Material and Methods

FHS Participants. As previously described[1], 1819 participants of the FHS Offspring Cohort 8 underwent physical examination. Individuals were categorized as hypertensive if they had systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or received treatment for hypertension. Individuals were considered diabetic if they had fasting blood glucose ≥126 mg/dL, non-fasting blood glucose ≥200 mg/dL, or treatment with insulin or hypoglycemic drugs. Finally, individuals were considered smokers if they smoked at least 1 cigarette a day in the year prior to examination[1]. The FHS protocol was approved by the Boston University Medical Center and the University of Massachusetts Medical School Institutional Review Boards and all participants signed informed consent.

Cell Culture. Meg-01 cells, a human megakaryoblast cell line (ATCC; CRL-2021), were cultured as previously described[2].

Mice. C57BL/6J (#000664), IL1R1$^{-/-}$ (B6.129S7-Il1r1^{tm1Imx}/J; #003245), and IL1β$^{-/-}$ (B6.129P2-P2rx7^{tm1Gab}/J; #005576) mice were purchased from Jackson Laboratories. Mice were maintained at Boston University School of Medicine. All studies were approved by the university's Institutional Animal Care and Use Committee. Mice were fed *ad libitum* a high fat diet (Bio-Serv; F3282) for up to 10 weeks.

Flow Cytometry. Intact human platelets and Meg-01 cells were stained with anti-human IL1R1 FITC conjugated antibodies or isotype controls (R&D Systems; FAB269F) and analyzed on a FACSCalibur flow cytometer using Cell Quest Software (BD Biosciences). For heterotypic aggregates, human whole blood was diluted with 1X PBS and then left untreated (resting) or treated with 10 μg/mL Pam3CSK4 (InvivoGen; tlrl-pms) or 10 ng/mL IL1β (R& D Systems; 201-LB) for 10 minutes at 37°C, while stirring at 1200 rpm. Samples were then dual-stained with anti-human CD41 FITC conjugated antibodies and anti-human CD14 PE-Cy7conjugated antibodies, or corresponding isotype controls (eBiosciences; 11-0419 and 25-0149, respectively) then analyzed by flow cytometry gating for neutrophils and measuring the percent that were positive for platelets[3]. For heterotypic aggregates in mice, whole blood was diluted with 1X PBS and then left untreated (resting) or treated with 10 ng/mL IL1β (R&D Systems; 401-ML) for 10 minutes at 37°C, while stirring at 1200 rpm. Samples were then stained with anti-mouse CD41 FITC conjugated antibodies and anti-mouse Ly6G PE-Cy7 conjugated antibodies, or corresponding isotype controls (eBioscience; 11-0411 and 25-5931, respectively). Analysis was done by flow cytometry as described above.

Western Blot Analysis. Western blots were run as previously described[2] and analyzed using ImageJ (NIH).

Ploidy. Bone marrow was isolated from C57BL/6J and IL1R1$^{-/-}$ mice as previously described[2]. Cells were treated daily with vehicle, 50 ng/mL mouse TPO (R&D Systems; 488-TPO), or 25 ng/mL IL1β for 3 days. Megakaryocyte ploidy was measured as previously described[2].

Meg-01 Adhesion. Meg-01 cells, treated with 25 ng/mL IL1β, were allowed to adhere to either fibronectin (10 μg/mL; Sigma; F0895) or fibrinogen (10 μg/mL; Enzyme Research Laboratories; FIB 3) for 1 h. The number of adherent cells was determined as previously described[2].

Gene Expression. Meg-01 cells were pretreated with DMSO, 50 μM LY294002 (Calbiochem; 440204), 50 μM U0126 (Enzo; BML-EI282), or 50 μM BAY11-7082 (Calbiochem; 196870) for 30 minutes, then treated with 25 ng/mL IL1β for 3 h at 37°C. Total RNA was isolated using RNeasy Mini Kit (Qiagen) and converted to cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Gene expression of GAPDH, COX2, MCP-1, NFκB1, IL1R1, IL1β, TLR2, CD41, and GP1b (Primers, probes from Applied Biosystems) was determined using TaqMan® Gene Expression Master Mix (Applied Biosystems) on an Applied Biosystems 7900 HT Fast Real-Time PCR System with SDS 2.2.2 Software.

Platelet RNA from FHS participants or mice was isolated using miRNeasy Mini Spin Columns (Qiagen). RNA was converted to cDNA as previously described. Pre-amplification was

performed using TaqMan® PreAmp Master Mix (Applied Biosystems), followed by real-time PCR using the BioMark™ System (Fluidigm, San Francisco, CA). The primers and probes were from Applied Biosystems.

Washed Platelet Aggregation. Human and mouse platelets were isolated from citrated whole blood as previously described[3]. Human protocols were approved by Boston University School of Medicine and the University of Massachusetts Medical School Institutional Review Boards. All donors signed informed consent. Platelets ($2x10^8$/mL), resuspended in a HEPES buffer (140 mM NaCl, 6 mM KCl, 2.4 mM $MgSO_4 \cdot 7H_2O$, 1.7 mM Na_2HPO_4, 6 mM Na Hepes, 0.35% BSA, 0.1% Dextrose, pH 7.4), were pretreated with 10 ng/mL IL1β in the presence of 1 mM $CaCl_2$, 2 mM $MgCl_2$, and 0.3 μM fibrinogen for 10 minutes at 37°C and then left untreated (Resting) or treated with either α-thrombin (human or mouse; Enzyme Research Laboratories; HT1002a and MIIa, respectively) or 10 μg/mL collagen (Chrono-Log; 385) for 10 minutes, at 37°C, while stirring at 1200 rpm. Percent aggregation was determined using a lumi-aggregometer (Bio/Data). Platelets were pretreated with SB203580 (0.2 μM; Enzo; BML-EI286), a p38 inhibitor, or vehicle (DMSO) for 5 minutes, prior to the addition of IL1β.

Platelet Adhesion. Human and mouse platelets were isolated as described above. $2x10^8$/mL platelets ($2x10^6$/mL for the mouse high fat diet studies), resuspended in HEPES buffer, were stained with calcein-AM (Invitrogen). Samples were left untreated (Resting), treated for 10 minutes with 10 ng/mL IL1β or thrombin, or pretreated with IL1β for 10 minutes then treated with thrombin for an additional 10 minutes at room temperature. Samples were then circulated over collagen-coated (100 μg/mL) or fibrinogen-coated (100 μg/mL) coverslips for 20 minutes in 1X HBSS without $CaCl_2$ and $MgCl_2$ at room temperature. Photographs were taken with a fluorescent microscope at 20X using Eclipse Ti-E Fluorescent Inverted Microscope (Nikon) and NIS Elements AR Software (Nikon) or an Olympus IX 70 Inverted Microscope with Metamorph Software. Analysis was done using ImageJ (NIH).

Platelet Samples for Western Blots. Human platelets were isolated as described above. $2x10^8$/mL were either left untreated or treated with 10 μg/mL Pam3CSK4 or increasing concentrations of IL1β (1-100 ng/mL). Other samples were pretreated with 10 ng/mL IL1β for 10 minutes then treated with either 0.5U/mL thrombin or 10 μg/mL collagen for an additional 10 minutes. P-p38 and total p38 levels were determined by western blot.

