Supplementary Data

Harrison et al.

Figure S1. Lipid extracts from EtOH + thermal burn injury-treated HaCaT cells does not affect IL-8 levels in PAF-R-negative KBM cells.

Figure S2. Dose-dependent effects of EtOH augmentation of thermal burn injury in HaCaT cells.

Figure S3. EtOH exposure augments PAF-R agonistic activity in response to thermal burn injury in primary cultures of human keratinocytes.

Figure S4. EtOH + thermal burn injury does not affect PAF-AH enzymatic activity in HaCaT cells.

Figure S5. TSI-01 pretreatment blocks augmentation of EtOH on PAF-R agonist formation in response to thermal burn injury in HaCaT cells.

Table SI Glycerophosphocholine species generated in response to EtOH or thermal burn alone, and EtOH plus thermal burn treatment of HaCaT cells.

Table SII. Direct incorporation of EtOH into PAF.

Table SIII. Ethanol + thermal burn injury modulates serum cytokine levels.

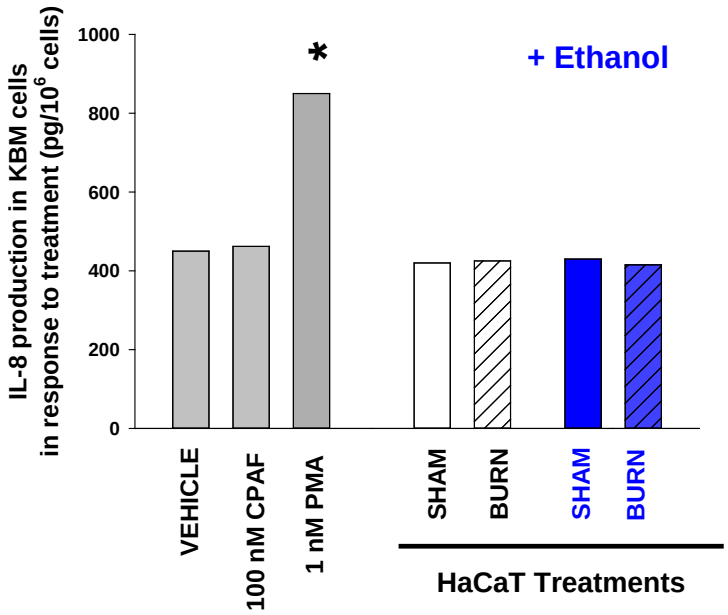


Figure S1. Lipid extracts from EtOH + thermal burn injury-treated HaCaT cells does not affect IL-8 levels in PAF-R-negative KBM cells.

HaCaT cells were subjected to 90^{0}C water bath x 2 min, or sham injury either under normal conditions or following a 30 min pre-incubation with 1% EtOH (BLUE). 5 min following injury treatment, the lipids were extracted. Lipid extracts from 5 x 10^6 HaCaT cells previously treated with thermal injury ± ethanol or 100nM CPAF, or 1 nM PMA as positive control were incubated with KBM cells and 6h later IL-8 measured in the supernatants. The data are the Mean ± SD IL-8 production in KBM cells (pg/10^6 KBM cells) from a single experiment representative of three separate experiments. *Denotes statistically significant (*P<0.05*) changes in levels of IL-8 from sham control values.

