Supplemental Data
Liu/Fahy et al.

Figure S1. Time course of MVP release in HaCaT cells in response to thermal burn injury.

Figure S2. Effect of thermal burn injury on viability in HaCaT cells.

Figure S3. Effect of thermal burn injury on MVP release in N/Tert human keratinocyte cell line.

Figure S4. Semi-quantification of Burn-MVP PAFR-agonistic activity using KBP cell release of IL-8 protein.

Table S1. Measurement of cytokines in MVP derived from HaCaT cells.

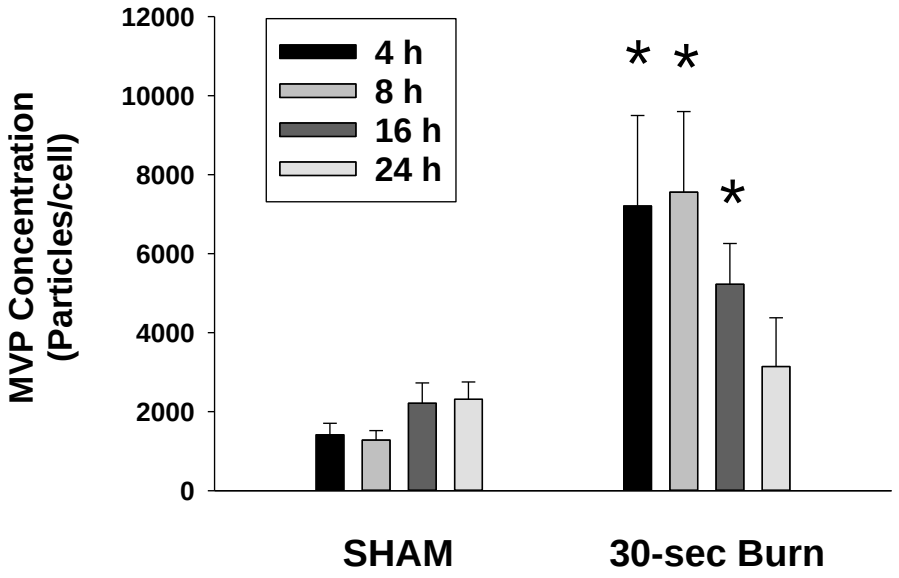

Figure S1. Time course of MVP release in HaCaT cells in response to thermal burn injury.
HaCaT cells were subjected to 90 ^{0}C water bath x 30 sec or untreated (Sham) control. At various times (4-24h), supernatant was removed and MVP measured. The data are the Mean ± SE MVP levels normalized to HaCaT cell numbers from 6-8 three separate experiments. *Denotes statistically significant (*P<0.05*) changes in levels of MVP levels from control values.

