Supporting Information

Bhatt et al. 10.1073/pnas.1205995109

SI Materials and Methods

Cell Isolation and Culture. Primary B cells were isolated by negative selection from donor buffy coats using the B-cell isolation kit II (Miltenyi) as per the manufacturer's directions. Cells were cultured at 37 °C with 5% CO_2 in filter-sterilized RPMI 1640 medium supplemented with 10% FBS, 100 U/mL each of penicillin and streptomycin, 0.075% $NaHCO_3$, and 0.05 mM 2-mercaptoethanol. Purity of the isolated B cells was determined by staining with phycoerythrin-conjugated anti-CD19 antibodies (Miltenyi) and analyzed on a MACSQuant VYB (Miltenyi). Purity ranged from 89% to 98%. Primary effusion lymphoma (PEL) cell lines were cultured in identical media and culture conditions as described (1). Lymphoblast cell line were generated by infecting freshly isolated primary human B cells with Epstein–Barr virus (EBV), using described protocols (2). Follicular lymphoma cells were cultured as described (3). CA46, a Burkitt lymphoma cell line, was purchased from ATCC and maintained in RPMI 1640 medium supplemented with 20% FBS and 100 U/mL penicillin and streptomycin. All cell lines were cultured in PEL growth medium for every experiment.

Chemical Compounds. C75, 2-deoxy-D-glucose (2DG), and lipopolysaccharide (LPS) were purchased from Sigma Aldrich. LY294002 was purchased from Calbiochem. C75 and LY294002 were suspended in DMSO, 2DG in H_2O, and LPS in PBS. All cells were treated for indicated time periods. For visualizing intracellular lipids, the lipophilic dye Nile Red (AAT Bioquest), was used per the manufacturer's direction. A Nikon Eclipse Ti microscope equipped with NIS Elements imaging software was used to acquire images.

Cell Viability Assays. To determine the susceptibility of both cell types to various inhibitors, 2×10^5 PEL or primary B cells were cultured in growth medium containing compounds at increasing concentrations, or the appropriate vehicle controls, for indicated lengths of time. Cell viability was determined in quadruplicate by trypan blue exclusion. Proliferation was measured by using the Cell Titer 96AQueous One Solution (Promega) and apoptosis was measured by using the ApoAlert Caspase-3 assay (Clontech), both according to the manufacturer's instructions, and absorbance was measured by using a FLUOstar OPTIMA (BMG Labtech) plate reader. Additionally, cell viability was also measured by using forward and side scatter parameters from flow cytometry performed on a MACSQuant VYB (Miltenyi).

Immunoblotting. After indicated treatments, cells were washed with ice-cold PBS and then lysed in a buffer containing 150 mM NaCl, 50 mM Tris·HCl at pH 8, 0.1% Nonidet P-40, 50 mM NaF, 30 mM β-glycerophosphate, 1 mM Na_3VO_4, and 1× Complete Protease Inhibitor mixture (Roche Diagnostics). Protein concentration was determined by Bradford assay, and equal amounts of proteins were separated by using SDS/PAGE, transferred onto Hybond-ECL nitrocellulose membranes (GE Healthcare), blocked, and incubated in appropriate antibodies overnight at 4 °C. The antibodies used were against fatty acid synthase, phospho-AKT Ser^{473} (Cell Signaling Technology), and Ku 70/80 as a loading control (a generous gift of Dale Ramsden, University of North Carolina, Chapel Hill, NC). Blots were incubated in appropriate secondary antibodies conjugated to horseradish peroxidase, and bands were visualized by using chemiluminescence (GE Corporation). Densitometry was performed by using NIH ImageJ.

Measurement of Glycolytic Flux. Glycolytic flux was measured as described (4). Briefly, 2×10^6 cells of each type were incubated with indicated compounds for 72 h, after which cells were washed twice in PBS and starved for 30 min by suspension in glucose-free Krebs solution, followed by a 1-h pulse with 10 μCi of D-[5-^{3}H](*N*)-glucose (Perkin-Elmer) and nonlabeled glucose, adjusted to a final glucose concentration of 10 mM. Equal volume of 0.2 M HCl was added to all samples to stop the reaction after which [^{3}H]-H_2O generated via glycolysis was separated from the D-[5-^{3}H](N)-glucose bolus by evaporation-mediated equilibration in sealed chambers. Levels of [^{3}H]-H_2O were measured on a liquid scintillation counter (Wallac), and glycolytic flux was determined by normalizing counts per million to the total protein input. These and all subsequently described data were analyzed by using a two-tailed type II Student's *t* test for significance; *P* values are indicated at appropriate locations within figure legends.

