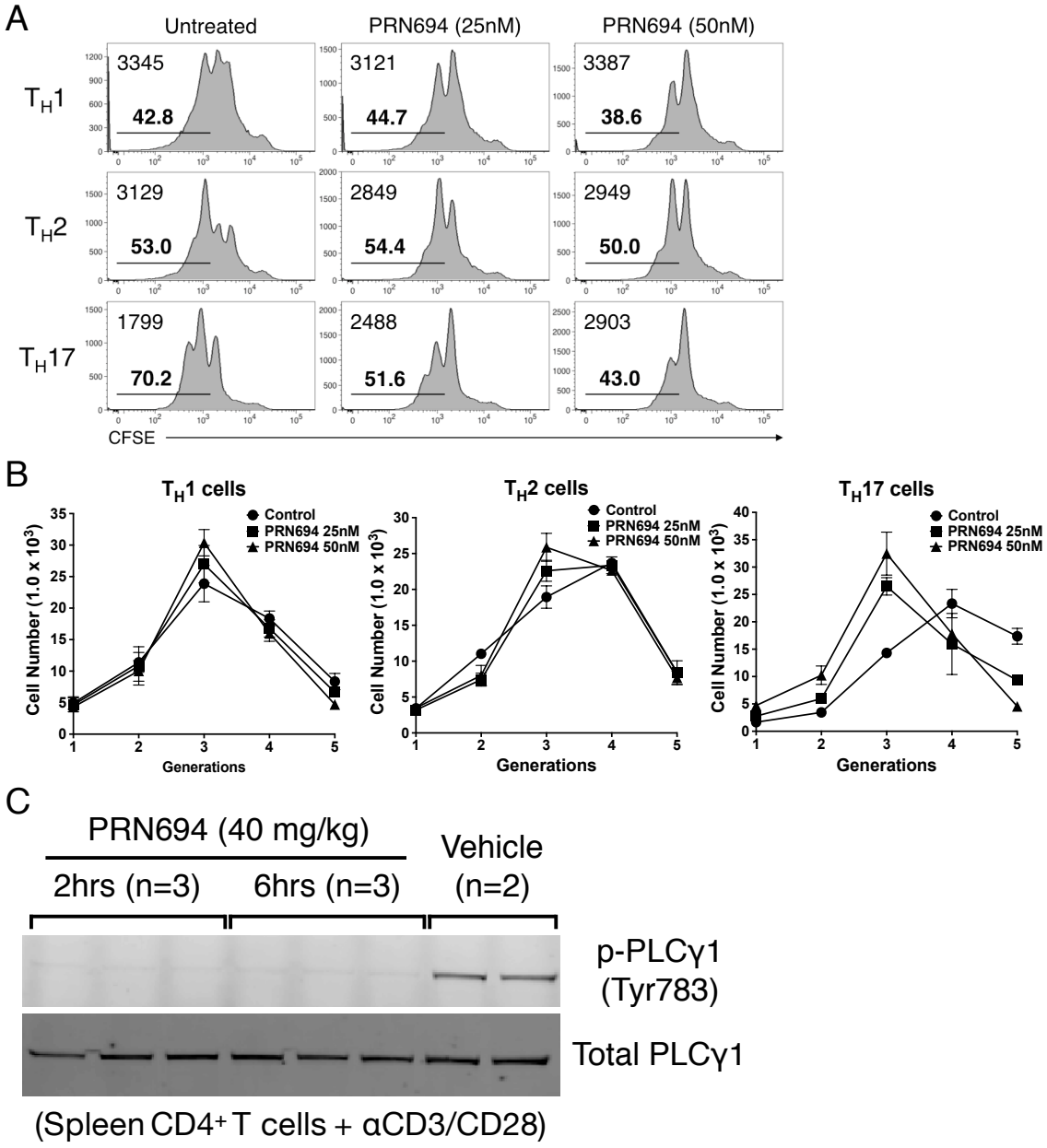
Supplemental Figure 1

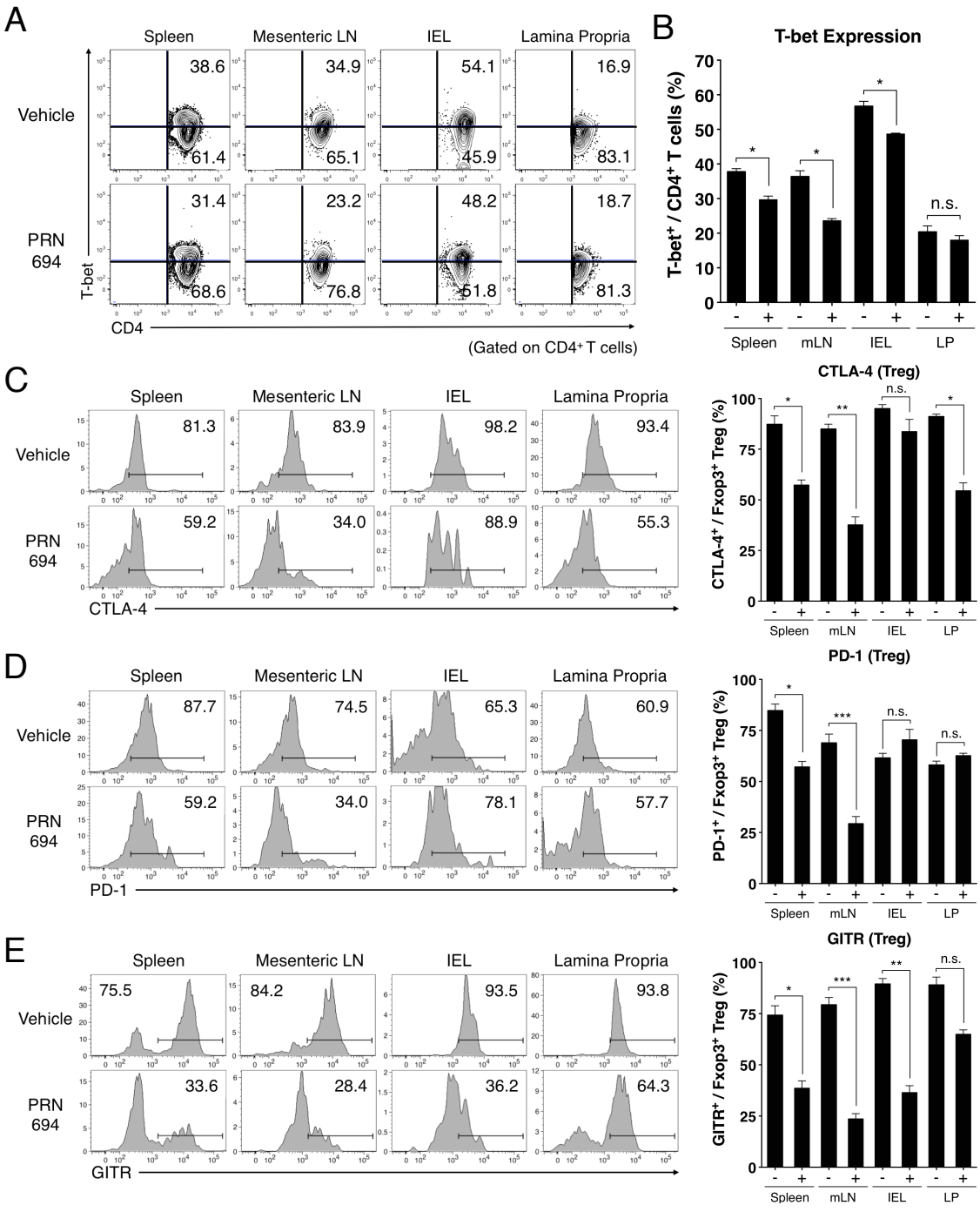


Supplemental Figure 1. PRN694 effect on CD4$^+$ T cell proliferation *in vitro* and phosphorylation of PLCγ1 *in vivo*.

