

SUPPLEMENTARY DATA**SUPPLEMENTARY TABLES**

Table S1. Read alignment statistics. The number of uniquely aligned reads per sample is provided for both ATAC-seq and RNA-seq datasets.

Table S2. Transcriptomic changes in PBMCs. PBMCs were isolated at day 0, and day 84 of 17 MS patients and 42 healthy individuals. RNA-seq analysis identified 13,041 expressed protein-coding genes (CPM > 1).

Table S3. Epigenomic changes in PBMCs. PBMCs were isolated at day 0, and day 84 of 10 MS patients and 29 healthy individuals. ATAC-seq analysis identified 18,998 prominent regions of accessible chromatin (CPM > 10).

Table S4. GO enrichment analysis of transcriptomic and epigenomic gene sets. GO Biological Process enrichment analyses were performed for MS-associated differentially expressed genes, vitamin D₃-responsive genes identified in each supplementation group, genes overlapping between RNA-seq and ATAC-seq analyses, genes associated with differentially accessible chromatin regions, and prioritized VDR-associated candidate target genes. For each gene set, significantly enriched GO terms are listed together with the corresponding GO identifier, gene count, enrichment statistics, and adjusted p-values.

SUPPLEMENTARY FIGURES

Figure S1. Clustering of MS-associated transcriptomic signatures at baseline and after vitamin D₃ supplementation. Heatmaps of the 1,480 genes differentially expressed between MS patients and healthy individuals (FDR < 0.05) at baseline (**A**, day 0) and after 84 days of supplementation (**B**, day 84). RNA-seq data were obtained from 17 MS patients and 42 healthy individuals. Expression values represent CPM-normalized counts subjected to per-gene min-max normalization (0-1 scaling). Genes were grouped into ten clusters based on hierarchical clustering using Euclidean distance and complete linkage. Samples are shown in the same order as in **Figure 2A**.

Figure S2. Expression of HLA genes in MS patients and healthy individuals. CPM-normalized expression levels of class I (*HLA-A*, *HLA-B*, *HLA-C*, *HLA-E*, *HLA-F*, *HLA-G*) and class II (*HLA-DMA*, *HLA-DMB*, *HLA-DOA*, *HLA-DOB*, *HLA-DPA1*, *HLA-DPB1*, *HLA-DQA1*, *HLA-DQB1*, *HLA-DQB2*, *HLA-DRA*, *HLA-DRB1*, *HLA-DRB5*) HLA genes in MS patients (n = 17) and healthy individuals (n = 42) at baseline. Boxes indicate median and interquartile range; dots represent individual samples.

Figure S3. Volcano plots of vitamin D-responsive genes identified by RNA-seq. Volcano plots showing differential gene expression between day 0 and day 84 in MS patients receiving regular vitamin D₃ supplementation (**A**, n = 7), and healthy individuals receiving monthly vitamin D₃ bolus supplementation (**B**, n = 42). The x-axis shows log₂FC and the y-axis shows -log₁₀(p-value). Red and blue dots indicate significantly upregulated and downregulated genes, respectively, defined by nominal p < 0.05 and |log₂FC| > 0.25; gray dots indicate non-significant genes. Numbers of regulated and non-significant genes are indicated in each panel.

Figure S4. Estimated immune-cell composition of PBMC samples. Estimated fractions of monocytes, T cells, B cells, and NK cells in PBMC samples from healthy individuals, MS patients receiving regular vitamin D₃ supplementation, and MS patients receiving daily high-dose vitamin D₃ supplementation at day 0 and day 84. Cell fractions were inferred from RNA-seq data using transcriptome-based

deconvolution. Boxes indicate median and interquartile range; dots represent individual samples. NS, not significant.

