

**Narciclasine improves outcome in sepsis among neonatal rats via inhibition of calprotectin
and alleviating inflammatory responses**

Manoj Kumar Kingsley¹, Ballambattu Vishnu Bhat ^{*1}, Bhawana Ashok Badhe², Benet Bosco
Dhas¹, Subhash Chandra Parija ³

Department of Neonatology¹, Pathology² and Microbiology³, JIPMER, Puducherry, India

SUPPLEMENTARY INFORMATION

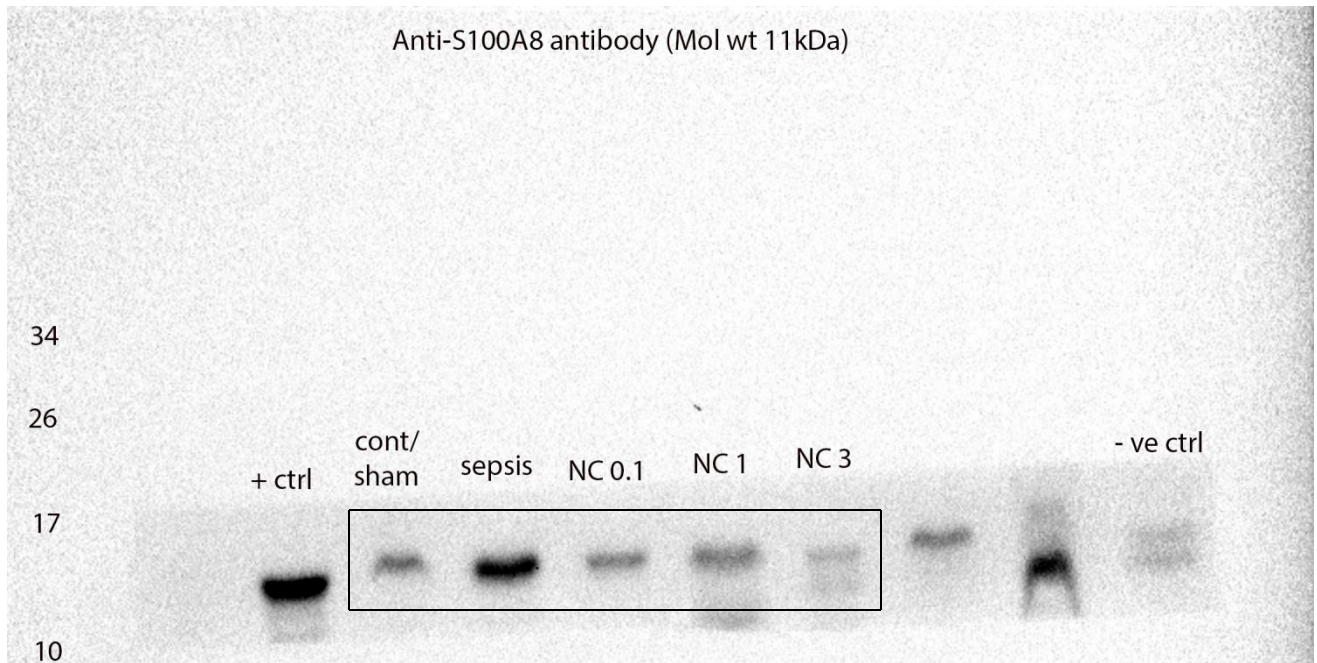
***¹Corresponding Author**

Ballambattu Vishnu Bhat,
Professor (Paediatrics), Pondicherry Institute of Medical Sciences,
Former HOD, Dept of Neonatology,
JIPMER, Puducherry-605006, India
Email : drvishnubhat@yahoo.com
Mobile: +91 9842351282

