

Calcitriol and its analogs establish the immunosuppressive microenvironment that drives metastasis in 4T1 mouse mammary gland cancer

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1 Supplementary Materials and Methods

1.1 Blood morphology

Complete blood count analysis was evaluated in each blood sample using the hematology analyzer Mythic 18 (C2 Diagnostics, Montpellier, France).

2 Supplementary Results

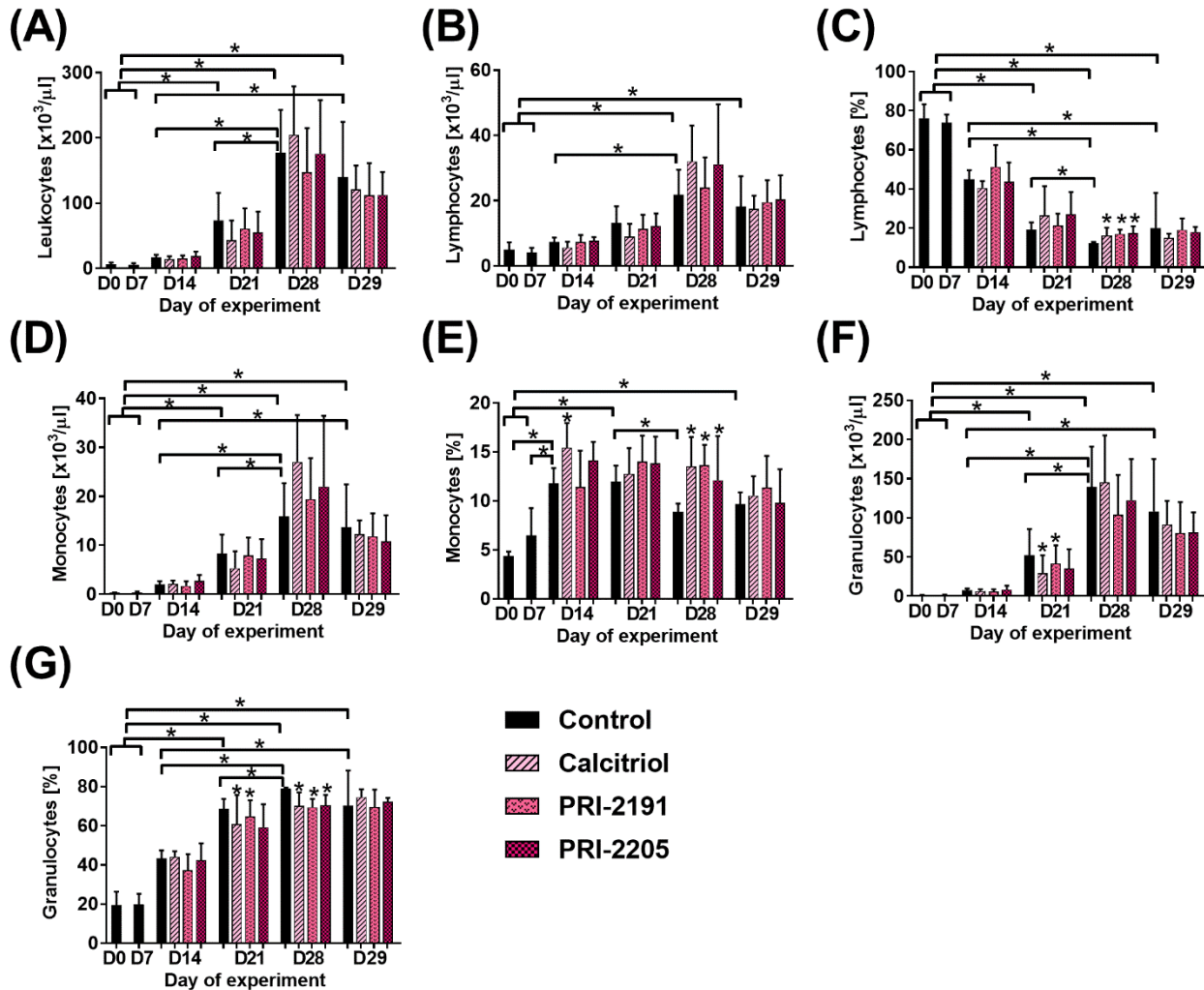
2.1 Phenotype of peripheral blood granulocytes

The population of Ly6G-6C positive cells with high SCC parameter (Ly6G-6C⁺SCC^{high}) was significantly higher in mice treated with calcitriol and with PRI-2205. Similar tendency was observed in mice treated with PRI-2191 (Figure S1A). Among the Ly6G-6C⁺SCC^{high} cell population, all compounds, but only PRI-2205 in a significant manner, decreased the percentage of CD54⁺ cells (Figure S1B). PRI-2205 also significantly decreased the percentage of CD184⁺ cells within Ly6G-6C⁺SCC^{high} population (Figure S1C).

3 Supplementary Figures and Tables

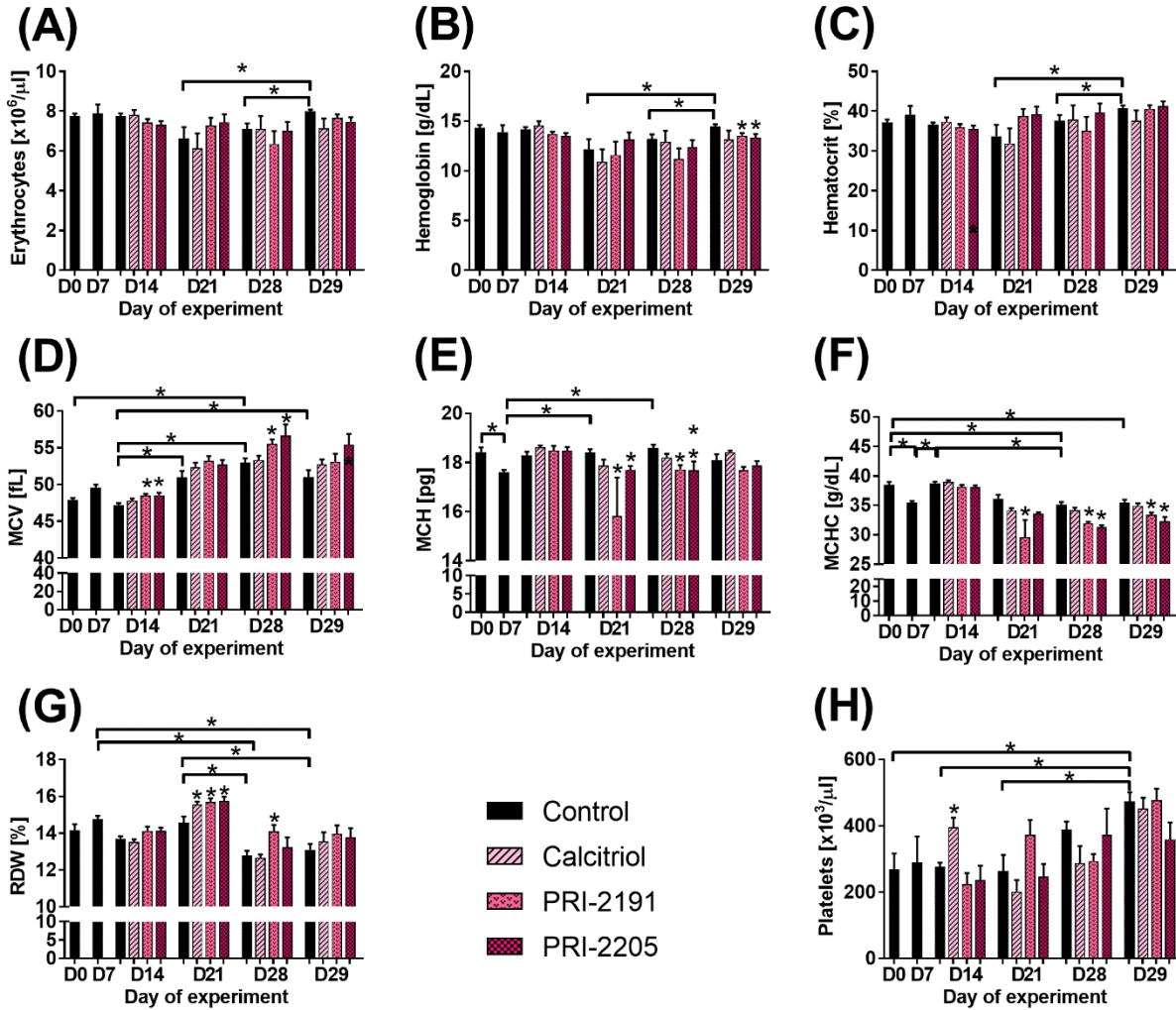
3.1 Supplementary Figures

Supplementary Figure S1. Calcitriol, PRI-2191, and PRI-2205 increased lymphocyte and monocyte percentage with a parallel decrease in granulocytes percentage in mice bearing 4T1 mammary gland tumors.



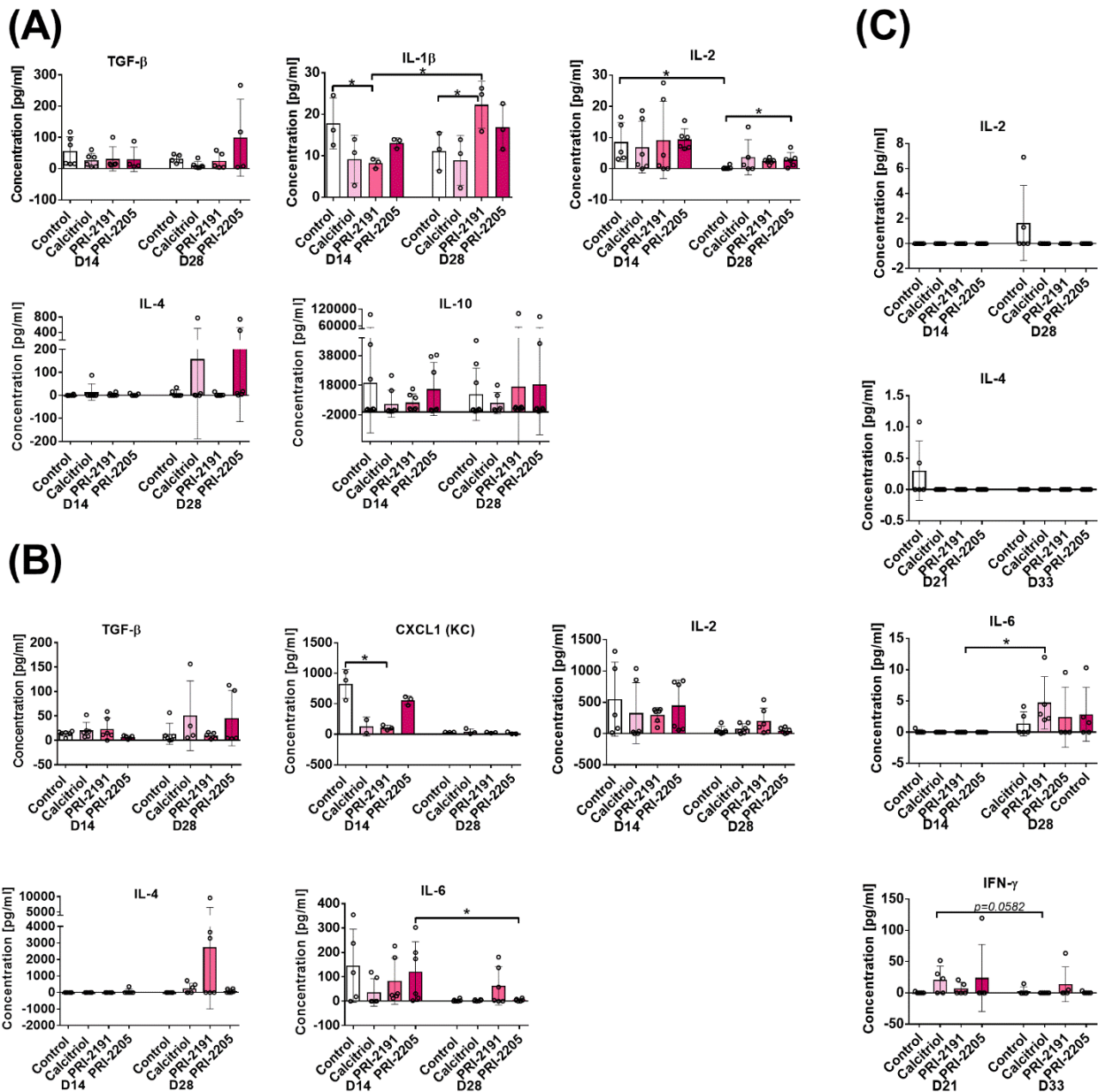
(A) General white blood cell count (WBC); the number (B) and the percentage (C) of: lymphocytes, (D) and (E) monocytes, (F) and (G) granulocytes. From day 7, vitamin D analogs were administered subcutaneously (s.c.) thrice a week. The single dose of compounds was as follows: calcitriol, 0.5 $\mu\text{g/kg}$; PRI-2191, 1.0 $\mu\text{g/kg}$; and PRI-2205, 10.0 $\mu\text{g/kg}$. Number of mice was 9–12 per group. The blood morphology was evaluated in each blood sample using the Mythic 18 automatic analyzer. Data are presented as mean $\pm$ SD. Statistical analysis: Kruskal–Wallis multiple comparison test. * $p < 0.05$.

Supplementary Figure S2. Selected blood morphological parameters of mice bearing 4T1 mammary gland tumors and treated with calcitriol and its analogs: PRI-2191 and PRI-2205.



