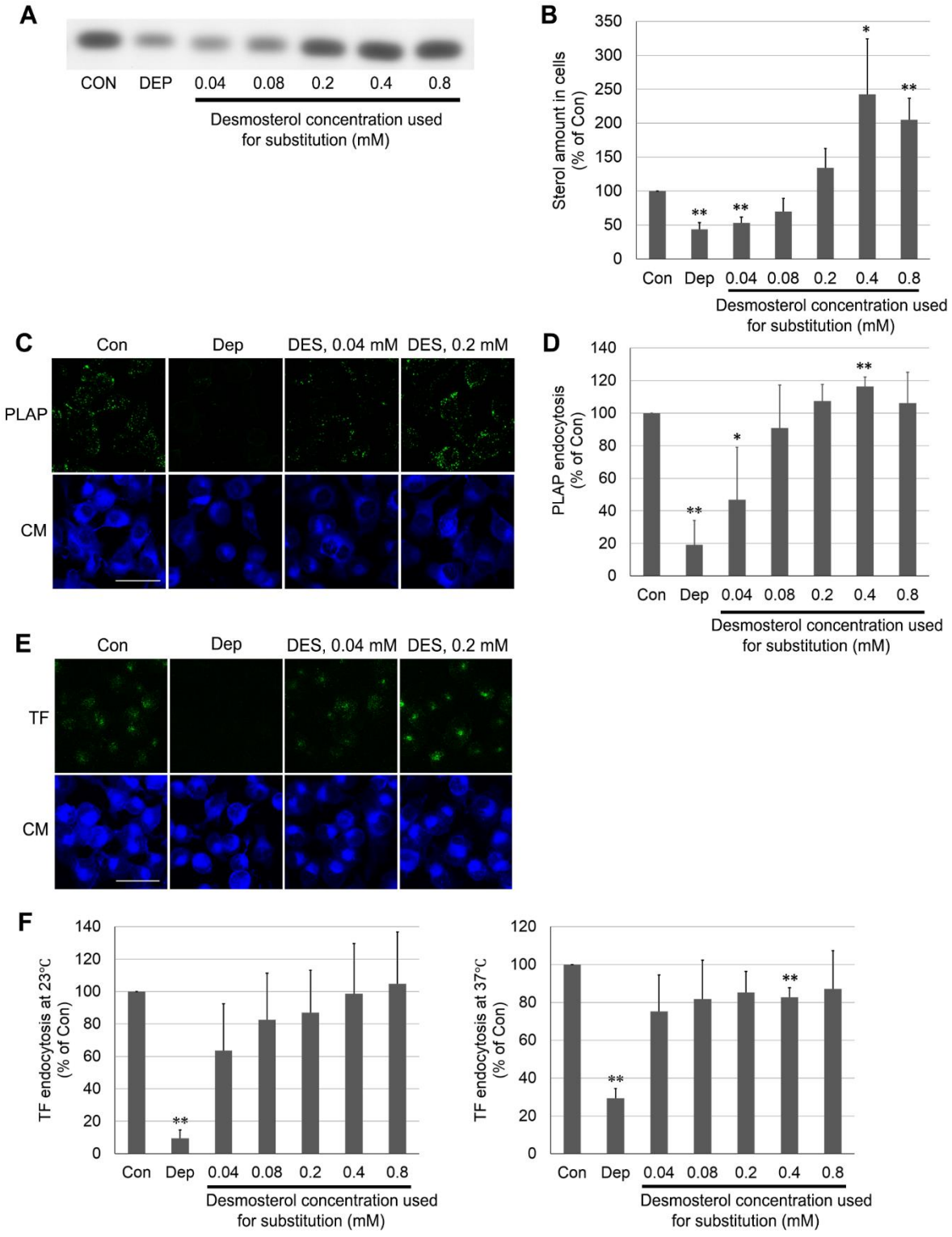

Fig. S1. Both PLAP- and TF-endocytosis are controlled by the amount of desmosterol. (A) HP-TLC plate showing cholesterol or mixture of cholesterol and desmosterol amounts in cells

