Supplementary Legends

**Table S1.** Serine hydrolase activity levels in aggressive versus nonaggressive cancer cell lines as determined by ABPP-MudPIT. Shown are average ± sem spectral counts for each serine hydrolase and significance of the difference between aggressive versus nonaggressive cells. Values for individual replicates are shown in **Tables S2 and S3.**

**Table S2.** Complete list of proteins identified in ABPP-MudPIT analysis of human cancer cell lines. Results shown are the individual spectral counts obtained for each identified protein (Tab 1) and an extracted list of serine hydrolases identified for each FP-biotin (3 replicates designated 1, 2, and 3) or no-probe (np, without FP-biotin) control proteomic sample (Tab 2).

**Table S3.** Complete list of all peptides and scoring for individual peptides identified in ABPP-MudPIT analysis of human cancer cell lines. Tables 3.1-3.6 represent ABPP-MudPIT experiments for each cell line: Table S3.1 contains 231MFP data, Table S3.2 contains MCF7 data, Table S3.3 contains C8161 data, Table S3.4 contains MUM2C data, Table S3.5 contains SKOV3 data, and Table 3.6 contains OVCAR3 data. Within each table are tabs that correspond to the individual replicates of the unsoluble or soluble proteomes (designated “us” or “sol”, respectively, on the tabs), or samples where FP-biotin probe was not included (designated “no-probe”). ABPP-MudPIT experiments were performed as described in Supplemental Methods.
Table S4. Levels of lipid metabolites in human cancer cell lines +/- MAGL inhibition, knockdown, or overexpression. Data are shown for melanoma, ovarian and breast cancer cell lines and are expressed as pmoles/1x10^6 cells (n=3-5/group). The “Abridged” tab contains a summary of the lipid quantitations, with average ± sem values for each lipid. The following “melanoma,” “ovarian,” and “breast” tabs contain values for individual replicates. Details on the protocols used for lipid quantitation can be found in the Supplementary Methods.

Figure S1. MAGL, KIAA1363 and lipolytic profiles across the panel of aggressive versus nonaggressive melanoma and ovarian cancer cell lines and explanation of mass balance discrepancy between elevated MAGs and reduced FFAs caused by treatment of cancer cells with the MAGL inhibitor JZL184. (A-C) Aggressive melanoma (C8161) and ovarian (SKOV3) cells show greater migration (A) and invasion (B) and faster tumor growth rates (C) compared to their nonaggressive counterparts (MUM2C and OVCAR3 cells, respectively). Similar data have been reported previously for these line and other aggressive lines used in this study (e.g., MUM2B and 231MFP) (Jessani et al., 2004; Jessani et al., 2002; Seftor et al., 2002). (D) Lipolytic profiles of cancer cell lines shows robust hydrolytic activity of MAGs in aggressive cancer cells compared to other lipolytic activities. In vitro free fatty acid (FFA) release from various lipid substrates was assayed. Cells were treated with DMSO or JZL184 in situ for 4 hr in serum-free media. Cell lysates (20 µg) were incubated with 100 µM lipid substrate for 1 hr at room temperature. Upper and lower panels show the same data with different y-axis scales (with and without MAG hydrolytic activity) for ease of comparison and visualization. Abbreviation for lipid substrates are as follows: TAG, triacylglycerol; DAG, diacylglycerol; MAG, monoacylglycerol; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PE phosphatidyl ethanolamine; LPE, lysophosphatidyl ethanolamine. *p<0.05, **p < 0.01 for
aggressive versus non-aggressive cancer line groups and ##p<0.01 for DMSO versus JZL184. Data are presented as means ± SEM; n = 3-15/group. (G-I) Elevations in lysophospholipids account for the mass balance discrepancy between elevated MAGs and reduced FFAs caused by treatment of cancer cells with the MAGL inhibitor JZL184. (E) JZL184 (1 µM, 4 hr) causes a 5500-6100 pmol/10^6 decrease in FFA levels in C8161 and SKOV3 cells. This reduction in FFA levels is similar in magnitude to the net increase in lysophosphatidylcholine (LPC) and lysophosphatidylethanolamine (LPE) levels observed in JZL184-treated cancer cells (4100-6500 pmol/10^6 cells). Complete lipidomic data on JZL184-treated cancer cells can be found in Table S4. (F) C17:0 MAG (20 µM, 1 hr) is converted to C17:0 LPC and C17:0 LPE by cancer cells, and this conversion is further enhanced by preincubation of cancer cells with JZL184 (1 µM, 4 hr). C17:0 MAG is also converted to C17:0 LPA, but, in this case, the conversion is blocked by JZL184. These data indicate that C17:0 MAG is directly converted to C17:0 LPC and C17:0 LPE in cancer cells, while conversion of C17:0 MAG to C17:0 LPA requires MAGL-dependent hydrolysis of C17:0 MAG to C17:0 FFA. C20:4 MAG (20 µM, 1 hr) is converted to prostaglandin E_2 (PGE2), and this conversion is blocked by JZL184. (G) C16:0 and C20:4 FFAs are converted to LPA and PGE2, respectively, by aggressive cancer cells. C8161 and SKOV3 cells were treated with d_2-C16:0 FFA or d_8-C20:4 FFA (10 µM, 4 hr) in serum-free media. d_2-C16:0 FFA was converted to d_2-C16:0 LPA and d_8-C20:4 FFA was converted to d_8-PGE_2. *p < 0.05, **p < 0.01 for comparison of control cells versus treated cells, #p < 0.05, ##p < 0.01 for comparison of JZL184/MAG treated cells versus MAG-treated cells alone. Data are presented as means ± SEM; n = 3-4/group.

