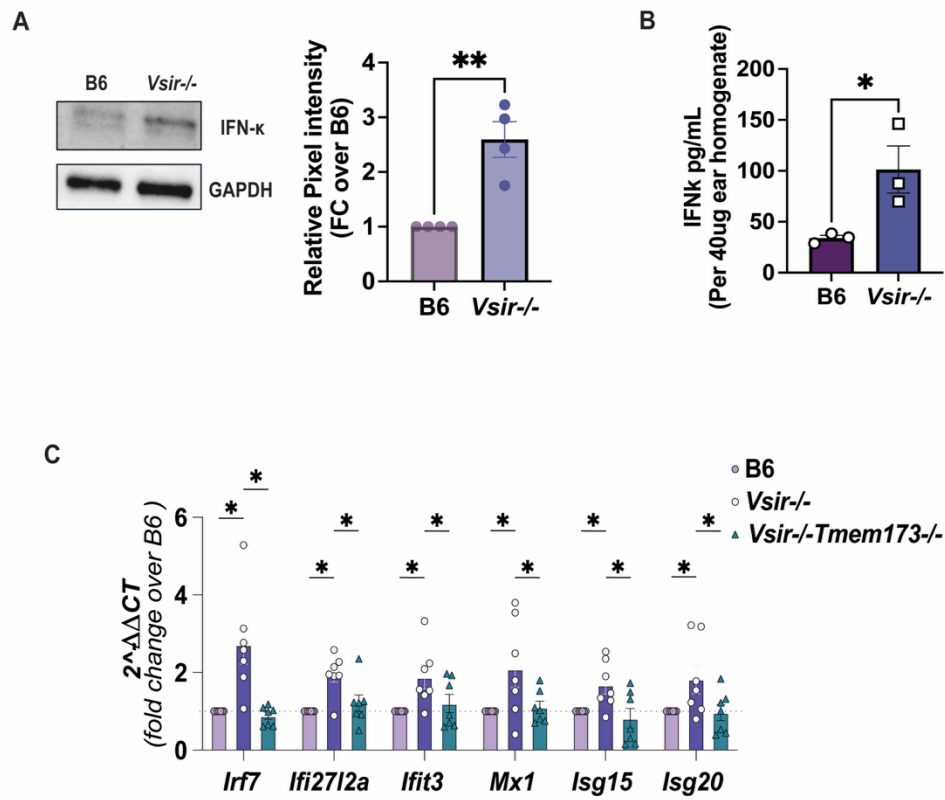
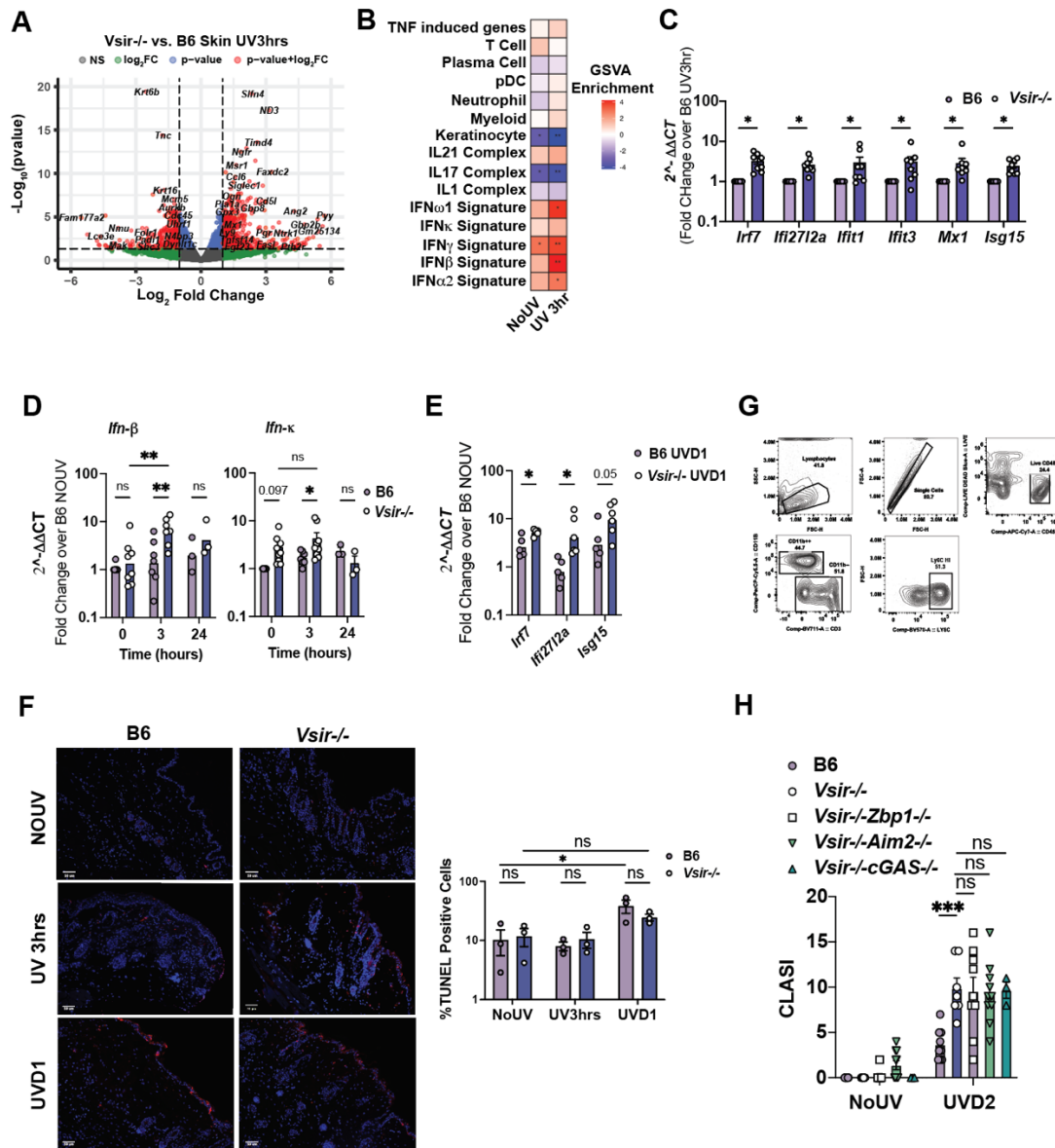


Supplemental Figures

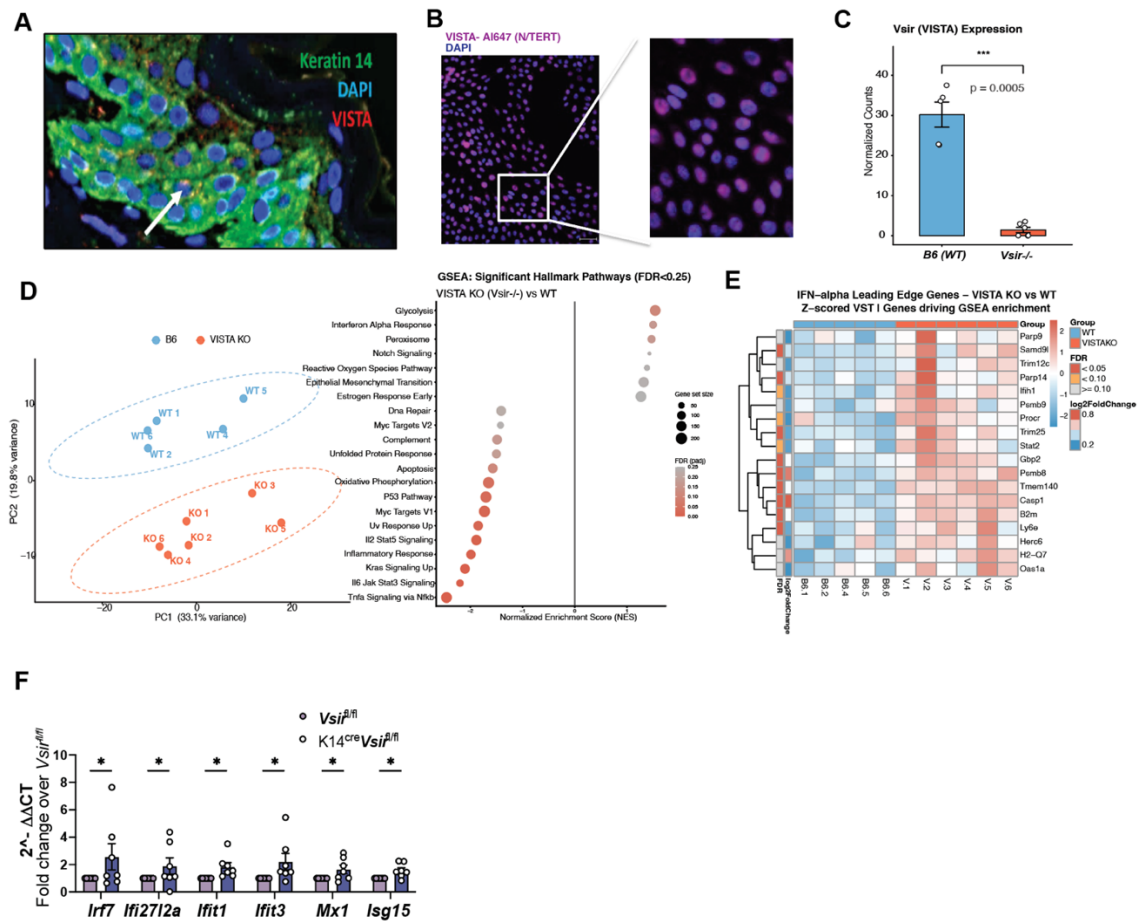


Supplemental Figure 1: IFN-κ and ISG levels in the skin of VISTA-deficient mice. (A) Representative IFN-κ immunoblot of B6 and *Vsir*^{-/-} mouse skin and ImageJ quantification relative to GAPDH compared by Mann-Whitney test (n=4; **p<0.01). **(B)** IFN-κ levels in B6 and *Vsir*^{-/-} mouse skin homogenates quantified by ELISA; significance was determined by independent t-test (n=3; *p<0.05). **(C)** Fold change in basal ISGs (*Irf7*, *Ifi2712a*, *Ifit3*, *Mx1*, *Isg15*, *Isg20*) normalized to 18s in *Vsir*^{-/-} and *Vsir*^{-/-}*Tmem173*^{-/-} mouse skin was compared using One-way ANOVA with post-hoc Tukey test, relative to B6 skin (n=7).

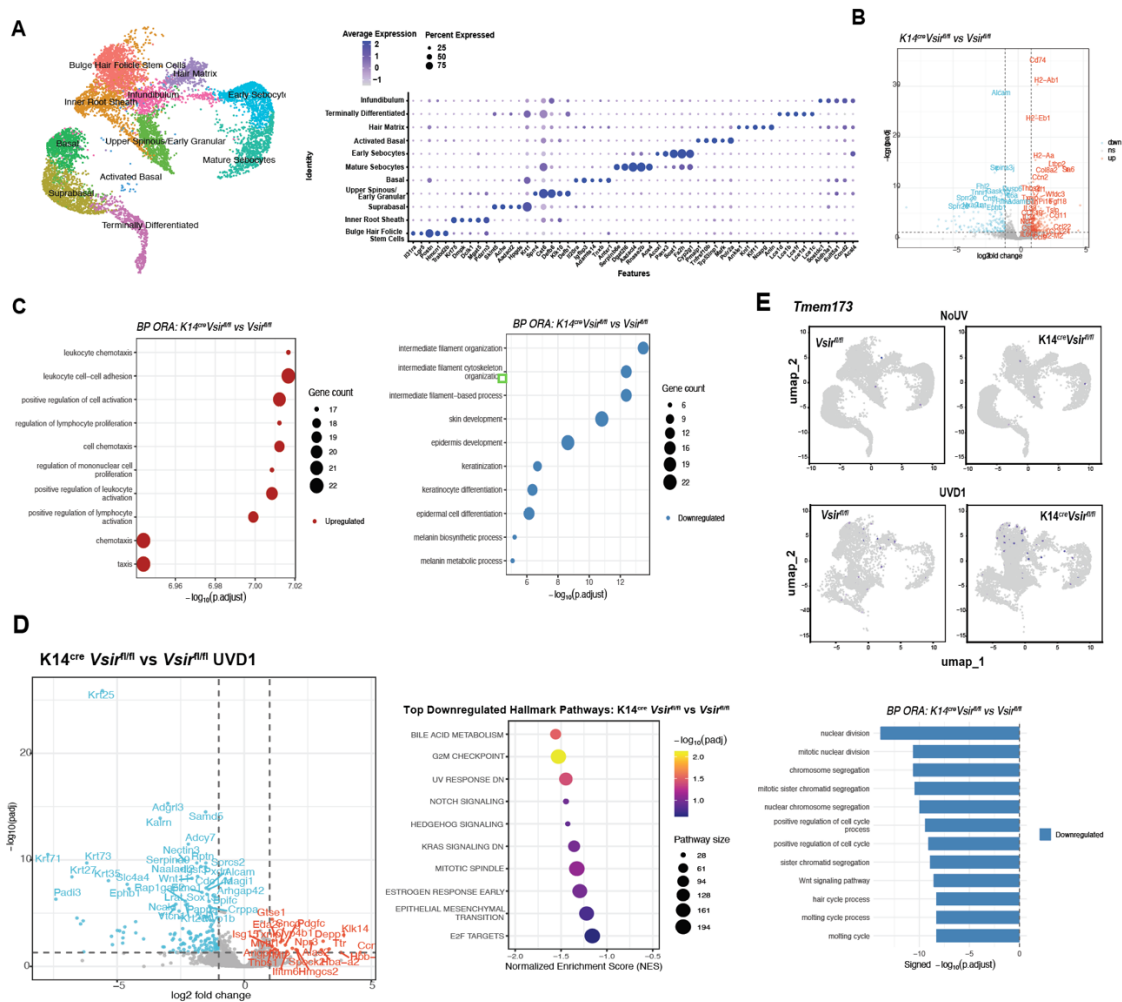


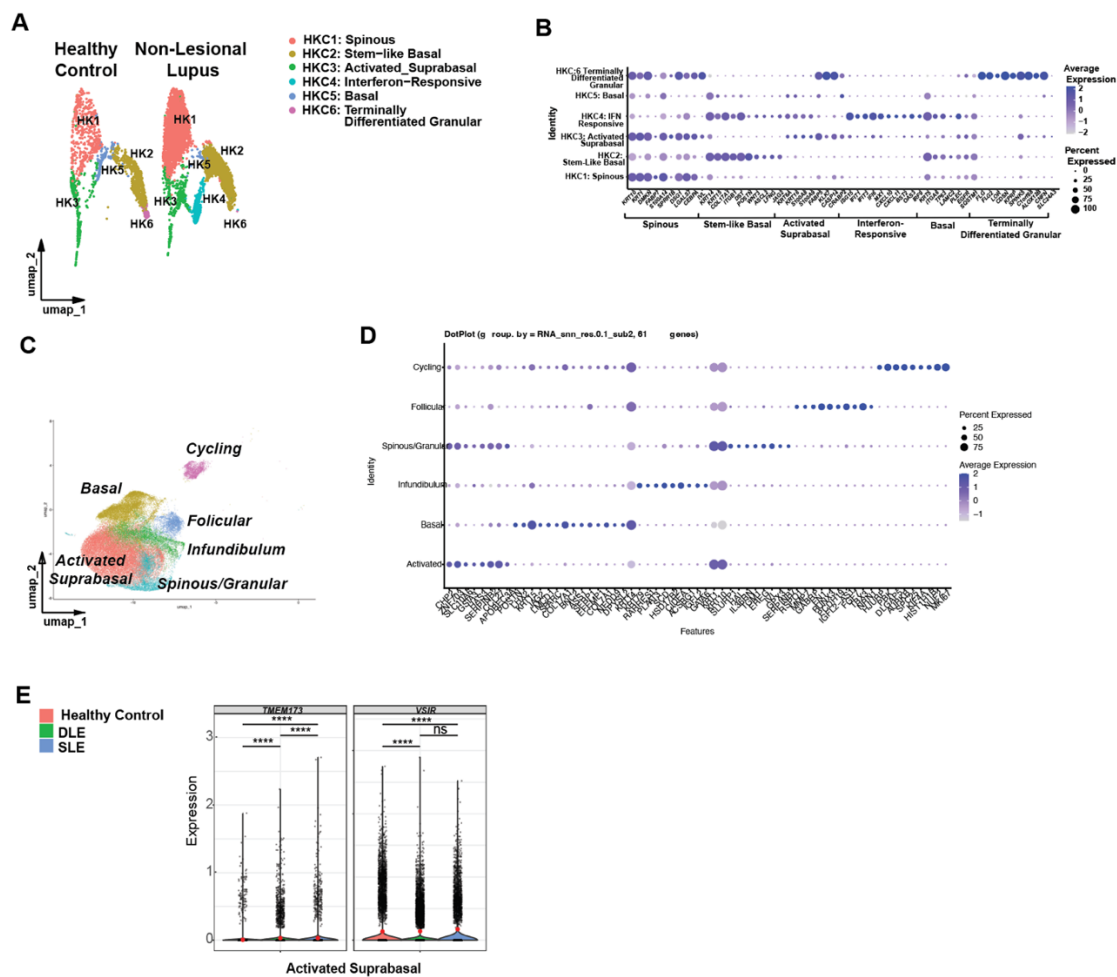