Measurement of Lipid Synthesis and Analysis of Newly Synthesized Lipid Classes. Lipid synthesis was measured as described (5). Briefly, 1×10^6 B cells were incubated with indicated compounds for 72 h; 12 h before harvest and subsequent analysis, growth medium of all cells was supplemented with D-[U-$^{14}C_6$]-glucose, after which cells were collected, washed three times with PBS, and then lysed by vortexing in 0.5% Nonidet P-40 in water. Lipids were extracted by sequentially mixing in 1 mL of methanol, 2 × 1 mL of $CHCl_3$ and 1 mL of H_2O, with extensive vortexing after addition of each solvent. To resolve aqueous and organic phases, tubes were centrifuged for 10 min at 2,000 × g in a tabletop centrifuge. The organic phase was transferred to a fresh tube, and excess $CHCl_3$ was evaporated. Extracted lipids were dissolved in 100 μL of $CHCl_3$ and counted by using scintillation fluid (Scintisafe) in a liquid scintillation counter (Wallac). Counts per million was normalized to the total protein input. Analysis of lipid components was performed by the University of North Carolina Nutrition and Obesity Research Center by using described methods (6).

Measurement of Fatty Acid Oxidation. Fatty acid oxidative capacity was determined by measuring 1-^{14}C-oleate oxidation to CO_2 as described (7). Briefly, 1×10^6 B cells were incubated with indicated compounds for 72 h, after which cells were resuspended in normal growth media supplemented with 12.5 mM Hepes at pH 7.4 and 500 μM 1-^{14}C-oleate (Perkin-Elmer) complexed with 0.5% BSA (Sigma) and incubated for 2 h at 37 °C. A result of 1-^{14}C-oleate oxidation is release of radiolabeled bicarbonate into the growth medium, which was collected at the end of 2 h and transferred to custom wells. Medium was acidified with $HClO_4$ and resulting $^{14}CO_2$ was captured in 1 M NaOH over 1 h and counted by using liquid scintillation (Wallac). Specific activity was calculated after normalization of counts per million to protein content.

Metabolic Profiling. Glycolytic and fatty acid metabolite profiles were obtained to assess the relative distribution of various intracellular and extracellular metabolites of PEL and primary B cells by culturing all cells for 3 d in growth media described above at a starting concentration of 1×10^6 cells per mL, after which cells were washed twice in PBS and cell pellets were flash frozen. The conditioned growth media of cultured cells was also collected after centrifugation and flash frozen. Further sample preparation, metabolic profiling, peak identification, and curation was performed by Metabolon using described methods (8).

Profiling of Fatty Acid Oxidation Intermediates. PEL (1×10^7) or primary B cells were cultured for 36 h in growth media supplemented with 1 μM L-carnitine, after which cells were placed in fresh media containing indicated compounds for an additional 72 h. Cells were harvested, washed in PBS and then snap frozen. Cell pellets were resuspended in ddH_2O and lysed on ice for 15 min, followed by sonication and centrifugation at 13,000 × g for 15 min at 4 °C. Supernatants were quantified for protein concentration and stored at −80 °C. Profiling of cell lysates by tandem MS was performed as described (9).

Bioinformatics. Hierarchical clustering and principal component analysis was conducted by using the R programming environment (version 2.13.2) package FactoMineR.

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Fig. S1. PEL cells display intracellular lipid droplets. BCBL-1 and primary B cells were stained with the lipophilic dye, Nile Red. Stained cells were visualized by using a Nikon Eclipse Ti microscope (Ex/Em = 552/636 nm, or bright field) at 10× magnification under identical settings. The Nikon Eclipse is equipped with NIS Elements imaging software. Fluorescence in PEL is attributed to the large amount of intracellular lipid, whereas the primary B cells do not exhibit the same degree of fluorescence.

Fig. S2. FAO levels do not significantly differ between PEL and primary B cells. PEL and primary B cells oxidize the radiolabeled fatty acid, ^{14}C-oleate, at similar rates. Data are normalized to total input protein and error bars are ± SEM.

