Supplementary Methods:

Relative Similarity Score to Humans:

Mean+2SD for adhesion to collagen, adhesion to fibrinogen, normalized spreading on fibrinogen, normalized spreading on collagen, and single platelet contraction forces were taken for each species to account for intraspecies variability. Each species was compared relative to human in each category and a final score was generated by summing the scores in each category.

Statistical Analysis:

Species differences by adhesion or spreading were assessed with a mixed model to account for the within subject correlation for any multiple experiment. Single platelet force was also analyzed at the subject level where average force was estimated with a mixed model, accounting for the within subject correlation among that subject's platelets. Tukey's adjusted comparisons were used to account for the multiple testing of pairwise comparisons from the significant models. Statistical analysis was performed using R 3.6.0 (https://www.R-project.org/).

Platelet Area:

Grayscale (1-layer) brightfield images of adhered platelets are converted to a black and white image using a binary threshold dependent on original imaging conditions, where platelets are represented as white regions of pixels. Blob detection algorithms are used to find these pixel regions and compute platelet area in pixels. Blob detection is done using python and the OpenCV (open source computer vision). OpenCV version 4.5.0 was used to uniformly apply the modules "cv2.threshold" with the "cv2.THRESH_BINARY" option to create a binary image, "cv2.SimpleBlobDetector_create" to utilize user-defined parameters dependent on imaging quality to perform blob analysis, and ".detect" to use the blob detector created to determine each cell's diameter. In this application, brightfield imaging of adhered cells creates connected pixel values that represent a cell. The blob detector created by the user outputs the diameter of each cell found, which can be used to calculate area for individual cells using a known pixel-to-um ratio specific to the microscope used. To normalize the data and actually calculate spreading area relative to original platelet size, platelet area was divided by platelet volumes. Experimental Platelet Volumes were used for pigs, sheep and dogs and known literature values of mice were used [28].

Platelet preparation:

Consent for human platelets was obtained according to GT IRB H15258 and blood collection for animal models was approved by the University of Georgia's Institutional Animal Care and Use Committee. For human platelets, blood was drawn into the anticoagulant acid-citrate-dextrose (ACD) solution 2. The sample was subsequently centrifuged at 150 G for 15 min without brake and the resulting platelet rich plasma (PRP) was centrifuged again with an additional 10% ACD by volume at 900G for 5min without brake. The supernatant, platelet poor plasma, was discarded and platelet pellet was resuspended into HEPES modified tyrodes buffer and was gel filtered into this same buffer. For dogs, pigs and sheep, blood was drawn into 3.2% citrate and PRP was prepared by centrifugation at 275G for 5 min (dog, pig) and 250G for 5 min (sheep) without brake. This platelet rich plasma was then centrifuged again with 10% ACD added by volume at 900G for 5 min without brake. The supernatant, platelet poor plasma, was discarded and platelet pellet was resuspended into HEPES modified tyrodes buffer and was gel filtered into this same buffer. For mice, blood was drawn into ACD and the sample was subsequently centrifuged at 100g for 5 min. PRP was then centrifuged again with 10% ACD at 900G for 5 min

without brake. The supernatant, platelet poor plasma, was discarded and platelet pellet was resuspended into HEPES modified tyrodes buffer and was gel filtered into this same buffer.

Platelet contraction: paired fibrinogen microdot system:

Pairs of fibrinogen microdots with a radius of 0.8 μm and separation of 4 μm were patterned on a polyacrylamide gel with a stiffness of 75 kPa. This system is akin to a spring, where the fibrinogen microdots displacement is linearly proportional to the applied platelet contractile force. These values for microdot size and spacing ensured that platelets preferentially attached to the microdots and were able to spread to neighboring microdot in the microdot pair. Platelet contraction was imaged on a Zeiss LSM 780/ELYRA PS1 confocal microscope using a 20x/0.8NA Plan Apochromat lens. Platelets were tagged with cell mask orange 554/567 (ThermoFisher) nm and Alexa Fluor 488 tagged fibrinogen was used for the microdot pairs. Images were analyzed using a MATLAB script which calculated the center to center distance between fibrinogen microdots. Because of the lithography produced high microdot pattern fidelity, uncontracted microdot pairs were utilized as an initial reference difference. Therefore, each contracted plated was compared to a nearby uncontracted reference. The current script is semi-automated where contracted platelets are identified and selected for analysis. This script is freely available at GitHub (https://github.com/davidrmyers/platelet-contraction). Traction forces (T) of individual platelets was calculated as:

