

Detection of degraded form of matricellular proteins *in vitro* and *in vivo*

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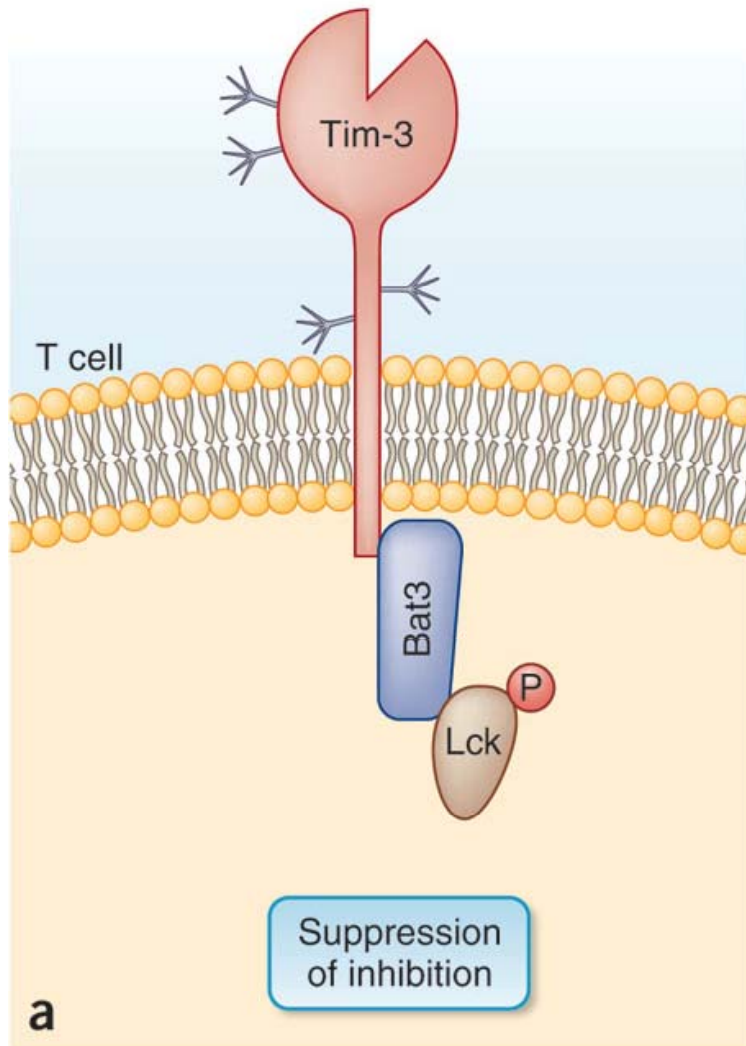
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Biological roles of matricellular proteins

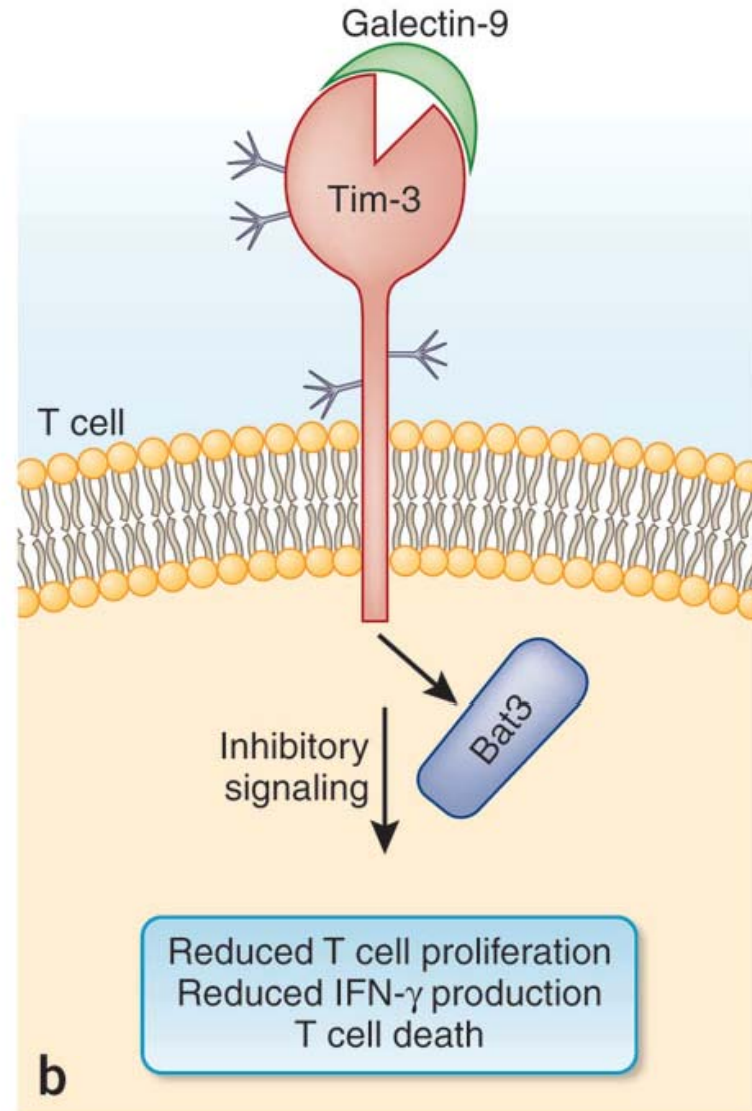
(Bornstein PJ. Cell Commun. Signal. (2009) 3:163)

Matricellular proteins are secreted into the extracellular environment, or matrix, but do not play a primary structural role in this location. Rather, these proteins modulate cell function by interacting with cell-surface receptors, proteases, hormones, and other bioeffector molecules, as well as with structural matrix proteins such as collagens.

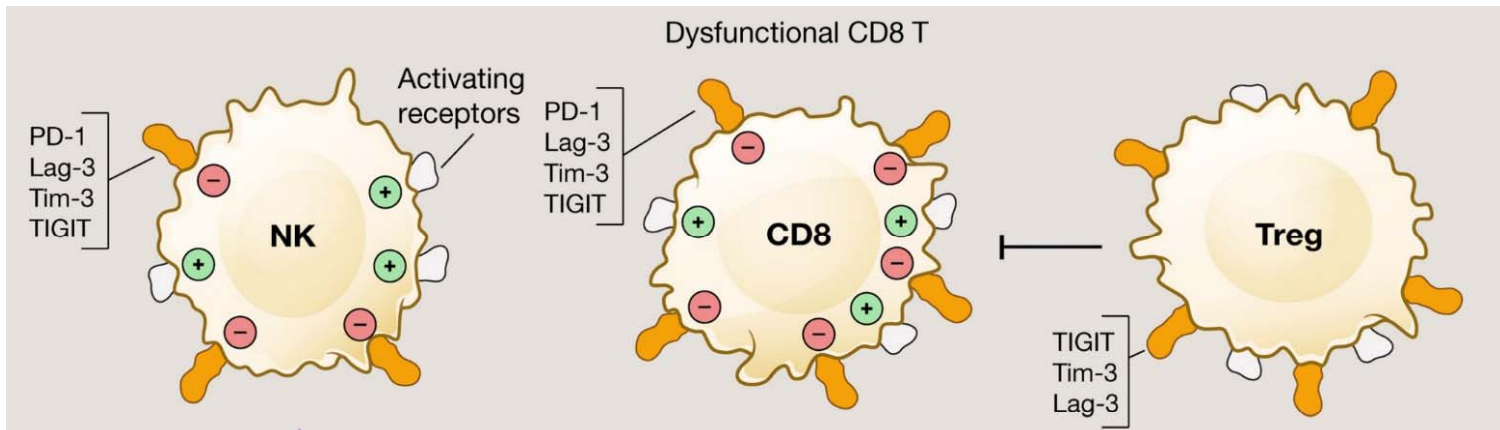
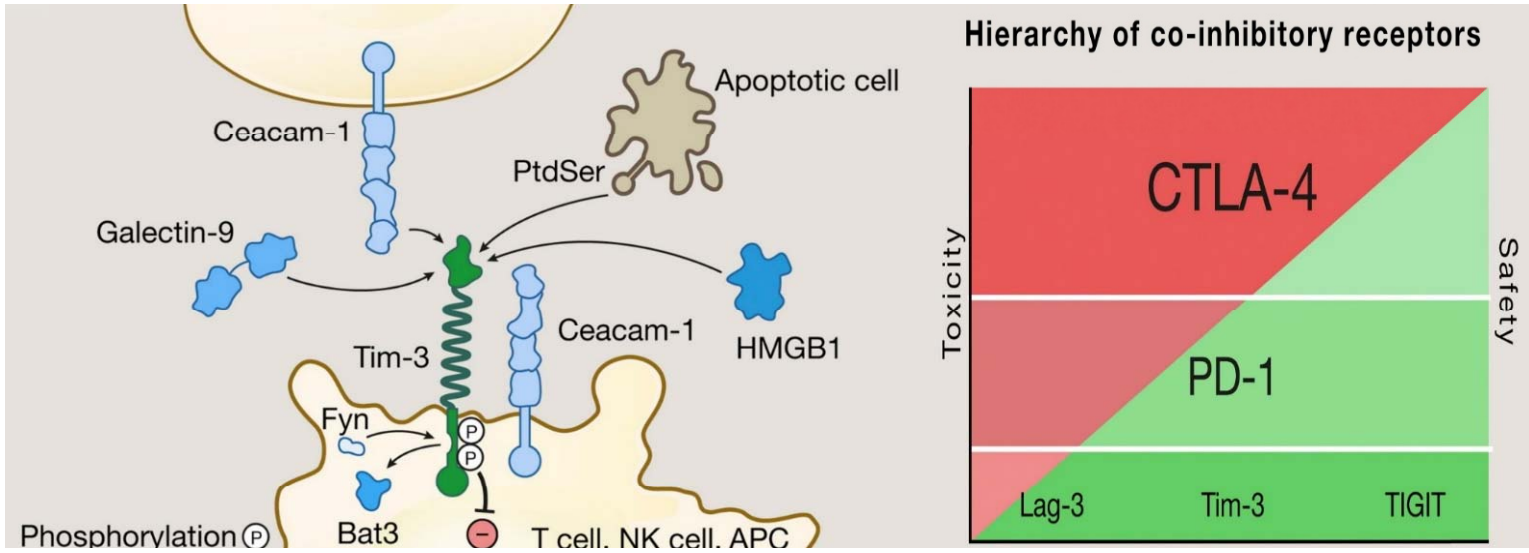
No galectin-9 binding