after cholesterol depletion or substitution with desmosterol. (B) Quantified total sterol amounts determined from HP-TLC band intensity. (C) Z-stack micrographs of PLAP endocytosis after cholesterol depletion or substitution with desmosterol. After sterol manipulation in cells, PLAP was clustered with antibody (green fluorescence), and clustered PLAP was internalized by incubation of cells at 37^{o}C for 20 min. Cell surface antibodies were washed off and cell membranes were counter-stained using CellMask (CM, blue fluorescence). (D) Internalized PLAP puncta per cell. In each experiment 3-4 images (total cell number ≥ 50) were used for puncta quantitation. (E) Z-stack micrographs of TF endocytosis at 23^{o}C after sterol manipulation in cells. After cholesterol depletion or substitution with desmosterol, cells were stained with AF488-TF (green fluorescence), cell surface stain washed off by acid wash and cells were counter-stained with CellMask. (F) TF endocytosis in cells quantitated from integrated green fluorescence intensity (Left: 23^{o}C, right: 37^{o}C). Endocytosis in a total of 50 cells from 2 or more images was analyzed in each sample. In B, D and F, average (mean value) and standard deviation from three independent experiments are shown (i.e. n=3). Values normalized to untreated control cells. Con: untreated control cells, Dep: cholesterol depleted cells, DES: desmosterol. Scale bar: 50 µm. $^{*}P<0.05$; $^{**}P<0.01$ compared to 'Con' (unpaired, two-tailed Student's *t*-test).

