SUPPLEMENTARY APPENDIX

The LSD1 inhibitor RN-1 recapitulates the fetal pattern of hemoglobin synthesis in baboons (P. anubis)

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Supplemental Figure 2. (A) Changes in MCV of anemic baboons treated with varying doses of RN-1. **(B)** Changes in MCHC in baboons treated with varying doses of RN-1. Animals were treated on days 1-5. **(C)** Relationship between HbF levels and changes in MCV in anemic baboons treated with varying doses of RN-1

Supplemental Figure 3. Difference in median fluorescence (PE-anti-HbF) in F cells of three non-anemic baboons pre-treatment and following treatment with RN-1 for either 10 weeks (n=1) or 6 weeks (n=2; mean $\pm$ SD).

Supplemental Figure 4. Changes in ANC, platelets, monocytes, absolute reticulocyte count, and WBC during treatment of a normal, non-anemic baboon with RN-1. Animals were treated 5d/wk starting on d1.

Supplemental Figure 5. MCV and MCHC levels of three non-anemic baboons treated with RN-1 for 10 weeks (PA 8696) or 6 weeks (PA8695, PA8698) on varying days.

Supplemental Methods

Globin Chain Synthesis

Peripheral blood (20 μL) from anemic animals was washed three times in PBS and incubated overnight at 37°C in 0.2 mL leucine-free α-minimum essential medium (MEM; Invitrogen, Carlsbad, CA, USA) containing 20% dialyzed fetal bovine serum, transferrin, ferric ammonium citrate, and 50 μCi/mL L-[4,5-^{3}H] leucine (Perkin Elmer). For non-anemic animals, a fraction enriched in reticulocytes was obtained by centrifugation of 7 ml whole blood on Percoll step gradients as previously described.[36] The reticulocyte rich fraction was centrifuged (500 g; 10 min) and washed three times with PBS. The reticulocyte-rich pellet radiolabeled with [^{3}H] leucine in leucine-free media as described above. Following radiolabeling, cells were washed three times in PBS (500 g; 10 min). Pellets were suspended in H_2O and cells lysed by multiple freeze-thaw cycles in dry ice-methanol baths. Lysates were filtered using 0.4 mm cellulose acetate syringe filter (Nalgene) and globin chain separation was achieved by high-performance liquid chromatography (HPLC) using a LithoCART 250-4 column (VWR) and a Spectra System HPLC (Thermo-Finnegan) with acetonitrile-methanol gradients.[37] Quantitation of radioactivity in collected fractions was determined by liquid scintillation counting using a Packard Tricarb 1600TR liquid scintillation analyzer. Results are expressed as $\gamma/\gamma+\beta$ chain ratios.

RNA analysis

RNA was isolated using the RNeasy Minikit (Qiagen #74104) and treated with RNase-free DNase I (Ambion #AM1906) according to the manufacturer's instructions. Purified RNA was used for the synthesis of cDNA using RevertAid First Strand CDNA Synthesis Kits (Thermo Scientific #K1622). Real time PCR analysis was conducted using custom designed primer-probe combinations were used for analysis of globin transcripts (Supplemental Table 1). Absolute numbers of globin transcripts were determined by extrapolation from standard curves prepared from the cloned amplicons.[38]

Bisulfite Sequence Analysis

For bisulfite sequence analysis, DNA was purified from washed cell pellets using QIAmp Blood DNA Minikits. Bisulfite modification was performed using Epitect Bisulfite kits (Qiagen #59104) according to the instructions of the manufacturer. Amplification of bisulfite modified DNA was performed as previously described. [35]with using the BG2 5'- AATAACCTTATCCTCCTCTATAAAATAACC and BG5 5'-GGTTGGTTAGTTTTGTTTTGATTAATAG primers.[35] Amplicons from three separate PCR reactions were pooled to reduce PCR bias and the amplicons cloned using Topo TA cloning kits (Life Technologies #K4575-01SC). DNA was isolated from individual clones and sequence analysis performed at the University of Illinois DNA Sequence Laboratory.

