Figure S1. Cytokine array screening.

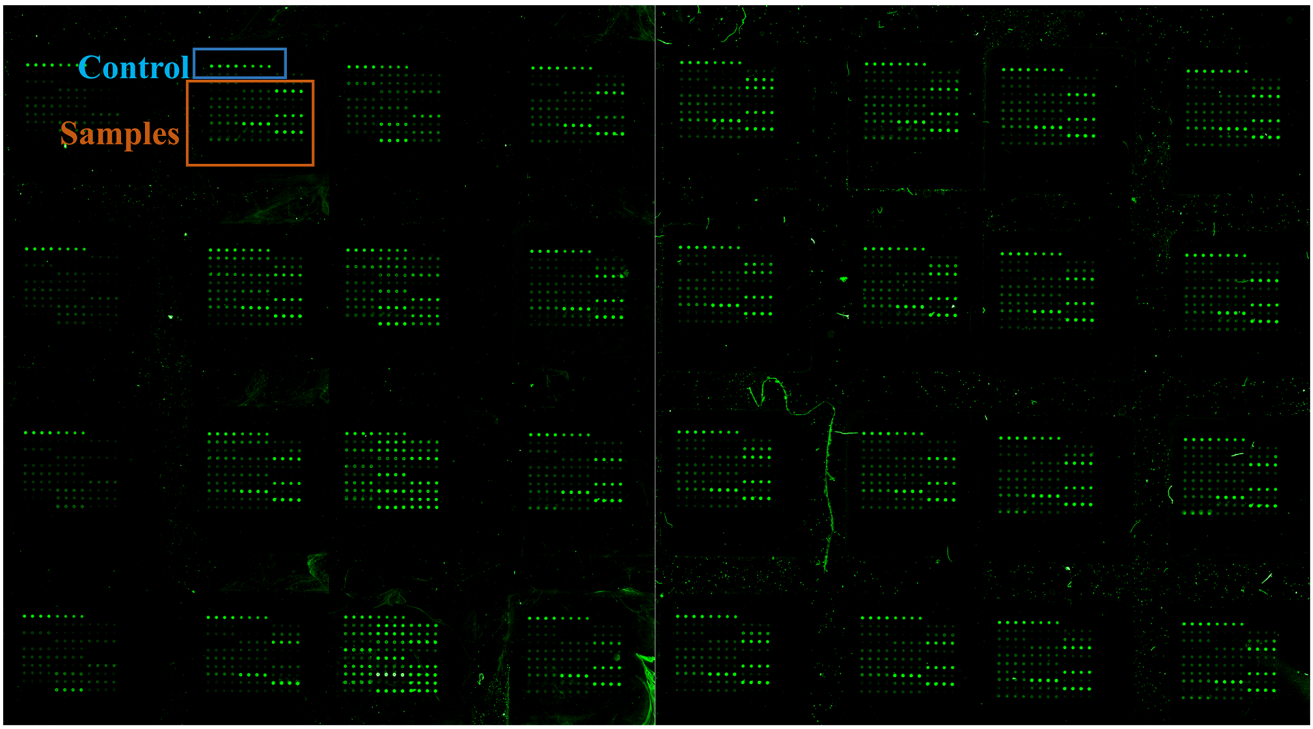

Figure S2. Screening results of bone loss-associated cytokines using protein array. Serum levels of 58 cytokines exhibited irregular changes following ovariectomy. (A) Serum levels of 30 cytokines.

