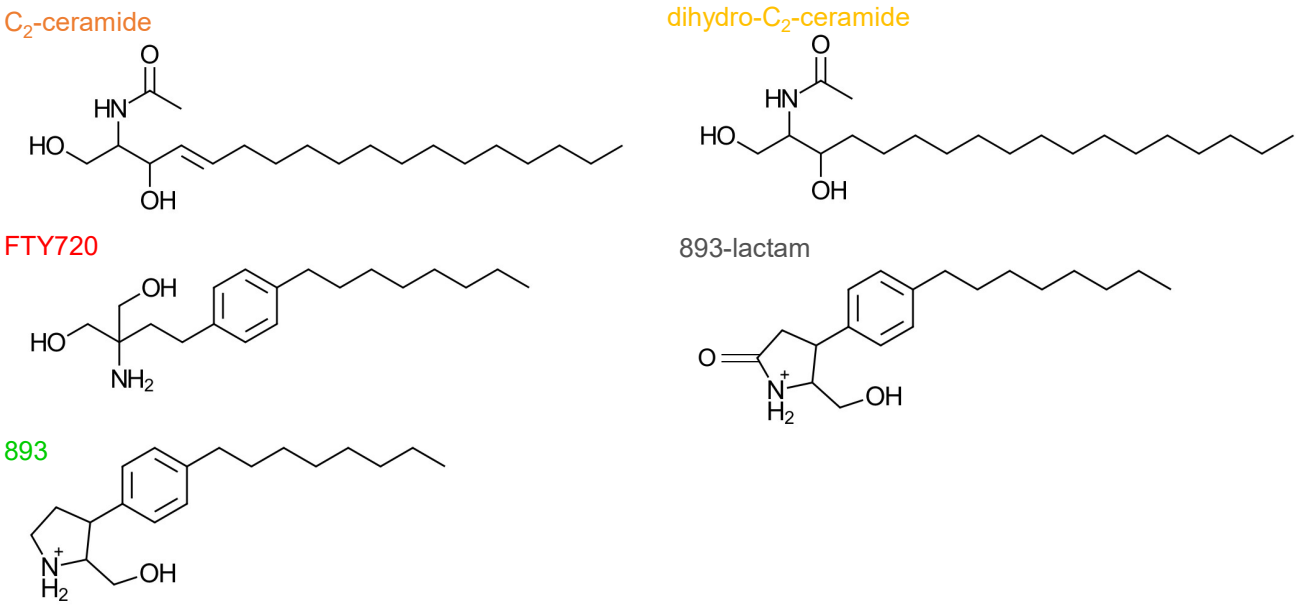
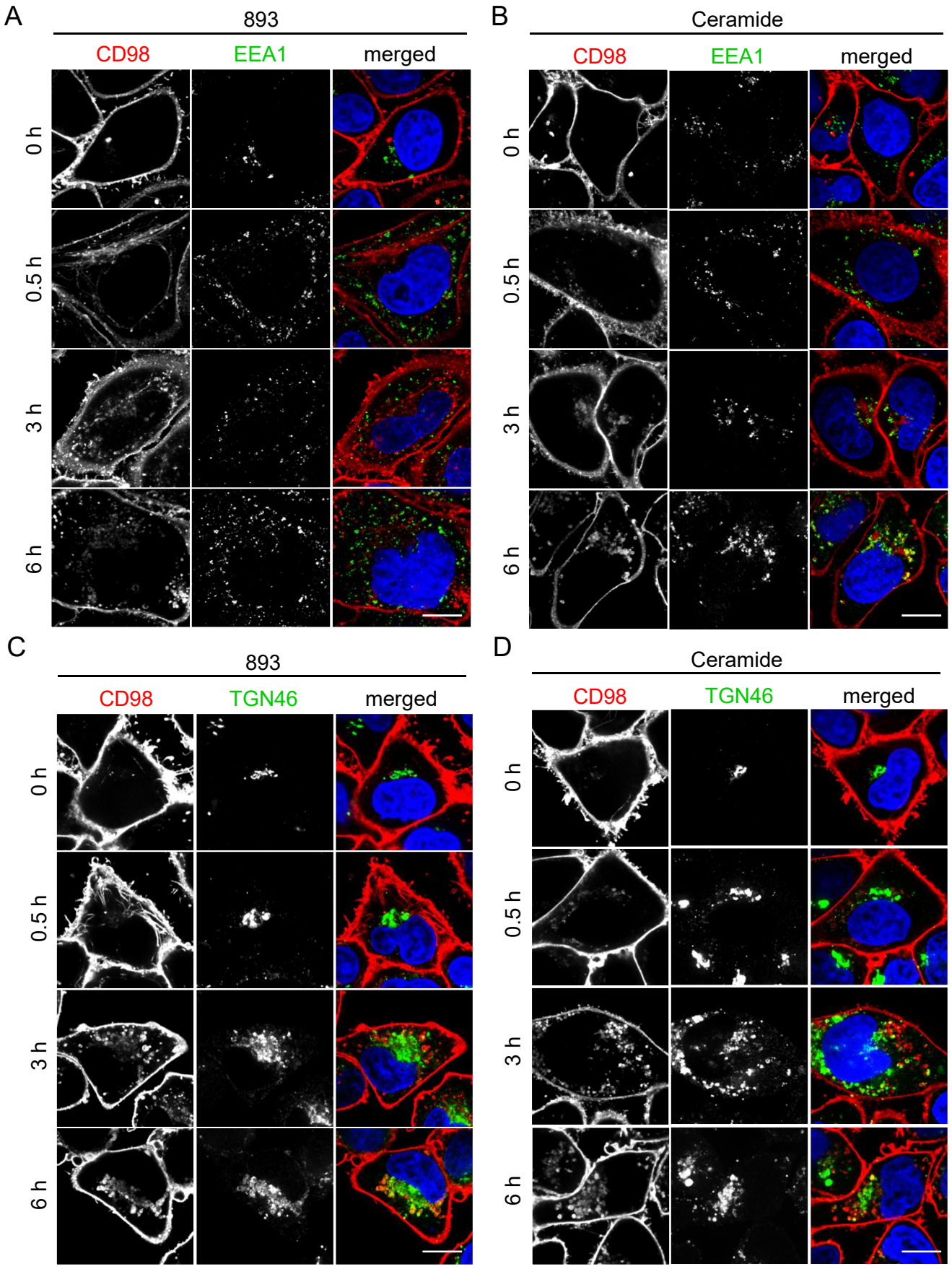
