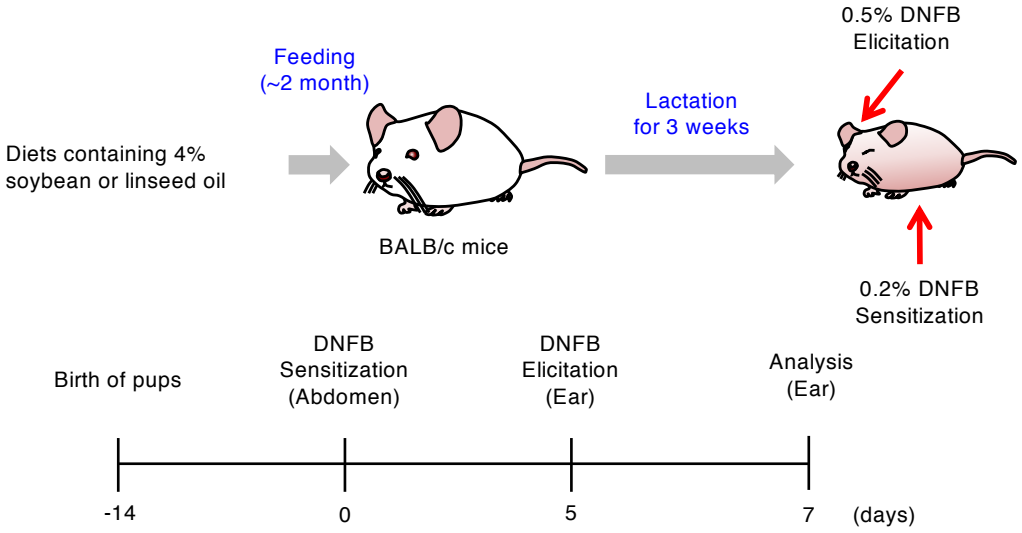
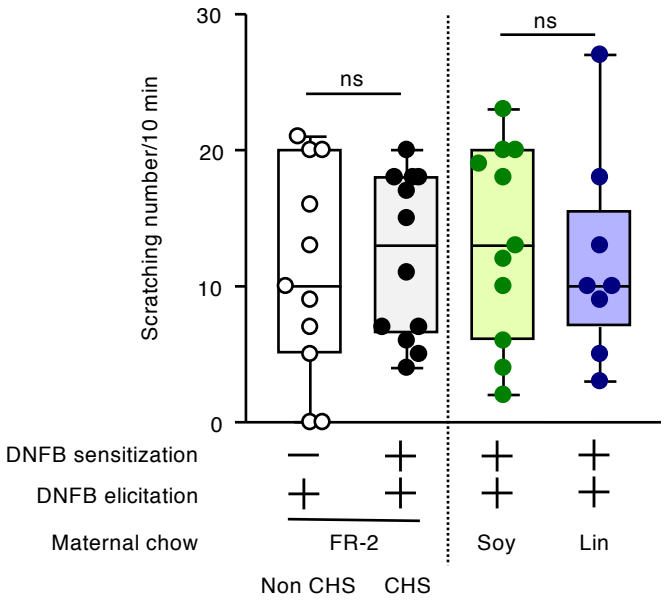
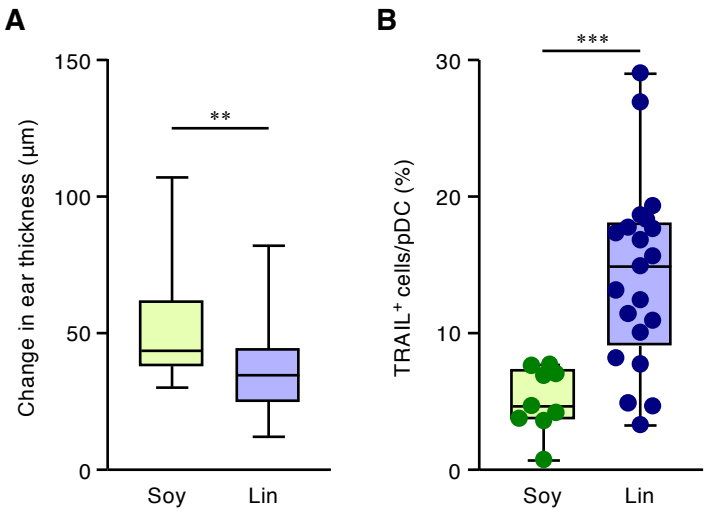
Supplemental Figure 1



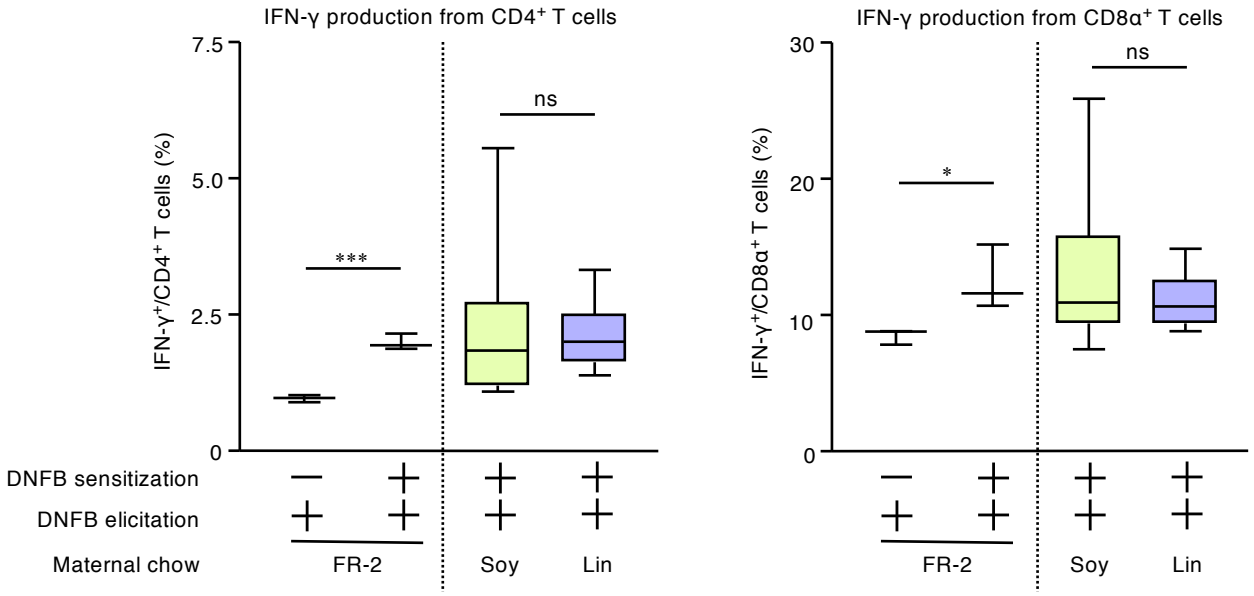
Supplemental Figure 1. Experimental protocol for the induction of DNFB-induced CHS in mouse pups.
Mouse dams were fed diets containing soybean oil or linseed oil for at least 2 months. After birth, pups were nursed by their dams for 2 weeks and then sensitized to DNFB. At day 5 after sensitization, infant mice were challenged with DNFB; CHS and immunologic responses were evaluated at 24 and 48 hours after challenge.