**Figure S2.** MAGL regulates MAG, FFA and cancer cell pathogenicity in aggressive cancer cell lines. (A, B) JZL184 treatment (1 µM, 4 hr) increases MAG (A) and reduces FFA levels (B) in 231MFP cells. (C) shMAGL probes selectively reduce the activity of
MAGL compared to other serine hydrolases in aggressive cancer cells C8161, SKOV3, and 231MFP as judged by activity-based protein profiling (ABPP). Serine hydrolase activities were labeled in whole cell proteomes with the activity-based probe FP-rhodamine and detected by SDS-PAGE and in-gel fluorescence scanning (fluorescent gel shown in grayscale). MAGL activity was reduced by >75 % with both shMAGL probes (shMAGL1, shMAGL2), as assessed by ABPP (for C8161, SKOV3, and 231MFP) and C20:4 MAG substrate assays (for 231MFP). (D, E) shMAGL cells show increased levels of MAGs (D) and decreased levels of FFAs (E) compared to shControl or parental 231MFP cells. (F-H) shMAGL cells show decreased migration (F), invasion (G), and cell survival (H) compared to shControl or parental 231MFP cells. The migratory defect was rescued by treatment with C16:0 FFA or C18:0 FFA (20 µM, 4 hr). (I) Acute blockade of MAGL by JZL184 (1 µM, 4 hr) impairs cancer cell migration. ** p < 0.01 for JZL184-treated or shMAGL cells versus their respective control groups. ## p < 0.01 for C16:0 or C18:0 FFA-treated versus DMSO-treated shMAGL groups. Data are presented as means ± SEM; n = 4-6/group.

**Figure S3.** In vivo treatment with JZL184 (40 mg/kg oral gavage, administered once daily for 30 days in 4 µL/g polyethylene glycol) inhibits tumor xenograft MAGL activity (A) and shMAGL tumors contained lower FFA levels compared to shControl cells (B). Vehicle or JZL184-treated SCID mouse tumor homogenates were incubated with DMSO or JZL184 (1 µM, 30 min) in vitro before addition of 100 µM C20:4 MAG for 30 min. *p<0.05, **p < 0.01 for JZL184-treated or shMAGL tumors versus vehicle or shControl tumors, respectively. Data are presented as means ± SEM; n = 4/group.