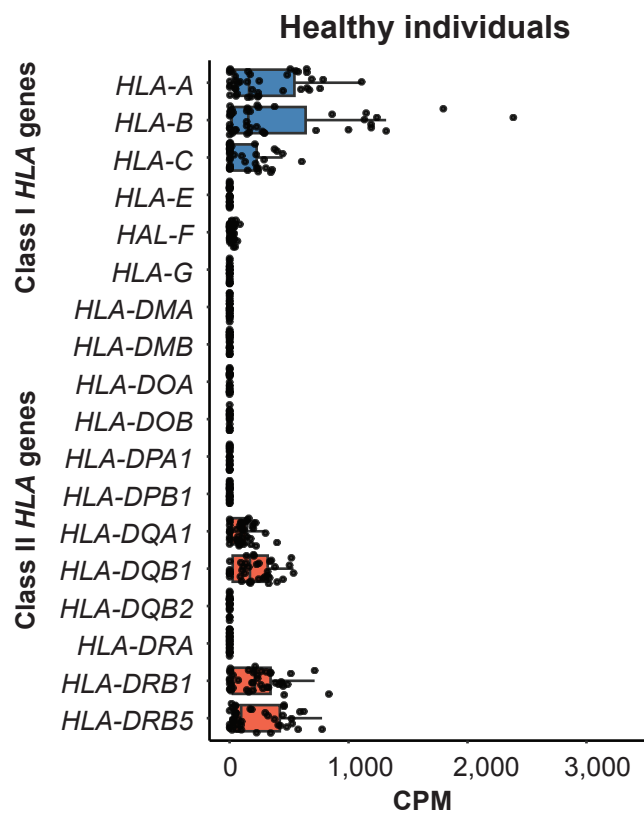
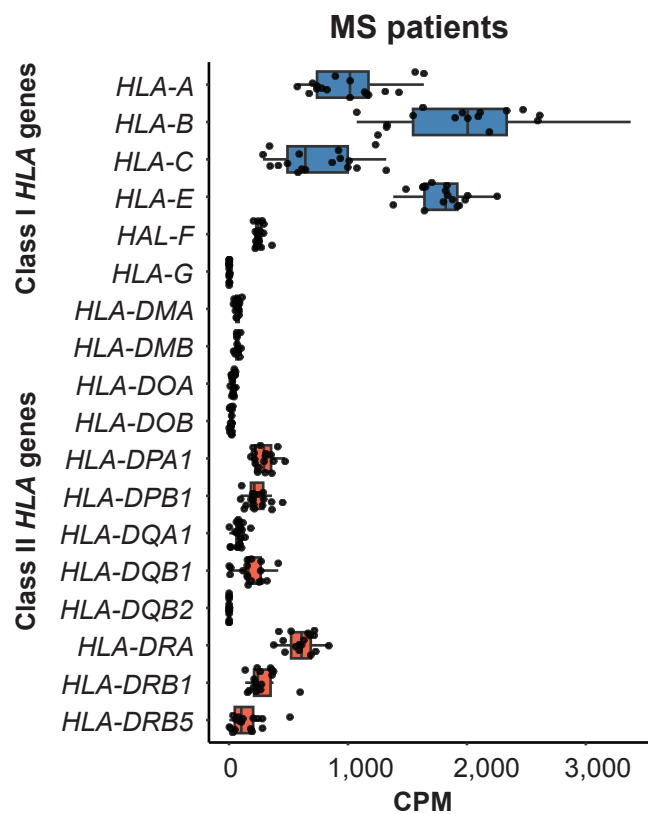
Figure S5. MDS scaling analysis of transcriptomic profiles before and after vitamin D₃ supplementation. MDS scaling plots based on RNA-seq expression profiles from MS patients receiving daily high-dose vitamin D₃ supplementation (**A**), MS patients receiving regular vitamin D₃ supplementation (**B**), and healthy individuals receiving monthly vitamin D₃ bolus supplementation (**C**). Analyses were performed using either all expressed genes (left panels) or vitamin D target genes (right panels). Blue circles indicate baseline samples (day 0) and red triangles indicate samples collected after 84 days of supplementation (day 84). Numbers identify individual participants.

