**Figure 1: Functional single cell analyses reveal the heterogeneity of primary and recurrent glioblastoma tumor microenvironments and co-occurrence of tumor subtypes and macrophage subsets**

(**A**) Schematic diagram of experimental design: Glioblastoma were initiated in the PDGFB-Ink4a/Arf KO (PDG-Ink4a) and PDGFB-p53 KD (PDG-p53) GEMM models (as described in Methods). Mice were assigned to primary control (treatment-naïve) or fractionated ionizing radiotherapy (RT- 5 x 2 Gy) groups. Tumors of comparable volumes (determined by MRI) were collected when animals reached the humane endpoint in the primary (n = 3 PDG-Ink4a, n = 4 PDG-p53) or recurrence post-RT (n = 3 PDG-Ink4a, n = 3 PDG-p53) treatment groups and processed into single-cell suspensions. CD45+ immune cells and CD45- cells were FACS-isolated and subjected to 10X Chromium single-cell RNA sequencing (scRNA-seq). Microglia (MG gated on CD45+,CD11b+, Ly6Clow, Ly6Glow, CD49dlow) and monocyte-derived macrophages (MDMs, gated on CD45+, CD11b+, Ly6Clow, Ly6Glow, CD49d+) were FACS-purified from independent cohorts of non-tumor bearing animals (only MG, n = 1 *Ntv-a*), primary (n = 1 PDG-Ink4a, n= 1 PDG-p53) or recurrent (n = 1 PDG-Ink4a, n= 1 PDG-p53) and subjected to 10X Chromium scRNA-seq to complement the pool of CD45+ cells. Independent cohort of PDG-Ink4a animals were sacrificed, and snap-frozen sections were processed for Visium 10X spatial transcriptomics (n = 4 PDG-Ink4a primary; n = 3 PDG-Ink4a recurrent, n = 3 PDG-Ink4a primary; n = 3 PDG-Ink4a recurrent). Published patient datasets, including Ivy GAP glioblastoma niche signatures13 and glioblastoma patient scRNA-seq130, were included in downstream analyses to assess the translational value of the mouse sequencing results.

(**B**) Uniform Manifold Approximation and Projection (UMAP) representation of CD45+ immune cell and CD45- glioblastoma cell populations from combined and integrated scRNA-seq mouse datasets (n = 18 containing 96,135 cells). Individual cells are colored according to the identified cell types (*DCs:* Dendritic Cells, *ECs:* Endothelial Cells).

(**C**) Visual representation of CD45- glioblastoma cancer cell (27,296 cells) subtype heterogeneity subjected to scRNA-seq (tumor cells from PDG-Ink4a primary samples are represented). The module scores (*NPC:* Neural-Progenitor like Cell, *OPC:* Oligodendrocyte-Progenitor like Cell, *MES:* Mesenchymal like Cell, *AC:* Astrocyte like Cell)16 of each individual cell are depicted on the axes. Individual cells are colored according to the assigned glioblastoma cellular subtype.

(**D**) Pie charts of the average fraction of the glioblastoma cell subtype (outer circle) identified in primary (P) and recurrent (R) PDG-Ink4a and PDG-p53 tumors representing the tumor subtypes depicted in (C), shown as a percentage of total glioblastoma cancer cells. Pseudo-location niche assignment of glioblastoma cancer cells (inner circle) is displayed as a percentage of cellular subtype across all samples subjected to scRNA-seq, based on the Ivy GAP dataset (*CT:* Cellular Tumor, *LE:* Leading Edge, *MVP:* Microvascular Proliferation, *PAN:* Pseudo-palisading cells Around Necrosis).13

(**E**) UMAP representation of MG and MDM clusters (52,688 cells) identified in the CD45+ population represented in (B). Individual cells are colored according to subsets identified by unsupervised clustering.

(**F**) Heatmap of Gene Set Enrichment Analysis (GSEA) scores of depicted pathways in MG and MDM clusters identified in (E). Data scaled between 0 (low activity = blue) and 1 (high activity = red) per column. Tumor-associated macrophage (TAM: MG and MDM) clusters are annotated per row, pathways associated with GSEA score are annotated per column.

(**G**) Pseudo-location niche assignment of macrophages as a percentage of total TAMs for each MG and MDM cluster. Data presentation follows each cluster depicted in (F) and is represented as mean ± S.E.M.

