Supplemental Materials

Sex-specific Platelet Activation through Protease-Activated Receptors Reverses in Myocardial Infarction

Beom Soo Kim [1¶], David A. Auerbach [2¶], Hamza Sadhra[1], Matthew Godwin[4],

Rohan Bhandari[4, 8], Frederick S. Ling[3], Amy Mohan[1], David I. Yule [5], Larry Wagner II[5], David Q. Rich,[1, 6,7] Sara Tura[1], Craig N. Morrell[1],

Livia Timpanaro-Perrotta[4], Arwa Younis[3], Ilan Goldenberg [3], and Scott J. Cameron, MD, PhD, RPVI [1,4,8*]

Author Affiliations:

[1]Aab Cardiovascular Research Institute, University of Rochester School of Medicine, Rochester, New York.
[2] Department of Pharmacology, SUNY Upstate Medical University, Syracuse, New York.
[3] Department of Medicine, Division of Cardiology, University of Rochester School of Medicine, Rochester, New York.
[4] Department of Cardiovascular and Metabolic Sciences, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio.
[5] Department of Pharmacology and Physiology, University of Rochester School of Medicine, Rochester, New York.
[6] Department of Public Health Sciences, University of Rochester School of Medicine, Rochester, New York.
[7] Department of Environmental Medicine, University of Rochester School of Medicine, Rochester, New York.
[8] Heart Vascular and Thoracic Institute, Department of Cardiovascular Medicine, Section of Vascular Medicine, Cleveland Clinic Foundation, Cleveland, Ohio 44195.

Major Resources Table

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
Mouse	Jaxson Laboratories	C57BL/6J	Male	https://www.jax.org/strain/000664
Mouse	Jaxson Laboratories	C57BL/6J	Female	https://www.jax.org/strain/000664

