

SUPPLEMENTARY MATERIAL

Laitano et al. Skeletal Muscle Interleukin-6 contributes to the innate immune response in septic mice

Table S1 Blood and Peritoneal Lavage Leukocyte Counts at 3 hours Post Cecal Slurry Injection.

Leukocytes	CRE			skmIL-6		
	Vehicle	Raloxifene	p-value	Vehicle	Raloxifene	p-value
FEMALE						
Blood						
Total Leukocytes (x10 ³ /μL)	1.75±1.09	1.92±1.05	0.73	2.14±1.37	1.83±0.49	0.48
% Neutrophils	44.3±11.7	51.2±7.9	0.14	44.7±8.0	44.8±5.7	0.99
% Lymphocytes	42.0±15.4	38.7±8.3	0.56	39.7±11.0	42.4±11.4	0.58
% Basophils	1.9±1.6	1.6±1.1	0.60	4.2±3.4	2.2±1.8	0.10
% Eosinophils	7.9±6.8	6.3±5.9	0.58	6.5±4.3	5.7±5.5	0.68
% Monocytes	3.9±2.1	5.2±2.3	0.22	4.9±4.1	5.0±3.5	0.94
Peritoneal Lavage						
Total Leukocytes (x10 ³ /μL)	1.1±0.5	1.2±0.4	0.47	1.64±0.6	1.8±0.6	0.47
% Neutrophils	38.1±14.5	37.5±8.6	0.90	37.6±5.9	43.0±14.3	0.29
% Lymphocytes	33.3±23	29.4±11.9	0.65	15.9±3.7	16.9±3.6	0.84
% Basophils	0.7±0.5	0.55±0.3	0.43	1.7±1.1	1.7±1.0	0.83
% Eosinophils	20.6±6.4	20.8±8.4	0.94	34.0±4.3	26.7±12.6	0.116
% Monocytes	7.5±1.9	10.9±1.7	0.19	11.6±4.3	10.8±2.5	0.64
MALE						
Blood						
Total Leukocytes (x10 ³ /μL)	3.2±1.2	2.7±0.8	0.33	3.38±1.2	2.89±1.0	0.31
% Neutrophils	56.5±13.8	61.1±8.5	0.39	59.6±4.7	56.7±5.5	0.20
% Lymphocytes	30.2±8.5	34.8±14.1	0.39	33.5±3.9	34.1±7.3	0.80
% Basophils	0.82±0.3	0.98±0.3	0.71	0.8±1.0	1.2±1.4	0.41
% Eosinophils	4.3±4.6	3.4±2.3	0.62	2.3±2.9	2.9±3.2	0.63
% Monocytes	4.5±0.5	3.5±1.4	0.17	3.9±1.7	5.0±1.6	0.12
Peritoneal Lavage						
Total Leukocytes	2.5±1.3	2.0±0.7	0.49	1.7±1.3	1.6±1.1	0.83
% Neutrophils	42.1±21.8	52.7±17.8	0.26	46.9±7.6	43.2±7.2	0.73
% Lymphocytes	39.7±7.0	23.4±6.6	0.11	23.7±20.2	13.4±10.5	0.21
% Basophils	0.4±0.3	0.4±0.2	0.48	1.0±0.6	1.5±0.6	0.57
% Eosinophils	14.5±4.4	10.9±3.7	0.07	23.3±5.1	23.3±5.1	0.97
% Monocytes	7.0±2.8	9.1±2.2	0.09	9.0±4.3	13.0±4.5	0.08

Mean +/- SD. p values T-tests within Strains, i.e. Cre only and SkmIL-6,

Vehicle vs. Ralox, n = 8-11 per group

Table S2 – Circulatory cytokine/chemokine concentrations for Female mice at 3h post septic shock.