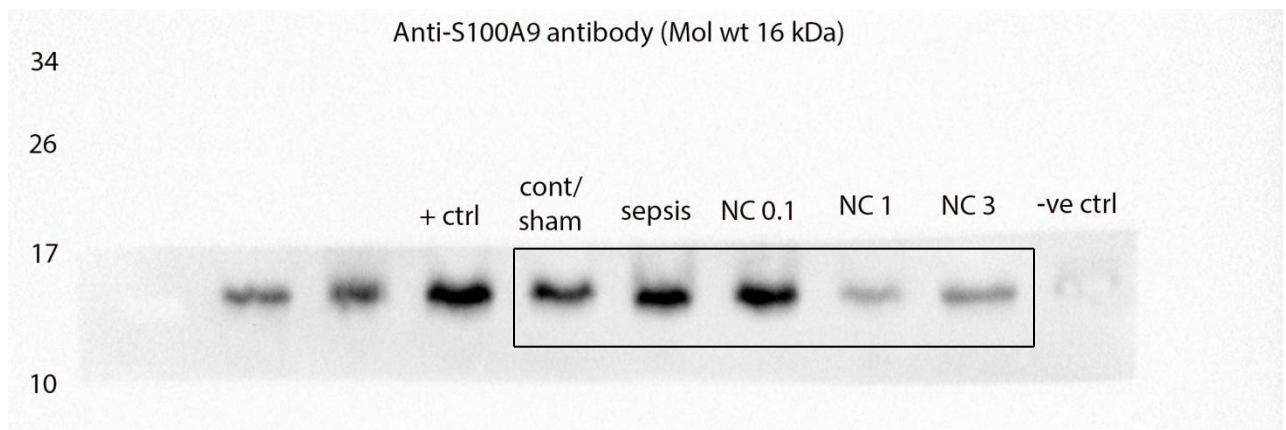
Supplementary Table S1. Primers used for qRT-PCR

Name of Gene	Primer	Primer sequence (5' → 3')
S100A8	Forward	GGCAACTGAACTGGAGAAGG
	Reverse	CCCACCCTTATCACCAACAC
S100A9	Forward	AGGACCTGGACACAAACCAG
	Reverse	GACTTGGTTGGGCAGATGTT
TNF- α	Forward	ACTCCCAGAAAAGCAAGCAA
	Reverse	CGAGCAGGAATGAGAAGAGG
IL-6	Forward	CCGGAGAGGAGACTTCACAG
	Reverse	ACAGTGCATCATCGCTGTTC
β -actin	Forward	AGCCATGTACGTAGCCATCC
	Reverse	CTCTCAGCTGTGGTGGTGAA