**Figure S4.** Ectopic expression of MAGL elevates FFA levels and enhances the in vitro and in vivo pathogenicity of OVCAR3 and MUM2C ovarian and melanoma cancer cells.
(A) MAGL overexpression (MAGL-OE, red bars) in OVCAR3 cells confirmed by ABPP (top panel), western blot (middle panel) and C20:4 MAG hydrolytic activity (bottom panel). Control and S122A cells (black bars) correspond to cancer cells infected with empty vector (EV) or a catalytically inactive MAGL mutant (S122A). Western analysis confirmed the overexpression of the S122A-MAGL mutant, which did not show any activity as judged by ABPP and C20:4 MAG hydrolysis assays. (B, C) MAGL-OE cells contain lower MAG (B) and higher FFA (C) levels compared to EV control and S122A cells. These metabolic effects were reversed by in situ treatment with JZL184 (1 µM, 4 hr, maroon bars). (D, E) MAGL-OE OVCAR3 cells show increased migration (D) and invasion (E) compared to EV and S122A control cells. This enhanced migration and invasion was reversed by JZL184 (1 µM, 4 hr). Representative migration panels are shown (D). (F) MAGL-OE MUM2C and OVCAR3 cells show significantly enhanced cell survival (24 h after serum starvation) compared to EV control cells. (G) The enhanced tumor growth rate of MAGL-OE MUM2C cells is similar to the tumor growth rate of aggressive C8161 melanoma cells. The C8161 and MUM2C data are derived from Figure 4E and Figure 5F, respectively (note that these experiments were performed concurrently to permit a direct comparison of the tumor growth rates). * p < 0.05, ** p < 0.01 for MAGL-OE- versus control groups. Data are presented as means ± SEM; n = 4-6/group.

Figure S5. Assessment of cannabinoid signaling, energetics, fatty acid beta oxidation or fatty acid synthesis in cancer aggressiveness. (A) FFA levels in shControl and shMAGL cancer cells were unchanged with CB1 (rimonabant, 1 µM, 4 hr) or CB2 (AM630, 1 µM, 4 hr) antagonist treatment. Data shown for C16:0 and C20:4 FFA. Similar results were observed for other FFAs (C18:0, C18:1). (B) The migratory defect in shMAGL cancer cells was not rescued by treatment with either the CB1 antagonist rimonabant or CB2
antagonist AM630 (1 µM, 4 hr), or co-treatment with both antagonists. **p < 0.01 for each shMAGL group versus shControl cells. Data are presented as means ± SEM; n = 6/group. (C, D) Levels of CB1 (C) and CB2 (D) receptor mRNA as determined by DNA microarray analysis. Left bar graph corresponds to data extracted from an analysis of the NCI60 panel of human cancer cell lines performed with a HU133APlus 2.0 Affymetrix array (Ross et al., 2000). Right bar graph corresponds to data extracted from an analysis of aggressive and non-aggressive cancer lines used in the current study performed with a HU133APlus 2.0 Affymetrix array. The SNB-19 cancer line, which contains high levels of CB1 receptor, is shown for comparison. (E-H) Increased (MAGL-OE) or decreased (shMAGL) expression of MAGL does not alter NAD⁺ (E), NADH (F), pyruvate (G), or lactate (H) levels in cancer cells. Data are presented as means ± SEM; n = 6/group. (I) Fatty acid β-oxidation inhibitors trimetazidine (3-ketoacyl coenzyme A thiolase inhibitor, 100 µM, 4 hr preincubation and coincubation with migration) or etomoxir (CPT1 inhibitor, 100 µM, 4 hr preincubation and coincubation with migration) did not affect migration of cancer cells. **p<0.01 for MAGL-OE versus control groups. Data are presented as means ± SEM, n=3/group. (J) Etoxomir treatment (100 µM, 4 hr) did not alter FFA levels in cancer cells with the exception of C20:4 FFA, which was elevated in C8161 and SKOV3 cells. * p < 0.05, ** p < 0.01 for shMAGL or MAGL-OE cells versus their respective control groups. # p < 0.05 for etoxomir-treated versus DMSO-treated control groups. Data are presented as means ± SEM; n = 3-4/group.

**Figure S6.** The effect of the EGFR kinase inhibitor tyrphostin on cancer cells. Tyrphostin decreased migration and cell survival (48 h, serum free) in C8161 (A, B) and SKOV3 (C, D) cells to a similar level as the migration and cell survival defects observed in shMAGL cancer cells. C8161 and SKOV3 shMAGL cells show enhanced sensitivity to tyrphostin-induced impairments in cell migration and survival (A, B). *p < 0.05, **p < 0.01 between
tyrphostin- versus vehicle-treated shControl or shMAGL cancer cells; \#p < 0.05, \#\#p < 0.01 between shControl and shMAGL cancer cells of the same treatment group. Data are presented as means ± SEM, n=4/group.

