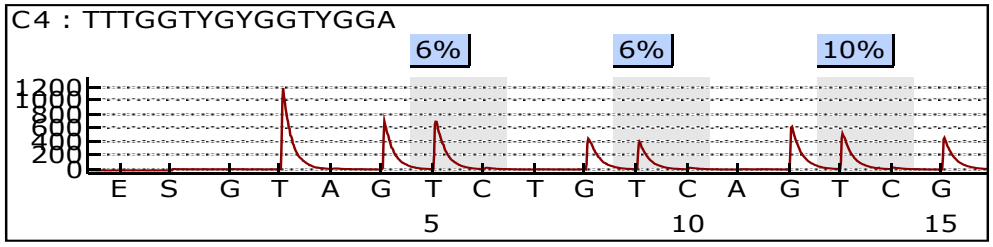
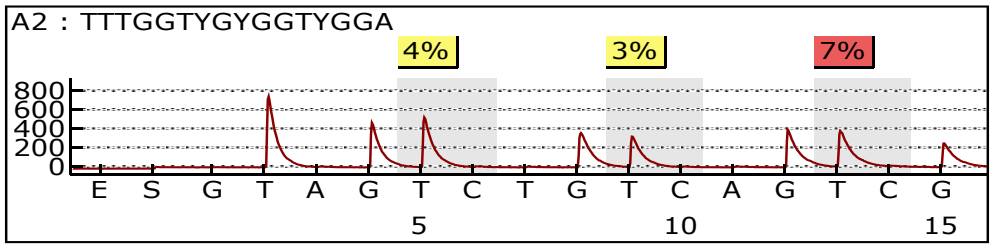
Supplemental Figure 1

a

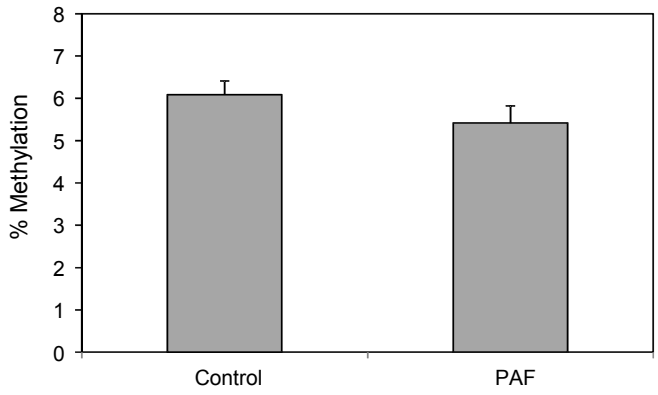
Control



PAF-treated



b



Comparison of the methylation status of individual CpG sites in the CXCR4 promoter in PAF-treated HMC-1 cells (10 mM; 24 hours). (**a**) Pyrosequencing profile. (**b**) Data expressed as % methylation Data are from two independent experiments and are expressed as the mean percentage of methylated CpG at each position in the CXCR4 promoter from control and PAF-treated samples.

Methylation analysis with pyrosequencing

500 ng to 1 mg of genomic DNA was treated with sodium bisulfite using the EZ DNA Methylation-Gold Kit (Zymo Research, Irvine, CA) according to protocol. The samples were eluted in 40 ml of M-Elution Buffer, and 2 ml (eq. to 25 ng of bisulfite-modified DNA) were used

for each PCR reaction. Both bisulfite conversion and pyrosequencing analysis were done at the DNA Methylation Analysis Core, M.D. Anderson Cancer Center.

PCR primers for the area proximal to the transcription start site of the genes of interest were designed using the Pyromark Assay Design SW 1.0 software (Qiagen, Hilden, Germany). In brief, a sequencing primer is identified within 1 to 5 base pairs near the CpG sites of interest, with an annealing temperature of 40±5 ^{o}C. After that, forward and reverse primers are identified upstream and downstream to the sequencing primer, with a target annealing temperature ranging from 50 to 60 °C and amplicon size ranging from 100 to 200 bp. Optimal annealing temperature for each primer was tested using gradient PCR. Controls for no-DNA template, high- (SssI-treated DNA), low- (WGA-amplified DNA), and partial-methylation (equimolar mixture of SssI-treated and WGA-amplified DNA), were included in each reaction. To account for biased representation of A/T rich regions in the whole genome, an additional control of WGA-amplified DNA that was SssI-treated prior to bisulfite conversion was also included.

PCR reactions were performed in a total volume of 15 µl, which was used entirely for each pyrosequencing reaction. PCR product purification was done with streptavidin-sepharose high-performance beads (GE Healthcare Life Sciences, Piscataway, NJ), and co-denaturation of the biotinylated PCR products; sequencing primer (3.6 pmol/reaction) was conducted following the PSQ96 sample preparation guide. Sequencing was performed on a PSQ HS 96 system (Biotage AB, Uppsala, Sweden) with the PyroMark Gold Q96 CDT Reagents (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The degree of methylation was calculated using the Pyro-Q CpG 1.0.9v software (Biotage AB, Uppsala, Sweden).
The primers used are reported below.

Oligo Name	Sequence 5' to 3'	Product Size	Sequence to Analyze
CXCR4/F	**AGATGAGGTGGTTATTGGAGTATTTAG**	**254**	TTTGGTYGYGGTYGGA
CXCR4/Rbio	**[Btn]CACCCCCAAACAACAAAATCCCCTAA**		
CXCR4/S	**TTGTAGTAGTTAATAAATTGAAGTT**		