

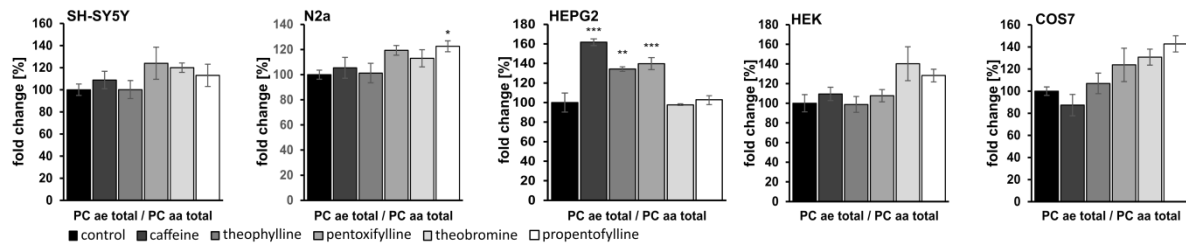
Supplementary Material

Mass spectrometry analysis of lipid changes induced by methylxanthines in different cell lines

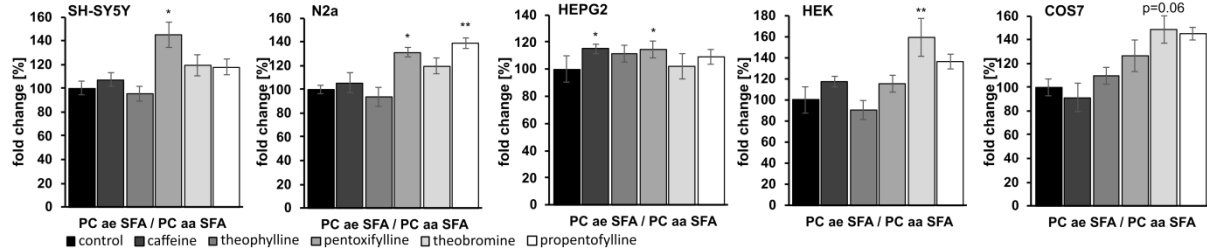
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Supplemental Figure S1

