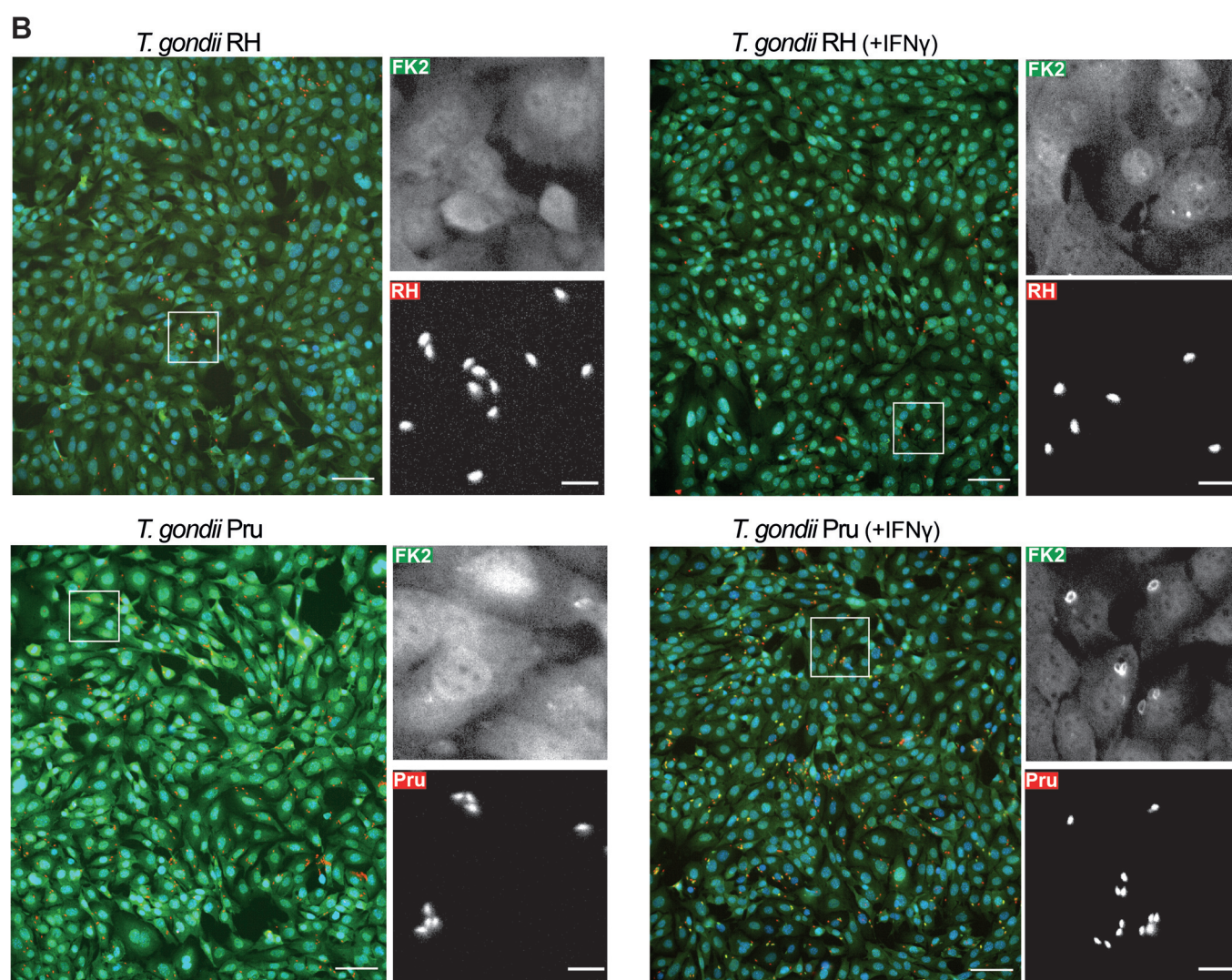
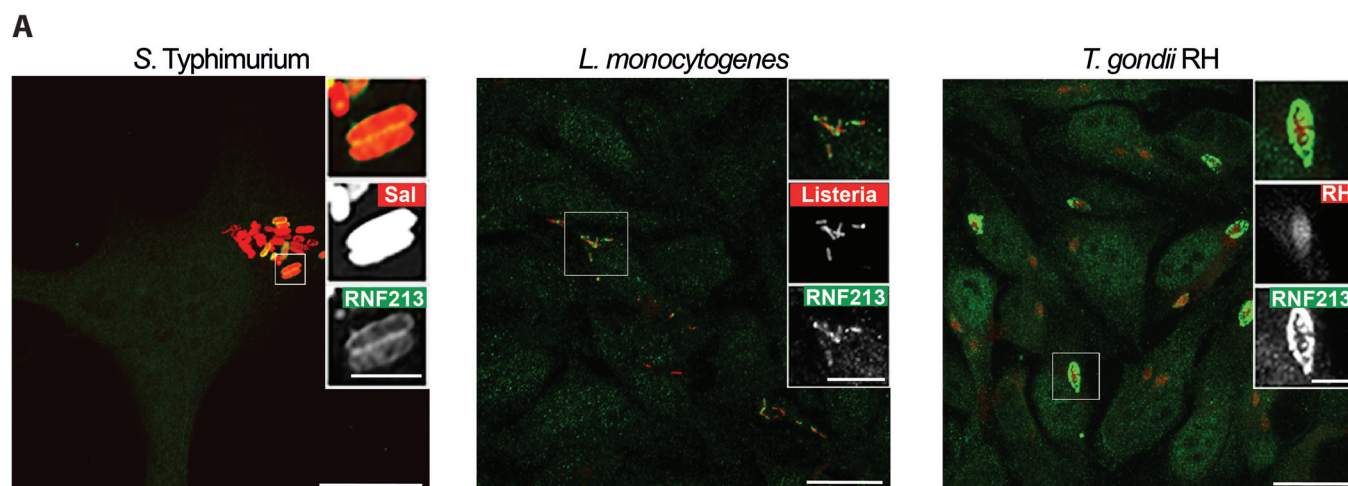
