

# **Supplementary Materials for General Direct Anticancer Effects of Deer Growing Antler Extract in Several Tumour Cell Lines, and Immune System-Mediated Effects in Xenograft Glioblastoma**

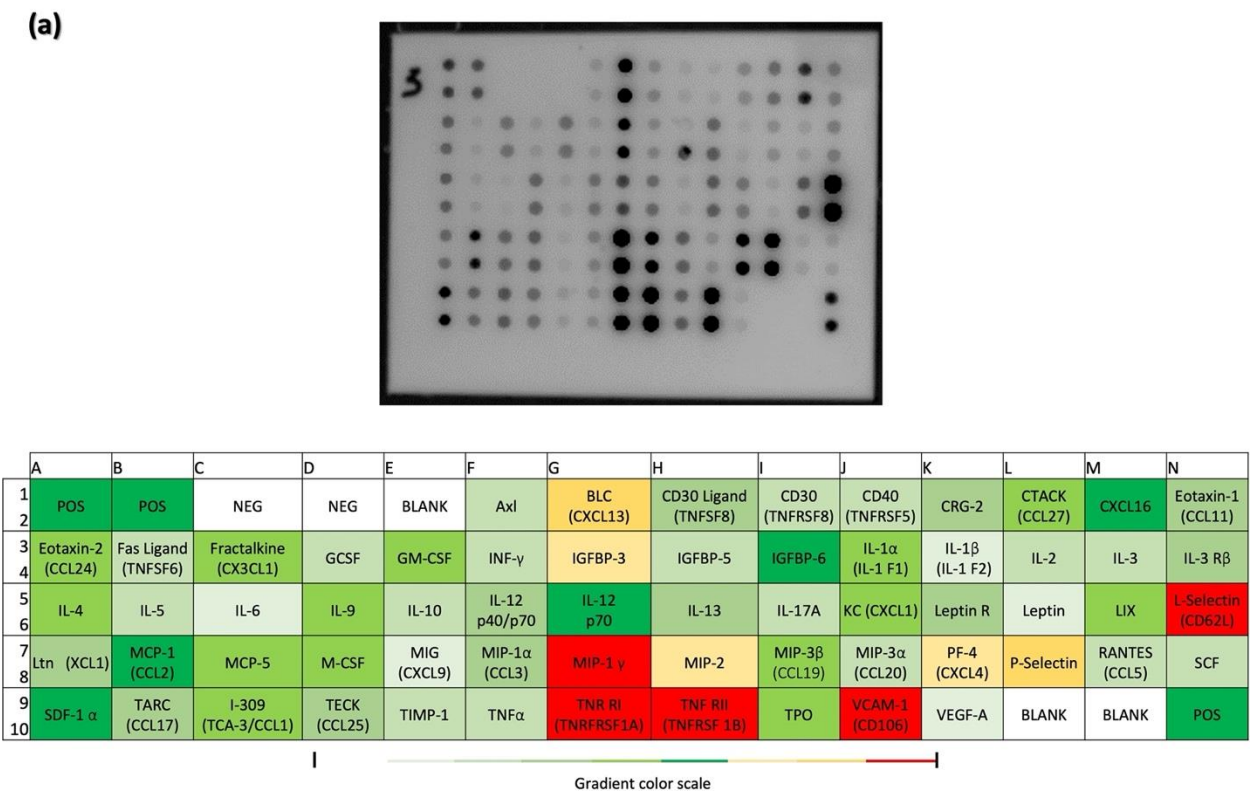
Alessandra Rossetti *et al.*

Corresponding author: Tomás Landete Castillejos, [Tomas.Landete@uclm.es](mailto:Tomas.Landete@uclm.es)

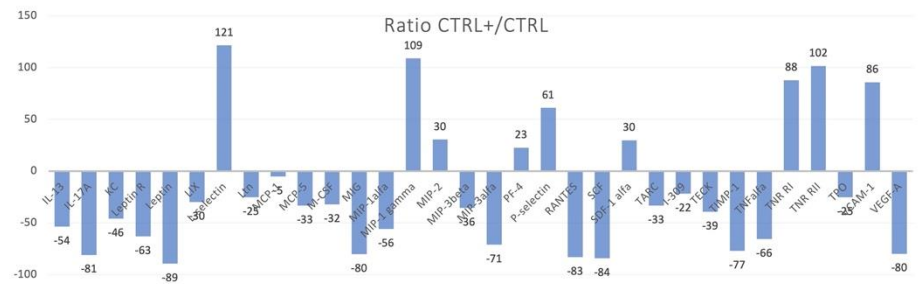
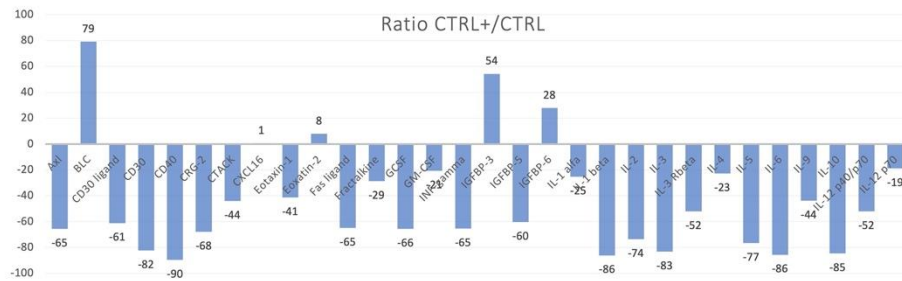
**This PDF file includes the following:**

Figure S1,  
Figure S2,  
Figure S3,  
Table S1.

