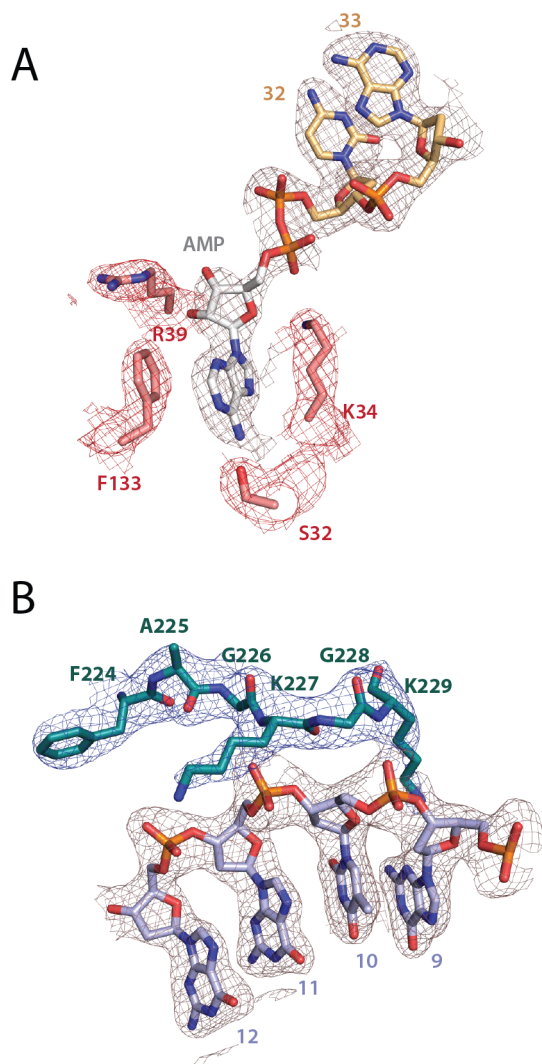


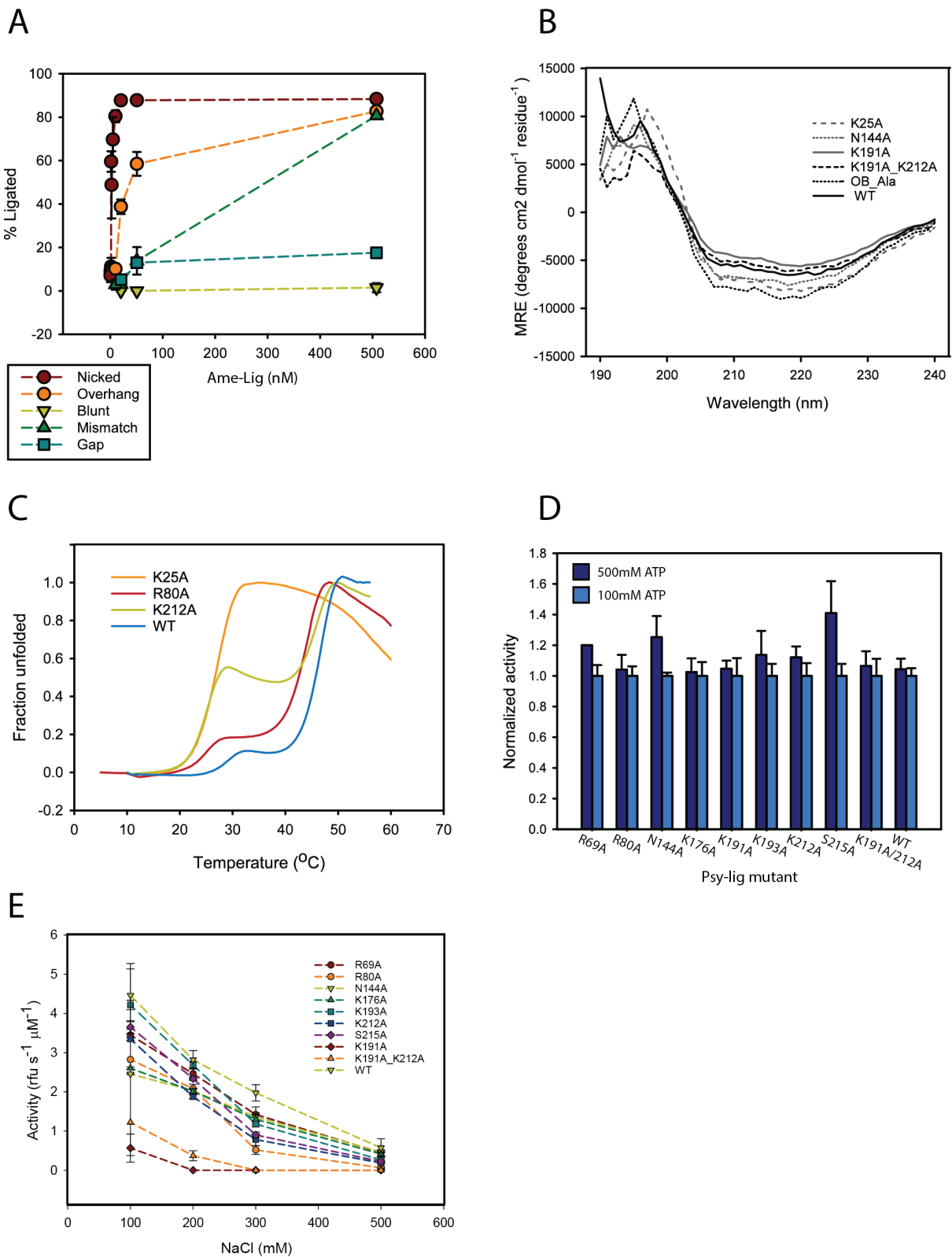
Supplementary information

Sup. Figure 1



Supplementary figure 1. Observed electron density maps for Ame-Lig bound to DNA. $2Fo - Fc$ maps are displayed at 1.0σ . (A) Adenylated nicked DNA (grey mesh, gold/ white sticks) in the active site of Ame-Lig (red mesh, salmon sticks). (B) Interaction site of the OB-domain GKGK motif (blue mesh, teal sticks) with complement-strand DNA (grey mesh, purple sticks).

