Supplemental Data

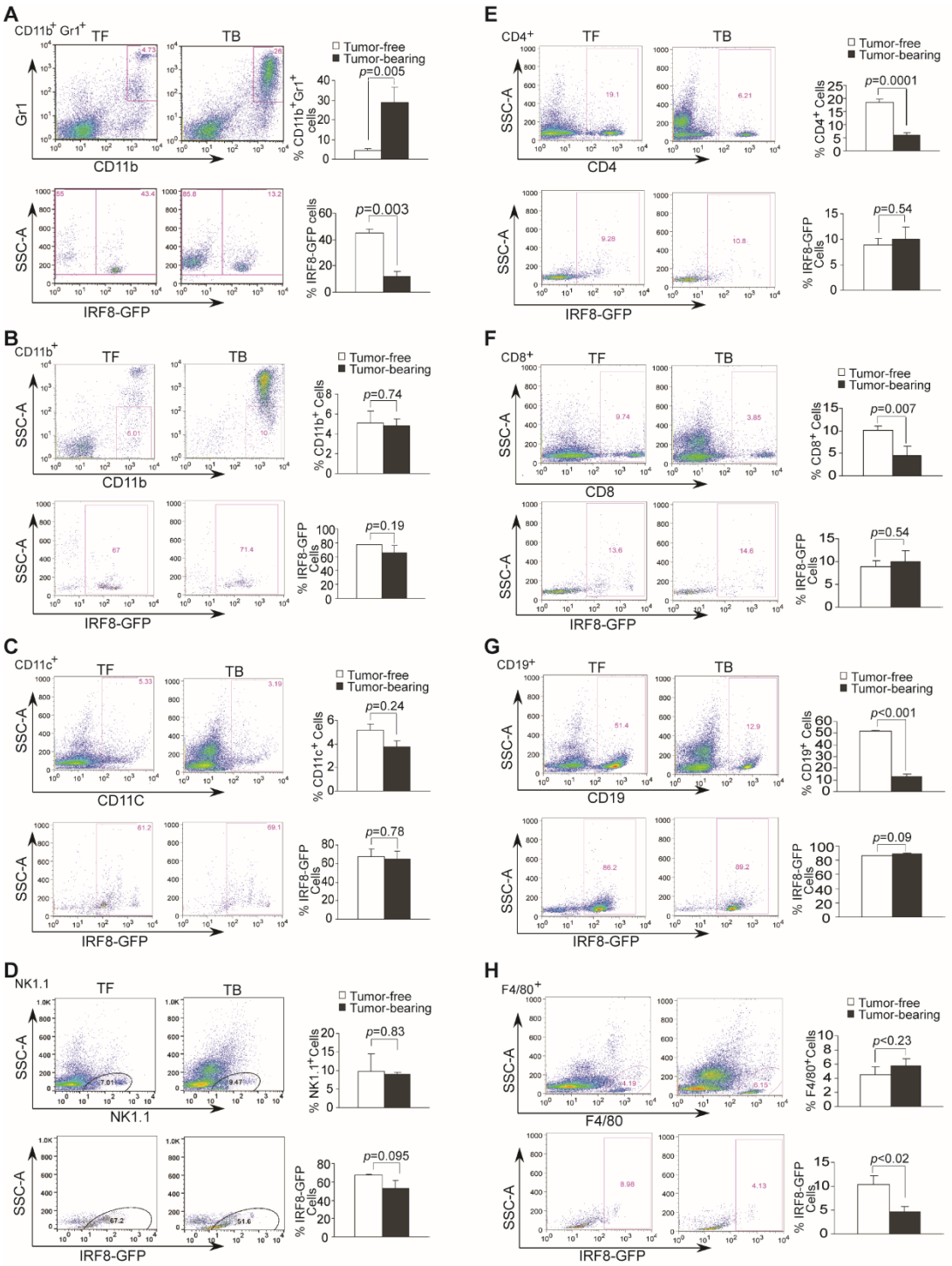

Figure S1. IRF8 expression profiles in subsets of immune cells in spleens of tumor-bearing mice. AT3 tumor cells were injected to the mammary gland of the IRF8-GFP reporter mice. Spleens were collected from tumor-free control tumor-free (TF) and tumor-bearing (TB) mice, stained with fluorescent dye-conjugated mAbs for CD11b and Gr1 (A & B), CD11c (C), NK1.1 (D), CD4 €, CD8 (F), CD19 (G), and F4/80 (H). The stained cells were analyzed by flow cytometry. The left two panels show dot plots of the respective immune cell subset phenotype (top) and GFP level of that immune cell subset (bottom). The right panel shows quantification of the respective immune cell subset (top) and GFP level of that immune cell subset (bottom). Column: Mean; Bar: SD.

