Supplementary Information

Accelerated resolution of inflammation underlies sex differences in inflammatory responses in humans

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Supplementary Tables

Table S1

The effect of typhoid on the expression of platelet activation markers and platelet activity in healthy volunteers. Data are shown for % P-selectin expression in males and females following PBS treatment and in response to ADP (3 and 10 µM) or collagen (3 and 10 µg/ml) at baseline, 8 hours and 32 hours post vaccine. For % platelet monocyte aggregates levels were measured at baseline, 8 hours and 32 hours post-vaccine. Data are shown as mean ± s.e.mean and statistical analysis conducted using two-way ANOVA to determine the influence of sex for n=10 male and n=12 female volunteers (2 samples lost due to technical difficulties). Statistical significance was tested using two-way ANOVA with Sidak's post-hoc analysis.

	Males			Females			Sex comparison
	Baseline	**8 hours**	**32 hours**	**Baseline**	**8 hours**	**32 hours**	**P Value**
% P-Selectin expression							
PBS	2.3 ± 1.4	0.7 ± 0.2	2.2 ± 1.2	1.0 ± 0.2	0.5 ± 0.1	0.8 ± 0.3	0.517
Collagen 1 µg/ml	2.2 ± 0.7	1.3 ± 0.3	2.6 ± 1.1	2.3 ± 0.7	1.1 ± 0.2	1.2 ± 0.2	0.567
Collagen 3 µg/ml	3.7 ± 0.9	3.2 ± 0.8	4.2 ± 1.6	3.8 ± 1.3	2.7 ± 0.6	2.2 ±0.4	0.448
ADP 3 µM	9.1 ± 2.6	6.8 ± 1.3	4.2 ± 1.6	8.9 ± 2.1	12.3 ± 3.7	10.8 ± 3.3	0.723
ADP 10 µM	10.9 ± 3.3	7.1 ± 1.8	12.5 ± 3.3	12.0 ± 2.5	12.9 ± 3.0	13.8 ± 3.6	0.587
% PMA expression	31.5 ± 4.2	34.2 ± 6.4	26.8 ± 4.3	27.8 ± 5.4	34.4 ± 6.0	33.1 ± 4.7	0.611
Platelet Aggregation (AU)							
PBS	7.2 ± 2.1	8.8 ± 3.0	8.2 ± 2.3	9.2 ± 2.0	13.5 ± 2.5	8.1 ± 2.3	0.268
Collagen 1 µg/ml	59.5 ± 3.4	61.6 ± 5.2	54.8 ± 4.0	60.2 ± 5.3	74.6 ± 5.1	61.3 ± 5.2	0.086
Collagen 3 µg/ml	78.9 ± 3.7	91.5 ± 4.9	75.1 ± 3.9	83.8 ± 5.9	99.6 ± 5.9	76.8 ± 6.5	0.245
ADP 3 µM	35.1 ± 4.1	37.8 ± 4.1	37.7 ± 5.4	46.3 ± 5.8	54.4 ± 5.1	43.2 ± 5.5	0.008
ADP 10 µM	47.8 ± 4.1	49.9 ± 4.3	43.9 ± 3.7	57.0 ± 4.4	68.6 ± 3.5	56.9 ± 4.8	0.0002

Table S2

Vascular measures at baseline and at 8 and 32 hours following typhoid vaccination Data are shown as mean ± SD with statistical significance determined using using two-way ANOVA with post-hoc Sidak's tests shown as * for P<0.05, **P <0.01, ***P <0.001, ****P <0.0001 for comparison of time-points between the sexes and no significant differences for within group comparisons to baseline.

