**Methods**

**Generation of myc-tagged WT and mutant hCD59 expression plasmids.** Total RNA was extracted from human white blood cell samples using TRI Reagent (Sigma, St Louis, MO, USA). cDNA generated by SuperScript II Reverse Transcriptase (Life Technologies, Carlsbad, CA, USA) from RNAs isolated from both a Cys64Tyr homozygous patient and a healthy individual were used as templates for PCR reactions using the following primers: Forward 5’-GGGCGCAAGCTTGTTCTGTGGACAATCACAATGGG -3’. Reverse 5’- AGTTTGTCTAGAGCTCTCCTGGTGTTGACTTAGG -3’. The 446bp PCR products were digested by HindIII-XbaI restriction enzymes (New England BioLabs, Ipswich, MA, USA) as per the manufacturer’s protocol and ligated into a pcDNA3 vector that was digested by the same restriction enzymes to create hCD59 and hCD59 Cys64Tyr plasmids. For myc tag insertion, a 30bp oligonucleotide (corresponding to the myc peptide sequence EQKLISEEDL) was inserted between the leader sequence of hCD59 and the N-terminal amino acid of the hCD59 (Fig. S1A) by PCR reactions using the following primer pairs: forward 5’ - GTCAATGGGAGTTTGTTTTGGC -3’, reverse 5’-CAGATCCTCTTCTGAGATGAGTTTTTGTTCGCTATGACCTGAATGGCAGAAG -3’; forward 5’-CTCATCTCAGAAGAGGATCTGCTGCAGTGCTACAACTGTCC -3’, reverse 5’-TCGAGGCTGATCAGCGAGC -3’. Based on the hCD59 and hCD59myc plasmids, we generated three additional mutant hCD59 plasmids; hCD59 Asp24Val, hCD59­ Asp24Valfs\* and hCD59 Ala16Alafs\* by site-directed mutagenesis using the following primer pairs for PCR reaction. hCD59 Asp24Val: forward 5’- GTTCATCTGATTTTGTTGCGTGTCTCATTAC-3’, reverse 5’-GTAATGAGACACGCAACAAAATCAGATGAAC-3’. hCD59­Asp24Valfs\*: forward 5’-GTTCATCTGATTTTGTGCGTGTCTCATTAC-3’, reverse 5’-TCGAGGCTGATCAGCGAGC-3’. hCD59 Ala16Alafs\*: forward 5’-GACTGCAAAACAGCGTCAATTGTTCATC-3’, reverse 5’-GATGAACAATTGACGCTGTTTTGCAGTC-3’. The hCD59 open reading frames for all the above constructs were confirmed by DNA sequencing. The first mutation in the Japanese case (Ala16Alafs\*) leads to a frameshift; thus, only the first mutation was expressed.

Expression of wild-type (WT) and mutant hCD59. Transfections of HEK-293T and COS7 cells were performed using CaPO4 and LipofectamineTM 2000 reagent (Invitrogen,Carlsbad, CA, USA), respectively.

***Immunofluorescence labeling.*** All seven anti-hCD59 antibodies were initially used for immunofluorescence labeling of live cells. 48 h after transfection, COS-7 cells grown on 13mm coverslips were washed with PBS supplemented containing calcium and magnesium (PBS++) and incubated for 45 min in 37°C with the above antibodies in DMEM, 1% FBS. Samples were then washed twice by PBS++ and fixed with 4% paraformaldehyde (PFA). After three additional washes, all samples were blocked for 1 h (PBS×1, 5% NGS, 0.1% glycine), incubated for 1 h at room temperature with myc tag antibodies diluted in blocking solution, washed, and incubated for 45 minutes at room temperature with secondary antibodies. Finally, coverslips were mounted with elvanol. Immunofluorescence labeling was also performed on permeabilised cells. After fixation, coverslips were blocked for 1 h (PBS×1, 5% NGS, 0.5% Triton X-100, 0.1% glycine), incubated for 1 h at room temperature with primary antibodies diluted in blocking solution with 0.1% Triton X-100, washed, and incubated again for 45 minutes at room temperature with secondary antibodies. Finally, slides were mounted with elvanol and analyzed.