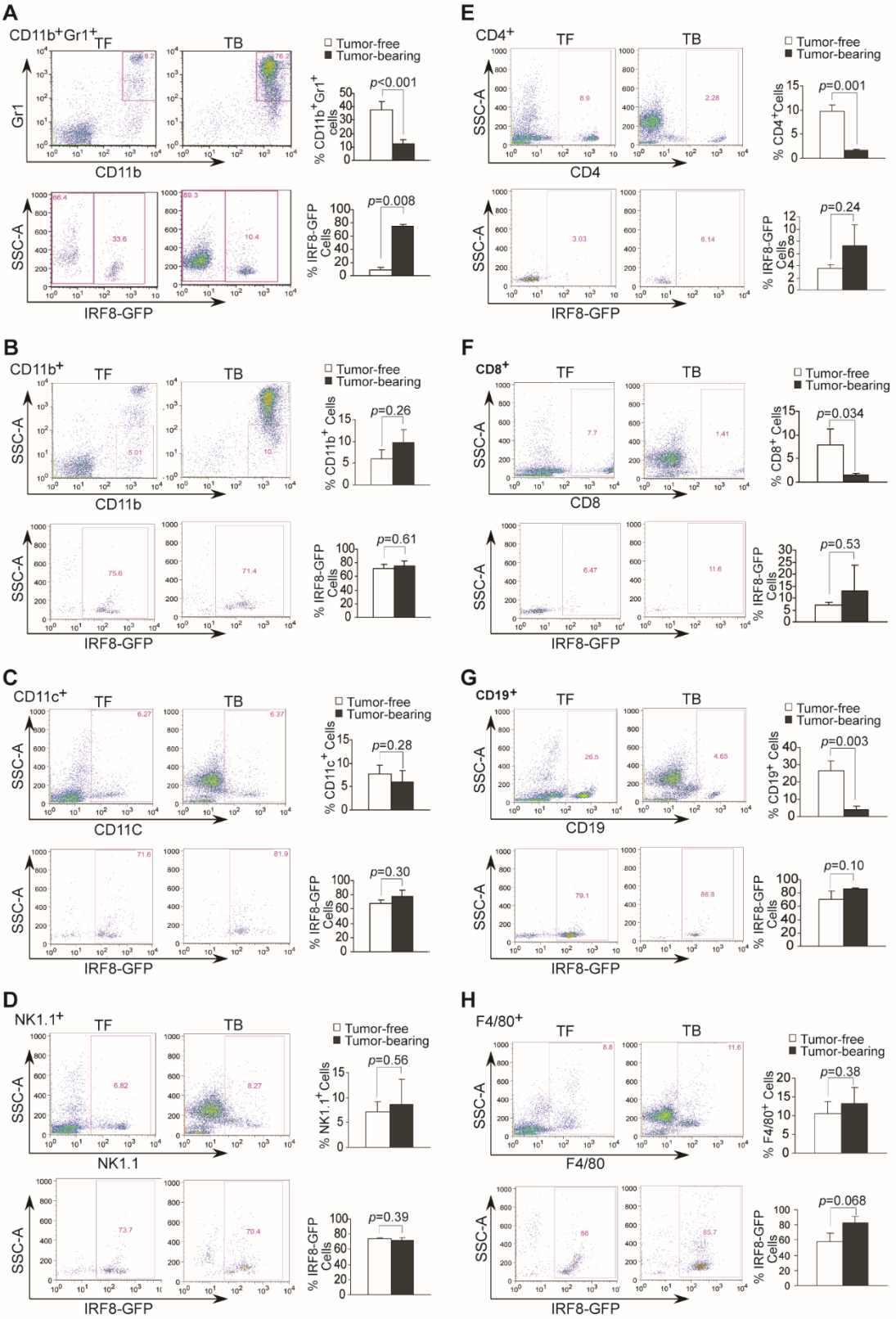

Figure S2. IRF8 expression profiles in subsets of immune cells in peripheral blood of tumor-bearing mice. AT3 tumor cells were injected to the mammary gland of the IRF8-GFP reporter mice. Peripheral blood samples were collected from tumor-free (TF) the tumor-bearing (TB) mice and analyzed as in Fig. S1.

