

1 **Supplementary Information**

2
3 **Megakaryocytes contain extranuclear histones and may be a source of platelet-associated**
4 **histones during sepsis.**

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17 Supplemental Methods and Materials

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20 **Methods and Materials**

21

22 **Cell culture**

23 Two sources of megakaryocytes were used for these experiments: Cord blood-derived CD34⁺
24 cells and a megakaryoblastic cell line (Meg-01). Cord blood CD34⁺ stem cells were purchased
25 and cultured in StemSpanII media with the megakaryocyte supplemental cytokines, according to
26 the culture and differentiation protocols from Stemcell Technologies (Stemcell Technologies,
27 Vancouver, BC, Canada). The Meg-01 cells was purchased and cultured in RPMI with 10% FBS,
28 according to the standard culture protocols from ATCC (American Type Culture Collection,
29 Manassas, VA). All cells were incubated in sterile conditions at 37°C.

30

31 **Platelet and white blood cell isolation**

32 For the evaluation of blood cells for the presence of histones, blood was acquired from Research
33 Blood Components, LLC (Boston, MA). As a positive control for histone staining, neutrophil
34 isolation was performed. For this, blood was collected in ACD BD Vacutainer tubes (Becton
35 Dickinson, Franklin Lakes, NJ, USA). Neutrophils were isolated from whole blood using a
36 negative-selection protocol. In brief, neutrophils were isolated using a density gradient with
37 HetaSep (Stemcell Technologies) and then purified with EasySep Human Neutrophil Kit
38 (Stemcell Technologies), following the manufacturer's protocol. Neutrophil purity was assessed
39 to be >98%, and cell count was performed using a hemocytometer. Neutrophils were
40 subsequently resuspended in IMDM with 20% FBS (Thermo Fisher Scientific). . Platelets were
41 prepared from blood collected into 2.9 ml 3.2% trisodium citrate Vacutainer tubes (Sarstedt,
42 Numbrecht, Germany). Platelet-rich plasma (PRP) was first prepared by centrifugation of the

43 whole blood at 210 g, 22°C, for 20 min. The PRP supernatant was gently pipetted into a new
44 tube and 20% volume ACD solution was added (Boston Bioproducts, Ashland, MA, USA) and
45 then centrifuged at 1900 g, 22°C, for 10 min. The platelet-poor plasma (PPP) was removed,
46 leaving a platelet pellet which was resuspended in HEPES-Tyrode buffer with 20% volume ACD
47 solution (Boston BioProducts).

48

49 **Immunofluorescence Imaging**

50 Both Meg-01 and cord-blood derived MKs (CB MKs) were fixed and stained for the evaluation
51 of cellular histone localization using immunofluorescence staining. Platelet pellets were prepared
52 as described in the 'Blood cell sample preparation' section above. CB MKs, at day 14 of
53 differentiation, and platelet pellets were fixed with 4% paraformaldehyde for 30 minutes and
54 then concentrated onto a poly-lysine coated slide using the Cytospin 4 cytocentrifuge (Thermo
55 Fisher Scientific), for 5 minutes at 1250 rpm, rinsed once with Deionized (DI) water and stored
56 at -80°C until staining and evaluation. For the LPS-stimulation of Meg-01 cells, Meg-01 cells
57 were co-incubated with various concentrations (30 pg/mL and 3 ug/mL) of *E. coli*
58 lipopolysaccharide (LPS) (O111:B4; Sigma Aldrich, St Louis, MO), for 60 minutes at 37°C on
59 poly-lysine coated slides (Sigma Aldrich, St Louis, MO, USA) and then fixed with 4%
60 paraformaldehyde (Santa Cruz Biotechnology, Dallas, TX, USA). The slides were then rinsed
61 with DI water and stored at -80°C until staining and evaluation. For staining, the slides were
62 thawed at room temperature and blocked with 5% donkey serum (Jackson ImmunoResearch) for
63 2 hours. The slides were rinsed three times with PBS and then treated with various combinations
64 of the following primary antibodies for two hours at room temperature: rat anti-Histone H3
65 (phospho S28) antibody (HTA28; Abcam, Cambridge, MA, USA) at 1:500, rabbit anti-Histone

66 H4 (39270; Active Motif, Carlsbad, Ca, USA), rabbit anti-Histone H2A.X (Active Motif), rabbit
67 anti-Histone 3 (ab1791; Abcam, Cambridge, MA, USA), and mouse anti-Histone 3 (96C10; Cell
68 Signaling Technology, Danvers, MA, USA) at 1:200. The slides were then rinsed three times
69 with PBS and incubated with various combinations of the following secondary antibodies: mouse
70 anti-human CD41 (GPIIb) 488 or 647 (Biolegend, San Diego, Ca, USA) at 1:250, donkey anti-
71 rabbit 488, donkey anti-rat 647, and donkey anti-mouse 568 (Life Technologies) at 1:500 for 30
72 minutes at room temperature. Slides were then rinsed three times with PBS and covered with
73 Vectashield antifade mounting medium with DAPI (Vector Labs, Burlingame, Ca, USA). The
74 cells were then imaged with one of two fluorescent microscopes: Life Technologies EVOS FL
75 (Thermo Fisher Scientific) or Nikon Eclipse 90i microscope (Nikon Instruments Inc., Melville,
76 NY).

77

78 **Histone-2B BacMam transfection of Meg-01 cells**

79 Meg-01 cells were transfected with GFP-Histone 2B (H2B) for localization of intracellular
80 histone. Meg-01 cells were transfected with CellLight Histone 2B-GFP (BacMam 2.0,
81 ThermoFisher Scientific) at 30 uL/mL for 48-72 hours at 37°C. Cell were then labeled with
82 MitoSox Red mitochondrial superoxide indicator (ThermoFisher Scientific) at 1:1000 Hoechst at
83 1:2000 for 30 minutes. Cells were pelleted at 1,900 x g for 10 minutes and rinsed twice with
84 PBS. Cells were then concentrated onto a poly-lysine coated slide using the Cytospin 4
85 cytocentrifuge (Thermo Fisher Scientific), for 5 minutes at 1250 rpm. Slides were rinsed with DI
86 water, cover slipped, and imaged on an EVOS fluorescent microscope.

