

Original Western Blot Images

A pomegranate-derived peptide Pug-4 alleviates nontypeable *Haemophilus influenzae*-induced inflammation by suppressing NF- κ B and NLRP3 inflammasome pathways

Pornpimon Jantaruk¹, Sittiruk Roytrakul², Anchalee Sistyayanarain¹ and Duangkamol Kunthalert^{1, 3*}

¹ Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand

² National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Thailand Science Park, Pathumthani, Thailand

³ Centre of Excellence in Medical Biotechnology, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand

*Corresponding author:

Duangkamol Kunthalert

Email address: duangkamol@nu.ac.th, kunthalertd@yahoo.com

Figure 5 Pug-4 peptide inhibited iNOS and COX-2 protein expression in NTHi-infected A549 cells.

Total proteins extraction

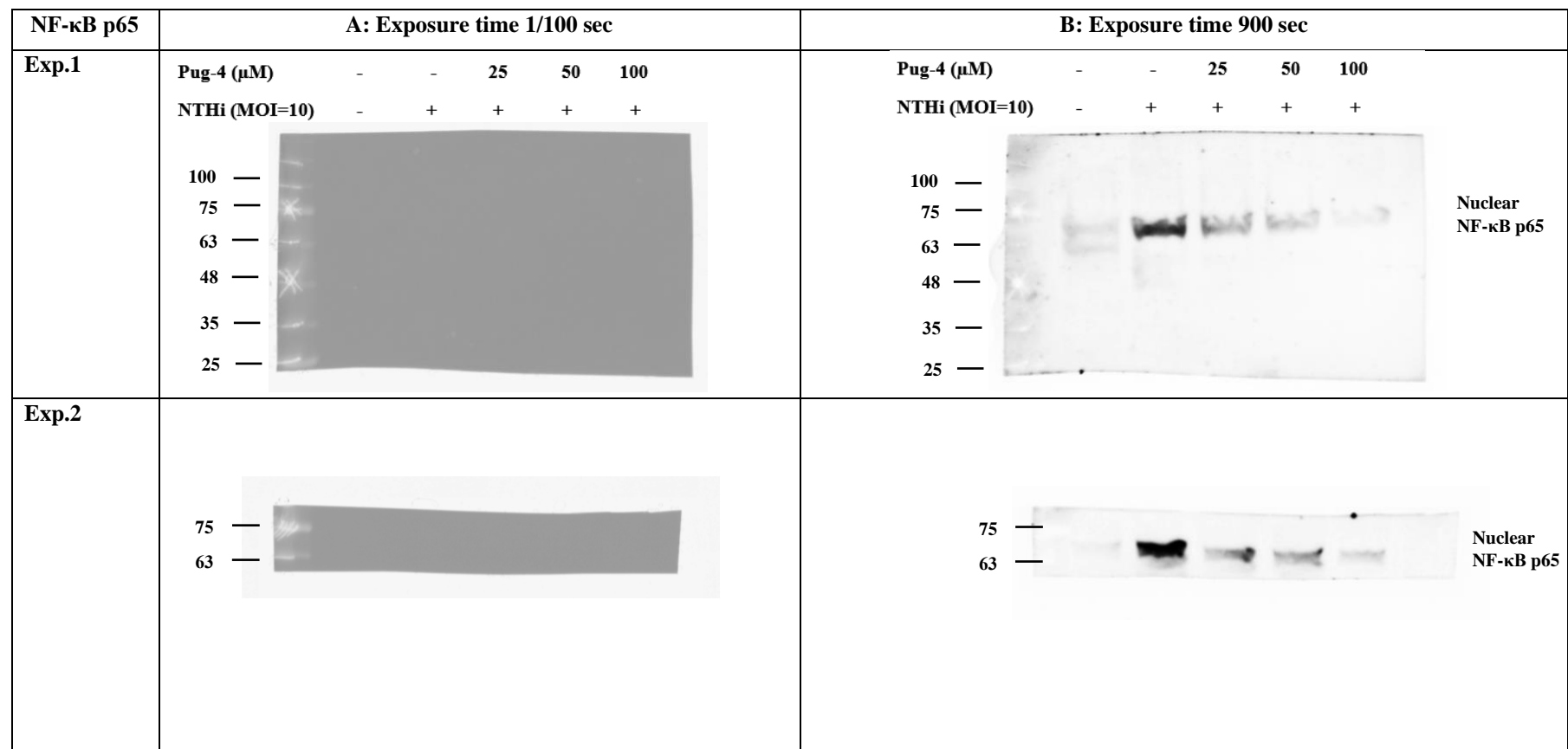
iNOS	A: Exposure time 1/100 sec					B: Exposure time 1200 sec						
Exp.1	Pug-4 (μM)	-	-	25	50	100	Pug-4 (μM)	-	-	25	50	100
	NTHi (MOI=10)	-	+	+	+	+	NTHi (MOI=10)	-	+	+	+	+
Exp.2												

