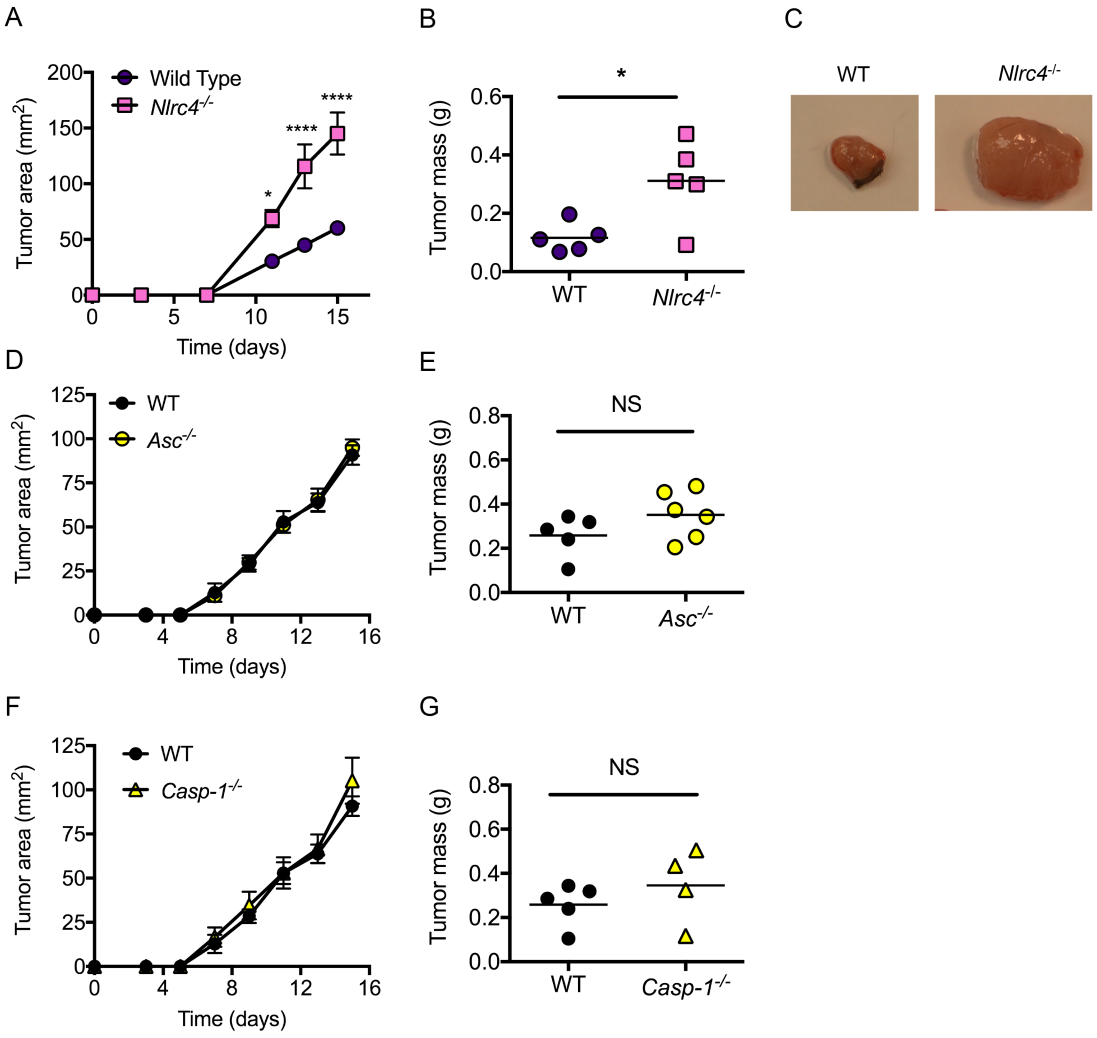
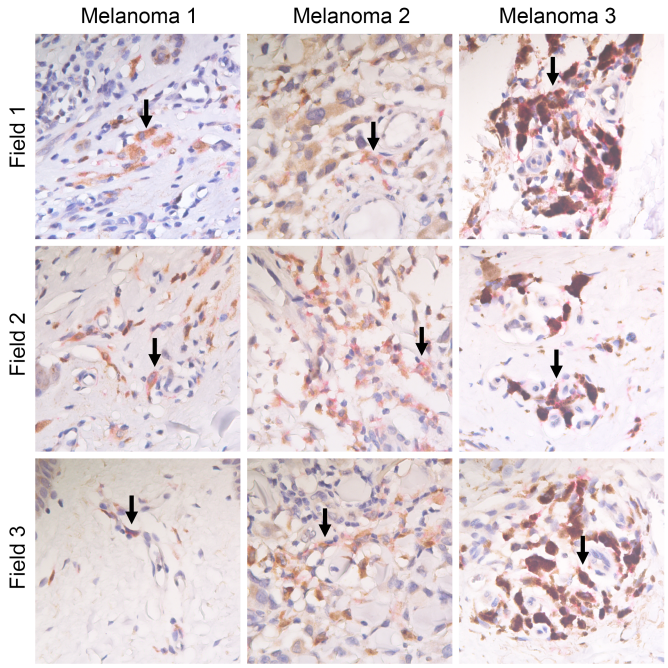
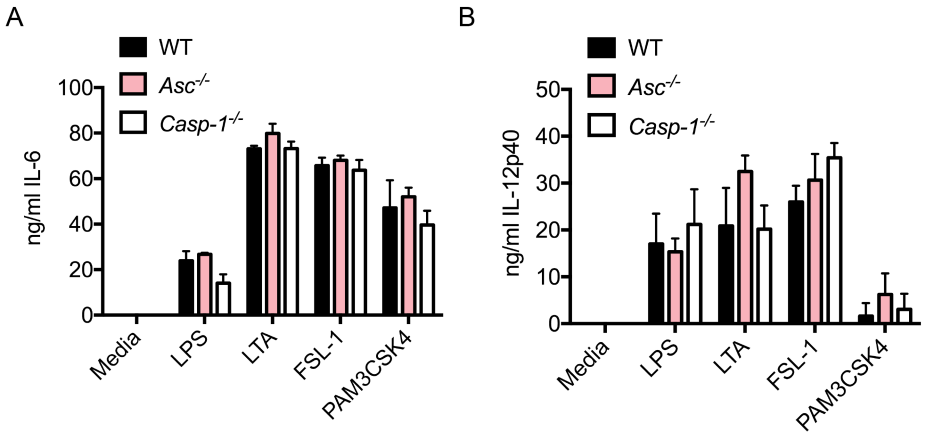


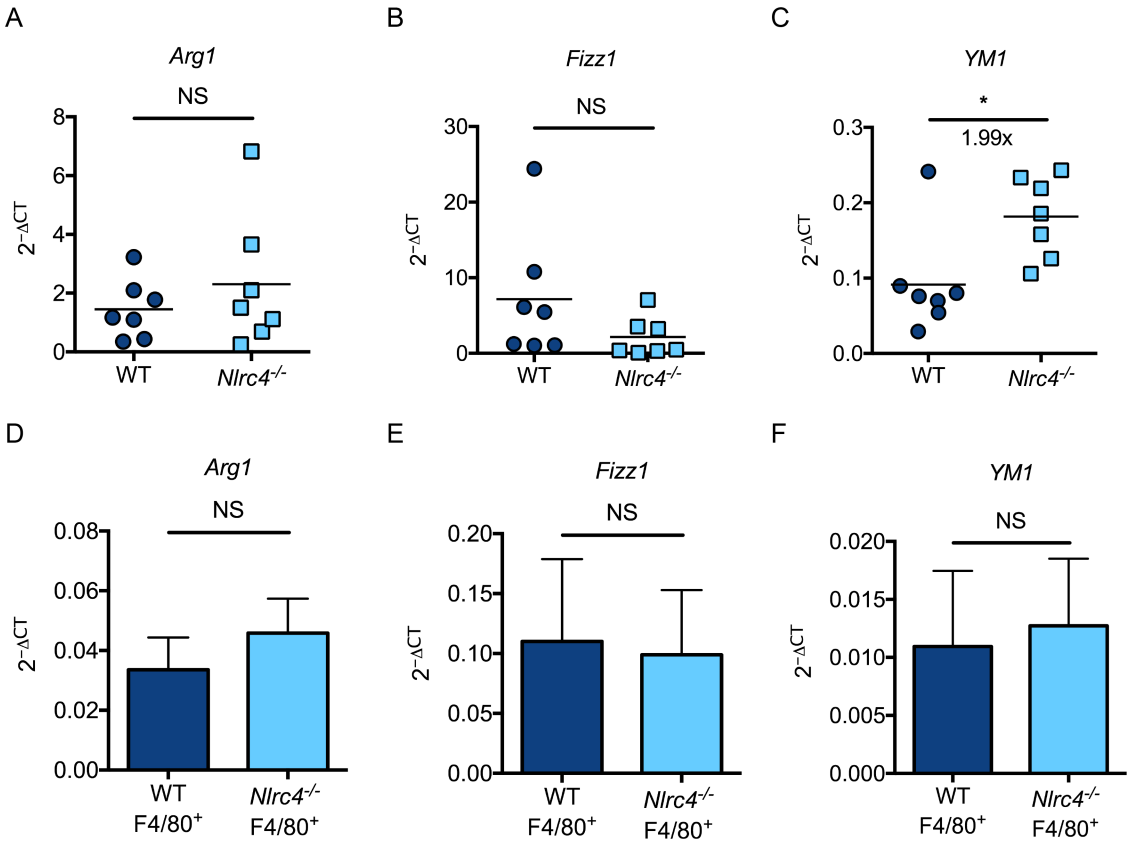
Supplemental Figure 1. NLRC4 protects against LLC tumor growth in vivo. (A-G) WT, *Nlrc4*$^{-/-}$, *Asc*$^{-/-}$, and *Casp-1*$^{-/-}$ mice were injected s.c. with 5 x 10^5 LLC