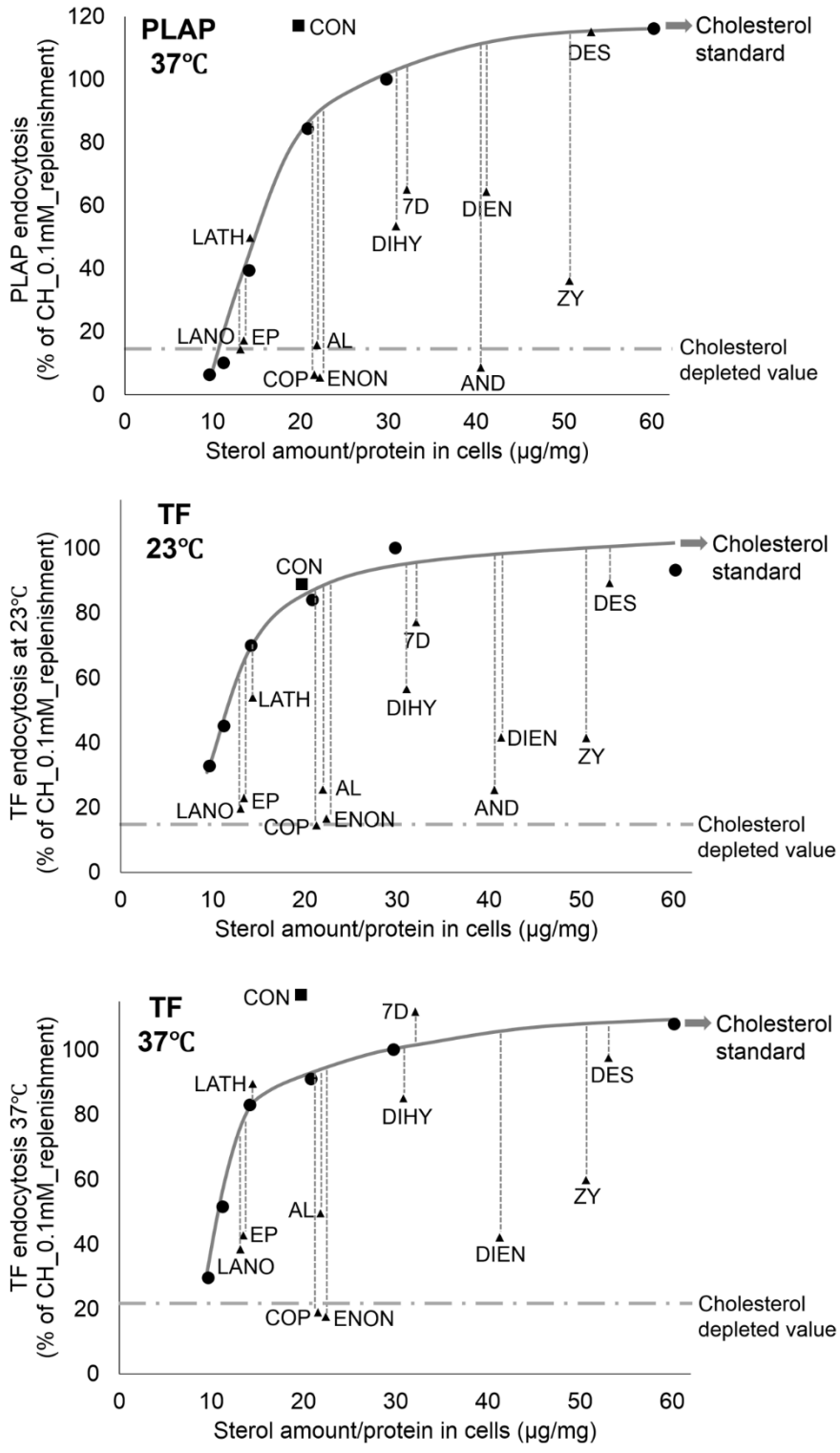

Fig. S2. Comparison of endocytosis level for different sterols compared to matching cholesterol levels. (●: cholesterol, ▲: substituted sterol). Gray dotted lines show the difference of endocytosis relative to cholesterol at the same total sterol amount. Dot-dash line shows baseline from cells after cholesterol depletion.