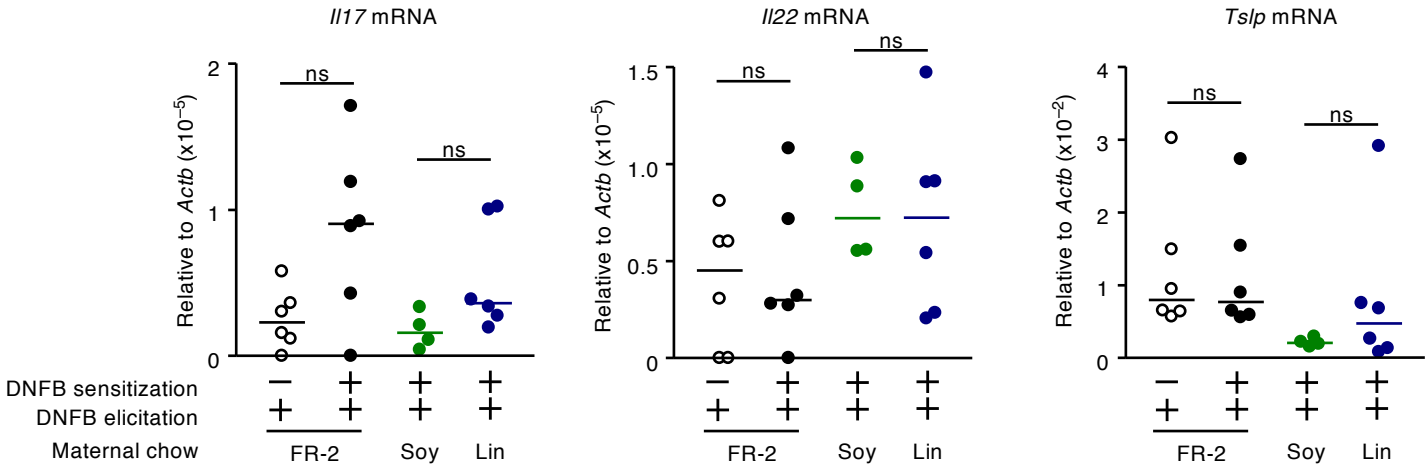
Supplemental Figure 2. No induction of ear scratching behavior of infant CHS models and no effects by maternal dietary oil intake
Dams were maintained on diets containing 4% soybean oil (Soy) or linseed oil (Lin), or purchased dams were maintained on FR-2 diet containing 4% soybean oil [FR-2 (Soy 4%)]; pups were nursed for 2 weeks and then sensitized to DNFB (day 0). At day 5, infants were challenged with DNFB, and ear scratching behavior was evaluated at 24 hours after challenge. Horizontal lines indicate median values. P values were obtained by using the two-tailed unpaired t-test (ns, not significant). The graph shows data from individual infant mice (n = 11 [non CHS], 12 [CHS], 11 [Soy] or 8 [Lin]); data were pooled from two representative independent experiments with reproducible results.



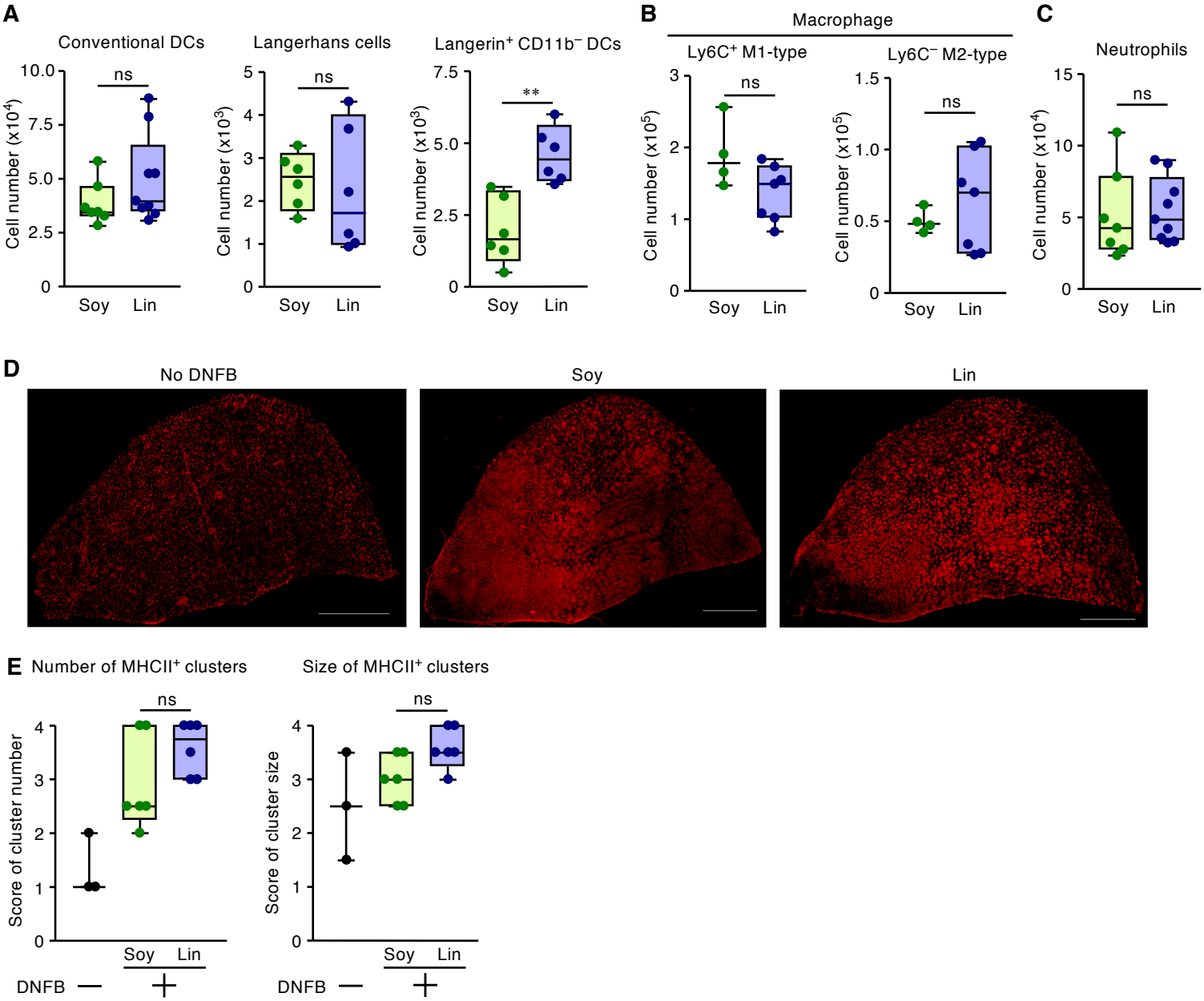
Supplemental Figure 3. Maternal dietary intake of linseed oil ameliorates Th2-type CHS in mouse pups.
Dams were maintained on diets containing 4% soybean oil (Soy) or linseed oil (Lin); pups were nursed for 2 weeks and then sensitized to FITC (days 0 and 1). At day 6, infants were challenged with FITC, and (**A**) ear swelling was evaluated as the difference in ear thickness between that at 24 hours after challenge and that before challenge. Horizontal lines indicate median values. P values were obtained by using the Mann–Whitney U-test (**, $P < 0.01$). The graph shows data from individual infant mice ($n = 22$ [Soy] or 42 [Lin]); data were pooled from three representative independent experiments with reproducible results. (**B**) The proportion of TRAIL-expressing dermal plasmacytoid dendritic cells (pDCs) among all dermal pDCs in pups was evaluated by using flow cytometry at 24 hours after FITC challenge. Horizontal lines indicate median values. *P* values were obtained by using the Mann–Whitney U-test (***, $P < 0.001$). Data from the left (i.e., challenged) ear of individual mice are shown and are representative of three independent experiments with reproducible results.