Galectin-9 binding



**A model to explain Bat3 repression of Tim-3 inhibition.
(Haining, Nature Med. 18: 1338,2012)**



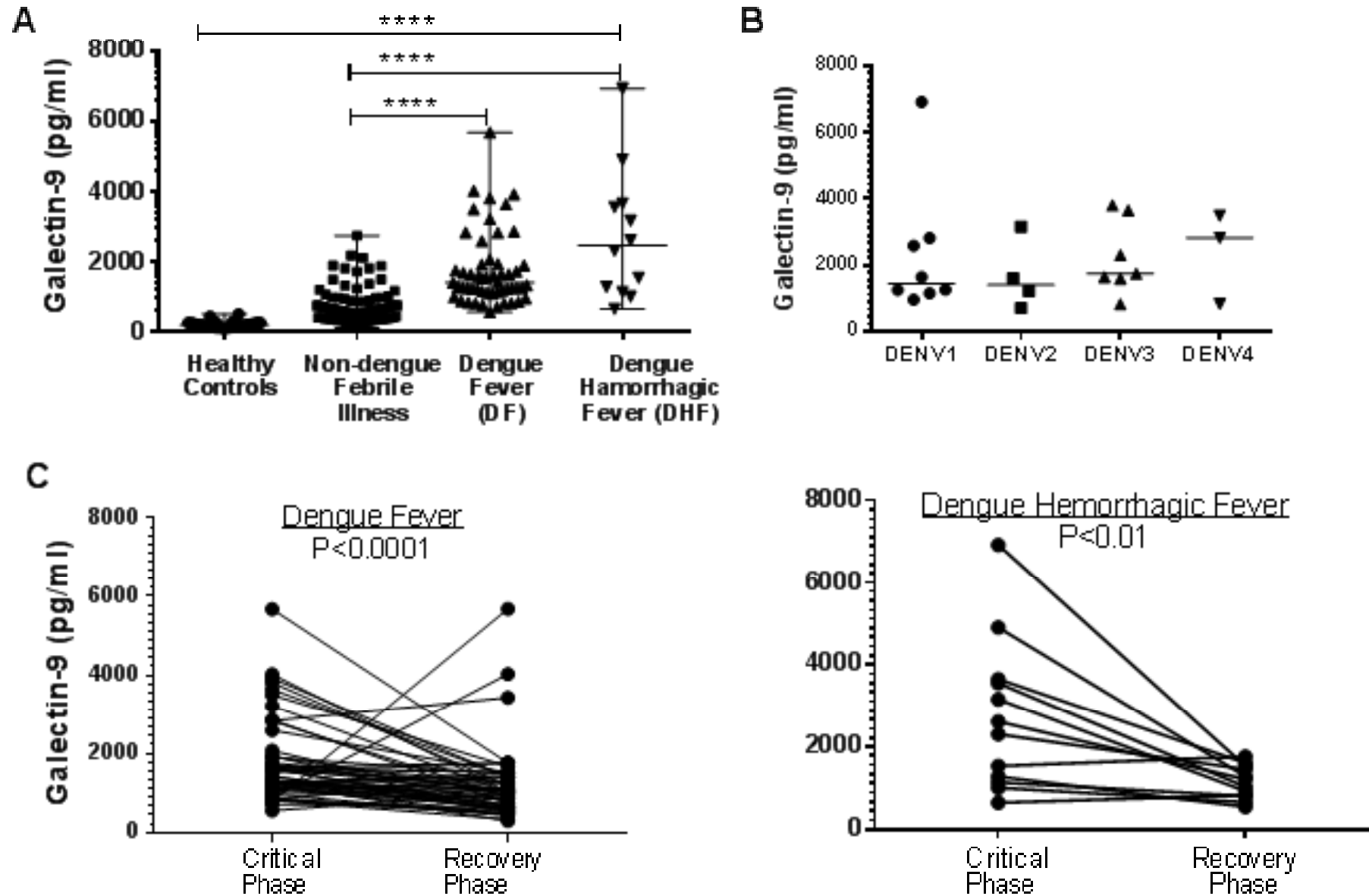
Gal-9 levels in plasma of different diseases

- PubMed search which have Healthy Control: HC value
- Publication year order

Journal	Serum/ plasma	Disease	Conc (pg/ml)		Comment
			HC	Patient	
Plos One 2010; 5: e9504	P	Chronic HCV infection	0	841	
Tohoku J Exp Med 2012; 228 : 157-161	P	Acute HIV-1 infection	46	2,300	
J Clin Virol. 2013; 58: 635-640	P	Dengue fever	196	1,407	
Retrovirology 2013; 10: 74	P	HIV-1 infection	160	1,000	
PLoS One 2014; 9: e92371	S	Pregnancy	111	1,978	Non-pregnant vs 3rd trimester
Arthritis Rheumatol 2014; 66: 2281-2289	P	Juvenile dermatomyositis	6,711	32,133	HC: disease in remission
AIDS Res. Hum. Retroviruses 2014; 30: 654-664	P	Chronic HIV-1 infection	54	326	
Tohoku J Exp Med 2014 ;232 :263-267	P	Influenza infection	14	184	
Int J Clin Exp Med 2015; 8: 8812-8819	S	Spontaneous abortion	372	611	HC: Non-pregnant
Cell Stem Cell 2015; 17: 341–352	S	Acute myeloid leukemia	22	392	
J Dermatol 2015; 42: 723–726	S	Atopic dermatitis	2,100	3,190	
Mediat Inflamm 2015; Article ID 457167:13	S	Coronary artery disease	3,566	3,284	
J Clin Neurosci 2016; 34: 193–197	P	Multiple sclerosis	26,560	37,110	
Malar J 2016; 15: 403	P	Malaria infection	348	923	HC: patients after mitigation
Int J Mol Sci 2016; 17: 832	S	Dengue fever	5,061	10,287	
Sci. Rep 2017; 7: 40994	S	Atherosclerotic stroke	6,490	7,230	
Hepatology 2017; 65: 18-31	S	Chronic HCV infection	0	146	
Int. J. Mol. Sci. 2017; 18: 19	P	Pulmonary tuberculosis	14	172	
J Invest Dermatol 2017; 137: 1850e1859	S	Systemic sclerosis	2,200	3,300	

