

## **Supplemental file for:**

# **Enhancing c-MYC degradation via 20S proteasome activation induces *in vivo* anti-tumor efficacy**

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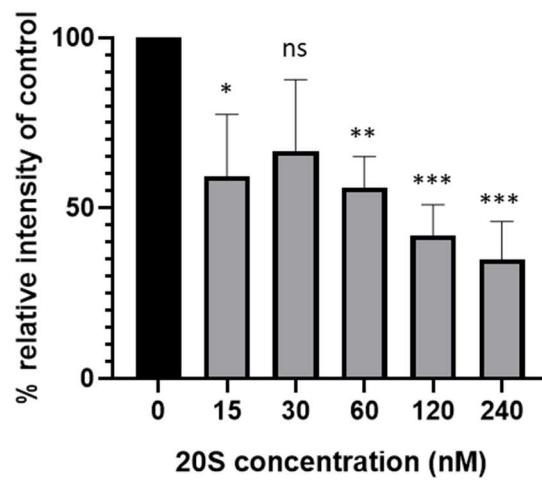
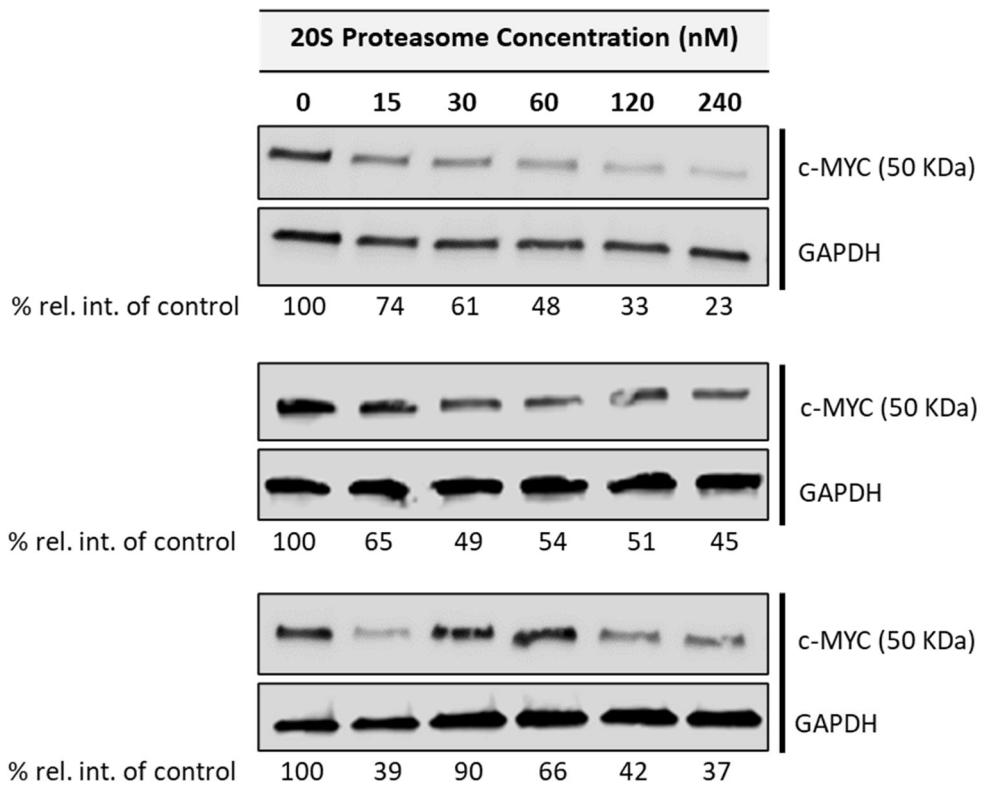
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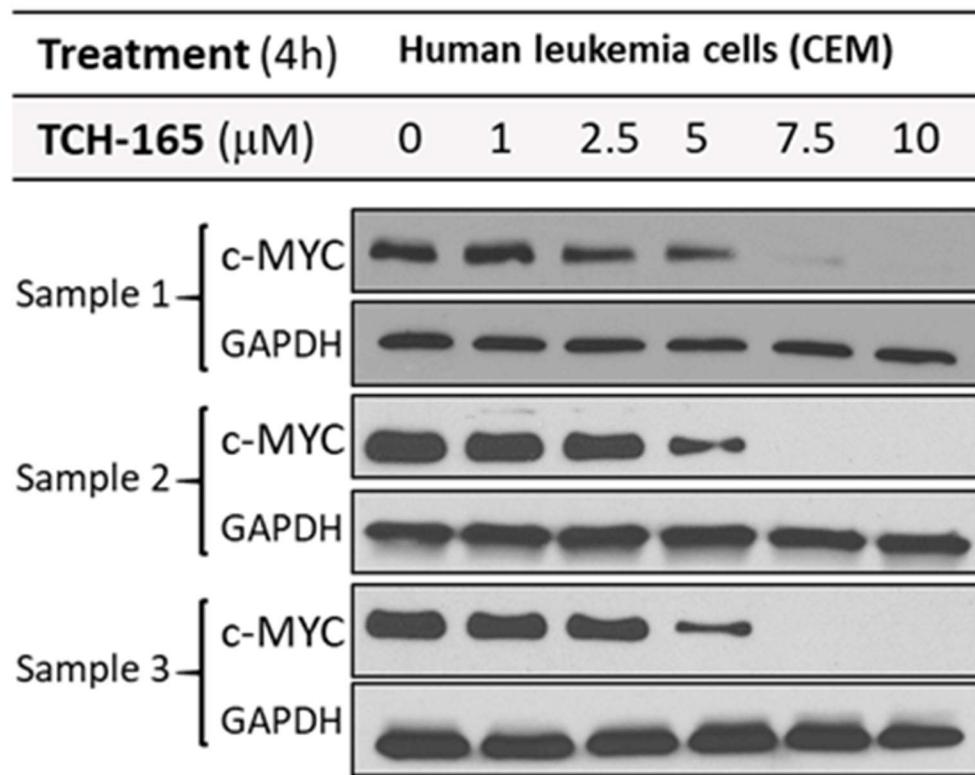