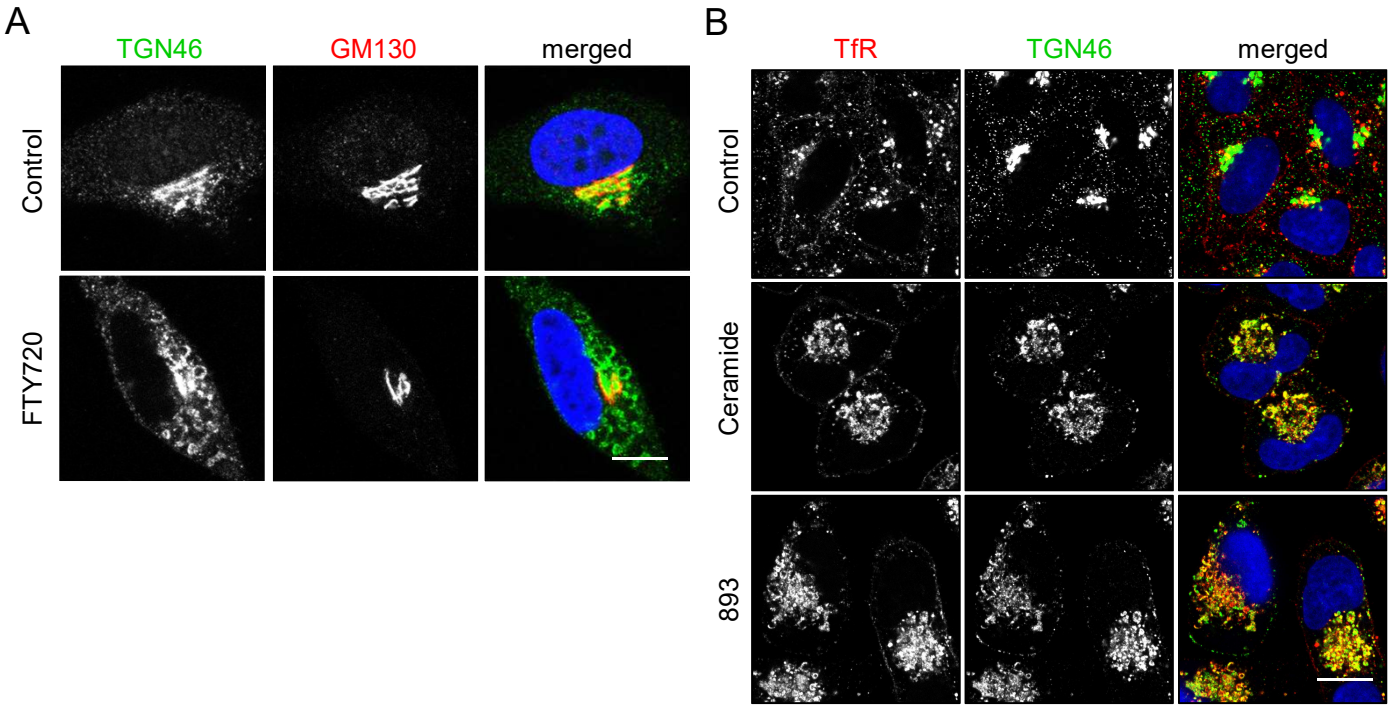


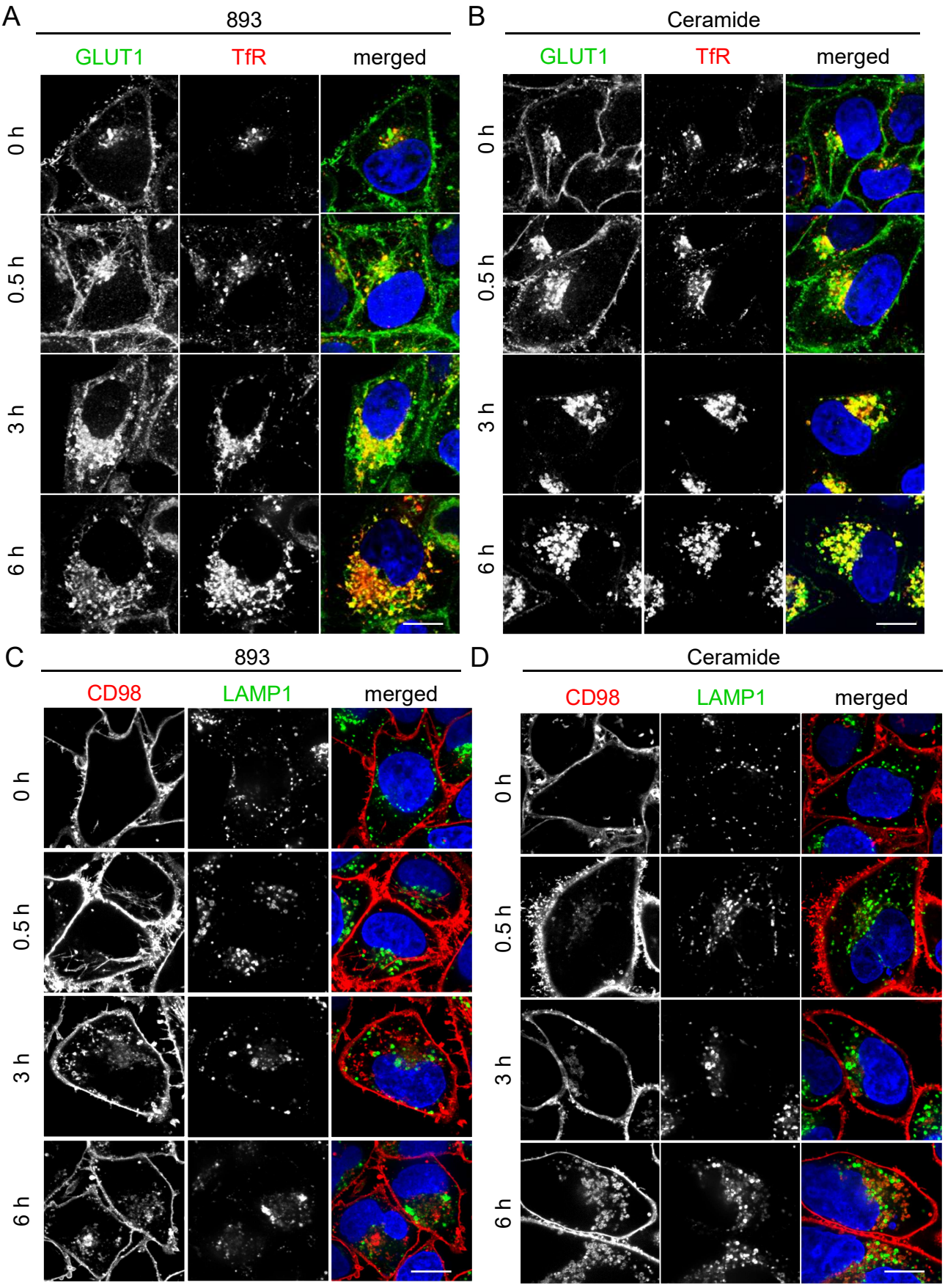
Supplemental Figure 1: Sphingolipids used in this study. Related to Figures 1-8. Structures of sphingolipids used in these studies.



