Supplemental Methods

Tissue analysis of inflammatory mediators. At the indicated day in the figures, the pups were weighed, euthanized and tissues collected. Peripheral blood was collected in heparin tubes and blood eosinophils were counted in a 1:10 dilution in Discomb stain (one part acetone, one part 2% aqueous eosin, and eight parts distilled water) [1, 2]. The eosin positive eosinophils were counted using a hemacytometer (catalog #0267110, Fisher Scientific) [3].

Serum was collected for analysis of antigen-specific antibodies. Skin punches and intestines were collected for histology or qPCR of mediators of inflammation. Neonate intestines were flushed with saline and fixed with 4% formaldehyde in PBS overnight followed by 30 minutes in 30% sucrose in PBS. The intestines were frozen in OCT and stored at -80^{o}C. Intestine tissue sections were stained with 0.1% toluidine blue (0.1% toluidine blue in 7% ethanol, 1% NaCl, ph2.3) for mast cells or with eosin and a light methyl-green counterstain (EMG stain) for eosinophils [3]. Paraffin-embedded skin tissue sections were processed for histology with toluidine blue for mast cells or with an eosin and light hematoxylin counterstain for eosinophils.

RNA Isolation and Quantitative Real-Time PCR. RNA extraction was performed using the RNeasy Mini Kit (QIAGEN), and cDNA was generated using the Applied Biosystems High-Capacity cDNA Archive Kit. The qRT-PCR analysis was performed with a 7500 Fast Real Time PCR System (Applied Biosystems). Reactions were set up with the SYBR™Green PCR Core Reagents (Invitrogen). Data were normalized to GAPDH, and mRNA expression fold-change relative to controls was calculated using the $2^{-\Delta\Delta Ct}$ method.

PNE-specific antibody, OVA-specific antibody, and MCPT-1 ELISAs. Serum anti-peanut IgE, IgG1, IgG2a and IgA were determined by ELISA. Briefly, high binding 96 well EIA/RIA plate

(Fisher Sci catalog #50-823-480) were coated with 50 µl of 10µg PE protein/ml in carbonate buffer for 2 hours at room temperature. Plates were washed with PBS-0.05%Tween and blocked with PBS + 3% BSA for 2 hours at room temperature. Plates were washed with PBS-Tween and incubated overnight with serum diluted as indicated in figures in PBS + 1% BSA. Plates were washed with PBS-0.05%Tween and incubated for 1 hour at room temperature with 100 µl of 2µg/ml biotin-labeled secondary antibody in PBS-1% essentially immunoglobulin-free BSA. Plates were washed with PBS-Tween and incubated with 100 µl /well streptavidin-HRP (1:1000 dilution in PBS, GE Healthcare, catalog #RPN1231-2ML). Plates were washed in TBS-Tween and incubated with 100 µl TMB substrate (eBioscience catalog#00-4201-56, 3,3',5,5'-Tetramethylbenzidine; HRP substrate). At the indicated times in the figures, the reaction was stopped with 100 µl 2M phosphoric acid and plates read at 450nm in a microtiter plate reader.

Serum anti-OVA IgE were determined by ELISA. Briefly, high binding 96 well EIA/RIA plate (Fisher Sci cat#50-823-480) were coated with 100 µl of 0.3 µg anti-IgE (clone R35-72, catalog #553413, BDPharMingen) in carbonate buffer overnight at 4^{o}C. Plates were washed with PBS-0.05%Tween and blocked with PBS-3% essentially immunoglobulin-free BSA (Sigma) for 2 hours at room temperature. Plates were washed with PBS-0.05%Tween and incubated overnight with anti-OVA IgE standard (catalog # MCA2259, ABDserotec) or serum as indicated in figures in PBS + 1% BSA. Plates were washed with PBS-0.05%Tween. Plates were incubated with 100 ul with 0.2 ug biotin-OVA for 1 hour at room temperature. Plates were washed with PBS-0.05%Tween and incubated with 100 µl /well streptavidin-HRP (1:1000 dilution in PBS, GE Healthcare, catalog #RPN1231-2ML). Plates were washed in TBS-Tween and incubated with 100 µl TMB substrate (eBioscience catalog#00-4201-56, 3,3',5,5'-Tetramethylbenzidine; HRP substrate). The reaction was stopped with 100 µl 2M phosphoric acid and plates read at 450nm in a microtiter plate reader.

