Supplemental Figure Legends

Supplemental Figure 1. *RANKL* expression is upregulated in response to treatment with $1,25(OH)_2D_3$. (A) $1,25(OH)_2D_3$ induces upregulation of human *RANKL* mRNA in MG63 cells in a dose-dependent manner. MG63 cells were treated for 36 hours with the indicated concentration of $1,25(OH)_2D_3$. RNA was isolated using TRI Reagent, reverse transcribed as described in Materials and Methods, and the resulting cDNA amplified via PCR using primers specific for *CYP24A1* (32 cycles), *RANKL* (32 cycles), or β-actin (23 cycles). (B, C) *RANKL* upregulation by $1,25(OH)_2D_3$ occurs in a time-dependent manner. MG63 cells were treated for the indicated time with 10^{-7} M $1,25(OH)_2D_3$. RNA was isolated, reverse transcribed, and the cDNA then analyzed using real time quantitative PCR. Values for *RANKL* (B) and *CYP24A1* (C) were normalized to β-actin values. Both plots are representative of at least two separate experiments.

Supplemental Figure 2. Identification of a functional VDRE within the hRLD2 region. (A) Wild type VDRE half-sites and DR3-type VDRE sequences identified in the hRLD2 region using the CONSITE transcription factor binding algorithm (http://mordor.cgb.ki.se/cgi-bin/CONSITE/consite) are indicated. Mutations were introduced into the wild type sequence and are designated M1-M3. (B) Mutations in the hRLD2 VDRE abrogate transcriptional response to treatment with $1,25(OH)_2D_3$. MG63 cells were transfected with pTK-hRLD2 reporter vectors (250 ng) containing either the putative wild type VDRE (WT) or one in which the possible half-sites had been mutated (M1-M3). Cells were also transfected with pcDNA-hVDR and pCH110-βgal expression vectors (50 ng), treated with the indicated concentration of $1,25(OH)_2D_3$ for 24 hours, and assayed for luciferase and β-gal activity. Luciferase activity was normalized to β-gal values and is displayed as the average of a triplicate set of transfections ± SEM. These results are representative of two separate experiments. (C) Electromobility shift competition assay (EMSA). The wild type probe described in part (A) was end-labeled with [γ-^{32}P]-dATP and incubated with 10 ng VDR, 5 ng RXR, 2 x 10^{-6} M $1,25(OH)_2D_3$, 150 mM KCl and either 0, 10-, or 100-fold molar excess of the indicated unlabeled probe. The resulting complexes were separated on 6% nondenaturing polyacrylamide gels, dried, and imaged by autoradiography. These results are representative of two similar experiments.

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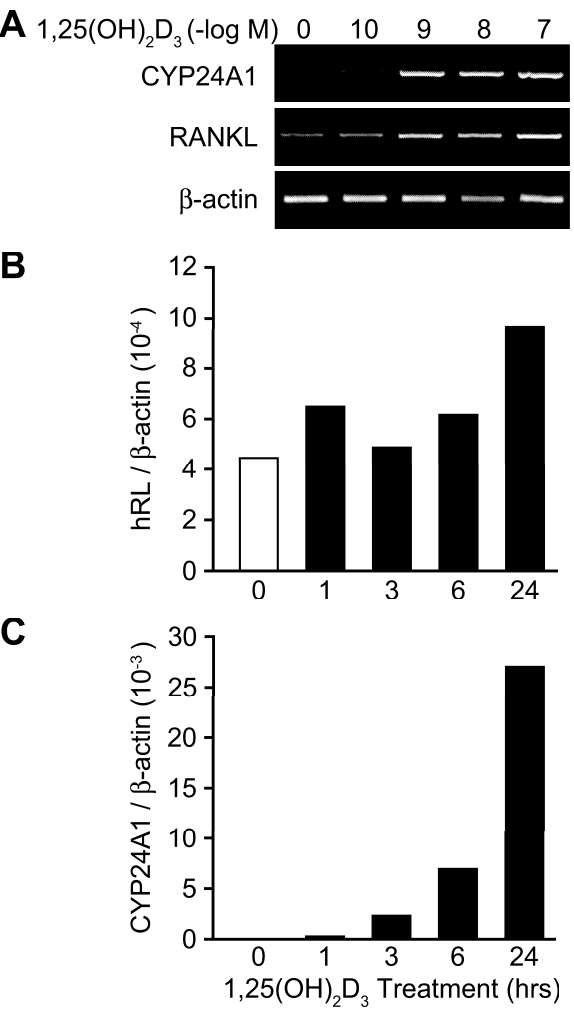

Supp. Figure 2
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