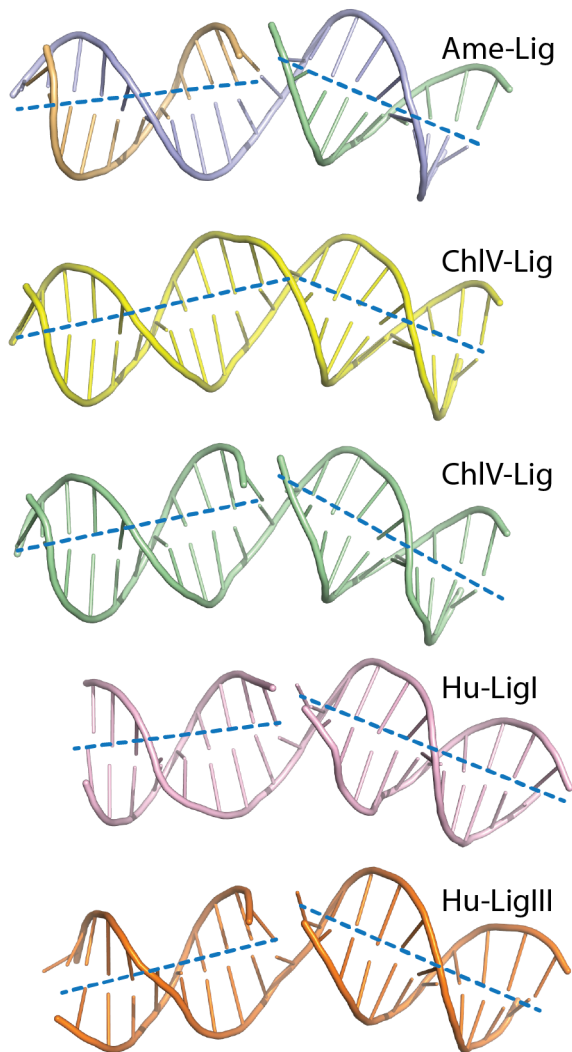
Sup. Figure 3



Supplementary figure 3. A) Ligase activity of Ame-Lig on various double-stranded substrates measured by denaturing urea-PAGE endpoint assay as described in (12). Nick and overhang substrates were tested for 15 minutes at 25 °C, other substrates were assayed at 15 °C for 2 hours (mismatch) or 6 hours (gapped and blunt). B) Representative CD spectra of Psy-Lig mutants. Data for remaining mutants is essentially identical. C) DSF unfolding data normalized to fraction folded for representative Psy-Lig mutants in the absence of added cofactor or substrate. D) Relative ligase activities of Psy-Lig mutants




measured by molecular beacon assay under standard (100 mM ATP) or high (500 mM) ATP conditions. E) Activities of *Psy-Lig* mutants with different concentrations NaCl measured by real-time molecular beacon assay.

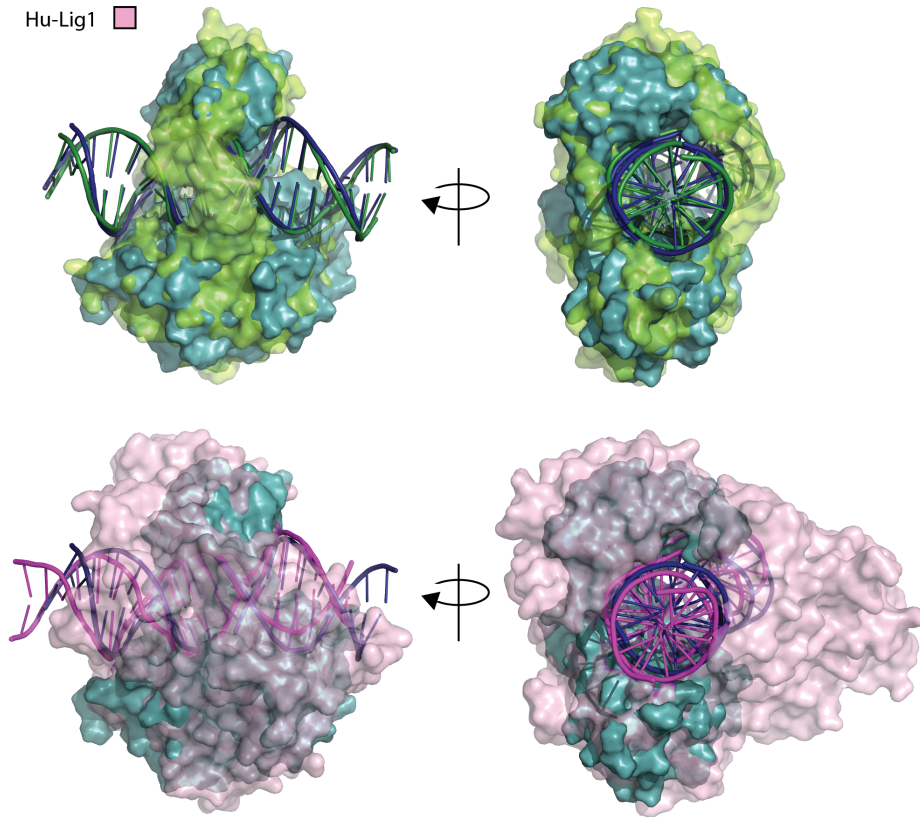
Sup. Figure 4



Supplementary figure 4. Cartoon representations of ligase-bound DNA showing the extent of bending. PDB files are as follows: *Ame-Lig*, 6gdr; *ChIV-Lig linear*, 2q2u; *ChIV-Lig nicked* 2q2t; *Hu-Lig1*, 3l2p; *Hu-lig3*, 1x9n.

