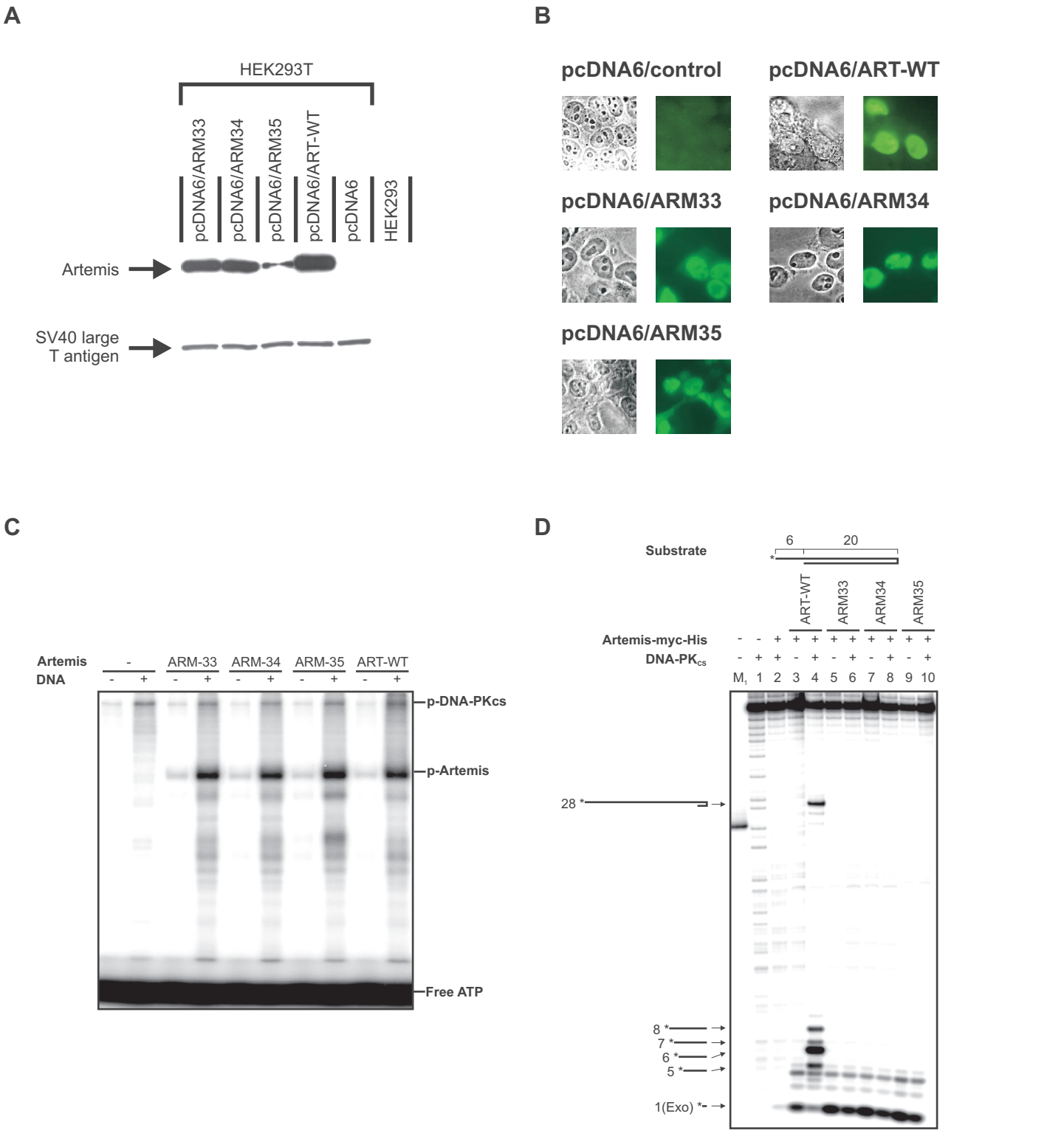

Figure Sup_3: Pannicke et al., ARTEMIS residues critical for enzymatic activity

Figure Sup_3. *In Vitro* Nuclease Assay of ARTEMIS Double Mutants

(A) HEK293T cells were transfected with pcDNA6 expression plasmids coding for myc-His fusion proteins of either wild-type (ART-WT) or double mutant (ARM33-35) ARTEMIS. As controls, HEK293 and HEK293T cells were transfected with the empty vector. The expression of the ARTEMIS-myc-His fusion proteins, as detected by immunoblot analysis, is shown in the upper panel; SV40 large T protein expression is shown below. (B) HEK293T cells were transfected with pcDNA6 expression plasmids coding for myc-His fusion proteins of wild-type (ART-WT) and double mutant (ARM33-35) ARTEMIS proteins. The subcellular localization of the myc-His fusion proteins was detected by an immunostaininig using a mouse anti-human myc antibody. An empty vector transfection served as a control. (C) Wild-type ARTEMIS and double mutant ARTEMIS proteins ARM33 to ARM35 were subjected to a DNA-PKcs phosphorylation assay as described for Figure 3. (D) The DNA-PK_{cs} dependent endonucleolytic activities, but not the exonucleolytic activity of ARTEMIS were abolished in ARM33 to ARM35. The hairpin opening assay was done as described in the legend of Figure 4. M marks the position of the 26 nt hairpin opening product.