Supplemental

Materials and Methods

Murine ear acute inflammation measurements

Wild-type mice and PAFR KO mice were treated with 10 μl of lipids extracted from 2.5 x 10^{11} HaCaT derived MVPs or vehicle (acetone), CPAF (100 μM), TPA (100 μM). Ear thickness was measured before and 2 hours post-treatment using Mitutuyo calipers. Differences in ear swelling was used as a surrogate for the inflammatory responses (S1).

Real-time PCR Primer Sequences

The following primer sequences were used: for EGFP, 5'-GAGCTGAAGGGCATCGACTTCAAG-3' (forward) and 5'-GGACTGGGTGCTCAGGTAGTGG-3' (reverse).

Murine genes: for IL-10, 5'-AGAATGCCAGCAACCTCTCCA-3' (forward) and 5'-TCCCACTTCCTTCCGATGGTTAC-3' (reverse); for TGF-beta, 5'-AGACGTGGGGACTTCTTGGC-3'(forward) and 5'-GTCCGGAATAGGGGCGTCTG-3' (reverse); for Interferon gama (IFN-r), 5'-TGGCATAGATGTGGAAGAAAAGA-3' (forward) and 5'-TGCAGGATTTTCATGTCACCAT-3' (reverse); for IL-12; 5'-CAGAAGCTAACCATCTCCTGGTTTG-3'(forward) and 5'-TCCGGAGTAATTTGGTGCTTCACAC-3' (reverse); for beta actin, 5'-

TGGAATCCTGTGGCATCCATGAAAC-3'(forward) and 5'-

TAAAACGCAGCTCAGTAACAGTCCG-3' (reverse). For murine Foxp3,

5'-CCTGGTTGTGAGAAGGTCTTCG-3' (forward) and 5'-

TGCTCCAGAGACTGCACCACTT-3' (reverse)

Reference for Supplemental.

S1. Sahu RP, Rezania S, Ocana JA, DaSilva-Arnold SC, Bradish JR, Richey JD, Warren SJ, Rashid B, Travers JB, Konger RL Topical application of a platelet activating factor receptor agonist suppresses phorbol ester-induced acute and chronic inflammation and has cancer chemopreventive activity in mouse skin. *PLoS One* **2014,** *9* (11), e111608.

SUPPLEMENTARY FIGURES

Supplementary Figure 1. UVB and CPAF induce MVP release in NTERT and primary human keratinocytes.

Supplementary Figure 2. Effect of UVB versus thermal burn injury on viability in HaCaT Keratinocytes.

Supplementary Figure 3. CPAF and UVB upregulates aSMase enzymatic activity in HaCaT Keratinocytes.

Supplementary Figure 4. Fluorescent images of CaSR expression in HaCaT, KBP and fibroblast cells.

Supplementary Figure 5. CaSR is a keratinocyte-selective marker for cell-derived MVPs.

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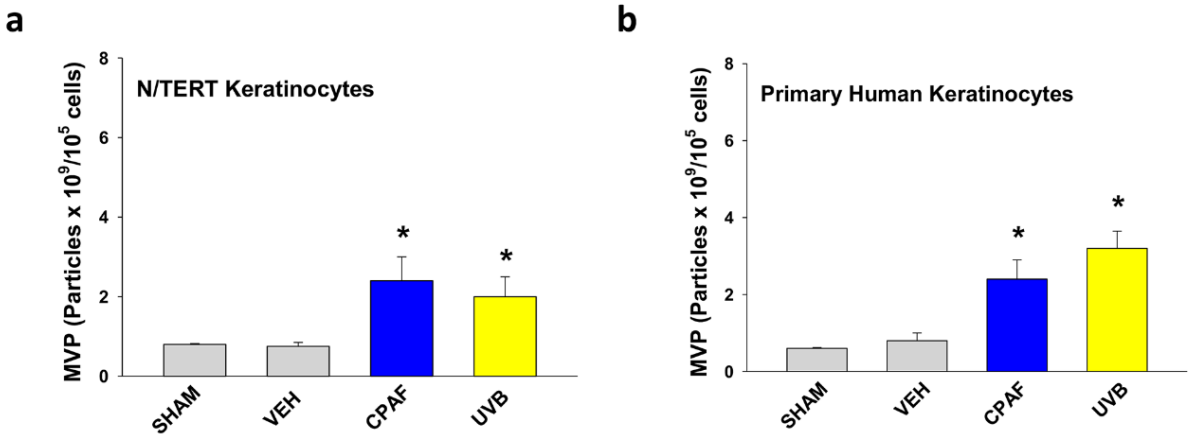
Supplementary Figure 9. Topical application of lipid extracts from UVB-MVP induce ear swelling via PAFR activation.

Supplementary Figure 10. UVB-MVP from wild-type but not *Spmd1-/-* mice contain PAFR-agonistic activity.

