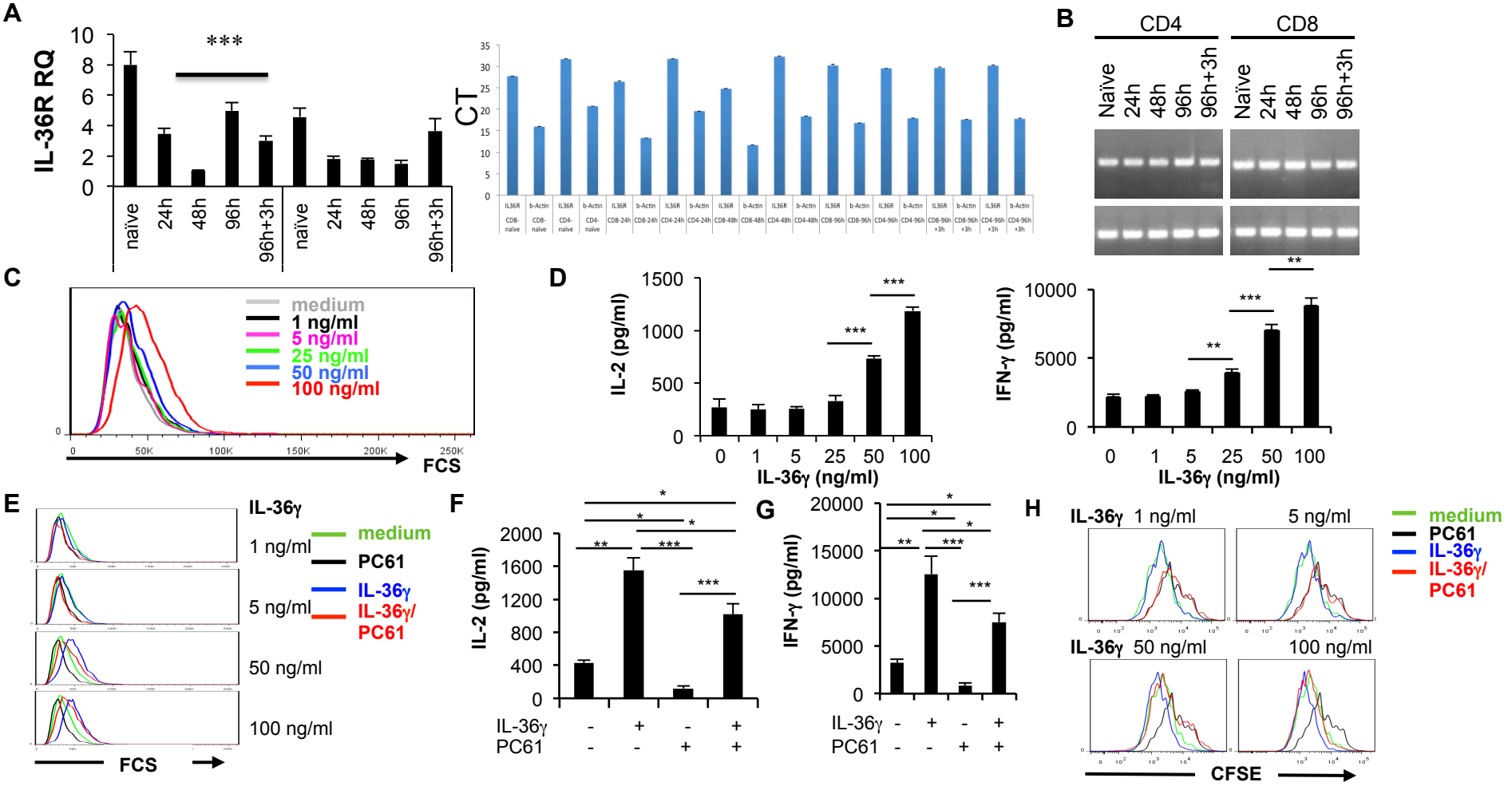

A
IL-36R RQ
***
naïve
24h
48h
96h
96h+3h
CT
B
CD4
CD8
Naïve
24h
48h
96h
96h+3h
**
C
medium
1 ng/ml
5 ng/ml
25 ng/ml
50 ng/ml
100 ng/ml
FCS
D
IL-2 (pg/ml)
IL-36γ (ng/ml)
IFN-γ (pg/ml)
E
IL-36γ
1 ng/ml
5 ng/ml
50 ng/ml
100 ng/ml
medium
PC61
IL-36γ
IL-36γ/ PC61
FCS
F
IL-2 (pg/ml)
IL-36γ
PC61
G
IFN-γ (pg/ml)
IL-36γ
PC61
H
IL-36γ 1 ng/ml
5 ng/ml
IL-36γ 50 ng/ml
100 ng/ml
medium
PC61
IL-36γ
IL-36γ/ PC61
CFSE