P.gingivalis Infection. Culturing and inoculation of *P. gingivalis* was done as previously described [4-6]. Briefly, *P. gingivalis* strain 381 was grown anaerobically on blood agar plates with hemin and menadione. Bacteria was collected and resuspended in sterile 1X PBS (pH 7.2). Mice were given 4% sulfamethoxazole in their drinking water for 2 weeks to clear oral micro flora. Mice were then challenged orally on the buccal surface of the maxilla 3 times for 1 week with $1x10^9$ CFU in 2% carboxymethyl cellulose. Mock-infected mice were treated with PBS and 2% carboxymethyl cellulose. Mice were sacrificed 24 h after the last oral challenge (Acute Infection) or 6 weeks (Chronic Infection). Blood cell counts were determined using a Coulter ® $A^c \cdot T$ Series Analyzer (Beckman Coulter). Heterotypic aggregates were measured from whole blood samples, stained with anti-mouse CD41 FITC conjugated and anti-mouse Ly6G PE-Cy7 conjugated antibodies, or corresponding isotype controls. Samples were analyzed on a flow cytometer gating for neutrophils and measuring the percent positive for platelets. Citrated plasma samples were pooled for each conditioned and then concentrated using centrifugal filters with a molecular weight cut off of 10kDa. Proform of IL1β were determined by western blot.

Statistics. Graphs consist of the average ± the standard deviation (SD). Some data are reported as the percent of No Treatment (NT), set to 100%, and analyzed using a one-sample t-test with a normal distribution, a theoretical mean of 100, and significance set at $p<0.05$. Other data are analyzed either by a Student's t-test or ANOVA, followed by a Tukey's Multiple Comparison Test. All statistics were done using GraphPad Prism 5.

Descriptive statistics for the FHS are displayed as mean ± SD for continuous variables and count (percentage) for categorical variables. The qRT-PCR results cycle threshold (Ct) values were corrected (∆Ct) by the geometric mean of three reference genes (β actin (ACTB), β2-microglobulin (B2M), glyceraldehyde-3-phosphate dehydrogenase (GAPDH)), previously found to be highly correlated[1]. Genes not expressed, *i.e.*, failing to surpass the Ct within a set period, were assigned the maximum Ct value allowed according to our qRT-PCR procedures (30 cycles). Multivariable linear regression models for gene expression as ∆Ct values were fitted adjusting the following potential confounding variables (all assessed at the same examination when RNA was collected): BMI, smoking status, total cholesterol, HDL cholesterol, triglycerides, systolic blood pressure, diastolic blood pressure, glucose level, diabetes, coronary heart disease, lipid-lowering therapy, hormone replacement therapy, antihypertensive therapy, and regular aspirin use (at least 3× per week). In addition, we estimated multivariable corrected models with BMI stratified by obesity status (BMI <25, ≥25 and <30, and ≥30 kg/m^2) to aid in the presentation of BMI associated expression values. To correct for the number of statistical comparisons conducted for each gene, that is, 6 regression models with 16 covariates, we employed false discovery rate (FDR) correction set at 5%. All gene expression analyses were done using Stata 10.1 statistical software.

References

1. Freedman JE, Larson MG, Tanriverdi K, O'Donnell CJ, Morin K, Hakanson AS, Vasan RS, Johnson AD, Iafrati MD, Benjamin EJ. Relation of platelet and leukocyte inflammatory transcripts to body mass index in the Framingham heart study. *Circulation*. 2010; 122:119-129.
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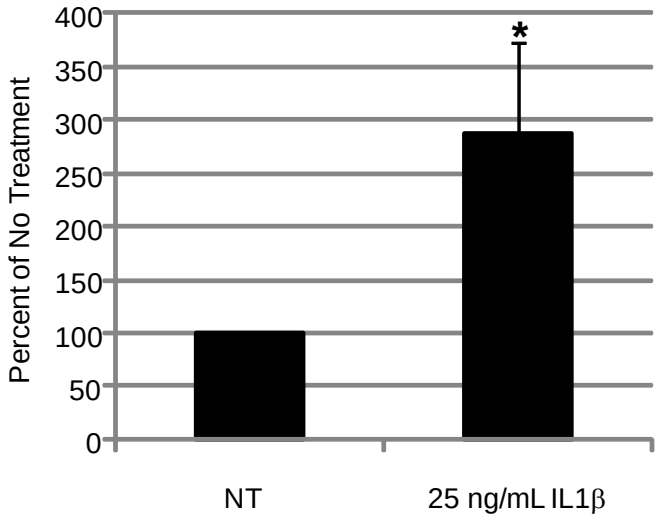
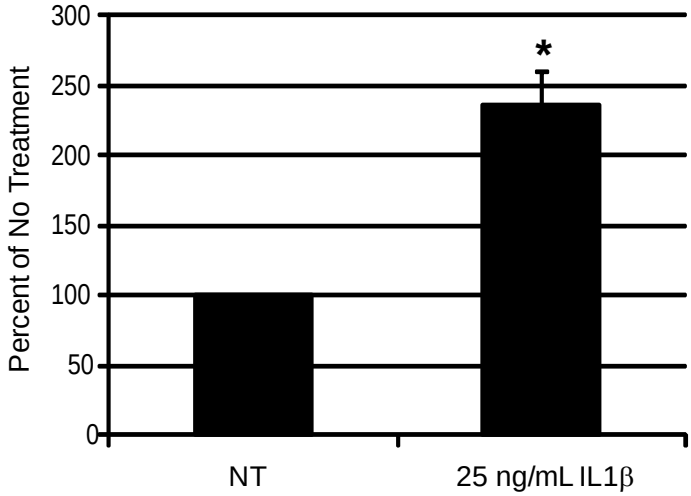
Supplemental Figure I

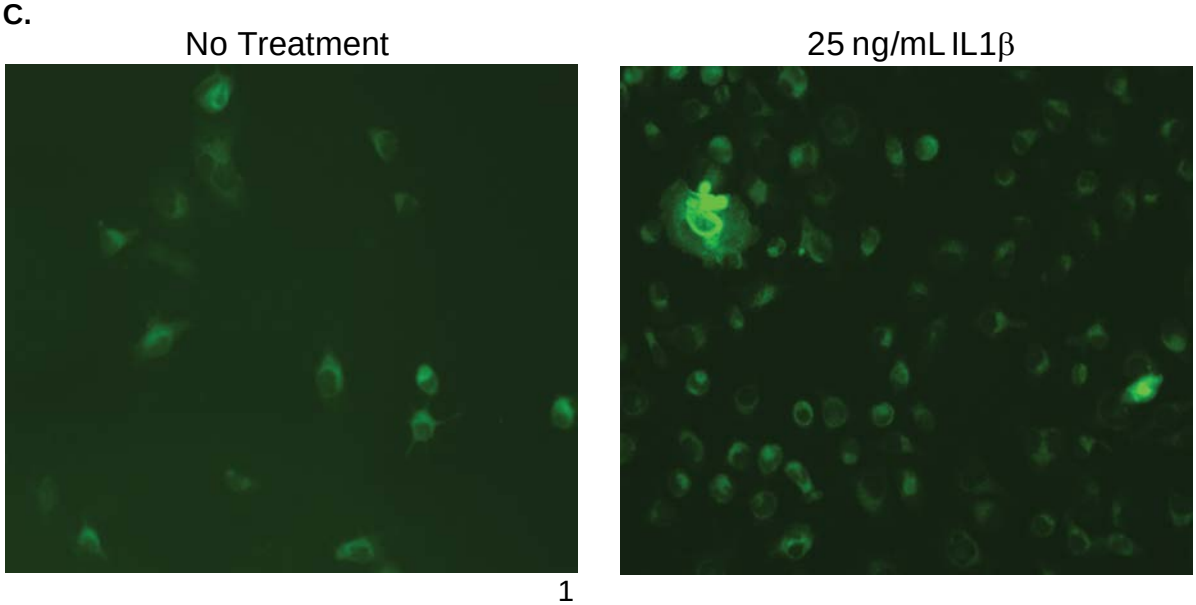
A.



