Supplemental Figure Legends

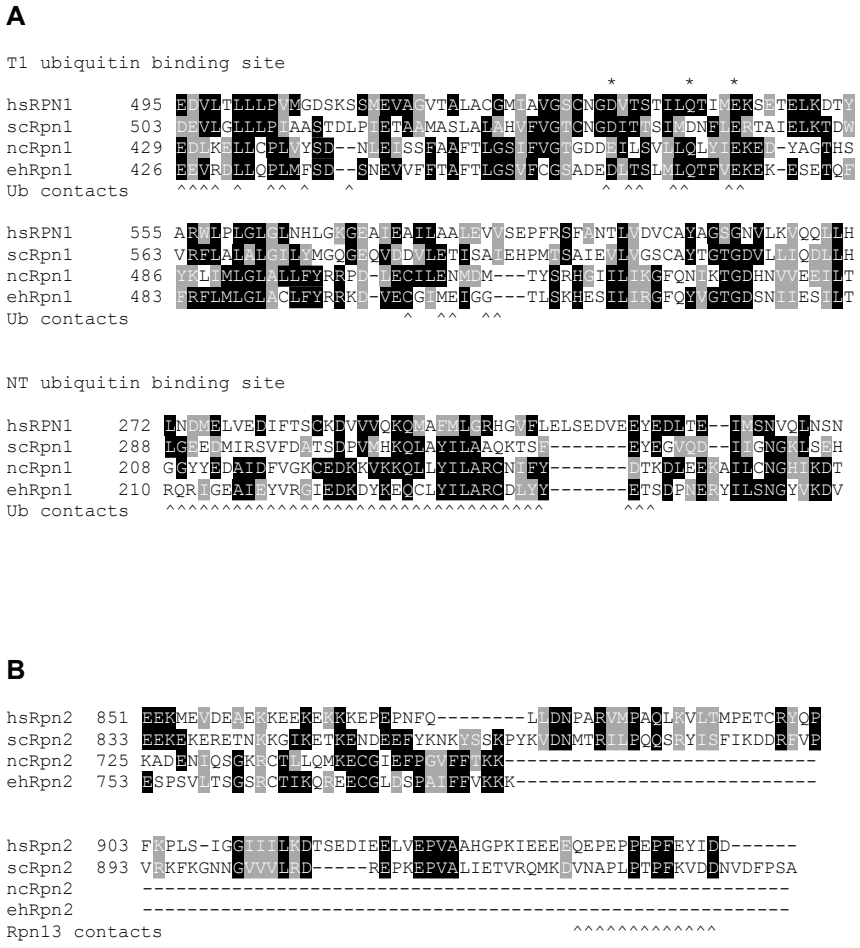

Figure S1. Comparisons of key Rpn1 and Rpn2 structural domains**.** (A) Sequence alignment of portions of the human, *S. cerevisiae*, *N. ceranae*, and *E. hellem* RPN1 orthologs surrounding reported ubiquitin binding sites T1 (top) and NT (bottom) [1,2]. ^, residues in *S. cerevisiae* Rpn1 reported to comprise the ubiquitin binding site. *, residues mutated in the ubiquitin binding-defective *S. cerevisiae* Rpn1 mutant *rpn1-ARR*. (B) Alignment of the C-termini of RPN2 orthologs as in (A). ^, residues in human RPN2 that bind RPN13.