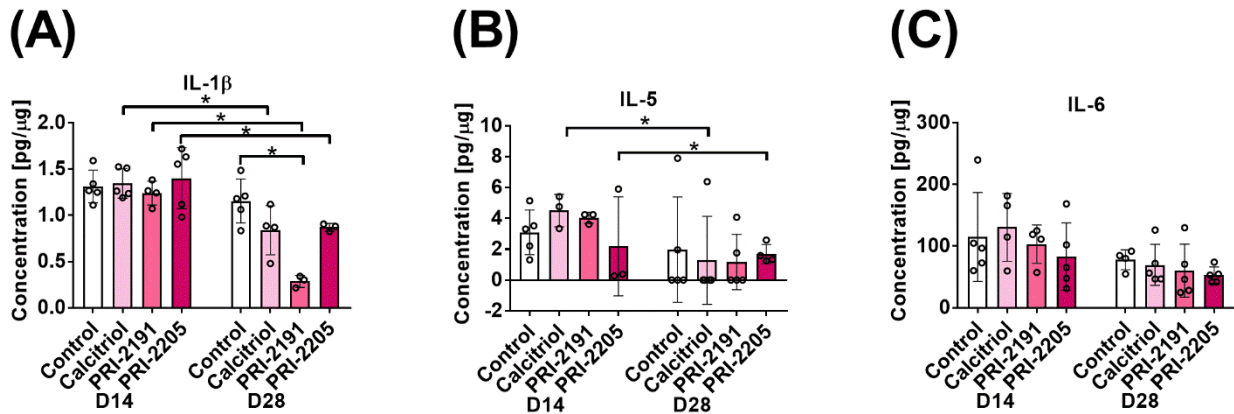
(A) Erythrocytes number; (B) hemoglobin (Hb); (C) hematocrit; (D) mean cell volume (MCV); (E) mean corpuscular hemoglobin (MCH); (F) mean corpuscular hemoglobin concentration (MCHC); (G) red distribution width (RDW); (H) platelets number. Mice were inoculated orthotopically with 4T1 cells on day 0. From day 7 (7 days after tumor inoculation), vitamin D analogs were administered subcutaneously (s.c.) thrice a week. The single dose of compounds were as follows: calcitriol, 0.5 $\mu\text{g/kg}$; PRI-2191, 1.0 $\mu\text{g/kg}$; and PRI-2205, 10.0 $\mu\text{g/kg}$. Number of mice were 6 per group. Data are presented as mean with standard deviation. Statistical analysis: Kruskal–Wallis multiple comparison test. * $P < 0.05$ as compare to control mice on appropriate day or as indicated.

Supplementary Figure S3. Selected cytokine levels in plasma and supernatants from lipopolisaccharide (LPS)- or Concanavalin A (ConA)-stimulated splenocytes.



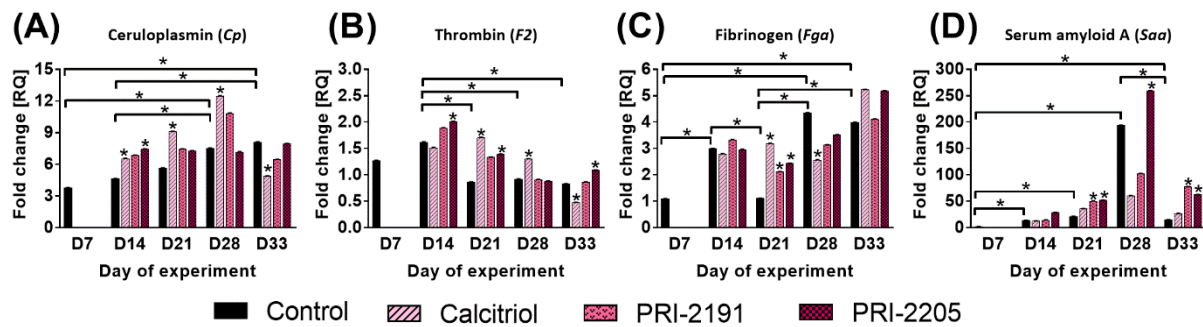
Supernatants obtained from spleen cells (harvested on days 14 and 28) stimulated with LPS (A) and ConA (B). Plasma from mice harvested on days 14 or 21 and 28 or 33 (C). Samples were analyzed with ELISA tests. Number of samples analyzed was 2–6 per group. Data are presented as mean $\pm$ SD and individual sample results. Statistical analysis: Kruskal–Wallis multiple comparison test. * $p < 0.05$.

Supplementary Figure S4. Expression of selected cytokines in tumor tissue.



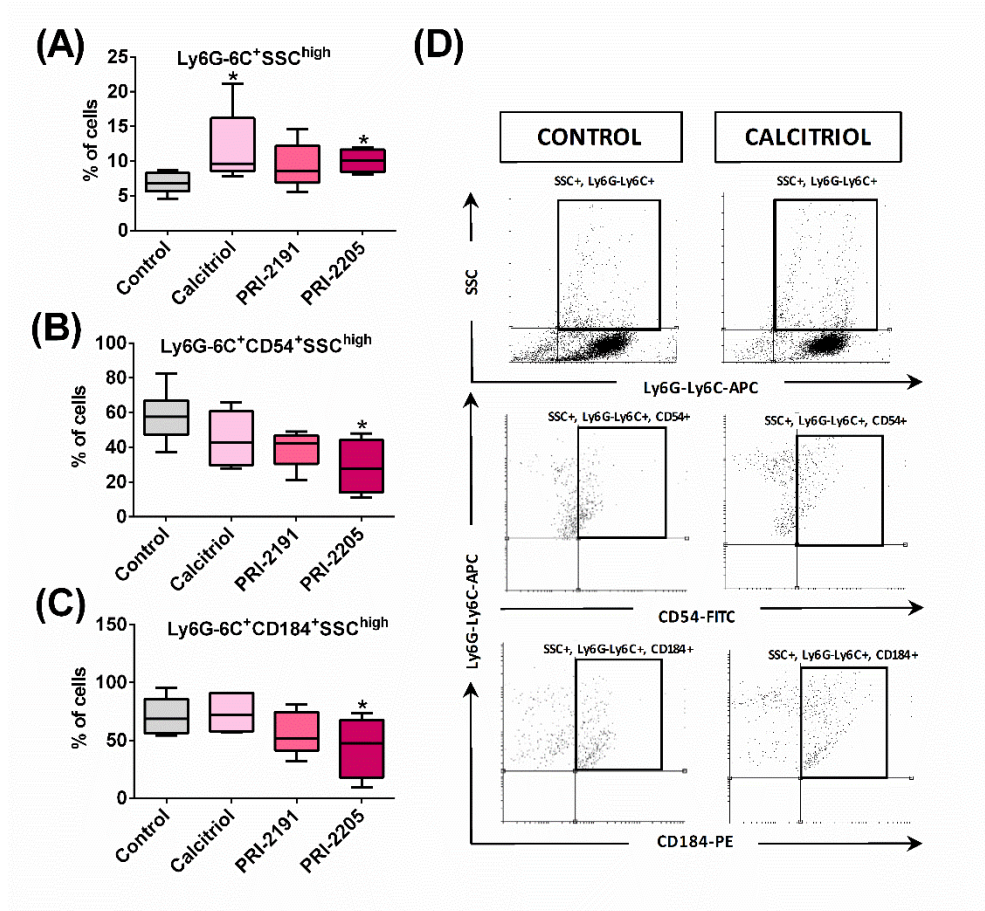
(A) IL-1 β . (B) IL-5. (C) IL-6. Tumors harvested on day 14 and 28 were homogenized and lysed. Supernatants were analyzed with ELISA tests. Number of samples analyzed was 3–5 per group. Data are presented as mean $\pm$ SD and individual sample results. Statistical analysis: Kruskal–Wallis multiple comparison test. * p <0.05.

Supplementary Figure S5. Increase in the mRNA of acute phase proteins in the liver of mice bearing 4T1 mammary gland tumors treated with calcitriol, PRI-2191, and PRI-2205.



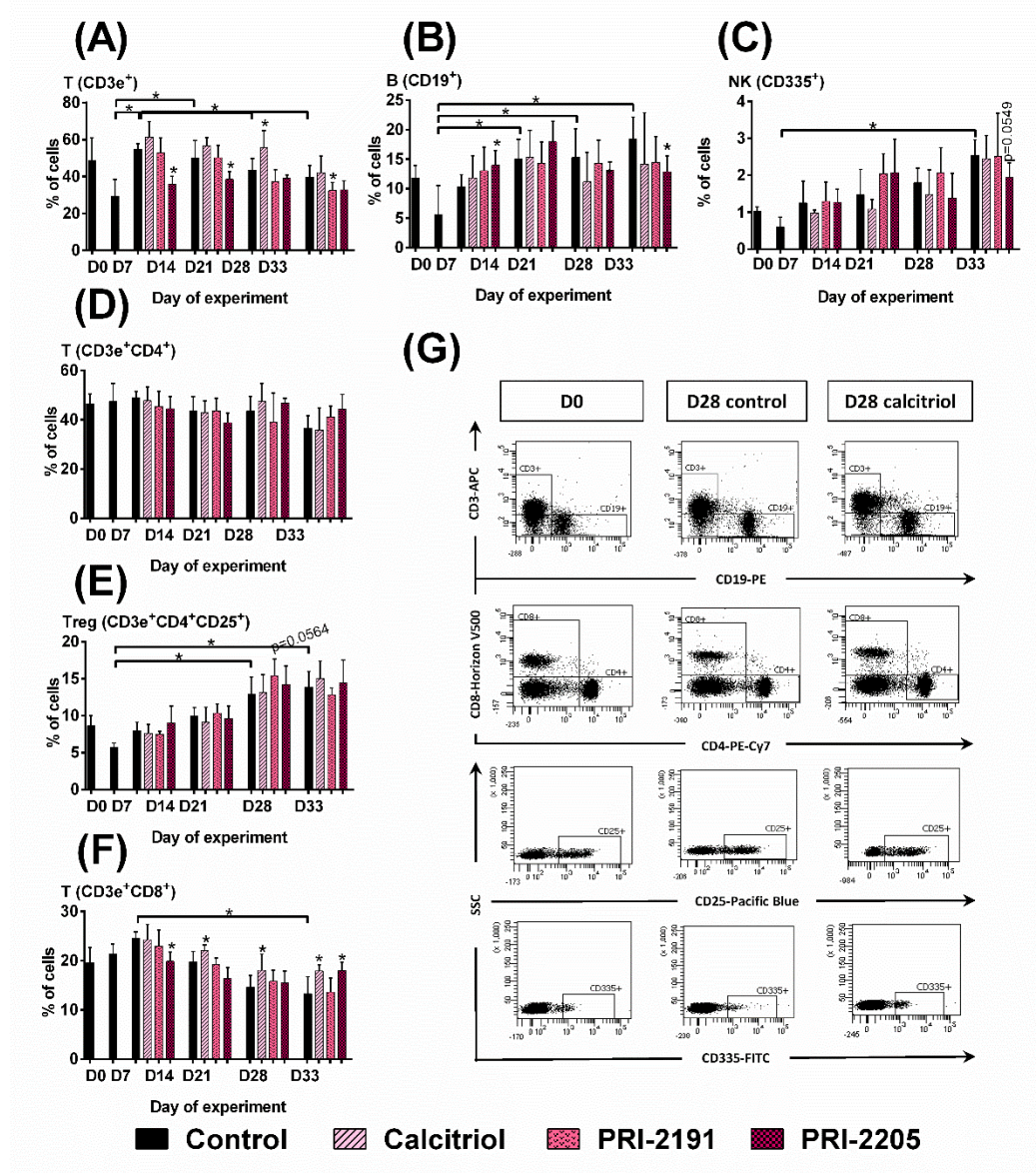
Real-time PCR analysis of four selected genes. (A) Ceruloplasmin (*Cp*), (B) Coagulation factor II, thrombin (*F2*), (C) Fibrinogen alpha chain (*Fga*), and (D) Serum amyloid A (*Saa*). Real-time PCR reaction was performed using specific primers coding following genes: *Cp* (Mm01289313_m1), *F2* (Mm00438843_m1), *Fga* (Mm00802584_m1), and *Saa* (Mm04208126_m1). Briefly, 50 ng of cDNA was used for a single reaction and each sample was performed in triplicate in a single experiment. Data were analyzed using comparative $\Delta\Delta C_t$ method by DataAssist 3.01 software in comparison to endogenous control: hypoxanthine phosphoribosyltransferase 1 (*Hprt1*, Mm00446968_m1). Mice were orthotopically inoculated with 4T1 cells on day 0. From day 7 (7 days after tumor inoculation), vitamin D analogs were administered subcutaneously (s.c.) thrice a week. A single dose of compounds were as follows: calcitriol, 0.5 μ g/kg; PRI-2191, 1.0 μ g/kg; and PRI-2205, 10.0 μ g/kg. Number of mice: 9–12 per group. Data are presented as mean $\pm$ SD. Statistical analysis: Kruskal–Wallis multiple comparison test. * p <0.05.

Supplementary Figure S6. Peripheral blood granulocytes phenotype in mice bearing 4T1 mammary gland tumors treated with calcitriol, PRI-2191, and PRI-2205 (day 28).



(A) $\text{Ly6G-6C}^+\text{SSC}^{\text{high}}$ mature granulocytes. (B) $\text{Ly6G-6C}^+\text{CD54}^+\text{SSC}^{\text{high}}$ (C) $\text{Ly6G-6C}^+\text{CD184}^+\text{SSC}^{\text{high}}$. (D) Representative dot plots of selected analysis performed on day 28. Data for calcitriol as example are shown. Number of samples analyzed was 4–6 per group. CD54^+ and CD184^+ granulocytes were gated in $\text{SSC}^{\text{high}} \text{Ly6G-Ly6C}^+$ population. Data were analyzed using the FACS Diva software. Data are presented as median line with min to max whiskers and 25–75% percentiles. Statistical analysis: Kruskal–Wallis multiple comparison test. * $p < 0.05$.

Supplementary Figure S7. Phenotype of regional lymph nodes lymphocytes in mice bearing 4T1 mammary gland tumors treated with calcitriol, PRI-2191, and PRI-2205.



(A) T lymphocytes CD3e⁺. (B) B lymphocytes CD19⁺. (C) NK cells CD335⁺. (D) TCD4⁺ lymphocytes. (E) TCD4⁺CD25⁺ lymphocytes. (F) TCD8⁺ lymphocytes. (G) Representative dot plots of selected analysis performed on day 28. Six samples per group was analyzed, except: D0 = 2 and D7 = 3. Data analysis was performed using Becton Dickinson FACS Fortessa cytometer with FACSDiva software. Shown data represent mean ± SD. Statistical analysis: Kruskal–Wallis multiple comparison test. *p < 0.05.

3.2 Supplementary Tables

Table S1. The fold change values of genes associated with precursor T cells differentiation in splenocytes samples from 4T1 tumor bearing mice treated with calcitriol or its analogs.

