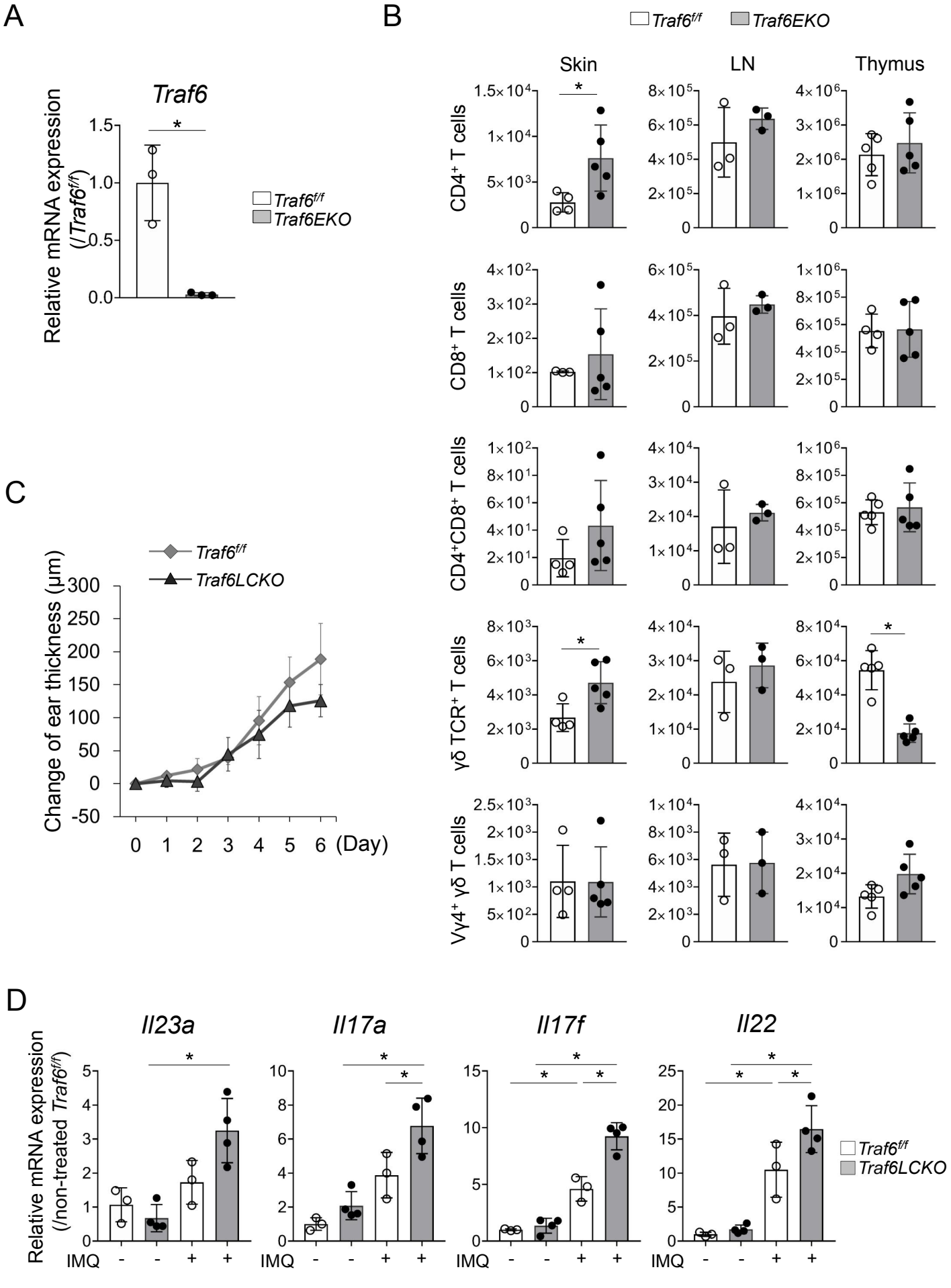