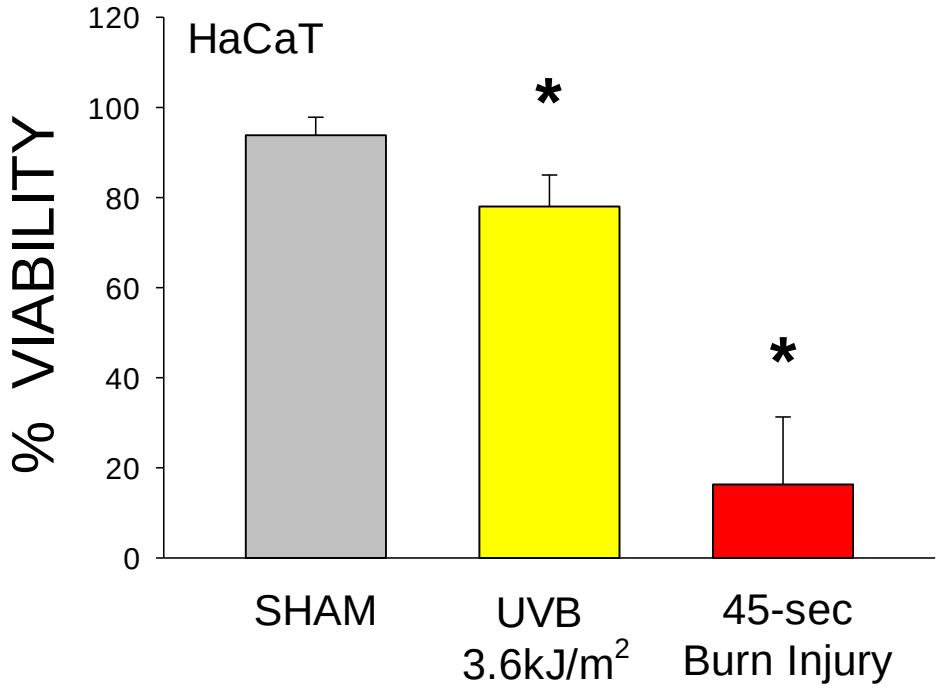
Supplementary Figure 11. UVB does not inhibit CHS reactions in *Spmd1-/-* mice.

Supplementary Figure 12. Topical imipramine blocks CHS reactions from UVB but not CPAF.

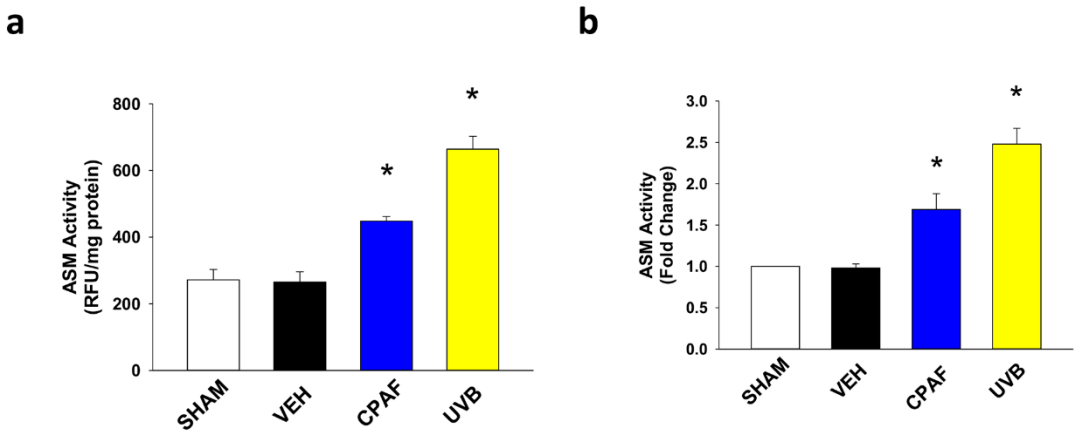
Supplementary Figure 13. MVP characterization by western blotting and transmission electron microscopy.



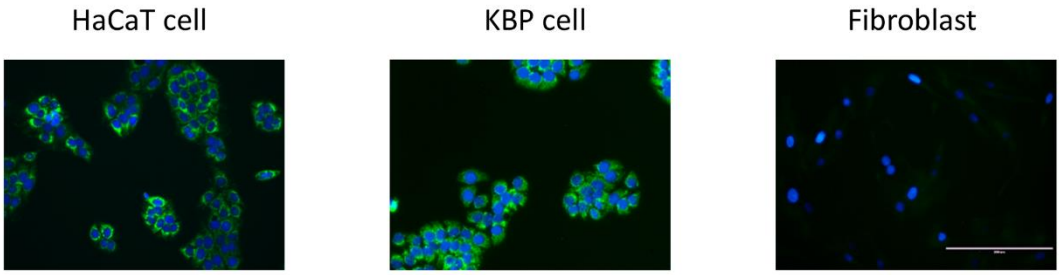
Supplementary Figure 1. UVB and CPAF induce MVP release in NTERT and primary human keratinocytes. a) N/TERT and **b)** Primary keratinocytes were treated with either vehicle (0.1% ethanol), CPAF (100 nM) or UVB (3,600 J/m^2). After 4 hours, MVP were collected and quantified. Data are mean ± SE of at least three independent experiments. *Denotes statistically significant differences ($p < 0.05$) from control using One-way ANOVA test.



