

1 **Supplemental Data**

2 **The sentinel against brain injury post-subarachnoid hemorrhage: efferocytosis**
3 **of erythrocytes by leptomeningeal lymphatic endothelial cells**

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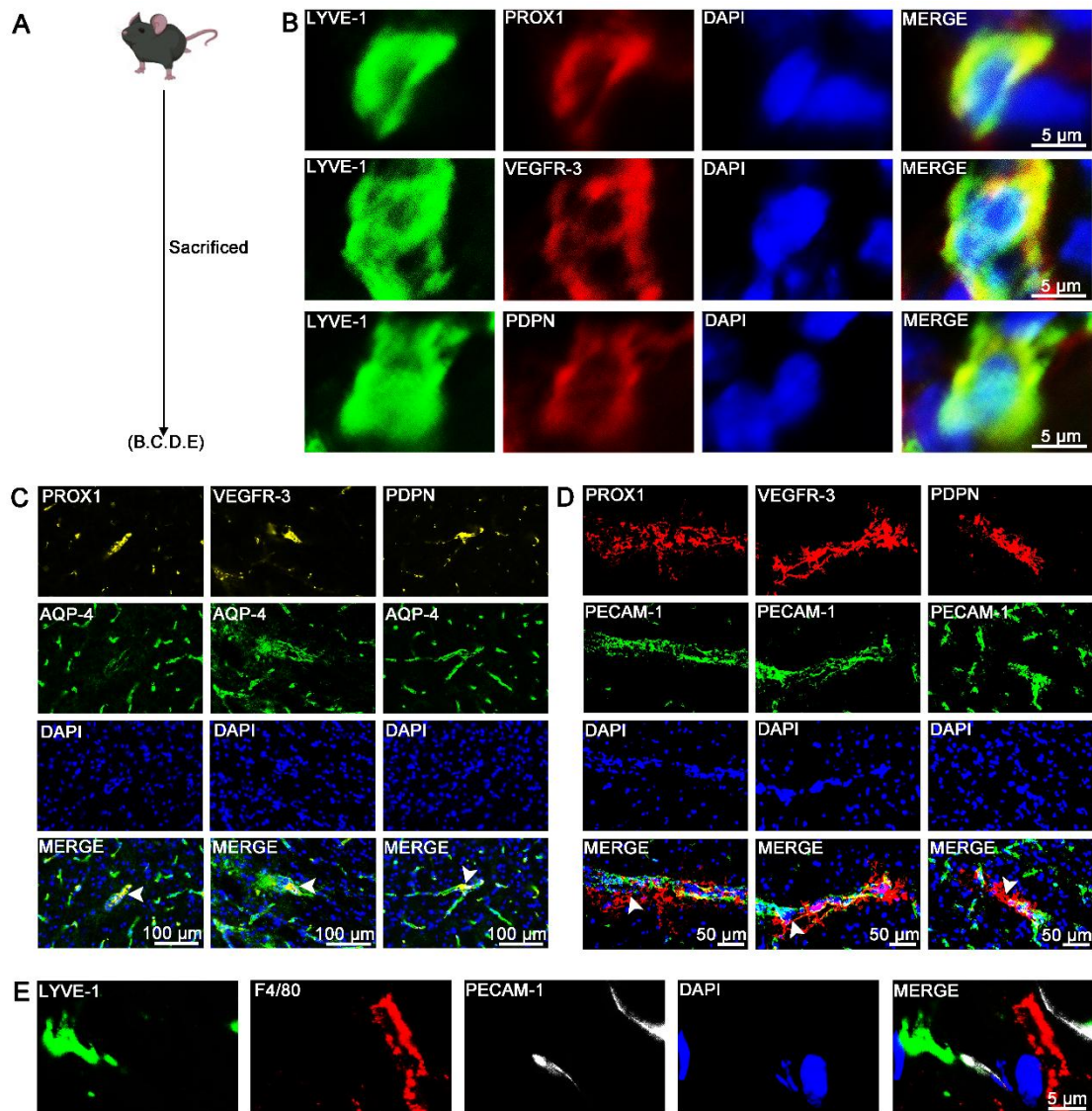
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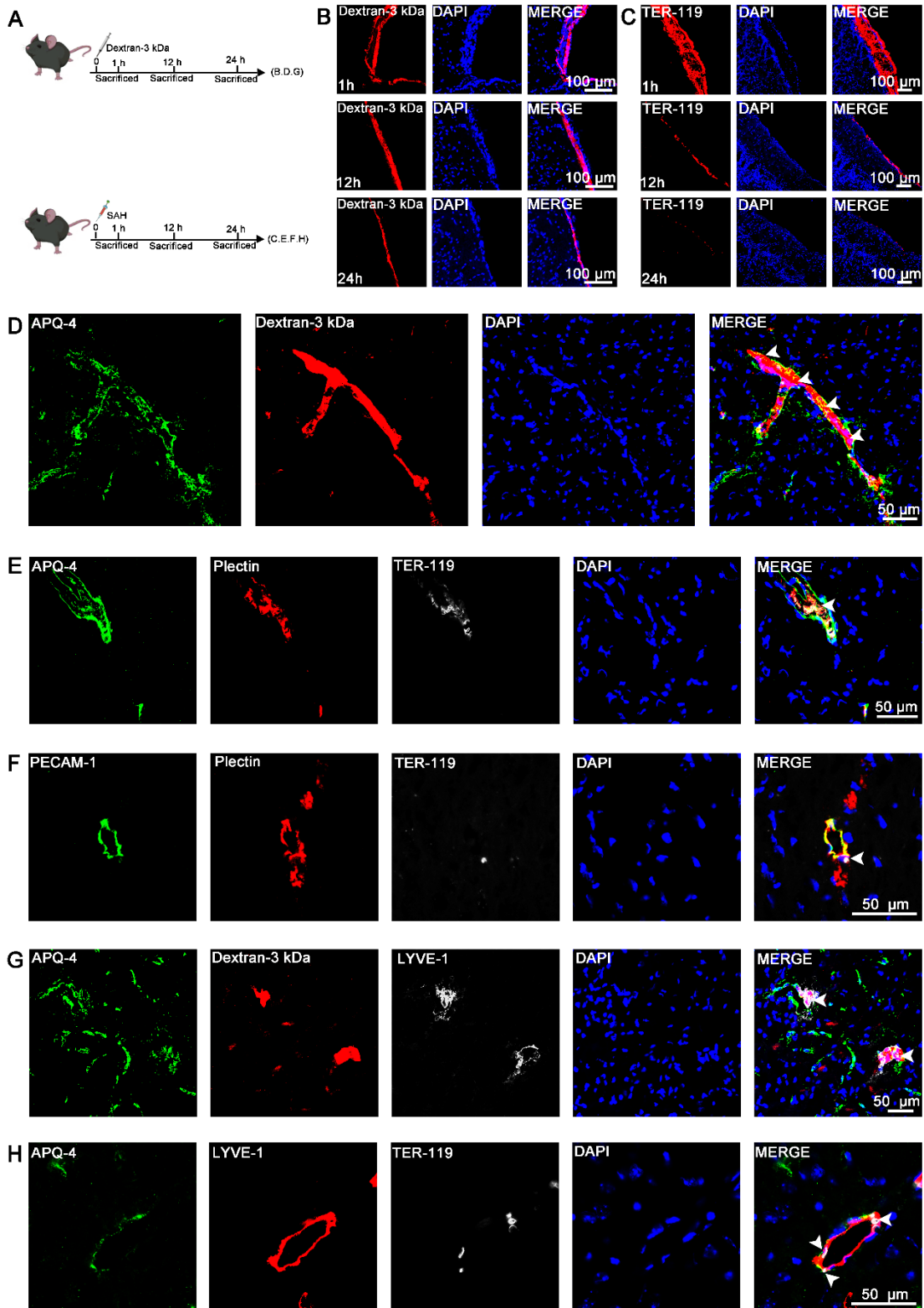


