## SUPPLEMENTAL MATERIAL

**Biggs et al., http://www.jem.org/cgi/content/full/jem.20091725/DC1**

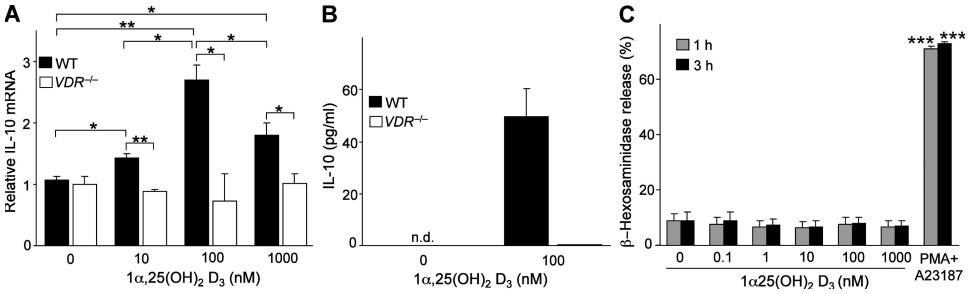


**Figure S1. $1\alpha,25(OH)_2D_3$ can increase IL-10 mRNA levels and induce release of IL-10 in WT BMCMCs, but not in *VDR*-deficient BMCMCs, but without inducing BMCMC degranulation.** (A) $1\alpha,25(OH)_2D_3$-induced increase in IL-10 mRNA levels in WT BMCMCs, but not in *VDR*-deficient BMCMCs. Quantitative real-time RT-PCR analysis of 4.5–5-wk-old BMCMCs, derived from B6J mice or $VDR^{-/-}$ mice, that were cultured ($2 \times 10^6$ cells/ml) in 0.1% BSA + IL-3 (5 ng/ml) for 6 h with 10–1,000 nM $1\alpha,25(OH)_2D_3$ or received no treatment as a control (0 nM). The y axis shows the values of mRNA expression relative to β-actin. *, $P < 0.05$ and **, $P < 0.01$ for indicated comparisons between groups. Data (mean + SEM) are from three independent experiments, in which the relative IL-10 mRNA expression was performed in triplicate, each of which gave similar results. (B) $1\alpha,25(OH)_2D_3$ does not induce IL-10 production by *VDR*-deficient BMCMCs. 5-wk-old WT or $VDR^{-/-}$ BMCMCs were cultured ($2 \times 10^6$ cells/ml) in DMEM containing 20% WEHI-3–conditioned medium for 24 h with or without 100 nM $1\alpha,25(OH)_2D_3$. IL-10 protein levels in the supernatants were measured by ELISA. Data (mean + SEM, $n = 3$ per group) are from the three independent experiments performed, each of which gave similar results (all measurements for each experiment were performed in duplicate). n.d., not detected (<30 pg/ml). The results for $VDR^{-/-}$ BMCMCs stimulated with 100 nM $1\alpha,25(OH)_2D_3$, while giving a signal in the assay, were below the stated lower limit of detection of this ELISA. (C) $1\alpha,25(OH)_2D_3$ does not induce BMCMC degranulation. WT (B6J) BMCMCs ($10^6$/ml) cultured in 0.1% BSA + IL-3 (5 ng/ml) were stimulated with 0.1–1,000 nM $1\alpha,25(OH)_2D_3$ (or, as a positive control, with 100 ng/ml PMA + 10 μM A23187) for 1 or 3 h, and the percentage degranulation was calculated based on release of β-hexosaminidase. Data are means + SEM ($n = 3$ per group) of three different experiments; all measurements for each experiment were performed in duplicate. ***, $P < 0.0001$, versus all concentrations (0–1,000 nM) of $1\alpha,25(OH)_2D_3$ for 1- or 3-h stimulations.