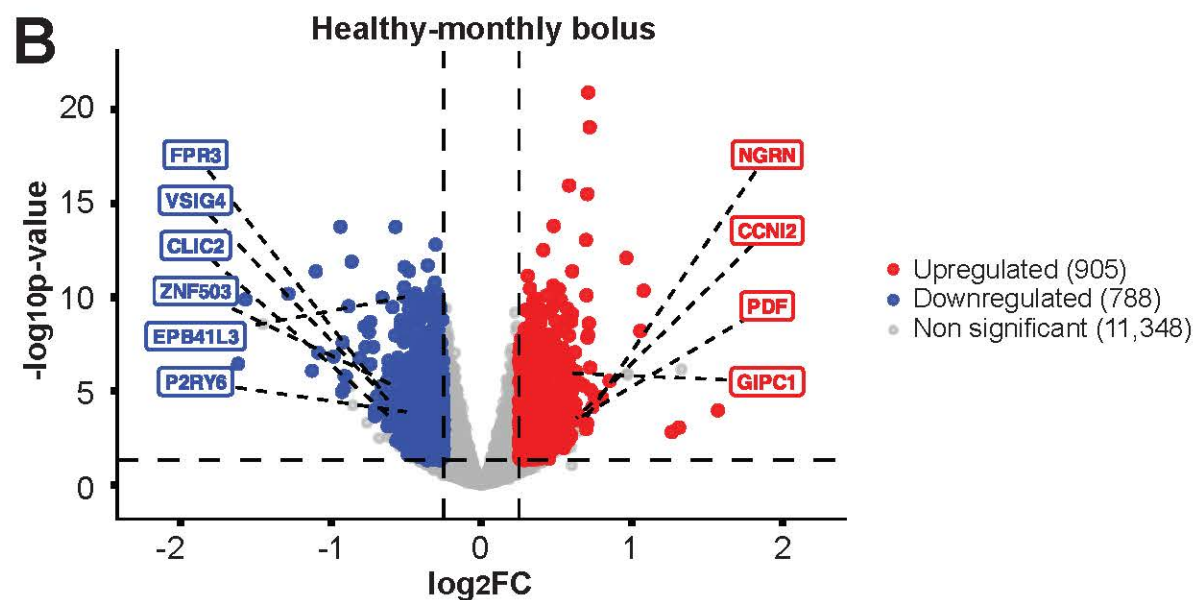
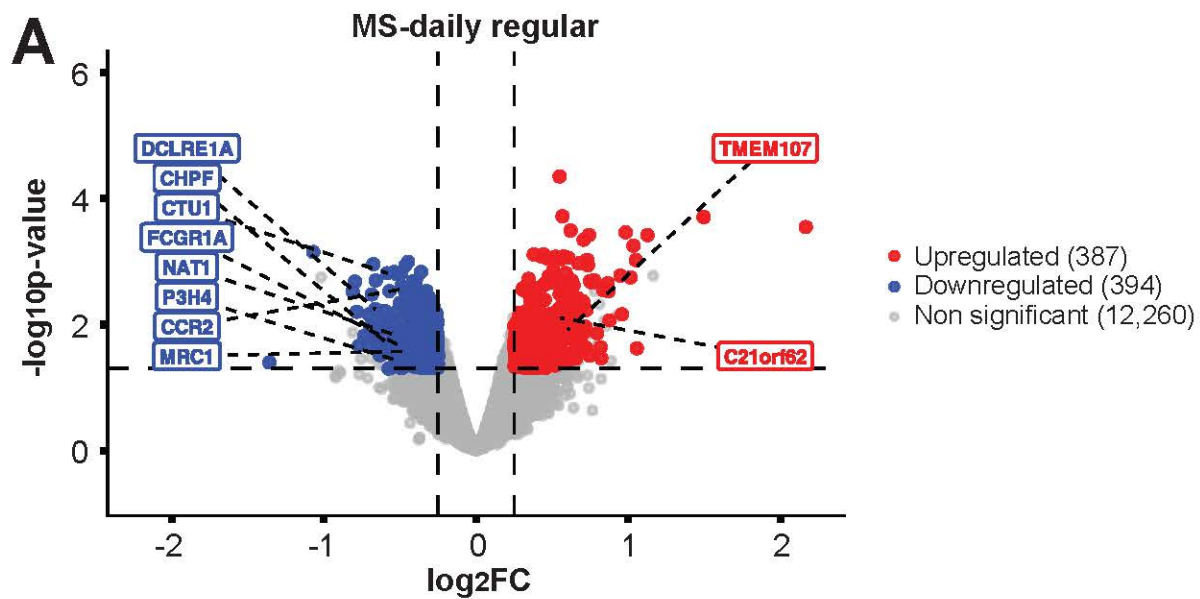
Figure S6. Correlation between changes in serum 25(OH)D₃ concentrations and gene expression. Correlation analysis between changes in serum 25(OH)D₃ concentrations ($\Delta 25(\text{OH})\text{D}_3$, day 84 minus day 0) and corresponding changes in gene expression ($\Delta \text{expression}$, day 84 minus day 0) in MS patients receiving daily high-dose vitamin D₃ supplementation. Selected genes showing Pearson correlation coefficients $|R| > 0.30$ are displayed. Each point represents one individual participant.

