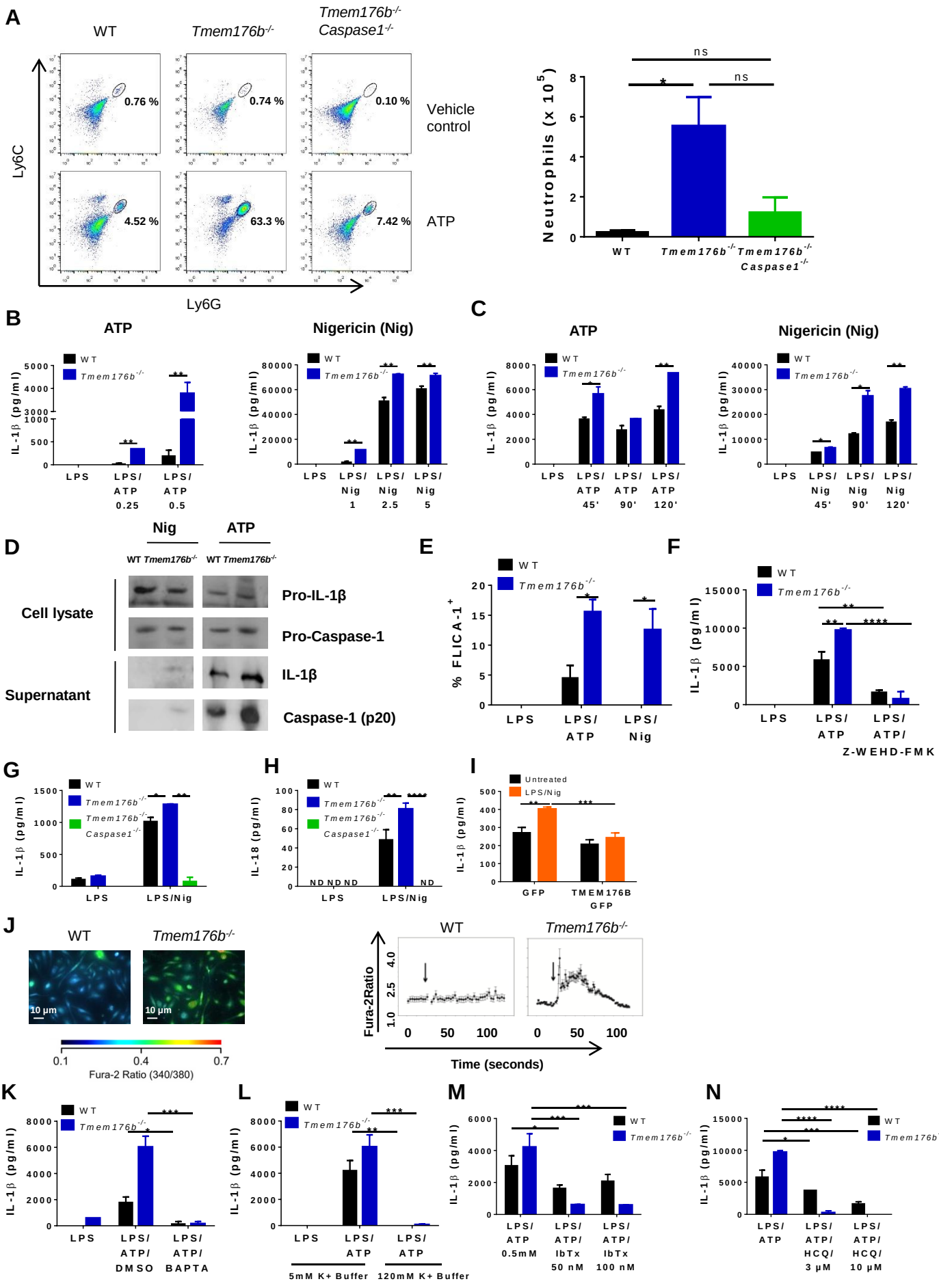

A
WT
Tmem176b-/-
Tmem176b-/- Caspase1-/-
0.76 %
0.74 %
0.10 %
4.52 %
63.3 %
7.42 %
Vehicle control
ATP
Ly6C
Ly6G
Neutrophils (x 10^5)
ns
*
ns
WT
Tmem176b-/-
Tmem176b-/- Caspase1-/-
B
ATP
Nigericin (Nig)
IL-1β (pg/ml)
WT
Tmem176b-/-
LPS
LPS/ ATP 0.25
LPS/ ATP 0.5
LPS/ Nig 1
LPS/ Nig 2.5
LPS/ Nig 5
C
ATP
Nigericin (Nig)
LPS/ ATP 45'
LPS/ ATP 90'
LPS/ ATP 120'
LPS/ Nig 45'
LPS/ Nig 90'
LPS/ Nig 120'
D
Nig
ATP
WT Tmem176b-/-
Cell lysate
Pro-IL-1β
Pro-Caspase-1
Supernatant
IL-1β
Caspase-1 (p20)
E
% FLICA-1+
LPS
LPS/ ATP
LPS/ Nig
F
IL-1β (pg/ml)
LPS/ ATP/ Z-WEHD-FMK
G
IL-1β (pg/ml)
Tmem176b-/- Caspase1-/-
LPS
LPS/Nig
H
IL-18 (pg/ml)
ND ND ND
ND
I
Untreated
LPS/Nig
IL-1β (pg/ml)
GFP
TMEM176B GFP
J
WT
Tmem176b-/-
10 µm
0.1
0.4
0.7
Fura-2 Ratio (340/380)
Fura-2Ratio
1.0
2.5
4.0
0
50
100
Time (seconds)
K
IL-1β (pg/ml)
LPS
LPS/ ATP/ DMSO
LPS/ ATP/ BAPTA
L
LPS/ ATP
5mM K+ Buffer
120mM K+ Buffer
M
LPS/ ATP 0.5mM
LPS/ ATP/ IbTx 50 nM
LPS/ ATP/ IbTx 100 nM
N
LPS/ ATP
LPS/ ATP/ HCQ/ 3 µM
LPS/ ATP/ HCQ/ 10 µM

FIGURE 2

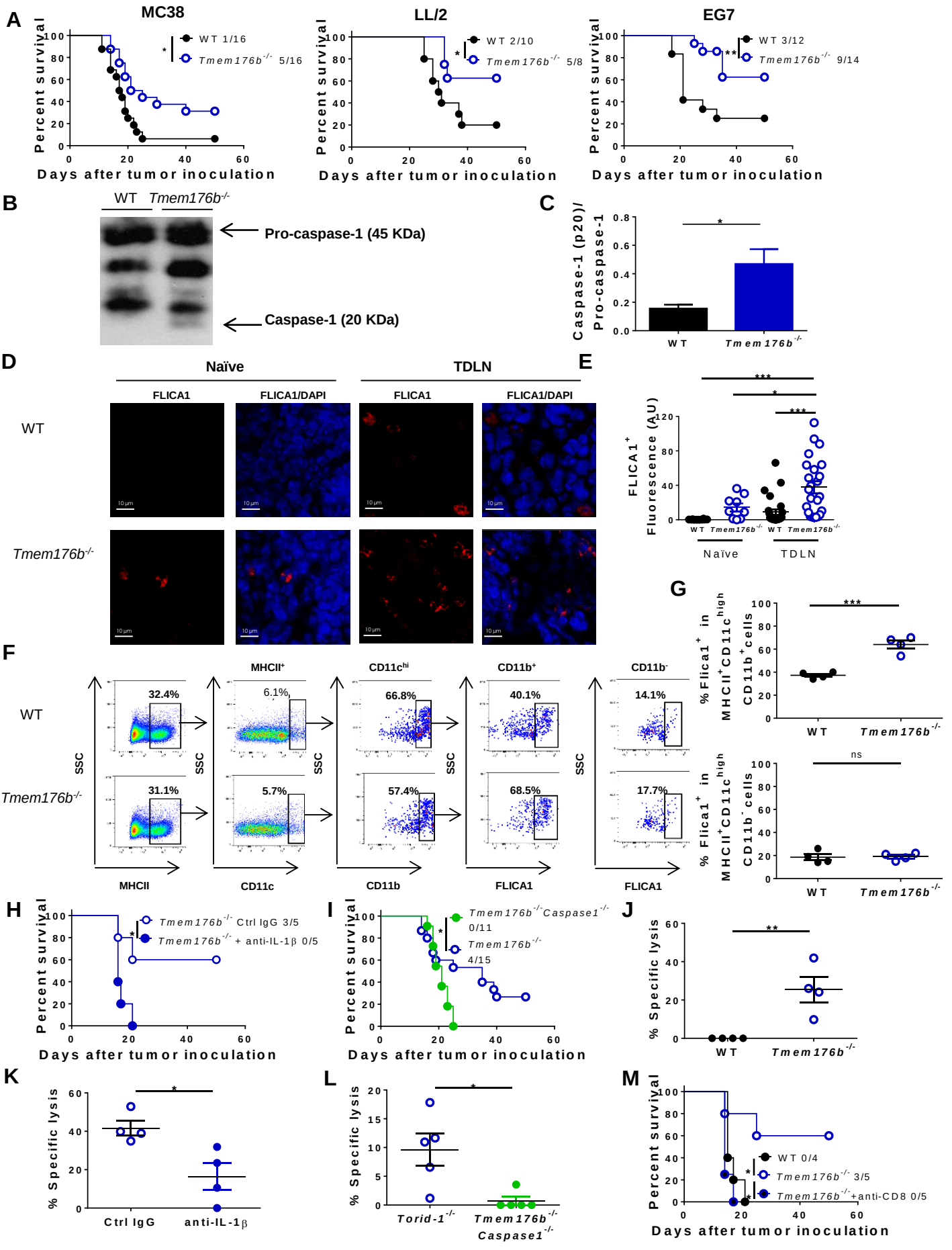

A
MC38
LL/2
EG7
Percent survival
Days after tumor inoculation
WT 1/16
Tmem176b-/- 5/16
WT 2/10
Tmem176b-/- 5/8
WT 3/12
Tmem176b-/- 9/14
B
WT
Tmem176b-/-
Pro-caspase-1 (45 KDa)
Caspase-1 (20 KDa)
C
Caspase-1 (p20)/ Pro-caspase-1
WT
Tmem176b-/-
D
Naïve
TDLN
FLICA1
FLICA1/DAPI
WT
Tmem176b-/-
E
FLICA1+ Fluorescence (AU)
Naïve
TDLN
F
MHCII+
CD11chi
CD11b+
CD11b-
32.4%
6.1%
66.8%
40.1%
14.1%
31.1%
5.7%
57.4%
68.5%
17.7%
SSC
MHCII
CD11c
CD11b
FLICA1
G
% Flica1+ in MHCII+CD11chigh CD11b+cells
% Flica1+ in MHCII+CD11chigh CD11b-cells
ns
H
Tmem176b-/- Ctrl IgG 3/5
Tmem176b-/- + anti-IL-1β 0/5
I
Tmem176b-/-Caspase1-/- 0/11
Tmem176b-/- 4/15
J
% Specific lysis
K
Ctrl IgG
anti-IL-1β
L
Torid-1-/-
Tmem176b-/- Caspase1-/-
M
WT 0/4
Tmem176b-/- 3/5
Tmem176b-/-+anti-CD8 0/5

