Supplementary information, Figure S1

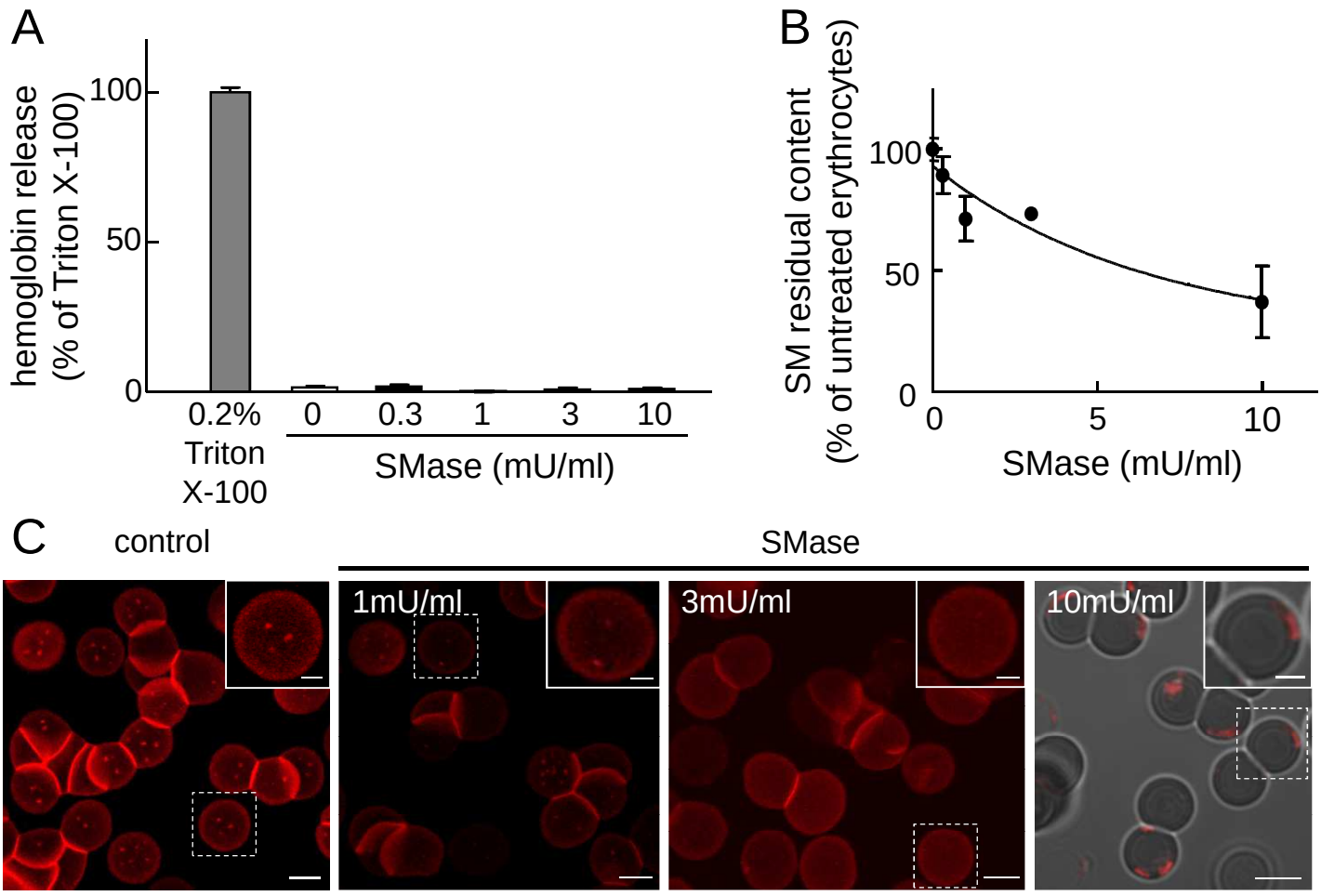

Figure S1 Lysenin* labels submicrometric domains which vanish upon endogenous SM depletion by sphingomyelinase (SMase). (A and B) Innocuity and efficacy of SMase on RBCs. RBCs were treated in suspension with the indicated concentrations of SMase at 20°C for 30min, then evaluated for toxicity (A; based on hemoglobin release, hemolysis upon 0.2% Triton X-100 set at 100%) and residual SM level (B). Data are means ± SEM and are expressed as percentage of Triton X-100 (A) or untreated erythrocytes (B). Hemoglobin release from SMase-treated erythrocytes was not significantly different from untreated RBCs (invisible error bars