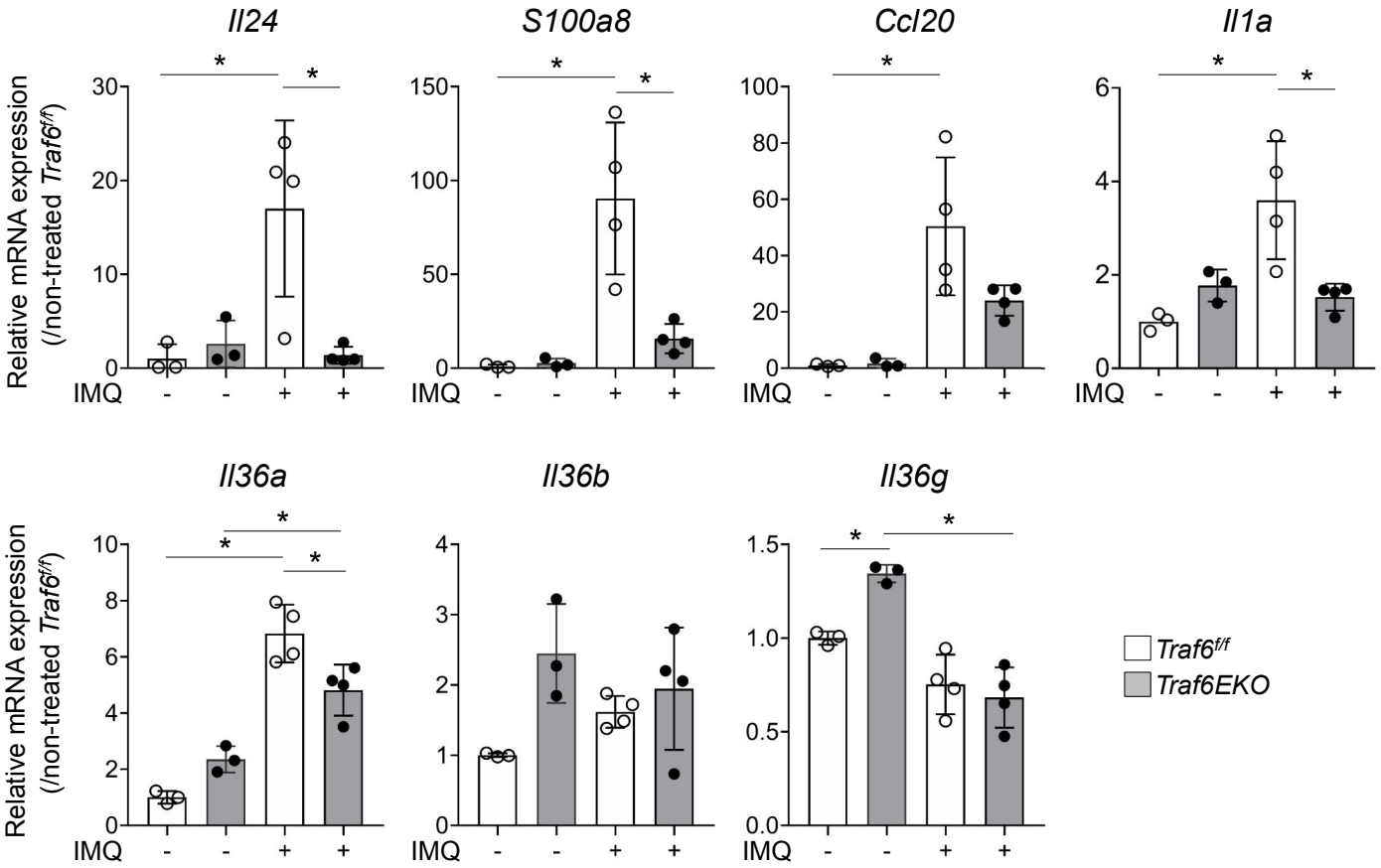
Figure S1.


