Mice Lacking Epidermal Retinol Dehydrogenases SDR16C5 and SDR16C6 Display Accelerated Hair Growth and Enlarged Meibomian Glands.

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SUPPORTING INFORMATION

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Table S1. A list of primers used for generating expression constructs and for screening and genotyping of knockout mouse strains.

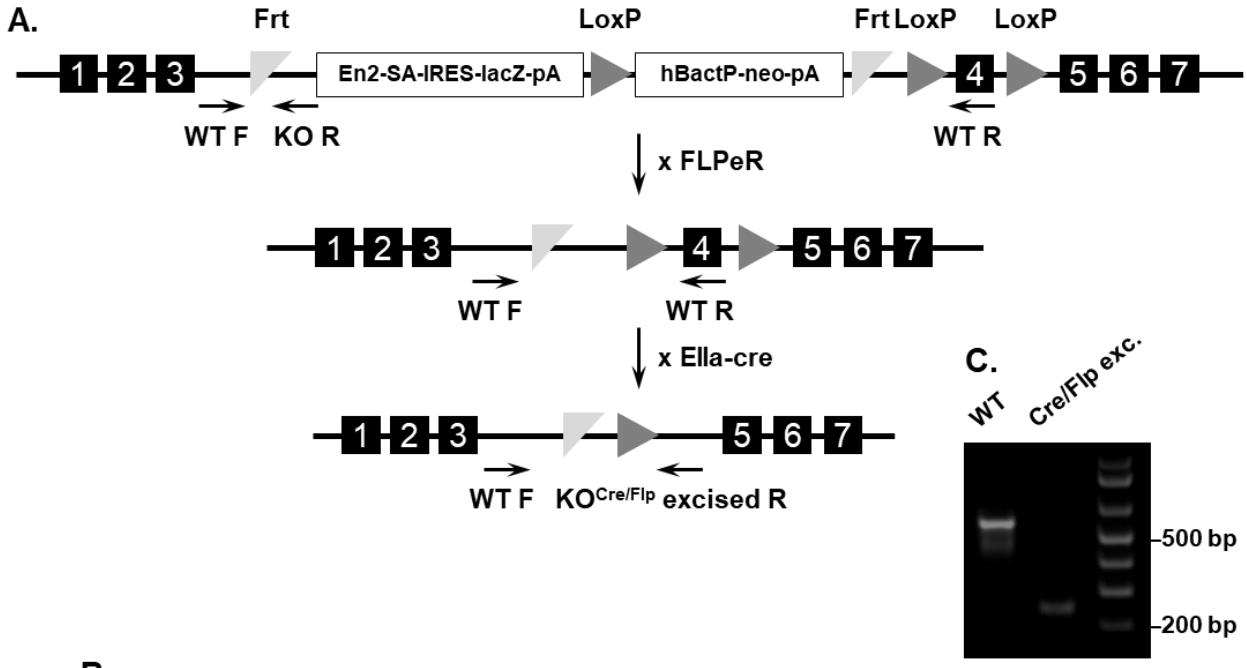
Primers for expression constructs		
Construct	Primer name	Primer sequence (restriction sites underlined)
mRDHE2/pBluescript II SK (-)	mE2_SalI_F	TTTGTCGACATGTCTCAAAACCTGGA
mRDHE2/pBluescript II SK (-)	mE2_XbaI_R	TTTTCTAGATTAAGTTTTCTTCTTTTG
mRDHE2-His/pVL1393	mE2_XbaI_F	AAATCTAGAATGTCTCAAAACCTGGA
mRDHE2-His/pVL1393	mE2_NotI_R	AATAATGCGGCCGCAGTTTTCTTCT
mRDHE2S-His/pVL1393	mE2S_BamHI_F	ATCGGATCCATGAATTCAGTGGCA
mRDHE2S-His/pVL1393	mE2S_NotI_R	ATCGCGGCCGCTTGCTCTTTCCTTT
mRDHE2-FLAG/pCMV-tag4A, mRDHE2-FLAG/pCS105	mE2_EcoRI_F	TTTGAATTCATGTCTCAAAACCTGGAA
mRDHE2-FLAG/pCMV-tag4A	mE2_XhoI_R	TTTCTCGAGAGTTTTCTTCTTTTGGCC
mRDHE2-FLAG/pCS105	FLAG_XbaI_R	TACTCTAGACTACTTATCGTCGTCATC
Primers for *Rdhe2s*$^{-/-}$ strain		
	Primer name	**Primer sequence**
Screening of ES cells by long-range PCR		
5’ arm	E2Sscreen1F	GGCATCTGGGATCTAACGTTTCTCCA
	E2Sscreen1R	AGCAGGCCACCCAACTGACCT
3’ arm	R2R	TCTATAGTCGCAGTAGGCGG
	Sdr16c6genoR2	GCTGGCAAGCTTCCTTTACAGCCA
Genotyping of *Rdhe2s*$^{-/-}$ “knockout first” strain		
WT allele	WT F	GGCATTTGTGGCATGGGAACTTATG
	WT R	GCAGGAAGGAAGGCCTTACAAGTC
“Knockout first” allele	WT F	GGCATTTGTGGCATGGGAACTTATG
	KO R (LAR3)	CACAACGGGTTCTTCTGTTAGTCC
Genotyping of *Rdhe2s*$^{-/-}$ strain		
WT allele	WT F	GGCATTTGTGGCATGGGAACTTATG
	WT R	GCAGGAAGGAAGGCCTTACAAGTC
KO (Flp, Cre-excised) allele	WT F	GGCATTTGTGGCATGGGAACTTATG
	KO$^{\text{FLP/CRE excised}}$R	ACTGATGGCGAGCTCAGACC
Primers for CRISPR-generated *Rdhe2*$^{-/-}$*;Rdhe2s*$^{-/-}$ founders		
	Primer name	**Primer sequence**
Screening of targeted exons:		
Exon 2, *Sdr16c6*	Sdr16c6ex2F	CAACATGAATTCAGTGGCAGACACA
	Sdr16c6ex2R	TGATCAGCAACTCTGTAGACCTC
Exon 5, *Sdr16c6*	Sdr16c6ex5F	ATACTCTGTCCTCAAGGATAAACC
	Sdr16c6ex5R	GAGGAAGAGCTCACTTACTTGGT
Exon 2, *Sdr16c5*	Sdr16c5ex2F	CCAGAACATGTCTCAAAACCTGG