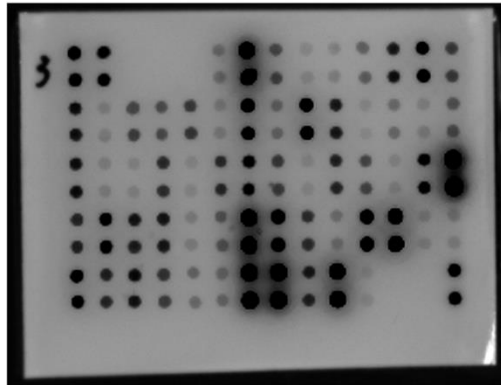
Figure S1. Mouse cytokine antibody array.



(b)



(c)



	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	POS	POS	NEG	NEG	BLANK	Axl	BLC (CXCL13)	CD30 Ligand (TNFSF8)	CD30 (TNFRSF8)	CD40 (TNFRSF5)	CRG-2	CTACK (CCL27)	CXCL16	Eotaxin-1 (CCL11)
2														
3	Eotaxin-2 (CCL24)	Fas Ligand (TNFSF6)	Fractalkine (CX3CL1)	G-CSF	GM-CSF	INF-γ	IGFBP-3	IGFBP-5	IGFBP-6	IL-1α (IL-1 F1)	IL-1β (IL-1 F2)	IL-2	IL-3	IL-3 Rβ
4														
5	IL-4	IL-5	IL-6	IL-9	IL-10	IL-12 p40/p70	IL-12 p70	IL-13	IL-17A	KC (CXCL1)	Leptin R	Leptin	LIX	L-Selectin (CD62L)
6														
7	Ltn (XCL1)	MCP-1 (CCL2)	MCP-5	M-CSF	MIG (CXCL9)	MIP-1α (CCL3)	MIP-1 γ	MIP-2	MIP-3β (CCL19)	MIP-3α (CCL20)	PF-4 (CXCL4)	P-Selectin	RANTES (CCL5)	SCF
8														
9	SDF-1 α	TARC (CCL17)	I-309 (TCA-3/CCL1)	TECK (CCL25)	TIMP-1	TNFα	TNR RI (TNFRSF1A)	TNF RII (TNFRSF 1B)	TPO	VCAM-1 (CD106)	VEGF-A	BLANK	BLANK	POS
10														

Gradient color scale

(d)

