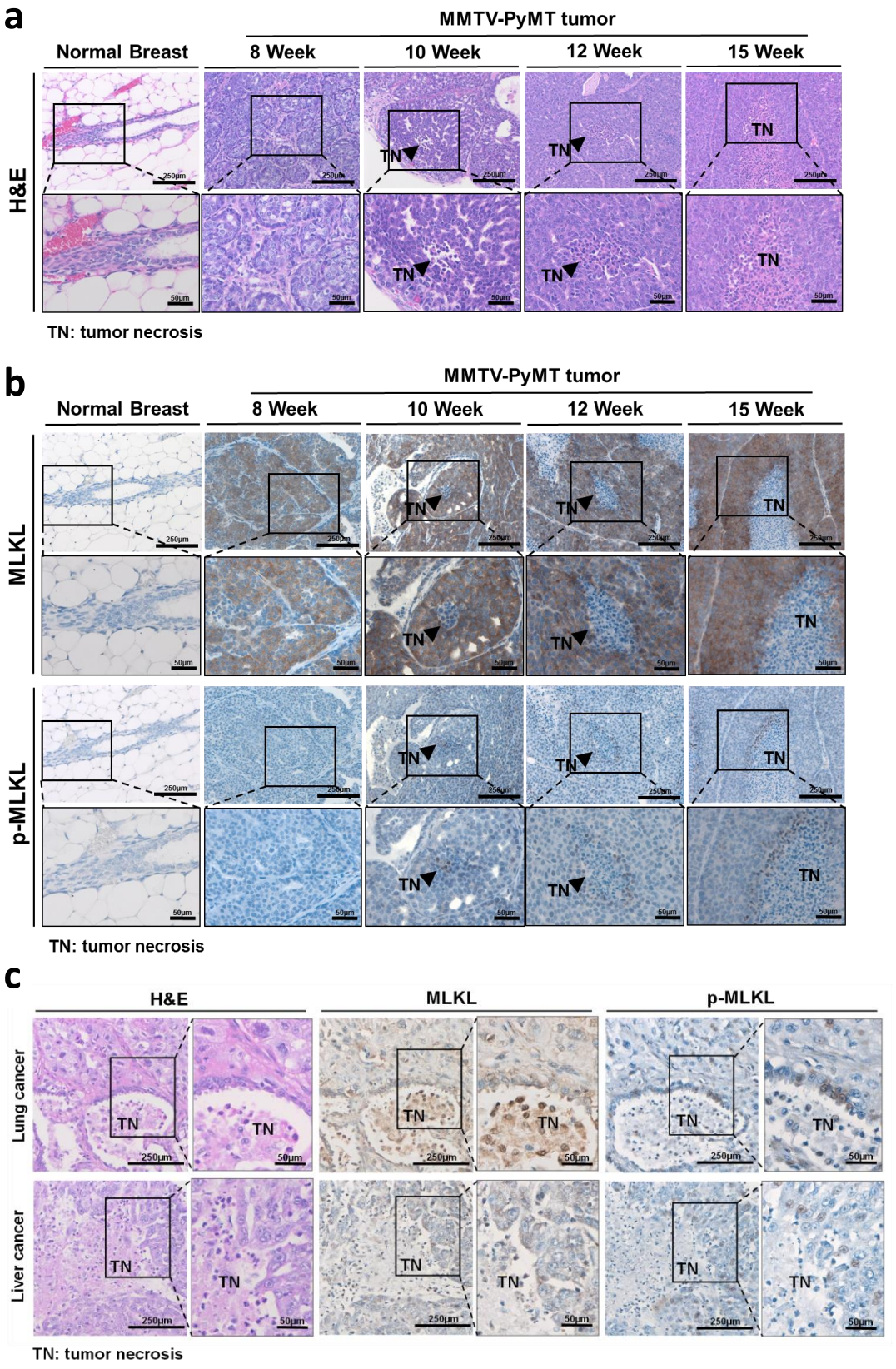
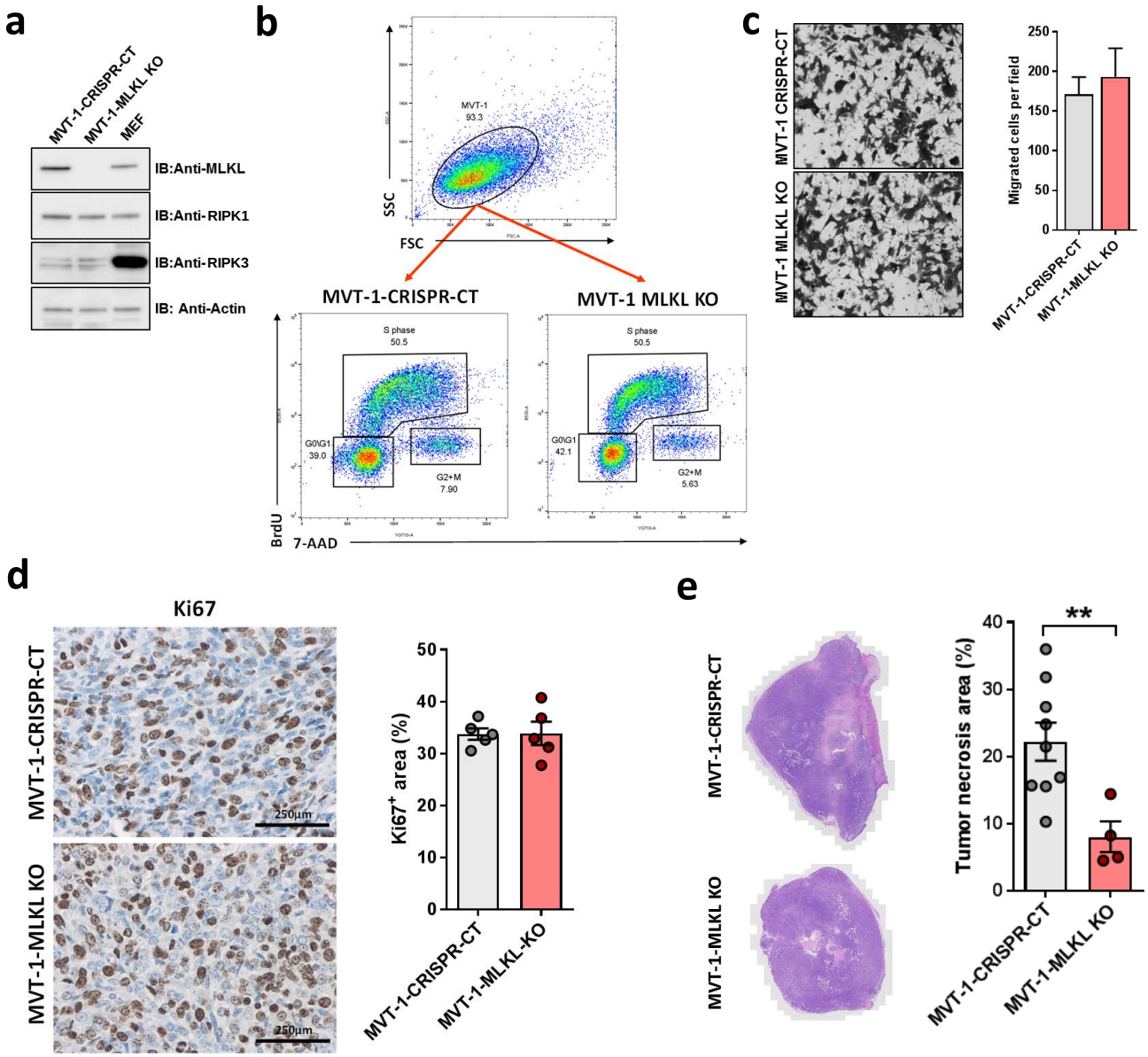