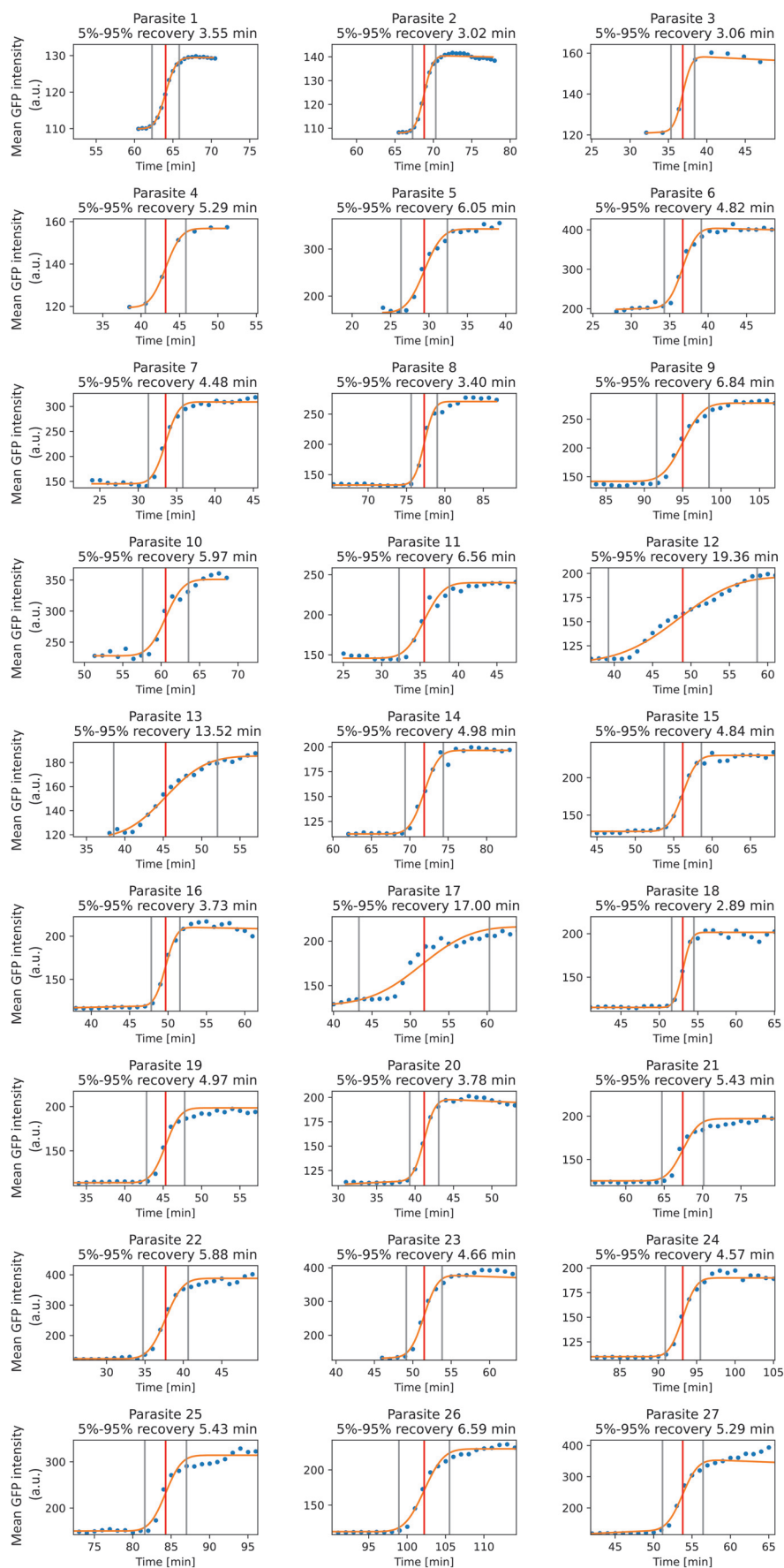


