Figure S5

A

mSCLC KP1 (GSEA Chemical & Genetic Perturbations Gene Set)

B

mSCLC KP1

hSCLC NHJ29

C

Neutral Lipids
mSCLC KP1

D
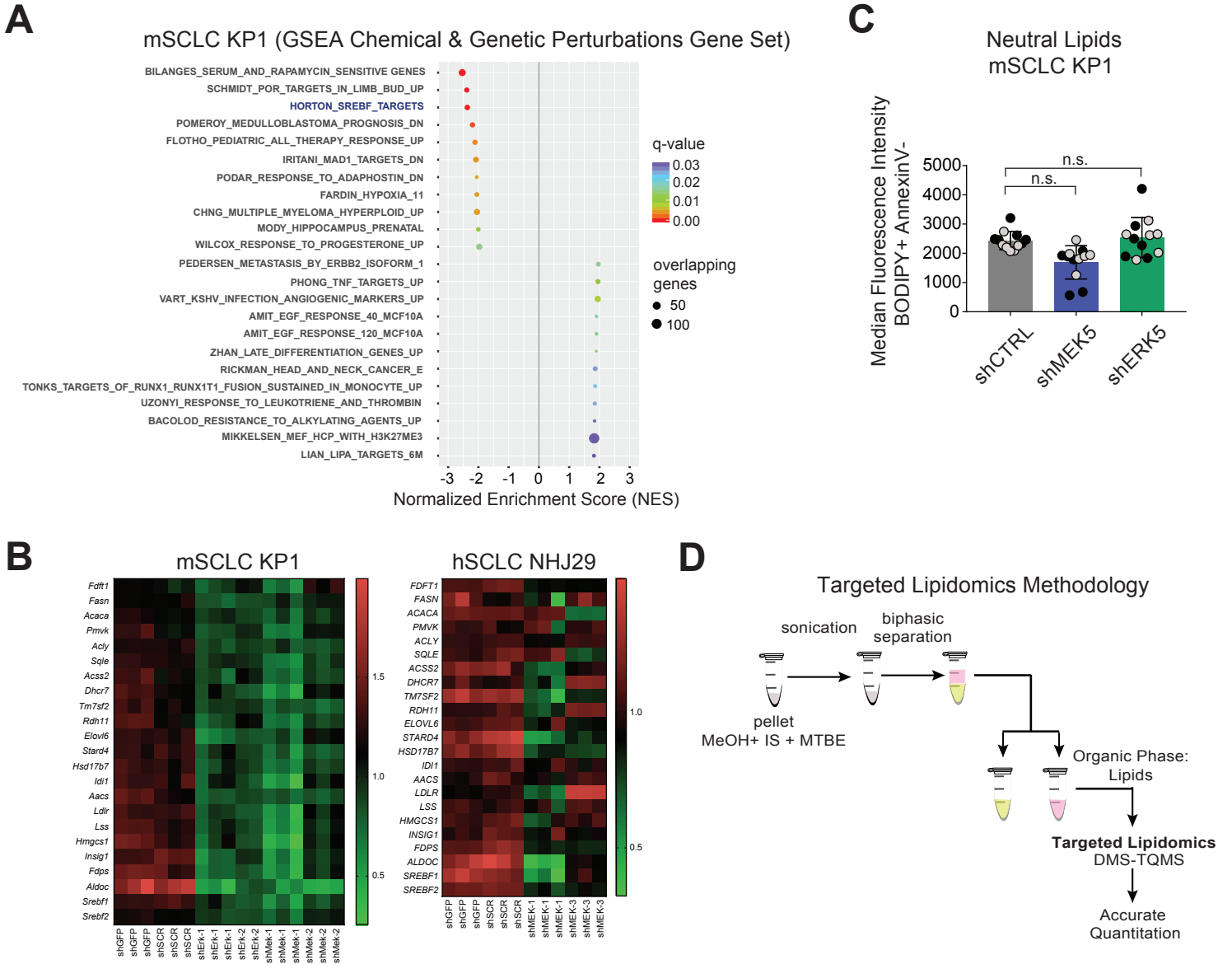

Targeted Lipidomics Methodology

Figure S5: Lipidomic profiling of SCLC cells

(A) Gene Set Enrichment Analysis (GSEA) using the CGP (Chemical & Genetic Perturbations) gene sets when the MEK5-ERK5 axis is downregulated in mSCLC KP1 cells compared to controls shows that SREBF targets are significantly disenriched; log2 fold change values averaged for shMEK5 and shERK5, respectively, each compared to shCTRLs (shGFP and shSCR), to focus analysis on genes controlled by both kinases; only enriched sets with q-values <0.03 are shown.