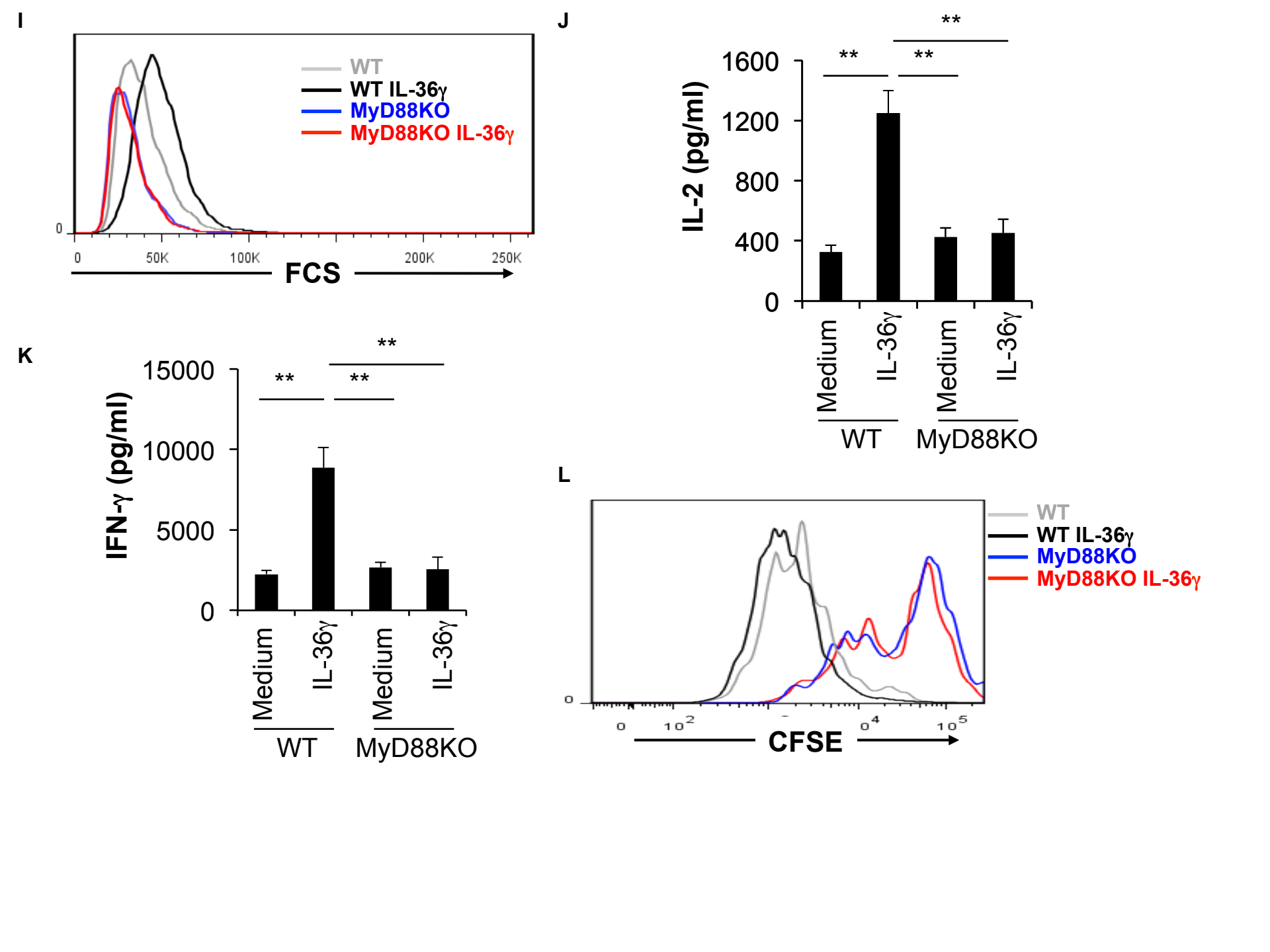

I
WT
WT IL-36γ
MyD88KO
MyD88KO IL-36γ
0
0
50K
100K
200K
250K
FCS
J
**
**
**
1600
1200
800
400
0
IL-2 (pg/ml)
Medium
IL-36γ
Medium
IL-36γ
WT
MyD88KO
K
**
**
**
15000
10000
5000
0
IFN-γ (pg/ml)
Medium
IL-36γ
Medium
IL-36γ
WT
MyD88KO
L
WT
WT IL-36γ
MyD88KO
MyD88KO IL-36γ
0
0
10^2
10^4
10^5
CFSE

