Supplementary Methods

Animals. Eight healthy adult (median age 2 years old; range 1-4) intact male mixed breed purpose-bred dogs were used (28.4 ± 5.6 kg). Dogs were loaned from the Francis Owen Blood Research Laboratory at the University of North Carolina at Chapel Hill or Laboratory Animal Resources at the North Carolina State College of Veterinary Medicine (NCSU).

To compare our experimental model with spontaneous canine ITP, blood samples were also obtained from dogs with naturally-occurring ITP (n=6) or thrombocytopenia of other causes (n=4) (Supplementary Table 1). These dogs were patients at the College of Veterinary Medicine, NCSU, from December 2011 through September 2012; owners consented to study enrollment. Enrollment criterion was a platelet count of $<30 \times 10^9$/l, the platelet range at which variable bleeding may occur. Exclusion criteria consisted of weight less than 10 lbs. or recent transfusion with a platelet-containing blood product. Diagnosis of primary ITP was based on thrombocytopenia in the absence of another identifiable cause.

All protocols were approved by the Institutional Animal Care and Use Committee of North Carolina State University.

Human ITP patients. Adult patients were diagnosed with chronic ITP according to international guidelines (Rodeghiero *et al*, 2009). Exclusion criterion was treatment with immunoglobulin or anti-D in the three months prior to inclusion. Blood was drawn at up to 3 serial visits into plain vacutainers; serum was prepared by centrifugation and stored at -80°C until further analysis. Platelet counts were measured in EDTA-anticoagulated blood.

Production and characterization of monoclonal antibodies. 2F9 is a murine monoclonal IgG2a recognizing canine GPIIb (Burstein, *et al*, 1991). The hybridoma was a gift of David Wilcox (Medical College of Wisconsin, Milwaukee, WI). The isotype control murine IgG2a anti-yellow fever (αYFA or CRL-1689.1) hybridoma was a gift of Gregg Dean (Colorado State University College of Veterinary Medicine, Fort Collins, CO) (Smithberg *et al*, 2008). Both antibodies were produced from their respective hybridomas by the Tissue Culture Facility, University of North Carolina, Chapel Hill.

Concentration of antibodies was determined using the BCA Protein Assay Kit (Pierce, Rockford, IL). The purity of the antibody preparations was checked by sodium dodecyl sulfate-

polyacrylamide gel electrophoresis (SDS-PAGE) and silver staining of the gel. Antibodies were determined to be mycoplasma-free using a rapid detection DNA probe test (Gen-Probe, San Diego, CA) and endotoxin-free using the Gel Clot LAL assay (Lonza, Walkersville, MD) to the limits of detection (100,000 organisms/ml and 0.03 EU/ml, respectively). Western blot analysis was performed to confirm the specific reactivity of 2F9 with GPIIb by using control dog platelets and frozen platelets previously prepared from Otterhounds with Glanzmann's thrombasthenia (Boudreaux *et al*, 2007). Platelets were prepared and lysed as described in Boudreaux et al (Boudreaux *et al*, 2007). Washed platelets were solubilized in Laemmli sample buffer containing 1 µg/mL pepstatin A, 5 mM EDTA, 1x Halt^{TM} protease and phosphatase inhibitor cocktail (Thermo Scientific, Rockford, IL). Oxidized or reduced (5 % βME, Sigma-Aldrich) platelet cell lysates (40 µg, 20 µg, and 10 µg protein per lane) were loaded onto pre-cast 10% polyacrylamide gels (Mini- PROTEAN TXG, BIO-RAD, Hercules, CA) and separated by SDS-PAGE (200 V, 30 min, RT) using 1x Tris-glycine SDS electrophoresis buffer (BIO-RAD) then electro-blotted to polyvinylidene difluoride (PVDF) membranes. PVDF membranes were blocked in 5% human serum albumin (Talecris, Clayton, NC) in PBS-T (0.2% Tween-20, Sigma-Aldrich). The membranes were first probed with 2F9 (1.0 µg/ml), washed, and then reacted with a goat anti-murine HRP antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) containing StepTactin HRP conjugate (BIO-RAD; 1:10,000 dilution) for detection of molecular weight markers (Precision Plus Protein™ WesternC™, BIO-RAD). Immuno- reactive platelet proteins on PVDF membranes were visualized by chemiluminescence using an ECL+Plus^{TM} kit (GE Healthcare Pittsburgh, PA) as directed. Digital images were captured and analyzed using a ChemiDoc™ XRS+ imaging system with Image Lab™ analysis software (BIO-RAD).

2F9 Dose Titration. One proof-of-concept dog received 300 µg/kg of 2F9, which induced absolute thrombocytopenia for 3 days (not shown). To model the clinical disease with variable bleeding tendencies at platelet counts below 30 x 10^9 platelets/l, we performed a dose-titration study in two dogs, aiming for a target platelet nadir of 5–30 x 10^9 platelets/l.

In the first animal, 2F9 was administered at a starting dose of 15 ng/kg i.v. The dose was increased by a factor of ten every 1–2 hours until the platelet count fell in the target range.

Platelet count was assessed hourly after antibody administration. Time zero was when the platelet count first fell into the target nadir range.

The second dog received a starting dose of 1.5 µg/kg. This dose was increased by a factor of ten until a decrease in platelet count was observed. The additional dose required to reach the target range was then calculated based on the amount of antibody given to achieve this initial decrease in platelet count.

Subsequently, three dogs were employed in a dose-repeatability study, receiving starting 2F9 doses of 50 µg/kg, based on the dose-titration study results; additional doses were given as needed and were based on their decrease in platelet count in response to the first 2F9 dose.

