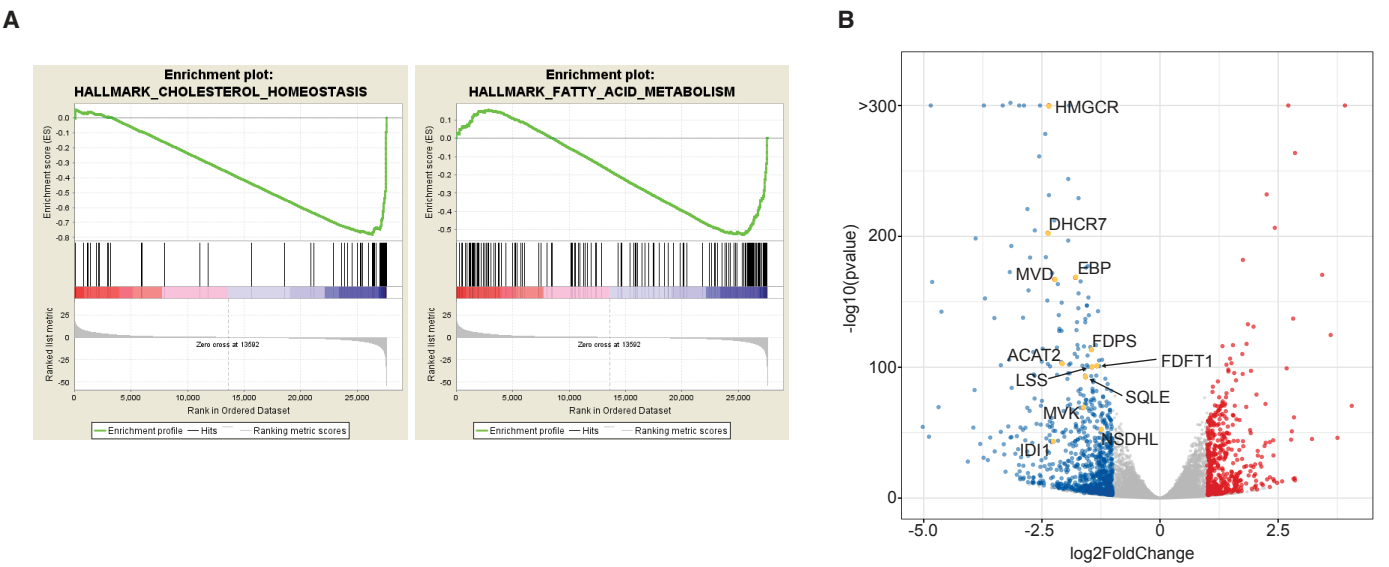
Supplementary Figure S1

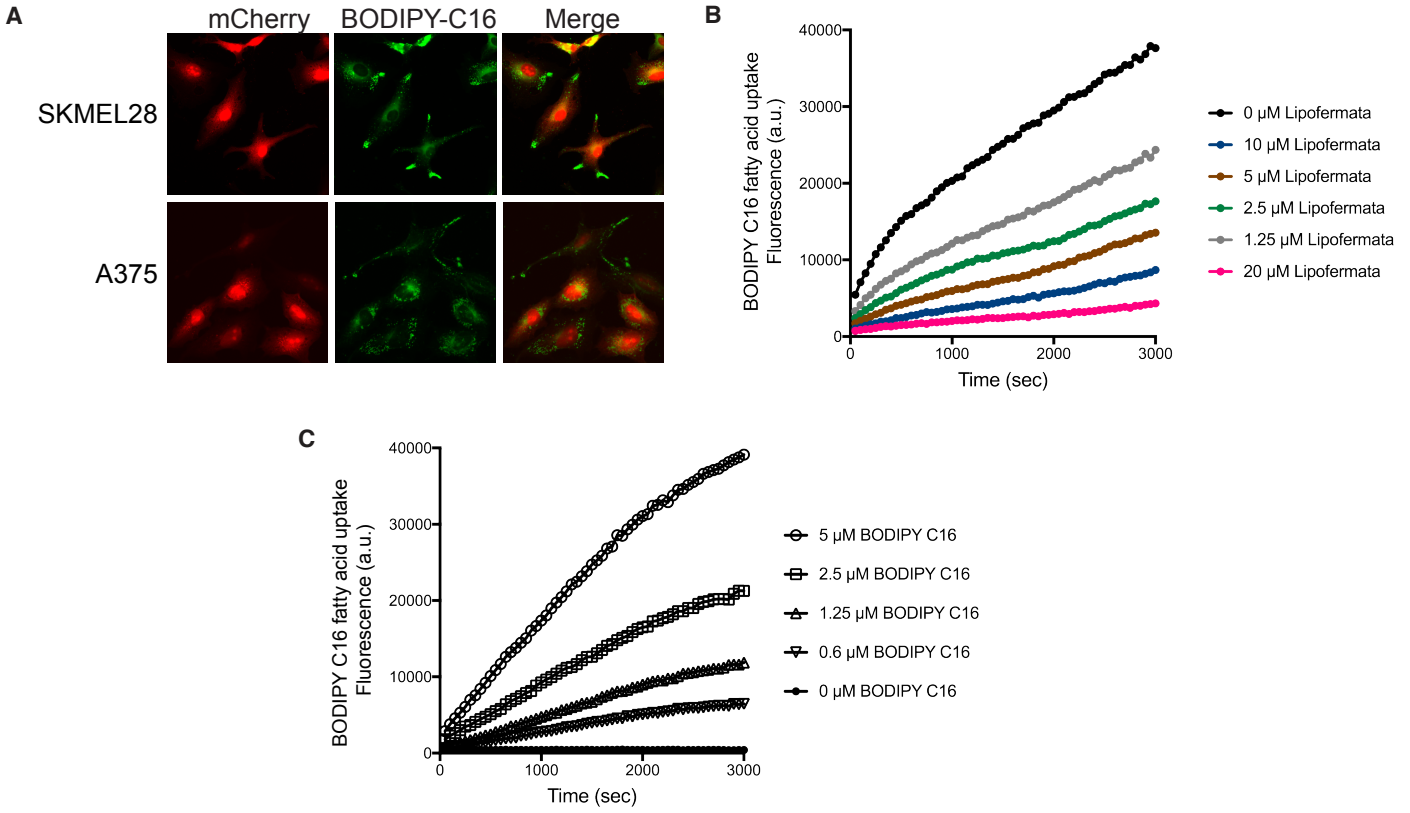


Supplementary Figure S1: RNA-seq analysis of human A375 melanoma cells in co-culture with adipocytes compared to monoculture controls.

(A) GSEA shows significant enrichment of hallmarks of cholesterol homeostasis and fatty acid metabolism in A375 cells co-cultured with 3T3L1 adipocytes, with strong downregulation of genes regulating *de novo* lipogenesis.