87

88 **Histone purification**

89 Histones were extracted from both isolated platelet pellets and Meg-01 cells for histone
90 quantification. For sample preparation, venous whole blood was collected in acid citrate dextrose
91 (ACD) tubes (Research Blood Components LLC, Boston, MA, USA). Platelet pellets were
92 isolated as described in the section above, 'Blood cell sample preparation'. Meg-01 cells were
93 pelleted down at 1900 x g for 10 minutes. For histone purification the Active Motif Histone
94 Purification Mini Kit (Active Motif, Carlsbad, CA) was utilized per the manufacturer's
95 instructions. Briefly, platelet and cell pellets were washed twice with hepes-tyrode buffer and
96 20% acid citrate dextrose buffer (Boston BioProducts, Boston, MA, USA). Pellets were then re-
97 suspended in ice-cold Extraction Buffer. Samples were homogenized in the Extraction Buffer for
98 30 minutes and then left overnight at 4°C. The samples were then spun at maximum speed
99 (18,000 x g) in a microfuge for 5 minutes at 4°C. The supernatant, containing the crude histones,
100 was then transferred to a new tube and neutralized with 5X Neutralization Buffer to a pH of 8.
101 One aliquot of crude histones was saved for downstream analysis at -80°C. Spin columns
102 provided by the kit were then equilibrated and the crude histones along with the Elution Buffer
103 were run through the columns at 500 x g for 3 minutes. One aliquot of the eluted histones was
104 saved for downstream analysis at -80°C. Histones were then precipitated with perchloric acid.
105 Briefly, histones were precipitated overnight at 4°C with 4% perchloric acid. The histones were
106 then spun down at maximum speed for 1 hour at 4°C. The pellet was washed with 1 mL cold 4%
107 perchloric acid, followed by two washes in ice cold acetone with 0.2% HCl, and then twice in ice
108 cold acetone. The resulting pellet was air dried for 10 minutes and then re-suspended in sterile
109 water. All histones and aliquots were stored at -80°C until the samples were analyzed.

110

111 **Histone quantification**

112 Histone quantification was performed using two methods: absorbance measurements and bead-
113 based ELISAs. For the absorbance measurements, both total protein and histone-specific
114 absorbances were performed as follows using the DS-11 Spectrophotometer (DeNovix Inc.,
115 Wilmington, DE, USA): 230 nm for total protein, 280 nm for histone quantification with the
116 following molecular weight extinction coefficients, as recommended by Active Motif (**Supp.**
117 **Table 1**). Histones purified from calf thymus (Sigma-Aldrich, St. Louis, MO, USA) were used
118 as a positive control at 2 mg/mL. For histone quantification using ELISAs the Luminex Histone
119 H3 PTM Multiplex Assay (Active Motif) was used according to the manufacturer's protocol. For
120 sample preparation, serial dilutions of Meg-01 and platelet pellet purified histones were
121 prepared. For the positive control, the purified histone 3 protein that was provided with the kit
122 was used in this assay. Both total Histone 3 (H3) and H3 post-translational modifications
123 (PTSMs) were evaluated for each sample (**Supp. Table 2**). Briefly, the samples were co-
124 incubated with multiple magnetic beads, which were conjugated to antibodies targeted to specific
125 H3 PTSMs, on a shaker plate for 2 hours. The beads were then washed 3 times, incubated with
126 biotinylated anti-H3 antibodies on a shaker plate for 30 minutes, washed 3 times and then
127 incubated with streptavidin-phycoerythrin for 30 minutes, followed by a final set of
128 washes. Samples were read by using a Flexmap 3D (Luminex Corp., Austin, TX). The standard
129 curve was generated by using 5-parametric-curve fitting with platform-specific software
130 (xPONENT Software Solutions, Luminex). Samples were run in duplicate on each plate for
131 quality control.

132

133 **Transmission electron microscopy and immune-gold labeling**

134 For transmission electron microscopy, cells were fixed in 2.5% gluteraldehyde, 3%
135 paraformaldehyde with 5% sucrose in 0.1M sodium cacodylate buffer (pH 7.4), scraped, pelleted
136 in a small eppendorf tube. Wash with 0.1M sodium cacodylate buffer 3x, post fixed in 1% OsO₄
137 in veronal-acetate buffer 1 hour. The pellet was stained overnight with 0.5% uranyl acetate in
138 veronal-acetate buffer, dehydrated and embedded in Embed 812 resin. Sections were cut on a
139 Leica Ultracut UCT microtome with a Diatome diamond knife at a thickness setting of 50 nm,
140 stained with uranyl acetate, and lead citrate. The sections were examined using a FEI Tecnai
141 spirit at 80KV.

142
143 For the immunogold labeling protocol, the protocol was as follows. (21) The cells were fix
144 mildly Using PLP (paraformaldehyde / lysine / sodium periodate) fixative for 4 hours. Cells were
145 pelleted and trimmed into small (<1mmsq) blocks. Infused with a cryo-protectant for at least one
146 hour (PVP/ sucrose). Blocks were mounted onto cryo-pins, and snap frozen in liquid nitrogen.
147 Ultrathin sections were cut at -110 degrees C with a Leica UC7 equipped with a FC7 cryo-stage
148 using a glass knife, and immunolabeled, stained and embedded using the Tokuyasu technique.
149 For the immunolabeling, rabbit anti-histone 4 antibody (39270; Active Motif) was used. The
150 material was examined using a FEI techni Spirit biotwin.¹

151

152 **Patient sample collection**

153 Discarded venous blood from patients diagnosed with sepsis, according to their medical records,
154 was collected and evaluated for platelet-associated histones in accordance with the collection
155 protocol approved by the Institutional Review Board (IRB protocol numbers, MGH No:
156 2014P002087; MIT No:150100681R001). Patient consent was not required for sample collection