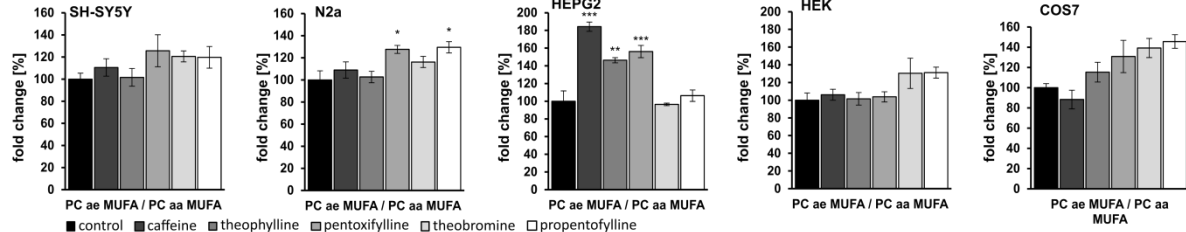
(A) PC ae / PC aa



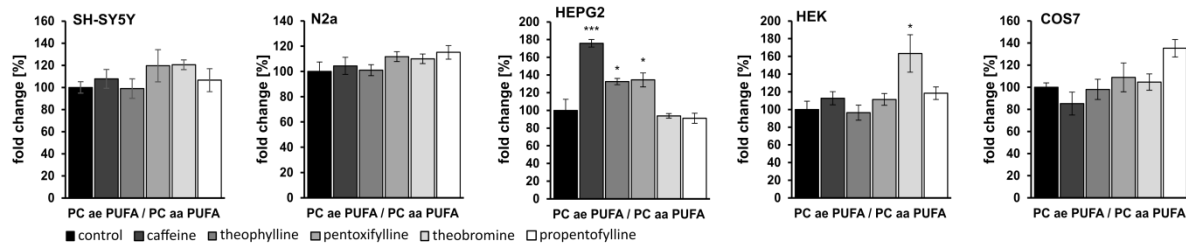
(B) PC ae SFA / PC aa SFA



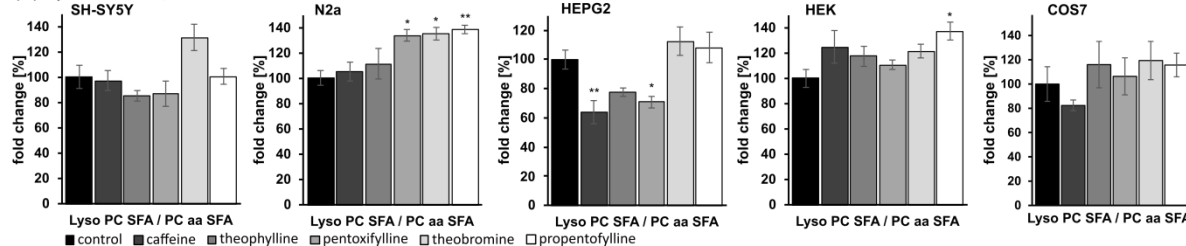
(C) PC ae MUFA / PC aa MUFA



(D) PC ae PUFA / PC aa PUFA

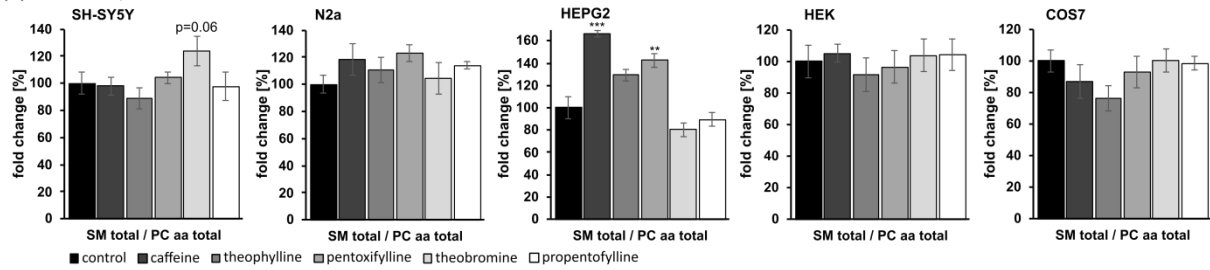


(E) Lyso PC SFA / PC aa SFA

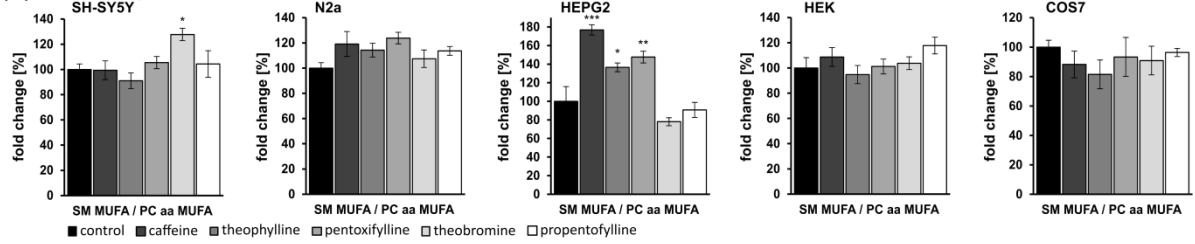


Supplemental Figure S1 (continued)