MCPT-1 in serum was measured by ELISA according to manufacturer's instructions (catalog #50-174-16, Fisher Scientific).

Endotoxin levels. Endotoxin was measured with modifications of the manufacturer's instructions for the Pierce Limulus Amebocyte Lysate (LAL) Chromogenic Endotoxin Quantification Kit (catalog #88282, Thermo Fisher Scientific). LAL can be activated by endotoxin or β-glucan [4]. Therefore, to specifically measure endotoxin, β-glucan activation of LAL [4] was blocked by addition of heat inactivated laminarin to the assay. Briefly, 5 mg/ml laminarin (catalog #L9634, Sigma) in 0.2 N NaOH was heat inactivated at 56^{o}C for 6 hr, stored at -20^{o}C until used, and then added to LAL in the endotoxin kit at a concentration of 20 µg/ml heat-inactivated laminarin.

Genotyping for *Flg*^{ft} and *Tmem79*^{ma}. Offspring of the mating of wild type C57BL/6 females with *Flg*$^{ft/ft}$/*Tmem79*$^{ma/ma}$ males were confirmed by genotyping. Briefly, for *Flg* PCR detection, murine genomic DNA was isolated from tail clips using the Quantabio *Extracta DNA Prep* kit (catalog #95091) and amplified with the primers: Forward: 5'-CATCTCCAGTCAGGGCTGACC-3', Reverse: 5'-GCTGCCTGTGGCCGGACTCG-3'. PCR amplification conditions were as follows: (96℃, 5 min) x1; (96℃ 30sec, 60℃ 30sec, 72℃ 30 sec) x35; (72℃, 5 mins) x1. Amplified transcripts were then digested with the restriction enzyme *Acc* I (New England Biolabs, catalog #R01611s) yielding either a wild type FLG band at 678bp or two fragments of the mutant FLG at 559bp and 134bp as determined by gel electrophoresis.

SDS/PAGE analysis of allergens. Peanut extract was incubated at room temperature with or without *Alternaria alternata* extract for 40 minutes in the same protein ration as applied to the neonatal mice. SDS-PAGE was performed with 10% Acrylamide gels. The gels were stained with SimplyBlue™ SafeStain (catalog #LC6060, Thermo Fisher) and imaged with an Odysey CLx (Licor).

SUPPLEMENT REFERENCES

1. Discombe G. Criteria of eosinophilia. Lancet. 1946; 1:195.
2. Copeland D, Grove DI. Effects of Toxoplasma gondii (Gleadle strain) on the host-parasite relationship in trichinosis. Int J Parasitol. 1979; 9:205-11.
3. Berdnikovs S, Abdala-Valencia H, Cook-Mills JM. Endothelial cell PTP1B regulates leukocyte recruitment during allergic inflammation. Am J Physiol Lung Cell Mol Physiol 2013; 304:L240-9.
4. Zhang GH, Baek L, Buchardt O, Koch C. Differential blocking of coagulation-activating pathways of Limulus amebocyte lysate. J Clin Microbiol. 1994; 32:1537-41.

Supplement Figure legends.

Supplement Figure 1. FT+/- Pups were treated with 3 skin applications of Alt/PNE as in Figure 1. A,B) Representative images of pup skin after gentle paper taping on PND3 and PND6 to gently remove dry shedding skin after birth. C) Representative image of pup skin on PND9 after shaving and then taping with paper tape to remove loose shaved fur.

Supplement Figure 2. OVA/Alt or OVA/HDM skin sensitization of FT+/- neonates induced responsiveness to anaphylaxis. (A) Timeline for mating and for FT+/- pup treatments. **(B)** Food allergen-induced temperature (^{o}C) changes on day 21. **(C)** Anti-OVA specific serum IgE,. N=8-10/group. *, $p<0.05$ as compared to the saline skin-sensitized groups.

Supplement Figure 3. There were no differences in response by gender and no differences in pup body temperature by gender prior to oral challenge. **(A)** These are the same data as from Figure 1 but separated here by gender. *, $p<0.05$ as compared to the saline skin-sensitized groups. **(B)** For pups from Figure 1, there was no differences in initial body temperature by gender or treatment just prior to oral challenge on PND21.

