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Supplemental Information

Hookworms Evade Host Immunity by Secreting a Deoxyribonuclease to Degrade Neutrophil Extracellular Traps

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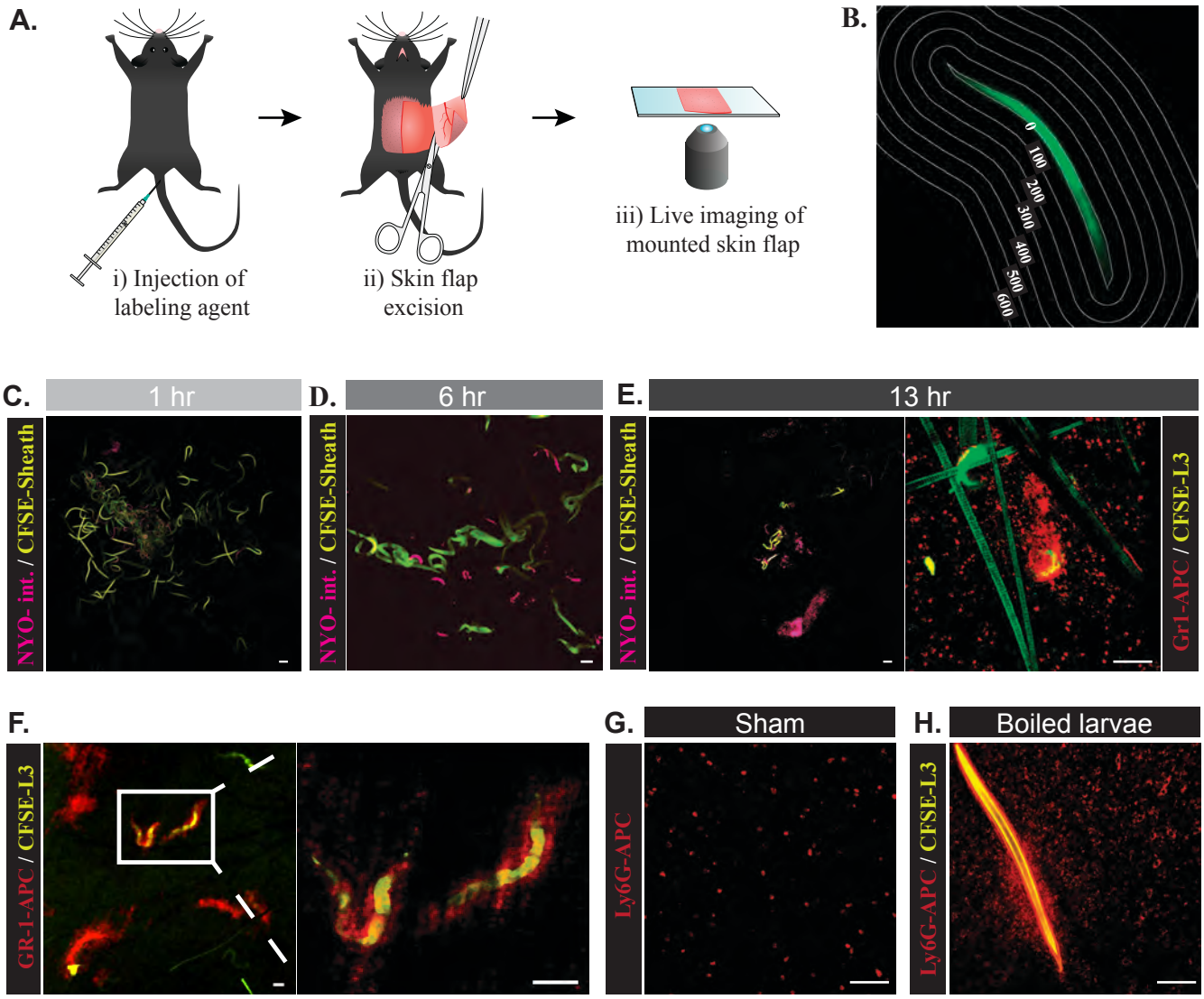

Figure S1. Myeloid cells swarm around hookworm larvae naturally penetrating the skin of mice. Related to Figure 1.

