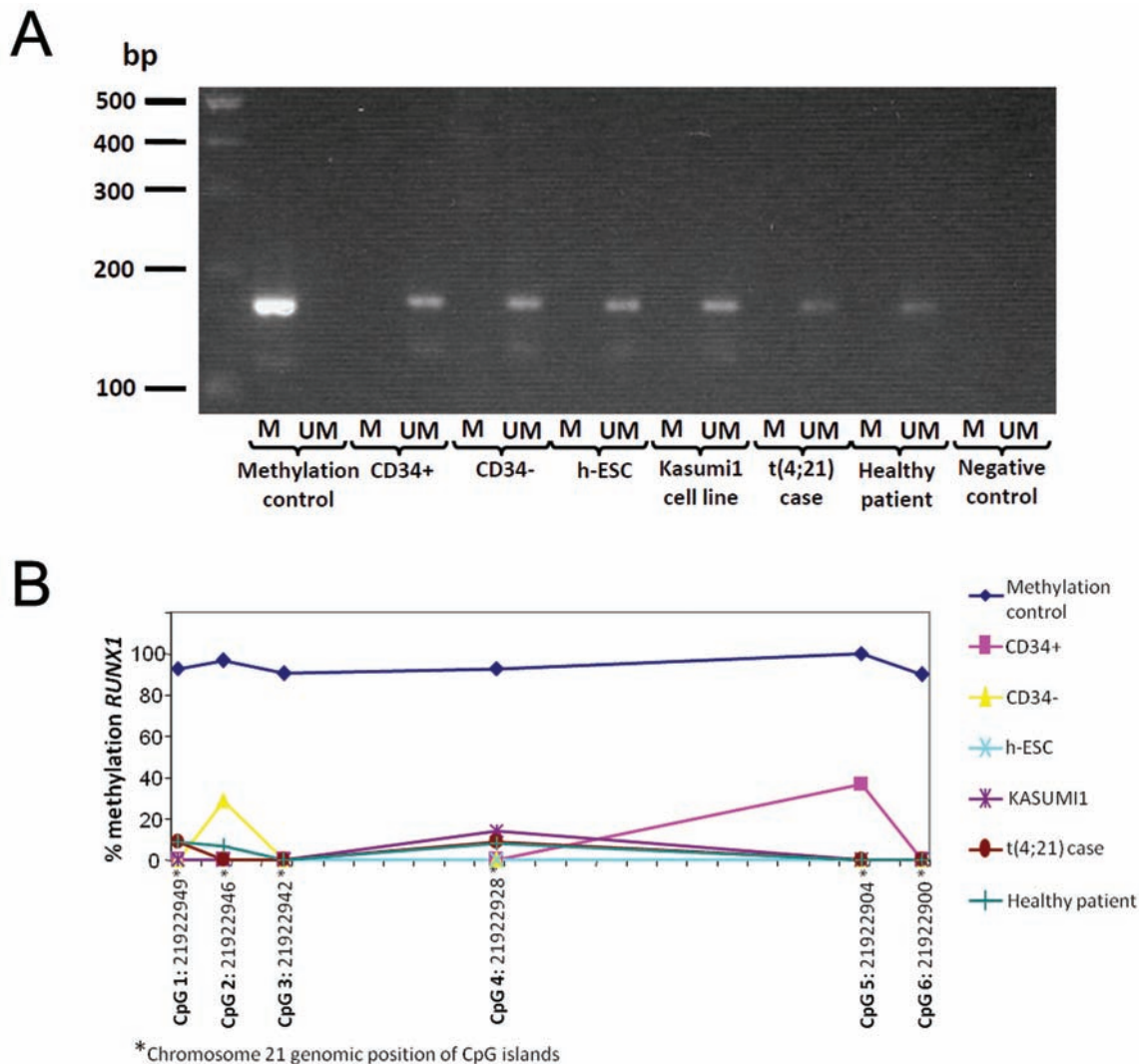


Abrogation of *RUNX1* gene expression in *de novo* myelodysplastic syndrome with t(4;21)(q21;q22)

