Tumor-induced reshuffling of lipid composition on the endoplasmic reticulum membrane sustains macrophage survival and pro-tumorigenic activity

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Tumor-associated macrophages (TAMs) display pro-tumorigenic phenotypes for supporting tumor progression in response to microenvironmental cues imposed by tumor and stromal cells. However, the underlying mechanisms by which tumor cells instruct TAM behavior remain elusive. Here, we uncover that tumor-cell-derived glucosylceramide stimulated unconventional endoplasmic reticulum (ER) stress responses by inducing reshuffling of lipid composition and saturation on the ER membrane in macrophages, which induced IRE1-mediated spliced XBP1 production and STAT3 activation. The cooperation of spliced XBP1 and STAT3 reinforced the pro-tumorigenic phenotype and expression of immunosuppressive genes. Ablation of XBP1 expression with genetic manipulation or ameliorating ER stress responses by facilitating LPCAT3-mediated incorporation of unsaturated lipids to the phosphatidylcholine hampered pro-tumorigenic phenotype and survival in TAMs. Together, we uncover the unexpected roles of tumor-cell-produced lipids that simultaneously orchestrate macrophage polarization and survival in tumors via induction of ER stress responses and reveal therapeutic targets for sustaining host antitumor immunity.

In the tumor microenvironment (TME), TAMs are one of the most abundant immune cell types and are characterized by heterogeneous and plastic features, giving rise to populations spanning from antitumorigenic towards pro-tumorigenic TAMs. Antitumorigenic TAMs are antigen-presenting cells expressing high levels of major histocompatibility complex II (MHCII) and capable of killing tumor cells with phagocytic activity. In addition, antitumorigenic TAMs can act as immunostimulatory cells to secrete proinflammatory cytokines for sustaining adaptive immunity. In contrast, pro-tumorigenic TAMs are proangiogenic and immunosuppressive cells characterized by low expression of MHCII but producing high levels of programmed death-ligand 1 (PD-L1) and anti-inflammatory cytokines. Microenvironmental factors imposed by the TME, including origins of tumors, metabolic contexts and cytokine milieu, have been suspected to tailor pro-tumorigenic features and mitigate anti-tumorigenic features, making macrophage plasticity an attractive target for therapeutic interventions. In support of this, targeting essential pro-tumorigenic features with macrophage-specific genetic ablations has been reported to result in tremendous effects on tumor progression and metastasis. Tumor cells have been suggested to use their oncogenic and metabolic pathways for creating microenvironmental cues that orchestrate differentiation and formation of immunosuppressive and pro-tumorigenic immune cells. Emerging evidence indicates that deregulated lipid metabolism not only enhances metastatic and invasive behavior of cancer cells, but also contributes to the generation of a lipid-enriched TME that hampers host antitumor immunity and sustains survival of suppressive cells. However, whether lipid production in tumor cells can polarize pro-tumorigenic TAMs remain largely elusive.

The ER stress response is an evolutionarily conserved mechanism to ensure survival or perish in response to stress-induced ER dysfunctions, including accumulation of misfolded proteins, impaired calcium homeostasis and altered lipid metabolism. These ER dysfunctions are sensed by three different proteins located in the ER membrane—activating transcription factor 6 (ATF6), protein kinase R-like ER kinase (PERK) and inositol-requiring enzyme 1 (IRE1)—to launch ER stress responses. The coordination of these ER stress signaling branches readjusts ER homeostasis through translational arrest, cellular macromolecule and mRNA and misfolded protein degradation, ER stress, especially IRE1-mediated production of the spliced form of X-box binding protein 1 (XBP1) and C/EBP homologous protein (CHOP), has recently emerged to be responsible for promoting T cell dysfunction, impairment of antigen-presenting capacity in dendritic cells and...
immunosuppressive phenotypes in myeloid-derived suppressor cells (MDSCs)\(^2\). In contrast, although IRE1 expression in TAMs has been shown to support suppressive features and expression of PD-L1\(^3\), how ER stress fine-tunes macrophage polarization, and which microenvironmental stimuli imposed by tumors are responsible for eliciting ER stress responses, remain largely unknown.

Here, we show that tumor-cell-produced β-glucosylceramide drives reshuffling of lipid composition on the ER membrane, leading to IRE1-dependent ER stress responses. As a result of coengagement of IRE1-XBP1 and IRE1–STAT3 signal branches, this specialized ER stress response facilitates pro-tumorigenic polarization in macrophages with a strong survival capacity in the TME. We further uncover that targeting IRE1-XBP1 and IRE1–STAT3 signal branches or preserving lipid composition of the ER membrane by genetic and pharmacological approaches effectively demolishes pro-tumorigenic TAMs and restrains tumor progression. These results highlight the unexplored mechanisms controlled by ER stress responses that allow tumor cells to manipulate macrophage properties in tumors, and indicate that targeting IRE1-mediated ER stress response and lipid reshuffling are promising strategies for reprogramming the TME.

Results

TAMs show high lipid content and ER stress responses. To gain insights into lipid metabolism in TAMs, we assessed lipid content and uptake in macrophages residing in tumors and spleen with BODIPY and Filipin III (hereafter Filipin) staining and uptake of BODIPY C12—a fluorescent lipid analog—in both the YUMM1.7 melanoma engraftment murine model and a genetically engineered murine melanoma model (referred to as Braf/Pten mice). In both models, TAMs increased neutral lipid (BODIPY 4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-s-indacene) staining and cholesterol (Filipin staining) content and uptake compared with splenic macrophages (Fig. 1a,b and Extended Data Fig. 1a–c). Moreover, lipid droplets appeared exclusively in the cytoplasm of TAMs and not in splenic macrophages (Fig. 1c). TAMs expressing arginase 1 (ARG1)—a marker enzyme rendering pro-tumorigenic features in macrophages—displayed a higher lipid content compared with ARG1–TAMs (Fig. 1d and Extended Data Fig. 1d), suggesting that aberrant lipid accumulation driven by the TME may skew pro-tumorigenic properties in TAMs. In addition to formation of lipid droplets, we found that the ER of TAMs was more extended and swollen—a morphological sign of ER stress responses—\(^4\) (Fig. 1e). In support of this, TAMs increased mRNA expression of typical ER stress-responsive genes, including Ern1 (Bip) and spliced Xbp1 (sXBP1), in both engrafted and inducible melanoma models (Fig. 2a and Extended Data Fig. 1e). Moreover, we confirmed that TAMs contained a higher percentage of populations expressing sXBP1 protein compared with splenic macrophages (Fig. 2b and Extended Data Fig. 1f), indicating that TAMs may engage ER stress responses. Since emerging studies suggested the crosstalk between IRE1-XBP1 and IRE1–STAT3 signal branches, we next examined the expression of sXBP1 with lipid content and ARG1 in TAMs. sXBP1\(^+\) TAMs had higher lipid content (Fig. 2c and Extended Data Fig. 1g) and represented most of the pro-tumorigenic TAM subset, characterized by ARG1 expression (Fig. 2d and Extended Data Fig. 1h,i). To confirm these findings in a more physiologically relevant setting, we compared the expression of sXBP1 and lipid content between TAMs and skin-resident macrophages from Braf/Pten melanoma-bearing mice and found that both parameters were higher in TAMs (Extended Data Fig. 1j–l). By computationally weighting ER stress responses of individual TAMs from the published single-cell RNA sequencing results of several human cancers, including colorectal cancer (CRC)\(^2\), lung adenocarcinoma without chronic obstructive pulmonary disease (LUAD-No COPD) and nonsmall cell lung carcinoma (NSCLC)\(^2\), and a cohort of murine sarcoma\(^2\), we observed that pro-tumorigenic TAMs displayed higher ER stress scores compared with anti-tumorigenic TAMs (Fig. 2e). Importantly, although TAMs expressed higher amounts of sXBP1, we observed only a slight increase in phosphorylated-PERK staining and decreased expression of ATF6 target genes, including Herpud1 and Derl1, compared with splenic macrophages (Extended Data Fig. 1m,n). These results indicate that TAMs preferentially engage XBP1-mediated ER stress responses. Taken together, our data highlight that ER stress and deregulated lipid metabolism may coordinate to tailor pro-tumorigenic features of TAMs.

IRE1-XBP1 reinforces macrophage pro-tumoral polarization. To investigate whether tumor-cell-derived factors could stimulate increased lipid content and ER stress responses in macrophages, we treated bone marrow (BM)-derived macrophages (BMDMs) with YUMM1.7 tumor-cell-derived conditioned medium (CM). Our results showed that CM promoted both lipid content and uptake (Fig. 3a and Extended Data Fig. 2a), accompanying elevated expression of pro-tumorigenic marker genes, including Arg1 and mannose receptor C-type 1 (Mrc1), but decreased expression of anti-tumorigenic marker genes (Fig. 3b and Extended Data Fig. 2b–d). Moreover, CM-treated BMDMs showed increased activity on suppressing proliferation of CD8\(^+\) T cells compared with naive macrophages (Extended Data Fig. 2e). Although interleukin(IL)-4/IL-13 neutralizing antibodies treatment abrogated IL-4/IL-13 induced alternative activation in BMDMs (Extended Data Fig. 2f), these neutralizing antibodies failed to prevent CM-induced expression of pro-tumorigenic marker genes (Extended Data Fig. 2g) and the amount of IL-4 and IL-13 in YUMM1.7 CM was lower than the detection limit (Extended Data Fig. 2h). In addition, CM derived from nontransformed murine embryonic fibroblasts (MEFs) was unable to increase lipid content and expression of pro-tumorigenic marker genes in BMDMs (Extended Data Fig. 2i–k). These results indicate that tumor cells could preferentially increase lipid content and pro-tumorigenic polarization in macrophages in an IL-4/IL-13-independent manner.