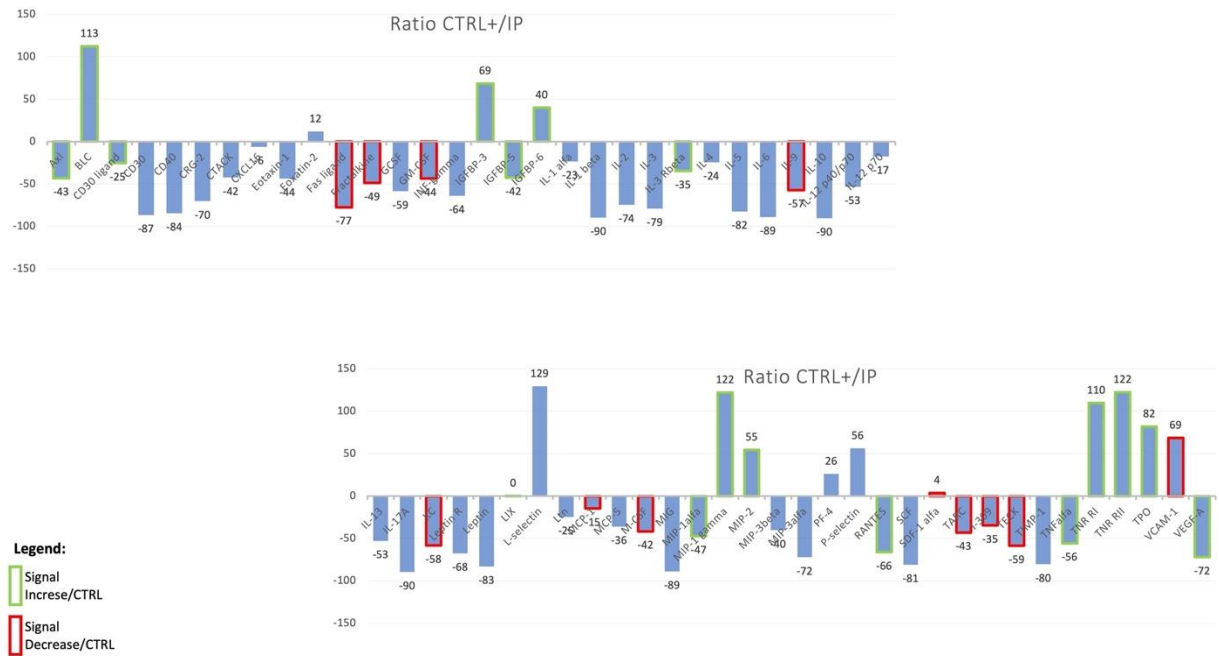
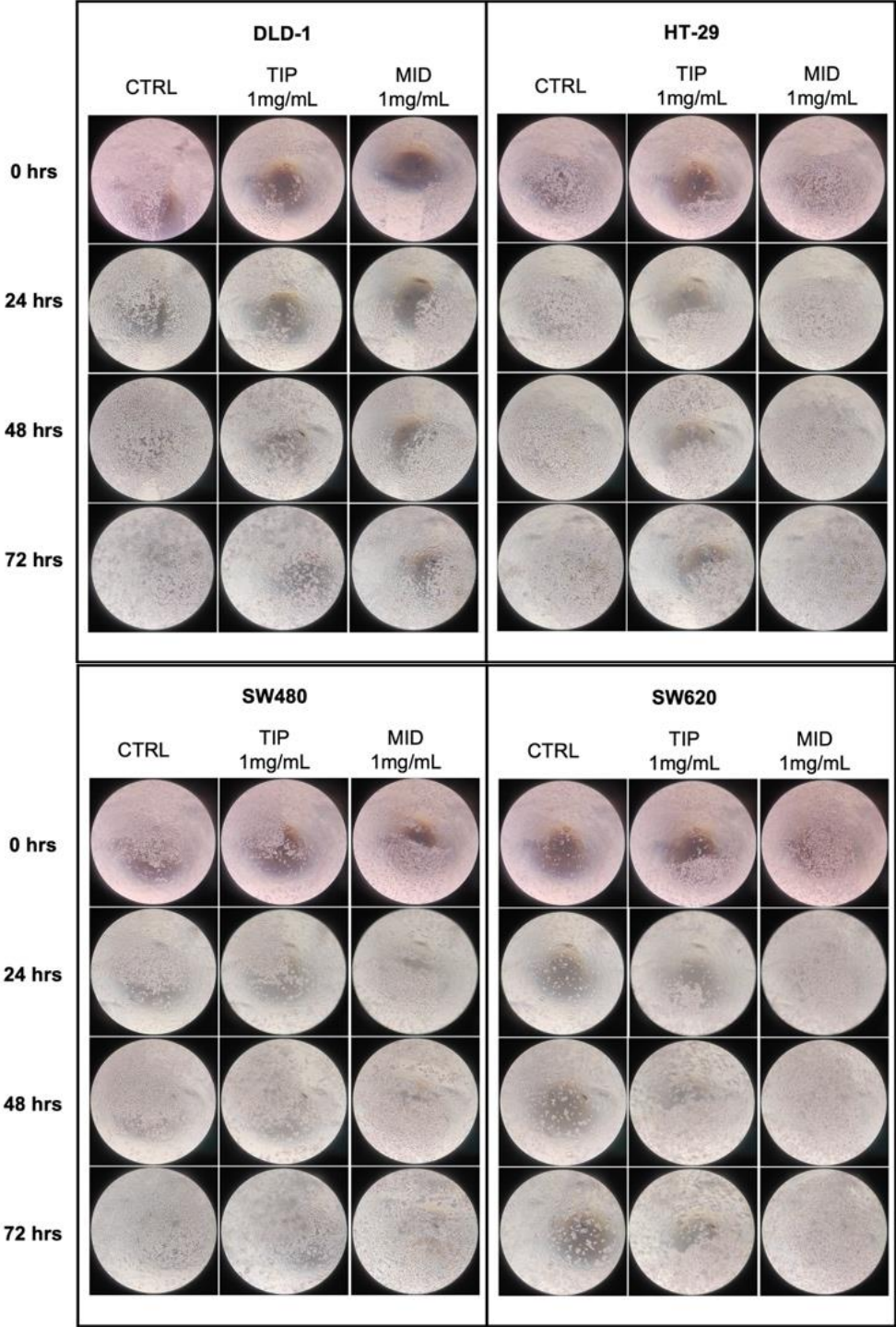
