Expanded View Figures

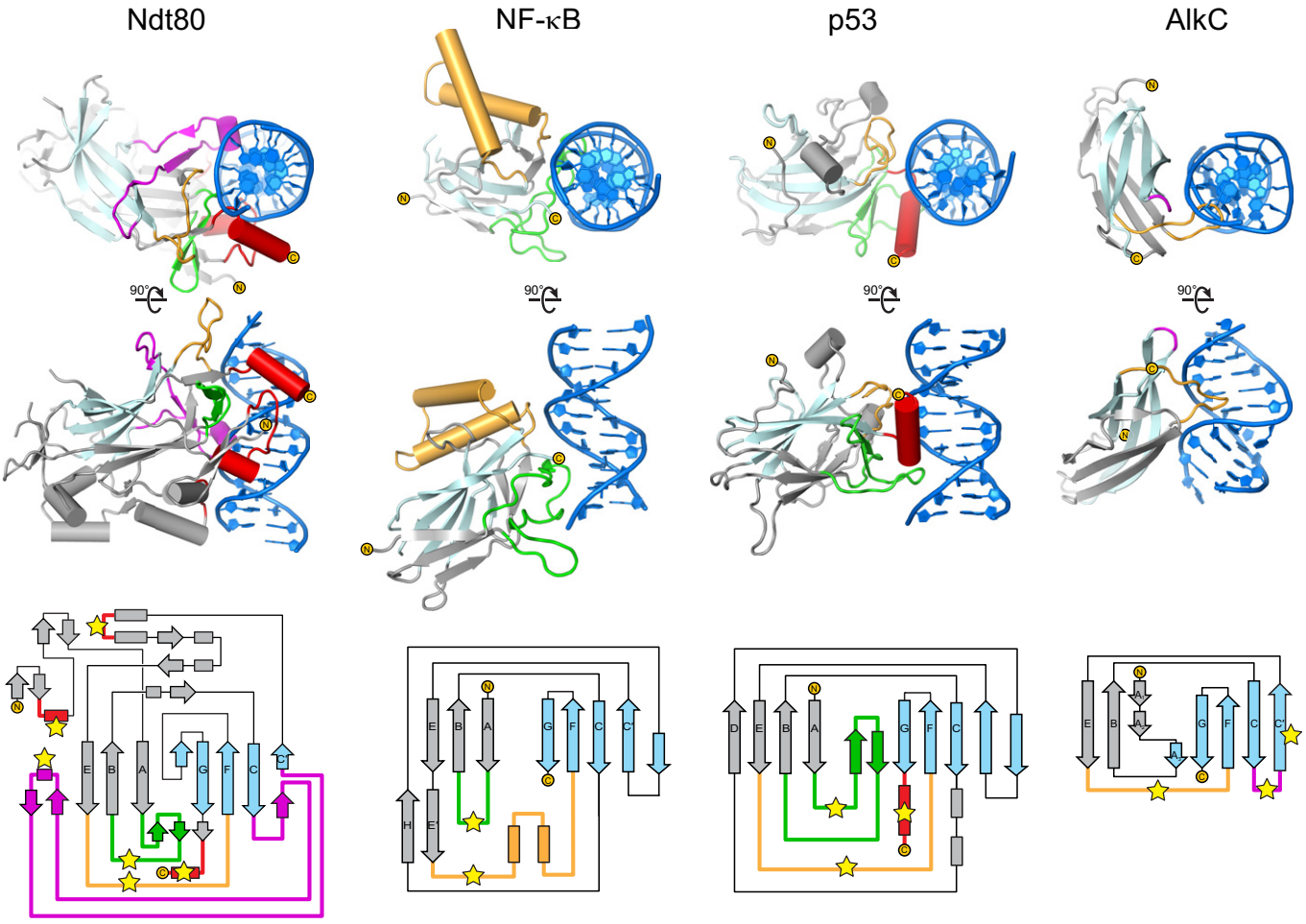

Figure EV1. Comparison of DNA binding Ig folds.

