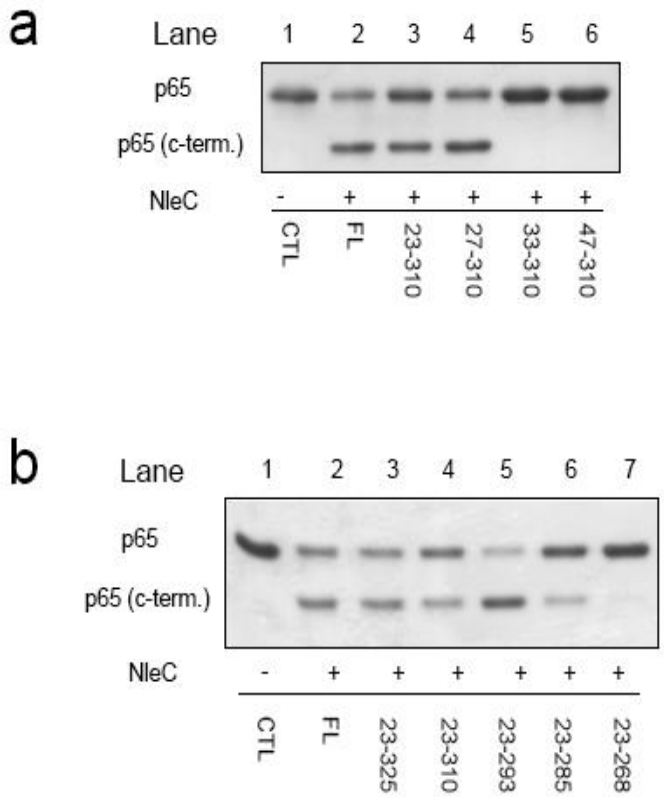
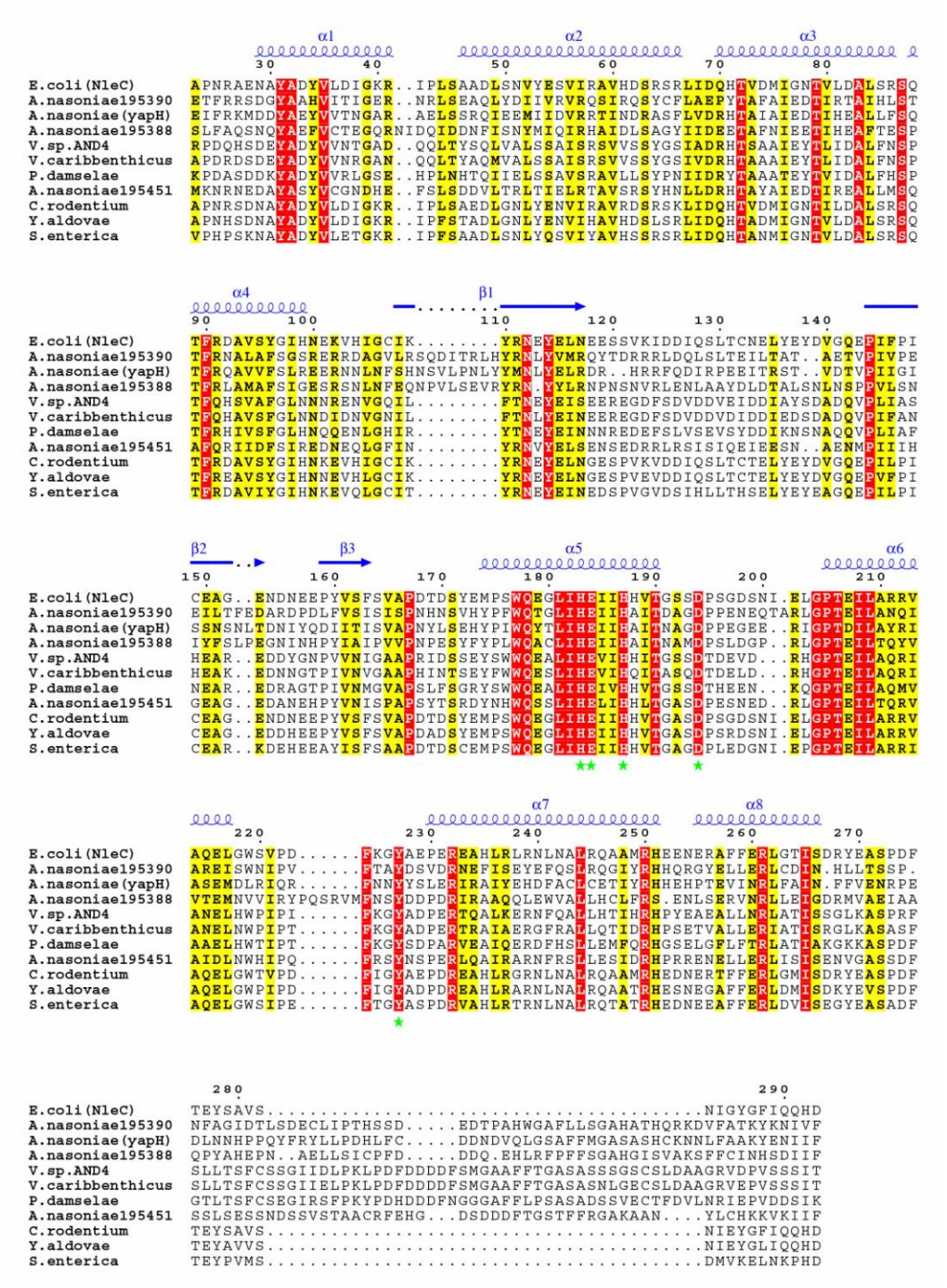
