

**Supplemental Materials for “Transmembrane Protein (Perfringolysin O) Association with Ordered Membrane Domains (Rafts) Depends Upon the Raft-Associating Properties of Protein-Bound Sterol” by Qingqing Lin and Erwin London**

**Supplemental Figure 1. PFO interaction with model membrane vesicles containing various lipid compositions.** (A-B) 1:1 (mol:mol) SM/DMoPC with sterol; (C-D) 1:1 DSPC/DPhPC with sterol; and (E-F) 1:1 SM/DPhPC with sterol. (A), (C) and (E) Trp fluorescence of 55 nM PFO interacting with MLVs (500  $\mu$ M total lipid) in PBS pH 5.1. (B), (D) and (F) Acrylodan fluorescence of 25 nM acrylodan-labeled PFO interacting with MLVs (500  $\mu$ M total lipid) in PBS pH 5.1. For vesicles containing a mixture of coprostanol and epicholesterol, the molar ratio of coprostanol to epicholesterol is 1:1. Average (mean) values and S.D. values from triplicates are shown.

**Supplemental Figure 2. Detection of domain formation in vesicles containing various lipid compositions by FRET.** (A) 1:1 SM/DMoPC with 45 mol% sterols; (B) 1:1 DSPC/DPhPC with 40 mol% sterols; (C) 1:1 SM/DPhPC with 40 mol% sterols. Samples were composed of MLVs containing 500  $\mu$ M lipids in PBS pH 5.1. For vesicles containing a mixture of coprostanol and epicholesterol, the molar ratio of coprostanol to epicholesterol is 1:1. F samples contained both FRET donor (0.05 mol% pyrene-DPPE) and FRET acceptor (2 mol% Rho-DOPE). Fo samples only contained FRET donor (0.05 mol% pyrene-DPPE). The ratio of donor fluorescence in the presence of acceptor to that in its absence (F/Fo) is graphed. Average (mean) values and S.D. values from triplicates are shown. Abbreviations: chol = cholesterol; epichol = epicholesterol; cop = coprostanol.

**Supplemental Figure 3. F/Fo values for FRET detection of PFO raft affinity in vesicles with co-existing Lo and Ld domains.** (A) F/Fo values for LW peptide, CT-B and PFO in MLVs (500  $\mu$ M total lipid) composed of DMoPC with 45 mol% cholesterol or 1:1 DSPC/DMoPC with 45 mol% cholesterol. (B) F/Fo values for LW peptide, CT-B and PFO in MLVs (500  $\mu$ M total lipid) composed of DMoPC with 45 mol% 1:1 coprostanol/epicholesterol or 1:1 DSPC/DMoPC with 45 mol% 1:1 coprostanol/epicholesterol. (C) F/Fo values for LW peptide, CT-B and PFO in MLVs (500  $\mu$ M total lipid) composed of DMoPC with 45 mol% cholesterol or 1:1 SM/DMoPC with 45 mol% cholesterol. (D) F/Fo values for LW peptide, CT-B and PFO in MLVs (500  $\mu$ M total lipid) composed of DMoPC with 45 mol% 1:1 coprostanol/epicholesterol or 1:1 SM/DMoPC with 45 mol% 1:1 coprostanol/epicholesterol. (E) F/Fo values for LW peptide, CT-B and PFO in MLVs (500  $\mu$ M total lipid) composed of DMoPC with 40 mol% cholesterol or 1:1 DSPC/DPhPC with 40 mol% cholesterol. (F) F/Fo values for LW peptide, CT-B and PFO in MLVs (500  $\mu$ M total lipid) composed of DMoPC with 40 mol% 1:1 coprostanol/epicholesterol or 1:1 DSPC/DPhPC with 40 mol% 1:1 coprostanol/epicholesterol. (G) F/Fo values for LW peptide, CT-B and PFO in MLVs (500  $\mu$ M total lipid) composed of DMoPC with 40 mol% cholesterol or 1:1 SM/DPhPC with 40 mol% cholesterol. (H) F/Fo values for LW peptide, CT-B and PFO in MLVs (500  $\mu$ M total lipid) composed of DMoPC with 40 mol% 1:1 coprostanol/epicholesterol or 1:1 DSPC/DPhPC with 40 mol% 1:1 coprostanol/epicholesterol. Samples were prepared in PBS pH 5.1. 2 mol% NBD-DPhPE was used as FRET acceptor. F/Fo is ratio of donor fluorescence in the presence of FRET acceptor to that in its absence. For vesicles containing a mixture of coprostanol and epicholesterol (B, D, E and F) in which PFO