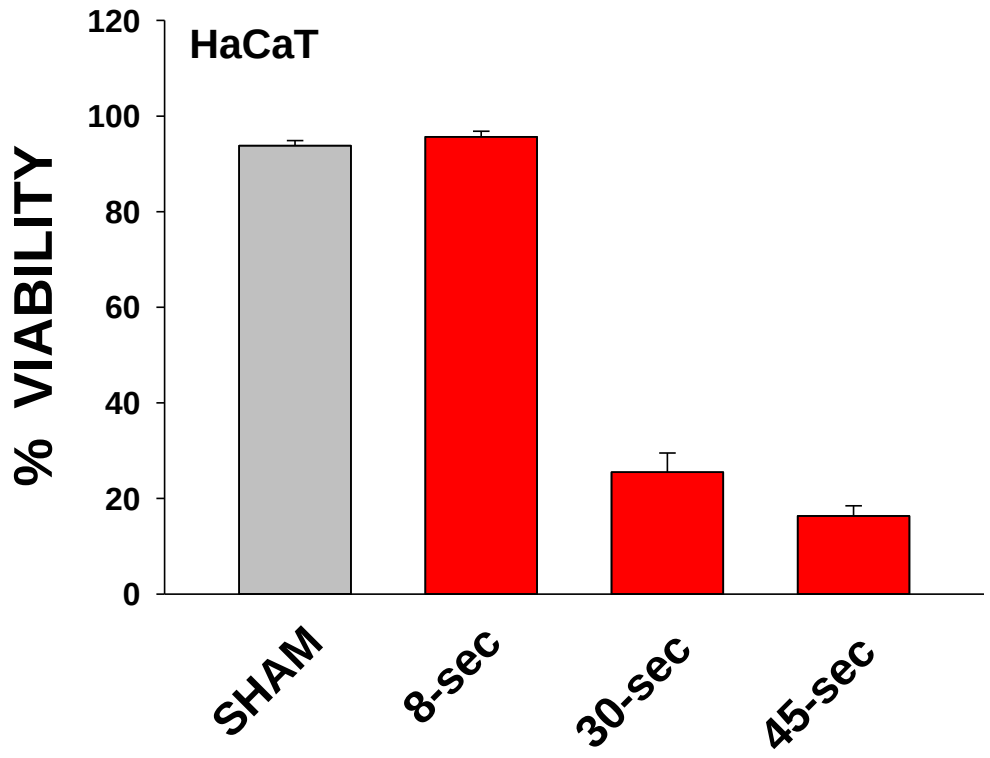

Figure S2. Effect of thermal burn injury on viability in HaCaT cells. HaCaT cells were subjected subjected to 90 ^{0}C water bath x 8, 30 or 45 sec, or no treatment (Sham). At 4h, the supernatants were removed, and cells were trypsinized. The total numbers of cells in supernatants + trypsinized cells 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 at least 6 separate experiments

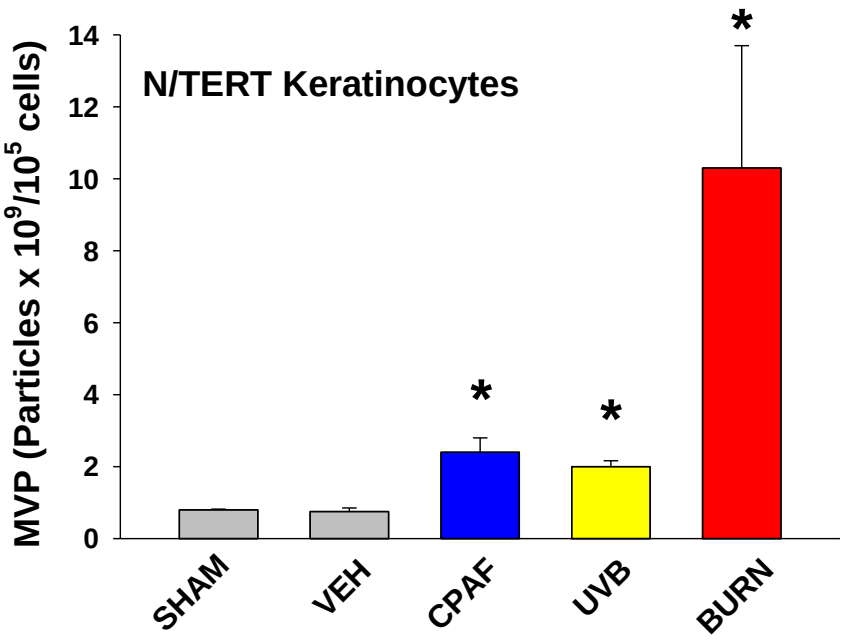

Figure S3. Effect of thermal burn injury on MVP release in N/Tert human keratinocyte cell line. N/Tert keratinocytes were subjected subjected to 90 ^{0}C water bath x 30 sec, or treatment with 3.6 KJ/m^{2} UVB, or 100nM CPAF, or 0.1% ethanol vehicle control. At 4h, the supernatant was removed and MVP measured. The data are the Mean ± SD MVP levels of duplicate samples normalized to 10^{5} cells from a single experiment representative of three separate experiments with similar results. *Denotes statistically significant (*P<0.05*) changes in levels of MVP levels from control values.

