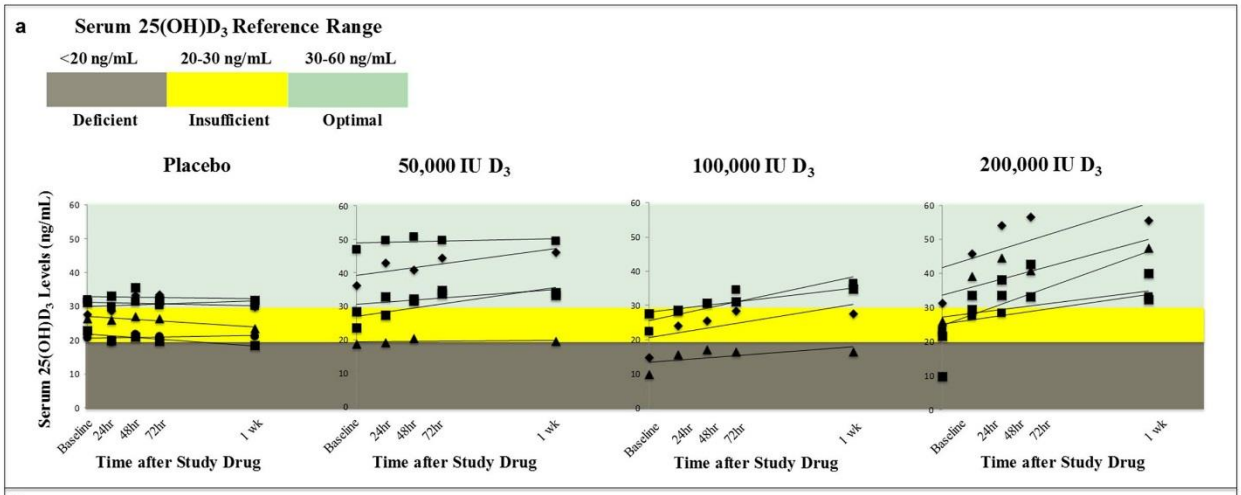



b

| Vitamin $D_3$ Metabolites | Placebo (n=6) | 50,000 IU $D_3$ (n=5) | | 100,000 IU $D_3$ (n=4) | | 200,000 IU $D_3$ (n=5) | |
|---|---|---|---|---|---|---|---|
| | Mean (SEM) | Mean (SEM) | *p* value | Mean (SEM) | *p* value | Mean (SEM) | *p* value |
| **25(OH)$D_3$ (ng/mL)** | | | | | | | |
| Δ24hr | -0.6 (0.8) | 3.6 (1.1) | 0.01 | 5.6 (1.7) | 0.01 | 12.8 (3.3) | 0.002 |
| Δ48hr | 1.6 (1.2) | 4.4 (1.0) | 0.11 | 7.2 (1.9) | 0.04 | 17.4 (4.1) | 0.003 |
| Δ72hr | 0.4 (1.3) | 6.9 (1.7) | 0.02 | 9.1 (2.4) | 0.01 | 21.1 (4.4) | 0.001 |
| Δ1wk | -1.0 (1.0) | 5.7 (1.9) | 0.01 | 10.3 (1.9) | 0.0004 | 19.3 (3.9) | 0.0004 |
| **1,25$(OH)_2D_3$ (pg/mL)** | | | | | | | |
| Δ24hr | -6.1 (4.6) | 3.8 (9.1) | 0.33 | 10.8 (8.6) | 0.09 | 17.9 (6.5) | 0.02 |
| Δ48hr | -6.8 (6.7) | -0.1 (4.4) | 0.45 | 16.3 (6.9) | 0.08 | 18.1 (5.8) | 0.03 |
| Δ72hr | -0.4 (5.0) | -0.2 (3.0) | 0.97 | 13.8 (8.1) | 0.15 | 16.8 (0.7) | 0.05 |
| Δ1wk | 2.3 (3.9) | -1.3 (7.5) | 0.67 | 6.6 (10.3) | 0.66 | 2.7 (7.1) | 0.56 |
| **24,25$(OH)_2D_3$ (ng/mL)** | | | | | | | |
| Δ24hr | 0.0 (0.2) | -0.5 (0.4) | 0.26 | 0.4 (0.1) | 0.14 | 0.6 (0.2) | 0.09 |
| Δ48hr | 0.1 (0.1) | -0.2 (0.4) | 0.57 | 0.4 (0.2) | 0.17 | 1.0 (0.20 | 0.002 |
| Δ72hr | 0.0 (0.1) | -0.1 (0.4) | 0.76 | 0.8 (0.2) | 0.01 | 1.7 (0.5) | 0.006 |
| Δ1wk | 0.0 (0.2) | 0.2 (0.4) | 0.62 | 1.0 (0.4) | 0.02 | 1.6 (0.6) | 0.02 |

