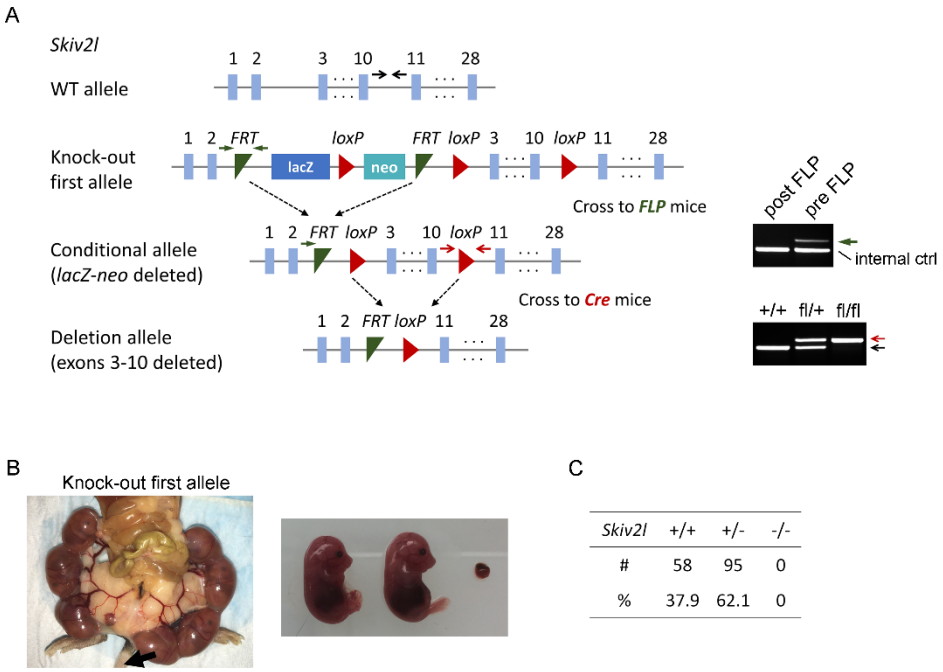
## Supplementary Figures

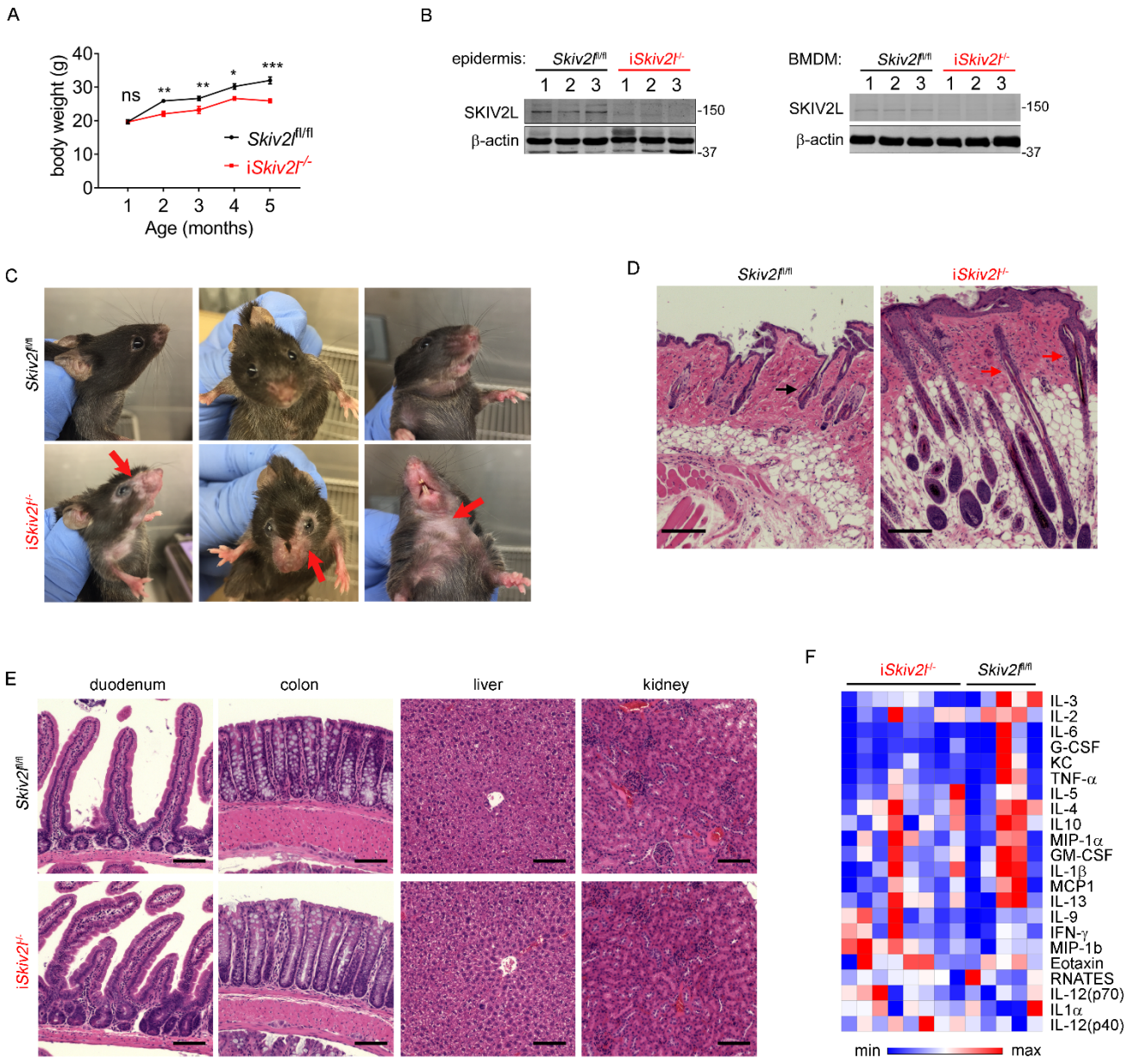



**Supplemental Figure 1. Generation of *Skiv2l* germline and conditional knockout mice**

(**A**) The breeding scheme of *Skiv2l*$^{fl/fl}$ mice and genotyping results of knockout-first and conditional alleles (right graphs).

(**B**) Representative embryos from intercrossing knockout-first-allele heterozygous (*Skiv2l*$^{+/-}$) female breeder mouse at embryonic day E13.5. Arrows indicated absorbed dead embryos. Morphologically normal embryos were either wild-type or heterozygotes.

(**C**) A summary of viable pups from *Skiv2l*$^{+/-}$ intercrossing.




**Supplemental Figure 2. Characterization of postnatal inducible whole-body *Skiv2l* knockout mice.**

(**A**) Body weight of i*Skiv2l*$^{-/-}$ male mice and *Skiv2l*$^{fl/fl}$ littermate controls. n=4-8 mice per genotype. Unpaired two-sided Student's *t*-test, **P* < 0.05, ***P* < 0.01, ****P* < 0.001. ns, not significant.

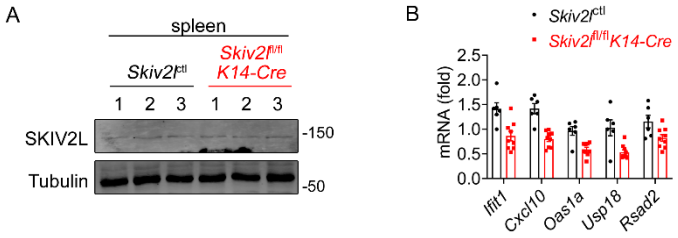
(**B**) Western blot analysis of SKIV2L in epidermis and BMDM from i*Skiv2l*$^{-/-}$ mice and *Skiv2l*$^{fl/fl}$ controls. n=3 mice per genotype.

(**C**) Skin lesion, alopecia of i*Skiv2l*$^{-/-}$ mice and *Skiv2l*$^{fl/fl}$ controls at 6 weeks of age.

