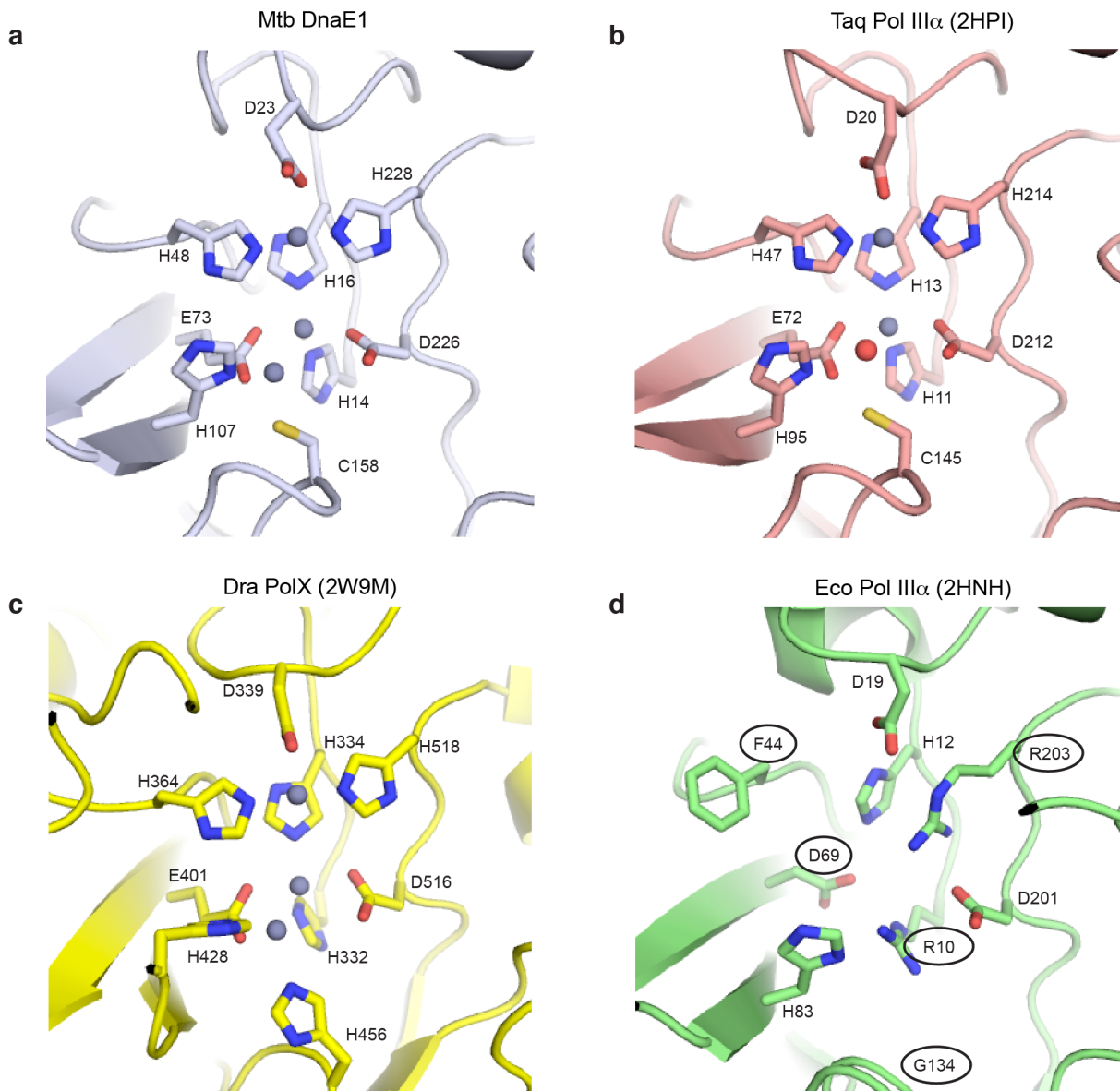


Supplementary Figure 1 | Stereo view of electron density and modeling of a DNA substrate into the crystal structure of Mtb DnaE1.

(a) Stereo view of a 2mFo-DFc electron density map contoured at 2.1σ (b) Modeling of DNA binding. The *Geobacillus kaustophilis* PolC-DNA complex¹ and *E. coli* PolIII α -DNA complex² were superimposed onto the structure of Mtb DnaE1 using the palm and fingers domain (residues 307-933) as a guide. Next, a B-form dsDNA model was manually aligned with the DNA from PolC and PolIII α .



Supplementary Figure 2 | Conserved features of the PHP-exonuclease active site. (a) PHP active site of Mtb DnaE1. The metal binding residues shown in stick, and Zn ions in grey spheres (b) PHP active site of Taq PolIII α ³. Note that one of the metal ions is modeled as a water molecule (shown in red sphere). (c) PHP active site from *Deinococcus radiodurans* PolX⁴ (d) PHP active site from *E. coli* PolIII α ⁵. The five residues that deviate from the consensus sequences are indicated with circles. Note that no metals are bound in the active site.