binding is not complete, corrected F/F<sub>0</sub> values are shown (see Fig. S4 and “Materials and Methods”). Average (mean) values and S.D. values from triplicates are shown.

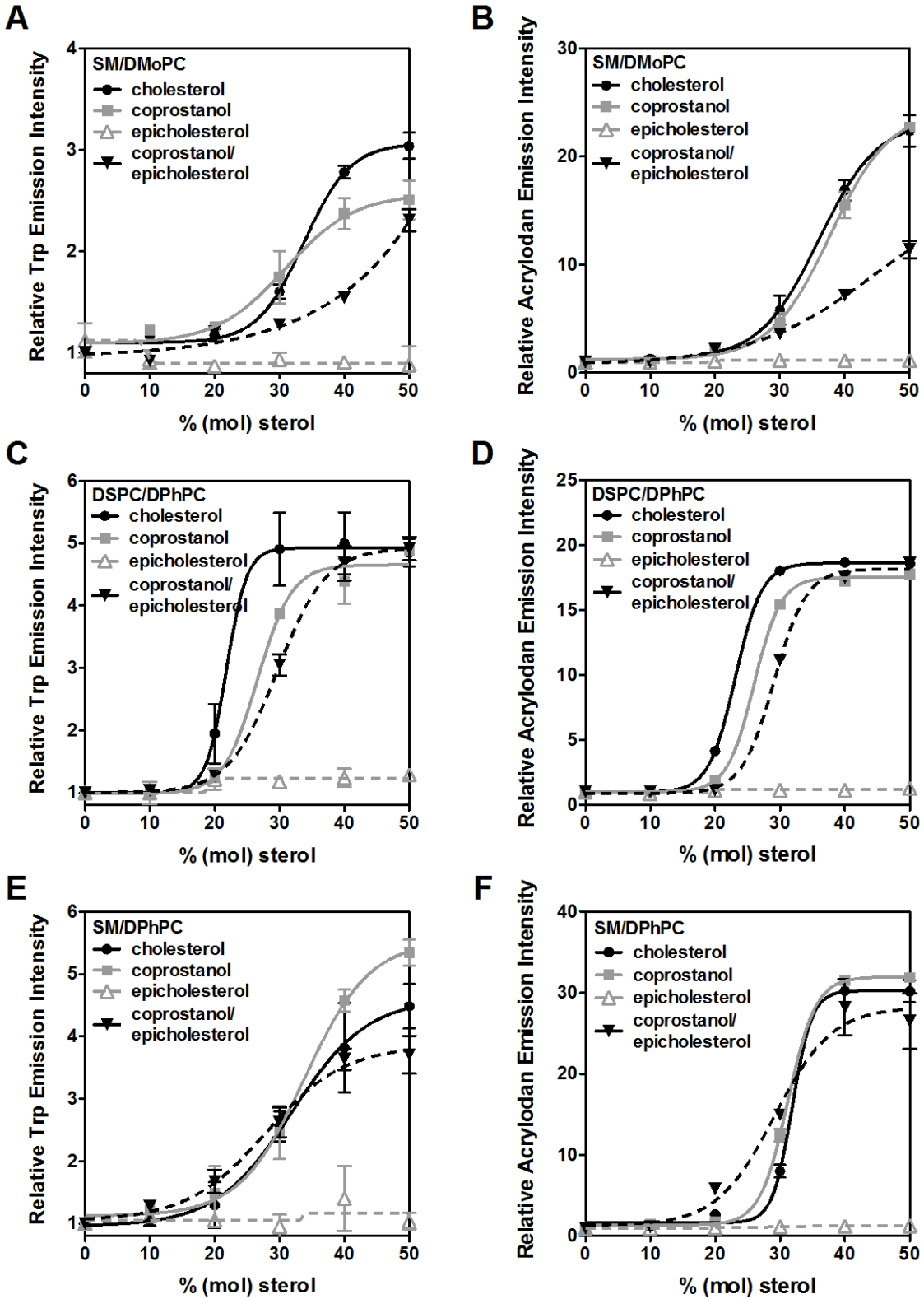
**Supplemental Figure 4. Binding of PFO to model membranes vesicles containing a mixture of coprostanol and epicholesterol.** Samples contained 1 ml of MLV (500 μM total lipid) and 25 nM BODIPY-labeled PFO in PBS pH 5.1. After centrifugation, supernatants were removed, and pellets were resuspended in 1 ml PBS pH 5.1. The BODIPY fluorescence was measured for both the supernatant and the pellet. Fraction of bound PFO =  $F_{\text{pellet}} / (F_{\text{pellet}} + F_{\text{supernatant}})$ . Average (mean) values and S.D. values from triplicates are shown. Abbreviations: epichol = epicholesterol; cop = coprostanol.