157 due to the use of discarded blood samples and all methods were carried out in accordance with
158 relevant guidelines and regulations. In brief, as blood was collected in EDTA tubes as part of
159 normal clinical treatment, once the clinical laboratory testing was completed, an aliquot of this
160 blood was used for experimental analysis. No blood draws and no extra blood tubes were
161 collected to complete this study. A patient was categorized as having sepsis when one of the
162 diagnoses for the patient was ‘sepsis’ according to their medical record and when there was a
163 confirmed infection via positive culture as part of their medical diagnosis. Twenty-one patient
164 samples were evaluated with 16 sepsis-positive samples (age 36-85 yrs., 4 females, 12 males)
165 collected from the Massachusetts General Hospital (Boston, MA) (**Supp. Table 6**) and 5 healthy
166 control samples purchased from Research Blood Components (Research Blood Components,
167 LLC, Boston, MA), age and sex not known. Platelet pellets were isolated as described in the
168 ‘Platelet isolation’ section from venous blood collected in vacutainer EDTA tubes and then
169 frozen at -80°C until analyzed. Flow cytometry was performed as described below, in the ‘Flow
170 cytometry’ section. For the assessment of histone expression, platelet pellets were either treated
171 with 4% paraformaldehyde fixation with 0.1% Triton™ X-100 or with 4% paraformaldehyde
172 alone. The pellets were washed prior to staining and evaluating. To assess for the effect of
173 freeze-thawing on the patient platelet pellets, one set of control samples (n=5) was divided into
174 two aliquots and one was run “fresh” without freeze-thawing and the other was freeze-thawed.
175 Both samples were pre-treated with 4% paraformaldehyde fixation with 0.1% Triton™ X-100
176 permeabilization. One set of samples was then frozen at -80°C. For the sepsis sample evaluation,
177 platelet pellets were pre-treated with 4% paraformaldehyde fixation with 0.1% Triton™ X-100
178 permeabilization, pellets were stored at -80°C until ready to evaluate. They were then thawed and

179 washed prior to staining and evaluation. Samples were evaluated as described in the section
180 below, 'Flow cytometry'.

181

182 **Flow cytometry**

183 Flow cytometry was performed to evaluate cells and platelets for histone expression. Cultured
184 Meg-01 cells, isolated platelets, and whole blood were fixed with 4% paraformaldehyde with or
185 without 0.1% Triton™ X-100 detergent (Sigma-Aldrich) for permeabilization for a total of 10
186 minutes. For the whole blood samples, platelets were isolated as described above in the 'Platelet
187 Isolation' section prior to fixing. After fixing, samples were then pelleted down at 1,900 x g for
188 10 minutes and re-suspended in PBS. Cells were first incubated with primary, unconjugated
189 antibodies, rabbit anti-Histone 3 (ab1791; Abcam) or rat anti-Histone H3 (phosphoS28) antibody
190 (HTA28; Abcam) at 1:400 for 20 minutes and were then washed twice with PBS. Cells were
191 then stained with a combination of conjugated primary antibodies and secondary antibodies for
192 15 minutes, including: mouse anti-human CD41 PacBlue, mouse anti-human CD45 594, mouse
193 anti-human CD162 PE, mouse anti-human CD62P PE, mouse anti-human CD61 FITC
194 (Biolegend, San Diego, Ca), and goat anti-rabbit 488 or donkey anti-rat 647 (Thermo Fisher
195 Scientific, Waltham, MA) at 1:200 for 15 minutes, with the exception of CD41 which was at a
196 concentration of 1:100 for 15 minutes. Cells were then stained with Draq5 (Thermo Fisher
197 Scientific, Waltham, MA) at a concentration of 1:10000 for 5 minutes. Data was obtained
198 through the Amnis ImageStreamX Mark II imaging flow cytometer and INSPIRE Software
199 (EMD Millipore, Billerica, MA). The accompanying IDEAS Software was used to perform data
200 analysis. Data is reported as the percent of the total cell population that stained positive for the
201 specific marker.

202

203 **Statistical analysis**

204 Platelet were evaluated both as ‘singles’ and as ‘clusters’ to parse between the more activated

205 platelets that are more likely to be present as clusters and individual platelets in circulation.

206 Statistics were performed using both Excel (Microsoft, Redmond, WA, USA) and GraphPad

207 Prism Software (GraphPad Software, Inc.). Either one-way ANOVA or student t-tests were

208 performed to compare between conditions. Pearson’s correlation was used to determine whether

209 there was a correlation present between platelet phenotype and platelet count or white blood cell

210 count. A p-value of <0.05 was considered significant.

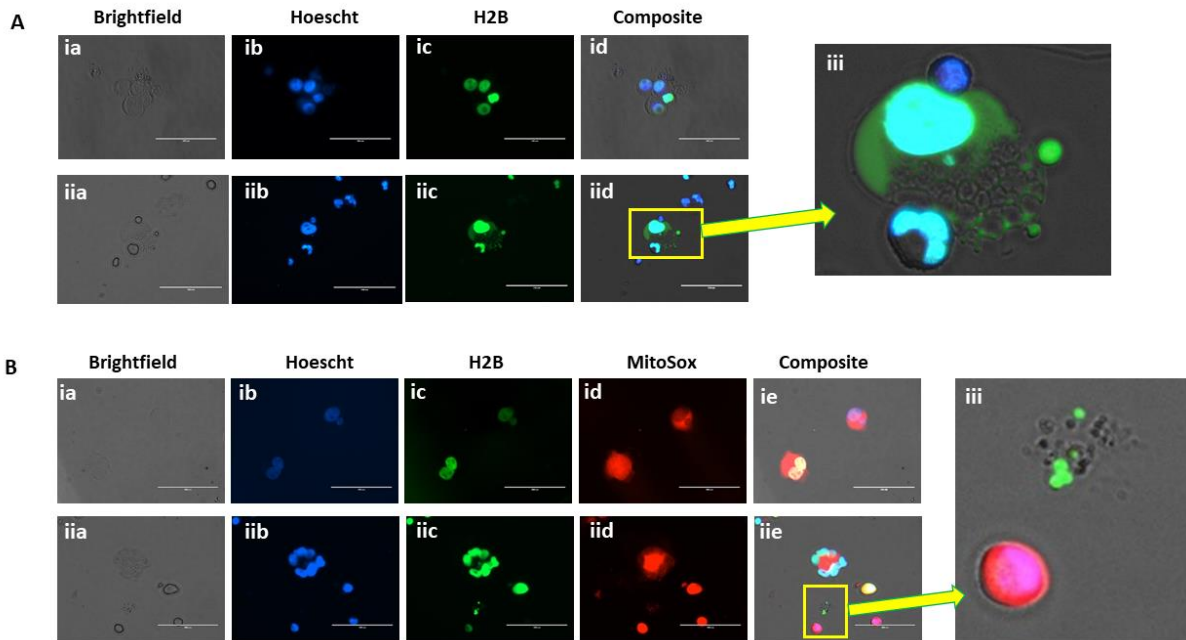
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212 **References:**