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28 **Figure S1. The identification of LLECs in mice.** (A) Schematic of the experimental
 29 design and procedures. (B) Representative immunofluorescence images showing
 30 LYVE-1-positive cells expressed PROX1, VEGFR-3 and PDPN *in vivo* (scale bars: 5
 31 μm). (C) Representative immunofluorescence images showing that PROX1-positive,
 32 VEGFR-3-positive, and PDPN-positive cells were located within AQP-4-positive
 33 perivascular spaces *in vivo* (indicated by white arrows; scale bars: 100 μm). (D)
 34 Representative immunofluorescence images revealing that PROX1-positive, VEGFR-
 35 3-positive, and PDPN-positive cells were peripheral to PECAM-1-positive vascular
 36 endothelial cells *in vivo* (indicated by white arrows; scale bars: 50 μm). (E)
 37 Representative immunofluorescence images showing LYVE-1-positive cells were
 38 peripheral to PECAM-1-positive vascular endothelial cells and distinct from F4/80-
 39 positive cells *in vivo* (scale bars: 5 μm). LYVE-1, a lymphatic endothelial cell marker;
 40 PROX1, a lymphatic endothelial cell marker; VEGFR-3, a lymphatic endothelial cell
 41 marker; PDPN, a lymphatic endothelial cell marker; AQP-4, a perivascular spaces

- 42 marker; PECAM-1, a vascular endothelial cell marker; F4/80, a macrophage marker;
43 DAPI, a nuclear marker.

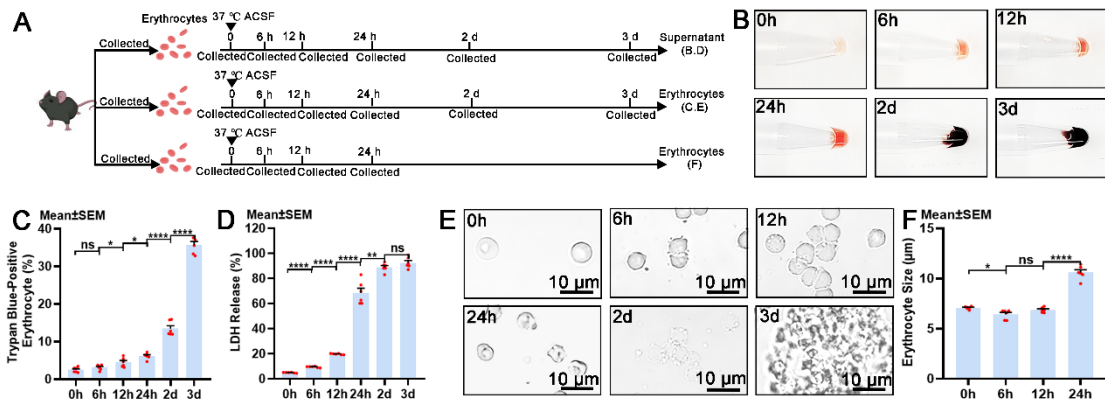
44 Supplemental Figure 2



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 46 **Figure S2. The distribution of dextran-3 kDa and erythrocytes in mice. (A)**
 47 **Schematic of the experimental design and procedures. (B)** Representative
 48 **immunofluorescence images showing the distribution of dextran-3 kDa at the surface**
 49 **of the brain after 1 hour, 12 hours and 24 hours *in vivo* (scale bars: 100 μ m). (C)**
 50 **Representative immunofluorescence images showing the distribution of TER-119-**

