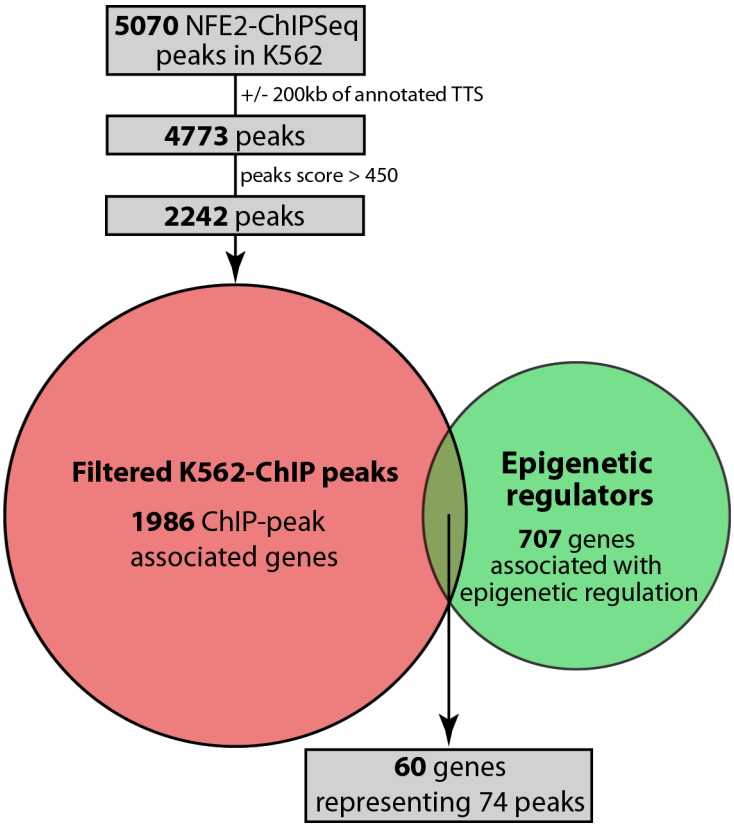
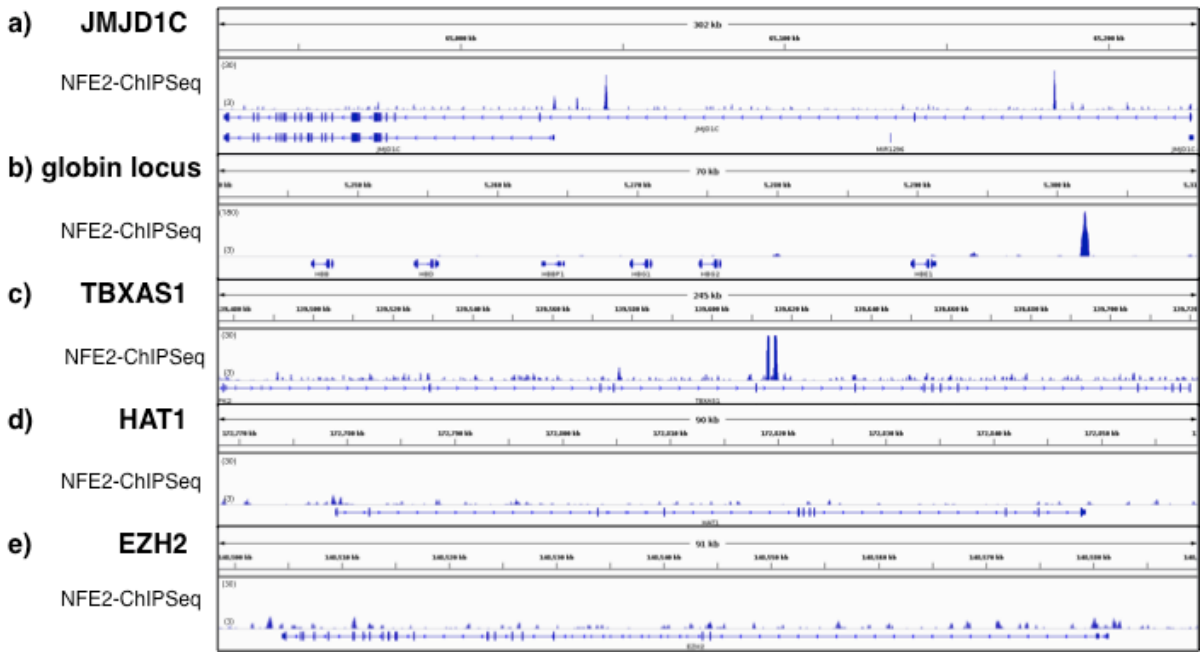
