Supplementary Material:

Escherichia coli proteins uL29 and ACP stabilize the Tn7-encoded TnsD and its DNA binding

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Figure S1. Available structures for TnsD complexes.

Figure S2. Characterization of apo and holo ACP.

Figure S3. Comparison of AlphaFold predictions for TnsD.

Figure S4. ACP$^{D39R/E42A}$ exists in its apo- and holo- forms.

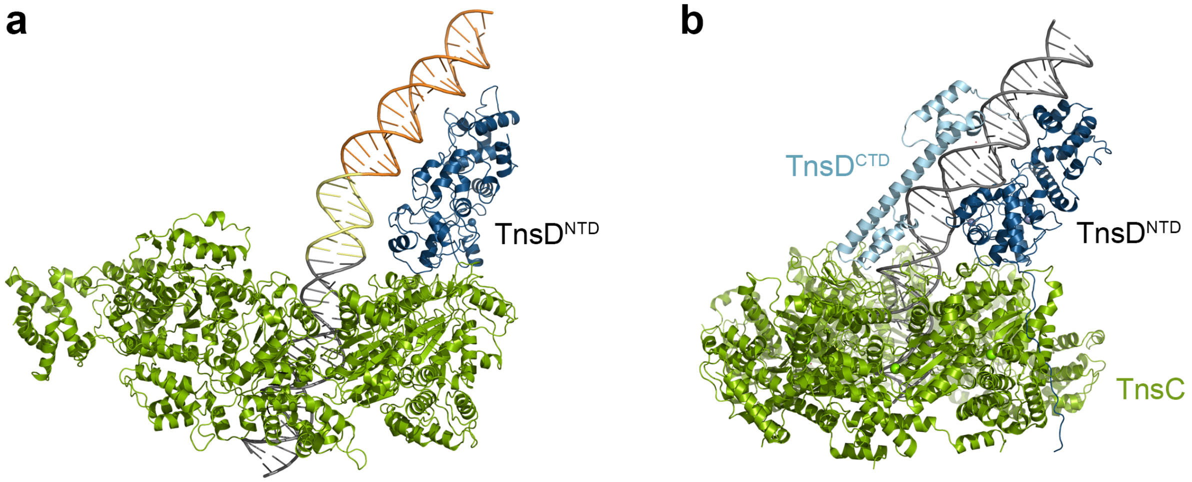

Figure S1. Available structures for TnsD complexes. (a) Cryo-EM reconstruction of TnsD^{NTD}:TnsC:DNA complex from the Tn7 element[1]. TnsD and TnsC are colored dark blue and green, respectively. (b) Cryo-EM reconstruction of the TnsD-targeting complex from a I-B2 CAST element[2]. The C-terminal domain of TnsD is shown in light blue, and TnsD^{NTD} and TnsC are shown as in (a).

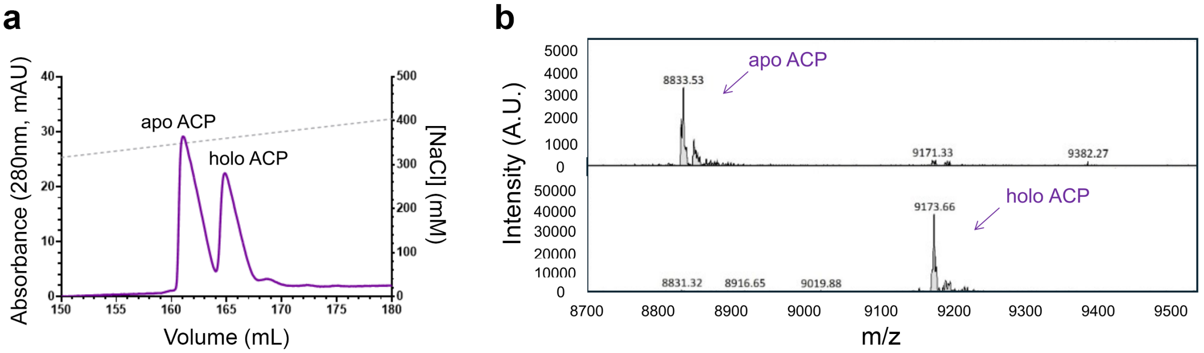

Figure S2. Characterization of apo and holo ACP. (a) Ionic exchange elution profile resolving the apo- and holo- forms of ACP. (b) ESI-Mass Spectrometry for apo (top) and holo (bottom) ACP. Molecular weights are reported in daltons, showing the expected 340.13 Da mass difference between the two forms.

