

# Extracellular vesicles secreted by *Nippostrongylus brasiliensis* contain vaccine candidates and protect against infection

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## INTRODUCTION

- Nippostrongylus brasiliensis* is a soil-transmitted nematode from rodents that has been extensively used as a model to study the immunobiology of gastrointestinal nematode infections because of the similarities in life cycle and morphology between this species and the human hookworms *Necator americanus* and *Ancylostoma duodenale*<sup>1</sup>.
- In a previous study we showed that *N. brasiliensis* secretes extracellular vesicles (EVs) that contain, at least, 81 different proteins, including immunomodulatory molecules and proteins previously used as vaccine candidates in other helminths<sup>2</sup>.
- In the present study we have cloned and expressed 5 different proteins present in the EVs from *N. brasiliensis* and tested their ability to protect against primary infections.

## MATERIALS AND METHODS

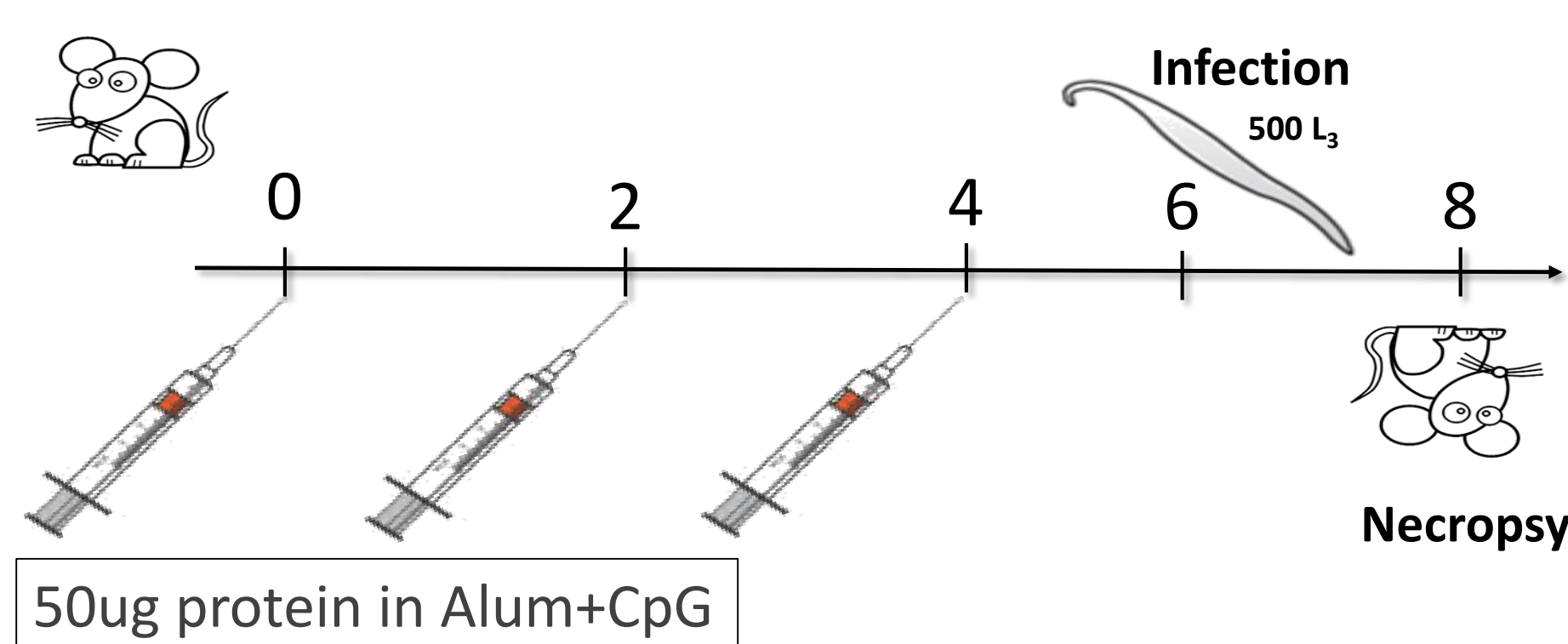
### Obtention and purification of EVs:

- EVs were obtained from cultured parasites by ultracentrifugation and purified using an Optiprep® density gradient to remove contaminants<sup>2</sup>.