A-E. Mice were given an iv. injection of 5 µg APC labeled anti-Ly6G mAb 1 hr before id. injection of 250 Nb L3 into the belly skin. Mice were sacrificed just prior to imaging and the area of skin surrounding the infection site excised. **A**. Schematic showing the experimental protocol and mounting of excised skin in a chamber containing fluorobrite medium and kept at 37°C, 5% C02. Imaging was conducted using a confocal microscope with the subcutaneous tissue facing upwards. **B.** Schematic showing the quantitation of cells and their estimated distance from concentric shaped larvae, used in the generation of data shown in Fig 1C&E. **C-E.** Prior to use in infection exp. Nb L3 were incubated with NYO-labeled beads to stain the worm intestinal lumen, the external sheath stained using CFSE. Images show larvae (pink), sheaths (green) and for some images Gr-1+ myeloid cells (red) within skin excised from mice at the indicated timepoints following infection. Scale bar=100 µm. Images are representative of 2 independent exp. (n=3/timepoint). **F.** Mice were given an iv. injection of 5 µg APC labeled anti-Ly6G mAb 1 hr before the first topical application of CFSE-labeled larvae to the depilated belly skin. 100 L3 were applied topically to the same site every 30 min for 6 hrs. 3 hrs after the last topical application of larvae, mice were sacrificed, skin from the infected site excised and imaged as detailed in A. n=3. Scale bar=100 µm. **G.** Mice were given an id. injection 10 µl of PBS and the skin around the injection site imaged 6 hrs later as detailed in A. **H.** Mice were given an id. injection 250 dead (boiled) Nb L3 in 10 µl of PBS and the skin around the injection site imaged 6 hrs later as detailed in A.