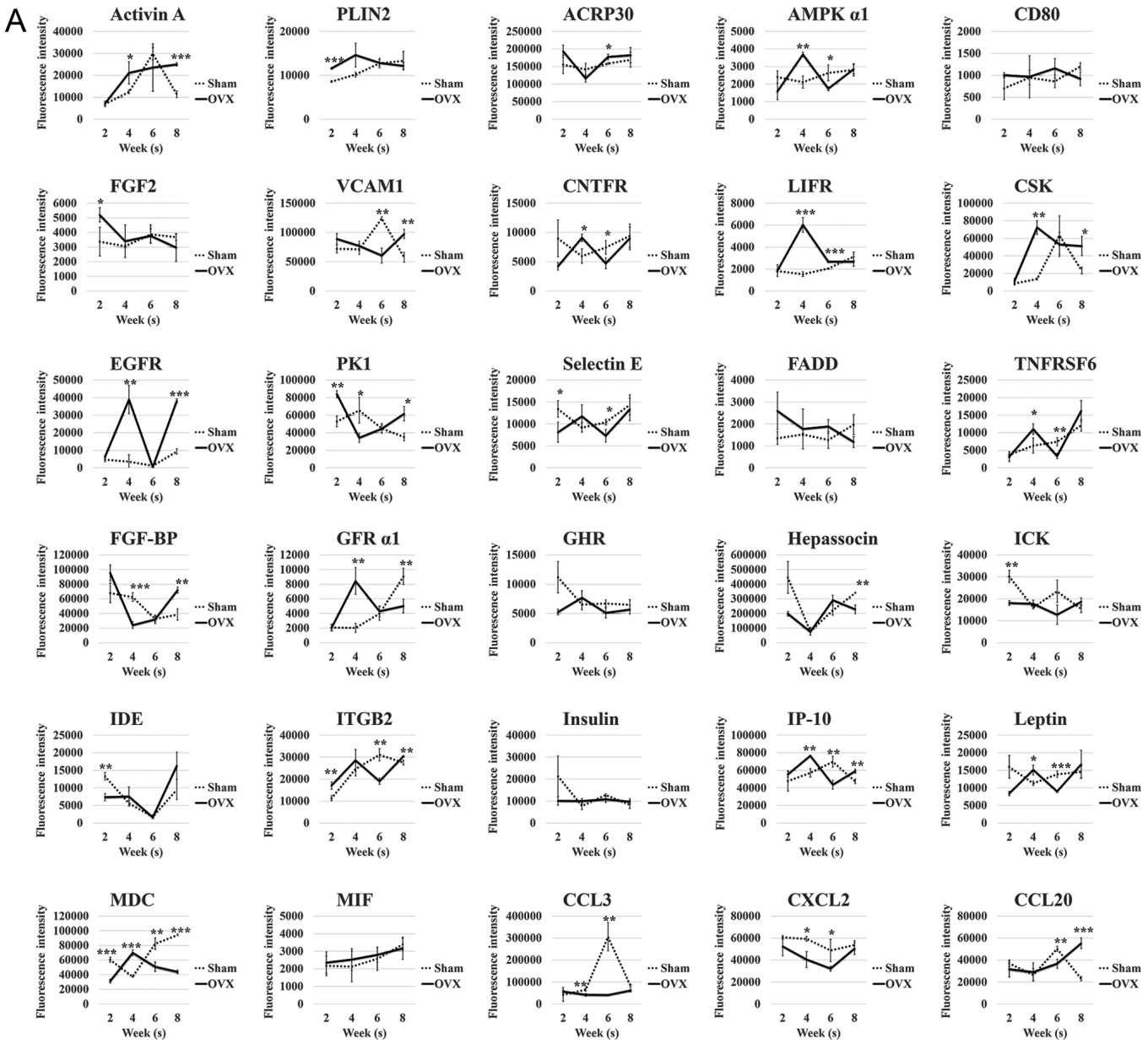

Figure S2. Continued. Screening results of bone loss-associated cytokines using protein array. Serum levels of 58 cytokines exhibited irregular changes following ovariectomy. (B) Serum levels of 28 cytokines. For definitions of gene names please see Table SI. *P<0.05, **P<0.01, ***P<0.001, ovariectomy vs. sham group (n=3).

