

Proteomics of anatomical sections of the gut of *Nosema*-infected Western honey bee (*Apis mellifera*) reveals different early responses to *Nosema* spp. isolates

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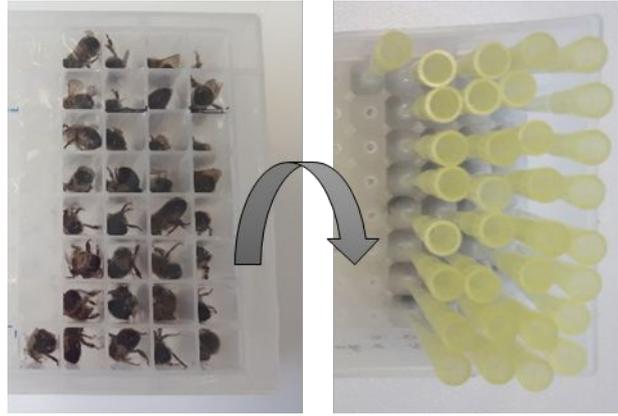
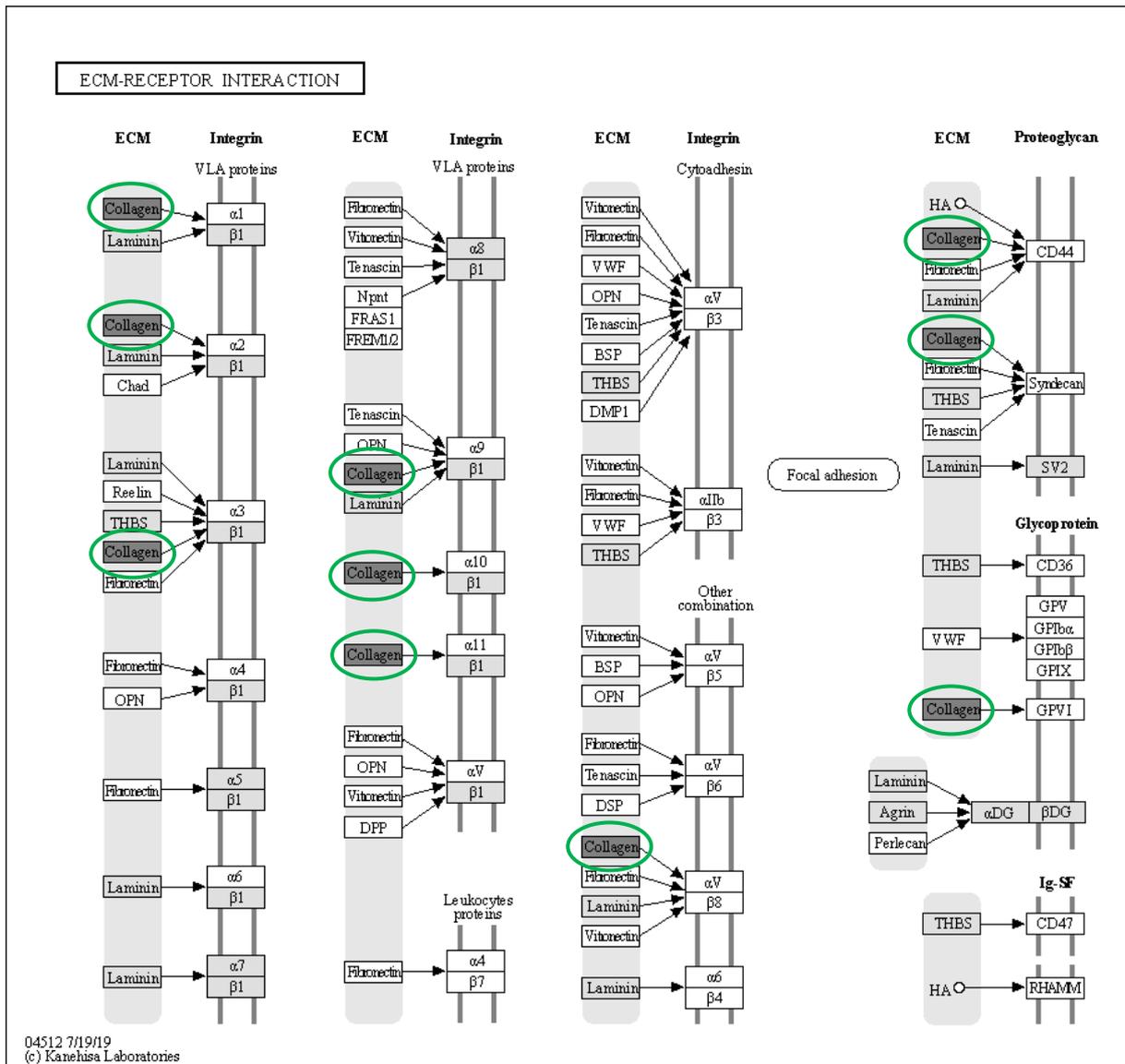