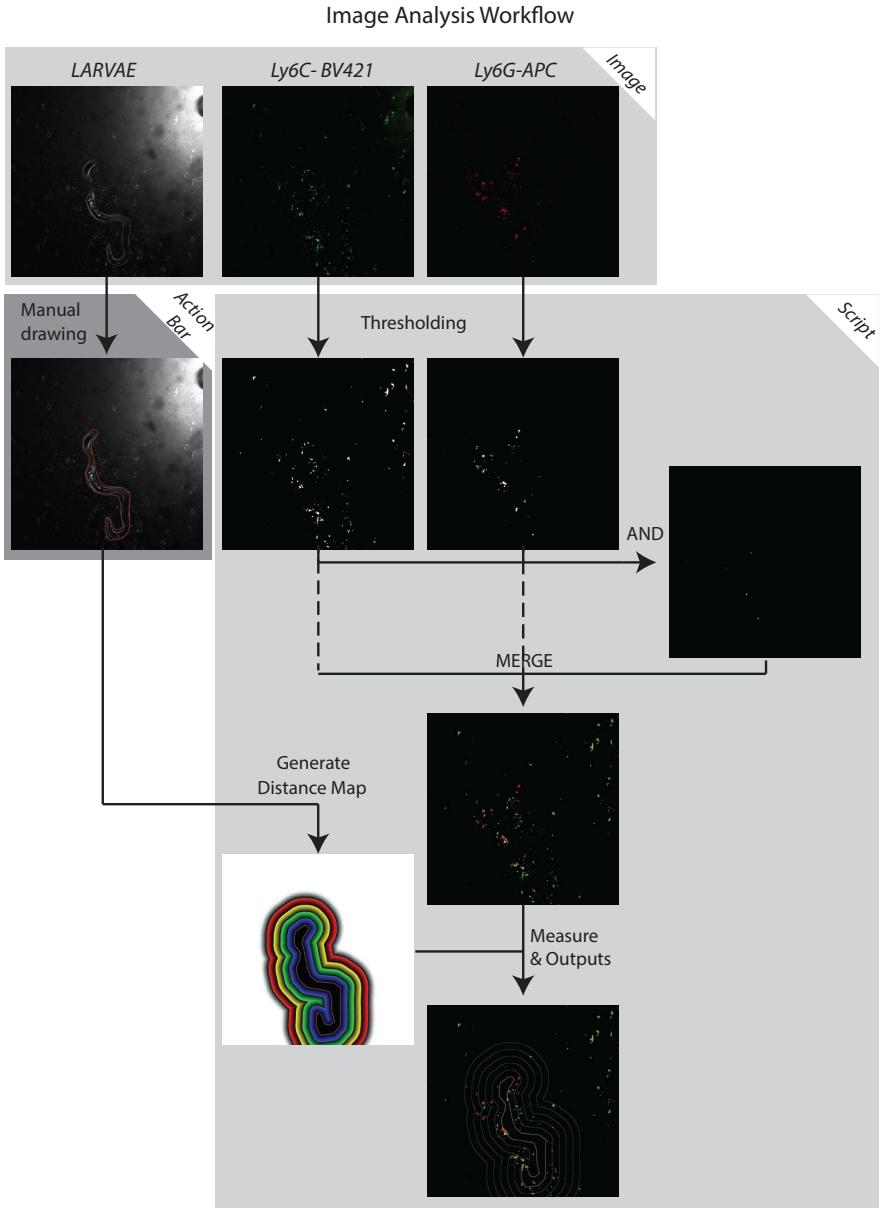

Figure S2. Analysis workflow of neutrophil and monocyte recruitment. Related to Figure 1.
The image analysis was performed with Fiji, using a generic ActionBar to define Region Of Interest (ROI) and a custom script (ImageJ macro language, available on demand) to automatically threshold and measure the images. The worm(s) outline(s) were manually drawn by an experimenter for each image, using the generic BIOP ActionBar "Multi Manual Select" that facilitates ROIs creation.
The two channels of interest (respectively Gr1 or Ly6C and Ly6G markers) were binarized using an automatic thresholding algorithm (respectively "Intermodes" for 1 hour time point and "Huang" for 6 hours time point, for both channels).The automatic thresholding algorithm was chosen based on the visual inspection of output images. The segmented images were denoised with a Median Filter (radius 2 pixels). The resulting masks of the two channels were used to generate a third mask using the Boolean operator AND (keeping the common positive pixels).
For each image, the experimenter-defined ROI(s) were used to create a distance map. This distance map was used to generate measurement bands, with a defined width (defined by experimenter to be 100 microns), iteratively expanded to a maximum value (defined by experimenter to be 600 microns). Finally, the positive areas of the masks were measured for each band and output as a table.
As an output image, the three masks (the two channels of interest and the resulting AND operation) were merged and saved as and RGB image for visualization as thumbnails in different Operating System independently of any software.

