Supplementary Figure 1

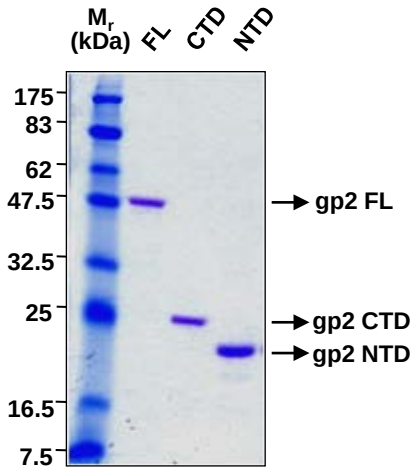

Figure S1 – Purified full-length (FL), N-terminal (NTD) and C-terminal (CTD) Gp2 domains.

Supplementary Figure 2

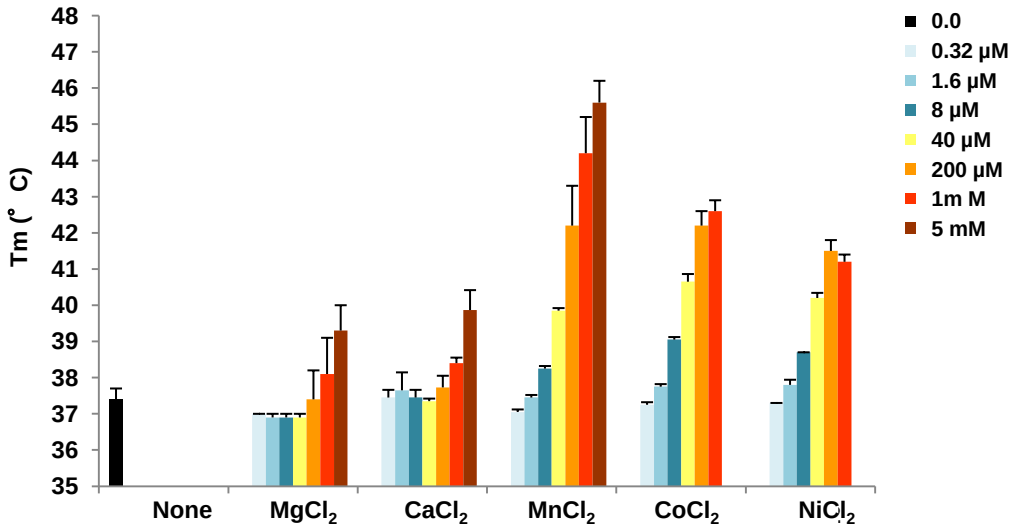

Figure S2 – A) Dose-dependent effect of divalent metal ions on gp2 thermal stability. The gp2 thermal stability was analyzed using a fluorescence based thermal shift assay in the absence (black bar) or in the presence of increasing concentrations of $MgCl_2$, $CaCl_2$, $MnCl_2$, $CoCl_2$, $NiCl_2$. The Tm values (°C) are the mean ± standard deviation from 3 independent experiments.