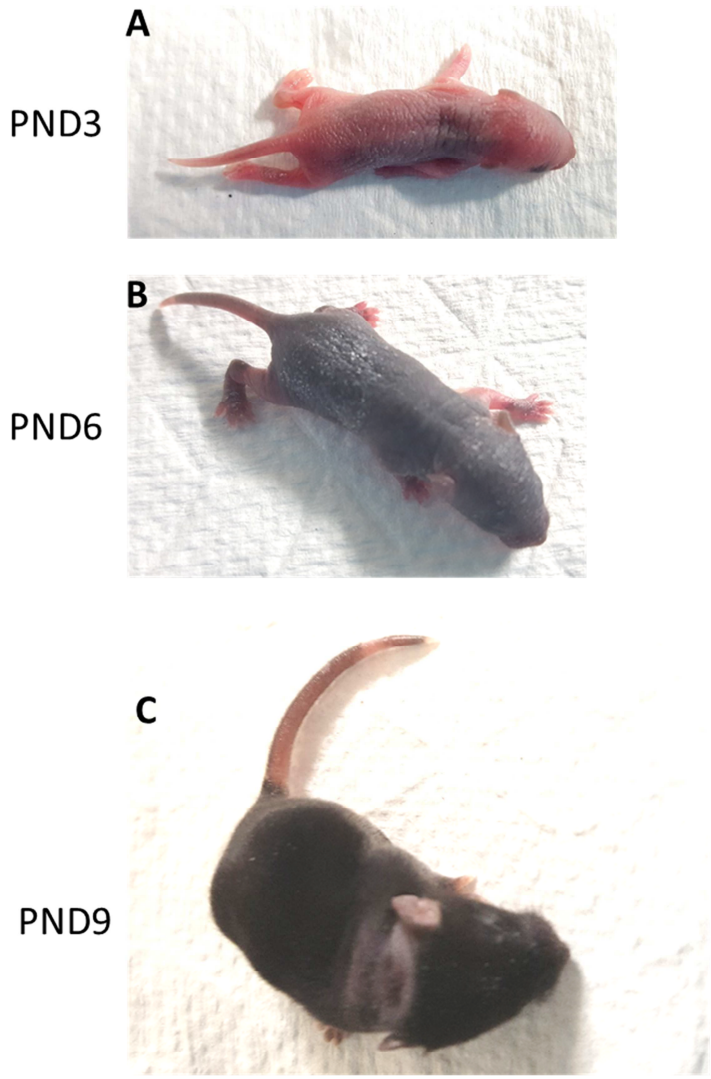
Supplement Figure 4. There was no indication of any new major band sizes after co-incubation of Alt with PNE or co-incubation of HDM with PNE. Co-incubation of PNE with **(A)** Alt or **(B)** HDM at room temperature for 20 or 45 minutes followed by SDS-PAGE and staining with simply blue. Indicated are the sizes for the major peanut proteins (AraH1, AraH3 acidic subunits, AraH3 basic subunit, AraH2, AraH6.

Supplement Figure 5. Endotoxin amounts in fecal pellets in mouse cage and in allergens for each skin treatment. Endotoxin was measured by the LAL chromogenic assay. Presented is endotoxin for each fecal pellet in cage bedding, 100 µg PNE, 10µg Alt, 10 µg HDM and 100 µg OVA, because these are the amounts applied to the skin at each treatment. *, $p<0.05$ as compared to other groups.

Supplemental Figure 6. The OVA allergic female mothers had OVA-specific serum IgE. These are allergic and saline-treated mothers that were treated as in Figure 2. Serum was collected on GD18 and OVA-specific IgE measured by ELISA. *, $p<0.05$.

Supplement Figure 7. Initial body temperature measured immediately before oral challenge increased with age but did not differ between pups of saline-treated and allergic mothers. Shown are initial body temperatures measured immediately before oral challenge for pups in Figure 3. *, <0.05 as compared to corresponding group with one fewer skin sensitization.

Supplement Figure 8. Data here are for ease of side-by-side comparisons for data from Figures 7 and 8. The data in figure 7 and 8 were from the same very large experiment. These are the data from Figure 7 groups 3&4 and Figure 8 groups 3&4. Oral pre-exposure to PNE to induce tolerance in FT+/- pups was less effective (Fig8 group 4) when their skin was pre-exposed to the ubiquitous environmental allergen Alt during oral pre-exposure to PNE as compared to the induction of tolerance with Oral PNE in the absence of Alt pre-exposure (Fig7 group 4). *, $p<0.05$ as compared to the group without the asterisk.



