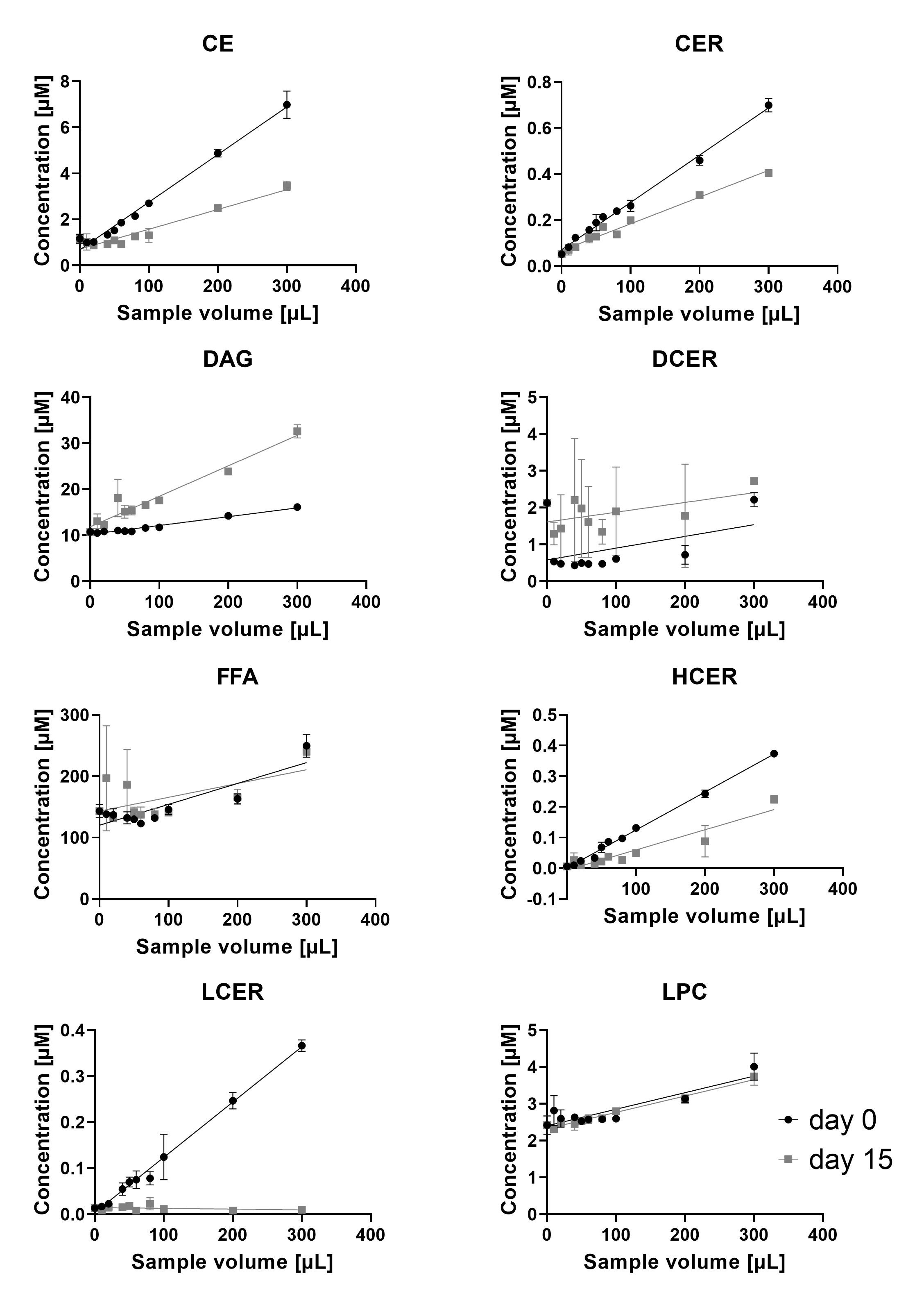
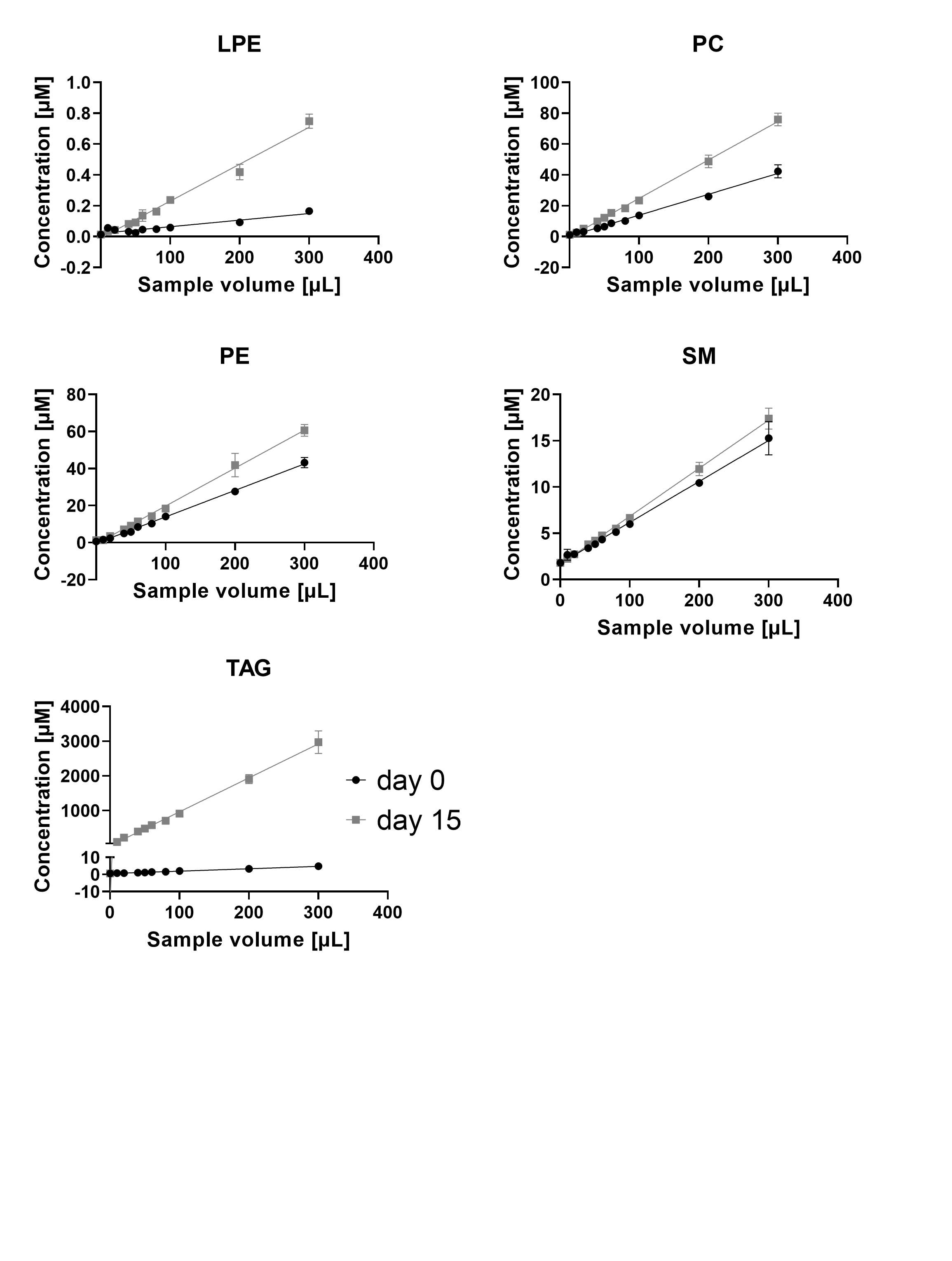
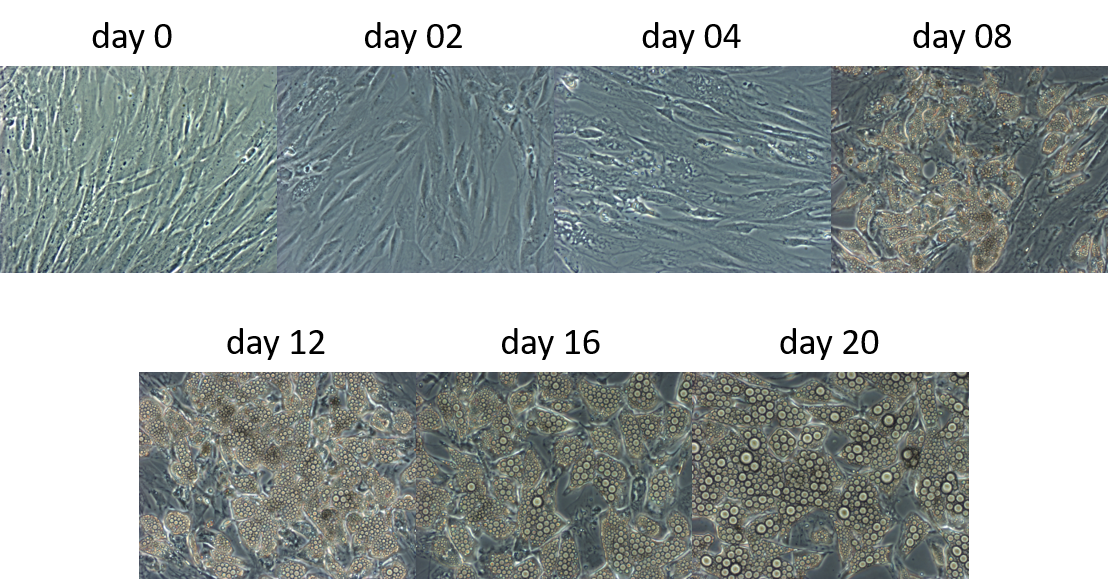
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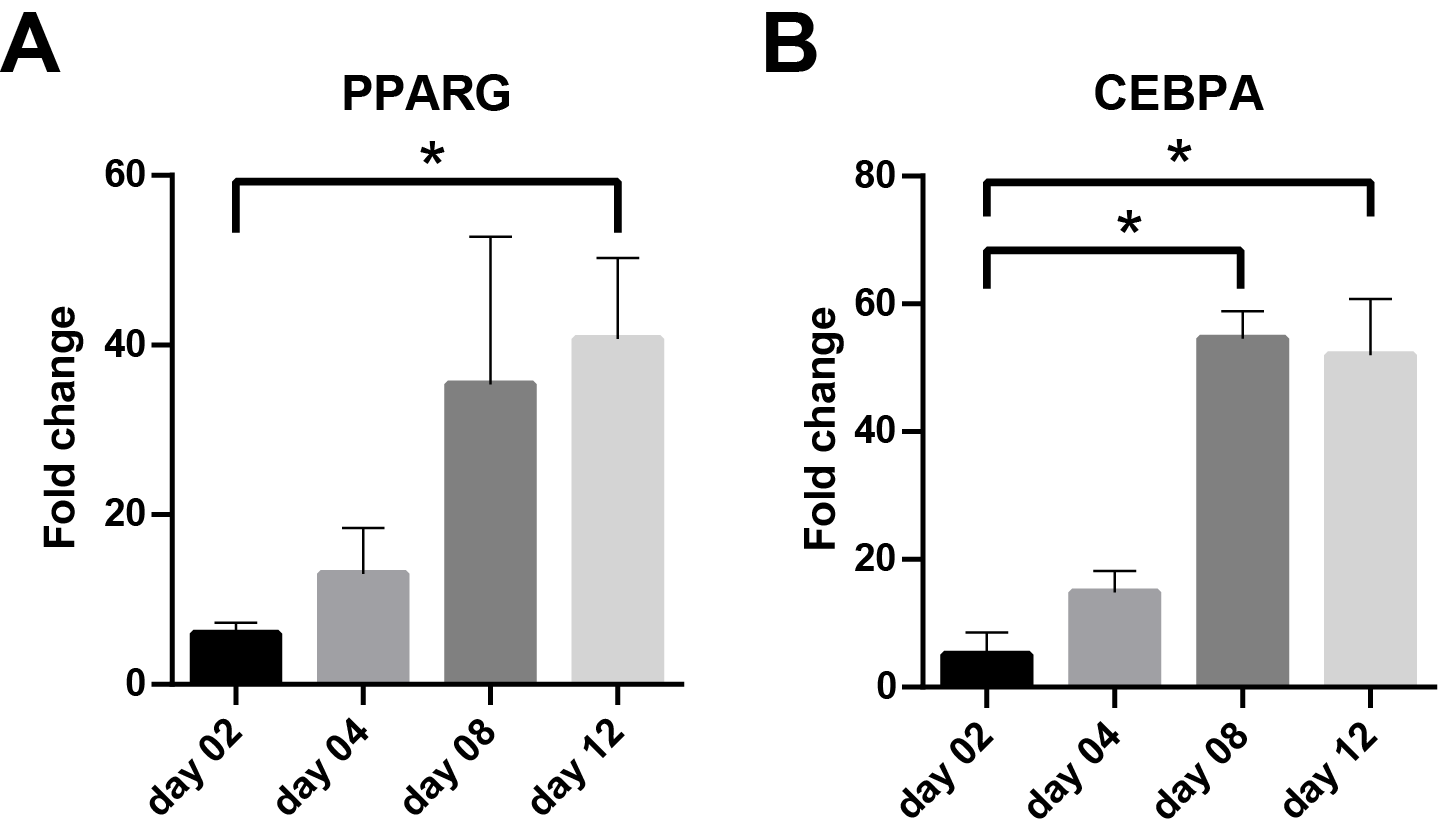


B

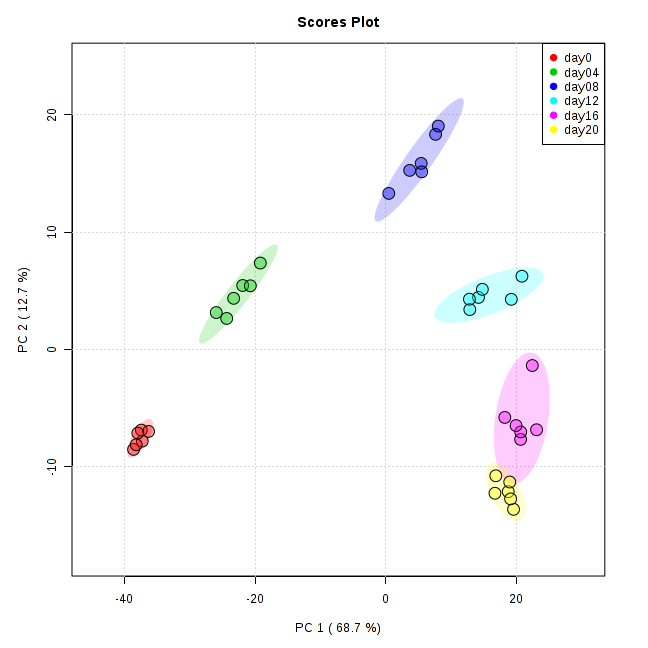
**Figure S1.** Analytical evaluation of the Lipidyzer™ method for undifferentiated and differentiated cells revealed strong linearity for most of the analyzed lipid classes**.** Nine different sample volumes of cell homogenates from day 0 (preadipocytes) or day 15 (lipid laden adipocytes) of differentiation, namely, 10, 20, 40, 50, 60, 80, 100, 200, and 300 µL were