Gene	Calcitriol		PRI-2191		PRI-2205	
	D21	D28	D21	D28	D21	D28
<i>Asb2</i>	2.403	0.399	2.244	0.736	1.734	0.172
<i>Cacna1f</i>	1.127	1.006	1.389	0.645	0.805	0.357
<i>Ccl11</i>	1.877	3.821	37.502	0.891	8.350	2.973
<i>Ccl5</i>	1.835	0.586	0.950	1.790	1.092	0.623
<i>Ccl7</i>	20.283	0.421	718.457	0.848	22.735	1.586
<i>Ccr3</i>	2.667	0.654	1.097	0.687	2.940	0.378
<i>Ccr4</i>	1.275	0.564	7.324	1.174	0.613	1.355
<i>Ccr6</i>	1.426	0.325	3.600	1.630	0.442	0.921
<i>Cebpb</i>	2.531	1.580	0.083	0.575	1.444	0.102
<i>Chd7</i>	1.914	1.520	4.638	0.509	0.721	0.153
<i>Csf2</i>	4.144	1.546	26.864	4.072	20.050	1.746
<i>Fasl</i>	1.314	0.900	12.786	1.173	1.175	1.293
<i>Fosl1</i>	2.358	1.023	1.893	0.706	1.974	0.866
<i>Foxp3</i>	2.958	1.096	8.829	0.896	1.304	0.681
<i>Gata3</i>	2.038	0.817	1.455	0.418	0.972	0.289
<i>Gata4</i>	0.718	0.784	37.502	1.226	1.249	3.015
<i>Gfi1</i>	0.547	1.851	0.322	0.357	0.305	1.817
<i>Ptgdr2</i>	4.452	0.554	26.461	0.647	3.382	0.404
<i>Havcr2</i>	2.714	1.288	4.150	0.948	1.377	1.249
<i>Hopx</i>	1.271	1.495	6.179	0.737	1.921	2.957
<i>Hoxa10</i>	0.377	6.067	5.354	1.882	0.144	2.556
<i>Hoxa3</i>	0.646	3.653	11.007	1.226	0.367	10.585
<i>Icos</i>	1.277	0.352	1.031	0.962	0.186	0.071
<i>Id2</i>	1.246	1.681	0.785	0.746	0.750	1.604
<i>Ifng</i>	2.876	0.981	2.366	1.601	2.248	0.927
<i>Igsf6</i>	0.861	1.243	0.958	0.808	0.994	1.458
<i>Ikzf2</i>	2.388	0.673	2.746	1.049	0.941	0.506
<i>Il12b</i>	6.414	0.081	37.502	2.584	8.184	0.312
<i>Il12rb2</i>	3.963	0.554	20.624	0.280	0.772	0.404
<i>Il13</i>	0.718	2.517	37.502	3.844	1.249	3.724
<i>Il13ra1</i>	1.651	0.966	1.598	0.709	1.314	1.015
<i>Il17a</i>	0.718	0.784	37.502	1.226	1.249	3.015
<i>Il17re</i>	1.424	1.964	8.803	1.013	1.677	8.485
<i>Il18</i>	1.652	0.495	9.805	0.887	3.525	1.815
<i>Il18r1</i>	0.932	1.442	1.428	1.055	0.723	2.551
<i>Il18rap</i>	0.735	1.677	0.544	0.666	1.330	1.463
<i>Il1r1</i>	4.356	0.628	12.066	0.787	2.024	0.909
<i>Il1r2</i>	0.984	2.934	1.211	0.844	1.068	3.136
<i>Il1rl1</i>	2.134	1.239	2.738	2.270	2.565	1.205

<i>Il2</i>	3.488	0.668	37.502	1.066	1.249	2.491
<i>Il21</i>	1.197	1.081	22.145	0.676	0.738	3.969
<i>Il2ra</i>	1.412	0.854	1.371	0.516	0.883	0.707
<i>Il4</i>	2.591	1.779	14.039	0.948	0.974	10.196
<i>Il4ra</i>	1.599	1.105	0.066	0.706	1.422	0.149
<i>Il5</i>	1.538	0.784	37.502	1.226	2.231	3.224
<i>Il9</i>	0.718	0.784	37.502	1.226	1.249	7.205
<i>Irf1</i>	1.680	0.618	2.446	0.918	1.355	0.381
<i>Irf4</i>	3.636	0.328	0.858	0.813	1.361	0.175
<i>Irf8</i>	2.265	0.851	0.151	0.631	1.015	0.150
<i>Jak1</i>	1.054	1.050	7.362	1.304	0.940	2.033
<i>Lrrc32</i>	0.961	1.156	32.662	3.991	1.603	3.706
<i>Maf</i>	1.845	0.639	0.484	0.577	1.778	0.912
<i>Myb</i>	0.613	1.400	0.368	0.624	0.633	3.182
<i>Nfatc1</i>	1.584	0.951	1.178	1.036	1.293	0.865
<i>Nfatc2</i>	2.119	0.745	3.025	1.024	1.202	0.617
<i>Nfatc2ip</i>	1.126	1.020	0.825	0.632	0.879	0.426
<i>Nr4a1</i>	11.904	0.539	4.153	0.552	8.755	0.054
<i>Nr4a3</i>	2.393	0.233	5.710	0.741	1.403	0.254
<i>Perp</i>	0.718	0.608	37.502	0.637	1.249	2.969
<i>Pkd2</i>	1.326	1.135	1.518	0.666	1.407	0.957
<i>Pou2f2</i>	2.685	0.697	1.369	0.814	1.637	0.289
<i>Pparg</i>	1.098	0.690	4.236	0.829	2.466	2.349
<i>Rel</i>	3.438	0.552	15.543	0.718	2.359	0.622
<i>Rora</i>	1.590	1.200	8.765	1.033	1.235	1.639
<i>Rorc</i>	2.178	0.999	15.387	0.860	1.544	2.010
<i>Runx1</i>	2.072	0.943	0.854	0.611	1.284	0.587
<i>Runx3</i>	1.937	0.968	0.316	0.651	0.539	0.786
<i>Socs1</i>	1.676	0.959	0.459	0.719	0.838	0.631
<i>Socs5</i>	1.366	0.972	0.543	0.824	0.940	0.471
<i>Stat1</i>	1.024	0.732	1.574	0.859	0.842	0.797
<i>Stat4</i>	0.917	1.076	0.833	1.299	0.988	2.120
<i>Stat6</i>	1.368	1.178	2.144	0.623	0.632	0.120
<i>Tbx21</i>	1.789	0.513	5.301	0.359	0.564	0.728
<i>Tgfb1</i>	4.189	0.517	2.687	0.760	1.785	0.155
<i>Tlr4</i>	1.242	0.927	0.929	0.723	1.010	0.728
<i>Tlr6</i>	1.240	1.042	0.200	0.917	1.826	1.030
<i>Tmed1</i>	1.356	1.356	1.478	0.600	1.625	0.312
<i>Tnf</i>	6.963	0.467	0.645	1.208	4.159	0.125
<i>Tnfrsf9</i>	1.631	0.941	1.920	4.069	0.418	0.372
<i>Tnfrsf11</i>	1.613	1.164	2.218	1.813	1.590	2.940
<i>Trp53inp1</i>	1.830	1.346	0.325	0.819	1.592	1.039
<i>Uts2</i>	1.508	2.004	37.502	1.950	1.559	3.015
<i>Zbtb7b</i>	0.885	1.207	0.207	0.465	0.644	0.586
<i>Zeb1</i>	0.718	0.784	37.502	1.226	1.249	3.015

Spleen specimens were collected on the days 21 and 28 (after inoculation with 4T1 cells) from mice treated with calcitriol or its analogs and control group receiving vehicle. Real-time PCR screening was performed using

Mouse T Helper Cell Differentiation RT² Profiler Array (Qiagen, Hilden, Germany) including 84 key genes and 5 housekeeping genes in the set. Data shows a mean relative quantification (RQ) values. Fold-change (RQ) of target genes was defined using double delta Ct method in reference to actin, beta (Actb) and beta-2 microglobulin (B2m) for splenocytes samples. Then the results were adjusted to the values obtained for the control group within the day 21 or 28th of the experiment for each treatment group. Data analysis was acquired using Qiagen online software suitable for purchased kit (Qiagen, Hilden, Germany). PCR amplification cycles were as follows 95 °C for 10 s and 58 °C for 45 s (50 cycles). We used 0,5 µg of cDNA (6 mice pooled per group) for a single reaction.

Table S2. Pixel densities of plasma cytokines acquired with *Mouse Cytokine Array Panel A* (A-E).

A.

Cytokine	D0		D7		Factor
	Mean	SD	Mean	SD	
BLC	6.519	1.503	10.188	1.918	1.563
C5/C5a	130.931	0.442	144.681	9.953	1.105
G-CSF	2.500	0.752	4.906	0.000	1.963
GM-CSF	2.794	0.124	1.482	1.061	0.530
I-309	2.894	0.301	1.069	1.150	0.369
Eotaxin	1.507	0.018	0.631	0.035	0.419
sICAM-1	101.213	2.289	93.100	2.678	0.920
IFN- γ	6.469	2.227	2.781	1.203	0.430
IL-1 α	6.681	0.017	2.700	0.503	0.404
IL-1 β	2.425	0.380	1.169	1.025	0.482
IL-1ra	9.488	1.759	2.331	2.157	0.246
IL-2	9.050	1.440	2.337	1.370	0.258
IL-3	2.737	0.680	3.669	1.326	1.341
IL-4	7.112	0.062	7.431	0.707	1.045
IL-5	2.356	0.089	2.831	0.743	1.202
IL-6	2.012	0.397	0.512	0.751	0.255
IL-7	2.838	0.045	0.525	0.556	0.185
IL-10	1.981	0.318	-0.088	0.981	-0.044
IL-13	3.494	0.336	2.062	1.546	0.590
IL-12p70	3.000	1.211	0.950	1.051	0.317
IL-16	5.769	0.442	2.543	0.371	0.441
IL-17	3.406	0.035	0.343	1.909	0.101
IL-23	4.050	1.388	1.881	1.114	0.465
IL-27	4.525	0.238	2.194	0.865	0.485
IP-20	3.144	0.760	3.031	0.211	0.964
I-TAC	4.225	0.628	2.569	1.645	0.608
KC	2.544	1.095	1.850	0.079	0.727
M-CSF	10.162	1.246	8.731	0.583	0.859
JE	21.588	0.309	6.194	1.803	0.287
MCP-5	3.356	1.573	0.606	0.265	0.181
MIG	2.163	0.115	0.912	0.521	0.422
MIP-1 α	2.425	1.565	1.688	0.786	0.696
MIP-1 β	3.469	0.248	0.531	0.601	0.153
MIP-2	3.125	0.416	0.350	0.893	0.112
RANTES	15.918	0.177	0.337	0.981	0.021
SDF-1	22.700	0.893	4.537	0.327	0.200
TARC	1.456	0.371	2.663	0.397	1.829
TIMP-1	4.356	0.000	4.232	0.354	0.971
TNF- α	1.844	0.407	0.625	0.840	0.339
TREM-1	1.813	0.981	1.237	0.080	0.682
REF	178.071	11.274	182.323	6.490	1.024

B.

Cytokine	CONTROL					
	D21			D33		
	Mean	SD	Factor	Mean	SD	Factor
BLC	35.313	5.020	5.417	40.250	4.110	6.175
C5/C5a	108.751	1.909	0.831	101.131	0.547	0.772
G-CSF	108.775	2.599	43.519	105.250	0.875	42.108
GM-CSF	8.225	4.119	2.944	4.119	2.563	1.474
I-309	4.588	0.584	1.585	1.925	0.327	0.665
Eotaxin	2.925	1.167	1.942	-0.100	0.574	-0.066
sICAM-1	109.181	8.742	1.079	85.200	0.911	0.842
IFN- γ	7.819	6.302	1.209	3.156	2.652	0.488
IL-1 α	15.951	0.530	2.387	8.619	0.124	1.290
IL-1 β	5.788	0.124	2.387	19.056	0.124	7.860
IL-1ra	52.294	0.981	5.512	59.031	0.549	6.222
IL-2	11.200	0.105	1.238	1.288	1.087	0.142
IL-3	7.812	1.008	2.854	3.956	1.202	1.445
IL-4	15.944	3.014	2.242	5.394	0.071	0.758
IL-5	8.319	0.698	3.531	4.013	0.274	1.703
IL-6	4.632	1.140	2.302	2.175	0.521	1.081
IL-7	4.363	0.601	1.537	3.112	0.008	1.097
IL-10	1.807	0.327	0.912	-0.526	0.363	-0.265
IL-13	13.182	4.800	3.773	1.481	0.247	0.424
IL-12p70	1.656	1.247	0.552	0.169	0.477	0.056
IL-16	18.750	1.026	3.250	22.281	0.158	3.863
IL-17	3.856	0.787	1.132	2.912	0.716	0.855
IL-23	6.126	1.061	1.513	0.719	0.530	0.178
IL-27	5.257	0.557	1.162	3.931	1.025	0.869
IP-20	7.800	0.600	2.481	6.713	1.264	2.135
I-TAC	10.857	0.734	2.570	12.994	2.280	3.075
KC	55.475	2.245	21.810	20.006	0.106	7.866
M-CSF	76.438	4.613	7.522	55.081	6.736	5.420
JE	35.644	2.890	1.651	30.819	0.018	1.428
MCP-5	5.787	1.856	1.724	3.200	1.440	0.953
MIG	4.375	0.336	2.023	1.675	0.133	0.775
MIP-1 α	2.200	0.105	0.907	0.519	0.442	0.214
MIP-1 β	1.369	0.238	0.395	1.594	0.194	0.459
MIP-2	2.375	0.777	0.760	1.594	0.336	0.510
RANTES	8.700	2.263	0.547	4.388	0.009	0.276
SDF-1	75.319	2.166	3.318	35.150	2.184	1.548
TARC	8.663	1.114	5.952	8.357	0.937	5.741
TIMP-1	57.838	8.256	13.278	66.481	3.871	15.262
TNF- α	17.838	0.264	9.676	13.462	0.239	7.302
TREM-1	20.363	1.838	11.234	26.013	4.110	14.352
REF	189.756	10.363	1.066	143.412	25.643	0.805

C.

