

MIR-144 Mediated NRF2 Gene Silencing Inhibits Fetal Hemoglobin Expression in Sickle Cell Disease

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Supplemental Material

Table S1. Summary of clinical phenotype data.				
Subject Number	Age (years)	Genotype	Hydroxyurea therapy	RBC transfusions*
001	5	HbSS	Never treated	None
003	11	HbSS	Never treated	None
08A	18	HbSS	Never treated	None
014	6	HbSS	Never treated	None
015	4	HbSS	Never treated	None
016	7	HbSS	Never treated	None
004	4	HbSS	Never treated	None
008	10	HbSS	Never treated	None
009	18	HbSS	Never treated	None
011	4	HbSS	Never treated	None
011A	26	HbSS	Never treated	None
012A	18	HbSS	Never treated	None

*No transfusion within 4 months of study enrollment.

Table S2. Summary of genes >1.5-fold up-regulated and down-regulated in the two SCD study groups.**A. miRNA genes up-regulated in Low HbF compared to High HbF**

	UniqueID	Mean of LFH	Mean of HFH	Fold
1.	hsa-miR-144-5p	1.09479787	0.13750352	7.961963
2.	hsa-miR-144-3p	1.071796715	0.13750352	7.794686
3.	hsa-miR-486-5p	0.865969221	0.13750352	6.297797
4.	hsa-miR-3940-5p	0.639115736	0.138339241	4.619916
5.	hsa-miR-660-3p	0.851470495	0.187613818	4.538421
6.	hsa-miR-1976	0.861867274	0.20058734	4.296718
7.	hsa-miR-363-3p	0.585060616	0.13750352	4.254877
8.	hsa-miR-3124-3p	0.564015831	0.13750352	4.101828
9.	hsa-miR-20a-5p	0.569472695	0.149379931	3.812244
10.	hsa-miR-32-5p	0.531557327	0.150052597	3.542473
11.	hsa-miR-106b-5p	0.807917714	0.23079437	3.500595
12.	hsa-miR-101-3p	0.635670707	0.187217072	3.395367
13.	hsa-miR-96-5p	0.603209342	0.177911858	3.390495
14.	hsa-miR-19b-3p	0.765209913	0.230056425	3.326184
15.	hsa-miR-4456	0.448119486	0.13750352	3.258967
16.	hsa-miR-4540	0.443508794	0.13750352	3.225436
17.	hsa-miR-3182	0.438093866	0.13750352	3.186056
18.	hsa-let-7b-5p	0.841458807	0.264309853	3.183607
19.	hsa-miR-3667-5p	0.439350694	0.139389185	3.151971
20.	hsa-miR-15a-5p	0.614141504	0.195854669	3.1357
21.	hsa-miR-142-3p	0.761057307	0.243364217	3.127236
22.	hsa-miR-424-5p	0.542625631	0.180081499	3.013223
23.	hsa-miR-3136-3p	0.404627373	0.13750352	2.942669
24.	hsa-miR-5000-3p	0.401119163	0.13750352	2.917156
25.	hsa-miR-3686	0.445757652	0.15533453	2.869662
26.	hsa-miR-4639-3p	0.447934757	0.158151155	2.83232
27.	hsa-miR-16-5p	0.438280617	0.155648388	2.815838
28.	hsa-miR-19a-3p	0.61979119	0.221802562	2.794337
29.	hsa-miR-192-5p	0.383849884	0.13750352	2.791564
30.	hsa-miR-182-5p	0.378368601	0.13750352	2.751701
31.	hsa-let-7f-5p	0.555343936	0.203320769	2.731368
32.	hsa-miR-3611	0.389109363	0.143840211	2.70515
33.	hsa-miR-1255a	0.365224272	0.13750352	2.656109
34.	hsv1-miR-H4-3p	0.384212226	0.146542562	2.621847
35.	hsa-miR-3680-5p	0.932082653	0.356540663	2.614239
36.	hsa-miR-4728-3p	0.729156837	0.283946574	2.567937

