SUPPLEMENTARY INFORMATION

Insights into eukaryotic primer synthesis from structures of the p48 subunit of human DNA primase

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Supplementary figure captions

Fig. S1. Crystal structure of p48ΔC. (A) Ribbon representation. (B) 2Fo-Fc (blue)/Fo-Fc (red and green) electron density map contoured at 1σ and 3σ, respectively. The alpha carbon traces in the asymmetric unit and in the symmetry mate are colored yellow and brown, respectively.

Fig. S2. Amino acid sequences of *Homo sapiens* (Hsa), *Mus musculus* (Mmu), *Saccharomyces cerevisiae* (Sce), *Schizosaccharomyces pombe* (Spo), *Pyrococcus horikoshii* (Pho), *Pyrococcus furiosus* (Pfu), and *Sulfolobus solfataricus* (Sso) aligned using T-Coffee. Identical residues are shaded with blue and conserved residues yellow. Residues investigated in this study are indicated by an asterisk; red denotes a lethal phenotype in yeast. Symbols for the secondary structures derived from our crystal structure of p48 (Hsa) are shown above the alignment, and dashed lines are drawn for the two disordered regions (residues 205-207 and 277-290) not observed in the structure.

Fig. S3. Contacts between the C-terminal helix and β strand 5 in the structure of p48. The side chains of the key residues involved in the interaction are shown in stick representation.

Fig. S4. Binding of UTP to the full-length primase heterodimer (p48/58) measured by ITC. UTP was titrated into a solution of 20 μM primase and 75 μM $MnCl_2$. The top and bottom panels show the raw heat change induced by UTP binding and the integrated raw data, respectively.

Fig. S5. Measurement of NTP binding to p48. ITC isothermograms for binding to p48 of ATP (A), CTP (B), and GTP (C). NTPs were titrated into a solution of 30 μM protein and 100 μM $MnCl_2$. The top and bottom panels show the raw heat change induced by ligand binding and the integrated raw data, respectively.

Fig. S6. Contacts between the zinc-binding motif and key residues required for catalysis in the structure of p48. The side chains of D111, T113, D117, and R304 are shown in ball and stick representation, with dotted lines drawn between interacting residues.

Fig. S7. Comparison of the UTP molecules in the structures of p48●UTP●Mn^{2+} and LigD(pol)●UTP●Mn^{2+} (PDB ID 3PKY) after best-fit superposition over all Cα atoms. The UTP molecules are colored in pink and cyan, respectively. The Mn^{2+} ions are shown as spheres.

Fig. S8. Comparison of the p48ΔL structure with p48 from the structure of p48/58N. Best-fit superposition of the structure of p48ΔL (PDB ID: 4LIK) in pink to the structure of p48 extracted from the complex with p58N (PDB ID: 4BPO) in cyan.