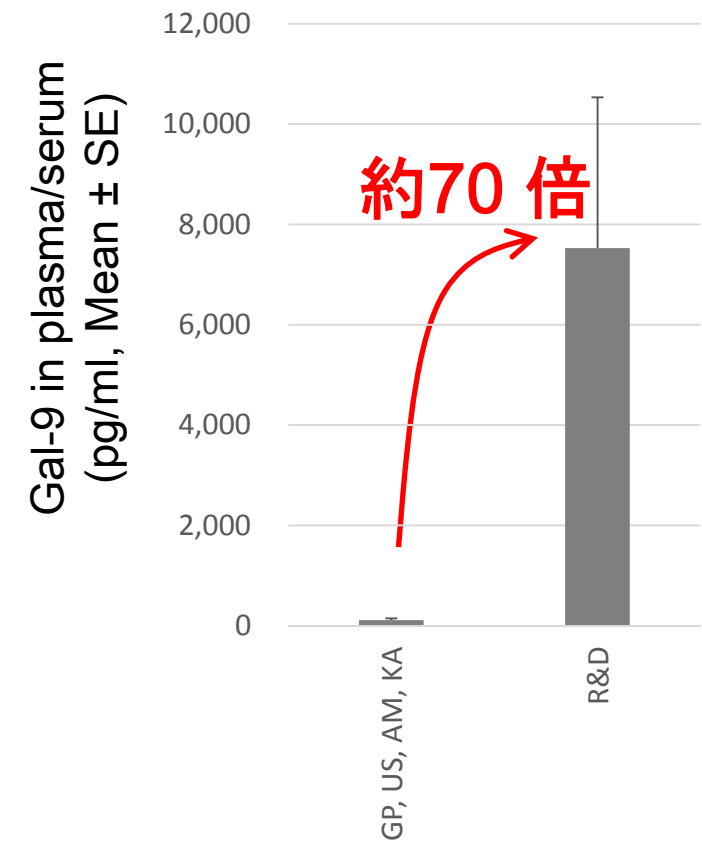
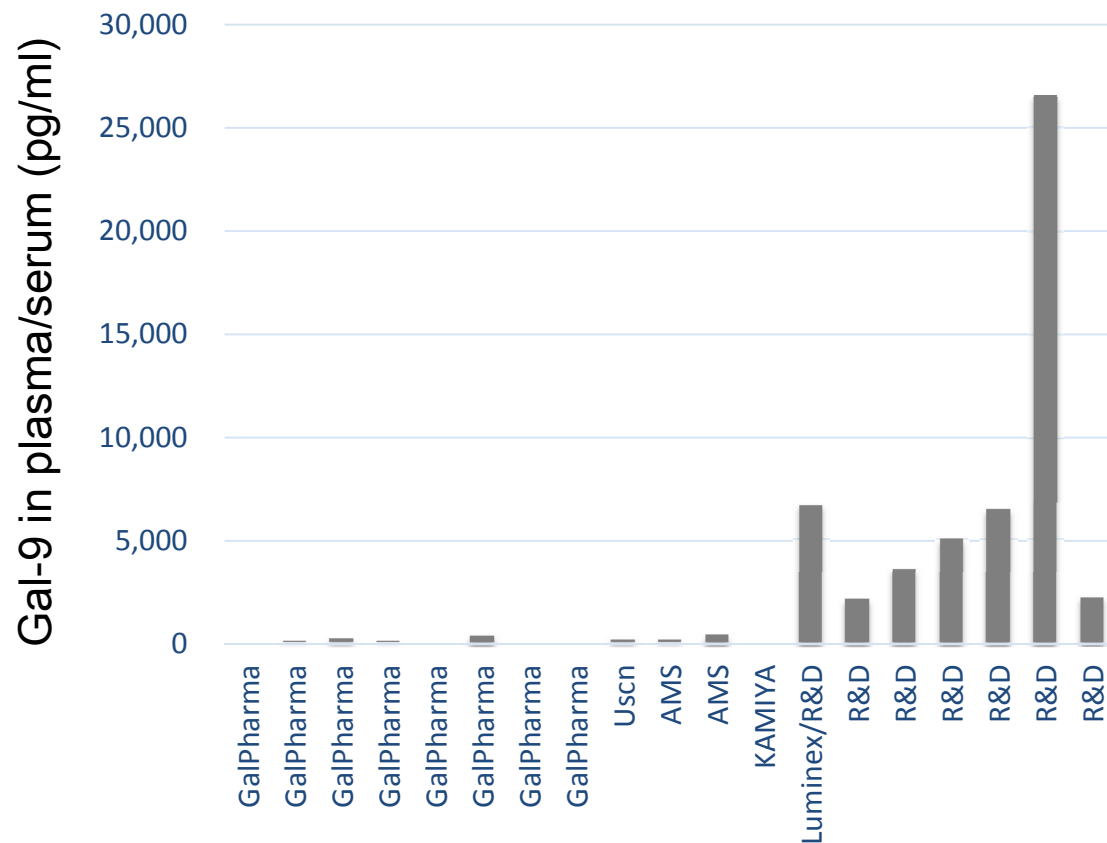
Plasma levels of galectin-9 in dengue virus infected individuals

Chagan-Yasutan H. J Clin Virol. 2013 Dec;58(4):635-40.

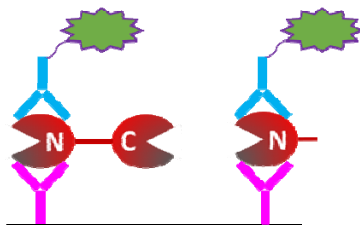
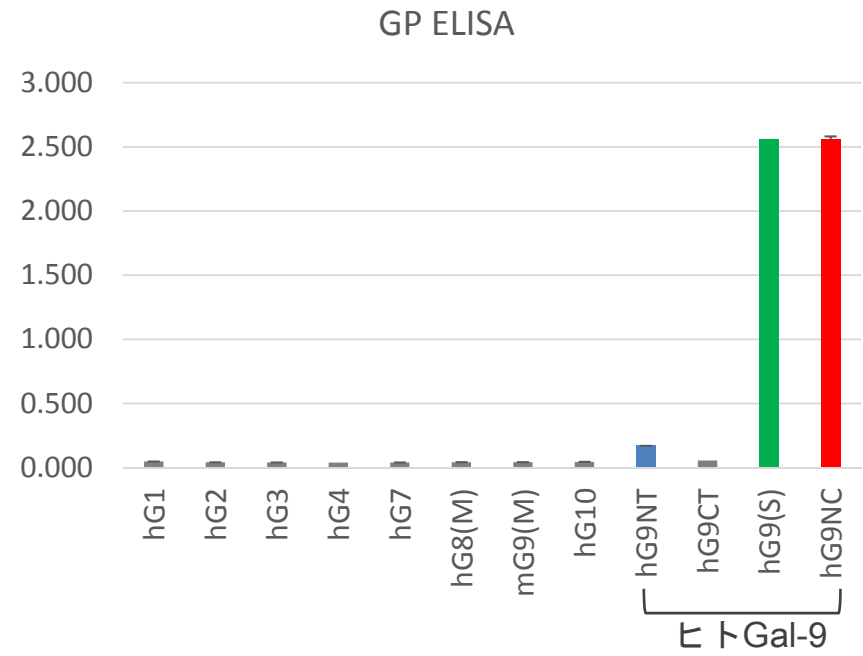
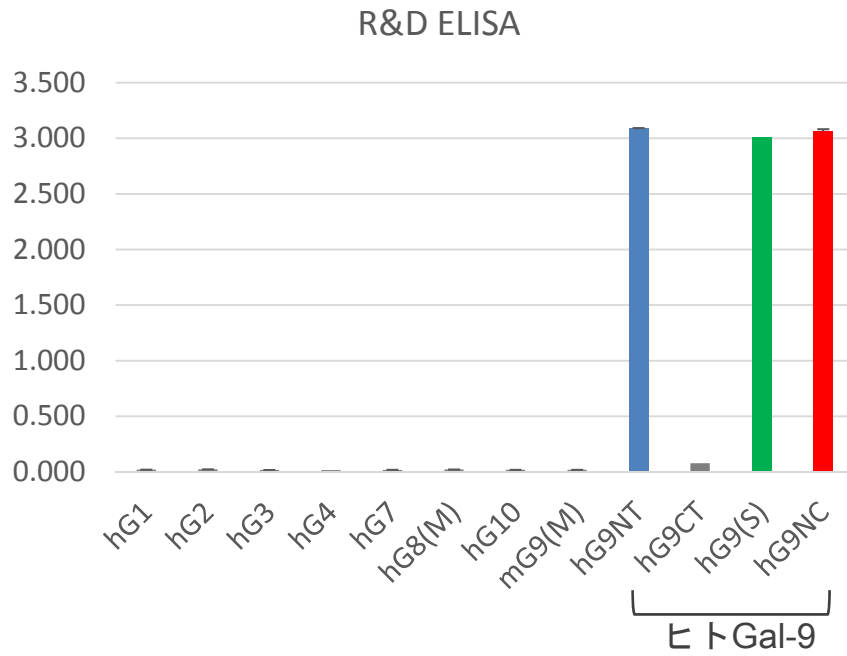


Gal-9 concentration in healthy individuals

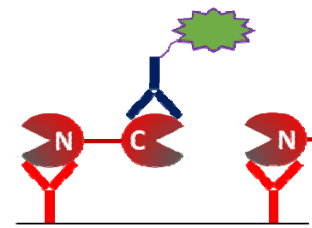
- R&D ELISA showed very high amount of Gal-9