Cytokine	CALCITRIOL					
	D21			D33		
	Mean	SD	Factor	Mean	SD	Factor
BLC	10.719	1.034	1.644	43.576	6.249	6.685
C5/C5a	79.194	14.098	0.605	107.594	24.130	0.822
G-CSF	81.063	3.147	32.431	68.725	2.130	27.495
GM-CSF	2.988	1.131	1.070	4.332	0.070	1.551
I-309	2.357	0.292	0.814	3.738	0.168	1.292
Eotaxin	1.719	0.044	1.141	2.532	0.689	1.680
sICAM-1	107.363	5.551	1.061	89.538	1.564	0.885
IFN- γ	6.125	5.568	0.947	3.788	1.264	0.586
IL-1 α	9.044	0.981	1.354	6.763	0.257	1.012
IL-1 β	3.156	0.150	1.302	12.663	0.151	5.223
IL-1ra	38.875	2.086	4.097	53.569	1.237	5.646
IL-2	2.675	0.972	0.296	3.307	0.689	0.365
IL-3	2.181	0.256	0.797	3.657	0.354	1.336
IL-4	5.656	0.911	0.795	5.026	0.928	0.707
IL-5	2.369	0.009	1.005	4.463	0.027	1.894
IL-6	0.937	0.390	0.466	5.482	0.230	2.724
IL-7	2.756	0.150	0.971	4.813	0.044	1.696
IL-10	1.082	0.026	0.546	2.213	0.009	1.117
IL-13	3.076	0.548	0.880	3.007	0.088	0.861
IL-12p70	1.137	0.742	0.379	1.888	0.397	0.629
IL-16	7.100	0.407	1.231	10.332	0.141	1.791
IL-17	1.256	0.168	0.369	3.363	0.221	0.987
IL-23	1.856	0.044	0.458	2.763	0.080	0.682
IL-27	4.500	0.460	0.995	3.938	0.080	0.870
IP-20	2.751	0.619	0.875	4.226	0.450	1.344
I-TAC	5.475	0.106	1.296	9.144	0.707	2.164
KC	18.132	3.120	7.129	11.688	0.593	4.595
M-CSF	39.081	0.946	3.846	25.513	3.014	2.511
JE	23.038	0.760	1.067	20.938	0.008	0.970
MCP-5	3.306	0.911	0.985	4.444	0.778	1.324
MIG	2.076	0.725	0.960	2.488	0.079	1.151
MIP-1 α	1.082	0.521	0.446	1.882	0.371	0.776
MIP-1 β	0.588	0.070	0.169	1.325	0.098	0.382
MIP-2	0.762	0.212	0.244	1.901	0.186	0.608
RANTES	2.257	0.238	0.142	5.757	0.159	0.362
SDF-1	22.882	2.131	1.008	25.169	0.832	1.109
TARC	1.375	0.177	0.945	6.126	0.221	4.209
TIMP-1	21.675	1.344	4.976	45.151	5.259	10.365
TNF- α	4.950	0.742	2.685	3.650	0.256	1.980
TREM-1	8.513	0.230	4.697	10.494	0.653	5.790
REF	169.198	11.558	0.950	166.946	13.607	0.938

D.

Cytokine	PRI-2191					
	D21			D33		
	Mean	SD	Factor	Mean	SD	Factor
BLC	12.937	1.493	1.985	23.650	1.291	3.628
C5/C5a	73.719	9.476	0.563	102.413	4.490	0.782
G-CSF	53.406	4.154	21.366	89.900	1.079	35.967
GM-CSF	3.156	1.184	1.130	3.169	0.787	1.134
I-309	2.413	0.221	0.834	1.350	0.247	0.467
Eotaxin	1.375	0.592	0.912	0.244	0.168	0.162
sICAM-1	99.937	0.822	0.987	88.450	1.256	0.874
IFN- γ	3.769	1.785	0.583	1.813	1.131	0.280
IL-1 α	6.594	0.247	0.987	6.532	0.221	0.978
IL-1 β	4.256	0.106	1.755	14.625	1.450	6.032
IL-1ra	22.037	0.133	2.323	31.644	1.493	3.335
IL-2	3.313	0.151	0.366	0.281	0.185	0.031
IL-3	4.400	1.512	1.608	1.600	0.177	0.585
IL-4	18.463	1.777	2.596	2.350	0.017	0.330
IL-5	6.212	0.751	2.637	1.806	0.027	0.767
IL-6	1.363	0.026	0.677	1.488	0.036	0.739
IL-7	2.250	0.026	0.793	2.038	0.318	0.718
IL-10	0.869	0.035	0.438	0.332	0.363	0.167
IL-13	6.044	1.290	1.730	1.613	0.124	0.462
IL-12p70	1.106	0.160	0.369	-0.075	0.460	-0.025
IL-16	8.431	0.194	1.461	13.088	0.265	2.269
IL-17	2.431	0.636	0.714	1.331	0.027	0.391
IL-23	2.219	0.301	0.548	0.581	0.327	0.143
IL-27	2.350	0.310	0.519	1.206	0.911	0.267
IP-20	3.800	0.080	1.209	3.081	0.593	0.980
I-TAC	6.488	0.380	1.536	8.025	0.443	1.899
KC	22.694	0.159	8.922	9.019	0.910	3.546
M-CSF	38.163	0.769	3.755	29.988	0.407	2.951
JE	22.307	1.715	1.033	19.007	2.696	0.880
MCP-5	2.737	1.069	0.816	2.681	0.557	0.799
MIG	2.838	0.026	1.312	2.194	0.522	1.015
MIP-1 α	1.800	0.150	0.742	0.556	0.027	0.229
MIP-1 β	0.993	0.070	0.286	0.175	0.106	0.050
MIP-2	1.381	0.530	0.442	0.713	0.425	0.228
RANTES	3.994	0.070	0.251	3.825	0.071	0.240
SDF-1	29.294	1.644	1.290	16.688	1.785	0.735
TARC	2.869	0.071	1.971	3.144	0.115	2.160
TIMP-1	24.681	1.131	5.666	22.813	0.672	5.237
TNF- α	9.038	0.186	4.902	5.588	0.035	3.031
TREM-1	11.644	0.442	6.424	19.450	0.354	10.731
REF	172.954	16.214	0.971	150.189	40.145	0.843

E.

Cytokine	PRI-2205					
	D21			D33		
	Mean	SD	Factor	Mean	SD	Factor
BLC	19.525	2.077	2.995	56.432	1.979	8.657
C5/C5a	107.007	2.988	0.817	100.313	15.513	0.766
G-CSF	98.944	1.167	39.586	99.307	1.132	39.731
GM-CSF	5.126	1.247	1.835	4.138	1.141	1.481
I-309	4.219	0.035	1.458	4.207	0.141	1.454
Eotaxin	2.713	0.380	1.801	1.844	0.018	1.224
sICAM-1	122.626	13.621	1.212	120.957	1.432	1.195
IFN- γ	4.950	2.820	0.765	2.600	1.140	0.402
IL-1 α	9.138	1.511	1.368	8.776	0.415	1.314
IL-1 β	4.256	0.071	1.755	18.695	1.432	7.711
IL-1ra	30.963	0.221	3.264	84.975	4.180	8.957
IL-2	3.813	0.539	0.421	1.875	0.522	0.207
IL-3	3.294	0.512	1.204	2.882	0.318	1.053
IL-4	5.695	0.088	0.801	3.463	0.291	0.487
IL-5	2.951	0.080	1.252	2.013	0.274	0.854
IL-6	1.419	0.372	0.705	1.119	0.300	0.556
IL-7	2.944	0.231	1.038	2.501	0.151	0.881
IL-10	1.094	0.159	0.552	0.726	0.097	0.366
IL-13	4.569	0.159	1.308	3.013	0.628	0.862
IL-12p70	0.919	0.460	0.306	0.663	0.256	0.221
IL-16	11.676	0.945	2.024	23.031	0.530	3.993
IL-17	2.088	0.027	0.613	2.007	0.053	0.589
IL-23	2.625	0.115	0.648	2.969	0.212	0.733
IL-27	2.000	0.115	0.442	1.376	0.238	0.304
IP-20	6.107	0.230	1.943	7.069	0.689	2.249
I-TAC	7.150	0.274	1.692	7.545	0.972	1.786
KC	24.538	1.989	9.647	13.875	0.663	5.455
M-CSF	59.413	0.716	5.847	43.625	0.327	4.293
JE	30.232	0.319	1.400	31.751	1.865	1.471
MCP-5	5.238	0.804	1.561	6.357	0.619	1.894
MIG	3.607	0.284	1.668	3.907	0.211	1.806
MIP-1 α	1.444	0.141	0.596	1.244	0.194	0.513
MIP-1 β	1.169	0.052	0.337	1.544	0.106	0.445
MIP-2	1.382	0.105	0.442	1.251	0.045	0.400
RANTES	4.563	0.574	0.287	7.232	1.149	0.454
SDF-1	45.550	2.413	2.007	37.688	0.787	1.660
TARC	2.894	0.371	1.988	3.988	0.699	2.740
TIMP-1	36.851	0.115	8.460	64.888	0.274	14.896
TNF- α	12.050	0.805	6.536	9.706	0.177	5.265
TREM-1	13.294	0.761	7.335	23.594	0.035	13.017
REF	183.781	10.745	1.032	171.892	16.768	0.965

Plasma specimens were collected on the days 0, 7, 21 and 33 (after inoculation with 4T1 cells) from mice treated with calcitriol or its analogs and control group receiving vehicle. Results were obtained using *Proteome Profiler*

Mouse Cytokine Array Kit, Panel A (R&D alp Systems. Inc. USA) according to the enclosed instruction. This array detects 40 mouse cytokines, chemokines, and acute phase proteins simultaneously. Pixel densities on developed X-ray film were collected using a multifunctional scanning device (Samsung SLC460) or Image Station 4000MM PRO (Carestream Health, Rochester, New York, USA) and image analysis software (ImageJ 1.48v). For each spot the final optical density level was determined as a factor acquired by subtracting the background optical level and dividing by values obtained from the untreated mice (Day 0).

Table S3. Pixel densities of cytokines contained in the supernatants from stimulated with lipopolisaccharide (LPS) splenocytes obtained using *Mouse Cytokine Array Panel A* (A-E).

A.

Cytokine	Day 0		Day 7		Factor
	Mean	SD	Mean	SD	
BLC	23.738	2.126	0.045	0.201	0.002
C5/C5a	5.514	0.433	-0.443	0.078	-0.080
G-CSF	81.026	4.795	5.761	0.837	0.071
GM-CSF	55.980	0.343	0.645	0.243	0.012
I-309	35.248	0.585	0.201	0.371	0.006
Eotaxin	1.832	0.564	-0.343	0.015	-0.187
sICAM-1	101.720	1.149	9.379	0.515	0.092
IFN-γ	34.038	3.560	0.550	0.239	0.016
IL-1α	70.394	2.505	2.563	0.518	0.036
IL-1β	65.182	0.888	3.630	0.016	0.056
IL-1ra	124.207	0.747	65.550	0.687	0.528
IL-2	54.187	6.538	1.617	0.378	0.030
IL-3	9.705	0.584	-0.473	0.049	-0.049
IL-4	8.972	0.560	-0.358	0.317	-0.040
IL-5	11.306	1.761	0.311	0.386	0.028
IL-6	74.049	0.567	0.548	0.170	0.007
IL-7	16.377	0.170	-0.075	0.155	-0.005
IL-10	72.831	4.371	2.771	0.279	0.038
IL-13	17.981	1.064	0.639	0.604	0.036
IL-12p70	32.327	4.586	1.731	0.280	0.054
IL-16	105.465	5.210	28.311	0.473	0.268
IL-17	43.160	5.823	1.840	0.059	0.043
IL-23	59.595	0.972	2.138	0.081	0.036
IL-27	18.794	2.734	0.859	1.161	0.046
IP-20	59.092	0.025	1.010	0.042	0.017
I-TAC	10.701	0.454	-0.554	0.077	-0.052
KC	131.671	5.156	71.225	1.881	0.541
M-CSF	55.022	16.395	0.251	0.541	0.005
JE	111.643	1.313	35.037	1.242	0.314
MCP-5	18.083	4.277	-0.259	0.091	-0.014

MIG	45.425	32.978	0.848	1.623	0.019
MIP-1α	136.452	0.658	120.183	13.653	0.881
MIP-1β	131.384	2.016	47.542	0.530	0.362
MIP-2	135.929	0.426	117.924	1.045	0.868
RANTES	133.400	0.702	66.125	0.808	0.496
SDF-1	86.462	7.575	10.235	2.345	0.118
TARC	3.405	1.497	-0.056	0.069	-0.016
TIMP-1	30.856	0.829	0.471	0.055	0.015
TNF-α	119.593	0.648	40.878	0.999	0.342
TREM-1	32.815	7.308	-0.044	0.295	-0.001
REF	135.998	0.349	130.278	1.557	0.958

B.