37.	hsa-let-7c-5p	0.597633099	0.238662377	2.504094
38.	hsa-miR-20b-5p	0.344011828	0.13750352	2.50184
39.	hsa-miR-3178	0.376580805	0.15156475	2.48462
40.	hsa-miR-140-5p	0.450189715	0.182744873	2.463488
41.	hsa-let-7a-5p	0.463280454	0.191771959	2.415788
42.	hsa-miR-5193	0.329797874	0.13750352	2.398469
43.	hsa-miR-3133	0.40738226	0.16986721	2.39824
44.	hsa-miR-4764-3p	0.523633371	0.221650819	2.362425
45.	hsa-miR-15b-5p	0.471860195	0.200196179	2.356989
46.	hsa-miR-362-3p	0.323882908	0.13750352	2.355452
47.	hsa-miR-3195	0.320663402	0.13750352	2.332038
48.	hsa-miR-197-3p	0.335016857	0.144325385	2.321261
49.	hsa-miR-190a-5p	0.68762134	0.297839147	2.3087
50.	hsa-miR-4290	0.313257769	0.13750352	2.27818
51.	hsa-miR-4742-3p	0.305071801	0.13750352	2.218647
52.	hsa-miR-373-5p	0.342954288	0.154890882	2.214167
53.	hsa-miR-185-5p	0.4776253	0.215715915	2.21414
54.	hsa-miR-4443	0.31517973	0.142648697	2.209482
55.	hsa-miR-106a-5p	0.377459993	0.172194016	2.192062
56.	hsa-miR-17-5p	0.349814489	0.16153767	2.165529
57.	hsa-miR-642b-5p	0.473200947	0.219803467	2.152837
58.	hsa-miR-664b-3p	0.290123314	0.13750352	2.109934
59.	hsa-let-7g-5p	0.423036719	0.203668522	2.077084
60.	hsa-miR-33b-5p	0.520613859	0.252659592	2.060535
61.	hsa-miR-28-3p	0.551039989	0.268697158	2.050785
62.	hsa-miR-550b-3p	0.28044952	0.13750352	2.039581
63.	hsa-miR-363-5p	0.273282001	0.13750352	1.987455
64.	hsa-miR-223-3p	0.70924748	0.35975493	1.971474
65.	hsa-miR-335-3p	0.295257881	0.150271013	1.964836
66.	hsa-miR-18b-5p	0.376082992	0.196184861	1.916983
67.	hsa-miR-5684	0.267029832	0.139317967	1.916693
68.	hsa-miR-1273g-3p	0.260580098	0.13750352	1.895079
69.	hsa-miR-374a-5p	0.301869228	0.161549548	1.868586
70.	hsa-miR-92a-3p	0.271638965	0.14560015	1.86565
71.	hsa-miR-7-5p	0.495201329	0.265618722	1.864331
72.	hsa-miR-548as-3p	0.333050797	0.179360357	1.856881
73.	hsa-miR-3148	0.251200219	0.13750352	1.826864
74.	hsa-miR-374a-3p	0.456135	0.253693201	1.797979
75.	hsa-miR-30b-5p	0.347093041	0.198833287	1.745649
76.	hsa-miR-3685	0.427613149	0.247670052	1.726544
77.	hsa-miR-767-5p	0.355502735	0.206478298	1.721744