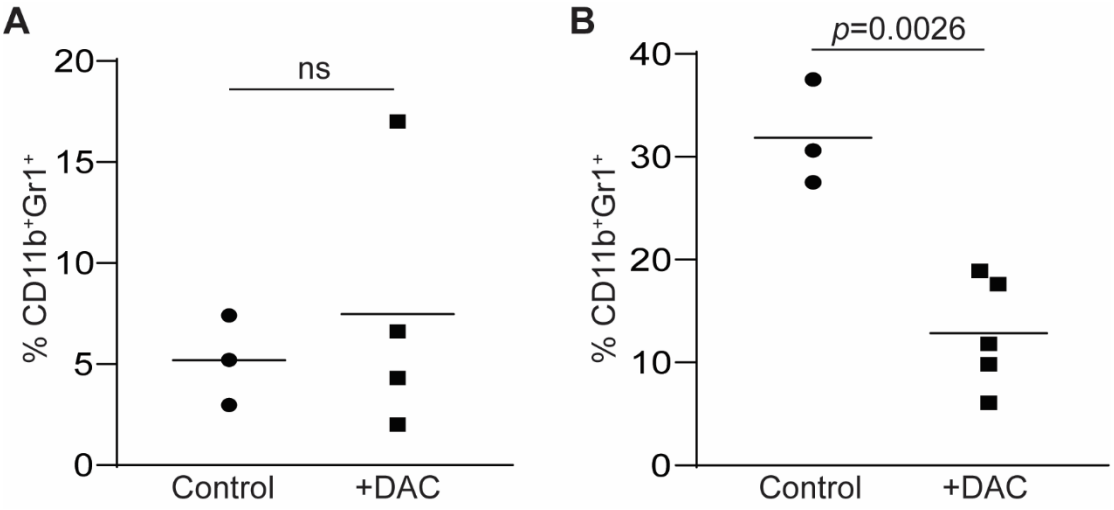

Figure S3. DNA methylation and MDSC accumulation in tumor-bearing mice. **A**. AT3 tumor cells ($2.5x10^5$ cells/mouse) were injected into mammary glands of C57BL/6 mice. Tumor-bearing mice were treated with saline (control, n=3) and DAC (1 mg/kg body weight, n=4) by i.v. injection at days 21-23 after tumor cell injection once every 2 days for 2 times. Mice were sacrificed 1 day after the last treatment. The tumors were digested with collagenase solution, stained with CD11b- and Gr1-specific antibodies and analyzed by flow cytometry. % $CD11b^+Gr1^+$ cells of the total tumor cells were quantified. **B**. AT3 tumor-bearing mice were treated with saline (control, n=3) and DAC (1 mg/kg body weight, n=5) as in A. Spleens cells were stained with CD11b- and Gr1-specific antibodies and analyzed by flow cytometry. % $CD11b^+Gr1^+$ cells of the total spleen cells were quantified.

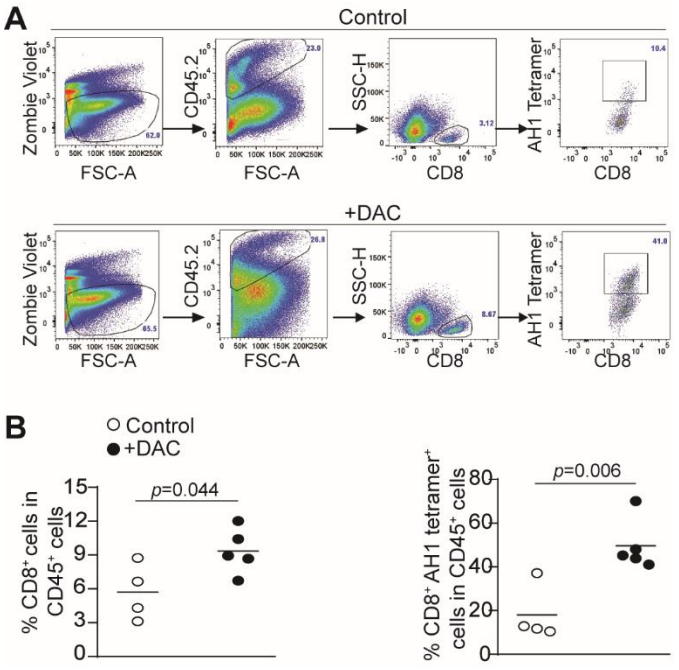

Figure S4. Inhibition of DNA methylation activates antigen-specific CTLs in tumor-bearing mice. **A**. CT26 cells ($1.5x10^5$) were injected s.c. to BALB/c mice. The tumor-bearing mice were treated with saline (control, n=4) and DAC (2 mg/kg body weight, n=5) at days 21 and 23 after tumor cell injection. Mice were sacrificed at day 24. Tumor tissues were collected, digested with collagenase to make single cells. The cells were then stained CD45.2-, and CD8-specific mAb, plus Zombie violet and AH1 tetramer. The live $CD45.2^+$ cells were dated and analyzed for $CD8^+$ and AH1 $tetramer^+$ cells. Shown are gating strategies. B. Quantification of $CD8^+$ and $CD8^+$AH1 $tetramer^+$ cells.