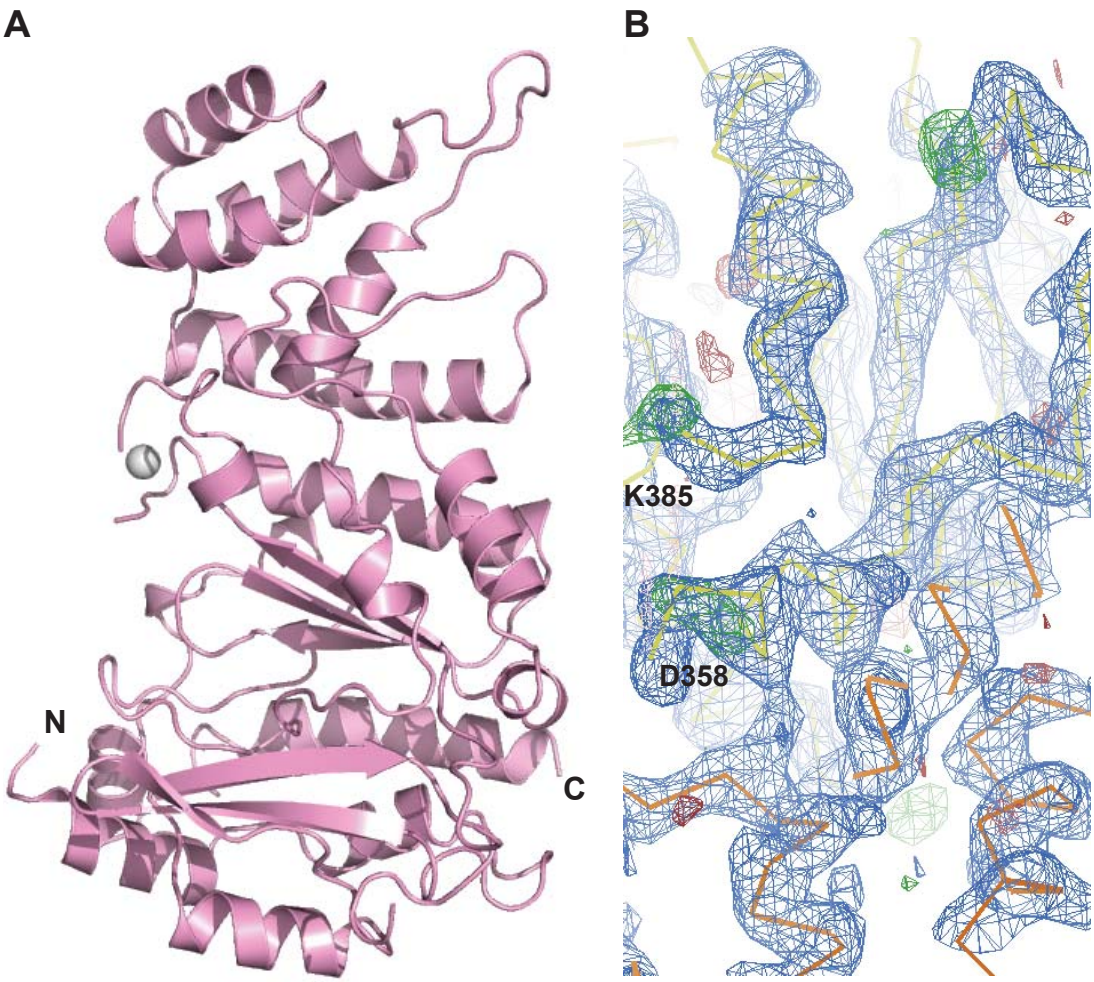


Fig. S1

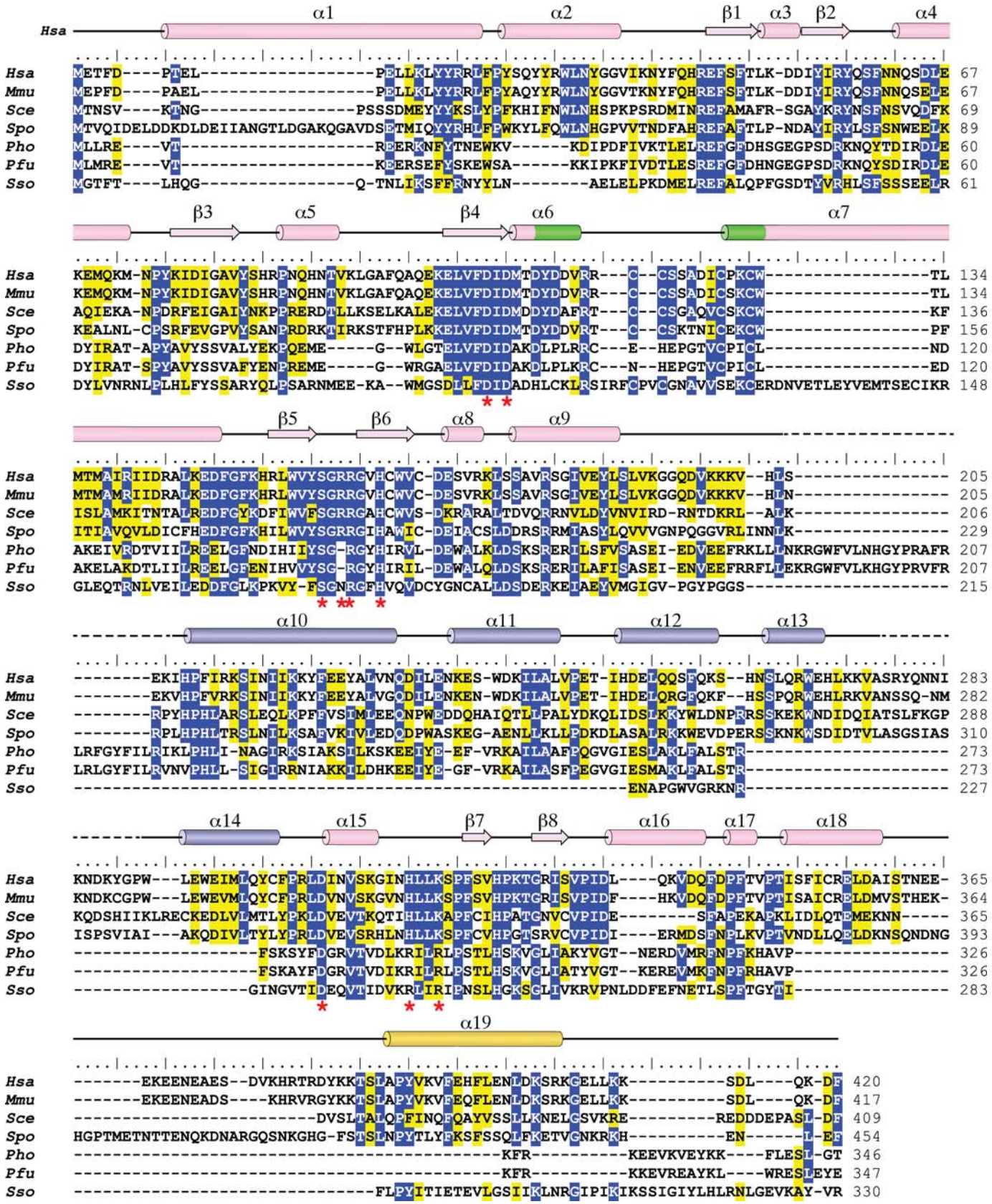

Hsa
α1 α2 β1 α3 β2 α4
Hsa METFD----PTEL----------PELLKLYYRRLFPYSQYYRWLNYGGVIKNYFQHREFSFTLK-DDIYIRYQSFNNQSDLE 67
Mmu MEPFD----PAEL----------PELLKLYYRRLFPYAQYYRWLNYGGVTKNYFQHREFSFTLK-DDIYIRYQSFNNQSELE 67
Sce MTNSV----KTNG----------PSSSDMEYYYKSLYPFKHIFNWLNHSPKPSRDMINREFAMAFR-SGAYKRYNSFNSVQDFK 69
Spo MTVQIDELDDKDLDEIIANGTLDGAKQGAVDSETMIQYYRHLFPWKYLFQWLNHGPVVTNDFAHREFAFTLP-NDAYIRYLSFSNWEELK 89
Pho MLLRE----VT----------REERKNFYTNEWKV------KDIPDFIVKTLELREFGFDHSGEGPSDRKNQYTDIRDLE 60
Pfu MLMRE----VT----------KEERSEFYSKEWSA------KKIPKFIVDTLESREFGFDHNGEGPSDRKNQYSDIRDLE 60
Sso MGTFT----LHQG----------Q-TNLIKSFFRNYYLN--------AELELPKDMELREFALQPFGSDTYVRHLSFSSSEELR 61
β3 α5 β4 α6 α7
Hsa KEMQKM-NPYKIDIGAVYSHRPNQHNTVKLGAFQAQEKELVFDIDMTDYDDVRR---C--CSSADICPKCW----------TL 134
Mmu KEMQKM-NPYKIDIGAVYSHRPNQHNTVKLGAFQAQEKELVFDIDMTDYDDVRR---C--CSSADICSKCW----------TL 134
Sce AQIEKA-NPDRFEIGAIYNKPPRERDTLLKSELKALEKELVFDIDMDDYDAFRT---C--CSGAQVCSKCW----------KF 136
Spo KEALNL-CPSRFEVGPVYSANPRDRKTIRKSTFHPLKKELVFDIDMTDYDDVRT---C--CSKTNICEKCW----------PF 156
Pho DYIRAT-APYAVYSSVALYEKPQEME-----G--WLGTELVFDIDAKDLPLRRC---E--HEPGTVCPICL----------ND 120
Pfu DYIRAT-SPYAVYSSVAFYENPREME-----G--WRGAELVFDIDAKDLPLKRC---N--HEPGTVCPICL----------ED 120