a

Mycobacterium tuberculosis H37Rv/73-142
Mycobacterium smegmatis str. MC2_155/75-144
Mycobacterium leprae TN/69-103
Rhodococcus pyridinivorans SB3094/68-102
Amycolicococcus subflavus DQ83-9a1/82-116
Gordonia bronchialis DSM 43247/68-102
Corynebacterium diptheriae HC02/70-104
Corynebacterium maris DSM 45190/70-104
Corynebacterium resistens DSM 45100/69-103
Corynebacterium kroppenstedtii DSM 44385/76-110
Corynebacterium terpenotabidum Y-11/69-103
Segniliparus rotundus DSM 44985/68-102
Frankia alni ACN14a/68-102
Thermomonospora curvata DSM 43183/68-102
Streptosporangium roseum DSM 43021/68-102
Catenulispora acidiphila DSM 44928/68-102
Kitasatospora setae KM-6054/769-103
Actinosynnema mirum DSM 43827/68-102
Saccharopolyspora erythraea NRRL 2338/69-107
Saccharomonospora viridis DSM 43017/69-107
Frankia sp. Cc13/69-146
Nocardioopsis dassonvillei subsp. *dassonvillei* DS
Streptomyces coelicolor A3
Kitasatospora setae KM-6054/68-107
Propionibacterium avidum 44067/73-110
Acidothermus cellulolyticus 11B/88-120
Pseudonocardia dioxanivorans CB1190/72-104
Arthrobacter sp. FB24/71-106
Micrococcus luteus NCTC 2665/72-107
Arthrobacter arilaitensis Re117/53-88
Renibacterium salmoninarum ATCC 33209/71-104
Rothia mucilaginosa DY-18/69-102
Kineococcus radiotolerans SRS30216 = ATCC BAA-14
Kribbella flavida DSM 17836/70-104
Microbacterium testaceum StLB037/53-88
Propionibacterium propionicum F0230a/99-132
Tropheryma whipplei str. Twist/68-101
Beutenbergia cavernae DSM 12333/73-108
Jonesia denitrificans DSM 20603/71-106
Isosporicola variabilis 225/82-117
Xylanimonas cellulolytica DSM 15894/70-106
Brachyacterium faecium DSM 4810/72-108
Mobiluncus curtisii ATCC 43063/69-106
Nocardioides sp. JS614/72-109
Bifidobacterium adolescentis ATCC 15703/69-105

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EAYTAPGS RFDTRRLWGD PSOKA . . . . DDVSGS GSYT
EAYTAPGS RFDTKRVTWGD PGQKG . . . . DDVSGS GAYT
EAYTAPGS RFDTRRIWGD PSOKA . . . . DDVSGS GAYT
EAYVAPES RFSTKRVTWGD PGQKS . . . . DDVSGS GAYT
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EAYVAPES RFETKRVTWGT RDQKD . . . . DDVSGS GAYT
EAYMAPGS RLHKRVRWGE PHQKS . . . . DDVSGS GAYL
EAYMAPES RFNKRVRWGT PDQKR . . . . DDVSGS GAYL
EAYTAPES RFNQNRVRWGE PEQKS . . . . DDVSGS GAYL
EAYMAPES RFNKRVRWGE AHQKR . . . . DDVSGS GAYL
EAYMAPES RLFKDRIRWGG PEQKR . . . . DDVAGGYYL
EAYLAPES RFSTKREFWGE PGQRS . . . . DDVSGS GAYT
EAYVAPES RLLKQVRWGE PHQKS . . . . DDVSGS GAYT
EAYMAPES RFHKKQVWGE PSQKR . . . . DDVSGGGLIN
EAYVAPES RHQKQVWGE PHQKR . . . . DDVSGGYYT
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EAYVAPES RFHKKQVWGN TGQRSFGE . G DDVSGG GAYT
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EAYVAPES RFHKKRVWGR . SSGSDSABEGGK . DDVSGG GAYL
EAYVAPES RRNRQVWGP . GGQRADGEGSK . DDVSGG GAYT
EAYVAPES RFLKQVFWAP . GGQRDPDGEKK . DDVSGG GAYT
EAYLAPGS RMRVQVFW . . . . GISRDDAEGGK . DDVSGG GAYT
EAYLAPGS RFDRRVSLAT . AGP . . . . . GDDVSGG GAYT
EAYLAPGS RHEKRRLTGG . QTV . . . . . SDDVSGG GAYT
EAYVAPES GTARQDRSRVWGE . ESQRK . . . . DDVSGG GAYT
EAYVAPES GQRTDKEKRWGE . PHQRK . . . . DDVSGG GAYT
EAYVAPES GTARDDKTRVWGD . ESQKG . . . . DDVSGG GLYN
EAYLAPES GTAHNDRTRVWGD . GSGK . . . . . DDVAGG GAYT
EAYLAPES GTHRTDRSRVWGD . GSR . . . . . DDVSGG GAYT
EAYLAPES GTARQDKTRVWGD . GSRG . . . . DDVSGA GAYT
EAYVAPES GTHRSERKRVWGD . NSGR . . . . DDVSGG GAYT
EAYVAPES GTHRSKTRVWGS . PEQQS . . . . DDVSGS GAYT
EAYLAPES GTHRTKRVWGD . GTG . . . . . DDVSGK GAYT
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EAYLAPES GTSRFQTRVWGE . QSORE . . . . DDVSGR GAYT
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EAYVAPES ETARQDPTRVWDTHRR . NNP . . . . DDVSGG GAYT