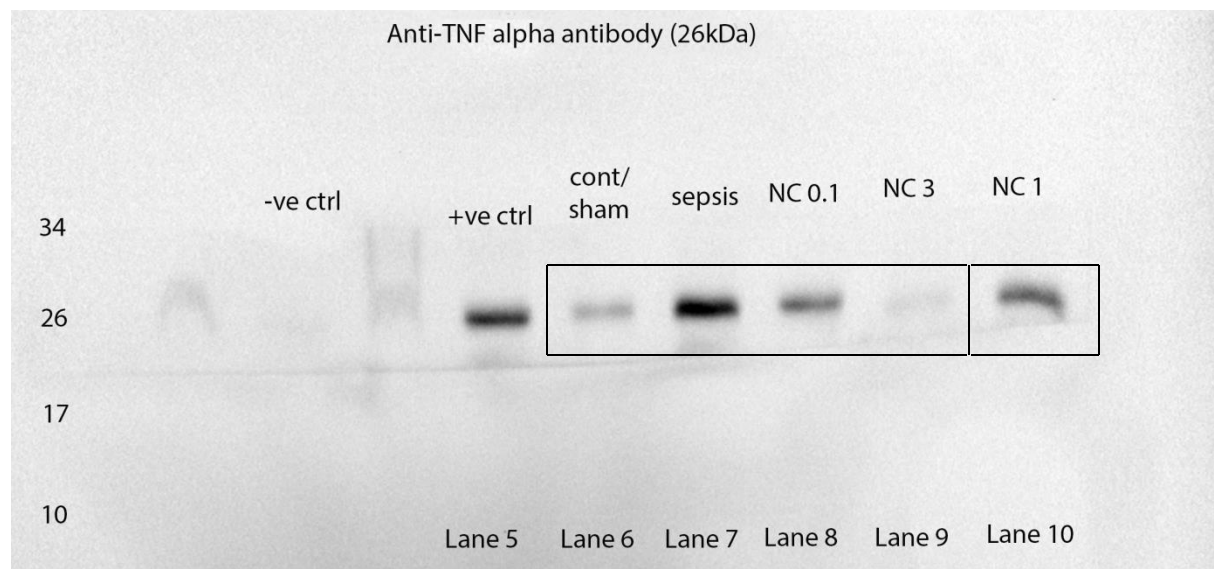
Full Length Western Blots



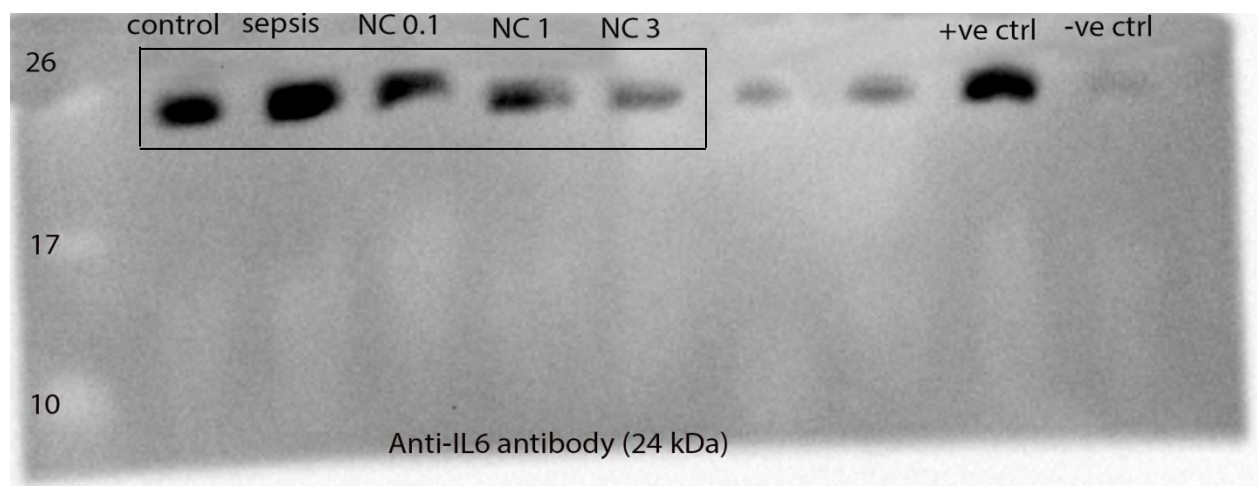
Supplementary Figure S1a shows the full length western blot for S100A8 protein expression in **Fig 4b**. The rectangle box indicates the areas of the western blots shown in the main figure (cont/sham – control group; sepsis group; NC 0.1 – sepsis + narciclasine 0.1 mg/kg group; NC 1 – sepsis+ narciclasine 1 mg/kg group; NC 3- sepsis + narciclasine 3 mg/kg group)



