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\textsuperscript{(SCC\textsubscript{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\textsuperscript{SCC\textsubscript{high}} cell population, all compounds, but only PRI-2205 in a significant manner, decreased the percentage of CD54\textsuperscript{+} cells (Figure S1B). PRI-2205 also significantly decreased the percentage of CD184\textsuperscript{+} cells within Ly6G-6C\textsuperscript{SCC\textsubscript{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 µg/kg; PRI-2191, 1.0 µg/kg; and PRI-2205, 10.0 µg/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 ± 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 µg/kg; PRI-2191, 1.0 µg/kg; and PRI-2205, 10.0 µg/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 lipopolysaccharide (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 ± 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-1β. (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 ± 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 ΔΔCt 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 µg/kg; PRI-2191, 1.0 µg/kg; and PRI-2205, 10.0 µg/kg. Number of mice: 9–12 per group. Data are presented as mean ± 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) Ly6G-6C⁺SSC\textsuperscript{high} mature granulocytes. (B) Ly6G-6C⁺CD54⁺SSC\textsuperscript{high} (C) Ly6G-6C⁺CD184⁺SSC\textsuperscript{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 SSC\textsuperscript{high} 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 CD3ε⁺. (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      |
| Asb2   | 2.403      | 0.399    | 2.244    | 0.736    | 1.734    | 0.172    |
| Cacna1f| 1.127      | 1.006    | 1.389    | 0.645    | 0.805    | 0.357    |
| Ccl11  | 1.877      | 3.821    | 37.502   | 0.891    | 8.350    | 2.973    |
| Ccl5   | 1.835      | 0.586    | 0.950    | 1.790    | 1.092    | 0.623    |
| Ccl7   | 20.283     | 0.421    | 718.457  | 0.848    | 22.735   | 1.586    |
| Ccr3   | 2.667      | 0.654    | 1.097    | 0.687    | 2.940    | 0.378    |
| Ccr4   | 1.275      | 0.564    | 7.324    | 1.174    | 0.613    | 1.355    |
| Ccr6   | 1.426      | 0.325    | 3.600    | 1.630    | 0.442    | 0.921    |
| Cebpb  | 2.531      | 1.580    | 0.083    | 0.575    | 1.444    | 0.102    |
| Chd7   | 1.914      | 1.520    | 4.638    | 0.509    | 0.721    | 0.153    |
| Csf2   | 4.144      | 1.546    | 26.864   | 4.072    | 20.050   | 1.746    |
| Fasl   | 1.314      | 0.900    | 12.786   | 1.173    | 1.175    | 1.293    |
| Fosl1  | 2.358      | 1.023    | 1.893    | 0.706    | 1.974    | 0.866    |
| Foxp3  | 2.958      | 1.096    | 8.829    | 0.896    | 1.304    | 0.681    |
| Gata3  | 2.038      | 0.817    | 1.455    | 0.418    | 0.972    | 0.289    |
| Gata4  | 0.718      | 0.784    | 37.502   | 1.226    | 1.249    | 3.015    |
| Gfi1   | 0.547      | 1.851    | 0.322    | 0.357    | 0.305    | 1.817    |
| Ptgdr2 | 4.452      | 0.554    | 26.461   | 0.647    | 3.382    | 0.404    |
| Havcr2 | 2.714      | 1.288    | 4.150    | 0.948    | 1.377    | 1.249    |
| Hopx   | 1.271      | 1.495    | 6.179    | 0.737    | 1.921    | 2.957    |
| Hoxa10 | 0.377      | 6.067    | 5.354    | 1.882    | 0.144    | 2.556    |
| Hoxa3  | 0.646      | 3.653    | 11.007   | 1.226    | 0.367    | 10.585   |
| Icos   | 1.277      | 0.352    | 1.031    | 0.962    | 0.186    | 0.071    |
| Id2    | 1.246      | 1.681    | 0.785    | 0.746    | 0.750    | 1.604    |
| Ifng   | 2.876      | 0.981    | 2.366    | 1.601    | 2.248    | 0.927    |
| Igsf6  | 0.861      | 1.243    | 0.958    | 0.808    | 0.994    | 1.458    |
| Ikzf2  | 2.388      | 0.673    | 2.746    | 1.049    | 0.941    | 0.506    |
| Il12b  | 6.414      | 0.081    | 37.502   | 2.584    | 8.184    | 0.312    |
| Il12rb2| 3.963      | 0.554    | 20.624   | 0.280    | 0.772    | 0.404    |
| Il13   | 0.718      | 2.517    | 37.502   | 3.844    | 1.249    | 3.724    |
| Il13ra1| 1.651      | 0.966    | 1.598    | 0.709    | 1.314    | 1.015    |
| Il17a  | 0.718      | 0.784    | 37.502   | 1.226    | 1.249    | 3.015    |
| Il17re | 1.424      | 1.964    | 8.803    | 1.013    | 1.677    | 8.485    |
| Il18   | 1.652      | 0.495    | 9.805    | 0.887    | 3.525    | 1.815    |
| Il18r1 | 0.932      | 1.442    | 1.428    | 1.055    | 0.723    | 2.551    |