(**D**) H&E staining analysis of hair follicles (HFs) in dorsal skin of 3-month-old i*Skiv2l*$^{-/-}$ mice and *Skiv2l*$^{fl/fl}$ littermate control. Black arrow, healthy HF without inflammation. Red arrow, dystrophic anagen HF with clustering inflammatory cells. Scale bar, 200 μm.

(**E**) H&E staining of indicated organs of i*Skiv2l*$^{-/-}$ mice and *Skiv2l*$^{fl/fl}$ controls at 3 months of age. Scale bar, 100 μm.

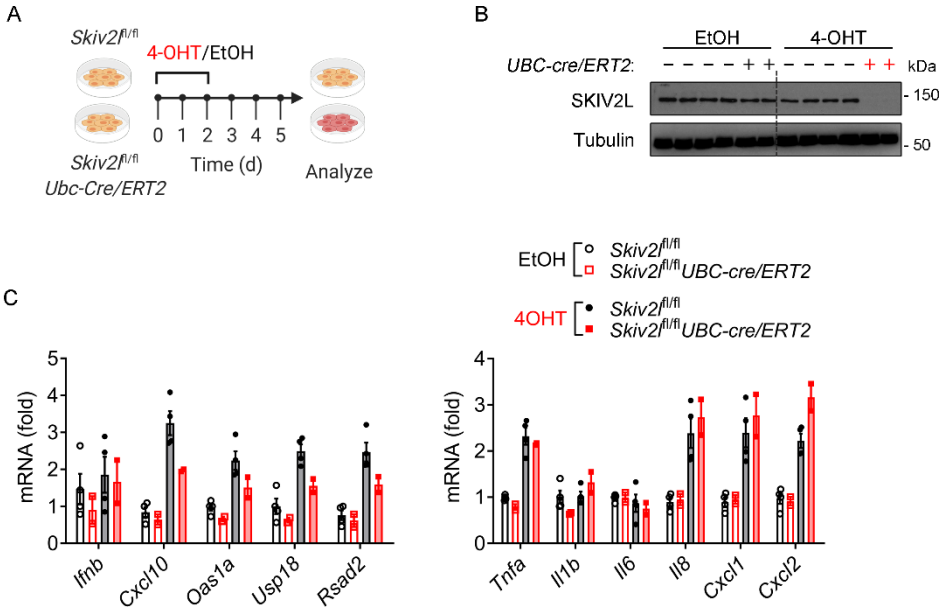
(**F**) Cytokine array analysis of serum of i*Skiv2l*$^{-/-}$ male mice (n=8) and sex-matched *Skiv2l*$^{fl/fl}$ littermates (n=5) at 2 months of age.




**Supplemental Figure 3. Germline keratinocyte-specific *Skiv2l* knockout mice do not show IFN signature in epidermis.**

(**A**) Western blot analysis of SKIV2L in spleen from *Skiv2l*$^{ctl}$ and *Skiv2l*$^{fl/fl}$*K14-Cre* P0 pup. n=3 mice per genotype.

(**B**) qPCR analysis of immune gene expression in P0 *Skiv2l*$^{ctl}$ (n=6) and *Skiv2l*$^{fl/fl}$*K14-Cre* (n=9) pup epidermis.

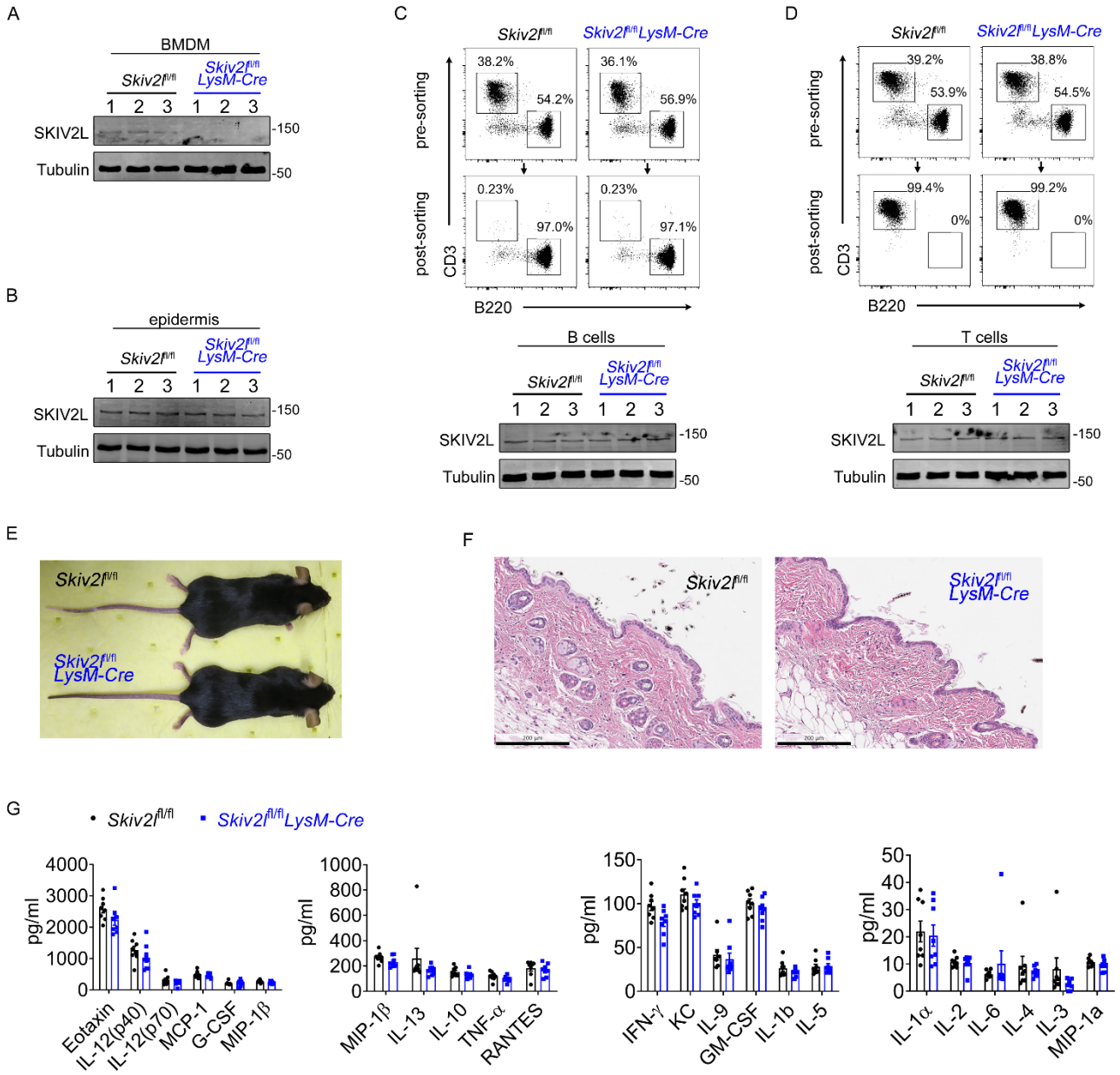



**Supplemental Figure 4. Ex vivo deletion of *Skiv2l* in primary keratinocytes does not trigger IFN response.**

(**A**) A schematic diagram showing experimental design for ex vitro deletion of *Skiv2l* in primary mouse neonatal keratinocytes. Primary mouse neonatal keratinocytes were isolated from *Skiv2l*$^{fl/fl}$*UBC-Cre/ERT2* P0 pups and *Skiv2l*$^{fl/fl}$ littermates, and treated with 4-hydroxytamoxifen (4-OHT) or vehicle (ethanol, EtOH) for 48 h followed by 72-h culture.

(**B**) Western blot analysis of SKIV2L in primary mouse neonatal keratinocytes from *Skiv2l*$^{fl/fl}$*UBC-Cre/ERT2* P0 pups and *Skiv2l*$^{fl/fl}$ littermates after 4-OHT treatment as in (**A**).

