SUPPLEMENTARY MATERIAL

TABLES

Supp table 1: Genes with hypermethylated promoters in MDS

Supp Table 2: Genes with hypomethylated promoters in MDS

FIGURES

Supp Figure 1: KLF3 hypermethylated by MassArray sequencing

Supp Figure 2: Pathway analysis of hypermethylated genes in MDS (Ingenuity)

Supplementary Table 1: Significantly hypermethylated refseq gene loci with mean log (HpaII/MspI) ratios

Symbol	Mean HELP ratio MDS	Mean HELP ratio Normals	p-value	Gene	Significantly underexpressed in MDS BM CD34+ cells*	Deleted in any MDS samples
ABCC1	-1.28	0.17	0.0136	ATP-binding cassette, sub-family C (CFTR/MRP), member 1		
ABCF3	-1.98	-0.82	0.0003	ATP-binding cassette, sub-family F (GCN20), member 3	+	+
ACAD8	-0.76	0.83	0.0009	acyl-Coenzyme A dehydrogenase family, member 8		
ADAM30	-2.24	-0.91	0.0039	ADAM metallopeptidase domain 30	+	+
ADAMTS15	-1.16	0.03	0.0070	ADAM metallopeptidase with thrombospondin type 1 motif, 15	NA	
ALDH3A1	-0.97	0.38	0.0009	aldehyde dehydrogenase 3 family, memberA1		
AMDHD2	-2.56	-1.44	0.0002	amidohydrolase domain containing 2		
ANGPTL2	-2.44	-0.57	0.0021	angiopoietin-like 2	+	
ANTXR1	-0.01	1.27	0.0090	anthrax toxin receptor 1		
ARHGEF4	-0.29	1.08	0.0037	Rho guanine nucleotide exchange factor (GEF) 4	+	
ARMC1	-0.07	1.01	0.0032	armadillo repeat containing 1		
ARRDC4	-1.12	-0.08	0.0034	arrestin domain containing 4	NA	
ATAD5	-1.79	-0.73	0.0001	ATPase family, AAA domain containing 5		
ATG2B	-2.41	-1.26	0.0030	ATG2 autophagy related 2 homolog B (S. cerevisiae)		+
ATP5S	-4.03	-2.87	0.0128	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit s (factor B)	+	
C11ORF30	-0.52	0.68	0.0002	chromosome 11 open reading frame 30	NA	
C14ORF126	-3.34	-2.23	0.0003	chromosome 14 open reading frame 126	NA	
C16ORF46	-3.14	-2.03	0.0001	chromosome 16 open reading frame 46	NA	
C16ORF73	-0.91	0.32	0.0397	chromosome 16 open reading frame 73	NA	
C1ORF113	0.10	1.26	0.0016	chromosome 1 open reading frame 113	NA	
C3ORF59	-1.90	-0.33	0.0006	chromosome 3 open reading frame 59	NA	+
C6ORF47	-0.70	0.33	0.0329	chromosome 6 open reading frame 47	+	
CA8	-3.51	-2.33	0.0287	carbonic anhydrase VIII		
CCDC115	-0.53	0.54	0.0445	coiled-coil domain containing 115	NA	
CCDC60	-0.35	0.70	0.0270	coiled-coil domain containing 60	NA	
CCRN4L	-1.65	-0.30	0.0000	CCR4 carbon catabolite repression 4-like (S. cerevisiae)	+	
CDADC1	-2.76	-1.74	0.0034	cytidine and dCMP deaminase		

