

Supplementary Materials for  
**ZFP750 affects the cutaneous barrier through regulating lipid metabolism**

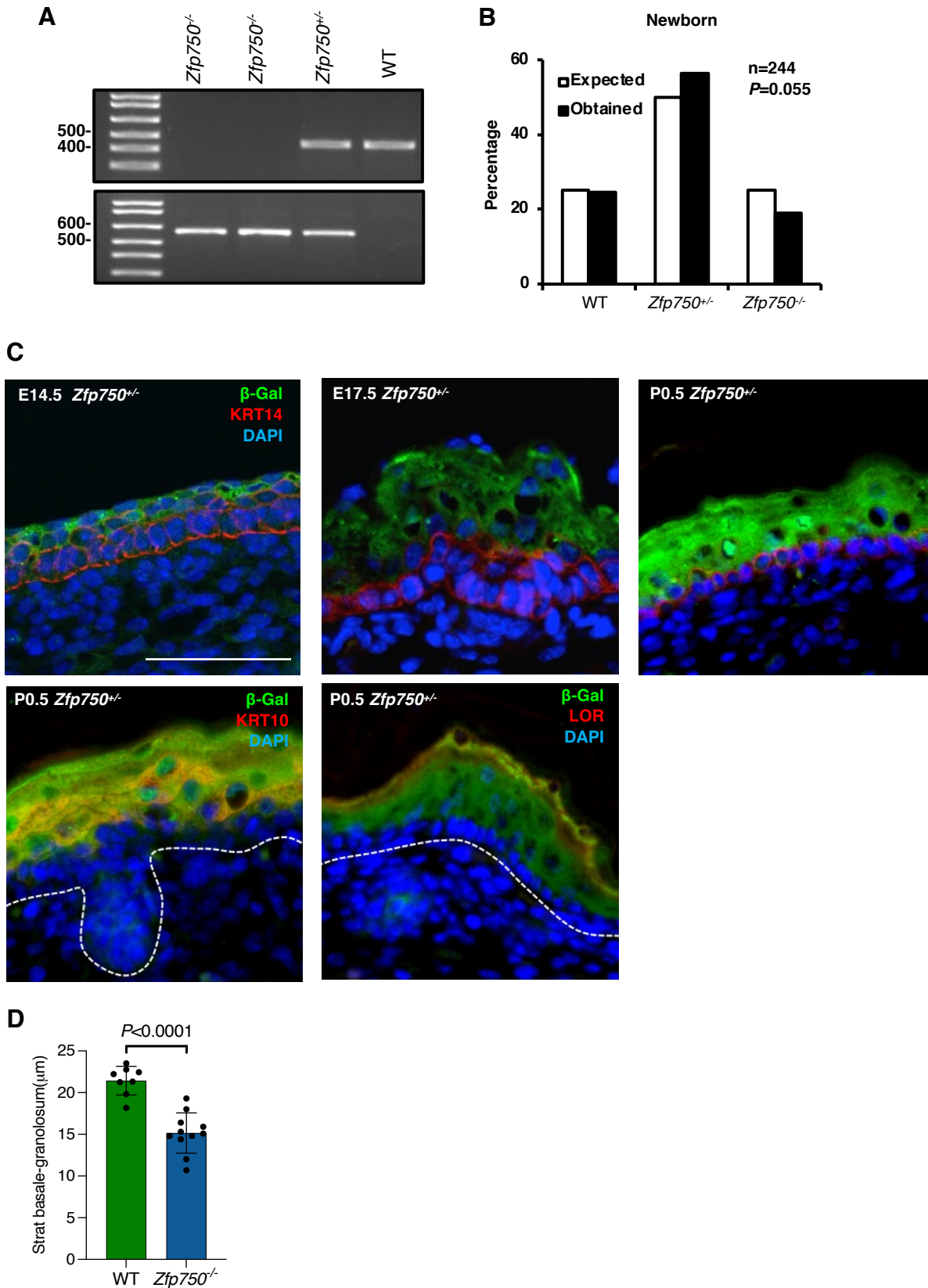