Chromatin Immunoprecipitation Analysis

Chromatin immunoprecipitation analysis was performed as previously described[39] with modifications. Fixation was conducted by incubation of cells in Iscove's media containing 1% fetal bovine serum and 1% formaldehyde (methanol-free,

Thermo Scientific #28906) for 10 minutes at room temperature. The fixation reaction was stopped by the addition of 0.125 M glycine followed by a second incubation at room temperature for 5 minutes. Cells were lysed in 200 μL cell lysis buffer. Chromatin shearing was performed using a Diagenode Bioruptor 300 sonicator (20 cycles; Power = High). Sheared chromatin (400-600bp) was diluted 1:5 in IP dilution buffer and pre-cleared overnight with normal rabbit serum. An equivalent of 10 μg chromatin lysate was used for each IP condition. Anti-Histone H3K4Me2 (07-030), anti-H3K4Me3 (17-614), anti-H3K9Me2 (17-648) and anti-H3K9ac (17-658) were obtained from Millipore. Anti-pol II (sc-899x) was obtained from Santa Cruz Biotech. Immunoprecipitation was performed overnight at 4^{o}C followed by incubation with RNase A followed by a second incubation with Proteinase K. DNA from the chromatin immunoprecipitates was purified using ChIP DNA and Concentrators (Zymo Research). Quantitative real-time PCR of triplicate samples was performed using primers specific for the necdin, ε-, γ-, and β-globin promoters and ε-, γ-, and β-globin IVSII regions (Supplemental Table 1) with SBYR Green reagent using a 7500 Real Time PCR instrument (Applied Biosystems).[5] Standard curves constructed for each primer set using dilutions of input DNA were used to determine the relative amount of specific sequences recovered in samples following immunoprecipitation. Results were expressed as the fraction of input.

Supplemental Table 1. Primer and Probe Sequences

RT-PCR primer-probes

Gene	Forward Primer	Reverse Primer	Probe
ε-globin	AACTTCAAGCTCCTGGGTAACG	GGGTGAACTCCCTGCCAAA	ATGGTGATTATTCTGGCTACT
γ-globin	CGGCAAGAAGGTGCTCACTT	GCCCTTGAGATCATCCAGGTT	CTTGGGAGATGCCG
β-globin	GCTGGTGGTCTACCCTTGGA	AGGAGAGGACAGATCCCCAAA	CCAGAGGTTCTTTGATTC

ChIP Primers

Gene Location	Forward Primer	Reverse Primer
Necdin	GAGCTCCTGGACGCAGAGG	GCAAAGTTAGGGTCGCTCAGA
ε-globin promoter	TCAGCC TTGACCAATGACTTTTAA	GTTCTGGCCCCCTGTTCTC
ε-globin IVS II	CAGAAATCATGGGTCGAGTTTG	TCAGGTTAGTCTTGTTATGCTCACT
γ-globin promoter	AGGGATGAAGAATAAAAGGAAGCA	CAGAAGCGAGTGTGTGGAACTG
γ-globin IVS II	GAGGGCAAAATGTCAGGCTTT	CCCAGCTTCCACCCAGAAT
β-globin promoter	GGGCTGAGGGTTTGAAGTCC	CCTTGGCTCTTCTGGCACTG
β-globin IVS II	TCCCCTTCTTTTCTATGATTAACTTCA	TTGTCTCTTCCCCATTCTAAACTGT

Supplemental Table 2. Experiments in Anemic Animals.

