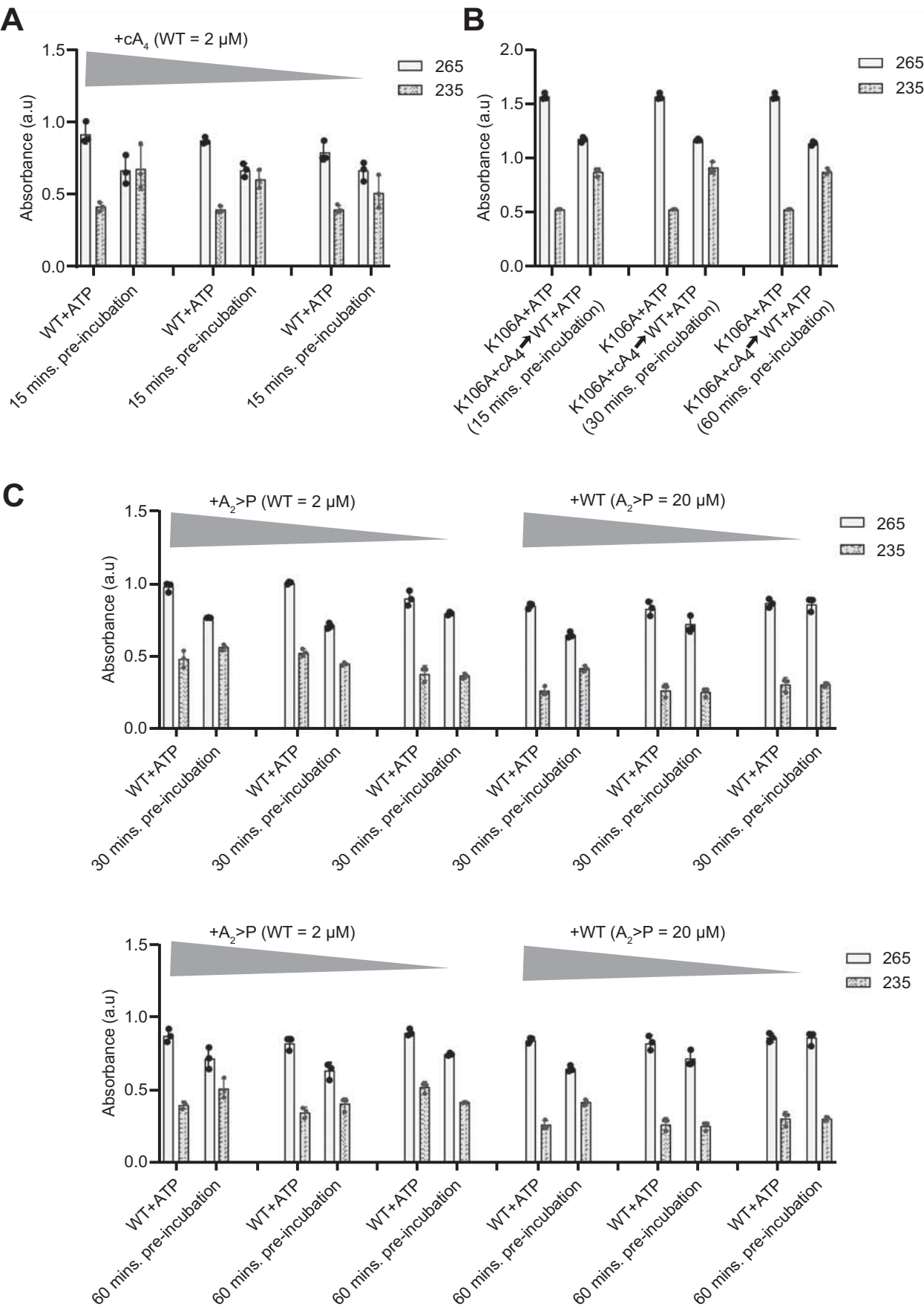


Expanded View Figures

Figure EV1. Concentration effects by cA_4 and $A_2 > P$ in their activation of *TaqCad1*.