FIGURE 3

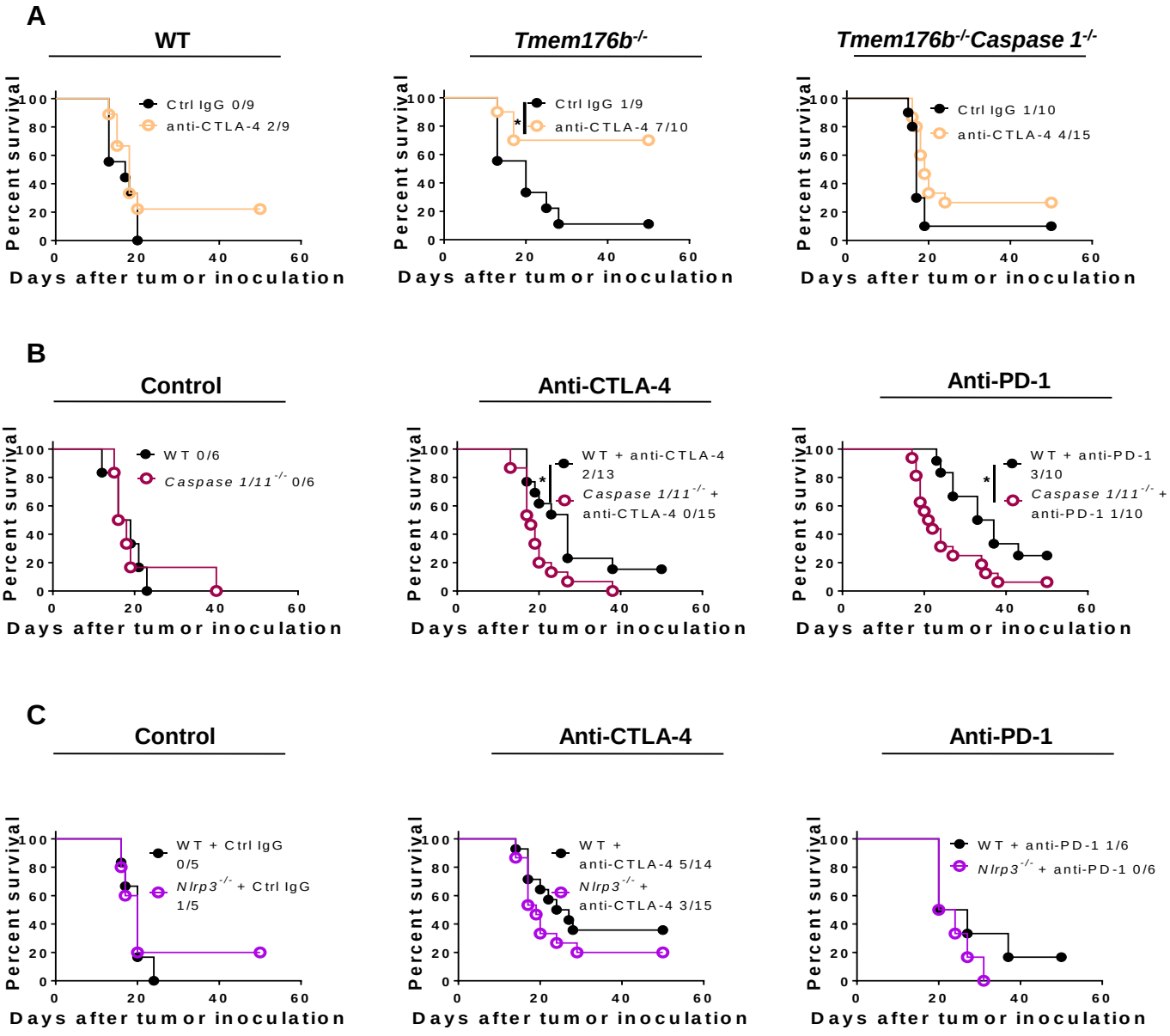

A
WT
Tmem176b-/-
Tmem176b-/-Caspase 1-/-
Percent survival
Days after tumor inoculation
Ctrl IgG 0/9
anti-CTLA-4 2/9
Ctrl IgG 1/9
anti-CTLA-4 7/10
Ctrl IgG 1/10
anti-CTLA-4 4/15
B
Control
Anti-CTLA-4
Anti-PD-1
WT 0/6
Caspase 1/11-/- 0/6
WT + anti-CTLA-4 2/13
Caspase 1/11-/- + anti-CTLA-4 0/15
WT + anti-PD-1 3/10
Caspase 1/11-/- + anti-PD-1 1/10
C
Control
Anti-CTLA-4
Anti-PD-1
WT + Ctrl IgG 0/5
Nlrp3-/- + Ctrl IgG 1/5
WT + anti-CTLA-4 5/14
Nlrp3-/- + anti-CTLA-4 3/15
WT + anti-PD-1 1/6
Nlrp3-/- + anti-PD-1 0/6

FIGURE 4

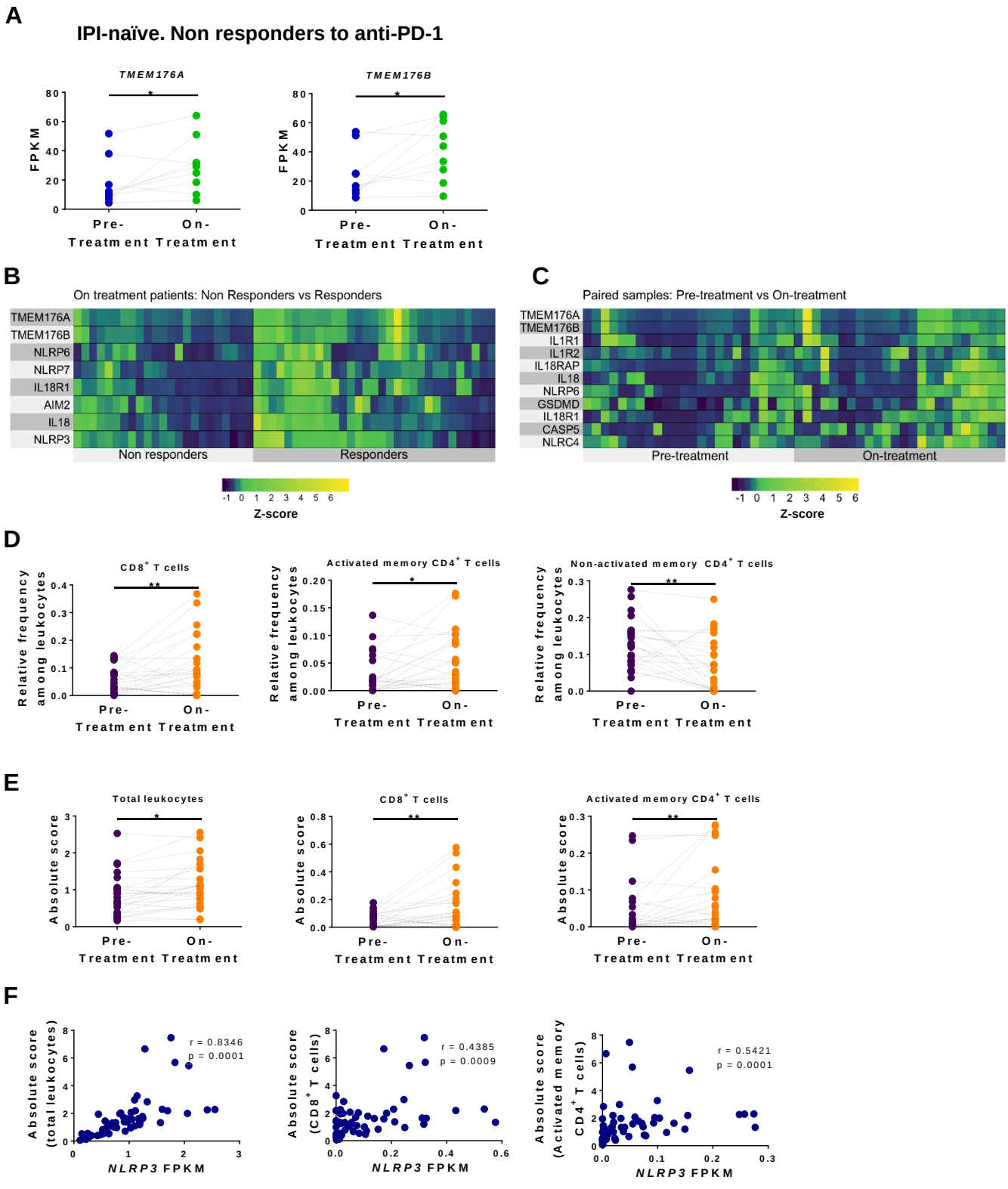

A
IPI-naïve. Non responders to anti-PD-1
TMEM176A
TMEM176B
FPKM
Pre-Treatment
On-Treatment
B
On treatment patients: Non Responders vs Responders
TMEM176A
TMEM176B
NLRP6
NLRP7
IL18R1
AIM2
IL18
NLRP3
Non responders
Responders
Z-score
C
Paired samples: Pre-treatment vs On-treatment
TMEM176A
TMEM176B
IL1R1
IL1R2
IL18RAP
IL18
NLRP6
GSDMD
IL18R1
CASP5
NLRC4
Pre-treatment
On-treatment
Z-score
D
CD8+ T cells
Activated memory CD4+ T cells
Non-activated memory CD4+ T cells
Relative frequency among leukocytes
E
Total leukocytes
CD8+ T cells
Activated memory CD4+ T cells
Absolute score
F
Absolute score (total leukocytes)
Absolute score (CD8+ T cells)
Absolute score (Activated memory CD4+ T cells)
NLRP3 FPKM
r = 0.8346
p = 0.0001
r = 0.4385
p = 0.0009
r = 0.5421
p = 0.0001

FIGURE 5

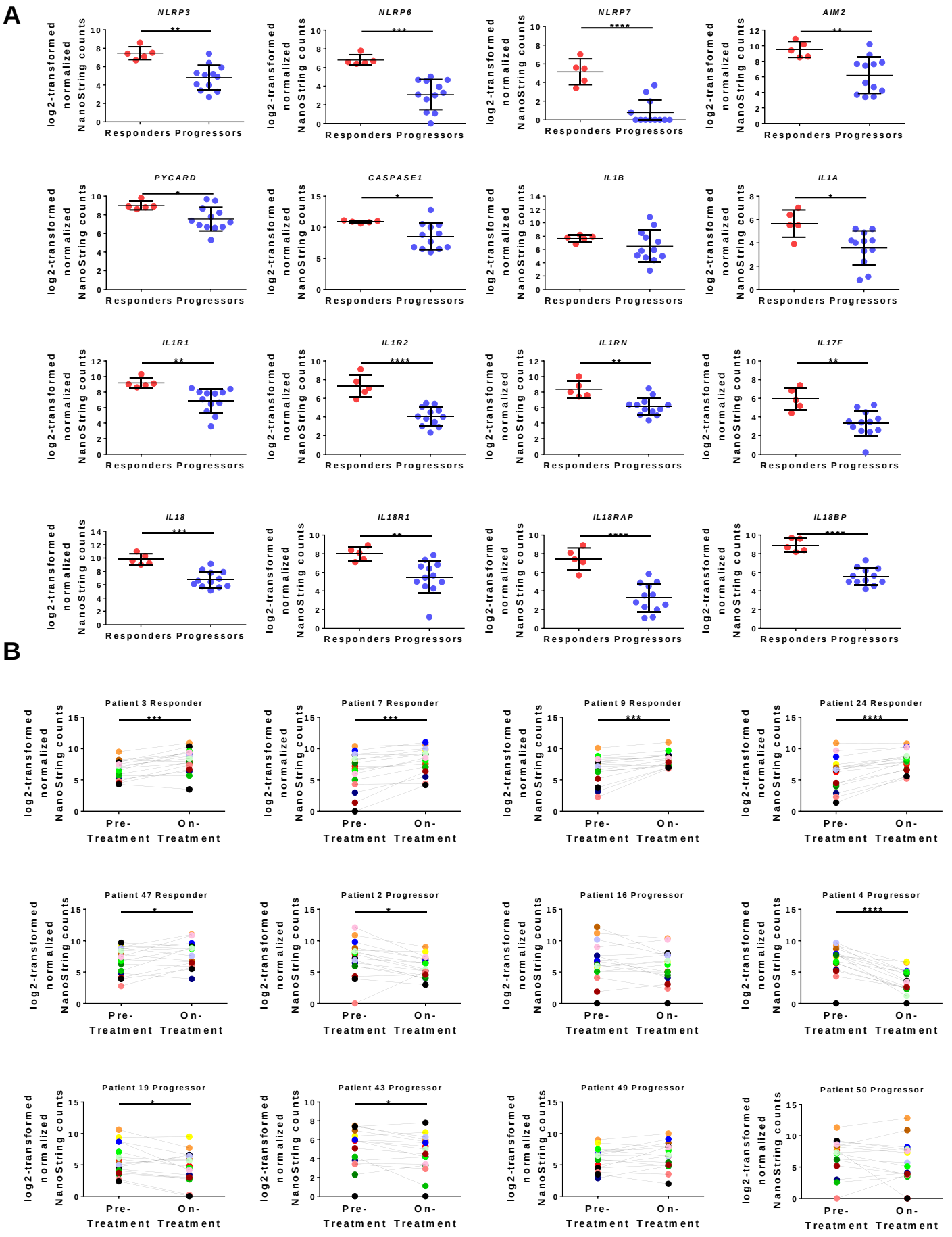
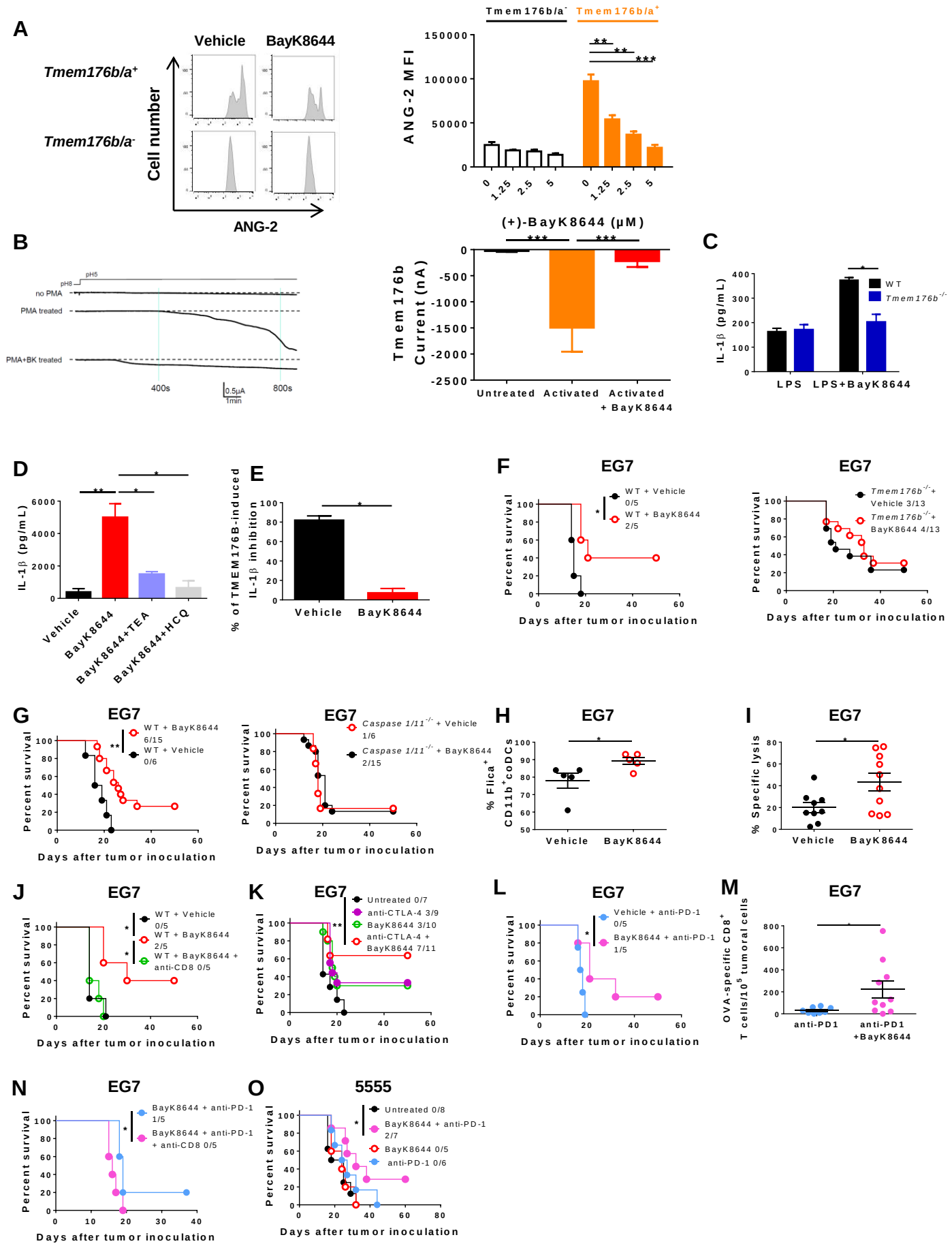