Cytokine	CONTROL					
	Day 21			Day 33		
	Mean	SD	Factor	Mean	SD	Factor
BLC	-0.897	0.004	-0.038	40.249	1.485	1.696
C5/C5a	-1.498	0.320	-0.272	16.813	0.697	3.049
G-CSF	62.953	5.863	0.777	129.958	5.928	1.604
GM-CSF	5.289	1.387	0.094	88.125	10.071	1.574
I-309	0.144	0.266	0.004	57.490	0.663	1.631
Eotaxin	-0.775	0.286	-0.423	12.282	0.857	6.706
sICAM-1	11.683	1.252	0.115	79.322	8.806	0.780
IFN-γ	0.418	0.230	0.012	37.058	4.029	1.089
IL-1α	41.006	2.080	0.583	96.893	4.443	1.376
IL-1β	77.673	1.078	1.192	126.021	2.314	1.933
IL-1ra	129.620	1.041	1.044	138.319	0.345	1.114
IL-2	39.297	43.091	0.725	63.048	12.612	1.164
IL-3	0.744	0.284	0.077	23.517	2.182	2.423
IL-4	-0.688	0.111	-0.077	24.120	0.416	2.688
IL-5	7.696	0.161	0.681	31.945	6.112	2.825
IL-6	43.243	0.483	0.584	119.355	5.639	1.612
IL-7	3.377	0.564	0.206	49.270	2.469	3.009
IL-10	58.105	0.875	0.798	112.557	4.528	1.545
IL-13	0.353	0.216	0.020	30.179	4.196	1.678
IL-12p70	9.321	0.943	0.288	25.600	4.332	0.792
IL-16	65.577	3.615	0.622	130.150	2.278	1.234
IL-17	13.103	2.432	0.304	85.816	7.847	1.988
IL-23	23.972	2.109	0.402	114.436	0.777	1.920
IL-27	3.715	3.411	0.198	34.448	11.399	1.833
IP-20	95.025	5.773	1.608	134.932	3.057	2.283
I-TAC	3.509	4.125	0.328	44.475	4.694	4.156
KC	114.070	9.272	0.866	136.731	1.455	1.038
M-CSF	16.740	12.671	0.304	82.967	13.839	1.508
JE	110.680	6.720	0.991	137.645	2.798	1.233
MCP-5	19.124	12.324	1.058	104.037	11.448	5.753
MIG	5.184	8.144	0.114	34.689	15.578	0.764

MIP-1α	124.862	2.483	0.915	138.259	0.735	1.013
MIP-1β	122.275	1.855	0.931	139.967	0.095	1.065
MIP-2	123.231	2.575	0.907	139.771	1.216	1.028
RANTES	118.067	2.157	0.885	138.355	0.370	1.037
SDF-1	33.734	22.956	0.390	80.278	12.758	0.928
TARC	3.262	1.727	0.958	30.755	4.536	9.032
TIMP-1	21.756	0.898	0.705	104.209	3.070	3.377
TNF-α	83.588	9.313	0.699	133.475	6.075	1.116
TREM-1	41.564	10.045	1.267	107.346	13.181	3.271
REF	117.227	6.539	0.862	139.261	0.602	1.024

C.

Cytokine	CALCITRIOL					
	Day 21			Day 33		
	Mean	SD	Factor	Mean	SD	Factor
BLC	14.644	0.342	0.617	11.067	0.516	0.466
C5/C5a	8.709	0.315	1.580	5.853	0.677	1.062
G-CSF	105.412	0.826	1.301	121.296	1.832	1.497
GM-CSF	50.630	0.303	0.904	60.817	1.724	1.086
I-309	31.048	1.619	0.881	26.361	0.919	0.748
Eotaxin	4.683	1.409	2.557	3.537	0.175	1.931
sICAM-1	75.841	2.232	0.746	63.663	0.775	0.626
IFN-γ	35.356	0.957	1.039	24.534	0.301	0.721
IL-1α	79.534	1.133	1.130	102.096	7.075	1.450
IL-1β	108.163	0.498	1.659	121.906	0.571	1.870
IL-1ra	137.018	1.547	1.103	137.518	0.344	1.107
IL-2	36.017	15.298	0.665	26.095	6.983	0.482
IL-3	11.482	0.645	1.183	5.579	0.651	0.575
IL-4	11.398	0.332	1.270	5.880	0.101	0.655
IL-5	25.857	0.433	2.287	18.462	10.945	1.633
IL-6	88.520	1.950	1.195	131.455	1.915	1.775
IL-7	34.407	0.012	2.101	37.198	5.612	2.271
IL-10	102.227	7.662	1.404	80.696	1.420	1.108
IL-13	23.740	6.194	1.320	34.898	3.450	1.941
IL-12p70	39.006	1.400	1.207	16.198	2.477	0.501
IL-16	126.816	3.970	1.202	126.957	0.655	1.204
IL-17	69.176	9.176	1.603	65.947	11.907	1.528
IL-23	94.952	10.462	1.593	72.408	5.806	1.215
IL-27	18.181	1.664	0.967	11.426	2.091	0.608
IP-20	126.821	1.500	2.146	109.271	3.434	1.849
I-TAC	20.682	1.537	1.933	6.561	0.979	0.613
KC	138.039	0.654	1.048	68.041	1.969	0.517
M-CSF	60.503	8.965	1.100	51.157	12.142	0.930
JE	138.504	0.083	1.241	140.285	0.744	1.257
MCP-5	62.477	12.436	3.455	73.380	18.259	4.058
MIG	43.675	32.420	0.961	32.230	19.721	0.710

MIP-1α	139.332	0.563	1.021	138.632	0.223	1.016
MIP-1β	140.568	0.396	1.070	139.671	0.295	1.063
MIP-2	140.664	0.011	1.035	95.301	8.422	0.701
RANTES	137.762	2.286	1.033	137.296	0.400	1.029
SDF-1	70.593	12.774	0.816	59.268	6.441	0.685
TARC	16.181	0.643	4.752	8.866	0.959	2.604
TIMP-1	59.710	0.934	1.935	52.598	0.454	1.705
TNF-α	125.770	4.511	1.052	126.441	7.226	1.057
TREM-1	74.956	9.144	2.284	70.558	10.769	2.150
REF	139.381	0.218	1.025	138.315	1.228	1.017

D.

Cytokine	PRI-2191					
	Day 21			Day 33		
	Mean	SD	Factor	Mean	SD	Factor
BLC	2.776	0.693	0.117	10.666	1.097	0.449
C5/C5a	1.772	0.091	0.321	4.183	0.855	0.759
G-CSF	60.192	4.340	0.743	94.889	2.193	1.171
GM-CSF	16.988	2.247	0.303	9.108	2.420	0.163
I-309	9.152	1.158	0.260	9.659	0.055	0.274
Eotaxin	2.819	0.303	1.539	4.288	0.528	2.341
sICAM-1	31.190	0.349	0.307	47.993	1.190	0.472
IFN-γ	5.538	0.376	0.163	5.235	0.519	0.154
IL-1α	29.717	0.426	0.422	45.406	3.761	0.645
IL-1β	72.399	6.403	1.111	100.828	2.266	1.547
IL-1ra	135.750	1.812	1.093	144.628	2.326	1.164
IL-2	7.784	4.562	0.144	8.032	4.586	0.148
IL-3	1.705	0.161	0.176	5.360	0.261	0.552
IL-4	2.318	0.163	0.258	6.401	0.732	0.713
IL-5	2.730	0.301	0.241	7.368	1.053	0.652
IL-6	26.862	2.949	0.363	38.931	0.110	0.526
IL-7	7.958	1.344	0.486	12.731	1.229	0.777
IL-10	76.837	4.441	1.055	97.113	5.599	1.333
IL-13	3.879	0.720	0.216	7.266	0.853	0.404
IL-12p70	16.630	3.757	0.514	4.636	0.057	0.143
IL-16	80.172	2.276	0.760	111.458	0.959	1.057
IL-17	19.959	1.897	0.462	19.127	2.979	0.443
IL-23	24.987	2.839	0.419	28.565	3.321	0.479
IL-27	4.325	1.663	0.230	4.296	1.293	0.229
IP-20	78.676	1.864	1.331	106.758	5.658	1.807
I-TAC	2.178	0.324	0.204	4.598	0.386	0.430
KC	86.618	15.143	0.658	123.545	1.126	0.938
M-CSF	19.014	2.169	0.346	11.705	3.752	0.213
JE	131.244	6.973	1.176	143.125	4.648	1.282
MCP-5	10.060	6.840	0.556	51.299	8.037	2.837
MIG	12.904	14.044	0.284	5.850	1.846	0.129
MIP-1α	143.602	0.934	1.052	119.366	9.129	0.875
MIP-1β	140.211	3.483	1.067	149.593	1.059	1.139

MIP-2	143.061	0.645	1.052	149.454	0.112	1.100
RANTES	138.197	1.986	1.036	142.195	3.965	1.066
SDF-1	46.739	8.979	0.541	48.983	11.902	0.567
TARC	2.312	0.542	0.679	6.136	1.146	1.802
TIMP-1	11.337	2.604	0.367	51.007	1.647	1.653
TNF-α	74.115	10.272	0.620	103.726	8.144	0.867
TREM-1	34.224	2.191	1.043	55.476	6.578	1.691
REF	143.687	0.636	1.057	143.200	0.716	1.053

E.

Cytokine	PRI-2205					
	Day 21			Day 33		
	Mean	SD	Factor	Mean	SD	Factor
BLC	3.286	0.084	0.138	3.750	0.354	0.158
C5/C5a	2.109	0.094	0.383	1.762	0.120	0.319
G-CSF	82.583	4.914	1.019	73.251	3.041	0.904
GM-CSF	16.169	0.068	0.289	19.561	0.137	0.349
I-309	2.662	0.058	0.076	2.639	0.115	0.075
Eotaxin	-0.648	0.353	-0.354	2.152	0.085	1.175
sICAM-1	37.650	1.421	0.370	39.604	3.356	0.389
IFN-γ	3.567	0.482	0.105	3.931	0.159	0.115
IL-1α	40.246	1.633	0.572	49.052	3.570	0.697
IL-1β	74.733	0.745	1.147	102.614	0.909	1.574
IL-1ra	121.997	5.846	0.982	141.772	1.662	1.141
IL-2	5.214	1.806	0.096	14.303	5.122	0.264
IL-3	2.932	0.396	0.302	3.733	0.024	0.385
IL-4	2.040	0.105	0.227	1.975	0.448	0.220
IL-5	12.436	1.683	1.100	2.775	0.087	0.245
IL-6	55.485	0.402	0.749	37.927	1.754	0.512
IL-7	8.533	1.777	0.521	9.596	1.621	0.586
IL-10	68.625	2.708	0.942	89.952	2.992	1.235
IL-13	3.270	0.898	0.182	4.574	1.725	0.254
IL-12p70	13.398	1.939	0.414	5.036	0.244	0.156
IL-16	89.897	1.066	0.852	103.306	4.699	0.980
IL-17	16.061	1.283	0.372	19.268	2.747	0.446
IL-23	30.755	1.270	0.516	44.801	1.926	0.752
IL-27	4.325	0.948	0.230	5.104	1.832	0.272
IP-20	78.240	0.540	1.324	120.390	3.420	2.037
I-TAC	2.494	0.424	0.233	2.442	0.405	0.228
KC	131.981	6.746	1.002	74.213	0.220	0.564
M-CSF	24.634	6.649	0.448	14.896	1.781	0.271
JE	134.656	0.076	1.206	134.001	3.113	1.200
MCP-5	8.113	6.913	0.449	66.237	16.965	3.663
MIG	19.404	22.149	0.427	5.916	5.411	0.130
MIP-1α	136.035	2.993	0.997	125.289	6.986	0.918
MIP-1β	137.810	0.503	1.049	141.594	0.791	1.078

MIP-2	136.390	1.242	1.003	134.307	1.081	0.988
RANTES	130.380	5.392	0.977	137.537	3.498	1.031
SDF-1	42.145	5.669	0.487	36.775	8.413	0.425
TARC	3.643	0.454	1.070	6.840	0.030	2.009
TIMP-1	9.209	0.192	0.298	37.433	0.808	1.213
TNF-α	109.950	3.451	0.919	98.079	13.105	0.820
TREM-1	49.217	5.267	1.500	56.408	3.613	1.719
REF	136.316	3.157	1.002	132.685	1.484	0.976

Spleen specimens were collected on the days 0, 7, 21 and 33 (after inoculation with 4T1 cells) from mice treated with calcitriol or its analogs and control group receiving vehicle and stimulated with lipopolisaccharide (LPS). Results were obtained using *Proteome Profiler Mouse Cytokine Array Kit, Panel A* (R&D alp Systems, Inc. USA) according to the enclosed instruction. This array detects 40 mouse cytokines, chemokines, and acute phase proteins simultaneously. Pixel densities on developed X-ray film were collected using a multifunctional scanning device (Samsung SLC460) or Image Station 4000MM PRO (Carestream Health, Rochester, New York, USA) and image analysis software (ImageJ 1.48v). For each spot the final optical density level was determined as a factor acquired by subtracting the background optical level and dividing by values obtained from the untreated mice (Day 0).

Table S4. Pixel densities of cytokines contained in the supernatants from stimulated with Concanavalin A (ConA) splenocytes obtained using *Mouse Cytokine Array Panel A*(A-E).

A.

Cytokine	Day 0		Day 7		Factor
	Mean	SD	Mean	SD	
BLC	24.093	0.862	2.192	0.351	0.091
C5/C5a	8.417	1.727	0.855	0.062	0.102
G-CSF	50.457	1.605	1.773	0.265	0.035
GM-CSF	97.510	5.016	14.988	0.163	0.154
I-309	28.431	0.304	0.901	0.525	0.032
Eotaxin	2.276	0.077	0.104	0.177	0.046
sICAM-1	92.882	6.220	7.951	0.736	0.086
IFN- γ	52.569	2.445	1.080	0.169	0.021
IL-1 α	62.158	0.045	4.668	0.593	0.075
IL-1 β	49.003	1.266	6.462	0.730	0.132
IL-1ra	114.766	8.124	74.239	3.758	0.647
IL-2	127.274	4.724	59.126	3.797	0.465
IL-3	111.816	5.187	23.161	1.334	0.207
IL-4	15.652	4.757	0.395	0.739	0.025
IL-5	6.058	0.721	0.775	0.733	0.128
IL-6	43.903	0.440	1.488	0.276	0.034
IL-7	14.703	3.291	3.180	1.543	0.216
IL-10	26.288	1.309	0.150	0.211	0.006
IL-13	42.653	3.433	0.379	0.518	0.009
IL-12p70	12.142	0.688	2.897	0.584	0.239
IL-16	99.521	4.315	32.383	0.738	0.325
IL-17	107.137	4.215	21.087	1.081	0.197
IL-23	46.775	6.078	4.856	0.489	0.104
IL-27	25.938	3.702	2.558	0.636	0.099
IP-20	94.847	2.882	19.585	1.882	0.206
I-TAC	12.013	1.787	0.269	0.076	0.022
KC	111.443	1.946	48.117	2.534	0.432
M-CSF	37.872	2.880	1.242	0.226	0.033
JE	97.958	1.675	49.514	0.665	0.505
MCP-5	8.066	1.554	0.744	0.473	0.092
MIG	24.013	7.953	1.336	1.095	0.056
MIP-1 α	137.543	1.469	103.000	6.341	0.749
MIP-1 β	101.336	5.985	47.186	1.578	0.466
MIP-2	131.549	3.461	87.477	1.523	0.665
RANTES	121.788	2.899	84.079	0.882	0.690
SDF-1	88.438	6.220	27.074	4.643	0.306
TARC	7.836	1.099	1.022	0.298	0.130
TIMP-1	24.295	0.123	0.791	0.386	0.033

TNF-α	108.639	2.019	32.804	1.766	0.302
TREM-1	23.288	4.076	1.256	0.048	0.054
REF	140.518	0.286	95.544	1.609	0.680

B.