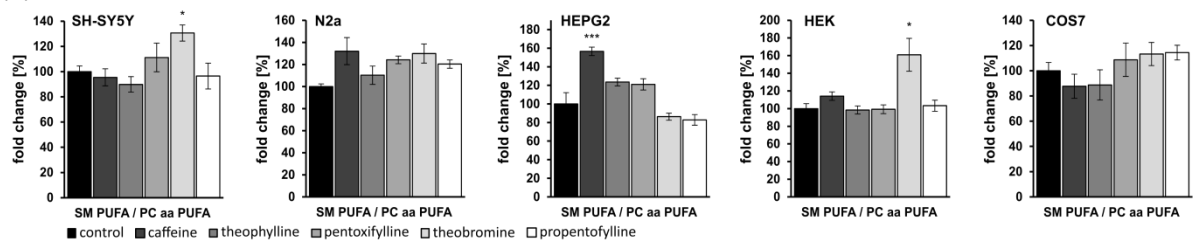
(F) SM total / PC aa total



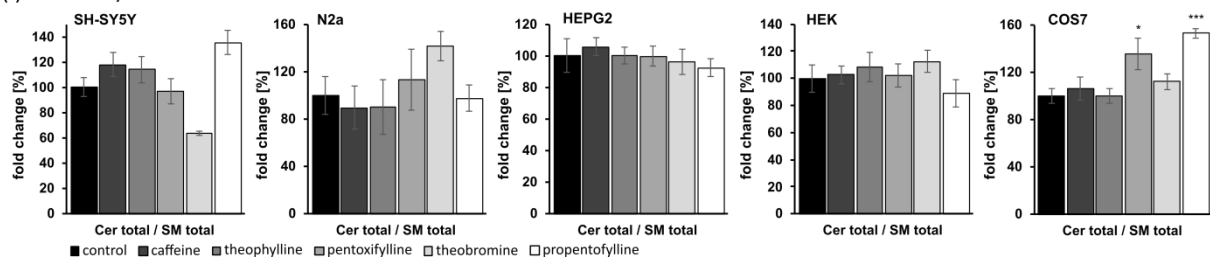
(G) SM MUFA / PC aa MUFA



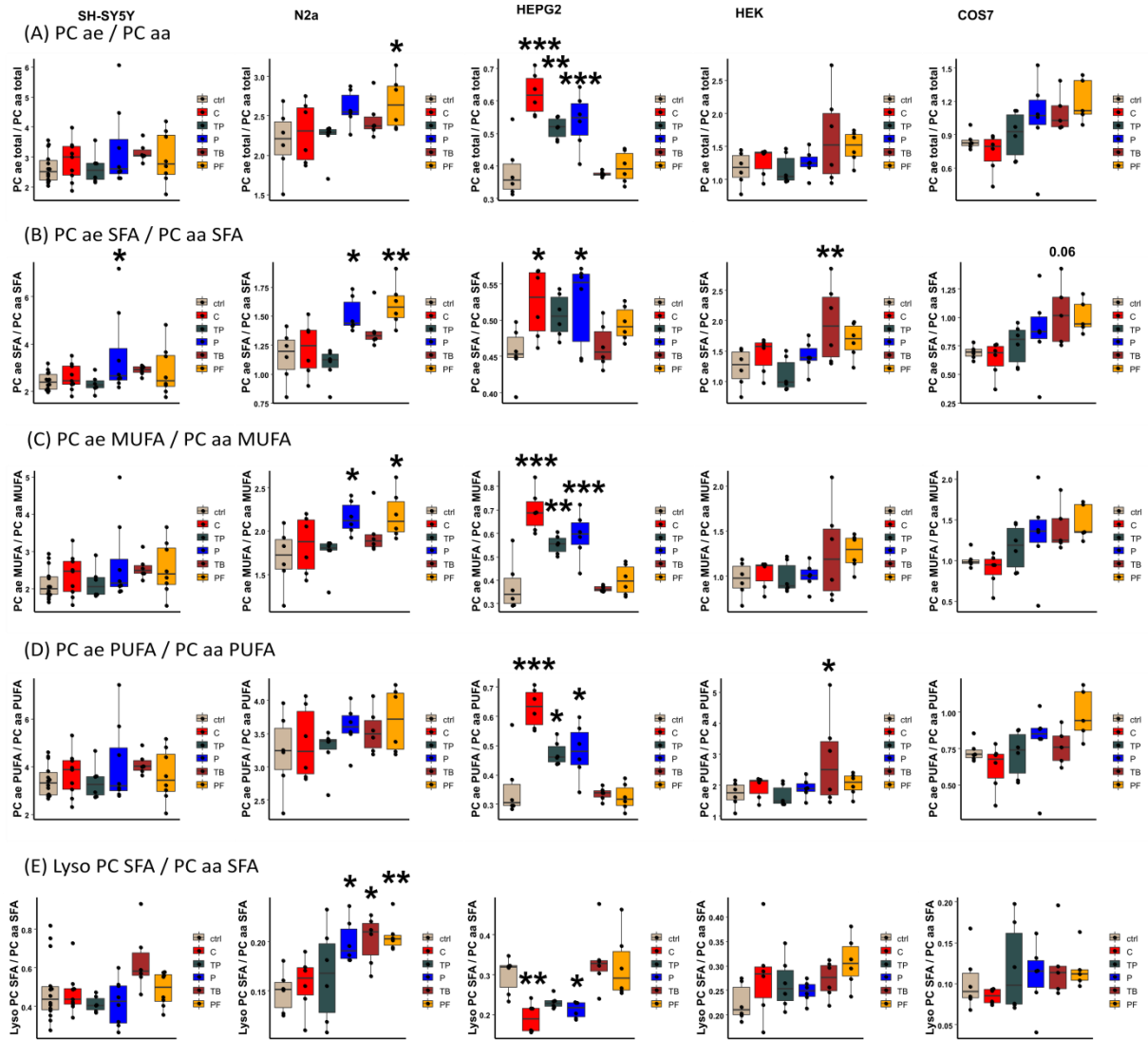
(H) SM PUFA / PC aa PUFA

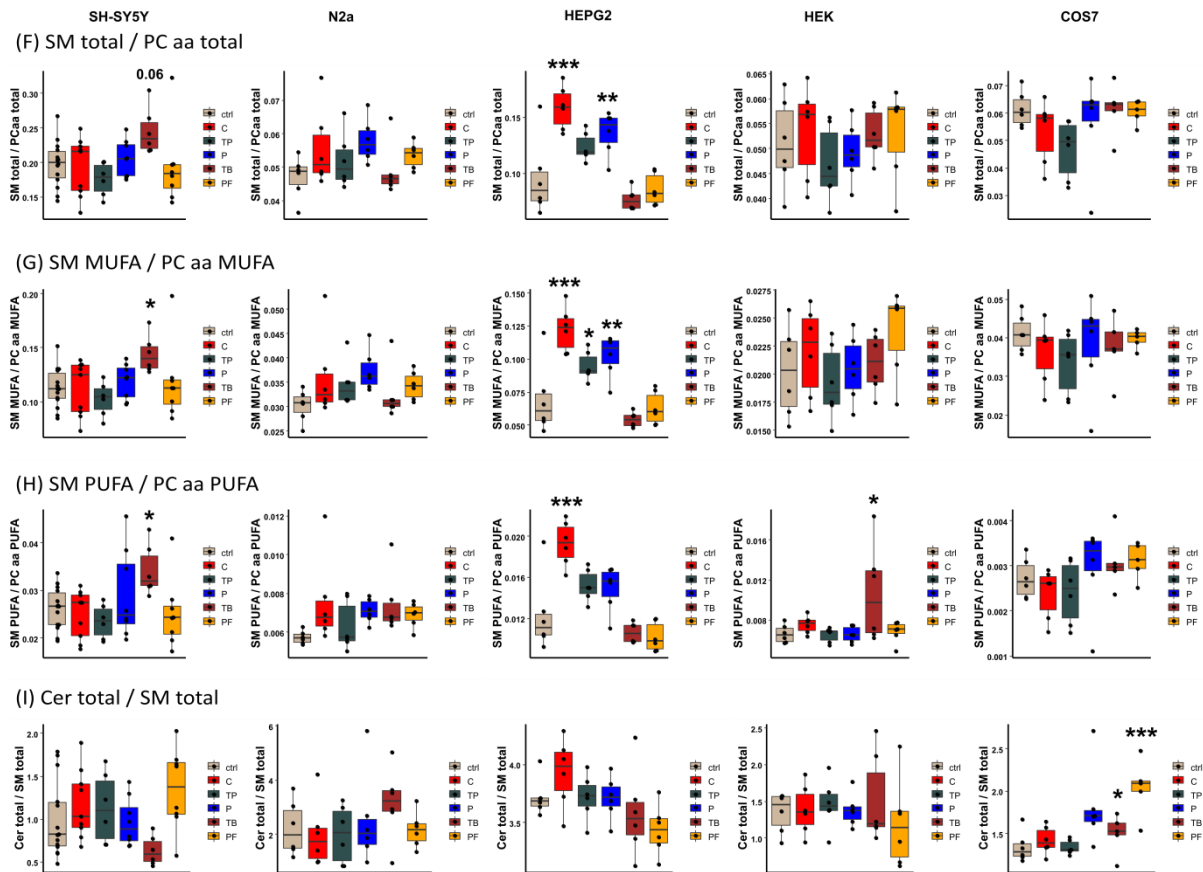


(I) Cer total / SM total



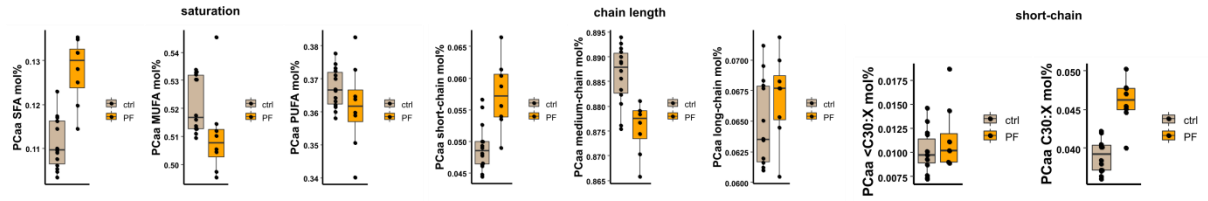
Supplementary Figure S1: Effect of different methylxanthines (caffeine, theophylline, pentoxifylline, theobromine, and propentofylline) on PCae / PCaa, Lyso PC/PCaa, SM/PCaa, and Cer/SM ratios in SH-SY5Y-, N2a-, HEPG2-, HEK-, and COS7 cells. Error bars represent the standard error of the mean (SEM) and statistical significance was set as * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$.



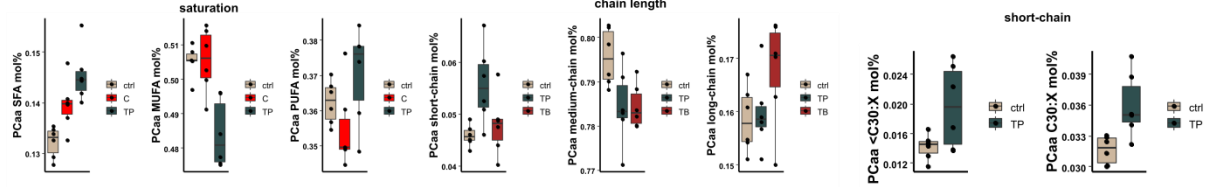


Supplementary Figure S2: Effect of different methylxanthines (caffeine, theophylline, pentoxifylline, theobromine, and propentofylline) on PCae / PCaa, Lyso PC/PCaa, SM/PCaa, and Cer/SM ratios in SH-SY5Y-, N2a-, HEPG2-, HEK-, and COS7 cells shown as boxplots. Error bars represent the standard error of the mean (SEM) and statistical significance was set as * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$.