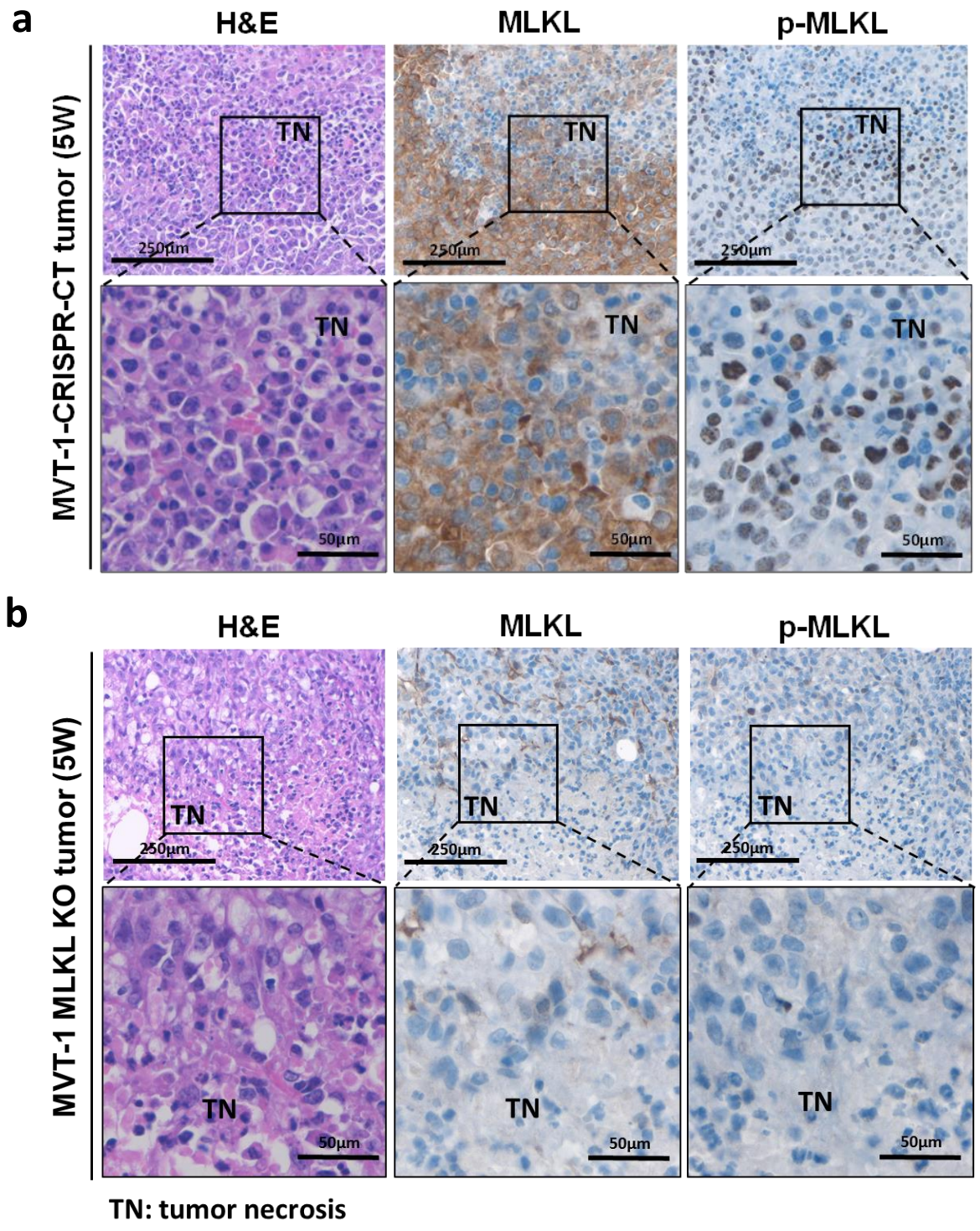