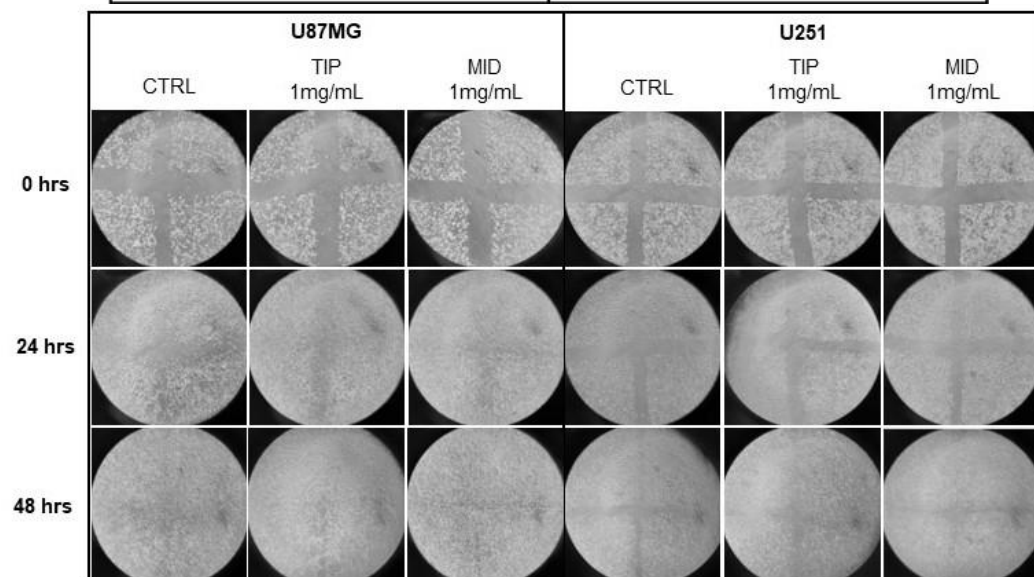
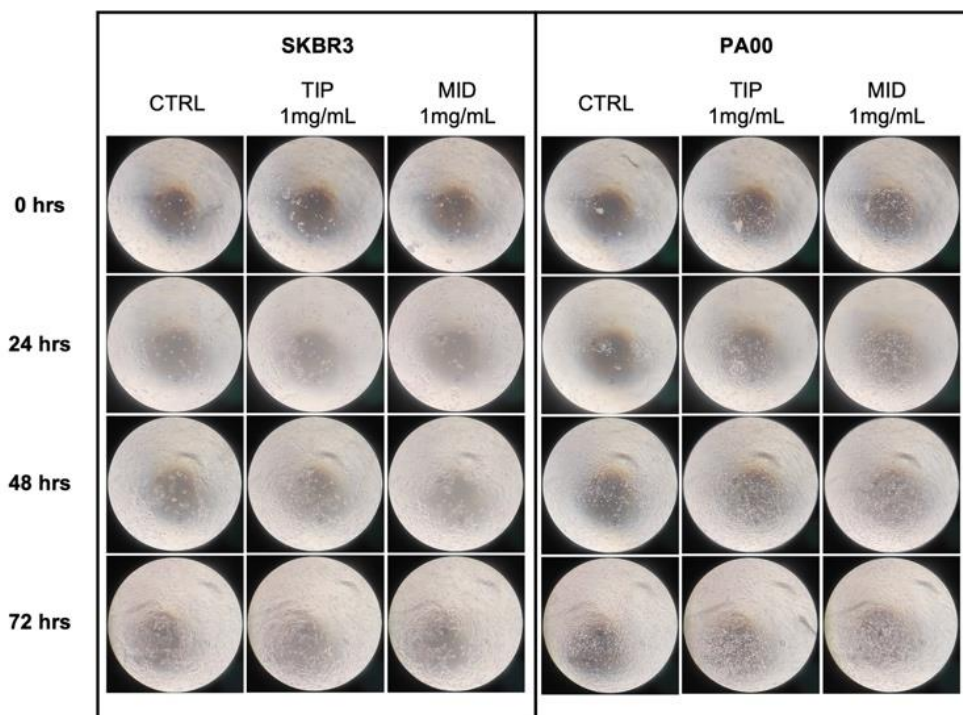