Fig. S3. B-NHL are susceptible to the FAS inhibitor, C75. CA46 (*A*) and SUDHL4 (*B*) B-NHL are susceptible to C75. C75 treatment leads to approximately 50% reduction in proliferation of these two lines, in contrast with >90% cell death of PEL, at the same dose (10 μg/mL). (*C*) C75 induces apoptosis in B-NHL.

Fig. S4. LPS-driven proliferation of primary B cells. (*A*) Glycolysis is minimally up-regulated in LPS-stimulated proliferating primary B cells, but the rates are lower than those of vehicle-treated PEL. Error bars are ± SEM. (*B*) FAS is not significantly up-regulated in LPS-stimulated primary B cells, and the rates of FAS are significantly lower than those seen in untreated PEL. Error bars are ±SEM. (*C*) PEL cell proliferation is not altered in response to LPS (10 μg/mL) stimulation (*Left*), whereas primary B cells (*Right*) proliferate upon LPS treatment over a period of 48 h. Error bars are ± SEM.

Fig. S5. PI3K inhibition of PEL. To determine a noncytotoxic dose of LY294002, the drug was added to cells at different concentrations ranging from 10 nM to 50 μM for 72 h. Cell death was measured by trypan blue exclusion and represented as percent of total cells counted.

Fig. S6. Overall levels of free carnitine and FAO intermediates differ between PEL and primary B cells. (*A* and *B*) Tandem mass spectrometric analysis was used to determine the relative intensities of carnitine and acyl-carnitine intermediates within PEL and primary B cells. (*A*) Levels of free carnitine (normalized to total cellular protein) in PEL are slightly lower than in primary B cells. Two different primary B-cell donors were combined for this analysis. FAS inhibition with C75 (open bars) decreases free carnitine in both PEL and primary B cells, however, the magnitude of decrease is greater in PEL compared with primary B cells. (*B* and *C*) Relative distribution of intracellular acyl-carnitines reveals that PEL (black bars) have higher levels of even-chained acyl-carnitine intermediates compared with primary B cells (gray bars). Further, higher levels of C_3 and C_5 acyl-carnitine intermediates (Fig. S3*B*) are found in primary B cells compared with PEL.

Fig. S7. FAS inhibition increases the susceptibility of PEL to the PI3K inhibitor, LY294002. PEL are sensitive to LY294002 in a dose-dependent manner, as measured by trypan blue exclusion. Addition of C75 significantly increases the number of dead cells, with maximal cell death when PEL are treated with 10 μM LY294002 and 10 μg/mL C75. For the comparison between PEL treated with only LY294002 vs. LY294002 plus C75: $P \leq 0.05$.

Table S1. De novo synthesized lipids and triglycerides synthesized by PEL and primary B cells

	^{14}C incorporation, cpm/10^6 cells								
Lipid class	BC-1	BC-3	BCBL-1	PEL average	PEL ratio of lipids to free FA	Donor 1	Donor 2	Primary B-cell average	Primary B-cell ratio of lipids to free FA
Phosphatidylcholine	121.5	146.4	192.7	153.5	24.3	11.9	6.8	9.3	4.4
Phosphatidylethanolamine	27.0	45.9	47.5	40.1	6.4	4.6	2.3	3.4	1.6
Phosphatidylinositol	9.5	9.9	12.3	10.6	1.7	2.1	1.5	1.8	0.8
Phosphatidylserine	24.3	43	44.5	37.2	5.9	4.7	2.5	3.6	1.7
Sphingomyelin	13.8	29.9	36.2	26.6	4.2	3.5	2.5	3.0	1.4
Triacylglycerol	52.3	72.6	41.9	55.6	8.8	4.6	3.1	3.8	1.8
1,2-Diacylglycerol	5.8	14.6	5.8	8.8	1.4	2.3	1.6	1.9	0.9
1,3-Diacylglycerol	6.0	7.2	5.0	6.1	0.9	1.4	1.4	1.4	0.7
Free fatty acids (FA)	4.5	8.1	6.4	6.3	1.0	2.3	2.0	2.1	1.0

TLC of extracted lipid fraction of both PEL and primary B cells reveals that a greater amount of phospholipids, triglycerides, and fatty acids (FA) are synthesized by PEL compared with primary B cell.