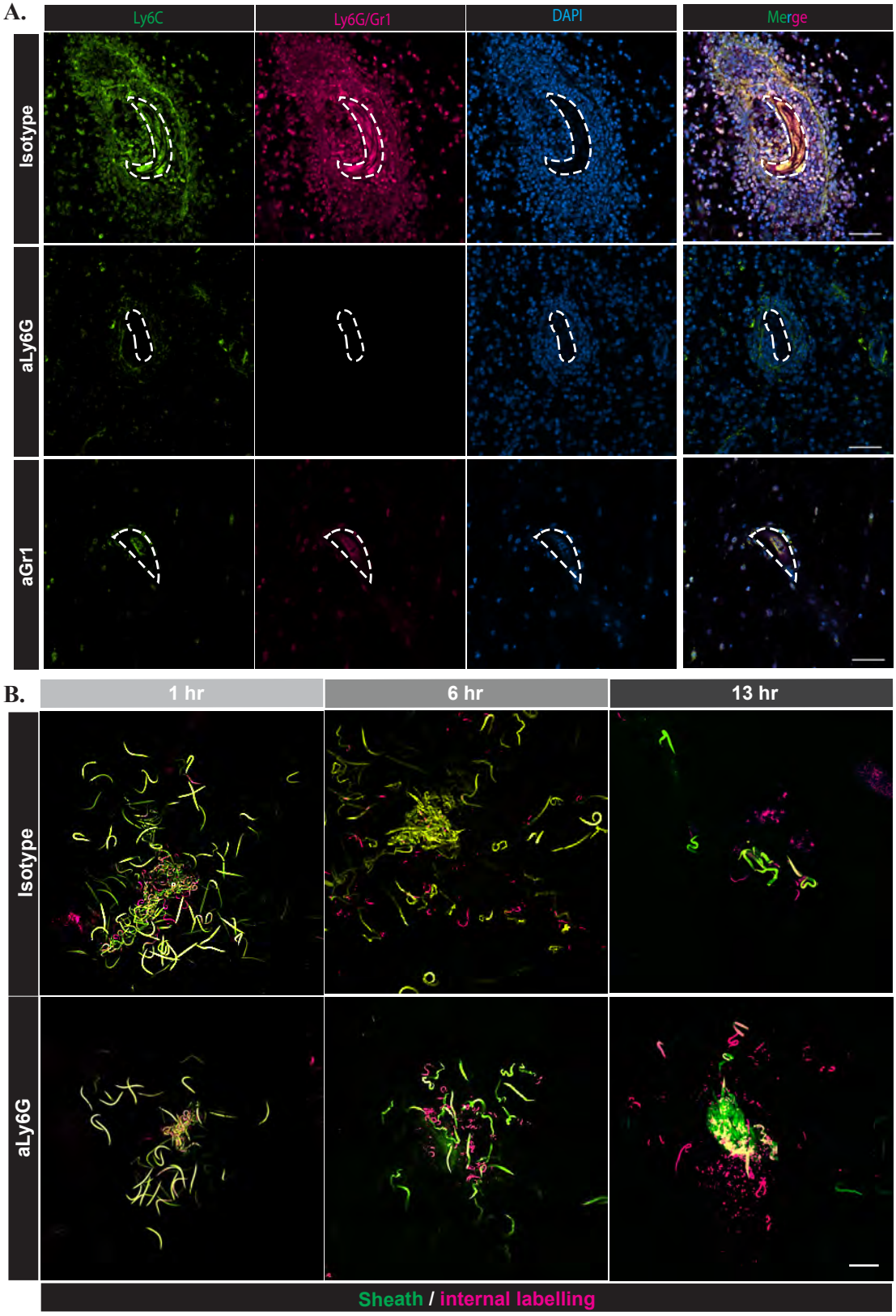

Figure S3. Neutrophil depletion efficiency in the skin. Related to Figure 2.

A. Mice were treated with 250 µg anti-Gr-1 mAb or 500 µg of anti-Ly6G mAb by ip. injection on day -1, 0 and 1. On day 0 mice were infected with 250 Nb L3 by id. injection and the skin surrounding the infected area removed 24 hrs later. Paraffin sections were stained as follow: All sections were stained anti-Ly6C (green) and counterstained with the DNA dye DAPI (blue). Those taken from mice treated with ic. mAb or anti-Gr-1 mAb were additionally stained with and anti-Ly6G (red) whilst those treated with anti-Ly6G were additionally stained with anti-Gr1 (red). Data are representative of images taken n=5/treatment group. Scale bar=50 µm. **B.** The dispersion of doubled-labeled larvae (CFSE for external labelling, NYO carboxybeads for internal labelling) was followed in the skin at 6 hr in condition described in **A.** The skin was imaged as described in FigS1A. Scale bar 400 um. Images representatives of 2 independent exp. n=3/time point.

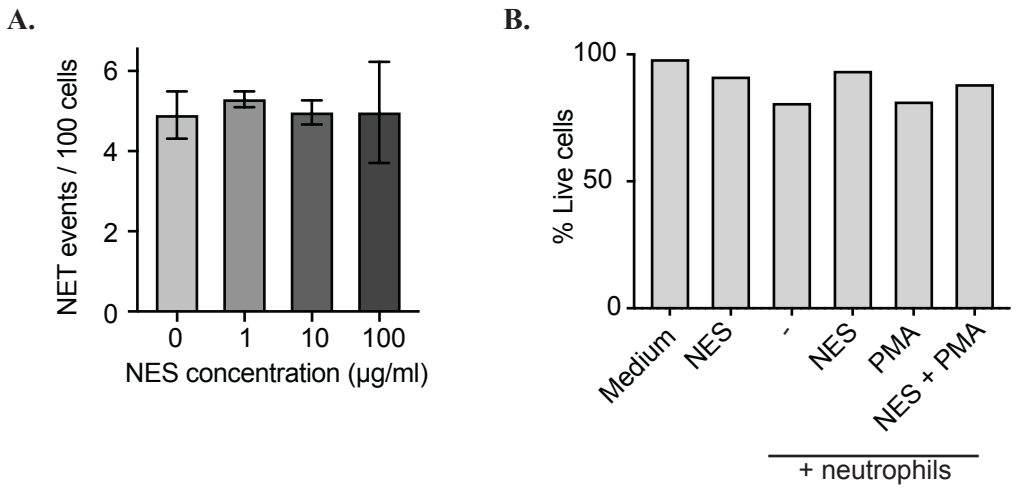

Figure S4. Nb-DNAse II, a NES protein, can degrade NETs. Related to Figure 3.