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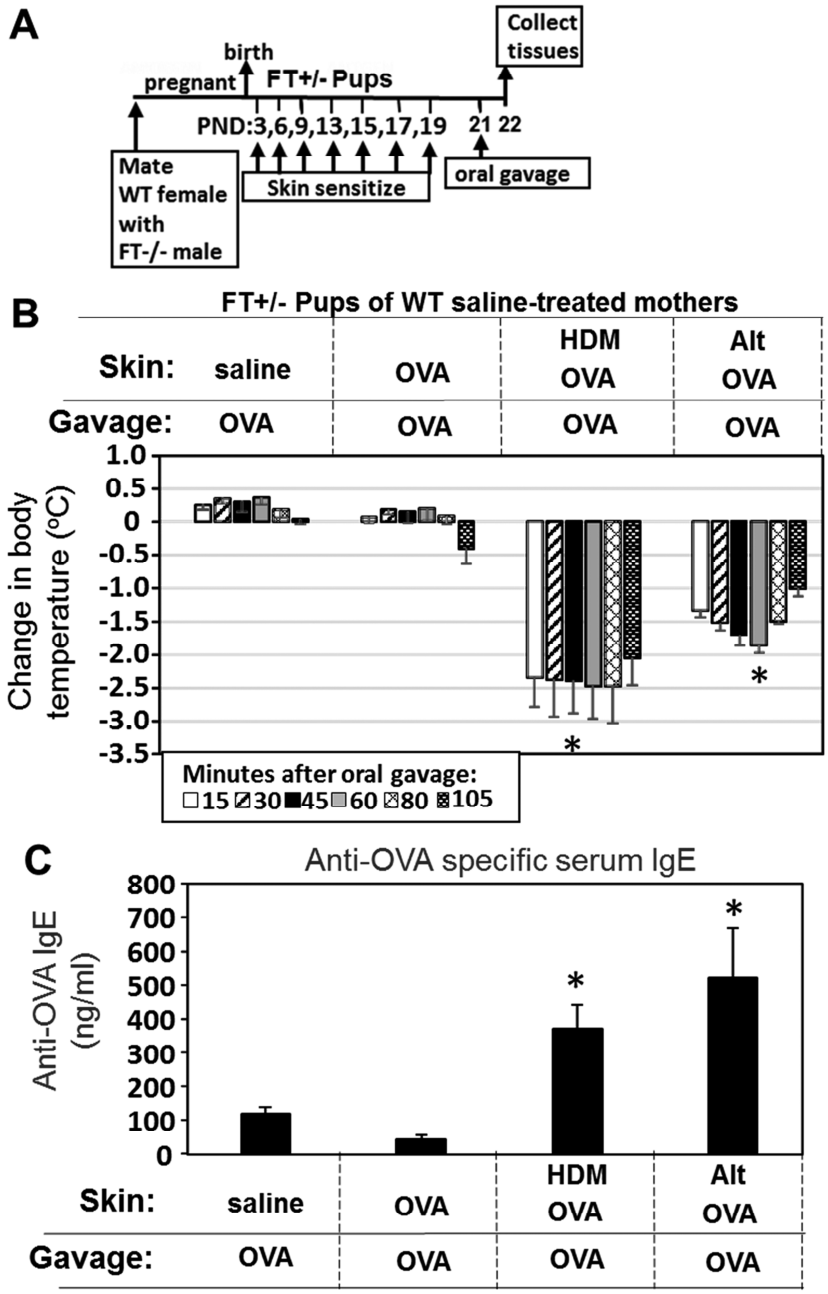

