Figure S1, related to Figure 1. Correlation scatter plots illustrating individual lipid species from various classes that exhibited statistically significant correlations to age. (A-B) Various species of O-acyl-ω-hydroxy fatty (OAHFA) acids displayed significant positive correlations to age; while **(C)** several poly-unsaturated phospholipids showed significant negative correlations to age. **(D)** Species of wax esters containing oleic-acid (FA 18:1) as the fatty acid moieties were positively correlated with age. Only lipid species demonstrating statistically significant ($p<0.05$) correlations with the respective clinical parameters were shown. Red eclipse indicates region within 95% confidence interval of the correlating parameters.

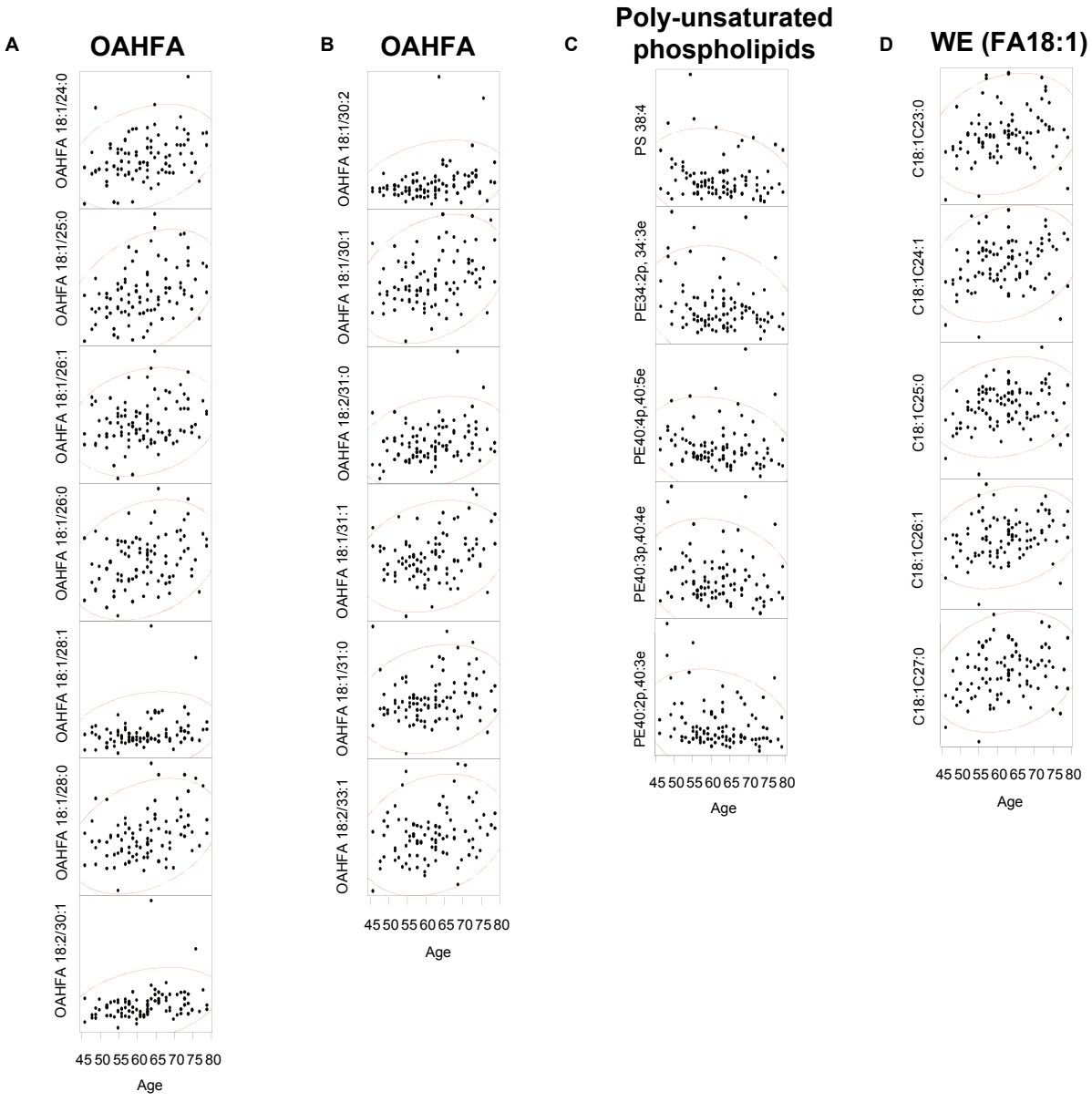


Figure S2, related to Figure 1. Heatmaps illustrating the correlations between lipid levels in tears with dry-eye associated clinical parameters including the Schirmer's length (Schir I), tear breakup time (TBUT), modified ocular surface disease index (OSDI) and Baylor's score for corneal staining. Correlation coefficients were plotted with red indicating a positive correlation and blue indicating a negative correlation. Statistical significance was denoted by the respective symbols. Correlations between **(A)** normalized levels and **(B)** molar concentrations of lipid classes with clinical parameters, respectively. The correlations between normalized levels of individual lipid species from selected lipid classes including **(C)** cholesteryl esters, **(D)** phosphatidylcholines, **(E)** phosphatidic acids and **(F)** phosphatidylglycerols were also presented. Individual species of PC, PA and PG were negatively correlated with Schir and TBUT; while positively correlated with OSDI and Baylor. The observed trend was consistent across the respective lipid classes, indicating that elevated levels of these species might be associated with dry eye disease pathogenesis.
0.05<p<0.10; *p<0.05; ** p<0.01; *** p<0.001.