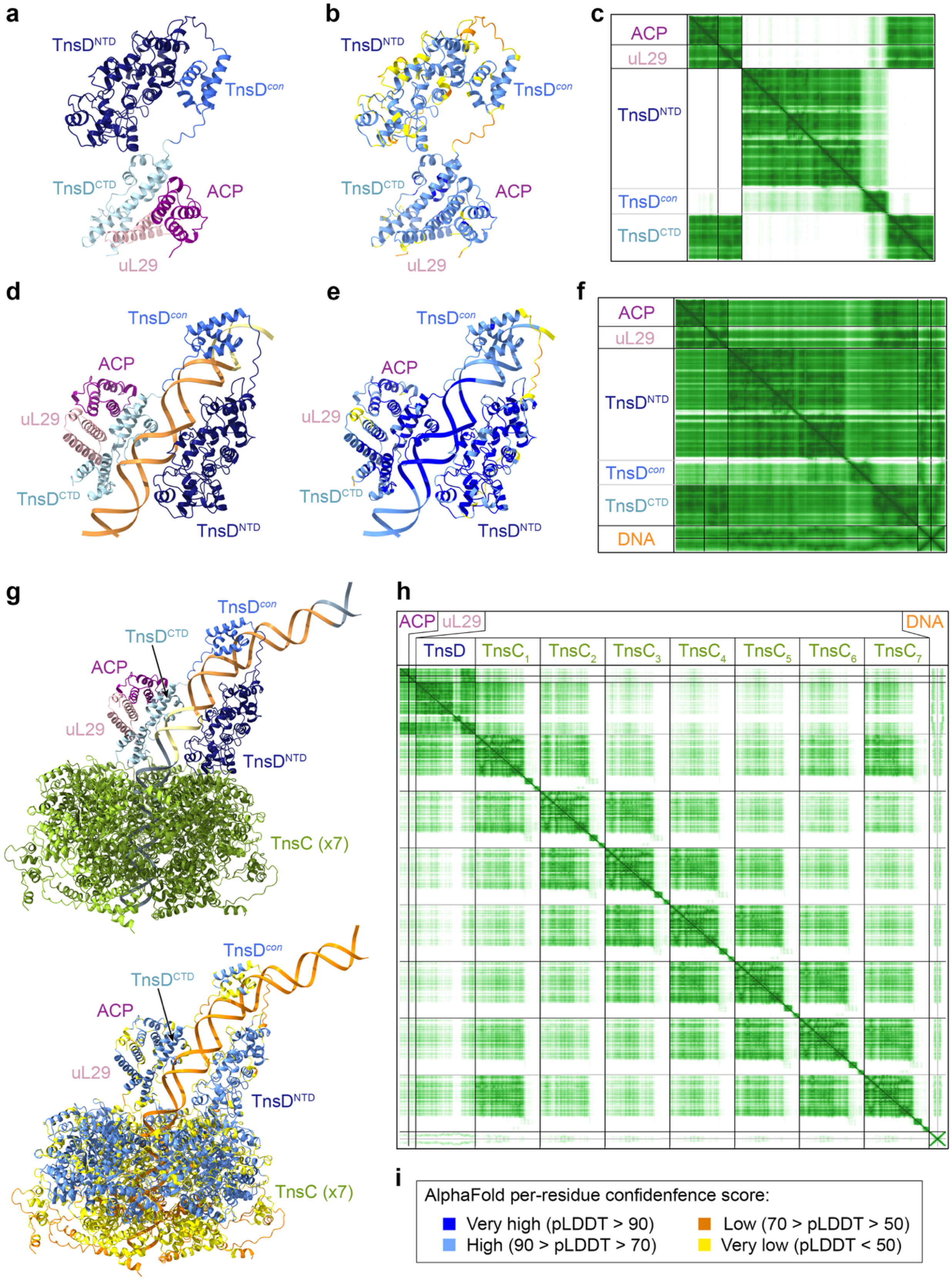

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Figure S3. Comparison of AlphaFold predictions for TnsD. (a) Prediction of the Tn7 TnsD:ACP:uL29 complex in the absence of DNA. TnsD is shown in shades of blue, ACP in magenta, and uL29 in pink, and labeled. (b) Prediction of the Tn7 TnsD:ACP:uL29 complex in the absence of DNA colored based on the local confidence of the prediction as defined by Alphafold. (c) Aligned Error (PAE) matrix for the TnsD:ACP:uL29 prediction. (d-e) Prediction of the TnsD:ACP:uL29 complex bound to the +23 to +58 fragment of *attTn7* site. Proteins are colored as in (a-b). In panel (d), DNA is color-coded based on high (orange) and low (yellow) protection upon binding as determined by Mitra *et al.