(**H**) Left and middle panel: dotplot depicting the differential abundance of TAM subsets between primary and recurrent PDG-Ink4a and PDG-p53 glioblastoma. Each dot represents a group of cells clustered in ‘neighborhoods’, with colors representing the logFC (grey = non-significant). Right panel: dot plots representing the correlation matrix between TAM subset and glioblastoma cellular subtype abundance (n = 13). Dot color and size correspond to the correlation coefficients. Statistics calculated with Kendall trend test (\* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001)

(**I**) Heatmap depicting the top 25 differentially expressed genes between GPNMBhigh (MG3 and MDM3) and GPNMBlow clusters (MG1-2, MG4, MDM1-2, MDM4) extracted from murine scRNA-seq dataset. The top 5 genes upregulated in the GPNMBhigh clusters were used to establish a gene module herein referred to as the GPNMBhigh gene signature (Table S5).

(**J**) Scatterplots depicting the correlation between GPNMBhigh TAMs as a percentage of total TAMs (quantification shown in Fig. S2H, I), and MES glioblastoma cells as a percentage of total tumor cells in each PDG-Ink4a and PDG-p53 primary and recurrent tumors subjected to scRNA-seq (n = 13) or as determined in glioblastoma patient sample scRNA-seq datasets (n = 22).17,130Pearson’s correlation coefficient and p-value are shown for each plot. Red line represents simple linear regression, black line bands represent 90% prediction.

**Figure 2: Spatial transcriptomic analyses of glioblastoma TME reveals co-localization between MES-like cancer cells and GPNMBhigh TAMs in hypoxic niches**

(**A**) Representative immunofluorescence (IF) staining performed on fresh-frozen brain sections from primary PDG-Ink4a tumor-bearing mice used in VISIUM 10X spatial transcriptomics. DAPI: nuclear stain (blue); IBA1: pan-macrophage (red); Pimonidazole: hypoxia (green); CD31: endothelial cells (white).

(**B**) Visualization of the classification methods used to assign the dominant niche and glioblastoma cellular subtype transcriptional activity per 10X spatial transcriptomics sequenced spot (see Methods).

(**C**) Spatial co-occurrence workflow: transcriptional modules from TAM subsets (as described in Figure S3A-B), glioblastoma cellular subtypes and microanatomical niches were assigned to each VISIUM 10X sequenced spot in order to infer correlation coefficients between each of the above components.

(**D**) Central panel: dot plot representing the correlation matrix between TAM subset transcriptional modules and either the glioblastoma cellular subtype (top) or Ivy GAP microanatomical niche transcriptional modules (bottom) across all VISIUM 10x samples (n = 13, n = 4 PDG-Ink4a primary, n = 3 PDG-Ink4a recurrent, n = 3 PDG-p53 primary, n = 3 PDG-p53 recurrent). The logFC of the occurrence of TAM subsets and tumor niches or glioblastoma cancer cell subtypes between primary and recurrent tumors are shown as a bar plot on the bottom and right side of the matrix, respectively. Dot color and size correspond to the correlation coefficient. Statistics calculated with Kendall trend test (\* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001).

(**E-G**) Representative visualization of VISIUM 10X spatial transcriptomic analyses in recurrent PDG-Ink4a glioblastoma, highlighting GPNMBhigh deserted (1) and enriched (2) areas. Spots assigned as TAM+ are classified as either GPNMBlow or GPNMBhigh and overlayed onto the corresponding IF image (E). Similarly, colors represent spots assigned to a glioblastoma cellular subtype (F) or microanatomical niche (G) as defined in (B).

**Figure 3: Lipid-laden macrophages display metabolic and immunosuppressive programs associated with loss of chromatin accessibility and promote glioblastoma relapse post radiotherapy**

(**A**) Schematic overview of lipid-laden macrophage phenotyping: MG and MDMs were FACS-isolated from single cell dissociated primary PDG-Ink4a glioblastoma based on their lipid-content (BODIPY) and granularity (SSC-A). FACS-purified LLMs (BODIPYhigh, SSC-Ahigh) from microglial (MG gated on CD45+,CD11b+, Ly6Clow, Ly6Glow, CD49dlow) and monocytic (MDMs, gated on CD45+, CD11b+, Ly6Clow, Ly6Glow, CD49d+) origin were collected (as depicted in Figure S4A) and processed for RNA-seq, ATAC-seq and immunophenotyping.

(**B**) Venn diagram of differentially expressed genes (DEGs) identified by RNA-seq in non-LLMs and LLMs from FACS-isolated MG and MDM subpopulations.