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b

Myxococcus xanthus DK 1622/66-85
Syntrophomonas wolfei subsp. *wolfei* str. Goettin
Syntrophothermus lipocalidus DSM 12680/67-88
Desulfotomaculum ruminis DSM 2154/70-91
Syntrophobolus glycolicus DSM 8271/65-86
Ruminiclostridium thermocellum ATCC 27405/68-89
Clostridium stercorarium subsp. *stercorarium* DSM
Alkaliphilus metalliredigens QYMF/66-87
Thermaerobacter marianensis DSM 12885/67-88
Clostridium saccharolyticum WMI/66-88
Halobacteroides halobius DSM 5150/67-86
Faecalibacterium prausnitzii L2-6/77-97
Oscillibacter valeriacigenes Sjm18-20/66-88
Clostridium acidurici 9a/68-89
Anaerococcus prevotii DSM 20548/68-88
Acidaminococcus fermentans DSM 20731/67-88
Eubacterium limosum KIST612/42-63
Syntrophobacter fumaroxidans MPOB/67-95
Geobacter uraniireducens Rf4/69-93
Geobacter hemidjiensis Bem/69-93
Geobacter sp. M18/69-93
Desulfomonile tiedjei DSM 6799/69-91
Desulfurispirillum indicum S5/66-88
Desulfurivibrio alkaliphilus AHT2/78-100
Desulfotalea psychrophila LSV54/91-112
Desulfobulbus propionicus DSM 2032/68-88
Desulfarculus baarsii DSM 2075/68-90
Candidatus Nitrospira defluvi/68-90
Thermodesulfobacterium yellowstonii DSM 11347/69-94
Syntrophus aciditrophicus SB/69-91
Deferribacter desulfuricans SSM1/68-113
Denitrovibrio acetiphilus DSM 12809/70-92
Paenibacillus sp. JDR-2/71-92
Treponema pallidum subsp. *pallidum* str. *Nichols*/
Spirochaeta smaragdinae DSM 11293/66-88
Sphaerochaeta globosa str. *Buddy*/78-101
Sphaerochaeta coccoides DSM 17374/67-89
Treponema succinifaciens DSM 2489/82-105
Lawsonia intracellularis PHE/MN1-00/67-87
Desulfobalobium retbaense DSM 5692/67-89
Desulfobacterium africanus str. *Walvis Bay*/67-87
Desulfococcus oleovorans Hxd3/73-94
Borrelia turicatae 91E135/70-92
Thermus aquaticus/70-93

