

Supplemental Figure Legends

A

T1 ubiquitin binding site

hsRPN1 495 EDVLTLLLP MGDSKSM EAVGVTA LACGM AVGSCNGDV TSTLTLOTMEKSE TELKDTY
scRpn1 503 DEVLGLLLP AA S TDLPE TATMA SLALAH FVG SCNGDITSTI DNFEETATIELKTD
ncRpn1 429 EDIKELLCP LMYS D---NLE SSFAAFTLGS FVGSGDDEITSVILQLYIEKEE YAGHS
ehRpn1 426 EFVRLLOLP MFSD---SNEVVFETAF TLGS FCGSADEDITSLMLQTYIEKK ESETQ
Ub contacts ^^^ ^

hsRPN1 555 ARRLPLGLG INHLG GATAEITAALELV SEFFRSFNTLL DVCA YAGSGNVLK QQLH
scRpn1 563 VRFLAALGLIL MGCGQGV DVLBTISAEHPMIAIEV L GSCAYTGTGDVLLQDLH
ncRpn1 486 YRLMLGLALLF YRRP IECILNMM ---TYSRH ILLKGFQNI RTGDNVVEEILT
ehRpn1 483 EFRMLMGLALCFYRRK IVECG MEIGG ---TLSKHESILIRGFQYGTGDSNIIESILT
Ub contacts ^

NT ubiquitin binding site

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hsRPN1      272  I N D M E L V E T F T S C K P V V Y Q K A F M L R H G V L E L S E D V E Y E D I T E -- I I S N V Q L N S N
scRpn1      288  L G E L M I R S V F D T S P F V H K Q A Y I L A A Q K T S E ----- E Y E G V O D -- I I G N G L S H
ncRpn1      208  G G Y Y E D A I F V G K C E D K Y K K Q L L Y I L A R C N I F Y ----- I T K D I E E A I I C N G A I K D T
ehRpn1      210  R Q R L G A A I Y V R I E D K D Y K E C L Y I L A R C D L Y Y ----- E T S P E N E Y I L S N G Y K D V
lib_contacts
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B

hsRpn2 851 EEKMEIDEEKKKEEKPEKKKEFEPNFQ-----LDNPARTMPACLVLMPETCRVOP
scRpn2 833 EEKEKERETNKGCIKETKENDEEFYKNKYSSKPYKVDMTRILPQCSRYISFIKDDRVE
ncRpn2 725 KADENIQSGRRCTLLQMKECGIEFPGVFFIKK-----
ehRpn2 753 ESPSVITSGSRETIKQEEECGLSPAIFFFVKK-----

hsRpn2 903 FPLSLIGGLIILMDTSEDIELVELPEVAAHGPKIEEELQEPEPPPEFFEYIDD-----
scRpn2 893 VKFKFGNGGVVVLND----REPKEPVALLETVRQMKLVNAPLPFFPFKVDLNVDFPSA
ncRpn2 -----
ehRpn2 -----

Rpn13 contacts ^^^^^^^^^^^^^^^

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.