B.

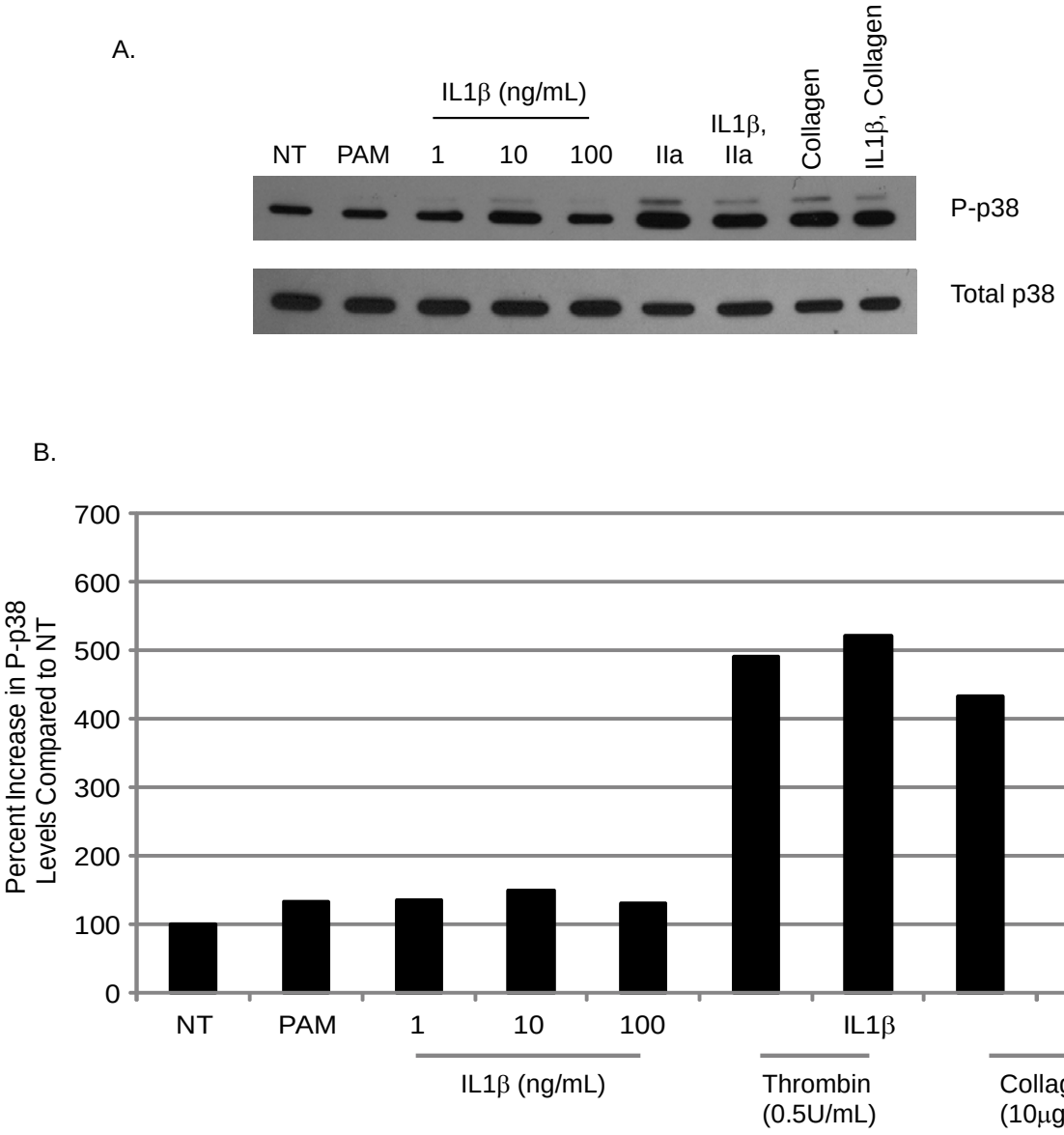


C.



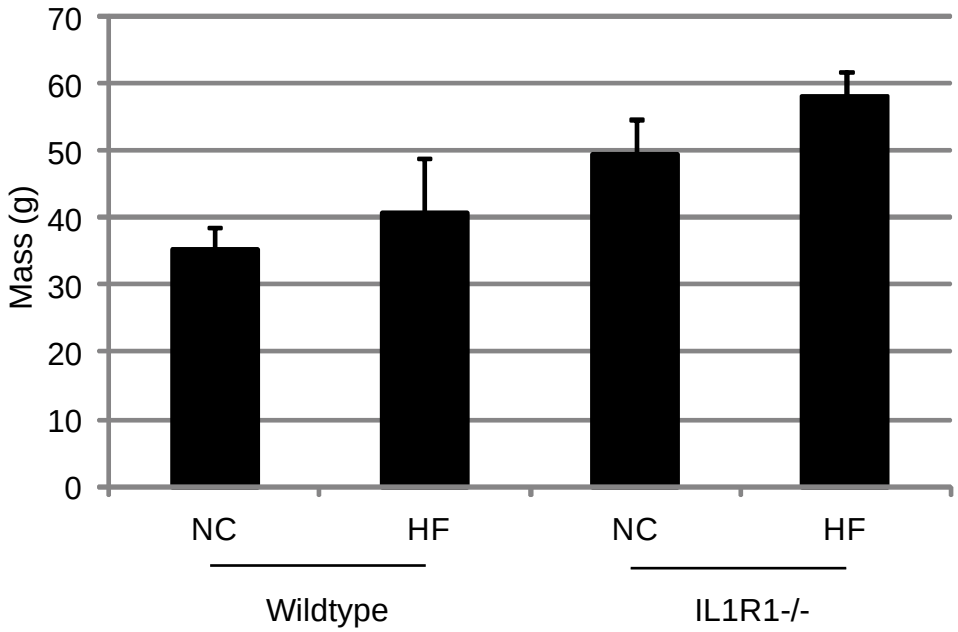
Supplemental Figure I: IL1R1 regulates megakaryocyte adhesion. Meg-01 cells were treated with 25 ng/mL IL1β for 3 hours then allowed to adhere onto fibrinogen (A) or fibronectin (B). n=4; *p<0.05. (C) Representative photographs of Meg-01 cells adherent to fibronectin and stained with anti-CD41 FITC conjugated antibodies.