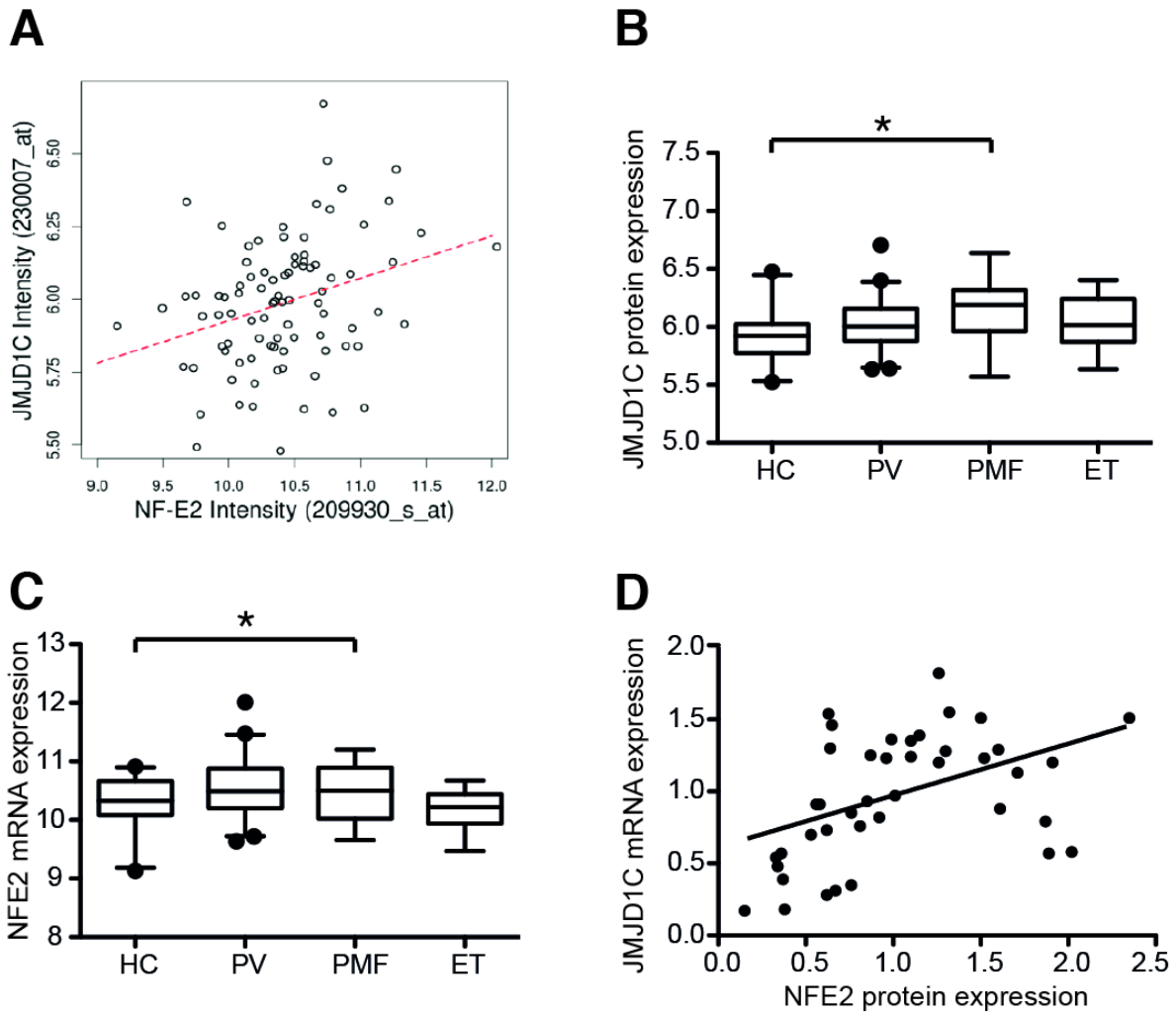
SUPPLEMENTAL MATERIAL