Cytokine/Chemokine (pg/ml)	CRE			skmIL-6		
	Vehicle	Raloxifene	p-value	Vehicle	Raloxifene	p-value
GM-CSF	309±76	260 ± 110	0.284	164±70	130±52	0.214
IFN-γ	13±4	18 ± 9	0.186	13±5	12±4	0.845
IL-1 α	711±294	908 ± 656	0.420	821±466	664±346	0.380
IL-1β	145±89	128 ± 78	0.662	112±111	70±68	0.294
IL-2	41±23	54 ± 49	0.493	29±21	21±14	0.309
IL-4	10 ± 8	33 ± 39	0.089	26±34	5±3	0.072
IL-5	235±127	250 ± 206	0.860	277±275	148±129	0.174
IL-6	60312±0	59356 ± 2868	0.362	12015±13047	7260± 0978	0.366
IL-7	13±1	15 ± 5	0.171	15±5	21±14	0.201
IL-9	1090±211	1075 ± 374	0.916	1029±459	1036±546	0.973
IL-10	8464±2237	13851 ± 1022	0.141	7776±9204	2395±2879	0.093
IL-12p40	96±0	96 ± 0	-	106±32	96±0	0.325
IL-12p70	224±66	240 ± 101	0.695	81±46	67±37	0.458
IL-13	394±115	350 ± 150	0.485	100±64	54±14	0.042*
IL-15	778±202	741 ± 340	0.777	657±449	439±226	0.166
IL-17	348±200	257 ± 234	0.380	167±175	90±114	0.231
CXCL10 (IP-10)	6302±2910	7069 ± 3096	0.586	4606±4482	3250±2585	0.395
CXCL1 (KC)	<i>saturated</i>	<i>Saturated</i>	-	51166±30246	49483±29880	0.8969
CCL2 (MCP-1)	16807±10387	11015±7133	0.170	10596±11652	7039±6997	0.3957
CCL3 (MIP-1α)	2298±803	2431±1016	0.757	1994±1999	1314±1042	0.3292
CCL4 (MIP-1β)	8699±4070	9604±5346	0.685	5518±6511	3662±3511	0.420
CXCL2 (MIP-2)	69837±11973	69321±10892	0.922	4120±34993	3083±30346	0.466
CCL5 (RANTES)	269±82	262±66	0.839	161±142	111±61	0.303
TNF-α	247±70	245±85	0.970	154±151	110±89	0.422

Mean +/- SD. p values T-tests within Strains, i.e. Cre only and SkmIL-6, Vehicle vs. Ralox, n = 8-11 per group

Table S3 – Circulatory cytokine/chemokine concentrations for Male mice at 3h post septic shock.

Cytokine/Chemokine (pg/ml)	CRE			skmIL-6		
	Vehicle	Raloxifene	p-value	Vehicle	Raloxifene	p-value
GM-CSF	305 ± 86	302 ± 112	0.952	113 ± 33	137 ± 56	0.244
IFN-γ	10 ± 1	16 ± 9	0.099	10 ± 2	11 ± 2	0.221
IL-1 α	536 ± 200	642 ± 180	0.241	456 ± 168	483 ± 200	0.746
IL-1β	133 ± 79	140 ± 101	0.869	38 ± 47	70 ± 103	0.371
IL-2	34 ± 16	46 ± 30	0.282	11 ± 1	26 ± 27	0.070
IL-4	4 ± 9	4 ± 1	0.187	4 ± 10	17 ± 45	0.329
IL-5	155 ± 86	158 ± 79	0.951	90 ± 106	127 ± 167	0.540
IL-6	58433 ± 5940	57280 ± 5968	0.678	3956 ± 6932	7351 ± 13308	0.461
IL-7	14 ± 5	16 ± 4	0.556	12 ± 0	12 ± 0	-
IL-9	765 ± 133	1064 ± 369	0.028*	787 ± 342	793 ± 294	0.964
IL-10	4372 ± 3131	4347 ± 3848	0.987	761 ± 1079	1099 ± 1777	0.595
IL-12p40	96 ± 0	96 ± 0	-	103 ± 24	96 ± 0	0.329
IL-12p70	200 ± 66	231 ± 83	0.385	42 ± 15	79 ± 70	0.102
IL-13	353 ± 98	358 ± 120	0.914	51 ± 19	63 ± 41	0.413
IL-15	637 ± 193	798 ± 289	0.167	251 ± 54	395 ± 178	0.01*
IL-17	457 ± 366	431 ± 325	0.869	102 ± 186	137 ± 262	0.722
CXCL10 (IP-10)	5044 ± 1992	5613 ± 2447	0.583	2675 ± 1710	1925 ± 1489	0.286
CXCL1 (KC)	71887 ± 9881	75012 ± 0	0.357	44982 ± 30589	38918 ± 29314	0.640
CCL2 (MCP-1)	12502 ± 6347	13133 ± 8048	0.850	6259 ± 7248	4499 ± 6033	0.542
CCL3 (MIP-1α)	1813 ± 602	2083 ± 971	0.470	674 ± 539	694 ± 723	0.942
CCL4 (MIP-1β)	6380 ± 2916	6925 ± 4006	0.737	1637 ± 1355	1716 ± 2345	0.924
CXCL2 (MIP-2)	54153 ± 19526	61998 ± 15696	0.351	24494 ± 26134	19522 ± 27673	0.669
CCL5 (RANTES)	210 ± 29	233 ± 71	0.361	86 ± 47	92 ± 47	0.745
TNF-α	246 ± 69	249 ± 94	0.937	93 ± 119	65 ± 57	0.489

