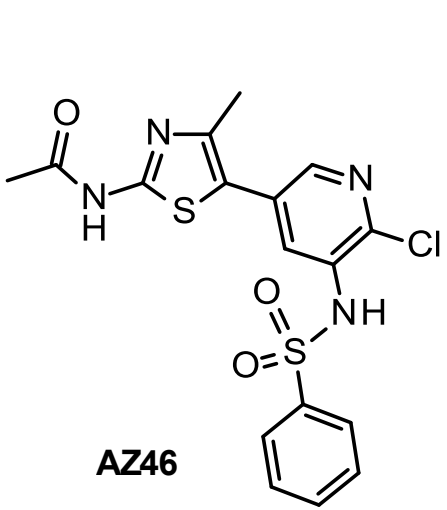
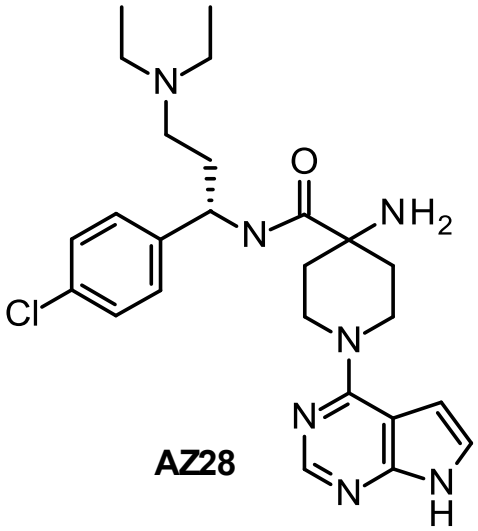
S. Figure 1. Chemical structure of drugs that inhibit metabolic enzymes FASN (AZ22), ACC (AZ52), DGAT (AZ17), PDHK (AZ30), and cell signalling kinase enzymes PI3K (AZ46), AKT (AZ28) and mTOR (AZ64).



AZ46



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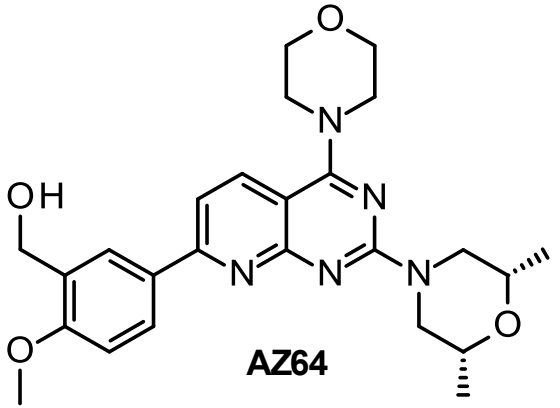
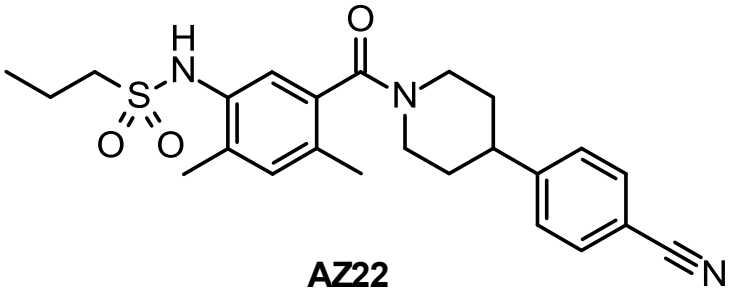
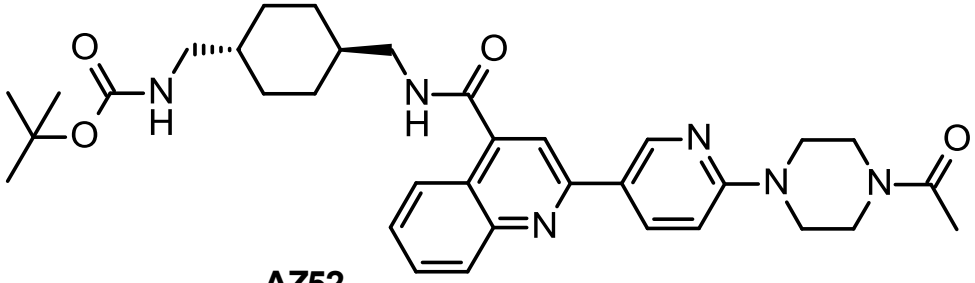


