

Supplementary Materials: Norovirus VPg binds RNA through a conserved N-terminal K/R basic patch

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NT* solubility tag removal: The protein was buffer exchanged into enterokinase cleavage buffer (20 mM Tris pH 8, 50 mM NaCl, 2mM CaCl₂) and cleaved using bovine enterokinase (New England Biolabs, Ipswich, MA, USA) at 16 U/mg protein for 4 hours. The enterokinase was removed using soybean trypsin inhibitor agarose (Sigma, St Louis, MO, USA) and the cleaved NT* tag was removed using Ni-NTA resin. The purified VPg proteins were then buffer exchanged into 50mM Tris, 150 mM NaCl and 10% glycerol and stored at -80°C .

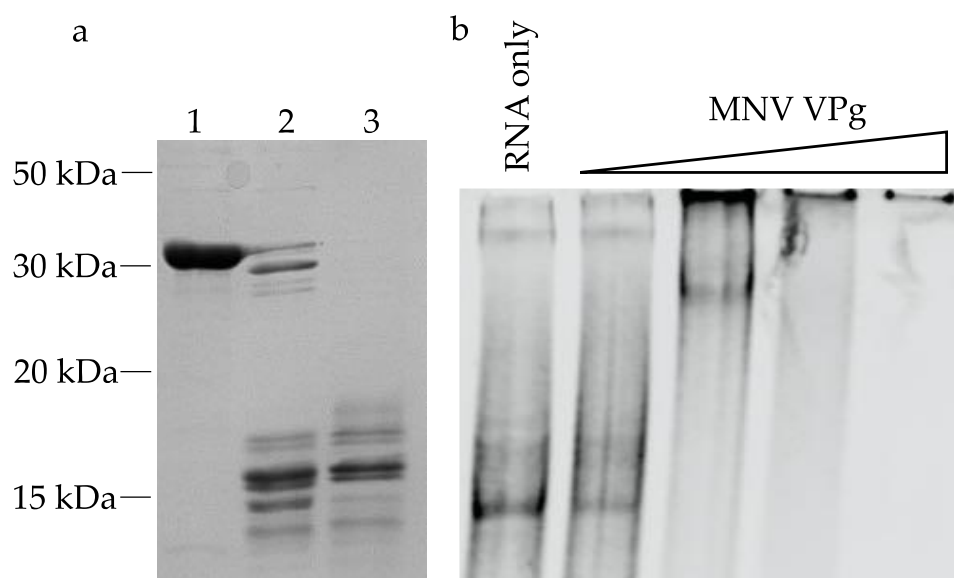


Figure S1. MNV VPg binds RNA. **(a)** NT*MNV VPg was digested with bovine enterokinase to remove the N-terminal fusion tags and analysed by SDS-PAGE gel followed by staining of proteins with Coomassie blue. 1; no enterokinase added to the protein, 2; enterokinase cleavage reaction, 3; purified MNV VPg sample. **(b)** MNV VPg was incubated with 100 nM of ATTO 680 labelled PP1 RNA for 30 min, separated on a 5% TBE gel and directly imaged in the 700 nm channel. MNV VPg was added to reactions at increasing concentrations; 2 μM , 5 μM , 10 μM and 20 μM .