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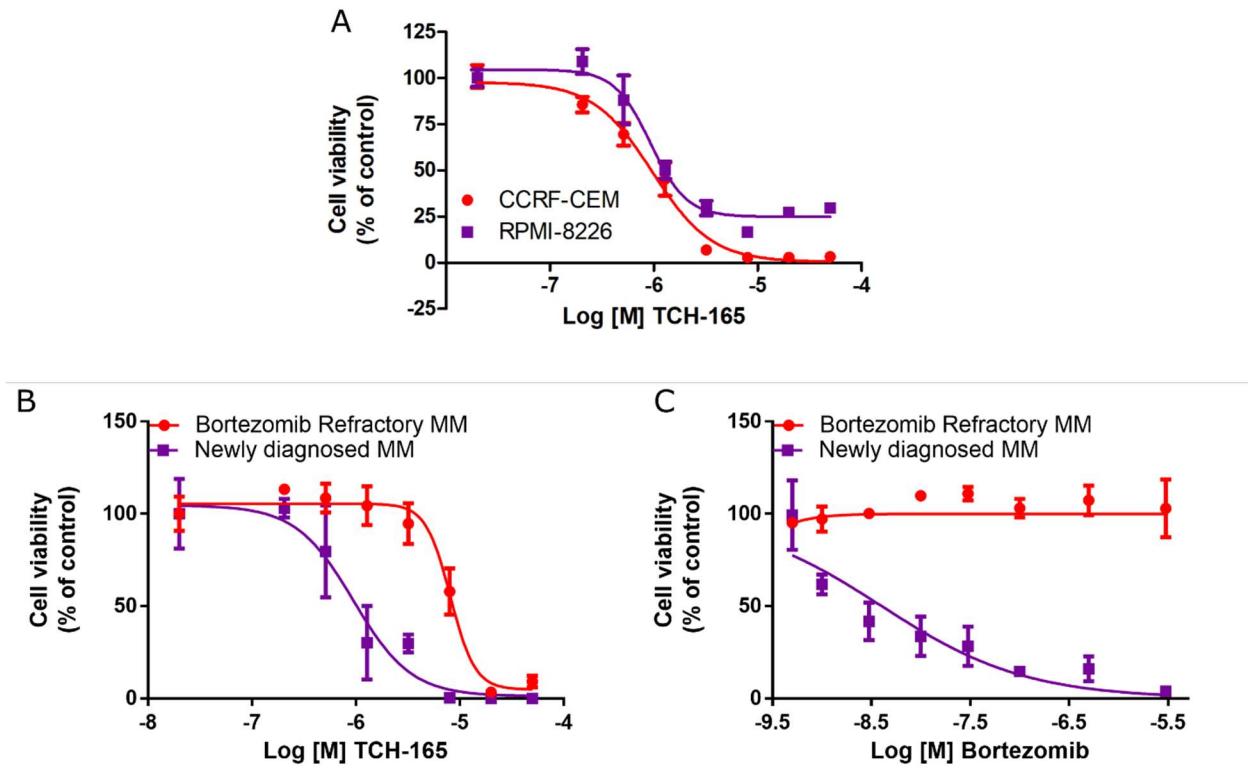
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**Fig. S1: c-MYC degradation by the 20S proteasome (n=3):** Immunoblot of c-MYC exposed to increasing concentrations of purified 20S proteasome after 48hr exposure.