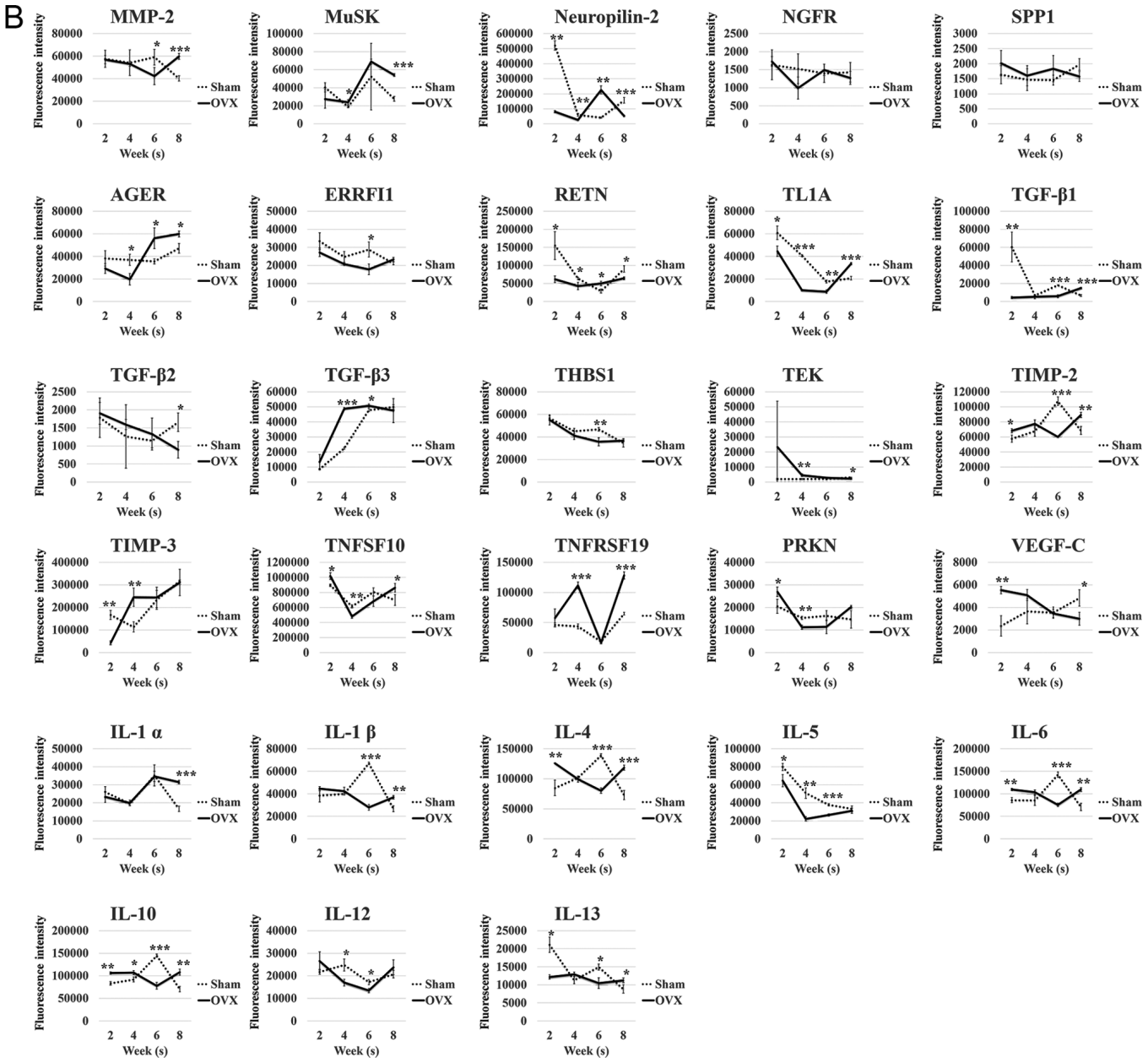

Figure S3. Correlation between serum cytokine expression levels and bone loss. For definitions of gene names please see Table SI.

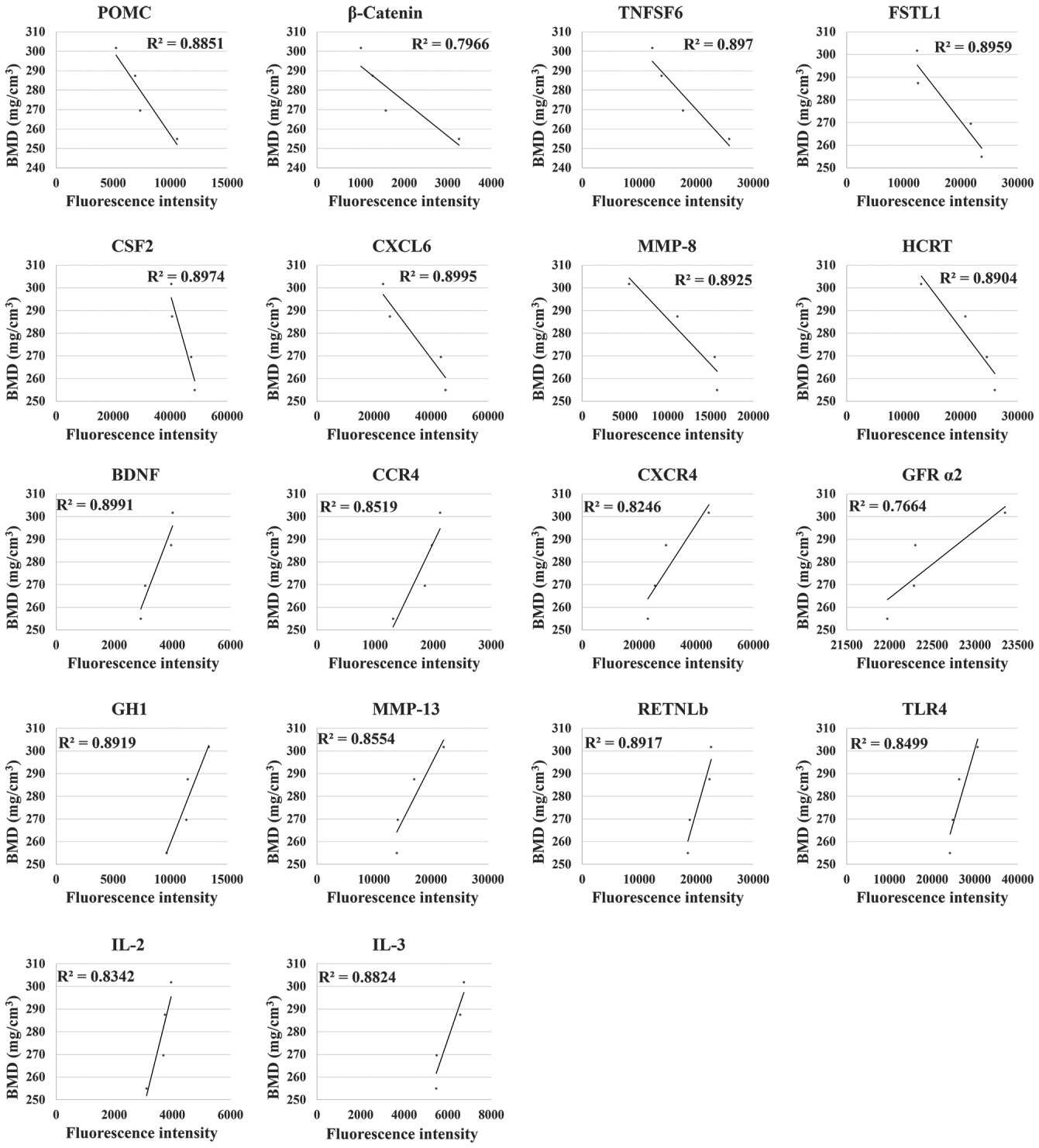

Figure S4. Pathways involving CCL2, CXCL1 and ILs. (A) Cytokine-cytokine receptor interaction pathway.