Supplemental Figure II



Supplemental Figure II: IL1β signals through p38 and enhances its phosphorylation in the presence of thrombin and collagen. Human platelets were left untreated (No Treatment – NT) or treated with 10 μg/mL Pam3CSK4 (PAM) or 1-100 ng/mL IL1β for 10 minutes. Other samples were pretreated with 10 ng/mL IL1β, then treated for an additional 10 minutes with either 0.5 U/mL thrombin or 10 μg/mL collagen. (A) Representative western blot of platelet samples probed for phospho- and total p38. (B) Graph of the densitometry of the above western blot.

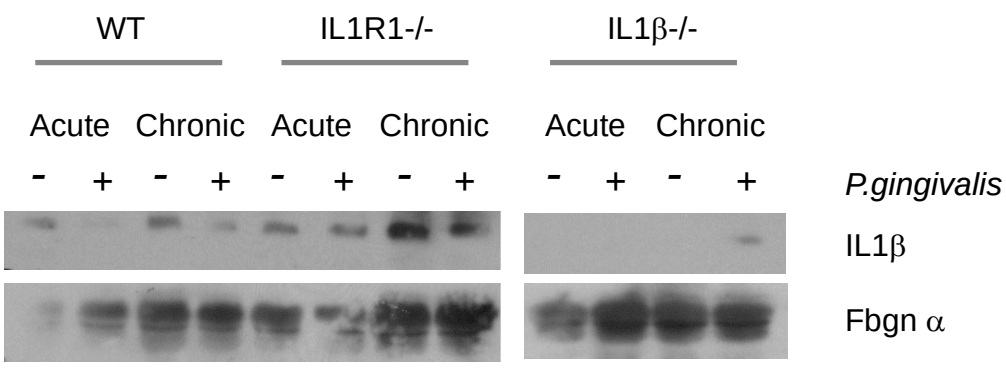
Supplemental Figure III



Supplemental Figure III: Mass of WT and IL1R1$^{-/-}$ mice on a normal chow or high fat diet. Wildtype and IL1R1$^{-/-}$ mice were placed on a normal chow (NC) or high fat diet (HF) diet for 10 weeks. Mass was measured at time of experiment; n=8-10 mice in each group.

Supplemental Figure IV

A.



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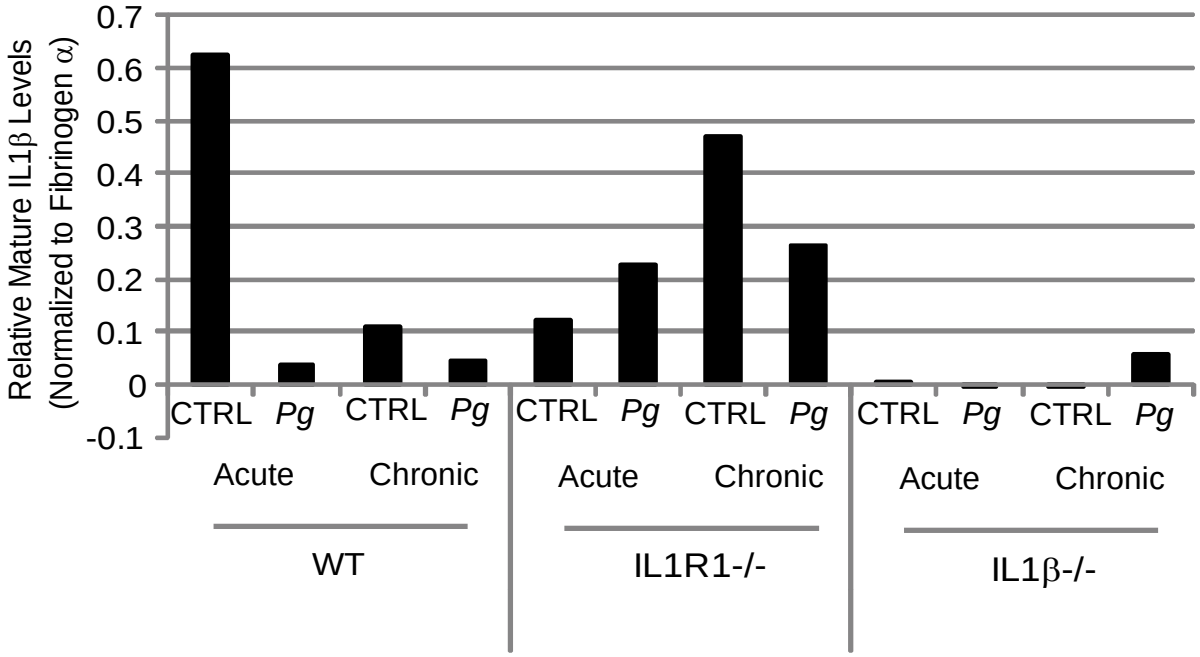
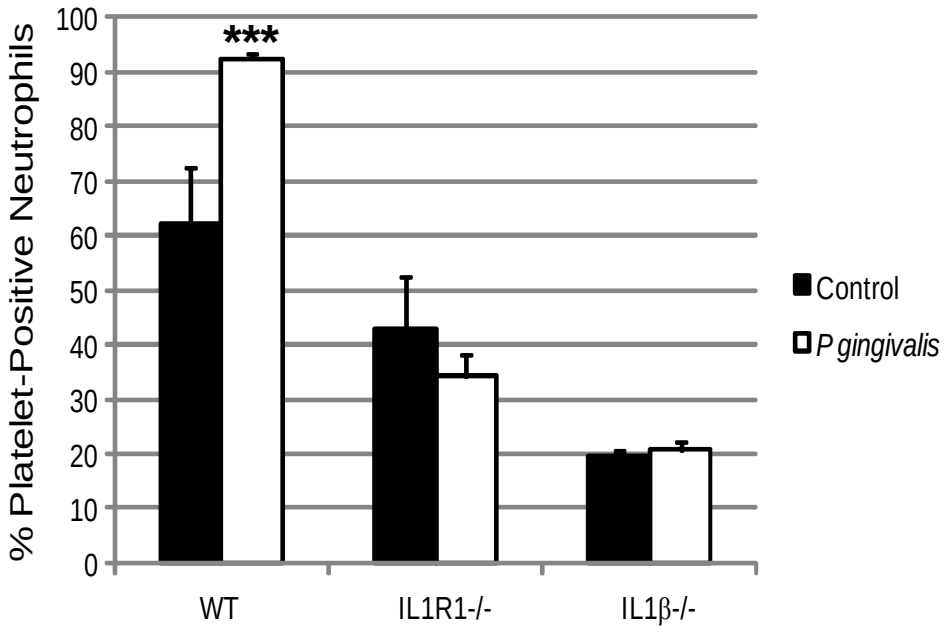
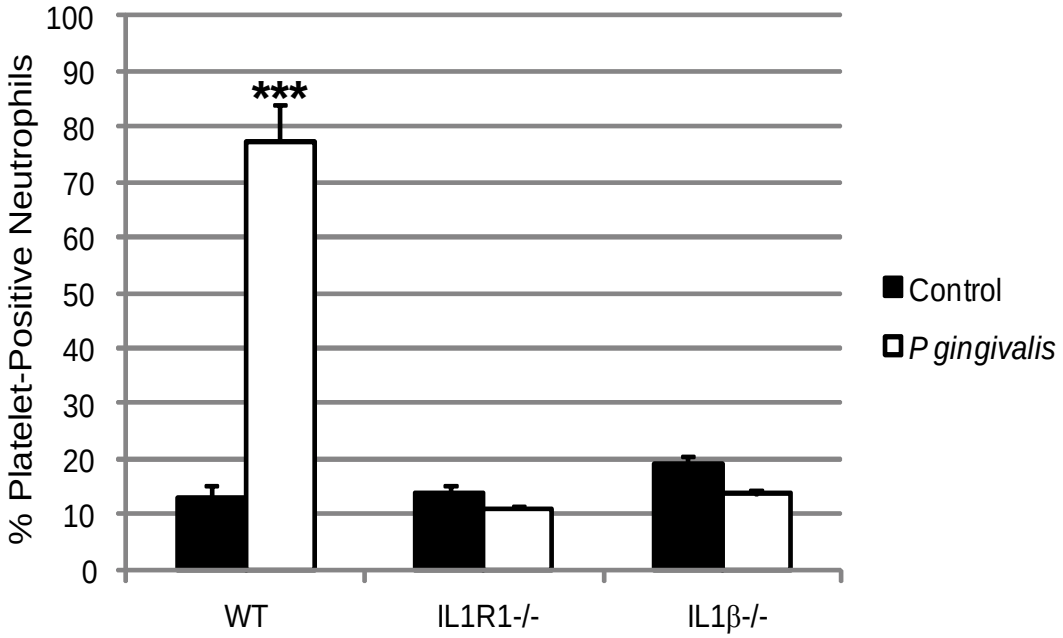