| Il18rap| 0.735      | 1.677    | 0.544    | 0.666    | 1.330    | 1.463    |
| Il1r1  | 4.356      | 0.628    | 12.066   | 0.787    | 2.024    | 0.909    |
| Il1r2  | 0.984      | 2.934    | 1.211    | 0.844    | 1.068    | 3.136    |
| Il1rl1 | 2.134      | 1.239    | 2.738    | 2.270    | 2.565    | 1.205    |
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 Mean | D0 SD | D7 Mean | D7 SD | Factor |
|----------|---------|-------|---------|-------|--------|
| 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-1α    | 6.681   | 0.017 | 2.700   | 0.503 | 0.404  |
| IL-1β    | 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-1α   | 2.425   | 1.565 | 1.688   | 0.786 | 0.696  |
| MIP-1β   | 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 | SD | Factor | D33 | SD | Factor |
|----------|---------|-----|----|--------|-----|----|--------|
| BLC      | 35.313  | 5.020 | 5.417 | 40.250 | 4.110 | 6.175 |
| CS/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-1α    | 15.951  | 0.530 | 2.387 | 8.619   | 0.124 | 1.290 |
| IL-1β    | 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-1α   | 2.200   | 0.105 | 0.907 | 0.519   | 0.442 | 0.214 |
| MIP-1β   | 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 |
| Cytokine   | D21 Mean | SD   | Factor | D33 Mean | SD   | Factor |
|------------|----------|------|--------|----------|------|--------|
| BLC        | 10.719   | 1.034| 1.644  | 43.576   | 6.249| 6.685  |
| CS/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-1α      | 9.044    | 0.981| 1.354  | 6.763    | 0.257| 1.012  |
| IL-1β      | 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-1α     | 1.082    | 0.521| 0.446  | 1.882    | 0.371| 0.776  |
| MIP-1β     | 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    | Mean D21 | SD  | Factor | Mean D33 | 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-1α       | 6.594    | 0.247 | 0.987  | 6.532    | 0.221 | 0.978  |
| IL-1β       | 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-1α      | 1.800    | 0.150 | 0.742  | 0.556    | 0.027 | 0.229  |
| MIP-1β      | 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        | D21 Mean | D21 SD | D21 Factor | D33 Mean | D33 SD | D33 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-1α           | 9.138    | 1.511  | 1.368      | 8.776    | 0.415  | 1.314      |
| IL-1β           | 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   | 0.216  | 5.847      | 53.586   | 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-1α          | 1.444    | 0.141  | 0.596      | 1.244    | 0.194  | 0.513      |
| MIP-1β          | 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.

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[15]
Mouse Cytokine Array Kit, Panel A (R&D 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).

| 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 |
| Cytokine   | Day 21 | Day 33 | Factor | Day 21 | Day 33 | Factor |
|------------|--------|--------|--------|--------|--------|--------|
| **Mean**   | **SD** | **Mean** | **SD** | **Factor** | **Mean** | **SD** | **Factor** |
| BLC        | -0.897 | 0.004  | -0.038 | 40.249 | 1.485  | 1.696  |
| CS/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  |
### C.

| Cytokine  | Day 21 | Day 33 |
|-----------|--------|--------|
| MIP-1α    | 124.862| 0.915  |
| MIP-1β    | 122.275| 0.931  |
| MIP-2     | 123.231| 0.907  |
| RANTES    | 118.067| 0.885  |
| SDF-1     | 33.734 | 0.390  |
| TARC      | 3.262  | 0.958  |
| TIMP-1    | 21.756 | 0.705  |
| TNF-α     | 83.588 | 0.699  |
| TREM-1    | 41.564 | 1.267  |
| REF       | 117.227| 0.862  |

| Cytokine  | Mean   | SD    | Factor | Mean   | SD    | Factor |
|-----------|--------|-------|--------|--------|-------|--------|
| BLC       | 14.644 | 0.342 | 0.617  | 11.067 | 0.516 | 0.466  |
| CS/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  |
| Cytokine  | 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| 5.659 | 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  |
| Cytokine  | PRI-2205 |   |   | Day 21 | Factor | Day 33 | Factor |
|-----------|----------|---|---|--------|--------|--------|--------|
|           | Mean   | SD |   |        |        | Mean   |        |
| 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  |
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 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).