Cytokine	CONTROL					
	Day 21			Day 33		
	Mean	SD	Factor	Mean	SD	Factor
BLC	1.964	0.124	0.082	9.132	0.640	0.379
C5/C5a	1.504	0.298	0.179	13.070	2.776	1.553
G-CSF	2.459	0.197	0.049	34.563	0.501	0.685
GM-CSF	3.760	0.549	0.039	33.974	1.755	0.348
I-309	2.062	0.645	0.073	40.241	0.247	1.415
Eotaxin	1.315	0.598	0.578	2.596	0.476	1.141
sICAM-1	2.678	0.098	0.029	73.587	3.337	0.792
IFN-γ	2.088	0.513	0.040	40.391	2.484	0.768
IL-1α	3.320	0.156	0.053	52.898	2.713	0.851
IL-1β	27.045	4.030	0.552	77.059	0.212	1.573
IL-1ra	116.177	3.326	1.012	131.521	3.677	1.146
IL-2	53.406	11.938	0.420	73.884	5.018	0.581
IL-3	19.171	1.611	0.171	12.788	5.773	0.114
IL-4	1.723	0.760	0.110	17.237	2.069	1.101
IL-5	0.876	0.020	0.145	1.412	0.675	0.233
IL-6	1.705	0.023	0.039	3.930	0.345	0.090
IL-7	2.017	0.055	0.137	28.324	0.960	1.926
IL-10	1.795	0.192	0.068	6.439	0.823	0.245
IL-13	1.308	0.497	0.031	15.238	0.235	0.357
IL-12p70	2.433	0.120	0.200	2.253	0.441	0.186
IL-16	28.853	2.651	0.290	122.546	8.124	1.231
IL-17	4.257	0.004	0.040	33.746	8.119	0.315
IL-23	10.800	1.223	0.231	66.402	0.091	1.420
IL-27	3.721	1.203	0.143	25.281	1.121	0.975
IP-20	87.991	0.165	0.928	73.708	0.723	0.777
I-TAC	3.394	3.031	0.282	14.531	2.326	1.210
KC	4.521	0.195	0.041	6.018	1.363	0.054
M-CSF	1.532	0.077	0.040	40.680	0.326	1.074
JE	76.260	2.775	0.778	134.581	0.633	1.374
MCP-5	9.063	4.183	1.124	37.650	25.427	4.668
MIG	1.305	0.681	0.054	16.966	0.221	0.707
MIP-1α	84.015	6.534	0.611	50.259	1.317	0.365
MIP-1β	87.521	0.823	0.864	44.651	0.512	0.441
MIP-2	82.005	1.203	0.623	28.013	0.211	0.213
RANTES	93.770	0.214	0.770	44.394	3.868	0.365
SDF-1	9.751	7.724	0.110	75.951	2.675	0.859
TARC	2.301	0.506	0.294	8.454	0.408	1.079
TIMP-1	1.189	0.992	0.049	41.542	2.132	1.710
TNF-α	6.130	0.508	0.056	58.223	1.808	0.536

TREM-1	2.662	0.046	0.114	78.023	1.206	3.350
REF	88.250	3.598	0.628	136.648	0.419	0.972

C.

Cytokine	CALCITRIOL					
	Day 21			Day 33		
	Mean	SD	Factor	Mean	SD	Factor
BLC	13.242	0.581	0.550	5.817	0.118	0.241
C5/C5a	10.658	2.647	1.266	1.799	0.083	0.214
G-CSF	11.465	0.349	0.227	5.917	0.489	0.117
GM-CSF	37.310	1.268	0.383	28.302	0.922	0.290
I-309	14.657	0.738	0.516	13.565	0.461	0.477
Eotaxin	1.897	0.210	0.833	0.049	0.074	0.022
sICAM-1	56.442	1.287	0.608	47.269	1.385	0.509
IFN-γ	27.064	0.423	0.515	24.701	1.400	0.470
IL-1α	36.810	0.101	0.592	37.452	1.046	0.603
IL-1β	58.780	0.642	1.200	59.075	3.366	1.206
IL-1ra	132.755	4.535	1.157	110.467	1.753	0.963
IL-2	90.919	8.637	0.714	70.303	2.147	0.552
IL-3	47.531	0.565	0.425	23.034	0.501	0.206
IL-4	9.493	2.686	0.607	2.411	0.479	0.154
IL-5	1.677	0.043	0.277	-0.301	0.013	-0.050
IL-6	4.554	0.491	0.104	3.810	0.375	0.087
IL-7	40.178	1.299	2.733	19.409	3.243	1.320
IL-10	10.304	0.421	0.392	3.323	1.002	0.126
IL-13	8.677	0.763	0.203	6.855	1.095	0.161
IL-12p70	2.599	0.228	0.214	0.846	0.211	0.070
IL-16	115.017	5.718	1.156	104.869	8.519	1.054
IL-17	33.365	1.054	0.311	29.288	5.408	0.273
IL-23	37.992	0.015	0.812	32.568	1.981	0.696
IL-27	13.614	1.388	0.525	7.515	0.559	0.290
IP-20	127.386	6.916	1.343	111.910	4.192	1.180
I-TAC	18.324	10.508	1.525	4.412	1.817	0.367
KC	22.240	0.293	0.200	1.918	0.146	0.017
M-CSF	33.146	0.282	0.875	13.195	3.215	0.348
JE	133.589	4.523	1.364	134.271	3.843	1.371
MCP-5	37.644	21.203	4.667	28.269	22.963	3.505
MIG	10.631	0.233	0.443	10.626	0.476	0.443
MIP-1α	61.942	0.710	0.450	32.127	0.808	0.234
MIP-1β	58.897	0.332	0.581	42.223	2.220	0.417
MIP-2	46.325	0.477	0.352	11.997	0.605	0.091
RANTES	85.271	1.199	0.700	48.638	1.066	0.399
SDF-1	60.584	4.829	0.685	59.313	0.689	0.671
TARC	9.118	0.339	1.164	7.971	0.105	1.017
TIMP-1	26.988	0.457	1.111	30.465	0.970	1.254
TNF-α	60.914	1.692	0.561	54.750	2.111	0.504

TREM-1	59.767	1.836	2.566	51.306	0.783	2.203
REF	135.487	1.611	0.964	137.360	1.259	0.978

D.

Cytokine	PRI-2191					
	Day 21			Day 33		
	Mean	SD	Factor	Mean	SD	Factor
BLC	2.382	0.446	0.099	2.220	0.082	0.092
C5/C5a	1.397	0.030	0.166	0.704	0.435	0.084
G-CSF	2.921	0.240	0.058	1.369	0.646	0.027
GM-CSF	13.741	0.917	0.141	3.130	0.062	0.032
I-309	2.824	0.249	0.099	3.283	0.199	0.115
Eotaxin	1.205	0.573	0.529	0.543	0.230	0.239
sICAM-1	21.056	0.353	0.227	24.149	0.798	0.260
IFN-γ	5.598	1.228	0.106	4.220	0.375	0.080
IL-1α	4.641	0.054	0.075	8.418	1.054	0.135
IL-1β	49.956	0.474	1.019	23.402	0.065	0.478
IL-1ra	126.837	7.783	1.105	121.442	6.689	1.058
IL-2	71.661	5.564	0.563	6.346	1.378	0.050
IL-3	43.191	0.405	0.386	2.682	0.710	0.024
IL-4	2.643	0.250	0.169	1.153	0.267	0.074
IL-5	0.221	0.110	0.036	-0.236	0.397	-0.039
IL-6	2.147	0.318	0.049	0.695	0.306	0.016
IL-7	24.555	3.367	1.670	3.358	0.101	0.228
IL-10	10.249	0.762	0.390	1.105	0.172	0.042
IL-13	3.331	0.152	0.078	1.877	0.021	0.044
IL-12p70	2.075	0.108	0.171	1.002	0.133	0.082
IL-16	78.181	0.857	0.786	95.354	6.852	0.958
IL-17	7.725	1.363	0.072	3.225	0.928	0.030
IL-23	17.526	2.184	0.375	10.943	2.028	0.234
IL-27	3.605	0.985	0.139	1.714	0.460	0.066
IP-20	128.356	1.276	1.353	28.399	0.038	0.299
I-TAC	3.305	2.157	0.275	1.534	0.143	0.128
KC	9.330	0.781	0.084	1.403	0.151	0.013
M-CSF	15.589	0.866	0.412	4.260	0.413	0.112
JE	138.875	1.716	1.418	88.865	4.107	0.907
MCP-5	11.493	6.698	1.425	2.180	1.597	0.270
MIG	2.702	0.726	0.113	1.910	0.414	0.080
MIP-1α	83.954	2.666	0.610	9.821	2.130	0.071
MIP-1β	73.258	1.255	0.723	7.792	1.104	0.077
MIP-2	51.434	0.275	0.391	3.847	0.584	0.029
RANTES	129.670	4.036	1.065	4.446	0.122	0.037
SDF-1	39.605	6.540	0.448	38.925	1.907	0.440
TARC	5.324	0.554	0.680	2.128	0.224	0.272
TIMP-1	5.970	0.411	0.246	4.093	0.076	0.168
TNF-α	52.882	0.093	0.487	7.961	0.772	0.073
TREM-1	34.659	0.778	1.488	37.152	0.299	1.595

REF	143.102	2.142	1.018	141.605	2.461	1.008
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E.

Cytokine	PRI-2205					
	Day 21			Day 33		
	Mean	SD	Factor	Mean	SD	Factor
BLC	3.735	0.036	0.155	2.766	0.434	0.115
C5/C5a	3.060	0.379	0.364	1.321	0.469	0.157
G-CSF	3.050	0.272	0.060	0.417	0.098	0.008
GM-CSF	7.629	0.124	0.078	5.018	0.469	0.051
I-309	1.841	0.459	0.065	1.201	0.039	0.042
Eotaxin	0.982	0.129	0.431	-1.242	0.321	-0.545
sICAM-1	36.483	0.395	0.393	22.499	0.110	0.242
IFN- γ	3.627	0.257	0.069	1.634	0.577	0.031
IL-1 α	10.298	1.227	0.166	2.724	0.721	0.044
IL-1 β	40.951	0.467	0.836	33.414	2.347	0.682
IL-1ra	121.343	3.577	1.057	102.794	0.736	0.896
IL-2	26.605	3.942	0.209	42.834	2.633	0.337
IL-3	4.897	0.593	0.044	13.702	0.957	0.123
IL-4	2.651	0.216	0.169	0.835	0.482	0.053
IL-5	1.368	0.132	0.226	-0.303	0.032	-0.050
IL-6	1.256	0.108	0.029	-0.121	0.191	-0.003
IL-7	13.928	5.160	0.947	7.919	3.287	0.539
IL-10	1.886	0.108	0.072	0.359	0.036	0.014
IL-13	1.932	0.018	0.045	0.809	0.246	0.019
IL-12p70	1.606	0.039	0.132	0.248	0.177	0.020
IL-16	85.388	1.729	0.858	78.826	2.741	0.792
IL-17	3.931	0.271	0.037	6.251	0.552	0.058
IL-23	8.259	1.692	0.177	13.252	0.078	0.283
IL-27	4.757	1.199	0.183	2.497	0.541	0.096
IP-20	90.386	0.190	0.953	126.311	1.622	1.332
I-TAC	2.355	0.602	0.196	2.863	2.019	0.238
KC	5.457	0.110	0.049	0.520	0.108	0.005
M-CSF	6.662	0.015	0.176	3.992	0.865	0.105
JE	123.732	0.270	1.263	133.561	3.678	1.363
MCP-5	2.731	1.761	0.339	11.038	10.554	1.368
MIG	1.606	0.227	0.067	1.165	0.049	0.049
MIP-1 α	23.294	1.662	0.169	12.280	0.373	0.089
MIP-1 β	11.896	0.218	0.117	21.272	0.694	0.210
MIP-2	14.046	0.075	0.107	2.650	0.457	0.020
RANTES	48.356	1.208	0.397	50.628	0.052	0.416
SDF-1	44.500	2.239	0.503	21.742	0.425	0.246
TARC	3.736	0.339	0.477	6.003	1.320	0.766
TIMP-1	4.959	1.086	0.204	3.856	1.096	0.159
TNF- α	30.002	1.435	0.276	15.887	0.671	0.146
TREM-1	40.215	1.474	1.727	44.962	0.285	1.931

REF	134.553	0.662	0.958	134.718	1.594	0.959
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Spleen specimens were collected on the days 0, 7, 21 and 33 (after inoculation with 4T1 cells) from mice treated with calcitriol or its analogs and control group receiving vehicle and stimulated with Concanavalin A (ConA). Results were obtained using *Proteome Profiler Mouse Cytokine Array Kit. Panel A* (R&D alp Systems. Inc. USA) according to the enclosed instruction. This array detects 40 mouse cytokines, chemokines, and acute phase proteins simultaneously. Pixel densities on developed X-ray film were collected using a multifunctional scanning device (Samsung SLC460) or Image Station 4000MM PRO (Carestream Health. Rochester. New York. USA) and image analysis software (ImageJ 1.48v). For each spot the final optical density level was determined as a factor acquired by subtracting the background optical level and dividing by values obtained from the untreated mice (Day 0).

Table S5. The fold change values of genes associated with precursor T cells differentiation in mouse lymph node samples from 4T1 tumor bearing mice treated with calcitriol or its analogs.

