

Supplementary Information

PAR2 absence completely rescues inflammation and ichthyosis caused by altered CAP1/Prss8 expression in mouse skin

¹Simona Frateschi, ^{2,3}Eric Camerer, ¹Giovanna Crisante, ¹Sarah Rieser, ^{1,11}Mathieu Membrez, ^{1,12}Roch-Philippe Charles, ⁴Friedrich Beermann, ⁵Jean-Christophe Stehle, ⁶Bernadette Breiden, ⁶Konrad Sandhoff, ⁵Samuel Rotman, ⁷Marek Haftek, ⁸Anne Wilson, ⁹Stephan Ryser, ¹⁰Martin Steinhoff, ²Shaun R. Coughlin & ^{1*}Edith Hummler

¹Department of Pharmacology and Toxicology, 1005 Lausanne, Switzerland

²Cardiovascular Research Institute, UCSF, San Francisco, CA 94158, USA

³Equipe Avenir, Inserm Unit 970, Paris Cardiovascular Research Center, 75006 Paris, France

⁴ISREC SV EPFL, 1015 Lausanne, Switzerland

⁵Pathology Department, 1011 Lausanne, Switzerland

⁶LIMES, Membrane Biology & Lipid Biochemistry Unit c/o Kekulé-Institut für Organische Chemie und Biochemie der Universität, 53121 Bonn, Germany

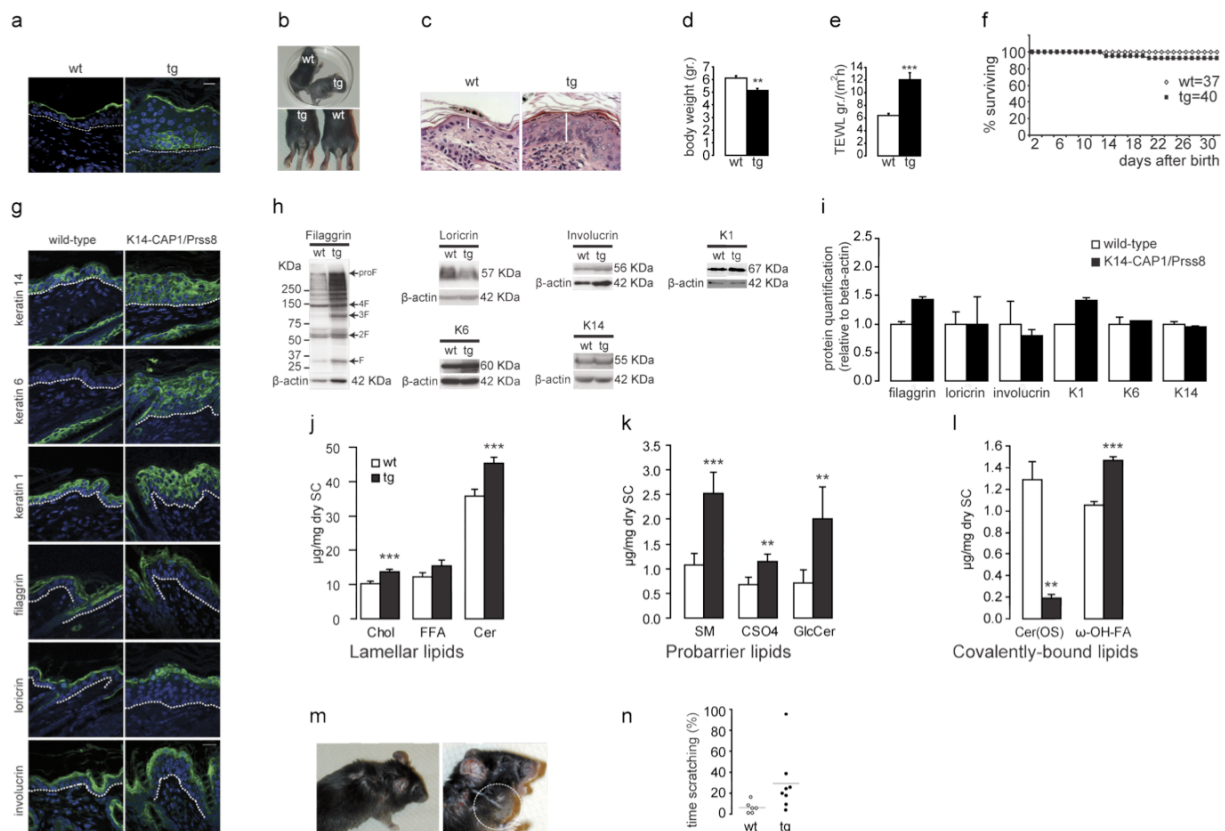
⁷University of Lyon, EA4169: Normal and Pathological Functions of the Skin Barrier, F-69437 Lyon, France