|     | MIP-2 | RANTES | SDF-1 | TIMP-1 | TNF-α | TARC | REF |
|-----|-------|--------|-------|--------|-------|------|-----|
| Day 0 | 136.390 | 130.380 | 42.145 | 3.643 | 109.950 | 49.217 | 136.316 |
| Day 7 | 1.242 | 5.392 | 5.669 | 0.454 | 3.451 | 5.267 | 3.157 |
| Day 21 | 1.003 | 0.977 | 0.487 | 1.070 | 0.919 | 1.500 | 1.002 |
| Day 33 | 134.307 | 137.537 | 36.775 | 6.840 | 98.079 | 56.408 | 132.685 |
|       | 1.081 | 3.498 | 8.413 | 0.030 | 13.105 | 3.613 | 1.484 |
|       | 0.988 | 1.031 | 0.425 | 2.009 | 0.820 | 1.719 | 0.976 |
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-1α    | 62.158| 0.045 | 4.668  | 0.593 | 0.075  |
| IL-1β    | 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| 1.266 | 6.462  | 0.730 | 0.132  |
| 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-1α   | 137.543| 1.469 | 103.000| 6.341 | 0.749  |
| MIP-1β   | 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  |
| Cytokine   | Day 21 | Day 33 |
|-----------|--------|--------|
|            | Mean   | SD     | Factor | Mean   | SD     | Factor |
| BLC       | 1.964  | 0.124  | 0.082  | 9.132  | 0.640  | 0.379  |
| CS/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  |
### C.

| Cytokine | 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 |
| Cytokine     | Day 21 |      | Day 33 |      |
|--------------|--------|------|--------|------|
| BLC          | Mean   | SD   | Factor | Mean  |
|              | 2.382  | 0.446| 0.099  | 2.220 |
| C5/C5a       | 1.397  | 0.030| 0.166  | 0.704 |
| G-CSF        | 2.921  | 0.240| 0.058  | 1.369 |
| GM-CSF       | 13.741 | 0.917| 0.141  | 3.130 |
| I-309        | 2.824  | 0.249| 0.099  | 3.283 |
| Eotaxin      | 1.205  | 0.573| 0.529  | 0.543 |
| sICAM-1      | 21.056 | 0.353| 0.227  | 24.149|
| IFN-γ        | 5.598  | 1.228| 0.106  | 4.220 |
| IL-1α        | 4.641  | 0.054| 0.075  | 8.418 |
| IL-1β        | 49.956 | 0.474| 1.019  | 23.402|
| IL-1ra       | 126.837| 7.783| 1.105  | 121.442|
| IL-2         | 71.661 | 5.564| 0.563  | 6.346 |
| IL-3         | 43.191 | 0.405| 0.386  | 2.682 |
| IL-4         | 2.643  | 0.250| 0.169  | 1.153 |
| IL-5         | 0.221  | 0.110| 0.036  | -0.236|
| IL-6         | 2.147  | 0.318| 0.049  | 0.695 |
| IL-7         | 24.555 | 3.367| 1.670  | 3.358 |
| IL-10        | 10.249 | 0.762| 0.390  | 1.105 |
| IL-13        | 3.331  | 0.152| 0.078  | 1.877 |
| IL-12p70     | 2.075  | 0.108| 0.171  | 1.002 |
| IL-16        | 78.181 | 0.857| 0.786  | 95.354|
| IL-17        | 7.725  | 1.363| 0.072  | 3.225 |
| IL-23        | 17.526 | 2.184| 0.375  | 10.943|
| IL-27        | 3.605  | 0.985| 0.139  | 1.714 |
| IP-20        | 128.356| 1.276| 1.353  | 28.399|
| I-TAC        | 3.305  | 2.157| 0.275  | 1.534 |
| KC           | 9.330  | 0.781| 0.084  | 1.403 |
| M-CSF        | 15.589 | 0.866| 0.412  | 4.260 |
| JE           | 138.875| 1.716| 1.418  | 88.865|
| MCP-5        | 11.493 | 6.698| 1.425  | 2.180 |
| MIG          | 2.702  | 0.726| 0.113  | 1.910 |
| MIP-1α       | 83.954 | 2.666| 0.610  | 9.821 |
| MIP-1β       | 73.258 | 1.255| 0.723  | 7.792 |
| MIP-2        | 51.434 | 0.275| 0.391  | 3.847 |
| RANTES       | 129.670| 4.036| 1.065  | 4.446 |
| SDF-1        | 39.605 | 6.540| 0.448  | 38.925|
| TARC         | 5.324  | 0.554| 0.680  | 2.128 |
| TIMP-1       | 5.970  | 0.411| 0.246  | 4.093 |
| TNF-α        | 52.882 | 0.093| 0.487  | 7.961 |
| TREM-1       | 34.659 | 0.778| 1.488  | 37.152|
| 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  |
| CS/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-1α         | 10.298   | 1.227    | 0.166  | 2.724    | 0.721    | 0.044  |
| IL-1β         | 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-1α        | 23.294   | 1.662    | 0.169  | 12.280   | 0.373    | 0.089  |
| MIP-1β        | 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  |
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.