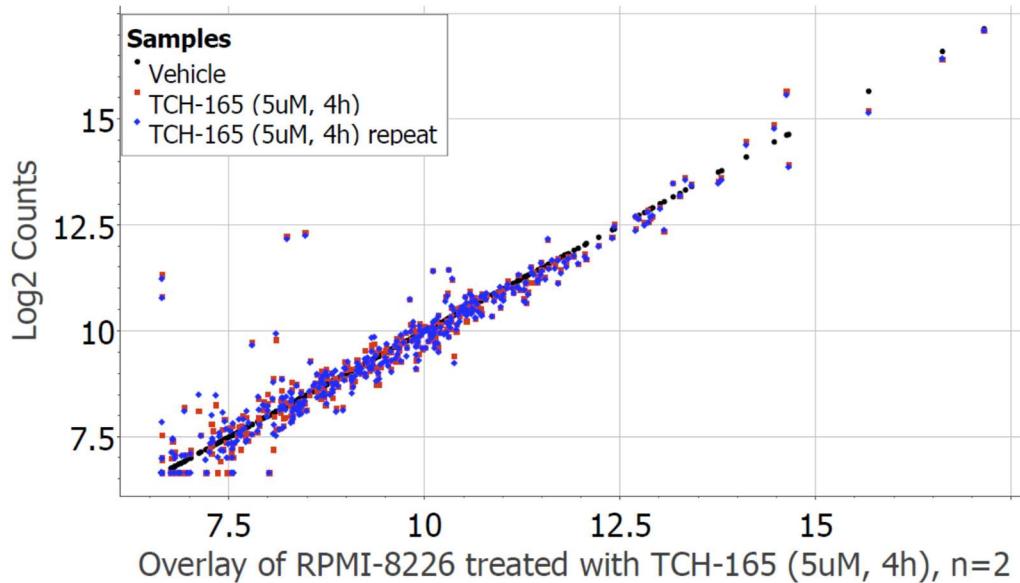


**Fig. S2: c-MYC degradation immunoblots (n=3):** Three different immunoblot of CCRF-CEM cell lysates, showing reproducible, concentration dependent reduction of c-MYC in human leukemia cells upon 4h treatment with various concentrations of TCH-165.