Table S2. Scaled mass spectrometry data for metabolites of glycolysis and FAS pathways normalized to protein content

			Primary B cells						PEL cell line				
Biochemical compound	Type	PubChem ID	PRIM B-1	PRIM B-2	PRIM B-3	PRIM B-4	PRIM B-5	PRIM B-6	BC-1	BCBL-1	BCP-1	JSC-1	VG-1
10-Heptadecenoate (17:1n7)	Lipid	5312435	2.154214	2.342948	2.142111	1.594887	1.302198	0.670551	0.388602	0.386392	0.408652	0.571206	0.725264
Adrenate (22:4n6)	Lipid	5282844	1.970749	4.090171	1.774704	2.307935	0.992950	1.007050	0.071169	0.038923	0.253323	0.092894	0.177979
Arachidonate (20:4n6)	Lipid	444899	0.971645	2.606871	2.682979	2.163501	1.417565	1.091183	0.084138	0.082987	0.108145	0.218607	0.270755
cis-vaccenate (18:1n7)	Lipid	5282761	1.028255	1.707979	1.000000	2.589450	2.732214	1.181014	0.638002	0.533335	0.944106	0.734640	0.730327
Dihomo-linoleate (20:2n6)	Lipid	6439848	1.058392	1.503024	1.429511	1.699742	0.941608	1.262077	0.502302	0.352439	0.333567	0.392245	0.366725
Dihomo-linolenate (20:3n3 or n6)	Lipid	5312529	0.843135	1.699267	1.633630	1.383610	1.167830	1.156865	0.159175	0.138249	0.185247	0.282637	0.260110
Docosahexaenoate (DHA; 22:6n3)	Lipid	445580	0.881393	1.818107	1.821204	2.446815	1.496151	0.839976	0.293674	0.308541	0.429882	0.763505	1.118607
Docosapentaenoate (n3 DPA; 22:5n3)	Lipid	—	0.991835	2.146026	1.919165	2.085257	1.345845	1.008165	0.174684	0.161990	0.236817	0.417872	0.816323
Linoleate (18:2n6)	Lipid	5280450	1.099654	1.351696	1.746969	1.615510	1.513593	1.086768	0.259622	0.259872	0.298921	0.417243	0.454039
Linolenate [alpha or gamma; (18:3n3 or 6)]	Lipid	—	1.365310	1.810361	2.407313	1.878679	1.840596	0.827205	0.293494	0.294118	0.404137	0.561291	0.518298
Oeate (18:1n9)	Lipid	445639	2.042494	1.068113	1.420366	1.713755	2.123259	0.875017	0.647002	0.708231	1.138020	0.730457	0.931887
2-Hydroxystearate	Lipid	69417	2.274826	1.879189	2.166177	1.966132	1.448567	0.551434	0.141471	0.163772	0.461971	0.356071	0.195178
Carnitine	Lipid	288	0.639972	1.653065	1.301053	0.806568	0.994267	1.259980	0.585440	0.881887	1.365614	1.198304	1.005733
CoA	Cofactors and vitamins	317	0.666142	0.666142	0.666142	0.666142	0.666142	0.666142	0.840945	1.000000	1.109555	0.666142	2.019305
Acetylcarnitine	Lipid	7045767	0.891397	0.778092	1.022919	0.726967	1.027963	1.114138	0.546011	0.977081	1.466425	1.030076	3.240323
Glucose	Carbohydrate	79025	7.136202	3.771906	2.066262	0.366332	1.385368	5.160871	1.144313	0.399013	0.169361	0.189251	0.855686
Glucose-6-phosphate (G6P)	Carbohydrate	—	0.206572	0.206572	0.206572	0.206572	0.206572	0.206572	3.159978	1.000000	0.206572	0.308069	1.701397
Isobar: fructose 1,6-diphosphate, glucose 1,6-diphosphate	Carbohydrate	—	0.609347	0.609347	0.609347	0.609347	0.609347	0.609347	3.333895	1.363272	0.816139	0.609347	1.183861
Malate	Energy	525	0.455786	0.455786	0.455786	0.455786	0.455786	0.455786	0.585008	1.161455	1.487411	0.455786	1.000000
1-Arachidonoylglycerophosphoethanolamine*	Lipid	—	0.230679	2.017211	2.590079	2.572300	0.560805	1.000000	0.283742	0.402849	0.409058	0.230679	1.698531