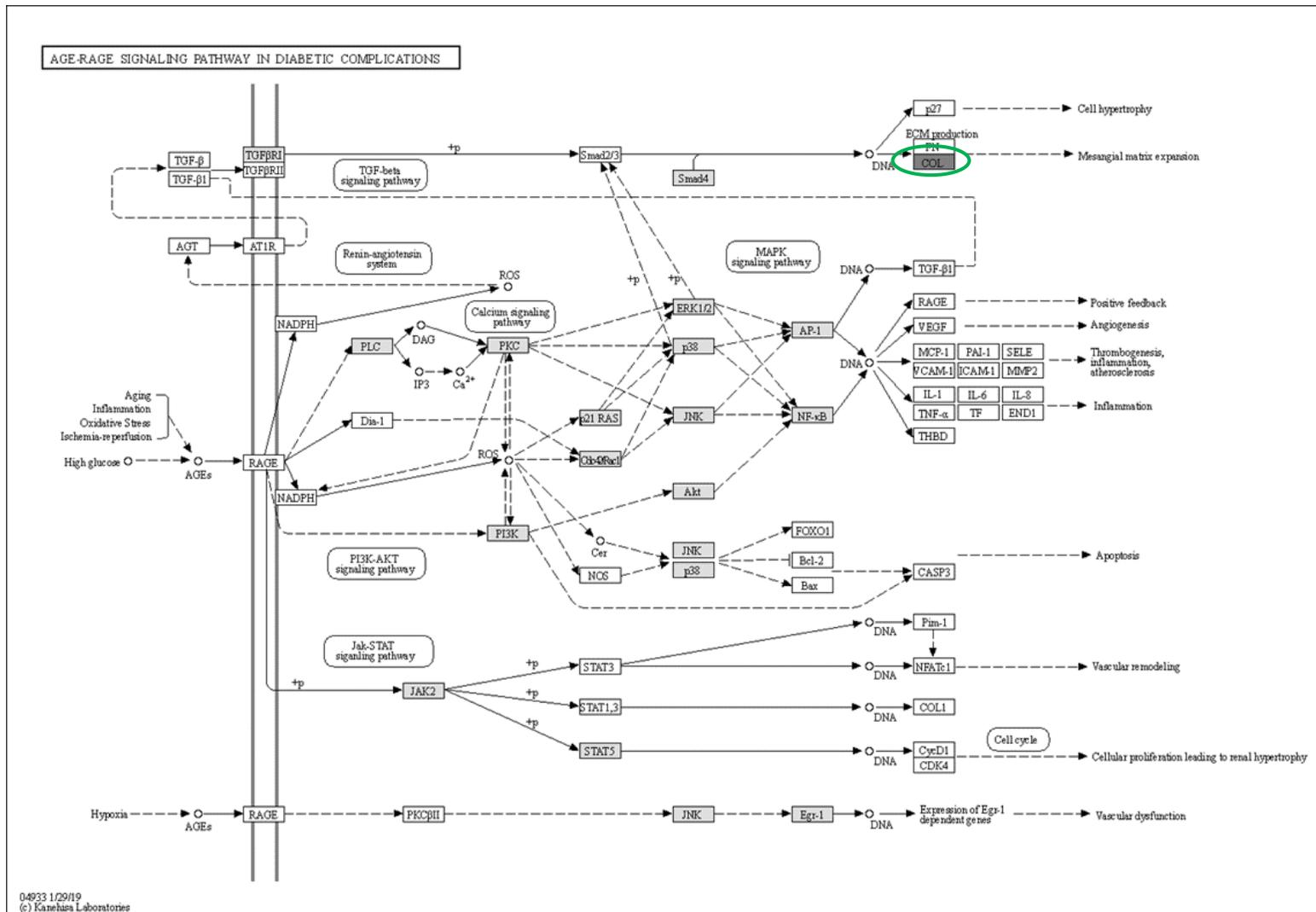
Figure S1: Experimental procedure to infect individual bees with a *Nosema* inoculum

