

Supplementary Information

Piezo1 expression in neutrophils regulates shear-induced NETosis

Sara Baratchi ^{1,2,3}, Habiba Danish ³, Chanly Chheang ¹, Ying Zhou ¹, Angela Huang ¹, Austin Lai ¹, Manijeh Khanmohammadi ³, Kylie M Quinn ³, Khashayar Khoshmanesh ⁴, Karlheinz Peter ^{1,2}

¹ Baker Heart and Diabetes Institute, Melbourne VIC 3004, Australia

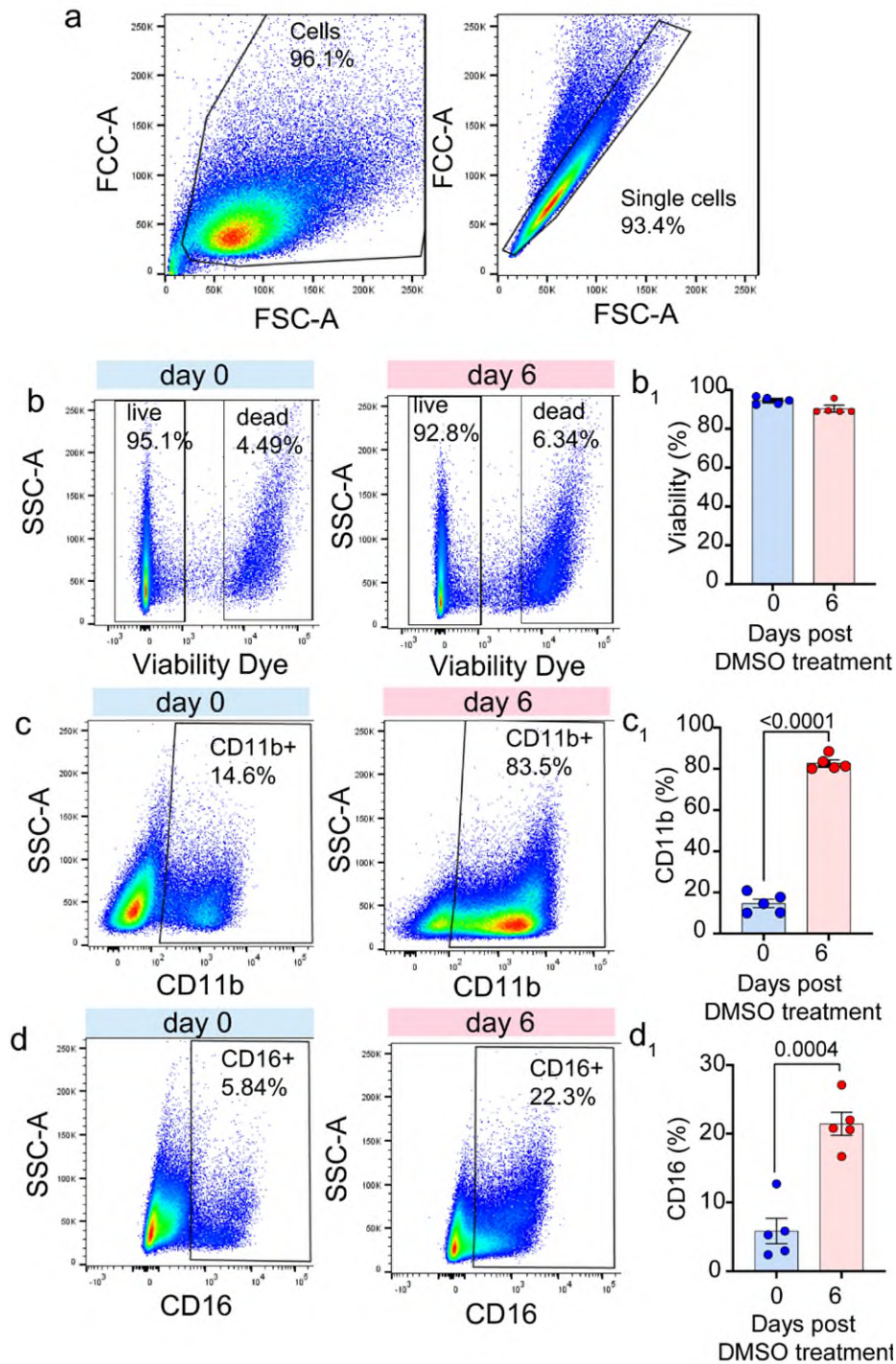
² Department of Cardiometabolic Health, University of Melbourne, Parkville VIC 3010, Australia

³ School of Health and Biomedical Sciences, RMIT University, Bundoora VIC 3082, Australia

⁴ School of Engineering, RMIT University, Melbourne VIC 3000, Australia

*** Corresponding author:**

A/Prof. Sara Baratchi: sara.baratchi@baker.edu.au



Supplementary Figure 1. Gating strategy and flow cytometry analysis of HL60 cells differentiation with DMSO. (a) Representative gating strategy for HL-60 cells. (b-d) Representative flow cytometry plots and (b₁-d₁) bar graphs of HL-60 cells before and post 6 days of differentiation assessed for viability (b-b₁), expression of (c-c₁) CD11b and (d-d₁) CD16. (b₁, c₁ and d₁: N=5 independent experiments and error bars show standard error, data is analysed with Student's t-test.

Supplementary Table 1. List of antibodies used in this study.

Name	Supplier name	Catalogue number	Lot number	Dilutions	Clone
PE anti-mouse/human CD11b	Biolegend	101208	B338668	1/100	<u>M1/70</u>
Alexa Fluor® 647 anti-human CD16	Biolegend	302020	B364586	1/100	3G8
Alexa Fluor® 488 anti-human CD11a	Biolegend	301216	B243729	1/100	HI111
PE Mouse Anti-Human CD62P	BD Bioscience	348107	3299627	1/100	AC1.2
Anti-Histone H3 (citrulline R2 + R8 + R17)	Abcam	Ab5103	1042692-1	1/300	Rabbit Polyclonal
Anti-Piezol	Novus Biologicals	NBP1-78537	D137790	1/100	Rabbit Polyclonal
Anti-GAPDH (H-12)	Santa Cruz Biotechnology	Sc-166574	C0217	1/2000	Mouse Monoclonal
Alexa Fluor™ 488 Goat-anti-Rabbit IgG (H+L)	Invitrogen	A11034	2379999	1/1000	-
m-IgGκ BP-HRP	Santa Cruz Biotechnology	Sc-516102	F2718	1/5000	-
mouse anti-rabbit IgG-HRP	Santa Cruz Biotechnology	Sc-2357	B0221	1/5000	-

Supplementary Table 2. List Dyes used in this study.

Dyes	Supplier name	Catalogue number	Lot number	Dilutions
Fluo-4, AM, cell permeant	Invitrogen	F14201	2591138	1/1000
Deoxyribonuclease I, Alexa Fluor™ 488 Conjugate	Invitrogen	D12371	2541660	1/500
Phalloidin–Atto 565	Merck	94072	BCCK1807	1/1000
Fixable Viability Dye eFluor™ 780	Thermofisher Scientific	65-0865-18	2633408	1/1000
DAPI stain	Cell Signalling	4083s	-	1/1000