Supplementary Materials and Methods

Mice

C57BL/6 Caspase-1$^{-/-}$ mice were obtained from Dr. Odilia Wijburg at the University of Melbourne Department of Microbiology and Immunology (Melbourne, Australia) (1). IL-1R1$^{-/-}$ mice were sourced from Dr. Helen Thomas at the St Vincent's Institute (Melbourne, Australia). C57BL/6 IL-18$^{-/-}$ mice were kindly provided by Dr. Shizuo Akira at the Osaka University (Japan) (2)

MCA-induced fibrosarcoma model

WT, caspase-1$^{-/-}$ or IL-1R1$^{-/-}$ mice were injected subcutaneously with 100 μl of corn oil containing MCA (100 or 400 μg) on the right hind flank. Some WT mice were treated with control Ig (cIg) or blocked of IL-1β, IL-1α or IL-1R by treatment with antibodies (early depletion: 250 μg of α-IL-1β, α-IL-1α or α-IL-1R was injected intraperitoneally (i.p.) at day -1, 0 and then weekly until week 4; late depletion: 250 μg of α-IL-1β, α-IL-1α or α-IL-1R was injected i.p. at day 28, 29 and then weekly until week 8. Development of fibrosarcomas was monitored weekly over the course of 300 days. Measurements were made with a caliper square as the product of two perpendicular diameters (mm^2) and individual mice are represented. Tumor growth rate (mm^2/day) was measured over the time from first detection to mouse sacrifice.

Experimental tumor models

WT or NLRP3$^{-/-}$ mice were inoculated subcutaneously in the right flank with 1 x 10^5 B16F10 melanoma cells. Tumor size was measured three times per week as above. In experimental metastasis experiments, WT or gene-targeted mice as indicated were injected intravenously

with B16F10 melanoma or RM-1 prostate carcinoma at different doses, from 1 x 10^4 to 5 x10^5 cells. Mice were sacrificed and lungs were harvested on day 14 post-inoculation. Some mice in the B16F10 melanoma experiments were treated with control Ig or anti-IL-1β, IL-1α or IL-1R (250 μg of α-IL-1β, α-IL-1α or α-IL-1R on day -1, 0 and 7 after tumor inoculation). Tumor nodules were counted with the aid of a dissection microscope.

Tumor-induced interstitial fluid (TIF) isolation

Lungs were harvested from the tumor bearing mice, minced finely and incubated in PBS for 2 hrs at 37°C. The supernatants were harvested for cytokines/chemokines measurement using Proteome Profiler Array kits (R&D Systems) or Cytometric Bead Array kits (BD Biosciences), according to the manufacturers' instructions.

Flow Cytometry

Single-cell suspensions were prepared from mouse organs and removal of erythrocytes was performed using ammonium chloride lysis as described previously (3). Cells were stained with anti-NK1.1-APC (PK136), anti-CD3-PacificBlue (145-2C11), anti-CD27-PE-Cy7 (Lg.7F9), and anti-CD11b-PE (M1/70). All antibodies were from eBioscience. Nonviable cells were excluded on the basis of staining with 7-aminoactinomycin D (BD Pharmingen). Datasets were analyzed using Flowjo software (Tree Star).

Cytotoxicity assays

NK cells were purified from naïve WT mice. $CD11b^+$ $Gr\text{-}1^+$ cells were harvested and processed from the $NLRP3^{-/-}$ mice at day 3 after B16F10 tumor inoculation as described above. NK cells and $CD11b^+$ $Gr\text{-}1^+$ cells were co-cultured with ^{51}Cr-labeled YAC-1 target cells for 4 hrs.

Supplementary Figures

Supplementary Figure 1. NLRP3 inflammasome promotes the formation of MCA-induced fibrosarcomas in BALB/c mice. BALB/c WT and BALB/c NLRP3$^{-/-}$ mice were injected with either 400 μg of MCA as indicated and subsequently monitored for tumor development over 250 days (n = 15-18 mice per group). Results are shown as survival curves defined as the percentage of tumor free mice at each time point. Statistical differences in tumor incidence were determined by Mantel Cox Log-rank test (***, P = 0.0008).

Supplementary Figure 2. Individual growth rates of tumors (mm^2/day) from mice depicted in scatter plots in Figure 1 C and D with the mean ± SEM shown for each group. Statistical difference was determined by Mann-Whitney U test (***, P<0.001).

Supplementary Figure 3. Caspase-1 inflammasome promotes MCA-induced carcinogenesis. (A-B) WT, caspase-1$^{-/-}$ and IL-1R1$^{-/-}$ mice were injected with either 100 μg or 400 μg of MCA as indicated and subsequently monitored for tumor development over 300 days (n = 10-15 mice per group). (C-D) WT mice were treated with control Ig (cIg) or depleted of IL-1β or IL-1α or blocked of IL-1R by treatment with antibodies (early depletion: 250 μg of α-IL-1β, α-IL-1α or α-IL-1R was injected i.p. at day -1, 0 and then weekly until week 4; late depletion: 250 μg of α-IL-1β, α-IL-1α or α-IL-1R was injected i.p. at day 28, 29 and then weekly until week 8). Results are shown as survival curves defined as the percentage of tumor free mice at each time point. Statistical differences in tumor incidence were determined by Mantel Cox Log-rank test (*<0.05; **, P<0.01; ***, P<0.001).

