Title

MiRNA182-5p regulates myeloid-erythroid ratio of chronic myeloid leukemia cells.

Deepak Arya1, 2, Sasikala P.S.1, Cecil Ross3, Dasaradhi Palakodeti4, Shang Li5, Sudhir Krishna1, #

Supplementary File: Table- 1, Table- 2, Figure S1-12

Supplementary table 1- Clinical details of CML patients

Sample	Disease	Response Status	BCR ABL
K562	CML	Blast	Positive
CC1	CML	Yes	48.52
CC2	CML	Yes	Negative
CC3	CML-fresh	Fresh CML	
CC4	CML fresh	Fresh CML	
CC5	CML-chronic	Yes	48.52
CN1	CML	No	10.88
CN2	CML	No	
CN3	CML-fresh	No	NEG
CB1	CML-BLAST	No	
CB2	CML	Yes	
CB3	CML		
NR1	CML	No	0.605
NR2	CML-NR	No	57.43
NR3	CML	No	25.62
NR4	CML	No	0.00354
NR5	CML	No	
NR6	CML-NR	Yes	
NR7	CML	No	
NR8	CML-blast	No	

Supplementary Table 2- Normalised fold change in the expression of miRNA182-5p.

MiRNA	Control (Normalised)	Imatinib (Normalised)
hsa-miR-941	1	0.068028171
hsa-miR-30c-5p	1	0.720134867
hsa-miR-19a-3p	1	1.267480223
hsa-miR-27b-3p	1	0.084736281
hsa-miR-1246	1	0.499664526
hsa-miR-642a-3p	1	0
hsa-miR-374b-5p	1	0.072930064
hsa-miR-130b-5p	1	0.008215668
hsa-let-7i-5p	1	0.00079515
hsa-miR-148b-3p	1	0.55020501
hsa-miR-1278	1	0
hsa-miR-374a-3p	1	0.072611937
hsa-miR-425-5p	1	0.125941609
hsa-miR-24-3p	1	0.086355795
hsa-miR-33a-5p	1	0.001261692
hsa-miR-1291	1	0.001186051
hsa-miR-15b-5p	1	0.075365058
hsa-let-7d-5p	1	0.00055199
hsa-miR-151a-5p	1	0.039158973
hsa-miR-22-3p	1	0.030651689
hsa-miR-18a-5p	1	0.313138471
hsa-miR-222-3p	1	0
hsa-miR-144-3p	1	0.008879411
hsa-miR-320b	1	0.003410919
hsa-miR-151a-3p	1	0.103428327
hsa-miR-330-3p	1	0.008772168
hsa-miR-125a-5p	1	0.049376705
hsa-miR-182-5p	1	2.998313088
hsa-let-7b-5p	1	0.000369092
hsa-miR-23a-3p	1	0.103891736
hsa-miR-148a-3p	1	0.630124942
hsa-miR-7-5p	1	0.093070343
hsa-miR-744-5p	1	0.000901427
hsa-miR-99b-5p	1	0.101314617
hsa-miR-92b-3p	1	0.002587935
hsa-miR-486-3p	1	0.004077553
hsa-miR-106b-5p	1	0.053452099
hsa-miR-423-3p	1	0.014604611
hsa-miR-221-3p	1	0
hsa-miR-16-5p	1	0.180895997
hsa-miR-186-5p	1	0.089614021
hsa-let-7c	1	0.003299537
hsa-miR-223-3p	1	0.005871449

hsa-miR-93-5p	1	0.449292322
hsa-miR-340-5p	1	0.005942312
hsa-miR-144-5p	1	0.478218307
hsa-miR-26a-5p	1	0.032706767
hsa-miR-29a-3p	1	0.01236458
hsa-miR-30e-5p	1	0.712356005
hsa-miR-26b-5p	1	0.008254059
hsa-miR-128	1	0.002599649
hsa-miR-224-5p	1	0.002482631
hsa-miR-142-5p	1	0.006297749
hsa-miR-486-5p	1	0.063843255
hsa-miR-130b-3p	1	0.023893347
hsa-miR-20a-5p	1	0.664997979
hsa-miR-10a-5p	1	0.120275996
hsa-miR-451a	1	0.493771868
hsa-miR-17-5p	1	0.226033823
hsa-miR-107	1	0.000908133
hsa-let-7g-5p	1	0
hsa-miR-17-3p	1	0.004599515
hsa-miR-192-5p	1	0.020082504
hsa-miR-452-5p	1	0.010758428
hsa-miR-30d-5p	1	0.086356953
hsa-miR-19b-3p	1	0.132467871
hsa-miR-25-5p	1	0.000126266
hsa-miR-146b-5p	1	0.091046425
hsa-miR-101-3p	1	0.066583124
hsa-miR-191-5p	1	0.018408132
hsa-miR-378a-3p	1	0.450659093
hsa-miR-92a-1-5p	1	0.00012145
hsa-let-7e-5p	1	0.00075418
hsa-miR-25-3p	1	0.004684743
hsa-miR-140-3p	1	0.0102982
hsa-miR-21-5p	1	0.023982188
hsa-miR-423-5p	1	0.00626765
hsa-miR-92a-3p	1	0.015070228
hsa-miR-103a-3p	1	0.016940454
hsa-let-7f-5p	1	0.001901265
hsa-miR-185-5p	1	0.001187326
hsa-let-7a-5p	1	0.000560282
hsa-miR-320a	1	0.000452567

Fig.S1 Effect of Imatinib on Bcr-Abl tyrosine kinase.