	Males (n=12)			Females (n=12)			
	Baseline	**8 hours**	**32 hours**	**Baseline**	**8 hours**	**32 hours**	**Sex comparison**
Ultrasound							
Baseline brachial artery diameter, mm	3.8 ± 0.5***	3.8 ± 0.5****	3.8 ± 0.4**	3.1 ± 0.3	3.1 ± 0.4	3.2 ± 0.4	<0.0001
Time to peak diameter, min	6.7 ± 0.5	6.7 ± 0.7	6.7 ± 0.8	6.7 ± 0.2	6.8 ± 0.2	6.8 ± 0.2	0.492
PWA							
Augmentation index, %	13.3 ± 1.9	13.8 ± 1.2	13.5 ± 1.7*	12.1 ± 1.4	11.6 ± 2.2	11.9 ± 1.9	0.24
PWV, m/s	6.7 ± 0.1	6.7 ± 0.1	6.7 ± 0.2*	6.3 ± 0.1	6.2 ± 0.2	6.2 ± 0.1	<0.0001

Table S3

Blood differentials and CRP levels in male and female healthy volunteers at baseline and post cantharidin-induced blister harvest. Data are shown as mean ± SD with statistical significance determined using using two-way ANOVA with post-hoc Sidak's tests (note there were no significant differences between the sexes or on the effects of cantharidin on circulating markers or within sex).

		Hb (g/dL)	Platelet count ($x10^9$/L)	WBC ($x10^{11}$/L)	Neutrophils ($x10^9$/L)	Lymphocytes ($x10^9$/L)	Monocytes ($x10^9$/L)	CRP (mg/L)
Male	**Baseline**	15.0 ± 0.3	234.7 ± 15.2	5.4 ± 0.3	3.0 ± 0.3	1.8 ± 0.1	0.4 ± 0.04	<5
	Post-blister	15.0 ± 0.3	242.2 ± 12.9	5.6 ± 0.4	3.2 ± 0.3	1.8 ± 0.1	0.4 ± 0.04	<5
Female	**Baseline**	13.1 ± 0.3	258.0 ± 12.0	6.3 ± 0.5	3.8 ± 0.4	1.9 ± 0.1	0.4 ± 0.02	<5
	Post-blister	12.7 ± 0.2	259.9 ± 12.2	6.0 ± 0.3	3.3 ± 0.2	2.0 ± 0.1	0.4 ± 0.02	<5
	Sex Comparison	<0.0001	0.123	0.109	0.133	0.133	0.859	0

Table S4 Cytokine/chemokine array of 24 hour cantharidin-induced blister supernatants in male and female healthy volunteers. Data are shown as mean ± SD with statistical significance determined using using two-way ANOVA with post-hoc Sidak's tests shown as ** for P <0.01 for comparison of time-points between the sexes and no significant differences for within group comparisons to baseline.

		IL-6 (pg/ml)	IL-10 (pg/ml)	CCL-5 (pg/ml)	CXCL-1 (pg/ml)	IL-8 (pg/ml)	MCP-1 (pg/ml)
Male	**24 h**	4361 ± 1182	6.6 ± 2.3	11.5 ± 1.8	1046 ± 606.6	5716 ± 1453	6139 ± 2141
	72 h	3250 ± 1178	48.1 ± 26.7	38.5 ± 26.0	224.5 ± 36.0	3675 ± 563.8	224.4 ± 26.86
Female	**24 h**	5087 ± 1697	18.0 ± 7.3	15.5 ± 2.7	4224 ± 1809	13383 ± 2771**	9658 ± 3113
	72 h	5240 ± 3007	30.6 ± 0.0	10.7 ± 0	380.4 ± 124.3	5511 ± 9376	302.6 ± 93.2
	Sex comparison	0.452	0.653	0.375	0.221	0.0484	0.527

Table S5 Blister exudate LM-SPM profiles. Exudates were collected from male (n=13) and female volunteers (n=11) 24h after cantharidin application and supernatants were profiling using LC-MS/MS based profiling. Results are mean ± s.e.m. with statistical significance determined using Student's 2-tailed unpaired t-test. - = below limit, limit ≈ 0.1 pg

