Neuroblastoma (NB) is a childhood cancer arising from sympatho-adrenal neural crest cells. MYCN amplification is found in half of high-risk NB patients; however, no available therapies directly target MYCN. Using multi-dimensional metabolic profiling in MYCN expression systems and primary patient tumors, we comprehensively characterized the metabolic landscape driven by MYCN in NB. MYCN amplification leads to glycerolipid accumulation by promoting fatty acid (FA) uptake and biosynthesis. We found that cells expressing amplified MYCN depend highly on FA uptake for survival. Mechanistically, MYCN directly upregulates FA transport protein 2 (FATP2), encoded by SLC27A2. Genetic depletion of SLC27A2 impairs NB survival, and pharmacological SLC27A2 inhibition selectively suppresses tumor growth, prolongs animal survival, and exerts synergistic anti-tumor effects when combined with conventional chemotherapies in multiple preclinical NB models. This study identifies FA uptake as a critical metabolic dependency for MYCN-amplified tumors. Inhibiting FA uptake is an effective approach for improving current treatment regimens.
N euroblastoma (NB), a childhood solid tumor of the sympathetic nervous system, accounts for 15% of total childhood cancer mortality. Despite multimodal therapies, the 5-year survival rate for high-risk patients with NB remains below 50%. Almost half of high-risk NB patients harbor the amplified MYCN oncogene, the primary oncogenic driver of NB, leading to resistance to therapy and poor clinical outcomes. Pleiotropic effects and the lack of enzymatic activity make directly targeting the MYCN oncoprotein challenging. Therefore, new strategies for disrupting MYCN oncogenic programming are critical for developing effective NB therapies. MYCN facilitates NB growth in complex microenvironments with limited nutrient availability by overriding hypoxia-inducible factor (HIF1α)-mediated cell cycle arrest and promoting intratumoral vascular development to facilitate nutrient access by interior tumor regions. However, the mechanisms underlying the MYCN reprogramming of NB tumors that permit nutrient recruitment from the microenvironment remain unknown.

Compared with normal cells, tumor cells are metabolically reprogrammed to promote biomass and energy production to support rapid cell growth. These metabolic changes are driven by oncogenic stimuli (e.g. phosphatidyl inositol 3-kinase [PI3K]–AKT–mechanistic target of rapamycin complex 1 [mTORC1], MYC, and RAS), loss of tumor suppressors de novo lipogenesis in oncogenesis, and increased growth demand is sustained through metabolic homeostasis and meet energy requirements (via lipid droplets as a FA reservoir). Under nutrient deprivation (FAs) assemble into triacylglycerols (TGs), which are stored in intratumoral vascular development to facilitate nutrient access by interior tumor regions. However, the mechanisms underlying the MYCN reprogramming of NB tumors that permit nutrient recruitment from the microenvironment remain unknown.

Lipids play essential roles in supporting tumor survival as membrane components, energy reservoirs, and signaling mediators. Glycerolipids, such as diacylglycerols (DGs), function as secondary messengers that directly bind protein kinase C, RAS guanyl nucleotide-releasing protein, and chimaerins to modulate downstream oncogenic signaling. DGs and fatty acids (FAs) assemble into triacylglycerols (TGs), which are stored in lipid droplets (LDs) as a FA reservoir. Under nutrient deprivation conditions, TGs are hydrolyzed, releasing FAs to maintain lipid homeostasis and meet energy requirements (via β-oxidation). Cancer cells actively obtain FAs through endogenous biosynthesis and uptake from the microenvironment. Endogenous lipigenic mechanisms include de novo synthesis from acetyl-CoA, FA desaturation, and elongation. Numerous studies support a role for de novo lipogenesis in oncogenesis. We and others have shown that MYC or MYCN [MYC(N)] promotes FA synthesis by cooperating with Mondo A (SREBP1) or directly activating transcription of key lipigenic enzymes, including acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), and stearoyl-CoA desaturase (SCD) differently altered metabolite levels in lipid, amino acid, carbohydrate, and nucleotide super pathways (p ≤ 0.05), respectively. Circle sizes represent the absolute log2 fold change (FC). Notably, MYCN differentially altered metabolite levels in lipid, amino acid, carbohydrate, and nucleotide super pathways (p ≤ 0.05), with lipid metabolism as the most represented category (>35% differential metabolites, median log2FC = 0.56, Supplementary Fig. 1a, right and bottom). These findings suggest that MYCN globally reprograms NB metabolism, particularly lipid metabolism. To identify metabolic changes in each subpathway, we used differential abundance analysis for three comparisons (MYCN...
KD vs. CTRL, MYCN-ON vs. MYCN-OFF, and MNA vs. non-MNA. An abundance score of 100 indicates that 100% of the subpathway metabolites are significantly upregulated \( (p \leq 0.05) \), whereas a score of \(-100\) indicates that 100% of the subpathway metabolites are significantly downregulated \( (p \leq 0.05) \). Significantly enriched subpathways (FDR < 0.25, Benjamini–Hochberg adjusted \( p \)-value) are marked with asterisks “*”. Subpathways with the same number of “*” designations were ranked by the average absolute differential abundance score. Notably, the DG group was the most differentially abundant pathway, consistently upregulated by MYCN in all three systems (differential abundance score: MYCN KD = −25; MYCN ON = 70; MNA = ...
Fig. 1 MYCN reprograms NB metabolism. a Untargeted metabolomics profiling workflow using UHPLC-MS/MS and GC-MS (Discovery HD4™ platform, Metabolon Inc.) in LANS cells (MYCN KD for 0, 72, and 96 h, n = 4 each), MYCN3 cells (MYCN-ON for 0, 48, and 72 h, n = 4 each), and primary tumors (MNA, n = 18; non-MNA, n = 18). b Metabolite classification network. Each circle represents a metabolite. Circle size indicates absolute log2FC of metabolite level in comparisons of MYCN KD 72 h vs. CTRL, MYCN-ON 72 h vs. MYCN-OFF and MNA vs. non-MNA. Red = upregulated metabolite; blue = downregulated metabolite (p ≤ 0.05). One-way ANOVA or Welch's two-sample t-test was used to compare metabolite levels between groups. c Metabolic changes within subpathways. Comparison groups are the same as in (b). Differential abundance scores were calculated from subpathways containing at least three measured metabolites. 100 or −100 = all metabolites in the subpathway are upregulated or downregulated (p ≤ 0.05). Circle size = number of differentially altered metabolites (p ≤ 0.05). *indicates FDR < 0.25, hypergeometric test with p-value adjusted by Benjamini-Hochberg procedure. d Pathway enrichment analysis using GSEA-based algorithm. Subpathways with FDR < 0.25 were selected and ranked by -log10(FDR). Red = upregulated subpathway; blue = downregulated subpathway. e Lipidomics profiling in LANS shMYCN (CTRL and MYCN KD for 72 h), MYCN3 [MYCN-OFF (∼DOX) and MYCN-ON (∼DOX) for 72 h] and SK-N-AS MYCN-ER™ [MYCN-OFF (∼4-OHT) and MYCN-ON (∼4-OHT) for 48 h] cells (n = 4 each). Two-sided unpaired t-test; p-value adjusted by Benjamini-Hochberg procedure to obtain FDR. Significantly altered lipids (FDR < 0.25) were selected for heatmap (color is scaled by Z-score: red = upregulated; blue = downregulated). Percentages of upregulated and downregulated lipid classes are shown in stacked bar graphs. DG diacylglycerol, TG triacylglycerol, PC phosphatidylcholine, PE phosphatidylethanolamine, PG phosphatidylglycerol, PI phosphatidylinositol, PS phosphatidylserine, CE cholesteryl ester, plasmenyl, PE plasmenyl phosphatidylethanolamine. KD knockdown, MNA MYCN-amplified, non-MNA non MYCN-amplified. Source data are provided in the Source Data file.