A
Traf6
Relative mRNA expression (/Traf6f/f)
Traf6f/f
Traf6EKO
B
Traf6f/f
Traf6EKO
Skin
LN
Thymus
CD4+ T cells
CD8+ T cells
CD4+CD8+ T cells
γδ TCR+ T cells
Vγ4+ γδ T cells
C
Traf6f/f
Traf6LCKO
Change of ear thickness (μm)
(Day)
D
Il23a
Il17a
Il17f
Il22
Relative mRNA expression (/non-treated Traf6f/f)
IMQ
Traf6f/f
Traf6LCKO

Supplemental Figure 1. The effect of keratinocyte-specific deletion of TRAF6 and the retained response in *Traf6LCKO* mice treated with IMQ.

(**A**) *Traf6* mRNA levels in keratinocytes from *Traf6*$^{f/f}$ mice and *Traf6EKO* mice (n = 3 per group). Results are shown as means ± SD, * $P < 0.05$ (two-tailed Student's *t*-test). The deletion efficiency of TRAF6 protein in keratinocytes from *Traf6EKO* mice was demonstrated by Western blot analysis (**Figure 4A**) (**B**) Flow cytometric quantification of $CD4^+$ T cells, $CD8^+$ T cells, $CD4^+$ $CD8^+$ T cells, γδ TCR^+ cells, γδ TCR^+ $Vγ4^+$ cells from ear, lymph node and thymus in *Traf6*$^{f/f}$ mice and *Traf6EKO* mice (n ≥ 3 per group). Results are shown as means ± SD, * $P < 0.05$ (two-tailed Student's *t*-test). (**C**) Time course of changes in ear thickness. *Traf6*$^{f/f}$ and *Traf6LCKO* mice (n ≥ 4 per group) were treated with IMQ for 6 consecutive days and ear thickness was measured daily before treatment. Results are shown as means ± SD, * $P < 0.05$ (two-tailed Student's *t*-test). (**D**) qPCR analysis of mRNA levels in the ear of *Traf6*$^{f/f}$ and *Traf6LCKO* mice treated with IMQ for 6 days (n ≥ 3). The results were normalized to *Gapdh* expression and are shown as means ± SD, * $P < 0.05$ (Tukey's multiple comparison test). Data are representative of five experiments (**A**), three experiments (**B**), or two experiments (**C and D**).