Mean +/- SD. p values T-tests within Strains, i.e. Cre only and SkmIL-6, Vehicle vs. Ralox, n = 8-11 per group

Supplement Figure S1

SkmlL-6 Strain: Blood Leukocytes

□ skmlL-6 strain cntrl
 ■ skmlL-6KD

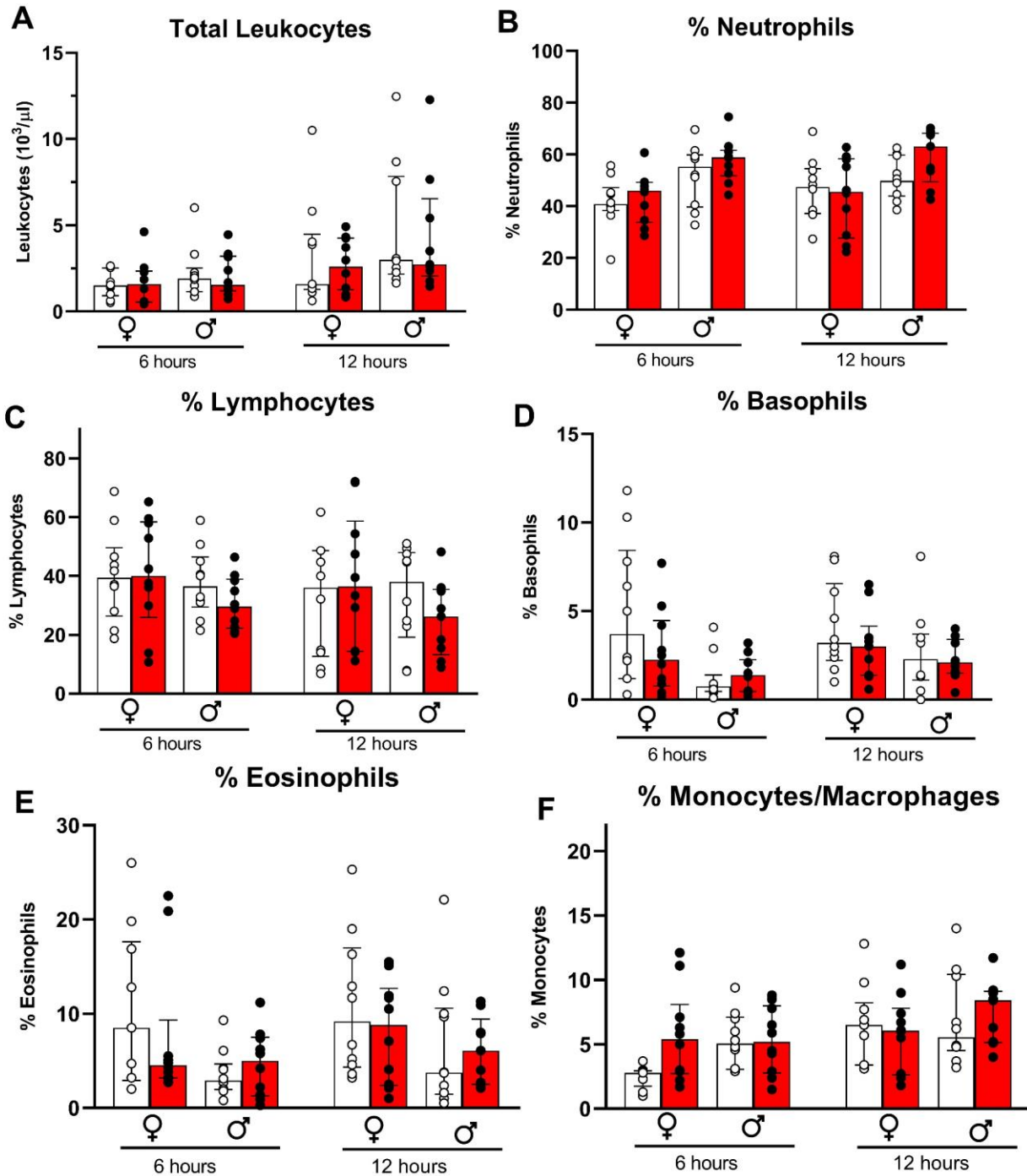


Figure S1. Blood leukocyte differential counts SkmlL-6 mice. White bars: skmlL-6 (vehicle treated/control) Closed bars skmlL-6KD (raloxifene treated) at 6 and 12 hours post EHS. (Medians +/- 25-75% quartiles).