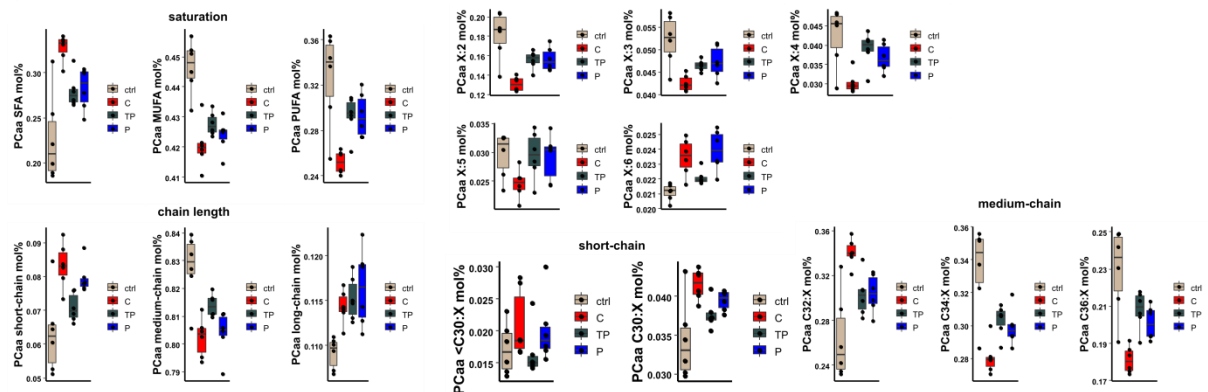
(A) SH-SY5Y



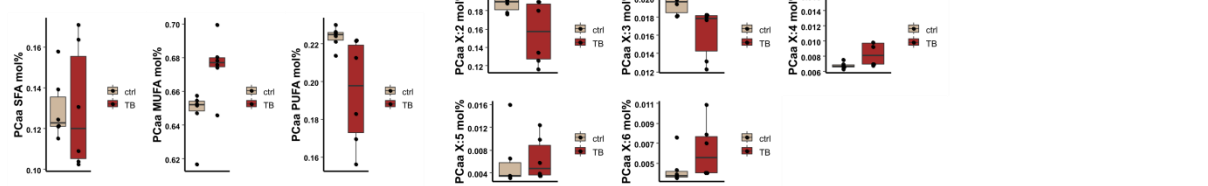
(B) N2a



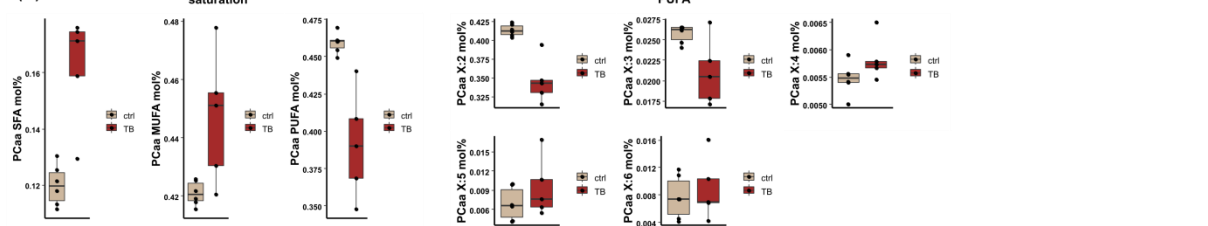
(C) HEPG2



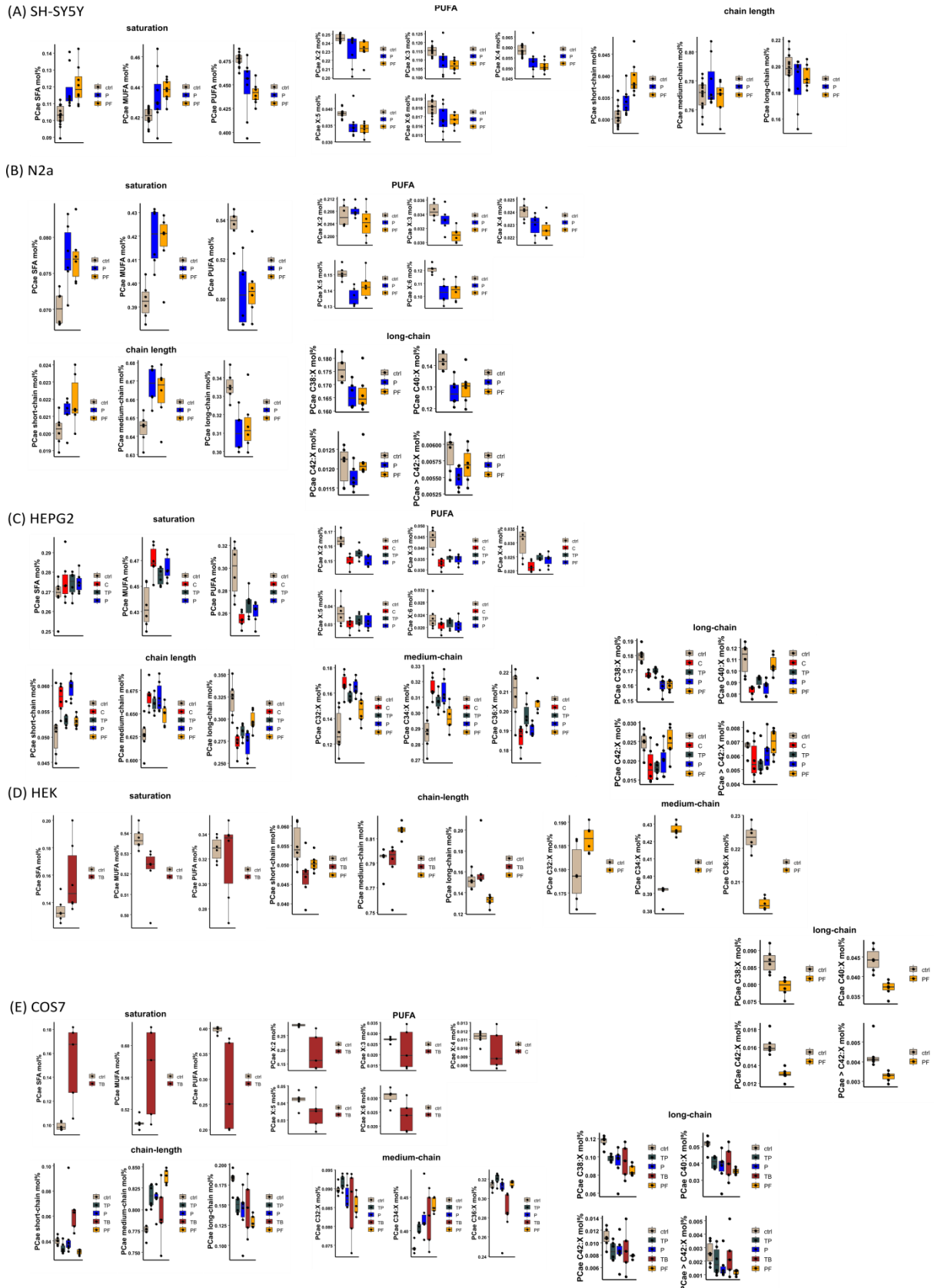
(D) HEK



(E) COS7

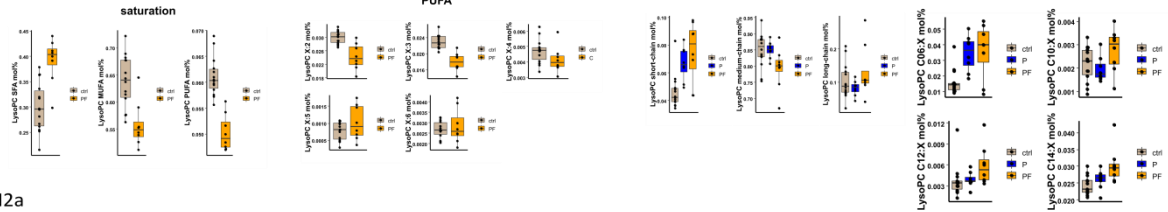


Supplementary Figure S3: Alterations of phosphatidylcholine (PCaa) levels in different cell lines (SH-SY5Y, N2a, HepG2, HEK and COS7) in presence of the analyzed MTXs caffeine (C), theophylline (TP), pentoxifylline (P), theobromine (TB) and propentofylline (PPF) shown as boxplots (in accordance to Figure 2).

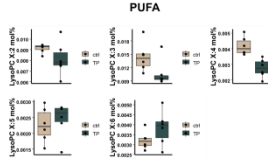


Supplementary Figure S4: Alterations of phosphatidylcholine plasmalogens (PCae) levels in different cell lines (SH-SY5Y, N2a, HepG2, HEK and COS7) in presence of the analyzed MTXs caffeine (C), theophylline (TP), pentoxifylline (P), theobromine (TB) and propentofylline (PPF) shown as boxplots (in accordance to Figure 3).

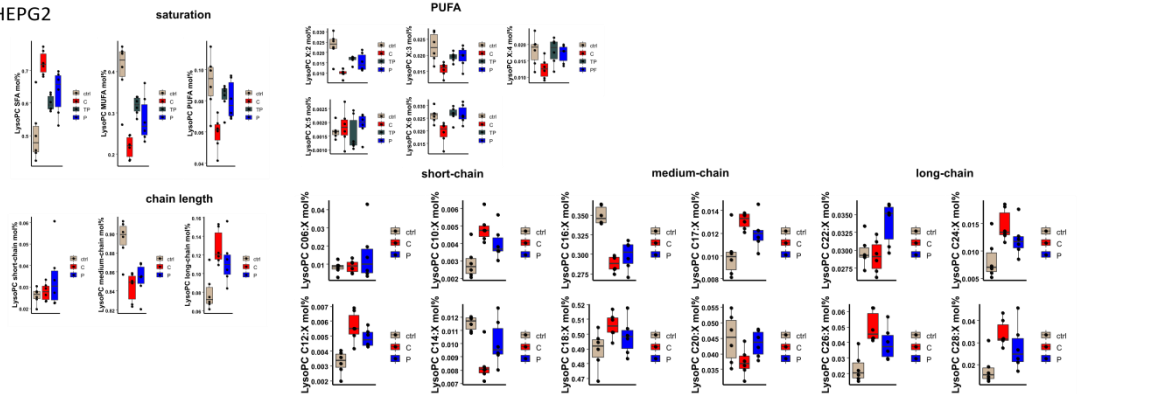
(A) SH-SY5Y



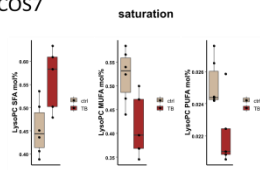
(B) N2a



(C) HEPG2

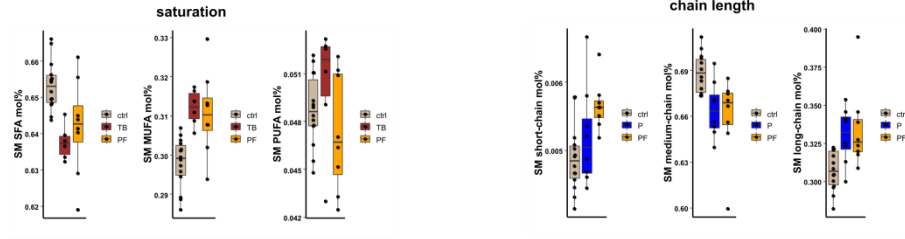


(D) COS7

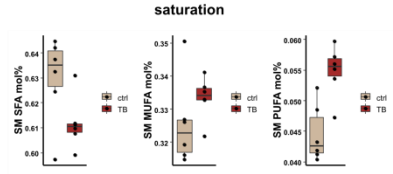


Supplementary Figure S5: Alterations of lyso-phosphatidylcholine (lyso-PC) levels in different cell lines (SH-SY5Y, N2a, HepG2, HEK and COS7) in presence of the analyzed MTXs caffeine (C), theophylline (TP), pentoxifylline (P), theobromine (TB) and propentofylline (PPF) shown as boxplots (in accordance to Figure 4).

