**Big Question**

Compare **DGAT1 vs. DGAT2** in **cancer (especially glioblastoma, GBM)** and how they influence the **immune response**.

**Approach**

1. **Manipulate DGAT1/2**
   * Use **KD/KO (knockdown/knockout)** with sgRNA (CRISPR) or shRNA.
   * Specifically target **DGAT1** and **DGAT2** separately.
2. **Cell Lines (GBM & others)**
   * Mouse GBM lines: **SB-28, CT-2A**.
   * Human GBM lines: **U305, G261, OV** (possibly ovarian, but here looks like GBM + others).
   * Also general cancer models.
3. **Readouts**
   * **Lipid storage**: LDs, triglycerides.
   * **Mitochondrial stress**: ROS, apoptosis.
   * **Tumor growth in vivo**: inject modified cells into **C57BL/6 mice**.
4. **Tumor vs. Immune**
   * Observe tumor growth (tumor shrinkage if DGAT1/2 are lost).
   * Track immune response: macrophages, microglia, T cells.
5. **Special Focus**
   * **Macrophages & microglia**: Does DGAT1/2 loss change their activation (M1 vs M2)?
   * **Cancer cells vs. immune cells**: Is DGAT1 more important for tumor survival? Is DGAT2 more relevant for immune cells?

**Expected Hypothesis Flow (based on notes + prior papers)**

* **DGAT1 loss in GBM cells** → fewer lipid droplets → more ROS → apoptosis → **smaller tumors**.
* **DGAT2 loss** → less clear (maybe compensates in some tissues; role in immune regulation less studied).
* **Immune cells (macrophages, microglia)**:
  + DGAT1 inhibition may lower inflammatory cytokines OR shift towards more Tregs (immunosuppression context-dependent).
* **In mice**: Compare tumor burden vs immune infiltration when DGAT1 or DGAT2 is knocked down.

**🗂️ How You Could Organize This Into a Workflow Diagram**

**Step 1: Gene Manipulation**

* sgRNA/shRNA → DGAT1↓ or DGAT2↓

**Step 2: Model Systems**

* In vitro: mouse/human GBM cell lines (SB-28, CT-2A, G261, U305, OV).
* In vivo: implant into C57BL/6 mice.

**Step 3: Measurements**

* Tumor growth.
* Lipid metabolism (LD formation, TGs).
* Stress & apoptosis (ROS, cytochrome c, caspases).
* Immune profiling (macrophages, microglia, T cells).

**Step 4: Compare DGAT1 vs DGAT2**

* Which one drives tumor growth more?
* Which one shapes immune cell behavior more?
* Are effects synergistic or distinct?

✅ This structure basically links **cancer metabolism (DGAT1/2) → tumor biology → immune response**.

**🧰 Master List: What You Might Need for DGAT1/2 Project**

**1.**

**Genetic Tools**

* **Lentiviral shRNA vectors**
  + pLKO.1-puro backbone (Sigma MISSION)
  + shDGAT1-1 / shDGAT1-2 (sequence in Supplement)
  + shDGAT2-1 / shDGAT2-2 (sequence in Supplement)
  + shNTC (non-target control)
* **Packaging plasmids**
  + psPAX2 (2nd gen packaging)
  + pMD2.G (VSV-G envelope)
* **Transfection reagents**
  + Polyethylenimine (PEI) or Lipofectamine 2000/3000
* **Concentration kit**
  + Lenti-X Concentrator (Takara)
* **Infection reagent**
  + Polybrene (8 µg/mL)

**2.**

**Pharmacological Compounds**

* **DGAT1 inhibitor (cell use):** A-922500 (20 µg/mL in vitro)
* **DGAT2 inhibitor:** PF-06424439 (20 µg/mL in vitro)
* **DGAT1 inhibitor (in vivo):** A-900225 (120 mg/kg/day PO; check vendor/availability)
* **Rescue controls**
  + N-acetyl-cysteine (NAC, 1 mM)
  + Etomoxir (CPT1 inhibitor, 6 µM)
  + Acylcarnitines (20 µM, mimic FA overload)