are included un the bars and the symbols). **(C) SM submicrometric domains vanish upon endogenous SM depletion.** RBCs were treated in suspension with the indicated SMase concentrations, incubated with lysenin*, attached onto poly-L-lysine-coverslips and observed by confocal microscopy. Scale bars; 5µm; insets, 2µm.

Supplementary information, Figure S2

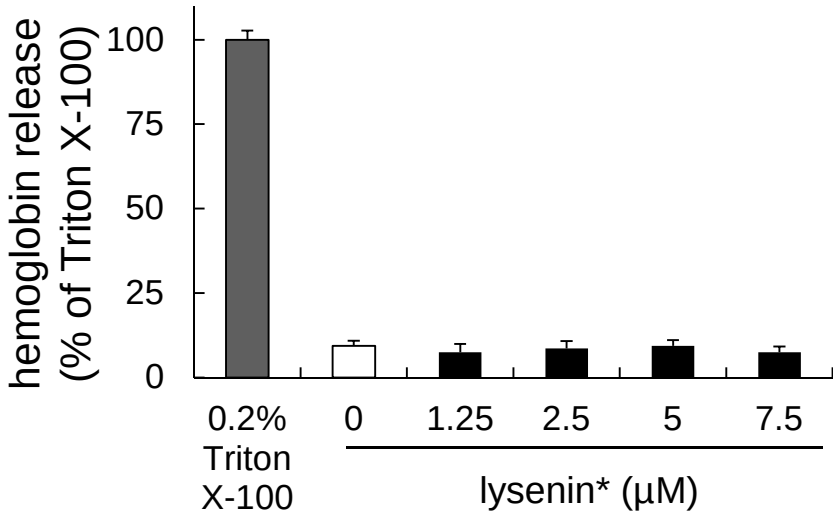

Figure S2 Innocuity of lysenin* for normal RBCs. RBCs were treated in suspension with the indicated concentrations of lysenin* at 37°C, then evaluated for toxicity (based on hemoglobin release, hemolysis upon 0.2% Triton X-100 set at 100%). Results are means ± SEM of 3-6 samples from 2 independent experiments.