Supplemental Figure S2

CRE-Mouse Strain: Peritoneal Leukocytes

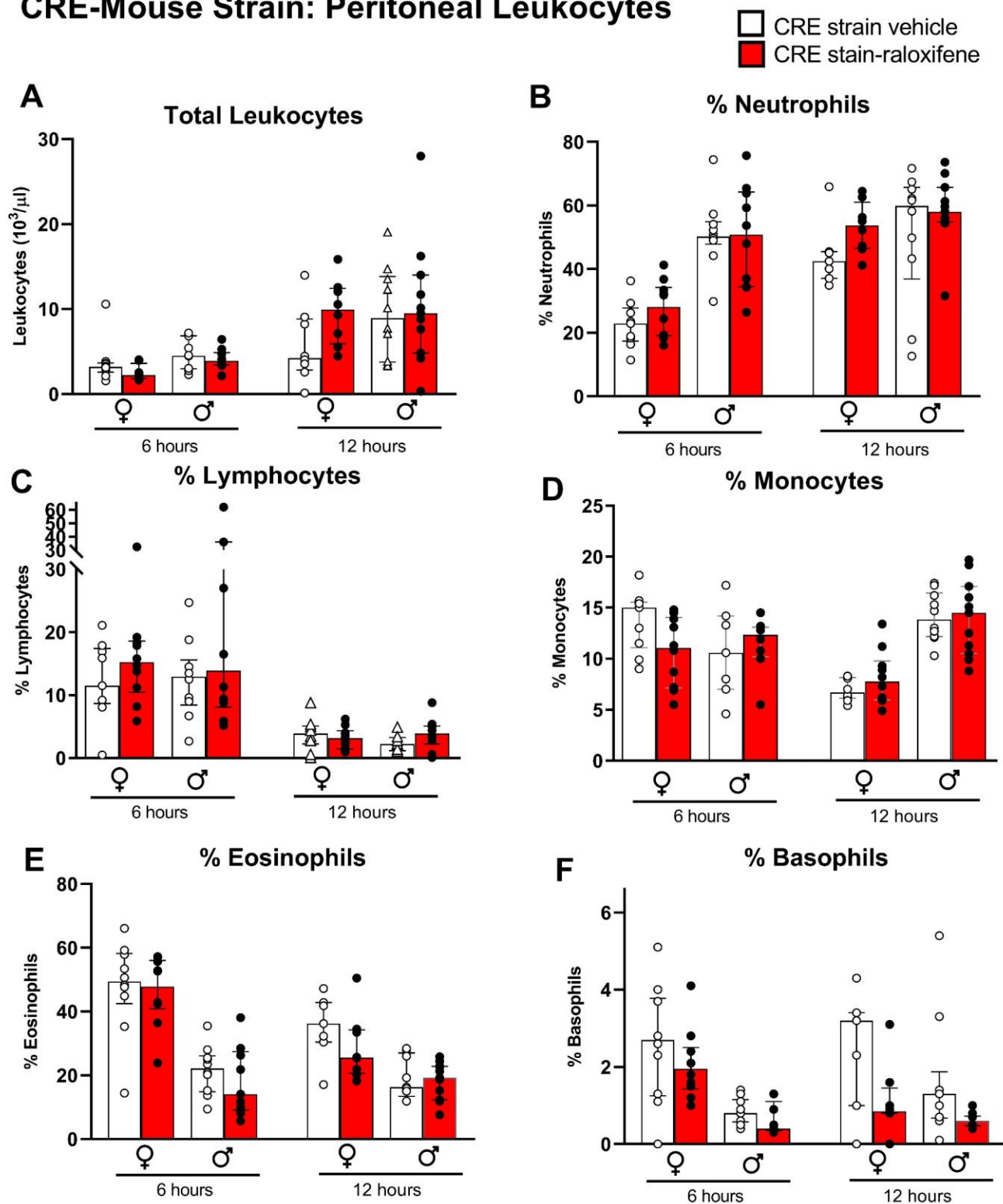


Figure S2. Peritoneal leukocyte differential counts in CRE mice. Open bars: CRE-mice (vehicle treated) against, Closed bar: CRE mic (raloxifene treated) at 6 and 12 hours post EHS. Median +/- 25-75% quartiles.