COX-2	A: Exposure time 1/100 sec					B: Exposure time 240 sec							
Exp.1-1	Pug-4 (μM)	-	-	25	50	100	Pug-4 (μM)	-	-	25	50	100	
	NTHi (MOI=10)	-	+	+	+	+	NTHi (MOI=10)	-	+	+	+	+	
Exp.2-1													
Exp.1-2													
Exp.2-2													


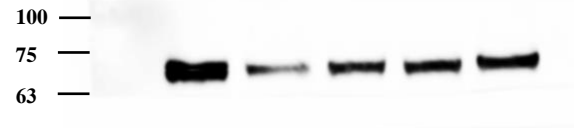


β -actin	A: Exposure time 1/100 sec						B: Exposure time 3 sec							
Exp.1	Pug-4 (μM)	-	-	25	50	100	Pug-4 (μM)	-	-	25	50	100		
	NTHi (MOI=10)	-	+	+	+	+	NTHi (MOI=10)	-	+	+	+	+		
	63 —							63 —						β -actin
	48 —							48 —						
	35 —							35 —						
	25 —							25 —						
	20 —							20 —						
	17 —							17 —						
Exp.2	63 —							63 —						β -actin
	48 —							48 —						
	35 —							35 —						
	25 —							25 —						
	20 —							20 —						
	17 —							17 —						
	11 —							11 —						

Figure 6 Pug-4 peptide inhibits NTHi-induced NF-κB activation in A549 cells.