A
NLRP3
NLRP6
NLRP7
AIM2
PYCARD
CASPASE1
IL1B
IL1A
IL1R1
IL1R2
IL1RN
IL17F
IL18
IL18R1
IL18RAP
IL18BP
log2-transformed normalized NanoString counts
Responders Progressors
B
Patient 3 Responder
Patient 7 Responder
Patient 9 Responder
Patient 24 Responder
Patient 47 Responder
Patient 2 Progressor
Patient 16 Progressor
Patient 4 Progressor
Patient 19 Progressor
Patient 43 Progressor
Patient 49 Progressor
Patient 50 Progressor
Pre-Treatment
On-Treatment

FIGURE 6


A
Vehicle
BayK8644
Tmem176b/a+
Tmem176b/a-
Cell number
ANG-2
Tmem176b/a-
Tmem176b/a+
ANG-2 MFI
150000
100000
50000
0
**
**
***
0
1.25
2.5
5
0
1.25
2.5
5
B
pH5
pH8
no PMA
PMA treated
PMA+BK treated
400s
0.5µA
1min
800s
(+)-BayK8644 (µM)
Tmem176b Current (nA)
***
***
0
-500
-1000
-1500
-2000
-2500
Untreated
Activated
Activated + BayK8644
C
IL-1β (pg/mL)
400
300
200
100
0
*
WT
Tmem176b-/-
LPS
LPS+BayK8644
D
IL-1β (pg/mL)
6000
4000
2000
0
**
*
Vehicle
BayK8644
BayK8644+TEA
BayK8644+HCQ
E
% of TMEM176B-induced IL-1β inhibition
100
80
60
40
20
0
*
Vehicle
BayK8644
F
EG7
Percent survival
WT + Vehicle 0/5
WT + BayK8644 2/5
*
Days after tumor inoculation
EG7
Percent survival
Tmem176b-/- + Vehicle 3/13
Tmem176b-/- + BayK8644 4/13
Days after tumor inoculation
G
EG7
Percent survival
WT + BayK8644 6/15
WT + Vehicle 0/6
**
Days after tumor inoculation
EG7
Percent survival
Caspase 1/11-/- + Vehicle 1/6
Caspase 1/11-/- + BayK8644 2/15
Days after tumor inoculation
H
EG7
% Flica+ CD11b+coDCs
*
Vehicle
BayK8644
I
EG7
% Specific lysis
*
Vehicle
BayK8644
J
EG7
Percent survival
WT + Vehicle 0/5
WT + BayK8644 2/5
WT + BayK8644 + anti-CD8 0/5
*
*
Days after tumor inoculation
K
EG7
Percent survival
Untreated 0/7
anti-CTLA-4 3/9
BayK8644 3/10
anti-CTLA-4 + BayK8644 7/11
**
Days after tumor inoculation
L
EG7
Percent survival
Vehicle + anti-PD-1 0/5
BayK8644 + anti-PD-1 1/5
*
Days after tumor inoculation
M
EG7
OVA-specific CD8+ T cells/10^5 tumoral cells
*
anti-PD1
anti-PD1 +BayK8644
N
EG7
Percent survival
BayK8644 + anti-PD-1 1/5
BayK8644 + anti-PD-1 + anti-CD8 0/5
*
Days after tumor inoculation
O
5555
Percent survival
Untreated 0/8
BayK8644 + anti-PD-1 2/7
BayK8644 0/5
anti-PD-1 0/6
*
Days after tumor inoculation

SUPPLEMENTAL INFORMATION

Figure S1. Related to Figure 1.

A) Sequence of genomic DNA (*Caspase-1* gene) from *Tmem176b*$^{-/-}$ and *Tmem176b*$^{-/-}$*Caspase1*$^{-/-}$ mice. *Tmem176b*$^{-/-}$ *Caspase1*$^{-/-}$ (double KO) mice were generated by deletion of the indicated bases in *Caspase-1* gene in *Tmem176b*$^{-/-}$ mice using the CRISPR/Cas9 strategy. Proteins sequences are shown in the lower part of the alignment. Right panel: Western blot confirming the absence of caspase-1 in *Tmem176b*$^{-/-}$*Caspase1*$^{-/-}$ splenocytes.

B) 6-8 weeks-old male WT and *Tmem176b*$^{-/-}$ mice were injected i.p. with 20 mg/kg ATP. 4 h later, peritoneal lavage was performed and absolute numbers of neutrophils ($CD11b^{+}Ly6G^{+}Ly6C^{int}$) were determined by flow cytometry. In the plots, $CD11b^{+}$ cells were analyzed for Ly6C and Ly6G expression. When indicated, the caspase-1 inhibitor Z-YVAD-CMK was injected i.p. at 5 mg/kg at the time of ATP treatment. At least six animals were studied in each group in two independent experiments. * $p<0.05$. ns: non significant. One-way ANOVA test. Left panel shows representative scatter dot plots and right panel shows quantification for the different groups.

C) WT and *Tmem176b*$^{-/-}$ bone marrow-derived DCs (BMDCs) were treated with LPS (0.25 µg/ml) during 4 h, then washed and treated with 500 µg/ml of aluminum particles for the indicated times (left panel). Dose-response experiments are shown in the central panel. Culture supernatants were harvested and IL-1β was determined by ELISA in the left and central panels. Right panel: caspase-1 activation was studied by flow cytometry using the FLICA1 reagent. BMDCs were stimulated for 3 h with LPS and then incubated in the presence or absence of 500 µg/ml aluminum particles during 45 min. * $p<0.05$; ** $p<0.01$ One-way ANOVA test. One experiment representative of three is shown.

D) WT BMDCs were primed for 3 h with LPS (0.25 µg/ml), then washed and left untreated or treated during 45 min with 5 µM nigericin. Cell lysates and culture supernatants were analyzed by Western blot for pro-caspase-1 and caspase-1 (p20) expression. One experiment representative of two is shown.

E) THP-1 cells were differentiated to macrophages by treatment for 48 h with 0.1 µM PMA. Cells were then electroporated with *GFP* or *GFP-TMEM176B-1* coding pcDNA1./8203 plasmids. Sixteen h later, cells were left untreated or treated for 3 h with 0.25 µg/ml LPS and then exposed for 2 h with 2.5 µM nigericin. Transfection efficiency was assessed by flow cytometry.

F) Cell viability was studied by analysis of propidium iodide staining by flow cytometry (right panel). One experiment representative of three is shown.

Figure S2. Related to Figure 2.

A) Stromal TMEM176B expression is associated with lower survival in human colon cancer patients. Ninety (90) samples from human colon carcinomas were assessed for TMEM176B expression (brown staining, counterstained with hematoxylin) by immunohistochemistry, Representative images for parenchyma and stroma depicting low and high expression are shown.

B) Survival analysis of colon cancer patients with high and low TMEM176B expression. High stromal TMEM176B expression was associated with worse overall survival. p=0.0194 Log-rank (Mantel-Cox) test. Parenchymal expression did not correlate with survival (p=0.55; Log-rank (Mantel-Cox) test). The staining and analysis were done by two independent researchers in a blinded fashion, ignoring the survival data for each sample.

C) Matrix of scatterplots showing correlations between *NLRP3, IL1B, IL18, TMEM176A and TMEM176B* gene expression in 420 macrophages from single cell RNA-Seq data from melanoma biopsies (Jerby-Arnon et al., 2018). Correlations were made using Spearman's correlation coefficient. Red lines indicate the local regression (LOESS) fit. P, P value; rho, Spearman's correlation coefficient.

D) 1 x 10^6 MC38 colon cancer cells, 1 x 10^5 LL2 lung cancer cells or 1 x 10^6 EG7 thymic lymphoma cells were s.c. injected into WT and *Tmem176b*$^{-/-}$ mice. Tumor growth was monitored every three days and measured in its longer and shorter diameter. Mice were euthanized when one of the diameters reached 2 cm. The ratio in the inset shows the number of animals developing tumors over the number of injected animals.

E) *Tmem176b* mRNA expression assessed by RT-PCR. The 249-bp band corresponds to the expected size of the specific amplified fragment. One experiment representative of two is shown.

F) Tmem176b-specific cell lysis was assessed by using the method described in the STAR METHODS section. WT naÏve splenocytes were loaded either with low or high doses of DDAO and injected i.v. in tumor-bearing WT and *Tmem176b*$^{-/-}$ animals 14 days after tumor inoculation. Four h after injection, the spleen was harvested and the ratio of low and high DDAO populations was studied to assess the percentage of specific cell death as explained in the STAR METHODS section. Not significant. Student's *t* test.

G) Analysis of caspase-1 activation by Western blot comparing tumors lysates from WT and *Tmem176b*$^{-/-}$ animals. One experiment representative of two is shown.

H) Left panel shows a representative scatter dot plot for MHCII and CD11c expression within TDLN to identify migratory and resident cDCs. The middle and right panels depict the percentage of FLICA1+ cells (expressing active caspase-1) within CD11b$^+$ resident and migratory cDCs respectively from WT and *Tmem176b*$^{-/-}$ animals. One experiment representative of two is shown. * $p<0.05$ Student's *t* test.