				domain containing 1		
CDC42SE1	-2.43	-1.18	0.0008	CDC42 small effector 1	+	+
CDR2L	-1.27	0.23	0.0000	cerebellar degeneration-related protein 2-like	NA	
CLEC4D	-2.32	-1.17	0.0061	C-type lectin domain family 4, member D	NA	
CXORF39	-1.69	-0.51	0.0139	chromosome X open reading frame 39	NA	
CXORF42	-2.48	-1.36	0.0017	chromosome X open reading frame 42	NA	
DAB2IP	-1.38	-0.15	0.0001	DAB2 interacting protein	NA	
DDAH2	-0.85	0.18	0.0007	dimethylarginine dimethylaminohydrolase 2	NA	
DNAH11	-1.65	-0.48	0.0181	dynein, axonemal, heavy chain 11	NA	
DOCK2	-1.76	-0.32	0.0137	dedicator of cytokinesis 2	+	
DOCK4	-3.03	-1.95	0.0004	dedicator of cytokinesis 4	+	+
DTX3L	-2.38	-1.08	0.0239	deltex 3-like (Drosophila)	NA	+
DUSP4	-0.81	0.26	0.0214	dual specificity phosphatase 4	+	+
ECHDC1	-0.13	1.06	0.0156	enoyl Coenzyme A hydratase domain containing 1		
EFCAB2	-0.83	0.34	0.0057	EF-hand calcium binding domain 2	+	
EFTUD1	-0.79	0.25	0.0000	elongation factor Tu GTP binding domain containing 1		
EIF2C3	-1.77	-0.56	0.0001	eukaryotic translation initiation factor 2C, 3		
EIF3A	-1.61	-0.44	0.0003	eukaryotic translation initiation factor 3, subunit A		
ENG	-0.89	0.17	0.0157	endoglin		
FAM177A1	-1.28	0.24	0.0004	family with sequence similarity 177, member A1	NA	
FARP1	-1.58	-0.33	0.0005	FERM, RhoGEF (ARHGEF) and pleckstrin domain protein 1 (chondrocyte-derived)		
GEMIN4	-2.36	-1.32	0.0047	gem (nuclear organelle) associated protein 4	NA	
GIT2	-2.09	-0.97	0.0320	G protein-coupled receptor kinase interacting ArfGAP 2	NA	
GLT25D2	-2.68	-1.38	0.0179	glycosyltransferase 25 domain containing 2		
GOLT1A	-2.16	-1.14	0.0015	golgi transport 1 homolog A (S. cerevisiae)	NA	+
GSPT1	-1.98	-0.97	0.0038	G1 to S phase transition 1		
H1FNT	-3.26	-2.12	0.0066	H1 histone family, member N, testis-specific	NA	
HCN1	-1.91	-0.50	0.0013	hyperpolarization activated cyclic nucleotide-gated potassium channel 1	NA	
HIST1H4J	-0.51	0.73	0.0006	histone cluster 1, H4j		+
HMGN3	-0.56	0.55	0.0095	high mobility group nucleosomal binding domain 3	NA	
HOXB3	-1.68	-0.67	0.0078	homeobox B3		+