**Supplemental Figure 5. Raw data (uncorrected for incomplete binding of PFO) for FRET assay of PFO raft affinity in vesicles with co-existing Lo and Ld domains.** (A) F/F<sub>0</sub> values and (B)  $C_{\text{LoLd}}/C_{\text{Ld}}$  ratios for LW peptide, CT-B and PFO in MLVs (500 μM total lipid) composed of DMOPC with 45 mol% 1:1 coprostanol/epicholesterol or 1:1 DSPC/DMoPC with 45 mol% 1:1 coprostanol/epicholesterol. (C) F/F<sub>0</sub> values and (D)  $C_{\text{LoLd}}/C_{\text{Ld}}$  ratios for LW peptide, CT-B and PFO in MLVs (500 μM total lipid) composed of DMOPC with 45 mol% 1:1 coprostanol/epicholesterol or 1:1 SM/DMoPC with 45 mol% 1:1 coprostanol/epicholesterol. (E) F/F<sub>0</sub> values and (F)  $C_{\text{LoLd}}/C_{\text{Ld}}$  ratios for LW peptide, CT-B and PFO in MLVs (500 μM total lipid) composed of DMOPC with 40 mol% 1:1 coprostanol/epicholesterol or 1:1 DSPC/DPhPC with 40 mol% 1:1 coprostanol/epicholesterol. (G) F/F<sub>0</sub> values and (H)  $C_{\text{LoLd}}/C_{\text{Ld}}$  ratios for LW peptide, CT-B and PFO in MLVs (500 μM total lipid) composed of DMOPC with 40 mol% 1:1 coprostanol/epicholesterol or 1:1 SM/DPhPC with 40 mol% 1:1 coprostanol/epicholesterol. Samples were prepared in PBS pH 5.1. 2 mol% NBD-DPhPE was used as FRET acceptor. The F/F<sub>0</sub> is ratio of donor fluorescence in the presence of FRET acceptor to that in its absence. The  $C_{\text{LoLd}}/C_{\text{Ld}}$  ratio represents the average local acceptor concentration of acceptor around the donor (protein) in vesicles containing Lo and Ld domains ( $C_{\text{LoLd}}$ ) relative to that in a homogenous bilayer (DMoPC with 40 mol% or 45 mol% sterol) lacking domains ( $C_{\text{Ld}}$ ) (see “Materials and Methods”). Average (mean) values and S.D. values from triplicates are shown.