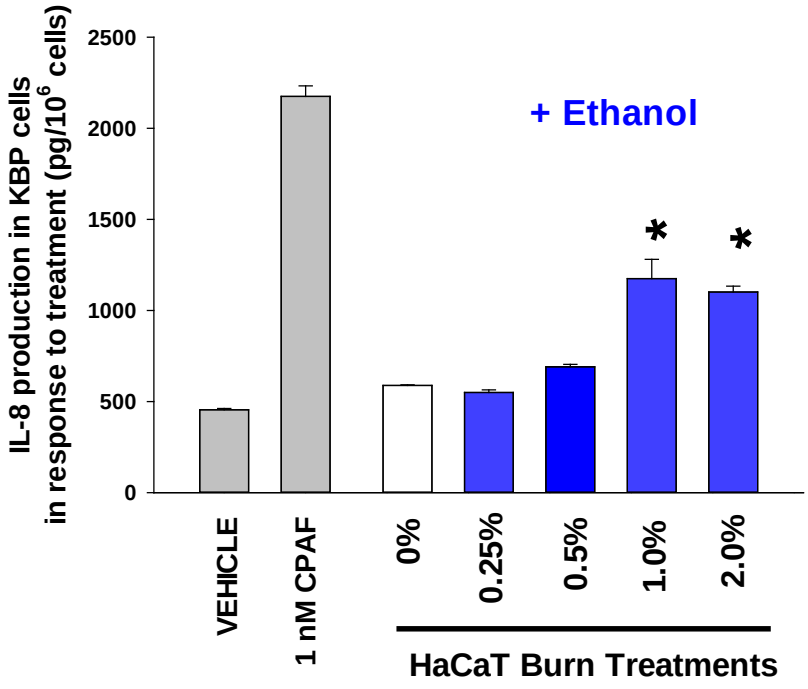


Figure S2. Dose-dependent effects of EtOH augmentation of thermal burn injury in HaCaT cells. HaCaT cells were subjected to 90^0C water bath x 2 min, or sham injury either under normal conditions or following a 30 min pre-incubation with various doses of EtOH (BLUE). 5 min following injury treatment, the lipids were extracted. Lipid extracts from 5 x 10^6 HaCaT cells previously treated with thermal injury ± ethanol or 1 nM CPAF, as positive control were incubated with KBP cells and 6h later IL-8 measured in the supernatants. The data are the Mean ± SD IL-8 production in KBP cells (pg/10^6 KBM cells) from a single experiment using duplicate values. *Denotes statistically significant (*P<0.05*) changes in levels of IL-8 from sham burn alone (with 0% EtOH) values.

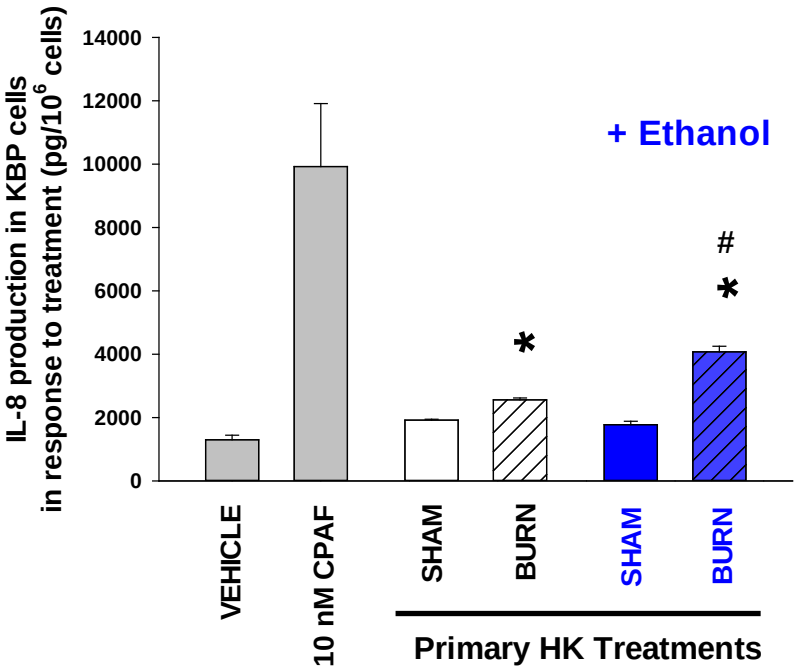


Figure S3. EtOH exposure augments PAF-R agonistic activity in response to thermal burn injury in primary cultures of human keratinocytes. Lipid extracts from 5 x 10^6 primary cultures of human foreskin-derived keratinocytes previously treated with thermal injury ± 1% ethanol or 1 nM CPAF as positive control were incubated with KBP cells and 6h later IL-8 measured in the supernatants as a surrogate for PAF-R activation. The data are the Mean ± SD IL-8 production in KBP cells (pg/10^6 KBP cells) of duplicates samples from a representative experiment from two separate experiments with qualitatively similar results. *Denotes statistically significant (*P<0.05*) changes in levels of PAF-R agonistic activity of keratinocytes treated with burn injury in comparison to unburned sham control values. # Denotes statistically significant (*P<0.05*) changes in levels of PAF-R agonistic activity of keratinocytes in comparison to keratinocytes treated with burn injury w/o EtOH exposure.