213 1. Tokuyasu K.T. Immunocytochemistry on ultrathin frozen sections. *Histochem. J.* **12**, 381-

214 403 (1980).

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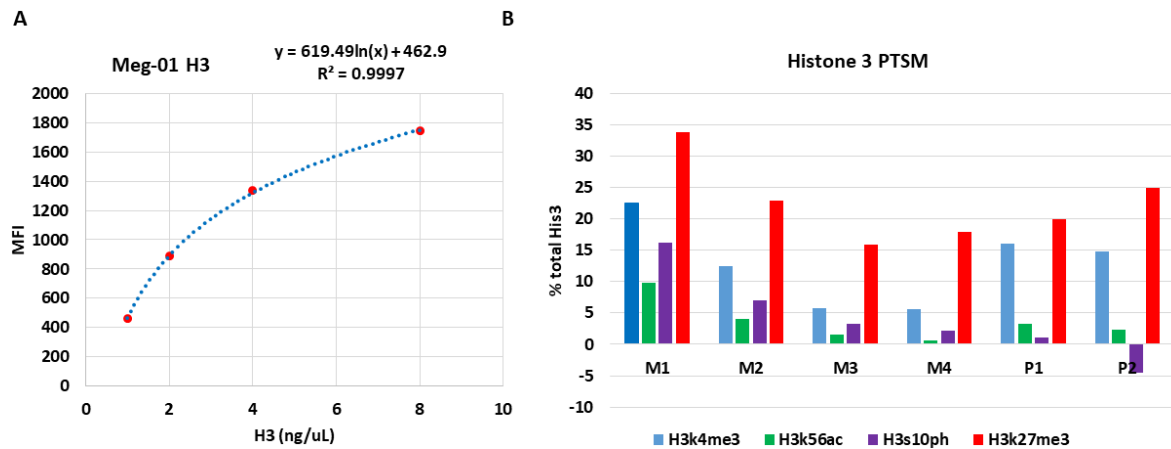
217 **Supplemental Figure 1: GFP-H2B expressing Meg-01 cells have extranuclear histones.**

218 Meg-01 cells were transfected with GFP-Histone 2B (H2B) BacMam to explore the cellular
 219 localization of H2B in megakaryocytes. (A) H2B was mostly observed to be restricted to the
 220 nucleus within Meg-01 cells (i); although, occasionally cells were noted to have some

221 extranuclear histones, such as the cells in the panel on the right (ii). (B) Cells were co-incubated
 222 with MitoSox red to show the cytoplasmic location of metabolically active mitochondria. While
 223 H2B was normally within the nucleus and within the boundaries of the cell membrane, isolated
 224 platelet-like particles with H2B expression were noted as well (ii).

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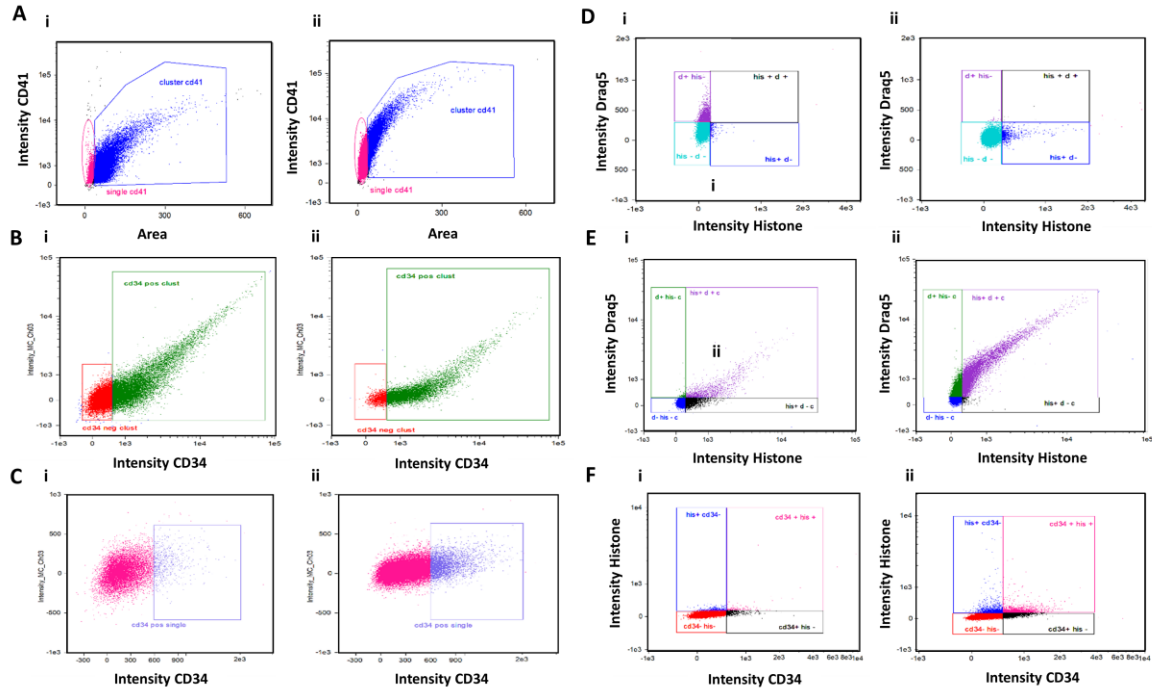
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228 **Supplementary Figure 2: Histone quantification using bead-based ELISA assays.**