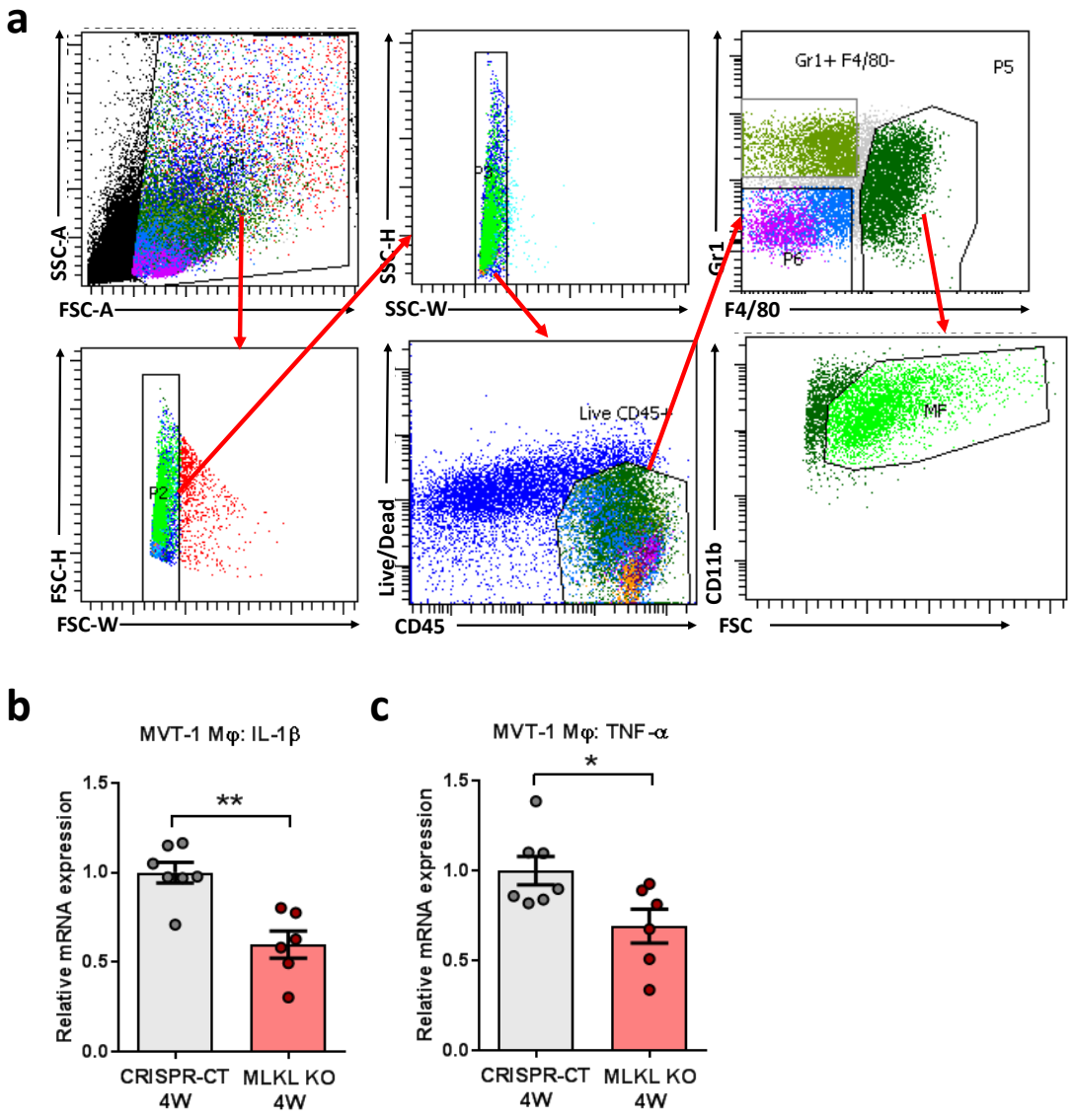
Supplementary Figure 1. Necroptosis in MMTV-PyMT breast tumors and human lung and liver cancers (a) Representative image of stained sections of normal or MMTV-PyMT tumor breast tissue. **(b)** Serial sections were stained with H&E or immunohistological staining with anti-MLKL or anti-pMLKL antibodies. **(c)** Representative image of stained sections of human lung and liver tumor tissue. Serial sections were stained with H&E or immunohistological staining with anti-MLKL or anti-pMLKL antibodies.