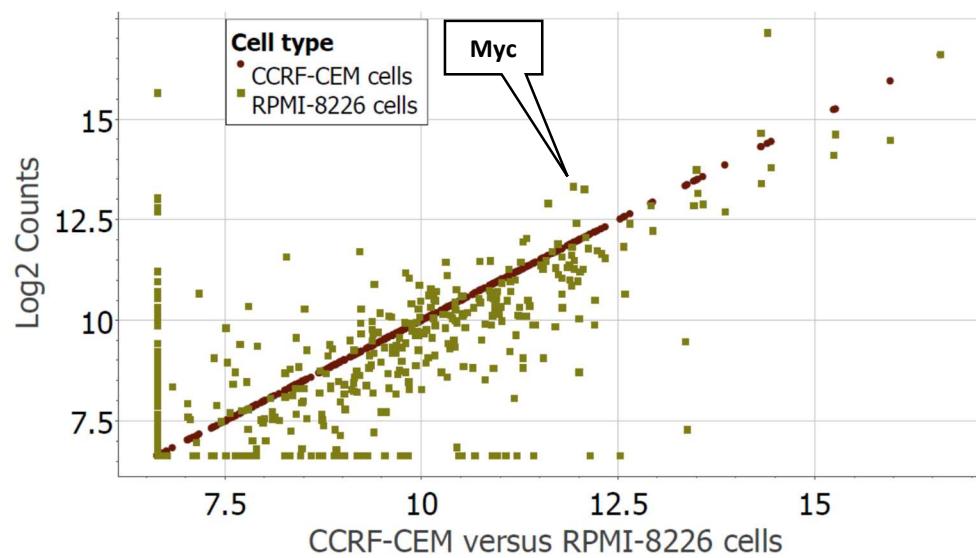


**Fig. S3: Concentration response curves for Fig. 1F.** **(A)** Viability of lymphoblastic leukemia (CCRF-CEM) and multiple myeloma (RPMI-8226) cells following treatment with TCH-165 for 72h. Multiple myeloma cells isolated from bone marrow aspirates of a newly diagnosed patient or a patient who is inherently resistant to bortezomib treatment were treated with TCH-165 **(B)** or Bortezomib **(C)** and cell viability measured after 72h

A



B



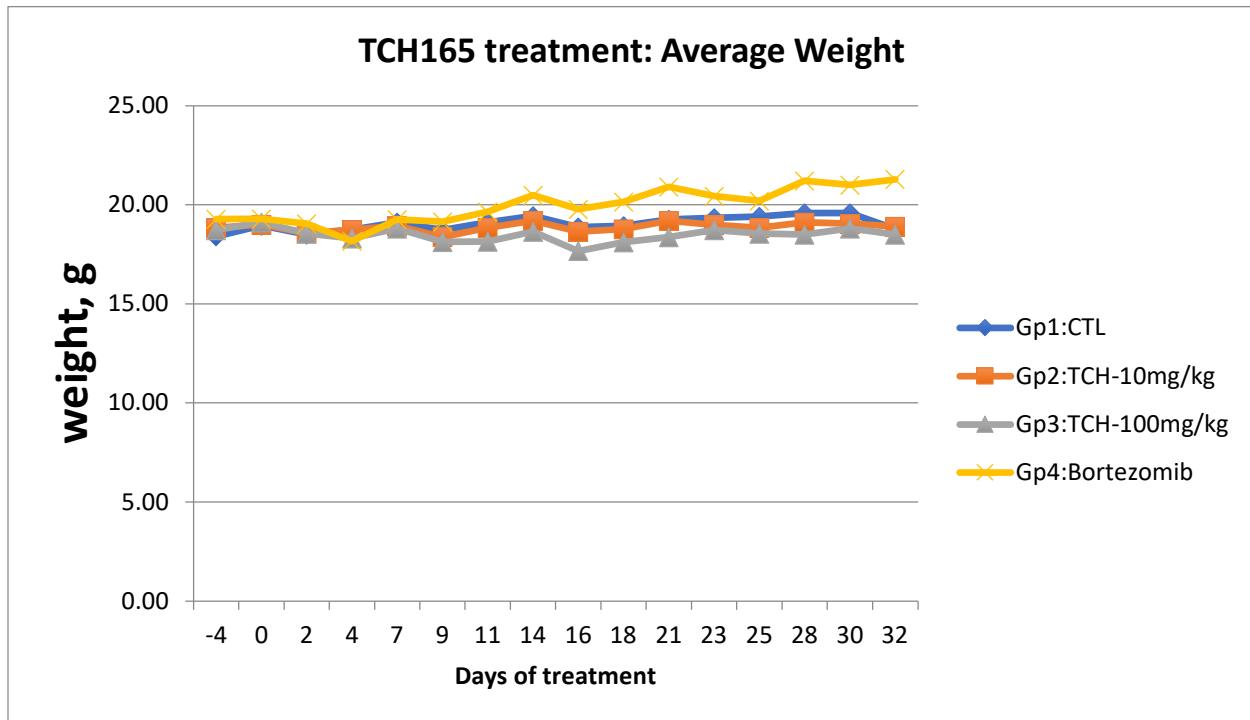
**Fig. S4: Gene expression profiles.** A. Scatter plot of the gene expression profile of CCRF-CEM cells compared to RPMI-8226 cells treated with vehicle for 4 hours. B. Scatter plot of RPMI-8226 cells treated with vehicle (4h) and treated with TCH-165 (5  $\mu$ M, 4h), in two independent experiments (n=2).