	Sdr16c5ex2R	GGTCAGCCACTCTGTACACTT
Exon 5, *Sdr16c5*	Sdr16c5ex5F	CAGACTATTGTGCAAGTAAATTCGC
	Sdr16c5ex5R	TGGGCAGAGAGTAAATTTGAATGCC
Screening for large deletions:		
Pair 1	Sdr16c6ex2F	CAACATGAATTCAGTGGCAGACACA
	Sdr16c6ex5R	GAGGAAGAGCTCACTTACTTGGT
Pair 2	Sdr16c6ex2F	CAACATGAATTCAGTGGCAGACACA
	Sdr16c5ex2R	GGTCAGCCACTCTGTACACTT
Pair 3	Sdr16c6ex2F	CAACATGAATTCAGTGGCAGACACA
	Sdr16c5ex5R	TGGGCAGAGAGTAAATTTGAATGCC
Pair 4	Sdr16c6ex5F	ATACTCTGTCCTCAAGGATAAACC
	Sdr16c5ex5R	TGGGCAGAGAGTAAATTTGAATGCC
Pair 5	Sdr16c5ex2F	CCAGAACATGTCTCAAAACCTGG
	Sdr16c5ex5R	TGGGCAGAGAGTAAATTTGAATGCC
Pair 6	Sdr16c5ex4F	GGTCTTCTTAGATGTATAAAGCCT
	Sdr16c5ex6R	CATGGAAACTGGTGCTGTTACCT
Additional exons:		
Exon 5, *Sdr16c5*	Sdr16c5ex3F	CCAGGTTAAGAAAGAAGTTGGTG
	Sdr16c5ex3R	CACCCATAAATGTGCTTTGAAATTGAC
Exon 4, *Sdr16c5*	Sdr16c5ex4F	GGTCTTCTTAGATGTATAAAGCCT
	Sdr16c5ex4R	ACACTGTGGGAGGCAGACC
Exon 6, *Sdr16c5*	Sdr16c5ex6F	TAGGTGTCCTACTCTGTTACCA
	Sdr16c5ex6R	CATGGAAACTGGTGCTGTTACCT
Exon 3, *Sdr16c6*	Sdr16c6ex3F	CTGACAATGCTCCCAGCAAG
	Sdr16c6ex3R	AATGCAGAGAATTCTGATGCTCAC
Exons 4 and 5, *Sdr16c6*	Sdr16c6ex4-5F	GCTGTATACTTCCTACTTTCCTGC
	Sdr16c6ex4-5R	TAAGAGTAGAGGAAGAGCTCACT
Exon 6, *Sdr16c6*	Sdr16c6ex6F	GAACCTCTGTGTTGTCATTCCAG
	Sdr16c6ex6R	TCTTGAGAGGCCCACATGATG
Exon 7, *Sdr16c6*	Sdr16c6ex7F	GGTGGTATGCTTCCATTCAGC
	Sdr16c6ex7R	GGCAGGATTATGAAAGGCCAG
Genotyping of DKO1 and DKO2 strains		
WT allele	Sdr16c5ex5F	CAGACTATTGTGCAAGTAAATTCGC
	Sdr16c5ex5R	TGGGCAGAGAGTAAATTTGAATGCC
DKO1 allele	Sdr16c6ex5F	ATACTCTGTCCTCAAGGATAAACC
	Sdr16c5ex5R	TGGGCAGAGAGTAAATTTGAATGCC
DKO2 allele	Sdr16c5intr4F4	CTTGAGATAATCAACTTGAAAGGAG
	Sdr16c5intr5R2	GAATGGGTCTGAATGGCATTACG