"WT + ATP" indicates the reaction in the absence of any ligand. *TaqCad1*-mediated reactions were dephosphorylated by calf intestinal alkaline phosphatase following heat deactivation. (A) UV absorption measurement results of the reaction of *TaqCad1* with ATP in the presence of various of cA_4 concentrations (0.2, 0.5, and 2 μ M). (B) UV absorption measurement results of the reaction of *TaqCad1* with ATP in the presence of various of products of 20 μ M cA_4 incubated with the K106A variant for 15, 30, and 60 min, respectively. (C) UV absorption measurement results of the reaction of *TaqCad1* with ATP in the presence of ring nuclease products resulted from incubating cA_4 at 5, 10, and 20 μ M, respectively, with 2 μ M WT *TaqCad1* for 30 (top) and 60 (bottom) min, respectively. For panels (A–C), data represent the mean $\pm$ standard deviation of the mean ($n = 3$ biological replicates). Source data are available online for this figure.



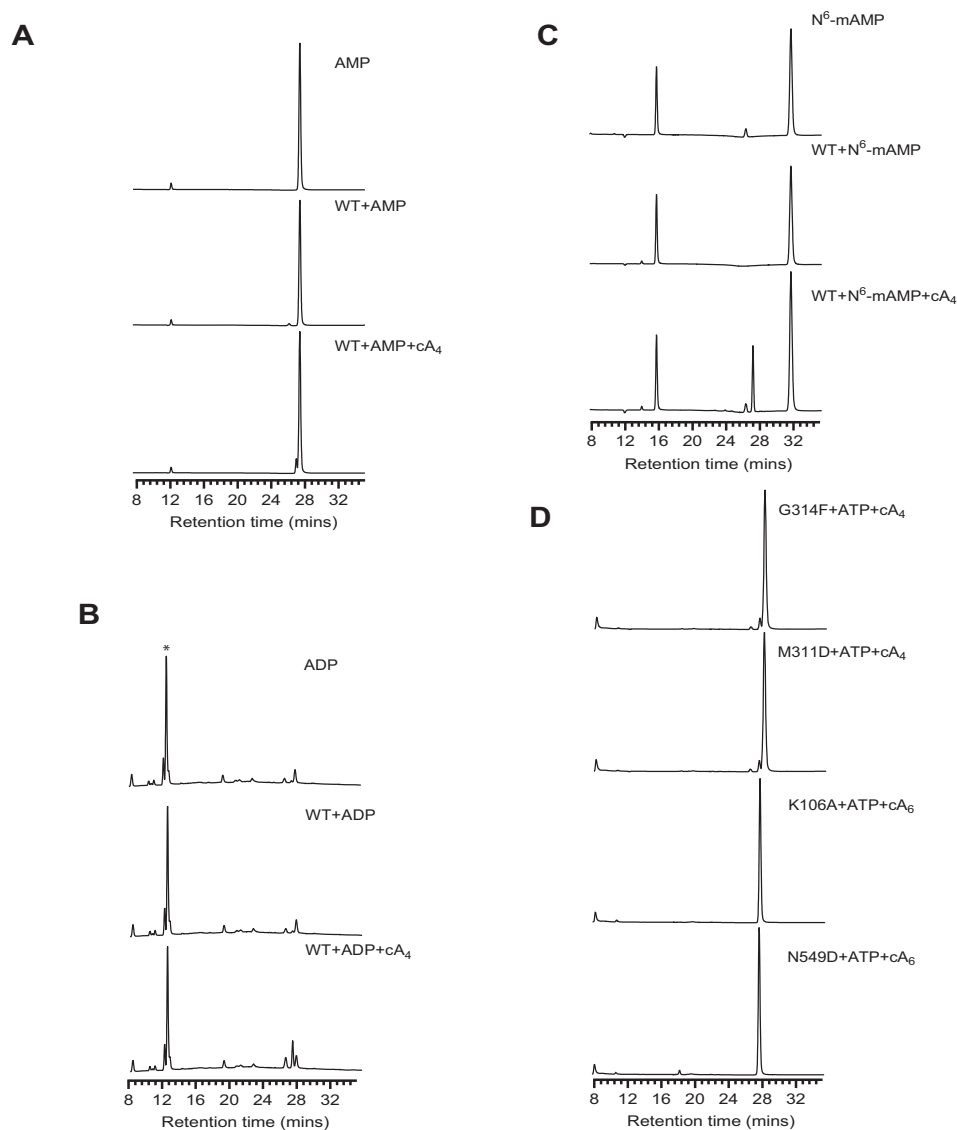
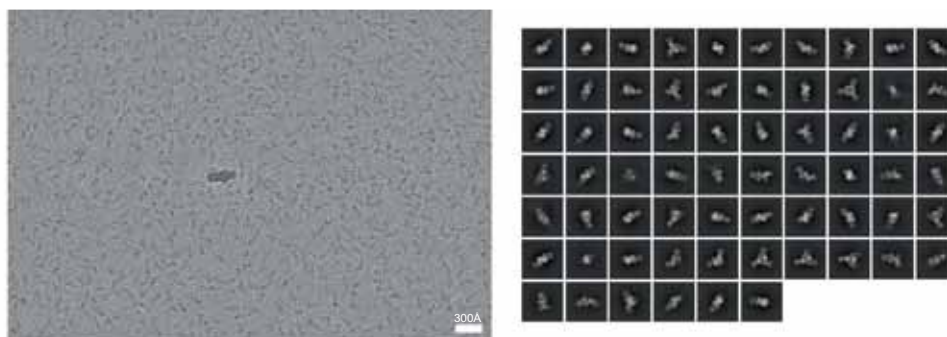