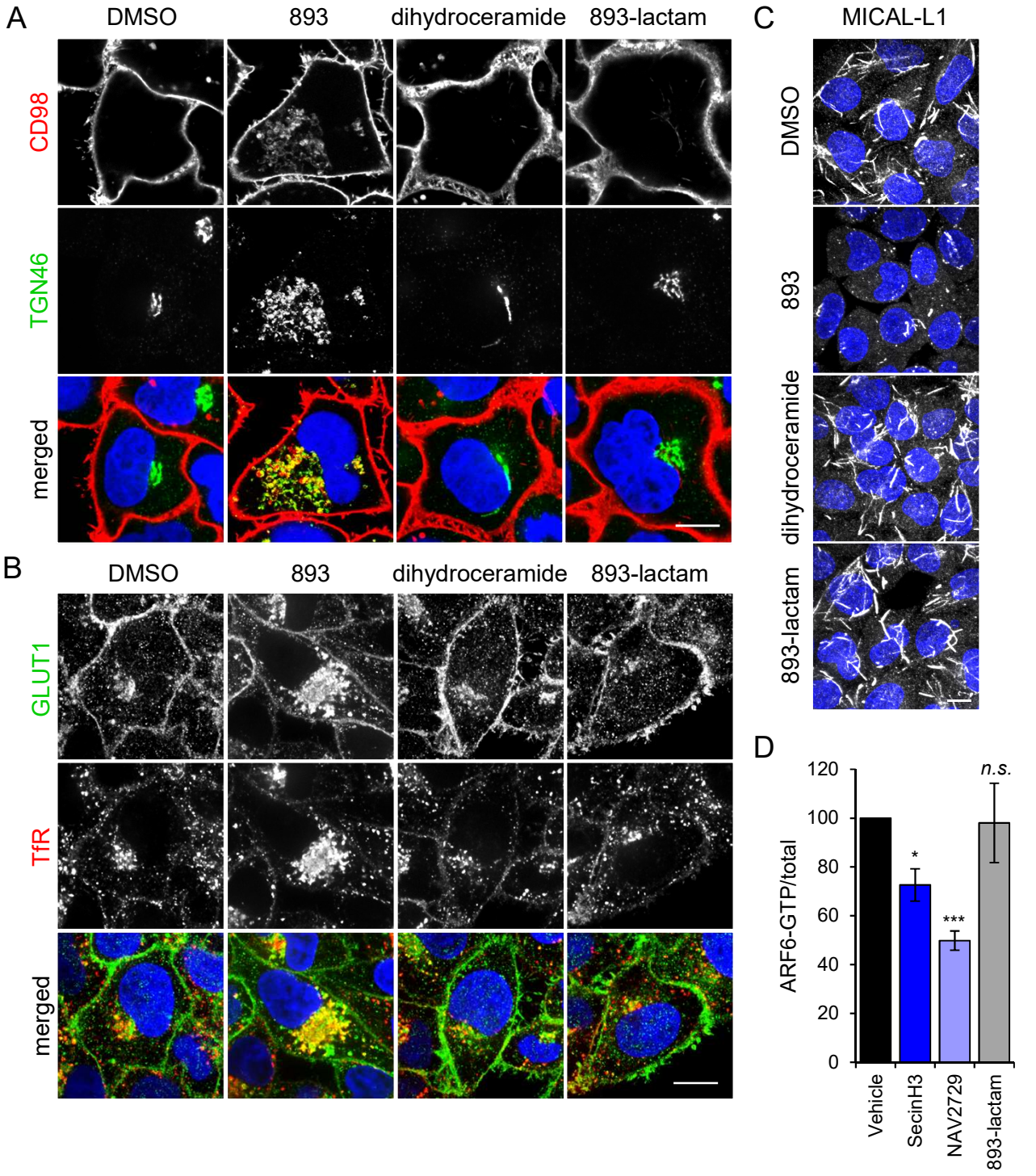
Supplemental Figure 2: Transporters do not enter the early endosome or colocalize with TGN46 until late timepoints. Related to Figure 1. (A-B) HeLa cells were treated with 893 (10 μM) or C_2-ceramide (50 μM) for 0.5, 3, or 6 h then stained for CD98 and EEA1. (C-D) HeLa cells were treated as in A&B and stained for CD98 and TGN46. Scale bar, 10 μm.