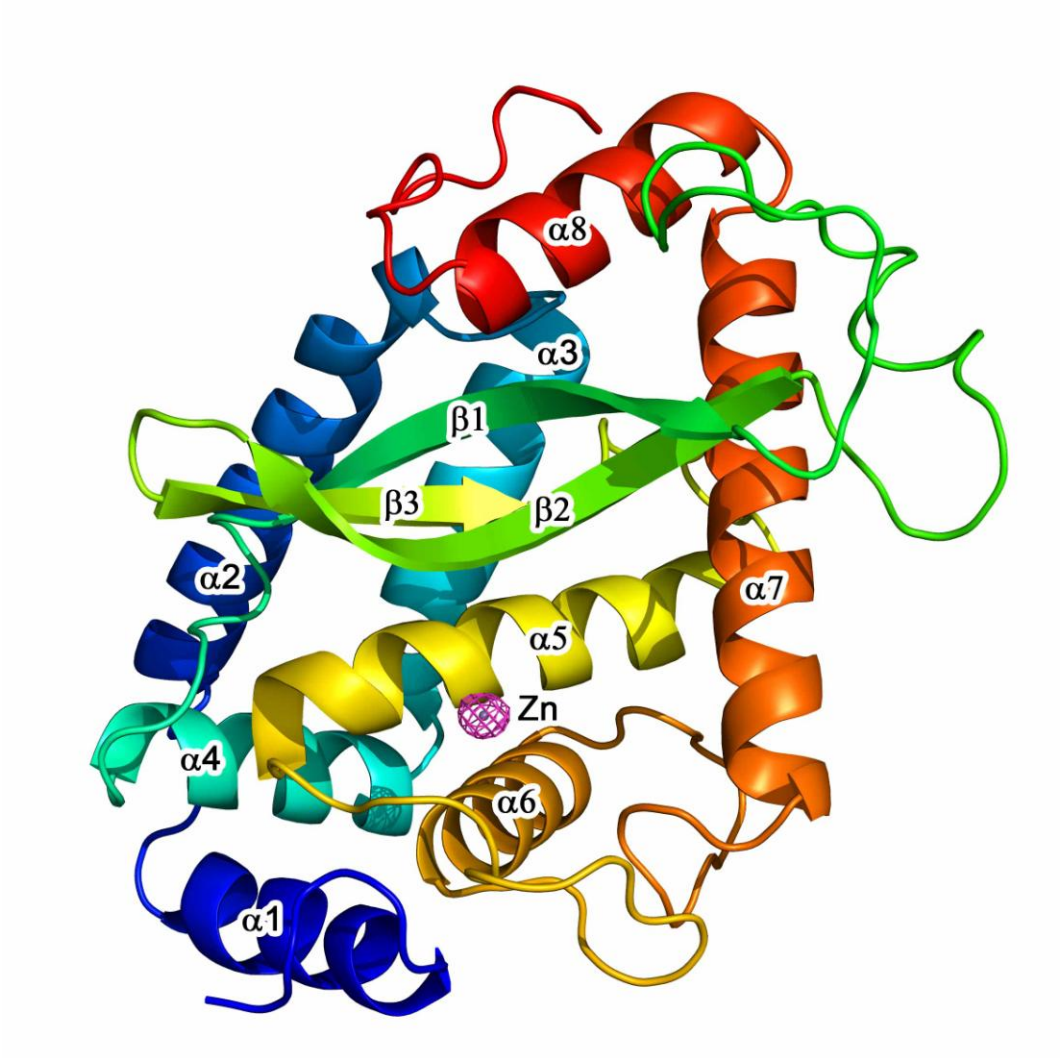