AZ64



AZ22



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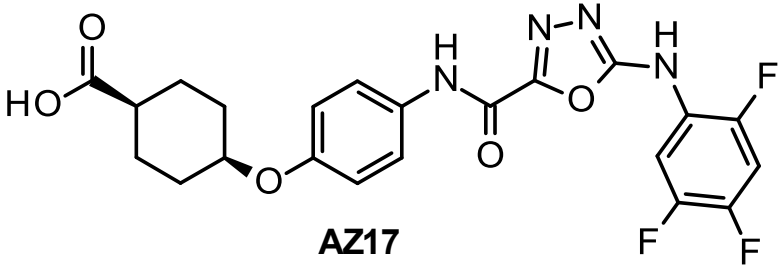
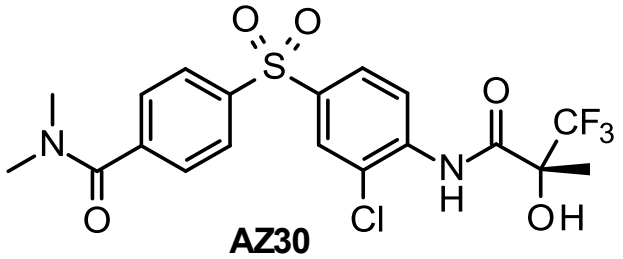
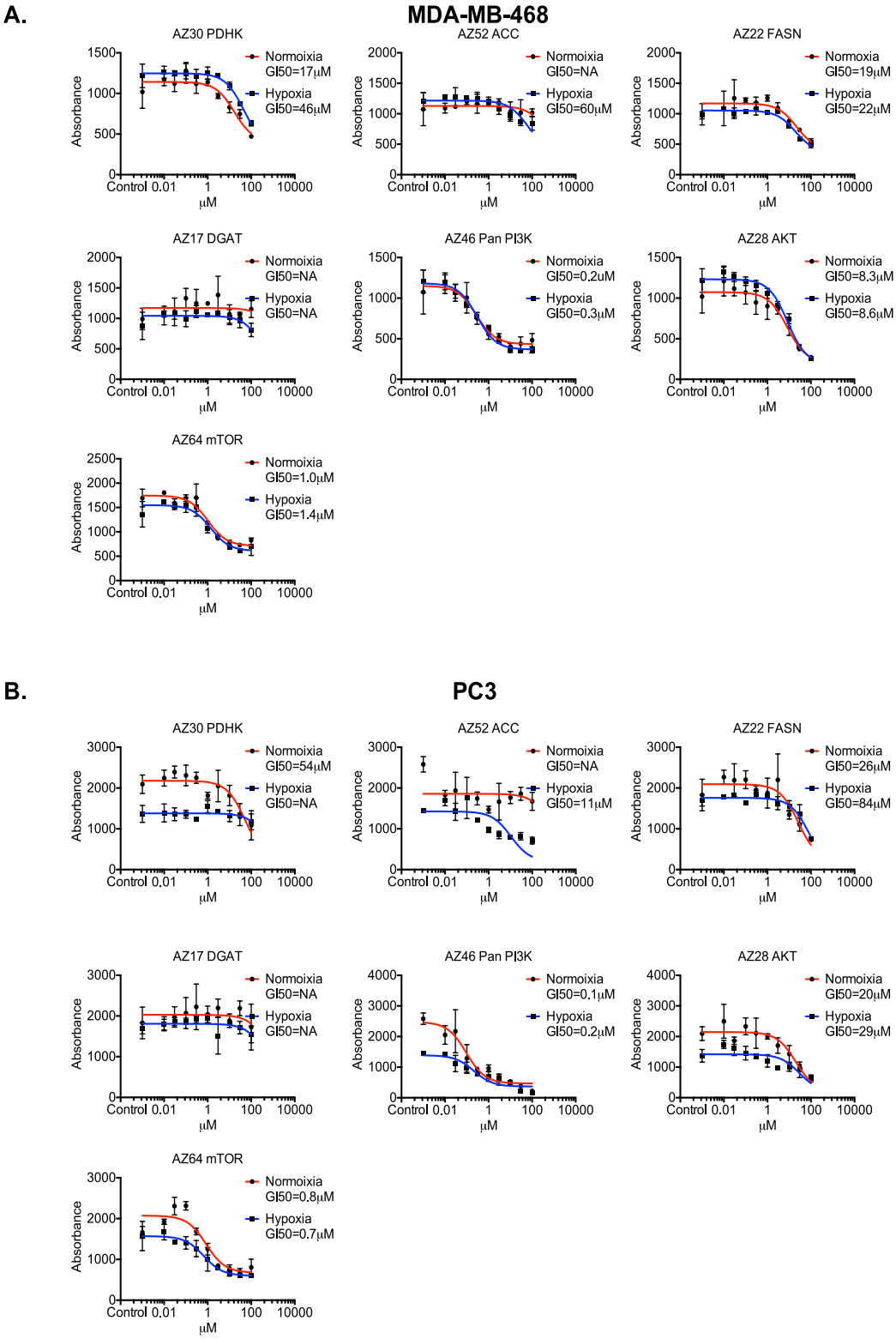