Supplemental Figure 4. Maternal dietary intake of linseed oil does not affect the splenic IFN-γ production from T cells in infant CHS. Pups were nursed for 2 weeks by dams maintained on diets containing 4% soybean oil (Soy) or linseed oil (Lin), or purchased dams were maintained on FR-2 diet containing 4% soybean oil [FR-2 (Soy 4%)], after which infant mice were DNFB sensitized to abdomen. Five days after sensitization, infants were challenged with DNFB to ear. Representative proportion of IFN-γ-producing $CD4^+$ and $CD8\alpha^+$ T cells in infant spleen at 48 hours after DNFB challenge to ear, as determined by using flow cytometry. The proportion of cells shown is for the spleen of each pup. Horizontal lines indicate median values. P values were obtained by using the two tailed unpaired t test (*, $P < 0.05$; ***, $P < 0.001$; ns, not significant). The graph shows data from individual infant mice ($n = 3$ [FR-2], 9 [Soy] or 8 [Lin]); Data were pooled from one or two representative independent experiments with reproducible results.

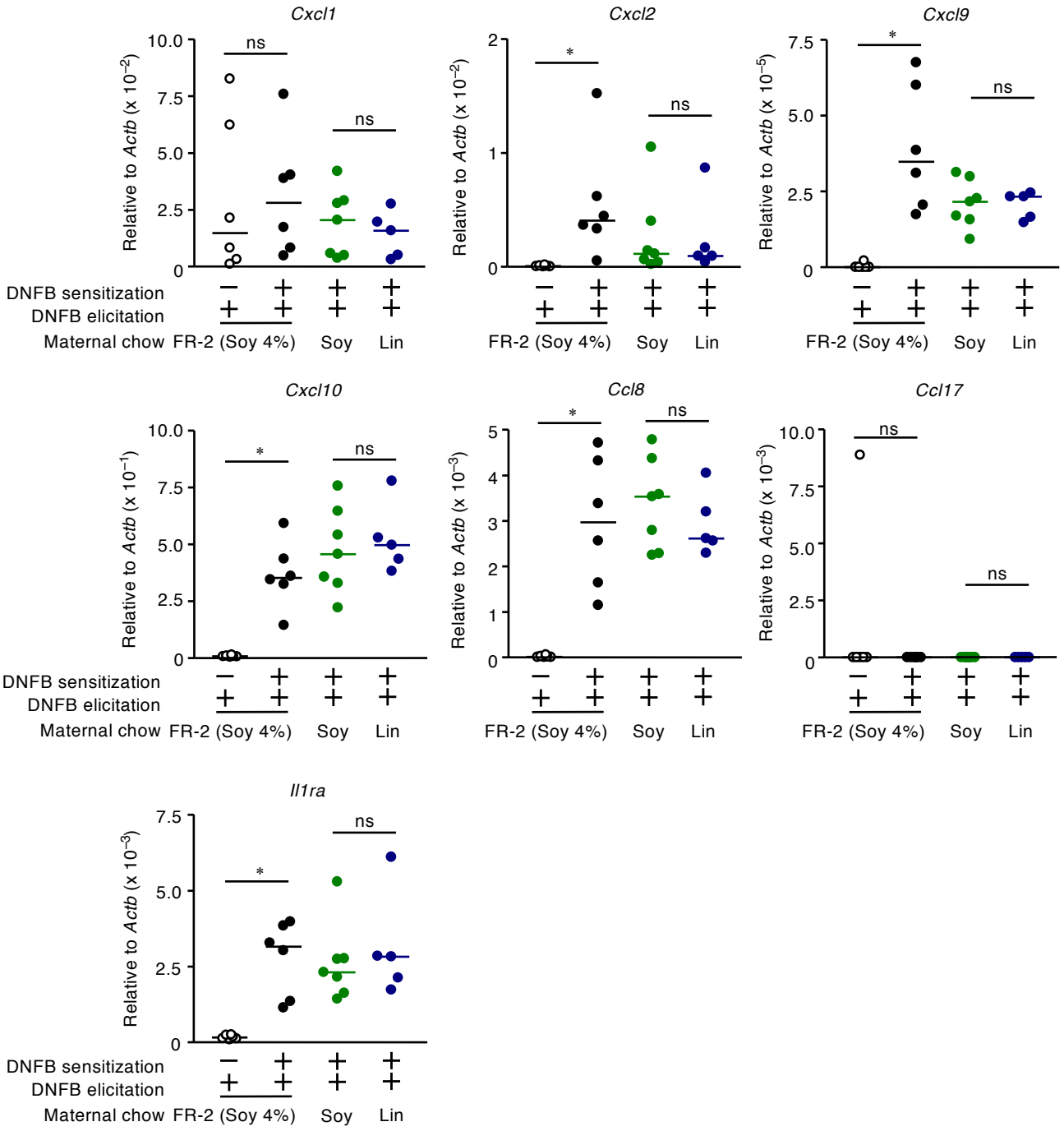


Supplemental Figure 5. Maternal dietary intake of linseed oil does not affect the mRNA levels of IL-17, IL-22, and TSLP in the ear skin of their pups in DNFB CHS
Dams were maintained on diets containing 4% soybean oil (Soy) or linseed oil (Lin), or purchased dams were maintained on FR-2 diet containing 4% soybean oil [FR-2 (Soy 4%)]; pups were nursed for 2 weeks and then sensitized to DNFB (day 0). At 48 hours after sensitized pups were challenged with DNFB, we evaluated the mRNA expression levels of *Il17, Il22,* and *Tslp* in the ear skin of 3-week-old pups. Horizontal lines indicate median values. P values were obtained by using the Mann–Whitney U-test (ns, not significant). The graph shows data from individual infant mice (n = 6 [FR-2], 4 [Soy] or 6 [Lin]). Data were pooled from one or two representative independent experiments with reproducible results.

