Targeting tumor-associated macrophages (TAMs) is a promising strategy to modify the immunosuppressive tumor microenvironment and improve cancer immunotherapy. Monoamine oxidase A (MAO-A) is an enzyme best known for its function in the brain; small molecule MAO inhibitors (MAOIs) are clinically used for treating neurological disorders. Here we observe MAO-A induction in mouse and human TAMs. MAO-A-deficient mice exhibit decreased TAM immunosuppressive functions corresponding with enhanced anti-tumor immunity. MAOI treatment induces TAM reprogramming and suppresses tumor growth in preclinical mouse syngeneic and human xenograft tumor models. Combining MAOI and anti-PD-1 treatments results in synergistic tumor suppression. Clinical data correlation studies associate high intratumoral MAOA expression with poor patient survival in a broad range of cancers. We further demonstrate that MAO-A promotes TAM immunosuppressive polarization via upregulating oxidative stress. Together, these data identify MAO-A as a critical regulator of TAMs and support repurposing MAOIs for TAM reprogramming to improve cancer immunotherapy.

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er the past decade, cancer immunotherapy has achieved significant breakthroughs. In particular, immune checkpoint blockade (ICB) therapy has yielded remarkable clinical responses and revolutionised the treatment of many cancers. So far, the FDA has approved cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed cell death protein 1/ligand 1 (PD-1/PD-L1) blockade therapies for treating more than ten different malignancies; however, only a small fraction of cancer patients respond to these therapies. Most ICB therapies work through enhancing antitumor CD8+ T-cell responses, which can be greatly limited by the immunosuppressive tumor microenvironment (TME). Tumor-associated macrophages (TAMs), a key component of the immunosuppressive TME, dampen T-cell antitumor reactivity in the majority of solid tumors. Growing evidence suggests that TAMs are responsible for inhibiting antitumor T-cell reactivity and limiting the ICB therapy efficacy, making TAMs potential targets for reversing the immunosuppressive TME and improving cancer immunotherapy.

In general, TAMs are considered to mature from bone marrow-derived circulating monocytes, these monocytes are recruited to the tumor sites, exposed to chemokines and growth factors in the TME, and subsequently differentiate into TAMs. There is also increasing evidence for tissue-resident macrophage–originated TAMs. Depending on the surrounding immune environment, macrophages can be polarized towards an immunostimulatory phenotype by pro-inflammatory stimuli (e.g., IFN-γ) or towards an immunosuppressive phenotype by anti-inflammatory stimuli (e.g., IL-4 and IL-13). Although a binary polarization system is commonly used in macrophage studies, in most large-scale transcriptome analyses, TAMs showed a continuum of phenotypes expressing both immunostimulatory and immunosuppressive markers in addition to the extreme ends of polarization. These mixed phenotypes and polarization states suggest the complexity of the TME and the residential TAM functionality. As a tumor develops, the enrichment of IL-4 and IL-13 produced by tumor cells and CD4+ T cells in the TME results in the polarization of TAMs towards an immunosuppressive phenotype, that promotes tumor growth, malignancy, and metastasis. In established solid tumors, TAMs predominantly exhibit an immunosuppressive phenotype, evidenced by their production of anti-inflammatory cytokines and arginase-1 (Arg1), as well as their expression of mannose receptor (CD206) and scavenger receptors. Through metabolising l-arginine via Arg1, TAMs can directly suppress cytotoxic CD8+ T-cell responses. Mannose receptor (CD206) expressed by TAMs can impair cytotoxicity of CD8+ T cells by suppressing CD45 phosphatase activity. In addition, TAMs can inhibit T-cell activities through immune checkpoint engagement by expressing the ligands of the inhibitory receptors PD-1 and CTLA-4. For example, PD-L1 and PD-L2 expressed on TAMs interact with PD-1 of T cells to directly inhibit TCR signalling, cytotoxic function, and proliferation of CD8+ T cells. These characteristics of TAMs make them potential targets for reversing the immunosuppressive TME to augment antitumor immunity.

Although the predominant phenotype of TAMs in established solid tumors is immunosuppressive, polarization is not fixed. Plasticity, one of the key features of TAMs, enables TAMs to change their phenotype in solid tumors and thereby providing a therapeutic window. Repolarizing/reprogramming TAMs from an immunosuppressive and tumor-promoting phenotype towards an immunostimulatory and tumoricidal phenotype has thus become an attractive strategy in immunotherapy. Preclinical and clinical studies are ongoing, evaluating TAM-repolarizing reagents (e.g., CD40 agonists, HDAC inhibitors, PI3K inhibitors, creatine, etc.) for improving ICB therapy; certain efficacies have been reported. Therefore, the search for new molecules regulating TAM polarization and the development of new combination treatments targeting TAM reprogramming is an active direction of current cancer immunotherapy studies.

Monoamine oxidase A (MAO-A) is an outer mitochondrial membrane-bound enzyme encoded by the X-linked MAOA gene. MAO-A is best known for its function in the brain, where it is involved in the degradation of a variety of monoamine neurotransmitters, including serotonin, dopamine, epinephrine, and norepinephrine. Through regulating the availability of serotonin, MAO-A modulates neuronal activities thereby influencing mood and behaviour in humans. Through regulating the availability of dopamine and the abundance of dopamine breakdown by-product hydrogen peroxide (H2O2; hence oxidative stress), MAO-A is involved in multiple neurodegenerative diseases, including Parkinson’s disease (PD). FDA-approved small-molecule MAO inhibitors (MAOIs) are currently available for the treatment of neurological disorders, including depression and PD.

In this study, we investigate the role of MAO-A in regulating TAM polarization and evaluate the possibility of repurposing MAOIs for reprogramming TAMs and improving cancer immunotherapy. We demonstrate that MAO-A promotes TAM immunosuppressive polarization and subsequent inhibition of antitumor immunity in mice via upregulating oxidative stress. MAOI treatment induces TAM reprogramming and suppresses tumor progression in preclinical mouse syngeneic and human xenograft tumor models. Combining MAOI and anti-PD-1 treatments result in synergistic tumor suppression. Clinical data correlation studies associate high intratumoral MAO-A expression with poor patient survival in a broad range of cancers. Together, these data identify MAO-A as a critical regulator of TAMs and support repurposing MAOIs for TAM reprogramming to improve cancer immunotherapy.

Results
MAO-A-deficient mice show reduced tumor growth associated with altered TAM polarization. In a search for new molecules regulating TAM reprogramming, we inoculated C57BL/6j mice with syngeneic B16-OVA melanoma tumors, isolated TAMs and assessed TAM gene expression profiles. Monocytes isolated from tumor-free and tumor-bearing mice were included as controls. In addition to changes in classical genes involved in regulating macrophage immune responses, we observed the induction of a Maa gene in TAMs (Fig. 1a), suggesting that MAO-A may be involved in modulating TAM activities.