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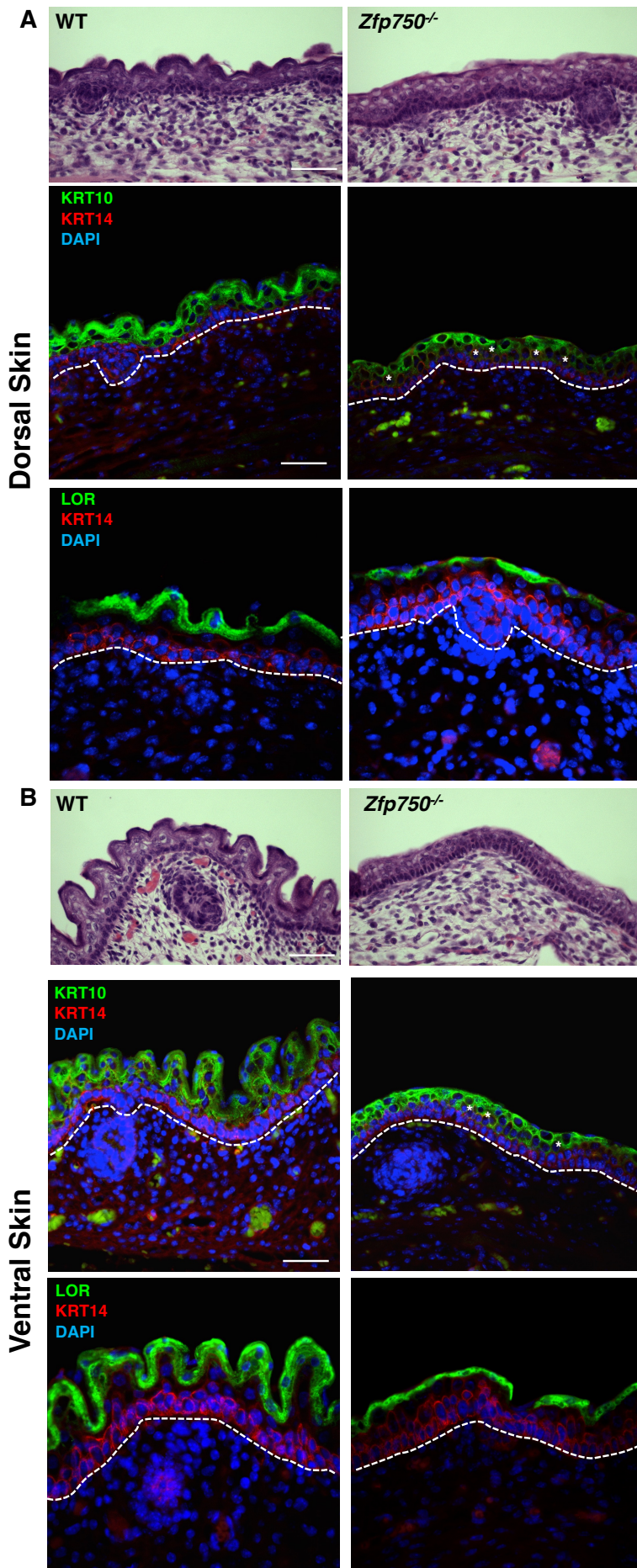
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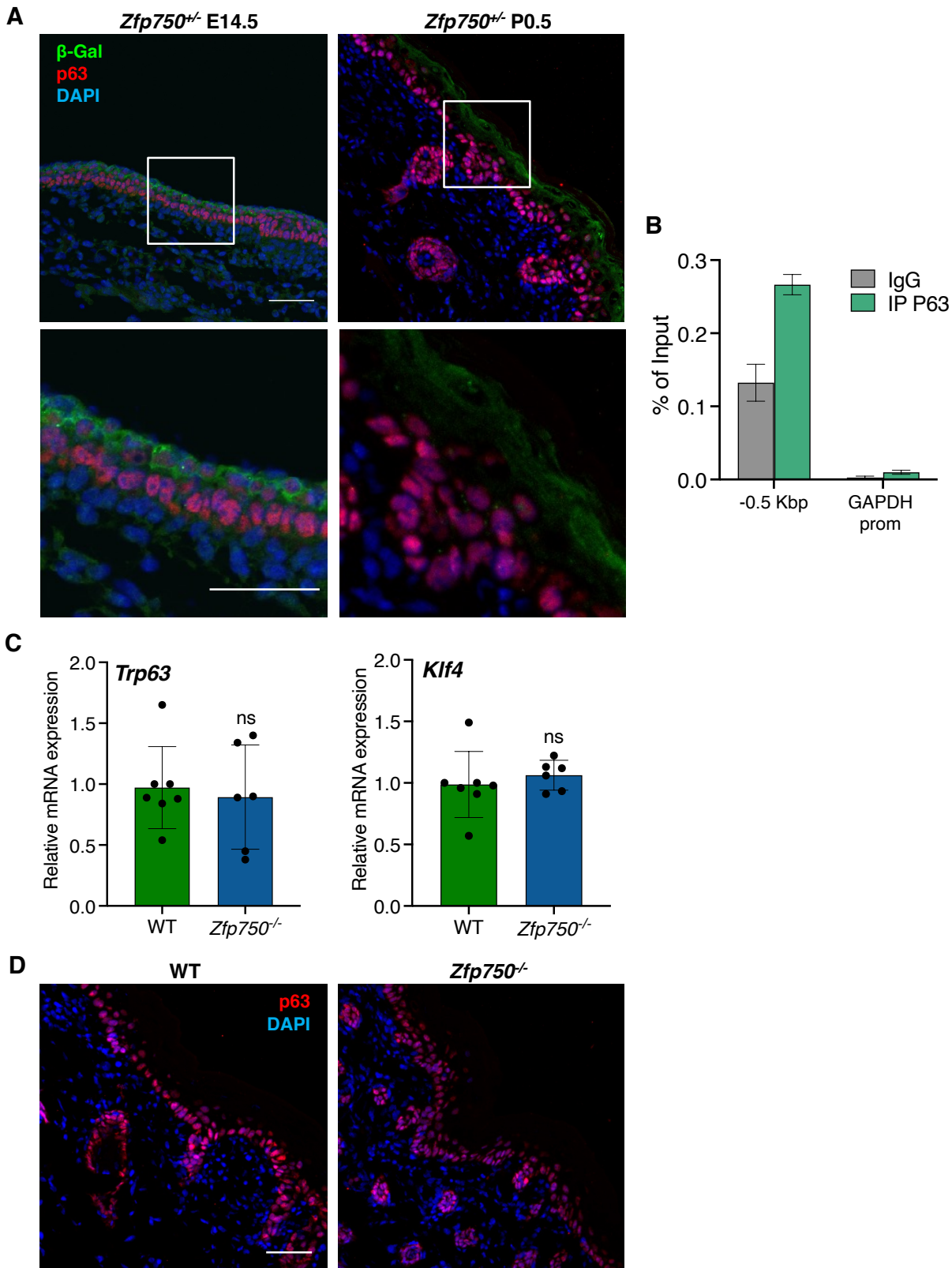