			Lipid mediators levels (pg/50µL)		
DHA bioactive metabolome	**Q1**	**Q3**	**Female**	**Male**	***p* value**
RvD1	375	215	1.6 ± 1.1	1.4 ± 0.8	0.317
RvD2	375	215	0.4 ± 0.7	0.1 ± 0.3	0.120
RvD3	375	147	0.0 ± 0.1	-	0.143
RvD4	375	101	-	-	
RvD5	359	199	0.3 ± 0.3	0.5 ± 0.5	0.176
RvD6	359	159	-	-	
17R-RvD1	375	215	1.8 ± 1.7	0.8 ± 0.9	0.051
17R-RvD3	375	147	0.0 ± 0.1	0.1 ± 0.1	0.246
PD1	359	153	**0.3 ± 0.6**	**-**	**0.046**
10S,17S-diHDHA (PDX)	359	153	0.7 ± 0.6	0.8 ± 0.9	0.463
MaR1	359	250	2.5 ± 6.4	0.9 ± 1.7	0.195
7S,14S-diHDHA	359	221	0.1 ± 0.3	0.3 ± 0.7	0.201
17-HDHA	343	245	22.7 ± 17.5	242.6 ± 589.5	0.115
14-HDHA	343	205	194.2 ± 108.2	163.8 ± 120.4	0.263
7-HDHA	343	141	2.7 ± 1.6	3.2 ± 2.7	0.282
4-HDHA	343	101	3.6 ± 1.7	4.6 ± 4.8	0.264
DHA	327	283	3031.3 ± 1309.2	2789.6 ± 1334.4	0.33
EPA bioactive metabolome					
RvE1	349	161	0.4 ± 1.0	0.4 ± 0.6	0.403
RvE2	333	199	1.0 ± 1.1	0.8 ± 1.2	0.344
RvE3	333	245	4.0 ± 6.6	1.6 ± 3.1	0.125
18-HEPE	317	259	2.1 ± 4.8	4.0 ± 9.6	0.288
15-HEPE	317	219	7.9 ± 11.3	101.7 ± 227.7	0.094
12-HEPE	317	179	20.1 ± 52.2	27.3 ± 70.8	0.390
5-HEPE	317	115	0.4 ± 1.2	1.6 ± 4.9	0.23.2
EPA	301	257	975.5 ± 622.1	1231.0 ± 857.7	0.210
AA bioactive metabolome					
LXA_4	351	115	-	-	
LXB_4	351	221	5.4 ± 3.6	3.5 ± 2.0	0.062
5S,15S-diHETE	335	115	15.0 ± 19.6	9.9 ± 6.6	0.193
AT-LXA_4	351	115	0.2 ± 0.3	0.2 ± 0.3	0.493
AT-LXB_4	351	221	0.6 ± 1.2	0.3 ± 0.5	0.198
LTB_4	335	195	**0.6 ± 0.3**	**1.2 ± 1.0**	**0.037**
PGD_2	351	189	**16.2 ± 12.9**	**8.9 ± 4.0**	**0.032**
PGE_2	351	189	228.1 ± 205.6	134.9 ± 72.2	0.070
$PGF_{2\alpha}$	353	193	1116.6 ± 550.0	808.9 ± 468.2	0.076
TxB_2	369	169	805.2 ± 796.4	426.8 ± 559.6	0.093
20-HETE	319	301	15.5 ± 11.9	19.9 ± 17.8	0.246
15-HETE	319	219	144.8 ± 211.0	848.2 ± 1605.3	0.082
12-HETE	319	179	551.2 ± 223.4	514.5 ± 311.8	0.374
5-HETE	319	115	9.3 ± 5.3	13.3 ± 8.6	0.098
AA	303	259	4320.9 ± 1980.6	4714.8 ± 2041.8	0.319