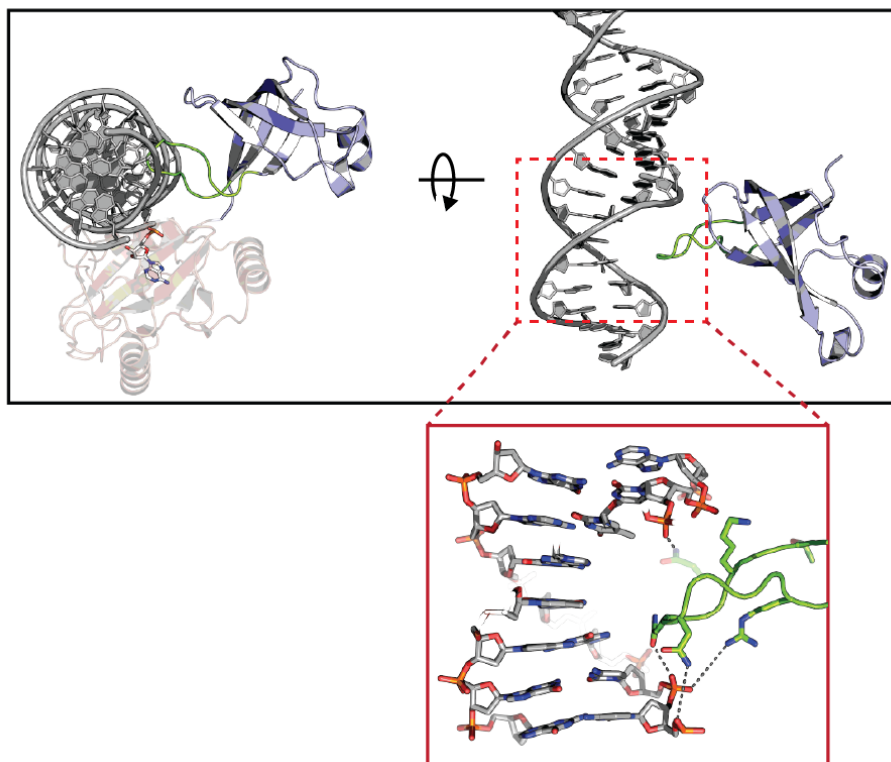
Sup. Figure 6

Ame-Lig 
ChIV-Lig 
Hu-Lig1 



Supplementary figure 5. Superimposition of Ame-Lig with the larger ChIV-Lig (upper) and Hu-Lig1 (lower) highlighting the complete encirclement of DNA by the latter structures. DNA is coloured dark blue for Ame-Lig, green for ChIV-Lig and dark pink for Hu-Lig1.

Sup. Figure 5



Supplementary figure 6. Superposition of the B conformation of the Psy-Lig enzyme-adenylate with the DNA-bound form of Ame-Lig by alignment of the ligase AD-domains. This places a loop on the back of the Psy-Lig OB domain (green) in the major groove of the DNA.

Sup. Table 1

Supplementary Table 1. Oligonucleotides used in experimental work. All were purchased from Sigma as reversed HPLC quality.