(A-B) CFSE-labeled naïve CD4$^+$ T cells were stimulated with anti-CD3/CD28 under different T_H polarization conditions for 72hr. Histograms are shown to analyze the

magnitude of $CD4^+$ T cell proliferation. Mean fluorescence intensity of CFSE (Top, left) and percentages of more-divided (diluted) $CD4^+$ T cells (Bottom, left) are shown (A). Calculated $CD4^+$ T cell numbers of each cell division (Generation #1-5) by FlowJo software are shown. Data from three experiments were compiled (B). (C) Splenic $CD4^+$ T cells isolated from vehicle-dosed (n=2) or PRN694-dosed (40 mg/kg) (2hr post-treatment, n=3; 6hr post-treatment, n=3) mice were stimulated with anti-CD3/CD28 for 5min and then lysed to examine the phosphorylation of PLCγ1 (Tyr783) by western blot. Both p-PLCγ1 (Top) and total PLCγ1 (Bottom) are shown.

Supplemental Figure 2



Supplemental Figure 2. Transcription factor and surface marker expression on transferred colitogenic CD4$^+$ T cells from vehicle-treated and PRN694-treated mice.

(A-B) Isolated $CD4^+$ T cells from each different organ of vehicle-treated or PRN694-treated mice at 7 weeks of post-transfer were analyzed for T-bet expression, and the percentages of $T\text{-}bet^+$ $CD4^+$ T cells are shown. Graph shows a compilation of data from 3-5 mice in each group. (C-E) Histograms show the expression of CTLA-4 (C), PD-1 (D), and GITR (E) on $CD4^+$ $Foxp3^+$ Treg cells isolated from spleen, mesenteric LN, intestinal epithelium (IEL), and lamina propria tissue of vehicle-treated (-) or PRN694-treated (+) mice. Graphs shown each panel represent the compiled data from 3-5 mice in each group.