Gene Name	Order selected by geNorm	SD after normalization
SLC4A1AP-mRNA	1	0.133
DHX16-mRNA	2	0.106
CNOT4-mRNA	3	0.12
PRPF38A-mRNA	4	0.0707
VPS33B-mRNA	5	0.0907
EIF2B4-mRNA	6	0.113
COG7-mRNA	7	0.102
MRPS5-mRNA	8	0.141
ACAD9-mRNA	9	0.111
SF3A3-mRNA	10	0.188
PIK3R4-mRNA	11	0.18
RBM45-mRNA	12	0.214
SAP130-mRNA	13	0.187
ZNF384-mRNA	14	0.205
MTMR14-mRNA	15	0.16
FTSJ2-mRNA	16	0.181
PIAS1-mRNA	17	0.189
NOL7-mRNA	18	0.2
TMUB2-mRNA	19	0.198
CNOT10-mRNA	20	0.21
GPATCH3-mRNA	21	0.223
TRIM39-mRNA	22	0.237
DNAJC14-mRNA	discarded	0.332
ZKSCAN5-mRNA	discarded	0.345
EDC3-mRNA	discarded	0.353
ERCC3-mRNA	discarded	0.367
FCF1-mRNA	discarded	0.375
NUBP1-mRNA	discarded	0.375
TLK2-mRNA	discarded	0.391
AGK-mRNA	discarded	0.411
TTC31-mRNA	discarded	0.426
ZNF143-mRNA	discarded	0.456
C10orf76-mRNA	discarded	0.518
AMMECR1L-mRNA	discarded	0.518
ZC3H14-mRNA	discarded	0.585
CC2D1B-mRNA	discarded	0.611
USP39-mRNA	discarded	0.631
HDAC3-mRNA	discarded	0.678
ZNF346-mRNA	discarded	0.763
DDX50-mRNA	discarded	1.13

