Title:

The effect of high doses of vitamin D supplementation on dengue virus replication, Toll-like receptor expression, and cytokine profiles on dendritic cells

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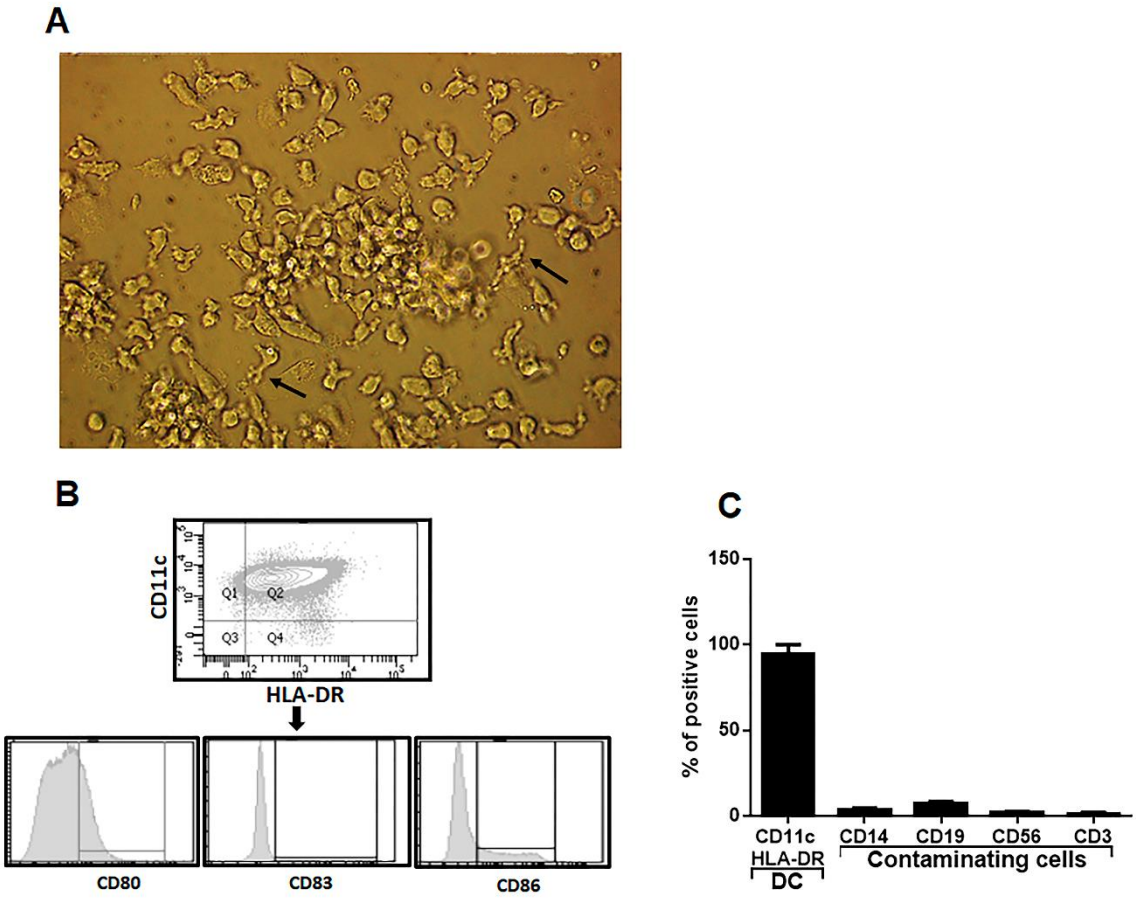
Supplementary 1. Primers and annealing temperature