Supplementary Figure 3

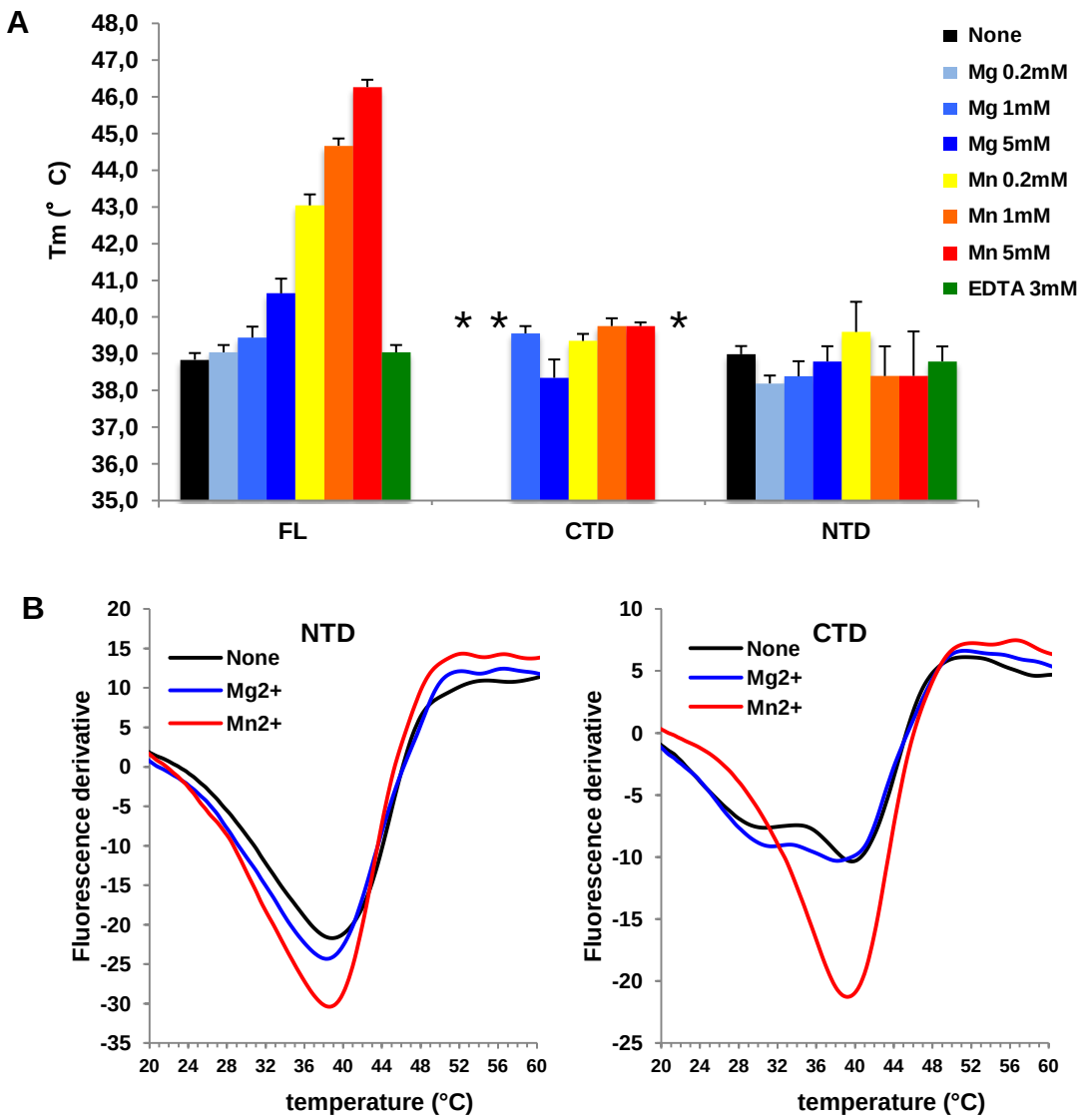

Figure S3 – Effect of Mg^{2+} and Mn^{2+} on the thermo stability of full-length (FL) gp2 and N-terminal (NTD) and C-terminal (CTD) gp2 domains. (**A**) Dose-dependent effect of Mg^{2+} and Mn^{2+} on gp2 domains thermal stability. The T_m values (°C) are the mean ± standard deviation from 3 independent experiments. * - the curves obtained show a complex denaturation behavior of the gp2 molecules population (see Fig S3B, right panel) hampering accurate determination of the T_m. (**B**) Graphics show the opposite of the derivatives of the fluorescence signal from the gp2 NTD (left panel) and CTD (right panel) melting curves (Fig. 4B) without metal ions (black) or in the presence of 200 µM of Mg^{2+} (bleu) or Mn^{2+} (red)**.**