56; Fig. 1c). Other MYCN-altered subpathways included: phospholipid (PL), glutamate, endocannabinoid, methionine, cysteine, S-adenosylmethionine (SAM) and taurine metabolism, and phosphatidylethanolamine (PC) (FDR < 0.25, Fig. 1c and Supplementary Fig. 1b). To further support our findings, we performed gene set enrichment analysis (GSEA; Fig. 1d and Supplementary Data 2). DG was the most enriched subpathway in MNA patients compared with non-MNA patients and was consistently induced by MYCN in both in vitro systems (FDR < 0.25, Fig. 1d). Polyamine metabolism was also upregulated in MNA patients, consistent with previous studies showing that MYCN activates polyamine metabolism.26 The fatty acyl carnitine group was highly enriched upon MYCN activation, suggesting the upregulation of FA oxidation, but no enrichment was observed in other systems. Collectively, these results suggest that MYCN alters lipid metabolism and promotes DGs accumulation in NB tumors.

To specifically evaluate the lipid metabolic changes induced by MYCN, we performed targeted lipidomics in LAN5 shMYCN, MYCN3 Tet-On cells, and SK-N-AS MYCN-ER™ cells, in which MYCN-mediated transcription is conditionally activated by 4-hydroxytamoxifen (4-OHT).23 Significantly altered lipids are represented in the heatmap (FDR < 0.25, Fig. 1e and Supplementary Data 3). All TGs (downstream of DGs) were significantly downregulated upon genetic depletion of MYCN (FDR < 0.25, Fig. 1e, left), whereas most DGs and TGs were upregulated upon MYCN induction (FDR < 0.25, Fig. 1e, middle and right). Because FAs are required for glycerolipid synthesis, we analyzed the FA chain compositions of MYCN-upregulated glycerolipids across the three systems. The FA chains 14:0, 16:0, 16:1, and 18:1 were the most represented (30%, Supplementary Fig. 1c), suggesting that these FAs contribute to glycerolipid synthesis. To examine how MYCN alters FA compositions, we applied mass spectrometry to profile FAs in MYCN3 Tet-On cells and TH-MYCN +/+ transgenic mice, in which neural crest-specific MYCN expression drives spontaneous NB formation recapitulating human disease.9 NB cells and tumors expressing high MYCN levels also contained high levels of 14:0, 16:1, or 18:1 (FDR < 0.25, Supplementary Fig. 1d–e), suggesting that MYCN upregulates these FAs for glycerolipid synthesis. In addition, MYCN increased the ratios of 14:1 to 14:0 and 16:1 to 16:0 (Supplementary Fig. 1d–e), thus MYCN also promotes FA desaturation. Collectively, these results indicate that MYCN changes the abundance of FAs required for glycerolipid accumulation.

MNA cell survival relies highly on FA uptake. FAs can be synthesized in cells or imported from the microenvironment. To dynamically examine how MYCN regulates FA synthesis, we added deuterated water (D2O) and [1-13C]16:0 to SK-N-AS MYCN-ER™ cell culture medium to trace de novo FA synthesis and desaturation, respectively. MYCN activation (4-OHT for 24 h) promoted de novo FA synthesis by increasing deuterium-labeling 16:0, and enhanced FA desaturation by increasing the ratios of [13C16]16:1 to [13C16]16:0 and [13C16]18:1 to [13C16]18:0 (p < 0.05, Fig. 2a). To determine the effects of MYCN on FA uptake, we traced changes in cell media supplemented with [13C16]16:0, the most abundant FA in mammalian cells, as an indicator of uptake. MYCN activation significantly enhanced FA uptake (p < 0.05, Fig. 2a), suggesting that MYCN promotes lipid accumulation by enhancing both FA synthesis and uptake. These findings were validated in a second MYCN system (MYCN Tet-On cells, p < 0.05, Supplementary Fig. 2a), indicating that MYCN enhances intracellular FA availability for lipid synthesis.

To assess the impact of FA synthesis and uptake on cell survival, we evaluated NB cell viability [MNA: LAN5, IMR32, SK-N-BE(2c); non-MNA: SH-SY5Y, SHEP, SK-N-AS] and normal cells (ARPE-19, C2C12, and HS-5) after treatment with two FA synthesis inhibitors (A939572, an SCD1 inhibitor and orlistat, a FASN inhibitor)22,34 or two FA uptake inhibitors (CB16.2 and CB5, which target FATP2)36 (Fig. 2b). NB cells were more sensitive to FA uptake inhibition (MNA IC50: 0.5–7.9 µM; non-MNA IC50: 1.4–10.4 µM) than to FA synthesis inhibition (MNA IC50: 7.6–60.7 µM; non-MNA IC50: 9.2–77.5 µM), suggesting that NB cells actively use exogenous FA for survival. CB16.2 was toxic to all tested normal cells, despite having the highest efficacy in NB cells. Conversely, CB5 was highly effective in NB cells and did not elicit cytotoxicity against normal cells. MYCN enhances SCD1 activity (Supplementary Figs. 1d–e and 2a), whereas A939572 suppresses SCD1 activity (p < 0.0001) and de novo FA synthesis (p < 0.05) in MNA cells (Supplementary Fig. 2b, left). However, SCD1 inhibition did not effectively inhibit cell growth and stimulated compensatory dose-dependent FA import from the media (p < 0.05; Supplementary Fig. 2b, right), suggesting that exogenous FA uptake may reduce cell sensitivity to FA synthesis inhibition. To test this hypothesis, we evaluated the viability of MNA cells in complete and delipidized media with and without A939572. The removal of exogenous lipids significantly enhanced the cytotoxicity of A939572 (IC50 from 41.0 µM to 2.2 µM), which was partially rescued by FAs supplementation (Supplementary Fig. 2c). Pharmacological inhibition of FA uptake via CB5 also enhanced the cytotoxic effects of A939572 (IC50 from 39.3 µM to 0.4 µM) and increased cell apoptosis (p < 0.05, Supplementary Fig. 2d). These results suggest that NB cells import exogenous FAs as a compensatory mechanism to evade FA synthesis inhibition.
Fig. 2 MNA cell survival relies on FA uptake. **a** Stable isotope tracing of FA synthesis and FA uptake in SK-N-AS MYCN-ER™ cells with or without 4-OHT (500 nM) for 24, 48, and 72 h. Mean ± SD (n = 3); two-sided unpaired t-test per time point. **b** Viability of NB and normal cells upon 72 h treatment with FA synthesis inhibitors (A939572, orlistat) and FA uptake inhibitors (CB16.2 and CB5). IC50 calculated in GraphPad Prism (7.01). Mean ± SD (n = 3). **c** Top, cell viability in complete media, delipidized media, and delipidized media supplemented with 0.025% FAs. MNA: LAN5 (0–6 day), IMR32 (0–4 day) and SK-N-BE(2c) (0–6 day); non-MNA: SHEP (0–6 day) and SK-N-AS (0–6 day). Mean ± SD (n = 3); two-way ANOVA with Dunnett’s multiple comparisons test. Bottom, Caspase 3/7 activity in complete media, delipidized media, and delipidized media supplemented with 0.025% FAs for 4 days. Mean ± SD (n = 3); one-way ANOVA with Dunnett’s multiple comparisons test. FC fold change, CM complete media, DLM delipidized media. Source data are provided in the Source Data file.
To determine whether MYCN-driven FA uptake supports cell survival, we examined the viability of three MNA (LAN5, IMR32, and SK-N-BE(2c)) and two non-MNA (SHEP and SK-N-AS) cell lines and SK-N-AS MYCN-ER cells with and without MYCN activation under complete and delipidized media conditions. Deprivation of exogenous lipids reduced the viability of MNA and MYCN-ER activated cells (+4-OHT) to a greater extent than non-MNA and MYCN-ER CTRL cells (–4-OHT) (Fig. 2c and Supplementary Fig. 2e). Moreover, FA supplementation partially restored viability of MNA and MYCN-ER activated cells (p < 0.05; Fig. 2c and Supplementary Fig. 2e), suggesting that exogenous FAs support MYCN-driven cell survival. Similarly, lipid deprivation induced cell apoptosis in MNA and MYCN-ER cells (Supplementary Fig. 3d). Altogether, our data indicate that FA transporters selectively promote cell survival in MYCN-driven cells. Collectively, our data indicate that MYCN-driven cell growth depends on exogenous FAs.