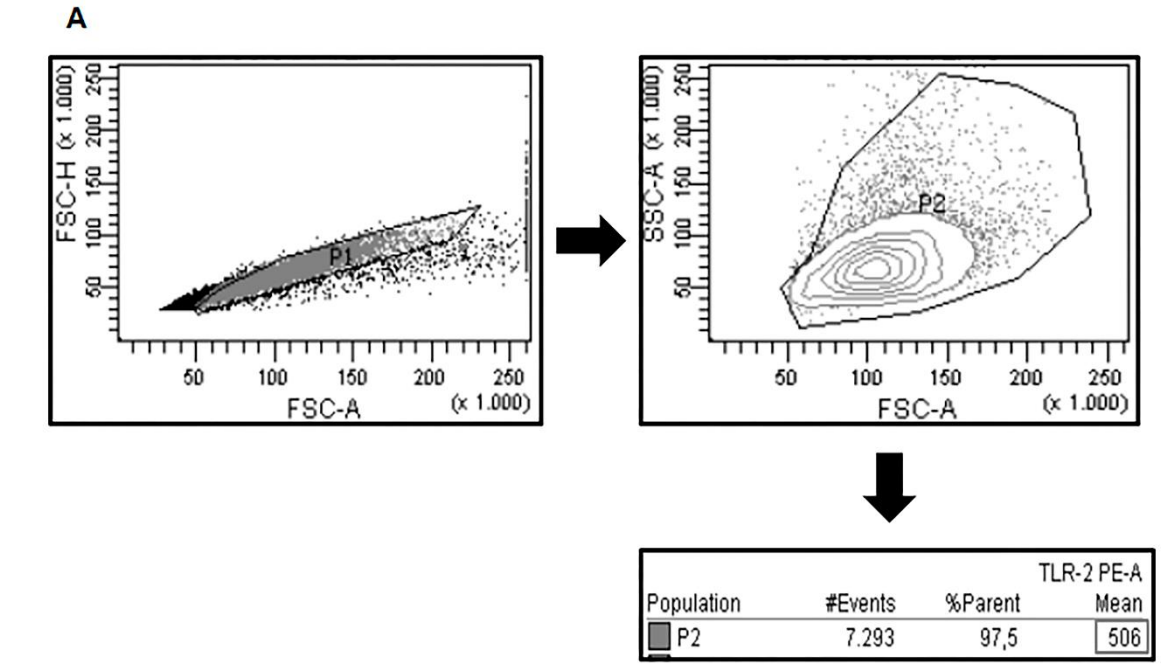
mRNA	Orientation	Primers 5'⟶ 3'	Annealing temperature (°C)
TLR2	Forward	GCTGCTCGGCGTTCTCTCAGG	65
	Reverse	TGTCCAGTGCTTCAACCCACAACT	
TLR3	Forward	ATTGGGTCTGGGAACATTTCTCTTC	63
	Reverse	GTGAGATTTAAACATTCCTCTTCGC	
TLR7	Forward	TCTACCTGGGCCAAAACTGTT	63
	Reverse	GGCACATGCTGAAGAGAGTTA	
TLR9	Forward	TTATGGACTTCCTGCTGGAGGTGC	63
	Reverse	CTGCGTTTTGTCGAAGACCA	
β-Actin	Forward	ATCTGGCACCACACCTTCTACAATGA	60
	Reverse	CGTCATACTCCTGCTTGCTGATCCAC	

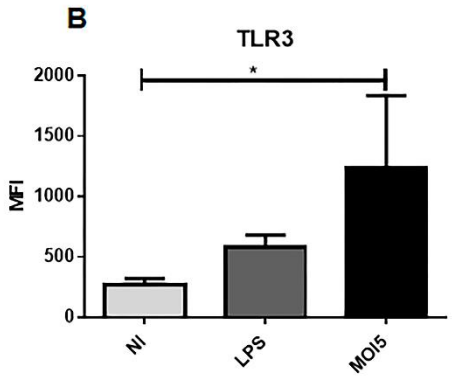
Supplementary 2

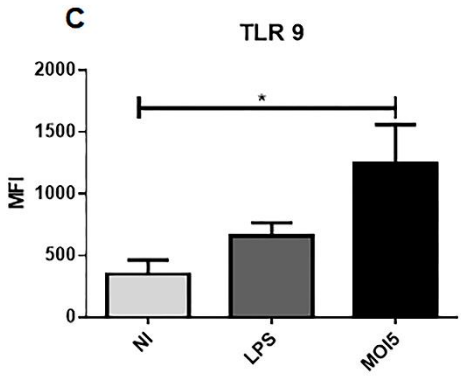


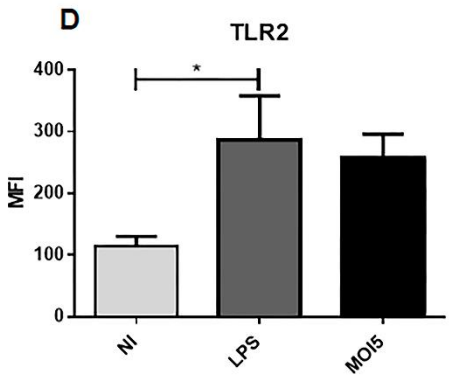
Supplementary 2. Morphology, phenotype and purity of MDDCs. CD14+ cells were differentiated for 6 days in the presence of 750 U / ml GM-CSF and 500 U / ml IL-4. In (A) the morphology of the cells is shown; they have extensions or dendrites (see arrows) and aggregate formation. Cells were harvested and stained to determine their phenotype by flow cytometry; (B) represents a representative example of 5 independent experiments on the way the flow cytometric analysis was done after the acquisition of 50,000 events. (C) Shows the accumulated 4 independent experiments and the percentage of expression of the evaluated markers and the purity of the MDDCs