Interestingly, CM-treated BMDM showed production of sXBP1, but minimal increases in PERK activation (based on mobility shift in immunoblot) and PERK downstream target, activating transcription factor 4 (ATF4) (Fig. 3c). Moreover, CM failed to stimulate expression of ATF6 target genes, including Herpud1 and Derl1 (Extended Data Fig. 2i), indicating preferential activation of the IRE1-XBP1 branch. Given that ER stress responses have been revealed recently to hamper antitumor immunity by modulating functionality of CD8\(^+\) T cells, dendritic cells and MDSCs\(^19,20\), we speculate that CM-mediated activation of IRE1-XBP1 signaling might support acquisition of pro-tumorigenic phenotypes in macrophages. To test this postulate, we treated CM-stimulated BMDMs with STF081030—an inhibitor of the endoribonuclease activity of IRE1 that can prevent production of sXBP1—and found that STF081030 effectively suppressed expression of sXBP1 and pro-tumorigenic marker genes caused by CM (Fig. 3d). Furthermore, STF081030 treatment effectively ameliorated this suppressive ability in CM-treated BMDMs (Fig. 3e) and CM-boosted lipid accumulation was also dampened by STF081030 (Extended Data Fig. 2m). To confirm the contribution of IRE1 in inducing pro-tumorigenic polarization, we transduced BMDMs generated from LysM-Cre Cas9 mice\(^2\) with lentivirus harboring either scramble guide RNAs (gRNAs) or IRE1-targeting gRNAs to generate control BMDMs or IRE1-deficient BMDMs, respectively. IRE1 expression was lower in IRE1-deficient BMDMs compared with control BMDMs (Extended Data Fig. 2n), and CM-induced pro-tumorigenic polarization was compromised in IRE1-deficient BMDMs (Fig. 3f), indicating that induction of IRE1 activity by CM reinforces pro-tumorigenic polarization in macrophages. Conventional ER stress inducers,
including tunicamycin and thapsigargin, were capable of inducing an ER stress response, but failed to polarize BMDMs towards a pro-tumorigenic phenotype (Fig. 3g and Extended Data Fig. 3g), implying that specialized signaling cascades controlled by sXBP1 rather than conventional ER stress response are needed for reinforcing immunosuppressive activities in macrophages in response to tumor-cell-derived factors.

**XBP1 remodels TAM phenotype and supports tumor progression.**

To further elucidate the contribution of XBP1 to pro-tumorigenic polarization in macrophages, we transduced BMDMs generated from LysM-Cre Cas9 mice with lentivirus harboring either scrambled gRNAs or XBP1-targeting gRNAs to generate control BMDMs or XBP1-deficient BMDMs, respectively. Both total and spliced XBP1 expression were decreased in XBP1-deficient BMDMs compared with control BMDMs (Extended Data Fig. 3a). We found that CM-induced expression of pro-tumorigenic marker genes was reduced in XBP1-deficient BMDM compared with control BMDMs (Fig. 4a). To investigate whether the expression of XBP1 modulates pro-tumorigenic features in TAMs, we first generated myeloid-cell-specific XBP1-deficient mice (designated as XBP1<sup>cko</sup>) by crossing Xbp1<sup>fl/fl</sup> mice with LysM-Cre mice; Xbp1<sup>fl/fl</sup> mice were referred to as wild-type (WT; XBP1<sup>wt</sup>) mice. We further found that suppressive activity as well as lipid accumulation were reduced in XBP1-deficient BMDM compared with WT BMDMs (Fig. 4b and Extended Data Fig. 3b). These data highlight a role for XBP1 in skewing macrophages towards pro-tumorigenic phenotype upon exposure to tumor-derived components. We next engrafted YUMM1.7 melanoma cells expressing ovalbumin peptide (YUMM1.7-OVA) into WT or XBP1<sup>cko</sup> mice and found that genetic ablation of Xbp1 in myeloid cells suppressed tumor growth (Fig. 4c,e) accompanied by a significant loss of macrophages from the TME (Extended Data Fig. 3c). We next applied anti-CSF1R antibody treatment to deplete macrophages in tumor-bearing WT or XBP1<sup>cko</sup> mice to examine whether macrophages are responsible for the differential tumor growth rates in WT and XBP1<sup>cko</sup> mice. WT and XBP1<sup>cko</sup> mice displayed similar tumor growth rates and tumor burdens upon treatment with anti-CSF1R antibody (Extended Data Fig. 3d–f), indicating that XBP1 expression is required for promoting tumor progression by supporting accumulation and survival of TAMs. In agreement with previous reports<sup>30,31</sup>, in Ly6G<sup>−</sup> myeloid cell populations, we observed a population of F4/80<sup>+</sup> macrophages that express intermediate levels of Ly6C (referred to as immature TAMs (iTAMs)), which was absent in the spleens of YUMM1.7-OVA-engrafted (Extended Data Fig. 3g) and melanoma-bearing Braf/Pten (Extended Data Fig. 3l) mice. iTAMs expressed much higher levels of sXBP1, PD-L1 and arginase 1 (ARG1) than mTAMs (Fig. 4e), indicating that ablation XBP1 could ameliorate pro-tumorigenic features in TAMs. Similar to YUMM1.7-OVA melanomas, we found that genetic ablation of XBP1 in myeloid cells suppressed growth of B16 melanoma overexpressing ovalbumin (Extended Data Fig. 3g–i) and led to a reduction trend in tumor growth of MC38 colon adenocarcinoma overexpressing ovalbumin (Extended Data Fig. 3j–n). Collectively, these results reveal that the expression of XBP1 propels TAMs towards pro-tumorigenic activation, rendering a survival advantage to tumors.

**STAT3 signal optimizes macrophage pro-tumoral polarization.**

Next, we sought to test whether expression of sXBP1 alone is sufficient to promote the M2 phenotype. Our results showed
upregulated by CM (Fig. 5b). We speculated that STAT3 activation in macrophages. A recent study revealed that STAT3 activation pathway might be required for skewing pro-tumorigenic polarization of M2 genes in control-treated BMDM (Fig. 5a), indicating that other signaling cascades that coordinate with the IRE1-XBP1 expression that IRE1-mediated production of sXBP1 is needed to rein force pro-tumorigenic and pro-tumorigenic macrophages from different human and murine cancer types. Data are pooled from at least two independent experiments. Each symbol represents one individual. All data are mean ± s.e.m and were analyzed by two-tailed, unpaired Student’s t-test (t-test), whiskers represent the 5th and 95th percentile values, box limits represent the 25th and 75th percentiles and the black line represents the median.

that STF083010 treatment suppressed CM-induced expression of pro-tumorigenic marker genes in control BMDMs, but not BMDMs overexpressing sXBP1 (Fig. 5a), supporting our conclusion that IRE1-mediated production of sXBP1 is needed to reinforce pro-tumorigenic activity in macrophages stimulated with CM. However, overexpression of sXBP1 was unable to induce the expression of M2 genes in control-treated BMDM (Fig. 5a), indicating that other signaling cascades that coordinate with the IRE1-XBP1 pathway might be required for skewing pro-tumorigenic polarization in macrophages. A recent study revealed that STAT3 activation promotes tumor progression by triggering cathepsin expression in TAMs42. Indeed, we found that phosphorylated STAT3 was strongly upregulated by CM (Fig. 5b). We speculated that STAT3 activation is also needed to reinforce pro-tumorigenic polarization in response to tumor-cell CM. In support of this postulate, genetic ablation of STAT3, or treatment with the STAT3 inhibitor Statmic, hampered expression of the MRC1 gene, but not that of ARG1, highlighting the possibility of a partial contribution of STAT3 activation to the suppressive activity of CM-treated BMDMs (Fig. 5c and Extended Data Fig. 4d). Moreover, neutralizing antibodies against IL-6 and IL-10 were unable to suppress CM-induced STAT3 phosphorylation (Fig. 5d) and the expression of pro-tumorigenic marker genes (Fig. 5e), while the doses of neutralizing antibodies we used...
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were able to abrogate the STAT3 phosphorylation induced by IL-6 and IL-10 (Fig. 5d). These results indicate that a tumor-cell-derived factor activates STAT3 for skewing pro-tumorigenic polarization in macrophages in an IL-6/IL-10-independent manner. IRE1 has been shown to support STAT3 activation in hepatocytes by forming a protein complex with STAT3 (ref. 34). By using a proximity ligation assay, we found that CM facilitated the IRE1–STAT3 interaction in BMDMs (Fig. 5f). We then tested whether IRE1 is needed for STAT3 phosphorylation and found that tumor-cell CM-induced STAT3 phosphorylation was dampened in IRE1-deficient BMDMs compared with control BMDMs (Fig. 5g). However, pharmacological inhibition of the endoribonuclease activity of IRE1 with STF083010 did not prevent CM-induced STAT3 phosphorylation, indicating that the effect is independent of sXBP1 production (Extended Data Fig. 4e). In contrast to CM, tunicamycin was not able to induce STAT3 phosphorylation, suggesting that CM leads to an unconventional and mild ER stress response that activates STAT3 in an IRE1-dependent manner (Extended Data Fig. 4f).