**Fig. S5.** House keeping genes used for normalization of gene expression

Group	PK Timepoint (h) Post-Initial Gavage	Animal #	Plasma Concentration (nM)	Mean Plasma Concentration (nM)	± Standard Deviation
1	0.5	1	447.6	355.1	181.5
		2	146.0		
		3	471.6		
2	1	4	198.0	620.8	398.6
		5	989.6		
		6	674.8		
3	2	7	819.2	932.3	184.5
		8	832.4		
		9	1145.2		
4	4	10	764.0	678.9	323.3
		11	951.2		
		12	321.6		
5	8	22	885.6	845.9	373.6
		23	1198.0		
		24	454.0		
6	9	16	1321.2	1170.9	130.6
		17	1107.2		
		18	1084.4		
7	10	19	646.0	911.6	282.7
		20	880.0		
		21	1208.8		
8	12	13	2138.4	1434.0	625.2
		14	1218.8		
		15	944.8		
9	16	25	840.4	471.1	330.5
		26	203.2		
		27	369.6		

**Fig. S6:** Pharmacokinetic data in mice. TCH-165 plasma concentrations after oral gavage 3:7 (v/v) propylene glycol: 5% D5W vehicle of male CD-1 mice.



**Fig. S7:** Weights of mice during tumor study.

Mean	Days	-17	-14	-11	-7	-3	0	2	4	7	9	11
Gp1: Control		10.60	15.43	43.95	41.76	38.49	63.25	70.62	90.73	137.86	163.48	212.01
Gp2:TCH-10mg/kg		9.68	16.32	30.88	45.85	36.36	58.33	55.23	70.72	89.68	98.72	103.98
Gp3:TCH-100mg/kg		11.18	19.37	32.31	37.50	38.99	49.70	47.20	54.74	73.95	74.03	79.59
Gp4:Bortezomib		10.30	8.87	18.10	28.61	37.38	47.25	69.28	52.54	62.66	64.45	104.43

14	16	18	21	23	25	28	30	32	35	37	39	42
241.02	319.30	346.02	466.65	478.21	561.67	786.61	841.01	900.52	958.86	1026.87	1122.13	1253.46
138.48	148.48	230.71	281.51	343.57	395.40	569.20	739.04	733.71	867.08	1028.36	1046.77	1395.61
84.60	96.08	112.96	107.12	142.69	145.59	169.57	177.03	198.99	239.73	252.08	270.05	304.43
122.46	126.51	137.34	158.57	204.03	264.35	287.39	323.49	363.80	396.01	510.98	617.56	771.41

**Fig. S8:** Tumor volume data from TCH-165 treated RPMI-8226 xenograft model using SCID mice.

**A.** Plasma concentration of TCH-165 at Day 1 and Day 5.    **B.** PK of TCH-165 at Day 1 and Day 5

	Dog 1	Dog 2	Dog 3	Mean	SD	N
<b>Pre-dose</b>	0	0	0	0	0	3
<b>1hr SD1</b>	25	0	23	16	14	3
<b>2hr SD1</b>	103	63	160	109	49	3
<b>4hr SD1</b>	183	121	97	134	44	3
<b>8hr SD1</b>	130	41	45	72	50	3
<b>9hr SD1</b>	534	453	33	340	269	3
<b>10hr SD1</b>	1444	1390	239	1024	681	3
<b>24hr SD1</b>	909	2199	1603	1570	645	3
<b>1hr SD5</b>	593	961	750	768	184	3
<b>2hr SD5</b>	605	881	792	759	141	3
<b>4hr SD5</b>	747	1017	595	786	214	3
<b>8hr SD5</b>	756	782	387	641	221	3
<b>9hr SD5</b>	841	873	350	688	293	3
<b>10hr SD5</b>	1622	1462	733	1272	474	3
<b>24hr SD5</b>	546	1225	1135	969	369	3

	Dog 1	Dog 2	Dog 3	Mean
AUC <sub>(0-24)</sub> Day 1 (nM)	18781	2631	13713	19772
Cmax Day 1 (nM)	1444	2199	1603	1570
Tmax Day 1 (nM)	10	24	24	24
AUC <sub>(0-24)</sub> Day 5 (nM)	22163	27221	18108	22494
Cmax Day 5 (nM)	1622	1462	1135	1272

**Fig. S9:** Pharmacokinetic data in dogs: **A.** Plasma concentration (nM) of TCH-165 following oral gavage (500mg BID) at Day 1 (SD1) and Day 5 (SD5). **B.** Pharmacokinetic parameter of TCH-165 (500mg BID).