**Supplemental Methods**

**Materials**

All cell lines, with the exception of C8161, MUM-2B, MUM-2C, and 231MFP, are part of the NCI60 panel of cancer cell lines and were obtained from the National Cancer Institute’s Developmental Therapeutics Program. The C8161, MUM-2B, and MUM-2C lines were provided by Mary Hendrix. The 231MFP cells were generated from explanted xenograft tumors of MDA-MB-231 cells, as described previously (Jessani et al., 2004). C15:0, C16:0, C18:0 and C18:1 FFAs, C18:1 FFA-OH and C16:0, C18:0, C18:1, and C20:4 MAGs were purchased from Sigma. C12:0 MAGE and C15:0 MAG were purchased from Alexis Biochemicals and Nu-check Prep, respectively. C20:4 FFA and PGE₂ were from Cayman Chemicals. Other representative lipid standards of various classes of lipids listed in Table S4 were purchased from Sigma, Cayman Chemicals, Nu-check Prep, or Avanti Lipids. FP-rhodamine and FP-biotin was synthesized by following previously described procedures. JZL184 was synthesized as previously detailed. The human MAGL antibody (against N terminal amino acids 1-121) was purchased from Novus Biologicals.

**Pharmacological Inhibition of MAGL in Cancer Cells**

Cancer cells were maintained in RPMI medium 1640 with 10% (v/v) fetal calf serum at 37°C in a humidified atmosphere of 5% CO₂/95% air. At 80% confluency, cells were trypsinized and counted using a hemocytometer, and 1 x 10⁶ cells were plated in 6 cm
dishes (subconfluent). A total of 20 hr after plating, cells were washed twice with PBS and inhibitors were incubated in serum-free RPMI media with JZL184 (1 µM) or vehicle (DMSO) at 0.1%. After incubation for 4 hr, the cells were harvested and analyzed by ABPP or LC-MS.

**Identification and Comparative Quantitiation of Serine Hydrolase Activities from Cancer Cell Proteomes by ABPP-MudPIT**

The soluble and unsoluble proteome fractions from each of the human cancer cell lines were generated as previously described (Jessani et al., 2002) and analyzed by ABPP-MudPIT (Jessani et al., 2005). Standard conditions for FP-Biotin proteome labeling reactions were as follows: proteomes were adjusted to a final protein concentration of 1.0 µg/µl and 1000 µg of proteome were labeled with 5 µM of FP-biotin for 2 hours at room temperature (RT). After incubation, the unsoluble proteome was solubilized with 1% Triton-X, rotated at 4ºC for 1 hour. Enrichment of FP-labeled proteins from the soluble and unsoluble proteome fractions was achieved as previously described (Kidd et al., 2001). The avidin enriched proteome was washed 2 times for 8 minutes with 1) 1% SDS, 2) 6M Urea, 3) 50 mM Tris pH 8.0 and finally resuspended in 200 µl 8M Urea 50 mM Tris pH 8.0. Samples were then prepared for on-bead digestion by reduction with 10 mM TCEP for 30 minutes at room temperature and alkylated with 12 mM of iodoacetamide for 30 minutes at room temperature in the dark. Digestions were performed for 12 hours at 37 °C with Trypsin (3 µLs of 0.5 µgs/ul in the presence of 2 mM CaCl₂ after samples were diluted to 2M Urea with 50 mM Tris pH 8.0. Lastly peptide samples were acidified to a final concentration of 5% formic acid. Digested peptide mixtures were loaded on to a biphasic (strong cation exchange/reverse phase) capillary column and analyzed by two-dimensional liquid chromatography (2D-LC) separation in combination with tandem mass spectrometry as previously described.
Peptides were eluted in a 5-step MudPIT experiment (using 0, 10, 25, 80 and 100% salt bumps) and data was collected in an ion trap mass spectrometer, LTQ (Thermo Scientific) set in a data-dependent acquisition mode with dynamic exclusion turned on (60s). Specifically, one full MS survey (ms1) scan was followed by 7 ms2 scans. The ms2 spectra data were extracted from the raw file using RAW Xtractor (version 1.9.1) which is publicly available (http://fields.scripps.edu/?q=content/download). ms2 spectra data were searched using the SEQUEST algorithm (Version 3.0) (Eng et al., 1994) against a custom made database containing the longest entry from v3.26 of the human IPI database associated with each Ensembl gene identifier resulting in a total of 22935 unique entries. Additionally, each of these entries was reversed and appended to the database for assessment of false-discovery rates. SEQUEST searches allowed for oxidation of methionine residues (16 Da), static modification of cysteine residues (57 Da-due to alkylation), no enzyme specificity and a mass tolerance set to ±1.5 Da for precursor mass and ±0.5 Da for product ion masses. The resulting ms2 spectra matches were assembled and filtered using DTASelect (version 2.0.27) (Cociorva and Yates, 2006). A quadratic discriminant analysis was used to achieve a maximum peptide false positive rate of 1% as previously described (Cociorva et al., 2007; Tabb et al., 2002)