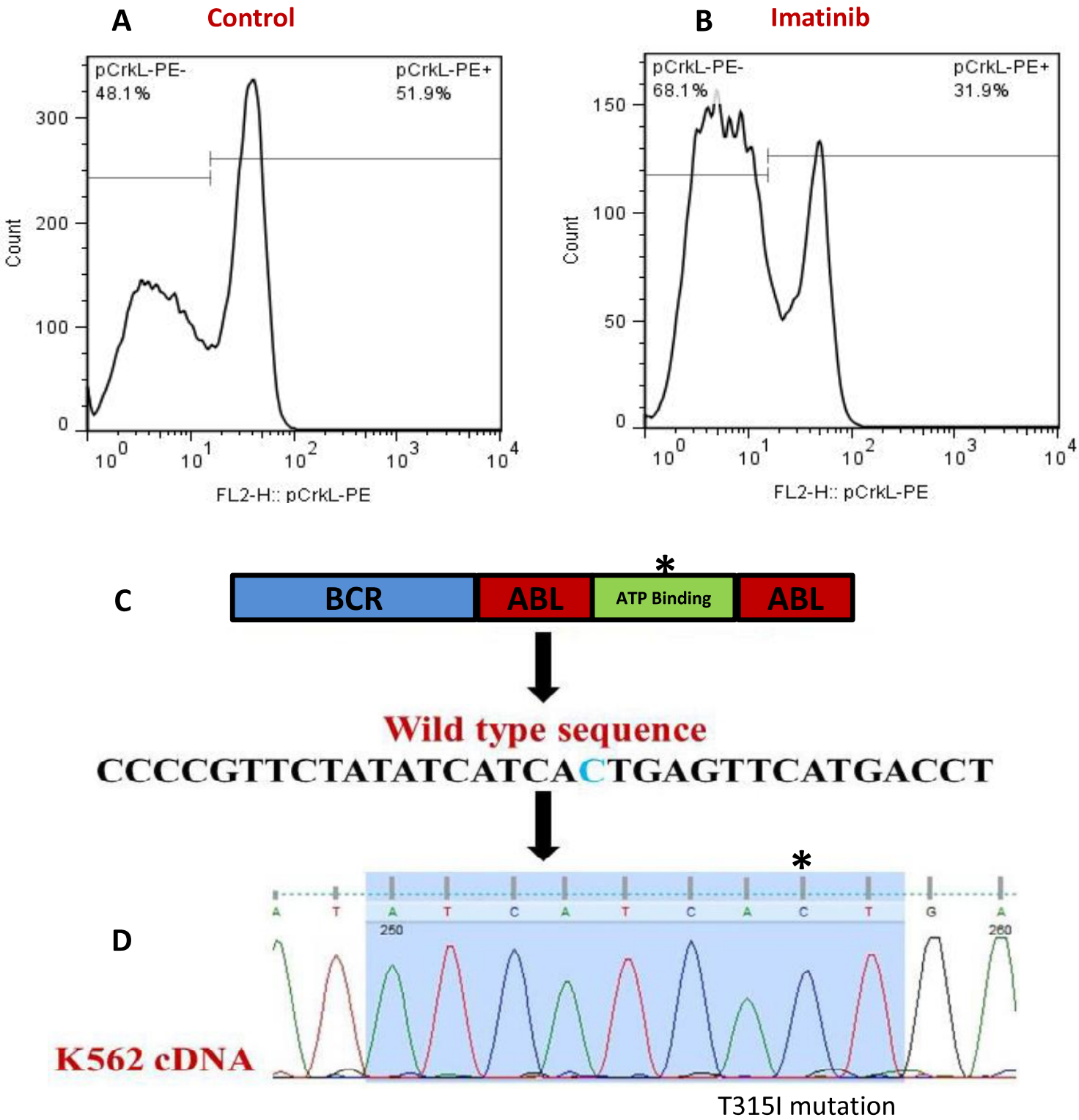
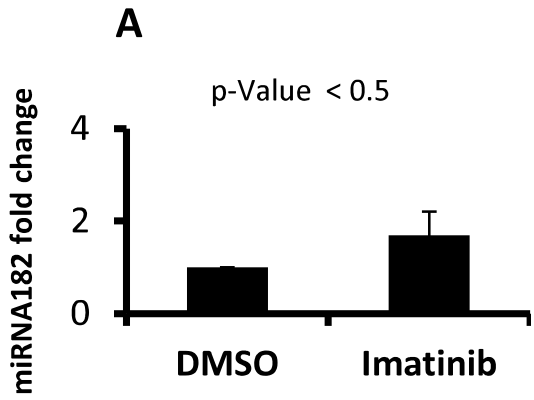
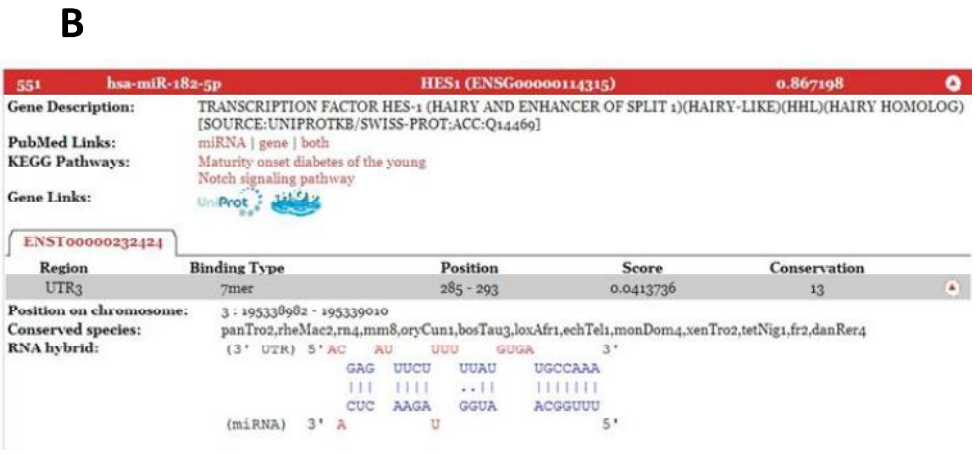


Fig.S2 MiRNA182-5p negatively correlates with Notch signalling target genes in the context of TKI resistance.