Supplemental Table 1

Analytes	Units	Myriad RBM LDD	Myriad RBM LLOQ	Samples	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
				PRN694	-			-			20nM			5μM		
				anti-CD3/CD28	-			+			+			+		
Alpha-1-Antitrypsin (AAT)	mg/mL	3.8E-08	8.2E-08		0.000004	0.000002	0.0000019	0.0000046	0.0000038	0.0000028	0.0000049	0.0000034	0.0000029	0.0000044	0.0000024	0.0000021
Alpha-2-Macroglobulin (A2Macro)	mg/mL	0.000098	0.000087		0.00045	0.00051	0.00052	0.00053	0.00052	0.00045	0.00053	0.00054	0.00053	0.00044	0.00053	0.00050
Beta-2-Microglobulin (B2M)	ug/mL	0.000045	0.000051		0.0066	0.012	0.0082	0.014	0.029	0.021	0.014	0.016	0.020	0.0047	0.012	0.0067
Brain-Derived Neurotrophic Factor (BDNF)	ng/mL	0.0046	0.0072		<0.0072	0.0096	<0.0072	<0.0072	<0.0072	<0.0072	<0.0072	<0.0072	<0.0072	<0.0072	<0.0072	<0.0072
C-Reactive Protein (CRP)	ug/mL	0.0000034	7.8E-06		<7.8E-06	<7.8E-06	<7.8E-06	<7.8E-06	<7.8E-06	<7.8E-06	<7.8E-06	<7.8E-06	<7.8E-06	<7.8E-06	<7.8E-06	<7.8E-06
Complement C3 (C3)	mg/mL	1.6E-08	1E-07		0.0000025	0.0000016	0.000001	0.0000024	0.0000019	0.00000086	0.0000025	0.0000016	0.00000097	0.0000026	0.0000014	0.00000074
Eotaxin-1	pg/mL	1.9	29		<29	<29	<29	<29	<29	<29	<29	<29	<29	<29	<29	<29
Factor VII	ng/mL	1.2	0.77		<0.77	<0.77	<0.77	<0.77	<0.77	<0.77	<0.77	<0.77	<0.77	<0.77	<0.77	<0.77
Ferritin (FRTN)	ng/mL	0.013	0.021		0.24	0.42	0.20	0.44	0.78	0.45	0.49	0.72	0.51	0.29	0.48	0.35
Fibrinogen	mg/mL	0.00000022	2.4E-07		0.000091	0.000047	0.000051	0.000082	0.000047	0.000058	0.000086	0.000047	0.000058	0.000087	0.000034	0.000052
Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)	pg/mL	3.2	18		<18	<18	<18	56	297	49	46	257	38	<18	<18	<18
Haptoglobin	mg/mL	0.00000012	3.2E-07		<3.2E-07	<3.2E-07	<3.2E-07	<3.2E-07	<3.2E-07	<3.2E-07	<3.2E-07	<3.2E-07	<3.2E-07	<3.2E-07	<3.2E-07	<3.2E-07
Intercellular Adhesion Molecule 1 (ICAM-1)	ng/mL	0.35	0.26		<0.26	<0.26	<0.26	<0.26	0.28	<0.26	<0.26	0.28	<0.26	<0.26	<0.26	<0.26
Interferon gamma (IFN-gamma)	pg/mL	0.49	0.30		<0.30	<0.30	<0.30	42	516	52	11	170	5.3	<0.30	<0.30	<0.30
Interleukin-1 alpha (IL-1 alpha)	ng/mL	0.00030	0.00040		<0.00040	<0.00040	<0.00040	0.0023	0.0054	0.0032	0.00085	0.0042	0.00047	<0.00040	<0.00040	<0.00040
Interleukin-1 beta (IL-1 beta)	pg/mL	0.30	0.57		2.8	.57	1.7	44	126	47	9.8	90	11	.57	.57	.57
Interleukin-1 receptor antagonist (IL-1ra)	pg/mL	9.9	19		<19	<19	<19	184	218	211	406	417	652	<19	46	<19
Interleukin-2 (IL-2)	pg/mL	1.0	1.7		1.7	1.7	1.7	594	1800	660	174	787	83	1.7	1.7	1.7
Interleukin-3 (IL-3)	ng/mL	0.0011	0.0032		<0.0032	<0.0032	<0.0032	0.0036	0.0059	0.0038	<0.0032	<0.0032	<0.0032	<0.0032	<0.0032	<0.0032
Interleukin-4 (IL-4)	pg/mL	4.4	5.9		5.9	5.9	5.9	75	131	86	33	97	16	5.9	5.9	5.9
Interleukin-5 (IL-5)	pg/mL	1.0	2.6		2.6	2.6	2.6	5.6	19	7.2	3.1	13	2.6	2.6	2.6	2.6
Interleukin-6 (IL-6)	pg/mL	0.84	2.2		22	2.2	13	104	192	117	18	58	88	2.2	2.2	3.7