| Gene     | Calcitriol | PRI-2191 | PRI-2205 |
|----------|------------|----------|----------|
|          | D14 | D28 | D14 | D28 | D14 | D28 |
| Asb2     | 0.775 | 2.091 | 1.100 | 2.101 | 1.205 | 2.058 |
| Cacna1f  | 2.292 | 1.937 | 1.057 | 1.933 | 4.895 | 2.025 |
| Ccl11    | 0.749 | 1.002 | 0.612 | 0.931 | 0.727 | 0.731 |
| Ccl5     | 1.371 | 1.141 | 1.676 | 1.165 | 1.636 | 0.782 |
| Ccl7     | 2.497 | 1.784 | 3.229 | 1.109 | 3.220 | 1.666 |
| Ccr3     | 0.718 | 0.417 | 0.707 | 0.527 | 0.969 | 0.491 |
| Ccr4     | 0.726 | 0.806 | 0.706 | 0.860 | 0.698 | 0.585 |
| Ccr6     | 0.914 | 0.607 | 0.798 | 0.792 | 1.036 | 0.738 |
| Cebpb    | 1.025 | 3.788 | 0.927 | 2.456 | 0.883 | 4.005 |
| Chd7     | 1.397 | 2.364 | 1.203 | 1.587 | 1.785 | 2.530 |
| Csf2     | 0.417 | 2.909 | 0.389 | 1.214 | 6.436 | 4.031 |
| Fasl     | 1.153 | 1.299 | 0.755 | 1.190 | 0.749 | 1.096 |
| Fosl1    | 1.811 | 1.635 | 0.363 | 3.318 | 1.448 | 8.437 |
| Foxp3    | 1.287 | 1.800 | 1.788 | 1.772 | 1.422 | 2.380 |
| Gata3    | 0.951 | 1.128 | 1.487 | 1.211 | 1.590 | 1.234 |
| Gata4    | 1.310 | 2.203 | 0.535 | 1.422 | 3.239 | 3.145 |
| Gfi1     | 1.297 | 1.343 | 1.192 | 0.930 | 1.021 | 1.899 |
| Ptgdr2   | 2.236 | 3.174 | 1.507 | 0.747 | 1.406 | 2.371 |
| Havcr2   | 0.976 | 0.666 | 0.590 | 1.008 | 0.941 | 1.077 |
| Hopx     | 0.663 | 1.243 | 0.776 | 1.010 | 1.093 | 1.236 |
| Hoxa10   | 0.195 | 1.356 | 0.176 | 1.540 | 0.284 | 2.526 |
| Hoxa3    | 0.604 | 2.118 | 0.342 | 0.685 | 0.893 | 1.187 |
| Icos     | 0.521 | 1.009 | 0.466 | 0.992 | 0.728 | 0.618 |
| Id2      | 0.918 | 1.076 | 0.984 | 0.987 | 0.967 | 0.913 |
| Ifng     | 1.300 | 1.104 | 1.516 | 1.323 | 1.492 | 1.351 |
| Igsf6    | 1.564 | 0.917 | 1.100 | 0.755 | 1.381 | 1.210 |
| Ikrf2    | 0.623 | 0.770 | 0.533 | 0.668 | 0.896 | 0.581 |
| Gene     | Tbx21 | Stat6 | Soc51 | Runx3 | Runx1 | Rorc | Rora | Pparg | Pou2f2 | Pkd2 | Perp | Nr4a1 | Nfatc1 | Maf | Lrrc32 | Jak1 | Irf4 | Irf8 | Ikr1 | Ikr2 | Ikr11 | Ii2 | Ii21 | Ii2r1 | Ii4 | Ii4ra | Ii5 | Ii9 | Ilr1 | Il2ra | Il4 | Il4ra | Il5 | Il9 | Il18rap | Il18r1 | Il18 | Il17re | Il17a | Il17a | Ii13ra1 | Ii13 | Ii13 | Ii18r1 | Ii18rap | Ii18r1 | Ii18r1 | Ii18rap | Il18r1 |
|----------|-------|-------|-------|-------|-------|------|------|-------|-------|------|------|-------|-------|-----|-------|------|------|------|------|------|-------|------|-----|-------|-------|-----|-------|------|-----|-------|-------|-----|-------|------|-----|-------|-------|-----|-------|------|-----|
|          | 0.695 | 0.945 | 0.991 | 0.816 | 2.235 | 0.844 |
| Tbx21    | 0.762 | 0.681 | 0.588 | 0.663 | 1.153 | 0.812 |
| Ii13     | 1.251 | 2.119 | 1.885 | 2.513 | 3.717 | 2.414 |
| Ii13ra1  | 1.607 | 0.777 | 1.484 | 0.944 | 1.152 | 1.069 |
| Ii13a    | 0.703 | 0.353 | 0.600 | 0.683 | 0.759 | 0.986 |
| Ii17re   | 1.641 | 1.221 | 2.015 | 1.573 | 1.544 | 1.148 |
| Ii18     | 1.692 | 1.710 | 1.353 | 1.123 | 1.521 | 1.336 |
| Ii18r1   | 1.204 | 1.230 | 1.122 | 0.934 | 1.053 | 1.029 |
| Ii18rap  | 1.082 | 1.317 | 0.589 | 0.672 | 0.968 | 1.508 |
| Ii1r1    | 0.575 | 0.837 | 0.827 | 0.764 | 0.703 | 0.848 |
| Ii1r2    | 1.458 | 3.719 | 1.821 | 0.974 | 1.628 | 3.206 |
| Ii1r11   | 0.506 | 0.718 | 0.709 | 0.976 | 0.844 | 0.761 |
| Ii2      | 2.488 | 0.723 | 1.564 | 0.667 | 2.298 | 1.428 |
| Ii21     | 1.910 | 1.165 | 1.100 | 0.682 | 2.784 | 1.242 |
| Ii2r1    | 1.035 | 0.934 | 1.086 | 0.871 | 1.341 | 0.733 |
| Ii4      | 2.083 | 1.665 | 1.804 | 1.380 | 2.069 | 0.860 |