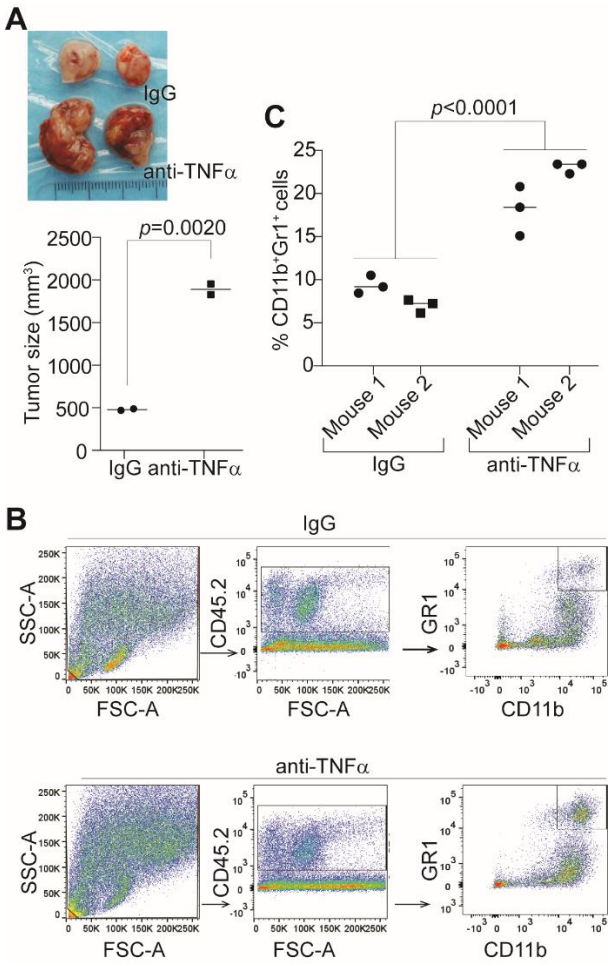

Figure S5. Neutralization of TNFα increases tumor growth and MDSC accumulation. A. AT3 cells ($2x10^5$ cell/mouse) were injected to the mammary gland of C57BL/6 mice. The tumor-bearing mice were treated with IgG (n=2, 200 μg/ml) and anti-TNFα mAb (200 μg/mouse, n=2), respectively, at day 8 after tumor cell injection every 3 days for 4 times. The mice were sacrificed 1 day after the last treatment. Shown are tumor image (top panel) and tumor size quantification (bottom panel). **B**. Tumor tissues were digested with collagenase to make single cells. Cells were then stained with CD45.2-, CD11b-, and Gr1-specific antibodies and analyzed by flow cytometry. All $CD45.2^+$ cells were gated and analyzed for $CD11b^+Gr1^+$ cells. Shown are gating strategy. **C**. Quantification of $CD11b^+Gr1^+$ cells as shown in B. Tumor from each mouse were analyzed for MDSCs in triplicates.