Interleukin-7 (IL-7)	pg/mL	2.1	1.8		<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	2.5	<1.8	<1.8	<1.8
Interleukin-8 (IL-8)	pg/mL	0.63	0.79		2630	489	1810	18500	15900	16700	14100	16100	19800	1100	1540	1650
Interleukin-10 (IL-10)	pg/mL	1.1	1.4		<1.4	<1.4	<1.4	59	97	97	18	29	9.4	<1.4	<1.4	<1.4
Interleukin-12 Subunit p40 (IL-12p40)	ng/mL	0.016	0.055		<0.055	<0.055	<0.055	<0.055	0.21	<0.055	<0.055	<0.055	<0.055	<0.055	<0.055	<0.055
Interleukin-12 Subunit p70 (IL-12p70)	pg/mL	5.9	9.7		<9.7	<9.7	<9.7	<9.7	132	10	<9.7	24	<9.7	<9.7	<9.7	<9.7
Interleukin-15 (IL-15)	ng/mL	0.12	0.079		<0.079	<0.079	<0.079	<0.079	<0.079	<0.079	<0.079	0.080	<0.079	<0.079	<0.079	<0.079
Interleukin-17 (IL-17)	pg/mL	1.2	0.99		.99	.99	.99	12	8.6	10	5.0	3.7	1.6	.99	.99	.99
Interleukin-18 (IL-18)	pg/mL	3.7	8.3		<8.3	<8.3	<8.3	<8.3	16	8.3	<8.3	12	<8.3	<8.3	<8.3	<8.3
Interleukin-23 (IL-23)	ng/mL	0.12	0.080		<0.080	<0.080	<0.080	<0.080	<0.080	<0.080	<0.080	<0.080	<0.080	<0.080	<0.080	<0.080
Macrophage Inflammatory Protein-1 alpha (MIP-1 alpha)	pg/mL	8.2	8.4		198	22	206	4790	5810	4040	6320	11800	5220	53	83	138
Macrophage Inflammatory Protein-1 beta (MIP-1 beta)	pg/mL	4.0	6.2		1350	257	1460	22600	27900	23500	20300	39300	17400	297	391	651
Matrix Metalloproteinase-2 (MMP-2)	ng/mL	1.3	0.87		1.4	<0.87	<0.87	6.1	7.9	7.7	5.0	9.9	7.1	<0.87	<0.87	<0.87
Matrix Metalloproteinase-3 (MMP-3)	ng/mL	0.0066	0.012		<0.012	<0.012	<0.012	<0.012	<0.012	<0.012	<0.012	<0.012	<0.012	<0.012	<0.012	<0.012
Matrix Metalloproteinase-9 (MMP-9)	ng/mL	5.0	7.9		<7.9	<7.9	<7.9	<7.9	<7.9	<7.9	<7.9	<7.9	<7.9	<7.9	<7.9	<7.9
Monocyte Chemotactic Protein 1 (MCP-1)	pg/mL	4.6	9.0		508	206	298	1710	2760	2680	1550	3690	2150	51	144	50
Stem Cell Factor (SCF)	pg/mL	18	23		<23	<23	<23	<23	<23	<23	<23	<23	<23	<23	<23	<23
T-Cell-Specific Protein RANTES (RANTES)	ng/mL	0.00018	0.0010		0.26	0.13	0.18	0.48	0.30	0.41	0.51	0.28	0.40	0.32	0.066	0.24
Tissue Inhibitor of Metalloproteinases 1 (TIMP-1)	ng/mL	0.010	0.016		3.0	2.1	2.0	2.1	1.9	1.8	2.2	1.6	2.1	1.1	1.3	0.75
Tumor Necrosis Factor alpha (TNF-alpha)	pg/mL	2.9	4.5		42	6.9	53	3830	7680	3270	2920	6880	2340	9.0	21	38
Tumor Necrosis Factor beta (TNF-beta)	pg/mL	2.3	1.9		<1.9	<1.9	<1.9	11	34	13	5.3	24	3.5	<1.9	<1.9	<1.9
Tumor necrosis factor receptor 2 (TNFR2)	ng/mL	0.0011	0.0057		0.077	0.071	0.053	0.21	0.24	0.22	0.23	0.26	0.26	0.053	0.097	0.052
Vascular Cell Adhesion Molecule-1 (VCAM-1)	ng/mL	0.0090	0.057		<0.057	<0.057	<0.057	<0.057	<0.057	<0.057	<0.057	<0.057	<0.057	<0.057	<0.057	<0.057
Vascular Endothelial Growth Factor (VEGF)	pg/mL	12	6.8		12	13	8.3	10	14	10	<6.8	10	<6.8	8.3	14	<6.8
Vitamin D-Binding Protein (VDBP)	ug/mL	0.00002	0.000028		0.00037	0.000078	0.00014	0.00039	0.00013	0.00016	0.00039	0.000078	0.00018	0.00036	0.000066	0.00014
von Willebrand Factor (vWF)	ug/mL	0.0016	0.0034		0.018	0.017	0.014	0.016	0.015	0.015	0.015	0.015	0.014	0.015	0.011	0.014