PDI staining was performed under different conditions that included methanol permeabilization (post-fixation, 5 minutes at -20°C); blocking solutions included PBS×1, 5% NGS, and 0.3% Triton X-100. First antibodies were incubated overnight at 4°C. Fluorescence images were obtained using a confocal microscope (LSM700, Carl Zeiss, Oberkochen, Germany) fitted with an ORCA-ER CCD camera (Hamamatsu Ltd., Hamamatsu, Japan). Images were acquired and processed using the Zen2012 (Zeiss) and Photoshop software (Adobe, San Jose, CA, USA).

Flow cytometry. All seven anti-hCD59 antibodies (MEM43, BRIC229, YTH53.1, A35, HC1, 1.39, rabbit polyclonal antibody) were tested for staining of lymphoblasts from both a Cys64Tyr homozygous patient and a healthy individual. Fluorophore-coupled secondary antibodies included RPE goat anti-mouse and rabbit IgG (Jackson Laboratories). Flow cytometry detection was performed using FACSCalibur (Becton Dickinson, Franklin Lakes, NJ, USA).

***Antibodies***. The following human CD59 antibodies were used in immunofluorescence-labeling assays of overexpressing COS7 cells and detected by flow cytometry and fluorescent microscopy: mouse monoclonal antibody (mAb) MEM43 – epitopes W40, R-53, F23 and K41; mouse mAb BRIC229 – epitope W40; mouse mAb YTH53.1 – epitopes W40 and R53; mouse mAb A35 – epitopes W40, R53, L54, K38; mouse mAb HC1 – epitopes L33, E56; mouse mAb 1.39 – unknown epitope; rabbit polyclonal antibody – multiple epitopes.[14](#_ENREF_14) All antibodies were obtained from Cardiff University. Additional antibodies that were used include mouse and rabbit antibodies against myc tag peptide (Merck), and rabbit polyclonal antibody against protein disulfide isomerase (PDI) (Cell Signaling, Danvers, MA, USA). Fluorophore-coupled secondary antibodies included Cy3-coupled anti-mouse, and anti-rabbit IgG, Cy5-coupled anti-mouse, and anti-rabbit IgG (Jackson Laboratories, Bar Harbor, ME, USA). Cell nuclei were stained using Dapi (Sigma).

***Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting****.* Transfected HEK-293T cells were lysed directly by X3 sample buffer. Cell lysates were separated on 15% polyacrylamide gels and transferred to polyvinylidene difluoride (PVDF) membranes. After blocking with 3% BSA, membranes were incubated with mouse anti-myc antibody followed by extensive washes and incubation with HRP-conjugated anti-mouse antibody. ECL reaction imaging was performed with the ChemiDoc MP System (BioRad, Hercules, CA, USA). For glycosylation pattern examination, hCD59 was initially immunoprecipitated from cell lysates using anti-myc antibodies followed by denaturing of the isolated immunocomplexes and deglycosylation of high mannose structures using endoglycosidase H (Endo H) (New England BioLabs) for 1 h at 37°C.

***In vitro cell lysis assay assessing MAC attack in WT and mutants.*** Transfections of CHO cells were done using LipofectamineTM 2000 reagent. 24 h post transfection, cells were harvested and replated on 24-well plates (3×105 cells in 1ml per well) and left until the plates were confluent. Cells were then washed twice with serum-free medium, incubated in 0.25 ml complete medium (7.5% FCS heat-inactivated) containing a 1/750 dilution of calcein-AM (Molecular Probes, Eugene, OR, USA, 1 mg/ml stock in DMSO) for 1 h at 37°C. After one washing with PBS, duplicate wells were incubated with 1/5 dilution of normal human serum (NHS) and 40μl of rabbit anti-CHO IgG. After incubation for 1 h at 37°C, all fluid was removed from the cells and transferred to 96-well plates for calcein measurement.

***Immunoprecipitation.*** For conditional medium and deglycosylation samples, 20μl of goat anti-mouse IgG-agarose beads (Sigma) were mixed with 4μl of mouse antibody against the myc-tag peptide by rotating for 30 minutes at room temperature. Samples were then added and rotated overnight at 4°C. After aspiration and washing with PBS and PBS 0.1% Triton X-100, 20μl of sample buffer was added to the pelleted beads and boiled for 4 minutes at 95°C prior to separation of the supernatants on SDS-PAGE.