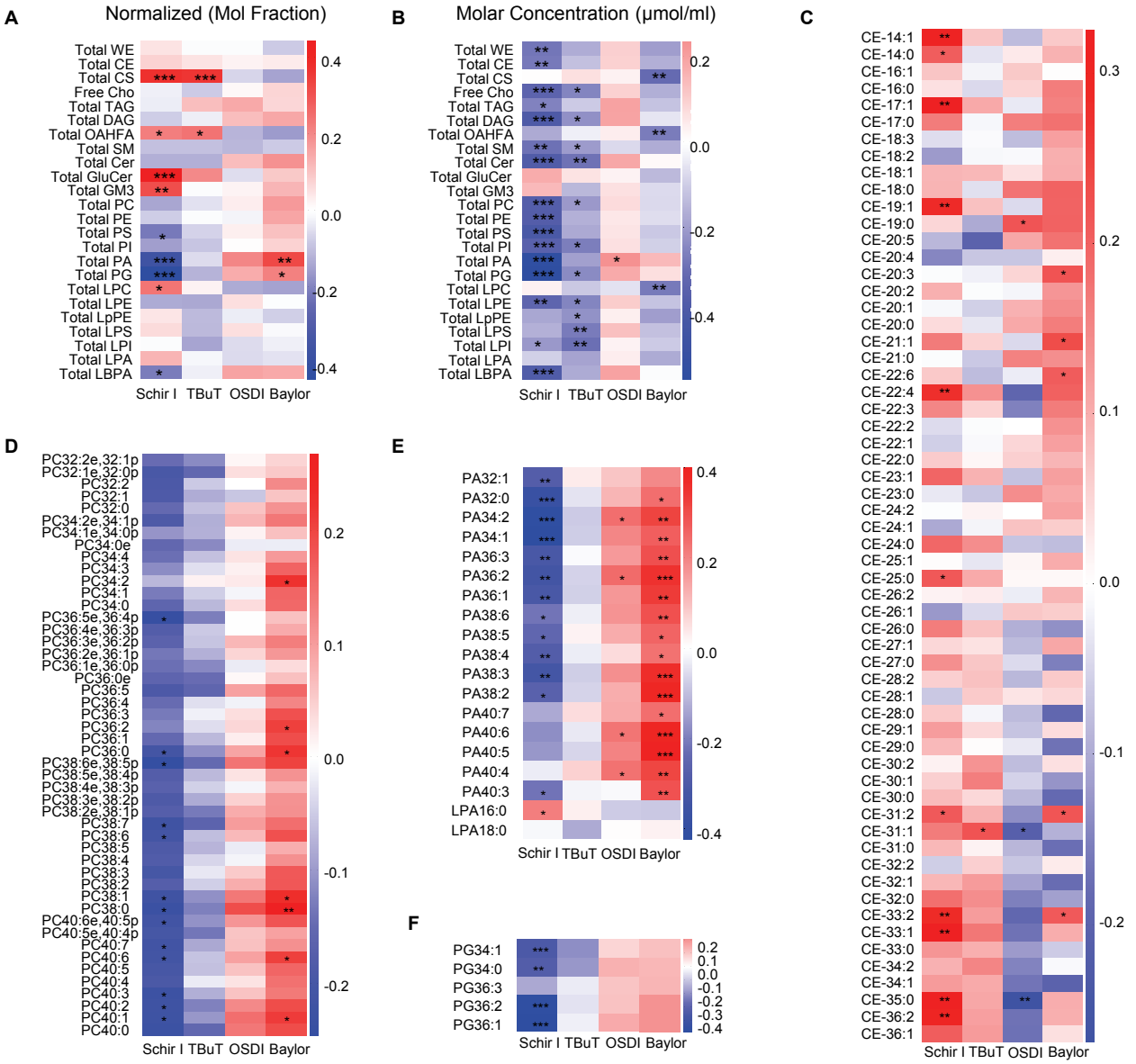


Figure S3, related to Figure 2. Clinical classification of study cohort. (A) Schematic diagram illustrating the stratification of subjects into various clinical groups. The comparison of dry eye-associated clinical parameters between various clinical groups including the **(B)** modified ocular surface disease index (OSDI); **(C)** Schirmer's length (Schir I); **(D)** tear breakup time (TBUT); **(E)** central corneal staining; **(F)** inferior corneal staining; **(G)** Baylor's score for corneal staining; **(H)** age and **(I)** total molar concentration of lipids detected in tears. Symptomatic score was used primarily to define dry eye as symptoms represent the essence of the disorder for patients. As tear film instability manifests as an imitating event in all dry eye subtypes, TBuT was used to identify asymptomatic individuals at risks of developing the disease.