⁸Ludwig Institute for Cancer Research, 1066 Epalinges, Switzerland

⁹Laboratory of Cutaneous Biology, CHUV, 1011 Lausanne, Switzerland

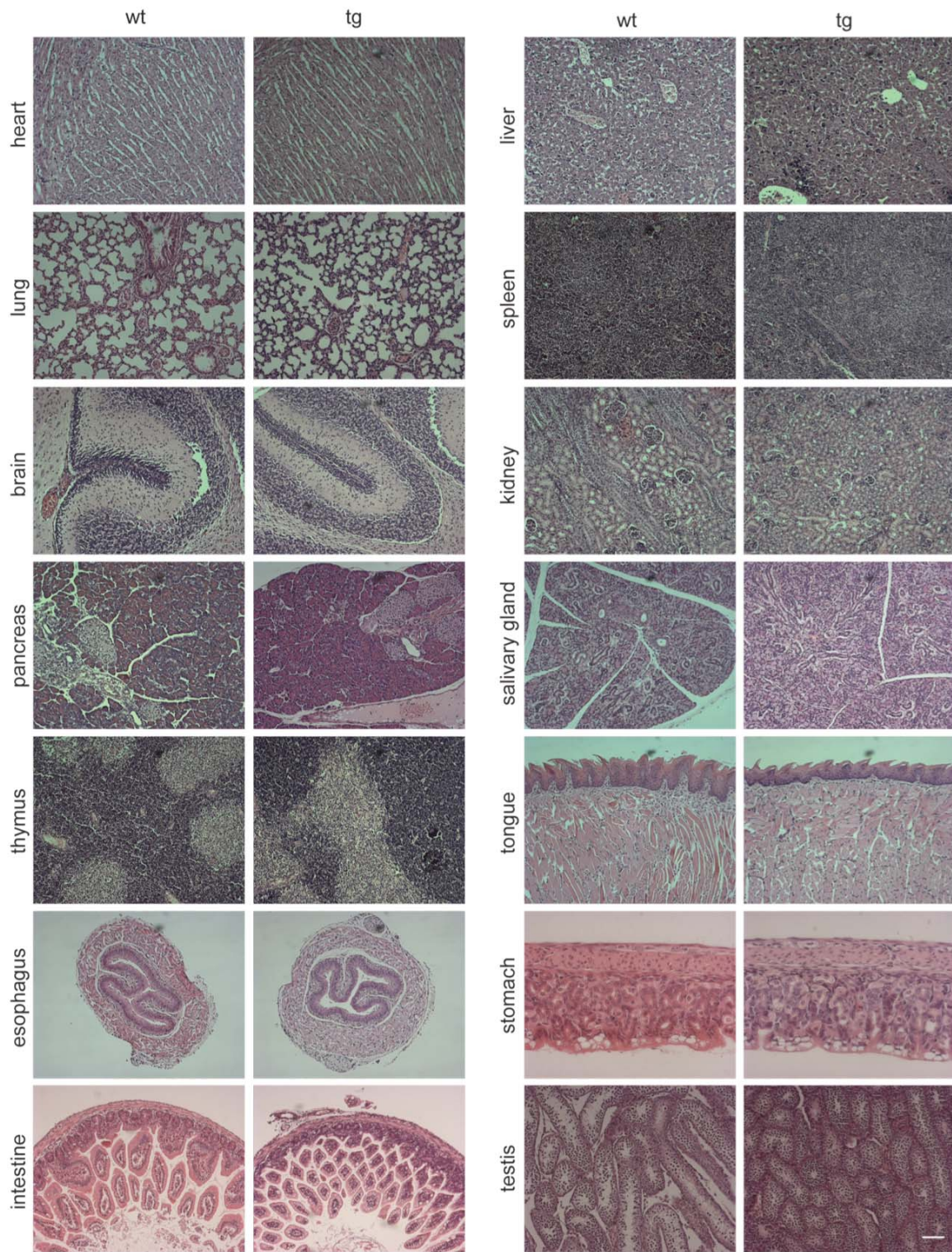
¹⁰Department of Dermatology, UCSF, San Francisco, CA 94115, USA