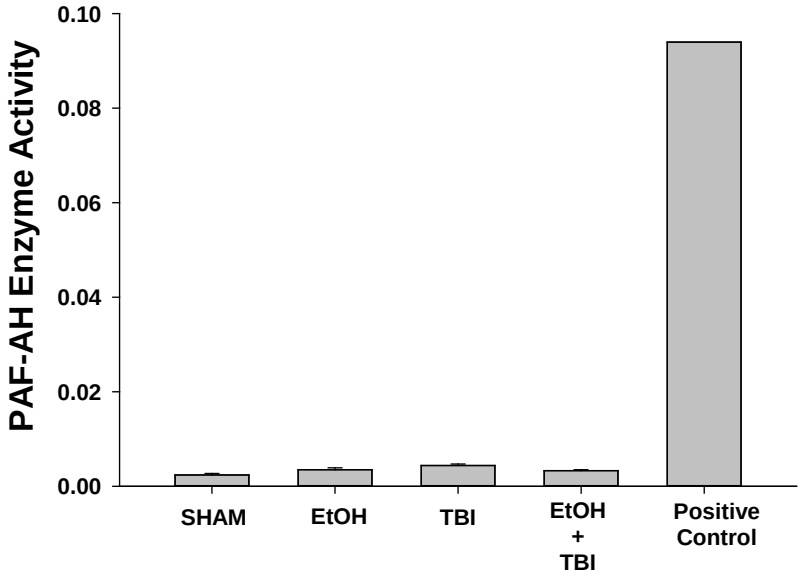


Figure S4. EtOH + thermal burn injury does not affect PAF-AH enzymatic activity in HaCaT cells. HaCaT cells were treated with 1% EtOH for 30 min. Cells were then subjected to thermal burn injury (TBI), and 5 min following injury the PAF-acetylhydrolase enzymatic activity was assessed. The data represent Mean ± SE PAF-AH activity (μmole/min/ml) from three separate experiments. Positive control is recombinant PAF-AH provided by the assay manufacturer.