78.	hsa-miRPlus-A1087	1.005028288	0.59570921	1.687112
79.	hsa-let-7i-5p	0.331813296	0.19744503	1.680535
81.	hsa-miR-3606-5p	0.304687783	0.182387548	1.670551
82.	hsa-miR-4306	0.351285994	0.215207716	1.632311
83.	hsa-miR-140-3p	0.366259317	0.228070048	1.605907
84.	hsa-miR-4286	0.298559616	0.186332467	1.602295
85.	hsa-miR-501-5p	0.606995771	0.379401689	1.599876
86.	hsa-miR-4508	0.340902343	0.22531308	1.513016
87.	hsa-miR-4297	0.308132519	0.203853903	1.511536
88.	SNORD12	0.576184829	0.381266619	1.511239
89.	hsa-miR-1827	0.300866216	0.200042297	1.504013
B. miRNA genes >1.5-fold down-regulation in Low HbF compared to High HbF				
90.	hsa-miR-548k	0.526617696	0.794086168	-1.5079
91.	hsa-miR-31-5p	0.24490121	0.370707192	-1.5137
92.	hsa-miR-744-5p	0.140254105	0.213169088	-1.51988
93.	hsa-miR-1275	0.13750352	0.209522737	-1.52376
94.	hsa-miR-371b-3p	0.321478665	0.497433307	-1.54733
95.	hsa-miR-204-3p	0.21927695	0.33958324	-1.54865
96.	SNORD49A	0.13750352	0.212963673	-1.54879
97.	hsa-miR-1913	0.710434849	1.10181878	-1.55091
98.	hsv1-miR-H1-3p	0.670087328	1.046449562	-1.56166
99.	hsa-miR-151a-5p	0.174738651	0.279452768	-1.59926
100.	hsa-miR-378c	0.309663246	0.49590637	-1.60144
101.	hsa-miR-335-5p	0.145147736	0.233306388	-1.60737
102.	hsa-miR-378e	0.14585572	0.237691623	-1.62964
103.	hsa-miR-652-3p	0.13750352	0.224599354	-1.63341
104.	hsa-miR-10b-3p	0.675632646	1.111300739	-1.64483
105.	hsa-miR-27b-3p	0.13750352	0.229083461	-1.66602
106.	hsv2-miR-H10	0.137633334	0.229621651	-1.66836
107.	hsa-miR-4328	0.13750352	0.229621651	-1.66993
108.	hsv2-miR-H24	0.416211476	0.695982724	-1.67219
109.	hsa-miR-4291	0.13750352	0.230693214	-1.67773
110.	ebv-miR-BHRF1-2-3p	0.156465024	0.263392128	-1.68339
111.	hsa-miR-26a-5p	0.13750352	0.233893851	-1.701
112.	hsa-miR-1321	0.139441729	0.238851691	-1.71291
113.	hsa-miR-4436b-3p	0.142664755	0.244546182	-1.71413
114.	hsa-miR-625-3p	0.16115209	0.276760668	-1.71739
115.	hsa-miR-143-3p	0.168386489	0.291325641	-1.7301
116.	hsa-miR-103a-3p	0.174743901	0.303572697	-1.73724
117.	hsa-miR-338-3p	0.353828117	0.615812806	-1.74043
118.	hsa-miR-505-5p	0.378450314	0.667859236	-1.76472

119.	hsa-miR-3675-3p	0.13750352	0.243094196	-1.76791
120.	hsa-miR-4284	0.13750352	0.243812146	-1.77313
121.	hsa-miR-152-3p	0.13750352	0.244345543	-1.77701
122.	hsa-miR-4646-3p	0.409116854	0.733000949	-1.79167
123.	hsa-miR-4723-3p	0.837650607	1.512273391	-1.80537
124.	hsa-miR-193a-5p	0.444121659	0.80830431	-1.82001
125.	hsa-miR-191-5p	0.173793256	0.317004027	-1.82403
126.	hsa-miR-664a-3p	0.374088317	0.684991255	-1.8311
127.	hsa-miR-550a-3-5p/hsa-miR-550a-5p	0.148494368	0.271950148	-1.83138
128.	hsa-miR-4769-3p	0.422235752	0.785390891	-1.86008
129.	hsa-miRPlus-A1015	0.13750352	0.259047076	-1.88393
130.	hsa-miR-3607-5p	0.13750352	0.260857873	-1.8971
131.	hsa-miR-3654	0.13750352	0.263831519	-1.91873
132.	hsa-miR-222-3p	0.13750352	0.265360201	-1.92984
133.	hsa-miR-425-5p	0.138201346	0.268205285	-1.94069
134.	hsa-miR-486-3p	0.202410509	0.393610631	-1.94462
135.	hsa-miR-483-5p	0.168386489	0.330458313	-1.9625
136.	hsa-miR-130b-3p	0.157796065	0.311579789	-1.97457
137.	hsa-miR-664b-5p	0.148922876	0.304547509	-2.045
138.	hsa-miR-151a-5p/hsa-miR-151b	0.13750352	0.283933627	-2.06492
139.	hsa-miR-4311	0.294961244	0.615186691	-2.08565
140.	hsa-miR-1281	0.427120537	0.910565893	-2.13187
141.	hsa-miR-4780	0.13750352	0.300081822	-2.18236
142.	SNORD118	0.13750352	0.300364802	-2.18442
143.	hsa-miR-1246	0.13750352	0.304528447	-2.2147
144.	hsa-miR-4687-5p	0.213586489	0.473090723	-2.21498
145.	hsa-miR-629-5p	0.229916503	0.517708336	-2.25172
146.	SNORD110	0.173667938	0.394852941	-2.27361
147.	hsa-miR-3653	0.14573139	0.332049218	-2.2785
148.	hsa-miR-4288	0.13750352	0.315991327	-2.29806
149.	hsa-miR-146a-5p	0.13750352	0.316849003	-2.3043
150.	hsa-miR-625-5p	0.13750352	0.318150538	-2.31376
151.	hsa-miR-2355-3p	0.13750352	0.3252191	-2.36517
152.	hsa-miR-29a-3p	0.13750352	0.327009529	-2.37819
153.	hsa-miR-1228-5p	0.235323404	0.577493943	-2.45404
154.	hsa-miR-146b-5p	0.170156375	0.427330308	-2.5114
155.	hsa-miR-377-3p	0.13750352	0.346272958	-2.51828
156.	hsa-miR-584-5p	0.186135734	0.469648461	-2.52315
157.	hsa-miR-145-5p	0.281574557	0.719547053	-2.55544
158.	hsa-miR-433-5p	0.187515914	0.479982163	-2.55969
159.	SNORD4A	0.13750352	0.356897029	-2.59555