To study the role of MAO-A in antitumor immunity in vivo, we used MAO-A-deficient mice that carry a hypomorphic MAO-A mutant. Although a degree of Maa expression leakage in the brain had been previously reported in these mice, analysis of their immune system showed nearly complete ablation of MAO-A expression in major lymphoid organs, including the spleen and bone marrow (BM) (Supplementary Fig. 1a). Since we focused on immune cells in this study, we denote these mice as Maa knockout (KO) mice. When challenged with B16-OVA melanoma cells (Fig. 1b), tumor growth in Maa KO mice was significantly suppressed compared to that in Maa wild-type (WT) mice (Fig. 1c, d). Although similar levels of TAMs (gated as CD45.2+CD11b+Ly6G−Ly6C−/lowF4/80+ cells) were detected in Maa WT and Maa KO mice (Supplementary Fig. 1b, c), compared to their WT counterparts, TAMs isolated from Maa KO mice exhibited a less immunosuppressive phenotype, indicated by their decreased expression of immunosuppressive markers (i.e., CD206; Fig. 1e), and their increased expression of immunostimulatory molecules (i.e., CD69, CD86 and MHC class
Fig. 1 MAO-A-deficient mice show reduced tumor growth associated with altered TAM polarization. a QPCR analyses of Maoa mRNA expression in TAMs isolated from wild-type mice-bearing B16-OVA tumors. Monocytes (Mo) were isolated from peripheral blood of tumor-free and tumor-bearing mice (**p < 0.001). N = 4. b–j Studying B16-OVA tumor growth in Maoa WT and Maoa KO mice. b Experimental design. c Tumor growth (**p = 0.0038, ***p < 0.001). d Tumor volume at day 18 (**p = 0.0038). e–h FACS analyses of CD206 (e) (**p < 0.001), CD69 (f) (**p < 0.001), CD86 (g) (**p = 0.0064) and I-Ab (h) (**p = 0.0275) expression on TAMs at day 18. WT, n = 9; KO, n = 8. MFI mean fluorescence intensity. i, j QPCR analyses of immunosuppressive (Mrc1, **p = 0.0041; Chi3l3, ***p < 0.001 and Arg1, *p = 0.0339; i) and immunostimulatory (Il6, ***p < 0.001; Ccl2, **p < 0.001 and Tnf, *p = 0.0036; j) signature genes mRNA expression in TAMs (n = 4). k–n scRNAseq analyses of tumor-infiltrating immune cells (TIIs) from Maoa WT and Maoa KO mice at day 14 post B16-OVA tumors challenge. k Uniform Manifold Approximation and Projection (UMAP) of single TIIs showing the formation of six cell clusters (TAM/Mono, T cell, NK cell, B cell, DC and pDC) from total CD45.2+ TIIs and five cell clusters (TAM_1, TAM_2, Mono_1, Mono_2 and Mono_3) from the TAM/Mono subpopulation. Each dot represents one single cell and is coloured according to cell types. Mono monocyte, NK natural killer cell, DC dendritic cell, pDC plasmacytoid dendritic cell. l UMAP of the TAM subpopulation, showing the formation of two clusters (TAM_1: Mrc1low Cd86high; and TAM_2: Mrc1high Cd86low). Each dot represents one single cell and is coloured according to cell clusters. Ratios of TAM_1:TAM_2 are presented. m, n Violin plots of immunosuppressive (Mrc1 and Chi3l3; m) and immunostimulatory (Ccl2, Cd7, Cd86, H2-Aa, and H2-Ab); n) signature genes expression in single TAMs. Each dot represents an individual cell. Representative of 1 (k–n), 3 (a), and 5 (b–j) experiments. Analysed by one-way ANOVA (a) or by Student’s t test (c–j). p values of violin plots are determined by Wilcoxon rank-sum test (m, n). Statistics are all two-sided. Source data are provided as a Source Data file.
II 1-Aβ; Fig. 1f–h). Further analysis showed that TAMs from Maoa KO mice expressed reduced levels of immunosuppression-associated genes (i.e., Mrc1, Chi3l3 and Arg1; Fig. 1i) and increased levels of pro-inflammatory cytokine genes (i.e., Il6, Tnfa and Ccl2; Fig. 1j). Corresponding to the altered TAM polarization in Maoa KO mice, tumor-infiltrating CD8+ T cells in these mice showed enhanced activation (i.e., increased production of Granzyme B; Fig. 2m) were observed in mice receiving Maoa KO BMDMs. Collectively, these in vivo studies demonstrate that MAO-A acts as an autonomous factor directly regulating TAM polarization, and thereby influencing T-cell antitumor reactivity and impacting tumor growth.

**MAO-A promotes macrophage immunosuppressive polarization**. To study MAO-A regulation of macrophage polarization, we cultured Maoa WT and KO BMDMs in vitro and polarized these macrophages towards an immunosuppressive phenotype by adding anti-inflammatory stimuli (i.e., IL-4 and IL-13; Fig. 3a). We observed a sharp induction of Maoa mRNA expression in Maoa WT BMDMs during M-CSF-induced macrophage differentiation; Maoa expression was then plateaued in matured BMDMs and maintained over IL-4/IL-13-induced immunosuppressive polarization (Fig. 3b, c). MAO-A expression was undetectable in Maoa KO BMDMs, confirming their Maoa-deficiency genotype (Fig. 3b, d). Compared to their wild-type counterpart, Maoa KO macrophages displayed a less immunosuppressive phenotype under IL-4/IL-13 stimulation, evidenced in their reduced expression of immunosuppressive markers (i.e., CD68, Fig. 3e) and signature genes (i.e., Chi3l3 and Arg1; Fig. 3f, g and Supplementary Fig. 3a). When tested in a macrophage/T-cell coculture assay (Fig. 3h), in agreement with their less immunosuppressive phenotype, IL-4/IL-13-polarized Maoa KO macrophages exhibited impaired suppression of wild-type CD8+ T cells under anti-CD3/CD28 stimulation, shown as their attenuated inhibition of CD8+ T-cell proliferation (Fig. 3i) and activation marker expression (i.e., upregulation of CD25 and CD44, and downregulation of CD62L; Fig. 3j, k and Supplementary Fig. 3b).

To verify whether MAO-A deficiency directly contributed to the alleviated immunosuppressive polarization of Maoa KO macrophages, we performed a rescue experiment. We constructed a MIG-Moaα retinoviral vector, used this vector to transduce Maoa KO BMDMs, and achieved overexpression of MAO-A in these macrophages (Fig. 3l–n and Supplementary Fig. 3c). MAO-A overexpression significantly exacerbated the immunosuppressive phenotype of IL-4/IL-13-polarized Maoa KO BMDMs (i.e., upregulation of immunosuppressive signature genes such as Chi3l3 and Arg1; Fig. 3o, p). Taken together, these results indicate that MAO-A acts as an autonomous factor promoting macrophage immunosuppressive polarization under anti-inflammatory stimuli.

**MAO-A directly regulates TAM polarization and influences TAM-associated T-cell antitumor reactivity**. In our Maoa KO mice tumor challenge study, MAO-A deficiency impacted both immune and non-immune cells (Fig. 1b). To determine whether MAO-A directly regulates immune cells, we conducted a BM transfer experiment wherein BM cells harvested from Maoa WT or KO mice were adoptively transferred into Boyj (CD45.1) WT-recipient mice followed by B16-OVA tumor challenge (Fig. 2a). In this experiment, MAO-A deficiency comparison was confined to immune cells. MAO-A deficiency in immune cells resulted in suppressed tumor growth (Fig. 2b, c), altered TAM polarization (i.e., downregulation of immunosuppressive markers such as CD206, Fig. 2d; and upregulation of immunostimulatory markers such as CD69, CD86 and MHC class II I-Ab; Fig. 2e) injection into Boyj WT-recipient mice to establish solid tumors (Fig. 2g). In this study, MAO-A-deficiency comparison was confined to TAMs. Suppressed tumor growth (Fig. 2h, i), downregulated expression of TAM immunosuppressive markers (i.e., CD206; Fig. 2j), upregulated expression of TAM immunostimulatory markers (i.e., CD69 and CD86; Fig. 2k, l) and enhanced tumor-infiltrating CD8+ T-cell reactivity (i.e., increased production of Granzyme B; Fig. 2m) were observed in mice receiving Maoa KO BMDMs. Collectively, these in vivo studies demonstrate that MAO-A acts as an autonomous factor directly regulating TAM polarization, and thereby influencing T-cell antitumor reactivity and impacting tumor growth.

**MAO-A promotes macrophage immunosuppressive polarization via ROS upregulation**. Next, we sought to investigate the molecular mechanisms regulating MAO-A promotion of macrophage immunosuppressive polarization. It has been reported that intracellular reactive oxygen species (ROS; hence, oxidative stress) elicit macrophage immunosuppressive features. MAO-A catalyzes the oxidative deamination of monoamines, thereby generating hydrogen peroxide (H2O2) as a by-product that can increase intracellular ROS levels. We, therefore, speculated that MAO-A might promote TAM immunosuppressive polarization in TME via upregulating ROS levels in TAMs (Fig. 4a).

To test this hypothesis, we directly measured ROS levels in TAMs isolated from Maoa WT and KO mice bearing B16-OVA tumors and detected significantly lower levels of ROS in Maoa KO TAMs (Fig. 4b, c). Measurement of ROS levels in vitro cultured Maoa WT and KO BMDMs also showed reduced levels of ROS in Maoa KO BMDMs, with or without IL-4/IL-13 stimulation, in agreement with the in vivo TAM results (Fig. 4d). Supplementation H2O2 to IL-4/IL-13-stimulated Maoa WT and KO BMDMs elevated their intracellular ROS to similar
levels (Supplementary Fig. 4a, b) and eliminated their differences in expression of immunosuppressive markers (i.e., CD206; Fig. 4e) and signature genes (i.e., \textit{Chi3l3} and \textit{Arg1}; Fig. 4f, g).

On the other hand, supplementation of tyramine, a substrate of MAO-A, increased ROS levels and upregulated the expression of immunosuppressive genes (i.e., \textit{Chi3l3} and \textit{Arg1}) in \textit{Maoa} \textit{WT} BMDMs but not in \textit{Maoa} \textit{KO} BMDMs (Fig. 4h–j). Taken together, these data indicate that MAO-A regulates macrophage immunosuppressive polarization via modulating macrophage intracellular ROS levels.