Sso DYLVNRNLPLHLFYSSARYQLPSARNMEE-KA--WMGSDLLFDIDADHLCKLRSIRFCPVCGNAVVSEKCERDNVETLEYVEMTSECIKR 148
β5 β6 α8 α9
Hsa MTMAIRIIDRALKEDFGFKHRLWVYSGRRGVHCWVC-DESVRKLSSAVRSGIVEYLSLVKGGQDVKKKV--HLS---------- 205
Mmu MTMAMRIIDRALKEDFGFKHRLWVYSGRRGVHCWVC-DESVRKLSSAVRSGIVEYLSLVKGGQDVKKKV--HLN---------- 205
Sce ISLAMKITNTALREDFGYKDFIWVFSGRRGAHCWVS-DKRARALTDVQRRNVLDYVNVIRD-RNTDKRL--ALK---------- 206
Spo ITIAVQVLDICFHEDFGFKHILWVYSGRRGIHAWIC-DEIACSLLDRSRRMIASYLQVVVGNPQGGVRLINNLK---------- 229
Pho AKEIVRDTVIILREELGFNDIHIIYSG-RGYHIRVL-DEWALKLDSKSRERILSFVSASEI-EDVEEFRKLLLNKRGWFVLNHGYPRAFR 207
Pfu AKELAKDTLIILREELGFENIHVVYSG-RGYHIRIL-DEWALQLDSKSRERILAFISASEI-ENVEEFRRFLLEKRGWFVLKHGYPRVFR 207
Sso GLEQTRNLVEILEDDFGLKPKVY-FSGNRGFHVQVDCYGNCALLDSDERKEIAEYVMGIGV-PGYPGGS---------- 215
α10 α11 α12 α13
Hsa --------EKIHPFIRKSINIIKKYEEEYALVNQDILENKES-WDKILALVPET-IHDELQQSFQKS--HNSLQRWEHLKKVASRYQNNI 283
Mmu --------EKVHPFVRKSINIIKKYEEEYALVGQDILENKEN-WDKILALVPET-IHDELQRGFQKF--HSSPQRWEHLRKVANSSQ-NM 282
Sce --------RPYHPHLARSLEQLKPFDVSIMLEENPWEDDQHAIQTLLPALYDKQLIDSLKKYWLDNPRRSSKEKWNDIDQIATSLFKGP 288
Spo --------RPLHPHLTRSLNILKSADVKIVLEDQDPWASKEG-AENLLKLLPDKDLASALRKKWEVDPERSSKNKWSDIDTVLASGSIAS 310
Pho LRFGYFILRIKLPHLI-NAGIRKSIAKSILKSKEEIYE-EF-VRKAILAAFPQGVGIESLAKLFALSTR---------- 273
