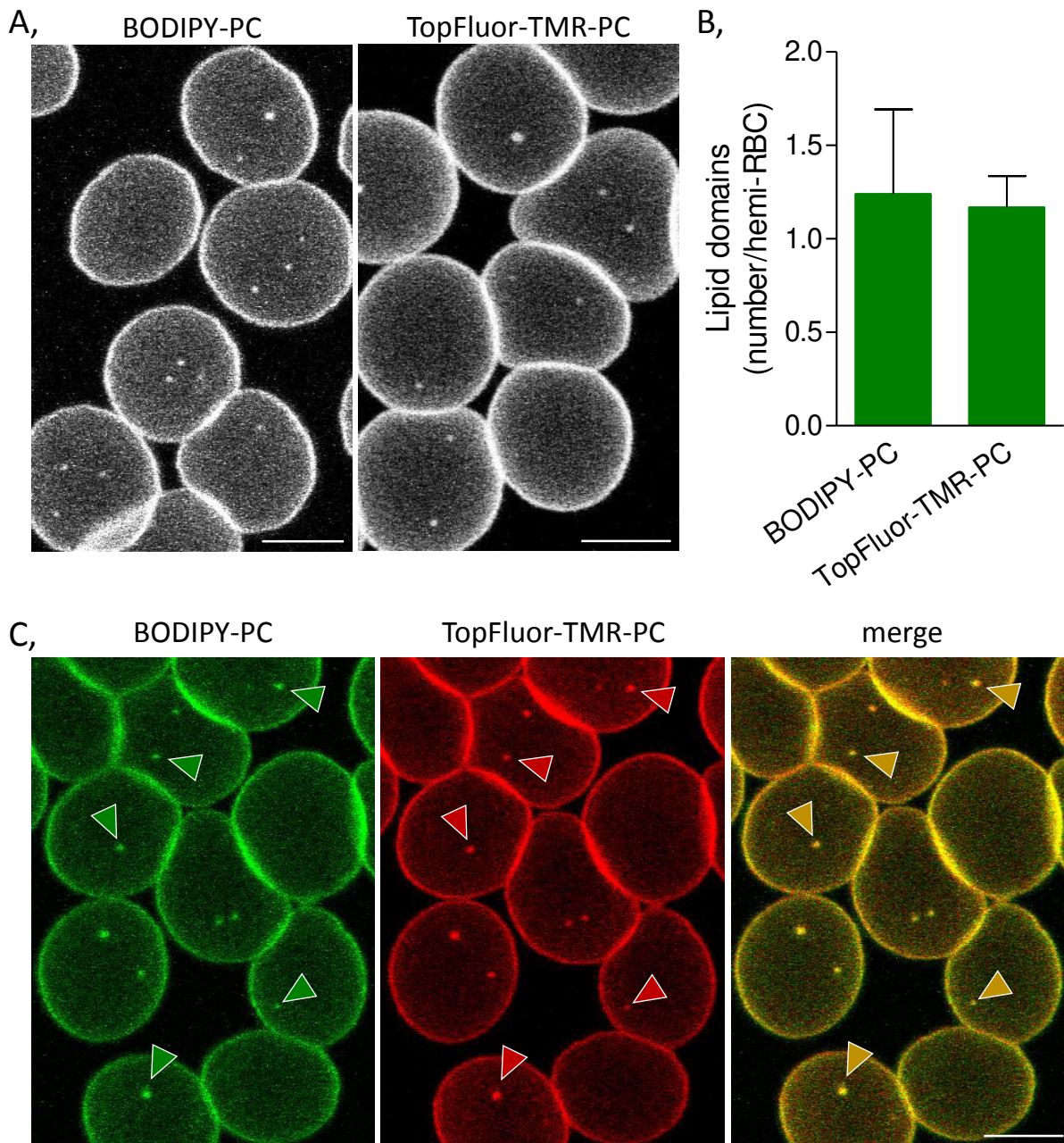


Supplementary Material

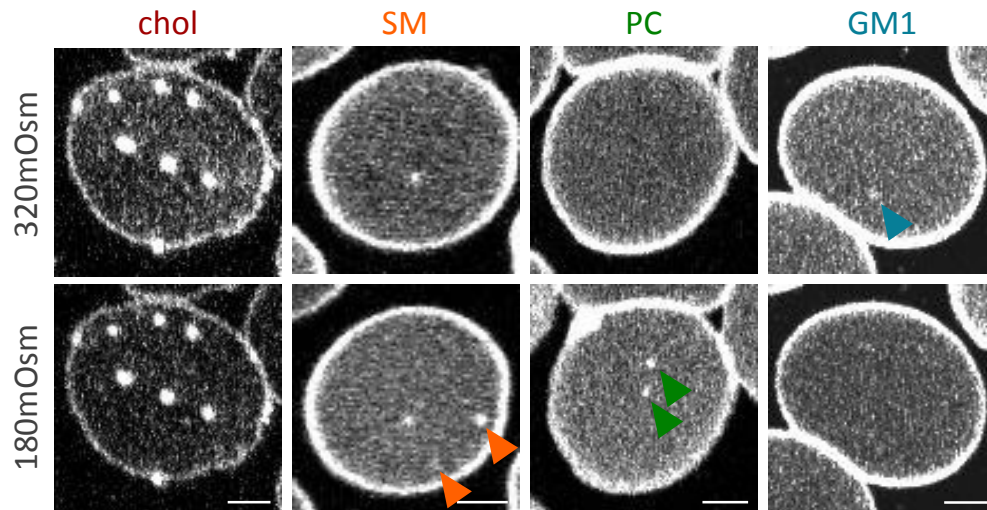
Spatial Relationship and Functional Relevance of Three Lipid Domain Populations at the Erythrocyte Surface