### Cloning and protein expression:

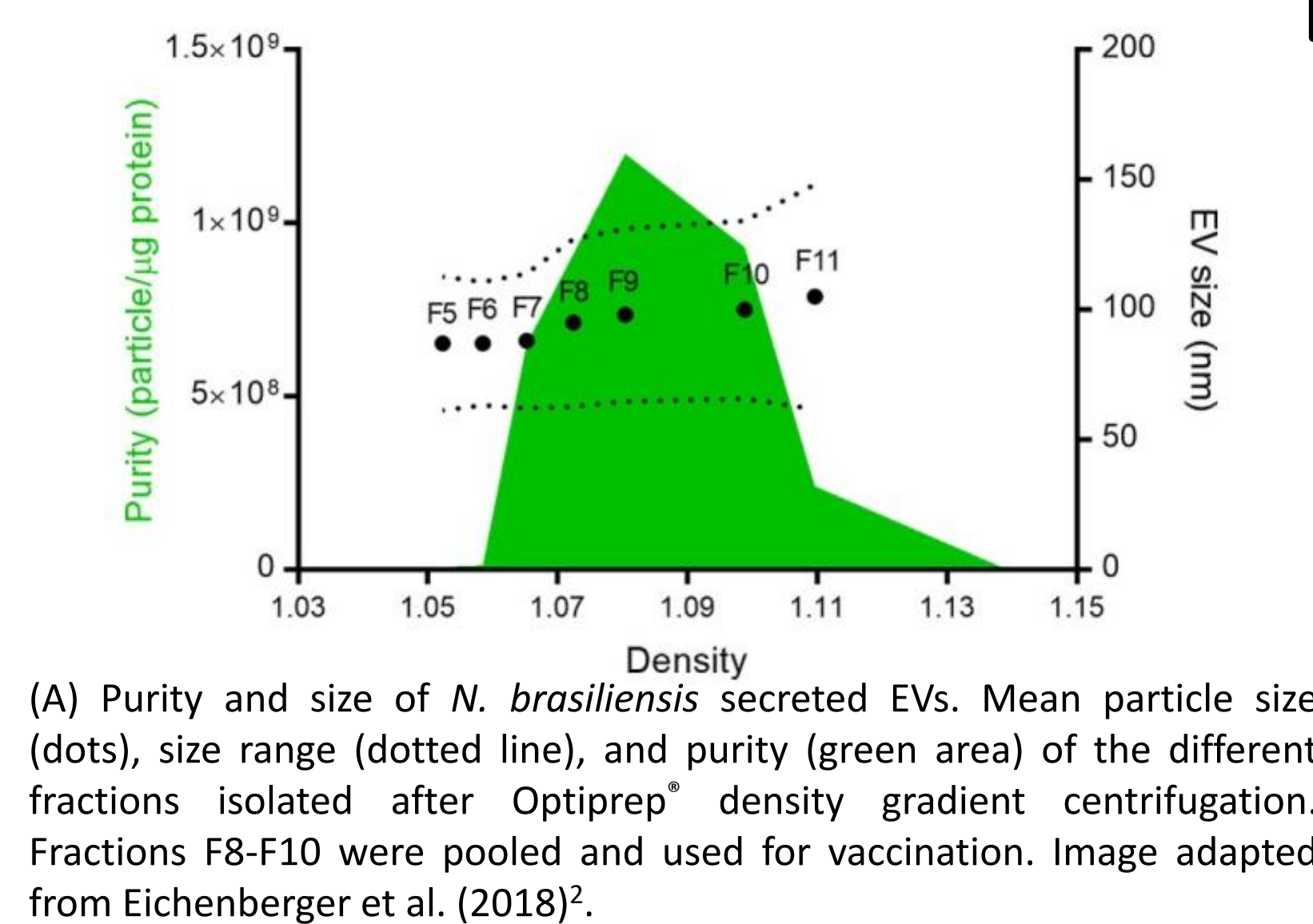
- Tetraspanin (TSP), Peroxiredoxin (PRX), Ferritin (FER) and Saposin (SAP) were cloned into pET32a vector and expressed in BL21 *Escherichia coli*.
- An SCP/TAPS protein (SCP) was cloned into pPICZalpha vector and expressed using *Pichia pastoris*.
- All proteins were purified with a His-Trap column using an ÄKTA pure system.