Exp	Animal	Dose mg/kg/d	Schedule	ANC (/μL)		Plt (X 10^3/μl)			Mono (/μL)		Abs Retics (X 10^9/L)		F cells (%)		F retics (%)		HbF synthesis (γ/γ+β)		HbF (%)	
				Pre	Nadir	Pre	Nadir	Peak	Pre	Peak	Pre	Nadir	Pre	Peak	Pre	Peak	Pre	Peak	Pre	Peak
1	8548	2.5	4d	3020	290	750	130	1350	84	483	323	209	28.5	59.2	34.6	92.3	ND	0.78	5.8	27.3
2	8549	0.5	5d	3160	570	772	372	1445	95	380	526	209	19.1	60.8	33.9	97.5	0.06	0.68	2.4	29.4
3	8000	0.25	5d	3620	870	353	122	362	322	532	467	249	20.3	47	45.7	80.7	0.15	0.49	4.1	20.6
4	8548	0.2	5d	3870	800	772	514	1092	110	204	280	51	36.9	54.8	36.9	89.2	0.04	0.52	3.7	20.5
5	8001	0.125	5d	11120	3242	476	400	596	243	483	253	244	13.5	21.8	22.9	31.7	ND	0.06	1.7	4.8
6	8549	0.125	10d	3150	870	804	663	1108	120	195	390	242	17.6	42.7	21	70	0.04	0.22	3.6	16
7	8000	0.25	2d/wk 4 wks	2580	2360	372	217	391	262	591	265	138	18.2	28.1	29.2	52.2	0.11	0.46	4.7	10.4
8	8548	0.5	2d/wk 4 wks	3830	570	896	1165	1165	98	198	360	200	22.2	53.6	24.1	86.4	0.13	0.51	6.2	22.1

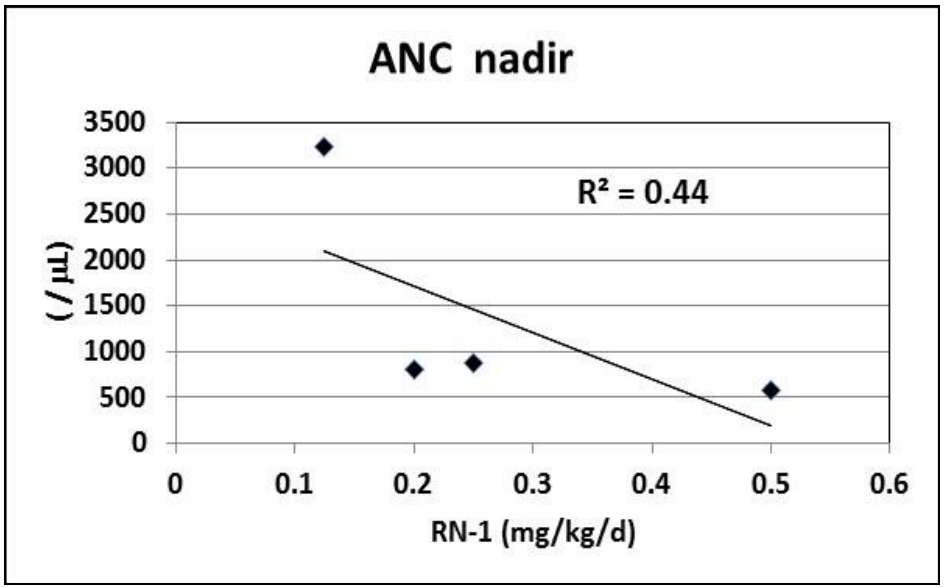
Supplemental Table 3. Effect on RN-1 on Globin mRNA

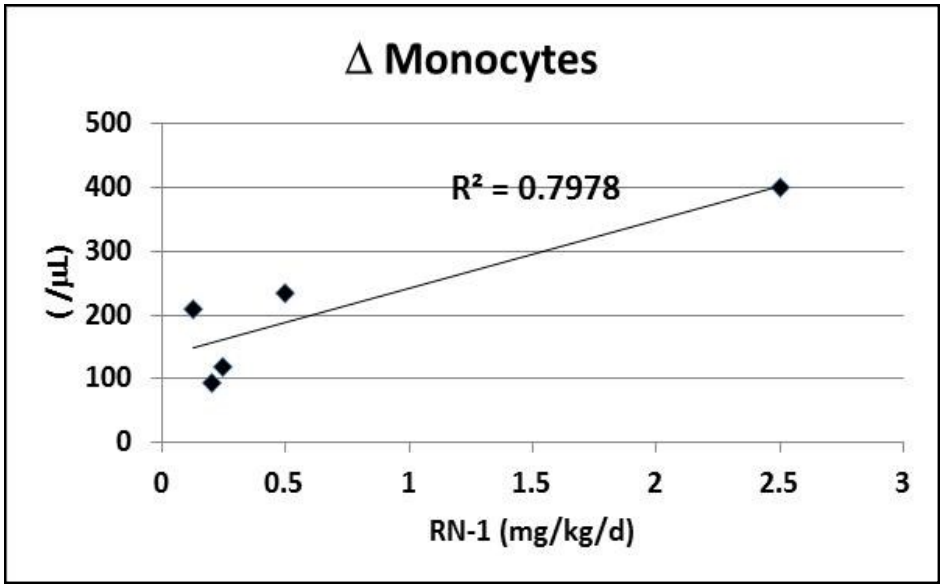
Exp	Animal	Dose (mg/kg/d)	γ-globin mRNA (γ/ε+γ+β)		ε-globin mRNA (γ/ε+γ+β)	
2	8549	0.5	0.07	0.52	0.007	0.016
4	8548	0.2	0.07	0.35	0.005	0.011
6	8549	0.125	0.09	0.30	0.002	0.003
Mean			0.08+0.01	0.39+0.12	0.004+0.002	0.001+0.008

