Supplemental Information

Ligand-independent vitamin D receptor actions essential for keratinocyte homeostasis in the skin

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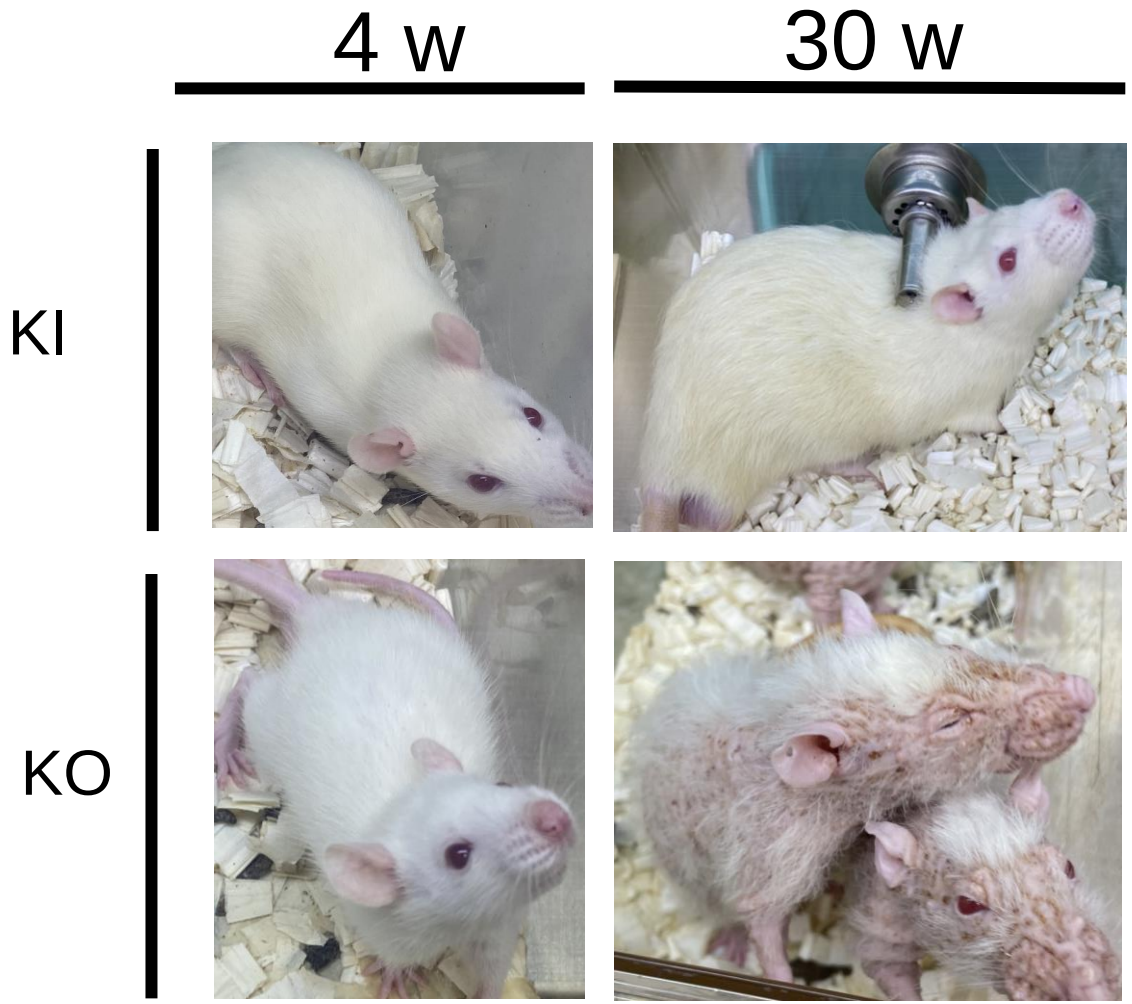
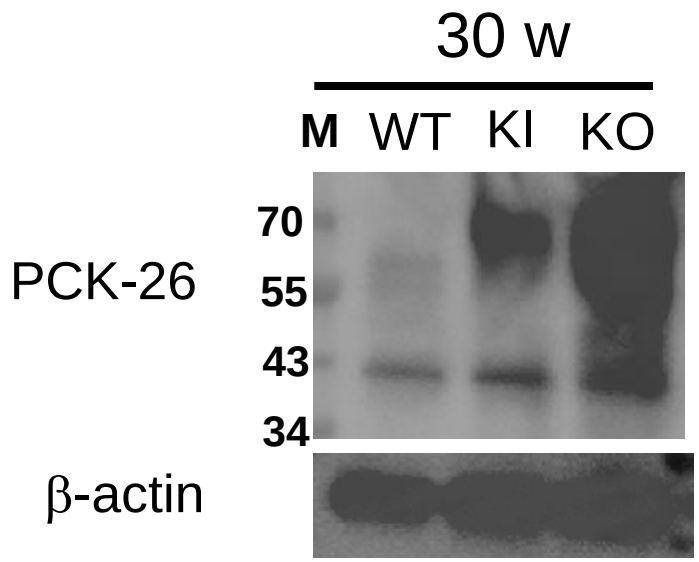