Table S6 Lipid mediator profiles in plasma from male or female volunteers following typhoid vaccination Plasma was collected and LM levels were assessed using LM-profiling. Results are expressed as pg/mL; mean ± s.e.m. ; n = 4 volunteers per group. - = below limit, limit ≈ 0.1 pg
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			Lipid mediators levels (pg/mL)		
DHA bioactive metabolome	**Q1**	**Q3**	**Male**	**Female**	***p* value**
RvD1	375	233	0.1 ± 0.0	0.1 ± 0.0	0.280
RvD2	375	141	0.3 ± 0.1	0.4 ± 0.2	0.312
RvD3	375	147	0.2 ± 0.1	0.1 ± 0.0	0.187
RvD4	375	101	0.5 ± 0.5	0.1 ± 0.1	0.230
RvD5	359	199	1.2 ± 0.2	2.0 ± 0.7	0.152
RvD6	359	101	0.3 ± 0.1	0.2 ± 0.1	0.340
17R-RvD1	375	141	0.1 ± 0.0	0.1 ± 0.1	0.312
17R-RvD3	375	147	0.2 ± 0.1	0.1 ± 0.0	0.187
PD1	359	153	0.2 ± 0.0	0.2 ± 0.1	0.296
17R-PD1	359	153	0.2 ± 0.1	0.1 ± 0.1	0.329
22-OH-PD1	375	153	0.4 ± 0.4	0.7 ± 0.7	0.343
10S,17S-diHDHA (PDX)	359	153	0.6 ± 0.0	0.9 ± 0.3	0.098
MaR1	359	221	-	-	
MaR2	359	191	2.9 ± 1.3	2.5 ± 1.0	0.395
22-OH-MaR1	375	221	0.4 ± 0.4	0.1 ± 0.2	0.247
7S,14S-diHDHA	359	221	-	-	0.232
4S,14S-diHDHA	359	101	0.3 ± 0.3	0.1 ± 0.1	0.178
EPA bioactive metabolome					
RvE1	349	161	0.2 ± 0.1	0.4 ± 0.1	0.118
RvE2	333	159	0.1 ± 0.1	-	0.369
RvE3	333	201	0.4 ± 0.2	0.9 ± 0.4	0.094
AA bioactive metabolome					
LXA_4	351	217	0.1 ± 0.0	0.1 ± 0.0	0.298
LXB_4	351	221	**5.2 ± 2.6**	**-**	**0.028**
5S,15S-diHETE	335	235	4.9 ± 2.6	1.1 ± 0.8	0.082
AT-LXA_4	351	217	0.2 ± 0.2	0.1 ± 0.1	0.317
AT-LXB_4	351	221	1.1 ± 0.6	1.2 ± 0.8	0.440
LTB_4	335	195	**0.6 ± 0.0**	**0.5 ± 0.0**	**0.006**
6-trans- LTB_4	335	195	0.1 ± 0.0	-	0.168
12-epi-6-trans- LTB_4	335	195	0.2 ± 0.1	-	0.099
5S,12S-diHETE	335	195	0.1 ± 0.0	0.1 ± 0.0	0.414
20-OH-LTB_4	351	195	0.1 ± 0.0	-	0.085
PGD_2	351	189	3.5 ± 1.8	4.4 ± 2.6	0.382
PGE_2	351	189	5.7 ± 2.4	6.9 ± 3.7	0.379
$PGF_{2\alpha}$	353	193	3.8 ± 1.0	6.0 ± 3.0	0.220
TxB_2	369	169	97.8 ± 36.3	108.7 ± 52.7	0.426