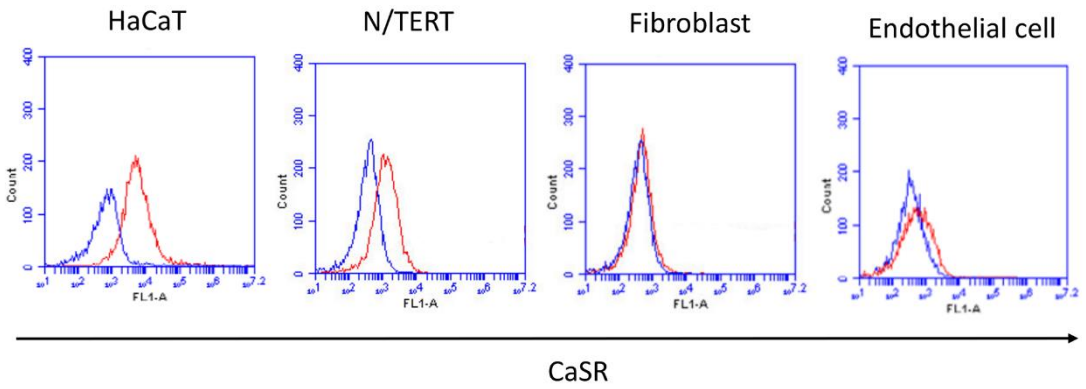
Supplementary Figure 2. Effect of UVB versus thermal burn injury on viability in HaCaT Keratinocytes. HaCaT cells were treated with either UVB (3,600 J/m^2), a thermal burn injury of 45 second exposure to 90 ^{o}C waterbath (as per reference # 28), or no treatment (SHAM). At 4h, the supernatants were removed, and cells were trypsinized. The total numbers of cells in supernatants + trypsinized cells were collected and stained with 0.4% trypan blue. Cell viability (%) were calculated by viable cells (non-stained cells) / total cell numbers x 100%. The data are the Mean ± SE % viable cells from 2 separate experiments. * Denotes statistically significant differences ($p < 0.05$) from untreated cells using One-way ANOVA test.



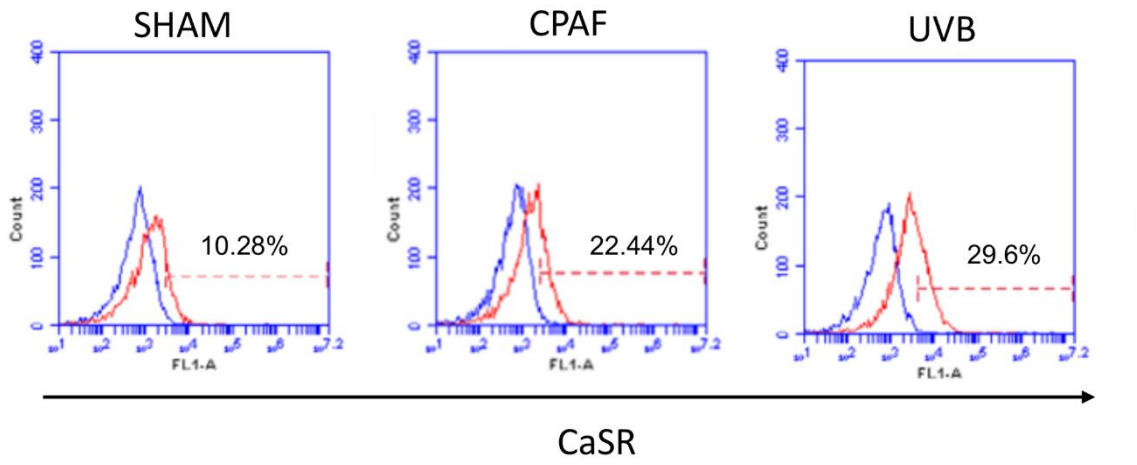
Supplementary Figure 3. CPAF and UVB upregulates aSMase enzymatic activity in HaCaT Keratinocytes. HaCaT cells were treated with either vehicle (0.1% ethanol), CPAF (100 nM) or UVB (3,600 J/m^2). After 4 hours, treated HaCaT cells were trypsinized and protein was extracted. Acid sphingomyelinase enzymatic activity was detected using the Acid Sphingomyelinase Activity Colorimetric Assay Kit (Abcam) using manufacturer's protocol. The aSMase activity was determined by measuring a colorimetric product with absorbance at 570 nm (A570) proportional to the enzymatic activity present. Data are mean ± SE of at least three independent experiments (N = 3 for CPAF; N= 4 for UVB). *Denotes statistically significant differences ($p < 0.05$) from control using One-way ANOVA test.



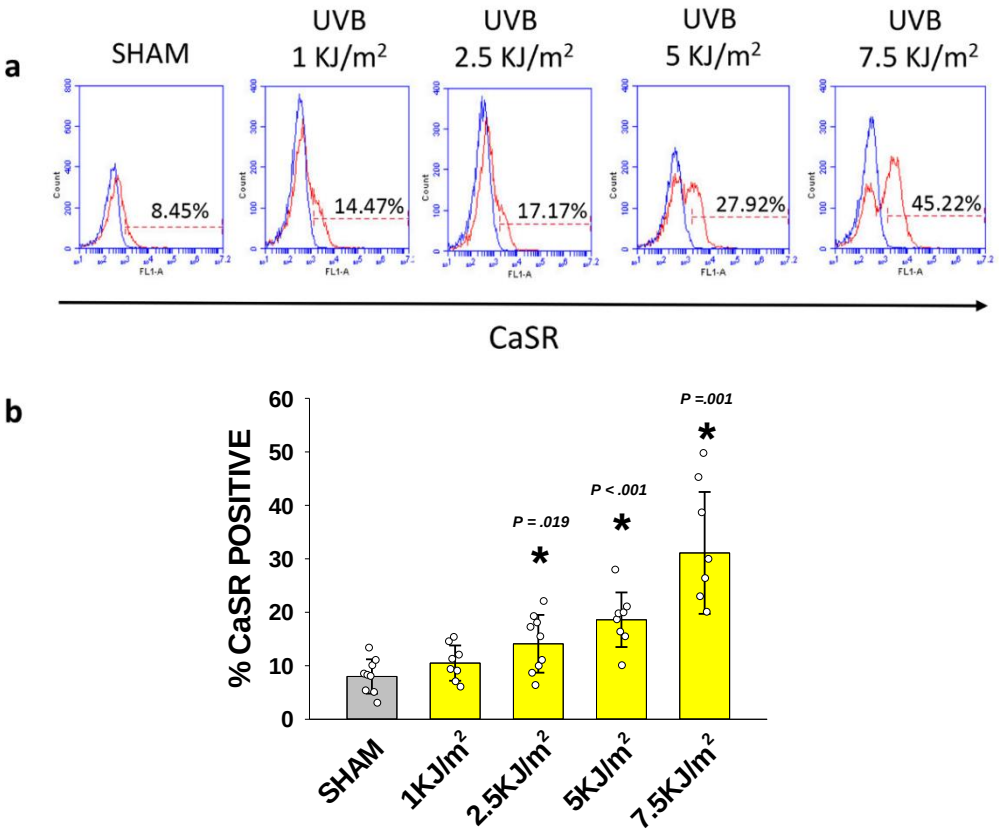
Supplementary Figure 4. Fluorescent images of CaSR expression in HaCaT, KBP and fibroblast cells. Human keratinocyte cell line HaCaT, the PAFR positive nasal epithelioid carcinoma cell line KBP and primary cultures of human dermal fibroblasts were stained with anti-CaSR antibody and Alexa fluor 488 for CaSR expression detection, and counterstained with DAPI. Data are representative of two-three separate experiments. Bar 200μm