(**C**) Stacked violin plots depicting TAM subset (from Figure 1E) transcriptional enrichment for gene signatures identified in RNA-seq analyses from FACS-purified non-LLMs and LLMs from microglial and monocytic origin (Table S7). Bold line represents the average signature score of the cluster most enriched for that same signature.

(**D**)Average peak profiles (top panel) and heatmaps (bottom panel) depicting the normalized ATAC-seq signals at differentially accessible chromatin regions in LLMs and non-LLMs from MG (left) and MDM (right) subpopulations.

(**E**) Boxplots depicting the logFC of gene expression (from bulk RNA-seq dataset) associated with higher and lower accessibility in promoter regions of LLMs compared to expression of the same genes in non-LLMs from MG (left) and MDM (right) subpopulations. Statistics: Wilcoxon signed-rank test.

(**F**) Bar plots depicting the -log10(FDR) of significantly enriched gene-sets based on gene expression associated with higher (top panel) or lower (bottom panel) promoter accessibility in LLMs compared to non-LLMs from the MDM subpopulation. Vertical line (-log10(FDR)=2.3) represent the significant threshold (see Methods).

(**G**)Normalized ATAC-seq peak signals at gene regions related to gained accessibility (*Abca1*) or loss of accessibility (*Dhcr24*, *H2-Eb1* and *Cd74*) in LLMs compared to non-LLMs from MDM subpopulations.

(**H**) Histogram depicting the flow cytometry mean fluorescent intensity (MFI) of H3K27me3 in LLMs and non-LLMs from MG (left) and MDM (right) subpopulations isolated from primary PDG-Ink4a tumor-bearing mice. Statistics: 2-way ANOVA with Sídák correction for multiple comparison.

(**I**) Histogram showing the log2FC of mean fluorescent intensity (MFI) for the indicated cell surface marker expression in LLMs versus non-LLMs from MG and MDM subpopulations isolated from primary PDG-Ink4a tumor-bearing mice. Statistics shown in Figure S5K.

(**J**) Flow cytometry quantification of LLMs (BODIPYhigh, SSC-Ahigh) as a percentage of MG (left panel) and MDMs (right panel) in primary or recurrent tumors isolated from PDG-Ink4a and primary PDGFA-NF1-P53 tumor-bearing mice (see Figure S4A for gating strategy). Statistics: Two-stage step-up unpaired t-test with Benjamini, Krieger and Yekutiel correction for multiple comparison.

(**K**) Kaplan-Meier curve showing animal survival over time. Treatment groups are control (DMSO); fractionated ionizing radiation (RT, 5x2 Gy) and DMSO; SSO (30mg/kg daily treatment); or RT + SSO. DMSO and SSO treatments were initiated 48h post last dose of RT in the combination groups. Mice were euthanized when symptomatic. Statistics: log-rank test was used to determine statistical significance in survival benefit of given treatment.

(**L**) Flow cytometry quantification of LLMs (BODIPYhigh, SSC-Ahigh) as a percentage of MG and MDMs in PDG-Ink4a tumors treated with RT + DMSO and RT + SSO at the trial endpoint. Statistics: two-way ANOVA with Sídák correction for multiple comparison. (**H-J, L**) Data are represented as mean ± S.E.M.

**Figure 4. LLM formation relies on myelin phagocytosis and subsequent sterol accumulation.**

(**A**) Normalized levels (sum of peaks) of depicted lipid classes determined by liquid chromatography mass spectrometry in FACS-purified LLMs and non-LLMs from primary PDG-Ink4a tumors. Lipid categories are referenced in Table S10. Statistics: two-way ANOVA with Sídák correction for multiple comparison.

(**B**) Histogram depicting the corrected area ratio of sterols determined by gas chromatography-mass spectrometry in FACS-purified LLMs and non-LLMs isolated from primary PDG-Ink4a tumor-bearing mice. Statistics: two-way ANOVA with Sídák correction for multiple comparison. Of note, different internal standards were used for cholesterol and its less abundant precursors.

(**C**) Concentration of desmosterol in FACS-purified LLMs and non-LLMs isolated from primary PDG-Ink4a tumor-bearing mice, assessed from the lipidomic analyses depicted in (B). Statistics: Two-tailed paired t-test.

(**D**)Histogram depicting the normalized expression of cholesterol synthesis pathway genes in FACS-purified LLMs and non-LLMs from the RNA-seq dataset presented in Figure 3B and S4B. Statistics: Two-tailed paired t-test.