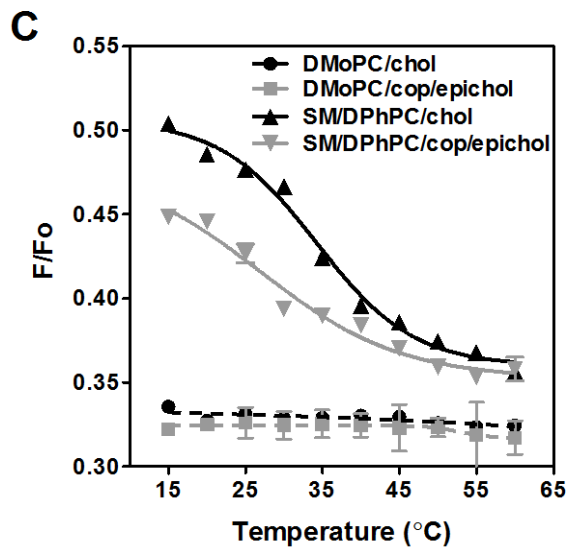
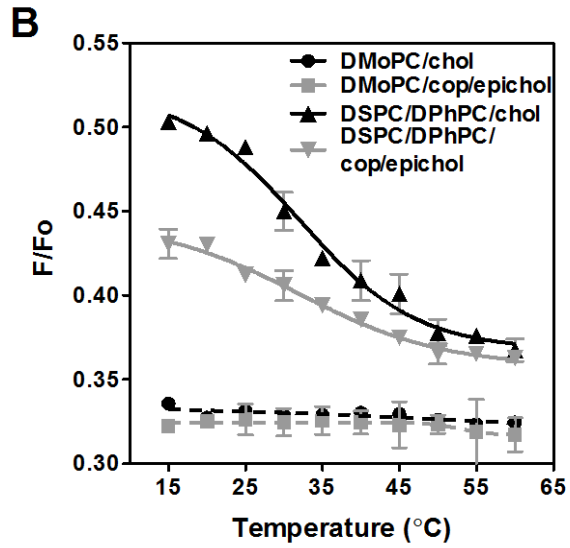
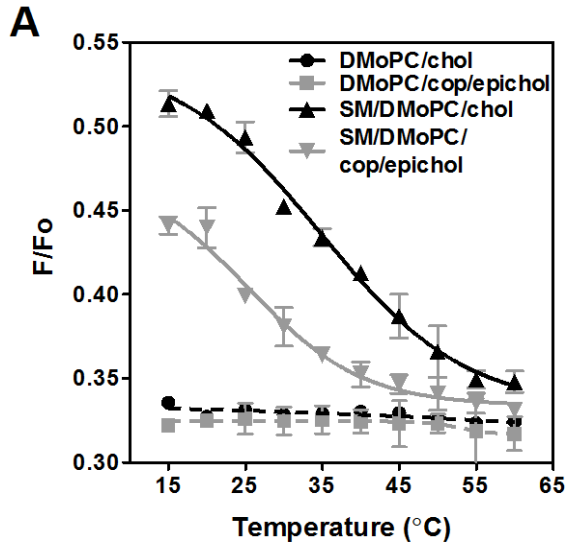
**Supplemental Figure 6. FRET-detected raft affinity of PFO in vesicles containing high-T<sub>m</sub> lipid, low-T<sub>m</sub> lipid and 1:2 coprostanol/epicholesterol** (A) F/F<sub>0</sub> values and (B)  $C_{\text{LoLd}}/C_{\text{Ld}}$  ratios for LW peptide, CT-B and PFO in MLVs (500 μM total lipid) composed of DMOPC with 45 mol% 1:2 coprostanol/epicholesterol or 1:1 DSPC/DMoPC with 45 mol% 1:2 coprostanol/epicholesterol. (C) F/F<sub>0</sub> values and (D)  $C_{\text{LoLd}}/C_{\text{Ld}}$  ratios for LW peptide, CT-B and PFO in MLVs (500 μM total lipid) composed of DMOPC with 40 mol% 1:2 coprostanol/epicholesterol or 1:1 DSPC/DPhPC with 40 mol% 1:2 coprostanol/epicholesterol. Samples were prepared in PBS pH 5.1. 2 mol% NBD-DPhPE was used as FRET acceptor. The F/F<sub>0</sub> is ratio of donor fluorescence in the presence of FRET acceptor to that in its absence. The  $C_{\text{LoLd}}/C_{\text{Ld}}$  ratio represents the average local acceptor concentration of acceptor around the donor (protein) in vesicles containing Lo and Ld domains ( $C_{\text{LoLd}}$ ) relative to that in a homogenous bilayer (DMoPC with 40 mol% or 45 mol% sterol) lacking domains ( $C_{\text{Ld}}$ ) (see “Materials and Methods”). F/F<sub>0</sub> and  $C_{\text{LoLd}}/C_{\text{Ld}}$  values shown are corrected for incomplete PFO binding to vesicles (see Fig. S4 and “Materials and Methods”). Average (mean) values and S.D. values from triplicates are shown.