The total proteomic data obtained (Table S2 and Table S3) were initially filtered for serine hydrolases using a manually assembled list of InterPro domain identifiers (IPR000120, IPR000379, IPR001031, IPR001087, IPR001466, IPR002641, IPR002642, IPR002921, IPR004177, IPR005181, IPR007751, IPR008262, IPR010662) that correspond to known members of this family. The resulting set of serine hydrolases were then filtered for enzymes that displayed > 10-fold higher spectral counts in FP-rhodamine-treated proteomes compared to "no-probe" control proteomes for at least one of the cancer cell lines examined. For these calculations, spectral count values from the
membrane and soluble proteomes were combined for each serine hydrolase from each cancer line. This analysis resulted in a list of ~50 serine hydrolase activities (Table S1). Previous studies have shown that comparative quantitation is best restricted to proteins that show an average of \( \geq 10 \) spectral counts in at least one of the two groups under comparison (Jessani et al. Nat Methods 2005). Using this filter (where comparison groups were defined as aggressive cancer lines compared to their non-aggressive counterparts), we identified ~35 serine hydrolase activities for comparative quantitation. Among these serine hydrolases, KIAA1363 and MAGL met the following criteria defined for aggressiveness-associated hydrolase activities: 1) average spectral counts > 2-fold in all three aggressive cancer lines relative to their non-aggressive counterparts, and 2) a \( p \)-value of < 0.01 for differences between each aggressive and non-aggressive cancer cell line pair.

**ABPP of Cancer Cell Proteomes**

For ABPP experiments, cell lysate proteomes were treated with 2 \( \mu \text{M} \) FP-rhodamine for 30 min at room temperature (50 \( \mu \text{l} \) total reaction volume). Reactions were quenched with one volume of standard 4x SDS/PAGE loading buffer (reducing), separated by SDS/PAGE (10\% acrylamide), and visualized in-gel with a Hitachi FMBio IIe flatbed fluorescence scanner (MiraiBio). Integrated band intensities were calculated for the labeled proteins and averaged from three independent cell samples to determine the level of each enzyme activity. IC50 values were determined from dose-response curves from three trials at each inhibitor concentration by using SigmaPlot 10.0. MAGL expression was also assessed by western blotting using standard procedures.

**Hydrolytic Activity Assays**
For hydrolytic activity assays, cells were treated \textit{in situ} with JZL184 (1 µM) for 4 h in serum-free RPMI media before harvesting cells by scraping. Cell lysates (20 µg) in Tris buffer were then incubated with lipid (100 µM, e.g. C20:4 MAG for MAGL activity) at room temperature for 30 min in a volume of 200 µl. Reactions were quenched with 600 µl 2:1 chloroform:methanol and 10 nmol of C15:0 FFA or C12:0 MAGE internal standard was added. The products were extracted into the organic layer which was extracted and directly injected into LC-MS. LC-MS settings were as previously described (Blankman et al., 2007). Product levels (e.g. C20:4 FFA for MAGL activity) were quantified in relation to internal standard levels and standard curves generated between varying lipid concentration versus constant internal standard levels. Specific activity was determined during the linear phase of enzymatic reactions (i.e., less than 20% substrate utilized).