The FA transporter SLC27A2 is a direct target of MYCN. To further elucidate how MYCN regulates FA import, we assessed the expression of membrane FA transporter genes (SLC27A1–6 and CD36) in multiple MYCN models. SLC27A2 was remarkably upregulated in SK-N-AS MYCN-ER cells upon MYCN activation (10 fold at 48 h, p = 0.0001, Fig. 3a) and in MYCN3 cells upon MYCN induction (p = 0.006, Supplementary Fig. 3a). Moreover, SLC27A2 was the only significantly downregulated transporter when MYCN was turned off in Tet-21/N cells (p = 0.01, Fig. 3b), suggesting that SLC27A2 is selectively regulated by MYCN.

To assess MYCN binding to promoter regions of membrane FA transporters (SLC27A1–6 and CD36), we performed MYCN ChIP-qPCR analysis in both MNA cells and MYCN Tet-Off cells (Tet-21/N). We found significant enrichment (>15 fold) of MYCN binding to the promoter region of SLC27A2 (chr15 [hg38]: 50,182,222-50,182,302), which contains a non-canonical E-box (CACCTG) in MNA cells (LAN5 and IMR32, Supplementary Fig. 3b–c and Supplementary Data 4). Moreover, turning off MYCN almost completely abrogated MYCN binding (Fig. 3c). We then asked whether c-MYC could also bind to SLC27A2. c-MYC ChIP-qPCR analysis in SH-SY5Y NB cells with high c-MYC expression showed that c-MYC binds to the same promoter region but with lower affinity (4 fold enrichment; Supplementary Fig. 3b–c). To determine whether MYCN promoter binding results in gene transcription, we fused a downstream luciferase gene to the promoters of SLC27A1, wild-type and mutant SLC27A2, and ODC1 (a known MYCN target) and evaluated luciferase activity. Turning off MYCN significantly reduced wild-type SLC27A2-fused luciferase activity (p = 0.01, Supplementary Fig. 3d). However, mutation of the non-canonical E-box (CACCTG to GAATTC) in the SLC27A2 promoter abrogated this effect (Supplementary Fig. 3d), suggesting that MYCN activates SLC27A2 transcription via binding to this region. Although MYCN also binds to the SLC27A1 promoter (Fig. 3c and Supplementary Fig. 3b), no changes in SLC27A1 transcription activity were detected when MYCN was turned off (Supplementary Fig. 3d). Altogether, our study identified SLC27A2 as a direct transcriptional target of MYCN.

Because MYCN selectively upregulates SLC27A2 expression, we next asked whether SLC27A2 expression correlates with MYCN expression, activity, or amplification status in NB patients. SLC27A2 expression positively correlated with MYCN expression and activity, as indicated by the summed expression score for 157 MYCN target genes (p < 0.05, Fig. 3d) in a large patient cohort (cohort 1: GSE45547, n = 649). By contrast, mRNA levels of other FA transporters did not correlate with MYCN expression or activity, although CD36 expression positively correlated with c-MYC expression (p < 0.05, Fig. 3d, left). High expression of SLC27A2 also correlated with MYCN amplification (p = 8.1e-15) and stage 4 disease (p < 0.01) (Fig. 3d), and strongly predicted poor overall (OS) and event-free survival (EFS) in patients of all stages (OS: p = 1.0e-3; EFS: p = 4.7e-6) and stage 3–4 high-risk patients (OS: p = 2.7e-4; EFS: p = 4.2e-2, Fig. 3e). Compared with other long-chain FA transport-associated genes (GO: 0015909, n = 75), SLC27A2 ranked top in predicting poor clinical outcomes (R2, Fig. 3f, red dots). These findings were validated in a second patient cohort (cohort 2: GSE85047, n = 283, Supplementary Fig. 3g). SLC27A2 is a critical transporter for NB survival. To explore potential for targeting SLC27A2, we compared SLC27A2 mRNA expression in three NB cohorts (Lastowska, GSE13136, n = 30; Miyama, GSE16237, n = 51; Versteeg, GSE16476, n = 88) with normal tissue cohort (ADrenal Gland, SN_ADGL, n = 13; Neural Crest, GSE14340, n = 5; Normal Various, GSE7307, n = 504 including 108 types of normal tissues; datasets generated from the same u133a2 platform and normalized by MA55.0). Median SLC27A2 expression was higher in MNA patients than in normal tissues (Supplementary Fig. 3h), suggesting that SLC27A2 could function as a therapeutic target in NB.

Genetic and pharmacological inhibition of SLC27A2 impairs NB survival. To determine the impact of SLC27A2 expression on cell survival, we analyzed survival outcomes in 789 cell lines after SLC27A2 knockout using the CRISPR (Avana) PublQV4V2 dataset (Broad Institute, USA) (49). The results were grouped according to primary disease and ranked using a dependency score. NB ranked tenth among 30 primary diseases (dependency score = −0.23), suggesting that NB cells depend on SLC27A2 for survival (Supplementary Fig. 4a).

To confirm whether SLC27A2 is required for NB survival, we genetically depleted SLC27A2 via shRNA-mediated gene silencing (two sequences) in MNA LAN5 cells (Fig. 4a), which express high basal SLC27A2 levels (Supplementary Fig. 4b). SLC27A2 KD in LAN5 cells effectively reduced FA uptake (p < 0.01, Fig. 4b) and impaired cell growth (p < 0.0001, Fig. 4c) and colony forming capacity (p < 0.05, Fig. 4d), suggesting that SLC27A2 is required for cell growth. This phenotypic outcome was confirmed in a second MNA cell line (IMR32) (Supplementary Fig. 4c) and was selective to MNA cells, as SLC27A2 depletion in non-MNA cells (SK-N-AS) did not affect FA uptake or cell growth (Supplementary Fig. 4c). To further examine whether SLC27A2 is required in vivo tumor growth, we orthotopically implanted MNA LAN5 shCTRL and shSLC27A2 cells into NCr nude mice. Silencing SLC27A2 significantly reduced tumor growth (assessed by MRI imaging, p = 0.048) and tumor weights (p = 0.01, Fig. 4e–f). These effects were associated to a significant reduction of intratumoral neutral lipids (Oil Red O staining, p = 0.003, Fig. 4g). Furthermore, depletion of SLC27A2 reduced tumor proliferation (Ki67 staining, p = 0.004, Supplementary Fig. 4d) and increased tumor apoptosis (cleaved Caspase-3 staining, p < 0.0001, Supplementary Fig. 4e) without altering MYCN expression (p = 0.4, Supplementary Fig. 4f). Lipidomic profiling of shCTRL (n = 8) and shSLC27A2 (n = 8) tumors demonstrated downregulation of the majority of DGs and TGs upon SLC27A2 KD (FDR < 0.25, absolute FC > 2, Fig. 4h and Supplementary Data 3), confirming that inhibition of SLC27A2 effectively reverses MYCN-induced glycerolipid accumulation. Collectively, these data indicate that SLC27A2 is required for NB cell survival and tumor growth.

To pharmacologically target FATP2 (encoded by SLC27A2), we used the small-molecule FATP2 inhibitor CB56, which elicits...
selective cytotoxicity against NB cells but spares normal cells (Fig. 2b). We first validated the ability of CB5 to block FA uptake in NB cells by both stable isotope tracing (Supplementary Fig. 2d) and staining with a fluorescent-labeled FA analog BODIPY™ 500/510 C1, C12. CB5 preferentially blocked FA uptake in MNA LAN5 cells (p < 0.05) compared with non-MNA SHEP cells (Fig. 4i). Because the FATP2 inhibitor CB16.2 also suppresses FATP1 activity41, we verified the specificity of CB5 for FATP2. Ectopic overexpression of both FATP1 and FATP2 increased FA uptake in MNA cells. However, CB5 only reduced FA uptake in FATP2-overexpressing cells (Supplementary Fig. 4g), suggesting that CB5 specifically targets FATP2 in NB. CB5 more effectively inhibited cell viability and induced apoptosis (determined by Caspase 3/7 activity) in MNA cells than in non-MNA cells,
Targeting FA transport effectively suppresses NB tumor growth. To determine the contribution of FA transport to tumor growth, we evaluated the anti-tumor activity of CB5 in multiple preclinical NB models. We orthotopically implanted MNA LN5 luciferase-expressing cells and non-MNA SK-N-AS cells into the renal capsule of NCr nude mice (Fig. 5a). After tumor engraftment, mice were randomly assigned to CTRL (vehicle) or CB5 (25 mg/kg, twice a day [b.i.d.], intraperitoneal injection [i.p.] for 2 weeks) treatment groups. CB5 significantly inhibited the growth of LN5 xenografts as measured by luciferase activity (p = 0.002) and tumor weights (p = 0.03, Fig. 5b and Supplementary Fig. 5b).