Three dogs served as controls. They received αYFA, the isotype control antibody, via infusion at the highest cumulative effective dose of 2F9 administered (167 µg/kg).

The first administration of each antibody was given as a slow intravenous infusion via a preplaced cephalic catheter through a syringe filter (Fisher Scientific, Dublin, Ireland) over 20 minutes. Subsequent doses were administered as intravenous boluses. Demeanor, temperature, pulse, and respiration were monitored throughout antibody infusion and before and after bolus antibody administration.

Blood sampling and processing. Dexmedetomidine (Pfizer, NY, NY) was administered intravenously through a peripheral catheter to effect (median 11.0 µg/kg; range 8.0-21.9 µg/kg) and a 19-gauge indwelling central catheter (BD Intracath; Becton Dickinson, Franklin Lakes, NJ) was placed in the jugular or lateral saphenous vein of each dog for blood sampling throughout the study (both control and 2F9 treated dogs). If needed, dogs were reversed with atipamezole (Antisedan; Pfizer) on completion of catheter placement.

Blood was sampled via indwelling catheters. Catheters were first flushed with 6 ml of 0.9% sodium chloride. A 5 ml catheter purge sample was then collected into 1 ml 0f 0.9% sodium chloride and set aside. Blood was collected into a plain syringe and transferred immediately to EDTA tube (Tyco Healthcare Group, Mansfield, MA) (2 ml) for platelet and complete blood count and an additive-free tube (Tyco) (3 ml) for serum preparation and into a syringe containing 0.38 % sodium citrate (final concentration; 4 ml) for plasma preparation. The initial purge sample was then returned to the dog and the catheter flushed with 6 ml of 0.9% sodium chloride. Sodium citrate blood was spun at room temperature at 2440 g for 15 minutes

and supernatant spun again at 3500 g for 15 minutes to generate platelet free plasma which was then stored at -80°C for future coagulation parameter analysis. Serum was generated after allowing the additive-free tube to clot at room temperature for a minimum of 20 minutes by centrifugation at 1000 g for 15 minutes. Serum was harvested and stored at - 80°C for future cytokine analysis.

In hospitalized canine patients with spontaneous ITP blood was drawn once on study enrollment.

Platelet counts and complete blood counts. Canine EDTA-anticoagulated whole blood was submitted for complete blood counts (CBC) to the Clinical Pathology Laboratory at NCSU. Automated counts were performed with an Advia 120 (Siemens Healthcare Diagnostics, Inc., Norwood, MA). Differential cellular counts were determined by examination of Wright-Giemsa stained slides by trained veterinary clinical pathology laboratory technologists. Automated platelet counts were always confirmed by comparison with a slide estimate. If automated platelet counts were below 10×10^9 platelets/l (limit of machine linearity), platelet count was instead determined by hemocytometer counts after red blood cell lysis in ammonium chloride (1:100 dilution, Acros Organics, Geel, Belgium). The diluted blood sample was allowed to settle for 10 minutes in a Neubauer hemocytometer (Hausser Scientific, Horsham, PA) and platelets were counted under a phase contrast microscope at 600x magnification.

Bleeding quantification. At baseline and time zero, while dogs were sedated with dexmedetomidine, buccal mucosal bleeding times (BMBTs) were performed with a template device (Surgicutt, Jorgensen Laboratories, Loveland, CO) according to previously described methods (Brooks & Catalfamo, 1993). If bleeding continued for over 15 minutes, the test was stopped and pressure was applied to the site.

At the time of each blood draw, bleeding scores were determined by history (for clinical patients), observation in the time period since the last bleeding score (in experimental dogs), and physical examination. The scoring system was adapted from the human ITP bleeding score that quantifies bleeding at 8 anatomic sites (Supplementary Table 2) (Page *et al*, 2007). The fecal occult blood test was performed using o-toluidine tablets (Hematest, Siemens Diagnostics,

Tarrytown, NY). Microscopic hematuria was assessed with urine dipsticks (Siemens).

Cytokine and chemokine analysis. Analytes evaluated via the multiplex assay included interleukin 2 (IL-2), IL-6, IL-7, IL-8, IL-10, IL-15, IL-18, canine orthologue of Chemokine (C-X-C motif) ligand 1 (CXCL1 orthologue or KC-like), Tumor Necrosis Factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-inducible protein 10 IP-10 (CXCL10), and interferon gamma (IFN-γ). Normal values were determined from the baseline values of the 8 healthy experimental dogs. For the multiplex cytokine assay, the dynamic range (lower and upper limits of detection in pg/ml) for cytokines were: IL-8 (218.306, 52841.549), IL-6 (11.867, 51919.926), IL-7 (10.194, 51200.999), IL-2 (11.954, 52343.73), IL-10 (11.857, 49655.509), IL-15 (10.161, 51199.813), IL-18 (11.333, 50535.442), CXCL1 orthologue (12.274, 41428.856), TNF-α (12.419, 53967.053), GM-CSF (12.649, 12452.237), IP-10 (12.289, 10347.254), IFN-γ (159.731, 50320.889), and MCP-1 (52.885, 2812.664) respectively.

In cases where cytokine concentrations were below the assay Lower Limit of Detection (LLOD), values were assigned that were one half of the LLOD (defined above) for the purpose of statistical analysis (Li *et al*, 2011).

ELISA absorbances were read using a VersaMax plate reader (Molecular Devices, Sunnyvale, CA), and curve analysis performed with Softmax Pro 5.