Pfu LRLGYFILRVNVPHLL-SIGIRRNIAKKILDHKEEIYE-GF-VRKAILASFPEGVGIESMAKLFALSTR---------- 273
Sso ----------ENAPGWVGRKNR---------- 227
α14 α15 β7 β8 α16 α17 α18
Hsa KNDKYGPW---LEWEIMLQYCFPRLDINVSKGINHLLKSPFSVHPKTGRISVPIDL----QKVDQEDPFTVPTISFICRELDAISTNEE- 365
Mmu KNDKCGPW---LEWEVMLQYCFPRLDVNVSKGVNHLLKSPFSVHPKTGRISVPIDF----HKVDQEDPFTVPTISAICRELDMVSTHEK- 364
Sce KQDSHIIKLRECKEDLVLMTLYPKLDVEVTKQTIHLLKAPFCIHPATGNVCVPIDE--------SFAPEKAPKLIDLQTEMEKNN----- 365
Spo ISPSVIAI---AKQDIVLTYLYPRLDVEVSRHLNHLLKSPFCVHPGTSRVCVPIDI----ERMDSFNPLKVPTVNDLLQELDKNSQNDNG 393
Pho --------FSKSYFDGRVTVDLKRILRLPSTLHSKVGLIAKYVGT--NERDVMRFNPFKHAVP---------- 326
Pfu --------FSKAYFDGRVTVDIKRILRLPSTLHSKVGLIATYVGT--KEREVMKFNPFRHAVP---------- 326
Sso --------GINGVTIDEQVTIDVKRLIRIPNSLHGKSGLIVKRVPNLDDFEFNETLSPFTGYTI---------- 283
α19
Hsa -------EKEENEAES--DVKHRTRDYKKTSLAPYVKVFEHFLENLDKSRKGELLKK----------SDL----QK-DF 420
Mmu -------EKEENEADS----KHRVRGYKKTSLAPYVKVFEQFLENLDKSRKGELLKK----------SDL----QK-DF 417
Sce ----------------------DVSLTALQPFINQFQAYVSSLLKNELGSVKRE---------REDDDEPASL-DF 409
Spo HGPTMETNTTENQKDNARGQSNKGHG-FSTSLNPYTLYFKSFSSQLFKETVGNKRKH----------EN------L-EF 454
Pho ----------KFR----------KEEVKVEYKK----FLESL-GT 346
Pfu ----------KFR----------KKEVREAYKL----WRESLEYE 347
Sso ----------FLPYITIETEVLGSIIKLNRGIPIKIKSSIGIYLHLRNLGEVKAY-VR 330

