SUPPLEMENTARY APPENDIX

In vitro and in vivo effects of short-term cold storage of platelets in PAS-C

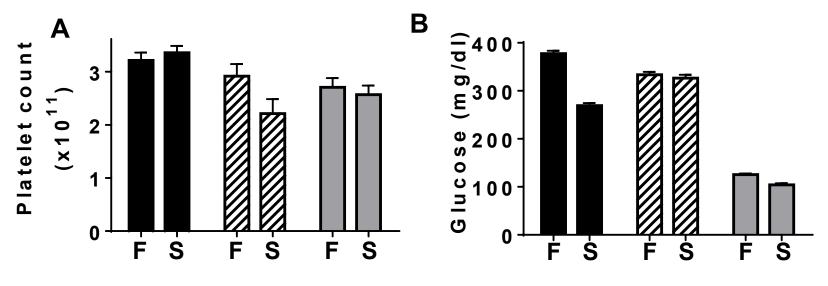
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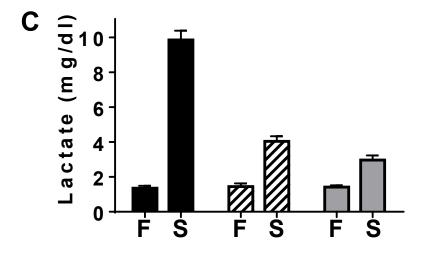
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Supplemental Figure 1