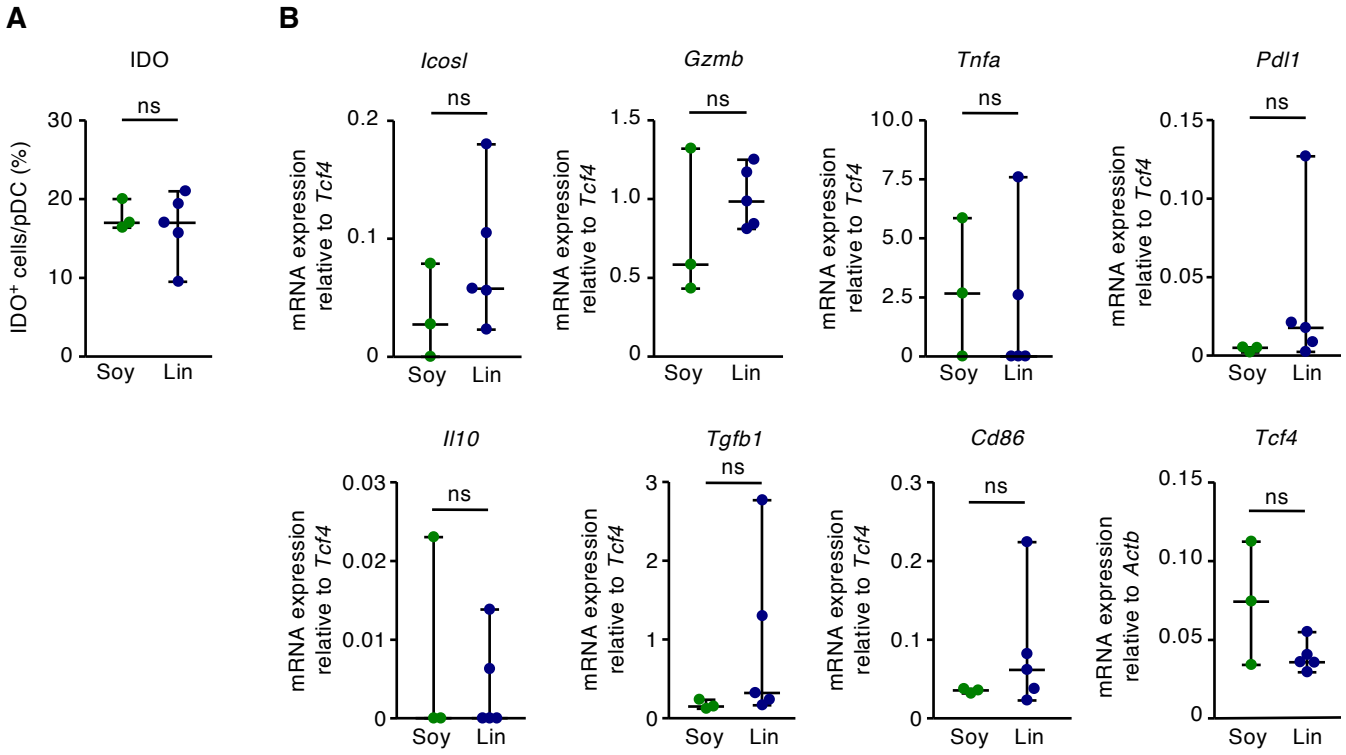


Supplemental Figure 6. Inflammatory and regulatory cells during the elicitation phase of infant CHS. At 48 hours after DNFB challenge, (**A**) CD11c$^+$ CD11b$^+$ conventional dendritic cells (DCs), Langerin$^+$ CD11b$^+$ Langerhans cells, and Langerin$^+$ CD11b$^-$ DCs; (**B**) Ly6C$^+$ (M1-type) and Ly6C$^-$ (M2-type) F4/80$^+$ CD11b$^+$ macrophages; and (**C**) Ly6G$^+$ CD11b$^+$ neutrophils in infant skin were counted. Cell counts were obtained from the left (i.e., challenged) ear of individual mice. (**D**) At 24 hours after DNFB challenge, whole mounts of infant ear skin were stained by using an anti-MHC class II monoclonal antibody (mAb). (**E**) The formation of inducible skin-associated lymphoid tissue was quantified by scoring according to the size and number of clusters. Horizontal lines indicate median values. *P* values were obtained by using the Mann–Whitney U-test (**, $P < 0.01$; ns, not significant). Data are representative of one to three independent experiments with reproducible results.