To infect a large number of bees individually with a *Nosema* inoculum, we have developed the following method. After 15 hours of fasting each bee was placed individually in a home-made “inoculated-96-well deep bottom plate”. It consists of a 96-well deep bottom plate with holes at the bottom. After placing the bees in the holes, the box is covered with a plastic film. The box is turned over, then 10-200 μL pipette tips containing the syrup are placed in front of each bee. Bees were either fed with 10 μL of 50% syrup containing 100,000 spores for the experimental bees, or fed with syrup alone for the control bees. The bees were kept in this device the time to consume all of the syrup. They were then removed from the device and then placed by experimental group in boxes with syrup *ad libitum* until tissues collection.

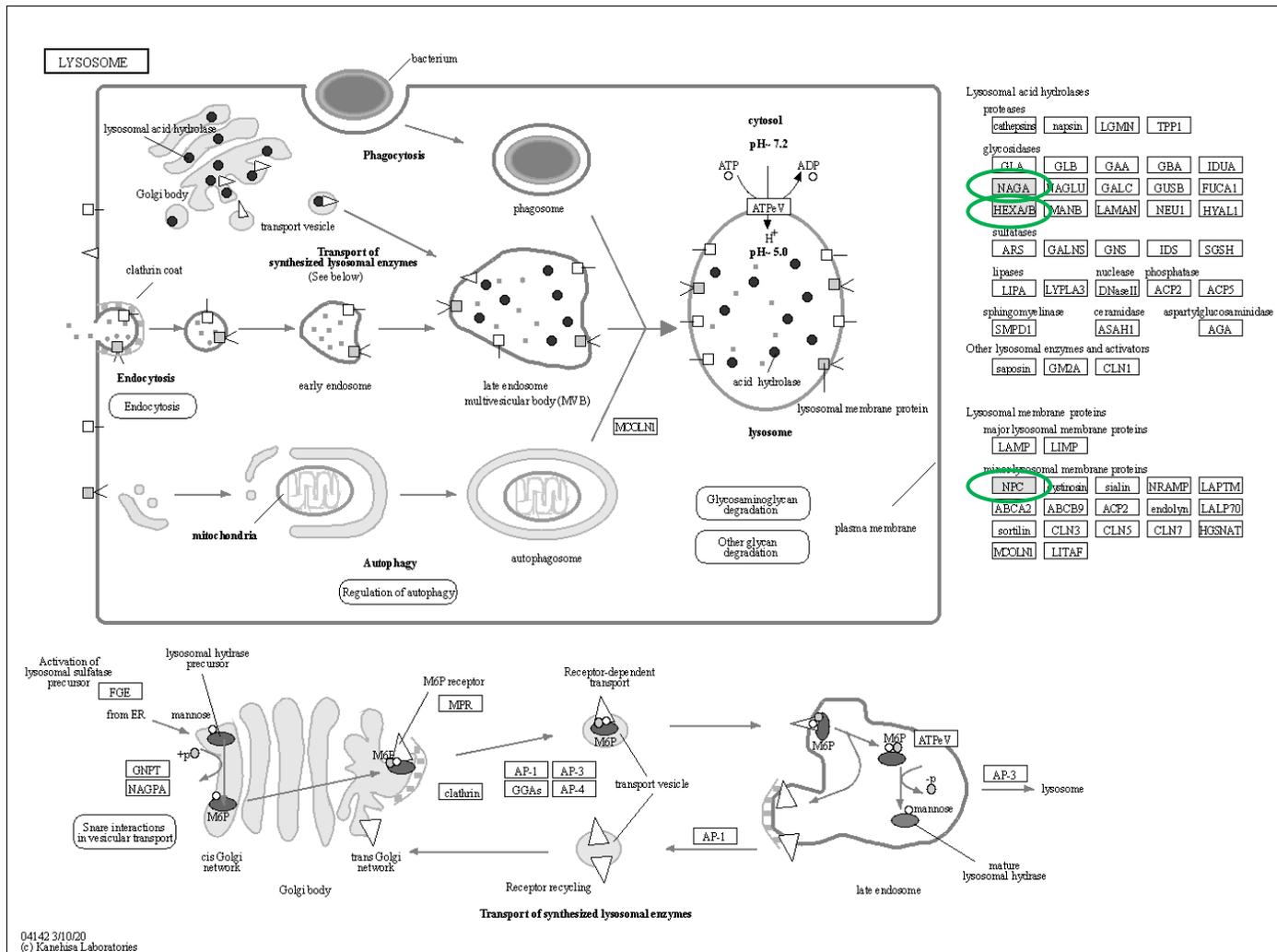
Figure S2: Mapping of the up-regulated proteins of *A. mellifera* in a context of infection by *Nosema* spp. isolates.