AZ17

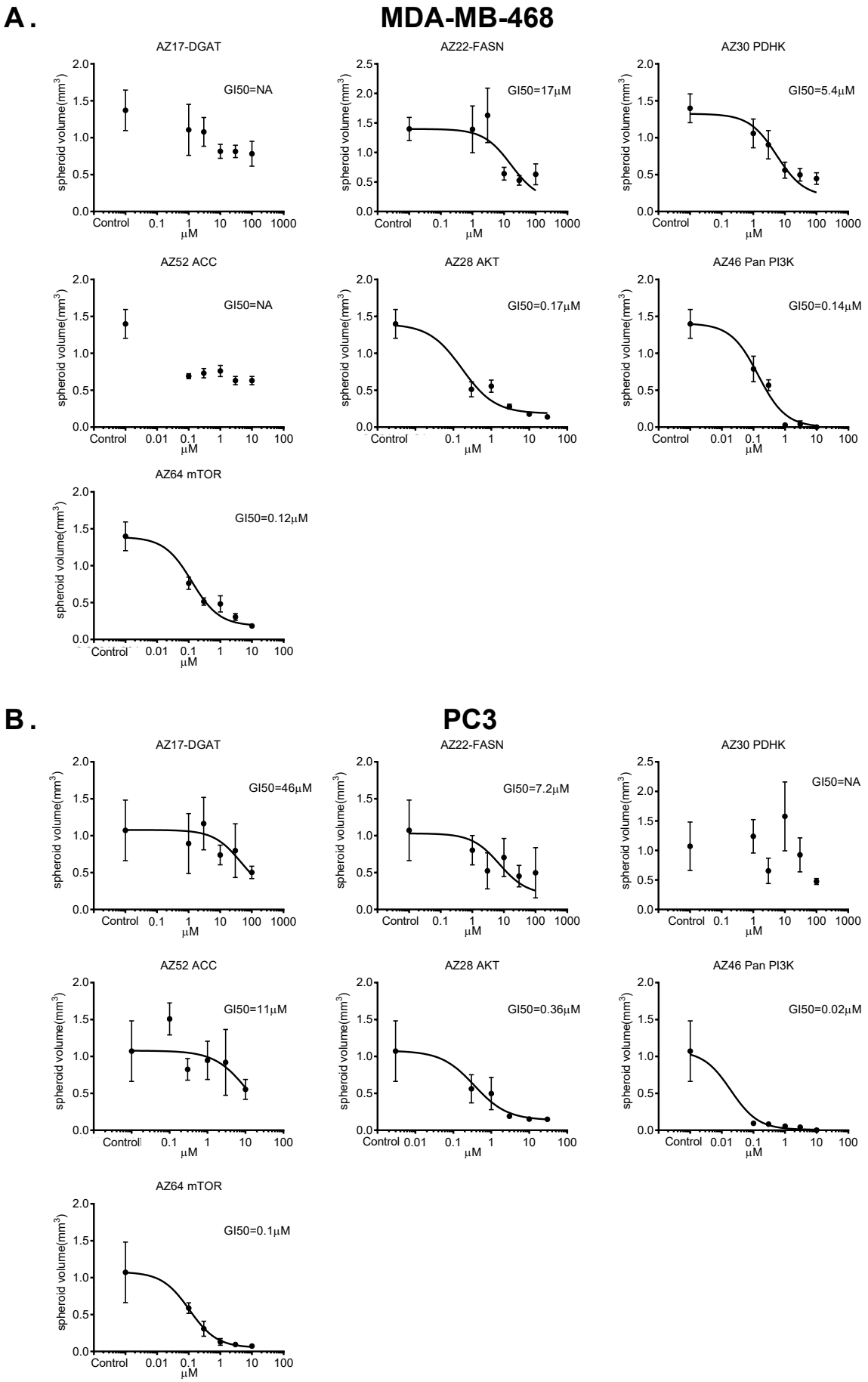


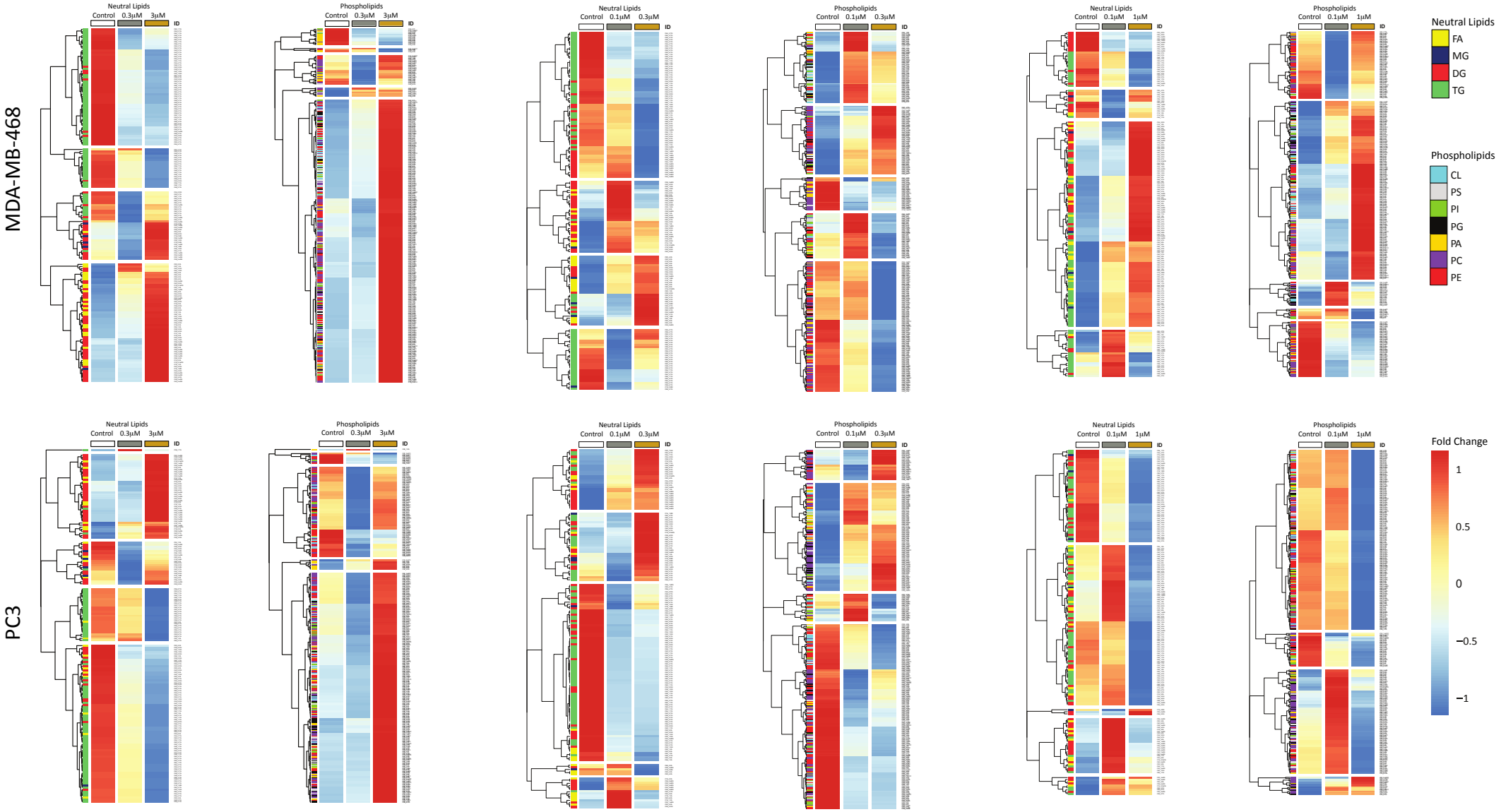
AZ30

S. Figure 2. Two dimensional CyQuant cell proliferation assay of (A) MDA-MB-468 and (B) PC3 cells following 3 days of treatment under normoxic and hypoxic conditions with inhibitors against metabolic enzymes PDHK, ACC, FASN and DGAT, and signalling enzymes PI3K, Akt and mTOR. Results were plotted in triplicate, errors bars represent standard deviation and GI50 values were calculated using Graph Prism.