Supplemental Figure S3

Male and Female Plasma Cytokines: SkmIL-6 mice (6 & 12 h)

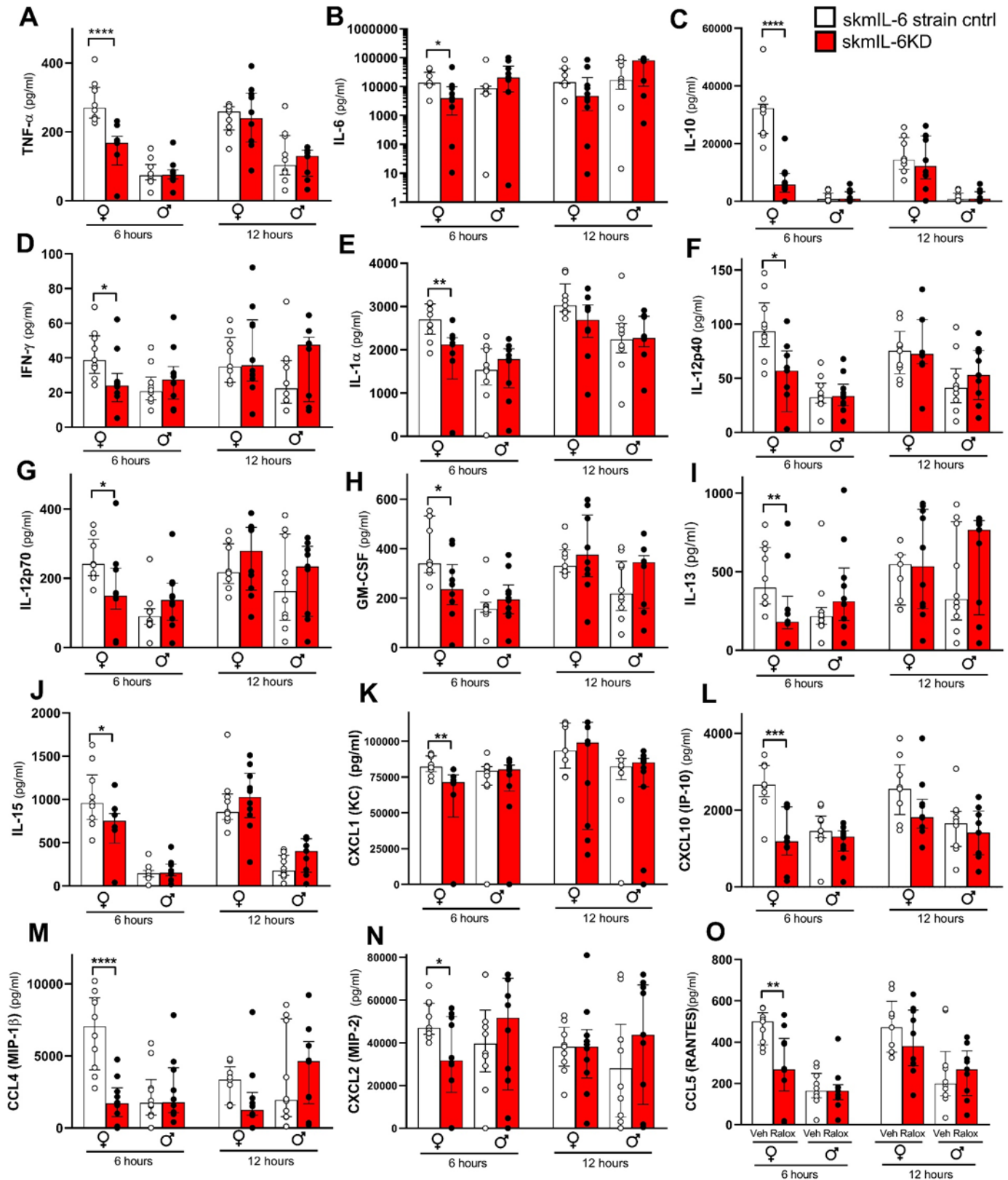


Figure S3. Effects of skmIL-6KD on circulating cytokines White bars = SkmIL-6KD strain match control. Red Bars = SkmIL-6 KD. Time points: 6 and 12 hours post cecal slurry. Following ANOVA, groups were tested by orthogonal comparisons within sex and strain matched controls. * = $P < 0.05$, ** = $P < 0.01$. $N = 9-10$ in each group. Data expressed as medians $\pm 25-75\%$ quartiles.