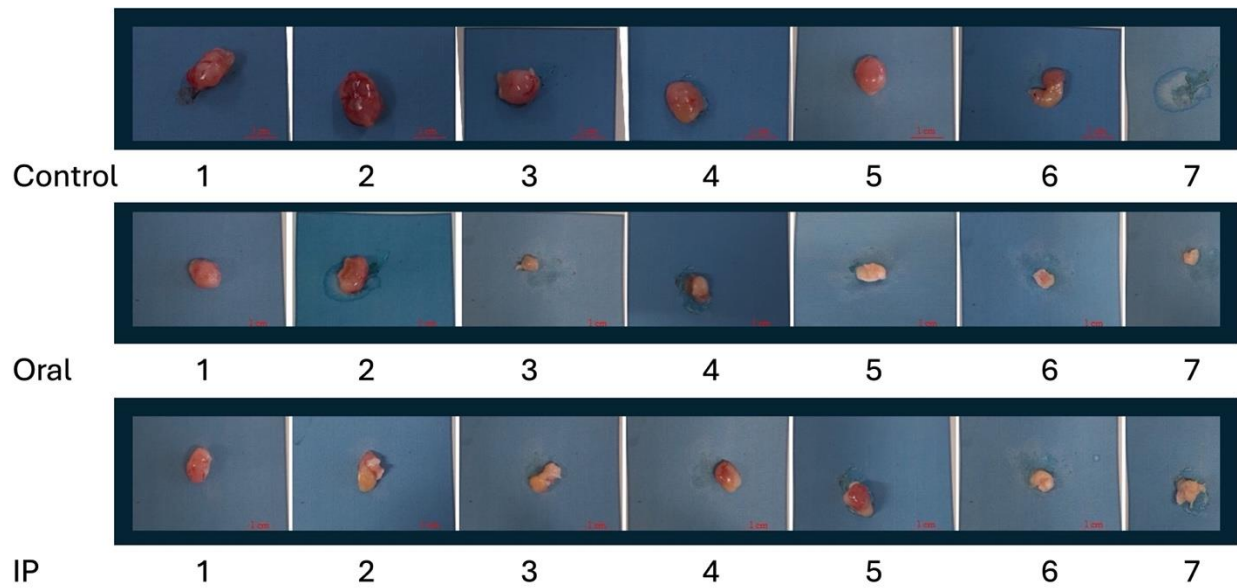
Figure S2. Images of scratch assay.