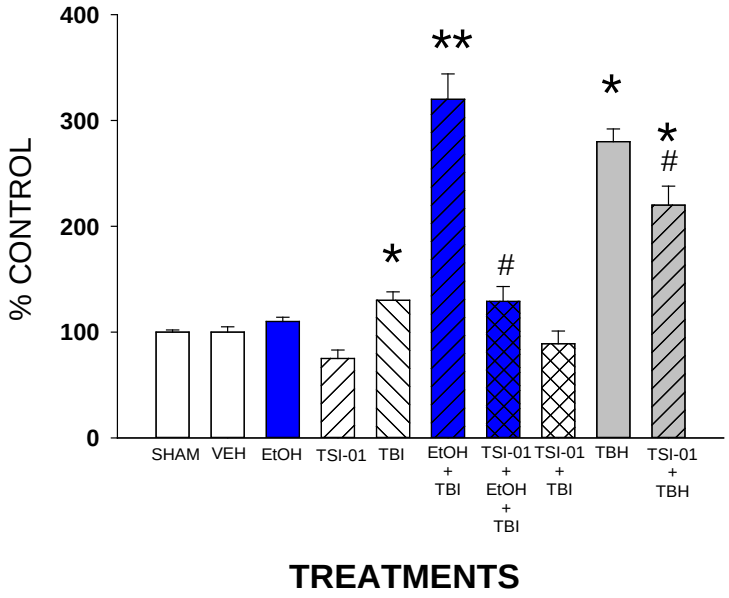
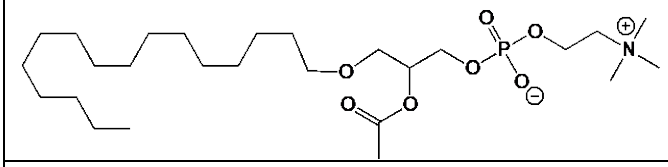
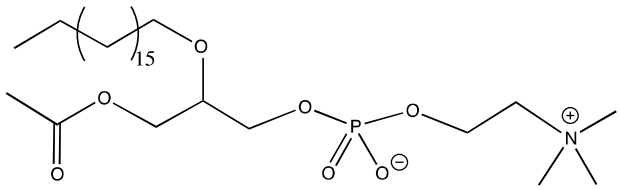
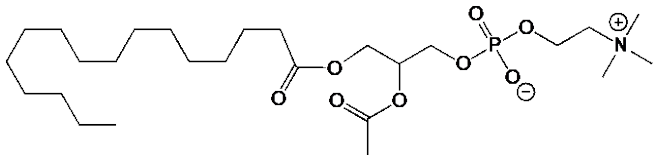
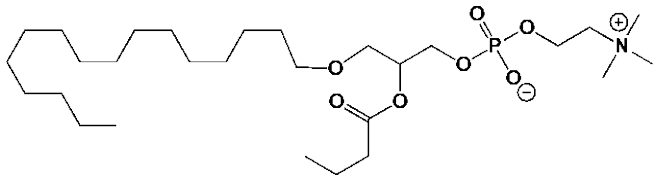
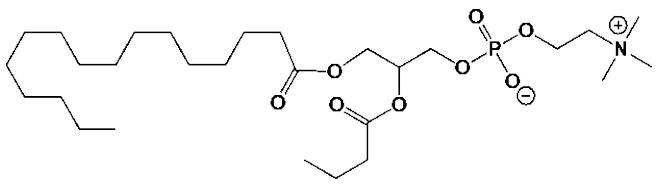
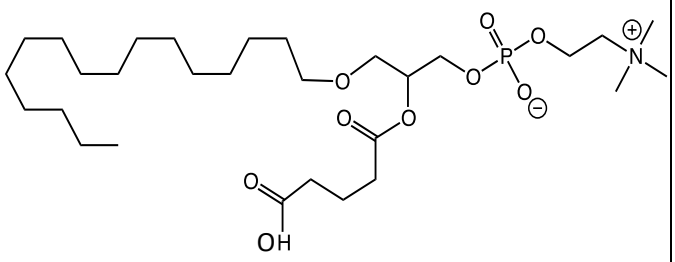
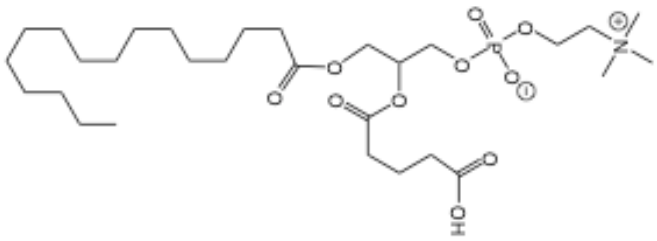
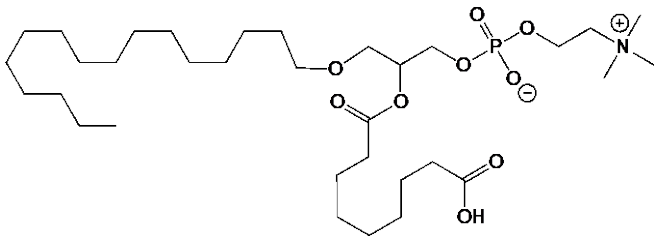
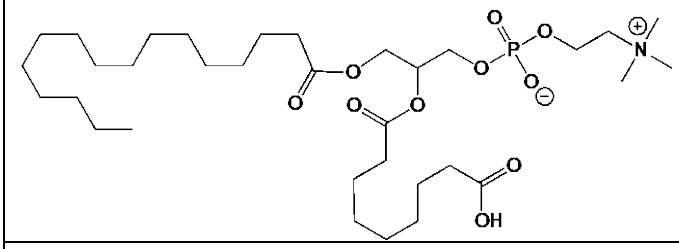
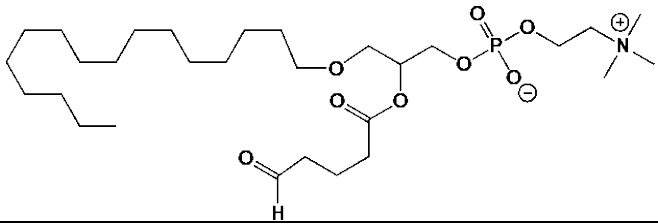


Figure S5. TSI-01 pretreatment blocks augmentation of EtOH on PAF-R agonist formation in response to thermal burn injury in HaCaT cells. HaCaT cells were untreated (SHAM), or pretreated with 60 μM TSI-01, or 0.1% DMSO vehicle (VEH) for 30 min before treatment with 1% EtOH (BLUE) for an additional 30 min. Cells were then subjected to thermal burn injury (TBI), or 100 μM tert-butyl hydroperoxide (TBH) and 5 min following injury the lipids were extracted and PAF-R agonistic activity determined as measurement of IL-8 released in KBP cells. The data are the Mean ± SD % sham control PAF-R agonists measured from IL-8 production in KBP cells (pg/10^6 KBP cells) from a single experiment representative of three separate experiments. ** Denotes statistically significant ($P<0.01$) changes in comparison to burn injury alone; *Denotes statistically significant ($P<0.05$) changes in comparison to SHAM treatment; # Denotes statistically significant ($P<0.05$) changes induced by TSI-01 treatment in comparison to no TSI-01.

