Supplementary Information

Loss of epidermal microRNA-149 sensitizes to skin inflammation

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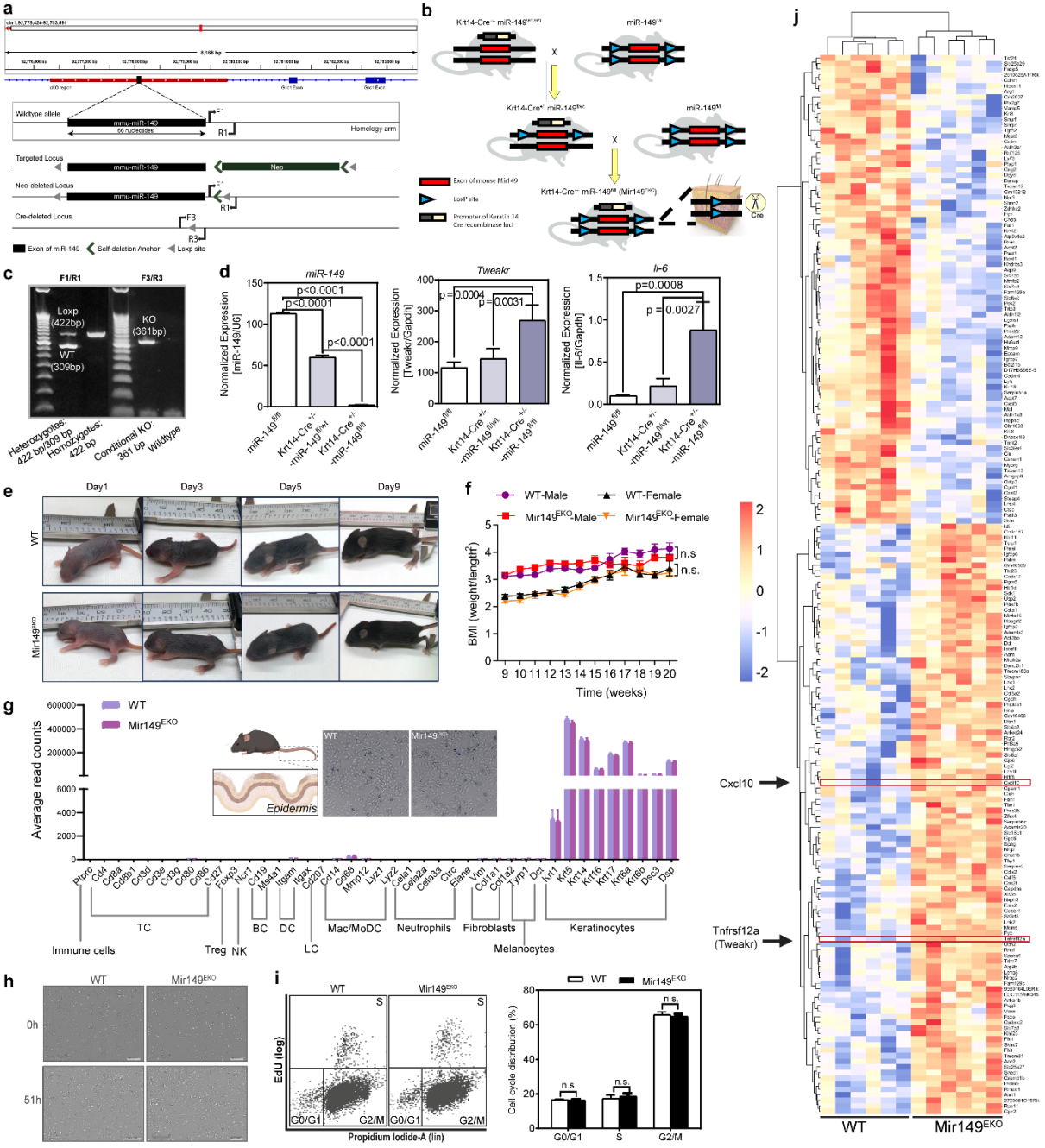
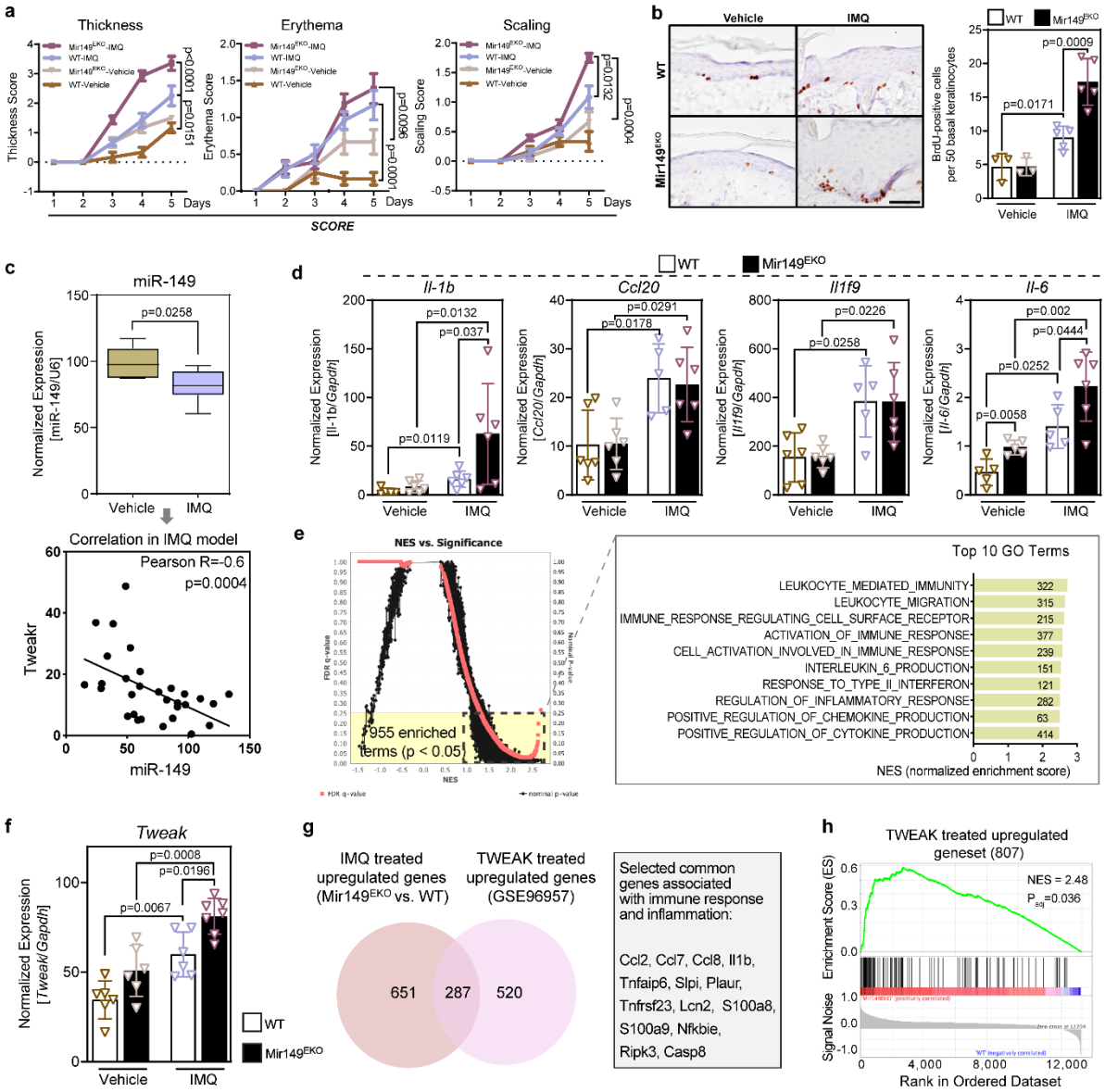
Supplementary Figure 1.