**Figure S3.** Images of xenograft tumours.



The tumours were removed after sacrifice and weighed for statistical comparison, which is shown in Figure 5 of the main text of the MS.

**Table S1.** List of functional annotations.

Category	Term	Count	%	P-value	Genes	Fold Enrichment	Benjamini	FDR
KEGG_PATHWAY	mm00600-Cytokine-cytokine receptor interaction	11	91.66666667	1.17E-14	IL10, CCL25, CD40, CSF2, IL5, CCL5, IL1B, LEP, F	28.28795662	<b>9.36E-13</b>	7.14E-13
GOTERM_BP_DIRECT	GO:0005123-cytokine activity	9	75	9.20E-14	IL10, CCL25, CSF2, IL5, CCL5, IL1B, FASLG, CXCL	59.36170213	<b>4.69E-12</b>	4.40E-12
GOTERM_BP_DIRECT	GO:0006954-inflammatory response	8	66.66666667	3.65E-10	CCL25, CD40, IL5, AXL, CCL5, IL1B, CXCL1, IL1	33.44006651	<b>1.69E-07</b>	1.56E-07
GOTERM_BP_DIRECT	GO:0006955-immune response	8	66.66666667	3.56E-09	IL10, CCL25, CSF2, IL5, CCL5, IL1B, FASLG, CXCL	25.96662111	<b>5.44E-07</b>	5.04E-07
GOTERM_BP_DIRECT	GO:007134-cellular response to interleukin-1	5	41.66666667	8.26E-08	CCL25, CD40, CCL5, CXCL1, IL17A	102.326911	<b>1.27E-05</b>	1.18E-05
GOTERM_BP_DIRECT	GO:0042531-positive regulation of tyrosine phosphorylation of STAT protein	4	33.33333333	3.58E-06	CD40, CSF2, CCL5, LEP	17.65497708	<b>4.40E-04</b>	3.75E-04
GOTERM_BP_DIRECT	GO:0043491-protein kinase B signaling	4	33.33333333	5.21E-06	CD40, AXL, CCL5, IL1B	103.174559	<b>4.82E-04</b>	4.46E-04
GOTERM_BP_DIRECT	GO:0036939-neurotrophin chemotaxis	4	33.33333333	1.05E-05	CCL25, CCL5, IL1B, CXCL1	81.7855285	<b>8.10E-04</b>	7.50E-04
GOTERM_BP_DIRECT	GO:0008264-positive regulation of cell proliferation	4	33.33333333	1.24E-05	CSF2, IL5, IL1B, LEP, FASLG, CXCL1	15.79199172	<b>8.15E-04</b>	7.55E-04
KEGG_PATHWAY	mm04672-cellular response to interferon gamma	4	33.33333333	1.63E-05	IL10, CCL25, CD40, IL5	69.85271318	<b>6.51E-04</b>	4.96E-04
KEGG_PATHWAY	mm05121-Inflammatory bowel disease	4	33.33333333	2.54E-05	CCL25, CCL5, FASLG, CXCL1	60.9666667	<b>0.001467734</b>	0.001359719
KEGG_PATHWAY	mm05130-Allograft rejection	4	33.33333333	4.92E-05	IL10, IL5, IL1B, IL17A	48.44623606	<b>0.001012802</b>	7.88E-04
GOTERM_BP_DIRECT	GO:0071350-cellular response to tumor necrosis factor	4	33.33333333	5.16E-05	IL10, CD40, IL5, FASLG	47.6724808	<b>0.001032802</b>	7.88E-04
GOTERM_BP_DIRECT	GO:0041112-positive regulation of IkappaB kinase/NF-kappaB signaling	4	33.33333333	6.04E-05	CCL25, CD40, CCL5, CXCL1	45.62131519	<b>0.001068789</b>	0.002872675
GOTERM_BP_DIRECT	GO:0048245-oncogenic chemotaxis	3	25	8.80E-05	CD40, IL1B, FASLG, CXCL1	41.81234401	<b>0.001434957</b>	0.002912217
KEGG_PATHWAY	mm05120-Antimutagen thyroid disease	3	25	8.77E-05	CCL25, CCL5, CXCL1	193.4519231	<b>0.003683197</b>	0.003412139
GOTERM_BP_DIRECT	GO:0048020-CCR chemokine receptor binding	3	33.33333333	1.02E-04	IL10, CD40, IL5, FASLG	38.02109705	<b>0.001627847</b>	0.001341232
GOTERM_BP_DIRECT	GO:0048247-lymphocyte chemotaxis	3	25	1.03E-04	CCL25, CCL5, CXCL1	178.8401538	<b>0.002614872</b>	0.0025636
GOTERM_BP_DIRECT	GO:0043544-positive regulation of GTPase activity	3	25	1.25E-04	CCL25, CCL5, CXCL1	16.25	<b>0.004666953</b>	0.004325907
KEGG_PATHWAY	mm00435-Rheumatoid arthritis	3	25	1.31E-04	CCL25, CD40, CCL5, CXCL1	35.1109284	<b>0.004666953</b>	0.004325907
GOTERM_BP_DIRECT	GO:001240-negative regulation of extrinsic apoptotic signaling pathway in absence of	3	25	1.36E-04	CSF2, CCL5, IL1B, IL17A	34.52494521	<b>0.007152469</b>	0.006134351
KEGG_PATHWAY	mm04657-IL-1 signaling pathway	3	25	1.51E-04	CSF2, IL1B, CXCL1	147.9338235	<b>0.004983248</b>	0.00461673
KEGG_PATHWAY	mm00567-IL-1 signaling pathway	3	33.33333333	1.65E-04	CSF2, IL5, IL1B, IL17A	32.29749104	<b>0.001761849</b>	0.001343431
KEGG_PATHWAY	mm00601-Viral protein interaction with cytokine and cytokine receptor	3	25	1.79E-04	IL10, CCL25, CCL5, CXCL1	16.41745386	<b>0.001761849</b>	0.001343431
GOTERM_BP_DIRECT	GO:0005248-oncogenic chemotaxis	3	25	1.79E-04	CCL25, CCL5, CXCL1	135.931892	<b>0.005516862</b>	0.00511086
KEGG_PATHWAY	mm05142-Chagas disease	4	33.33333333	2.24E-04	IL10, CCL5, IL1B, FASLG	29.1031232	<b>0.001995288</b>	0.001512504
GOTERM_BP_DIRECT	GO:0070314-positive regulation of ERM1 and ERM2 cascade	3	33.33333333	2.36E-04	CCL25, CCL5, IL1B, CXCL1	26.78254649	<b>0.005516862</b>	0.00511086
GOTERM_BP_DIRECT	GO:0050796-regulation of insulin secretion	3	25	2.42E-04	CCL5, IL1B, LEP	116.7093002	<b>0.006516599</b>	0.006037023