### Vaccination strategy:

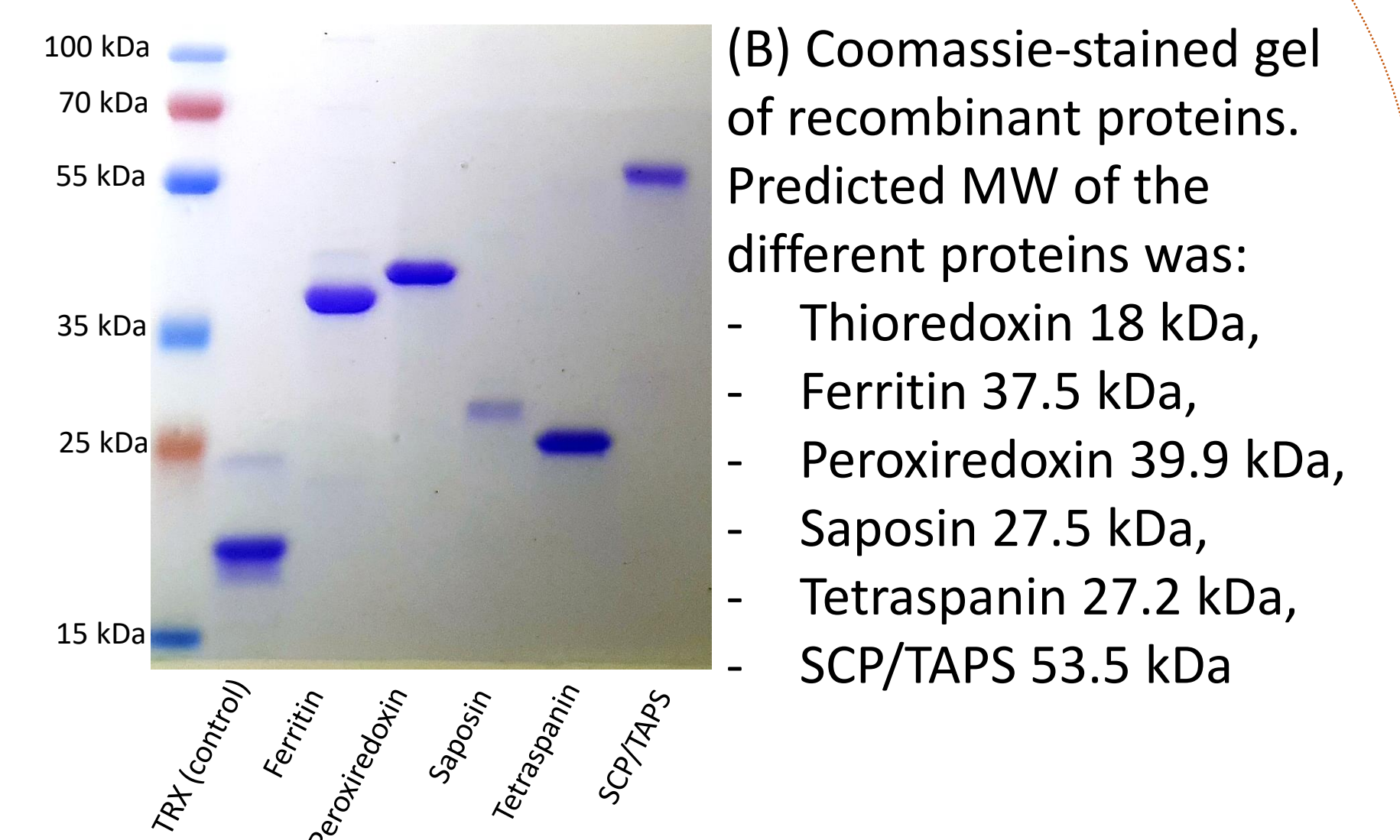


## RESULTS

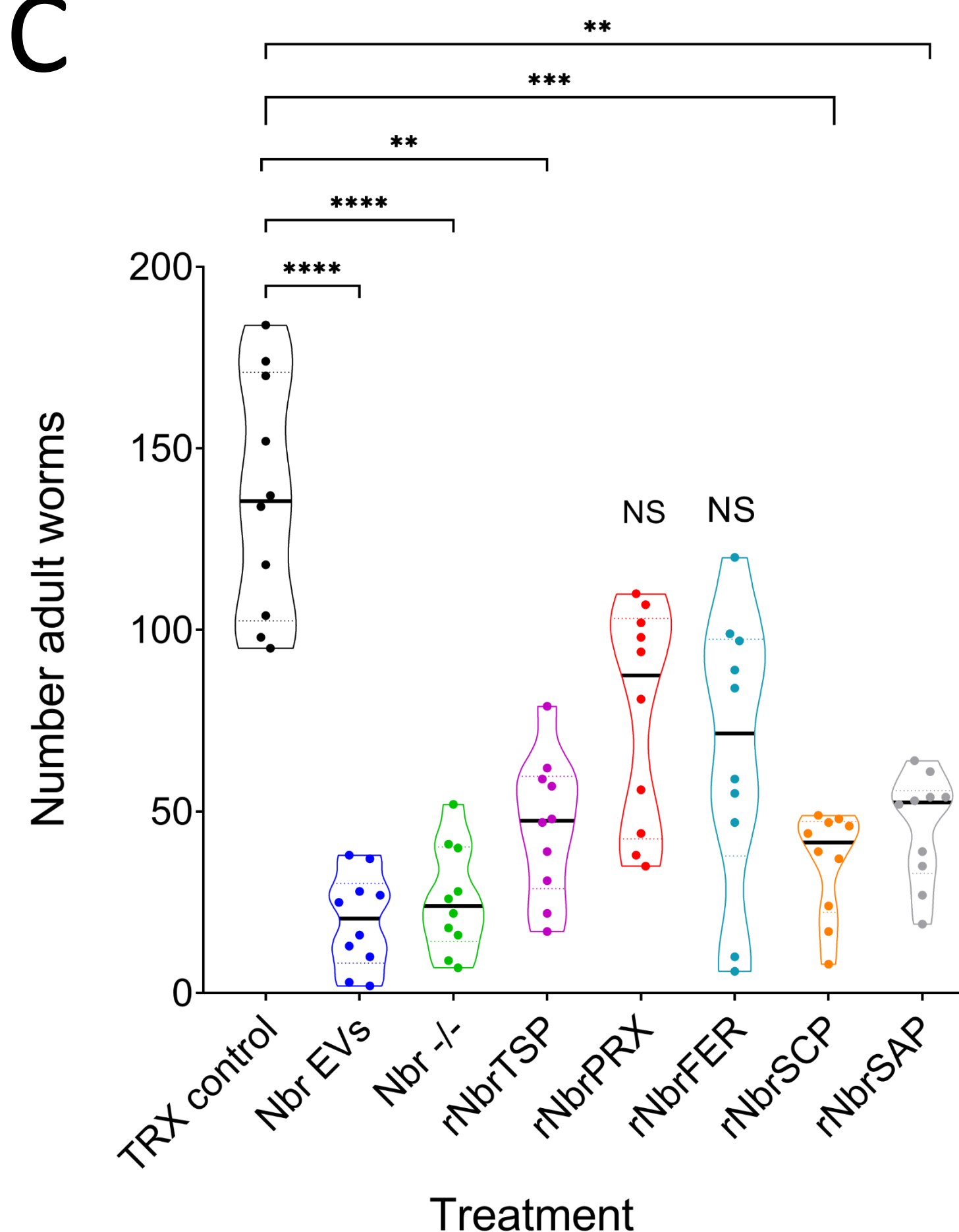