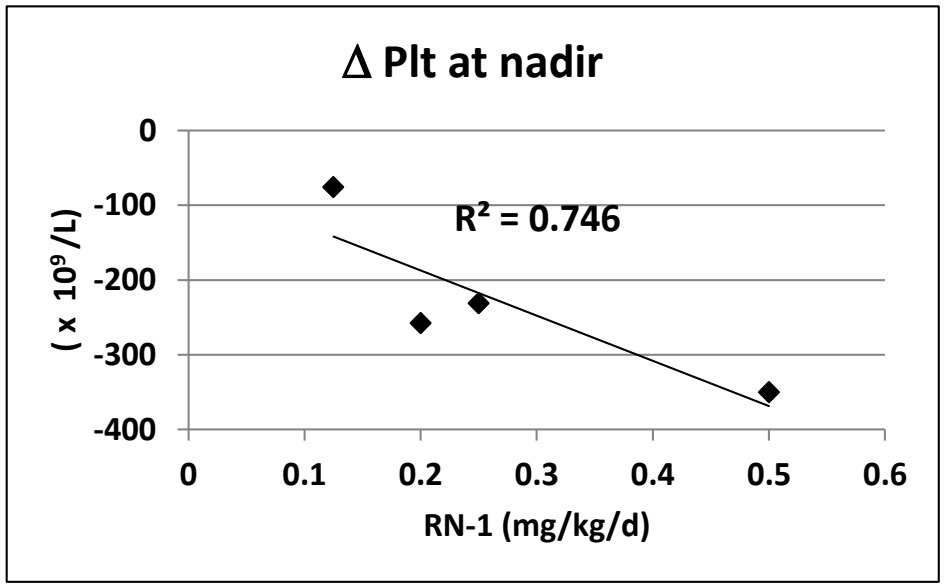
Supplemental Table 4. Experiments in Non-Anemic Animals.