However, CB5 did not reduce tumor volumes and weights of SK-N-AS xenografts (Fig. 5c and Supplementary Fig. 5c), suggesting that CB5 preferentially inhibits MNA tumors. Notably, CB5 treatment did not cause toxicity assessed by mouse general clinical conditions and weight changes during treatment (Supplementary Fig. 5b–c).

The TH-MYCN transgenic model is an aggressive MYCN-induced de novo NB model. To assess the anti-cancer activity of CB5, we generated an orthotopic allograft model of NB by implanting a TH-MYCN+/+ tumor into NCr nude mice (Fig. 5d). Tumors developed after 2 weeks, at which time mice were randomly assigned to CTRL (vehicle) or CB5 (25 mg/kg, b.i.d.) treatment groups. MRI was performed on treatment days 1 and 14 to monitor tumor growth. CB5 significantly reduced tumor volumes (p = 0.006, Fig. 5e and Supplementary Fig. 5d) and weights (p = 0.01, Fig. 5f) in this model, and no signs of toxicity were observed during the study (Supplementary Fig. 5d). Moreover, mice responsive to CB5 treatment showed lower neutral lipid levels than CTRL mice (p = 0.004, Fig. 5g), suggesting that CB5 blocks lipid accumulation in vivo. This is likely due to the CB5-mediated inhibition of MYCN and MYCN-targeted FA synthesis and transport protein expression (SCD1, ACC, and FATP2; Supplementary Fig. 5d). Two tumors escaped CB5 treatment (Fig. 5f).

One caveat to using nude mice as preclinical models is that they lack an immune microenvironment. To evaluate the efficacy of blocking FA transport in the presence of an intact immune microenvironment, we generated a TH-MYCN+/+–derived orthotopic syngeneic mouse model (Fig. 5h) by implanting a TH-MYCN+/+ tumor into the renal capsule of wild-type immunocompetent 129×1/svj mice. After 2 weeks, mice were treated with either CTRL (vehicle) or CB5 (30 mg/kg, b.i.d. i.p.) for 2 weeks. CB5 treatment blocked tumor growth (Fig. 5h, p < 0.0001) without apparent toxicity (Supplementary Fig. 5e) in this model. To evaluate the long-term effects of blocking FA uptake in MNA NBs, we used a patient-derived orthotopic xenograft model (Fig. 5i). Cells prepared from a primary MNA stage 4 NB tumor (P0) were implanted into the renal capsule of NOG mice (P1). The tumor was then passaged in NOG mice to P4 and in NCr nude mice to P6. In this model, tumors initiate approximately 5 weeks after implantation and mice succumb to disease burden 8–10 weeks after implantation. We asked whether blocking FA uptake through long-term CB5 treatment could prevent MNA tumor development and prolong animal survival. CB5 treatment was applied 2 weeks after implantation when tumors had not initiated. Mice received CTRL (vehicle) or CB5 (25 mg/kg, b.i.d. i.p.) for 6 weeks and tumor growth was monitored by MRI. CB5 did not prevent tumor initiation (Fig. 5i). However, chronic CB5 treatment significantly prolonged animal survival (p = 0.004, Fig. 5i) without notable toxicity (Supplementary Fig. 5f), suggesting that blocking FA uptake can suppress primary MNA tumor growth and extend survival.

Targeting FA transport sensitizes NB to conventional chemotherapies. Elevated lipid metabolism promotes acquired resistance to chemotherapy. Targeting FA synthesis (FASN), oxidation (CPT1), or uptake (CD36) sensitizes cancer cells to chemotherapy in adult models. Thus, we asked whether FATP2 inhibition enhanced the anti-tumor activity of conventional chemotherapies, such as etoposide (VP16, a topoisomerase II inhibitor) or temozolomide (TMZ, a DNA alkylating agent), which are used as induction or relapse therapies. We first evaluated the synergy between CB5 and either VP16 or TMZ by assessing cell viability under single and combination treatment conditions, assigning a synergy score using the Bliss model (>10 indicates synergy). CB5 synergizes with both VP16 and TMZ in MNA cells (Fig. 6a and Supplementary Fig. 6a–b). In addition,
both CB5 + VP16 and CB5 + TMZ combinations increased Caspase-mediated apoptosis compared with single-drug treatments in MNA cells (p < 0.05, Fig. 6b and Supplementary Fig. 6c), suggesting that these combination therapies are highly effective in the setting of MYCN amplification.

Because the CB5 + VP16 combination showed promising in vitro synergistic effects, we assessed the anti-tumor activity of this combination in MNA LAN5-derived orthotopic xenografts (Fig. 6c). Combination therapy (CB5: 15 mg/kg, b.i.d., i.p. 6 days/week and VP16: 8 mg/kg, i.p. 3 days/week) markedly reduced final tumor weights compared with monotherapies (p < 0.0005, Fig. 6c). Moreover, CB5 + VP16 demonstrated enhanced effects on tumor cell proliferation and apoptosis (p ≤ 0.05, Supplementary Fig. 6d–e) without further reducing MYCN expression.
Fig. 4 Suppressing FA uptake impairs NB cell survival. a Silencing SLC27A2 in LAN5 cells. Two shSLC27A2 GIPZ vectors tested, with empty GIPZ vector as control. Mean ± SEM (n = 3). b FA uptake in LAN5 shCTRL and shSLC27A2 cells. Cells stained with the FA analog BODIPY™ 558/568 C12 and quantified as CTCF by ImageJ. Mean±SD (n = 3). c Cell growth in LAN5 shCTRL and shSLC27A2 cells. Mean ± SD (n = 3). d Clonogenic assay in LAN5 shCTRL and shSLC27A2 cells. Mean ± SD (n = 3); e-h LAN5 shCTRL and shSLC27A2 orthotopic xenograft model. e Tumor volumes at weeks 3 and 4 post-implantation. CTRL = 15; shSLC27A2 = 14; two-way ANOVA with Sidak’s multiple comparisons test. Tumor weights at week 5 post-implantation. Mean ± SEM (CTRL = 13, shSLC27A2 = 8); two-sided unpaired Mann–Whitney test. g Oil Red O staining of intratumoral lipids. Mean ± SEM (CTRL = 6, shSLC27A2 = 8); two-sided unpaired Mann–Whitney test. h Lipidomics profiling of shCTRL and shSLC27A2 tumors (n = 8 each). Lipids (FDR < 0.25, absolute FC > 2) are shown in the heatmap (color scaled by Z-score: red = upregulated; blue = downregulated). MGDG monogalactosyldiacylglycerol, DGDG digalactosyldiacylglycerol, dhCER dihydroceramides, CL cardiolipin, SM sphingomyelin, PA phosphatidic acid, lyso.PC lysophosphatidylcholine, lyso.PE lysoosphatidylethanolamine, plasmanylPC plasmennylphosphatidylcholine, all other abbreviations consistent with Fig. 1e. i FA uptake following CB5 treatment in LAN5 and SHEP cells (0–15 μM, 5 min). Cells stained with BODIPY™ 500/510 C12 and quantified as CTCF by ImageJ. Mean ± SD (n = 3). j Caspase 3/7 activity of NB and normal cells after CB5 treatment (0–10 μM, 24 h). Mean ± SD (n = 3). k Apoptosis, p53/p21, and MYCN/c-MYC protein expression in LAN5, IMR32, and SHEP with CB5 (0–20 μM, 16–24 h). VP16 (10 μM, 24 h) = positive CTRL. Representative blots from three independent experiments are shown. FC fold change, Arb. Unit arbitrary unit. a, b, d, and i, one-way ANOVA with Dunnell’s multiple comparisons test; c and j, two-way ANOVA with Dunnell’s multiple comparisons test. Source data are provided in the Source Data file.

compared with CB5 alone (Supplementary Fig. 6f) and with no evidence of body weight loss or normal organ toxicity (Supplementary Fig. 6g). These data suggest that blocking FA transport effectively enhances tumor responses to VP16. To then determine the long-term effects of combination therapy on animal survival, we used our MNA patient-derived orthotopic xenograft model (Fig. 6d). Mice were subjected to single or combination therapy (CB5: 15 mg/kg, b.i.d, i.p. 6 days/week and VP16: 6 mg/kg, i.p. 3 days/week) for 5 weeks. Animals treated with CB5 + VP16 combination therapy survived significantly longer than those receiving single-drug therapies (p < 0.05, Fig. 6d). No treatments caused significant body weight loss or clinical signs of toxicity (Supplementary Fig. 6h). Collectively, our data suggest that blocking MYCN-induced metabolic reprogramming effectively enhances the anti-tumor effects of conventional chemotherapy.