Fibrinogen and D-dimer analysis. Clottable (Clauss) fibrinogen was measured using an automated clot-detection instrument (STA Compact, Diagnostica Stago, Parsippany, NJ), as previously described (Stokol *et al*, 2000). Pooled canine plasma (prepared from 20 healthy adult dogs) was used as the assay standard. D-dimer concentration was measured via a previously described quantitative, immunoturbidometric method (Delgado *et al*, 2009) using a commercial kit and the manufacturer's human D-dimer standards (HemosIL, D-dimer Calibrator, Instrumentation Laboratory, Bedford, MA).

Supplementary Tables

Supplementary Table 1. Signalment of naturally-occurring canine thrombocytopenia cases.

Dog Number	Breed	Age (yrs)	Sex*	Diagnosis	Platelet Count (x 10^9/l)	Treatments prior to sampling
1	Doberman Pinscher	6	FS	Primary ITP	<0.5	Vincristine (0.02 mg/kg IV once) Prednisone (1.2 mg/kg PO BID) Doxycycline (6 mg/kg PO BID) Azathioprine (1.8 mg/kg PO SID) GI protectants IV fluids
2	Labradoodle	6	MC	Other (pancytopenia of unknown cause)	<0.5	Packed RBC transfusion (12.5 ml/kg) Ciprofloxacin (10 mg/kg IV SID) Doxycycline (4.6 mg/kg IV BID) GI protectants Dexamethasone (0.1 mg/kg IV BID) Vincristine (0.02 mg/kg IV once) Ampicillin sulbactam (22 mg/kg IV TID) IV fluids
3	Greyhound	3	FS	Primary ITP	<0.5	Doxycycline (5.4 mg/kg PO BID) Vincristine (0.02 mg/kg IV once) Prednisone (0.9 mg/kg PO BID)

4	Maltese	10	MC	Primary ITP	0.5	Vincristine (0.02 mg/kg IV once) Doxycycline (4.7 mg/kg PO BID) Dexamethasone (0.15 mg/kg IV BID) Medications for previous controlled heart failure (pimobendan, furosemide, spironolactone, enalapril), Soloxine for hypothyroidism, Denamarin and ursodiol
5	Rottweiler	8	MC	Primary ITP	10	Packed RBC transfusions (11.0 ml/kg then 10 ml/kg) Fresh whole blood (4.6 ml/kg, 72 hours before sampling) Dexamethasone (0.14 mg/kg IV BID) Azathioprine (2 mg/kg PO SID) Doxycycline (4.6 mg/kg PO SID) Ciprofloxacin 28mg/kg PO SID) Ampicillin sulbactam (22 mg/kg IV TID) Intravenous immunoglobulin (0.5 g/kg IV once) Vincristine (0.02 mg/kg IV once) Lidocaine CRI Sotalol (2.2 mg/kg PO BID) GI protectants IV fluids
6	Labrador Retriever	9	FS	Other (lymphoma and suspected chemotherapy-induced thrombocytopenia)	26	Ampicillin sulbactam (22 mg/kg IV TID) Enrofloxacin (10 m/kg IV SID) Doxycycline (5 mg/kg IV BID) Dexamethasone (0.05 mg/kg PO SID) Filgrastim (5 mcg/kg SQ once 48 hours prior to sampling) IV fluids GI protectants

7	Soft Coated Wheaten Terrier	4	FS	Other (suspected myelodysplastic syndrome)	37	Intravenous immunoglobulin (1 g/kg IV once) Packed RBC transfusion (9 ml/kg) Prednisolone (1.1 mg/kg PO BID) Cyclosporine (5.7 mg/kg PO BID) Aspirin (4.6mg/kg PO SID) Amoxicillin/ clavulanic acid (14.2 mg/kg PO BID) GI protectants
8	Cocker Spaniel	5	FS	Primary ITP	16	Vincristine (0.02 mg/kg IV once) Prednisolone (1.3 mg/kg PO BID) Doxycycline (5 mg/kg PO BID) Luflunomide (2 mg/kg PO SID) GI protectants Ursodiol
9	German Shepherd	4	MC	Secondary ITP (Ehrlichiosis)	16	Vincristine (0.02 mg/kg IV once) Prednisone (1 mg/kg PO BID) Doxycycline (5 mg/kg PO BID) Ciprofloxacin 27 mg/kg PO SID) Intravenous immunoglobulin (0.4 mg/kg IV once 11 days prior to sampling)
10	Irish Setter	12	FS	Other (Hemangiosarcoma and DIC)	29	Splenectomy for bleeding splenic hemangiosarcoma Packed RBC transfusions (20ml/kg) Lidocaine CRI Fentanyl CRI IV fluids and colloids (Voluven)

*FS indicates spayed female; MC, neutered male.

Supplementary Table 2. ITP bleeding score. Bleeding was graded at 8 different anatomic sites and the grade at each site summed for a total bleeding score out of a possible 16. This bleeding score was adapted from a human ITP bleeding score that has been validated for clinical use (Page *et al*, 2007).