$$T = \frac{2\pi Ga\ (x_s - x_f)}{2 - v}$$

Where G is the shear modulus, a is the microdot radius, v is Poisson's ratio and x_s is the starting distance and x_f is the final distance post contraction. Since we measure the displacement of the platelet contractive microdot relative to the starting reference distance, we are able to calculate applied force by a platelet.

Supplementary Tables and Figures:

Table S1. Relative similarity to humans in each single platelet biophysical assay

According to this scoring method, Mice have the most similar platelets to that of humans, as they were the only species to not drastically differ from humans in any category. Because porcine platelets had drastically different adhesion patterns to that of all the other species, they had the least similar platelets to humans.

Species	Adhesion to Collagen	Adhesion to Fibrinogen	Spreading on Collagen	Spreading on Fibrinogen	Single Platelet Forces	Relativity Sum	Percent Similarity
Human	1	1	1	1	1	5	100%
Mouse	0.224	1.164	1.529	1.01	0.842	4.773	95.4%
Dog	0.206	1.075	0.774	0.747	1.762	4.566	91.2%
Pig	11.566	0.049	0.953	1.124	0.840	14.397	34.7%
Sheep	0.189	1.578	2.184	2.283	0.702	7.077	70.6%

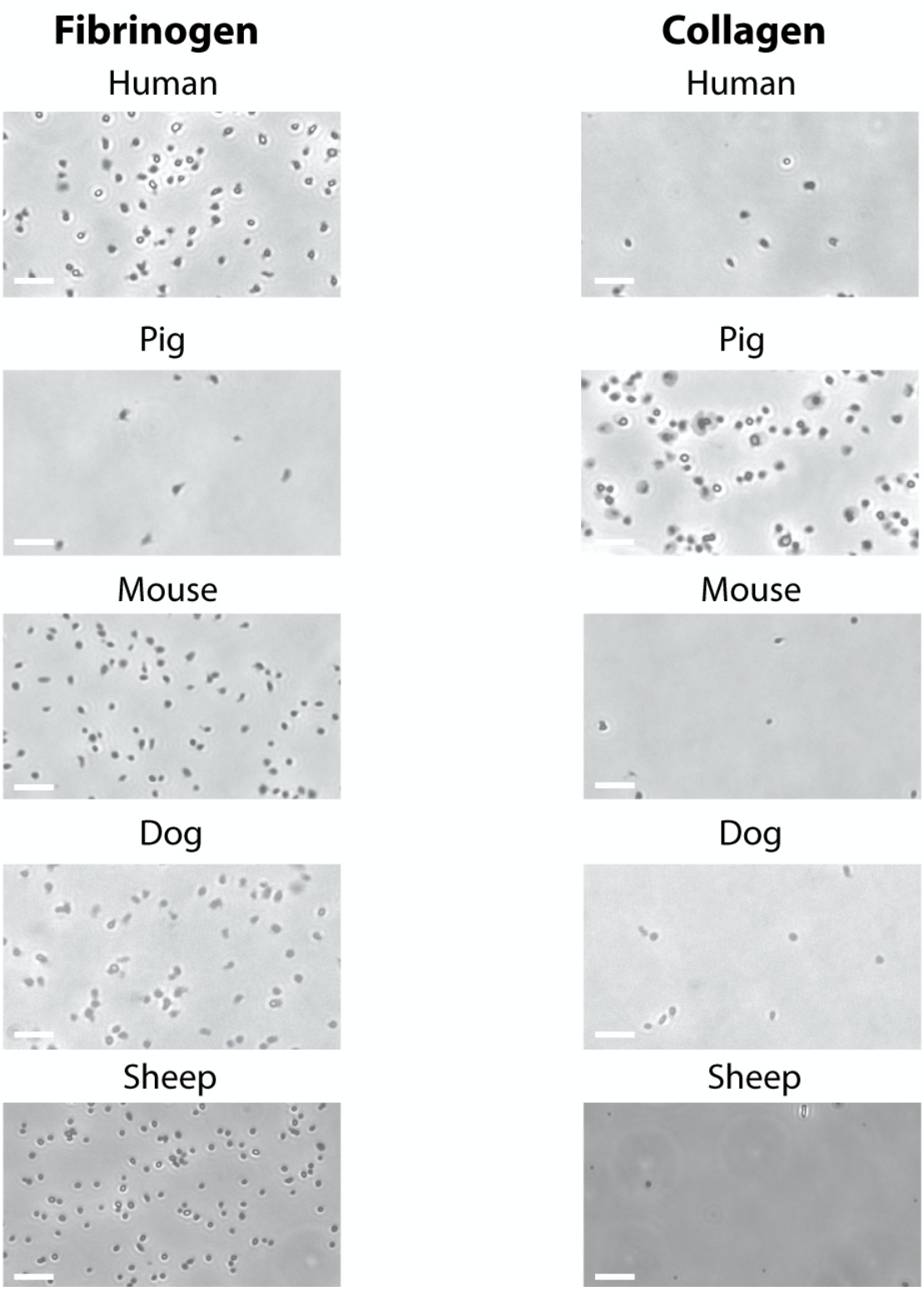

Figure S1. Brightfield Images of Platelet Adhesion on Fibrinogen or Collagen
Platelet adhesion of various species on both collagen and fibrinogen. Brightfield images were taken at 30X magnification. Scale Bars indicate 10 μm.