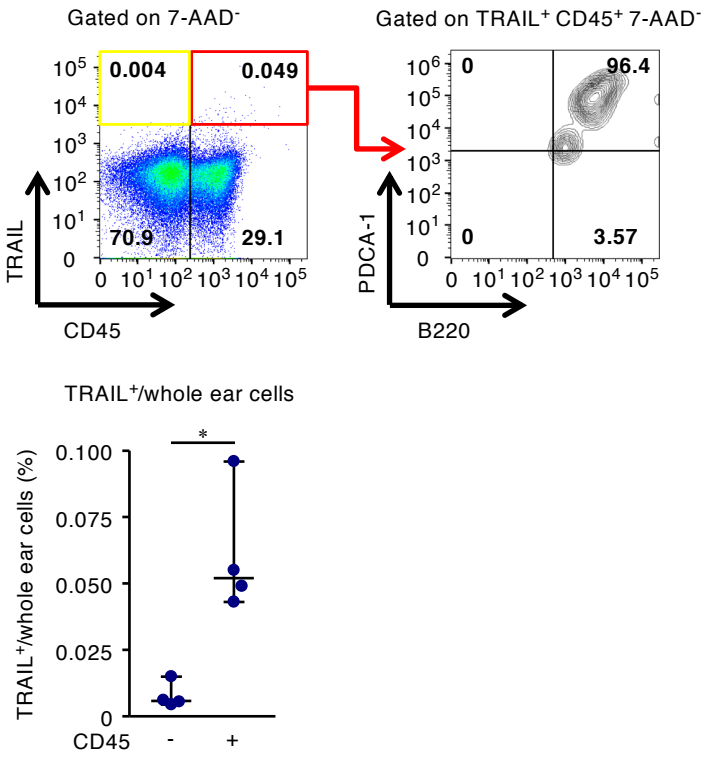
Supplemental Figure 7



Supplemental Figure 7. Maternal dietary intake of linseed oil do not affect chemokines production and IL-1Ra production in epidermal cells of their pups.
Pups were nursed for 2 weeks by dams maintained on diets containing 4% soybean oil (Soy) or linseed oil (Lin), or purchased dams were maintained on FR-2 diet containing 4% soybean oil [FR-2 (Soy 4%)], after which infant mice were DNFB sensitized to abdomen. At day 5, infants were challenged with DNFB. The mRNA expression levels of TRAIL receptor genes (*Cxcl1, Cxcl2, Cxcl9, Cxcl10, Ccl8, Ccl17 and Il1ra*) in the epidermal sheet in CHS of 3-week-old pups in Soy or Lin. Horizontal lines indicate median values. P values were obtained by using the Mann–Whitney U-test (ns, not significant). The graph shows data from individual infant mice ($n = 6$ [FR-2], 11 [Soy] or 8 [Lin]); data were pooled from two representative independent experiments with reproducible results.

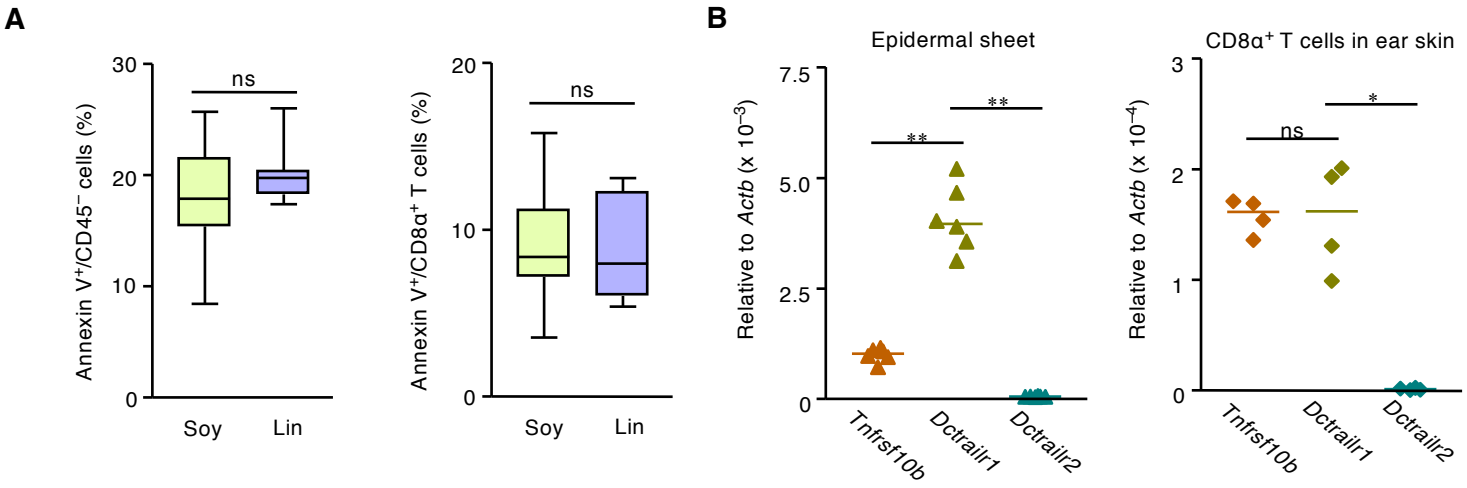


Supplementary Figure 8. The proportion of indoleamine 2,3-dioxygenase (IDO)-positive pDCs and the mRNA levels of *Icosl*, *Gzmb*, *Tnfa*, and *Tcf4* in pDCs in a murine model of infant CHS. At 48 hours after sensitized pups were challenged with DNFB, we evaluated (**A**) the proportion of IDO$^+$ cells among dermal pDCs and (**B**) the mRNA levels of *Icosl*, *Gzmb*, *Tnfa*, *Pdl1*, *Il10*, *Tgfb1*, *Cd86* and *Tcf4* expressed in dermal pDCs. Horizontal lines indicate median values. *P* values were obtained by using the Mann–Whitney U-test (ns, not significant).