**Fig S1: Generation of ZFP750 constitutive knockout (KO) mouse. (A)** PCR genotyping of ear snip DNA isolated from Wild-Type (WT), ZFP750<sup>+/-</sup> and ZFP750<sup>-/-</sup> mice. WT bands= 437 bp and KO bands= 594 bp. **(B)** The observed distribution of frequencies follows the expected Mendelian ratio. Differences are not statistically significant (n=244

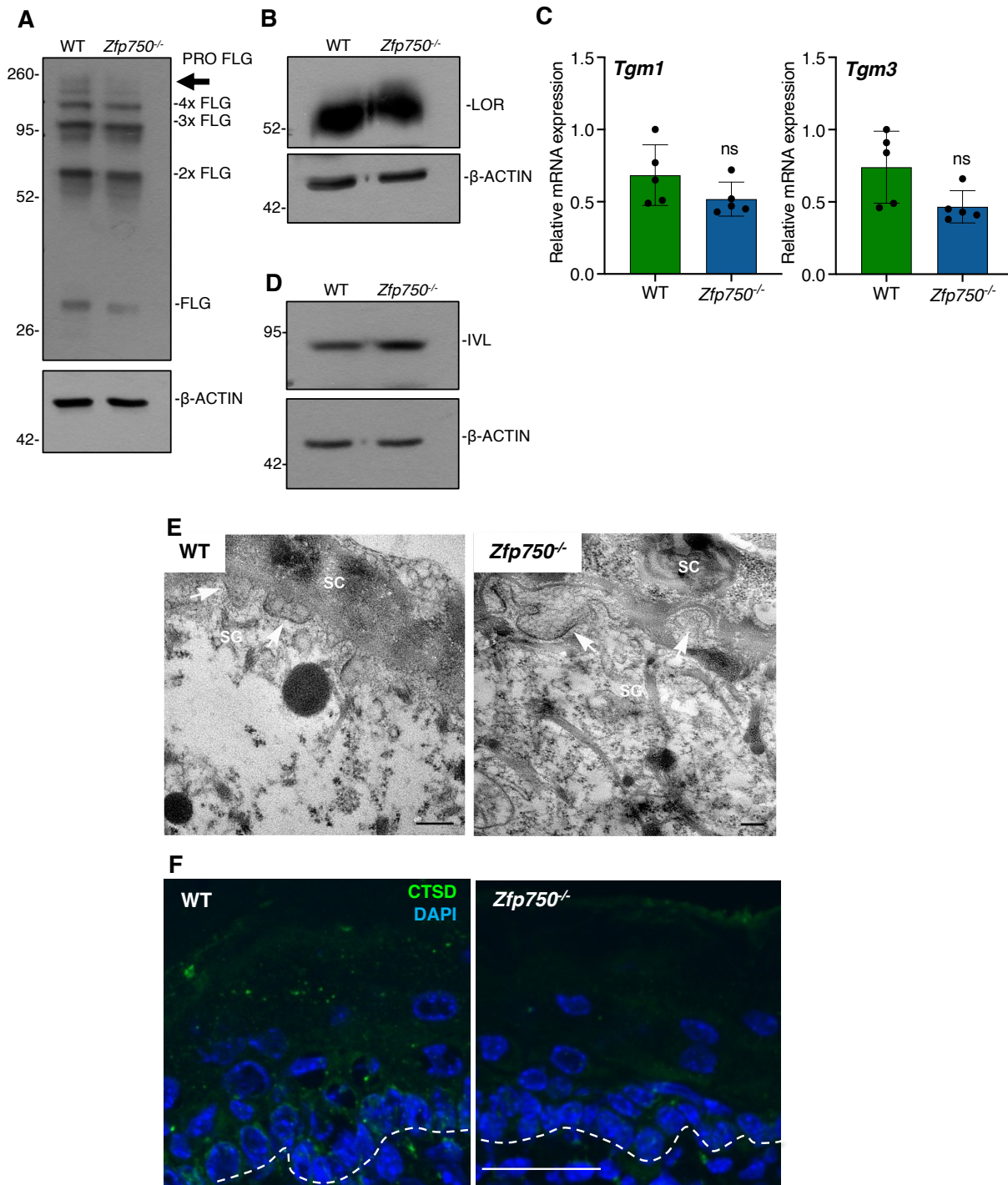
mice;  $P=0.055$ ). (C) ZFP750 expression in the epidermis of the indicated mice during development (Bar: 50  $\mu\text{m}$ ). (D) Epidermal thickness