analyzed in triplicates each. For most of the lipid classes we received satisfying linearity for both sample types (coefficient of determination of the linear regression > 0.9; see Supplement Table S2). Only dihydroceramides (DCER) and free fatty acids (FFA) showed insufficient linearity for both sample types. These two lipid classes were therefore excluded from further evaluation. The results for CE, CER, DAG, DCER, FFA, HCER, LCER, and LPC (1A) and LPE, PC, PE, SM, and TAG (1B) are shown separately.



**Figure S2.** Representative microscopic images of SGBS cells showed successful differentiation based on morphological changes and a strong increase in number and size of lipid droplets. Undifferentiated cells have a long and flat fibroblast-like morphology. During cell differentiation, the cells contract and turn into more oval shaped cells with increasing storage of lipids into lipid droplets. During maturation, the size of the lipid droplets increases. Magnification in all images was 100-fold.

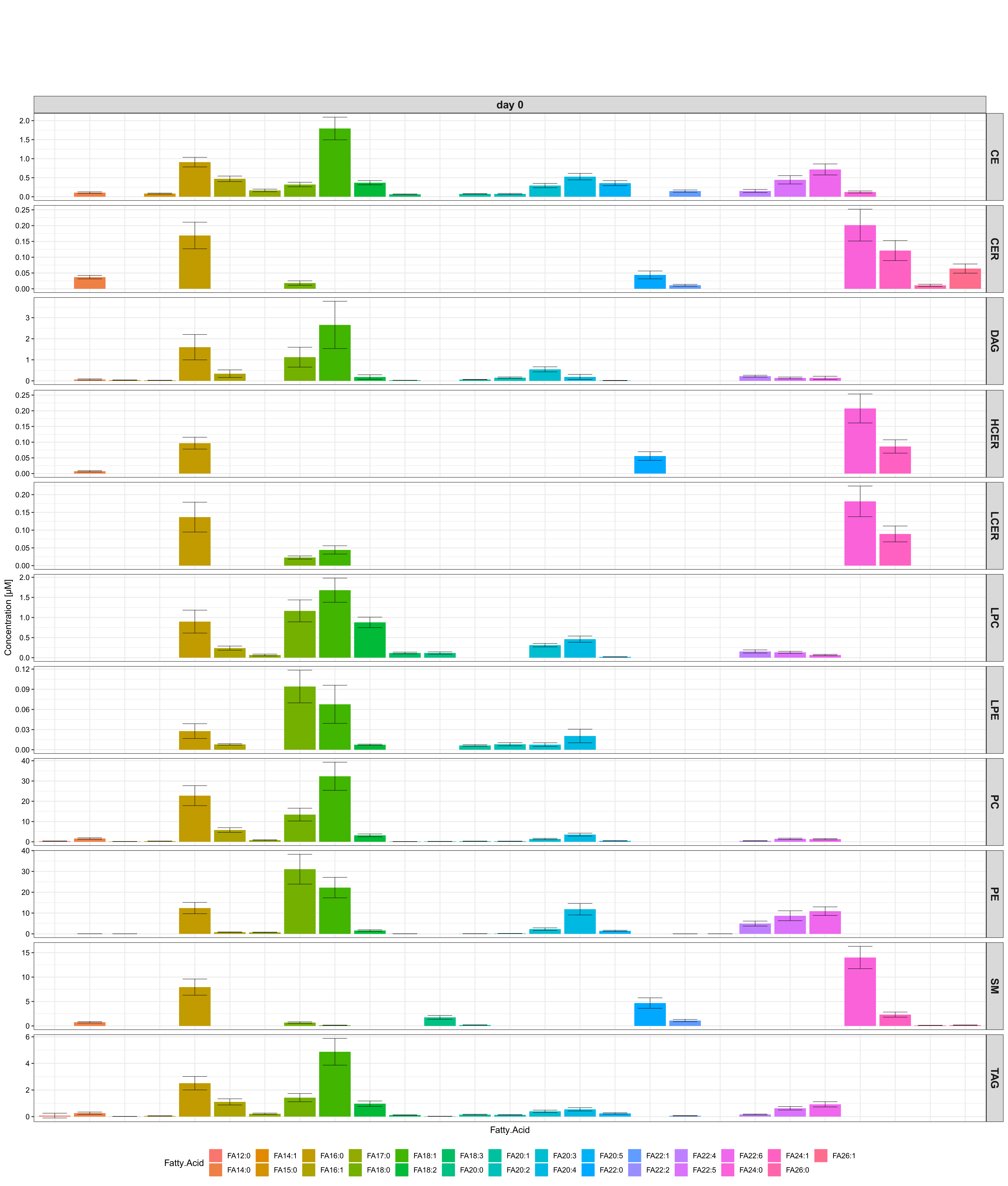


**Figure S3.** Transcripts of the main adipogenic transcription factors PPAR (PPARG) and C/EBP (CEBPA) were highly upregulated during adipogenesis. Relative mRNA expression determined by qRT-PCR is shown as fold