Supplemental Table 1. Inhibition of cytokines upon anti-CD3/CD28 stimulation of human PBMC by PRN694 using Human InflammationMap® v.1.0 biomarker panel.

Shown are the levels of cytokine measured at each condition. The least detectable dose (LDD) was determined as the mean + 3 standard deviations of 20 blank readings. The LLOQ (Lower Limit of Quantitation) is the lowest concentration of an analyte in a sample that can be reliably detected.

Supplemental Table 2

Analytes	Units	Myriad RBM LDD	Myriad RBM LLOQ	Samples	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6	Mouse 7	Mouse 8	Mouse 9	Mouse 10	Mouse 11	Mouse 12
				PRN694	-			-			+			+		
				anti-CD3	-			+			1hr			6hr		
Apolipoprotein A-I (Apo A-I)	ug/mL	2.3	20		357	543	291	353	244	289	315	312	263	250	193	307
C-Reactive Protein Mouse (CRP Mouse)	ug/mL	0.75	2.1		5.6	4.9	3.8	7.0	5.5	4.4	4.0	4.7	4.3	6.4	7.2	8.0
CD40 (CD40)	pg/mL	6.7	13		44	47	38	48	56	81	61	69	62	48	72	116
CD40 Ligand (CD40-L)	pg/mL	370	428		1040	787	443	1480	1310	1840	1130	1480	1310	3740	3150	2960
Eotaxin	pg/mL	3.5	6.2		953	1100	1200	2770	3050	3910	2160	3160	2270	6940	8140	6050
Epidermal Growth Factor Mouse (EGF Mouse)	pg/mL	18	15		20	17	20	35	40	44	31	29	35	51	60	49
Factor VII	ng/mL	25	19		25	38	27	54	52	68	38	51	47	82	96	70
Fibrinogen	ug/mL	231	510		73100	89600	70100	66000	76900	62300	73900	92600	74100	75900	80600	117000
Fibroblast Growth Factor 9 (FGF-9)	ng/mL	2.4	2.9		6.6	4.2	5.2	16	16	18	9.3	14	14	12	14	16
Fibroblast Growth Factor basic (FGF-basic)	ng/mL	12	15		<15	<15	<15	<15	<15	<15	<15	<15	<15	<15	<15	<15
Granulocyte Chemotactic Protein-2 Mouse (GCP-2 Mouse)	ng/mL	0.16	0.49		1.4	1.4	1.3	3.6	3.4	3.2	2.2	2.4	1.8	4.1	4.8	4.1
Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)	pg/mL	4.1	6.8		<6.8	<6.8	<6.8	77	281	114	18	33	37	19	32	19
Growth Hormone (GH)	ng/mL	1.9	1.6		5.7	3.0	3.0	3.0	3.5	3.5	3.5	3.3	3.0	6.0	4.2	2.6
Growth-Regulated Alpha Protein (KC/GRO)	ng/mL	0.012	0.026		0.48	0.74	1.3	14	11	14	5.0	7.4	7.0	6.4	8.0	6.2
Haptoglobin	ug/mL	118	121		131	160	126	125	125	125	127	125	127	144	146	150
Immunoglobulin A (IgA)	ug/mL	18	19		258	1460	117	419	342	169	368	144	431	218	258	346
Insulin	uIU/mL	4.1	6.3		<6.3	<6.3	<6.3	<6.3	<6.3	<6.3	<6.3	<6.3	<6.3	<6.3	<6.3	<6.3
Interferon gamma (IFN-gamma)	pg/mL	15	38		<38	<38	<38	128	152	98	<38	40	47	54	86	80