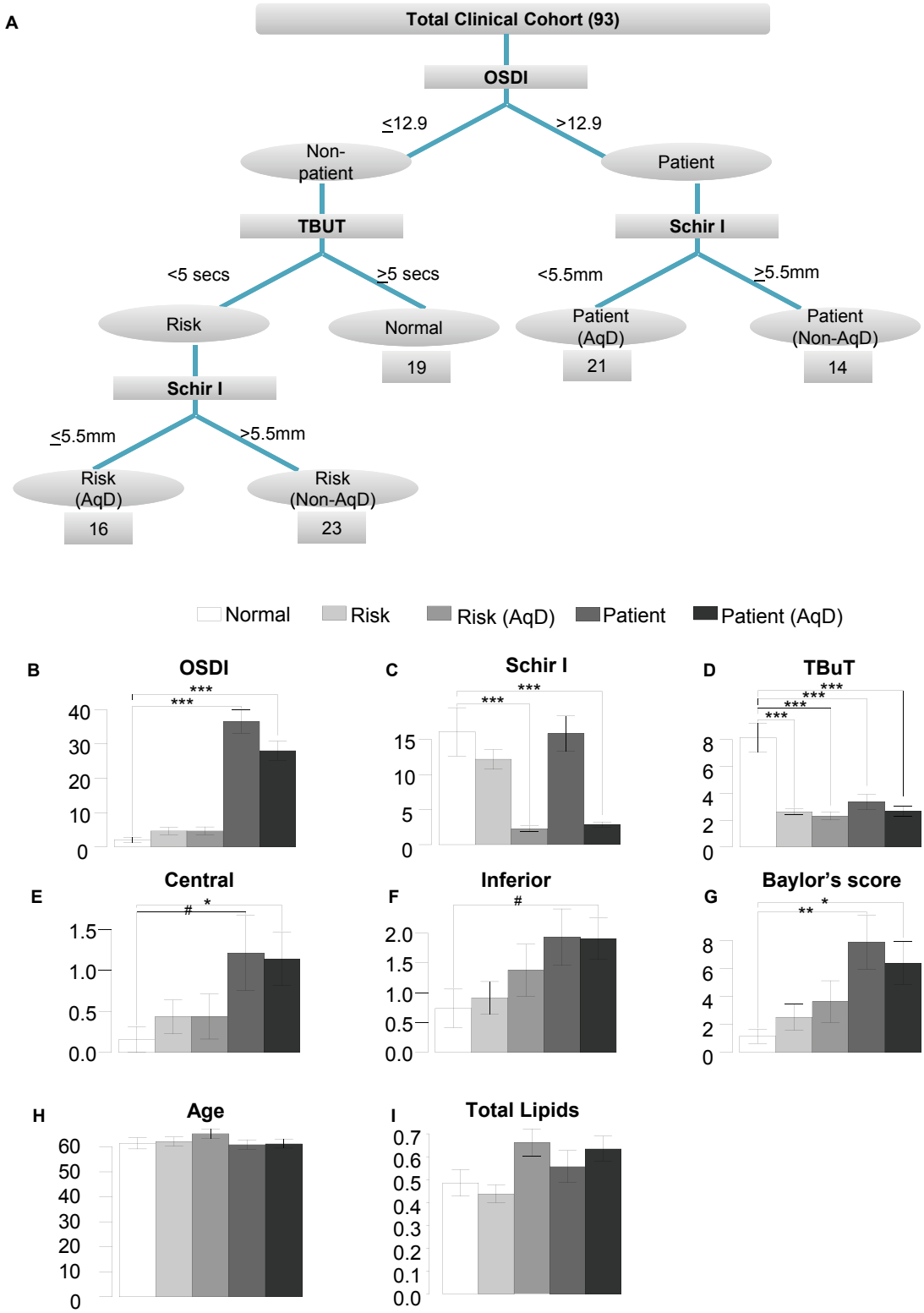


Figure S4, related to Figure 2. Comparison of the levels of lipid classes among various clinical groups. (A) Neutral lipids including waxes (WE), cholesteryl esters (CE), triacylglycerides (TAG), diacylglycerides (DAG), free cholesterols (Free Cho); **(B)** O-acyl-ω-hydroxy-fatty acids (OAHFA), cholesteryl sulfate (CS), and sphingolipids including sphingomyelins (SM), ceramides (Cer), glucosylceramides (GluCer), and monosialo-dihexosyl gangliosides (GM3); **(C)** phospholipids including phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylserines (PS), phosphatidylinositols (PI), phosphatidic acids (PA), phosphatidylglycerols (PG) and lyso-biphosphatidic acids (LBPA); and **(D)** lyso-phospholipids including lyso-PC (LPC), lyso-PE (LPE), lyso-plasmalogen PE (LpPE), lyso-PS (LPS), lyso-PI (LPI) and lyso-PA (LPA).