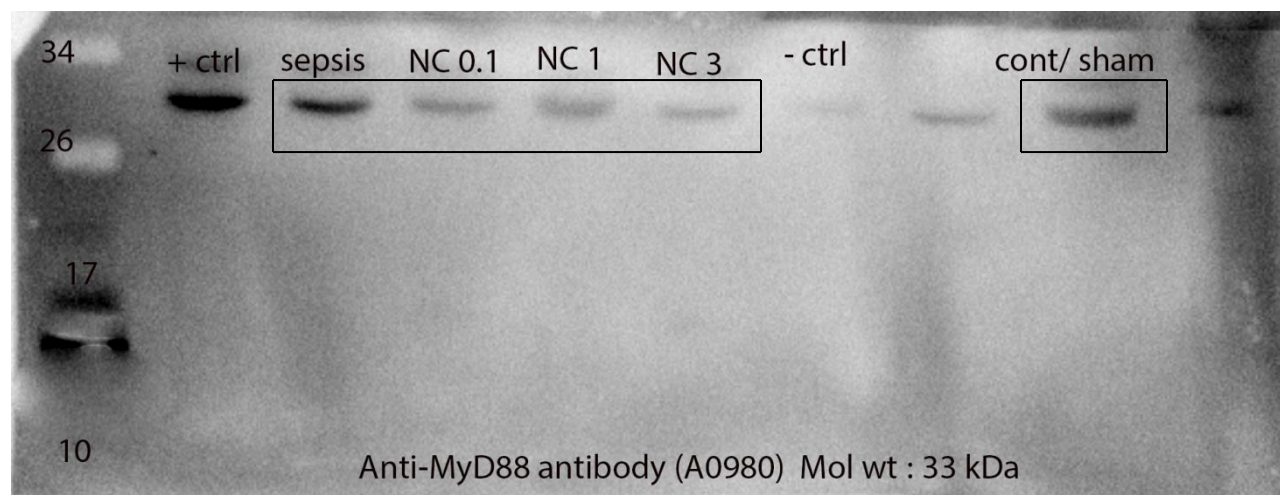
Supplementary Figure S1b shows the full length western blot for S100A9 protein expression in **Fig 4b**. The rectangle box indicates the areas of the western blots shown in the main figure (cont/sham – control group; sepsis group; NC 0.1 – sepsis + narciclasine 0.1 mg/kg group; NC 1 – sepsis+ narciclasine 1 mg/kg group; NC 3- sepsis + narciclasine 3 mg/kg group)