GOTERM_BP_DIRECT	GO:0032725-positive regulation of interleukin-12 production	3	25	2.42E-04	CD40, LEP, IL17A	113.1312	<b>0.005516599</b>	0.005037023
KEGG_PATHWAY	mm04668-IL-1 signaling pathway	3	33.33333333	2.48E-04	CSF2, CCL5, CXCL1	80.8113294	<b>0.00235667</b>	0.001786189
GOTERM_BP_DIRECT	GO:0008039-chemokine activity	3	25	2.97E-04	CCL25, CCL5, CXCL1	105.6818182	<b>0.005044808</b>	0.004694589
GOTERM_BP_DIRECT	GO:0030335-positive regulation of cell migration	4	33.33333333	3.56E-04	CSF2, CCL5, IL1B, CXCL1	23.02163184	<b>0.008283015</b>	0.007893279
GOTERM_BP_DIRECT	GO:0070938-mediated signaling pathway	3	25	3.69E-04	CCL25, CCL5, CXCL1	94.8999414	<b>0.008283015</b>	0.007893279
KEGG_PATHWAY	mm05130-Asthma	3	25	4.00E-04	IL10, CD40, IL5	90.11	<b>0.002911111</b>	0.002719722
GOTERM_BP_DIRECT	GO:0046424-positive regulation of JAK-STAT cascade	3	25	4.73E-04	IL10, IL5, LEP	83.82916667	<b>0.004012802</b>	0.003965740
BIOCARTA	in_cytokinePathway-The Role of Interleukin-10 in the Chemokine Network of Allergic	3	25	5.02E-04	CSF2, IL5, IL1B, IL17A	14.44444444	<b>0.01586326</b>	0.014486514
GOTERM_BP_DIRECT	GO:0032720-negative regulation of tumor necrosis factor production	3	25	6.80E-04	IL10, AXL, CXCL1	69.85763889	<b>0.014286804</b>	0.013234727
GOTERM_BP_DIRECT	GO:0042102-positive regulation of cell proliferation	3	25	8.39E-04	CCL5, IL1B, LEP	62.781875	<b>0.005085202</b>	0.004781547
KEGG_PATHWAY	mm01143-African trypanosomiasis	3	25	8.80E-04	IL10, IL1B, CXCL1	57.7623051	<b>0.006095315</b>	0.005647677
KEGG_PATHWAY	mm00430-JAK-STAT signaling pathway	4	33.33333333	9.00E-04	IL10, CSF2, IL5, LEP	17.56530214	<b>0.006095315</b>	0.005647677
REACTOME_PATHWAY	R-MMU_380108-Chemokine receptors bind chemokines	3	25	0.001070486	CCL25, CCL5, CXCL1	52.7654231	<b>0.005425285</b>	0.049340987
BIOCARTA	in_cytokinePathway-Angiogenesis Dependent T Cell Activation	3	25	0.001481335	IL10, IL1B, CXCL1	43.86666667	<b>0.023318021</b>	0.020529206
GOTERM_BP_DIRECT	GO:0032735-positive regulation of interleukin-6 production	3	25	0.001606077	IL1B, LEP, IL17A	45.31306306	<b>0.030916964</b>	0.02864170
KEGG_PATHWAY	mm05147-Lipid and atherosclerosis	4	33.33333333	0.001943993	CD40, CCL5, IL1B, FASLG	13.989642	<b>0.001188504</b>	0.00470257
KEGG_PATHWAY	mm05144-Malaria	25	208.3333333	0.002053611	IL10, IL5, IL1B	39.52179282	<b>0.011117485</b>	0.010617667
GOTERM_BP_DIRECT	GO:0051091-positive regulation of sequence-specific DNA binding transcription factor	3	25	0.002222666	IL10, IL5, IL1B	38.39503817	<b>0.00164665</b>	0.017308824
GOTERM_BP_DIRECT	GO:0051097-positive regulation of protein kinase B signaling	3	25	0.002360349	AXL, LEP, CXCL1	38.10416667	<b>0.00164665</b>	0.017308824
GOTERM_BP_DIRECT	GO:0009135-chemotaxis	3	25	0.003793993	IL10, IL1B, CXCL1	36.9845558	<b>0.01149171</b>	0.010793816
GOTERM_BP_DIRECT	GO:0050999-regulation of nitric-oxide synthase activity	2	16.66666667	0.003731078	IL1B, LEP	67.06333333	<b>0.045061794</b>	0.041345558
KEGG_PATHWAY	mm05163-Human cytomegalovirus infection	4	33.33333333	0.003151271	CCL5, IL1B, FASLG, CXCL1	11.7373092	<b>0.005780553</b>	0.021207094
GOTERM_BP_DIRECT	GO:0019211-cytokine-mediated signaling pathway	25	208.3333333	0.003297769	IL5, IL1B, CXCL1	114.559375	<b>0.009755738</b>	0.009409554
GOTERM_BP_DIRECT	GO:0045576-positive regulation of angiogenesis	3	25	0.003297769	CD40, CCL5, IL1B	31.4539375	<b>0.009755738</b>	0.009409554
GOTERM_BP_DIRECT	GO:0045944-positive regulation of transcription from RNA polymerase II promoter	5	41.66666667	0.003338096	IL10, CD40, IL1B, CXCL1, IL17A	6.76041667	<b>0.009755738</b>	0.009409554
GOTERM_BP_DIRECT	GO:0051091-positive regulation of transcription factor activity	3	25	0.003432035	IL10, IL5, IL1B	39.52179282	<b>0.004758186</b>	0.02864170
GOTERM_BP_DIRECT	GO:0051092-positive regulation of NF-kappaB transcription factor activity	3	25	0.003503541	CD40, IL1B, CXCL1	30.48333333	<b>0.005056706</b>	0.04664860
GOTERM_BP_DIRECT	GO:0010625-positive regulation of fever generation	2	16.66666667	0.003821525	CCL5, IL1B	47.93238095	<b>0.005192782</b>	0.048106257
GOTERM_BP_DIRECT	GO:0012744-positive regulation of interleukin-23 production	3	16.66666667	0.003821525	CCL5, IL17A	79.03238095	<b>0.005192782</b>	0.048106257
GOTERM_BP_DIRECT	GO:0043006-negative regulation of apoptosis	4	33.33333333	0.004761285	IL10, AXL, LEP, CXCL1	10.16111111	<b>0.006284895</b>	0.005822271
KEGG_PATHWAY	mm04640-Hematopoietic cell lineage	3	25	0.005573035	CSF2, IL5, IL1B	22.9642553	<b>0.02611929</b>	0.019969505
