

Supplementary information:

**A Transgenic Mouse Model for Reticulated Platelet Detection Reveals Expansion After Myocardial Ischemia/Reperfusion**

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**Supplementary Table 1: Comprehensive list of materials and reagents.** This table lists all the reagents and materials used in the study.

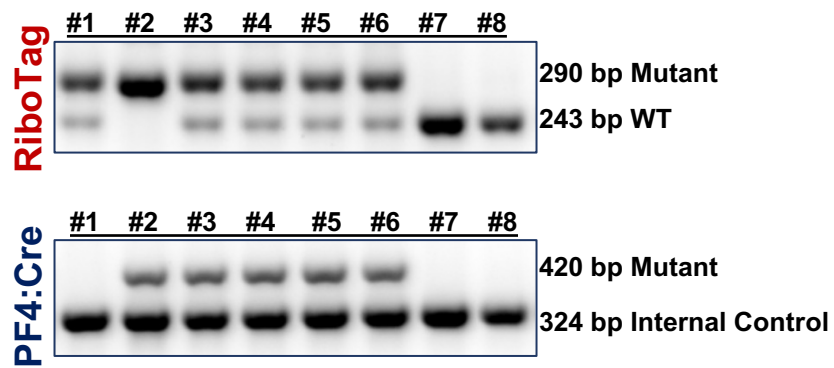
<b>Reagent</b>	<b>Company</b>	<b>Catalog No.</b>
Extract-N-Amp Tissue PCR Kit	Sigma-Aldrich	XNAT2
KAPA2G Fast HotStart PCR Kit	Merck	KK5523
Ethidium bromide	Sigma-Aldrich	E7637
Ketaset (Ketamine 100 mg/ml)	Zoetis	365437
Rompun (Xylazine 2%)	Bayer	770-081
Isoflurane	Piramal Critical Care	PZN 09714675
Glucose 5%	B.Braun	2355740
Unfractionated heparin	Ratiopharm	25000
Clexane (Enoxaparin Sodium)	Sanofi	00955644
Dulbecco's phosphate-buffered saline with calcium and magnesium (DPBS+/+)	ThermoFisher Scientific	14040091
Murine Protease-Activated Receptor-4 Agonist	AnaSpec	AS-60778
Prostaglandin I <sub>2</sub> (sodium salt)	Cayman	18220
Phosflow Lyse/Fix Buffer 5X	BD Biosciences	558049
Triton X-100	Sigma	T8787
Fetal Bovine Serum	Sigma	F4135
Bovine Serum Albumin	Sigma	10775835001
Rat anti-mouse CD42b-DyLight 649	Emfret	M040-3
Rat anti-mouse CD41/61-PE	Emfret	M023-2
Rat anti-mouse CD62P-PE	Emfret	M130-2
Rat anti-mouse CD41- Brilliant Violet 421	BioLegend	133912
Mouse anti-mouse/rat CD62P-PE/Cy7	BioLegend	148310
Mouse anti-HA.11-Brilliant Violet 421	BioLegend	682405
Rat anti-mouse CD45-APC	BioLegend	109814
Mouse IgG2a, k isotype-PE/Cy7	BioLegend	400254
Rat IgG1, κ isotype-Brilliant Violet 421	BioLegend	400439
Rat IgG isotype-PE	Emfret	P190-2
Thiazole Orange - 10 mM in DMSO	BIOZOL	BOT-40077
SYTO 13 Green Fluorescent Nucleic Acid Stain	ThermoFisher Scientific	S7575
Portex Fine Bore Tube	Smith's Medical	800/100/100
PERMAHAND Silk Suture 8-0	Ethicon	W8703
PERMAHAND Silk Suture 6-0	Ethicon	EH7435G
β-Mercaptoethanol	PanReac AppliChem	APA11080100

**Supplementary Table 2. Primer sequences and PCR conditions for genotyping of Pf4-Cre and RiboTag alleles.** This table lists the oligonucleotide sequences used to detect the Pf4-Cre transgene and the RiboTag (Rpl22<sup>HA</sup> floxed) allele, including expected amplicon sizes for wild-type (WT) and mutant alleles.