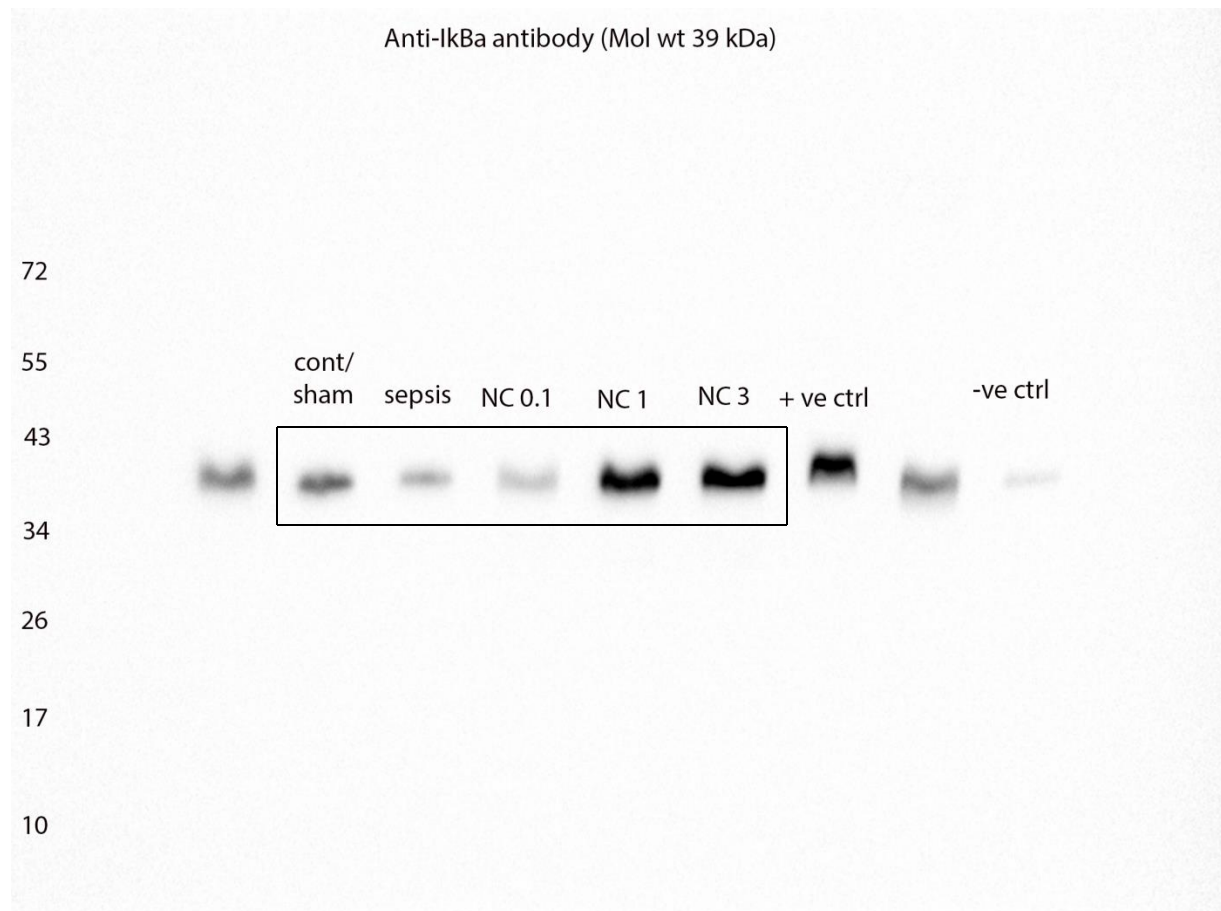
Supplementary Figure S2a shows the full length western blot for TNF- α protein expression in **Fig 6d** of the main manuscript. Note that the lanes 6-9 and tenth lane was cropped out to make a composite image according to the group wise order in **Fig 6d**. The rectangle boxes indicate the areas of the western blots shown in the main figure (cont/sham – control group; sepsis group; NC 0.1 – sepsis + narciclasine 0.1 mg/kg group; NC 1 – sepsis+ narciclasine 1 mg/kg group; NC 3- sepsis + narciclasine 3 mg/kg group)



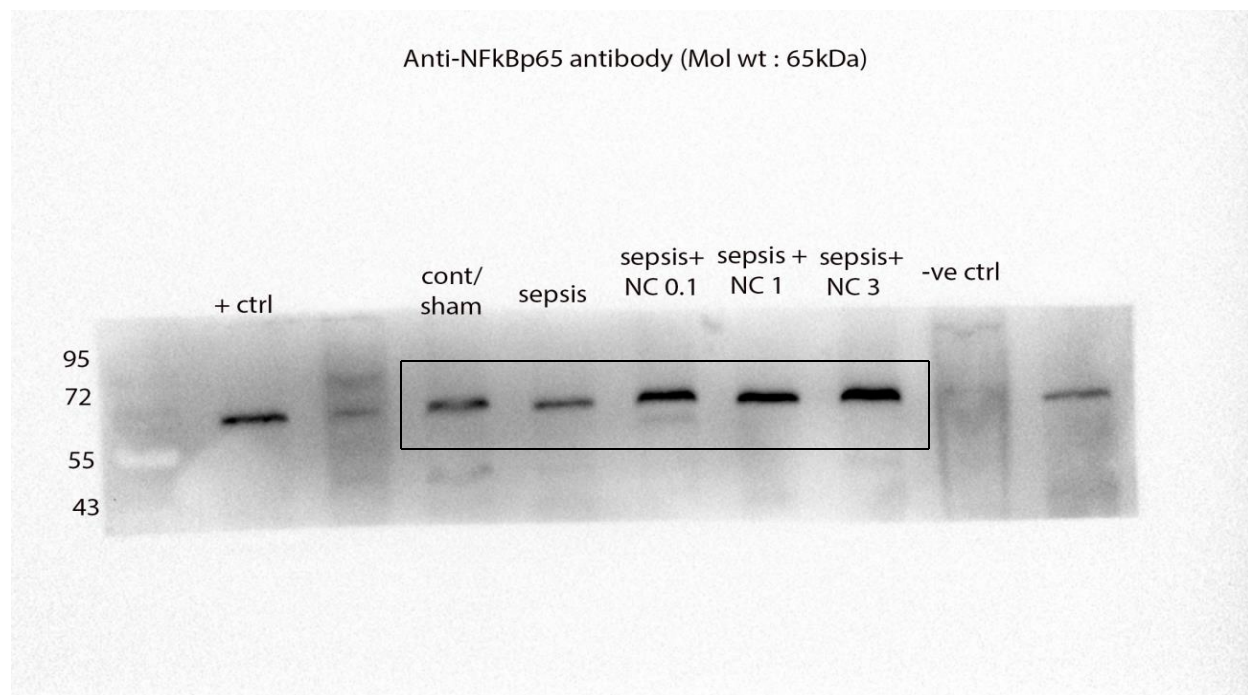
Supplementary Figure S2b shows the full length western blot for IL-6 protein expression in **Fig 6d** of the main manuscript. The rectangle box indicates the areas of the western blots shown in the main figure (control group; sepsis group; NC 0.1 – sepsis + narciclasine 0.1 mg/kg group; NC 1 – sepsis+ narciclasine 1 mg/kg group; NC 3- sepsis + narciclasine 3 mg/kg group)



