

# **Different Mode of Induction of Galectin-9 and Osteopontin in THP-1 Cells upon Dengue Virus Infection *In Vitro***

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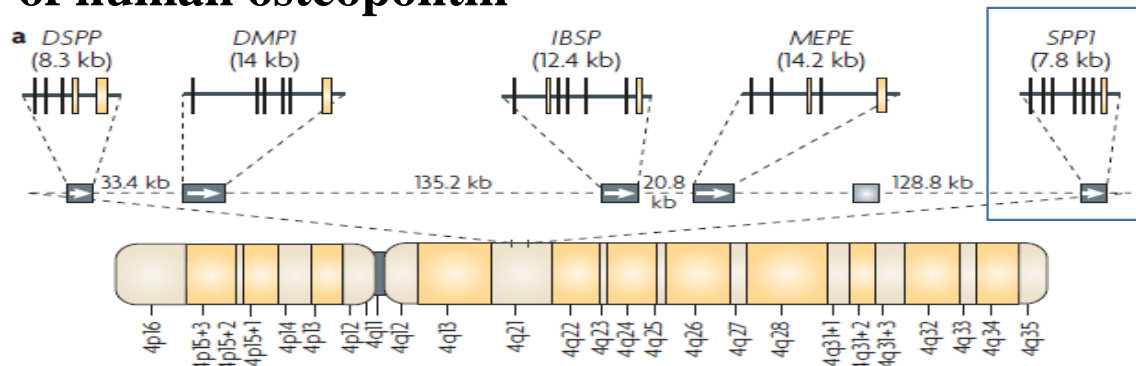
## **MCPs in infectious diseases.**

A family of secreted extracellular matrix (ECM) proteins which influence on cell-matrix interactions was termed as “matricellular” proteins (MCPs).

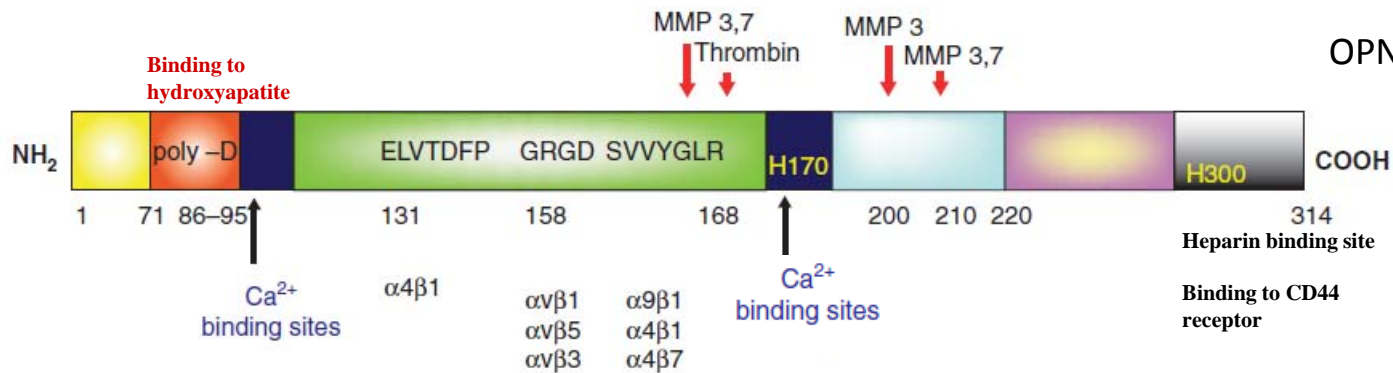
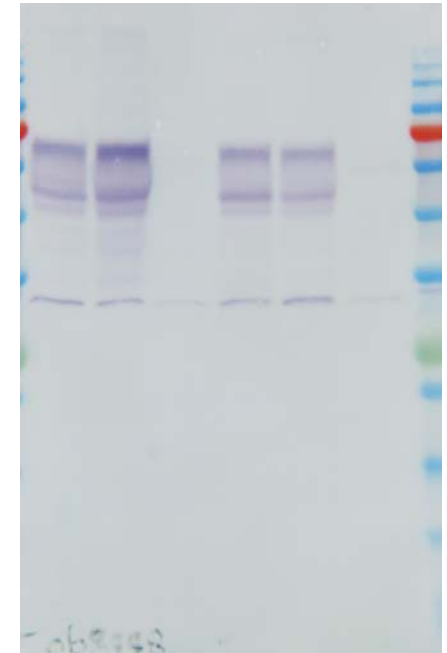
MCPs include connective-tissue growth factors, galectins and osteopontin (OPN).

The multitasking aspects of MCPs are derived from the different structural proteins, cell-surface receptors, proteases, and cytokines with which these proteins come into contact in the local environment of various tissues.

