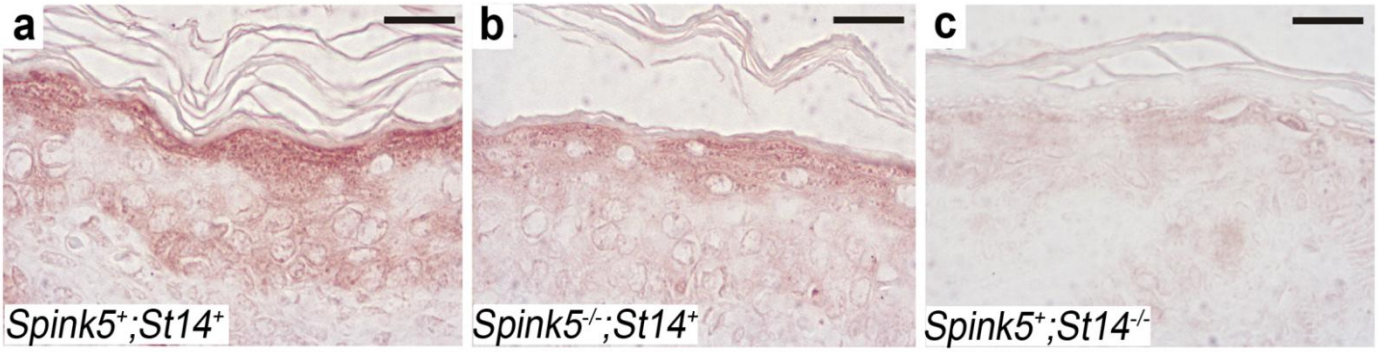
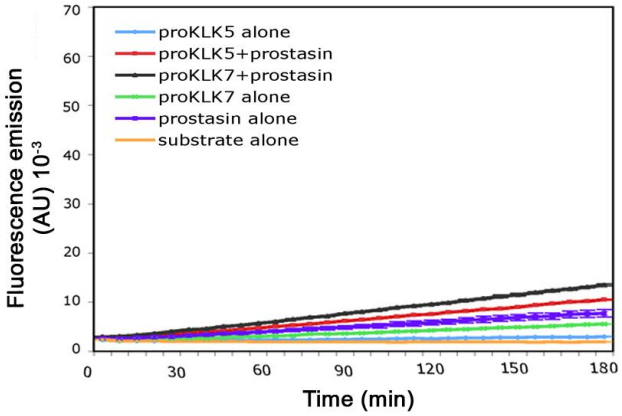
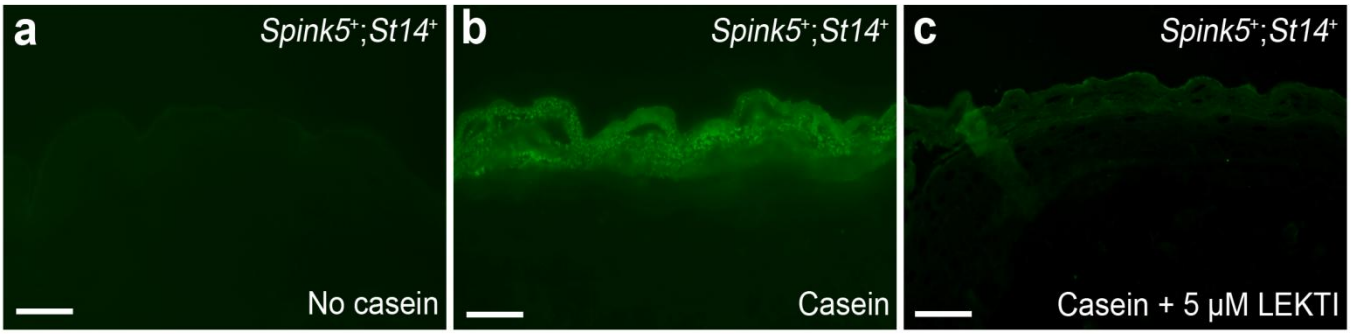