Supplemental Figure 2. UV-induced IFN-I response is elevated in *Vsig-/-* mouse skin and skin injury is not dependent on ZBPI, AIM2, or cGAS. (A) Differentially expressed genes in VISTA-deficient (*Vsig-/-*) mouse compared to wild-type B6 skin, 3 hours post UVB (FDR adjusted p value < 0.01 and -1 < Log₂FC > 1). (B) Comparative gene set variation analysis (GSEA) showing hedges G effect size of pathway enrichment in *Vsig-/-* skin before and 3 hours post-UVB compared to B6 skin by Welch's t-test (n=3; *p<0.05, **p<0.01). (C) Fold change in ISG expression in *Vsig-/-* vs. B6 mouse skin 3 hours post UVB exposure (500mJ/cm²) compared by multiple Mann-Whitney tests, controlled for false discovery by Benjamini, Krieger Yekutieli test (n=8, *p<0.05). (D) Fold change increase in Ifn-β and Ifn-κ gene expression in *Vsig-/-* compared to base B6 mouse skin by two-way ANOVA with

post-hoc Tukey test (n=3-9, *p<0.05, **p<0.01). **(E)** Fold change of ISG expression in B6 and *Vsir*^{-/-} skin at 24 hours after UV compared with baseline B6 by multiple Mann Whitney U tests (n=5-6, *p<0.05). **(F)** TUNEL staining of B6 and *Vsir*^{-/-} skin at baseline, 3 hours, and 1-day post-UV and quantification of TUNEL+ cells by ImageJ determined by two-way ANOVA with post-hoc Tukey test (n=3; *p<0.05, ns = not significant). **(G)** Representative gating strategy for identifying Ly6C^{hi} monocytes in mouse skin. **(H)** CLASI scores of B6, *Vsir*^{-/-}, *Vsir*^{-/-}*Zbp1*^{-/-}, *Vsir*^{-/-}*Aim2*^{-/-} and *Vsir*^{-/-}*cGAS*^{-/-} were compared by two-way ANOVA with post-hoc Tukey test ***p<0.001, ****p<0.0001, n=3-10). Data compiled from 2 independent experiments.

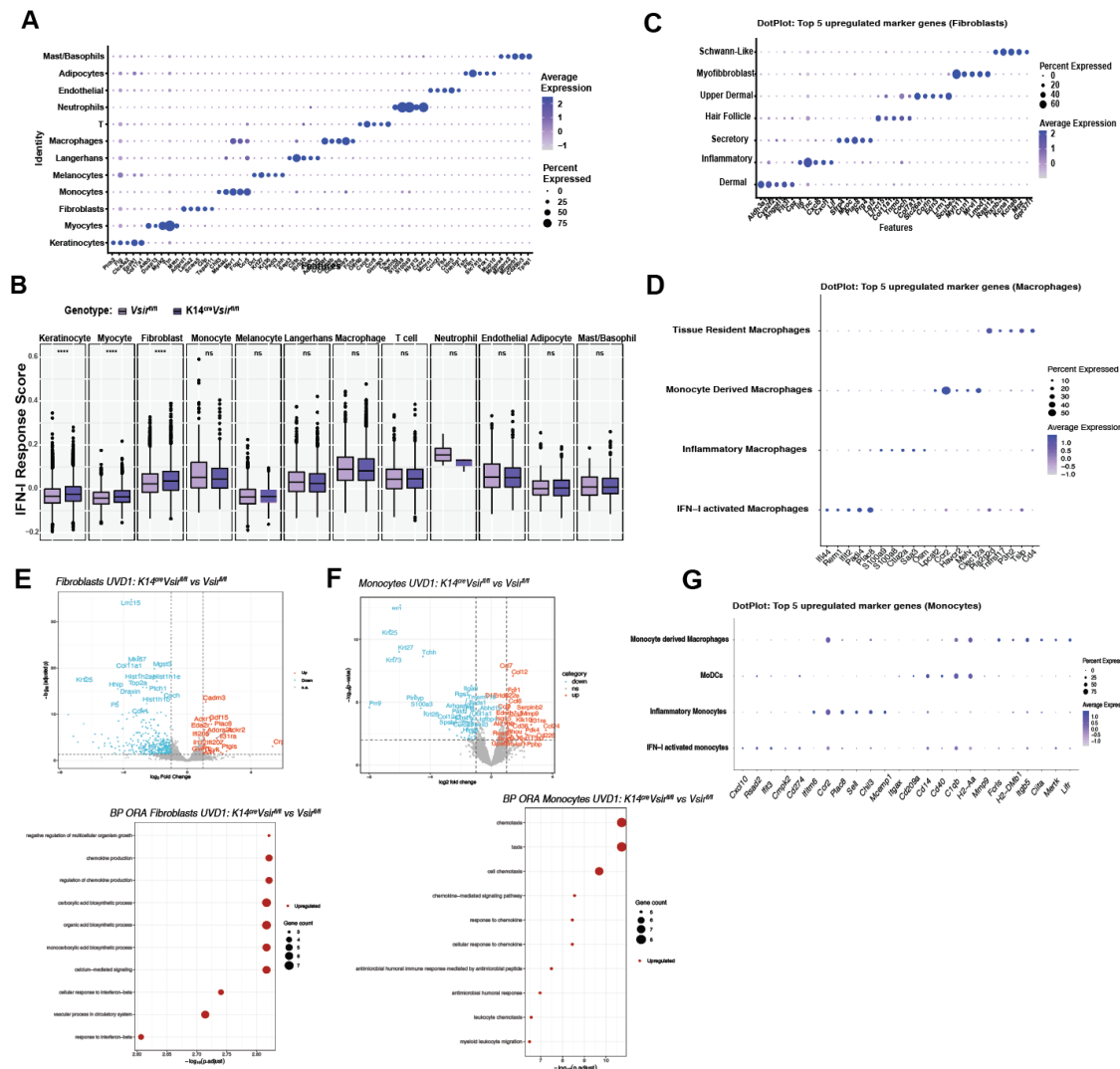


Supplemental Figure 3. Loss of keratinocyte VISTA increases basal IFN-I signaling and disrupts keratinocyte differentiation. (A) Mouse epidermis stained for VISTA (red), Keratin-14 (green), and DAPI (blue). (B) Immunofluorescence staining of VISTA (red) in human keratinocytes (NTERTs). (C) *Vsir* expression in epidermal B6 and *Vsir*^{-/-} mouse keratinocyte samples in the bulk RNAseq analysis. (D) PCA plot demonstrating distinct clustering of WT vs *Vsir*^{-/-} primary mouse keratinocytes and Top Hallmark pathways enriched and reduced in keratinocytes in the absence of VISTA (GSEA). (E) Heatmap depicting per-sample normalized gene expression in the Hallmark IFN-alpha Response pathway. (F) Fold change in ISGs in K14^{Cre} *Vsir*^{fl/fl} skin at baseline compared to *Vsir*^{fl/fl} skin by multiple Mann-Whitney tests, corrected with Benjamini, Krieger Yekutieli test (n=7, *p<0.05).

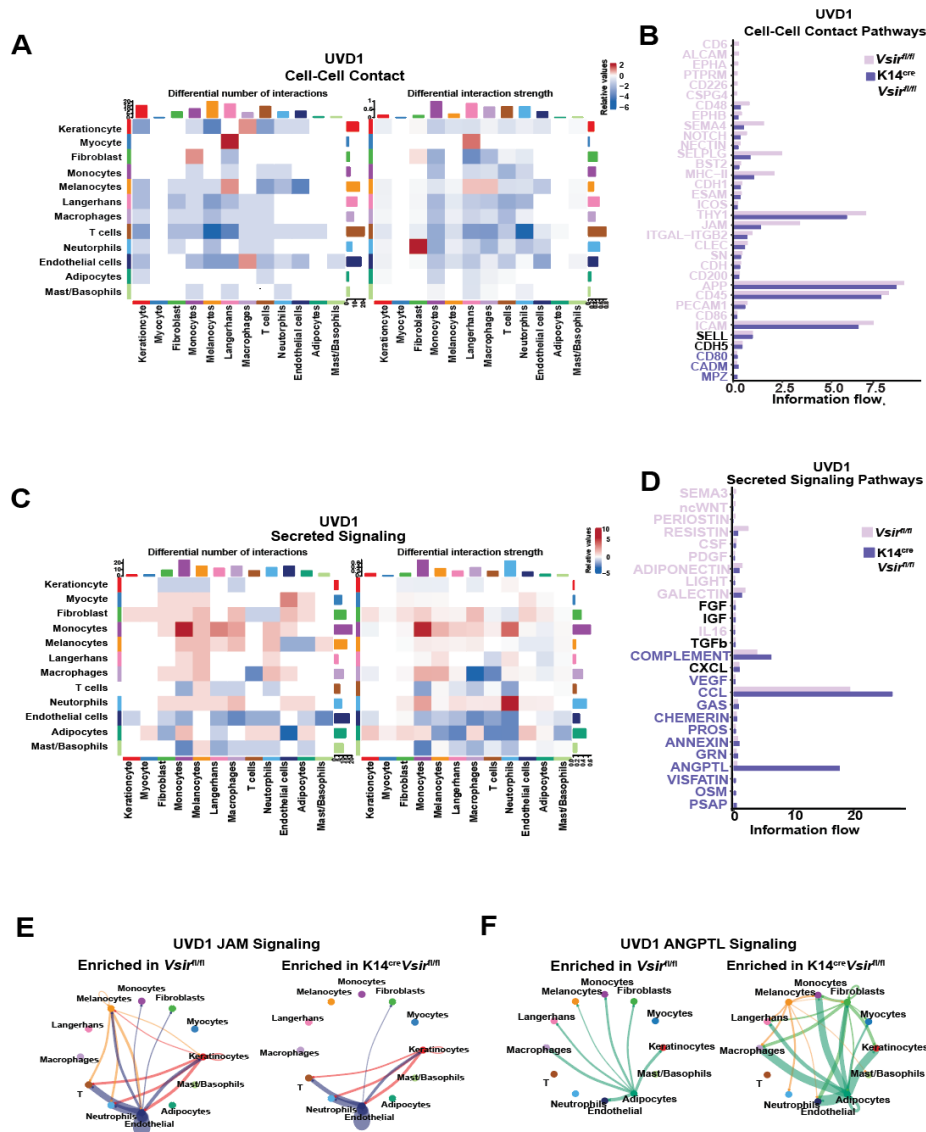




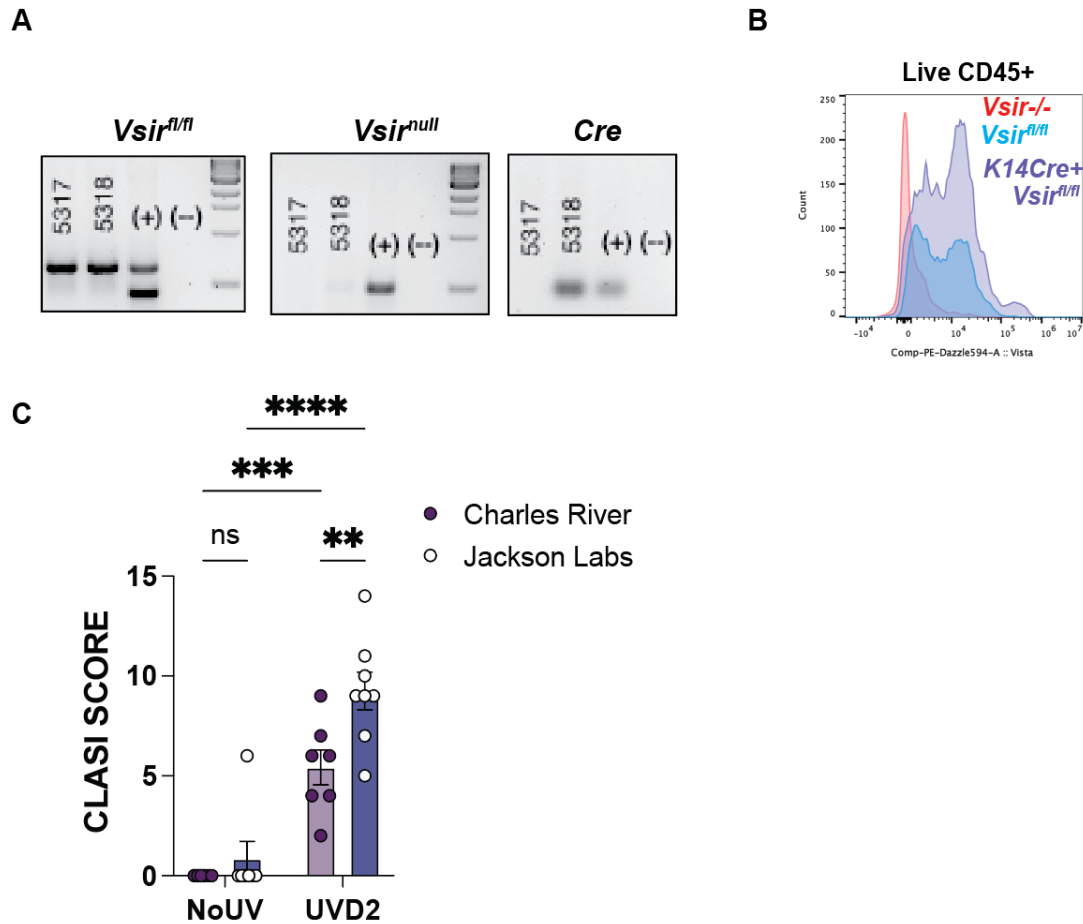
