Supplemental Methods

S1. Sickle cell disease donor blood collection

Whole blood was collected either from adult (>18 years) and pediatric SCD patients of the Augusta University Medical Center in EDTA Vacutainer® tubes under an Augusta University IRB approved protocol or from adult SCD donors at Biogen in heparin Vacutainer® tubes under a Western IRB approved protocol (WIRB #1138926). Blood donations following informed consent were from SCD subjects at steady state who had not received a blood transfusion within 3 months prior to the day of blood draw. SCD donor characteristics are detailed in Table S1. The peripheral blood mononuclear cell (PBMC) fraction was isolated using a Ficoll gradient.

S2. Mouse study of MMF exposure

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Augusta University. Normal C57/BL/6 mice were purchased from Jackson Laboratory, Bar Harbor, Maine. The Townes SCD mice were generated as previously described (Wu 2006) and homozygous HbSS mice bred from heterozygous HbAS "sickle trait" breeder pairs provided by Dr. Timothy Townes, University of Alabama at Birmingham School of Medicine. Male and female SCD mice (between 16 to 59 weeks old) or C57/BL/6 mice (25 weeks old) were treated by i.p. injection with 50 mg/kg DMF (#242926, Sigma-Aldrich) in 0.08% hydroxypropyl methyl cellulose (HPMC) in order to assess plasma levels of MMF after a single injection. This exposure study tested plasma levels of MMF 15 minutes after i.p. injection and included 3 Townes HbSS, 3 Townes HbAA, 3 Townes HbAS, and 3 wild-type C57BL/6 mice. A minimum of 80 µL of plasma is required for MMF analysis and 200 µL of whole blood was collected after warming the tail prior to bleeding. Ice-cold 1.5 mL conical microcentrifuge tubes were prepared containing lithium heparin and sodium fluoride (NaF) to collect 200 µL of blood. MMF was stabilized from ester hydrolysis by rapid chilling of the blood sample and addition of the esterase inhibitor NaF (10 mg/tube). Mouse blood collection tubes were prepared by addition of 120 µL of 250 mg/mL stock solution of NaF in water to a standard opened 3 mL "green top" lithium heparin blood collection tube with mixing to dissolve the heparin. The resulting solution was divided

into 15 microfuge tubes for collecting mouse blood (~10 µL solution per tube). Whole blood was stored on ice prior to preparation of plasma fraction by centrifugation (5000 rpm, 10 minute) then plasma frozen immediately on dry ice and maintained frozen (< 70 °C) prior to shipment on dry-ice to Biogen for analysis. MMF levels in mouse plasma samples (Table S2) were quantified following protein precipitation, extraction of MMF and LCMS as compared to a standard curve (LLOQ = 10 ng/mL).

Table S1 - Characteristics of SCD donors for PBMC erythroid progenitor studies

Donor	**Site**	**Sex**	**Age** (years)	**SCD Type**	**Hemoglobin** (g/dL)	**Reticulocytes** (%)	**Platelets** (10^3/µL)
Adult08A	Augusta[2]	NA[3]	>18	SS	7.4	8.8	192
Adult10A	Augusta	NA	>18	SS	6.5	10.5	467
PED011	Augusta	M	4	SS	7.6	12.0	570
PED012	Augusta	F	12	SS	8.6	9.2	458
PED026	Augusta	M	5	SS	8.7	9.0	363
PED027	Augusta	M	6	SS	8.2	8.4	416
1.1	Biogen	F	30	SS	8.7	11.8	221
2.1[1]	Biogen	F	21	SS	6.5	11.5	468
3.1[1]	Biogen	F	31	SS	6.3	12.4	343
4.1[1]	Biogen	F	36	SS	3.7	6.2	249
5.1	Biogen	F	31	SS	7.0	16.6	224
6.1[1]	Biogen	M	19	SS	9.9	8.2	362
6.2[1]	Biogen	M	19	SS	10.1	11.2	363
7.1[1]	Biogen	F	48	SS	8.7	11.5	614

[1]Donor reported use of hydroxyurea during consent interview. [2]Augusta University. [3]Data not available.