	Pre Dose			Day 6		
	Dog 001	Dog 002	Dog 003	Dog 001	Dog 002	Dog 003
Hemolysis		Normal	Normal	Normal		
Lipemia		Normal	Normal	Normal		
Icterus		Normal	Normal	Normal		
Total Protein	g/dL	6.7	6.4	6.8		
RBC	x 10.e6/uL	7.1	6.8	7		
Hgb	g/dL	16.1	15.9	16.3		
Hct	%	47	46	47		
HCT Spun	%	48	44	45		
MCV	fL	66	67	68		
MCH	pg	23	23	23		
MCHC	g/dL	34.0	35.0	34.0		
CHCM	g/dL	33*	34	34		
RDW	%	13**	13**	13**		
Platelet	x10.e3/uL	287	269	188		
MPV	fL	10.4	9.4	13.7		
WBC	x10.e3/uL	9.7a	10.9	8.2		
Seg Neut #	x10.e3/uL	5.7	7.1	5.7		
Neutrophil #	x10.e3/uL	NA	NA	NA		
Band Neutrophil #	x10.e3/uL	0.1	0.2**	0.1		
Lymphocyte #	x10.e3/uL	2.8	3.1	1.6		
Monocyte #	x10.e3/uL	0.6	0.3	0.8		
Eosinophil #	x10.e3/uL	0.5	0.2	0		
Basophil #	x10.e3/uL	0	0	0		
LUC #	x10.e3/uL	NA	NA	NA		
Neutrophil Pct	%	NA	NA	NA		
Seg Neut Pct	%	59	65	70		
Band Neut Pct	%	1	2	1		
Lymphocyte Pct	%	29	28	19		
Monocyte Pct	%	6	3	10		
Eosinophil Pct	%	5	2	0		
Basophil Pct	%	0	0	0		
LUC Pct	%	1	NA	NA		
NRBC	/100 WBC	1	NA	NA		
NRBC #	x10.e3/uL	0.1	NA	NA		
Reactive Lymphs		NA	NA	NA		
Platelet Clump		NA	NA	present		
Platelet Comment		NA	NA	b		

\* Low Result

\*\* High Result

a - WBC corrected for nucleated RBCs

b - Platelet concentration should be considered a minimum value and the MPV may be falsely increased due to platelet clumping.

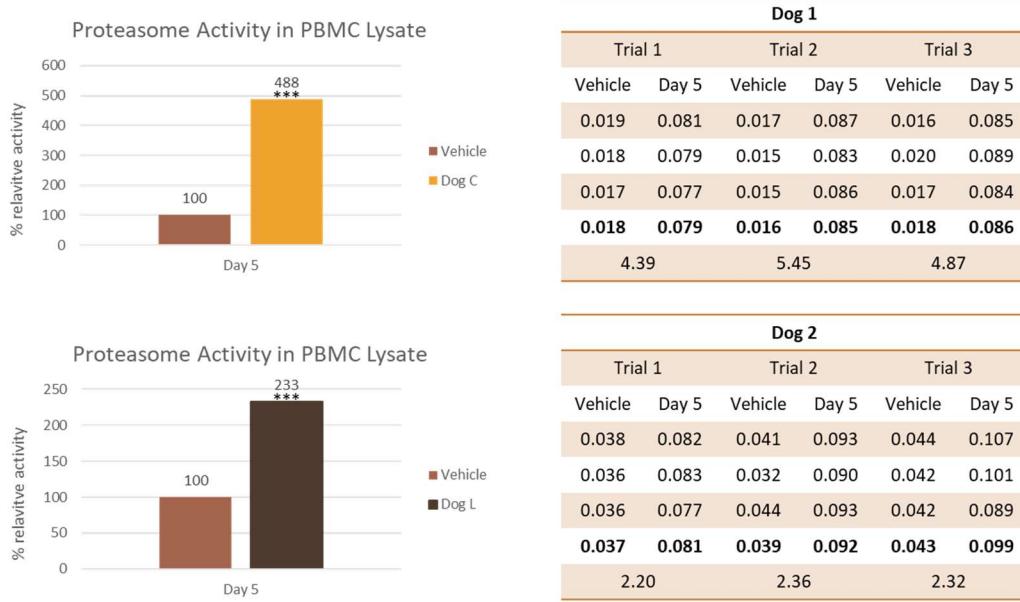
**Fig. S10:** Blood count panel of treated versus untreated dogs