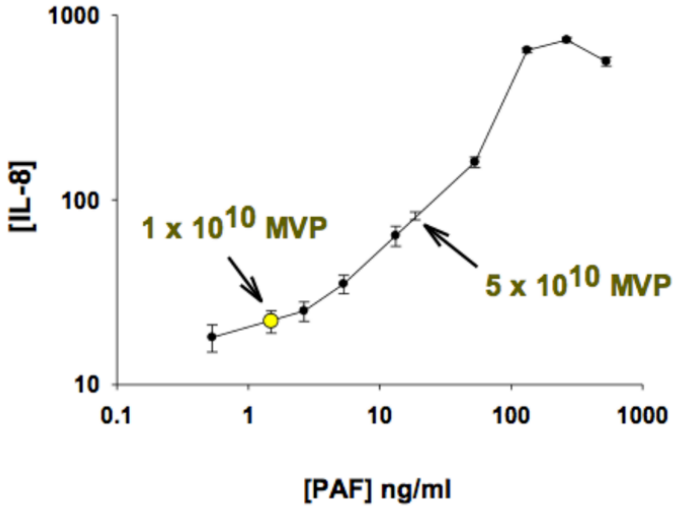
Supplementary Figure 5. CaSR is a keratinocyte-selective marker for cell-derived MVPs. HaCaT, N/TERT, human primary cultures of dermal fibroblasts and HUVEC endothelial cell-derived MVPs were stained with CaSR-FITC antibody, and CaSR expression were detected by flow cytometry. MVPs were stained with either isotype control (blue line) or CaSR antibody (red line). CaSR-positive MVPs were determined using flow cytometry by CaSR-stained MVPs exclude the isotype control-stained MVPs. Data are representative from three separate experiments.



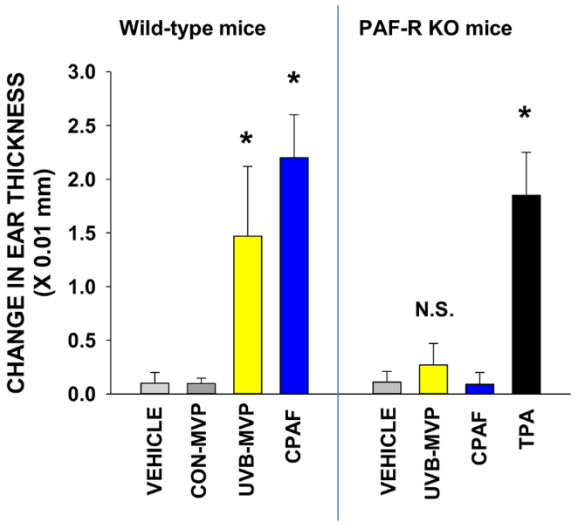
Supplementary Figure 6. UVB- and CPAF-induced blister MVP are CaSR positive. Suction blisters were formed on human explant skin from discarded abdominoplasty surgeries and treated with either topical CPAF (100 nM) or UVB (2,500 J/m^2), MVPs were isolated from blister fluids 4 hours after treatment as per our previously published protocol [11]. Blister fluid MVPs were stained with either isotype control (blue line) or CaSR antibody (red line). CaSR-positive MVPs were determined using flow cytometry by CaSR-stained MVPs exclude the isotype control stained MVPs. Data are representative from three separate experiments. Numbers of MVP measured in the various treatments were as follows (mean ± [SE] x 10^7/mg): Sham 2.3 ± [0.6]; UVB 21 ± [8.2]; CPAF18 ± [5.2].