Supplementary Figure 2. MLKL KO does not affect MVT-1 cell proliferation and migration in vitro and in vivo but does suppress tumor necrosis. (a) Western blot analysis of MVT-1–CRISPR-CT and MVT-1 MLKL KO cells. Cell lysates of untreated MEFs serve as control. The blot was probed with antibodies as indicated. **(b)** Proliferation and cell cycle of MVT-1–CRISPR-CT and MVT-1 MLKL KO cells was analyzed by BrdU uptake and 7-AAD staining in flow cytometry. **(c)** Migration of MVT-1–CRISPR-CT and MVT-1 MLKL KO cells was analyzed by a trans-well assay. Representative image (left panel) and migrated cells per field were counted (right panel). **(d)** Representative image of Ki67 stained 4 week old tumors from mice injected with MVT-1–CRISPR-CT or MVT-1 MLKL KO cells. Ki67 positive area was determined using ImageJ software. Scale bar, 250 µm. **(e)** Representative image of H&E stained tumors (left panel) at 5 weeks from mice injected with MVT-1-CRISPR-CT or MVT-1-MLKL KO cells. Right panel shows the percentage of tumor necrosis area (right panel) of total tumor from mice at 5 weeks. **indicates significance $p<0.01$.



Supplementary Figure 3. Necroptosis happens in tumor cells at tumor death areas. (a-b) Representative image of stained sections of tumors at 5 weeks from mice injected with MVT-1-CRISPR-CT (**a**) or MVT-1-MLKL KO (**b**) cells. Serial sections were stained with H&E or immunohistological staining with anti-MLKL or anti-pMLKL antibodies.



