SUPPLEMENTARY MATERIALS AND METHODS

Tumor models and cell cultures

3LL-R Lewis lung carcinoma cells or TS/A breast carcinoma were harvested and single-cell suspensions of $3 \times 10^6$ in 200 µl of PBS were injected subcutaneously into the right flank of syngeneic C57Bl/6 mice or Balb/c respectively. 3LL-R cells were generated in house from C57BL/6 Lewis Lung carcinoma as previously described (1). TS/A cells were a kind gift of Vincenzo Bronte (University of Verona, Verona, Italy).

3LL-R and TS/A cell lines were maintained in Roswell Park Memorial Institute-1640 medium (RPMI; Sigma) supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS; Gibco), 300 µg/ml L-glutamine (Gibco), 100 units/ml penicillin and 100 µg/ml streptomycin (Gibco) and monthly tested for the presence of mycoplasma. For TAM cultures, this medium was supplemented with 1mM non-essential amino acids (Invitrogen), 1mM sodium pyruvate (Invitrogen) and 0.02 mM 2-mercaptoethanol (Invitrogen).

Measurement of IL-34, M-CSF, GM-CSF and CCL2 production

Briefly, for M-CSF, GM-CSF and CCL2 96-well plates (NUNC Maxisorp, eBioscience) were coated overnight with capture antibodies for the respective cytokines using coating buffer (8.34 g NaHCO$_3$ in 1L H$_2$O). All incubations were at room temperature protected from light. The following day, wells were blocked with 300 µl blocking buffer (PBS 1% BSA) for two hours. Subsequently, plates were washed and serial standard dilutions and samples were loaded on the 96-well plate and incubated for two hours. After washing, 100 µl of detection antibody diluted in blocking buffer was added for
one hour. After washing, streptavidin-HRP was added and plates were incubated for 20 minutes. Substrate solution (TMB Substrate Reagent Set; BD OptEIA™) was added and plates were incubated for 15 minutes, after which 2N H₂SO₄ was added to stop the color-reaction. Plates were read on a microplate-reader at 450 nm. Optical densities of the standard curve were plotted using a linear fit and analyzed with Microplate Manager. For IL-34, pre-coated plates were used (Quantikine ELISA kit R&D systems). A similar protocol was followed, using the reagents provided in the kit.

Intravital Imaging

To assess the proportion of intravascular and extravascular monocytes in tumors, 3LL-R tumor-bearing CX3CR1-GFP⁺⁻ reporter mice were anesthetized followed by tracheotomy and controlled respiration with 2.5% isoflurane in 100% O₂. Tumors were surgically exposed and carefully dissected from the surrounding connective tissue, preventing operation induced tissue traumata, followed by extracorporal immobilization of the tumor on a custom-made stage and embedding in 3% PBS agarose. Subsequently, mice were injected intravenously with 300 µg isotype Ab or α-M-CSFR Ab and with 1µg Evans blue (Sigma Aldrich) per gram body weight to visualize the blood vessels. Intravital microscopy was performed using a LaVision multiphoton laser-scanning microscope (BioTec, Bielefeld, Germany) and images were analyzed using Imaris 7.7 (Bitplane, Zurich, Switzerland).
**Bromodeoxyuridine labeling**

To assess the amount of BrdU⁺ cells, tumor single-cell suspension were pre-incubated for 20 min with rat anti-mouse CD16/CD32 (2.4G2, BD Biosciences) to prevent aspecific binding and subsequently with fluorescently-labeled Ab for extracellular cell-surface markers for 20 min on melting ice protected from light. After washing with staining buffer, cells were fixed for 25 min on melting ice with 100 µl Cytofix/Cytoperm buffer (BD Biosciences). Cells were washed with Perm-wash buffer (BD Biosciences) and incubated for 10 min with 100 µl Cytofix/Cytoperm PLUS buffer (BD Biosciences) on melting ice. Cells were again washed with Perm-wash and incubated for 5 additional minutes with Cytofix/Cytoperm buffer on melting ice. To ensure an optimal working of the BrdU labeling, cells were incubated for 1h at 37°C with 30 µg DNAse (Worthington) diluted in PBS. After washing with Perm-wash buffer, cells were incubated with anti-BrdU Ab diluted in Perm-wash (1:50) for 20 min at room temperature. Cells were washed with Perm-wash and BrdU signals were measured via flow cytometry.

**Mixed Leukocyte Reaction assays**

For Mixed Leukocyte Reaction (MLR) assays, T cells were purified from Balb/c splenocytes, by first immunodepleting CD11c⁺ and CD19⁺ cells using a MACS LD column with anti-CD11c and anti-CD19 microbeads (Miltenyi biotech) and subsequently positively selecting CD4⁺ or CD8⁺ T cells using anti-CD4 or anti-CD8 microbeads (Miltenyi biotech). 2x10⁵ purified Balb/c T cells were cultured in round-bottom 96-well plates with 5x10⁴ MHC-II⁺ or MHC-II⁺ TAM subsets sorted from 14-days old 3LL-R tumor-bearing mice, treated with either isotype or α-M-CSFR every
two days from day 0 past tumor inoculation until sacrifice (day 14 p.i). Three days later, $^3$H-thymidine was added and cell proliferation was measured after another 18h culture as counts per minute (cpm) on a Wallac 1450 Liquid Scintillation Counter.
SUPPLEMENTARY REFERENCES

List of abbreviations

BM: Bone marrow
BrdU: bromodeoxyuridine
CCL: C-C chemokine ligand
GFP: Green fluorescent protein
GM-CSF(R): Granulocyte-macrophage colony stimulating factor (receptor)
Imm: immature
Iv: intravascular
M-CSF(R): Macrophage colony stimulating factor (receptor)
MFI: Median Fluorescence Intensity
MLR: mixed leukocyte reaction
Mo: monocytes
MΦ: macrophage
TAM: tumor-associated macrophage
TADC: tumor-associated dendritic cell