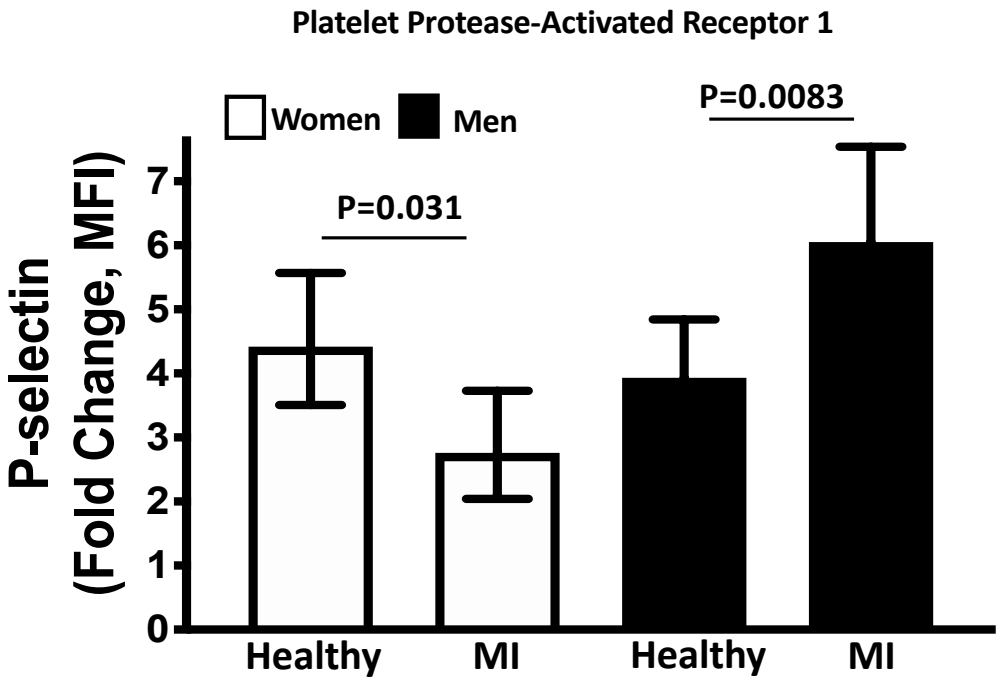
Antibodies

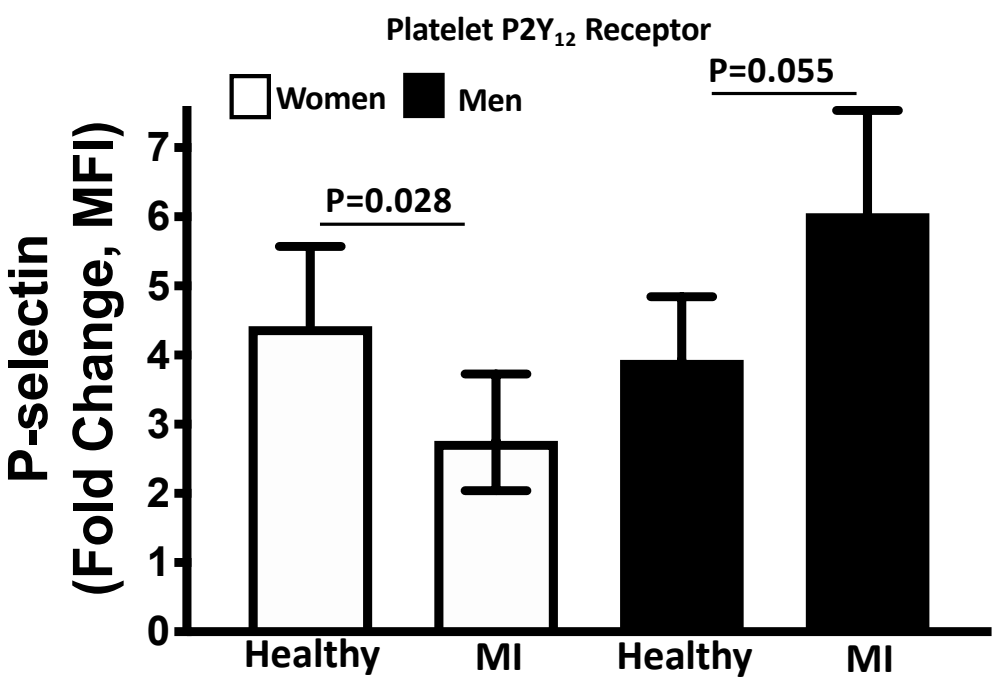
Target antigen	Vendor or Source	Catalog #	Working concentration	Persistent ID / URL
CD62P-PE	Thermo Fisher Waltham	12-0628-42	0.25μg/mL	https://www.thermofisher.com/antibody/product/CD62P-P-Selectin-Antibody-clone-AK-4-Monoclonal/12-0628-42
CD62P-FITC	BD Pharmingen	553744	5μg/mL	https://www.bdbiosciences.com/eu/applications/research/t-cell-immunology/regulatory-t-cells/surface-markers/mouse/fitc-rat-anti-mouse-cd62p-rb4034/p/553744
Gα(q)	Cell Signaling Technologies	14373	59.0 ng/ml	https://www.cellsignal.com/products/primary-antibodies/ga-q-d5v1b-rabbit-mab/14373?Ntk=Products&Ntt=14373
GAPDH	Cell Signaling Technologies	5174	6.8 ng/ml	https://www.cellsignal.com/products/primary-antibodies/gapdh-d16h11-xp-rabbit-mab/5174?Ntk=Products&Ntt=5174
α-Tubulin	Cell Signaling Technologies	3873	355.0 ng/ml	https://www.cellsignal.com/products/primary-antibodies/a-tubulin-dm1a-mouse-mab/3873?Ntk=Products&Ntt=3873
PLCγ1	Cell Signaling Technologies	2822	102.0 ng/ml	https://www.cellsignal.com/products/primary-antibodies/plcg1-antibody/2822?Ntk=Products&Ntt=2822
P-PLCγ1 (Y783)	Cell Signaling Technologies	14008	260.0 ng/ml	https://www.cellsignal.com/products/primary-antibodies/phospho-plcg1-tyr783-d6m9s-rabbit-mab/14008?Ntk=Products&Ntt=14008

Data and Code Availability

Description	Source / Repository	Persistent ID / URL
SAS Code – Platelet	SAS stored on a password protected cloud drive for access to collaborators upon request.	Box.com