Supplemental Figure IV

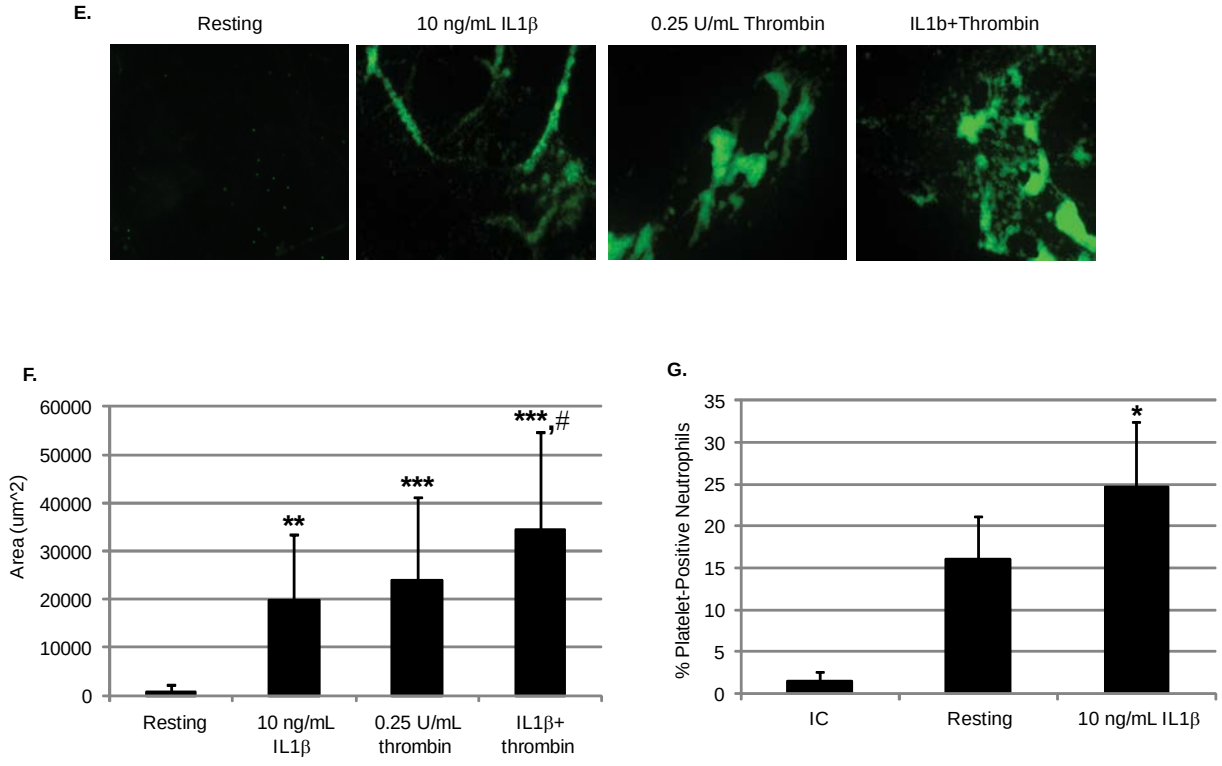
C.



D.



Supplemental Figure IV



Supplemental Figure IV: IL1β affects heterotypic aggregate formation in the setting of infection. Whole blood from wildtype, IL1R1$^{-/-}$, IL1β$^{-/-}$ mice either infected with vehicle (control) or *P. gingivalis* for 24 h (Acute) or 6 weeks (Chronic). (A) Western blot on concentrated plasma samples collected from mice were analyzed for the proform of IL1β and the loading control, fibrinogen α. (B) Graph of the densitometry of the above western blot. Whole blood was analyzed for platelet-positive neutrophils at the Acute (C) and Chronic (D) timepoints. n=3 mice in each group. (E) Representative photographs of isolated IL1β$^{-/-}$ mouse platelets treated with IL1β, thrombin, or IL1β then thrombin adherent to collagen. (F) Quantification of the area covered by platelets in each photograph. n=10 mice; ** p<0.01, *** p<0.001 compared to Resting; # p<0.05 compared to IL1β alone. (G) The percent of platelet-positive neutrophils in whole blood from IL1β$^{-/-}$ mice were treated with IL1β. n=6; * p<0.05 compared to Resting.

Supplemental Tables

Supplemental Table I: Mass and Blood Cell Counts from Mice in Acute and Chronic Infection Experiments.