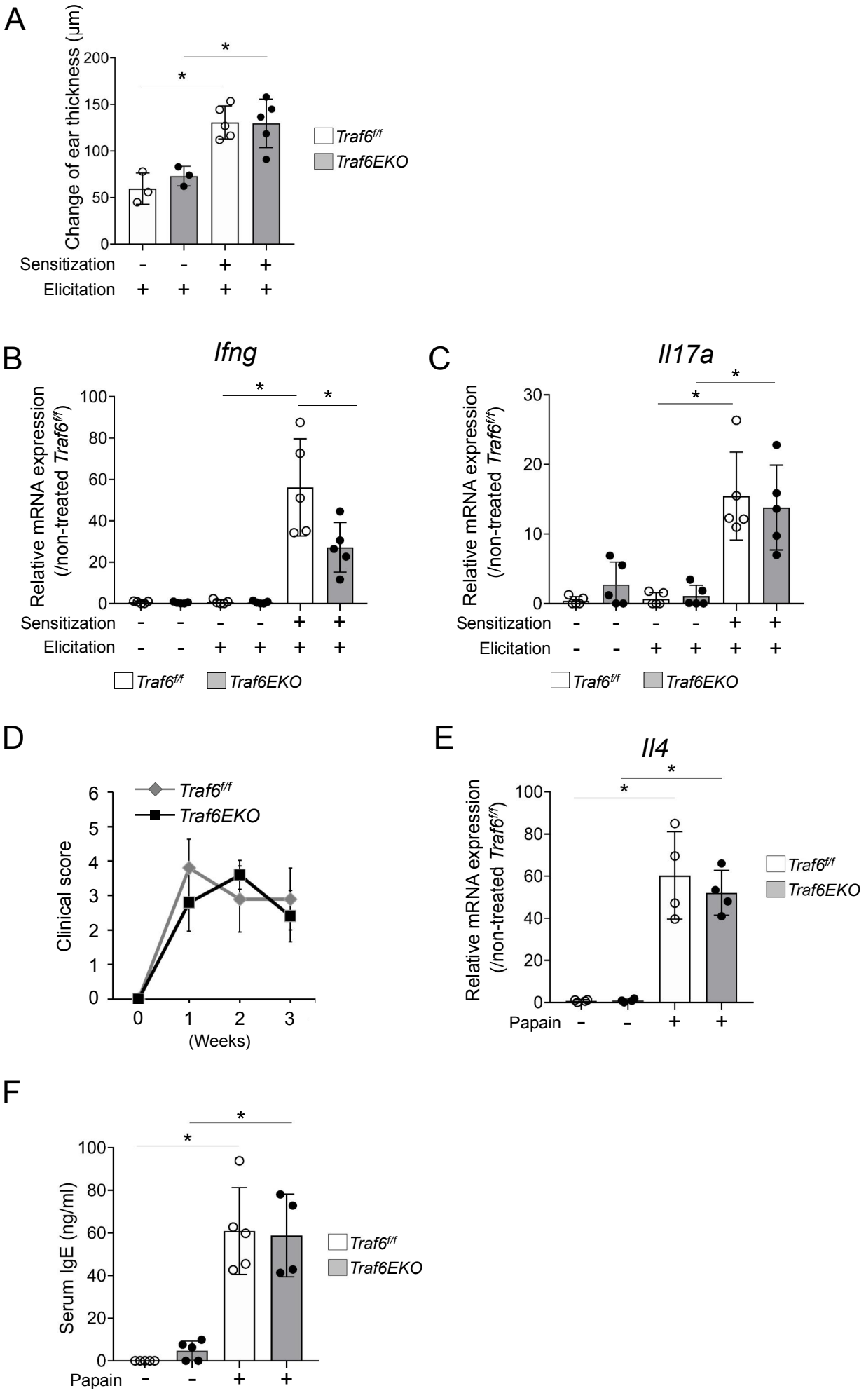
Figure S2.


Il24
S100a8
Ccl20
Il1a
Il36a
Il36b
Il36g
Relative mRNA expression (/non-treated Traf6f/f)
IMQ
Traf6f/f
Traf6EKO

Supplemental Figure 2. Additional gene expression analysis of IMQ-treated epidermis in *Traf6EKO* mice.

qPCR analysis of psoriasis-related gene expression levels in the epidermis of the IMQ-treated mouse ear on day 2 ($n \geq 3$ per group). The results were normalized to *Gapdh* expression and are shown as means ± SD, * $P < 0.05$ (Tukey's multiple comparison test). Data are representative of three experiments.

Figure S3.