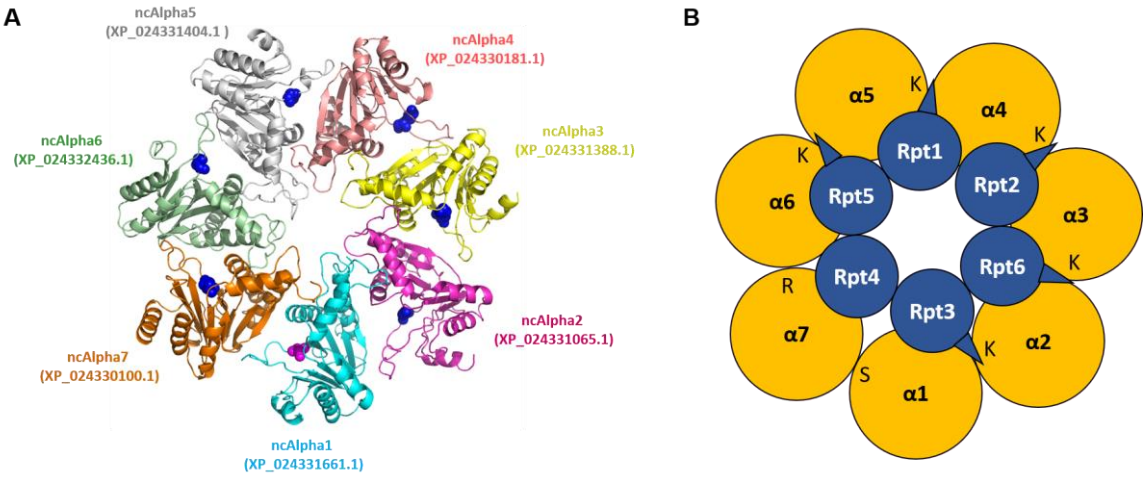

Figure S2. *N. ceranae* α ring predictions. (**A**) Homology model of the *N. ceranae* α ring and hypothesized subunit assignments. Pocket lysines (α2-α6) and arginines (α7) are shown as blue spheres, and the α1 serine 61 in the equivalent position is shown as magenta spheres. (**B**) Cartoon model showing the anticipated register of the RP Rpt subunits on the α ring. The pocket amino acid contributed to a given α-α interface by each α subunit is shown. Rpt subunits contributing Hb-Y-X or Hb-Y-X-like motifs are shown with triangular tails pointing into their respective α subunit pockets.

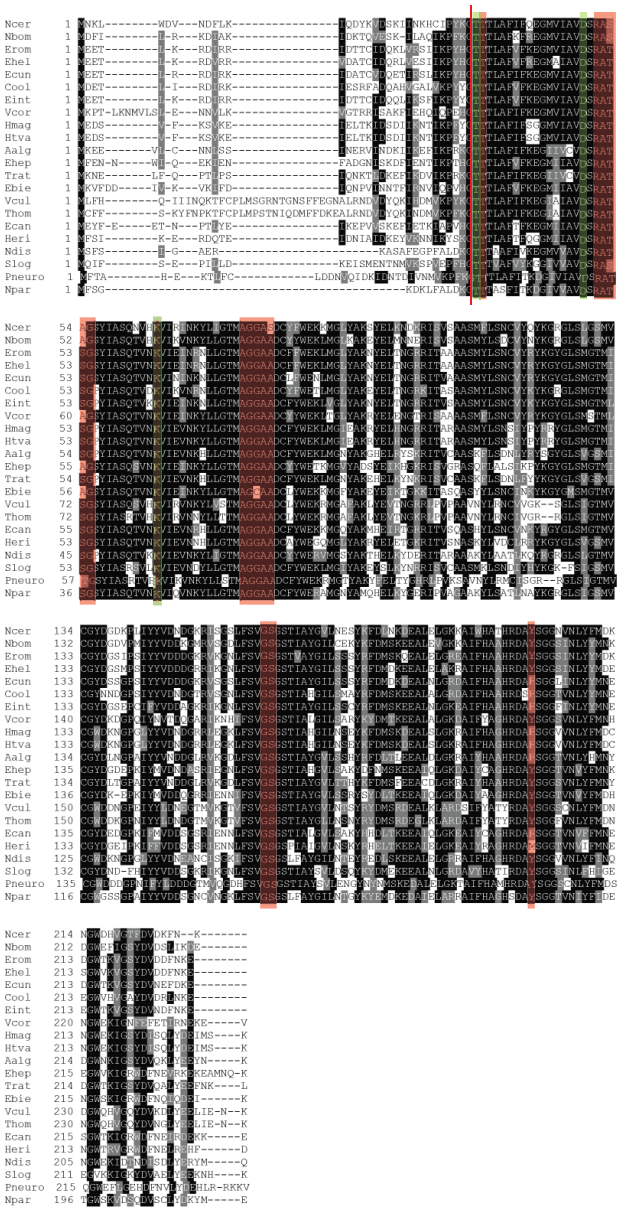

Figure S3. Sequence alignment of the 20S proteasome subunit β5 subunit from available microsporidia genomes. Amino acids from the catalytic triad (T1, D17, and K33 numbered from processed *H. sapiens* protein [3]) are boxed in green and amino acids critical for binding proteasome inhibitors (T2, R19, A20, T21, A22, G23, K33, A46, G47, G48, A49, A50, G129, S130, Y169, numbered from processed *H. sapiens* protein [4]) are boxed in red. The processing site for generating the mature protein is denoted with a red line.