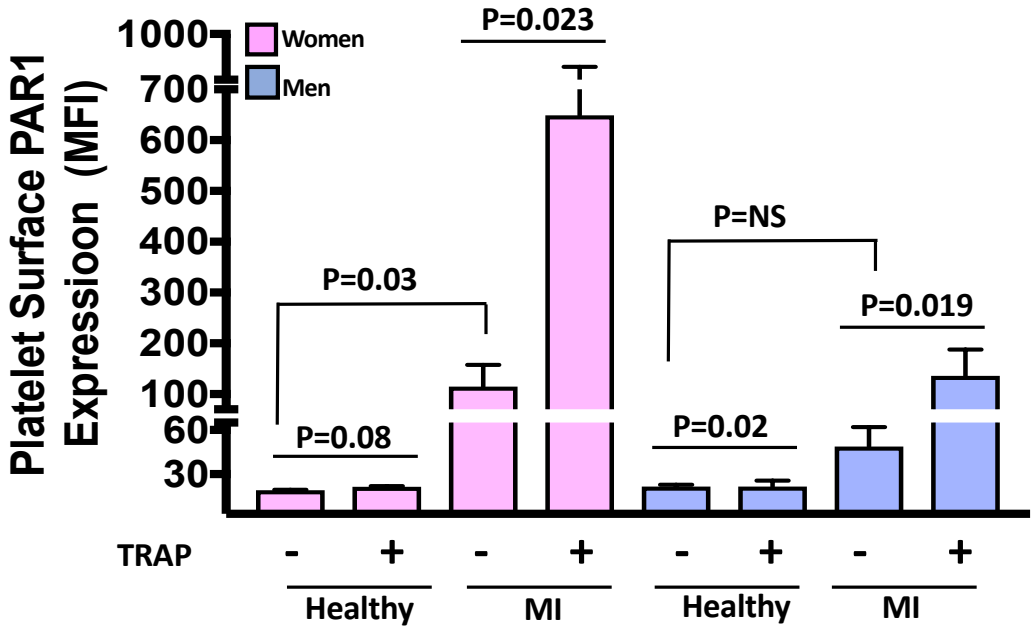
Platelet Activation (Healthy vs. MI)





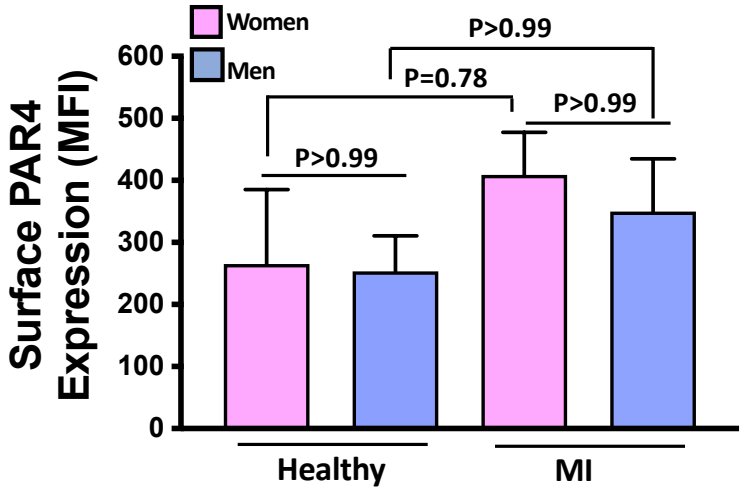
SI: Agonist-mediated platelet activation in health and after MI. Blood was drawn from healthy women and men and compared to blood drawn from patients with MI (STEMI + NSTEMI) as soon as they were diagnosed (prior to coronary angiography, and prior to loading with a $P2Y_{12}$ receptor antagonist). Platelet rich plasma was isolated and platelets were stimulated for 15 mins with an agonist for platelet PAR1 (TRAP-6, 10 μM) or the $P2Y_{12}$ receptor (ADP, 10 μM). Platelets were labeled with a tagged antibody for p-selectin, and then analyzed by flow cytometry. Platelet function is represented as mean fold change in surface P-selectin by median fluorescence intensity (MFI) from baseline ± 95% C.I. Differences between women and men for each agonist was assessed by the Mann-Whitney U test. Level of significance is noted above the graph.