Supplementary Figures

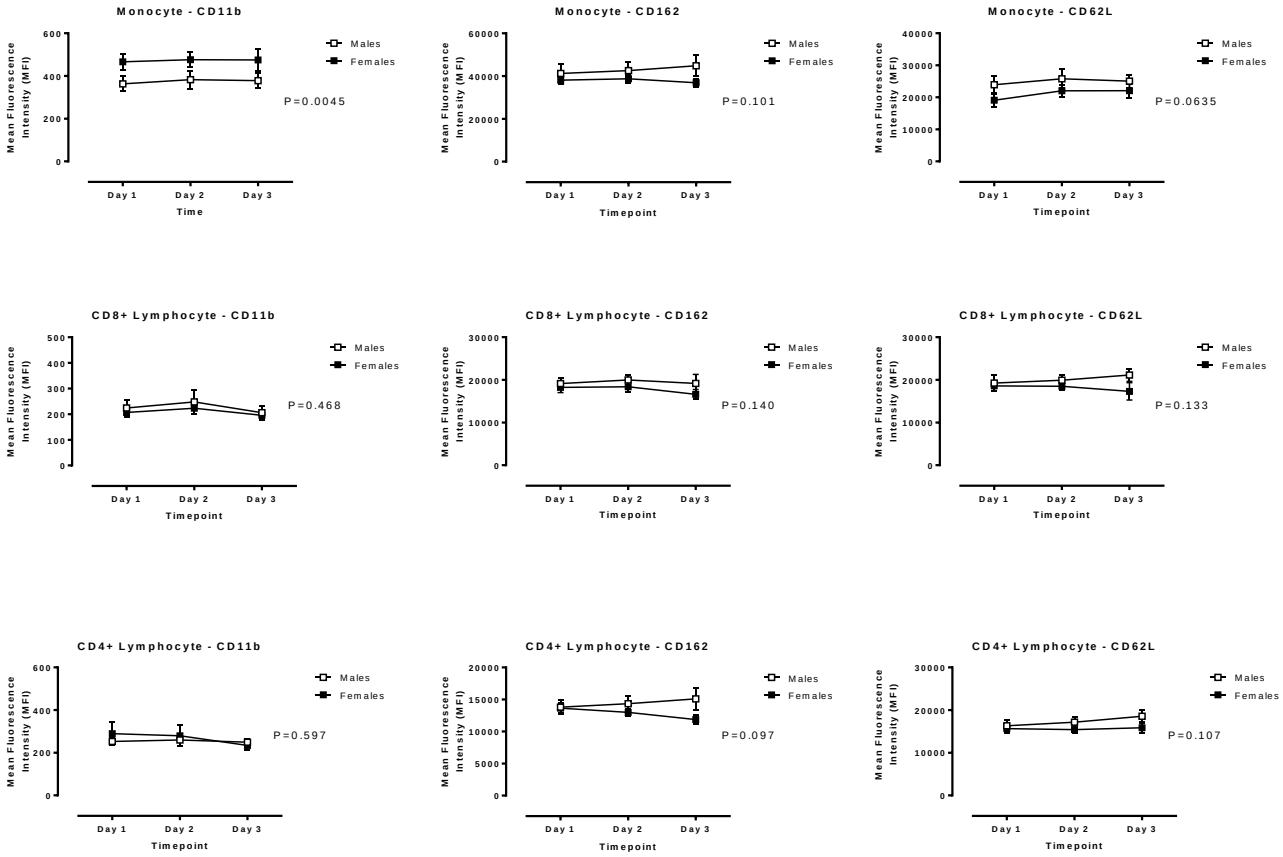

Figure S1 – Changes in expression of CD11b, CD62L and CD162 on monocytes, CD4+ and CD8+ lymphocytes in male and female healthy volunteers measured at baseline, 8 hours and 32 hours following typhoid vaccine. Data expressed as mean ± s.e.mean for n=12 for each sex for all the panels. Statistical significance determined using two-way ANOVA with Sidak's post-hoc analysis for all the panels. MFI = Median fluorescence intensity.