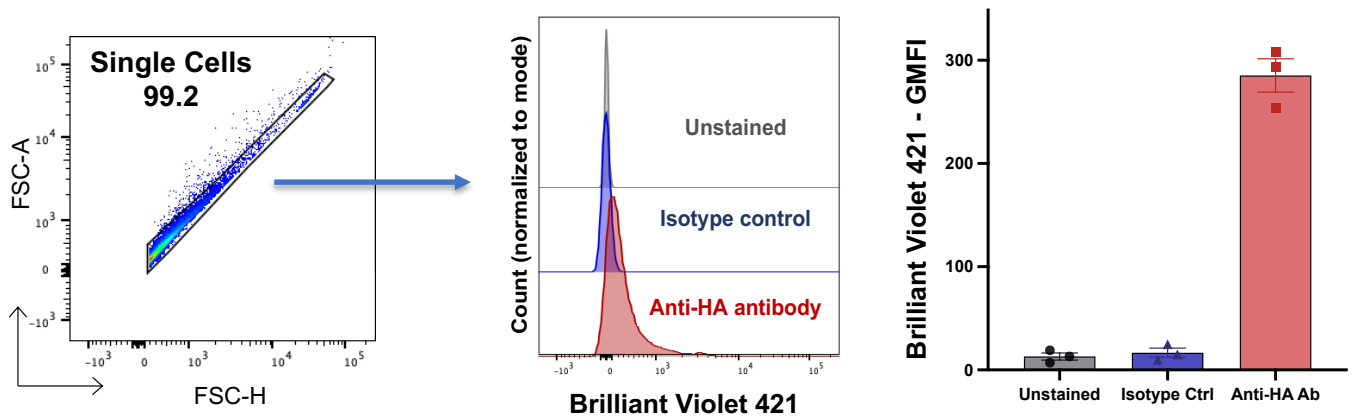
Target	Primer Name	Sequence (5'–3')	Expected Band Size
Pf4-Cre	19003 oIMR 7338 oIMR 7339 oIMR 8969	CCA AGT CCT ACT GTT TCT CAC TC CTA GGC CAC AGA ATT GAA AGA TCT GTA GGT GGA AAT TCT AGC ATC ATC C TGC ACA GTC AGC AGG TT	Transgene 420 bp Internal control 324 bp
RiboTag	Forward 9508 Reverse 9509	AAG AAA GTC TGC TGG TTT AGT G CTG GTC ATC TGC CTG GGC AGA T	Mutant = 290 bp Wildtype = 243bp Heterozygote = 290bp and 243bp

**PCR Conditions (KAPA2G Fast HotStart PCR Kit):** For both RiboTag and Pf4:Cre

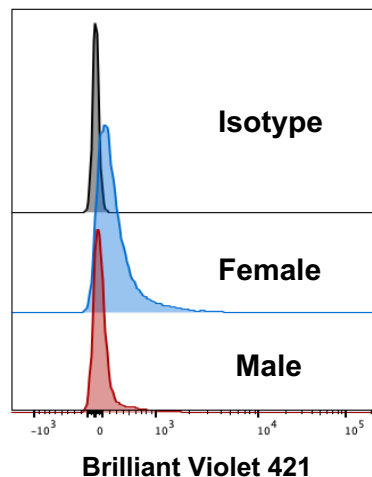
Step	Temperature (°C)	Time	Description
0	99	–	Lid temperature
1	94	2 min	Initial polymerase activation
2	94	20 sec	Denaturation
3	60	15 sec	Annealing
4	68	10 sec	Extension
<b>Repeat steps 2–4 for 10 cycles</b>			
5	94	15 sec	Denaturation
6	60	15 sec	Annealing
7	72	10 sec	Extension
<b>Repeat steps 5–7 for 28 cycles</b>			
8	72	2 min	Final extension
9	10	∞ (hold)	Hold temperature



**Fig. S1: Genotyping of Pf4-Cre:RiboTag mice confirms dual allele presence for platelet-specific hemagglutinin-tag expression.** Representative PCR genotyping results from individual mice. **Top gel:** RiboTag floxed allele detected by a ~290 bp band. **Bottom gel:** Pf4-Cre transgene detected by a ~420 bp band. DNA was extracted from ear punch tissue, and PCR products were visualized. Pf4: Platelet factor 4, WT: Wild Type.