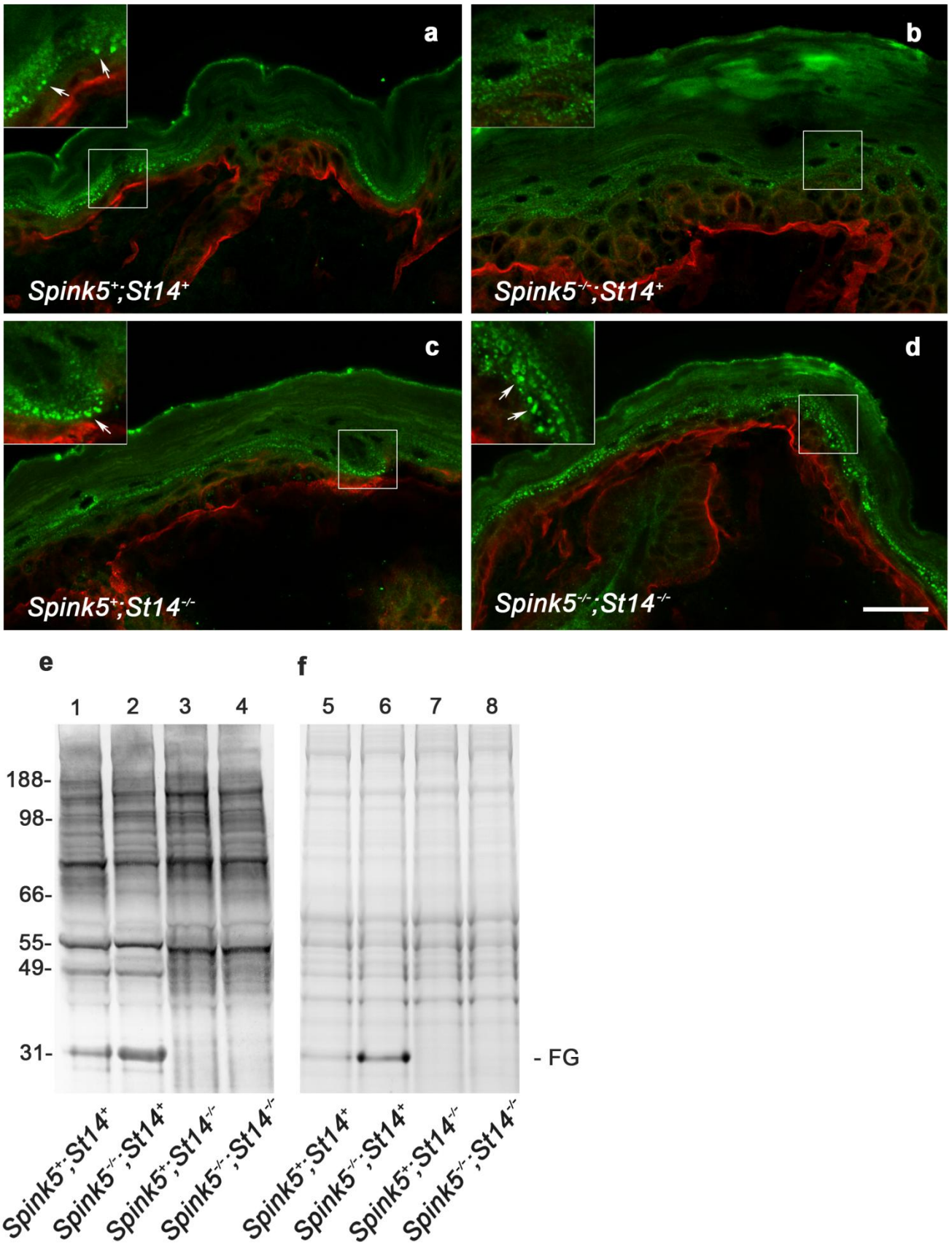
Supplementary Figure 1. Matriptase expression is retained in LEKTI-deficient epidermis. Skin sections from newborn LEKTI- and matriptase-sufficient (*Spink5*$^{+}$;*St14*$^{+}$) (**a**), LEKTI-deficient (*Spink5*$^{-/-}$;*St14*$^{+}$) (**b**), and matriptase-deficient (*Spink5*$^{+}$;*St14*$^{-/-}$) (**c**) mice were immunostained with antibodies against matriptase. Matriptase expression is retained in the suprabasal epidermis of LEKTI-deficient skin. Size bar, 25 mm.



Supplementary Figure 2. Prostasin is not an efficient activator of epidermal pro-kallikrein-related peptidases. Recombinant pro-kallikrein-related peptidase 5 (8 nM) or pro-kallikrein-related peptidase 7 (0.4 mM) were incubated for 30 min with and without 0.4 mM recombinant prostasin. The fluorogenic kallikrein-selective peptide Boc-Val-Pro-Arg-AMC was then added and fluorescence development was recorded over time. Low hydrolytic activity and additive effects of mixing prostasin with pro-kallikrein-related peptidase 5 are observed. The experiment was performed in triplicate and data are shown as mean fluorescence ± standard error of the mean.



