Supplemental Table 1. Patient characteristics for samples for TLR1, 2 and 6 RNA expression analysis (N=103).

Characteristics		**No. of Patients**	**%**
Age, years			
Median	68		
Range	33-92		
Sex			
Male		59	57%
Female		44	43%
BM blast, %			
Median	4		
Range	0-26		
WBC, X10^9/ L			
Median	4		
Range	1-83.5		
Hemoglobin, g/dL			
Median	10		
Range	6.4-15		
Platelet, X10^9/ L			
Median	74		
Range	4-1040		
Neutrophil, X10^9/L			
Median	50		
Range	5- 94		
Dx			
5q-		2	1.9%
RA/ RARS/ RCMD/ RCMD-/ Rs/ MDS-U		46	44.7%
RAEB		36	35.0%
RAEBT		4	3.9%
CMML		15	14.6%
IPSS			
H		10	9.9%
I-2		23	22.8%
I-1		39	38.6%
L		29	28.7%
NA		2	
Cytogenetic			
Dip, -Y		70	68.6%
5/ 7-		17	16.7%
Other		14	13.7%
IM		1	1.0%
ND		1	

Supplemental Table 2. Patient characteristics for samples for TLR2-F217S sequencing (N=149).

Characteristics			No. of Patients	%
Age, years				
	Median	69		
	Range	28-89		
Sex				
	Male		99	66
	Female		50	34
BM blast, %				
	Median	7		
	Range	0-52		
WBC, X10^9/ L				
	Median	3		
	Range	0.7-116.1		
Hemoglobin, g/dL				
	Median	10		
	Range	6.8-15		
Platelet, X10^9/ L				
	Median	68		
	Range	1-954		
Neutrophil, X10^9/L				
	Median	49		
	Range	0-96		
Ferritin, υg/L				
	Median	574		
	Range	11-21197		
Dx				
	5q-		3	2
	RA/ RARS/ RCMD/ RCMD-/ Rs/ MDS-U		57	38
	RAEB		63	42
	RAEBT		8	5
	CMML		18	12
IPSS				
	H		16	11
	I-2		44	30
	I-1		50	34
	L		37	25
	NA		2	1
Cytogenetic				
	Dip, -Y		91	61
	5/ 7-		31	21
	Other		23	15
	IM		4	3

Supplemental Table 3. Patient characteristics for BM CD34+ cells used for TLR2 inhibition

sample ID	Age	Sex	Race	CG Group	IPSS	BM BL	WBC	Plt	Hgb	Neut
lower-risk #1	73	M	W	Dip	INT-1	5	2.1	108	10	36
lower-risk #2	79	F	W	-5/5q-	Low	2	2.2	277	9.9	68
lower-risk #3	78	M	W	Dip	Low	2	4	126	11.1	31
lower-risk #4	66	M	W	Dip	Low	3	5.4	52	13.7	63
higher-risk #1	80	M	W	Mis	High	19	63.5	33	8.1	20
higher-risk #2	81	F	W	+8	INT-2	10	2.2	123	9.4	29
higher-risk #3	64	M	W	Dip	INT-2	15	3.6	24	10.4	37

Supplemental Figure 1

A

GENE	chr	location (hg18)	SNP	sample ID
TLR2	chr4	154845515	CCC-CTC	WBM40
TLR2	chr4	154845415	AGC-CGC	WBM51
TLR2	chr4	154844159	TTT-TCT	WBM57
TLR4	chr9	119515069	TGC-TAC	WBM69

