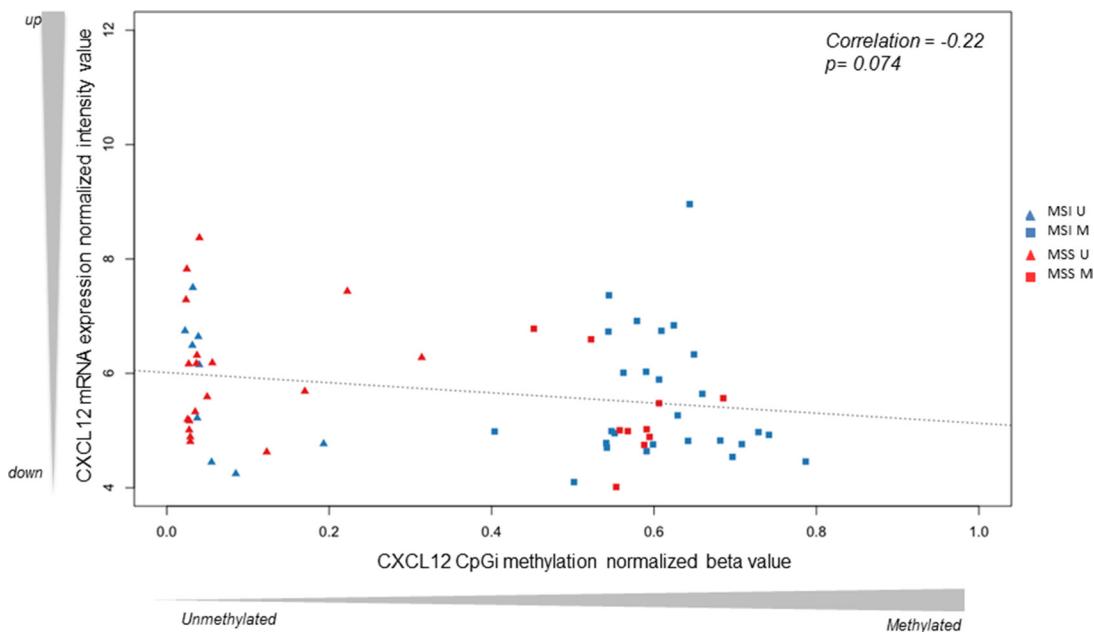
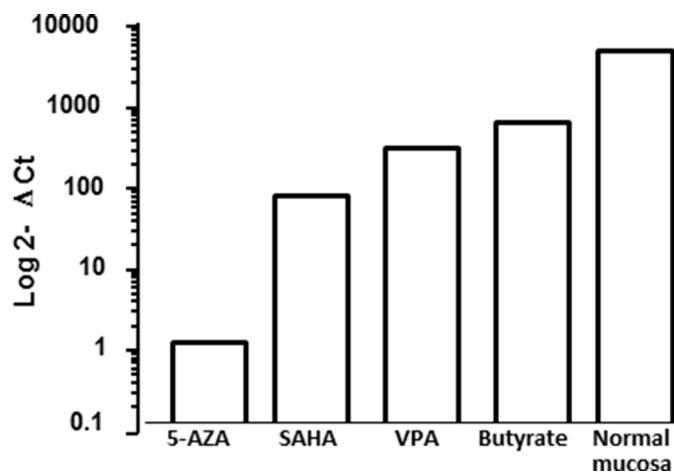


Histone hypoacetylation contributes to CXCL12 downregulation in colon cancer: impact on tumor growth and cell migration

Supplementary Materials



Supplementary Figure 1: Correlation between CXCL12 promoter methylation and mRNA expression. y-axis corresponds to Affymetrix log₂ normalized intensity values of CXCL12 probe set 209687_at and x-axis to β -values of the CpG site cg17267805.



Supplementary Figure 2: Respective roles of methylation and acetylation in CXCL12 mRNA reexpression.
SW480 cells were treated with 5-AZA (2.5 μ M), SAHA (50 μ M), VPA (1 mM) and butyrate (10 mM) for 24 h. The mean Ct for CXCL12 was calculated for each treatment, normalized with PBGD as control and the results were expressed as 2- Δ Ct. Experiments have been repeated in triplicate. Results are representative of three sets of independent samples per group.