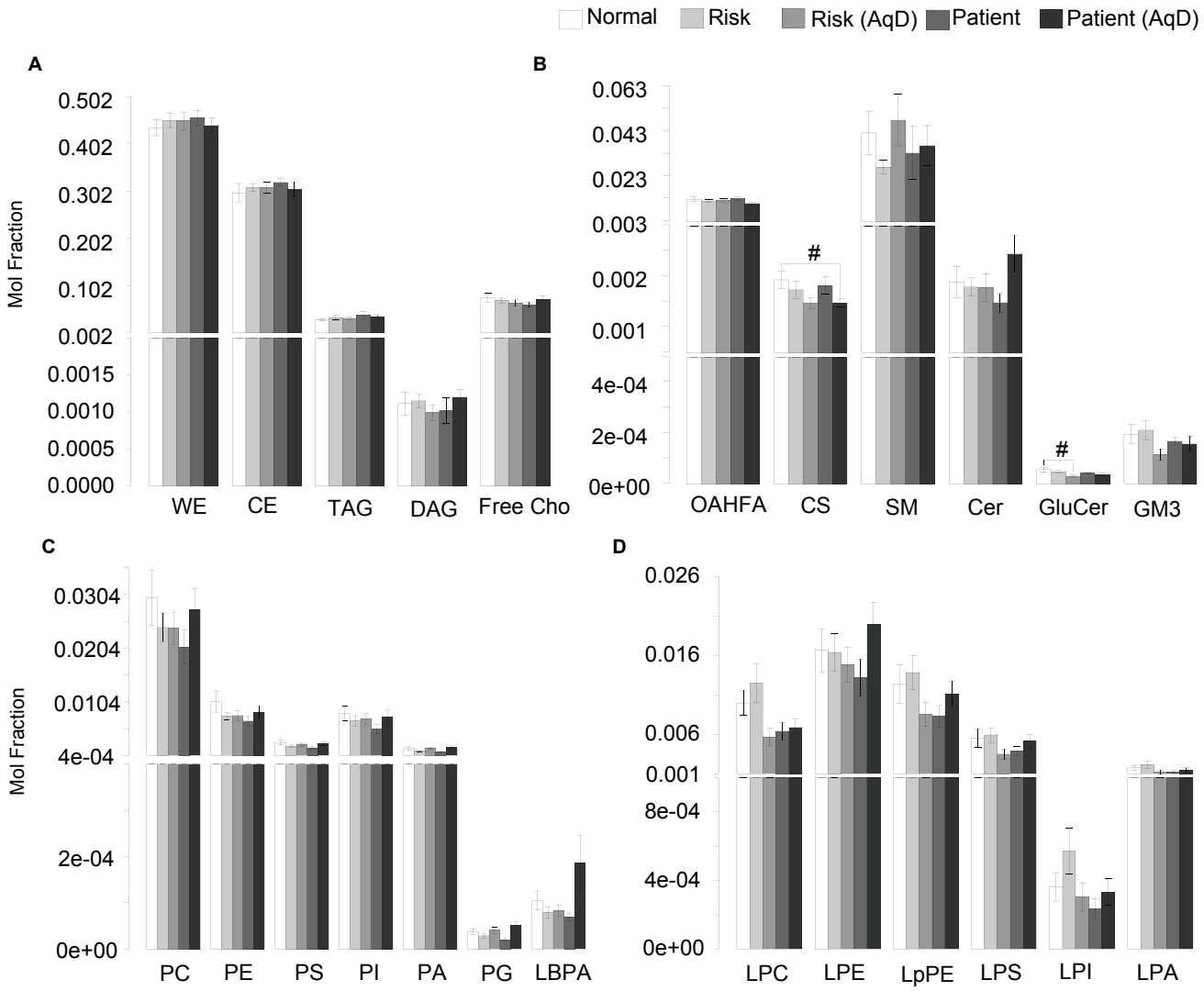


Figure S5, related to Figure 3. Correlations of individual wax esters with DES clinical signs and symptoms. Heatmaps summarizing the correlations between individual wax ester species of **(A)** low molecular mass (range: 490.5-588.6) and **(B)** medium to high molecular mass (range: 590.6-716.6) with clinical parameters for DES. Values were plotted as correlation coefficients. Red indicates positive correlation while blue indicates negative correlation. R: Individuals at risk; RA: Individuals at risk with aqueous deficiency; P: Patients (non-aqueous-deficient); PA: Patients with aqueous deficiency; N: Normal.