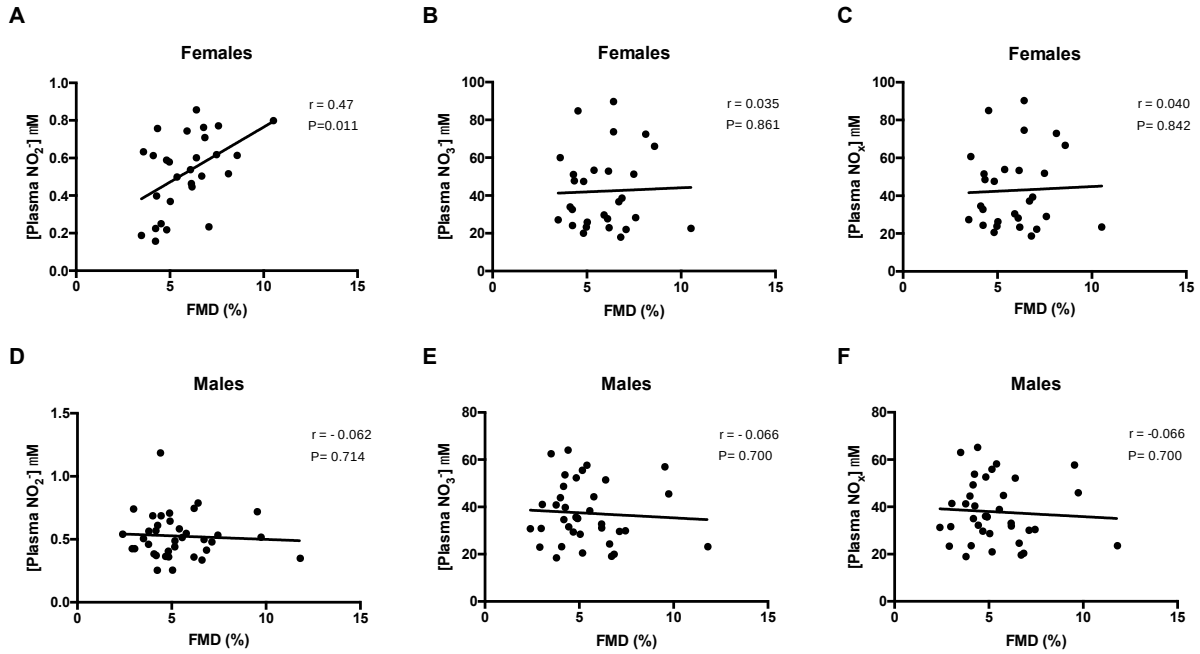

Figure S2 Correlations between FMD responses in healthy volunteers and levels of plasma NO metabolites NO_2^-, NO_3^- and NO_X in females (A-C) and males (D-F). Data from pre-vaccine baseline and 8 and 32 hours post-vaccine are collated. All graphs show Pearson linear regression, and statistical significance of r-value for all the panels.