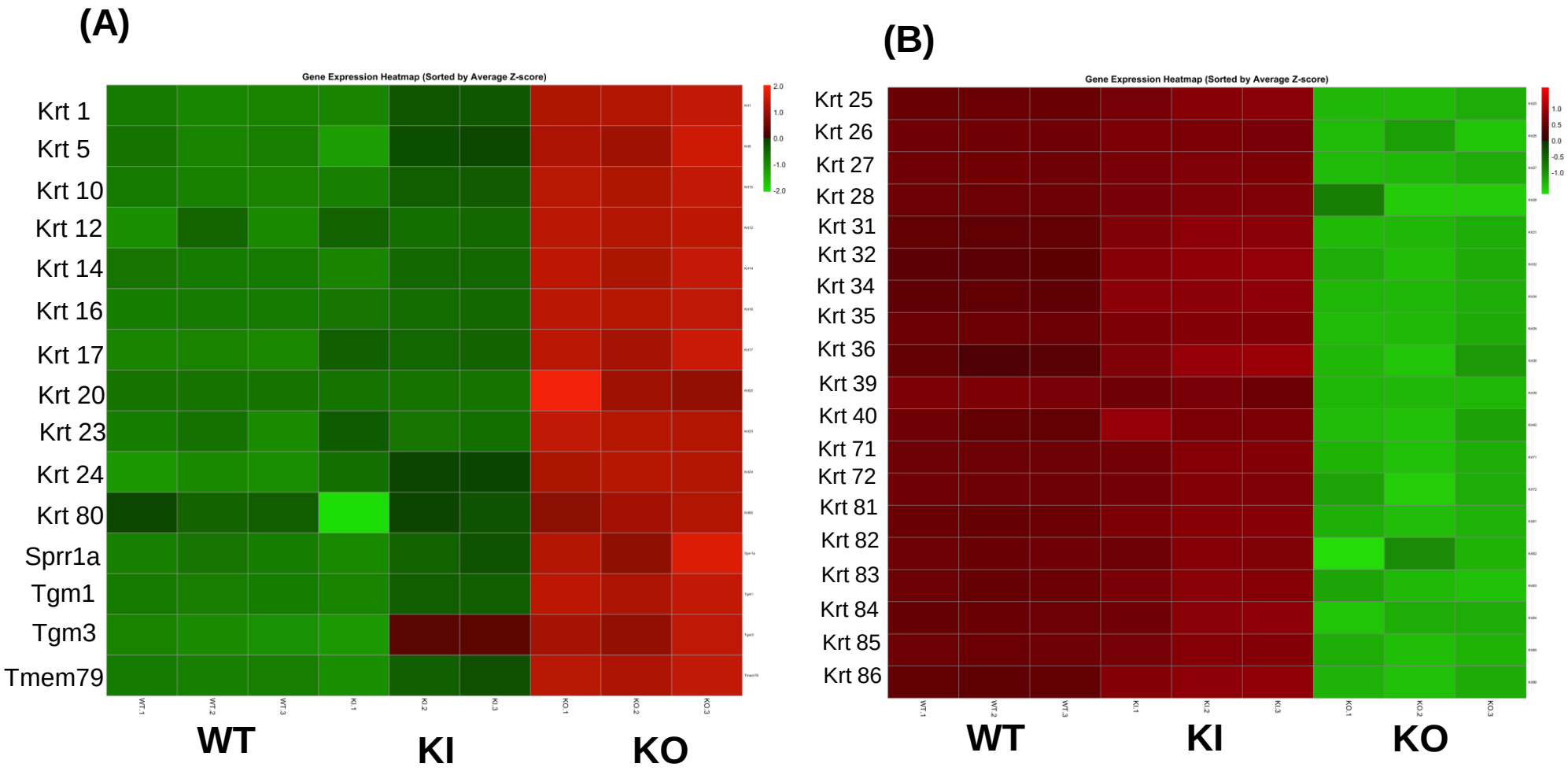
Supplemental Fig. 1 Comparison of the appearance of 4-week-old and 30-week-old KI and KO Rats.