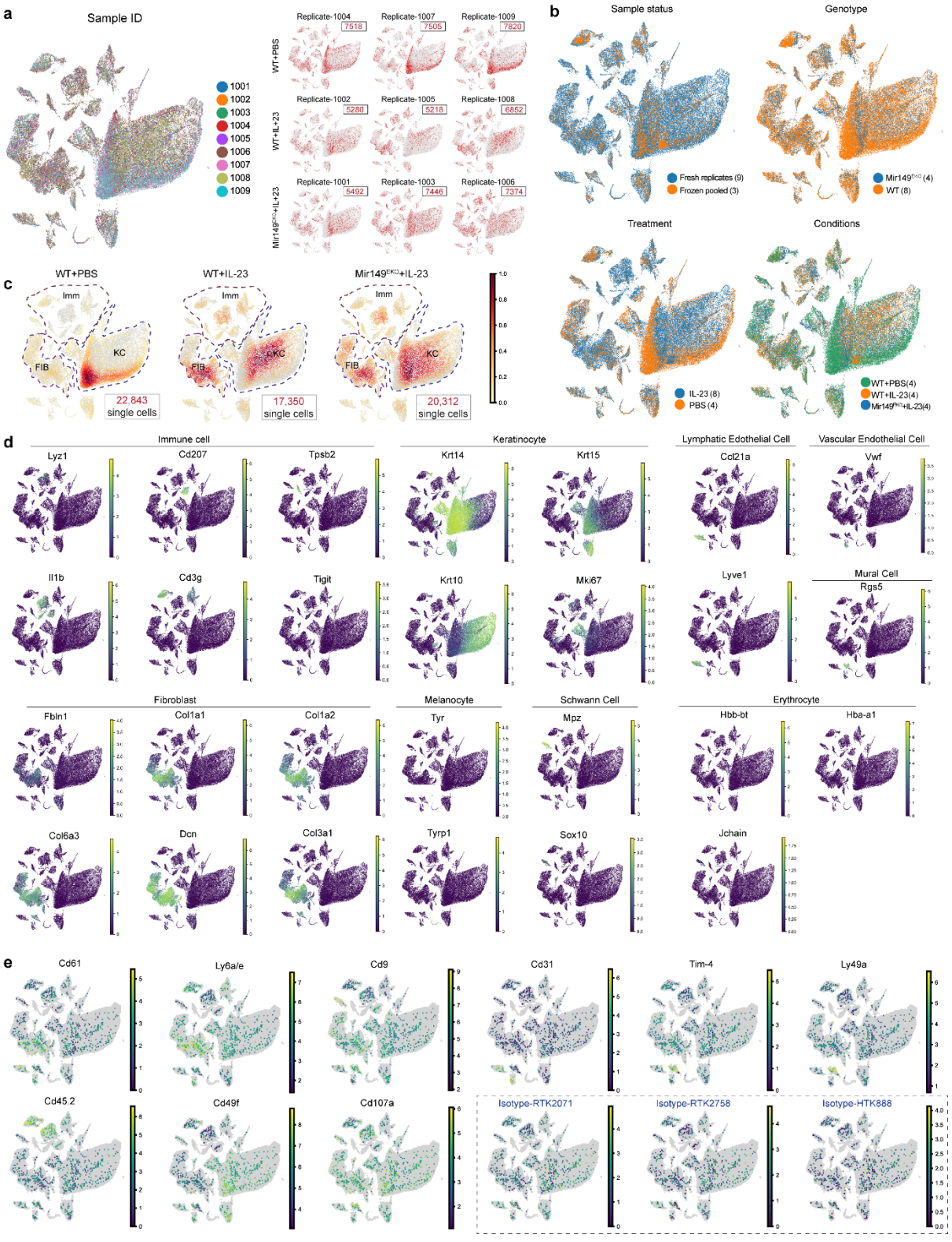
Supplementary Figure 1. Generation and characterization of epidermis-specific miR-149 knockout mice. **a**. Schematic representation of genotyping primers and targeting strategy. **b**. Conditional knockout model of miR-149 in epidermal basal keratinocytes generated by crossing *Mir149*$^{fl/fl}$ mice with Krt14-Cre mice. **c**. miR-149 genotyping with F1/R1 and F3/R3 primers, showing 422 bp band for WT and 361 bp for knockout alleles. **d**. qRT-PCR analysis of miR-149, Tweakr, and Il-6 expression in epidermis from WT and *Mir149*^{EKO} mice. **e**. Skin phenotypic progression in WT and *Mir149*^{EKO} mice from day 1 to day 9. **f**. Body mass index (BMI) of WT and *Mir149*^{EKO} mice from 9 to 20 weeks (n = 6). **g**. Representative images and marker gene expression in keratinocytes isolated from WT and *Mir149*^{EKO} mice. **h**. IncuCyte live-cell analysis of primary keratinocyte growth (n = 6). **i**. EdU cell cycle assay analysis of cell cycle progression (n = 6). **j**. Unsupervised heatmap of gene expression differences between *Mir149*^{EKO} and WT mice (n = 6). The data were analyzed by one-way ANOVA (**d**) and unpaired two-tailed student's t-test (**i**). Data are presented as mean ± SD (**d, g, i**) or mean ± SEM (**f**). Source data are provided as a Source Data file.