The JAK-Stat6 signalling pathway plays a key role in mediating IL-4/IL-13-induced immunosuppressive polarization of TAMs in TME\textsuperscript{37,38}. After IL-4/IL-13 stimulation, JAK is phosphorylated and subsequently phosphorylates Stat6; phosphorylated Stat6 dimerises and migrates to the nucleus, where it binds to the promoters of IL-4 and IL-13 responsive genes including those involved in macrophage immunosuppressive functions\textsuperscript{39}. ROS has been reported to promote JAK and Stat6 phosphorylation in a variety of cell types\textsuperscript{40,41}. Since we observed decreased ROS levels in \textit{Maoa} KO macrophages compared to those in \textit{Maoa} WT macrophages (Fig. 4b, c), we postulated that MAO-A may impact macrophage polarization through upregulating ROS levels and thereby sensitising the JAK-Stat6 signalling pathway. Indeed, direct analysis of TAMs isolated from B16-OVA tumor-bearing \textit{Maoa} \textit{WT} and \textit{Maoa} \textit{KO} mice confirmed that compared to wild-type TAMs, \textit{Maoa} \textit{DEFicient TAMs showed reduced Stat6 activation (i.e., reduced Stat6 phosphorylation; Fig. 4k, l). Further analysis of IL-4/IL-13-induced JAK-Stat6 signalling pathway in \textit{Maoa} KO BMDMs compared to that in \textit{Maoa} WT BMDMs showed significantly reduced JAK-Stat6 signalling (i.e., reduced JAK1, JAK2, JAK3 and Stat6 phosphorylation; Fig. 4m). Supplementing H$_2$O$_2$ to IL-4/IL-13-stimulated \textit{Maoa} WT and KO BMDMs increased their JAK-Stat6 signalling to similar levels (i.e., comparable JAK1, JAK2, JAK3 and Stat6 phosphorylation;
and four (followed by FACS sorting of GFP

Source data are provided as a Source Data

control BMDMs, IL-4/IL-13 IL-4 and IL-13-polarized BMDMs, ns not signifi-

cation of MAO-A as a

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Fig. 3 MAO-A promotes macrophage immunosuppressive polarization. a–g. Studying the in vitro differentiation and IL-4/IL-13-induced polarization of Maa WT (WT) and Maa KO (KO) BMDMs. a Experimental design. b. c. FACS analyses of Maa mRNA expression over the 6-day BMDM differentiation culture (b) (**p < 0.001) and IL-4/IL-13-induced polarization (c) (n = 6). d Western blot analyses of MAO-A protein expression in the indicated BMDMs. Source data are provided as a Source Data file. e. f. g. FACS analyses of CD206 expression on the indicated BMDMs (***p < 0.001). h. i. j. k. l. m. n. o. p. qPCR analyses of CD8+ T cells (identified as TCRβ+CD4+CD8+ cells) (**p < 0.001). j,k. FACS analyses of CD25 (1:2, **p < 0.001; 1:4, **p < 0.001; 1:8, *p = 0.0038) and CD62L (k) (**p < 0.001) expression on CD8+ T cells. l–p. Studying the IL-4/IL-13-induced polarization of Maa KO BMDMs with Maa-overexpression (n = 3). In vitro-cultured Maa KO BMDMs were transduced with either a MIG-Maa retrovector or a MIG mock retrovector, polarized with IL-4/IL-13, followed by FACS sorting of GFP+ Maa KO BMDMs for further analyses. I Schematics of the MIG and MIG-Maa retrovectors. m. n. qPCR analyses of prior-to-sorting Maa KO BMDMs, showing retrovector transduction efficiency (measured as %GFP+ cells). o. p. qPCR analyses of sorted GFP+ Maa KO BMDMs, showing the mRNA expression of Maa (m) (**p < 0.001), Chi3l3 (o) (**p = 0.0038), and Arg1 (p) (**p < 0.001). Representative of three (h–k, l–p) and four (a–g) experiments. ns not significant. Analysed by one-way ANOVA (b), two-way ANOVA (e, g, i, k) or by Student’s t test (c, n–p). Statistics are all two-sided. Source data are provided as a Source Data file.

Fig. 4m), corresponding to their comparable high levels of ROS (Supplementary Fig. 4a, b). These data indicate that MAO-A promotes macrophage immunosuppressive polarization via ROS-sensitised JAK-Stat6 pathway activation.

Collectively, these in vivo and in vitro data support a working model that MAO-A promotes TAM immunosuppressive polarization in TME, at least partly through upregulating TAM intracellular ROS levels and thereby enhancing the IL-4/IL-13-induced JAK-Stat6 signalling pathway.

**MAO-A blockade for cancer immunotherapy—synergistic mouse tumor model studies.** The identification of MAO-A as a key regulator of TAM immunosuppressive polarization makes MAO-A a promising drug target for cancer immunotherapy. Because of the known functions of MAO-A in the brain, small-molecule MAOIs have been developed and clinically utilised for treating various neurological disorders, making it a highly feasible and attractive approach to repurpose these established MAOI drugs for cancer immunotherapy31,42. In an in vitro WT BMDM IL-4/IL-13-induced polarization culture (Fig. 5a), addition of multiple MAOIs efficiently reduced ROS levels in BMDMs (Fig. 5b) and suppressed their immunosuppressive polarization, evidenced by their decreased expression of immunosuppressive markers (i.e., CD206; Fig. 5c) and immunosuppressive genes (i.e., Chi3l3 and Arg1; Fig. 5d, e). Notably, the MAOIs that we tested
**Fig. 4 MAO-A promotes macrophage immunosuppressive polarization via ROS upregulation.**

a Schematics showing MAO-A breaks down monoamines and generates hydrogen peroxide (H$_2$O$_2$) as a by-product, thereby increasing reactive oxygen species (ROS) levels in a TAM.
b,c Studying the in vivo ROS levels in TAMs isolated from Maoa WT and Maoa KO mice-bearing B16-OVA tumors ($n=4$).
b Experimental design.
c FACS analyses of ROS levels in TAMs at day 18. TAMs were gated as the CD45.2$^+$ CD11b$^+$ Ly6G$^-$ Ly6C$^-$/low F4/80$^+$ cells of total TIIs (**$p=0.0088$).
d Experimental design.
e FACS analyses of ROS levels in in vitro-cultured Maoa WT and Maoa KO BMDMs, without or with IL-4/IL-13 polarization ($n=4$). NC no cytokine, IL-4/IL-13 IL-4/IL-13-polarized. *$p<0.05$, **$p<0.01$ and ***$p<0.001$.
f,g QPCR analyses of Chi3l3 (f) and Arg1 (g) mRNA expression. *$p<0.05$, **$p<0.01$ and ***$p<0.001$.
h,j Study of IL-4/IL-13-polarized Maoa WT and Maoa KO BMDMs, with or without tyramine supplement ($n=3$). e FACS analyses of CD206 expression. f,j QPCR analyses of Chi3l3 (f) and Arg1 (j) mRNA expression. *$p<0.05$, **$p<0.01$ and ***$p<0.001$.
i,k Study of TAMs isolated from Maoa WT and Maoa KO mice-bearing B16-OVA tumors at day 18 (combined from five mice per group). k Experimental design. I Western blot analyses of TAMs. TAMs were FACS sorted as the DAPI$^-$ CD45.2$^+$ CD11b$^+$ Ly6G$^-$ Ly6C$^-$/low F4/80$^+$ cells from total TILs. Source data are provided as a Source Data file.

**m** Western blot analyses of JAK-Stat6 signalling in Maoa WT and Maoa KO BMDMs, with or without IL-4/IL-13 polarization and H$_2$O$_2$ treatment. BMDMs were treated with H$_2$O$_2$ for 30 min prior to IL-4/IL-13 stimulation for another 30 min. Representative of three experiments. Analysed by two-way ANOVA (d-j) or by Student’s t test (c).

