Supplemental Methods
Collection of patient samples
Anonymized samples were obtained from two patients with normal erythropoiesis who had previously undergone splenectomy. Consecutive eligible sickle cell disease patients who were attending for an automated exchange gave informed written consent (IRAS project ID: 59193). A sample was drawn at the same time that the needle was inserted at the start of the exchange and blood was collected into EDTA before transportation.

Confocal Microscopy
All washes and dilutions were performed in Buffer A (PBS pH 7.4 containing 5mg/ml BSA (Park Scientific, Nottingham, UK) and 1mg/ml glucose). Cells were seeded on 0.01% (w/v) Poly-L-lysine (Sigma) coated coverslips and incubated for 30 minutes at 37°C in 5% CO₂. Reticulocytes were fixed in 1% paraformaldehyde (TAAB, Aldermaston, UK) and permeabilised in 0.05% saponin (Sigma). After permeabilisation all subsequent washes and antibody dilutions were carried out in Buffer A containing 0.005% saponin. Mature erythrocytes were fixed in 1% formaldehyde plus 0.0075% glutaraldehyde (Sigma-Aldrich, Gillingham, UK) and permeabilized in 0.1% (w/v) Triton X-100 (Sigma-Aldrich). Samples were imaged at 22°C using 40x oil immersion lenses (magnification 101.97µm at zoom 3.8, 1.25 NA) on a Leica DMI 6000 inverted microscope with phase contrast connected to a Leica TCS SP5 confocal imaging system (Leica, Wetzlar, Germany). Images were obtained using Leica LAS AF software and subsequently processed using Adobe Photoshop (Adobe, San Jose, CA) and Volocity (Perkin Elmer, MA, USA).

For live cell imaging, 4µl packed cells were suspended in 396µl Buffer A and 25µl of this used per test. Cells were incubated either with directly conjugated antibodies to the cytoplasmic domains of GPA (BRIC 163-FITC) and AE1 (BRIC 155-FITC) IBGRL, Bristol, UK (all with a 1:50 dilution) or with the Annexin-V-FLUOS staining kit (Roche) for PS detection (with a 1:50 dilution of Annexin V FITC in the supplied Annexin V binding buffer). The cells were washed once in 1ml Buffer A, resuspended in 200µl Buffer A, then added to an IbiTreat µ-slide 8 well microscopy chamber (Ibidi GmbH, Germany). The slides were imaged on the Leica SP5 confocal system using the environmental chamber for temperature control at 37°C and CO₂ enrichment. For vesicle quantitation analysis, random fields were imaged and vesicles from 0.8µm to 1.8µm were counted.

Antibodies
Anti-GPA antibodies BRIC256, BRIC163 and R10 and anti-AE1 antibodies BRIC155 and BRAC18 (all IBGRL, Bristol, UK). Organelle markers used were LC3 (MBL, Nagoya, Japan), giantin (Covance Inc, NJ, USA) and Mitotracker® Deep Red FM (Life technologies, Paisley, UK). PS was stained using the Annexin-V-FLUOS Staining Kit (Roche, West Sussex, UK). The Conversion antibody used was F’ab’ fragment rabbit anti–mouse IgG (Jackson ImmunoResearch Laboratories) Secondary antibodies used
were Alexa fluor® 488, 546 and 633 (Life Technologies) diluted in 4% (w/v) normal goat serum. Directly conjugated antibodies were made using Alexa-Fluor conjugation kit (Life Technologies).

**Fluorescence Activated Cell sorting**
Live cells were stained with the Annexin-V-FLUOS staining kit for PS detection, washed and the sorted for positivity on the Becton Dickinson InFlux Cell Sorter at the Flow Cytometry facility at the University of Bristol, using BD Sortware 1.2 software and the 86μm nozzle to sort.

**Trypsin treatment.**
Filtered reticulocytes or whole blood samples were washed 3 times with PBS prior to resuspension in a 1:5 ratio of packed cells to trypsin (2.5mg/ml) (Sigma-Aldrich) PBS solution and incubated with rotation at 37°C for 30 minutes. Cells were removed from the trypsin PBS by 4 washes with Buffer A.
Supplemental figure 1. Peripheral red cells treated to stain PS uniformly. (A) Red cells from peripheral blood were treated with NEM followed by Ca\(^{2+}\) and ionomycin as per Kuypers et al. 1996\(^{13}\) (but for 15 minutes rather than 60 minutes) and stained with Annexin V-FITC (green). PS stained uniformly over the cell in the Ca\(^{2+}\) ionomycin treated cells as observed in Kuypers et al. 1996\(^{13}\).
(B) Red cells from peripheral blood that had not been treated with NEM, Ca\(^{2+}\) and ionomycin were stained with Annexin V-FITC (green). PS stained in a vesicle in untreated cells.

All scale bars are 5µm.
Supplemental figure 2. SCD red cells sorted for PS fluorescence.
(A) SCD red cells were labelled for PS with Annexin V-FITC. PS positive and PS negative red cells were obtained by flow cytometric sorting. PS positive cells are gated in blue.
(B) PS positivity is shown on the x-axis (log scale) with the positively sorted cells highlighted in blue and the negatively sorted cells in green with the central negative gate shown in pink.