I) Lymph nodes from naive mice or tumor-bearing animals (TDLN; harvested 14 d after EG7 cell injection) were immunostained with anti-Tmem176b (red) and anti-CD11b (Cyan) antibodies. Nuclei were stained with DAPI (blue). The white arrows indicate Tmem176b$^+$ CD11b^{int} cells. At least three animals were studied in each group.

J) TCRβ$^+$ CD4$^+$ RORγt$^+$ T cells were assessed by FACS in the TDLN from EG7-bearing WT and *Tmem176b*$^{-/-}$ mice. Relative (left panel) and absolute numbers (central panel) were determined. The right panel shows relative cell numbers in *Tmem176b*$^{-/-}$ animals treated with control IgG or anti-IL-1β neutralizing antibody. * $p<0.05$ Student's *t* test.

K) EG7-bearing WT and *Tmem176b*$^{-/-}$ animals were euthanized 14 days after tumor cell inoculation. TDLN cells were re-stimulated *in vitro* with 10μM OVA peptide 323-339 (ISQAVHAAHAEINEAGR). IL-17A$^+$ CD4$^+$ T cells were assessed by flow cytometry. One experiment representative of three is shown. * $p<0.05$; ** $p<0.01$ Two-way ANOVA test.

L) EG7-bearing *Tmem176b*$^{-/-}$ mice were treated with control IgG or anti-IL-17A neutralizing antibody and mouse survival was studied. $p=0.0593$. Log-rank (Mantel-Cox) test.

Figure S3. Related to Figure 2.

A) EG7 tumors and tumor-draining lymph nodes (TDLN) from WT and *Tmem176b*$^{-/-}$ mice were harvested 14 days after tumor cell injection (n=5 per group). Quantitative RT-PCR was performed for the indicated genes. * $p<0.05$ Student's *t* test.

B) EG7 tumors from WT and *Tmem176b*$^{-/-}$ mice were harvested 14 days after tumor cell injection (at least n=5 per group). Tumors were disaggregated with collagenase D and cell suspensions were stained with the following antibodies and analyzed by flow cytometry (Infiltrating cells: TCRVβ12^{-} (EG7 cells are TCRVβ12^{+}); NK: TCRVβ12^{-} TCRβ^{-}NK1.1^{+}; NKT: TCRVβ12^{-}TCRβ^{+}NK1.1^{+}; TγδCD27^{+}: TCRVβ12^{-}TCRγδ^{+}CD27^{+}; Tγδ :TCRVβ12^{+}TCRγδ^{+}; Th17: TCRVβ12^{+}TCRβ^{+}CD4^{+}RORγt^{+} B: TCRVβ12^{-}TCRβ^{-}CD19^{+}; MDSCs: TCRβ^{-}CD11b^{+}Gr1^{+}. Student's *t* test.

Figure S4. Related to Figure 2.

A) Representative flow cytometry analysis of total and OVA (SIINFEKL peptide)-specific CD8^{+} T cells within the tumor microenvironment. TCRVβ12 staining was used to identify tumoral EG7 T cells. Representative of three experiments.

B) Determination of the frequency of total and OVA-specific CD8^{+} T cells in WT and *Tmem176b*$^{-/-}$ mice studied in A. * $p<0.05$ (Student's *t* test).

C) Assessment of intratumoral regulatory T cells (Tregs) and CD8/Treg ratio within the tumor microenvironment. * $p<0.05$ (Student's *t* test).

D) Tumor-infiltrating T cells were purified by negative selection and re-stimulated *in vitro* in the presence of LPS-treated BMDCs (1/10 ratio) with SIINFEKL peptide. Proliferation of CD8^{+} T cells was determined by flow cytometry by analyzing DDAO dilution. Four WT and four *Tmem176b*$^{-/-}$ animals were studied. * $p<0.05$; ** $p<0.01$. Student's *t* test.

E) Representative histograms of *in vivo* T-cell cytotoxicity against OVA tumoral antigen in experiments shown in Figure 2G.

F) Percentage of CD107a (degranulation marker) studied by flow cytometry within CD8^{+} T cells infiltrating tumors in *Tmem176b*$^{-/-}$ and *Tmem176b*$^{-/-}$*Caspase 1*$^{-/-}$ mice. * $p<0.05$. Student's *t* test.

Figure S5. Related to Figure 2.

Tumor-draining lymph nodes from EG7-bearing WT and *Tmem176b*$^{-/-}$ animals were harvested 14 days after tumor inoculation. Different lymphocyte populations were analyzed by flow cytometry.

A) Representative scatter dot plots indicating the frequency of TCRαβ, CD4, CD8 and Foxp3.

B) Percentage and absolute numbers of different lymphocyte populations. Student's *t* test. * $p<0.05$.

Figure S6. Related to Figure 5.

The log2-transformed normalized NanoString counts from melanoma tumor biopsies for the indicated inflammasome-related genes is shown for melanoma patients from the Chen *et al*. 2016 cohort analyzed in Figure 5. Biopsies were obtained before anti-CTLA-4 therapy in A and B.

A) Patients were classified as responders and progressors to anti-CTLA-4 therapy according to clinical outcome as defined by Chen *et al* (2016). * $p<0.05$. Non-paired Student's *t* test.

B) Patients progressing to anti-CTLA-4 therapy were then treated with anti-PD-1 antibodies. Based on their clinical outcome (with regards to anti-PD-1 therapy), they were classified as responders and progressors. * p<0.05; ** p<0.01. Non-paired Student's *t* test.

Figure S7. Related to Figure 5.

The log2-transformed normalized NanoString counts from melanoma tumor biopsies for the indicated inflammasome-related genes is shown for melanoma patients from the Chen *et al*. 2016 cohort analyzed (in Figure 5). Non-paired Student's *t* test.

A) Tumor biopsies were obtained before anti-PD-1 therapy in patients not responding to anti-CTLA-4 antibodies. In the figure, responders and progressors were classified according to their clinical outcome in response to anti-PD-1 therapy.

B) Tumor biopsies were obtained during the anti-CTLA-4 therapy (first 2-3 months). In the figure responders and progressors were classified according to their clinical outcome in response to anti-CTLA-4 therapy.

C) Color code to identify the genes studied in Figure 5B.

Figure S8. Related to Figure 6.

A) CHO-7 cells were transfected with *Tmem176b* and *Tmem176a*-mcherry-coding pcDNA1.3 plasmids. Cells were then loaded with the Na^+-sensitive fluorescent dye Asante NaTRIUM Green 2 (ANG-2). The graph indicates quantification of ANG-2 mean fluorescence intensity (MFI) subtracting in each condition the MFI obtained in Na^+-free buffer. Untreated and (-) BayK8644-treated cells were studied. One experiment representative of three is shown. ** p<0.01; *** p<0.001. Two-way ANOVA test.

B) BMDCs were treated for 3 h with 0.25 µg/ml LPS. Cells were washed and then treated with 2 mM ATP, 2.5 µM BayK8644 or both stimuli. Cell lysates and precipitated culture supernatants were electrophoresed, blotted and analyzed using an anti-IL-1β antibody. One experiment representative of four is shown.

C) Western blot analysis of pro-caspase-1 and caspase-1 expression in BMDCs (supernatants) treated as follows. 1: LPS; 2: LPS/ATP; 3: LPS/verapamil + ATP; 4: LPS/nifedipine + ATP; 5: LPS/diltiazem + ATP; 6: LPS/DMSO + ATP; 7: LPS/ATP medium standard K^+; 8: LPS/BayK8644 medium standard K^+; 9: LPS/ATP medium high K^+; 10: LPS/BayK medium high K^+. One experiment representative of two is shown.

D) BMDCs were treated with 0.5 µM BayK8644 for two h and then stained with FLICA1 to determine active caspase-1 by flow cytometry. Student's *t* test. * p<0.05. One experiment representative of three is shown.

E) EG7 tumor cells were untreated or treated *in vitro* with vehicle (ethanol) or with (+) BayK8644 (10 µM). Apoptosis was determined by analyzing active caspase-3/7. The grey histogram shows unstained conditions and the dotted line shows caspase-3/7 staining. One experiment representative of three is shown.

F) WT mice were inoculated with EG7 tumor cells and left untreated or treated with BayK8644 in the absence or presence of anti-CD8 depleting antibody. Growth of individual tumors is shown.

G-H) Growth of individual tumors (G) and survival (H) of BALB/c mice injected s.c with $1x10^5$ CT26 colon cancer cells. Mice were treated daily i.p with vehicle or 1 mg/kg BayK8644 at days 3-15 after tumor cell inoculation. * p<0.05; Log-rank (Mantel-Cox) test.

I) Absolute numbers (left panel) and percentage (right panel) of TCRβ$^+$CD4$^+$RORγt$^+$ T cells within TDLN from tumor (EG7)-bearing mice treated with anti-PD-1 or anti-PD-1 + BayK8644. 250 μg anti-PD-1 antibody was injected i.p at days 6, 9 and 12 after tumor inoculation. BayK8644 was injected every day since day 9 (in mice had established tumors) until day 21. * p<0.05 Student's *t* test.

J-K) Growth of individual tumors (J) and survival (K) of C57BL/6 mice injected s.c. with 1 x 10^5 LL/2 lung tumor cells. WT mice were injected with LL/2 cells and then treated with 250 μg anti-PD-1 antibody at days 6, 9 and 12 after tumor inoculation. BayK8644 was injected daily since day 9 (tumors were 10-20 mm^2 in surface) until day 21. In this therapeutic protocol BayK8644 monotherapy showed no anti-tumoral effect. ns: non significant. Log-rank (Mantel-Cox) test.

L-M) Growth of individual tumors (L) and survival (M) of C57BL/6 mice injected s.c with 1 x 10^6 MC38 colon cancer cells. WT mice were injected with MC38 cells and then treated with 250 μg anti-PD-1 antibody at days 6, 9 and 12 after tumor inoculation. BayK8644 was injected daily since day 9 (tumors were 10-20 mm^2 in surface) until day 21. In this therapeutic protocol BayK8644 monotherapy showed no significant anti-tumoral effect. ns: non significant. Log-rank (Mantel-Cox) test

N) WT mice were inoculated with 5555 melanoma cells and left untreated or treated either with anti-PD-1 antibody (days 6, 9 and 12), BayK8644 (days 9-21) or both. All animals had established tumors when BayK8644 treatment was started. Growth of individual tumors is shown.

O) C57BL/6 mice were s.c injected with 2.5 x 10^5 5555 melanoma cells. Ten days after tumor cell inoculation, animals were treated with control IgG, anti-CTLA-4 + anti-PD-1 or anti-CTLA-4 + anti-PD-1 + BayK8644. Mice were sacrificed when one of the tumor diameters reached 2 cm. Mice survival was monitored. Statistical significance was determined through the Log-rank (Mantel-Cox) test. ns: non significant. Control IgG vs anti-CTLA-4 + anti-PD-1 p= 0.0057; Control IgG vs anti-CTLA-4 + anti-PD-1 + BayK8644 p<0.0001; anti-CTLA-4 + anti-PD-1 vs anti-CTLA4 + anti-PD-1 + BayK8644, ns.

Figure S9. Related to discussion section of the manuscript.

WT and *Caspase1/11*$^{-/-}$ BMDCs were left untreated (NT) or were treated with LPS (0.25 μg/ml for 3 h), washed and exposed to ATP (0.5 mM for 2 h). A-B) Data are representative of two independent experiments. ns: non significant;
* p<0.05; *** p<0,001. Two-way ANOVA test.

A) *Tmem176b*, *Tnfa* and *IL6* mRNA expression was assessed by qRT-PCR.

B) Annexin V/7AAD staining of WT BMDCs left untreated (NT) or treated with LPS+ATP. The numbers indicate the percentage of cells in each quadrant.