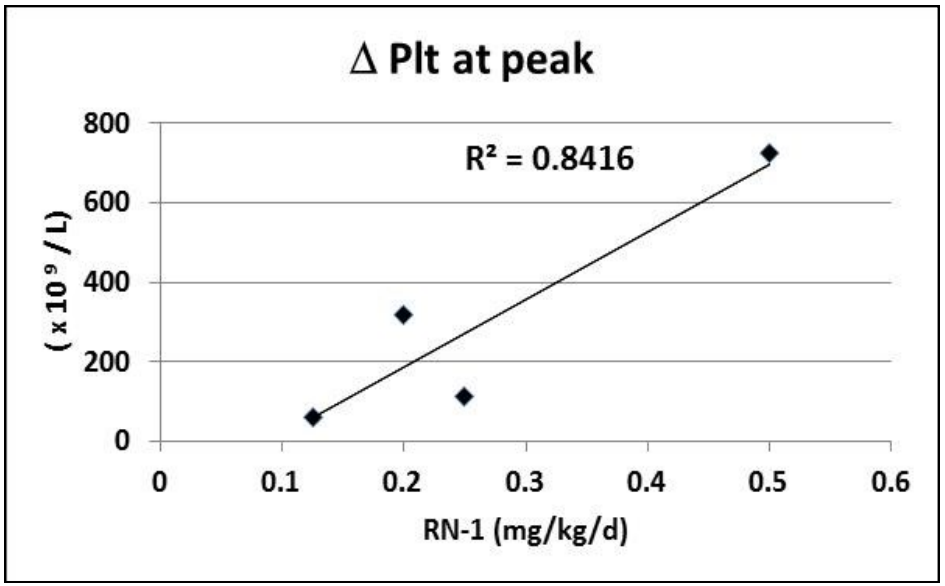
Exp	Animal	Dose mg/kg/d	Schedule	ANC (/μL)		Plt (X 10^3/μL)			Mono (/μL)		Abs Retics (X 10^9/L		F cells (%)		F retics (%)		HbF synthesis (γ/γ+β)		HbF (%)	
				Pre	Nadir	Pre	Nadir	Peak	Pre	Peak	Pre	Peak	Pre	Peak	Pre	Peak	Pre	Peak	Pre	Peak
9	8549	0.5	5d	7130	1910	394	169	1244	175	1269	39.7	105	ND	ND	ND	ND	0.03	0.71	1.2	2.1
10	8549	0.25	5d	3350	1910	377	219	839	236	720	53.6	135	ND	ND	ND	ND	0.02	0.26	1.6	2.6
11	8698	0.25	5d/wk 2 wks	4190	1210	249	68	1340	151	719	30.5	105	2.0	9.2	ND	ND	0.01	0.24	0.6	2.3
12	8696	0.2-0.25	5d/wk 10wks	3720	1440	328	64	288	106	378	35.8	112	3.5	29.0	2.4	74.4	ND	0.16	1.5	4.8
13	8695	0.25	5d/wk 6wks	2110	1610	351	198	302	100	293	29.3	48.7	3.1	17.4	4.8	76.6	ND	ND	1.2	4.1
14	8698	0.25	5d/wk 6wks	2690	1250	224	90	187	161	460	25.4	43.6	2.0	17.3	15.8	75.6	ND	ND	1.0	4.3

Supplemental Figure 1



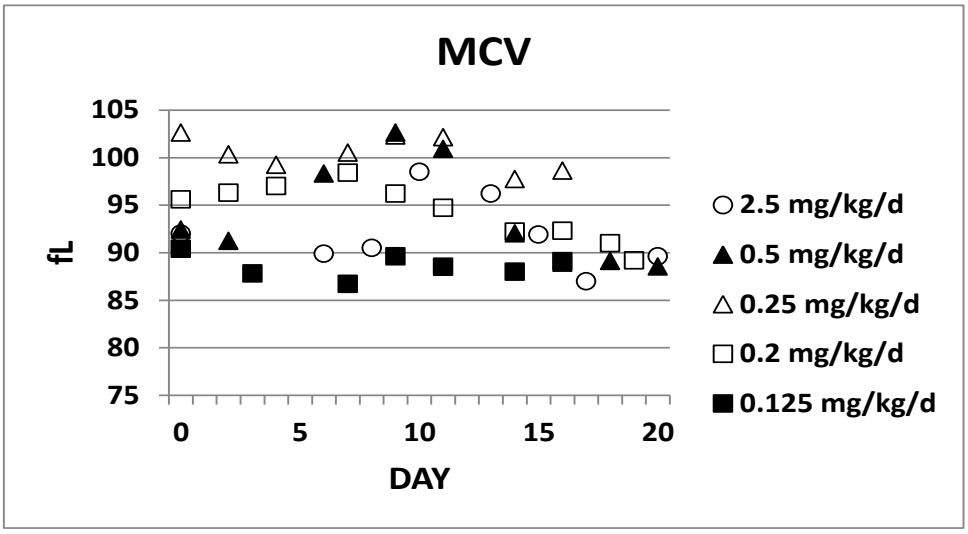






Supplemental Figure 2

A.