Discussion

Using a variety of biochemical and analytical approaches, we comprehensively characterized the metabolic landscape of NB tumors and defined MYCN amplification as a major driver of distinct metabolic adaptations. Our metabolic screening and analyses in human tumors and cell lines across various models revealed that MYCN induces a consistent accumulation of glycerolipids. Glycerolipids function as secondary messengers that activate downstream oncogenic signaling and serve as FA reservoir for energy storage and the prevention of toxic FAs accumulation to support tumor growth29. MYCN induction or amplification resulted in a distinct glycerolipid signature mainly characterized by a robust increase in DGs. FAs can be funneled into various metabolic pathways to synthesize more complex lipid species, including DGs (which are precursors of TGs), contributing to the structural diversity of the cellular lipids. MYCN may directly regulate glycerolipid synthesis and degradation, for example, by upregulating diacylglycerol-acyltransferase 2 (DGAT2) to store excess FAs in TGs and LDs (Supplementary Fig. 7). DGAT1 and DGAT2 catalyze the esterification of acyl-CoA with DGs to form TGs54. Although their roles in oncogenesis remain largely unexplored, targeting DGAT1 to block FA storage induces severe oxidative stress in glioblastoma35. Future investigations remain necessary to elucidate how MYCN maintains lipid homeostasis and protects NB cells from oxidative damage. Here we show that targeting FA uptake effectively inhibits MYCN-induced glycerolipid accumulation and tumor growth, suggesting that FA transport is critical for these functions.

Cancer tissues show aberrant activation of de novo lipogenesis, and inhibition of enzymes within the FA biosynthesis pathway can block tumor growth30. FA biosynthesis contributes to cancer by providing the building blocks for biological membranes and ATP synthesis during nutrient depletion, in addition to regulating membrane trafficking and signaling pathways critical for cell survival, such as phosphatidylinositol-3,4,5-trisphosphate and sphingolipids56,57. MYC(N) dynamically alters de novo lipogenesis, lipid storage, and β-oxidation to generate ATP. Inhibition of MYC(N) induces LDs formation as a consequence of impaired FA oxidation58. We and others have also shown that MYC(N) promotes FA biosynthesis and aberrant activation of SREBP118 in cancer, including NB22,23, and genetic and pharmacological interference with FA biosynthesis blocks MYC-driven tumor growth18. Moreover, ACC or FASN inhibition partially blocks cancer growth in a subcutaneous NB model22. However, the long-term efficacy and normal tissue toxicity associated with this approach remain largely unknown. We found that inhibiting FA biosynthesis via the SCD1 inhibitor A939572 triggered compensatory FA uptake, suggesting that NB cells can utilize exogenous lipids when precursors are limited or FA synthesis is impaired. We demonstrated that blocking FA uptake either through exogenous lipid deprivation or pharmacological FATP2 inhibition remarkably reduced the viability of MYCN-induced cells, promoted apoptosis, and enhanced sensitivity to FA biosynthesis inhibition. These effects could be partially rescued by FA supplementation, indicating that MYCN-driven cells depend on FA uptake for survival. The dependency on exogenous FAs is emerging in other cancers. In prostate cancer and melanoma, the membrane transporters CD36 and FATP1 respectively promote lipid accumulation and tumor progression38,41. Moreover, melanoma cells use FATP2 to acquire lipids from aged fibroblasts, conferring resistance to targeted therapy42. In NB, we found that oncogenic MYCN drives FA uptake to maintain tumor growth. By screening mRNA expression and MYCN binding to membrane FA transporters, we identified the FA transporter gene SLC27A2 (encoding FATP2) as a direct MYCN target required for NB survival. SLC27A2 expression in NB patients uniquely correlates with MYCN expression and activity. Moreover, high SLC27A2 expression strongly predicts poor clinical outcomes when compared to other FA transporters. Collectively, these data suggest that MNA NB depends on SLC27A2-mediated FA uptake, making SLC27A2 an attractive therapeutic target for high-risk disease.

SLC27A2 is necessary for NB survival, as both genetic interference and pharmacological inhibition via the small-molecule inhibitor CB5 impaired MYCN-induced tumor growth. Supporting the selectivity of targeting SLC27A2 in NB, normal cells were not affected by CB5 treatment, suggesting the possibility of new therapeutic opportunities. We demonstrated the anti-tumor
activity of CB5 in multiple MYCN-induced preclinical NB models, including cell line-derived orthotopic models, both allograft and syngeneic models, and patient-derived models, suggesting that FA uptake via \( SLC27A2 \) represents an intrinsic vulnerability of MYCN-driven tumors that can be targeted therapeutically. \( SLC27A2 \) is also expressed in immune cells, such as oncogenic polymorphonuclear myeloid-derived suppressor cells, and \( SLC27A2 \) inhibition blocks immune-suppressive activity in these cells, delaying tumor progression. Future studies remain necessary to determine whether \( SLC27A2 \) inhibition also interferes with immune cell functions and their metabolic dependencies to prohibit tumor growth.

MYCN-induced drug resistance limits the anti-tumor effects of conventional chemotherapy, and thus remains a major clinical
obstacle. Moreover, metabolic reprogramming and altered lipid metabolism also promote acquired drug resistance. Increased FASN, CPT1B, and CD36 expression correlate with poor drug response in cancer patients. We found that targeting a key MYCN-dependent metabolic vulnerability, the FA uptake pathway, strongly enhanced the clinical activity of cytotoxic agents that inhibit DNA synthesis, such as VP16 and TMZ. Because FA-derived acetyl-CoA can fuel the TCA cycle to generate aspartate, the nucleotide synthesis precursor, we speculate that inhibiting FA uptake may limit nucleotide reservoirs for DNA synthesis. Future studies are needed to test whether FA uptake contributes to FA oxidation and nucleotide synthesis and investigate the molecular mechanisms underlying the observed synergy between CB5 and conventional chemotherapy.

In conclusion, we uncovered that MYCN promotes glycerolipid accumulation in NB. MYCN drives FA uptake by directly upregulating SLC27A2, which is required for glycerolipid synthesis and MYCN-induced cell survival. Genetic and pharmacological interference (via CB5) of SLC27A2 blocks NB tumor growth. Moreover, CB5 prolongs animal survival, and synergizes with conventional chemotherapy in multiple preclinical NB models. Our study shows that MYCN-driven tumors rely heavily on FA uptake for survival, suggesting that FA uptake may represent a promising therapeutic target in high-risk MNA patients.

Methods

Patient sample analyses. Frozen primary tumors (MNA, n = 18; non-MNA, n = 18) were provided by the Research Tissue Support Service (RTSS) at Texas Children’s Hospital (TCH). Patient clinical information is summarized in Supplementary Data 1. This study was approved by the Institutional Review Board for Human Subject Research at Baylor College of Medicine and Affiliated Hospitals (BCM IRB, H-42596, H-6650). Informed consent was obtained from all participants. Compensation was not provided.