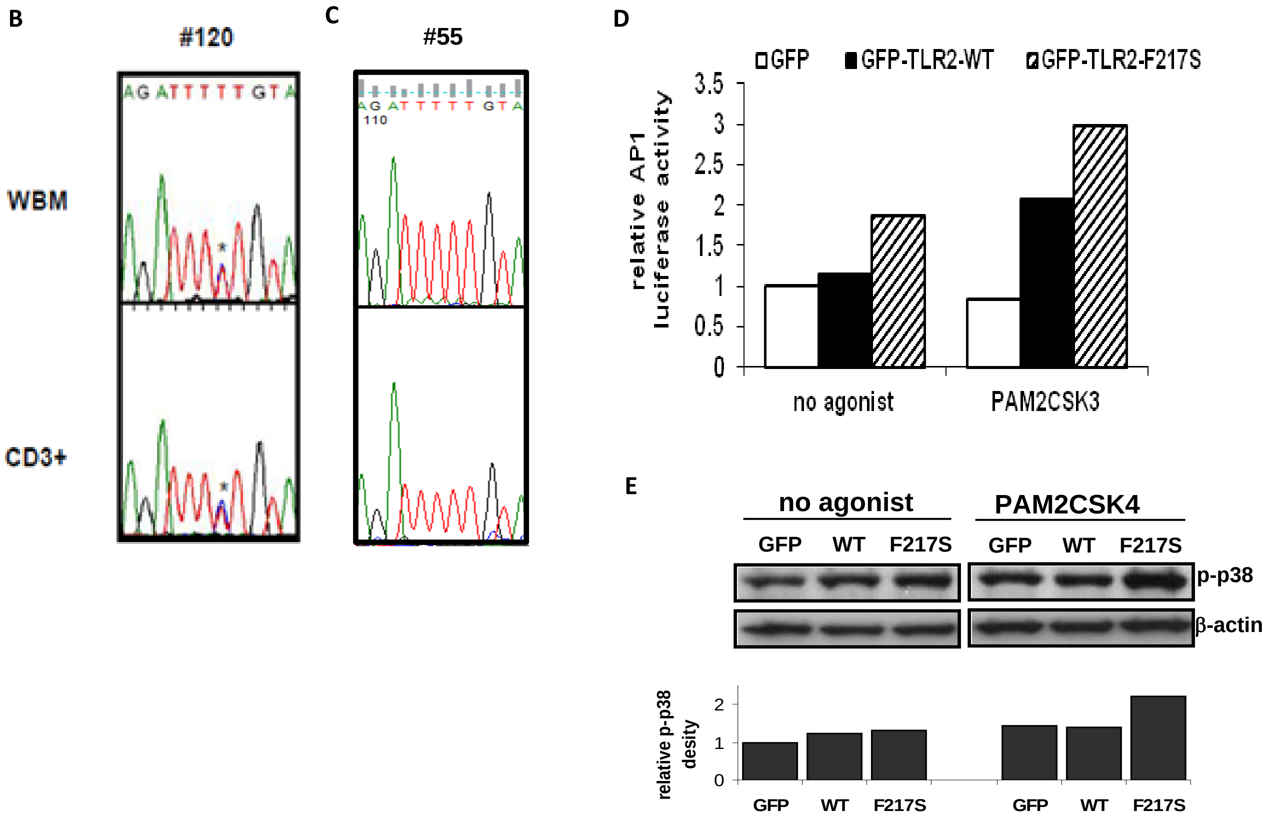

Figure S1. Mutational analysis of TLRs in MDS. (a) Identification of three rare SNPs of TLR1 and one rare SNP of TLR4 by captured deep sequencing in MDS BM-MNCs. (b) The presence of TLR2-F217S in both BM-MNC and BM-CD3+ cells of one case of MDS. (c) Lack of TLR2-F217S in BM-MNC or BM-CD3+ cells of another case of MDS. (d) Luciferase analysis of activation of AP1 by WT and F217S TLR2 in transfected 293T cells. Cells were exposed to either no agonist or to PAM2CSK4. (e) Western blot characterization of p38 phosphorylation in 293T cells transfected with either WT or F217S TLR2 and treated with no agonist, MALP2 or PAM2CSK4. Top, Western blot analysis of p-p38; Bottom, Quantification of p-p38 band densities.

