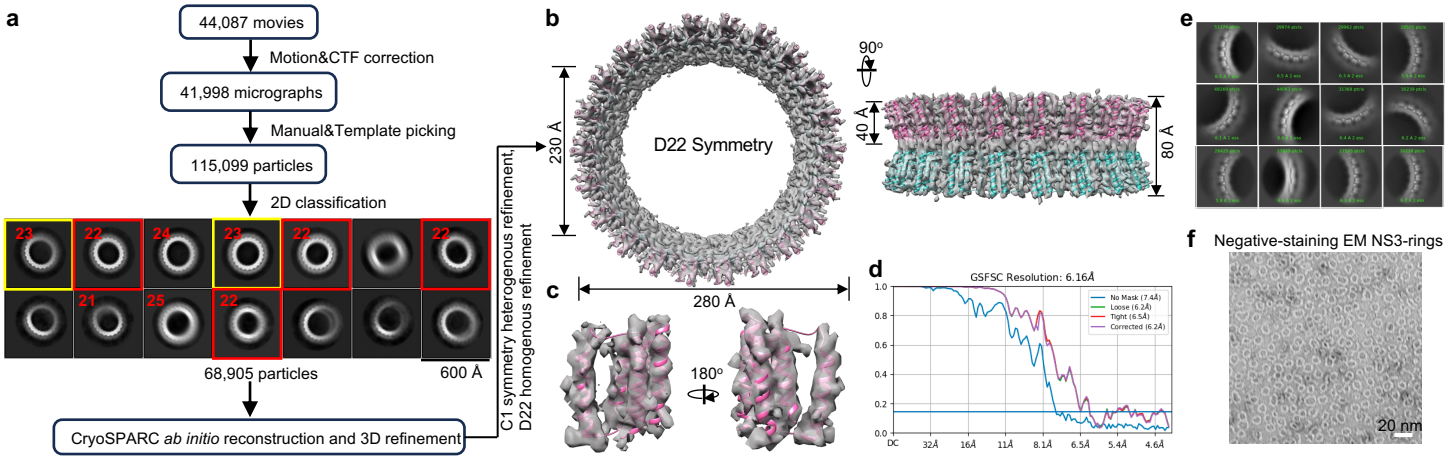


**Figure 1. Cryo-EM structural determination of the norovirus NS3 ring-like structures.** (**a**) Chart flow for cryo-EM structural determination of NS3 rings and representative 2D class averages. Symmetries of 2D class averages are labeled. The 22- and 23- fold major populations are boxed. (**b**) Final electron density map of NS3 ring superimposed with atomic model was shown in two views. (**c**) NS3 MLKL-like domain fitted in the cryo-EM map viewed for individual helices. (**d**) Gold-standard Fourier shell correlation (FSC) curves calculated from two independently refined half-maps, indicate an overall resolution of 6.16 Å for NS3 ring. (**e**) 2D classification of NS3 ring segments. (**f**) A representative negative staining EM image of NS3 rings reconstituted in the presence of cardiolipin-liposomes.

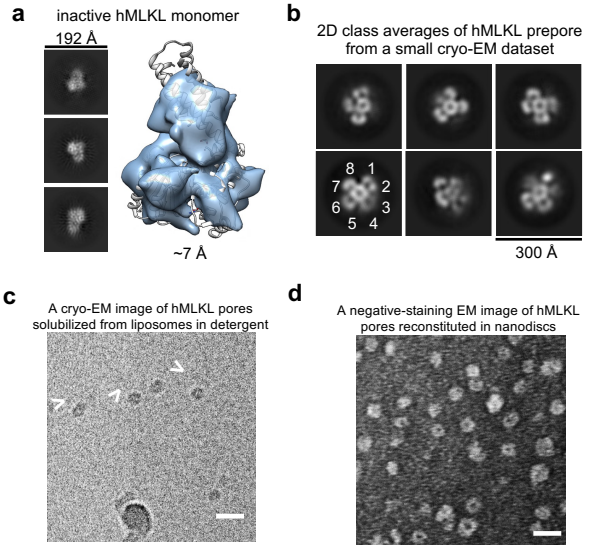


**Figure 2. Structural elucidation of human MLKL in multiple functional states.** (**a**) Cryo-EM 2D averages of monomeric full-length hMLKL and the cryo-EM map of hMLKL monomer superimposed with the crystal structure of full-length murine MLKL (PDB: 4BTF). (**b**) 2D class averages showed that hMLKL prepore is an flower-shaped octamer with a central ring likely formed by NEDs and four dimeric pskD domains flanking the central ring as petals. (**c**) A representative cryo-EM image of detergent-solubilized hMLKL pores. (**d**) A representative negative staining EM image of hMLKL pores reconstituted in nanodiscs. Scale bars: 20 nm.

a

| Description of proteins | Taxonomy | sequence identity | Liposome leakage activity |
|---|---|---|---|
| Non-specific serine/threonine protein kinase (tbMLKL) | Thiotrichales bacterium | 21.5 | Yes |
| Protein kinase domain-containing protein | Thiotrichales bacterium | 20.9 | TBD |
| Protein kinase domain-containing protein (csMLKL) | Coxiella sp. | 19.5 | Yes |
| Uncharacterized protein | Gammaproteobacteria bacterium | 18.53 | TBD |
| Uncharacterized protein | Rhizobiaceae bacterium | 10.1 | TBD |
| Rubrerythrin diiron-binding domain-containing protein | Candidatus Methylomirabilota bacterium | 9 | TBD |

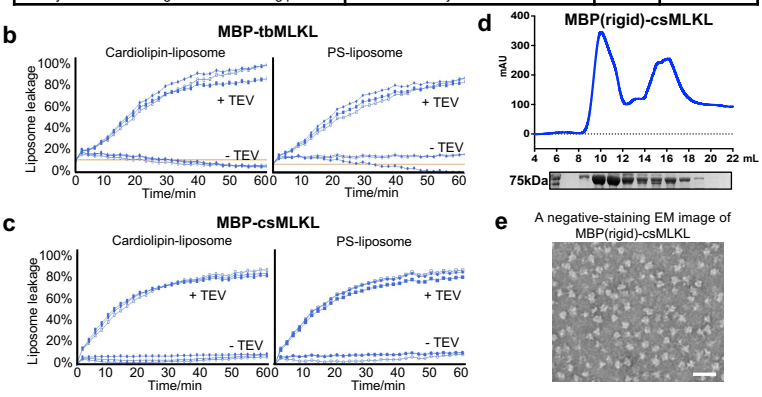


**Figure 3. Identification and structural elucidation of bacterial MLKL-like proteins.** (**a**) Identification of bacterial MLKL-like proteins using Foldseek. (**b** and **c**) Assessing the liposome leakage activity MLKLs from *Thiotrichales bacterium* (**b**) and *Coxiella sp.* (**c**) with cardiolipin- and PS-liposomes. (**d**) Size exclusion chromatography analysis of MBP(rigid)-csMLKL with a superdex 200 column. (**e**) A representative negative staining EM image of hMLKL pores reconstituted MBP(rigid)-csMLKL. Scale bar: 20 nm.