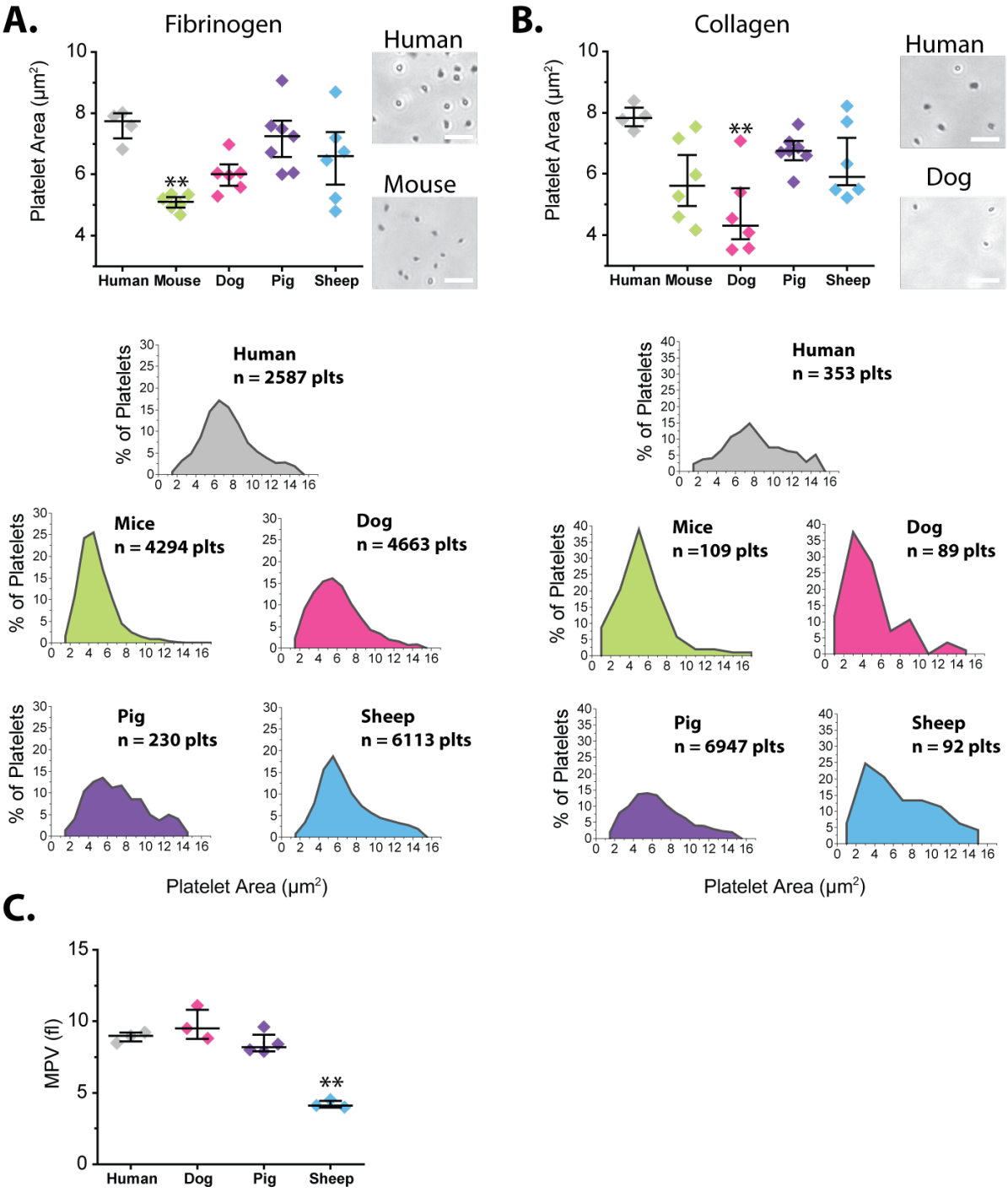

Figure S2. Non-normalized platelet area and Mean Platelet Volumes
Non-normalized platelet areas on both fibrinogen (A) and collagen (B). Histogram plots show the platelet area profile of each species on fibrinogen and collagen. (C) Experimental mean platelet volumes for Humans (n=3), Dogs (n=3), Pigs (n=4) and Sheep (n=3) were used as they match literature values [27]. All species were compared to humans and statistical significance was determined by One-way ANOVA followed by Tukey's multiple comparisons test. **P ≤ .01.

