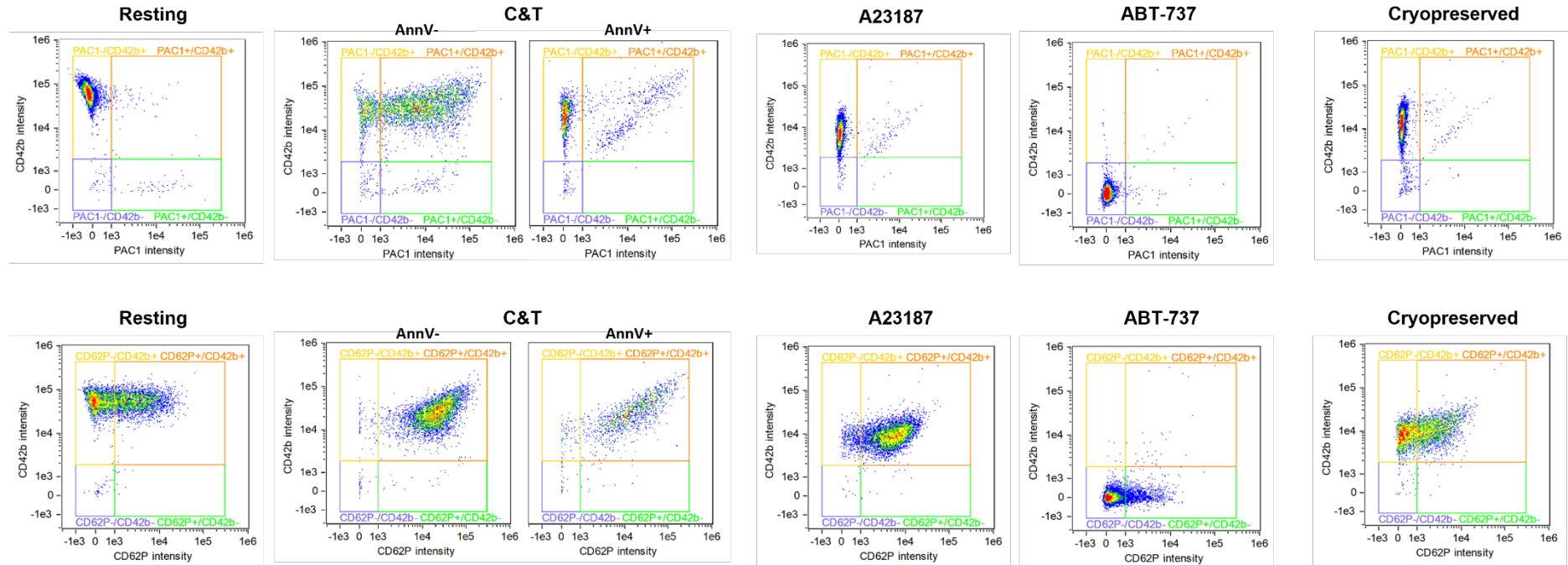


**Identification of platelet subpopulations in cryopreserved platelet components using multi-colour imaging flow cytometry**

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**Supplementary Fig. S1. Representative scatterplots of platelet subpopulations from the five treatment groups.** Fresh components were either unstimulated (resting), or stimulated with collagen and thrombin (C&T), A23187 or ABT-737. Cryopreserved platelet components were thawed and reconstituted in plasma. The platelets were distinguished based on brightfield area and aspect ratio intensity and then the subpopulations were defined based on annexin-V (AnnV), PAC1 and CD42b (upper panels) and annexin-V, CD62P and CD42b (lower panels). Representative images of the fluorescence profile of platelet population in the treatment groups are presented.

**Supplementary Table S1. Time course of phenotype following ABT-737 stimulation.**

ABT-737 treatment	% subpopulations							
	AnnV-				AnnV+			
	CD42b <sup>+</sup> CD62P <sup>-</sup>	CD42b <sup>+</sup> CD62P <sup>+</sup>	CD42b <sup>-</sup> CD62P <sup>-</sup>	CD42b <sup>-</sup> CD62P <sup>+</sup>	CD42b <sup>+</sup> CD62P <sup>-</sup>	CD42b <sup>+</sup> CD62P <sup>+</sup>	CD42b <sup>-</sup> CD62P <sup>-</sup>	CD42b <sup>-</sup> CD62P <sup>+</sup>
2 hours	0.4	0.2	5.7	1.7	1.8	2.4	70.2	17.2
4 hours	0.2	0.0	1.4	0.4	0.2	0.2	90.3	7.1
Subpopulation	<b>Resting</b>	<b>Aggregatory</b>	N/A	N/A	<b>Novel</b>	<b>Procoagulant</b>	<b>Apoptotic</b>	N/A

Shaded cells indicate the dominant phenotype(s) within the treatment group

N/A indicates a minority phenotype not associated with a subpopulation

Novel indicates a phenotype not aligned with the known subpopulations

**Supplementary Table S2. Antibodies and dyes used for imaging flow cytometry**

<b>Antibody/dye</b>	<b>Target</b>	<b>Manufacturer</b>	<b>Catalogue number</b>	<b>Clone</b>	<b>Stock concentration</b>	<b>Dilution prior to adding to staining tube</b>	<b>Volume added to 50 µL staining tube (µL)</b>
Annexin-V-APC	Phosphatidylserine externalisation	Biolegend, San Diego, CA, USA	640941	N/A	8 µg/mL	N/A	1
CD61-FITC	GPIIIa (surface glycoprotein)	Dako, Glostrup, Denmark	F0803	Y2/51	0.1 µg/mL	1/20	1
CD41/CD61-FITC	Activated GPIIb/IIIa (activation marker)	BD Biosciences, San Jose, CA, USA	340507	PAC-1	25 µg/mL	N/A	1
CD42b-PE	GPIbα (surface glycoprotein)	BD Biosciences, San Jose, CA, USA	555473	HIP1	25 µg/mL	N/A	1
GPVI-PE	GPVI (surface glycoprotein)	BD Biosciences, San Jose, CA, USA	565241	HY101	50 µg/mL	1/2	1
CD62P-FITC	Exposed P-selectin (activation marker)	BD Biosciences, San Jose, CA, USA	550866	AC1.2	50 µg/mL	N/A	1
TMRE	Mitochondrial membrane polarisation status	Abcam, Cambridge, United Kingdom	ab113852	N/A	1 mM	1/200	1

Abbreviations: allophycocyanin (APC); fluorescein isothiocyanate (FITC); no dilution was performed (N/A); phycoerythrin (PE); tetramethylrhodamine ethyl ester (TMRE).