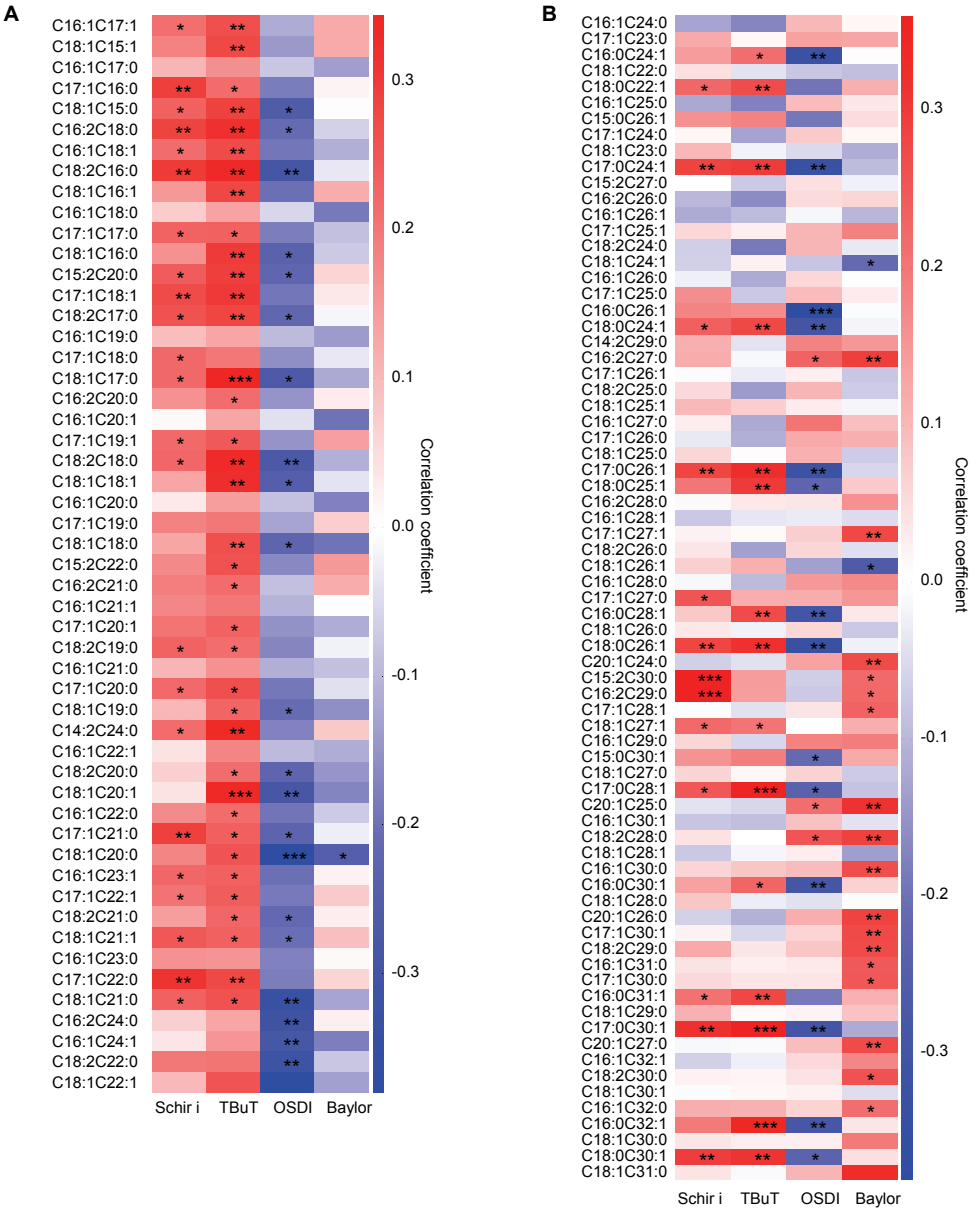


Figure S6, related to Figure 4. Classification of clinical cohort based on molar concentrations of lipids. (A) Dot plots illustrating the distribution of total lipid levels in the study cohort. The study cohort was stratified into three groups using thresholds defined by the 25th and 75th percentiles of the cohort based on total lipid levels in molar concentration (molml^{-1}). The comparison of dry eye-associated clinical parameters between the low, medium and high groups defined by total lipid levels, including the **(B)** modified ocular surface disease index (OSDI); **(C)** Schirmer's length (Schir); **(D)** tear breakup time (TBUT); **(E)** central corneal staining; **(F)** inferior corneal staining; **(G)** Baylor's score for corneal staining; **(H)** age were shown.