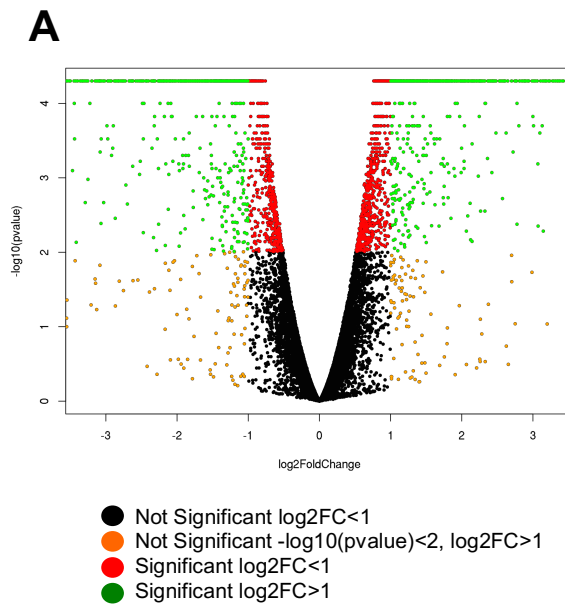
**Fig S2: Genetic deletion of ZFP750 alters embryonic epidermal differentiation.** (A and B) Both dorsal and ventral skin was isolated from E17.5 WT and ZFP750<sup>-/-</sup> mice and stained with the indicated differentiation markers. Asterisks indicate keratinocytes expressing both Krt10 and Krt14 markers. (Bar: 50μm).



**Fig S3: The p63/ZNF750/KLF4 axis is partially conserved in mouse.** (A) Co-expression analysis of p63 and ZFP750 ( $\beta$ -Gal) during epidermal development. A representative micrograph is shown ( $n=3$ ), (Bar:  $50\mu\text{m}$ ). (B) ChIP assay showing that endogenous murine p63 binds the promoter of ZFP750. The *Gapdh* promoter is used a negative control region. A representative experiment is shown. Bars represent the mean of three technical replicates ( $n=3$ , PCR runs)  $\pm$  SD. (C and D) mRNA levels of *Trp63* and *Klf4* in the epidermis isolated from WT and ZFP750<sup>-/-</sup> mice evaluated by qPCR. Data are normalized to  $\beta$ -Actin and relative to WT. Bars represent the mean  $\pm$  SD ( $n=6$  mice/genotype). A representative micrograph is shown ( $n=4$ ), (Bar:  $50\mu\text{m}$ )