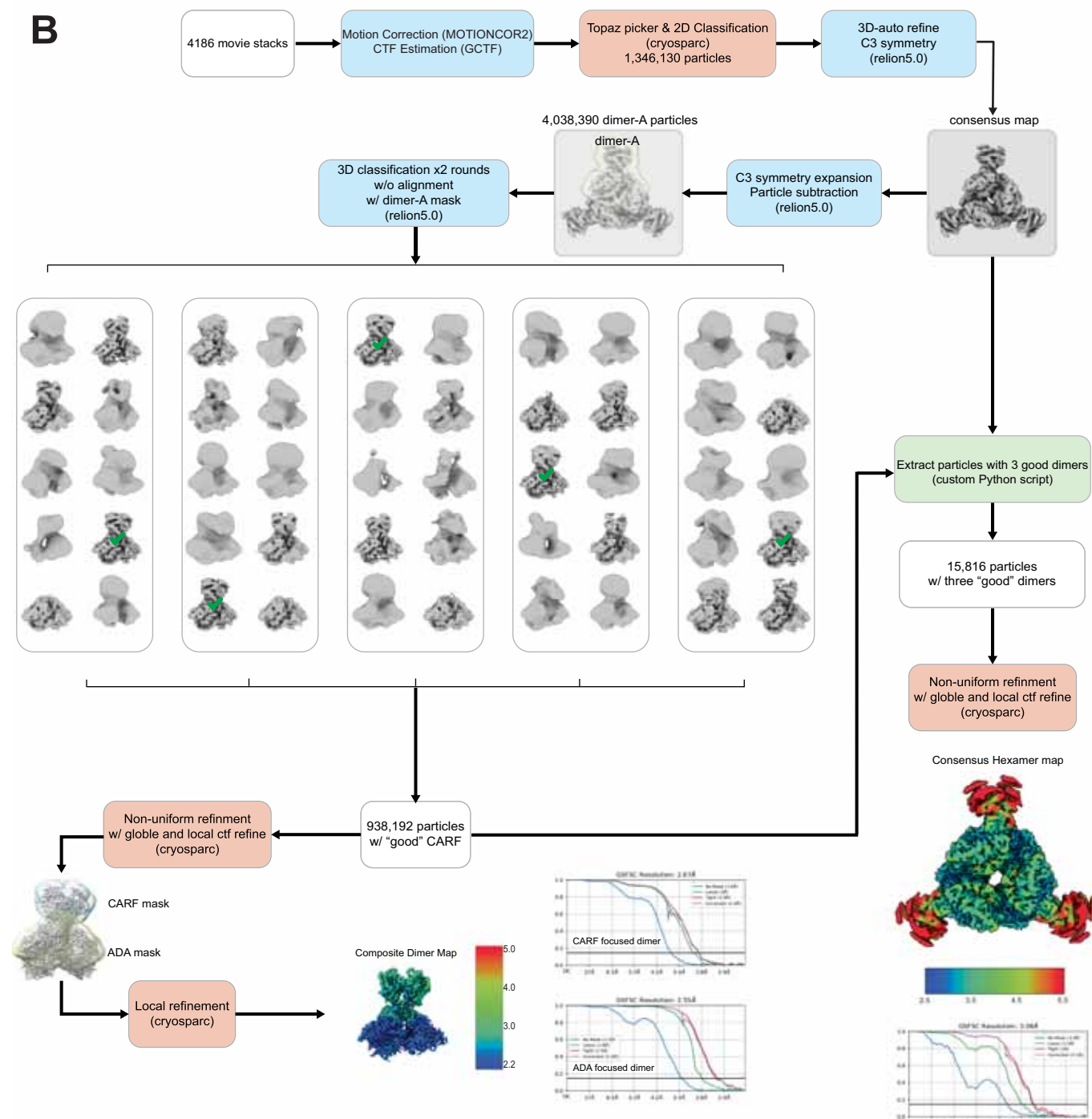
Figure EV2. Additional deamination assays for *TaqCad1*.