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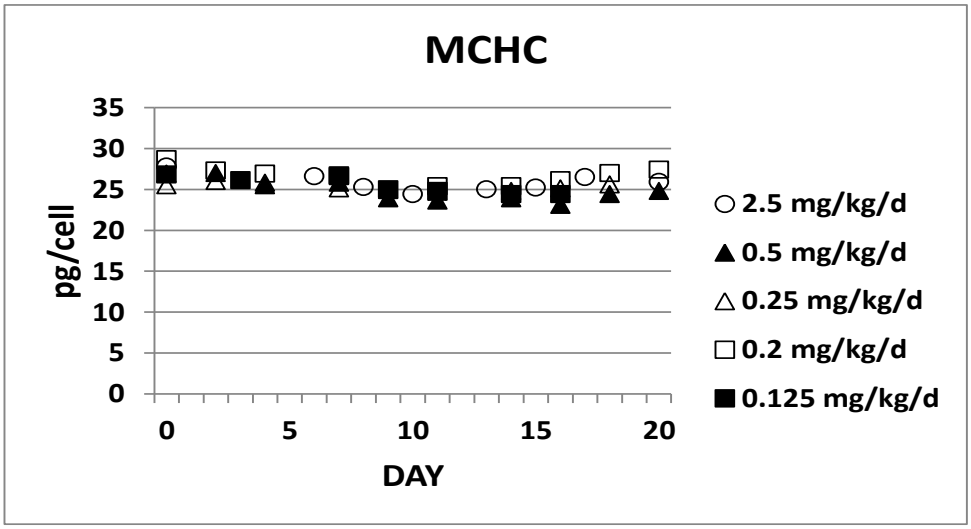