**Fig. S1. Serum Vitamin $D_3$ Metabolites**

Panel A presents the serum concentration of 25(OH)$D_3$ (ng/mL) for each participant at baseline, as well as 24hr, 48hr, 72hr, and 1 week after administration of the study drug in the investigative phase of the study. Linear regression best-fit lines are presented for each participant. Panel B presents the change in serum concentration for the vitamin $D_3$ metabolites 25(OH)$D_3$ (ng/mL), 1,25$(OH)_2D_3$ (pg/mL), and 24,25$(OH)_2D_3$ (ng/mL) at each time point after administration of the study drug in the investigative phase of the study. The difference in serum concentration between each time point and baseline are presented. Statistical comparisons are between vitamin $D_3$ treatment groups and the placebo group.

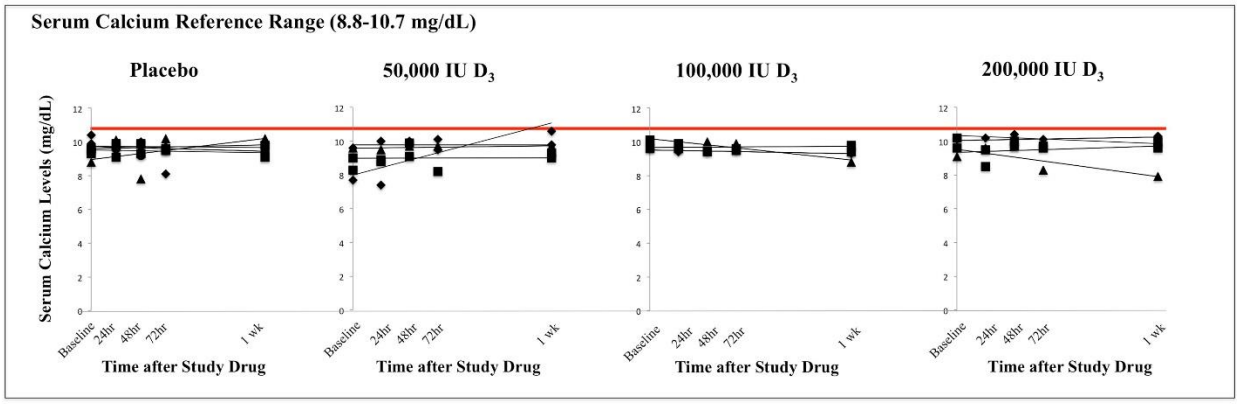



**Fig. S2. Serum Calcium Levels**

The serum calcium concentration (mg/dL) is presented for each participant at baseline, as well as 24hr, 48hr, 72hr, and 1 week after administration of the study drug in the investigative phase of the study. Linear regression best-fit lines are presented for each participant. The solid red line indicates the upper limit of the reference range for total serum calcium (10.7 mg/dL).