Site	**Bleeding grade**		
	0	***1***	***2***
Skin (PE)	None	≤1 bruise, scattered petechiae	>1 bruise and/or diffuse petechiae
Oral mucosa (PE)	None	Petechiae present, but ≤10	>10 petechiae and/or gingival bleeding
Intraocular (PE)	None	N/A	Hyphema
Epistaxis (observed)	None	Bleeding <5 min (per episode)	Bleeding ≥ 5 min (per episode)
Gastrointestinal (observed)	None	Occult blood (stool o-toluidine test)	Gross blood
Urinary (observed)	None	Microscopic (positive on dipstick)	Macroscopic
Pulmonary (suspected/observed)	None	N/A	Yes
Intracranial hemorrhage (suspected/observed)	None	N/A	Yes
Total Score (out of possible 16)			

Supplementary Table 3. 2F9 dose titration and repeatability study data. After each 2F9 dose, platelet count was measured 1 hour (and sometimes 2 hours) later, before an additional dose was given. Dogs 1 and 2 were dose titration dogs. Dogs 3-5 were dose repeatability dogs. In the dose repeatability dogs, an initial dose was given, and based on the subsequent platelet count decrease, the estimated additional antibody required to reach the target platelet nadir was calculated. A sample calculation is shown for dog 5:

Dose 2 = Desired Δ Platelet count with Dose 2 * Dose 1/(Dose 1 induced Δ Platelet count)
= (47 x 10^9 platelets/l-10 x 10^9 platelets/l)* 1405 µg/142 x 10^9 platelets /l
= 366 µg

Dog	Dose number	Dose (µg) (Dose µg/kg)	Starting platelet count (x 10^9/l)	Platelet count 1 hour after dose (x 10^9/l) (plt count 2 hrs after dose)	Δ Platelet count (x 10^9/l) 1 hour after dose (Δ plt count 2 hrs after dose)
1	1	0.34 (0.015)	302	239 (clumped) (234, clumped)	63 (68)*
	2	0.497 (0.022)	239	250	11
	3	3.4 (0.15)	250	252	2
	4	34 (1.5)	252	231	21
	5	340 (15.0)	231	232	1
	6	3405 (150.0)	232	28	204
	Total dose	3783 (166.7)			
2	1	32.7 (1.5)	305	333 (338)	28 (33)
	2	327 (15.0)	333	284 (244)	49 (89)
	3	273 (12.5)	217	145 (135)	72 (82)
	4	311 (14.3)	135	62 (48)	73 (87)
	5	69 (3.2)	43	57	14
	6	115 (5.3)	57	14	43
	Total dose	1127.7 (51.7)			
3	1	1196 (32.7)	203	82	121
	2	624 (17.0)	82	26 (14)	56 (68)
	Total dose	1820 (49.7)			
4	1	1245 (50)	243	73	170

	2	405 (16.3)	73	38	35
	3	150 (6.0)	38	32	6
	4	176 (7.1)	34	11	23
	Total dose	1976 (79.4)			
5	1	1405 (50)	189	47	142
	2	366 (13.0)	47	11	36
	Total dose	1771 (63.0)			

Δ signifies change in

*Δ platelet probably due to clumping artifact

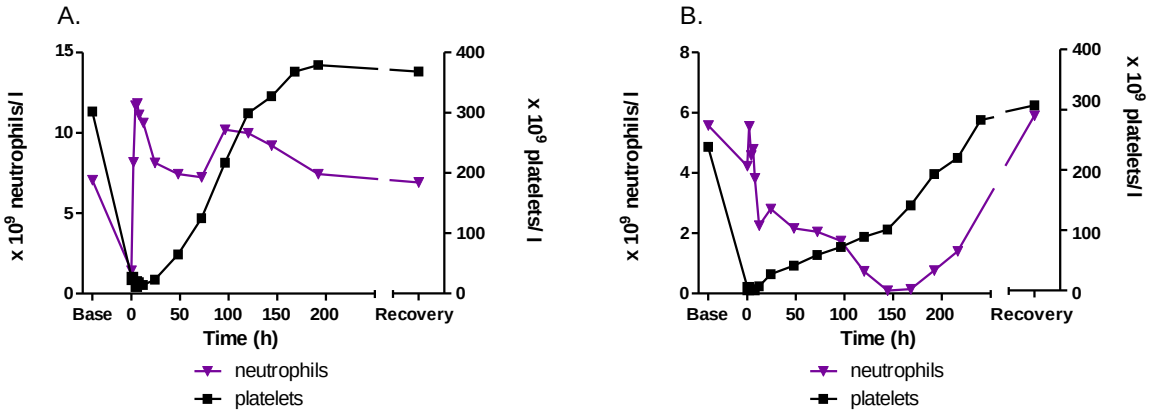
Supplementary Table 4. Experimental ITP dogs and dogs with spontaneous primary ITP have similar cytokine/chemokine profiles and other laboratory parameters. Comparison of laboratory parameters [median (range)] between dogs with naturally-occurring thrombocytopenia (primary ITP, secondary ITP, and thrombocytopenia due to other causes), experimental dogs and time-matched controls, and healthy dogs. Number (n) in each group is indicated at top of column or in individual cell if different than column header. The secondary ITP case was not employed in statistical comparisons. In the paired analyses (exact Wilcoxon rank sum test), healthy dogs were not compared with control or experimental dogs since healthy dogs were these same dogs at baseline; instead control and experimental were compared to each other at matching time points. NS = not statistically significant; S = significant as shown in relevant cells.