# Gene structure and domain organization of human osteopontin



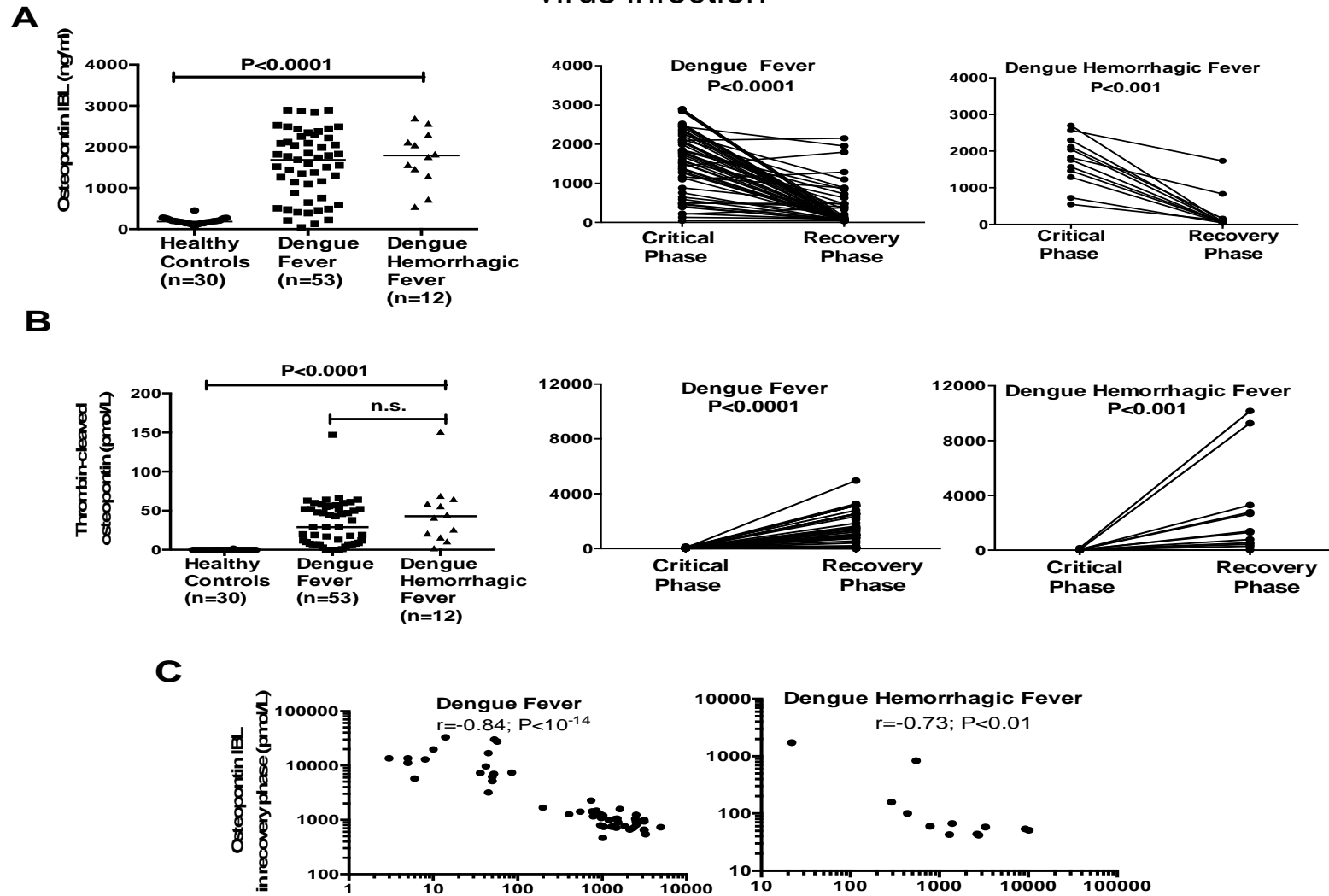
Bellahcène A et al., Nature, 2008



OPN in THP-1 cells

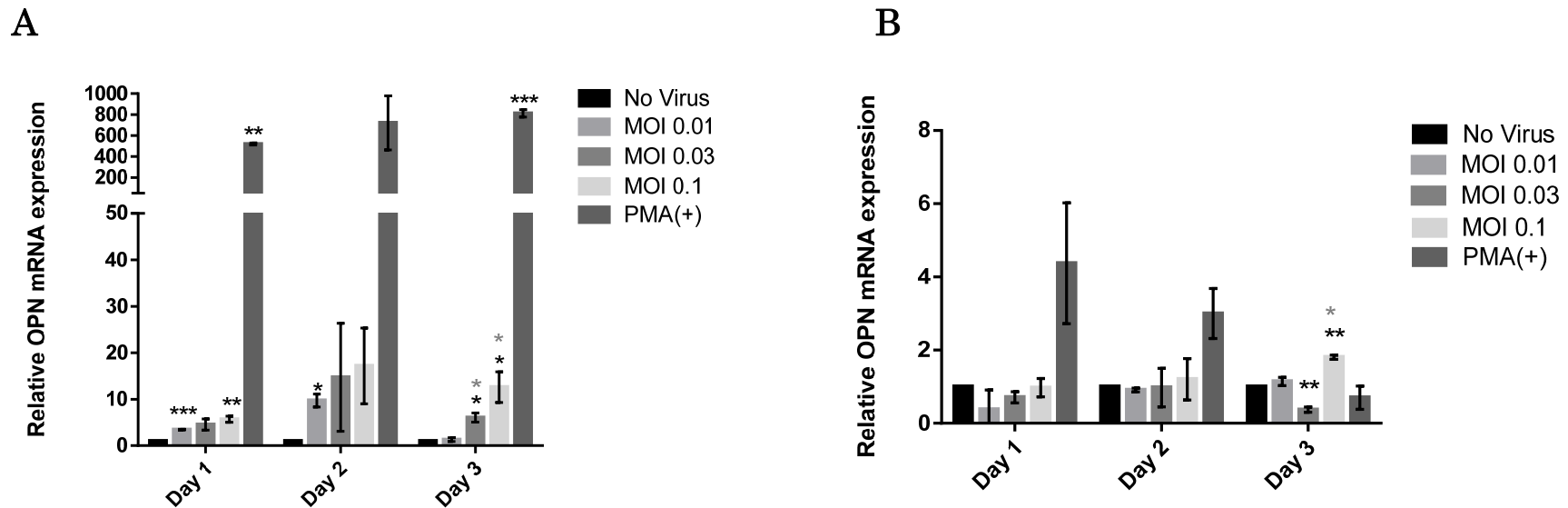
Ahmed et al., Expert Opin. Ther. Targets, 2001