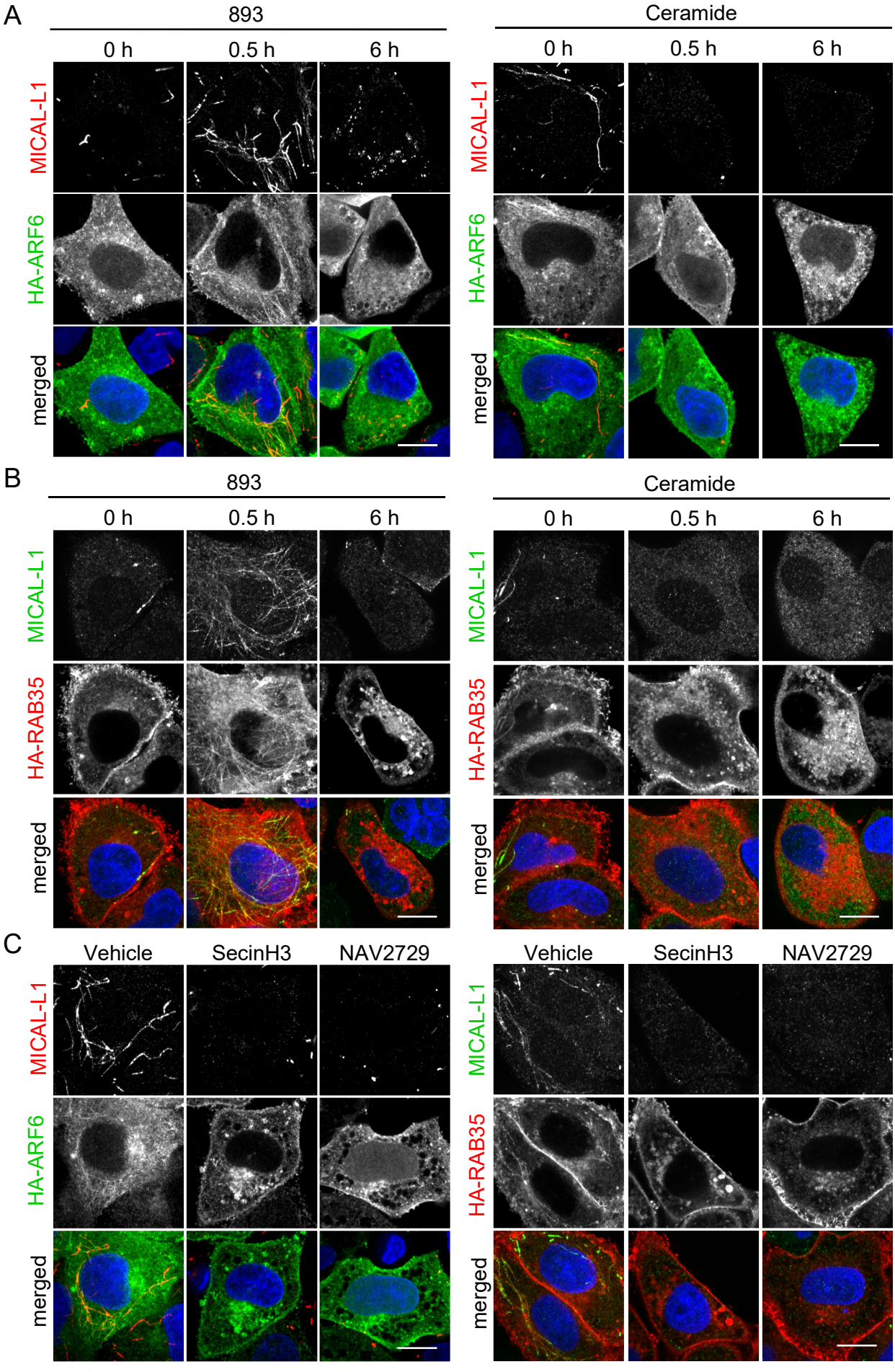
Supplemental Figure 3: TGN46 is mislocalized in sphingolipid-treated cells. Related to Figure 1. (A) HeLa cells were treated with FTY720 (5 μM) for 12 h and stained for TGN46 and GM130. (B) HeLa cells were treated with vehicle, C_2-ceramide (50 μM), or 893 (10 μM) for 12 h and stained for TfR and TGN46. Scale bar, 10 μm.