(A) HPLC profiles following *TaqCad1* reaction with adenosine monophosphate (AMP) in the presence and absence of cA_4 . (B) HPLC profiles following *TaqCad1* reaction with adenosine diphosphate (ADP) in the presence and absence of cA_4 . The asterisk indicates a nonspecific peak present in the unreacted ADP sample (top). (C) HPLC profiles following *TaqCad1* reaction with N^6 -methyl-adenosine monophosphate (N^6 m-AMP) in the presence and absence of cA_4 . (D) HPLC profiles of the reaction products following incubating ATP with *TaqCad1* variants (G314F, M311D, N549D, or K106A) in the presence and absence of cA_4 or cA_6 . The reaction products were treated with CIP. The profiles of adenosine and inosine are included for comparison. Dash lines indicate the elution positions of inosine and adenosine.

A



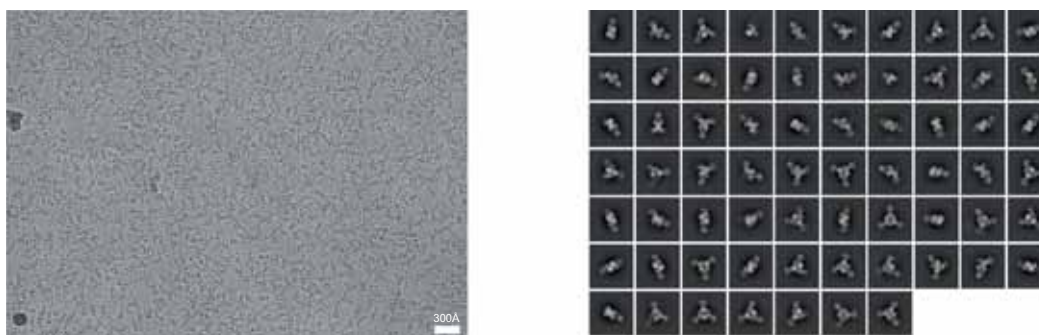
B



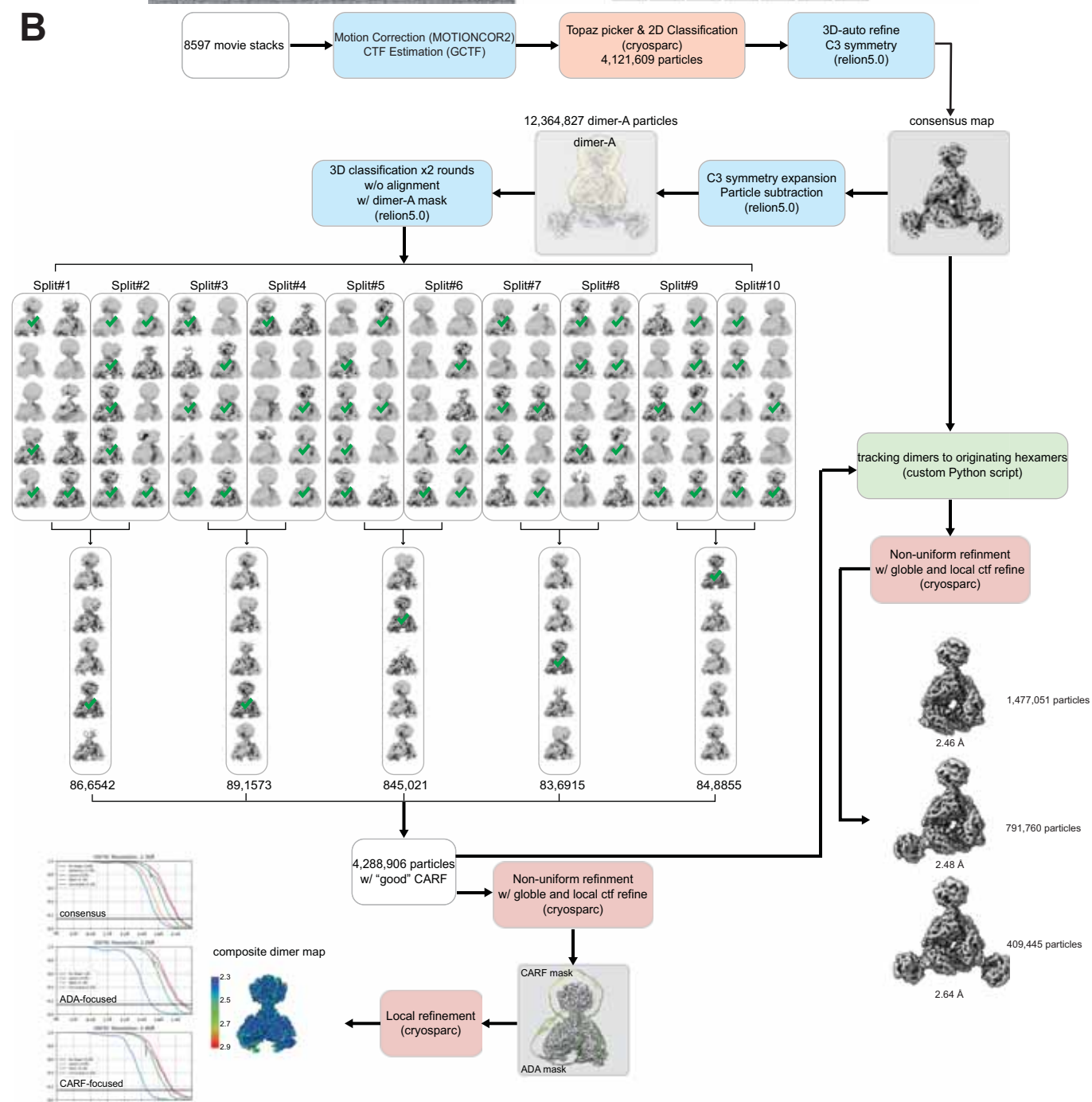
◀ Figure EV3. Data collection, processing, and 3D reconstruction of the cryoEM structures of apo TaqCad1.

(A) Example micrograph and 2D class averages (scale bar 300 Å). (B) Data collection, particle selection, and reconstruction flowchart. Two reconstructions were made for the individual dimers and the intact hexamer, respectively. The dimer was reconstructed from a C3 symmetry expansion, leading to over 4 million particles. 3D classification was performed to select the best dimers that were then reconstructed for the CARF and ADA domains, respectively, that were combined to form the composite map. The best hexamer containing three intact dimers was reconstructed without focused refinement. Final maps used for refinement are shown with local resolutions and their Fourier shell correlation (FSC) curves. 0.143 FSC cutoff was used for resolution estimation.