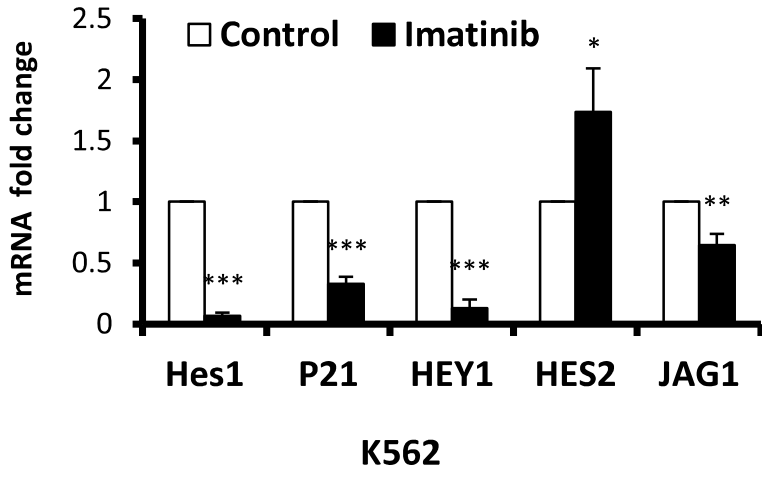




C

Conserved

	predicted consequential pairing of target region (top) and miRNA (bottom)	seed match	site-type contribution	3' pairing contribution	local AU contribution	position contribution	TA contribution	SPS contribution	context+ score	context+ score percentile	conserved branch length	PCT
Position 288-294 of HES1 3' UTR	5' ...UUCUUUUUUAUGUGAUGCCAAAG...	7mer-1A	-0.074	0.001	-0.011	-0.041	0.010	-0.027	-0.14	70	2.419	0.74
hsa-miR-182	3' UCACACUCAAGAUGGUAACGGUUU											



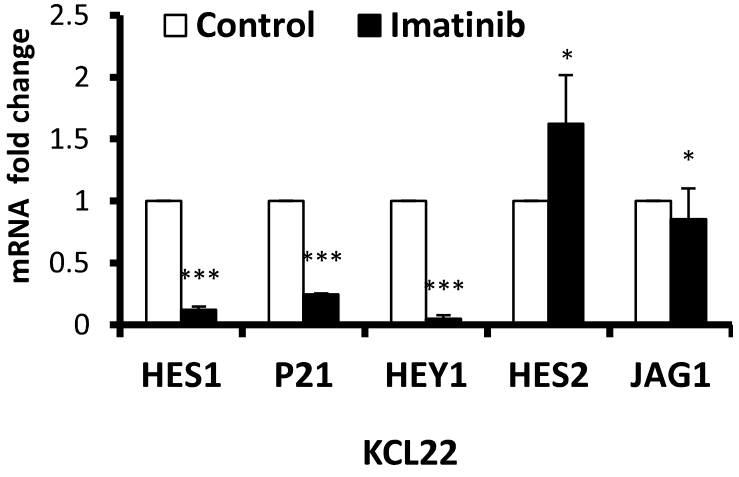
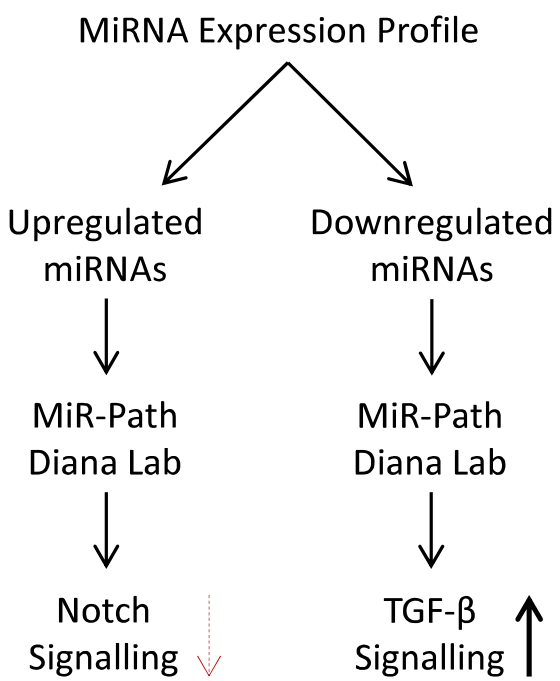


Fig.S3 Analysis of miRNA182 signalling components.