Supplementary Figures



Supplementary Figure S1

Phenotypical characterization of *K14-CAP1/Prss8* transgenic animals from line 1. (a) Immunofluorescence showing transgene-driven CAP1/Prss8 expression (green) to basal layer. Nuclei were counterstained with DAPI (blue). The white bar represents 20 μm. (b) Macroscopic appearance of *K14-CAP1/Prss8* transgenic mice at one and two weeks of age. (c) H&E-stained skin sections from two week old *K14-CAP1/Prss8* transgenics. The white bars indicate the thickness of the epidermis. The black bar represents 20 μm. (d) Body weight values in two week old *K14-CAP1/Prss8* transgenic mice compared to littermate controls. (e) Trans epidermal water loss measurements in transgenic compared to littermates. $n \geq 13$ mice/group. ** $p < 0.01$, *** $p < 0.001$. (f) Survival curves of *K14-CAP1/Prss8* transgenic mice versus littermate controls. (g) Immunohistochemistry of keratin-14, -6, -1, filaggrin, loricrin and involucrin (green). White dotted lines indicate the basal membrane. Nuclei were counterstained with DAPI (blue). The pictures are representative of three animals analyzed per genotype. The white bar represents 20 μm. (h) Western blot analyses of filaggrin, loricrin, involucrin, keratin-1, keratin-6 and keratin-14 from wild-type and transgenic skin. (i) Quantification of western blot analyses relative to beta-actin. $n \geq 3$ mice/group. (j) Unbound stratum corneum ceramide (Cer) and (k) probarrier lipids (sphingomyelin (SM), cholesterol sulfate (CSO4), and glucosylceramide (GlcCer) quantifications. (l) Covalently-bound lipids: ceramide (OS) level (Cer(OS) is merely 14% of control in *K14-CAP1/Prss8* transgenic mice. $n \geq 3$ mice/group; (j-l) ** $p < 0.01$, *** $p < 0.001$). (m) Adult *K14-CAP1/Prss8* transgenic mice display skin lesions and swelled lymph nodes (white dotted circle). (n) Dot plot indicating the duration of the scratching behavior expressed in percentage. Each dot represents a single event per animal ($p < 0.01$). Bars indicate the average of the time scratching. $n \geq 3$ mice/group. All data are presented as mean \pm S.E.M.



Supplementary Figure S2

Hematoxylin-eosin staining of two week old *K14-CAP1/Prss8* versus wild-type littermates from line 2. Histopathological analyses of heart, liver, lung, spleen, brain, kidney, pancreas, salivary gland, thymus, tongue, oesophagus, stomach, intestine, uterus and testis. Bar represents 10 μ m; $n \geq 3$ mice/group.

Supplementary Table S1

Composition of the free extractable stratum corneum ceramides

Genotypes	<i>K14-CAPI/Prss8</i>		<i>K14-CAPI/Prss8</i>	
	Line 1	wild-type	Line 2	wild-type
Cer (EOS)	4.44±0.45 ^{***}	1.94±0.23	3.99±0.40 ^{***}	1.63±0.23
Cer(C26-NS)	15.61±0.57 ^{**}	12.08±0.91	15.09±0.87 ^{**}	11.62±0.59
X2	3.15±0.04 ^{**}	2.07±0.04	3.08±0.10 ^{***}	1.60±0.34
Cer(C16-NS)	13.41±0.61 [*]	11.65±0.59	13.41±0.92 [*]	10.89±0.79
Cer (NP)	2.82±0.40	2.66±0.30	2.63±0.27	2.49±0.33
Cer(C26-AS)	3.59±0.23	3.25±0.29	3.19±0.27 [*]	2.45±0.36
Cer(C16-AS)	2.43±0.23	2.44±0.37	2.39±0.19	2.24±0.24
X1	0.10±0.06	0.11±0.03	0.03±0.01	0.11±0.09
Cer(C26-NH)	1.17±0.09 ^{***}	0.50±0.11	1.09±0.06 ^{**}	0.67±0.10
Cer(C16-NH)	0.34±0.04	0.29±0.09	0.32±0.04	0.30±0.07

Data are presented as mean ± S.E.M. and expressed in ng/mg of dry stratum corneum.

*p<0.05, **p<0.01, ***p<0.001.

Supplementary Table S2 Primers list

Primer names and sequences used for genotyping, semi-quantitative RT-PCR (semi-q. RT-PCR) and quantitative RT-PCR (q. RT-PCR) analyses.