Table S2. CRISPR target sequences.

	Target sequence
***Sdr16c6*, Exon 2**	
CRISPR1	AACTAAAGTGGCCCCGTGGC *TGG*
CRISPR2	CAATATGGAAACCTGTAGAC *TGG*
***Sdr16c6*, Exon 5**	
CRISPR1	TCATTATCATAGTTAATTCA *AGG*
CRISPR2	TGCCCCTATTTCATCAAAAC *TGG*
***Sdr16c5*, Exon 2**	
CRISPR1	TCACCGGCAACGTTCTTCCG *TGG*
CRISPR2	ATAGTGCTCATAACAGGTGC *TGG*
***Sdr16c5*, Exon 5**	
CRISPR1	CATAGATTCTGCAAATCCAA *GGG*
CRISPR2	CACTTGCCAAGAAACAATGG *GGG*

Nucleotides in italics represent the protospacer adjacent motif (PAM), the sequence required for CRISPR/Cas9.



B.

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WT RDHE2S     AAT GCC ATT TCC CAC TTC TGG ACT TGT AAG GCC TTC CTT CCT GCC ATG GTT
               N   A   I   S   H   F   W   T   C   K   A   F   L   P   A   M   V   -161
Cre/Flp exc.  AAT GCC ATT TCT CAC TTC TGG ATT ATT CTT CAT GGA ATT TGC AGC CTT TGG
               N   A   I   S   H   F   W   I   I   L   H   G   I   C   S   L   W

WT RDHE2S     AAA GCT AAC CAT GGT CAC CTG GTC TGT ATT TCA AGC ATA GCA GGA CTC GTT
               K   A   N   H   G   H   L   V   C   I   S   S   I   A   G   L   V   -178
Cre/Flp exc.  CTT TAC CGA GTC TCT CTT CCT TGG ATT AAC TAT AAT AAT GAA AAC TAA AGT
               L   Y   R   V   S   L   P   W   I   N   Y   N   N   E   N   *   S

WT RDHE2S     GGT ATT AAT GGA CTG TCA GAT TAT TCT TCA AGC AAA TTT GCA GCC TTT GGC
               G   I   N   G   L   S   D   Y   S   S   S   K   F   A   A   F   G   -195
Cre/Flp exc.  TAA AAG TAC CAT CGA TTG CCC CTA TTT CAT CAA AAC TGG AAT GTT TGA AGG
               *   K   Y   H   R   L   P   L   F   H   Q   N   W   N   V   *   R
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Figure S1. Generation of *Rdhe2s*$^{-/-}$ mice. A. Diagram of targeting construct for production of *Rdhe2s*$^{-/-}$ mice. Mice carrying the "knockout first" *Rdhe2s* mutant allele (***Sdr16c6***$^{\text{tm1a(KOMP)Wtsi}}$) were mated with FLPeR mice carrying a gene encoding FLP recombinase. The progeny was mated with EIIa-cre mice, which carry a gene encoding Cre recombinase. **B.** Sequences of wild type and null alleles of *Rdhe2s* beginning at codon for residue N145. The mutant sequence is underlined starting at residue 152. Catalytic residues are shown in white on black background. The null allele contains a frameshift mutation and aberrant stop codon at L177 (indicated by *), resulting in a truncated protein that lacks catalytic residues. **C.** Gel electrophoresis of PCR products confirming the Cre and Frt excision. Wild type allele was amplified using primers WT F and WT R; null allele was amplified using primers WT F and KO$^{\text{FLP/CRE excised}}$R.