Figure S1 related to figure 1. IL-36γ effect on $CD8^+$ T cells. (A) Naïve $CD4^+$ and $CD8^+$ T ($CD44^-$ $CD62L^+$) cells were purified from spleen cells of C57BL/6 mice by FACS sorting. T cells were then stimulated in the Th1 condition for the time indicated. (96h +3h indicates that T cells were cultured for 96 h and subsequently re-stimulated with anti-CD3 for 3 h). Total RNA was subjected quantitative RT-PCR analysis (A) and RT-PCR (B) for IL-36R. RQ (relative quantification values) were calculated based on ΔΔCT method. (C and D) Naïve pmel-1 $CD8^+$ T cells were stimulated with plate-bound 5 μg/ml CD3 and 2.5 μg/ml CD28 mAbs in the presence or absence of IL-36γ at various concentrations as indicated. (C) Cell sizes (FSC) were determined by flow cytometry at 72 h.(D) The level of IL-2 and IFN-γ at 24 h after stimulation was measured by ELISA. (E to H) Naïve $CD8^+$ T cells were stimulated with plate-bound 5 μg/ml CD3 and 2.5 μg/ml CD28 mAbs in the presence or absence of mouse IL-36γ at various concentrations as indicated. CD25 mAbs (clone PC61, 20 μg/ml) were added to cultures to block IL-2 signaling. (E) Cell sizes (FSC) were determined by flow cytometry at 72 h. IL-2 (F) or IFN-γ (G) secretion was determined by ELISA at 24 h. (H) Proliferation was determined by the CFSE-dilution assay at 72 h. Data are shown as mean ± SEM. *** $p < 0.001$, ** $p < 0.01$, * $p<0.05$. two-tailed unpaired Student's t-test. (I- L) Naïve $CD8^+$ T cells were isolated from WT or $Myd88^{-/-}$ B6 mice. They were subsequently stimulated with plate-bound 5 μg/ml CD3 and 2.5 μg/ml CD28 mAbs in the presence or absence of 100 ng/ml IL-36γ for 72 h. (I) Cell sizes (FSC) were determined by flow cytometry. (J) IL-2 and (K) IFN-γ production was determined by ELISA. Data are shown as mean ± SEM. ** $p < 0.01$ two-tailed unpaired Student's t-test. (L) Proliferation was determined by the CFSE-dilution assay.