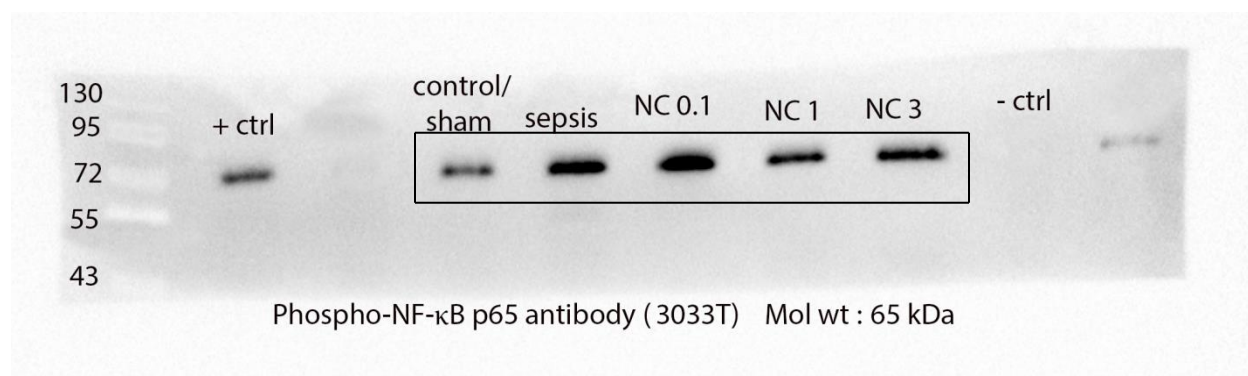
Supplementary Figure S3a shows the full length western blot for MyD88 protein expression in **Fig 9b** of the main manuscript. Note that the lanes 3-6 and ninth lane was cropped out to make a composite image according to the group wise order in **Fig 9b**. The rectangle boxes indicate the areas of the western blots shown in the main figure (cont/sham – control group; sepsis group; NC 0.1 – sepsis + narciclasine 0.1 mg/kg group; NC 1 – sepsis+ narciclasine 1 mg/kg group; NC 3- sepsis + narciclasine 3 mg/kg group)