cells. (A, D, F) Tumor area (length x width) was measured every 2-3 days and (B, E, G) tumors mass was determined 15 days post-inoculation. (C) Representative images of WT and *Nlrc4*$^{-/-}$ LLC tumors. n=4-5 mice per group. (A, D, F) Error bars represent SEM. (B, E, G) * $p \leq 0.05$ and NS denotes not significant by unpaired two-tailed Student's *t*-test. (A) * $p \leq 0.05$ and **** $p \leq 0.0001$ by two-way ANOVA with Sidak's multiple comparisons test.



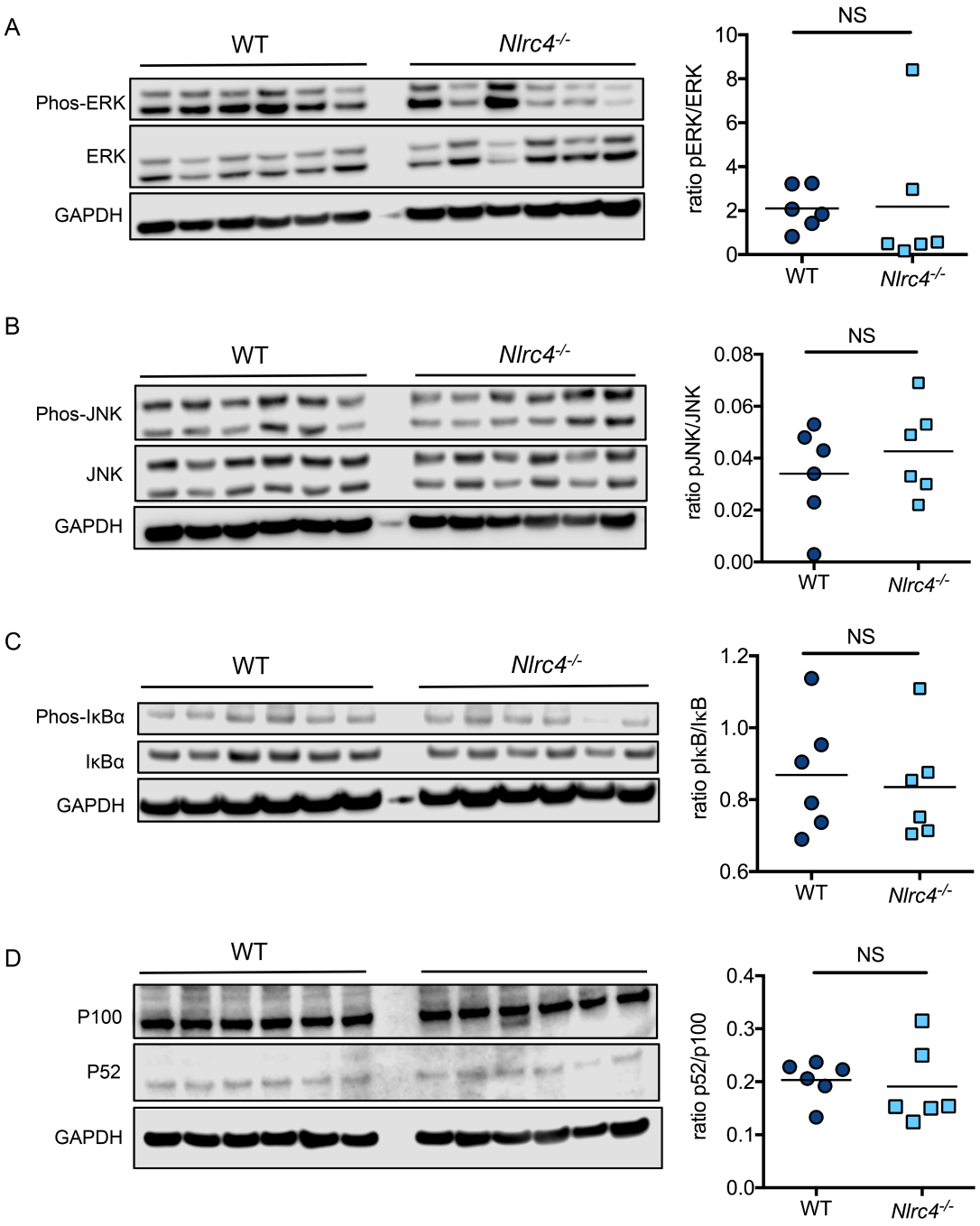
Supplemental Figure 2. $CD163^{+}$ macrophages in primary invasive melanomas co-stain with NLRC4. The three primary invasive melanomas used in Figure 2 were stained for CD163 using 3,3'-Diaminobenzidine (brown) and NLRC4 using Permanent Red (red). Three representative sections per melanoma are shown with an arrow identifying the clearest example of $CD163^{+}NLRC4^{+}$ tumor-associated macrophages in each panel.



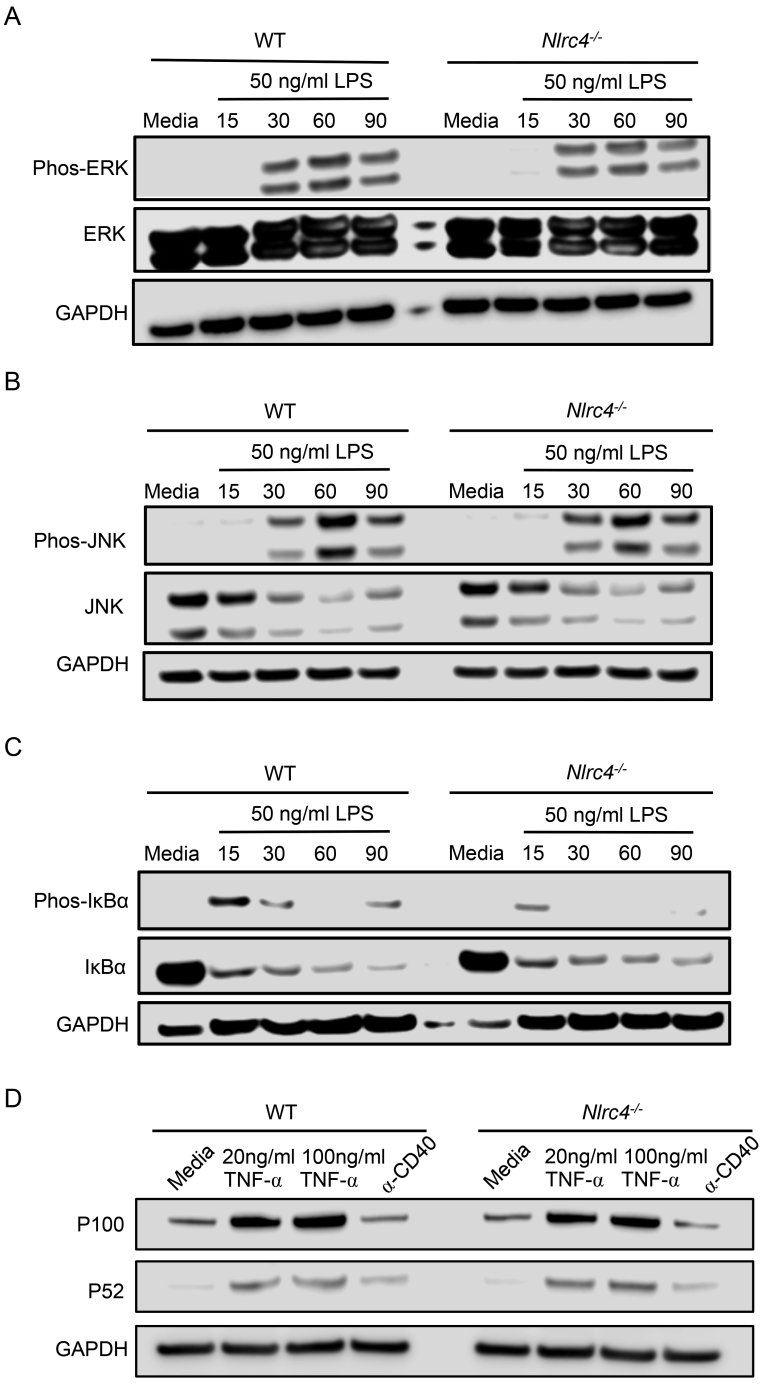
Supplemental Figure 3. IL-6 and IL-12p40 production are intact in *Asc*$^{-/-}$ and *Casp1*$^{-/-}$ BMDM. (A, B) WT, *Asc*$^{-/-}$, and *Casp-1*$^{-/-}$ BMDM were challenged with 50 ng/ml LPS, 50 μg/ml LTA, 100 ng/ml FSL-1, and 1 μg/ml Pam3CSK4. 20 hours later IL-6 and IL-12p40 levels in the supernatants was determined by ELISA. Determinations were performed in triplicate and are representative of 2 independent experiments. Error bars represent SD.