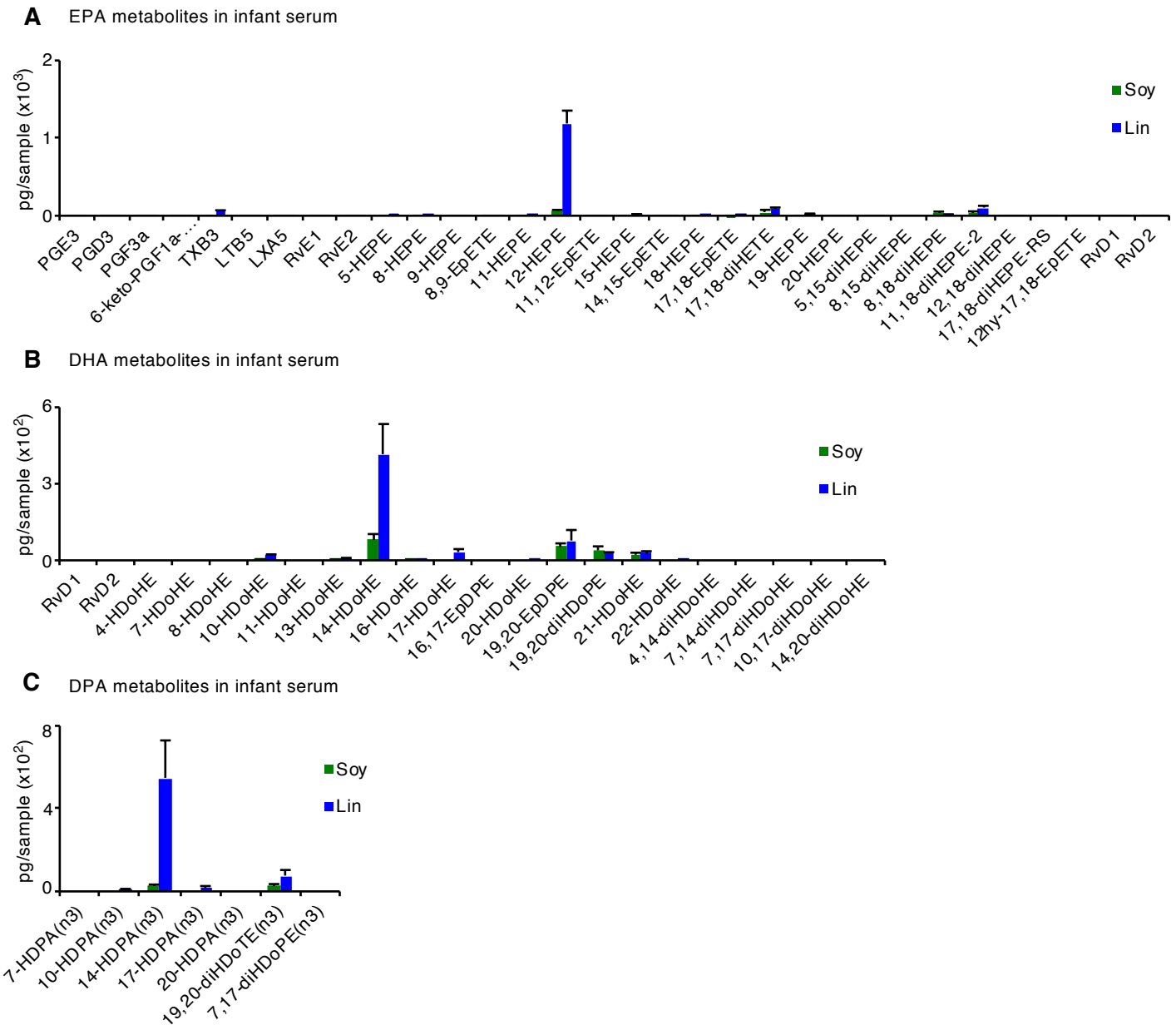


Supplemental Figure 9. Characteristics of the main subset of TRAIL-expressing cells induced by maternal intake of linseed oil

Representative plots of flow cytometric analysis and the proportions of TRAIL-expressing $CD45^{-}$ or $CD45^{+}$ cells after the induction of CHS are shown. Representative plots of the flow cytometric analysis of PDCA-1 and B220 gated on $TRAIL^{+}$ $CD45^{+}$ cells are shown. Horizontal lines indicate median values. *P* values were obtained by using the Mann–Whitney U-test (*, $P < 0.05$).

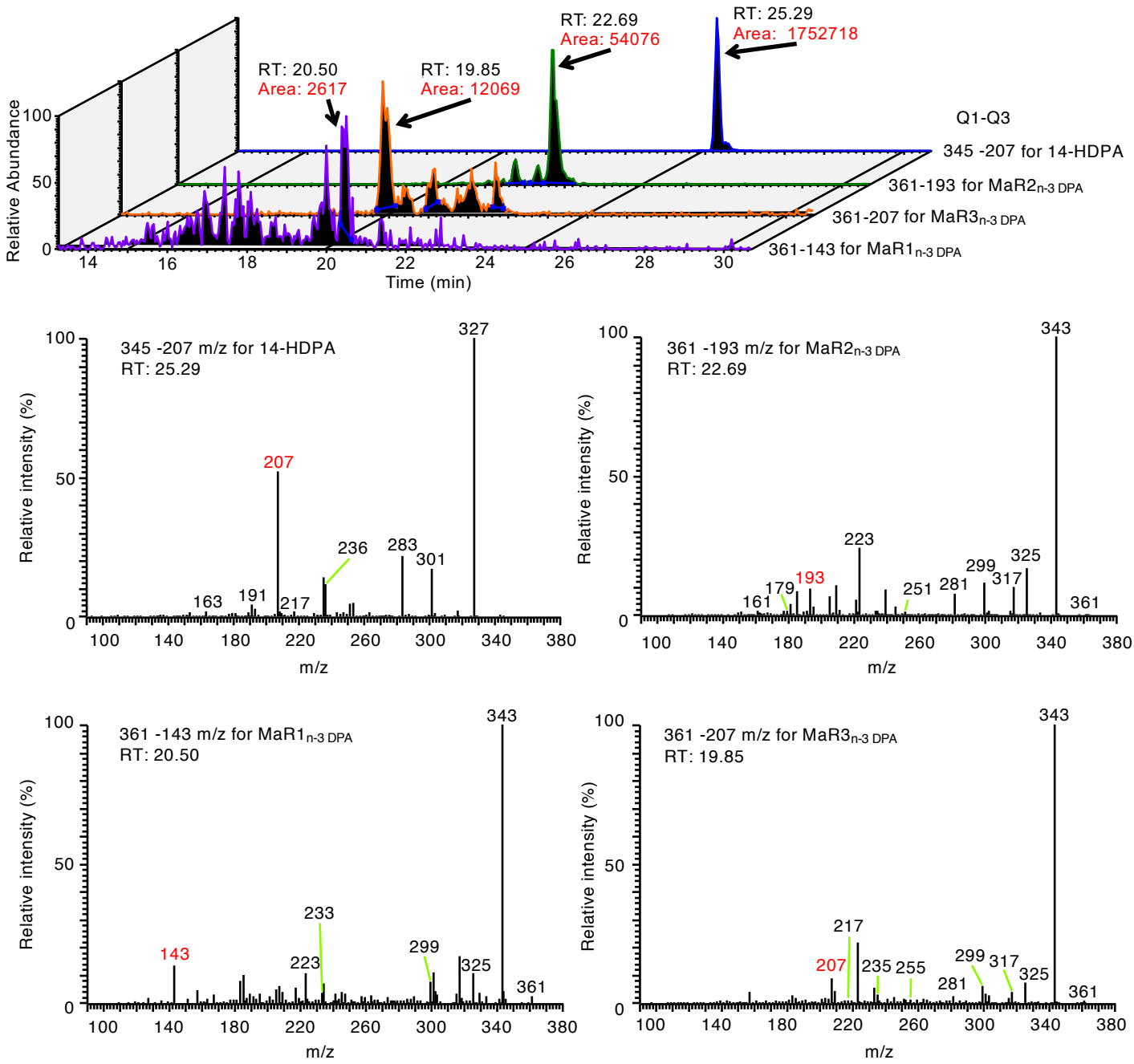


Supplemental Figure 10. Maternal dietary intake of linseed oil do not affect apoptosis in epidermal cells and/or in CD8α⁺ T cells of their pups.
(A and B) Dams were maintained on diets containing 4% soybean oil (Soy) or linseed oil (Lin); pups were nursed for 2 weeks and then sensitized to DNFB (day 0). At day 5, infants were challenged with DNFB. (A) Representative proportion of Annexin V⁺ in CD45⁻ or CD8α⁺ T cells in infant ear skin at 48 hours after DNFB challenge to ear, as determined by using flow cytometry. (B) The mRNA expression levels of TRAIL receptor genes (*Tnfrsf10b*, *Dctrailr1*, and *Dctrailr2*) in the epidermal sheet and sorted dermal CD8α⁺ T cells in CHS of 3-week-old pups. Horizontal lines indicate median values. P values were obtained by using the Mann–Whitney U-test (*, $P < 0.05$; **, $P < 0.01$; ns, not significant).



