

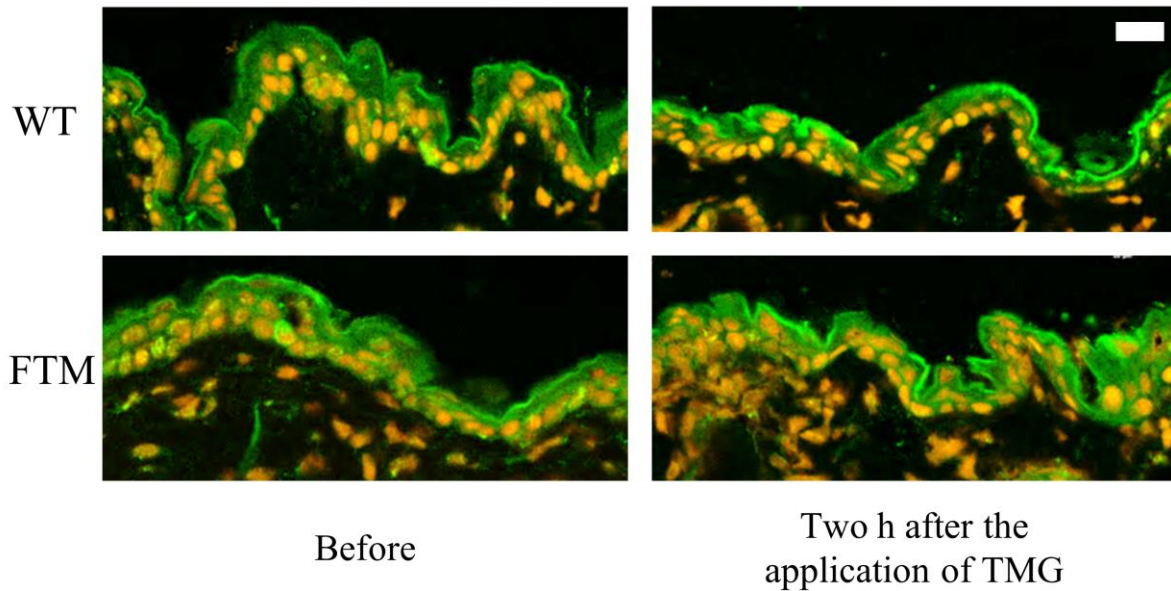
Supplementary data

Defective maintenance of pH of stratum corneum is correlated with preferential emergence and exacerbation of atopic-dermatitis-like dermatitis in flaky-tail mice

Sakai et al.