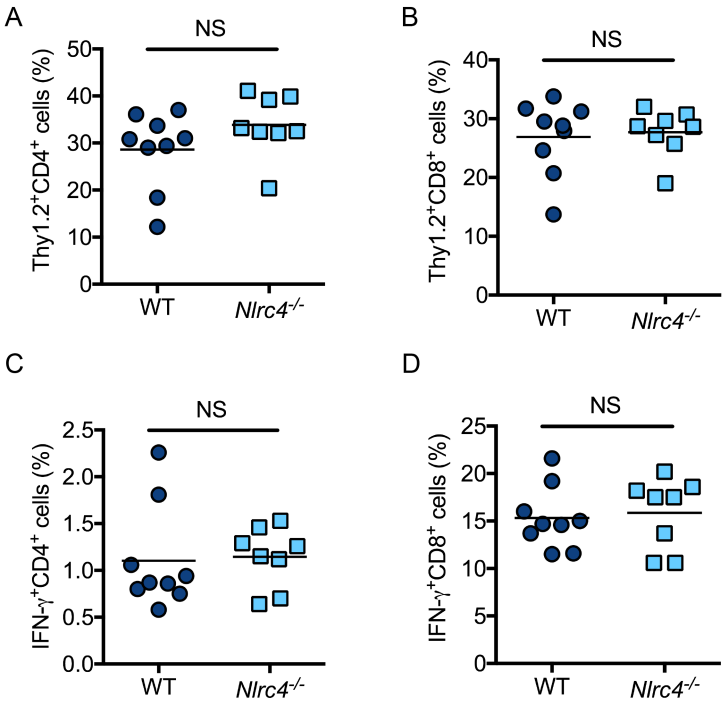
Supplemental Figure 4. Expression of M2 macrophage associated genes are similar in tumors and tumor-associated macrophages from WT and *Nlrc4*$^{-/-}$ mice. (A-C) WT and *Nlrc4*$^{-/-}$ mice were injected s.c. with 1 x 10^5 B16F10 cells. Day 14 post-inoculation total RNA was isolated from homogenized tumors and used to determine expression of *Arg1* (A), *Fizz1* (B), and *Ym1* (C). Data are pooled from two independent experiments with n=7 mice per group. (D-F) WT and *Nlrc4*$^{-/-}$ mice were injected s.c. with 1 x 10^5 B16F10 cells; 14 days post-inoculation tumors were harvested, pooled, and CD45.2$^+$F4/80$^+$ cells were FACs sorted. RNA was isolated from CD45.2$^+$F4/80$^+$ cells and used to determine expression of *Arg1* (D), *Fizz1* (E), and *Ym1* (F) by qPCR. Data are pooled from three independent experiments each with n≥5 pooled tumors per group. Error bars represent SEM. * $p \leq 0.05$ and NS denotes not significant by unpaired two-tailed Student's *t*-test.



Supplemental Figure 5. Evaluation of MAPK and NF-κB signaling pathways in tumors from NLRC4-deficient mice. (A-D) B16F10 tumors from WT and *Nlrc4*$^{-/-}$ mice day 14 post-inoculation were homogenized and immunoblotted with antibodies against phospho-p44/42 MAPK (Erk1/2) and p44/42 MAPK (Erk1/2) (A), phospho-SAPK/JNK and SAPK/JNK (B), phospho-IκBα and IκBα (C), NF-κB2 p52/p100 (D), and GAPDH (A-D)**.** Densitometry of the ratio of phosphorylated to total protein or p52 to p100 are depicted. Each lane represents a tumor from an individual mouse. NS denotes not significant by unpaired two-tailed Student's *t*-test.



Supplemental Figure 6. Comparison of MAPK and NF-κB signaling pathways in WT and *Nlrc4*$^{-/-}$ BMDM. (A-C) WT and *Nlrc4*$^{-/-}$ BMDM were challenged for 15, 30, 60 and 90 minutes with 50 ng/ml LPS. Cell lysates were immunoblotted with antibodies against phospho-p44/42 MAPK (Erk1/2) and p44/42 MAPK (Erk1/2) (A), phospho-SAPK/JNK and SAPK/JNK (B), phospho-IκBα and IκBα (C), and GAPDH (A-C)**.** (D) WT and *Nlrc4*$^{-/-}$ BMDM were challenged with 20 ng/ml TNF-α, 100 ng/ml TNF-α, or 10 μg/ml anti-CD40 antibody for 18 hours. Cell lysates were immunoblotted with antibodies against NF-κB2 p52/p100 and GAPDH. Data are representative of 3 independent experiments.



Supplemental Figure 7. IFN-γ production by $CD4^+$ and $CD8^+$ T cells is not altered in the tumor draining lymph node of *Nlrc4*$^{-/-}$ mice. (A-D) WT and *Nlrc4*$^{-/-}$ mice were injected s.c. with 1 x 10^5 B16F10 cells and at day 14 post-inoculation tumor draining lymph nodes were harvested for analysis by flow cytometry. Frequency of $CD4^+Thy1.2^+$ cells (A) and $CD8^+Thy1.2^+$ cells (B) from the tumor draining lymph node is shown. (C, D) Cells from the tumor draining lymph node were stimulated with PMA and ionomycin followed by intracellular cytokine staining for IFN-γ. Frequency of $CD4^+$ IFN-γ^+ T cells (C) and $CD8^+$ IFN-γ^+ T cells (D) are shown. n≥8 mice per group. NS denotes not significant by unpaired two-tailed Student's *t*-test.