Laboratory Parameter	Healthy (n=8)	Primary ITP (n=5)	Secondary ITP (n=1)	Thrombocytopenia (other) (n=4)	Experimental ITP at time 0 (n=5)	Experimental ITP at 24 hr (n=5)	Control at time 0 (n=3)	Control at 24 h (n=3)	Differences
GM-CSF (pg/ml)	34.06 (3.57-1135.58)	59.53 (22.82-145.65)	5664.22	1.91 (1.91-68.41)	6.32 (3.57-1022.53)	6.32 (0.25-698.8)	74.31 (6.32-163.99)	98.6 (16.88-129.88)	NS
IFN-gamma (pg/ml)	132.67 (79.87-347.16)	35.46 (35.46-283.3)	1890.82	35.46 (35.46-153.02)	212.3 (42.84-562.2)	173.26 (79.87-878.1)	79.87 (79.87-132.67)	42.84 (42.83-287.13)	NS
IL-2 (pg/ml)	27.61 (5.98-581.42)	55.97 (26.78-67.87)	4513.74	14.89 (2.05-42.41)	6.03 (5.98-456.68)	6.03 (5.98-329.25)	48.00 (10.56-73.77)	55.99 (43.98-106.83)	NS
IL-6 (pg/ml)	43.86 (2.71-328.49)	39.97 (4.59-103.58)	4369.50	29.25 (14.36-76.59)	21.37 (2.71-407.39)	5.93 (5.93-238.87)	43.89 (24.27-65.38)	65.38 (54.73-96.54)	NS
IL-10 (pg/ml)	5.93 (5.93-445.10)	4.06 (2.14-37.62)	175.16	21.54 (2.14-66.64)	5.93 (5.93-1,142.66)	5.93 (5.93-133.25)	5.93 (5.93-5.93)	5.93 (1.05-5.93)	NS
IL-8 (pg/ml)	3724.23(1954.34-6881.63)	74** (20.55-373.53) ***P=0.0016 vs. healthy*	446.83	249.40* (37.75-2613.42) **P=0.0162 vs. healthy*	109.15* (109.15-692.03) **P=0.0357 vs. control 0*	242.72* (71.93-1594.39) **P=0.0357 vs. control 24*	4354.90 (2609.74-5527.64)	3102.75 (2984.24-5684.68)	S

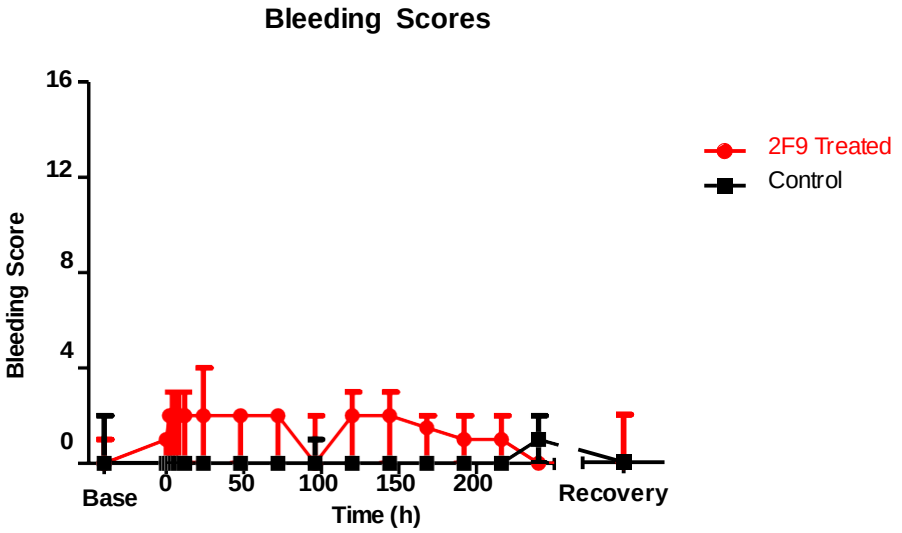
MCP-1 (pg/ml)	225.80 (26.43-704.06)	161.53 (74.33-185.19)	1016.47	305.31 (163.97-1,430.81)	227.50 (26.43-2,741.65)	86.13 (26.43-512.86)	256.63 (129.28-365.59)	319.93 (158.56-432.63)	NS
TNF-alpha (pg/ml)	6.21 (6.21-17.39)	9.65 (2.09-36.03)	1181.03	3.58 (2.09-6.58)	6.21 (0.86-15.32)	6.21 (0.00-6.21)	6.21 (6.21-6.21)	6.21 (6.21-6.21)	NS
IL-7 (pg/ml)	43.73 (5.10-297.33)	42.02 (9.71-80.99)	6970.06	7.48 (2.04-62.67)	5.10 (5.10-216.32)	21.74 (5.10-155.28)	102.18 (5.10-201.01)	191.46 (5.10-201.01)	NS
IL-15 (pg/ml)	161.48 (5.08-2,255.74)	85.76 (10.04-215.79)	9356.16	39.45 (23.50-177.69)	92.60 (5.08-2,450.65)	30.48 (5.08-1,443.25)	197.53 (137.00-412.32)	379.44 (197.53-488.03)	NS
IP-10 (pg/ml)	6.14 (2.93-24.53)	20.56 (4.41-35.04)	19.65	1.98 (1.98-45.26)	6.14 (0.82-18.17)	6.54 (1.39-21.38)	6.14 (6.14-22.09)	6.14 (6.14-27.94)	NS
CXCL-1 orthologue (pg/ml)	320.10 (84.78-617.93)	150.61 (104.80-1,330.12)	717.70	712.79 (100.24-1,408.88)	157.26 (37.77-785.26)	82.41 (30.09-151.32)	248.21 (89.52-354.10)	162.07 (97.75-420.09)	NS
IL-18 (pg/ml)	86.00 (0.58-1,600.21)	95.45 (83.60-237.63)	8761.73	128.36 (20.62-236.83)	20.19 (6.25-1,705.85)	42.25 (11.85-1,219.44)	77.85 (5.67-148.55)	88.76 (6.25-186.46)	NS
Fibrinogen (mg/dl)	341.50 (295.00-473.00)	621.00 (322.00-1,146.00); n=4		820.50 (790.00-851.00); n=2	389.00 (270.00-471.00)	389.00 (323.00-500.00)	373.00 (340.00-550.00)	414.00 (336.00-519.00)	NS
D-dimers (ng/ml)	163.00 (0.00-290.00)	212.50 (78.00-411.00); n=4		2,335.50 (649.00-4,022.00); n=2	231.00 (109.00-2,450.00)	133.00 (0.00-456.00)	224.00 (125.00-269.00)	269.00 (149.00-273.00)	NS
WBC (cells x 10^9/l)	8.98 (6.92-11.73)	14.52 (6.72-40.23)	15.38	1.31 (0.35-35.71)	8.19 (3.66-14.16)	9.32 (4.94-12.93)	7.45 (7.26-11.07)	7.28 (7.24-10.28)	NS

