

Name of miRNA	Average Fluorescence Ratio of Calcitriol/Control	Standard Deviation
miR-125a	0.486	0.021
miR-133b	0.585	0.1
miR-139	0.837	0.1
miR-154	0.366	0.061
miR-196a	0.669	0.021
miR-26a	0.715	0.057
miR-296	0.686	0.071
miR-30b	0.47	0.071
miR-33	0.709	0.044
miR-491	0.471	0.093
miR-499	0.553	0.042
miR-517a	0.648	0.017
miR-518a	0.37	0.06
miR-532	0.667	0.148
miR-549	0.526	0.058
miR-553	0.834	0.016
miR-557	0.365	0.004
miR-571	0.62	0.147
miR-579	0.627	0.139
miR-591	0.57	0.055
miR-595	0.613	0.079
miR-597	0.555	0.062
miR-600	0.57	0.155
miR-608	0.456	0.004

miR-637	0.533	0.015
miR-646	0.73	0.158
miR-662	0.8	0.234
miR-9-AS	0.452	0.008

The fold of changes in miRNA levels in calcitriol-treated cells comparing to control cells is indicated by the ratio of fluorescence of the two samples. For example, a ratio of 0.452 indicates 54.8% decrease of the miRNA in the calcitriol-treated cells.

List of miRNAs that are detected but not significantly affected by Calcitriol

Name of miRNA	Average Fluorescence Ratio of Calcitriol/Control	Standard Deviation
miR-30e-5p	0.905	0.089
miR-452-AS	0.906	0.172
miR-30c	0.929	0.101
miR-370	0.964	0.142
miR-663	0.991	0.055
miR-519a	1.018	0.128
miR-299-5p	1.028	0.017
miR-568	1.044	0.254
miR-562	1.052	0.097
miR-106b	1.111	0.162
miR-589	1.149	0.015
miR-619	1.154	0.022

List of miRNAs Not Detected in This Study

hsa_let_7a
hsa_let_7b
hsa_let_7c
hsa_let_7d
hsa_let_7e
hsa_let_7f
hsa_let_7i
hsa_miR_1
hsa_miR_100
hsa_miR_101
hsa_miR_103
hsa_miR_105
hsa_miR_106a
hsa_miR_107
hsa_miR_10a
hsa_miR_10b
hsa_miR_122a
hsa_miR_124a
hsa_miR_125b
hsa_miR_126
hsa_miR_126_AS
hsa_miR_127
hsa_miR_128a
hsa_miR_128b
hsa_miR_129
hsa_miR_130a
hsa_miR_130b
hsa_miR_132
hsa_miR_133a
hsa_miR_134
hsa_miR_135a
hsa_miR_136
hsa_miR_137
hsa_miR_138
hsa_miR_140
hsa_miR_141
hsa_miR_142_3p
hsa_miR_142_5p
hsa_miR_143
hsa_miR_144
hsa_miR_145
hsa_miR_146a
hsa_miR_146b

hsa_miR_147
hsa_miR_148a
hsa_miR_148b
hsa_miR_149
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hsa_miR_154_AS
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hsa_miR_15a
hsa_miR_15b
hsa_miR_16
hsa_miR_17_3p
hsa_miR_17_5p
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hsa_miR_99a
hsa_miR_99b

Supplementary Table 2

ID	TUMOR OR NON TUMOR	GRADE	PATH STAGE	NODES POSITIVE	NODES EXAM	LEFT OR RIGHT COLON
NT1	non tumor colon mucosa					Left Colon
NT2	non tumor colon mucosa					Left Colon
NT3	non tumor colon mucosa					Right colon
NT4	non tumor colon mucosa					Left Colon
NT5	non tumor colon mucosa					Colon NOS
NT6	non tumor colon mucosa					Left Colon
T1	Adenocarcinoma	Moderately differentiated	2	0	25	Left Colon
T10	Adenocarcinoma	Moderately differentiated	4	0	13	Right colon
T11	Adenocarcinoma	Moderately differentiated	4	0	19	Right colon
T12	Adenocarcinoma	Moderately differentiated	3	6	20	Left Colon
T13	Adenocarcinoma	Moderately differentiated	4	2	14	Left Colon
T14	Adenocarcinoma	Poorly differentiated	2	0	11	Left Colon
T15	Adenocarcinoma	Moderately differentiated	4	9	55	Right colon
T16	Adenocarcinoma	Moderately differentiated	4	9	55	Right colon
T17	Adenocarcinoma	Moderately differentiated	2	0	33	Transverse colon
T18	Adenocarcinoma	Moderately differentiated	4	0	22	Left Colon
T2	Adenocarcinoma	Moderately differentiated	2	0	30	Left Colon
T3	Adenocarcinoma	Moderately differentiated	3	10	11	Right colon
T4	Adenocarcinoma	Poorly differentiated	3	3	18	Transverse colon
T5	Adenocarcinoma	Moderately differentiated	4	4	20	Right colon
T6	Adenocarcinoma	Moderately differentiated	4	9	55	Right colon
T7	Adenocarcinoma	Moderately differentiated	3	1	15	Right colon
T8	Adenocarcinoma	Moderately differentiated	4	0	14	Left Colon
T9	Adenocarcinoma	Moderately differentiated	1	0	7	Left Colon