Table S2 - Plasma levels of MMF following intraperitoneal (i.p.) injection of DMF[1]

Type of Mouse[2]	ID	Gender	Age (weeks)	MMF Plasma Concentration	
				(µg/mL)	(µM)
Townes SCD HbSS	35F	female	59	13.5	104
	67-1F	female	32	14.5	112
	67-3F	female	32	9.0	69
			Average[3]:	12.3 ± 2.9	95 ± 23
Townes SCD HbAA	60-1F	female	30	22.3	172
	61-3F	female	30	5.57	43
	61-4F	female	30	6.04	46.5
			Average:	11.3 ± 9.5	87 ± 73
Townes SCD HbAS	41-2M	male	42	11.0	85
	41-3M	male	42	6.24	48
	41-4M	male	42	6.02	46
			Average:	7.8 ± 2.8	60 ± 22
C57BL/6	41-1F	female	25	12.0	92
	41-2F	female	25	22.4	172
	41-4F	female	25	21.3	164
			Average:	18.6 ± 5.7	143 ± 44

[1]All mice were dosed by intraperitoneal injection of 50 mg/kg DMF in 0.08% hydroxypropyl methyl cellulose (HPMC, #56340, Sigma-Aldrich) and after 15 minutes a sample of blood (200 µL) was drawn, processed and frozen prior to shipment to Biogen for LCMS analysis. [2]HbSS = homozygous for HbS, HbAA = homozygous for HbA (normal hemoglobin), HbAS = heterozygous for HbS and HbA. [3]mean ± SD

Table S3 - Complete blood count (CBC) of mice at 6 weeks in chronic i.p. study[1]

Parameter	Vehicle	DMF	p-value
Hemoglobin (g/dL)	6.83 ± 0.86[2]	8.23 ± 0.45	0.004[3]
RBC (x10^6/µL)	7.26 ± 0.85	8.61 ± 0.60	0.007
RDW (%)	28.9 ± 1.33	25.4 ± 1.58	0.001
HDW (g/dL)	7.25 ± 0.57	6.48 ± 0.51	0.028
WBC (x10^3/µL)	31.7 ± 8.74	26.0 ± 4.59	0.186
Hematocrit (%)	32.5 ± 4.30	36.4 ± 2.30	0.077
Neutrophils (x10^3/µL)	3.87 ± 1.54	4.41 ± 1.02	0.518
MCV (fL)	89.6 ± 4.89	84.7 ± 3.61	0.069
MCH (pg)	18.9 ± 0.69	19.1 ± 0.67	0.619
MCHC (pg/dL)	42.1 ± 1.54	45.1 ± 1.15	0.003

[1]Townes SS mice were administered 50 mg/kg DMF (n = 7) or 0.08 % HPMC (n = 8) as vehicle (i.p., BID for 6 weeks) and whole blood samples collected at week 6 were subjected hematological analysis using the Advia analyzer. [2]Values are mean ± SD. [3]p-values calculated by student t-test, 2-tailed comparing vehicle with DMF treated mice.

Figure S1

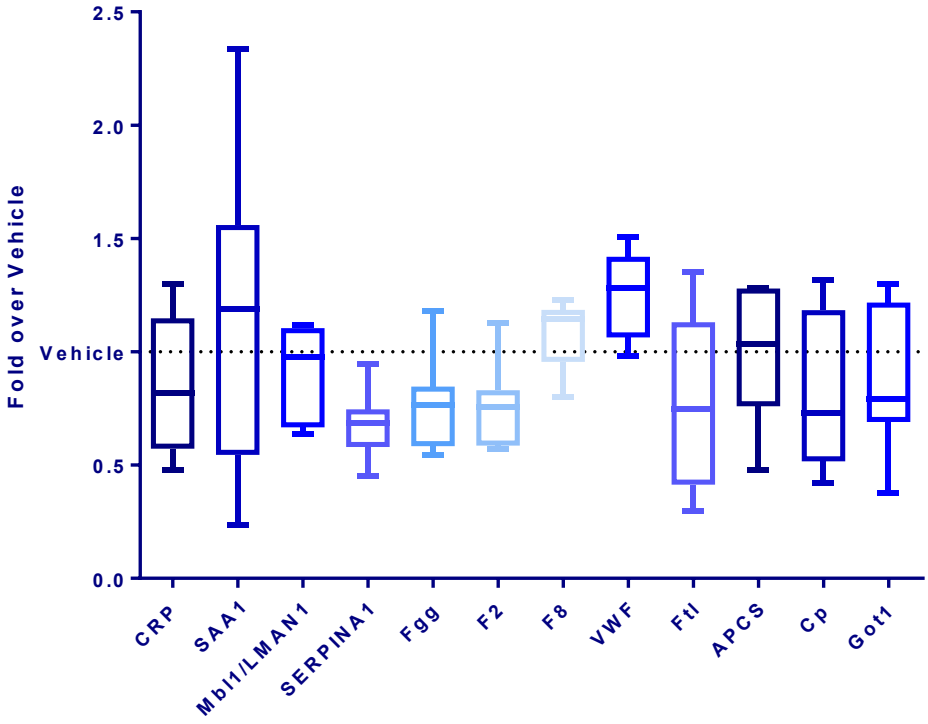

Figure S2