Expanded View Figures

Figure EV1. RNF213 accumulates on phylogenetically distant pathogens.

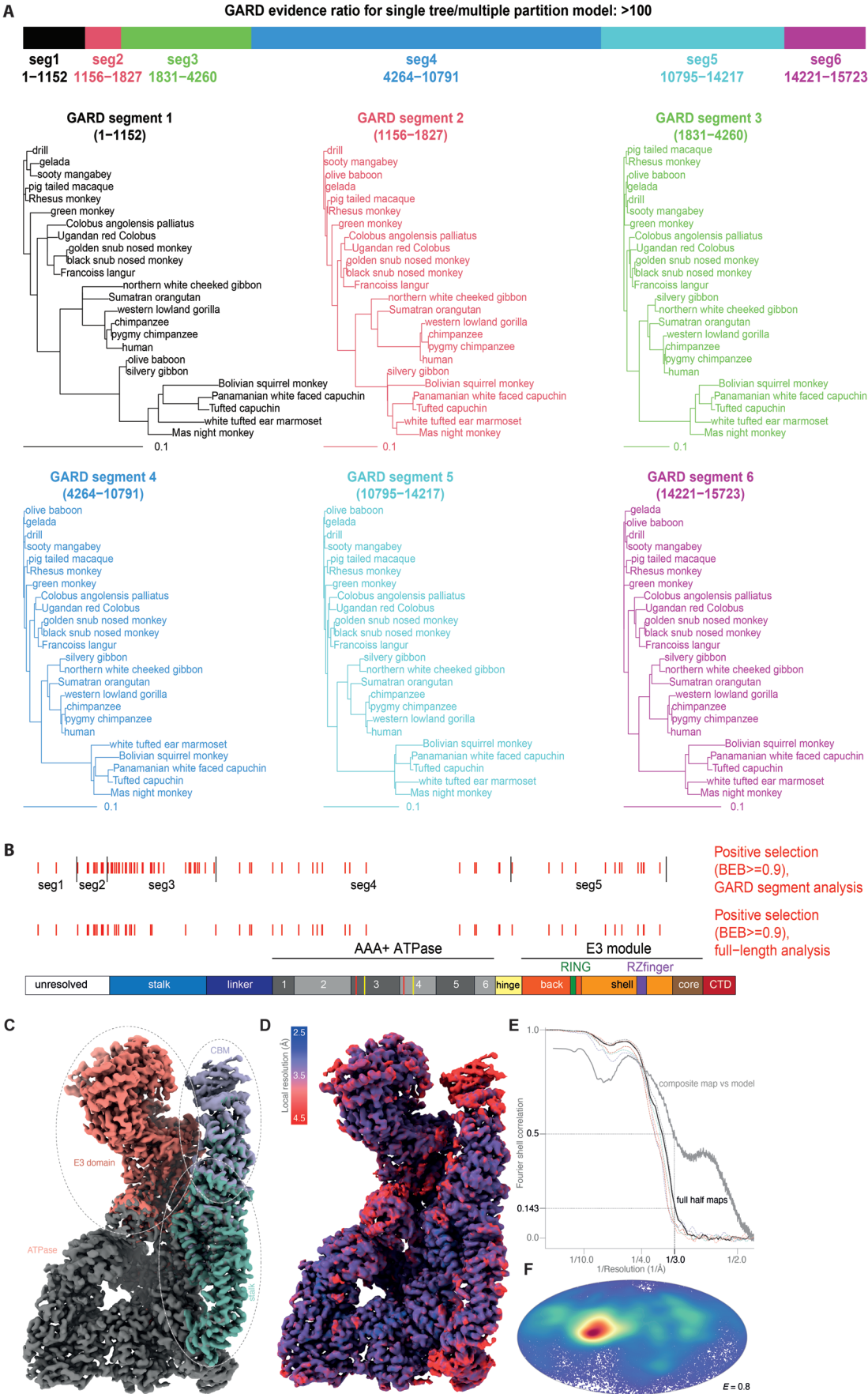
(A) Confocal micrographs of HeLa cells infected with *S. Typhimurium* for 4 h and *L. monocytogenes* or *T. gondii* infected for 6 h. Cells were stained with anti-RNF213 antibody. Scale bar 20 μm (magnification box scale bar; 5 μm). (B) Confocal micrographs representative of quantifications shown in Fig. 1E. MEFs stimulated with IFN γ as indicated, infected with Tomato-expressing *T. gondii* Type I RH or Type II Pru for 1 h and stained with anti-ubiquitin (FK2) antibody and DAPI. Regions marked with white borders in the main images are shown magnified on the right. Scale bar 80 μm (magnification box, scale bar; 20 μm). Source data are available online for this figure.