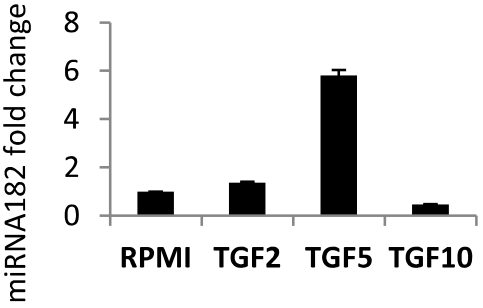
A



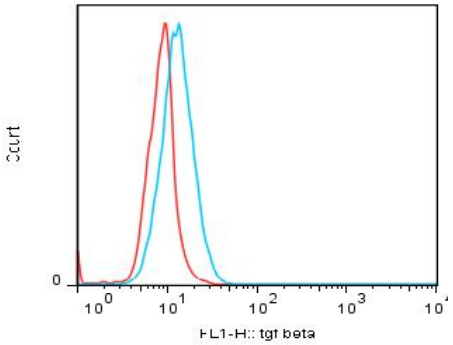
B

#	KEGG pathway	p-value	#genes	#miRNAs
1.	Fatty acid biosynthesis (hsa00061)	<1e-16	1 see genes	2
2.	ECM-receptor interaction (hsa04512)	<1e-16	12 see genes	6
3.	Glycosaminoglycan biosynthesis - heparan sulfate (hsa00534)	<1e-16	7 see genes	7
4.	Mucin type O-Glycan biosynthesis (hsa00512)	1.776357e-15	7 see genes	5
5.	TGF-beta signaling pathway (hsa04350)	1.133326e-09	35 see genes	2
6.	Prion diseases (hsa05020)	4.753938e-08	7 see genes	1
7.	Lysine degradation (hsa00310)	3.177965e-05	3 see genes	1
8.	Axon guidance (hsa04360)	7.888913e-05	27 see genes	4
9.	Endocytosis (hsa04144)	0.0003580835	57 see genes	4

C



D



E

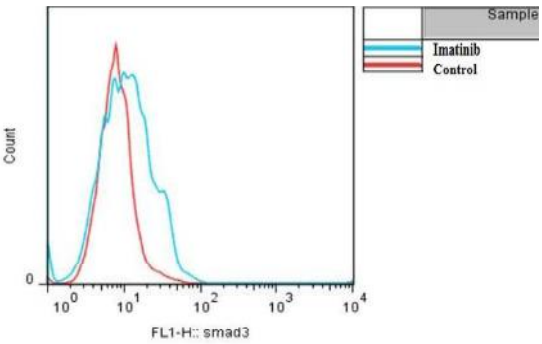
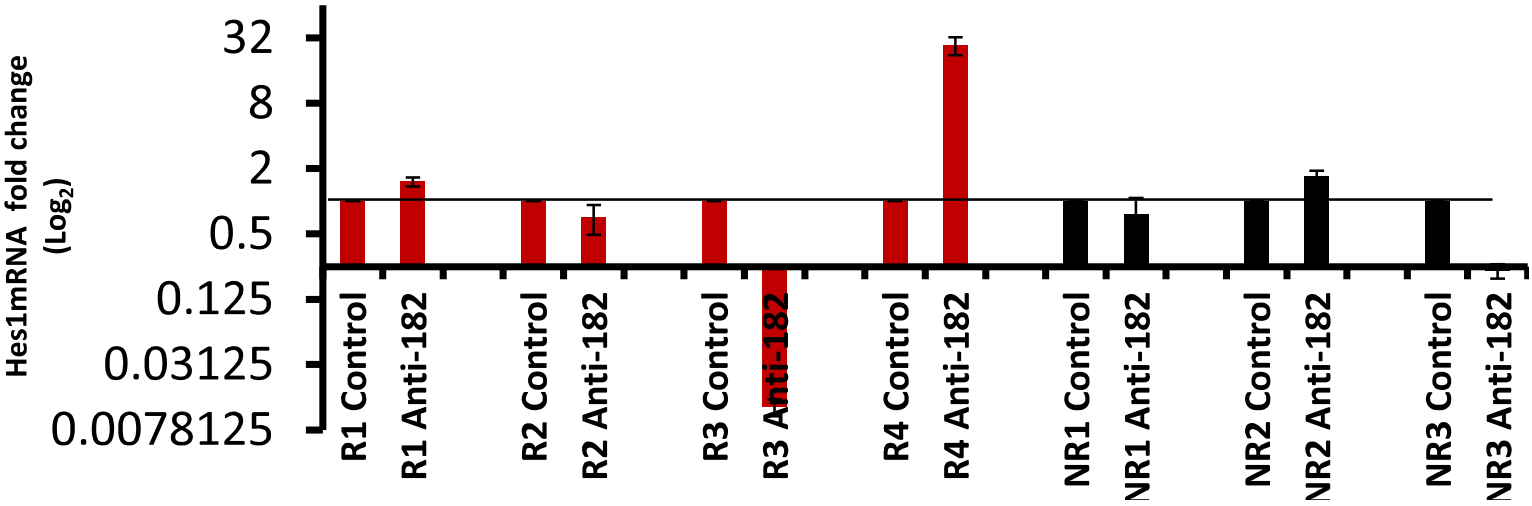
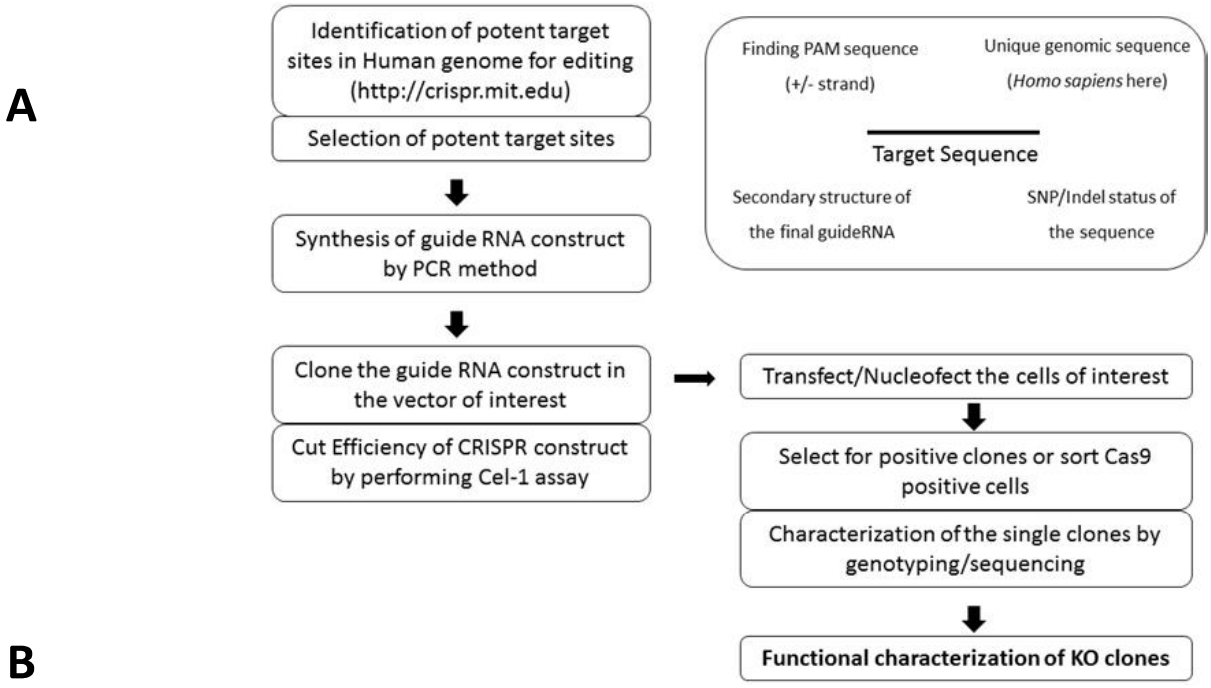