HSPG2	-2.02	-1.01	0.0003	heparan sulfate proteoglycan 2		
IMP4	-0.53	0.54	0.0445	IMP4, U3 small nucleolar ribonucleoprotein, homolog (yeast)		
IQGAP2	-1.07	-0.01	0.0265	IQ motif containing GTPase activating protein 2	+	
ITPK1	-3.48	-2.39	0.0001	inositol 1,3,4-triphosphate 5/6 kinase	+	+
KCNN2	0.3	0.09	0.04	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 2		+
JMJD2A	-0.50	0.77	0.0003	jumonji domain containing 2A	+	+
JMJD2B	-0.62	0.58	0.0017	jumonji domain containing 2B	+	+
JPH4	-3.26	-2.15	0.0006	junctophilin 4	NA	
KIAA0232	-2.37	-1.37	0.0113	KIAA0232	+	
KLF3	-0.796	0.163	0.005	Kruppel like factor 3	NA	+
LEPREL1	-0.56	0.55	0.0013	leprecan-like 1		
LMLN	-1.88	-0.71	0.0257	leishmanolysin-like (metallopeptidase M8 family)	NA	
LOC100129034	-0.92	0.16	0.0060	hypothetical protein LOC100129034	NA	
LOC100131654	-1.35	-0.31	0.0115	hypothetical protein LOC100131654	NA	
LOC646903	-1.15	-0.14	0.0107	hypothetical LOC646903	NA	
LTA4H	-1.63	-0.62	0.0018	leukotriene A4 hydrolase		
MBP	-0.07	1.04	0.0003	myelin basic protein		
MDS1	-0.88	0.38	0.0174	myelodysplasia syndrome 1		+
MED12	-1.00	0.86	0.0024	mediator complex subunit 12	+	
MGC24125	-1.80	-0.42	0.0046	hypothetical protein MGC24125	NA	
MRP63	-0.20	1.35	0.0382	mitochondrial ribosomal protein 63	+	
MST152	-2.04	-0.94	0.0099	MSTP152	NA	
MT1X	-1.31	-0.16	0.0029	metallothionein 1X		
MTCH1	-1.00	0.13	0.0307	mitochondrial carrier homolog 1 (C. elegans)	+	
NICN1	-1.03	-0.01	0.0001	nicolin 1	NA	+
NME5	-0.95	0.19	0.0033	non-metastatic cells 5, protein expressed in (nucleoside-diphosphate kinase)	+	
N-PAC	-1.26	-0.19	0.0083	cytokine-like nuclear factor n-pac	+	
NUP107	-2.14	-1.09	0.0069	nucleoporin 107kDa		
NXT2	-2.72	-1.66	0.0051	nuclear transport factor 2-like export factor 2		+
OBFC1	-2.73	-0.37	0.0486	oligonucleotide/oligosaccharide-binding fold containing 1	+	
ODC1	-1.97	-0.94	0.0001	ornithine decarboxylase 1		
OXTR	-2.51	-1.22	0.0046	oxytocin receptor	+	
PACS2	-1.29	-0.16	0.0000	phosphofurin acidic cluster sorting protein 2		
PAPSS2	-1.60	-0.38	0.0002	3'-phosphoadenosine 5'-phosphosulfate synthase 2		
PCDH12	-1.01	0.10	0.0129	protocadherin 12		
PCDHB11	-2.10	-0.73	0.0015	protocadherin beta 11	NA	
PLAGL2	-0.32	0.76	0.0060	pleiomorphic adenoma gene-like 2	+	

PGGT1B	-3.5	-2.1	0.04	protein geranylgeranyltransferase type I, beta subunit		+
PP13439	-1.64	-0.60	0.0060	hypothetical LOC100128046	NA	
PPARGC1A	-0.66	0.38	0.0142	peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	+	
PPP1R15B	-2.59	-1.30	0.0000	protein phosphatase 1, regulatory (inhibitor) subunit 15B	NA	+
PRKAR1A	-0.44	0.63	0.0011	protein kinase, cAMP-dependent, regulatory, type I, alpha (tissue specific extinguisher 1)	NA	+
PRPF8	-0.11	1.22	0.0048	PRP8 pre-mRNA processing factor 8 homolog (S. cerevisiae)		
PRES	-1.4	-0.3	0.04	prestin (motor protein)		+
RAB2A	-1.84	0.58	0.0052	RAB2A, member RAS oncogene family	+	
RAB36	-2.62	-1.45	0.0009	RAB36, member RAS oncogene family	+	
RALGPS1	-2.40	-1.39	0.0036	Ral GEF with PH domain and SH3 binding motif 1		
REXO1	-2.27	-0.07	0.0013	REX1, RNA exonuclease 1 homolog (S. cerevisiae)		
RHBDD3	0.02	1.24	0.0016	rhomboid domain containing 3		
RNF135	-1.66	-0.56	0.0092	ring finger protein 135	NA	
RPL39	-1.52	0.07	0.0394	ribosomal protein L39		
RUNX3	-1.25	0.15	0.0075	runt-related transcription factor 3	+	+
SEZ6	-1.11	-0.06	0.0021	seizure related 6 homolog (mouse)	+	
SFMBT2	-0.64	0.87	0.0012	Scm-like with four mbt domains 2	NA	
SH2B3	-1.36	-0.22	0.0090	SH2B adaptor protein 3	+	
SHE	-2.54	-1.44	0.0020	Src homology 2 domain containing E	NA	+
SLC35D1	-2.55	-1.43	0.0030	solute carrier family 35 (UDP-glucuronic acid/UDP-N-acetylgalactosamine dual transporter), member D1	+	
SMG6	-2.70	-1.61	0.0002	Smg-6 homolog, nonsense mediated mRNA decay factor (C. elegans)	+	
SNRPF	-0.73	0.59	0.0061	small nuclear ribonucleoprotein polypeptide F		+
SNX33	-0.55	0.59	0.0048	sorting nexin 33	NA	
STRN	-2.79	-1.70	0.0078	striatin, calmodulin binding protein	+	
SV2A	-1.19	-0.02	0.0007	synaptic vesicle glycoprotein 2A		
SYK	-1.42	-0.16	0.0008	spleen tyrosine kinase		
SYNE1	-1.45	-0.40	0.0033	spectrin repeat containing, nuclear envelope 1		
TKT	-1.92	-0.90	0.0000	transketolase	+	
TM7SF4	-2.50	-1.24	0.0092	transmembrane 7 superfamily member 4		
TM9SF2	-1.01	0.26	0.0037	transmembrane 9 superfamily member 2	NA	
TMEM99	-0.32	1.02	0.0004	transmembrane protein 99	NA	
TNFAIP9	-2.3	-0.7	0.03	tumor necrosis factor, alpha-induced		+

