

Supplementary information

NO Synthesis in Immune-Challenged Locust Hemocytes and Potential Signaling to the CNS

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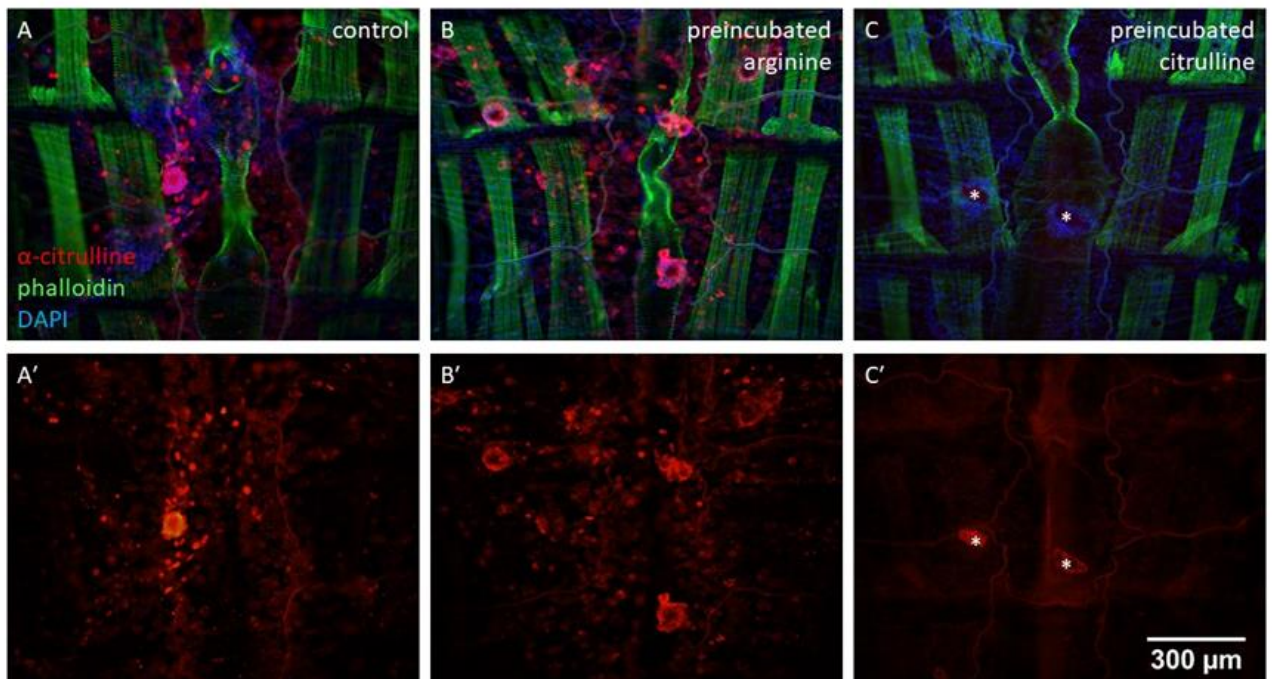


Figure S1 Testing of preincubated anti-citrulline antibody with BSA-conjugated amino acids on dorsal abdomens of first instar locust injected with 5×10^5 CFU heat-inactivated *E. coli* after 24h **A** control **B** preincubated with BSA-conjugated arginine 100mM (1:200) **C** preincubated with BSA-conjugated citrulline 100mM (1:200), asterisks indicate capsules.

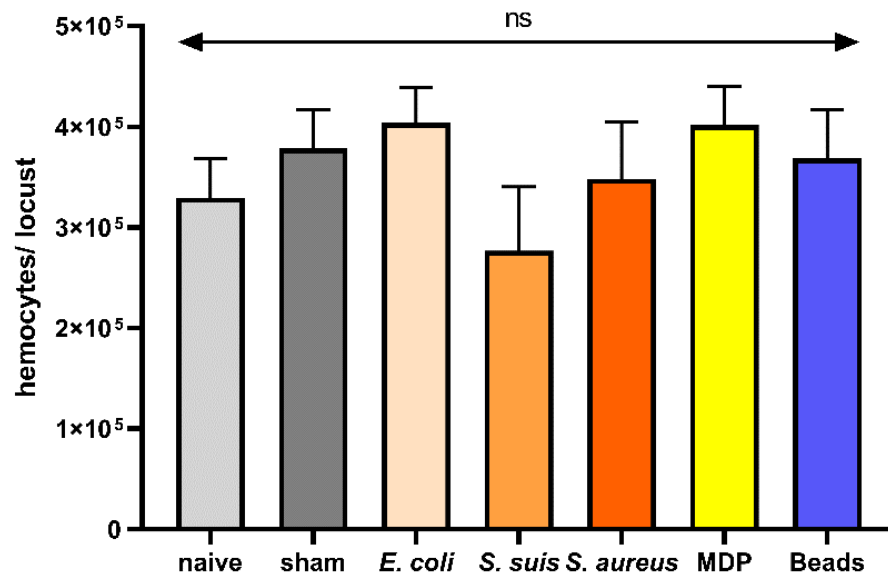


Figure S2 Average numbers of hemocytes gained from adult locusts of different treatment groups after hemocyte preparation. Naive: $329,389 \pm 39,257$ hemocytes per locust ($n=15$, \pm SEM), sham: $378,785 \pm 38,210$ hemocytes per locust ($n=16$, \pm SEM), *E. coli*: $403,802 \pm 35,439$ hemocytes per locust ($n=16$, \pm SEM), *S. suis*: $276,694 \pm 63,512$ hemocytes per locust ($n=6$, \pm SEM), *S. aureus*: $348,333 \pm 56,789$ hemocytes per locust ($n=3$, \pm SEM), MDP: $401,750 \pm 38,366$ hemocytes per locust ($n=3$, \pm SEM), PMMA beads (2 μ m): $368,834 \pm 47,834$ hemocytes per locust ($n=2$, \pm SEM), statistical test: Kruskal Wallis-test with Dunn's multiple comparison test. (ns) not significant

Table S1 Number of preparations with citrulline-positive hemocytes, with capsules and total preparations of first instar locust abdomens: sham injected, 5×10^5 CFU heat-inactivated *E. coli* injected, 5 mM D-NMMA and 5×10^5 CFU heat-inactivated *E. coli* injected and 5 mM L-NMMA and 5×10^5 CFU heat-inactivated *E. coli* injected.