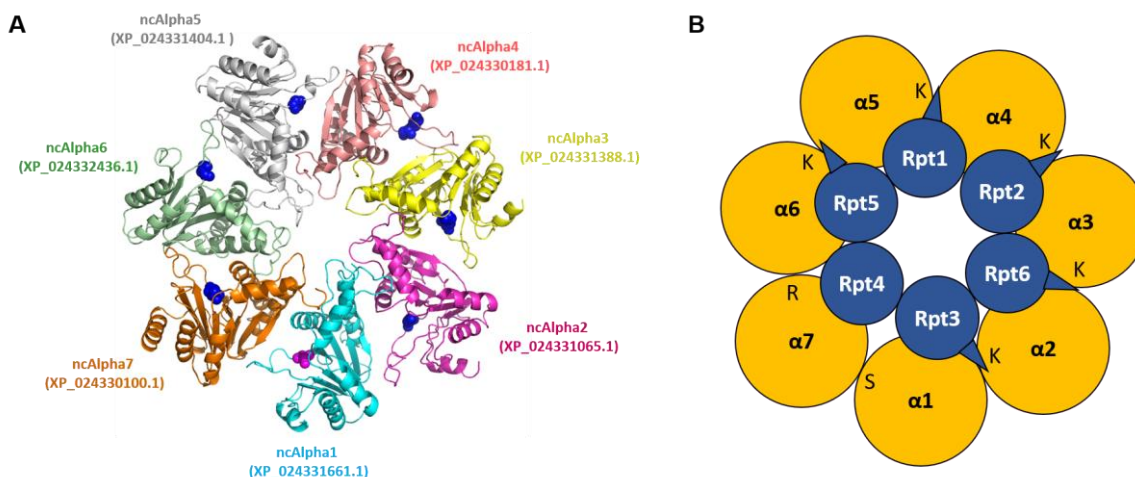


Figure S2. *N. ceranae* α ring predictions. (A) Homology model of the *N. ceranae* α ring and hypothesized subunit assignments. Pocket lysines ($\alpha 2$ - $\alpha 6$) and arginines ($\alpha 7$) are shown as blue spheres, and the $\alpha 1$ serine 61 in the equivalent position is shown as magenta spheres. (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.

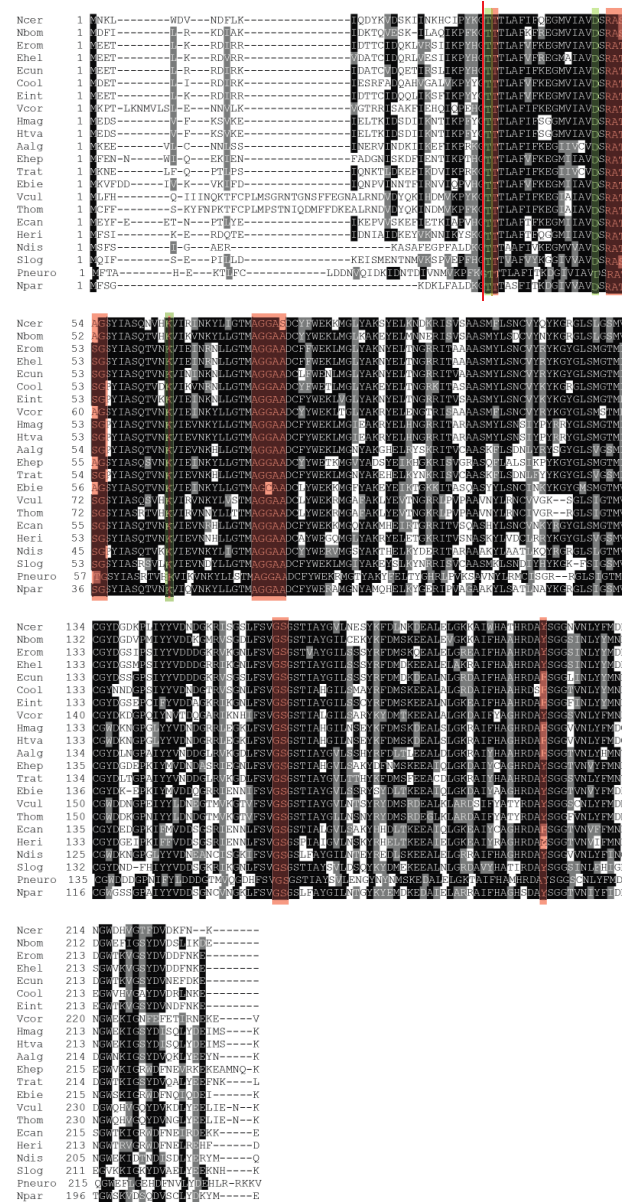


Figure S3. Sequence alignment of the 20S proteasome subunit $\beta 5$ subunit from available microsporidia genomes. Amino acids from the catalytic triad (T1, D17, and K33 numbered from processed *H. sapiens* protein [3]) are boxed in green and amino acids critical for binding proteasome inhibitors (T2, 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.