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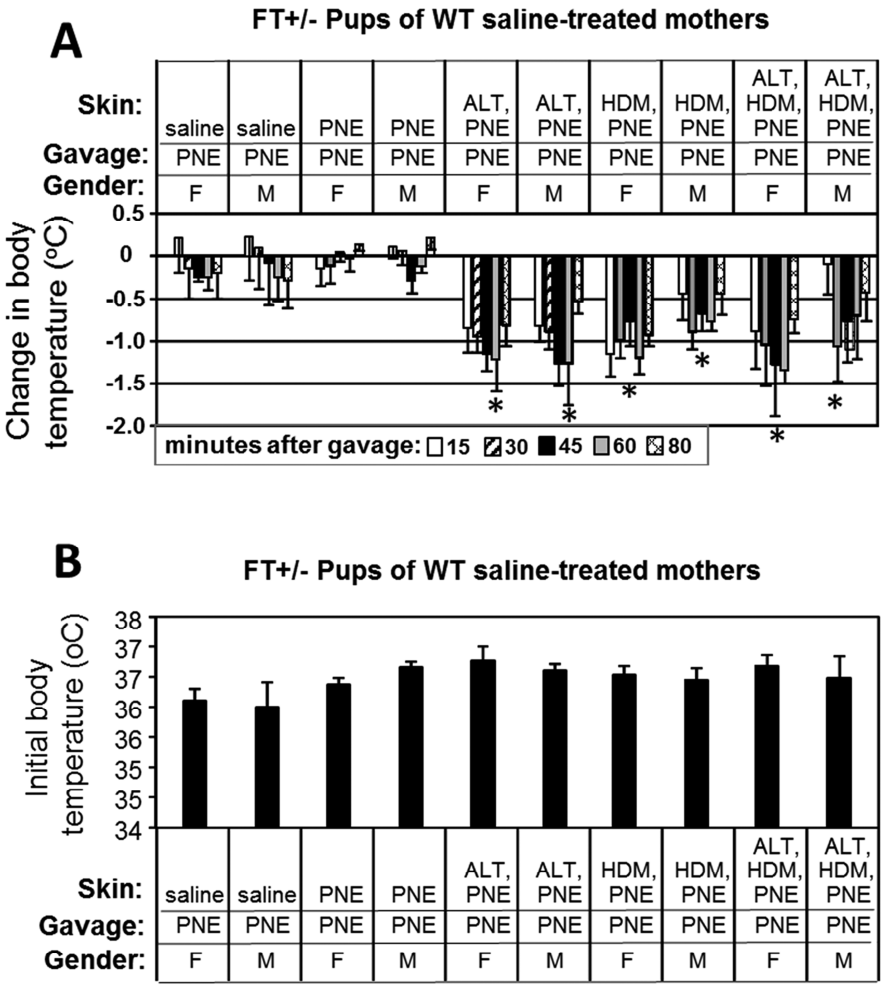

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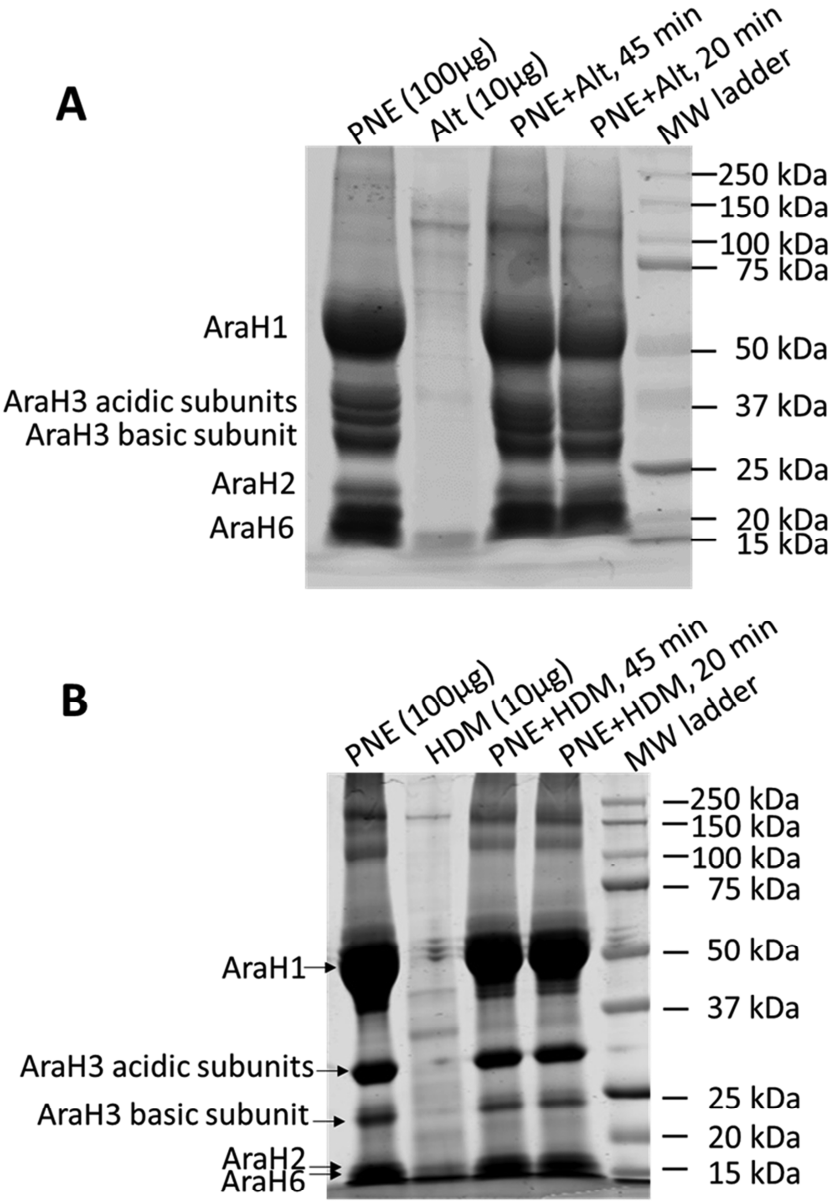