Fig. 3 | Tumor cells drive pro-tumorigenic polarization in BMDMs via IRe1. a, BMDMs were cultured in regular culture medium (Ctrl) or in the presence of YUMM1.7 melanoma cell CM for 18 h. Flow cytometry analysis was performed to analyze lipid content with anti-CD3 and anti-CD28 alone or in coculture with BMDM previously exposed to CM or CM in presence of 50 µM STF083030 (CM+STF) or control vehicle (CM). Data are representative results of three independent experiments. b, Immunoblot analysis of lipid expression with TUNICAMYCIN and THAPSIGARGIN as positive controls. Data are representative results of two independent experiments. c, qPCR analysis of mRNA expression of indicated genes in BMDMs treated with TUNICAMYCIN, THAPSIGARGIN or YUMM1.7 CM for 18 h. Flow cytometry analysis was performed to analyze lipid content with anti-CD3 and anti-CD28 alone or in coculture with BMDM previously exposed to CM or CM in presence of 50 µM STF083030 for 24 h, at a ratio of 2:1 (n=3 per group). Data are representative results of three independent experiments. f, qPCR analysis of mRNA expression of indicated genes in control or IRE1-deficient BMDMs transfected with YUMM1.7 CM. Data are pooled from two independent experiments from four independent repeats (n=5 or 6 per group). g, qPCR analysis of mRNA expression of indicated genes in BMDM treated with tunicamycin, thapsigargin or YUMM1.7 CM for 18 h. Flow cytometry analysis was performed to analyze lipid content with anti-CD3 and anti-CD28 alone or in coculture with BMDM previously exposed to CM or CM in presence of 50 µM STF083030 for 24 h, at a ratio of 2:1 (n=3 per group). Data are representative results of three independent experiments with n=3 per group. All data are mean ± s.e.m. and were analyzed by two-tailed, unpaired Student’s t-test (a, b, d, f, g) or one-way ANOVA with Tukey’s multiple comparison test (e).
Taken together, our results indicate that a CM-triggered IRE1 signal leads to pro-tumorigenic polarization in macrophages by simultaneously stimulating STAT3 activation and production of sXBP1.

Glucosylceramide sensing by macrophage inducible Ca²⁺-dependent lectin receptor tailors macrophage activation. Since aberrant lipid metabolism has been suggested to elicit an ER stress response, we postulated that lipids generated by tumor cells may be responsible for the induction of pro-tumorigenic features in macrophages. To test this, we removed lipids, including cholesterol and fatty acids, from YUMM1.7 melanoma cell CM with a lipid removal agent (Extended Data Fig. 5a,b). We found that lipid removal abolished CM-triggered production of sXBP1, STAT3 phosphorylation and the expression of pro-tumorigenic marker genes (Fig. 6a–c). By examining the transcriptomic changes induced by CM treatment, we found that most genes responsible for pro-tumorigenic activity in macrophages were upregulated by lipid removal (Fig. 6d). Together, these results indicate that lipids produced by tumor cells may control the engagement of ER stress responses for polarizing macrophages.

**Fig. 4 | Deletion of XBP1 in TAMs suppresses tumor growth.** a, qRT-PCR analysis of indicated genes in Cas9-expressing BMDM transduced with retrovirus expressing gRNA targeting control (CTRL) or XBP1 sequence and exposed to Ctrl or CM (n = 6 per group). Data are pooled from two independent experiments, repeated four times. b, Proliferation of CFSE-labeled T cells activated with anti-CD3 and anti-CD28 alone or in coculture with BMDM isolated from XBP1wt or XBP1cKO mice and previously exposed to CM at a ratio of 2:1 (n = 6 per group). Data are representative of three independent experiments. c, Tumor growth (c) and tumor weight (d) of YUMM1.7-OVA melanoma from XBP1wt (n = 3 per group) or XBP1cKO (n = 9) mice. Data are pooled from two independent experiments. Each symbol represents one individual. All data are mean ± s.e.m. and were analyzed by two-tailed, unpaired Student’s t-test (a, c-e) or one-way ANOVA with Tukey’s multiple comparison test (b).
Fig. 5 | Activation of IRE1-STAT3 signal supports CM-induced polarization. **a**, qPCR analysis of mRNA expression of indicated genes in BMDMs stimulated with YUMM1.7 CM in the presence of 50 μM STF081030 (CM + STF) or vehicle (CM) BMDMs transduced with retrovirus expressing empty vector (EV) or xBP1 as indicated in Methods (n = 6 per group for EV-CM, EV-CM-STF, XBP1-Ctrl and XBP1-CM-STF, n = 5 for EV-Ctrl and n = 5 for XBP1-CM). Data are pooled from two independent experiments repeated four times. **b**, Immunoblots of BMDMs treated with CM for indicated duration. **c**, Immunoblots of BMDMs treated with indicated treatments. Ctrl, control medium or YUMM1.7 CM (n = 5 per group). BMDMs were transduced with retrovirus expressing control or STAT3-targeting gRNAs. Data are representative results of three independent experiments. **d**, Immunoblots of BMDMs treated with CM in the absence or presence of 0.25 μg ml^{-1} anti-IL-6 and 0.25 μg ml^{-1} anti-IL-10. Data are representative results of three independent experiments. **e**, qPCR analysis of mRNA expression of indicated genes in BMDMs treated with control medium or YUMM1.7 CM (n = 5 per group). BMDMs were transduced with retrovirus expressing control or IRE1-targeting gRNAs. Data are pooled from two independent experiments repeated four times. **f**, Immunoblots of indicated proteins in BMDMs treated with indicated treatments. Ctrl, control medium; CM, YUMM1.7 conditioned medium; IL-6, IL-6 treatment (10 ng ml^{-1}); IL-10: IL-10 treatment (10 ng ml^{-1}). Anti-IL-6/IL-10 antibodies were added alone or in combination (0.25 μg ml^{-1} anti-IL-6 and 0.25 μg ml^{-1} anti-IL-10). Data are representative results of three independent experiments. **g**, Representative images (left) and quantification (right) of proximity ligation assay (PLA) of BMDM exposed to Ctrl or CM for 18 h. Scale bar, 10 μM. Data are representative results of two independent experiments: Ctrl (n = 22), CM (n = 35). **f**, Immunoblots (left) and quantification (right) (n = 3 per group) of indicated proteins in control or IRE1-targeting gRNA expressing BMDMs stimulated with control medium (Ctrl) or YUMM1.7 CM (CM). Data are representative results of three independent experiments. All data are mean ± s.e.m. and were analyzed by two-tailed, paired Student's t-test (**a, c, e, f**) or one-way ANOVA with Sidak’s multiple comparison test (**g**).
a neutralizing anti-CD36 antibody failed to prevent CM-induced expression of sXBP1 and pro-tumorigenic marker genes (Extended Data Fig. 5e). Altogether, these results indicate that cholesterol produced by tumor cells is not involved in tumor-cell CM-mediated pro-tumorigenic activation in macrophages.

By examining transcriptomic analysis with a particular focus on genes involved in lipid recognition and binding that were significantly upregulated by tumor-cell CM, we found that macrophage inducible Ca²⁺-dependent lectin receptor (Mincle), also known as C-Type lectin domain family 4 member E (Clec4e), was significantly induced by CM. The induction of Mincle protein expression in BMDMs treated with CM was further validated by flow cytometry (Fig. 6e). In addition to acting as a pattern recognition receptor to tailor macrophage activities, Mincle has been reported recently to induce ER stress responses and facilitate lipid accumulation by inhibiting cholesterol efflux in macrophages residing in atheromas and during kidney injuries. To test whether Mincle-mediated lipid recognition is responsible for CM-induced changes in macrophages, we stimulated BMDMs with YUMM1.7 CM in the absence or presence of anti-Mincle antibody, which can block lipid recognition ability of Mincle. We found that anti-Mincle antibody treatment compromised activity of CM-stimulated BMDMs on hampering CD8⁺ T cell proliferation and lipid accumulation (Fig. 6f and Extended Data Fig. 5f). In addition, the CM-induced expression of sXBP1, pro-tumorigenic marker genes and STAT3 phosphorylation were ameliorated by anti-Mincle antibody treatment (Fig. 6g,h).

Using a genetic approach, we further confirmed that CM induced less pro-tumorigenic polarization, including declined STAT3 activation, expression of sXBP1, pro-tumorigenic genes and suppressive activity, in Mincle-deficient BMDMs (Mincle-KO) compared with WT BMDMs (Extended Data Fig. 5g,i). Together, these results indicate that Mincle-mediated lipid recognition is responsible for CM-induced pro-tumorigenic polarization in macrophages. Since β-glucosylceramide and cholesterol sulfate are the known endogenous ligands of Mincle and derived from HMG-CoA-deficient tumor cells remained effective on modulating Mincle expression and during kidney injuries. To test whether Mincle-mediated lipid recognition is responsible for CM-induced changes in macrophages, we stimulated BMDMs with YUMM1.7 CM in the absence or presence of anti-Mincle antibody, which can block lipid recognition ability of Mincle. We found that anti-Mincle antibody treatment compromised activity of CM-stimulated BMDMs on hampering CD8⁺ T cell proliferation and lipid accumulation (Fig. 6f and Extended Data Fig. 5f). In addition, the CM-induced expression of sXBP1, pro-tumorigenic marker genes and STAT3 phosphorylation were ameliorated by anti-Mincle antibody treatment (Fig. 6g,h).

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Moreover, anti-Mincle antibody did not further suppress expression of pro-tumorigenic genes in BMDMs stimulated with CM derived from UGCG-deficient YUMM1.7 cells (Extended Data Fig. 5k), suggesting that β-glucosylceramide is the bioactive component sensed by Mincle in macrophages. We also found that tumor interstitial fluids in engrafted YUMM1.7 melanomas and inducible Braf/ Pten melanomas contained more β-glucosylceramide compared with serum in tumor-bearing mice (Extended Data Fig. 5l–m). Moreover, UGCG deficiency in YUMM1.7 melanoma cells led to declined tumor growth (Fig. 6l–m) accompanied by a decreased ratio of iTAMs to mTAMs (Fig. 6n). Altogether, these results imply that β-glucosylceramide produced by tumor cells triggers ER stress responses in a Mincle-dependent manner for unleashing pro-tumorigenic activities in TAMs.

### Disturbed lipid composition on ER membrane activates sXBP1

Since Mincle activation has been shown to inhibit cholesterol efflux and TAMs accumulate higher levels of cholesterol, we speculated that CM may promote accumulation of intracellular cholesterol by stimulating cholesterol synthesis. In support of this, we found that CM increased intracellular cholesterol in BMDMs as measured by Fillipin III staining. However, treating statin to block cholesterol synthesis and anti-Mincle antibody effectively hampered CM-induced accumulation of cholesterol (Fig. 7a).