RT Plasma

4 °C Plasma

4 °C PAS/Plasma

F: Fresh

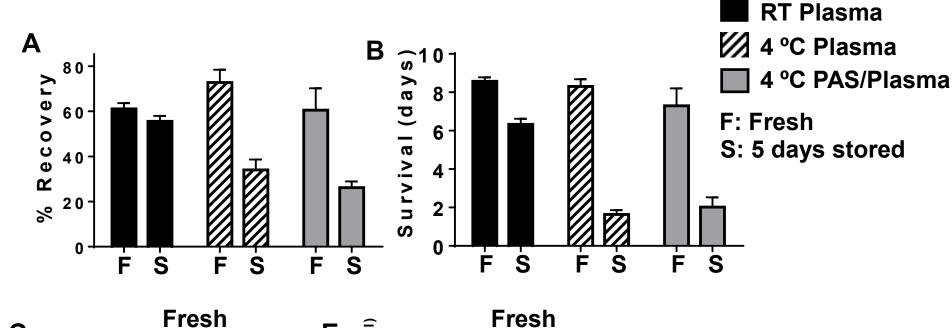
S: 5 days stored

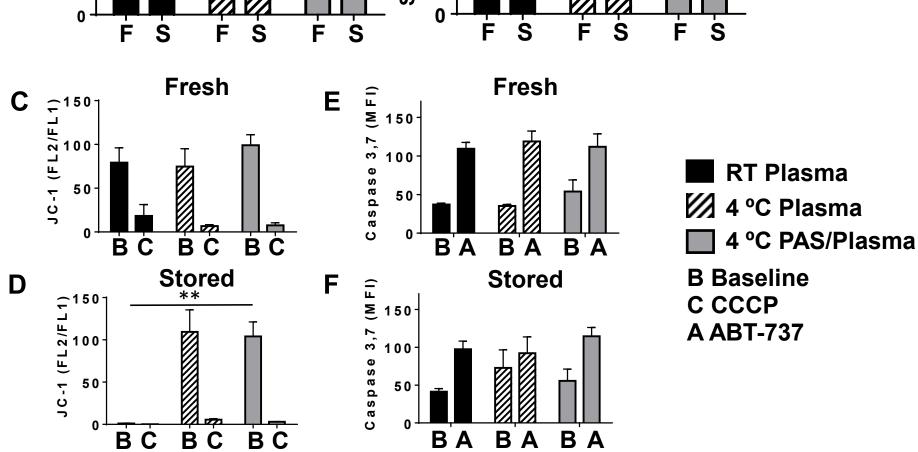
Supplemental Figure 2

C

В

В

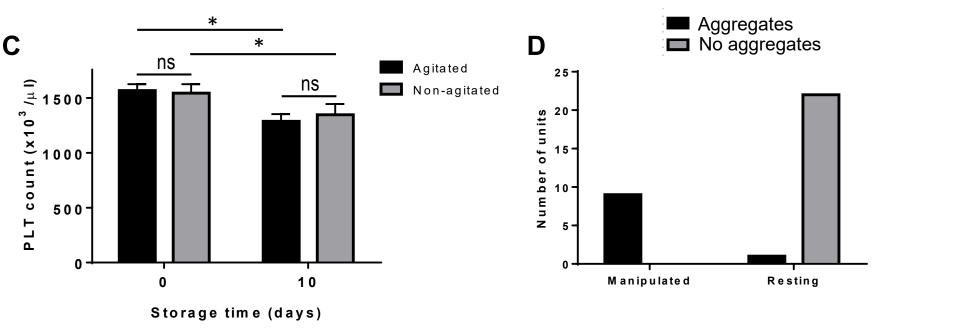




В

A

Supplemental Figure 3 RT Plasma Annexin V P-selectin 4 °C Plasma В Α 10 4 °C PAS/Plasma Annexin V (%) 60 8 F: Fresh D62P+ S: 5 days stored 6 40 4 20 C 2 0 S S S F F F F S S F S F



Supplemental Figure Legends

Supplemental Figure 1: In vitro platelet characteristics (absolute data)

P-RSP (Plasma, 22 °C, solid black bars), P-CSP (Plasma, 4 °C, diagonally striped bars), PAS-CSP (PAS-C/plasma, 4 °C, solid grey bars) were tested fresh (*F*) or after storage for 5 days (*S*). **(A)** Platelet count measured by ABX Hemanalyzer. (P-RSP n=18, P-CSP n=5, PAS-CSP n=6) **(B)** Glucose P-RSP n=6, P-CSP n=5, PAS-CSP n=6, and **(C)** Lactate measured by blood gas analyzer P-RSP n=6, P-CSP n=5, PAS-CSP n=6. Data shown as mean + SEM.

Supplemental Figure 2: In vivo platelet characteristics and in vitro apoptosis parameters (absolute data)

P-RSP (Plasma, 22 °C, solid black bars), P-CSP (Plasma, 4 °C, diagonally striped bars), PAS-CSP (PAS-C/plasma, 4 °C, solid grey bars) were tested fresh (*F*) or after storage for 5 days (*S*). (**A**) Recovery of transfused platelets after 2 hours, (P-RSP n=21, P-CSP n=5, PAS-CSP n=5) (**B**) survival of transfused platelets. Data shown as percentage of fresh mean + SEM, (P-RSP n=21, P-CSP n=5, PAS-CSP n=5). (**C**) Platelet mitochondrial membrane potential measured by JC-1 dye red (FL2) to green (FL-1) ratio tested on fresh platelets, (P-RSP n=9, P-CSP n=5, PAS-CSP n=6) (**D**) Platelet mitochondrial membrane potential measured by JC-1 dye red (FL2) to green (FL-1) ratio tested on 5 day-stored platelets (P-RSP n=7, P-CSP n=5, PAS-CSP n=5), (**E**) Caspase 3,7 activation measured by flow cytometry on fresh platelets (P-RSP n=5, P-CSP n=5, PAS-CSP n=6) (**F**) Caspase 3,7 activation measured by flow cytometry on 5 day-stored platelets (P-RSP n=5, P-CSP n=5). Data shown as percentage of fresh mean + SEM.

Supplemental Figure 3: In vitro activation (absolute data) and aggregate storage data

P RSP (Plasma, 22 °C, solid black bars), P-CSP (Plasma, 4 °C, diagonally striped bars), PAS-CSP (PAS-C/plasma, 4 °C, solid grey bars) were tested fresh or after storage for 5 days. **(A)** Platelet phosphatidylserine exposure, measure by Annexin V binding by flow cytometry (all groups n=4). **(B)** Platelet alpha-degranulation measured by P-selectin binding by flow cytometry (all groups n=4). **(C)** Platelets were stored at 4 °C for 10 days either with (black bars) or without agitation (grey bars), and the platelet count was measured by ABX. n=2, Data shown as mean ± SEM **(D)** Occurrence of visual aggregates (black bars) or no visual aggregates (grey bars) in resting or manipulated units during 4 °C storage. "Resting" included units without any interference during storage time (no sampling/rewarming) (n=23) (reference (11)). "Manipulated" included units with frequent sampling and inadvertent rewarming (n=9).