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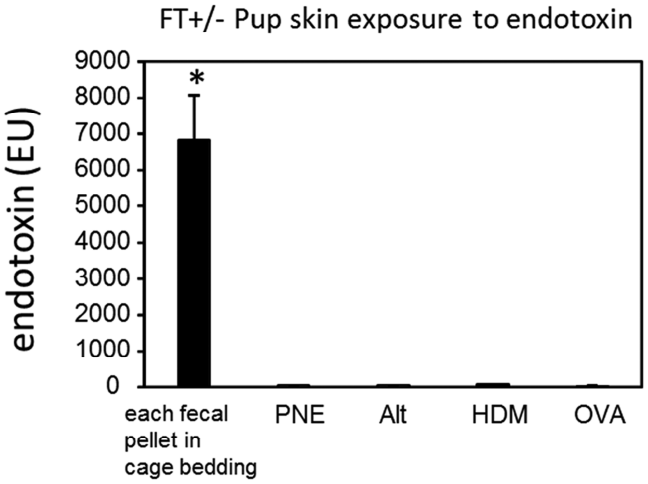

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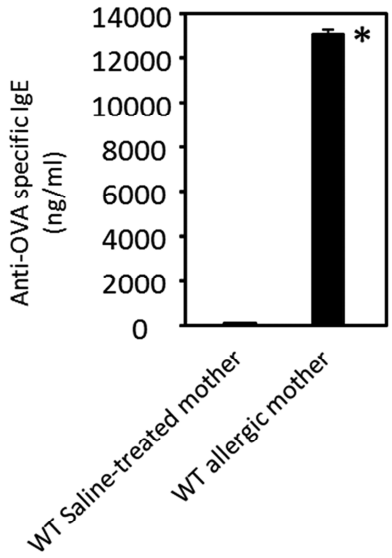