(B) Volcano plot depicting differentially expressed genes between A375 cells in mono-culture and in coculture with 3T3L1 cells. Log2 fold change is shown on the x-axis and –log10(pvalue) on the y-axis. Differentially expressed genes are shown in red (up in co-culture cells) and blue (down in co-culture cells) (log2 fold change ± 1, FDR 5%). Select lipid-related genes are highlighted in yellow.

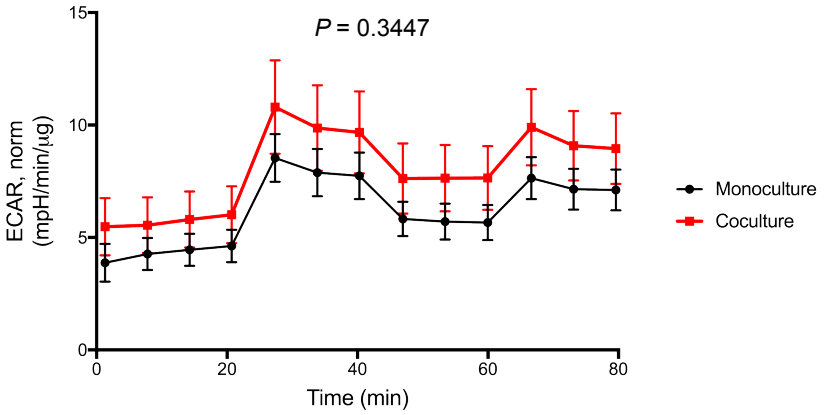
Supplementary Figure S2



Supplementary Figure S2: Lipid transfer and melanoma cell uptake of long chain fatty acids