change. For each time point, the mean of four samples was normalized to expression of TBP and day 0 of differentiation. \**p* < 0.01.

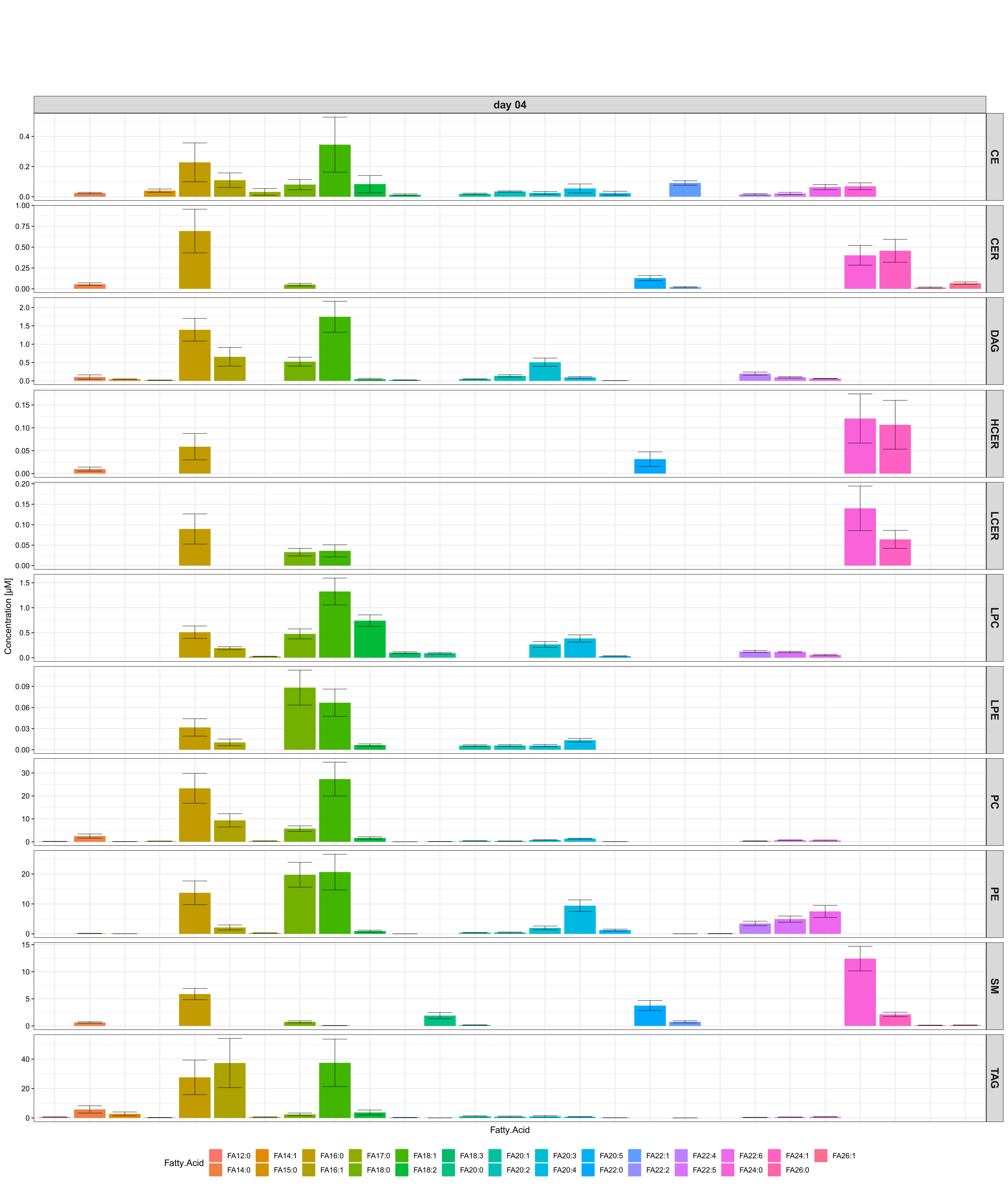


**Figure S4.** Principal component analysis (PCA) score plot showing very clear clustering of lipid species regarding the different time points of adipogenesis. The color code for the data points of the different days of adipogenesis is shown in the box inside the figure. Illustrated are also the 95 % confidence intervals of each group. The first principal component separated the data set for 68.7%, PC2 for 12.7%. Each time point consisted of six samples.