Platelet Surface PAR1 Expression



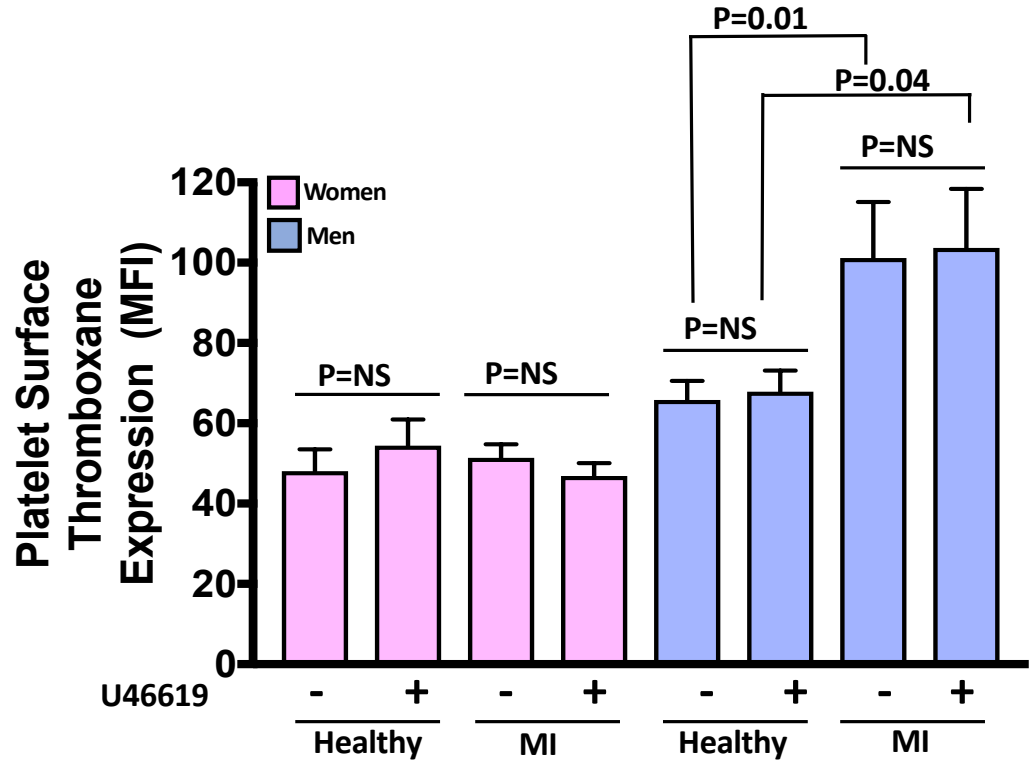
SII: Platelet surface receptor expression after M.I. Blood was drawn patients as soon as they were diagnosed with NSTEMI, prior to coronary angiography, and prior to loading with a $P2Y_{12}$ receptor antagonist. Platelet rich plasma was isolated and platelets were stimulated for 15 mins with an agonist for PAR1 (TRAP-6, 10 μM). Platelets were labeled with a FITC-tagged antibody for PAR1, and analyzed by flow cytometry. Platelet surface receptor density is represented as mean fluorescence intensity (MFI) ± SEM. Differences between groups was assessed by the Kruskal-Wallis test followed by Dunn's post test. Level of significance is noted above the graph.

Platelet Surface PAR4 Expression



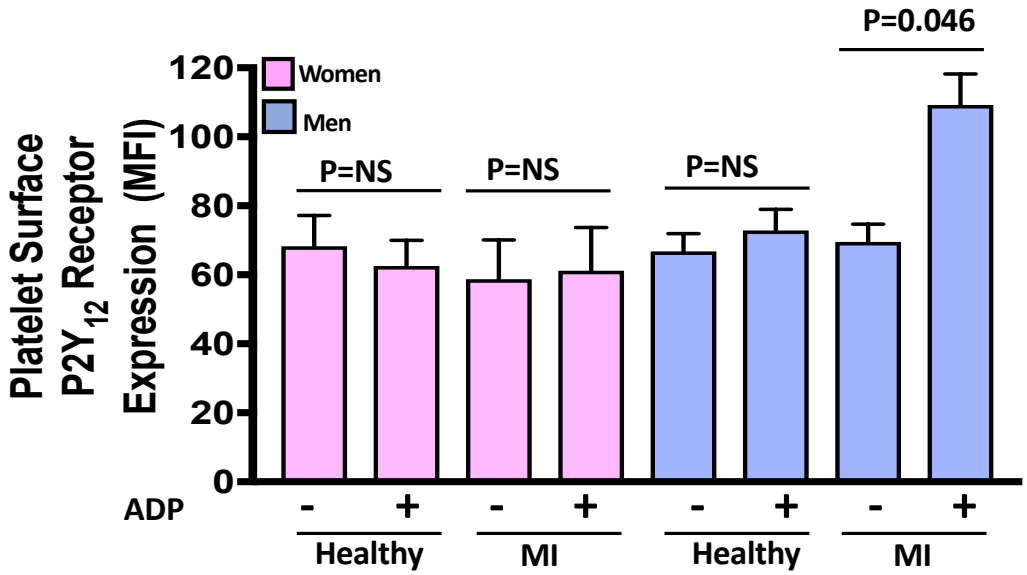
SIII: Platelet surface PAR4 receptor expression after M.I. Blood was drawn patients as soon as they were diagnosed with NSTEMI, prior to coronary angiography, and prior to loading with a $P2Y_{12}$ receptor antagonist. Platelet rich plasma was isolated and platelets were labeled with a FITC-tagged antibody for PAR4, and analyzed by flow cytometry. Platelet surface receptor density is represented as mean fluorescence intensity (MFI) ± SEM. Differences between groups was assessed by the Kruskal-Wallis test followed by Dunn's post test. Level of significance is noted above the graph.