HCT (%)	48.80 (45.90-51.70)	40.50 (21.10-58.70)	46.6	20.70** (15.70-29.10) ***P=0.0040 vs. healthy*	45.70 (32.90-47.60)	37.80 (31.50-48.70)	43.40 (42.10-45.70)	44.80 (41.30-46.90)	S
Platelets (cells x 10^9/l)	209.50 (183.00-329.00)	0.50** (0.00-16.00) ***P=0.0016 vs. healthy*	16	26.50** (0.00-29.00) ***P=0.0040 vs. healthy*	14.00* (11.00-28.00) **P=0.0347 vs control 0*	32.00* (23.00-36.00) **P=0.0119 vs. pITP *P=0.0357 vs. control 24*	208.00 (174.00-211.00)	189.00 (174.00-189.00)	S
Bleeding score	0.00 (0.00-2.00)	3.00** (1.00-8.00) *P=0.0055 vs. healthy*	1	3.50** (2.00-10.00) *P=0.0064 vs. healthy*	1.00* (0.00-1.00) *P=0.0254 vs. pITP*	2.00* (0.00-4.00) *P=0.0306 vs. control 24*	0.00 (0.00-0.00)	0.00 (0.00-0.00)	S

Supplementary Figures



Supplementary Figure 1. **Two model ITP dogs developed neutropenia.** A. One dog developed very transient neutropenia immediately after 2F9 infusion. B. The second dog developed severe delayed neutropenia. We confirmed that the dog with prolonged neutropenia was not septic. We were unable to detect 2F9 or canine IgG, IgM, or complement binding of the dog's neutrophils (data not shown). The neutropenia resolved following administration of GM-CSF (Neupogen, Amgen, Thousand Oaks, CA). 2F9 does weakly bind neutrophils in the presence, but not in the absence, of platelets (data not shown). This is believed to be due to a nonspecific interaction of the Fc portion of platelet-bound 2F9 with neutrophil Fc receptors. The neutropenia may have been the result of this non-specific 2F9 neutrophil binding leading to neutrophil destruction via off-target complement fixation or phagocytosis of immune-complex opsonized neutrophils. The very nature of the mechanism of platelet depletion (platelet-antibody complexes) means neutrophils will be activated via their Fc receptor to some degree, likely enough to marginate, and with complement fixation, perhaps enough to deplete them. The delayed neutropenia may have also been the result of generalized stimulation of the reticuloendothelial system secondary to 2F9 administration.



Supplementary Figure 2. Bleeding scores of 2F9-treated compared to control dogs. Maximum bleeding score was 16. Bleeding was variable but mild in 2F9-treated dogs. Data shown as median and range.

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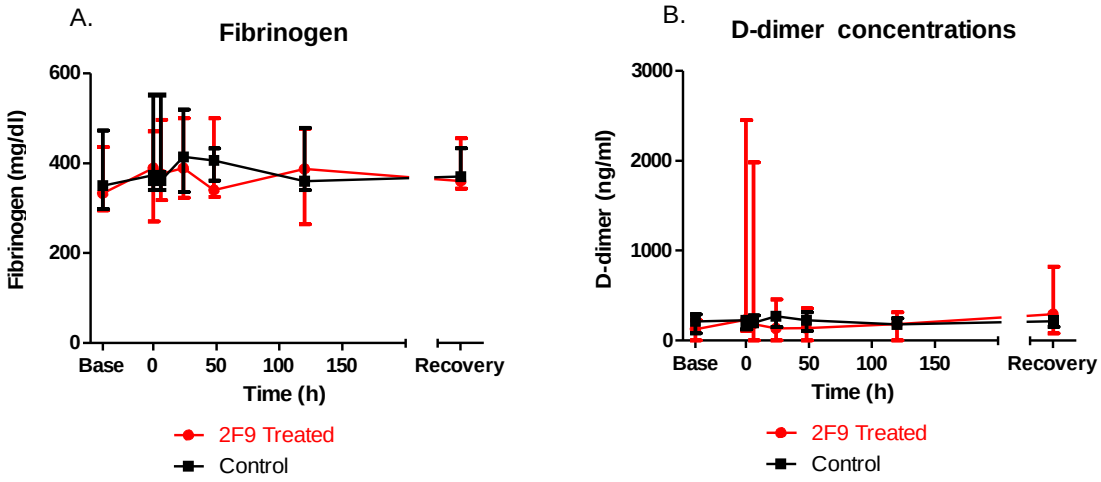
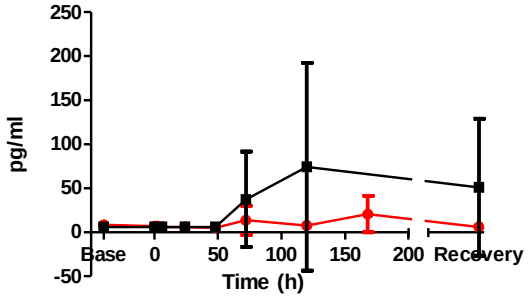
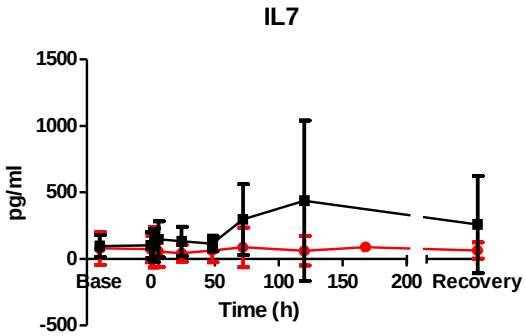


Supplementary Figure 3. Canine ITP model is not prothrombotic. A. Fibrinogen (P=0.268) and B. D-dimers (P=0.457) were not significantly different between 2F9-treated and control dogs. Data shown as median and range.