# Elevated plasma Levels of osteopontin and N-half osteopontin in dengue virus infection



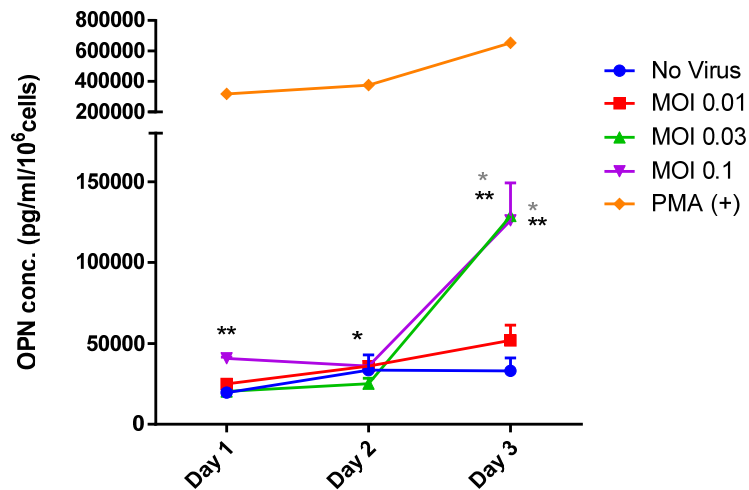
# Experiment conditions

Cell line	THP-1	293T
Cell type	Human monocytes	Embrionic kidney
Growth medium	RPMI-1640 10% FBS	DMEM 10% FBS
Cell number (at infection time)	2 x 10E5 cells	3.2 x 10E5 cells
Type of cell culture flasks/dishes/plates/tubes	Cell culture tube	12-wells tissue culture plates
Total volume of medium	1 mL	2 mL
Virus type	DENV 3 (MOI 0.001, 0.003, 0.005); 1, 3, and 5 days incubation	
Purification kit	Maxwell 16 LEV SimplyRNA Cells Kit	
ELISA kit	Human OPN DuoSet ELISA R&D Systems	
RT-PCR kit	RNA Ultrasense One-Step Quantitative RT-PCR System	

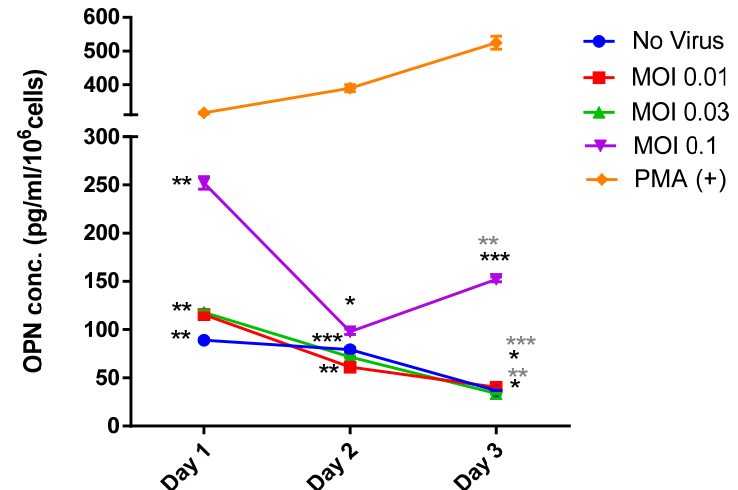


**Figure 1. MOI- and time-dependent change in *OPN* mRNA expression upon DENV infection.** (A) THP-1 and (B) 293T cells were infected with DENV-3 at various MOIs (range: 0.01–0.1). PMA was used as a positive control. Cells were harvested daily (1–3 days). Total RNA was prepared from cell lysates and *OPN* levels were determined by RT-qPCR. *GAPDH* was used as reference gene to normalize the expression level. The table summarizes statistical analysis, \* $P < 0.005$ , \*\* $P < 0.001$ , \*\*\* $P < 0.0005$  vs. control (uninfected cells; unpaired two-tailed t test). Data represent mean  $\pm$  SEM. § and § § represent MOI- and time-dependent variables