Supplementary Figure 4. Analysis of tumor cell necroptosis-induced inflammatory response. (a) Gating strategy for flow sorting of tumor infiltrating macrophages. Starting from the FSC/SSC plot of magnetically purified $CD45^+$ cells (upper left), red arrows show the directionality of sub-gating of cells. FSC/SSC and different markers are indicated at the left and bottom of the dot plots. Macrophages (MF) were sorted and used for subsequent experiments. **(b-c)** Relative expression of IL-1β (b) and TNF- α (c) in macrophages ($CD45^+Gr1^-F4/80^+CD11b^+$) were assessed by RT-PCR.

Methods

Mice

Female FVB/NJ and MMTV-PyMT mice were purchased from The Jackson Laboratory. All animal experiments were performed under protocols approved by the National Cancer Institute Animal Care and Use Committee and followed NIH guidelines. For orthotopic model, MVT-1 cells (syngeneic mouse mammary cancer cell line derived from c-Myc/vegf tumor explants) were suspended in 100 µl Matrigel Matrix (Corning) solution (diluted 1:1 with PBS) and then injected (2×10^6/mouse) into the right inguinal mammary fat pad of FVB/NJ mice. Tumor volume was monitored every week upon inoculation.

Clinical tumor samples

Clinical breast, lung and liver tumor samples were obtained from LCRC Biospecimen Core in accordance with their regulations. Formalin fixation was used to stabilize protein in fresh tissue, and prevent autolysis and putrefaction. Fresh surgical resection specimens were fixed in 10% neutral buffered formalin and embedded in paraffin blocks can be sectioned, mounted on slides, and stained with Hematoxylin and Eosin (H&E) for tumor morphology. Unstained sections used for immunohistochemical analyses of protein expression.

Additional clinical late-stage breast tumor samples were obtained from the Xinqiao Hospital, Chongqing, China. This study was approved by the Research Ethics Committee of Xinqiao Hospital, Chongqing, China. Informed consent was obtained from all patients. The collected tissues were either formalin-fixed or stored at -80°C for western blot analysis.

Cell culture

Mouse mammary cancer cell line MVT-1, mouse embryonic fibroblasts (MEFs) and human colorectal adenocarcinoma cell line HT-29 have been described[1, 2] and maintained in DMEM

containing 10% FBS. We used CRISPR technology to generate MLKL knockout MVT-1 cell line. The lentiviral sgRNA vector targeting MLKL was constructed by ligation of hybridized oligos into LentiCRISPR (pXPR_001, GeCKO) vector: Oligo1 (caccgcgtctaggaaaccgtgtgca) and Oligo 2 (aaactgcacacggtttcctagacgc). Then 293T cells were transfected with sgRNA vector, PAX2 (Addgene) and pCMV-VSV-g (Addgene) for 24 hours. MVT-1 cells were then infected with lentivirus-containing supernatants containing polybrene (Millipore) for 24h. After two weeks of selection with 2 μg/ml puromycin (Sigma-Aldrich), MVT-1-MLKL KO cells were used for subsequent experiments. For positive controls, MEF or HT-29 cells were treated with TNF-α (30 ng/ml), Smac (10 nM) and z-VAD-fmk (20 μM) for 4h to induce necroptosis.

Transwell migration assay

To assess tumor cell migration activity, 2×10^4 MVT-1-CRISPR-CT or MVT-1-MLKL KO cells were cultured in serum-free DMEM in the upper compartment of a transwell insert (8 μm pore size, Corning). The lower compartment was filled with DMEM containing 10% FBS. After 12 hours, cells in the upper compartment were removed and the transwell membrane was stained with 0.5% crystal violet (Sigma-Aldrich). Migrated cells on the membrane were counted under a light microscope (Zeiss).