1-Arachidonoylglycerophosphoinositol*	Lipid	—	0.553519	0.553519	0.553519	0.553519	0.553519	0.553519	0.968149	0.553519	0.749567	1.031851	3.033269
1-Oleoylglycerophosphoethanolamine	Lipid	9547071	0.227386	0.227386	0.227386	0.227386	0.227386	0.227386	1.599367	1.000000	0.967465	0.815195	2.707747
1-Oleoylglycerophosphoinositol*	Lipid	—	0.760707	0.760707	0.760707	0.760707	0.760707	0.760707	0.760707	1.000000	1.610265	0.919150	1.365526
1-Palmitoylglycerol (1-monopalmitin)	Lipid	14900	2.934504	2.473959	0.393936	1.758260	0.895583	1.846332	0.604346	0.724260	0.743110	0.393936	1.000000
1-Palmitoylglycerophosphocholine	Lipid	86554	20.396284	0.136747	4.037944	33.142727	0.136747	1.089359	0.136747	0.159513	0.454525	0.183257	2.619025
1-Palmitoylglycerophosphoethanolamine	Lipid	9547069	0.899064	0.899064	0.899064	0.899064	0.899064	0.899064	2.034674	0.913648	0.935041	0.899064	4.045914
1-Palmitoylglycerophosphoinositol*	Lipid	—	0.683694	0.683694	0.683694	0.683694	0.683694	0.683694	1.214195	1.150388	1.000000	0.683694	0.761693
1-Palmitoylplasmenylethanolamine*	Lipid	—	1.156021	1.854534	1.370189	0.634664	0.483241	0.954234	1.391208	0.559061	0.534737	1.045766	0.302165
1-Stearoylglycerophosphocholine	Lipid	497299	6.651697	0.035097	1.796015	7.817932	0.035097	0.035097	0.035097	0.035097	0.079610	0.035097	0.203985
1-Stearoylglycerophosphoethanolamine	Lipid	9547068	0.942180	2.685918	2.180839	2.212741	0.850304	0.824761	1.057820	0.784227	0.696988	0.474447	1.102602
1-Stearoylglycerophosphoinositol	Lipid	—	0.481612	0.481612	1.344767	1.841572	0.745977	0.998880	1.291571	0.481612	0.649268	0.913844	1.001120
2-Oleoylglycerophosphoethanolamine*	Lipid	—	0.758856	0.758856	0.758856	0.758856	0.758856	0.758856	1.027633	0.818893	1.000000	0.758856	3.476123
2-Oleoylglycerophosphoinositol*	Lipid	—	0.457303	0.457303	0.457303	0.457303	0.457303	2.740765	0.457303	0.504259	1.296641	0.904644	1.095356
Lactate	Carbohydrate	612	0.598431	0.913886	1.192845	1.152137	0.735923	0.732343	1.564702	1.117737	1.774399	1.067171	0.931858
Isobar: fructose 1,6-diphosphate, glucose 1,6-diphosphate-media	Carbohydrate	—	0.609347	0.609347	0.609347	0.609347	0.609347	0.609347	3.333895	1.363272	0.816139	0.609347	1.183861
Dihydroxyacetone phosphate (DHAP)	Carbohydrate	4643300	0.774646	0.774646	0.774646	0.774646	0.774646	0.774646	1.850655	0.873661	0.774646	0.774646	1.126339
Lactate-media	Carbohydrate	612	0.311439	0.435180	0.594924	0.736026	1.022632	1.000000	1.396906	1.447225	1.391265	1.395947	1.469370
Pyruvate-media	Carbohydrate	107735	0.752238	0.627062	0.717811	0.789492	0.565593	1.210508	3.193524	3.515419	3.030925	0.569251	4.679479
Glucose-media	Carbohydrate	79025	1.969532	1.602706	1.556455	1.427756	0.592489	0.960880	0.147572	0.020101	0.001989	0.000713	0.006450

Metabolites not assigned a PubChem ID are indicated with a "—" in that column. Compounds with an asterisk "*" next to their names are those identified with confidence by Metabolon, Inc. but are not officially based on a standard.