| Ii4ra    | 1.734 | 2.054 | 1.365 | 1.676 | 1.345 | 1.735 |
| Ii5      | 0.567 | 1.701 | 0.371 | 0.840 | 2.141 | 2.642 |
| Ii9      | 0.215 | 3.524 | 0.111 | 4.618 | 0.429 | 5.840 |
| Irf1     | 1.046 | 1.099 | 1.068 | 0.985 | 0.967 | 0.885 |
| Irf4     | 0.739 | 1.165 | 0.747 | 0.776 | 1.141 | 0.654 |
| Irf8     | 1.283 | 1.395 | 1.070 | 1.165 | 1.005 | 0.927 |
| Jak1     | 1.514 | 1.038 | 1.476 | 1.043 | 1.323 | 1.063 |
| Lrcc32   | 0.904 | 2.533 | 0.758 | 1.657 | 0.772 | 1.642 |
| Maf      | 1.097 | 1.386 | 1.022 | 1.170 | 0.922 | 1.237 |
| Myb      | 1.215 | 0.888 | 1.289 | 0.857 | 1.577 | 0.727 |
| Nfatc1   | 1.381 | 1.007 | 1.261 | 0.933 | 1.412 | 0.970 |
| Nfatc2   | 0.938 | 0.961 | 0.995 | 0.918 | 0.827 | 0.885 |
| Nfatc2ip | 1.528 | 1.342 | 1.439 | 1.098 | 1.268 | 1.047 |
| Nr4a1    | 1.624 | 1.431 | 1.592 | 1.061 | 1.384 | 1.027 |
| Nr4a3    | 0.235 | 0.930 | 0.206 | 0.734 | 0.208 | 1.081 |
| Perp     | 0.981 | 1.163 | 1.693 | 0.591 | 0.845 | 1.408 |
| Pkd2     | 0.828 | 1.424 | 0.775 | 1.284 | 0.901 | 1.644 |
| Pou2f2   | 1.429 | 1.462 | 1.456 | 1.105 | 1.397 | 0.976 |
| Pparg    | 0.635 | 1.240 | 0.904 | 1.053 | 0.610 | 1.032 |
| Rel      | 1.834 | 1.102 | 1.565 | 0.834 | 1.451 | 0.834 |
| Rora     | 1.603 | 1.238 | 1.792 | 1.012 | 1.200 | 1.043 |
| Rorc     | 1.159 | 1.515 | 1.361 | 1.278 | 1.566 | 1.829 |
| Runx1    | 1.373 | 1.035 | 1.266 | 1.027 | 1.345 | 1.097 |
| Runx3    | 0.687 | 1.466 | 0.660 | 1.180 | 0.696 | 1.171 |
| Socs1    | 5.505 | 1.069 | 1.424 | 1.211 | 1.531 | 1.079 |
| Socs5    | 2.017 | 0.794 | 1.521 | 1.093 | 1.203 | 1.245 |
| Stat1    | 1.656 | 0.722 | 2.029 | 0.833 | 1.841 | 0.768 |
| Stat4    | 0.929 | 1.478 | 1.052 | 1.066 | 1.154 | 0.849 |
| Stat6    | 1.647 | 1.422 | 1.119 | 1.200 | 1.117 | 1.142 |
| Tbx21    | 0.404 | 1.571 | 0.296 | 0.811 | 0.770 | 1.193 |
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     |
| Adams1  | 0.1021  | 6.9524     | 4.0763   | 0.2026   | 2.6365  | 0.3105  | 0.2591  | 0.1217  |
| Aldh3a1 | 0.1081  | 3.4041     | 5.2648   | 0.1759   | 1.8660  | 0.4573  | 0.0288  | 0.1350  |
| Angpt1  | 0.0175  | 5.3426     | 14.3275  | 0.4190   | 0.8559  | 0.0481  | 1.7566  | 0.0040  |
| Angpt14 | 0.0717  | 2.8635     | 1.6292   | 0.1172   | 0.1740  | 0.0533  | 0.1094  | 0.0794  |
| Casp8   | 0.1609  | 4.4852     | 17.1495  | 0.1560   | 0.8823  | 0.5039  | 0.2987  | 0.4391  |
| Cd2h   | 0.1950  | 4.4482     | 3.0244   | 0.0991   | 0.7191  | 1.3455  | 1.5076  | 0.0743  |
| Cdh6    | 0.6451  | 4.0217     | 11.8376  | 0.3184   | 2.3374  | 0.3950  | 1.1163  | 0.9422  |
| Col4a2  | 0.2231  | 14.0953    | 9.9344   | 2.2378   | 2.1428  | 0.2715  | 0.8251  | 1.1612  |
| Col6a1  | 0.5293  | 1.9947     | 1.4182   | 0.0779   | 0.4406  | 0.2262  | 0.4336  | 0.4766  |
| Cst7    | 0.6212  | 5.4917     | 5.7955   | 15.5825  | 5.5213  | 3.8085  | 1.8882  | 13.6300 |
| Ctgf    | 0.0301  | 0.5168     | 0.4273   | 0.0283   | 0.0541  | 0.0781  | 0.1498  | 0.0664  |
| Ctsb    | 0.2542  | 3.1115     | 8.4300   | 0.2027   | 0.3488  | 0.1780  | 0.6045  | 1.0403  |
| Ctsl1   | 0.0322  | 2.0009     | 3.4083   | 0.0294   | 0.0803  | 0.2343  | 0.9123  | 0.0512  |
| Cxcl1   | 0.9050  | 9.8453     | 9.4692   | 6.1423   | 6.4151  | 3.6930  | 2.2256  | 21.3126 |
| Drg1    | 0.0147  | 0.8098     | 0.1756   | 0.0353   | 0.0795  | 0.0511  | 0.0683  | 0.0875  |
| Fli1    | 0.0923  | 1.6656     | 9.5041   | 0.2455   | 0.7573  | 0.3315  | 1.1368  | 0.1928  |