160.	hsa-miR-199a-5p	0.13750352	0.357352403	-2.59886
161.	hsa-miR-3620-3p	0.215832199	0.562310979	-2.60532
162.	hsa-miR-125b-5p	0.189632659	0.494794813	-2.60923
163.	hsv1-miR-H7-3p	0.152291045	0.399610154	-2.62399
164.	hsa-miR-484	0.177101562	0.466798219	-2.63577
165.	hsa-miR-3607-3p	0.13750352	0.366763932	-2.66731
166.	SNORD48	0.138984049	0.37306532	-2.68423
167.	SNORD32	0.160333171	0.439352353	-2.74025
168.	hsa-miR-3924	0.16562598	0.466167492	-2.81458
169.	hsa-miR-376c-3p	0.13750352	0.391407728	-2.84653
170.	hsa-miR-5010-3p	0.251063605	0.728442465	-2.90143
171.	hsa-miR-4312	0.300465718	0.880775273	-2.93137
172.	SNORD2	0.13750352	0.41564969	-3.02283
172.	hsa-miR-874-5p	0.148360446	0.488450413	-3.29232
173.	hsa-miR-2116-3p	0.13750352	0.457817979	-3.3295
175.	hsa-miR-5701	0.13750352	0.4606099	-3.3498
176.	SNORD10	0.142228305	0.493075406	-3.46679
177.	hsa-miR-1	0.13750352	0.479908777	-3.49016
178.	SNORD6	0.13750352	0.510241464	-3.71075
179.	SNORD38B	0.13750352	0.523304125	-3.80575
180.	SNORD13	0.13750352	0.526691621	-3.83039