(A) Transfer of BODIPY C16 fluorescent fatty acid from adipocytes to melanoma cells. 3T3L1 adipocytes were grown alone on the top portion of a Transwell and lipid droplets were labeled with BODIPY. After washing away extracellular BODIPY, Transwell insert containing adipocytes were transferred to a new well containing SKMEL28-mCherry or A375-mCherry cells. n=3 independent experiments, representative images are shown.
(B) BODIPY C16 real time fatty acid uptake in A375 cells treated with lipofermata. Representative graph is shown from n=3 independent experiments.
(C) BODIPY C16 real time fatty acid uptake in A375 cells with various concentrations of BODIPY C16. Representative graph is shown from n=3 independent experiments.

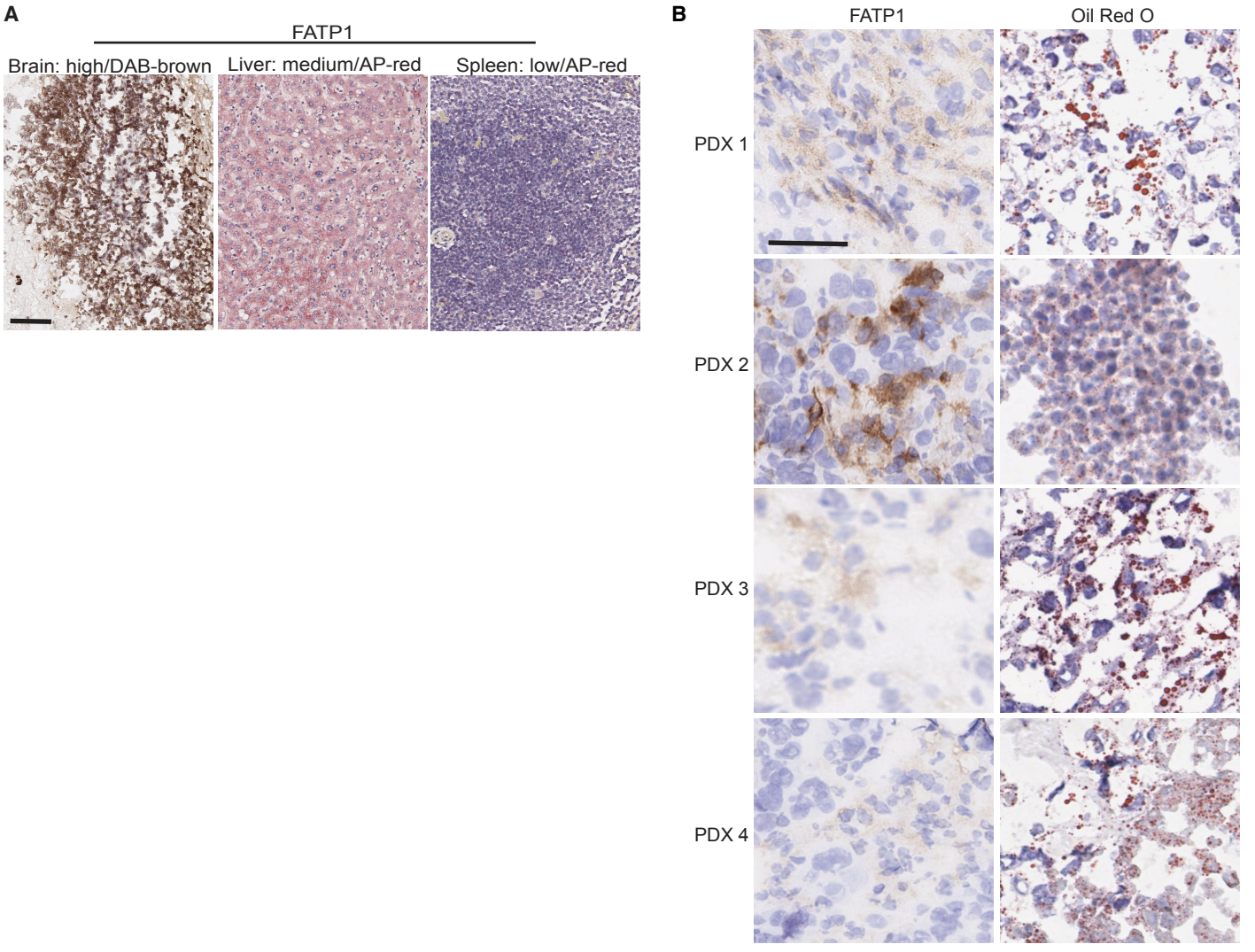
Supplementary Figure S3



Supplementary Figure S3: Adipocytes do not increase glycolysis in melanoma cells.

