Supplementary data

Supplementary methods

RBC concentrates collection and storage

SAGM units were collected using a five-fold NPT blood bag system (Macopharma, Tourcoing, France) with LEUCOFLEX LCRD2 leukoreduction filter was used for collection, leukofiltration of whole blood and separation of plasma. RBC concentrates were obtained from routine blood center inventory (n=17) or were deemed not suitable for clinical use (n=18). In the latter case, blood was specifically donated for non-therapeutic use by donors with contraindications for allogeneic blood donation (e.g., recent tattoo or piercing, travel, dental care).
AS-3 units were collected using the Leukotrap® RC System with RC2D Filter (Haemonetics). The AS-1 unit was collected using BIOFLEX RC Red Cell Leukocyte reduction filter in PL2209 plastic bags (Fenwal). Both used the red cell filtration method.

Imaging flow cytometry analysis on human samples

Imaging flow cytometry using ImageStream X Mark II (AMNIS part of EMD Millipore) was performed to determine RBC dimensions and morphology by using brightfield images (60X magnification) processed with computer software (IDEAS v6.2, AMNIS). Focused cells and single cells were respectively selected using the features gradient RMS and aspect ratio versus area. Front views were selected and analyzed by the mask "Object" and the features of circularity, perimeter and area. At least 6000 front views of focused single RBC/condition were analyzed. SME proportion was determined independently for each donor, using the nadir of the bimodal frequency histograms as the gating boundary. Stored RBC were suspended at 1% hematocrit just before acquisition (INSPIRE software, AMNIS) in a Krebs-albumin solution (Krebs-Henseleit buffer, Sigma-Aldrich) modified with 2 g of glucose, 2.1 g of sodium bicarbonate, 0.175 g of calcium chloride dehydrate, and 5 g of lipid-rich bovine serum albumin (Albu-MAX II, Thermo Fisher Scientific) for 1 L of sterile water (pH 7.4).

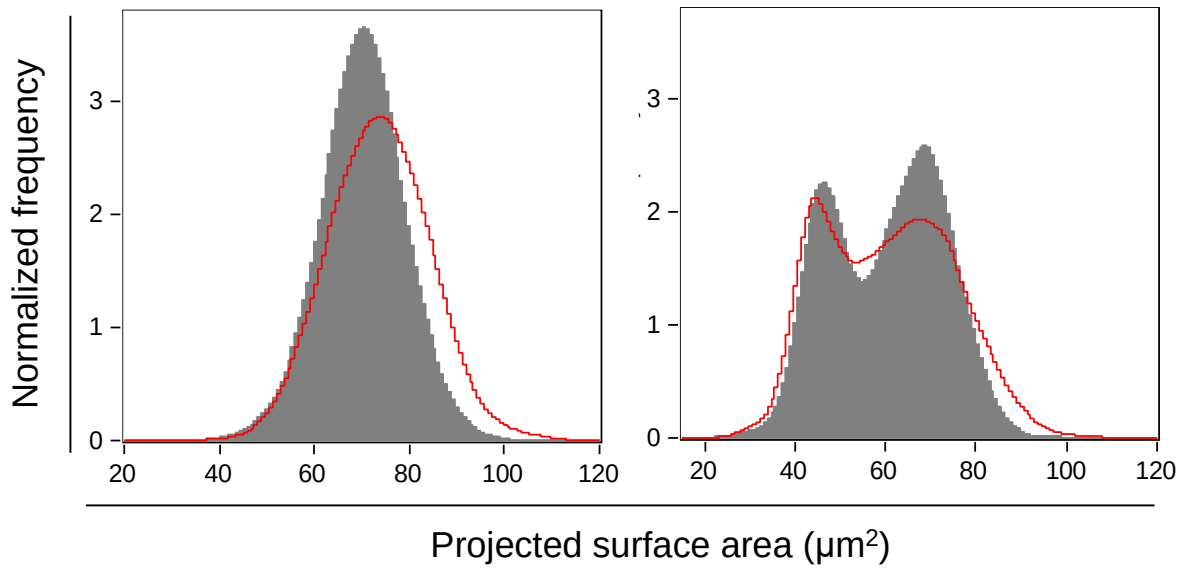
In vivo erythrophagocytosis

Spleen and liver cells were dissociated mechanically using dedicated dissociation kits and gentleMACS dissociator (Miltenyi Biotec). Bone marrow cells were obtained by flushing tibia and femur with PBS/2% FBS/EDTA 2mM. RBC were removed from the suspension using ACK lysing buffer (Invitrogen). Cells were first incubated with anti-mouse CD16/CD32 clone 2.4G2 to block IgG receptors (BD). Then, cells were stained at 4°C using a panel of antibodies.