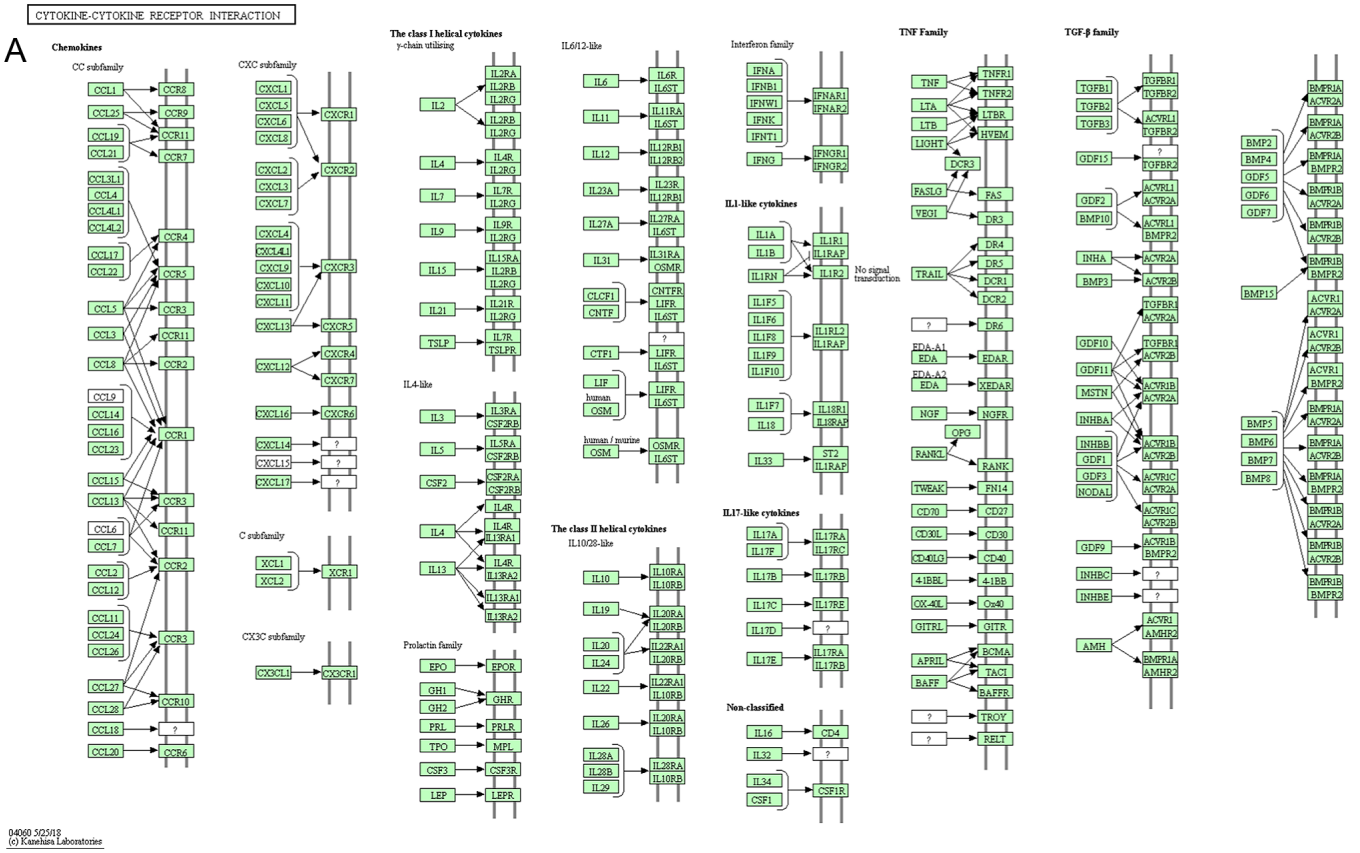

Figure S4. Continued. Pathways involving CCL2, CXCL1 and ILs. (B) Influenza A pathway.