Supplementary Figure 2.



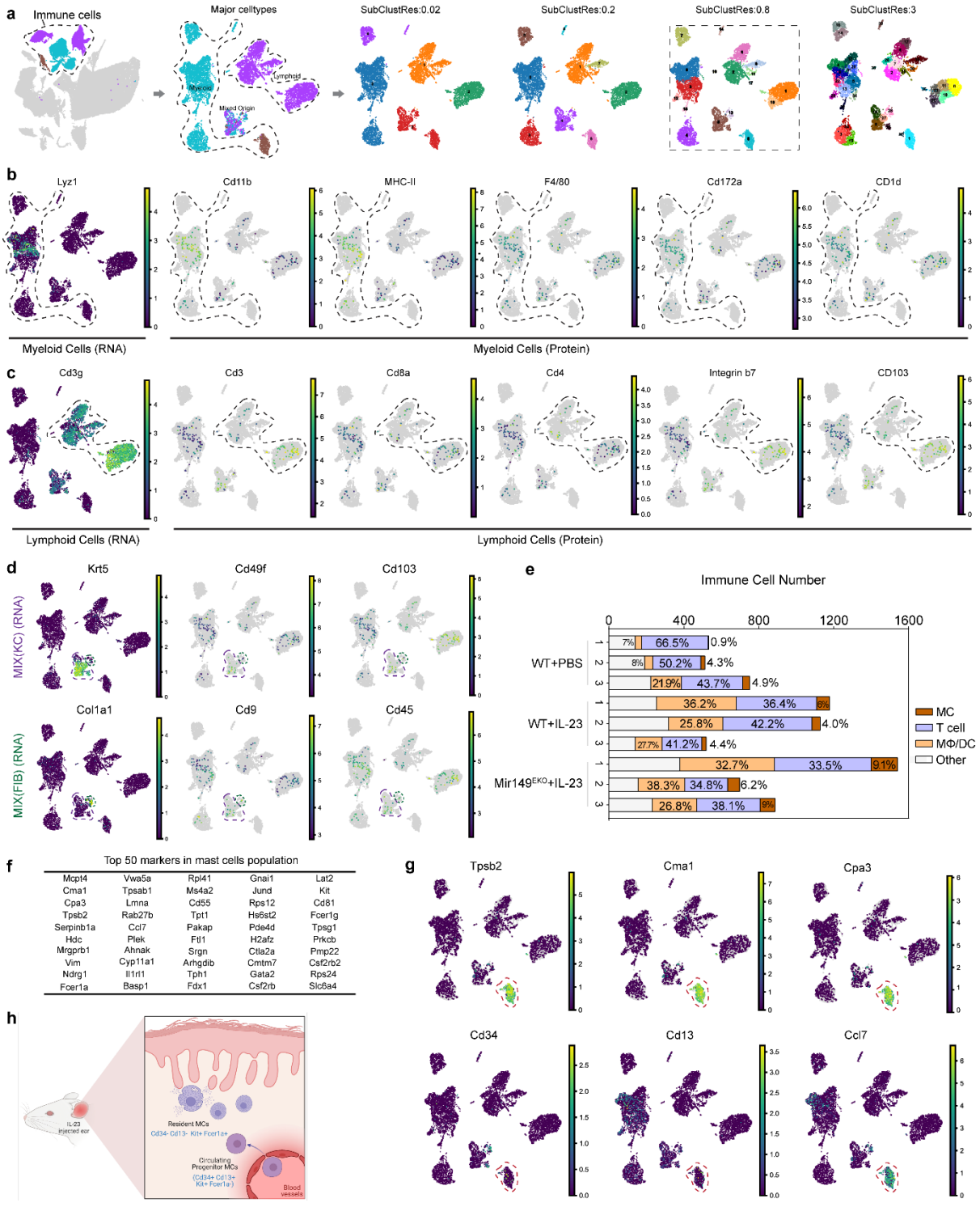
Supplementary Figure 2. Deletion of *Mir149* in the epidermis exacerbates IMQ-induced psoriasis-like skin inflammation and induces transcriptomic changes with enrichment of a TWEAK-associated signature. a. Severity scores for skin thickness, erythema, and scaling in IMQ-treated *Mir149*^{EKO} (n=7) and WT (n=6) mice. **b**. BrdU incorporation (brown) indicates increased epidermal proliferation in IMQ-treated *Mir149*^{EKO} mice. **c**. qRT-PCR for miR-149 in IMQ-treated WT skin and a negative correlation between Tweakr and miR-149 in an extended cohort of mouse skin samples from the IMQ model. **d**. qRT-PCR for psoriasis-associated inflammatory mediators (Il-1b, Ccl20, Il1f9, Il-6) (n = 6-7). **e**. GSEA identifies 955 upregulated gene sets in IMQ-treated *Mir149*^{EKO} skin, with the top 10 shown. **f**. qRT-PCR for Tweak expression in vehicle or IMQ-treated WT (n = 6) and *Mir149*^{EKO} (n = 7) skin. **g**. Intersection analysis of Tweak-upregulated genes (GSE96957) and DEGs from IMQ-treated *Mir149*^{EKO} skin, highlighting 8 common genes. **h**. GSEA shows enrichment of TWEAK-upregulated gene sets in IMQ-treated *Mir149*^{EKO} skin. The data were analyzed by unpaired two-tailed student's t-test (**c**) and one-way ANOVA (**b**, **d**, **f**) and two-way ANOVA (**a**). Data are presented as mean ± SD (**b-d, f**) or mean ± SEM (**a**). Scale bar = 50 µm. Source data are provided as a Source Data file.