(**E**) Pie chart depicting the average fraction of each major lipid species present in myelin isolated from PDG-Ink4a primary tumors. Data for this graph was extracted from Nuclear Magnetic Resonance (NMR) analysis to quantify cholesterol (red), and Lipidyzer analysis which quantified other lipid classes (grey). Data shown as mean ± S.D.

(**F**) Electron microscopy representative images depicting *in vivo* engulfment of myelin debris by macrophages (Iba1+ immunogold) in primary PDG-Ink4a tumors. High-magnification images (box 1.2 and 2.2, from images 1.1 and 2.1 respectively) show phagosomes containing myelin adjacent to lipid accumulation. Bottom panels are schematic annotated illustrations corresponding to the electron microscopy images.

(**G**) IF staining was performed on fresh-frozen brain sections from primary PDG-Ink4a tumor-bearing mice and LLM distribution was assessed in relation to the presence of myelin in the TME. Representative IF image of single and merged staining of myelin-engulfing LLM. DAPI: nuclear stain (blue); IBA1: pan-macrophage (magenta); PLIN2: lipid droplets (white); MBP: myelin-basic protein (yellow). Large insert shows 3D surface rendering of an IBA1+ cell containing MBP and PLIN2+ lipid droplets, depicted on a single 2D image. Smaller inserts show single channel 2D maximum projection of z-stack.

(**H**)Raindrop plot depicting the quantification of mean MBP intensity in TAMs classified as (non-)LLMs (IBA1+ PLIN2- or PLIN2+, respectively) in primary PDG-Ink4a tumors (as presented in Figure S7B). Each dot represents individual TAMs (IBA1+) and size corresponds to cell area, as quantified by IF. Boxplot, violin plot and average of mean MBP are given for each group. Statistics: pairwise comparison for analyzing multiple population means in pairs.

(**I**) Visual IF image representation of an IBA1+PLIN2+ LLM in primary PDG-Ink4a tumor and the associated distance to myelin used to annotate individual TAMs (IBA1+) as MBP+ (minimal distance to MBP < 0 pixels), close to MBP (min. dis. to MBP 0-5 pixels) or far from MBP (min. dis. to MBP > 5 pixels).

**(J)** Raindrop plot depicting the quantification of the total number of PLIN2+ lipid droplets present in each TAM, correlated to their minimal distance to MBP+ staining (colocalization = MBP+, close to MBP or far from MBP) in primary PDG-Ink4a tumors. Dots each represent individual TAMs (IBA1+), as quantified by IF. Boxplot, violin plot and average of number of PLIN2+ lipid droplets are given for each group. Statistics: pairwise comparison for analyzing multiple population means in pairs.

(**K**) Flow cytometry quantification of SSC-Ahigh, BODIPYhigh LLMs as a percentage of total dTomato+ BMDMs exposed to: control media (10% FCS DMEM); dissociated PDG-Ink4a primary glioblastoma with or without myelin; tumor-derived myelin alone (as depicted in Figure S7F). Statistics: One-way ANOVA with Sídák correction for multiple comparison.

(**L**) Venn diagram depicting the differentially expressed genes (Table S11) identified in RNA-seq from FACS-purified dTomato+ BMDMs used in (K).

(**M**) Bar plots depicting the p-values of relevant pathways specific to Myelin/TME synergistic genes (1) and TME-regulated gene sets inhibited by myelin addition to BMDMs(2) from (K). Colors represent pathways that belong to genes which are either down- (beige) or up-regulated (red) within each signature (Table S11). Genes driving pathway enrichment are stated within each respective bar. Statistics: Fisher’s exact test in combination with the Benjamini-Hochberg method for correction of multiple hypotheses testing.

(**A-D, K**) Data are represented as mean + S.E.M.

**Figure 5. LXR pathway activation in LLMs stimulates lipid exchange within the TME.**

(**A**) Seahorse glycolytic activity assay was performed on glioblastoma cell lines generated from primary/OPC-like (blue, n = 4) or recurrent/MES-like (red, n = 4) PDG-Ink4a tumors. Graph depicts modification of extracellular acidification rate (ECAR) over time upon injection of drugs interfering with glycolytic activity, as indicated.

(**B**) Histogram depicting the relative fold-change of carboxyfluorescein succinimidyl ester (CFSE)-labeled myelin uptake (MFI) in BMDMs exposed to OPC-like or MES-like TCM compared to control media for 24h prior to labeling. Statistics: Friedman test with Dunn’s correction for multiple comparison.