229 Histones extracted and purified from Meg-01 cells and platelet pellets were quantified. (A) Meg-
 230 01 cell histone-extract serial dilutions were run on the ELISA assay. His3 concentration based on
 231 absorbance measurements were used to extrapolate the histone concentration input (ng/uL) in
 232 each dilution. These concentrations were then plotted against the mean fluorescence intensity
 233 (MFI) reading from the ELISA assay. The points were then fit with a logarithmic best-fit line,
 234 which has an R^2 value of >0.99 . (B) The Meg-01 and platelet histone extracts were also assessed
 235 for various H3 PTSMs. These are expressed in terms of % of total H3 based on MFI readings.
 236 M1, Meg-01 at a 1:250 dilution; M2, Meg-01 at a 1:500 dilution; M3, Meg-01 at a 1:1000
 237 dilution; M4, Meg-01 at a 1:2000 dilution; P1, Platelet at a 1:25 dilution; P2, Platelet at a 1:50
 238 dilution.



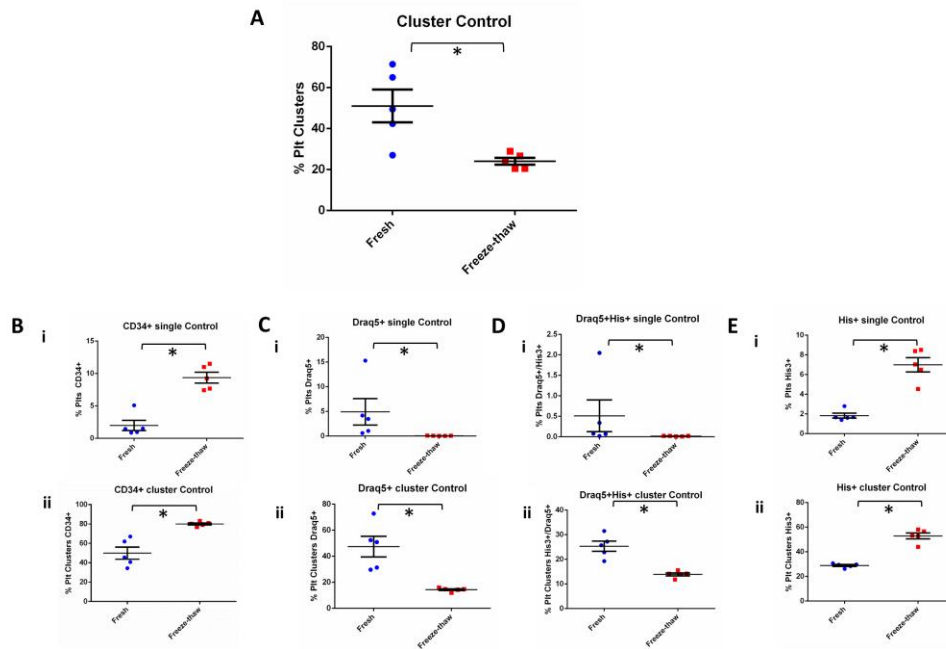
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240 **Supplementary Figure 3: Flow cytometry comparison of fresh versus freeze-thawed**
 241 **platelets.**

242 Flow cytometry was performed on both fresh and freeze-thawed platelets, in order to control for
 243 the effects of storage conditions on the patient platelet analysis. (A-F) Representative scatter
 244 plots of flow cytometry results comparing fresh platelets (i) and freeze-thawed platelets (ii). (A)
 245 CD41 compared to area of cell identifies single versus clusters of CD41 platelets; (B) CD34
 246 intensity in CD41 positive clusters; (C) CD34 intensity in CD41 positive singlets; (D) Intensity of
 247 Draq5 and Histone in CD41 positive singlets; (E) Intensity of Draq5 and Histone in CD41
 248 positive clusters; (F) Intensity of CD34 and Histone in CD41 positive singlets.

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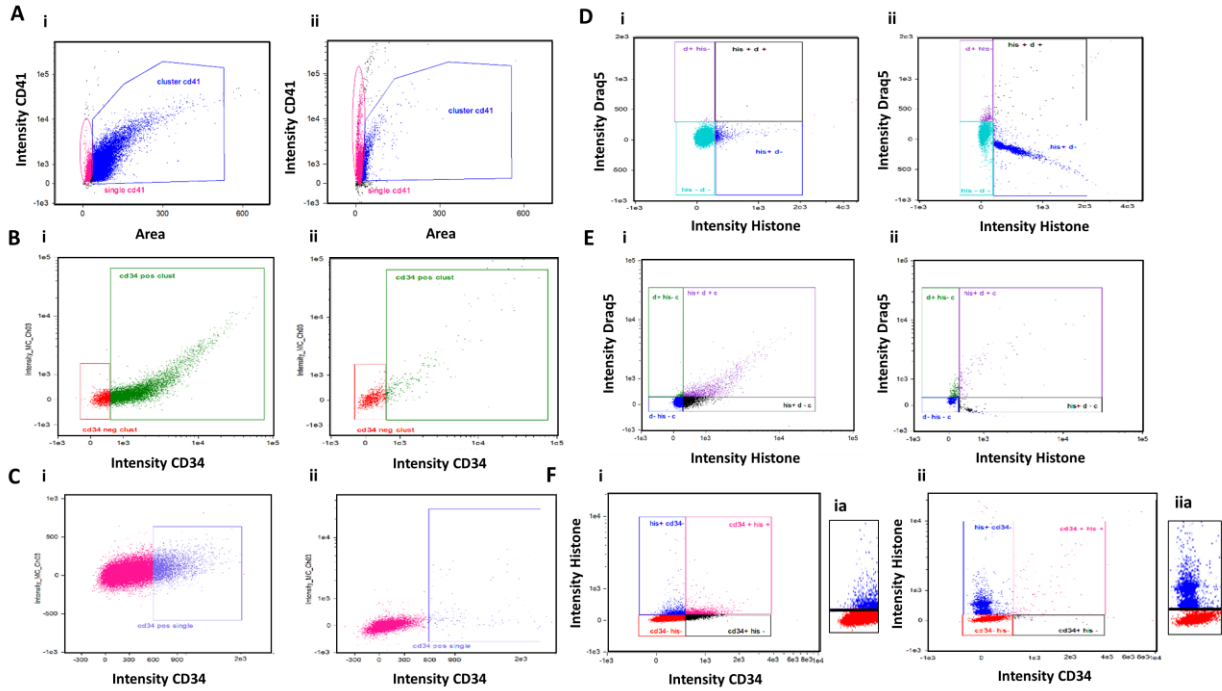
251