Fig. S2

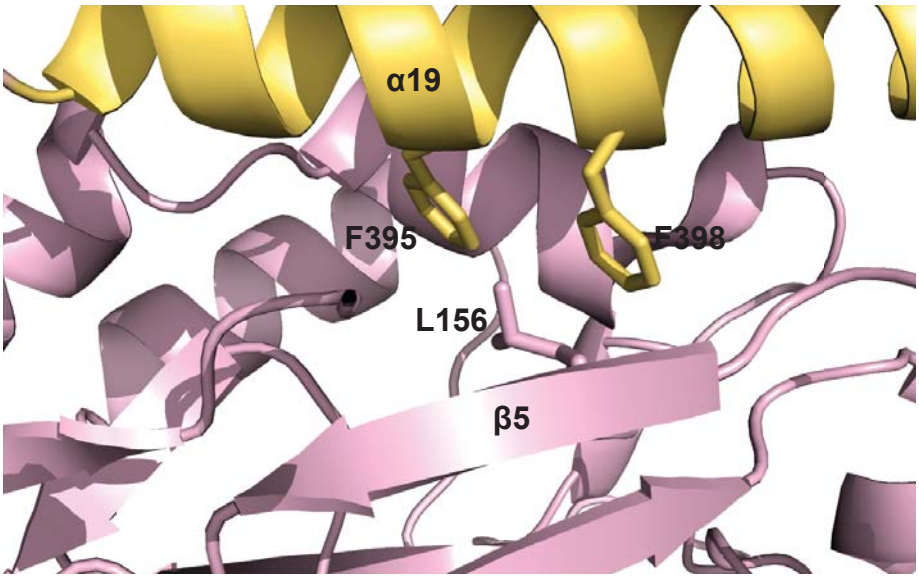
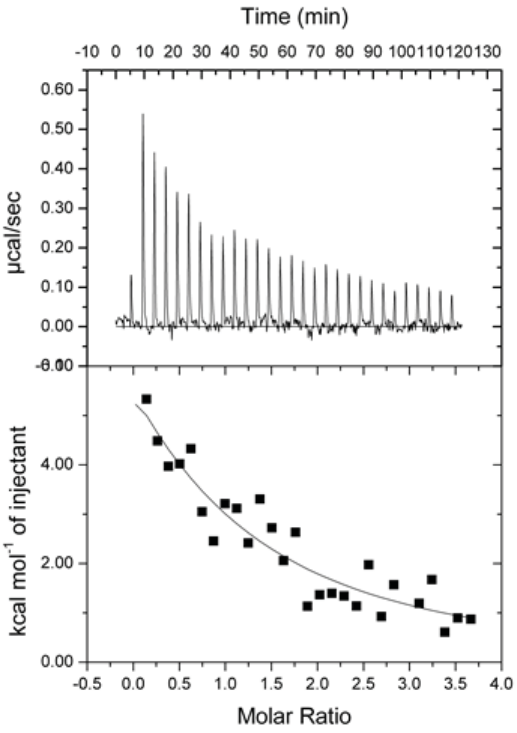