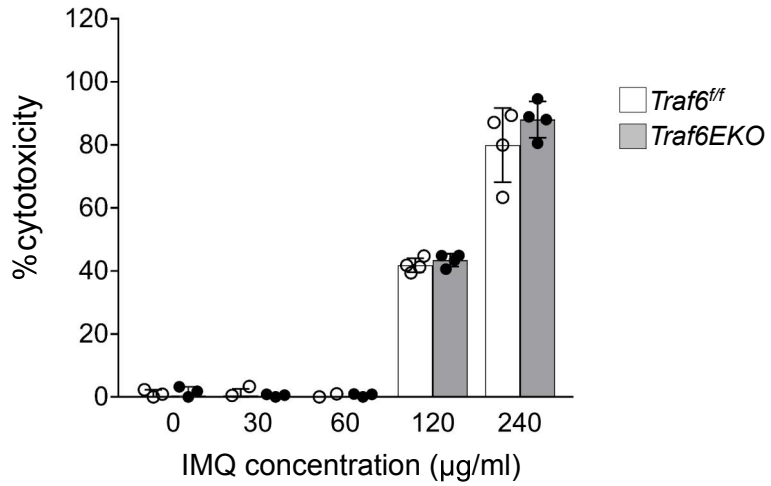

A
Change of ear thickness (μm)
200
150
100
50
0
*
*
Traf6f/f
Traf6EKO
Sensitization - - + +
Elicitation + + + +
B
Ifng
Relative mRNA expression (/non-treated Traf6f/f)
100
80
60
40
20
0
*
*
Sensitization - - - - + +
Elicitation - - + + + +
Traf6f/f
Traf6EKO
C
Il17a
Relative mRNA expression (/non-treated Traf6f/f)
30
20
10
0
*
*
Sensitization - - - - + +
Elicitation - - + + + +
Traf6f/f
Traf6EKO
D
Traf6f/f
Traf6EKO
Clinical score
6
5
4
3
2
1
0
0 1 2 3
(Weeks)
E
Il4
Relative mRNA expression (/non-treated Traf6f/f)
100
80
60
40
20
0
*
*
Papain - - + +
Traf6f/f
Traf6EKO
F
Serum IgE (ng/ml)
100
80
60
40
20
0
*
*
Papain - - + +
Traf6f/f
Traf6EKO

Supplemental Figure 3. Additional response of *Traf6EKO* mice in several types of skin inflammation.

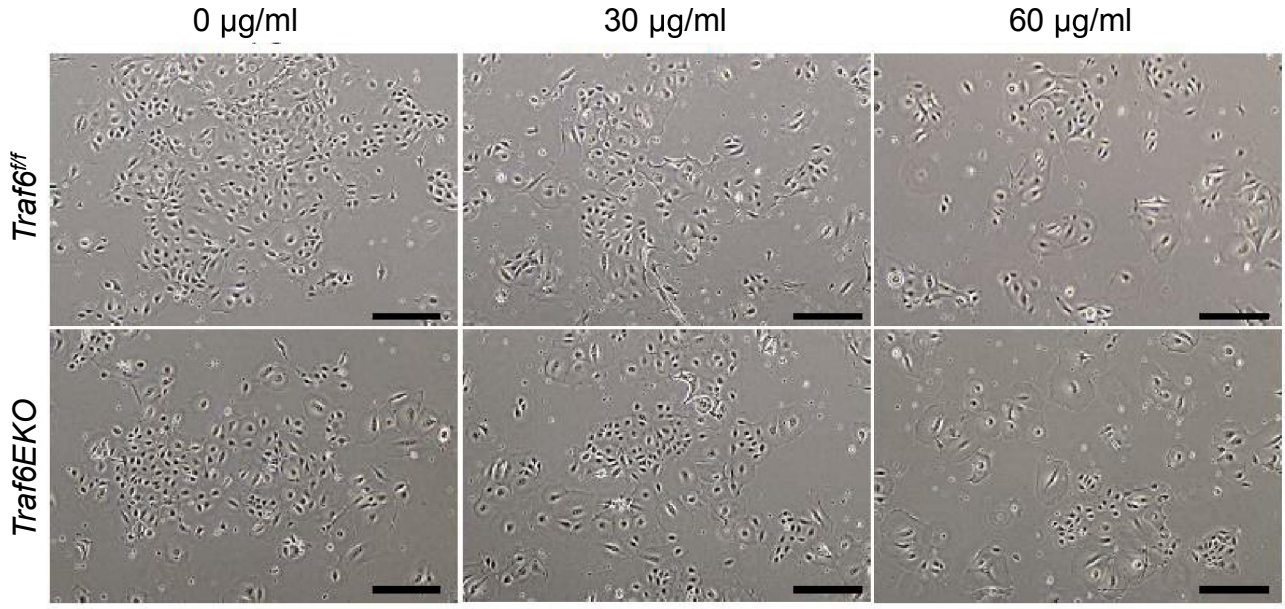
(**A**) Changes in ear thickness in a DNFB-induced contact hypersensitivity (CHS) model. *Traf6*$^{f/f}$ and *Traf6EKO* mice (n ≥ 3) were sensitized with DNFB or vehicle, and the ear thickness was measured 24 h after elicitation. (**B and C**) qPCR analysis of mRNA levels of *Ifng* (**B**) and *Il17a* (**C**) in the CHS-elicited ears of *Traf6*$^{f/f}$ and *Traf6EKO* mice (n ≥ 4). (**D**) Clinical severity scores of *Traf6*$^{f/f}$ and *Traf6EKO* mice treated with papain (n ≥ 4). (**E**) qPCR analysis of mRNA levels of *Il4* in the draining lymph nodes of *Traf6*$^{f/f}$ and *Traf6EKO* mice 5 days after epicutaneous administration with papain (n ≥ 4). (**F**) Serum IgE levels of *Traf6*$^{f/f}$ and *Traf6EKO* mice were determined by enzyme-linked immunosorbent assay after 3-week treatment with papain (n ≥ 4). Results are shown as means ± SD, * $P < 0.05$ (Tukey's multiple comparison test). Data are representative of three experiments (**A–F**).