**Human Primary Ovarian Tumors**

Patients were diagnosed and treated for ovarian tumors at Brigham and Women’s Hospital and Dana-Farber Cancer Center, Boston, MA. All patient-derived biologic specimens were collected and archived under protocols approved by the Human Subjects Committee of the Brigham and Women’s Hospital. The histopathologic diagnosis was determined by the gynecological pathologists at Brigham and Women’s Hospital. The tumors were classified and graded according to the International Federation of Gynecology and Obstetrics (FIGO) system. For this work, 10 benign and 13 high-grade malignant ovarian tumor samples were used for the MAGL activity and metabolite measurements. The benign cases included benign cysts, ovarian fibromas and benign serous cystadenomas, whereas the malignant cases were all high-grade papillary serous carcinomas. Fresh tumor tissues were cut with scalpels into 2-5 mm pieces, individually wrapped in aluminum foil, snap-frozen in liquid nitrogen and kept at –80 °C freezer. MAGL activity and FFA levels were measured as described above.
RNA Interference Studies in Human Cancer Cell Lines

RNA interference studies were conducted as described previously (Chiang et al., 2006). Briefly, short-hairpin RNA constructs were subcloned into the pLP-RetroQ acceptor system, and retrovirus was generated by using the AmphiPack-293 Cell Line (Clontech). Hairpin oligonucleotides utilized for RNA interference studies were: for MAGL (shMAGL1), 5’-CAACTTTCAAGGTCCTTGC-3’ and (shMAGL2), 5’-AGACTACCTGGGCTTCTCCT-3’; for the shControl (shDPPIV), 5’-GATTCTTCTGGGACTGCTG-3’. The shControl construct was designed to target a distinct serine hydrolase that was not consistently altered between aggressive and non-aggressive cancer cells (DPPIV). Using an shControl construct that targets an endogenous protein (Rather than a non-functional scrambled construct) controls for non-specific effects due to general activation of the RNA interference machinery (Minn et al., 2005). Virus containing supernatant from 1–6 d was collected, concentrated by ultracentrifugation, and, in the presence of 10 µg/ml polybrene, used to stably infect cells for 48 hr. Infection was followed by 3 days of selection in medium containing puromycin (1 µg/ml for C8161, MUM2C, SKOV3, and 231MFP and 0.3 µg/ml for OVCAR3), as the retroviral vector contained this selection marker. Infected cells were expanded and tested for the loss of enzyme activity by ABPP and C20:4 MAG hydrolytic activity.

Overexpression Studies in Human Cancer Cell Lines

Stable MAGL overexpression was achieved by subcloning the MAGL gene into the pMSCVpuro vector (Clontech), generating retrovirus using the AmphiPack-293 Cell Line, as described above with the RNA interference studies. The human MAGL construct was generated by PCR with primers 5’-

GCTCTCGAGGCGCCATGCCAGGAAAGTTCTC-3’ and 5’-
AGCTGAATTTCAGGGTGGGACGCAGTTCTG-3'. PCR products were subcloned into the pMSCVpuro (Clontech) by using XhoI and EcoRI restriction sites.

**Cell Migration, Cell Survival, and Invasion Studies**

Migration assays were performed in Transwell chambers (Corning) with 8 µm pore-sized membranes coated with 10 µg/ml collagen for 4 h at 37°C. A total of 24 hr before the start of the migration assay, cancer cell lines were plated at a concentration of 1 x 10⁶ cells per 6 cm dish. At the start of the migration assay, cells were harvested by washing two times with PBS and were then serum starved in serum-free media for 4 hr. Inhibitor, lipid or vehicle (0.1 % DMSO) were preincubated with the cells during this 4h time. Serum-starved cells were trypsinized, spun at 1400 x g for 3 min, resuspended, and counted. 50,000 cells were seeded in the upper chamber of the transwells in 200 µl serum-free media (containing inhibitor, lipid or vehicle). Inhibitor, lipid or vehicle was also added to the lower chamber, and cells were allowed to migrate for 5 h (for C8161 and 231MFP cells) or 20 hr (SKOV3, MUM2C and OVCAR3 cells). The filters were then fixed and stained with Diff-Quik (Dade Behring). Cells that had not migrated through the chamber were removed with a cotton ball. The cells that migrated were counted at a magnification of 400x, and 4 fields were independently counted from each migration chamber. An average of cells in 4 fields for one migration chamber represents n=1.