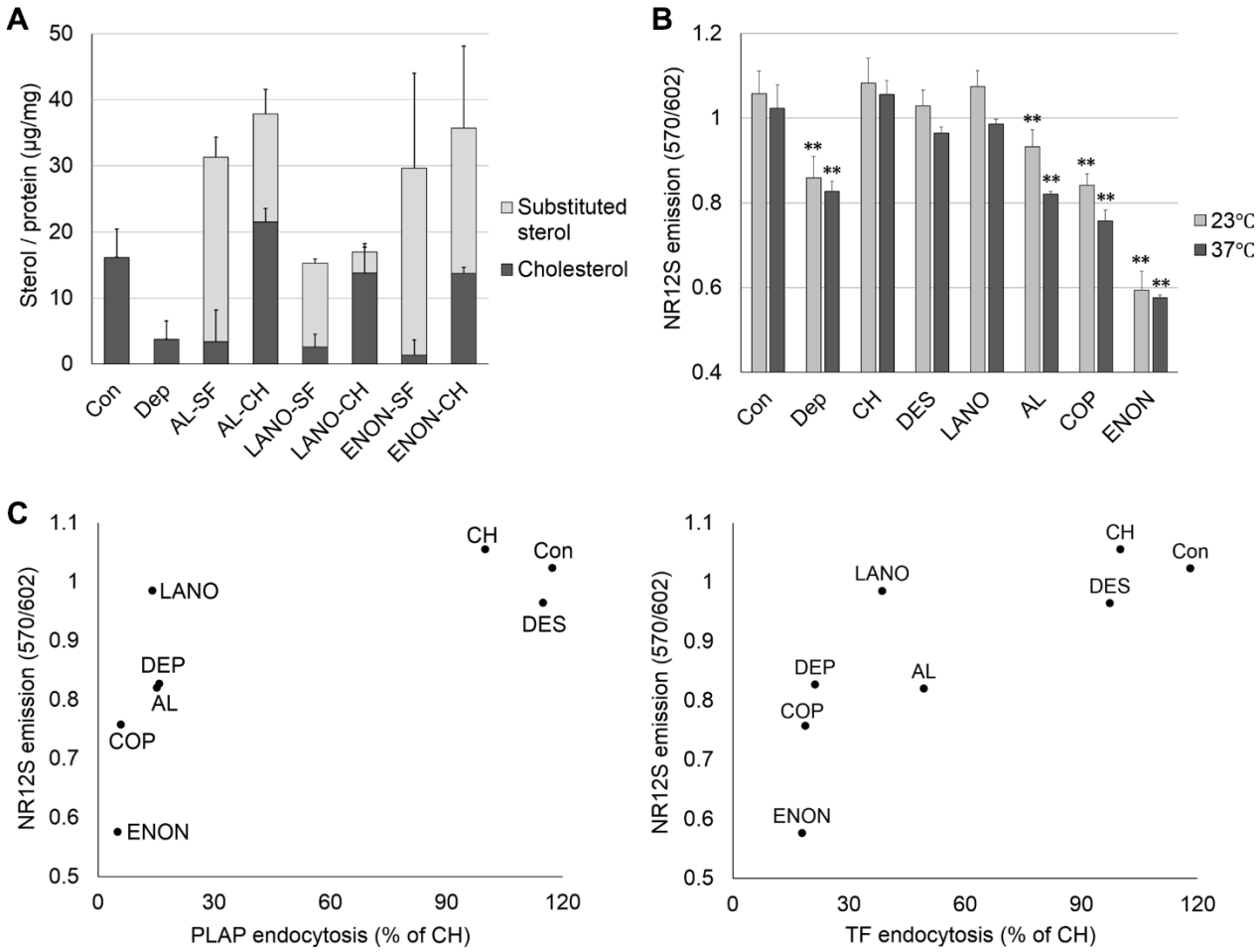

Fig. S3. Susceptibility of different sterols to removal by a second round of exchange and effect of exchange upon lipid packing.

(A) The amount of cholesterol and substituted sterol was determined by HP-TLC after cholesterol depletion or sterol substitution followed by a second step of substitution with cholesterol or serum free medium. AL: allocholesterol used in first substitution step, LANO: lanosterol used in first substitution step, ENON: 4-cholesten-3-one used in first substitution step, CH: cholesterol used in second substitution step; SF: serum-free media used in second substitution step control. Average (mean value) and standard deviations are shown (n=3, except n=2 for AL-SF and AL-CH). (B) NR12S emission ratio (570/602) in sterol-manipulated cells. After cholesterol-depletion or sterol substitution, 40 nM NR12S was added into the cells. The NR12S emission at 570 and 602 nm was measured (excitation at 520 nm) and emission intensity ratio of 570/602 was calculated. Con: untreated control cells, Dep: cholesterol depleted cells; CH: cholesterol replenished cells. Substitutions carried out with: DES: desmosterol, LANO: lanosterol, AL: allocholesterol, COP: coprostanol, ENON: 4-cholesten-3-one. Average (mean value) and standard deviations are shown (i.e. n≥3). **P<0.01 compared to 'Con' (unpaired, two-tailed Student's t-test). (C) Average NR12S emission ratio (570/602) vs. average endocytosis level of PLAP (at 37°C) or TF (at 37°C) (see Fig. 3).