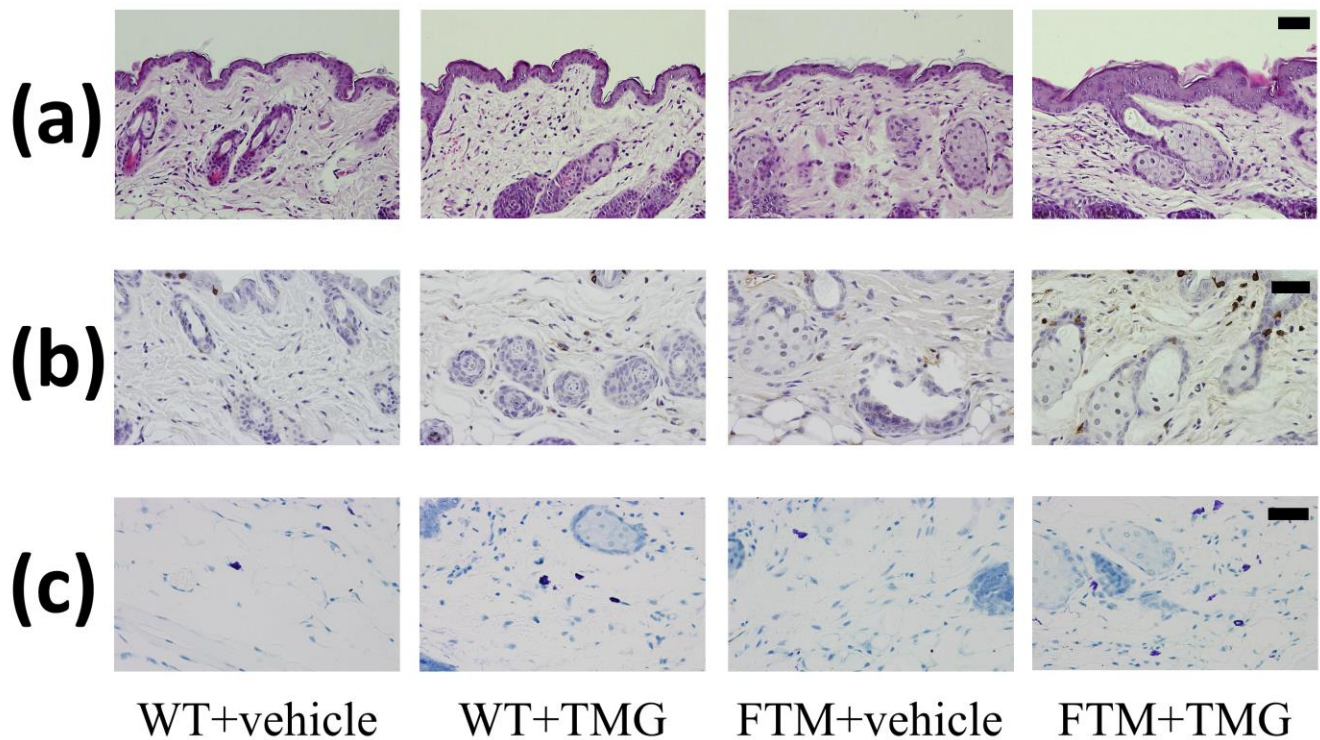
Supplementary Figures S1-5

sPLA2



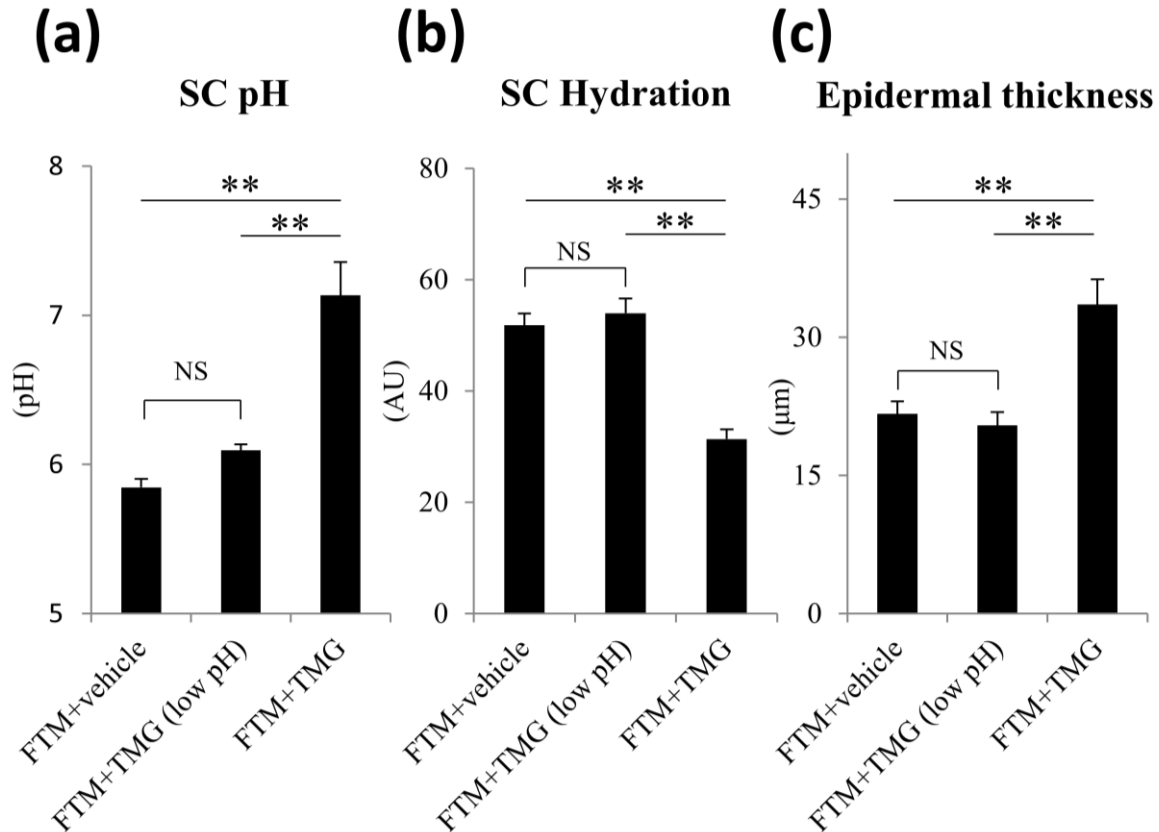
Supplementary Figure S1: The expression of sPLA2 before and after application of TMG