The top two rows show orthogonal views of DNA-bound structures of Ig-like domains from three eukaryotic transcription factors Ndt80 (PDB 1MNN), NF-κB (PDB 1A3Q), and p53 (PDB 1TUP), compared to that of PfAlkC (this work). Topology diagrams of each structure are shown at the bottom and are colored the same as the crystal structures. The two β-sheets within the Ig core are colored gray (A, B, D, E, E′, and H) and pale cyan (C, C′, F, and G). Regions contacting the DNA are colored green (AB-loop), magenta (CC′-loop), orange (EF-loop), and red. Points of contact with the DNA in are marked with yellow stars in the topology diagram.

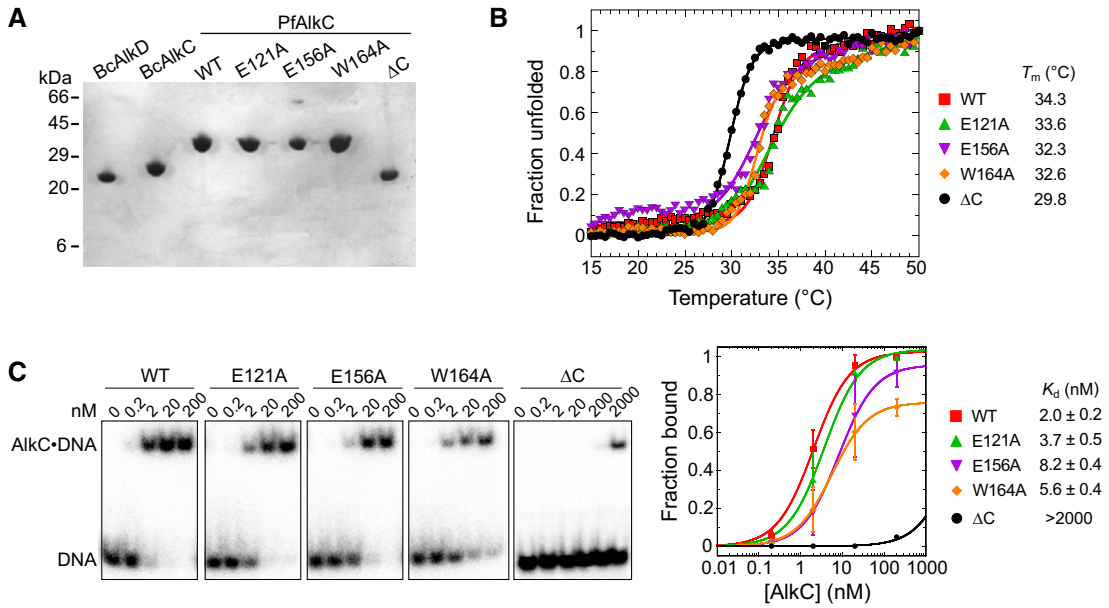

Figure EV2. Characterization of PfAlkC proteins used in this study.

A SDS–PAGE of purified proteins, stained with Coomassie Blue.

B Thermal denaturation of wild-type and PfAlkC mutants monitored by circular dichroism. The fraction of unfolded protein was expressed as the normalized molar ellipticity at 222 nm. Melting temperatures (T_m) were derived by fitting the data to the equation (*fraction unfolded*) = $1/(1 + e^{(Tm-T)/k})$, where k denotes the cooperativity of the transition. Although PfAlkCΔC has a lower T_m than the full-length proteins, it is properly folded at 21°C at which biochemical assays were performed.

C Electrophoretic mobility shift assay for PfAlkC mutants binding to 1 nM THF-DNA of the sequence ^{32}P-d(GACCACTACACT(THF)ATTCCTAACAAC)/d(GTTGTTAGGAAT(T) AGTGTAGTGGTC) in 25 mM HEPES (pH 7.5), 50 mM KCl, 5 mM DTT, 5% glycerol, and 0.05 mg/ml BSA at 21°C for 30 min. Concentrations of PfAlkC are shown at the top of the representative gels. Electrophoretic separation was carried out on a Novex™ TBE gel (ThermoFisher Scientific). Quantitation is plotted on the right, in which each value is the mean ± SD (n = 3). Equilibrium dissociation constants (K_d) were extracted by fitting the data to a two-state binding model.