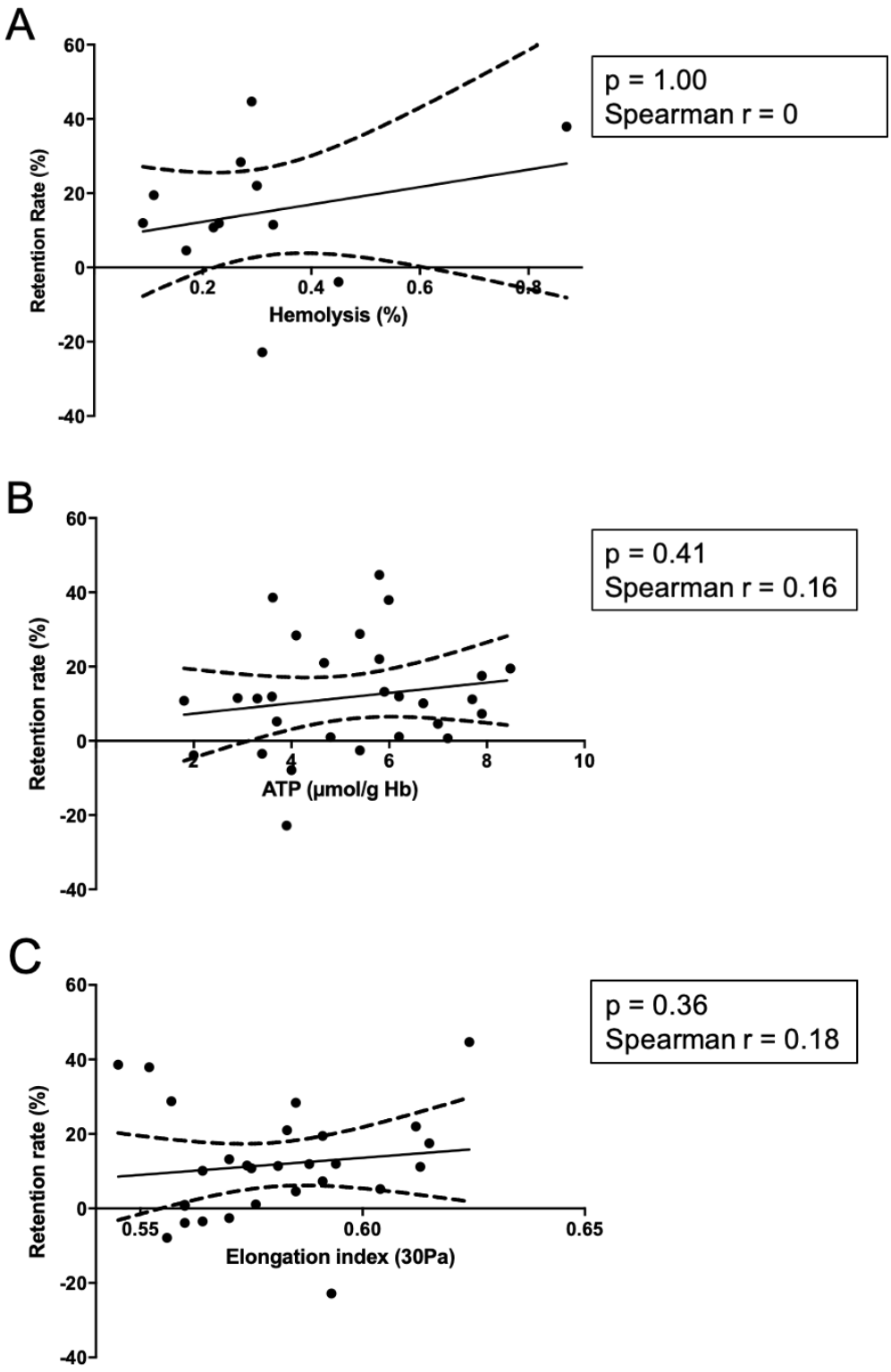
Antibody	Clone	Fluorochrome	Provider
CD3ε	145-2C11	APC	Sony
CD19	6D5	APC	Sony
NK1.1	PK136	APC	Sony
F4/80	BM8	eF450	Ebioscience
CD11b	M1/70	AF700	BD
Ly-6G	1A8	PerCP	Sony
Ly-6C	HK1.4	PeCy7	Sony