| Gpi     | 0.6091  | 7.4262     | 9.5362   | 24.3252  | 4.8662  | 4.1971  | 1.8743  | 10.1971 |
| Gsn     | 0.9534  | 6.6025     | 9.7069   | 1.0058   | 4.4323  | 0.5571  | 1.6766  | 0.8818  |
| Id1     | 0.3805  | 4.7843     | 13.5532  | 0.3334   | 1.2883  | 0.4716  | 0.5435  | 0.3495  |
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 ΔΔCT 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 & Score for metastases [median (min-max)]
\hline
Day of experiment & Control & Calcitriol & PRI-2191 & PRI-2205
\hline
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)*
\hline

*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 |
|--------|-------------|
| Asb2   | Ankyrin repeat and SOCS box-containing 2 |
| Cacna1f| Calcium channel, voltage-dependent, alpha 1F subunit |
| Ccl11  | Chemokine (C-C motif) ligand 11 |
| Ccl5   | Chemokine (C-C motif) ligand 5 |
| Ccl7   | Chemokine (C-C motif) ligand 7 |
| Ccr3   | Chemokine (C-C motif) receptor 3 |
| Ccr4   | Chemokine (C-C motif) receptor 4 |
| Ccr6   | Chemokine (C-C motif) receptor 6 |
| Cebpb  | CCAAT/enhancer binding protein (C/EBP), beta |
| Chd7   | Chromodomain helicase DNA binding protein 7 |
| Csf2   | Colony stimulating factor 2 (granulocyte-macrophage) |
| Fasl   | Fas ligand (TNF superfamily, member 6) |
| Fosl1  | Fos-like antigen 1 |
| Foxp3  | Forkhead box P3 |
| Gata3  | GATA binding protein 3 |
| Gata4  | GATA binding protein 4 |
| Gfi1   | Growth factor independent 1 |
| Ptgdr2 | G protein-coupled receptor 44 |
| Hacr2  | Hepatitis A virus cellular receptor 2 |
| Hopx   | HOP homeobox |
| Hoxa10 | Homeobox A10 |
| Hoxa3  | Homeobox A3 |
|  **Gene**  | **Description**                                      |
|----------|-----------------------------------------------------|
| **Icos** | Inducible T-cell co-stimulator                     |
| **Id2**  | Inhibitor of DNA binding 2                         |
| **Ifng** | Interferon gamma                                   |
| **Igsf6**| Immunoglobulin superfamily, member 6               |
| **Ikzf2**| IKAROS family zinc finger 2                        |
| **Il12b**| Interleukin 12B                                    |
| **Il12rb2**| Interleukin 12 receptor, beta 2                  |
| **Il13** | Interleukin 13                                     |
| **Il13ra1**| Interleukin 13 receptor, alpha 1              |
| **Il17a**| Interleukin 17A                                    |
| **Il17e**| Interleukin 17 receptor E                           |
| **Il18** | Interleukin 18                                     |
| **Il18r1**| Interleukin 18 receptor 1                          |
| **Il18rap**| Interleukin 18 receptor accessory protein         |
| **Il1r1**| Interleukin 1 receptor, type I                     |
| **Il1r2**| Interleukin 1 receptor, type II                    |
| **Il1rl1**| Interleukin 1 receptor-like 1                     |
| **Il2**  | Interleukin 2                                      |
| **Il21** | Interleukin 21                                     |
| **Il2ra**| Interleukin 2 receptor, alpha chain                |
| **Il4**  | Interleukin 4                                      |
| **Il4ra**| Interleukin 4 receptor, alpha                      |
| **Il5**  | Interleukin 5                                      |
| **Il9**  | Interleukin 9                                      |
| **Irf1** | Interferon regulatory factor 1                     |