Statistics are all two-sided. Source data are provided as a Source Data file.
include phenelzine, clorgyline, moclobemide, and pirilnole, covering the major categories of established MAOIs classified on the basis of whether they are non-selective or selective for MAO-A, and whether their effect is reversible (Fig. 5a)31,43. Among these MAOIs, phenelzine (trade name: Nardil) is clinically available in the United States42. In the following studies, we chose phenelzine as a representative to study the possibility of repurposing MAOIs for cancer immunotherapy, using two syngeneic

Fig. 5 MAO-A blockade for cancer immunotherapy—syngeneic mouse tumor model studies. a–e Studying the effect of MAOI treatment on IL-4/IL-13-induced BMDM polarization in vitro (n = 4). a Experimental design. Wild-type BMDMs were stimulated with IL-4/IL-13 with or without MAOI treatment. MAOIs (monoamine oxidase inhibitors) studied were phenelzine (Phe; 20 μM), clorgyline (Clo; 20 μM), moclobemide (Moc; 200 μM), and pirilnole (Pir; 20 μM). NT no MAOI treatment. b FACS analyses of ROS levels in BMDMs. c FACS analyses of CD206 expression on BMDMs. d, e QPCR analyses of Chi3l3 (d) and Arg1 (e) mRNA expression in BMDMs. **p < 0.001. f–j Studying the TAM-related cancer immunotherapy potential of MAOI treatment in a B16-OVA melanogenic syngeneic mouse tumor model. f Experimental design. B6 wild-type mice were treated with clodronate liposomes (Clod) to serve as TAM-depleted experimental mice or with vehicle liposomes (Veh) to serve as TAM-intact control mice. Phe phenelzine treatment, NT no phenelzine treatment. g Tumor growth. h Tumor volume at day 18 (**p < 0.001). I FACS analyses of CD206 expression on TAMs of TAM-intact experimental mice (p = 0.0164). J FACS analyses of intracellular Granzyme B production in tumor-infiltrating CD8+ T cells of all experimental mice (NT, *p = 0.0257; Veh, **p = 0.0025). Veh NT, n = 7; Veh Phe, n = 8; Clod NT, n = 7; Clod Phe, n = 7. k–o Studying the cancer therapy potential of MAOI treatment in combination with anti-PD-1 treatment in the B16-OVA melanoma and MC38 colon cancer syngeneic mouse tumor models (n = 5). k Experimental design. Tumor-bearing mice were treated with anti-PD-1 antibody (aPD-1) or isotype control (Iso), together with or without phenelzine (Phe) treatment. NT no Phe treatment. l B16-OVA tumor growth. m B16-OVA tumor volume at day 18. n MC38 tumor growth. o MC38 tumor volume at day 27. *p < 0.05, **p < 0.01 and ***p < 0.001. Representative of three experiments. Analysed by one-way ANOVA (b–e, h, j, m, o) or by Student’s t test (l). Statistics are all two-sided. Source data are provided as a Source Data file.
mouse tumor models: a B16-OVA melanoma model and a MC38 colon cancer model. Of note, phenelzine is a non-selective irreversible MAOI that inhibits both MAO-A and its isoenzyme MAO-B; however, because mouse macrophages predominantly express MAO-A over MAO-B, phenelzine treatment mainly regulates TAM reprogramming via inhibiting MAO-A in these tumor models (Supplementary Fig. 5a).

First, we studied the therapeutic potential of phenelzine in a B16-OVA tumor prevention model (Fig. 5f). Phenelzine treatment effectively suppressed B16-OVA tumor growth in B6 wild-type mice (Fig. 5g, h). No tumor growth difference was observed when we depleted TAMs in experimental mice via a clodronate liposome treatment, indicating that phenelzine suppressed tumor growth via modulating TAMs (Fig. 5g, h and Supplementary Fig. 5b). Correspondingly, TAMs isolated from phenelzine-treated mice displayed a less immunosuppressive phenotype, evidenced by their decreased expression of immunosuppressive markers (i.e., CD206; Fig. 5i) and signature genes (i.e., Chi3L3 and Arg1; Supplementary Fig. 5c) while increased expression of immunostimulatory markers (i.e., CD69, CD86 and I-Ab; Supplementary Fig. 5d–f), that was correlated with an enhanced antitumor reactivity of tumor-infiltrating CD8+ T cells (i.e., increased production of Granzyme B; Fig. 5j) in these mice. Further studies showed that phenelzine treatment also effectively suppressed the progression of pre-established solid tumors in both B16-OVA and MC38 models (Supplementary Fig. 5g–k).

Notably, similar to that for the B16-OVA tumor model, direct challenge of Maaal WT and KO mice with MC38 tumor cells also resulted in a significantly suppressed tumor growth in Maaal KO mice, confirming a general impact of MAO-A-deficiency on tumor growth for multiple tumor models (Fig. 1b–d and Supplementary Fig. 5l–n).

Next, we evaluated the potential of phenelzine for combination therapy, in particular combining with other ICB therapies, such as PD-1/PD-L1 blockade therapy (Fig. 5k). Although most ICB therapies target CD8+ T cells, these cells are in fact closely regulated by TAMs in the TME, making targeting TAMs another potential avenue for immunotherapy. In both B16-OVA and MC38 tumor models, phenelzine treatment significantly suppressed the progression of pre-established solid tumors at a level comparable to the anti-PD-1 treatment; importantly, the combination of phenelzine and anti-PD-1 treatments yielded synergistic tumor suppression efficacy (Fig. 5l–o). These tumor suppression effects of phenelzine were due to immunomodulation but not direct tumor inhibition, because phenelzine treatment did not suppress the growth of B16-OVA and MC38 tumors in immunodeficient NSG mice (Supplementary Fig. 5o–s).

Collectively, these syngeneic mouse tumor model studies provided proof-of-principle evidence for the cancer immunotherapy potential of MAOIs via targeting TAM reprogramming and thereby enhancing antitumor T-cell responses.

MAO-A blockade for cancer immunotherapy—human TAM and clinical data correlation studies. To explore the translational potential of MAO-A blockade therapy, we first studied MAO-A regulation of human macrophage polarization. Using a Tumor Immune Dysfunction and Exclusion (TIDE) computational method, we analysed the gene expression signatures of in vitro-cultured immunostimulatory M1-like and immunosuppressive M2-like human monocyte-derived macrophages (MDMs) (GSE35449). Interestingly, among all immune checkpoint, immunostimulatory, and immunosuppressive genes examined, MAOA ranked as the top gene with the most dramatically elevated expression in M2-like MDMs (i.e., 7.28 M2/M1 log-fold change; Fig. 6a), suggesting a possible role of MAO-A in promoting human macrophage immunosuppressive polarization. Time-course analysis of MDM culture confirmed an upregulation of MAO-A gene and protein expression during macrophage differentiation that was further upregulated post-IL-4/IL-13-induced immunosuppressive polarization (Fig. 6b–d). Blockade of MAO-A using phenelzine significantly inhibited IL-4/IL-13-induced immunosuppressive polarization of MDMs, evidenced by their decreased expression of immunosuppressive markers (i.e., CD206 and CD273; Fig. 6e and Supplementary Fig. 6a) and signature genes (i.e., ALOX15 and CD200R1; Fig. 6f, g). Collectively, these in vitro data suggest that MAO-A is highly expressed in human macrophages especially during their immunosuppressive polarization, and that MAO-A blockade has the potential to reprogram human macrophage polarization.

To directly evaluate whether MAOIs could reprogram human TAM polarization in vivo, we established a human tumor/TAM xenograft NSG mouse model. A375 human melanoma cells were mixed with monocytes sorted from healthy donor peripheral blood mononuclear cells (PBMCs), and s.c. injected into NSG mice to form solid tumors, with or without phenelzine treatment after inoculation (Fig. 6h). Phenelzine treatment effectively suppressed immunosuppressive polarization of human TAMs (gated as hCD45+hCD11b+hCD14–; Supplementary Fig. 6b), supported by their decreased expression of immunosuppressive markers (i.e., CD206 and CD273; Fig. 6i, j).

Next, we studied whether MAOI-induced human TAM reprogramming could impact human T-cell antitumor reactivity, using a 3D human tumor/TAM/T-cell organoid culture (Fig. 6k). NY-ESO-1, a well-recognised tumour antigen commonly expressed in a large variety of human cancers, was chosen as the model tumour antigen. An A375 human melanoma cell line was engineered to co-express NY-ESO-1 as well as its matching MHC molecule, HLA-A2, to serve as the human tumor target (denoted as A375-A2-ESO; Supplementary Fig. 6c, d). NY-ESO-1-specific human CD8+ T cells were generated by transducing healthy donor peripheral blood CD8+ T cells with a Retro/ESO-TCR retroviral vector encoding a NY-ESO-1-specific TCR (clone 3A1; denoted as ESO-TCR); the resulting T cells, denoted as ESO-T cells, expressed ESO-TCRs and specifically targeted A375-A2-ESO tumor cells, thereby modelling the tumor-specific human CD8+ T cells (Supplementary Fig. 6e, f). Human MDMs were cultured from healthy donor PBMCs, followed by IL-4/IL-13 stimulation to induce immunosuppressive polarization in the presence or absence of phenelzine treatment (Fig. 6k). The A375-A2-ESO human melanoma cells, ESO-T cells, and IL-4/IL-13-polarized MDMs were mixed at a 2:2:1 ratio and placed in a 3D tumor organoid culture mimicking TME (Fig. 6k). IL-4/IL-13-polarized MDMs effectively suppressed ESO-T-cell-mediated killing of A375-A2-ESO tumor cells; this immunosuppressive effect was largely alleviated by phenelzine treatment during MDM polarization (Fig. 6l). Accordingly, ESO-T cells co-cultured with phenelzine-treated MDMs, compared to those co-cultured with non-phenelzine-treated MDMs, showed an enhancement in T-cell activation (i.e., increased cell number, increased CD25 expression, and decreased CD62L expression; Fig. 6m and Supplementary Fig. 6g). Collectively, these data suggest that MAOI-induced human TAM reprogramming has the potential to improve antitumor T-cell responses.