**Supplemental Figure 7. Stick/skeletal representation of coprostanol.** Arrow indicates bend at 5-6 carbon-carbon bond in B ring. Carbon is black. Oxygen is red. Hydrogen (only a few are shown) is gray.

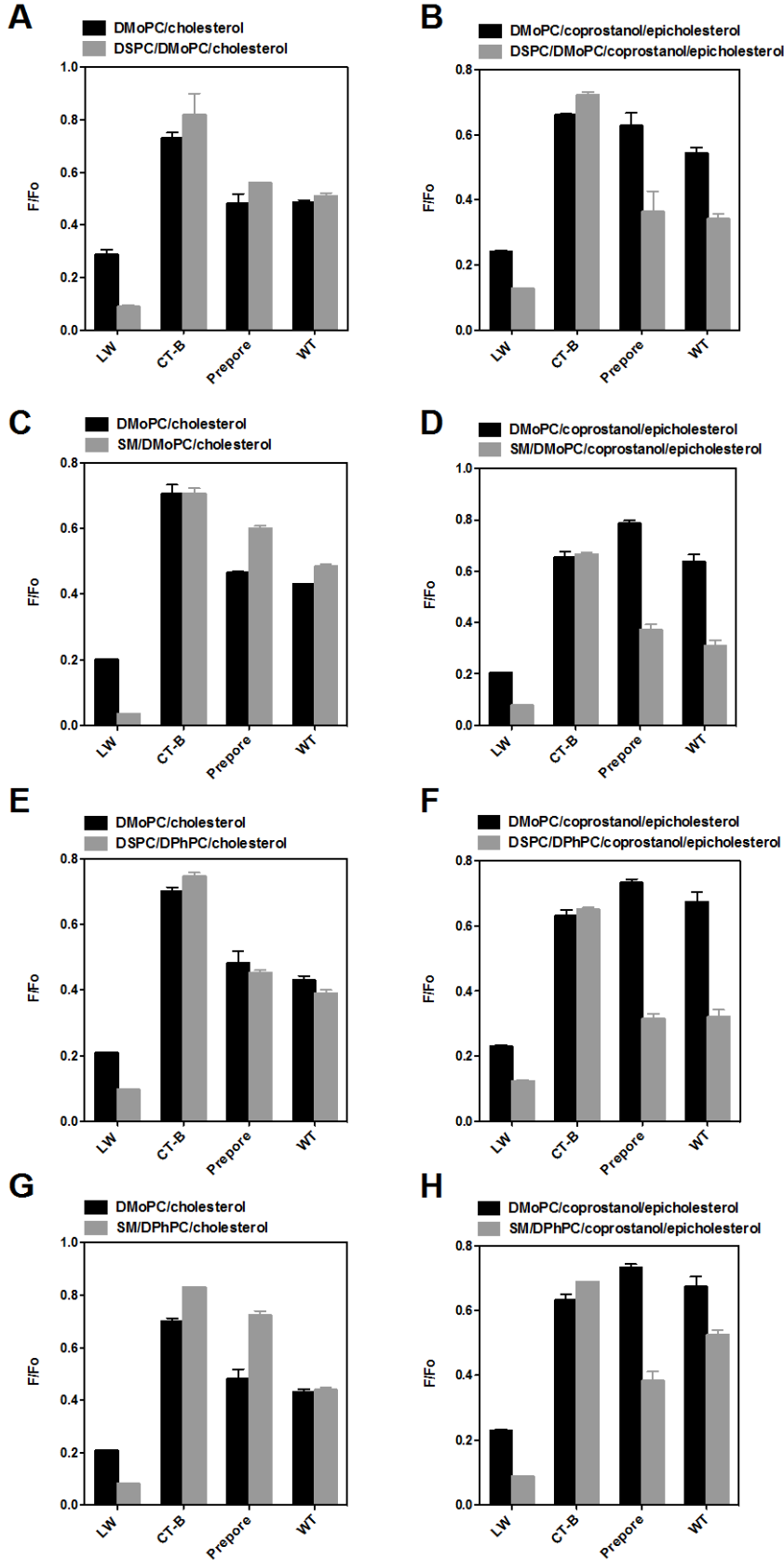
Supplemental Figure 1