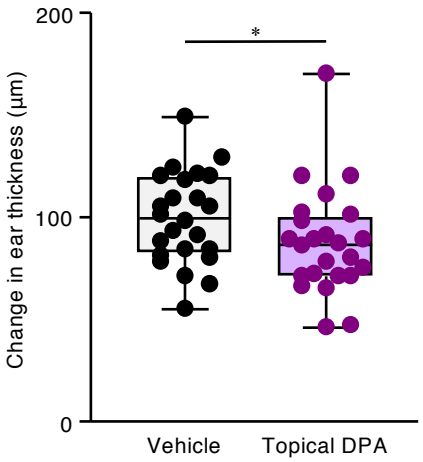
Supplemental Figure 11. EPA, DHA, and DPA metabolites in infant serum due to maternal intake of linseed oil or soybean oil.
Serum was collected from 2-week-old pups for the measurement of metabolites of (**A**) EPA, (**B**) DHA, and (**C**) DPA. Results are expressed as mean ± SEM. Source data are provided as a Source Data file.

Supplemental Figure 12

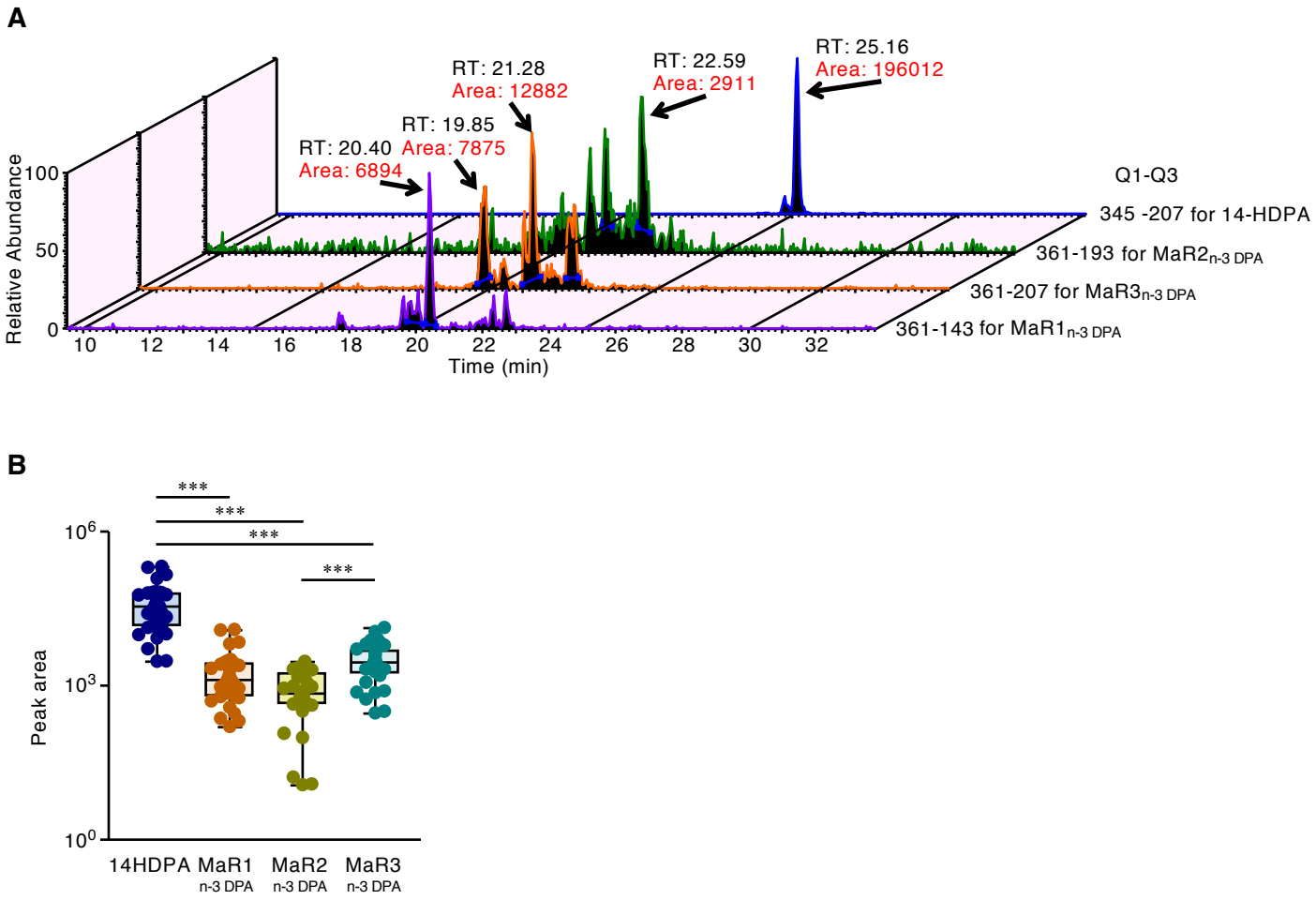


Supplemental Figure 12. Mass spectrometric profiles of DPA-derived 14-lipoxygenation products

To generate 14-lipoxygenation products, DPA was combined with recombinant 12-LOX protein, and the lipid fraction was evaluated through mass spectrometry. Representative chromatographs obtained by multiple reaction monitoring of the parent ion (Q1) and a diagnostic daughter ion (Q3) during MS/MS for the 14-lipoxygenation products 14-HDPA, $MaR1_{n\text{-}3\ DPA}$, $MaR2_{n\text{-}3\ DPA}$, and $MaR3_{n\text{-}3\ DPA}$ are shown.