51 positive erythrocytes at the surface of the brain after 1 hour, 12 hours and 24 hours *in*
52 *vivo* (scale bars: 100 μm). **(D)** Representative immunofluorescence images showing
53 that dextran-3 kDa were located within AQP-4-positive perivascular spaces *in vivo*
54 (indicated by white arrows; scale bars: 50 μm). **(E)** Representative immunofluorescence
55 images showing that TER-119-positive erythrocyte were located within AQP-4-
56 positive perivascular spaces and plectin-positive pia mater *in vivo* (indicated by white
57 arrows; scale bars: 50 μm). **(F)** Representative immunofluorescence images showing
58 that TER-119-positive erythrocytes were peripheral to PECAM-1-positive vascular
59 endothelial cells and plectin-positive pia mater *in vivo* (indicated by white arrows; scale
60 bars: 50 μm). **(G)** Representative immunofluorescence images showing that LYVE-1-
61 positive cells were located within AQP-4-positive perivascular spaces and
62 phagocytized dextran-3 kDa (indicated by white arrows; scale bars: 50 μm). **(H)**
63 Representative immunofluorescence images showing that LYVE-1-positive cells were
64 located within AQP-4-positive perivascular spaces and phagocytized TER-119-positive
65 erythrocytes (indicated by white arrows; scale bars: 50 μm). LYVE-1, a lymphatic
66 endothelial cell marker; TER-119, an erythrocyte marker; AQP-4, a perivascular spaces
67 marker; PECAM-1, a vascular endothelial cell marker; Plectin, a pia mater maker;
68 DAPI, a nuclear marker.