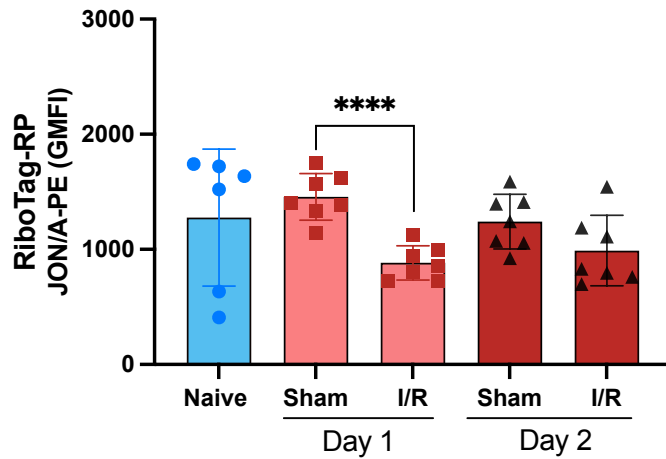
**Fig. S2: Specific detection of HA-tagged ribosomes in Pf4-Cre:RiboTag mouse whole blood using flow cytometry.** (Left) Single-cell events were gated based on FSC-A vs FSC-H profiles. (Middle) Histogram overlay of Brilliant Violet 421 fluorescence comparing unstained (gray), isotype control (blue), and anti-HA antibody (red)-stained samples. (Right) Quantification of geometric mean fluorescence intensity (GMFI) reveals a significant increase in anti-HA signal relative to controls. Data represent biological replicates ( $n = 3$ ); each point represents an individual mouse. Error bars = SD.



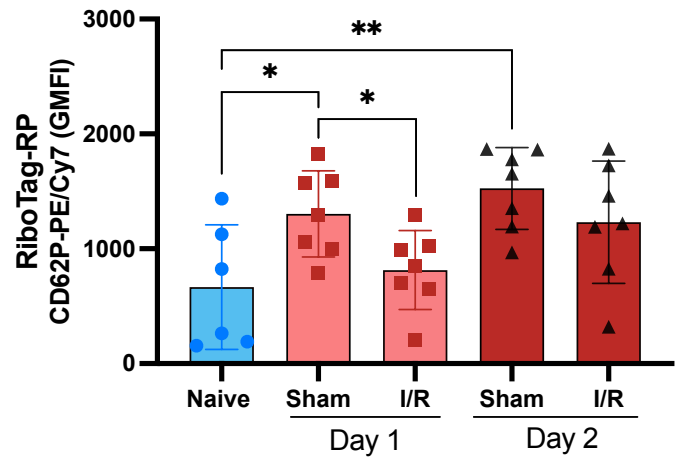
**Fig. S3: Comparison of RiboTag signal in total platelets between male and female Mice.** RiboTag (HA) expression was measured in the total platelet population from male and female Pf4-Cre:Rpl22-HA mice showing that the RiboTag system labels platelets consistently in both male and female mice.

# Reticulated Platelets Agonist-Responsiveness Dynamics Following I/R Injury

## Activated GPIIb/IIIa



## P-selectin



**Fig. S4: Flow cytometry analysis of surface activation marker expression on reticulated platelets (RP) at baseline and Days 1–2 after sham or I/R surgery.** Expression of activated GPIIb/IIIa (JON/A binding), and P-selectin (CD62P) was assessed under activated (PAR-4 stimulation) condition. Data are shown as geometric mean fluorescence intensity (GMFI), mean  $\pm$  SD ( $n = 6 - 7$  mice per group; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\*\* $p < 0.0001$ ).