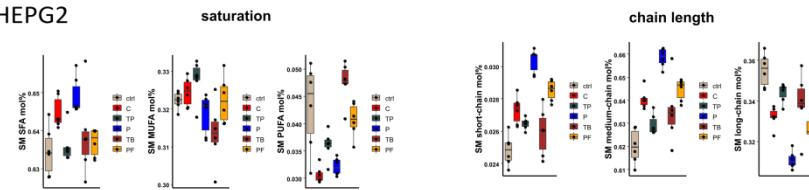
(A) SH-SY5Y



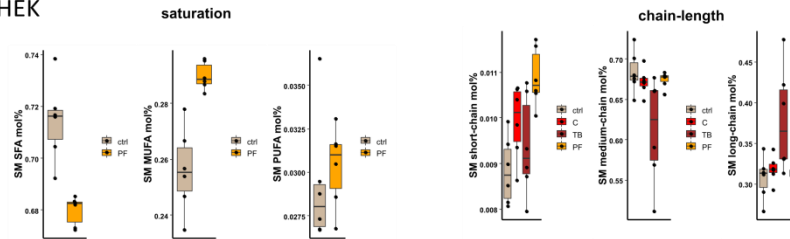
(B) N2a



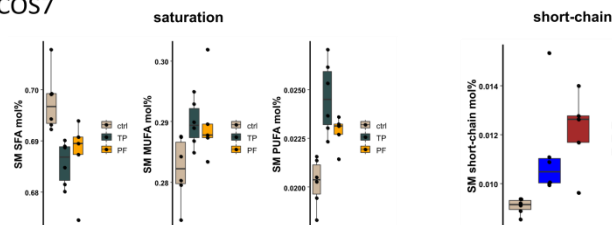
(C) HEPG2



(D) HEK

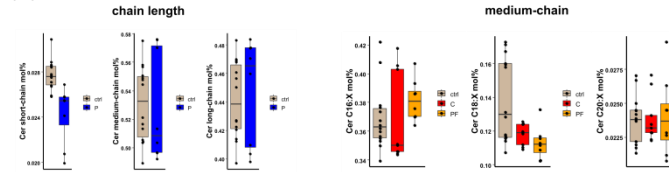


(E) COS7

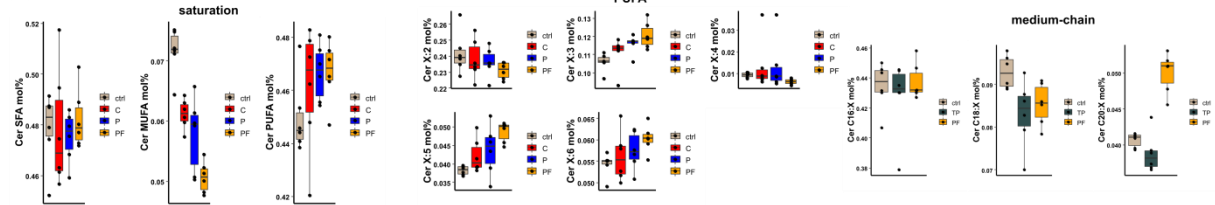


Supplementary Figure S6: Alterations of sphingomyelin (SM) levels in different cell lines (SH-SY5Y, N2a, HepG2, HEK and COS7) in presence of the analyzed MTXs caffeine (C), theophylline (TP), pentoxifylline (P), theobromine (TB) and propentofylline (PPF) shown as boxplots (in accordance to Figure 5).