S2A: Mapping of the pathway ECM-receptor interaction (KEGG Pathways number ame04512). In a presence of *Nosema* spores the two Collagen IV NC1 domain-containing proteins of *A. mellifera* up-regulated are involved in the protein processing in endoplasmic reticulum pathway (in green circles).

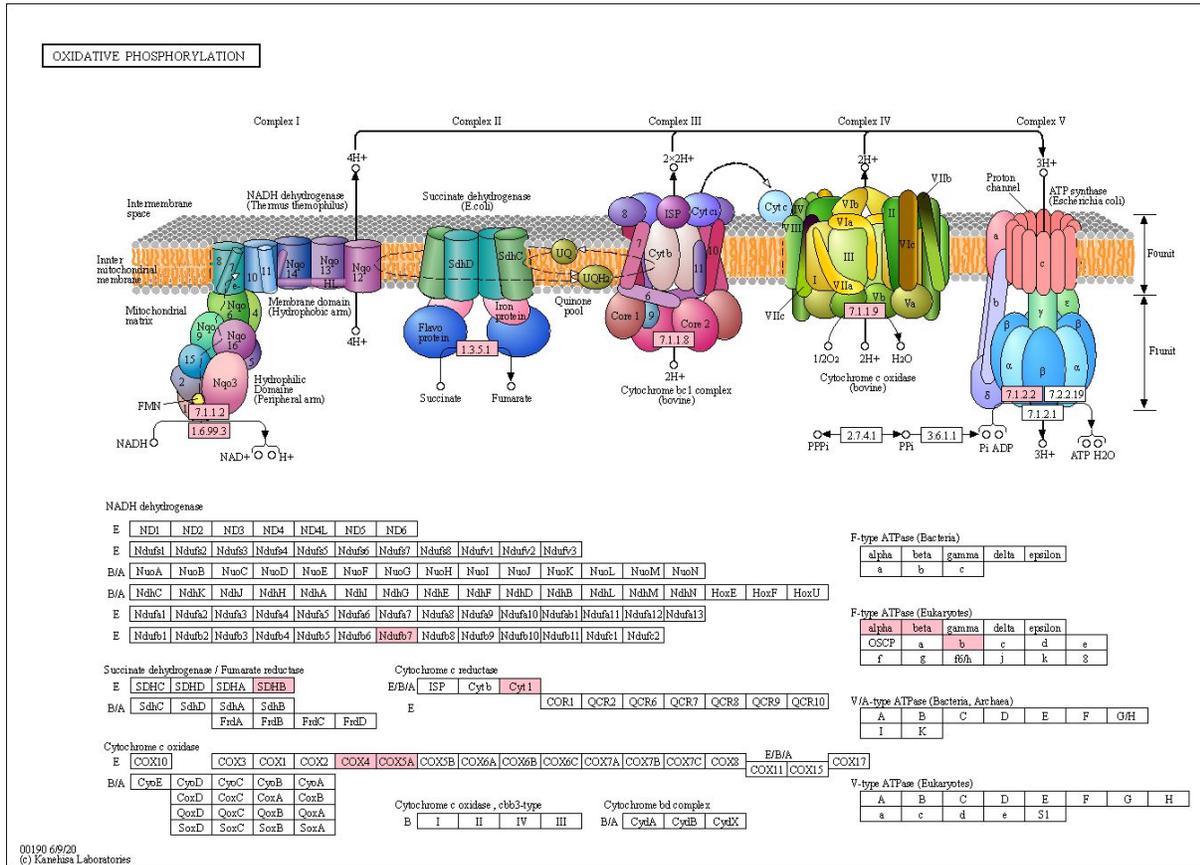


S2B: Mapping of the pathway AGE-RAGE signaling pathway in diabetic complications (KEGG Pathways number ame04933). In a presence of *Nosema* spores the two Collagen IV