Supplementary Information for

Persistent mirusvirus infection in the marine protist *Aurantiochytrium*

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Supplementary Information

Viral glycoside hydrolases and sulfatases with glycoprotein-like domains

Our analyses uncovered two distinct classes of putative carbohydrate-active enzymes encoded on the AurliV-1 episome that share intriguing architectural and evolutionary similarities. The first are arylsulfatases with appended low-complexity, glycoprotein-like domains similar to BLLF1 envelope proteins of herpesviruses (Figure S5). The second are three alpha-galactosidases, also bearing predicted viral glycoprotein-like domains at

their C-termini (Figure S6). Both sets of enzymes form monophyletic groups that appear to have emerged via ancient co-option of host-derived genes, followed by viral genome integration.

Due to their low-complexity sequence, it is difficult to know whether all or some of the glycoprotein-like domains detected in these proteins are true homologs of viral glycoproteins. However, their proline/threonine-rich composition suggests that they could serve similar functions in protein targeting, structural support, or interactions with host surfaces. Because thraustochytrid cell walls are rich in sulfated polysaccharides and complex sugars, viral arylsulfatases and alpha-galactosidases may aid mirusviruses in remodeling the host extracellular environment to facilitate virion release or infection. These enzymes thus potentially parallel the well-documented role of bacteriophage-encoded cell wall-degrading enzymes, representing a convergent strategy to overcome host barriers in a eukaryotic context.

The finding that mirusvirus genomes carry both sulfatases and glycoside hydrolases with appended glycoprotein-like domains highlights a broader theme of viral adaptation. This convergence—observed in two distinct enzyme families—suggests that persistent, intimate virus-host relationships can drive the incorporation and modification of host-like metabolic functions within viral genomes. Whether these enzymes enhance viral fitness, mediate host-virus co-evolution, or serve in virus particle maturation or release remains an open question. However, the repeated appearance of these composite proteins supports the view that mirusviruses are active participants in sculpting their host environments through metabolic innovation.