Supplemental Figure 5. Keratinocyte subcluster annotation and *VSIR*/*TMEM173* expression in human lupus skin datasets. (A) UMAP of keratinocyte subclusters from healthy control and non-lesional lupus skin (GSE186476), annotated into six subpopulations (HKC1–6). **(B)** Dot plot showing scaled average expression and percent expressed of subcluster marker genes across HKC1–6. **(C)** UMAP of keratinocyte subclusters from a second human dataset comprising healthy control, discoid lupus erythematosus (DLE), and systemic lupus erythematosus (SLE) skin, with stacked bar plots showing disease condition composition across keratinocyte subpopulations (GSE179633). **(D)** Dot plot of marker gene expression across keratinocyte subclusters in the DLE/SLE dataset. **(E)** Violin plots of *TMEM173* (STING) and *VSIR* (VISTA) expression within the suprabasal keratinocyte population across healthy, DLE, and SLE conditions. Statistical comparisons by Wilcoxon rank-sum test; **** $p < 0.0001$, ns = not significant.



Supplemental Figure 6. Gene signatures define skin immune and stromal cells in the absence of keratinocyte VISTA. (A) The top 5 expressed genes across immune cell clusters were selected based on gene set enrichment analysis. (B) IFN- γ response gene signature scores in different cell subsets from $K14^{Cre}Vs1^{fl/fl}$ and $Vs1^{fl/fl}$ skin at baseline (NoUV) were compared by multiple paired cluster-wise Wilcoxon tests corrected by Benjamini-Hochberg ($n=4$ mice per genotype; $**p<0.01$, $***p<0.001$, ns = not significant). (C-D) The top 5 expressed genes across clusters within (C) fibroblasts and (D) macrophages were selected based on gene set enrichment analysis. Dot size represents the percent of subcluster expressed in each cell and color demonstrates normalized expression of each gene. (E-F) Differentially expressed genes and top GO:Biological processes pathways (ORA) in $K14^{Cre}Vs1^{fl/fl}$ versus $Vs1^{fl/fl}$ in (E) fibroblasts and (F) monocytes one day after UV. (G) The top 5 expressed genes across clusters within monocytes.