At 4 weeks of age, both groups exhibit nearly identical appearances. However, by 30 weeks of age, KO rats show significant hair loss and skin abnormalities.

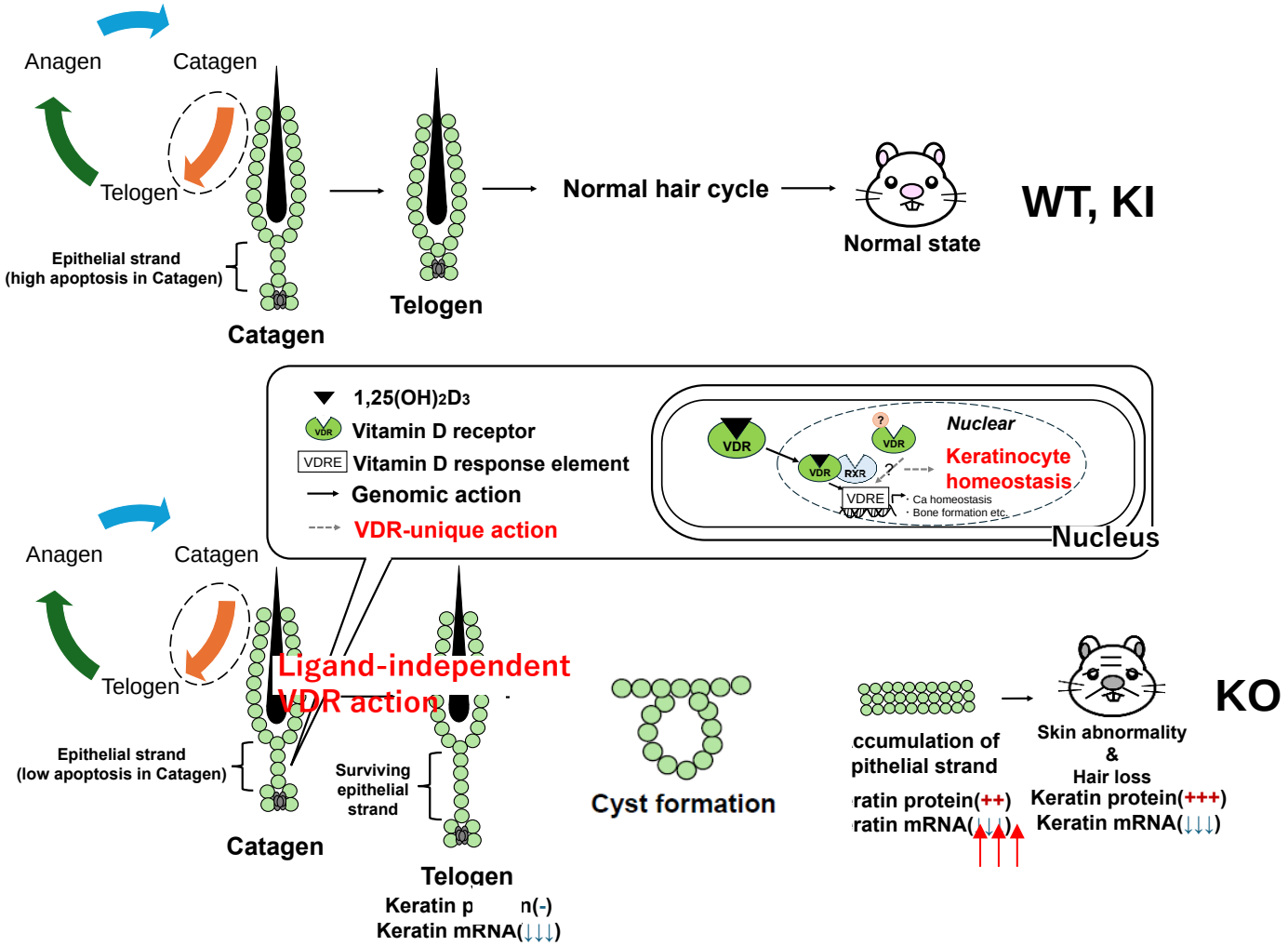


Supplemental Fig. 2 Western blot analysis using the PCK26 antibody, which reacts with Krt1, Krt5, and Krt8.

In 30-week-old rats, band intensity increases in the order of WT, KI, and KO, with KO rats exhibiting marked expression of skin keratins.