| **Irf4** | Interferon regulatory factor 4                     |
| **Irf8** | Interferon regulatory factor 8                     |
| **Jak1** | Janus kinase 1                                     |
| **Lrrc32**| Leucine rich repeat containing 32                  |
| **Maf**  | Avian musculoaponeurotic fibrosarcoma (v-maf) AS42 oncogene homolog |
| **Myb**  | Myeloblastosis oncogene                            |
| **Nfats1**| Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 |
| **Nfats2**| Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2 |
| **Nfats2ip**| Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2 interacting protein |
| **Nr4a1**| Nuclear receptor subfamily 4, group A, member 1    |
| **Nr4a3**| Nuclear receptor subfamily 4, group A, member 3    |
| **Perp** | PERP, TP53 apoptosis effector                       |
| **Pkd2** | Polycystic kidney disease 2                        |
| **Pou2f2**| POU domain, class 2, transcription factor 2        |
| **Pparg**| Peroxisome proliferator activated receptor gamma   |
| **Rel**  | Reticuloendotheliosis oncogene                     |
| **Rora** | RAR-related orphan receptor alpha                  |
| **Rorc** | RAR-related orphan receptor gamma                  |
| **Runx1**| Runt related transcription factor 1                |
| **Runx3**| Runt related transcription factor 3                |
| **Socs1**| Suppressor of cytokine signaling 1                 |
| Symbol | Description |
|--------|-------------|
| **Socs5** | Suppressor of cytokine signaling 5 |
| **Stat1** | Signal transducer and activator of transcription 1 |
| **Stat4** | Signal transducer and activator of transcription 4 |
| **Stat6** | Signal transducer and activator of transcription 6 |
| **Tbx21** | T-box 21 |
| **Tgif1** | TGFβ-induced factor homeobox 1 |
| **Tlr4** | Toll-like receptor 4 |
| **Tlr6** | Toll-like receptor 6 |
| **Tmed1** | Transmembrane emp24 domain containing 1 |
| **Tnf** | Tumor necrosis factor |
| **Tnfrsf9** | Tumor necrosis factor receptor superfamily, member 9 |
| **Tnfsf11** | Tumor necrosis factor (ligand) superfamily, member 11 |
| **Trp53inp1** | Transformation related protein 53 inducible nuclear protein 1 |
| **Uts2** | Urotensin 2 |
| **Zbtb7b** | Zinc finger and BTB domain containing 7B |
| **Zeb1** | 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 |
|--------|-------------|
| **Adamts1** | ADAM metallopeptidase |
| **Aldh3a1** | Aldehyde dehydrogenase 3 family, member A1 |
| **Angpt1** | Angiopoietin 1 |
| **Angptl4** | Angiopoietin-like 4 |
| **Casp8** | Caspase 8, apoptosis-related cysteine peptidase |
| **Ccne2** | Cyclin E2 |
| **Ccr7** | Chemokine (C-C motif) receptor 7 |
| **Cd44** | CD44 molecule (Indian blood group) |
| **Cd82** | CD82 antigen |
| **Cdh1** | Cadherin 1, type 1, E-cadherin (epithelial) |
| **Cdh11** | Cadherin 11, type 2, OB-cadherin (osteoblast) |
| **Cdh2** | Cadherin 2, type 1, N-cadherin |
| **Cdh6** | Cadherin 6 |
| **Cldsn7** | Claudin 7 |
| **Col1a1** | Collagen, type I, alpha 1 |
| **Col4a2** | Collagen, type IV, alpha 2 |
| **Col16a1** | Collagen, type VI, alpha 1 |
| **Csf1** | Colony stimulating factor 1 |
| **Csf2** | Colony stimulating factor 2 |
| **Csf3** | Colony stimulating factor 3 |
| **Cst7** | Cystatin F (leukocystatin) |
| **Ctgf** | Connective tissue growth factor |
| **Ctsb** | Cathepsin B |
| **Ctsd** | Cathepsin D |
| **Ctsk** | Cathepsin K |