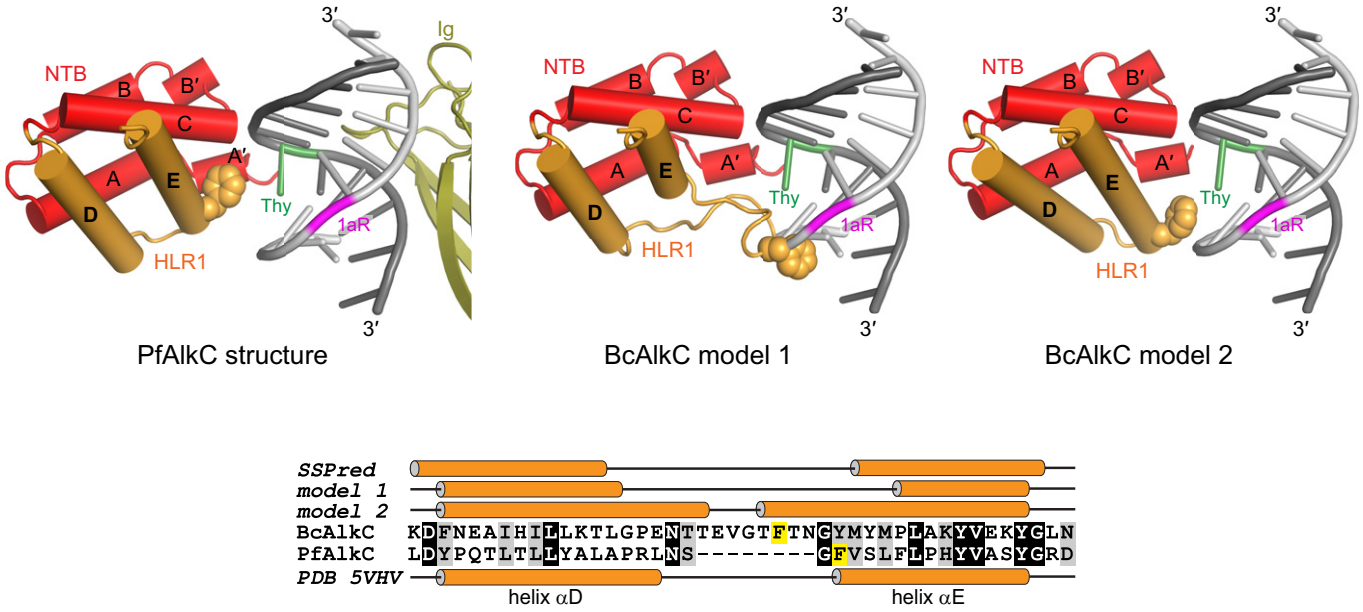

Figure EV3. AlkCα proteins contain an insertion that may stabilize DNA bend in the absence of Ig-like domain.

Two homology models for BcAlkC (center and right) are shown superimposed against the 1aR-DNA from the PfAlkC/1aR-DNA crystal structure (left). Only the N-terminal helical bundle (NTB, red), HEAT-like repeat 1 (HLR1, orange), and Ig-like domain (olive) are shown for clarity. A phenylalanine side chain at the N-terminus of helix αE in PfAlkC and in the 8-residue insertion of BcAlkC is shown in spheres and highlighted yellow in the sequence alignment at the bottom. The secondary structural elements from the three models and from a secondary structure prediction of BcAlkC are shown against the sequences for each protein. The homology models were generated in SWISS-MODEL (https://swissmodel.expasy.org/) using either the sequence alignment shown in Appendix Fig S1 (model 1) or generated by SWISS-MODEL (model 2). In both models, the insertion makes contacts to the 1aR strand, either as a loop (model 1) or as an N-terminal extension to helix αE (model 2).