**3.**

**Western Blot Antibodies**

* **DGAT1** — Santa Cruz sc-32861
* **CPT1A** — Abcam ab128568
* **CPT1B** — Abcam ab104662
* **Cytochrome c** — BD 556433
* **Cleaved Caspase-3 (Asp175)** — CST 9661
* **Cleaved Caspase-9 (Asp330)** — CST 9501
* **PARP (46D11)** — CST 9532
* **BiP/GRP78 (C50B12)** — CST 3177
* **CHOP (L63F7)** — CST 2895
* **COX IV (3E11)** — CST 4850
* **PDIA1 (H-17)** — Santa Cruz sc-30932
* **β-Actin (loading control)** — Sigma A1978
* **TIP47 / Perilipin-3 (LD marker)** — Abcam ab47638

**Secondary antibodies**

* Anti-mouse HRP — CST 7076
* Anti-rabbit HRP — CST 7074
* Alexa Fluor secondaries (488, 568) — Thermo A-11034, A-11036, A-11004

**4.**

**qPCR Setup**

* **Housekeeping gene:** 36B4 (RPLP0)
* **Target primers** (from Table S1 of paper — order exact sequences):
  + DGAT1
  + DGAT2
  + CPT1A
  + CHOP
  + BiP
  + (plus any cytokines you want to test: IL-6, TNFα, IFNβ, etc.)
* **Reagents**
  + TRIzol (RNA extraction)
  + iScript cDNA synthesis kit
  + SYBR Green PCR Master Mix (ABI)
* **Instrument:** ABI 7900HT

**5.**

**Dyes, Stains, Kits**

* **BODIPY493/503** — lipid droplet staining (IF, microscopy)
* **MitoTracker Red** — mitochondrial morphology
* **CellROX Deep Red** — ROS detection
* **ECL reagent** — for WB detection
* **Seahorse XF reagents** — Oligomycin, FCCP, Rotenone/Antimycin A (for OCR assays)

**6.**

**Cell Models**

* **Human GBM lines**: U251, U87/EGFRvIII
* **Patient-derived GBM**: GBM30, GBM30-luc (for intracranial BLI)
* **Other lines mentioned in PI’s notes**: SB-28, CT-2A, G261, M005 (mouse glioma lines)

**7.**

**Animal Models**

* **C57BL/6 mice** — for syngeneic models (SB-28, CT-2A)
* **Nude or NOD-SCID mice** — for human xenografts (U87, GBM30)
* **Intracranial implantation** setup — stereotactic injector + luciferase imaging system (if using luc-tagged cells)

**The Story of DGAT1 in Immunology**

Think of **DGAT1** as a **storage manager** in immune cells. Its main job is to **pack fatty acids into triglycerides and store them safely in lipid droplets (LDs)** — like putting extra food into a fridge so it doesn’t spoil.

This storage affects how immune cells behave in inflammation, infection, and disease.

**🦠 1.**

**Macrophages: The Big Eaters**

* Normally, macrophages don’t store many fats.
* But when they sense **danger (LPS, IFN-γ)**, they **build lipid droplets** to handle stress.
* **DGAT1 is needed** for these triglyceride stores.
* If you block DGAT1:
  + Less TG and LDs form.
  + Less **pro-inflammatory cytokine** production (weaker “alarm”).
  + Also less **PGE2**, a lipid messenger.
* In a mouse sepsis model, blocking DGAT1 **reduced inflammation**.

👉 **Takeaway**: DGAT1 helps macrophages build up energy and signals for a strong inflammatory response. Blocking it calms inflammation.

**🌬️ 2.**

**Innate Lymphoid Cells (ILCs): The Barrier Guards**

* ILC2s, found in skin, lung, and gut, need fatty acids to survive stress.
* During infection or allergen exposure (e.g., fungus *Alternaria*), ILC2s **store fats in lipid droplets**.
* This storage depends on DGAT1.
* If DGAT1 is blocked, ILC2s can’t store fats → they die from **lipotoxicity**.

👉 **Takeaway**: DGAT1 protects ILC2s in inflamed tissues; without it, they are more vulnerable.