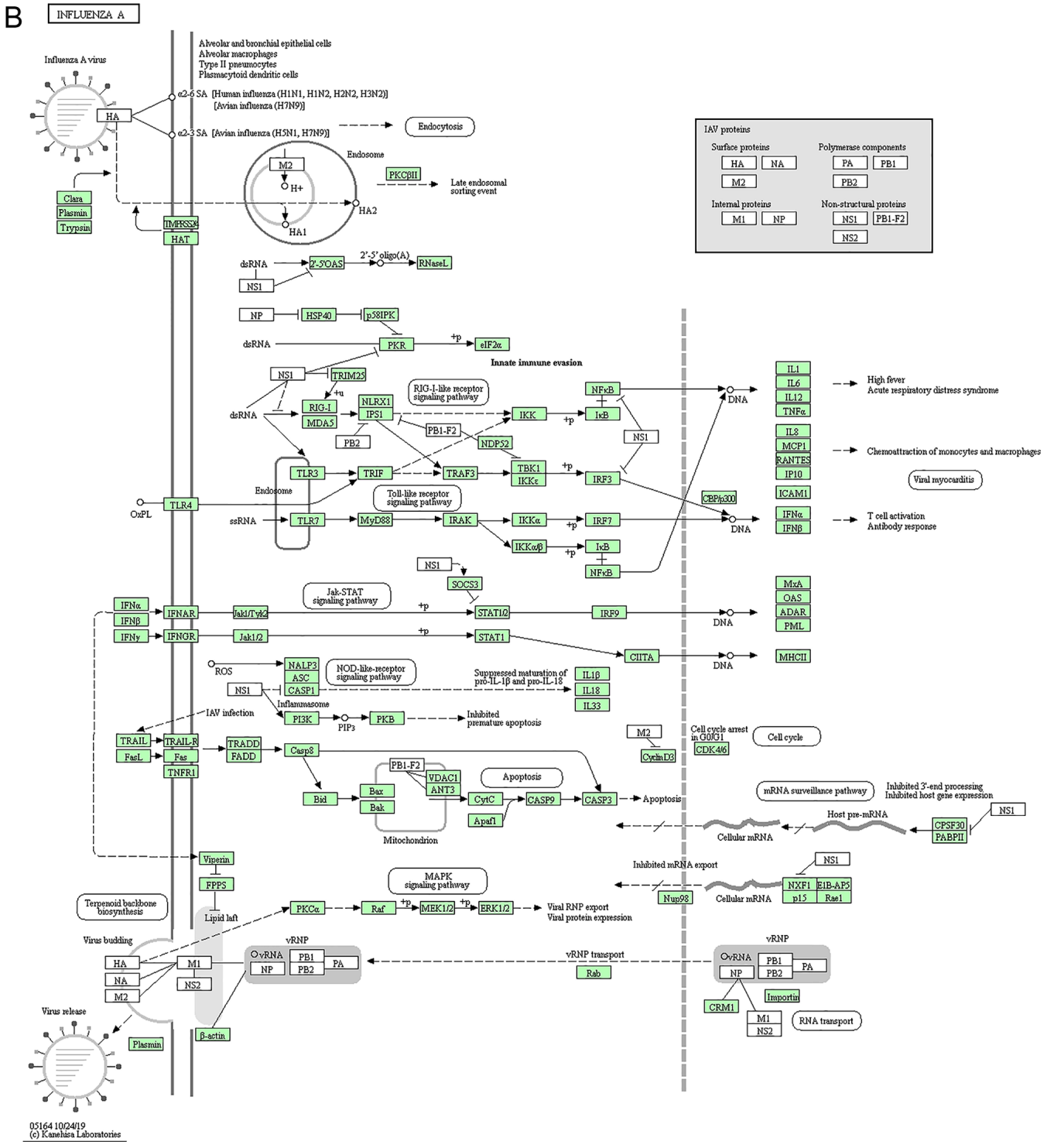

Figure S4. Continued. Pathways involving CCL2, CXCL1 and ILs. (C) NOD-like receptor signaling pathway. For full definitions of gene names please see Table SI. CCL2, C-C motif chemokine ligand 2; CXCL1, C-X-C motif chemokine ligand 1; IL, interleukin; TNF, tumor necrosis factor; TGF, transforming growth factor.