				protein 9		
TOMM20L	-1.89	-0.53	0.0031	translocase of outer mitochondrial membrane 20 homolog (yeast)-like	NA	
TREML1	-2.16	-1.02	0.0009	triggering receptor expressed on myeloid cells-like 1	NA	
TSFM	-0.03	1.00	0.0103	Ts translation elongation factor, mitochondrial		
UBAP2	-1.65	-0.64	0.0250	ubiquitin associated protein 2		
UBE3C	-0.65	0.43	0.0014	ubiquitin protein ligase E3C	+	
UNKL	-2.10	-1.08	0.0010	unkempt homolog (Drosophila)-like		
VAMP3	-1.47	-0.33	0.0014	vesicle-associated membrane protein 3 (cellubrevin)		
WDFY3	-0.97	0.15	0.0014	WD repeat and FYVE domain containing 3		
XPA	-0.84	0.31	0.0089	xeroderma pigmentosum, complementation group A	+	
ZCCHC17	-2.06	-0.78	0.0010	zinc finger, CCHC domain containing 17	NA	
ZFX	-2.78	-1.77	0.0406	zinc finger protein, X-linked		
ZNF502	-2.65	-1.39	0.0425	zinc finger protein 502	NA	
ZNF540	-2.59	-1.58	0.0302	zinc finger protein 540	NA	
ZNF764	-0.27	0.94	0.0042	zinc finger protein 764	+	
ZNF831	-0.78	0.50	0.0309	zinc finger protein 831	NA	