(B) Data from the normalized RNA-seq analysis (FPM values) with MEK5 and ERK5 knockdown cells and downregulation of the SREBP pathway targets (fold-changes are shown in Figure 4A).

(C) Results of BODIPY staining performed on shMEK5, shERK5 and control samples in mSCLC KP1 cells grown in reduced (2%) media show no difference between neutral lipid amounts after suppression of the MEK5-ERK5 axis; Mean fluorescence intensity of BODIPY staining is plotted, and AnnexinV positive cells were excluded from the analysis; grey or black dots represent two independent shRNAs per group; n=6 replicates per hairpin, n.s., p>0,05 by t-test following one-way ANOVA p=0.009, *** (significant differences between means).

(D) Schematic of targeted lipidomics profiling of mSCLC cells. MeOH, methanol; IS, internal standards; MTBE, methyl tertiary-butyl ether; DMS-TQMS, Differential Mobility Spectrometry-Triple Quadrupole Mass Spectrometry.
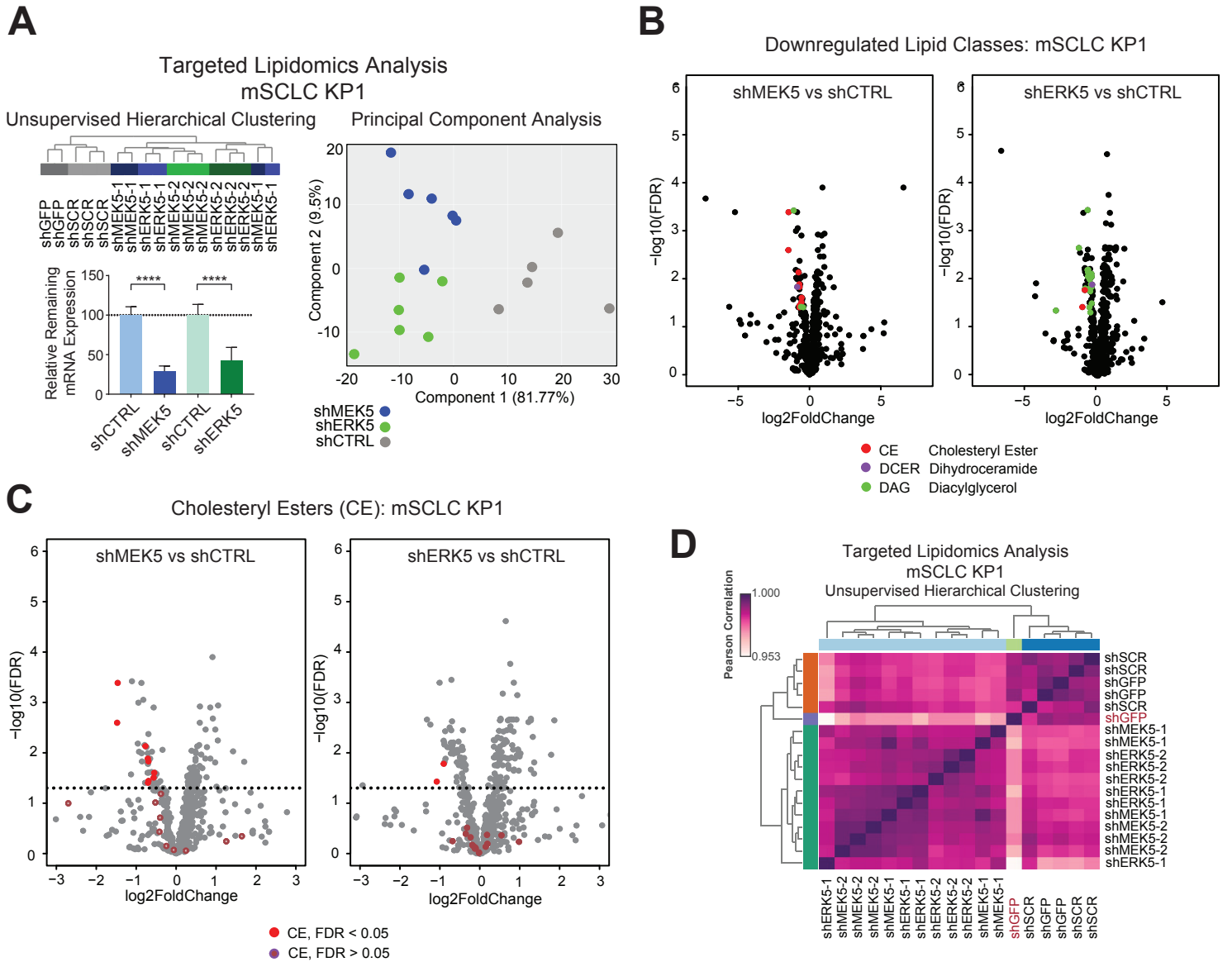
Figure S6