A



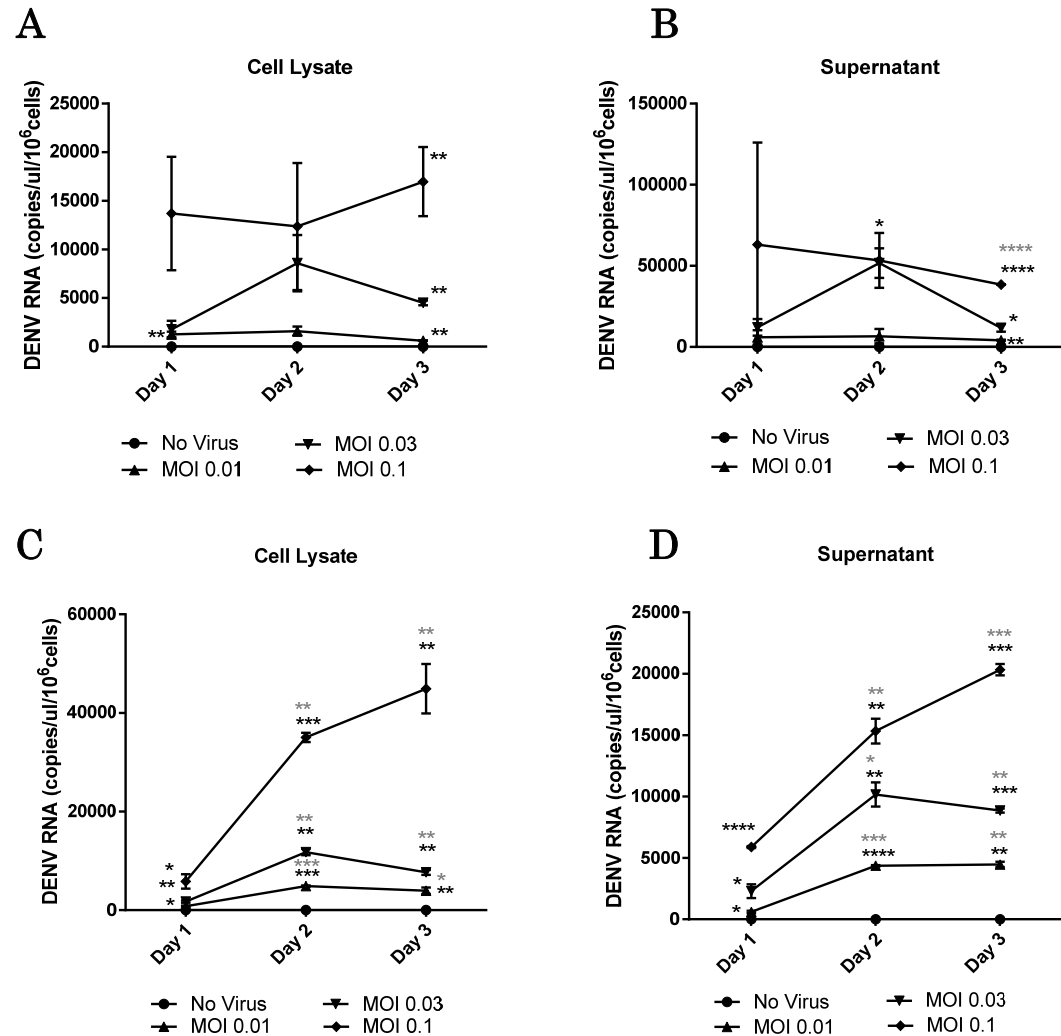
B



**MOI- and time-dependent change in OPN protein expression upon DENV infection.** (A) THP-1 and (B) 293T cells were infected with DENV-3 at various MOIs (range: 0.01–0.1). PMA was used as a positive control. Normalized OPN levels in each sample are expressed as pg/ml/10<sup>6</sup> cells., \*P < 0.005, \*\*P < 0.001, \*\*\*P < 0.0005 vs. control (uninfected cells; unpaired two-tailed t test)

### Figure 3. DENV RNA copy number in infected cell lines.

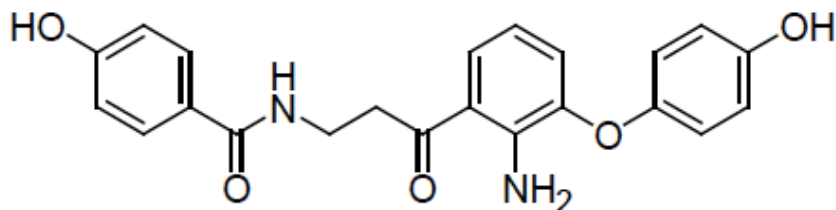
(A, B) THP-1 and (C, D) 293T cells were left uninfected or infected with DENV-3 at various MOIs (range: 0.01 – 0.1). Total RNA was extracted from cell lysates and the culture supernatant and DENV genome copy number was determined by RT-qPCR., \*P < 0.005, \*\*P < 0.001, \*\*\*P < 0.0005, \*\*\*\*P < 0.0001 vs. control..