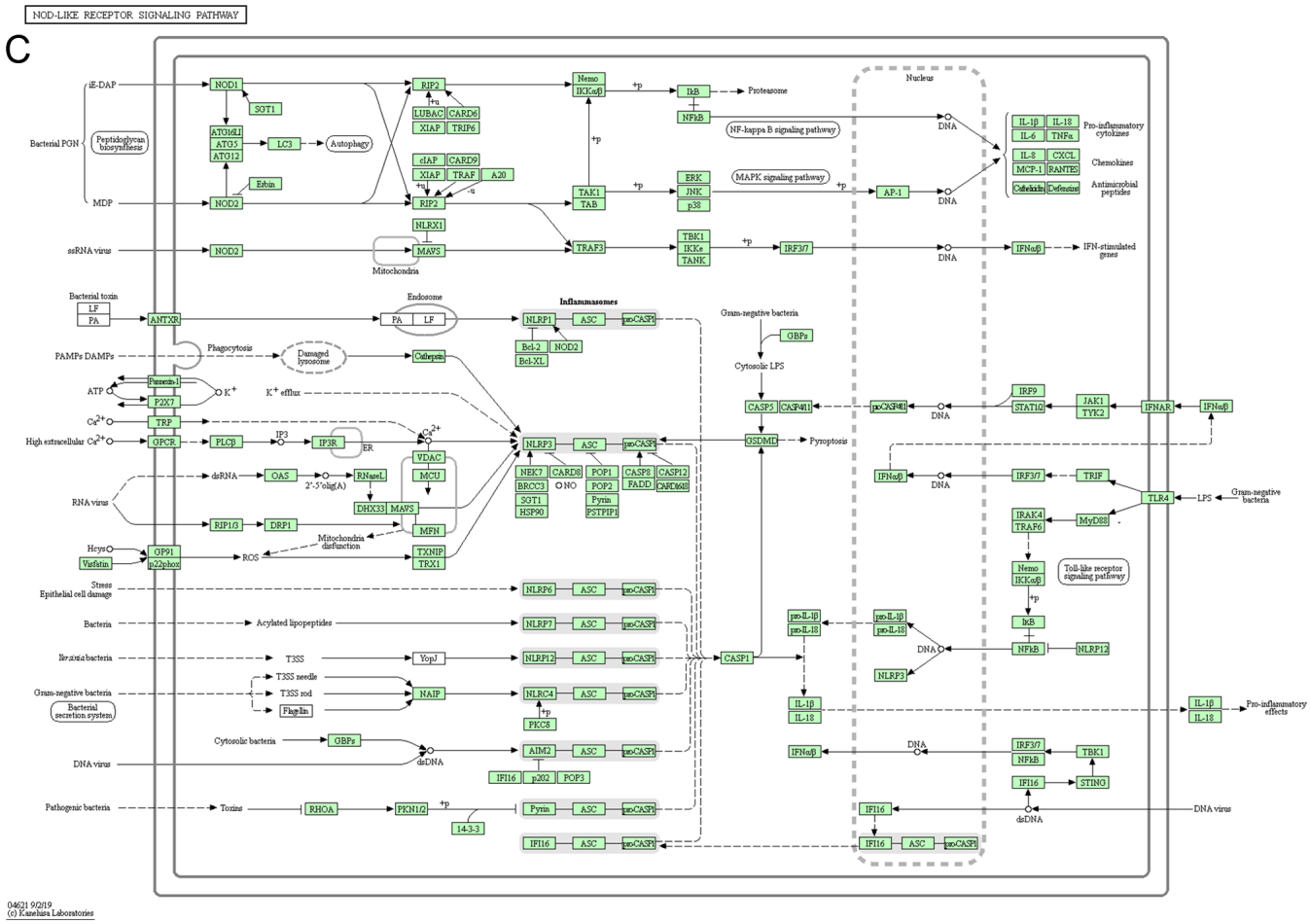

Table SI. Information on the total 78 cytokines.

Gene	ID	Full name
POMC	24664	Proopiomelanocortin
PLIN2	298199	Perilipin 2
ACRP30	246253	Adipoq adiponectin, C1Q and collagen domain containing
AMPK α 1	65248	5'-AMP-activated protein kinase catalytic subunit α-1
CD80	25408	Cd80 molecule
BDNF	24225	Brain-derived neurotrophic factor
β-Catenin	84353	Catenin β-1; Ctnnb1
FGF2	54250	Fibroblast growth factor 2
CCR4	171054	C-C motif chemokine receptor 4
VCAM1	25361	Vascular cell adhesion molecule 1
CNTFR	313173	Ciliary neurotrophic factor receptor subunit α
LIFR	81680	LIF receptor α
CSK	315707	C-terminal Src kinase
CXCR4	60628	C-X-C motif chemokine receptor 4
EGFR	24329	Epidermal growth factor receptor
PK1	192205	Prokineticin 1
Selectin E	25544	Selectin E
FADD	24329	Epidermal growth factor receptor
TNFRSF6	246097	Fas cell surface death receptor
FGF-BP	64535	Fibroblast growth factor binding protein 1
FSTL1	79210	Follistatin-like 1
GFR α-1	25454	GDNF family receptor α 1
GFR a4	66023	GDNF family receptor α 4
CSF2	116630	Colony stimulating factor 2
GH1	24391	Growth hormone 1
GHR	25235	Growth hormone receptor
ICK	84411	Intestinal cell kinase
IDE	25700	Insulin degrading enzyme
ITGb2	309684	Integrin subunit β 2
IP-10	245920	Cxcl10 C-X-C motif chemokine ligand 10
Leptin	25608	Leptin
CXCL6	60665	C-X-C motif chemokine ligand 6
CCL2 (MCP-1)	24770	C-C motif chemokine ligand 2
MDC	117551	Chemokine (C-C motif) ligand 22; Ccl22
MIF	81683	Macrophage migration inhibitory factor
CCL3	25542	C-C motif chemokine ligand 3
CXCL2	114105	C-X-C motif chemokine ligand 2
CCL20	29538	C-C motif chemokine ligand 20
MMP-2	81686	Matrix metallopeptidase 2
MMP-8	63849	Matrix metallopeptidase 8
MMP-13	171052	Matrix metallopeptidase 13
MuSK	81725	Muscle associated receptor tyrosine kinase
Neuropilin-2	81527	Neuropilin 2
NGFR	24596	Nerve growth factor receptor
HCRT	25723	Hypocretin neuropeptide precursor
SPP1	25353	Secreted phosphoprotein 1
AGER	81722	Advanced glycosylation end product-specific receptor
ERRFI1	313729	ERBB receptor feedback inhibitor 1
RETNLb	498074	Resistin like β
RETN	246250	Resistin
TL1A	252878	Tumor necrosis factor ligand superfamily member 15
TGF-β1	59086	Tgfb1 transforming growth factor, β-1
TGF-β2	81809	Transforming growth factor β-2; Tgfb2
TGF-β3	25717	Transforming growth factor, β-3
THBS1	445442	Thrombospondin 1
TIE-2	89804	TEK receptor tyrosine kinase
TIMP-2	29543	TIMP metallopeptidase inhibitor 2
TIMP-3	25358	TIMP metallopeptidase inhibitor 3
TLR4	29260	Toll-like receptor 4

Table SI. Continued.