Both ELISA is specific



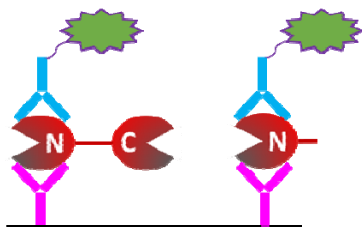
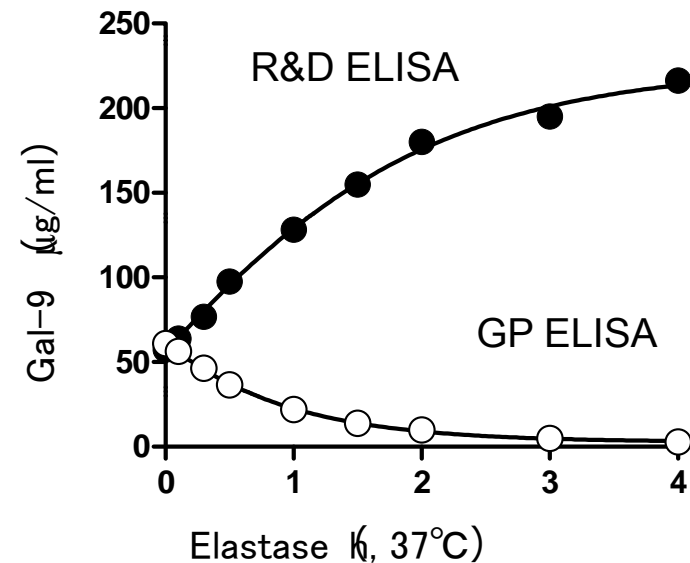
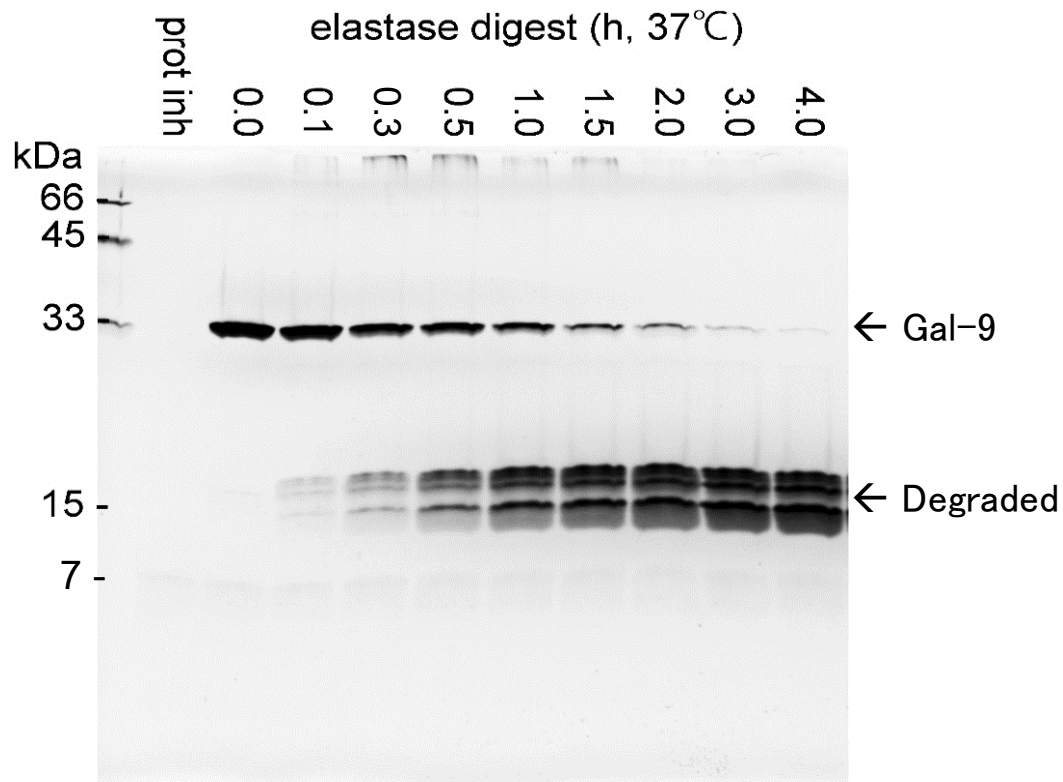
R&D
ELISA
Detect N-
CRD



GP ELISA
Detect Full
Gal-9

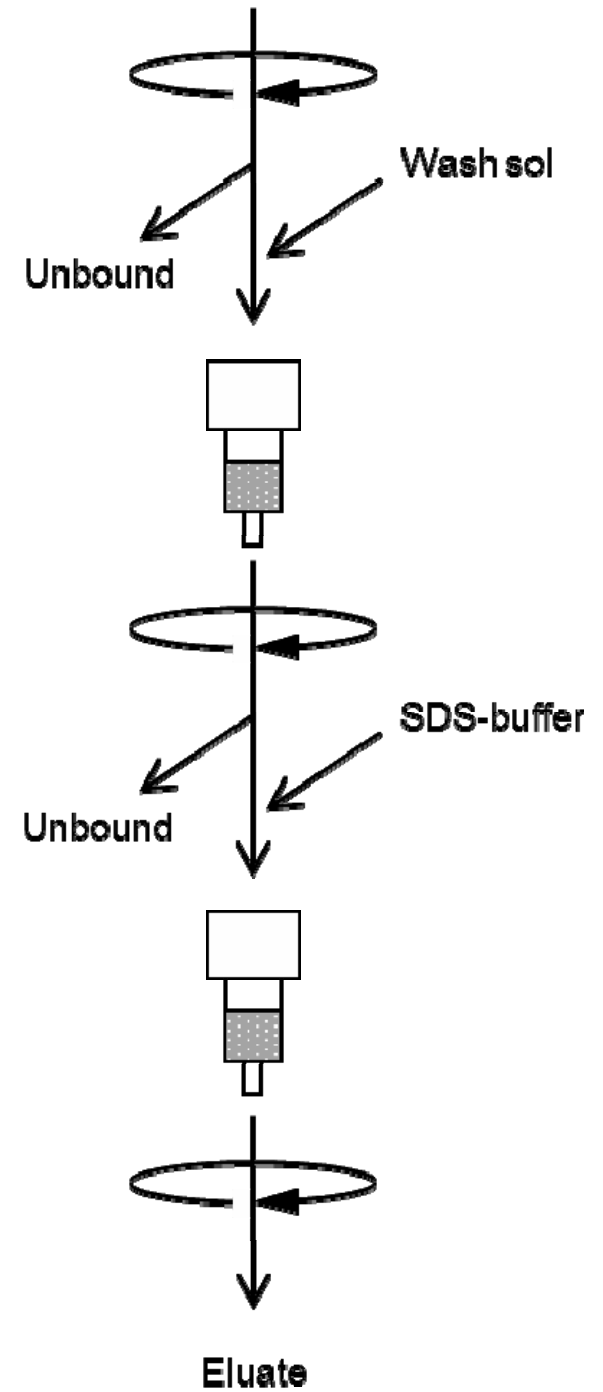
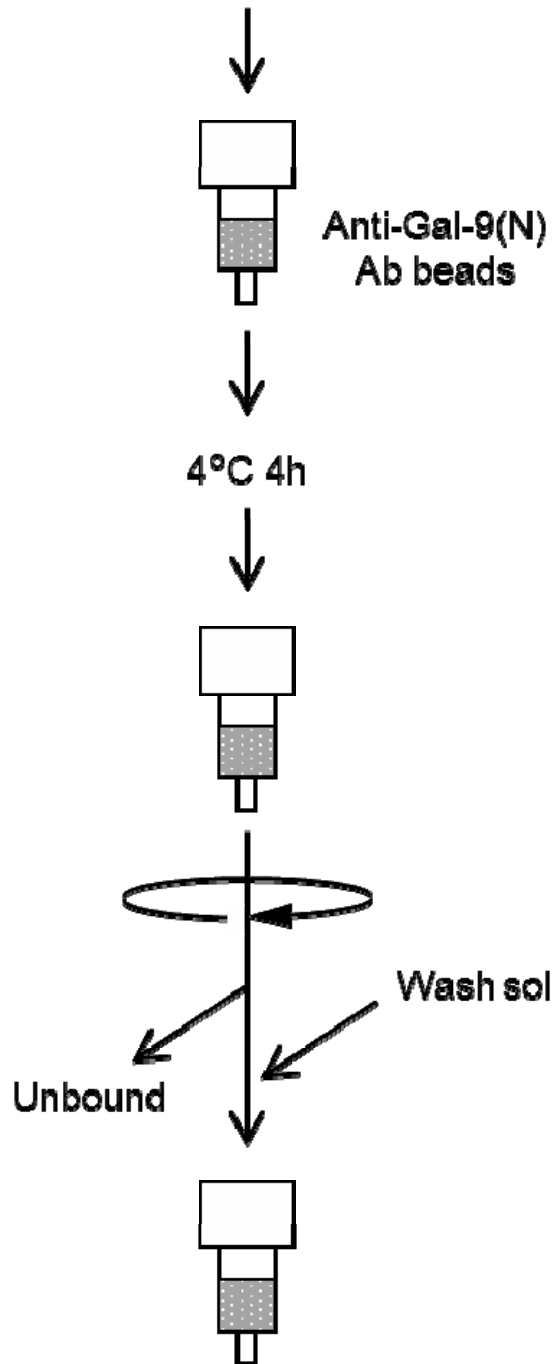
Elevation of Gal-9 in R&D ELISA also by degradation

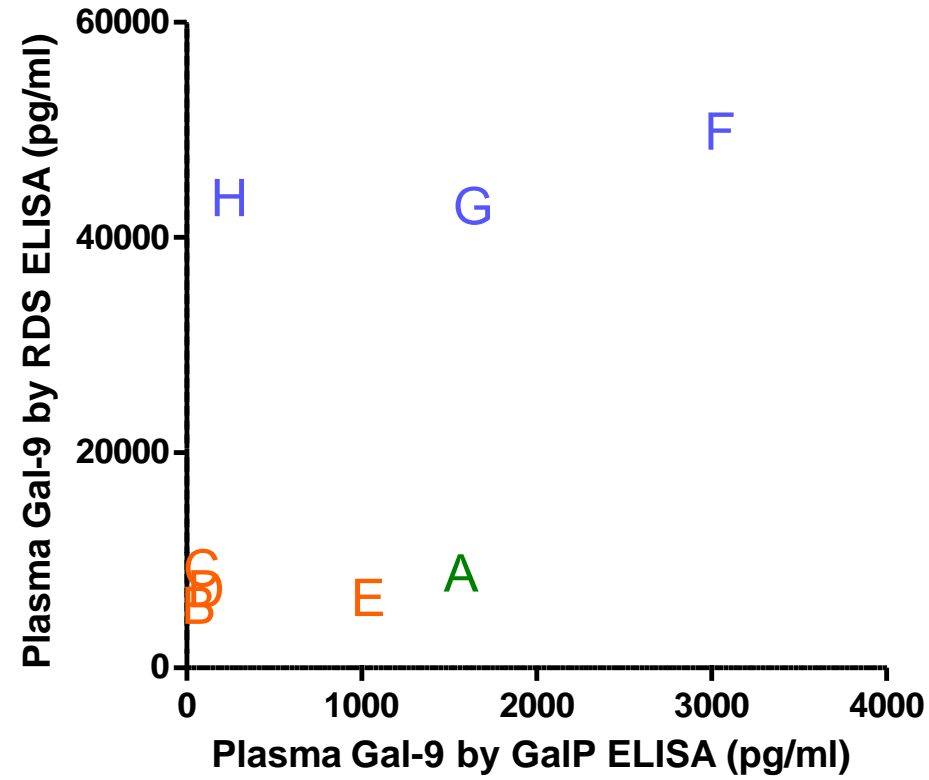
- Gal-9(S) was digested by proteases.