Extracellular acidification rate (ECAR) did not differ between FACS-isolated A375-GFP cells in monoculture or after co-culture with 3T3L1 adipocytes for 7 days. ECAR was measured under basal conditions followed by the sequential addition of oligomycin (1 µM), FCCP (2 µM), rotenone (1 µM) or antimycin A (1 µM). Representative experiment is shown. Error bars indicate s.e.m. n= 4 independent experiments. Two-way ANOVA with repeated measures.

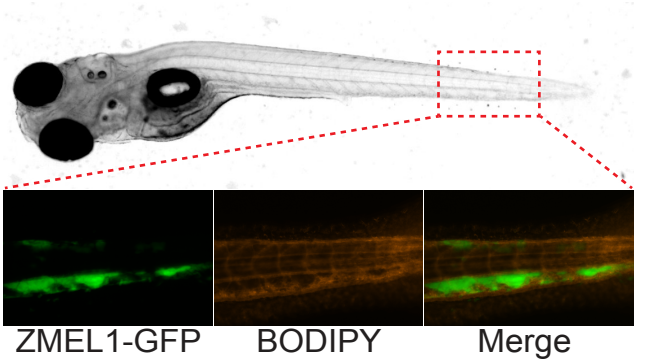
Supplementary Figure S4



Supplementary Figure S4: Endogenous FATP expression in control tissues and patient-derived xenografts

(A) Immunohistochemistry of FATP1 on human brain, liver, spleen and kidney tissues used as positive and negative controls for quantifying FATP1 IHC of TMAs and PDXs. Scale bar is 100μm.

(B) Immunohistochemistry of FATP1 and Oil Red O on 4 patient-derived xenograft samples. Scale bar is 50μm.