Supplementary Table 1: CIMP profile and CXCL12 methylation in colon cancer cells

| | CACNA1G | IGF2 | NEUROG1 | RUNX3 | SOCS1 | CXCL12 | CIMP |
|------------------|---------|------|---------|-------|-------|--------|------------------|
| HCT116 | M | M | M | U | M | M | CIMP-High |
| SW480 | U | U | U | U | U | U | CIMP-Low |
| Caco2/TC7 | U | U | M | U | M | M | CIMP-Low |

M = methylated; U = Unmethylated.

Supplementary Table 2: Characteristics of the 26 carcinomas of the validation cohort for PCAF and CXCL12 expression

| Carcinomas | | <i>n</i> = 26 |
|----------------------------|----------------|---------------|
| <i>Mean age ± S.D</i> | 67 ± 12 | |
| <i>Men:Women</i> | 15/11 | 26 |
| <i>Localization</i> | Proximal colon | 8 |
| | Distal colon | 18 |
| <i>UICC classification</i> | In situ Tis) | |
| | I | 2 |
| | II | 10 |
| | III | 8 |
| | IV | 6 |
| <i>MSS/MSI/CIMP</i> | 26/0/0 | 26 |

Supplementary Table 3: Primer sequences for MS-PCR

| PRIMERS | | SEQUENCES |
|---------|------------|-------------------------------|
| CACN1G | CACN1G-UF | FAM- GTTTGGTTTTTGTGTTGTGT |
| | CACN1G-UR | CACCCTCTAAAACAACCTCAC |
| | CACN1G-MF | FAM - TCGGTTTCGTTCGCGT |
| | CACN1G-MR | CTCTCGAAACGACTTCGCCG |
| IGF2 | IGF2-UF | VIC- TTGGAGTGGTTTGGTGTGTT |
| | IGF2-UR | CAAACCAACACCCAACTCAA |
| | IGF2-MF | VIC-GCGGTTTCGGTGTGTTATC |
| | IGF2-MR | CGCGAACGCCAACTCGA |
| NEUROG1 | NEUROG1-UF | FAM-TATTGTTGGTTAATGGTGGTG |
| | NEUROG1-UR | ACATACCTCAACCAACTAACAC |
| | NEUROG1-MF | FAM-TCGGTTAACGGCGGC |
| | NEUROG1-MR | TACCTCGACCGCTAACCGC |
| RUNX3 | RUNX3-UF | NED-GTTTATGGAAATATGTATAATAGTG |
| | RUNX3-UR | CCCACTCTCACAAACAACAACA |
| | RUNX3-MF | NED-CGGGAATACGTATAATAGCG |
| | RUNX3-MR | GCTTCTCGCGACAACGACG |
| SOCS1 | SOCS1-UF | PET-TATTTTTGGTGTGTGATAGTTG |
| | SOCS1-UR | AAACAACCAACCTAAAAATACACA |
| | SOCS1-MF | PET -ATTTTTGGTGCACGATAGTC |
| | SOCS1-MR | GACCGACCTAAAAATACACGC |
| CXCL12 | CXCL12-UF | FAM-AAGGTTGGAGTGTATTGTGTTTG |
| | CXCL12-UR | AAAACCAAAATACTAACCATACCA |
| | CXCL12-MF | FAM-CGGAGCGTATTGCCTTC |
| | CXCL12-MR | CCGAATACTAACCGTAACCG |

Supplementary Table 4: Primer sequences for chromatin immunoprecipitation

| Primer sequences (5'-3') | |
|--------------------------|-----------------------|
| CXCL12-F | CGCTTAAGGTCTCAGTCTC |
| CXCL12-R | CTCCTCCGCTCCCTCTGT |
| CXCL12 Exon 2-F | GGAAGCCCGTCAGCCTG |
| CXCL12 Exon 2-R | ACAATCTGAAGGGCACAGTTG |