LYMPH NODES						
Gene	Calcitriol		PRI-2191		PRI-2205	
	D14	D28	D14	D28	D14	D28
<i>Asb2</i>	0.775	2.091	1.100	2.101	1.205	2.058
<i>Cacna1f</i>	2.292	1.937	1.057	1.933	4.895	2.025
<i>Ccl11</i>	0.749	1.002	0.612	0.931	0.727	0.731
<i>Ccl5</i>	1.371	1.141	1.676	1.165	1.636	0.782
<i>Ccl7</i>	2.497	1.784	3.229	1.109	3.220	1.666
<i>Ccr3</i>	0.718	0.417	0.707	0.527	0.969	0.491
<i>Ccr4</i>	0.726	0.806	0.706	0.860	0.698	0.585
<i>Ccr6</i>	0.914	0.607	0.798	0.792	1.036	0.738
<i>Cebpb</i>	1.025	3.788	0.927	2.456	0.883	4.005
<i>Chd7</i>	1.397	2.364	1.203	1.587	1.785	2.530
<i>Csf2</i>	0.417	2.909	0.389	1.214	6.436	4.031
<i>Fasl</i>	1.153	1.299	0.755	1.190	0.749	1.096
<i>Fosl1</i>	1.811	1.635	0.363	3.318	1.448	8.437
<i>Foxp3</i>	1.287	1.800	1.788	1.772	1.422	2.380
<i>Gata3</i>	0.951	1.128	1.487	1.211	1.590	1.234
<i>Gata4</i>	1.310	2.203	0.535	1.422	3.239	3.145
<i>Gfi1</i>	1.297	1.343	1.192	0.930	1.021	1.899
<i>Ptgdr2</i>	2.236	3.174	1.507	0.747	1.406	2.371
<i>Havcr2</i>	0.976	0.666	0.590	1.008	0.941	1.077
<i>Hopx</i>	0.663	1.243	0.776	1.010	1.093	1.236
<i>Hoxa10</i>	0.195	1.356	0.176	1.540	0.284	2.526
<i>Hoxa3</i>	0.604	2.118	0.342	0.685	0.893	1.187
<i>Icos</i>	0.521	1.009	0.466	0.992	0.728	0.618
<i>Id2</i>	0.918	1.076	0.984	0.987	0.967	0.913
<i>Ifng</i>	1.300	1.104	1.516	1.323	1.492	1.351
<i>Igsf6</i>	1.564	0.917	1.100	0.755	1.381	1.210
<i>Ikzf2</i>	0.623	0.770	0.533	0.668	0.896	0.581

<i>Il12b</i>	0.695	0.945	0.991	0.816	2.235	0.844
<i>Il12rb2</i>	0.762	0.681	0.588	0.663	1.153	0.812
<i>Il13</i>	1.251	2.119	1.885	2.513	3.717	2.414
<i>Il13ra1</i>	1.607	0.777	1.484	0.944	1.152	1.069
<i>Il17a</i>	0.703	0.353	0.600	0.683	0.759	0.986
<i>Il17re</i>	1.641	1.221	2.015	1.573	1.544	1.148
<i>Il18</i>	1.692	1.710	1.353	1.123	1.521	1.336
<i>Il18r1</i>	1.204	1.230	1.122	0.934	1.053	1.029
<i>Il18rap</i>	1.082	1.317	0.589	0.672	0.968	1.508
<i>Il1r1</i>	0.575	0.837	0.827	0.764	0.703	0.848
<i>Il1r2</i>	1.458	3.719	1.821	0.974	1.628	3.206
<i>Il1r1l1</i>	0.506	0.718	0.709	0.976	0.844	0.761
<i>Il2</i>	2.488	0.723	1.564	0.667	2.298	1.428
<i>Il21</i>	1.910	1.165	1.100	0.682	2.784	1.242
<i>Il2ra</i>	1.035	0.934	1.086	0.871	1.341	0.733
<i>Il4</i>	2.083	1.665	1.804	1.380	2.069	0.860
<i>Il4ra</i>	1.734	2.054	1.365	1.676	1.345	1.735
<i>Il5</i>	0.567	1.701	0.371	0.840	2.141	2.642
<i>Il9</i>	0.215	3.524	0.111	4.618	0.429	5.840
<i>Irf1</i>	1.046	1.099	1.068	0.985	0.967	0.885
<i>Irf4</i>	0.739	1.165	0.747	0.776	1.141	0.654
<i>Irf8</i>	1.283	1.395	1.070	1.165	1.005	0.927
<i>Jak1</i>	1.514	1.038	1.476	1.043	1.323	1.063
<i>Lrrc32</i>	0.904	2.533	0.758	1.657	0.772	1.642
<i>Maf</i>	1.097	1.386	1.022	1.170	0.922	1.237
<i>Myb</i>	1.215	0.888	1.289	0.857	1.577	0.727
<i>Nfatc1</i>	1.381	1.007	1.261	0.933	1.412	0.970
<i>Nfatc2</i>	0.938	0.961	0.995	0.918	0.827	0.885
<i>Nfatc2ip</i>	1.528	1.342	1.439	1.098	1.268	1.047
<i>Nr4a1</i>	1.624	1.431	1.592	1.061	1.384	1.027
<i>Nr4a3</i>	0.235	0.930	0.206	0.734	0.208	1.081
<i>Perp</i>	0.981	1.163	1.693	0.591	0.845	1.408
<i>Pkd2</i>	0.828	1.424	0.775	1.284	0.901	1.644
<i>Pou2f2</i>	1.429	1.462	1.456	1.105	1.397	0.976
<i>Pparg</i>	0.635	1.240	0.904	1.053	0.610	1.032
<i>Rel</i>	1.834	1.102	1.565	0.834	1.451	0.834
<i>Rora</i>	1.603	1.238	1.792	1.012	1.200	1.043
<i>Rorc</i>	1.159	1.515	1.361	1.278	1.566	1.829
<i>Runx1</i>	1.373	1.035	1.266	1.027	1.345	1.097
<i>Runx3</i>	0.687	1.466	0.660	1.180	0.696	1.171
<i>Socs1</i>	5.505	1.069	1.424	1.211	1.531	1.079
<i>Socs5</i>	2.017	0.794	1.521	1.093	1.203	1.245
<i>Stat1</i>	1.656	0.722	2.029	0.833	1.841	0.768
<i>Stat4</i>	0.929	1.478	1.052	1.066	1.154	0.849
<i>Stat6</i>	1.647	1.422	1.119	1.200	1.117	1.142
<i>Tbx21</i>	0.404	1.571	0.296	0.811	0.770	1.193

<i>Tgfb1</i>	1.452	0.743	0.940	0.664	1.092	1.285
<i>Tlr4</i>	1.469	0.736	1.030	0.768	0.944	1.151
<i>Tlr6</i>	0.550	1.306	0.675	0.873	0.650	1.290
<i>Tmed1</i>	0.881	1.407	0.976	1.154	0.796	1.321
<i>Tnf</i>	0.506	0.997	0.503	1.374	0.399	0.985
<i>Tnfrsf9</i>	0.963	1.283	1.083	1.067	1.505	1.006
<i>Tnfsf11</i>	1.552	0.820	1.164	0.877	1.560	0.480
<i>Trp53inp1</i>	0.674	0.921	0.545	0.679	0.712	0.863
<i>Uts2</i>	3.795	3.326	0.644	2.161	5.583	3.080
<i>Zbtb7b</i>	1.560	0.916	1.459	0.910	1.585	1.120
<i>Zeb1</i>	2.058	2.361	1.918	2.349	1.862	3.430

Lymph node samples were collected from mice treated with calcitriol or its analogs and control group receiving vehicle on the days 21 and 28 (after inoculation with 4T1 cells). Real-time PCR screening was performed using *Mouse T Helper Cell Differentiation RT² Profiler Array* (Qiagen. Hilden. Germany) including 84 key genes and 5 housekeeping genes in the set (Table S1). Data presented as a mean relative quantification (RQ) values. Fold-change (RQ) of target cDNA was defined using double delta Ct method in reference to beta-2 microglobulin (*B2m*), glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) and heat shock protein 90 alpha, class B member 1 (*Hsp90ab1*). Next the results were adjusted to the values obtained for the control group within the day 14 or 28th of the experiment for each treatment group. Data analysis was acquired using Qiagen online software suitable for purchased kit (Qiagen. Hilden. Germany). PCR amplification cycles were as follows 95 °C for 10 s and 58 °C for 45 s (50 cycles). We used 0.5 µg of cDNA (6 mice pooled per group) for a single reaction.

Table S6. The expression of genes associated with tumor invasion or metastasis evaluated in 4T1 lung tissue after treatment with calcitriol or its analogs.

GENE	Control		Calcitriol		PRI-2191		PRI-2205	
	D14	D28	D14	D28	D14	D28	D14	D28
<i>Adamts1</i>	0.1021	6.9524	4.0763	0.2026	2.6365	0.3105	0.2591	0.1217
<i>Aldh3a1</i>	0.1081	3.4041	5.2648	0.1759	1.8660	0.4573	0.0288	0.1350
<i>Angpt1</i>	0.0175	5.3426	14.3275	0.4190	0.8559	0.0481	1.7566	0.0040
<i>Angpt14</i>	0.0717	2.8635	1.6292	0.1172	0.1740	0.0533	0.1094	0.0794
<i>Casp8</i>	0.1609	4.4852	17.1495	0.1560	0.8823	0.5039	0.2987	0.4391
<i>Cdh2</i>	0.1950	4.4482	3.0244	0.0991	0.7191	1.3455	1.5076	0.0743
<i>Cdh6</i>	0.6451	4.0217	11.8376	0.3184	2.3374	0.3950	1.1163	0.9422
<i>Col4a2</i>	0.2231	14.0953	9.9344	2.2378	2.1428	0.2715	0.8251	1.1612
<i>Col6a1</i>	0.5293	1.9947	1.4182	0.0779	0.4406	0.2262	0.4336	0.4766
<i>Cst7</i>	0.6212	5.4917	5.7955	15.5825	5.5213	3.8085	1.8882	13.6300
<i>Ctgf</i>	0.0301	0.5168	0.4273	0.0283	0.0541	0.0781	0.1498	0.0664
<i>Ctsb</i>	0.2542	3.1115	8.4300	0.2027	0.3488	0.1780	0.6045	1.0043
<i>Ctsl1</i>	0.0322	2.0009	3.4083	0.0294	0.0803	0.2343	0.9123	0.0512
<i>Cxcl1</i>	0.9050	9.8453	9.4692	6.1423	6.4151	3.6930	2.2256	21.3126
<i>Drg1</i>	0.0147	0.8098	0.1756	0.0353	0.0795	0.0511	0.0683	0.0875
<i>Flt1</i>	0.0923	1.6656	9.5041	0.2455	0.7573	0.3315	1.1368	0.1928
<i>Gpi</i>	0.6091	7.4262	9.5362	24.3252	4.8662	4.1971	1.8743	10.1971
<i>Gsn</i>	0.9534	6.6025	9.7069	1.0058	4.4323	0.5571	1.6766	0.8818
<i>Id1</i>	0.3805	4.7843	13.5532	0.3334	1.2883	0.4716	0.5435	0.3495

<i>Isg20</i>	0.1123	1.8314	7.3532	0.0350	0.2886	0.1615	0.2314	0.1935
<i>Map2k4</i>	0.1032	3.2856	1.4784	0.1501	0.5141	0.3991	0.0467	0.2073
<i>Mcam</i>	0.0836	0.2120	0.4795	0.0286	0.1504	0.0245	0.3727	0.0273
<i>Metap2</i>	14.3950	9.1724	9.7487	4.6765	9.9921	0.0137	35.8166	36.8244
<i>Mmp11</i>	0.7830	2.7765	22.9619	0.3264	2.9887	0.3795	1.1443	0.7011
<i>Mmp13</i>	0.0420	1.7269	0.7483	0.1373	0.2746	3.5925	0.0657	0.3389
<i>Mmp14</i>	1.0173	1.6715	7.7031	0.1365	1.4630	0.0857	0.4762	1.0691
<i>Mmp2</i>	0.1803	0.7334	12.0421	0.1514	0.4155	0.0196	0.5275	0.1447
<i>Nedc9</i>	2.7636	8.6074	1.0877	0.0755	0.2595	0.0271	0.3969	0.1664
<i>Nf2</i>	0.1619	9.4709	1.4281	11.2397	0.2890	0.1596	0.2174	0.1802
<i>Nme2</i>	0.6354	9.8238	20.6674	1.0054	1.9418	0.2143	0.0024	0.0029
<i>Pax5</i>	0.8642	21.6493	9.1668	1.5501	6.4063	0.2890	1.7699	1.6823
<i>Pdgfa</i>	0.2329	21.1707	5.2079	0.2951	1.1809	0.0557	0.3843	0.6058
<i>Plaur</i>	0.1704	9.6868	0.8387	0.4348	0.1407	0.0939	0.2691	0.5652
<i>Serpin1</i>	0.76645	35.42515	6.8383	2.2784	1.6462	0.14405	1.1322	3.2355
<i>Sparc</i>	0.04625	0.1872	3.94765	0.02625	0.1818	0.0016	0.0625	0.0975
<i>Spp1</i>	0.14845	0.4796	0.4975	0.162	0.721	2.6722	2.266	2.2923
<i>Src</i>	0.1781	1.7142	2.14515	0.1282	0.3674	0.0278	0.2587	0.3649
<i>Tgfb1</i>	0.287	1.49485	0.952	0.2028	1.03565	0.44	0.1180	0.4342
<i>Timp1</i>	0.1075	32.78185	0.32545	2.77745	0.2614	0.41765	0.2206	1.0214
<i>Timp2</i>	0.1829	0.13365	10.02855	2.58465	0.0857	0.10945	0.1581	0.0648
<i>Timp3</i>	0.3003	0.1151	26.33775	0.0773	0.55415	0.0976	0.0228	0.0862
<i>Timp4</i>	0.3497	0.21445	0.62505	0.12745	1.1912	0.09305	0.1347	0.0988
<i>Vegfa</i>	0.64185	2.3577	10.97125	2.1168	0.9339	0.2302	0.526	0.229

Lung specimens were collected on the days 14 and 28 (after inoculation with 4T1 cells) from mice treated with calcitriol or its analogs and control group receiving vehicle. Real-time PCR screening was performed using the mouse tumor invasion/metastasis PCR array library (MTIM-1). From 88 genes available in this array (Table S2) the expression for 45 genes was not detected in 4T1 lung tissue. Data presented as a mean relative quantification (RQ) values (calculated from duplicate). Fold-change (RQ) of target cDNA was determined by calculating the differences in $\Delta\Delta CT$ values in reference to ribosomal protein L13A (*Rpl13a*) and adjusted to the values obtained for the untreated mice (named D0) for each treatment group. Data analysis was performed by DataAssist v 3.01 software. All PCR amplification cycles were performed at 95 °C for 10 s and 58 °C for 45 s (50 cycles). We used 25 ng of cDNA for a single reaction and each test was performed in duplicate.

Table S7. The number of lung metastases in mice bearing 4T1 mammary gland cancer cells and treated with calcitriol or its analogs: PRI-2191 and PRI-2205.