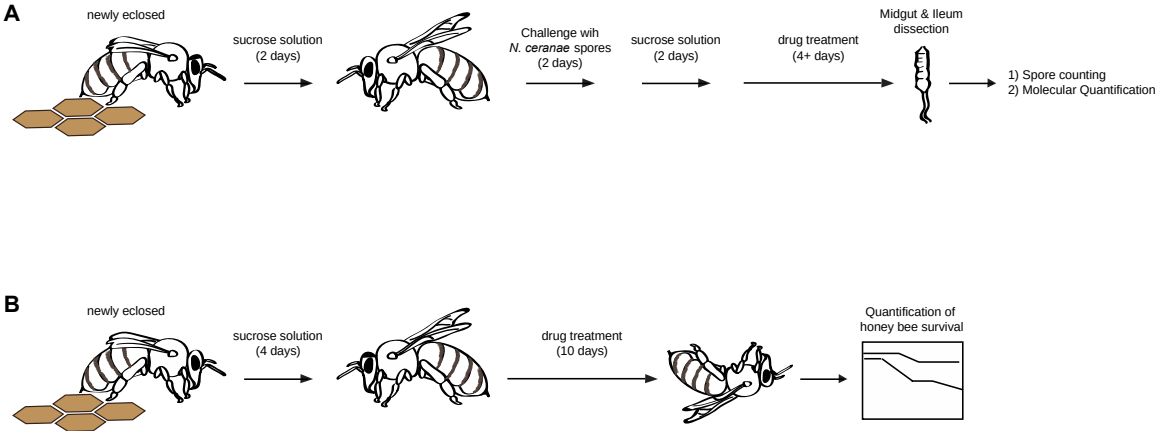

Figure S4. Schematic of *N. ceranae* infection of newly eclosed bees. On day two post-eclosion, *N. ceranae* spores ($5x10^6$ / mL) were fed to bees in sucrose solution *ad libitum* for 48 hours. At 4 days post-infection, honey bees in individual cages were fed sucrose solution containing one of the pharmacologic agents at the indicated doses or vehicle control alone. After 4 days of drug feeding, honey bee midguts were dissected, and infection levels were assessed by spores counting and qPCR. (A) Schematic of survival experiments with newly eclosed bees. At 4 days post-eclosion, honey bees in individual cages were fed sucrose solution containing one of the pharmacologic agents at the indicated doses or vehicle control alone (B).

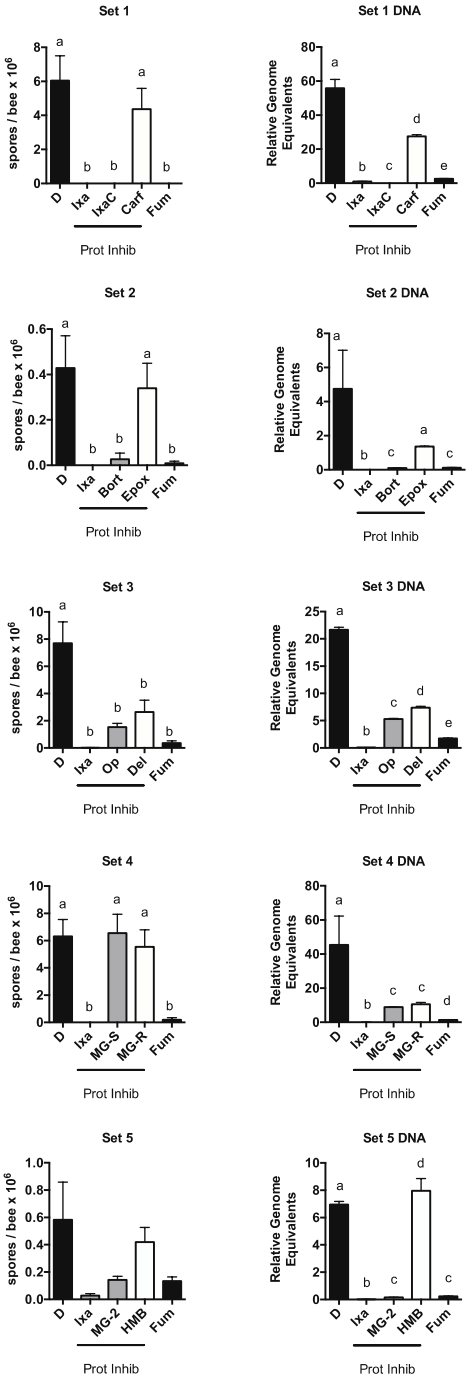

Figure S5. Diverse proteasome inhibitors reduce *N. ceranae* infection in newly eclosed bees. *N. ceranae* levels in midguts of infected newly eclosed bees fed sucrose solution containing DMSO or the indicated compounds for 4 days as determined by spore count using light microscopy (left) or by qPCR (right) $a \neq b$, $p < 0.05$.

References

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