Supplementary information, Figure S3

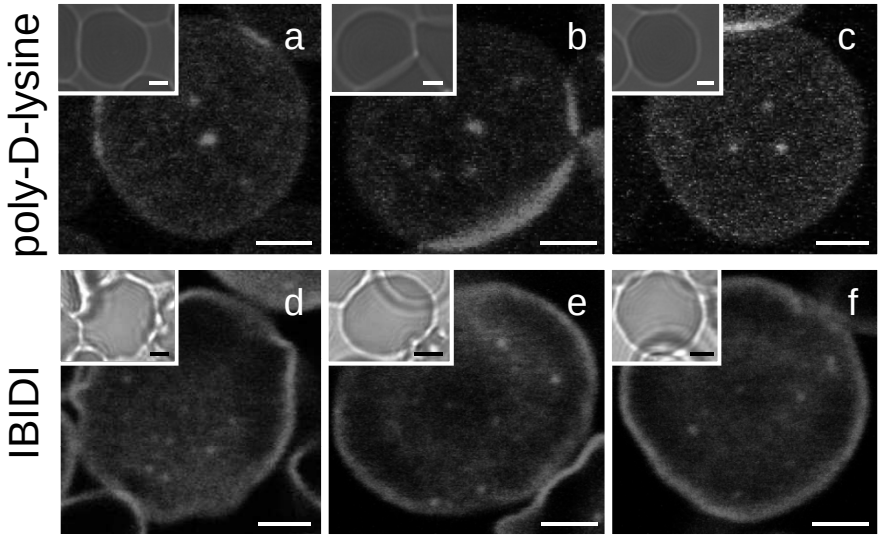

Figure S3 Evidence for SM submicrometric domains on RBCs attached onto poly-D-lysine-coated coverslips and on barely-attached RBCs. Erythrocytes labelled in suspension with lysenin* were either immobilized on poly-D-lysine-coated coverslips and placed in Lab-Tek chambers (a-c; 1 experiment), or laid down on IBIDI chambers (d-f; 4 independent experiments). Scale bars, 2µm.

Supplementary information, Figure S4

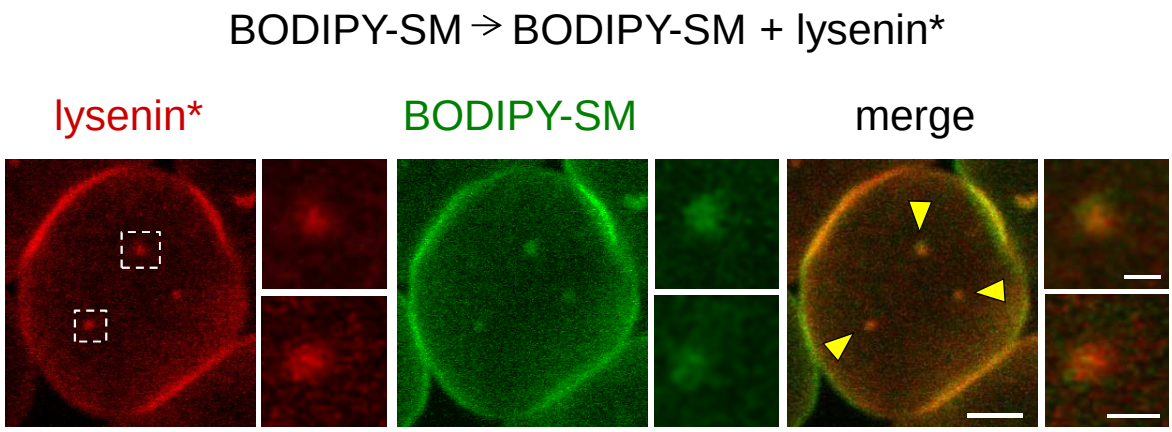

Figure S4 Perfect co-localization between BODIPY-SM and lysenin* domains in RBCs labelled with BODIPY-SM then with lysenin*. RBCs were labelled in suspension with exogenous BODIPY-SM then with lysenin* in the presence of BODIPY-SM, before spreading onto poly-L-lysine-coated coverslips. Yellow arrowheads, domains labelled by BODIPY-SM and lysenin*. Scale bar, 2µm; insets, 0.5µm.