Figure S1. Related to Figure 1

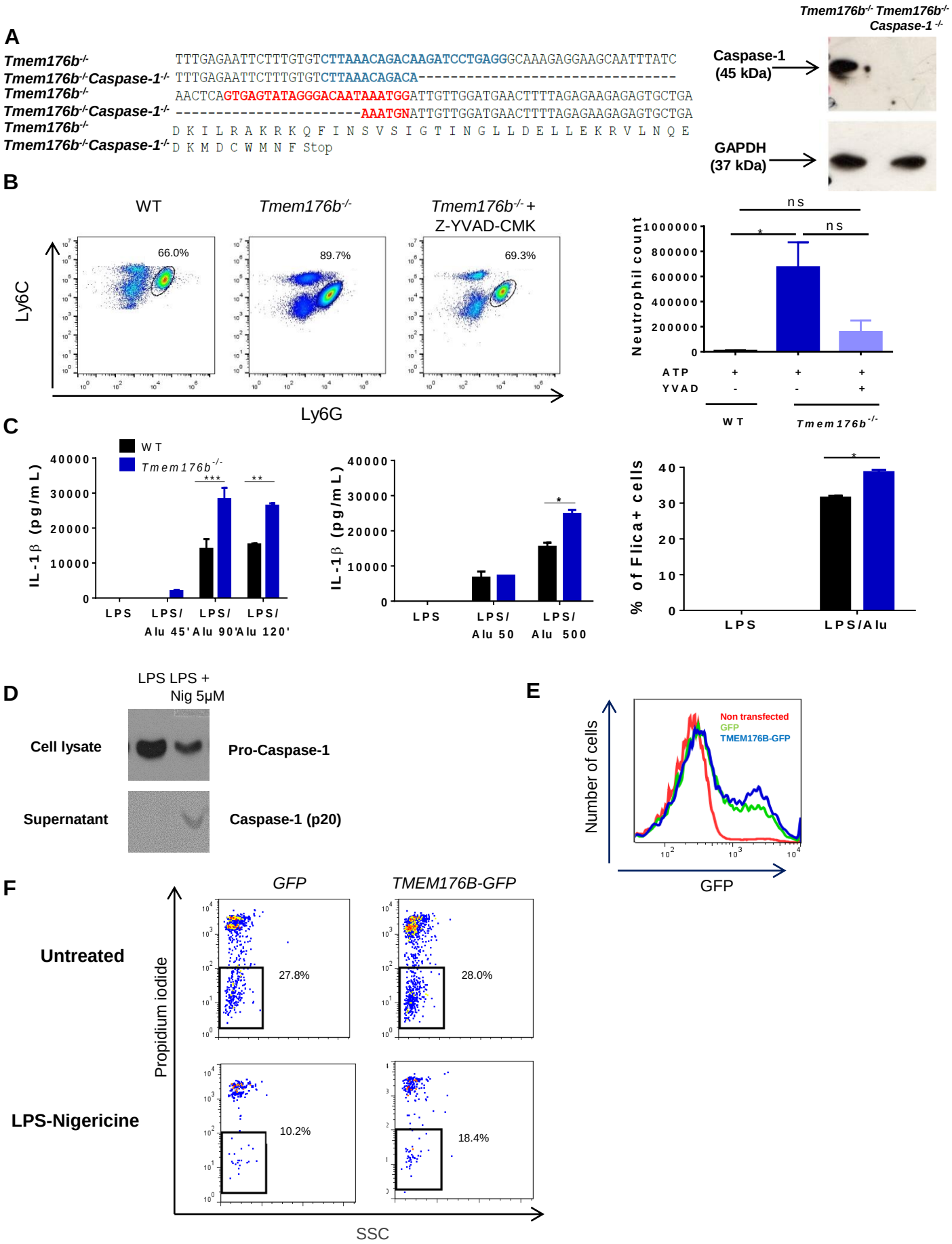


Figure S2. Related to Figure 2.

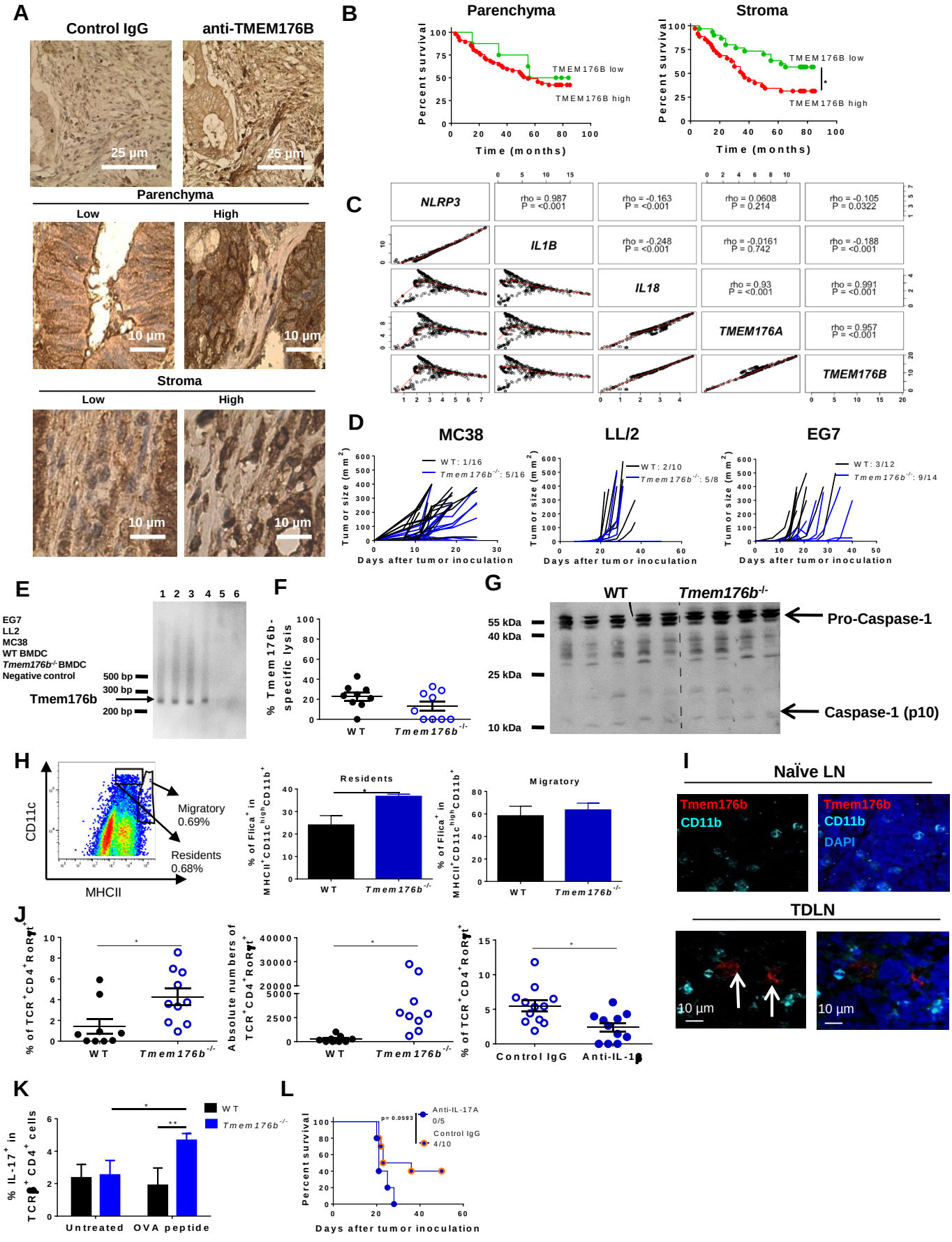


Figure S3. Related to Figure 2.

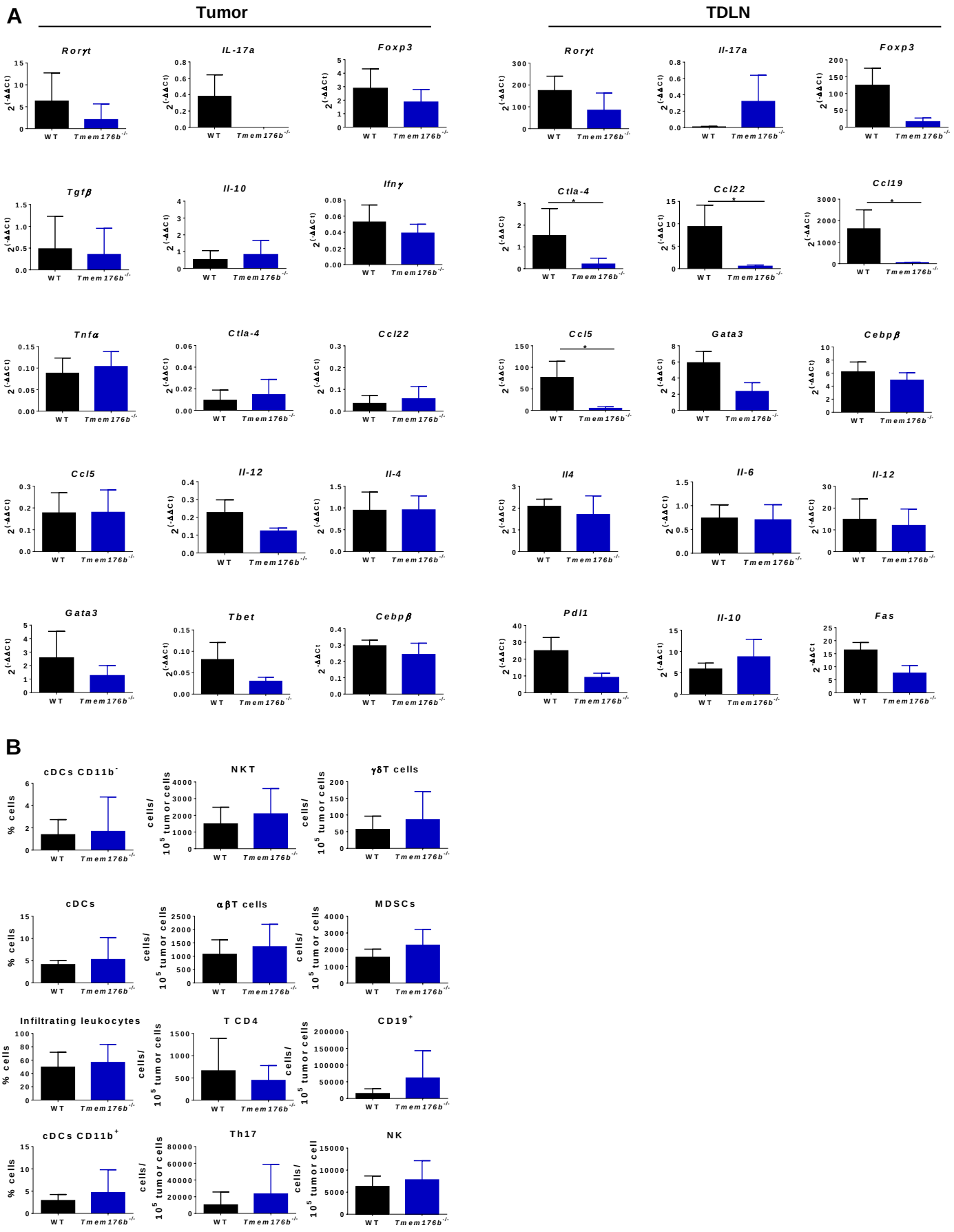


Figure S4. Related to Figure 2.