		Pre-Dose			24h Post Day 5 AM Dose		
		Dog 001	Dog 002	Dog 003	Dog 001	Dog 002	Dog 003
<b>Urea Nitrogen</b>	<i>mg/dL</i>	20	16	21	17	14	18
<b>Creatinine</b>	<i>mg/dL</i>	1	0.8	0.9	0.8	0.6	0.7
<b>Sodium</b>	<i>mmol/L</i>	148	148	147	146	144	146
<b>Potassium</b>	<i>mmol/L</i>	4.5	4.4	4.7	4.7	4.6	4.4
<b>Chloride</b>	<i>mmol/L</i>	111	111	106	110	112	110
<b>TCO2</b>	<i>mmol/L</i>	23	24	27	22	18	22
<b>Anion Gap</b>	<i>mmol/L</i>	18	17	19	19	19	18
<b>Na/K Ratio</b>		33	34	31	31	31	33
<b>Osmolarity Calc</b>	<i>mOs/L</i>	308	307	306	303	298	303
<b>Glucose</b>	<i>mg/dL</i>	88	92	87	91	83	87
<b>Calcium</b>	<i>mg/dL</i>	10.1	10.0	10.2	10.3	10.3	9.9
<b>Magnesium</b>	<i>mg/dL</i>	1.9	1.9	2	1.8	1.6 *	1.7
<b>Phosphorus</b>	<i>mg/dL</i>	4.4	4.6	4.9	4.3	4.4	4.3
<b>Iron</b>	<i>ug/dL</i>	126	147	108*	276**	284**	307**
<b>Total Protein</b>	<i>g/dL</i>	5.9	5.7	5.9	5.4	5.0*	5.0*
<b>Albumin</b>	<i>g/dL</i>	3.3	3.2	3.2	3.1	3	2.8
<b>Globulin Calc</b>	<i>g/dL</i>	2.6	2.5	2.7	2.3	2.0*	2.2*
<b>Total Bili</b>	<i>mg/dL</i>	0.2	0.2	0.2	0.1	0.2	0.1
<b>Direct Bili</b>	<i>mg/dL</i>	0	0	0	0	0	0
<b>Indirect Bili</b>	<i>mg/dL</i>	0.2	0.2	0.2	0.1	0.2	0.1
<b>Amylase</b>	<i>U/L</i>	612	457	590	495	505	516
<b>ALP</b>	<i>U/L</i>	59	32	35	66	71	47
<b>ALT</b>	<i>U/L</i>	19*	34	24	25	44	23
<b>AST</b>	<i>U/L</i>	26	37	34	23	32	24
<b>Chol</b>	<i>mg/dL</i>	111	330**	125	184	140	190
<b>CK</b>	<i>U/L</i>	180	136	203	78	241**	83
<b>Hemolysis</b>		Normal	Normal	Normal	Normal	Slight	Normal
<b>Icterus</b>		Normal	Normal	Normal	Normal	Normal	Normal
<b>Lipemia</b>		Normal	Normal	Normal	Normal	Normal	Normal

\* Low Result

\*\* High Result

**Fig. S11:** Clinical chemistry panel of treated versus untreated dogs



**Fig. S12** Target engagement study in treated and untreated dogs