GOTERM_BP_DIRECT	GO:0030434-negative regulation of vascoconstriction	3	25	0.005952867	LEP, CXCL1, CXCL1	43.81333333	<b>0.027999664</b>	0.02611929
KEGG_PATHWAY	mm04620-Toll like receptor signaling pathway	3	25	0.006282703	CD40, CCL5, IL1B	22.5275	<b>0.027923124</b>	0.021291382
KEGG_PATHWAY	mm04607-T cell receptor signaling pathway	3	25	0.00665397	IL10, CSF2, IL5	21.8713922	<b>0.028016716</b>	0.021367240
BIOCARTA	in_cytokinePathway-Cytokine Network	3	25	0.007067495	IL10, IL5, IL1B, IL17A	69.41239841	<b>0.007381494</b>	0.006290272
GOTERM_BP_DIRECT	GO:0071803-positive regulation of podosome assembly	2	16.66666667	0.007086446	CSF2, IL5	257.9338074	<b>0.008486958</b>	0.007974097
KEGG_PATHWAY	mm05146-Anemia/hem	3	25	0.007164336	IL10, CSF2, IL5	21.05737832	<b>0.028057343</b>	0.021851224
GOTERM_BP_DIRECT	GO:0042573-chemokine receptor binding	3	16.66666667	0.007663414	CCL25, CCL5, CXCL1	35.8451538	<b>0.027368562</b>	0.026174414
GOTERM_BP_DIRECT	GO:0070328-dendritic cell differentiation	3	16.66666667	0.008713405	CSF2, AXL	209.5729167	<b>0.030363888</b>	0.005750202
GOTERM_BP_DIRECT	GO:0001934-positive regulation of protein phosphorylation	3	25	0.008725524	CD40, IL1B, LEP	10.58203333	<b>0.030363888</b>	0.005750202
BIOCARTA	in_cytokinePathway-Cytokines and Inflammatory Responses	3	25	0.009121388	IL10, IL5, IL1B, IL17A	16.48717949	<b>0.030628621</b>	0.02864170
GOTERM_BP_DIRECT	GO:0071232-cellular response to lipopolysaccharide	3	25	0.01115226	IL10, IL1B, IL17A	16.76833333	<b>0.128737964</b>	0.119263491
GOTERM_BP_DIRECT	GO:0020035-positive regulation of endothelial cell apoptotic process	2	16.66666667	0.01142474	CD40, FASLG	159.6746032	<b>0.128774964</b>	0.119263491
REACTOME_PATHWAY	R-MMU_375276-Tyrosine ligand-binding receptor	3	25	0.012094035	CCL25, CCL5, CXCL1	13.8189962	<b>0.296381874</b>	0.285001804
KEGG_PATHWAY	mm05156-Alcoholic liver disease	3	25	0.01218847	IL1B, FASLG, IL17A	15.97695035	<b>0.046437513</b>	0.035480604
GOTERM_BP_DIRECT	GO:0010759-positive regulation of macrophage chemotaxis	2	16.66666667	0.015897419	CCL5, CXCL1	134.2266667	<b>0.149404414</b>	0.138462742
KEGG_PATHWAY	mm05155-Non-alcoholic fatty liver disease	3	25	0.016747632	IL10, IL1B, FASLG	14.44070513	<b>0.005375734</b>	0.004800859
GOTERM_BP_DIRECT	GO:0002676-positive regulation of immunoglobulin production	2	16.66666667	0.016284878	IL10, IL5	111.7722222	<b>0.172719465</b>	0.160088059
GOTERM_BP_DIRECT	GO:1900745-positive regulation of p38MAPK cascade	2	16.66666667	0.021632325	IL1B, LEP	108.1666667	<b>0.172719465</b>	0.160088059
GOTERM_BP_DIRECT	GO:0059040-basophilic migration	3	16.66666667	0.021632325	IL10, IL5, IL1B	108.1666667	<b>0.172719465</b>	0.160088059
KEGG_PATHWAY	mm05164-Influenza A	3	25	0.01799748	CCL5, IL1B, FASLG	13.016763	<b>0.005651409</b>	0.004730526
GOTERM_BP_DIRECT	GO:0014344-response to interferon gamma	2	16.66666667	0.04317655	CD40, CCL5	96.62249002	<b>0.1812821</b>	0.167900352
GOTERM_BP_DIRECT	GO:0045621-positive regulation of coelomic differentiation	2	16.66666667	0.04317655	CCL5, IL1B	96.62249002	<b>0.1812821</b>	0.167900352
GOTERM_BP_DIRECT	GO:0010934-macrophage cytokine production	2	16.66666667	0.030405976	AXL, IL1B	90.62612133	<b>0.192377173</b>	0.17873579
GOTERM_BP_DIRECT	GO:0030106-platelet activation	2	16.66666667	0.031122851	AXL, CXCL1	85.97863248	<b>0.199158329</b>	0.184048055
GOTERM_BP_DIRECT	GO:0042825-defense response to protozoan	2	16.66666667	0.031659911	IL10, CD40	81.82916667	<b>0.200129996</b>	0.185481018
KEGG_PATHWAY	mm05062-Chemokine signaling pathway	3	25	0.021889495	CCL25, CCL5, CXCL1	11.7373092	<b>0.072962884</b>	0.055053801
GOTERM_BP_DIRECT	GO:0008118-negative regulation of cytokine production	2	16.66666667	0.022195901	IL10, AXL	81.7855285	<b>0.20100414</b>	0.186384433
GOTERM_BP_DIRECT	GO:0012889-negative regulation of interferon-gamma production	2	16.66666667	0.022370764	IL10, AXL	79.83730159	<b>0.201058099</b>	0.187891676
GOTERM_BP_DIRECT	GO:0031663-lipopolysaccharide-mediated signaling pathway	2	16.66666667	0.023260201	CCL5, IL1B	77.98026104	<b>0.202811036</b>	0.18785548
GOTERM_BP_DIRECT	GO:0043056-chemotactant activity	2	16.66666667	0.024404949	CCL5, CXCL1	72.5	<b>0.198979979</b>	0.195078411
GOTERM_BP_DIRECT	GO:0010957-negative regulation of anaphage	2	16.66666667	0.024879911	IL1B, LEP	62.11784662	<b>0.172719465</b>	0.167900352
REACTOME_PATHWAY	R-MMU_418594-G alpha (i) signaling events	3	25	0.025227851	CCL25, CCL5, CXCL1	10.39902676	<b>0.030563097</b>	0.02864170