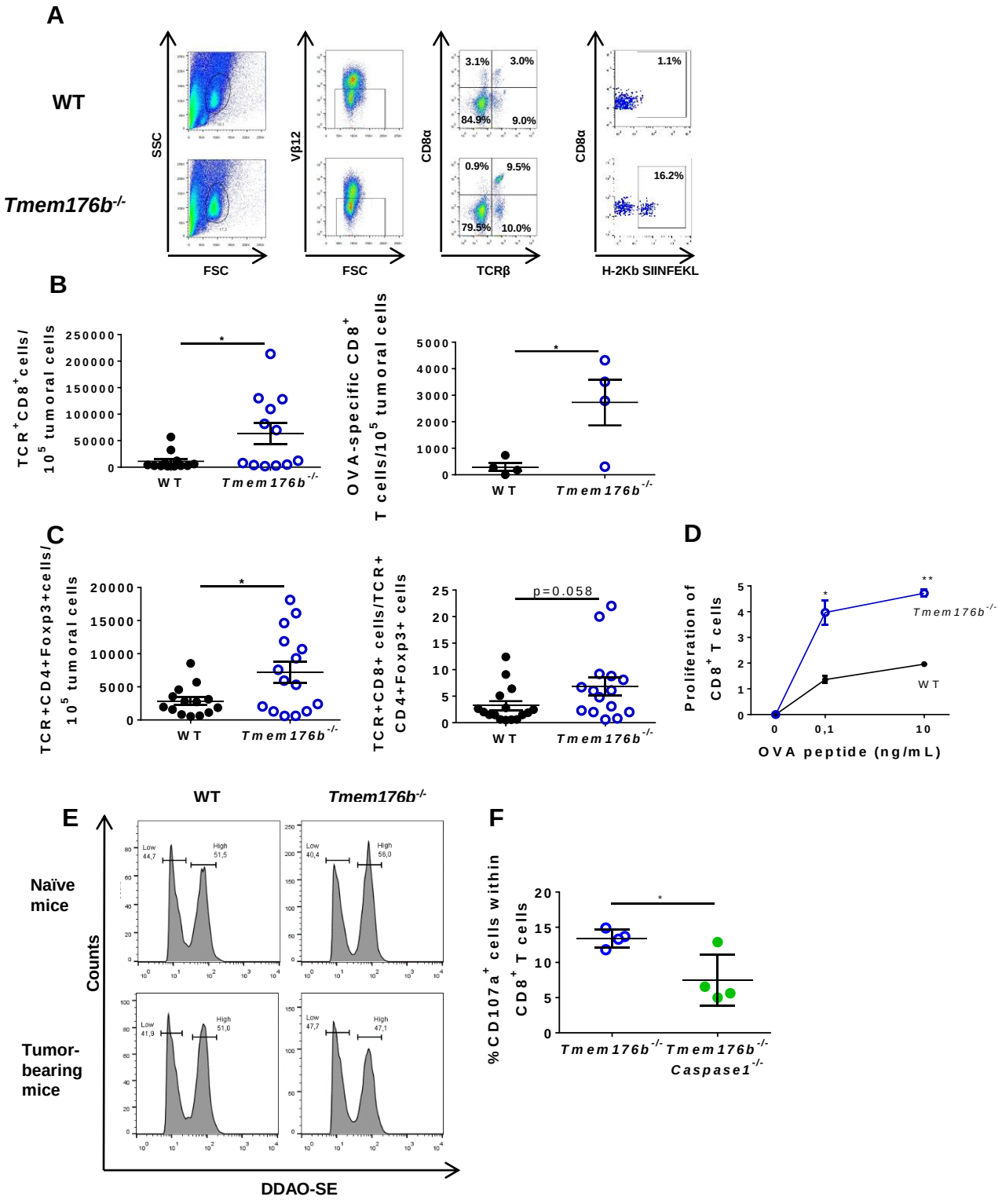


Figure S5. Related to Figure 2.

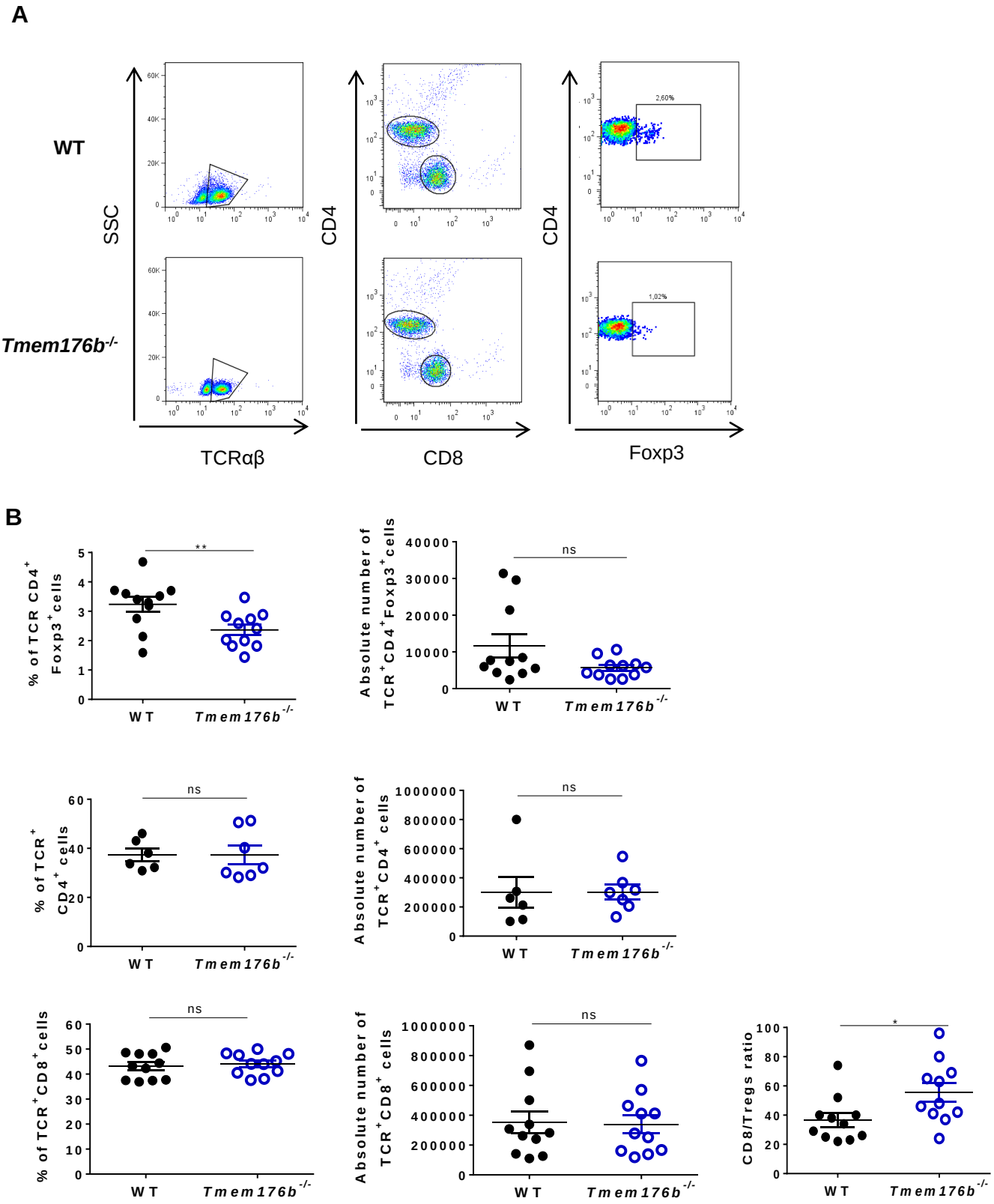


Figure S6. Related to Figure 4.

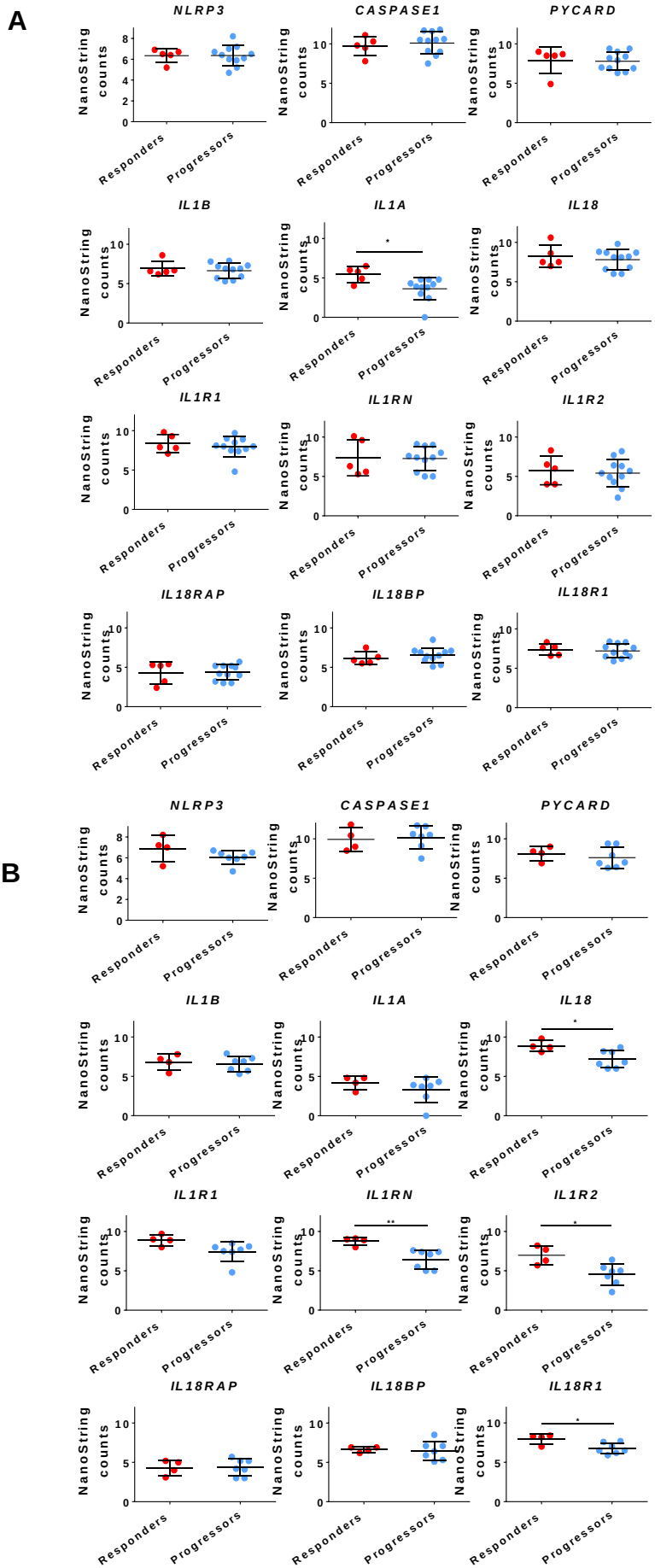


Figure S7. Related to Figure 4.

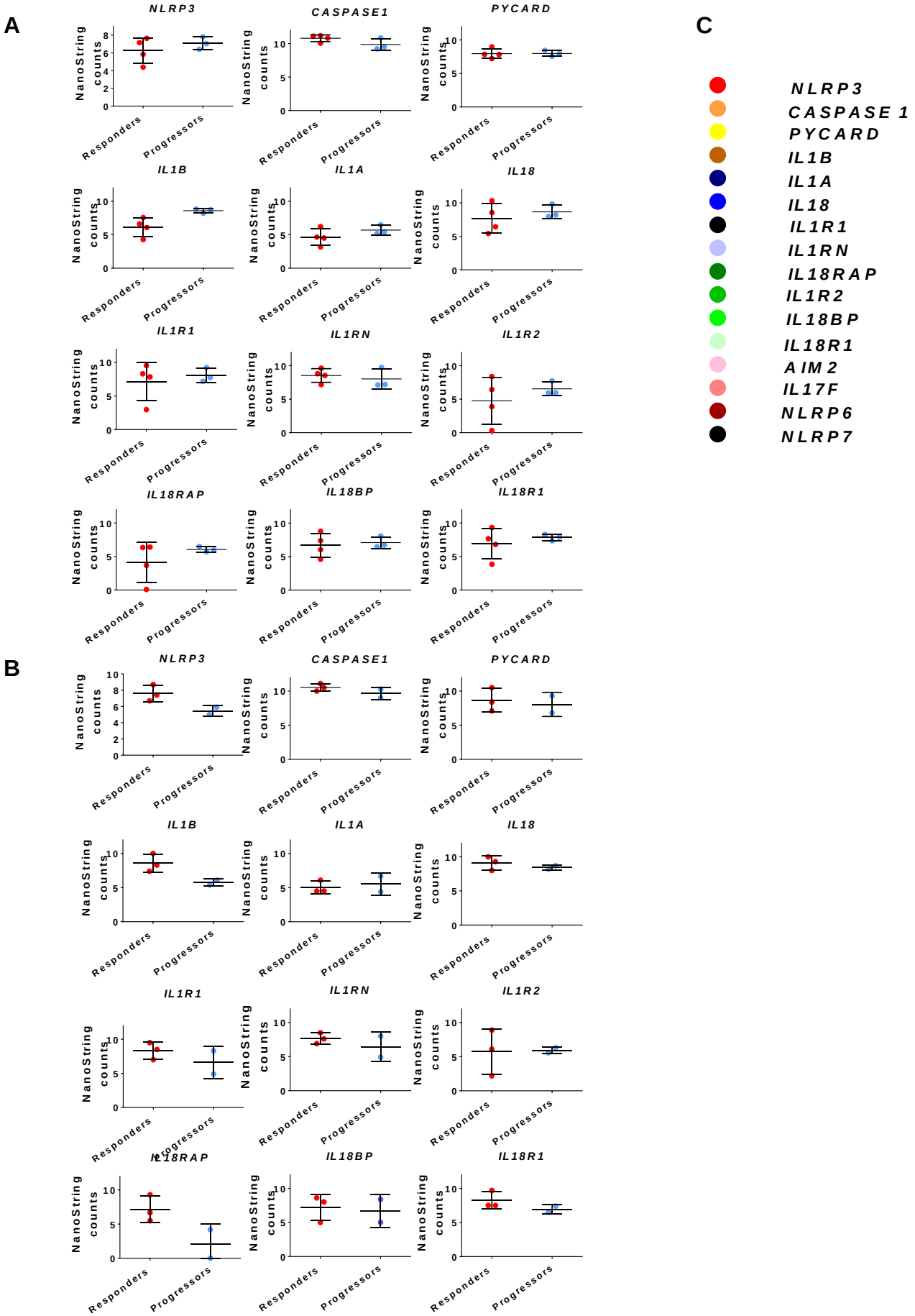


Figure S8. Related to Figure 6.