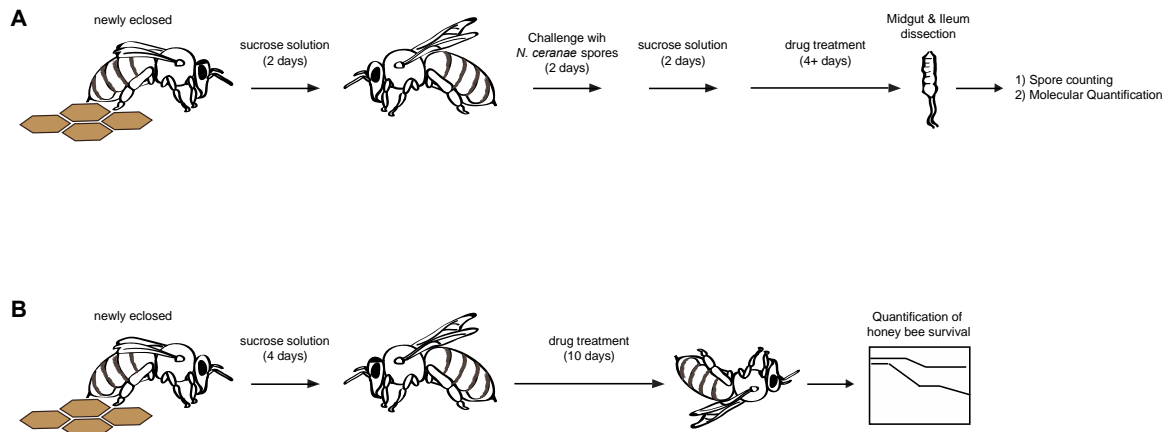


Figure S4. Schematic of *N. ceranae* infection of newly eclosed bees. On day two post-eclosion, *N. ceranae* spores (5×10^6 / mL) were fed to bees in sucrose solution *ad libitum* for 48 hours. At 4 days post-infection, honey bees in individual cages were fed sucrose solution containing one of the pharmacologic 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).

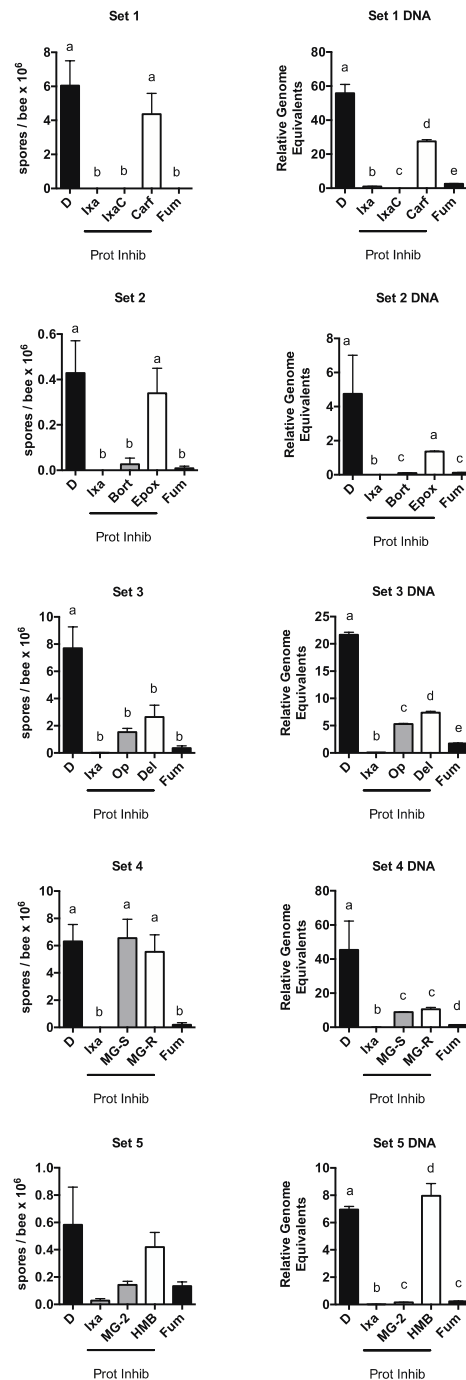


Figure S5. Diverse proteasome inhibitors reduce *N. ceranae* infection in newly eclosed bees. *N. ceranae* levels in midguts of infected newly eclosed bees fed sucrose solution containing DMSO or the indicated compounds for 4 days as determined by spore count using light microscopy (left) or by qPCR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

References

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4. Schrader, J.; Henneberg, F.; Mata, R. A.; Tittmann, K.; Schneider, T. R.; Stark, H.; Bourenkov, G.; Chari, A. The inhibition mechanism of human 20S proteasomes enables next-generation inhibitor design. *Science* **2016**, *353*, 594–598.