R&D
ELISA
Detect N-

- R&D ELISA detect N-CRD. The elevation is seen by elastase digestion.
- Cell Physiol Biochem. 2018;50(5):1856-1868. doi: 10.1159/000494866. Epub 2018 Nov 5.

A**Plasma 150 μ l
(+/- Recombinant Gal-9)**

B**C**

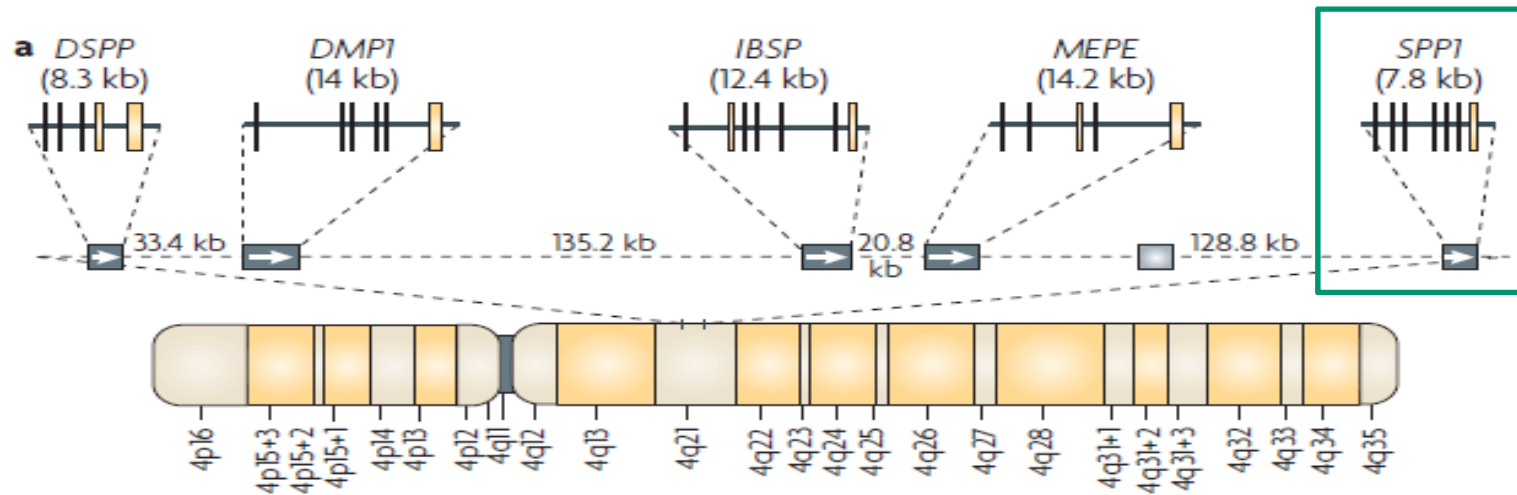
Summary of Gal-9 and 9-deg.

1. Gal-9 modulate immune responses to appropriate responses.
2. Soluble Gal-9 levels is high in many infectious diseases and leukemia.
3. The levels of Dengue is the highest using Gal-pharmer kit.
4. The levels of normal healthy carriers are different depending on the kits.

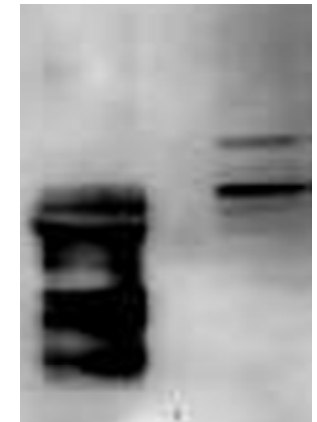
R&D shows very high levels in normal healthy individuals.

5. R&D elisa detects N-Card of Gal-9, while Galpharmer elisa detects full size.
6. By Affinity chromatography, we could also detect degraded Gal-9 (9-deg) in febrile patients.
7. Further analysis is necessary to conclude which one (Full size or 9-deg) correlate disease severity of dengue.

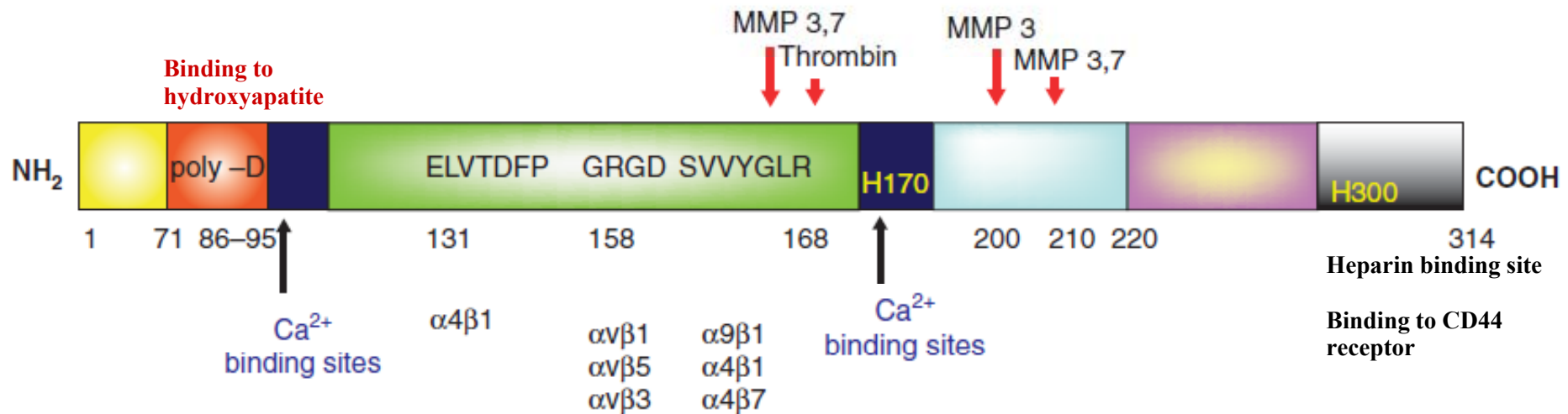
Gene structure and domain organization of human **osteopontin**