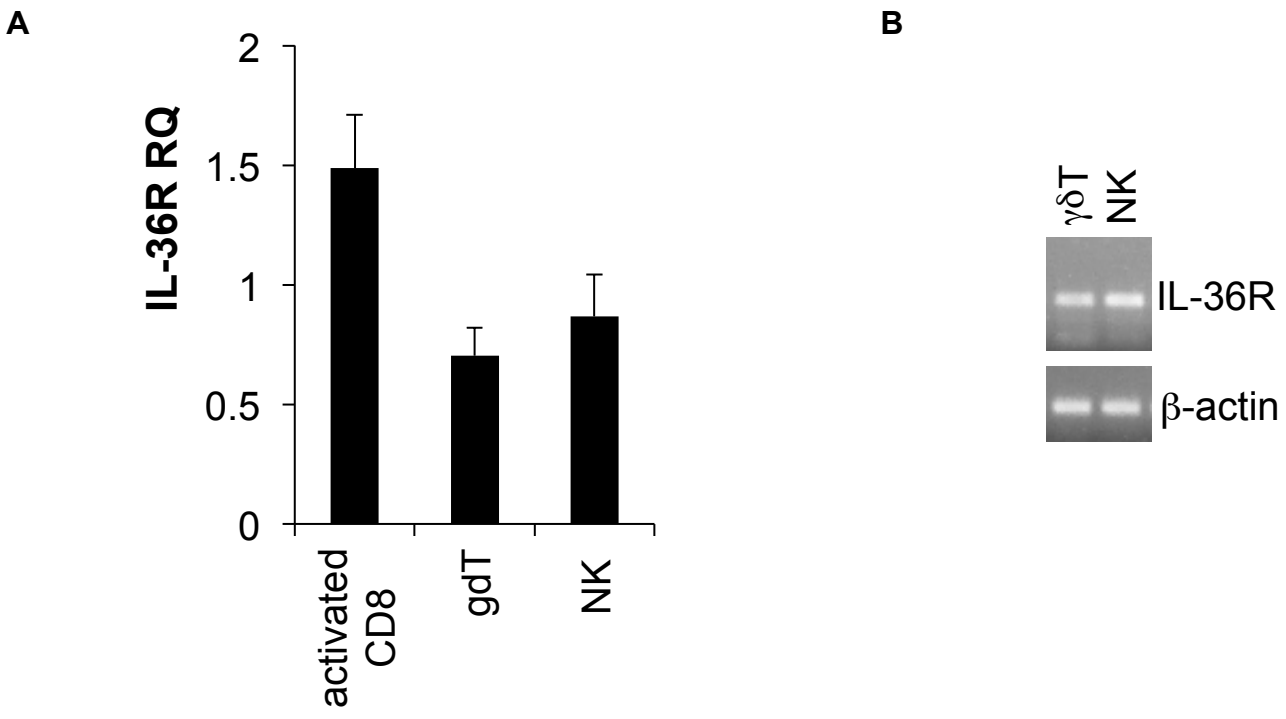


Figure S2 related to figure 2. IL-36R is expressed in NK and γδ T cells. Naïve CD8$^+$ T (CD44$^-$ CD62L$^+$), NK (DX5$^+$) and γδ T cells were purified from spleen cells of C57BL/6 mice by FACS sorting. T cells were then stimulated in the Th1 condition for 96 h. NK cells were cultured in the presence of IL-2 for 72 h and γδ T cells were stimulated with CD3 mAbs and IL-2 for 72 h. Total RNA was purified and subjected to (A) quantitative RT-PCR analysis for IL-36R. RQ (relative quantification values) were calculated based on ΔΔCT method. Data are shown as mean ± SEM. Data are shown as mean ± SEM. (B) RT-PCR analysis.