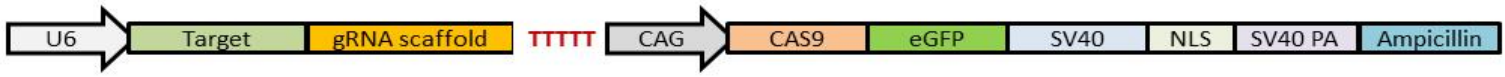
Fig.S4 Effect of anti-miRNA182-5p on Hes1 expression in primary CML cells.

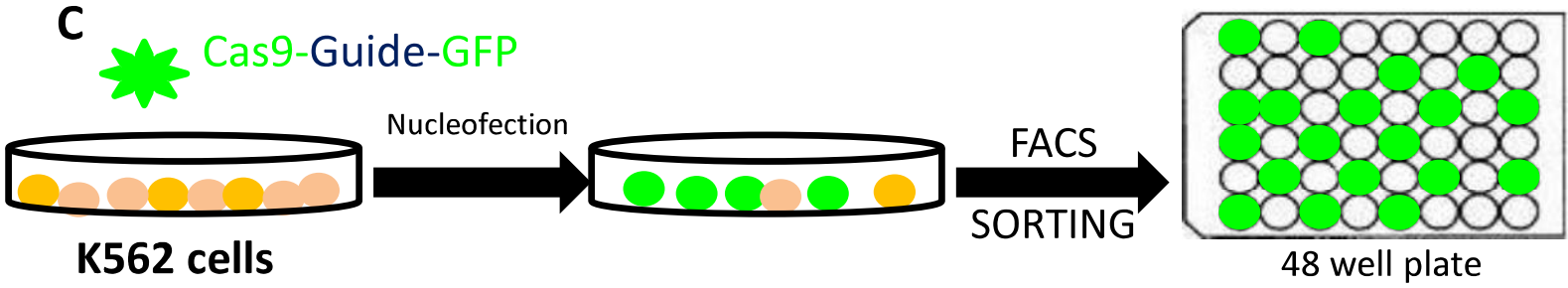


R: responder, NR: non-responder

Fig.S5 Experimental layout of *MIR182* knockout in K562 cells.