(**C**) qPCR analysis of IFNs, IFN-stimulated genes ISGs, inflammatory cytokine, and chemokine mRNA expression in neonatal keratinocytes of *Skiv2l*$^{fl/fl}$*UBC-Cre/ERT2* mouse and littermate after 4-OHT treatment as in (**A**).




**Supplemental Figure 5. Germline myeloid-specific *Skiv2l* knockout does not cause skin disease or systemic inflammation.**

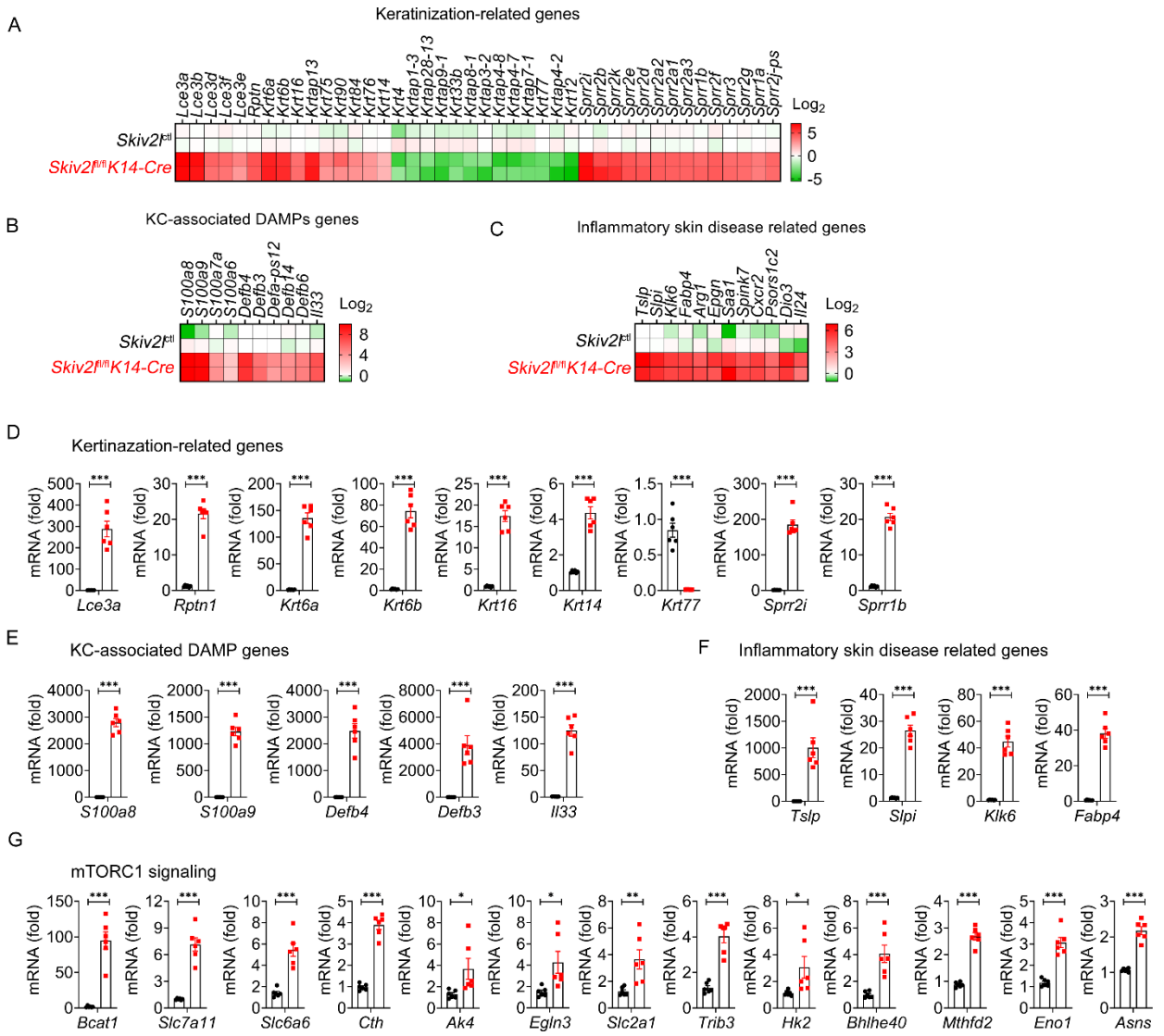
(**A, B**) Western blot analysis of SKIV2L in BMDM (**A**) or epidermis (**B**) of *Skiv2l*$^{fl/fl}$*LysM-Cre* mice and *Skiv2l*$^{fl/fl}$ littermate control. n=3 mice per genotype.

(**C, D**) Western blot analysis of SKIV2L in sorted splenic B cells (**C**) or T cells (**D**) of *Skiv2l*$^{fl/fl}$*LysM-Cre* mice and *Skiv2l*$^{fl/fl}$ littermate control. Flow cytometry analysis of sorting purity is shown in upper panels. n=3 mice per genotype.

(**E**) Skin and hair appearance of 7-month-old *Skiv2l*$^{fl/fl}$*LysM-Cre* (germline myeloid-specific *Skiv2l* knockout) mice and *Skiv2l*$^{fl/fl}$ littermates.

(**F**) H&E staining of dorsal skin of 7-month-old *Skiv2l*$^{fl/fl}$*LysM-Cre* mice and *Skiv2l*$^{fl/fl}$ littermates. Scale bar, 200 μm.

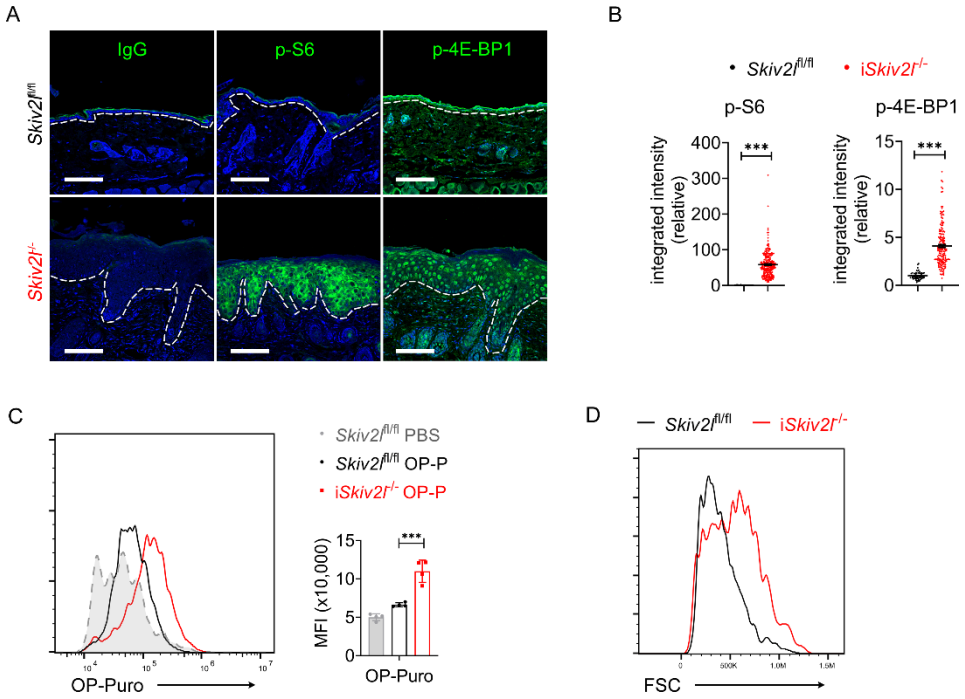
(**G**) Serum cytokine analysis of 7-month-old *Skiv2l*$^{fl/fl}$*LysM-Cre* female mice and *Skiv2l*$^{fl/fl}$ littermates. n=9 per genotype.




**Supplemental Figure 6. Dysregulated pathways in epidermis of germline keratinocyte-specific *Skiv2l* knockout mice.**

(**A-C**) A heatmap showing mRNA expression of keratinization-related genes, keratinocyte-associated DAMPs genes and inflammatory skin disease related genes in *Skiv2l*$^{ctl}$ and *Skiv2l*$^{fl/fl}$*K14-Cre* (germline keratinocyte-specific *Skiv2l* knockout) P0 epidermis (n=2 mice per genotype). Relative mRNA level of each gene is shown after normalizing FPKM of each sample to that of the average value of *Skiv2l*$^{ctl}$ samples.