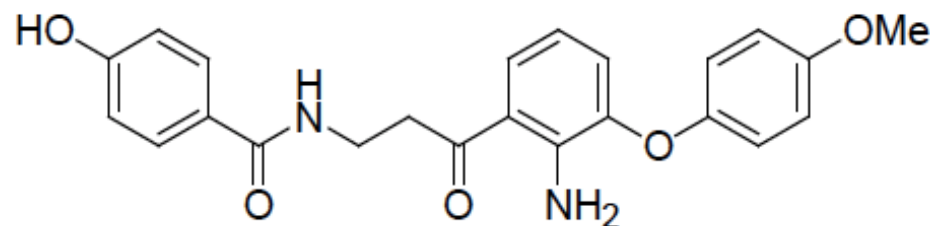


## A glimpse of chemical compounds 40 and 62

- A new aromatic amide, brefelamide, was isolated from methanol extracts of the fruiting bodies of *Dictyostelium brefeldianum* and *D. giganteum* (slime molds).

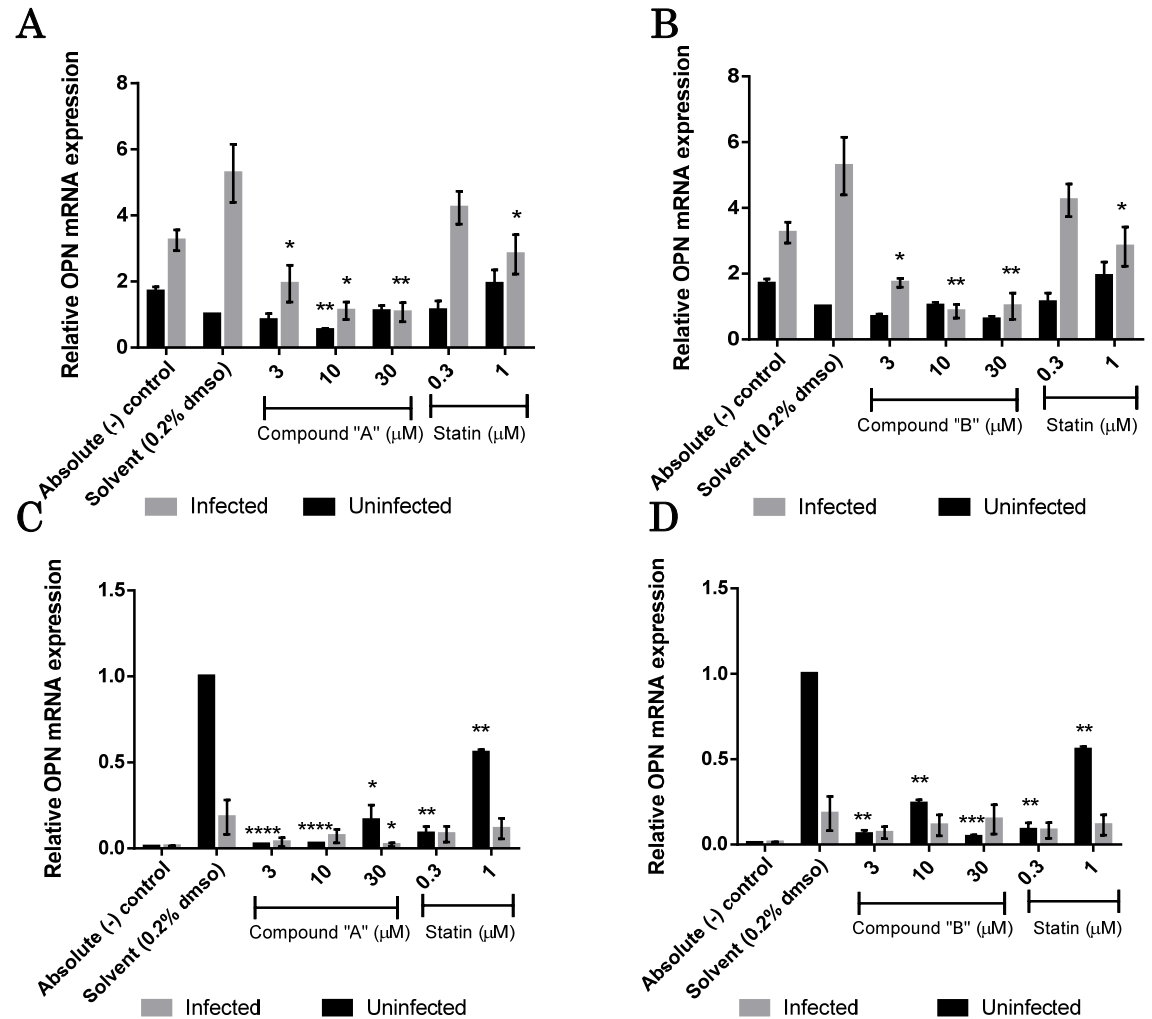


Compound A



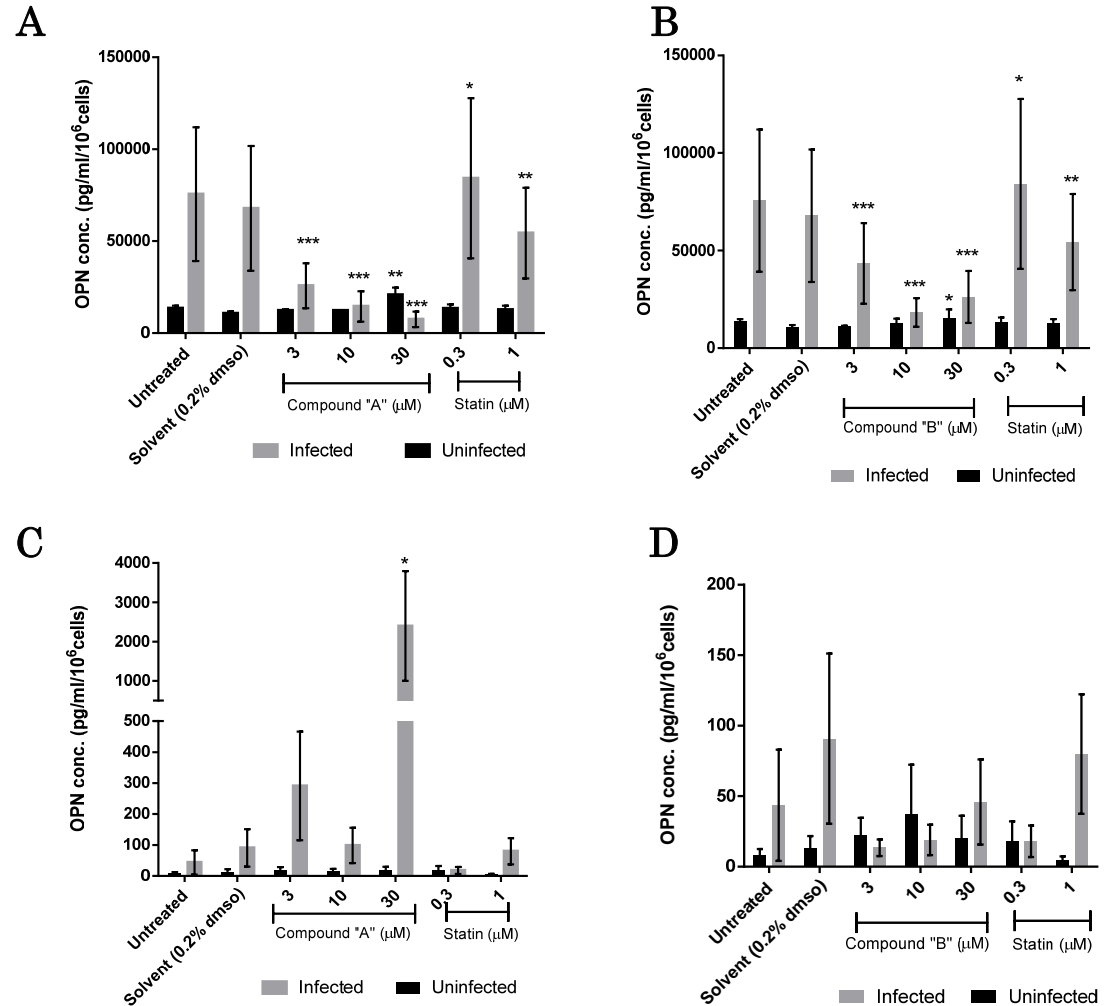
Compound B

**Figure 4. Changes in *OPN* mRNA expression by treatment with compounds A and B in DENV-infected cells.** Uninfected and DENV-infected THP-1 (A,B) or 293T (C,D) cells were left untreated or treated with compound A or compound B (range: 3–30  $\mu$ M), or statin (range: 0.3–1  $\mu$ M) for 72 h. \* $P < 0.005$ , \*\* $P < 0.001$ , \*\*\* $P < 0.0005$ , \*\*\*\* $P < 0.0001$  vs. control (0.2% DMSO; unpaired two-tailed t test). Data represent mean  $\pm$  SEM.