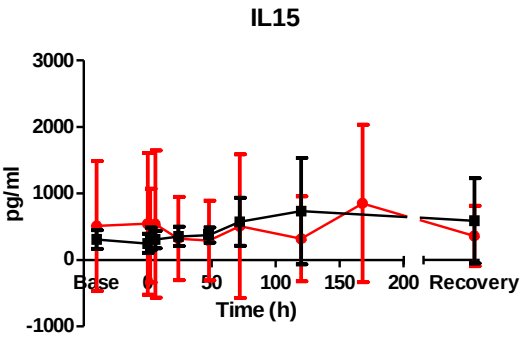
A.


TNFalpha
pg/ml
250
200
150
100
50
0
-50
Base
0
50
100
150
200
Recovery
Time (h)

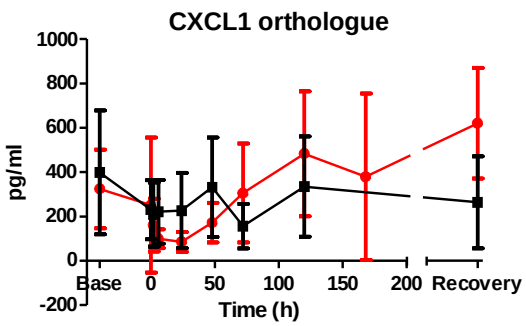
B.


IL7
pg/ml
1500
1000
500
0
-500
Base
0
50
100
150
200
Recovery
Time (h)

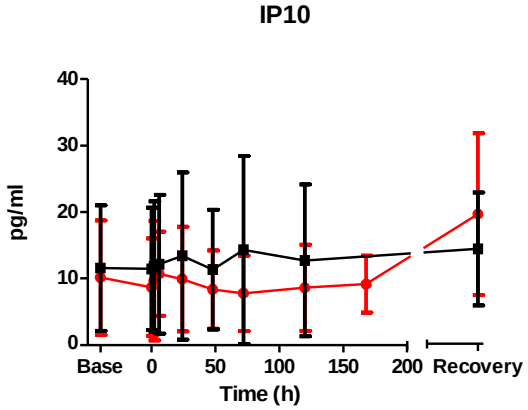
C.


IL15
pg/ml
3000
2000
1000
0
-1000
Base
0
50
100
150
200
Recovery
Time (h)

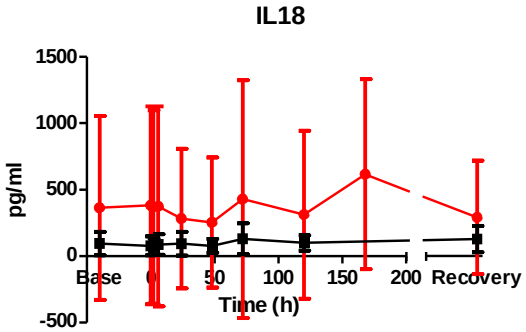
D.