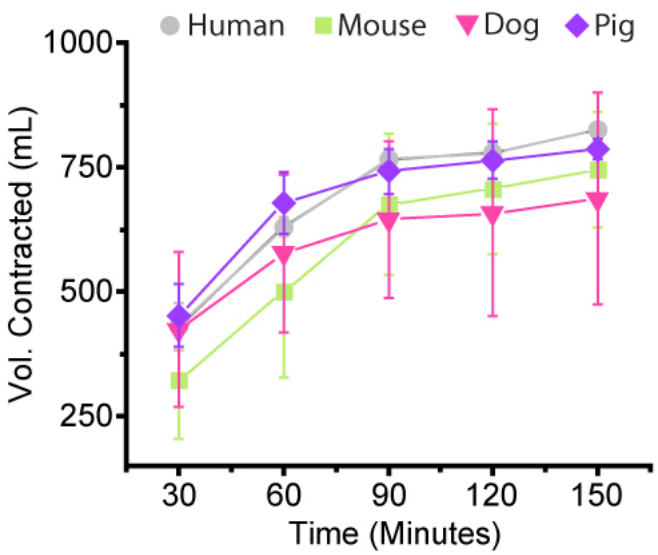

Figure S3. Bulk clot contraction versus Time

Bulk clot contraction versus time in Humans (n=2), Mice (n=3), Dog (n=3), Pig (n=4). When compared to humans, no significant differences were seen for any species at any time point. Bulk data is shown as mean ± SD. All species were compared to humans and statistical significance was determined by One-way ANOVA followed by Tukey's multiple comparisons test