In addition, blocking cholesterol synthesis with statin prevented CM-induced expression of sXBP1 and pro-tumorigenic marker genes in BMDMs (Fig. 7b). Anti-Mincle treatment failed to further suppress CM-induced expression of sXBP1 and pro-tumorigenic marker genes in BMDMs treated with statin. These results indicate that Mincle activation orchestrates pro-tumorigenic polarization in macrophages by enhancing cholesterol synthesis. IRE1 contains a transmembrane domain that can sense lipid imbalance and induces its dimerization and activation, and increased cholesterol accumulation might reshuffle lipid composition of the ER membrane towards a low phosphatidylincholine to phosphatidyl-ethanolamine ratio (PC/PE ratio) and decreased polyunsaturated fatty acid (PUFA), which can decrease ER membrane fluidity, as a result of a disturbed cholesterol-sensing mechanism. Thus, we hypothesized that tumor-cell CM triggers IRE1/XBP1 activation by reshuffling lipid composition on the ER membrane. Lipid profiling of the ER membrane showed that the PC/PE ratio was decreased significantly in CM-treated BMDMs compared with the control group (Fig. 7c). Furthermore, we observed decreased abundance of polyunsaturated PC, but elevated abundance of saturated PC, in CM-stimulated BMDMs, especially palmitoyl (16:0) and linoleoyl (18:2)-containing PC (Fig. 7d,e). We next attempted to rescue the disturbed lipid composition on the ER membrane by overexpressing lysophosphatidylcholine acyltransferase 3 (LPCAT3)—an

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**Fig. 6** | Mincle-dependent glucosylceramide sensing pathway tailors macrophage activation. a–c, Immunoblots of indicated proteins (a, b) and qPCR analysis of indicated genes (c) in BMDMs treated with control medium (Ctrl), CM or CM without lipids (wo Lip) for the indicated time points (a) or for 18 h (b, c) (n = 3 sample per group in c). Data are representative results of three independent experiments. d, Heatmap of mRNA expression of pro-tumorigenic genes upon exposure of CM or CM without lipids, in BMDMs (n = 3 per group). e, Quantitative plot of Mincle protein expression in BMDMs treated with control medium (Ctrl) or CM (n = 3). Data are representative results of three independent experiments. f, Proliferation of CFSE-labeled T cells activated with anti-CD3 and anti-CD28 alone or in coculture with BMDM previously treated with CM or with CM in presence of 5 μg ml⁻¹ anti-Mincle antibody at a ratio of 2:1 for 72 h (n = 3). Data are representative of three independent experiments. g, h, qPCR analysis of indicated genes (g) (n = 5 or 6) and immunoblots of indicated proteins (left) with quantification (right) (h) (n = 3) in BMDMs cultured with control medium (Ctrl) or CM in the absence or presence of 5 μg ml⁻¹ anti-Mincle antibody. Data are pooled from two independent experiments repeated four times (g) or three (h) times. i, Immunoblot of indicated proteins in YUMM1.7 cells shCTRL or shUGCG. j, qPCR analysis of indicated genes in BMDMs exposed to CM derived from YUMM1.7 shCTRL or YUMM1.7 shUGCG (n = 6). Data are pooled from four independent experiments repeated four times. k, Immunoblots of indicated proteins (left) and quantification (right) of BMDMs cultured with CM from shCTRL or shUGCG YUMM1.7 (n = 3). l, Tumor growth curve (l) and tumor weight (m) of YUMM1.7 shCTRL and YUMM1.7 shUGCG tumors in coengrafted mice (n = 10). Ratio of iTAMs to mTAMs from the indicated tumors of tumor-bearing mice (n = 10). Data are pooled from two independent experiments. Each symbol represents one individual. Data are mean ± s.e.m. and were analyzed by two-tailed (c, e, g, j), unpaired Student’s t-test or paired t-test (m, n) and one-way ANOVA with Tukey’s multiple comparison test (f, h) and repeated measures one-way ANOVA with Sidak’s multiple comparison test (k).
enzyme responsible for synthesizing PC that contains preferentially unsaturated FA as acyl chains and has been shown to restrict lipid-overloading-induced ER stress responses in hepatocytes. In support of our postulate, LPCAT3 overexpression reduced both the PC/PE ratio and the abundance of unsaturated PC on the ER membrane in CM-stimulated BMDMs (Fig. 7f–h). Upon analyzing...
Fig. 7 | CM causes reshuffling of lipid composition and saturation of ER membrane. a, MFI of Filipin staining in BMDMs treated with CM or CM in the presence of simvastatin (10 μM) or anti-Mincle antibody (5 μg/ml) (n = 3). b, qPCR analysis of indicated genes in BMDMs stimulated with control medium (Ctrl) or CM alone or with anti-Mincle antibody (5 μg/ml) (n = 3). Data are representative results of three independent experiments. Data are mean ± s.e.m and were analyzed by two-tailed, unpaired Student’s t-test (a, b) and one-way ANOVA with Sidak’s multiple comparison test (d, e). In box plots (d, e), whiskers represent the minimum and maximum values, box limits represent the minimum and maximum values and the black line represents the median.
As previous studies showed that expression of LPCAT3 is controlled by the liver X receptor (LXR)\(^{16,17}\), we sought to induce expression of LPCAT3 pharmacologically with the LXR agonist GW3965, and investigate whether this treatment can be exploited to tailor functionality of TAMs. Treatment with GW3965 resulted in LPCAT3 induction in CM-treated BMDMs (Fig. 8a) accompanied by reduced expression of sXBP1 and STAT3 phosphorylation (Fig. 8b,c). In addition, GW3965 restrained the expression of Arg1 and Mrcl induced by YUMM1.7 CM (Fig. 8d) and partially hampered the suppressing activity of CM-treated BMDMs towards CD8\(^+\) T cells (Fig. 8e), indicating that GW3965 can prevent the engagement of ER stress-mediated pro-tumorigenic polarization in response to tumor-cell-derived stimulation as we observed in the BMDM overexpressing LPCAT3. In addition, GW3965 failed to ameliorate CM-induced sXBP1 expression and STAT3 activation as well as suppressive activity on controlling CD8\(^+\) T cell proliferation, in LPCAT3-deficient BMDMs (Extended Data Fig. 6a–c).

ER morphology by electron microscopy, we observed that LPCAT3 overexpression reduced extension of the ER membrane in melanoma cell CM, but not regular culture media (Fig. 7i), indicating that forcing LPCAT3 expression could ameliorate CM-induced ER stress in macrophages. Furthermore, LPCAT3 overexpression could prevent CM-induced expression of sXBP1 and pro-tumorigenic marker genes in BMDMs (Fig. 7j–l). Collectively, these data show that tumor-induced reshuffling of lipid composition on the ER membrane is critical for launching the ER stress-mediated pro-tumorigenic polarization and that LPCAT3-driven lipid metabolism could be a promising strategy to tailor macrophage behavior by intervening in this unique ER stress induction mechanism.

**LXR agonist controls tumor burden via LPCAT3 in macrophages.** As previous studies showed that expression of LPCAT3 is controlled by the liver X receptor (LXR)\(^{16,17}\), we sought to induce expression of LPCAT3 pharmacologically with the LXR agonist GW3965, and investigate whether this treatment can be exploited to tailor functionality of TAMs. Treatment with GW3965 resulted in LPCAT3 induction in CM-treated BMDMs (Fig. 8a) accompanied by reduced expression of sXBP1 and STAT3 phosphorylation (Fig. 8b,c). In addition, GW3965 restrained the expression of Arg1 and Mrcl induced by YUMM1.7 CM (Fig. 8d) and partially hampered the suppressing activity of CM-treated BMDMs towards CD8\(^+\) T cells (Fig. 8e), indicating that GW3965 can prevent the engagement of ER stress-mediated pro-tumorigenic polarization in response to tumor-cell-derived stimulation as we observed in the BMDM overexpressing LPCAT3. In addition, GW3965 failed to ameliorate CM-induced sXBP1 expression and STAT3 activation as well as suppressive activity on controlling CD8\(^+\) T cell proliferation, in LPCAT3-deficient BMDMs (Extended Data Fig. 6a–c).
that can sustain their metabolic needs in the TME. Thus, it is also expressing the highest level of sXBP1. Moreover, pro-tumorigenic polarization, but decreased survival of iTAMs (the TAM population). LPCAT3 expression in macrophages results in less pro-tumorigenic immortality can restrain pro-tumorigenic features and survival in TAMs.

Discussion

ER stress has emerged as a critical regulatory circuit to modulate immune cells in the TME; however, the stimuli imposed by tumor cells for eliciting ER stress in macrophages, and how ER stress tailors functionalities of TAMs, remain elusive. Here, we show that tumor cells promote a Mincle-mediated ER stress response to orchestrate pro-tumorigenic polarization in TAMs. We further demonstrate that reshuffling of lipid composition on the ER membrane simultaneously activates IRE1-XBP1 and IRE1–STAT3 signal branches to reinforce pro-tumorigenic properties and survival in macrophages. Moreover, promoting PC synthesis and incorporation of unsaturated fatty acids into PC on the ER membrane to improve fluidity can restrain pro-tumorigenic features and survival in TAMs. Together, our findings unravel the underexplored mechanisms by which tumor cells modulate macrophage behavior, and warrant development of treatments targeting lipid metabolism in macrophages for cancer therapy.