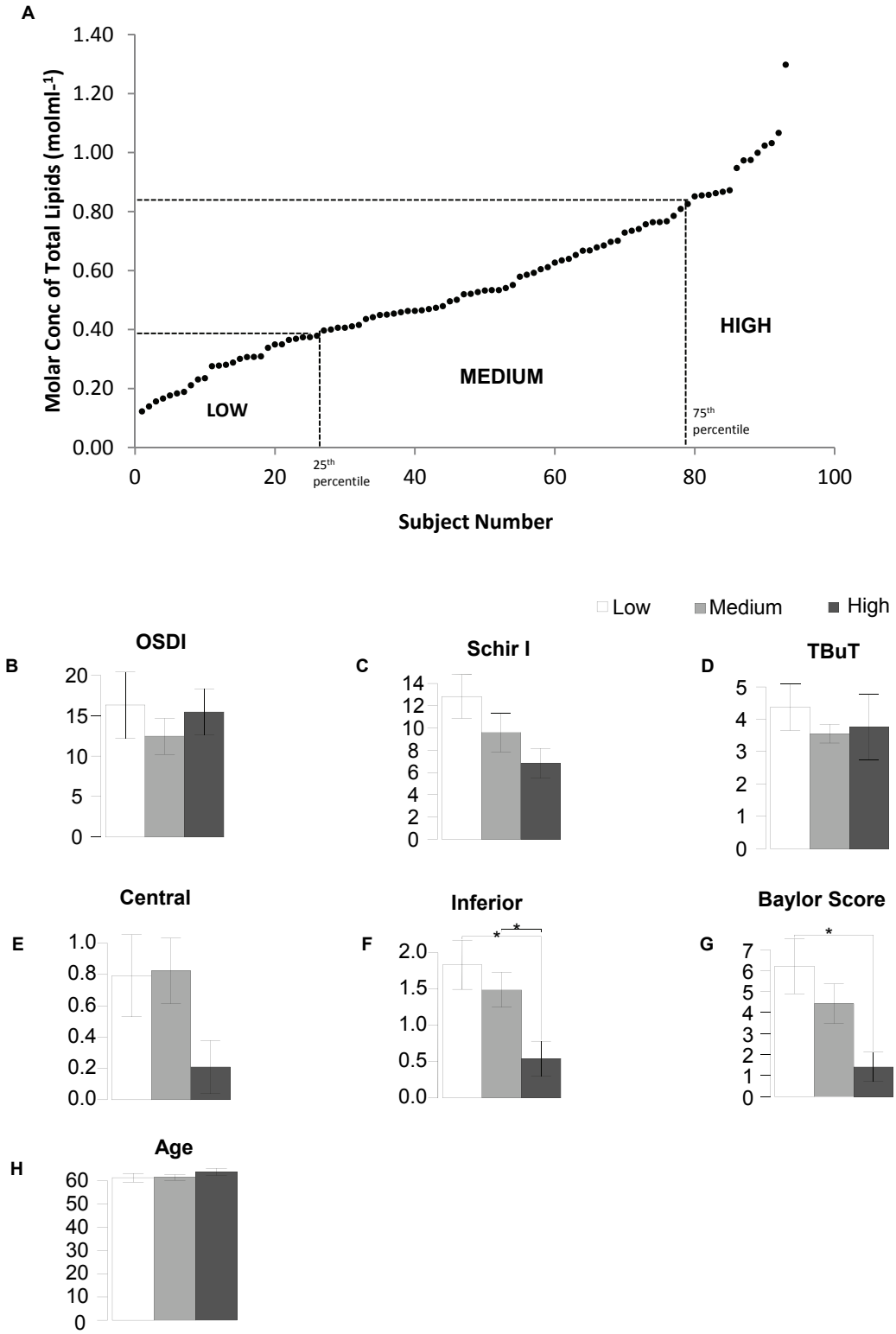