Bellahcène A et al., Nature, 2008



Human rOPN and OPN in A549 cells



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



Antibodies

Thrombin Cleavage

Aspartate Domain

$\alpha 9 / \alpha 4$ Binding Domain

Heparin Binding Domain

$\alpha V \beta 3$ Binding Domain

Calcium Binding Domain

NH₂

COOH

O-17

10A16

1B20

Human # 18625

Mouse # 18621

Rat # 18628

Human

Mouse
Rat

R G D S V V Y G L R

R G D S L A Y G L R

Human # 10011

Human # 10191

34E3

N-Half # 11108

ELISA Kits

Human
27158

O-17

10A16

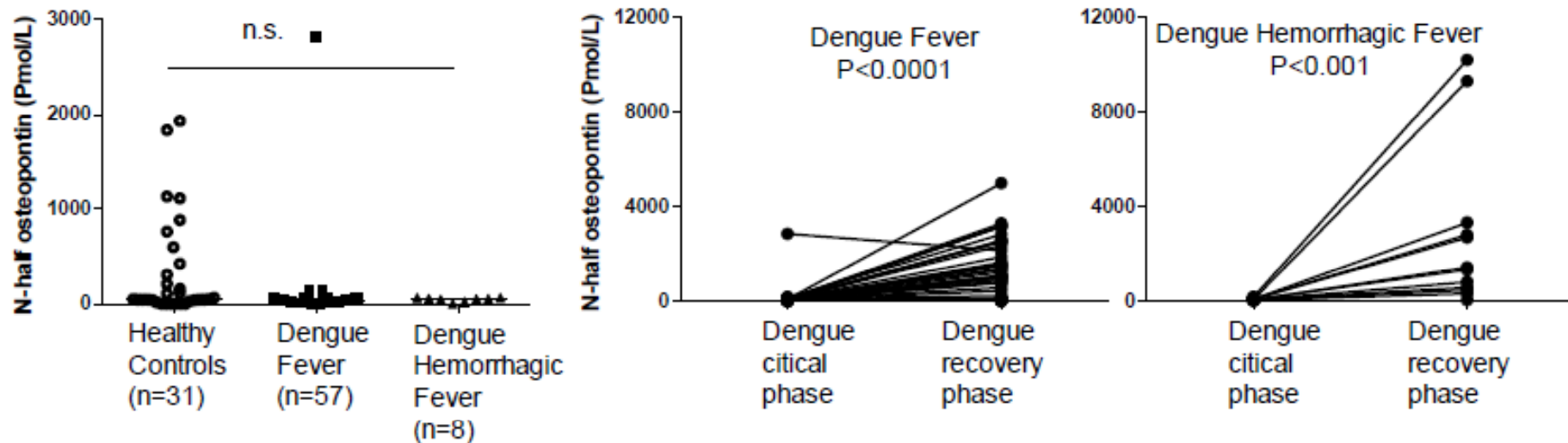
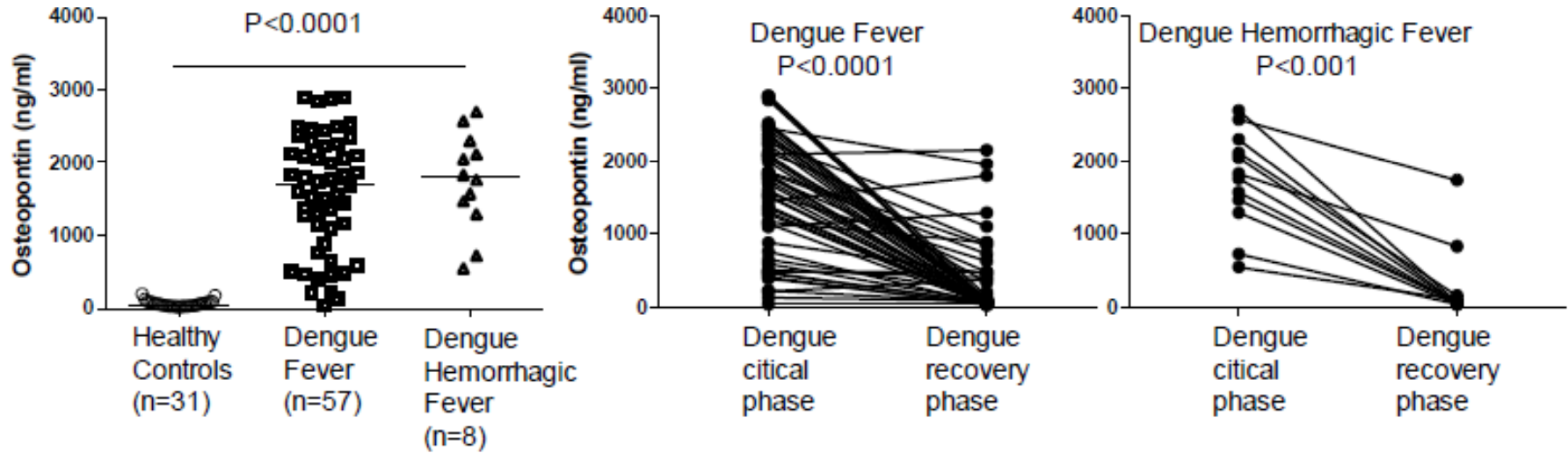
Human
27258

O-17

34E3

Plasma levels of OPN and N-half OPN in DENV infection

Chagan-Yasutan H Thromb Res. 2014 Aug;134(2)



Summary

- Plasma levels of OPN increased in critical phase and decreased in recovery phase, and reflect disease severity.
- In contrast, plasma N-half OPN levels are low in critical and increased in recovery phase.
- N-half OPN levels in recovery phase correlated more with TAT1 than TAT2, indicated partial involvement of thrombin in their generation.
- Further study of mechanism on N-half OPN production in recovery phase should be performed.

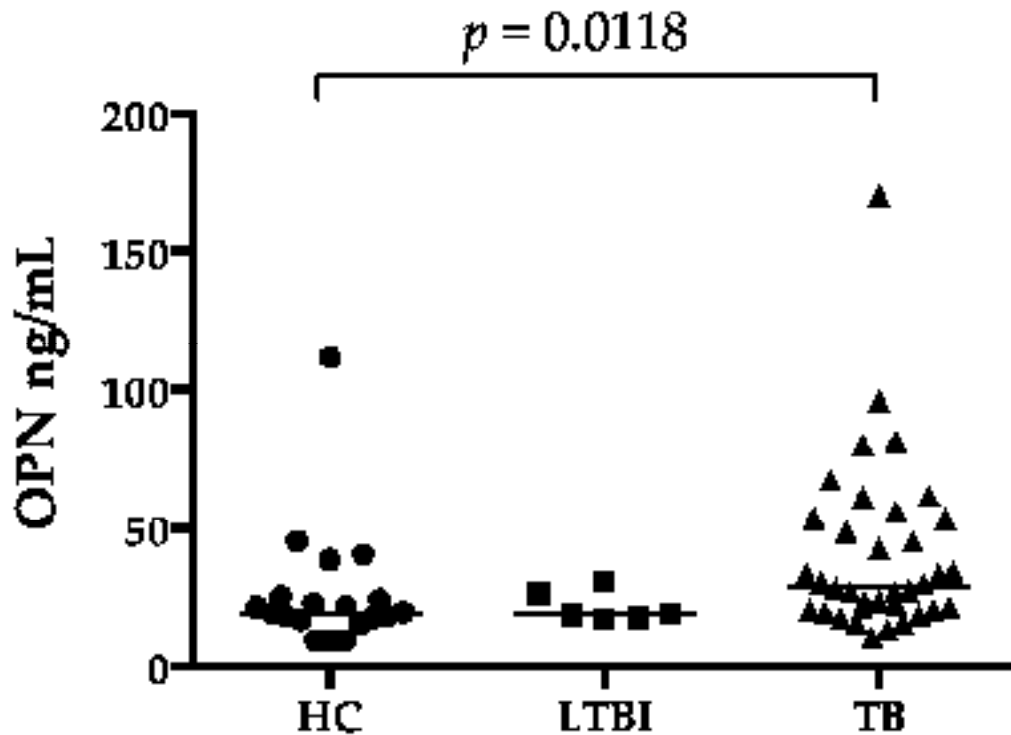
Background

Correlation of OPN, Gal-9 and sCD44 with other laboratory parameters and biomarkers in TB patients (r: correlation, *P*: *P* volume, Ns: not significant).

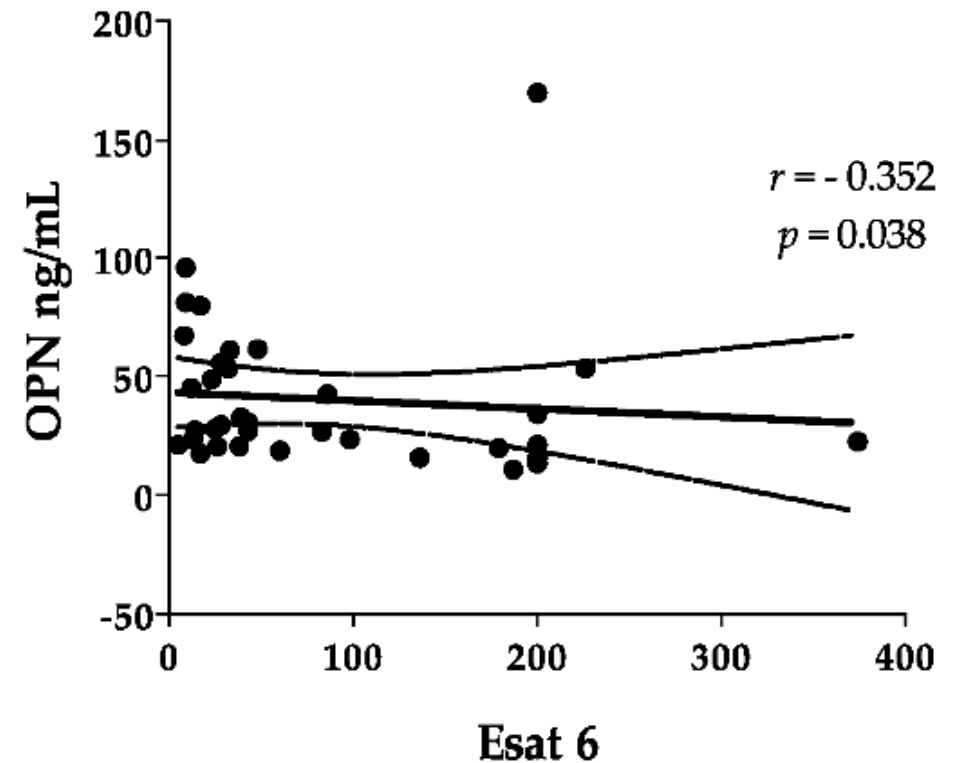
Measurements	OPN	sCD44	Gal-9
	r (<i>P</i>)	r (<i>P</i>)	r (<i>P</i>)
WBC (10 ³ /μl)	Ns	0.388 (0.019)	Ns
Neutrophil (%)	0.517 (<0.0001)	Ns	Ns
Lymphocyte (%)	-0.569 (<0.0001)	-0.558 (<0.0001)	Ns
CRP (mg/dl)	0.757 (<0.0001)	0.534 (0.001)	Ns
IL-8 (pg/ml)	0.474 (0.013)	0.524 (0.005)	Ns
IP-10 (pg/ml)	0.420 (0.029)	0.542 (0.003)	Ns
TNFα	Ns	0.446 (0.020)	Ns
ALT	Ns	Ns	0.375 (0.024)
Cre	Ns	Ns	0.377 (0.023)