Figure S4.

A


120
100
80
60
40
20
0
% cytotoxicity
0
30
60
120
240
IMQ concentration (μg/ml)
Traf6f/f
Traf6EKO

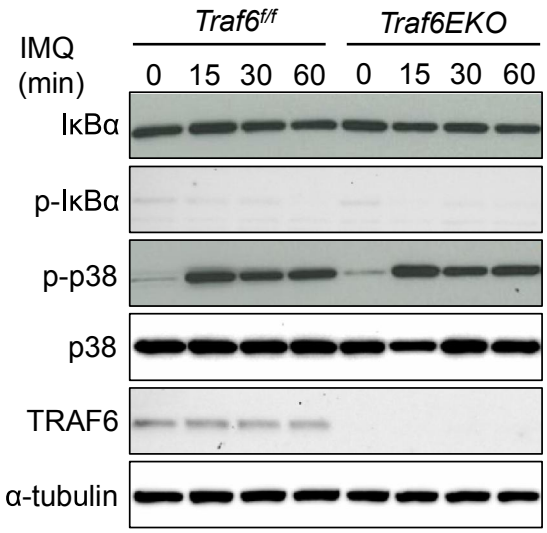
B


0 μg/ml
30 μg/ml
60 μg/ml
Traf6f/f
Traf6EKO

Supplemental Figure 4. The impact of TRAF6 deficiency on keratinocyte cell death.

(**A**) LDH release assay of cytotoxicity. Culture supernatants of *Traf6*$^{f/f}$ and *Traf6EKO* keratinocytes were collected after 2 h exposure to 0–120 μg/ml IMQ (n ≥ 3). Bars indicate means ± SD of the percentiles of cytotoxicity. * $P < 0.05$ (two-tailed Student's *t*-test). (**B**) Photomicrographs of comparison of cell morphology between *Traf6*$^{f/f}$ and *Traf6EKO* keratinocytes at different concentration of IMQ. Primary cultured keratinocytes from *Traf6*$^{f/f}$ and *Traf6EKO* mice were stimulated with IMQ (0–60 μg/ml) for 2 h. Data are representative of three experiments (**A and B**).

Figure S5.


Traf6f/f
Traf6EKO
IMQ (min)
0 15 30 60 0 15 30 60
IκBα
p-IκBα
p-p38
p38
TRAF6
α-tubulin

Supplemental Figure 5. Additional Western blot analysis of IMQ-treated TRAF6-deficient keratinocytes.

Western blot analysis of keratinocytes from *Traf6EKO* mice and *Traf6*$^{f/f}$ mice stimulated with IMQ at the indicated time point. Total cell lysates were subjected to SDS-PAGE followed by immunoblotting.

Supplemental Table 2. KEGG pathways on genes which were upregulated by IMQ treatment in *Traf6*$^{f/f}$ mice compared to *Traf6EKO* mice.