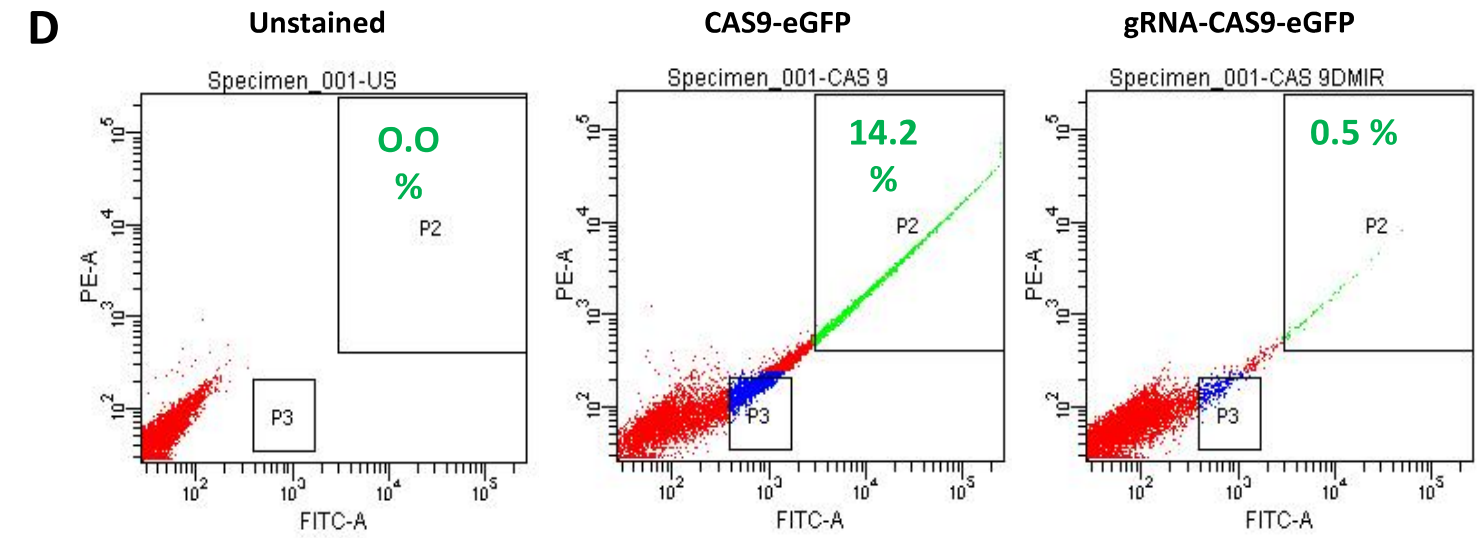
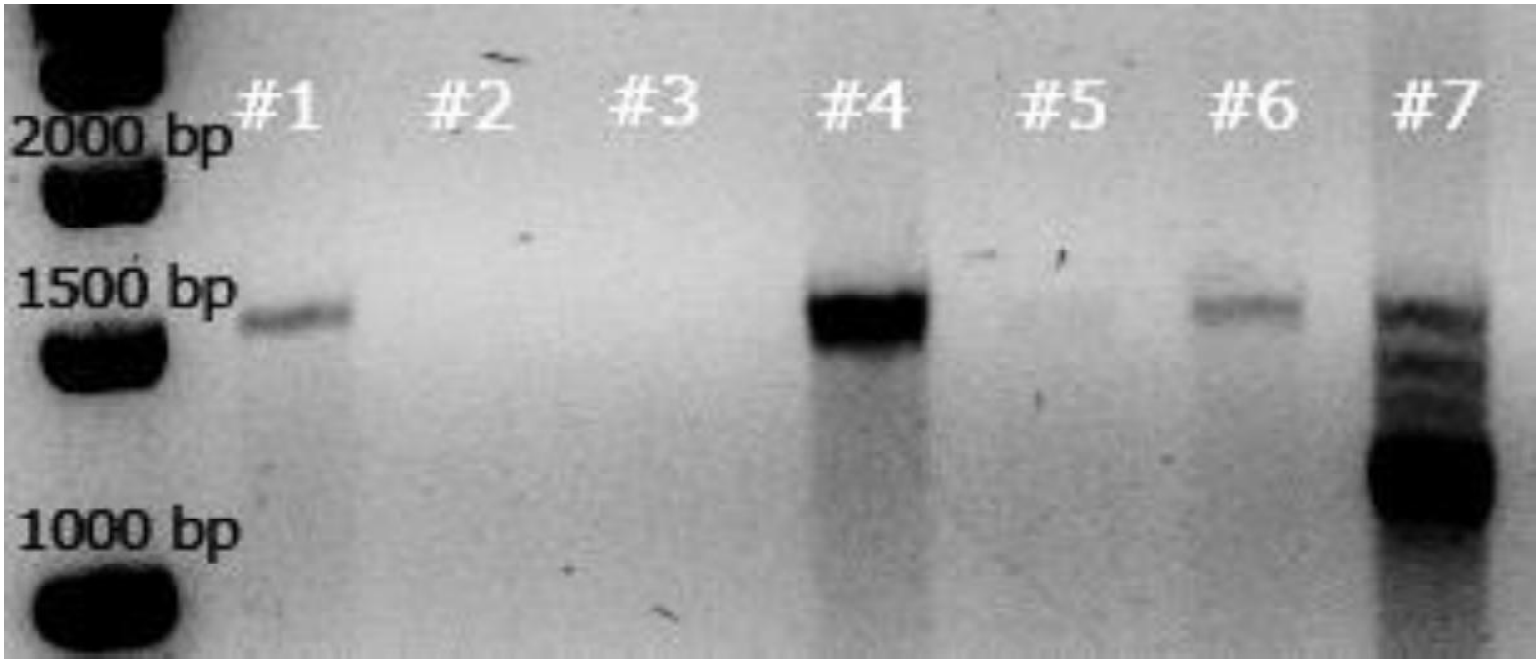


Fig.S6 CRISPR mediated *MIR182* knockout in HEK293 cells.

A



B

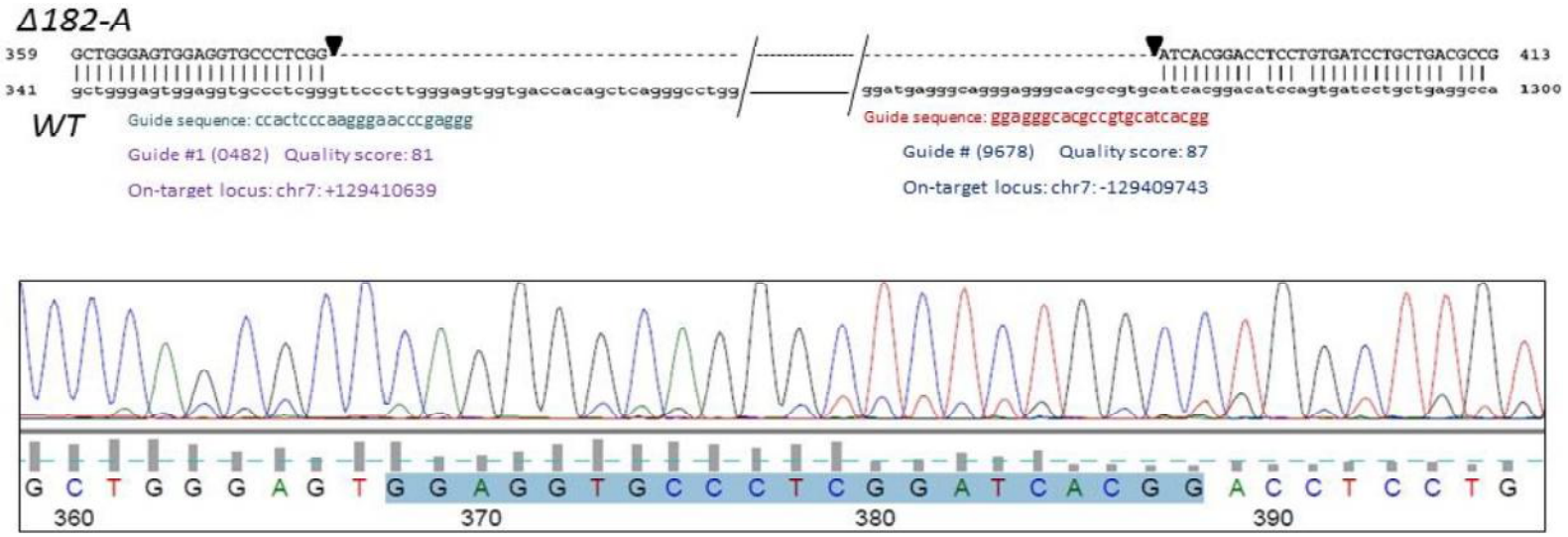


Fig.S7 Effect of modulation in expression of miRNA182-5p on differentiation potential of K562 cells.