Our results show that myeloid cell-specific XBP1fl/fl mice contain fewer macrophages in tumor immune infiltrates and display a differential TAM profile compared with WT mice. Since sXBP1 is known to promote PC synthesis for ameliorating amplitudes of ER stress and supporting cell survival in response to perturbations of ER homeostasis, it is likely that the high expression of sXBP1 in macrophages promotes their survival and PC synthesis when encountering metabolic perturbations imposed by the TME. As a trade-off of sXBP1-supported survival, the accumulation of sXBP1 can launch pro-tumorigenic polarization in macrophages. In support of this possibility, our results show that LXR agonist-induced LPCAT3 expression in macrophages results in less pro-tumorigenic polarization, but decreased survival of iTAMs (the TAM population expressing the highest level of sXBP1). Moreover, pro-tumorigenic TAMs have been speculated to engage in unique metabolic programs that can sustain their metabolic needs in the TME. Thus, it is also likely that the metabolic properties engaged during pro-tumorigenic polarization may coordinate with sXBP1-mediated metabolic regulations to fine-tune the amplitude of ER stress to ensure survival of macrophages in response to metabolic insults such as lipid overloading and glucose deprivation in tumors. Therefore, it is of interest to examine the differences in metabolic properties between iTAMs and mTAMs and to explore how these differentially engaged metabolic processes can support survival of TAMs in coordination with sXBP1-mediated regulations. Understanding these questions will lead to the development of new interventions targeting pro-tumorigenic TAMs but preserving antitumorigenic TAMs.

Although activation of IRE1-XBP1 and IRE1–STAT3 signal branches are critical for skewing pro-tumorigenic macrophages, it remains unknown whether and how sXBP1 and STAT3 work synergistically to induce a functional switch towards a pro-tumorigenic phenotype. In addition to modulating transcriptional programming, STAT3 has been shown to modulate metabolic reprogramming in numerous cell types via nontranscriptional events. Thus, it is possible that both STAT3-mediated metabolic reprogramming and transcriptional modulations can be involved in the coordination with XBP1 in tailoring macrophage polarization. Moreover, our results show that CM promotes IRE1–STAT3 protein complex formation and that genetic ablation of IRE1 abolishes CM-induced STAT3 activation. However, IL-6 has been shown to activate IRE1 via a STAT3-dependent manner, which in turn promotes the secretion of cathepsin protease. Thus, these findings also highlight that a positive feedback loop between IRE1 and STAT3 may exist in TAMs by integrating tumor-cell-derived β-glucosylceramide and IL-6 produced by stromal and tumor cells. Thus, identifying the molecular mechanisms controlled by the synergistic actions between XBP1 and STAT3 would provide critical information for targeting, and even reprogramming, pro-tumorigenic TAMs. IRE1 has been shown recently to support suppressive activity of polymorphonuclear MDSCs in tumors, which further underlines the therapeutic potential of harnessing the IRE1 targeting strategy for cancer treatment.

Our results reveal that TAMs preferentially engage in the production of sXBP1 and show less activation of PERK and ATF6 signal branches. Moreover, although conventional ER stress inducers, including tunicamycin and thapsigargin, stimulate all three signal branches of ER stress, tunicamycin and thapsigargin fail to promote pro-tumorigenic polarization in macrophages. In fact, it has been suspected that ER stress induced by disturbed lipid homeostasis on the ER membrane is different from ER stress induced by conventional ER stress inducers, such as tunicamycin and thapsigargin, on modulating cellular behavior. In contrast to conventional ER stress inducer tunicamycin, which activates all three ER stress signal pathways (IRE1, ATF6 and PERK), we find that CM stimulation has no impact on the expression of ATF6 target genes. Moreover, CM treatment, but not tunicamycin stimulation, induces STAT3 phosphorylation. These results indicate that CM may induce low grade ER stress that can preferentially engage the IRE1-XBP1 axis and have less impact on PERK and ATF6. As a result of this specialized engagement of the signaling axis, it is likely that CM can orchestrate pro-tumorigenic polarization in BMDMs. In contrast, conventional ER stress inducers simultaneously activate all three signaling arms; integration of three signal axes may avoid pro-tumorigenic polarization but triggers survival and translational arrest in BMDMs by inducing a different array of downstream events. Together, these findings imply that specialized ER stress-sensing mechanisms that can differentiate amplitude and stress initiation events may participate in tailoring differentiation and survival in macrophages. Therefore, elucidating how ER stress responses are engaged in dictating cellular programs in macrophages, as well as in other immune cells, represents an important avenue for tailoring ER stress responses in cancer treatments.
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Tumor engraftment, BM transplantation and in vivo treatment. For the oncogene-driven melanoma model, 3-week-old Braf/PTEN mice were treated with 4-hydroxystaurosporine on the skin surface, as described previously16, to induce melanoma engraftment. For tumor engraftment, 5 × 10^6 cells of YUMM1.7, or 1 × 10^6 of YUMM1.7-OVA or 2 × 10^6 of YUMM1.7 shCTRL and shUGCG tumor cells, 2 × 10^6 of B16-OVA and 7 × 10^6 of MC38-OVA were injected subcutaneously in 50 μl PBS. Tumor growth was measured every 2–3 days after tumor engraftment or the indicated treatments. For BM transplantation experiments, 7-week-old C57BL/6 mice were irradiated with 9 Gy. Subsequently, mice were injected with either LPCAT3^+/+ or LysM-Cre LPCAT3^−/− mice were injected intravenously. Tumor experiments were initiated 7 days post-BM reconstitution. For in vivo treatment, day 7 post-engraftment, YUMM1.7-OVA-bearing mice were administered daily with either 10% DMSO or LXR agonist (GW3965) (10 mg per kg body weight); SelleckChem) by intraperitoneal (i.p.) injection. For CSF1R blockade, anti-CSF1R Ab (Clone InVivoMab) at 50 μg per kg was injected i.p. every 3 days starting from day −1 to tumor engraftment. All experiments were performed according to Swiss federal regulations and approved by the veterinary authority of Canton Vaud, Switzerland.

Cell lines and in vitro culture. The YUMM1.7 melanoma cell line was provided by M. Bosenberg as described previously16. MEFs were provided by F. Martinou. The YUMM1.7-OVA and B16-OVA cell line was established as described before16 and were cultured in high-glucose DMEM (Life Technologies) with 10% FBS (Gibco) and 100 μM penicillin-streptomycin (ThermoFisher Scientific) and puromycin (InvivoGen). The YUMM1.7 shCTRL and shUGCG were stably established by transduction of the parental cell line with lentivirus PLKO.1 (Addgene) carrying short hairpins targeting scrambler or UGGCG sequence and were maintained in high-glucose DMEM with 10% FBS, 100 μM penicillin-streptomycin and puromycin. The MC38-OVA were provided by P. Romero. BM cells were collected and cultured in high-glucose DMEM supplemented with 10% FBS and 20% L929 cell culture supernatant for macrophage differentiation for 6 days. On day 6, differentiated BMDMs were seeded with DMEM (without L929 cell culture supernatant) for 4 h. Then, cells were stimulated as described in the figure legends. For the experiments with BMDMs generated from Mincle-KO mice, CD11b^+ M cells (70% density) with DMEM plus 10% FBS for 24 h. Then, culture medium was collected and filtered through a 0.22 μm filter to remove cell debris and used as CM. To generate CM without lipids, CM collected as described above was treated with Cleanascant reagent (Biotech Support Group) according to the manufacturer’s instructions.

Plasmids and reagents. The retroviral vectors MSCV-xBPI-Thy1.1 and MSCV-LPCAT3-thy1.1 were constructed by PCR cloning, sXBP1 cDNA was amplified by PCR from the vector pFLAG.XBP1pCMV2 (Addgene) and LPCAT3 cDNA was amplified from the vector LPCAT3-pCXN2 (ref. 17) (kindly provided by J. Miyazaki). Both cDNAs were inserted into MSCV-Thy1.1 between BglII and SalI restriction sites. MSCV-Thy1.1 was a gift from S. Kaech (Salk Institute for Biological Studies). For CRISPR-Cas9-mediated depletion, guide RNA sequences (gRNAs) were cloned into pSUPER-pU6-Thy1.1 plasmid using BsiWI. The retroviral vector pSUPER-pU6-Thy1.1 backbone vector was kindly provided by P. Romero, University of Lausanne. The short hairpins against shCTRL or UGGCG (shUGCG) sequence were inserted into the lentiviral vector PLKO.1 purchased from Addgene (catalog no. 50942, BioLegend), anti-IL-6 Ab (catalog no. 504902, BioLegend), anti-IL-10 Ab (catalog no. 504902, BioLegend), anti-IL-6 Ab (catalog no. Q3-695); anti-CD206, 1:100 (catalog no. MMR); anti-ARG1, 1:100 (catalog no. M5/114.15.2); anti-sXBP1, 1:50 (catalog no. 509402, BioLegend) and anti-CD36 Ab (clone CRF D-2712 (ref. 58) provided by R. Silverstein at the Medical Biological Studies). For CRISPR–Cas9-mediated depletion, guide RNAs (gRNAs) were designed as CD45+CD3 Lyt2CD11b+ F480+Ly6C+; iTAMs were defined as CD45+CD3 Lyt2CD11b+ F480+Ly6C+; the following antibodies were used: anti-CD45.2, 1:100 (catalog no. Ali4A2); anti-CD3e, 1:100 (catalog no. 17A2); anti-Gr1, 1:100 (catalog no. RB8-6C3); anti-Ly6G, 1:200 (catalog no. 1A8); anti-Ly61b, 1:200 (catalog no. M170); anti-Ly6C, 1:2000 (catalog no. HK1.4); anti-F4/80, 1:100 (catalog no. BMB, anti-PD-L1 (catalog no. CD274), 1:100 (catalog no. MI15); anti-MHCII (catalog no. IAd-IEd), 1:2000 (catalog no. M5/114.15.2); anti-sXBP1, 1:50 (catalog no. Q3-695); anti-CD206, 1:100 (catalog no. MMR); anti-ARG1, 1:100 (catalog no. A1exF5) and anti-phosphoPERK (catalog no. Thr980), 1:100 (catalog no. BS33309). These antibodies were purchased from BioLegend, BD Biosciences or Invitrogen.