Cell survival assays were performed using the Cell Proliferation Reagent WST-1 (Roche) as previously described (Roca et al., 2008; Siddiqui et al., 2005). Cells were washed twice in PBS, harvested by trypsinization, washed twice in PBS, centrifuged at 1400 x g and resuspended in serum-free media. 20,000 cells were plated in 200 µl in 96-well plates in serum-free media 0, 24 or 48 h prior to addition of WST-1 (20 µl) for 4 h at 37°C/5% CO₂. Absorbance was measured at 450 nm using a spectrophotometer.
Invasion assays were conducted using the BD Matrigel Invasion Chambers per the manufacturer's protocol.

**Tumor Xenograft Studies**

Human cancer xenografts were established by transplanting cancer cell lines ectopically into the flank of C.B17 SCID mice (Taconic Farms). Briefly, cells were washed two times with PBS, trypsinized, and harvested in serum-containing medium. Next, the harvested cells were washed two times with serum-free medium and resuspended at a concentration of \(2.0 \times 10^4\) cells/µl and 100 µl was injected. Growth of the tumors was measured every 3 days with calipers. For HFD studies, mice were placed on a 60 kcal % fat diet (Research Diets), two weeks prior to cancer cell injections. Mice were weighed every 3-6 days. For chronic JZL184 treatment studies, mice were treated with JZL184 or vehicle once daily (at approximately the same time everyday) by oral gavage in polyethylene glycol 300 (4 µL/g). The treatments were initiated immediately after ectopic injection of cancer cells.

**Lipid Measurements in Cancer Cells**

Lipid measurements were conducted in cancer cells grown in serum-free media for 4 hrs to minimize the contribution of serum-derived lipids to the cellular profiles. Cancer cells \((1 \times 10^6\) cells/6 cm dish, 80 % confluency) were washed with twice with phosphate buffer saline (PBS), isolated by centrifugation at \(1,400 \times g\), and dounce-homogenized in 4 ml of a 2:1:1 mixture of chloroform:methanol:Tris buffer. Samples were homogenized in the presence of the following synthetic standards: C12:0 MAGE (10 nmol), C15:0 FFA (10 nmol). Organic and aqueous layers were separated by centrifugation at \(2000 \times g\) for 5 min and the organic layer was collected. The aqueous layer was acidified (for metabolites such as LPA) by adding 5 % formic acid, followed by
the addition of 2 ml chloroform. The mixture was vortexed, and the organic layers were combined, dried down under \( \text{N}_2 \) and dissolved in 100 µl chloroform, of which 30 µl was analyzed by LC-MS.

LC-MS analysis was performed by using an Agilent 1100 LC-MSD SL instrument. LC separation was achieved with a Gemini reverse-phase C18 column (50 mm x 4.6 mm with 5 µm diameter particles) from Phenomenex together with a precolumn (C18, 3.5 mm, 2 mm x 20 mm). Mobile phase A was composed of a 95:5 ratio of water:methanol, and mobile phase B consisted of 2-propanol, methanol, and water in a 60:35:5 ratio. Solvent modifiers such as 0.1% formic acid and 0.1% ammonium hydroxide were used to assist ion formation as well as to improve the LC resolution in both positive and negative ionization modes, respectively. The flow rate for each run started at 0.1 ml/min for 5 min, to alleviate backpressure associated with injecting chloroform. The gradient started at 0% B and increased linearly to 100% B over the course of 45 min with a flow rate of 0.4 ml/min, followed by an isocratic gradient of 100% B for 17 min at 0.5 ml/min before equilibrating for 8 min at 0% B with a flow rate of 0.5 ml/min. MS analysis was performed with an electrospray ionization (ESI) source. The capillary voltage was set to 3.0 kV, and the fragmentor voltage was set to 100 V. The drying gas temperature was 350\(^\circ\)C, the drying gas flow rate was 10 L/min, and the nebulizer pressure was 35 psi.