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EAYVAGPKGREDR . . . . . SEKV . . . . . SH
EAYVAP.RSRLDK . . . . . EARI . . . . . DN . . . . LY
EAYVAP.RHRFDR . . . . . TPKV . . . . . DA . . . . LY
EAYVAR.RTMADK . . . . . VPKM . . . . . DQ . . . . PY
EAYVAP.GKLTEK . . . . . NAGR . . . . . DK . . . . NY
EAYTAK.GSRFDK . . . . . QGGW . . . . . SD . . . . PG
EAYTAA.RTRFRD . . . . . EAGI . . . . . SE . . . . QG
EAYTAA.RGMTDK . . . . . DPKV . . . . . DK . . . . QG
EAYLAR.RTRHRD . . . . . EPKV . . . . . DQ . . . . SY
EAYVTS.GSRFDR . . . . . ETAG . . . . . GE . . . . YY
EAYVTE.D.YQ . . . . . KQPR . . . . . N . . . . LN
EAYVAT.RTRFDK . . . . . VNK . . . . . I . . . . NN
EAYVAPGRTRFQK . . . . . QHEF . . . . . AE . . . . SR
EAYVTK.GNYMEK . . . . . DKSK . . . . . E . . . . QY
EAYVSE.N.DH . . . . . TIKE . . . . . AS . . . . RY
EAYITS.GSHLDK . . . . . SLE . . . . . TR . . . . LY
EAYVSP.RRLDQK . . . . . EGN . . . . . TN . . . . PY
EAYLAP.RSRHRH . . . . . GRAEGSG . . . . TG . . . . EED . RNY
EAYIAP.GSRFSK . . . . . ESGN . . . . . GH . . . . EST . SSY
EAYIAP.QDRFLK . . . . . QAPS . . . . . SP . . . . GQA . SSY
EAYIAP.HSRTLK . . . . . QAPT . . . . . AA . . . . DPV . TSY
EAYIAP.ESRFDH . . . . . NAKA . . . . . GQ . . . . VA . YY
EAYVSP.GSREK . . . . . NAEG . . . . . VY . . . . EG . . . . AY
EAYVAP.ADRRDK . . . . . SAKS . . . . . AG . . . . AA . . . . AH
EAYIAP.GDMREQ . . . . . K . . . . . KI . . . . . NG . . . . QT . . . . AY
EAYLAE.NGMEIH . . . . . D . . . . . RS . . . . . AG . . . . HN . . . . F
EAYVAP.GDRRDK . . . . . EHRP . . . . . GH . . . . ET . . . . AF
EAYMAP.GSRLEK . . . . . NSHL . . . . . AH . . . . ND . . . . YY