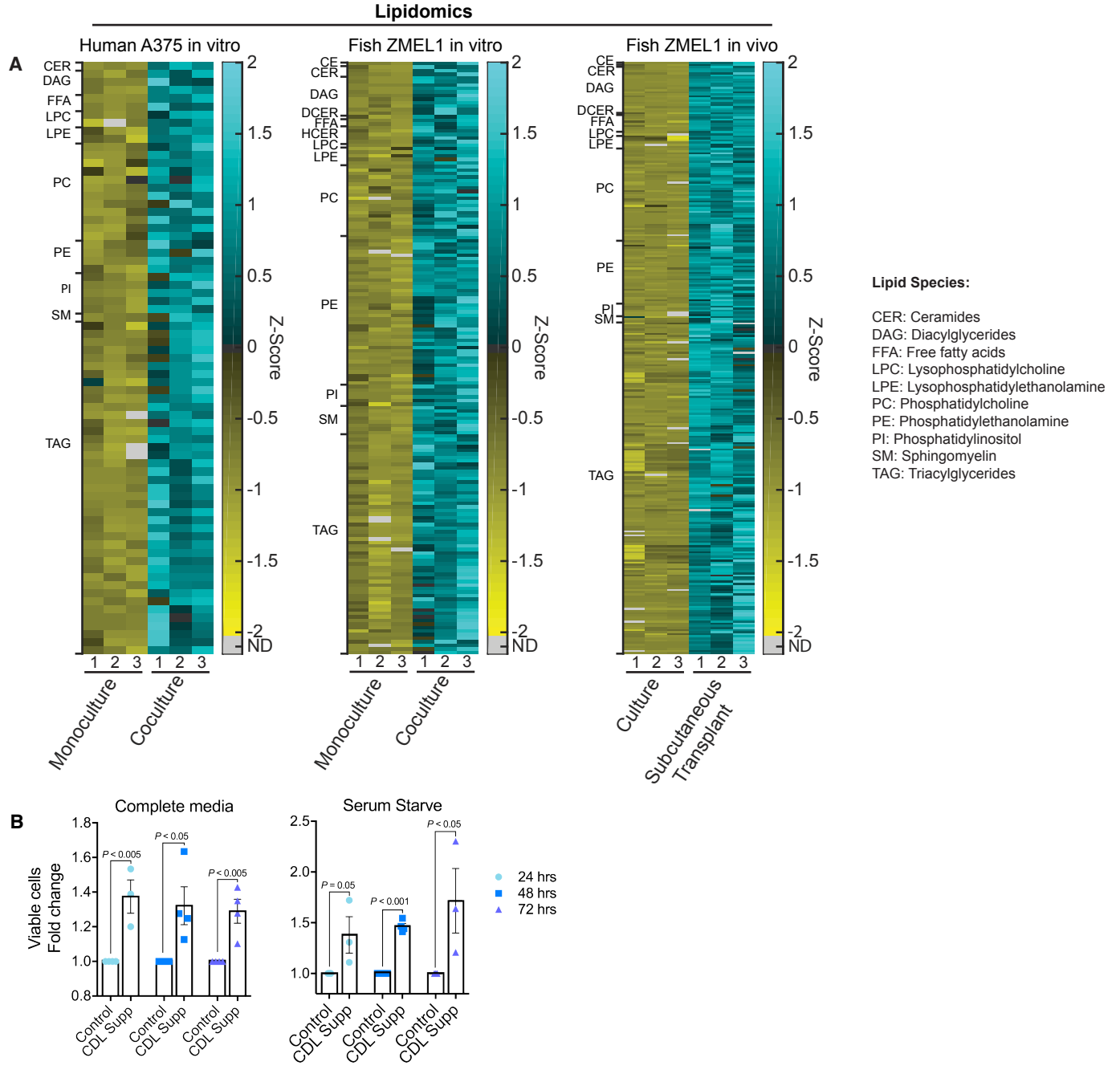
	# Fish w/ lipid-laden tumors	# Total fish examined	%
Adipocyte-positive subcutaneous	16	21	76
Adipocyte-negative tail	3	29	10

P < 0.001, Chi-square

Supplementary Figure S5: Absence of lipid-laden tumor cells in in vivo setting devoid of adipocytes

Casper embryo devoid of adipocytes with ZMEL1-GFP cells. Fish is stained with BODIPY 558/568 to visualize lipids. Insets show tails with GFP+ tumor cells and BODIPY 558/568 staining. Representative images are shown. Double positive cells were observed in n=3/29 transplanted fish. Table summarizes quantification of adult transplants (Figure 4C) with tumors next to adipocytes versus embryo transplants with tumors in areas devoid of adipocytes.