Proteins from Nuclear extraction



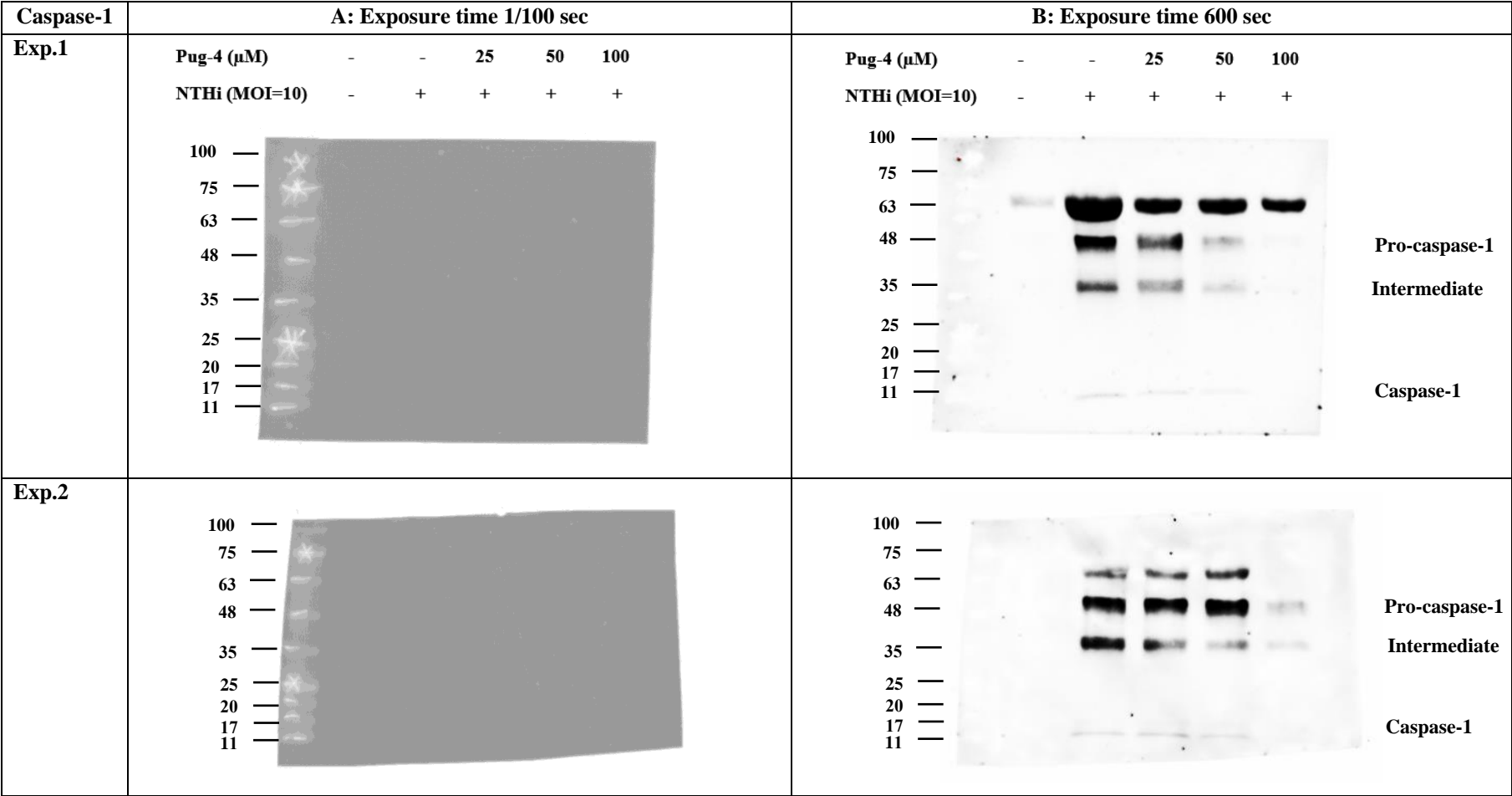
Proteins from Cytoplasmic extraction

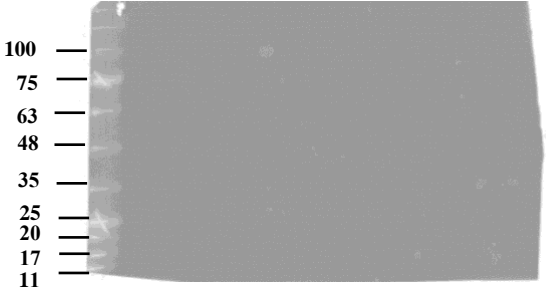
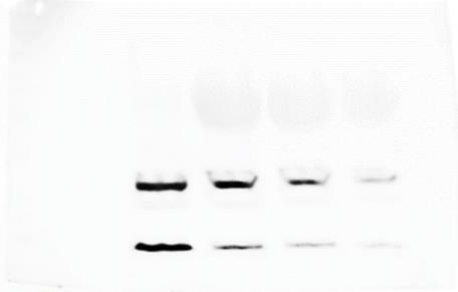

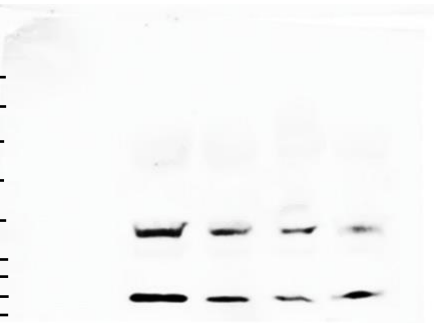
NF-κB p65	A: Exposure time 1/100 sec	B: Exposure time 240 sec																								
<p>Exp.1</p>	<table border="1"> <tr> <td>Pug-4 (μM)</td> <td>-</td> <td>-</td> <td>25</td> <td>50</td> <td>100</td> </tr> <tr> <td>NTHi (MOI=10)</td> <td>-</td> <td>+</td> <td>+</td> <td>+</td> <td>+</td> </tr> </table> 	Pug-4 (μM)	-	-	25	50	100	NTHi (MOI=10)	-	+	+	+	+	<table border="1"> <tr> <td>Pug-4 (μM)</td> <td>-</td> <td>-</td> <td>25</td> <td>50</td> <td>100</td> </tr> <tr> <td>NTHi (MOI=10)</td> <td>-</td> <td>+</td> <td>+</td> <td>+</td> <td>+</td> </tr> </table>  <p>Cytoplasmic NF-κB p65</p>	Pug-4 (μM)	-	-	25	50	100	NTHi (MOI=10)	-	+	+	+	+
Pug-4 (μM)	-	-	25	50	100																					
NTHi (MOI=10)	-	+	+	+	+																					
Pug-4 (μM)	-	-	25	50	100																					
NTHi (MOI=10)	-	+	+	+	+																					
<p>Exp.2</p>		 <p>Cytoplasmic NF-κB p65</p>																								