Supplemental Figure 4: Nutrient transporters are trapped with other recycled cargo at late timepoints. Related to Figure 1. (A-B) HeLa cells were treated with 893 (10 μM) or C_2-ceramide (50 μM) for 0.5, 3, or 6 h then stained for GLUT1 and the transferrin receptor (TfR). (C-D) HeLa cells were treated as in A&B and stained for CD98 and LAMP1. Scale bar, 10 μm.



Supplemental Figure 5: Structurally-similar sphingolipids that do not activate PP2A or reduce ARF6-GTP levels fail to cause nutrient transporter trafficking defects. Related to Figure 5. (A-C) HeLa cells were treated with 893 (10 μM), C_2-dihydroceramide (50 μM), or 893-lactam (10 μM) for 12 h and then stained for CD98 and TGN46 (in A) or for GLUT1 and TfR (in B) or for MICAL-L1 (in C). (D) ARF6-GTP levels normalized to total in HeLa cells treated with SecinH3 (30 μM), NAV2729 (25 μM), or 893-lactam (10 μM) for 3 h. Means ± SEM shown, n ≥ 4 for SecinH3 and NAV2729; n = 2 for 893-lactam. Using ordinary one-way ANOVA, n.s. = not significant; * = p≤ 0.05; *** = p≤ 0.001. Dunnett's test was used to correct for multiple comparisons.



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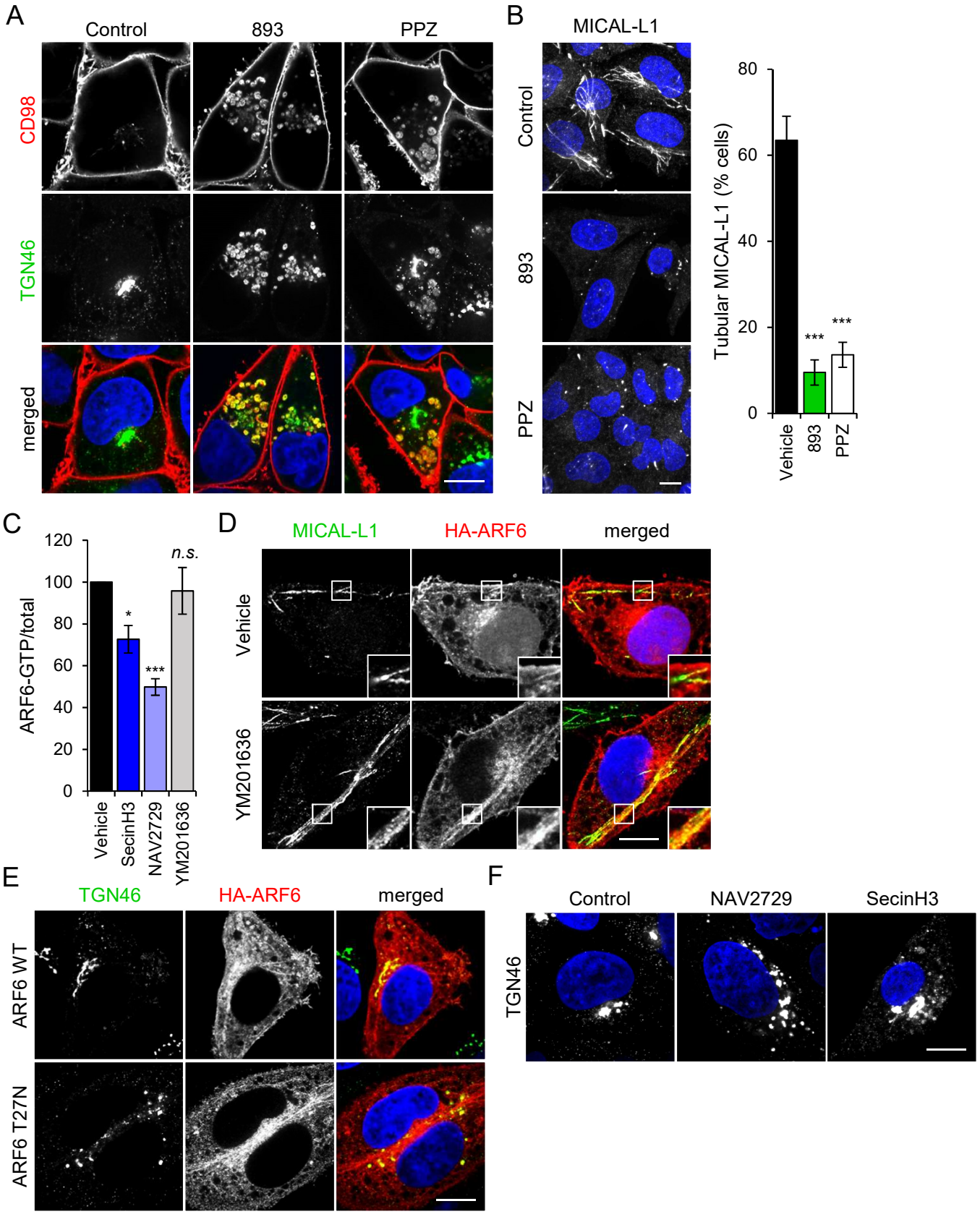
Supplemental Figure 6: ARF6 inhibition by sphingolipids is sufficient to block recruitment of ARF6, RAB35, and their scaffold MICAL-L1 to the tubular recycling endosome. Related to Figure 6. (A) HeLa cells transfected with HA-ARF6 were treated with 893 (10 μM) or C_2-ceramide (50 