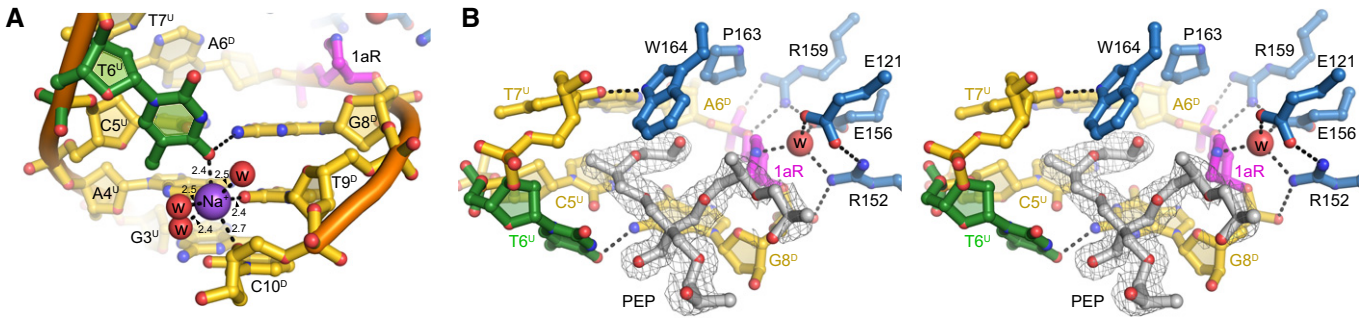

Figure EV4. Crystallographic features of the active site.

A Na^+ ion coordination in the 1aR-DNA structure. Na^+ and water oxygens are shown as purple and red spheres, respectively. 1aR is magenta, and the opposite thymidine T6 is green. Superscripts in nucleotide labels refer to the 1aR-containing, damaged (D) strand or the opposite, undamaged (U) strand. Hydrogen bonds are shown as dashed lines with interatomic distances in Ångstroms.

B Stereo-views of the PfAlkC/1aR-DNA active site. Active site residues are blue, and DNA is gold/magenta/green. Pentaerythritol propoxylate (PEP) sequestered from the solvent is in silver and superimposed against composite omit electron density contoured at 1σ. The putative catalytic water is shown as a red sphere. One arm of the PEP projects into the DNA kink between the 1aR and the flanking base pairs and thus may limit rotation of the 1aR ring back toward the DNA. The other two polymeric PEP arms project outward to solvent. The fourth PEP arm is not present; its terminal hydroxyl group forms a hydrogen bond to the displaced $T6^U$ thymine base. The position of the thymine is thus affected by the presence of the PEP in addition to its coordination by the Na^+ ion. However, even in the absence of these stabilizing contacts, the $T6^U$ thymidine would be displaced into the minor groove as a result of the kink in the DNA. The space occupied by the PEP molecule would be occupied by the excised base both before and immediately after cleavage.