Publicly available patient data were retrieved from R2: Genomics Analysis and Visualization Platform (http://r2.amc.nl). Cohort 1 (Kocak, GSE45547, n = 44) data included CCGA gene expression profiles from 449 NB tumors based on 44 K oligonucleotide microarrays. Survival annotations are available for 479 patients. Two weeks later mice were treated with vehicle or CB5 (25 mg/kg, b.i.d., 6 days/week) for 2 weeks. Tumor volumes (MRI) and weights after treatment. Mean ± SEM (CTRL = 6, CBS = 8); two-way ANOVA with Sidak’s multiple comparisons test (left); two-sided unpaired Mann-Whitney test (right). c. SK-N-AS tumor volumes (MRI) and weights after treatment. Mean ± SEM (CTRL = 8, CBS = 8): two-way ANOVA with Sidak’s multiple comparisons test (left); two-sided unpaired Mann-Whitney test (right). d. TH-MYCN1/4/5-deleted orthotopic allograft model. D Cells from one TH-MYCN1/4/5 tumor were orthotopically implanted in NCr nude mice. Two weeks later mice were treated with vehicle or CB5 (25 mg/kg, b.i.d., 6 days/week) for 2 weeks. e. Tumor volumes (MRI) on treatment days 1 and 14. Tumors were framed and quantified; representative images and mean ± SEM are shown (CTRL = 10, CBS = 9). f. Tumor weights at treatment day 14. Mean ± SEM (CTRL = 10, CBS = 9); two-sided unpaired Mann-Whitney test. g. Oil Red O staining of intratumoral lipids. Mean ± SEM (CTRL = 6, CBS = 5 responsive tumors); two-sided unpaired Mann-Whitney test. h. Cells from one TH-MYCN1/4/5 tumor were orthotopically implanted in syngeneic 129 × 1/svj wild-type mice. Two weeks later mice were treated with vehicle or CB5 (30 mg/kg, b.i.d., 6 days/week) for 2 weeks. i. Tumors were weighed on treatment day 14. Mean ± SEM (CTRL = 14, CBS = 13); two-sided unpaired Mann-Whitney test. j. Cells from one MNA patient tumor (P6) were orthotopically implanted in NCr nude mice, and 2 weeks later mice were treated with vehicle or CB5 (25 mg/kg, b.i.d., 6 days/week) for 6 weeks. Kaplan-Meier survival analyzed by log-rank test. Arb. Unit arbitrary unit, Px treatment period. Source data are provided in the Source Data file.

Fig. 5 Suppressing FA uptake exerts anti-tumor effects in multiple preclinical models. a, c NB cell line-derived orthotopic xenograft model. a. LANS or SK-N-AS cells were orthotopically implanted in NCr nude mice. Two weeks later mice were treated with vehicle or CBS (25 mg/kg, b.i.d., 6 days/week) for 2 weeks. b. LANS tumor sizes (IVIS) and weights after treatment. Mean ± SEM (CTRL = 6, CBS = 6); two-way ANOVA with Sidak’s multiple comparisons test (left); two-sided unpaired Mann-Whitney test (right). c. SK-N-AS tumor volumes (MRI) and weights after treatment. Mean ± SEM (CTRL = 8, CBS = 8); two-way ANOVA with Sidak’s multiple comparisons test (left); two-sided unpaired Mann-Whitney test (right). d- g TH-MYCN1/4/5-deleted orthotopic allograft model. D Cells from one TH-MYCN1/4/5 tumor were orthotopically implanted in NCr nude mice. Two weeks later mice were treated with vehicle or CBS (25 mg/kg, b.i.d., 6 days/week) for 2 weeks. e. Tumor volumes (MRI) on treatment days 1 and 14. Tumors were framed and quantified; representative images and mean ± SEM are shown (CTRL = 10, CBS = 9). f. Tumor weights at treatment day 14. Mean ± SEM (CTRL = 10, CBS = 9); two-sided unpaired Mann-Whitney test. g. Oil Red O staining of intratumoral lipids. Mean ± SEM (CTRL = 6, CBS = 5 responsive tumors); two-sided unpaired Mann-Whitney test. h. Cells from one TH-MYCN1/4/5 tumor were orthotopically implanted in syngeneic 129 × 1/svj wild-type mice. Two weeks later mice were treated with vehicle or CB5 (30 mg/kg, b.i.d., 6 days/week) for 2 weeks. i. Tumors were weighed on treatment day 14. Mean ± SEM (CTRL = 14, CBS = 13); two-sided unpaired Mann-Whitney test. j. Cells from one MNA patient tumor (P6) were orthotopically implanted in NCr nude mice, and 2 weeks later mice were treated with vehicle or CB5 (25 mg/kg, b.i.d., 6 days/week) for 6 weeks. Kaplan-Meier survival analyzed by log-rank test. Arb. Unit arbitrary unit, Px treatment period. Source data are provided in the Source Data file.
Fig. 6 Suppressing FA uptake promotes the efficacy of conventional chemotherapies. a Synergy analyses in MNA (LAN5 and IMR32) and non-MNA (SHEP) cells treated with CB5 (0.4–2.5 µM), VP16 (5–50 nM) and their combination for 72 h. Heatmap represents mean Bliss scores from three independent experiments. Bliss score > 10 indicates synergy. b Cell viability and Caspase 3/7 activity after single and combination treatments. Cells were treated with CTRL, CB5 (3 µM), VP16 (80 nM), or their combination for 72 h. Mean ± SD (n = 3); one-way ANOVA with Tukey’s multiple comparisons test. c Anti-tumor activity of CB5 + VP16 combination therapy in LAN5-derived orthotopic xenografts. LAN5 cells were implanted in the renal capsule of NCr nude mice. Two weeks after implantation, mice were treated with CTRL, CB5 (15 mg/kg, b.i.d. 6 days/week), VP16 (8 mg/kg daily, 3 days/week) or their combination for 2 weeks. Tumor weights after 2 weeks of treatment are shown. Mean ± SEM (CTRL = 11, CB5 = 11, VP16 = 10, CB5 + VP16 = 12); two-sided unpaired Mann-Whitney test. d Survival analysis in patient-derived orthotopic xenografts. Cells prepared from one MNA patient tumor (P8) were implanted in the renal capsule of NCr nude mice. Five and half weeks after implantation, mice were treated with CTRL (vehicle), CB5 (15 mg/kg, b.i.d. 6 days/week), VP16 (6 mg/kg daily, 3 days/week), or their combination for 5 weeks. Survival was plotted as a Kaplan-Meier curve and analyzed by the log-rank test (CTRL = 12, CB5 = 10, VP16 = 10, CB5 + VP16 = 11). Px treatment period; FC fold change. Source data are provided in the Source Data file.
Mouse models. In vivo studies were approved by the BCM Institutional Animal Care and Use Committee (AN7089 and AN6190). Mice were housed at the TCH Animal Facility in a 70%–80% humidity (60%-70%) and a specific pathogen-free environment under a 14 h:10 h light/dark cycle. Mice were fed standard chow diet (LabDiet, 3002096-704) ad libitum. The maximal tumor size permitted was 1.5 cm by diameter, and this size was not exceeded in this study. TH-MYCN transgenic mice: TH-MYCN−/− mice (129sv/j, kindly provided by the Weiss lab (University of California San Francisco, CA, USA), were bred and mated to produce offspring. Two pups were genotyped to identify TH-MYCN−/−, TH-MYCN+/−, and TH-MYCN+/+ mice. TH-MYCN−/− (129sv/j) mice develop tumors (almost 100% incidence) at week 3 and succumb to disease by week 7–8.

In vivo functional assays

Cell viability: Cell viability was determined using a Cell Counting Kit-8 (CCK, Dojindo, Molecular Technologies, MD, USA, CK04-05) according to the manufacturer’s instructions. For synergy studies, cells were treated with different doses of two drugs either alone or in combination. Cell viability was converted to inhibition % = 100−(viability%) and synergy scores were calculated using the Bliss model (>1 indicates synergy and <1 indicates antagonism) [13]. Synergy heatmaps were prepared in GraphPad Prism (7.0). Caspase 3/7 activity assay: Caspase 3/7 activity was measured using a Caspase-Glo assay kit (Promega, WI, USA, G8091) as previously described [14].

FA uptake assay: Real-time FA uptake was determined using a QBT Fatty Acid Uptake Assay Kit (Molecular Devices, CA, USA, R6132) according to the manufacturer’s instructions. Cells were serum-starved for 48 h before FA uptake was induced by FA synthesis inhibition, cells were serum-starved for 48 h, treated with 0–15 µM A993975 for 15 min, and a proprietary assay reagent was added. Plates were read at λm = 485 nm and λs = 515 nm every 30 s for 30 min. To determine drug specificity, IMR32 FATP1 and FATP2-overexpressing cells were serum-starved and induced (DOX 20 ng/mL for 48 h or 72 h) before adding the assay reagent containing C5 (0–6 µM). Plates were read at λm = 485 nm and λs = 515 nm every 30 sec for 100 min. FA uptake imaging was performed by fluorescence staining of an FA analog (BODIPY™ 558/568 C12, D3835 and BODIPY™ 550/510 C1, C12, D3823, Thermofisher) using an Olympus IX71 (Olympus, Japan). The staining solution contained 10 µM fluorescein-dye, 5 µM BSA and 0.2% trypan blue in phenol red-free delipidated RPMI media, modified from Li et al. [15]. Images were quantified by ImageJ: corrected total cell fluorescence (CTCF) = integrated density (area of selected cell-mean fluorescence of background readings) [16].