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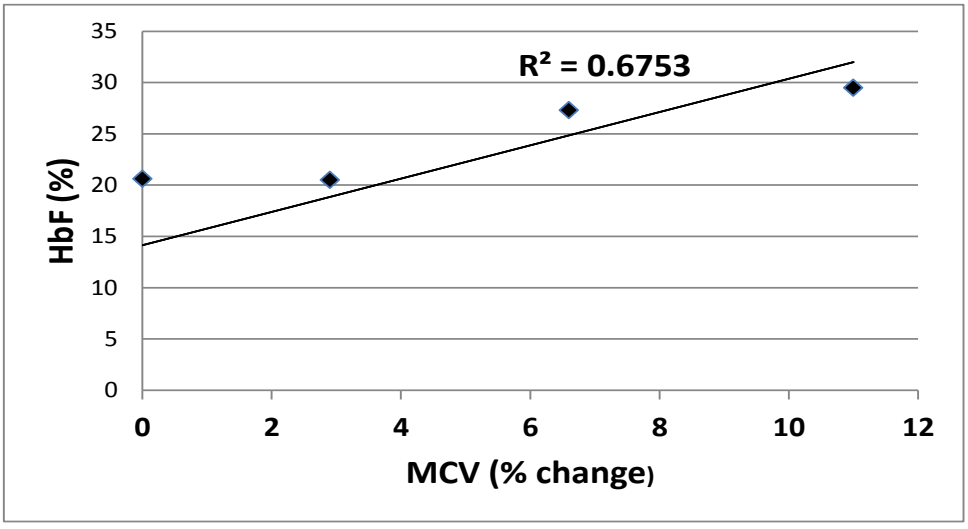
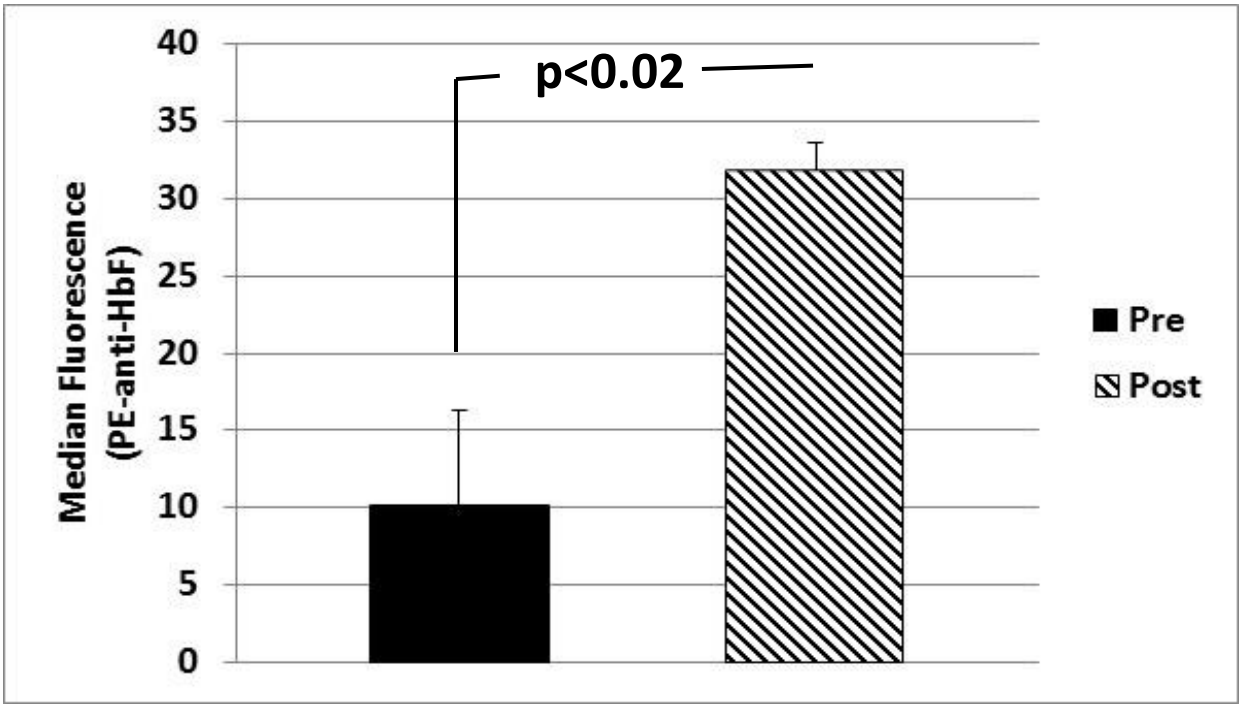
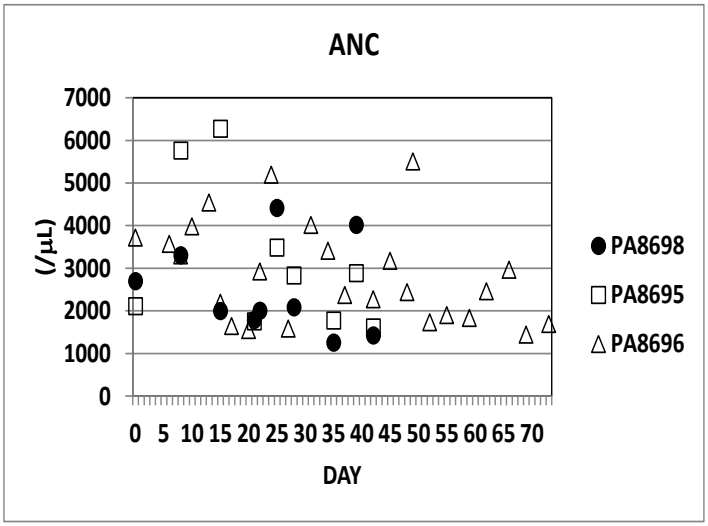
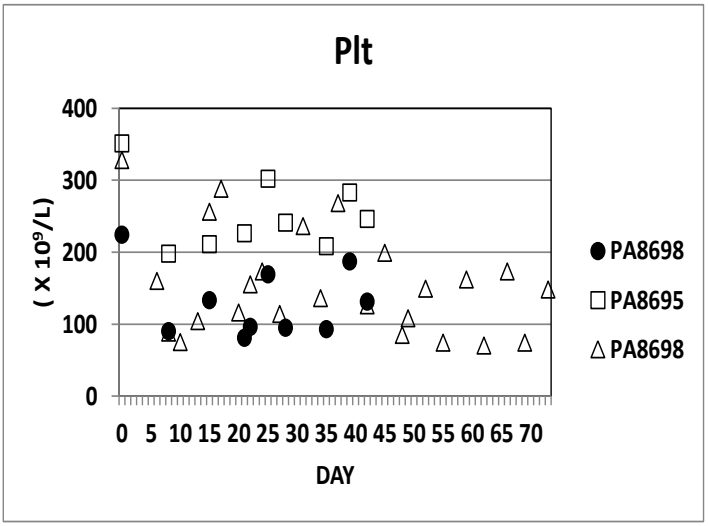