Gene	ID	Full name
TNFSF10	246775	Tumor necrosis factor superfamily member 10
TNFRSF19	290300	Tumor necrosis factor receptor superfamily member 19
PRKN	56816	Parkin RBR E3 ubiquitin protein ligase
VEGF-C	114111	Vascular endothelial growth factor C
CXCL1 (IP10)	81503	C-X-C motif chemokine ligand 1
IL-1α	24493	Interleukin 1α [*Rattus norvegicus*]
IL-1β	24494	Interleukin 1β
IL-2	116562	Interleukin 2
IL-3	24495	Interleukin 3
IL-4	287287	Interleukin 4
IL-5	81809	Interleukin 5
IL-6	24498	Interleukin 6
IL-10	25325	Interleukin 10
IL-12	64546	Interleukin 12
IL-13	116553	Interleukin 13
CCL2(Homo)	6347	C-C motif chemokine ligand 2
CXCL1(Homo)	2919	C-X-C motif chemokine ligand 1

Table SII. Information on the human subjects.

Case no.	Age, years	Sex	BMD (mg/cm^3)	T-value
1	51	F	604	-3.2
2	63	F	648	-3.6
3	58	F	670	-3.4
4	65	F	696	-3.2
5	52	F	746	-2.7
6	51	F	770	-2.5
7	64	M	788	-2.8
8	60	M	794	-2.7
9	60	F	797	-2.3
10	46	M	810	-2.6
11	51	F	840	-1.9
12	60	F	885	-1.5
13	15	F	890	-1.4
14	45	F	936	-1.0
15	49	F	939	-1.0
16	75	F	944	-0.9
17	49	F	954	-0.8
18	39	F	1024	-0.6
19	57	F	1024	-0.2
20	35	F	1064	0.2
21	51	M	1098	0.1
22	56	F	1157	1.0
23	61	M	1176	0.8
24	56	M	1262	1.6

BMD, bone mineral density; M, male; F, female.

Table SIII. Data of the cytokine array for the 20 cytokines.

Protein	Group	2 weeks	4 weeks	6 weeks	8 weeks
POMC	Sham	4,878±867	4,259±519	4,701±913	4,360±290
	OVX	5,253±513	6,937±1,340	7,366±331	10,607±180
	P-value	0.553922525	0.032051485	0.008953289	5.88801×10^{-6}
β-Catenin	Sham	812±353	895±80	812±75	950±271
	OVX	1,008±327	1,279±298	1,578±422	3,260±328
	P-value	0.519970799	0.196036752	0.01688277	0.000576887
TNFSF6	Sham	11,295±2,574	11,020±1,848	11,623±2,211	11,957±609
	OVX	12,292±1,493	13,899±1,612	17,663±2,314	25,789±1,921
	P-value	0.592667136	0.111779767	0.030840114	0.003454509
FSTL1	Sham	12,750±1,888	11,620±1,027	12,237±262	12,966±826
	OVX	12,367±185	12,551±1,267	21,806±1,168	23,699±1,425
	P-value	0.759415789	0.378831501	0.000157604	0.000351396
CSF2	Sham	32,760±1,486	33,144±3,338	34,026±2,269	32,485±1,651
	OVX	40,411±1,830	40,673±5,754	47,356±1,503	48,574±1,231
	P-value	0.004921701	0.121504351	0.001058506	0.000172702
CXCL6	Sham	22,421±3,747	22,180±1,347	21,033±2,307	23,883±580
	OVX	23,210±8,332	25,560±1,995	43,475±2,521	45,050±3,967
	P-value	0.888310629	0.071860555	0.000340632	0.000793545
CXCL1	Sham	1,030±145	938±156	1,017±187	990±103
	OVX	1,273±133	1,473±176	2,112±109	2,440±143
	P-value	0.032117882	0.003963775	7.92296E-05	0.00075878
CCL2	Sham	100,822±7,176	99,837±4,802	101,320±7,797	101,457±6,812
	OVX	105,591±8,968	121986±16,564	128,204±3,728	137,280±1,337
	P-value	0.511760515	0.090159146	0.005737362	0.000866515
MMP-8	Sham	5,698±163	5,071±334	4,821±472	5505±658
	OVX	5,472±598	11,123±3,129	15,512±2,202	15777±593
	P-value	0.561928696	0.077127916	0.001193473	3.63198×10^{-5}
HCRT	Sham	5,255±675	6,346±1,311	5,505±237	5,917±1,282
	OVX	13,093±4,391	20,842±2,823	24,591±3,262	25,980±2,123
	P value	0.03782636	0.001282226	0.000539123	0.00015058
BDNF	Sham	4,045±828	4,739±460	4,667±561	4,020±797
	OVX	4,008±451	3,958±766	3,064±317	2,915±338
	P-value	0.949976405	0.204730748	0.012567757	0.091463731
CCR4	Sham	2,220±690	2,498±331	2,342±598	2,416±321
	OVX	2,119±267	1,974±592	1,857±34	1,312±482
	P-value	0.824575692	0.252396343	0.295425111	0.029770909
CXCR4	Sham	57,959±6,202	56,943±7,159	58,554±6,913	57,159±6,084
	OVX	44,428±3,776	29,379±4,935	25,494±1,983	23,024±762
	P-value	0.032049253	0.005360481	0.001347984	0.000647144
GFR α2	Sham	25,568±5,320	24,990±1,364	25,676±1,628	25,325±874
	OVX	23,353±1,776	22,304±2,252	22,288±522	21,978±453
	P-value	0.531610052	0.15192174	0.026456257	0.004159744
GH1	Sham	14,926±341	14,703±1,474	14,667±482	13,656±387
	OVX	13,381±3,477	11,538±1,478	11,422±142	9,697±1,431
	P-value	0.486522396	0.058450185	0.000363725	0.009831271
MMP-13	Sham	27,814±3,679	26,245±3,441	26,130±1,714	26,078±2,346
	OVX	22,204±1,838	17,052±467	14,167±465	13,998±787
	P-value	0.077451697	0.010139433	0.000308545	0.001071581
RETNLb	Sham	29,836±2,103	30,380±3,060	30,616±6,947	31,950±7,285
	OVX	22,628±3,032	22,341±3,297	18,870±5,602	18,513±1,236
	P-value	0.027692509	0.036388789	0.084824563	0.034520573
TLR4	Sham	35,364±3,488	34,171±4,123	35,447±5,281	34,142±4,129
	OVX	30,620±1,032	26,331±3,257	24,845±3,221	24,180±4,535
	P-value	0.086785342	0.06104018	0.041204218	0.04816884
IL-2	Sham	4,296±272	4,457±906	4,652±253	4,468±227
	OVX	3,958±390	3,747±290	3,693±592	3,110±345
	P-value	0.28525603	0.265893076	0.061378911	0.004709672
IL-3	Sham	8,485±1,158	8,498±128	7,971±759	8,307±330
	OVX	6,742±883	6,575±1448	5,490±1,059	5,477±1,255
	P-value	0.106834153	0.083705083	0.030006226	0.019487738

