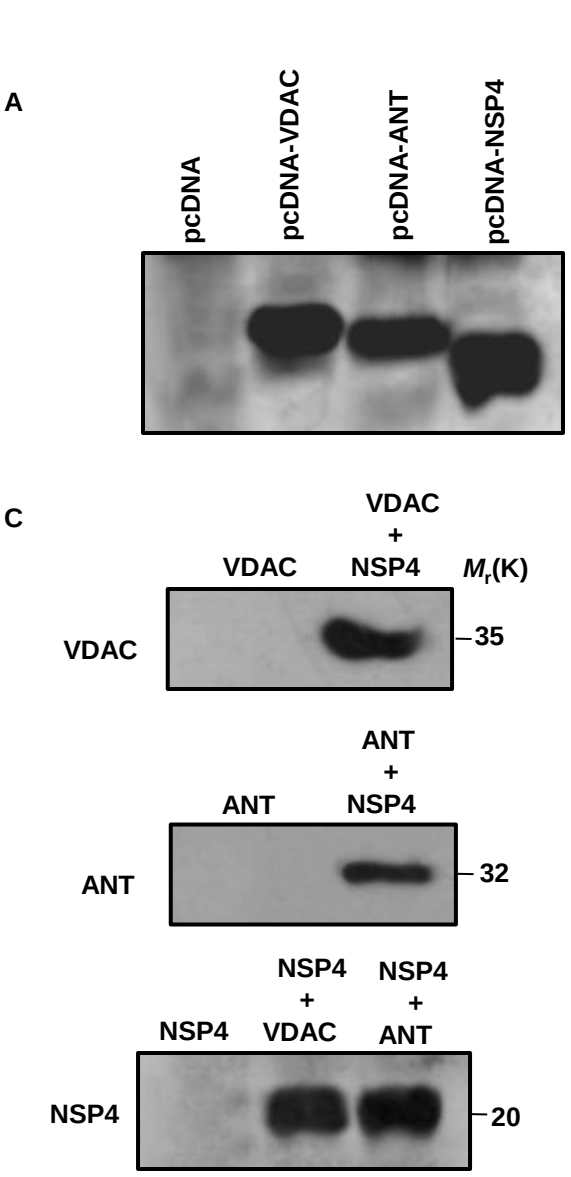

A) pcDNSP4, pcDVDAC1 and pcDANT3 were subjected to invitro coupled transcription and translation (IVT) in presence of TranscendTMbiotinylated-lysyl t-RNA and the products were separated by SDS PAGE and immunoblotted using streptavidin-HRP.

B) Recombinant protein were purified on Ni^{2+} -NTA magnetic agarose beads under native conditions and immunoblot analysis were performed using antibody against NSP4, VDAC and ANT.

C) Purified VDAC1 and ANT3 were subjected to enterokinase treatment (EK: substrate ratio=1:42 at 37°C in 50mM Tris, pH 7.6; reaction was stopped by adding 1mM PMSF and heating at 95°C for 10min and then passed through the Ni^{2+} -NTA column) to remove His tag. Recombinant native NSP4 (~5µg) were previously immobilized on Ni2+ by overnight incubation in HEPES-buffered saline (HBS) at 4°C. After extensive washing in PBS/0.3% Tween to remove unbound protein, NSP4 proteins were incubated with VDAC (10µg) or ANT (10µg) for 4h at 4°C in HBS. Beads were washed extensively with HBS to remove non-specifically bound proteins. Remaining proteins were separated by 4 × sample buffer and then analyzed by SDS-PAGE and immunoblotted for VDAC (upper panel) or ANT (middle panel). Similarly, in reciprocal experiments, recombinant VDAC or ANT (~5µg) (with His tag) were pre-immobilized on Ni2+, followed by binding and immunoblotting of NSP4 (enterokinase treated) (10µg) protein (lower panel). In both ways NSP4 showed interaction with VDAC1 and ANT3.