Real-time qPCR and western blotting

qPCR analysis. Total RNA isolated using an RNeasy Mini Kit (Qiagen, MD, USA, 74104) was directly mixed with the reagents supplied in the Quantitect SYBR® Green RT-PCR Kit (Qiagen, 204243) and subjected to one-step RT-PCR performed on StepOnePlus® Real-Time PCR System (Thermo Scientific, Primer). Sequences are listed in Supplementary Data 4. Western blotting: Cells and tumors were lysed with RIPA buffer (Sigma, 27815) for 20 min at room temperature and rinsed with distilled water to remove excess dye. The plate was then imaged using a FluorChem R System (ProteinSimple, CA, USA) and the number of colonies was counted using alpha View-FluorChem Q software (3.4.0.0).

Metabolomics, lipidomics, and FA analyses

Global metabolomics. Cells (MYCN KD 0.7, 22.6; MYCN-ON 0.4, 48.2; n = 4 per condition) and primary tumor samples (MNA, n = 18; non-MNA, n = 18) were submitted to Metabolon Inc. for untargeted metabolomic profiling (DiscoveryHD Metabolic Platform, n = 545 compound library, NC, USA). One-way ANOVA was used to compare metabolite levels between groups. Welch’s two-sample t-test was used to compare metabolite levels between MNA and non-MNA primary tumors (p-value ≤ 0.05). The metabolite classification network is presented by Cytoscape 3.8.2 using subpathways as the source nodes and metabolites as the target nodes. Target node sizes indicate the absolute log FC of metabolite levels between MYCN KD 72 h vs. CTRL, MYCN-ON 72 h vs. MYCN-OF and MYCN KD vs. non-MNA. Red upregulated metabolites; blue downregulated metabolites (p ≤ 0.05); gray: no significant change (p > 0.05). To determine metabolic changes in each subpathway, a differential abundance score was calculated for each condition (adapted from Hakimi et al. [17]). Differential abundance score = (# of significantly increased metabolites – # of significantly decreased metabolites) / # of metabolites measured × 100. Differential abundance scores were calculated in subpathways containing at least three differentially altered metabolites in at least one condition (p ≤ 0.05). A score of 100 indicates that all
metabolites in a subpathway are upregulated; a score of −100 indicates that all metabolites in a subpathway are downregulated (p < 0.05). To identify subpathway enrichment, two enrichment algorithms were used. Algorithm 1: Over-representation analysis using the hypergeometric distribution with p-values adjusted by the Benjamini–Hochberg procedure. “*” Indicates that a subpathway is significantly enriched in one comparison group (adjusted p-value < 0.25), and subpathways were ranked by the number of “*” designations. Subpathways with the same number of “*” designations were ranked by the average absolute differential abundance score from three comparison groups. Algorithm 2: GSEA values were adjusted using the Benjamini–Hochberg procedure to obtain FDR. Changes with FDR < 0.25 (SK-N-AS MYCN-ERα cells; LAN5 shCTRL and shSLC27A2) tumors are presented in heat maps (R software 4.0.4).

FA profiling. Intracellular total FAs were analyzed by either LC-MS (n = 4 per group) or GC–MS (n = 6 per group) for each metabolite. For LC-MS analysis, extracted FAs were analyzed by high-performance LC coupled with Agilent 6495 QQQ MS (Agilent Technologies, CA, USA) using ESI negative ionization via single-reaction monitoring. Data analyzed with Agilent mass hunter quantitative software (10.0) were normalized with an internal standard and log2 transformed per sample. FA concentrations and FA enrichment were measured using perfluoroisobutyric derivatization and GC-MS negative chemical ionization as previously reported29, with a slight modification: an SP-2380 column (Supelco, PA, USA) was used to improve the separation of palmitoleic acid (16:1(n=7) and sapienic acid (16:1(n=10) peaks. Data were acquired in selective ion-monitoring mode and standard peak areas were determined. The ratio of the areas from all (M0–M2) analyte-derived ions relative to that of the internal standard tridecanoic acid (m/z, 213.2135) was calculated. Ratios were compared with the calibration curves of serial FA concentrations to determine each FA concentration. Student’s t-test was used to compare FA levels between groups. p-values were adjusted using the Benjamini–Hochberg procedure to obtain FDR. Changes with FDR < 0.25 were selected for data presentation.

FA tracing. Briefly, deuterated water (D2O) (99 atom%, Cambridge Isotope Laboratory, MA, USA) was used to determine de novo lipogenesis23. [U-13C] palmitic acid (16:0) potassium salt (99 atom%13C, Cambridge Isotope Laboratories) was used to determine FA desaturase activity (ratio of 13C15:0 mono-insaturates to [13C2]saturated FA and FA uptake (original [13C2]FAs in medium—remaining [13C1]FAs in medium [13C2]FAs). FAs were analyzed by GC-MS and normalized by the total protein contents of each sample.

Chromatin immunoprecipitation (ChIP) and luciferase activity assay MYCN GEP. Briefly, 1x107 cells were cross-linked with 1% formaldehyde, and the reaction was stopped with 0.125 M glycine before harvest. DNA was sheared by sonicating using Bioruptor PLUS (Diagenode, NJ, USA). A small aliquot of sheared material was retained as input and the remaining sample was immunoprecipitated using 5 μg ChIP-grade antibodies (MYCN, Santa Cruz, sc-53993, clone MYCN3 cells) and with absolute log2FC > 1 and FDR < 0.25 (SK-N-AS MYCN-ERα cells; LAN5 shCTRL and shSLC27A2) tumors are presented in heat maps (R software 4.0.4).

References

1. Park, J. R., Eggert, A. & Caron, H. Neuroblastoma: biology, prognosis, and treatment. Hematol./Oncol. Clin. North Am. 24, 65–86 (2010).
2. Brodeur, G. M., Seeger, R. C., Schwab, M., Varmus, H. E. & Bishop, J. M. Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. Science 224, 1121–1124 (1984).
3. Weiss, W. A., Aldape, K., Mohapatra, G., Feuerstein, B. G. & Bishop, J. M. Targeted expression of MYCN causes neuroblastoma in transgenic mice. EMBO J. 16, 2985–2995 (1997).
4. Wolpaw, A. J. et al. Drugging the ‘undruggable’ MYCN oncogene: An Epigenetic approach. Nuc. Acid. Res. 16, 2023–2033 (2016).
5. Chong, W. et al. Determining the optimal dose and schedule of MYC inhibition for treatment of N-Myc-dependent neuroblastomas. J. Clin. Oncol.: Off. J. Am. Soc. Clin. Oncol. 39, 2994–3003 (2021).
6. Park, J. R., Eggert, A., Caron, H., Neuroblastoma: biology, prognosis, and treatment. Hematol./Oncol. Clin. North Am. 24, 65–86 (2010).
7. Brodeur, G. M., Seeger, R. C., Schwab, M., Varmus, H. E. & Bishop, J. M. Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. Science 224, 1121–1124 (1984).
8. Weiss, W. A., Aldape, K., Mohapatra, G., Feuerstein, B. G. & Bishop, J. M. Targeted expression of MYCN causes neuroblastoma in transgenic mice. EMBO J. 16, 2985–2995 (1997).
9. Wolpaw, A. J. et al. Drugging the ‘undruggable’ MYCN oncogene: An Epigenetic approach. Nuc. Acid. Res. 16, 2023–2033 (2016).
10. Chong, W. et al. Determining the optimal dose and schedule of MYC inhibition for treatment of N-Myc-dependent neuroblastomas. J. Clin. Oncol.: Off. J. Am. Soc. Clin. Oncol. 39, 2994–3003 (2021).
11. Park, J. R., Eggert, A., Caron, H., Neuroblastoma: biology, prognosis, and treatment. Hematol./Oncol. Clin. North Am. 24, 65–86 (2010).
12. Brodeur, G. M., Seeger, R. C., Schwab, M., Varmus, H. E. & Bishop, J. M. Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. Science 224, 1121–1124 (1984).
13. Weiss, W. A., Aldape, K., Mohapatra, G., Feuerstein, B. G. & Bishop, J. M. Targeted expression of MYCN causes neuroblastoma in transgenic mice. EMBO J. 16, 2985–2995 (1997).
14. Wolpaw, A. J. et al. Drugging the ‘undruggable’ MYCN oncogene: An Epigenetic approach. Nuc. Acid. Res. 16, 2023–2033 (2016).
15. Chong, W. et al. Determining the optimal dose and schedule of MYC inhibition for treatment of N-Myc-dependent neuroblastomas. J. Clin. Oncol.: Off. J. Am. Soc. Clin. Oncol. 39, 2994–3003 (2021).
10. Kim, J. W. et al. Evaluation of myc E-box phylogenetic footprints in glycolytic genes by chromatin immunoprecipitation assays. Mol. Cell. Biol. 24, 5923–5936 (2004).