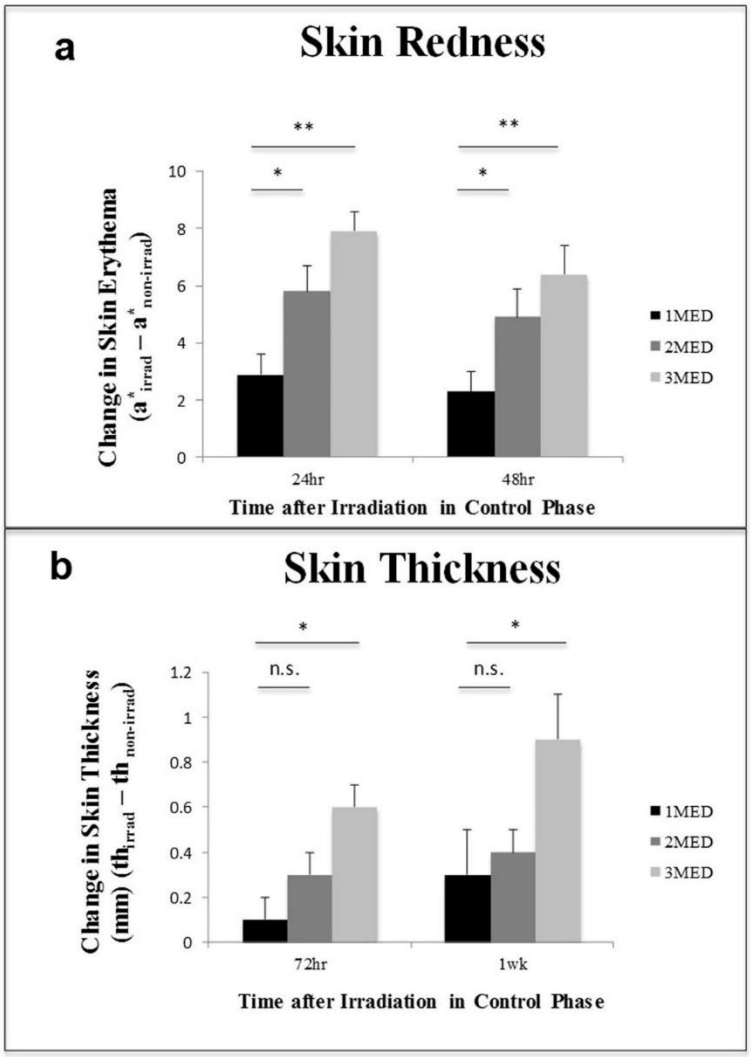



**Fig. S3. Dose-Dependent Relationship Between MED and Non-Invasive Clinical Outcomes**

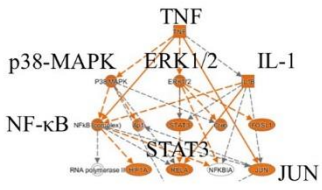
Panel A presents the difference in skin redness between irradiated and non-irradiated skin ($a^*_{irrad} - a^*_{non\text{-}irrad}$) 24hr and 48hr after exposure to 1MED, 2MED, and 3MED. Panel B presents the difference in skin thickness between irradiated and non-irradiated skin ($th_{irrad} - th_{non\text{-}irrad}$) 72hr and 1 week after exposure to 1MED, 2MED, and 3MED. Bars represent the mean, and error bars represent the standard error of the mean for all participants in each MED group (n=20). Statistical comparisons are between the 2MED or 3MED groups and the 1MED group at each time point. * $p<0.05$; ** $p<0.005$; n.s., non-significant.

**a**

| Genes | Vitamin $D_3$ Non-Responders (n=11) Log FC | *p* value | Vitamin $D_3$ Responders (n=7) Log FC | *p* value |
|---|---|---|---|---|
| *TGM3* (transglutaminase 3) | -0.82 | 0.006 | 1.65 | 0.046 |
| *KRT78* (keratin 78) | -0.33 | 0.215 | 1.60 | 0.046 |
| *CALML5* (calmodulin-like 5) | -0.75 | 0.011 | 1.30 | 0.046 |
| *CDSN* (corneodesmosin) | -0.24 | 0.320 | 1.26 | 0.046 |
| *KRT80* (keratin 80) | -0.02 | 0.957 | 1.20 | 0.046 |
| *TGM5* (transglutaminase 5) | -1.02 | 0.002 | 1.11 | 0.046 |
| *ARG1* (arginase 1) | -0.88 | 0.016 | 1.48 | 0.046 |
| *MMP1* (matrix metallopeptidase 1) | 3.20 | 0.001 | -0.92 | 0.451 |
| *MMP3* (matrix metallopeptidase 3) | 2.59 | 0.001 | -0.78 | 0.402 |
| IL1A (interleukin 1 alpha) | 1.77 | 0.001 | -0.07 | 0.926 |
| *CCL2* (chemokine (C-C motif) ligand 2) | 1.43 | 0.002 | -0.68 | 0.404 |
| *GSTA4* (glutathione S-transferase alpha 4) | -0.78 | 0.003 | 0.58 | 0.166 |
| *MGST2* (microsomal glutathione S-transferase 2) | -0.54 | 0.006 | 0.24 | 0.490 |