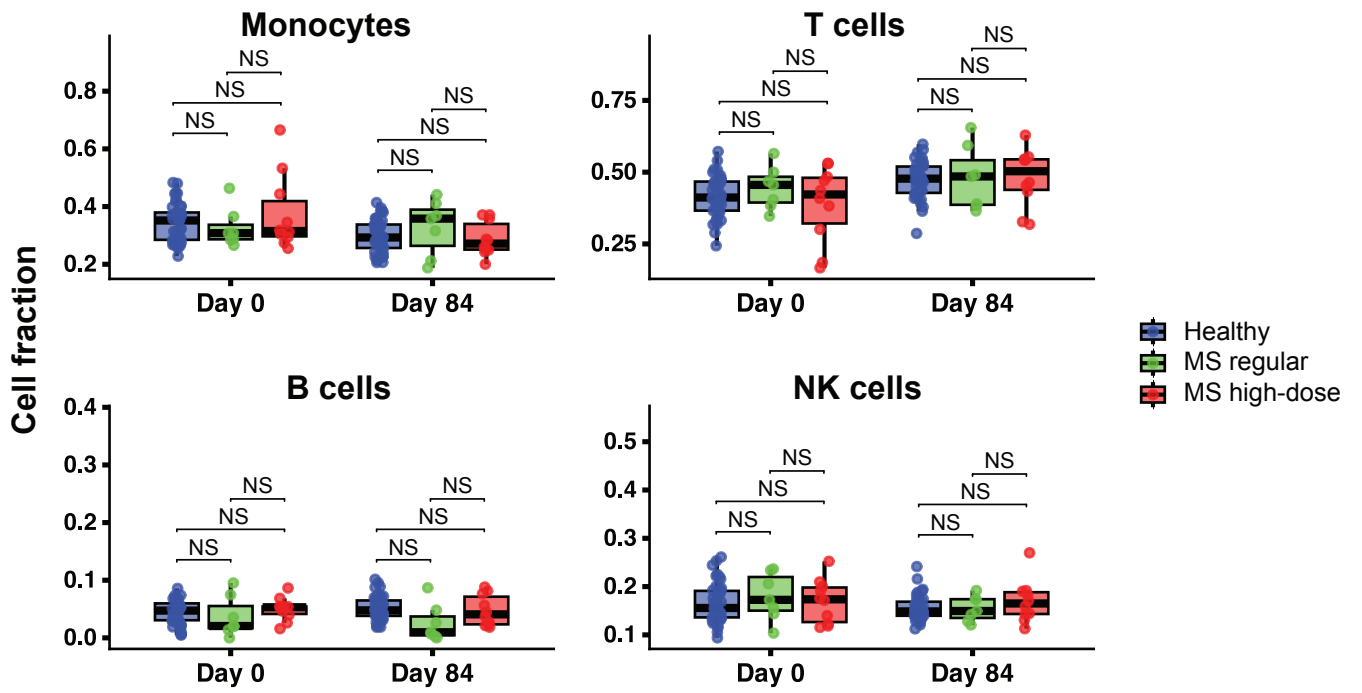
Figure S7. STRING protein-protein interaction network of MS-associated vitamin D-responsive genes. The network comprises 45 genes identified by overlap between the 478 vitamin D-responsive genes detected in MS patients receiving daily high-dose vitamin D₃ supplementation and the 1,480 genes differentially expressed between MS patients and healthy individuals at baseline. Nodes represent proteins encoded by the overlapping genes, and edges indicate known or predicted protein-protein interactions retrieved from the STRING database. Selected immune-regulatory and MS-relevant genes discussed in the manuscript are highlighted in red (*CR1*, *HLA-DPB1*, and *ZAP70*). The network is presented as a descriptive visualization of functional relationships among MS-associated vitamin D-responsive genes and does not imply causal interactions.

Figure S8. Global chromatin accessibility profiles in MS patients and healthy individuals. Heatmap showing normalized ATAC-seq accessibility values for 18,998 chromatin regions with CPM > 10 in PBMC samples from MS patients (n = 10) and healthy individuals (n = 29) at baseline. Accessibility values were normalized using the DiffBind workflow. Peaks and samples were hierarchically clustered using Euclidean distance and complete linkage. Red and blue colors indicate increased and decreased chromatin accessibility, respectively.