NC1 domain-containing proteins of *A. mellifera* up-regulated are involved in the protein processing in endoplasmic reticulum pathway (in green).

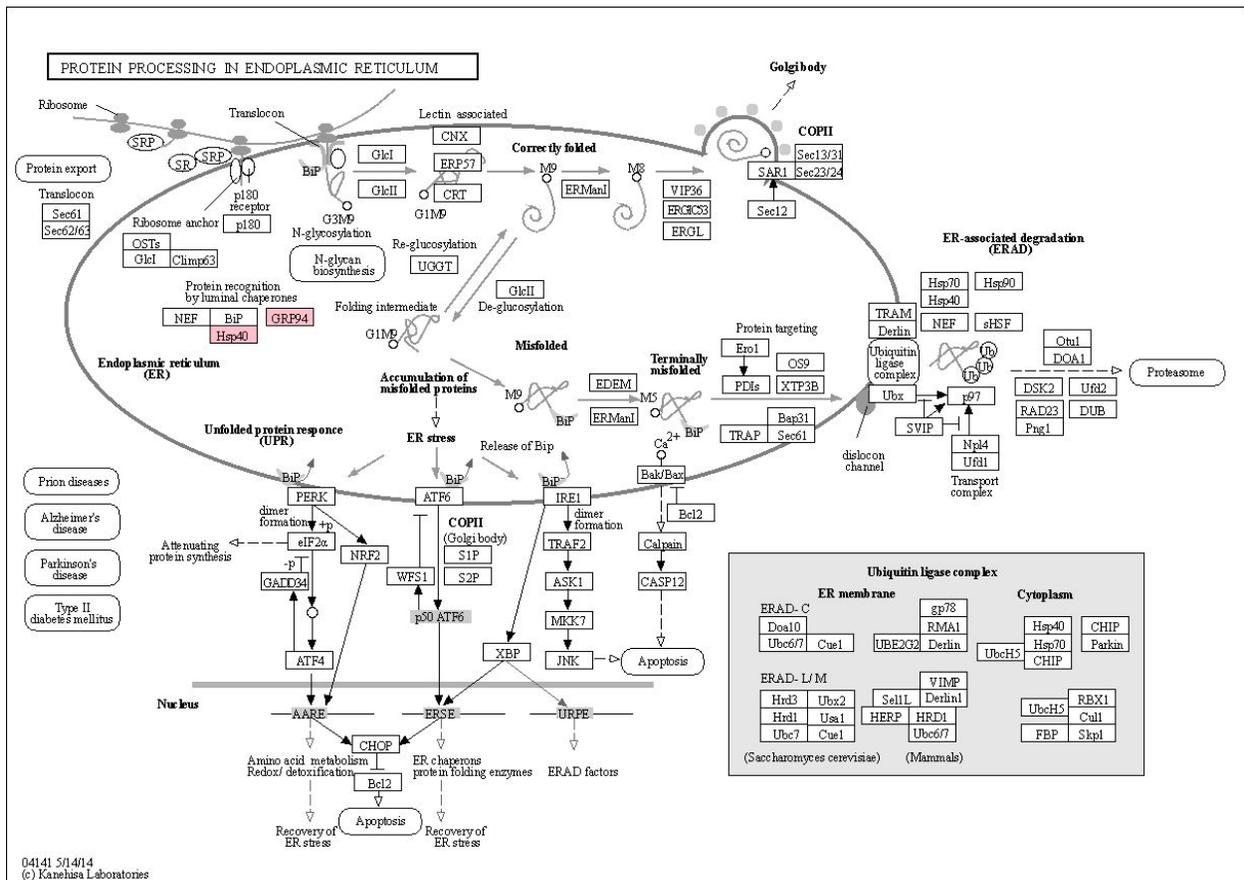


S2C: Mapping of the lysosome pathway (KEGG Pathways number ame04933). In a presence of *Nosema* spores the three proteins (Alpha-galactosidase, Beta-hexosaminidase and ML domain-containing protein) of *A. mellifera* up-regulated are involved in the protein processing in endoplasmic reticulum pathway (in green).