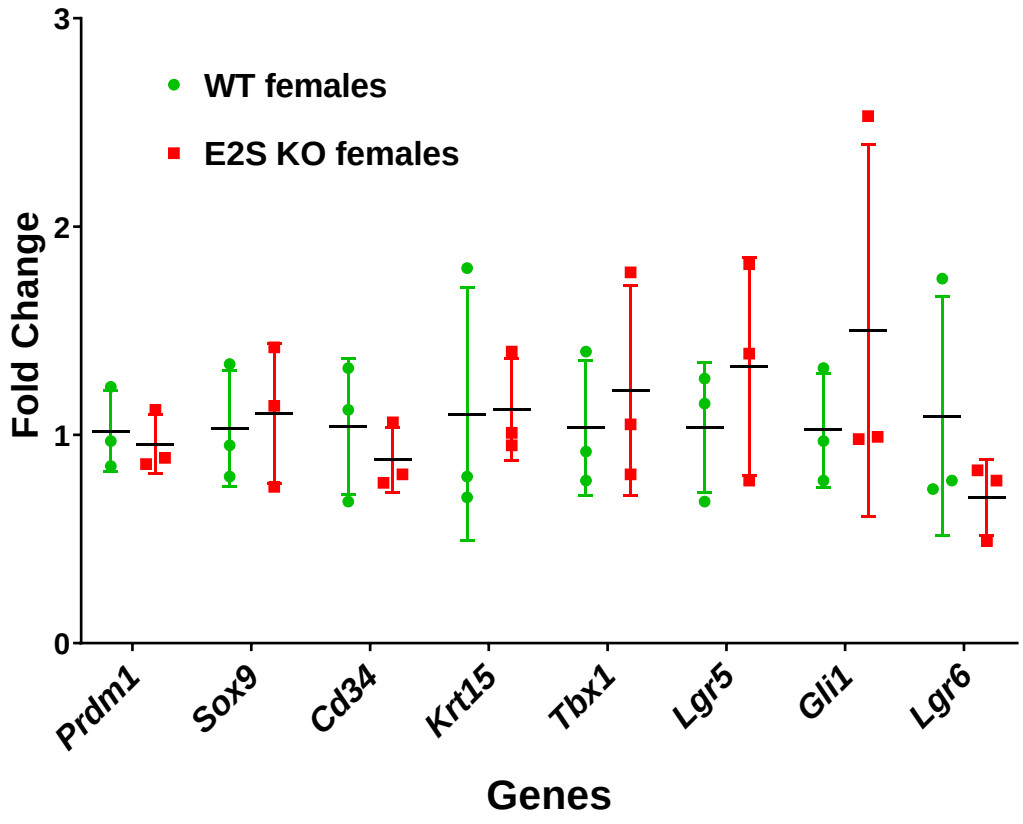

Figure S2. QPCR analysis of genes expressed in hair follicles and sebaceous glands. QPCR was performed using skin from *Rdhe2s*$^{-/-}$ (E2S KO) female mice (n=3) and their littermates (n=3) (~10 weeks old) fed chow diet as described under *Experimental Procedures*. The differences in gene expression were not statistically significant. Expression levels were normalized per *Hprt*.

Figure S3. Protein sequences encoded by CRISPR-Cas9 – generated alleles

RDHE2S:

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                        10        20        30        40        50        60        70        80
WT   RDHE2S  MNSVADTAIFFGKFLYYFLESLVFKVIPKRKKDVSGEIVLITGAGSGLGRLLAIHFASHGATLVLWDINQEGNMETCRLV
DKO1 RDHE2S  MNSVADTAIFFGKFLYYFLESLVFKVIPKRKKDVSGEIVLITGAGSGLGRLLAIHFASHGATLVLWDINQEGNMETCRLV
DKO2 RDHE2S  MNSVADTAIFFGKFLYYFLESLVFKVIPKRKKDVSGEIVLITGAGSGLGRLLAIHFASHGATLVLWDINQEGNMETCQTK
                       90        100       110       120       130       140       150       160
WT   RDHE2S  KQKGDVKVFAYKCDCSSRIEVYRVADQVKEEVGDVTILINNAGVVTGKSFLNTPDHLVEKSFLVNAISHFWTCKAFLPAM
DKO1 RDHE2S  KQKGDVKVFAYKCDCSSRIEVYRVADQVKEEVGDVTILINNAGVVTGKSFLNTPDHLVEKSFLVNAISHFWTCKAFLPAM
DKO2 RDHE2S  R*
                      170       180       190       200       210       220       230       240
WT   RDHE2S  VKANHGHLVCISSIAGLVGINGLSDYSSSKFAAFGFAESLFLELTMIMKTKVKSTIVCPYFIKTGMFEGCTTKYPLLLPL
DKO1 RDHE2S  VKANHGHLVCISSIAGLVGINGLSDYSSSKFAAFGFAESLFLDLVLR*
DKO2 RDHE2S
                       250       260       270       280       290       300       310
WT   RDHE2S  LEQEYVAQKIFNAILEEQVYLIIPKFAYVALFLKQIISPKMMIALGEYLGVDTCMTSFTGRVKAEELQMETKRKEQ*
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RDHE2:

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                        10        20        30        40        50        60        70        80
WT   RDHE2   MSQNLESVKNLLVFLGKSLLSVLEALLFHVISKPRKNVAGEIVLITGAGSGLGRLLALQFARLGAVLVLWDVNKEANDET
DKO2 RDHE2   MSQNLESVKNLLVFLGKSLLSVLEALLFHVISKPRKNVAGEIVLITGAGSGLGRLLALQFARLGAVLVLWDVNKEANDET
                        90       100       110       120       130       140       150       160
WT   RDHE2   HQLAREAGAARVHAYTCDCSRREEVYRVADQVKKEVGDVSILINNAGIVTGRNFLDCPDDLMEKSFDVNFKAHLWMYKAF
DKO2 RDHE2   HQLAREAGAARVHAYTCDCSRREEVYRVADQVKKEVGDVSILINNAGIVTGRNFLDCPDDLMEKSFDVNFKAHLWMYKAF
                       170       180       190       200       210       220       230       240
WT   RDHE2   LPAMIANNHGHLVCISSSAGLIGVNGLSDYCASKFAALGFAESMFIETLAKKQWGIKTTIVCPFFIKTGMFEGCTTKCPT
DKO2 RDHE2   LPAMIANNHGHLVCISSSAGLIGVNGLSGVLLCYQFWIQNMQLGKS*
                       250       260       270       280       290       300       310
WT   RDHE2   LLPILDPEYAVRKIIDAILQEQLYLYMPKFLYFIVFLKSILPIKTGILIADYLGVFHMTEGFTGQKKKT*
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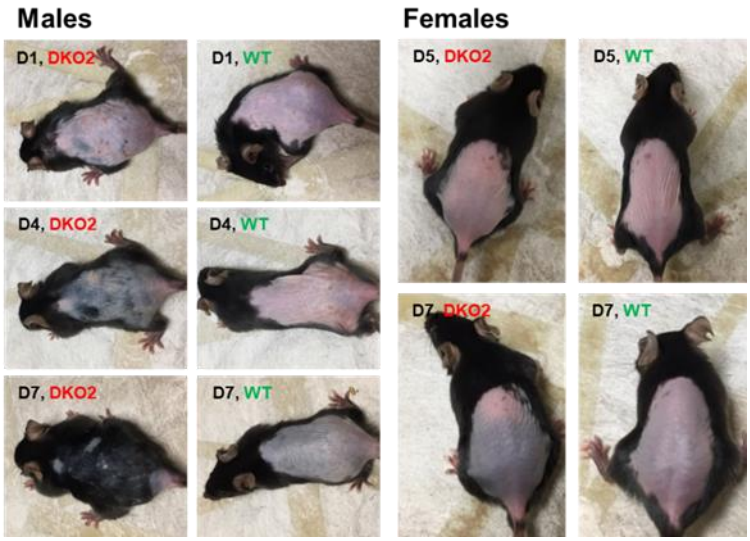

Figure S4. Hair regrowth in DKO and WT mice on vitamin A deficient diet. DKO2 mice of both sexes and their WT littermates of the same sex were shaved at ~10 weeks of age. Male mice were photographed on Days 1, 4 and 7 after shaving. Female mice were photographed on Days 5 and 7 after shaving.