Supplemental Fig. 3 Heatmap of gene expression for hair keratin and keratinization-related gene groups (A) and epidermal keratin gene groups (B), color-coded by Z-score.



Supplemental Fig. 4 Abnormal differentiation and proliferation of keratinocytes in KO rats due to disruption in hair cycle maintenance.

In WT and KI rats, the normal hair cycle is maintained, but in KO rats, there is hair loss, cyst formation, abnormal proliferation of epidermal keratinocytes, accompanied by a significant increase in the expression of skin keratins and a marked decrease in hair keratin expression.

Suppl Table S1 Biological processes in which proteins are dramatically increased or decreased in VDR-KO rats compared with WT rats

Proteins that are increased in VDR-KO compared to WT.

Biological Process	false discovery rate
Keratinization	6.44E-06
RNA processing	1.13E-05
Peptide cross-linking	5.81E-05
Regulation of water loss via skin	5.81E-05
Water homeostasis	0.00011
mRNA processing	0.00013
Regulation of cell activation	0.00027
Establishment of skin barrier	0.00027
Defense response to other organism	0.00027
Chronic inflammatory response	0.00034
Regulation of body fluid levels	0.00039
Innate immune response	0.00053
mRNA metabolic process	0.00088
Multicellular organismal water homeostasis	0.00089
Regulation of leukocyte activation	0.0027
Blood coagulation	0.0031
Skeletal system development	0.0032
Hemopoiesis	0.0034
Granulocyte chemotaxis	0.0035
Neutrophil chemotaxis	0.0036

Proteins that are decreased in VDR-KO compared to WT.

Biological Process	false discovery rate
Generation of precursor metabolites and energy	5.70E-17
Energy derivation by oxidation of organic compounds	5.70E-17
Cellular respiration	1.57E-16
ATP metabolic process	4.99E-16
Oxidative phosphorylation	2.03E-15
Mitochondrion organization	3.59E-11
Muscle contraction	1.80E-10
Respiratory electron transport chain	1.80E-10
Muscle system process	5.85E-10
Translation	6.94E-10
Aerobic respiration	1.01E-09
Peptide biosynthetic process	1.75E-09
ATP synthesis coupled electron transport	9.38E-09
Striated muscle contraction	2.80E-08
Electron transport chain	9.20E-08
Mitochondrial transport	1.77E-07
Mitochondrial atp synthesis coupled electron transport	6.64E-07
Cardiac muscle contraction	1.04E-06
Amide biosynthetic process	1.27E-06
Mitochondrial transmembrane transport	2.48E-06

Back skin samples were collected from one female WT and one female KO rat, following the method described in the Materials and Methods section, and proteome analysis was performed according to the method of Hara et al. [37]. Proteins on keratinization is dramatically increased in KO rats consistent with RNAseq analysis and Western blot analysis (Fig. 6 and suppl. Fig. 2)

Suppl Table S2 Cellular components in which proteins are dramatically increased or decreased in VDR-KO rats compared with WT rats

Proteins that are increased in VDR-KO compared to WT.

Cellular component	false discovery rate
Keratin filament	0.0000316
Cornified envelope	0.0000332
Chromosome	0.0019
Lysosome	0.0024
Spliceosomal complex	0.0045
Site of polarized growth	0.0045
Growth cone	0.0052
Integral component of synaptic membrane	0.0061

Proteins that are decreased in VDR-KO compared to WT.

Cellular component	false discovery rate
Mitochondrial inner membrane	2.36E-26
Mitochondrial protein complex	4.17E-26
Organelle inner membrane	1.18E-23
Inner mitochondrial membrane protein complex	3.03E-20
Oxidoreductase complex	2.88E-15
Respirasome	3.11E-15
Mitochondrial matrix	3.52E-15
Mitochondrial respirasome	2.02E-14

Proteins on keratin filament and cornified envelop were dramatically increased in VDR-KO rats.
In contrast, mitochondrial proteins are dramatically decreased in KO rats.

Suppl Table S3　Proteins related with keratinization and cornified envelope formation abundantly expressed in VDR-KO rat compared with WT rat

Proteins related with keratinization	VDR-KO/WT (log2 value)	Fold
Sprr1a	12.92	7750
Invo	11.23	2402
Tgm3	9.01	541
Tgm1	8.61	391
Krt80	7.78	220
Tmem79	7.53	185
Krt24	7.41	170

Proteins on keratinization were dramatically increased in KO rats consistent with RNAseq analysis (Fig. 11).