Background

Osteopontin in tuberculosis



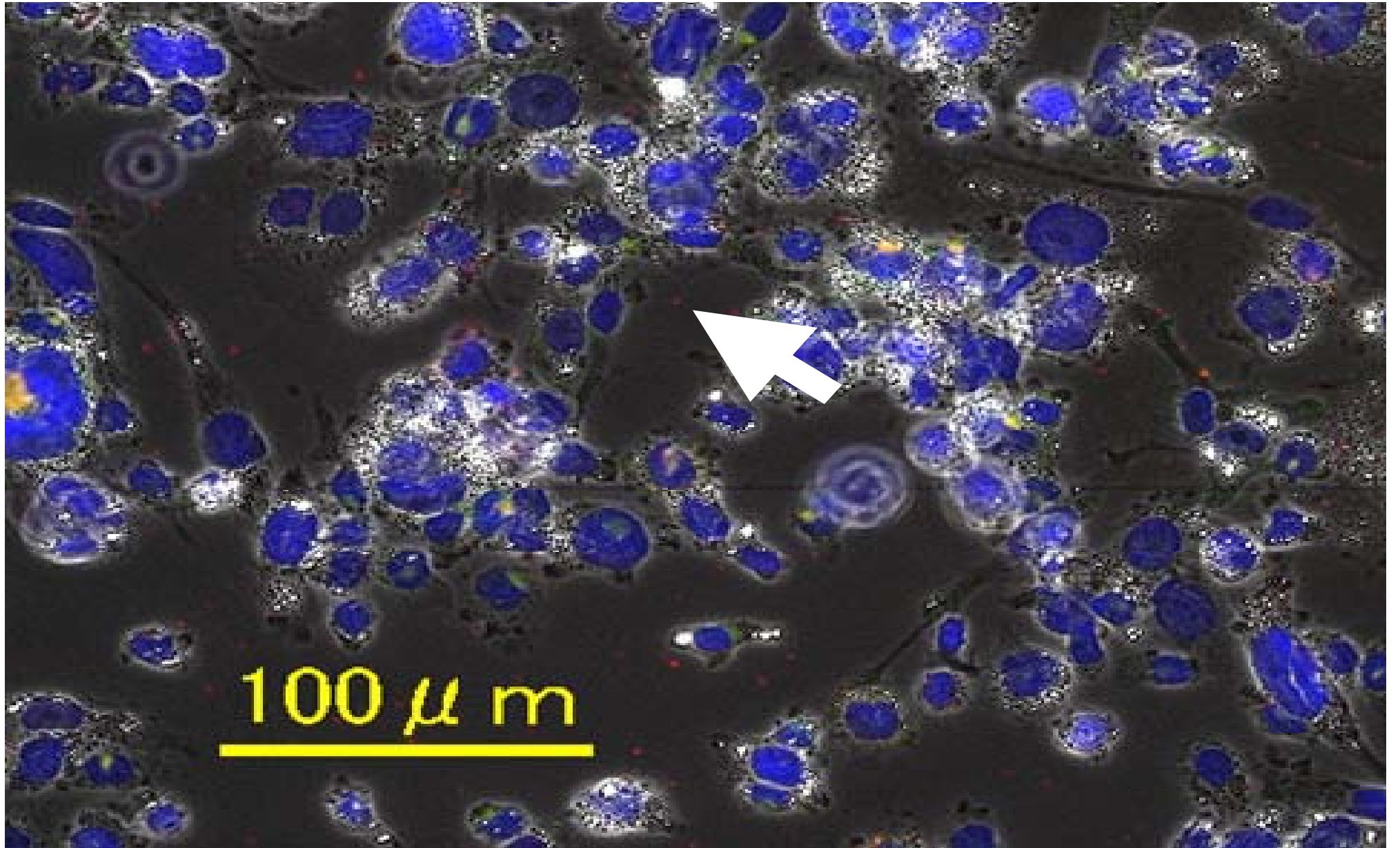
Plasma levels of Ud-OPN(R&D)



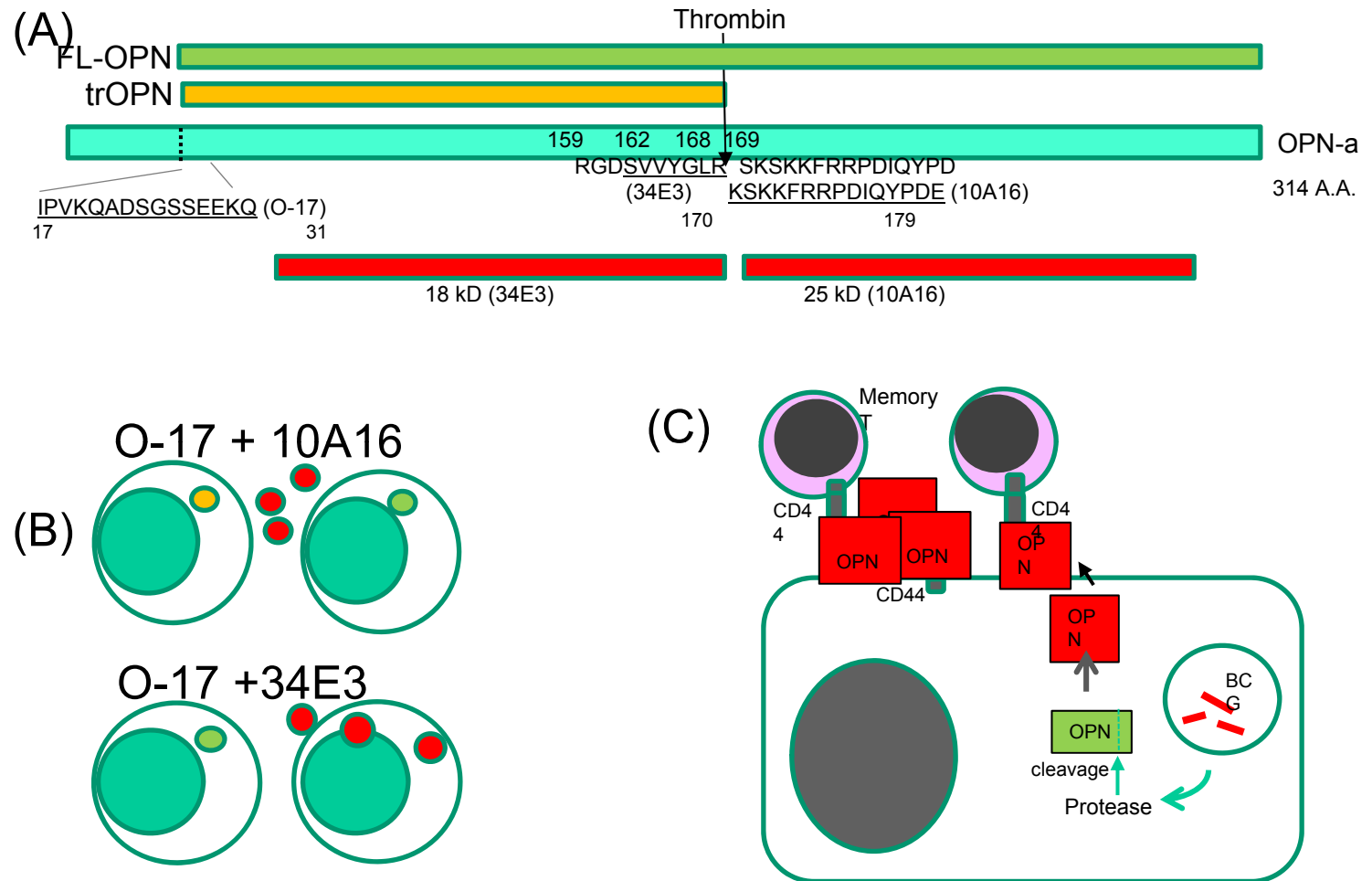
OPN & memory T cell

(r: correlation, P: P value, Ns: not significant)

Int. J. Mol. Sci. **2017**, *18*(1), 19 Shiratori B et. al



Conclusion

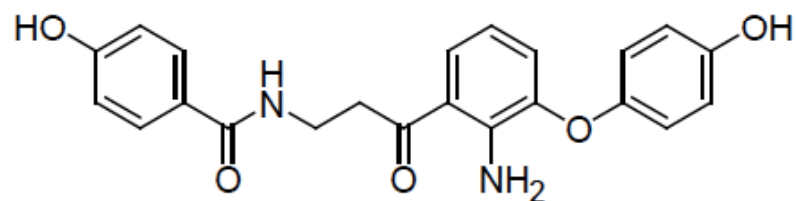


Localization of the cleaved osteopontin and the functional effect.