EAYVAP.QSRFDK . . . . . INKDPGM . . . . PE . . . . EA . . . . SF
EAYVAP.KTRFEK . . . . . TGGG . . . . . IG . . . . DT . . . . AR
EAYVAP.DSRFNK . . . . . NYSK . . . . . KE . . . . DK . . . . SY
EAYVAP.DSRFNT . . . . . TYER . . . . . GO . . . . ER . . . . NH
EAYFTA.GSRFEK . . . . . GTRK . . . . . D . . . . NP . . . . IY
EAYVAP.ESRFDK . . . . . SEHT . . . . . IG . . . . RR . . . . YY
EAYVAP.GSRHVK . . . . . NGSE . . . . . KG . . . . NR . . . . YQ
EAYCNP.AGHTER . . . . . PAPG . . . . . GRKN . . . . QY
EAYVAP.ESHTEK . . . . . EGVE . . . . . G . . . . KPR . . . . AY
EAYVAV.GSHTER . . . . . NDVP . . . . . FGRS . . . . YF
EAYVTK.DHADK . . . . . LSK . . . . . FS . . . . KA . . . . RH
EAYVAP.GDRRTK . . . . . HTDK . . . . . DI . . . . RS . . . . GY
EAYVAG.EGGVE . . . . . KGP . . . . . G . . . . AK . . . . RH
EAYVAP.RSITDR . . . . . TPMDH . . . . . DG . . . . LS . . . . .
EAYMSS.TSKHIK . . . . . KNDE . . . . . LG . . . . RP . . . . YF
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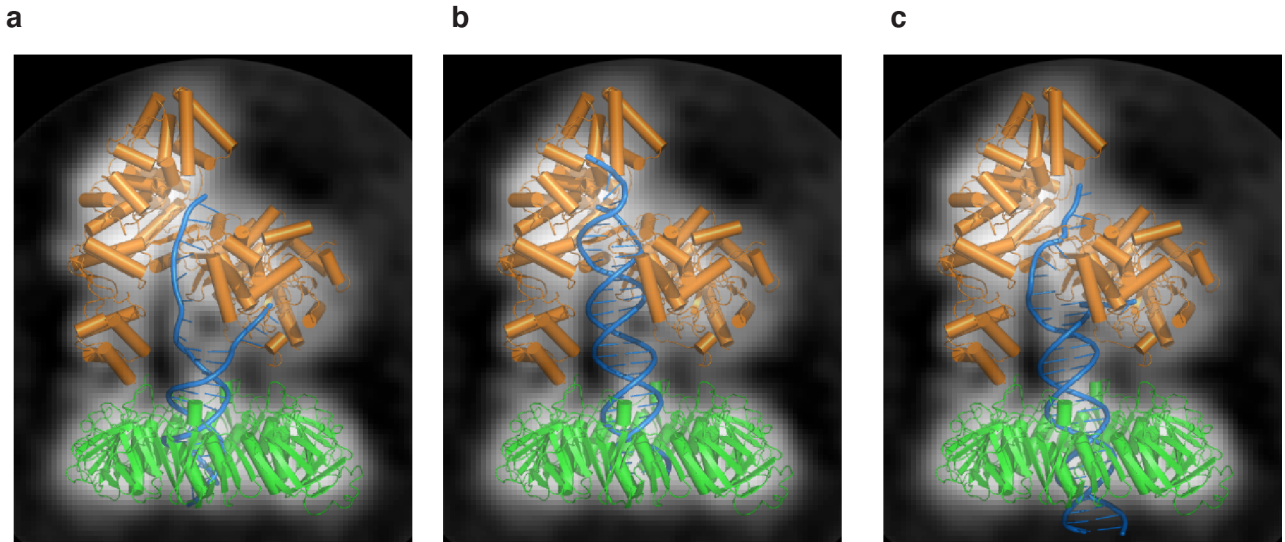
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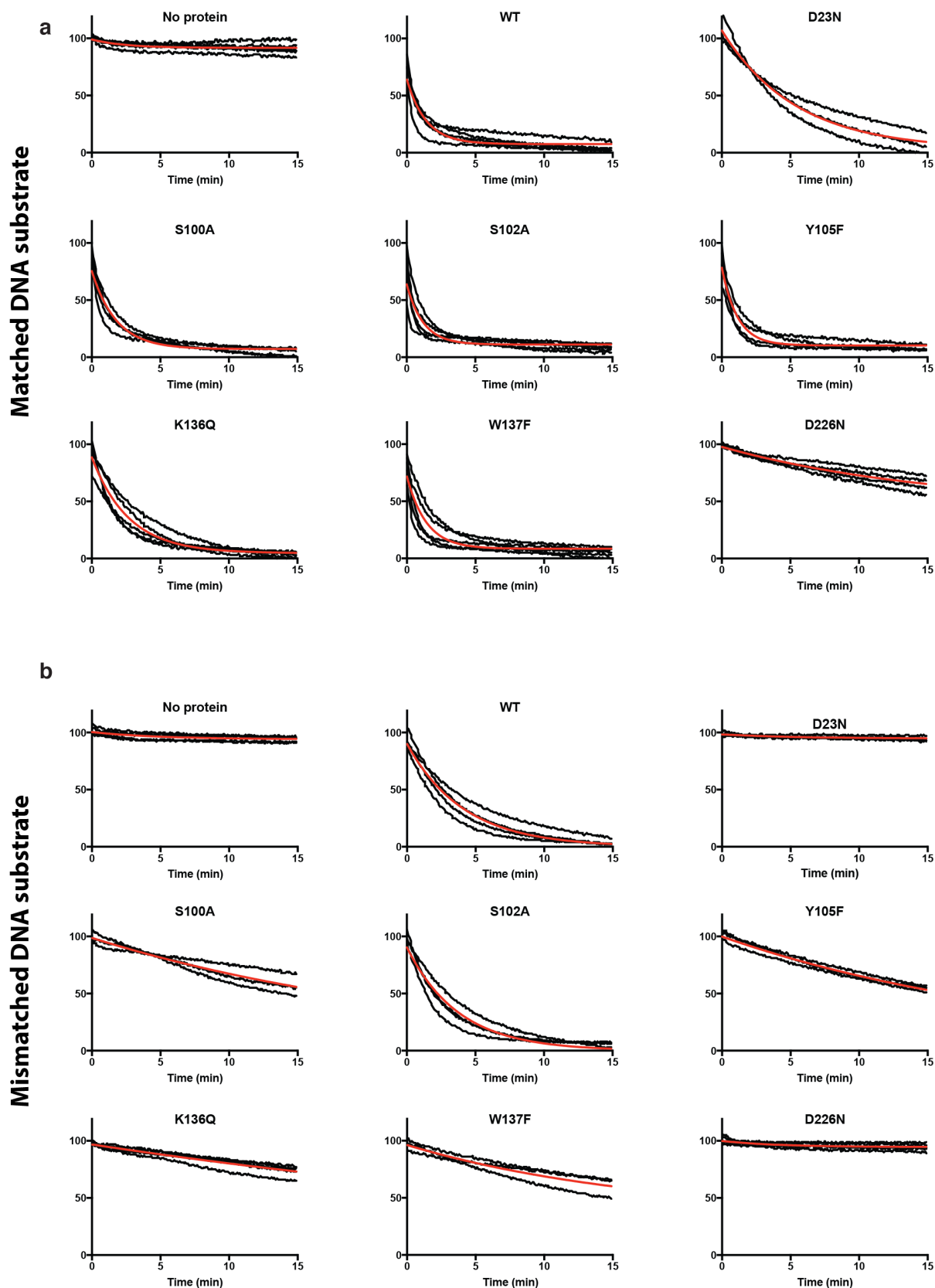
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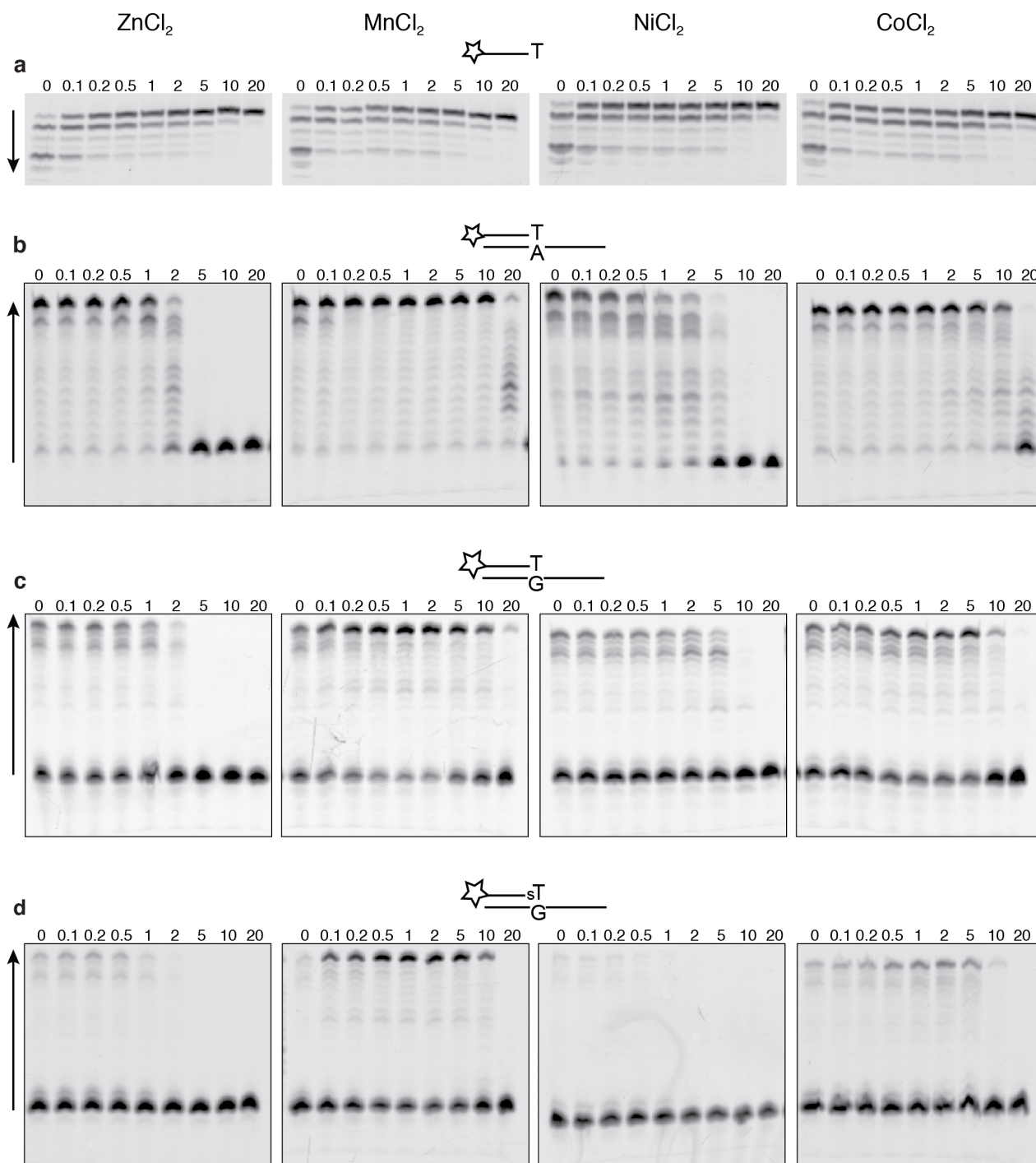
Supplementary Figure 3 | Sequence conservation of the long loop in the PHP-exonuclease. Alignment of the long loop in the PHP-exonuclease, equivalent to glutamate 73 to histidine 107 from Mtb DnaE1 (see Sup. Fig. 2). Blue arrows on top indicate the β -strands flanking the loop. (a) 45 sequences from Actinobacteria (b) 45 sequences from δ -proteobacteria and Firmicutes (c) The equivalent sequence from *E. coli* PolIII α , which is an inactive PHP-exonuclease.