Platelet Surface Thromboxane Receptor Expression

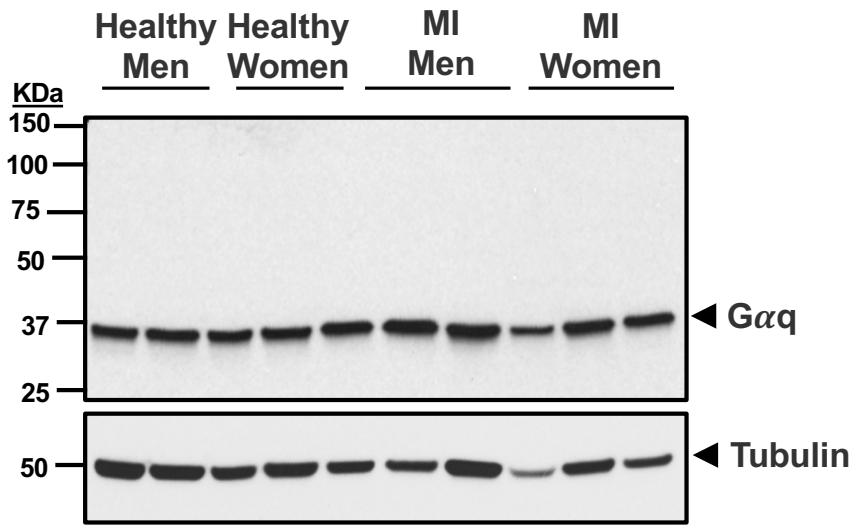


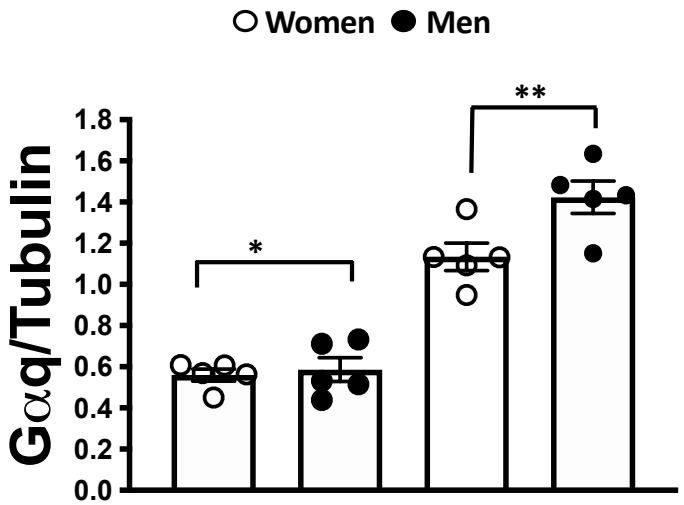
SIV: Platelet surface receptor expression after M.I. Blood was drawn patients as soon as they were diagnosed with NSTEMI, prior to coronary angiography, and prior to loading with a $P2Y_{12}$ receptor antagonist. Platelet rich plasma was isolated and platelets were stimulated for 15 mins with an agonist for the thromboxane receptor (U46619, 10 μM). Platelets were labeled with a FITC-tagged antibody for thromboxane receptor, and analyzed by flow cytometry. Platelet surface receptor density is represented as mean fluorescence intensity (MFI) ± SEM. Differences between groups was assessed by the Kruskal-Wallis test followed by Dunn's post test. Level of significance is noted above the graph.