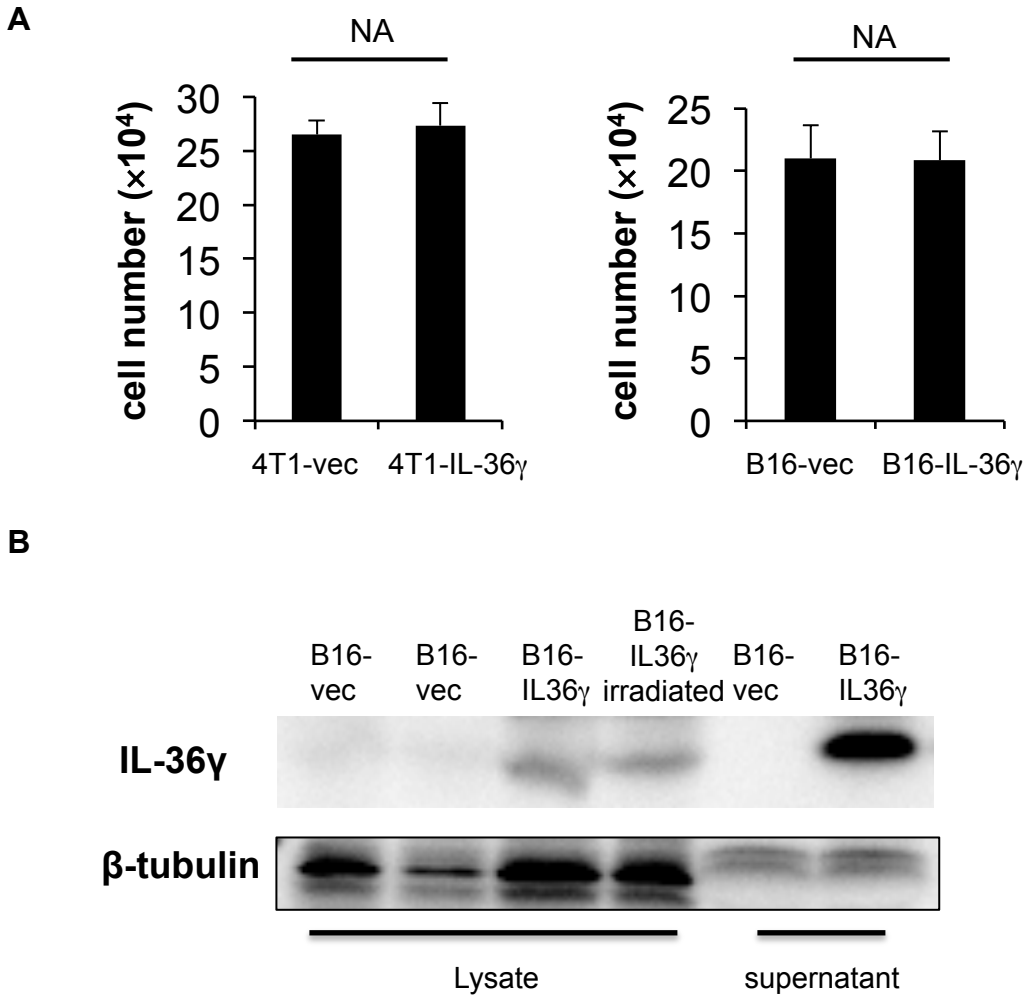
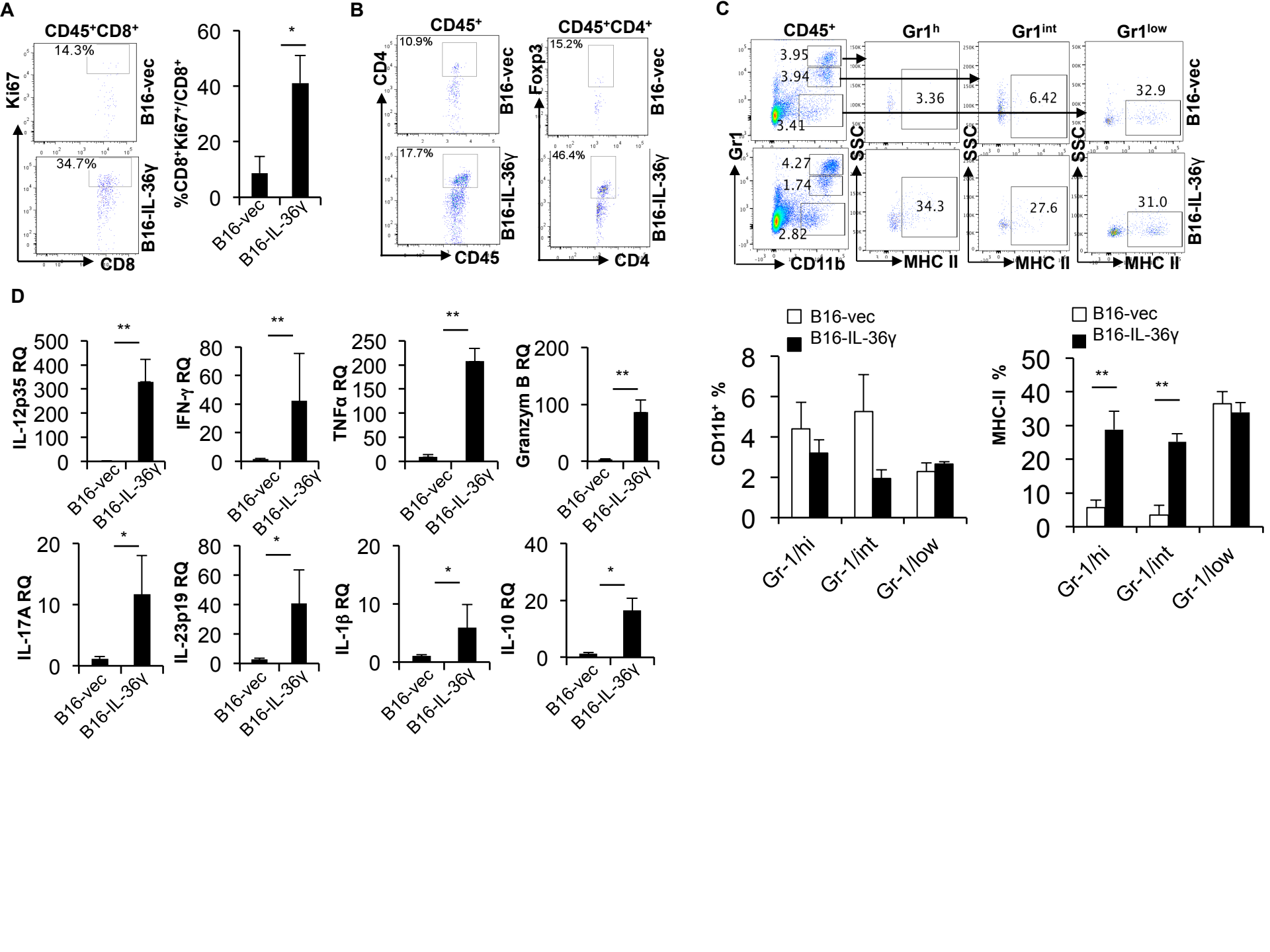


Figure S3 related to figure 3. Analysis of IL36γ-expressing cell lines.
(A) The growth of IL-36γ transfectants were comparable to the control cells. 4×10^4 4T1-vec, 4T1-IL-36γ B16-vec or B16-IL-36γ, cells were seeded into 24 wells with triplicates. The cells were cultured for 2 days, the number of live cells was determined by live cell counting after cells were stained with Trypan blue. Data (mean ±SEM) are representative of three independent experiments. (B) Detection of IL-36γ protein by Western blot. Supernatants were harvested from B16-vec or B16-IL-36γ cell culture and concentrated 10 times. Cell lysates were made from B16-vec or B16-IL-36γ cells. Western blot was performed using rabbit anti-IL1F9 antibody ((aa5-149) LS-C294790; LifeSpan Biosciences, Inc.)