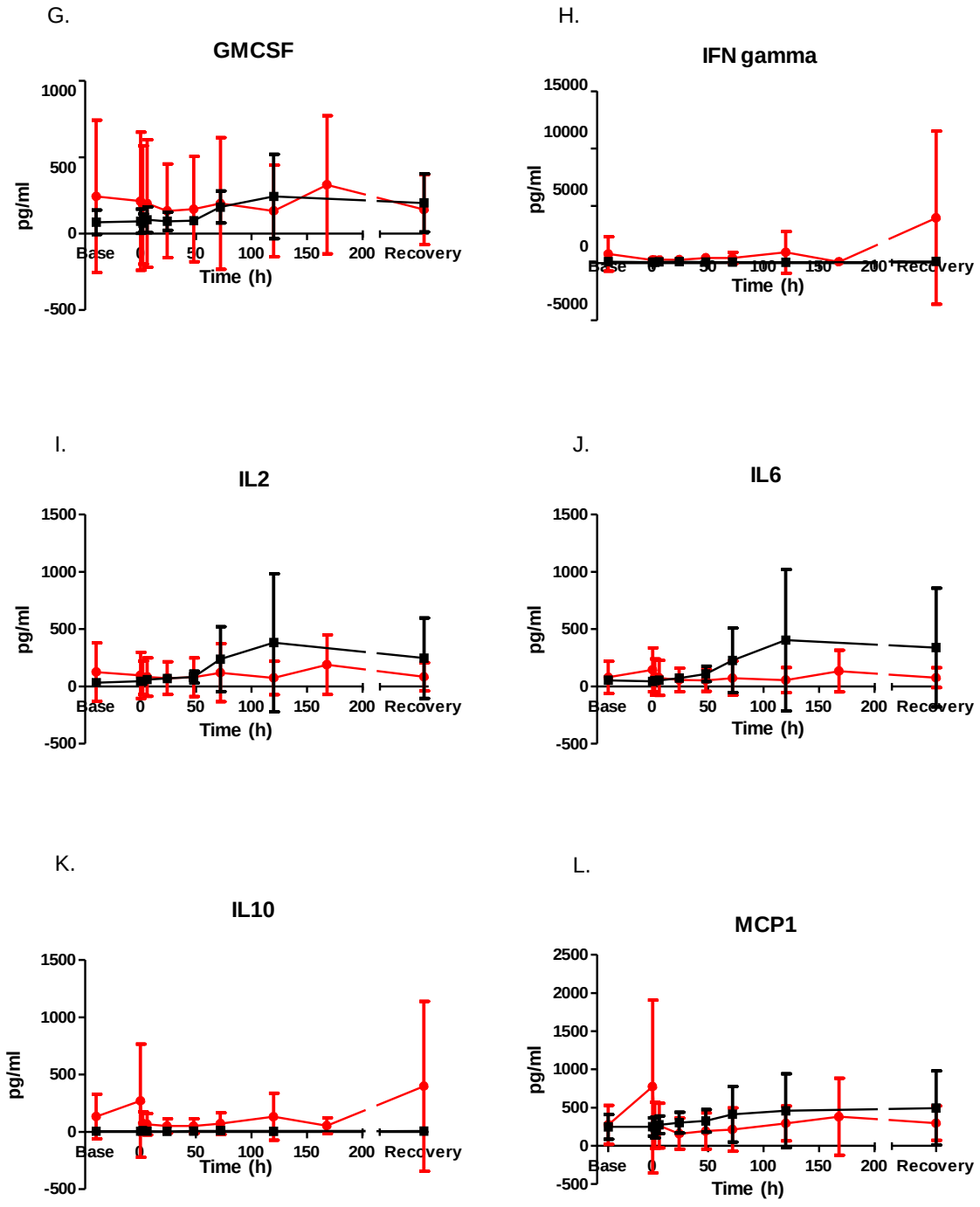

CXCL1 orthologue
pg/ml
1000
800
600
400
200
0
-200
Base
0
50
100
150
200
Recovery
Time (h)

E.


IP10
pg/ml
40
30
20
10
0
Base
0
50
100
150
200
Recovery
Time (h)

F.


IL18
pg/ml
1500
1000
500
0
-500
Base
0
50
100
150
200
Recovery
Time (h)



Supplementary Figure 4. Canine ITP model is not pro-inflammatory. In the measured chemokines and cytokines, over time there are no significant differences between 2F9-treated (●——●) (n=5) and control dogs (■——■) (n=3) (mean ± SD). Measured cytokines and their P-values comparing treated and control dogs are as follows A. TNFα (P=0.157) B. IL-7 (P=0.018, not significant with Bonferroni correction) C. IL-15 (P=0.903) D. CXCL-1 orthologue

(P=0.345) E. IP-10 (P=0.589) F. IL-18 (P=0.069) G. GM-CSF (P=0.358) H. INF γ (P=0.412) I. IL-2 (P=0.603) J. IL-6 (P=0.330) K. IL-10 (P=0.086) L. MCP-1 (P=0.928).

References:

Boudreaux, M.K., Catalfamo, J.L. & Klok, M. (2007) Calcium-diacylglycerol guanine nucleotide exchange factor I gene mutations associated with loss of function in canine platelets. *Translational Research,* **150,** 81-92.

Brooks, M. & Catalfamo, J. (1993) Buccal mucosa bleeding time is prolonged in canine models of primary hemostatic disorders. *Thrombosis and Haemostasis,* **70,** 777-780.

Burstein, S.A., Friese, P., Downs, T. & Epstein, R.E. (1991) Canine megakaryocytopoiesis: analysis utilizing a monoclonal antibody to a 140-kd dog platelet protein. *Experimental Hematology,* **19,** 47-52.

Delgado, M.A., Monreal, L., Armengou, L., Rios, J. & Segura, D. (2009) Peritoneal D-dimer concentration for assessing peritoneal fibrinolytic activity in horses with colic. *Journal of Veterinary Internal Medicine,* **23,** 882-889.

Li, J., Birkenheuer, A.J., Marr, H.S., Levy, M.G., Yoder, J.A. & Nordone, S.K. (2011) Expression and function of triggering receptor expressed on myeloid cells-1 (TREM-1) on canine neutrophils. *Developmental & Comparative Immunology,* **35,** 872-880.

Page, L.K., Psaila, B., Provan, D., Michael Hamilton, J., Jenkins, J.M., Elish, A.S., Lesser, M.L. & Bussel, J.B. (2007) The immune thrombocytopenic purpura (ITP) bleeding score: assessment of bleeding in patients with ITP. *British Journal of Haematology,* **138,** 245-248.

Rodeghiero, F., Stasi, R., Gernsheimer, T., Michel, M., Provan, D., Arnold, D.M., Bussel, J.B., Cines, D.B., Chong, B.H., Cooper, N., Godeau, B., Lechner, K., Mazzucconi, M.G., McMillan, R., Sanz, M.A., Imbach, P., Blanchette, V., Kuhne, T., Ruggeri, M. & George, J.N. (2009) Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. *Blood,* **113,** 2386-2393.

Smithberg, S.R., Fogle, J.E., Mexas, A.M., Reckling, S.K., Lankford, S.M., Tompkins, M.B. & Dean, G.A. (2008) In vivo depletion of CD4+CD25+ regulatory T cells in cats. *Journal of Immunological Methods,* **329,** 81-91.

Stokol, T., Brooks, M.B. & Erb, H.N. (2000) Effect of citrate concentration on coagulation test results in dogs. *Journal of the American Veterinary Medical Association,* **217,** 1672-1677.