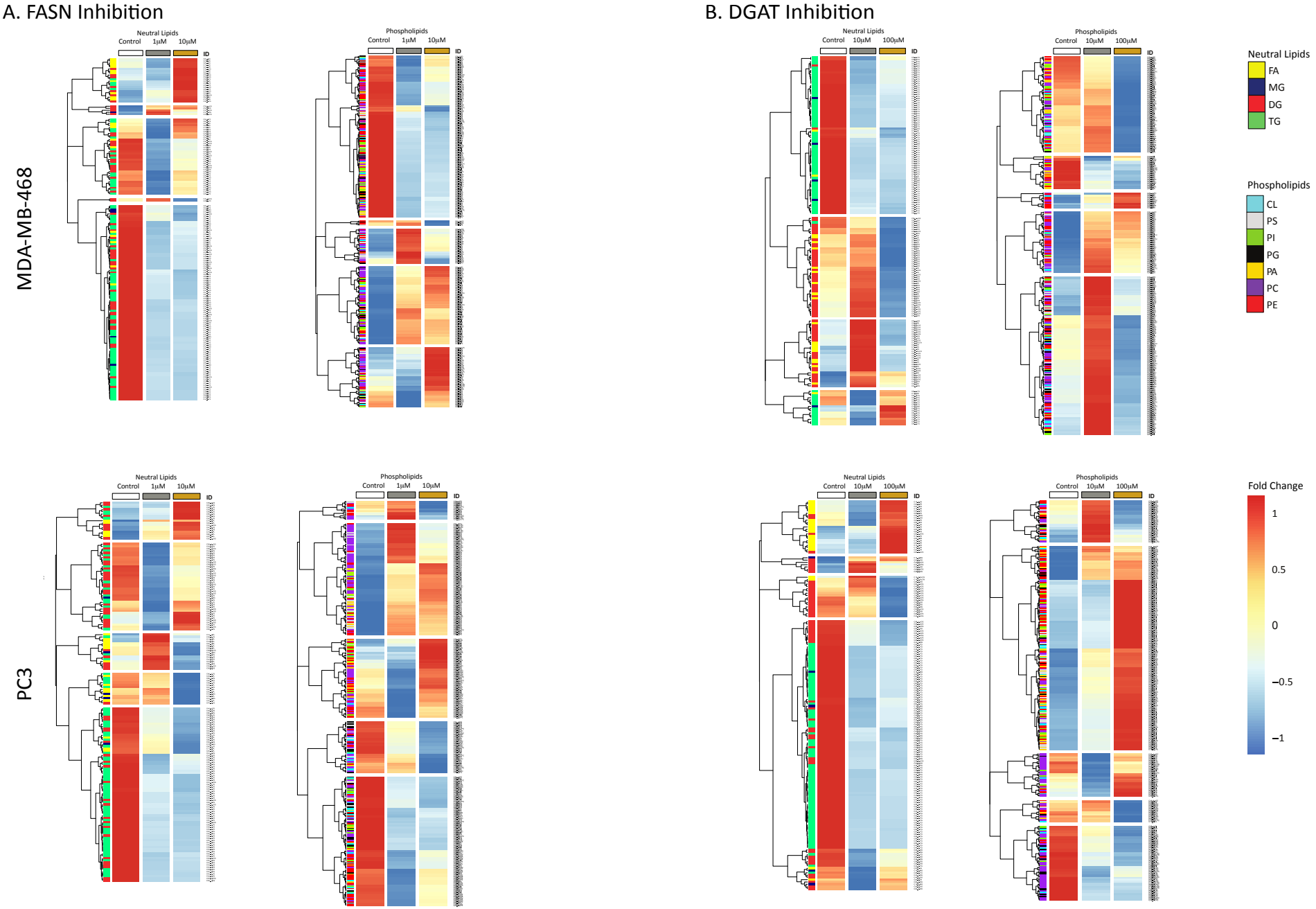


S. Figure 3. GI50 calculation of spheroids treated with inhibitors. (A) MDA-MB-468 on day 8 and (B) PC3 spheroids on day 12 treated with inhibitors against metabolic enzymes PDHK, ACC, FASN and DGAT, and signalling enzymes PI3K, Akt and mTOR. Between 5 and 15 spheroids were measured per condition, errors bars represent standard deviation and GI50 values were calculated using Graph Prism.

