

## **Calcitriol Analogues Decrease Lung Metastasis but Impair Bone Metabolism in Aged Ovariectomized Mice Bearing 4T1 Mammary Gland Tumours**

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# SUPPLEMENTARY DATA

**Supplemental Table 1.** List of genes included in *Mouse T Helper Cell Differentiation RT<sup>2</sup> 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>Ptgd2</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

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<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>Tgfb1</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>Tnfsf11</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

**Supplemental Table 2.** Fold change values of genes associated with mouse T helper cell differentiation in tumour samples from 4T1 tumour bearing mice treated with calcitriol or its analogues PRI-2191 and PRI-2205.

Gene	Tumour tissue					
	Calcitriol		PRI-2191		PRI-2205	
	Mean	SD	Mean	SD	Mean	SD
<i>Asb2</i>	2.674	1.600	1.224	0.737	1.410	0.792
<i>Cacna1f</i>	1.264	0.542	1.612	0.514	1.099	0.318
<i>Ccl11</i>	2.094	1.646	2.061	1.826	3.438	2.277
<i>Ccl5</i>	0.694	0.226	0.913	0.508	1.305	0.538
<i>Ccl7</i>	0.735	0.140	0.609	0.111	0.808	0.138
<i>Ccr3</i>	0.932	0.198	0.756	0.203	0.879	0.209
<i>Ccr4</i>	1.128	0.274	1.118	0.213	1.398	0.335
<i>Ccr6</i>	5.510	5.185	7.018	10.445	3.269	2.945
<i>Cebpb</i>	0.838	0.068	0.978	0.075	0.936	0.077
<i>Chd7</i>	0.780	0.061	0.942	0.077	1.375	0.118
<i>Csf2</i>	1.985	0.418	1.401	0.399	1.306	0.296
<i>Fasl</i>	0.694	0.232	0.504	0.215	1.216	0.575
<i>Fosl1</i>	0.666	0.268	0.676	0.171	1.064	0.280
<i>Foxp3</i>	1.266	0.573	1.417	0.463	1.274	0.470
<i>Gata3</i>	0.776	0.052	0.728	0.052	1.149	0.071
<i>Gata4</i>	0.630	0.110	1.028	0.193	1.117	0.198
<i>Gfi1</i>	1.105	0.605	1.665	0.816	1.476	0.489
<i>Ptger2</i>	1.589	0.775	2.296	0.977	3.400	1.885