**🧑‍🤝‍🧑 3.**

**T Cells: The Decision-Makers**

* T cells need fatty acids for different roles:
  + Effector T cells (Th1, Th2, Th17) = **fat builders**.
  + Memory T cells & Tregs = **fat burners**.
* DGAT1 influences **retinoid metabolism**:
  + Normally DGAT1 stores vitamin A as **retinyl esters**.
  + Without DGAT1, more **retinoic acid (RA)** forms.
* RA tilts the balance toward **more Tregs** (regulatory T cells).
* In a mouse MS model (EAE):
  + DGAT1 KO or inhibitor treatment = fewer symptoms, more Tregs.
* But results are mixed:
  + Some studies show blocking DGAT1 increases Tregs.
  + Others show it decreases FoxP3+ Treg numbers.
  + Th17 response (IL-17 secretion) is also variably affected.

👉 **Takeaway**: DGAT1 affects how T cells differentiate by controlling retinoid levels, which shifts the immune balance.

**🧪 4.**

**Neutrophils: The First Responders**

* Neutrophils express the **highest DGAT1 levels** among immune cells.
* They normally store triglycerides and form lipid droplets when activated.
* In psoriasis models:
  + DGAT1 deficiency did **not stop lipid droplet accumulation**, but…
  + It reduced **neutrophil migration** to inflamed skin.
* Why? Because without DGAT1, more RA builds up → RA acts like a **signal jammer** and **slows neutrophil chemotaxis**.

👉 **Takeaway**: DGAT1 doesn’t just store fat — it controls RA levels, which decides how actively neutrophils move to inflammation sites.

**🎯 Overall Message**

DGAT1 is more than a fat-storage enzyme — it’s an **immunomodulator**.

* In **macrophages**, it fuels pro-inflammatory cytokines.
* In **ILC2s**, it protects against lipotoxicity.
* In **T cells**, it tilts the balance toward Tregs or effector cells, depending on RA levels.
* In **neutrophils**, it regulates migration to inflamed tissues.

👉 Whether DGAT1’s role is **good or bad** depends on the context:

* Blocking it can **reduce harmful inflammation** (sepsis, autoimmunity).
* But it may also **weaken protective responses** (tumor killing, pathogen clearance).

**1.**

**DGAT1 is up in cancers**

* Many tumors (glioblastoma, ovarian, breast, gastric, pancreatic, etc.) show **higher DGAT1 expression**.
* More DGAT1 = more triglycerides and lipid droplets (LDs).
* Patients with **high DGAT1** generally have a **worse prognosis** (poorer survival).

👉 Analogy: DGAT1 acts like a “storage manager” that lets tumors safely stash away fat. The more storage space they have, the more aggressively they grow.

**2.**

**Why tumors need DGAT1**

* Cancer cells take in and make lots of fatty acids (fuel + building blocks).
* Without DGAT1, these free fatty acids would overwhelm cells → toxic.
* With DGAT1, tumors **pack them into droplets**, avoiding lipotoxicity and oxidative stress.
* This **protects tumor mitochondria**, keeping the cancer alive and resistant to stress/therapy.

**3.**

**DGAT1 and immune suppression**

Here’s where it gets interesting — lipid droplets aren’t just helping tumor cells, they’re also **messing with immune cells**:

* **Tumor microenvironment = fatty, stressful**
  + Excess fatty acids spill over into immune cells infiltrating the tumor.
  + This overload **impairs immune attack** (CD8+ T cells, NK cells).
  + At the same time, it **boosts suppressor cells** (Tregs, TAMs, MDSCs).
* **Specific immune cells affected**:
  + **TAMs (tumor-associated macrophages, CD206+)**:
    - Load up on LDs.
    - Become more suppressive (anti-inflammatory M2-like).
    - Promote tumor growth & metastasis.
  + **Dendritic cells**:
    - Accumulated lipids block peptide–MHC class I presentation.
    - Means **CD8+ T cells don’t get activated** → weak anti-tumor immunity.
  + **MDSCs (myeloid-derived suppressor cells)**:
    - High DGAT1 expression → more lipid droplets → stronger suppression of T cell activity.