(**D-G**) qRT-PCR analysis of representative gene expression in *Skiv2l*$^{ctl}$ and *Skiv2l*$^{fl/fl}$*K14-Cre* P0 epidermis. n=6 mice per genotype. Two-sided Student's *t*-test, **P*<0.05, ***P*<0.01, ****P* < 0.001.

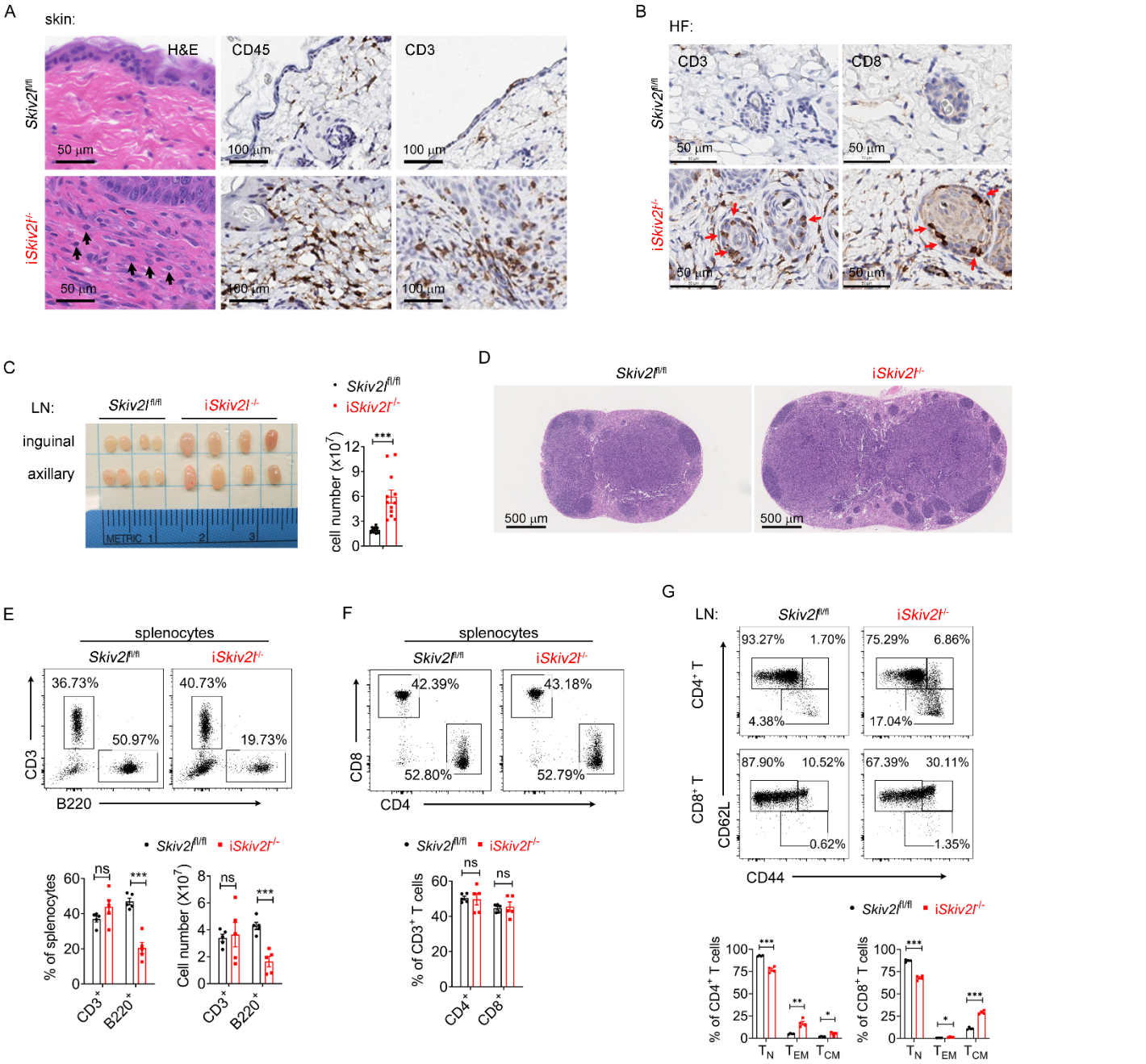



**Supplemental Figure 7. Aberrant activation of the mTORC1 pathway in epidermis of postnatal whole-body inducible *Skiv2l* knockout mice.**

(**A, B**) Fluorescent immunohistochemistry analysis of phospho-S6 ribosomal protein (S235/236) and phospho-4E-BP1 (T37/46) in dorsal skin of i*Skiv2l*$^{-/-}$ and *Skiv2l*$^{fl/fl}$ mice at 3 months of age (**A**). Dashed line, epidermal-dermal junction. Scale bar, 50 μm. Quantification of pS6 or p4E-BP1 fluorescence intensity per cell (>60 cells each genotype) is shown in (**B**). Two-sided unpaired Student's *t*-test, ***$P$<0.001.

(**C**) Global protein translation measured by OP-Puro incorporation in keratinocyte of i*Skiv2l*$^{-/-}$ and *Skiv2l*$^{fl/fl}$ mice in vivo. n=4 mice per group. *Skiv2l*$^{fl/fl}$ mice were injected with OP-Puro or PBS and analyzed 1 h after administration. One-way ANOVA with post hoc Tukey's multiple comparison, ***$P$ <0.001.

(**D**) Flow cytometry analysis of keratinocyte cell size (indicated by FSC). Keratinocytes were isolated from i*Skiv2l*$^{-/-}$ and *Skiv2l*$^{fl/fl}$ mice and analyzed by FACS.