Fig. S3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0.60
0.50
0.40
0.30
0.20
0.10
0.00
kcal mol-1 of injectant
4.00
2.00
0.00
-0.5 0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0
Molar Ratio

Fig. S4

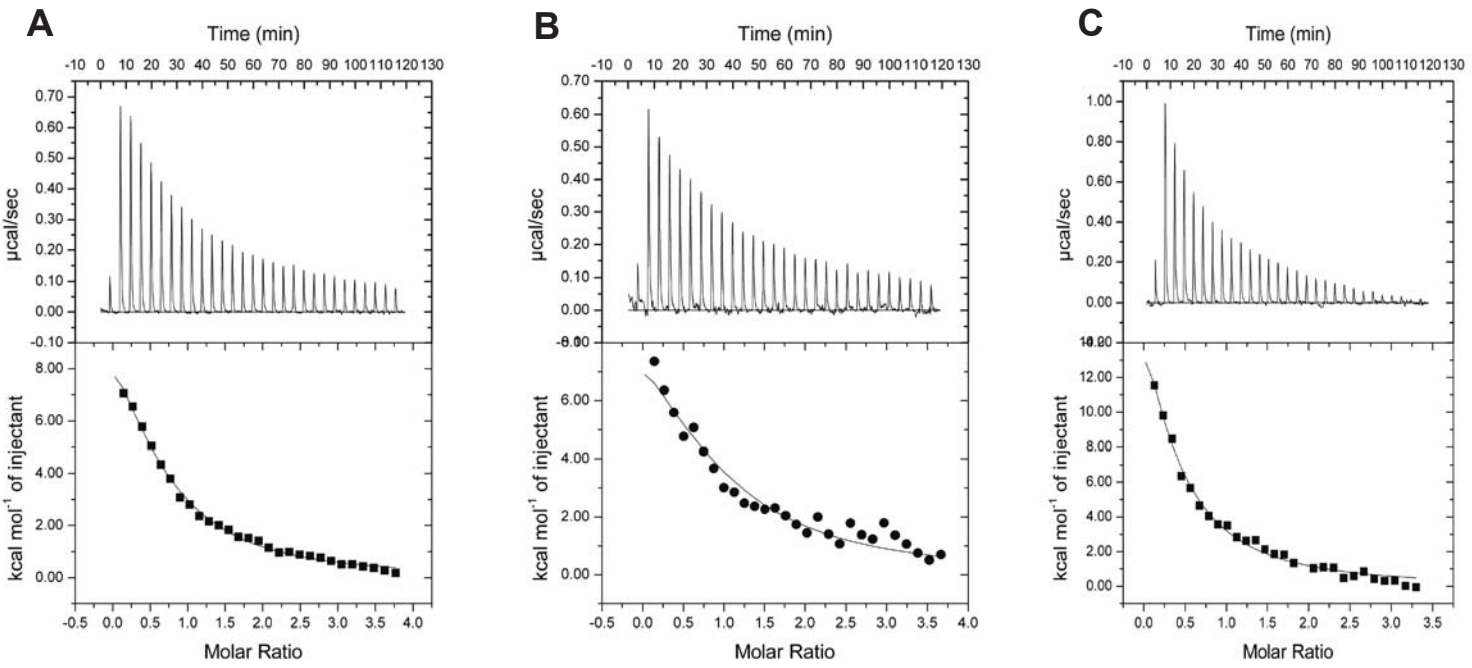

Fig. S5