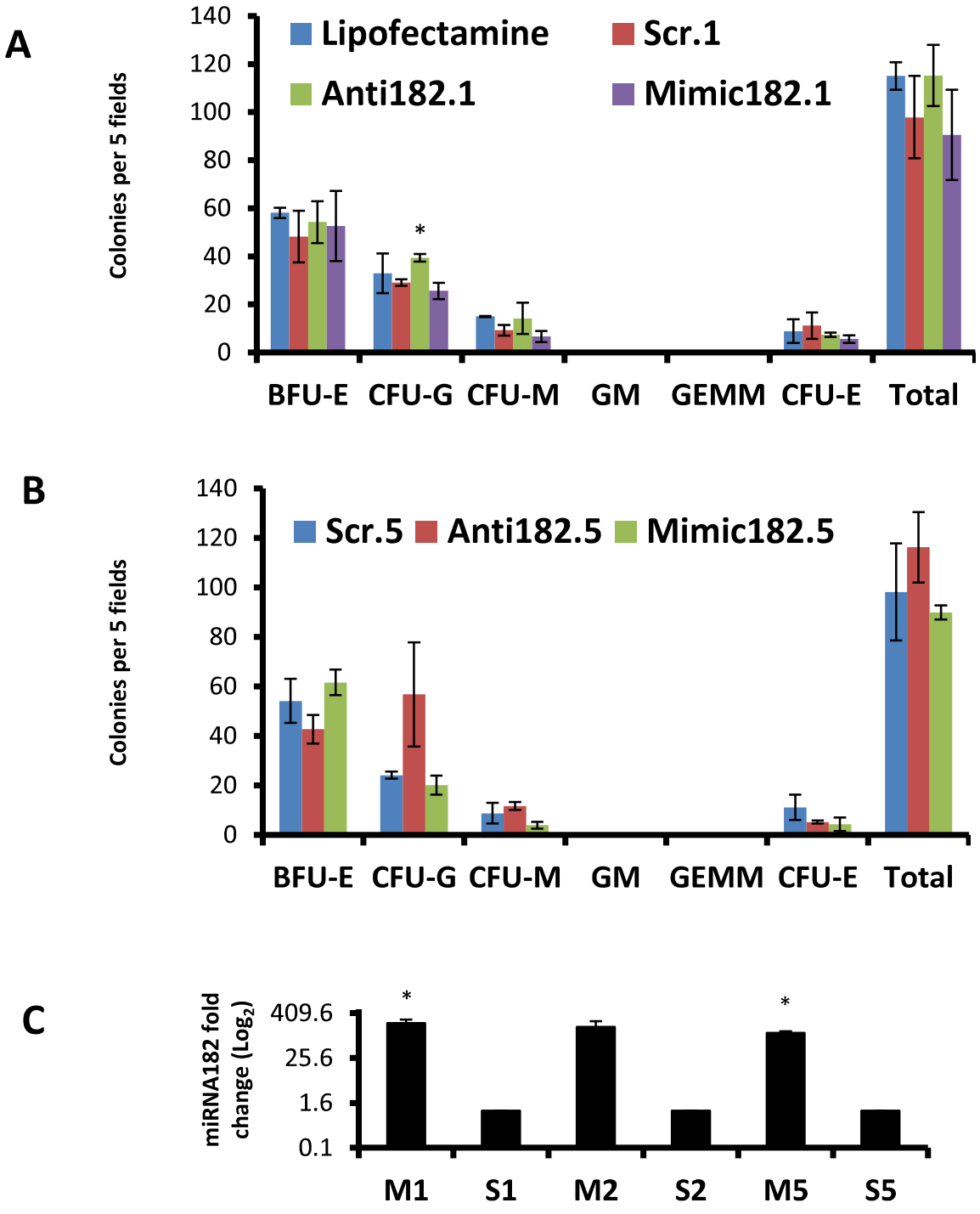


Fig.S8 Off-target analysis of CRISPR guideRNAs targeting *MIR182* locus.

Guide #4 (0425)

Quality score: 88

Guide sequence: GAAGGACCTTGTCGCAGTTG**CGG**

On-target locus: chr7:-129410425

Number of off-target sites: 90 (25 are in genes)

Sequence	score	mismatches	UCSC gene	locus
TGAAGGCCTTGTCGCAGTTGGGG	0.8	4MMs [1:2:4:6]	NM_020807	chr16:+58030598
TAAAAACCTTGTGGCAGTTGCAG	0.6	4MMs [1:4:5:13]		chr3:-106872028
GTAGAACGTTGTTGCAGTTGTAG	0.6	4MMs [2:5:8:13]		chr8:+83102115
GTAGGCTCTCGTCGCAGTTGAAG	0.5	4MMs [2:6:7:10]	NR_029427	chrX:-48463337
GCAGGACCTTGTTGCAATTGAAG	0.5	3MMs [2:13:17]		chr5:-27227006

Guide #1 (0482)