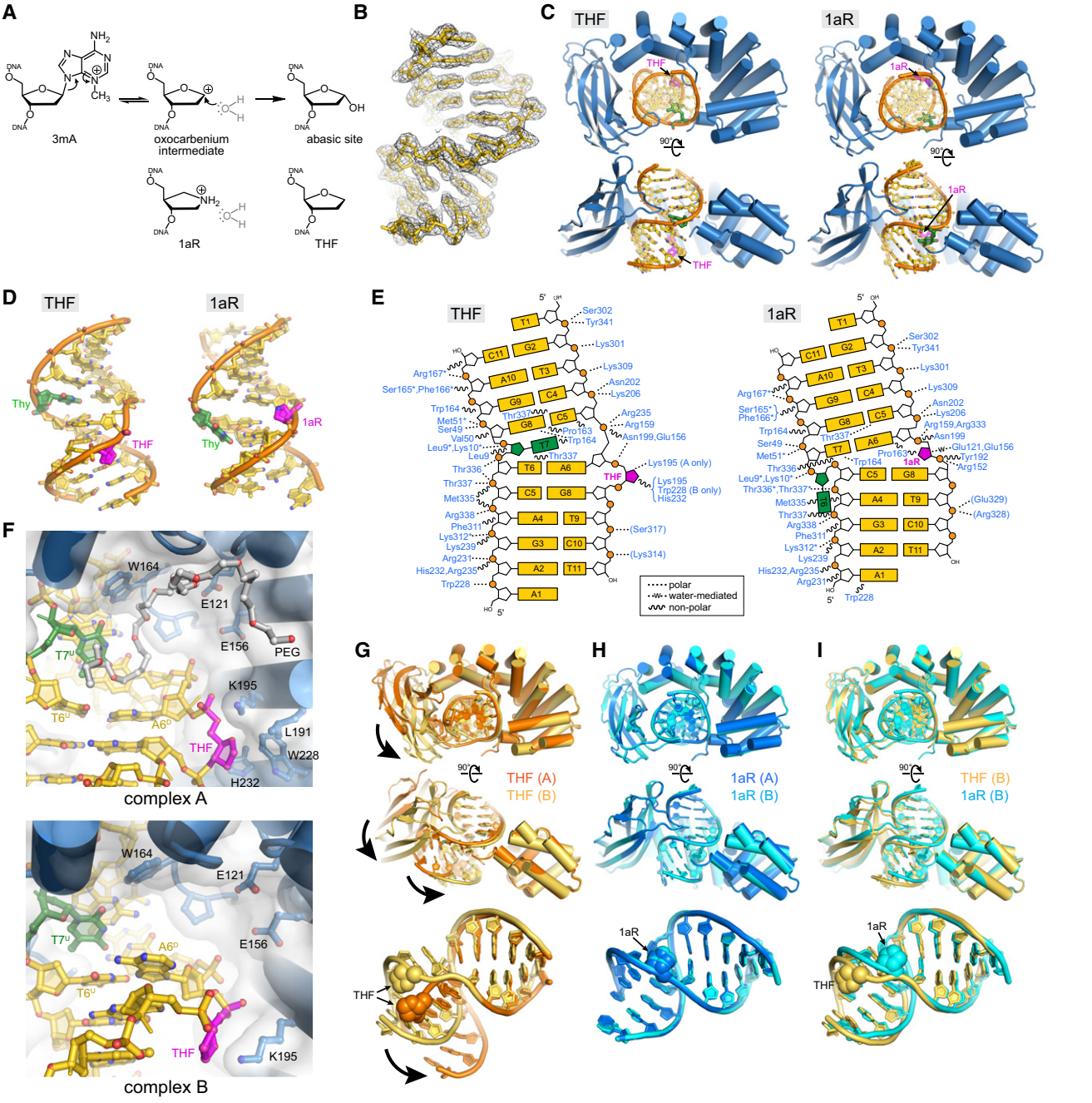

Figure EV5. Structure of the PfAlkC/THF-DNA complex and its comparison to PfAlkC/1aR-DNA.

A DNA glycosylase-catalyzed reaction together with chemical structures of intermediate and product mimetics used in this study.

B Annealed composite omit electron density contoured to 1σ is superposed onto the THF-DNA crystallographic model.

C Two views of the crystal structures, with protein in blue, DNA in gold, THF/1aR in magenta, and the thymidine opposite THF/1aR in green.

D DNA structures extracted from the complexes.

E Schematic of protein–DNA contacts. Contacts to the protein backbone are marked with an asterisk, and symmetry-related contacts are in parentheses. PfAlkC maintains similar contacts with the DNA in THF and 1aR complexes relative to the position of the DNA bend and not to the position of the 1aR/THF.

F Details of THF-DNA bound outside of the PfAlkC active site. Both complexes in the crystallographic asymmetric unit are shown. A PEG 4,000 molecule (white carbons) fills the void in the catalytic pocket in complex A.

G Superposition of the two protomers in the THF complex. Bold arrows highlight the difference in positions of the Ig-like domain relative to the HLR domain and in the two DNA conformations.

H Superposition of the two 1aR complexes.

I Superposition of one protomer from each of the THF (gold) and 1aR (cyan) complexes.