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Online Supplementary Figure S1. Methylation Specific PCR and pyrosequencing. (A) Methylation-specific PCR was carried out to assess *RUNX1* proximal promoter methylation using specific *RUNX1* promoter primers for methylation and unmethylation (MF: 5'- TTCGGTTGCGTATAGTAGC; MR: 5'- ATAAAC-CCGACCGAAAAAAT; UMF: 5'- GTTTGGTTGTGTATAGTAGT; UMR: 5'- TATAAACCAACCAAAAAAAT). For methylation-specific PCR reaction, we included universal methylated DNA (CpGenome Universal Methylated DNA, Millipore, Temecula, CA, USA) as positive control, and normal blood DNA as negative control. Quality controls for bisulphite conversion were performed for each reaction. (B) The second methylation analysis of the *RUNX1* proximal promoter was realized by bisulphite genomic pyrosequencing of the corresponding six CpG islands located within the fragment amplified by MSP. Following sodium bisulphite treatment, the specific region was PCR amplified using specific primers for modified DNA (Forward: 5'- GGTTAGTATGATAGTTGGATG and Reverse: 5'- CTATAAATAAAAATACCTAAAAATAAAC) and PCR products were sequenced. The positive control also corresponds to the universal methylated DNA. In both analyses we included DNA from CD34⁺ and CD34⁻ cells isolated from cord blood, AND2 human embryonic stem cell line, AML Kasumi1 cell line, the studied case and DNA from the patient in remission. No methylation within the *RUNX1* proximal promoter was observed in any of these cases. We conclude that this epigenetic mechanism is not involved in the inactivation of the remaining gene copy in the patient.

Online Supplementary Table S1. Deleted genes on chromosome 4 and 21 revealed by aCGH.

Ch4		Description
6.6Mb	CXCL9	chemokine (C-X-C motif) ligand 9
	CXCL10	chemokine (C-X-C motif) ligand 10
	CXCL11	chemokine (C-X-C motif) ligand 11
	ASAH1	N-acylethanolamine acid amidase
	SDAD1	SDA1 domain containing 1
	ART3	ADP-ribosyltransferase 3
	GENX-3414	starch binding domain 1
	FLJ25770	coiled-coil domain containing 158
	ShrmL	shroom family member 3
	SEPT11	septin 11
	CCNI	cyclin I
	CCNG2	cyclin G2
	CXCL13	chemokine (C-X-C motif) ligand 13
	CNOT6L	CCR4-NOT transcription complex, subunit 6-like
	MRPL1	mitochondrial ribosomal protein L1
	FRAS1	Fraser syndrome 1
	ANXA3	annexin A3
	BMP2K	BMP2 inducible kinase
	PAQR3	progesterin and adipoQ receptor family member III
	GK2	glycerol kinase 2
	GDEP	gene differentially expressed in prostate (non-coding RNA)
	ANTXR2	anthrax toxin receptor 2
	PRDM8	PR domain containing 8
	FGF5	fibroblast growth factor 5
	MGC35043	uncharacterized protein C4orf22
	PRKG2	protein kinase, cGMP-dependent, type II
BMP3	bone morphogenetic protein 3	
RASGEF1B	RasGEF domain family, member 1B	
HNRNPD	heterogeneous nuclear ribonucleoprotein D	
HNRPDL	heterogeneous nuclear ribonucleoprotein D-like	
MASA	enolase-phosphatase 1	
SCD5	stearoyl-CoA desaturase 5	
Ch21		Description
2.3Mb	KCNE2	potassium voltage-gated channel, Isk-related family, member 2
	KCNE1	potassium voltage-gated channel, Isk-related family, member 1
	C21orf51	family with sequence similarity 165, member B
	DSCR1	regulator of calcineurin 1
	CLIC6	chloride intracellular channel 6
	RUNX1	runt-related transcription factor 1
	C21orf18	SET domain containing 4
	CBR1	carbonyl reductase 1
	CBR3	carbonyl reductase 3
	C21orf51	family with sequence similarity 165, member B
	CHAF1B	chromatin assembly factor 1, subunit B (p60)
	ZCWCC3	MORC family CW-type zinc finger 3