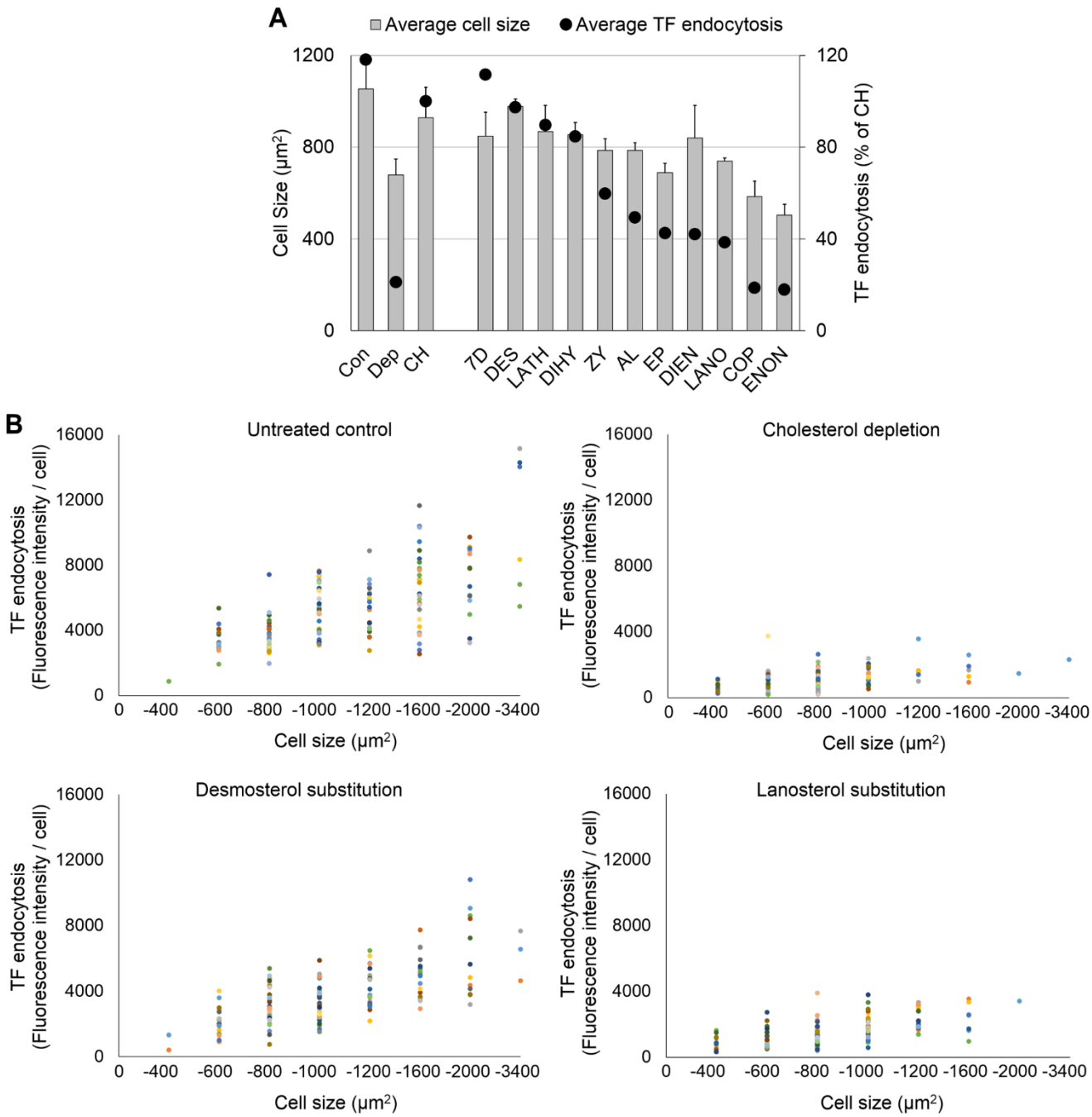

Fig. S4. Variation in apparent cell size and TF endocytosis. (A) Average (mean) apparent cell size (as judged by cross-sectional area) and TF endocytosis levels with different sterols. TF endocytosis values are relative to cholesterol-replenished cells. Gray bar: average cell size, black circle: average TF endocytosis. Average (mean value) and (for cell size) standard deviation are shown from independent experiments (see Figure 3D). (B) Graphs showing cell size and TF endocytosis levels for individual cells. Cells were divided into bins of different cell size ranges and AF488-TF fluorescence intensity in each cell is shown.