(**C**) Histogram depicting the relative fold-change of BODIPY MFI in myelin-exposed BMDMs over control (no myelin). Statistics: Friedman test with Dunn’s correction for multiple comparison.

(**D**) Histogram depicting the relative fold-change of H3K27me3 MFI in BMDM monoculture exposed to control media (no myelin) or myelin for 24, 48 or 72 hours, and in BMDMs co-cultured with MES-like glioblastoma cells for 72 hours. Statistics: one-way ANOVA with Sídák correction for multiple comparison.

(**E-F**)Graph depicting therelative expression of genes associated with the LXR pathway assessed by RT-qPCR in BMDMs exposed to (E) control media or MES-like TCM, and (F) MES-like TCM ± myelin for 1 or 4 hours. Statistics: two-way ANOVA with Sídák correction for multiple comparison.

(**G**) Dot plot displaying the expression levels of the lipid export genes *Abca1*, *Abcg1*, *Apoc1* and *Apoe*, and lipoprotein receptor gene *Vldlr*, in the most abundant cell types identified in the murine scRNA-seq dataset (Figure 1B). Dot color represents the average expression; size depicts percentage of cells expressing each specific gene.

(**H**) Histogram depicting the content of free cholesterol quantified in the supernatant of BMDMs previously exposed to myelin for 24h *in vitro*. Statistics: two-tailed paired t-test.

(**I**) Experimental design illustrating the collection of tumor interstitial fluid (TIF) from glioblastoma whole tumors (see Methods).

(**J**) Relative levels (sum of peaks) of total lipids detected in the TIF of non-tumor bearing brain (Ntb), primary and recurrent PDG-Ink4a tumors. Statistics: two-way ANOVA with Sídák correction for multiple comparison.

(**K**) Fold-change in cholesteryl ester (CE) levels quantified in the TIF of primary/OPC-like and recurrent/MES-like PDG-Ink4a tumors compared to Ntb tissue (control). Statistics: two-way ANOVA with Sídák correction for multiple comparison.

(**A-F, H, J, K**) Data are represented as mean ± S.E.M**.**

**Figure 6. LLM-mediated lipid export fuels MES-like cell malignancy in the lipid-scarce glioblastoma TME**

(**A**)Radar plot depicting normalized GSEA scores (between 0 and 1 based on each pathway) of the represented metabolic pathways (KEGG) between OPC, MES, NPC and AC glioblastoma cells extracted from murine scRNA-seq datasets of primary and recurrent PDG-Ink4a driven glioblastoma. Grey area represents the average activity in each metabolic pathway across all tumor cells.

(**B**) Dot plot displaying the expression levels of cholesterol *de novo* biosynthesis and import genes in glioblastoma cell subtypes from the murine scRNA-seq dataset (Figure 1E). Dot color represents the average expression; size depicts percentage of cells expressing each specific gene.

(**C**) Histogram depicting the flow cytometry MFI quantification of cholesterol content in OPC-like or MES-like glioblastoma cells at the indicated time points post co-culture with BMDMs previously loaded with clickable cholesterol overnight (experimental design depicted in Figure S11A). Statistics: two-way ANOVA with Sídák correction for multiple comparison.

(**D**) Volcano plot displaying the log2 fold-change on the x-axis and -log10 p-value on the y-axis of depicted lipid classes quantified by NMR and Lipidyzer in MES-like tumor cells in mono- (left panel) or co-culture with TAMs (right panel) concurrent to 24 hours of continuous myelin exposure compared to control (no addition of myelin), as shown in Figure S11B. Colors correspond to significantly increased (red) lipid classes within MES-like tumor cells. Lipid categories are referenced in Table S10. No significantly decreased lipid species were identified.

(**E**) Histogram representing the cholesterol and triglyceride (TG) levels quantified using NMR and Lipidyzer respectively in MES-like glioblastoma cells at indicated time points post-incubation with myelin debris in mono- (grey) or co-culture with BMDMs (red). Statistics: two-way ANOVA with Sídák correction for multiple comparison.

(**F-G**) To determine the fate of cholesterol esters taken up by glioblastoma cells, U13C-FA18:1 labelled cholesteryl oleate (CE13C-FA) was synthesized and incubated with MES-like glioblastoma cells for 24 hours (n = 3). **(F)** Pie chart represents the relative distribution (based on lipid ng/µg protein) of labelled 13C among lipid classes quantifiable 24 hours after administration of CE13C-FA. Percentages depict the average fraction of the respective lipid classes. **(G)** Histogram depicting the quantification of metabolized CE13C-FA in MES-like glioblastoma cancer cells per lipid species (ng/µg protein) in which U13C-FA18:1 was detected.