Skin samples were obtained from WT and FTM, before (at steady-state) or 2 h after the application of TMG. Immunohistochemical staining was performed as described in the text. Green staining of epidermis represents expression of sPLA2 and red counterstaining represents nuclear staining with propidium iodide. There was no difference between the both mice at steady state, and before and after the application of TMG. Scale bar = 20 μ m.



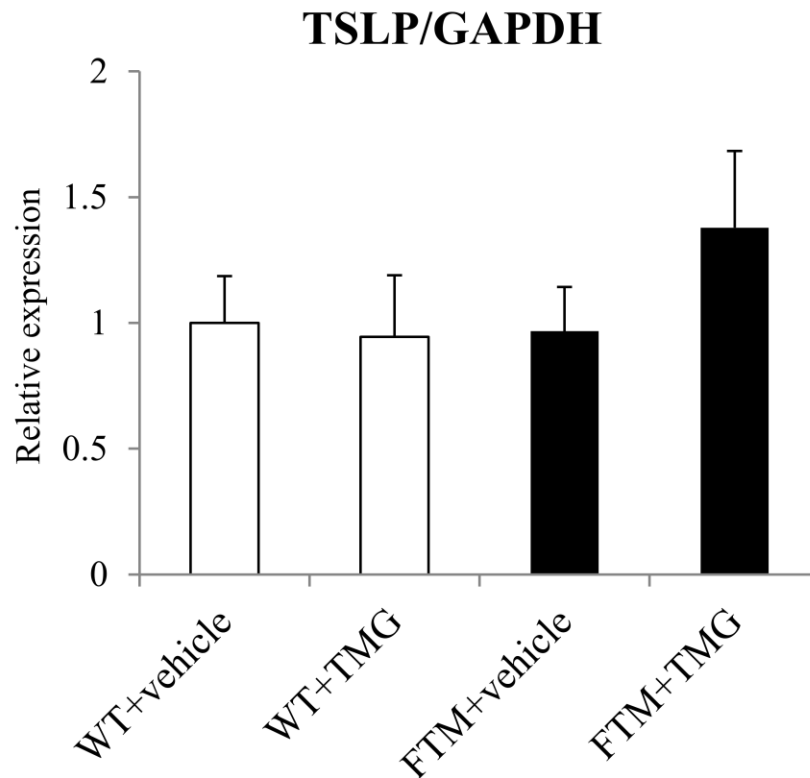
Supplementary Figure S2: Skin morphology after two weeks of daily application of TMG or vehicle

(a-c) Skin samples were obtained from WT and FTM after two weeks of daily application of TMG or vehicle. Hematoxylin and eosin staining and immunohistochemical stainings were performed as described in the text. Epidermis thickened (a), and number of CD3-positive cells (b) and mast cells (c) increased only in TMG-treated FTM. Scale bar = 50 μ m.