Interferon gamma Induced Protein 10 (IP-10)	pg/mL	6.6	7.1		292	1740	165	2940	3910	2780	737	2010	1490	3450	3470	4190
Interleukin-1 alpha (IL-1 alpha)	pg/mL	108	132		156	<132	<132	327	312	265	219	281	281	327	296	357
Interleukin-1 beta (IL-1 beta)	ng/mL	6.5	3.5		<3.5	<3.5	<3.5	<3.5	<3.5	<3.5	<3.5	<3.5	<3.5	<3.5	<3.5	<3.5
Interleukin-2 (IL-2)	pg/mL	25	49		<49	<49	<49	428	761	541	<49	<49	<49	69	146	<49
Interleukin-3 (IL-3)	pg/mL	2.9	4.3		<4.3	<4.3	<4.3	9.4	14	8.4	<4.3	4.5	5.4	<4.3	6.4	5.4
Interleukin-4 (IL-4)	pg/mL	52	68		<68	<68	<68	106	633	224	<68	73	87	<68	100	<68
Interleukin-5 (IL-5)	ng/mL	0.47	0.75		.75	.75	.75	0.92	0.76	0.82	1.3	<0.75	<0.75	3.6	3.7	0.87
Interleukin-6 (IL-6)	pg/mL	2.6	3.8		45	21	17	700	1260	461	206	307	152	119	510	269
Interleukin-7 (IL-7)	ng/mL	0.086	0.12		0.14	<0.12	0.14	0.54	0.48	0.69	0.24	0.36	0.39	0.55	0.48	0.53
Interleukin-10 (IL-10)	pg/mL	91	220		220	220	220	762	1090	979	271	440	504	440	590	376
Interleukin-11 (IL-11)	pg/mL	40	70		70	70	70	122	122	110	76	110	110	103	122	155
Interleukin-12 Subunit p70 (IL-12p70)	ng/mL	0.12	0.14		.14	.14	.14	0.40	0.45	0.25	0.14	0.17	0.25	0.22	0.33	0.31
Interleukin-17A (IL-17A)	ng/mL	0.0071	0.0073		<0.0073	<0.0073	<0.0073	0.16	0.16	0.13	0.029	0.037	0.042	0.042	0.056	0.045
Interleukin-18 (IL-18)	ng/mL	6.2	8.0		14	14	12	14	12	14	16	14	14	16	18	20
Leptin	ng/mL	0.026	0.053		1.1	0.87	0.44	0.25	0.58	0.90	1.3	1.0	1.3	0.92	0.64	1.7
Leukemia Inhibitory Factor (LIF)	pg/mL	218	463		487	<463	<463	1530	1250	1530	583	1010	869	1810	2360	1710
Lymphotactin	pg/mL	19	21		93	75	67	1120	1340	1420	346	517	508	713	989	919
Macrophage Colony-Stimulating Factor-1 (M-CSF-1)	ng/mL	0.033	0.051		5.7	7.4	5.5	6.1	5.4	5.1	5.9	5.1	5.8	6.7	7.3	7.0
Macrophage-Derived Chemokine (MDC)	pg/mL	34	55		1280	1220	910	2180	3000	2900	1500	1960	1500	3870	4270	3640
Macrophage Inflammatory Protein-1alpha (MIP-1 alpha)	ng/mL	4.1	3.3		3.3	3.3	3.3	4.2	3.3	4.2	3.7	3.3	3.3	5.6	6.1	6.1
Macrophage Inflammatory Protein-1 beta (MIP-1 beta)	pg/mL	47	53		1590	960	714	20100	22800	25200	5960	9790	10600	10200	17100	12700
Macrophage Inflammatory Protein-1 gamma (MIP-1 gamma)	ng/mL	0.45	0.67		10	11	12	14	14	11	13	13	12	19	17	18
Macrophage Inflammatory Protein-2 (MIP-2)	pg/mL	5.3	11		52	32	36	5210	3360	3420	819	2270	1040	711	1380	1520