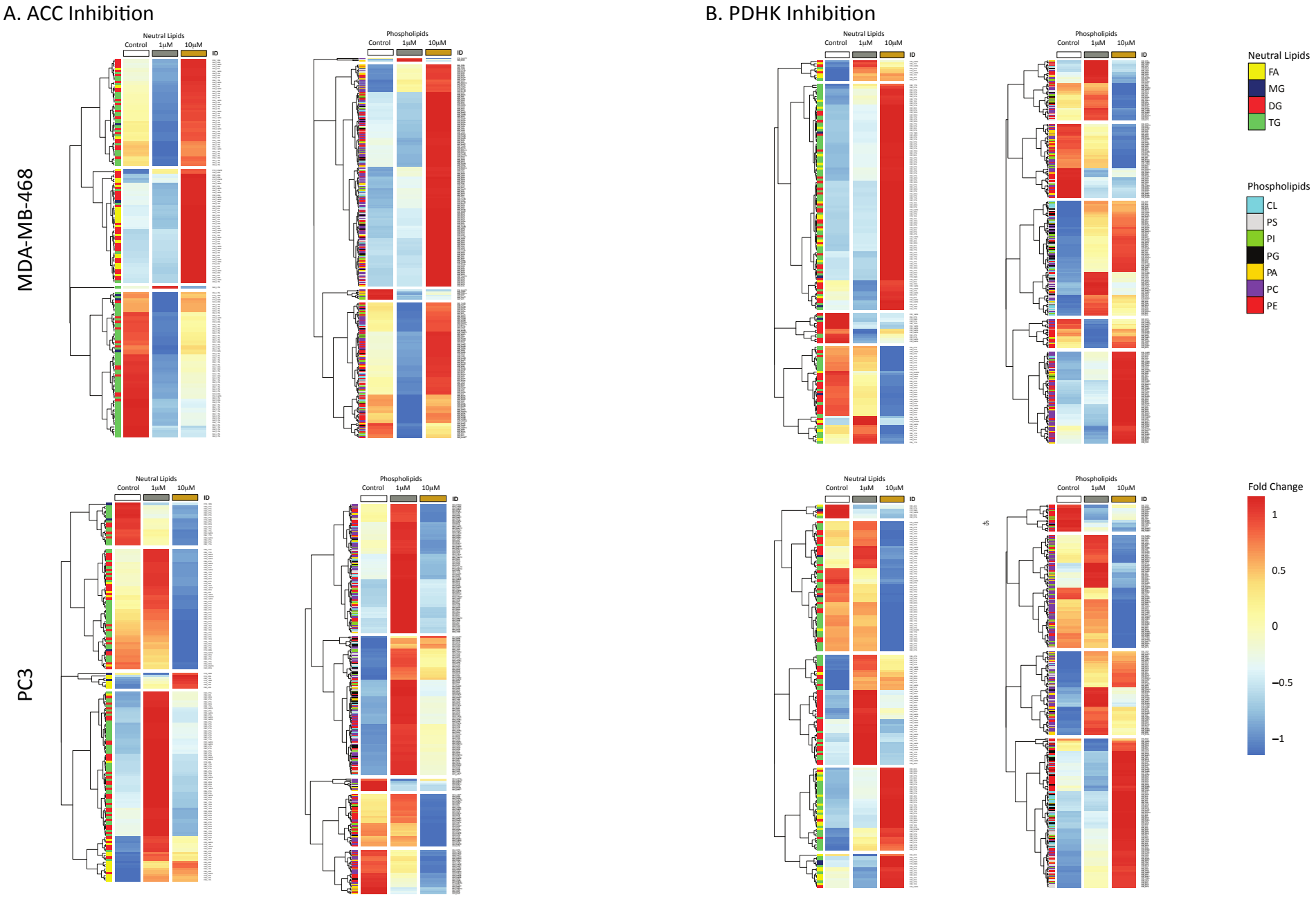




S. Figure 4. Clustered heat map analysis of lipids in MDA-MB-468 and PC3 spheroids treated for 4 days with A. AKT, B. PI3K and C. mTOR inhibitor at different concentrations. All Lipids were compared against DMSO control. Side covariates indicate lipid families, and lipids within each family are sorted by name.