Fatty acid uptake and lipid content measurement. To determine fatty acid uptake, cells were cultured in RPMI medium containing 2% FBS, DMSO (1 μg ml−1, Sigma–Aldrich) and collagenase (1 mg ml−1, Sigma–Aldrich), followed by digestion at 37 °C for 50 min. After digestion, the samples were filtered through a 70-μm cell strainer. We performed leukocyte enrichment by density gradient centrifugation (800 g, 30 min) at 25% with 40% and 80% Percoll (GE Healthcare). Isolated cells were incubated with Fc receptor-blocking anti-CD16/32 (93) antibodies (BioLegend) at 4 °C for 10 min, washed and stained for surface markers for 30 min on ice. The intracellular staining procedure was then performed as described16. Fluorescence-activated cell sorting (FACS) analyses were performed using an LSR II flow cytometer (BD Biosciences) with BD FACSDiva software (v.8.0.1). We performed data analysis using FlowJo. mTAMs were defined as CD45+CD3 Lyt2CD11b+F480+Ly6C−; iTAMs were defined as CD45+CD3 Lyt2CD11b+F480+Ly6C+. The following antibodies were used: anti-CD45.2, 1:100 (catalog no. Ali4A2); anti-CD3e, 1:100 (catalog no. 17A2); anti-Gr1, 1:100 (catalog no. RB8-6C3); anti-Ly6G, 1:200 (catalog no. 1A8); anti-Ly61b, 1:200 (catalog no. M170); anti-Ly6C, 1:2000 (catalog no. HK1.4); anti-F4/80, 1:100 (catalog no. BMB, anti-PD-L1 (catalog no. CD274), 1:100 (catalog no. MI15); anti-MHCII (catalog no. IAd-IEd), 1:2000 (catalog no. M5/114.15.2); anti-sXBP1, 1:50 (catalog no. Q3-695); anti-CD206, 1:100 (catalog no. MMR); anti-ARG1, 1:100 (catalog no. A1exF5) and anti-phosphoPERK (catalog no. Thr980), 1:100 (catalog no. BS33309). These antibodies were purchased from BioLegend, BD Biosciences or Invitrogen.

Electron microscopy. Sorted cells were fixed in 2.5% glutaraldehyde solution (EMS) at 4 °C for 2 h, then postfixed with 1% osmium tetroxide (EMS)/1.5% potassium ferrocyanide (Sigma) for 1 h at room temperature. After several washes and dehydration in acetone (Sigma), cells were then embedded in Epon resin (Sigma). Sections of 50 nm were prepared on a Leica Ultracut microtome (Leica Microsystems), followed by poststaining with 4% uranyl acetate (Sigma) and Reynolds’ lead citrate (Sigma). Images were recorded with a transmission electron microscope Philips CM100 (ThermoFisher Scientific) at an acceleration voltage of 80 kV with a TemCam-F416 digital camera (TVIPS). Analysis and quantification were performed using ImageJ software. For assessing ER extension, each dot represents the total length of the ER compartment assessed by analyzing the length of each selected line of ER in the region of interest manager.

RNA sequencing. The RNA sequencing (RNA-seq) data was processed using the standard RNA-seq analysis pipeline as described previously1. Briefly, we performed read alignment using tophat2 v.2.1.0 (parameters ‘--no-novel-junctions’ and ‘--G’ when specifying the genome file), with read alignment using tophat2 v.2.1.0 (parameters ‘--no-novel-junctions’ and ‘--G’ when specifying the genome file), with
Articles

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of re-equilibration to the initial chromatographic conditions. The flow rate was applied for 2 min, then from 20% to 80% B for 3 min, followed by 3 min for 15 min to pellet the mitochondria. The supernatants were subjected to centrifugation at 6,000 g for 15 min. Supernatants from both centrifugation steps were then collected.

The microsomal compartment of the cells was isolated through ultracentrifugation as previously described. Briefly, the cells were collected in PBS and, after centrifugation, were resuspended with ice-cold 250-μLSTDMP buffer (250 mM sucrose, 50 mM Tris-Cl (pH 7.4), 5 mM MgCl2) in the presence of protease inhibitors and homogenized for a minimum of 5 min using a tight-fitting pestle. The solution was transferred to a tube and centrifuged at 800 g for 15 min at 4°C. The supernatant was transferred to a new tube and pellets were resuspended in five volumes of 250-μLSTDMP buffers and rehomogenized for 10 min in the homogenizer. The solution was then centrifuged at 800 g for 15 min. Supernatants from both centrifugation steps were then collected and subjected to centrifugation at 6,000 g for 15 min to pellet the mitochondria. The remaining supernatants were subjected to ultracentrifugation for 1 h at 100,000 g in a swing-bucket ultracentrifuge to collect pellets representing the microsomal (ER) fraction. Microsomal pellets were then resuspended in methanol:water (80:20) and then 25 μL of the resulting solution was extracted with 125 μL of butanol:methanol (1:1) by vortexing. The extracts were centrifuged for 15 min at 4,000 g at 4°C (Hermle) and the resulting supernatant was collected and transferred to liquid chromatography-mass spectrometry (LC-MS) vials for injection. Cell extracts were analyzed by hydrophilic interaction liquid chromatography coupled to tandem mass spectrometry (HILIC–MS/MS) in both positive and negative ionization modes using a Q-TRAP 6500 plus LC-MS/MS instrument (Sciex). Lipid separation was carried out on an Acquity BEH Amide, 2.1 mm × 50 mm, 1.7 μm inner diameter column (Waters). The mobile phase was composed of A = 10 mM ammonium acetate in acetoni-trile:water (95:5) and B = 10 mM ammonium acetate in acetonitrile:water (50:50). The linear gradient elution from 0.1% to 2% B was applied for 2 min, then from 20% to 80% B for 3 min, followed by 3 min of re-equilibration to the initial chromatographic conditions. The flow rate was 600 μL/min, column temperature 45°C and sample injection volume 2 μL. Optimized electrospray ionization (ESI) parameters were selected as follows: ion spray (IS) voltage 5,500 V in positive mode and −4,500 V in negative mode, curtain gas 35 psi, nebulizer gas (GS1) 50 psi, auxiliary gas (GS2) 60 psi and source temperature 550°C. Nitrogen was used as the nebulizer and collision gas. Optimized compound-dependent parameters were used for data acquisition in scheduled multiple reaction monitoring mode. Pooled quality control (PQC) samples (replicates of the entire sample set) were analyzed periodically (every four to five samples) throughout the overall analytical run, to assess the quality of the data, correct the signal intensity drift and remove peaks with poor reproducibility (coefficient of variation >30%). In addition, a series of diluted quality controls (QC) were prepared by dilution with butanol:methanol:100% QC, 50% QC, 25% QC, 12.5% QC and 6.25% QC. QC samples were analyzed at the beginning and at the end of the sample batch. This QC dilution series served as a linearity filter to remove the features which do not respond linearly (correlation with dilution factor <0.65). Raw LC–MS/MS data was processed using the MultiQuant Software (v.3.0.3, Scieix). Relative quantification of metabolites was based on extracted ion chromatogram areas for the monitored multiple reaction monitoring transitions. The obtained tables (containing peaks areas of detected metabolites across a data set) were exported to “R” software (http://cran.r-project.org/) where signal intensity drift was corrected in the LOWESS/Spline normalization program followed by noise filtering (coefficient of variation (QC features) >30%) and visual inspection of the linear response.

Measurement of β-glucosidase activity. Serum and tumor interstitial fluid were isolated from YUMM1.7 or MEF cells using the kit LEGENDplex murine Th Cytokine panel 12-plex, according to the manufacturer’s instructions. Samples were acquired on LSRII and analyzed using LEGENDplex software (BioLegend). Cytokine and fatty acids in CM and without lipids were measured using a Total Cholesterol and Cholesteryl Ester Colorimetric/Fluorometric Assay Kit (Biovision) and Free Fatty Acid Quantification Colorimetric/Fluorometric Kit (Biovision), respectively. Quantification was performed following the manufacturer’s instructions.

Proximity ligation assay. BMDMs from indicated treatment conditions were fixed with 4% paraformaldehyde at 37°C for 15 mins and permeabilized with 0.2% Triton in PBS at room temperature for another 15 min. Duolink in Situ Red Starter Kit Mouse/Rabbit was used according to the manufacturer’s instructions. Anti-ST3 (catalog no. 124H) Mouse Ab and anti-IRE1 rabbit polyclonal Ab (catalog no. 37073) were used as primary antibodies. Images were acquired with a confocal microscope (Zeiss catalog no. LSM800) and quantified using ImageJ software.

Data analysis and statistics. Biological replicates and presentation in each figure are shown as mean ± s.e.m. as mentioned in the figure legends. Statistical significance was determined by using two-tailed, unpaired, Student’s t-test, paired t-test, ordinary one-way analysis of variance (ANOVA) corrected for Sidak’s or Tukey’s multiple comparison test, or multiple repeated one-way ANOVA with Bonferroni’s multiple comparison as mentioned in the figure legends. No statistical method was used to predetermine sample size, but our sample sizes are similar to those reported in previous publications. Data distribution was assumed to be normal, but this was not formally tested. Unless when restricted by the genotype, animals and cell plates were assigned randomly to experimental conditions. Data collection and analysis were not performed blind to the conditions of the experiments. No data exclusion was performed.

In this study, further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

RNA-seq results are available in the Gene Expression Omnibus database under accession code (GSE166735). Other data are available from the corresponding author upon request. Source data are provided with this paper.