Histology analysis

Human and mouse tissues were fixed at 4 °C with 4% formalin for 72 hours, followed by PBS rinsing, dehydrating and paraffin-embedding. The sections were routinely stained with haematoxylin and eosin as described[3]. Tumor necrosis was designated on H&E-stained slides as areas of dark-haematoxylin-stained necrotic tumor cells immediately adjacent to light-haematoxylin-stained viable tissues[4]. The quantitation of tumor necrotic/death area was conducted using ImageJ and represented as the percentage of tumor necrotic/death area within whole tumor.

To detect MLKL and p-MLKL expression in tumors by immunohistochemistry, 4 μm paraffin sections were subjected to antigen retrieval with sodium citrate buffer (pH 6.0) and blocked with 2% normal goat serum, followed by overnight incubation with anti-mouse/human MLKL antibody (Clone EPR17514; Abcam), anti-mouse p-MLKL antibody (Clone EPR9515(2); Abcam), or anti-human p-MLKL antibody (Clone EPR9514; Abcam). Signals were developed using VECTASTIN ABC Elite kit (Vector Laboratories) and DAB Substrate Kit (Vector Laboratories). To detect apoptotic tumor cells, TUNEL assay was conducted on paraffin sections by using an In situ Apoptosis Detection Kit (Abcam). For the quantitation of metastatic burden, paraffin-embedded lung tissues were sectioned 400 μm apart throughout the whole lung followed by H&E staining. The frequency of the metastatic foci was counted manually in a blinded fashion, and the size of the foci were determined by using ZEN software (ZEISS).

Western blot

Tumor tissues and *in vitro* cultured cells were lysed in RIPA buffer and M2 buffer, respectively. Tumor and cell lysates were separated by either non-reducing (for MLKL oligomerization) or reducing (except for MLKL oligomerization) SDS-PAGE, followed by probing with anti-mouse/human MLKL antibody (Clone EPR17514; Abcam), anti-human p-MLKL antibody (Clone EPR9514; Abcam), anti-mouse p-MLKL antibody (Clone EPR9515(2); Abcam), anti-mouse/human RIPK1 antibody (Clone 38/RIP; BD Transduction Laboratories), anti-mouse/human RIPK3 antibody (Rabbit pAb; Abcam), or anti-mouse/human Actin antibody (Rabbit pAb; Sigma-Aldrich). Signals were developed by using enhanced chemiluminescence kit (Bio-Rad).

Fluorescence activated cell sorting

Tumor-infiltrating macrophages were isolated through magnetic isolation followed by fluorescence activated cell sorting. Briefly, cells were blocked with anti-mouse CD16/32 antibody

(Clone 93; Biolegend), and stained with Fixable Aqua Dead cell staining dye (Thermo Fisher) plus fluorochrome-labeled antibodies that included APC-labeled anti-mouse CD45 (Clone 30-F11; Biolegend). Subsequently, total $CD45^+$ tumor-infiltrating leukocytes were magnetically isolated by incubating with anti-APC magnetic beads (Miltenyi Biotec). Macrophages were then sorted from total $CD45^+$ cells by using a BD FACSAria Fusion sorter (BD Biosciences). Gating strategies for the sorting can be found in Supplementary information, Figure. S4a.

Quantitative RT-PCR

200-300,000 sorted macrophages were subjected to RNA extraction using RNeasy Mini Kit (Qiagen). cDNA synthesis was conducted using Superscript III First-strand synthesis kit (Invitrogen). Predesigned qPCR Assays for β-Actin, IL-1β and TNF-α (Integrated DNA Technologies) were used and relative mRNA expression was measured using SensiFAST Probe Hi-ROX Mix (Bioline) on QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific). $2^{-\Delta\Delta CT}$ method was used to quantify fold induction and each cDNA was normalized by β-Actin expression.

Statistical analysis

All data were analyzed with the Graphpad Prism 7 software. Student's t-test and One-way analysis of variance (ANOVA) was used to determine the statistical significance of differences between groups. Differences with P values < 0.05 were considered significant.

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