Supplementary Figure 3.



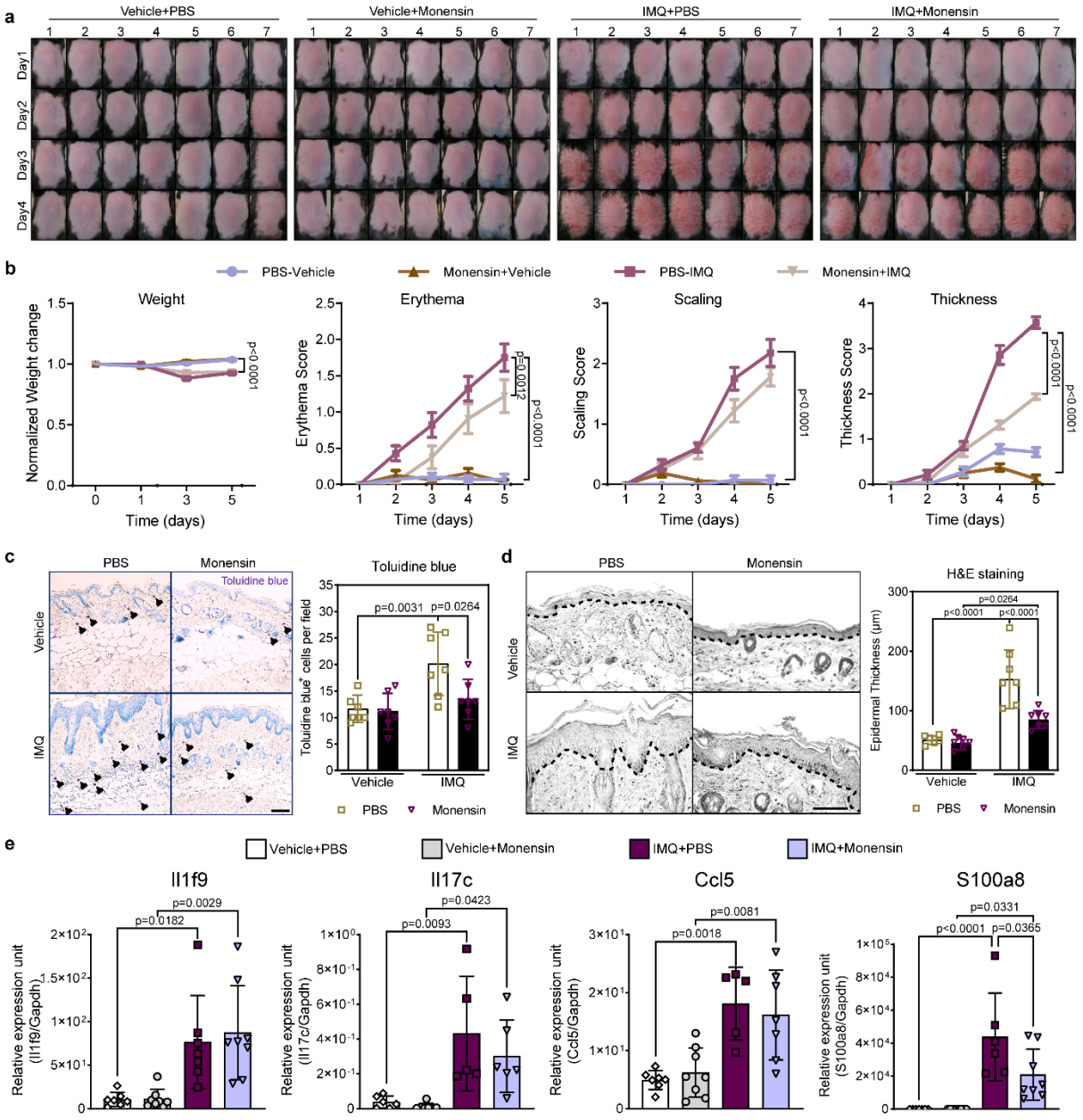
Supplementary Figure 3. Single-cell analysis of cell distribution, marker gene expression, and surface protein profiling among major cell populations after the first level of clustering. a. UMAP plot for all cells from nine mouse samples, shown together or separately, with cell numbers from each sample indicated. **b**. UMAP plot showing batch effects across sample status, genotype, treatment, and conditions. **c**. Feature plot visualizing immune population distribution among WT cells with PBS or IL-23 and *Mir149*^{EKO} cells with IL-23. **d**. Feature plot showing expression of marker genes for immune cells, keratinocytes, fibroblasts, melanocytes, Schwann cells, mural cells, endothelial cells, and erythrocytes. **e**. Distribution of selected cell surface proteins (Cd61, Ly6a/e, Cd9, Cd31, Tim-4, Ly49a, Cd45.2, Cd107a) along with 3 isotype controls. Source data are provided as a Source Data file.