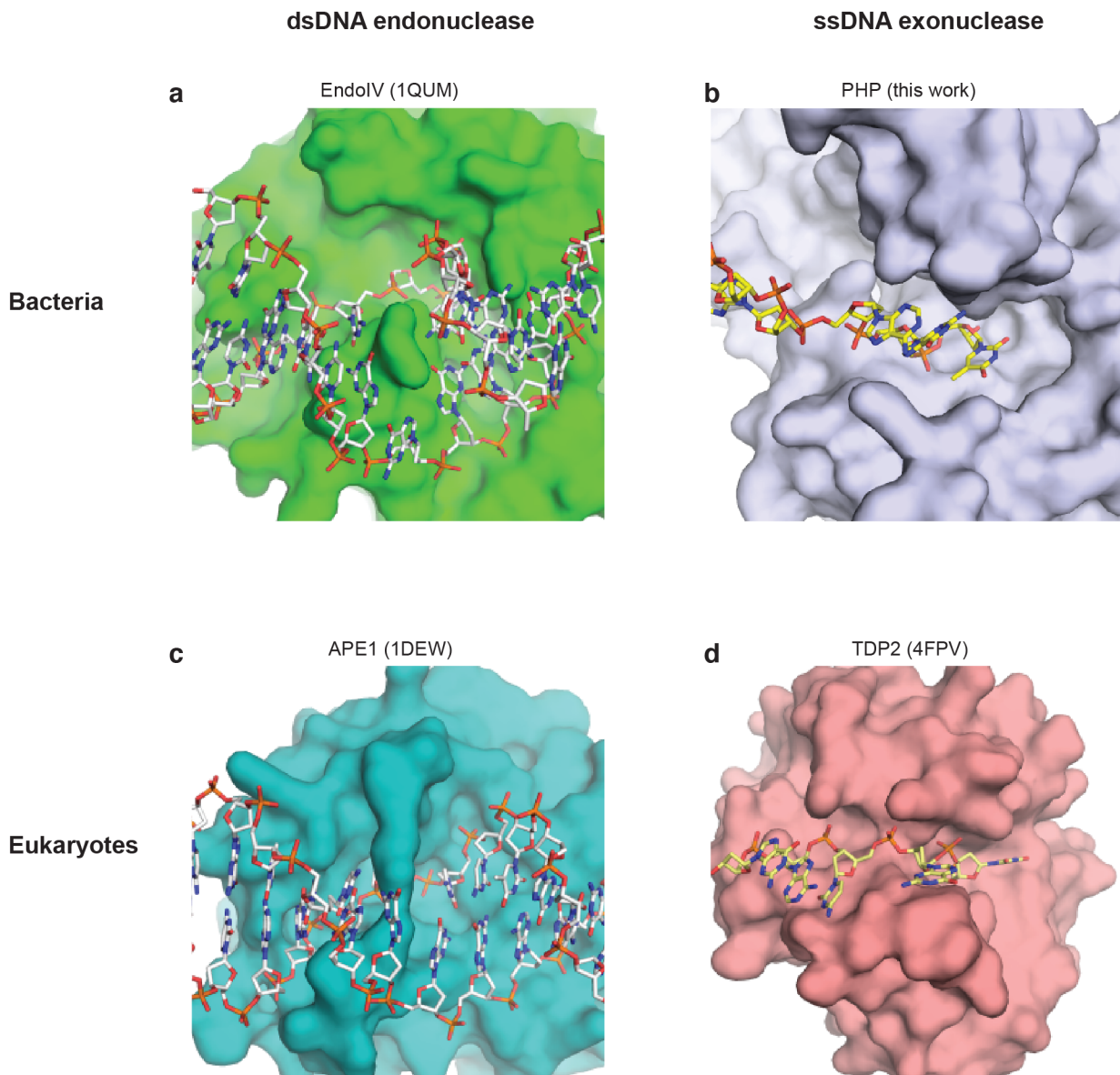
Supplementary Figure 4 | Modeling of the DNA into the 2D cryo-EM class. Different DNA models were fitted into the 2D cryo-EM class. **(a)** Model with the primer strand entering the PHP-exonuclease between short β -hairpin and long loop in the PHP domain (see Fig. 3). **(b)** DNA modeled in the polymerase mode (see Supplementary Fig. 1) **(c)** Model with the primer strand entering the PHP-exonuclease via the narrow groove between polymerase active site and exonuclease active site (see Fig. 2d).