A
CD45+CD8+
14.3%
34.7%
Ki67
CD8
B16-vec
B16-IL-36γ
%CD8+Ki67+/CD8+
B
CD45+
CD45+CD4+
10.9%
17.7%
15.2%
46.4%
CD4
Foxp3
CD45
C
CD45+
Gr1h
Gr1int
Gr1low
3.95
3.94
3.41
3.36
6.42
32.9
4.27
1.74
2.82
34.3
27.6
31.0
Gr1
SSC
CD11b
MHC II
D
IL-12p35 RQ
IFN-γ RQ
TNFα RQ
Granzym B RQ
IL-17A RQ
IL-23p19 RQ
IL-1β RQ
IL-10 RQ
CD11b+ %
MHC-II %
Gr-1/hi
Gr-1/int
Gr-1/low

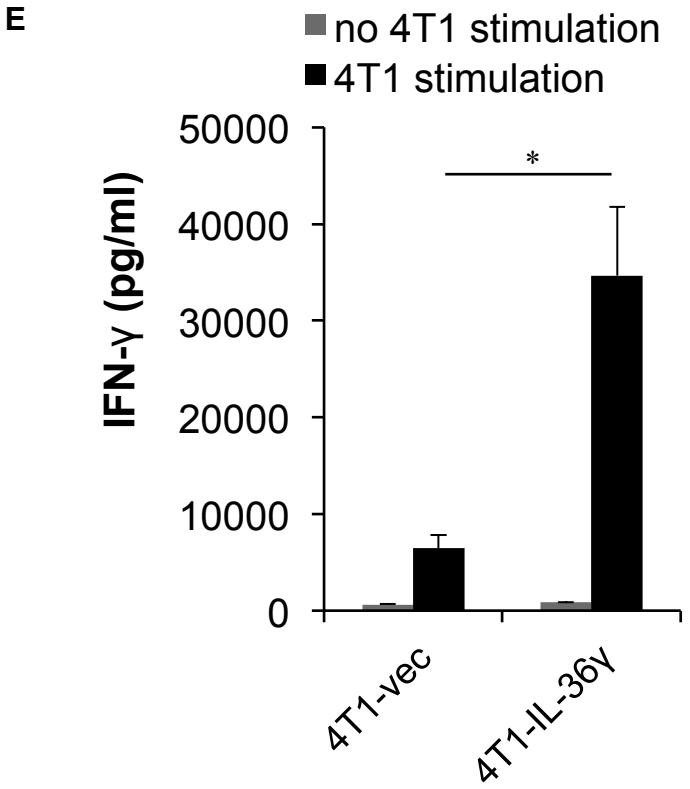


Figure S4 related to Figure 4. Tumoral expression of IL-36γ influence antitumor immune responses. 1x10^5 B16-vector or B16-IL-36γ cells were injected i.d. into B6 mice. On day 24, tumors were resected and processed to generate single cell suspensions. (A) Representative flow cytometric plots and percentages of CD8$^+$ Ki67$^+$ populations from the CD8$^+$ in tumor microenvironment are shown. (B) Representative flow cytometric plots and percentages of CD4$^+$ and Treg cells in the tumor microenvironment are shown. (C) Representative flow cytometric analysis of CD11b$^+$ sub-populations and percentages of CD11b$^+$ subpopulations of CD45$^+$ immune cells and percentages of MHC class II expression on three CD11b$^+$ sub-populations. (D) Tumors were resected and total RNA was extracted and subjected to RT-QPCR analysis. RQ (relative quantification values) were calculated based on ΔΔCT method. Results are shown as mean ± SEM of four independent experiments. One tumor was used in each experiment. *p < 0.05 and ** p <0.01, two-tailed unpaired Student's t-test. (E) Tumor antigen-specific CD8$^+$ T cell responses were enhanced by IL-36γ. 1x10^5 4T1-vector or 4T1-IL-36γ cells were injected into the mammary fat pad of BALB/c mice. 30 days after inoculation, CD8$^+$ T cells were purified from the spleens of these mice and re-stimulated with irradiated 4T1 cells for 72h in the presence of irradiated APC. The level of IFN-γ was then measured by ELISA. Results are shown as mean ± SEM of three independent experiments. One pair of mice were used in each experiment. *p < 0.05, two-tailed unpaired Student's t-test.