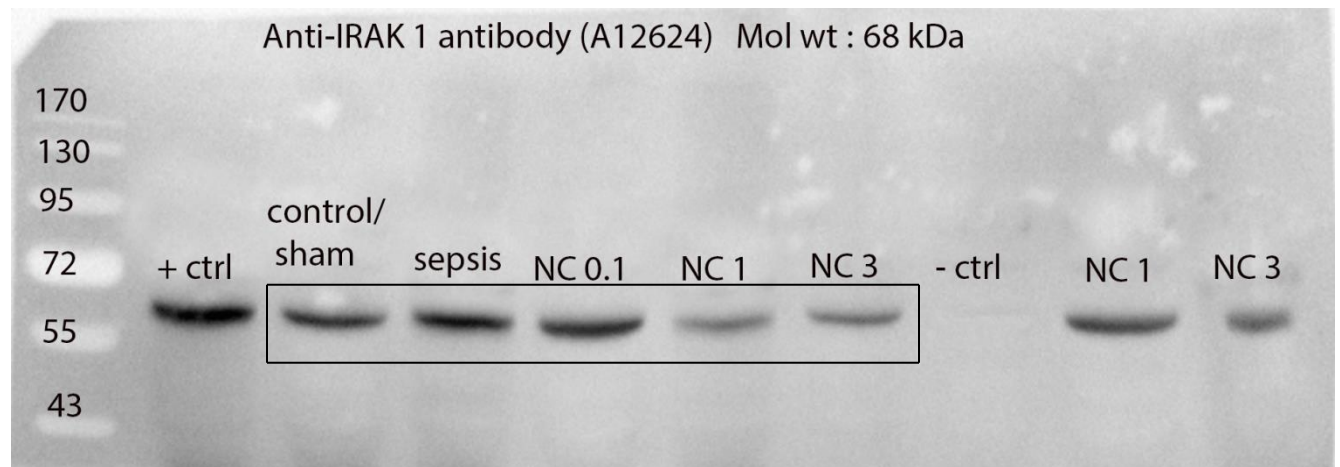
Supplementary Figure S3b shows the full length western blot for I κ B α protein expression in **Fig 9b** of the main manuscript. The rectangle box indicates the areas of the western blots shown in the main figure (cont/sham – control group; sepsis group; NC 0.1 – sepsis + narciclasine 0.1 mg/kg group; NC 1 – sepsis+ narciclasine 1 mg/kg group; NC 3- sepsis + narciclasine 3 mg/kg group)