Supplemental Figure 7. Keratinocyte VISTA deficiency alters cell-cell communication in the skin after UV exposure. Ligand receptor analysis (Cell-Chat) showing changes in the number and strength of (A-B) Cell-Cell contact and (C-D) secreted signaling interactions in *K14^{Cre}Vsiir^{fl/fl}* compared to *Vsiir^{fl/fl}* skin, 1 day after UV. Interaction strength is calculated as average expression of the sender (ligand) multiplied by receiver (receptor) genes. (B, D) Information flow of (B) cell-cell contact and (D) secreted signaling pathways in *K14^{Cre}Vsiir^{fl/fl}* skin 1 day after UV. Overall information flow is calculated as the sum of probabilities (edges) that denote an interaction between cell types and significant differences in pathway information flow are determined by permutation testing. Pathways upregulated in *K14^{Cre}Vsiir^{fl/fl}* are labeled in purple and pathways decreased in *K14^{Cre}Vsiir^{fl/fl}* are labeled in pink. (E-F) Circos plots showing (E) change in cell-cell contact signaling pathway JAM and (F) secreted signaling pathway ANGPTL in *K14^{Cre}Vsiir^{fl/fl}* vs *Vsiir^{fl/fl}* skin cells 1 day after UV.



Supplemental Figure 8. Genotyping and UV-induced CLASI scores from different C57BL6 background strains. (A) Representative DNA gels of genotyping *Vsir^{fl/fl}* mice for loxp sites, aberrant cre recombination and cre expression. (B) Mean fluorescence intensity of VISTA on CD45+ cells from *Vsir^{-/-}*, *Vsir^{fl/fl}*, and *KcreVsir^{fl/fl}* skin. (C) CLASI scores two days following one dose of UVB (D2, 500 mJ/cm²) were compared between WT B6 mice raised in our facility that were originally from Charles River or Jackson Laboratory using two-way ANOVA with a post hoc Tukey test (n=6-8, *p<0.05, ***p<0.001).