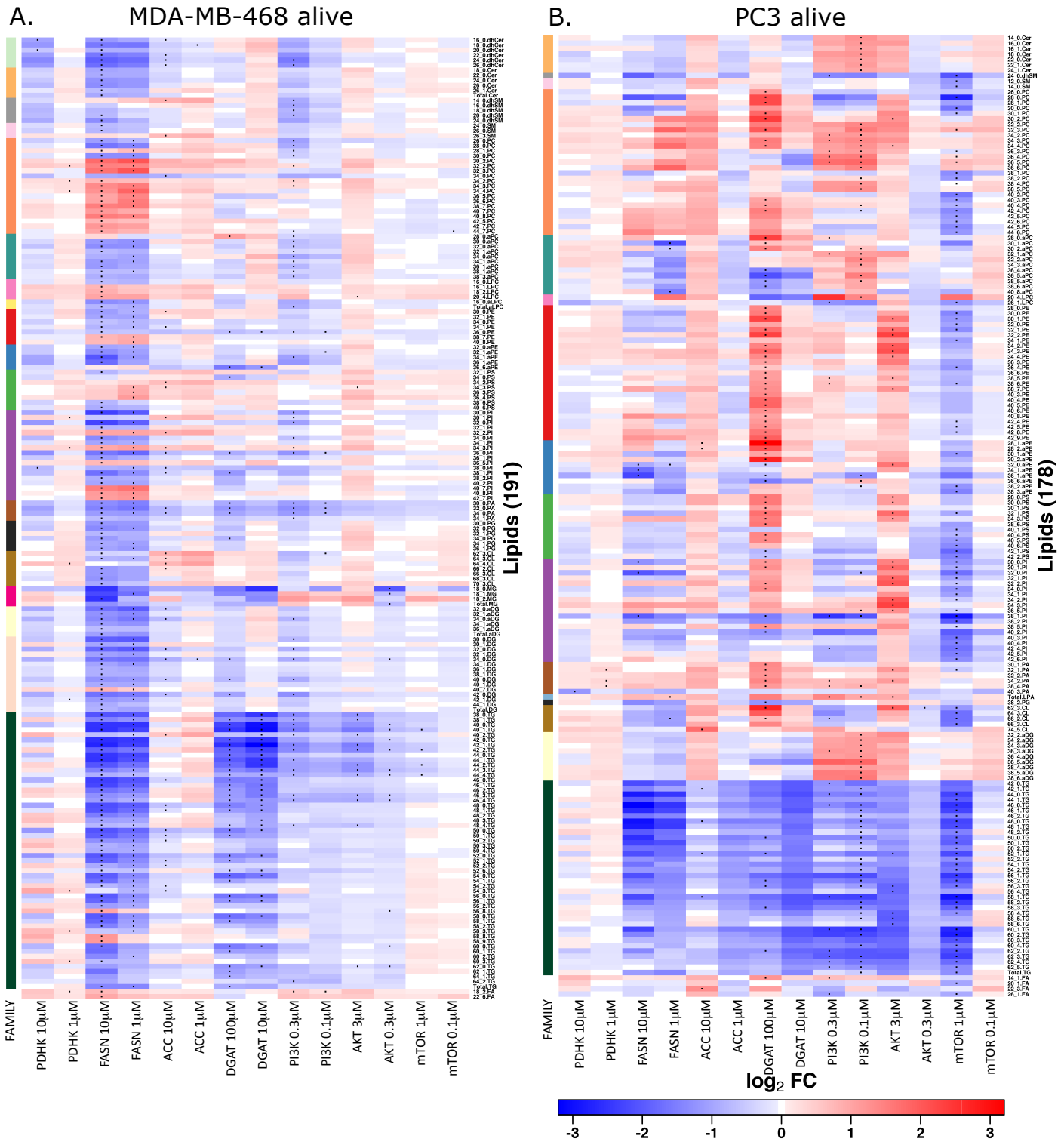


S. Figure 5. Clustered heat map analysis of lipids in MDA-MB-468 and PC3 spheroids treated for 4 days with A. FASN and B. DGAT inhibitor at different concentrations. All Lipids were compared against DMSO control. Side covariates indicate lipid families, and lipids within each family are sorted by name.



S. Figure 6. Clustered heat map analysis of lipids in MDA-MB-468 and PC3 spheroids treated for 4 days with A. ACC and B. PDHK inhibitor at different concentrations. All Lipids were compared against DMSO control. Side covariates indicate lipid families, and lipids within each family are sorted by name.

S. Figure 7. Significant differential abundance analysis of lipids as a heat map. A. MDA-MB-468 and B. PC3 spheroids were treated for 4 days with inhibitors against metabolic enzymes PDHK, ACC, FASN and DGAT, and kinases PI3K, AKT and mTOR at two different concentrations. All Lipids were compared against DMSO control, normalised to viable cells. Only lipids that were differentially abundant ($p<0.05$) following treatment by at least one drug (see dots in heat map) are epresented. Side covariates indicate lipid families, and lipids within each family are sorted by name. Paired t-test was used where 3 replicat es were available. Only fold change is reported where 2 samples were available. ($p<0.05$ and absolute $log2FC>1$).



S. Figure 8. Analysis of saturated, mono-unsaturated and poly-unsaturated phospholipids in MDA-MB-468 and PC3 spheroids. Levels of PC, PE, PI, PS, and SM in MDA-MB-468 and PC3 spheroids treated with inhibitors against metabolic enzymes PDHK, ACC, FASN and DGAT, and signalling enzymes PI3K, Akt and mTOR at two different concentrations. Data were converted to $\log_2$ scale for each replicate. Abundance of saturated, monounsaturated and polyunsaturated members within each family were then compared using one-way ANOVA, followed by Tukey Post-hoc test for pair-wise comparison (* $p<0.05$).