Immune and Macrophage Marker Reference

Marker	Cell type / function
TREM2	Lipid-associated macrophage marker, immunosuppressive TAM phenotype
PD-L1 (CD274)	Immune checkpoint ligand, inhibits T cell activation
CD206 (MRC1)	M2-like macrophage marker, tissue repair/immunosuppressive
CD163	Scavenger receptor, M2-like immunosuppressive macrophages
CD16 (FcγRIII)	Monocyte subset marker, also on NK cells; non-classical monocytes
CD4	Helper T cells (Th1/Th2/Th17/Tregs)
CD8	Cytotoxic/killer T cells
CD25 (IL-2Rα)	High on Tregs, IL-2 receptor component
Foxp3	Transcription factor, master regulator of Tregs
CCR6	Chemokine receptor for CCL20, on T cells (esp. Tregs, Th17), DCs, B cells

Figure 1 – LLM accumulation & correlation with disease progression

Figure 1 – Panel A–B

Flow cytometry: more BODIPY+CD14+ macrophages in tumor (~45%) vs nontumor (~17%).

Figure 1 – Panel C–D

Confocal microscopy: tumors enriched in LD-laden macrophages.

Figure 1 – Panel E

CD68+ macrophages express PLIN2 → confirms identity of LLMs.

Figure 1 – Panel F

qPCR: lipid-associated macrophage genes (TREM2, APOC1, LIPA) ↑ correlate with LLM levels.

Figure 1 – Panel G–H

Flow/t-SNE: BODIPY+ macrophages show ↑ TREM2, PD-L1, CD206, CD163 (immunosuppressive).

Figure 1 – Panel I

IHC: fewer CD8+ T cells when LLM infiltration is high.

Figure 1 – Panel J–K

Clinical correlation: more LLMs → larger tumors & higher TNM stage.

Figure 1 – Panel L

Kaplan–Meier: high LLMs → shorter recurrence-free survival.

Figure 2 – Tumor-induced lipid reprogramming

Figure 2 – Panel A–C

Monocytes exposed to tumor supernatant (TSN) vs MED (control). Flow cytometry: ↑ LD formation with TSN.

Figure 2 – Panel D

Confocal microscopy: LDs visible in TSN-treated cells.

Figure 2 – Panel E–F

PLIN2 expression (qPCR, WB) ↑ with TSN → confirms LD biogenesis.

Figure 2 – Panel G

Electron microscopy: electron-dense LDs in TSN-treated monocytes.

Figure 2 – Panel H

Lipidomics heatmap: broad lipid profile reprogramming by TSN.

Figure 2 – Panel I–J

Volcano plots: ↑ triglycerides (TAGs), ↑ DAGs in TSN-treated cells.

Figure 2 – Panel K

↓ phosphatidylcholine, ↑ lysophosphatidylcholine → evidence of lipid reshuffling.

Figure 2 – Panel L

Ceramide species ↑ in TSN-treated cells → stress-driven lipid remodeling.

Figure 3 – Sources of fatty acids for LDs

Figure 3 – Panel A

Schematic: three FA sources for LDs → de novo synthesis, lipid reshuffling (PLA2), uptake from tumor lipids.

Figure 3 – Panel B

PLA2 inhibitors (AACOCF3, Darapladib) ↓ LD formation → reshuffling contributes.

Figure 3 – Panel C–E

C12-BODIPY uptake assays: TSN drives lipid uptake & LD formation, dose-dependent.

Figure 3 – Panel F

Delipidated TSN loses ability to induce LDs → external tumor lipids required.

Figure 3 – Panel G

Endocytosis inhibitors (cytochalasin D, latrunculin A) ↓ LDs → uptake via endocytosis.

Figure 3 – Panel H

Neutralizing Abs: only anti-TNFα reduces LDs → TNFα is key cytokine.

Figure 3 – Panel I–J

PLA2 inhibition still effective even with TNFα blockade → independent pathways. TNFα effect lost with delipidated TSN → TNFα promotes uptake.

Figure 3 – Panel K–M

Recombinant TNFα increases lipid uptake (C12-BODIPY) in dose-dependent manner → TNFα drives FA uptake.

Figure 4 – DGAT1/2 mediate LDs and survival

Figure 4 – Panel A

qPCR: DGAT1 & DGAT2 higher in tumor macrophages vs nontumor.

Figure 4 – Panel B–C

TSN ↑ DGAT1/2 expression (WB, qPCR).

Figure 4 – Panel D–E

Immunofluorescence: DGAT1 highly expressed on CD68+ macrophages in tumors.

Figure 4 – Panel F–G

DGAT1i or DGAT2i alone → small effect. Both together → strong reduction in LDs.

Figure 4 – Panel H–I

Annexin V/PI assay: BODIPY+ macrophages resist apoptosis; DGAT inhibition ↑ apoptosis.

Figure 4 – Panel J

Cleaved caspase-3 staining: LD+ macrophages have lower apoptosis in vivo.

Figure 5 – DGATs promote CCL20 secretion

Figure 5 – Panel A

Volcano plot (RNA-seq): TSN vs MED → many DEGs; CCL20 strongly upregulated.

Figure 5 – Panel B

Volcano plot: TSN+DGATi vs TSN → subset of genes reduced; CCL20 most affected.

Figure 5 – Panel C

Venn diagram overlap: genes induced by TSN but reduced by DGAT inhibition → LD-dependent genes. CCL20 strongest hit.

Figure 5 – Panel D

qPCR + ELISA: CCL20 ↑ with TSN, reduced by DGAT inhibition.

Figure 5 – Panel E

Immunofluorescence: CCL20 staining brighter with TSN, suppressed by DGATi.

Figure 5 – Panel F–G

Patient macrophages: tumor TAMs secrete more CCL20; reduced with DGATi.

Figure 5 – Panel H

Correlation: LLM levels positively correlate with CCL20 expression.

Figure 6 – LLM-derived CCL20 recruits CCR6+ Tregs

Figure 6 – Panel A

Flow: ↑ CCR6+ immune cells in tumor vs nontumor.

Figure 6 – Panel B

Subset analysis: in tumors, >60% of CCR6+ cells are CD4+ T cells.

Figure 6 – Panel C

CCR6+CD4+ cells express more CD25; confocal shows CCR6+ cells also Foxp3+ → Tregs.

Figure 6 – Panel D–E

Correlation: macrophage CCL20 expression positively correlates with CCR6+ cells.

Figure 6 – Panel F–G

Correlation: LLM levels correlate with CCR6+ cells and Foxp3+ Tregs in tumor tissues.

Figure 6 – Panel H

Transwell chemotaxis: TSN-treated macrophage medium recruits CCR6+ cells; effect blocked by DGAT inhibition.

Figure 6 – Panel I–M

Mouse model: DGAT1i+2i ↓ LLMs, ↓ CCR6+ cells, ↓ Foxp3+ Tregs, ↓ tumor growth.