252 **Supplementary Figure 4: Flow cytometry comparison of fresh versus freeze-thawed**
 253 **platelets.**

254 Flow cytometry was performed on both fresh and freeze-thawed platelets, in order to control for
 255 the effects of storage conditions on the patient platelet analysis. (A-E) Scatter plots with mean
 256 and standard error bars comparing fresh compared to freeze-thawed platelets for both single
 257 platelets (i) and clustered platelets (ii). Student unpaired t-test was performed, with significance
 258 defined as $p \leq 0.05$ according to a paired student t-test for all samples.

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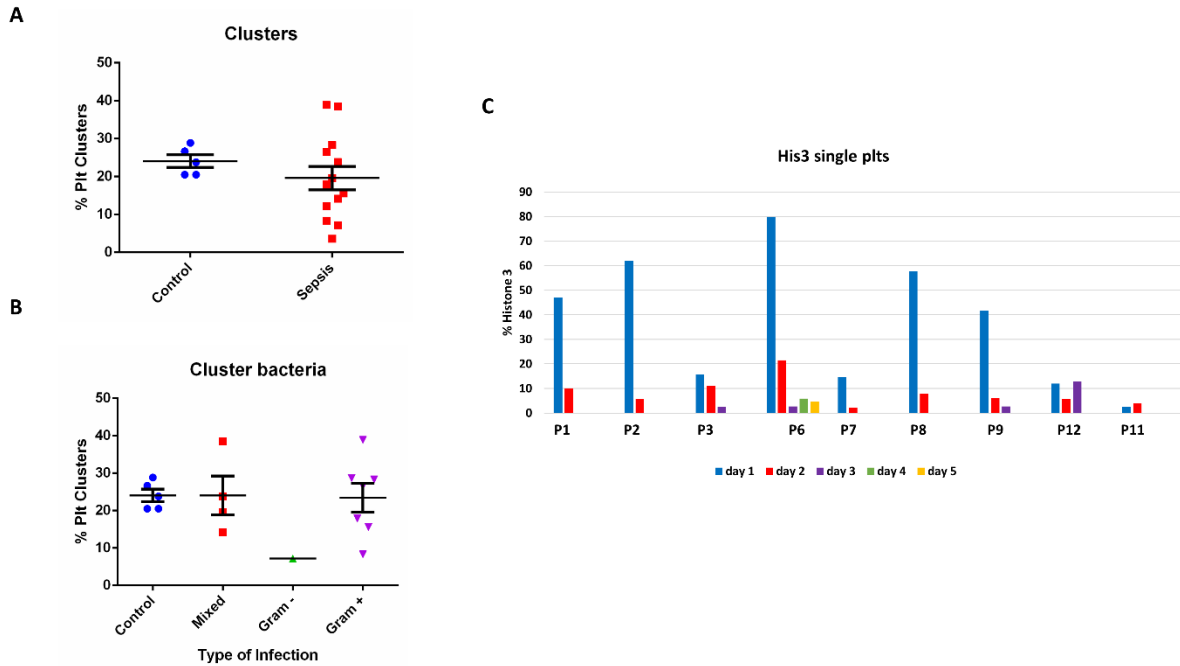


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262 **Supplementary Figure 5: Flow cytometry analysis of platelets from patients diagnosed with**
 263 **sepsis.**

264 Flow cytometry was performed on platelets isolated from patients patient with and without
 265 sepsis. (A-F) Representative scatter plots of flow cytometry results comparing platelets from
 266 control patients (i) and platelets from patients diagnosed with sepsis (ii). (A) CD41 compared to
 267 area of cell identifies single versus clusters of CD41 platelets; (B) CD34 intensity in CD41
 268 positive clusters; (C) CD34 intensity in CD41 positive singlets; (D) Intensity of DraQ5 and
 269 Histone in CD41 positive singlets; (E) Intensity of DraQ5 and Histone in CD41 positive clusters;
 270 (F) Intensity of CD34 and Histone in CD41 positive singlets. A magnified example of single
 271 platelet-associated His3 signal is represented for both the control patient (Fia) and the sepsis
 272 patient (Fiia)

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276 **Supplementary Figure 6: Platelet phenotype is associated with the diagnosis of sepsis and**
 277 **the type of infection.**