**b**

| Canonical Pathways | Overlap (%) | *p* value | Overlap (%) | *p* value |
|---|---|---|---|---|
| Granulocyte Adhesion and Diapedesis | 18.5 | $7.1 \times 10^{-9}$ | N/A | N/A |
| IL-6 Signaling | 19.5 | $1.8 \times 10^{-7}$ | N/A | N/A |
| Agranulocyte Adhesion and Diapedesis | 16.4 | $3.1 \times 10^{-7}$ | N/A | N/A |
| Arginase Pathway | N/A | N/A | 25.0 | $6.3 \times 10^{-3}$ |
| **Up-Stream Regulators** | **Predicted** | ***p* value** | **Predicted** | ***p* value** |
| TNF-α | Activated | $3.1 \times 10^{-28}$ | N/A | NS |
| ERK | Activated | $1.7 \times 10^{-16}$ | N/A | NS |
| **Networks** | **Pro-Inflammatory** | | **Barrier Repair** | |



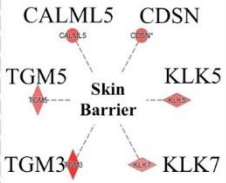




**Fig. S4. Genes, Canonical Pathways, and Networks Characterizing Participants from Cluster 1 and Cluster 2**

Panel A presents significantly differentially expressed genes between the two clusters, with blue shading highlighting genes involved in skin barrier repair, and red shading highlighting genes involved in skin inflammation. Panel B presents the significantly represented canonical pathways, predicted up-stream regulators, and molecular networks, as determined by ingenuity pathway analyses, characterizing participants from cluster 1 and cluster 2.

## Supplementary materials and methods

### Enrollment, allocation, and follow-up

The Investigational Drug Services at University Hospitals Cleveland Medical Center was responsible for generating the random allocation sequence and assigning participants to interventions. Participants were blocked randomized into one of four groups using a balanced assignment, with a target of five participants successfully completing treatment in each group. Groups were closed to enrollment once the target was reached, and recruitment continued until every group had reached its target. The random allocation sequence was concealed from all investigators until interventions were assigned. Participants, investigators, and care providers were all blinded to interventions.

Participants were recruited through advertisements placed in local newspapers, hospital and university bulletins, and posters, and by referral from other study participants. Exclusion criteria included Fitzpatrick skin types IV to VI, concurrent use of more than 800 IU of vitamin $D_3$ daily, chronic medical conditions, current or recent use of photosensitive medications, and women who were pregnant, nursing, or who anticipating becoming pregnant during the study period. Two Fitzpatrick skin type IV participants were excluded. Three participants withdrew before receiving the study drug, including one participant with a vagal response during a blood draw, and two participants who voluntarily withdrew. Two participants withdrew after receiving the study drug, including one participant with a vagal response during a blood draw, and one participant who voluntarily withdrew. Vitamin $D_3$ was administered as 50,000 IU capsules in doses of 50,000, 100,000, and 200,000 IU. Single vitamin $D_3$ doses up to 500,000 IU are considered safe and

capable of raising serum vitamin $D_3$ to therapeutic concentrations (Ilahi et al., 2008, Sanders et al., 2010). Adverse events were assessed, graded, and reported at each visit.

**Determination of the minimal erythema dose**

The MED is the amount of UVR that will produce minimally perceptible skin erythema a few hours after UVR exposure (Heckman et al., 2013). MED testing was performed by exposing eight $1cm^2$ areas on sun-protected buttock skin to increasing doses of UVR using plastic holed templates to ensure that adjacent skin was not irradiated. Total body exposure of a fair-skinned individual to one MED of UVR is approximately equal to the ingestion of vitamin $D_3$ at a dose of 10,000 to 25,000 IU (Holick, 2001). Thus, exposure of $1cm^2$ of skin in this protocol is unlikely to have contributed to an appreciable rise in serum vitamin $D_3$. The duration of irradiation was based on the participants' Fitzpatrick skin type, as previously described (Fitzpatrick, 1988). Erythema at each site was quantified 24hr after irradiation using a chromameter (CR300; Minolta, Ramsey, NJ). The erythema of unexposed skin at each time point served as a baseline. Spectrophotometers measure darkness ($L^*$), hue ($b^*$), and redness ($a^*$) of the skin) (Heckman et al., 2013). Larger $a^*$ values indicate greater erythema. A linear regression best-fit line was calculated from a plot of the erythema of exposed minus unexposed skin ($a^*_{exp} - a^*_{unexp}$) versus the log of the UVR exposure time. The MED is formally defined as the smallest UVR dose capable of producing $a^*_{exp} - a^*_{unexp}$ greater than or equal to 2.5. Solving the linear regression equation for the inverse log of x when $\Delta a^*$ of 2.5 yielded the exposure time in seconds that was required to produce the MED. The ultraviolet B (UVB) irradiance ($W/cm^2$) is the UVB output of a particular SSR devise, and was determined for each participant using a radiometer prior to MED testing. The MED dose ($mJ/cm^2$) of UVB is the product of the irradiance ($mJ/sec*cm^2$) and exposure time (sec). The MED dose of