		6h				24h			
		sham	<i>E. coli</i>	D-NMMA + <i>E. coli</i>	L-NMMA + <i>E. coli</i>	sham	<i>E. coli</i>	D-NMMA + <i>E. coli</i>	L-NMMA + <i>E. coli</i>
dorsal	with citrulline positive hemocytes	0	0	2	0	0	23	21	1
	with capsules	0	5	9	6	0	24	23	23
ventral	with citrulline positive hemocytes	0	0	6	0	0	24	14	1
	with capsules	0	2	3	2	1	13	7	3
total preparations		8	8	9	8	24	24	24	23

Table S2 Number of preparations with citrulline-positive hemocytes, with capsules and total preparations of first instar locust abdomens: naive locusts, LB injected, 5×10^5 CFU heat-inactivated *E. coli* injected, 5×10^5 CFU heat-inactivated *S. suis* injected and 5×10^5 CFU heat-inactivated *S. aureus* injected.

<i>S. suis</i>		6h				24h			
		naive	sham	<i>E. coli</i>	<i>S. suis</i>	naive	sham	<i>E. coli</i>	<i>S. suis</i>
dorsal	with citrulline positive hemocytes	1	2	3	5	0	6	35	33
	with capsules	0	1	13	10	0	1	35	25
ventral	with citrulline positive hemocytes	0	1	1	1	0	5	34	19
	with capsules	0	0	2	2	0	1	13	4
total preparations		12	20	20	20	18	36	36	36

<i>S. aureus</i>		6h				24h			
		naive	sham	<i>E. coli</i>	<i>S. aureus</i>	naive	sham	<i>E. coli</i>	<i>S. aureus</i>
dorsal	with citrulline positive hemocytes	1	10	9	11	0	10	18	8
	with capsules	0	1	8	1	0	1	17	4
ventral	with citrulline positive hemocytes	0	1	0	1	0	9	17	7
	with capsules	0	0	1	1	0	1	7	2
total preparations		12	12	12	12	18	18	18	18

Table S3 Number of preparations with citrulline-positive hemocytes, with capsules and total preparations of first instar locust abdomens: A) LPS injection, B) MDP injection, C) PMMA beads injection, D) RVFV injection.

A) LPS		6h			24h		
		sham	LPS 10 ng	LPS 100 ng	sham	LPS 10 ng	LPS 100 ng
dorsal	with citrulline positive hemocytes	0	0	0	0	1	0
	with capsules	0	0	0	0	0	0
ventral	with citrulline positive hemocytes	0	0	0	4	5	4
	with capsules	0	0	0	0	0	0
total preparations		16	16	16	24	24	24

B) MDP		6h				24h			
		naive	sham	MDP	<i>E. coli</i>	naive	sham	MDP	<i>E. coli</i>
dorsal	with citrulline positive hemocytes	0	1	2	10	2	9	3	17
	with capsules	0	0	0	10	0	0	10	1
ventral	with citrulline positive hemocytes	0	1	0	3	1	7	0	17
	with capsules	0	0	0	4	0	0	0	9
total preparations		12	12	12	12	18	18	18	18

C) beads		6h				24h			
		sham	<i>E. coli</i>	beads 2 µm	beads 20 µm	sham	<i>E. coli</i>	beads 2 µm	beads 20 µm
dorsal	with citrulline positive hemocytes	1	0	0	0	1	18	1	1
	with capsules	0	11	0	0	1	17	0	1
ventral	with citrulline positive hemocytes	0	4	0	0	0	17	1	0
	with capsules	0	5	0	0	0	13	0	0
total preparations		12	12	12	12	18	18	18	18

D) RVFV		6h				24h			
		naive	sham	RVFV	<i>E. coli</i>	naive	sham	RVFV	<i>E. coli</i>
dorsal	with citrulline positive hemocytes	1	0	0	0	1	1	0	16
	with capsules	0	0	0	11	1	0	1	16
ventral	with citrulline positive hemocytes	0	0	0	1	1	0	0	15
	with capsules	0	0	0	6	0	0	0	10
total preparations		12	12	12	12	18	18	18	18

Table S4 Numbers of adult locust primary hemocyte culture. Raw data can be found in a separate file (Table S4_Raw data.xlsx).

trial	n(biologicals replicates)	treatment	n(locusts)	n(culture wells)	n(images)
<i>S. suis</i>	6	naïve	18	18	95
		sham	18	18	96
		<i>S. suis</i>	18	18	95
		<i>E. coli</i>	18	18	94
<i>S. aureus</i>	3	naïve	9	6	60
		sham	9	6	60
		<i>S. aureus</i>	9	6	60
		<i>E. coli</i>	9	6	60
MDP	3	naïve	9	6	60
		sham	9	6	60
		MDP	9	6	60
		<i>E. coli</i>	9	6	60
beads	3	naïve	9	12	36
		sham	9	12	36
		beads	9	12	37
		<i>E. coli</i>	9	12	35
total			180	168	1004