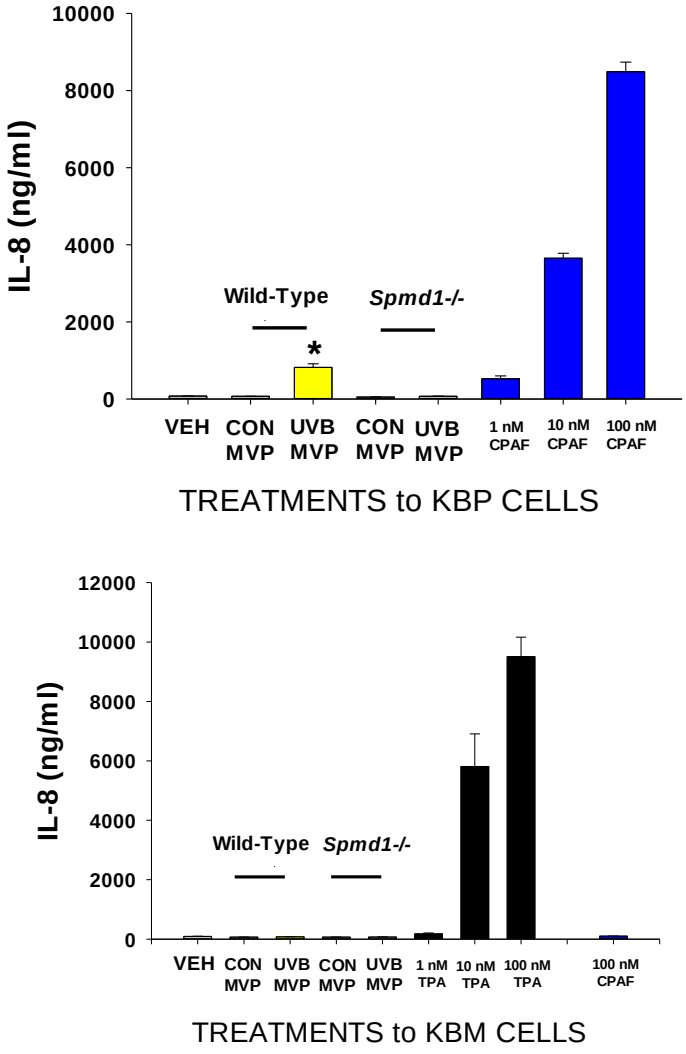
Supplementary Figure 7. Increased murine blood plasma MVPs following UVB irradiation are CaSR positive. **a)** Examples of CaSR expression on blood plasma MVPs isolated from wild type mice treated with various UVB fluences (from Figure 2e) measured by flow cytometry. MVPs were stained with either isotype control (blue line) or CaSR antibody (red line). CaSR-positive MVPs were determined using flow cytometry by CaSR stained MVPs excluding the isotype control stained MVPs. **b)** Percentage of the CaSR-positive MVP in wild-type mice blood plasma post treatment. Data are mean ± SD using at least 7 mice per group. *Denotes statistically significant ($p < 0.05$) differences from sham control using One-way ANOVA test.



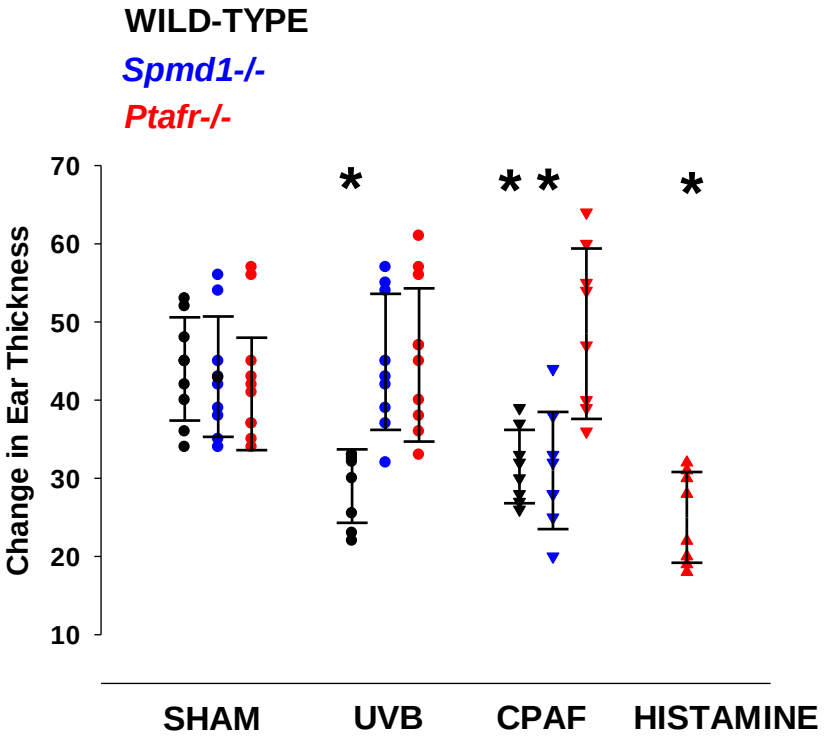
Supplementary Figure 8. Semi-quantification of UVB-MVP PAFR-agonistic activity using KBP cell release of IL-8 protein. PAFR-positive KBP cells were incubated with various concentrations of PAF (1-hexadecyl-2-acetyl-GPC) or lipid extracts derived from 1 x 10^{10} and 5 x 10^{10} MVP collected from 3,600 J/m^2 UVB treated HaCaT keratinocytes for 120 minutes. Four hours after treatment of KBP cells, supernatants were collected, and IL-8 levels measured by ELISA. The data were the Mean ± SD IL-8 levels of duplicate samples normalized to 10^5 cells from a single experiment. The amounts of PAF activity were approximately 1.5 ng/ml for 1 x 10^{10} and 18 ng/ml for 5 x 10^{10} MVP. Given that the latter value appears to be in the linear range of the assay, it is more likely the most accurate value. Neither PAF nor UVB-MVP generated IL-8 release in PAFR-negative KBM cells, with TPA as positive control (*not shown*).