Supplemental Table 1. B16F10 tumors from *Nlrc4*$^{-/-}$ mice have decreased cytokine and chemokine expression. Full table of cytokines and chemokines from Qiagen RT^2 profiler PCR array on B16F10 tumors from WT and *Nlrc4*$^{-/-}$ mice. Select cytokines and chemokines are shown in Figure 5.

Cytokine/ Chemokine	Descriptive name	Wild Type $2^{-\Delta CT}$	*Nlrc4*$^{-/-}$ $2^{-\Delta CT}$	Fold change relative to *Nlrc4*$^{-/-}$
Cxcl5	Chemokine (C-X-C motif) ligand 5	0.00159	0.00008	18.92516
Cxcl9	Chemokine (C-X-C motif) ligand 9	0.00812	0.00044	18.54929
Cxcl13	Chemokine (C-X-C motif) ligand 13	0.00301	0.00039	7.81542
Cxcl16	Chemokine (C-X-C motif) ligand 16	0.02418	0.00384	6.29930
Ifng	Interferon gamma	0.00097	0.00019	5.15934
Ccl11	Chemokine (C-C motif) ligand 11	0.00532	0.00108	4.90841
Ccl5	Chemokine (C-C motif) ligand 5	0.02279	0.00503	4.52788
Ccl4	Chemokine (C-C motif) ligand 4	0.00929	0.00223	4.16989
Tnfsf10	Tumor necrosis factor (ligand) superfamily, member 10	0.00082	0.00021	3.85346
Fasl	Fas ligand	0.00043	0.00012	3.70580
Tnf	Tumor necrosis factor	0.00167	0.00047	3.53300
Il1rn	Interleukin 1 receptor antagonist	0.00729	0.00207	3.52900
Xcl1	Chemokine (C motif) ligand 1	0.00183	0.00052	3.49379
Cxcl12	Chemokine (C-X-C motif) ligand 12	0.00843	0.00265	3.17839
Il12b	Interleukin 12B	0.00091	0.00029	3.16666
Il16	Interleukin 16	0.00137	0.00044	3.11653
Il27	Interleukin 27	0.00079	0.00028	2.87949
Il10	Interleukin 10	0.00060	0.00023	0.00023
Ccl3	Chemokine (C-C motif) ligand 3	0.00555	0.00212	2.61188
Ccl7	Chemokine (C-C motif) ligand 7	0.04146	0.01715	2.41752
Ltb	Lymphotoxin B	0.00209	0.00087	2.40942
Ccl12	Chemokine (C-C motif) ligand 12	0.01751	0.00800	2.18919
Cxcl10	Chemokine (C-X-C motif) ligand 10	0.12356	0.05665	2.18108
Il11	Interleukin 11	0.00062	0.00030	2.09779
Ccl22	Chemokine (C-C motif) ligand 22	0.00218	0.00104	2.08825
Tnfsf13b	Tumor necrosis factor (ligand) superfamily, member 13b	0.00046	0.00023	2.01492
Cx3cl1	Chemokine (C-X3-C motif) ligand 1	0.00263	0.00132	2.00060
Tnfrsf11b	Tumor necrosis factor receptor superfamily, member 11b	0.00621	0.00318	1.95353
Spp1	Secreted phosphoprotein 1	0.05356	0.02813	1.90386
Tnfsf11	Tumor necrosis factor (ligand) superfamily, member 11	0.00038	0.00021	1.86315

Il1a	Interleukin 1 alpha	0.00084	0.00045	1.84969
Ccl1	Chemokine (C-C motif) ligand 1	0.00042	0.00023	1.84633
Lif	Leukemia inhibitory factor	0.00074	0.00041	1.78131
Cd70	CD70 antigen	0.00033	0.00018	1.78027
Csf1	Colony stimulating factor 1	0.00567	0.00321	1.76902
CD40lg	CD40 ligand	0.00014	0.00008	1.71351