*[3] (f) PAE matrix for the TnsD:ACP:uL29:DNA prediction. (g-h) Alphafold prediction of the Tn7 TnsD-mediated targeting complex colored as in (a-b) with TnsC shown in green (top) and based on the pLDDT (bottom). (f) PAE matrix for the TnsD:ACP:uL29:TnsC:DNA prediction. (i) Color-key for the local confidence scores (pLDDT) used in panels (b), (e), and (g).

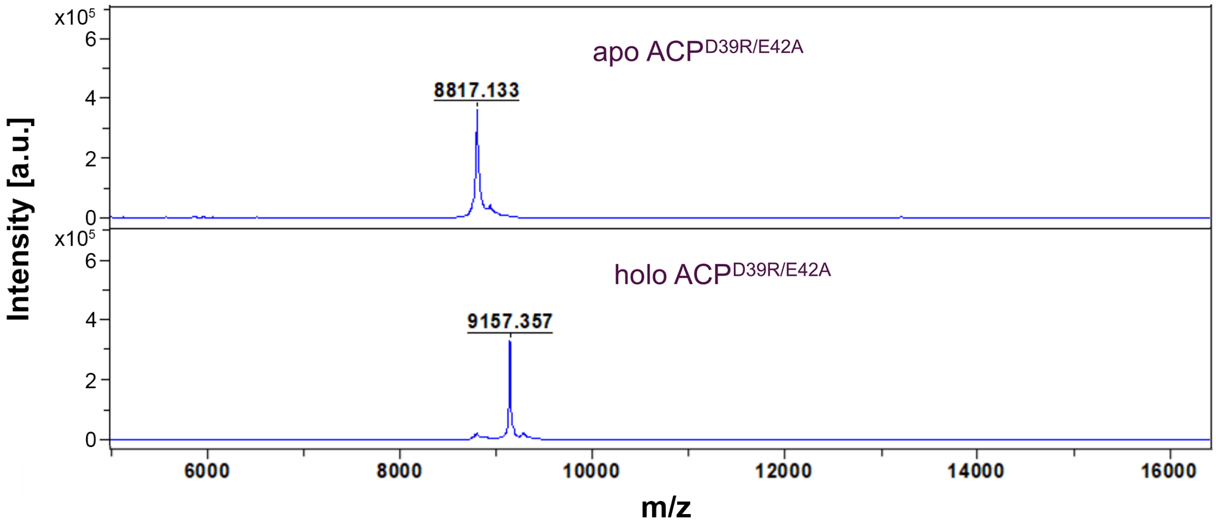

Figure S4. ACP$^{D39R/E42A}$ exists in its apo- and holo- forms. MALDI-Mass Spectrometry for apo (top) and holo (bottom) ACP$^{D39R/E42A}$ showing a 340.2 Da mass difference between the two forms.

References:

1. Shen, Y., et al., *Assembly of the Tn7 targeting complex by a regulated stepwise process.* Mol Cell, 2024. **84**(12): p. 2368-2381 e6.
2. Wang, S., et al., *Structure of TnsABCD transpososome reveals mechanisms of targeted DNA transposition.* Cell, 2024. **187**(24): p. 6865-6881 e16.
3. Mitra, R., et al., *Characterization of the TnsD-attTn7 complex that promotes site-specific insertion of Tn7.* Mob DNA, 2010. **1**(1): p. 18.