Macrophages ($CD3^-/CD19^-/NK1.1^-/Ly\text{-}6G^-/F4/80^{hi}/CD11b^{lo}$), monocytes ($CD3^-/CD19^-/NK1.1^-/Ly\text{-}6G^-/F4/80^-/CD11b^{lo}/Ly\text{-}6C^-$), inflammatory monocytes ($CD3^-/CD19^-/NK1.1^-/Ly\text{-}6G^-/F4/80^-/CD11b^{lo}/Ly\text{-}6C^+$), and granulocytes ($CD3^-/CD19^-/NK1.1^-/Ly\text{-}6G^+$) were analyzed. Stained samples were analyzed by flow cytometry and background fluorescence from non-transfused mice was subtracted for each cell type.

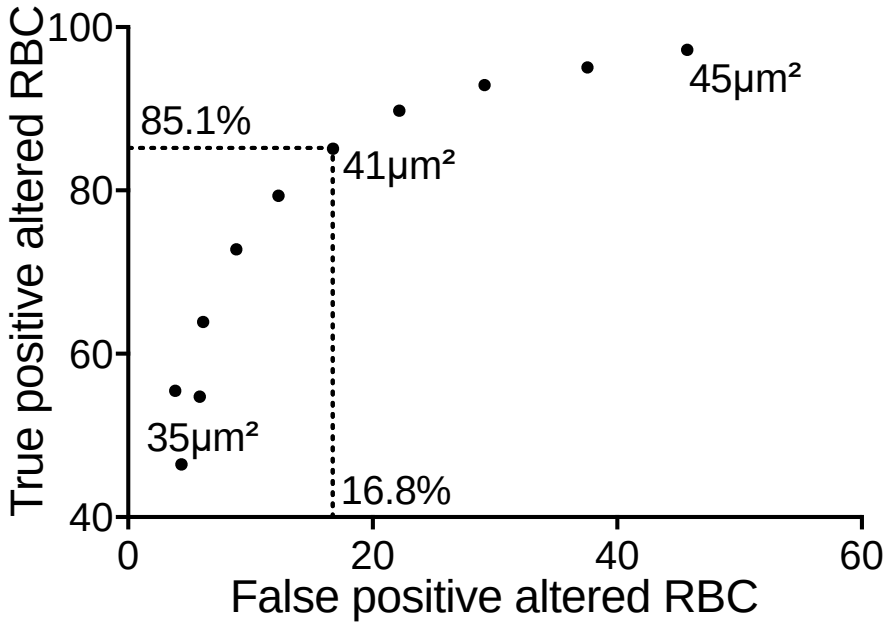
Supplementary figures



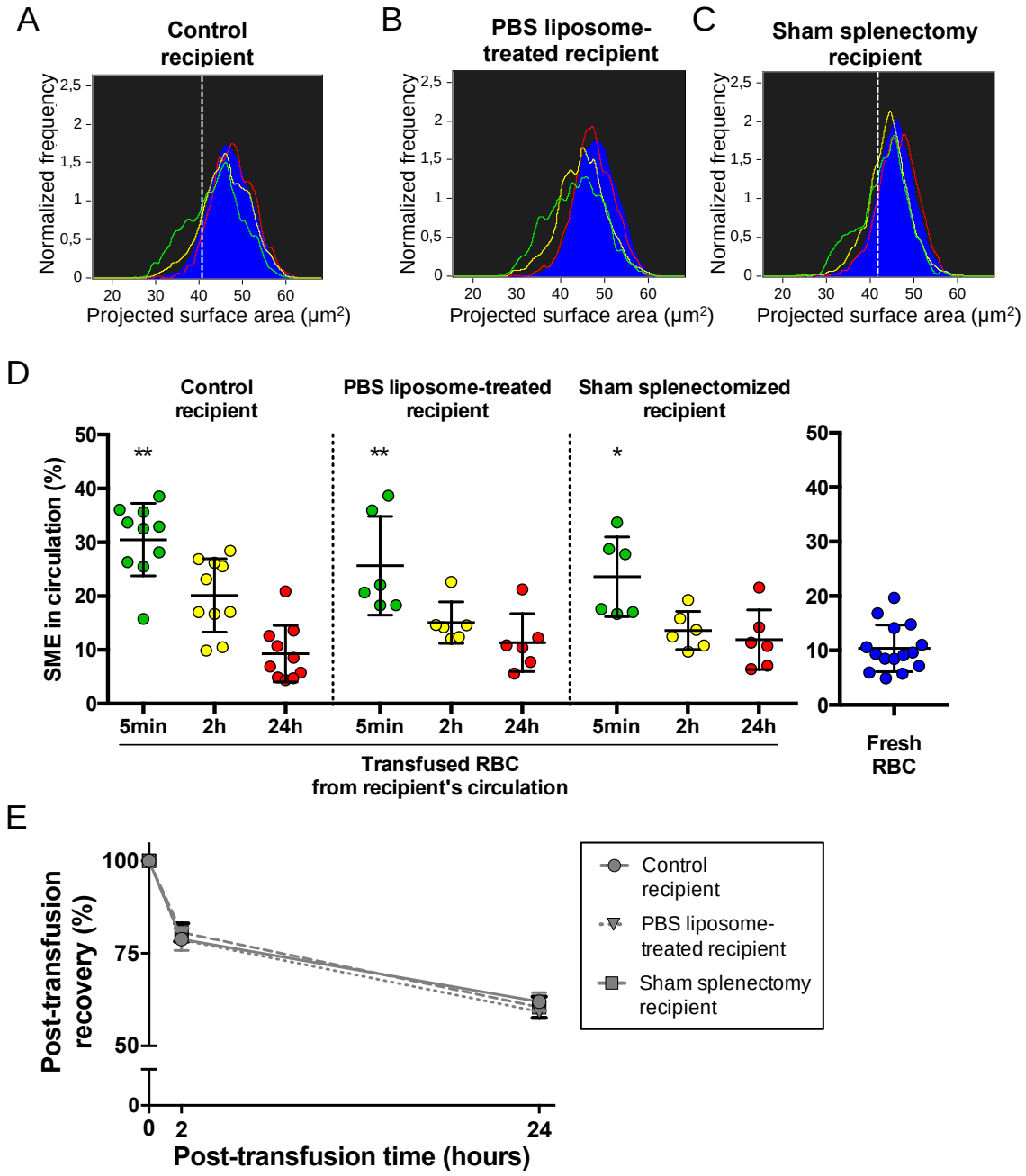
Supplementary Figure 1: RBC morphology after resuspension in fresh plasma. Projected surface area on normalized frequency plot for fresh (left panel) or stored RBC (right panel) is similar when a Krebs-albumin solution (in gray) or neat fresh plasma (in red) is used as a resuspension medium for imaging flow cytometry analysis (15min incubation before acquisition) .



Supplementary Figure 2: Correlation between spleen retention rate and classical markers of the storage lesion. Correlation between mean retention rate in human spleens perfused *ex vivo* and storage hemolysis (A), intracellular ATP level (B), and elongation index measured at 30 Pascal (Pa) by LORRCA (C) in the RBC concentrate before perfusion is shown.



Supplementary Figure 3: ROC analysis to determine optimal cutoffs for quantifying the proportion of morphologically altered mouse RBC by Imaging Flow Cytometry. A threshold set at 41 µm^2 enables identification of altered RBC with a specificity of 83.2% and a sensitivity of 85.1%.



Supplementary Figure 4: Comparison of the transfused RBC morphology and transfusion recovery in the circulation of different control recipient mouse subgroups. Representative normalized frequency plot of projected surface area for long-stored mouse RBC, as observed at 5 min (green line), 2h (yellow line), and 24h (red line) after transfusion to a control (A), PBS liposome-treated recipients (B) or sham splenectomized (C). Control RBC from a non-transfused mouse (blue) are shown as a reference. The dashed white vertical line defines the gating of SME. (D) Declining proportion of SME in the circulation following transfusion is similar in sham splenectomized, PBS liposome-treated and control recipients (n = a minimum of 6 mice/group). (E) Transfusion recovery is similar in sham splenectomized, PBS liposome-treated and control recipients (n = a minimum of 6 mice/group). In D and E, data are presented as mean ± SEM. In D, *P<0,05, **P<0,01 when compared to the "Fresh RBC" condition by a Kruskal-Wallis test.