Supplementary Figure 4.



Supplementary Figure 4. Single-cell transcriptome and cell surface protein expression reveal the distribution of immune cells in control or IL-23-injected ear skin. a. UMAP plot visualization depicting the process of sub-clustering for all immune cell populations at varying resolutions, with 0.8 highlighted as the final chosen resolution. (**b-d**). Feature plot displaying the expression of selected RNA markers (Lyz1, Cd3g, Krt5, Col1a1) and cell surface proteins in two major lineages: myeloid (Cd11b, MHC-II, F4/80, Cd172a, and Cd1d) and lymphoid cells (Cd3, Cd8a, Cd4, Integrin β7, and Cd103), as well as two mixed populations (Cd49f, Cd103, Cd9, Cd45). **e**. Bar plot quantifying cell numbers and proportions of mast cells, macrophages/dendritic cells, and T cells from individual donors. **f**. Table showing the top 50 makers for mast cells. **g**. Feature plot displaying the expression of selected RNA markers (Tpsb2, Cma1, Cpa3, Cd34, Cd13, Ccl7) within mast cells. **h**. Illustration graph showing the resident mast cells and bloodstream derived progenitor mast cells in IL-23 injected ear skin. Source data are provided as a Source Data file.

Supplementary Figure 5.



Supplementary Figure 5. Intraperitoneal injection of monensin ameliorates IMQ-induced psoriasis-like skin inflammation in mice. a. Macroscopic evaluation of vehicle- or IMQ-treated mice, with or without monensin injection, from day 1 to day 4. **b.** Mouse weight and skin scores represent skin thickness, erythema and scaling evaluation on vehicle or IMQ-treated mice with (n=8) or without monensin (n=7) injection. **c.** Toluidine blue staining and quantification of mouse skin with or without monensin treatment (n=7). **d.** H&E staining and quantification of epidermal thickness in vehicle- or IMQ-treated mouse skin (n=7). **e.** The expression of Il1f9, Il17c, Ccl5, and S100a8 was detected by qRT-PCR in vehicle- or IMQ-treated mice with (n=8) or without Monensin (n=7) injection. The data were analyzed by one-way ANOVA (**c-e**) and two-way ANOVA (**b**). Data are presented as mean ± SD (**c-e**) or mean ± SEM (**b**). Scale bar = 100 μm. Source data are provided as a Source Data file.