Day of experiment	No of metastases [median (min-max)]			
	Control	Calcitriol	PRI-2191	PRI-2205
D21	3 (1.0-4.5)	3 (0-6.0)	3 (0.5-6.0)	3.5 (0.5-12.0)*
D28	11 (3.0-68.0)	14 (6.0-29.0)	25 (13.0-60.0)*	17.25 (0-83.0)

D33	12 (5.0-25.5)	18 (3.0-34.0)	18.5 (4.5-63.0)	19 (4.0-44.0)
Day of experiment	Score for metastases [median (min-max)]			
	Control	Calcitriol	PRI-2191	PRI-2205
D14	0 (0-1)	0 (0-0)	0.5 (0-1)	0 (0-1)
D21	1 (0-2)	1 (1-1)	1 (0-2)	1 (0-1)
D28	2 (1-4)	2 (1-3)	3 (1-4)	4 (1-4)
D33	1 (0-4)	4 (4-4)*	4 (3-4)*	4 (3-4)*

*p<0.05. Statistical analysis: Kruskal-Wallis multiple comparison test. Number of lung metastases was counted in lungs macroscopically by two independent examiners. Score for metastases was performed by pathologist in H&E stained lungs on days 14, 21, 28 and 33: 0, no metastasis detected; +, 1-3 metastatic foci; ++, 4-7 foci; +++, 8-10 foci; +++, >10 metastatic foci in lungs. Mice were inoculated orthotopically with 4T1 cells on day 0. From day 7 (7 days after tumor inoculation), vitamin D analogs were administered subcutaneously (s.c.) thrice a week. The single dose of compounds were as follows: calcitriol, 0.5 µg/kg; PRI-2191, 1.0 µg/kg; and PRI-2205, 10.0 µg/kg. Number of mice: 9-13 per group. [Data from: Anisiewicz, A. *et al.* Unfavorable effect of calcitriol and its low-calcemic analogs on metastasis of 4T1 mouse mammary gland cancer. *Int. J. Oncol.* **52**, 103–126 (2018)].

Table S8. List of genes included in Mouse T Helper Cell Differentiation RT² Profiler Array.

Symbol	Description
<i>Asb2</i>	Ankyrin repeat and SOCS box-containing 2
<i>Cacna1f</i>	Calcium channel, voltage-dependent, alpha 1F subunit
<i>Ccl11</i>	Chemokine (C-C motif) ligand 11
<i>Ccl5</i>	Chemokine (C-C motif) ligand 5
<i>Ccl7</i>	Chemokine (C-C motif) ligand 7
<i>Ccr3</i>	Chemokine (C-C motif) receptor 3
<i>Ccr4</i>	Chemokine (C-C motif) receptor 4
<i>Ccr6</i>	Chemokine (C-C motif) receptor 6
<i>Cebpb</i>	CCAAT/enhancer binding protein (C/EBP), beta
<i>Chd7</i>	Chromodomain helicase DNA binding protein 7
<i>Csf2</i>	Colony stimulating factor 2 (granulocyte-macrophage)
<i>Fasl</i>	Fas ligand (TNF superfamily, member 6)
<i>Fosl1</i>	Fos-like antigen 1
<i>Foxp3</i>	Forkhead box P3
<i>Gata3</i>	GATA binding protein 3
<i>Gata4</i>	GATA binding protein 4
<i>Gfi1</i>	Growth factor independent 1
<i>Ptgdr2</i>	G protein-coupled receptor 44
<i>Havcr2</i>	Hepatitis A virus cellular receptor 2
<i>Hopx</i>	HOP homeobox
<i>Hoxa10</i>	Homeobox A10
<i>Hoxa3</i>	Homeobox A3

<i>Icos</i>	Inducible T-cell co-stimulator
<i>Id2</i>	Inhibitor of DNA binding 2
<i>Ifng</i>	Interferon gamma
<i>Igsf6</i>	Immunoglobulin superfamily, member 6
<i>Ikzf2</i>	IKAROS family zinc finger 2
<i>Il12b</i>	Interleukin 12B
<i>Il12rb2</i>	Interleukin 12 receptor, beta 2
<i>Il13</i>	Interleukin 13
<i>Il13ra1</i>	Interleukin 13 receptor, alpha 1
<i>Il17a</i>	Interleukin 17A
<i>Il17re</i>	Interleukin 17 receptor E
<i>Il18</i>	Interleukin 18
<i>Il18r1</i>	Interleukin 18 receptor 1
<i>Il18rap</i>	Interleukin 18 receptor accessory protein
<i>Il1r1</i>	Interleukin 1 receptor, type I
<i>Il1r2</i>	Interleukin 1 receptor, type II
<i>Il1rl1</i>	Interleukin 1 receptor-like 1
<i>Il2</i>	Interleukin 2
<i>Il21</i>	Interleukin 21
<i>Il2ra</i>	Interleukin 2 receptor, alpha chain
<i>Il4</i>	Interleukin 4
<i>Il4ra</i>	Interleukin 4 receptor, alpha
<i>Il5</i>	Interleukin 5
<i>Il9</i>	Interleukin 9
<i>Irf1</i>	Interferon regulatory factor 1
<i>Irf4</i>	Interferon regulatory factor 4
<i>Irf8</i>	Interferon regulatory factor 8
<i>Jak1</i>	Janus kinase 1
<i>Lrrc32</i>	Leucine rich repeat containing 32
<i>Maf</i>	Avian musculoaponeurotic fibrosarcoma (v-maf) AS42 oncogene homolog
<i>Myb</i>	Myeloblastosis oncogene
<i>Nfatc1</i>	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1
<i>Nfatc2</i>	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2
<i>Nfatc2ip</i>	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2 interacting protein
<i>Nr4a1</i>	Nuclear receptor subfamily 4, group A, member 1
<i>Nr4a3</i>	Nuclear receptor subfamily 4, group A, member 3
<i>Perp</i>	PERP, TP53 apoptosis effector
<i>Pkd2</i>	Polycystic kidney disease 2
<i>Pou2f2</i>	POU domain, class 2, transcription factor 2
<i>Pparg</i>	Peroxisome proliferator activated receptor gamma
<i>Rel</i>	Reticuloendotheliosis oncogene
<i>Rora</i>	RAR-related orphan receptor alpha
<i>Rorc</i>	RAR-related orphan receptor gamma
<i>Runx1</i>	Runt related transcription factor 1
<i>Runx3</i>	Runt related transcription factor 3
<i>Socs1</i>	Suppressor of cytokine signaling 1

<i>Socs5</i>	Suppressor of cytokine signaling 5
<i>Stat1</i>	Signal transducer and activator of transcription 1
<i>Stat4</i>	Signal transducer and activator of transcription 4
<i>Stat6</i>	Signal transducer and activator of transcription 6
<i>Tbx21</i>	T-box 21
<i>Tgif1</i>	TGFB-induced factor homeobox 1
<i>Tlr4</i>	Toll-like receptor 4
<i>Tlr6</i>	Toll-like receptor 6
<i>Tmed1</i>	Transmembrane emp24 domain containing 1
<i>Tnf</i>	Tumor necrosis factor
<i>Tnfrsf9</i>	Tumor necrosis factor receptor superfamily, member 9
<i>Tnfrsf11</i>	Tumor necrosis factor (ligand) superfamily, member 11
<i>Trp53inp1</i>	Transformation related protein 53 inducible nuclear protein 1
<i>Uts2</i>	Urotensin 2
<i>Zbtb7b</i>	Zinc finger and BTB domain containing 7B
<i>Zeb1</i>	Zinc finger E-box binding homeobox 1

Table S9. List of genes evaluated using mouse tumor invasion/metastasis PCR array library (MTIM-1).

Symbol	Description
<i>Adamts1</i>	ADAM metallopeptidase
<i>Aldh3a1</i>	Aldehyde dehydrogenase 3 family, member A1
<i>Angpt1</i>	Angiopoietin 1
<i>Angptl4</i>	Angiopoietin-like 4
<i>Casp8</i>	Caspase 8, apoptosis-related cysteine peptidase
<i>Ccne2</i>	Cyclin E2
<i>Ccr7</i>	Chemokine (C-C motif) receptor 7
<i>Cd44</i>	CD44 molecule (Indian blood group)
<i>Cd82</i>	CD82 antigen
<i>Cdh1</i>	Cadherin 1, type 1, E-cadherin (epithelial)
<i>Cdh11</i>	Cadherin 11, type 2, OB-cadherin (osteoblast)
<i>Cdh2</i>	Cadherin 2, type 1, N-cadherin
<i>Cdh6</i>	Cadherin 6
<i>Cldn7</i>	Claudin 7
<i>Col1a1</i>	Collagen, type I, alpha 1
<i>Col4a2</i>	Collagen, type IV, alpha 2
<i>Col6a1</i>	Collagen, type VI, alpha 1
<i>Csf1</i>	Colony stimulating factor 1
<i>Csf2</i>	Colony stimulating factor 2
<i>Csf3</i>	Colony stimulating factor 3
<i>Cst7</i>	Cystatin F (leukocystatin)
<i>Ctgf</i>	Connective tissue growth factor
<i>Ctsb</i>	Cathepsin B
<i>Ctsd</i>	Cathepsin D
<i>Ctsk</i>	Cathepsin K
<i>Ctsl1</i>	Cathepsin L-like 3

<i>Cxcl1</i>	Chemokine (C-X-C motif) ligand 1
<i>Cxcl13</i>	Chemokine (C-X-C motif) ligand 13
<i>Cxcr4</i>	Chemokine (C-X-C motif) receptor 4
<i>Cxcr6</i>	Chemokine (C-X-C motif) receptor 6
<i>Drg1</i>	Developmentally regulated GTP binding protein 1
<i>Ereg</i>	Epiregulin
<i>Fgf8</i>	Fibroblast growth factor 8 (androgen-induced)
<i>Flt1</i>	Fms-related tyrosine kinase
<i>Flt4</i>	Fms-related tyrosine kinase 4
<i>Gpi</i>	Glucose phosphate isomerase
<i>Gsn</i>	Gelsolin (amyloidosis, Finnish type)
<i>Hgf</i>	Hepatocyte growth factor (hepapoietin A; scatter factor)
<i>Hif1a</i>	Hypoxia inducible factor 1, alpha subunit
<i>Hmgb1</i>	High-mobility group box 1
<i>Id1</i>	Inhibitor of DNA binding 1
<i>Il13ra2</i>	Interleukin 13 receptor, alpha 2
<i>Isg20</i>	Interferon stimulated exonuclease gene 20kDa
<i>Jag1</i>	Jagged 1 (Alagille syndrome)
<i>Kiss1</i>	KiSS-1 metastasis-suppressor
<i>Klrc2</i>	Killer cell lectin-like receptor subfamily C, member 2
<i>Kynu</i>	Kynureninase (L-kynurenine hydrolase)
<i>Ltbp1</i>	Latent transforming growth factor beta binding protein 1
<i>Map2k4</i>	Mitogen-activated protein kinase kinase 4
<i>Map2k5</i>	Mitogen-activated protein kinase kinase 5
<i>Map2k7</i>	Mitogen-activated protein kinase kinase 7
<i>Mcam</i>	Melanoma cell adhesion molecule
<i>Met</i>	Met proto-oncogene (hepatocyte growth factor receptor)
<i>Metap2</i>	Methionyl aminopeptidase 2
<i>Mmp1</i>	Matrix metalloproteinase 1 (interstitial collagenase)
<i>Mmp10</i>	Matrix metalloproteinase 10 (stromelysin 2)
<i>Mmp11</i>	Matrix metalloproteinase 11 (stromelysin 3)
<i>Mmp13</i>	Matrix metalloproteinase 13 (collagenase 3)
<i>Mmp14</i>	Matrix metalloproteinase 14 (membrane-inserted)
<i>Mmp2</i>	Matrix metalloproteinase 2
<i>Mmp7</i>	Matrix metalloproteinase 7 (matrilysin, uterine)
<i>Myc</i>	V-myc myelocytomatosis viral oncogene homolog
<i>Nedd9</i>	Neural precursor cell expressed, dev. down-regulated 9
<i>Nf2</i>	Neurofibromin 2 (merlin)
<i>Nme1</i>	Non-metastatic cells 1, protein (NM23A)
<i>Nme2</i>	Non-metastatic cells 2, protein
<i>Nme4</i>	Non-metastatic cells 4, protein
<i>Pax5</i>	Paired box 5
<i>Pdgfa</i>	Platelet-derived growth factor alpha polypeptide
<i>Plaur</i>	Plasminogen activator, urokinase receptor
<i>Ptgs2</i>	Prostaglandin-endoperoxide synthase 2
<i>Runx1</i>	Runt-related transcription factor 1
<i>Serpine1</i>	Serpin peptidase inhibitor, clade E
<i>Serpinb5</i>	Serpin peptidase inhibitor, clade B5

<i>Sox4</i>	SRY (sex determining region Y)-box 4
<i>Sparc</i>	Secreted protein, acidic, cysteine-rich (osteonectin)
<i>Spp1</i>	Secreted phosphoprotein 1
<i>Src</i>	V-src sarcoma viral oncogene homolog (avian)
<i>Tff1</i>	Trefoil factor 1
<i>Tgfb1</i>	Transforming growth factor, beta 1
<i>Timp1</i>	TIMP metalloproteinase inhibitor 1
<i>Timp2</i>	TIMP metalloproteinase inhibitor 2
<i>Timp3</i>	TIMP metalloproteinase inhibitor 3
<i>Timp4</i>	TIMP metalloproteinase inhibitor 4
<i>Tnc</i>	Tenascin C (hexabrachion)
<i>Tp53</i>	Tumor protein p53
<i>Vegfa</i>	Vascular endothelial growth factor A
<i>Vegfb</i>	Vascular endothelial growth factor B
<i>Actb</i>	Actin, beta
<i>B2m</i>	Beta-2-microglobulin
<i>Gapd</i>	Glyceraldehyde-3-phosphate dehydrogenase
<i>Gusb</i>	Glucuronidase, beta
<i>Hprt1</i>	Hypoxanthine phosphoribosyltransferase 1
<i>Pgk</i>	Phosphoglycerate kinase 1
<i>Ppia</i>	Peptidylprolyl isomerase A
<i>Rpl13a</i>	Ribosomal protein L13a