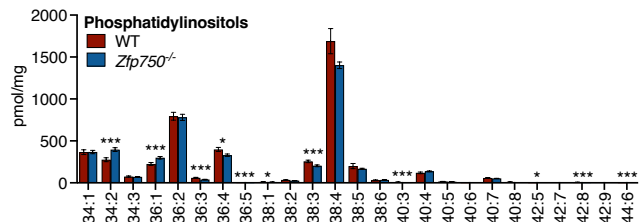
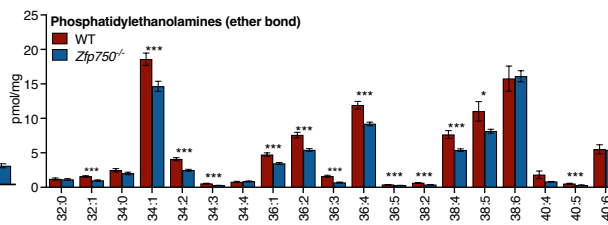
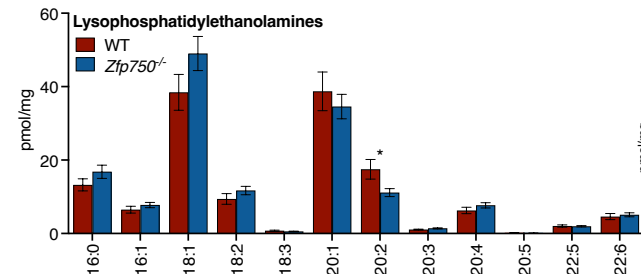
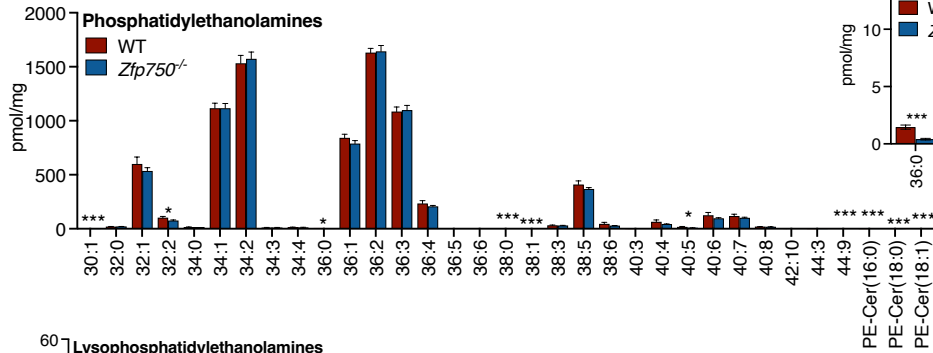
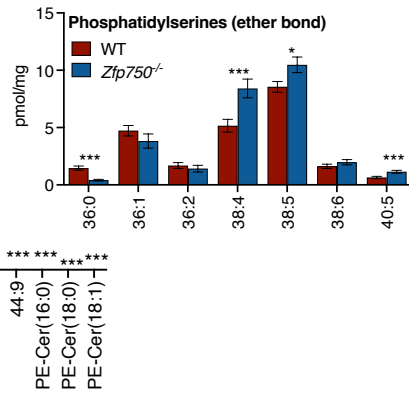
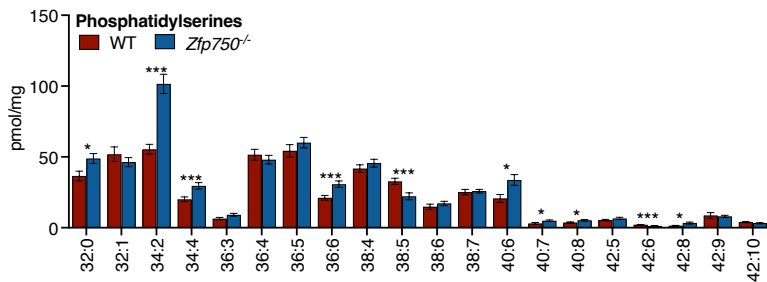
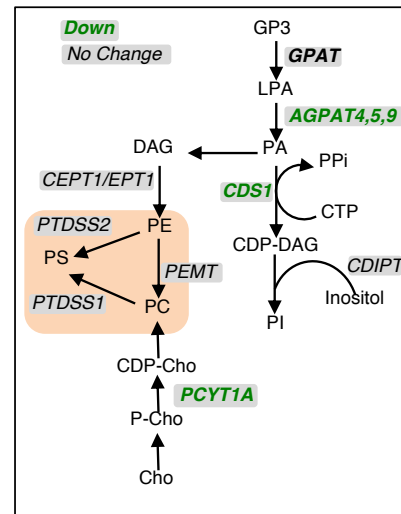
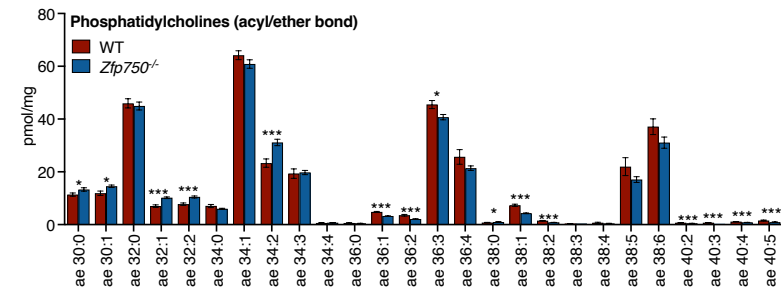
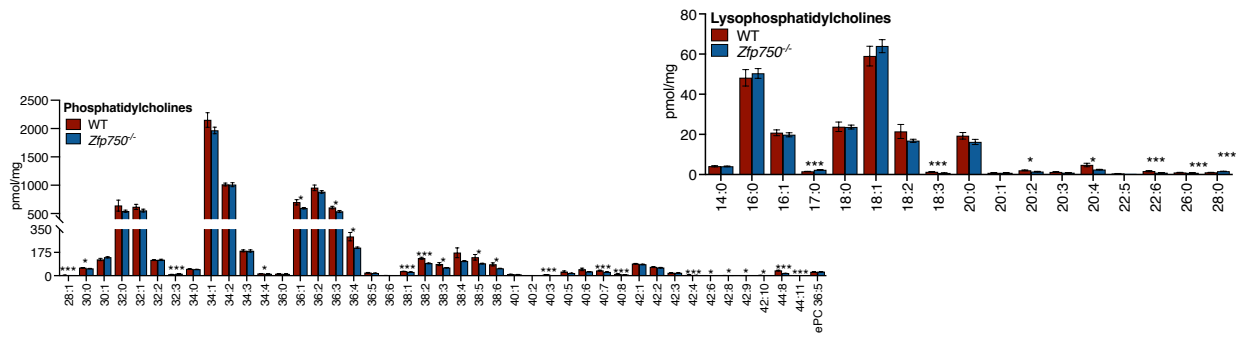