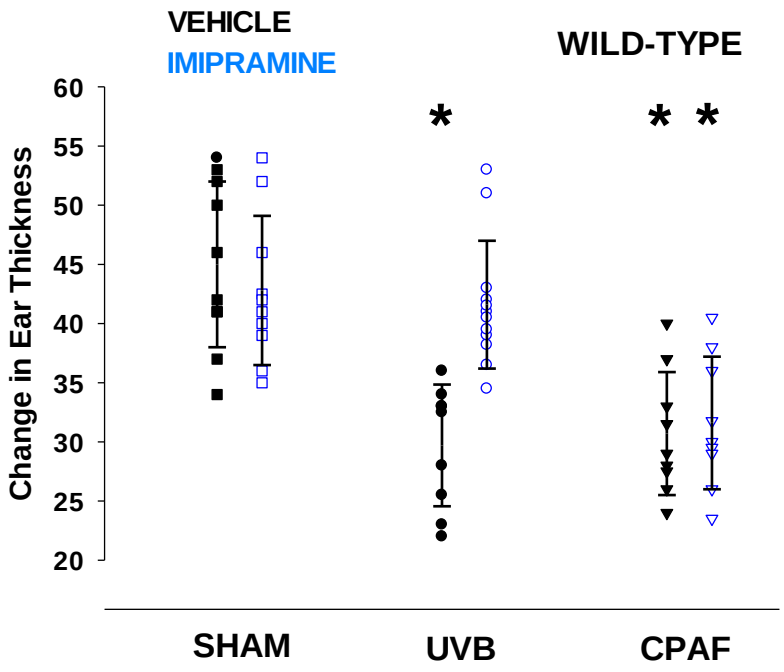
Supplementary Figure 9. Topical application of lipid extracts from UVB-MVP induce ear swelling via PAFR activation. 10 μl of MVP lipids extracted from 2.5 x 10^{11} control or 3,600J/m^2 UVB-treated HaCaT keratinocyte-derived MVPs at 120 minutes or acetone vehicle were added to wild-type or PAFR-deficient mice ear. Ear thickness were measured before and 2 hours post-treatment as an indirect measure of skin inflammation (S1). 100 μM CPAF and 100 μM TPA were used as control treatments in wild-type mice and PAFR-deficient mice. Data are mean ± SE changes in ear thickness from three separate experiments. *Denotes statistically significant ($p < 0.05$) differences from vehicle control using One-way ANOVA test.



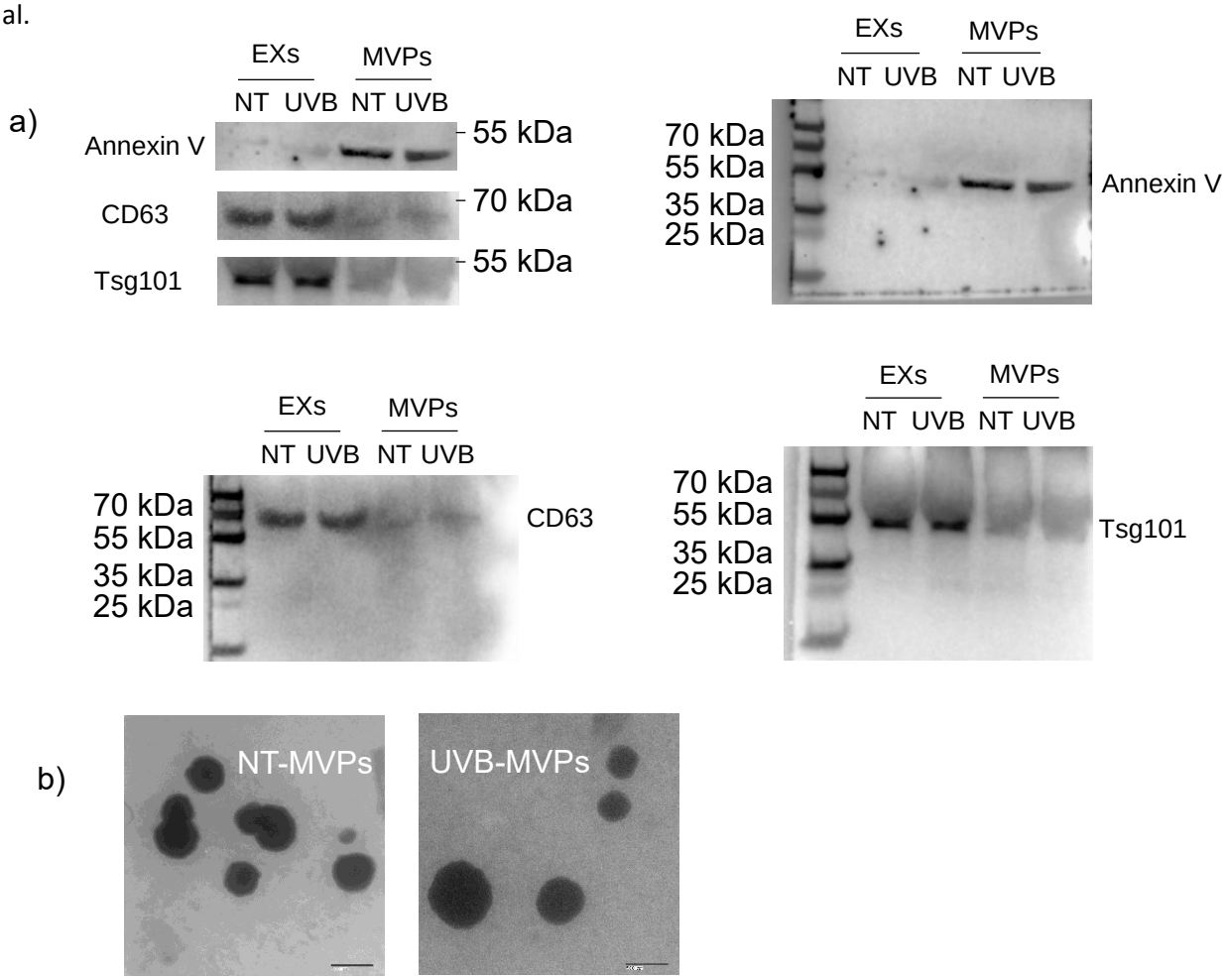
Supplementary Figure 10. UVB-MVP from wild-type but not aSMase KO mice contain PAFR-agonistic activity. Groups of 4 wild-type and aSMase KO mice (*Spmd1-/-*) were treated with 7.5 kJ/m2 UVB or no treatment (CON) to the entire shaved back skin. At 4 h post treatment, the mice were sacrificed, exsanguinated, and MVP and exosome fractions isolated (exosome fractions consisted of an additional 120,000 x g for 70 minutes. Lipid extracts were obtained from the MVP or exosome fractions and were given to PAFR-positive KBP cells or PAFR-negative KBM cells and 8h later the supernatants harvested and IL-8 release measure by ELISA. CPAF or TPA were used as positive controls. The data depicted are mean ± SE IL-8 levels from duplicate experiments from UVB-MVP. Treatment of KBP/KBM cells with lipid extracts from the exosome fractions did not result in increased levels of IL-8 release (*data not shown*). *Denotes statistically significant ($p < 0.05$) differences from control using One-way ANOVA test.