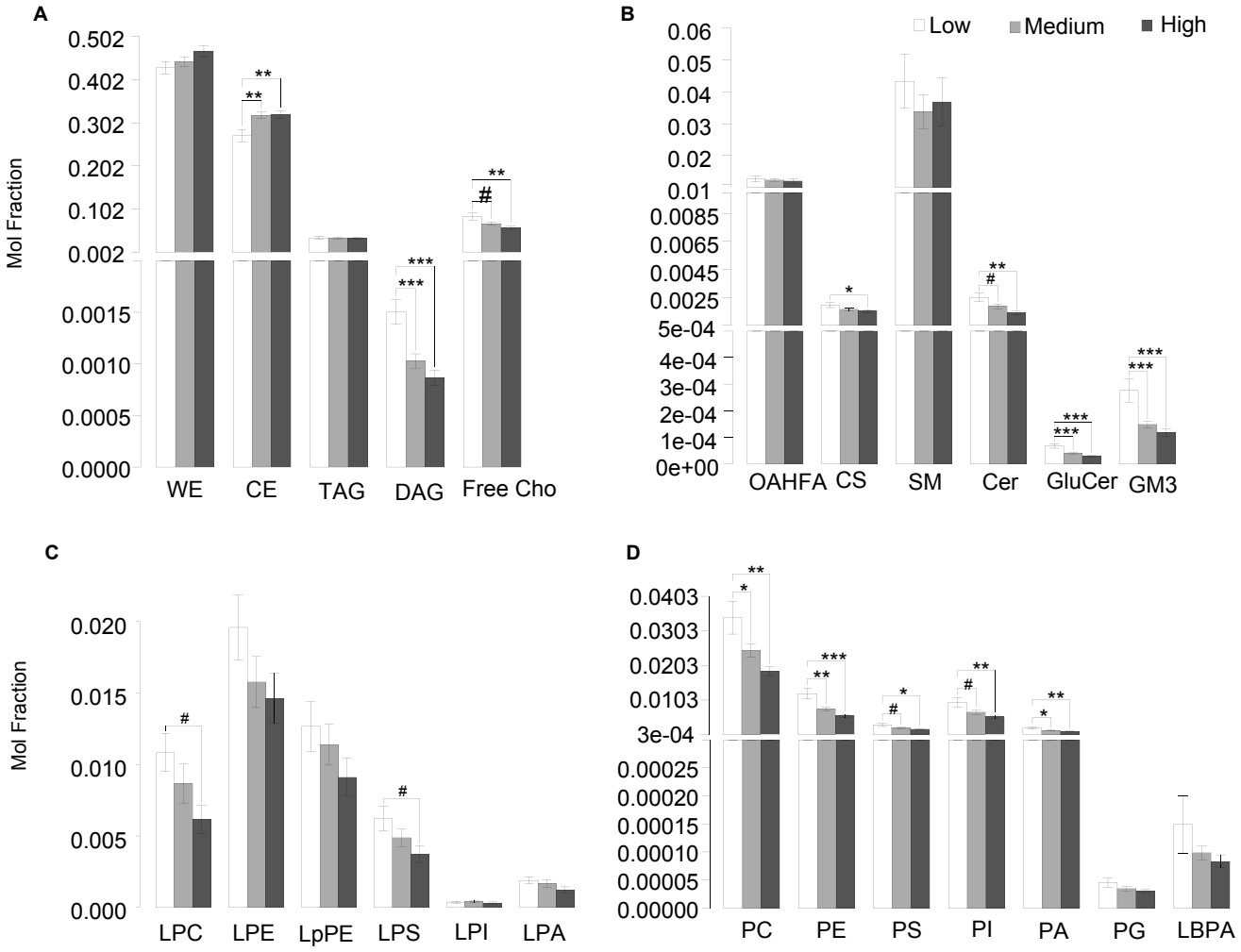