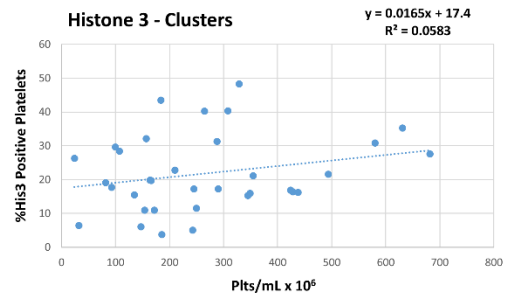
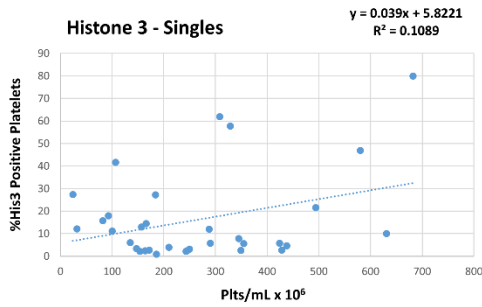
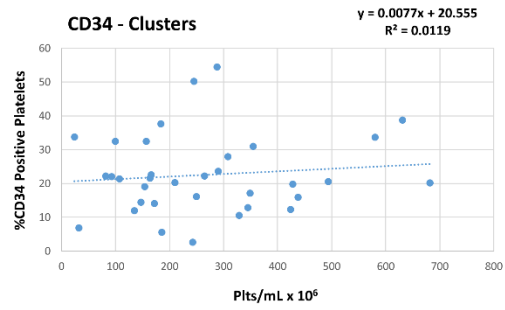
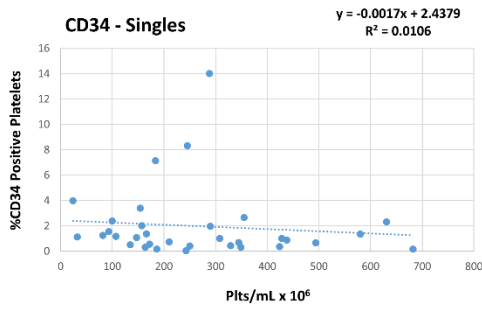
278 Flow cytometry was performed to evaluate platelet phenotype from patients diagnosed with
 279 sepsis. (A) Platelet phenotype was compared for patients with and without sepsis. (B) Platelet
 280 phenotype was also compared for patients with different types of infection: Gram +, Gram -, or
 281 Mixed. (C) Platelet were evaluated on multiple days of hospitalization. His3⁺ platelets appeared
 282 to be highest in patients with acute sepsis and on day 1 of evaluation and decreased as the patient
 283 was treated (P1,2,3,6,7,8,9). Patients with chronic sepsis that had been in the hospital for a
 284 prolonged period did not have the same changes in His3⁺ platelets and these patients did not
 285 recover. For A, student t-test was performed and for B, one-way ANOVA testing was performed
 286 comparing the type of infection to the control group, with significance being defined as $p < 0.05$

287 (*) Statistics were not performed on the Gram – group as there was only one patient in this
288 group.

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294 **Supplemental Figure 7: Relationship of platelet count and PAH**

295 Flow cytometry of platelets from patients with sepsis were plotted against the platelet count. %

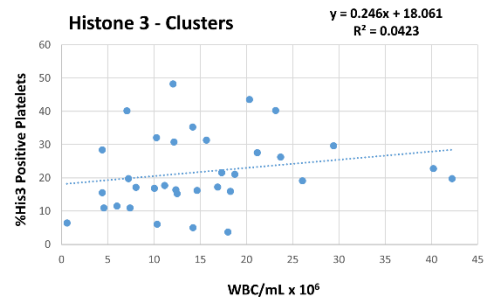
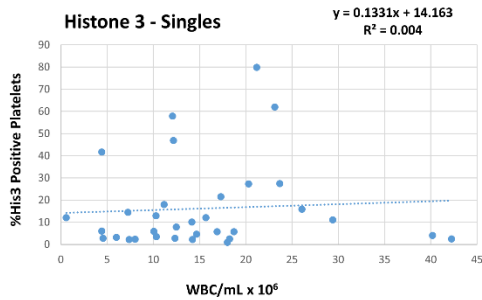
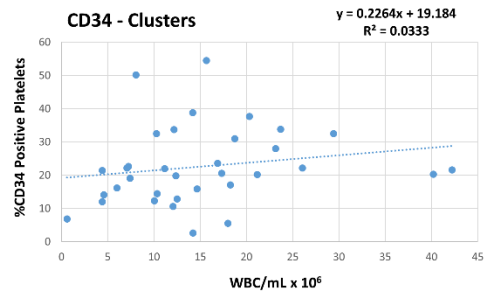
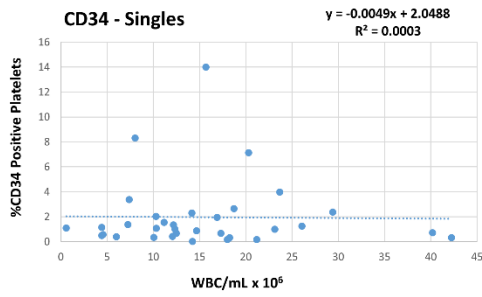
296 CD34 and % Histone 3-positive single platelets (A & B) and % CD34 and % Histone 3-positive

297 platelet clusters showed minimal to no relationship with patient platelet count. Best-fit line

298 (linear) is plotted.

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300



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302

303 **Supplemental Figure 8: Relationship of white blood cell count and PAH**

304 Flow cytometry of platelets from patients with sepsis were plotted against the white blood cell

305 count. % CD34 and % Histone 3-positive single platelets (A & B) and % CD34 and % Histone 3-

306 positive platelet clusters showed minimal to no relationship with patient white blood cell count.

