Supplementary Fig. 1. Infection of three *C. elegans* strains used for spatially restricted enzymatic tagging. Animals infected with *N. parisii* stained with a FISH probe (red) specific for *Nematocida* rRNA.
Supplementary Fig. 2. Biotin labeling of infected *C. elegans* displays specificity for the host intestinal tissue. Dissected intestines stained with anti-GFP antibody (green), with DAPI for DNA (blue) and with Alexa Fluor 568 streptavidin to label biotin. Left, GFP only. Middle, GFP-APX-NES Right, GFP-APX-NLS. CEN, *C. elegans* intestinal nuclei. Labeled arrows indicate representative *C. elegans* nuclei, which are large blue circles of DNA. NPN, *N. parisii* nuclei. Labeled arrows indicate representative *N. parisii* nuclei, which are small blue circles of DNA.
Supplementary Fig. 3. The number of *C. elegans* and microsporidia proteins identified through spatially restricted enzymatic tagging. *C. elegans* (blue) or *Nematocida* (orange) proteins identified from animals infected with either *N. parisii* or *N. sp. 1*. Numbers represent the total proteins with at least 1 peptide identification from either GFP, GFP-APX-NES, or GFP-APX-NLS.

Supplementary Fig. 4. Properties of identified *C. elegans* intestinal proteins. A. Comparison of identified *C. elegans* proteins specific to the nucleus (118 proteins) to those specific to the cytoplasm (114 proteins). GO term analysis for the compartments of the nucleus and the cytoplasm was performed using PANTHER. B. Properties of 891 *C. elegans* proteins identified in the intestine with spatially restricted enzymatic tagging, compared to the *C. elegans* genome. TM, transmembrane. SP, signal peptide.
Supplementary Fig. 5. *N. parisii* proteins in the no APX sample are not enriched in the same properties as host-exposed proteins. **A.** Comparison of mRNA expression levels of the 72 identified host-exposed proteins (orange), 392 proteins identified in the No APX sample (green), and the rest of *N. parisii* genome (blue). Expression data are from animals infected for 30 hours at 25°C. **B.** Comparison of the 72 identified host-exposed proteins (orange), 392 proteins identified in the No APX sample (green), and the genome (blue). Left, categorization by properties enriched in host-exposed proteins. TM, transmembrane. SP, signal peptide. Right, categorization by conservation class. The percentage of the *N. parisii* genome, no APX proteins, and host-exposed proteins in each category are shown.
Supplementary Fig. 6. Phylogenetic tree of 21 microsporidia species.
Phylogenetic tree of 21 microsporidia species and *R. allomycis* as an outgroup. Tree was inferred using RAxML 8.2.4 using the PROTGAMMALG model and 1000 bootstrap replicates. Bootstrap support is indicated next to each node. Scale bar indicates changes per site. Strains are in parentheses. Tree was created using FigTree 1.4.2. (http://tree.bio.ed.ac.uk/software/figtree/).
Supplementary Fig. 7. Properties of *N. sp. 1* host-exposed proteins. Identified host-exposed proteins (orange) were compared to all proteins in the genome (blue). SP/TM, signal peptide or transmembrane domain. The percentage of the *N. sp. 1* genome and the *N. sp. 1* host-exposed proteins in each category are shown. Enrichment p-values (one-side Fisher’s exact test) are listed below each category.

Supplementary Fig. 8. Properties of *Spraguea lophii* proteins identified from germinated spores. Proteins identified from germinated *S. lophii* spores (orange) were compared to all proteins in the genome (blue). SP, signal peptide. The percentage of the *S. lophii* genome and the *S. lophii* secreted proteins in each category are shown. Enrichment p-values (one-side Fisher’s exact test) are listed below each category. Proteins identified from germinated *S. lophii* spores is taken from Campbell et al.
Supplementary Fig. 9. Evidence of duplication of large gene family proteins.  

A. Percentage of large gene family proteins in *N. parisii* at chromosomal ends (See methods).  

B. Percentage of large gene family proteins in *N. parisii* that are adjacent to each other (See methods).
Supplementary Fig. 10. Pfam domains in predicted host-exposed proteins that are not part of large gene families. Pfam domains with at least 8 occurrences in predicted host-exposed proteins that are not members of large gene families are shown.
Supplementary Table 1. Strains used in this study.

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<th>Tissue (promoter)</th>
<th>Protein</th>
<th>Subcellular location (localization tag)</th>
<th>Strain nomenclature</th>
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Supplementary Table 2. Predicted signal peptidase-processed N-terminal peptides.

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[a] "-" is the N-terminal, "," is N-terminal acetylation, "[a]" is oxidized Methionine and ",X.X" is the trypsinization site.

[b] The number of peptide counts in each sample.

Supplementary Table 3. Summary of genome assembly statistics.

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<th>Proteins</th>
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