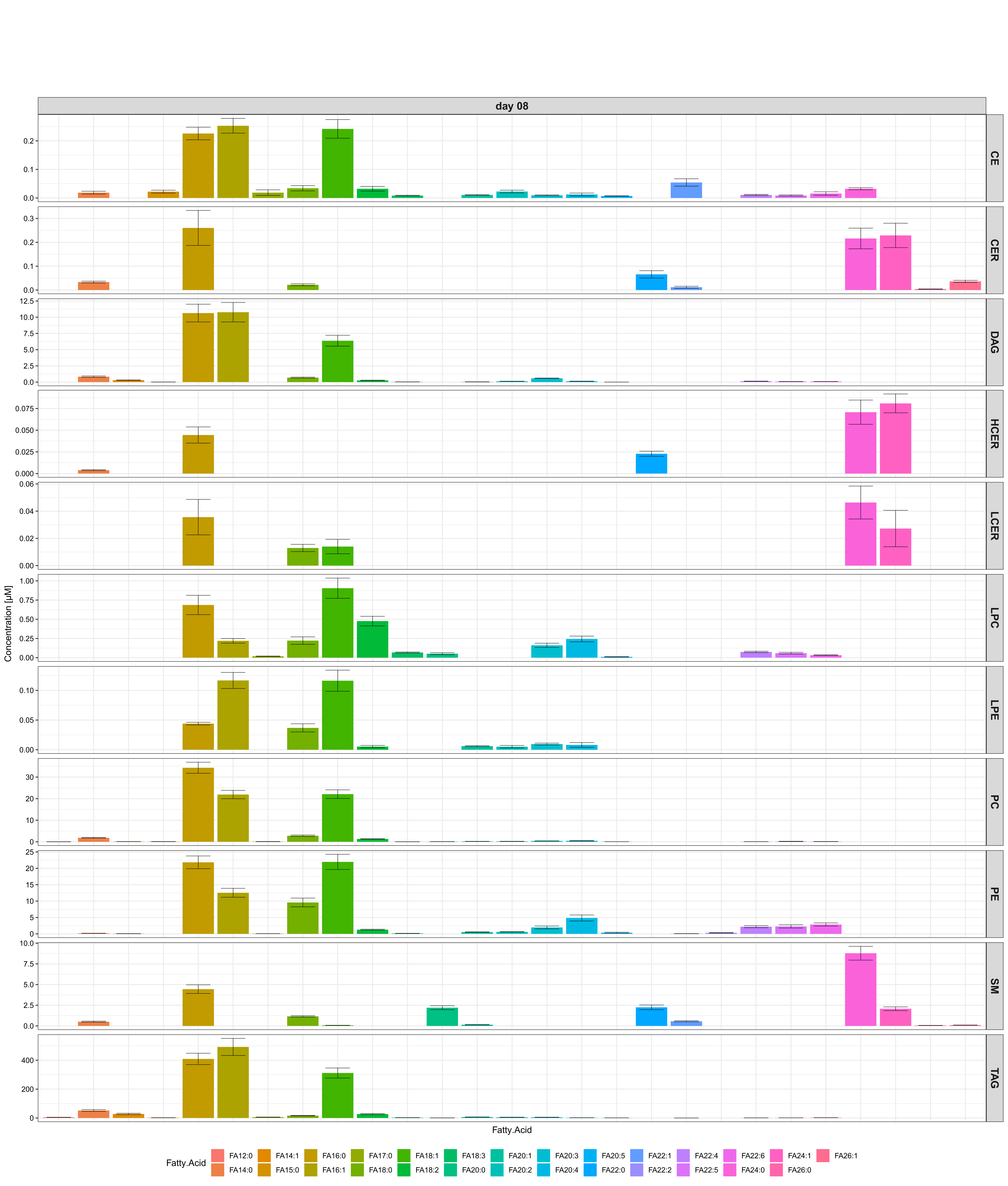
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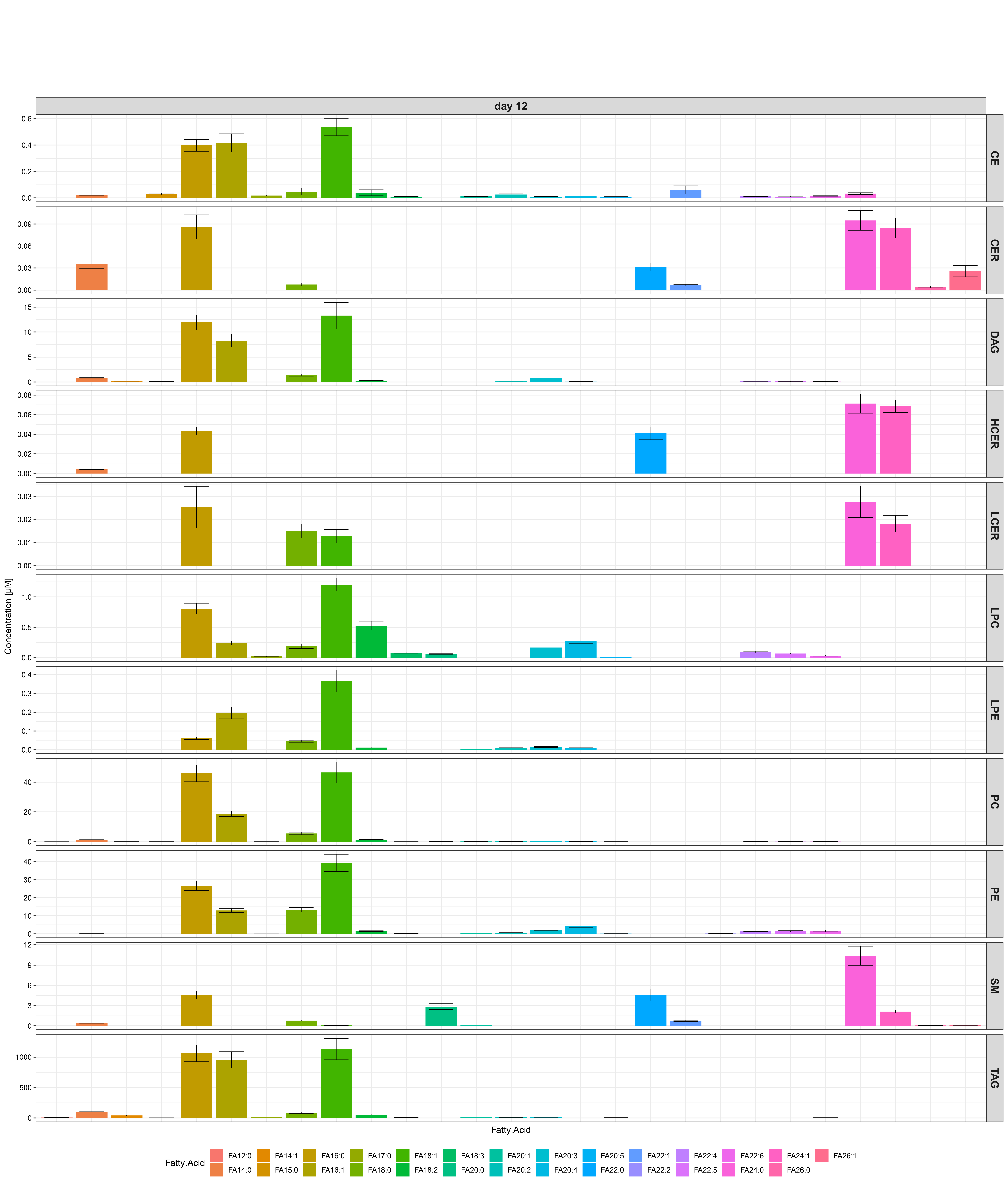
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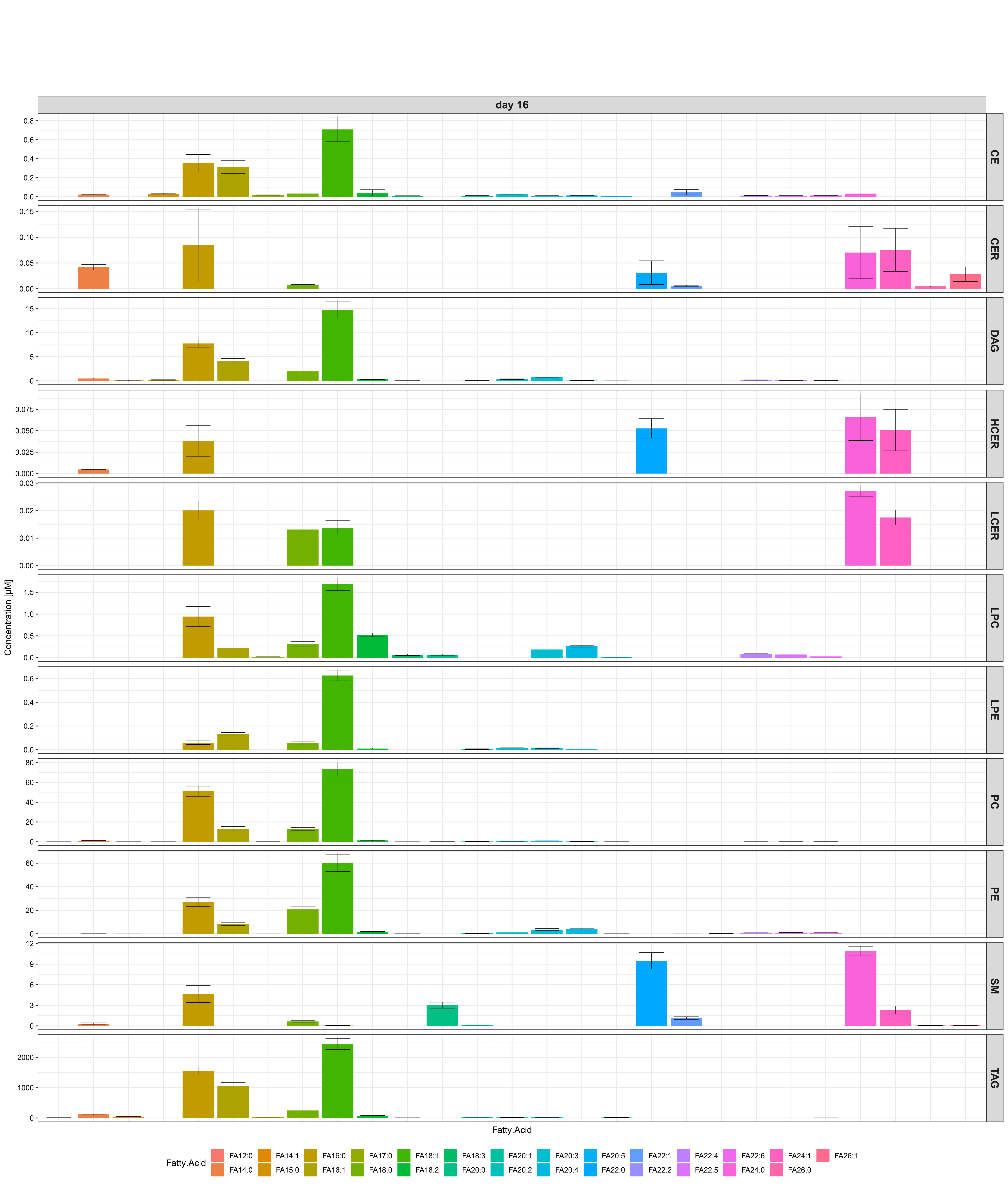
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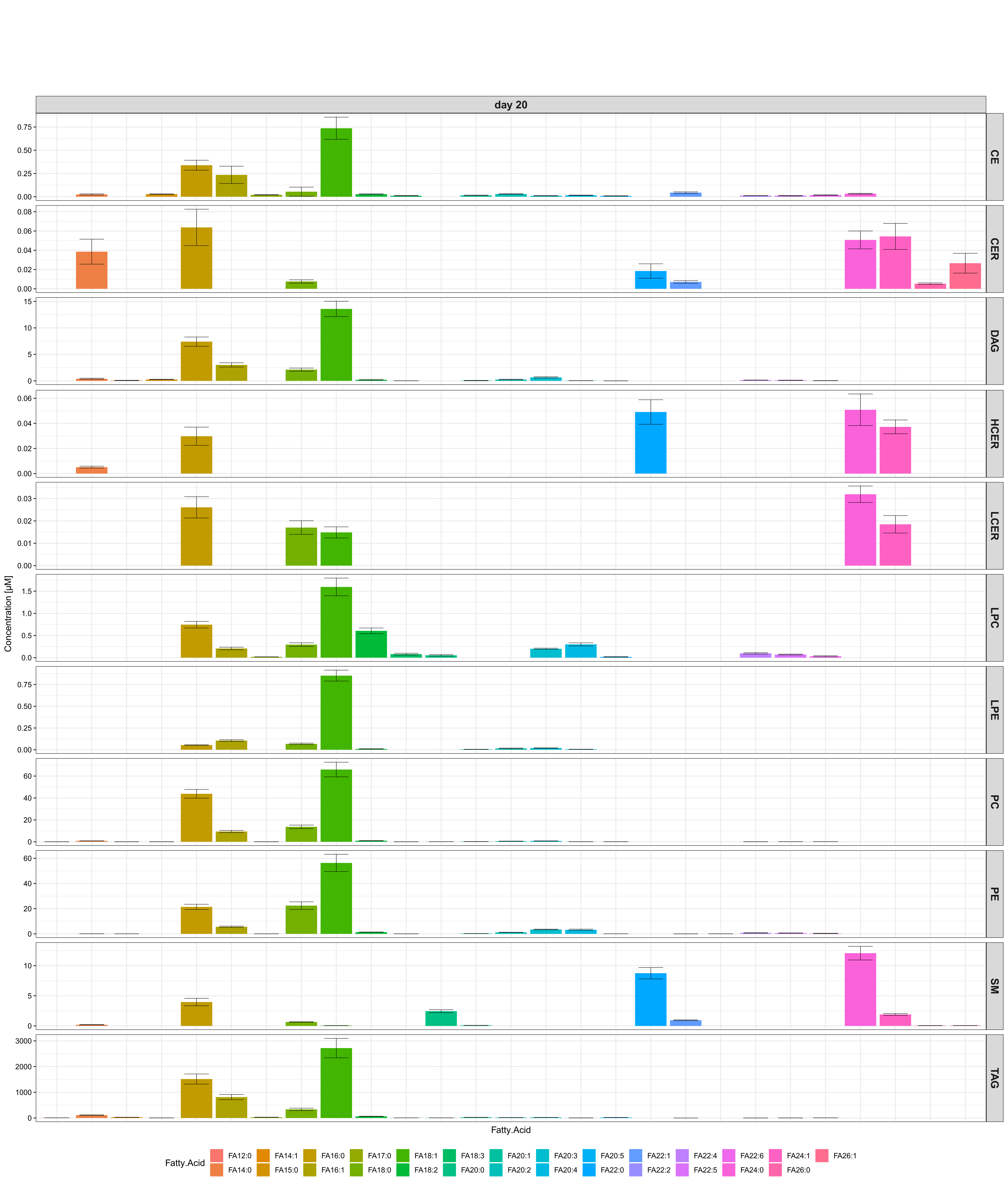
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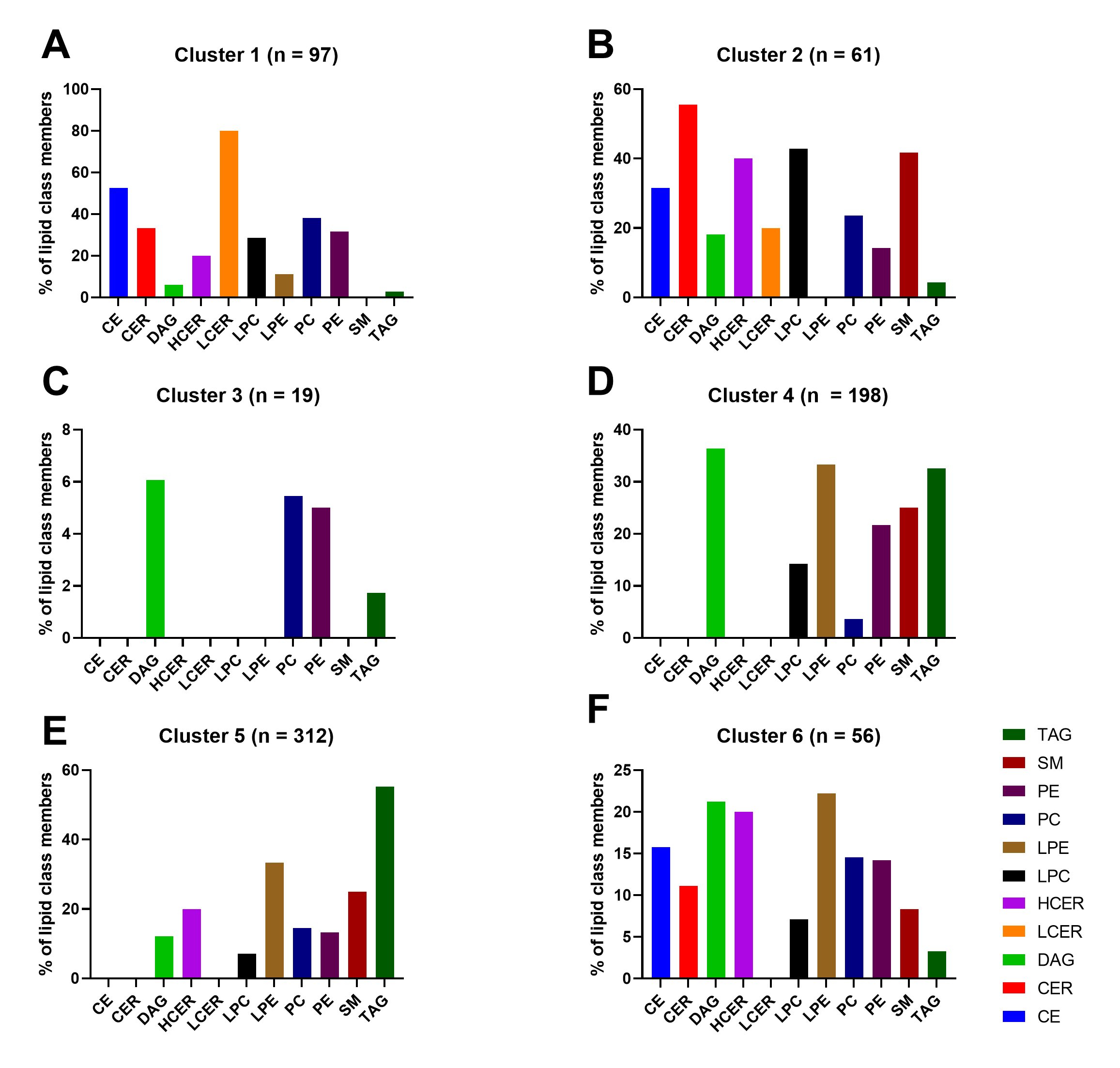
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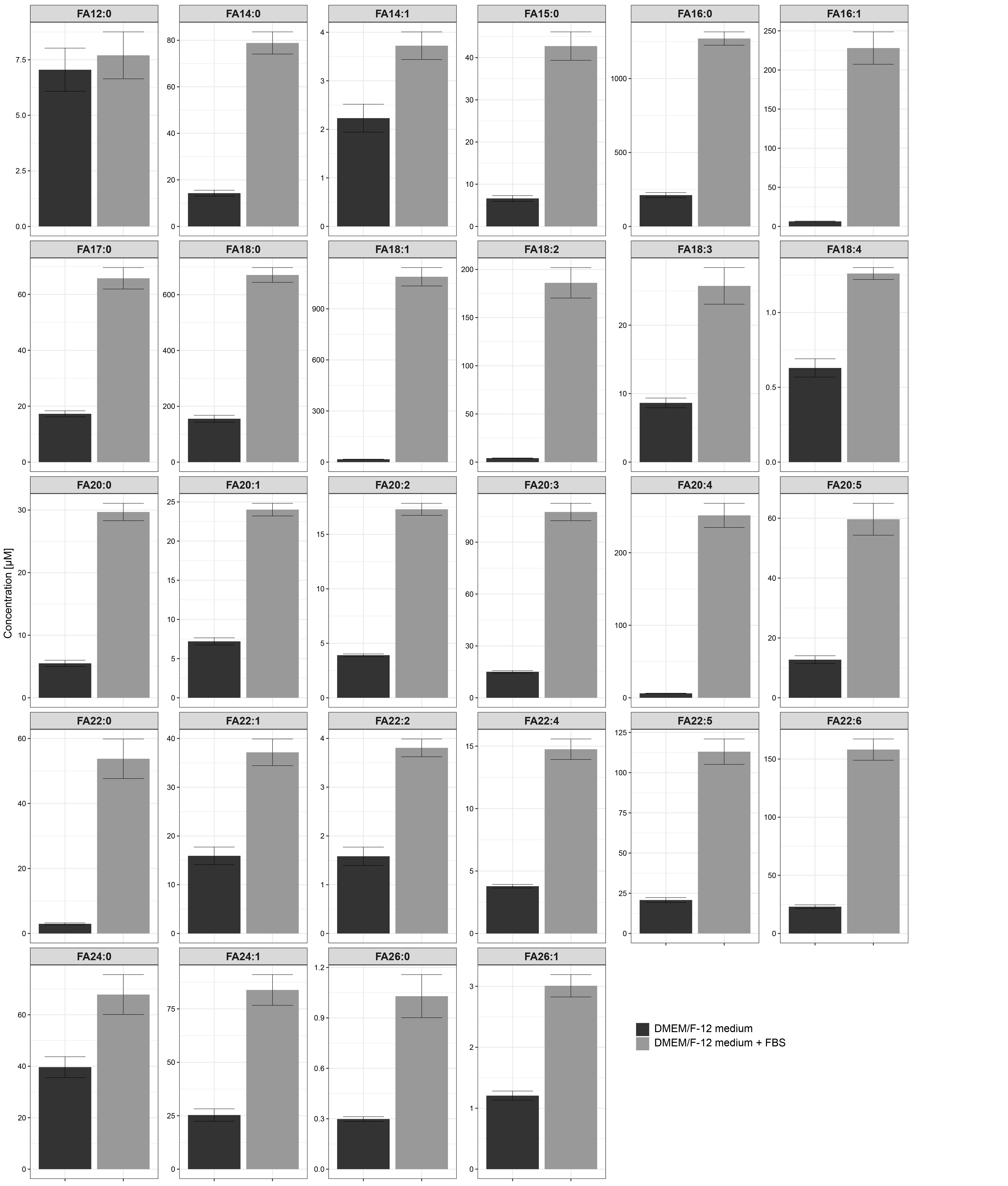
F



**Figure S5.** Fatty acid concentrations and compositions changed markedly during adipogenesis in all 11 lipid classes.At the start of differentiation, the lipids had a very heterogeneous side chain distribution with high concentration levels of long- (LCFA) and very long-chain fatty acids (VLCFA). The concentrations of the VLCFA decreased during adipogenesis in all classes, with the exception of the class SM. The concentration courses of the LCFA were more complex because their levels increased markedly during adipogenesis within the classes DAG, LPE, PC, PE, and TAG, but decreased in the class CE. The FA concentration course of the SM differed strongly from the other classes because the VLCFA remained at high levels during adipogenesis. The results for day 0 (**A**), day 4 (**B**), day 8 (**C**), day 12 (**D**), day 16 (**E**), and day 20 (**F**) are separately shown.



**Figure S6.** Clusters from the spearman’s rank correlation analysis consisted of distinct lipid class compositions.Panels A–F show the lipid class compositions for each cluster normalized to the species’ total number per class over all clusters.



**Figure S7.** Polyunsaturated and very long-chain fatty acids were highly concentrated in FBS-containing medium used for cultivation before differentiation start. For analysis, 300 µL medium was used. *n* = 6 per group.