👉 Analogy: Tumor lipids are like “sugar-coated bullets” — they disarm the soldiers (CD8+ T cells, NK cells) and instead feed the “peacekeepers” (TAMs, Tregs, MDSCs) who calm the fight.

**4.**

**What happens if you block DGAT1 (A922500 inhibitor)?**

* In tumor cells:
  + LD formation blocked → fatty acids pile into mitochondria → ROS burst → apoptosis → smaller tumors.
* In immune cells:
  + **TAMs**: fewer LDs → less mitochondrial respiration → less suppressive → can’t protect tumor.
  + **CD8+ T cells**: infiltration ↑ and activity ↑ (better killing).
  + **MDSCs**: neutral lipid load ↓ → weaker immunosuppressive activity.

👉 Blocking DGAT1 both **kills cancer cells** *and* **frees immune cells** from suppression.

**5.**

**Big picture**

* DGAT1 is a **double enabler**:
  + Helps tumor cells survive fatty environments.
  + Turns infiltrating immune cells into **suppressors instead of fighters**.
* Inhibition of DGAT1:
  + **Weakens tumors directly** (lipotoxicity).
  + **Strengthens immune response indirectly** (fewer suppressive TAMs/MDSCs, more active CD8+ T cells).

👉 That’s why DGAT1 inhibitors are being considered not just as “metabolic drugs” but also as **immunotherapy helpers**.

Oleszycka, E., Kwiecień, K., Grygier, B., Cichy, J., & Kwiecińska, P. (2025). The many faces of DGAT1. *Life Sciences*, *362*, 123322. <https://doi.org/10.1016/j.lfs.2024.123322>

# DATA BASE

## Phase 1: Foundational Expression Analysis

To find out where and when your gene is expressed, you'll need to query large-scale transcriptomic datasets.

#### Key Databases and Portals:

* **Gene Expression Omnibus (GEO)**: A massive, publicly accessible repository from the NCBI that archives high-throughput gene expression and other functional genomics data submitted by researchers worldwide. It's a great place to find datasets for specific diseases or cell types.
* **The Cancer Genome Atlas (TCGA)**: An essential resource for cancer research. It contains genomic, epigenomic, and transcriptomic data, along with clinical information, for over 30 types of cancer. You can access this data through:
  + **cBioPortal**: An easy-to-use portal for exploring TCGA and other cancer genomics datasets.
  + **UCSC Xena**: A powerful browser for visualizing TCGA and other public genomics data.
* **Genotype-Tissue Expression (GTEx)**: A fantastic resource for understanding gene expression across a wide variety of healthy human tissues. This helps establish a baseline for your gene's normal function.
* **Human Cell Atlas (HCA) & Single Cell Expression Atlas**: These are the go-to resources for single-cell RNA sequencing data. They allow you to investigate your gene's expression with incredible resolution, pinpointing its activity in very specific immune cell subtypes (e.g., Th17 cells vs. regulatory T cells).
* **Database of Immune Cell Expression (DICE)**: A specific database that profiles gene expression in 15 sorted immune cell types, making it highly relevant for immunological questions.

## Phase 2: Functional and Pathway Enrichment

These databases provide the functional annotations needed to interpret your gene lists from DGE or co-expression analyses.

#### Key Databases and Portals:

* **Gene Ontology (GO) Consortium**: The definitive source for Gene Ontology terms. Analysis tools like DAVID, Metascape, or Enrichr use GO annotations to determine what biological processes or functions are overrepresented in your gene list.
* **KEGG (Kyoto Encyclopedia of Genes and Genomes)**: A widely used, manually curated database of biological pathways. It's excellent for seeing if your gene network is involved in canonical signaling pathways like MAPK or Toll-like receptor signaling.
* **Reactome**: An open-source, peer-reviewed pathway database. It provides detailed, map-like visualizations of molecular pathways, offering a very granular look at biological processes.
* **MSigDB (Molecular Signatures Database)**: A comprehensive collection of annotated gene sets. It's the backend for the popular GSEA (Gene Set Enrichment Analysis) tool and is invaluable for discovering the functional themes in your data.