Platelet Surface P2Y12 Receptor Expression

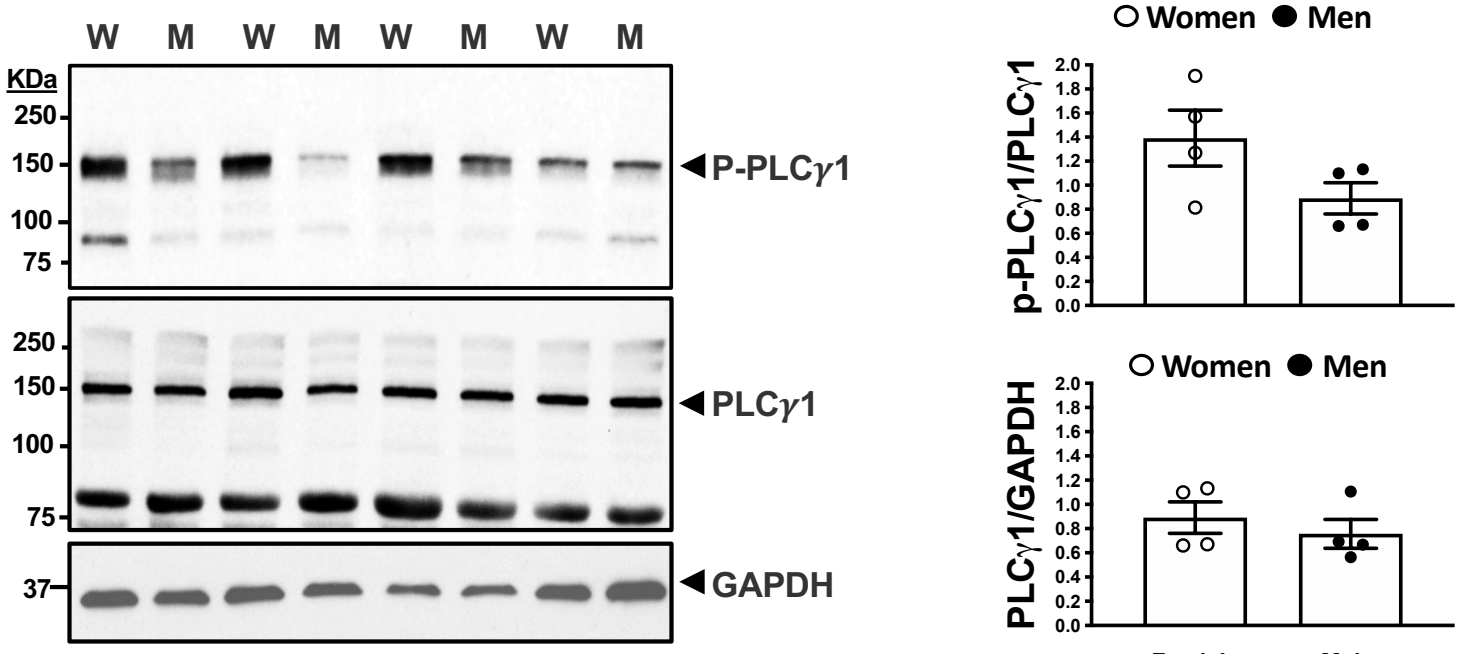


SV: Platelet surface receptor expression after M.I. Blood was drawn patients as soon as they were diagnosed with NSTEMI, prior to coronary angiography, and prior to loading with a $P2Y_{12}$ receptor antagonist. Platelet rich plasma was isolated and platelets were stimulated for 15 mins with an agonist for the thromboxane receptor (U46619, 10 μM). Platelets were labeled with a FITC-tagged antibody for the $P2Y_{12}$ receptor, and analyzed by flow cytometry. Platelet surface receptor density is represented as mean fluorescence intensity (MFI) ± SEM. Differences between groups was assessed by the Kruskal-Wallis test followed by Dunn's post test. Level of significance is noted above the graph.