Supplementary Figure 5 | Real time polymerase assays on matched and mismatched DNA. Real-time primer extension assay in which the intensity of a 5' placed fluorophore is quenched by the incorporation of nucleotides in the bottom strand. **(a)** Primer extensions on a matched DNA substrate **(b)** Primer extension on mismatched DNA substrate. A single exponential decay was fitted to three to five independently measured experiments. Experimental curves in black, fitted curve in red.



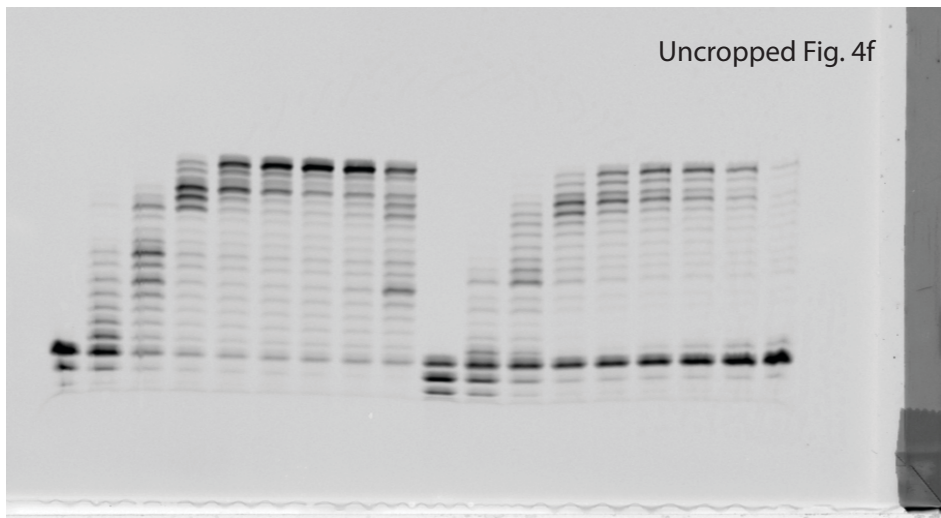
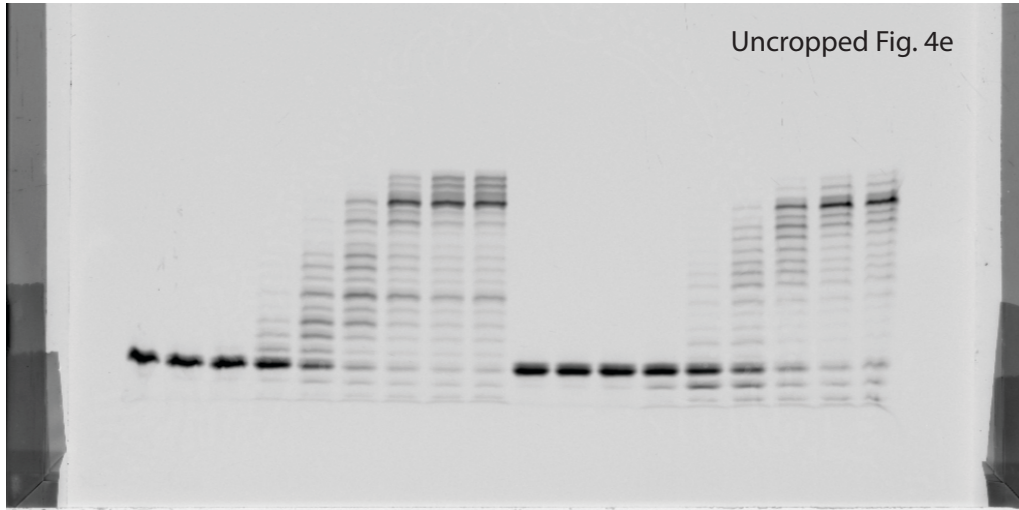
Supplementary Figure 6 | Exonuclease and polymerase inhibition by diverse metals. Different metals inhibit the PHP-exonuclease and polymerase at mM concentrations (a) Exonuclease activity on a single stranded DNA substrate (b) Polymerase activity on a matched substrate (c) Polymerase activity on a mismatched DNA substrate (d) Polymerase activity on a non-cleavable mismatched DNA substrate. Note that MnCl_2 enables the polymerase to extend from a mismatch, but inhibits the exonuclease. All reactions contained 2 mM Mg and increasing amounts of the indicated metal (0-20 mM). See main Fig. 4e-f for more details. Uncropped gels are shown in Supplementary Fig. 8.