**Fig S4: *Zfp750* deletion alters the epidermal differentiation program and lipid composition.** Immunoblot analysis of Filaggrin (FLG) (A), Loricrin (B). Epidermis from *Zfp750*<sup>-/-</sup> mice shows low levels of FLG monomer when compared to WT (C) mRNA levels of Transglutaminase (*Tgm*) 1 and 3 in the epidermis isolated from WT and *ZFP750*<sup>-/-</sup> mice evaluated by qPCR. Data are normalized to β-Actin and relative to WT. Bars represent the mean ± SD (n=5 mice/genotype). ns, not significant. (D) Immunoblot analysis of Involucrin (IVL). β-Actin is used as loading control. A representative immunoblot is shown (n=2). (E) Representative transmission electron micrograph of WT and *Zfp750*<sup>-/-</sup> skin (F) Immunofluorescence analysis of Cathepsin D (CTSD), a marker of lamellar bodies, reveals the presence of spots highly positive for CTSD in WT mice when compared to *ZFP750*<sup>-/-</sup> mice. Nuclei stained with DAPI.



**Fig S5: Transcriptional deregulation after *Zfp750* deletion.** (A) Volcano plot of the RNA sequencing analysis. Significant,  $P < 0.05$ . not significant,  $P > 0.05$ . FC, Fold change.





**Fig S6: *Zfp750* genetic deletion results in the impairment of epidermal phospholipids composition.** Mass spectrometry analysis of the indicated phospholipid species extracted from the epidermis isolated from newborn WT and *ZFP750*<sup>-/-</sup> mice. Bars represent the mean  $\pm$  SE (n=13 mice/genotype). GP3 Glyceraldehyde 3-phosphate; LPA Lysophosphatidic acid; PA Phosphatidic acid; CDP, Cytidine diphosphate; CTP Cytidine triphosphate; Cho Choline; P-Cho Phosphocholine PI Phosphatidylinositol; PE, Phosphatidylethanolamines; PS, Phosphatidylserines; PC Phosphatidylcholine; PPi, pyrophosphate; CEPT1/EPT1, choline/ ethanolaminephosphotransferase 1; PEMT, Phosphatidylethanolamine N-methyltransferase; PTDSS, Phosphatidylserine Synthase 1; CDIPT, CDP-diacylglycerol-inositol 3-phosphatidyltransferase; CDS CDP-Diacylglycerol Synthases; AGPAT, 1-acylglycerol-3-phosphate-O-acyltransferase; PCYT1A choline-phosphate cytidyltransferase.

**Table S1. Primers for genotyping**

<b>Gene</b>	<b>Forward Primer</b>	<b>Reverse Primer</b>
WT allele	GGAAGGGAAGGGCAAATCTA	TGTTTGGGGCTTCTGATAGG
Targeted allele	GGCGACTTCCAGTTCAACAT	AAGTTTAACGCCAGCCTGAG

**Table S2. Primers for Real-Time PCR (mouse)**

<b>Gene</b>	<b>Forward Primer</b>	<b>Reverse Primer</b>
<i>Zfp750</i>	GCAAAGGAGGATGCCAAAGAGAAC	GCTGAATGTCTAACCCCACTGTCCG
<i>Trp63</i> (DN isoform)	CCTGGAAGCAGAAAAGAGGAGAGC	TGTGCGTGGTCTGTGTTGTAGG
<i>Krt14</i>	AGCGGCAAGAGTGAGATTTCT	CCTCCAGTTATTCTCCAGGG
<i>Involucrin</i> ( <i>Ivl</i> )	TCTCCCTCCTGTGAGTTTGTGG	CAGTGAAGACCTGGCATTGTGTAGG
<i>Filaggrin</i> ( <i>Flg</i> )	GCAAGTGGTCAGGGAGGATAT	GGAACGATATACCTGGAGATGC
<i>Loricrin</i> ( <i>Lor</i> )	GGTTGCAACGGAGACAACA	CATGAGAAAGTTAAGCCCATCG
<i>Klf4</i>	GTGCCCCGACTAACCGTT	GTGGTTGAACTCCTCGGTCT
<i>Actb</i>	CCTTGTCCTGTATGCCTCTGGTC	GAACCGCTCGTTGCCAATAGT
<i>Smpd1</i>	CAAATTCAGTGCCATAGCGCC	TGACTGGCACACATCTAGTGG
<i>Smpd3</i>	TCTACCTCCTCGACCAGCAC	TGCTGCTCCAGTTTGTTCATC
<i>Degs1</i>	ATGGGCCTCTGAACTTGCTC	TGCGGGAGGTCATGCTAGTA
<i>Degs2</i>	CACCACGACTTCCCCAGTATC	ACACTTGCGCTTAACCCCTGG
<i>Sptlc1</i>	CTCAGGCACGGTACTTGGAC	CAGTGACCACAACCCTGATG
<i>Elov6</i>	CAGCAAAGCACCCGAACTA	AGGAGCACAGTGATGTGGTG
<i>Elov7</i>	CTATTCTCAGTCGCCAAGAGC	CAGCTCGATGAATTTGGAGAA
<i>Dgat2</i>	GCTGGTGCCCTACTCCAAG	CCAGCTTGGGGACAGTGA
<i>Sgpl1</i>	GGATGACTTGTTCCTCTTCA	TTTCCGTTCCCCCAGAAG
<i>Kdsr</i>	GGTGCATGTGATCTCCAGTA	CAATAGCAATGCACTTCCCAA
<i>Krt2</i>	GGAAATCAGCGAGTTGAACC	ATCTCCACATCCAGGGACAG
<i>Krt6a</i>	GAGCAGATCAAGACCCTCAACA	CAATGATGCTGTCCAGCTGTCTG
<i>Krt6b</i>	GATCAAGACCCTCAACAACAAGT	GCTGATGTAAGTCTCAACATAGG
<i>Spr1b</i>	CCACACTACCTGTCTCCATA	TGTCACAGGGTGTCTTGACT
<i>Spr2b</i>	ACCAGCCCATTACAGGGAGA	CTGCTGCTGGTGGTAAGACAT
<i>Spr2d</i>	CTGGTACTCAAGGCCGAGAC	CAGGGCACTTTGGTGGAG
<i>Tgm1</i>	ACCACCACAGTGCTCCGATG	CCACACGTGGAAGTTCCAAAC
<i>Tgm3</i>	GCCATTGCTATTGCCAGTCTG	CTCATAAAGACATCCGCTTGC
<i>Tgm5</i>	CAGCCCAGGAGCCAGAAG	GGCCTCGGCGGACAAC
<i>Tbp</i>	CACGCACAAGTGCCTTGATT	GGAACGATATACCTGGAGATGC