## Phase 3: Network and Regulatory Analysis

To build interaction networks and understand how your gene is regulated, you'll rely on databases that curate interaction and transcription factor data.

#### Key Databases and Portals:

* **STRING**: A powerful and user-friendly database for protein-protein interactions. It integrates information from experimental data, computational predictions, and public text mining, providing a comprehensive view of your protein's interaction network.
* **BioGRID (Biological General Repository for Interaction Datasets)**: A large database that curates protein and genetic interactions from published literature. It's a great source for high-quality, experimentally validated interactions.
* **ENCODE (Encyclopedia of DNA Elements)**: A massive project that has mapped functional elements in the human genome. It's the best place to find experimental data on where transcription factors bind (from ChIP-seq experiments) and which regions of chromatin are "open" or active (from ATAC-seq). This helps predict which factors regulate your gene.
* **JASPAR**: An open-access database containing curated, non-redundant transcription factor binding profiles (motifs). You can use it to scan the promoter of your gene to predict which transcription factors might bind there.

## Phase 4: Clinical and Multi-Omics Integration

To link your gene to clinical outcomes and other layers of biological data, you'll often return to the large cancer portals or specialized databases.

#### Key Databases and Portals:

* **cBioPortal & UCSC Xena**: As mentioned before, these are ideal for survival analysis. They seamlessly integrate gene expression data from TCGA with clinical data, allowing you to quickly generate Kaplan-Meier survival plots.
* **COSMIC (Catalogue of Somatic Mutations in Cancer)**: The world's largest and most comprehensive resource for exploring the impact of somatic mutations in human cancer. You can check if your gene is frequently mutated in certain cancer types.
* **ClinVar**: A database from the NCBI that aggregates information about genomic variations and their relationships to human health. It's useful for seeing if variants in your gene are associated with any known diseases.
* **Roadmap Epigenomics Project**: Similar to ENCODE, this project provides a public resource of human epigenomic data, which can be used to understand the epigenetic regulation of your gene in various cell types.

# DATA BASE

# A. Bulk tumors (TCGA-GBM/CGGA): “Is DGAT1/2 linked to immune suppression & poor outcome?”

**Datasets**: TCGA-GBM (and LGG for breadth), CGGA.  
**Core variables**: DGAT1, DGAT2 expression; survival; IDH status; MGMT methylation.

**Analyses**

1. **Survival & clinicopath**
   * Split tumors into **DGAT1-high vs low** (quartiles).
   * Kaplan–Meier & multivariable Cox (adjust IDH, age, MGMT).
   * Expectation: DGAT1-high → **worse prognosis** (multiple cancers reported). [BioMed Central](https://translational-medicine.biomedcentral.com/articles/10.1186/s12967-024-05084-z?utm_source=chatgpt.com)
2. **Immune infiltration deconvolution**
   * Run **CIBERSORTx/xCell/TIMER2** on bulk RNA-seq.
   * Test if DGAT1-high tumors show **↑TAMs (M2/CD206⁺)**, **↑MDSCs**, **↓CD8 T cells**.
   * Rationale: LD-loaded **TAMs** are immunosuppressive and abundant in cancers; **DGAT1i reduces CD206⁺ TAMs and raises CD8 T cells** in vivo. [PMC](https://pmc.ncbi.nlm.nih.gov/articles/PMC6835560/?utm_source=chatgpt.com)
3. **Pathway & signature scores**
   * ssGSEA/GSVA for gene sets: **lipid droplet program** (PLIN2/PLIN3/TIP47), **FAO** (CPT1A set), **ER stress** (BiP/CHOP), **ROS**, **T-cell dysfunction/exclusion** (TIDE or published sets).
   * Hypotheses from GBM paper: DGAT1-high ↔ **LD↑, FAO↑ (CPT1A↑), ROS buffering, ER stress signaling**. [PMC](https://pmc.ncbi.nlm.nih.gov/articles/PMC7415721/?utm_source=chatgpt.com)
   * TAM suppression mechanisms: **TAM M2** signatures ↑ with DGAT1-high. [PMC](https://pmc.ncbi.nlm.nih.gov/articles/PMC6835560/?utm_source=chatgpt.com)
4. **Cytokine/chemokine environment**
   * Correlate DGAT1 with **IL10, TGFB1, CCL2, CSF1, MRC1 (CD206), ARG1**, **S100A8/A9** (MDSC).
   * Look for inverse correlation with **CD8A, GZMB, IFNG**.
   * Mechanistic support: LD-rich myeloid cells suppress CD8 activity and presentation. [Nature+1](https://www.nature.com/articles/s41467-017-02186-9?utm_source=chatgpt.com)
5. **Contrast with DGAT2**
   * Repeat 1–4 for **DGAT2**; add **SREBP-1 lipogenesis** signatures.
   * Expect distinct biology: **DGAT2 inhibition lowers SREBP-1 activity via ER-PE**, so DGAT2-high may track **lipogenesis** rather than LD-buffering per se. [Cell](https://www.cell.com/cell-metabolism/fulltext/S1550-4131%2824%2900011-1?utm_source=chatgpt.com)