Infection	Acute						Chronic					
Mouse Model	WT		IL1R1$^{-/-}$		IL1$\beta^{-/-}$		WT		IL1R1$^{-/-}$		IL1$\beta^{-/-}$	
P.gingivalis	-	+	-	+	-	+	-	+	-	+	-	+
Mass (g)	28.3±*0.6*	25.7±*1.5*	25.0±*0.0*	27.3±*0.6*	35.0±*1.7*	24.0±*0.0*	34.4±*2.0*	32.4±*3.0*	32.6±*2.0*	31.8±*1.5*	32.2±*1.0*	33.1±*4.2*
WBC (per L*g)	20.29x10^8 ±*5.9x10^7*	26.63x10^8 ±*3.6x10^7*	2.89x10^8 ±*10.2x10^7*	3.76x10^8 ±*5.4x10^7*	2.88x10^8 ±7.2x10^7	2.42x10^8 ±*11.2x10^7*	2.13x10^8 ±*12.0x10^7*	2.22x10^8 ±*8.5x10^7*	1.34x10^8 ±*5.4x10^7*	1.43x10^8 ±*7.7x10^7*	2.55x10^8 ±*8.8x10^7*	0.84x10^8 ±*6.7x10^7*
Platelet (per μL*g)	26477 ±*11384*	29832 ±*3145*	28493 ±*6499*	26170 ±*1880*	19739 ±*12493*	11250 ±*6737*	19838 ±*8616*	24376 ±*9539*	27765 ±*8165*	20954 ±*8438*	16879 ±*11168*	19943 ±*3152*

White blood cells (WBC) and platelet concentrations were determined from whole blood samples and normalized for mass.

Supplemental Table II: Average Gene Expression Ct and ΔCt for WT and IL1R1$^{-/-}$ Mouse Megakaryocytes.

	Ct		ΔCt	
	WT	IL1R1$^{-/-}$	WT	IL1R1$^{-/-}$
COX2	22.83 ± 5.50	25.64 ± 4.09	7.95 ± 4.13	9.57 ± 2.16
MCP-1	27.96 ± 2.04	25.13 ± 4.37	13.08 ± 3.41	9.22 ± 1.83
NFkB1	18.89 ± 3.68	19.96 ±1.89	4.01 ± 2.31	5.50 ± 0.85
TLR2	21.44 ± 4.32	24.48 ± 4.11	7.56 ± 4.36	8.52 ± 1.76
IL1b	19.48 ± 4.33	21.53 ± 3.83	4.60 ± 2.95	5.62 ± 1.37
IL1R1	23.41 ± 4.93	23.80 ± 4.24	8.53 ± 3.56	7.90 ± 1.76
CD41	14.34 ± 1.34	15.69 ± 2.05	-0.55 ± 0.03	-0.22 ± 0.65
GP1b	21.24 ± 4.30	22.16 ± 4.08	6.36 ± 2.93	6.24 ± 1.90
GAPDH	14.88 ± 1.37	15.92 ± 2.63		

Ct and ΔCt ± Standard Deviation.

Supplemental Table III. Clinical correlates of gene expression in platelets. Results from adjusted multi-variable adjustment of BMI-Score

	IL18	IL1β	IRAK1	MMP9	MYD88	NLRP2
	β±se, *p*-value	β±se, *p*-value	β±se, *p*-value	β±se, *p*-value	β±se, *p*-value	β±se, *p*-value
Age, y	0.003±0.010, *p*=0.8032	0.004±0.010, *p*=0.7265	-0.001±0.010, *p*=0.9087	0.055±0.010, *p*<0.0001**	0.011±0.009, *p*=0.2464	0.005±0.011, *p*=0.6602
Female Sex	-0.008±0.162, *p*=0.9589	-0.053±0.163, *p*=0.7472	0.247±0.156, *p*=0.1151	0.281±0.157, *p*=0.0739	0.356±0.147, *p*=0.0157	0.092±0.169, *p*=0.5866
BMI, kg/m^2	0.025±0.015, *p*=0.0917	0.029±0.015, *p*=0.0490*	0.002±0.014, *p*=0.9111	0.051±0.014, *p*=0.0003**	0.030±0.013, *p*=0.0253*	0.022±0.015, *p*=0.1539
Diabetes	0.295±0.269, *p*=0.2723	0.301±0.272, *p*=0.2684	-0.003±0.260, *p*=0.9900	0.400±0.261, *p*=0.1258	0.457±0.245, *p*=0.0625	0.348±0.281, *p*=0.2165
Total Cholesterol, mg/100mL	0.001±0.003, *p*=0.7778	0.000±0.003, *p*=0.8847	0.000±0.002, *p*=0.8929	0.002±0.002, *p*=0.3322	0.003±0.002, *p*=0.1602	0.002±0.003, *p*=0.4219
HDL Cholesterol mg/100mL	0.006±0.005, *p*=0.2932	0.006±0.005, *p*=0.2318	0.002±0.005, *p*=0.7274	-0.013±0.005, *p*=0.0101	-0.002±0.005, *p*=0.7325	0.005±0.006, *p*=0.3435
Triglyceride, mg/100mL	-0.002±0.001, *p*=0.1961	-0.002±0.001, *p*=0.2138	-0.001±0.001, *p*=0.3240	-0.001±0.001, *p*=0.6334	-0.001±0.001, *p*=0.3545	-0.002±0.001, *p*=0.1031
Antihypertensive Treatment	0.302±0.161, *p*=0.0618	0.359±0.163, *p*=0.0278	0.148±0.156, *p*=0.3431	0.187±0.157, *p*=0.2322	0.291±0.147, *p*=0.0482	0.441±0.169, *p*=0.0091
Systolic Blood Pressure, mmHg	0.004±0.005, *p*=0.4719	0.004±0.005, *p*=0.4062	0.007±0.005, *p*=0.1594	-0.004±0.005, *p*=0.4804	0.002±0.005, *p*=0.6521	0.002±0.006, *p*=0.6542
Diastolic Blood Pressure, mmHg	0.001±0.009, *p*=0.8953	-0.004±0.009, *p*=0.6752	-0.012±0.009, *p*=0.1639	-0.010±0.009, *p*=0.2549	-0.005±0.008, *p*=0.5099	-0.002±0.009, *p*=0.8640
Glucose, mg/dL	-0.000±0.004, *p*=0.9335	-0.002±0.004, *p*=0.5208	0.002±0.004, *p*=0.6366	-0.003±0.004, *p*=0.4457	-0.005±0.003, *p*=0.1155	-0.002±0.004, *p*=0.5708
Lipid Treatment	-0.488±0.171, *p*=0.0043	-0.342±0.172, *p*=0.0476	-0.480±0.165, *p*=0.0037	-0.210±0.166, *p*=0.2044	-0.158±0.156, *p*=0.3110	-0.307±0.179, *p*=0.0855
Prevalent CHD	-0.011±0.244, *p*=0.9641	0.171±0.247, *p*=0.4880	0.220±0.236, *p*=0.3529	0.399±0.237, *p*=0.0923	0.100±0.223, *p*=0.6521	-0.018±0.256, *p*=0.9437
Aspirin (3/week)	0.030±0.154, *p*=0.8455	-0.132±0.155, *p*=0.3943	0.152±0.149, *p*=0.3068	-0.144±0.149, *p*=0.3343	-0.134±0.140, *p*=0.3377	-0.000±0.161, *p*=0.9995
Current HRT	0.285±0.311, *p*=0.3600	0.027±0.314, *p*=0.9304	-0.116±0.301, *p*=0.6998	0.187±0.302, *p*=0.5354	0.054±0.284, *p*=0.8491	0.180±0.326, *p*=0.5798
Smoker	-0.090±0.255, *p*=0.7231	-0.009±0.258, *p*=0.9730	-0.110±0.247, *p*=0.6573	0.107±0.248, *p*=0.6671	-0.168±0.232, *p*=0.4699	-0.130±0.267, *p*=0.6263

n=1819. Associations significant at *p*<0.05 are marked with an * and those significant after correction for multiple comparisons are marked with **.