Supplementary Figure S6

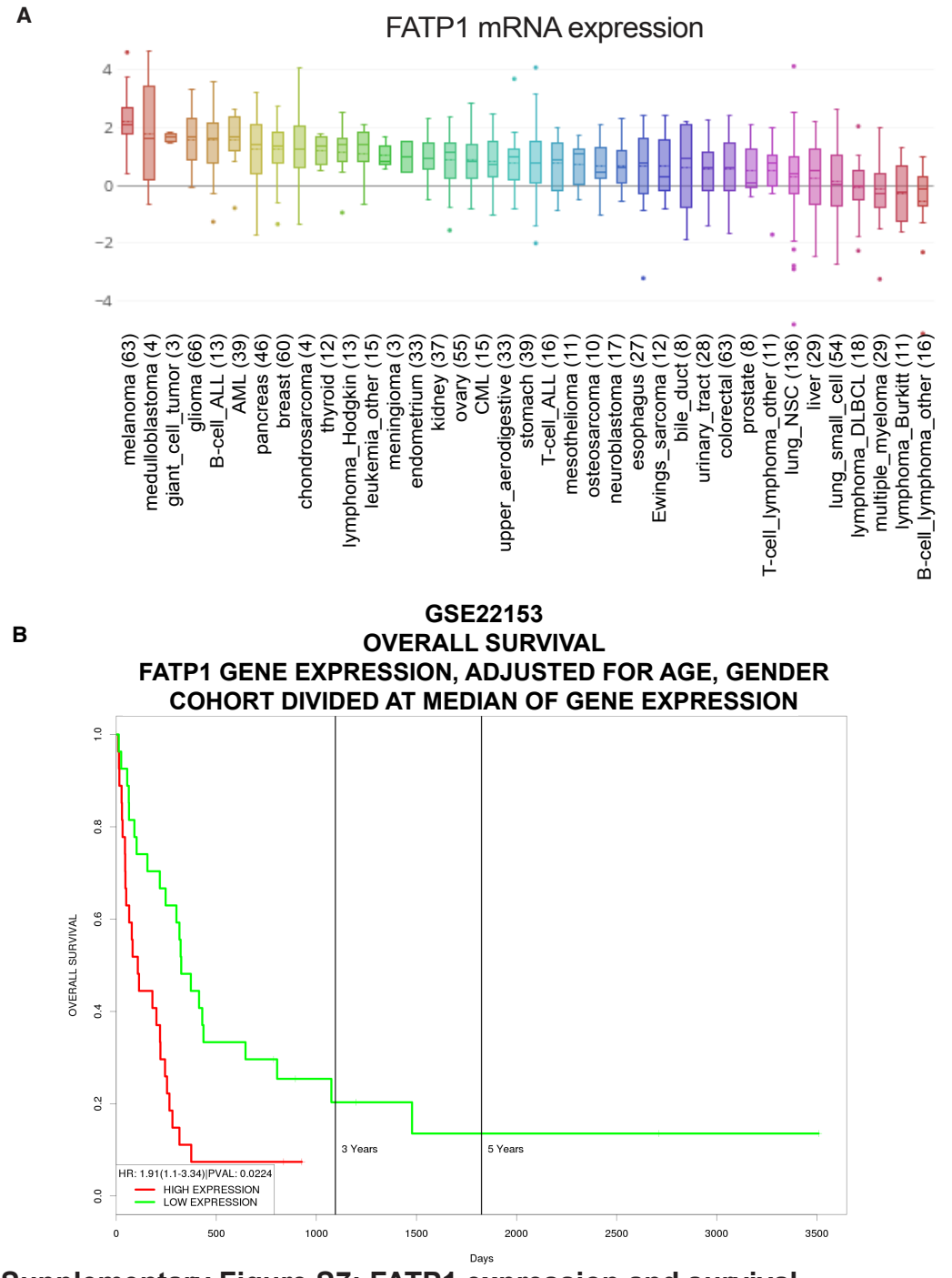


Supplementary Figure S6: Lipidomics reveals specific lipid species transferred from adipocytes to melanoma cells

(A) Heatmaps of lipidomics concentration z-scores from human A375 (left panel) or zebrafish ZMEL1 cells grown either in monoculture or co-culture with 3T3L1 adipocytes (center panel) and from zebrafish ZMEL1 cells grown either in culture or in subcutaneous transplants in zebrafish (right panel). Lipid species that were not detected (ND) are indicated. Classes of lipid species are indicated on the left of each heatmap (n=3 biological replicates for each group).

(B) Quantification of viable A375 cells after 24, 48 or 72 hours of growth in complete or serum-free control media or complete or serum-free media supplemented with commercially purchased, chemically-defined lipid mixture. n=3 independent experiments. Two-tailed, unpaired T-test.

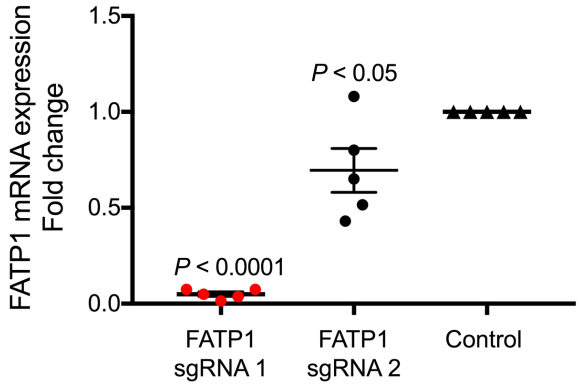
Supplementary Figure S7



Supplementary Figure S7: FATP1 expression and survival

(A) *FATP1* expression across 1019 cancer cell lines. Box-and-whisker plots show the distribution of mRNA expression for each cancer subtype, ordered by median *FATP1* expression level (line), the inter-quartile range (box) and up to 1.5X the inter-quartile range (bars). Sample numbers (n) are indicated in parentheses.