Supplemental Figure 1: Strategy for Identifying Epigenetic Regulators as Novel Target Genes.
ChIPseq analysis of NFE2 chromatin binding in K562 cells, provided by the ENCODE Consortium, was used to identify 5070 sites bound by this transcription factor [1]. Of these 4773 were within 200 kb of a transcriptional start site [2] and a subset of 2242 were considered highly significant (peak score above 450). These peaks were associated with 1986 genes, which were subsequently compared to a list of 707 epigenetic modifiers, published by Plaas and colleagues [3,4]. 60 epigenetic regulators that constitute potential novel NFE2 target genes were identified and are listed in Supplemental Table 1.

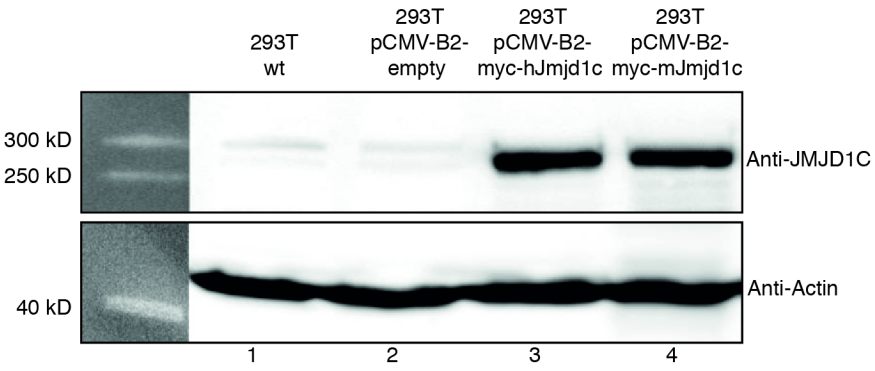


Supplemental Figure 2: NFE2 chromatin binding analyzed by ChIP-seq at various gene loci.
Visualization of NFE2 chromatin binding by ChIP-Seq at the following gene loci (ENCODE data) **(a)** JMJD1C, **(b and c)** beta-globin and TBXAS1 (known NF-E2 target genes as positive controls), **(d and e)** HAT1 and EZH2, (epigenetic enzymes, which do not show NF-E2 enrichment as negative controls).



Supplemental Figure 3. Analysis of protein and mRNA expression levels of NFE2 and JMJD1C.

(A) Published expression data from DNA microarrays from 69 MPN patients (41 PV / 19 ET / 9 PMF) and 21 healthy controls (HC) was used[5]. Statistical significance was determined using Spearman's correlation coefficient, p=0.0035. (B) Box plots of mRNA expression levels of JMJD1C (B) and NFE2 (C). Whiskers depict the 95% confidence interval. The nonparametric Kruskal-Wallis test yielded significant results indicating that samples originate from distinct distributions (p=0.036 and p=0.05 for JMJD1C and NF-E2, respectively). Statistical significance between groups was assessed using the Wilcoxon signed-rank test, *p<0.05. In total, 35 patients (50.7%) for JMJD1C and 30 (43.5%) patients for NFE2 showed mRNA expression levels above the 95% confidence interval of the HC. The comparably low elevation of NFE2 expression levels in this study contradicts results from multiple previous publications [6,7,8]. (D) Correlation of JMJD1C and NFE2 protein levels. Statistical significance was determined using Spearman's correlation coefficient, p=0.0005. See Supplemental Table 6 for patient characteristics.