Ccl19	Chemokine (C-C motif) ligand 19	0.00631	0.00369	1.71161
Mstn	Myostatin	0.00066	0.00040	1.66271
Ccl20	Chemokine (C-C motif) ligand 20	0.00028	0.00017	1.65709
Csf3	Colony stimulating factor 3	0.00033	0.00020	1.64588
Il7	Interleukin 7	0.00029	0.00018	1.64310
Il15	Interleukin 15	0.00125	0.00078	1.59683
Il18	Interleukin 18	0.00543	0.00347	1.56592
Ccl2	Chemokine (C-C motif) ligand 2	0.02886	0.01848	1.56165
Csf2	Colony stimulating factor 2	0.00027	0.00018	1.54081
Il17a	Interleukin 17A	0.00008	0.00006	1.47224
Il12a	Interleukin 12A	0.00024	0.00017	1.45716
Il24	Interleukin 24	0.00028	0.00019	1.41821
Il21	Interleukin 21	0.00014	0.00010	1.38019
Hc	Hemolytic complement	0.00034	0.00025	1.35328
Il5	Interleukin 5	0.00039	0.00029	1.34847
Bmp2	Bone morphogenetic protein 2	0.00078	0.00058	1.33497
Lta	Lymphotoxin A	0.00044	0.00033	1.33224
Il17f	Interleukin 17F	0.00033	0.00025	1.32738
Il2	Interleukin 2	0.00023	0.00017	1.30518
Ifna2	Interferon alpha 2	0.00033	0.00026	1.29424
Il22	Interleukin 22	0.00034	0.00027	1.28006
Bmp6	Bone morphogenetic protein 6	0.00035	0.00028	1.25181
Il3	Interleukin 3	0.00031	0.00026	1.22028
Il9	Interleukin 9	0.00019	0.00016	1.20566
Il13	Interleukin 13	0.00028	0.00023	1.19427
Il4	Interleukin 4	0.00043	0.00037	1.16699
Thpo	Thrombopoietin	0.00037	0.00032	1.14787
Nodal	Nodal	0.00023	0.00021	1.13964
Cxcl11	Chemokine (C-X-C motif) ligand 11	0.00033	0.00030	1.12890

Il6	Interleukin 6	0.00057	0.00054	1.06581
Cntf	Ciliary neurotrophic factor	0.00072	0.00070	1.03102
Ccl17	Chemokine (C-C motif) ligand 17	0.00112	0.00114	-1.01912
Ctf1	Cardiotrophin 1	0.00035	0.00037	-1.05448
Bmp4	Bone morphogenetic protein 4	0.00115	0.00135	-1.17356
Tgfb2	Transforming growth factor, beta 2	0.00249	0.00293	-1.17921
Bmp7	Bone morphogenetic protein 7	0.00069	0.00083	-1.19182
Pf4	Platelet factor 4	0.01994	0.02467	-1.23679
Il23a	Interleukin 23, alpha subunit p19	0.00036	0.00046	-1.28299
Cxcl1	Chemokine (C-X-C motif) ligand 1	0.00033	0.00045	-1.33535
Ccl24	Chemokine (C-C motif) ligand 24	0.00021	0.00028	-1.34812
Mif	Macrophage migration inhibitory factor	5.39932	7.54029	-1.39653
Gpil	Glucose phophate isomerase 1	1.01432	1.54162	-1.51985
Osm	Oncostatin M	0.00079	0.00124	-1.57051
Vegfa	Vascular endothelial growth factor A	0.02908	0.04833	-1.66193
Cxcl3	Chemokine (C-X-C motif) ligand 3	0.00037	0.00064	-1.75844
Adipoq	Adiponectin, C1Q and collagen domain containing	0.00158	0.00294	-1.85462
Il1b	Interleukin 1 beta	0.00227	0.00456	-2.00574
Ppbp	Pro-platelet basic protein	0.00051	0.00336	-6.59844