Quality score: 81

Guide sequence: CCACTCCCAAGGGAACCCGA**GGG**

On-target locus: chr7:+129410639

Number of off-target sites: 145 (15 are in genes)

sequence	score	mismatches	UCSC gene	locus
TGACACCCAGGGGAACCCGATGG	1.3	4MMs [1:2:5:10]		chr10:+72837071
CTGCTCTGAAGGGAACCCGAGGG	0.9	4MMs [2:3:7:8]		chr7:-47843799
TGACTCCAGAGGGAACCCGAAAG	0.9	4MMs [1:2:8:9]		chr17:+3868097
CCAAACCTCAGGGAACCCGAGGG	0.8	4MMs [4:5:8:9]		chr10:+98478197
CCAATCCATCGGGAACCCGAGGG	0.8	4MMs [4:8:9:10]		chr6:-30449530

Guide #2 (9678)

Quality score: 87

Guide sequence: GGAGGGCACGCCGTGCATCA**CGG**

On-target locus: chr7:-129409743

Number of off-target sites: 83 (22 are in genes)

sequence	score	mismatches	UCSC gene	locus
GGAGGGCAGGCCGTGCATCCAGG	2.4	2MMs [9:20]		chr20:+43221228
CCAGGGCAGTCCGTGCATCAGAG	0.8	4MMs [1:2:9:10]		chr17:+48274952
GGAGGCCTCGCCGTGCGTCACAG	0.7	3MMs [6:8:17]	NM_001040285	chr16:-50187013
GGCTTGCACGCCCTGCATCAAAG	0.6	4MMs [3:4:5:13]		chr17:+6113729
GGAGGGCAGTCCCTGCATCAGGG	0.5	3MMs [9:10:13]		chr11:-134043507

Fig.S9 TKI resistance is associated with a shift in ME% of K562 cells.

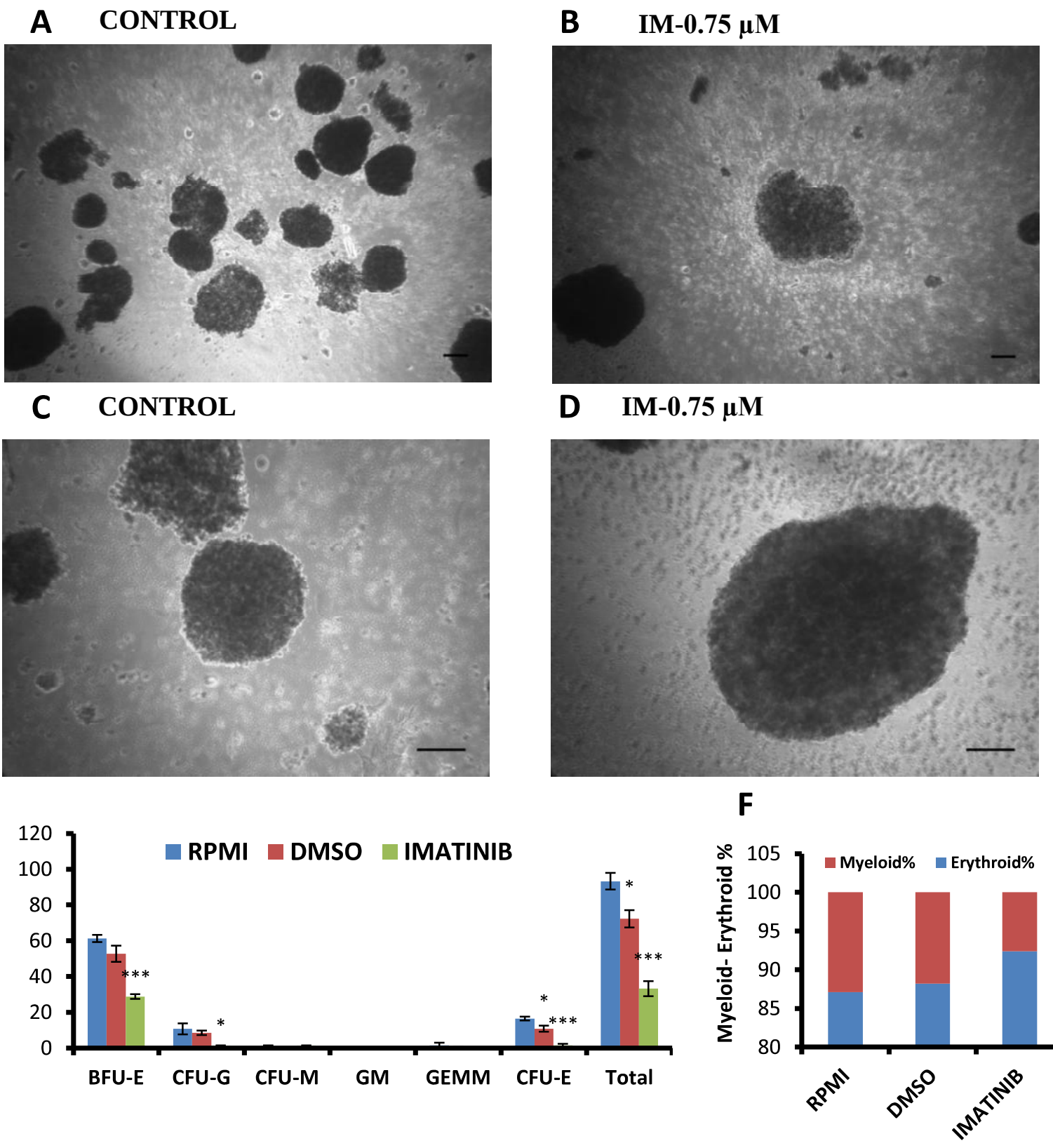


Fig. S10 Proliferation potential of *Δ182* cells.