**A**



**B**



**C**

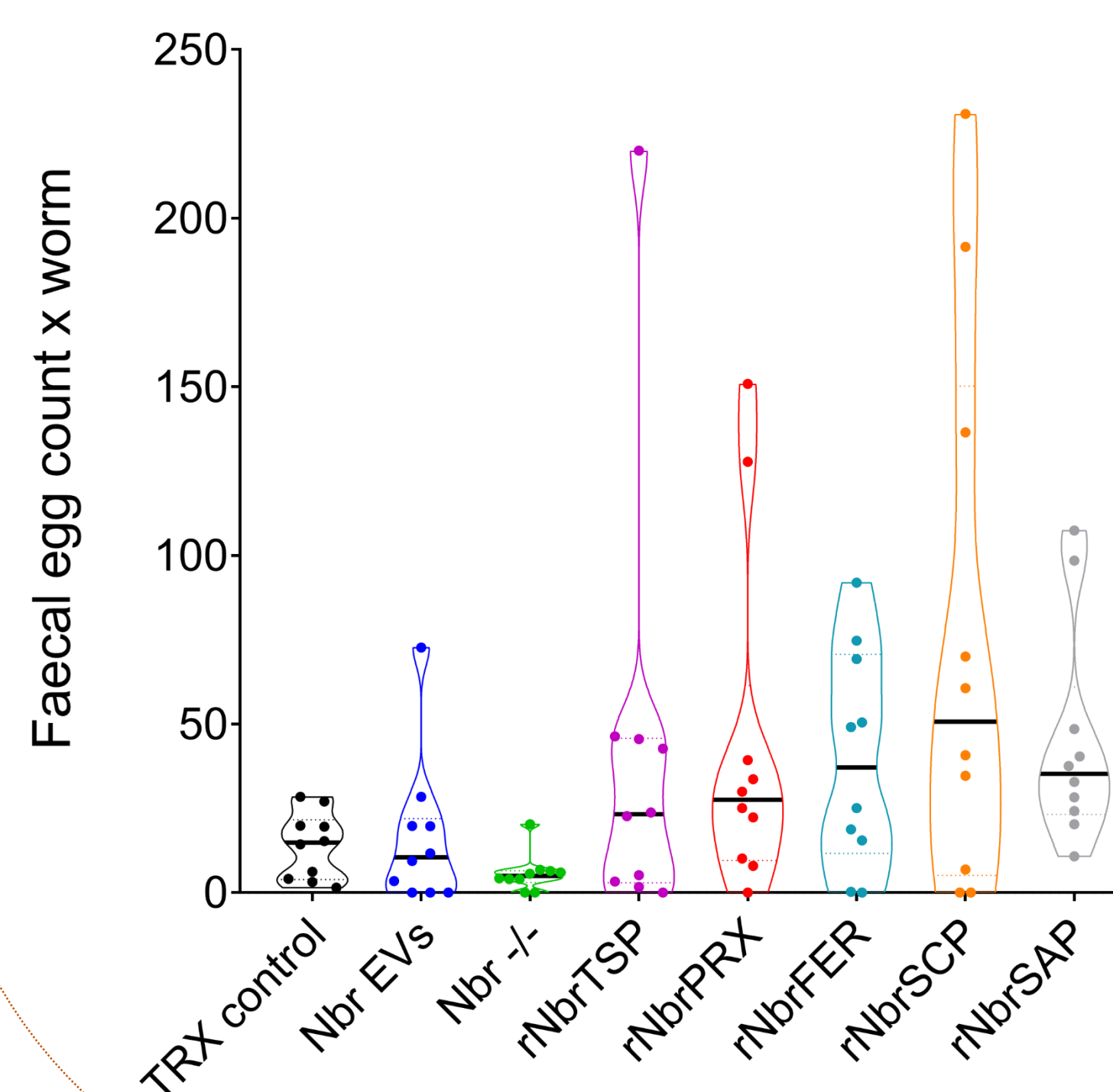


EVs, secreted ES products not containing EVs (Nbr -/-) and recombinant proteins TSP, PRX, FER, SCP and SAP conferred protection against a *N. brasiliensis* challenge infection.