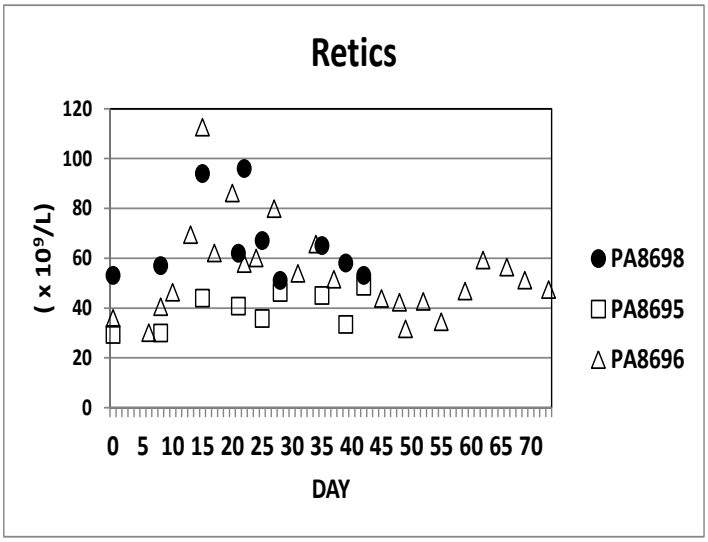
Supplemental Figure 3

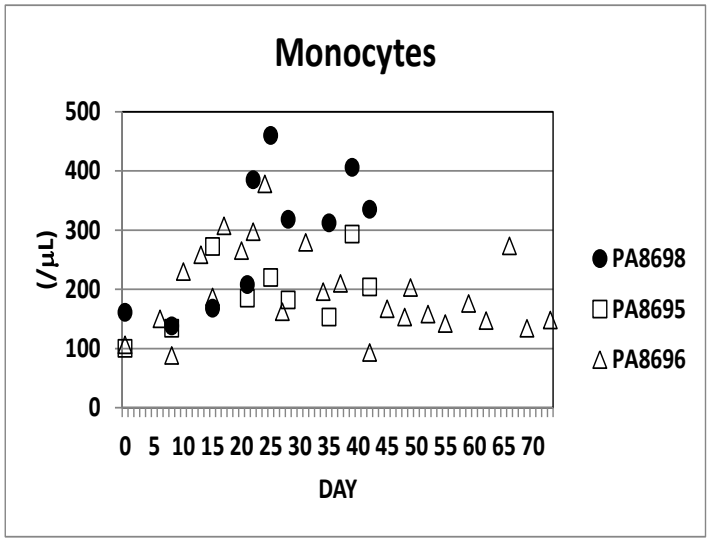


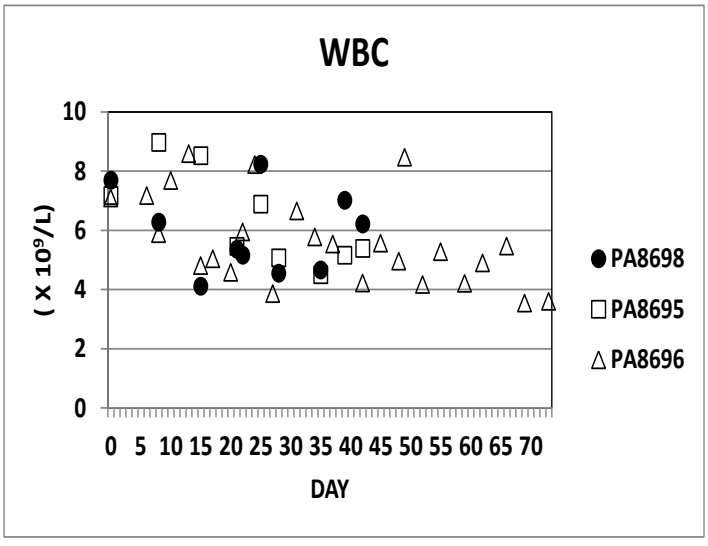
Supplemental Figure 4











Supplemental Figure 5