69 **Supplemental Figure 3**

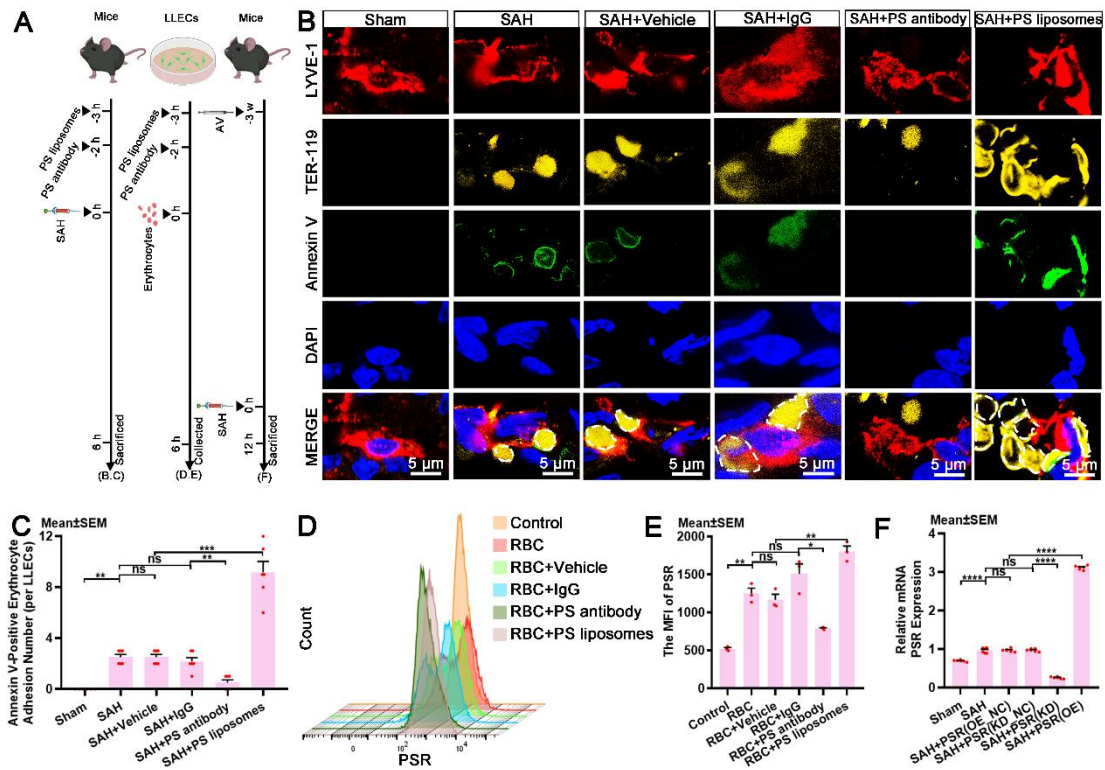


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71 **Figure S3. The cellular dynamics of membrane disruption in erythrocytes in**
 72 **ACSF. (A)** Schematic of the experimental design and procedures. **(B)** Observation of
 73 the supernatant of erythrocytes in ACSF. **(C)** Quantification of the percentage of trypan
 74 blue-positive erythrocyte in ACSF at various time points by trypan blue staining (n =
 75 6). **(D)** Quantification of percentage of LDH release from erythrocytes in ACSF at
 76 various time points by LDH release assays (n = 6). **(E)** Optical microscope images
 77 showing the morphological characteristics of erythrocytes in ACSF at various time
 78 points (scale bars: 10 µm). **(F)** Quantification of erythrocyte size in ACSF at various
 79 time points by size analysis (n = 6). The data are presented as the means ± SEM; *P <
 80 0.05, **p < 0.01, ***p < 0.005; ****p < 0.001; ns, not significant.