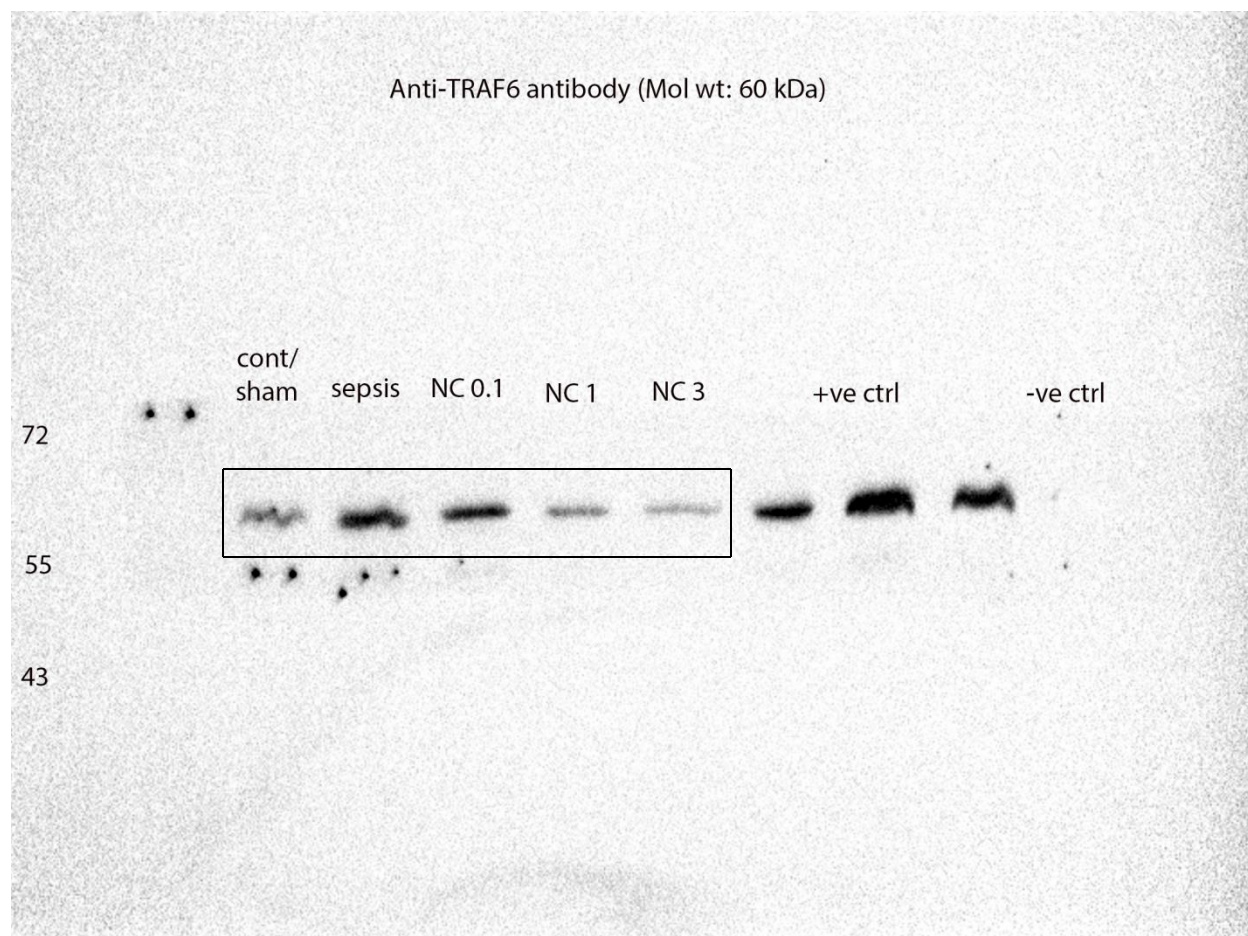
Supplementary Figure S3c shows the full length western blot for NFκBp65 protein expression in **Fig 9b** of the main manuscript. The rectangle box indicates the areas of the western blots shown in the main figure (cont/sham – control group; sepsis group; NC 0.1 – sepsis + narciclasine 0.1 mg/kg group; NC 1 – sepsis+ narciclasine 1 mg/kg group; NC 3- sepsis + narciclasine 3 mg/kg group)



Supplementary Figure S3d shows the full length western blot for phospho-NFκBp65 protein expression in **Fig 9b** of the main manuscript. The rectangle box indicates the areas of the western blots shown in the main figure (control/sham – control group; sepsis group; NC 0.1 – sepsis + narciclasine 0.1 mg/kg group; NC 1 – sepsis+ narciclasine 1 mg/kg group; NC 3- sepsis + narciclasine 3 mg/kg group)



Supplementary Figure S3e shows the full length western blot for IRAK-1 protein expression in **Fig 9b** of the main manuscript. The rectangle box indicates the areas of the western blots shown in the main figure (cont/sham – control group; sepsis group; NC 0.1 – sepsis + narciclasine 0.1 mg/kg group; NC 1 – sepsis+ narciclasine 1 mg/kg group; NC 3- sepsis + narciclasine 3 mg/kg group)



Supplementary Figure S3f shows the full length western blot image for TRAF 6 protein expression in **Fig 9b** of the main manuscript. The rectangle box indicates the areas of the western blots shown in the main figure (cont/sham – control group; sepsis group; NC 0.1 – sepsis + narciclasine 0.1 mg/kg group; NC 1 – sepsis+ narciclasine 1 mg/kg group; NC 3- sepsis + narciclasine 3 mg/kg group)