OVX, ovariectomized group.

Table SIV. Linear regression of 18 serum cytokines. They were negatively correlated with the BMD of the ovariectomized rats (P>0.05).

Linear regression: Y(BMD)=A+B*X(fluorescence intensity)

Protein	Parameter	Value	Standard error	T-value	P-value
POMC	Intercept(A)	343.2179	17.05318	20.12632	0.05918
	Slope(B)	-0.0086	0.00219	-3.92599	
β-Catenin	Intercept(A)	330.5735	11.59145	28.51875	0.05182
	Slope(B)	-0.03354	0.00708	-4.73532	
TNFSF6	Intercept(A)	334.3883	14.01226	23.86398	0.05291
	Slope(B)	-0.00322	7.71E-04	-4.17293	
FSTL1	Intercept(A)	333.3495	12.68295	26.28328	0.05534
	Slope(B)	-0.00308	6.79E-04	-4.53499	
CSF2	Intercept(A)	465.4552	39.48617	11.7878	0.05146
	Slope(B)	-0.0042	8.84E-04	-4.75734	
CXCL6	Intercept(A)	336.1081	14.20872	23.65507	0.05156
	Slope(B)	-0.00168	3.97E-04	-4.23179	
MMP-8	Intercept(A)	326.2745	12.45099	26.20469	0.05527
	Slope(B)	-0.004	9.82E-04	-4.07511	
HCRT	Intercept(A)	349.0575	14.44247	24.16883	0.05726
	Slope(B)	-0.00339	6.73E-04	-5.03453	
BDNF	Intercept(A)	154.4238	23.82054	6.4828	0.05438
	Slope(B)	0.03581	0.00682	5.25286	
CCR4	Intercept(A)	181.1199	29.07687	6.229	0.07702
	Slope(B)	0.05357	0.0158	3.3917	
CXCR4	Intercept(A)	219.1679	20.00716	10.95447	0.09191
	Slope(B)	0.00194	6.31E-04	3.06659	
GFR α2	Intercept(A)	-391.811	261.69888	-1.49718	0.12456
	Slope(B)	0.02981	0.01164	2.56159	
GH1	Intercept(A)	130.6701	36.59066	3.57113	0.05559
	Slope(B)	0.01283	0.00316	4.0625	
MMP-13	Intercept(A)	192.6269	20.08656	9.58984	0.05098
	Slope(B)	0.00501	0.00115	4.35055	
RETNLb	Intercept(A)	97.43197	44.78896	2.17536	0.05572
	Slope(B)	0.00879	0.00217	4.05713	
TLR4	Intercept(A)	105.7058	51.53563	2.05112	0.07809
	Slope(B)	0.00652	0.00194	3.36544	
IL-2	Intercept(A)	91.91937	59.00991	1.55769	0.08668
	Slope(B)	0.0514	0.01621	3.17161	
IL-3	Intercept(A)	107.1946	44.39677	2.41447	0.06064
	Slope(B)	0.0282	0.00728	3.87387	

BMD, bone mineral density.