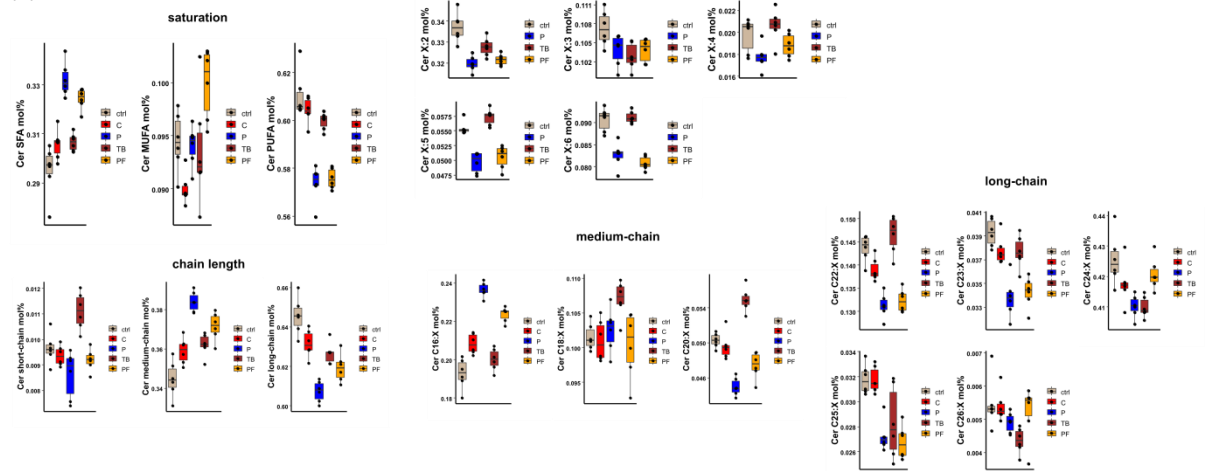
(A) SH-SY5Y



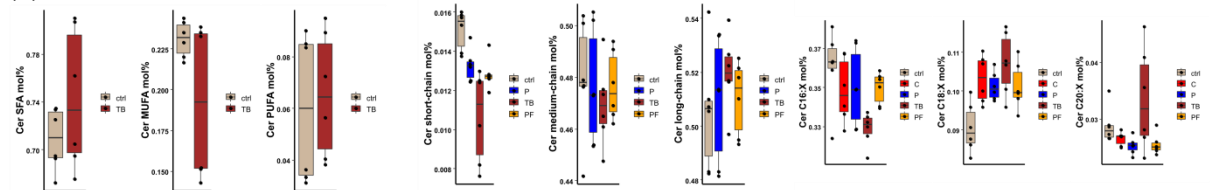
(B) N2a



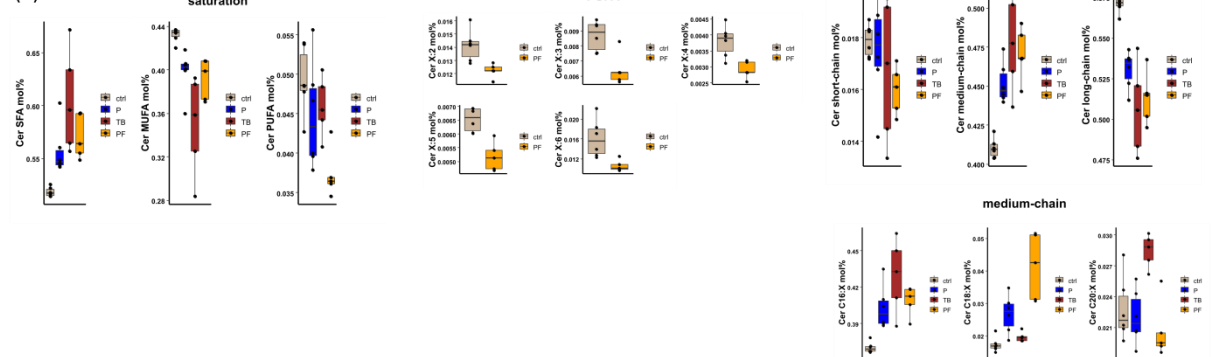
(C) HEPG2



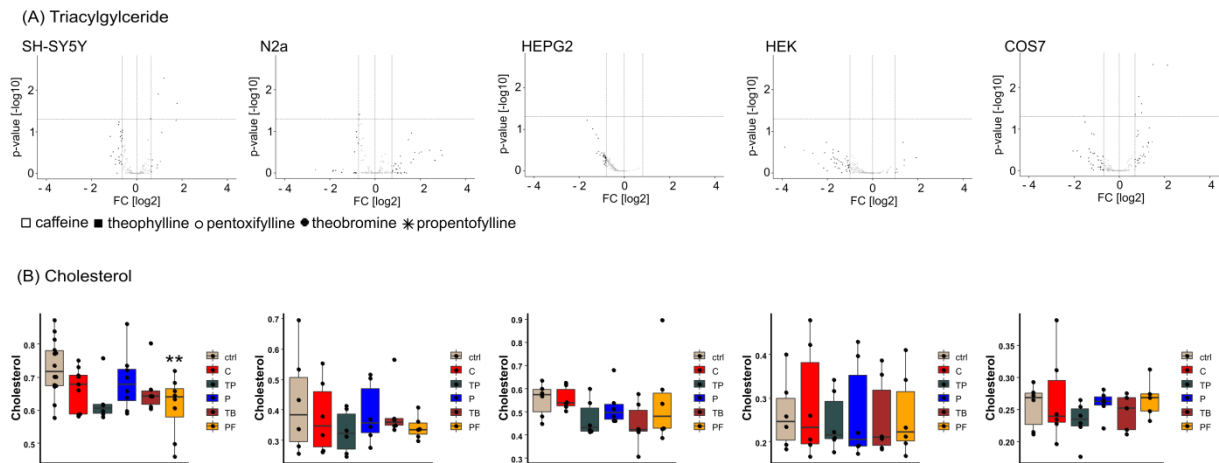
(D) HEK



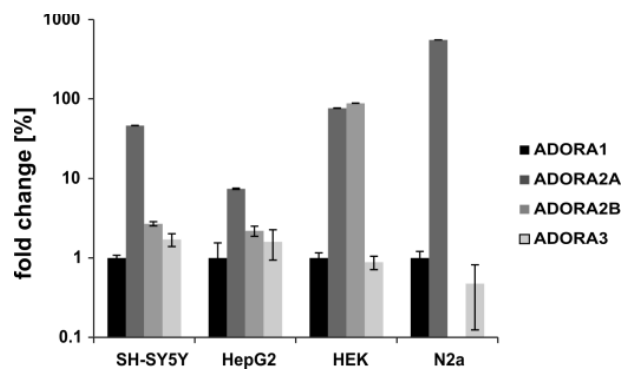
(E) COS7



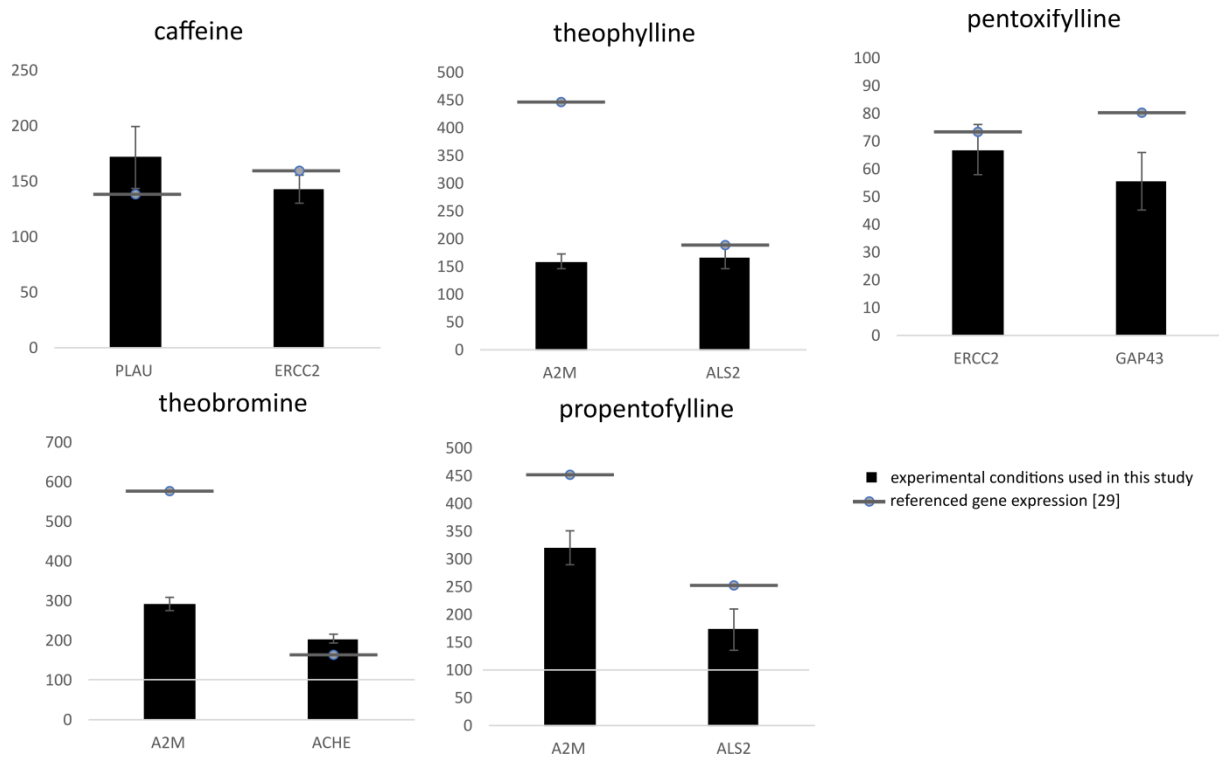
Supplementary Figure S7: Alterations of ceramide (Cer) levels in different cell lines (SH-SY5Y, N2a, HepG2, HEK and COS7) in presence of the analyzed MTXs caffeine (C), theophylline (TP), pentoxifylline (P), theobromine (TB) and propentofylline (PPF) shown as boxplots (in accordance to Figure 6).