To study MAOA gene expression in primary human TAMs, we collected fresh ovarian cancer tumor samples from patients, isolated TAMs (sorted as DAPI–hCD45+hCD11b–hTCRαβ+hCD14–; Supplementary Fig. 6f), and assessed their MAOA gene expression. Primary human monocytes isolated from healthy donor PBMCs (sorted as DAPI–hCD45+hCD11b–hTCRαβ–hCD14+ cells; Sup-
plementary Fig. 6i) were included as controls. Like mouse TAMs, human TAMs expressed high levels of MAOA gene, confirming MAO-A as a valid drug target in human TAMs (Figs. 1aa and 6n).

Lastly, we conducted clinical data correlation studies to investigate whether intratumoral MAOA gene expression is correlated with clinical outcomes in cancer patients, using the TIDE computational method. Intratumoral MAOA expression level was negatively correlated with patient survival in multiple cancer patient cohorts spanning ovarian cancer (Fig. 6o)48, lymphoma (Fig. 6p)49, and breast cancer (Fig. 6q)50. Moreover, analysis of a melanoma patient cohort receiving anti-PD-1 treatment showed that high levels of intratumoral MAOA expression largely abrogated the survival benefit offered by the PD-1 treatment, suggesting that combining MAO-A blockade
therapy with PD-1/PD-L1 blockade therapy may provide synergistic therapeutic benefits through modulating TAM polarization and thereby changing the immunosuppressive TME and improving antitumor immunity (Fig. 6f)\textsuperscript{31}. Of note, these whole-tumor lysate transcriptome data analyses could not localise the MAOA expression to a specific cell type (e.g., TAMs); future studies of quality transcriptome data generated from single cells or sorted TAMs are needed to obtain such information. Meanwhile, other intratumoral immune cells may also express MAOA and can mediate MAO-A-regulated antitumor immunity. For instance, human T cells have been indicated to express MAOA and have direct effector antitumor immunity, there has been considerable efforts in developing immunotherapies, as exemplified by our current finding of this “MAO-A-ROS axis” regulation of TAM polarization in the TME.

Considering the importance of TAMs in regulating antitumor immunity, there has been considerable efforts in developing cancer therapeutic strategies targeting TAMs. These strategies can be roughly divided into two categories: (1) those which deplete TAMs, and (2) those which alter TAM immunosuppressive activities\textsuperscript{26}. The first category includes strategies targeting TAM recruitment and survival, such as blocking the CCL2-CCR2 axis thereby preventing monocyte mobilisation from the bone marrow and recruitment into inflammatory sites, or blocking the CSF1-CSF1R axis thereby inducing apoptosis of TAMs, or blocking the CXCL12-CXCR4 and angiopoietin 2 (ANG2)-TIE2 axes thereby depleting TIE2\(^+\) macrophages that are critical for tumor angiogenesis\textsuperscript{26,54}. However, an intrinsic downside of depleting TAMs is the loss of their innate immunostimulatory role as the primary phagocytes and professional antigen-presenting cells (APCs) in solid tumors. Reprogramming or repolarizing immunosuppressive TAMs towards an immunostimulatory phenotype therefore can be an attractive direction; this second category of TAM-repolarizing strategies includes those reprogramming TAMs via CD40 agonists, HDAC inhibitors, P13K inhibitors, and creatine\textsuperscript{26,35–58}. Many of these TAM reprogramming strategies are currently under active clinical evaluation\textsuperscript{39}. Notably, CD40 agonists work through activating CD40L-downstream NF-kB pathway\textsuperscript{56,59}; HDAC inhibitors work through altering histone modifications\textsuperscript{55,60}; P13K inhibitors work through stimulating NF-kB activation while inhibiting C/EBP\(\beta\) activation\textsuperscript{57,61}, and creatine uptake works through regulating cytokine responses\textsuperscript{58}. Our discovery of MAO-A as a critical regulator of TAM polarization through modulating oxidative stress provides a drug target and a mechanism of action (MOA) for expanding TAM-repolarizing strategies.

Compared to many new therapeutic candidates, MAO-A is unique in that it is already an established drug target due to its known functions in the brain\textsuperscript{42}. In fact, small-molecule MAOIs...
have been developed to block MAO-A enzymatic activity in the brain and are clinically used for treating various neurological disorders. Notably, some MAOIs cross-inhibit the MAO-A isoenzyme MAO-B, that co-expressed with MAO-A in the brain (Supplementary Fig. 7). However, in human macrophages, especially in M2-like immunosuppressive macrophages, MAO-A is the dominant form (i.e., the expression of MAOA was about 40-fold higher than that of MAOB in M2-like human macrophages; Supplementary Fig. 8). Of course, other tissues and cells may express significant levels of MAO-B that is subjected to MAO1 inhibition and may mediate part of the MAO1-induced therapeutical effects. In our studies, we tested multiple clinically approved MAOIs (phenelzine, clorgyline, moclobemide, and pirlindole) and demonstrated their efficacy in regulating macrophage ROS levels and immunosuppressive polarization, pointing to the possibility of repurposing these drugs for cancer immunotherapy (Figs. 5 and 6). Developing new cancer drugs is an economically and speedy pathway to novel cancer therapies because of reported side effects and the introduction of other disease conditions will be interesting topics for future research.

MAOIs had been used extensively over two decades after their introduction in the 1950s, but since then their use has declined because of reported side effects and the introduction of other classes of antidepressant drugs. However, these MAOI side effects may be manageable. For instance, a claimed major side effect of MAOIs is the risk of triggering tyramine-induced hypertensive crisis when patients eat tyramine-rich foods such as aged cheese (hence, “cheese effects”), which has led to cumbersome food restrictions. The development of reversible and increasingly MAO-B-selective MAOI agents administered via a transdermal delivery system (i.e., the EMSAM selegiline transdermal system) has largely avoided the tyramine related “cheese effects” and thereby can relieve food restrictions and improve the overall safety of MAOIs. Interest in MAOIs as a major class of antidepressants is reviving, and repurposing MAOIs for cancer immunotherapy can be an attractive application of these potent drugs. Moreover, many cancer patients suffer from depression and anxiety; these overwhelming emotional changes can negatively interfere with the quality of life and cancer treatment efficacy of cancer patients. Repurposing MAOIs for cancer immunotherapy thus may provide cancer patients with antidepressant and antitumor dual benefits, making this therapeutic strategy particularly attractive. Nonetheless, caution about drug-food and drug-drug interactions are still relevant for MAOIs’ cancer therapy application, evidenced by the side effects (e.g., hypertension) observed in a recent Phase 2 trial of MAOI phenelzine in biochemical recurrent prostate cancer. Because preclinical evidence largely supports combinatorial approaches being necessary to achieve significant antitumor efficacy, most TAM-targeting strategies currently under clinical evaluation are tested in combination with standard chemotherapy or radiation therapy or in combination with T-cell-directed ICB therapies such as PD-1 or/and PD-L1 blockade therapy. In our study, we found that MAOI treatment synergised with anti-PD-1 treatment in suppressing syngeneic mouse tumor growth (Fig. 5k-o), and that intratumoral MAOA gene expression levels dictated poor patient survival in melanoma patients receiving anti-PD-1 therapy (Fig. 6r). These data highlight the promise of MAOI treatment as a valuable component for combination cancer therapies.

Interestingly, MAO-A upregulation has been detected in cancerous tissues compared to normal tissues and MAO-A expression has been associated with cancer metastases and decreased cancer-related patient survival for several cancers, including prostate cancer, lung cancer, breast cancer, glioma and lymphoma. Together with our finding, these studies suggest the possible multifaceted function of MAO-A in promoting certain cancers, through the direct promotion of tumor development and indirect suppression of antitumor immunity. Consequently, MAO-A blockade therapy may provide dual therapeutic benefits for these cancers, by both suppressing tumor metastasis and enhancing antitumor immunity.