11. Kamphorst, J. J. et al. Hypoxic and Ras-transformed cells support growth by scavenging unsaturated fatty acids from lysophospholipids. Proc. Natl Acad. Sci. USA 110, 8882–8887 (2013).

12. Bensad, K. et al. TIGAR, a p53-inducible regulator of glycolysis and apoptosis. Cell 126, 127–130 (2006).

13. Dang, L. et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature 462, 739–744 (2009).

14. Stine, Z. E., Walton, Z. E., Altman, B. J., Hsieh, A. L. & Dang, C. V. MYC, metabolism, and cancer. Cancer Discov. 5, 1024–1039 (2015).

15. Osthus, R. C. et al. Deregulation of glucose transporter 1 and glycolytic gene expression by c-Myc. Biol. Chem. 275, 21797–21800 (2000).

16. Wise, D. R. et al. Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. Proc. Natl Acad. Sci. USA 105, 18782–18787 (2008).

17. Kim, J., Lee, J. H. & Iyer, V. R. Global identification of Myc target genes reveals its direct role in mitochondrial biogenesis and its E-box usage in vivo. PLoS One 3, e7998 (2008).

18. Gouw, A. M. et al. The MYC oncogene cooperates with sterol-regulated element-binding protein to regulate lipogenesis essential for neoplastic growth. Cell Metab. 30, 556–572 e555 (2019).

19. Schlosser, I. et al. for a c-Myc in the regulation of ribosomal RNA processing. Nucleic Acids Res. 31, 6148–6156 (2003).

20. Manna, S. et al. Direct role of nucleotide metabolism in C-MYC-dependent proliferation of melanoma cells. Cell Cycle 7, 2392–2400 (2008).

21. Olijnyk, G. et al. MYCN-enhanced oxidative and glycolytic metabolism reveals vulnerabilities for targeting neuroblastoma. Science 21, 188–204 (2019).

22. Ruiz-Pérez, M. V. et al. Inhibition of fatty acid synthesis induces differentiation and reduces tumor burden in childhood neuroblastoma. iScience 24, 102128 (2021).

23. Moreno-Smith, M. et al. Restoration of the molecular clock is tumor suppressive in neuroblastoma. Nat. Commun. 12, 4006 (2021).

24. Ren, P. et al. ATF4 and N-Myc coordinate glutamine metabolism in MYCN-expression by c-Myc. Cell Metab. 30, 556–572 e555 (2019).

25. Xia, Y. et al. Metabolic reprogramming by MYCN confers dependence on the fatty acid transporters FATP2. Cancer Discov. 10, 1282–1295 (2020).

26. Gamble, L. et al. Inhibition of polyamine synthesis and uptake reduces sensitivity to an ROS augmenting agent. Cell death Dis. 9, 220 (2018).

27. Ding, Y. et al. MYCN and PRC1 cooperatively repress docosahexaenoic acid synthesis in neuroblastoma via ELOVL2. J. Exp. Clin. cancer Res.: CR 38, 498 (2019).

28. Griner, E. M. & Kazanietz, M. G. Protein kinase C and other diacylglycerol effectors in cancer. J. Biol. Chem. 282, 7033–7039 (2007).

29. Kazantzis, M. & Stahl, A. Fatty acid transport proteins, implications in physiology and disease. Biochimica et biophysica acta 1821, 852–857 (2012).

30. Wastney, M. E. & Dvorak, A. M. Endothelial cell hyaluronan secretion: a TEAD4-MYCN effector. Am. Assoc. Cancer Res. 74, 765–767 (2013).

31. Rajbhandari, P. et al. Cross-cohort analysis identifies a TEAD4-MYCN positive feedback loop as the core regulatory element of high-risk neuroblastoma. Cancer Discov. 8, 582–599 (2018).

32. Subramanian, A. et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl Acad. Sci. USA 102, 15545–15550 (2005).

33. Mohammadi, M. A. & Haymond, M. W. Regulation of lipid synthesis genes and milk fat production in human mammary epithelial cells during secretory differentiation. Am. J. Physiol. Endocrinol. Metab. 305, E700–E716 (2013).

34. Liu, P. Y. et al. The long noncoding RNA IncN81 promotes tumorigenesis by interacting with ribosomal protein RPL35. Nat. Commun. 10, 5026 (2019).
Acknowledgements
We acknowledge Philip Bocock and Scott McGaloch from Metabolon, Inc. for their support with sample preparation and data analysis. We thank Alessandro Baldan for providing primary samples (RTSS, TCH), Brian J. Altman (University of Rochester) for providing SK-N-AS MYCN-ER™ cells, Ronald Bernardi (BCM, now at Genentech) for providing SK-N-BE(2c) and LAN5 shMYCN cells, Michele Redell (BCM) for providing HS-5 cells, Joel R Neilon (BCM) for providing C2C12 cells, and Huda Zoghbi (BCM) for providing ARPE-19 cells. We thank our master student Nathan Drolet (BCM), summer student Kevin Rodriguez (University of Houston) and visiting post-doc Davide Leardini (University of Bologna) for their technical support. We also thank the Cell-Based Assay Screening Service (BCM), the Stable Isotope Core Laboratory (CNRC, BCM), the Metabolomics Core (BCM) and the Pathology Core (TCH) for their technical support. L.T. is supported by Kate Amato Foundation. E.B. is supported by NIH R01 (R01-CA222224), DOD (W81XWH-19-1-0556), and CPRIT (RP180851). P.S. is partially funded by European Commission, HORIZON 2020 (826121). Y.Z. is partially supported by NIH grant R01CA224304. K.B.G. and A.B. are supported by NIH (NCI/NIDCR 1U01DE028233), St. Baldrick’s Research Grant (714511) and the Russell and Glenda Gordy Center for Innovative Therapies at Texas Children’s Cancer Center. N.P. is supported by NIH/NCI R01CA220297 and NIH/NCI R01CA216426. G.P. is supported by Italian Association for Research on Cancer (AIRC2020-IG24341). T.D.P. and C.C. are partially supported by the NCI P30 shared resource grant (CA125123), CPRIT (RP170005 and RP200504), and NIEHS P30 (ES030285) and P42 (ES0327725) grants. This project is supported by CPRIT Proteomics and Metabolomics Core Facility (RP210227), NIH (P30 CA125123) and Dan L. Duncan Cancer Center.

Author contributions
L.T. and E.B. formulated the ideas and aims of this study. L.T., M.A.M., G.M., M.M.-S., A.B., B.E.H., A.B.W., and S.A. performed the experiments. L.T., M.A.M., G.M., M.M.-S., T.D.P., B.Z., A.B.W., Z.Z., J.H.F., J.H., C.C., and E.B. analyzed the data. P.S., Y.Z., K.B.G., N.P., G.P., C.C., and E.B. supervised the study. L.T. and E.B. wrote the manuscript with input from all authors.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-022-31331-2.

Correspondence and requests for materials should be addressed to Eveline Barbieri.

Peer review information Nature Communications thanks the anonymous reviewers for their contribution to the peer review of this work. Peer reviewer reports are available.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2022