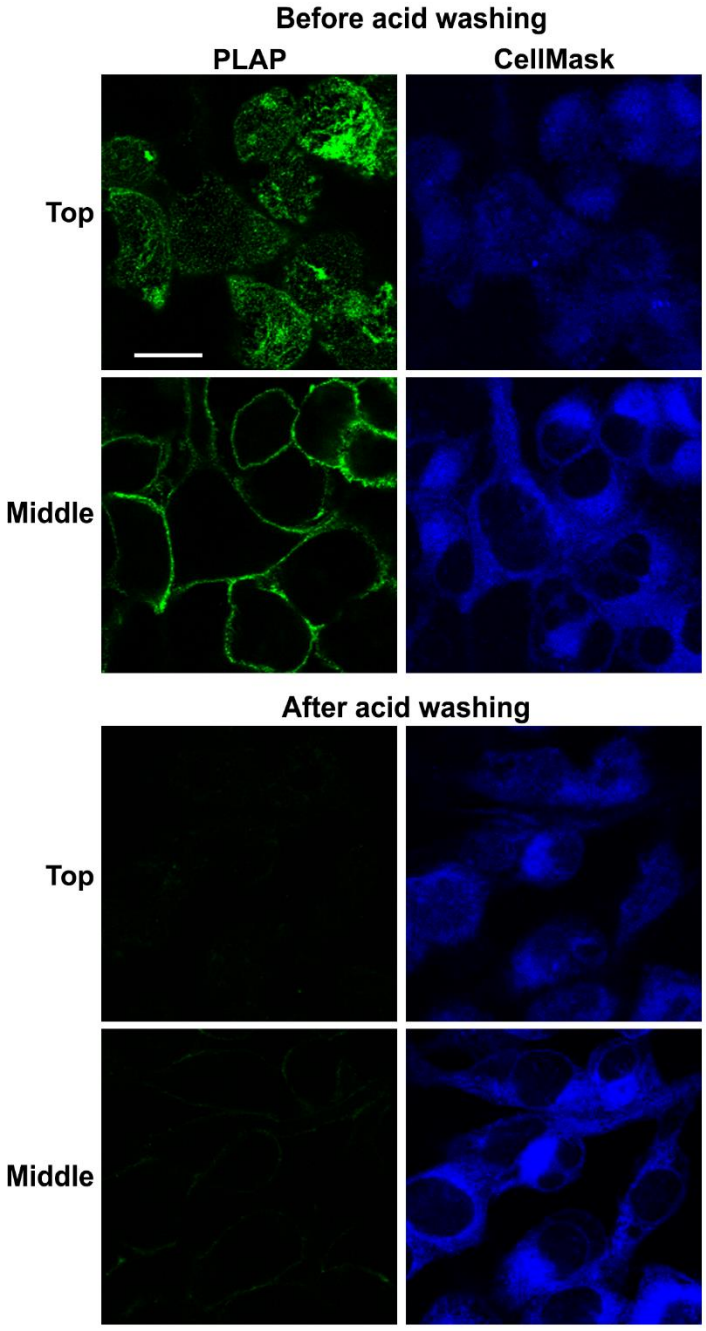

Fig. S5. Removal of PLAP and fluorescent antibody binding by acid washing. Images of PLAP immunofluorescence at 4°C (i.e without endocytosis). Upper four panels show cells without acid washing, lower four panels show cells after acid washing. Top refers to confocal sections at the upper surface of the cells. Middle refers to equatorial confocal sections. Scale bar: 20 µm.

	i) Raft forming ability			ii) 3β-hydroxyl group		iii) Double bond between 5-6 carbons		iv) Cholesterol tail structure	
	(+++)	(+)	(–)	O	X	O	X	Same	Different
7-Dehydrocholesterol	●			●		●		●	
Cholesterol	●			●		●		●	
Dihydrocholesterol	●			●			●	●	
Epicholesterol	●				●	●		●	
Lathosterol	●			●			●	●	
Desmosterol		●		●		●			●
Allosterol		●		●			●	●	
Choesta-4,6-dien-3-ol		●		●			●	●	
Lanosterol		●		●			●		●
Zymosterol		●		●			●		●
4-cholesten-3-one			●		●		●	●	
Androstenol			●	●		●			●
Coprostanol			●	●			●	●	

Table S1. Categories dividing sterols depend on the 4 properties. The sterols were divided into categories according to 4 kinds of properties; i) raft forming ability (Megha et al., 2006; Xu and London, 2000; Wang et al., 2004; Xu et al., 2001; Beattie et al., 2005): raft promoting sterols (+++), intermediate (+), or raft disrupting sterols (-), ii) 3β-hydroxyl group: with (O) or without (X), iii) double bond between position 5-6 carbons: with (O) or without (X), iv) cholesterol tail structure: having same tail structure as cholesterol (same) or different tail structure (different). These properties were analyzed for the relationship with endocytosis efficiency of PLAP and TF.