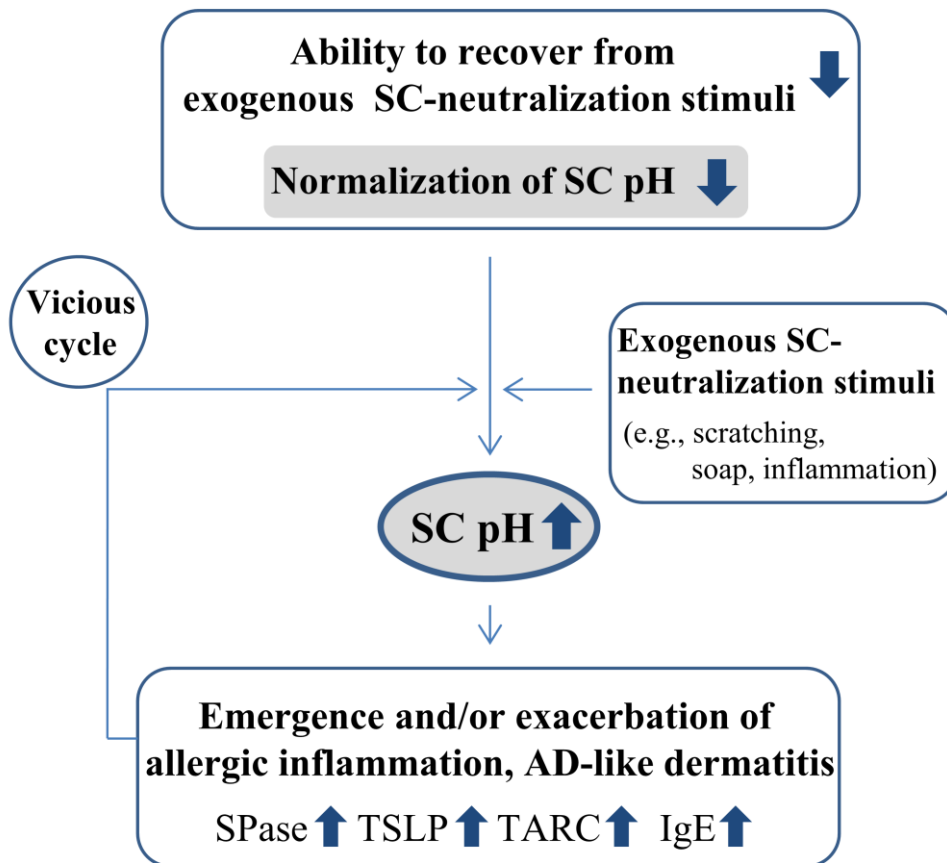
Supplementary Figure S3: Low pH-TMG did not induce substantial skin inflammation

We subjected FTM to two weeks of daily application of vehicle, TMG or low pH-TMG (TMG [low pH]). The pH of low pH-TMG was adjusted into that of vehicle (pH value is about 5.70), as described in the text. (a) Application of low pH-TMG did not elevate SC pH at the end of the treatment period. n=6 to 9; (b) SC hydration was not reduced in low pH-TMG-treated FTM. n=8 to 11; (c) Hematoxylin and eosin staining were performed as described in the text. Epidermal thickness did not increase in low pH-TMG-treated FTM. n=12. These results suggested that neutralization of SC pH is the major factor of skin inflammation in this study. Error bars equal to means±SEMs. ** p < 0.01. NS, Not significant. AU, Arbitrary units.



Supplementary Figure S4: Expression of TSLP in whole skin analyzed by real-time PCR after two weeks of daily application of TMG or vehicle

Whole skin samples were obtained from WT and FTM after two weeks of daily application of TMG or vehicle. Relative expression of TSLP in the skin was examined by real-time PCR as described in the text. n=5 to 8. The real-time PCR showed about 35 % increase in TSLP expression compared to that in vehicle-treated WT only in TMG-treated FTM, supporting the results from the immunohistochemical analysis (Fig. 5), although the difference has not reached a statistical significance. Error bars equal to means±SEMs.



Supplementary Figure S5: Proposal for a key role of failure to maintain acidity of SC in the pathogenesis of allergic inflammation and AD-like dermatitis.

In the proposed model, increased sensitivity to an exogenous SC-neutralization stimulus can induce persistent elevation of SC pH by several stimuli such as scratching, use of soap and inflammation, with resultant induction of emergence and/or exacerbation of allergic inflammation including AD-like dermatitis via elevation of SPase activity in epidermis and serum levels of TSLP, TARC, and IgE. Furthermore, skin inflammation increase SC pH, creating a vicious cycle.