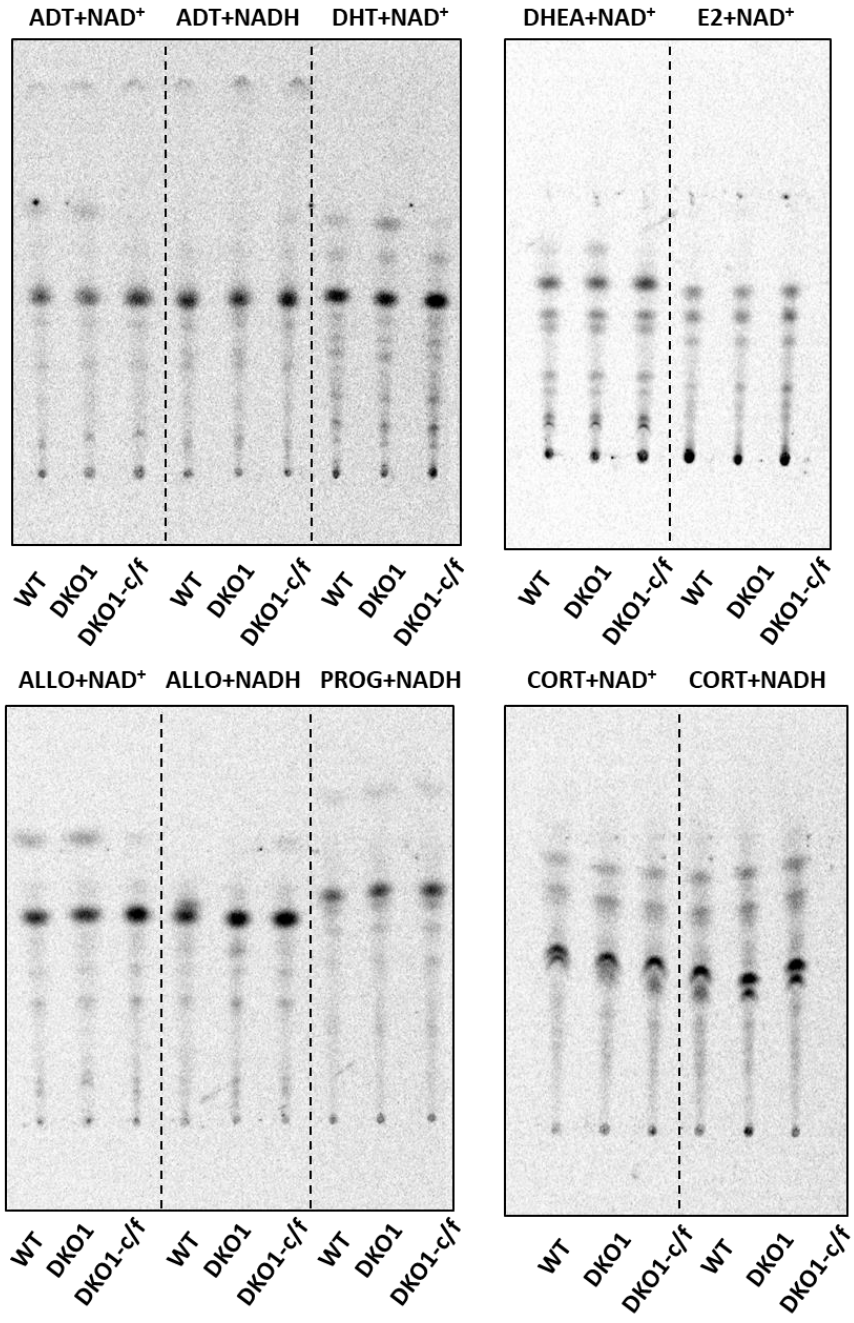

Figure S5. Thin-layer chromatography analysis of reaction products derived from incubation of skin microsomes with steroid substrates. Skin microsomal fractions of WT and DKO animals (90 µg) were incubated with 3 µM [^{3}H]-labeled steroids solubilized with bovine serum albumin in the presence or absence of 1 mM NAD(H) cofactor as described in [90]. Incubation was performed in 300-µl reaction volume for 75 min at 37 °C and steroids were extracted, separated by thin layer chromatography and visualized as described before [90]. Abbreviations are as follows: ADT, androsterone; DHT, dihydrotestosterone; DHEA, dehydroepiandrosterone; E2, estradiol; PROG, progesterone; CORT, corticosterone.