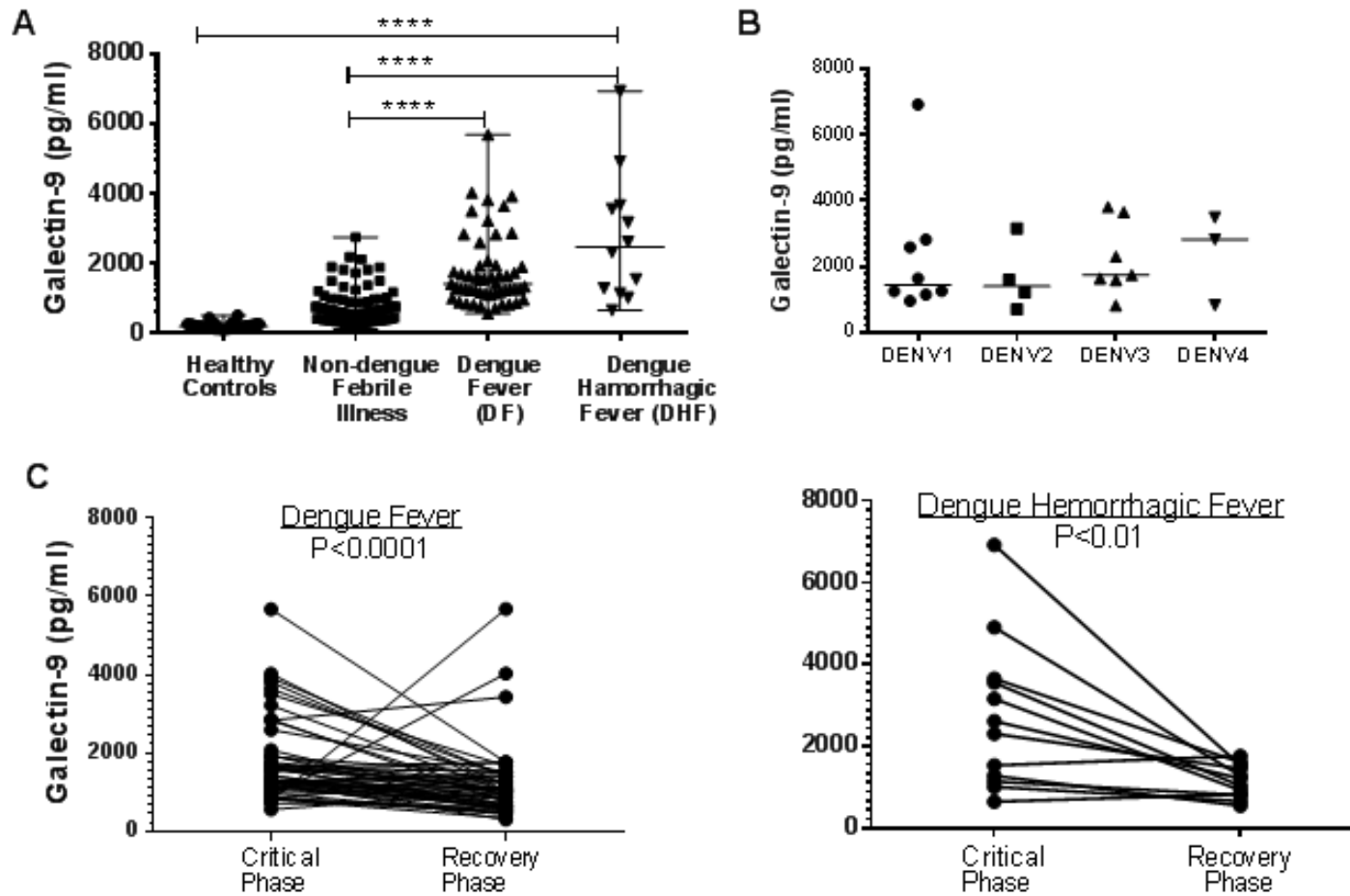
## Figure 5. Effect of compounds A and B on OPN protein level in DENV-infected cells.

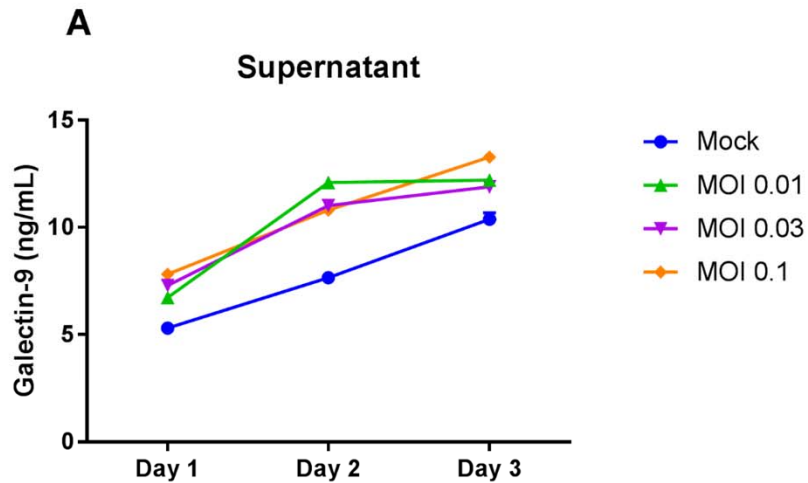
Uninfected and DENV-infected THP-1 (A,B) or 293T (C,D) cells were left untreated or treated with compound A or compound B (range: 3–30  $\mu\text{M}$ ), or statin (range: 0.3–1  $\mu\text{M}$ ) for 72 h. \* $P < 0.005$ , \*\* $P < 0.001$ , \*\*\* $P < 0.0005$  vs. control (0.2% DMSO; unpaired two-tailed t test). Data represent mean  $\pm$  SEM.



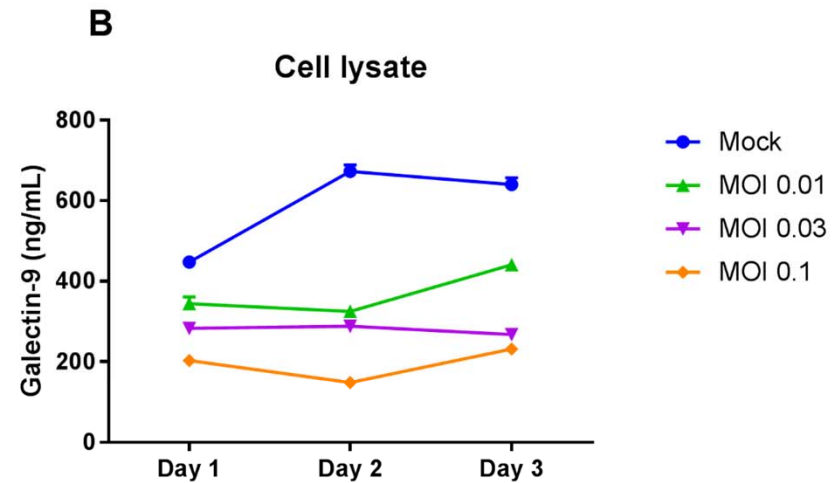
# Plasma levels of galectin-9 in dengue virus infected individuals