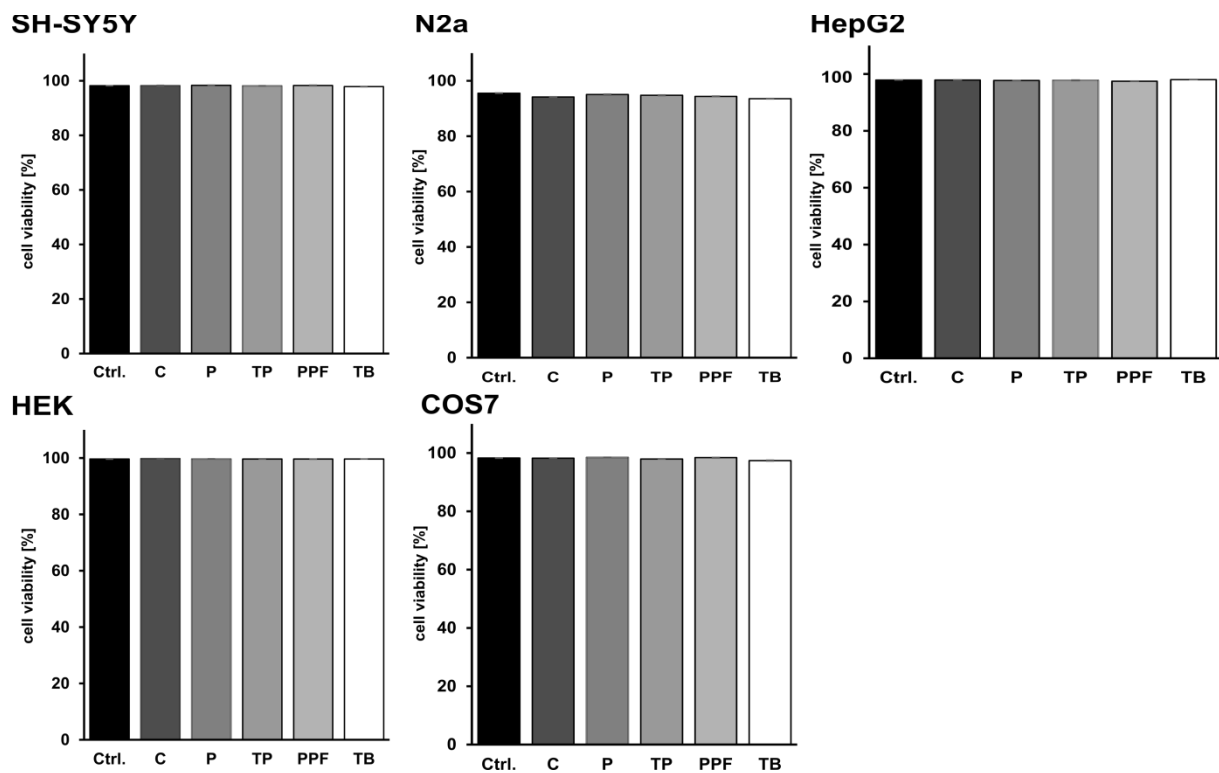
Supplementary Figure S8: Effect of different methylxanthines (caffeine, theophylline, pentoxifylline, theobromine, and propentofylline) on TAG and cholesterol levels in SH-SY5Y-, N2a-, HEPG2-, HEK-, and COS7 cells shown as volcano plots and boxplots. Error bars represent the standard error of the mean (SEM) and statistical significance was set as * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$.



Supplementary Figure S9: Gene expression pattern of ADORA1, ADORA2A, ADORA2B and ADORA3 in SH-SY5Y, HepG2, HEK and N2a cells analyzed by qPCR measurement. Error bars represent the standard error of the mean (SEM).



Supplementary Figure S10: Verification of the biological effects of the methylxanthines on known alterations in gene expressions (as reported in [29]) compared to the effects on gene expression under the experimental conditions used in this study. Error bars represent the standard error of the mean (SEM).



Supplementary Figure S11: Cell viability after treatment with different methylxanthines or solvent control in SH-SY5Y, N2a, HepG2, HEK and COS7 cells. Error bars represent the standard error of the mean (SEM).

Supplemental Table 1. Number of biological replicates for each analyzed cell line and each incubated methylxanthine.

Cell line	Methylxanthine	Biological replicates
SH-SY5Y	Solvent control	n=14
	Caffeine	n=9
	Theophylline	n=6
	Pentoxifylline	n=8
	Theobromine	n=6
	Propentofylline	n=8
N2a	Solvent control	n=6
	Caffeine	n=6
	Theophylline	n=6
	Pentoxifylline	n=6
	Theobromine	n=6
	Propentofylline	n=6
HEK	Solvent control	n=6
	Caffeine	n=6
	Theophylline	n=6
	Pentoxifylline	n=6
	Theobromine	n=6
	Propentofylline	n=6
HepG2	Solvent control	n=6
	Caffeine	n=6
	Theophylline	n=6
	Pentoxifylline	n=6
	Theobromine	n=6
	Propentofylline	n=6
COS7	Solvent control	n=6
	Caffeine	n=6

	Theophylline	n=6
	Pentoxifylline	n=6
	Theobromine	n=5
	Propentofylline	n=5