**Table S3. Primers for Real-Time PCR (human)**

<b>Gene</b>	<b>Forward Primer</b>	<b>Reverse Primer</b>
ZNF750	AGCTCGCCTGAGTGTGAC	TGCAGACTCTGGCCTGTA
DEGS1	GGGAAGACTTCGAGTGGGTC	CCAAGTGGGTGAGAACCATCA
SPTLC1	GGAAGGGATTCTGATCCTCTGG	GAGGTTCTGGTTGCCACTCT
KDSR	TGCTGTACATGGTGTCTCCG	CGATAGCAATGCACTTCCCG
DEGS2	AGCGACTTCGAGTGGGTCTA	GCCTTGATGGCCGGGTA
SMPD1	TGCCCAATCTGCAAAGGTCT	GGACCATGGATTGGCACACG
SMPD3	TCATGGACGTGGCCTATCAC	CCCGACGATTCTTTGGTCT
DGAT2	TCCAGTCAAACACCAGCCAA	AGTGGGTCCTGTCTTCCCTT
ELOVL6	AAACGTGCGGGCACTAAGA	TCATTGGGGCTGATCTTCGG
ELOVL7	TTTGTGATGTCTGGCTGGGG	AGTAATAAAGCCAGCAGGTACG
TBP	TCAAACCCAGAATTGTCTCCTTAT	CCTGAATCCCTTTAGAATAGGGTAG

**Table S4. Primers for ChIP qPCR**

<b>Gene (Human)</b>	<b>Forward Primer</b>	<b>Reverse Primer</b>
SMPD3	GTTGGAAAAGTGGATCAGGG	GATGGTGGTACACTACTTCT
DGAT2	CCTTGGAAGCAGGGCTCAG	ACTTGCTTGTGGGGGAACC
DEGS1	GGGCGGCAGGTTTATCTCA	GTTCTGAGCTTCGGTGACTC
DEGS2	CGAAGGCTCTGATTTTGAAGCT	ACCAGCCATCACTTTACAGATCAG
ELOVL6	CACCCGGTCTCTCTATGAAAC	ATCCCTCGTGTCTTCTCTTTC
ELOVL7	AGTCCCGAGAGTCAGGG	CAGGTGCCCGCCGAG
Gene desert	AAGAGGCCCTTCTCTATGC	TGTGATTAATCTCGACTCCAAGA
<b>Gene (Mouse)</b>	<b>Forward Primer</b>	<b>Reverse Primer</b>
Zfp750 promoter	AAGGTGAACACACTGCCGA	GGAGTTTTGTTGAGGTCACCG