In summary, here we identified MAO-A as a critical molecule regulating TAM immunosuppressive polarization and thereby modulating antitumor immunity, and demonstrated the potential of repurposing established MAO1 antidepressants for cancer immunotherapy. Future clinical studies are encouraged to investigate the clinical correlations between MAO1 treatment and clinical outcomes in cancer patients and to explore the possibility of repurposing MAOIs for combination cancer therapies. Meanwhile, the immune regulatory function of MAO-A certainly goes beyond regulating TAM polarization. Notably, in another recent study, we have identified MAO-A as an immune checkpoint restraining antitumor T-cell immunity through controlling intratumoral T-cell autocrine serotonin signalling. MAO-A’s capacity to regulate various components of antitumor immunity is attractive, suggesting that MAO-A blockade may be multi-functional for cancer immunotherapy. It is also likely that MAO-A regulates immune reactions to other diseases such as infectious diseases and autoimmune diseases. Studying the roles of MAO-A in regulating various immune cells under different health and disease conditions will be interesting topics for future research.

Methods

**Mice.** C57BL/6J (B6), B6.SJL–PtprcPepcJBoyJ (CD45.1, BoyJ), 129S–Masotm1Shih/J (Masotm1Shih/J (Maso KO)) and NOD.Cg-Pkrdcl−1Jd2ytm1Vjls6J (NSG) mice were purchased from the Jackson Laboratory (Bar Harbor). Meso KO mice were backcrossed with C57BL/6J mice for more than nine generations at the University of California, Los Angeles (UCLA). Eight- to twelve-week-old female mice were used for all experiments unless otherwise indicated. Due to ethical reasons, we ended experiments before tumor volume surpassed 1000 mm3. All mice experiments were repeated at least three times unless specifically mentioned. Replicates of each individual experiment are stated in its figure legends. All animals were maintained at the UCLA animal facilities and all animal experiments have complied with all relevant ethical regulations approved by the Institutional Animal Care and Use Committee of UCLA.

**Human tumor samples.** All human tumor samples were obtained following institutional guidelines under protocols approved by the institutional review boards (IRBs) at the UCLA Medical Center. Primary human ovarian cancer tumor samples were obtained from the operating room at the UCLA Medical Center from patients who had undergone surgery and have complied with all relevant ethical regulations using IRB-approved protocols (IRB# 10-000772). Tumors specimens were brought back to the laboratory for further analyses. Detailed samples information is provided in Supplementary Table 1, including diagnosis and staging.

**Cell lines and viral vectors.** The B16-OVA mouse melanoma cell line and the PG13 retroviral packaging cell line were provided by Dr. Pin Wang (University of Southern California, CA). The MC38 mouse colon adenocarcinoma cell line was provided by M. Rosenberg (Yale). The HEK 293T and Phoenix ECO retroviral packaging cell lines, the A375 human melanoma cell line, and the L929 mouse connective tissue cell line were purchased from the American Type Culture Collection (ATCC). The A375-A2-ESO cell line was previously reported. The Phoenix-Eco-MIG, Phoenix-Eco-MIG-Maotm, and PG13-ESO-TCR stable virus-producing cell lines were generated in this study. The MIG (MSCV-IRES-GFP) retroviral vector was reported previously. The MIG-Maotm and Retro/ESO-TCR retroviral vectors were generated in this study.

**Syngeneic mouse tumor models.** B16-OVA melanoma cells (1 x 106 per animal) or MC38 colon cancer cells (5 x 106 per animal) were subcutaneously (s.c.) injected into experimental mice to form solid tumors. In some experiments, mice received an intraperitoneal (i.p.) injection of phenelzine (30 mg/kg/day) to block MAO-A activity. In some experiments, mice received i.p. injection of clodronate liposomes (200 µl/animal, twice per week) to deplete TAMs; mice received i.p. injection of vehicle liposomes (200 µl/animal, twice per week) were included as controls. In some experiments, mice received i.p. injection of anti-mouse PD-1 antibodies (300 µg/animal, twice per week) to block PD-1; mice received i.p. injection of isotype antibodies was included as controls. During an experiment, tumor growth was...
Bone marrow (BM) transfer mouse tumor model. BM cells were collected from femurs and tibias of Maoa WT and Maoa KO donor mice, and were separately transferred into BoyJ (CD45.1) wild-type recipient mice that were preconditioned with whole-body irradiation (1200 rad). Recipient mice were maintained on antibiotic water (Amoxil, 0.25 mg/ml) for 4 weeks after BM transplantation. Per-iodical bleedings were performed to monitor immune cell reconstitution using flow cytometry. Tumor inoculation started at 12 weeks post BM transfer when recipient mice were fully immune reconstituted. B16-OVA mouse melanoma cells were s.c. injected into recipient mice to form solid tumors (1 × 10^6 cells per animal). Tumor growth was monitored twice per week by measuring tumor size using a Fisherbrand™ Traceable™ digital caliper; tumor volumes were calculated by formula 1/2 × L × W^2. At the end of an experiment, tumor-infiltrating immune cells were isolated for analysis using flow cytometry.

Syngeneic mouse tumor-TAM co-inoculation model. Bone marrow cells were collected from Maoa WT and Maoa KO mice and were cultured in vitro to generate bone marrow-derived macrophages (BMDMs). B16-OVA tumor cells (1 × 10^6 cells per mouse) and BMDMs (5 × 10^5 cells per mouse) were mixed and s.c. injected into BoyJ mice to form solid tumors. Tumor growth was monitored twice per week by measuring tumor size using a Fisherbrand™ Traceable™ digital caliper; tumor volumes were calculated by formula 1/2 × L × W^2. At the end of an experiment, tumors were collected and tumor-infiltrating immune cells were isolated for analysis using flow cytometry.

Xenograft human tumor-TAM co-inoculation model. Human peripheral blood mononuclear cells (PBMCs) of healthy donors were obtained from the CFAR Gene and Cellular Therapy Core Laboratory at UCLA, without identification information under federal and state regulations. Human monocytes were isolated from healthy donor PBMCs via magnetic-activated cell sorting (MACS) using human C14 microbeads (Miltenyi Biotec, 130-050-201) followed by fluorescence-activated cell sorting (FACS; sorted as hCD45^+CD11b^+CD14^+ cells) using a FACSaria II flow cytometer (BD Biosciences). Human A375 melanoma cells (1 × 10^6 cells per animal) and purified human monocytes (5 × 10^5 cells per animal) were mixed and s.c. injected into NSG mice to form solid tumors. Some experimental animals received i.p. injection of MAOI (phenelzine, 30 mg/kg/day) to block MAO-A activity. At the end of an experiment, tumor-associated immune cells were isolated for analysis using flow cytometry.

Tumor-infiltrating immune cell (TII) isolation and analysis. Solid tumors were collected from experimental mice at the termination of a tumor experiment. Tumor tissues were cut into pieces and suspended against cold ammonium chloride buffer to lyse red blood cells according to a standard protocol (Cold Spring Harbor Protocols). The resulting TII isolates were then used for further analysis.

In some experiments, TII isolates were sorted via FACS using a FACSaria II flow cytometer (BD Biosciences) to purify TAMs (sorted as DAPI^-CD45^+CD11b^+Ly6G^-Ly6C^-/lowF4/80^+ cells), which were then subjected to QPCR analysis of Maoa mRNA expression in TAMs.

In some experiments, TII isolates were sorted via FACS using a FACSAria II flow cytometer (BD Biosciences) to purify immune cells (sorted as DAPI^-CD45^+ cells), which were then subjected to scRNASeq analysis of gene expression profiling of TII.

In some experiments, TII isolates were directly analysed using MACSQuant Analyzer 10 Flow Cytometer (Miltenyi Biotec) to study the cell surface marker expression of TAMS (pre-gated as CD45.2^-CD11b^+Ly6G^-Ly6C^-/lowF4/80^+ cells) and the intracellular effector molecule production of CD8^+ T cells (pre-gated as CD45.2^+TCCR^+CD8^+ cells).

Mouse monocyte isolation. Peripheral blood samples collected from experimental mice were treated with Tris-buffered ammonium chloride buffer to lyse red blood cells according to a standard protocol (Cold Spring Harbor Protocols). The resulting mononuclear cells were sorted via FACS using a FACSaria II flow cytometer (BD Biosciences) to purify monocytes (sorted as DAPI^-CD45.2^-CD11b^+Ly6G^-Ly6C^- cells), which were then subjected to QPCR analysis of Maoa mRNA expression.