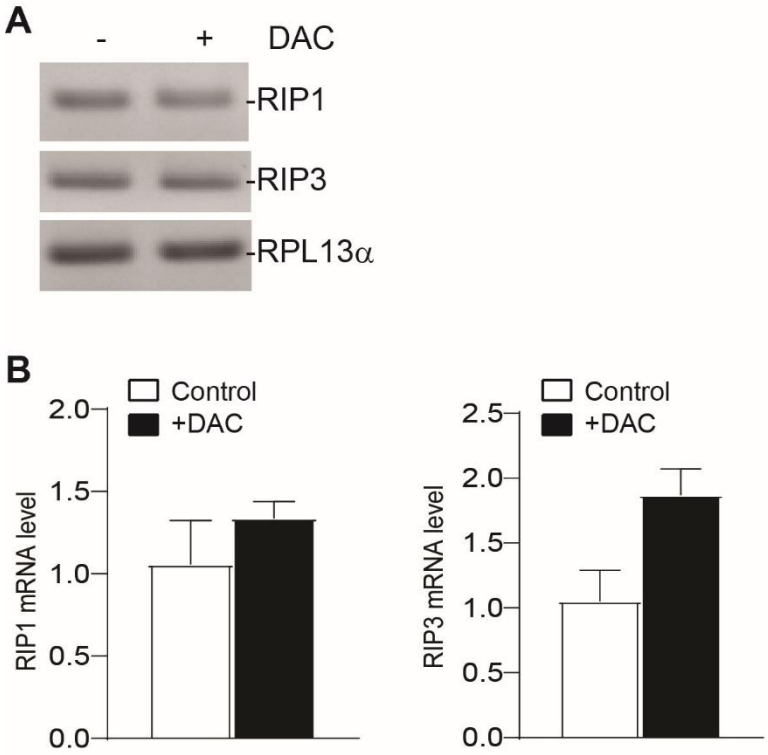

Figure S6. RIP1 and RIP3 are expressed in MDSCs. A. J774M cells were treated with DAC (1 μM) for 3 days and analyzed by semi-quantitative PCR for RIP1 and RIP3 expression level. RPL13α was used as normalization control. **B**. J774M cells were treated as in A and analyzed by qPCR for RIP1 and RIP3 expression with RPL13α as internal control. The expression level of untreated cells was arbitrarily set at 1.

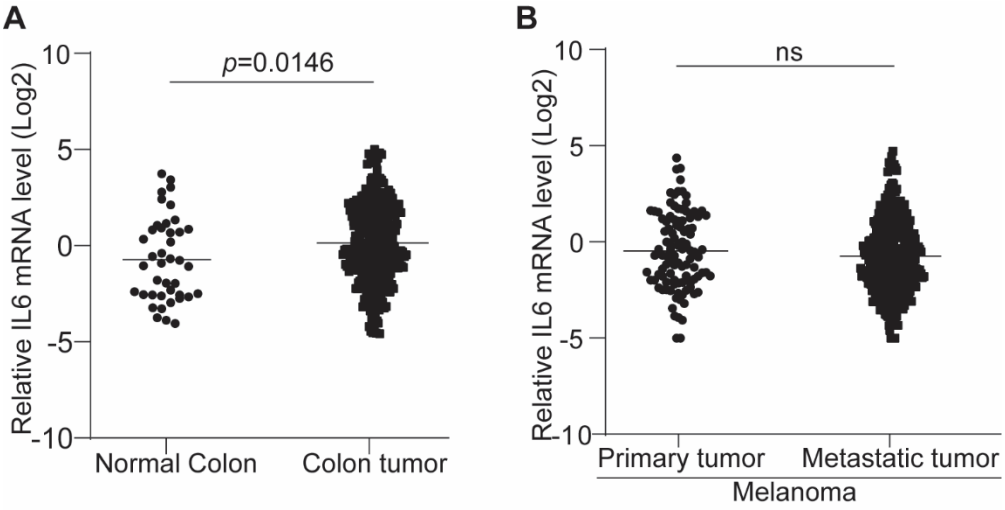

Figure S7. IL6 expression level in human colon carcinoma and melanoma. A. Data sets of the IL6 mRNA levels in human colon carcinoma (n=286) and normal colon tissues (n=41) were extracted from TCGA database and plotted. Two-tail *t* tests were used to determine differences with $p<0.05$ as being statistically significant. **B**. Data sets of the IL6 mRNA levels in human primary melanoma (n=103) and metastatic melanoma (n=368) were extracted from TCGA database and plotted. Two-tail *t* tests were used to determine differences with $p<0.05$ as being statistically significant. ns: not significant.

Table S1. Colorectal Cancer patient data*

Patient #	Gender	Ethinicity	Age	Type	Stage	Tumor	Treatment **
1	M	Black	52	Colon	4	Metastatic	FOLFOX+Avastin
2	F	Black	56	Colon	4	Metastatic	FOLFOX+Avastin
3	F	Black	63	Colon	4	Metastatic	FOLFOX+Avastin
4	F	White	80	Colon	4	Metastatic	FOLFOX+Avastin
5	F	White	39	Rectal	3	Primary	Xeloda

* Patient information for Figure 7D & E. Blood samples were collected 7 days after the last chemotherapy

**FOLFOX: folinic acid + 5'-fluorouracil +oxaliplatin

Table S2. Colon Cancer patient data*

Patient #	Gender	Ethinicity	Age	Type	Stage	Tumor	Treatment **
6	M	White	74	Colon	3	Primary	FOLFOX
7	M	White	45	Colon	3	Primary	FOLFOX
8	F	Black	56	Colon	3	Primary	XELODA
9	M	Black	57	Colon	3	Primary	XELOX
10	F	White	60	Colon	4	Metastatic	FOLFOX /Avastin

* Patient information for Figure 7F & G. Blood samples were collected 7 days after the last chemotherapy

**FOLFOX: folinic acid + 5'-fluorouracil +oxaliplatin