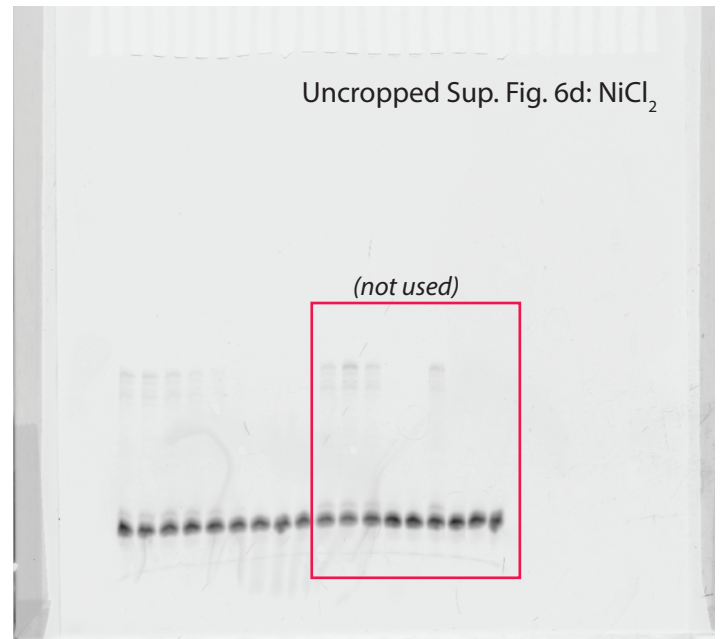
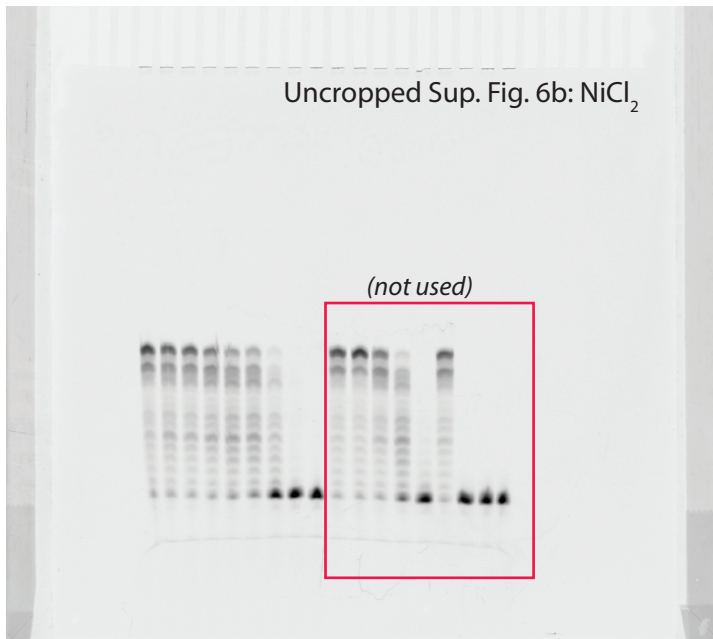
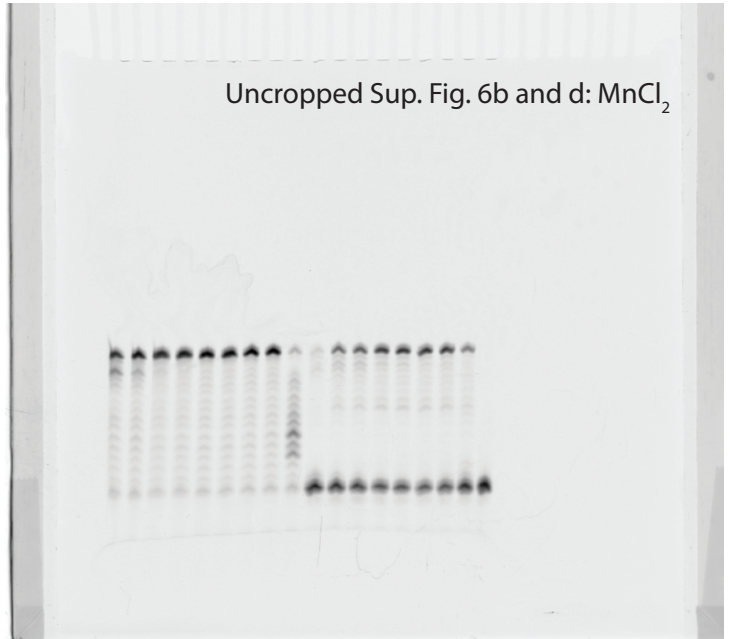
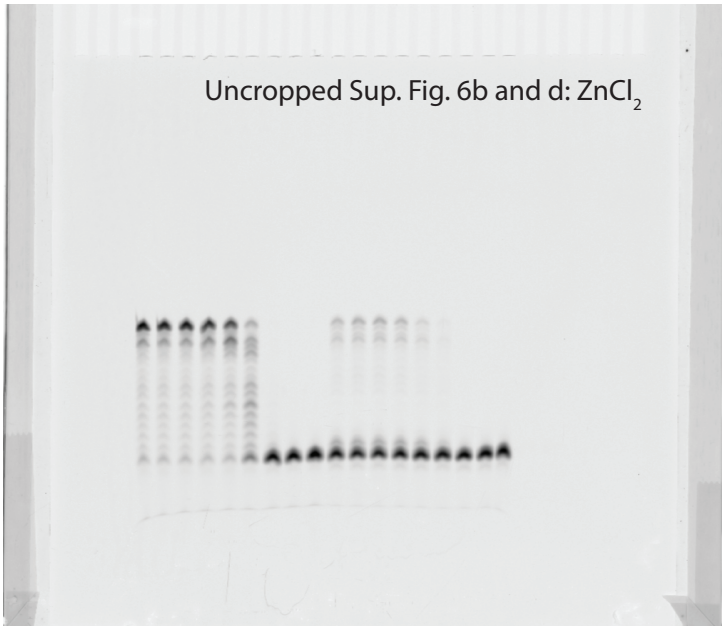
Supplementary Figure 7 | DNA binding in the endonuclease/exonuclease pairs EndoIV/PHP and APE1/TDP2 (a) dsDNA with an abasic site bound by *E. coli* Endo IV¹¹ (b) Modeling of ssDNA binding in PHP domain of Mtb DnaE1 (c) dsDNA with an abasic site bound by human APE1¹² (d) ssDNA binding in the zebrafish TDP2¹³.

Supplementary References

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Supplementary Figure 8 | Uncropped gels for Figures 3e-f and Supplementary Figure 6a



Supplementary Figure 8 (continued) | Uncropped gels for Supplementary Figure 6b and 6d