Louise Conrard^a Amaury Stommen^a Anne-Sophie Cloos^a Jan Steinkühler^b Rumiana
Dimova^b Hélène Pollet^a Donatienne Tyteca^a

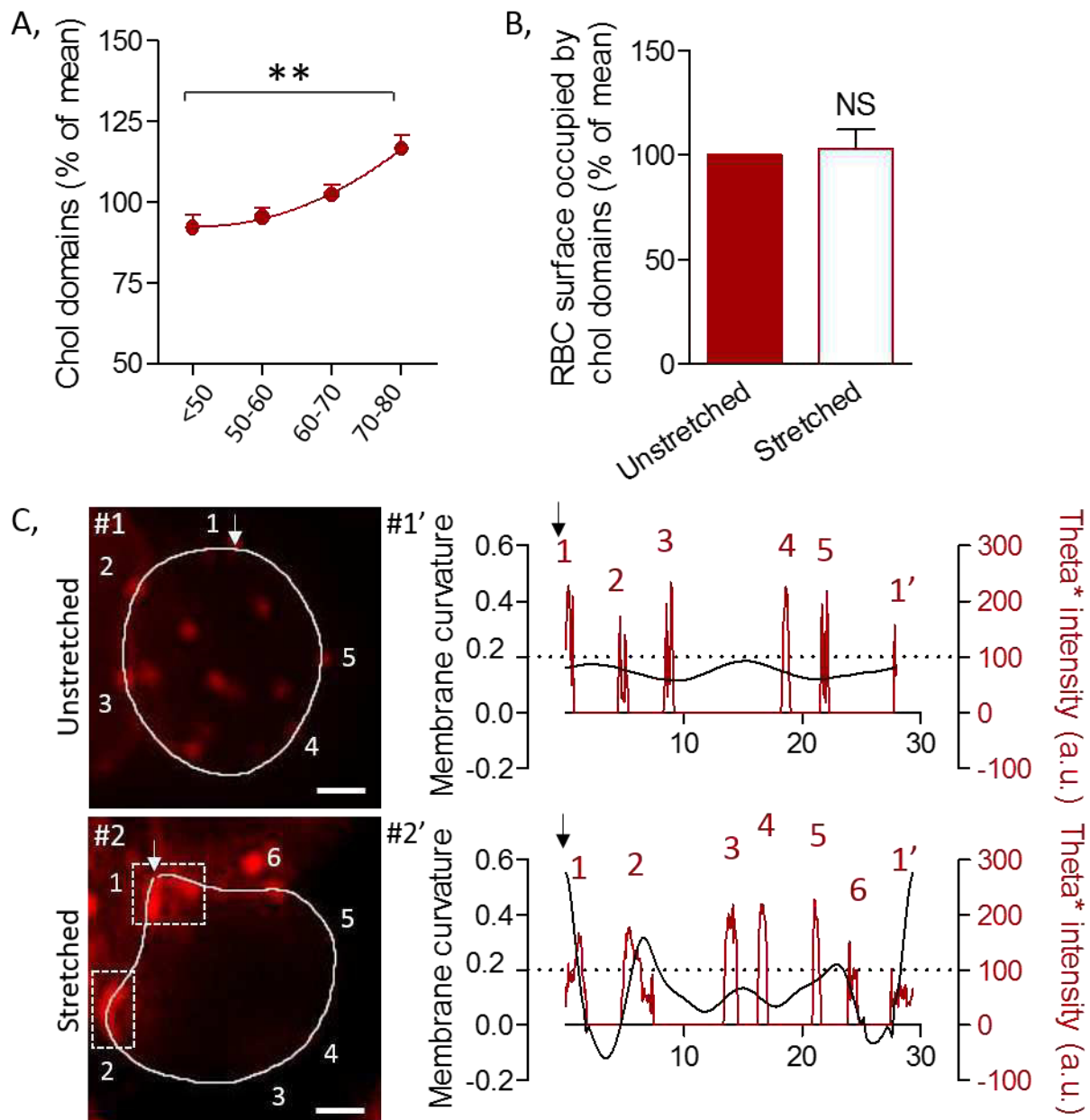
^aCELL Unit, de Duve Institute & Université catholique de Louvain, Brussels, Belgium, ^bTheory and Bio-Systems, Max
Planck Institute of Colloids and Interfaces, Science Park Golm, Potsdam, Germany