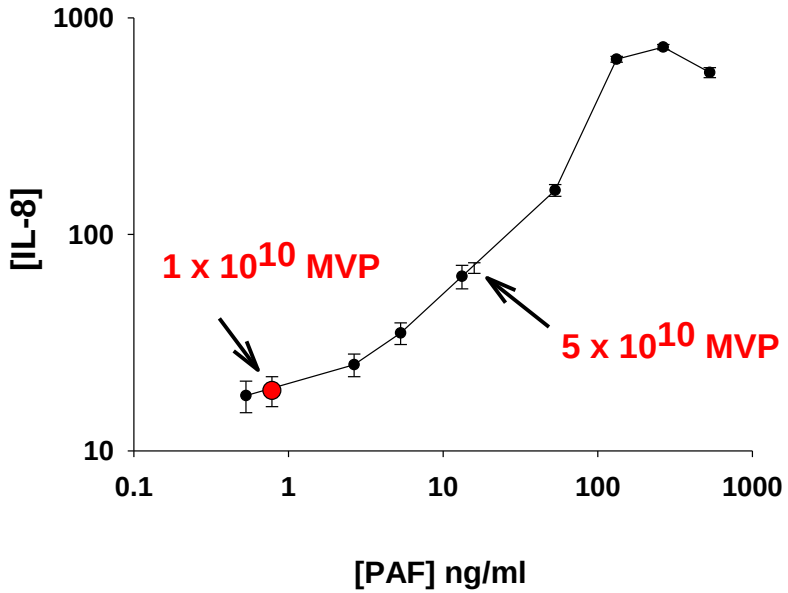

Figure S4. Semi-quantification of Burn-MVP PAFR-agonistic activity using KBP cell release of IL-8 protein. PAFR-positive KBP cells were incubated with various concentrations of PAF (1-hexadecyl-2-acetyl-GPC) or lipid extracts derived from 1 x 10^{10} and 5 x 10^{10} MVP derived from a 45 sec treatment of HaCaT cells collected 4 h post-burn. 6 h after treatment of KBP cells, supernatants were removed and IL-8 levels measured by ELISA. The data are 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 0.8 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. PAF nor Burn-MVP did not generate IL-8 release in PAFR-negative KBM cells, with TPA as positive control (not shown).

Cytokine	Control	Burn
IL-1β	0.1594 (0.02131)	0.003643 *** (0.0002774)
IL-1ra	352.8 (61.89)	35.85 *** (8.525)
IL-2	0.3279 (0.1308)	0.006765 * (0.002059)
IL-4	OOR<	OOR<
IL-5	0.2954 (0.07864)	0.014 ** (0.002963)
IL-6	0.1256 (0.02356)	0.005432 *** (0.00211)
IL-7	0.05519 (0.007471)	0.003416 ** (0.0006275)
IL-8	26.6 (7.147)	0.7774 ** (0.4324)
IL-9	0.1867 (0.0636)	0.01667 (0.006667)
IL-10	0.2063 (0.02703)	0.01041 *** (0.002639)
IL-12	0.03774 (0.007148)	0.002588 ** (0.0006549)
IL-13	OOR<	OOR<
IL-15	4.163 (0.6595)	0.2867 *** (0.0768)
IL-17	0.3062 (0.134)	0.01247 (0.003944)
Eotaxin	OOR<	OOR<
FGF basic	5.594 (2.19)	0.2308 * (0.05491)
G-CSF	2.865 (0.825)	0.2757 *** (0.109)
GM-CSF	22.55 (11.43)	0.8677 (0.3642)
IFN-γ	0.5703 (0.1687)	0.01851 ** (0.004947)
IP-10	14.94 (2.942)	0.1087 ** (0.0003385)
MCP-1	47.33 (10.31)	0.5124 *** (0.1713)
MIP-1α	OOR<	OOR<
PDGF-bb	2.356 (0.6948)	0.05833 * (0.006874)
MIP-1β	OOR<	OOR<
RANTES	26.08 (6.535)	0.8218 *** (0.1934)
TNF-α	3.363 (0.9502)	0.5469 ** (0.3701)
VEGF	7.217 (2.64)	1.011 * (0.1094)

Table S1. Measurement of cytokines in MVP derived from HaCaT cells. HaCaT cells were incubated with either no treatment (sham control or subjected to a 30 sec thermal burn injury. 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 Mean cytokine levels in pg/ml (± SE) normalized to MVP numbers from three separate experiments. OOR< represents data below detection range. Statistically significant differences relative to Control (*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$).