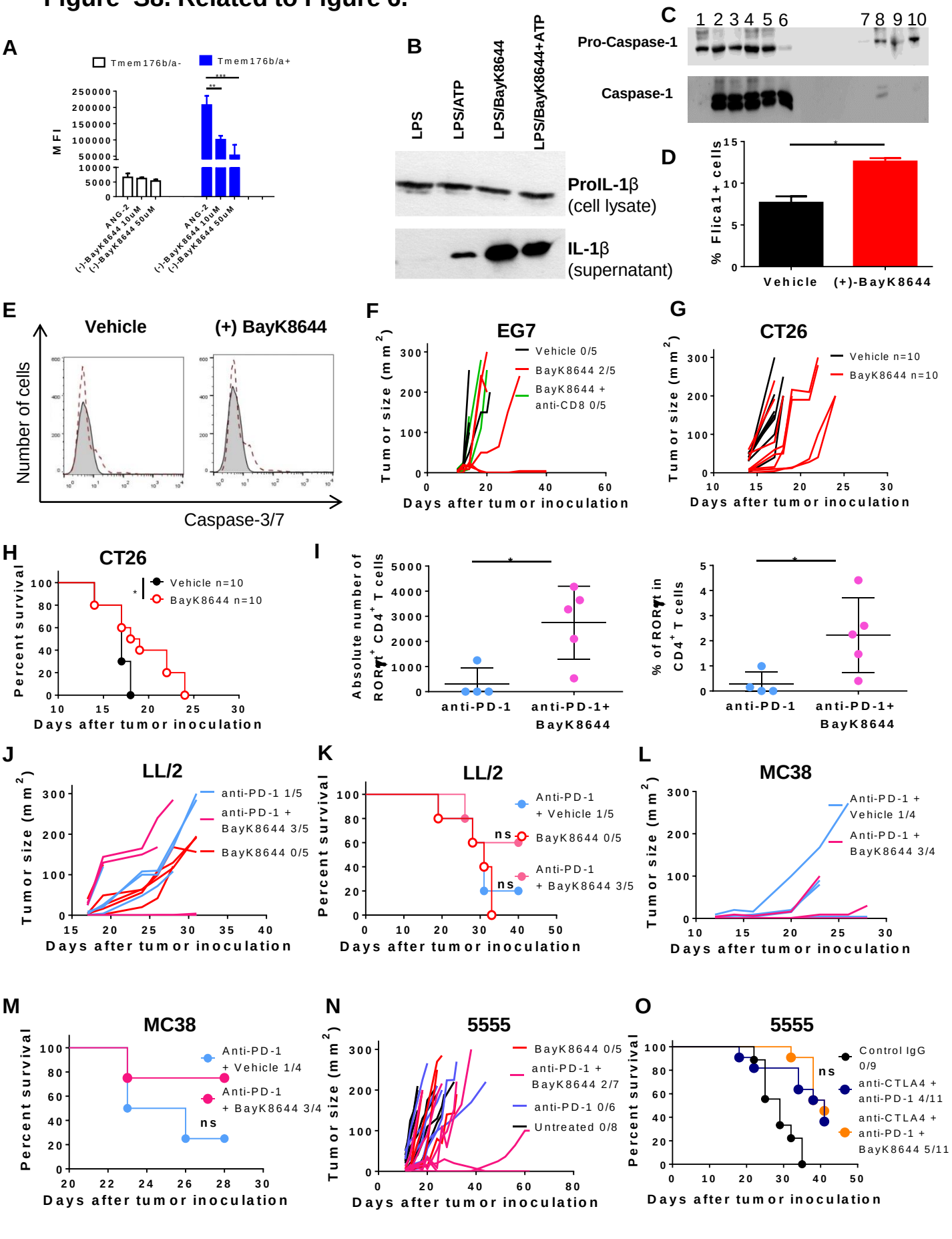
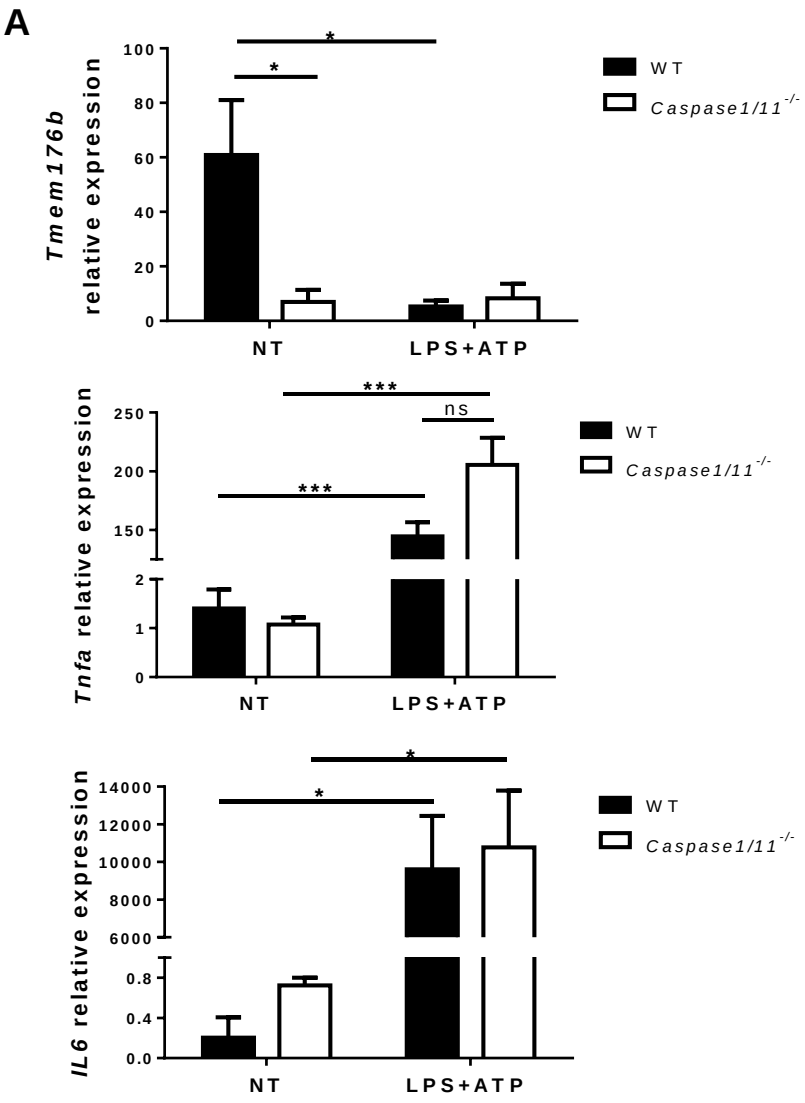


Figure S9. Related to discussion section of the manuscript.



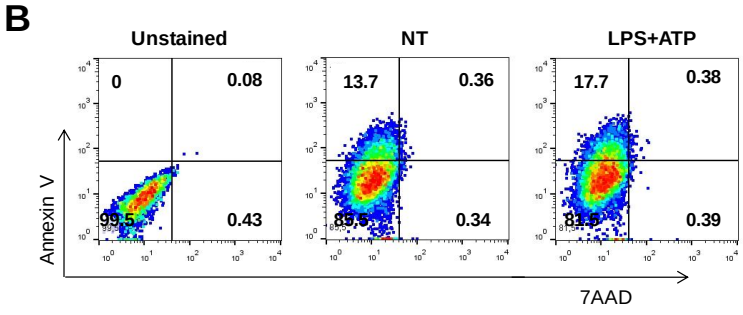


Table S1. Related to Figure 4. Analysis of data from Riaz *et al*. 2017. Paired analysis of inflammasome-associated gene expression profile in non-responders on/pre-treatment (anti-PD-1 antibody). IPI naïve patients

Gene	p_value	fdr	fc[a]	p_value_log2	fdr_log2
TMEM176B	0.0390625	0.46875	0.7567829	0.029506455	0.37796936
TMEM176A	0.0390625	0.46875	0.72357185	0.048297486	0.37796936
CASP4	0.06761715	0.46875	0.3790591	0.111900929	0.44760372
IL18R1	0.08848316	0.46875	-0.42848366	0.175189823	0.54545455
NLRP6	0.09765625	0.46875	0.64605376	0.09765625	0.44760372
IL1RN	0.12890625	0.4921875	0.66854228	0.062994893	0.37796936
IL1RAP	0.1640625	0.4921875	0.3397688	0.25	0.54545455
IL1R2	0.1640625	0.4921875	0.62915261	0.053073014	0.37796936
IL1B	0.203125	0.54166667	0.39703187	0.31477899	0.62955798
CASP5	0.25	0.54545455	0.32829776	0.220629385	0.54545455
NLRP12	0.25	0.54545455	-0.78174959	0.25	0.54545455
AIM2	0.359375	0.71875	0.3707348	0.214068788	0.54545455
PYCARD	0.43022486	0.79426128	-0.17641233	0.477043954	0.74553571
GSDMD	0.49609375	0.85044643	0.07568183	0.588683156	0.74553571
IL18RAP	0.58736276	0.86979167	0.32970506	0.577301487	0.74553571
SIRT3	0.65103296	0.86979167	-0.06294952	0.607275156	0.74553571
IL1A	0.65234375	0.86979167	-0.01555178	0.65234375	0.74553571
IL18	0.65234375	0.86979167	0.14703032	0.55410695	0.74553571
ABHD5	0.8916341	1	0.01940535	0.786760725	0.85828443
CASP1	0.91015625	1	0.20761326	0.604608002	0.74553571
IL1R1	0.93720565	1	-0.02012097	0.65234375	0.74553571
NLRP7	0.94418251	1	0.16525862	0.833634883	0.86987988
NLRC4	0.95868982	1	0.01288561	0.645871386	0.74553571
NLRP3	1	1	-0.12646354	0.901745055	0.90174505

a: fc=FC=Log2(on-treatment/pre-treatment)

Table S2. Related to Figure 4. Analysis of data from Riaz *et al*. 2017. Inflammasome-related gene expression profile at pre-treatment stage (Anti-PD-1). Bulk patients

Gene	p_value	fdr	fc[a]	p_value_log2	fdr_log2
IL18	0.26886624	0.9610583	-0.06039552	0.44313385	0.90452546
AIM2	0.30215351	0.9610583	-1.18605245	0.302153513	0.90452546
ABHD5	0.30818719	0.9610583	0.16813754	0.382450457	0.90452546
NLRP7	0.32975435	0.9610583	-0.65461498	0.329754349	0.90452546
NLRP6	0.36689235	0.9610583	-0.39904646	0.366892345	0.90452546
TMEM176A	0.43219326	0.9610583	-0.25246754	0.511560587	0.90452546
TMEM176B	0.50555314	0.9610583	-0.19952116	0.574971819	0.90452546
IL1RAP	0.51837548	0.9610583	-0.30412707	0.403383302	0.90452546
IL1R2	0.54450591	0.9610583	0.38787846	0.544505907	0.90452546
NLRP3	0.57126261	0.9610583	-0.195523	0.571262605	0.90452546
CASP1	0.5848672	0.9610583	0.1042929	0.571883087	0.90452546
IL1A	0.60894254	0.9610583	0.87696368	0.608942545	0.90452546
IL1B	0.62654098	0.9610583	0.51189721	0.626540976	0.90452546
IL1R1	0.62654098	0.9610583	0.50606896	0.600290705	0.90452546
IL18R1	0.63778549	0.9610583	-0.45389818	0.437045786	0.90452546
IL1RN	0.64070554	0.9610583	1.45651703	0.640705535	0.90452546
NLRP12	0.77138887	0.99608145	0.65653567	0.771388868	0.91889441
SIRT3	0.78870657	0.99608145	0.08908715	0.530108715	0.90452546
CASP5	0.80403261	0.99608145	-0.1828214	0.804032606	0.91889441
NLRC4	0.89734932	0.99608145	0.24808789	0.72127875	0.91889441
IL18RAP	0.91224132	0.99608145	0.18853179	0.912241316	0.9592106
GSDMD	0.91307466	0.99608145	-0.0650615	0.775336521	0.91889441
PYCARD	0.97625031	1	0.43830075	0.919243494	0.9592106
CASP4	1	1	0.04520392	1	1

a: fc=Log2(non responders/responders)

Table S3. Related to Figure 4. Analysis of data from Riaz *et al*. 2017. Inflammasome gene expression at pre-treatment stage (Anti-PD-1). IPI naïve patients.