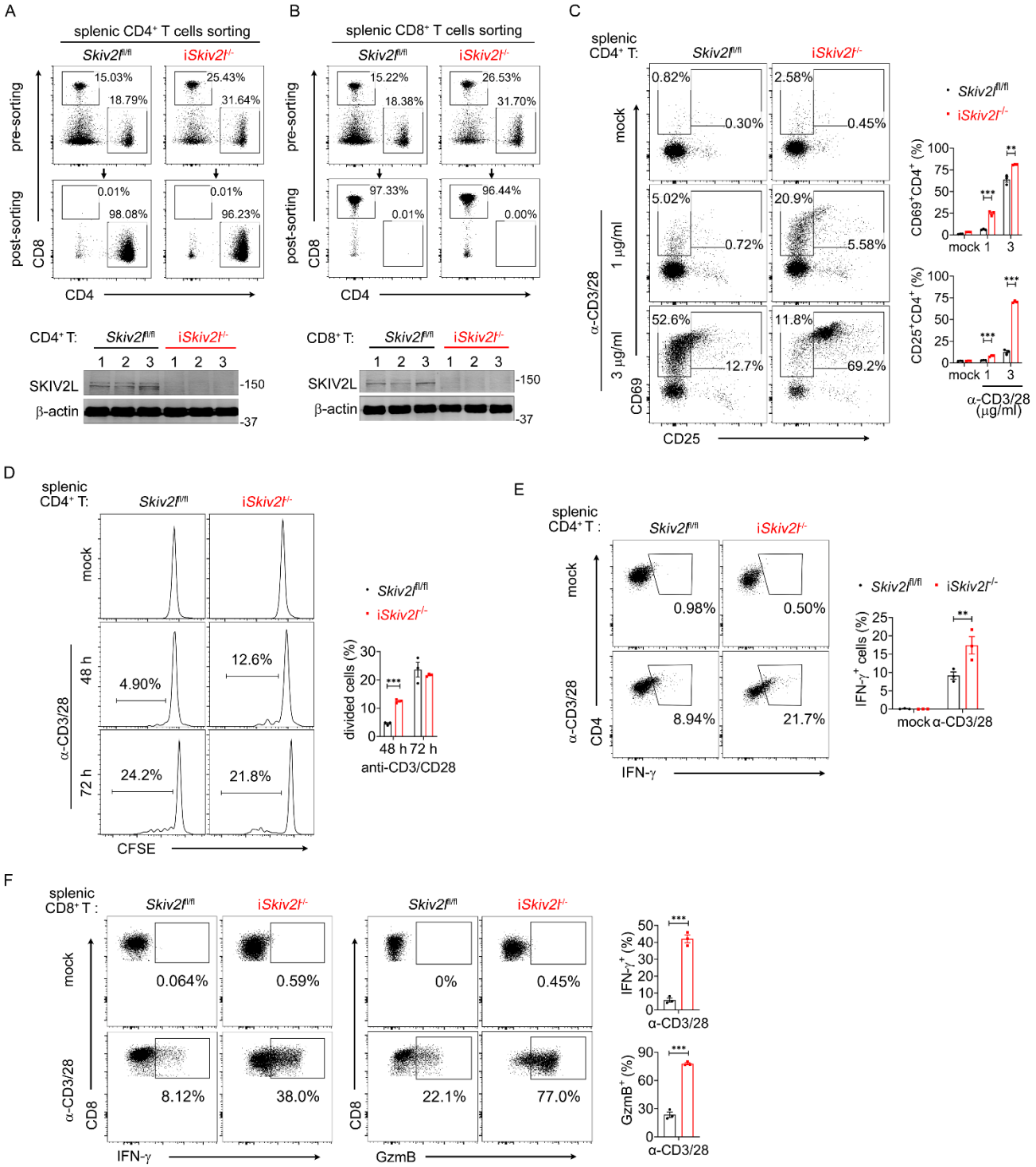

**Supplemental Figure 8. Disruption of immune homeostasis in postnatal whole-body inducible *Skiv2l* knockout mice.**

(**A**) H&E staining and immunohistochemistry analysis of leukocytes (CD45) and T cells (CD3) in dorsal skin of i*Skiv2l*$^{-/-}$ mice and *Skiv2l*$^{fl/fl}$ littermate control (3-month-old). Black arrows, immune infiltrates in dermis. The H&E staining images of *Skiv2l*$^{fl/fl}$ (top left) and i*Skiv2l*$^{-/-}$ (bottom left) are different crops of images shown in **Figure 1C** (top right and bottom right, respectively).

(**B**) Immunohistochemistry analysis of T cells (CD3, CD8) in hair follicles (HF) of 3-month-old i*Skiv2l*$^{-/-}$ mice and *Skiv2l*$^{fl/fl}$ littermate control. Red arrows, immune infiltrates. Scale bar, 50 μm.

(**C**) Skin-draining inguinal and axillary lymph nodes and their cell numbers (lymph nodes from one side were counted). n=12 mice per genotype. Two-sided Student's *t*-test, ***$P$<0.001.

(**D**) H&E staining of inguinal lymph nodes of i*Skiv2l*$^{-/-}$ mice and *Skiv2l*$^{fl/fl}$ littermate controls (3-month-old).
(**E**) Flow cytometry analysis of CD3$^{+}$ T cells and B220$^{+}$ B cells in splenocytes of i*Skiv2l*$^{-/-}$ mice and *Skiv2l*$^{fl/fl}$ controls. n=5 mice per genotype. Two-sided Student's *t*-test, ***$P$<0.001, ns, not significant.
(**F**) Flow cytometry analysis of CD4$^{+}$ T cells and CD8$^{+}$ B cells in spelnocytes (gated on CD3$^{+}$) of i*Skiv2l*$^{-/-}$ mice and *Skiv2l*$^{fl/fl}$ controls. n=5 mice per genotype. Two-sided Student's *t*-test; ns, not significant.
(**G**) Flow cytometry analysis of i*Skiv2l*$^{-/-}$ and *Skiv2l*$^{fl/fl}$ inguinal lymph node T cells. Numbers adjacent to each gate indicate the percentage of each population. n=3 mice per genotype. Two-sided Student's *t*-test, *$P$<0.05, **$P$<0.01, ***$P$<0.001.



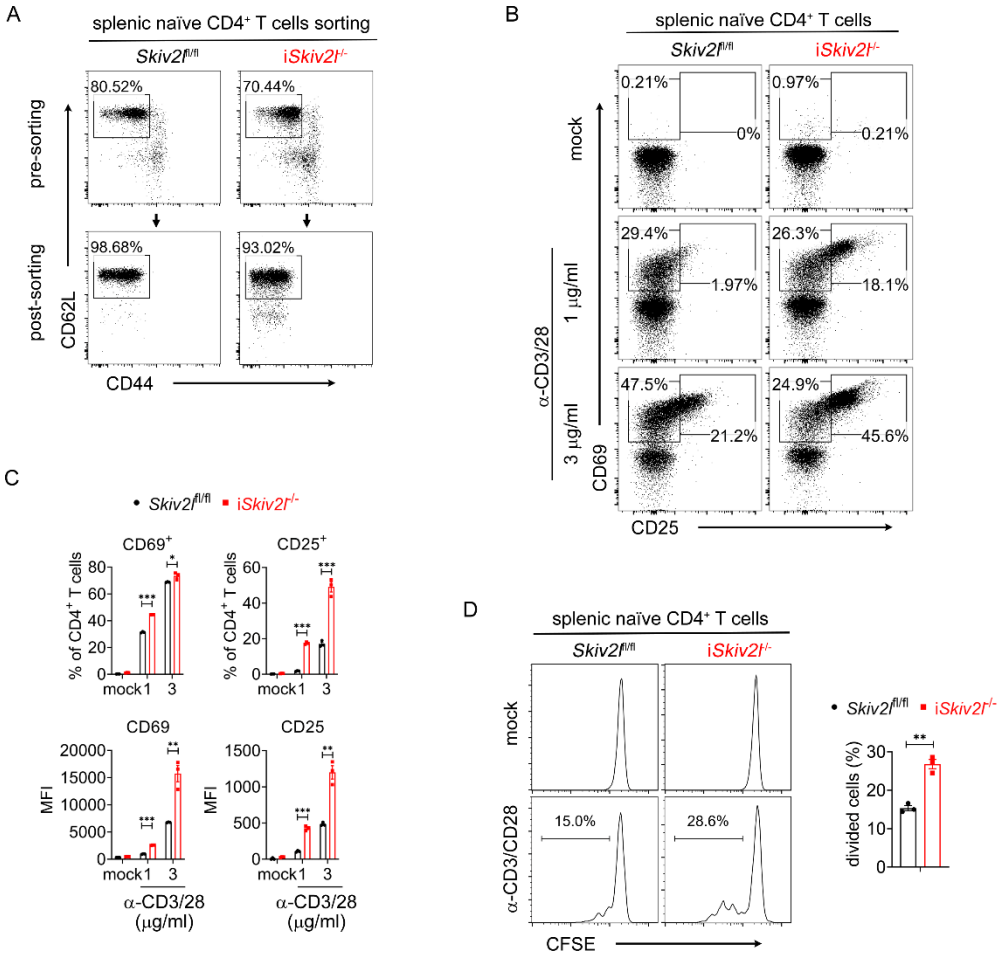

**Supplemental Figure 9. T-cell hyperactivation in postnatal inducible whole-body *Skiv2l* knockout mice.**

(**A, B**) Western blot analysis of SKIV2L in sorted splenic CD4$^+$ (**A**) or CD8$^+$ T cells (**B**) of i*Skiv2l*$^{-/-}$ mice and *Skiv2l*$^{fl/fl}$ littermate controls. Flow cytometry analysis of sorting purity is shown in upper panels. n=3 mice per genotype.

(**C**) Expression of activation markers of i*Skiv2l*$^{-/-}$ and *Skiv2l*$^{fl/fl}$ splenic CD4$^+$ T cells stimulated with indicated concentration of anti-CD3 and anti-CD28 antibodies for 16 h. n=3 per genotype. Two-sided Student's *t*-test, ***P*<0.01, ****P*<0.001.