Supplementary Figure S1. NleC construct design. a, Identification of a minimalist of N-terminal truncation that retains the same protease activity. Deletion of the amino acids 1-22 or 1-26 in NleC had no detectable effect on its protease activity (lanes 3-4). Deletion of six additional residues or more in NleC abolished its peptidase activity (lanes 5-6). b, Identification of a minimalist of C-terminal truncation that retains the same protease activity. Deletion of the amino acids 294-330 had no detectable effect on its protease activity (lane 5). Deletion of more addition residues than that starting from 294 will abolish or cripple the protease activity. Crystals were finally generated for the NleC fragment 23-293.

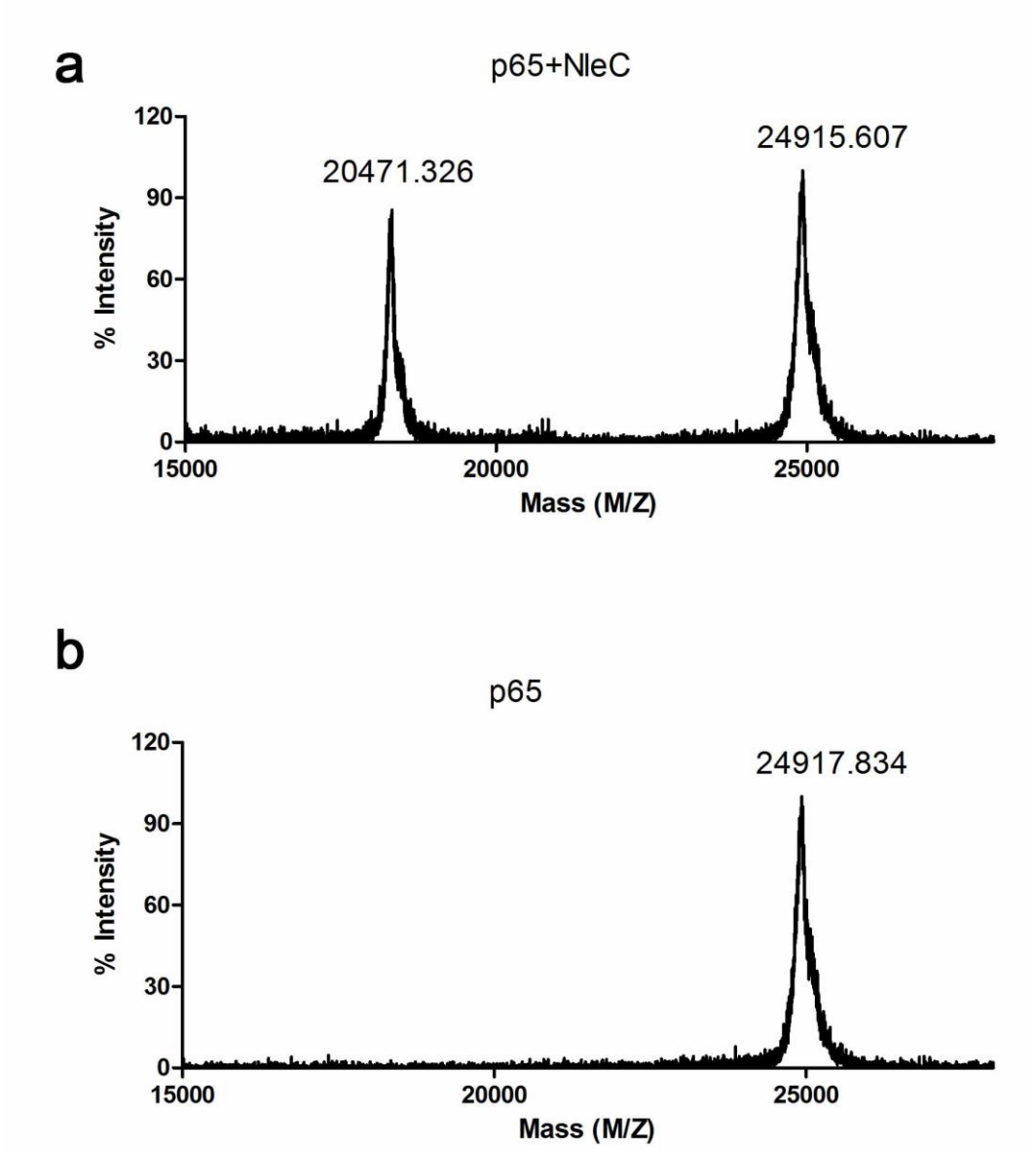


Supplementary Figure S2. Sequence and structural features of NleC. Secondary structural elements of NleC are indicated above the sequences. Invariant and highly conserved amino acids are highlighted in red and yellow, respectively. The zinc-binding ligands and active site residue D184 are indicated by green stars. The aligned sequences are from: *Escherichia coli*, MER 195406 (NleC); *Arsenophonus*

nasoniae, MER 195390; *Arsenophonus nasoniae*, MER195425 (yapH protein); *Arsenophonus nasoniae*, MER 195388; *Vibrio sp. AND4*, MER 195393; *Vibrio caribbenthicus*, MER 195392; *Photobacterium damselae*, MER 195394; *Arsenophonus nasoniae*, MER 195451; *Citrobacter rodentium*, MER 195404; *Yersinia aldovae*; MER 195398; *Salmonella enterica*, MER 195395. Sequence alignment was carried out with ClustalW2 (Thompson *et al.*, 2002).



Supplementary Figure S3. The anomalous difference map of the bound zinc atom at 5.0 σ level is shown as a magenta mesh. The structural polymer of NleC is rainbow-colored, with N-terminus in blue and C-terminus in red.



Supplementary Figure S4. MALDI-TOF/TOF CID spectrum of p65 and the cleavage products after incubation with NleC. The result indicates that NleC cleaved p65 within its conserved DNA-binding domain just after residue C38..

References

Thompson, J. D., Gibson, T. & Higgins, D. G. (2002). *Current protocols in bioinformatics*, 2.3. 1-2.3. 22.