A

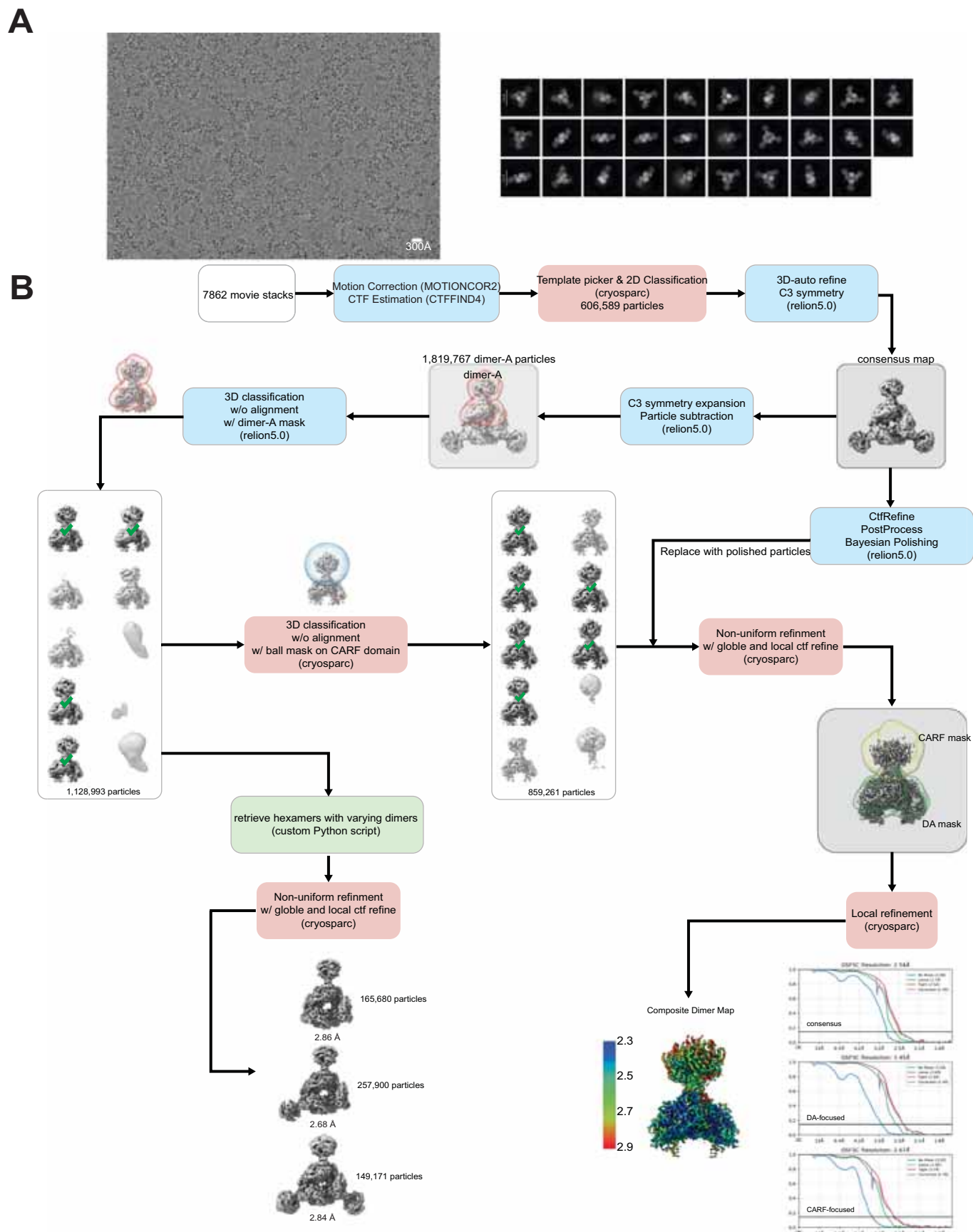


B



**Figure EV4. Data collection, processing, and 3D reconstruction of the cryoEM structures of *TaqCad1* incubated with *cA₄*.**

(A) Example micrograph and 2D class averages (scale bar 300 Å). (B) Data collection, particle selection, and reconstruction flowchart. Two reconstructions were made for the individual dimers and the intact hexamer, respectively. The dimer was reconstructed from a C3 symmetry expansion, leading to over 4 million particles. 3D classification was performed to select the best dimers that were then reconstructed for the CARF and ADA domains, respectively, that were combined to form the composite map. The hexamers containing one, two, or three intact dimers were reconstructed without focused refinement. Final maps used for refinement are shown with local resolutions and their Fourier shell correlation (FSC) curves. 0.143 FSC cutoff was used for resolution estimation.



**Figure EV5. Data collection, processing, and 3D reconstruction of the cryoEM structures of *TaqCad1* incubated with *cA₄* and ATP.**

(A) Example micrograph and 2D class averages (scale bar 300 Å). (B) Data collection, particle selection, and reconstruction flowchart. Two reconstructions were made for the individual dimers and the intact hexamer, respectively. The dimer was reconstructed from a C3 symmetry expansion, leading to over 4 million particles. 3D classification was performed to select the best dimers that were then reconstructed for the CARF and ADA domains, respectively, that were combined to form the composite map. The hexamers containing one, two, or three intact dimers were reconstructed without focused refinement. Final maps used for refinement are shown with local resolutions and their Fourier shell correlation (FSC) curves. 0.143 FSC cutoff was used for resolution estimation.