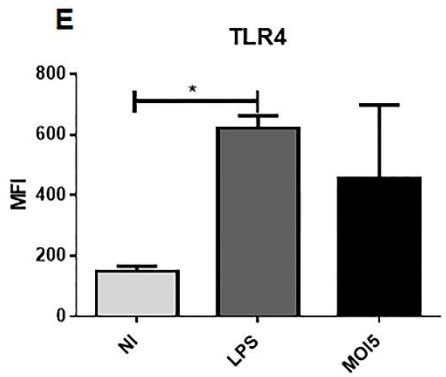
Supplementary 3











Supplementary 3. DENV-2 infection of the MDDCs induces alteration in the expression of TLRs. In (A) we show the gatting strategy to determine the expression density by quantifying the mean fluorescence intensity (MFI) of TLRs in the population. The MDDCs were stimulated with LPS (10ng / ml) or infected with DENV-2 (MOI 5) for 48 hours. After the stimulation the MDDCs were collected and the MFI for (B) TLR3, (C) TLR9, (D) TLR2, (E) and TLR4 was determined by flow cytometry using as control the MFI emitted by uninfected MDDCs after 48 hours of incubation. To determine the statistical differences between the groups we used a Kruskal-Wallis test, the error bars show the median and interquartile range. * $p < 0.05$ **, $p < 0.01$, *** $p < 0.001$; $n = 3$

Supplementary 4

A. Possible VDRE in the TLR3 gene sequence

Model ID	Model name	Score	Relative score	Start	End	Strand	predicted site sequence
MA0074.1	RXRA::VDR	10.826	0.804366715572581	1552	1566	1	GGATCAAGAGGTTAA
MA0693.1	Vdr	3.469	0.808082688078768	2802	2817	1	AAGGTTGCAAAGTTTA
MA0693.1	Vdr	5.109	0.821798938757165	3122	3137	-1	AAATTCAGTAAGTTTA
MA0693.1	Vdr	3.070	0.804745624651523	9147	9162	1	AAGTTTATGGAGGTAA
MA0074.1	RXRA::VDR	11.280	0.813526734251219	9597	9611	-1	AGATCATCGGGTACC
MA0693.1	Vdr	4.494	0.816655344752766	12009	12024	1	GGGGGCATTGGTGTCA
MA0074.1	RXRA::VDR	11.070	0.809289721206034	16367	16381	-1	GGTTCAATGACTTTA
MA0693.1	Vdr	7.309	0.840198787228185	16367	16382	-1	AGGTTCAATGACTTTA
MA0693.1	Vdr	14.085	0.896870320518928	16629	16644	-1	GAGTTCAAAGGGGGCA
MA0693.1	Vdr	8.129	0.847056912567384	16742	16757	1	CAGTACATCGAGTTCT

B. Possible VDRE in the TLR7 gene sequence

Model ID	Model name	Score	Relative score	Start	End	Strand	predicted site sequence
MA0693.1	Vdr	5.577	0.825713088341	4226	4241	1	GAGTTCACTAGATTTG
MA0693.1	Vdr	5.055	0.821347306112876	11654	11669	-1	AGGTTCCCAGAGTTGA
MA0074.1	RXRA::VDR	11.070	0.809289721206034	12030	12044	-1	GAGTTAATGGGTACA
MA0693.1	Vdr	2.609	0.800890020040096	15447	15462	1	AGGTCCGCTTGGTGCA
MA0693.1	Vdr	6.992	0.837547536334861	17575	17590	1	GGCTGCACCCAGTTCA
MA0074.1	RXRA::VDR	11.251	0.812941622925932	17857	17871	-1	GGGTCAGGGAATTCC
MA0693.1	Vdr	4.181	0.814037548129389	17929	17944	-1	CAGGACAGTGGGTGCA

C. Possible VDRE in the TLR9 gene sequence

Model ID	Model name	Score	Relative score	Start	End	Strand	predicted site sequence
MA0074.1	RXRA::VDR	11.421	0.816371585867272	1997	2011	-1	GGGGCACAGACTTCA
MA0074.1	RXRA::VDR	12.006	0.828174693636002	3047	3061	-1	GGTTGATGAAGTTCA
MA0693.1	Vdr	5.847	0.827971251562443	3047	3062	-1	TGGTTGATGAAGTTCA
MA0693.1	Vdr	2.529	0.80022093464115	4151	4166	-1	AAGTCCATAAAGGCCG

Supplementary 4. Prediction of possible VDRE in the TLR3, 7 and 9 gene sequence. With the help of the JASPAR database, VDRE prediction was performed on the promoter or coding sequence of the TLRs regulated by VitD supplementation, finding 10 possible binding sites for TLR3 (A), 7 for TLR7 (B) and 4 for TLR9 (C)