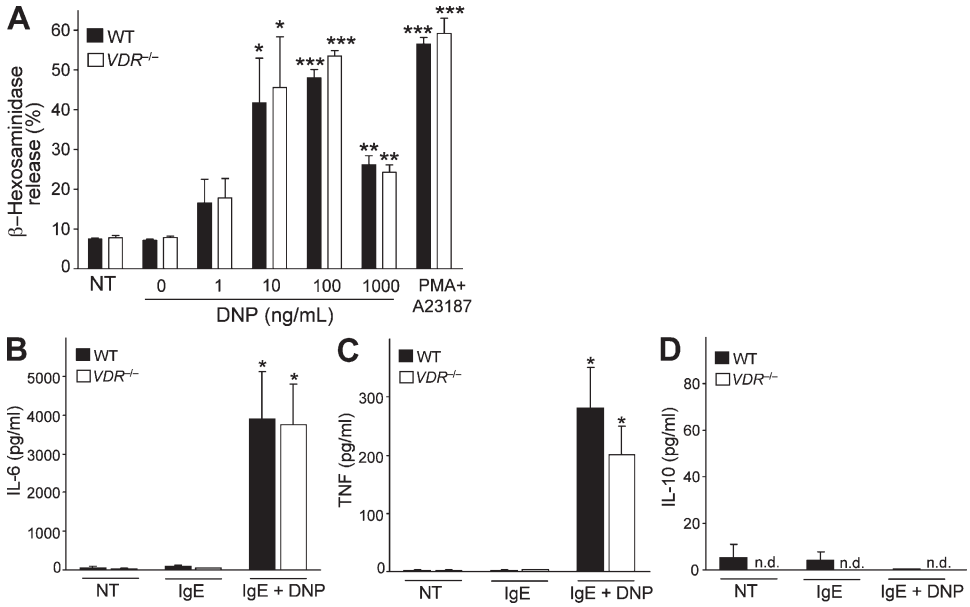


**Figure S2. VDR-deficient and WT mast cells exhibit similar levels of IgE- and antigen-dependent degranulation and cytokine production.** (A) *VDR*$^{-/-}$ and WT BMCMCs (5 wk old) were preloaded for 16 h with IgE anti-DNP (2 µg/ml) in cDMEM containing 20% WEHI-3–conditioned medium, stimulated with DNP-HSA at the concentrations indicated (or, as a positive control, with 100 ng/ml PMA + 10 µM A23187) for 1 h, and the percentage degranulation was calculated based on release of β-hexosaminidase. For measurement of concentrations of IL-6 (B), TNF (C), and IL-10 (D) in culture supernatants, BMCMCs were preloaded with IgE-DNP as above, stimulated with 20 ng/ml DNP-HSA for 6 h, and cytokine protein levels were measured by ELISA. Data are expressed as mean + SEM of three different experiments (A, C, and D; $n = 3$ per group) or five different experiments (B; $n = 5$ per group); all measurements for each experiment were performed in duplicate. *, $P < 0.05$; **, $P < 0.001$; ***, $P < 0.0001$, versus no treatment (NT) or versus 0 ng/ml DNP-HSA (IgE preloaded only) in the same group of BMCMCs. n.d., not detected (<30 pg/ml). In D, the results for WT BMCMCs, although giving a signal in the assay, were below the stated lower limit of detection of this ELISA.

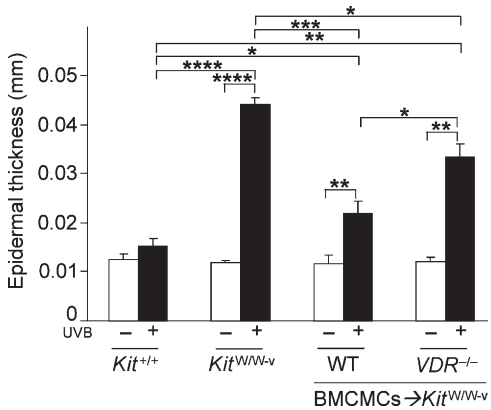


**Figure S3. Influence of mast cell VDR expression on ability of mast cells to limit epidermal thickness associated with chronic low-dose UVB irradiation of ear skin.** Epidermal thickness was measured in cross sections of ear skin sampled at control sites not treated with UVB irradiation (– UVB) or ears treated with 15 exposures to 2 kJ/m$^2$ UVB irradiation (+ UVB) of WBB6F$_1$-*Kit*$^{+/+}$ (WT) mice, mast cell–deficient WBB6F$_1$-*Kit*$^{W/W\text{-}v}$ (*Kit*$^{W/W\text{-}v}$) mice, WT BMCMC→*Kit*$^{W/W\text{-}v}$, and *VDR*$^{-/-}$ BMCMC→*Kit*$^{W/W\text{-}v}$ mice. Data (mean + SEM, $n = 9$–12 per group) are from three experiments, three to four mice/group per experiment. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$ for the indicated comparisons between groups of UVB-irradiated mice, or control ears (no UVB treatment) versus UVB-irradiated ears in the same group of mice.

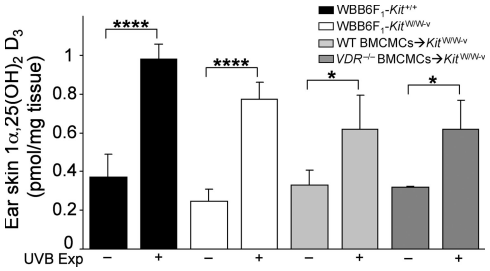


**Figure S4. Levels of $1\alpha,25(OH)_2D_3$ in ear skin are increased 24 h after the fifth exposure to UVB irradiation.** $1\alpha,25(OH)_2D_3$ levels in ear skin lysates sampled from WBB6F$_1$-*Kit*$^{+/+}$ (WT) mice, mast cell–deficient WBB6F$_1$-*Kit*$^{W/W\text{-}v}$ (*Kit*$^{W/W\text{-}v}$) mice, WT BMCMC→*Kit*$^{W/W\text{-}v}$ mice, and *VDR*$^{-/-}$ BMCMC→*Kit*$^{W/W\text{-}v}$ mice which either were not treated with UVB irradiation (– UVB) or were sacrificed 24 h after the last of 5 exposures to 2 kJ/m$^2$ UVB irradiation. Data (mean + SD, $n$ = 3–5 mice per group) are from the one experiment performed. *, $P < 0.05$; ****, $P < 0.0001$ for the comparisons indicated by the brackets.