Mouse bone marrow-derived macrophages (BMDM) culture and polarization. To generate BMDMs, BM cells were collected from femurs and tibias of Maoa WT mice and Maoa KO mice, and were cultured in C10 medium containing 20% of LB29-conditioned medium in a 10-cm dish (2 × 10^5 cells per ml; 12 ml per dish) for 6 days. At day 6, the resulting BMDMs were collected and reseeded into a 10-cm dish (10 × 10^5 cells per ml; 12 ml per dish) for 48 h, in the presence or absence of recombinant murine IL-4 (10 ng/ml) (Peprotech, 200-04) and IL-13 (10 ng/ml) (Peprotech, 200-13) to induce BDMD immuno- suppressive polarization.

In some experiments, MAOIs were added to the Maoa WT BMDM polarization culture 30 min prior to adding recombinant murine IL-4 and IL-13, to block MAO-A activity during BMDM polarization. MAOIs studied were phenelzine (Phe, 20 μM) (Sigma-Aldrich), clorgyline (Clo, 20 μM) (Sigma-Aldrich), moclobemide (Moc, 200 μM) (Sigma-Aldrich), and pirindole (Pir, 20 μM) (R&D Systems). At 24 h after IL-4/IL-13 stimulation, BMDMs were collected for flow cytometry and QPCR analysis.

In some experiments, H_2O_2 (100 μM) were added to the Maoa WT and Maoa KO BMDM polarization culture 30 min prior to adding recombinant murine IL-4 and IL-13. At 24 h after IL-4/IL-13 stimulation, BMDMs were collected for flow cytometry and QPCR analysis.

Macrophage suppressive function assay. IL-4/IL-13 polarized Maoa WT and Maoa KO BMDMs were mixed with splenocytes harvested from B6 wild-type mice at various ratios (1:1, 1:2, 1:4, and 1:8) in a 24-well plate (5 × 10^5 splenocytes/ml/well), in the presence of plate-bound anti-mouse CD3ε (5 μg/ml) and soluble anti-mouse CD28 (1 μg/ml) for 2 days. At the end of a culture, cells were collected for flow cytometry analysis.

MIG-Maoa retroviral vector construction, production, and macrophage transduction. MIG retroviral vector was reported previously77–79. Codon-optimized Maoa cDNA (synthesised by IDT) was inserted into a MIG retroviral vector to generate the MIG-Maoa retroviral vector. Vsv-g-pseudotyped MIG and MIG-Maoa retroviruses were produced using HEK 293T virus packaging cells following a standard calcium precipitation method77,78, and then were used to transduce Phoenix-ECO cells to generate stable cell lines producing ECO-pseudotyped MIG or MIG-Maoa retroviruses (denoted as Phoenix-ECO-MIG and Phoenix-ECO-MIG-Maoa cells, respectively). For virus production, Phoenix-ECO-MIG and Phoenix-ECO-MIG-Maoa cells were seeded at a density of 0.8 × 10^6 cells per ml in D10 medium, and cultured in a 15-cm dish (30 ml per dish) for 2 days. Virus supernatants were then collected and used for macrophage transduction.

BM cells harvested from Maoa WT and Maoa KO mice were cultured in a six-well plate in C10 medium containing 20% LB29-conditioned medium (4 × 10^5 cells/ml; 2 ml/well) for 6 days, to differentiate into BMDMs. From day 1 to day 5, cells were spin-infected daily with virus supernatants supplemented with polybrene (0.1 μg/ml) at 660 ng at 35 °C for 90 min. At day 6, recombinant murine IL-4 (10 ng/ml) and IL-13 (10 ng/ml) were added to the BMDM polarization culture 30 min prior to adding recombinant human IL-4 (10 ng/ml) (Peprotech, 214-14) and human IL-13 (10 ng/ml) (Peprotech, 214-13) to induce MDM immunosuppressive polarization. In some experiments, MAOIs were added to the BMDM polarization culture 30 min prior to adding recombinant murine IL-4 and IL-13. At 24 h after IL-4/IL-13 stimulation, BMDMs were collected for flow cytometry and QPCR analysis or for setting up the 3D human tumor organoid culture experiments.

Human monocyte-derived macrophage (MDM) culture and polarization. Human peripheral blood mononuclear cells (PBMCs) of healthy donors were obtained from the CFAR Gene and Cellular Therapy Core Laboratory at UCLA, without identification information under federal and state regulations. Human monocytes were isolated from healthy donor PBMCs by adherence. Briefly, PBMCs were suspended in serum-free RPMI 1640 media (Corning Cellgro, 10-040-CV) at 10 × 10^6 cells/ml. In total, 12.5 μl of the cell suspension were added to each 10-cm dish and incubated for an hour in a humidified 37 °C, 5% CO_2 incubator. Medium that contained non-adherent cells was discarded. Dishes were washed twice and adherent monocytes were cultured in C10 media with human M-CSF (10 ng/ml) (Peprotech, 300-25) for 6 days to generate MDMs. At day 6, the resulting MDMs were collected and reseeded into 1-well plate in C10 medium (1 × 10^6 cells/ml; 2 ml/well) for 48 h, in the presence or absence of recombinant human IL-4 (10 ng/ml) (Peprotech, 214-14) and human IL-13 (10 ng/ml) (Peprotech, 214-13) to induce MDM immunosuppressive polarization. In some experiments, MAOIs (phenelzine, 20 μM) were added to the MDM polarization culture 30 min prior to adding recombinant murine IL-4 and IL-13, to block MAO-A activity during MDM polarization. Polarized MDMs were then collected and used for flow cytometry and QPCR analysis or for setting up the 3D human tumor organoid culture experiments.

Human NY-ESO-1-specific TCR-engineered CD8^- T (ESO-T) cells. The Retro- ESO-TCR vector was constructed by inserting into the parental pMSGV vector a synthetic gene encoding an HLA-A2-restricted, NY-ESO-1 tumor antigen-specific human CD8 TCR (clone 3A1)55,56. Vsv-g-pseudotyped Retro-ESO-TCR retrovirus...
were generated by transfecting HEK 293T cells following a standard calcium precipitation protocol and an ultracentrifugation concentration protocol; the viruses were then harvested and stored. Recombinant single packaging cell line producing GALV-pseudotyped Retro/ESO-TCR retroviruses (denoted as the PG13-ESO-TCR cell line). For virus production, the PG13-ESO-TCR cells were seeded at a density of 0.8 × 10^6 cells per ml in D10 medium, and cultured in a 15-cm dish (30 ml per dish) for 2 days; virus supernatants were then harvested and stored at −80°C for future use. Healthy donor PBMCs were cultured in a 12-well plate in C10 medium (1 × 10^6 cells/ml/well) for 2 days, stimulated with Dynabeads™ Human T-Activator CD3/CD28 (10 μl/ml) (Gibco, 11161D1) and recombinant human IL-2 (20 ng/ ml) (PeproTech). After 2 days, dynabeads were removed and cells were spin-infected with frozen-thawed Retro/ESO-TCR retroviral supernatants supplemented with polybrene (10 μg/ml) at 660 μg at 30 °C for 90 min following an established protocol75. Transduced human CD8+ T cells (denoted as ESO-T cells) were expanded for another 6–8 days in C10 medium containing recombinant human IL-2 (20 ng/ml/well) (PeproTech). After 2 days, dynabeads were removed and cells were spin-infected with frozen-thawed Retro/ESO-TCR retroviral supernatants supplemented with polybrene (10 μg/ml) at 660 μg at 30 °C for 90 min following an established protocol75. Transduced human CD8+ T cells (denoted as ESO-T cells) were expanded for another 6–8 days in C10 medium containing recombinant human IL-2 (20 ng/ml/well) (PeproTech). After 2 days, dynabeads were removed and cells were spin-infected with frozen-thawed Retro/ESO-TCR retroviral supernatants supplemented with polybrene (10 μg/ml) at 660 μg at 30 °C for 90 min following an established protocol75.

To study cell surface marker expression, cells were stained with Fixable Viability Dye followed by Fc blocking and surface marker staining, following a standard protocol. Data were acquired using a BD Flow cytometer. Intracellular staining was performed following the manufacturer’s instructions. To perform intracellular staining, cells were fixed with BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit (BD Biosciences, 55474) following the manufacturer’s instructions.