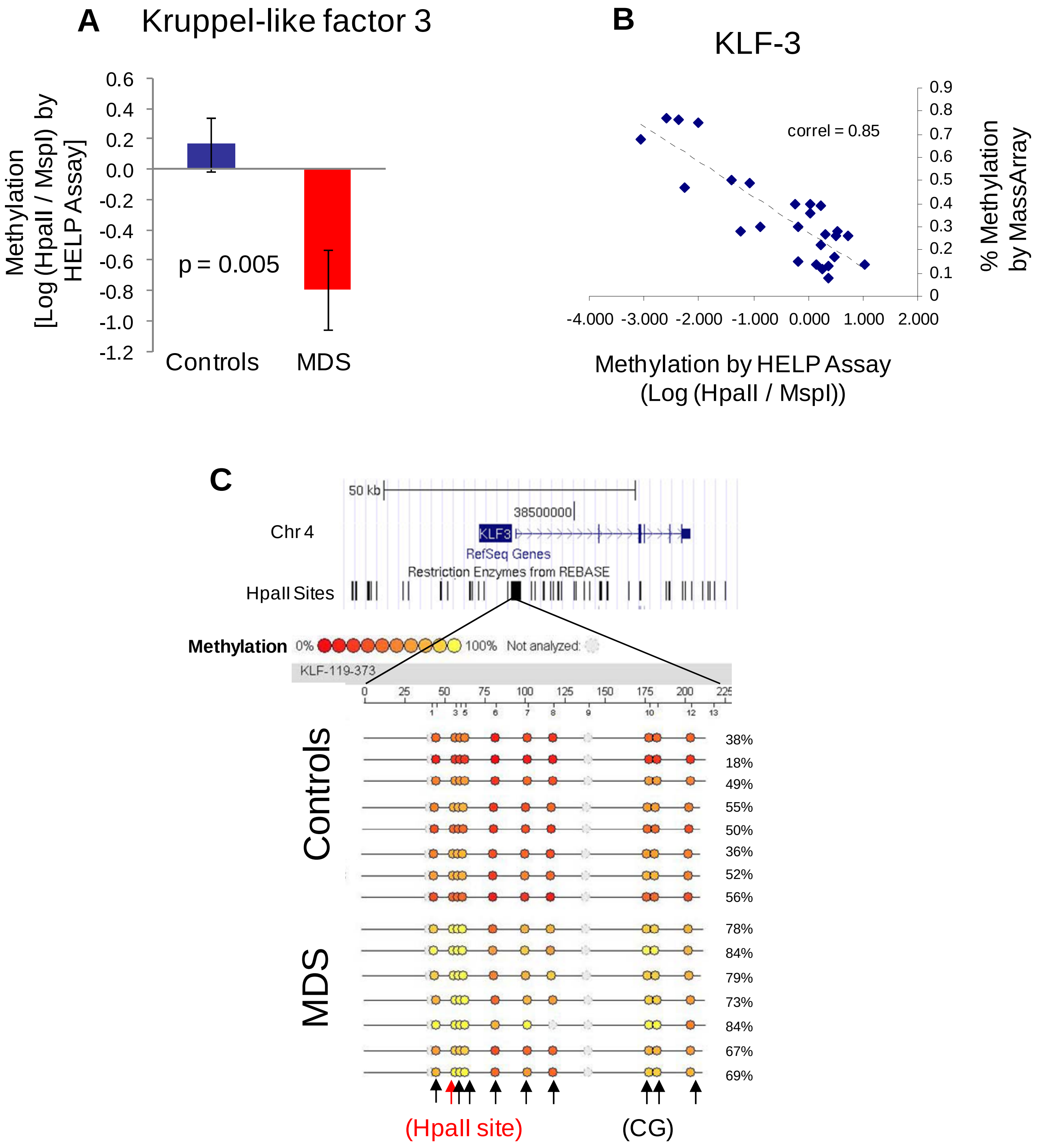
*Genes that were underexpressed in MDS bone marrow CD34+ gene expression data are indicated with +. NA refers to genes not found in the meta-analytical dataset.

A significantly higher proportion of the hypermethylated genes were found to be underexpressed in the bone marrow gene expression dataset (Proportions test, $P<0.05$, 41% underexpressed and 21% overexpressed in MDS).

Supplementary Table 2: Significantly hypomethylated loci with mean log (HpaII/MspI) ratios

Symbol	Mean HELP ratio MDS	Mean HELP ratio Normals	p-value	Gene
IGF2R	0.359626	-1.03951	0.026736	insulin-like growth factor 2 receptor
KIAA1239	-1.85303	-2.88467	0.021872	KIAA1239
LOC100131490	1.241382	0.234222	0.00222	hypothetical LOC100131490
MTMR9	-1.25005	-2.67926	0.041733	myotubularin related protein 9
PON3	-0.58988	-2.60105	0.002136	paraoxonase 3
SEC23B	-1.52286	-2.69926	0.009693	Sec23 homolog B (S. cerevisiae)
SGK269	-1.46313	-2.82733	0.009747	NKF3 kinase family member
SLC47A1	-0.97891	-2.266	0.005183	solute carrier family 47, member 1
ZNF219	-1.77894	-2.80356	0.038723	zinc finger protein 219

Supp Figure 1: Hypermethylation of genes seen in the array are validated by MassArray : The mean methylation (log2(HpaII/MspI)) for promoters of KLF3 reveals significant hypermethylation in MDS samples when compared to controls. Correlation between results of quantitative methylation obtained after MassArray sequencing reveals good correlation with the HELP assay data (B). MassArray epigrams reveal sequencing results of multiple CGs in the KLF3 promoter. MDS patients have hypermethylation of multiple CGs when compared to controls as evident from change in color from red to yellow. The black arrows represent CG dicnucleotides and the red arrow represents an HpaII site (CCGG) (C).



Supplementary Figure 2 : Functional pathways involved by hypermethylated genes in MDS