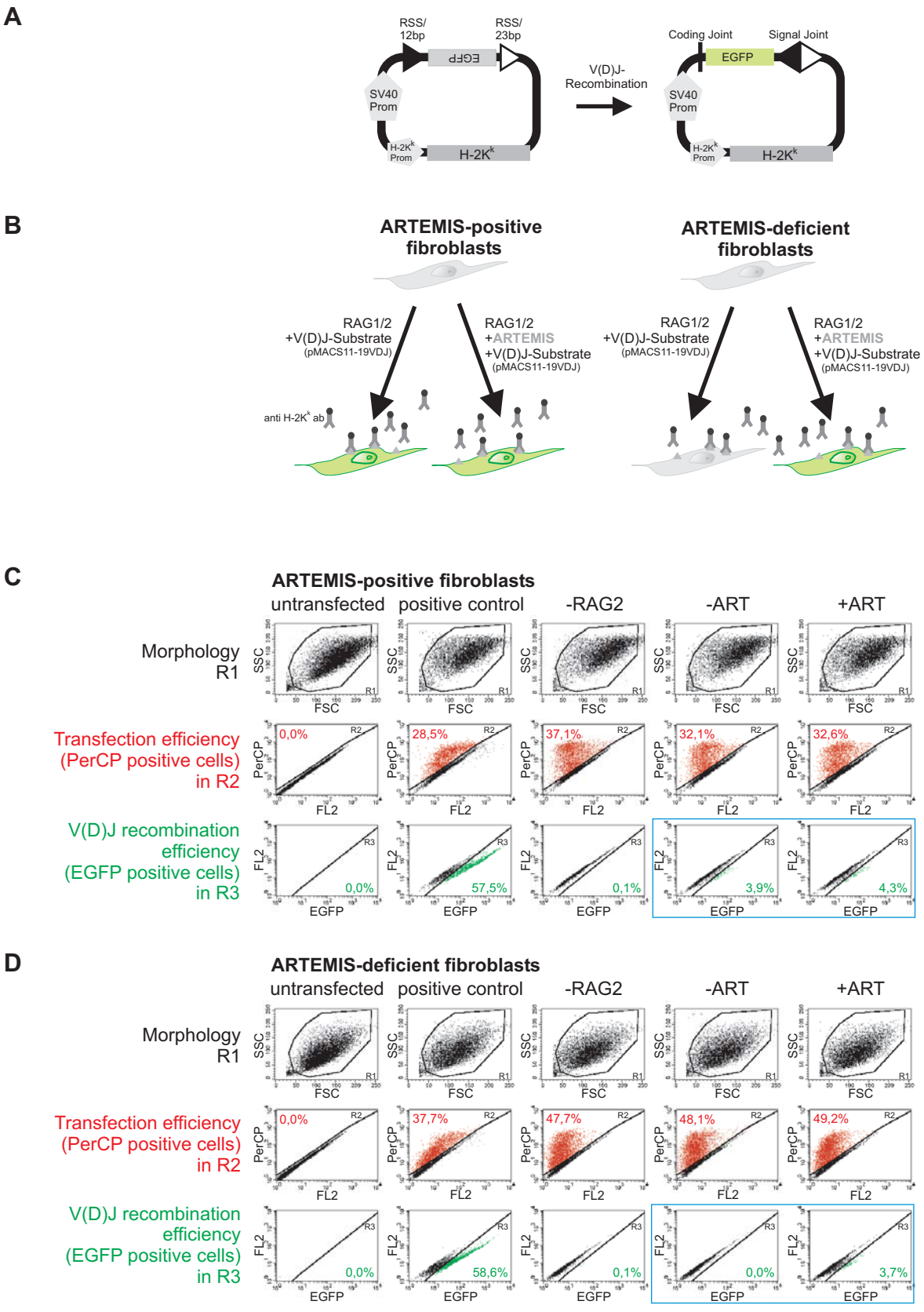

Figure Sup_2: Pannicke et al., ARTEMIS residues critical for enzymatic activity

Figure Sup_2. *In Vivo* V(D)J Recombination Assay in Human Primary Dermal Fibroblasts

(A) In the inversional V(D)J recombination substrate plasmid pMACS/11-19VDJ, an ORF for the Enhanced Green Fluorescence Protein (EGFP) is located between two RSSs. Upon a recombination between the 12- and the 23-RSS, the EGFP ORF is inverted and comes under the control of the SV40 promoter; V(D)J recombination-positive cells can thus be quantified by FACS analysis due to their green fluorescence. The constitutively expressed mouse MHC class I gene H-2K^k located on the substrate vector allows the FACS-determination of transfected cells by immunostaining with an anti H-2K^k antibody. (B) Normal human control fibroblasts transfected with expression plasmids for the recombination activating genes 1 and 2 (RAG1/2) and with the V(D)J recombination substrate pMACS/11-19VDJ show the capability to perform V(D)J recombination independently of the co-transfection of an ARTEMIS expression plasmid. The capability for V(D)J recombination in ARTEMIS-deficient fibroblasts is strictly dependent on co-transfection of an ARTEMIS expression plasmid. ARTEMIS-positive control fibroblasts (C) or ARTEMIS-deficient fibroblasts (D) were transfected with pre-recombined control plasmid alone (positive control), or co-transfected with expression plasmids coding for RAG1, RAG2 and wild-type ARTEMIS (+ART) and the V(D)J recombination substrate pMACS11-19VDJ. As additional controls, the same co-transfections were done without the expression plasmids coding for RAG2 (-RAG2) or wild-type ARTEMIS (-ART). For control of autofluorescence untransfected cells were used. Evaluations of the FACS analyses were performed in three steps. At first, living fibroblasts were selected according to their morphology (R1). Secondly, transfected living cells, which expressed MHC class I protein H-2K^k on their surface were stained with an PerCP-conjugated anti H-2K^k antibody. The stained cells were detected in fluorescence

channel 3 (650nm longpass). Finally, fibroblasts containing recombined V(D)J substrate plasmids were detected in the subpopulation of transfected living cells, due to their green fluorescence (EGFP expression) in fluorescence channel 1 (EGFP) (530nm +/-15nm). For each assay $5x10^4$ cells were analysed. Percentages of transfected and EGFP-positive cells are quoted. Measurements discriminating the V(D)J recombination capabilities of the ARTEMIS-positive and ARTEMIS-defective cell lines are highlighted in blue.