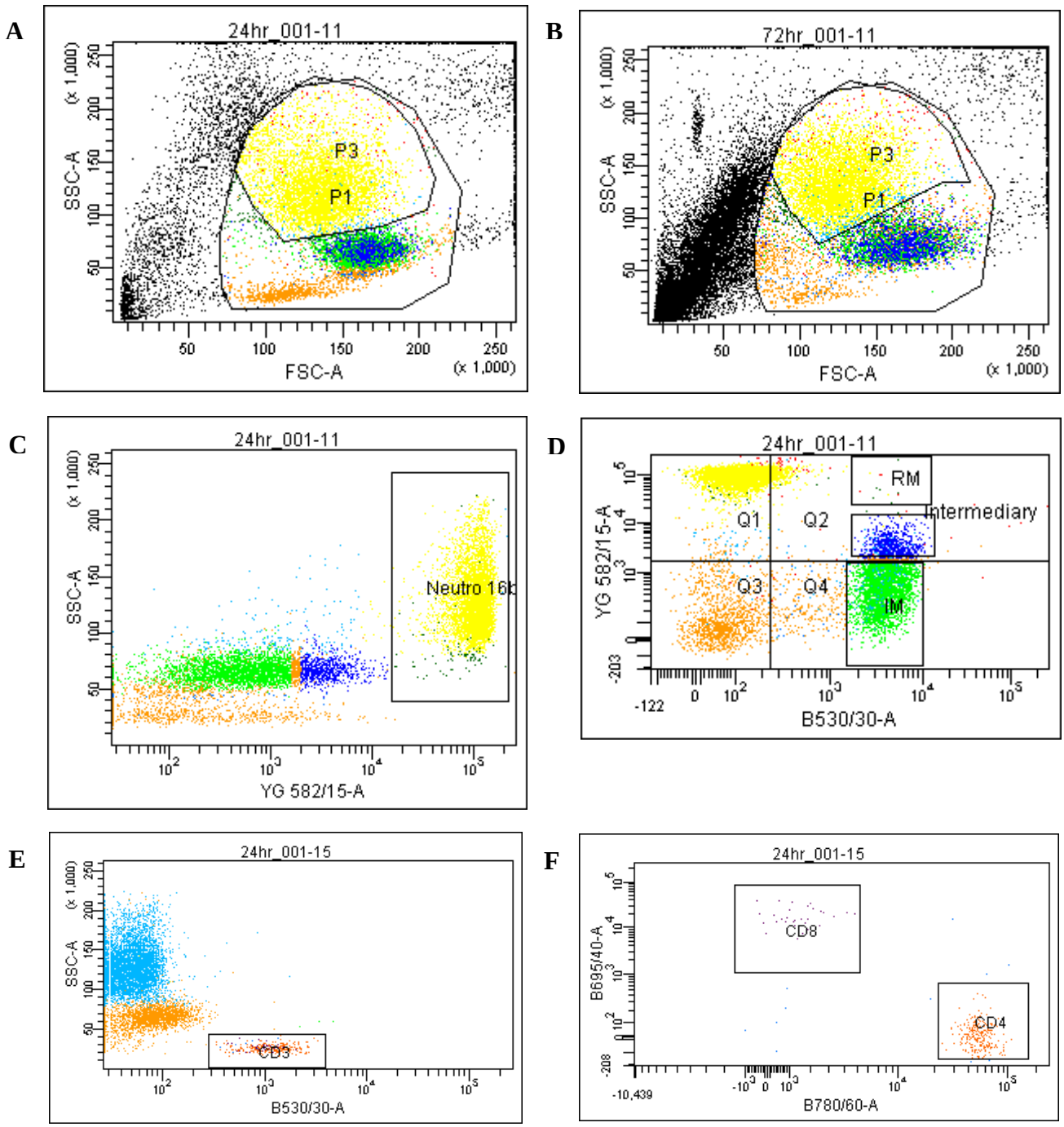

Figure S3 – Representative dot plots to identify leucocyte sub-populations collected in cantharidin induced blister fluid in healthy volunteers: A-B) Characteristic FSC/SSC dot plot from a 24hrs and 72hrs blister fluid respectively; **C)** Density dot plot of neutrophils (yellow-gated cells) confirmed by anti-CD16b+ binding; **D)** Density dot plot of monocytes showing presence of CD14+/CD16- classical inflammatory (IM) monocytes (green-gated), CD14+/CD16+ intermediate monocytes (blue-gated) and a fraction of CD14lo/CD16++ resident (RM) monocytes confirmed by appropriate binding patterns with anti-CD14 and/or anti-CD16; **E)** Density dot plot of lymphocytes (red-gated cells) confirmed by anti-CD3 binding and **F)** Density dot plot showing T-cell sub-types with anti-CD4+ (orange-gated cells) and anti-CD8+ (blue-gated cells) binding.