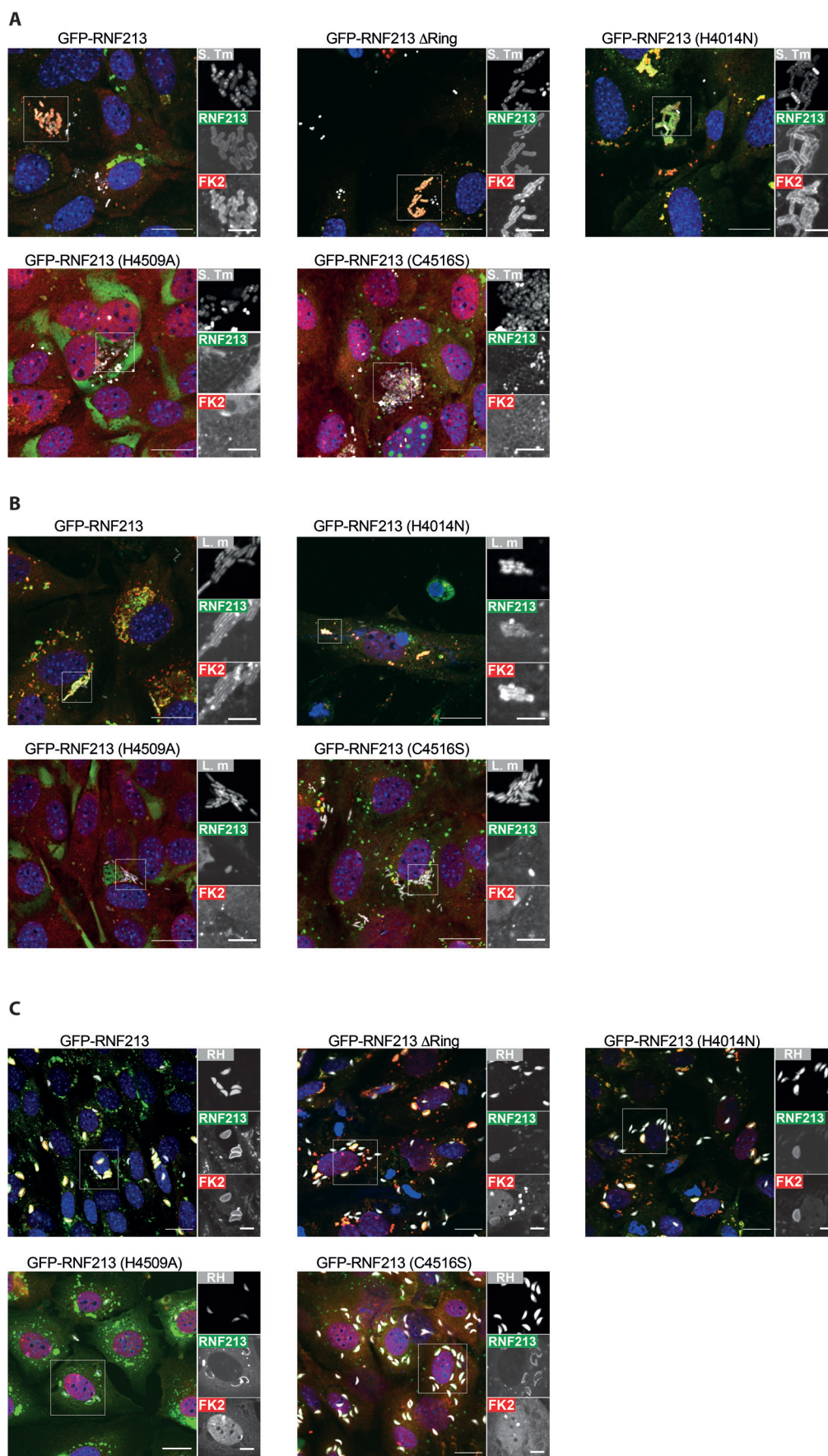
**Figure EV2. Kinetics of GFP-RNF213 accumulation on individual *T. gondii* vacuoles.**