Supplementary Table S-I

Glycerophosphocholine Species	Sham-Treated (pg/10^6 cells)	EtOH Treated (pg/10^6 cells)	Thermal Burn Injury -Treated (pg/10^6 cells)	EtOH +TBI-Treated (pg/10^6 cells)
16ePAF 	8.5(3.7)	10.0(3.7)	507(125)	2575(955)
18ePAF 	2.0(2.7)	0.6(1.3)	56.4(27.9)	307(155)
PAcPC 	0.4(0.4)	0.2(0.3)	ND	0.9(1.2)
BPAF 	5.3(1.2)	5.3(1.3)	5.3(1.2)	5.3(1.3)
PBPC 	5.4(1.3)	5.4(1.2)	5.3(1.2)	5.3(1.2)

GPAF 	7.2(15.4)	5.4(7.5)	3.7(4.8)	3.5(5.5)
PGPC 	4.8(10.2)	6.9(9.6)	3.3(5.6)	5.8(10.3)
AzPAF 	122(93)	134(70)	119(52)	125(73)
PAzPC 	491(505)	472(289)	350(118)	448(262)
OVPAF 	7.6(2.5)	7.4(2.0)	7.7(2.7)	7.7(2.1)
POVPC				7.0(1.3)

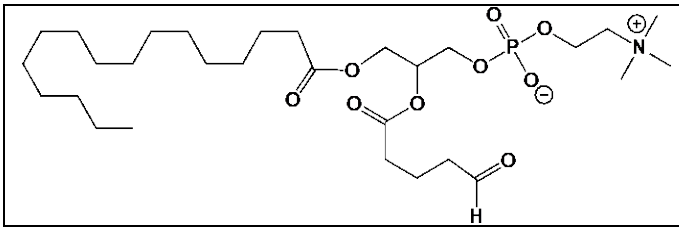
	6.9(1.6)	7.0(1.6)	6.8(1.8)	

Table Supplementary S-I. Glycerophosphocholine species generated in response to EtOH or thermal burn alone, and EtOH plus thermal burn treatment of HaCaT cells. HaCaT cells were treated with HBSS alone or 1% EtOH,in HBSS for 30 min, followed by TBI or sham treatment as outlined in the manuscript. 5 min after treatment, the lipids extracted. Lipid extracts were analyzed by mass spectrometry using deuterium-labeled internal standards (see supplementary online methods). The data are listed as structures and amounts of different GPCs in vehicle and treated cells as pg per 10^6 cells from 5 separate experiments.

TREATMENT	C16 PAF ($ng/10^6$ cells)	D3/D0 Ratio
CH_3CH_2OH	4.7 (1.8)	0 (0)
CH_3CH_2OH: CD_3CH_2OH (1:1)	5.3 (1.3)	0.045 (0.007)
CD_3CH_2OH	4.8 (1.3)	0.084 (0.017)

Table S-2. Direct incorporation of EtOH into PAF. HaCaT cells were incubated with either 1% EtOH, deuterium-labelled EtOH, or 1:1 EtOH:D3 EtOH for 30 min, then treated with thermal burn injury. 5 min following burn injury, lipids were extracted and 1-hexadecyl-2-acetyl-GPC (D0) and 1-hexadecyl-2-D_3acetyl-GPC measured by mass spectrometry as described in Figure 2. The data are the Mean (± SEM) PAF levels ($ng/10^6$ HaCaT cells) and fraction of D3-PAF to PAF from four separate experiments.