Figures S3: Mapping of the down-regulated proteins of *A. mellifera* in a context of infection by *Nosema spp.* isolates.



S3A: Mapping of the pathway oxidative phosphorylation (KEGG Pathways number ame00190). In a presence of *Nosema* spores the eight proteins (ATP synthase subunit b, cytochrome c oxidase subunit 5A, ATP synthase subunit alpha, cytochrome c oxidase subunit 4 isoform 1, cytochrome c1, succinate dehydrogenase iron-sulfur subunit, ATP synthase subunit beta, NADH dehydrogenase 1 beta subcomplex subunit 7) of *A. mellifera* down-regulated are involved in the oxidative phosphorylation pathway (in red).



S3B: Mapping of the pathway protein processing in endoplasmic reticulum (KEGG Pathways number ame04141). In a presence of *Nosema* spores the two proteins (HATPase_c domain-containing protein and Uncharacterized protein with the accession number A0A088ABL3) of *A. mellifera* down-regulated are involved in the protein processing in endoplasmic reticulum pathway (in red).

Table S1: PCR results of the four *Nosema* isolates.

Nosema species were confirmed by PCR analysis. The presence of *Nosema* was scored as 1 and 0 for its absence.

<i>Apis mellifera</i>			
Condition of <i>Nosema</i> infection	Apiary	PCR confirmation	
		<i>N. ceranae</i>	<i>N. apis</i>
<i>N. ceranae</i> from <i>A. cerana</i> from Thailand (NC1)	1	1	0
	2	1	0
	3	1	0
<i>N. ceranae</i> from <i>A. mellifera</i> from Thailand (NC2)	1	1	0
	2	1	0
	3	1	0
<i>N. ceranae</i> from <i>A. mellifera</i> from France (NF)	1	1	0
	2	1	0
	3	1	0
<i>N. apis</i> (NA)	1	0	1
	2	0	1
	3	0	1

Table S2: *Nosema* spores counted for each experimental modality.

<i>Apis mellifera</i>					
<i>Nosema</i> spp.	Apiary	Result	MEAN	SD	SE
		<i>N.ceranae</i> from <i>A. cerana</i> from Thailand (NC1)	1	27,600,000 28,300,000 20,200,000	25,366,666.67
	2	22,900,000 25,700,000 24,700,000	24,433,333.33	1,418,919.77	819,213.71
	3	22,700,000 22,100,000 25,000,000	23,266,666.67	1,530,795.00	883,804.90
<i>N.ceranae</i> from <i>A. mellifera</i> from Thailand (NC2)	1	51,700,000 44,000,000 35,500,000	43,733,333.33	8,103,291.51	4,678,437.53
	2	41,100,000 43,300,000 49,700,000	44,700,000.00	4,467,661.58	2,579,405.61
	3	46,900,000 48,800,000 51,300,000	49,000,000.00	2,206,807.64	1,274,100.99
<i>N. ceranae</i> from <i>A. mellifera</i> from France (NF)	1	26,000,000 24,200,000 25,000,000	25,066,666.67	901,849.950	520,683.311
	2	26,300,000 27,400,000 23,600,000	25,766,666.67	1,955,334.58	1,128,912.94
	3	26,700,000 26,300,000 22,500,000	25,166,666.67	2,318,045.15	1,338,323.99
<i>N. apis</i> (NA)	1	26,000,000 17,700,000 17,400,000	20,366,666.7	4,880,915.21	2,817,997.71
	2	16,100,000 17,700,000 18,600,000	17,466,666.7	1,266,227.99	731,057.073
	3	13,400,000 16,800,000 16,300,000	15,500,000.00	1,835,755.97	1,059,874.20