Kinetics of GFP-RNF213 accumulation on *T. gondii* vacuoles. Dots represent the measured GFP intensity. An error function was fitted (orange line) to calculate the 5% and 95% of maximum coat formation (vertical grey lines) as well as the midpoint (vertical red line). Time required for coat completion (5% to 95% of maximum fluorescence) is annotated above the graphs. $n = 6$ biological experiments; parasites 1 and 2 from experiment 1; parasites 3 and 4 from experiment 2; parasites 5 to 10 from experiment 3; parasite 11 from experiment 4; parasites 12 to 21 from experiment 5; parasites 22 to 27 from experiment 6. Acquisition started at 60 min, 30 min, 23 min, 24 min, 30 min and 19 min p.i, respectively. Experiments 1 and 2 were acquired on a Nikon iSIM swept field high speed inverted microscope with a 100X super resolution Apo TIRF oil objective, experiments 3 to 6 on a Nikon X1 Spinning Disk inverted microscope with a 40x/1.3NA Oil lens. Source data are available online for this figure.



◀ **Figure EV3. Positive selection in GARD-identified RNF213 segments and cryo-EM analysis of RNF213.**

(A) The GARD algorithm detects evidence of possible recombination among RNF213 simian primate orthologs, with an evidence ratio of >100. Schematic of the 6 segments identified by GARD, and the phylogenies for each segment according to GARD. Differences between segment topologies are mostly subtle, in basal branches of the tree. (B) Repeat of codeml analysis, performed individually on each of the 6 GARD segment alignments. Segments 1–5 all showed evidence of positive selection (Appendix Table S1). Indicated with red tick marks are residues with high probability (Bayes Empirical Bayes posterior probability ≥ 0.9) of evolving under positive selection identified by GARD segment and full-length analysis, as indicated. Analysing the 6 GARD segments separately appears to increase the statistical power of codeml to identify rapidly evolving sites: across the 5 segments, a total of 86 sites have $\geq 90\%$ posterior probability (Bayes Empirical Bayes) of evolving under positive selection, in contrast to 59 sites when analyzing the full-length alignment. (C) Composite map of RNF213 created by local refinement of individual color-coded domains. (D) Composite map of RNF213 coloured by local resolution, calculated using ResMap (Kucukelbir et al, 2014). (E) Fourier shell correlation between the two consensus half-maps (solid black line), and between the composite map and the refined atomic model (solid grey line), is plotted as a function of resolution. The Fourier shell correlations for each pair of locally refined half maps are shown with the dashed lines, coloured according to the domains in (A). (F) Orientation distribution of the RNF213 particles used in the consensus refinement shown on a Mollweide projection plot, coloured in blue to red from low to high density, and has an efficiency $E = 0.8$, calculated using cryoEF (Naydenova and Russo, 2017).



**Figure EV4. Enzymatic activity in RNF213 is required for coat formation on pathogens.**

Confocal micrographs representative of quantifications shown in Fig. 4D–F. RNF213^{KO} MEFs complemented with the indicated GFP-RNF213 alleles and stained with anti-ubiquitin antibody (FK2) and DAPI at 4 h post-infection with mCherry-expressing *S. Typhimurium* (A), 6 h post-infection with mCherry-expressing *L. monocytogenes* Δ ActA (B) and 1 h post-infection with Tomato-expressing *T. gondii* RH Type I strain (C). Regions marked with white borders in the main images are shown magnified on the right. Scale bar, 20 μ m (magnification box scale bar, 10 μ m). Source data are available online for this figure.