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<i>Haver2</i>	0.879	0.253	1.133	0.339	0.910	0.236
<i>Hopx</i>	0.640	0.175	0.822	0.290	0.938	0.297
<i>Hoxa10</i>	1.379	0.161	1.095	0.116	1.529	0.179
<i>Hoxa3</i>	0.725	0.052	0.623	0.062	1.119	0.072
<i>Icos</i>	0.795	0.387	0.961	0.457	1.320	0.735
<i>Id2</i>	0.983	0.408	1.021	0.508	0.779	0.325
<i>Ifng</i>	0.594	0.273	1.178	0.762	1.982	1.496
<i>Igsf6</i>	0.837	0.283	0.787	0.255	0.790	0.269
<i>Ikzf2</i>	1.058	0.150	0.606	0.131	1.114	0.202
<i>Il12b</i>	1.153	0.581	0.839	0.492	2.059	1.274
<i>Il12rb2</i>	1.173	0.501	1.301	0.482	2.708	1.004
<i>Il13</i>	1.510	0.440	1.933	0.464	1.397	0.333
<i>Il13ra1</i>	1.865	0.505	0.920	0.129	0.898	0.117
<i>Il17a</i>	0.815	0.135	1.271	0.225	1.911	0.401
<i>Il17re</i>	5.205	1.105	1.011	0.248	4.802	1.318
<i>Il18</i>	1.057	0.146	0.944	0.124	1.098	0.143
<i>Il18r1</i>	1.061	0.428	1.201	0.541	1.122	0.298
<i>Il18rap</i>	1.580	1.148	1.109	0.635	1.865	1.965
<i>Il1r1</i>	0.810	0.120	0.709	0.097	0.909	0.140
<i>Il1r2</i>	1.352	1.108	1.197	0.596	0.892	0.485
<i>Il1rl1</i>	1.390	0.714	1.251	0.490	2.234	0.973
<i>Il2</i>	0.980	0.254	1.578	0.375	1.905	0.558
<i>Il21</i>	0.671	0.254	0.817	0.186	1.216	0.434
<i>Il2ra</i>	0.827	0.244	1.046	0.309	0.938	0.231
<i>Il4</i>	0.969	0.250	1.015	0.383	0.904	0.276
<i>Il4ra</i>	0.797	0.110	0.808	0.117	1.061	0.125
<i>Il5</i>	1.616	1.307	2.619	1.890	1.824	1.366
<i>Il9</i>	3.534	2.300	2.045	1.567	4.154	3.750
<i>Irf1</i>	0.709	0.046	0.755	0.053	0.831	0.048
<i>Irf4</i>	1.230	0.451	1.942	0.946	1.680	0.855
<i>Irf8</i>	0.778	0.108	1.114	0.162	1.026	0.138
<i>Jak1</i>	0.867	0.060	1.097	0.071	0.998	0.065
<i>Lrrc32</i>	0.680	0.191	1.126	0.395	0.987	0.237
<i>Maf</i>	0.879	0.204	0.913	0.176	1.050	0.184
<i>Myb</i>	1.689	0.619	1.394	0.428	2.895	1.287
<i>Nfatc1</i>	0.775	0.071	0.791	0.084	0.861	0.072
<i>Nfatc2</i>	1.077	0.189	0.961	0.159	1.080	0.184
<i>Nfatc2ip</i>	0.795	0.096	0.870	0.095	1.147	0.126
<i>Nr4a1</i>	0.745	0.087	0.784	0.090	0.887	0.086
<i>Nr4a3</i>	0.701	0.263	0.780	0.279	1.144	0.477
<i>Perp</i>	2.566	0.682	1.826	0.631	3.476	2.073
<i>Pkd2</i>	0.734	0.027	0.628	0.026	0.898	0.032
<i>Pou2f2</i>	1.215	0.357	1.355	0.453	1.706	0.536
<i>Pparg</i>	1.829	0.656	4.033	1.631	1.526	0.456
<i>Rel</i>	0.971	0.214	0.881	0.183	1.124	0.216
<i>Rora</i>	1.080	0.230	1.329	0.288	1.468	0.312
<i>Rorc</i>	1.137	0.103	1.300	0.106	1.215	0.102
<i>Runx1</i>	0.786	0.052	0.693	0.046	0.930	0.055
<i>Runx3</i>	0.733	0.141	0.694	0.142	1.162	0.230
<i>Soes1</i>	0.689	0.128	0.932	0.256	0.967	0.194
<i>Soes5</i>	1.184	0.231	1.169	0.223	1.253	0.210
<i>Stat1</i>	0.583	0.096	0.731	0.189	1.121	0.324
<i>Stat4</i>	0.653	0.191	0.836	0.410	1.338	0.590
<i>Stat6</i>	0.690	0.115	1.027	0.234	1.581	0.236
<i>Tbx21</i>	0.589	0.183	1.213	0.466	1.296	0.404
<i>Tgif1</i>	0.989	0.253	0.595	0.171	1.090	0.322
<i>Tlr4</i>	0.949	0.214	1.072	0.220	1.019	0.209
<i>Tlr6</i>	0.984	0.473	0.677	0.299	0.907	0.409
<i>Tmed1</i>	0.679	0.062	0.599	0.058	0.806	0.064

## SUPPLEMENTARY DATA

<i>Tnf</i>	0.834	0.182	0.518	0.133	1.609	0.752
<i>Tnfrsf9</i>	0.476	0.123	0.572	0.090	0.550	0.094
<i>Tnfsf11</i>	1.529	0.528	3.798	1.707	4.840	3.454
<i>Trp53inp1</i>	1.072	0.226	0.725	0.131	0.902	0.182
<i>Uts2</i>	0.716	0.328	0.692	0.250	1.135	0.480
<i>Zbtb7b</i>	0.840	0.078	0.854	0.085	1.024	0.088
<i>Zeb1</i>	0.849	0.155	1.325	0.257	1.204	0.283

Tumour tissue specimens were collected on the day 21 (after inoculation with 4T1 cells) from 60 week old mice treated with calcitriol or its analogues and control group receiving vehicle for vitamin D compounds. *Mouse T Helper Cell Differentiation RT<sup>2</sup> Profiler Array* (Qiagen, Hilden, Germany) was used for Real-time PCR screening (84 key genes and 5 housekeeping genes). Data shows a mean fold change values and standard deviation for each group. Fold-change (RQ) of target genes was defined using double delta Ct method in reference to the heat shock protein 90 alpha (cytosolic), class B member 1 (*Hsp90ab1*) for tumour samples. Then the results were adjusted to the values obtained for the control group within the day 21 of the experiment for each treatment group. Data analysis was acquired using Qiagen online software (Qiagen, Hilden, Germany). PCR amplification cycles were as follows 95°C for 10 s and 58°C for 45 s (50 cycles). Each reaction contained 0.5 µg of cDNA (for each mouse in the group; 3 or 4 mice per group).