Macrophage Inflammatory Protein-3 beta (MIP-3 beta)	ng/mL	0.36	1.2		2.1	2.5	1.5	3.1	4.0	4.5	2.5	3.4	2.4	6.2	7.0	5.4
Matrix Metalloproteinase-9 (MMP-9)	ng/mL	9.9	14		66	66	48	168	162	125	109	100	104	100	111	106
Monocyte Chemotactic Protein 1 (MCP-1)	pg/mL	4.9	7.9		1810	1460	1550	4390	3630	4680	3170	3490	3780	4110	4250	4650
Monocyte Chemotactic Protein 3 (MCP-3)	pg/mL	4.2	4.4		1800	1470	2060	3820	4150	4170	3110	3100	3610	4210	4250	4430
Monocyte Chemotactic Protein-5 (MCP-5)	pg/mL	1.7	2.3		172	68	265	1070	1180	676	381	374	739	985	1320	1080
Myeloperoxidase (MPO)	ng/mL	0.48	0.66		102	102	61	115	140	146	100	112	104	191	182	174
Myoglobin	ng/mL	5.7	6.0		81	15	9.6	380	64	32	109	45	13	103	58	156
Oncostatin-M (OSM)	ng/mL	0.097	0.21		0.42	0.22	0.27	1.0	0.96	1.1	0.62	1.0	0.88	0.98	1.2	1.2
Plasminogen Activator Inhibitor 1 (PAI-1)	ng/mL	0.015	0.034		1.5	9.2	0.73	9.0	13	7.6	3.4	10	9.3	11	12	11
Resistin	ng/mL	0.013	0.039		<0.039	<0.039	<0.039	<0.039	<0.039	<0.039	<0.039	<0.039	<0.039	<0.039	<0.039	<0.039
Serum Amyloid P-Component (SAP)	ug/mL	6.4	9.0		45	45	42	56	49	42	39	47	48	42	53	62
Stem Cell Factor (SCF)	pg/mL	153	290		1230	934	1110	3010	2470	2740	2010	2270	2410	2780	3130	2940
T-Cell-Specific Protein RANTES (RANTES)	pg/mL	0.026	0.028		0.031	<0.028	<0.028	0.27	0.20	0.29	0.059	0.049	0.092	0.10	0.18	0.12
Thrombopoietin	ng/mL	30	60		<60	<60	76	68	<60	60	68	68	60	84	99	95
Tissue Inhibitor of Metalloproteinases 1 Mouse (TIMP-1 Mouse)	ng/mL	0.021	0.084		1.5	1.6	1.3	1.8	2.0	1.2	1.2	1.3	1.1	4.6	4.5	2.7
Tumor Necrosis Factor alpha (TNF-alpha)	ng/mL	0.029	0.11		<0.11	<0.11	<0.11	0.28	0.23	0.25	0.17	0.16	0.22	0.20	0.23	0.23
Vascular Cell Adhesion Molecule-1 (VCAM-1)	ng/mL	3.3	5.4		732	894	682	970	953	814	1040	1090	877	933	1060	1360
Vascular Endothelial Growth Factor A (VEGF-A)	pg/mL	222	158		717	474	756	2080	1960	2280	1230	1750	1690	2000	2360	2320
von Willebrand factor (vWF)	ng/mL	27	45		345	351	457	385	589	446	671	578	731	639	677	655

Supplemental Table 2. Inhibition of cytokines upon anti-CD3 stimulation *in vivo* by PRN694 using RodentMap® v.3.0 biomarker panel.

Shown are the levels of cytokine measured at each condition. The least detectable dose (LDD) was determined as the mean + 3 standard deviations of 20 blank readings. The

LLOQ (Lower Limit of Quantitation) is the lowest concentration of an analyte in a sample that can be reliably detected.