Supplemental Table IV. Clinical correlates of gene expression in platelets. Results from adjusted multi-variable adjustment of BMI Stratification.

	IL18	IL1β	IRAK1	MMP9	MYD88	NLRP3
	β±se, *p*-value	β±se, *p*-value	β±se, *p*-value	β±se, *p*-value	β±se, *p*-value	β±se, *p*-value
Age, y	0.002±0.010, *p*=0.8516	0.003±0.010, *p*=0.7808	-0.001±0.010, *p*=0.9169	0.053±0.010, *p*<0.0001 **	0.010±0.009, *p*=0.2699	0.004±0.011, *p*=0.6759
Female Sex	0.003±0.163, *p*=0.9873	-0.043±0.164, *p*=0.7915	0.260±0.157, *p*=0.0988	0.280±0.158, *p*=0.0768	0.358±0.148, *p*=0.0158*	0.113±0.170, *p*=0.5068
Overweight (25≤BMI<30)	0.224±0.185, *p*=0.2243	0.226±0.186, *p*=0.2246	0.164±0.179, *p*=0.3589	0.113±0.180, *p*=0.5295	0.176±0.168, *p*=0.2950	0.354±0.193, *p*=0.0666
Obese (BMI≥30)	0.355±0.204, *p*=0.0826	0.404±0.206, *p*=0.0504	0.108±0.198, *p*=0.5836	0.439±0.199, *p*=0.0273 *	0.425±0.186, *p*=0.0224*	0.430±0.214, *p*=0.0441*
Lipid Treatment	-0.499±0.171, *p*=0.0035*	-0.353±0.173, *p*=0.0410*	-0.489±0.165, *p*=0.0032*	-0.214±0.166, *p*=0.1985	-0.167±0.156, *p*=0.2828	-0.326±0.179, *p*=0.0686
Total Cholesterol, mg/100mL	0.001±0.003, *p*=0.7870	0.000±0.003, *p*=0.8846	0.000±0.002, *p*=0.9356	0.003±0.003, *p*=0.2913	0.003±0.002, *p*=0.1533	0.002±0.003, *p*=0.4568
HDL Cholesterol mg/100mL	0.006±0.005, *p*=0.2772	0.006±0.005, *p*=0.2276	0.003±0.005, *p*=0.6152	-0.015±0.005, *p*=0.0037*	-0.002±0.005, *p*=0.7336	0.006±0.006, *p*=0.2511
Triglyceride, mg/100mL	-0.002±0.001, *p*=0.1948	-0.002±0.001, *p*=0.2113	-0.001±0.001, *p*=0.3243	-0.001±0.001, *p*=0.6488	-0.001±0.001, *p*=0.3421	-0.002±0.001, *p*=0.1006
Antihypertensive Treatment	0.302±0.161, *p*=0.0609	0.362±0.163, *p*=0.0261*	0.134±0.156, *p*=0.3909	0.232±0.157, *p*=0.1393	0.295±0.147, *p*=0.0446*	0.424±0.169, *p*=0.0120*
Systolic Blood Pressure, mmHg	0.004±0.005, *p*=0.4950	0.004±0.005, *p*=0.4218	0.007±0.005, *p*=0.1797	-0.003±0.005, *p*=0.5122	0.002±0.005, *p*=0.6499	0.002±0.006, *p*=0.7152
Diastolic Blood Pressure, mmHg	0.001±0.009, *p*=0.9251	-0.004±0.009, *p*=0.6476	-0.013±0.009, *p*=0.1545	-0.010±0.009, *p*=0.2679	-0.006±0.008, *p*=0.4798	-0.002±0.009, *p*=0.8088
Glucose, mg/dL	-0.000±0.004, *p*=0.9503	-0.002±0.004, *p*=0.5408	0.002±0.004, *p*=0.6603	-0.002±0.004, *p*=0.5452	-0.005±0.003, *p*=0.1231	-0.002±0.004, *p*=0.5540
Diabetes	0.322±0.269, *p*=0.2322	0.327±0.272, *p*=0.2287	0.011±0.260, *p*=0.9659	0.440±0.262, *p*=0.0932	0.472±0.245, *p*=0.0544	0.380±0.282, *p*=0.1772
Prevalent CHD	-0.013±0.244, p=0.9565	0.169±0.247, *p*=0.4928	0.217±0.236, *p*=0.3585	0.395±0.238, *p*=0.0966	0.102±0.223, *p*=0.6482	-0.021±0.256, *p*=0.9341
Aspirin (3/week)	0.036±0.154, *p*=0.8137	-0.126±0.155, *p*=0.4179	0.157±0.149, *p*=0.2913	-0.143±0.149, *p*=0.3383	-0.129±0.140, *p*=0.3579	0.011±0.161, *p*=0.9467
Current HRT	0.300±0.312, *p*=0.3358	0.043±0.315, *p*=0.8910	-0.106±0.301, *p*=0.7248	0.193±0.303, *p*=0.5237	0.068±0.284, *p*=0.8097	0.204±0.326, *p*=0.5310
Smoker	-0.091±0.255, *p*=0.7212	-0.011±0.258, *p*=0.9654	-0.101±0.247, *p*=0.6814	0.080±0.248, *p*=0.7461	-0.171±0.232, *p*=0.4620	-0.120±0.267, *p*=0.6518

n=1819. Associations significant at $p<0.05$ are marked with an * and those significant after correction for multiple comparisons are marked with **.

Supplemental Table V. Association of Serum Biomarker levels and Platelet Gene Expression. Results from adjusted multi-variable adjustment of BMI-Scores.

	IL18	IL1β	IRAK1	MMP9	MYD88	NLRP3
	β±se, *p*-value	β±se, *p*-value	β±se, *p*-value	β±se, *p*-value	β±se, *p*-value	β±se, *p*-value
CRP	0.017±0.070, *p*=0.8132	0.087±0.071, *p*=0.2217	0.062±0.068, *p*=0.3622	0.492±0.067, *p*<0.0001**	0.207±0.064, *p*=0.0012*	0.066±0.073, *p*=0.3703
ICAM1	-0.530±0.263, *p*=0.0440*	-0.275±0.266, *p*=0.3005	-0.081±0.255, *p*=0.7507	0.685±0.253, *p*=0.0070*	-0.218±0.240, *p*=0.3627	-0.672±0.275, *p*=0.0146*
IL6	-0.061±0.112, *p*=0.5830	0.168±0.113, *p*=0.1382	0.090±0.108, *p*=0.4057	0.737±0.107, *p*<0.0001**	0.174±0.102, *p*=0.0877	0.079±0.117, *p*=0.5019
MCP1	-0.380±0.232, *p*=0.1015	-0.089±0.234, *p*=0.7051	0.070±0.225, *p*=0.7574	0.302±0.224, *p*=0.1779	0.178±0.211, *p*=0.3991	-0.416±0.242, *p*=0.0865
OPG	0.771±0.255, *p*=0.0026**	0.667±0.258, *p*=0.0097*	0.511±0.247, *p*=0.0390*	-0.062±0.248, *p*=0.8042	0.514±0.233, *p*=0.0275*	0.837±0.267, *p*=0.0017*
PSELECTIN	-0.604±0.224, *p*=0.0072*	-0.494±0.227, *p*=0.0294*	-0.467±0.217, *p*=0.0318*	0.688±0.218, *p*=0.0016*	-0.176±0.205, *p*=0.3910	-0.476±0.235, *p*=0.0431*
TNFR	-0.130±0.154, *p*=0.3998	0.014±0.155, *p*=0.9262	0.098±0.149, *p*=0.5112	0.514±0.149, *p*=0.0006**	0.042±0.140, *p*=0.7651	-0.166±0.161, *p*=0.3034

Associations significant at $p<0.05$ are marked with an * and those significant after correction for multiple comparisons are marked with **.