A. Human peripheral neutrophils were stimulated with C. albicans hyphae to induce NET formation and increasing concentrations of NES extract added to the cultures. NET events were counted dynamically using live cell microscopy and the number of NET events occurring over 14 hrs were counted per 1500 cells per condition. Data are representative of 2 independent experiment. **B.** Human peripheral neutrophils were stimulated with or without PMA and 30 µg/ml of NES. Supernatants of neutrophil cultures were then added to human peripheral monocytes and monocyte viability was measured by Sytox staining. Control monocytes were incubated in fresh medium with or without NES. All values were normalized against monocytes that had not received a medium change.

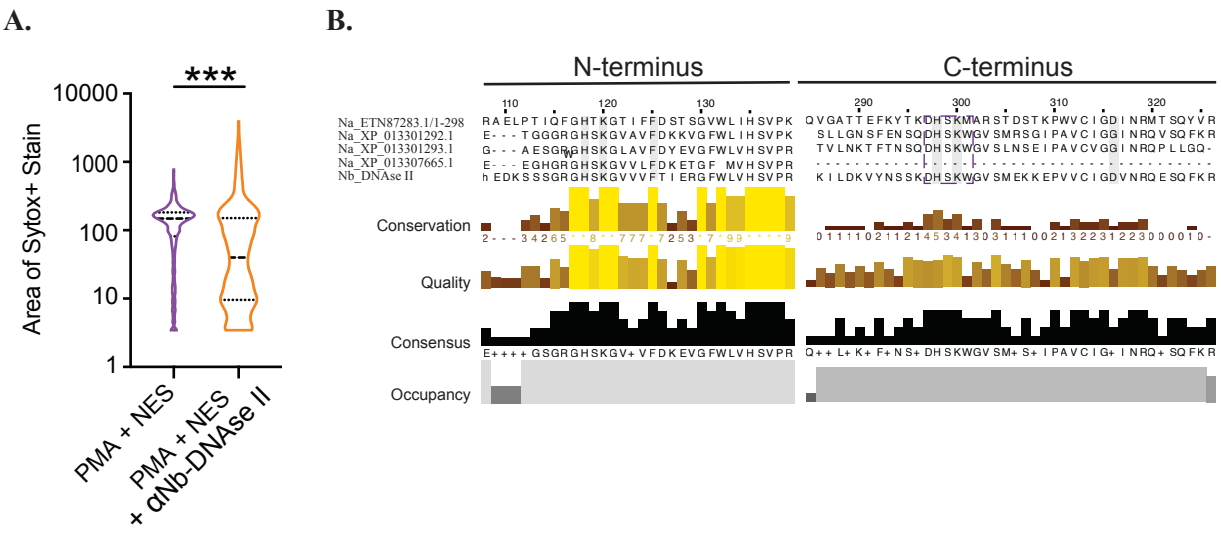

Figure S5. A conserved DNAse II in hookworms. Related to Figure 5.

A. Human peripheral blood neutrophils were stimulated with 100 nM PMA to induce NET formation in the presence of 10 µg/mL of NES with or without antisera raised against Nb-DNAse II (1:50). NETs were visualized and their area quantified as described in Fig3a. More than 1000 events were recorded for each condition. **B.** Amino acid sequence alignment of Nb-DNAse II and the corresponding regions of Na DNase homologues. The catalytic site contains two copies of a variant HKD motif where the aspartic acid residue is variable; the motifs are distributed in both the N- and C-terminal domains (shown in gray box). Nb sequence (m.13872) is available in Sup Text 1.

> m.13872_N. brasiliensis_DNAseII
SCKNMEGKDVDWFAAVKLPSNVDERKGRTFAYYDSTQTGWKFSPLPINSTDSAIGATVKQLYDSDNSY
HLKIAYNDDHPhghEDKSSSGRGHSKGVVVFTIERGFWLVHSVPRFPDPEKYDYPESGSKFAQSFICLTLSS
DFLPDISQYLRYSQVTPFVMNLPENHKLLAPYLVDVQAKKSLGRAdtKFTSTHSYQTMGGKRFTILAKHKK
FNNDLWHDFIALYFKTPMAVETWRNGaAKNVGTQCGVGYNVYDITTVKILDKVYNSSKDHSKWGVSM
EKKEPVVCIGDVNRQESQFKRGGGAVCMEDVKLWNTFHDSVKSYLNC

Data S1. *N. brasiliensis* DNAse II protein sequence. Related to Figure 5.