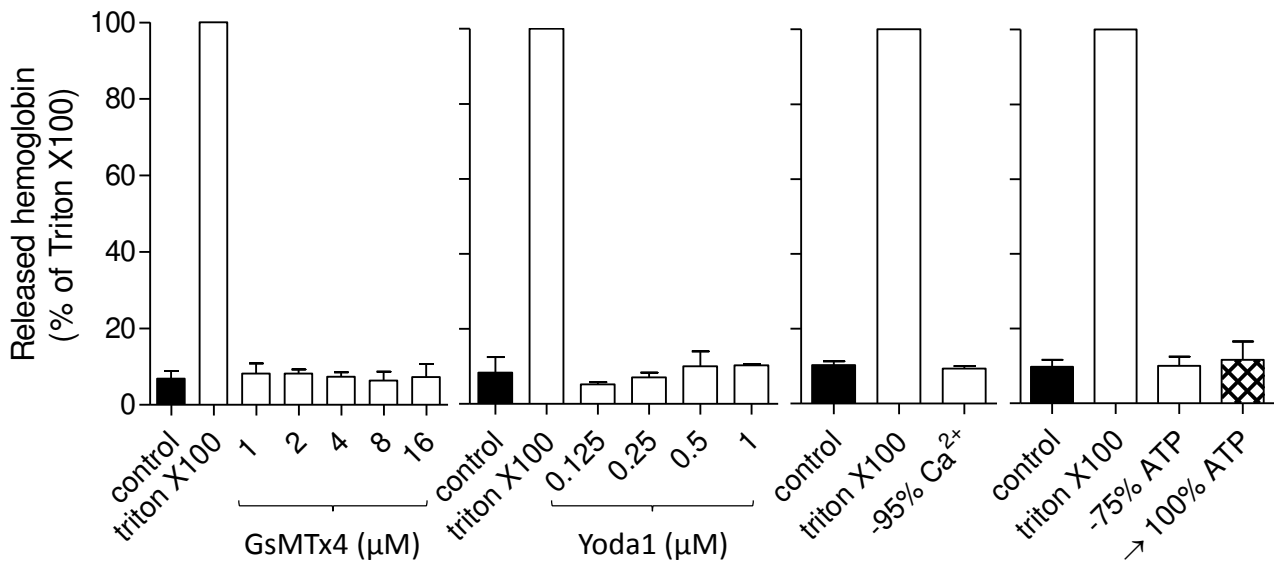
Suppl. Fig 1 Plasma membrane insertion of BODIPY-PC or TopFluor-TMR-PC at trace level reveals domains that are comparable and that perfectly co-localize. RBCs were spread and labeled with either BODIPY-PC or TopFluor-TMR-PC (A,B) or both probes (C) at 37°C. (A) Representative images of simple labeling. (B) Quantification of lipid domains per hemi-RBC. Means \pm SEM from 2-3 experiments where >480 RBCs were counted. (C) Representative images of double-labeled RBCs. All scale bars 5 μ m.



Suppl. Fig 2 Lipid domains differentially respond to osmolarity modulation. RBCs were spread in a microfluidic chamber (CellAsic ONIX), labeled with theta* or BODIPY-polar lipids in an iso-osmolar medium at 37°C and imaged (320 mOsm, top images). A hypo-osmolar flow (180 mOsm, bottom images) was then applied and the same RBCs were imaged after 3 min. Orange and green arrow heads show SM- and PC-enriched domains that appear under hypo-osmolar flow; blue arrow head shows a GM1-enriched domain that disappears under hypo-osmolar flow. *Representative images of >2 independent experiences. Scale bars 2 μ m.*



Suppl. Fig 3 Chol-enriched domains are modulated in size and distribution under RBC mechanical stimulation. (A) Chol-enriched domain abundance at 20°C upon increased spreading on PLL. (B, C) RBCs labeled for chol at 20°C and stretched in PDMS chambers. (B) Chol-enriched domain surface occupation of hemi-RBC in unstretched condition and under stretching in PDMS chambers. (C) Recruitment of chol-enriched domains in increased curvature areas of the RBC edges upon stretching (#2, #2' vs #1, #1'). *Panels B and C are adapted from [28]. Scales bars 2 μ m.*



Suppl. Fig 4 Pharmacological agent innocuity. Released hemoglobin by RBCs either untreated (full bars) or incubated in (i) GsMTx4-containing medium, (ii) Yoda1-containing medium, (iii) Ca²⁺-free medium supplemented with EGTA followed or not by repletion in Ca²⁺-containing medium, and (iv) glucose-depleted medium followed or not by repletion in glucose-containing medium. Results are expressed as percentage of full hemolysis obtained by 0.5 % Triton X100.