Supplemental Figure 4. Validation of the JMJD1C Antibody. 293T cells were left untransduced (lane 1), transduced with an empty vector (lane 2) or with vectors encoding human or murine JMJD1C cDNAs (lanes 3 and 4, respectively). Whole cell extracts were subjected to Western Blotting and interrogated with an anti-JMJD1C antibody (top) and subsequently with an antibody to actin (bottom) to assure equal loading.

Supplemental Table 1: ChIP Antibodies.

NFE2	Santa Cruz, sc-291
control anti-IgG	Cell Signaling, #2729
H3K9me2	Activ Motif, 39753
HP1α	Cell Signaling, C7F11
H3Y41ph	Abcam, 26310
JMJD1C	Abcam, AB31215

Supplemental Table 2: Primers used for DNA amplification after Chromatin Immunoprecipitation.

JMJD1C -120 bp FP	5' CTTGAGCTTTCAGTCTCGCC 3'
JMJD1C -120 bp RP	5' TCACAGGTGCTGATGGAGTC 3'
JMJD1C -16 kb FP	5' TGACATTCAGGTTCTGCTCC 3'
JMJD1C -16 kb RP	5' GGGCCTACACCATTGATAAC 3'
JMJD1C -154 kb FP	5' CTTCATACATTGATGATGGGAAC 3'
JMJD1C -154 kb RP	5' CTCTGTTGCTGGACATTTGG 3'
JMJD1C +3.2 kb control FP	5' AGATTGCAGTGAGTCAACCTG 3'
JMJD1C +3,2 kb control RP	5' ATTGTGTTTCCCCTTTCTCTTATTC 3'
myogenin control FP	5' AGGGGCTGCTGAGAAATGAAAAC 3'
myogenin control RP	5' ATATAGCCAACGCCACAGAAACCT 3'
NFE2 1A Promotor FP	5' CGTCTGAAGCCCTCGGCCTGAG 3'
NFE2 1A Promotor RP	5' AGAAACGCAGACCTGGCTGGACTC 3'
NFE2 1F Promotor FP	5' TTCCAAGATGATGACAGTATATGC 3'
NFE2 1F Promotor RP	5' CTGATTGTATGGGTTAAGGTATGG 3'
NFE2 -5kb Enhancer FP	5' CAGAAAATTAGCTGGGCATCTTGGC 3'
NFE2 -5kb Enhancer RP	5' GCTTGGGTCCTGGCATTTATTGGC 3'

Supplemental Table 3: Antibodies used for Immunoblotting.

NFE2	Goerttler et al., 2005
JMJD1C	Abcam, AB31215
JMJD1C	Merck Millipore, 17-10262
SHP-1	Cell Signaling, C14H6
JAK2	Cell Signaling, 3230
phospho-JAK2	QCB inc., 44-426
HP1α	Cell Signaling, C7F11
H3K9me3	Millipore, 07-442
H3K9me2	Activ motif, 39753
H3K9me1	Millipore, 07-450
β-ACTIN	Sigma-Aldrich, Clone AC-15
HISTONE 3	Abcam, AB1791
GAPDH	Sigma-Aldrich, Clone 71.1
PU.1	Cell Signaling Technology, 2266

Supplemental Table 4: Primers used for quantitative RT-PCR.

B2-microglobulin FP	5’ CTTTCTGGTGCTTGTCTCACTGAC 3’
B2-microglobulin RP	5’ GGTGGCGTGAGTATACTTGAATTTG 3’
B2-microglobulin Probe	5’ ATCCAGAAAACCCC 3’

Supplemental Table 5: Potential Novel Target Genes

For each peak, the Peak Signal Value reported by ENCODE is given *(https://genome.ucsc.edu/encode).* Peak Signal Values range: 0 – 1000.