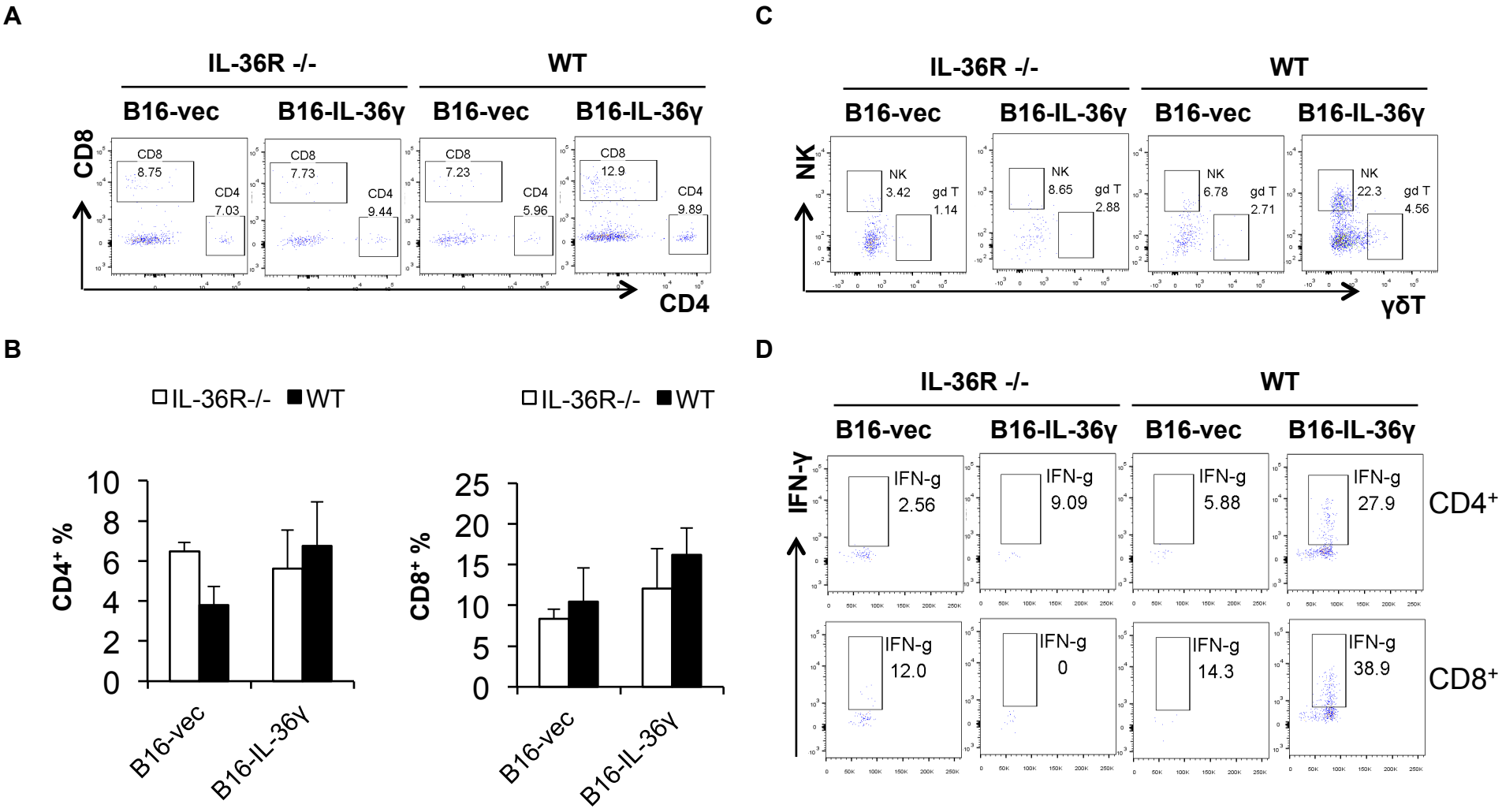
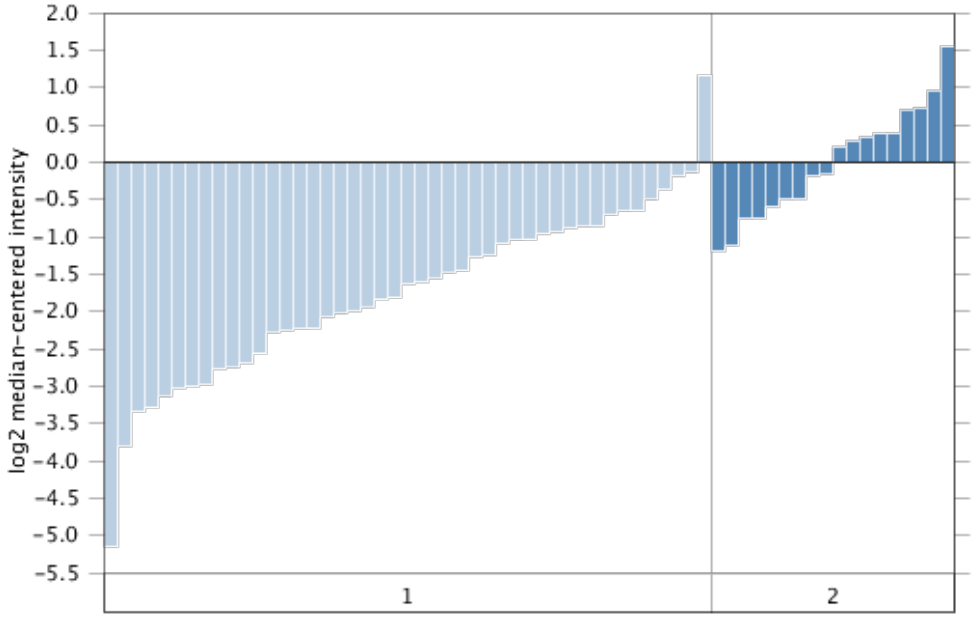