307 Best-fit line (linear) is plotted.

308

309 **Tables:**

310

311 **Supplementary Table 1: Histone protein measurement.**

Histone	MW (kDa)	E/1000
Histone 2A	13.960	4.05
Histone 2B	13.774	6.07
Histone 3	15.273	4.04
Histone 4	11.236	5.40

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313

314 **Supplementary Table 2: Histone protein quantification**

Sample	Total Protein (OD) ¹	Total Protein ¹ (mg/mL)	H2A ² (mg/mL)	H2B ³ (mg/mL)	H3 ⁴ (mg/mL)	H4 ⁵ (mg/mL)
Calf thymus	0.57 ⁷	10.35 ⁶	2.81	1.8	3.3	1.84
Meg-01 cells	2.97 ⁷	7.07	1.76	1.4	2	1.12
Platelet 1	0.074	0.18	0.27	0.62	0.44	0.18
Platelet 2	0.064	0.15	0.36	0.18	0.34	0.29

1. 230 nm
2. 280 nm; MW (kDa), 13.960; E/1000, 4.05
3. 280 nm; MW (kDa), 13.774; E/1000, 6.07
4. 280 nm; MW (kDa), 15.273; E/1000, 4.04
5. 280 nm; MW (kDa), 11.236; E/1000, 5.40
6. Expected concentration is 10 mg/mL total histones
7. Sample diluted 1:10

317 **Supplementary Table 3: Histone post-translational modifications**

Histone Modification	Residue	Modification	Functions
H3k4me3	Lys4	Tri-methylation	Euchromatin, transcriptional activation
H3k56ac	Lys56	Acetylation	DNA damage repair, chromatin assembly
H3s10ph	Ser10	Phosphorylation	Mitosis, immediate early gene activation
H3k27me3	Lys27	Tri-methylation	Transcriptional silencing
H3s28ph	Ser28	Phosphorylation	Mitosis
Cit H3	N/A	Citrullination	Catalyzed by PAD4
H2A.X	N/A	N/A	DNA damage repair, response to double strand DNA breaks

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320 **Supplementary Table 4: Histone post-translational modification quantification**

Sample	Total H3 ¹	H3k4me3 ¹	H3k56ac ¹	H3s10ph ¹	H3k27me3 ¹
Positive Control ² (1:25)	3278.5	3360	2006	2170	2196
Blank	18	9	13	14	10
Meg-01 (1:250)	1760	401	185	296.5	598
Meg-01 (1:500)	1354	175.5	66	108	315
Meg-01 (1:1000)	909	60.5	27	43	151.5
Meg-01 (1:2000)	477	35	16	24	92
Platelet (1:25)	108	23.5	16	15	28
Platelet (1:50)	62	15.5	14	12	21

1. All measurements are provided as MFI (mean fluorescent intensity), with the exception of the LOD row.
2. Positive control of HeLa cell extract, estimated to be 0.5 mg/mL total protein; information provided by Active Motif.

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322

323 **Supplementary Table 5: Histone post-translational modification percentages**

Sample	Total H3 ¹	H3k4me3 ²	H3k56ac ²	H3s10ph ²	H3k27me3 ²
Meg-01 (1:250)	200.0 / 1970.9	22.5	9.9	16.2	33.8
Meg-01 (1:500)	100.0 / 2046.7	12.5	4.0	7.0	22.8
Meg-01 (1:1000)	50.0 / 1995.8	5.8	1.6	3.3	15.9
Meg-01 (1:2000)	25.0 / 1987.4	5.7	0.7	2.2	17.9
Platelet (1:25)	13.75 / 0.46 ³	16.1	3.3	1.1	20.0
Platelet (1:50)	12.75 / 0.42 ³	14.8	2.3	-4.5	25.0
LOD ⁴	2	30	7.8	8	3

1. Total input per well in ng / Concentration in units of ng/uL of stock protein
2. % of total H3 based on MFI
3. LOD, Limit of detection. Based on purified histones input. Total ng input.

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326 **Supplementary Table 6: Sepsis patient descriptive statistics**

Patient ID	Signalment (sex/age)	Infectious Organisms	Source of Infection	Bacterial Classification ¹
1	F/82	- <i>Klebsiella pneumoniae</i> - Influenza	- Urine	Gram -
2	M/36	- <i>Pseudomonas aeruginosa</i> ²	- Urine	Gram -
3	M/67	- <i>Enterococcus faecalis</i> - Yeast - <i>Staphylococcus aureus</i> - <i>Streptococcus pneumoniae</i>	- Lungs - Lungs - Lungs - Lungs	Gram +
4	M/73	- <i>Pseudomonas aeruginosa</i> - <i>Enterococcus faecium</i> - Yeast	- Lungs/Urine - Lungs - Lungs	Mixed
5	M/85	- <i>Enterococcus faecium</i> - <i>Candida albicans</i> - Coagulase negative <i>Staphylococcus</i> - Yeast	- Wound - Wound - Lungs - Lungs	Gram +
6	M/49	- <i>B-haemolytic Streptococcus A</i> - <i>Staphylococcus aureus</i> - <i>Aspergillus fumigatus</i> - <i>Enterococcus faecium</i> - Influenza	- Lungs - Lungs - Lungs - Lungs	Gram +
7	M/69	- <i>Streptococcus pneumoniae</i> - Multi-drug resistant <i>Staphylococcus aureus</i> - Influenza	- Lungs - Lungs - Blood - Blood	Gram +
8	M/71	- <i>Enterococcus faecalis</i> - <i>Enterobacter aerogenes</i> - <i>Corynebacterium</i>	- Blood - Lungs - Lungs	Mixed
9	M/62	- Multi-drug resistant <i>Staphylococcus aureus</i> - <i>Enterococcus aerogenes</i>	- Blood - Blood	Gram +
10	M/61	- <i>Staphylococcus aureus</i>	- Lungs	Gram +
11	M/77	- <i>Streptococcus intermedius</i>	- Pleural fluid	Gram -
12	M/59	- <i>Klebsiella pneumoniae</i> - <i>Proteus mirabilis</i> - <i>Enterococcus</i>	- Blood/Lungs - Blood - Blood	Mixed
13	F/76	- Metapneumovirus - No positive bacterial culture	- N/A	N/A
14	F/73	- <i>Escherichia coli</i> - <i>Staphylococcus epidermidis</i>	- Blood and Urine - Blood	Mixed
15	F/67	- <i>Escherichia coli</i> - <i>Staphylococcus epidermidis</i>	- Blood/Urine - Urine	Gram +
16	M/81	- <i>Enterococcus faecalis</i>	- Blood	Gram -

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329 1. Gram positive, Gram negative or mixed infection (Gram positive and Gram negative bacteria)

330 2. Culture was negative prior to blood sample collection

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