(**D**) T cell proliferation analysis by CFSE dilution assay. i*Skiv2l*$^{-/-}$ and *Skiv2l*$^{fl/fl}$ splenic CD4$^{+}$ T cells were stained with CFSE then stimulated with anti-CD3 and anti-CD28 (3 μg/ml) for indicated times. n=3 per genotype. Two-sided Student's *t*-test, ***$P$<0.001.

(**E, F**) Intracellular IFN-γ or granzyme B (GzmB) staining of i*Skiv2l*$^{-/-}$ and *Skiv2l*$^{fl/fl}$ splenic CD4$^{+}$ T (**E**) and CD8$^{+}$ T cells (**F**) after stimulation with anti-CD3 and anti-CD28 (3 μg/ml). Two-sided Student's *t*-test, **$P$<0.01, ***$P$<0.001.

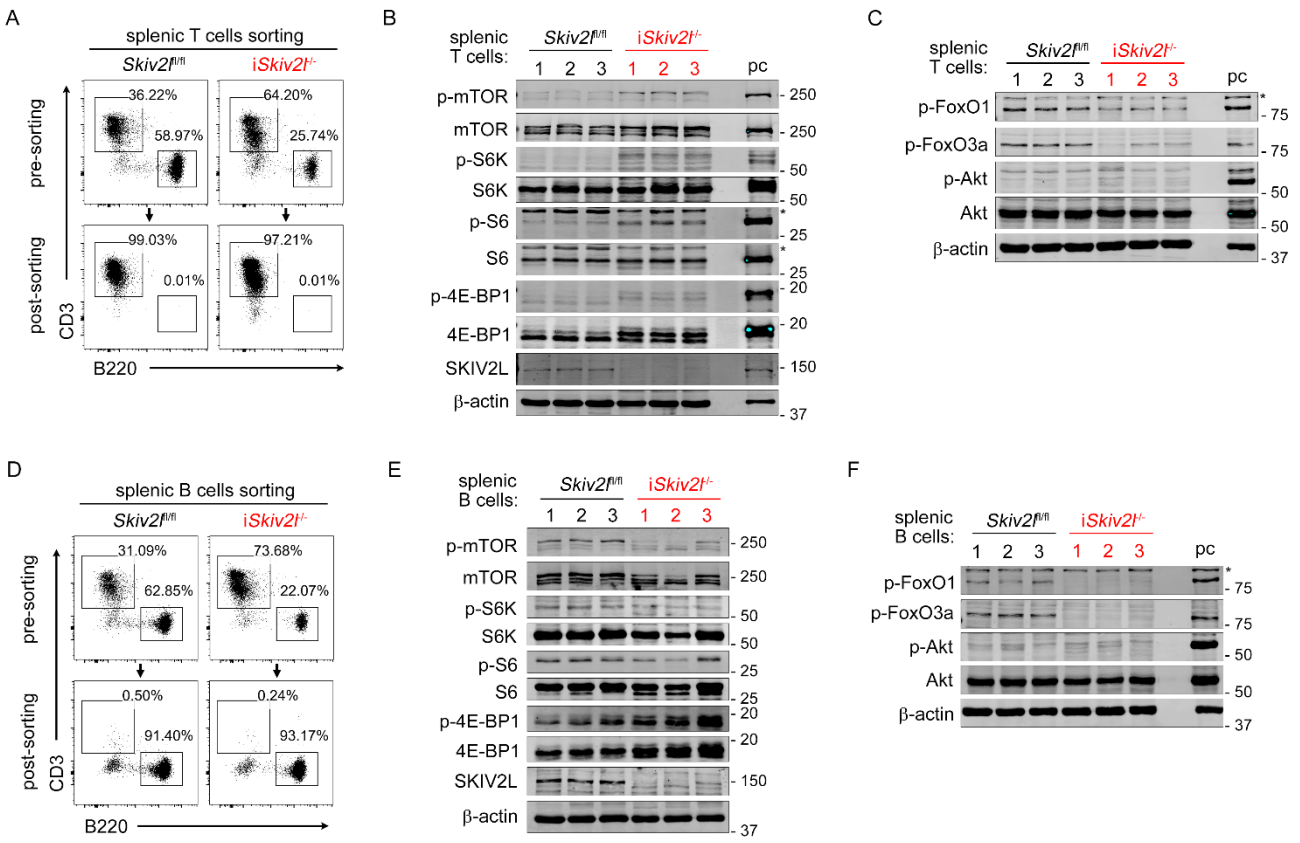



**Supplemental Figure 10. Hyperactive response of *Skiv2l*-deficient naïve CD4$^+$ T cells.**

(**A**) Flow cytometry analysis of naïve CD4$^+$ T cells purity after sorting from i*Skiv2l*$^{-/-}$ and *Skiv2l*$^{fl/fl}$ splenocytes.

(**B, C**) Expression of activation markers CD69 and CD25 of i*Skiv2l*$^{-/-}$ and *Skiv2l*$^{fl/fl}$ naïve CD4$^+$ T cells stimulated with indicated concentration of anti-CD3 and anti-CD28 antibodies for 16 h. n=3 per genotype. Two-sided Student's *t*-test, **P*<0.05, ***P*<0.01, ****P*<0.001.

(**D**) T cell proliferation analysis by CFSE dilution assay. i*Skiv2l*$^{-/-}$ and *Skiv2l*$^{fl/fl}$ naïve CD4$^+$ T cells were stained with CFSE then stimulated with anti-CD3 and anti-CD28 (3 μg/ml) for 72 h. n=3 per genotype. Two-sided Student's *t*-test, ***P*<0.01.