Western blot (WB). Total protein was extracted using a RIPA lysis buffer (PIERCE, Thermo Fisher Scientific) supplemented with protease and inhibitor cocktail ( complète Mini (one tablet/10 ml) (Sigma-Aldrich, 4906845001), then transferred to pre-cooled Eppendorf tubes. The lysed solution was kept on ice for 30 min and then centrifuged at 15,000×g for 5 min at 4 °C. Supernatants were collected and protein concentrations were quantified using a BCA protein assay (PIERCE, Thermo Fisher Scientific, 23253). Equal amounts of protein were loaded and separated by 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to an Immunobilon-P PVDF Membrane (Millipore). The membranes were blocked with a SuperBlock® T20 (TBS) Blocking Buffer (Thermo Fisher Scientific, 37536). Antibodies were diluted in 5% nonfat milk dissolved in washing buffer TRST (20 mM Tris–HCl, 150 mM NaCl, 0.1% Tween-20).

Primary antibodies against mouse Stat6, p-Stat6 (Tyr641), JAK2, p-JAK2 (Tyr1034/1035), JAK2, p-JAK2 (Tyr1034/1035), JAK2, p-JAK3 (Tyr980/981), HRP–labelled anti-rabbit secondary antibodies, and HRP–labelled anti-mouse secondary antibodies were purchased from Cell signaling Technology. 15-2 (clone HCD14), CD206 (clone DREG-56), and human Fc Receptor Blocking Solution (TruStain FCX™, 422302) were added to the mixture. Fixable Viability Dye eFlour 566 was purchased from Thermo Fisher Scientific. DAPI (Thermo Fisher Scientific) was included to exclude dead cells in FACS sorting.

Stained cells were analyzed using a MACSQuant Analyzer 10 flow cytometer (Miltenyi Biotec); data were analysed using a FlowJo software (BD Biosciences).

Detailed reagent information is provided in Supplementary Table 2.

**Western blot (WB).** Total protein was extracted using a RIPA lysis buffer (PIERCE, Thermo Fisher Scientific) supplemented with protease and inhibitor cocktail ( complète Mini (one tablet/10 ml) (Sigma-Aldrich, 4906845001), then transferred to pre-cooled Eppendorf tubes. The lysed solution was kept on ice for 30 min and then centrifuged at 15,000×g for 5 min at 4 °C. Supernatants were collected and protein concentrations were quantified using a BCA protein assay (PIERCE, Thermo Fisher Scientific, 23253). Equal amounts of protein were loaded and separated by 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to an Immunobilon-P PVDF Membrane (Millipore). The membranes were blocked with a SuperBlock® T20 (TBS) Blocking Buffer (Thermo Fisher Scientific, 37536). Antibodies were diluted in 5% nonfat milk dissolved in washing buffer TRST (20 mM Tris–HCl, 150 mM NaCl, 0.1% Tween-20).

Primary antibodies against mouse Stat6, p-Stat6 (Tyr641), JAK1, p-JAK1 (Tyr1034/1035), JAK2, p-JAK2 (Tyr1034/1035), JAK2, p-JAK3 (Tyr980/981), HRP–labelled anti-rabbit secondary antibodies, and HRP–labelled anti-mouse secondary antibodies were purchased from Cell signaling Technology. 15-2 (clone HCD14), CD206 (clone DREG-56), and human Fc Receptor Blocking Solution (TruStain FCX™, 422302) were added to the mixture. Fixable Viability Dye eFlour 566 was purchased from Thermo Fisher Scientific. DAPI (Thermo Fisher Scientific) was included to exclude dead cells in FACS sorting.

**Quantitative real-time PCR (QPCR).** The total RNA was extracted from cells using TRIzol reagent (Invitrogen, Thermo Fisher Scientific, 15596018) following the manufacturer’s instructions. SuperScript III First-strand (Thermo Fisher Scientific, 18808051) was used for reverse transcription. QPCR was performed using a KAPA SYBR FAST qPCR Master Mix (Kapa Biosystems) and a 7500 Real-time PCR System (Applied Biosystems) according to the manufacturer’s instructions. Housekeeping gene Ube2d2 was used as an internal control for mouse immune cells and ACTB was used as an internal control for human immune cells. The relative expression of a target gene was calculated using the 2^(-ΔΔCt) method. All primers used in this study are listed in Supplementary Table 3.

**Reactive oxygen species (ROS) measurement.** Cells were stained with surface marker antibodies, washed with PBS, then resuspended in pre-warmed PBS (1 × 10^6 cells/ml/tube) containing 1 μM CM-H2DCFDA (Thermo Fisher Scientific). Cells were immediately washed with cold PBS followed by flow cytometry analysis. ROS levels were measured by oxidation of the CM-H2DCFDA probes that can be read out as the fluorescence intensity at the FITC/488 channel of a flow cytometer.

**Single-cell RNA sequencing (scRNAseq).** scRNAseq was used to analyse the gene expression profiles of TILs. B16-OVA tumors were harvested from Moos WT and Moos KO mice to prepare TIL suspensions (ten tumors were combined for each group). TIL suspensions were then sorted using a FACSAria II flow cytometer to purify immune cells (gated as DAPI− CD45.2+ cells). Sorted TILs were immediately delivered to the Technology Center for Genomics & Bioinformatics (TGB) facility at UCL for library construction and sequencing. Cells were stained with trypan blue (Thermo Fisher Scientific, T1024X) and counted using a Cell Count II automated cell counter (Thermo Fisher Scientific). 10,000 TILs from each experimental group were loaded on the Chromium platform (10X Genomics) and libraries were constructed using a Chromium Single Cell 3' library & Gel Bead Kit V2 (10X Genomics, PN-120237) according to the manufacturer’s instructions. Libraries were sequenced on an Illumina NovaSeq 6000 System, using a NovaSeq 6000 S2 Reagent Kit (100 cycles; 20012862, Illumina). Data analysis was performed using a Cellranger Software Suite (10X Genomics). BCL files were extracted from the sequencer and used as inputs for the cellranger pipeline to generate the digital expression matrix for each sample. Then cellranger agg command was used to generate the two samples into one digital expression matrix. The digital expression matrix was analysed using Seurat, an R package designed for single-cell RNA sequencing. Specifically, cells were first filtered to have at least 300 UMIs (unique molecular
Tumor immune dysfunction and exclusion (TIDE) computational method. TIDE analyses were conducted as previously described (http://tide.dfci.harvard.edu)\(^45\). Two functions of the TIDE computational method were used: (1) the prioritisation function and (2) the survival correlation function. The prioritisation function of TIDE was used to rank a target gene by its immune dysfunction/risk score, which for TAMs, was calculated as its gene expression log-fold change of M2-like/M1-like MDMs\(^45\). A transcriptome dataset (GSE153615)\(^49\), breast cancer (GSE9893)\(^50\), and melanoma (PRJEB23709)\(^51\). The human macrophage transcriptome dataset is publicly available from the GEO database. Patient cohort data of PD-1/PD-L1 blockade therapy were obtained through the Kaplan–Meier plots. \(^54\). The Kaplan–Meier plots were compared by the LogRank test. \(^55\) The Kaplan–Meier plot of the overall patient survival for ovarian cancer, lymphoma, and melanoma with different MAOA levels, the p value was calculated by two-sided Wald test in a Cox-PH regression.

Statistical analysis. GraphPad Prism 6 (GraphPad Software) was used for the graphic representation and statistical analysis of the data. All data were presented as the mean ± standard error of the mean (SEM). A two-tailed Student’s t test was used for comparison between two groups. Multiple comparisons were performed using an ordinary one-way ANOVA followed by Tukey’s multiple comparisons test or a two-way ANOVA followed by Sidak’s multiple comparisons test. p < 0.05 was considered statistically significant. ns not significant; *p < 0.05; **p < 0.01; ***p < 0.001. For the Kaplan–Meier plot of the overall patient survival for ovarian cancer, lymphoma, breast cancer, and melanoma with different MAOA levels, the p value was calculated by two-sided Wald test in a Cox-PH regression.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. The scRNAseq datasets generated in this study have been deposited in the GEO database under accession code GSE153615. Patient cohorts data analysed, including ovarian cancer (GSE26712)\(^48\), lymphoma (GSE10846)\(^49\), breast cancer (GSE9893)\(^50\), and melanoma (P35430) were publicly available from the GEO database. Patient cohort data of PD-1/PD-L1 blockade therapy in melanoma is publicly available from NCBI BioProject database (P35430). The human macrophage transcriptome dataset is publicly available from the GEO database (GSE153615). TIDE computational method analyses were conducted on TIDE website (http://tide.dfci.harvard.edu)\(^45\). The remaining data associated with this study are presented in the Article or Supplementary Information. Further information and requests may be directed to and will be fulfilled by the corresponding author, Lili Yang (liliyang@ucsd.edu). Source data are provided with this paper.

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