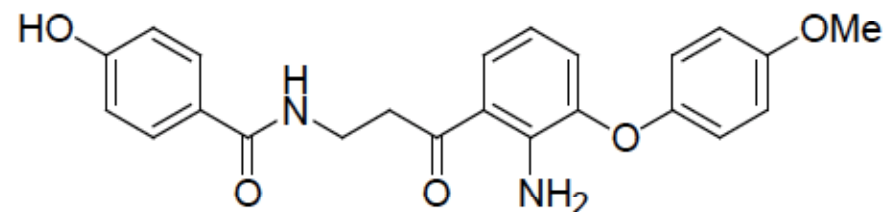
(A) Antibody epitopes and cleaved fragments of osteopontin. (B) Localization of osteopontin cleaved fragments. (C) Functional hypothesis of extracellular osteopontin fragments.

A glimpse of chemical compounds 40(A) and 62(B)

- A new aromatic amide, brefelamide, was isolated from methanol extracts of the fruiting bodies of *Dictyostelium brefeldianum* and *D. giganteum*.
- Vegetative cells of *D. discoideum* grow as single amoebae by eating bacteria; however, when starved, they start a developmental program of morphogenesis and gather to form a slug-shaped multicellular aggregate.



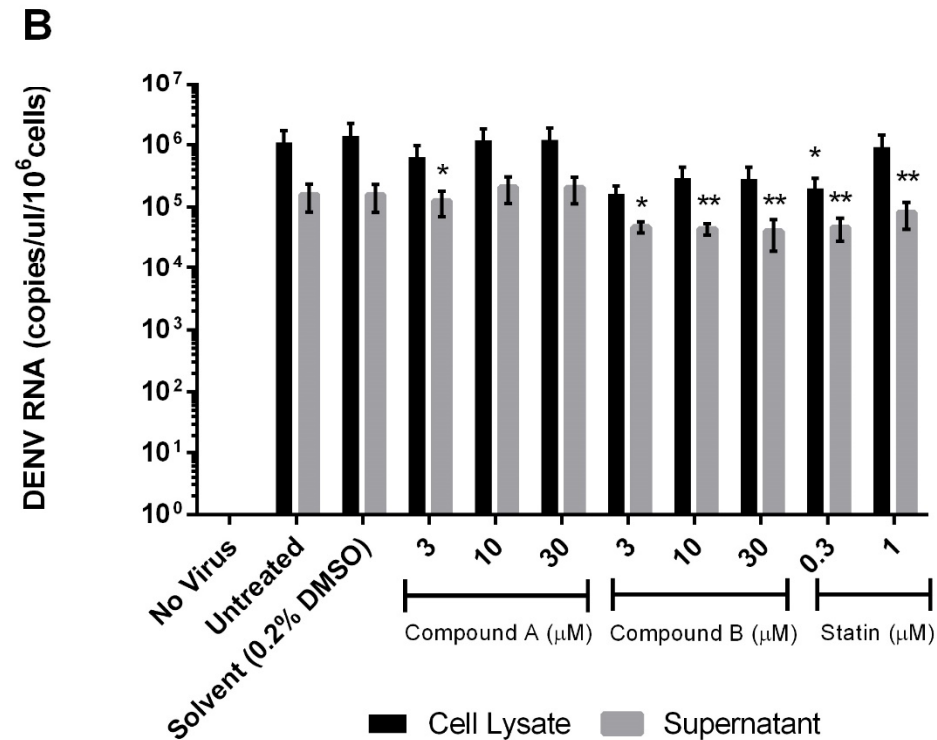
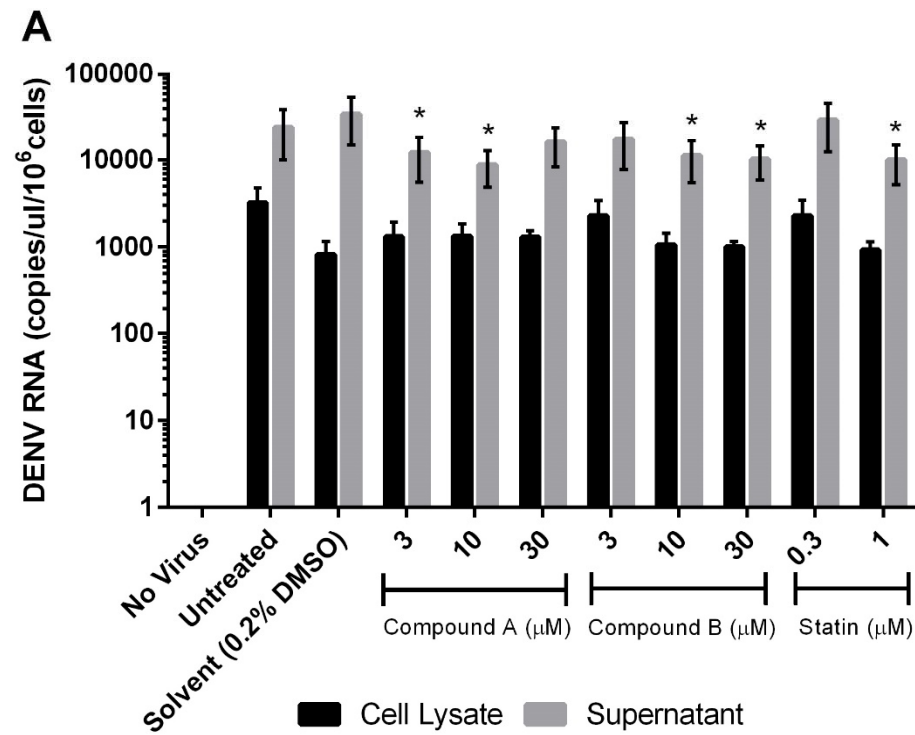
No. 40



No. 62

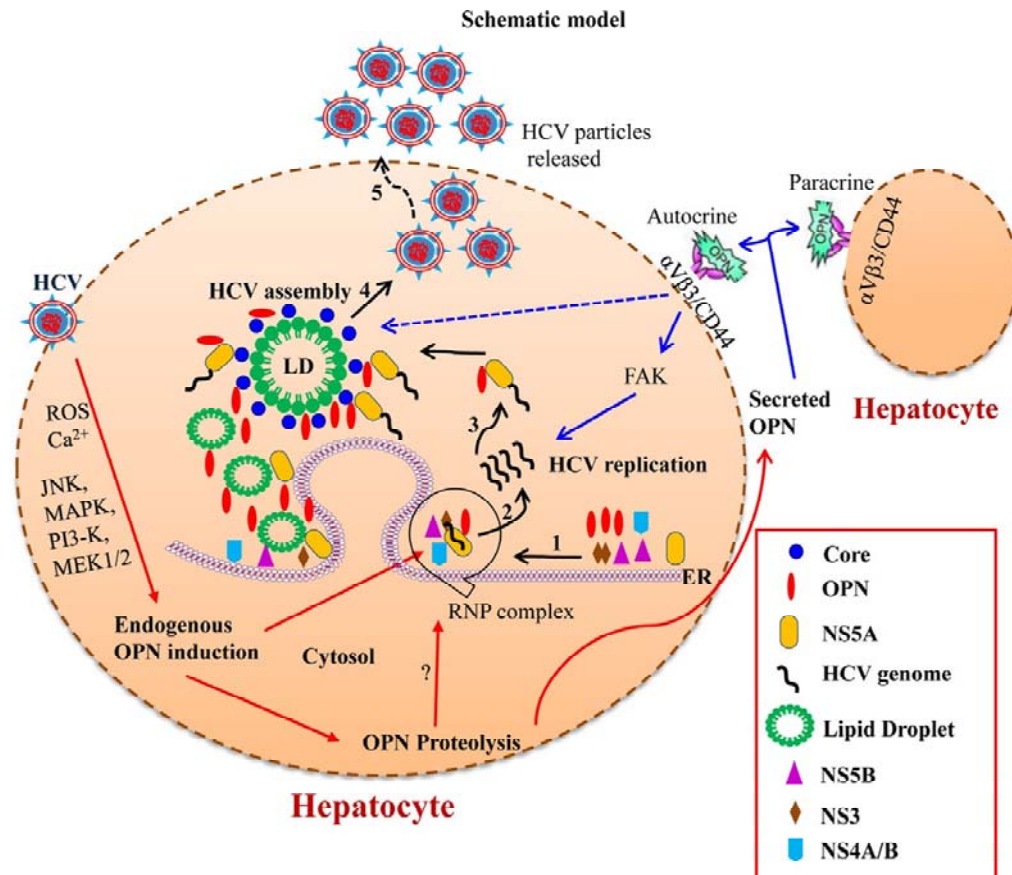
Inhibition of DENV replication by compounds.

Frontiers Immunol. 2016



Inhibition of OPN transcription by new compounds

Comp #	OPNluc clC50 (uM)
B	10.2
C	1
D	1.1
E	1.2



Model illustrating the role of OPN in HCV replication and assembly. Recently, we have shown the precursor form of the OPN is cleaved via activation of calpain proteases, and the cleaved forms are secreted out of the cells . In this study, we show that activated endogenous OPN interacts with HCV nonstructural proteins (NS3, NS4A/B, NS5A, and NS5B) in the ER to facilitate HCV replication (steps 1 and 2). In addition, OPN also interacts with HCV NS5A, core, and LDs to regulate HCV assembly process (steps 3 and 4).

J Virol. 2018 Jun 13;92

Acknowledgements

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