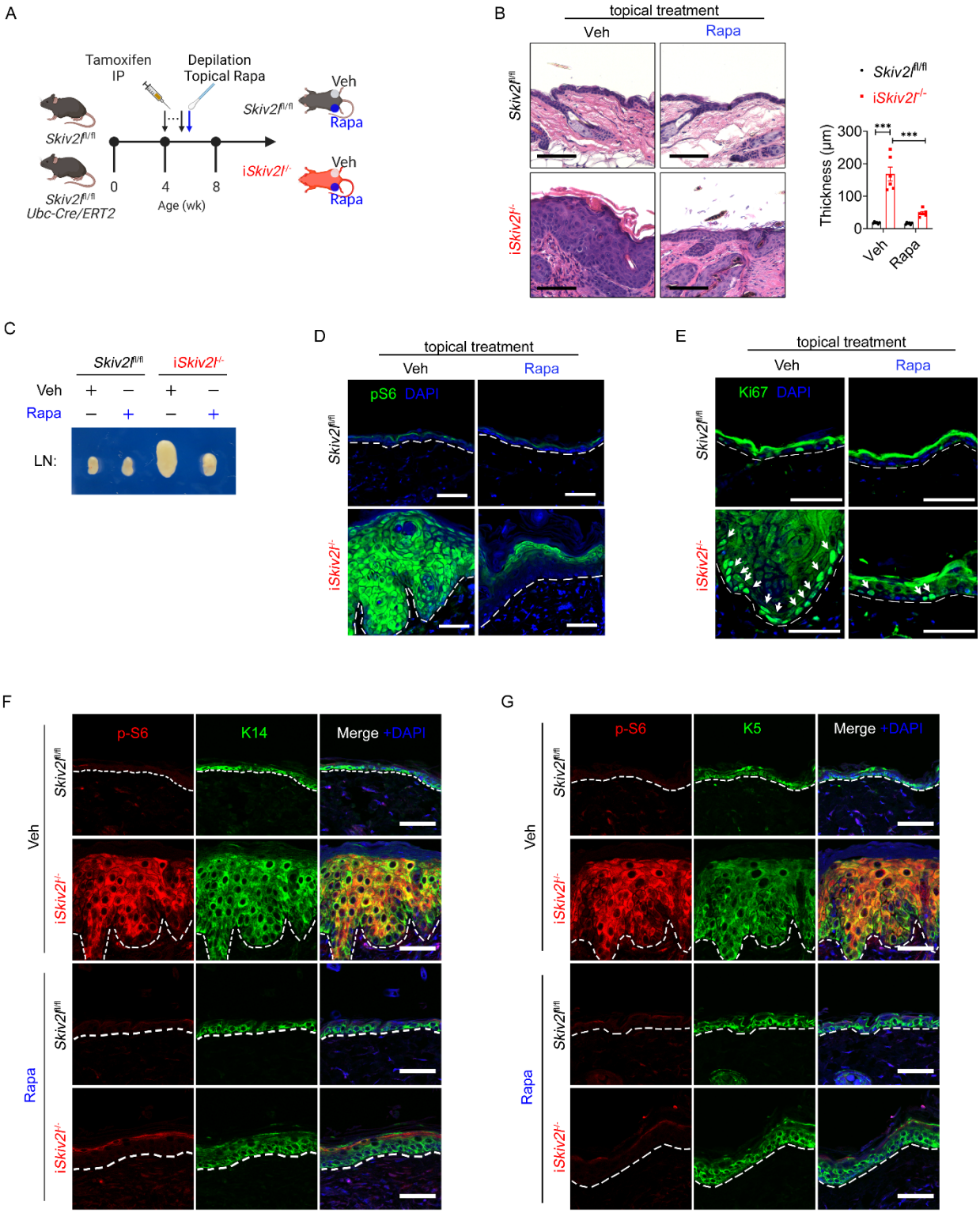

**Supplemental Figure 11. Activation of the mTORC1 pathway in T cells of postnatal inducible whole-body *Skiv2l* knockout mice.**

(**A**) Flow cytometry analysis of T cells purity after sorting of i*Skiv2l*$^{-/-}$ and *Skiv2l*$^{fl/fl}$ splenocytes.

(**B, C**) Western blot analysis of mTORC1 (**B**) and mTORC2 (**C**) pathway in sorted splenic T cells of i*Skiv2l*$^{-/-}$ mice and *Skiv2l*$^{fl/fl}$ controls. n=3 mice per genotype. Whole cell lysate of B16 melanoma cells was used as positive control (pc). *, non-specific band.

(**D**) Flow cytometry analysis of B cells purity after sorting of i*Skiv2l*$^{-/-}$ and *Skiv2l*$^{fl/fl}$ splenocytes.

(**E, F**) Western blot analysis of mTORC1 (**E**) and mTORC2 (**F**) pathway in sorted splenic B cells of i*Skiv2l*$^{-/-}$ mice and *Skiv2l*$^{fl/fl}$ controls. n=3 mice per genotype. Whole cell lysate for B16 melanoma cells was used as positive control (pc). *, non-specific band.




**Supplemental Figure 12. Topical rapamycin treatment ameliorates skin pathology of postnatal whole-body inducible *Skiv2l* knockout mice**

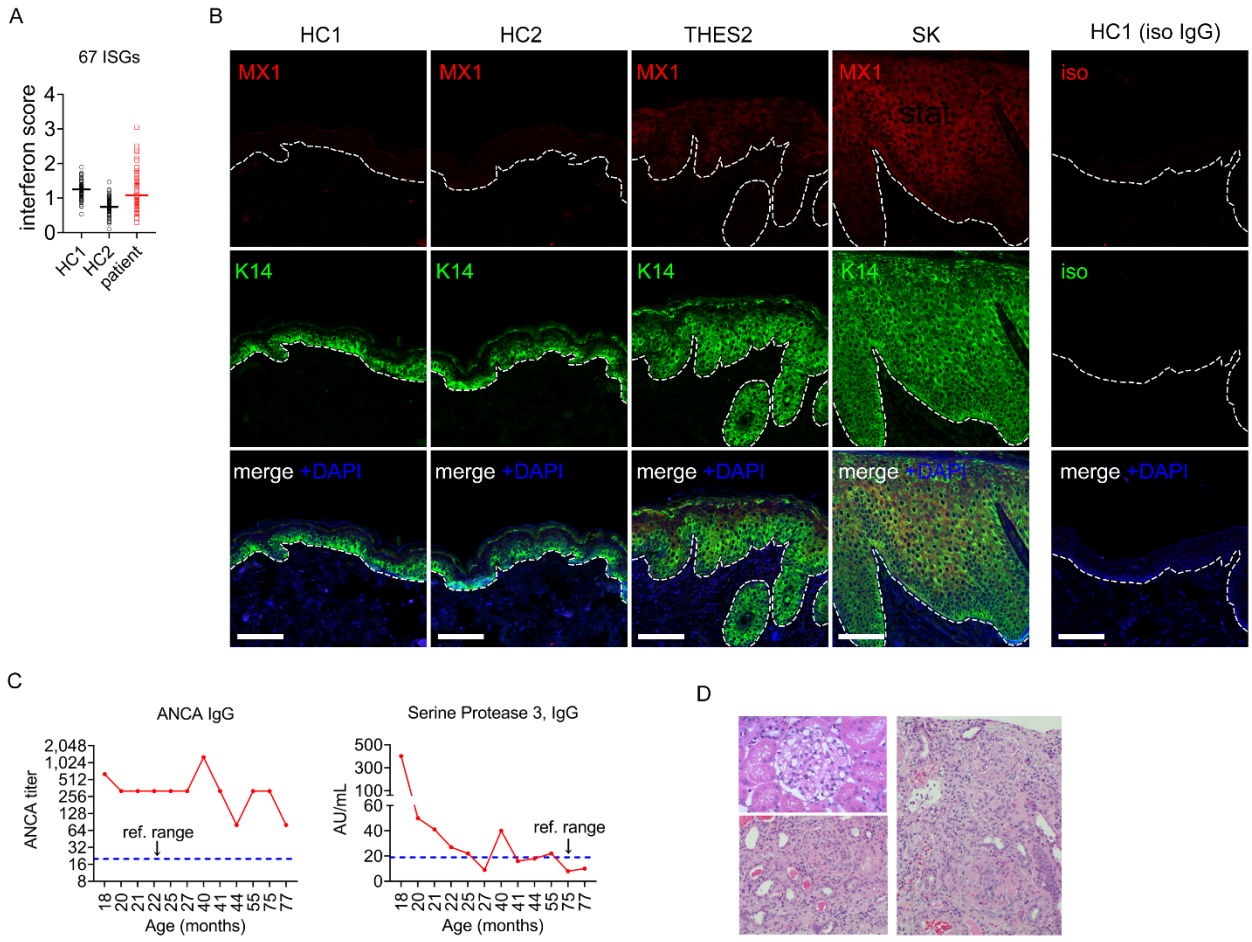
(**A**) A schematic diagram of experimental design for topical rapamycin treatment of i*Skiv2l*$^{-/-}$ mice and *Skiv2l*$^{fl/fl}$ controls after tamoxifen injection and depilation.

(**B**) H&E staining of i*Skiv2l*$^{-/-}$ and *Skiv2l*$^{fl/fl}$ mice skin topically treated with rapamycin or vehicle as above (**A**). Scale bar, 50 μm. Quantification of epidermal thickness is shown on the right bar graph. n=6 mice per group. Two-way ANOVA with post hoc Tukey's multiple comparisons test, ***$P$<0.001.

(**C**) Skin-draining inguinal and axillary lymph nodes of i*Skiv2l*$^{-/-}$ and *Skiv2l*$^{fl/fl}$ mice skin topically treated with rapamycin or vehicle.

(**D, E**) Fluorescent immunohistochemistry analysis of phospho-S6 ribosomal protein (S235/236) (**D**) and proliferation marker Ki67 (**E**) in i*Skiv2l*$^{-/-}$ and *Skiv2l*$^{fl/fl}$ mouse skin treated with rapamycin or vehicle. White arrows in (**E**), Ki67 positive cells. Dashed line, epidermal-dermal junction. Scale bar, 50 μm.