Supplementary information, Figure S5

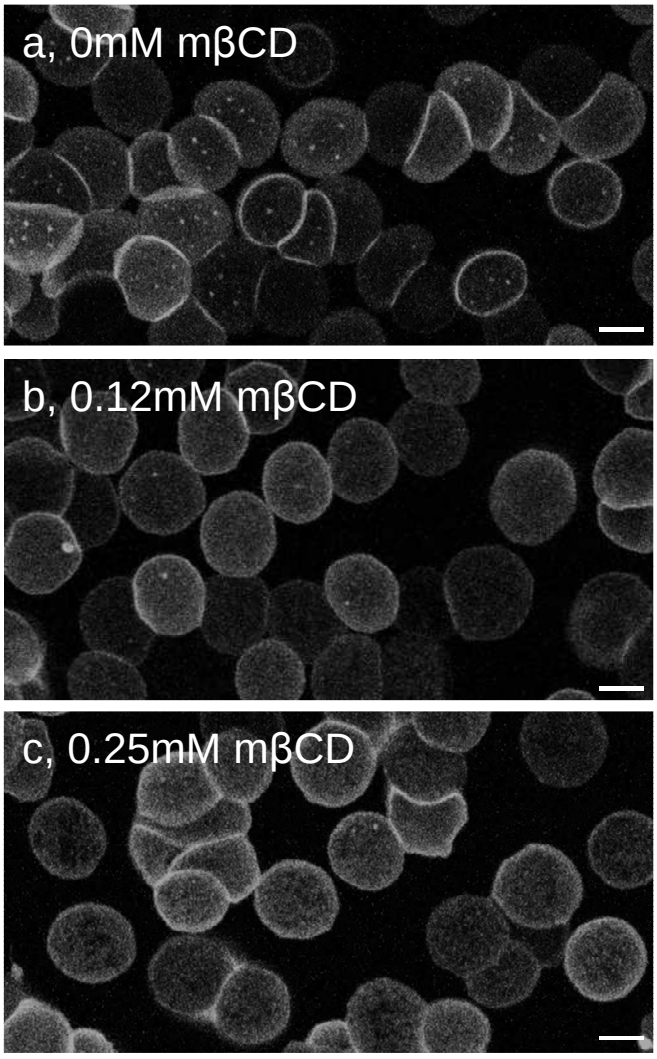

Figure S5 Endogenous SM submicrometric domains depend on cholesterol: low magnifications of Figure 5A. RBCs were treated in suspension with the indicated mβCD concentrations, incubated with lysenin*, attached-spread onto poly-L-lysine-coverslips and observed by confocal microscopy using identical image acquisition settings. Representative images of 6 independent experiments. Scale bars, 5µm.

Supplementary information, Figure S6

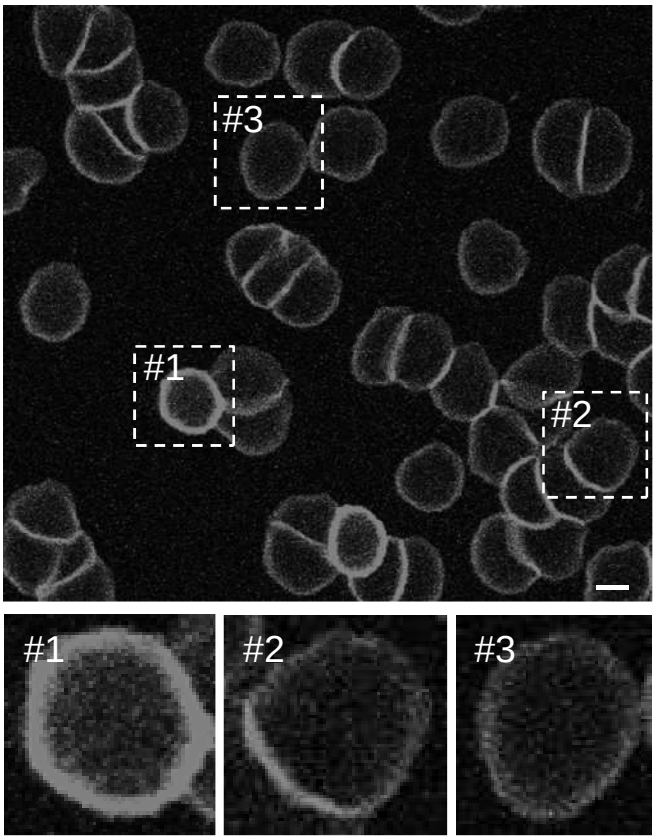

Figure S6 Regardless of spreading stage, RBC membrane is homogeneously labelled by TMA-DPH. RBCs were spread onto poly-L-lysine-coated coverslips, labelled with TMA-DPH and examined by two-photon microscopy at 20°C. This led to three stages of RBC spreading (#1, #2, #3), all of which are homogeneously labelled by TMA-DPH. Scale bar, 5µm.