Supplementary Figure 11. UVB does not inhibit CHS reactions in aSMase deficient mice. As per Figure 5a in manuscript, groups of 8-10 wild-type mice, aSMase KO mice (*Spmd1-/-)* and PAFR KO mice (*Ptafr-/-*) were either injected with 250 ng CPAF i.p., 1 μg histamine s.c., or exposed to 7,500 J/m^2 UVB on shaved lower back. The mice then underwent sensitization with chemical DNFB on un-irradiated back skin followed by ear elicitation. Decreased ear thickness change was indicated as a suppressed immune response compared to sham-treated mice. The data are the changes in ear thickness (x 0.1 mm) after 24 h post elicitation from baseline values for each mouse. Black symbols represent wild-type mice; Blue symbols represent aSMase KO mice (*Spmd1-/-*); Red symbols represent PAFR KO mice (*Ptafr-/-*). Error bars represent SD. * Statistically significant ($p < .05$) compared to Sham values by One-way ANOVA test.



Supplementary Figure 12. Topical imipramine blocks CHS reactions from UVB but not CPAF. As per Figure 5b in manuscript, groups of 9-12 Wild-type mice were treated with UVB to lower back skin, or i.p. CPAF and then treated with topical imipramine (500 μM) for 3 days. The mice then underwent sensitization with chemical DNFB on un-irradiated back skin followed by ear elicitation. Decreased ear thickness change was indicated as a suppressed immune response compared to sham-treated mice. The data are the changes in ear thickness (x 0.1 mm) after 24 h post elicitation from baseline values for each mouse. Black symbols represent vehicle; Blue (unfilled) symbols represent treatment with imipramine. Error bars represent SD. * Statistically significant ($p < .05$) compared to sham control values by One-way ANOVA test.



Supplementary Figure 13. MVP characterization by western blotting and transmission electron microscopy.

HaCaT keratinocytes in non-treatment (NT) or were treated with 3,600 J/m^2 UVB. MVPs and exosomes (EXs) were isolated from cell supernatant 4 hours post-treatment. **a)**. Representative western bands showing MVPs expressing MVP specific marker Annexin V but negative for EX specific markers CD63 and Tsg 101. along with individual blots. Protein lysates were prepared using cell lysis buffer supplemented with protease inhibitor cocktail and electrophoresed through SDS-PAGE gel and transferred onto PVDF membranes. The membranes were blocked with 5% nonfat milk for 1 hr at room temperature and incubated with primary antibody against Annexin V (Santa cruz, 1:200) or CD63 (Abcam, 1:200) and Tsg101 (Abcam, 1 ug/ml) at 4°C overnight. On the next day, membranes were washed and incubated with horseradish-peroxidase-conjugated anti-goat or anti-rabbit IgG (1: 40000; Jackson Immuno Research Labs) for 1 hr at room temperature. Blots were developed with enhanced chemiluminescence developing solutions. **b)**. Representative transmission electron microscopy images showing the shape and size of MVPs collected from the cell supernatant of non-treated or UVB-treated cells. Scale bar is 500 nm. All MVPS were fixed with 2% glutaraldehyde and post-fixed with 1% osmium (Electron Microscopy Science), then embedded with Spurr resin (Sigma), and baked at 60°C according to the manufacturer's instruction. Ultrathin sections (60–80 nm) were prepared with MT7000, mounted on 300-mesh copper grids, and stained with uranyl acetate and lead citrate. All samples were examined with an EM 208 (Philips) transmission electron microscope at an accelerating voltage of 70 KV. All studies were from two independent experiments with similar results.