(**F, G**) Fluorescent immunohistochemistry analysis of phospho-S6 ribosomal protein (S235/236) and keratinocyte maker K14 (**F**) or K5 (**G**) in i*Skiv2l*$^{-/-}$ and *Skiv2l*$^{fl/fl}$ mouse skin treated with rapamycin or vehicle. Dashed line, epidermal-dermal junction. Dashed line, epidermal-dermal junction. Scale bar, 50 μm.




**Supplemental Figure 13. Immunological parameters of the THES2 patient.**

(**A**) Interferon score from average expression of 67 ISGs in PBMCs from the THES2 patient and two unrelated healthy controls (HC).

(**B**) Fluorescent immunohistochemistry analysis of MX1 and K14 in skin biopsies from healthy controls, THES2, and an unrelated case of seborrheic keratosis (SK). Dashed line, epidermal-dermal junction. Scale bar, 100 µm. The skin biopsy of THES2 is from the same patient as presented in **Figure 7E**.

(**C**) Titers of anti-neutrophil cytoplasmic antibody (ANCA) and serine protease 3 IgG antibodies in THES2 patient. Patient's titers are shown in solid red line and reference range are in dashed blue line.

(**D**) H&E staining of kidney biopsy at 17-month old indicates pauci-immune necrotizing and crescentic glomerulonephritis (NCGN), necrotizing arteriolitis. acute tubulointerstitial nephritis, global glomerulosclerosis, interstitial fibrosis and tubular atrophy.

**Supplemental Table 1. qRT-PCR primer sequences**

| *Gene* | Primer | Sequence 5'-3' |
|---|---|---|
| *Ifit1* | Fwd | TTCACATGGAAGCTGCTATTTGAAA |
| | Rev | TGCTCAGCTGCTCGCTCTGGATCAA |
| *Cxcl10* | Fwd | GGGATCCCTCTCGCAAGGACGGTCC |
| | Rev | ACGCTTTCATTAAATTCTTGATGGT |
| *Oas1a* | Fwd | GGATGGCATAGATTCTGGGA |
| | Rev | CTGCATCAGGAGGTGGAGTT |
| *Usp18* | Fwd | GTGTCCGTGATCTGGTCCTT |
| | Rev | CTGCAGAAATACAACGTGCC |
| *Rsad2* | Fwd | GGACGCTTCATGGTGTTATTTG |
| | Rev | TGATTGGTCGCCTGTTTATCT |
| *Ifnb* | Fwd | CTGCGTTCCTGCTGTGCTTCTCCA |
| | Rev | TTCTCCGTCATCTCCATAGGGATC |
| *Tnfa* | Fwd | CTACCTTGTTGCCTCCTCTTT |
| | Rev | GAGCAGAGGTTCAGTGATGTAG |
| *Il1b* | Fwd | GGTACATCAGCACCTCACAA |
| | Rev | TTAGAAACAGTCCAGCCCATAC |
| *Il6* | Fwd | CACAAGTCCGGAGAGGAGAC |
| | Rev | CAGAATTGCCATTGCACAAC |
| *Cxcl1* | Fwd | CGAAGTCATAGCCACACTCAA |
| | Rev | GAGCAGTCTGTCTTCTTTCTCC |
| *Cxcl2* | Fwd | TAAGCACCGAGGAGAGTAGAA |
| | Rev | GTCCAAGGGTTACTCACAACA |
| *Lce3a* | Fwd | GTCTTGGGCTCTGTGTTCTT |
| | Rev | CATGGTTGGACACAGGTGAT |
| *Rptn* | Fwd | GAAGGAACACGGAGCCATAAA |
| | Rev | CCTTCAGACTGATTGTGGTGAG |
| *Krt6a* | Fwd | CAACATCATAACCCTCCCTGTC |
| | Rev | GAGGAAGCCAAGAGCATCAA |
| *Krt6b* | Fwd | GTCTCTGAGTTGCCTGGTAAAG |
| | Rev | CCAGGCCATTGGAAACTAGAA |
| *Krt16* | Fwd | CTCCTCTGGACAGTCCTATTCT |
| | Rev | GTCCCTGGAACTCTGACTTTG |
| *Krt14* | Fwd | GAGCGGCAAGAGTGAGATTT |
| | Rev | CTTTGGTCTCCTCCAGGTTATTC |
| *Krt77* | Fwd | GCTGCTTTCATGGGCAAATC |
| | Rev | GGGACAGCTCCGTATCAAATAG |
| *Sprr2i* | Fwd | TCCACATAGCACCTCCTTCTA |
| | Rev | ATTCTCTGCAGGCCCTTTAC |
| *Sprr1b* | Fwd | CCATATACCAGGCTCATCCATC |
| | Rev | GGCTGTTTCACTTGTTGCTC |

| | | |
|---|---|---|
| *S100a8* | Fwd | CTTTGTCAGCTCCGTCTTCA |
| | Rev | TGTAGAGGGCATGGTGATTTC |
| *S100a9* | Fwd | GAGGAGTGTATGATGCTGATGG |
| | Rev | GTCACATGGCTGACCTCTTAAT |
| *Defb4* | Fwd | CGAAGAACCAGCAAGATGAATAAA |
| | Rev | CTAGAACTGGAGTTAGAGAAGGTAATC |
| *Il33* | Fwd | GGGTACCAAGCATGAAGAGAA |
| | Rev | GTCAACAGACGCAGCAAATG |
| *Tslp* | Fwd | TCATGACCTGACTGGAGATTTG |
| | Rev | AGCCAGGGATAGGATTGAGA |
| *Slpi* | Fwd | CGCCTCCTGGTAAAGACATAAA |
| | Rev | GGAGCACCGTGAAAGGTAAA |
| *Klk6* | Fwd | AGAGCACAGAACACTGCTAAAT |
| | Rev | CATCTGCTAACCACACCCATAG |
| *Fabp4* | Fwd | GCTCCTCCTCGAAGGTTTAC |
| | Rev | CCCACTCCCACTTCTTTCAT |
| *Bcat1* | Fwd | GTGGTAGCATTCGTGGGTAATA |
| | Rev | TCCTAAGCGCTGGGATTAAAG |
| *Slc7a11* | Fwd | GGGAAGGTGATGGCTGTATTT |
| | Rev | CACCACTCAGCATCTGTGTATC |
| *Slc6a6* | Fwd | CTGCCAAGTGACCCTCTAATC |
| | Rev | TAACACGAGAGCAACCAGAAATA |
| *Cth* | Fwd | GGTCCGGATGGAGAAACATT |
| | Rev | AGAGGGTAGCCCAGGATAAA |
| *Ak4* | Fwd | CCCTTCACAGGAACGAGTATAG |
| | Rev | CCTGTGGAAGGTGGGATAAA |
| *Egln3* | Fwd | GCCCAGGACTGCTTCTTATT |
| | Rev | TGGCATCTGTCACCAACTTTA |
| *Slc2a1* | Fwd | AAGACTGCTGCTCAGATCTATTC |
| | Rev | GGAGATAGGAGAGTGGCTGATA |
| *Trib3* | Fwd | GTCTCAGCTTTCTCCTGACTTT |
| | Rev | CACTAGGCACAGGAACGAATAA |
| *Hk2* | Fwd | CTCGGCCTTGTGAGTAGATAGA |
| | Rev | CGCGGGTGAATGAGGTATTT |
| *Bhlhe40* | Fwd | CTGCTGCCTTGCTTTCTTTC |
| | Rev | CTGAATCTTCTCTGTGGGTCTG |
| *Mthfd2* | Fwd | GTGCTTGGACCAGTACTCTATG |
| | Rev | CCAGCCACTACCACATTCTT |
| *Eno1* | Fwd | GATGGACGGCACAGAGAATAAA |
| | Rev | TCAGCAATGTGGCGGTAAA |
| *Asns* | Fwd | CAGTGTCTGAGTGCGATGAA |
| | Rev | CAAGCGGTGAAAGCCAAAG |

| | | |
|---|---|---|
| *Actb* | Fwd | GAGGTATCCTGACCCTGAAGTA |
| | Rev | CACACGCAGCTCATTGTAGA |