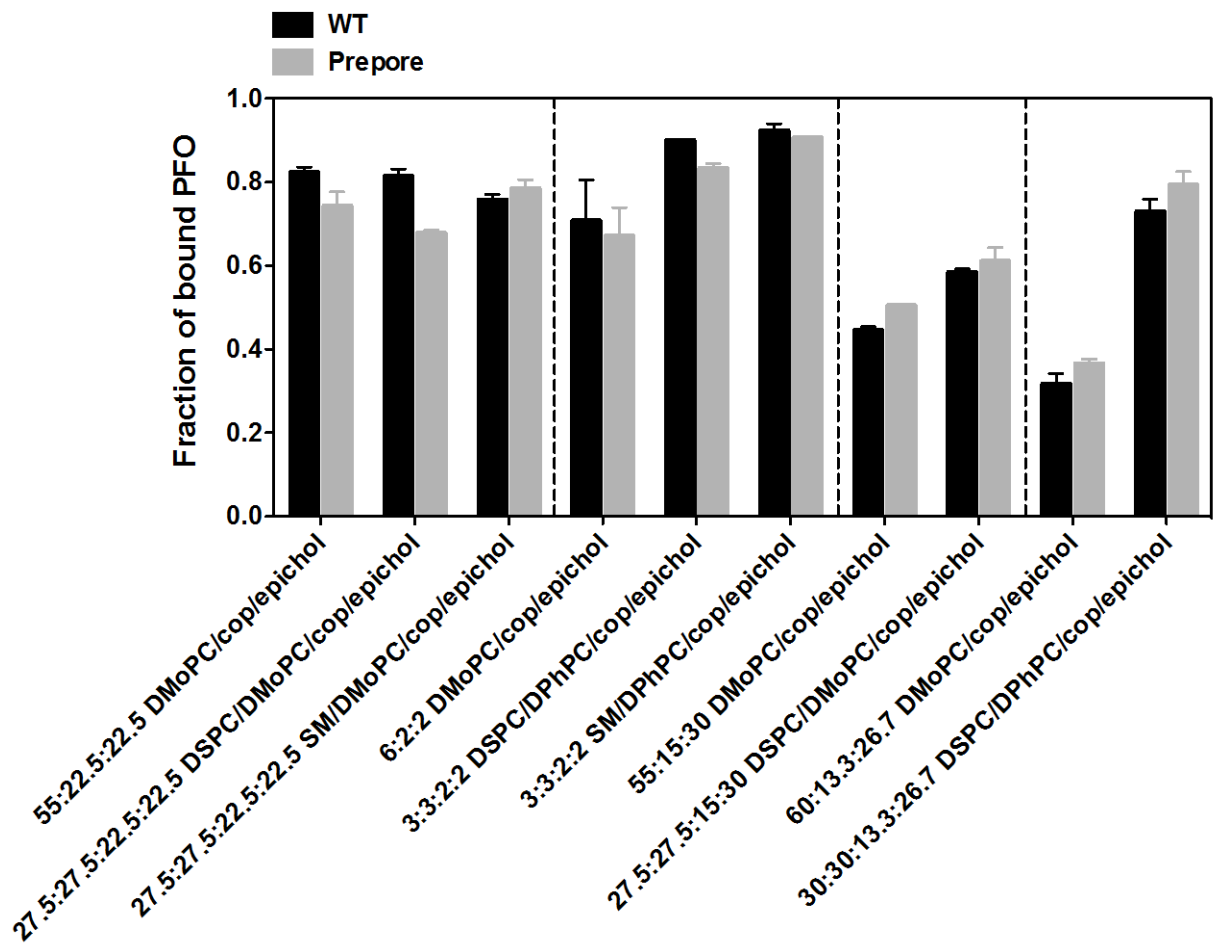
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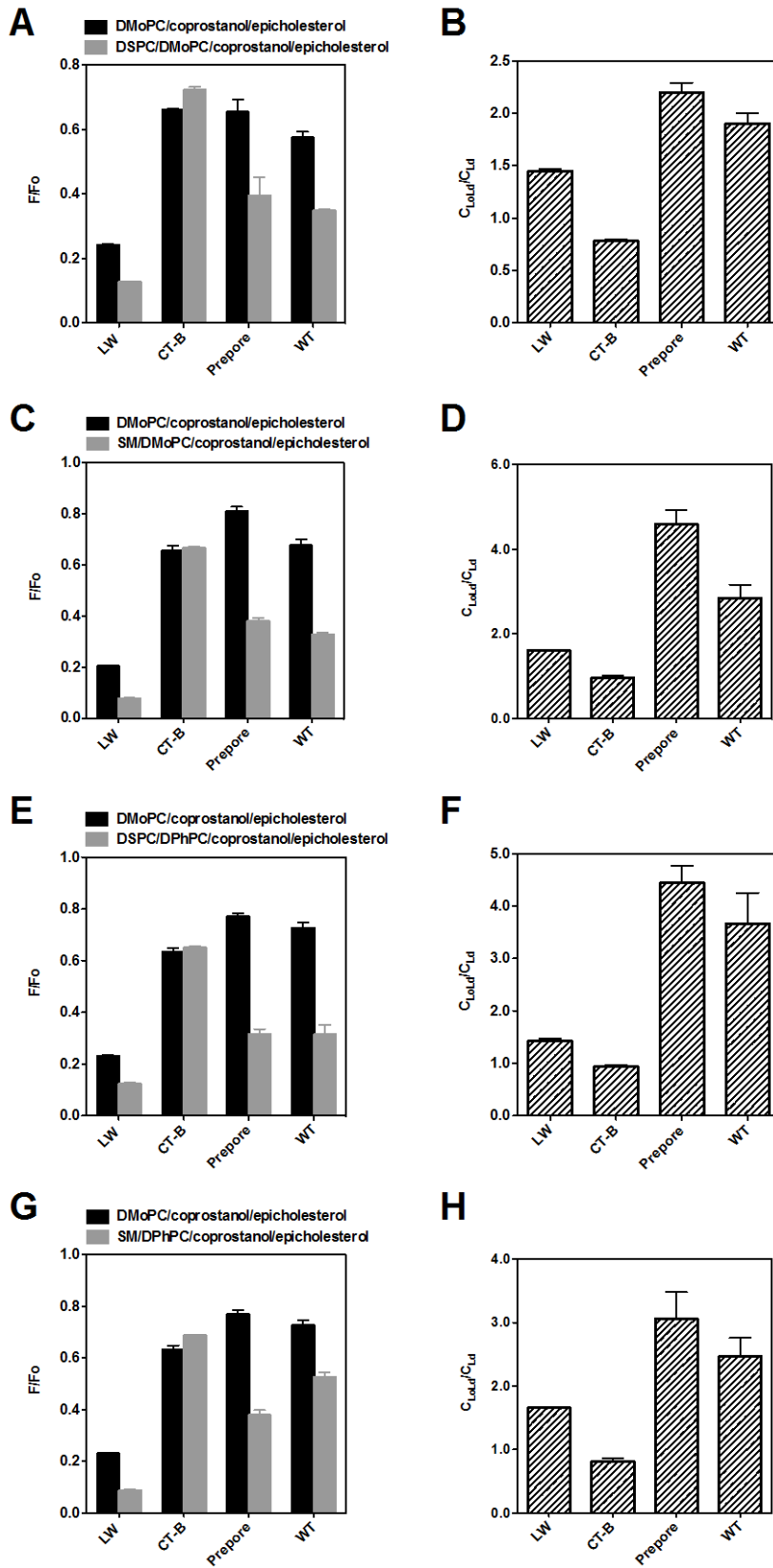
supplemental Figure 3