ID	Description	Gene count	*P* value	Genes
mmu04060	Cytokine-cytokine receptor interaction	10	2.9E-03	*Ccl3, Ccl2, Ccl20, Clcf1, Ccr1, Il1b, Csf3r, Cxcr2, Ccl7, Il1a*
mmu04668	TNF signaling pathway	7	4.1E-03	*Ccl2, Ptgs2, Ccl20, Socs3, Cxcl2, Bcl3, Il1b*
mmu00590	Arachidonic acid metabolism	5	4.2E-02	*Ptgs2, Ptges, Pla2g4b, Alox8, Pla2g4e*
mmu05323	Rheumatoid arthritis	5	4.4E-02	*Ccl3, Ccl2, Ccl20, Il1b, Il1a*
mmu04914	Progesterone-mediated oocyte maturation	5	4.5E-02	*Cdk1, Ccnb2, Plk1, Bub1, Ccna2*
mmu04110	Cell cycle	6	4.6E-02	*Cdk1, Ccnb2, Plk1, Bub1, Cdc20, Ccna2*
mmu04062	Chemokine signaling pathway	7	4.6E-02	*Ccl3, Ccl2, Ccl20, Ccr1, Cxcl2, Cxcr2, Ccl7*

Supplemental Table 3. Primer sequences used for quantitative RT-PCR analyses.

Gene Symbol	Forward primer (5'>3')	Reverse primer (5'>3')
Gapdh	GGCCTCACCCCATTTGATGT	CATGTTCCAGTATGACTCCACTC
Il1b	TTGACGGACCCCAAAAGATG	TGGACAGCCCAGGTCAAAG
Il4	GGTCTCAACCCCCAGCTAGT	GCCGATGATCTCTCTCAAGTGAT
Il6	CACTTCACAAGTCGGAGGCT	TGCCATTGCACAACTCTTTTCT
Il12b	GGTGTAACCAGAAAGGTGCG	TAGCGATCCTGAGCTTGCAC
Il23a	GACCCACAAGGACTCAAGGA	CATGGGGCTATCAGGGAGTA
Il17a	CTCCAGAAGGCCCTCAGACTAC	GGGTCTTCATTGCGGTGG
Il17f	AATCCTGGTCCTTCGGAGGG	GCCAACTTTTAGGAGCATCTTCT
Il19	GCCAACTCTTTCCTCTGCGT	GGTGGCTTCCTGACTGCAGT
Il22	ATGAGTTTTTCCCTTATGGGGAC	GCTGGAAGTTGGACACCTCAA
Il24	GAGCCTGCCCAACTTTTTGTG	TGTGTTGAAGAAAGGGCCAGT
Saa1	GCGAGCCTACACTGACATGAAG	CCCCCGAGCATGGAAGTATT
Il36a	AGCAGCATCACCTTCGCTTAG	GTGTCCAGATATTGGCATGGG
Il36b	CTTCGATCCCAGAGACAAGACT	ATTCGGTTCCCACATTTGAA
Il36g	CAGGTGTGGATCTTTCGTAATCA	CATGGGAGGATAGTCACGCTG
Ccl20	GCCTCTCGTACATACAGACGC	CCAGTTCTGCTTTGGATCAGC
Tnf	CTGGGACAGTGACCTGGACTGT	ACTCTCCCTTTGCAGAACTCAGG
Cxcl1	ACTGCACCCAAACCGAAGTC	TGGGGACACCTTTTAGCATCTT
Defb3	ATTTCTCCTGGTGCTGCTGT	GGAACTCCACAACTGCCAAT
S100a8	AAATCACCATGCCCTCTACAAG	CCCACTTTTATCACCATCGCAA
S100a9	GCACAGTTGGCAACCTTTATG	TGATTGTCCTGGTTTGTGTCC
Ifng	GAACTGGCAAAAGGATGGTGA	TGTGGGTTGTTGACCTCAAAC