Supplementary information, Figure S7

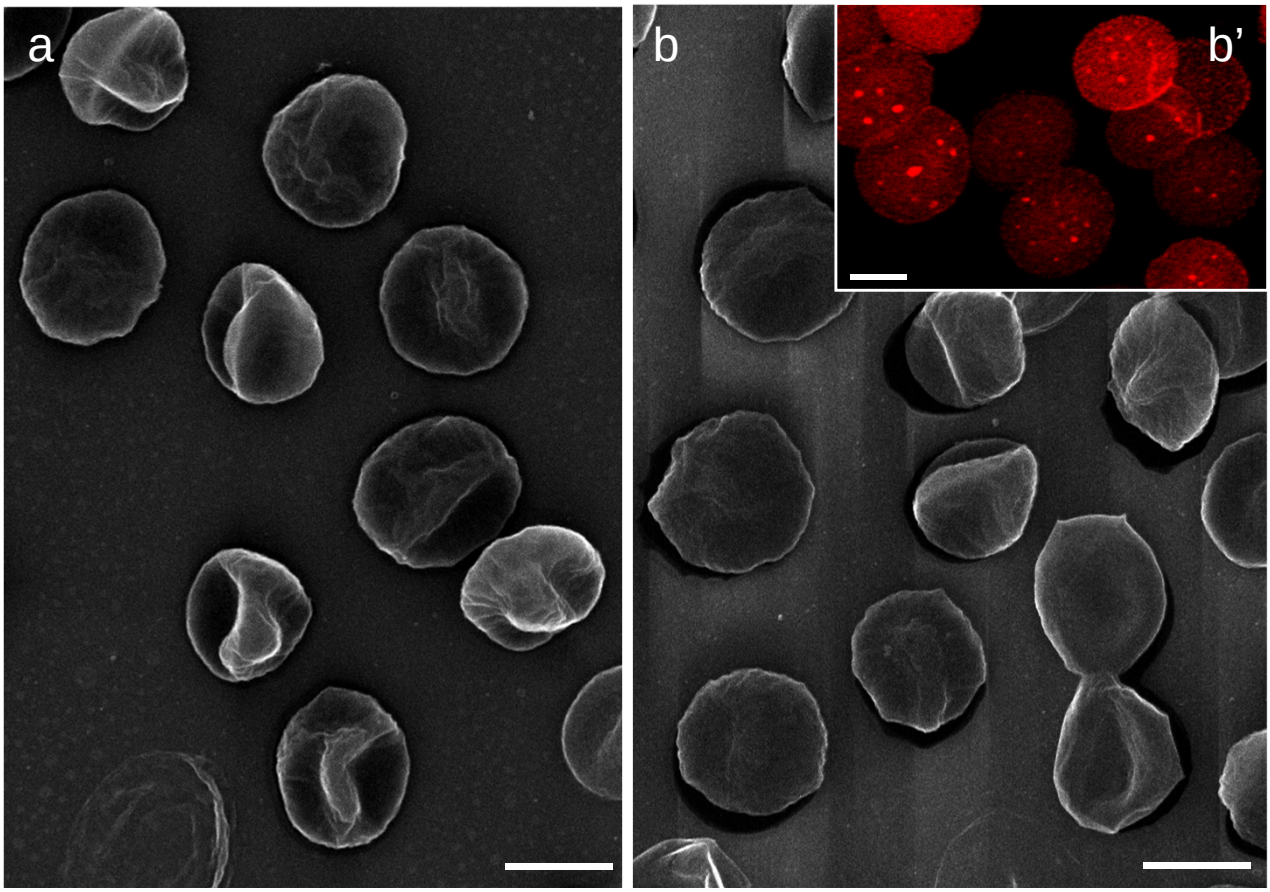

Figure S7 Scanning electron microscopy of erythrocytes attached on poly-L-lysine and labelled or not with Lysenin*. RBCs were incubated (b,b’) or not (a) with lysenin* in suspension, washed, fixed with dimethylsuberimidate (DMS) and processed either for scanning electron microscopy (a,b) or for confocal microscopy (b’). Notice that fixation with DMS preserves submicrometric domains (b’) and that lysenin* labelling does not detectably alter the smooth plasma membrane (compare panels a and b). Scale bar, 5µm.