Purpose	Name	Sequence	Modification
Psy-lig R69A	R69A_FD	atggcgagttatggattgctgctcaacagttcgaaacg	
	R69A_BK	cggttcgaactgtgagcagcaatccataactcgccat	
Psy-lig K191A	K191A_BK	cacctaacagaccttcatattgacctgcaccggcaatagg	
	K191A_FD	cctattgccgggtgaggcaaatatgaaggtctgttagtg	
Psy-lig K193A	K193A_BK	cacctaacagaccttcatatgcgcttaccggcaatagg	
	K193A_FD	cctattgccgggtaaaggcgcatatgaaggtctgttagtg	
Psy-lig K212A	K212A_BK	gagaatccagagccgattgcaaggttacgccttcctc	
	K212A_FD	gaggaaggcgtaaccttcgcaatcggtctggattctc	
Psy-lig S215A	S215A_BK	ctcatcggagaatccagcgccgatttgaaggta	
	S215A_FD	taacctcaaaatcggcgctggattcctcgatgag	
Psy-lig K25A	K25A_FD	cgcacgaattccatcaagtgcctcactcacgtagtactgt	
	K25A_BK	acagtactacgtgagtgaggcactgatggaattcgtgcg	
Psy-lig R80A	R80A_corr_FD	ctgattatcctgcgtggcagcaatgccgtaacg	
	R80A_corr_BK	cgtagcggcattgctgccacgcaggataatcag	
Psy-lig N144A	N144A_FD	ttcccatgacgacctggcagtaagtgaacagtg	
	N144A_BK	gcactgttcgactactggccaaggtcgatgggcaa	
Psy-lig K176A	K176_FD	tcgcttcggcatcctcaaatgccttcagtttcatcagatcac	
	K176_BK	gtgatctgatgaactgaaggcatttgaggatccgaagcga	
Ame-Lig D-Loop	Ama_5' fragment_FD	gctcgggccccaaataat	

	Ama_5' fragment_BK	accggatccaacaatgcttgcgg	
	Ama_3' fragment_FD	gcattgttgatccggtgcaac	
	Ama_3' fragment_BK	cgcagaagcttgcgcaaacg	
Nicked duplex for crystallization	Oligo1_Xtal_21_5P	cac tat cgg aa	5' Phosphate
	Oligo_2Xtal_21_3OH	att gcg acc	
	Oligo_3Xtal_21_compl	ttc cga tag tgg ggt cgc aat	
MST / EMSA assay	MST_5-prime_nick	tccgaattcgagctccgctcg	5' Phosphate
	MST_3-prime_nick	aggccatggctgatatcgga	5' FAM (6-Carboxyfluorescein)
	EMSA_linear	aggccatggctgatatcgatccgaattcgagctccgctcg	
	MST_EMSA_Complement	cgacggagctcgaattcgatccgatatcagccatggcct	

Sup. Table 2

Supplementary Table 2. List of Gene identifiers used to make sequence logo in Figure 2D. Lig Es were identified by downloading all sequences in the NCBI non-redundant database possessing only pfam domains O1068 and 14743 and no other large N- or C-terminal extensions (greater than 40 amino acids). Only sequences from bacteria were considered, and sequences were further curated by removing those with insert regions corresponding to the Chlorella Virus latch.

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Sup. Table 3

Supplementary Table 3. Functional conservation of interactions between the linker region and catalytic domain in a selection of Lig E proteins. For details of interactions see Figure 5 of main text. Name abbreviations are: Hin-lig, *Haemophilus influenzae*; Nme-lig, *Neisseria meningitidis*; Vch-lig, *Vibrio cholera*; Vib-lig, *Aliivibrio salomicida*.

Protein	Polar interactions					Hydrophobic interactions			
	N144 - K176	K176 - E152	E178-N10			Y11	F140	F177	F247
Psy-Lig	N144	K176	E152	E178	N10	Y11	F140	F177	F247
Ama-Lig	E	Q	E	E	Q	Y	E	F	F
Hin-Lig	A	T	E	R	T	Y	Y	A	F
Nme-Lig	K	S	E	Y	E	Y	M	Q	F
Vch-Lig	D	R	E	Q	N	Y	L	H	F
Vib-Lig	E	K	E	Y	S	Y	L	H	F
Par-Lig	E	P	E	L	V	Y	T	Y	F