Supplementary Figure 3. Epidermal *in situ* proteolytic activity is inhibited by recombinant LEKTI. Skin cryosections (5 µM) from newborn wildtype mice were incubated either with buffer (**a**), with fluorescence-quenched casein alone (**b**) or with fluorescence-quenched casein and 5 µM of recombinant LEKTI D6-9 (**c**). The proteolytic activity was strongly inhibited upon the addition of recombinant LEKTI.


a
Spink5+;St14+
b
Spink5-/-;St14+
c
Spink5+;St14-/-
d
Spink5-/-;St14-/-
e
f
1
2
3
4
5
6
7
8
188-
98-
66-
55-
49-
31-
- FG
Spink5+;St14+
Spink5-/-;St14+
Spink5+;St14-/-
Spink5-/-;St14-/-
Spink5+;St14+
Spink5-/-;St14+
Spink5+;St14-/-
Spink5-/-;St14-/-

Supplementary Figure 4. Loss of matriptase restores profilaggrin granules and blocks excessive profilaggrin processing in LEKTI-deficient epidermis. Combined profilaggrin/filaggrin (green) and basal-layer marker α6-integrin (red) immunofluorescence of epidermis from newborn LEKTI- and matriptase-sufficient (*Spink5*$^{+}$;*St14*$^{+}$) (**a**), LEKTI-deficient (*Spink5*$^{-/-}$;*St14*$^{+}$) (**b**), matriptase-deficient (*Spink5*$^{+}$;*St14*$^{-/-}$) (**c**), and combined LEKTI- and matriptase-deficient (*Spink5*$^{-/-}$;*St14*$^{-/-}$) (**d**) mice. Profilaggrin-granules in LEKTI-sufficient epidermis (examples with arrows in inset in **a**) are absent in LEKTI-deficient epidermis (inset in **b**), but are restored after simultaneous matriptase ablation (examples with arrows in inset in **d**). Size bars all frames, 50 mm. (**e**) Profilaggrin/filaggrin Western blot and (**f**) Coomassie brilliant blue staining of epidermal extracts from newborn LEKTI and matriptase-sufficient (*Spink5*$^{+}$;*St14*$^{+}$) (lanes 1 and 5), LEKTI-deficient (*Spink5*$^{-/-}$;*St14*$^{+}$) (lanes 2 and 6), matriptase-deficient (*Spink5*$^{+}$;*St14*$^{-/-}$) (lanes 3 and 7), and combined LEKTI and matriptase-deficient (*Spink5*$^{-/-}$;*St14*$^{-/-}$) (lanes 4 and 8) mice from a single litter of mice, showing that excessive proteolytic processing of profilaggrin in LEKTI-deficient epidermis is blocked by simultaneous matriptase deficiency. Molecular weight standards (kDa) are indicated at left and the position of processed filaggrin monomer (FG) is indicated at right.

Supplementary Table 1. Primer sequences

Amplification of full length mouse pro-kallikrein-related peptidase 5	
proKLK5f	5'ATAGGATCCATGGCGAGGACCGGA 3'
proKLK5r	5'ATAGAATTCTTAGTGGTGGTGGTGGTGGTGGTTGGA GTTCATGGT 3'
Amplification of full length mouse pro-kallikrein-related peptidase 7	
proKLK7f	5'ATAGGATCCATGGGAGTCTGGCTC 3'
proKLK7r	5'ATAGAATTCTTAGTGGTGGTGGTGGTGGCGATG GGTTTTCAT 3'
qPCR of inflammation-associated genes	
TNFα forward	5' CAGCCTCTTCTCATTCCTGC 3'
TNFα reverse	5' AGGGTCTGGGCCATAGAACT 3'
INFγ forward	5' AGCGGCTGACTGAACTCAGATTGTAG 3'
INFγ reverse	5' GTCACAGTTTTCAGCTGTATAGGG 3'
Il-1β forward	5' GGGCCTCAAAGGAAAGAATC 3'
Il-1β reverse	5' TACCAGTTGGGGAACTCTGC 3'
Il-6 forward	5' TTCCATCCAGTTGCCTTCTT 3'
Il-6 reverse	5' TTCTGCAAGTGCATCATCGT 3'