Genes are sorted in the following order:
The two genes containing three potential NFE2 peaks, ordered by the sum of the peak values, followed by the ten genes containing two potential NFE2 peaks, ordered by the sum of the peak values, followed by the remaining genes containing one NFE2 peak, ordered by the peak value [2].

GeneID	Peak value 1	Peak value 2	Peak value 3	Sum of peak values
SMYD3	1000	1000	488	2488
SETMAR	719	670	480	1869
ATXN7L1	961	501		1462
CHD6	1000	899		1899
EED	808	471		1279
NCOR2	641	458		1099
SMARCD3	556	529		1085
NR3C1	579	496		1075
TBL1XR1	536	506		1042
TRIM27	528	457		985
JMJD1C	495	457		952
CENPO	456	453		909
BRD2	1000			1000
KAT5	1000			1000
NIPBL	1000			1000
HIST1H4H	885			885
PRDM6	875			875
CHD9	845			845
USP25	842			842
UBE2H	790			790
PPP2R2D	761			761
ADNP2	749			749
TET2	747			747
ZMYND8	745			745
UBR2	707			707
GTF2A1	696			696
HLCS	641			641
HIST1H1C	625			625
SALL1	566			566
PPP2R2C	564			564
EP400	553			553
PRDM1	550			550
IDH1	545			545
TRIM33	542			542
KBTBD7	536			536
ZZZ3	534			534
ZNF518B	526			526
EHMT1	521			521
SIRT1	513			513
MAP3K8	507			507
ZFPM2	507			507

JAZF1	505			505
HIST1H4B	504			504
RPS6KA5	500			500
BTAF1	497			497
DPF1	497			497
FLYWCH1	497			497
GADD45A	492			492
PPP2R5A	492			492
SNAI2	485			485
MYT1L	484			484
BRWD1	480			480
YEATS2	480			480
CREBBP	471			471
PADI4	464			464
SENP7	459			459
SETD8	459			459
BRD7	458			458
PIAS4	457			457
TAF4	457			457

Supplemental Table 6: Patient characteristics

UPN: unique patient number; HB: hemoglobin, PLT: platelet count, WBC: white blood cell count; Follow up: from time of diagnosis to sample acquisition; cytoreductive treatment at the time of sample acquisition. PV patients were, in addition, treated by phlebotomy and ASS, according to international guidelines.

UPN	Diagnosis	Driver mutation	HB (g/dl)	WBC (G/l)	PLT (G/l)	Follow up	cytoreductive treatment
1457	PV	$JAK2^{V617F}$	7.9	25.9	48	21 years	-
1732			10.8	8.1	678	15 years	Hydroxyurea + Anagrelide
3984			16.4	6.4	517	12 years	Hydroxyurea
5183			15.6	8.1	567	16 years	Hydroxyurea
5936			14.0	6.2	448	17 years	Hydroxyurea
6739			14.9	25.6	502	12 years	-
10582			21.9	11.58	339	1 year	-
10528			18.2	8.97	369	4 years	-
10559			8.6	16.44	254	19 years	Ruxolitinib
10584			18.5	7.68	584	at diagnosis	-
2804	ET	$JAK2^{V617F}$	15.4	10	985	18 years	Anagrelide
2750			17.1	10.4	1062	17 years	Anagrelide
10604			12	11	531	4.5 years	Anagrelide
10709		CALR	12.8	5.4	1163	7 years	Hydroxyurea
2860	PMF	$JAK2^{V617F}$	16.5	13.1	821	5.5 years	Hydroxyurea
2912			14.6	8.2	598	3 years	-
2867		CALR	10.1	7.6	575	5 years	Hydroxyurea
2941			14.9	7.72	869	at diagnosis	-
10561			11.9	5.84	185	3 years	Ruxolitinib
10696			13.9	12.88	713	at diagnosis	-

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