Supplemental Figure 4

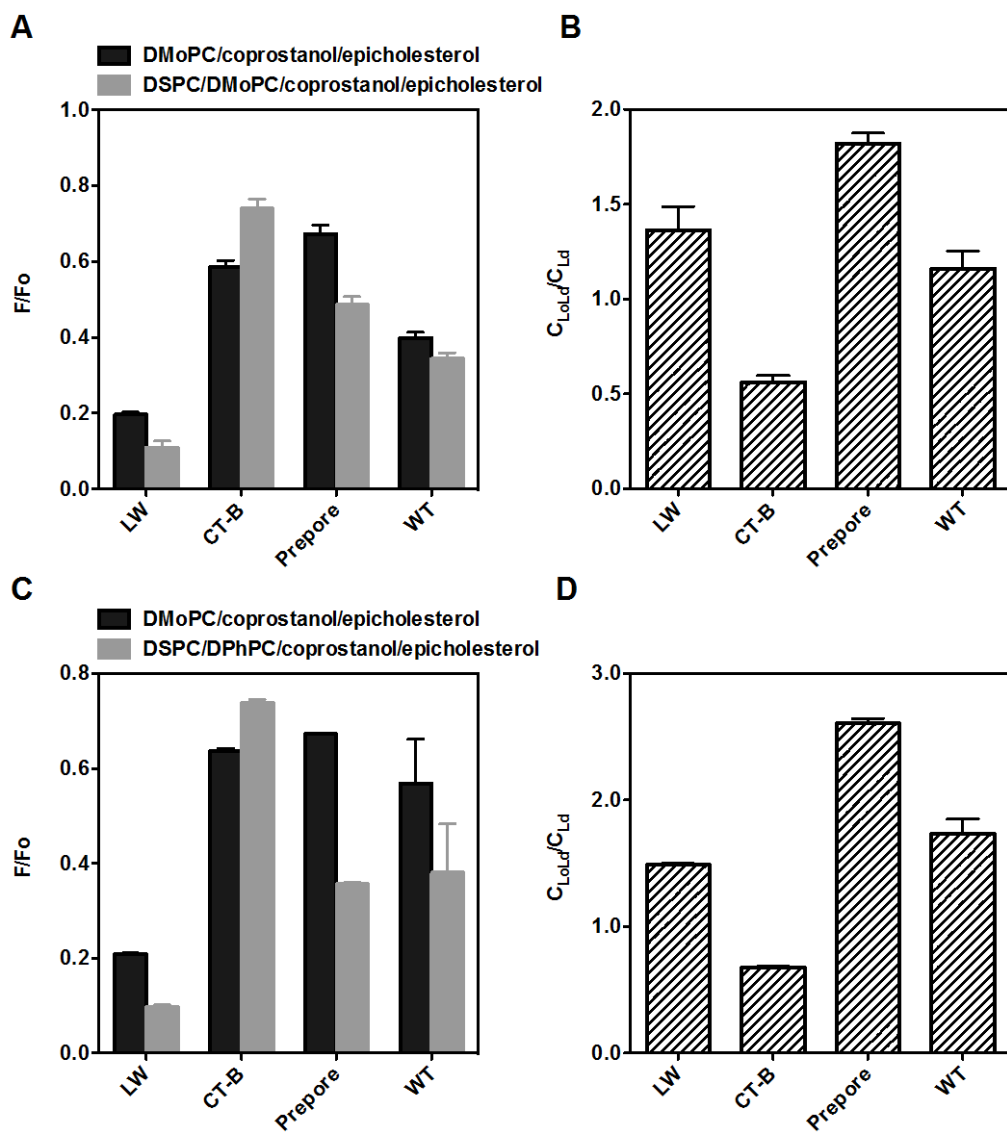


## Supplemental Figure 5





Supplemental Figure 6



Supplemental Figure 7