Lipidomic analysis was performed in both targeted and untargeted mode by LC-MS analysis. Standard lipid classes were quantitated by targeting using selected ion monitoring (SIM) (except for very high-abundance metabolites which were quantitated by untargeted analysis) and identities of metabolites were confirmed by coelution of metabolites with their respective standards and mass accuracy to within 10 ppm as determined with an accurate mass Agilent 6520 Accurate Mass QTOF-MS-MS. The standard lipid metabolites that were quantitated are listed in Table S4. Lipidomic analysis was performed in both targeted and untargeted mode by LC-MS analysis.
Standard lipid classes were quantitated by targeting using selected ion monitoring (SIM) (except for very high-abundance metabolites which were quantitated by untargeted analysis) and identities of metabolites were confirmed by coelution of metabolites with their respective standards and mass accuracy to within 10 ppm as determined with an accurate mass Agilent 6520 Accurate Mass QTOF-MS-MS. The standard lipid metabolites that were quantitated are listed in Table S4. These lipids were quantified by measuring the area under the peak and were normalized to an internal standard (C12:0 MAGE in positive mode or C15:0 FFA in negative mode). Absolute quantitation of each lipid species in pmoles/1x10\(^6\) cells was based on generation of a standard curve of a representative lipid from each class analyzed against 10 nmoles of C12:0 MAGE and/or C15:0 FFA, where the relative extraction efficiencies of lipids and standards were also taken into account. MAGs were also quantified based on a C15:0 MAG (instead of C12:0 MAGE) internal standard and identical results were obtained. There was less than 5 % degradation of MAG to fatty acid acyl anion species at the MS interface. Since we quantified MAG levels against an unnatural C15:0 MAG species (which also had the same degradation pattern as endogenous MAG species), the minimal levels of MAG degradation at the interface did not interfere with absolute MAG quantitation. The dynamic linear ranges for MAG and FFA quantitation were 0.6 –10,000 pmoles and 1 – 10,000 pmoles, respectively. Untargeted data were collected on an Agilent 1100 LC-MSD SL instrument using a mass range of 200–1200 Da and were exported as common data format (.CDF) files for computational analysis. Differentially expressed metabolites between sample pairs were identified by using the XCMS analyte profiling software (http://metlin/download), which aligns and quantifies the relative signal intensities of mass peaks from multiple LC-MS traces (Smith et al., 2006). Significant inhibitor, shMAGL or MAGL-OE-sensitive peak changes were confirmed by manual quantification
by using the area under the peak normalized to the internal standards (C12 MAGE for positive mode and C15:0 FFA for negative mode).

**Supplemental References**


Figure S2

A. monoacylglycerols

B. fatty acids

C. C8161, SKOV3, 231MFP

D. monoacylglycerols

E. fatty acids

F. migration

G. invasion

H. cell survival

I. C8161, SKOV3 migration
Figure S5

A) C8161 and SKOV3 cell lines were treated with control or MAgL shRNA. The levels of C20:4 FFA and C16:0 FFA were measured using mass spectrometry.

B) Migration assays were performed using control or MAgL shRNA treated C8161 and SKOV3 cells. The number of migrated cells was counted.

C) CB1 expression levels were measured using qPCR in C8161 and SKOV3 cells treated with control or MAgL shRNA.

D) CB2 expression levels were measured using qPCR in C8161 and SKOV3 cells treated with control or MAgL shRNA.

E) NAD⁺ levels were measured using the NAD⁺/NADH assay kit in C8161 and SKOV3 cells treated with control or MAgL shRNA.

F) NADH levels were measured using the NAD⁺/NADH assay kit in C8161 and SKOV3 cells treated with control or MAgL shRNA.

G) Pyruvate levels were measured using the pyruvate assay kit in C8161 and SKOV3 cells treated with control or MAgL shRNA.

H) Lactate levels were measured using the lactate assay kit in C8161 and SKOV3 cells treated with control or MAgL shRNA.

I) Migration assays were performed using control or MAgL shRNA treated C8161 and SKOV3 cells. The number of migrated cells was counted.

J) Fatty acid levels were measured using mass spectrometry in C8161 and SKOV3 cells treated with control or MAgL shRNA.