(**H**) Histogram depicting the percentage of OPC-like and MES-like glioblastoma cells in S-phase (EdU+) after 48h co-culture with TCM pre-conditioned BMDMs, previously loaded with myelin (3h) or not (as described in Figure S11A). Statistics: two-way ANOVA with Sídák correction for multiple comparison.

(**I**) Electron microscopy representative images depicting the contact points between MES-like glioblastoma tumor cells (T) and TCM pre-conditioned myelin-loaded BMDMs (M) in co-culture for 24h. Box selection on panel 1 and 2 are magnified to visualize lipid droplets (LD), contact points through coated vesicles (CV) (2 and 4) and encapsulated myelin debris (MD) (3). Magnification: left panel 4800X; middle panel 13000X; right panels 18500X.

(**J**)Histogram depicting the percentage of MES-like glioblastoma cells in S phase (EdU+) assessed after 48h of co-culture with TCM pre-conditioned BMDMs previously loaded with myelin (3h) or not, in the presence of the CD36 inhibitor SSO and/or the ABCA1 inhibitor valspodar (ABCA1i). Statistics: mixed-effects analysis with Sídák correction for multiple comparison.

(**K**)Quantification of viable glioblastoma cells (ZombieNIR-, AnnexinV-) as a percentage of total tumor cells (CD45-CD11b- cells) in *ex vivo* assays (as described in Figure S10E). PDG-Ink4a glioblastoma from tumor-bearing mice were dissociated into single cell preparations, in which myeloid cells were either maintained (+CD11b+) or *ex vivo* depleted (-CD11b+). Glioblastoma single cell preparations were cultured in media supplemented with lipid-free FBS, in the presence of the ABCA1 inhibitor valspodar (ABCA1i) or LXR inhibitor GSK2033 (LXRi). Statistics: two-way ANOVA with Sídák correction for multiple comparison.

(**C, E-H, J, K**) Data are represented as mean + S.E.M.

**Figure 7. Lipid-laden macrophages predict glioblastoma** **patients’** **clinical outcome and correlate with poor response to immunotherapy**

(**A**) Kaplan-Meier curves of primary IDHWT glioblastoma patients from the TCGA64 and GLASS datasets65 stratified by low and high enrichment for the LLM signature derived from Figure 1I (LLMhigh: ssGSEA score from a tumor ≥ the 3rd quantile of the whole ssGSEA score across all tumors; LLMlow: ssGSEA score from a tumor ≤ the 1st quantile of the whole ssGSEA score across all tumors- see Methods). Statistics: Log-rank test.

(**B**)UMAP representation of cancer-specific macrophages, colors represent macrophages from each depicted tumor type gathered from published scRNA-seq datasets.17,66,130

(**C**) UMAP representation of the LLMsig enrichment score in pan-cancer macrophage clusters. Data scaled between 0 (low LLMsig score = grey) and ≥2.3 (high LLMsig score = blue).

(**D**) Left panel: schematic overview of pan-cancer TAM analysis. Right panel: violin plot depicting the average LLM signature scores in TAMs from cancer types highlighted in the left panel.

(**E**) Histogram depicting the percentage of TAMs positive for the LLM signature in scRNA-seq datasets of treatment-naïve, recurrent and neoadjuvant anti-PD-1-treated glioblastoma patient samples.69 Statistics: one-way ANOVA with Sídák correction for multiple comparison.

(**F**) Quantification of the percentage of TAMs assigned as non-LLMs or LLMs based on the LLMsig enrichment score in melanoma patients prior to immunotherapy with anti-PD-1 and/or anti-CTLA4, in scRNA-seq datasets of responder and non-responder patients.67 Statistics: Two-tailed paired t-test.

(**G**) Receiver operating characteristic (ROC) curves of the different random forest models performed to compare the predictive value of tumor mutational burden (TMB), LLMs, non-LLMs and total macrophages as a percentage of CD45+ cells to predict ICB response in melanoma. The diagonal dashed line indicates random performance. Axes depict false-positive rate (x-axis) and true-positive rate (y-axis). Statistics: unpaired two-sided bootstrap test.

(**E, F**) Data are represented as mean + S.E.M.