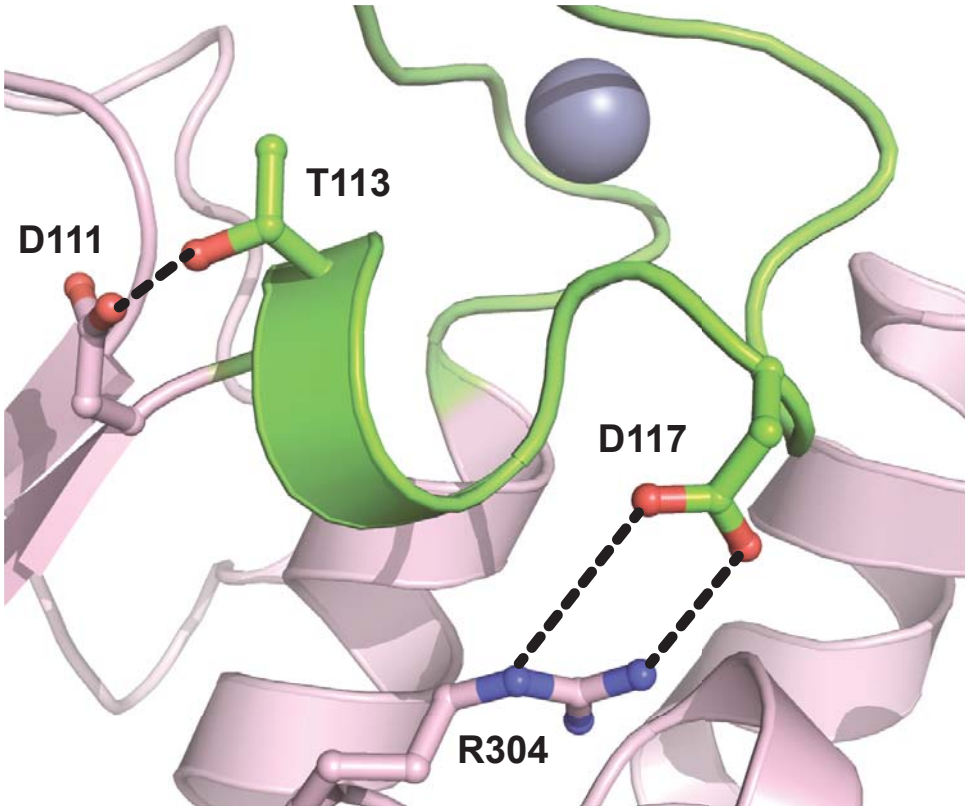

Fig. S6

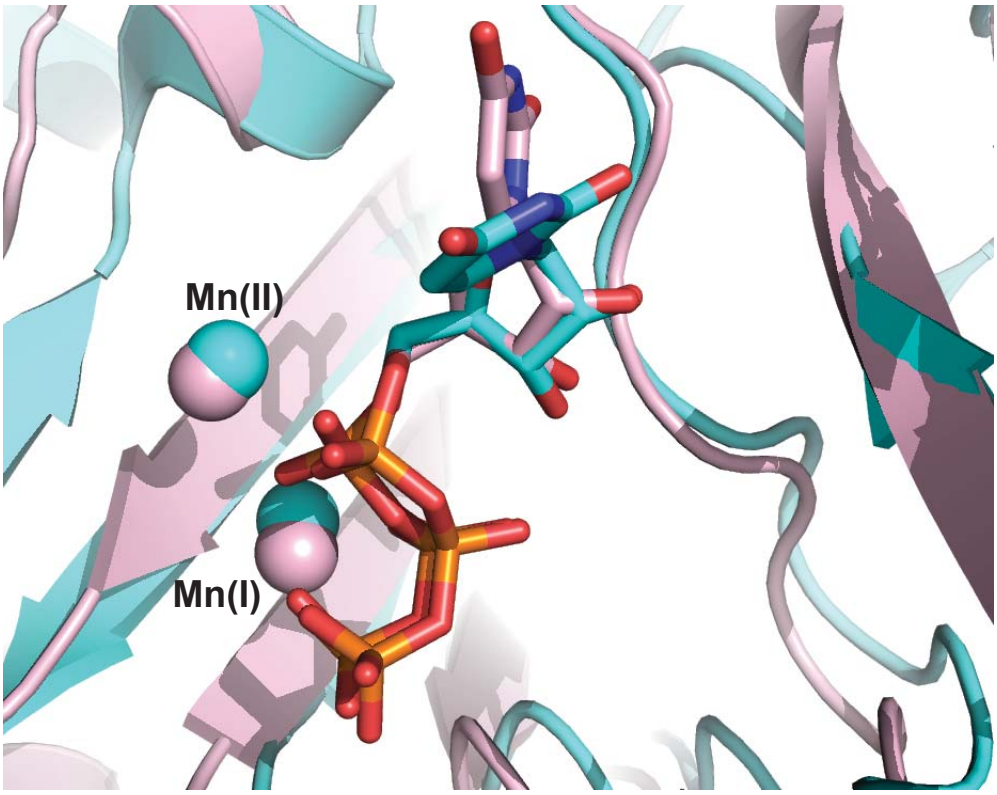

Fig. S7

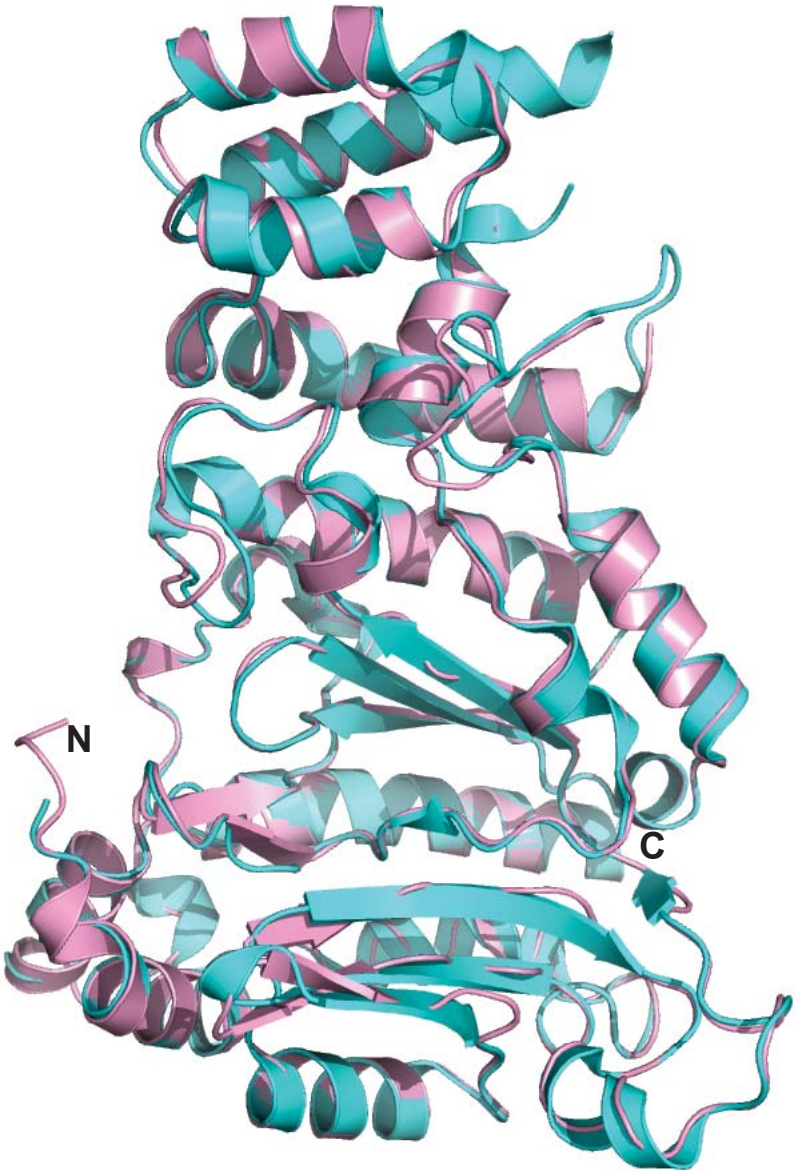

Fig. S8