μM) for 0.5 or 6 h and then stained for MICAL-L1 and HA tag. (B) HeLa cells transfected with HA-RAB35 were treated with 893(10 μM) or C_2-ceramide (50 μM) for 0.5 or 6 h and then stained for MICAL-L1 and HA tag. (C) HeLa cells transfected with HA-ARF6 or HA-RAB35 were treated with SecinH3 (30 μM) or NAV2729 (12.5 μM) for 6 h and then stained for MICAL-L1 and HA tag. Scale bar, 10 μm.



Supplemental Figure 7: PP2A activation or ARF6 inhibition but not PIKfyve inhibition is sufficient to disrupt endocytic recycling. Related to Figure 6. (A-B) HeLa cells were treated with 893 (10 μM) or perphenazine (PPZ; 12.5 μM) for 16 h and then stained for CD98 and TGN46 (A) or MICAL-L1 (B). The percent of cells per field of view with tubular MICAL-L1 staining is shown in (B). (C) ARF6-GTP normalized to total in HeLa cells treated with SecinH3 (30 μM), NAV2729 (25 μM), or YM201636 (800 nM) for 1 h. (D) HeLa cells transfected with HA-ARF6 were treated with YM201636 (800 nM) for 1 h and then stained for MICAL-L1 and HA tag. (E) HeLa cells transfected with HA-ARF6 wildtype (WT) or the dominant negative ARF6 mutant (HA-ARF6 T27N) were stained for TGN46 and HA tag. (F) HeLa cells were treated with SecinH3 (30 μM) or NAV2729 (12.5 μM) for 16 h and then stained for TGN46. Scale bar, 10 μm. Means ± SEM shown (B,C), n ≥ 4. Using ordinary one-way ANOVA (B,C), n.s. = not significant; * = p≤ 0.05; ** = p≤ 0.01; *** = p≤ 0.001. Dunnett's test was used to correct for multiple comparisons.