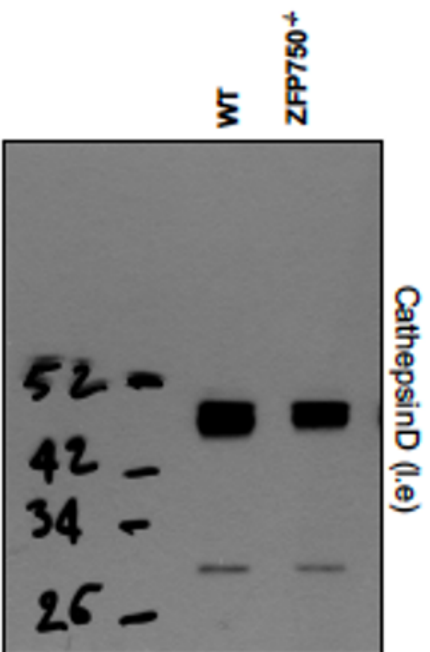
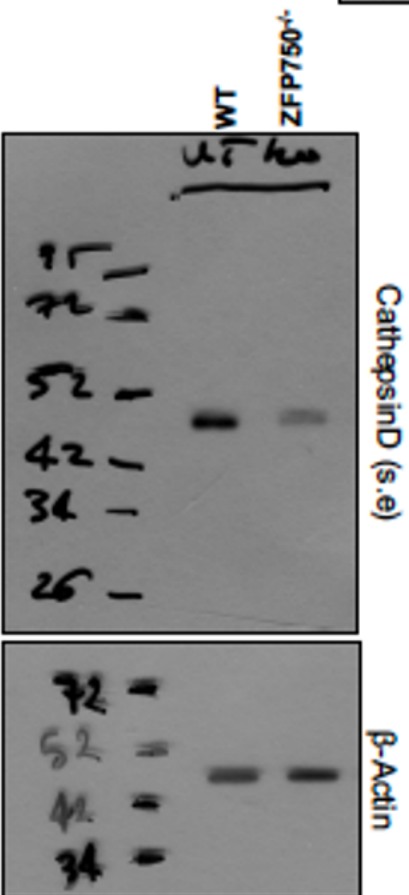
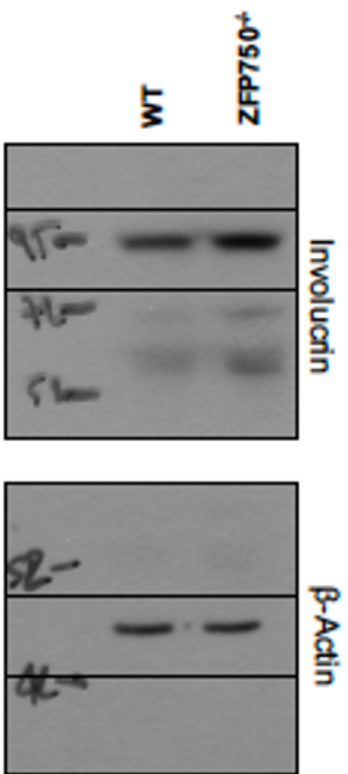
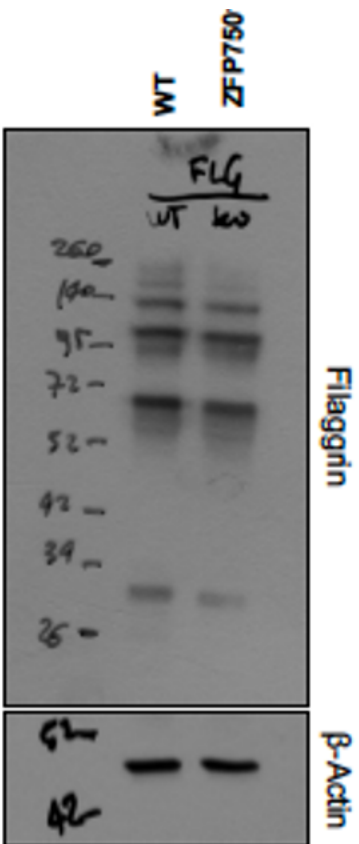
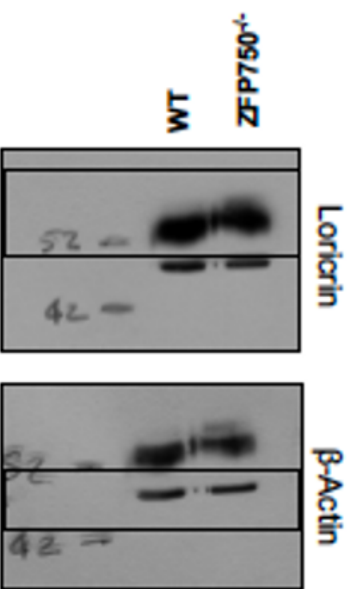
**Table S5. siRNA**

<b>Target transcript</b>	<b>Company</b>	<b>Cat. No.</b>
ON-TARGETplus Non-targeting Pool	Dharmacon	D-001810-10
ZNF750	Qiagen	SI04370485

**Table S6. Antibodies**

<b><i>Antibody</i></b>	<b><i>Source</i></b>	<b><i>Cat. No.</i></b>
beta-Galactosidase	Abcam	ab9361
Cathepsin D (CTSD)	Abcam	ab75852
Filaggrin	Biologend	905801
GlucosylCeramide/Ceramide	GlycoBiotech	RAS0011
Involucrin	Biologend	924401
Keratin 10	Covance	PRB-159P
Keratin 14	Biologend	905301
Keratin 14-LL02	Abcam	ab7800
Ki-67	Cell Signaling Technology	12202
Loricrin	Biologend	905101
P63	Cell signalling technology	13109
ZNF750	Sigma	HPA023012
ZO1	Invitrogen	402200
$\beta$ -Actin	Sigma	A-5441

**Supplementary Materials (Uncropped Western Blot scans)**



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