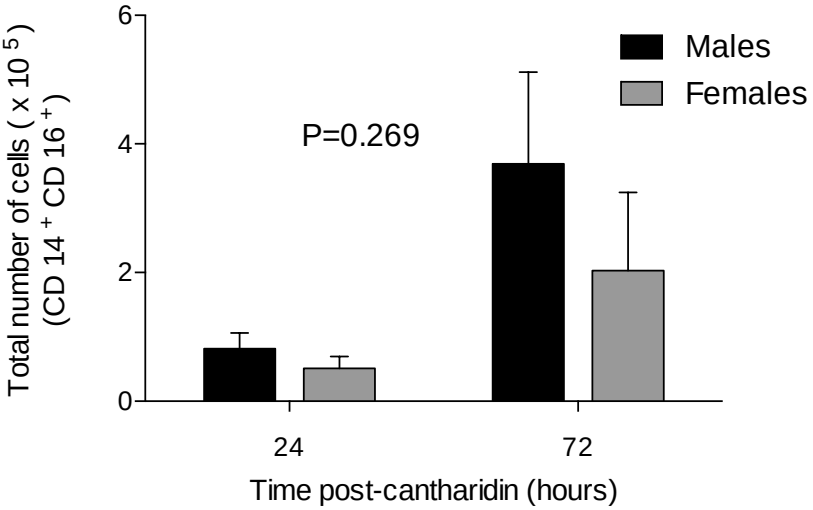

Figure S4 – Intermediate Monocyte count in cantharidin-induced blister fluid at 24 hours and 72 hours post application in healthy volunteers (male n=13-15, female n=14-16). Data shown as mean ± s.e.mean with statistical significance determined using two-way ANOVA to compare between sexes on each day; not significant.

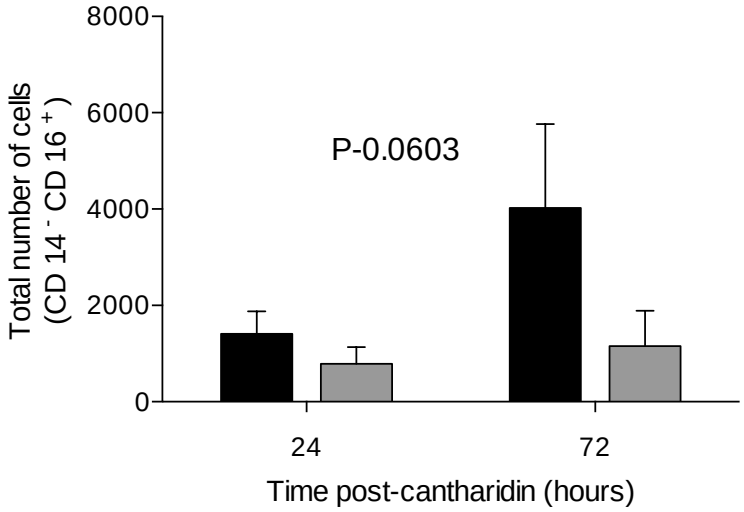

Figure S5 – Resident Monocyte in cantharidin-induced blister fluid at 24 hours and 72 hours post application in healthy volunteers (male n=13-15, female n=14-16). Data shown as mean ± s.e.mean with statistical significance determined using two-way ANOVA to compare between sexes on each day; not significant.

Figure S6. Distinct plasma LM-SPM profiles in males and females following typhoid vaccination. Plasma was collected for healthy volunteers following typhoid vaccine administration and LM levels were assessed using LM-profiling (see methods for details). (A) MS-MS fragmentation and diagnostic ions employed for LXB_4 and RvE1 identification. m/z, mass-to-charge ratio; M-H, molecular ion. (B) *top panel* 2-dimentional score plot of human plasma LM-SPM profiles. *Bottom panel* 2-dimentional loading plot. Grey ellipse in the score plot denotes 95% confidence regions. Results are representative n=4 male and n=4 female healthy volunteers. (C-D) Cumulative levels for each of the bioactive LM families identified. Results are mean±s.e.m n=4 volunteers per group. * $p<0.05$ vs Male volunteer plasma values.