Supplemental Figure 2

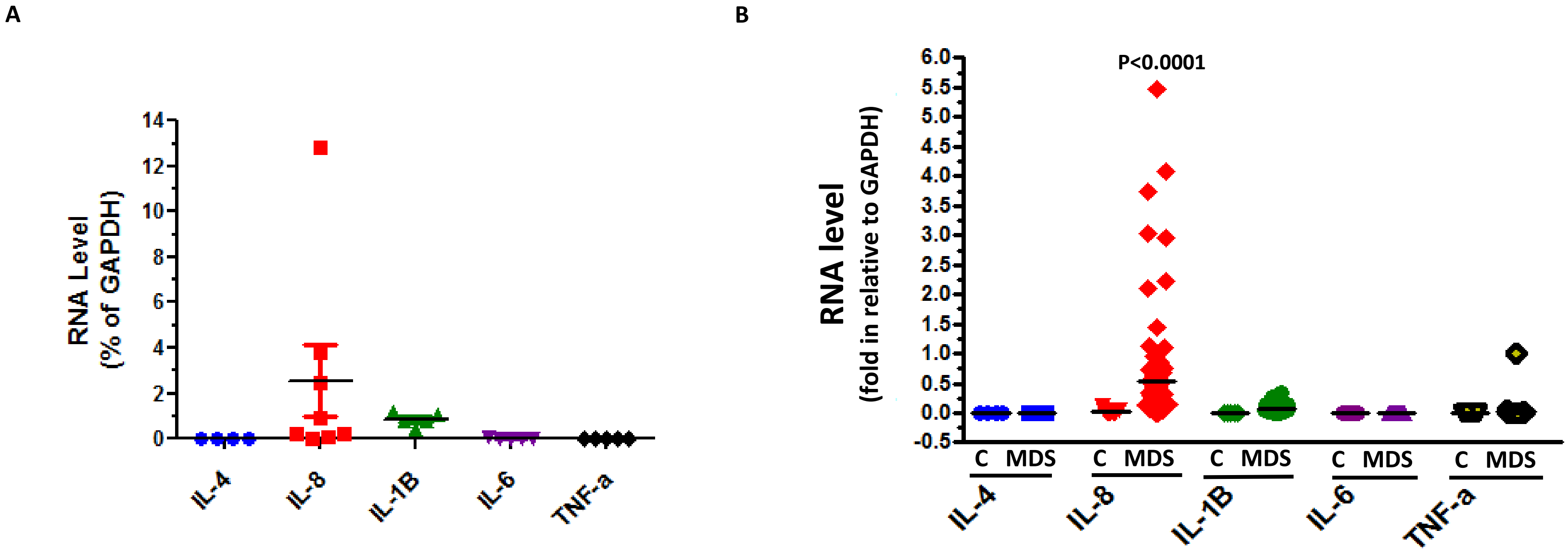

Figure S2. Overexpression of IL-8 in MDS BM CD34+ cells. (a) Q-RTPCR analysis of multiple genes of inflammatory cytokines in normal BM CD34+ cells. (b) Q-RTPCR analysis of multiple genes of inflammatory cytokines in MDS BM CD34+ cells in comparison to controls.

Supplemental Figure 3

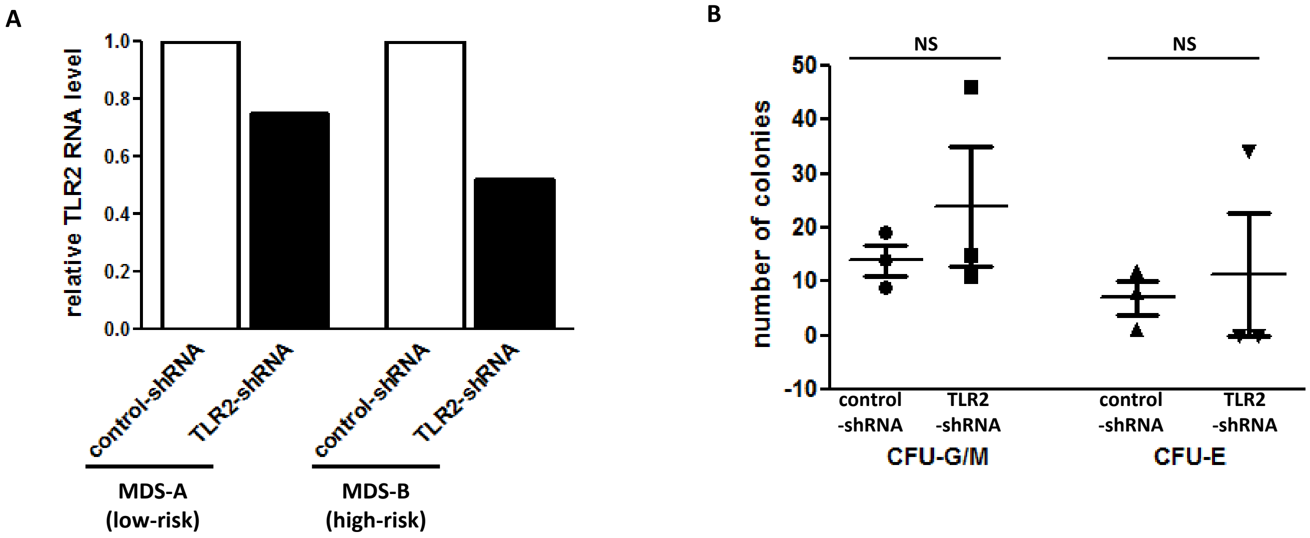

Figure S3. Inhibition of TLR2 in primary MDS BM CD34+ cells. (a) Q-PCR analysis of TLR2 RNA expression after transduction of shRNA into two primary MDS BM CD34+ cells. (b) Methocult medium supported colony formation assays revealed no significant difference for the formation of CFU-G/M or CFU-E in BM CD34+ cells of higher-risk MDS (intermediate-2 and high-risk) two weeks after transduction of TLR2-shRNA and control shRNA.

Supplemental Figure 4

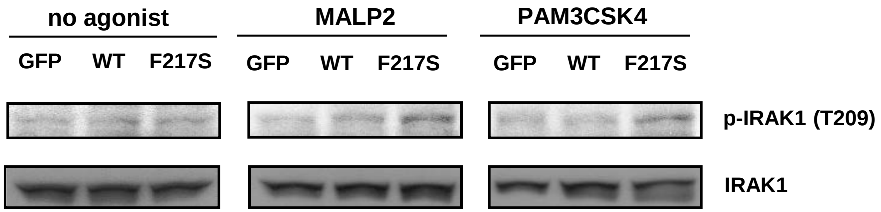

Figure S4. Western blot characterization of IRAK1 phospho-T209 in 293T cells transfected with either WT or F217S TLR2 and treated with no agonist, MALP2 or PAM3CSK4. Top, Western blot analysis of p-T209 of IRAK1; Bottom, Western blot analysis of IRAK1.