Supplementary Figure 4. Host NLRP3 does not affect B16F10 subcutaneous growth. Groups of 5 - 9 WT and NLRP3$^{-/-}$ mice were injected subcutaneously with 1 x 10^5 B16F10 tumor cells. Tumor measurements were made as indicated with a caliper square and recorded as the product of two perpendicular diameters (mm^2)(mean $\pm$ SEM).

Supplementary Figure 5. Caspase-1 and IL-1 do not affect the development of lung metastasis. Groups of 5-10 WT, NLRP3$^{-/-}$, caspase-1$^{-/-}$, IL-18$^{-/-}$ and IL-1R1$^{-/-}$ were injected i.v. with (A) 2 x 10^5 B16F10 cells, (B) 5 x 10^4 or 1 x 10^5 RM-1 cells, (C) 5 x 10^5 B16F10, or (D) 5 x 10^5 B16 cells. In C and D, mice were additionally treated with α-IL-1β, α-IL-1α or α-IL-1R (100 μg i.p.) on day 0, day -1 and day 7 relative to tumor inoculation. Fourteen days after tumor inoculation, the lungs of these mice were harvested and fixed, and the colonies counted under a dissecting microscope. Symbols represent the number of tumor colonies in the lung from individual mice. Mann-Whitney *U* test was used to compare differences between mice (**, $P<0.01$).

Supplementary Figure 6. Normal NK cell homeostasis in NLRP3$^{-/-}$ mice. (A-B) Bone marrow, lymph node, lung and spleen from WT and NLRP3$^{-/-}$ mice were processed for FACS analysis of NK cell subsets as described previously (3). NK cells (NK1.1$^+$ CD3$^-$) are present in similar percentages in WT and NLRP3$^{-/-}$ mice and exhibit similar NK cell subsets (as determined by CD27 and CD11b) in NLRP3$^{-/-}$ mice. Data shown are representative of two independent experiments each consisting of three mice per group. (C) Splenic NK cells were purified and cultured in the presence of recombinant cytokines for 16 hrs. IFN-γ in the

supernatant was determined by CBA. Results are pooled for two independent experiments using triplicate wells. (D) IL-2-activated purified NK cells were tested for cytotoxic activity against YAC-1 in a standard 4 hr ^{51}Cr release assay. Each data point is the mean ± SEM of two independent experiments done in triplicate for each effector/target ratio.

Supplementary Figure 7. CD11b^{+} Gr-1^{int} cells express NLRP3. (A) An eGFP cassette was inserted into the NLRP3 gene during the generation of NLRP3$^{-/-}$ mice as described previously (4). Thus, analysis of eGFP expression in different cell types can be used to determine the NLRP3 expression. (B) Morphology and Giemsa staining of cytospun Gr-1^{bright}, Gr-1^{int} and Gr-1^{dim} cells isolated from the lungs of B16F10-bearing NLRP3$^{-/-}$ mice. Cells were sorted as CD45.2^{+} CD11b^{+} Gr-1$^{bright/int/dim}$.

Supplementary Figure 8. CD11b^{+} Gr-1^{int} cells do not suppress or enhance NK cell cytotoxic function. (A) FACS-sorted CD11b^{+} Gr-1^{+} cells from tumor-bearing NLRP3$^{-/-}$ mice were cultured with NK cells from naive WT mice (1:1 ratio) and ^{51}Cr-labeled YAC-1 targets cells for 4 hrs (NK:YAC-1 - 10:1 ratio). Each data is the mean ± SEM of two independent experiments. (B) Cytokine arrays were incubated with the TIF that was harvested and prepared from the B16F10-bearing WT or NLRP3$^{-/-}$ mice. Red boxes indicate major spot density differences for CXCL9 (position D7) and RANTES (position D11) between WT and NLRP3$^{-/-}$ mice amongst those detected by the Proteome Profiler Array kit.

Supplementary Figure 9. Cytokine profile of CD11b^{+} Gr-1^{int} cells. Cells were sorted as CD45.2^{+} CD11b^{+} Gr-1$^{bright/int/dim}$ from the lungs of B16F10-bearing NLRP3$^{-/-}$ mice and

cultured in the presence of PMA and Ionomycin for 12 hrs. Cytokines in the supernatant were determined by CBA. Results are pooled for two independent experiments using triplicate wells. Statistical difference was determined by Mann-Whitney U test (*<0.05; **, P<0.01; ***, P<0.001).

References

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