| **Ctsl1** | Cathepsin L-like 3 |
| Gene  | Description |
|-------|-------------|
| Cxcl1 | Chemokine (C-X-C motif) ligand 1 |
| Cxcl13 | Chemokine (C-X-C motif) ligand 13 |
| Cxcr4 | Chemokine (C-X-C motif) receptor 4 |
| Cxcr6 | Chemokine (C-X-C motif) receptor 6 |
| Drg1 | Developmentally regulated GTP binding protein 1 |
| Ereg | Epiregulin |
| Fgf8 | Fibroblast growth factor 8 (androgen-induced) |
| Flt1 | Fms-related tyrosine kinase |
| Flt4 | Fms-related tyrosine kinase 4 |
| Gpi | Glucose phosphate isomerase |
| Gsn | Gelsolin (amyloidosis, Finnish type) |
| Hgf | Hepatocyte growth factor (hepapoietin A; scatter factor) |
| Hif1a | Hypoxia inducible factor 1, alpha subunit |
| Hmgb1 | High-mobility group box 1 |
| Id1 | Inhibitor of DNA binding 1 |
| Il13ra2 | Interleukin 13 receptor, alpha 2 |
| Isg20 | Interferon stimulated exonuclease gene 20kDa |
| Jag1 | Jagged 1 (Alagille syndrome) |
| Kiss1 | KiSS-1 metastasis-suppressor |
| Klrc2 | Killer cell lectin-like receptor subfamily C, member 2 |
| Kynu | Kynureninase (L-kynurenine hydrolase) |
| Ltbp1 | Latent transforming growth factor beta binding protein 1 |
| Map2k4 | Mitogen-activated protein kinase kinase 4 |
| Map2k5 | Mitogen-activated protein kinase kinase 5 |
| Map2k7 | Mitogen-activated protein kinase kinase 7 |
| Mcam | Melanoma cell adhesion molecule |
| Met | Met proto-oncogene (hepatocyte growth factor receptor) |
| Metap2 | Methionyl aminopeptidase 2 |
| Mmp1 | Matrix metalloproteinase 1 (interstitial collagenase) |
| Mmp10 | Matrix metalloproteinase 10 (stromelysin 2) |
| Mmp11 | Matrix metalloproteinase 11 (stromelysin 3) |
| Mmp13 | Matrix metalloproteinase 13 (collagenase 3) |
| Mmp14 | Matrix metalloproteinase 14 (membrane-inserted) |
| Mmp2 | Matrix metalloproteinase 2 |
| Mmp7 | Matrix metalloproteinase 7 (matrilysin, uterine) |
| Myc | V-myc myelocytomatosis viral oncogene homolog |
| Nedd9 | Neural precursor cell expressed, dev. down-regulated 9 |
| Nf2 | Neurofibromin 2 (merlin) |
| Nme1 | Non-metastatic cells 1, protein (NM23A) |
| Nme2 | Non-metastatic cells 2, protein |
| Nme4 | Non-metastatic cells 4, protein |
| Pax5 | Paired box 5 |
| Pdgfa | Platelet-derived growth factor alpha polypeptide |
| Plaur | Plasminogen activator, urokinase receptor |
| Ptgs2 | Prostaglandin-endoperoxide synthase 2 |
| Runx1 | Runx-related transcription factor 1 |
| Serpine1 | Serpin peptidase inhibitor, clade E |
| Serpinb5 | Serpin peptidase inhibitor, clade B5 |
| Protein | Description |
|---------|-------------|
| Sox4 | SRY (sex determining region Y)-box 4 |
| Sparc | Secreted protein, acidic, cysteine-rich (osteonectin) |
| Spp1 | Secreted phosphoprotein 1 |
| Src | V-src sarcoma viral oncogene homolog (avian) |
| Tff1 | Trefoil factor 1 |
| Tgfβ1 | Transforming growth factor, beta 1 |
| Timp1 | TIMP metalloproteinase inhibitor 1 |
| Timp2 | TIMP metalloproteinase inhibitor 2 |
| Timp3 | TIMP metalloproteinase inhibitor 3 |
| Timp4 | TIMP metalloproteinase inhibitor 4 |
| Tnc | Tenascin C (hexabrachion) |
| Tp53 | Tumor protein p53 |
| Vegfa | Vascular endothelial growth factor A |
| Vegfb | Vascular endothelial growth factor B |
| Actb | Actin, beta |
| B2m | Beta-2-microglobulin |
| Gapd | Glyceraldehyde-3-phosphate dehydrogenase |
| Gusb | Glucuronidase, beta |
| Hprt1 | Hypoxanthine phosphoribosyltransferase 1 |
| Pkg | Phosphoglycerate kinase 1 |
| Ppia | Peptidylprolyl isomerase A |
| Rpl13a | Ribosomal protein L13a |