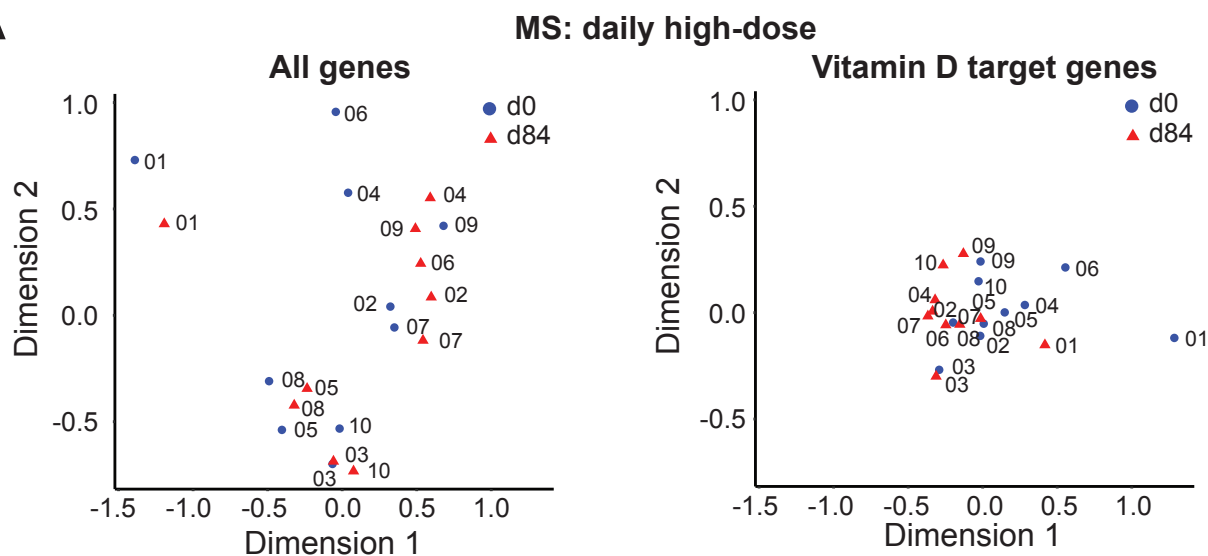
Figure S9. Differential chromatin accessibility across ATAC-seq comparisons. Volcano plots showing differential chromatin accessibility ($\log_2FC$ versus $-\log_{10}(p\text{-value})$) for MS patients receiving daily high-dose vitamin D₃ supplementation (**A**, n = 6 paired samples), MS patients receiving regular vitamin D₃ supplementation (**B**, n = 4 paired samples), healthy individuals receiving monthly vitamin D₃ bolus supplementation (**C**, n = 29 paired samples), and the baseline comparison between MS patients (n = 10) and healthy individuals (n = 29) (**D**). Differential accessibility was assessed using DiffBind with DESeq2-based statistical testing. Red dots indicate regions with increased accessibility (opening), blue dots indicate regions with decreased accessibility (closing), and gray dots indicate non-significant regions. The number of FDR-significant regions (FDR < 0.05) is indicated in each panel. No FDR-significant accessibility changes were observed in the longitudinal MS analyses, whereas robust accessibility differences were detected between MS patients and healthy individuals and following monthly vitamin D₃ bolus supplementation in healthy individuals.



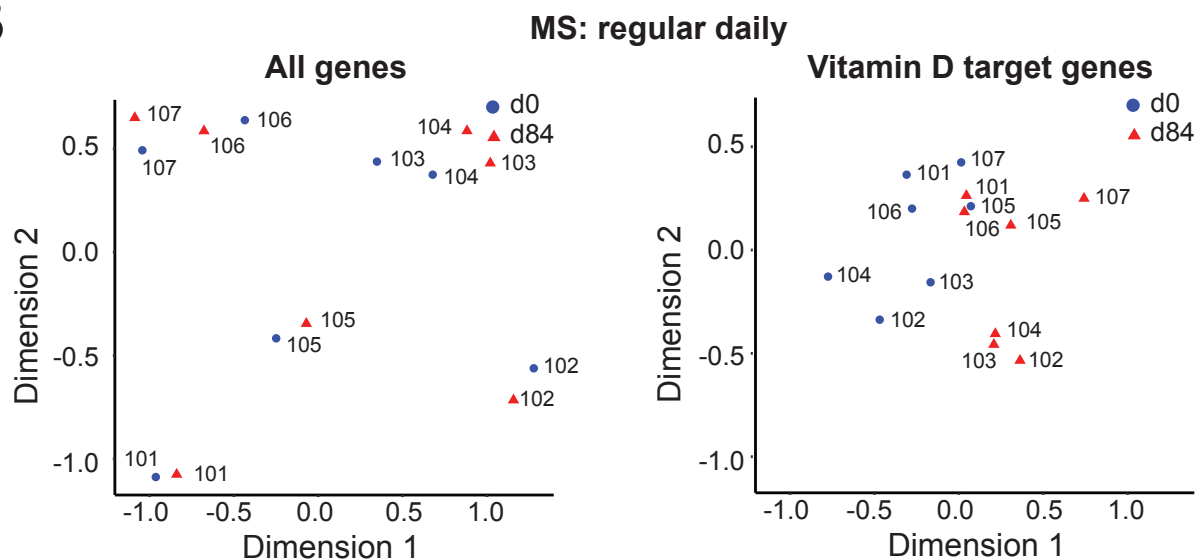




A



B



C