SUPPLEMENTARY TABLES

Supplementary Table 1. Physiologic fluences of UVB generate UVB-MVP in human skin.

Supplementary Table 2. Medical narrow band UVB phototherapy generates UVB-MVPs in human blood plasma.

Supplementary Table 3. Measurement of cytokines and chemokines in MVPs derived from HaCaT keratinocytes.

Supplementary Table 1. Physiologic fluences of UVB generate UVB-MVP in human skin.

Subject #	Age	Control MVP x 10^{11} / g (Fold)	UVB MVP x 10^{11} / g (Fold)
1	28	**5.1 (1.0)**	**8.1 (1.6)**
2	26	**10.8 (1.0)**	**1.6 (0.15)**
3	25	**0.95 (1.0)**	**1.6 (1.7)**
4	29	**2.8 (1.0)**	**9.4 (3.3)**
5	24	**6.2 (1.0)**	**12.0 (1.9)**
6	27	**3.3 (1.0)**	**5.8 (1.8)**
7	23	**9.5 (1.0)**	**23.3 (2.5)**
8	29	**0.96 (1.0)**	**1.5 (1.5)**
Mean fold change (± SEM)		**1.0**	**1.8 (± 0.31) P = 0.02**

Eight healthy adult male donors with Fitzpatrick Skin Types I and II were treated with 1000 J/m^2 UVB on volar forearm skin, and after 4 hours, 5 mm punch biopsies were obtained from UVB- and SHAM control treated skin. The tissue was weighed, and MVPs were isolated. Data are presented as particle levels ± SE normalized to tissue sample weight (fold change).

Supplementary Table 2. Medical narrow band UVB phototherapy generates UVB-MVPs in human blood plasma.

Subject #	Clinical Diagnosis	Gender/Age/ Fitz Skin Type	nUVB (KJ/m^2)	Pre-RX MVP X 10^9 / ml	2h Post-RX MVP X 10^9 / ml	4h Post-RX MVP X 10^9 / ml
1	Pruritus	Female/63/IV	14.5	9.9	20.7	12.7
2	Vitiligo	Male/22/IV	11.3	44.2	81.4	96.6
3	Vitiligo	Female/25/V	11.5	34.0	28.0	49.4
4	Dermatitis	Female/65/II	9.1	9.7	30.4	39.8
5	Dermatitis	Female/78/II	15.5	6.7	5.0	18.5
6	Dermatitis	Male/72/III	13.0	2.4	3.3	4.3
7	Dermatitis	Male/25/II	13.4	5.0	6.8	17.6
8	Psoriasis	Female/30/II	7.5	6.0	7.2	12.8

Eight patients who were undergoing chronic narrow-band UVB therapy for various skin disorders who had completed more than 20 treatments (and thus on a stable but high UVB fluence) were enrolled in this study. Blood were collected from patients before a treatment, then 2 and 4 hours after phototherapy. MVPs were collected from blood plasma. Data were presented as particle levels normalized to blood plasma volume (10^9 particles per ml of blood plasma).

Supplementary Table 3. Measurement of cytokines and chemokines in MVPs derived from HaCaT keratinocytes.

	NT	CPAF	UVB
IL-1β	0.15 (0.03)	0.031 (0.01)	0.027 (0.002)
IL-1ra	334 (60)	362 (80)	883 (208)
IL-2	0.31 (0.10)	0.11 (0.02)	0.05 (0.01)
IL-5	0.35 (0.06)	0.23 (0.07)	0.16 (0.04)
IL-6	0.127 (0.012)	0.122(0.035)	0.04 (0.01)
IL-7	0.06 (0.01)	0.021 (0.002)	0.005 (0.002)
IL-8	25 (7)	42 (13)	7 (4)
IL-9	0.20 (0.06)	0.33 (0.22)	0.27 (0.13)
IL-10	0.21 (0.03)	0.19 (0.02)	0.12 (0.02)
IL-12	0.038 (0.008)	0.047 (0.01)	0.03 (0.02)
IL-15	4.1 (0.3)	3.5 (0.6)	4.2 (1.3)
IL-17	0.32 (0.12)	0.23 (0.07)	0.13 (0.06)
FGF basic	5.7 (2.1)	1.2 (0.4)	1.2 (0.4)
G-CSF	2.9 (0.7)	1.9 (0.1)	3.3 (1.4)
GM-CSF	20 (10)	3.5 (1.1)	3.7 (1.9)
IFN-γ	0.6 (0.2)	0.6 (0.2)	0.5 (0.2)
IP-10	15 (3)	5.1 (0.7)	0.71 (0.05)
MCP-1(MCAF)	42 (9)	38 (8)	10 (3)
PDGF-bb	2.5 (0.6)	2.1 (0.7)	1.9 (0.5)
RANTES	28 (7)	25 (5)	8.4 (2.0)
TNF-α	3.4 (0.9)	5.7 (2.2)	2.2 (0.8)
VEGF	7.1 (2.5)	23 (7)	14 (2)

HaCaT keratinocytes were incubated with either no treatment (NT), CPAF (100 nM) or UVB (3,600 J/m^2). MVP were collected 4 h later and quantified, then subjected to multiplex cytokine analysis via a Bio-RAD human Bio-Plex cytokine assay. The data are the cytokine levels in pg/ml (SD) normalized to MVP numbers from four separate experiments. See Figure 4a for graphical depiction of fold changes in relevant cytokines.