A

Targeted Lipidomics Analysis mSCLC KP1

Unsupervised Hierarchical Clustering

shGFP
shGFP
shSCR
shSCR
shMEK5-1
shMEK5-1
shERK5-1
shMEK5-2
shMEK5-2
shERK5-2
shERK5-2
shERK5-2
shERK5-1

Relative Remaining mRNA Expression
150
100
50
0
**** ****
shCTRL shMEK5 shCTRL shERK5

Principal Component Analysis

Component 2 (9.5%)
20
10
0
-10
-20 -10 0 10 20 30
Component 1 (81.77%)

shMEK5
shERK5
shCTRL

B

Downregulated Lipid Classes: mSCLC KP1

shMEK5 vs shCTRL

-log10(FDR)
6
5
4
3
2
1
0
-5 0 5
log2FoldChange

shERK5 vs shCTRL

-log10(FDR)
6
5
4
3
2
1
0
-5 0 5
log2FoldChange

CE — Cholesteryl Ester
DCER — Dihydroceramide
DAG — Diacylglycerol

C

Cholesteryl Esters (CE): mSCLC KP1

shMEK5 vs shCTRL

-log10(FDR)
6
5
4
3
2
1
0
-3 -2 -1 0 1 2 3
log2FoldChange

shERK5 vs shCTRL

-log10(FDR)
6
5
4
3
2
1
0
-3 -2 -1 0 1 2 3
log2FoldChange

CE, FDR < 0.05
CE, FDR > 0.05

D

Targeted Lipidomics Analysis mSCLC KP1 Unsupervised Hierarchical Clustering

Pearson Correlation
1.000
0.953

shSCR
shSCR
shGFP
shGFP
shSCR
shGFP
shMEK5-1
shMEK5-1
shERK5-2
shERK5-2
shERK5-2
shERK5-1
shERK5-1
shMEK5-1
shMEK5-2
shMEK5-2
shERK5-1

shERK5-1
shMEK5-2
shMEK5-2
shMEK5-1
shERK5-1
shERK5-2
shERK5-2
shERK5-2
shMEK5-1
shGFP
shSCR
shGFP
shGFP
shSCR
shSCR

Figure S6: Lipid species reduced after MEK5-ERK5 axis suppression

(A) Targeted lipidomics profiles of mSCLC KP1 cells subjected to unsupervised hierarchical clustering of lipid species abundances (top left) and Principal Component Analysis (right left) separates cells with MEK5 and ERK5 knockdown from those with control hairpins; relative remaining mRNA levels of MEK5 and ERK5 in mSCLC KP1 cells (bottom left) before subjection to lipidomic profiling; mRNA levels for each gene are normalized to the average expression level of that gene in the shCTRLs (shGFP and shSCR samples) with two independent shRNAs per group and n=2-3 independent replicates per hairpin; **** signifies p<0.0001.

(B) Significantly changing lipid species (FDR < 0.05) between shMEK5 and shCTRL cells (left) or between shERK5 and shCTRL cells (right) are shown in volcano plots with -log10(FDR) on the Y axis) and log2 fold change on the X axis; significantly changing lipid species (FDR < 0.05) from the CE (cholesteryl ester), DCER (dihydroceramide) or DAG (diacylglycerol) classes are shown as colored dots (colors corresponding to different lipid classes are shown in the legend at bottom).

(C) All detected cholesteryl esters (CEs), and their log2 fold changes between shMEK5 and shCTRL cells (left) and between shERK5 and shCTRL cells (right) are shown in volcano plots with -log10(FDR) on the Y axis and log2 fold change on the X axis; bright red dots specify significantly changing CEs (FDR < 0.05), while darker red dots below the FDR cutoff line do not reach significance (FDR > 0.05).

(D) Unsupervised clustering of sample correlations for targeted lipidomics data in KP1 mSCLC cells. The correlation heatmap depicts the Pearson correlation of lipid abundances across samples for pairwise complete observations on the log10 scale. Distance trees are calculated with the complete linkage method (hclust). The colored bars indicate the three main clusters. The excluded outlier sample (shGFP) is marked in red.