J Clin Virol 2013



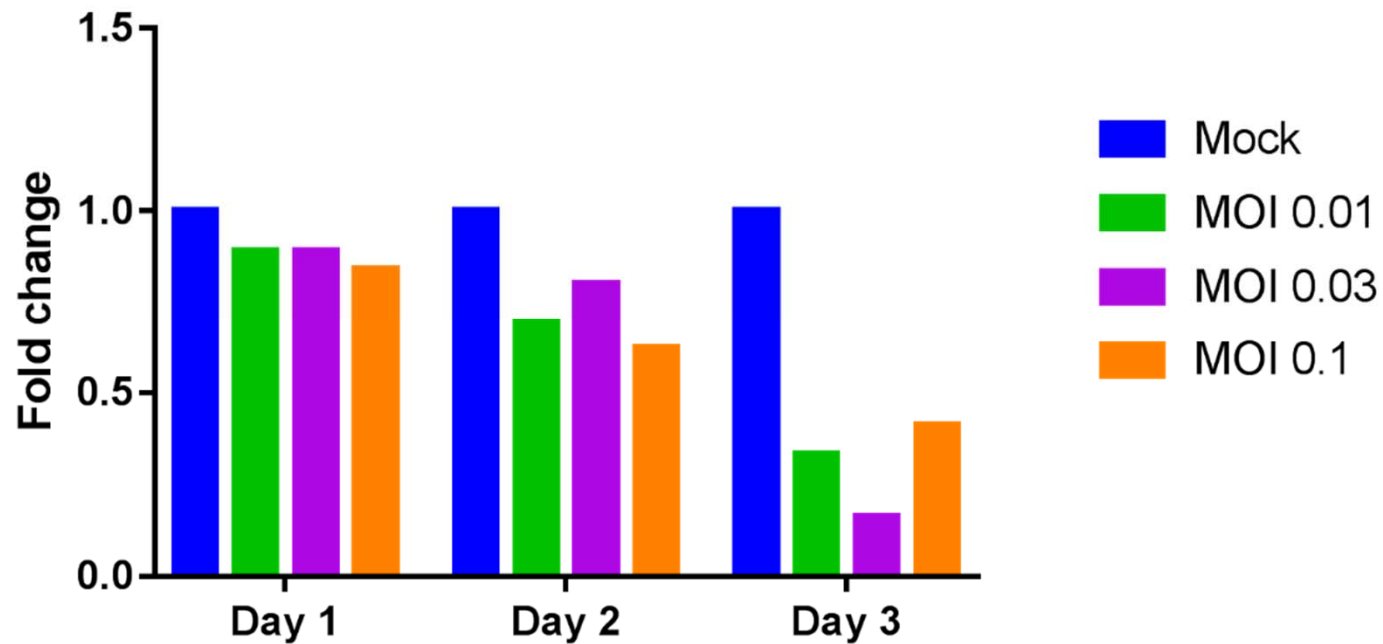


		Mock	MOI 0.01	MOI 0.03	MOI 0.1
Day 1	§	-	**	**	***
	§§	-	-	-	-
Day 2	§	-	***	**	**
	§§	***	***	**	**
Day 3	§	-	*	ns	*
	§§	**	**	**	**



		Mock	MOI 0.01	MOI 0.03	MOI 0.1
Day 1	§	-	*	**	***
	§§	-	-	-	-
Day 2	§	-	**	**	***
	§§	**	ns	ns	**
Day 3	§	-	**	**	**
	§§	**	*	ns	ns

Galectin-9 levels in dengue virus-infected THP-1 cells. Cells were mock-infected or infected with dengue virus-3 (DENV-3) at varying doses. Cells were incubated and harvested after 1, 2 and 3 days post-infection. Extracellular (**A**) and intracellular (**B**) galectin-9 was assayed using ELISA. Unpaired *t*-test was used to calculate significance (ns = no significance, \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$  and \*\*\*\*  $p \leq 0.0001$ ) vs. control.



Relative expression of galectin-9 mRNA. THP-1 cells were uninfected, or infected with DENV-3 at varying doses. Cells were incubated and harvested after 1, 2 and 3 days post-infection. Total RNA from cell lysates was extracted and reverse transcription quantitative RT-PCR was used to determine the expression of gal-9 mRNAs. The mRNA levels of gal-9 were normalized to GAPDH mRNA.

# Conclusion

- THP-1 constitutively expressed OPN mRNA and protein which was enhanced by DENV-3 infection.
- Brefelamide and its derivative isolated from slime mold fruiting bodies suppressed OPN production in DENV-3 infected THP-1 cells.
- DENV-3 infection increased gal-9 levels in the supernatant, while reduced in the cells.
- Decreased expression of *LGALS9* mRNA, together implicated gal-9 as a potential DAMP in DENV infection.
- MCP proteins are released by distinct mechanisms in THP-1 cells by DENV infection.

# Acknowledgement

- We thank Prof. Vivek Nerurkar (Hawaii University, USA) for providing plaque assay protocol for DENV infection.
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