Figure S5 related to figure 5. Analysis of TIL in WT and IL-36R -/- mice. 1x10^5 B16-vector (B16-vec) or B16-IL-36γ cells were injected intradermally into WT and IL-36R -/- mice. The tumors were resected on day 17 and TIL were subjected to the flow cytometric analysis. (A) Representative flow cytometric analysis of CD4, CD8 T cells among CD45$^+$ TIL. (B) Percentages of the CD4$^+$ and CD8$^+$ T cells within the CD45$^+$ TIL. (C) Representative flow cytometric analysis of NK and γδ T cells among CD45$^+$ TIL. (D) Representative flow cytometric analysis of IFN-γ$^+$ T cells among CD4$^+$ and CD8$^+$ TIL.



1. Cutaneous Melanoma (45)
2. Melanoma Precursor (18)

Figure S6 related to figure 8. IL-36γ expression in melanoma and melanoma precursor. The data is generated after search oncomine database (www.oncomine.com) (Rhodes et al., 2004). The result is based on Talantov D et al's paper titled: Novel genes associated with malignant melanoma but not benign melanocytic lesions (Talantov et al., 2005).

Supplemental experimental procedures:

List of RT-QPCR primers

Genes	Forward	Reverse
IL-36R	AAACACCTAGCAAAAGCCCAG	AGACTGCCCGATTTTCCTATG
IFN-γ	CCTGCGGCCTAGCTCTGAG	GCCATGAGGAAGAGCTGCA
IL-12p35	CAATCACGCTACCTCCTCTTTT	CAGTGCAGGAATAATGTTTC
Granzyme B	CCACTCTCGACCCTACATGG	GGCCCCCAAAGTGACATTTATT
IL-17α	CTCCAGAAGGCCCTCAGACTAC	GGGTCTTCATTGCGGTGG
IL-1β	CTGAACTCAACTGTGAAATG	CGAGATTTGAAGCTGGATGC
IL-23p19	TGGCATCGAGAAACTGTGAGA	TCAGTTCGTATTGGTAGTCCTGTTA
TNFα	ATGAGCACAGAAAGCATGATC	TACAGGCTTGTCACTCGAATT
IL-10	TGTCAAATTCATTCATGGCCT	ATCGATTTCTCCCCTGTGAA
β-actin	GAAATCGTGCGTGACATCAAA	TGTAGTTTCATGGATGCCACAG

Supplemental reference

Rhodes, D. R., Yu, J., Shanker, K., Deshpande, N., Varambally, R., Ghosh, D., Barrette, T., Pandey, A., and Chinnaiyan, A. M. (2004). ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia (New York, NY) *6*, 1-6.

Talantov, D., Mazumder, A., Yu, J. X., Briggs, T., Jiang, Y., Backus, J., Atkins, D., and Wang, Y. (2005). Novel genes associated with malignant melanoma but not benign melanocytic lesions. Clinical cancer research : an official journal of the American Association for Cancer Research *11*, 7234-7242.