**Deliverables**: forest plots (Cox), volcano of correlations, heatmaps of signature scores across DGAT1 quartiles, immune-cell proportion boxplots.

# B. Single-cell RNA-seq (GBM): “Which cells use DGAT1, and with what programs?”

**Datasets**: public GBM scRNA-seq atlases (e.g., Neftel et al. 2019), plus any in-house.  
**Focus cells**: malignant, **myeloid (TAMs/microglia)**, T/NK, DC.

**Analyses**

1. **Cell-type localization**
   * Map **DGAT1** and **LD genes (PLIN2/3)** across clusters.
   * Expect DGAT1 in tumor cells and **myeloid** subsets that are suppressive/TAM-like. [PMC](https://pmc.ncbi.nlm.nih.gov/articles/PMC6835560/?utm_source=chatgpt.com)
2. **Myeloid state scoring**
   * Score **M2/TAM (CD206/MRC1, MSR1, APOE)** vs M1 programs; compute **OXPHOS/FAO** scores.
   * Test if **DGAT1-high myeloid cells** have **LD↑ and suppressive programs**. (LDs drive TAM suppression and respiration.) [PMC](https://pmc.ncbi.nlm.nih.gov/articles/PMC6835560/?utm_source=chatgpt.com)
3. **Antigen presentation in DC-like cells**
   * In DC/mono-DC clusters, correlate DGAT1/LD program with **cross-presentation markers** (**TAP1/2, B2M, HLA-A/B/C**) and with **defect signatures** described for **oxidized-lipid lipid bodies** impairing MHC-I trafficking. [Nature+1](https://www.nature.com/articles/s41467-017-02186-9?utm_source=chatgpt.com)
4. **T-cell states**
   * Score **exhaustion/dysfunction** (PDCD1, LAG3, TOX), **cytotoxicity** (GZMB/PRF1), and **Treg** (FOXP3/IL2RA).
   * If spatial data are available, ask whether DGAT1-high TAM niches co-localize with **exhausted CD8s** (TIDE-like phenotype).

**Deliverables**: UMAPs with DGAT1 & LD score overlays; violin plots of state scores per cluster; cell–cell correlation matrix.

# C. Ligand–receptor crosstalk: “How might DGAT1-high cells reprogram others?”

**Approach**: **CellPhoneDB / NicheNet / LIANA** on scRNA-seq.

* Identify **myeloid→T-cell** and **tumor→myeloid** ligand–receptor pairs enriched in **DGAT1-high** neighborhoods (e.g., **CSF1–CSF1R**, **CCL2–CCR2**, **TGFB1–TGFBR**, **PD-L1–PD-1**).
* Tie to literature that lipid-rich myeloid cells **impair cross-presentation** and bolster suppressive circuits. [Nature](https://www.nature.com/articles/s41467-017-02186-9?utm_source=chatgpt.com)

**Deliverables**: chord plots of top L–R pairs; ranked list of interventions (CSF1R, CCR2, PD-1 combos) you could pair with **DGAT1i** later.