Supplementary Figure 4

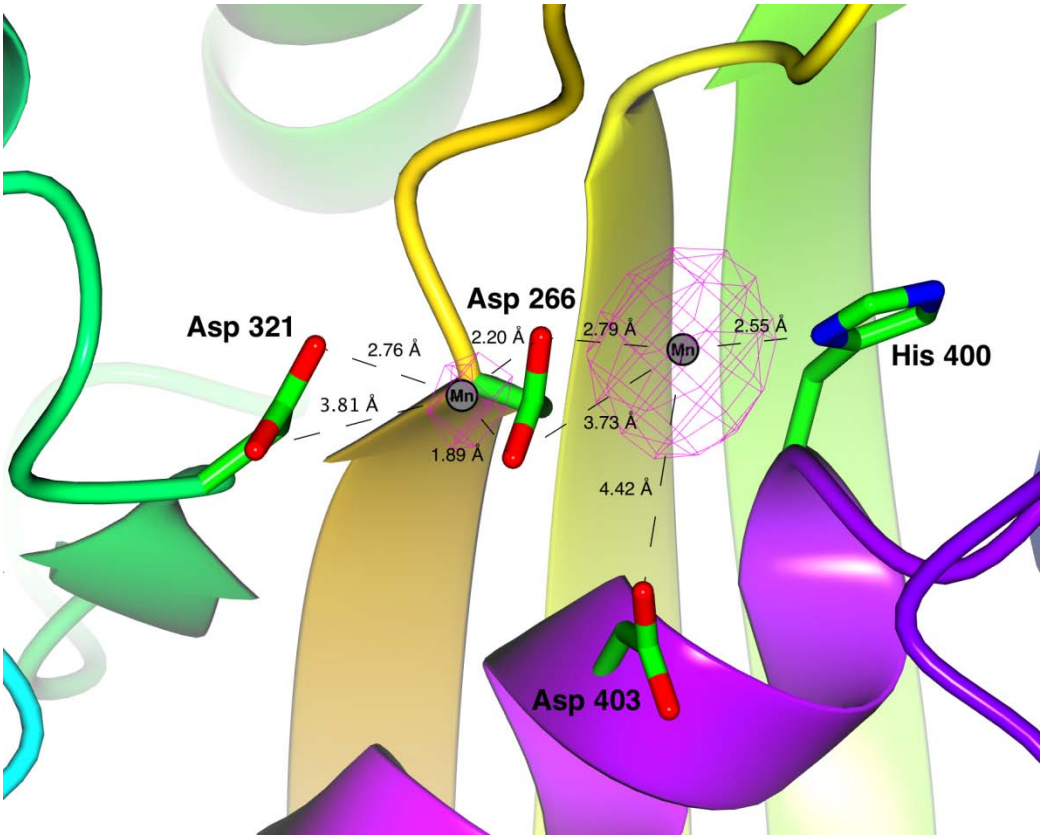

Figure S4 – The active site of the gp2 nuclease showing coordination distances to the bound Mn ions. The anomalous difference density from the Mn scattering (magenta) is contoured at 4.0 sigma. PDB code: 2wc9

Supplementary Figure 5

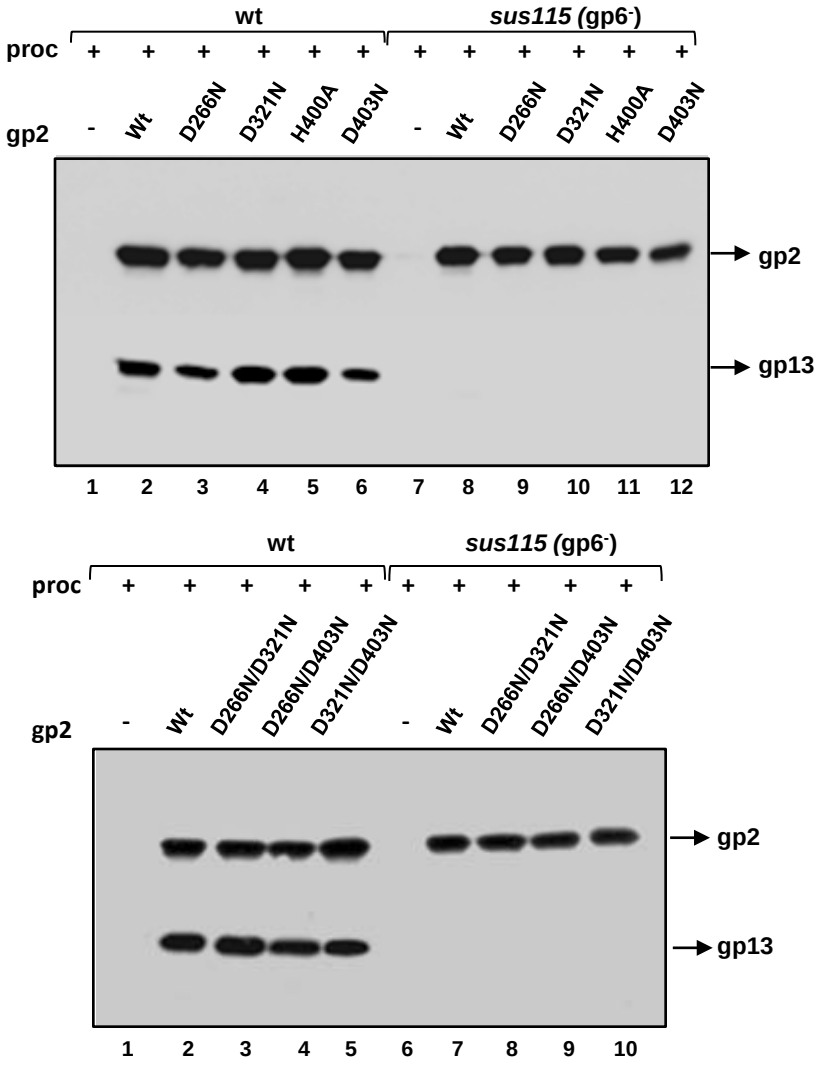

Figure S5 – Nuclease mutations do not affect gp2-portal binding. Specific binding of wt, gp2 single (upper panel) or double (lower panel) nuclease mutants to the procapsid portal vertex was analyzed by affinity pull-down assays with purified wt or portal-less procapsids (*sus115*, gp6$^-$), as detailed under Material and methods. Proteins present in the beads fraction were resolved by SDS-PAGE followed by Western blotting. Blots were incubated sequentially with anti-SPP1 (which recognizes the major head protein gp13) and anti-gp2 antibodies. The positions of the major head protein as well as gp2 are indicated.