Gene	p_value	fdr	fc[a]	p_value_log2	fdr_log2
IL1RAP	0.04919459	1	-0.84507093	0.068028757	0.97593334
CASP1	0.14290646	1	0.8346638	0.215221623	0.97593334
IL1R2	0.1895867	1	-0.27886838	0.189586695	0.97593334
ABHD5	0.2409666	1	0.28218828	0.336421084	0.97593334
NLRC4	0.25253619	1	0.57558152	0.427551872	0.97593334
PYCARD	0.28754702	1	0.786833	0.278688796	0.97593334
IL18R1	0.44913681	1	0.06558208	0.687783063	0.97593334
NLRP6	0.51709317	1	-0.31920005	0.517093172	0.97593334
IL1A	0.5180268	1	-1.37787825	0.518026796	0.97593334
AIM2	0.52539868	1	-1.62832169	0.285701311	0.97593334
IL1R1	0.66298319	1	0.20863927	0.525398683	0.97593334
NLRP3	0.69470252	1	0.02702505	0.694702525	0.97593334
IL1B	0.69470252	1	-1.04185706	0.694702525	0.97593334
GSDMD	0.69470252	1	-0.01225233	0.871411967	0.97593334
TMEM176A	0.73989814	1	-0.25420257	0.739898142	0.97593334
IL18RAP	0.73989814	1	0.22563589	0.739898142	0.97593334
NLRP12	0.73989814	1	1.41454798	0.739898142	0.97593334
CASP5	0.78594874	1	-0.32849091	0.78594874	0.97593334
NLRP7	0.92607981	1	-0.18311634	0.926079813	0.97593334
CASP4	0.96607684	1	-0.01321584	0.871781457	0.97593334
IL1RN	0.97593334	1	0.45616083	0.956178957	0.97593334
IL18	0.97593334	1	0.46255714	0.975933341	0.97593334
SIRT3	1	1	0.12306673	0.586578059	0.97593334
TMEM176B	1	1	-0.20950591	0.749503358	0.97593334

a: fc=Log2(non responders/responders)

Table S4. Related to Figure 4. Analysis of data from Riaz *et al.* 2017. Inflammasome gene expression at pre-treatment stage (Anti-PD-1). IPI progressors patients.

Gene	p_value	fdr	fc[a]	p_value_log2	fdr_log2
NLRP7	0.11883877	0.87847857	-1.43340439	0.118838768	0.93807971
IL18	0.1981639	0.87847857	-0.41133656	0.237145531	0.93807971
IL18R1	0.27567576	0.87847857	-0.80848563	0.193730961	0.93807971
PYCARD	0.30505426	0.87847857	0.20522215	0.324660564	0.93807971
TMEM176A	0.44302914	0.87847857	-0.0569624	0.69003012	0.93807971
AIM2	0.49979366	0.87847857	-0.75123062	0.429215461	0.93807971
NLRP12	0.53313639	0.87847857	-0.11519728	0.533136387	0.93807971
TMEM176B	0.53995284	0.87847857	-0.01448501	0.756637021	0.93807971
IL1RN	0.53995284	0.87847857	2.1700564	0.388204956	0.93807971
NLRP3	0.60978261	0.87847857	-0.41559361	0.609782609	0.93807971
NLRP6	0.62191817	0.87847857	-0.37938013	0.621918166	0.93807971
SIRT3	0.66060345	0.87847857	0.09644427	0.60716929	0.93807971
IL18RAP	0.6777879	0.87847857	0.20423474	0.9658985	0.9658985
IL1B	0.68321676	0.87847857	2.29490109	0.68321676	0.93807971
NLRC4	0.72110363	0.87847857	-0.02883275	0.826361869	0.93807971
CASP1	0.72110363	0.87847857	-0.17541481	0.89899306	0.93807971
CASP4	0.72110363	0.87847857	0.15641347	0.721103627	0.93807971
IL1A	0.7546836	0.87847857	2.81678569	0.7546836	0.93807971
ABHD5	0.75627594	0.87847857	0.07287696	0.756880106	0.93807971
IL1R2	0.7988051	0.87847857	0.84095489	0.798805099	0.93807971
GSDMD	0.83902191	0.87847857	-0.05960373	0.71436234	0.93807971
IL1R1	0.87847857	0.87847857	0.77439692	0.828883091	0.93807971
IL1RAP	0.87847857	0.87847857	0.17601368	0.878478572	0.93807971
CASP5	0.87847857	0.87847857	-0.04991744	0.878478572	0.93807971

a: fc=Log2(non responders/responders)

Table S5. Related to Figure 4. Analysis of data from Riaz *et al*. 2017. Inflammasome-related gene expression at on-treatment stage (Anti-PD-1). IPI naïve patients.

Gene	p_value	fdr	fc[a]	p_value_log2	fdr_log2
TMEM176B	0,00390625	0,0625	1,84751874	0,00390625	0,0625
GSDMD	0,00541809	0,0625	0,63057312	0,00541809	0,0625
TMEM176A	0,0078125	0,0625	1,7844506	0,0078125	0,0625
NLRP6	0,01824504	0,07943254	0,80397817	0,01824504	0,07943254
IL18R1	0,01890336	0,07943254	0,88988129	0,01890336	0,07943254
IL1RAP	0,01985814	0,07943254	-0,890935	0,01985814	0,07943254
IL18RAP	0,02734375	0,09375	1,08256634	0,02734375	0,09375
CASP1	0,0546875	0,16193182	0,93111062	0,0546875	0,16193182
IL18	0,07344048	0,16193182	1,17698945	0,07344048	0,16193182
NLRP7	0,07421875	0,16193182	0,98046218	0,07421875	0,16193182
IL1R1	0,07421875	0,16193182	0,90803425	0,07421875	0,16193182
CASP4	0,0846027	0,1692054	0,60492518	0,0846027	0,1692054
PYCARD	0,15770119	0,29114067	0,60839068	0,15770119	0,29114067
NLRC4	0,25	0,42160536	0,91383868	0,25	0,42160536
NLRP3	0,26670139	0,42160536	0,55527122	0,26670139	0,42160536
CASP5	0,28107024	0,42160536	0,6440894	0,28107024	0,42160536
AIM2	0,30078125	0,42463235	0,22745745	0,30078125	0,42463235
IL1A	0,359375	0,47916667	0,67605275	0,359375	0,47916667
IL1R2	0,42578125	0,53782895	0,82922261	0,42578125	0,53782895
NLRP12	0,5226743	0,62720916	0,47842529	0,5226743	0,62720916
ABHD5	0,74473826	0,85112944	-0,0715091	0,74473826	0,85112944
IL1RN	0,8203125	0,89488636	0,98637591	0,8203125	0,89488636
SIRT3	0,97073348	1	0,00631734	0,97073348	1
IL1B	1	1	0,92505986	1	1

a: fc=Log2(non responders/responders)

Table S6. Related to Figure 4. Analysis of data from Riaz *et al*. 2017. Paired analysis of Inflammasome-related gene expression in responders (Anti-PD-1) on/pre-treatment stage. IPI naïve patients.

Gene	p_value	fdr	fc	p_value_log2	fdr_log2
IL18R1	0,02143359	0,51440626	-0,4859683	0,021433594	0,51440626
IL1RAP	0,12932599	0,86453951	0,17706561	0,129325989	0,864539513
NLRP12	0,22875214	0,86453951	-0,066435	0,228752136	0,864539513
NLRP7	0,28588144	0,86453951	-0,2509041	0,285881445	0,864539513
IL1R2	0,30379486	0,86453951	0,16216661	0,303794861	0,864539513
GSDMD	0,30379486	0,86453951	0,00292277	0,303794861	0,864539513
ABHD5	0,36921692	0,86453951	0,21108858	0,369216919	0,864539513
CASP4	0,36921692	0,86453951	0,0423972	0,369216919	0,864539513
NLRP3	0,43614622	0,86453951	-0,1577218	0,43614622	0,864539513
IL1RN	0,44229889	0,86453951	0,32536957	0,442298889	0,864539513
IL18RAP	0,44229889	0,86453951	0,16380521	0,442298889	0,864539513
SIRT3	0,46035442	0,86453951	-0,0661062	0,460354416	0,864539513
IL1B	0,46829224	0,86453951	0,21193413	0,468292236	0,864539513
IL18	0,5508728	0,88139648	-0,0953584	0,550872803	0,881396484
TMEM176A	0,5508728	0,88139648	-0,422569	0,550872803	0,881396484
IL1R1	0,60945892	0,90792501	0,02509495	0,609458923	0,907925011
NLRC4	0,75676165	0,90792501	-0,0565318	0,756761647	0,907925011
PYCARD	0,76602936	0,90792501	-0,4921455	0,766029358	0,907925011
TMEM176B	0,79870605	0,90792501	-0,3214791	0,798706055	0,907925011
CASP1	0,79914976	0,90792501	0,03520439	0,799149764	0,907925011
AIM2	0,80196793	0,90792501	0,06147296	0,801967933	0,907925011
NLRP6	0,83226459	0,90792501	-0,080559	0,832264593	0,907925011
IL1A	0,88706869	0,9256369	-0,0472519	0,887068694	0,925636898
CASP5	1	1	-0,2518302	1	1

a: fc=FC=Log2(on-treatment/pre-treatment)

Table S7. Mouse oligonucleotides used in this study.

Gene	Primer forward	Primer reverse
Rorγt	GGA GGA CAG GGA GCC AAG TT	AGT AGG CCA CAT TAC ACT GCT
Il17a	AGT CCA GGG AGA GCT TCA TCT	TCT TCA TTG CGG TGG AGA GTC
Foxp3	TCC AAG TCT CGT CTG AAG GC	GCG AAA GTG GCA GAG AGG TA
Tgfβ1	TGA CGT CAC TGG AGT TGT ACG G	GGT TCA TGT CAT GGA TGG TGC
Il10	CCA AGC CTT ATC GGA AAT GA	TTT TCA CAG GGG AGA AAT CG
Ifnγ	TGG CTC TGC AGG ATT TTC ATG	TCA AGT GGC ATA GAT GTG GAA GAA
Tnfa	TGG GAG TAG ACA AGG TAC AAC CC	CAT CTT CTC AAA ATT CGA GTG ACA A
Ctla4	CTG AAG GTT GGG TCA CCT GT	TGG ACT CCG GAG GTA CAA AG
Ccl22	CAC CCT CTG CCA TCA CGT TT	CCT GGG ATC GGC ACA GAT AT
Ccl5	ACT CCC TGC TGC TTT GCC TAC	GAG GTT CCT TCG AGT GAC A
Il12	GGA AGC ACG GCA GCA GAA TA	AAC TTG AGG GAG AAG TAG GAA TGG
Il4	GGT CTC AAC CCC CAG CTA GT	GCC GAT GAT CTC TCT CAA GTG AT
Gata3	AGG ATG TCC CTG CTC TCC TT	GCC TGC GGA CTC TAC CAT AA
Tbet	GTC TGG GAA GCT GAG AGT CG	CTT TCC ACA CTG CAC CCA CT
Cebpβ	GGA GAC GCA GCA CAA GGT	AGC TGC TTG AAC AAG TTC CG
Ccl19	GAC CTT CCC AGC CCC AAC T	CGG AAG GCT TTC ACG ATG TT
Il6	GAG GAT ACC ACT CCC AAC AGA CC	AAG TGC ATC ATC GTT GTT CAT ACA
Fas	AGT TTC ATG AAC CCG CCT C	GCA GAC ATG CTG TGG ATC TG
Pdl1	ATG CTC AGA AGT GGC TGG AT	TGC TGC ATA ATC AGC TAC GG
Tmem176b	ACT CCA GCT AGA ATT GCC ACA G	CAT CAG CAT CCA CAT CCA CC
Gapdh	CTA CAG CAA CAG GGT GGT GG	TAT GGG GGT CTG GGA TGG