Supplemental Table VI. Full Model Results for BMI-Score and Serum Biomarkers Predicting MMP9 Platelet Gene Expression

	CRP	ICAM1	IL6	MCP1	OPG	PSELECTIN	TNFR
	β±se, *p*-value	β±se, *p*-value	β±se, *p*-value	β±se, *p*-value	β±se, *p*-value	β±se, *p*-value	β±se, *p*-value
Serum Biomarker	0.492±0.067, *p*<0.0001**	0.685±0.253, *p*=0.0070*	0.737±0.107, *p*<0.0001**	0.302±0.224, *p*=0.1779	-0.062±0.248, *p*=0.8042	0.688±0.218, *p*=0.0016*	0.514±0.149, *p*=0.0006**
Age, y	0.045±0.010, *p*<0.0001**	0.056±0.010, *p*=0.0000**	0.043±0.010, *p*<0.0001**	0.056±0.010, *p*<0.0001**	0.056±0.010, *p*<0.0001**	0.053±0.010, *p*=0.0001**	0.050±0.010, *p*<0.0001**
Female Sex	0.176±0.155, *p*=0.2582	0.234±0.160, *p*=0.1434	0.282±0.157, *p*=0.0725	0.289±0.159, *p*=0.0691	0.285±0.158, *p*=0.0714	0.329±0.157, *p*=0.0367*	0.268±0.157, *p*=0.0869
BMI, kg/m^2	0.018±0.015, *p*=0.2368	0.049±0.014, *p*=0.0006**	0.032±0.015, p=0.0290*	0.050±0.014, *p*=0.0006**	0.051±0.014, *p*=0.0004**	0.050±0.014, *p*=0.0005**	0.048±0.014, *p*=0.0008**
Lipid Treatment	-0.104±0.164, *p*=0.5243	-0.193±0.168, *p*=0.2507	-0.126±0.166, *p*=0.4463	-0.183±0.168, *p*=0.2759	-0.214±0.166, *p*=0.1986	-0.212±0.165, *p*=0.2004	-0.174±0.165, *p*=0.2920
Total Cholesterol, mg/100mL	0.003±0.002, *p*=0.3007	0.003±0.003, *p*=0.3024	0.004±0.002, *p*=0.1449	0.003±0.003, *p*=0.3190	0.002±0.003, *p*=0.3434	0.002±0.002, *p*=0.3591	0.003±0.003, *p*=0.2433
HDL Cholesterol mg/100mL	-0.011±0.005, *p*=0.0307*	-0.012±0.005, *p*=0.0182*	-0.012±0.005, *p*=0.0180*	-0.014±0.005, *p*=0.0057*	-0.013±0.005, *p*=0.0103*	-0.013±0.005, *p*=0.0088*	-0.012±0.005, *p*=0.0206*
Triglyceride, mg/100mL	-0.001±0.001, *p*=0.5507	-0.001±0.001, *p*=0.6100	-0.001±0.001, *p*=0.5481	-0.001±0.001, *p*=0.5230	-0.001±0.001, *p*=0.6389	-0.001±0.001, *p*=0.3810	-0.000±0.001, *p*=0.7233
Antihypertensive Treatment	0.142±0.155, *p*=0.3570	0.138±0.159, *p*=0.3846	0.044±0.157, *p*=0.7823	0.143±0.159, *p*=0.3667	0.189±0.157, *p*=0.2289	0.170±0.156, *p*=0.2774	0.155±0.156, *p*=0.3207
Systolic Blood Pressure, mmHg	-0.004±0.005, *p*=0.3931	-0.003±0.005, *p*=0.5105	-0.004±0.005, *p*=0.4553	-0.003±0.005, *p*=0.6304	-0.004±0.005, *p*=0.4889	-0.004±0.005, *p*=0.4658	-0.004±0.005, *p*=0.4098
Diastolic Blood Pressure, mmHg	-0.008±0.009, *p*=0.3639	-0.011±0.009, *p*=0.2199	-0.008±0.009, *p*=0.3719	-0.011±0.009, *p*=0.2107	-0.010±0.009, *p*=0.2511	-0.011±0.009, *p*=0.2286	-0.008±0.009, *p*=0.3835
Glucose, mg/dL	-0.004±0.004, *p*=0.2210	-0.003±0.004, *p*=0.3850	-0.004±0.004, *p*=0.3248	-0.003±0.004, *p*=0.3878	-0.003±0.004, *p*=0.4526	-0.003±0.004, *p*=0.4363	-0.002±0.004, *p*=0.5654
Diabetes	0.512±0.258, *p*=0.0473*	0.358±0.261, *p*=0.1693	0.366±0.258, *p*=0.1558	0.363±0.261, *p*=0.1651	0.405±0.262, *p*=0.1222	0.372±0.261, *p*=0.1535	0.332±0.261, *p*=0.2037
Prevalent CHD	0.439±0.234, *p*=0.0609	0.385±0.239, *p*=0.1074	0.293±0.237, *p*=0.2166	0.403±0.240, *p*=0.0928	0.404±0.238, *p*=0.0898	0.415±0.237, *p*=0.0793	0.397±0.236, *p*=0.0930
Aspirin (3/week)	-0.036±0.148, *p*=0.8063	-0.123±0.151, *p*=0.4138	-0.056±0.149, *p*=0.7102	-0.130±0.151, *p*=0.3913	-0.146±0.149, *p*=0.3294	-0.106±0.149, *p*=0.4778	-0.123±0.149, *p*=0.4080
Current HRT	0.046±0.298, *p*=0.8767	0.119±0.306, *p*=0.6964	0.111±0.302, *p*=0.7124	0.102±0.306, *p*=0.7398	0.188±0.302, *p*=0.5339	0.199±0.301, *p*=0.5094	0.218±0.301, *p*=0.4685
Smoker	-0.118±0.246, *p*=0.6321	-0.041±0.258, *p*=0.8735	-0.117±0.252, *p*=0.6411	0.117±0.254, *p*=0.6446	0.113±0.249, *p*=0.6501	0.023±0.248, *p*=0.9247	0.078±0.247, *p*=0.7506

Associations significant at *p*<0.05 are marked with an * and those significant after correction for multiple comparisons are marked with **.