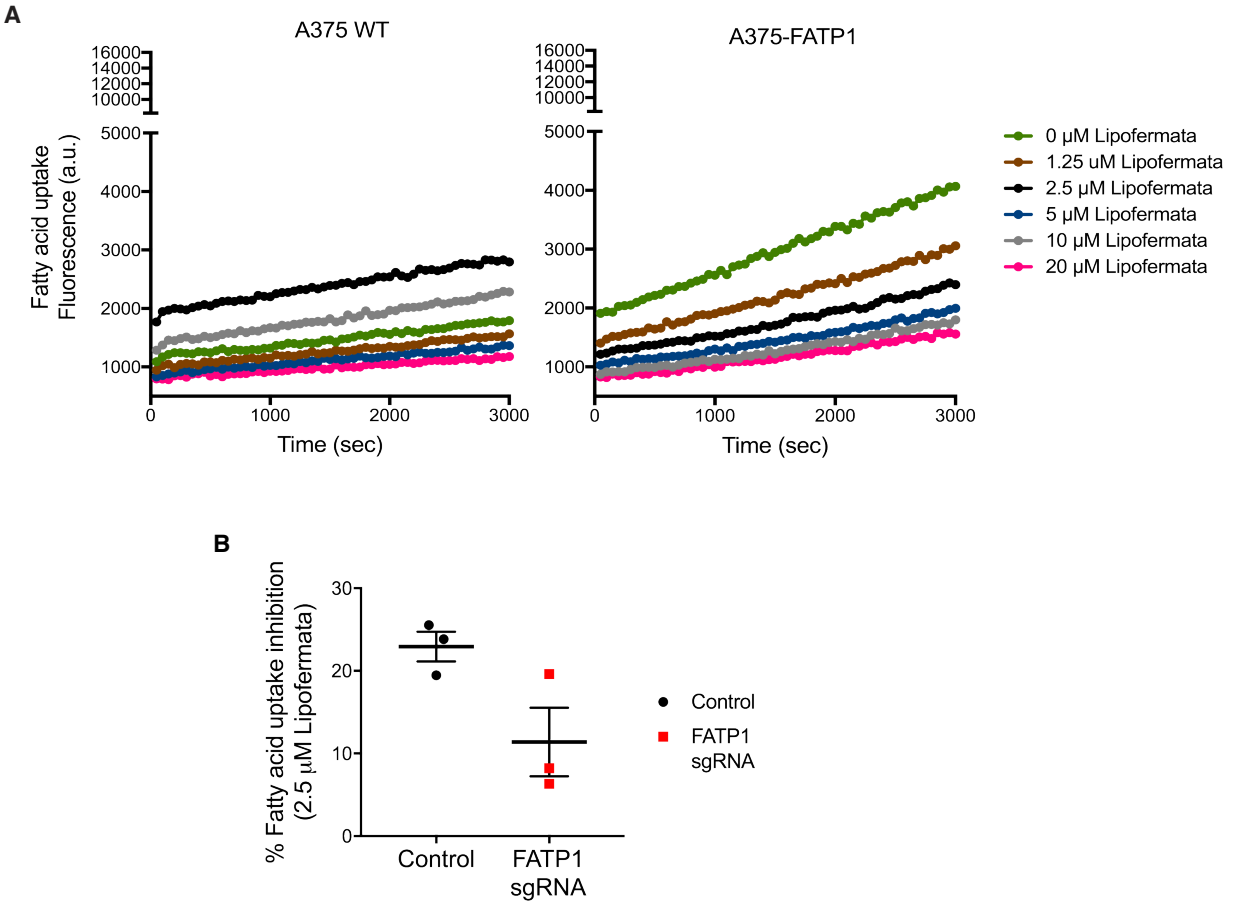
(B) Kaplan-Meier survival plot comparing stage IV melanoma patients in the BSE22153 dataset with high or low expression of FATP1, adjusted for age and gender. The X and Y axes represent follow-up time in days and the fraction of survival, respectively. The associated log-rank p-value is 0.0224.