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82 Supplemental Figure 4

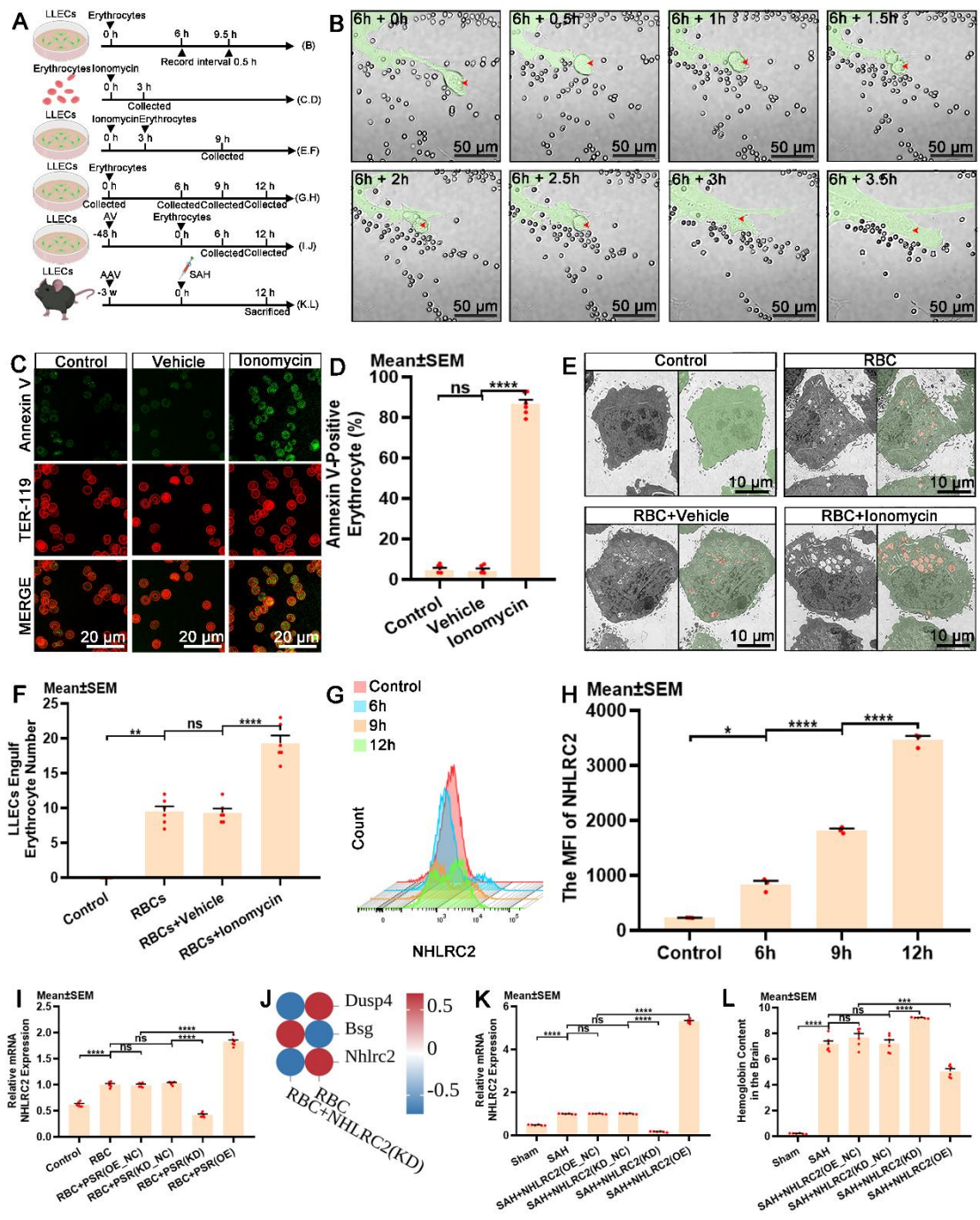


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84 **Figure S4. PS modulates the recognition of apoptotic erythrocytes by LLECs and**
 85 **regulates the expression of PSR. (A)** Schematic of the experimental design and
 86 procedures. **(B)** Representative immunofluorescence images showing the number and
 87 adhesion status of Annexin V-positive, TER-119-positive erythrocytes and LYVE-1-
 88 positive LLECs *in vivo* (scale bars: 5 μm). **(C)** Quantification of Annexin V-positive
 89 erythrocyte adhesion number (per LLECs) by immunofluorescence staining (n = 6). **(D)**
 90 Representative flow cytometry histograms displaying the expression of PSR *in vitro*.
 91 **(E)** Quantification of the MFI of PSR by flow cytometry analysis (n = 3). **(F)**
 92 Quantification of the mRNA expression of PSR by RT-qPCR (n = 6). Annexin V, an
 93 apoptosis marker; TER-119, an erythrocyte marker; LYVE-1, a lymphatic endothelial
 94 cell marker; DAPI, a nuclear marker. The data are presented as the means ± SEM; *P
 95 < 0.05, **p < 0.01, ***p < 0.005; ****p < 0.001; ns, not significant.

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97 Supplemental Figure 5



98

99 **Figure S5. LLECs engulf apoptotic erythrocytes and express NHLRC2.** (A)
 100 Schematic of the experimental design and procedures. (B) Time-lapse images
 101 documenting the dynamic process by which LLECs engulf erythrocytes (LLECs are
 102 indicated by green pseudocolor; engulfed erythrocytes are indicated by red arrows;
 103 scale bars: 50 μ m). (C) Representative immunofluorescence images showing the
 104 expression of Annexin V-positive and TER-119-positive erythrocytes. (D)
 105 Quantification of the percentage of Annexin V-positive erythrocyte by
 106 immunofluorescence staining (n = 6). (E) Representative TEM images depicting the

107 engulfment interactions between LLECs and erythrocytes *in vitro* (LLECs are indicated
108 by green pseudocolor; erythrocytes are indicated by red pseudocolor; scale bars: 10 μ m).
109 **(F)** Quantification of the number of erythrocytes LLECs engulfing by TEM (n = 6). **(G)**
110 Representative flow cytometry histograms displaying the expression of NHLRC2 *in*
111 *vitro*. **(H)** Quantification of the MFI of NHLRC2 by flow cytometry analysis (n = 3).
112 **(I)** Quantification of the mRNA expression of NHLRC2 by RT-qPCR (n = 6). **(J)** DEGs
113 analysis between the RBC+NHLRC2(KD) vs RBC group in efferocytosis gene set. **(K)**
114 Quantification of the mRNA expression of NHLRC2 by RT-qPCR (n = 6). **(L)**
115 Quantification of the hemoglobin content in the brain by hemoglobin content detection
116 (n = 6). Annexin V, an apoptosis marker; TER-119, an erythrocyte marker. The data
117 are presented as the means \pm SEM; *P < 0.05, **p < 0.01, ***p < 0.005; ****p < 0.001;
118 ns, not significant.

119 **Video S1. Related to Figure S5B.** Time-lapse images document the dynamic process
120 by which LLECs engulfed erythrocytes between 6 to 9.5 hours post-co-incubation.