UVB ($mJ/cm^2$) for each participant was calculated using the measured UVB irradiance of the device and the MED exposure time as calculated above.

**RNA expression and tissue microarray**

RNA (100 ng) was isolated using the Qiagen RNeasy Lipid Tissue Mini Kit (Qiagen Inc., Valencia, CA). TNF-α, iNOS, and arginase-1 mRNA expression was quantified using TaqMan Gene Expression Assays and the TaqMan RNA-to-$C_T$ 1-Step (Life Technologies, Grand Island, NY), as previously described (Au et al., 2015). Gene expression was normalized to the 18s RNA housekeeping gene. Samples were analyzed using a Step-One System (Biosystems, Grand Island, NY) based on the manufacturer's recommendations. For microarray analyses, the gene set of differentially expressed genes was restricted to transcripts with a fold change threshold ($T_{interv}$ / $T_{control}$) $\geq 1.5$ or $\leq 1.5$, thereby detecting genes that were reliably and differentially changed by study drug intervention.

**Immunofluorescence staining**

Primary antibodies, including mouse monoclonal CD163 (EDHu-1, Bio-Rad, Hercules, CA), and rabbit polyclonal arginase-1 (Novus Biologicals, Littleton, CO) were diluted 1:50 and 1:100 respectively in 10% goat serum in phosphate buffered saline. Arginase-1 was incubated for 1hr at room temperature, and CD163 was incubated overnight at 4 degrees Celsius. Secondary antibodies, including goat anti-mouse (Alexa Fluor 488) and goat anti-rabbit (Alexa Fluor 647) (Thermo Fisher, Grand Island, NY), were diluted 1:2000 in phosphate buffered saline. Eight micrometer tissue sections were imaged using a Leica DMI 6000 B inverted microscopy (Leica Microsystems, Wetzlar, Germany) with a Retiga Aqua blue camera (Q-Imaging, Vancouver,

British Columbia), and subsequently analyzed using Metamorph Imaging Software (Molecular Devices, Downinton, PA).

**Statistical analysis**

All participants completing both phases of the study and receiving the study drug were included in the per-protocol analysis. All statistical comparisons were made between the vitamin $D_3$ treatment groups and the placebo group. Significance of the canonical pathways represents the likelihood that genes in the differentially expressed gene set map to a particular process or pathway more than expected by random chance alone. The Bonferroni correction for multiple comparisons was not utilized given the small overall number of planned comparisons in the primary outcome analyses, as well as the exploratory nature of the post-hoc analyses performed to guide further investigation.

**Supplementary References**


Au L, Meisch JP, Das LM, Binko AM, Boxer RS, Wen AM, et al. Suppression of Hyperactive Immune Responses Protects against Nitrogen Mustard Injury. The Journal of investigative dermatology 2015;135(12):2971-81.

Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. Archives of dermatology 1988;124(6):869-71.

Heckman CJ, Chandler R, Kloss JD, Benson A, Rooney D, Munshi T, et al. Minimal Erythema Dose (MED) testing. Journal of visualized experiments : JoVE 2013(75):e50175.

Holick MF. Sunlight "D"ilemma: risk of skin cancer or bone disease and muscle weakness. Lancet (London, England) 2001;357(9249):4-6.

Ilahi M, Armas LA, Heaney RP. Pharmacokinetics of a single, large dose of cholecalciferol. The American journal of clinical nutrition 2008;87(3):688-91.

Sanders KM, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D, et al. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. Jama 2010;303(18):1815-22.