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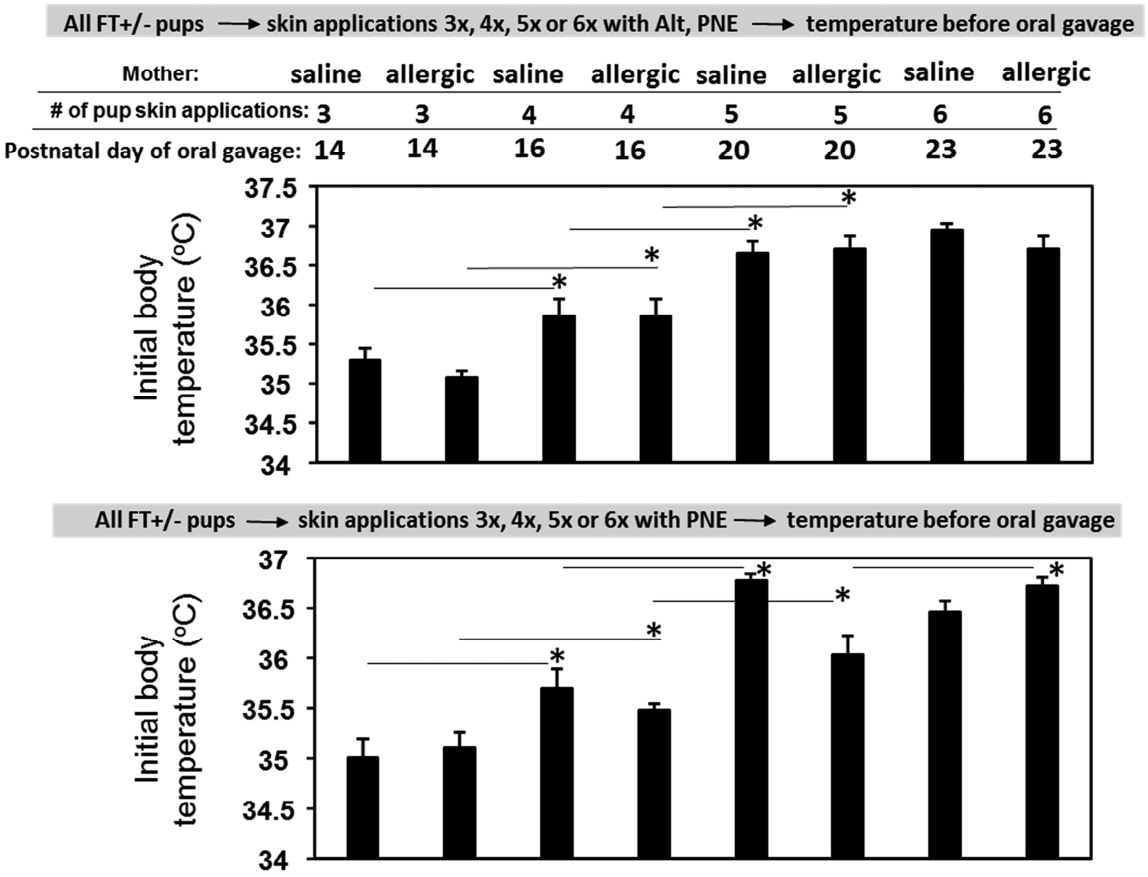

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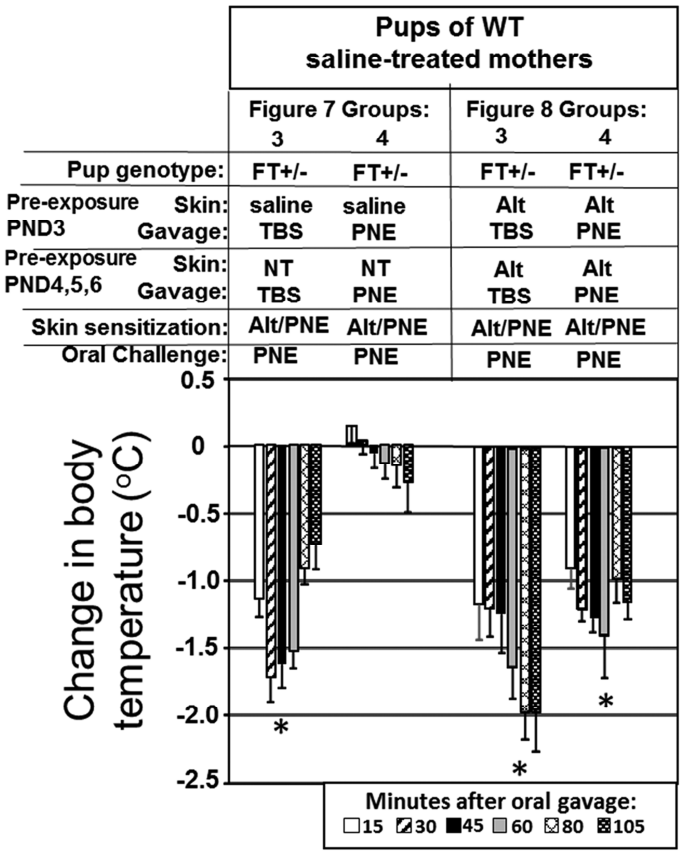

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