Figure S7, related to Figures 4. Comparison of the levels of lipid classes among low, medium and high groups defined by total lipid levels. (A) Neutral lipids including waxes (WE), cholesteryl esters (CE), triacylglycerides (TAG), diacylglycerides (DAG), free cholesterols (Free Cho); **(B)** O-acyl-ω-hydroxy-fatty acids (OAHFA), cholesteryl sulfate (CS), and sphingolipids including sphingomyelins (SM), ceramides (Cer), glucosylceramides (GluCer), and monosialo-dihexosyl gangliosides (GM3); **(C)** phospholipids including phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylserines (PS), phosphatidylinositols (PI), phosphatidic acids (PA), phosphatidylglycerols (PG) and lyso-biphosphatidic acids (LBPA); and **(D)** lyso-phospholipids including lyso-PC (LPC), lyso-PE (LPE), lyso-plasmalogen PE (LpPE), lyso-PS (LPS), lyso-PI (LPI) and lyso-PA (LPA).



Supplementary Figure S8, related to Figure 4. Compositional alterations in tear lipids with varying amounts of total lipids. Subjects were stratified into three groups (*i.e.* low, medium and high) based on the total absolute concentrations of lipids detected using the 25th and 75th percentiles as cutoffs. Changes in the molar fractions of selected lipid classes including **(A)** DAG; **(B)** CE; and **(C)** PC amongst the three groups were observed. The Schirmer's lengths for selected subjects in the low-lipid category with elevated CS and GluCer were indicated with dotted lines. Blue: Subjects without aqueous-deficiency; Red: Subjects with aqueous-deficiency.

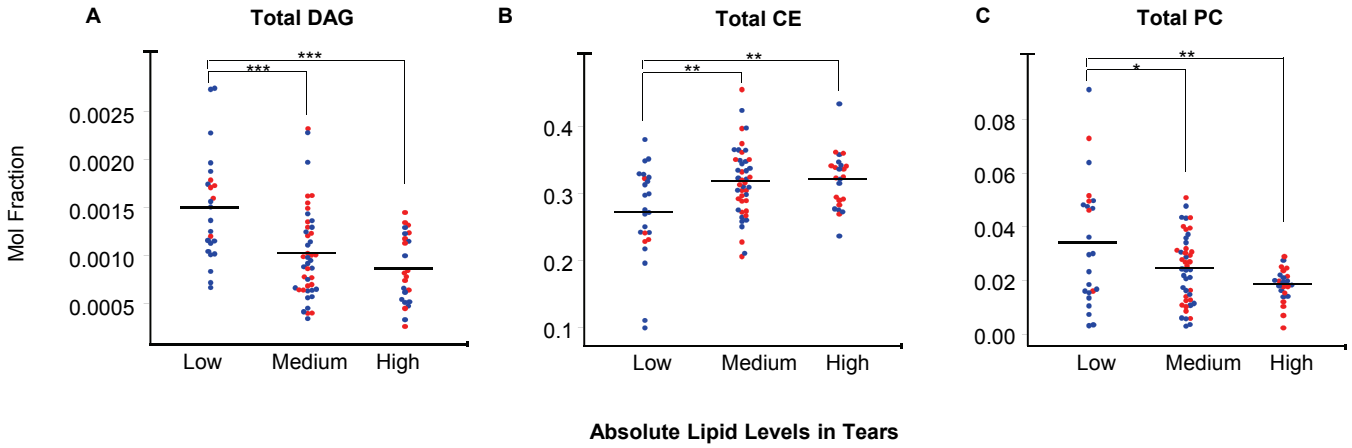


Table S1, related to Figures 1-3 (see attached spreadsheet). Demographic and clinical information of the dry eye cohort used for derivation of dry eye biomarkers. A total of 93 subjects were recruited and stratified into various clinical groups according to the criteria described in Figure S11.

Table S2 (see attached spread sheet), related to Fig. 2. P-values and q-values for ANOVA of wax ester species. Only species with both p- and q-values <0.05 were considered statistically significant and marked in Figure 4.