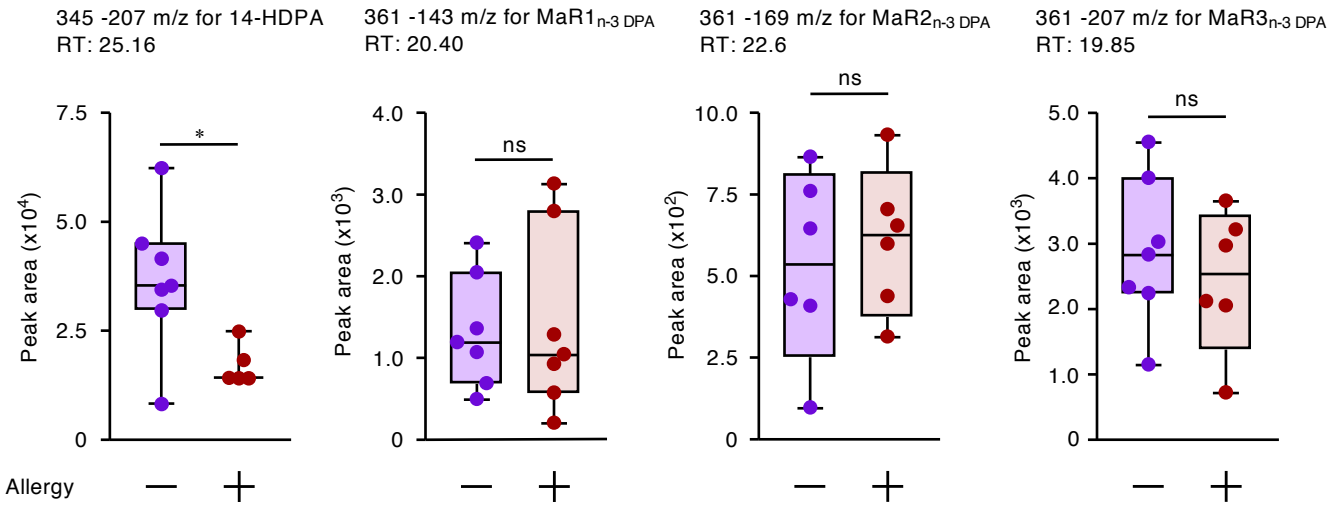


Supplemental Figure 13. Topical treatment of DPA inhibits the development of infant CHS.
Infant mice were topically treated with DPA (10 μg/skin of abdomen or each ear) or vehicle (Veh) at 30 minutes before DNFB sensitization and elicitation; ear swelling was calculated as the difference between ear thickness before and at 48 hours after DNFB challenge. Horizontal lines indicate median values. *P* values were obtained by using the Mann–Whitney U-test (*, $P < 0.05$). The graph shows data from individual infant mice and data were pooled from two independent experiments with reproducible results.



Supplemental Figure 14. Detection and measurement of 14-lipoxygenation products of DPA in human breast milk

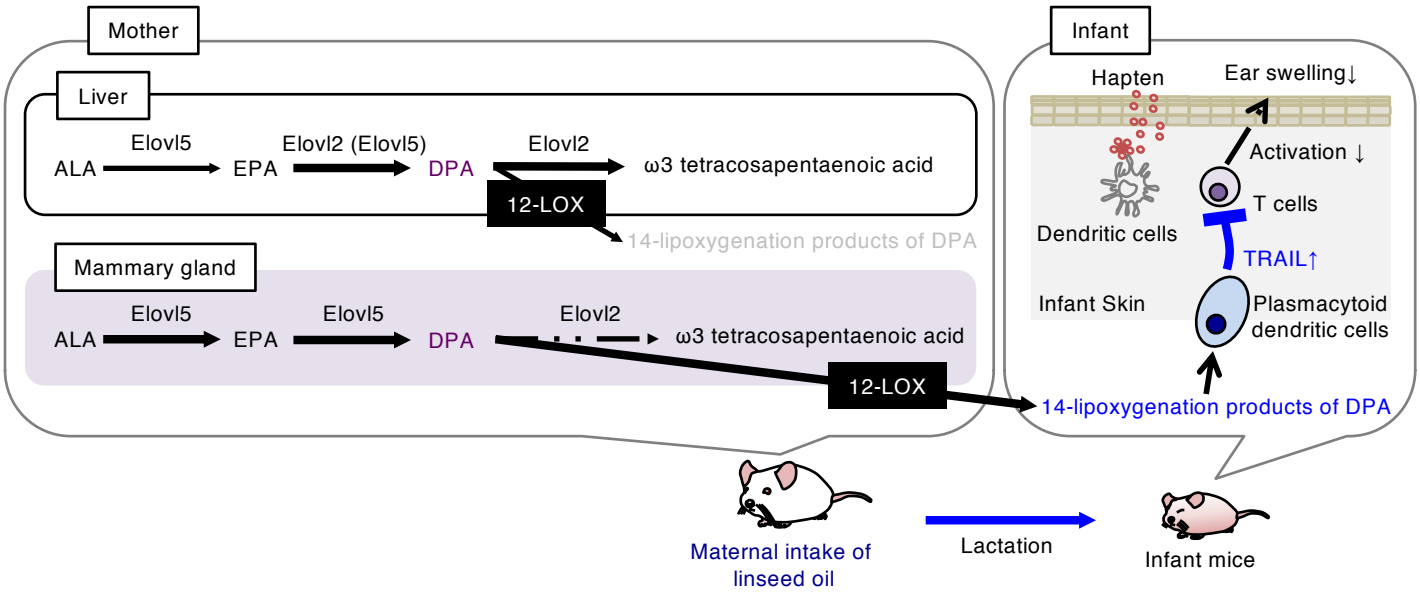
Human breast milk samples ($n = 34$) were collected within 1 month after childbirth, and 14-lipoxygenation products of DPA were identified by lipidomics analysis. (**A**) Representative chromatographs obtained by multiple reaction monitoring of the parent ion (Q1) and a diagnostic daughter ion (Q3) during MS/MS; representative MS/MS spectra used to identify the 14-lipoxygenation products 14-HDPA, $MaR1_{n\text{-}3\ DPA}$, $MaR2_{n\text{-}3\ DPA}$, and $MaR3_{n\text{-}3\ DPA}$ are shown. (**B**) The peak areas of these four 14-lipoxygenation products were compared with each other; samples were not differentiated in regard to infant allergic status. Horizontal lines indicate median values. *P* values were obtained by using Dunn's multiple-comparison test (***, $P < 0.001$). Source data are provided as a Source Data file.



Supplemental Figure 15. Human breast milk from mothers of allergic infants contains low amounts of 14-lipoxygenation products of DPA

Human breast milk samples were collected within 1 month after childbirth, and 14-lipoxygenation products of DPA were identified by lipidomics analysis. Allergy (atopic dermatitis and food allergy) onset in sensitized infants (age, 1 year or younger) was diagnosed by a pediatrician. The peak area of each 14-lipoxygenation product in breast milk was compared between mothers whose infants were allergic compared with those who were not. Horizontal lines indicate median values. P values were obtained by using the Mann–Whitney U-test (*, $P < 0.05$; ns, not significant). The graph shows data from individual maternal milk samples.

Supplemental Figure 16



Supplemental Figure 16. Regulation of skin inflammation in mouse pups through the metabolism of dietary lipid in breast milk.

Maternally ingested linseed oil preferentially yields 14-lipoxygenation products of DPA in the dams' milk, which is transferred to nursing pups. In pups, these products induce the expression of TRAIL on dermal pDCs. In turn, TRAIL down-regulates the activation of T cells and thus suppresses infant CHS.