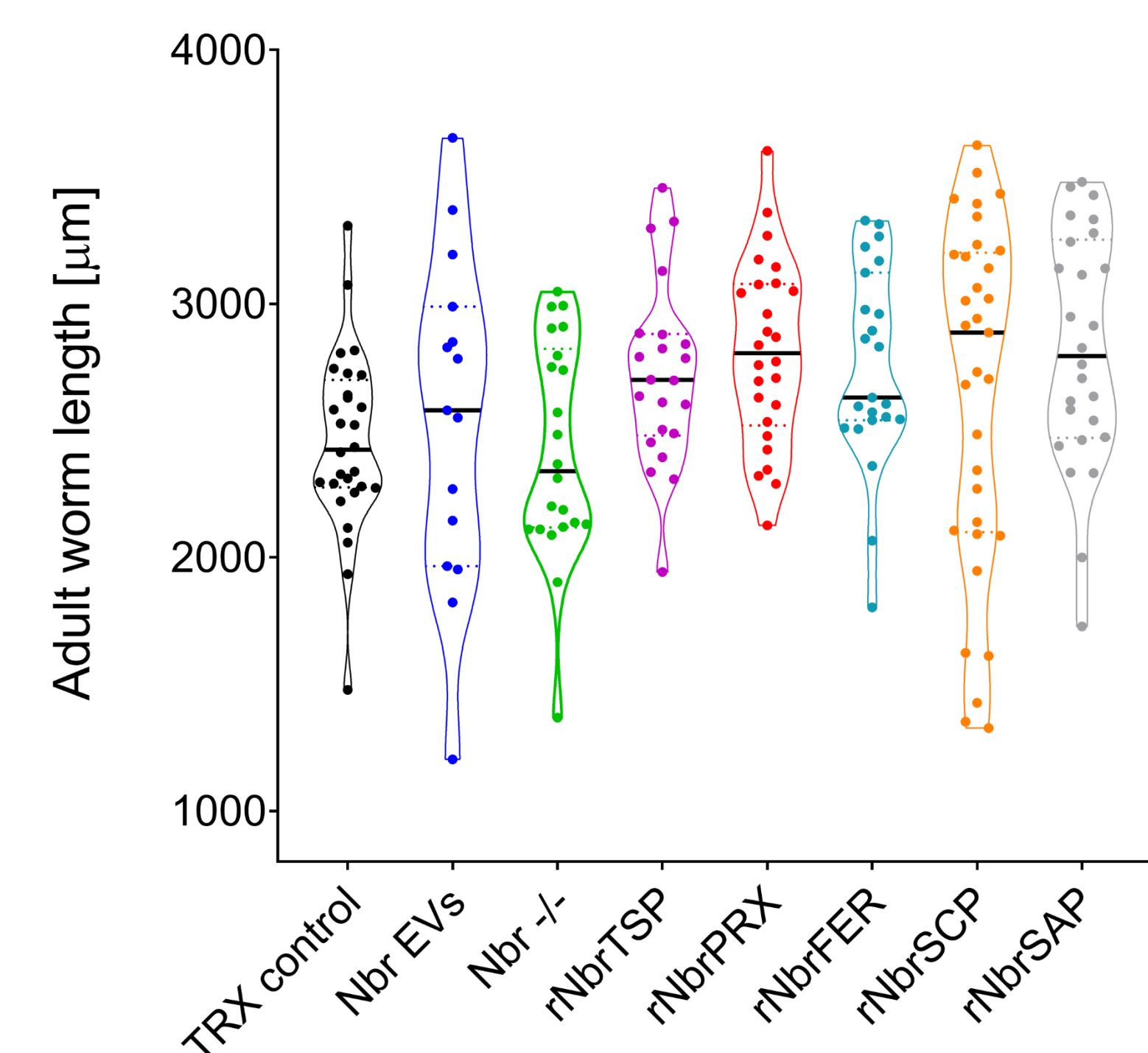
Significant reduction in the number of worms with all vaccinations except for PRX and FER (C).

No significant changes observed in the number of eggs (D) and size from recovered worms (E).

**D**



**E**



## CONCLUSIONS

- EVs and EV proteins confer significant protection against a primary infection with *N. brasiliensis*
- The study of EVs could provide important information for the development of novel vaccine strategies against nematode infections and should be explored further



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### REFERENCES

- <sup>1</sup> Camberis, M., Gros, G. and Urban, J. (2003), Animal Model of *Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus*. Current Protocols in Immunology, 55: 19.12.1-19.12.27. doi:10.1002/0471142735.im1912s55
- <sup>2</sup> Eichenberger RM, Ryan S, Jones L, et al. Hookworm Secreted Extracellular Vesicles Interact With Host Cells and Prevent Inducible Colitis in Mice. *Front Immunol*. 2018;9:850. Published 2018 Apr 30. doi:10.3389/fimmu.2018.00850