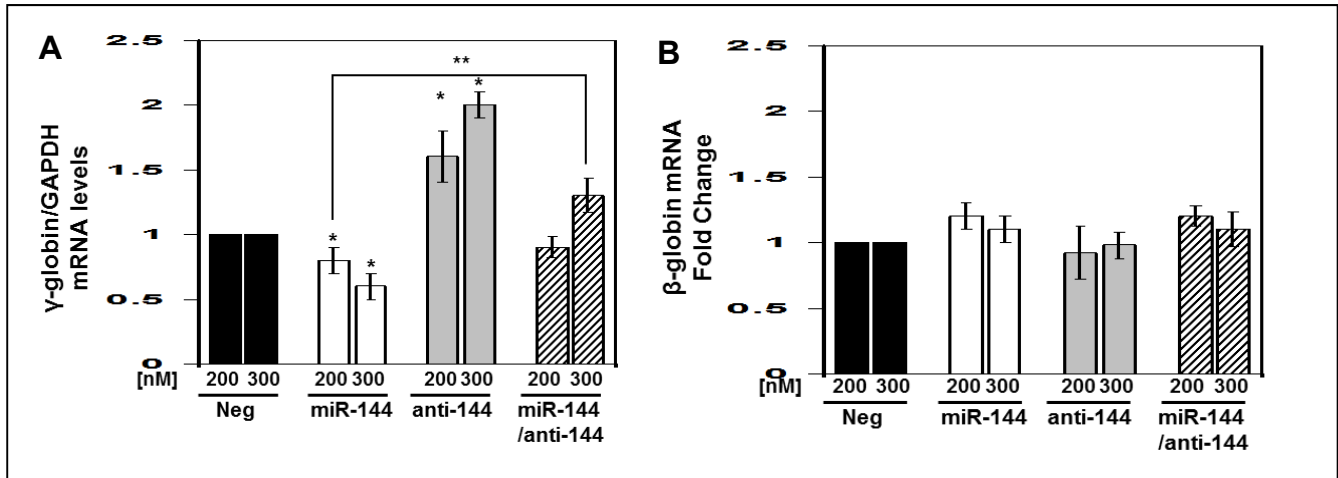


Fig. S1. MiR-144 antagomir has no effect on β -globin gene expression. CD34⁺ stem cells were cultured as described in Materials and Method and previously published (Li B et al. *Haematologica* pii: haematol.2017.185967, 2018). On day 8 erythroid progenitors were treated with negative (Neg) miRNA mimic control, miR-144 mimic, antagomir (anti-miR-144) or combination for 48 hours. Total RNA was isolated for RT-qPCR analysis for γ -globin (A) and β -globin (B) expression normalized by GAPDH (See Methods). Data are shown at the mean \pm standard error of the mean.

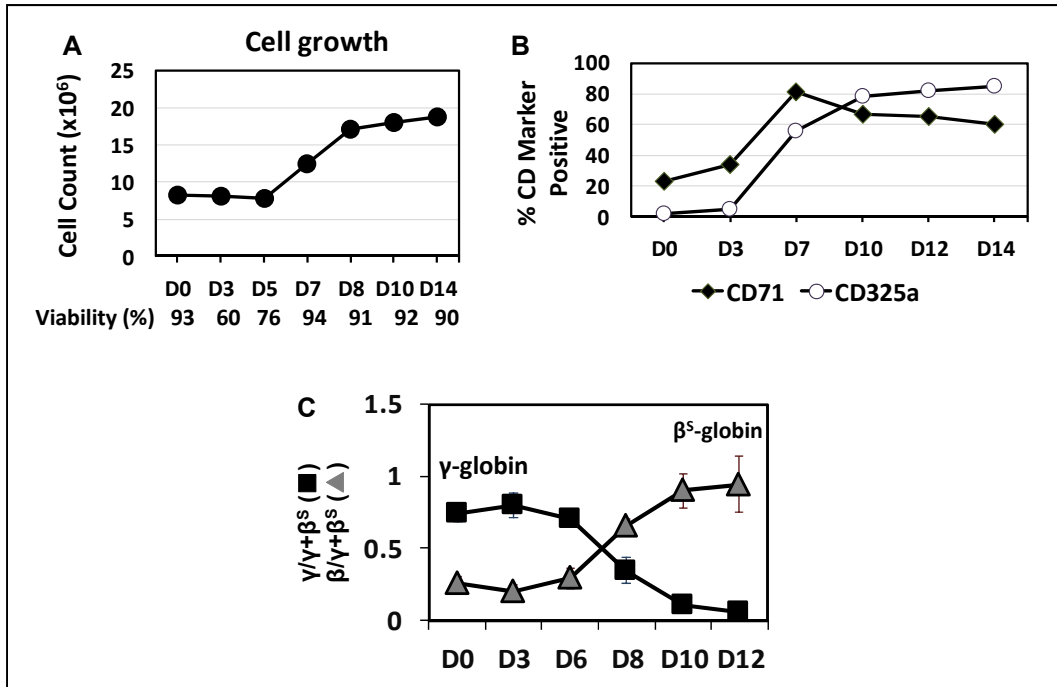


Fig.S2 Characterization of sickle erythroid progenitor growth in primary culture. After obtaining IRB approval, blood samples were collected from sickle cell patients and processed by ficoll histopaque separation of peripheral blood mononuclear cells (PBMCs) that were stored live in DMSO at -80°C. The PBMCs were grown in the two-phase culture system (See Methods) and then cells were harvested at day 0 through day 12 for cell growth and viability by Trypan blue exclusion (**A**). Cells were harvested for flow cytometry after staining with FITC-CD71 and PE-CD235a antibodies (**B**). To confirm globin gene switching in culture (**C**), RNA was harvested at each time point and RT-qPCR completed with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as internal control (See Methods).