Supplemental Figure S4

Male and Female Plasma Cytokines: Cre mice (6 & 12 h)

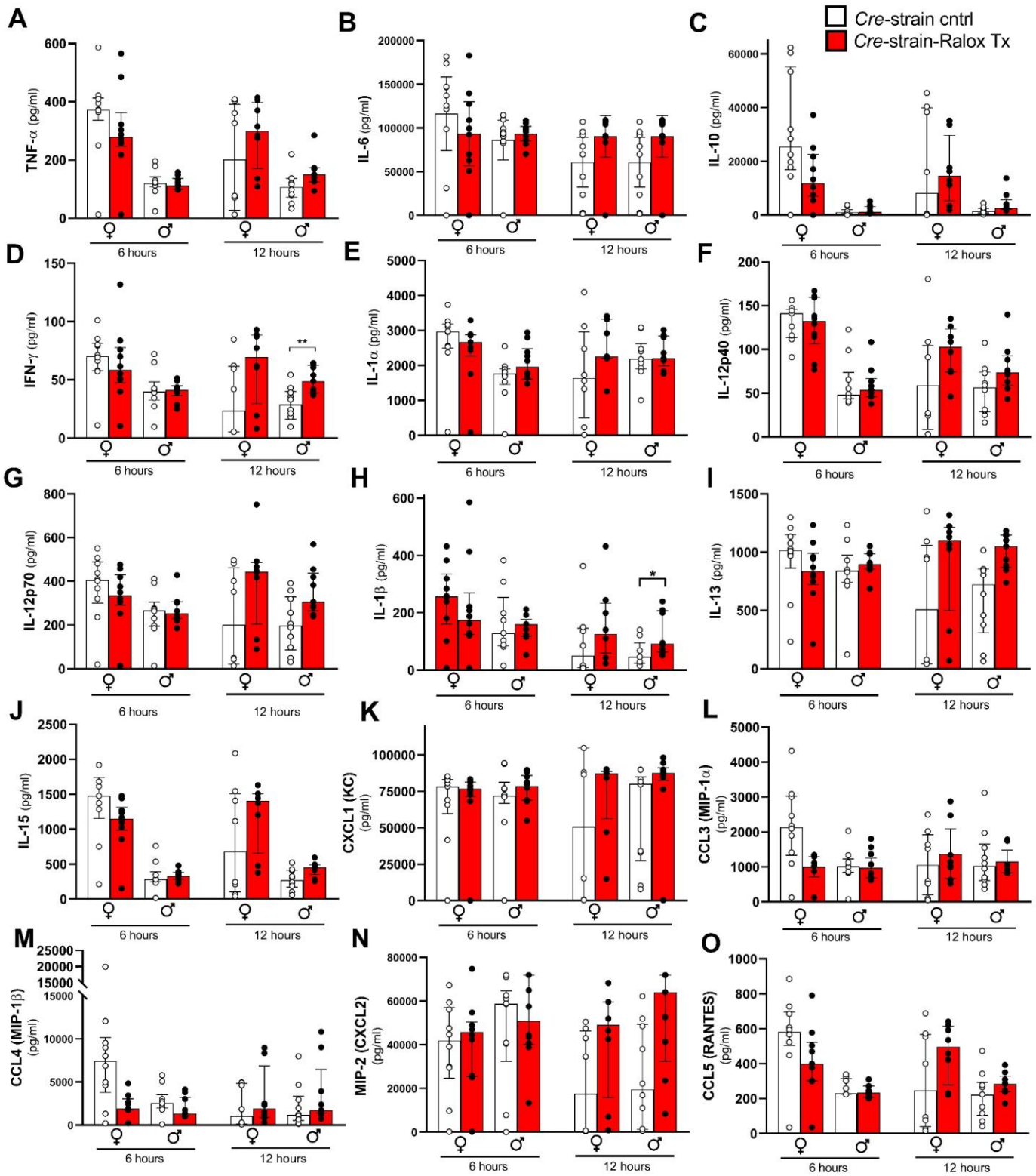


Fig. S4. CRE mice, effects of CRE induction with raloxifene on circulating cytokines/chemokines. White bars = control CRE mice (vehicle treated); Red bars = CRE mice treated with raloxifene. Time points: 6 and 12 hours post cesal slurry. Following ANOVA, groups were tested by orthogonal comparisons within sex and strain matched controls. * = $P < 0.05$, ** = $P < 0.01$. $N = 9-10$ in each group. Medians \pm 25-75% quartiles.