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Author contributions
G.D.C. and P.-C.H. designed the research. G.D.C., Y.-R.Y. and C.-H.T. performed in vivo experiments. G.D.C., Y.-R.Y. and X.L. performed in vitro experiments. H.G.-A. and J.I. performed lipidomic experiments and analysis. F.F. performed electron microscopy analyses. L.Z., M.F. and L.N.R. performed western blots. X.X. and P.P. performed computational analysis of single-cell RNA sequencing. Z.X. and J.W.L. performed computational analysis of single-cell RNA sequencing of human and murine tumor cohorts. S.C.-C.H. and F.M. provided feedback and advice. G.D.C. and P.-C.H. wrote the manuscript.

Competing interests
P.-C.H. is scientific advisory for Elixiron Immunotherapeutics, Acepodia and Novartis. P.-C.H. also receives research support from Roche and Elixiron. J.W.L. is a paid advisor to Restoration Foodworks. The remaining authors declare no competing interests.

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Correspondence and requests for materials should be addressed to Ping-Chih Ho.
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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | TAMs display high lipid content and ER stress responses in the inducible Braf/Pten melanoma model. a, Representative histogram (left) and quantitative plot (right) of BODIPY C12 staining in splenic macrophages (n = 10) or tumor-associated macrophages (TAMs) (n = 11) from YUMM1.7 melanoma bearing mice. Data are pooled from two independent experiment. b–h, Analysis of Braf/Pten tumor-bearing mice seven weeks after tamoxifen administration. b, c, Representative histogram (left) and quantitative plot (right) of BODIPY staining (b) and BODIPY C12 staining (c) in splenic macrophages or TAMs isolated from Braf/Pten tumor-bearing mice (n = 5 per group). d, Representative histogram (left) and quantitative plots (right) of BODIPY staining in TAMs gated based on ARG1 expression (n = 10 per group). e, qPCR analysis of mRNA expression of the indicated genes in splenic macrophages and TAMs isolated from Braf/Pten tumor-bearing mice (n = 4 per group). f, Representative histogram (left) and quantitative plot of the abundance (right) of sXBP1+ subset among splenic macrophages and TAMs in Braf/Pten tumor-bearing mice (n = 10 per group). g, Representative histogram (left) and quantitative plot (right) of BODIPY staining in TAMs gated based on sXBP1 expression (n = 10 per group). h, Representative histogram (left) and quantitative plot of the abundance (right) of sXBP1+ cells among ARG1+ and ARG1− TAMs (n = 10 per group). i, Gating strategy applied to define ARG1+ macrophages from spleen and tumor of YUMM1.7 tumor-bearing mice. j–l, Representative histogram (j) and quantitative plot of BODIPY staining (k) or sXBP1 (l) in skin-resident macrophages or TAMs from Braf/Pten tumor-bearing mice (n = 10 per group). m, Representative histogram (left) and quantitative plot (right) of pPERK staining in splenic macrophages or tumor-associated macrophages (TAMs) (n = 9 per group) of YUMM1.7 melanoma bearing mice. Data are pooled from two independent experiments. n, qRT-PCR of the indicated genes from sorted splenic macrophages and TAMs (n = 8 per group) isolated from YUMM1.7 tumor-bearing mice. Data are representative of two independent experiments (b, c, e). Each symbol represents one individual. All data are mean ± s.e.m and were analyzed by two-tailed, unpaired Student's t-test or paired t-test (d, g, h).
Extended Data Fig. 2 | Tumor cells reinforce protumorigenic polarization in macrophages via an IL-4/IL-13 independent manner. 

a, Representative histogram (left) and quantitative plot (right) of BODIPY FL C12 staining in BMDM cultured in DMEM (Ctrl) or YUMM1.7 CM (n = 4 per group). Data are representative of two independent experiments. 

b, c, Representative histogram (left) and quantitative plot (right) of ARG1 (b) and MRCl (c) expression in BMDMs cultured in DMEM or CM (n = 3 per group). Data are representative of three independent experiments. 

d, qPCR analysis of mRNA expression of the indicated genes in BMDMs cultured in DMEM or CM for 18 h (n = 3 per group). Data are representative of two independent experiments. 

e, Proliferation of CFSE-labeled T cells activated with anti-CD3 and anti-CD28 alone or in co-culture with BMDM Naïve or previously exposed to CM in a ratio 2:1 (n = 6). Data are pooled of two independent experiments. 

f, qPCR analysis of Arg1 mRNA expression in BMDMs treated with IL-4 and IL-13 (10 ng/ml) in the absence or presence of 0.25 μg/ml anti-IL-4 and 0.25 μg/ml anti-IL-13 neutralizing antibodies for 18 h (n = 3 per group). 

g, qPCR analysis of mRNA expression of indicated genes in BMDMs treated with CM in the absence or presence of 0.25 μg/ml anti-IL-4 and 0.25 μg/ml anti-IL-13 neutralizing antibody for 18 h (n = 3 per group). Data are representative results of three independent experiments. 

h, Multiplex cytokine array was used to determine the concentration of IL-13 (left) and IL-4 (right) in CM from YUMM1.7 and MEF cells. Stand0 to Stand7 show the increased concentration detected by the standard provided by the kit (n = 4). Data are representative of two independent experiments. 

i-k, Representative histogram (left) and quantitative plot (right) of BODIPY staining (i), and protein expression of ARG1 (j), and MRCl (k) in BMDMs stimulated with regular culture medium (Ctrl) or CM from YUMM1.7 (CM) or MEF (CM MEF) (n = 3 per group). Data are representative results of three independent experiments. 

l, qPCR analysis of indicated genes in BMDMs exposed to CM or Tunicamycin for 18 h (n = 3 per group). Data are representative results of three independent experiments. 

m, Quantification of BODIPY staining in BMDMs treated with CM in the absence or presence of 50 μM STF083010; Ctrl (n = 7), CM (n = 7), CM-STF (n = 6). Data are pooled from three independent experiments. 

n, Immunoblots of indicated proteins in BMDM transduced with retrovirus expressing scramble or IRE1-targeting gRNAs. 

o, qPCR analysis of BIP and sXBP1 mRNA expression in BMDMs treated with 1 μg/ml of Tunicamycin, 1 μM thapsigargin and CM for 16 h (n = 3 per group). Data are representative results of three independent experiments. Data are mean ± s.e.m. were analyzed by two-tailed, unpaired Student's t-test or one-way ANOVA with Tukey's multiple comparison test (e).
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | XBP1 supports protumorigenic polarization in response to cancer-derived stimuli. a, Immunoblots of indicated proteins in control or XBP1-deficient BMDMs stimulated with or without 1 μM thapsigargin for 6 h. Data are representative results of two independent experiments. b, Quantification of BODIPY staining in BMDMs generated from WT (XBP1wt) or KO (XBP1cKO) mice cultured stimulated with regular culture medium (Ctrl) or YUMM1.7 CM (n = 12 per group). Data are pooled from three independent experiments. c, Percentages of TAMs (F4/80+ CD11b+ Gr1−) among CD45+ cells in melanomas from tumor-bearing XBP1wt (n = 5) and XBP1cKO mice (n = 6). Data are representative results of three independent experiments. d, e, Tumor growth (d) and tumor weight (e) of YUMM1.7-OVA melanoma from WT and XBP1cKO mice treated with PBS or with anti-CSF1R as indicated in the methods; XBP1wt PBS (n = 13), XBP1cKO PBS mice (n = 12), XBP1wt αCSF1R (n = 12), XBP1cKO αCSF1R (n = 13). Data are pooled from three independent experiments. f, Percentages of TAMs (F4/80+ cells gated on CD11b+ Gr1−) among CD45+ cells in the experiment showed in d, e (n = 8 per group for XBP1wt PBS, XBP1cKO PBS, XBP1wt αCSF1R; n = 9 for XBP1cKO αCSF1R). Data are pooled from two independent experiments. g, Representative plots of iTAMs and mTAMs populations in tumor and spleen of YUMM1.7 tumor-bearing. h–k, Representative histograms (up) and quantitative plots (down) of PDL1 (h), MHCII (i), sXBP1 (j) and ARG1 (k) expression in iTAMs and mTAMs from YUMM1.7 tumor-bearing mice (n = 6). Data are pooled from two independent experiments. l, Representative plots of iTAMs and mTAMs populations in tumor and spleen of Braf/Pten melanoma-bearing mice. m–p, Representative histograms (up) and quantitative plots (down) of PDL1 (m), MHCII (n), sXBP1 (o) and ARG1 (p) expression in iTAMs and mTAMs from Braf/Pten melanoma-bearing mice (n = 5). Data are representative of two independent experiments. Each symbol represents one individual. q–t, Tumor growth (q) and tumor weight (r) of B16-OVA melanoma and tumor growth (s) and tumor weight (t) of MC38-OVA colon adenocarcinoma in XBP1wt (n = 9 for B16-OVA and n = 8 for MC38-OVA) or XBP1cKO (n = 10 for B16-OVA and n = 7 for MC38-OVA) mice. Data are pooled from two independent experiments. Data are mean ± s.e.m. were analyzed by two-tailed, unpaired Student’s t-test.
Extended Data Fig. 4 | STAT3 is required for CM-induced protumorigenic polarization. 

a, Immunoblots of indicated proteins in BMDMs expressing scramble or STAT3-targeting gRNAs treated with 10 ng/ml IL-6 for 6 h. Data are representative results of two independent experiments. 

b, c, Immunoblots of indicated proteins (b) and qPCR analysis of mRNA expression of indicated genes (c) in BMDMs treated with CM in the presence of vehicle (CM) or 10 μM Stattic (CM + Stattic) (n = 6 for Ctrl and n = 5 for CM and CM + Stattic). Data are representative results of three independent experiments. 

d, Multiplex cytokine array was used to determine the concentration of IL-10 (left) and IL-6 (right) in CM from YUMM1.7 and MEF cells. Stand0 to Stand7 show the increased concentration detected by the standard provided by the kit (n = 4). Data are representative results of two independent experiments. 