Cytokine	Control	EtOH	TBI	EtOH/TBI
Eotaxin (wt)	1256 (665)	983 (215)	1256 (532)	1292 (340)
Eotaxin (-/-)	896 (464)	650 (130)	775 (339)	715 (145)
G-CSF (wt)	225 (138)	341 (272)	467 (287)	985$^{\#\dagger}$ (796)
G-CSF (-/-)	185 (70)	212 (106)	412 (207)	394 (208)
GM-CSF (wt)	93 (33)	119 (5)	102 (28)	127$^{\#}$ (6)
GM-CSF (-/-)	94 (48)	109 (20)	104 (20)	116 (11)
IL-1α (wt)	10.1 (4.3)	8.0 (3.1)	7.3 (1.7)	8.2 (3.8)
IL-1α (-/-)	10.8 (9.7)	14.1 (4.8)	14.1 (4.3)	14.4 (3.3)
IL-1β (wt)	42 (27)	37 (21)	34 (16)	39 (17)
IL-1β (-/-)	30 (17)	41 (32)	29 (48)	22.22 (14)
IL-2 (wt)	37 (7.8)	29 (13)	45 (29)	35 (16)
IL-2 (-/-)	27 (21)	37 (11)	19.8 (2.3)	24.1 (11)
IL-3 (wt)	46 (12)	55 (1.3)	48 (7.8)	52 (2.6)
IL-3 (-/-)	54 (4)	50 (3.6)	54 (5.1)	53 (2.6)
IL-4 (wt)	32 (9.7)	38 (2.1)	33 (8.7)	40 (2.9)
IL-4 (-/-)	40 (5.9)	38 (4.3)	39 (7.1)	42 (2.9)
IL-5 (wt)	42 (25)	27 (16)	36 (22)	57† (17)
IL-5 (-/-)	35 (31)	47 (15)	28 (21)	29 (16)
IL-6 (wt)	21 (14)	25 (4.7)	57$^{\#}$ (16)	97$^{\#\dagger*}$ (38)
IL-6 (-/-)	15 (6.4)	31 (36)	61$^{\#}$ (22)	69$^{\#\dagger}$ (26)
IL-9 (wt)	123 (30)	143 (4.3)	138 (13)	141 (7.4)
IL-9 (-/-)	153$^{\#}$ (6.7)	135† (7.7)	148 (10.3)	145 (6.7)
IL-10 (wt)	82 (41)	89 (42)	79 (33)	119 (31)
IL-10 (-/-)	72 (47)	88 (35)	98 (12)	98 (14)
IL-12(p70) (wt)	323 (114)	392 (26)	375 (64)	382 (26)
IL-12(p70) (-/-)	350 (169)	378 (35)	418 (43)	399 (46)
IL-12(p40) (wt)	150 (37)	153 (21)	150$^{\#*}$ (31)	199 (47)
IL-12(p40) (-/-)	110 (35)	97 (22)	100 (23)	100 (22)
IL-13 (wt)	62 (108)	20 (5.1)	18 (2.5)	29 (3)
IL-13 (-/-)	144 (252)	85 (141)	102 (193)	26 (6.5)
IL-17 (wt)	62 (20)	55 (6.2)	71 (26)	48* (6.1)
IL-17 (-/-)	52 (20)	45 (6.2)	45 (15)	43 (4.9)
INFγ (wt)	60 (29)	79 (2.2)	63 (27)	76 (5.8)
INFγ (-/-)	59 (29)	70 (13)	70 (12)	79 (5.9)
KC (wt)	39 (12)	53 (18)	158$^{\#\dagger}$ (97)	177$^{\#\dagger}$ (46)
KC (-/-)	22 (6.3)	35 (17)	86$^{\#\dagger}$ (24)	84$^{\#\dagger}$ (30)
MCP-1 (wt)	190 (95)	223 (83)	216 (67)	388$^{\#\dagger*}$ (152)
MCP-1 (-/-)	304 (167)	370 (235)	280 (267)	227 (141)
MIP-1α (wt)	13.1 (5.9)	17.1 (0.8)	12.9 (5.7)	16 (1.1)

MIP-1α (-/-)	13 (3.6)	15.5 (2.1)	14.8 (3)	15.8 (0.8)
MIP-1β (wt)	55 (30)	80 (9.3)	54 (30)	90#* (9.4)
MIP-1β (-/-)	54 (35)	71 (20)	61 (16)	77 (15)
RANTES (wt)	35 (2.4)	32 (2.5)	31 (3.6)	32 (4.7)
RANTES (-/-)	31 (6.5)	27 (2.3)	27 (2.3)	27 (1.7)
TNFα (wt)	357 (215)	542 (51)	364 (237)	587#* (57)
TNFα (-/-)	339 (247)	436 (156)	376 (109)	512 (147)

Table S-3. Ethanol-thermal burn injury modulates serum cytokine levels. Serum harvested from 6-8 wild-type (wt) or pafr -/- (-/-) mice/treatment were analyzed for cytokine protein expression via a Bio-Rad Mouse Bio-Plex cytokine assay. Data represents the mean of detectable serum protein in pg/mL (+/- Standard Deviation). Statistically significant differences relative to Control (#), EtOH (†) or TBI (*) samples were determined by 1-way ANOVA with Dunnett's post-hoc test ($p < 0.05$).