allele	name	sequence 5' - 3'
genotyping		
<i>K14-CAP1/Prss8</i>	beta globin sens 2	CCTCTGCTAACCATGTTTCATGC
	K14 CAP1/Prss8 as 2	GTTGCCATCGTAGGTGATGCTG
PAR2 k.o.	myo 50s	TTACGTCCATCGTGGACAGC
	myo 51as	TGGGCTGGGTGTTAGTCTTA
	NEO371_R	CGCATTGCATCAGCCATGATGG
	NEO_57	GGAGAGGCTATT CGGCTATGAC
	PAR2_GR_F	GAGGGCTATCCGACTCATCATC
<i>Grhl3^{PAR2/+}</i>	PAR2_GR_R	CTGCCTCTGGGTTTT GATTAGG
	GrhP_comm_F	CACCCCTCAGCTAAGAAGGAA
	GrhP_wt_R	CCCTTT GGCAAGAGGAGAGAAA
	GrhP_ki_R	CTGGGTTTCCAATCTGCCAATAAG
semi-q. RT-PCR		
CAP1/Prss8	CAP1/Prss8tg_171_F	TTCCAATTTACCCGAGCA
	CAP1/Prss8tg_299_R	CAGAAGGCTGGTTTTGAAGG
PAR2	PAR2_RT_222_F	TGGGAGGTATCACCC TTCTG
	PAR2_RT_222_R	AGACGACCGGAAGAAAGACA
IL 1 alpha	IL1a_S	TGCCATTGACCATCTCTCTCT
	IL1a_AS	TGATCTGGGTTGGATGGTCT
IL 1 beta	IL1b_S	AGTTGACGGACCCCAAAAG
	IL1b_AS	CCATGAGTCACAGAGGATGG
MMP9	MMP9_S	GACGGCAAATTTGGTTTCTG
	MMP9_AS	ACAGC TCTCCTGCCGAGTT
TSLP	TSLP_F	AAGTTCGAGCAAATCGAGGA
	TSLP_R	GGACTTCTTGTGCCATTTCC
beta-actin	beta_actin_S	AGCCATGTACGTAGCCATCC
	beta_actin_AS	CTCTCAGCTGTGGTGGTGAA
q. RT-PCR		
TSLP	SF_TSLP_F_taq	GCTTGTCTCCTGAAAATCGAGTACT
	SF_TSLP_probe_taq	TACTCTCAATCCTATCCCTGGCTGCCCT
	SF_TSLP_R_taq	CTCCGGGCAAATGTTTTGTC
CAP1	TAQRPC7_F	CCCATCTGCCTCCCTGC
	TAQRPC7_probe	CCAATGCCTCCTTTCCCAACGGC
	TAQRPC7_R	CCATCCCGTGACAGTACAGTGA
beta-actin	TAQRPC12_F	AGGTCATCACTATTGGCAACGA
	TAQRPC12_probe	TGCCACAGGATTCCATACCCAAGAAGG
	TAQRPC12_R	CACTTCATGATGGAATTGAATGTAGT

Supplementary Methods

K14-CAP1/Prss8 cloning strategy

The human keratin 14 promoter, the rabbit beta globin intron and the human growth hormone polyadenylation signal were contained in the pBluescript II KS+ vector resulting in a plasmid named pBHR2(SmaI)bis. pBHR2(SmaI)bis carried a unique *Sma* I restriction site between the rabbit beta globin intron and the human growth hormone polyadenylation signal, and unique *Cla* I and *Not* I restriction sites upstream the keratin 14 promoter and downstream the poly A, respectively. pT7mCAP1 plasmid consisted of the mouse CAP1/Prss8 coding sequence contained in the pT7 blue vector. CAP1/Prss8 coding sequence could be released by *Sma* I and *Spe* I digestions. The insertion of the mouse CAP1/Prss8 coding sequence into the unique *Sma* I site of pBHR2(SmaI)bis, was obtained by blunting the *Spe* I extremity and ligating the resulting insert into the *Sma* I site of pBHR2(SmaI). The insert orientation was controlled by *Cla* I/*Sma* I digestions. The resulting pKRmCAP1 plasmid was finally cut by *Cla* I and *Not* I in order to release the K14 promoter - intron - CAP1/Prss8 - poly A fragment that was thereafter used for pronuclear injections.

Grhl3^{PAR2/+} cloning strategy

To generate the *Grhl3*^{PAR2/+} mouse line, we introduced the cDNA encoding the mouse PAR2 in frame into the start ATG of the *Grhl3* gene as predicted by the Ensembl database (www.ensembl.org). To facilitate detection of expression directly and later, to address the effects of PAR2 mediated excision on target cells, an internal ribosomal entry sequence (IRES) followed by a nuclear localization signal and lacZ gene were introduced downstream of the PAR2 gene. An FRT-flanked Neo cassette was positioned 5' of the PAR2, and a thymidine kinase (tk) cassette was positioned outside the homologous arms (amplified by PCR from E14 ES cell DNA) for negative selection. The targeting construct was introduced into E14 ES cells (129SVJ) by electroporation and, after positive and negative selection, homologous recombination was confirmed by Southern blotting with probes positioned both 5' and 3' of the homologous arms. Chimeric males derived from blastocyst injections of positive clones into C57BL/6 recipients were mated with C57 FLPe females to remove the neo gene.