β-actin	A: Exposure time 1/100 sec					B: Exposure time 3 sec							
Exp.1	Pug-4 (μM)	-	-	25	50	100	Pug-4 (μM)	-	-	25	50	100	
	NTHi (MOI=10)	-	+	+	+	+	NTHi (MOI=10)	-	+	+	+	+	
											β-actin		
Exp.2											β-actin		


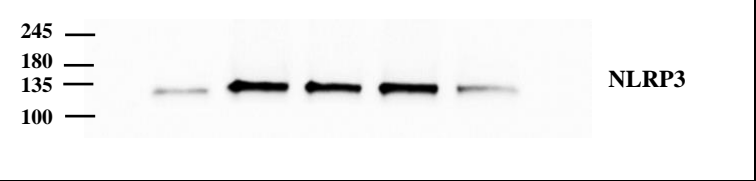

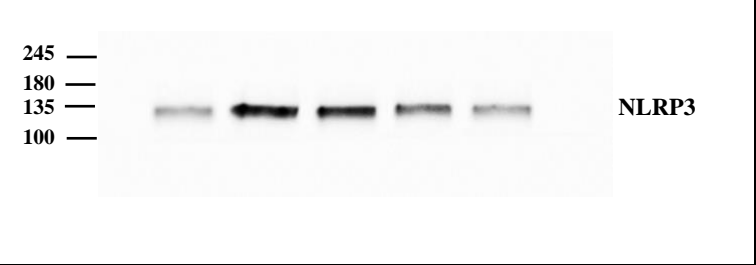
Figure 7 Pug-4 peptide suppressed NTHi-induced activation of NLRP3 inflammasome pathway in A549 cells.

Proteins from supernatant



IL-1 β	A: Exposure time 1/100 sec	B: Exposure time 600 sec
Exp.1	<p data-bbox="387 256 936 284">Pug-4 (μM) - - 25 50 100</p> <p data-bbox="387 300 936 327">NTHi (MOI=10) - + + + +</p> 	<p data-bbox="1182 256 1731 284">Pug-4 (μM) - - 25 50 100</p> <p data-bbox="1182 300 1731 327">NTHi (MOI=10) - + + + +</p>  <p data-bbox="1816 496 1928 523">Pro-IL-1β</p> <p data-bbox="1816 560 1883 587">IL-1β</p>
Exp.2		 <p data-bbox="1794 895 1906 922">Pro-IL-1β</p> <p data-bbox="1794 959 1861 986">IL-1β</p>

Proteins from cell lysates

NLRP3	A: Exposure time 1/100 sec					B: Exposure time 240 sec							
Exp.1	Pug-4 (μM)	-	-	25	50	100	Pug-4 (μM)	-	-	25	50	100	
	NTHi (MOI=10)	-	+	+	+	+	NTHi (MOI=10)	-	+	+	+	+	
	245 — 180 — 135 — 100 —						245 — 180 — 135 — 100 —						NLRP3
Exp.2	245 — 180 — 135 — 100 —						245 — 180 — 135 — 100 —						NLRP3

β -actin	A: Exposure time 1/100 sec						B: Exposure time 3 sec						
Exp.1	Pug-4 (μM)	-	-	25	50	100	Pug-4 (μM)	-	-	25	50	100	β -actin
	NTHi (MOI=10)	-	+	+	+	+	NTHi (MOI=10)	-	+	+	+	+	
		63 —					63 —						
		48 —					48 —						
		35 —					35 —						
		25 —					25 —						
		20 —					20 —						
		17 —					17 —						
Exp.2		63 —					63 —						β -actin
		48 —					48 —						
		35 —					35 —						
		25 —					25 —						
		20 —					20 —						
		17 —					17 —						
		11 —					11 —						