e, Immunoblot of indicated proteins in BMDMs treated with control media (Ctrl), cancer cell conditioned media (CM) or CM plus 50 μM STF081030 for 18 h. Data are representative of two independent experiments. 

f, Immunoblot of BMDMs treated with control vehicle (Ctrl), cancer cell conditioned media (CM) or tunicamycin (1 μg/ml; Tuni) for the indicated time points. Data are representative of two independent experiments. All data are mean ± s.e.m and were analyzed by two-tailed, unpaired Student’s t-test.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | β-glucosylceramide, rather than cholesterol, is released by tumor cells to mediate the protumorigenic polarization in macrophages. a, b, Quantification results of Cholesterol (a) (n = 6 per group) and fatty acids (b) (n = 4 per group) on YUMM1.7 CM prior (Yumm1.7) and after treatment with lipid removal reagent (Y1.7 w.o. Lipids). Data are pooled from two independent experiments. c, Immunoblot of YUMM1.7 cells stably transduced with lentivirus expressing short hairpin RNA against scramble or HMGCR sequence. d, qPCR analysis of indicated genes in BMDMs exposed to CM isolated from YUMM1.7 shCTRL or from YUMM1.7 shHMGCR (n = 3). Data are representative of three independent experiments. e, qPCR analysis of mRNA expression of indicated genes in BMDMs treated with CM in the absence or presence of 1 μg/ml α-CD36 antibody (n = 3 per group). Data are representative results of two independent experiments. f, Quantification of BODIPY staining in BMDM cultured with regular culture medium (Ctrl) or with YUMM1.7 CM in the absence or presence of 5 μg/ml α-Mincle antibody; Ctrl (n = 9), CM (n = 9), CMα-Mincle (n = 8). Data are pooled from three independent experiments. g, h, Immunoblot and quantification of the indicated proteins (g) (n = 4 per group) and qPCR analysis of mRNA expression of ARG1 and MRC1 (h) of WT or Mincle-KO BMDMs exposed to regular culture medium (Ctrl) or or with YUMM1.7 CM for 18 h (n = 9 per group). Data are pooled from three independent experiments. i, Proliferation of CFSE-labeled T cells activated with anti-CD3 and anti-CD28 alone or co-cultured with WT or Mincle-KO BMDMs previously treated with CM in a ratio 2:1 for 72 h; T cells (n = 6), WT (n = 8), KO (n = 9). Data are pooled from three independent experiments. j, Quantification result of indicated β-glucosylceramide levels from CM derived from YUMM1.7 shCTRL and YUMM1.7 shUGCG cells (n = 3 per group). k, qPCR analysis of the indicated genes in BMDMs treated with Ctrl or CM derived from YUMM1.7 shCTRL and YUMM1.7 shUGCG cells alone or in presence of α-Mincle antibody (5 μg/ml) (n = 3). Data are representative of two independent experiments. l–m, Quantification result of indicated β-glucosylceramide levels in serum and tumor interstitial fluid (TIF) isolated from YUMM1.7 melanoma-bearing mice (l) or Braf/Pten melanoma-bearing mice (m) (n = 5 per group). All data are mean ± s.e.m and were analyzed by two-tailed, unpaired Student's t-test (a–f), paired Student's t-test (g, j, l–m), one-way ANOVA with Sidak’s multiple comparison test (i), one-way ANOVA with Tukey’s multiple comparison test (k).
Extended Data Fig. 6 | Macrophage-specific ablation of LPCAT3 abolishes GW3965 anti-tumor responses. a, b, qPCR analysis of mRNA expression of sXBP1 (a) and immunoblot and quantification (b) of indicated proteins in WT or LPCAT3-KO BMDMs exposed to regular culture medium (Ctrl) and YUMM1.7 CM in absence or presence of 3μM GW3965 (n=9 per group for qPCR and n=3 per group for immunoblots). Data are pooled from three independent experiments. c, Proliferation of CFSE-labeled T cells activated with anti-CD3 and anti-CD28 alone or co-cultured with WT or LPCAT3-KO BMDMs previously treated with YUMM1.7 CM in the absence or presence of GW3965 in a ratio 2:1 for 72 h (n=3 per group). Data are representative of three independent experiments. d, Illustration of experimental design for bone marrow transplantation. e, qPCR analysis of exon 3 of LPCAT3 gene in LPCAT3<sup>fl/fl</sup> (WT) and LysM-Cre LPCAT3<sup>fl/fl</sup> (KO) mice (n=15). f, Bone marrow was isolated from WT and KO chimeric tumor-bearing mice and the abundance of indicated immune cells was measured by flow cytometry (n=4). g, Percentage of mTAMs among CD11b<sup>+</sup> tumor-infiltrating myeloid cells from YUMM1.7-OVA melanoma treated with either control vehicle or GW3965 in mice transplanted with BM cells from LPCAT3<sup>fl/fl</sup> (WT) and LysM-Cre LPCAT3<sup>fl/fl</sup> (KO) mice (WT + Vehicle: n=9; WT + GW3965: n=10; KO + Vehicle: n=10; KO + GW3965: n=9). Data are pooled from two independent experiments. Each symbol represents one individual. All data are mean ± s.e.m and were analyzed by two-tailed, unpaired Student’s t-test (a, e-g), RM one-way ANOVA with Bonferroni’s multiple comparison test (b), and ordinary one-way ANOVA with Tukey’s multiple comparison test (c).
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Sample size
Sample size were chosen based on prior experience and prior published studies with similar experimental layout (see references in Method section).

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No data exclusions

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All experiments have been reproduced at least 2 or 3 times with similar results. In many instances, the experiments have been pooled.

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Unless when restricted by genotype of the animals, animals and in vitro experimental groups were randomly assigned to different treatment groups.

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For in vivo tumor experiments, the analysis was not blinded because the genotype of the mice was previously known.

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Materials & experimental systems

n/a Involved in the study
☐ ☐ Antibodies
☐ ☐ Eukaryotic cell lines
☐ ☐ Palaeontology and archaeology
☐ ☐ Animals and other organisms
☐ ☐ Human research participants
☐ ☐ Clinical data
☐ ☐ Dual use research of concern

Methods

n/a Involved in the study
☐ ☐ ChiP-seq
☐ ☐ Flow cytometry
☐ ☐ MRI-based neuroimaging

Antibodies

Antibodies used
Western blot antibody:
- anti-XBP1 [O2C1F-Cell Signaling],
- anti-XBP1 [61395-Biolegend],
- phospho-Y705 STAT3 [AP0070-Abclonal],
- STAT3 [A1192-Abclonal],
- monoclonal anti-bactin, clone AC-74 [A2228-Sigma],
- anti-Tubulin [AG-27B-005 Adipogen],
- anti-UGCG (ab124296 Abcam),
- anti-PERK [C33E10-Cell Signaling],
- anti-IRE1 [14C10-Cell Signaling],
- anti-AIF4 [sc-200, Santa Cruz].
- PLA antibodies.
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)
The original cell line YUMM1.7 melanoma cell line was provided by Marcus Bosenberg [ATCC]. The YUMM1.7-OVA melanoma cell lines were generated as described in methods. Phoenix cell line, B16-OVA and MC38-OVA were provided by Pedro Romero, University of Lausanne.

Authentication
None of the cell lines were authenticated in these studies, but low passage number cell lines were utilized.

Mycoplasma contamination
All the cell lines are mycoplasma-free. They have been tested for mycoplasma contamination regularly.

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No commonly misidentified cell lines were used.

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Laboratory animals
All the mouse experiments were carried out with sex and age matched [male and female] groups. All animal were used between the ages of 3-7 weeks. For bone marrow chimerism experiment mice were irradiated at 7 weeks and kept in experiment until week 16. The strains and source of mice:

- C57BL/6j (WT), LysM-Cre (B6.129p2-Lys2tm1[cre]flo/+), B6 Cas9 (B6.129(Cg)-Gtl[ROSA]26Sortm1.1[CAG-cas9*-EGFP]flo/h): purchased form the Jackson Laboratory.
- B6aF1; Tyr::CreER; Ptenflox-5 (Braf/Pten) mice were obtained form Marcus Bosenberg, Yale University. XSP1 fil/m mice were provided by Laurie Gilmcher and Gakhan Hotamisligil.

All mice housed in conventional animal facility of University of Lausanne were kept in individually ventilated cages, between 19-23 °C with 45-65% humidity and a 12hour dark/light cycle.

Wild animals
No wild animals were involved.

Field-collected samples
No samples were collected from the field.

Ethics oversight
All experiments were conducted according to Swiss federal regulations and approved by the veterinary authority of Canton Vaud.

Flow Cytometry

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a `group` is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.
Methodology

Sample preparation

For TAMs, Tumors were minced in RPMI with 2% FBS, collagenase IV (0.5 mg/mL, Sigma-Aldrich), and DNase (1 μg/mL, Sigma-Aldrich) and digested at 37°C for 45mins. The digested samples were then filtered through cell strainer and washed with FACS buffer (PBS with 2% FBS and 2mM EDTA). Next, viable cells in tumor single cell suspensions were further enriched by density gradient centrifugation (8000g, 30min) at room temperature with 40% and 80% percoll (GE healthcare) and collected from the interphase of the gradient. For BMDM, cells were scraped, collected and washed in FACS buffer before staining.

Instrument

Cells were analyzed on LSRII flow cytometers (BD Biosciences)

Software

BD FACSDiva was used for data acquisition. Flowjo v10.6.1 was used for data analysis.

Cell population abundance

All analysis have been performed on a population that included between 1000 and 20000 macrophages within tumor and spleen samples, depending on initial material abundance.

Gating strategy

TAMs and splenic macrophages have been defined as CD45+Gr1-CD11b+F4/80+, immature TAMs as CD45+Ly6G-CD11b+F4/80+Ly6C-, mature TAMs as CD45+Ly6G-CD11b+F4/80+Ly6C-.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.