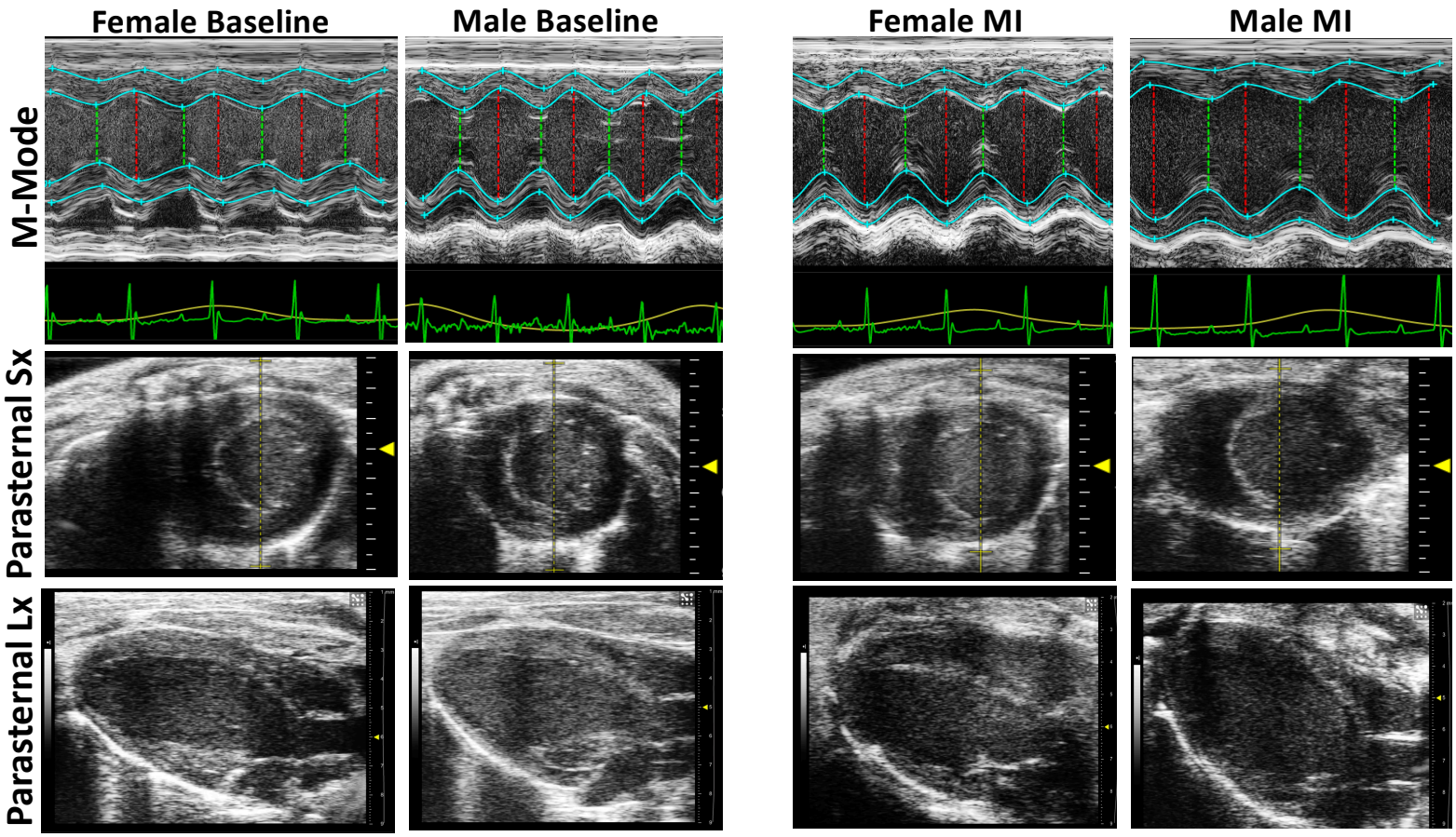




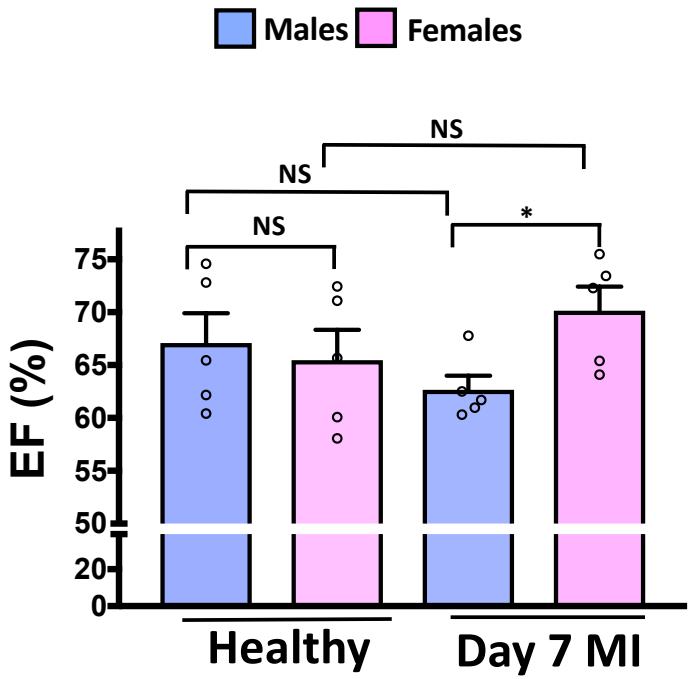
SVI (A) Platelet GPCR Signaling in NSTEMI. Platelets from healthy men and women evaluated for Gαq expression by immunoblotting as mean Gαq /tubulin ± SEM, n=5 (representative samples of all groups shown on same gel). *P=0.6913 between groups by t-test. Platelets were isolated from men and women at the time of NSTEMI and evaluated for for Gαq expression as mean Gαq /tubulin ± SEM, n=5 in each group. **P=0.023 between groups by t-test.

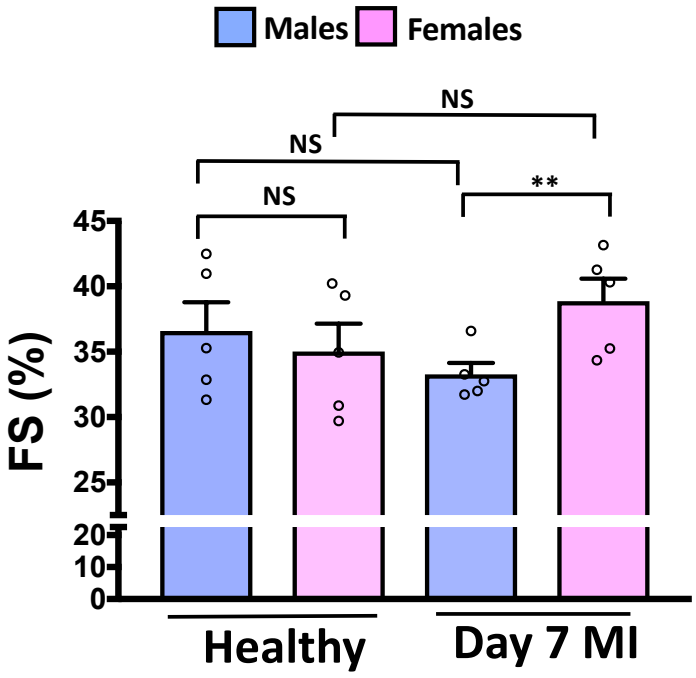


SVII Platelet PLCγ signaling with NSTEMI Platelets were isolated from men (M) and women (W) at the time of NSTEMI. Western blotting was conducted for activated (phosphorylated) PLCγ1 and expressed as the ratio of p-PLCγ1/ PLCγ1 ± SEM, n=4, P= NS between groups by t-test. The expression of platelet PLCγ1 was expressed as mean PLCγ1/GAPDH ± SEM, n=4, P= NS by t-test between groups.



SVIII: **LV Performance in mice before and after MI:** Male and female C57/BL6 mice underwent sham surgery or cryoablation of the LAD. Systolic performance was evaluated by echocardiography as left ventricular ejection fraction (LVEF) or fractional shortening (FS) by echocardiography. Representative baseline and images three days post-MI images are shown using sonographic windows for the parasternal short axis, the parasternal long axis, and by M-Mode. LAD=left anterior descending coronary artery.





SIX Left Ventricular Performance before and following MI: Male and female C57/BL6 mice underwent sham surgery or cryoablation of the LAD. Systolic performance was evaluated by echocardiography as left ventricular ejection fraction (LVEF) or fractional shortening (FS) by echocardiography . Data are presented as mean ± SEM for n=5 mice, * P =0.0021 or **P=0.020 by student's t-test between analyzed groups.