# D. Retinoid/Treg axis (DGAT1-specific): “Is RA-Treg signaling visible?”

DGAT1 controls retinol esterification; loss increases **retinoic acid (RA)** and favors **Treg differentiation** (context-dependent). [PNAS](https://www.pnas.org/doi/10.1073/pnas.1817669116?utm_source=chatgpt.com)

**Analyses**

* Build an **RA signaling score** (ALDH1A1/2, RAR target genes) per cell/tumor.
* Test **DGAT1 vs RA score** correlations in **T cells** and **myeloid** subsets.
* At bulk level, associate **DGAT1** with **Treg signatures** vs **CD8 activity** to anticipate immunomodulation risks/benefits.

# E. “Mechanism-first” meta-tests you can pre-register

Directly echo the mechanistic papers so reviewers see the logic:

1. **DGAT1-high tumors have more LD program, FAO/CPT1A, and immunosuppressive myeloid signatures; fewer effective CD8 signatures.**  
   Justification: GBM DGAT1 biology + LD-TAM literature. [PMC+1](https://pmc.ncbi.nlm.nih.gov/articles/PMC7415721/?utm_source=chatgpt.com)
2. **DGAT1-high myeloid cells show M2/TAM markers and higher OXPHOS, while DC-like cells with high LD program show impaired cross-presentation signatures.**  
   Justification: TAM LDs drive suppression; oxidized-lipid bodies block DC cross-presentation. [PMC+1](https://pmc.ncbi.nlm.nih.gov/articles/PMC6835560/?utm_source=chatgpt.com)
3. **DGAT2 tracks lipogenesis/SREBP-1 modules more than immune suppression modules.**  
   Justification: DGAT2→ER-PE→blocks SREBP-1 cleavage (opposite direction mechanistically). [Cell](https://www.cell.com/cell-metabolism/fulltext/S1550-4131%2824%2900011-1?utm_source=chatgpt.com)

# F. Tools & minimal pipelines

* **Bulk**: TCGAbiolinks → DESeq2/edgeR → GSVA/ssGSEA → CIBERSORTx/xCell/TIMER2 → survival (survival/survminer).
* **scRNA-seq**: Seurat/Scanpy → DoubletFinder/Scrublet → Azimuth for cell types → module scoring (AddModuleScore/Scanpy.tl.score\_genes) → NicheNet/CellPhoneDB for L–R.
* **Spatial (if available)**: Visium/SpatialDE to see **DGAT1-high TAM neighborhoods** vs **CD8 exclusion**.

# G. “Short list” of figures for your pre-bench slide deck

1. **DGAT1 in GBM bulk**: KM survival + TAM/CD8 proportions vs DGAT1 quartiles.
2. **DGAT1 in scRNA-seq**: UMAP showing DGAT1 in tumor + myeloid; violin of LD/OXPHOS/TAM scores.
3. **DC cross-presentation**: negative correlation of LD score with pMHC-I genes in DC-like cells. [Nature](https://www.nature.com/articles/s41467-017-02186-9?utm_source=chatgpt.com)
4. **L–R map**: tumor/myeloid → T-cell suppressive circuits enriched in DGAT1-high contexts.
5. **DGAT2 foil**: ridge plot showing SREBP-1 module tracks DGAT2, not DGAT1. [Cell](https://www.cell.com/cell-metabolism/fulltext/S1550-4131%2824%2900011-1?utm_source=chatgpt.com)

## Why this is credible to reviewers/PI

* **Anchor**: GBM DGAT1 paper shows LD buffering → FAO/ROS/apoptosis; that’s the tumor-intrinsic half. [PMC](https://pmc.ncbi.nlm.nih.gov/articles/PMC7415721/?utm_source=chatgpt.com)
* **Immune half**: Independent papers show **LDs drive TAM suppression**, **lipids block DC cross-presentation**, **DGAT1 affects Treg/RA** and **ILC2** lipid programs—so bulk/scRNA patterns you test are **mechanistically expected**. [Cell+3PMC+3Nature+3](https://pmc.ncbi.nlm.nih.gov/articles/PMC6835560/?utm_source=chatgpt.com)