Supplementary Figure S8: Validation of FATP1 knockdown

qPCR analysis of FATP1 expression in A375 FATP1 CRISPR knockout cell lines. FATP1 expression was calculated as fold change compared to Control 2. Two-tailed unpaired T-test, n=3 independent experiments.

Supplementary Figure S9

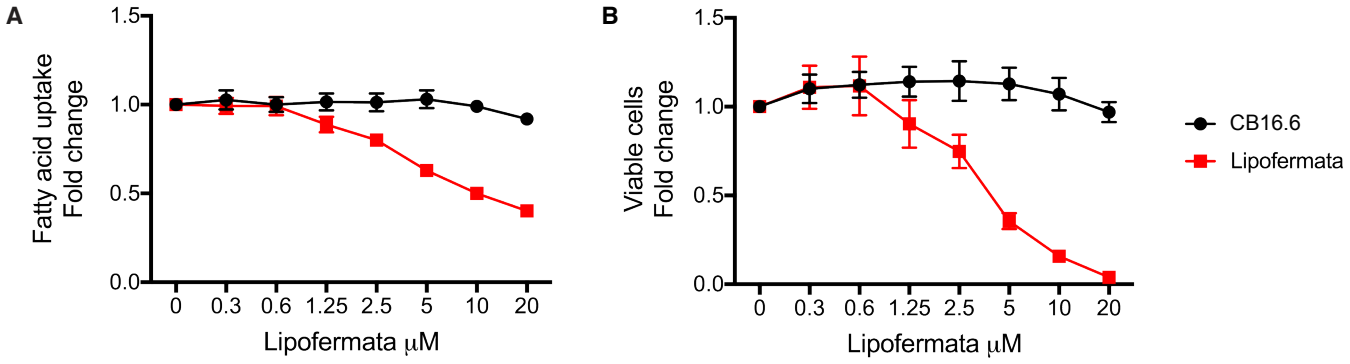


Supplementary Figure S9: Lipofermata targets FATP1

(A) QBT timed lipid uptake in Lipofermata-treated A375 overexpressing FATP1 compared to control cells. Graph represents mean from n=3 independent experiments. Error bars are s.e.m. Area under the curve (AUC) was calculated for each curve and differences were compared by 95% confidence intervals.

(B) Quantification of the inhibition of fatty acid uptake in A375 FATP1 CRISPR cells treated with 2.5 µM lipofermata compared to A375 control cells. Two-tailed unpaired T-test, n=3 independent experiments.

Supplementary Figure S10

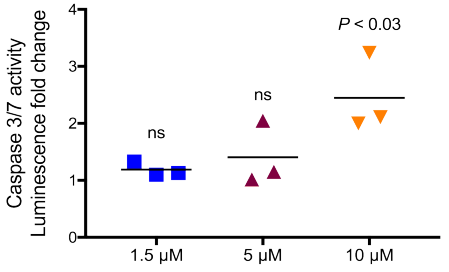


Supplementary Figure S10: Compounds that are structurally similar to Lipofermata do not cause decreases in lipid uptake or increased cell death

(A) Cell Titer Glo assay for cell viability on A375 cells treated with varying doses of CB16.6 and Lipofermata for 72 hours. Data represents mean fold change ± s.e.m., n=3 independent experiments.

(B) Lipid uptake assay in A375 cells treated with varying doses of CB16.6 or Lipofermata. Data represents mean fold change ± s.e.m., n=3 independent experiments.

Supplementary Figure S11



Supplementary Figure S11: Measurement of apoptosis in Lipofermata-treated cells

Caspase 3/7 Glo assay for measurement of apoptosis on A375 cells treated with 1, 5 or 10µM of Lipofermata for 24 hours. Mean is shown. Two-tailed Student's T-test, n=3 independent experiments.